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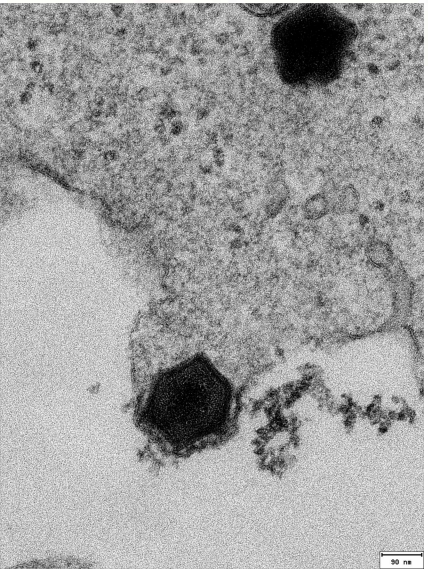
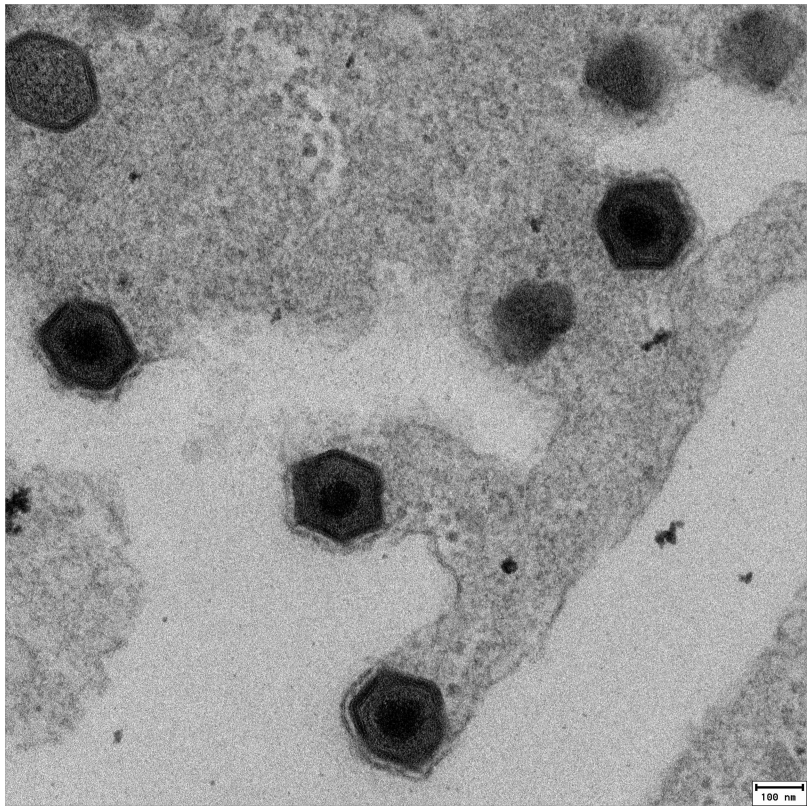
CENTRO DE BIOLOGÍA MOLECULAR
CSIC UAM

DR. YOLANDA REVILLA



你好！

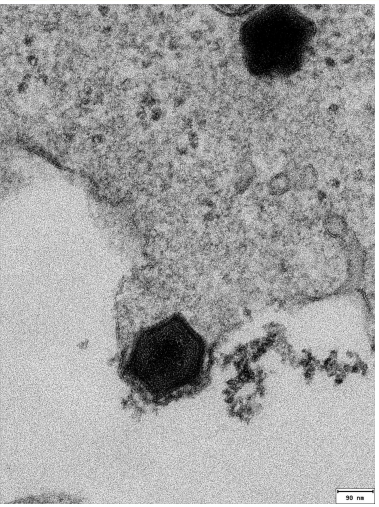
非常感谢你的到来



ASFV

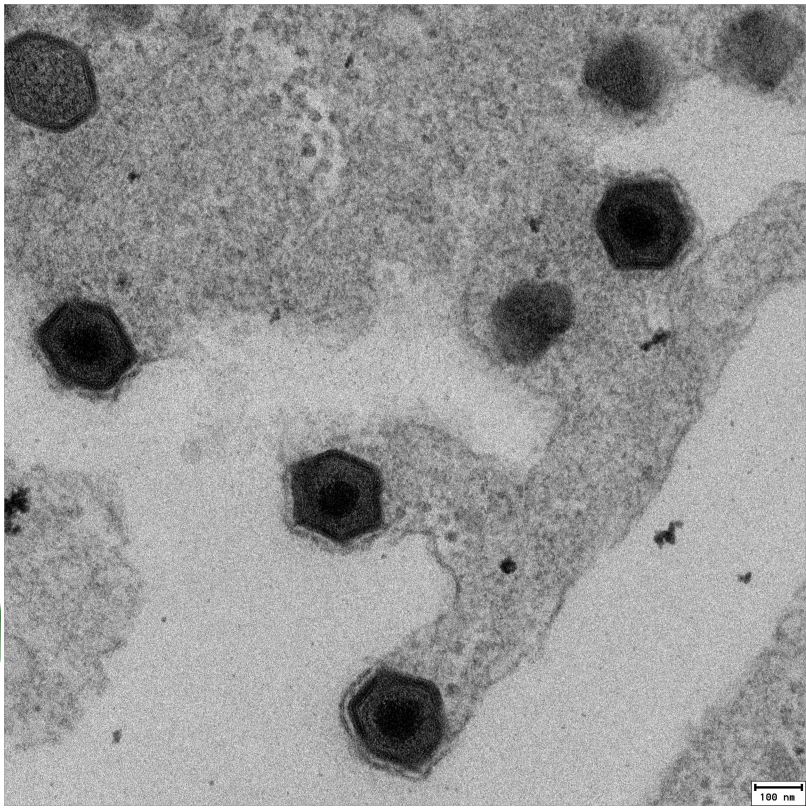
VIRULENCE

NO VACCINE

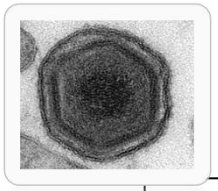


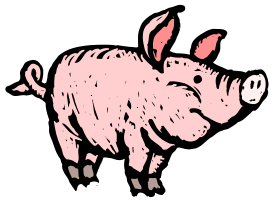
ASFV

ATTENUATION



ASFV virulence vs attenuation





VIRUS

HOST

Highly virulent strains

Chinese strain, Georgia, Armenia, Current circulating virus in UE, Other virulent strains E70, Ba71, OURT88/1, Malawi

Acute infection
100% mortality

Subacute infection Some mortality in young swine

NaturallyAttenuated strains

OURT88/3, NH/P68, Latvian Strain
(Gallardo et al 2019)

Persistent infection
Clinical lesions
chronic ASFV

Naturally attenuated strains protect against homologous and heterologous challenge (NH/P68 vs Lisbon60 and Armenia, CBMSO-CISA, Revilla, 2018).

Understanding ASFV virulence

Ba71V, Georgia-V, adapted strains, do not infect or protect animals from virulent challenge.

ASFV Tropism, Mechanisms and Genes Interfering Host Response Pathways: Actors for ASF Vaccine development

Yolanda Revilla

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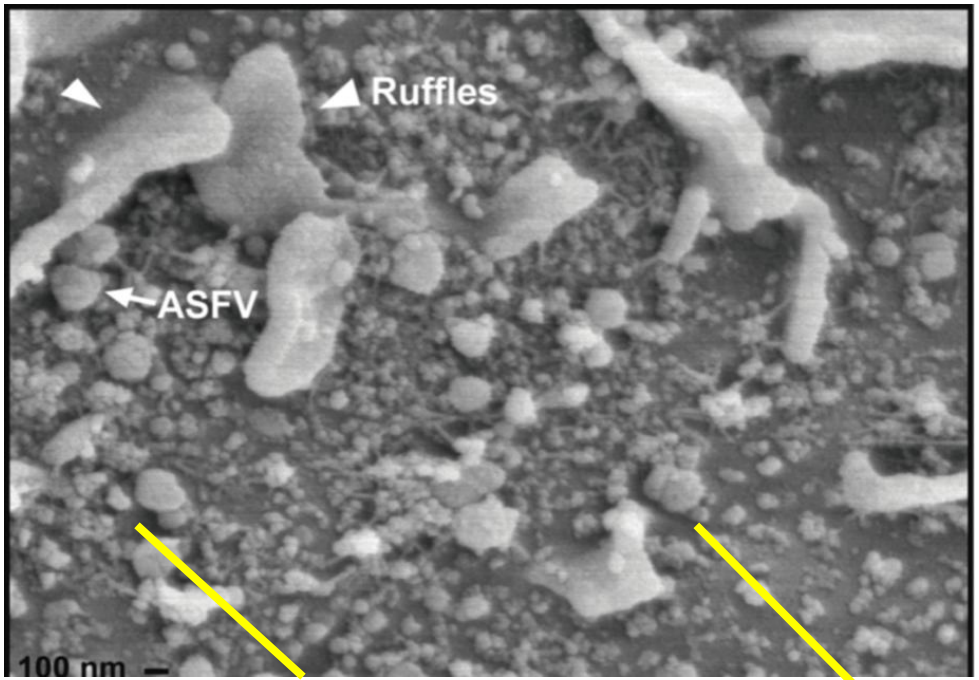
Endocytosis –mediated Entry in Macrophages



Cell membrane protrusions

ASFV GENES AND MECHANISMS INVOLVED IN VIRULENCE

- Approaching the viral and cellular factors, and the molecular mechanism(s) involved in ASFV virulence and attenuation

Important for Vaccine Development

- Rational design of “Live Attenuated Vaccines” by deletion of specific genes involved in “virulence” to generate new attenuated, safer strains.

GENOMIC REGIONS WHICH USUALLY REARRANGE DURING NATURAL ATTENUATION

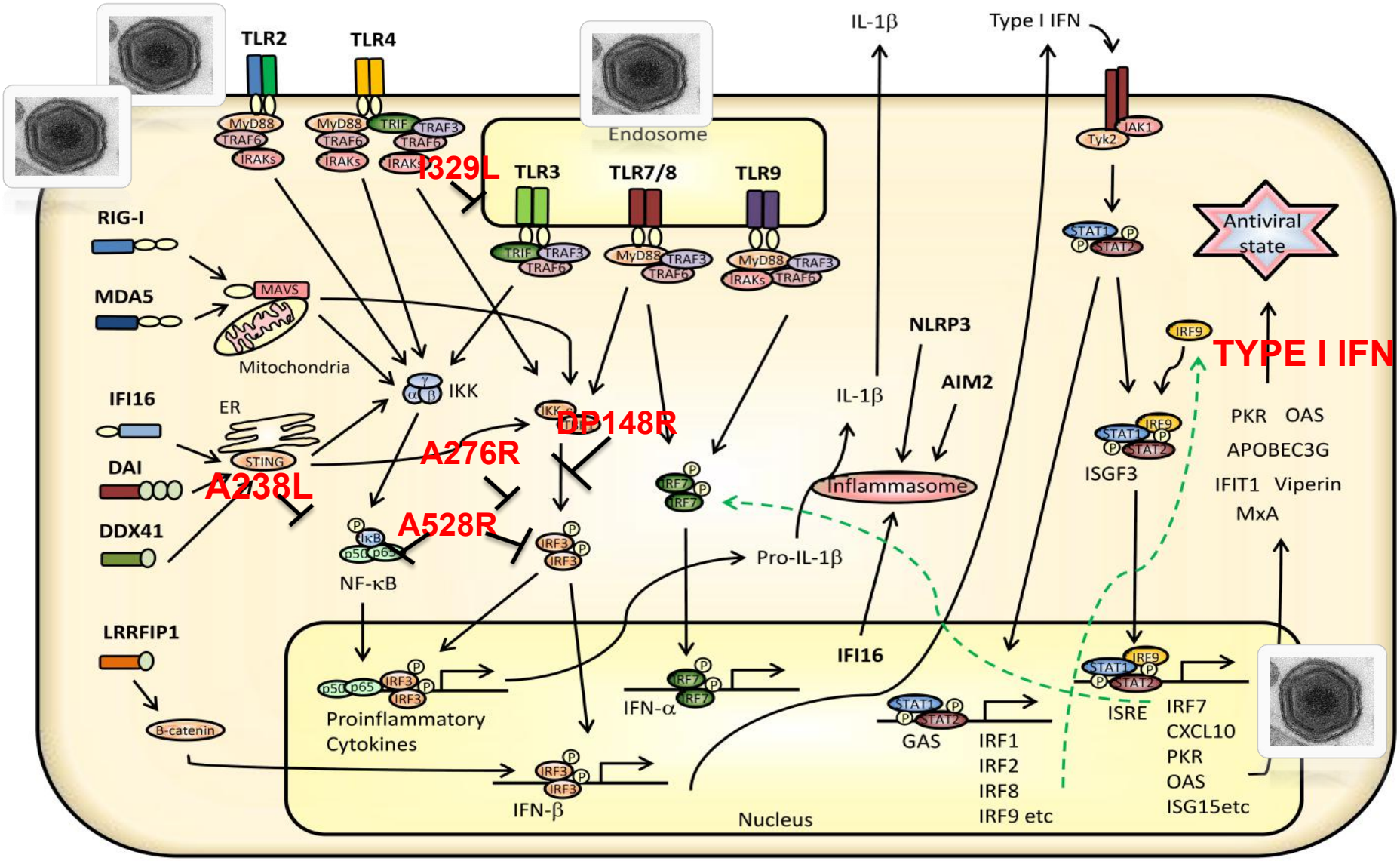
1) Central region of the genome: EP402R is altered in attenuated strains

i.e. CD2v protein is absent in attenuated strains

2) Left and right parts of the viral genome encoding for IFN-modulating genes

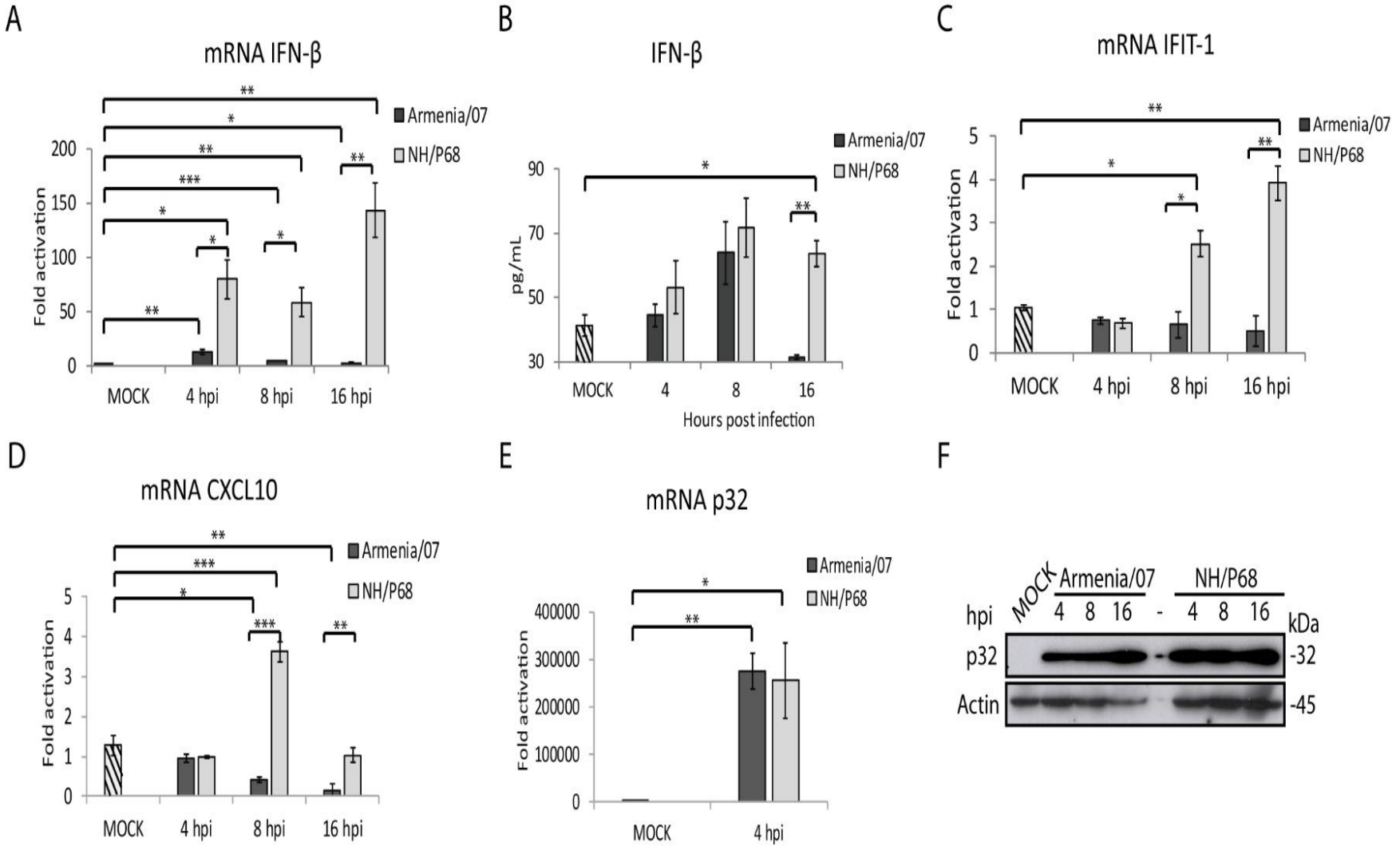
ROLE OF TYPE I IFN IN VIRULENCE

Type I IFN modulation by virulent ASFV strains



García Belmonte R, Pérez D, Richt J, Revilla Y. J. Virology, April 2019

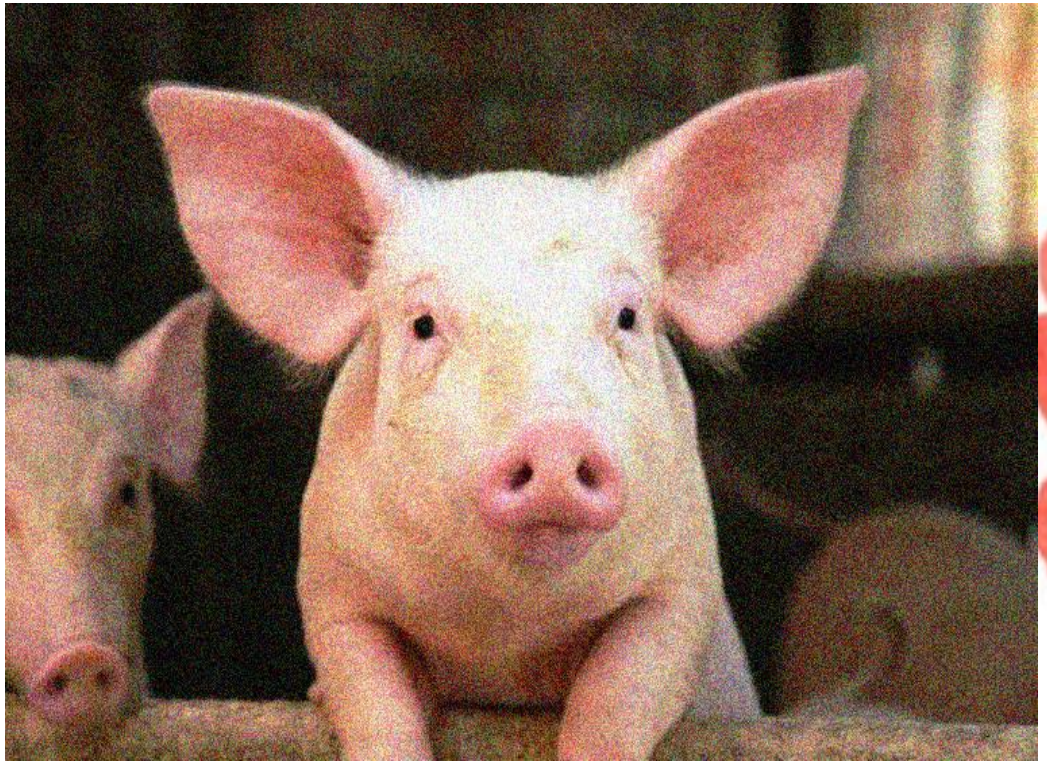
Virulent ASFV Armenia/07 infection inhibits mRNA and secretion of type I IFN-β by controlling STING

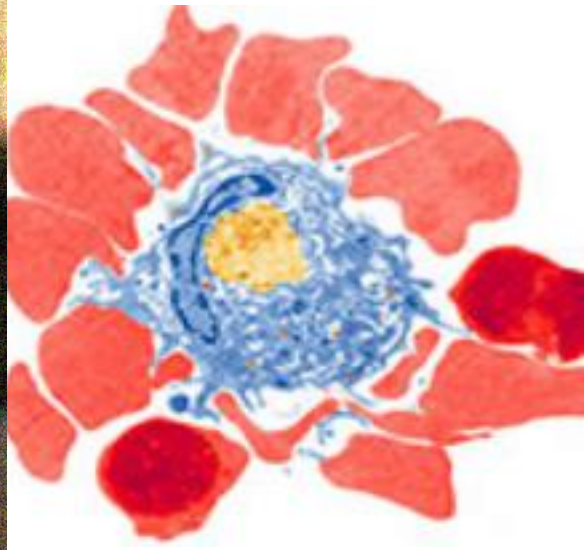


García Belmonte R, Pérez D, Richt J, Revilla Y. J. Virology, April 2019

ASFV GENES INVOLVED IN VIRULENCE

NATURAL ATTENUATION OF THE VIRULENT STRAINS USUALLY INVOLVES RREARANGEMENT OF SEQUENCES ENCODING ASFV CD2v PROTEIN AND MGFS





Daniel Pérez Núñez (Y. Revilla's Group)

July 11th 2018

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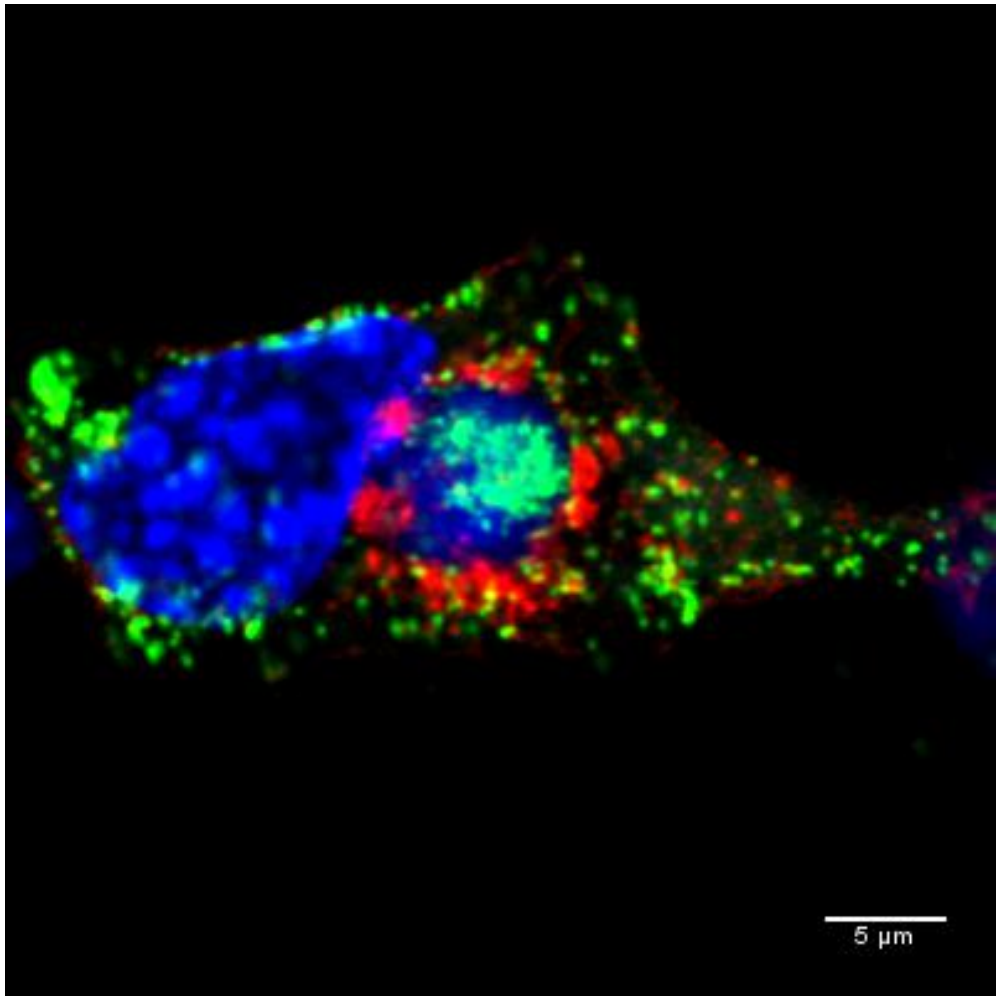
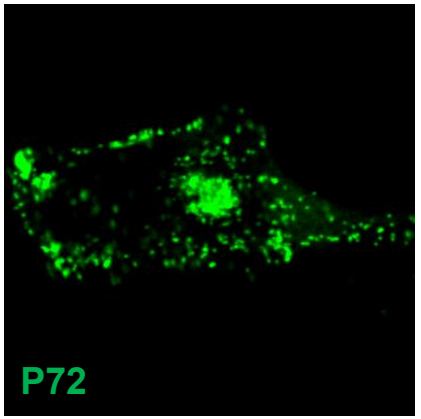
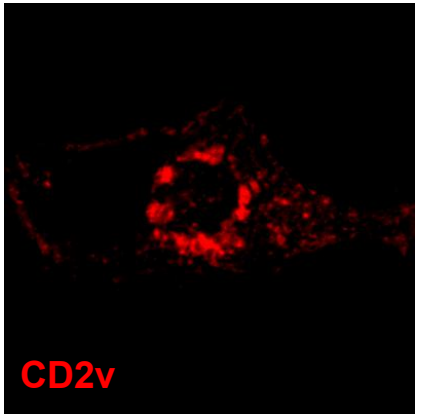
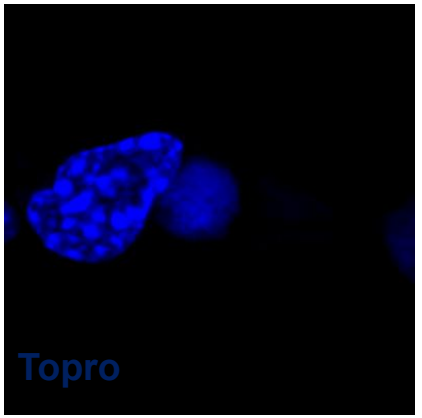
EP402R (CD2v) localizes around viral factory

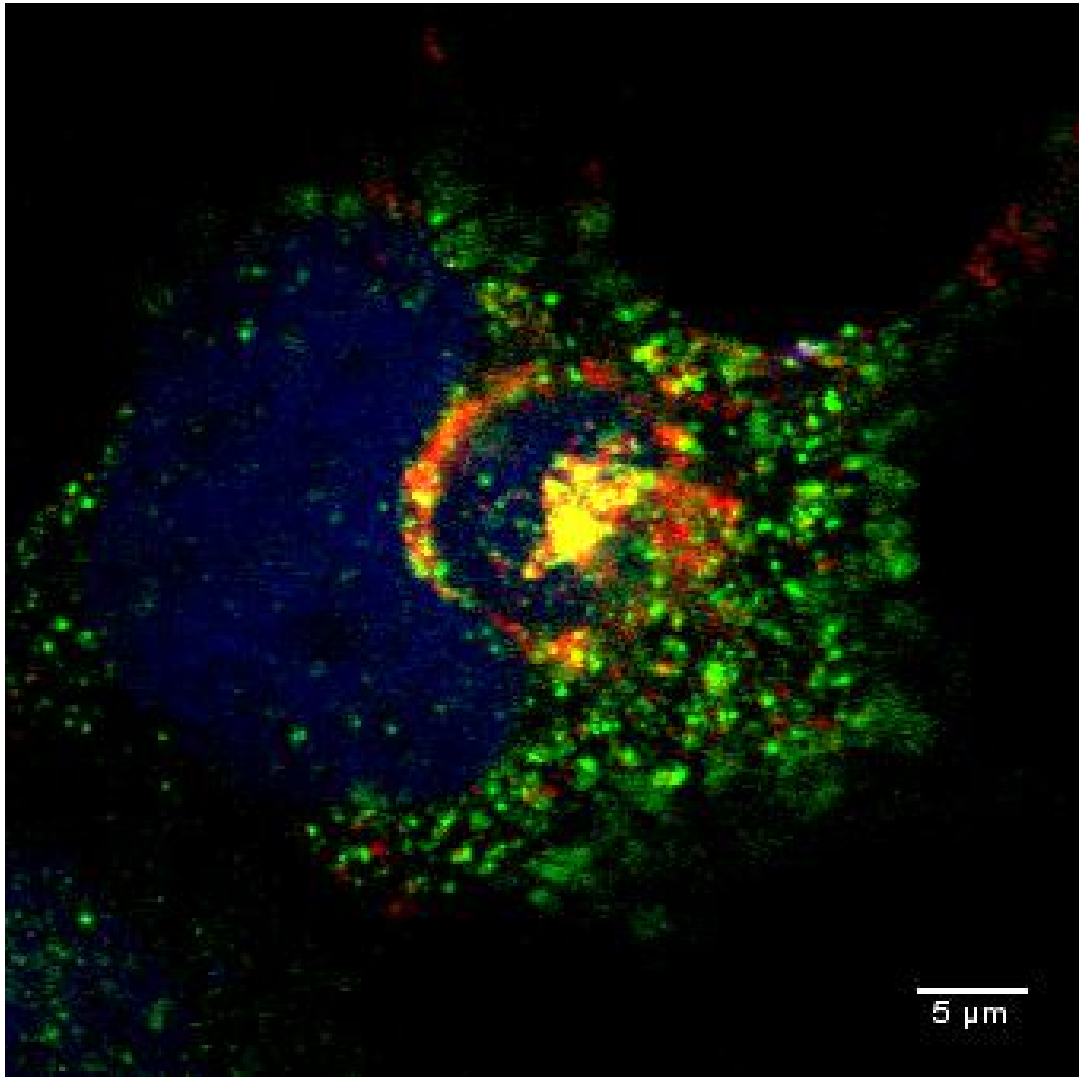


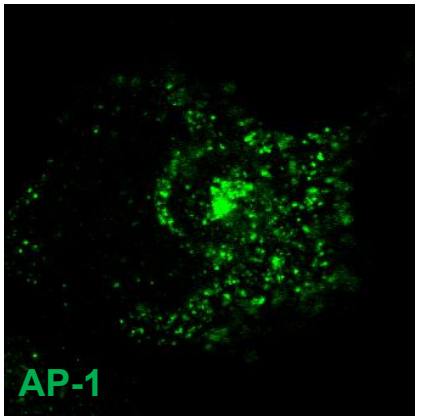


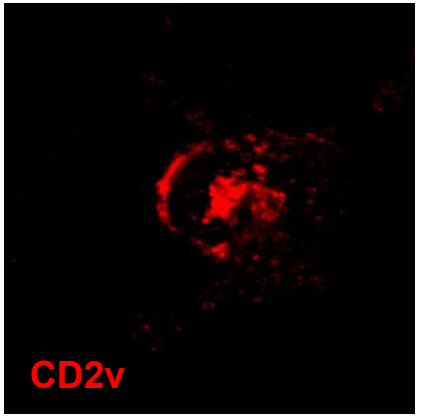


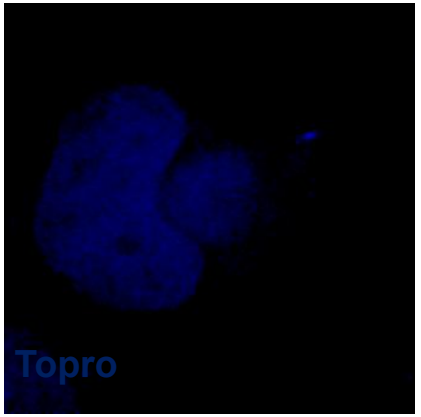


CD2v localization in ASFV infected cells





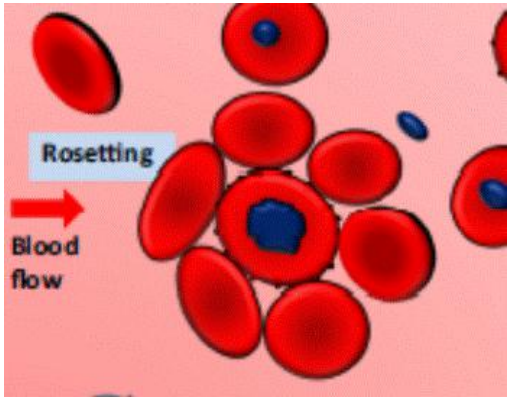


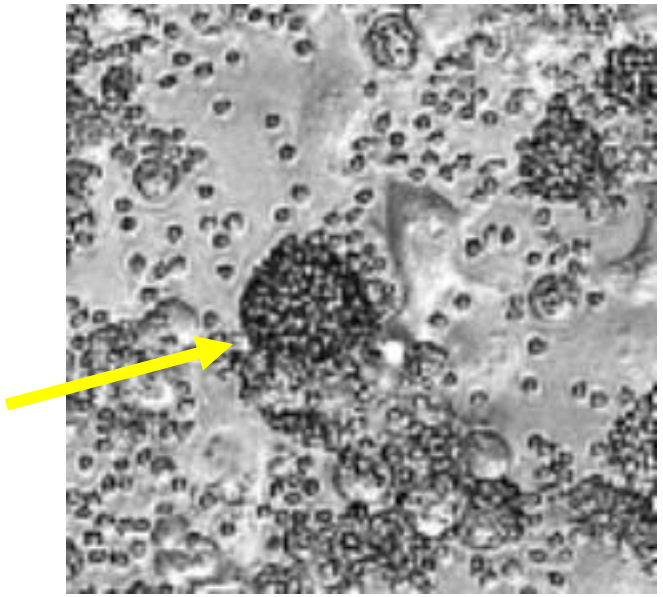


CD2v co-localizes with the adaptor protein AP-1

Hemadsorption: Role in viral pathogenesis?

- Adherence of red blood cells to the surface of an infected cell
- Phenomenon related to pathogenesis and infectivity in many pathogens
- Involved in evasion of host-immune system by unknown mechanism

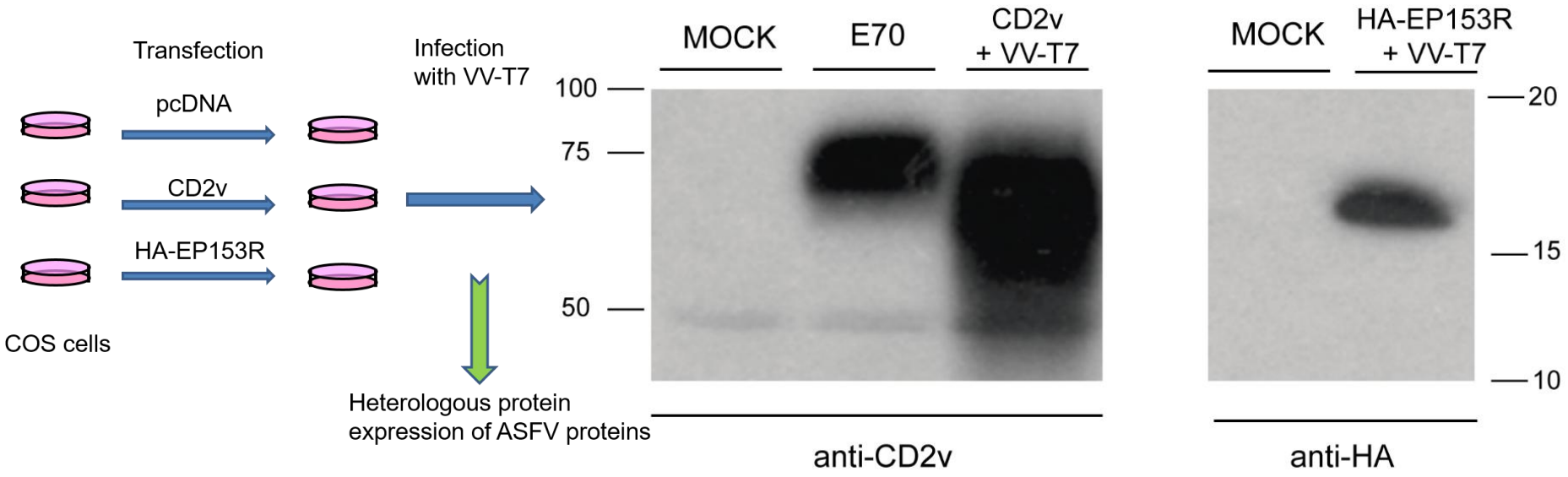




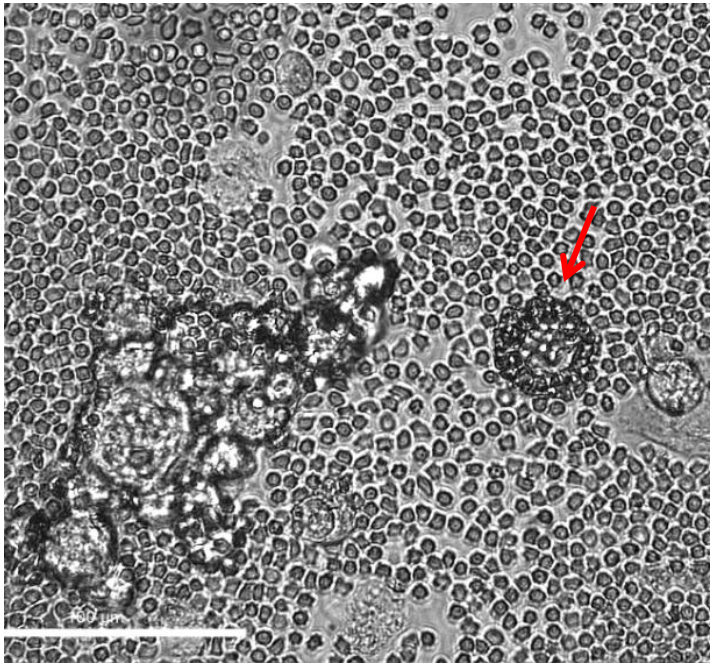
ASFV-rosette: ASFV-infected cell surrounded by RBC

Adapted from Yam, et al. Three is a Crowd – New Insigths into Rosetting in Plasmodium falciparum. Trends in Parasitol 2017

Role of CD2v in ASFV-depending rosette formation by heterologous expresión of CD2v in COS cells



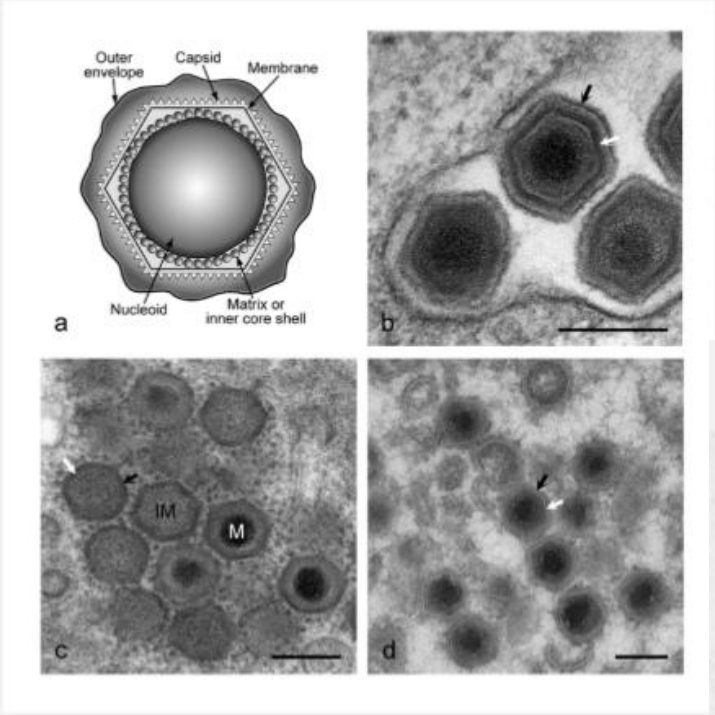
Transfected CD2 mediates rossette formation and is sufficient for hemadsorption



Why are ASF succesful vaccines not available so far?

1. High complexity of the virus

ASFV structure

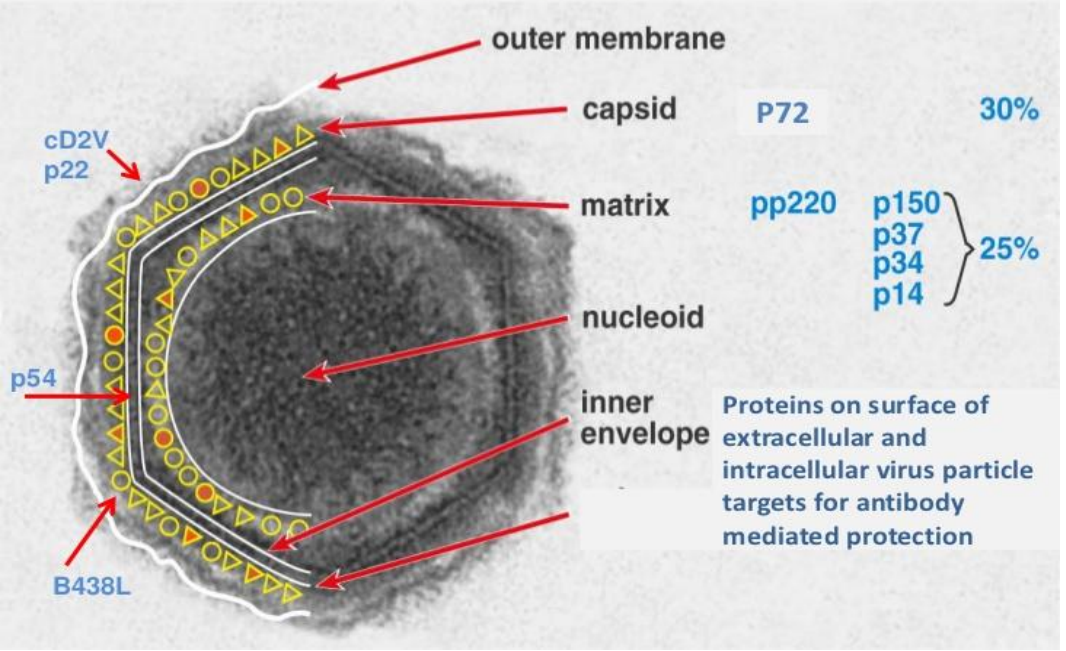


- ASFV virions have a complex multilayer structure
- More than 50 proteins are present
- Extracellular and intracellular mature virions are both infectious

Pippa Hawes IAH

2. Inactivated virions do not induce protection

Virus Particle



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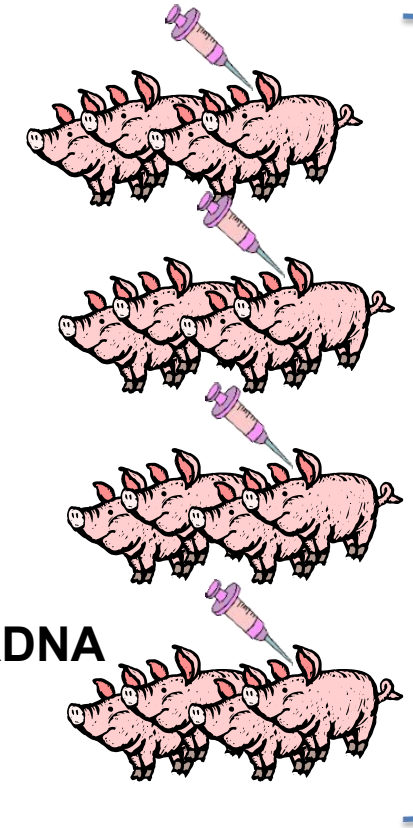
RECENT EXPERIMENTS OF VACCINATION

Vaccination of pigs by using combination of several viral recombinant proteins and DNAs.

KSU

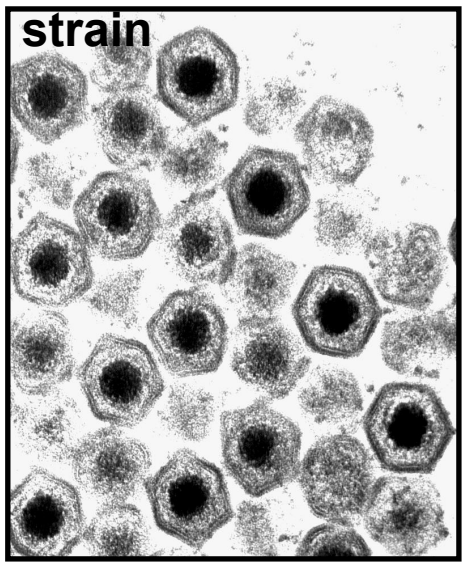
Year 2016

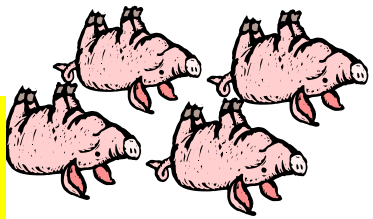
- •p35 protein + CD2vDNA
- •81 % inhibition *in vitro*
- • p15 protein + p72DNA
- •62 % inhibition *in vitro*
- • p54 protein + p32 DNA
- •27 % inhibition *in vitro*
- • CD2v protein + CP312RDNA
- •19 % inhibition in vitro



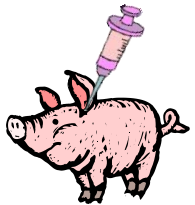
+

Virulent, circulating ASFV Armenia strain





100% Died !!



FOCUSING LAVs AS EMERGENCY TOOLS FOR AFFECTED AREAS

GENERATING LAVs FROM THE VIRULENT, EU CIRCULATING GENOTYPE 2 ARMENIA 07STRAIN BY USING CRISPR/Cas9 TECHNOLOGY. GFP GREEN MARKER INCORPORATED IN THE VIRAL GENOME

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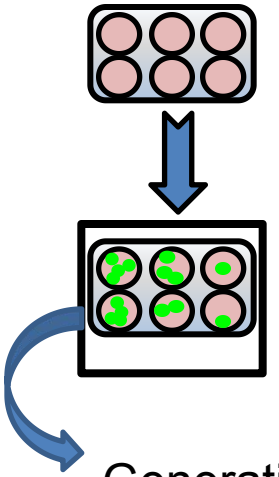
Generación of the recombinant vaccine lacking CD2 from ASFV/Armenia07 by CRISPR

Generation



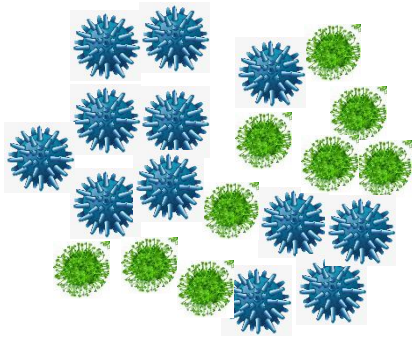
COS cells

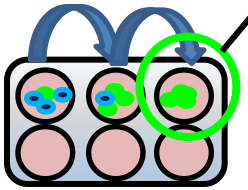
Transfection and Infection (WT virus)

Generation of recombinant virus (GFP+)

ARMENIA07deltaCD2

Purification

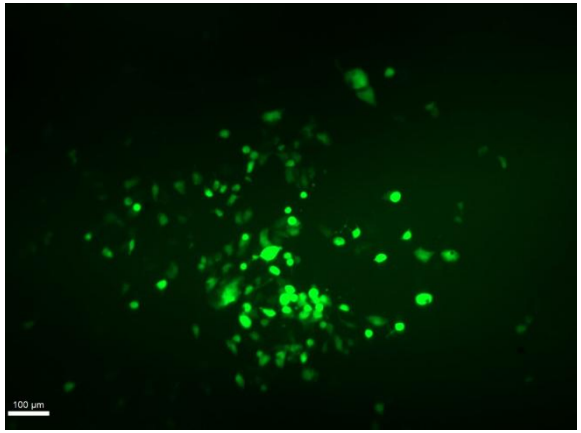




Isolation of plates

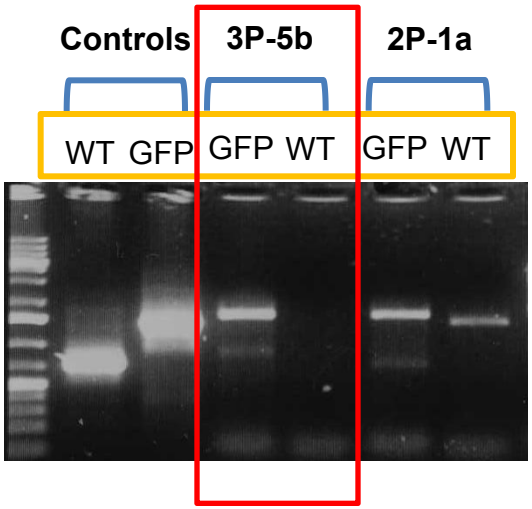
Verification of absence of WT virus:

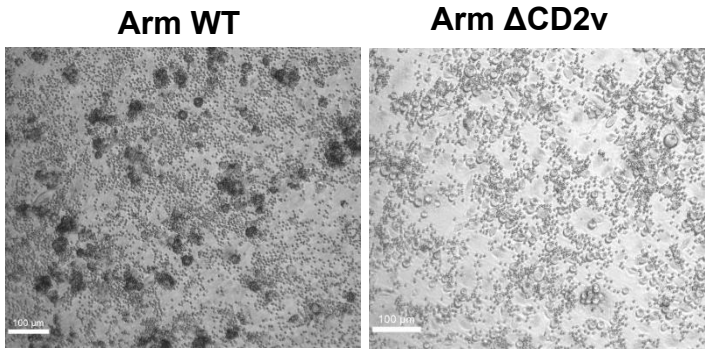
- PCR (GFP vs WT gene)
- WB
- HAD (CD2v dependent)
- NGS
- qPCR



Generation of recombinant ASFV Arm-ΔCD2v by CRISPR

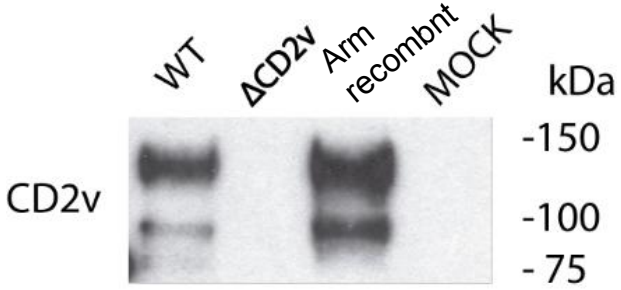
Purification





Verification of absence of WT virus:

- PCR (GFP vs WT gene)
- WB
- HAD (CD2v dependent)
- NGS



IN VIVO TESTING OF DELETION MUTANTS FROM VIRULENT STRAINS AS VACCINE PROTOTYPES IN VACCINATION/CHALLENGE MODELS

CURRENTLY ON GOING TRIAL IN PIGS BY VACCINATING WITH THE ARMENIA07ΔCD2V AND OTHER RECENTLY PREPARED VACCINE PROTOTYPES

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KSU-CEEZAD

THE VACCINE CHALLENGE FROM NATURALLY ATTENUTED STRAINS: OUR SECOND APPROACH

ASFV NHV/P68 Naturally ATTENUATED STRAIN

AS MODEL FOR SAFE VACCINE DEVELOPMENT BY GENERATING RECOMBINANT VIRUSES LACKING OF SELECTED GENES

AVOIDING THE CLINIC SYMPTOMS AND THE VIREMIA PRODUCED BY THE PARENTAL VIRUS

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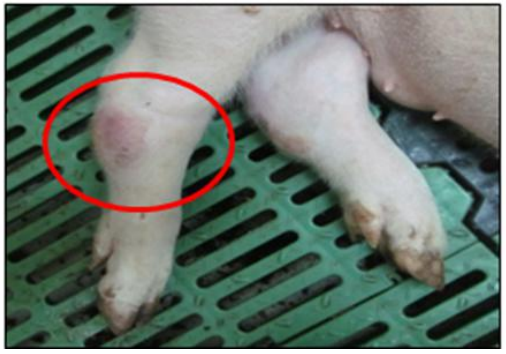
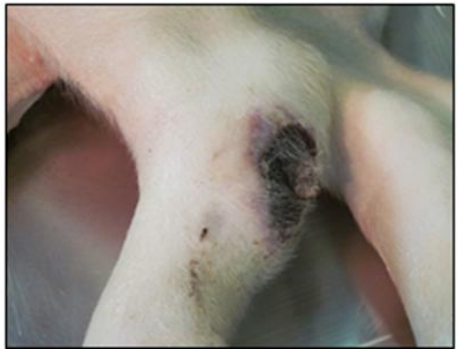
WE HAVE FOUND THAT:

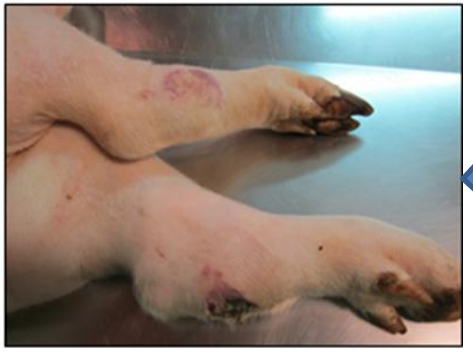
The NHV/P68wt strain, grown in porcine alveolar macrophages (PAM) FULLY protected to the vaccinated pigs against lethal challenge with virulent strains, including Armenia07 (100% of protection)

DISADVANTAGES

1.Side effects related to chronic forms of ASF







2. Some low viremia peaks. ENHANCING OF SAFETY REQUIRED!

3. INDUSTRIALLY VACCINE PRODUCTION further impaired by the fact of it should be generated in primary MACROPHAGES cultures, since not a fully productive ASFV cell line exists YET

1. THE NH/P68 ATTENUATED AS MODEL FOR VACCINE PROTOTYPES

ARTICLE IN PRESS

Vaccine xxx (2018) xxx–xxx

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Vaccine

journal homepage: www.elsevier.com/locate/vaccine

African swine fever virus (ASFV) protection mediated by NH/P68 and NH/P68 recombinant live-attenuated viruses

Carmina Gallardo [b,1], Elena G. Sánchez [a,1], Daniel Pérez-Núñez [a], Marisa Nogal [a], Patricia de León [a], Ángel L. Carrascosa [a], Raquel Nieto [b], Alejandro Soler [b], María Luisa Arias [b], Yolanda Revilla [a,*]

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[b] *European Union Reference Laboratory for ASF, Centro de Investigación en Sanidad Animal (INIA-CISA), Madrid, Spain*

SOME CONCLUSIONS ABOUT NH/P68 LAVs :

-OUR MODELS CAN PROTECT AGAINST ARMENIA AND OTHER VIRULENT VIRUS BUT STILL INDUCE CLINICAL SIDE EFFECTS.

-APPART FROM THE FACT THAT WE STILL NEED MORE STUDIES TO SELECT THE GENES CANDIDATES TO BE DELETED, THE MAIN DRAWBACK WE FOUND IS THAT NO IDEAL CELLULAR SOURCE TO INDUSTRIALLY PRODUCE LAVs FROM ATTENUATED STRAINS IS AVAILABLE SO FAR.

-MODERATE LOSS OF PROTECTION WAS FOUND WERE DELETION MUTANTS WERE ACHIEVED IN COS CELLS

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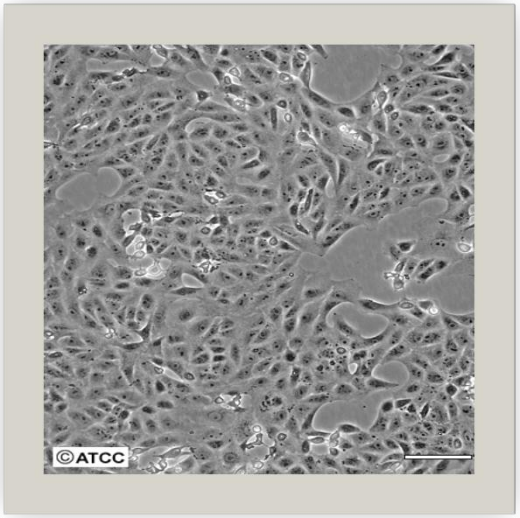
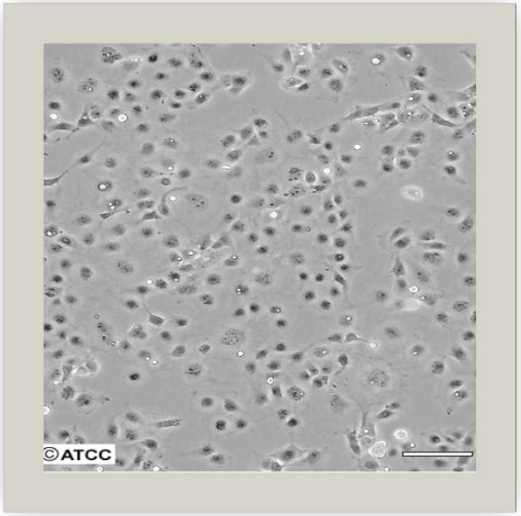
Monocytes and tissue macrophages are the NATURAL target of ASFV, but primary cultures are not a good option for vaccine production

WSL



ASFV- NATURALLY ATTENUATED PROTOTYPES

COS



VIRULENT STRAINS
Armenia07, Sardinian

SCIENTIFIC REPORTS

OPEN

Phenotyping and susceptibility of established porcine cells lines to African Swine Fever Virus infection and viral production

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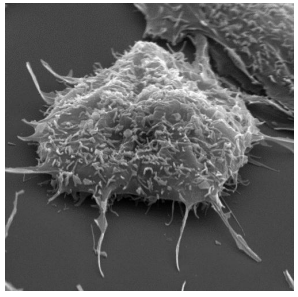
Accepted: 1 August 2017
Published online: 04 September 2017

Elena G. Sánchez[1], Elena Riera[1], Marisa Nogal[1], Carmina Gallardo[5], Paloma Fernández[1], Raquel Bello-Morales[3], José Antonio López-Guerrero[3], Carol G. Chitko-McKown[4], Jürgen A. Richt[2] & Yolanda Revilla[1]

NEXT: CRISPR AND LENTIVIRUS-MEDIATED MODIFICATION OF SELECTED CELL FACTORS FROM COS AND WSL CELLS TO IMPROVE LAV VACCINE DEVELOPMENT AND PRODUCTION

WSL CELL



非常感谢您的关注!　¡Gracias! THANK YOU!

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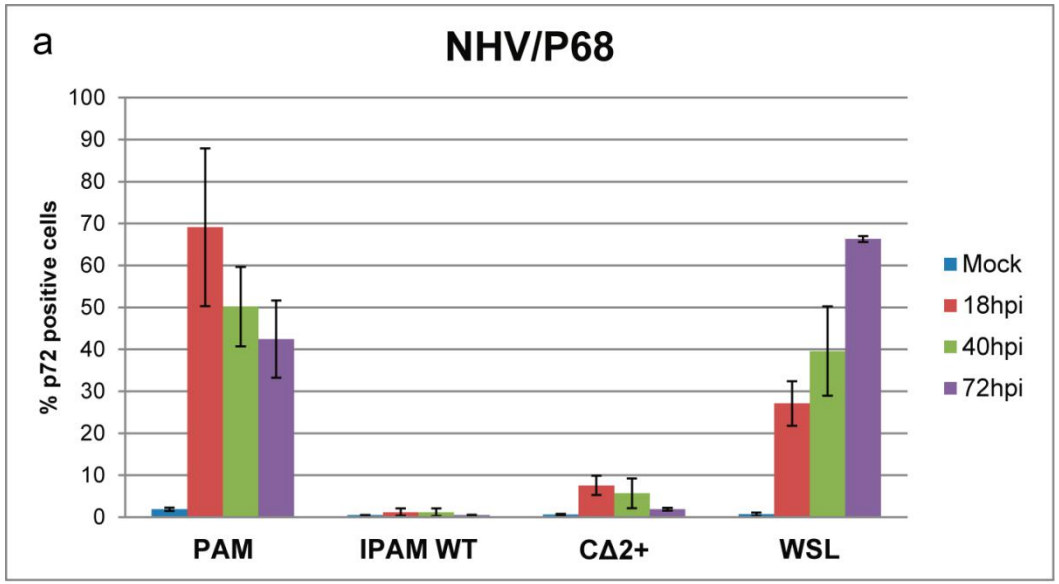
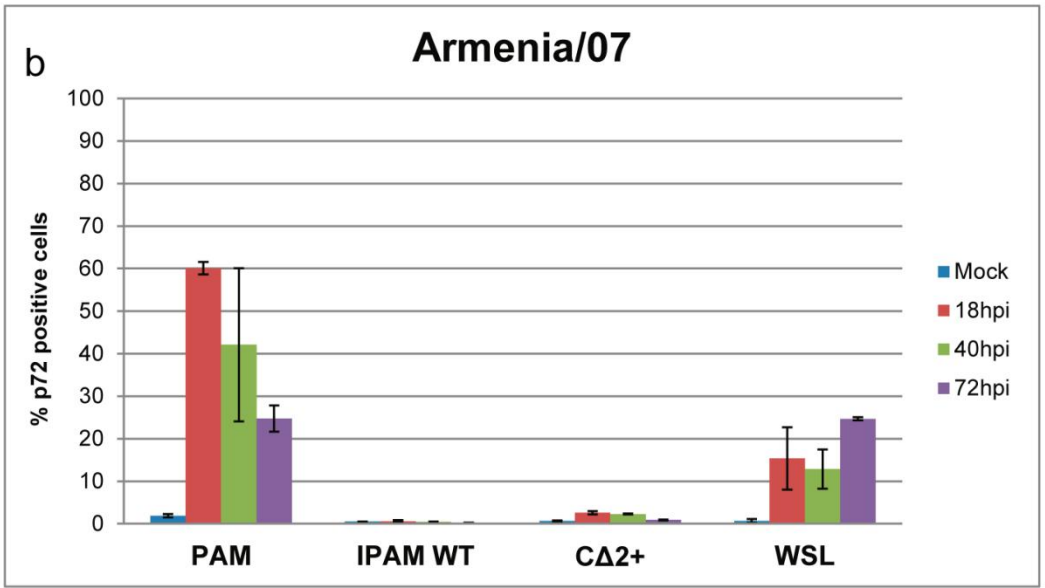
FACS analysis of p72 expression in PAM, IPAM-WT, CΔ2+ and WSL infected cells.

Cells were infected with NHV/P68 (a) or Armenia/07 (b) (MOI=1) and at 18, 40 and 72 hpi, cells were processed for FACS analysis. The percentage of infected cells was detected with a MoAb anti p72-17LD3 antibody (n ≥ 2, performed in duplicate; mean ±S.D.)





CONSTRUCTION OF DELETION MUTANTS from the NHV/P68 ASFV genome

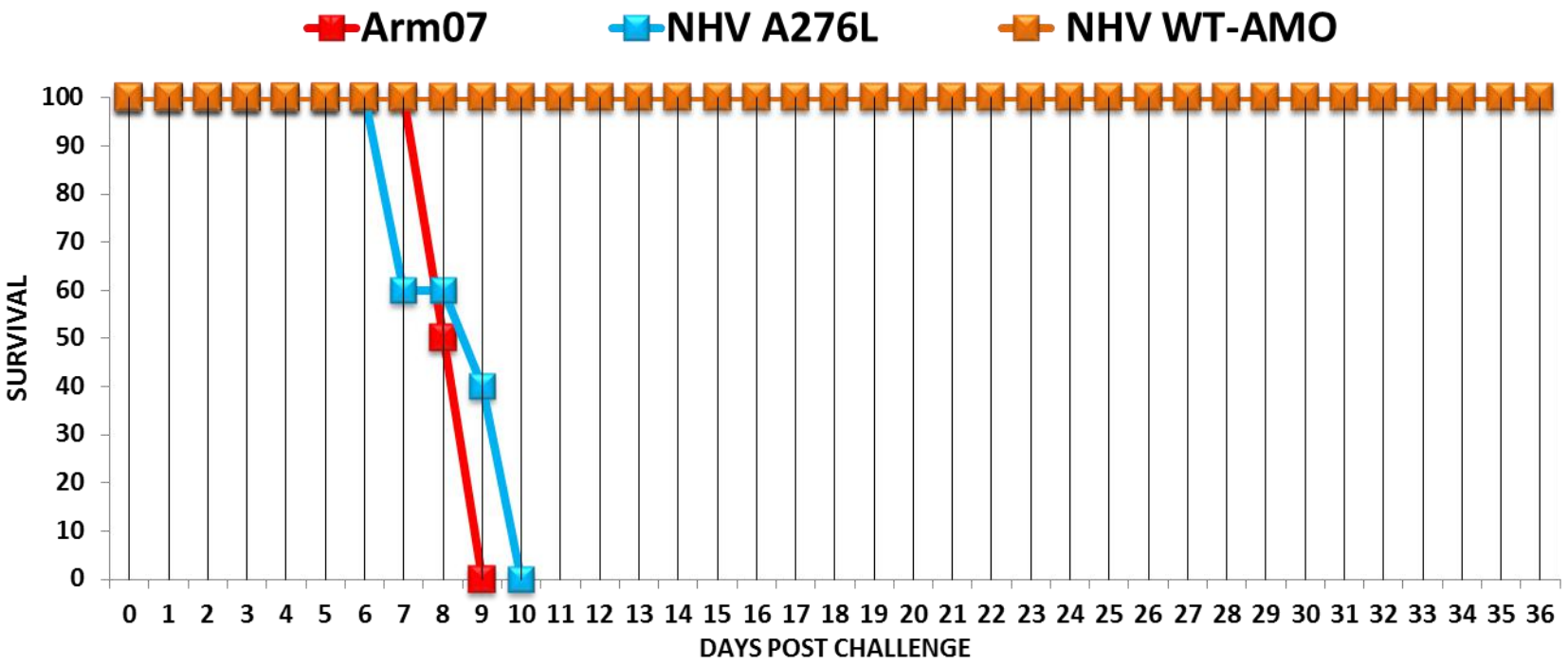
1.NHV/P68 -ΔA238L→ *A238L* protein inhibits key pathways involved in activating transcription of immunomodulatory genes

2.NHV/P68 -ΔA276R → *A276R* gene inhibits the induction of IFN-β

The main goal was to assess whether the NHV/P68Δ A238L and NHV/P68 Δ A276R were able to induce protective immunity in pigs against a lethal challenge and their ability to reduce the adverse clinical signs produced by the attenuated strain.

RESULTS: THE CHALLENGE D29PI

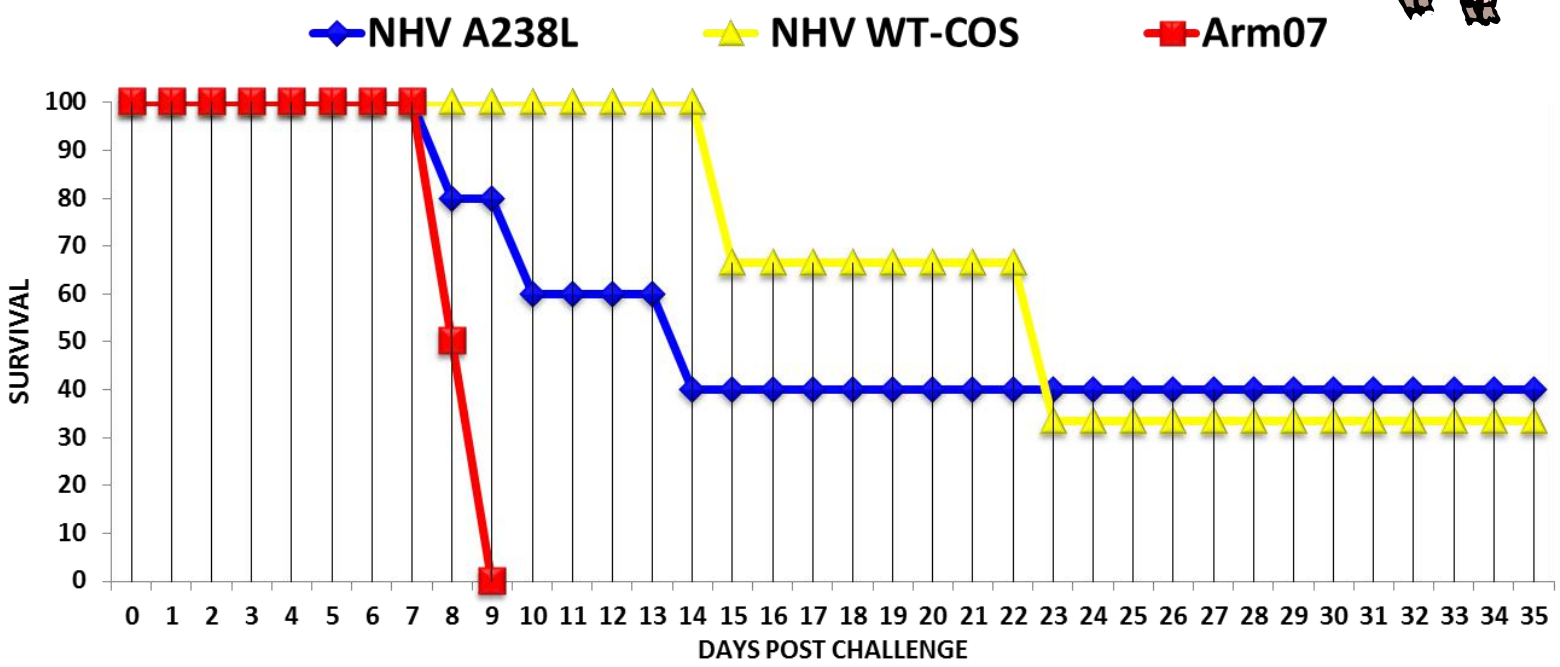
GROUP 2 NHV-PAM → After challenge Armenia (d29pi).



- NHVΔA276L 0 % survival (0/5) slaughtered between days 7 - 10 pc-
- **NHV-PAM CONTROL 100% SURVIVAL (2/2)**
- Armenia Controls : 0% Survival , slaughtered 8 -9 dpi.

RESULTS: THE CHALLENGE D29PI

GROUP 1 NHV-COS →SURVIVAL RATE



- Armenia Controls : 0% SURVIVAL at 8 -9 días post inoculation
- NHV-ΔA238L-COS 40 % survival (2/5) →slaughtered at day 8 and 14 pc.
- NHV-COS-control 33,4% survival (1/3) → slaughtered days 15-23 pc.