FMDV control of innate immunity

Teresa de los Santos

Plum Island Animal Disease Center. ARS-USDA

- Viruses are the most abundant biological entities on Earth: one to two orders of magnitude higher than cells

- Coevolution: viruses exploit all conceivable replication-expression strategies within a host that is indispensable for survival

➢ *How has FMDV* become *such a successful pathogen?*

FMDV favorable evolutionary advantages

- FMDV causes an acute infection of ~ 100% morbidity but disease resolves relatively quickly inducing strong adaptive immunity

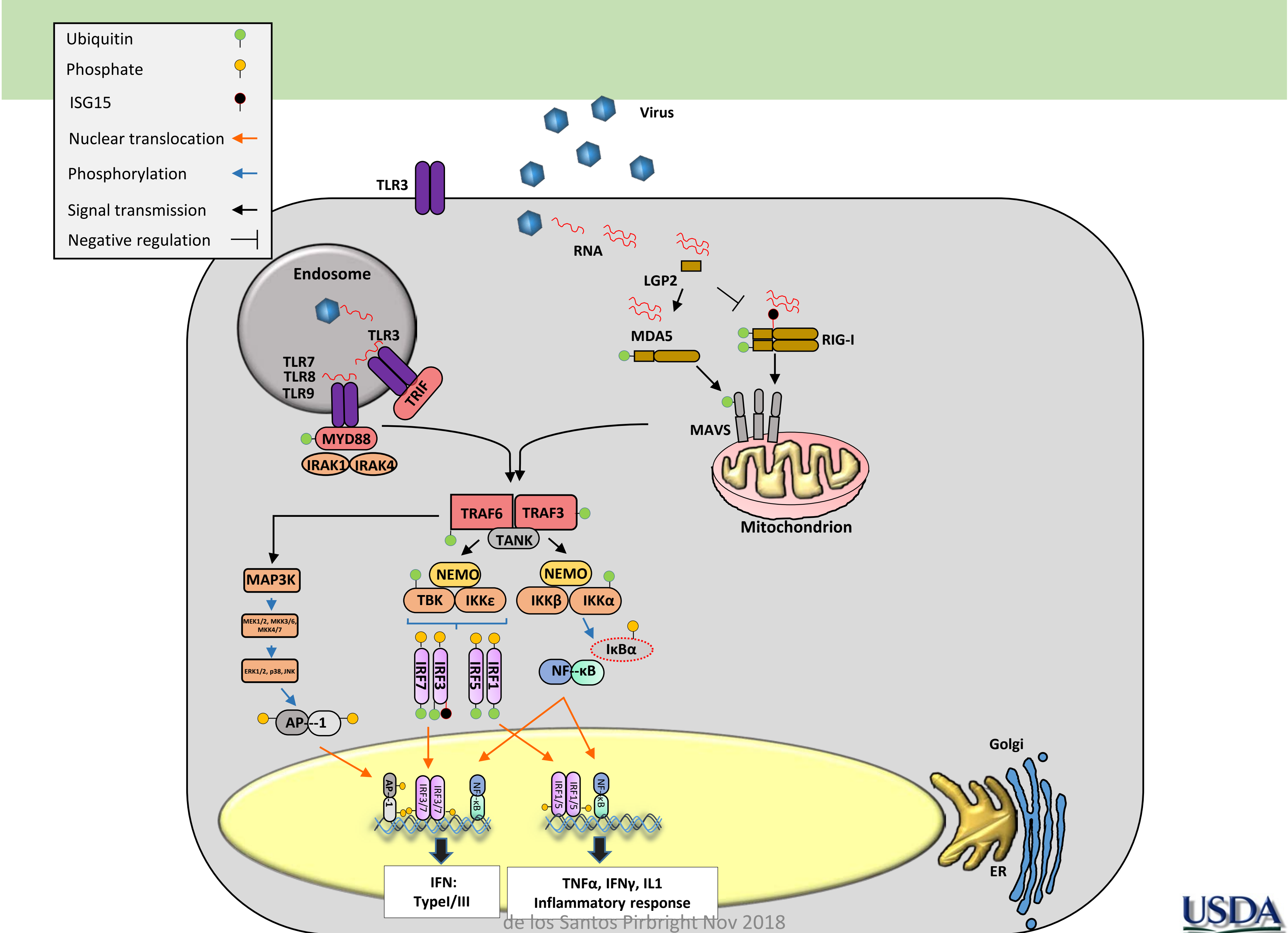
- FMDV contains an error prone polymerase (quasispecies and easiness to mutate for immune escape).

- FMDV displays flexibility on receptor usage (heterodimeric integrins, heparan sulphate, JMJD6)

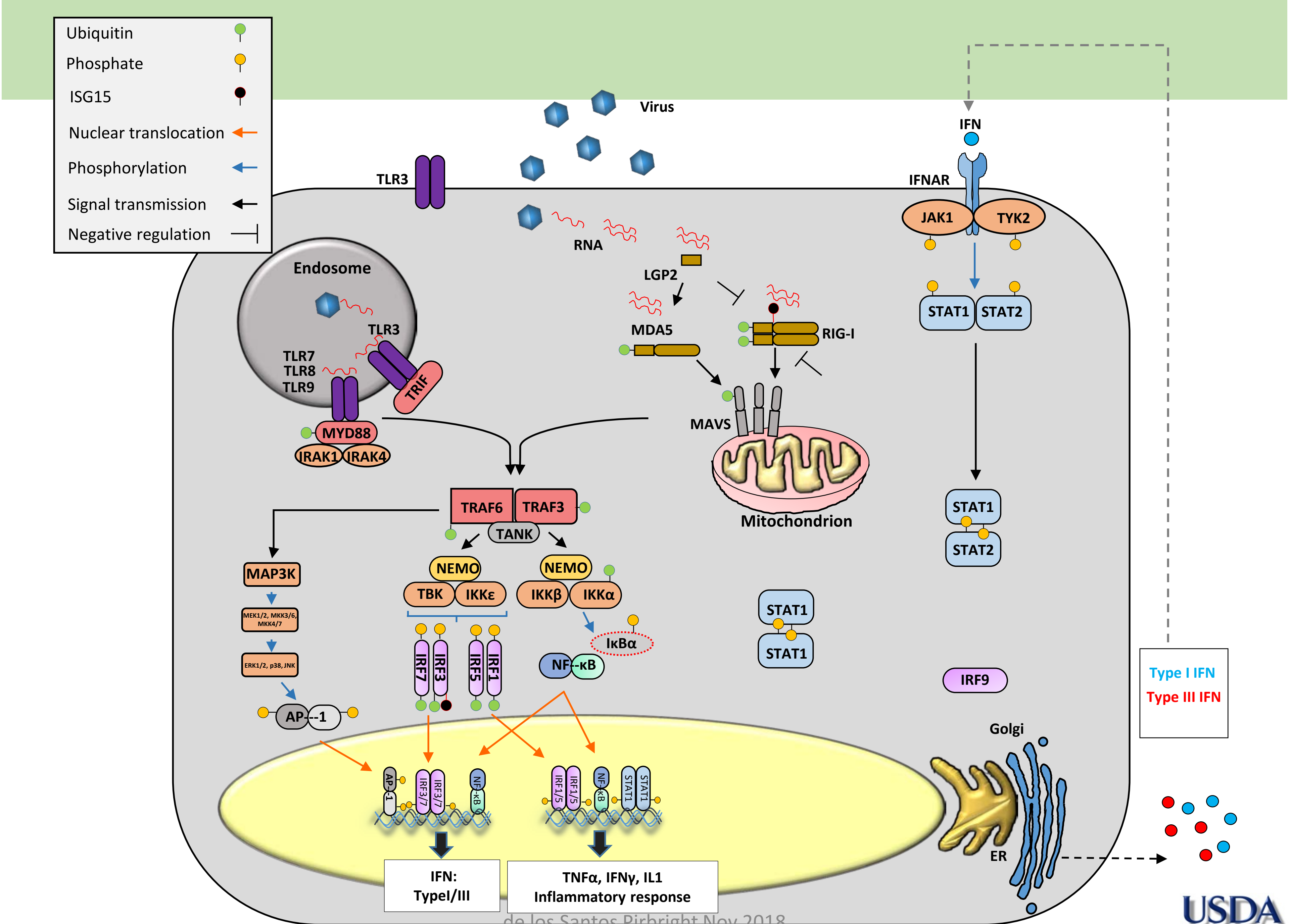
- It can basically use its entire genome to counteract the host immediate innate response to infection

USDA

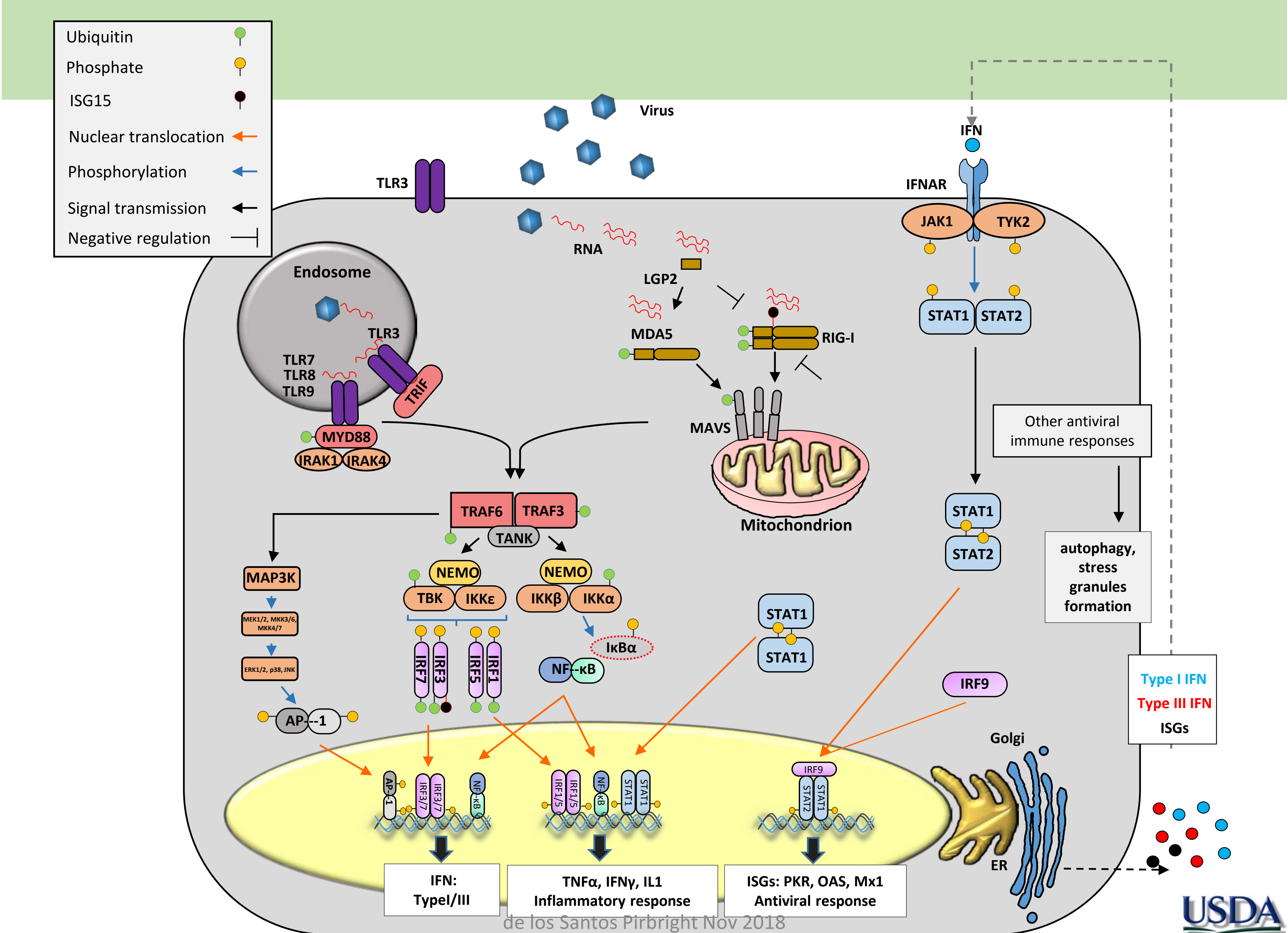
How is FMDV recognized by the host innate immune system?

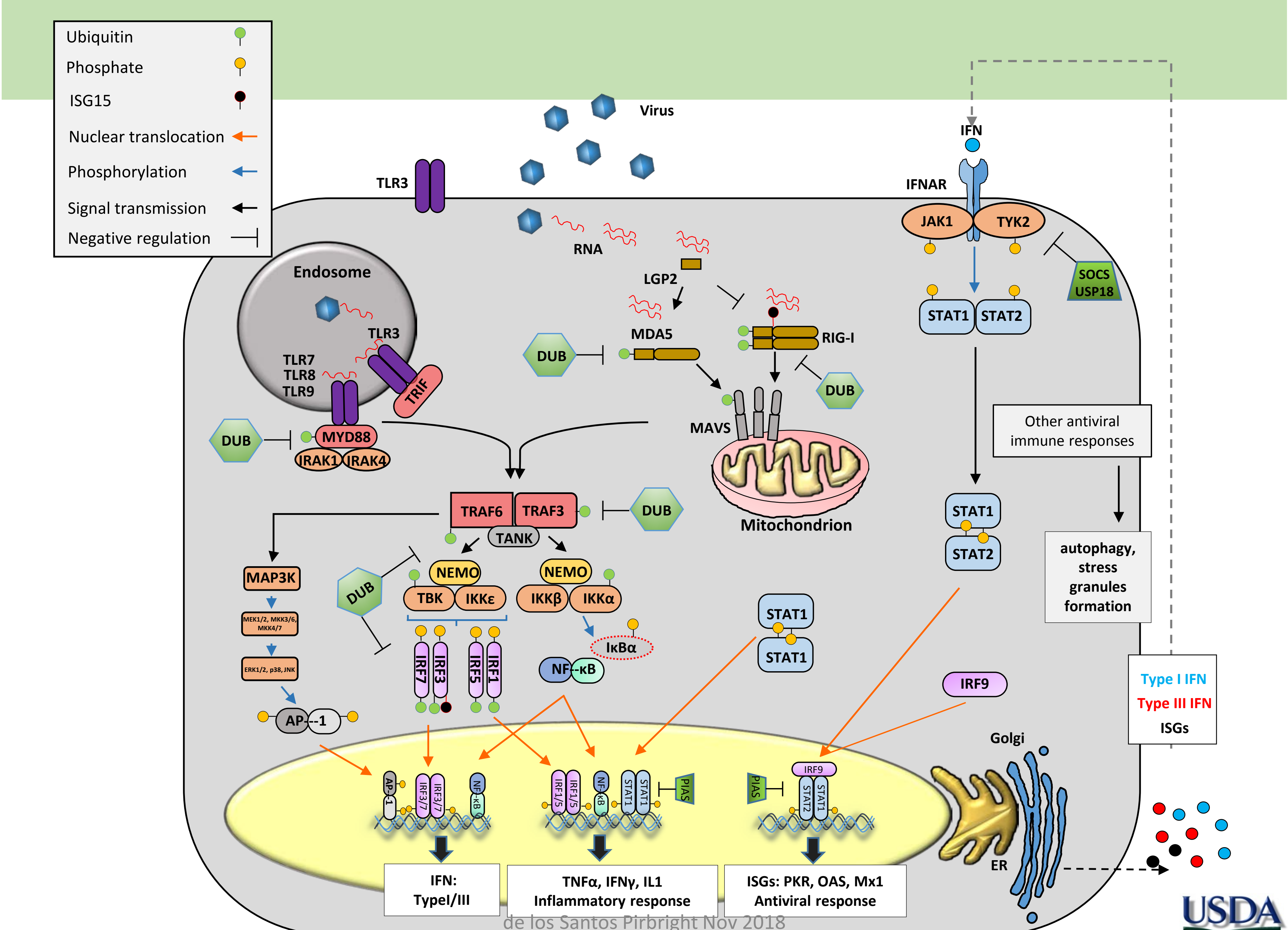


Ubiquitin
Phosphate
ISG15
Nuclear translocation
Phosphorylation
Signal transmission
Negative regulation
Virus
TLR3
RNA
LGP2
MDA5
RIG-I
Endosome
TLR3
TLR7
TLR8
TLR9
TRIF
MYD88
IRAK1
IRAK4
MAVS
Mitochondrion
TRAF6
TRAF3
TANK
MAP3K
MEK1/2, MKK3/6, MKK4/7
ERK1/2, p38, JNK
AP--1
NEMO
TBK
IKKε
NEMO
IKKβ
IKKα
IκBα
NF--κB
IRF7
IRF3
IRF5
IRF1
AP-1
IRF3/7
IRF3/7
NF-κB
IRF1/5
IRF1/5
NF-κB
IFN:
TypeI/III
TNFα, IFNγ, IL1
Inflammatory response
Golgi
ER
de los Santos Pirbright Nov 2018



Ubiquitin
Phosphate
ISG15
Nuclear translocation
Phosphorylation
Signal transmission
Negative regulation
Virus
TLR3
IFN
IFNAR
JAK1
TYK2
RNA
LGP2
MDA5
RIG-I
Endosome
TLR3
TLR7
TLR8
TLR9
TRIF
MYD88
IRAK1
IRAK4
MAVS
Mitochondrion
STAT1
STAT2
TRAF6
TRAF3
TANK
MAP3K
MEK1/2, MKK3/6, MKK4/7
ERK1/2, p38, JNK
AP-1
NEMO
TBK
IKKε
IKKβ
IKKα
IκBα
NF-κB
IRF7
IRF3
IRF5
IRF1
IRF9
IRF3/7
IRF1/5
Golgi
ER
Type I IFN
Type III IFN
IFN:
TypeI/III
TNFα, IFNγ, IL1
Inflammatory response


Ubiquitin
Phosphate
ISG15
Nuclear translocation
Phosphorylation
Signal transmission
Negative regulation
Virus
TLR3
IFN
IFNAR
JAK1
TYK2
RNA
LGP2
MDA5
RIG-I
Endosome
TLR3
TLR7
TLR8
TLR9
TRIF
MYD88
IRAK1
IRAK4
MAVS
Mitochondrion
STAT1
STAT2
Other antiviral immune responses
autophagy, stress granules formation
TRAF6
TRAF3
TANK
MAP3K
MEK1/2, MKK3/6, MKK4/7
ERK1/2, p38, JNK
AP-1
NEMO
TBK
IKKε
NEMO
IKKβ
IKKα
IκBα
NF-κB
IRF7
IRF3
IRF5
IRF1
STAT1
STAT1
IRF9
Type I IFN
Type III IFN
ISGs
Golgi
ER
IRF3/7
IRF1/5
IFN: TypeI/III
TNFα, IFNγ, IL1 Inflammatory response
ISGs: PKR, OAS, Mx1 Antiviral response
de los Santos Pirbright Nov 2018



Ubiquitin
Phosphate
ISG15
Nuclear translocation
Phosphorylation
Signal transmission
Negative regulation
Virus
TLR3
RNA
LGP2
MDA5
RIG-I
DUB
MAVS
Mitochondrion
Endosome
TLR3
TLR7
TLR8
TLR9
TRIF
MYD88
IRAK1
IRAK4
TRAF6
TRAF3
TANK
NEMO
TBK
IKKε
IKKβ
IKKα
IκBα
NF-κB
MAP3K
MEK1/2, MKK3/6, MKK4/7
ERK1/2, p38, JNK
AP-1
IRF7
IRF3
IRF5
IRF1
IFN
IFNAR
JAK1
TYK2
SOCS USP18
STAT1
STAT2
Other antiviral immune responses
autophagy, stress granules formation
IRF9
Type I IFN
Type III IFN
ISGs
Golgi
ER
IRF3/7
IRF1/5
PIAS
IFN: TypeI/III
TNFα, IFNγ, IL1 Inflammatory response
ISGs: PKR, OAS, Mx1 Antiviral response
de los Santos Pirbright Nov 2018

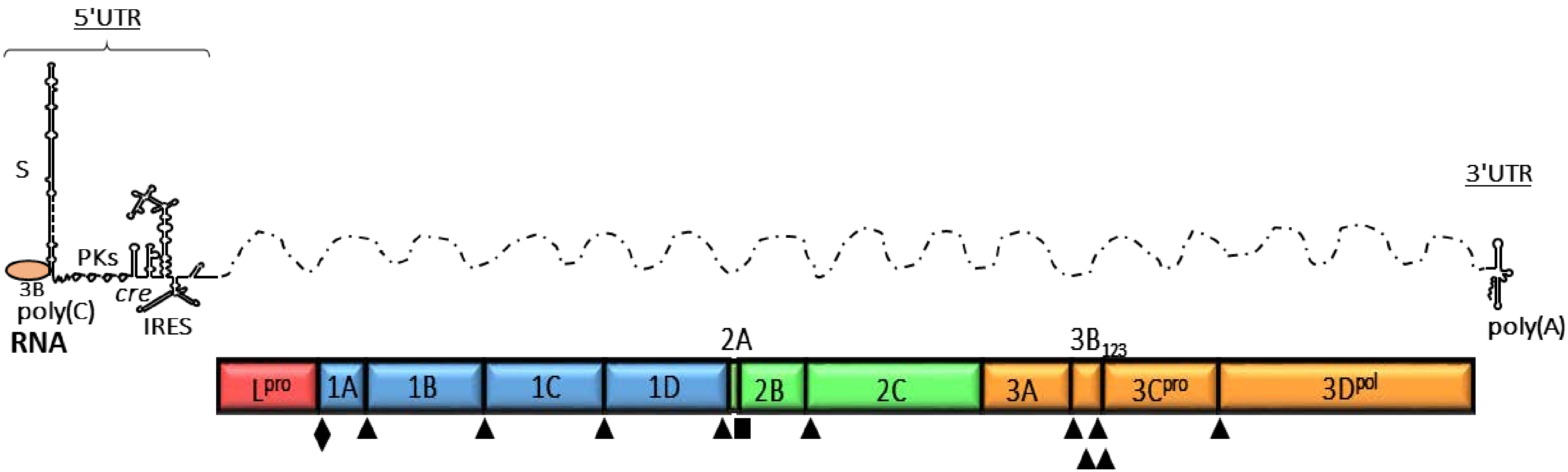
Additional cellular processes also affect innate immunity

Stress granules: organelles that accumulate host RNA and translation machinery upon stress. SGs may also serve as a platform for RIG-I dependent recognition of foreign RNA. MDA5 has also been detected in SGs.
Viruses usually block SG formation to usurp the translation machinery

Autophagy: cell recycling mechanism/self-eating/ programmed cell survival. Energy favorable.
IFN enables autophagy. Activation of Myd88 and TRIFs allow for binding to Beclin1 and positively regulate autophagy (positive loop).
TLR7 dependent induction of IFN in pDCs requires ATG5 (in turn FMDV takes advantage of autophagy and virus endocytosis and replication are stimulated)

Apoptosis: the process of cell death that leads to cytoskeletal disintegration, metabolic unbalance and genomic fragmentation**.**
Apoptosis is induced upon Picornavirus infection but it is abortive. Completion of apoptosis leads to elimination of infected cells, a host innate response against infection.

FMDV battle against the IFN response



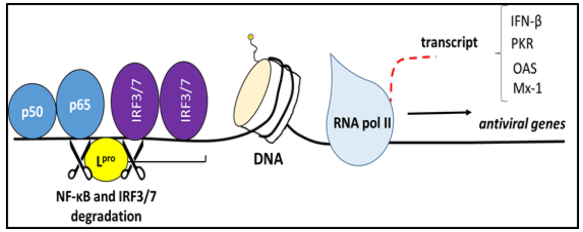
~ 8 kb

14 proteins

2 UTRs

At least 8 viral proteins: L, 2B, 2C, 3A, 3C, VP0, VP1, VP3 and UTRs have been shown as involved in the modulation or control of the host innate response !!!! Couldn't be more efficient..

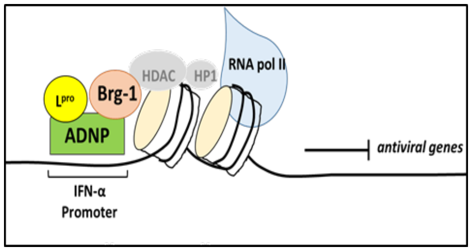
Leader: a security protein



NF-κB and IRF3/7 degradation

Reduced IFN transcription

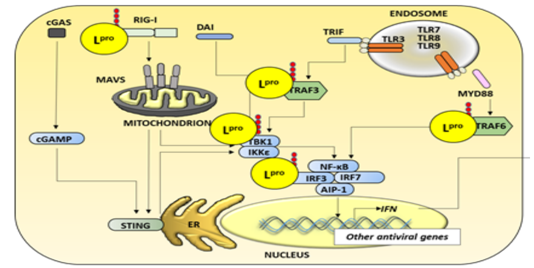
(delosSantos '06;'07;'09; Wang '10)



Chromatin remodeling

IFN transcription repression

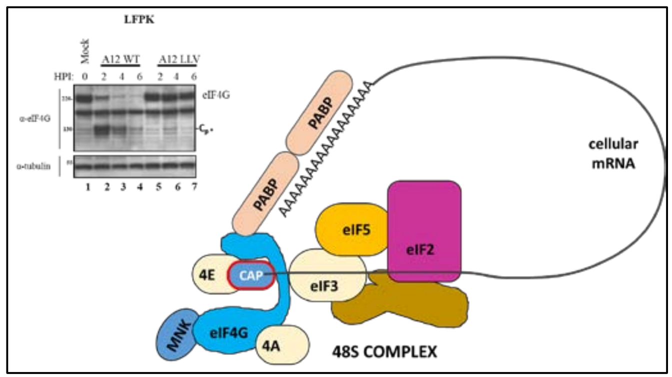
(Medina '17)



De-Ubiquitination

Inactive RIGI, TRAF3/6, IRF3/7

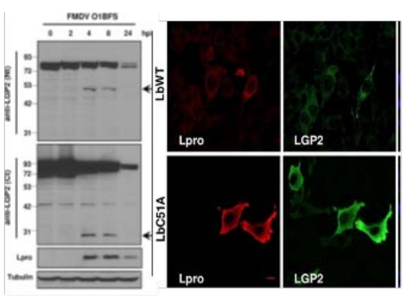
(Wang '11)



eIF-4G cleavage

Translation shut off

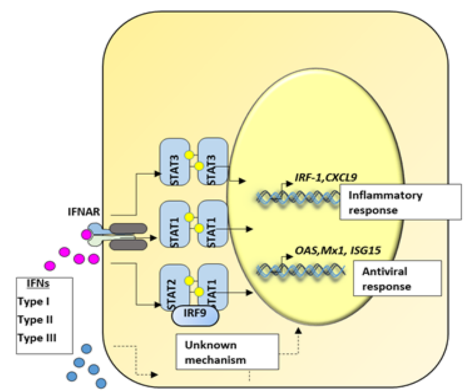
(Devaney '88)



LGP2 cleavage

Reduced IFN transcription

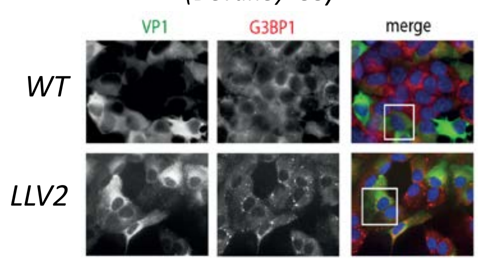
(Rodriguez-Pulido '18)



De-ISGylation

Affects IFN paracrine signaling?

(Swatek '18)



Interference with SG formation

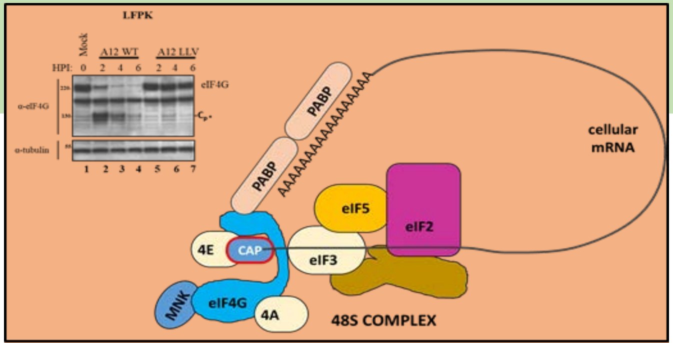
Affects stress response

(Visser, subm)

Journal of Virology, Dec. 1999, p. 9891–9898 Vol. 73, No. 12
0022-538X/99/$04.00+0

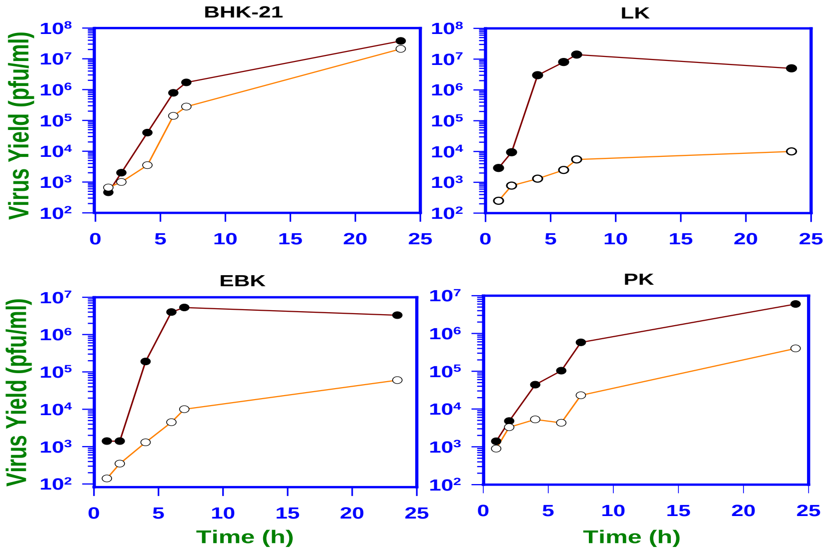
Ability of Foot-and-Mouth Disease Virus To Form Plaques in Cell Culture Is Associated with Suppression of Alpha/Beta Interferon

JARASVECH CHINSANGARAM, MARIA E. PICCONE,† and MARVIN J. GRUBMAN*
Plum Island Animal Disease Center, North Atlantic Area, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York 11944



eIF-4G cleavage

Translation shut off
(Devaney '88)



Cell supernatant source	Inducing virus	MOI	Antiviral units
EBK	None		<2
	LLV2	1	32
	WT	1	ND
	LLV2	10	>64
	WT	10	8
LK	None		<2
	LLV2	1	>64
	WT	1	4
	LLV2	10	64
	WT	10	ND
PK	None		<2
	LLV2	1	16
	WT	1	<2
	LLV2	10	32
	WT	10	<2
BHK	None		<2
	LLV2	1	<2
	WT	1	-
	LLV2	10	<2
	WT	10	-

Journal of Virology, June 2001, p. 5498–5503 Vol. 75, No. 12
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.12.5498–5503.2001

Inhibition of L-Deleted Foot-and-Mouth Disease Virus Replication by Alpha/Beta Interferon Involves Double-Stranded RNA-Dependent Protein Kinase

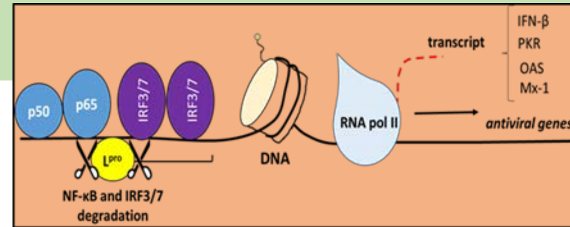
JARASVECH CHINSANGARAM, MARLA KOSTER, and MARVIN J. GRUBMAN*

USDA

JOURNAL OF VIROLOGY, Feb. 2006, p. 1906–1914 Vol. 80, No. 4
0022-538X/06/$08.00+0 doi:10.1128/JVI.80.4.1906–1914.2006

The Leader Proteinase of Foot-and-Mouth Disease Virus Inhibits the Induction of Beta Interferon mRNA and Blocks the Host Innate Immune Response

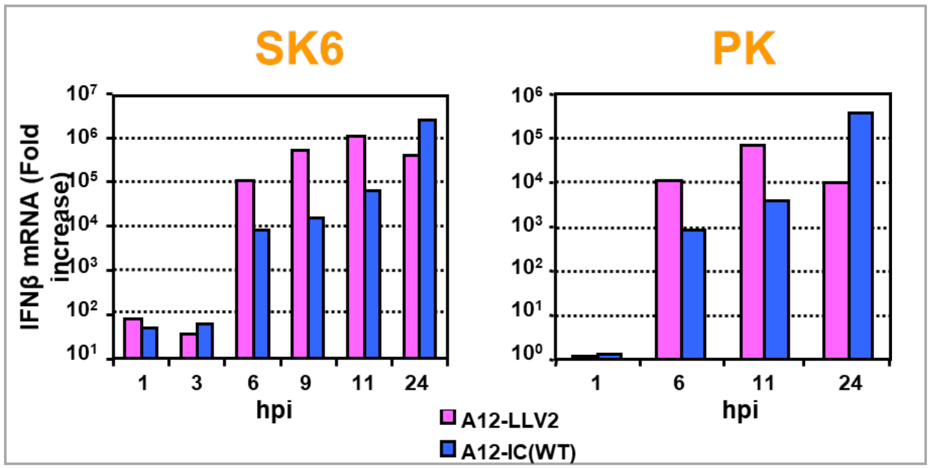
Teresa de los Santos,† Sonia de Avila Botton,†‡ Rudi Weiblen,‡ and Marvin J. Grubman*
Plum Island Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York 11944

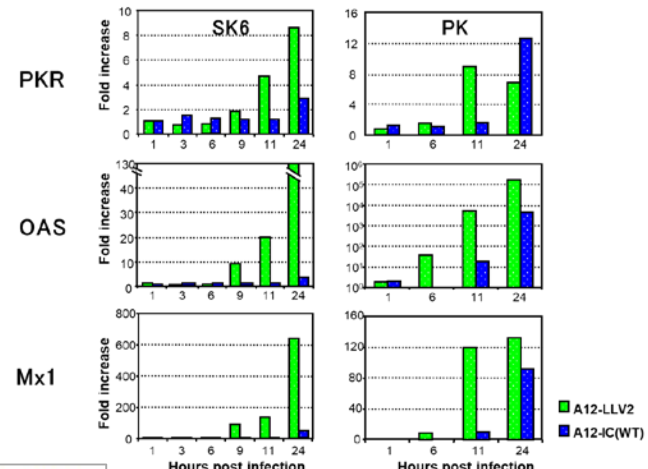


NF-κB and IRF3/7 degradation

Reduced IFN transcription

(delosSantos '06;'07;'09; Wang '10)

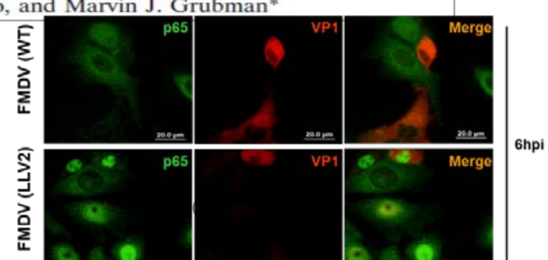


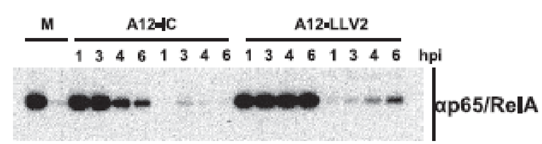


JOURNAL OF VIROLOGY, Dec. 2007, p. 12803–12815 Vol. 81, No. 23
0022-538X/07/$08.00+0 doi:10.1128/JVI.01467-07

Degradation of Nuclear Factor Kappa B during Foot-and-Mouth Disease Virus Infection▽

Teresa de los Santos, Fayna Diaz-San Segundo, and Marvin J. Grubman*

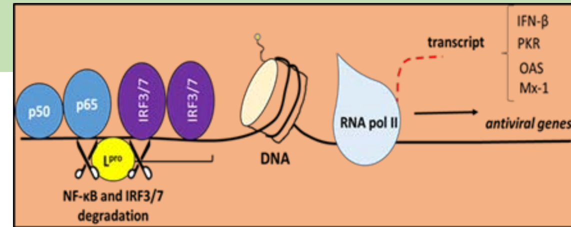




SAP

A Conserved Domain in the Leader Proteinase of Foot-and-Mouth Disease Virus Is Required for Proper Subcellular Localization and Function

Teresa de los Santos,* Fayna Diaz-San Segundo, James Zhu, Marla Koster, Camila C. A. Dias, and Marvin J. Grubman



NF-κB and IRF3/7 degradation

Reduced IFN transcription

(delosSantos '06;'07;'09; Wang '10)

Biochemical and Biophysical Research Communications 399 (2010) 72–78

Contents lists available at ScienceDirect

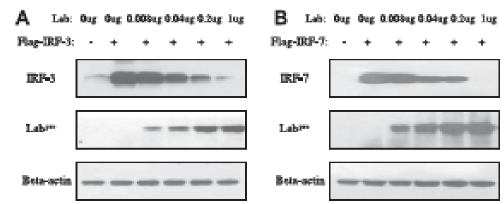
Biochemical and Biophysical Research Communications

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

Foot-and-mouth disease virus leader proteinase inhibits dsRNA-induced type I interferon transcription by decreasing interferon regulatory factor 3/7 in protein levels

Dang Wang, Liurong Fang, Rui Luo, Rui Ye, Ying Fang, Lilan Xie, Huanchun Chen, Shaobo Xiao *

Division of Animal Infectious Diseases, State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, PR China



39 differentially expressed genes containing promoters with canonical NF-κB and IRF binding sites

	P	Q	R	S	T	U	V
1	Matrix	TFBS	Name	Threshold	Hits	Probability	
2	M00043	I$DL_01	dorsal	0.89	37	2.91E-12	
3	M00052	V$NFKAPPAB65_01	NF-kappaB (p65)	0.84	160	1.05E-11	
4	M00053	V$CREL_01	c-Rel	0.83	293	1.14E-15	
5	M00062	V$IRF1_01	IRF-1	0.85	78	1.61E-11	
6	M00063	V$IRF2_01	IRF-2	0.86	41	2.30E-10	
7	M00171	I$ADF1_Q6	Adf-1	0.86	229	5.61E-28	
8	M00194	V$NFKB_Q6	NF-kappaB	0.78	365	2.16E-18	
9	M00208	V$NFKB_C	NF-kappaB binding site	0.84	95	4.74E-15	
10	M00253	V$CAP_01	transcription initiation	0.98	193	1.97E-06	
11	M00258	V$ISRE_01	ISGF-3	0.85	64	2.37E-21	
12	M00051	V$NFKAPPAB50_01	NF-kappaB (p50)	0.79	191	[illegible]	
13	M00054	V$NFKAPPAB_01	NF-kappaB	0.87	112	[illegible]	
14							

Virology 404 (2010) 32–40

Contents lists available at ScienceDirect

Virology

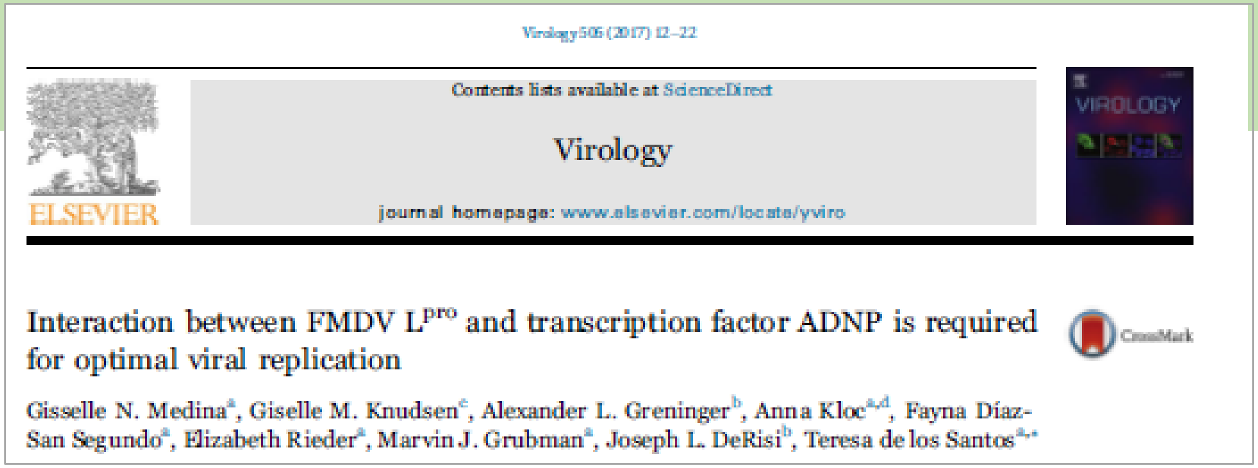
journal homepage: www.elsevier.com/locate/yviro

Differential gene expression in bovine cells infected with wild type and leaderless foot-and-mouth disease virus

James Zhu [a], Marcelo Weiss [a,b], Marvin J. Grubman [a], Teresa de los Santos [a,*]

[a] Plum Island Animal Disease Center, North Atlantic Area, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York 11944, USA

[b] Oak Ridge Institute for Science and Education, PIADC Research Participation Program, Oak Ridge, TN 37831, USA



Virology 506 (2017) 12–22

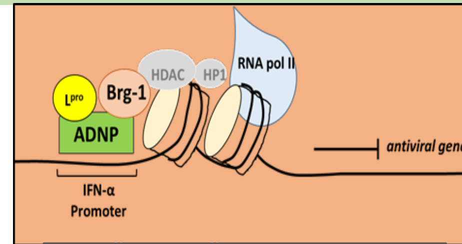
Contents lists available at ScienceDirect

Virology

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

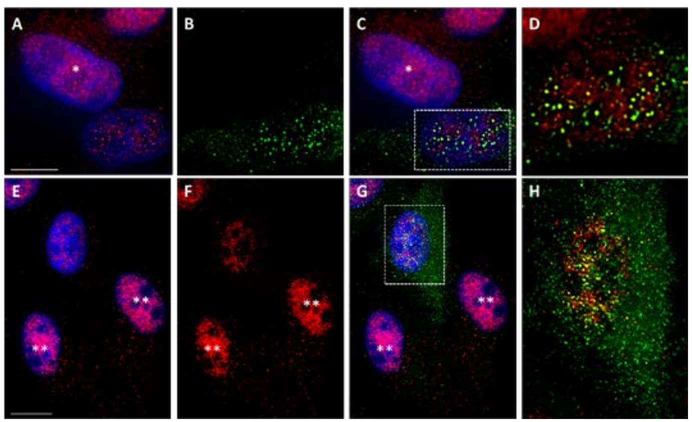
Interaction between FMDV L[pro] and transcription factor ADNP is required for optimal viral replication

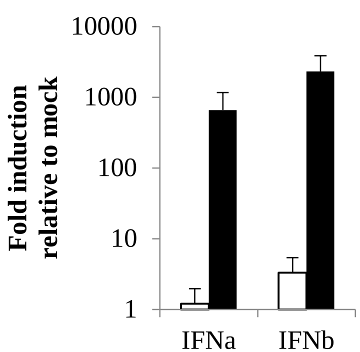
Gisselle N. Medina[a], Giselle M. Knudsen[c], Alexander L. Greninger[b], Anna Kloc[a,d], Fayna Díaz-San Segundo[a], Elizabeth Rieder[a], Marvin J. Grubman[a], Joseph L. DeRisi[b], Teresa de los Santos[a,*]

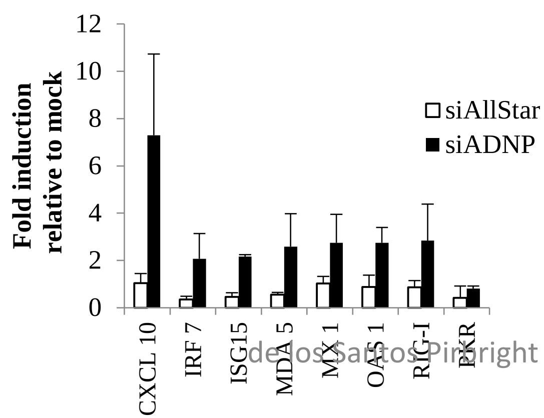


Chromatin remodeling
IFN transcription repression
Medina '17

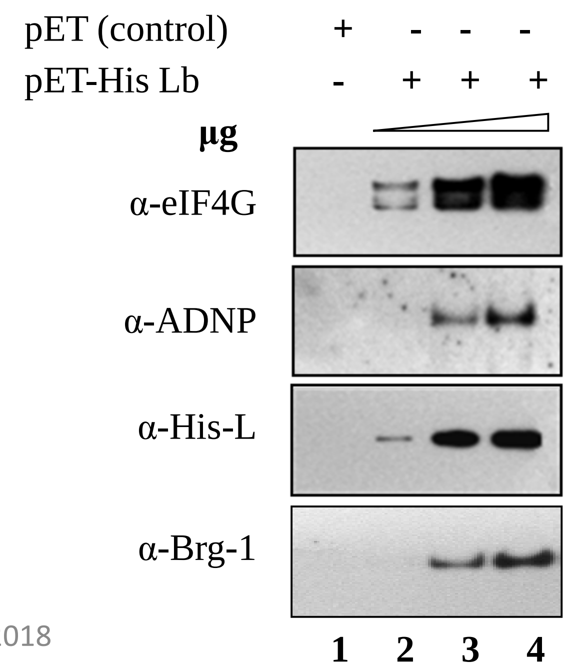
ADNP colocalizes with Lpro







Lpro interacts with ADNP and BRG1 (SNF/SWI complex)

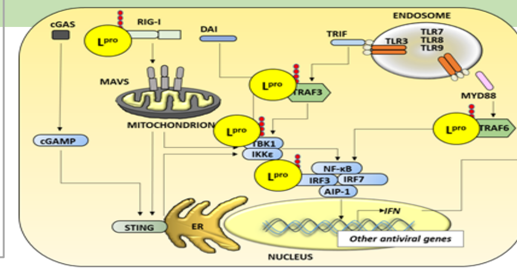


JOURNAL OF VIROLOGY, Apr. 2011, p. 3758–3766
0022-538X/11/$12.00 doi:10.1128/JVI.02589-10

Vol. 85, No. 8

The Leader Proteinase of Foot-and-Mouth Disease Virus Negatively Regulates the Type I Interferon Pathway by Acting as a Viral Deubiquitinase

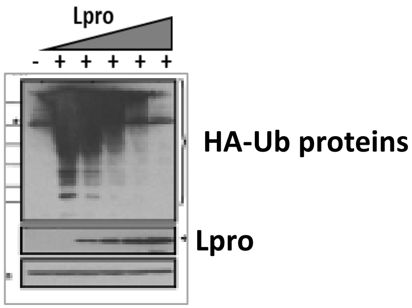
Dang Wang,[1] Liurong Fang,[1] Ping Li,[1] Li Sun,[2] Jinxiu Fan,[1] Qingye Zhang,[3] Rui Luo,[1] Xiangtao Liu,[4] Kui Li,[5] Huanchun Chen,[1*] and Shaobo Xiao[1*]

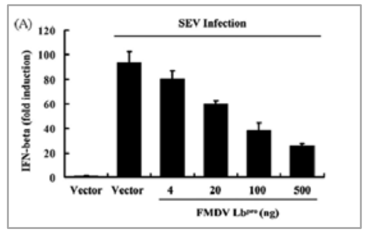


De-Ubiquitination

Inactive RIGI, TRAF3/6, IRF3/7

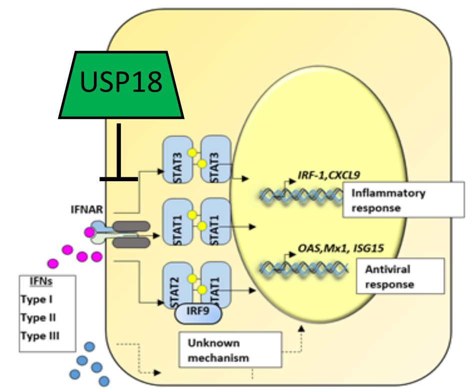
(Wang '11)





Irreversible inactivation of ISG15 by a viral leader protease enables alternative infection detection strategies

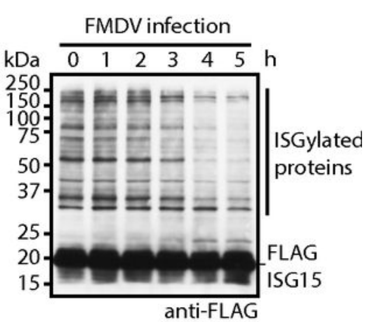
Kirby N. Swatek[a], Martina Aumayr[b,1], Jonathan N. Pruneda[a,1], Linda J. Visser[c,1], Stephen Berryman[d], Anja F. Kueck[a], Paul P. Geurink[e], Huib Ovaa[e], Frank J. M. van Kuppeveld[c], Tobias J. Tuthill[d], Tim Skern[b], and David Komander[a,2]

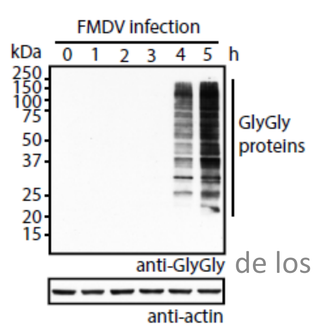


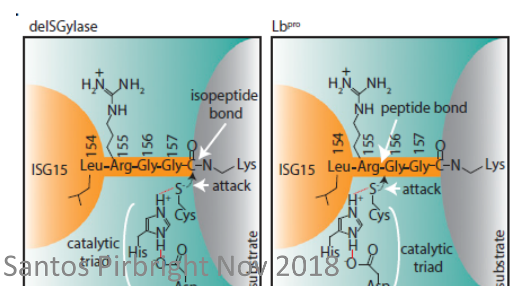
De-ISGylation

Affects IFN paracrine signaling?

(Swatek '18)

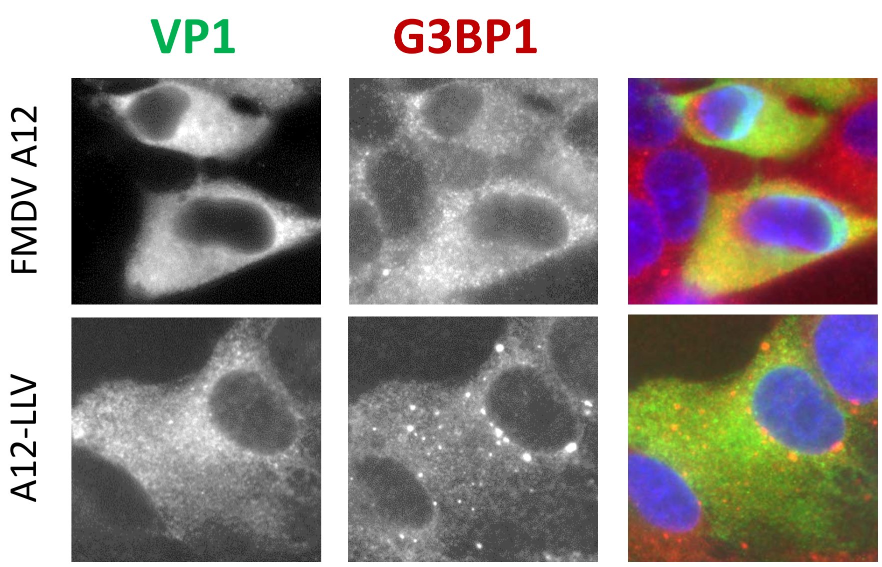


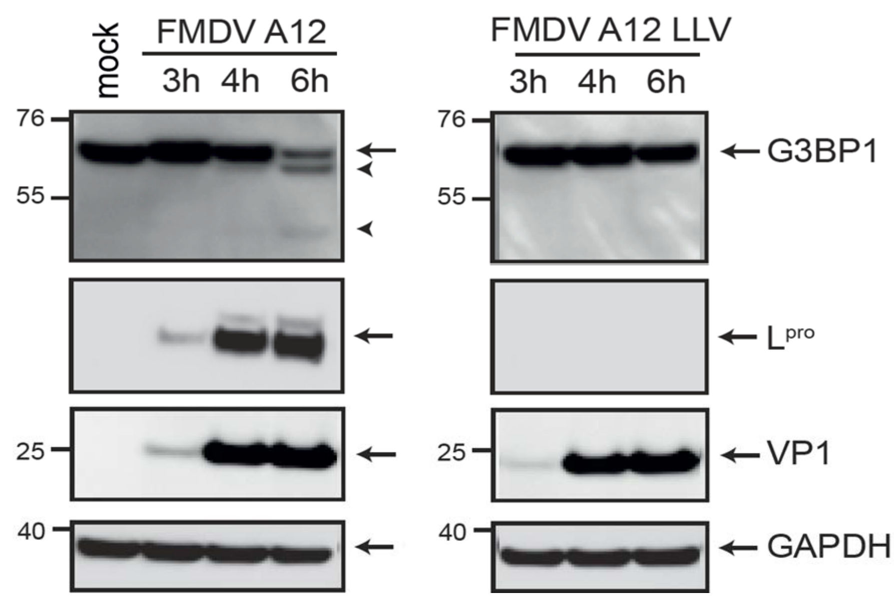




L^{pro} suppresses SG formation and cleaves G3BP1/2

Visser et al, 2018 submitted





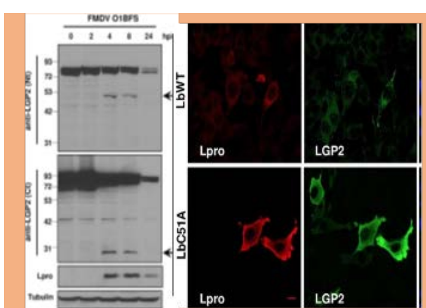

PLOS | PATHOGENS

June 29, 2018

RESEARCH ARTICLE

Innate immune sensor LGP2 is cleaved by the Leader protease of foot-and-mouth disease virus

Miguel Rodríguez Pulido[1], María Teresa Sánchez-Aparicio[2,3], Encarnación Martínez-Salas[1], Adolfo García-Sastre[2,3,4], Francisco Sobrino[1], Margarita Sáiz[1]*



LGP2 cleavage
Reduced IFN transcription
(Rodriguez-Pulido '18)

Leader: a security protein

Dispensable for virus replication but is directly associated to virulence and pathogenicity in cells and in cattle and swine

1. Cleaves eIF4G shutting off host cell translation (IFN and ISG)
2. Induces degradation of NF-κB, IRF3 and IRF7 reducing transcription of IFN, inflammatory cytokines and ISGs
3. Removes Ubiquitin from key signal molecules in IFN induction: RIG1, TRAF3/6, TBK1
4. Interacts with transcription factors ADNP and BRG1, involved in the epigenetic control of IFN expression (transcriptional repression)
5. Interacts and causes degradation of LGP2 affecting IFN induction and favoring FMDV replication
6. Removes ISG15 modifier possibly affecting innate responses
7. Cleaves G3BP1/2 to prevent SG formation possibly affecting innate responses

USDA

FMDV factor	Affected process	Viral counter-mechanism
L^{pro}	Translation and transcription	• eIF4G1 cleavage (Devaney 1988; Kirchweger 1994) • Gemin5 cleavage (Pineiro 2012) • Decreased amounts of IFNβ (de los Santos 2007) • Degradation of NF-κB (de los Santos 2007) • Modulation of PKR (Chinsangaram 2001; de los Santos 2006) • Inhibition of RANTES (de los Santos 2006; Wang 2010) • Induction of ADNP binding to IFNα promoter to disrupt the expression of IFN and ISGs (Medina 2017) • Deubiquitination of proteins involved in innate immunity signaling (RIG-I, TBK1, TRAF3, TRAF6) (Wang 2011) • DeISGylation (Swatek 2018) • Modulation of IFNβ expression through interaction with LGP2 (Rodriguez-Pulido 2018)
2B+2C and or 2BC	Membrane rearrangements, secretion and trafficking, autophagy and modulation of ISGs expression	• Membrane rearrangements (Teterina 2006; Monaghan 2004) • Inhibition of MHC class I surface expression and secretion of antiviral cytokines (Moffat et al., 2005, 2007; Sanz-Parra et al., 1998) • Modulation of cytopathogenicity (Arias 2010) • Induction of autophagy (O'Donnell 2011; Berryman 2012, Gladue 2012) • Alteration of Ca2+ concentrations leading to autophagy (Ao 2015) • Interaction with RIG-I to suppress expression of ISGs and GBP1 (Zhu 2016) • Interaction with LGP2 (Zhu 2017; Rodriguez-Pulido 2018) • Induction of apoptosis via interaction with Nmi (Wang 2012b) • Interaction with IFN-induced protein IF35 (Zheng 2014)

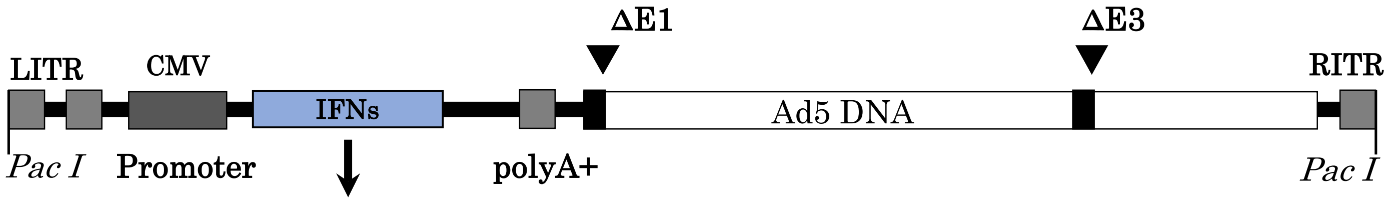
FMDV factors	Affected process	Viral counter-mechanism
3A	Membranes and innate immunity signaling factors	• Interaction with membranes (Gonzales-Magaldi 2014, Lotufo 2018) • Inhibition of RLR (RIG-I, MDA5, MAVS)-mediated IFNβ induction (Li 2016a)
3C^pro^	Transcription, translation and autophagy	• Histone H3 cleavage (Grigera 1984; Tesar 1990; Falk 1990) • eIF4G and eIF4A cleavage (Belsham 2000) • Sam68 cleavage (Lawrence 2012) • NEMO cleavage (Wang 2012a) • Reduction of the endogenous levels of PKR (Li 2017) • Interference of JAK-STAT signaling pathway (Du 2014) • Degradation of autophagy proteins ATG5 and ATG12 (Fan 2017) • Cleavage of G3BP1 (SG marker) (Galan 2017; Ye 2018)
VP1, VP2, VP3	Suppression of innate immune signaling responses (type I IFN) and autophagy	• Interaction with the cellular protein sorcin to downregulate transcription of IFNα/β and NF-κB (Li 2013) • Downregulation of TNFα and NF-κB (Ho 2014; Wang 2016) • Induction of autophagy (Sun 2018) • Inhibition of STAT phosphorylation (Li 2016b) • Blockage of IRF3 phosphorylation and dimerization (Li 2016b) • Capsid assembly requires chaperone Hsp90 (Newman 2017). This interaction may affect PRR innate signaling (Binder 2014, Andino 2018).
Untranslated regions	Modulation of innate immune signaling	• 5'UTR can stimulate type I IFN responses: Mx-1, IFNβ, IL-6, TNFα, IRF7 (Rodriguez-Pulido 2011, Kloc 2017). • 3'UTR can trigger an antiviral state via IFNβ (Rodriguez-Pulido 2011)

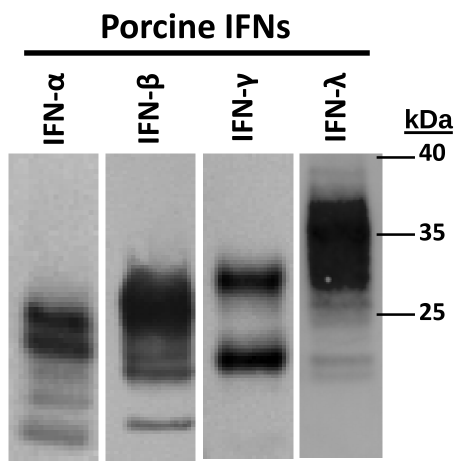
USDA

How can we use this wealth of information to develop or improve FMD countermeasures?

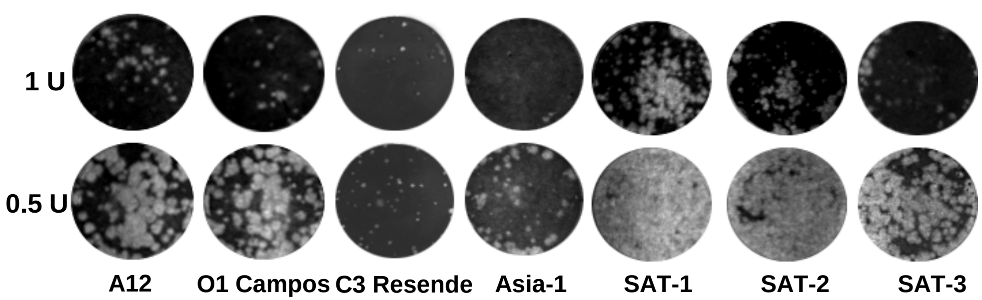
Can treatment with IFN prevent FMD?

Ad5-IFN blocks FMDV infection *in vitro*



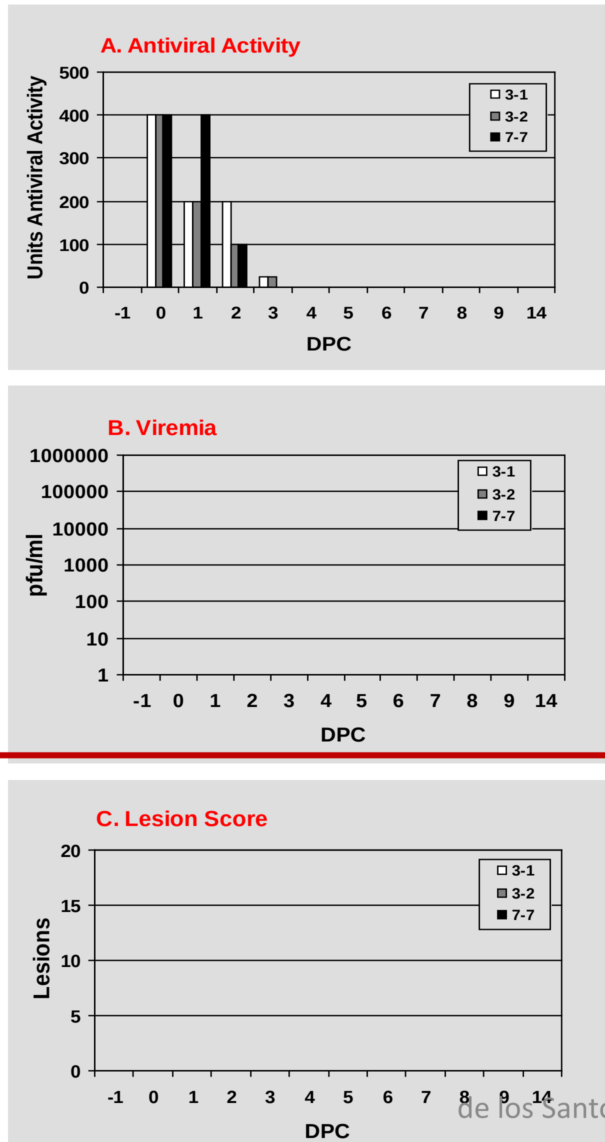


- ✓ **Type I IFN**
  (*Chinsangaram 2003; Moraes 2003; Dias 2011)*
- ✓ **Type II IFN**
  (*Moraes 2007; Kim 2014)*
- ✓ **Type III IFN**
  (*Perez-Martin 2014; Perez-Martin 2016; Diaz-San Segundo 2017)*

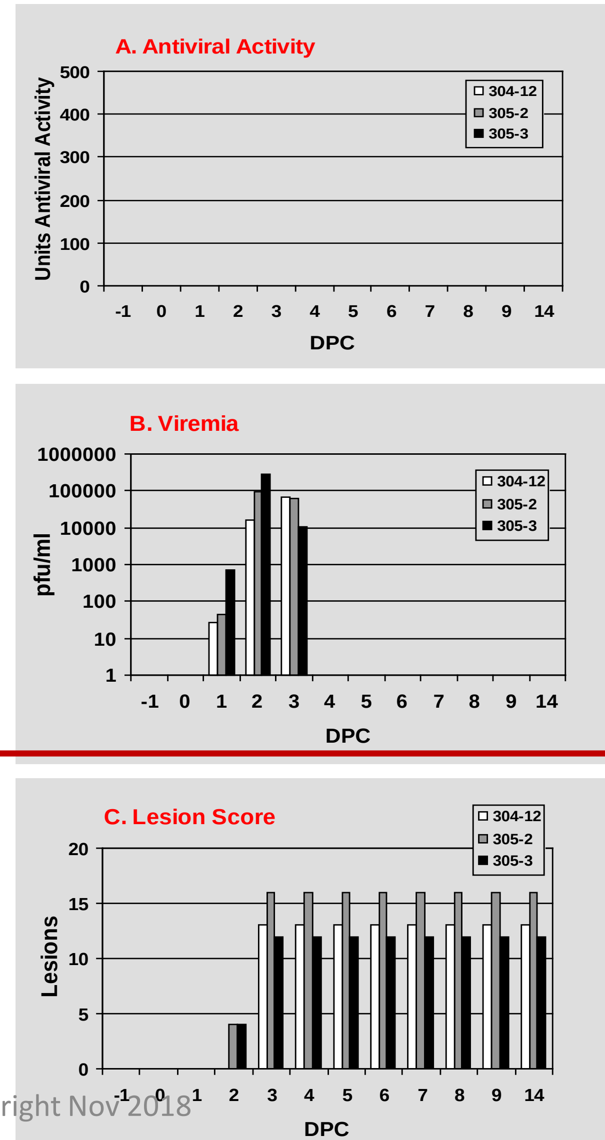


Ad5-poIFNα protects swine against FMD

Ad5-pIFNα 10^9 pfu

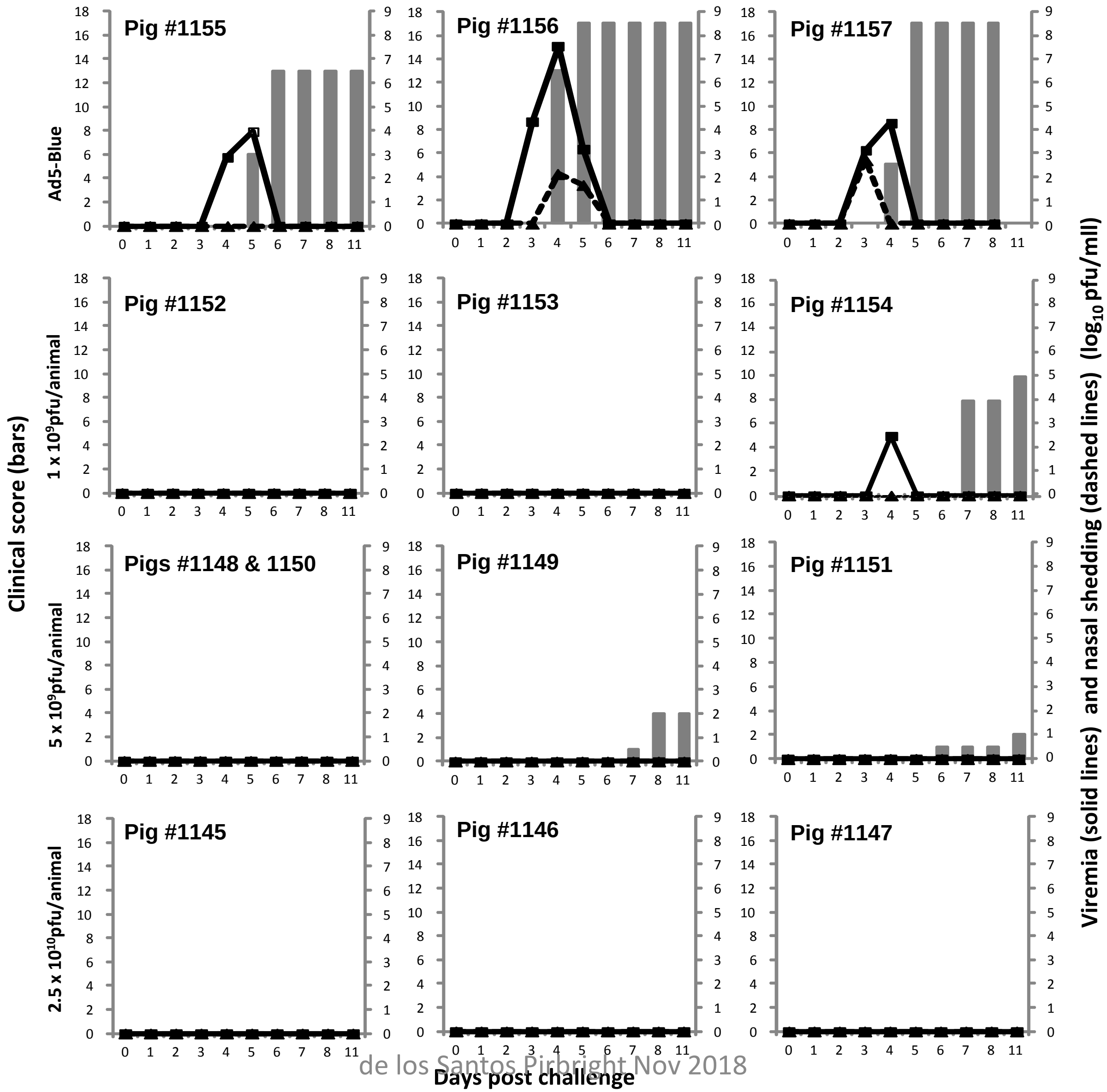


Ad5-blue 10^9 pfu



Chinsangaram 2003
Moraes 2003
Dias 2011

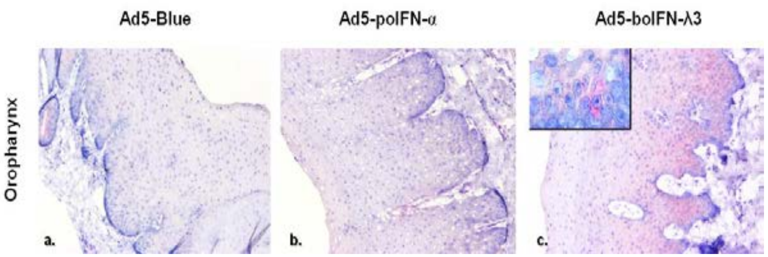
Ad5-poIFNλ protects swine against FMD



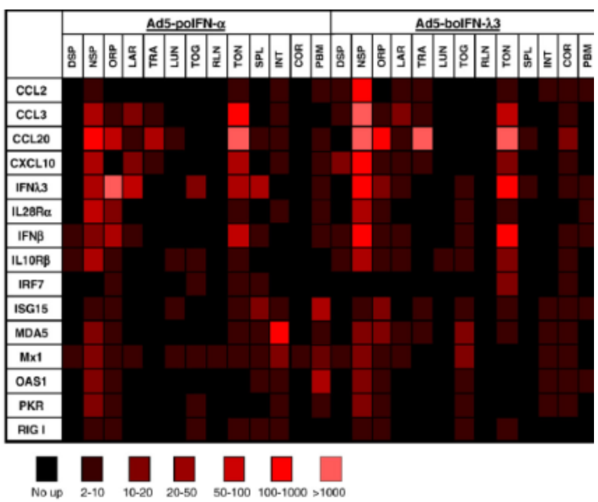
de los Santos Pirbright Nov 2018

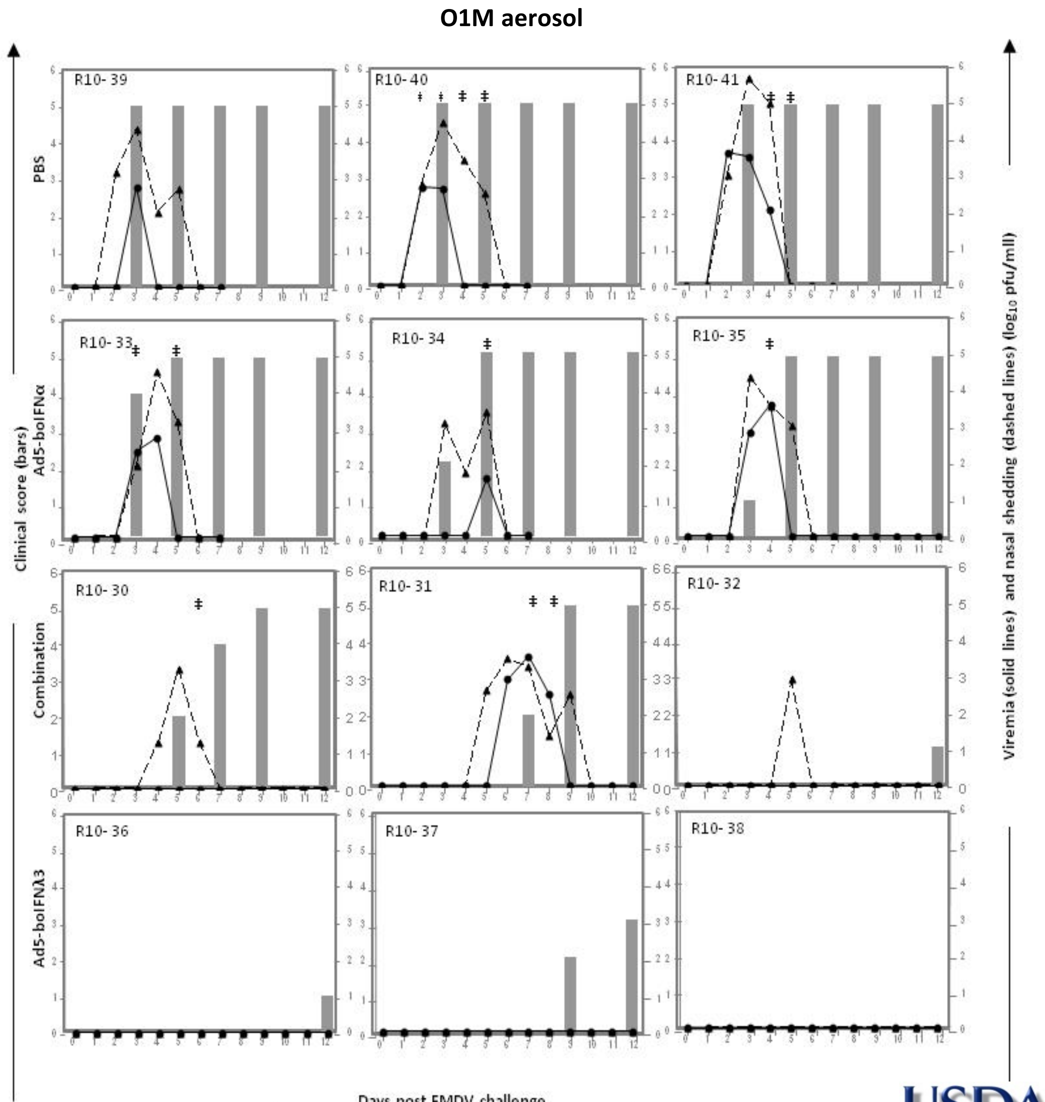
Perez-Martin, Diaz-San Segundo, et al 2014

Ad5-boIFNλ3 protects cattle against FMD



Mx1 staining





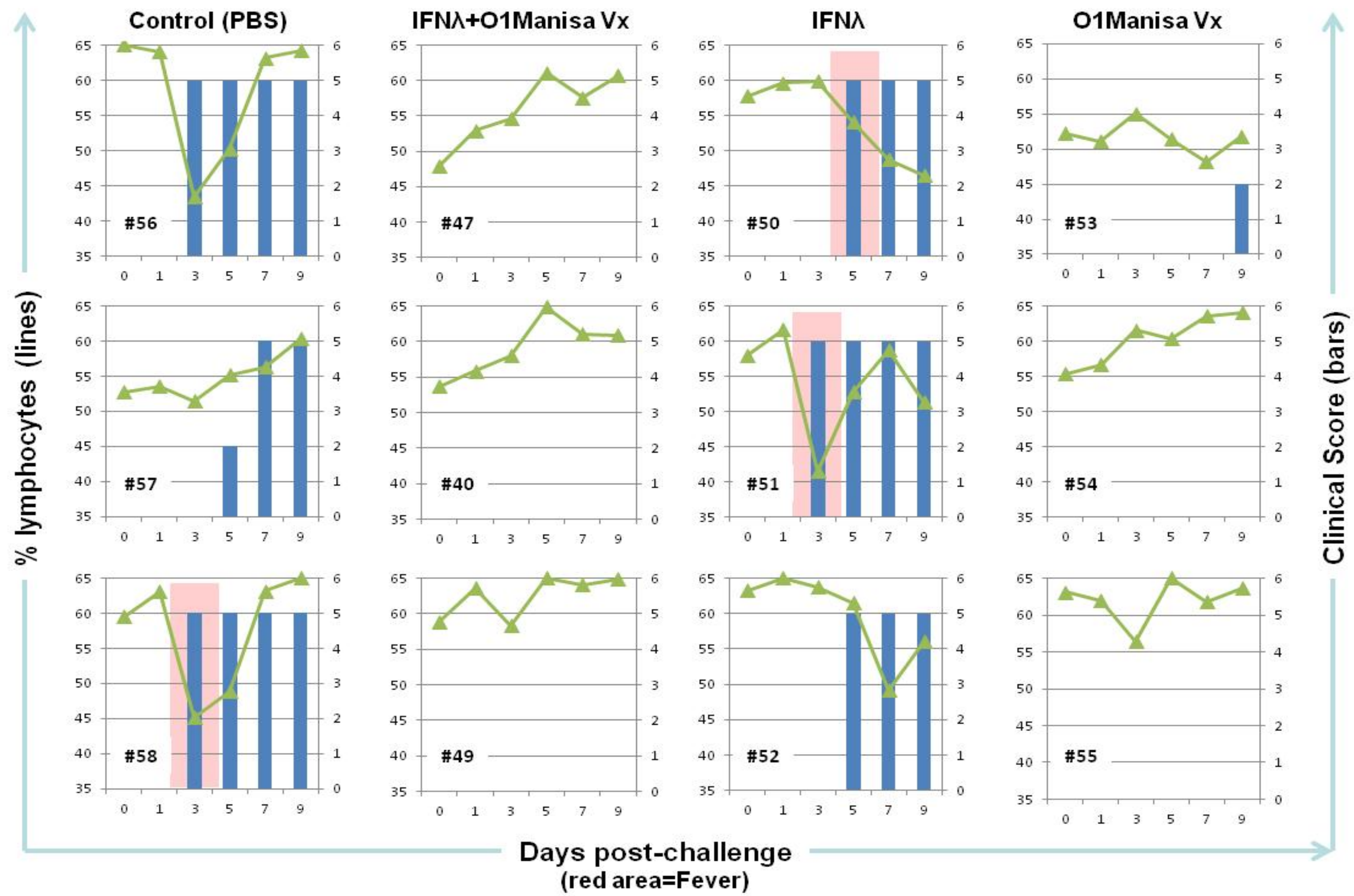
Diaz San-Segundo 2011; Perez-Martin 2012

Ad5-poIFNα and Ad5-FMD protect swine against FMD

Group	IFN (U/ml)	Viremia/dpc	Clinical score/dpc
Control			
9987	52	$2.0x10^5/1$	14/2
9988	1,867	$1.0x10^3/1$	13/3
9989	0	$6.1x10^4/1$	14/2
Ad5-A24			
9981	0	0/0	2/3
9982	395	0/0	6/3
9983	0	0/3	9/3
Ad5-IFNα			
9975	13,718	0/0	0/0
9976	15,431	0/0	0/0
9977	16,059	0/0	0/0
Ad5-IFNα +Ad5A24			
9972	17,390	0/0	0/0
9973	18,631	0/0	0/0
9974	17,654	0/0	0/0

Moraes 2003

Ad5-boIFNλ3 and Ad5-FMD protect cattle against FMD



Diaz San Segundo 2017

Biotherapeutics protect against FMD !

- Ad5- type I, II and III IFNs protect swine against FMD
- Combination treatment of Ad5-poIFNα and Ad5-FMD can fully protect swine as early as 1 day post inoculation and for 5 days (Ad5-FMD fully protects swine at 7dpi)
- Inducers of innate immunity such as Poly ICLC , VRPs and IRF7/3(5D) can protect by themselves against FMD
- ISGs including PKR, OAS, IP10 and increased numbers of DCs and NK cells in draining lymph nodes are responsible for the IFN-mediated protection against FMD in swine

- Ad5-type III IFN protects cattle against FMD
- Combination treatment of Ad5-boIFNλ and Ad5-FMD can fully protect cattle against FMD

Thank you

Gisselle Medina
Paul Azzinaro
Joseph Gutkoska
Aishwarya Mogulothu

Fayna Diaz-San Segundo

Luis Rodriguez

Jonathan Arzt/Carolina Stenfeldt
Bill Golde
James Zhu
Elizabeth Rieder
Manuel Borca
Penny Berry

PIADC Animal Research Unit

Marvin J. Grubman

Eva Perez-Martin
Mauro Moraes
Lek Chinsangaram
Josh Wu
Camila Dias
Diego Sturza
Elizabeth Ramirez-Medina
Marcelo Weiss
Marla Koster

Happy 60th Pirbright birthday!