Identification of African Swine Fever Virus antigens for development of an efficacious subunit vaccine

by

Michelle Dawn Zajac

B.S., Kansas State University, 2009
M.S., Kansas State University, 2019

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology
College of Veterinary Medicine

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Abstract

African Swine Fever (ASF) is a virulent disease in domestic swine and wild boar that is caused by the African Swine Fever Virus (ASFV), a complex enveloped DNA virus in the family Asfarviridae. Epidemics caused by the ASFV have an overwhelming economic influence on impacted areas and jeopardize swine commerce globally with nearly 100% mortality in naïve populations. There is no vaccine or treatment available and current control measures focus on the use of antiquated regulatory methods such as quarantine, limiting transport, and depopulation of affected swine. Previous studies have shown that some ASFV mutants can confer protection, but safety and vaccine virus scale-up need to be addressed. Development of a subunit vaccine would be more attractive: however, the actual protective antigen(s) have not yet been identified. The first study aimed to identify ASFV subunit vaccine candidates that contain CD8+ T cell epitopes by using lymphocytes from pigs that had been immunized with an antigen cocktail, which included the largest ASFV protein (pp220 polyprotein) that is processed to generate p5, p34, p14, p37, and p150 individual proteins. The results from this study showed that four predicted *SLA*-I binding nonamer peptides (p34$^{161-169}$, p37$^{859-867}$, p150$^{1363-1371}$, and p150$^{1463-1471}$) elicited robust IFN-γ+ responses in peripheral blood mononuclear cells (PBMCs) and splenocytes. These peptides are highly conserved among different ASFV genomes. The role played by these epitopes in immune protection will need to be determined in challenge studies. In the second study, an adjuvanted or non-adjuvanted adenovirus-based subunit vaccine candidate containing nearly all of the proteins in the ASFV proteome (which included pp220 antigens) was evaluated in pigs in a homologous prime-boost-boost immunization regimen followed by challenge using a natural transmission model. The results of this second study showed that this adenovirus-vectored ASFV vaccine cocktail induced robust antibody responses in swine, but only one pig survived. Future studies will

entail the development of a porcine-specific granzyme B (PGB) monoclonal antibody to screen PBMCs from immunized pigs to identify novel IFN-γ+/granzyme B+ T cell epitopes present in ASFV vaccine candidates by using flow cytometric analyses following intracellular staining. More screening is required to identify additional novel IFN-γ+/granzyme B+ T cell epitopes for inclusion in a rationally designed prototype subunit vaccine to be tested for protective efficacy in pigs. Altogether, the knowledge generated in these and future studies will inform the design of an efficacious ASFV subunit vaccine that is needed to safeguard the pork industry against the risk posed by ASFV.

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Dedication

I would like to dedicate this work to my daughter, Alexis Zajac. May you forge ahead towards all of your dreams with full force and always believe in your potential.

Love,

Mom

Chapter 1 – Current Progress and Limitations in ASFV Vaccine Development

1.1 African Swine Fever Virus (ASFV)

African Swine Fever (ASF) disease is the result of infection with a large, complex *Asfivirus* in the family *Asfarviridae* (Dixon et al. 2020; Alonso et al. 2018). The causative agent, African Swine Fever Virus (ASFV) is a double-stranded enveloped DNA virus transmitted via soft-bodied ticks or nose-to-nose contact in both domestic and wild pigs (Dixon et al. 2020; Alonso et al. 2018). Typical disease presentation ranges from mild clinical symptoms to rapid death and hemorrhage. The complexity of ASFV and its ability to encode for much of its replication machinery has led to difficulty in combating this viral disease (Duan et al. 2022; Cackett et al. 2020; Wang et al. 2021). The ASFV replicates in the host cell cytoplasm and it targets macrophages and other mononuclear cells as the primary sites of infection (Martins et al. 1993). This is key to one of the main mechanisms involved in viral evasion resulting in dysregulation of the type I interferon response and exhaustion of the cytotoxic T-lymphocyte (CTL) subset responsible, partly, for viral clearance (Martins et al. 1993; Yu et al. 2023). It is important to understand this for rational and targeted delivery of vaccine material to antigen-presenting cells (APCs) (Alvarez et al. 2013). This approach leads to the critical induction of the memory response and expansion of ASFV-specific lymphocytes responsible for both the cellular (T-cell driven) and humoral (antibody-driven) immune responses (Alvarez et al. 2013; Argilaguet et al. 2012).

Since ASFV surveillance began in 2005, it has spread from Africa to Georgia and Eastern Europe (2007), the European Union (2014), Asia (2018), Oceania (2019-2020), the Americas (2021), and more recently increased its territory in Asia and Europe (2022) (Dixon et al. 2020; Spinard et al. 2023; Gallardo et al. 2023). A majority of the most severe outbreaks and disease

have recently been caused by the genotype II strains (Ankhanbaatar et al. 2021; Beltran-Alcrudo et al. 2019; Calkins and Scasta 2020). The ASFV is highly stable, allowing it to remain viable and infectious under multiple harsh natural conditions making this virus capable of effortless transmission, thus, stringent biosecurity and biocontainment must be followed to counteract transmission (Lewis et al. 2000).

The accomplishment of whole genome sequencing from multiple isolates has increased ASFV knowledge and improved the predictive capabilities for the design and generation of novel vaccines and efforts toward innovative control approaches (Yáñez et al. 1995; Blome, Gabriel, and Beer 2014; Portugal et al. 2015; Herrera-Uribe et al. 2018; Zhu and Meng 2020). Control of ASFV and development of an effective vaccine will require identification of protective antigens as well as understanding viral infection and evasion mechanisms (Borca et al. 1998; Correia, Ventura, and Parkhouse 2013; Takamatsu et al. 2013). There are twenty-four ASFV genotypes identified so far, as determined by the C-terminal sequence of its p72 surface protein transcribed from the *B646L* gene ("African Swine Fever" 2019; Qu et al. 2022; Cackett et al. 2020). Greater than sixty structural proteins and over one hundred proteins associated with infection have been identified, which complicates vaccine development (Alejo et al. 2018; Sanchez et al. 2012; Montaner-Tarbes et al. 2019; Wöhnke et al. 2022). Presently, mass culling of affected and neighboring swine herds followed by appropriate sanitation of farms are the only countermeasures for controlling the spread of the disease (Dixon et al. 2020; "African Swine Fever" 2019). Historically, vaccine efforts have remained largely unsuccessful with no current vaccine or treatment available (Chathuranga and Lee 2023). Despite this, numerous prototype vaccines including novel ASFV antigens and strategies are in various stages of evaluation and development.

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1.2 Recent ASFV Vaccines

Multiple ASFV mutants, natural isolates or generated by targeted gene deletion, have been tested for their protective efficacy in domestic and wild swine. These viral strains have low virulence due to loss of specific DNA sequences associated with transmission and infectivity, but they retain ability to stimulate a protective immune response (Leitão et al. 2001) (Arias et al. 2017; Revilla, Perez-Nunez, and Richt 2018; Borca et al. 2021; Zhang et al. 2021; Ramirez-Medina et al. 2022; Lopez et al. 2020; Monteagudo et al. 2017; Attreed et al. 2022; Sanford et al. 2016; Neilan et al. 2002; Abrams et al. 2013; Gallardo et al. 2018; Fernandez-Sainz et al. 2019; O'Donnell et al. 2017; Chen et al. 2020; Tran et al. 2021; Barasona et al. 2021; Teklue et al. 2020; Gladue and Borca 2022). Inactivation traditionally involves the employment of heat or chemical treatment to abrogate infectivity while retaining the immunogenic protein characteristics (Cadenas-Fernandez et al. 2021; Pikalo et al. 2022). Direct delivery methods of known antigens can be through the administration of recombinant DNA that result in ASFV gene expression, or through packaging these nucleic acids into a non-pathogenic live vector for delivery (Abrams et al. 2013; Gómez-Puertas et al. 1998; Barderas et al. 2001; Neilan et al. 2004; Ruiz-Gonzalvo, RodríGuez, and Escribano 1996; Argilaguet et al. 2013; Argilaguet et al. 2011; Lacasta et al. 2014; Netherton et al. 2019; Lokhandwala et al. 2019; Murgia et al. 2019; Jancovich et al. 2018; Goatley et al. 2020).

1.2.1 Inactivated ASFV vaccines

Attempts to establish an immunization strategy using inactivated ASFV antigens have proven to be largely unproductive with little to no protection despite the induction of robust ASFV-specific antibodies (Blome, Gabriel, and Beer 2014; Cadenas-Fernandez et al. 2021; Pikalo et al. 2022). One study revealed that immunization with an inactivated Armenia08 strain using adjuvants was

not protective after homologous challenge despite the robust production of specific antibodies (Blome, Gabriel, and Beer 2014). Another efficacy study reported a similar negative survival outcome using inactivated Pol16/DP/OUT21 formulated in multiple adjuvants. (Cadenas-Fernandez et al. 2021). More recently, an alternative study utilized gamma-irradiated Estonia 2014 (with adjuvant) as the immunogen and determined protective efficacy using a heterologous virus for challenge (Pikalo et al. 2022). This study revealed that again, while post-immunization IgG antibodies were present, no protection was achieved with a notable increase in clinical disease presentation (Pikalo et al. 2022). These outcomes suggest that antibodies may cause an enhancement of disease. This information brings to light the ongoing knowledge gap of the functions, types, and mechanisms of ASFV-specific antibodies in conferring protection that still needs to be addressed (Sánchez-Cordón et al. 2018).

1.2.2 Live attenuated ASFV vaccines (LAVs)

One potential way to circumvent vaccine ineffectiveness associated with stimulating antibodies alone is to target stimulation of both the humoral and cellular immune responses. Live attenuated vaccines can simultaneously activate these immune responses due to their ability to replicate in the host and also encode a majority of the native genes that act as antigenic targets (Lacasta et al. 2015; Carlson et al. 2016). While vaccination with live attenuated virus under laboratory conditions has failed to yield the desired outcome, promising results from isolates obtained from the field suggest that further investigation is warranted (Lacasta et al. 2015; Balysheva et al. 2015; Krug et al. 2015; Leitão et al. 2001; Gallardo et al. 2018; Portugal et al. 2015). Field isolates with varying virulence ranging from low to high levels result in a broad presentation of clinical symptoms and disease (Blome, Gabriel, and Beer 2013). Wild boar and domestic pigs are

susceptible, and they display anywhere between peracute form of ASF with sudden onset of death to the chronic form with little to no signs of disease (Blome, Gabriel, and Beer 2013). One finding consistent across multiple studies that tested low virulent strains of ASFV revealed the correlation between increased cellular activity and a robust antibody response with a positive survival outcome (Leitão et al. 2001; Onisk et al. 1994; Zheng, He, and Baker 1997; Oura et al. 2005; de León, Bustos, and Carrascosa 2013; Lokhandwala et al. 2019; Borca et al. 2021; Tran et al. 2022). One specific example is immunization with the low-virulent NH/P68 (NHV) strain that can confer protection against challenge with the highly virulent Lisboa60 (L60) (Leitão et al. 2001). While there is a risk of reversion to virulence when employing naturally attenuated strains as vaccines and the possibility of increased post-vaccination side effects, there is a need for further studies to address these limitations in order to generate a safe and efficacious live attenuated ASFV vaccine (Gallardo et al. 2018).

To circumvent this increased risk of reversion, gene deletion techniques are the next logical steps in vaccine development. Numerous ASFV gene deletion mutants have been developed and tested for their ability to elicit immune protection from disease. Genes associated with virulence and replication, such as DP96R and DP71L, are rational targets for ASFV deletions (Gallardo et al. 2018; Abrams et al. 2013). Genes associated with the reduction of IFN-γ (A276R, DP148R, MGF360, MGF530/505, I329L, and K205R) are also rational targets (Gallardo et al. 2018; Reis et al. 2016; Reis et al. 2017; Correia et al. 2023; Lokhandwala et al. 2017; Wang et al. 2022). These deletions could lead to generation of progeny virus that retains the ability to elicit a cytotoxic T-cell (CTL) response against ASFV. The encoded proteins from these genes are involved in a range of immune processes including but not limited to: hemadsorption of red blood cells on infected target cells; inhibiting apoptosis and transcription; endonuclease activity; as well as other

processes such as oxidative phosphorylation that all impact viral pathogenesis (Dixon et al. 2013; Lewis et al. 2000; Gómez-Puertas et al. 1998). Immunization of pigs with an EP402R gene deletion mutant resulted in a notable reduction in tissue viremia following homologous and heterologous challenge (Argilaguet et al. 2013; Monteagudo et al. 2017). In some instances, immunization with certain gene deletion mutants resulted in viremia but limited protection upon challenge (Abrams et al. 2013; Fernandez-Sainz et al. 2019). Variable results were also noted when MGF360, MGF505, or B119GL genes were deleted from ASFV Georgia 2007/1 (O'Donnell, Holinka, Gladue, et al. 2015; O'Donnell, Holinka, Krug, et al. 2015; O'Donnell et al. 2016; O'Donnell et al. ; Wang et al. 2018; Lewis et al. 2000). Individual deletion mutants induced protection against homologous challenge, but reduced protection was noted with deletion of MGF360/505 and B119GL. Enhanced protection was noted when DP96R/UK (inhibits the cGAS-STING pathway) and B119GL (oxidative phosphorylation/growth in macrophages) were deleted (O'Donnell, Holinka, Gladue, et al. 2015; O'Donnell, Holinka, Krug, et al. 2015; O'Donnell et al. 2016; O'Donnell et al. ; Wang et al. 2018; Lewis et al. 2000). The success of the latter gene deletion is likely due to a reduction in viral fitness in host macrophages and the ability to retain the functionality of the IFN-β pathway for crosstalk between the innate and adaptive immune systems. Data suggests that the variability in the protective potential is strain specific as noted in the outcomes obtained from Thymidine kinase gene deletions comparing Malawi (protective) and Georgia 2007/1 (non-protective) strains (Moore et al. 1998; Sanford et al. 2016; Monteagudo et al. 2017).

Recent work by Borca et. al. reported the first deletion mutant (ASFV-G-ΔI177L) capable of stimulating a robust sterilizing immunity in domestic pigs (Borca et al. 2020; Borca et al. 2021; Tran et al. 2021; Attreed et al. 2022). Despite claims of work towards commercializing this

prototype vaccine by two Vietnamese companies, no vaccine was manufactured (Urbano and Ferreira 2022). A derivative strain (ASFV-G-ΔI177L/ΔLVR) capable of replication in swine epithelial cells was developed with similar immunogenicity and efficacy leading to commercial vaccine development of NAVET-ASFVAC by NAVETCO (Borca et al. 2021; Tran et al. 2022). Unfortunately, this vaccine was pulled from the market less than 6 months post-release due to adverse reactions and deaths in domestic pigs. While unconfirmed, it was likely due to incorrect vaccine usage. Additional vaccine development efforts have resulted in generation of promising candidates (ASFV-G-Δ9GL, ASFV-G-ΔMGF, and ASFV-G-Δ9GL/ΔUK) (Gladue et al. 2020; Ramirez-Medina et al. 2022; Deutschmann et al. 2022; O'Donnell et al. 2016; Silva et al. 2022). More recently, complete protection was achieved using the following ASFV gene deletion mutant strains: ASFV-SY18-ΔCD2v/ΔUK; SY18ΔI226R; ASFV-G-ΔA137R; and ASFV-G-ΔE184L (Teklue et al. 2020; Zhang et al. 2021; Gladue et al. 2020; Ramirez-Medina et al. 2022). The ASFV-G-ΔE184L mutant is DIVA compatible, which would make it more attractive for deployment in countries that are still free of ASFV (Ramirez-Medina et al. 2022). While progress has been made in development of live attenuated ASFV prototype vaccines, safety and vaccine virus scalability are still limitations that need to be addressed.

1.2.3 Protein-based Subunit Vaccines

Protein-based subunit vaccines consist of defined antigen(s) that are used to elicit protective immune responses and offer increased safety compared to live attenuated pathogens (Gaudreault and Richt 2019). The target antigen can be generated as a purified recombinant protein that is formulated in adjuvant and used to immunize target host(s). The key ASFV antigens that have been evaluated using this methodology include, p54, p30, p72, and CD2v, and similar to the LAVs,

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they also vary in their immunogenicity and protective capabilities when used individually or in combined formulations (Angulo, Vinuela, and Alcami 1993; Gomez-Puertas et al. 1996; Ruiz-Gonzalvo, RodrÍGuez, and Escribano 1996; Gómez-Puertas et al. 1998; Neilan et al. 2004; Burmakina et al. 2016). The most positive data arose from studies that were conducted using baculovirus-expressed p54/p30 or CD2v proteins of which both achieved some degree of protection despite contradictory immunogenicity data (Barderas et al. 2001; Ruiz-Gonzalvo, RodrÍGuez, and Escribano 1996). Another candidate vaccine formulated using p30, p54, and p72 antigens did not confer protection following challenge despite induction of strong antigen-specific antibodies (Neilan et al. 2004).

1.2.3.1 DNA-based subunit vaccine candidates

This approach involves immunization with recombinant mammalian expression plasmid DNA constructs encoding vaccine target antigen. DNA vaccines have increased stability, are scalable, do not use infectious vectors for delivery, and are capable of stimulating B-, T-helper, and CTL cellular responses (Argilaguet et al. 2008; Takamatsu et al. 2013). Several ASFV antigens (p30, p54, hemagglutinin extracellular domain) have been tested in a DNA vaccine format with partial protection following challenge (Argilaguet et al. 2012; Argilaguet et al. 2011). Interestingly, increased protection and cellular immune responses were noted when these genes were ubiquitinated (Argilaguet et al. 2011; Argilaguet et al. 2012). Following these encouraging results, a large (4,000 fragments) DNA expression library, again fused to ubiquitin, conferred partial protection against virulent challenge (Lacasta et al. 2014). A combination DNA-recombinant protein prime-boost immunization strategy followed by challenge with the Armenia 2007 strain did not confer protection, and early death and increased pathology were noted (Sunwoo et al.

2019). In contrast, a recent investigation targeting ASFV genes M448R and MGF505-7R improved protection in pigs when administered using a DNA prime-attenuated virus boost approach (Bosch-Camós et al. 2021). There is evidence to support the utility of DNA or protein vaccination, but the mode of delivery lacks consistent immunogenicity and protective efficacy.

1.2.3.2 Live-vectored subunit vaccine candidates

This vaccine development approach involves *in vivo* antigen delivery using a live vector expressing vaccine target antigen. Live-vectored ASFV subunit vaccines have been developed using Baculovirus (BacMam), modified *Vaccinia virus* Ankara (MVA), Alphavirus, Adenovirus Type 5, and more recently poxvirus and *Lactobacillus* (Argilaguet et al. 2013; Lopera-Madrid et al. 2017; Murgia et al. 2019; Lokhandwala et al. 2016b; Lokhandwala et al. 2017; Lokhandwala et al. 2019; Netherton et al. 2019; Zhang et al. 2022; Lopera-Madrid et al. 2021; Zajac et al. 2022). Several live-vectored antigen cocktails have been tested for immunogenicity and safety with increased antibody production and T-cell responses noted (Lopera-Madrid et al. 2017; Murgia et al. 2019; Netherton et al. 2019; Zhang et al. 2022; Lopera-Madrid et al. 2021). These studies did not challenge the vaccinees and therefore, efficacy was not determined and thus, further studies are needed. Immunization of pigs with MVA- and adenovirus-vectored subunit vaccine candidates elicited robust humoral and cellular immune responses, but variable levels of protection was noted following challenge (Argilaguet et al. 2013; Lokhandwala et al. 2016; Lokhandwala et al. 2017; Lokhandwala et al. 2019; Netherton et al. 2019). In one study, an increase in protection was associated with low antibody response, while high antibody levels were associated with poor survival which would be consistent with antibody-dependent enhancement (Lokhandwala et al. 2019). Other studies have shown that prime-boost immunization with multiple ASFV antigens

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resulted in a significant reduction of viremia post-challenge (Jancovich et al. 2018; Netherton et al. 2019). Even though no protection was noted, this methodology warrants further investigation to identify the protective ASFV antigens required to develop an efficacious subunit vaccine (Netherton et al. 2019). Alternative vectors have been explored, and are in early developmental phases, but show great promise as novel delivery systems for ASFV antigens (Lopera-Madrid et al. 2021; Zhang et al. 2022).

1.3 Future directions

Improvements are necessary for continued progress in the research and development of a successful ASFV vaccine. To date, much is still unknown and remains to be tested for the most efficacious immunization route, prime-boost vaccine schedules, challenge models, and protective antigen targets. Multiple studies support mucosal routes of vaccination versus the traditional intradermal inoculation, and these are more appealing for the mass immunization of wild boar populations (Sánchez-Cordón et al. 2017; Barasona et al. 2019; Deutschmann et al. 2022; Bosch-Camós et al. 2022; Borca et al. 2021). Similarly, mimicking the natural route of ASFV transmission and infectious dose are equally important in designing an appropriate challenge efficacy study to assess the level of protection induced by immunization. Typically, immunization and challenge are achieved through direct needle inoculation; however, while ASF is an arthropod-associated disease, many endemic areas do not support the survival of these tick populations and transmission occurs via pig to pig contact, oral-fecal route or spread through contaminated fomites and feed (Guinat et al. 2016; Sánchez-Cordón et al. 2017; Niederwerder et al. 2019). Empirical identification of the antigen(s) that elicit protective immunity against ASF is yet to be determined. Bioinformatic approaches can predict and narrow CTL target antigens for further analysis in

immunization and challenge experiments and are critical in the development of a safe and efficacious ASFV vaccine (Bosch-Camós et al. 2021; Mima et al. 2020; Herrera and Bisa 2021; Imdhiyas et al. 2022; Dixon et al. 2013; Lokhandwala et al. 2016; Zajac et al. 2022).

1.4 Conclusion

Food security and animal health depend on the reduction in high swine mortality through prevention, diagnosis, and control of ASFV infection and spread. Whole genome sequencing and comparison of natural mutants versus gene deletion mutants has generated new knowledge regarding the factors involved in viral survival and spread. Computer-aided *in silico* prediction is a useful tool that can inform prioritization of the ASFV antigens that can induce protective immunity. Specifically, CD8+ T-cell epitope identification is lacking as well as the determination of the protective role of IgG antibodies. Given the recent negative outcomes from live attenuated ASFV vaccines, a safer alternative remains to be developed. Live-vectored subunit vaccine candidates have the best potential due to the relative ease of scale-up, ability to include multiple antigens, induction of antibody and T cell responses, and DIVA capability. Understanding the epidemiology, transmission, and correlates of protection is key to the successful design and deployment of a rationally designed ASFV vaccine.

Table 1.1 Naturally attenuated ASFV strains used for vaccination.

ASFV Immunization Strain	Homologous	Heterologous #1	Heterologous #2	Survival	Reference
				Infection strain(s)	
OURT88/3	OURT88/3			100%	(Abrams et al. 2013)
		OURT88/1	OURT88/1	75%	(King et al. 2011)
		OURT88/1	Benin 97/1	100%	
		OURT88/1	Uganda 65	100%	
NH/P68		L60		100%	(Leitão et al. 2001; Gallardo et al. 2018)
				100%	
			Armenia 07	100%	(Gallardo et al. 2018)
Pol16/DP/OUT21	Pol16/DP/OUT21 x2			0%	(Cadenas-Fernandez et al. 2021)
Estonia 2014		Armenia 2008		0%	(Pikalo et al. 2022)
Lv17/WB/Rie1				50%	(Gallardo et al. 2018)
		Armenia 2007		92%	(Barasona et al. 2019)
Congo KK-262	K49			20%	(Burmakina et al. 2016)

Table 1.2 ASFV vaccines attenuated by gene-deletion.

ASFV Strain	Deleted gene(s)	Challenge strain(s)			Protection	Reference
		Homologous	Heterologous #1	Heterologous #2		
OURT/88/3	DP71L and DP96R	OURT88/3ΔDP2			Partial (66%)	(Abrams et al. 2013)
NH/p68	A238L		L60	Armenia 07	Full	(Gallardo et al. 2018)
			Armenia 07		None	
	EP153R		L60	Armenia 07	Full	
			Armenia 07		None	
	A224L		L60	Armenia 07	Full	
			Armenia 07		Partial (50%)	
Benin97/1	MGF	Benin 97/1			Partial	(Reis et al. 2016)
	DP148R	Benin 97/1			Partial (94%)	(Reis et al. 2017)
Georgia 07/1	9GL	Georgia 07/1			Partial (80%)	(O'Donnell, Holinka, Gladue, et al. 2015)
	MGF	Georgia 07/1			Partial (50%)	(Deutschmann et al. 2022)
	9GL & UK	Georgia 07/1			Full	(O'Donnell et al. 2016)
	NL	Georgia 07/1			Partial (80%)	(Ramirez-Medina et al. 2020)
	UK	Georgia 07/1			None	
	9GL, NL, and UK	Georgia 07/1			None	
	9GL & CD2v	Georgia 07/1			None	(Gladue et al. 2020)
	9GL, CD2v, & EP153R	Georgia 07/1			None	
		Georgia 07/1			Full	(Zhu et al. 2019)
	I177L		TTKN/ASFV/DN/2019		Partial (80%)	(Tran et al. 2021)
	I177L & LVR	Georgia 07/1			Full	(Borca et al. 2021)
Georgia 2010	A137R	Georgia 2010			Full	(Gladue et al. 2020)
	E184L	Georgia 2010			Partial (60%)	(Ramirez-Medina et al. 2022)
Ba71	EP402R		E75		Partial (72%)	(Monteagudo et al. 2017)
			Georgia 07/1		Full	
SY18	I226R	SY18			Full	(Zhang et al. 2021)
	CD2v & UK	SY18			Full	(Teklue et al. 2020)
Arm/07/CBM/c2	EP402R (CD2v) & A238L	Arm/07/CBM/c2			Full	(Pérez-Núñez et al. 2022)

UK=DP96R; CD2v=EP402R; MGF=Multigene family; 9GL=B119L; NL=DP171L; C-type Lectin=EP153R

Table 1.3 ASFV antigen-based vaccine formulations.

Immunogen Type	ASFV proteins /genes	Expression system or vector		Total Doses	Challenge	Protection	Reference
		Prime	Boost				
Protein	CD2v	Baculovirus		3	E75	Partial	(Ruiz-Gonzalvo, RodrÍGuez, and Escribano 1996)
	p30 or p54	Baculovirus		1	E75	Full	(Gómez-Puertas et al. 1998)
	p30 & p54 (chimeric)	Baculovirus		5	E75	Partial	(Barderas et al. 2001)
	p22, p30, p54, & p72	Baculovirus		1	Pr4	None	(Neilan et al. 2004)
Vectored	CD2v & C-type Lectin	France FK-32/135		2 & 5	K49	Full	(Burmakina et al. 2016)
	p30, p54, & sHA	BacMam-sHAPQ		2 & 4	E75	Partial	(Argilaguet et al. 2013)
	p72, CD2v, & EP153R	MVA		2	immunogenicity only	IFN-γ T-cell+	(Lopera-Madrid et al. 2017)
	7 and 12 antigen cocktails	Adenovirus		2	immunogenicity only	IFN-γ T-cell+	(Lokhandwala et al. 2016b; Lokhandwala et al. 2017)
	7 antigen cocktail	Adenovirus				Partial	
	7 antigen cocktail	Adenovirus		2	Georgia 2007/1	None	(Lokhandwala et al. 2019)
	12 antigen cocktail	Adenovirus				None	
	p30, p54 and pHA-72	Alphavirus (x2)	Attenuated OURT88/3	3	immunogenicity only	antibody correlation	(Murgia et al. 2019)
	18 antigen cocktail	Adenovirus	MVA	2	OURT88/1	IFN-γ T-cell+	(Netherton et al. 2019)
	pp220	Adenovirus		2	immunogenicity only	IFN-γ T-cell+	(Zajac et al. 2022)
DNA Subunit	DNA expression library	DNA		1	E75	Partial	(Lacasta et al. 2014)
	p54/E183L, p30/CP204L	DNA		3	E75	None	(Argilaguet et al. 2012)
	Ubiquitin-CD2v/pEP402R-p54/E183L-p30/CP204L	DNA		2 & 4	E75	Partial	(Argilaguet et al. 2012)
Combination	47 antigen pool	DNA	MVA	2	Georgia 2007/1	Partial	(Jancovich et al. 2018)
	p15, p35, p54, and +/−p17 & p32, p72, CD2v, and +/−p17	DNA & protein		3	Arm07	None	(Sunwoo et al. 2019)
	M448R, MGF505-7R & BA71ΔCD2	DNA (x2)	Attenuated BA71ΔCD2	3	Georgia 2007/1	Partial	(Bosch-Camós et al. 2021)

MVA=Modified Vaccinia Virus Ankara; CD2v=EP402R; C-type Lectin=EP153R

1.5 References:

Abrams, C. C., L. Goatley, E. Fishbourne, D. Chapman, L. Cooke, C. A. Oura, C. L. Netherton, H. H. Takamatsu, and L. K. Dixon. 2013. 'Deletion of virulence associated genes from attenuated African swine fever virus isolate OUR T88/3 decreases its ability to protect against challenge with virulent virus', *Virology*, 443: 99-105.

Alejo, Alí, Tania Matamoros, Milagros Guerra, and Germán Andrés. 2018. 'A Proteomic Atlas of the African Swine Fever Virus Particle', *Journal of Virology*, 92.

Alonso, Covadonga, Manuel Borca, Linda Dixon, Yolanda Revilla, Fernando Rodriguez, and Jose M. Escribano. 2018. 'ICTV Virus Taxonomy Profile: Asfarviridae', *Journal of General Virology*, 99: 613-14.

Alvarez, B., T. Poderoso, F. Alonso, A. Ezquerra, J. Domínguez, and C. Revilla. 2013. 'Antigen targeting to APC: from mice to veterinary species', *Dev Comp Immunol*, 41: 153-63.

Angulo, Ana, Eladio Vinuela, and Antonio Alcami. 1993. 'Inhibition of African swine fever virus binding and infectivity by purified recombinant virus attachment protein p12', *Journal of Virology*, 67: 5463-71.

Ankhanbaatar, Ulaankhuu, Tserenchimed Sainnokhoi, Buyantogtokh Khanui, Gerelmaa Ulziibat, Tserenjav Jargalsaikhan, Dulam Purevtseren, Tirumala Bharani K. Settypalli, John Flannery, William G. Dundon, Ganzorig Basan, Carrie Batten, Giovanni Cattoli, and Charles E. Lamien. 2021. 'African swine fever virus genotype II in Mongolia, 2019', *Transboundary and Emerging Diseases*, 68: 2787-94.

African Swine Fever. Paris: World Organization for Animal Health (2019).

Argilaguet, J., C Gallardo, M Nofrarías, J Pujols, M. Pérez-Filgueira, and M. Blanco-Fuertes. 2008. "DNA vaccines against African swine fever virus: Relevance of humoral and cellular responses in protection." In. Durban.

Argilaguet, J.M., E. Pérez-Martín, M. Nofrarías, C. Gallardo, F. Accensi, A. Lacasta, M. Mora, M. Ballester, I. Galindo-Cardiel, and S. López-Soria. 2012. 'DNA Vaccination Partially Protects against African Swine Fever Virus Lethal Challenge in the Absence of Antibodies', *PLoS One*, 7: e40942.

Argilaguet, JM, E Pérez-Martín, C Gallardo, FJ Salguero, B Borrego, A Lacasta, F Accensi, I Díaz, M Nofrarías, and J Pujols. 2011. 'Enhancing DNA immunization by targeting ASFV antigens to SLA-II bearing cells', *Vaccine*, 29: 5379-85.

Argilaguet, Jordi M., Eva Pérez-Martín, Sergio López, Martin Goethe, J. M. Escribano, Katrin Giesow, Günther M. Keil, and Fernando Rodríguez. 2013. 'BacMam immunization partially protects pigs against sublethal challenge with African swine fever virus', *Antiviral Research*, 98: 61-65.

Arias, Marisa, Ana De la Torre, Linda Dixon, Carmina Gallardo, Ferran Jori, Alberto Laddomada, Carlos Martins, R Michael Parkhouse, Yolanda Revilla, and Fernando Rodriguez. 2017. 'Approaches and perspectives for development of African swine fever virus vaccines', *Vaccines*, 5: 35.

Attreed, Sarah E., Christina Silva, Sophia Abbott, Elizabeth Ramirez-Medina, Nallely Espinoza, Manuel V. Borca, Douglas P. Gladue, and Fayna Diaz-San Segundo. 2022. 'A Highly Effective African Swine Fever Virus Vaccine Elicits a Memory T Cell Response in Vaccinated Swine', *Pathogens*, 11: 1438.

Balysheva, V. I., E. Y. Prudnikova, T. V. Galnbek, and V. M. Balyshev. 2015. '[Continuous cell subline A4C2/9K and its application to the african swine fever virus study]', *Vopr Virusol*, 60: 43-7.

Barasona, J. A., E. Cadenas-Fernández, A. Kosowska, S. Barroso-Arévalo, B. Rivera, R. Sánchez, N. Porras, C. Gallardo, and J. M. Sánchez-Vizcaíno. 2021. 'Safety of African Swine Fever Vaccine Candidate Lv17/WB/Rie1 in Wild Boar: Overdose and Repeated Doses', *Front Immunol*, 12: 761753.

Barasona, Jose A, Carmina Gallardo, Estefanía Cadenas-Fernández, Cristina Jurado, Belén Rivera, Antonio Rodríguez-Bertos, Marisa Arias, and Jose M Sánchez-Vizcaíno. 2019. 'First oral vaccination of Eurasian wild boar against African swine fever virus genotype II', *Frontiers in Veterinary Science*, 6: 137.

Barderas, MG, F Rodriguez, P Gomez-Puertas, M Aviles, F Beitia, C Alonso, and JM Escribano. 2001. 'Antigenic and immunogenic properties of a chimera of two immunodominant African swine fever virus proteins', *Archives of Virology*, 146: 1681-91.

Beltran-Alcrudo, Daniel, John R. Falco, Eran Raizman, and Klaas Dietze. 2019. 'Transboundary spread of pig diseases: the role of international trade and travel', *BMC Veterinary Research*, 15.

Blome, Sandra, Claudia Gabriel, and Martin Beer. 2013. 'Pathogenesis of African swine fever in domestic pigs and European wild boar', *Virus Research*, 173: 122-30.

Blome, Sandra, Claudia Gabriel, and Martin Beer. 2014. 'Modern adjuvants do not enhance the efficacy of an inactivated African swine fever virus vaccine preparation', *Vaccine*, 32: 3879-82.

Borca, M. V., C. Carrillo, L. Zsak, W. W. Laegreid, G. F. Kutish, J. G. Neilan, T. G. Burrage, and D. L. Rock. 1998. 'Deletion of a CD2-like gene, 8-DR, from African swine fever virus affects viral infection in domestic swine', *J Virol*, 72: 2881-9.

Borca, M. V., E. Ramirez-Medina, E. Silva, E. Vuono, A. Rai, S. Pruitt, L. G. Holinka, L. Velazquez-Salinas, J. Zhu, and D. P. Gladue. 2020. 'Development of a Highly Effective

African Swine Fever Virus Vaccine by Deletion of the I177L Gene Results in Sterile Immunity against the Current Epidemic Eurasia Strain', *J Virol*, 94.

Borca, Manuel V., Elizabeth Ramirez-Medina, Ediane Silva, Elizabeth Vuono, Ayushi Rai, Sarah Pruitt, Nallely Espinoza, Lauro Velazquez-Salinas, Cyril G. Gay, and Douglas P. Gladue. 2021. 'ASFV-G-ΔI177L as an Effective Oral Nasal Vaccine against the Eurasia Strain of Africa Swine Fever', *Viruses*, 13: 765.

Bosch-Camós, Laia, Uxía Alonso, Anna Esteve-Codina, Chia-Yu Chang, Beatriz Martín-Mur, Francesc Accensi, Marta Muñoz, María J. Navas, Marc Dabad, Enric Vidal, Sonia Pina-Pedrero, Patricia Pleguezuelos, Ginevra Caratù, María L. Salas, Lihong Liu, Stanimira Bataklieva, Boris Gavrilov, Fernando Rodríguez, and Jordi Argilaguet. 2022. 'Cross-protection against African swine fever virus upon intranasal vaccination is associated with an adaptive-innate immune crosstalk', *PLOS Pathogens*, 18: e1010931.

Bosch-Camós, Laia, Elisabet López, Javier Collado, María J. Navas, Miguel Blanco-Fuertes, Sonia Pina-Pedrero, Francesc Accensi, Maria Luisa Salas, Egbert Mundt, Veljko Nikolin, and Fernando Rodríguez. 2021. 'M448R and MGF505-7R: Two African Swine Fever Virus Antigens Commonly Recognized by ASFV-Specific T-Cells and with Protective Potential', *Vaccines*, 9: 508.

Burmakina, G., A. Malogolovkin, E. R. Tulman, L. Zsak, G. Delhon, D. G. Diel, N. M. Shobogorov, Y. P. Morgunov, S. Y. Morgunov, G. F. Kutish, D. Kolbasov, and D. L. Rock. 2016. 'African swine fever virus serotype-specific proteins are significant protective antigens for African swine fever', *J Gen Virol*, 97: 1670-75.

Cackett, G., D. Matelska, M. Sýkora, R. Portugal, M. Malecki, J. Bähler, L. Dixon, and F. Werner. 2020. 'The African Swine Fever Virus Transcriptome', *J Virol*, 94.

Cadenas-Fernandez, E., J. M. Sanchez-Vizcaino, E. van den Born, A. Kosowska, E. van Kilsdonk, P. Fernandez-Pacheco, C. Gallardo, M. Arias, and J. A. Barasona. 2021. 'High Doses of Inactivated African Swine Fever Virus Are Safe, but Do Not Confer Protection against a Virulent Challenge', *Vaccines (Basel)*, 9.

Calkins, Craig M., and J. Derek Scasta. 2020. 'Transboundary Animal Diseases (TADs) affecting domestic and wild African ungulates: African swine fever, foot and mouth disease, Rift Valley fever (1996–2018)', *Research in Veterinary Science*, 131: 69-77.

Carlson, J., V. O'Donnell, M. Alfano, L. Velazquez Salinas, L. G. Holinka, P. W. Krug, D. P. Gladue, S. Higgs, and M. V. Borca. 2016. 'Association of the Host Immune Response with Protection Using a Live Attenuated African Swine Fever Virus Model', *Viruses*, 8.

Chathuranga, Kiramage, and Jong-Soo Lee. 2023. 'African Swine Fever Virus (ASFV): Immunity and Vaccine Development', *Vaccines*, 11: 199.

Chen, W., D. Zhao, X. He, R. Liu, Z. Wang, X. Zhang, F. Li, D. Shan, H. Chen, J. Zhang, L. Wang, Z. Wen, X. Wang, Y. Guan, J. Liu, and Z. Bu. 2020. 'A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs', *Sci China Life Sci*, 63: 623-34.

Correia, S., P. L. Moura, S. Ventura, A. Leitão, and R. M. E. Parkhouse. 2023. 'I329L: A Dual Action Viral Antagonist of TLR Activation Encoded by the African Swine Fever Virus (ASFV)', *Viruses*, 15.

Correia, S., S. Ventura, and R. M. Parkhouse. 2013. 'Identification and utility of innate immune system evasion mechanisms of ASFV', *Virus Res*, 173: 87-100.

de León, P., M. J. Bustos, and A. L. Carrascosa. 2013. 'Laboratory methods to study African swine fever virus', *Virus Res*, 173: 168-79.

Deutschmann, Paul, Tessa Carrau, Julia Sehl-Ewert, Jan Hendrik Forth, Elisenda Viaplana, Jose Carlos Mancera, Alicia Urniza, Martin Beer, and Sandra Blome. 2022. 'Taking a Promising Vaccine Candidate Further: Efficacy of ASFV-G-ΔMGF after Intramuscular Vaccination of Domestic Pigs and Oral Vaccination of Wild Boar', *Pathogens*, 11: 996.

Dixon, Linda K, David AG Chapman, Christopher L Netherton, and Chris Upton. 2013. 'African swine fever virus replication and genomics', *Virus Research*, 173: 3-14.

Dixon, Linda K., Karl Stahl, Ferran Jori, Laurence Vial, and Dirk U. Pfeiffer. 2020. 'African Swine Fever Epidemiology and Control', *Annual Review of Animal Biosciences*, 8: 221-46.

Duan, Xianghan, Yi Ru, Wenping Yang, Jingjing Ren, Rongzeng Hao, Xiaodong Qin, Dan Li, and Haixue Zheng. 2022. 'Research progress on the proteins involved in African swine fever virus infection and replication', *Frontiers in Immunology*: 3916.

Fernandez-Sainz, Ignacio, Tyler D. Gavitt, Marla Koster, Elizabeth Ramirez-Medina, Yelitza Y. Rodriguez, Ping Wu, Lawrence K. Silbart, Teresa de Los Santos, and Steven M. Szczepanek. 2019. 'The VP1 G-H loop hypervariable epitope contributes to protective immunity against Foot and Mouth Disease Virus in swine', *Vaccine*, 37: 3435-42.

Gallardo, C., E. G. Sánchez, D. Pérez-Núñez, M. Nogal, P. de León, L. Carrascosa Á, R. Nieto, A. Soler, M. L. Arias, and Y. Revilla. 2018. 'African swine fever virus (ASFV) protection mediated by NH/P68 and NH/P68 recombinant live-attenuated viruses', *Vaccine*, 36: 2694-704.

Gallardo, Carmina, Nadia Casado, Alejandro Soler, Igor Djadjovski, Laura Krivko, Encarnación Madueño, Raquel Nieto, Covadonga Perez, Alicia Simon, Emiliya Ivanova, Daniel Donescu, Vesna Milicevik, Eleni Chondrokouki, Imbi Nurmoja, Maciej Frant, Francesco Feliziani, Petr Václavek, Simona Pileviciene, and Arias Marisa. 2023. 'A multi gene-

approach genotyping method identifies 24 genetic clusters within the genotype II-European African swine fever viruses circulating from 2007 to 2022', *Frontiers in Veterinary Science*, 10.

Gaudreault, N. N., and J. A. Richt. 2019. 'Subunit Vaccine Approaches for African Swine Fever Virus', *Vaccines (Basel)*, 7.

Gladue, D. P., V. O'Donnell, E. Ramirez-Medina, A. Rai, S. Pruitt, E. A. Vuono, E. Silva, L. Velazquez-Salinas, and M. V. Borca. 2020. 'Deletion of CD2-Like (CD2v) and C-Type Lectin-Like (EP153R) Genes from African Swine Fever Virus Georgia-9GL Abrogates Its Effectiveness as an Experimental Vaccine', *Viruses*, 12.

Gladue, Douglas P., and Manuel V. Borca. 2022. 'Recombinant ASF Live Attenuated Virus Strains as Experimental Vaccine Candidates', *Viruses*, 14: 878.

Goatley, Lynnette C., Ana L. Reis, Raquel Portugal, Hannah Goldswain, Gareth L. Shimmon, Zoe Hargreaves, Chak-Sum Ho, María Montoya, Pedro J. Sánchez-Cordón, Geraldine Taylor, Linda K. Dixon, and Christopher L. Netherton. 2020. 'A Pool of Eight Virally Vectored African Swine Fever Antigens Protect Pigs against Fatal Disease', *Vaccines*, 8.

Gomez-Puertas, Paulino, Fernando Rodriguez, Jose M Oviedo, Fernando Ramiro-Ibanez, Francisco Ruiz-Gonzalvo, Covadonga Alonso, and Jose M Escribano. 1996. 'Neutralizing antibodies to different proteins of African swine fever virus inhibit both virus attachment and internalization', *Journal of Virology*, 70: 5689-94.

Gómez-Puertas, Paulino, Fernando Rodríguez, José M. Oviedo, Alejandro Brun, Covadonga Alonso, and José M. Escribano. 1998. 'The African Swine Fever Virus Proteins p54 and p30 Are Involved in Two Distinct Steps of Virus Attachment and Both Contribute to the Antibody-Mediated Protective Immune Response', *Virology*, 243: 461-71.

Guinat, C., A. Gogin, S. Blome, G. Keil, R. Pollin, D. U. Pfeiffer, and L. Dixon. 2016. 'Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions', *Vet Rec*, 178: 262-7.

Herrera-Uribe, J., A. Jimenez-Marin, A. Lacasta, P. L. Monteagudo, S. Pina-Pedrero, F. Rodriguez, A. Moreno, and J. J. Garrido. 2018. 'Comparative proteomic analysis reveals different responses in porcine lymph nodes to virulent and attenuated homologous African swine fever virus strains', *Vet Res*, 49: 90.

Herrera, Leana Rich De Mesa, and Elizabeth Paulino Bisa. 2021. 'In silico analysis of highly conserved cytotoxic T-cell epitopes in the structural proteins of African swine fever virus', *Veterinary World*: 2625-33.

Imdhiyas, M., S. Sen, N. Barman, L. Buragohain, Y. Malik, and S. Kumar. 2022. 'Computational analysis of immunogenic epitopes in the p30 and p54 proteins of African swine fever virus', *J Biomol Struct Dyn*: 1-10.

Jancovich, J. K., D. Chapman, D. T. Hansen, M. D. Robida, A. Loskutov, F. Craciunescu, A. Borovkov, K. Kibler, L. Goatley, K. King, C. L. Netherton, G. Taylor, B. Jacobs, K. Sykes, and L. K. Dixon. 2018. 'Immunization of Pigs by DNA Prime and Recombinant Vaccinia Virus Boost To Identify and Rank African Swine Fever Virus Immunogenic and Protective Proteins', *J Virol*, 92.

Krug, Peter W, Lauren G Holinka, Vivian O'Donnell, Bo Reese, Brenton Sanford, Ignacio Fernandez-Sainz, Douglas P Gladue, Jonathan Arzt, Luis Rodriguez, and Guillermo R Risatti. 2015. 'The progressive adaptation of a georgian isolate of African swine fever virus to vero cells leads to a gradual attenuation of virulence in swine corresponding to major modifications of the viral genome', *Journal of Virology*, 89: 2324-32.

Lacasta, Anna, María Ballester, Paula L. Monteagudo, Javier M. Rodríguez, María L. Salas, Francesc Accensi, Sonia Pina-Pedrero, Albert Bensaid, Jordi Argilaguet, and Sergio López-Soria. 2014. 'Expression Library Immunization Can Confer Protection against Lethal Challenge with African Swine Fever Virus', *Journal of Virology*, 88: 13322-32.

Lacasta, Anna, Paula L Monteagudo, Ángeles Jiménez-Marín, Francesc Accensi, María Ballester, Jordi Argilaguet, Iván Galindo-Cardiel, Joaquim Segalés, María L Salas, and Javier Domínguez. 2015. 'Live attenuated African swine fever viruses as ideal tools to dissect the mechanisms involved in viral pathogenesis and immune protection', *Veterinary Research*, 46: 1-16.

Leitão, Alexandre, Clara Cartaxeiro, Ricardo Coelho, Benedita Cruz, RME Parkhouse, Fernando C Portugal, José D Vigário, and Carlos LV Martins. 2001. 'The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response', *Journal of General Virology*, 82: 513-23.

Lewis, T., L. Zsak, T. G. Burrage, Z. Lu, G. F. Kutish, J. G. Neilan, and D. L. Rock. 2000. 'An African swine fever virus ERV1-ALR homologue, 9GL, affects virion maturation and viral growth in macrophages and viral virulence in swine', *J Virol*, 74: 1275-85.

Lokhandwala, S., V. Petrovan, L. Popescu, N. Sangewar, C. Elijah, A. Stoian, M. Olcha, L. Ennen, J. Bray, R. P. Bishop, S. D. Waghela, M. Sheahan, R. R. R. Rowland, and W. Mwangi. 2019. 'Adenovirus-vectored African Swine Fever Virus antigen cocktails are immunogenic but not protective against intranasal challenge with Georgia 2007/1 isolate', *Vet Microbiol*, 235: 10-20.

Lokhandwala, S., S. D. Waghela, J. Bray, C. L. Martin, N. Sangewar, C. Charendoff, R. Shetti, C. Ashley, C. H. Chen, L. R. Berghman, D. Mwangi, P. J. Dominowski, D. L. Foss, S. Rai, S. Vora, L. Gabbert, T. G. Burrage, D. Brake, J. Neilan, and W. Mwangi. 2016. 'Induction of Robust Immune Responses in Swine by Using a Cocktail of Adenovirus-Vectored African Swine Fever Virus Antigens', *Clin Vaccine Immunol*, 23: 888-900.

Lokhandwala, S., S. D. Waghela, J. Bray, N. Sangewar, C. Charendoff, C. L. Martin, W. S. Hassan, T. Koynarski, L. Gabbert, T. G. Burrage, D. Brake, J. Neilan, and W. Mwangi. 2017. 'Adenovirus-vectored novel African Swine Fever Virus antigens elicit robust immune responses in swine', *PLoS One*, 12: e0177007.

Lopera-Madrid, J., L. G. Medina-Magües, D. P. Gladue, M. V. Borca, and J. E. Osorio. 2021. 'Optimization in the expression of ASFV proteins for the development of subunit vaccines using poxviruses as delivery vectors', *Sci Rep*, 11: 23476.

Lopera-Madrid, Jaime, Jorge E Osorio, Yongqun He, Zuoshuang Xiang, L Garry Adams, Richard C Laughlin, Waithaka Mwangi, Sandesh Subramanya, John Neilan, and David Brake. 2017. 'Safety and immunogenicity of mammalian cell derived and Modified Vaccinia Ankara vectored African swine fever subunit antigens in swine', *Veterinary Immunology and Immunopathology*, 185: 20-33.

Lopez, E., J. van Heerden, L. Bosch-Camos, F. Accensi, M. J. Navas, P. Lopez-Monteagudo, J. Argilaguet, C. Gallardo, S. Pina-Pedrero, M. L. Salas, J. Salt, and F. Rodriguez. 2020. 'Live Attenuated African Swine Fever Viruses as Ideal Tools to Dissect the Mechanisms Involved in Cross-Protection', *Viruses*, 12.

Martins, C. L. V., M. J. P. Lawman, T. Scholl, C. A. Mebus, and J. K. Lunney. 1993. 'African swine fever virus specific porcine cytotoxic T cell activity', *Archives of Virology*, 129: 211-25.

Mima, K. A., E. I. Katorkina, S. A. Katorkin, S. Z. Tsybanov, and A. S. Malogolovkin. 2020. '[In silico prediction of B- and T-cell epitopes in the CD2v protein of african swine fever virus (African swine fever virus, Asfivirus, Asfarviridae).]', *Vopr Virusol*, 65: 103-12.

Montaner-Tarbes, S., M. Pujol, T. Jabbar, P. Hawes, D. Chapman, H. D. Portillo, L. Fraile, P. J. Sanchez-Cordon, L. Dixon, and M. Montoya. 2019. 'Serum-Derived Extracellular Vesicles from African Swine Fever Virus-Infected Pigs Selectively Recruit Viral and Porcine Proteins', *Viruses*, 11.

Monteagudo, Paula L, Anna Lacasta, Elisabeth López, Laia Bosch, Javier Collado, Sonia Pina-Pedrero, Florencia Correa-Fiz, Francesc Accensi, María Jesús Navas, and Enric Vidal. 2017. 'BA71ΔCD2: a new recombinant live attenuated African swine fever virus with cross-protective capabilities', *Journal of Virology*, 91: e01058-17.

Moore, D. M., L. Zsak, J. G. Neilan, Z. Lu, and D. L. Rock. 1998. 'The African swine fever virus thymidine kinase gene is required for efficient replication in swine macrophages and for virulence in swine', *J Virol*, 72: 10310-5.

Murgia, Maria V, Mark Mogler, Andrea Certoma, Diane Green, Paul Monaghan, David T Williams, Raymond RR Rowland, and Natasha N Gaudreault. 2019. 'Evaluation of an African swine fever (ASF) vaccine strategy incorporating priming with an alphavirus-

expressed antigen followed by boosting with attenuated ASF virus', *Archives of Virology*, 164: 359-70.

Neilan, J. G., L. Zsak, Z. Lu, T. G. Burrage, G. F. Kutish, and D. L. Rock. 2004. 'Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection', *Virology*, 319: 337-42.

Neilan, J. G., L. Zsak, Z. Lu, G. F. Kutish, C. L. Afonso, and D. L. Rock. 2002. 'Novel swine virulence determinant in the left variable region of the African swine fever virus genome', *J Virol*, 76: 3095-104.

Netherton, Christopher L., Lynnette C. Goatley, Ana Luisa Reis, Raquel Portugal, Rachel H. Nash, Sophie B. Morgan, Lynden Gault, Raquel Nieto, Veronica Norlin, and Carmina Gallardo. 2019. 'Identification and immunogenicity of African swine fever virus antigens', *Frontiers in Immunology*, 10: 1318.

Niederwerder, M. C., A. M. M. Stoian, R. R. R. Rowland, S. S. Dritz, V. Petrovan, L. A. Constance, J. T. Gebhardt, M. Olcha, C. K. Jones, J. C. Woodworth, Y. Fang, J. Liang, and T. J. Hefley. 2019. 'Infectious Dose of African Swine Fever Virus When Consumed Naturally in Liquid or Feed', *Emerg Infect Dis*, 25: 891-97.

O'Donnell, V., G. R. Risatti, L. G. Holinka, P. W. Krug, J. Carlson, L. Velazquez-Salinas, P. A. Azzinaro, D. P. Gladue, and M. V. Borca. 2017. 'Simultaneous Deletion of the 9GL and UK Genes from the African Swine Fever Virus Georgia 2007 Isolate Offers Increased Safety and Protection against Homologous Challenge', *J Virol*, 91.

O'Donnell, Vivian, Lauren G Holinka, Douglas P Gladue, Brenton Sanford, Peter W Krug, Xiqiang Lu, Jonathan Arzt, Bo Reese, Consuelo Carrillo, and Guillermo R Risatti. 2015. 'African swine fever virus Georgia isolate harboring deletions of MGF360 and MGF505 genes is attenuated in swine and confers protection against challenge with virulent parental virus', *Journal of Virology*, 89: 6048-56.

O'Donnell, Vivian, Lauren G Holinka, Peter W Krug, Douglas P Gladue, Jolene Carlson, Brenton Sanford, Marialexia Alfano, Edward Kramer, Zhiqiang Lu, and Jonathan Arzt. 2015. 'African swine fever virus Georgia 2007 with a deletion of virulence-associated gene 9GL (B119L), when administered at low doses, leads to virus attenuation in swine and induces an effective protection against homologous challenge', *Journal of Virology*, 89: 8556-66.

O'Donnell, Vivian, Lauren G Holinka, Brenton Sanford, Peter W Krug, Jolene Carlson, Juan M Pacheco, Bo Reese, Guillermo R Risatti, Douglas P Gladue, and Manuel V Borca. 2016. 'African swine fever virus Georgia isolate harboring deletions of 9GL and MGF360/505 genes is highly attenuated in swine but does not confer protection against parental virus challenge', *Virus Research*, 221: 8-14.

Onisk, D. V., M. V. Borca, S. Kutish, E. Kramer, P. Irusta, and D. L. Rock. 1994. 'Passively Transferred African Swine Fever Virus Antibodies Protect Swine against Lethal Infection', *Virology*, 198: 350-54.

Oura, C. A., M. S. Denyer, H. Takamatsu, and R. M. Parkhouse. 2005. 'In vivo depletion of CD8+ T lymphocytes abrogates protective immunity to African swine fever virus', *J Gen Virol*, 86: 2445-50.

Pikalo, Jutta, Luca Porfiri, Valerij Akimkin, Hanna Roszyk, Katrin Pannhorst, Richard Thiga Kangethe, Viskam Wijewardana, Julia Sehl-Ewert, Martin Beer, Giovanni Cattoli, and Sandra Blome. 2022. 'Vaccination With a Gamma Irradiation-Inactivated African Swine Fever Virus Is Safe But Does Not Protect Against a Challenge', *Frontiers in Immunology*, 13.

Portugal, R., J. Coelho, D. Höper, N. S. Little, C. Smithson, C. Upton, C. Martins, A. Leitão, and G. M. Keil. 2015. 'Related strains of African swine fever virus with different virulence: genome comparison and analysis', *J Gen Virol*, 96: 408-19.

Qu, Hailong, Shengqiang Ge, Yongqiang Zhang, Xiaodong Wu, and Zhiliang Wang. 2022. 'A systematic review of genotypes and serogroups of African swine fever virus', *Virus Genes*, 58: 77-87.

Ramirez-Medina, Elizabeth, Elizabeth Vuono, Sarah Pruitt, Ayushi Rai, Nallely Espinoza, Alyssa Valladares, Edward Spinard, Ediane Silva, Lauro Velazquez-Salinas, Douglas P. Gladue, and Manuel V. Borca. 2022. 'ASFV Gene A151R Is Involved in the Process of Virulence in Domestic Swine', *Viruses*, 14: 1834.

Reis, A. L., C. C. Abrams, L. C. Goatley, C. Netherton, D. G. Chapman, P. Sanchez-Cordon, and L. K. Dixon. 2016. 'Deletion of African swine fever virus interferon inhibitors from the genome of a virulent isolate reduces virulence in domestic pigs and induces a protective response', *Vaccine*, 34: 4698-705.

Reis, Ana L, Lynnette C Goatley, Tamara Jabbar, Pedro J Sanchez-Cordon, Christopher L Netherton, David AG Chapman, and Linda K Dixon. 2017. 'Deletion of the African swine fever virus gene DP148R does not reduce virus replication in culture but reduces virus virulence in pigs and induces high levels of protection against challenge', *Journal of Virology*, 91: e01428-17.

Revilla, Yolanda, Daniel Perez-Nunez, and Juergen A Richt. 2018. 'African swine fever virus biology and vaccine approaches', *Advances in virus research*, 100: 41-74.

Ruiz-Gonzalvo, F., F. RodrÍGuez, and J. M. Escribano. 1996. 'Functional and Immunological Properties of the Baculovirus-Expressed Hemagglutinin of African Swine Fever Virus', *Virology*, 218: 285-89.

Sánchez-Cordón, P. J., D. Chapman, T. Jabbar, A. L. Reis, L. Goatley, C. L. Netherton, G. Taylor, M. Montoya, and L. Dixon. 2017. 'Different routes and doses influence protection in pigs immunised with the naturally attenuated African swine fever virus isolate OURT88/3', *Antiviral Res*, 138: 1-8.

Sánchez-Cordón, PJ, M Montoya, AL Reis, and LK Dixon. 2018. 'African swine fever: A re-emerging viral disease threatening the global pig industry', *The Veterinary Journal*, 233: 41-48.

Sanchez, E. G., A. Quintas, D. Perez-Nunez, M. Nogal, S. Barroso, A. L. Carrascosa, and Y. Revilla. 2012. 'African swine fever virus uses macropinocytosis to enter host cells', *PLoS Pathog*, 8: e1002754.

Sanford, B., L. G. Holinka, V. O'Donnell, P. W. Krug, J. Carlson, M. Alfano, C. Carrillo, P. Wu, A. Lowe, G. R. Risatti, D. P. Gladue, and M. V. Borca. 2016. 'Deletion of the thymidine kinase gene induces complete attenuation of the Georgia isolate of African swine fever virus', *Virus Res*, 213: 165-71.

Silva, Ediane B., Peter W. Krug, Elizabeth Ramirez-Medina, Alyssa Valladares, Ayushi Rai, Nallely Espinoza, Douglas P. Gladue, and Manuel V. Borca. 2022. 'The Presence of Virus Neutralizing Antibodies Is Highly Associated with Protection against Virulent Challenge in Domestic Pigs Immunized with ASFV live Attenuated Vaccine Candidates', *Pathogens*, 11: 1311.

Spinard, E., V. O'Donnell, E. Vuono, A. Rai, C. Davis, E. Ramirez-Medina, N. Espinoza, A. Valladares, M. V. Borca, and D. P. Gladue. 2023. 'Full genome sequence for the African swine fever virus outbreak in the Dominican Republic in 1980', *Sci Rep*, 13: 1024.

Sunwoo, Sun-Young, Daniel Pérez-Núñez, Igor Morozov, Elena Sánchez, Natasha Gaudreault, Jessie Trujillo, Lina Mur, Marisa Nogal, Daniel Madden, Kinga Urbaniak, In Kim, Wenjun Ma, Yolanda Revilla, and Juergen Richt. 2019. 'DNA-Protein Vaccination Strategy Does Not Protect from Challenge with African Swine Fever Virus Armenia 2007 Strain', *Vaccines*, 7: 12.

Takamatsu, Haru-Hisa, Michael S. Denyer, Anna Lacasta, Catrina M. A. Stirling, Jordi M. Argilaguet, Christopher L. Netherton, Chris A. L. Oura, Carlos Martins, and Fernando Rodríguez. 2013. 'Cellular immunity in ASFV responses', *Virus Research*, 173: 110-21.

Teklue, T., T. Wang, Y. Luo, R. Hu, Y. Sun, and H. J. Qiu. 2020. 'Generation and Evaluation of an African Swine Fever Virus Mutant with Deletion of the CD2v and UK Genes', *Vaccines (Basel)*, 8.

Tran, X. H., T. T. P. Le, Q. H. Nguyen, T. T. Do, V. D. Nguyen, C. G. Gay, M. V. Borca, and D. P. Gladue. 2021. 'African swine fever virus vaccine candidate ASFV-G-DeltaI177L efficiently protects European and native pig breeds against circulating Vietnamese field strain', *Transbound Emerg Dis*.

Tran, X. H., L. T. T. Phuong, N. Q. Huy, D. T. Thuy, V. D. Nguyen, P. H. Quang, Q. V. Ngôn, A. Rai, C. G. Gay, D. P. Gladue, and M. V. Borca. 2022. 'Evaluation of the Safety Profile of the ASFV Vaccine Candidate ASFV-G-ΔI177L', *Viruses*, 14.

Urbano, Ana Catarina, and Fernando Ferreira. 2022. 'African swine fever control and prevention: an update on vaccine development', *Emerging microbes & infections*, 11: 2021-33.

Wang, G., M. Xie, W. Wu, and Z. Chen. 2021. 'Structures and Functional Diversities of ASFV Proteins', *Viruses*, 13.

Wang, Xixi, Jing Wu, Yingtong Wu, Hongjun Chen, Shoufeng Zhang, Jinxiang Li, Ting Xin, Hong Jia, Shaohua Hou, and Yitong Jiang. 2018. 'Inhibition of cGAS-STING-TBK1 signaling pathway by DP96R of ASFV China 2018/1', *Biochemical and biophysical research communications*, 506: 437-43.

Wang, Yang, Shuai Cui, Ting Xin, Xixi Wang, Hainan Yu, Shiyu Chen, Yajun Jiang, Xintao Gao, Yitong Jiang, Xiaoyu Guo, Hong Jia, and Hongfei Zhu. 2022. 'African Swine Fever Virus MGF360-14L Negatively Regulates Type I Interferon Signaling by Targeting IRF3', *Frontiers in cellular and infection microbiology.*, 11.

Wöhnke, Elisabeth, Gwenny Cackett, Finn Werner, Sandra Blome, Thomas C. Mettenleiter, and Axel Karger. 2022. 'Proteome Analysis of Swine Macrophages after Infection with Two Genotype II African Swine Fever Isolates of Different Pathogenicity', *Viruses*, 14: 2140.

Yáñez, R. J., J. M. Rodríguez, M. L. Nogal, L. Yuste, C. Enríquez, J. F. Rodriguez, and E. Viñuela. 1995. 'Analysis of the complete nucleotide sequence of African swine fever virus', *Virology*, 208: 249-78.

Yu, L., Z. Zhu, J. Deng, K. Tian, and X. Li. 2023. 'Antagonisms of ASFV towards Host Defense Mechanisms: Knowledge Gaps in Viral Immune Evasion and Pathogenesis', *Viruses*, 15.

Zajac, Michelle D., Neha Sangewar, Shehnaz Lokhandwala, Jocelyne Bray, Huldah Sang, Jayden McCall, Richard P. Bishop, Suryakant D. Waghela, Rakshith Kumar, Tae Kim, and Waithaka Mwangi. 2022. 'Adenovirus-Vectored African Swine Fever Virus pp220 Induces Robust Antibody, IFN-γ, and CTL Responses in Pigs', *Frontiers in Veterinary Science*, 9.

Zhang, H., S. Zhao, H. Zhang, Y. Shen, P. Zhang, H. Shan, and X. Cai. 2022. 'Orally administered recombinant Lactobacillus expressing African swine fever virus antigens that induced immunity responses', *Front Microbiol*, 13: 1103327.

Zhang, J., Y. Zhang, T. Chen, J. Yang, H. Yue, L. Wang, X. Zhou, Y. Qi, X. Han, J. Ke, S. Wang, J. Yang, F. Miao, S. Zhang, F. Zhang, Y. Wang, M. Li, and R. Hu. 2021. 'Deletion of the L7L-L11L Genes Attenuates ASFV and Induces Protection against Homologous Challenge', *Viruses*, 13.

Zheng, Zhi-Ming, Pei-Jun He, and Carl C Baker. 1997. 'Structural, functional, and protein binding analyses of bovine papillomavirus type 1 exonic splicing enhancers', *Journal of Virology*, 71: 9096-107.

Zhu, Z., and G. Meng. 2020. 'ASFVdb: an integrative resource for genomic and proteomic analyses of African swine fever virus', *Database (Oxford)*, 2020.

Chapter 2 - Adenovirus-vectored African Swine Fever Virus pp220 induces robust antibody, IFN-γ, and CTL responses in pigs

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Abstract

African Swine Fever Virus (ASFV) poses a serious threat to the pork industry worldwide, however, there is no safe vaccine or treatment available. Development of an efficacious subunit vaccine will require identification of protective antigens. The ASFV pp220 polyprotein is essential for virus structural integrity. This polyprotein is processed to generate p5, p34, p14, p37, and p150 individual proteins. Immunization of pigs with a cocktail of adenoviruses expressing the proteins induced significant IgG, IFN-γ-secreting cells, and cytotoxic T lymphocyte responses. Four predicted *SLA*-I binding nonamer peptides, namely p34$^{161\text{-}169}$, p37$^{859\text{-}867}$, p150$^{1363\text{-}1371}$, and p150$^{1463\text{-}1471}$, recalled strong IFN-γ$^+$ PBMC and splenocyte responses. Notably, peptide p34$^{161\text{-}169}$ was recognized by PBMCs isolated from 7/10 pigs and by splenocytes isolated from 8/10 pigs. Peptides p37$^{859\text{-}867}$ and p150$^{1363\text{-}1371}$ stimulated recall IFN-γ$^+$ responses in PBMCs and splenocytes isolated from 8/10 pigs, whereas peptide p150$^{1463\text{-}1471}$ recalled responses in PBMCs and splenocytes isolated from 7/10 and 9/10 pigs, respectively. The results demonstrate that the pp220 polyprotein contains multiple epitopes that induce robust immune responses in pigs. Importantly, these epitopes are 100% conserved among different ASFV genotypes and were predicted to bind multiple *SLA*-I alleles. The outcomes suggest that pp220 is a promising candidate for inclusion in a prototype subunit vaccine.

2.1 Introduction

High-consequence transboundary animal diseases, such as African Swine Fever [ASF], have an enormous socio-economic impact on both animal and public health sectors (Ebwanga, Ghogomu, and Paeshuyse 2021; Gogin et al. 2013; Fasina et al. 2012; Chenais et al. 2017). Development and deployment of rationally designed treatments and vaccines is crucial in combating and preventing the effects of such diseases ("ASF Situation Report" 2021). Since the introduction of the African Swine Fever Virus [ASFV] into Georgia from Africa, the virus has spread to Europe, Asia and Oceania, and more recently the Dominican Republic and Haiti. In some countries, the disease has become endemic to the extent of endangering food security (Kim et al. 2019; Liu et al. 2020; Sugiura et al. 2020; Zhao et al. 2019; Zhou et al. 2019; Gale, Bowen, and Perrin 2021; Gonzales et al. 2021; Paulino-Ramirez 2021). Since there is no vaccine, surveillance by testing with subsequent removal of infected and in-contact animals, and enhanced biosecurity measures are the primary control and elimination methods for ASF ("Infection" 2021; "ASF Situation Report" 2021). These methods are moderately effective, but not ideal, since they are expensive and labor-intensive. Thus, development of a safe and efficacious vaccine is a high priority (Lewis and Roth 2021).

The ASFV is a complex double-stranded DNA virus in the family *Asfarviridae* with the genome encoding more than 150 ORFs. More than twenty ASFV genotypes have been reported so far based on the c-terminal sequence of the gene encoding the p72 capsid protein (Achenbach et al. 2017; Alonso et al. 2018). The virus can infect all members of the Suidae family, but clinical manifestations of a hemorrhagic disease only occur in wild boars and domestic pigs (*Sus scrofa*) of all ages and sexes. The virus has been detected in oral/nasal secretions, blood, feces, urine, along with raw meat or carcasses of infected pigs ("Infection" 2021; "ASF Situation Report" 2021).

Ancestrally, transmission occurs via a sylvatic cycle involving the *Ornithodoros* ticks and African wild suids. However, once domestic or feral pigs are infected, transmission occurs primarily by contact and ingestion of contaminated feed, pork products or soil. Infection with highly virulent ASFV isolates can be lethal to nearly 100% of infected pigs in naïve populations (Pietschmann et al. 2016; Carlson et al. 2020; Dixon et al. 2020; "Infection" 2021; "African Swine Fever" 2019; Juszkiewicz et al. 2020). Pigs that recover from infection with ASFV of low to moderate virulence; and animals vaccinated with attenuated strains or gene-deletion mutants are protected to varying degrees against either homologous or heterologous virulent strains (Ruiz-Gonzalvo F 1983; King et al. 2011; Hamdy and Dardiri 1984; Lewis et al. 2000; Leitao et al. 2001; Lacasta et al. 2015). Development of a subunit vaccine requires definition of correlates of protection and identification of cognate antigen(s). Most naïve animals infected with highly virulent ASFV succumb to the disease before the immune system can intervene (Rodriguez-Bertos et al. 2020; Zhuo et al. 2021). Macrophages, monocytes and to some extent DCs support ASFV replication, and the impaired APC function are potential mechanisms of immune evasion (Schäfer et al. 2022; Wang et al. 2020; Franzoni et al. 2022). Infection occurs via the upper respiratory tract where the virus replicates in tonsils and draining lymph nodes in the head and neck region. Cross-talk between innate and adaptive immune responses is facilitated in the lymph nodes, which makes the regions they drain ideal sites for immunization with ASF vaccines (Herrera-Uribe et al. 2018; Salguero 2020; Sehl et al. 2020; Woodruff et al. 2014). The draining lymph nodes are key to the development of mature B cells, cytotoxic T lymphocytes (CTLs), and natural killer (NK) cells, which are involved in clearance of infected cells (Bosch-Camós, López, Navas, et al. 2021; Franzoni et al. 2022; Gerner, Käser, and Saalmüller 2009; De Pelsmaeker et al. 2019; Butler, Wertz, and Sinkora 2017).

Several studies have demonstrated a role for both ASFV-specific antibodies and cellular immunity in protection. However, conflicting data have generated the view that high levels of circulating antibodies do not correlate with protection (Netherton, Goatley, Reis, Portugal, Nash, Morgan, Gault, Nieto, Norlin, Gallardo, Ho, Sanchez-Cordon, et al. 2019; Carlson, O'Donnell, et al. 2016; Onisk et al. 1994; Neilan et al. 2004; Escribano, Galindo, and Alonso 2013). Experiments conducted using porcine PBMCs have demonstrated cross-protection between differing ASFV strains, which is associated with an increase in IFN-γ producing cells (Takamatsu et al. 2013; Dixon, Sun, and Roberts 2019). In the early stages of infection, clearance of virus-infected cells generally requires $CD8^+$ T cell activation (Schäfer et al. 2022; De Pelsmaeker et al. 2019). Depletion of T cells in pigs immunized with a low virulence ASFV isolate resulted in lack of protection following challenge with a virulent strain, which suggests that $CD8^+$ T cells are required for protection (Oura et al. 2005). A connection between IFN-γ secretion and $CD8^+$ T-cell activity has been observed in several studies in response to antigenic stimulation or natural infection. Thus, IFN-γ response and CTL activities are logical indicators of immune responses to vaccination (Franzoni et al. 2022; Gao et al. 2018; Hühr et al. 2020). However, IFN-γ levels may not reflect protection since the cytokine can be produced by macrophages, $CD4^+$, $CD8^+$, $γδ^+$ T cells, innate B cells, and NK cells on antigen activation (Schäfer et al. 2022; Franzoni et al. 2022; Carlson, O'Donnell, et al. 2016).

Induction and expansion of CTLs by either high or low virulence ASFV strains is still little understood. However, CTL responses probably provide the best immune readout for protection induced by ASFV antigens (Argilaguet et al. 2012; Bosch-Camós, López, Navas, et al. 2021; Burleson, Burleson, and Dietert 2010; Lokhandwala et al. 2017). Several structural, non-structural, multi-gene-encoded, and uncharacterized ASFV antigens have been evaluated for their potential

to elicit protective immunity, however, they did not induce adequate protection to justify development as candidate vaccines, without further research (Argilaguet et al. 2012; Netherton, Goatley, Reis, Portugal, Nash, Morgan, Gault, Nieto, Norlin, Gallardo, Ho, Sánchez-Cordón, et al. 2019; Sunwoo et al. 2019; Cadenas-Fernandez et al. 2020; Lokhandwala et al. 2019).

The pp220 (pCP2475L) and pp62 (pCP530R) are two major polyproteins that are cleaved into mature structural proteins for formation of the core shell and make up about a third of the ASFV protein mass (Andres, Alejo, et al. 2002). The pp220 polyprotein is initially processed into a p150 protein and a pp90 preprotein. The latter is cleaved into p34 protein and a precursor pp55 protein, from which p5, p14, and p37 proteins are eventually generated (Simon-Mateo, Andres, and Vinuela 1993; Alejo et al. 2018). The cytosol of infected cells contains processed forms of pp220 and all the pp220 proteins are also found in the mature virions (Alejo et al. 2018; Heath, Windsor, and Wileman 2003). Both the p14 and p37 proteins have been localized to the cellular nuclei, however, p37 is also found in the cytoplasm, which implies a role in nucleocytoplasmic transport of viral DNA and its protection from DNA sensors of the inflammasomes, which is critical for ASFV replication in the viral factories within the cells (Eulalio et al. 2006; Eulalio et al. 2004; Andrés et al. 2020; Andres, Garcia-Escudero, et al. 2002; Andres, Simon-Mateo, and Vinuela 1993, 1997; Andres, Alejo, et al. 2002; Suarez, Salas, and Rodriguez 2010). The abundance of the pp220 antigens in the cytosol means that the antigens are amenable for breakdown by the proteasome, which results in generation of peptides that could be loaded onto MHC-I molecules for presentation to CD8^{+} T cells. In the current study, immunization of pigs with an adenovirus-vectored pp220 polyproteins (ASFV Georgia 2007/1) using two different adjuvants induced antigen-specific antibodies, strong IFN-γ responses, and CTL responses. Lymphocytes from the pigs were used to map T cell epitopes by screening peptides identified by *in silico*

prediction using well-characterized *SLA-I* alleles. Empirical identification and validation of ASFV antigens containing CD8^{+} T cell epitopes, as performed in this study, will be important to inform future subunit vaccine development.

2.2 Materials and Methods

2.2.1 Plasmid and virus construction: p5-34-14-37, p150-I, and p150-II

Three polypeptide sequences from the ASFV pp220 polyprotein (Georgia 2007/1: Genebank Accession FR682468) were designed and used to generate expression constructs. The polypeptide sequences were designated p5-34-14-37, p150-I and p150-II (p150 was split into two due to its large size). Genes encoding the polypeptides were synthesized (GenScript) and then cloned into pcDNA3.3-TOPO TA (K8300001, Invitrogen). Following validation of protein expression, the genes were subcloned into pAd/CMV/V5-DESTGateway (V49320, Invitrogen) for generation of recombinant adenovirus. The genes were also subcloned into pFastBac HBM TOPO (A11338, Invitrogen) for generation of baculovirus that were used to produce recombinant proteins needed for immune response readouts. Replication-incompetent adenoviruses encoding ASFV proteins, Ad-p5-34-14-37, Ad-p150-I, and Ad-p150-II were generated using the Invitrogen ViraPower Adenoviral Expression System (K493000). An adenovirus encoding Luciferase (Ad-Luc) was also generated to serve as negative control. Quality control and validation of protein expression was confirmed by immunocytometric analyses as previously described (Lokhandwala et al. 2017; Lokhandwala et al. 2016; Lokhandwala et al. 2019). Viral titers, in infectious focus units per mL (IFU/ml), was determined by immunoassay as previously described (Lokhandwala et al. 2017; Lokhandwala et al. 2016; Lokhandwala et al. 2019). To generate recombinant antigens, Bacmids encoding HA-tagged p5-34-14-37, p150-I or p150-II were transfected into Sf-9 insect cells to

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produce recombinant baculoviruses and protein expression was confirmed by immunocytometric analyses as previously described (Lokhandwala et al. 2017; Lokhandwala et al. 2016; Lokhandwala et al. 2019). A single clone of each recombinant baculovirus was amplified, titrated, and used to infect High-Five cells (B85502, Invitrogen) to express recombinant proteins that were purified by using Anti-HA Agarose affinity purification gel (Sigma).

2.2.2 Validation of Protein Expression

2.2.2.1 Immunocytometry

Protein expression validation and quality control were assayed by immunocytometric analyses as previously described (Lokhandwala et al. 2017; Lokhandwala et al. 2016; Lokhandwala et al. 2019). Briefly, duplicate 12-well plates of Human Embryonic Kidney (HEK) 293A cells were transfected (plasmids), mock transfected (negative controls), or infected (adenoviruses containing each respective construct) with luciferase serving as the negative control for infection. At 48 hr. post-transfection and 24 hr. post-infection, the cell monolayers were fixed with cold methanol, rinsed with 1x PBS, blocked for 1 hr. at room temperature with 1x PBS plus 5% fetal bovine serum (blocking buffer) and then probed with a 1:200 dilution of ASFV-specific convalescent swine serum (E.J. Kramer, Plum Island Animal Disease Center) (Lokhandwala et al. 2016). For the cells probed with the convalescent serum, goat anti-porcine IgG-AP conjugate (Southern Biotech), diluted 1:1000 in blocking buffer, was used as the secondary antibody. Cell staining was visualized with Fast-Red TR/Naphthol AS-MX (Sigma, F4523) AP substrate.

2.2.2.2 Western blot

To generate proteins, HEK 293A cells were transfected as above in section 2.2.2.1 and cells were washed at 48 hr. post-transfection with 1x PBS then lysed in RIPA buffer supplemented with protease inhibitor (Sigma). Following clarification by centrifugation supernatants were prepared under reducing conditions in Laemmli buffer (Bio-Rad) containing 10% β-Mercaptoethanol followed by heat denaturation at 95°C for 5 minutes, fractionated by SDS-PAGE using a 7.5% Acrylamide/bis gel (ProtoGel, National Diagnostics), transferred to PVDF membranes (Amersham), and blocked for 1.5 hrs. at room temperature in SuperBlock (PBS) Blocking Buffer (ThermoFisher). The membranes were incubated with a 1:10,000 dilution of ASFV-specific convalescent swine serum followed by exposure to a 1:8,000 dilution of horseradish peroxidase-conjugated swine secondary antibody followed by chemiluminescence development (Pierce Chemiluminescent Plus Substrate Kit, Invitrogen) and detection using the Invitrogen iBright 1500 imaging system. Purified proteins TMSP7 and p62 that were previously validated served as the negative and positive controls respectively.

2.2.3 Immunization of pigs

A cocktail of Ad-pp220 consisting of 10^{11} ifu of Ad-p5-34-14-37, Ad-p150-I, and Ad-p150-II (total 3 x 10^{11} ifu) formulated in an adjuvant was used to immunize pigs intramuscularly as previously described (Lokhandwala et al. 2016). The pigs were boosted with the same dose and via the same route fourteen weeks post-priming. Control pigs received 3 x 10^{11} ifu of the Ad-Luc virus. Each treatment group contained randomly selected age-matched commercial piglets (n=5) that received either: 1) Ad-pp220 cocktail plus ENABL® adjuvant (from BenchMark Biolabs); 2)

Ad-pp220 cocktail plus an experimental adjuvant ZTS-01, from Zoetis; or 3) Ad-Luc plus ENABL® adjuvant (Table 1). The study was terminated after 8 weeks post-boost.

2.2.4 Sample Collection

During acclimatization of piglets, skin biopsies were collected using 4-mm tissue punches (3785707; American Screening Corp.) and processed to generate skin fibroblasts for use as autologous CTL targets. Blood was collected in EDTA-treated or untreated vacutainers tubes once before immunization and then weekly post-prime and post-boost for isolation of peripheral blood mononuclear cells (PBMCs) and serum, respectively. Spleens were collected for isolation of splenocytes beginning on day 8 post-boost (Figure 2) as previously described (Lokhandwala et al. 2016).

2.2.5 Enzyme-linked Immunosorbent Assay (ELISA)

Antigen-specific IgG responses were evaluated by ELISA using Costar (3590) 96-well plates coated with 2.5 µg/mL of the affinity-purified antigens in 0.5% bicarbonate buffer as previously described (Lokhandwala et al. 2017; Lokhandwala et al. 2016). The antigen-coated wells were blocked with 10% nonfat dry milk in PBST (1x PBS + 0.1% Tween 20) prior to the addition of 1:100 diluted serum samples for the screening assay and two-fold serial dilution for endpoint titer determination. Plates were incubated for 1 hr. at 37°C, washed 6x using PBST before adding 1:5000 dilution of anti-porcine IgG-POD (peroxidase) antibody (114-035-003, Jackson Immuno-Research) to each well. The plates were further incubated at 37°C for 1 hr. and then washed 6x with PBST and 3x with PBS. Peroxidase activity was measured by adding Sure Blue tetramethylbenzidine (TMB) substrate (53-00-02, KPL). The reaction was stopped with 1N HCl, the optical density (OD) was measured at 430 nm using a spectrophotometer (BioTek Epoch).

End-point titers were calculated by comparing the mean OD of the post-boost serum to that of the baseline at day zero post-immunization (DPI) for each animal. A positive result was determined by selecting the mean value which was higher than the cognate DPI 0 plus 3x the standard deviation (SD).

2.2.6 Immunohistochemistry

Paraffin embedded formalin-fixed spleen tissue from a high titer (as determined by qPCR) animal receiving ASFV challenge from a subsequent study was used to ensure that the positive signal represented was authentic. Naïve and ASFV-infected tissues were used for IHC following two 5-minutes dewaxing in Xylene (Sigma) and rehydration using gradient ethanol (2x 100%, 90%, 80%) followed by distilled water (all 5 minute each). Antigen retrieval was achieved using a 0.1% Protease solution (Sigma) at 37°C for 30 minutes followed by washing (2x distilled water, 3x 0.1% PBST). Slides were blocked using 5% goat sera (diluted in 0.1% PBST) incubated at room temperature for 40 minutes. Following a quick wash in 0.1% PBST, a 0.5% solution of Bovine Serum Albumin (BSA) was added for a secondary blocking step at room temperature for 30 minutes. Primary antibody was tested at 1:200-1:10,000 with the most ideal dilution being 1:2,500 for ASFV-specific convalescent sera and 1:250 for swine sera from immunized pigs (0.5% BSA also used as mock for FITC only control). Primary antibody was added following a quick wash (0.1% PBST) and incubated overnight at 4°C. Slides were washed 3x for 7 minutes each in 0.1% PBST. Secondary antibody was diluted at 1:200 in 0.5% BSA (goat anti-swine IgG-FITC, Jackson ImmunoResearch) and incubated for 1 hr. at room temperature. Ten-minute washes in 0.1% PBST were repeated a total of 3x. DAPI staining, and mounting were performed as per the manufacturer's instructions for the VectaTrueVIEW Autofluorescence Quenching Kit w/DAPI (VectorLabs; SP-

8500). The use of the Autofluorescence Quenching Kit aided in reducing the potential for autofluorescence and background for clear IFA readouts. Slides were visualized and images acquired using an Olympus fluorescent microscope paired with CellSens software.

2.2.7 Peptide Prediction and Selection

NetMHCpan version 2.8 data base (http://www.cbs.dtu.dk/services/NetMHCpan-2.8/) was initially used for in silico prediction of nonamer peptides from the ASFV pp220 polyprotein that can bind strongly (percent rank less than 0.5; the default setting for targeting MHC class I binders) to all available Swine Leukocyte Antigen class I (SLA-I) alleles within this comprehensive software database to generate a peptide library as previously described (Sangewar et al. 2021). After sorting the predicted nonamers based on their predicted scores, a total of eighty-eight putative epitopes were selected and synthesized (Peptide 2.0, Inc). Conservation of the putative epitopes among ASFV genotypes was determined by multi-sequence alignment of the available pp220 polypeptide sequences. The crude nonamer peptides were reconstituted in ultrapure sterile water with 25% DMSO at 10 mg/mL concentration and stored in aliquots at -80°C until use in EliSpot assays.

2.2.8 IFN-γ EliSpot Assay

The number of IFN-γ-secreting T-cells was determined by Porcine IFN-γ EliSpot BASIC kit (3130-2A, MabTech) using PBMCs and splenocytes pulsed with peptides as previously described (Lokhandwala et al. 2017; Lokhandwala et al. 2016). Briefly, each sample was assayed in triplicate in MultiScreen-HA 96-well plates (MAIPS4510, Millipore) with 2.5×10^5 cells/mL cells pulsed with 2.5 µg/mL of each peptide in cRPMI 1640 media. Peptide screening was carried out using

four pools [A-D] containing eighteen 9-mer peptides and a final pool [E] contained the remaining 16 peptides (Table 2). Reactive pools were then tested at the individual peptide level at the same concentration indicated above. For each test, positive and negative controls were Phytohemagglutinin (PHA) mitogen at a concentration of 5 µg/mL and media alone, respectively. After a 48-hr. incubation at 37°C in 5% CO_2 atmosphere, plates were developed as per the MabTech protocol, the membranes air dried in the dark, and spots detected using EliSpot reader (MabTech) and AID software (version 3.4; AutoImmun Diagnostica, Strasburg, Germany). Data is presented as Spot Forming Cells (SFC)/10^6 PBMCs or splenocytes based upon the mean number of peptide-specific IFN-γ producing cells after subtracting the negative control mean counts as background.

2.2.9 CTL Chromium Release Assay

Lytic activity of antigen-specific cells was determined by using the traditional ^{51}Cr release assay as previously described (Lokhandwala et al. 2016). To generate effectors, PBMCs collected at four weeks post-boost were seeded at a density of 4 x 10^6 cells/mL per well in a 24-well plate in 1 ml RPMI 1640 medium (12-167Q, Lonza) containing 45% Click's medium (9195, Irvine Scientific), 10% fetal bovine serum (FBS), 50mM Mercaptoethanol, 200mM GlutaMAX (35050061, Gibco), 50 µg/mL Gentamicin, and Penicillin (100 IU/mL)/Streptomycin (100 µg/mL). The PBMCs were stimulated with each adenovirus at a MOI of 1,000. Ten days post-stimulation, the cells were harvested, viable cells were purified by Ficoll-Histopaque centrifugation, washed with 1x PBS and then resuspended in complete RPMI for use as effector cells. For generation of target cells, skin punch biopsies were minced using sterile technique to generate primary skin fibroblasts which were cultured in 1 mL of Dulbecco's modified Eagle's medium (DMEM) with 10% FBS, 200mM

GlutaMAX, 50 µg/mL Gentamicin, and Penicillin (100 IU/mL)/Streptomycin (100 µg/mL) per well in 12-well plates, as previously described (Lokhandwala et al. 2016). Twenty-four hrs. prior to the ^{51}Cr release assay, autologous skin fibroblasts were transfected with the plasmid construct encoding target antigen using Gene-In transfection reagent (GST-1000, MTI-Global Stem) as per manufacturer's instructions. To prepare the transfected fibroblasts as target cells, the fibroblasts were detached with Accutase, rinsed 3x with DMEM supplemented with 10% FBS and then labeled with 100 µCi of Na_2 $^{51}CrO_4$ (Perkin Elmer) per 10^6 cells for 1 hr. at 37°C in 5% CO2. The labeled fibroblasts were washed 3x and resuspended in cRPMI 1640 medium. The ^{51}Cr release assay was performed in duplicates at effector-to-target (E:T) ratios of 25:1 and 50:1 in a final volume of 100 µL/well using a round-bottom 96-well plate. Following a 6-hr. incubation at 37°C in 5% CO_2, the cells were centrifuged for 4 minutes at 1,000 rpm and supernatants were collected to measure chromium release. Spontaneous (targets without effectors) and maximum chromium release (lysis with 5% Triton-X detergent solution) were also measured for all target cells. A plasmid construct encoding a Foot and Mouth Disease Virus (FMDV) VP1 and 3D polymerase chimeric antigen was used as negative control. Chromium release percent specific lysis values were determined as previously described (Ceppi et al. 2005).

2.2.10 Statistical Analysis

GraphPad Prism, version 6.05 with significance (P-value) of 0.05 was used to analyze all data. A one-way ANOVA followed by the Tukey's multiple-comparison test was used to compare the IgG titers of each immunization group. The IFN-γ responses between the treatment groups and the negative control group was determined by one-way ANOVA followed by Bonferroni's multiple-comparison test.

2.2.11 Ethics Statement

Texas A&M University Institutional Animal Care and Use Committee (IACUC) (permit# 2009067) approved Animal Use Protocol 2012-59 that follows the regulations, policies, and guidelines put forth by the Animal Welfare Act, United Stated Department of Agriculture (USDA) Animal Care Resource Guide, and the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals. All protocols outlined in this document were followed including use of clinical scoring for daily monitoring and assessment of animal health. Termination was performed using a lethal dose of sodium pentobarbital and confirmation of euthanasia by lack of heartbeat.

2.3 Results

2.3.1 Design, expression, and validation of pp220 constructs

Three recombinant plasmid and adenovirus constructs encoding the components of the pp220 polyprotein from the ASFV Georgia 2007/1 isolate, designated p5-p34-p14-p37, p150-I, and p150-II (each combined encode for the entire pp220 antigen) (Figure 1), were validated for protein expression in transfected and adenovirus-infected HEK 293A cells using ASFV-specific convalescent swine serum (Figure 2A). Authenticity of the antigens was validated by Western Blot using ASFV-specific convalescent serum. Previously validated purified ASFV p62 antigen served as a positive control, whereas an irrelevant antigen, TMSP7, was used as a negative control (Figure 2B).

2.3.2 Ad-pp220 cocktail primed strong IgG responses

Following prime-boost immunization (Table 1), pp220-specific immune responses were evaluated in pigs at defined timepoints (Figure 3). All pigs immunized with the Ad-pp220 cocktail seroconverted and had detectable post-prime pp220-specific IgG responses (Figure 4A). The highest mean IgG responses against all the three pp220 antigens were observed in pigs immunized with the Ad-pp220 cocktail formulated in ZTS-01 adjuvant (Figure 4A). Both treatment groups, pp220-ENABL® ($p < 0.01$) and pp220-ZTS-01 ($p < 0.001$), had significantly higher p5-p34-p14-p37-specific IgG responses than the negative control group, Ad-Luc-ENABL® (Figure 4A). The Ad-pp220 cocktail formulated in ENABL® adjuvant elicited low levels of post-prime IgG responses against p150-I in pigs (Figure 4A). However, the Ad-pp220-ZTS-01 treatment group had significantly higher mean IgG responses against p150-I compared to the Ad-pp220-ENABL® ($p < 0.001$) treatment group and the Ad-Luc-ENABL® control group ($p < 0.0001$) (Figure 5A). Similar to the p5-p34-p14-p37-specific responses, significantly higher p150-II-specific mean IgG responses were primed in the Ad-pp220-ENABL® ($p < 0.001$) and the Ad-pp220-ZTS-01 ($p < 0.0001$) treatment groups compared to those in the Ad-Luc-ENABL® negative control group (Figure 4A). Following boosting, significantly higher ($p < 0.0001$) IgG responses were recalled against all the three pp220 antigens in pigs from both the treatment groups compared to the negative controls (Figure 4B).

Post-boost, pp220-specific IgG end-point titers elicited in the pigs primed with the Ad-pp220-ENABL® and the Ad-pp220-ZTS-01 formulations were higher than the IgG titers detected in the ASFV-specific convalescent porcine serum (Figure 5). All pigs in both treatment groups developed high levels of IgG titers, in the range of 0.1×10^6 to 4.0×10^6, against p5-p34-p14-p37, p150-I, and p150-II antigens (Figure 5). In comparison, IgG titers detected in the ASFV-specific

convalescent serum for p5-p34-p14-p37, p150-I, and p150-II antigens were 1: 2.5 x 10^5, 1: 3.2 x 10^4, and 1: 3.2 x 10^4, respectively (Figure 5).

2.3.3 Antibodies induced by the Ad-pp220 cocktail recognize wildtype ASFV

Sera from the pigs immunized with the adenovirus-vectored pp220 antigens (Ad-pp220-ENABL and Ad-pp220-ZTS-01) recognized cells infected with wildtype ASFV (Georgia 2007/1) following immunohistochemical analysis of ASFV-infected spleen tissue slides using sera obtained two weeks post-boost (Figure 6). The ASFV-specific convalescent serum served as a positive control, whereas negative control sera from the mock-immunized pigs as well as secondary FITC controls did not result in antigen detection. The IHC outcome confirmed that immunization with the adenovirus-vectored pp220 antigens elicited ASFV-specific antibody responses (Figure 6).

2.3.4 Ad-pp220 cocktail induced IFN-γ responses

The Ad-pp220 cocktail formulated in ENABL® adjuvant elicited the highest cellular IFN-γ responses against pp220 antigens in pigs (Figure 7). Post-prime, the mean p5-p34-p14-p37- ($p < 0.0001$) and p150-I-specific ($p < 0.001$) IFN-γ responses detected in PBMCs from the Ad-pp220-ENABL® treatment group were significantly higher than those detected in the Ad-pp220-ZTS-01 treatment group and the Ad-Luc-ENABL® control group (Figure 7A). Pigs in the Ad-pp220-ENABL® treatment group also had the highest post-prime mean IFN-γ response detected in PBMCs against the p150-II antigen. However, no significant differences were detected among the treatment and negative control groups (Figure 7A).

After boosting, p5-p34-p14-p37- ($p < 0.05$) and p150-I-specific ($p < 0.001$) IFN-γ responses in PBMCs were significantly expanded in the Ad-pp220-ENABL®-immunized pigs compared to

the IFN-γ responses in the Ad-pp220-ZTS-01-immunized pigs (Figure 7B). Mean IFN-γ responses against p5-p34-p14-p37 ($p < 0.01$) and p150-I ($p < 0.0001$) antigens in the Ad-pp220-ENABL® treatment group were also significantly higher than the responses in the negative control Ad-Luc-ENABL® group (Figure 7B). Surprisingly, very low levels of post-boost IFN-γ responses against the p150-II antigen were detected in PBMCs from the Ad-pp220-ENABL®-immunized pigs, suggesting that the post-prime responses did not amplify after boosting (Figure 7B). Pigs in the Ad-pp220-ZTS-01 treatment group had no detectable post-boost p150-II-specific responses in PBMCs (Figure 7B).

Consistent recall IFN-γ responses against the p5-p34-p14-p37 and p150-I antigens were detected in the splenocytes from the Ad-pp220-ENABL®- and the Ad-pp220-ZTS-01-immunized pigs (Figure 7C). Significantly higher mean p5-p34-p14-p37-specific IFN-γ$^+$ splenocytes were recalled in pigs from the Ad-pp220-ENABL® ($p < 0.001$) and the Ad-pp220-ZTS-01 ($p < 0.05$) treatment groups compared to the pigs in the negative control Ad-Luc-ENABL® group (Figure 7C). Mean IFN-γ response recalled in splenocytes against the p150-I antigen in the Ad-pp220-ENABL®-immunized pigs was significantly higher than the responses detected in the pigs from the Ad-pp220-ZTS-01 ($p < 0.01$) and the Ad-Luc-ENABL® ($p < 0.001$) groups (Figure 7C). Pigs in the Ad-pp220-ZTS-01 treatment group also had p150-I-specific recall IFN-γ$^+$ splenocytes, however, this response was not significantly higher than that detected in the Ad-Luc-ENABL®-immunized pigs (Figure 7C). Similar to the post-boost responses detected in PBMCs, very low levels of p150-II-specific IFN-γ$^+$ splenocytes were detected in the two treatment groups (Figure 7C).

2.3.5 Cytotoxic T-lymphocytes (CTLs) responses were elicited against pp220 antigens

Post-boost, PBMCs collected from the pigs immunized with the Ad-pp220-ENABL® and the Ad-pp220-ZTS-01 formulations showed strong lytic activities against autologous skin fibroblasts expressing the pp220 antigens (Figure 8). Mean background lytic activity against the negative control FMDV antigen in both the treatment groups was at or below 20% (Figure 8). In the Ad-pp220-ENABL® group, 3/5 pigs had p5-p34-p14-p37-specific lytic responses that were higher than the FMDV negative control antigen at both tested effector-to-target ratios (25:1 and 50:1), whereas 3/5 and 2/5 pigs had detectable p150-I-specific lytic responses at the 25:1 and the 50:1 ratio, respectively (Figure 8A). One Ad-pp220-ENABL®-immunized pig had a 100% specific lytic response against the p150-I antigen at the 50:1 ratio (Figure 8A). Against the p150-II antigen, 3/5 and 2/5 Ad-pp220-ENABL®-immunized pigs had lytic activity above the FMDV negative control antigen at the 25:1 and 50:1 ratio, respectively (Figure 8A).

Lytic activity against the p5-p34-p14-p37 antigen was detected in 2/5 and 3/5 pigs from the Ad-pp220-ZTS-01 treatment group, at the 25:1 and 50:1 ratio, respectively (Figure 8B). For the p150-I antigen, high levels of lytic responses were detected in 4/5 pigs from the Ad-pp220-ZTS-01 treatment group at both effector-to-target ratios used (Figure 8B). Notably, one Ad-pp220-ZTS-01-immunized pig (number 37) had a consistently high response (>80% specific lysis) against the p150-I antigen at the 25:1 and 50:1 ratio (Figure 8B). In the Ad-pp220-ZTS-01-immunized pigs, 2/5 pigs had consistently high p150-II-specific lytic responses at both the effector-to-target ratios used (Figure 8B). Two pigs (numbers 37 and 93) had consistently high lytic responses against all the three antigens at both the effector-to-target ratios tested (Figure 8B). Overall, the Ad-pp220 cocktail formulated in ZTS-01 adjuvant primed stronger and consistent CTL responses in pigs

against all the three pp220 antigens that were detectable at the lower effector to target ratio (Figure 8B).

2.3.6 IFN-γ-inducing nonamer peptides were identified within ASFV pp220

Five pools of predicted SLA-I binding nonamer peptides from the ASFV (Georgia 2007/1) pp220 polyprotein (Pools A-E) were screened for their ability to stimulate IFN-γ responses in PBMCs and splenocytes from the pigs immunized with the Ad-pp220-ENABL® formulation since this group had the highest pp220-specific cellular IFN-γ responses (Table 2, Figures 7, and 9). The peptide pools A, B, and C stimulated high levels of IFN-γ responses in terminal PBMCs (Figure 9A) as well as splenocytes (Figure 9B) from a majority of the Ad-pp220-ENABL®-immunized pigs. Individual peptides from the three selected pools were then evaluated for their ability to stimulate pp220-specific recall IFN-γ$^+$ responses in PBMCs and splenocytes from pigs in the Ad-pp220-ENABL® and the Ad-pp220-ZTS-01 treatment groups.

Four IFN-γ inducing peptides, namely p34$^{161-169}$, p37$^{859-867}$, p150$^{1363-1371}$, and p150$^{1463-1471}$, recalled high numbers of IFN-γ+ PBMCs and splenocytes in the pp220-immunized pigs (Figure 10). The first peptide, p34$^{161-169}$, was recognized by PBMCs (Figure 10A) as well as splenocytes (Figure 10B) from 4/5 pigs belonging to the Ad-pp220-ENABL® treatment group. This peptide was also recognized by PBMCs isolated from 3/5 pigs (Figure 10A) and splenocytes from 4/5 pigs (Figure 10B) immunized with the Ad-pp220-ZTS-01 formulation. The second peptide, p37$^{859-867}$, stimulated recall IFN-γ$^+$ responses in PBMCs (Figure 10A) and splenocytes (Figure 10B) from 5/5 and 4/5 pigs, respectively, from the Ad-pp220-ENABL® treatment group. This peptide was also recognized by PBMCs from 2/5 pigs (Figure 10A) and splenocytes from 4/5 pigs (Figure 10B) immunized with the Ad-pp220-ZTS-01. The third peptide, p150$^{1363-1371}$, recalled IFN-γ$^+$ PBMC

and splenocyte responses in 4/5 Ad-pp220-ENABL®- and Ad-pp220-ZTS-01-immunized pigs (Figure 10), whereas the fourth peptide, p150[1463-1471], recalled IFN-γ^+ PBMCs in 4/5 and 3/5 (Figure 10A) and IFN-γ^+ splenocytes in 5/5 and 4/5 pigs (Figure 10B) in the Ad-pp220-ENABL® and Ad-pp220-ZTS-01 treatment groups, respectively. Interestingly, these four epitopes are 100% conserved among different ASFV genotypes isolated from domestic pigs, wild boars, Warthog, and ticks (Supplemental Figure 1 and Supplemental Table 1). In addition, in silico analyses showed that the peptides bind strongly to multiple SLA-I alleles (Supplemental Table 2).

2.4 Discussion

The development of efficacious ASFV subunit vaccines is hindered by the lack of definition of the correlates of immune protection and identification of protective antigens (Sang et al. 2020). Since ASFV mutants can confer immune protection (Barasona et al. 2019; Borca et al. 2020), identification of the protective determinants will allow development of rationally designed prototype subunit vaccines. In this study, pigs were immunized with a cocktail of three adenoviruses encoding the pp220 polyprotein (Ad-p5-p34-p14-p37, Ad-p150-I, and Ad-p150-II). The pp220 polyprotein and pp62 are key components of the ASFV core shell and the processing of these proteins requires the presence of the major capsid protein p72 (Andres, Alejo, et al. 2002). The adenovirus cocktail formulated in adjuvant induced robust pp220 antigen-and wildtype ASFV specific IgG responses (Figure 5 and Figure 6). The endpoint titers of the p5-p34-p14-p37, p150-I, and p150-II antigen-specific IgG titers primed and expanded by both adjuvants are unprecedented and were significantly higher than those detected in the convalescent serum (Figure 5). These outcomes were consistent with previous antibody responses against all the three antigens in sera from pigs immunized with adenovirus cocktail formulated with BioMize (ENABL)

adjuvant. However, strong antibody responses were only observed against p5-p34-p14-p37 in the sera from pigs immunized with the cocktail formulated with ZTS-01 adjuvant (Lokhandwala et al. 2019). Whether anti-pp220 antibodies have a protective function is yet to be determined empirically by challenge. The role of ASFV-specific antibodies in protection is contentious as neutralization of virus has been reported, but it may not be mutually exclusive for protection, and this may relate to the target antigens or subtype of immunoglobulin being measured (Onisk et al. 1994; Ruiz-Gonzalvo F 1983; Ruiz Gonzalvo et al. 1986; Leitao et al. 2001). A previous study showed that, even though pigs succumbed to the disease following challenge, the pigs that had significantly lower antigen-specific IgG response had better survival rate and lesser clinical scores (Lokhandwala et al. 2019). An immune-mediated enhancement (ADE) of the disease may explain the higher clinical scores observed in the pigs that had high antibody responses than those of the control pigs. Other studies have reported similar findings, and no alternative explanation of the underlying mechanism for enhanced disease has been outlined (Jancovich et al. 2018; Lokhandwala et al. 2016; Blome, Gabriel, and Beer 2014).

The adenovirus cocktail formulated in adjuvant induced strong IFN-γ-secreting cells following intramuscular immunization of pigs. The ENABL-adjuvanted recombinant adenovirus cocktail generated a significantly higher mean number of antigen-specific IFN-γ secreting cells than the ZTS-01 adjuvanted adenovirus cocktail in response to the p5-p34-p14-p37 and the p150-I antigens. However, both adjuvants elicited poor IFN-γ responses against the p150-II antigen. This trend was observed in PBMCs post-priming and post-boost, as well as in splenocytes at study termination (Figure 7). The outcome suggests that the p5-p34-p14-p37 and the p150-I antigens are rich in IFN-γ-inducing epitopes. Strong recall IFN-$γ^+$ responses to adenovirus-vectored ASFV antigens have previously been observed (Lokhandwala et al. 2017; Jancovich et al. 2018).

Cytokine response to ASFV infection is highly dependent on the antigen, genotype, level of attenuation, and dose of the virus (Karalyan et al. 2012; Leitao et al. 2001; Lacasta et al. 2015). The level of protection of immunized or recovered pigs following ASFV challenge is associated with the frequency of ASFV-specific T cells producing IFN-γ (Sun et al. 2021). The importance of IFN-γ in immune protection is further supported by the demonstration that ASFV antigen-specific CD8^{+} T cells and/or CD4^{+}CD8^{+} T cells with cytotoxic ability produce high levels of IFN-γ in response to attenuated virus and can be related to cross-protection between different isolates (Netherton, Goatley, Reis, Portugal, Nash, Morgan, Gault, Nieto, Norlin, and Gallardo 2019; King et al. 2011; Oura et al. 2005). It has also been shown that immunization of pigs with a pool of 8 live-vectored ASFV antigens induced high IFN-γ spot forming cells and conferred 100% survival of animal post-challenge (Goatley et al. 2020).

Immunization of pigs with the adenovirus cocktail also induced strong CTL responses. However, the cocktail formulated in the ZTS-01 adjuvant primed unprecedented, stronger and more consistent CTL responses against all the pp220 antigens (Figure 8). This outcome suggests that CTL epitopes are present in the p5-p34-p14-p37, p150-I, and p150-II antigens. This outcome also suggests that these antigens may play a role in eliciting protective immunity, but this will need to be determined empirically. Immunization of pigs with a five-antigen cocktail that included adenoviruses expressing the pp220 antigens conferred protection in 5/9 pigs following challenge (Lokhandwala et al. 2019). Induction of CTLs capable of eliminating infected cells could be the key to complete protection since ASFV-infected cells are cleared specifically by CTLs induced by live attenuated ASFV (Schäfer et al. 2022; Franzoni et al. 2022). It has previously been shown that, CD8^{+} cells from pigs that recovered from ASFV infection are cytotoxic to macrophages infected with vaccinia virus expressing p32 antigen (Alonso et al. 1997). It has also been shown

that pigs immunized with an avirulent isolate are immune to challenge with the corresponding virulent strain. However, when such pigs are depleted of CD8^{+} lymphocytes, they develop severe ASF and succumb to the disease upon challenge, suggesting that the CD8^{+} T cells are involved in reducing viremia (Oura et al. 2005). Several studies support the role of cellular immunity in protection against ASFV wherein specific T cell responses were present in the absence of measurable antibodies (Argilaguet et al. 2012; Lacasta et al. 2014).

The IFN-γ EliSpot assay is commonly used to enumerate antigen-specific IFN-γ^{+} T cells following stimulation with one or multiple peptide antigens (Schmittel, Keilholz, and Scheibenbogen 1997; Barabas et al. 2017; Mwangi 2007.; Sangewar et al. 2020), and epitopes presented in the context of MHC I can be identified *ex vivo* (Anthony and Lehmann 2003). A CTL epitope in ASFV p72 antigen was previously mapped using the cumbersome procedure of expressing peptides in a plasmid vector and transfecting target cells (Leitao et al. 1998). Assessment of T-cell responses against CD2v (EP402R) and C-type lectin proteins conducted using 15-mer overlapping peptides showed that 6 of the 132 total predicted peptides resulted in a high frequency of IFN-γ producing cells (Burmakina et al. 2019). Bioinformatic platforms in conjunction with EliSpot and CTL assays provide a more practical approach to map key epitopes that may be useful for vaccine development (De Groot et al. 2003; Martin, Sbai, and De Groot 2003; Sangewar et al. 2020; Herrera and Bisa 2021). The application of in silico screening of sequence data combined with experimental methods to develop synthetic vaccines based on defined epitopes presents a theoretical advantage over traditional approaches to vaccine design (Palatnik-de-Sousa, Soares, and Rosa 2018). Multiple IFN-γ[+]-inducing epitopes were identified by screening predicted strong SLA-I binding nonamer peptides using the IFN-γ EliSpot assay. Out of the eighty-eight putative epitopes, four peptides, namely p34[161-169], p37[859-867], p150[1363-1371], and

p150$^{1463-1471}$, recalled very strong IFN-γ$^+$ responses in PBMC and splenocytes from pigs immunized with the Ad-pp220 cocktail formulated in either ENABL or ZTS-01 adjuvants (Figure 10). The p34$^{161-169}$ and p37$^{859-867}$ peptides are present in the p5-p34-p14-p37 antigen, whereas the p150$^{1363-1371}$ and p150$^{1463-1471}$ peptides are present in the p150-I antigen, which might explain the poor IFN-γ responses against the p150-II antigen (Figure 7). Thus, multiple T cell epitopes are present in the pp220 polyprotein that can induce robust IFN-γ$^+$ responses in domestic pigs. In addition, the epitopes, are 100% conserved among different ASFV genotypes isolated from Suids and ticks (Supplemental Figure 1 and Supplemental Table 1). Furthermore, the peptides bind strongly, in silico, to multiple SLA-I alleles, (Supplemental Table 2). Future challenge studies will determine whether these peptides are also CTL epitopes produced from natural infection and whether they play a role in protection. The peptide ITKTFVNNI (number 68 in Table 2) was also previously identified by Bosch-Camos et al. and assessed for immunogenicity in pigs when expressed using a plasmid vector; however, it did not elicit an immune response (Bosch-Camós, López, Collado, et al. 2021). More recent prediction data indicated peptides IADAINQEF, QIYKTLLEY, and SLYPTQFDY (numbers 2, 4-5 in Table 2) which are highly conserved cytotoxic T-cell epitopes in the ASFV genome (Herrera and Bisa 2021). The high level of conservation and binding to multiple alleles suggests that the epitopes identified in this study are ideal for inclusion in a prototype subunit vaccine since they have potential to elicit broad immune responses in outbred pigs. Overall, the use of bioinformatics tools to predict epitopes from the large ASFV proteome followed by empirical identification of relevant determinants that have potential to contribute to immune protection is a rational subunit vaccine development approach (Ros-Lucas et al. 2020; Herrera and Bisa 2021).

In conclusion, the results generated in this study demonstrate that the pp220 ASFV polyprotein induced ASFV-specific antibody responses as well as antigen-specific IFN-γ^+ cellular and CTL responses. These immune responses are important in the clearance of ASFV, given that ASFV-infected cells are cleared by CTLs induced by live attenuated ASFV and inhibition of IFN-γ has been tied to persistence and replication of ASFV particles (Fan et al. 2020; Lacasta et al. 2015; Zhuo et al. 2021; Schäfer et al. 2022; Franzoni et al. 2022). Since attenuated ASFV can confer protection, future studies will entail empirical identification of novel antigens that induce IFN-γ^+ and CTL responses, and evaluation of their protective potential to allow selection of a minimal number of validated antigens for the development of a rationally designed ASFV subunit vaccine.

2.5 References

Achenbach, J. E., C. Gallardo, E. Nieto-Pelegrín, B. Rivera-Arroyo, T. Degefa-Negi, M. Arias, S. Jenberie, D. D. Mulisa, D. Gizaw, and E. Gelaye. 2017. 'Identification of a new genotype of African swine fever virus in domestic pigs from Ethiopia', *Transboundary and Emerging Diseases*, 64: 1393-404.

Alejo, A., T. Matamoros, M. Guerra, and G. Andrés. 2018. 'A Proteomic Atlas of the African Swine Fever Virus Particle', *J Virol*, 92.

Alonso, Covadonga, Manuel Borca, Linda Dixon, Yolanda Revilla, Fernando Rodriguez, Jose M. Escribano, and ICTV Report Consortium. 2018. 'ICTV Virus Taxonomy Profile: Asfarviridae', *Journal of General Virology*, 99: 613-14.

Alonso, F., J. Dominguez, E. Vinuela, and Y. Revilla. 1997. 'African swine fever virus-specific cytotoxic T lymphocytes recognize the 32 kDa immediate early protein (vp32)', *Virus Res*, 49: 123-30.

Andres, G., A. Alejo, J. Salas, and M. L. Salas. 2002. 'African swine fever virus polyproteins pp220 and pp62 assemble into the core shell', *J Virol*, 76: 12473-82.

Andrés, G., D. Charro, T. Matamoros, R. S. Dillard, and N. G. A. Abrescia. 2020. 'The cryo-EM structure of African swine fever virus unravels a unique architecture comprising two icosahedral protein capsids and two lipoprotein membranes', *J Biol Chem*, 295: 1-12.

Andres, G., R. Garcia-Escudero, M. L. Salas, and J. M. Rodriguez. 2002. 'Repression of African swine fever virus polyprotein pp220-encoding gene leads to the assembly of icosahedral core-less particles', *J Virol*, 76: 2654-66.

Andres, G., C. Simon-Mateo, and E. Vinuela. 1993. 'Characterization of two African swine fever virus 220-kDa proteins: a precursor of the major structural protein p150 and an oligomer of phosphoprotein p32', *Virology*, 194: 284-93.

Andres, G., C. Simon-Mateo, and E. Vinuela. 1997. 'Assembly of African swine fever virus: role of polyprotein pp220', *J Virol*, 71: 2331-41.

African Swine Fever. Paris: World Organization for Animal Health (2019).

African Swine Fever (ASF) Situation Report. Paris: World Organisation for Animal Health (2021).

Anthony, Donald D., and Paul V. Lehmann. 2003. 'T-cell epitope mapping using the ELISPOT approach', *Methods*, 29: 260-69.

Argilaguet, Jordi M, Eva Pérez-Martín, Miquel Nofrarías, Carmina Gallardo, Francesc Accensi, Anna Lacasta, Mercedes Mora, Maria Ballester, Ivan Galindo-Cardiel, and Sergio López-

Soria. 2012. 'DNA vaccination partially protects against African swine fever virus lethal challenge in the absence of antibodies', *PLOS ONE*, 7: e40942.

Barabas, Sascha, Theresa Spindler, Richard Kiener, Charlotte Tonar, Tamara Lugner, Julia Batzilla, Hanna Bendfeldt, Anne Rascle, Benedikt Asbach, Ralf Wagner, and Ludwig Deml. 2017. 'An optimized IFN-γ ELISpot assay for the sensitive and standardized monitoring of CMV protein-reactive effector cells of cell-mediated immunity', *BMC Immunology*, 18: 14.

Barasona, Jose A, Carmina Gallardo, Estefanía Cadenas-Fernández, Cristina Jurado, Belén Rivera, Antonio Rodríguez-Bertos, Marisa Arias, and Jose M Sánchez-Vizcaíno. 2019. 'First oral vaccination of Eurasian wild boar against African swine fever virus genotype II', *Frontiers in Veterinary Science*, 6: 137.

Blome, Sandra, Claudia Gabriel, and Martin Beer. 2014. 'Modern adjuvants do not enhance the efficacy of an inactivated African swine fever virus vaccine preparation', *Vaccine*, 32: 3879-82.

Borca, M. V., E. Ramirez-Medina, E. Silva, E. Vuono, A. Rai, S. Pruitt, L. G. Holinka, L. Velazquez-Salinas, J. Zhu, and D. P. Gladue. 2020. 'Development of a Highly Effective African Swine Fever Virus Vaccine by Deletion of the I177L Gene Results in Sterile Immunity against the Current Epidemic Eurasia Strain', *J Virol*, 94.

Bosch-Camós, L., E. López, J. Collado, M. J. Navas, M. Blanco-Fuertes, S. Pina-Pedrero, F. Accensi, M. L. Salas, E. Mundt, V. Nikolin, and F. Rodríguez. 2021. 'M448R and MGF505-7R: Two African Swine Fever Virus Antigens Commonly Recognized by ASFV-Specific T-Cells and with Protective Potential', *Vaccines (Basel)*, 9.

Bosch-Camós, Laia, Elisabet López, María J. Navas, Sonia Pina-Pedrero, Francesc Accensi, Florencia Correa-Fiz, Chankyu Park, Montserrat Carrascal, Javier Domínguez, Maria L. Salas, Veljko Nikolin, Javier Collado, and Fernando Rodríguez. 2021. 'Identification of Promiscuous African Swine Fever Virus T-Cell Determinants Using a Multiple Technical Approach', *Vaccines*, 9.

Burleson, Gary R., Florence G. Burleson, and Rodney R. Dietert. 2010. 'The cytotoxic T lymphocyte assay for evaluating cell-mediated immune function.' in, *Immunotoxicity Testing* (Springer).

Burmakina, G., A. Malogolovkin, E. R. Tulman, W. Xu, G. Delhon, D. Kolbasov, and D. L. Rock. 2019. 'Identification of T-cell epitopes in African swine fever virus CD2v and C-type lectin proteins', *J Gen Virol*, 100: 259-65.

Butler, J. E., Nancy Wertz, and Marek Sinkora. 2017. 'Antibody repertoire development in swine', *Annual review of animal biosciences*, 5: 255-79.

Cadenas-Fernandez, E., J. M. Sanchez-Vizcaino, A. Kosowska, B. Rivera, F. Mayoral-Alegre, A. Rodriguez-Bertos, J. Yao, J. Bray, S. Lokhandwala, W. Mwangi, and J. A. Barasona.

2020. 'Adenovirus-vectored African Swine Fever Virus Antigens Cocktail Is Not Protective against Virulent Arm07 Isolate in Eurasian Wild Boar', *Pathogens*, 9.

Carlson, J., V. O'Donnell, M. Alfano, L. Velazquez Salinas, L. G. Holinka, P. W. Krug, D. P. Gladue, S. Higgs, and M. V. Borca. 2016. 'Association of the Host Immune Response with Protection Using a Live Attenuated African Swine Fever Virus Model', *Viruses*, 8.

Carlson, Jolene, Melina Fischer, Laura Zani, Michael Eschbaumer, Walter Fuchs, Thomas Mettenleiter, Martin Beer, and Sandra Blome. 2020. 'Stability of African swine fever virus in soil and options to mitigate the potential transmission risk', *Pathogens*, 9: 977.

Carlson, Jolene, Vivian O'Donnell, Marialexia Alfano, Lauro Velazquez Salinas, Lauren G. Holinka, Peter W. Krug, Douglas P. Gladue, Stephen Higgs, and Manuel V. Borca. 2016. 'Association of the Host Immune Response with Protection Using a Live Attenuated African Swine Fever Virus Model', *Viruses*, 8: 291.

Ceppi, M., M. G. M. de Bruin, T. Seuberlich, C. Balmelli, S. Pascolo, N. Ruggli, D. Wienhold, J. D. Tratschin, K. C. McCullough, and A. Summerfield. 2005. 'Identification of classical swine fever virus protein E2 as a target for cytotoxic T cells by using mRNA-transfected antigen-presenting cells', *Journal of General Virology*, 86: 2525-34.

Chenais, Erika, Sofia Boqvist, Ulf Emanuelson, Claudia Von Brömssen, Emily Ouma, Tonny Aliro, Charles Masembe, Karl Ståhl, and Susanna Sternberg-Lewerin. 2017. 'Quantitative assessment of social and economic impact of African swine fever outbreaks in northern Uganda', *Preventive Veterinary Medicine*, 144: 134-48.

De Groot, Anne S., Vishvanath Nene, Nagendra R. Hegde, S. Srikumaran, James Rayner, and William Martin. 2003. 'T cell epitope identification for bovine vaccines: an epitope mapping method for BoLA A-11', *International Journal for Parasitology*, 33: 641-53.

De Pelsmaeker, Steffi, Bert Devriendt, Nick De Regge, and Herman W. Favoreel. 2019. 'Porcine NK cells stimulate proliferation of pseudorabies virus-experienced CD8+ and CD4+ CD8+ T cells', *Frontiers in Immunology*, 9: 3188.

Dixon, L. K., H. Sun, and H. Roberts. 2019. 'African swine fever', *Antiviral research*, 165: 34-41.

Dixon, Linda K., Karl Stahl, Ferran Jori, Laurence Vial, and Dirk U. Pfeiffer. 2020. 'African swine fever epidemiology and control', *Annual review of animal biosciences*, 8: 221-46.

Ebwanga, Ebanja Joseph, Stephen Mbigha Ghogomu, and Jan Paeshuyse. 2021. 'African Swine Fever in Cameroon: A Review', *Pathogens*, 10: 421.

Escribano, José M., Inmaculada Galindo, and Covadonga Alonso. 2013. 'Antibody-mediated neutralization of African swine fever virus: Myths and facts', *Virus Research*, 173: 101-09.

Eulalio, A., I. Nunes-Correia, A. L. Carvalho, C. Faro, V. Citovsky, J. Salas, M. L. Salas, S. Simoes, and M. C. de Lima. 2006. 'Nuclear export of African swine fever virus p37 protein occurs through two distinct pathways and is mediated by three independent signals', *J Virol*, 80: 1393-404.

Eulalio, A., I. Nunes-Correia, A. L. Carvalho, C. Faro, V. Citovsky, S. SimõEs, and M. C. Pedroso De Lima. 2004. 'Two African Swine Fever Virus Proteins Derived from a Common Precursor Exhibit Different Nucleocytoplasmic Transport Activities', *Journal of Virology*, 78: 9731-39.

Fan, W., P. Jiao, H. Zhang, T. Chen, X. Zhou, Y. Qi, L. Sun, Y. Shang, H. Zhu, R. Hu, W. Liu, and J. Li. 2020. 'Inhibition of African Swine Fever Virus Replication by Porcine Type I and Type II Interferons', *Front Microbiol*, 11: 1203.

Fasina, F. O., D. D. Lazarus, B. T. Spencer, A. A. Makinde, and A. D. S. Bastos. 2012. 'Cost Implications of African Swine Fever in Smallholder Farrow-to-Finish Units: Economic Benefits of Disease Prevention Through Biosecurity', *Transboundary and Emerging Diseases*, 59: 244-55.

Franzoni, Giulia, Susanna Zinellu, Tania Carta, Chiara Grazia De Ciucis, Floriana Fruscione, Antonio Anfossi, Mauro Ledda, Simon P. Graham, Silvia Dei Giudici, Elisabetta Razzuoli, and Annalisa Oggiano. 2022. 'Analyses of the Impact of Immunosuppressive Cytokines on Porcine Macrophage Responses and Susceptibility to Infection to African Swine Fever Viruses', *Pathogens*, 11: 166.

Gale, Paul, Joe Bowen, and Lauren Perrin. 2021. "African Swine Fever in the Dominican Republic and Haiti." In *Animal diseases: international and UK monitoring*. United Kingdom: Department for Environment, Food and Rural Affairs, Animal and Plant Health Agency, Advice Services - International Disease Monitoring.

Gao, Feng-Shan, Xiao-Xin Zhai, Ping Jiang, Qiang Zhang, Hua Gao, Zi-Bin Li, Yong Han, Jie Yang, and Zong-hui Zhang. 2018. 'Identification of two novel foot-and-mouth disease virus cytotoxic T lymphocyte epitopes that can bind six SLA-I proteins', *Gene*, 653: 91-101.

Gerner, Wilhelm, Tobias Käser, and Armin Saalmüller. 2009. 'Porcine T lymphocytes and NK cells – An update', *Developmental & Comparative Immunology*, 33: 310-20.

Goatley, Lynnette C., Ana L. Reis, Raquel Portugal, Hannah Goldswain, Gareth L. Shimmon, Zoe Hargreaves, Chak-Sum Ho, María Montoya, Pedro J. Sánchez-Cordón, Geraldine Taylor, Linda K. Dixon, and Christopher L. Netherton. 2020. 'A Pool of Eight Virally Vectored African Swine Fever Antigens Protect Pigs against Fatal Disease', *Vaccines*, 8.

Gogin, A., V. Gerasimov, A. Malogolovkin, and D. Kolbasov. 2013. 'African swine fever in the North Caucasus region and the Russian Federation in years 2007–2012', *Virus Research*, 173: 198-203.

Gonzales, W., C. Moreno, U. Duran, N. Henao, M. Bencosme, P. Lora, R. Reyes, R. Núñez, A. De Gracia, and A. M. Perez. 2021. 'African swine fever in the Dominican Republic', *Transbound Emerg Dis*.

Hamdy, F. M., and A. H. Dardiri. 1984. 'Clinical and immunologic responses of pigs to African swine fever virus isolated from the Western Hemisphere', *American journal of veterinary research*, 45: 711-14.

Heath, Colin M., Miriam Windsor, and Thomas Wileman. 2003. 'Membrane Association Facilitates the Correct Processing of pp220 during Production of the Major Matrix Proteins of African Swine Fever Virus', *J. Virol.*, 77: 1682-90.

Herrera-Uribe, Júber, Ángeles Jiménez-Marín, Anna Lacasta, Paula L. Monteagudo, Sonia Pina-Pedrero, Fernando Rodríguez, Ángela Moreno, and Juan J. Garrido. 2018. 'Comparative proteomic analysis reveals different responses in porcine lymph nodes to virulent and attenuated homologous African swine fever virus strains', *Veterinary Research*, 49: 1-15.

Herrera, Leana Rich De Mesa, and Elizabeth Paulino Bisa. 2021. 'In silico analysis of highly conserved cytotoxic T-cell epitopes in the structural proteins of African swine fever virus', *Veterinary World*: 2625-33.

Hühr, Jane, Alexander Schäfer, Theresa Schwaiger, Laura Zani, Julia Sehl, Thomas C. Mettenleiter, Sandra Blome, and Ulrike Blohm. 2020. 'Impaired T-cell responses in domestic pigs and wild boar upon infection with a highly virulent African swine fever virus strain', *Transboundary and Emerging Diseases*, 67: 3016-32.

Infection with African Swine Fever Virus Terrestrial Animal Health Code. Paris: World Organization for Animal Health (2021).

Jancovich, James K., Dave Chapman, Debra T. Hansen, Mark D. Robida, Andrey Loskutov, Felicia Craciunescu, Alex Borovkov, Karen Kibler, Lynnette Goatley, Katherine King, Christopher L. Netherton, Geraldine Taylor, Bertram Jacobs, Kathryn Sykes, and Linda K. Dixon. 2018. 'Immunization of Pigs by DNA Prime and Recombinant Vaccinia Virus Boost To Identify and Rank African Swine Fever Virus Immunogenic and Protective Proteins', *Journal of Virology*, 92.

Juszkiewicz, Małgorzata, Marek Walczak, Natalia Mazur-Panasiuk, and Grzegorz Woźniakowski. 2020. 'Effectiveness of chemical compounds used against African swine fever virus in commercial available disinfectants', *Pathogens*, 9: 878.

Karalyan, Z., H. Zakaryan, Kh Sargsyan, H. Voskanyan, H. Arzumanyan, H. Avagyan, and E. Karalova. 2012. 'Interferon status and white blood cells during infection with African swine fever virus in vivo', *Veterinary Immunology and Immunopathology*, 145: 551-55.

Kim, Hyun-Joo, Min-Jung Lee, Soo-Kyoung Lee, Da-young Kim, Sang-Ji Seo, Hae-Eun Kang, and Hyang-Mi Nam. 2019. 'African swine fever virus in pork brought into South Korea by travelers from China, August 2018', *Emerging infectious diseases*, 25: 1231.

King, K., D. Chapman, J.M. Argilaguet, E. Fishbourne, E. Hutet, R. Cariolet, G. Hutchings, C.A.L. Oura, C.L. Netherton, and K. Moffat. 2011. 'Protection of European domestic pigs from virulent African isolates of African swine fever virus by experimental immunisation', *Vaccine*, 29: 4593-600.

Lacasta, A., P. L. Monteagudo, A. Jimenez-Marin, F. Accensi, M. Ballester, J. Argilaguet, I. Galindo-Cardiel, J. Segales, M. L. Salas, J. Dominguez, A. Moreno, J. J. Garrido, and F. Rodriguez. 2015. 'Live attenuated African swine fever viruses as ideal tools to dissect the mechanisms involved in viral pathogenesis and immune protection', *Vet Res*, 46: 135.

Lacasta, Anna, María Ballester, Paula L. Monteagudo, Javier M. Rodríguez, María L. Salas, Francesc Accensi, Sonia Pina-Pedrero, Albert Bensaid, Jordi Argilaguet, and Sergio López-Soria. 2014. 'Expression Library Immunization Can Confer Protection against Lethal Challenge with African Swine Fever Virus', *Journal of Virology*, 88: 13322-32.

Leitao, A., A. Malur, P. Cornelis, and C. L. Martins. 1998. 'Identification of a 25-aminoacid sequence from the major African swine fever virus structural protein VP72 recognised by porcine cytotoxic T lymphocytes using a lipoprotein based expression system', *J Virol Methods*, 75: 113-9.

Leitao, Alexandre, Clara Cartaxeiro, Ricardo Coelho, Benedita Cruz, R. M. E. Parkhouse, Fernando C. Portugal, Jose D. Vigario, and Carlos L. V. Martins. 2001. 'The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response', *J Gen Virol*, 82: 513-23.

Lewis, Charles E., and James A. Roth. 2021. 'Challenges in Having Vaccines Available to Control Transboundary Diseases of Livestock', *Current Issues in Molecular Biology*, 42.

Lewis, T., L. Zsak, T. G. Burrage, Z. Lu, G. F. Kutish, J. G. Neilan, and D. L. Rock. 2000. 'An African swine fever virus ERV1-ALR homologue, 9GL, affects virion maturation and viral growth in macrophages and viral virulence in swine', *J Virol*, 74: 1275-85.

Liu, Jinling, Bangzuo Liu, Baiqiang Shan, Shu Wei, Tongqing An, Guoshun Shen, and Zeliang Chen. 2020. 'Prevalence of African Swine Fever in China, 2018-2019', *Journal of medical virology*, 92: 1023-34.

Lokhandwala, S., V. Petrovan, L. Popescu, N. Sangewar, C. Elijah, A. Stoian, M. Olcha, L. Ennen, J. Bray, R. P. Bishop, S. D. Waghela, M. Sheahan, R. R. R. Rowland, and W. Mwangi. 2019. 'Adenovirus-vectored African Swine Fever Virus antigen cocktails are immunogenic but not protective against intranasal challenge with Georgia 2007/1 isolate', *Vet Microbiol*, 235: 10-20.

Lokhandwala, S., S. D. Waghela, J. Bray, N. Sangewar, C. Charendoff, C. L. Martin, W. S. Hassan, T. Koynarski, L. Gabbert, T. G. Burrage, D. Brake, J. Neilan, and W. Mwangi. 2017. 'Adenovirus-vectored novel African Swine Fever Virus antigens elicit robust immune responses in swine', *PLoS One*, 12: e0177007.

Lokhandwala, Shehnaz, Suryakant D. Waghela, Jocelyn Bray, Cameron L. Martin, Neha Sangewar, Chloe Charendoff, Rashmi Shetti, Clay Ashley, Chang-Hsin Chen, Luc R. Berghman, Duncan Mwangi, Paul J. Dominowski, Dennis L. Foss, Sharath Rai, Shaunak Vora, Lindsay Gabbert, Thomas G. Burrage, David Brake, John Neilan, and Waithaka Mwangi. 2016. 'Induction of Robust Immune Responses in Swine by Using a Cocktail of Adenovirus-Vectored African Swine Fever Virus Antigens', *Clinical and Vaccine Immunology*, 23: 888-900.

Martin, William, Hakima Sbai, and Anne S. De Groot. 2003. 'Bioinformatics tools for identifying class I-restricted epitopes', *Methods*, 29: 289-98.

Mwangi, W., W. C. Brown, G. A. Splitter, C. J. Davies, C. J. Howard, J. C. Hope, Y. Aida, Y. Zhuang, B. J. Hunter, and G. H. Palmer. 2007. 'DNA vaccine construct incorporating intercellular trafficking and intracellular targeting motifs effectively primes and induces memory B and T cell responses in outbred animals.', *Clin. Vaccine Immunol.*, 14: 304-11.

Neilan, J. G., L. Zsak, Z. Lu, T. G. Burrage, G. F. Kutish, and D. L. Rock. 2004. 'Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection', *Virology*, 319: 337-42.

Netherton, C. L., L. C. Goatley, A. L. Reis, R. Portugal, R. H. Nash, S. B. Morgan, L. Gault, R. Nieto, V. Norlin, C. Gallardo, C. S. Ho, P. J. Sanchez-Cordon, G. Taylor, and L. K. Dixon. 2019. 'Identification and Immunogenicity of African Swine Fever Virus Antigens', *Front Immunol*, 10: 1318.

Netherton, Christopher L., Lynnette C. Goatley, Ana Luisa Reis, Raquel Portugal, Rachel H. Nash, Sophie B. Morgan, Lynden Gault, Raquel Nieto, Veronica Norlin, and Carmina Gallardo. 2019. 'Identification and immunogenicity of African swine fever virus antigens', *Frontiers in Immunology*, 10: 1318.

Netherton, Christopher L., Lynnette C. Goatley, Ana Luisa Reis, Raquel Portugal, Rachel H. Nash, Sophie B. Morgan, Lynden Gault, Raquel Nieto, Veronica Norlin, Carmina Gallardo, Chak-Sum Ho, Pedro J. Sánchez-Cordón, Geraldine Taylor, and Linda K. Dixon. 2019. 'Identification and Immunogenicity of African Swine Fever Virus Antigens', *Frontiers in Immunology*, 10.

Onisk, D. V., M. V. Borca, S. Kutish, E. Kramer, P. Irusta, and D. L. Rock. 1994. 'Passively Transferred African Swine Fever Virus Antibodies Protect Swine against Lethal Infection', *Virology*, 198: 350-54.

Oura, C. A., M. S. Denyer, H. Takamatsu, and R. M. Parkhouse. 2005. 'In vivo depletion of CD8+ T lymphocytes abrogates protective immunity to African swine fever virus', *J Gen Virol*, 86: 2445-50.

Palatnik-de-Sousa, Clarisa Beatriz, Irene da Silva Soares, and Daniela Santoro Rosa. 2018. 'Editorial: Epitope Discovery and Synthetic Vaccine Design', *Frontiers in Immunology*, 9: 826.

Paulino-Ramirez, Robert. 2021. 'Food Security and Research Agenda in African Swine Fever Virus: a new Arbovirus Threat in the Dominican Republic', *InterAmerican Journal of Medicine and Health*, 4: e202101028.

Pietschmann, Jana, Lina Mur, Sandra Blome, Martin Beer, Ricardo Pérez-Sánchez, Ana Oleaga, and José Manuel Sánchez-Vizcaíno. 2016. 'African swine fever virus transmission cycles in Central Europe: Evaluation of wild boar-soft tick contacts through detection of antibodies against Ornithodoros erraticus saliva antigen', *BMC Veterinary Research*, 12: 1-5.

Rodriguez-Bertos, A., E. Cadenas-Fernandez, A. Rebollada-Merino, N. Porras-Gonzalez, F. J. Mayoral-Alegre, L. Barreno, A. Kosowska, I. Tome-Sanchez, J. A. Barasona, and J. M. Sanchez-Vizcaino. 2020. 'Clinical Course and Gross Pathological Findings in Wild Boar Infected with a Highly Virulent Strain of African Swine Fever Virus Genotype II', *Pathogens*, 9.

Ros-Lucas, Albert, Florencia Correa-Fiz, Laia Bosch-Camós, Fernando Rodriguez, and Julio Alonso-Padilla. 2020. 'Computational Analysis of African Swine Fever Virus Protein Space for the Design of an Epitope-Based Vaccine Ensemble', *Pathogens*, 9.

Ruiz-Gonzalvo F, Carnero ME, Bruyel V. 1983. "Immunological responses of pigs to partially attenuated African swine fever virus and their resistance to virulent homologous and heterologous viruses." In *FAO/CEC Expert consultation in African Swine Fever Research*, edited by Wilkinson PJ, pp 206–16. Sardinia.

Ruiz Gonzalvo, F., M. E. Carnero, C. Caballero, and J. Martinez. 1986. 'Inhibition of African swine fever infection in the presence of immune sera in vivo and in vitro', *Am J Vet Res*, 47: 1249-52.

Salguero, Francisco J. 2020. 'Comparative Pathology and Pathogenesis of African Swine Fever Infection in Swine', *Frontiers in Veterinary Science*, 7: 282.

Sang, H., G. Miller, S. Lokhandwala, N. Sangewar, S. D. Waghela, R. P. Bishop, and W. Mwangi. 2020. 'Progress Toward Development of Effective and Safe African Swine Fever Virus Vaccines', *Front Vet Sci*, 7: 84.

Sangewar, N., S. D. Waghela, J. Yao, H. Sang, J. Bray, and W. Mwangi. 2021. 'Novel Potent IFN-γ-Inducing CD8(+) T Cell Epitopes Conserved among Diverse Bovine Viral Diarrhea Virus Strains', *J Immunol*, 206: 1709-18.

Sangewar, Neha, Wisam Hassan, Shehnaz Lokhandwala, Jocelyn Bray, Rachel Reith, Mary Markland, Huldah Sang, Jianxiu Yao, Bailey Fritz, and Suryakant D. Waghela. 2020. 'Mosaic Bovine Viral Diarrhea Virus Antigens Elicit Cross-Protective Immunity in Calves', *Frontiers in Immunology*, 11.

Schäfer, Alexander, Giulia Franzoni, Christopher L. Netherton, Luise Hartmann, Sandra Blome, and Ulrike Blohm. 2022. 'Adaptive Cellular Immunity against African Swine Fever Virus Infections', *Pathogens*, 11: 274.

Schmittel, A., U. Keilholz, and C. Scheibenbogen. 1997. 'Evaluation of the interferon-gamma ELISPOT-assay for quantification of peptide specific T lymphocytes from peripheral blood', *J Immunol Methods*, 210: 167-74.

Sehl, Julia, Jutta Pikalo, Alexander Schäfer, Kati Franzke, Katrin Pannhorst, Ahmed Elnagar, Ulrike Blohm, Sandra Blome, and Angele Breithaupt. 2020. 'Comparative pathology of domestic pigs and wild boar infected with the moderately virulent African swine fever virus strain "Estonia 2014"', *Pathogens*, 9: 662.

Simon-Mateo, C., G. Andres, and E. Vinuela. 1993. 'Polyprotein processing in African swine fever virus: a novel gene expression strategy for a DNA virus', *EMBO J*, 12: 2977-87.

Suarez, C., M. L. Salas, and J. M. Rodriguez. 2010. 'African swine fever virus polyprotein pp62 is essential for viral core development', *J Virol*, 84: 176-87.

Sugiura, Katsuaki, Zhihao Lei, Caitlin Holley, and Takeshi Haga. 2020. 'Assessing the risk of ASFV entry into Japan through pork products illegally brought in by air passengers from China and fed to pigs in Japan', *PLOS One*, 15: e0232132.

Sun, Wenqiang, He Zhang, Wenhui Fan, Lihong He, Teng Chen, Xintao Zhou, Yu Qi, Lei Sun, Rongliang Hu, Tingrong Luo, Wenjun Liu, and Jing Li. 2021. 'Evaluation of Cellular Immunity with ASFV Infection by Swine Leukocyte Antigen (SLA)—Peptide Tetramers', *Viruses*, 13: 2264.

Sunwoo, Sun-Young, Daniel Pérez-Núñez, Igor Morozov, Elena G Sánchez, Natasha N Gaudreault, Jessie D Trujillo, Lina Mur, Marisa Nogal, Daniel Madden, and Kinga Urbaniak. 2019. 'DNA-Protein Vaccination Strategy Does Not Protect from Challenge with African Swine Fever Virus Armenia 2007 Strain', *Vaccines*, 7: 12.

Takamatsu, Haru-Hisa, Michael S. Denyer, Anna Lacasta, Catrina M. A. Stirling, Jordi M. Argilaguet, Christopher L. Netherton, Chris A. L. Oura, Carlos Martins, and Fernando Rodríguez. 2013. 'Cellular immunity in ASFV responses', *Virus Research*, 173: 110-21.

Wang, Jun, Xin-Jin Shi, Hai-Wei Sun, and Hong-Jun Chen. 2020. 'Insights into African swine fever virus immunoevasion strategies', *Journal of Integrative Agriculture*, 19: 11-22.

Woodruff, Matthew C., Balthasar A. Heesters, Caroline N. Herndon, Joanna R. Groom, Paul G. Thomas, Andrew D. Luster, Shannon J. Turley, and Michael C. Carroll. 2014. 'Trans-nodal migration of resident dendritic cells into medullary interfollicular regions initiates immunity to influenza vaccine', *Journal of Experimental Medicine*, 211: 1611-21.

Zhao, Dongming, Renqiang Liu, Xianfeng Zhang, Fang Li, Jingfei Wang, Jiwen Zhang, Xing Liu, Lulu Wang, Jiaoer Zhang, and Xinzhou Wu. 2019. 'Replication and virulence in pigs of the first African swine fever virus isolated in China', *Emerging microbes & infections*, 8: 438-47.

Zhou, Lin, Y. W. Evan, Shujin Wang, and Changpo Sun. 2019. 'African swine fever epidemic in China', *The Veterinary Record*, 184: 713.

Zhuo, Yisha, Zeheng Guo, Tongtong Ba, Cheng Zhang, Lihua He, Cuiping Zeng, and Hanchuan Dai. 2021. 'African Swine Fever Virus MGF360-12L Inhibits Type I Interferon Production by Blocking the Interaction of Importin α and NF-κB Signaling Pathway', *Virologica Sinica*, 36: 176-86.

Table 2.1. Swine Immunization Protocol

Groups	Swine ID	Immunogen (prime-boost dose per pig)	Adjuvant
Ad-pp220-ENABL	34 41 43 46 48	Ad-pp220 cocktail: Ad-p5-34-14-37 (10^{11} ifu), Ad-p150-I (10^{11} ifu) and Ad-p150-II (10^{11} ifu)	ENABL
Ad-pp220-ZTS-01	31 37 93 94 96	Ad-pp220 cocktail: Ad-p5-34-14-37 (10^{11} ifu), Ad-p150-I (10^{11} ifu) and Ad-p150-II (10^{11} ifu)	ZTS-01
Ad-Luc-ENABL	32 38 39 44 45	Ad-Luciferase (3 X 10^{11} ifu)	ENABL

Table 2.2. Predicted *SLA-I* binding peptides from ASFV pp220 (Georgia 2007/1).

Pool A		Pool B		Pool C		Pool D		Pool E	
Peptide ID	Sequence	Peptide ID	Sequence	Peptide ID	Sequence	Peptide ID	Sequence	Peptide ID	Sequence
1	AINTFMYYY	19	SQWDLVQKF	37	INMRHHTSY	55	YSFEEIACL	73	YVYKTPRWL
2	QIYKTLLEY	20	YGIQNNRSM	38	KSMAAKIFI	56	RRLLNEQNL	74	VSAENIAEF
3	RVFSRLVFY	21	IGMNAVYSL	39	LTTETLFAW	57	LRLRLNLEL	75	FYTHAIQAL
4	SLYPTQFDY	22	SLSNFQALK	40	ETEDVFFTF	58	ASICRQIVL	76	EAMQWFMTM
5	IADAINQEF	23	YTHAIQALR	41	NTLSYWDNI	59	EQYGRVFSR	77	IAASVANKI
6	SAMEVLHEL	24	FIINIRSFK	42	KEIALTPNI	60	RRFYRALEG	78	MAAKIFIVL
7	RLDRKHILM	25	GMNAVYSLR	43	RQMVPMSPL	61	TRLIRNLIF	79	AVNLLRQTF
8	ALDLSLIGF	26	LTHGLRAEY	44	FEHFYTHAI	62	NALMRSIPL	80	KLIQGSESL
9	YTDIVQKKY	27	IYQHFNLEY	45	REFMLKLLI	63	RLLRLRLN	81	GLISLIDSL
10	TVSAIELEY	28	SYWDNIALR	46	SYEENYATI	64	RYRLYGSDY	82	YYYYVAQIY
11	HIDKNIIQY	29	AGYMSRIFR	47	VMMYNENTF	65	SRLLQIIDF	83	VFNQLIASY
12	LLSKGNAGY	30	LMADTKYFL	48	RTMNDFGMM	66	FYWLEEHLI	84	IYLNLINAF
13	KTLQDVISF	31	MMMVFNQLI	49	IQNNRSMMM	67	YDPLLYPNL	85	NYRANLPLF
14	AGAQLTALF	32	STQAYNDFL	50	TLAQVFESF	68	ITKTFVNNI	86	NYDYSFEEI
15	SLMADTKYF	33	NTFMYYYYV	51	SMMMVFNQL	69	ALIHFVNEI	87	LYDSCSRLL
16	AQEENTLSY	34	TLFAWIVPY	52	NIYNYDYSF	70	LIASYITRF	88	LMPFSLSLY
17	MPFSLSLYY	35	AVMEMGYAH	53	YATILGDAI	71	YINSLTHGL		
18	YTENSVLTY	36	INMRLSMVY	54	YPDPTTEAA	72	YVAQIYSNL		

Table 2.3. IFN-γ inducing nonamer peptides from ASFV pp220 (Georgia 2007/1).

Peptide ID	pp220 Peptide	Sequence	Predicted *SLA*-I Allele
26	p34$^{161-169}$	LTHGLRAEY	SLA-2*01:01
38	p37$^{859-867}$	KSMAAKIFI	SLA-2*05:01
11	p150$^{1363-1371}$	HIDKNIIQY	SLA-1*04:01
3	p150$^{1463-1471}$	RVFSRLVFY	SLA-1*02:01
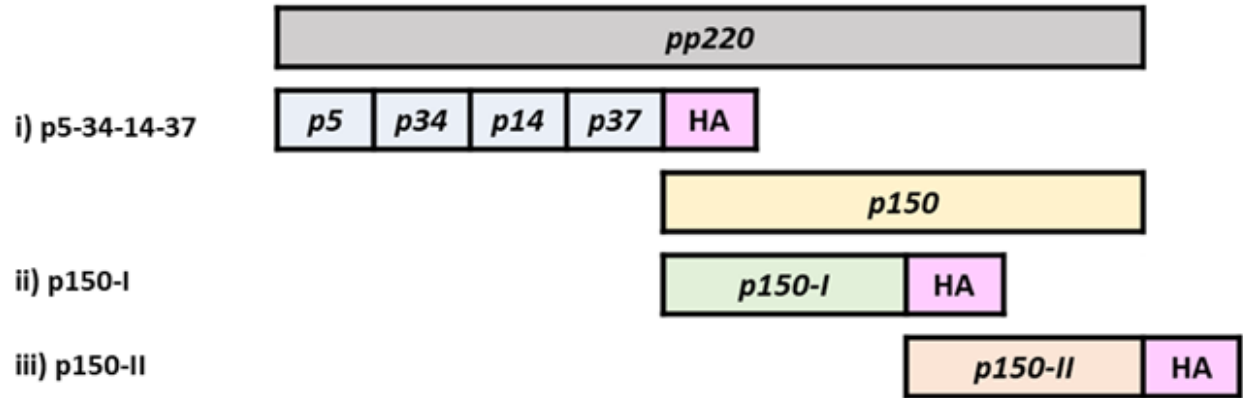
Figure 2.1. ASFV pp220 expression constructs.

 Illustration of synthetic genes encoding ASFV pp220 polyprotein. p5-34-14-37 constitute genes encoding structural proteins p5, p34, p14, and p37. Due to its large size, the sequence encoding p150 was split into two genes: p150-I; and p150-II. Synthetic genes had an HA tag added in-frame at the 3' end for tracking protein expression.
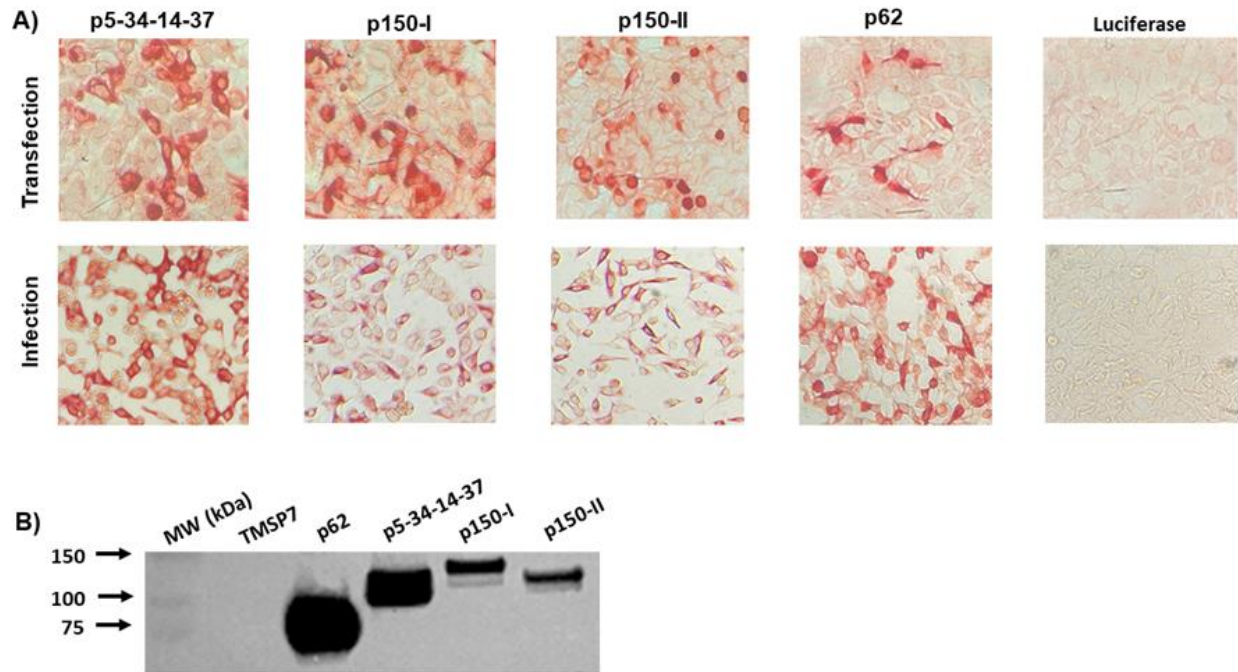
Figure 2.2. ASFV pp220 construct antigen expression.

(**A**) Antigen expression was evaluated by immunostaining of HEK 293A cells transfected with pcDNA or infected with adenovirus encoding each pp220 construct, and (**B**) Western Blot, using proteins produced by transfected HEK 293A cells, probed with ASFV-specific convalescent porcine serum. Recombinant ASFV p62 antigen served as a positive control, whereas an irrelevant recombinant antigen, TMSP7, served as a negative control.
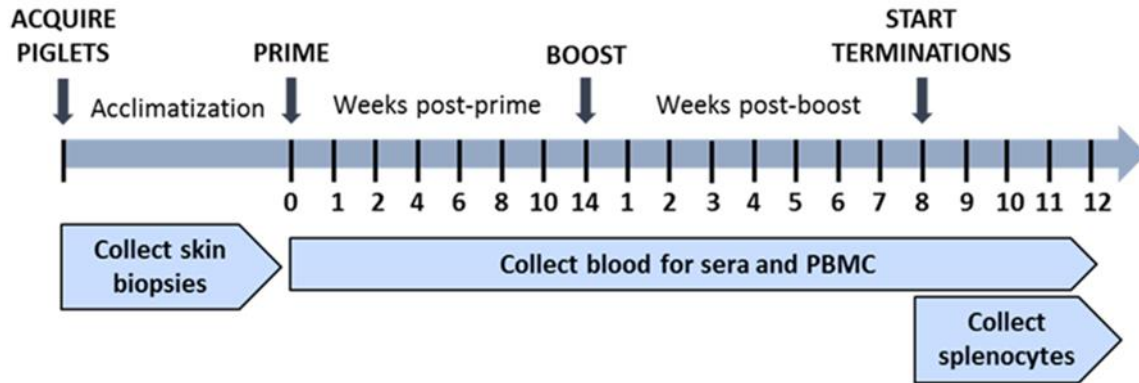
Figure 2.3. In vivo study timeline.

Piglets were acclimatized, and skin biopsies were collected prior to immunization. Piglets in treatment groups were primed at week 0 and then boosted at week 14 post-prime with the Ad-pp220 cocktail as shown in Table 1. Negative control piglets were similarly primed and boosted, but with Ad-Luciferase. Pigs from all the groups were terminated after week 8 post-boost. Blood samples were collected weekly post-prime and post-boost for PBMCs and sera. During termination, blood samples were collected, and spleens were harvested.
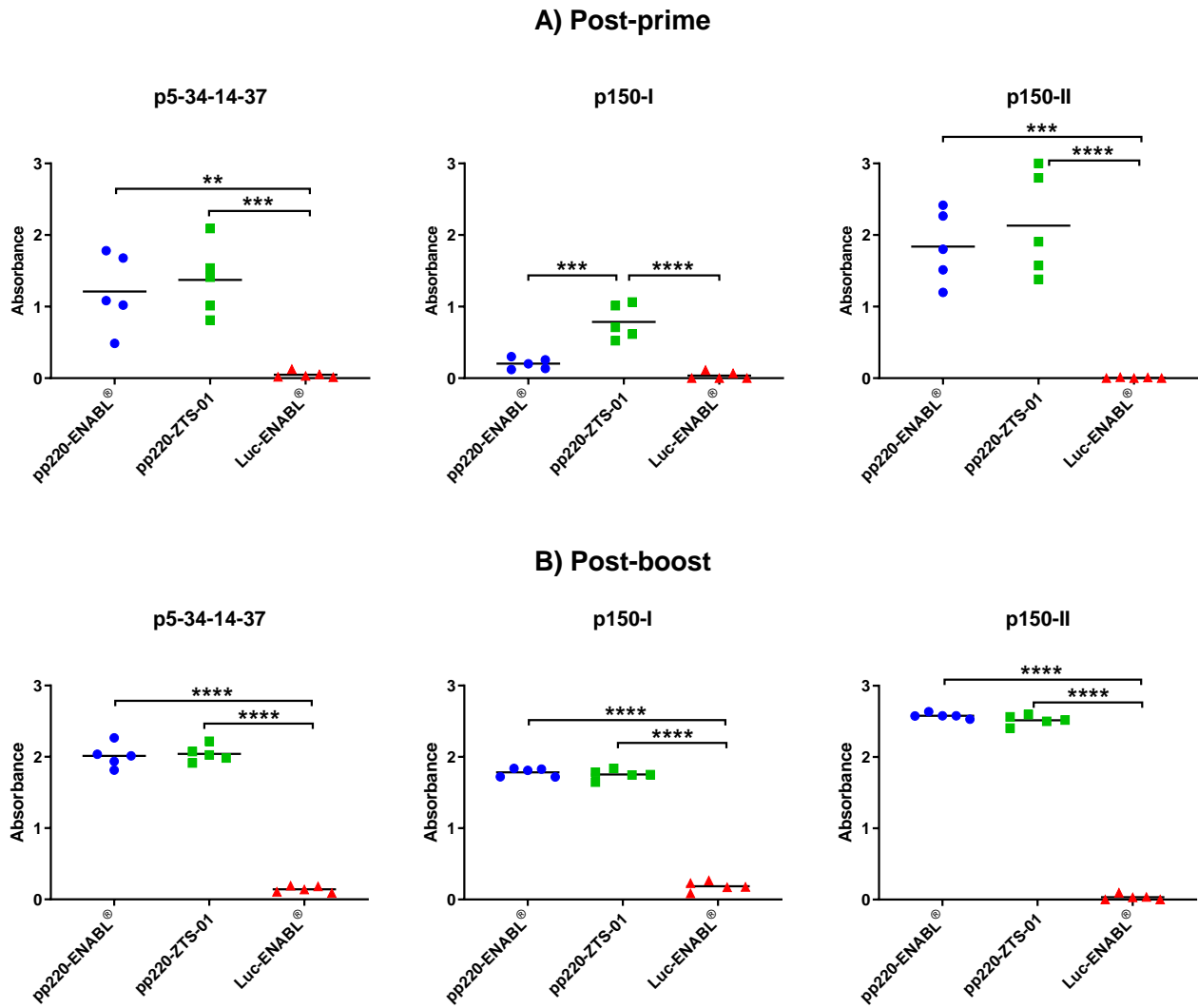
Figure 2.4. Antibody responses against pp220 antigens.

IgG responses against p5-34-14-37, p150-I and p150-II in (**A**) week 4 post-prime sera; and (**B**) week 1 post-boost sera were evaluated by ELISA. Mean responses for the groups are denoted by bars and statistically significant differences between groups is denoted by asterisks (**$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$).
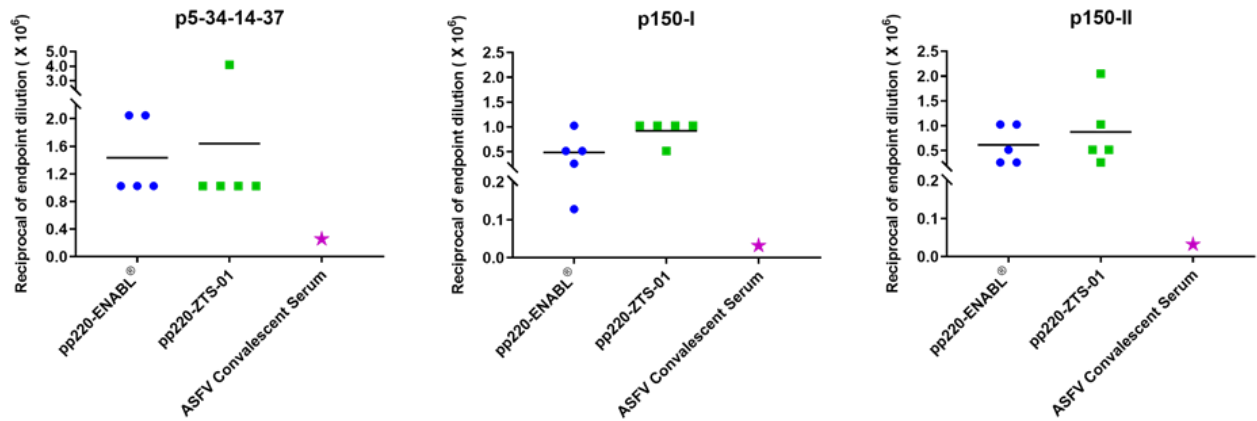
Figure 2.5. Antibody titers for pp220 antigens.

IgG end-point titers were determined by ELISA for p5-34-14-37, p150-I, and p150-II in sera from week 1 post-boost and in ASFV-specific convalescent porcine serum. Mean IgG titers for the groups are denoted by bars, whereas IgG titers in the convalescent serum are denoted by the pink star.
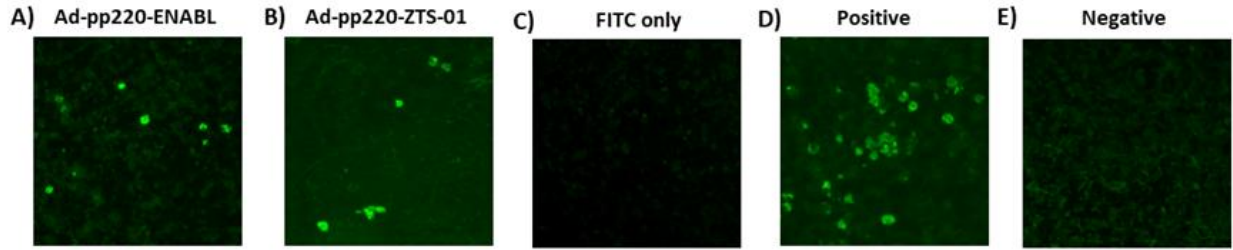
Figure 2.6. Validation of Induced anti-pp220 antibodies.

Authenticity of the antibodies elicited by the adenovirus-vectored pp220 antigens was confirmed by Immunohistochemistry. Formalin-fixed ASFV (Georgia 2007/1) infected swine spleen tissues were probed with sera that was obtained from each group two weeks post-boost: (**A**) Ad-pp220-ENABL; (**B**) Ad-pp220-ZTS-01; (**C**) Secondary (FITC) antibody control: secondary antibody is probed in the absence of primary sera, (**D**) Positive control serum: ASFV specific convalescent swine serum, and (**E**) Negative control serum: Ad-Luc-ENABL.
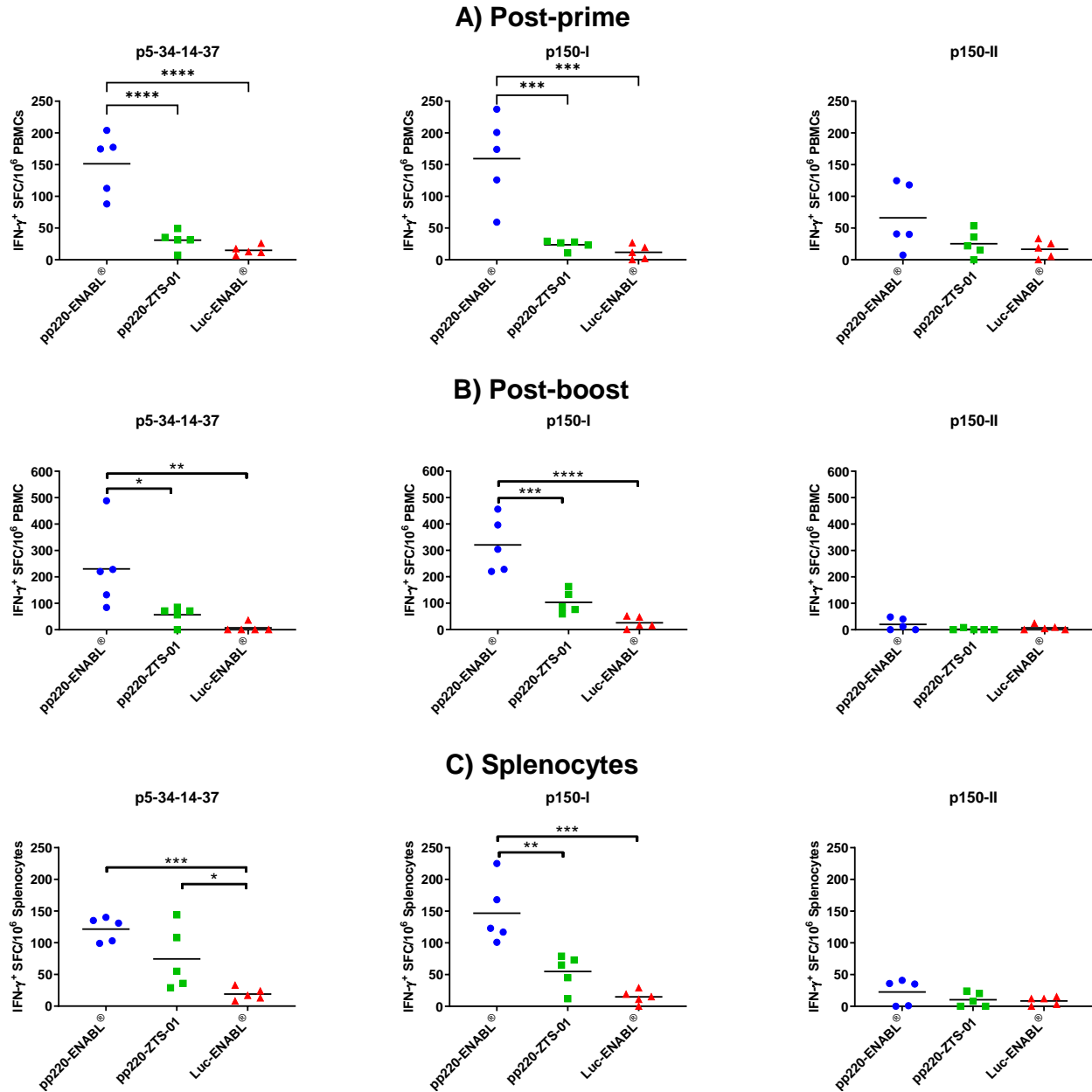
Figure 2.7. IFN-γ responses against pp220 antigens.

p5-34-14-37, p150-I- and p150-II-specific IFN-γ responses were detected by EliSpot assay using PBMCs from (**A**) Two weeks post-priming; (**B**) One-week post-boost; and (**C**) in splenocytes. Data is presented as Spot Forming Cells (SFC)/10^6 PBMCs or splenocytes. Medium alone served as the negative control, and the data shown is minus media background counts. Mean responses for the groups are denoted by bars, and statistically significant differences between groups are denoted by asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$).
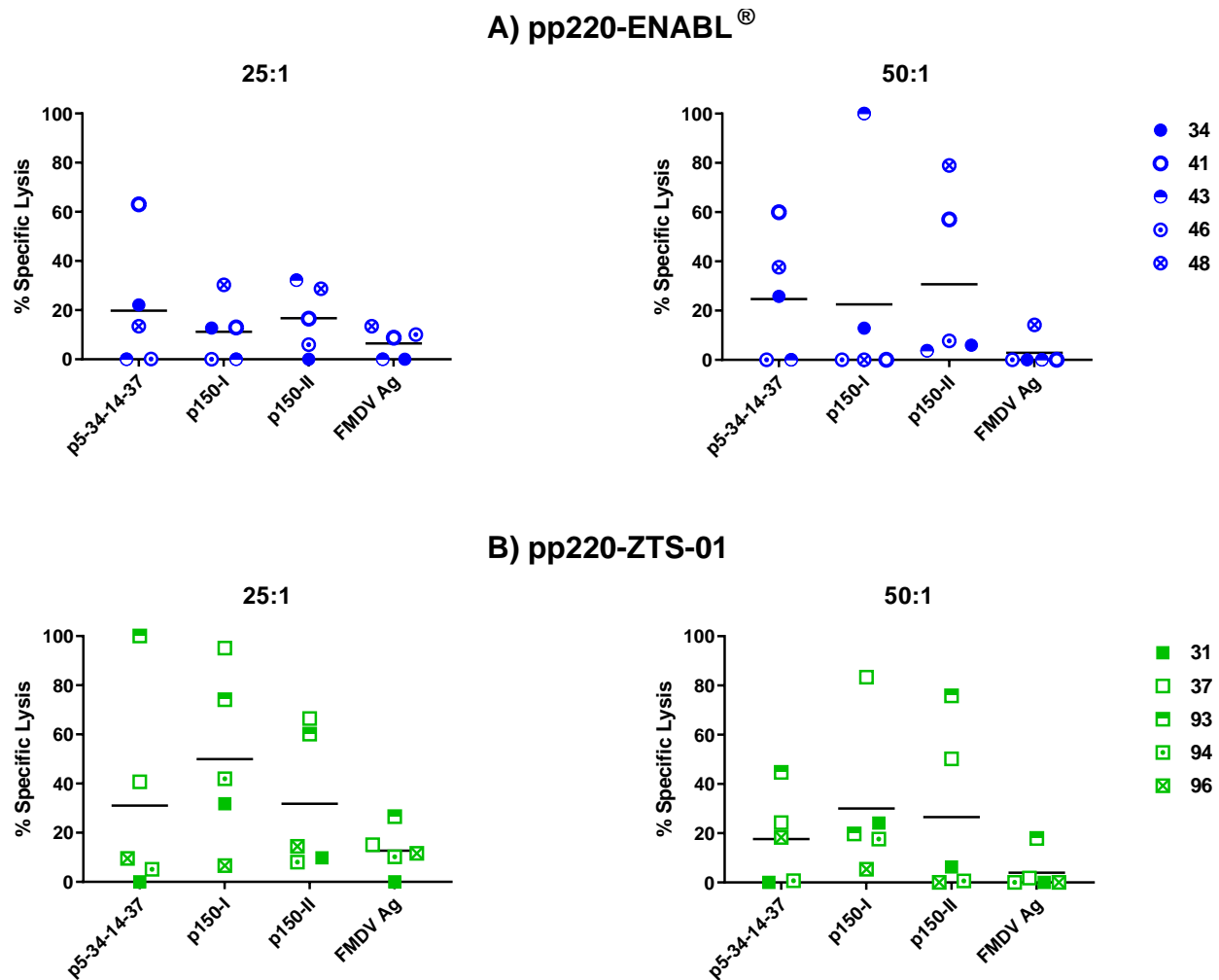
Figure 2.8. Cytotoxic T-lymphocyte (CTL) responses against the pp220 antigens.

At four weeks post-boost, p5-34-14-37-, p150-I-, and p150-II-specific CTL responses in PBMCs collected from (**A**) Ad-pp220-ENABL®-immunized; or (**B**) Ad-pp220-ZTS-01-immunized pigs were evaluated at effector to target ratios of 25:1 and 50:1 using the standard ^{51}Cr release assay. Data are represented as the percent specific lysis against each antigen and a negative-control FMDV antigen (Ag). Mean responses for each antigen are denoted by bars.
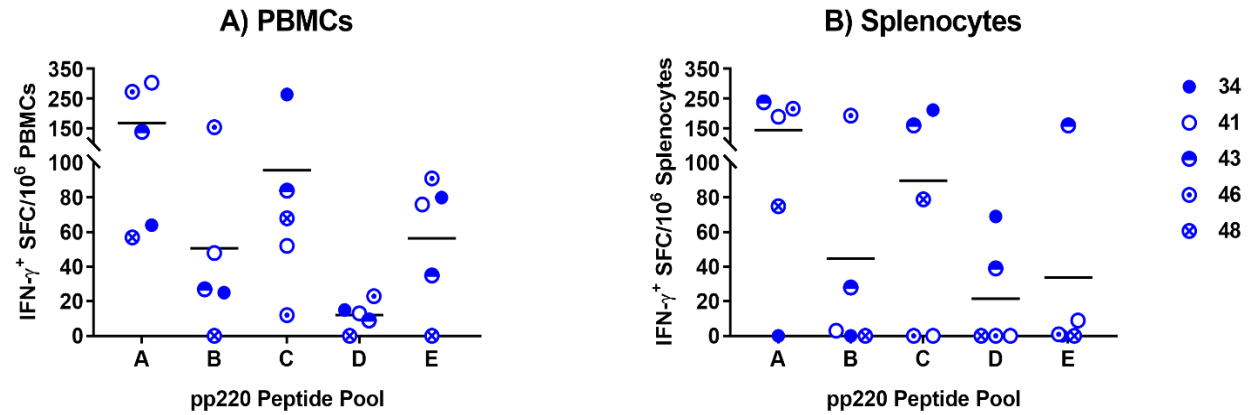
Figure 2.9. Screening of the predicted pp220 peptide pools.

Five pools (Pool A-E) of predicted *SLA I*-binding peptides from the ASFV (Georgia 2007/1) pp220 polyprotein (Table 2) were used to stimulate (**A**) PBMCs or (**B**) splenocytes isolated from pigs immunized with the Ad-pp220-ENABL® formulation, which were then evaluated for antigen-specific recall IFN-γ responses by EliSpot. Data is presented as Spot Forming Cells (SFC)/10^6 PBMCs or splenocytes for each pig. Medium alone served as the negative control, and the data shown is minus media background counts.
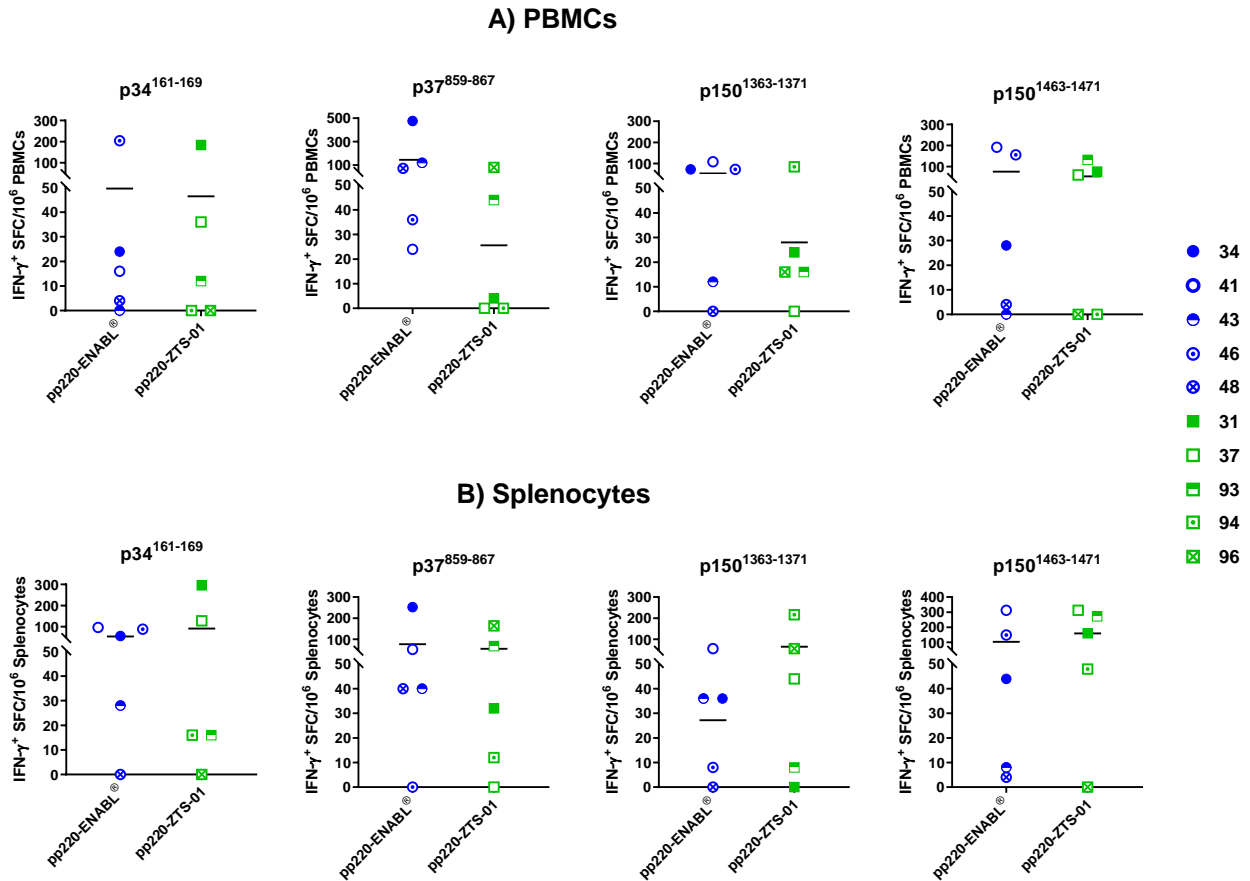
Figure 2.10. IFN-γ-inducing peptides from ASFV pp220.

Four IFN-γ-inducing nonamer peptides from ASFV pp220 that stimulated recall IFN-γ responses in (**A**) PBMCs or (**B**) splenocytes isolated from pigs immunized with the Ad-pp220-ENABL® or the Ad-pp220-ZTS-01 formulation were identified by EliSpot (Table 3). Data for each pig is presented as Spot Forming Cells (SFC)/10⁶ PBMCs or splenocytes. Medium alone served as the negative control and the data shown is minus media background counts. Mean responses for the two groups are denoted by bars.

Supplementary Table 2.1 Alignment of polyprotein 220 (CP2475) amino acid sequences of the ASFV isolates listed in Supplementary Table 2.2. Conservation of the IFN-γ-inducing nonamer peptides is highlighted in bright green color.

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YP_0099272 : MGNRGSSTSSRPLPSSEANIYAKLQDHIQRQTRPFSGGGYFNGGGDKNPVQHIKDYHIDSVSSKAKLRIIEGIIRAIAKIGFKVDTKQPIEDILKDIKKQLPDPRAGSTFVKNAEKQETVCKMIADAINQEFIDLGQDKLIDTTEGAASI : 150
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VFV48026.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QOW03225.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QOW03039.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
VVW94185.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
CAD0059543  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QXP50051.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
CAD7112603  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
AXZ96054.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QED90535.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
VVW94190.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QEY87898.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QGV56805.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QPB67618.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QUQ60377.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QUQ60197.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
SPS73515.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QDL88123.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QIA61506.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QTZ19752.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QIM07715.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QIM07948.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QIM08183.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
YP_0097024  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
YP_0097038  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
YP_0097023  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
YP_0097026  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
YP_0097035  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QZK26792.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
YP_0097036  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
QID21266.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAPPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
P0CA04.1    : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
P0CA02.1    : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQISILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300
YP_0097029  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLSDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLKTDEVDIIKILNDTNSVLGTKNFGKVLSYTLCNLGIAATVANKINKA : 300
AXB50010.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLSDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLKTDEVDIIKILNDTNSVLGTKNFGKVLSYTLCNLGIAATVANKINKA : 300
AXB49666.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLSDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLKTDEVDIIKILNDTNSVLGTKNFGKVLSYTLCNLGIAATVANKINKA : 300
CAD7112308  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLSDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLKTDEVDIIKILNDTNSVLGTKNFGKVLSYTLCNLGIAATVANKINKA : 300
QRY19118.1  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLSDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLKTDEVDIIKILNDTNSVLGTKNFGKVLSYTLCNLGIAATVANKINKA : 300
P0CA01.1    : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLSDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLKTDEVDIIKILNDTNSVLGTKNFGKVLSYTLCNLGIAATVANKINKA : 300
YP_0097028  : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLSDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLKTDEVDIIKILNDTNSVLGTKNFGKVLSYTLCNLGIAATVANKINKA : 300
P0CA03.1    : CRQIVLYINSLTHGLRAEYLDVHGSIENTLENIKLLNDAIKQLHERMVTEVTKAAPNEEVINAVTMIEAVYRRLLNEQNLQINILTNFIDNILTPTQKELDKLQTDEVDIIKLLNDTNSVLGTKNFGKVLSYTLCNLGIAASVANKINKA : 300

                     *         320       *         340       *         360       *         380       *         400       *         420       *         440       *
YP_0099272  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
AZP54139.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QIE06881.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QGJ83454.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
AYW34063.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QLF78620.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QTP96400.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QBH90765.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QOY24398.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QSG73791.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QTE18752.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
VFV48026.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QOW03225.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QOW03039.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
VVW94185.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
CAD0059543  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
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QXP50051.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
CAD7112603  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
AXZ96054.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QED90535.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
VVW94190.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QEY87898.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QGV56805.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QPB67618.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QUQ60377.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QUQ60197.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
SPS73515.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QDL88123.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QIA61506.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QTZ19752.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QIM07715.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QIM07948.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QIM08183.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
YP_0097024 : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
YP_0097038 : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
YP_0097023 : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
YP_0097026 : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
YP_0097035 : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QZK26792.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
YP_0097036 : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
QID21266.1  : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
P0CA04.1    : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
P0CA02.1    : LQKVGLKVEQYLQSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENNCAKKGGDEVKTPLDKRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
YP_0097029 : LQRVGLKVEQYLHSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENNCAKKGGDEEKTPLDKRMEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGMKLVKEIALTPNITKLRDALSRINDMGTIALDISLIGF : 450
AXB50010.1  : LQRVGLKVEQYLHSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENNCAKKGGDEEKTPLDKRMEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGMKLVKEIALTPNITKLRDALSRINDMGTIALDISLIGF : 450
AXB49666.1  : LQRVGLKVEQYLHSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENNCAKKGGDEEKTPLDKRMEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGMKLVKEIALTPNITKLRDALSRINDMGTIALDISLIGF : 450
CAD7112308  : LQRVGLKVEQYLHSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENNCAKKGGDEEKTPLDKRMEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGMKLVKEIALTPNITKLRDALSRINDMGTIALDISLIGF : 450
QRY19118.1  : LQRVGLKVEQYLHSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENNCAKKGGDEEKTPLDKRMEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGMKLVKEIALTPNITKLRDALSRINDMGTIALDISLIGF : 450
P0CA01.1    : LQRVGLKVEQYLHSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENNCAKKGGDCEKTPLDKRMEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGMKLVKEIALTPNITKLRDALSRINDMGTIALDISLIGF : 450
YP_0097028 : LQRVGLKVEQYLHSKNWAEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENNCAKKGGDEEKTPLDKRMEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGMKLVKEIALTPNITKLRDALSRINDMGTIALDISLIGF : 450
P0CA03.1    : LQRVGLKVEQYLQSKNWEEFDKELDLKRFSGLVSAENIAEFEKAVNLLRQTFNERHKILENSCAKKGGDEEKTPLDRRIEAQRLDRKHIILMEFLNKSTQAYNDFLENVKKIGIKLVKEIALTPNITRLRDALSRINDMGTIALDISLIGF : 450
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                   460       *       480       *       500       *       520       *       540       *       560       *       580       *       600
YP_0099272 : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
AZP54139.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QIE06881.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QGJ83454.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
AYW34063.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QLF78620.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QTP96400.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QBH90765.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QOY24398.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QSG73791.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QTE18752.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
VFV48026.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QOW03225.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QOW03039.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
VVW94185.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
CAD0059543  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QXP50051.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
CAD7112603  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
AXZ96054.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QED90535.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
VVW94190.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QEY87898.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QGV56805.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QPB67618.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QUQ60377.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QUQ60197.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
SPS73515.1  : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEEIGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
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QDL88123.1 : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QIA61506.1 : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QTZ19752.1 : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QIM07715.1 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QIM07948.1 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QIM08183.1 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
YP_0097024 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
YP_0097038 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
YP_0097023 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
YP_0097026 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
YP_0097035 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QZK26792.1 : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
YP_0097036 : YTNAAAREERETFLTQFMLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QID21266.1 : YTNAAAREERETFLTQFMLVKNVLEEQSKIDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
P0CA04.1   : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
P0CA02.1   : YTNAAAREERETFLTQLTLVKNVLEEISKTDPNFKNLYDSCFRLLQIIDFYTDIVQKKYGGGEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
YP_0097029 : YNNAAAREERETFLIQLTLVKNVLEELAKTDPNFKNLYDSCFRLLQIIDFYTDIVQKKYGGGEDCECTKVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
AXB50010.1 : YNNAAAREERETFLIQLTLVKNVLEELAKTDPNFKNLYDSCFRLLQIIDFYTDIVQKKYGGGEDCECTKVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
AXB49666.1 : YNNAAAREERETFLIQLTLVKNVLEELAKTDPNFKNLYDSCFRLLQIIDFYTDIVQKKYGGGEDCECTKVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
CAD7112308 : YNNAAAREERETFLIQLTLVKNVLEELAKTDPNFKNLYDSCFRLLQIIDFYTDIVQKKYGGGEDCECTKVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
QRY19118.1 : YNNAAAREERETFLIQLTLVKNVLEELAKTDPNFKNLYDSCFRLLQIIDFYTDIVQKKYGGGEDCECTKVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
P0CA01.1   : YNNAAAREERETFLIQLTLVKNVLEELAKTDPNFKNLYDSCFRLLQIIDFYTDIVQKKYGGGEDCECTKVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
YP_0097028 : YNNAAAREERETFLIQLTLVKNVLEELAKTDPNFKNLYDSCFRLLQIIDFYTDIVQKKYGGGEDCECTKVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600
P0CA03.1   : YTNAAAREERETFLTQLMLVKNVLEEQSKTDPNFKNLYDSCSRLLQIIDFYTDIVQKKYGGEEDCECTRVGGAALTVEELGLSKAARSQVDLNQAINTFMYYYVAQIYSNLTHNKQEFQSYEENYATILGDAIAGRLMQLDTEKNARIN : 600

                    *         620       *         640       *         660       *         680       *         700       *         720       *         740       *
YP_0099272 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
AZP54139.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QIE06881.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QGJ83454.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
AYW34063.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QLF78620.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QTP96400.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QBH90765.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QOY24398.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QSG73791.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QTE18752.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
VFV48026.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QOW03225.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QOW03039.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
VVW94185.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
CAD0059543 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QXP50051.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
CAD7112603 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
AXZ96054.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QED90535.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
VVW94190.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QEY87898.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QGV56805.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QPB67618.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QUQ60377.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QUQ60197.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
SPS73515.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QDL88123.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QIA61506.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QTZ19752.1 : SPAVDIARGHVGPNPGGAQEVDWKATVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTETTGDTLAQVFESFPTSTGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QIM07715.1 : SPAVDIARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QIM07948.1 : SPAVDIARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QIM08183.1 : SPAVDIARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
YP_0097024 : SPAVDIARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
YP_0097038 : SPAVDIARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
YP_0097023 : SPAVDIARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
YP_0097026 : SPAVDIARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
YP_0097035 : SPAVDIARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDIYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
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QZK26792.1  : SPAVDLARGHVGPNPGGAQEADWKAAVSAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
YP_0097036  : SPAVDLARGHVGPNPGGAQEADWKATVSAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
QID21266.1  : SPAVDLARGHVGPNPGGAQEEDWKATVSAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
P0CA04.1    : SPAVDLARGHVGPNPGGAQEEDWKATVSAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750
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YP_0097029  : SPAVDLARGHVGPNPGGAQEVDWKATISAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRGVGTLRPVRASQAKNIRDLIG : 750
AXB50010.1  : SPAVDLARGHVGPNPGGAQEVDWKATISAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRGVGTLRPVRASQAKNIRDLIG : 750
AXB49666.1  : SPAVDLARGHVGPNPGGAQEVDWKATISAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRGVGTLRPVRASQAKNIRDLIG : 750
CAD7112308  : SPAVDLARGHVGPNPGGAQEVDWKATISAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRGVGTLRPVRASQAKNIRDLIG : 750
QRY19118.1  : SPAVDLARGHVGPNPGGAQEIDWKATISAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRGVGTLRPVRASQAKNIRDLIG : 750
P0CA01.1    : SPAVDLARGHVGPNPGGAQEVDWKATISAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRGVGTLRPVRASQAKNIRDLIG : 750
YP_0097028  : SPAVDLARGHVGPNPGGAQEVDWKATISAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRGVGTLRPVRASQAKNIRDLIG : 750
P0CA03.1    : SPAVDLARGHVGPNPGGAQEVDWKAAVSAIELEYDVKRRFYRALEGLDLYLKNITKTFVNNIDSIQTVQQMLDGVRIIGRWFTEATGDTLAQVFESFPTSAGNDSNVFTDNAPAGHYYEKVAAEIQQGRSVGTLRPVRASQAKNIRDLIG : 750

                       760         *         780         *         800         *         820         *         840         *         860         *         880         *         900
YP_0099272  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
AZP54139.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QIE06881.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QGJ83454.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
AYW34063.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QLF78620.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QTP96400.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QBH90765.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QOY24398.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QSG73791.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QTE18752.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
VFV48026.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QOW03225.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QOW03039.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
VVW94185.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
CAD0059543  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QXP50051.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
CAD7112603  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
AXZ96054.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QED90535.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
VVW94190.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QEY87898.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QGV56805.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QPB67618.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QUQ60377.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QUQ60197.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
SPS73515.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QDL88123.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QIA61506.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QTZ19752.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QIM07715.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QIM07948.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QIM08183.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
YP_0097024  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
YP_0097038  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
YP_0097023  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
YP_0097026  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
YP_0097035  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QZK26792.1  : RSLSNFQALKNIINAFARIGDMLGGEELROTVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
YP_0097036  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QID21266.1  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
P0CA04.1    : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
P0CA02.1    : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYLQHSALSVGLKNLNQSEIGGORMALAQTAEEAAQRVYLSTVRVNDALSRWETEDMFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
YP_0097029  : RSLSNFQALKNIINAFARIGDMLGGEELROMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQTQIGGORVALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
AXB50010.1  : RSLSNFQALKNIINAFARIGDMLGGEELROTVPMSPLQIYKTLLEYIQHSALSVGLKNLNQTQIGGORVALAQTAEEASQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
AXB49666.1  : RSLSNFQALKNIINAFARIGDMLGGEELROTVPMSPLQIYKTLLEYIQHSALSVGLKNLNQTQIGGORVALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
CAD7112308  : RSLSNFQALKNIINAFARIGDMLGGEELROTVPMSPLQIYKTLLEYIQHSALSVGLKNLNQTQIGGORVALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
QRY19118.1  : RSLSNFQALKNIINAFARIGDMLGGEELROTVPMSPLQIYKTLLEYIQHSALSVGLKNLNQTQIGGORVALAQTAEEASQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
P0CA01.1    : RSLSNFQALKNIINAFARIGDMLGGEELROTVPMSPLQIYKTLLEYIQHSALSVGLKNLNQTQIGGORVALAQTAEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
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YP_0097028 : RSLSNFQALKNIINAFARIGDMLGGEELRQTVPMSPLQIYKTLLEYIQHSALSVGLKNLNQTQIGGQRVALAQTAEEASQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900
P0CA03.1   : RSLSNFQALKNIINAFARIGDMLGGEELRQMVPMSPLQIYKTLLEYIQHSALSVGLKNLNQSEIGGQRVALARTPEEAAQRVYLSTVRVNDALSTRWETEDVFFTFMLKSMAAKIFIVLGIYDMFERPEPVYKLIPTRMILGGADELEPE : 900


                  *        920        *        940        *        960        *        980        *       1000        *       1020        *       1040        *
YP_0099272 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
AZP54139.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QIE06881.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QGJ83454.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
AYW34063.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QLF78620.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QTP96400.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QBH90765.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QOY24398.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QSG73791.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QTE18752.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
VFV48026.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QOW03225.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QOW03039.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
VVW94185.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
CAD0059543 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QXP50051.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
CAD7112603 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
AXZ96054.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QED90535.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
VVW94190.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QEY87898.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QGV56805.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QPB67618.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QUQ60377.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QUQ60197.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
SPS73515.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QDL88123.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QIA61506.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QTZ19752.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QIM07715.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QIM07948.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QIM08183.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
YP_0097024 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
YP_0097038 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
YP_0097023 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
YP_0097026 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
YP_0097035 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QZK26792.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
YP_0097036 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QID21266.1 : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYIQS : 1050
P0CA04.1   : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
P0CA02.1   : VIPEAAELYFRLPRLAEFYQKLFSFRDGNVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
YP_0097029 : VIPEAAGLYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRVIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEAVKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
AXB50010.1 : VIPEAAGLYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRVIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEAVKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
AXB49666.1 : VIPEAAGLYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRVIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEAVKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
CAD7112308 : VIPEAAGLYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRVIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEAVKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
QRY19118.1 : VIPEAAGLYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRVIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEAVKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
P0CA01.1   : VIPEAAGLYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRVIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEAVKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
YP_0097028 : VIPEAAGLYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRVIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEAVKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050
P0CA03.1   : VIPEAAELYFRLPRLAEFYQKLFSFRDENVQISMLPELEGIFSGLIRIIFMRPIELINIGDYSETEIRQLIKEINVIYQHFNLEYGEQEATKKALIHFVNEINRRFGVITRTEWEKFQRIVQEARTMNDFGMMNQTNYSILPDEDGYTQS : 1050


             1060        *       1080        *       1100        *       1120        *       1140        *       1160        *       1180        *       1200
YP_0099272 : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
AZP54139.1 : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QIE06881.1 : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QGJ83454.1 : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
AYW34063.1 : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QLF78620.1 : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
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QTP96400.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QBH90765.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QOY24398.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QSG73791.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QTE18752.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
VFV48026.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QOW03225.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QOW03039.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
VVW94185.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
CAD0059543  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QXP50051.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
CAD7112603  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
AXZ96054.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
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QEY87898.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QGV56805.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
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QUQ60377.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QUQ60197.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
SPS73515.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QDL88123.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QIA61506.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QTZ19752.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QIM07715.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QIM07948.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QIM08183.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
YP_0097024  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
YP_0097038  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
YP_0097023  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
YP_0097026  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
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QZK26792.1  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
YP_0097036  : SQLLPSDRFISPSTQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
QID21266.1  : SQLLPSDRFISPSTQPSPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
P0CA04.1    : SQLLPSDRFIGPSSQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKISYQELIRQAINELKKDHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200
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AXB49666.1  : SQLLPSDRFIGPSSQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIQQATNELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNTFRNNIKALDLDMIQKS : 1200
CAD7112308  : SQLLPSDRFIGPSSQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIQQATNELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNTFRNNIKALDLDTIQKS : 1200
QRY19118.1  : SQLLPSDRFIGPSSQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIQQATNELKKEHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNTFRNNIKALDLDTIQKS : 1200
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P0CA03.1    : SQLLPSDRFIGPSSQPTPKWRPALYNIDSVDVQTGMLQPNSQWDIVQKFRKQLSEMFEDPSLQQELGKVSYQELIRQAINELKKDHTDKIQIVSKLIQGSESLADTDVNKIFLFHETVITGLNLLSAIYVLLNNFRNNIKGLDLDTIQKS : 1200


                      *         1220      *         1240      *         1260      *         1280      *         1300      *         1320      *         1340      *
YP_0099272  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
AZP54139.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QIE06881.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
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AYW34063.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QLF78620.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QTP96400.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QBH90765.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QOY24398.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QSG73791.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QTE18752.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
VFV48026.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QOW03225.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QOW03039.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
VVW94185.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
CAD0059543  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QXP50051.1  : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTELFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
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CAD7112603 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
AXZ96054.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QED90535.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
VVW94190.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QEY87898.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QGV56805.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
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QUQ60377.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QUQ60197.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
SPS73515.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QDL88123.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QIA61506.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QTZ19752.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAAAAQDRNLTTETLFAWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1350
QIM07715.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVVKENDVRLSRVYPDPTTNATAPQDQNLV-TETLFAWIVPYVGIPAGGGVRAEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1349
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YP_0097024 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVVKENDVRLSRVYPDPTTNATAPQDQNLV-TETLFAWIVPYVGIPAGGGVRAEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1349
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YP_0097023 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVVKENDVRLSRVYPDPTTNATAPQDQNLV-TETLFAWIVPYVGIPAGGGVRAEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1349
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QZK26792.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVIKEINMRLSMVYPDPTTEAATVQDRNLT-TETLFVWIVPYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAHVHLDFTGLISLIDSLM : 1349
YP_0097036 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVVKENDVRLSRVYPDPTTNATAPQDQNLV-TETLFAWIVPYVGIPAGGGVRAEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1349
QID21266.1 : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVVKENDARLSRVYPDPTTNATAPQDQNLV-TETLFAWIVPYVGIPAGGGVRAEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1349
P0CA04.1   : IIEWLRETQAANVNRANLIDWLGRKHGAISEIRNPGLVVKENDARLSRVYPDPTTN-ATAPQDQNLV-TETLFAWIVPYVGIPAGGGVRAEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1349
P0CA02.1   : IIEWLRETQPPDVNHANLIDWLGRKHGAISEIRNPGLVVKANNARLSEVYPDPTTD-ANTPQDRNLTTETLFAWIVQYVGIPAGGGVRPEQELAARYLVDNQRIMQLLLTNIFEITSSFNKMIQVRFPETSTAHVHLDFTGLISLIDSLM : 1349
YP_0097029 : IVEWLRETQAANVNHANLIDWLGRRHGDISEIRNPGLVIKANDARLSMVYPDPTTDPTLPQDQNLT-TETLFAWIEQYVGIPAGGGIRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKLVQVRFPETSTAHVHLDFTGLISLIDSLM : 1349
AXB50010.1 : IVEWLRETQAANVNHANLIDWLGRRHGDISEIRNPGLVIKANDARLSMVYPDPTTDPTLPQDQNLT-TETLFAWIEQYVGIPAGGGIRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKLVQVRFPETSTAHVHLDFTGLISLIDSLM : 1349
AXB49666.1 : IVEWLRETQAANVNHANLIDWLGRRHGDISEIRNPGLVIKANDARLSMVYPDPTTDPTLPQDQNLT-TETLFAWIEQYVGIPAGGGIRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKLVQVRFPETSTAHVHLDFTGLISLIDSLM : 1349
CAD7112308 : IIEWLRETQAANVNRANLIDWLGRRHGDISEIRNPGLVIKANDARLSEVYPDPTTDATAPLDRNLV-TETLFAWFTRFVGIPADGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKLVQVRFPETSTAHVHLDFTGLISLIDSLM : 1349
QRY19118.1 : IIEWLRETQAANVNRANLIDWLGRRHGDISEIRNPGLVIKANDARLSEVYPDPTTDATAPLDRNLV-TETLFAWFTRFVGIPADGGVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKLVQVRFPETSTAHVHLDFTGLISLIDSLM : 1349
P0CA01.1   : IIEWLRETQAANVNRANLIDWLGRRHGDISEIRNPGLVIKANDARLSEVYPDPTTDATAPLDRNLV-TETLFAWFTRFVGIPADGAVRPEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKLVQVRFPETSTAHVHLDFTGLISLIDSLM : 1349
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P0CA03.1   : IIEWLRET-PANVNHANLIDWLGRKHGAISEIRNPGLVIKEINMRLSEVYPDPTTE-ANVPQDRNLTTETLFAWIVPYVGIPAGGGVRAEQELAARYLVDNQRIMQLLLTNIFEMTSSFNKMVQVRFPETSTAQVHLDFTGLISLIDSLM : 1348
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AZP54139.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QIE06881.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QGJ83454.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
AYW34063.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QLF78620.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QTP96400.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKSYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QBH90765.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QOY24398.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QSG73791.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QTE18752.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
VFV48026.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QOW03225.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QOW03039.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
VVW94185.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
CAD0059543 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QXP50051.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
CAD7112603 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
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VVW94190.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QEY87898.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QGV56805.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
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QUQ60377.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
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SPS73515.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
QDL88123.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELGEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
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QIA61506.1 : ADTKYFLDLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELCLEGVNQIINKTYTLLTKPYNVLQLRGGAQRRDAANIQINNNPQSSERFEQYGRVFSRLVFYDALENNSGLRVEQVALGDFRLSNLIRTNN : 1500
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P0CA04.1   : ADTKYFLNLLRPHIDKNIIQYYENRSNPGSFYWLEEHLIDKLIKPPTDAGGRPLPGGELCLEGVNQIINKTYTLLTKPYNVLQLQGGAQRRDAANIQINNNPQPSERFEQYGRVFSRLVFYDALENNSGLRVEQVVLGDFRLSNLIRTNN : 1499
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                  *         1520      *         1540      *         1560      *         1580      *         1600      *         1620      *         1640      *
YP_0099272 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
AZP54139.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
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QOY24398.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
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QTE18752.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
VFV48026.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
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QOW03039.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
VVW94185.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
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QXP50051.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
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QGV56805.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
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QUQ60197.1 : AQEENTLSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLIASYITRFYDAPSGKIYLNLINAFANGNFSQAVEMGYAHPDLARNNNVFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1648
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QIM08183.1 : AQEENTLSYWDNMA--PRTYANVNDAANNLRRYRLYGSDYGIQNNRSMMMVFNQLVASYIARFYDAPSGKIYLNLINAFANGNFSQAVMELGYTHPDLARDNTAFGHRGDPTEQSVLLLSLGLMLQRLIKDTNRQGLSQHLISTLTEIPI : 1647
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QZK26792.1 : AQEENALSYWDNIA--LRTYANVNDAANNLRRYRLYGSDYGIRNNRSMMMVFNQLVASYIARFYDAPSGKIYLNLINAFANGNFSQAVMELGYTHPDLARDNTAFGHRGDPTEQSVLLLSLGLMLQRLIKDTNRQGLSQHLISTLTEIPI : 1647
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YP_0097029 : AQEENALSFWTAVAPAPRAYANVNNAANN-RRYRLYGSDYGIRNNRSMMVFNQLVASYIARFYDAPSGKIYLNLINAPANGNFSQAVMELGYAHPDLARDNIAFGHRGDPTEQSVLLLSLGLMLQRLIKDTNRQGLSQHLISTLTEIPI : 1648
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AXB49666.1 : AQEENALSFWTAVA--PRAYANVNNAANNLRRYRLYGSDYGIRNNRSMMVFNQLVASYIARFYDAPSGKIYLNLINAPANGNFSQAVMELGYAHPDLARDNIAFGHRGDPTEQSVLLLSLGLMLQRLIKDTNRQGLSQHLISTLTEIPI : 1649
CAD7112308 : AQEENALSFWTAVA--PRAYANVNNAANNLRRYRLYGSDYGIRNNRSMMVFNQLVASYIARFYDAPSGKIYLNLINAPANGNFSQAVMELGYAHPDLARDNTAFGHRGDPTEQSVLLLSLGLMLQRLIKDTNRQGLSQHLISTLTEIPI : 1647
QRY19118.1 : AQEENALSFWTAVA--PRAYANVNDAANNLRRYRLYGSDYGIRNNRSMMVFNQLVASYIARFYDAPSGKIYLNLINTPANGNFSQAVMEYGYAHPDLARDNTAFGHRGDPTEQSVLLLSLGLMLQRLIKDTNRQGLSQHLISTLTEIPI : 1647
P0CA01.1   : AQEENALSFWTAVA--PRAYANVNDAANNLRRYRLYGSDYGIRNNRSMMVFNQLVASYIARFYDAPSGKIYLNLINTPANGNFSQAVMELGYAHPDLARDNTAFGHRGDPTEQSVLLLSLGLMLQRLIKDTNRQGLSQHLISTLTEIPI : 1647
YP_0097028 : AQEENALSFWTAVA--PRTYANVNDAANNLRRYRLYGSDYGIRNNRSMMVFNQLVASYIARFYDAPSGKIYLNLINAPANGNFSQAVMELGYAHPDLARDNTAFGHRGDPTEQSVLLLSLGLMLQRLIKDTNRQGLSQHLISTLTEIPI : 1647
P0CA03.1   : AQEENALSYWDNIA--LRTYANVNDAANNLRRYRLYGSDHGIQNNRSMMVFNQLVASYIARFYDAPSGKIYLNLINAPANGNFSQAVMEMGYAHPDLARNNNAFGHRGDPTEQSVLLLSLGLILQRLIKDTNRQGLSQHLISTLTEIPI : 1646
                                                                                                                     f

                  1660         *        1680        *       1700        *       1720        *       1740        *       1760        *       1780        *       1800
YP_0099272 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
AZP54139.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QIE06881.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QGJ83454.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
AYW34063.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QLF78620.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QTP96400.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
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QOY24398.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QSG73791.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QTE18752.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
VFV48026.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QOW03225.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QOW03039.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
VVW94185.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
CAD0059543 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QXP50051.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
CAD7112603 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
AXZ96054.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QED90535.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
VVW94190.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QEY87898.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QGV56805.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QPB67618.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QUQ60377.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QUQ60197.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
SPS73515.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QDL88123.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QIA61506.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QTZ19752.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNN-NVPATGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QIM07715.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QIM07948.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QIM08183.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
YP_0097024 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
YP_0097038 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
YP_0097023 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
YP_0097026 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
YP_0097035 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QZK26792.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNNINVPTTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDIVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
YP_0097036 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSVIYYNNNINVPMTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDVVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
QID21266.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSIIYYNNN-NVPTTGLSVGQAALRGIGGVFRPNVTLMPLGDAQNTSDIVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1796
P0CA04.1   : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLMGLLGANNDSIIYYNNN-NVPMTGLSVGQAAMRGIGGVFRPNVTLMPLGDAQNTSDIVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1796
P0CA02.1   : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLRANNDSVIYYNNN-NVPMTGLSISQAAMRGIGGVFRPNVTLMPLGDAQNTSDIVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDFPIYLETEEHFIQNYMS : 1796
YP_0097029 : YLKENYRANLPLYNKMFNILISQGELLKQFIQYTKVQLARPNLMALLGANNDAIIYYNN-INVPNTGLSIGQAALRGIGSVFRPNITLMPLGDAQNNTDIVRKRLIAVINGVIRGSLTTANSAMEVLHELTDFPIYLETEEHFIQNYMS : 1797
AXB50010.1 : YLKENYRANLPLYNKMFNILISQGELLKQFIQYTKVQLARPNLMALLGANNDAIIYYNN-INVPNTGLSIGQAALRGIGSVFRPDITLMPLGDAQNNTDIVRKRLIAVINGVIRGSLTTANSAMEVLHELTDFPIYFETEEHFIQNYMS : 1798
AXB49666.1 : YLKENYRANLPLYNKMFNILISQGELLKQFIQYTKVQLARPNLMALLGANNDAIIYYNN-INVPNTGLSIGQAALRGIGSVFRPDITLMPLGDAQNNTDIVRKRLIAVINGVIRGSLTTANSAMEVLHELTDFPIYFETEEHFIQNYMS : 1798
CAD7112308 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTKVQLARPNLTALLGANNDSIIYYNN-NVPNTGLTIGQAALRGIGSVFRPDITLMPLGNAQNNTDIVRKRLIAVINGIIRGSLTTANSAMEVLHELTDFPIYFETEEHFIQNYMS : 1796
QRY19118.1 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTKVQLARPNLTALLGANNDSIIYYNN-NVPNTGLTVGQAALRGIGSVFRPDITLMPLGNAQNNTDVVRKRLIAVINGIIRGSLTTANSAMEVLHELTDFPIYFETEEHFIQNYMS : 1796
P0CA01.1   : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTKVQLARPNLTALLGANNDSIIYYNN-NVPNTGLTVGQAALRGIGSVFRPDITLMPLGNAQNNTDVVRKRLIAVINGIIRGSLTTANSAMEVLHELTDFPIYFETEEHFIQNYMS : 1796
YP_0097028 : YLKENYRANLPLFNKMFNILISQGELLKQFIQYTKVQLARPNLTALLGANNDSIIYYNN-NVPNTGLTVGQAALRGIGSVFRPDITLMPLGNAQNNTDVVRKRLIAVINGIIRGSLTTANSAMEVLHELTDFPIYFETEEHFIQNYMS : 1796
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P0CA03.1    : YLKENYRANLPFNKMFNILISQGELLKQFIQYTNVQLARPNLTALLGANNDSVIYYNNNINVPMTGLSVGQAAMRGIGGVFRPNVTLMPLGDACSNTSDIVRKRLVAVIDGIIRGSHTLADSAMEVLHELTDHPIYLETEEHFIQNYMS : 1796
                                                                                                                                    i

                        *        1820        *        1840        *        1860        *        1880        *        1900        *        1920        *        1940        *
YP_0099272  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
AZP54139.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QIE06881.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QGJ83454.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
AYW34063.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QLF78620.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QTP96400.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QBH90765.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QOY24398.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QSG73791.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QTE18752.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
VFV48026.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QOW03225.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QOW03039.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
VVW94185.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
CAD0059543  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QXP50051.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
CAD7112603  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
AXZ96054.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QED90535.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
VVW94190.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QEY87898.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QGV56805.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QPB67618.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QUQ60377.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QUQ60197.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
SPS73515.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QDL88123.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QIA61506.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QTZ19752.1  : RYNKEPLMPFSLSLYYLHDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QIM07715.1  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAHEQITPTRFEHFYTHAIQALRFIVNIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QIM07948.1  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAHEQITPTRFEHFYTHAIQALRFIVNIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QIM08183.1  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIVNIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
YP_0097024  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIVNIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
YP_0097038  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAHEQITPTRFEHFYTHAIQALRFIVNIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
YP_0097023  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIVNIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
YP_0097026  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIVNIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
YP_0097035  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAHEQITPTRFEHFYTHAIQALRFIVNIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QZK26792.1  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGDDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
YP_0097036  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
QID21266.1  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1946
P0CA04.1    : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMMYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1946
P0CA02.1    : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIVNIRSFKTVMTYNENTFGGVNLISEDRNDKPIITAGIGMNAVYSLRKT : 1946
YP_0097029  : RYNKEPLMPFSLSLYYLRDIRMENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYIHAIQALRFIINIRSFKTVMTYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1947
AXB50010.1  : RYNKEPLMPFSLSLYYLRDIRMENNEVYDPLLYPNLESGSPEFKILYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYIHAIQALRFIINIRSFKTVMTYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1948
AXB49666.1  : RYNKEPLMPFSLSLYYLRDIRMENNEVYDPLLYPNLESGSPEFKILYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYIHAIQALRFIINIRSFKTVMTYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1948
CAD7112308  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKILYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYIHAIQALRFIINIRSFKTVMTYNENTFGGVNLIGEDRDDKPIITEGIGMNAVYSLRKT : 1946
QRY19118.1  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKILYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYIHAIQALRFIINIRSFKTVMTYNENTFGGVNLIGEDRDDKPIITEGIGMNAVYSLRKT : 1946
P0CA01.1    : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKILYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYIHAIQALRFIINIRSFKTVMTYNENTFGGVNLIGEDRDDKPIITEGIGMNAVYSLRKT : 1946
YP_0097028  : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKILYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYIHAIQALRFIINIRSFKTVMTYNENTFGGVNLISEDRDDKPIITEGIGMNAVYSLRKT : 1946
P0CA03.1    : RYNKEPLMPFSLSLYYLRDIRIENNEVYDPLLYPNLESGSPEFKLLYGTRKLLGNDPVQLSDMPGVQLIMKNYNETVVAREQITPTRFEHFYTHAIQALRFIINIRSFKTVMTYNENTFGGVNLISENRDDKPIITAGIGMNAVYSLRKT : 1946

                     1960        *        1980        *        2000        *        2020        *        2040        *        2060        *        2080        *        2100
YP_0099272  : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
AZP54139.1  : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QIE06881.1  : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QGJ83454.1  : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
AYW34063.1  : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QLF78620.1  : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QTP96400.1  : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
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QBH90765.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QOY24398.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QSG73791.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QTE18752.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
VFV48026.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QOW03225.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QOW03039.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
VVW94185.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
CAD0059543 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QXP50051.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
CAD7112603 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
AXZ96054.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QED90535.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
VVW94190.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QEY87898.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QGV56805.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QPB67618.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QUQ60377.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QUQ60197.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
SPS73515.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QDL88123.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QIA61506.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QTZ19752.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QIM07715.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELMSKGSAGYMSR : 2097
QIM07948.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELMSKGSAGYMSR : 2097
QIM08183.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELMSKGSAGYMSR : 2097
YP_0097024 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELMSKGSAGYMSR : 2097
YP_0097038 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELMSKGSAGYMSR : 2097
YP_0097023 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELMSKGSAGYMSR : 2097
YP_0097026 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELMSKGSAGYMSR : 2097
YP_0097035 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELMSKGSAGYMSR : 2097
Q2K26792.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
YP_0097036 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2097
QID21266.1 : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2096
P0CA04.1   : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2096
P0CA02.1   : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTTAPQPDVAEVINIPNRPPINTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2096
YP_0097029 : LQDVISFVESSYQEEQINNIHKIVSPKSQTRSLGSNRERERIFNLFDMNIMPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTGAPQPDVAVVINIPNRPPMNTREFMIKLLINPYVTVSIQYGNELLSRGNAGYMSR : 2097
AXB50010.1 : LQDVISFVESSYQEEQINNIHKIVSPKSQTRSLGSNRERERIFNLFDMNIMPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTGAPQPDVAVVINIPNRPPMNTREFMIKLLINPYVTVSIQYGNELLSRGNAGYMSR : 2098
AXB49666.1 : LQDVISFVESSYQEEQINNIHKIVSPKSQTRSLGSNRERERIFNLFDMNIMPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTGAPQPDVAVVINIPNRPPMNTREFMIKLLINPYVTVSIQYGNELLSRGNAGYMSR : 2098
CAD7112308 : LQDVISFVESSYQEEQINHIHKIVSPRSQTRSLGSNRERERIFNLFDMNIMPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTAAPQPDVAQVINIPNRPPMNTREFMIKLLINPYVTVSITQYGNELLFRGNTGYMSR : 2096
QRY19118.1 : LQDVISFVESSYQEEQINNIHKIVSPRSQTRSLGSNRERERIFNLFDMNIMPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTAAPQPDVAQVINIPNRPPMNTREFMIKLLINPYVTVSITQYGNELLFRGNTGYMSR : 2096
P0CA01.1   : LQDVISFVESSYQEEQINNIHKIVSPRSQTRSLGSNRERERIFNLFDMNIMPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTAAPQPDVAQVINIPNRPPMNTREFMIKLLINPYVTVSITQYGNELLFRGNTGYMSR : 2096
YP_0097028 : LQDVISFVESSYQEEQINNIHKIVSPRSQTRSLGSNRERERIFNLFDMNIMPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLDTAAPQPDVAQVINIPNRPPMNTREFMIKLLINPYVTVSITQYGNELLFRGNTGYMSR : 2096
P0CA03.1   : LQDVISFVESSYQEEQINHIHKIVSPKGQTRTLGSNRERERIFNLFDMNIIPINVNALMRSIPLANIYNYDYSFEEIACLMYGISAEKVRSLNTAAPQPDIAEVINIPNRPPMNTREFMIKLLINPYVSVSITQYGNELLSKGNAGYMSR : 2096
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              *         2120        *        2140        *        2160        *        2180        *        2200        *        2220        *        2240        *
YP_0099272 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
AZP54139.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QIE06881.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QGJ83454.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
AYW34063.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QLF78620.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QTP96400.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QBH90765.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QOY24398.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QSG73791.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QTE18752.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
VFV48026.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QOW03225.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QOW03039.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
VVW94185.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
CAD0059543 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QXP50051.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
CAD7112603 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
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AXZ96054.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QED90535.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
VVW94190.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QEY87898.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QGV56805.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QPB67618.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QUQ60377.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QUQ60197.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
SPS73515.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QDL88123.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QIA61506.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QTZ19752.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2246
QIM07715.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
QIM07948.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
QIM08183.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
YP_0097024 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
YP_0097038 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
YP_0097023 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
YP_0097026 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
YP_0097035 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
QZK26792.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGRERWGHPMSIYINQALHELVRTIR-LAETVRGLRNVIDRNQIVGELNAFRTQLENTRRENNLVQTPEIQNNPTPEIIAAVQNWGQQYRGQITNLIDLIG : 2246
YP_0097036 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPLSEYINQALHELVRTIR-IPQKIRVLRNIIVKNQLIADLTTIREQLVSMRREVENMIQTPEIQNNPTPEVIAAAQNWTQQYRARVDTLINFIG : 2246
QID21266.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGRERWGHPMSIYINQALHELVRTIR-LAETVRGLRNVIDRNQIVGELNAFRTQLENTRRENNLVQTPEIQNNPTPEIIAAVQNWGQQYRGQITNLIDLIG : 2245
P0CA04.1   : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIQNNPTPEIIAAIQNWVQQYRGQITNLIDLIG : 2245
P0CA02.1   : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTLREVNNLVQTPEIQNNPTPEIIAAIQNWVQQYRAQITDLIDLIG : 2245
YP_0097029 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGRERWGHPMSIYINQALHEVVRTIR-LAETVRGLRNVIDRNQIIDELNAFRTQLEDTRREVDNLLQTPEIQNNVIPEIIAAVQNWGQQYRGQITDLIDLIG : 2246
AXB50010.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGRERWGHPMSIYINQALHEVVRTIR-LAETVRGLRNVIDRNQIIDELNAFRTQLEDTRREVDNLLQTPEIQNNVIPEIIAAVQNWGQQYRGQITDLIDLIG : 2247
AXB49666.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGRERWGHPMSIYINQALHEVVRTIR-LAETVRGLRNVIDRNQIIDELNAFRTQLEDTRREVDNLLQTPEIQNNVIPEIIAAVQNWGQQYRGQITDLIDLIG : 2247
CAD7112308 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPFSDYINQALHELVRTIR-IPQNIRVLRNIMVKNQLIADLAAIREQLVRMRREVENMVQTPEIQNNPTPEVIAAAQTWAQQYRARVDFLINFIG : 2245
QRY19118.1 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPFSDYINQALHELVRTIR-IPQNIRVLRNIMVKNQLIADLAAIREQLVRMRREVENMVQTPEIQNNPTPEVIAAAQTWAQQYRARVDFLINFIG : 2245
P0CA01.1   : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPFSDYINQALHELVRTIIPONIRVLRNIMVKNQLIADLAAIREQLVRMRREVENMVQTPEIQNNPTPEVIAAAQTWAQQYRARVDFLINFIG : 2246
YP_0097028 : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPGLAAGIQRGREQWGQPFSDYINQALHELVRTIR-IPQNIRVLRNIMVKNQLIADLAAIREQLVRMRREVENMVQTPEIQNNPTPEVIAAAQTWAQQYRARVDFLINFIG : 2245
P0CA03.1   : IFRGDNALNMGRPKFLSDQIFNKVLFGSLYPTQFDYDEAGPSLAAGIQRGRERWGHPMSIYINQALHEIVRTIR-LAETVRGLRNVIDRNQIIGELNAFRTQLEDTRREVNNLIQTPEIRNNPTPEIIAAVQNWGQQYRGQITDLIDLIG : 2245
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AZP54139.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMHHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QIE06881.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QGJ83454.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
AYW34063.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QLF78620.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QTP96400.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QBH90765.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QOY24398.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QSG73791.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QTE18752.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
VFV48026.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QOW03225.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
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VVW94185.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
CAD0059543 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
QXP50051.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
CAD7112603 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
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QEY87898.1 : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
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SPS73515.1  : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
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QIA61506.1  : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPVLIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
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QIM08183.1  : NIGQPNSML-DLIQTITPVTVRAQLGVIFNRHGIPVPHPRQILQTDDEATQWFMTNILNIPAIIMTPFTDLANDIRTFLETLERYVFNVPRWLGPSTGRVARAPVRMAPRD-MRHPISYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
YP_0097024  : NIGQPNSML-DLIQTITPVTVRAQLGVIFNRHGIPVPHPRQILQTDDEATQWFMTNILNIPAIIMTPFTDLANDIRTFLETLERYVFNVPRWLGPSTGRVARAPVRMAPRD-MRHPISYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
YP_0097038  : NIGQPNSML-DLIQTITPVTVRAQLGVIFNRHGIPVPHPRQILQTDDEATQWFMTNILNIPAIIMTPFTDLANDIRTFLETLERYVFNVPRWLGPSTGRVARAPVRMAPRD-MRHPISYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
YP_0097023  : NIGQPNSML-DLIQTITPVTVRAQLGVIFNRHGIPVPHPRQILQTDDEATQWFMTNILNIPAIIMTPFTDLANDIRTFLETLERYVFNVPRWLGPSTGRVARAPVRMAPRD-MRHPISYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
YP_0097026  : NIGQPNSML-DLIQTITPVTVRAQLGVIFNRHGIPVPHPRQILQTDDEATQWFMTNILNIPAIIMTPFTDLANDIRTFLETLERYVFNVPRWLGPSTGRVARAPVRMAPRD-MRHPISYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
YP_0097035  : NIGQPNSML-DLIQTITPVTVRAQLGVIFNRHGIPVPHPRQILQTDDEATQWFMTNILNIPAIIMTPFTDLANDIRTFLETLERYVFNVPRWLGPSTGRVARAPVRMAPRD-MRHPISYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
QZK26792.1  : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPILIAPFMLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHPTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2395
YP_0097036  : NIGQPNSML-DLIQTITPVTVRAQLGVIFNRHGIPVPHPRQILQTDDEATQWFMTNILNIPAIIMTPFTDLANDIRTFLETLERYVFNVPRWLGPSTGRVARAPVRMAPRD-MRHPISYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
QID21266.1  : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQALQNDIEAMQWFMTMVINHPPILIAPFMLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHPTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
P0CA04.1    : NAGQANSMI-NLIQNITPQTAGAQLTALFNIRGLPAPPPRQPLQNDIEAIQWFMTIVINHPPILIAPFMLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
P0CA02.1    : NAGQANSMI-SLIQNINVQTAGAQLTALFNIRGLPAPPPRQPLQNDIEAIQWFMTIVINHPPILIAPFMLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
YP_0097029  : NAGQGGNSMIPLIQIITAQTAAAQLTALFNRRGIPAPVPRQQLQNDIEALQWFMTMVINHPPIAPFMLVNNLKDFLDTLEQYVYRNPRWLGPRTARIAHPPVGMAPGNI-RYPTSYTENSVLTYIAEQNQEEGPWSIVKQVGVGIQK : 2395
AXB50010.1  : NAGQGGNSMIPLIQIITAQTAAAQLTALFNRRGIPAPVPRQQLQNDIEALQWFMTMVINHPPILIAPFMLVNNLKDFLDTLEQYVYRNPRWLGPRTARIAHPPVGMAPGNI-RYPTSYTENSVLTYIAEQNQEEGPWSIVKQVGVGIQK : 2396
AXB49666.1  : NAGQGGNSMIPLIQIITAQTAAAQLTALFNRRGIPAPVPRQQLQNDIEALQWFMTMVINHPPILIAPFMLVNNLKDFLDTLEQYVYRNPRWLGPRTARIAHPPVGMAPGNI-RYPTSYTENSVLTYIAEQNQEEGPWSIVKQVGVGIQK : 2396
CAD7112308  : NVQQPNSLI-QLIQNITPLTVRAQLTTVFIRHGLPVPDPDQALQTDDEATQWFMTNIINHPITMTPFTDLADDIRTFLETLERYVFNVPRWLGPSTGRVARVPVNMAPGN-MRYRTSYTENSVLTYIAEQNQEEGPWSIVKQVGVGIQK : 2393
QRY19118.1  : NVQQPNSLI-QLIQNITPLTVRAQLTTVFIRHGLPVPDPDQALQTDDEATQWFMTNIINQPITMIIPFTDLADDIRTFLETLERYVFNVPRWLGPSTGRVARVPVNMAPGN-MRYRTSYTENSVLTYIAEQNQEEGPWSIVKQVGVGIQK : 2393
P0CA01.1    : NAQQPNSLI-QLIQNITPLTVRAQLTTVFIRHGLPVPDPDQALQTDDEATQWFMTNIINQPITMLFTDLADDIRIFLETMERYVFNVPRWLGPSTGRVARVPVNMAPGNI-RYRTSYTENNVLTYIAEQNQEEGPWSIVKQVGVGIQK : 2394
YP_0097028  : NAQQPNSLI-QLIQNITPLTVRAQLTTVFIRHGLPVPDPDQALQTDDEATQWFMTNIINQPITMTPFTDLADDIRIFLETLERYVFNVPRWLGPSTGRVARVPVNMAPGN-MRYRTSYTENSVLTYIAEQNQEEGPWSIVKQVGVGIQK : 2393
P0CA03.1    : NVGQANSMI-NLIQNITPQTAGAQLIALFNIRGLPAPPPRQVIQNDIEAMQWFMTMVINHPPILIAPFMLLVNNLKEFLNTLERYVYKTPRWLGPGTARIAQPPVGMAPGINMRHHTSYTENSVLTYITEQNREEGPWSIVKQVGVGIQK : 2394
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                       *        2420        *        2440        *        2460        *        2480
YP_0099272  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
AZP54139.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QIE06881.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QGJ83454.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
AYW34063.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QLF78620.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QTP96400.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QBH90765.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QOY24398.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QSG73791.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QTE18752.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
VFV48026.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QOW03225.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QOW03039.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
VVW94185.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
CAD0059543  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QXP50051.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
CAD7112603  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
AXZ96054.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QED90535.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
VVW94190.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QEY87898.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QGV56805.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QPB67618.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QUQ60377.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QUQ60197.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
SPS73515.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QDL88123.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QIA61506.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QTZ19752.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
QIM07715.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
QIM07948.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
QIM08183.1  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
YP_0097024  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
YP_0097038  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
YP_0097023  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
YP_0097026  : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
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YP_0097035 : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
QZK26792.1 : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2476
YP_0097036 : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
QID21266.1 : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
P0CA04.1   : PTLVHIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
P0CA02.1   : PTLVQIGKDRFDTRLIRNLIFITNVQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
YP_0097029 : PALIQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
AXB50010.1 : PALIQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2477
AXB49666.1 : PALIQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2477
CAD7112308 : PALIQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2474
QRY19118.1 : PALIQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2474
P0CA01.1   : PALIQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPNHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
YP_0097028 : PALIQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2474
P0CA03.1   : PTLVQIGKDRFDTRLIRNLIFITNIQRLLRLRLNLELSQFRNVLVSPDHIINPSITEYGFSITGPSETFSDKQYDSDIRIL : 2475
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Supplementary Table 2.2 List of ASFV isolates used as the source of the pp220 sequences (https://www.ncbi.nlm.nih.gov/) and aligned in the Supplementary Table 2.1.

Accession #	Isolate; Country	Genotype; Source of isolation	References
NC_044959.2 YP_009927216/ CAD2068454	ASFV Georgia 2007/1 Georgia	Genotype II Domestic Pig	(Chapman et al., 2011)
MH910496.1 AZP54139.1	Georgia 2008/2 Georgia	Genotype II; Domestic Pig	(Farlow et al., 2018)
MN393476.1 QIE06881.1	ASFV Wuhan 2019-1 China	Genotype II Domestic pig	(Xiong, Zhang, Yu, & Wei, 2019)
MN172368.1 QGJ83454	China/CAS19-01/2019 China	Genotype II Domestic pig	(Jia et al., 2020)
MK128995.1 AYW34063.1	China/2018/AnhuiXCGQ China	Genotype II Domestic pig	(Bao et al., 2019)
MT496893.1 QLF78620.1	GZ201801 China	Genotype II Domestic pig - serum	(Tran et al., 2022)
MW521382.1 QTP96400.1	HuB20 China	Genotype II Domestic pig	(Tran et al., 2022)
MK333181.1 QBH90765.1	DB/LN/2018 China	Genotype II Domestic Pig - Blood	(Wen et al., 2019)
MT180393.1 QOY24398.1	ASFV_NgheAn_2019 Vietnam	Genotype II Domestic pig	(Nguyen et al., 2021)
MW465755.1 QSG73791.1	VNUA-ASFV-05L1/HaNam/VN/2020 Vietnam	Genotype II Domestic pig -spleen	(Truong et al., 2021)
MW396979.1 QTE18752.1	ASFV/Timor-Leste/2019/1 Timor-Leste	Genotype II Domestic pig	(Mileto et al., 2021)
LR536725.1 VFV48026.1	ASFV Belgium 2018/1 Belgium	Genotype II Domestic Pig	(Jan H. Forth et al., 2019)

MT847623.2	Pol19_53050_C1959/19	Genotype II	(Mazur-Panasiuk, 2020)
QOW03225.1	Poland	Domestic pig	
MT847622.1	Pol17_31177_O81	Genotype II	(Mazur-Panasiuk, 2020)
QOW03039.1	Poland	Domestic pig	
LR722599.1	ASFV Moldova 2017/1	Genotype II	(J. H. Forth et al., 2019)
VVW94185.1	Moldova	Domestic pig - spleen	
LR813622.1	Tanzania/Rukwa/2017/1	Genotype II	(Njau et al., 2021)
CAD0059543.1	SW Tanzania	Domestic pig	
MW856068.1	MAL/19/Karonga	Genotype II	(Hakizimana et al., 2020)
QXP50051.1	Malawi	Domestic pig	
LR899193.1	ASFV Germany 2020/1	Genotype II	(Sauter-Louis, 2021)
CAD7112603.1	Germany	Wild Boar	
MG939587.1	Pol17_03029_C201	Genotype II	(Mazur-Panasiuk, Woźniakowski, & Niemczuk, 2019)
AXZ96054.1	Poland	Wild boar	
MK543947.1	Belgium/Etalle/wb/2018	Genotype II	(Garigliany et al., 2019; Gilliaux et al., 2019)
QED90535	Belgium	Wild Boar	
LR722600.1	ASFV CzechRepublic 2017/1	Genotype II	(Forth, 2020)
VVW94190.1	Czech Republic	Wild Boar	
MK628478.1	ASFV/LT14/1490	Genotype II	(Gallardo et al., 2014)
QEY87898.1	Lithuania	Wild Boar - Blood	
MN715134.1	ASFV_HU_2018	Genotype II	(Olasz et al., 2019)
QGV56805.1	Hungary	Wild Boar - alveolar macrophages	
MT459800.1	ASFV/Kabardino-Balkaria 19/WB-964	Genotype II	(Malogolovkin, Yelsukova, Gallardo, Tsybanov, & Kolbasov, 2012; Mazloum et al., 2021)
QPB67618.1	Russia	Wild Boar - spleen	
MW306191.1	ASFV/Primorsky 19/WB-6723	Genotype II	(Mazloum et al., 2021)
QUQ60377.1	Russia	Wild Boar - spleen	

MW306190.1 QUQ60197.1	ASFV/Amur 19/WB-6905 Russia	Genotype II Wild Boar - spleen	(Mazloum et al., 2021)
LS478113.1 SPS73515.1	Estonia 2014 Estonia	Genotype II Wild Boar	(Nurmoja et al., 2020; Zani et al., 2018)
MK645909.1 QDL88123.1	ASFV-wbBS01 China	Genotype II Wild Boar	(Bao et al., 2019);(Njau et al., 2021)
MK940252.1 QIA61506.1	CN/2019/InnerMongolia-AES01- China	Genotype II Wild Boar	(Tran et al., 2022)
MW701371.1 QTZ19752.1	ASFV-G-deltaI177LdeltaLVR Plum Island	synthetic construct – deletion mutant of Georgia2007//1	(Borca, 2021)
MN270973.1 QIM07715.1	85/Ca/1985 Italy: Cagliari, Sardinia	Genotype I Domestic pig	(Torresi et al., 2020)
MN270974.1 QIM07948.1	141/Nu/1990 Italy: Nuoro, Sardinia	Genotype I Domestic pig	(Nix, Gallardo, Hutchings, Blanco, & Dixon, 2006; Torresi et al., 2020)
MN270975.1 QIM08183.1	142/Nu/1995 Italy: Nuoro, Sardinia	Genotype I Domestic pig	(Torresi et al., 2020)
NC_044942.1 YP_009702497.1	BA71V Spain: Badajoz	Genotype I Domestic pig - spleen	(Yanez, 1995)
NC_044958.1 YP_009703860.1	E75 Spain	Genotype I Domestic pig - spleen	(de Villiers et al., 2010)
NC_044941.1 YP_009702339.1	L60 Portugal	Genotype I Domestic pig	(Bastos et al., 2003)
NC_044943.1 YP_009702658.1	NHV Portugal	Genotype I Domestic pig	(Portugal et al., 2015)
NC_044956.1	Benin 97/1 Benin	Genotype I Domestic Pig	(Bastos et al., 2003)

YP_009703544.1/ CAN10191			
MZ202520.1 QZK26792.1	K49 Zaire - Katanga	Genotype I Serogroup 2	(Bastos et al., 2003)
NC_044957.1 YP_009703699.1	OURT 88/3 Portugal	Genotype I Tick	(Boinas, Hutchings, Dixon, & Wilkinson, 2004)
MN913970.1 QID21266.1	Liv13/33 (OmLF2) Zambia, Livingstone	Genotype I Tick	(Chastagner et al., 2020)
gi|229891462 AY261366.1 P0CA04.1	Namibia/Wart80/1980 Namibia	Genotype IV Warthog	(Bastos et al., 2003; Zsak et al., 2005)
AY261361.1 P0CA02.1	Malawi LIL 20/1 Malawi	Genotype VIII; Haplotype 8a Ticks	(Haresnape, 1989)
NC_044946.1 YP_009702990.1	Ken06.Bus Kenya	Genotype IX Domestic Pig	(Bishop et al., 2015; Gallardo et al., 2009)
MH025920.1 AXB50010.1	R35 Uganda: Tororo district	Genotype IX Domestic pig Blood	(Masembe et al., 2018)
MH025918.1 AXB49666.1	R25 Uganda: Tororo district	Genotype IX Domestic pig Blood	(Masembe et al., 2018)
LR899131.1 CAD7112308.1	ASFV Ken.rie1	Genotype X Domestic Pig - Blood	(Forth et al., 2020)
MT956648.1 QRY19118.1	Uvira B53 Kivu – DR Congo	Genotype X Serogroup 7 Domestic pig - spleen	(Bisimwa et al., 2020)
AY261360.1 P0CA01.1gi|229891459	KEN-50/1950 Kenya	Genotype X Domestic Pig	Kutish G.F. and Rock D.L, 2003

NC_044945.1 YP_009702825.1 HM745253	Ken05/Tk1 Kenya	Genotype X Ticks	(Bishop et al., 2015; Gallardo et al., 2009)
gi|229891461 AY261363.1 P0CA03.1	Pretoriuskop Pr4/1996 South Africa	Genotype XX Haplotype 20a Ticks	(Kleiboeker, Burrage, Scoles, Fish, & Rock, 1998)

Supplementary Table 2.3 Defined IFN-γ-inducing pp220 peptides are predicted to bind to multiple *SLA-I* alleles.

Peptide	Sequence	Aff(nM)	Bind Level	*SLA-I* Allele
p34[161-169]	LTHGLRAEY	1486.83	SB	SLA-1*02:01
p34[161-169]	LTHGLRAEY	1486.83	SB	SLA-1*02:02
p34[161-169]	LTHGLRAEY	270.87	SB	SLA-1*07:01
p34[161-169]	LTHGLRAEY	270.87	SB	SLA-1*07:02
p34[161-169]	LTHGLRAEY	1495.02	SB	SLA-1*08:01
p34[161-169]	LTHGLRAEY	538.61	SB	SLA-1*LWH
p34[161-169]	LTHGLRAEY	2054.49	SB	SLA-2*01:01
p34[161-169]	LTHGLRAEY	1397.84	SB	SLA-2*01:02
p34[161-169]	LTHGLRAEY	2046.26	SB	SLA-2*03:02
p34[161-169]	LTHGLRAEY	306.17	SB	SLA-2*04:01
p34[161-169]	LTHGLRAEY	1828.05	SB	SLA-2*04:02
p34[161-169]	LTHGLRAEY	1903.42	SB	SLA-2*10:01
p34[161-169]	LTHGLRAEY	1005.50	SB	SLA-2*10:02
p34[161-169]	LTHGLRAEY	451.91	SB	SLA-2*HB:01
p34[161-169]	LTHGLRAEY	4407.40	SB	SLA-2*LWH:AA
p34[161-169]	LTHGLRAEY	3744.41	SB	SLA-2*TPK:AA
p37[859-867]	KSMAAKIFI	1177.68	SB	SLA-2*05:01
p37[859-867]	KSMAAKIFI	7913.59	SB	SLA-2*12:01
p37[859-867]	KSMAAKIFI	1586.28	SB	SLA-2*YC:AA
p37[859-867]	KSMAAKIFI	1201.01	SB	SLA-3*01:01
p37[859-867]	KSMAAKIFI	2335.79	SB	SLA-3*06:02
p150[1363-1371]	HIDKNIIQY	9776.79	SB	SLA-1*01:01
p150[1363-1371]	HIDKNIIQY	520.01	SB	SLA-1*02:01
p150[1363-1371]	HIDKNIIQY	520.01	SB	SLA-1*02:02
p150[1363-1371]	HIDKNIIQY	13.25	SB	SLA-1*04:01
p150[1363-1371]	HIDKNIIQY	926.75	SB	SLA-1*06:01
p150[1363-1371]	HIDKNIIQY	420.20	SB	SLA-1*07:01
p150[1363-1371]	HIDKNIIQY	420.20	SB	SLA-1*07:02
p150[1363-1371]	HIDKNIIQY	554.29	SB	SLA-1*08:01
p150[1363-1371]	HIDKNIIQY	3350.95	SB	SLA-1*12:01
p150[1363-1371]	HIDKNIIQY	303.44	SB	SLA-1*13:01
p150[1363-1371]	HIDKNIIQY	2759.77	SB	SLA-1*HB:01
p150[1363-1371]	HIDKNIIQY	2038.59	SB	SLA-1*HB:02

Peptide	Sequence	Aff(nM)	Bind Level	*SLA-I* Allele
p150[1363-1371]	HIDKNIIQY	2038.59	SB	SLA-1*HB:03
p150[1363-1371]	HIDKNIIQY	2759.77	SB	SLA-1*HB:04
p150[1363-1371]	HIDKNIIQY	1181.03	SB	SLA-1*LWH
p150[1363-1371]	HIDKNIIQY	3227.75	SB	SLA-1*YC
p150[1363-1371]	HIDKNIIQY	13.25	SB	SLA-1*YDL:01
p150[1363-1371]	HIDKNIIQY	7612.26	SB	SLA-2*01:01
p150[1363-1371]	HIDKNIIQY	3376.90	SB	SLA-2*01:02
p150[1363-1371]	HIDKNIIQY	1348.91	SB	SLA-2*03:02
p150[1363-1371]	HIDKNIIQY	6358.65	SB	SLA-2*04:01
p150[1363-1371]	HIDKNIIQY	5207.98	SB	SLA-2*10:01
p150[1363-1371]	HIDKNIIQY	220.50	SB	SLA-2*10:02
p150[1363-1371]	HIDKNIIQY	213.00	SB	SLA-2*HB:01
p150[1363-1371]	HIDKNIIQY	8411.18	SB	SLA-2*LWH:AA
p150[1363-1371]	HIDKNIIQY	14014.36	SB	SLA-2*TPK:AA
p150[1363-1371]	HIDKNIIQY	13.25	SB	SLA-2*YDL:AA
p150[1363-1371]	HIDKNIIQY	13.25	SB	SLA-2*YDL:02
p150[1363-1371]	HIDKNIIQY	20574.55	SB	SLA-3*03:02
p150[1363-1371]	HIDKNIIQY	2759.77	SB	SLA-3*LWH
p150[1463-1471]	RVFSRLVFY	168.64	SB	SLA-1*02:01
p150[1463-1471]	RVFSRLVFY	168.64	SB	SLA-1*02:02
p150[1463-1471]	RVFSRLVFY	157.58	SB	SLA-1*04:01
p150[1463-1471]	RVFSRLVFY	1041.78	SB	SLA-1*05:01
p150[1463-1471]	RVFSRLVFY	1255.39	SB	SLA-1*06:01
p150[1463-1471]	RVFSRLVFY	84.92	SB	SLA-1*07:01
p150[1463-1471]	RVFSRLVFY	84.92	SB	SLA-1*07:02
p150[1463-1471]	RVFSRLVFY	402.78	SB	SLA-1*08:01
p150[1463-1471]	RVFSRLVFY	319.31	SB	SLA-1*12:01
p150[1463-1471]	RVFSRLVFY	1212.39	SB	SLA-1*13:01
p150[1463-1471]	RVFSRLVFY	1181.63	SB	SLA-1*HB:01
p150[1463-1471]	RVFSRLVFY	808.38	SB	SLA-1*HB:02
p150[1463-1471]	RVFSRLVFY	808.38	SB	SLA-1*HB:03
p150[1463-1471]	RVFSRLVFY	1181.63	SB	SLA-1*HB:04
p150[1463-1471]	RVFSRLVFY	248.15	SB	SLA-1*LWH
p150[1463-1471]	RVFSRLVFY	813.92	SB	SLA-1*YC
p150[1463-1471]	RVFSRLVFY	157.58	SB	SLA-1*YDL:01
p150[1463-1471]	RVFSRLVFY	548.67	SB	SLA-2*01:01

Peptide	Sequence	Aff(nM)	Bind Level	*SLA-I* Allele
p150[1463-1471]	RVFSRLVFY	130.67	SB	SLA-2*01:02
p150[1463-1471]	RVFSRLVFY	463.66	SB	SLA-2*03:02
p150[1463-1471]	RVFSRLVFY	314.89	SB	SLA-2*04:01
p150[1463-1471]	RVFSRLVFY	1264.22	SB	SLA-2*04:02
p150[1463-1471]	RVFSRLVFY	730.71	SB	SLA-2*10:01
p150[1463-1471]	RVFSRLVFY	108.09	SB	SLA-2*10:02
p150[1463-1471]	RVFSRLVFY	61.50	SB	SLA-2*HB:01
p150[1463-1471]	RVFSRLVFY	1138.60	SB	SLA-2*LWH:AA
p150[1463-1471]	RVFSRLVFY	5781.27	SB	SLA-2*TPK:AA
p150[1463-1471]	RVFSRLVFY	157.58	SB	SLA-2*YDL:AA
p150[1463-1471]	RVFSRLVFY	814.61	SB	SLA-2*YDY:AA
p150[1463-1471]	RVFSRLVFY	157.58	SB	SLA-2*YDL:02
p150[1463-1471]	RVFSRLVFY	2479.14	SB	SLA-3*03:01
p150[1463-1471]	RVFSRLVFY	3692.27	SB	SLA-3*03:02
p150[1463-1471]	RVFSRLVFY	2479.14	SB	SLA-3*03:03
p150[1463-1471]	RVFSRLVFY	3223.21	SB	SLA-*03:04
p150[1463-1471]	RVFSRLVFY	1229.98	SB	SLA-3*04:01
p150[1463-1471]	RVFSRLVFY	814.61	SB	SLA-3*06:01
p150[1463-1471]	RVFSRLVFY	4138.02	SB	SLA-3*06:02
p150[1463-1471]	RVFSRLVFY	1871.42	SB	SLA-3*07:01
p150[1463-1471]	RVFSRLVFY	814.61	SB	SLA-3*CDY
p150[1463-1471]	RVFSRLVFY	1181.63	SB	SLA-3*LWH
p150[1463-1471]	RVFSRLVFY	1301.51	SB	SLA-3*YC
p150[1463-1471]	RVFSRLVFY	814.61	SB	SLA-3*YDY:01
p150[1463-1471]	RVFSRLVFY	814.61	SB	SLA-3*YDY:02
p150[1463-1471]	RVFSRLVFY	1871.42	SB	SLA-3*YTH

The mapped IFN-γ-inducing nanomer peptides from ASFV pp220 (Georgia 2007/1) were predicted for *SLA-I* binding using NetMHCpan version 4.1 (https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1). Input type used was "Peptide" with the threshold for strong binders set at less than 0.5% Rank and for weak binders less than 2% Rank, but greater than 0.5% as are the default binding thresholds proven for MHC class I. Binding affinity and sorting were applied to filter for strong (SB) allele binders shown in the above table.

Chapter 3 - Immunization of pigs with replication-incompetent Adenovirus-vectored African Swine Fever Virus multiantigens induced humoral immune responses but no protection following contact challenge

Abstract

African Swine Fever Virus (ASFV) is a pathogen of great economic importance given that it continues to threaten the pork industry worldwide, but there is no safe vaccine or treatment available. Development of a vaccine is feasible since immunization of pigs with some live attenuated ASFV vaccine candidates can confer protection, but safety concerns and virus scalability are challenges that need to be addressed. Identification of protective ASFV antigens is needed to inform development of efficacious subunit vaccines. In this study, replication-incompetent adenovirus-vectored multicistronic ASFV antigen expression constructs that covered close to a 100% of the ASFV proteome, were generated and validated using ASFV convalescent serum. Immunization of pigs with a cocktail of the expression constructs, designated Ad5-ASFV, alone or formulated with either Montanide ISA-201 (Ad5-ASFV ISA-201) or BioMize adjuvant (Ad5-ASFV BioMize), primed strong B cell responses as judged by p62-specific IgG responses. Notably, the Ad5-ASFV and the Ad5-ASFV ISA-201, but not the Ad5-ASFV BioMize, immunogens primed significantly ($p<0.0001$) higher p62-specific IgG responses compared to Ad5-Luciferase formulated with Montanide ISA 201 adjuvant (Ad5-Luc ISA-201). The p62-specific IgG responses underwent significant ($p<0.0001$) recall in all the vaccinees after boosting and the induced antibodies strongly recognized ASFV (Georgia 2007/1)-infected primary swine cells. However, following challenge by contact spreaders, only one pig that had been immunized with the Ad5-ASFV cocktail survived. The survivor had no typical clinical symptoms, but it had viral loads and lesions consistent with chronic ASF. The outcome suggests that *in vivo* antigen expression, but not the antigen content, might be, in part, the limitation of this immunization approach since the replication-incompetent adenovirus does not amplify *in vivo* to effectively

prime and expand protective immunity. Addressing the *in vivo* antigen delivery limitation will likely yield promising outcomes.

3.1 Introduction

African Swine Fever (ASF) is a virulent disease in domestic swine and wild boar that is caused by the African Swine Fever Virus (ASFV) (Dixon et al. 2020). The ASFV is a complex enveloped DNA virus in the family Asfarviridae (Alonso et al. 2018). Epidemics caused by the ASFV have an overwhelming negative economic impact on the affected regions and jeopardize swine commerce globally with nearly 100% mortality in naïve populations ("ASF Situation Report" 2022; Ebwanga, Ghogomu, and Paeshuyse 2021). The global spread of ASFV (Georgia 2007/1) has occurred rapidly since its introduction from Africa and into the Russian Federation in the same year (Rowlands et al. 2008). Since then, the spread has occurred in several countries including Belgium (2018), the People's Republic of China (2018), the Dominican Republic and Haiti (2021), and has recently spread to Italy (2022) with additional reports in Northern Macedonia and Thailand (Kolbasov et al. 2018; "ASF Situation Report 2022).

Eradication of ASFV is not currently achievable given its presence in domestic and wild suids in many countries including Sub-Saharan African where it is also present in ticks (Dixon et al. 2020). Management of this pathogen is a much more feasible option. Control of ASFV dissemination traditionally has been through stomping out practices, and implementation of biosafety and security measures (Sánchez-Cordón et al. 2018; "ASF Situation Report" 2022; "Infection" 2022). Despite encouraging outcomes from studies that have evaluated vaccine candidates, including attenuated and inactivated ASFV, there are still safety concerns, such as the possibility of reversion, and poor efficacy (Gladue and Borca 2022; Tran et al. 2021; Xie et al.

2022; Deutschmann et al. 2022; Ding et al. 2022; Liu et al. 2023). These vaccine development approaches need to overcome multiple challenges including poor induction of protective immunity, shedding of vaccine virus, increased post-vaccination reactions, and unpredictability in the effects these viral modifications will have (Urbano and Ferreira 2022). Most recently, the modified live vaccine NAVET-ASFVAC, a joint commercial venture between USDA/ARS, the Vietnamese Ministry of Agriculture, and the Vietnamese Navetco Company, was suspended by Vietnamese agricultural officials less than two months after the launch of a 600,000-dose pilot vaccination program. It is believed that deaths in vaccinated swine are attributed to incorrect product utilization, which is currently under investigation (Tran et al. 2022; Borca et al. 2021; McDowell et al. 2022).

Subunit ASFV vaccines have historically included limited antigens with varied success, ranging from a complete lack of protection to full protection in a limited number of immunized animals (Gómez-Puertas et al. 1998; Barderas et al. 2001; Neilan et al. 2004). Antigen delivery platforms such as DNA vaccines and recombinant proteins or a combination of the two administered with or without adjuvant, have been evaluated (Argilaguet et al. 2012; Lacasta et al. 2014; Sunwoo et al. 2019). These approaches can induce robust immune responses, but variable protective efficacy has been reported (Argilaguet et al. 2012; Lacasta et al. 2014; Sunwoo et al. 2019). More recent innovations incorporate viral vectors for antigen delivery that stimulate strong and specific cellular immune responses and, in some instances, they have been shown to confer partial protection from ASF (Lokhandwala et al. 2016; Lokhandwala et al. 2017; Lokhandwala et al. 2019; Jancovich et al. 2018). Replication-deficient adenovirus type 5 (Ad5) and recombinant *Vaccinia virus* (rVACV) have recently been demonstrated as the most promising vector platforms for ASFV vaccination (Lokhandwala et al. 2019; Jancovich et al. 2018). However, challenges

remain such as: 1) determination of correlates of immune protection; 2) identification of protective antigens; and 3) development of an efficacious formulation and determination of the most appropriate immunization route.

Development of an efficacious ASFV subunit vaccine has not been successful due, in part, to the large viral genome which encodes more than 150 proteins and the protective antigens are yet to be identified (Dixon et al. 2013). While numerous immunodominant ASFV antigens have been characterized to date, empirical determination as to which antigen is required for protection has yet to be resolved (Kollnberger et al. 2002; Argilaguet et al. 2012; Lokhandwala et al. 2016; Lokhandwala et al. 2017; Lokhandwala et al. 2019; Dixon et al. 2013; Carlson et al. 2016). Some experimental vaccines utilizing this approach were able to achieve delayed viremia and death (and in some cases limited protection from disease) using one to multiple ASFV antigens (Argilaguet et al. 2013; Neilan et al. 2004; Lokhandwala et al. 2019). Recombinant *Vaccinia virus* encoding a combination of ASFV antigens (formulated without adjuvant) generated promising results with reduced blood and tissue viremia, even though protection from infection was not achieved following challenge (Jancovich et al. 2018). Available data suggest that, the design of a viral vectored vaccine for ASFV is expected to require the inclusion of multiple protective antigens. The major drawback to this design is in the time needed to: 1) empirically determine which antigen(s) to be included; 2) generate the expression constructs; and 3) test and determine the most efficacious formulation in domestic swine. Despite these limitations, the use of defined ASFV antigens for vaccination may offer a safer immunization option and has shown great potential in stimulating antigen-specific immune responses when packaged in a viral vector (Jancovich et al. 2018; Netherton, Goatley, et al. 2019; Lokhandwala et al. 2019; Murgia et al. 2019). Delivery of multiple antigens can be achieved by use of multicistronic expression cassettes that utilize the 2A

cleavage motif to allow generation of multiple independent antigens from a single mRNA molecule (Shaimardanova et al. 2019; Luke and Ryan 2018; Meas, Mekvichitsaeng, and Roshorm 2021).

In the current study, safety, immunogenicity, and protective efficacy of a replication-incompetent Ad5-vectored prototype subunit vaccine encoding ASFV multicistronic expression cassettes was evaluated in piglets. The experimental vaccines were formulated with no adjuvant or with either Montanide ISA-201 or BioMize adjuvant and used to immunize domestic piglets in a homologous prime-boost strategy. Vaccine efficacy was evaluated using a natural ASFV transmission model by exposure to comingled naïve ASFV-infected spreaders (Argilaguet et al. 2013; Burmakina et al. 2016; Gallardo et al. 2018; Jancovich et al. 2018 Argilaguet et al. 2013; Burmakina et al. 2016; Gallardo et al. 2018; Jancovich et al. 2018Guinat et al. 2016; Lokhandwala et al. 2019).

3.2 Materials and Methods

3.2.1 Generation of Recombinant Plasmid and Adenovirus Constructs

Selected ASFV Georgia 2007/1 open-reading-frames (Gene bank Accession FR682468) were used to design and generate multicistronic expression cassettes (Table 1). The pp220 polypeptide was split into two due to its large size. The polypeptide sequences were used to generate codon-optimized synthetic genes (GenScript, NJ, USA) that were cloned into pcDNA3.3 vector (Invitrogen, K8300001, CA, USA) with N-terminal HA and C-terminal FLAG tags, respectively. Protein expression by the recombinant plasmid constructs was evaluated by immunocytometric analyses as previously described (Lokhandwala et al. 2017; Lokhandwala et al. 2019; Lokhandwala et al. 2016; Zajac et al. 2022). The outcome was used to select the best expressers

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that were used as templates to PCR amplify the genes, which were then used to assemble cognate recombinant replication-incompetent adenoviruses using the Invitrogen ViraPower Adenoviral Expression System (K493000, CA, USA) as previously described (Lokhandwala et al. 2017; Lokhandwala et al. 2019; Lokhandwala et al. 2016; Zajac et al. 2022). A recombinant replication-incompetent adenovirus expressing luciferase (Ad5-Luciferase) was similarly generated. The recombinant adenoviruses were scaled up and viral titers (IFU/mL) were determined by immunocytometric analyses as previously described (Lokhandwala et al. 2016; Lokhandwala et al. 2017).

3.2.2 Validation of Protein Expression by the Recombinant Ad5 virus Constructs

Flow cytometry was used to evaluate protein expression by each recombinant adenovirus construct. Briefly, Human Embryonic Kidney (HEK) 293A cells were infected with Ad5-ASFV constructs and harvested after 24 hours using 2 mM EDTA to lift cells from the bottom of the culture vessel. Cells were then distributed at 1×10^6 cells per 5 mL polystyrene snap cap tube for each construct, followed by washing 2X using the Cyto-Fast™ Fix/Perm Buffer Set (BioLegend, 426803, CA, USA). Briefly, following the manufacturer's instructions, 1 mL wash buffer was added to each tube, gently vortexed, and centrifuged at 900 rpm for 5 minutes, after which the supernatant was discarded. Duplicate tubes were probed with a 1:200 dilution of ASFV-specific convalescent swine sera (26-28, 42) for 20 minutes at 4°C (kept in the dark). After washing as above, the cells were then probed with goat anti-porcine IgG FITC secondary antibody (Jackson ImmunoResearch, 114-095-003, PA, USA) diluted 1:250 in wash buffer for 20 minutes in the dark at 4°C. The cells were washed and fixed as per the manufacturer's instructions and resuspended in 2% goat sera (prepared in 1X PBS) before data acquisition using the BD LSRFortessa™ flow

cytometer and BD FACSDiva™ followed by analyses using FlowJo software (BD Biosciences, OR, USA). Negative mock-infected cells (media alone) were included and probed with ASFV convalescent serum and secondary antibody as controls for infection and gating.

3.2.2.1 Immunization of pigs

Twenty-eight piglets were acquired from a commercial vendor, housed at the BSL-2 Large Animal Research Center (LARC) at Kansas State University (KSU), and acclimatized for one week before immunization (Figure 1). The Ad5-ASFV cocktail (10^{10} ifu/construct, 4.1×10^{11} ifu total) was formulated with either 1X Phosphate Buffered Saline (PBS), Montanide ISA-201™ (Seppic, NJ, USA), or BioMize (VaxLiant, NE, USA) adjuvant and used to immunize pigs (n=5) intramuscularly as shown in Figure 2 and Table 3, and as previously described (Lokhandwala et al. 2019). Negative control pigs were similarly immunized, but with an equivalent dose of the Ad5-Luciferase (4.1×10^{11} ifu total) formulated with Montanide ISA-201 adjuvant. The pigs were boosted twice with the same priming dose and cognate formulation via the same route, three- and seven weeks post-priming. Each group had an additional 2 naïve pigs that were included to serve as contact spreaders in the challenge phase (Fig. 2 and Table 3).

3.2.2.2 Sample collection and clinical scoring post-immunization

During the immunization phase, blood, body temperatures, and weights were collected before vaccination and weekly thereafter. Following immunization, vaccine safety and tolerability was determined by observing the pigs daily and the following parameters were monitored and recorded: body weight, injection site reaction, rectal temperature, coughing, nasal and ocular discharges, and signs for depression.

3.2.3 Evaluation of Antibody Responses

Immunogenicity of the Ad5-ASFV immunogens in pigs was determined by tracking p62-specific IgG responses by ELISA since this antigen is highly immunogenic and sufficient amounts of mammalian cell-expressed recombinant protein can readily be generated (Lokhandwala et al. 2019). The ELISA was conducted using a p62 concentration of 1 µg/mL (100 µL/well) to coat microplates and a 1:100 dilution of serum samples as previously described (Lokhandwala et al. 2016; Lokhandwala et al. 2017; Lokhandwala et al. 2019; Zajac et al. 2022). In brief, plates were first blocked with 5% nonfat dry milk (diluted in PBST: 1X PBS + 0.1% Tween 20) followed by the addition of diluted serum (in triplicate) for 1 hour at 37°C. After 6 repeated washes (using PBST), a 1:5000 dilution of anti-porcine IgG-POD (Jackson ImmunoResearch, 114-035-003, PA, USA) secondary antibody was added to each well and the plates incubated at 37°C for 1 hour. The plates were washed 6X with PBST followed by 3X with PBS, and the POD colorimetric reaction was developed by adding Sure Blue tetramethylbenzidine (TMB) substrate (53-00-02, KPL, MA, USA). A 1N HCl solution was added to stop color development after 10 min. and the optical density (OD) was measured at 430 nm using a BioTek Epoch spectrophotometer (VT, USA). An irrelevant antigen, TMSP7, was used as a background control to establish the baseline for each sample which was subtracted from the p62 OD value. Additionally, a previously validated ASFV convalescent serum was included in each plate as a positive control alongside a validated naïve swine serum.

3.2.4 Validation of Ad5-ASFV-induced antibodies

Recognition of wild type ASFV by the antibodies induced by the Ad5-ASFV immunogens was determined by indirect fluorescence antibody (IFA) using ASFV (Georgia 2007/1)-infected and mock-infected naïve swine peripheral blood mononuclear cells (PBMCs) as previously described with the following modifications (Lokhandwala et al. 2016; Lokhandwala et al. 2017). Briefly, PBMCs were thawed from liquid nitrogen, washed using cold complete RPMI (cRPMI) plated at a density of 1 x 10^6 cells/well in a 96-well plate, and incubated overnight at 37°C. The cells were infected using a MOI of 0.001 of ASFV (Georgia 2007/1) prepared in cRPMI and incubated at 37°C for 1 hour after which, infection media was discarded and replaced with fresh cRPMI. Non-infected cells were included to serve as negative controls. After 48 hours, the cells were washed twice using 1X PBS, fixed using ice-cold methanol (100%) and air dried before blocking.

To perform the IFA, the plates were washed 2X using 1X PBS and incubated with blocking buffer (2% bovine serum albumin (BSA) in 1X PBS) for 1 hour at 37°C. After blocking, the cells were probed with a 1:20 dilution of serum (from 1-week post-boost 2) prepared in the blocking buffer for 1 hour at 37°C. ASFV-specific convalescent serum diluted at 1:500 was used as a positive control, and a 1:20 dilution of normal swine serum (Vector Laboratories, S-4000-20, CA, USA) was used as a negative control. Following three rinses with 1X PBS, the wells were then incubated with 1:200 goat anti-swine IgG FITC (Jackson ImmunoResearch, 114-095-003, PA, USA) alongside additional FITC only wells as secondary only controls for 45 min. at 37°C. Two more washes were performed using 1X PBS followed by the addition of 100 µL to each well before microscopic examination. The cells were visualized and images were acquired using an EVOS fluorescent imaging system (ThermoFisher, MA, USA).

3.2.4.1 ASFV Challenge

Two weeks after the final boost, the pigs were moved to the ABSL-3Ag biocontainment facility within the BRI on the KSU campus, acclimatized, and then challenged by exposure to contact spreaders infected with ASFV (Georgia 2007/1) (Figure 2). The two contact spreaders in each group were inoculated intramuscularly (IM) with a dose of 10^2 TCID$_{50}$/mL as determined by our previous study (Lokhandwala et al. 2019). The challenge of the comingled vaccinees in each group (n=5) occurred via the natural transmission where infection occurs through direct contact with the infected spreaders or virus shedding in the pens which mimic the natural course of infection in field settings (Guinat et al. 2016; Lokhandwala et al. 2019).

3.2.4.2 Sample collection and clinical scoring post-challenge

Prior to initiation of challenge in the ABSL-3Ag biocontainment, baseline nasal swabs, whole blood, and blood for serum were collected. Following challenge, blood, nasal swabs, weights, and rectal temperatures were collected on days 0, 5, 7, 11, and 14 with clinical signs monitored twice daily using a scoring rubric (Table 4). The surviving pig had additional collections on days 18, 21, 25, 28, 32, 35, and 37. Pigs that developed severe ASF were humanely euthanized followed by necropsy. The following tissues were collected: cranial mediastinal, gastrohepatic, mandibular, mesenteric, and renal lymph nodes; kidney, liver, lung, spleen, and tonsil.

3.2.5 Tissue Pathology

Formalin-fixed tissues were processed for histological analysis using standard procedures (37). Histological lesions were evaluated and scored for all animals utilizing the standardized parameters established previously by Galindo-Cardiel et al. with adaptations as per Sunwoo et al.

(Sunwoo et al. 2019; Galindo-Cardiel et al. 2013). Systematic histological assessment was performed blindly on the spleen, tonsil, mandibular, cranial mediastinal, mesenteric, gastrohepatic, renal lymph nodes, lung, liver, and kidney. The scoring matrix was as follows: absent (0) or minimal (1), mild (2), moderate (3), or severe (4). Lesion categories scored included: tissue necrosis (such as infarction) and cellular necrosis (such as lymphocytolysis); fibrin thrombi, fibrin deposition in tissue or fibrinoid degeneration of vessels, congestion and/or hemorrhage, and inflammation such as infiltrates of macrophages, eosinophils, or neutrophils. Overall total organ and lymphoid organ score ranges are represented as minimal (0-30); mild (31-60); moderate (61-90); or severe (above 90). The total score ranges for viscera (lung, liver, and kidney tissue) are indicated as minimal (0-10); mild (11-20); moderate (21-30); or severe (above 30).

3.2.6 Evaluation of ASFV Viremia

The detection and quantification of the ASFV genome in blood, swabs, and tissue samples from all pigs was performed using DNA purification followed by viral load assessment using a validated ASFV p72-specific quantitative real-time PCR (qPCR) assay. Briefly, ASFV DNA was extracted and purified from samples using the automated magnetic bead KingFisher Flex equipment (ThermoFisher, 5400610, MA, USA) that utilize the Total DNA Extraction Kit (GeneReach, Taiwan) as previously described (Sunwoo et al. 2019). Quantitative real-time PCR for the detection of the ASFV gene encoding the p72 antigen (primers: Integrated DNA Technology, IA, USA, and probe: ThermoFisher, MA, USA) was performed in duplicate wells using PerfeCTa® FastMix®II (Quanta Biosciences, MA, USA) and following reagent and cycle parameters as previously described on the Stratagene Mx3005p Real-Time PCR Detection System (Agilent, CA, USA) (Sunwoo et al. 2019; McDowell et al. 2022).

For the quantification of ASFV copy number (CN), serial dilutions (10X) of the positive control (ASFV p72 plasmid) were used to generate an 8-point standard curve (10^9 to 10^1 copies) using 16 qPCR well replicates performed as 2 scientific replicates. The ASFV p72 CN/reaction was mathematically determined using the mean cycle threshold value (Ct) and the slope and intercept of the DNA standard curve. A cycle threshold (Ct) cutoff of 38 for both PCR replicates was used as the positive cut-off. Each extraction and qPCR run included a standardized ASFV sample for the extraction and PCR positive control as well as a negative control sample (molecular grade water) as an extraction negative and PCR no template control.

3.2.7 Statistical analysis

GraphPad Prism, version 9.5.0, was used to analyze the data as follows: the percent survival significance was determined by the Mantel-Cox test; antibody responses were assessed using the Ordinary one-way ANOVA followed by Tukey's multiple comparisons test, and virus titers and histological scores were analyzed using the two-way ANOVA followed by Tukey's multiple comparisons test. Each comparison evaluated the treatment groups against the mock-immunized negative control (Ad5-Luciferase) group as well as each immunization group against each other (Ad5-ASFV No Adjuvant vs Ad5-ASFV ISA-201 and Ad5-ASFV BioMize; Ad5-ASFV ISA-201 vs Ad5-ASFV BioMize). For histological evaluation, all groups including the contact spreaders were compared for statistical significance. FlowJo, version 10.8.1, was used to gate and calculate the protein expression in the cells infected with the recombinant Ad5 virus constructs.

3.2.8 Ethics statement

Kansas State University Institutional Animal Care and Use Committee (IACUC) (Protocol # 3871 and #4411) and Institutional Biosafety Committee (IBC) (registration #1481) follows the regulations, policies, and guidelines put forth by the Animal Welfare Act, United States Department of Agriculture (USDA) Animal Care Resource Guide, and the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals. All protocols outlined in this document were followed including the use of clinical scoring for daily monitoring and assessment of animal health with weight and temperature included in this evaluation.

3.3 Results

3.3.1 Design, expression, and validation of ASFV antigen expression constructs

Recombinant pcDNA3 plasmid constructs encoding forty-one ASFV expression cassettes, with HA and FLAG tags at the 5' and 3' ends, respectively, were generated (Table 1). The pp220 polyprotein was split into two constructs due to its large size, whereas one construct was generated for each one of the next two largest antigens, NP1450L and G1340L (Table 1). The pcDNA3 constructs expressed the encoded antigens as judged by immunocytometric analyses of transfected HEK293A cells probed with tag-specific mAbs or ASFV convalescent serum (data not shown). The ASFV antigen expression cassettes from the pcDNA3 constructs were used to generate recombinant replication-incompetent adenoviruses (Table 1) and protein expression was confirmed by flow cytometric analyses of infected HEK293A cells probed with the ASFV convalescent serum (Fig. 1). Protein expression by the recombinant adenoviruses was heterogenous with low, medium, and high expression levels noted (Fig. 1 and Table 2). Notable

low expressers include Ad5-05 and Ad5-13, medium expressers include Ad5-04 and Ad5-29, whereas Ad5-09 and Ad5-19 were high expressers (Table 2).

3.3.2 The Ad5-ASFV cocktail was well tolerated

Homologous prime-boost immunization of piglets with a cocktail of the recombinant adenoviruses expressing multiple ASFV antigens, designated Ad5-ASFV, alone or formulated in adjuvant (Fig. 2 and Table 3), was well tolerated since there was no adverse effect observed. Notably, the vaccinees, the negative controls, and the comingled non-immunized piglets that were included to serve as contact spreaders at the challenge stage were healthy, had normal weight gain and maintained normal body temperatures throughout the immunization phase (Fig. 3).

3.3.3 Ad5-ASFV cocktail induced strong antibody responses that recognized ASFV

Immunization of piglets with the Ad5-ASFV cocktail, but not the negative control Ad5-Luciferase formulated in Montanide ISA-201, primed ASFV-specific humoral immune responses in all the vaccinees as judged by p62-specific IgG responses, which were used to evaluate immunogenicity (Figs. 4A and B). Compared to the negative control Ad5-Luciferase Montanide ISA-201 immunogen, the priming dose of the Ad5-ASFV cocktail alone or formulated with the Montanide ISA-201 adjuvant elicited significantly ($p<0.0001$) higher p62-specific IgG responses, but the IgG responses primed by the Ad5-ASFV cocktail formulated in BioMize adjuvant was not significant (Fig. 4B). The p62-specific IgG responses primed by the Ad5-ASFV cocktail alone or the Ad5-ASFV cocktail formulated with the Montanide ISA-201 adjuvant were significantly ($p=0.002$ and $p=0.005$, respectively) higher than the p62-specific IgG responses induced by the Ad5-ASFV cocktail formulated in BioMize adjuvant (Fig. 4B). The IgG responses primed by all the three

formulations were significantly (p<0.0001) amplified in all the vaccinees following the second booster dose (Fig. 4C). Compared to the p62-specific IgG responses induced by the priming dose, the second booster dose significantly (p<0.0001) amplified the primary response (Fig. 4B and C). However, following boosting, there was no significant difference in p62-specific IgG responses between the pigs immunized with the Ad5-ASFV cocktail alone and the pigs immunized with the Ad5-ASFV cocktail formulated with either the Montanide ISA-201 or the BioMize adjuvant, suggesting that these adjuvants did not enhance p62-specific IgG responses (Fig. 4C). Immunization of piglets with the Ad5-ASFV cocktail, but not the Ad5-Luciferase immunogen, elicited antibodies that strongly recognized primary swine cells infected with wildtype ASFV (Georgia 2007/1) as judged by IFA using post-boost sera (Fig. 5).

3.3.4 Ad5-ASFV Cocktail did not confer protection

Following challenge by comingling with naïve contact spreaders, which had received IM injection of the ASFV (Georgia 2007/1), the mean pig weights in all the groups was stable for one week (Fig. 6A). As expected, the contact spreaders developed high fever, rapid onset of high viremia in blood as well as nasal fluids, and severe clinical disease by the time they were terminated on day 8 post-challenge (Figs. 6B and 7). Histopathology of two representative contact spreaders showed tissue lesions that were consistent with severe acute ASF (Fig. 8). Multiple organ and tissue samples had high virus loads, which was consistent with acute ASF observed in naïve pigs and these values were statistically significant (p<0.0001) compared to all other groups (Fig. 9). One week after the initiation of contact challenge, the pigs immunized with the Ad5-ASFV cocktail formulated in BioMize adjuvant had rapid weight loss that was accompanied by elevated rectal temperature, viremia in blood and nasal fluid, and they were terminated after developing severe

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clinical disease (Figs. 6 and 7). BioMize immunized pigs had significantly lesser (p=0.0439) viremia in blood as compared to the contact spreaders (Fig. 9). Histopathology of one representative pig from this group revealed lesions that were consistent with moderate subacute ASF (Fig. 10), but all the tissue samples collected at termination had high levels of ASFV (Fig. 9). The mean weight of the negative control pigs immunized with the Ad5-Luciferase Montanide ISA-201 formulation and the pigs immunized with the Ad5-ASFV cocktail formulated in Montanide ISA-201 remained unchanged post-challenge, but the pigs developed high fever in the second week and severe clinical disease as well as high viremia in blood and nasal fluids that necessitated termination (Figs. 6 and 7). Viremia in blood was found to be significantly (p=0.0417) lower for the Montanide ISA-201 pigs compared to contact spreaders. (Fig 9). High levels of ASFV were also detected in multiple organs and tissues (Fig. 9).

Although the mean weight of the pigs immunized with the Ad5-ASFV cocktail without adjuvant was stable for eleven days, it declined thereafter as the pigs developed high fever and rapid onset of viremia in blood and nasal fluids that peaked on day seven after the initiation of contact challenge (Figs. 7A and B). Four out of the five vaccinees developed clinical disease that necessitated termination by day fourteen and histopathology of one representative pig revealed lesions that were consistent with moderate subacute ASF (Figs. 7C and 10). However, tissue samples collected from the four pigs at termination had high levels of ASFV that mirrored outcomes from the other treatment groups (Fig. 9). It was noted that, compared to the other groups, pigs from this group had the lowest mean viremia in the spleen (Fig. 9). It was also noted that the pigs immunized with the Ad5-ASFV formulated with or without adjuvant, had the lowest mean viremia in mandibular lymph nodes (Fig. 9). Overall, there was no significant difference in total

tissue viral load in the pigs immunized with the Ad5-ASFV formulated with or without adjuvant, when compared to the Ad5-Luciferase negative controls (Fig. 9).

3.3.5 Survivor had chronic ASF

One of the five pigs (number 6892) immunized with the Ad5-ASFV cocktail without adjuvant seroconverted and had rapid increase in antibody response within the first two weeks, but unlike the other four vaccinees in this group, boosting did not significantly amplify p62-specific IgG responses (Fig. 11A). Interestingly, this pig had the lowest post-boost p62-specific IgG responses compared to the other vaccinees in this group (Fig. 11A). Following exposure to contact spreaders, viremia in blood and nasal fluid peaked on day seven in this pig, whereas fever peaked on the eleventh day (Fig. 11B). The weight of the pig declined from day seven, but on day fourteen when the other four vaccinees in this group were terminated due to severe clinical disease, the pig started to gain weight and sustained weight gain until the study was terminated at thirty-nine days after initiation of contact challenge (Figs. 7C and 11B). The weight gain was accompanied by resolution of fever and gradual decrease of viremia in blood until study termination (Fig. 11B). Although the pig was clinically healthy at termination, it had recurrent episodes of high viremia in nasal fluid (Fig. 11B). Necropsy revealed that the pig had evident inflammation and marked thickening of the epicardium with an accumulation of fibrino-hemorrhagic fluid in the pericardial sac due to chronic-active pericarditis (Fig. 12). In addition, histopathology revealed lesions consistent with moderate subacute ASF, but viremia in tissue samples collected at termination had much lower levels of ASFV (tonsil, 3.9 x 10^2 CN/mL; mandibular LN, 2.2 x 10^3 CN/mL; cranial mediastinal LN, 7.7 x 10^4 CN/mL; mesenteric LN, 2.9 x 10^3 CN/mL; gastrohepatic LN, 3.9 x 10^3 CN/mL; renal LN, 5.7 x 10^2 CN/mL; spleen, 2.0 x 10^4 CN/mL; lung, 3.6 x 10^3 CN/mL; kidney, 9.5 x 10^5 CN/mL; liver,

1.1 x 10^4 CN/mL) compared to the average tissue viremia detected in the contact spreaders (tonsil, 1.9 x 10^6 CN/mL; mandibular LN, 3.0 x 10^6 CN/mL; cranial mediastinal LN, 1.8 x 10^6 CN/mL; mesenteric LN, 6.3 x 10^5 CN/mL; gastrohepatic LN, 1.8 x 10^6 CN/mL; renal LN, 1.4 x 10^6 CN/mL; spleen, 1.7 x 10^7 CN/mL; lung, 1.1 x 10^6 CN/mL; kidney, 1.1 x 10^5 CN/mL; liver, 7.9 x 10^6 CN/mL) as well as the other treatment and control pigs (Figs. 9 and 11C). The viremia in tissue samples for the survivor was significantly lower than the contact spreaders in the mandibular LN (p=0.0012), cranial mediastinal LN (p=0.0029), gastrohepatic LN (p=0.0344), and liver (p=0.0088) (Figs. 9 and 11C). Significantly lower viremia in the mandibular LN was also found compared to the Montanide ISA-201 (p=0.005) group, and the four no adjuvant pigs (p=0.0421) who succumbed earlier in the study (Figs. 9 and 11C).

3.3.6 Clinical disease and histopathology at termination

Following initiation of challenge by contact with naïve ASFV-infected pigs, which were terminated on day eight, the mean survival of the negative control pigs (Ad5-Luciferase Montanide ISA-201 formulation) was 11.4 days compared to 12.2 to 17 days for Ad5-ASFV vaccinees (Ad5-ASFV cocktail without adjuvant, Ad5-ASFV cocktail Montanide ISA-201 formulation, and Ad5-ASFV cocktail BioMize formulation) with the exemption of one vaccinee that survived (Ad5-ASFV cocktail without adjuvant) until study termination on day thirty nine (Fig. 7C). Typical symptoms for all animals that succumbed to the disease consisted of high fever, lethargy, reddening of the skin, anorexia and weight loss. Analysis of clinical presentation of ASF at termination showed that the vaccinees which were immunized with the Ad5-ASFV cocktail formulated with Montanide ISA-201 or BioMize had an average clinical score of 3.2, while the vaccinees that were immunized with the Ad5-ASFV cocktail without adjuvant had average clinical

score of 3.4 (Table 5). Although these scores were less than the average clinical score of 3.6 for the naïve contact spreaders, the difference was not significant (Table 5). The negative controls (Ad5-Luciferase Montanide ISA-201 formulation) were a notable exemption with an average clinical score of 1.4 and the lone survivor (Ad5-ASFV cocktail without adjuvant) that had no clinical symptoms at termination (Table 5).

Compared to the contact spreaders, the mean histopathological scores of lymphoid tissues from the Ad5-Luc controls and the vaccinees immunized with the Ad5-ASFV formulated with or without adjuvant were significantly (p<0.0001) lower (Fig. 13A). It was also noted that the mean histopathological scores of lymphoid tissues from the pigs immunized with Ad5-ASFV without adjuvant or the Ad5-ASFV Montanide ISA-201 vaccinees were significantly (p<0.0001) lower than the mean scores for lymphoid tissues from the Ad5-Luc controls and the Ad5-ASFV BioMize vaccinees (Fig. 13A). There was no difference between the mean scores for the lymphoid tissues from the pigs immunized with the Ad5-ASFV without adjuvant and the Ad5-ASFV Montanide ISA-201 vaccinees (Fig. 13A). The mean histopathological scores for visceral organs (liver, lungs, kidney, and heart) from the contact spreaders were significantly (p=0.0014) higher than the mean score for Ad5-Luc Montanide ISA-201 controls and the Ad5-ASFV Montanide ISA-201 vaccinees as well as the mean score for the vaccinees that received the Ad5-ASFV without adjuvant (p=0.0057) (Fig. 13B). There was no difference between the contact spreaders and the Ad5-ASFV BioMize vaccinees (Fig. 13B). However, analyses of individual lymphoid tissues as well as visceral organs showed that there was extreme variation in histopathological scores between the groups and among the controls as well as the vaccinees (Figs. 14 and 15).

3.4 Discussion

Promising results have been obtained from recent studies that evaluated protective efficacy of ASFV prototype vaccines, primarily attenuated virus (Gladue and Borca 2022; Tran et al. 2021; Xie et al. 2022; Deutschmann et al. 2022; Ding et al. 2022; Liu et al. 2023). Despite these advancements, the need for safe and more efficacious ASFV vaccines has been made increasingly evident given the poor performance of the vaccine candidates, the threat posed by continued virus global spread, and significant economic losses associated with this disease (Urbano and Ferreira 2022; Nguyen-Thi et al. 2021). This study evaluated safety, tolerability and efficacy of experimental subunit vaccines that were formulated, with and without adjuvant, using a cocktail of forty-one replication-deficient adenovirus-vectored multicistronic expression cassettes encoding ASFV antigens. Since protective ASFV antigens are yet to be identified, immunization with a cocktail of the multicistronic expression cassettes was a strategy to deliver nearly 100% of the ASFV Georgia 2007/1 proteome to mimic antigen delivery by some ASFV mutants that have been shown to confer protection and if successful, this approach could lead to identification of the protective determinants needed for the development of rationally designed prototype subunit vaccines (Gladue and Borca 2022; Tran et al. 2021; Xie et al. 2022; Deutschmann et al. 2022; Ding et al. 2022; Liu et al. 2023). The multicistronic expression cassettes were stable after multiple passages of the recombinant adenoviruses as judged by expression of the FLAG tag at the C-terminal of the last gene in the cassette. More importantly, the proteins expressed by the recombinant adenoviruses were shown to be authentic ASFV antigens as judged by flow cytometric analyses using ASFV convalescent serum (Fig. 1 and Table 1). However, *in vitro* expression of the ASFV antigens by the recombinant adenoviruses was heterogenous ranging from low (e.g. Ad5-05, Ad5-13), medium (e.g. Ad5-18, Ad5-39), to high (e.g. Ad5-09, Ad5-19)

expression (Fig. 1 and Table 1). This outcome could have been influenced by the gene combination or arrangement in each cassette, but it was noted that protein expression by the majority of the cassettes was comparable or better than the positive control recombinant adenovirus encoding the p62 antigen alone (Fig. 1 and Table 1). It is possible that the heterogenous expression of the ASFV antigens could have had an impact on the magnitude of priming B cell and T cell responses, but this study did not ascertain whether this was reflected in the resultant immune responses.

Three doses of a cocktail of the recombinant adenoviruses expressing the ASFV antigens (Ad5-ASFV), formulated with or without adjuvant, were well tolerated since all the pigs in the treatment groups remained healthy throughout the immunization phase (Fig. 3). The Ad5-Luciferase negative control construct formulated with the Montanide ISA-201 adjuvant (Ad5-Luc ISA-201), was also well tolerated (Fig. 3). Notably, the priming dose of the Ad5-ASFV cocktail alone or formulated with the Montanide ISA-201 adjuvant (Ad5-ASFV ISA-201), but not the Ad5-ASFV cocktail formulated in BioMize adjuvant (Ad5-ASFV BioMize), elicited significant (p<0.0001) p62-specific IgG responses compared to the Ad5-Luc ISA-201 negative control immunogen (Fig. 4B). Boosting significantly (p<0.0001) recalled p62-specific IgG responses in the vaccinees, but there was no difference in p62-specific IgG responses between the pigs immunized with the Ad5-ASFV cocktail alone and the pigs immunized with either the Ad5-ASFV ISA-201 or the Ad5-ASFV BioMize formulations, suggesting that the adjuvants were not necessary (Fig. 4C). This outcome cannot be used to imply that the adjuvants did not have an effect on the antibody isotype or T cell responses and/or functions of the induced effectors since these were not evaluated in this study. More importantly, the induced antibodies strongly recognized ASFV-infected, but not uninfected, primary swine cells (Fig. 5). This outcome confirmed that the Ad5-ASFV immunogens generated using synthetic genes induced authentic

IgG responses, which was consistent with previous findings in domestic pigs and wild boars (Pikalo et al. 2022; Obradovic et al. 2021; Blome, Gabriel, and Beer 2014; Lokhandwala et al. 2016; Lokhandwala et al. 2017; Zajac et al. 2022; Cadenas-Fernandez et al. 2020) .

Although homologous prime-boost immunization of pigs induced and significantly (p<0.0001) expanded antibody responses as judged by tracking p62-specific IgGs, all the controls and the vaccinees, with one exemption, succumbed to ASF within fourteen days following exposure to comingled naïve contact spreaders that had received IM injection of ASFV (Georgia 2007/1) (Figs. 6 and 7). The challenge by contact to infected pigs, as opposed to needle challenge, is the ideal model for natural infection given that transmission of ASFV by ticks is limited to Sub-Saharan Africa, while in other regions of the world, the spread of virus to naïve pigs is by ingestion of contaminated materials or exposure to infected pigs (Guinat et al. 2016). In this natural ASFV transmission model, the infection may occur via the mucosal route through direct animal to animal contact or oral-fecal route, likely with multiple low-dose repeated exposures more representative of non-tick ASFV transmission (Guinat et al. 2016). This challenge model has previously been shown to be effective by other investigators using ASFV (Arm07 genotype II isolate) (Cadenas-Fernandez et al. 2020). Seven days after the initiation of the challenge, the pigs immunized with the Ad5-ASFV cocktail formulated with or without adjuvant, lost weight, developed high fever and viremia in blood as well as in nasal fluids (Figs. 6, 7A and B). The pigs were terminated by day fourteen after they developed clinical ASF (Fig. 7C). There was no significant difference in clinical presentation of ASF at termination between the naïve contact spreaders and the treatment groups (Table 5). The Ad5-Luc ISA-201 controls with an average clinical score of 1.4 and the survivor (immunized with the Ad5-ASFV cocktail without adjuvant) which had no clinical symptoms at termination, were the only exemptions (Table 5). The low clinical score noted for the

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Ad5-Luc controls could have been due to the presentation of peracute ASF where animals are viremic but can show little to no outward signs of disease (Li et al. 2022; Salguero 2020).

Histopathology of representative pigs revealed lesions that were consistent with moderate subacute ASF (Figs. 7C and 10). These outcomes were consistent with the observations made from the naïve spreaders as well as the Ad5-Luc ISA-201 negative controls (Figs. 6 - 8, 10 and 12). At termination, the mean viremia in tissues from the naïve contact spreaders was significantly ($p<0.0001$) higher compared to the mean viremia in tissues from the Ad5-Luc ISA-201 negative controls and the pigs immunized with the Ad5-ASFV cocktail alone or formulated in adjuvant (Fig. 9). However, there was no significant difference in tissue viral load in the Ad5-ASFV vaccinees when compared to the Ad5-Luc negative controls (Fig. 9). In contrast, it was observed that the mean histopathological scores of tissues from the contact spreaders were higher than the mean scores for the Ad5-Luc controls and the Ad5-ASFV vaccinees (Figs. 13 and 14). However, at the individual level, there was wide variation in total tissue histopathological scores between the treatment groups and among the individual control and vaccinated pigs (Figs. 14 and 15). Overall, the outcome showed that immunization of pigs with the experimental Ad5-ASFV formulation was not protective even though the immunogens induced antibody responses that recognized ASFV-infected cells. However, only anti-p62 antibody responses were evaluated and therefore, it is not known whether the vaccinees mounted antibodies against the other antigens included in the cocktail. Although ASFV neutralization has been reported, the role of antibodies in protection against ASFV infection is yet to be determined, but it might depend on the target antigens or subtype of immunoglobulin induced (Onisk et al. 1994; Ruiz-Gonzalvo F 1983; Ruiz Gonzalvo et al. 1986; Leitão et al. 2001).

One pig (number 6892) immunized with the Ad5-ASFV cocktail without adjuvant was the only survivor (Fig. 7C). Although the pig mounted a rapid increase in p62-specific IgG responses after priming, there was no significant increase in antibody response after boosting and it had the lowest antibody responses compared to the other four pigs in this group (Fig. 11A). It was also noted that viremia peaked one week after initiation of contact challenge, followed by weight loss and high fever that peaked on the twelfth day (Fig. 11B). By day fourteen when all the other pigs in this group were terminated, the pig started to gain weight and sustained it for the next twenty-five days during which time the fever resolved and viremia in blood gradually decreased, but there were recurrent episodes of high viremia in nasal fluid until study termination (Figs. 7C and 11B). The pig did not exhibit typical ASF clinical symptoms, but necropsy revealed that it had chronic pericarditis and lesions consistent with moderate subacute ASF (Table 5 and Fig. 12). At termination, tissue samples had the lowest amount of ASFV compared to the average viremia detected in the contact spreaders as well as the pigs from all the other groups (Figs. 9 and 11C). The recurring virus shedding, low viremia in tissues, and the lesions observed were consistent with outcomes reported in pigs with chronic ASF (Sehl-Ewert et al. 2022; Sehl et al. 2020; Salguero 2020; Lai et al. 2022; Pornthummawat et al. 2021; Rodriguez-Bertos et al. 2020; Lohse et al. 2022). Although this pig had the lowest p62-specific antibodies, B cell responses against other antigens included in the Ad5-ASFV cocktail were not determined and in addition, T cell responses were not evaluated. A previous study has shown that, following immunization, vaccinees that mounted the lowest, but not the highest, antigen-specific IgG responses had better survival rate and lower clinical scores (Lokhandwala et al. 2019). It is possible that the survivors mounted low antibody responses that were directed against protective determinants, whereas the non-survivors had strong responses against non-protective antigens. It is also likely that the sole survivor had unique genetic

traits that enabled it to resolve the virus which would be consistent with the observation that infection of naïve pigs with the most virulent ASFV isolates does not always result in 100% mortality (Reiner 2009; Proudfoot, Lillico, and Tait-Burkard 2019; Pastoret et al. 2012; Netherton, Connell, et al. 2019).

In conclusion, the results from this study showed that immunization of pigs with a cocktail of forty-one multicistronic ASFV antigen expression constructs, formulated with and without adjuvant, primed humoral immune responses as judged by p62-specific IgGs, which underwent significant recall after boosting. However, none of the Ad5-ASFV formulation conferred protection upon challenge except one sole survivor that had been immunized with the Ad5-ASFV cocktail without adjuvant. Even though the survivor had no typical clinical symptoms, it had viral loads and lesions consistent with chronic ASF. Given the near 100% coverage of the ASFV proteome, the outcome from this study suggests that *in vivo* antigen expression, but not the antigen content, might be the limitation of this immunization approach. This could be due, in part, to the fact that the replication-incompetent adenovirus does not mimic replication of attenuated ASFV since it does not amplify and persist in pigs to effectively prime and expand protective immunity. Addressing this limitation of the *in vivo* ASFV antigen delivery will likely yield promising outcomes.

Data Availability Statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics Statement

The animal study was reviewed and approved by Texas A&M University Institutional Animal Care and Use Committee (IACUC) (permit# 2009067) approved Animal Use Protocol 2012-59 that follows the regulations, policies, and guidelines put forth by the Animal Welfare Act, United States Department of Agriculture (USDA) Animal Care Resource Guide, and the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals.

3.5 References

Alonso, Covadonga, Manuel Borca, Linda Dixon, Yolanda Revilla, Fernando Rodriguez, and Jose M. Escribano. 2018. 'ICTV Virus Taxonomy Profile: Asfarviridae', *Journal of General Virology*, 99: 613-14.

African Swine Fever (ASF) Situation Report. Paris: World Organisation for Animal Health (2022).

Argilaguet, J.M., E. Pérez-Martín, M. Nofrarías, C. Gallardo, F. Accensi, A. Lacasta, M. Mora, M. Ballester, I. Galindo-Cardiel, and S. López-Soria. 2012. 'DNA Vaccination Partially Protects against African Swine Fever Virus Lethal Challenge in the Absence of Antibodies', *PLoS One*, 7: e40942.

Argilaguet, Jordi M., Eva Pérez-Martín, Sergio López, Martin Goethe, J. M. Escribano, Katrin Giesow, Günther M. Keil, and Fernando Rodríguez. 2013. 'BacMam immunization partially protects pigs against sublethal challenge with African swine fever virus', *Antiviral Research*, 98: 61-65.

Barderas, MG, F Rodriguez, P Gomez-Puertas, M Aviles, F Beitia, C Alonso, and JM Escribano. 2001. 'Antigenic and immunogenic properties of a chimera of two immunodominant African swine fever virus proteins', *Archives of Virology*, 146: 1681-91.

Blome, Sandra, Claudia Gabriel, and Martin Beer. 2014. 'Modern adjuvants do not enhance the efficacy of an inactivated African swine fever virus vaccine preparation', *Vaccine*, 32: 3879-82.

Borca, Manuel V., Elizabeth Ramirez-Medina, Ediane Silva, Elizabeth Vuono, Ayushi Rai, Sarah Pruitt, Nallely Espinoza, Lauro Velazquez-Salinas, Cyril G. Gay, and Douglas P. Gladue. 2021. 'ASFV-G-ΔI177L as an Effective Oral Nasal Vaccine against the Eurasia Strain of Africa Swine Fever', *Viruses*, 13: 765.

Burmakina, G., A. Malogolovkin, E. R. Tulman, L. Zsak, G. Delhon, D. G. Diel, N. M. Shobogorov, Y. P. Morgunov, S. Y. Morgunov, G. F. Kutish, D. Kolbasov, and D. L. Rock. 2016. 'African swine fever virus serotype-specific proteins are significant protective antigens for African swine fever', *J Gen Virol*, 97: 1670-75.

Cadenas-Fernandez, E., J. M. Sanchez-Vizcaino, A. Kosowska, B. Rivera, F. Mayoral-Alegre, A. Rodriguez-Bertos, J. Yao, J. Bray, S. Lokhandwala, W. Mwangi, and J. A. Barasona. 2020. 'Adenovirus-vectored African Swine Fever Virus Antigens Cocktail Is Not Protective against Virulent Arm07 Isolate in Eurasian Wild Boar', *Pathogens*, 9.

Carlson, J., V. O'Donnell, M. Alfano, L. Velazquez Salinas, L. G. Holinka, P. W. Krug, D. P. Gladue, S. Higgs, and M. V. Borca. 2016. 'Association of the Host Immune Response with Protection Using a Live Attenuated African Swine Fever Virus Model', *Viruses*, 8.

Deutschmann, Paul, Tessa Carrau, Julia Sehl-Ewert, Jan Hendrik Forth, Elisenda Viaplana, Jose Carlos Mancera, Alicia Urniza, Martin Beer, and Sandra Blome. 2022. 'Taking a Promising Vaccine Candidate Further: Efficacy of ASFV-G-ΔMGF after Intramuscular Vaccination of Domestic Pigs and Oral Vaccination of Wild Boar', *Pathogens*, 11: 996.

Ding, Mingyang, Wen Dang, Huanan Liu, Keshan Zhang, Fan Xu, Hong Tian, Huaguo Huang, Zhengwang Shi, Yongjie Sunkang, Xiaodong Qin, Yong Zhang, and Haixue Zheng. 2022. 'Sequential Deletions of Interferon Inhibitors MGF110-9L and MGF505-7R Result in Sterile Immunity against the Eurasia Strain of Africa Swine Fever', *Journal of Virology*, 96: e01192-22.

Dixon, Linda K, David AG Chapman, Christopher L Netherton, and Chris Upton. 2013. 'African swine fever virus replication and genomics', *Virus Research*, 173: 3-14.

Dixon, Linda K., Karl Stahl, Ferran Jori, Laurence Vial, and Dirk U. Pfeiffer. 2020. 'African Swine Fever Epidemiology and Control', *Annual Review of Animal Biosciences*, 8: 221-46.

Ebwanga, Ebanja Joseph, Stephen Mbigha Ghogomu, and Jan Paeshuyse. 2021. 'African Swine Fever in Cameroon: A Review', *Pathogens*, 10: 421.

Galindo-Cardiel, I., M. Ballester, D. Solanes, M. Nofrarías, S. López-Soria, J. M. Argilaguet, A. Lacasta, F. Accensi, F. Rodríguez, and J. Segalés. 2013. 'Standardization of pathological investigations in the framework of experimental ASFV infections', *Virus Res*, 173: 180-90.

Gallardo, C., E. G. Sánchez, D. Pérez-Núñez, M. Nogal, P. de León, L. Carrascosa Á, R. Nieto, A. Soler, M. L. Arias, and Y. Revilla. 2018. 'African swine fever virus (ASFV) protection mediated by NH/P68 and NH/P68 recombinant live-attenuated viruses', *Vaccine*, 36: 2694-704.

Gladue, Douglas P., and Manuel V. Borca. 2022. 'Recombinant ASF Live Attenuated Virus Strains as Experimental Vaccine Candidates', *Viruses*, 14: 878.

Gómez-Puertas, Paulino, Fernando Rodríguez, José M. Oviedo, Alejandro Brun, Covadonga Alonso, and José M. Escribano. 1998. 'The African Swine Fever Virus Proteins p54 and p30 Are Involved in Two Distinct Steps of Virus Attachment and Both Contribute to the Antibody-Mediated Protective Immune Response', *Virology*, 243: 461-71.

Guinat, C., A. Gogin, S. Blome, G. Keil, R. Pollin, D. U. Pfeiffer, and L. Dixon. 2016. 'Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions', *Vet Rec*, 178: 262-7.

Infection with African Swine Fever Virus Terrestrial Animal Health Code. Paris: World Organization for Animal Health (2022).

Jancovich, J. K., D. Chapman, D. T. Hansen, M. D. Robida, A. Loskutov, F. Craciunescu, A. Borovkov, K. Kibler, L. Goatley, K. King, C. L. Netherton, G. Taylor, B. Jacobs, K. Sykes, and L. K. Dixon. 2018. 'Immunization of Pigs by DNA Prime and Recombinant Vaccinia Virus Boost To Identify and Rank African Swine Fever Virus Immunogenic and Protective Proteins', *J Virol*, 92.

Kolbasov, Denis, Ilya Titov, Sodnom Tsybanov, Andrey Gogin, and Alexander Malogolovkin. 2018. 'African swine fever virus, Siberia, Russia, 2017', *Emerging infectious diseases*, 24: 796.

Kollnberger, SD, B Gutierrez-Castañeda, M Foster-Cuevas, A Corteyn, and RME Parkhouse. 2002. 'Identification of the principal serological immunodeterminants of African swine fever virus by screening a virus cDNA library with antibody', *Journal of General Virology*, 83: 1331-42.

Lacasta, Anna, María Ballester, Paula L. Monteagudo, Javier M. Rodríguez, María L. Salas, Francesc Accensi, Sonia Pina-Pedrero, Albert Bensaid, Jordi Argilaguet, and Sergio López-Soria. 2014. 'Expression Library Immunization Can Confer Protection against Lethal Challenge with African Swine Fever Virus', *Journal of Virology*, 88: 13322-32.

Lai, Danh Cong, Taehwan Oh, Hien The Nguyen, and Duy Tien Do. 2022. 'The study of antigen carrying and lesions observed in pigs that survived post African swine fever virus infection', *Tropical Animal Health and Production*, 54: 264.

Leitão, Alexandre, Clara Cartaxeiro, Ricardo Coelho, Benedita Cruz, RME Parkhouse, Fernando C Portugal, José D Vigário, and Carlos LV Martins. 2001. 'The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response', *Journal of General Virology*, 82: 513-23.

Li, Z., W. Chen, Z. Qiu, Y. Li, J. Fan, K. Wu, X. Li, M. Zhao, H. Ding, S. Fan, and J. Chen. 2022. 'African Swine Fever Virus: A Review', *Life (Basel)*, 12.

Liu, Y., Z. Xie, Y. Li, Y. Song, D. Di, J. Liu, L. Gong, Z. Chen, J. Wu, Z. Ye, J. Liu, W. Yu, L. Lv, Q. Zhong, C. Tian, Q. Song, H. Wang, and H. Chen. 2023. 'Evaluation of an I177L gene-based five-gene-deleted African swine fever virus as a live attenuated vaccine in pigs', *Emerg Microbes Infect*, 12: 2148560.

Lohse, L., J. Nielsen, Å Uttenthal, A. S. Olesen, B. Strandbygaard, T. B. Rasmussen, G. J. Belsham, and A. Bøtner. 2022. 'Experimental Infections of Pigs with African Swine Fever Virus (Genotype II); Studies in Young Animals and Pregnant Sows', *Viruses*, 14.

Lokhandwala, S., V. Petrovan, L. Popescu, N. Sangewar, C. Elijah, A. Stoian, M. Olcha, L. Ennen, J. Bray, R. P. Bishop, S. D. Waghela, M. Sheahan, R. R. R. Rowland, and W. Mwangi. 2019. 'Adenovirus-vectored African Swine Fever Virus antigen cocktails are

immunogenic but not protective against intranasal challenge with Georgia 2007/1 isolate', *Vet Microbiol*, 235: 10-20.

Lokhandwala, S., S. D. Waghela, J. Bray, C. L. Martin, N. Sangewar, C. Charendoff, R. Shetti, C. Ashley, C. H. Chen, L. R. Berghman, D. Mwangi, P. J. Dominowski, D. L. Foss, S. Rai, S. Vora, L. Gabbert, T. G. Burrage, D. Brake, J. Neilan, and W. Mwangi. 2016. 'Induction of Robust Immune Responses in Swine by Using a Cocktail of Adenovirus-Vectored African Swine Fever Virus Antigens', *Clin Vaccine Immunol*, 23: 888-900.

Lokhandwala, S., S. D. Waghela, J. Bray, N. Sangewar, C. Charendoff, C. L. Martin, W. S. Hassan, T. Koynarski, L. Gabbert, T. G. Burrage, D. Brake, J. Neilan, and W. Mwangi. 2017. 'Adenovirus-vectored novel African Swine Fever Virus antigens elicit robust immune responses in swine', *PLoS One*, 12: e0177007.

Luke, Garry A, and Martin D Ryan. 2018. 'Using the 2A protein coexpression system: Multicistronic 2A vectors expressing gene (s) of interest and reporter proteins.' in, *Reporter Gene Assays* (Springer).

McDowell, Chester D., Dashzeveg Bold, Jessie D. Trujillo, David A. Meekins, Cassidy Keating, Konner Cool, Taeyong Kwon, Daniel W. Madden, Bianca L. Artiaga, Velmurugan Balaraman, Ulaankhuu Ankhanbaatar, Batsukh Zayat, Jamie Retallick, Kimberly Dodd, Chungwon J. Chung, Igor Morozov, Natasha N. Gaudreault, Jayme A. Souza-Neto, and Jürgen A. Richt. 2022. 'Experimental Infection of Domestic Pigs with African Swine Fever Virus Isolated in 2019 in Mongolia', *Viruses*, 14: 2698.

Meas, Sochanwattey, Phenjun Mekvichitsaeng, and Yaowaluck Maprang Roshorm. 2021. 'Co-expression of self-cleaved multiple proteins derived from Porcine Reproductive and Respiratory Syndrome Virus by bi-cistronic and tri-cistronic DNA vaccines', *Protein Expression and Purification*, 177: 105763.

Murgia, Maria V, Mark Mogler, Andrea Certoma, Diane Green, Paul Monaghan, David T Williams, Raymond RR Rowland, and Natasha N Gaudreault. 2019. 'Evaluation of an African swine fever (ASF) vaccine strategy incorporating priming with an alphavirus-expressed antigen followed by boosting with attenuated ASF virus', *Archives of Virology*, 164: 359-70.

Neilan, J. G., L. Zsak, Z. Lu, T. G. Burrage, G. F. Kutish, and D. L. Rock. 2004. 'Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection', *Virology*, 319: 337-42.

Netherton, Christopher L., Samuel Connell, Camilla T. O. Benfield, and Linda K. Dixon. 2019. 'The Genetics of Life and Death: Virus-Host Interactions Underpinning Resistance to African Swine Fever, a Viral Hemorrhagic Disease', *Frontiers in Genetics*, 10.

Netherton, Christopher L., Lynnette C. Goatley, Ana Luisa Reis, Raquel Portugal, Rachel H. Nash, Sophie B. Morgan, Lynden Gault, Raquel Nieto, Veronica Norlin, and Carmina

Gallardo. 2019. 'Identification and immunogenicity of African swine fever virus antigens', *Frontiers in Immunology*, 10: 1318.

Nguyen-Thi, Thinh, Linh Pham-Thi-Ngoc, Que Nguyen-Ngoc, Sinh Dang-Xuan, Hu Suk Lee, Hung Nguyen-Viet, Pawin Padungtod, Thuy Nguyen-Thu, Thuy Nguyen-Thi, Thang Tran-Cong, and Karl M. Rich. 2021. 'An Assessment of the Economic Impacts of the 2019 African Swine Fever Outbreaks in Vietnam', *Frontiers in Veterinary Science*, 8.

Obradovic, M. R., L. Corsaut, D. Dolbec, M. Gottschalk, and M. Segura. 2021. 'Experimental evaluation of protection and immunogenicity of Streptococcus suis bacterin-based vaccines formulated with different commercial adjuvants in weaned piglets', *Vet Res*, 52: 133.

Onisk, D. V., M. V. Borca, S. Kutish, E. Kramer, P. Irusta, and D. L. Rock. 1994. 'Passively Transferred African Swine Fever Virus Antibodies Protect Swine against Lethal Infection', *Virology*, 198: 350-54.

Pastoret, S., H. Ameels, F. Bossiroy, A. Decreux, F. De Longueville, A. Thomas, and D. Desmecht. 2012. 'Detection of disease resistance and susceptibility alleles in pigs using oligonucleotide microarray hybridization', *J Vet Diagn Invest*, 24: 479-88.

Pikalo, Jutta, Luca Porfiri, Valerij Akimkin, Hanna Roszyk, Katrin Pannhorst, Richard Thiga Kangethe, Viskam Wijewardana, Julia Sehl-Ewert, Martin Beer, Giovanni Cattoli, and Sandra Blome. 2022. 'Vaccination With a Gamma Irradiation-Inactivated African Swine Fever Virus Is Safe But Does Not Protect Against a Challenge', *Frontiers in Immunology*, 13.

Pornthummawat, Apisit, Quang Lam Truong, Nguyen Thi Hoa, Nguyen Thi Lan, Uda Zahli Izzati, Mathurot Suwanruengsri, Phawut Nueangphuet, Takuya Hirai, and Ryoji Yamaguchi. 2021. 'Pathological lesions and presence of viral antigens in four surviving pigs in African swine fever outbreak farms in Vietnam', *Journal of Veterinary Medical Science*, 83: 1653-60.

Proudfoot, Chris, Simon Lillico, and Christine Tait-Burkard. 2019. 'Genome editing for disease resistance in pigs and chickens', *Animal Frontiers*, 9: 6-12.

Reiner, Gerald. 2009. 'Investigations on genetic disease resistance in swine—A contribution to the reduction of pain, suffering and damage in farm animals', *Applied Animal Behaviour Science*, 118: 217-21.

Rodriguez-Bertos, A., E. Cadenas-Fernandez, A. Rebollada-Merino, N. Porras-Gonzalez, F. J. Mayoral-Alegre, L. Barreno, A. Kosowska, I. Tome-Sanchez, J. A. Barasona, and J. M. Sanchez-Vizcaino. 2020. 'Clinical Course and Gross Pathological Findings in Wild Boar Infected with a Highly Virulent Strain of African Swine Fever Virus Genotype II', *Pathogens*, 9.

Rowlands, R. J., V. Michaud, L. Heath, G. Hutchings, C. Oura, W. Vosloo, R. Dwarka, T. Onashvili, E. Albina, and L. K. Dixon. 2008. 'African swine fever virus isolate, Georgia, 2007', *Emerg Infect Dis*, 14: 1870-4.

Ruiz-Gonzalvo F, Carnero ME, Bruyel V. 1983. "Immunological responses of pigs to partially attenuated African swine fever virus and their resistance to virulent homologous and heterologous viruses." In *FAO/CEC Expert consultation in African Swine Fever Research*, edited by Wilkinson PJ, pp 206–16. Sardinia.

Ruiz Gonzalvo, F., M. E. Carnero, C. Caballero, and J. Martinez. 1986. 'Inhibition of African swine fever infection in the presence of immune sera in vivo and in vitro', *Am J Vet Res*, 47: 1249-52.

Salguero, F. J. 2020. 'Comparative Pathology and Pathogenesis of African Swine Fever Infection in Swine', *Front Vet Sci*, 7: 282.

Sánchez-Cordón, PJ, M Montoya, AL Reis, and LK Dixon. 2018. 'African swine fever: A re-emerging viral disease threatening the global pig industry', *The Veterinary Journal*, 233: 41-48.

Sehl-Ewert, J., P. Deutschmann, A. Breithaupt, and S. Blome. 2022. 'Pathology of African Swine Fever in Wild Boar Carcasses Naturally Infected with German Virus Variants', *Pathogens*, 11.

Sehl, Julia, Jutta Pikalo, Alexander Schäfer, Kati Franzke, Katrin Pannhorst, Ahmed Elnagar, Ulrike Blohm, Sandra Blome, and Angele Breithaupt. 2020. 'Comparative pathology of domestic pigs and wild boar infected with the moderately virulent African swine fever virus strain "Estonia 2014"', *Pathogens*, 9: 662.

Shaimardanova, A. A., K. V. Kitaeva, Abdrakhmanova, II, V. M. Chernov, C. S. Rutland, A. A. Rizvanov, D. S. Chulpanova, and V. V. Solovyeva. 2019. 'Production and Application of Multicistronic Constructs for Various Human Disease Therapies', *Pharmaceutics*, 11.

Sunwoo, Sun-Young, Daniel Pérez-Núñez, Igor Morozov, Elena Sánchez, Natasha Gaudreault, Jessie Trujillo, Lina Mur, Marisa Nogal, Daniel Madden, Kinga Urbaniak, In Kim, Wenjun Ma, Yolanda Revilla, and Juergen Richt. 2019. 'DNA-Protein Vaccination Strategy Does Not Protect from Challenge with African Swine Fever Virus Armenia 2007 Strain', *Vaccines*, 7: 12.

Tran, X. H., T. T. P. Le, Q. H. Nguyen, T. T. Do, V. D. Nguyen, C. G. Gay, M. V. Borca, and D. P. Gladue. 2021. 'African swine fever virus vaccine candidate ASFV-G-DeltaI177L efficiently protects European and native pig breeds against circulating Vietnamese field strain', *Transbound Emerg Dis*.

Tran, X. H., L. T. T. Phuong, N. Q. Huy, D. T. Thuy, V. D. Nguyen, P. H. Quang, Q. V. Ngôn, A. Rai, C. G. Gay, D. P. Gladue, and M. V. Borca. 2022. 'Evaluation of the Safety Profile of the ASFV Vaccine Candidate ASFV-G-ΔI177L', *Viruses*, 14.

Urbano, Ana Catarina, and Fernando Ferreira. 2022. 'African swine fever control and prevention: an update on vaccine development', *Emerging microbes & infections*, 11: 2021-33.

Xie, Zhenhua, Yingnan Liu, Dongdong Di, Jingyi Liu, Lang Gong, Zongyan Chen, Yao Li, Wanqi Yu, Lu Lv, Qiuping Zhong, Yingying Song, Xinxin Liao, Qingqing Song, Heng Wang, and Hongjun Chen. 2022. 'Protection Evaluation of a Five-Gene-Deleted African Swine Fever Virus Vaccine Candidate Against Homologous Challenge', *Frontiers in Microbiology*, 13.

Zajac, Michelle D., Neha Sangewar, Shehnaz Lokhandwala, Jocelyne Bray, Huldah Sang, Jayden McCall, Richard P. Bishop, Suryakant D. Waghela, Rakshith Kumar, Tae Kim, and Waithaka Mwangi. 2022. 'Adenovirus-Vectored African Swine Fever Virus pp220 Induces Robust Antibody, IFN-γ, and CTL Responses in Pigs', *Frontiers in Veterinary Science*, 9.

Table 3.1 Ad5-ASFV constructs.

Construct	ASFV Antigens	Construct	ASFV Antigens
Ad5-01	CP2475 (p220) (1-1256aa)	Ad5-22	B962L, H233R, E75, H171R
Ad5-02	CP2475 (p220) (1190-2476aa)	Ad5-23	C962R, MGF505-3R, C147L
Ad5-03	p72, p15, B602L,	Ad5-24	A859L, B318L, B169L
Ad5-04	p62, p32, p54, EP153R, p10	Ad5-25	C717R, H359L, F317L
Ad5-05	K205R, A104R, EP402R, A151R, B119L, K196R, BA71V-CP80R	Ad5-26	MGF505-10R, B475L, MGF360-4L
Ad5-06	B438L, R298L, NP419L, K145R	Ad5-27	MGF505-2R, B407L, MGF360-13L, E111R
Ad5-07	B385R, CP312R, F165R	Ad5-28	K421R, EP424R, D339L, S183L
Ad5-08	F778R, S273R	Ad5-29	EP296R, B263R, C257L, I243L, A179L, B117L
Ad5-09	NP868R, H339R	Ad5-30	Q706L, D205R, E184L, I177L, C84L, L60L, DP60R
Ad5-10	I329L, A224L, MGF505-6R	Ad5-31	MGF505-10R, MGF300-1L, E199L, DP96R, EP84R, DP79L, DP71L, X69R
Ad5-11	C475L, B354L, D345L, H124R	Ad5-32	MGF505-11L, I267L, I196L, C129R, MGF100-1R, MGF110-3L
Ad5-12	C315R, MGF505-7R, MGF300-1L	Ad5-33	MGF505-7R, E301R, MGF110-1L, I215L, O174L
Ad5-13	MGF360-6L, MGF360-12L, MGF300-4L, D205R		
Ad5-14	MGF360-4L, MGF360-15R, A238L, H240R, B125R	Ad5-34	QP509L, QP383R, MGF360-16R, I226R
Ad5-15	NP1450L	Ad5-35	MGF505-4R, MGF360-9L, MGF360-8L, A240L
Ad5-16	G1340L	Ad5-36	MGF505-9R, EP364R, E248R, A137R, D129L
Ad5-17	M1249L, A118R, 173R	Ad5-37	MGF505-5R-MGF360-3L-MGF360-18R-DP238L-DP63R
Ad5-18	EP1242L, I9R, C62L,	Ad5-38	M448R-E423R-MGF505-1R
Ad5-19	G1211R, I7L, L83L,	Ad5-39	MGF505-6R-CP123L-I10L-I8L-MGF110-12L-I9R-L11L-MGF110-7L-MGF110-2L
Ad5-20	P1192R, EP152R, E66L,	Ad5-40	MGF360-4L-MGF360-2L-MGF360-11L-MGF110-9L
Ad5-21	D1133L, E165R, C122R	Ad5-41	MGF505-5R, DP238L
Ad5-22	F1055L, E146L, I8L		

Table 3.2 ASFV Antigen expression.

	Construct	% Positive cells
	None (negative control)	0.07
	Ad5-p62 (positive control)	2.97
Low ASFV antigen Expression	Ad5-05	0.53
	Ad5-13	0.75
	Ad5-34	1.53
	Ad5-03	1.71
	Ad5-01	1.92
Medium ASFV antigen Expression	Ad5-04	2.38
	Ad5-29	2.87
	Ad5-11	3.08
	Ad5-10	3.14
	Ad5-18	3.29
	Ad5-39	3.44
High ASFV antigen Expression	Ad5-21	4.55
	Ad5-09	7.78
	Ad5-19	8.92

Values are expressed as a percent of the total parental cell population as measured using ASFV convalescent serum by the Ad5-ASFV constructs using flow cytometric analyses.

Table 3.3 Immunization protocol.

Groups	Pig ID	Dose per Construct	Total Dose per Pig	Adjuvant
CS* Group 1	6908 6886	none		
Luc-ISA-201	6894 6909 6896 6897 6888	4.1×10^{11} ifu	4.1×10^{11} ifu	Montanide ISA-201™
CS* Group 2	6915 6901	none		
ASFV-No Adjuvant	6898 6903 6893 6892 6900	1×10^{10} ifu	4.1×10^{11} ifu	No Adjuvant
CS* Group 3	6887 6906	none		
ASFV-ISA-201	6884 6885 6883 6902 6910	1×10^{10} ifu	4.1×10^{11} ifu	Montanide ISA-201™
CS* Group 4	6912 6907	none		
ASFV-BioMize	6913 6891 6890 6899 6904	1×10^{10} ifu	4.1×10^{11} ifu	BioMize

Gray shading indicates no inoculum or adjuvant received and direct intramuscular challenge.
*CS: Contact Spreaders

Table 3.4 Clinical Score Rubric.

Parameter	Score	Description
Breathing	0	Normal breathing, no coughing/sneezing
	1	Slightly labored breathing, mild coughing/sneezing
	2	Labored, increased rate, moderate coughing/sneezing
	3	Highly labored, rapid rate, frequent coughing/sneezing
Liveliness	0	Attentive (curious, stands up immediately)
	1	Slightly reduced (stands up hesitantly but without help)
	2	Tired, get up only when forced to, lies down again
	3	Dormant, will not stand up
Walking	0	Well-coordinated movements
	1	Stiffness while standing up, afterward normal
	2	Distinct lameness, able to walk
	3	Massive lameness, unable to walk
Skin	0	Evenly light pink, flat hair coat
	1	Reddened skin areas
	2	Purple/blue discolored areas (ears, legs)
	3	Black-red discoloration of the skin
Eyes/conjunctiva	0	Light pink
	1	Reddened, clear secretion
	2	Highly inflamed, turbid secretion
	3	Highly inflamed, secretions prevent eyes from opening
Body Condition [detection of ribs, back bones, hip bones, and pin bones]	0	Ideal, smooth, and rounded with an even feel
	1	Mildly Thin, easy to feel with pressure
	2	Moderately Thin, easy to feel with pressure, and beginning to see
	3	Emaciated, obvious/easy to see
Feces	0	normal
	1	None
	2	Diarrhea
	3	Mucus or bloody diarrhea

Maximum score: 21

Table 3.5 Terminal clinical scores.

Groups	Pig ID	Adjuvant	Terminal Clinical Score	Group Mean Clinical Score
Contact Spreaders	6908	none	1	3.6
	6886		6	
	6915		4	
	6901		4	
	6887		4	
	6906		4	
	6912		3	
	6907		3	
Luc-ISA-201	6894	Montanide ISA-201™	1	1.4
	6909		2	
	6896		1	
	6897		2	
	6888		1	
ASFV-No Adjuvant	6898	No Adjuvant	9	3.4
	6903		2	
	6893		3	
	6892		0	
	6900		3	
ASFV-ISA-201	6884	Montanide ISA-201™	9	3.2
	6885		1	
	6883		1	
	6902		2	
	6910		3	
ASFV-BioMize	6913	BioMize	5	3.2
	6891		1	
	6890		2	
	6899		2	
	6904		6	
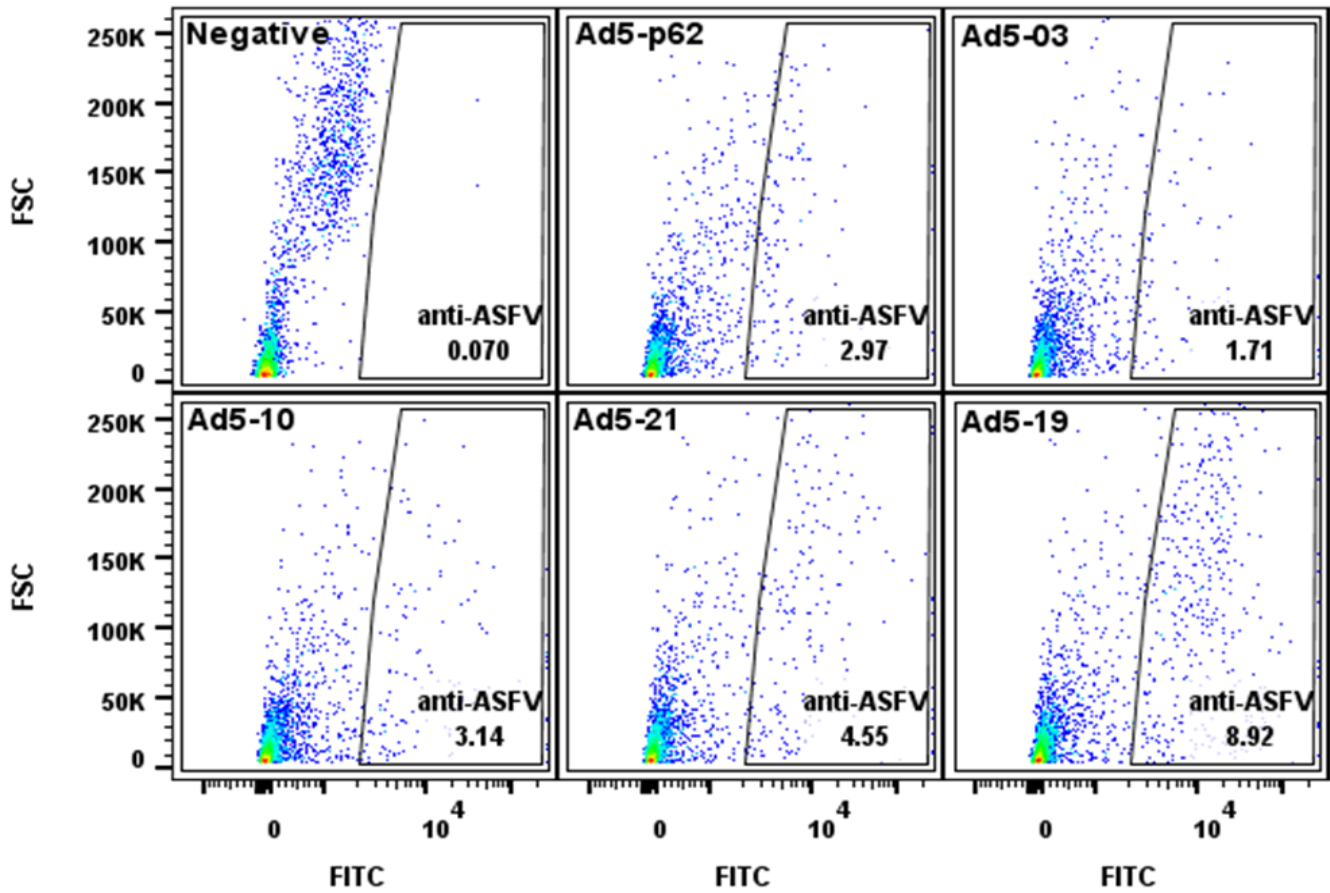
Figure 3.1 Flow cytometric analyses of protein expression by the Ad5-constructs.

Dot plot presentation of forward scatter (FSC) versus FITC for HEK293A cells infected with representative low (Ad5-03), medium (Ad5-10 and Ad5-21), and high (Ad5-19) antigen expressing Ad5-ASFV constructs followed by staining using ASFV convalescent serum. Ad5-p62 served as the positive control and noninfected cells served as the negative control. Protein expression by representative Ad5-ASFV constructs is presented in Table 3.1.
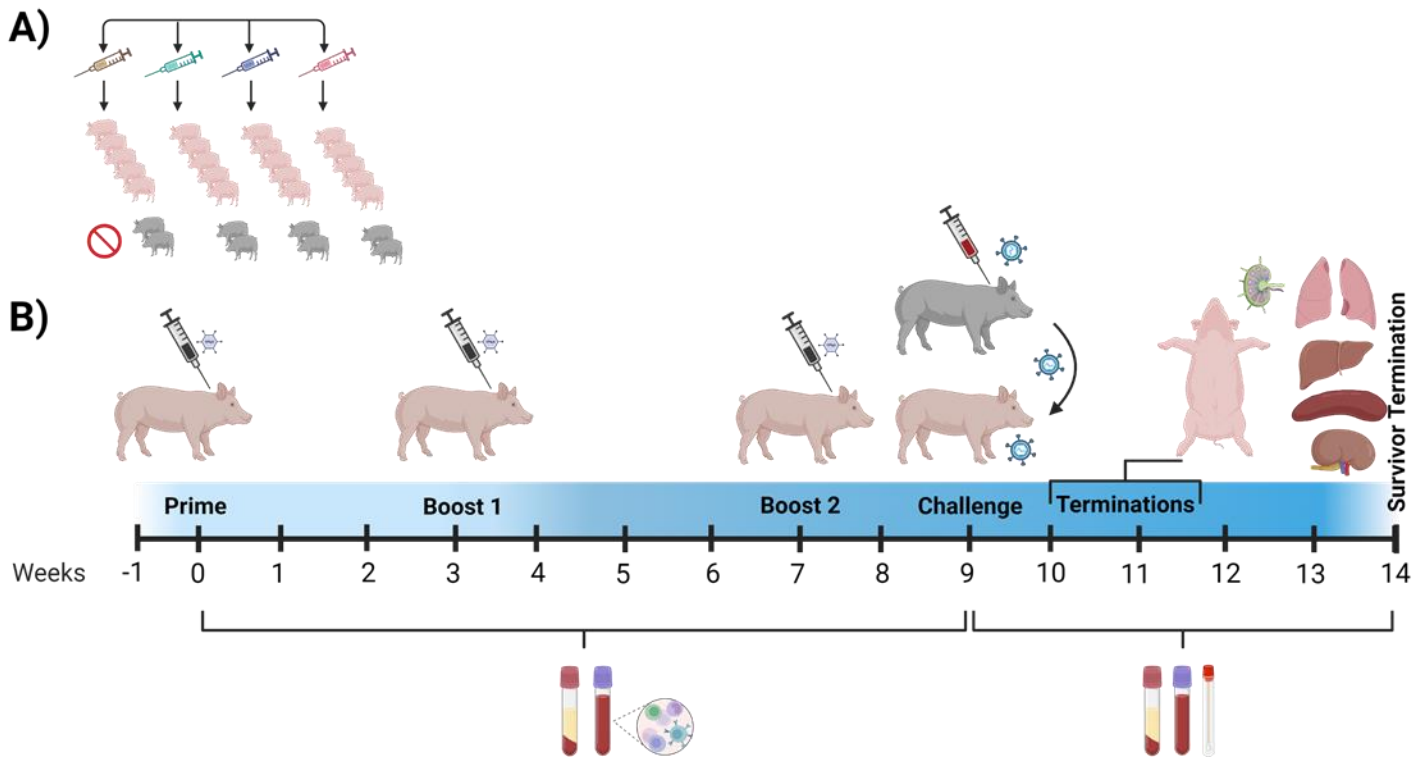
Figure 3.2 Study design and timeline.

(**A**) Pigs were randomly assigned to four treatment groups: 1) Ad5-Luciferase plus Montanide ISA-201™ adjuvant (Luc-ISA-201); 2) Ad5-ASFV cocktail (ASFV-No Adjuvant); 3) Ad5-ASFV cocktail plus Montanide ISA-201™ adjuvant (ASFV-ISA-201); or 4) Ad5-ASFV cocktail plus BioMize adjuvant (ASFV-BioMize). (**B**) Animals were acclimatized for one week and baseline serum and PBMC samples were collected with weekly collections thereafter. Pigs in all the treatment groups were primed after acclimatization followed by boosting at weeks 3 and 7 post-priming as shown in Table 2. The pigs were challenged by contact with comingled ASFV-infected naïve pigs at week 9. Blood and nasal swabs were collected until termination. The survivor from the ASFV-No Adjuvant group was terminated 37 days post-challenge. Created with BioRender.com (accessed on 9 March 2023).
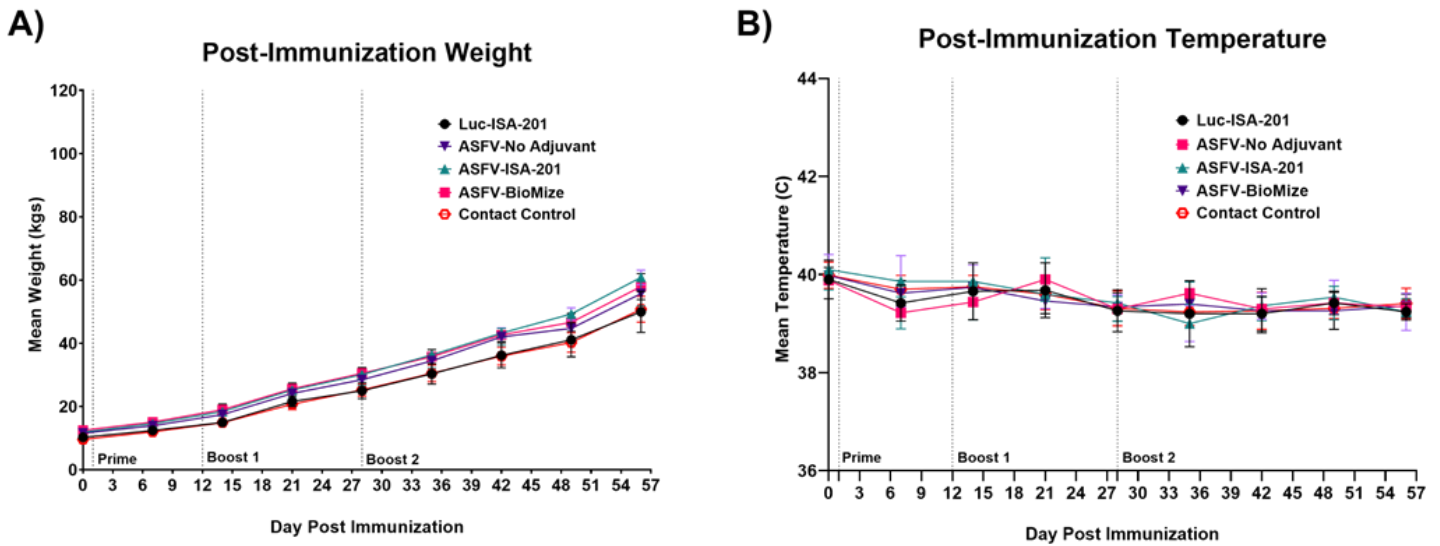
Figure 3.3 Post-immunization body temperatures and weights.

(**A**) Weekly weight gain and (**B**) temperature change post-immunization. Average weekly weight gain and temperature change as clinical indicators of immunization effects for each group were plotted as group averages. Prime-boost immunizations are indicated on the days of administration. Contact Spreaders were not immunized and are indicated with a red open octagon.
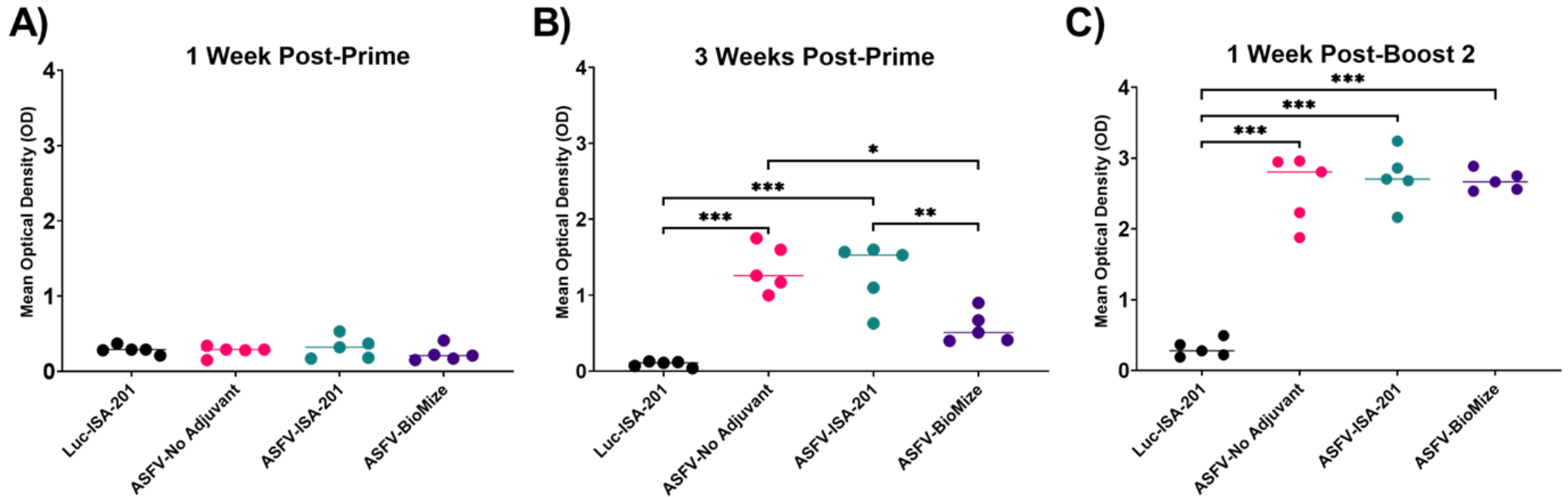
Figure 3.4 Ad5-ASFV elicited antibody responses.

Following priming and boosting, recombinant p62 antigen was used to track IgG responses by ELISA using sera from blood collected: (**A**) one-week post-priming; (**B**) three weeks post-priming; or (**C**) one week after the second boost. Mean responses for the treatment groups are denoted by bars and statistically significant differences between the groups are denoted by asterisks, *p=0.002, **p=0.005, ***p< 0.0001, any groups not denoted with an asterisk were not statistically significant.
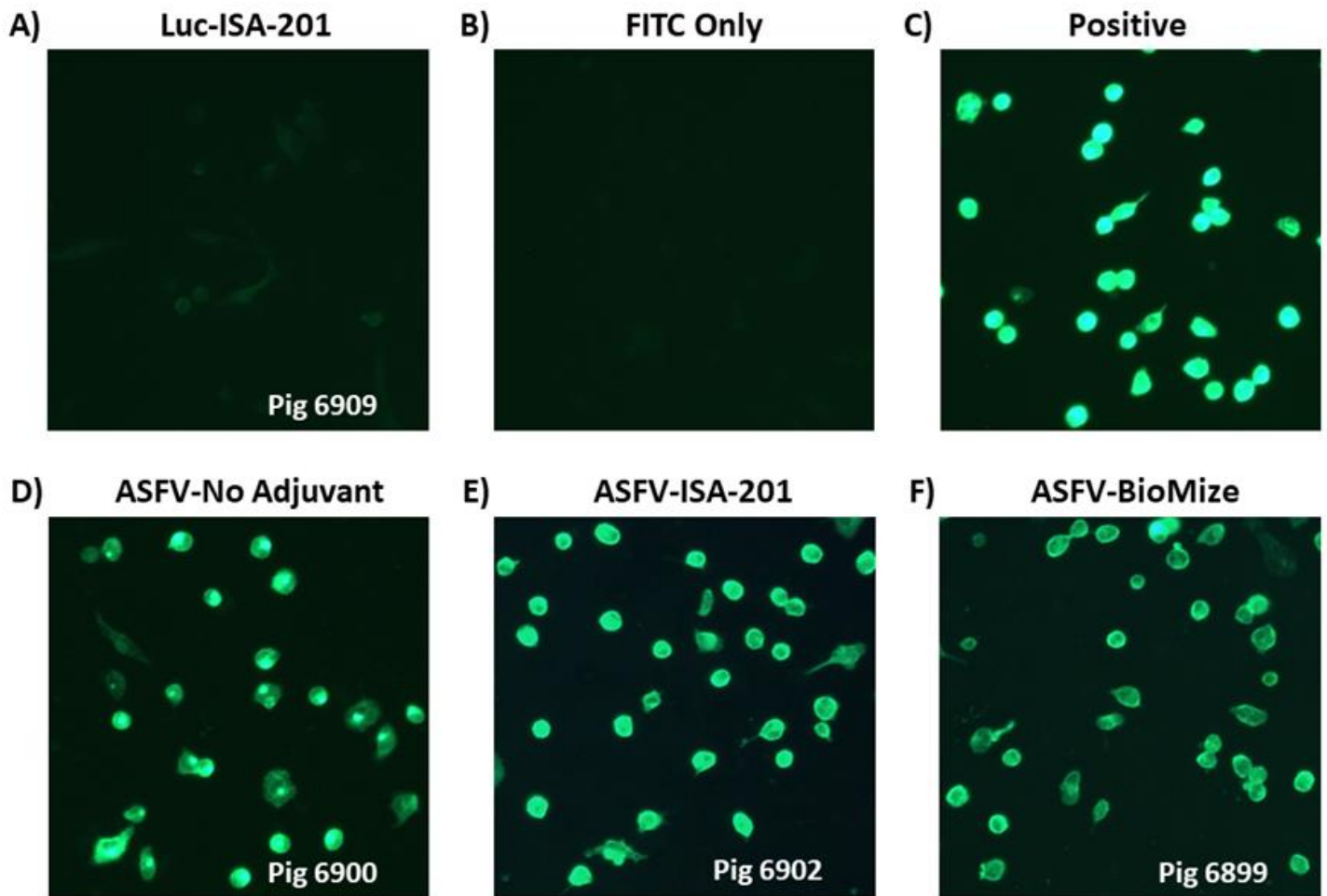
Figure 3.5 Antibodies elicited by Ad5-ASFV recognized ASFV-infected cells.

Antibodies induced by the Ad5-ASFV formulations recognized ASFV-infected swine PBMCs as judged by IFA using sera from blood collected one-week after the second boost. Data for representative pigs from the negative control and the vaccinees is shown.
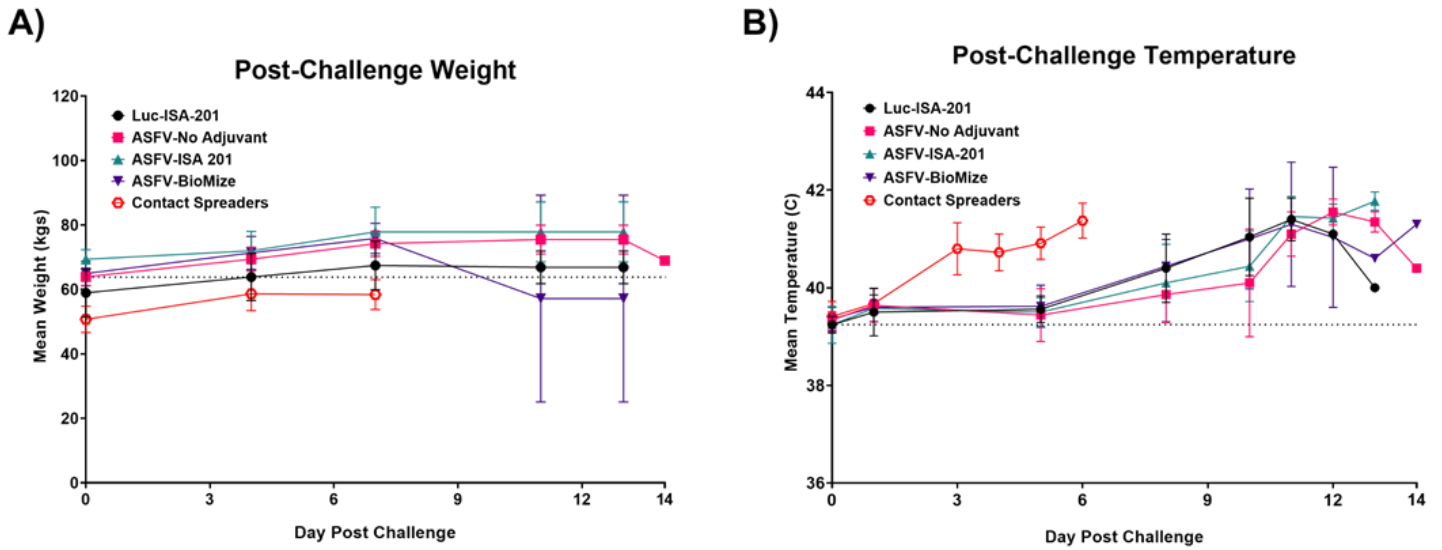
Figure 3.6 Post-challenge body temperatures and weights.

Post-challenge weights (**A**) and temperatures (**B**) were collected bi-weekly and plotted as group means. Contact Spreaders were not immunized and are indicated with a red open octagon. ASFV-No Adjuvant 14 DPC weight indicates the surviving animal only. Parameters were monitored as a clinical indicator of ASFV infection and disease progression for each group.
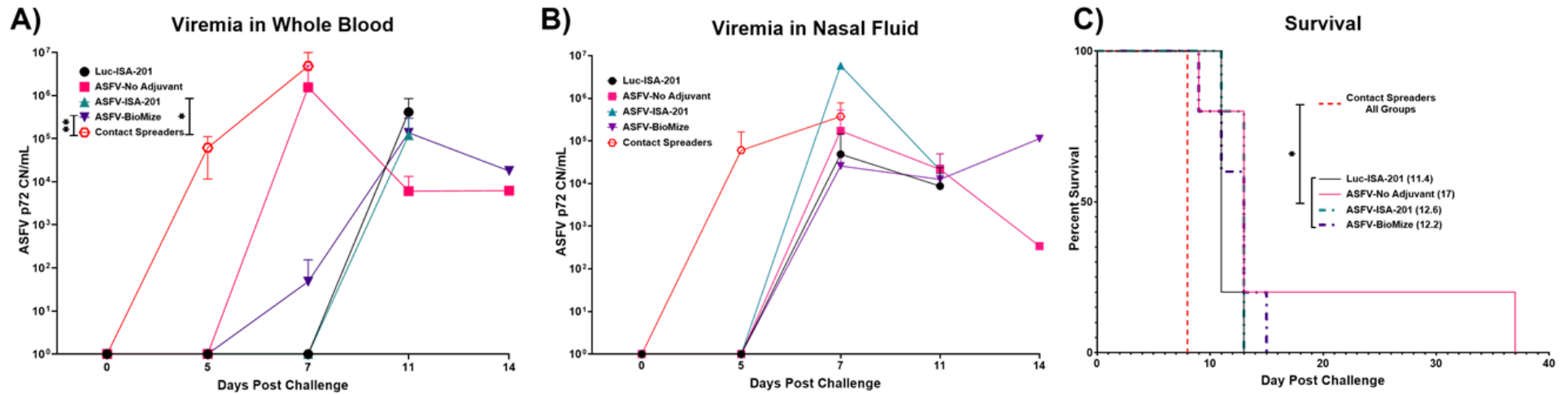
Figure 3.7 . Survival and viremia post-challenge.

Viremia (CN/mL) in (**A**) blood and (**B**) nasal swabs from contact non-immunized spreaders on days 0, 5, and 7; and blood from the treatment groups on days 0, 5, 7, 11, and 14 which includes the day of euthanasia for the animals that developed severe clinical disease. The mean viremia was significantly different between the contact spreaders and Montanide ISA-201 (*p=0.417) and BioMize (**p=0.0439) treatment groups, any groups not denoted with an asterisk were not statistically significant and the survivor is not represented here. (**C**) Pigs were monitored and euthanized based on the severity of the disease. All the non-immunized contact spreaders (CS) are shown in red with dashed lines. Average survival is indicated in parenthesis for each group

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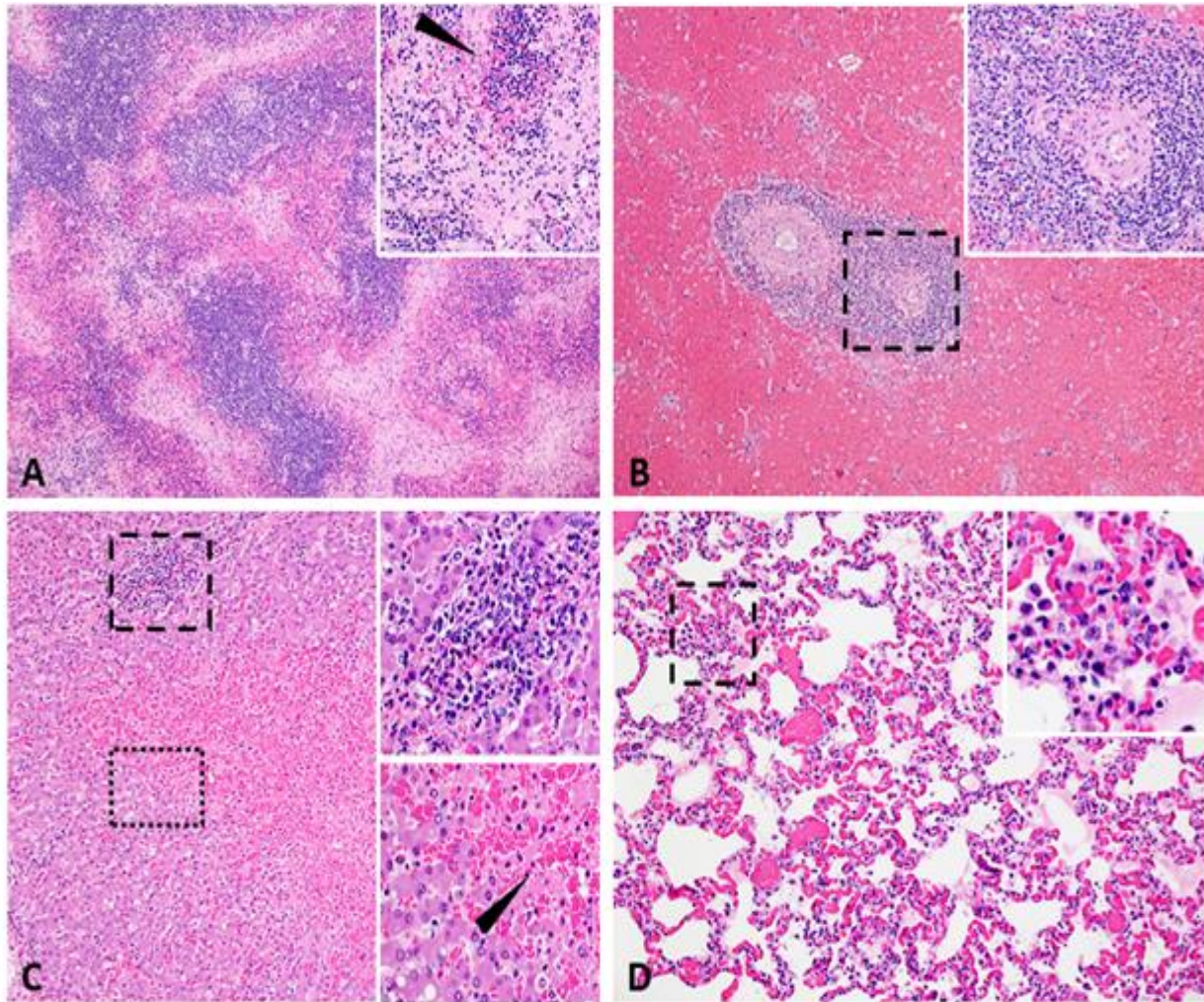
Figure 3.8 Lesions characteristic of severe acute ASF.

Representative histopathology of acute ASF in pigs infected intramuscularly with ASFV strain Georgia 2007/1: Contact Spreaders 6889 (**A-C**) and 6901 (**D**). (**A**) Severe lymphoid depletion, lymphocytolysis, and follicular loss accompanied by edema, fibrin, and hemorrhage. (**Insert A**) Vascular fibrinous degeneration (arrow), lymphocytolysis, edema, and hemorrhage (submandibular lymph node). (**B**) Marked splenic lymphoid necrosis and loss of periarteriolar sheaths with by marked diffuse red pulp hemorrhage. (**Insert B**) Macrophages, plasma cells, degenerate lymphocytes, eosinophils, and fibrin mats remain as periarteriolar sheaths (spleen). (**C**) Severe centrilobular necrosis of hepatocytes and marked congestion of sinusoids accompanied by moderate non-suppurative inflammation in portal regions. (**Upper Insert C**) Inflammation in portal regions consists of macrophages, degenerative lymphocytes, and lesser numbers of plasma cells and eosinophils. The inflammation extends beyond the portal plate into adjacent hepatic cords forming small clusters associated with hepatocyte necrosis. (**Lower insert C**) Junction of centrilobular hepatocyte necrosis and viable hepatocytes (liver). Necrotic hepatocytes are shrunken with hypereosinophilic cytoplasm and pyknotic nuclei, arranged in irregular cords (arrow). (**D**) Alveolar septa are congested and variably thickened by mononuclear cell infiltrates. Alveolar spaces irregularly contain aggregates of large foamy macrophages and degenerate inflammatory cells, fibrin, and edema (**Insert D**) (lung).

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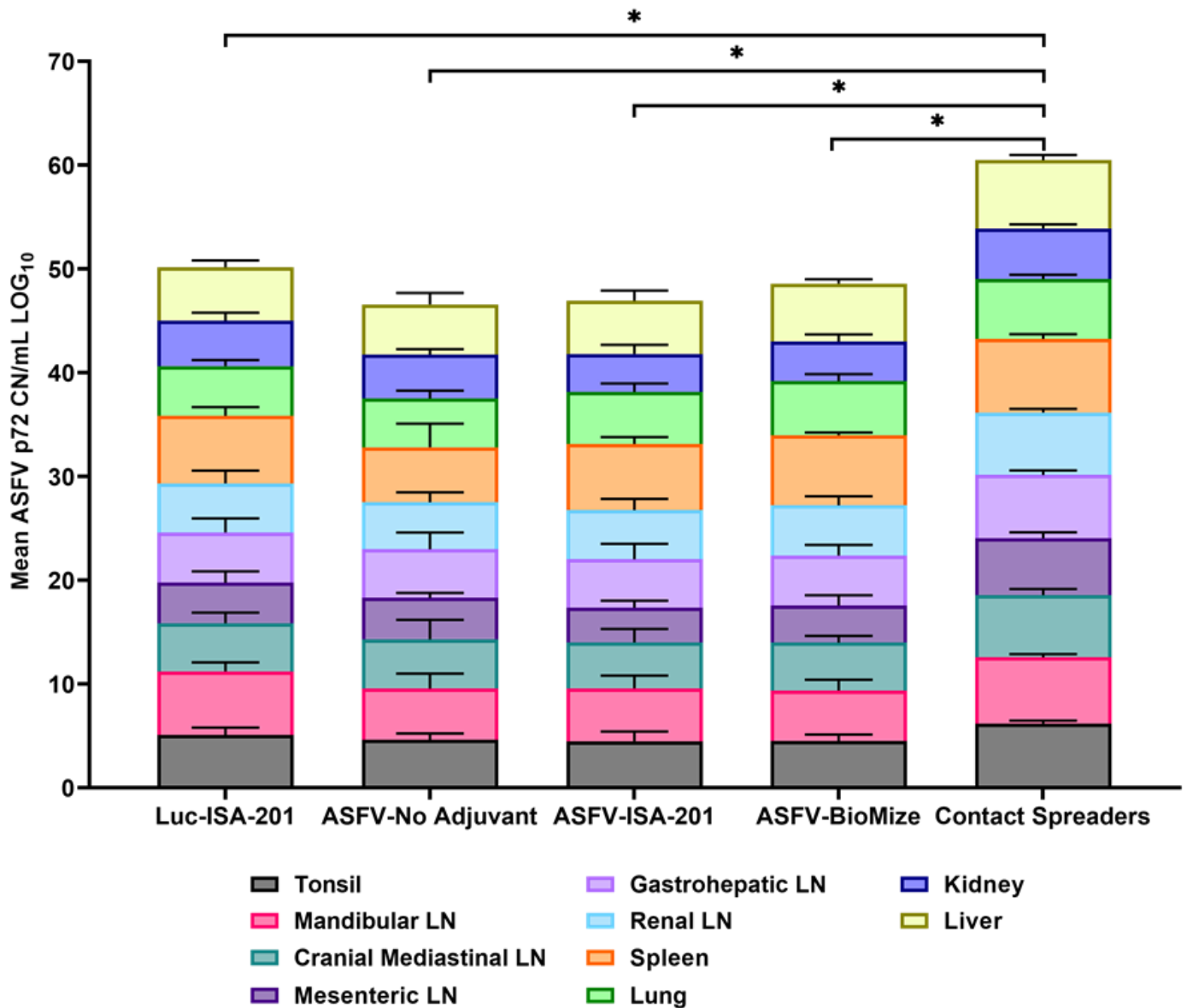
Figure 3.9 Viremia in infected tissue.

Tissue CN/mL was quantified by qPCR from the samples collected on the day of euthanasia for the vaccinees and Contact Spreaders. Respective tissue types are denoted by separate colors. Each plotted value is stacked and represents the mean for each tissue. The Log base 10 was calculated for each mean CN/mL and plotted in a stacked bar graph. Total mean ASFV p72 genomic DNA was statistically significant different between the contact spreaders and all other groups denoted by asterisks, *p< 0.0001, any groups not denoted with an asterisk were not statistically significant.
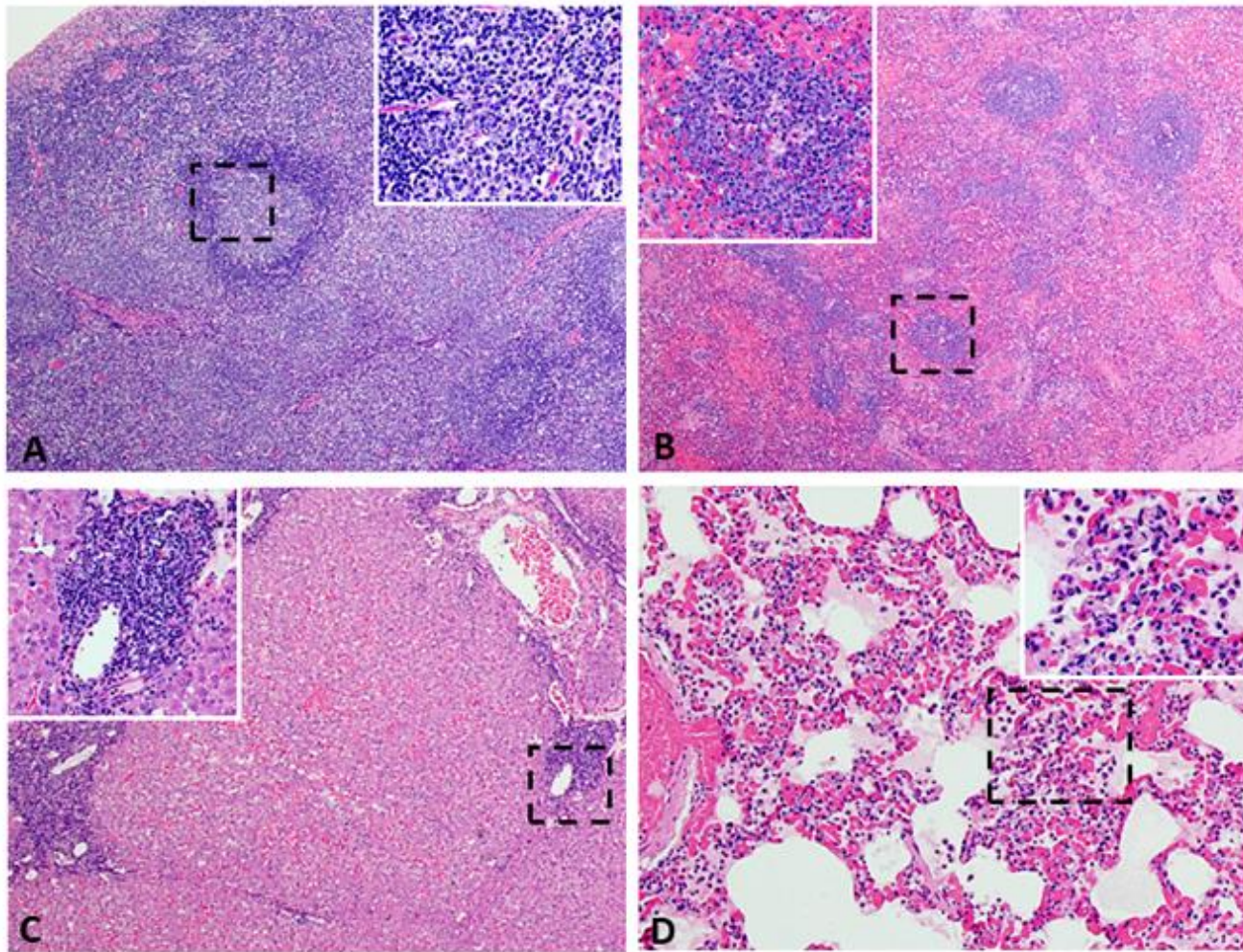
Figure 3.10 Lesions characteristic of moderate subacute ASF.

Representative ASFV histopathology in pigs vaccinated with Ad5-ASFV constructs and then challenged by contact. Data from two representative pigs [6903: ASFV-No Adjuvant (**A-C**) and 6904: ASFV- BioMize (**D**)] are shown. (**A**) Minimal lymphoid depletion with variable lymphocytolysis (**Insert A**) and expansion of cords and sinuses with large foamy macrophages, plasma cells, and eosinophils (submandibular lymph node). (**B**) Mild to moderate splenic lymphoid necrosis and thinning of periarteriolar sheaths accompanied by multifocal hemorrhage of the red pulp. (**Insert B**) Lymphocytolysis and necrotic cellular debris, macrophages, plasma cells, and eosinophils (**Insert B**) (spleen). (**C**) Moderate to severe, non-suppurative periportal hepatitis. Inflammation in portal regions consists of macrophages, degenerative lymphocytes, plasma cells, and lesser numbers of eosinophils (**Insert C**). (**D**) Alveolar septa are markedly congested and variably thickened by mononuclear cell infiltration and cellular debris. Alveolar spaces multifocally contain aggregates of large foamy macrophages and degenerate inflammatory cells, fibrin, and edema (**Insert D**) (lung).

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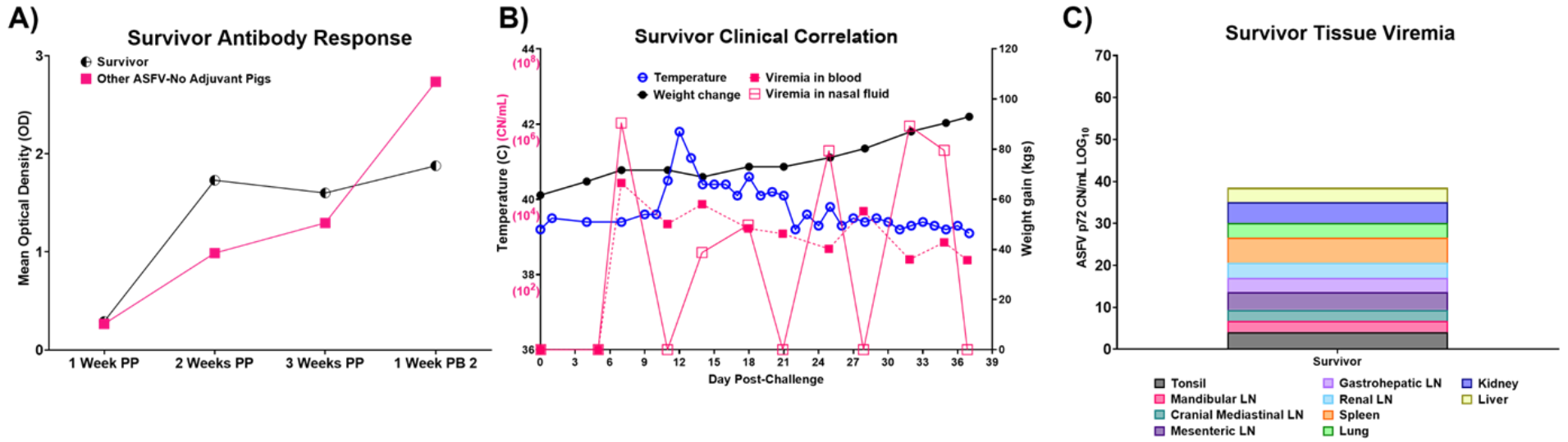
Figure 3.11 Ad5-ASFV-vaccinee that survived.

(**A**) Antibody response by the lone survivor that was immunized with the Ad5-ASFV without adjuvant. The half-circle indicates the p62-specific IgG responses by the survivor compared to the mean IgG responses (solid square) by the other four group mates that succumbed after challenge. Post-prime is indicated by PP and post-boost by PB. (**B**) Temperature, weight change, and viremia in blood and nasal fluid post-challenge. (**C**) Total tissue viremia expressed in Log base 10 of the CN/mL in a stacked bar graph.
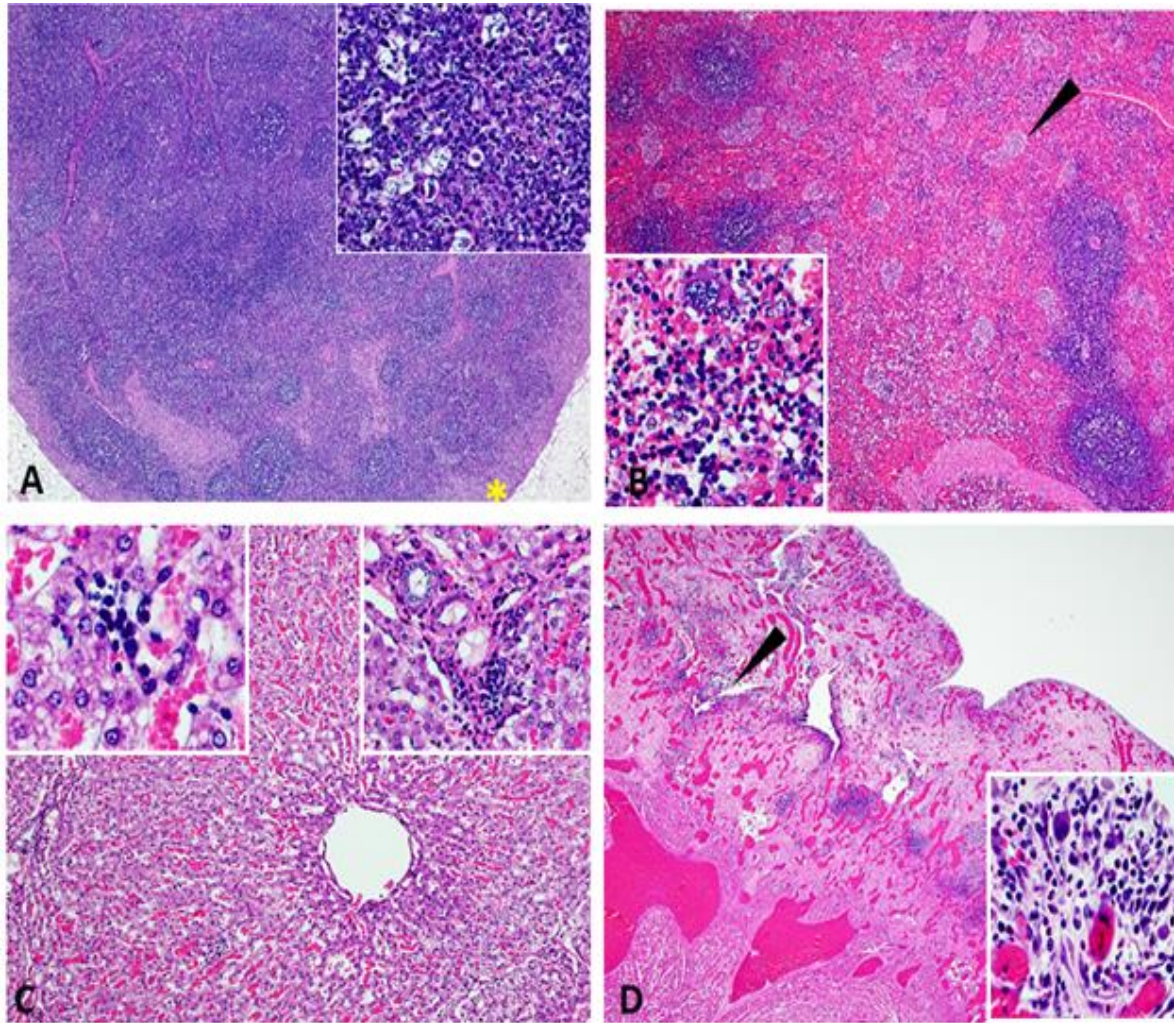
Figure 3.12 Lesions observed in chronic ASF.

Atypical ASF histopathology in the survivor pig (6892) vaccinated with ASFV-No Adjuvant then challenged by contact. (**A**) Moderate lymphoid hyperplasia with the expansion of follicles and cords. Variable yet infrequent lymphocytolysis occurs within germinal centers (**Insert A**) (lymph node). (**B**) Moderate splenic lymphoid hyperplasia with the expansion of periarteriolar sheaths accompanied by hyperplasia of the reticular cells forming nodular bundles dispersed within the red pulp (arrow) and expansion of the red pulp with myeloid cell clusters (**Insert B**) (spleen). (**C**) Moderate, non-suppurative hepatitis with moderate hepatocyte atrophy. Marked thinned hepatic cords are comprised of large irregularly arranged atrophied hepatocytes with foamy to vacuolated cytoplasm. Variable fibrosis occurs among cords and widens portal plates while multifocal, small aggregates of cellular debris, phagocytic cells, lymphocytes, and plasma cells occur among hepatic cords and in portal regions (**left and right Insert B**, respectively) (liver). (**D**) Left ventricular myocardium (lower left) overlaid by markedly thickened and inflamed epicardium. Dense highly vascular fibrotic granulation tissue extends from the myocardium to the outer surface and contains numerous irregular clefts and cavitations with mixed inflammatory cells within these spaces and scattered among the edematous to fibrotic stroma (arrow). The outermost surface of the granulation tissue consists of a delicate network of capillaries, reactive fibroblasts, and numerous, often multinucleate, large histiocytic-like cells admixed with lymphocytes and plasma cells and variable numbers of eosinophils (**Insert D**) (heart).
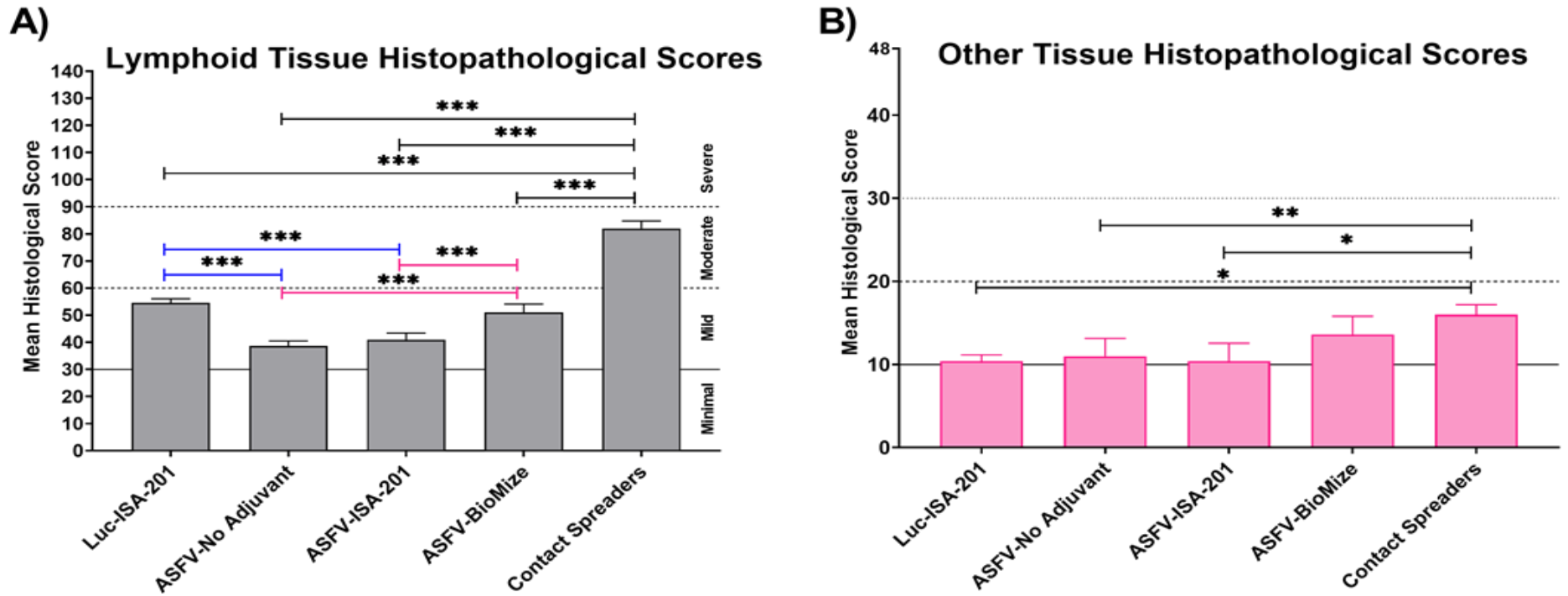
Figure 3.13 Tissue histopathology.

Mean histological scores per group for (**A**) lymphoid tissue and (**B**) other tissues (liver, lungs, kidney, heart). Group comparisons are indicated by *p=0.0014, **p=0.0057, ***p<0.0001, and any groups not denoted with an asterisk were not statistically significant.

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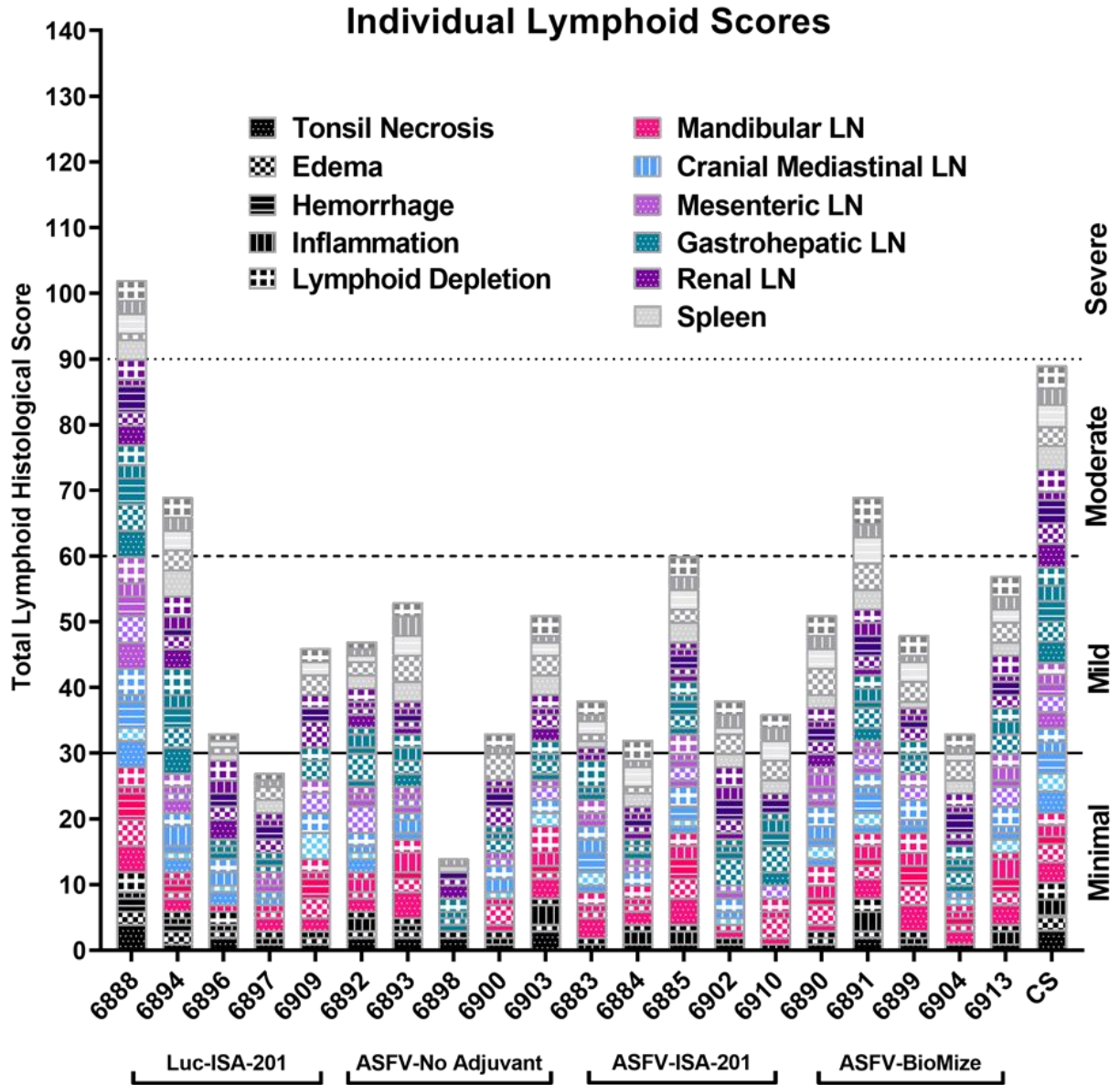
Figure 3.14 Lymphoid tissue histopathology.

Individual lymphoid tissue histological scores for each pig in the treatment and control groups, whereas the Contact Spreaders (CS) are plotted as group mean. Histological scores are stacked and reflected as the overall total per animal.
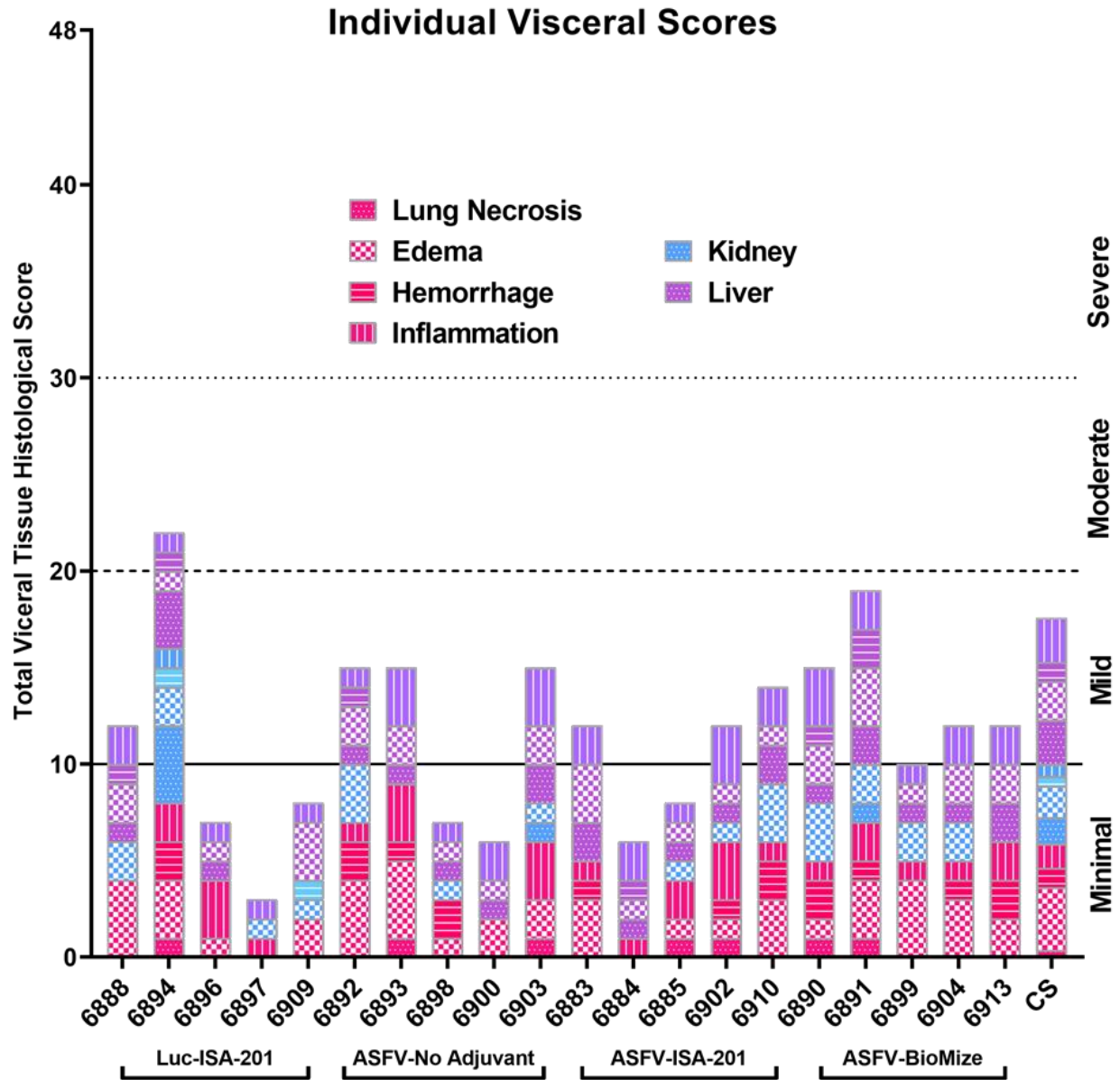
Figure 3.15 Visceral tissue histopathology.

Individual visceral tissue histological scores for each pig in the treatment and control groups, whereas the Contact Spreaders (CS) are plotted as group mean. Histological scores are stacked and reflected as the overall total per animal.

Chapter 4 - Conclusion

There is no safe and efficacious treatment for ASFV and thus swine production is at risk. Lack of knowledge needed to inform development of a safe vaccine has contributed to a significant delay in achieving this goal (Dixon et al. 2020). Additionally, due to its Federal Select Agent status, difficulties in performing efficacy studies are also a consideration in this shortcoming. Traditionally, antibody and neutralization responses have been one of the immunological measures for vaccination success. However, confounding studies for ASFV have supported both disease enhancement and the potential protective role of ASFV-specific IgG- antibodies (Neilan et al. 2004; Pérez-Núñez et al. 2019; Zhang et al. 2021; Zsak et al. 1993). Immunogenicity studies looking specifically at the CD8+ CTL response have shown utility in the identification of ASFV antigens that induce a strong cellular immune response without the disease enhancement effects and are hypothesized to play a key role in ASF clinical disease reduction and prevention (Lokhandwala et al. 2017; Afonso et al. 2004; Argilaguet et al. 2012; Childerstone et al. 1998; Lokhandwala et al. 2016; Bosch-Camos et al. 2021; Netherton, Goatley, Reis, Portugal, Nash, Morgan, Gault, Nieto, Norlin, Gallardo, et al. 2019; Zhu et al. 2019). Prototype ASFV immunogens show great promise in the protective efficacy elicited using live-attenuated versions of the virus, specifically gene-deletion mutants (Borca et al. 2021; Tran et al. 2022). Some studies have also correlated a robust CTL response to this success (Attreed et al. 2022; Franzoni et al. 2022; Schäfer et al. 2022). Safety is of utmost importance and despite the protective results achieved using virus attenuation, vaccine-related side effects or reversion are clear drawbacks of this vaccine development approach as proven by the recent failure of the NAVET-ASFVAC commercial vaccine. Thus, live-vectored subunit vaccine technology warrants further development as this delivery method has no chance of reversion to virulence while inducing

comparably robust cellular immune results (Netherton, Goatley, Reis, Portugal, Nash, Morgan, Gault, Nieto, Norlin, and Gallardo 2019; Lokhandwala et al. 2016; Lokhandwala et al. 2017; Zajac et al. 2022). Hence, the three main gaps in the field of ASFV vaccine immunology are: 1) lack of knowledge regarding correlates of protection; 2) identification of protective antigens; and 3) identification of an effective antigen delivery vector capable of inducing protective immunity in pigs. Addressing these limitations is expected to result in development of a safe and DIVA-compatible ASFV vaccine for protection of domestic pigs and wild boar and thereby increase productivity of the swine industry.

4.1 References:

Afonso, C. L., M. E. Piccone, K. M. Zaffuto, J. Neilan, G. F. Kutish, Z. Lu, C. A. Balinsky, T. R. Gibb, T. J. Bean, L. Zsak, and D. L. Rock. 2004. 'African swine fever virus multigene family 360 and 530 genes affect host interferon response', *J Virol*, 78: 1858-64.

Argilaguet, Jordi M., Eva Pérez-Martín, Miquel Nofrarías, Carmina Gallardo, Francesc Accensi, Anna Lacasta, Mercedes Mora, Maria Ballester, Ivan Galindo-Cardiel, Sergio López-Soria, José M. Escribano, Pedro A. Reche, and Fernando Rodríguez. 2012. 'DNA Vaccination Partially Protects against African Swine Fever Virus Lethal Challenge in the Absence of Antibodies', *PLoS One*, 7: e40942.

Attreed, Sarah E., Christina Silva, Sophia Abbott, Elizabeth Ramirez-Medina, Nallely Espinoza, Manuel V. Borca, Douglas P. Gladue, and Fayna Diaz-San Segundo. 2022. 'A Highly Effective African Swine Fever Virus Vaccine Elicits a Memory T Cell Response in Vaccinated Swine', *Pathogens*, 11: 1438.

Borca, Manuel V., Elizabeth Ramirez-Medina, Ediane Silva, Elizabeth Vuono, Ayushi Rai, Sarah Pruitt, Nallely Espinoza, Lauro Velazquez-Salinas, Cyril G. Gay, and Douglas P. Gladue. 2021. 'ASFV-G-ΔI177L as an Effective Oral Nasal Vaccine against the Eurasia Strain of Africa Swine Fever', *Viruses*, 13: 765.

Bosch-Camos, L., E. Lopez, M. J. Navas, S. Pina-Pedrero, F. Accensi, F. Correa-Fiz, C. Park, M. Carrascal, J. Dominguez, M. L. Salas, V. Nikolin, J. Collado, and F. Rodriguez. 2021. 'Identification of Promiscuous African Swine Fever Virus T-Cell Determinants Using a Multiple Technical Approach', *Vaccines (Basel)*, 9.

Childerstone, A., H. Takamatsu, H. Yang, M. Denyer, and R. M. Parkhouse. 1998. 'Modulation of T cell and monocyte function in the spleen following infection of pigs with African swine fever virus', *Vet Immunol Immunopathol*, 62: 281-96.

Dixon, Linda K., Karl Stahl, Ferran Jori, Laurence Vial, and Dirk U. Pfeiffer. 2020. 'African Swine Fever Epidemiology and Control', *Annual Review of Animal Biosciences*, 8: 221-46.

Franzoni, Giulia, Susanna Zinellu, Tania Carta, Chiara Grazia De Ciucis, Floriana Fruscione, Antonio Anfossi, Mauro Ledda, Simon P. Graham, Silvia Dei Giudici, Elisabetta Razzuoli, and Annalisa Oggiano. 2022. 'Analyses of the Impact of Immunosuppressive Cytokines on Porcine Macrophage Responses and Susceptibility to Infection to African Swine Fever Viruses', *Pathogens*, 11: 166.

Lokhandwala, S., S. D. Waghela, J. Bray, C. L. Martin, N. Sangewar, C. Charendoff, R. Shetti, C. Ashley, C. H. Chen, L. R. Berghman, D. Mwangi, P. J. Dominowski, D. L. Foss, S. Rai, S. Vora, L. Gabbert, T. G. Burrage, D. Brake, J. Neilan, and W. Mwangi. 2016. 'Induction of Robust Immune Responses in Swine by Using a Cocktail of Adenovirus-Vectored African Swine Fever Virus Antigens', *Clin Vaccine Immunol*, 23: 888-900.

Lokhandwala, S., S. D. Waghela, J. Bray, N. Sangewar, C. Charendoff, C. L. Martin, W. S. Hassan, T. Koynarski, L. Gabbert, T. G. Burrage, D. Brake, J. Neilan, and W. Mwangi. 2017. 'Adenovirus-vectored novel African Swine Fever Virus antigens elicit robust immune responses in swine', *PLoS One*, 12: e0177007.

Neilan, J. G., L. Zsak, Z. Lu, T. G. Burrage, G. F. Kutish, and D. L. Rock. 2004. 'Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection', *Virology*, 319: 337-42.

Netherton, C. L., L. C. Goatley, A. L. Reis, R. Portugal, R. H. Nash, S. B. Morgan, L. Gault, R. Nieto, V. Norlin, C. Gallardo, C. S. Ho, P. J. Sanchez-Cordon, G. Taylor, and L. K. Dixon. 2019. 'Identification and Immunogenicity of African Swine Fever Virus Antigens', *Front Immunol*, 10: 1318.

Netherton, Christopher L., Lynnette C. Goatley, Ana Luisa Reis, Raquel Portugal, Rachel H. Nash, Sophie B. Morgan, Lynden Gault, Raquel Nieto, Veronica Norlin, and Carmina Gallardo. 2019. 'Identification and immunogenicity of African swine fever virus antigens', *Frontiers in Immunology*, 10: 1318.

Pérez-Núñez, Daniel, Sun-Young Sunwoo, Elena G. Sánchez, Nicholas Haley, Raquel García-Belmonte, Marisa Nogal, Igor Morozov, Daniel Madden, Natasha N. Gaudreault, Lina Mur, Vinay Shivanna, Juergen A. Richt, and Yolanda Revilla. 2019. 'Evaluation of a viral DNA-protein immunization strategy against African swine fever in domestic pigs', *Veterinary Immunology and Immunopathology*, 208: 34-43.

Schäfer, Alexander, Giulia Franzoni, Christopher L. Netherton, Luise Hartmann, Sandra Blome, and Ulrike Blohm. 2022. 'Adaptive Cellular Immunity against African Swine Fever Virus Infections', *Pathogens*, 11: 274.

Tran, X. H., L. T. T. Phuong, N. Q. Huy, D. T. Thuy, V. D. Nguyen, P. H. Quang, Q. V. Ngôn, A. Rai, C. G. Gay, D. P. Gladue, and M. V. Borca. 2022. 'Evaluation of the Safety Profile of the ASFV Vaccine Candidate ASFV-G-ΔI177L', *Viruses*, 14.

Zajac, Michelle D., Neha Sangewar, Shehnaz Lokhandwala, Jocelyne Bray, Huldah Sang, Jayden McCall, Richard P. Bishop, Suryakant D. Waghela, Rakshith Kumar, Tae Kim, and Waithaka Mwangi. 2022. 'Adenovirus-Vectored African Swine Fever Virus pp220 Induces Robust Antibody, IFN-γ, and CTL Responses in Pigs', *Frontiers in Veterinary Science*, 9.

Zhang, G., W. Liu, Z. Gao, S. Yang, G. Zhou, Y. Chang, Y. Ma, X. Liang, J. Shao, and H. Chang. 2021. 'Antigenicity and immunogenicity of recombinant proteins comprising African swine fever virus proteins p30 and p54 fused to a cell-penetrating peptide', *Int Immunopharmacol*, 101: 108251.

Zhu, J. J., P. Ramanathan, E. A. Bishop, V. O'Donnell, D. P. Gladue, and M. V. Borca. 2019.
'Mechanisms of African swine fever virus pathogenesis and immune evasion inferred
from gene expression changes in infected swine macrophages', *PLoS One*, 14: e0223955.

Zsak, L., D. V. Onisk, C. L. Afonso, and D. L. Rock. 1993. 'Virulent African Swine Fever Virus
Isolates Are Neutralized by Swine Immune Serum and by Monoclonal Antibodies
Recognizing a 72-kDa Viral Protein', *Virology*, 196: 596-602.

Chapter 5 Future Directions

Great strides have been made in improving ASFV diagnostic techniques as well as in monitoring the immune response to vaccination and infection. Much of the focus has been on molecular techniques such as real-time quantitative polymerase chain reaction (RT-qPCR), genotyping, and improved modes of detection. Detection of ASFV alone lacks correlation to which arm of the immune system is responsible for vaccination and infection outcomes. For this, methodologies in immune response readouts are key and in order to wholly assess the immune response due to vaccination, development of contemporary readout platforms is required for ASFV. Despite confounding results in the role neutralizing antibodies have in protection, improvements have been made in the assessment of ASFV-specific antibodies and the cellular correlation to this response (Sereda et al. 2022). Cellular immunity, specifically IFN-γ producing CD8+ lymphocytes, has been directly associated with protection against ASFV (Lithgow et al. 2014; Takamatsu et al. 2013). Traditionally, cellular immune readout techniques such as IFN-γ EliSpot and chromium (^{51}Cr) release assays have been used in the assessment of CD8+ T-cell response (Svitek et al. 2022). These, however, typically require a combinatorial approach to fully phenotype and verify the cytotoxic T lymphocyte (CTL) status. More modern techniques involve the use of a cocktail of fluorescent probes to be able to fully phenotype and classify the CTL response by flow cytometry (Svitek et al. 2022). Flow cytometric analysis also allows the quantification of specific effector molecules and cytokines that facilitate the CTL response such as perforin and granzyme B (Chávez-Galán et al. 2009). Studies assessing the granzyme B response (as a measure of CTL) in humans and mice are more frequently performed due to the readily available antibodies for these two species (Tröscher et al. 2022; Xu et al. 2022; Sun et al. 2021). Additional improvements in the assessment of the antigen-specific memory response involve bioinformatic prediction of major

histocompatibility complex type I-restricted Swine Leukocyte Antigen (*SLA*-I) epitopes (Reynisson et al. 2020). When these predicted nonamer peptides are presented to CD8+ T lymphocytes the antigen-specific memory response is triggered and amplification of this cell subset is measurable (Schäfer et al. 2022). Tetrameric MHC-peptide complexes have aided in a more rapid and direct characterization of antigen-specific CTLs simultaneously (Sims, Willberg, and Klenerman 2010). Computational prediction methodologies are key to narrowing the vast selection of ASFV-specific antigenic targets and have proven utility in identifying ASFV specific CTLs (Netherton et al. 2019; Sun et al. 2021; Schäfer et al. 2022; Ros-Lucas et al. 2020; Bosch-Camos et al. 2021; Herrera and Bisa 2021). These techniques combined have the potential to completely redefine the knowledge related to ASFV pathogenesis and vaccine development through rapid, empirical determination of ASFV antigen function(s) and how those relate to specific immune phenotypes.

Despite these strides, vaccine development is limited by the availability of porcine-specific antibodies for these targeted readouts. In response to this, our laboratory has developed a porcine-specific granzyme B monoclonal antibody. Preliminary assay validation (not published) demonstrates the utility of classifying the more targeted CD8αβ+granzyme B+ cell subsets using porcine peripheral blood monocytes (PBMCs) and splenocytes from immunized ASFV-challenged animals in combination with ASFV-specific epitope mapping techniques using *SLA*-I restricted computationally predicted peptides (Zajac et al. 2022). This antibody paired with flow cytometric assay development will allow for rapid, high throughput screening ideal for use in high-level containment facilities for the assessment of the memory CTL response in ASFV-infected animals. The knowledge gained from this will be instrumental in identifying relevant determinants that have the protective potential for rational ASFV subunit vaccine development. A protective subunit

vaccine is required to build onto the tools available for swine health and the ability to control this

economically devastating disease and completely change the outlook of future outbreaks.

5.1 References:

Bosch-Camos, L., E. Lopez, M. J. Navas, S. Pina-Pedrero, F. Accensi, F. Correa-Fiz, C. Park, M. Carrascal, J. Dominguez, M. L. Salas, V. Nikolin, J. Collado, and F. Rodriguez. 2021. 'Identification of Promiscuous African Swine Fever Virus T-Cell Determinants Using a Multiple Technical Approach', *Vaccines (Basel)*, 9.

Chávez-Galán, L., M. C. Arenas-Del Angel, E. Zenteno, R. Chávez, and R. Lascurain. 2009. 'Cell Death Mechanisms Induced by Cytotoxic Lymphocytes', *Cellular & Molecular Immunology*, 6: 15-25.

Herrera, Leana Rich De Mesa, and Elizabeth Paulino Bisa. 2021. 'In silico analysis of highly conserved cytotoxic T-cell epitopes in the structural proteins of African swine fever virus', *Veterinary World*: 2625-33.

Lithgow, P., H. Takamatsu, D. Werling, L. Dixon, and D. Chapman. 2014. 'Correlation of cell surface marker expression with African swine fever virus infection', *Vet Microbiol*, 168: 413-9.

Netherton, Christopher L., Lynnette C. Goatley, Ana Luisa Reis, Raquel Portugal, Rachel H. Nash, Sophie B. Morgan, Lynden Gault, Raquel Nieto, Veronica Norlin, and Carmina Gallardo. 2019. 'Identification and immunogenicity of African swine fever virus antigens', *Frontiers in Immunology*, 10: 1318.

Reynisson, Birkir, Bruno Alvarez, Sinu Paul, Bjoern Peters, and Morten Nielsen. 2020. 'NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data', *Nucleic Acids Research*, 48: W449-W54.

Ros-Lucas, A., F. Correa-Fiz, L. Bosch-Camos, F. Rodriguez, and J. Alonso-Padilla. 2020. 'Computational Analysis of African Swine Fever Virus Protein Space for the Design of an Epitope-Based Vaccine Ensemble', *Pathogens*, 9.

Schäfer, Alexander, Giulia Franzoni, Christopher L. Netherton, Luise Hartmann, Sandra Blome, and Ulrike Blohm. 2022. 'Adaptive Cellular Immunity against African Swine Fever Virus Infections', *Pathogens*, 11: 274.

Sereda, A. D., A. S. Kazakova, V. V. Dmitrenko, and D. V. Kolbasov. 2022. 'Search for additional tests for immunobiological evaluation of the candidate vaccines against African swine fever', *PLoS One*, 17: e0265819.

Sims, Stuart, Christian Willberg, and Paul Klenerman. 2010. 'MHC–peptide tetramers for the analysis of antigen-specific T cells', *Expert Review of Vaccines*, 9: 765-74.

Sun, Wenqiang, He Zhang, Wenhui Fan, Lihong He, Teng Chen, Xintao Zhou, Yu Qi, Lei Sun, Rongliang Hu, Tingrong Luo, Wenjun Liu, and Jing Li. 2021. 'Evaluation of Cellular

Immunity with ASFV Infection by Swine Leukocyte Antigen (SLA)—Peptide Tetramers', *Viruses*, 13: 2264.

Svitek, N., E. L. N. Taracha, R. Saya, E. Awino, V. Nene, and L. Steinaa. 2022. 'Analysis of the Cellular Immune Responses to Vaccines', *Methods Mol Biol*, 2465: 283-301.

Takamatsu, Haru-Hisa, Michael S. Denyer, Anna Lacasta, Catrina M. A. Stirling, Jordi M. Argilaguet, Christopher L. Netherton, Chris A. L. Oura, Carlos Martins, and Fernando Rodríguez. 2013. 'Cellular immunity in ASFV responses', *Virus Research*, 173: 110-21.

Tröscher, A. R., K. M. Mair, L. V. de Juan, U. Köck, A. Steinmaurer, H. Baier, A. Becker, I. Blümcke, M. Finzel, C. Geis, R. Höftberger, C. Mawrin, T. J. von Oertzen, J. Pitsch, R. Surges, B. Voges, S. Weis, M. Winklehner, F. Woermann, J. Bauer, and C. G. Bien. 2022. 'Temporal lobe epilepsy with GAD antibodies: neurons killed by T cells not by complement membrane attack complex', *Brain*.

Xu, L., N. Liu, W. Zhan, Y. Deng, Z. Chen, X. Liu, G. Gao, Q. Chen, Z. Liu, and G. Liang. 2022. 'Granzyme B Turns Nanoparticle Fluorescence "On" for Imaging Cytotoxic T Lymphocyte Activity in Vivo', *ACS Nano*, 16: 19328-34.

Zajac, Michelle D., Neha Sangewar, Shehnaz Lokhandwala, Jocelyne Bray, Huldah Sang, Jayden McCall, Richard P. Bishop, Suryakant D. Waghela, Rakshith Kumar, Tae Kim, and Waithaka Mwangi. 2022. 'Adenovirus-Vectored African Swine Fever Virus pp220 Induces Robust Antibody, IFN-γ, and CTL Responses in Pigs', *Frontiers in Veterinary Science*, 9.