Global Foot-and-Mouth Disease Research Alliance

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CSIC

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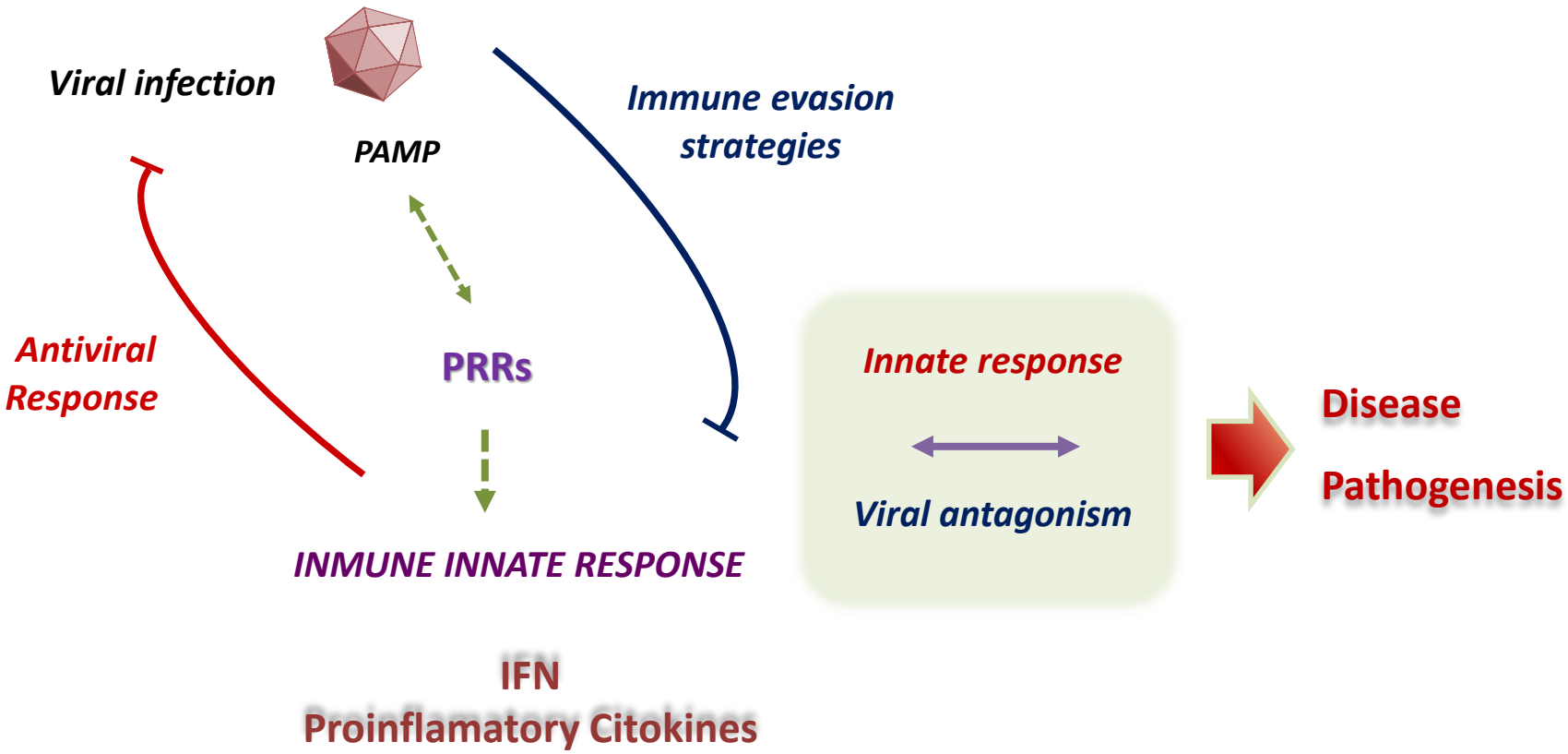
Session 6 - Virology

FMDV UNDERMINES THE HOST ANTIVIRAL RESPONSE BY CLEAVAGE OF KEY INNATE IMMUNE SENSORS

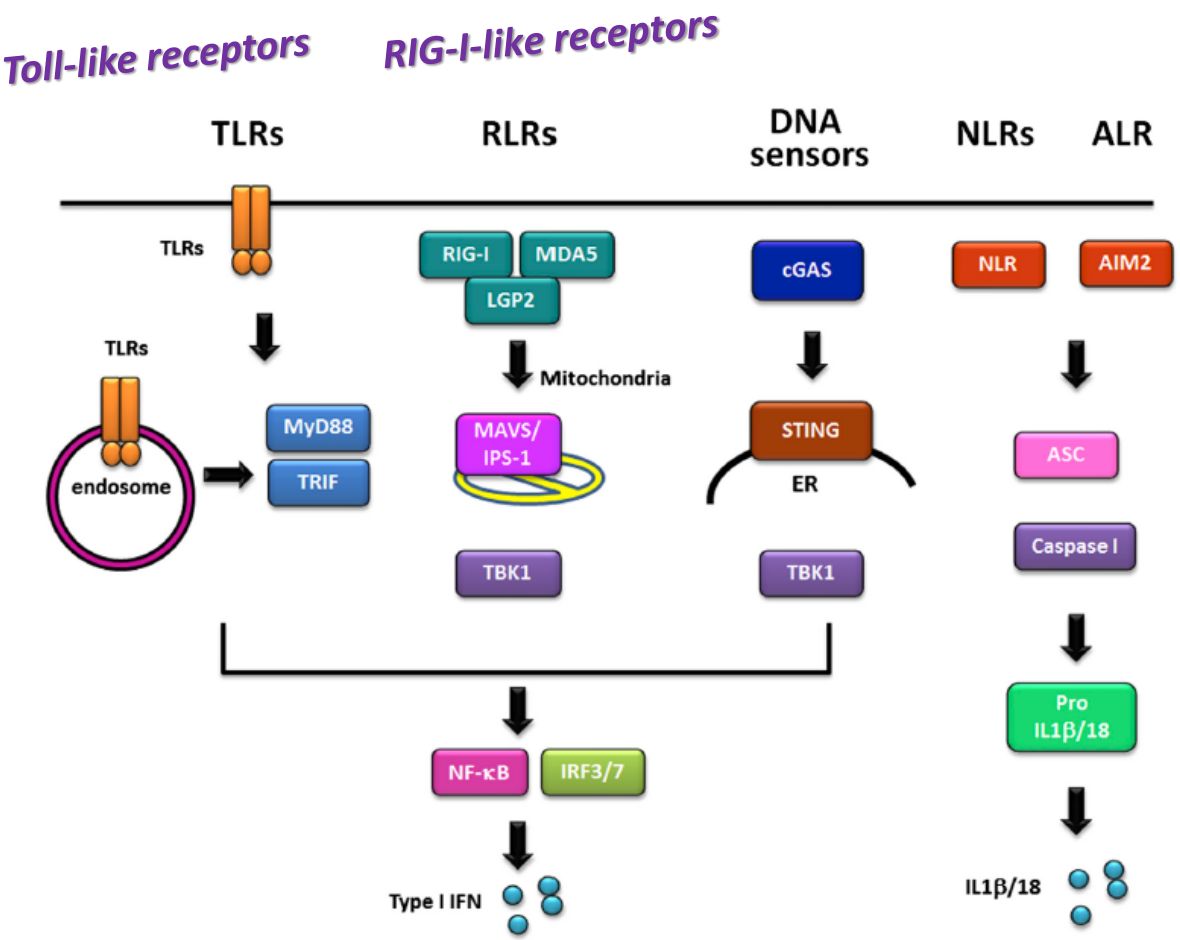
LESSONS LEARNED FROM THE FOE

Margarita Sáiz

Viral interplay with the innate immune system

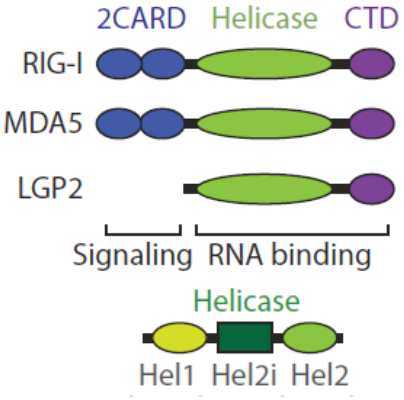


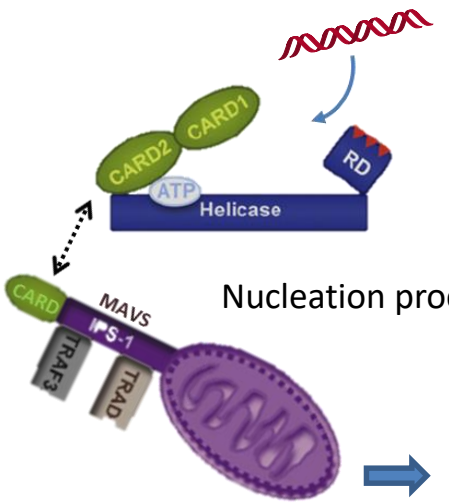
Pattern-Recognition Receptors (PRRs)



(Ma & Damania, Cell Host & Microbe 19, 2016)

RIG-I-like Receptors (RLRs) and FMDV





Nucleation process and filament assembly

Recognition preferences

- **RIG-I** - *PAMP: 5´-ppp blunt short dsRNA*
- **MDA5** - *PAMP: long dsRNA (≥ 0.5-1 Kb)*
- **LGP2** - *PAMP: dsRNA any length, high affinity*

(Repressor of RIG-I - and enhancer of MDA5- signaling, respectively)

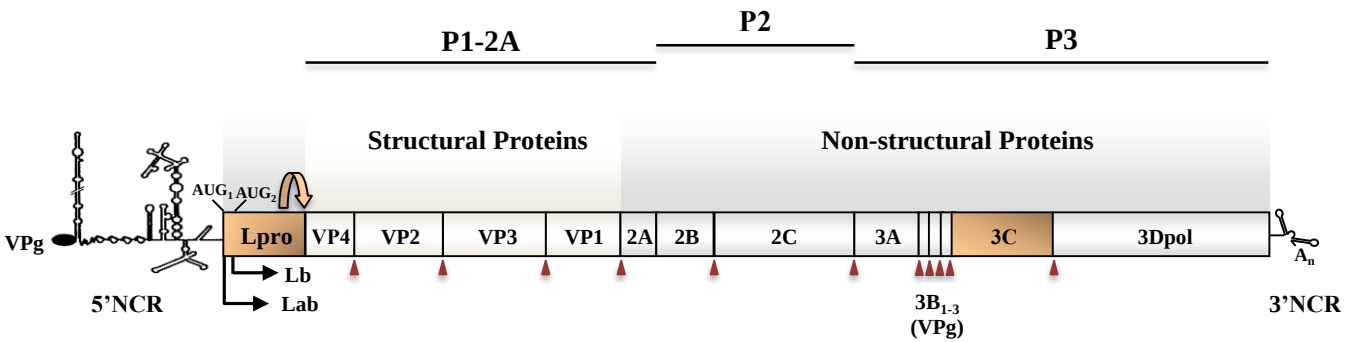
➡ Recruitment of downstream signaling molecules

Sensing of the FMDV genome

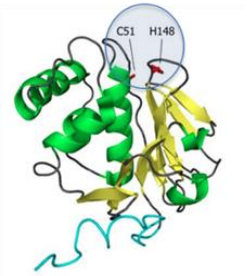
Type-I IFN induction during picornavirus/FMDV infections has been linked to MDA5

- Transfection of RIG-I$^{-/-}$, MDA5$^{-/-}$, or MAVS$^{-/-}$ MEFs with RNA of equine rhinitis A **aphthovirus** (ERAV) induced an MDA5- and MAVS-dependent, but RIG-I-independent, IFN-β response (Feng et al 2012)
- IFN-β mRNA induction during **FMDV** infection was only reduced significantly in MDA5 silenced porcine PK-15 cells (Husser et al 2011)
- Overexpression of LGP2 can inhibit **FMDV** replication in PK-15 cells (Zhu et al 2017)

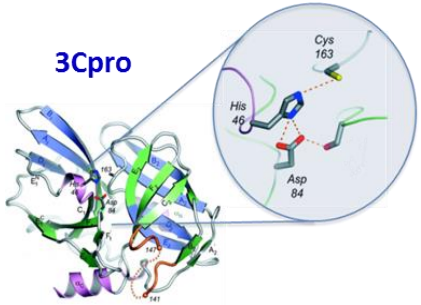
FMDV proteases are actively involved in immune evasion



Lpro



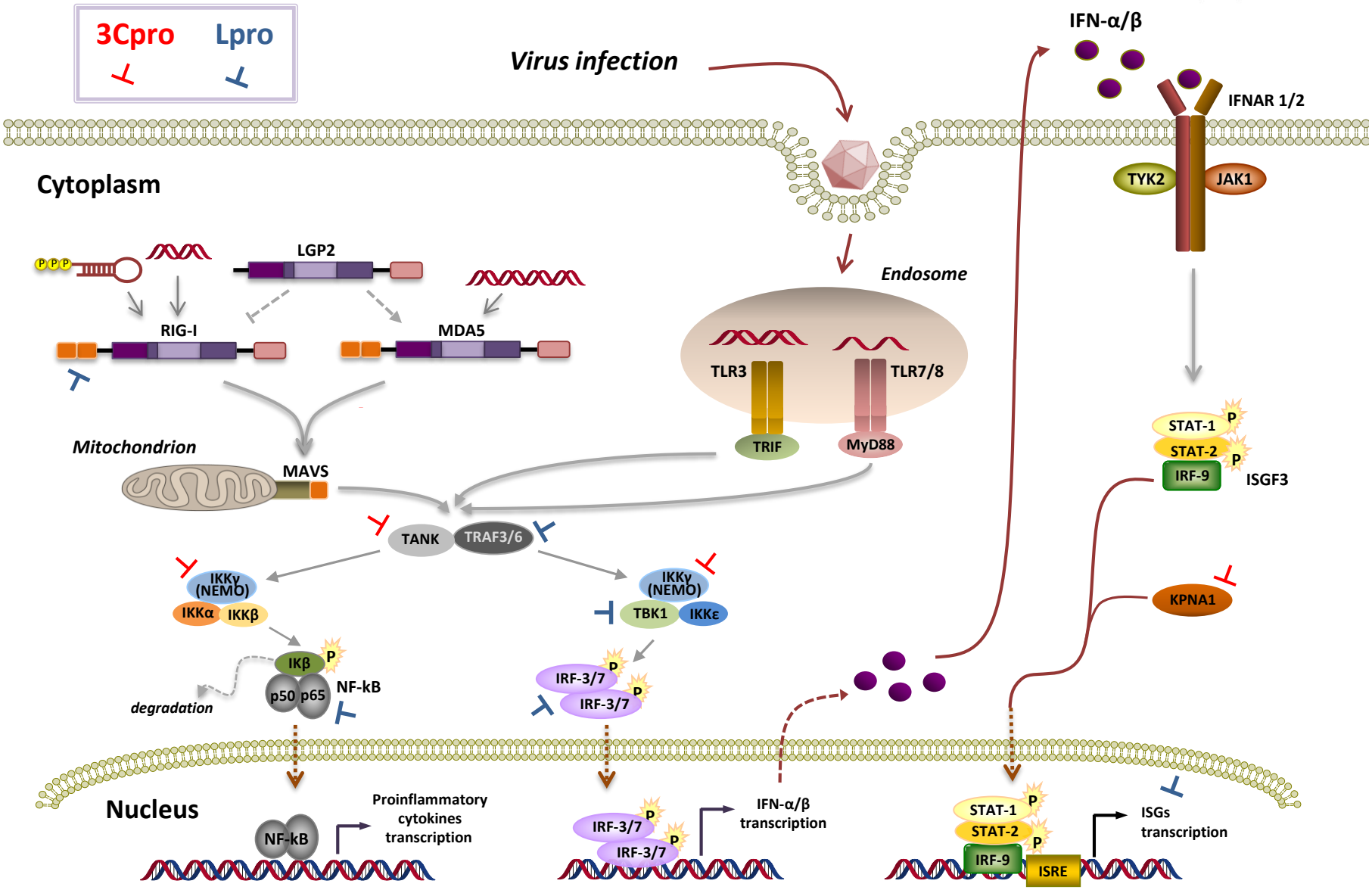
3Cpro



By general mechanisms:

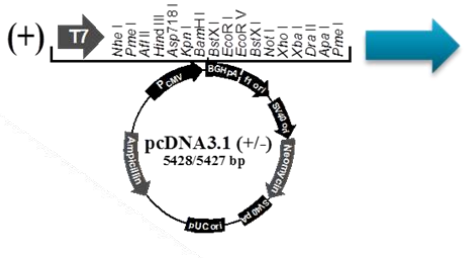
Cap-dependent translation shut-off
Host transcription regulation
DeISGylation
Deubiquitination ….

… and specific mechanisms affecting crucial steps in antiviral response

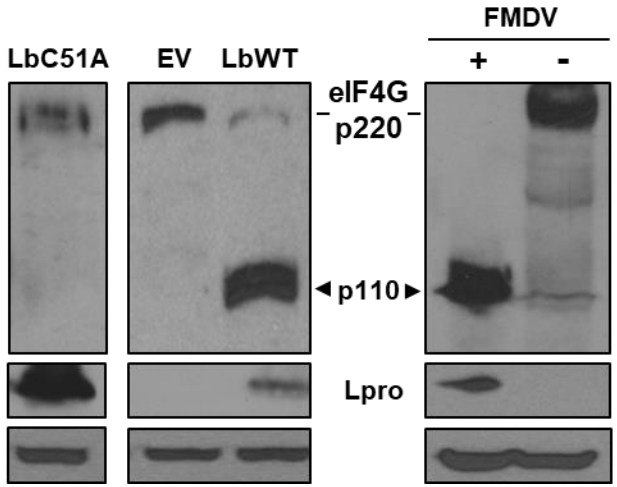


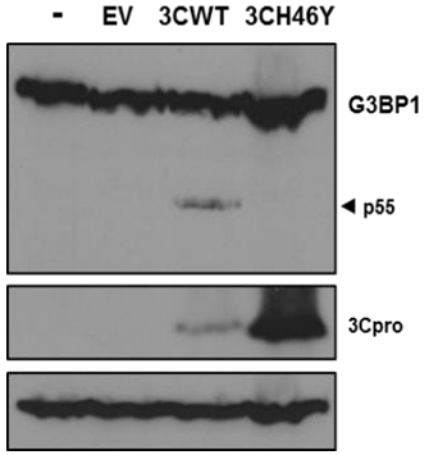
Testing the effect of FMDV proteases on innate sensors and adaptor proteins

- **Expression of wt and catalytically inactive forms of FMDV Lpro and 3Cpro**



Lb WT
Lb C51A — *Piccone et al 1995*, *Roberts & Belsham 1995*
3C WT
3C H46Y — *Grubman et al 1995*



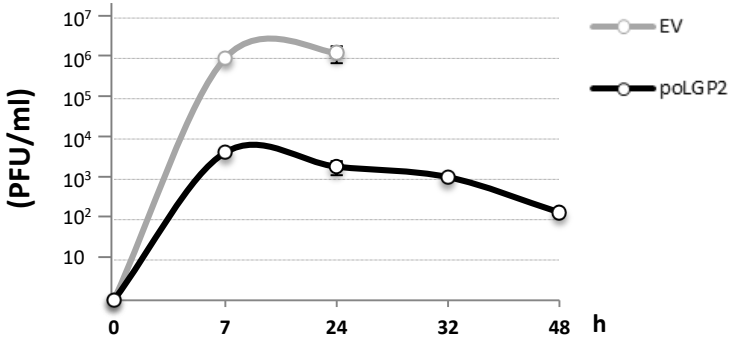


- **Co-transfection experiments with plasmids expressing different proteins relevant for antiviral immunity**
  - **Analysis of the effect of expression of those proteins on FMDV infection**

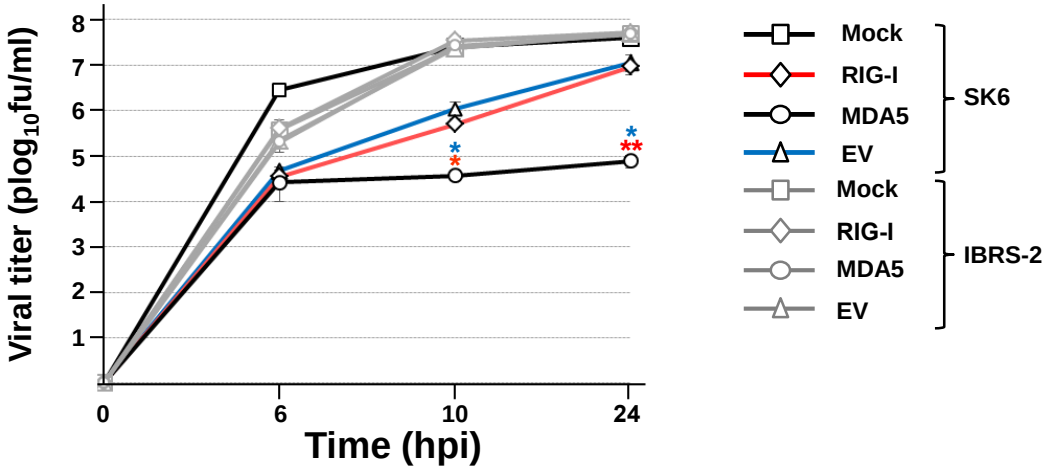
FMDV and LGP2

Why LGP2?

- No information related to FMDV
- Apparently not a key target for viral antagonism
- Less studied among RLRs, “undersetimated”
- **Strong inhibitory effect** on FMDV infection



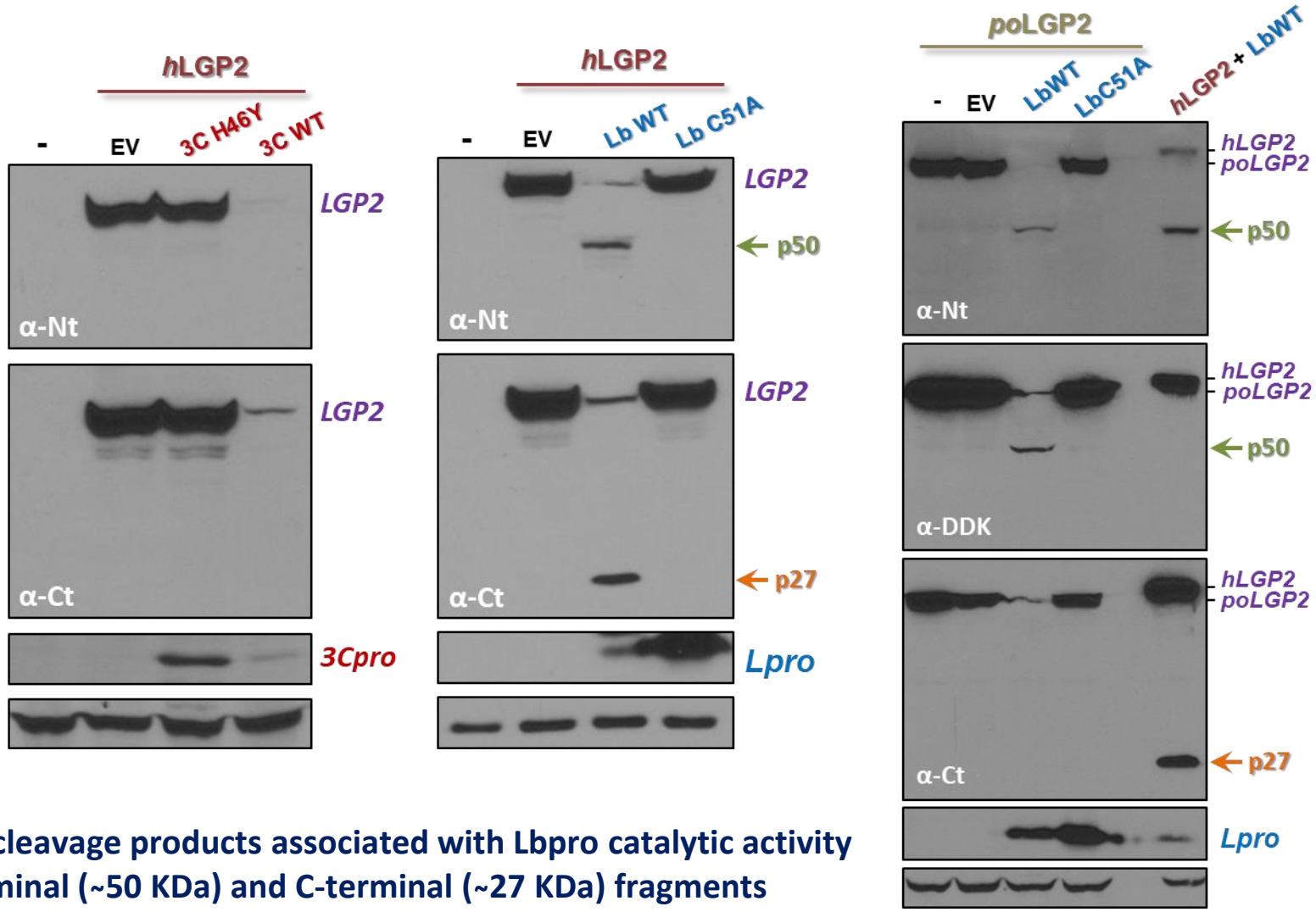
SK-6 cells infected with FMDV (MOI 5)



Foot-and-mouth disease virus infection inhibits LGP2 protein expression to exaggerate inflammatory response and promote viral replication

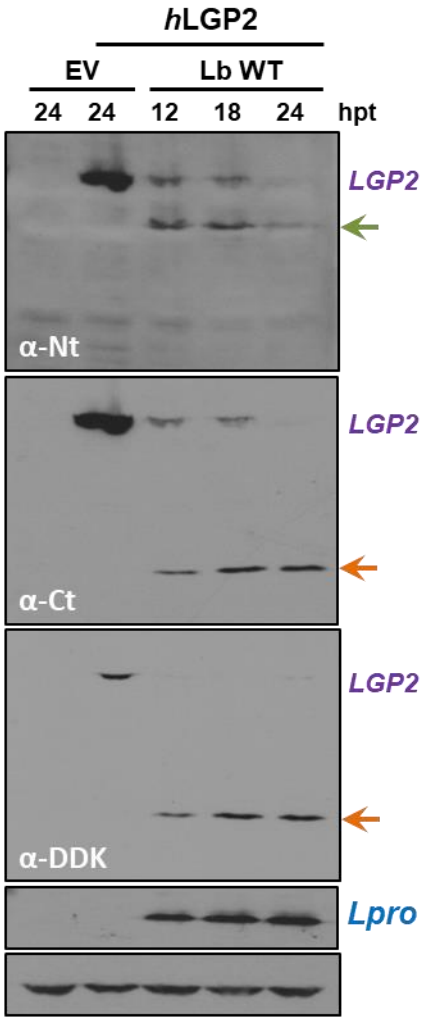
Zixiang Zhu[1], Chuntian Li[1], Xiaoli Du[1], Guoqing Wang[1], Weijun Cao[1], Fan Yang[1], Huanhuan Feng[1], Xiangle Zhang[1], Zhengwang Shi[1], Huanan Liu[1], Hong Tian[1], Dan Li[1], Keshan Zhang[1], Xiangtao Liu[1] and Haixue Zheng[*,1]

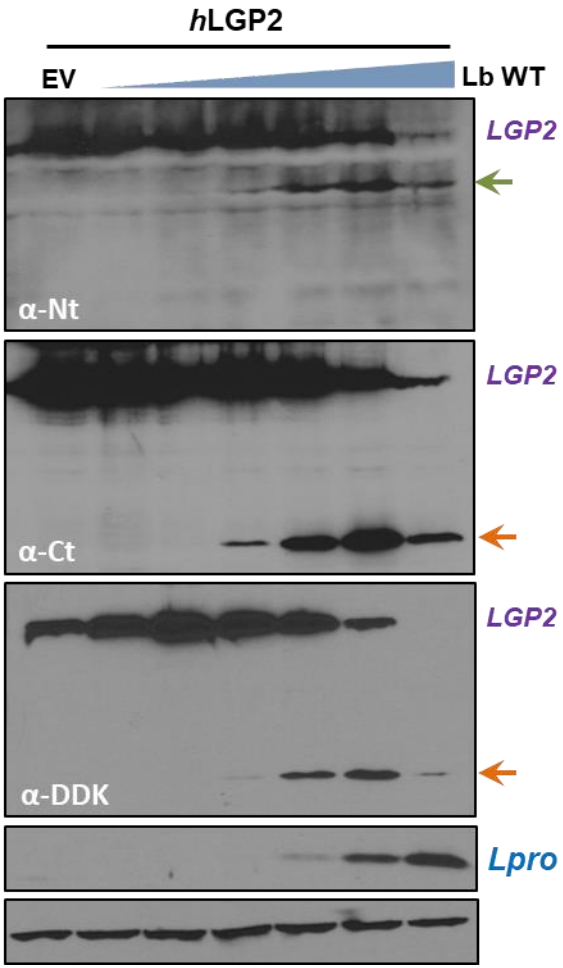
FMDV proteases and LGP2

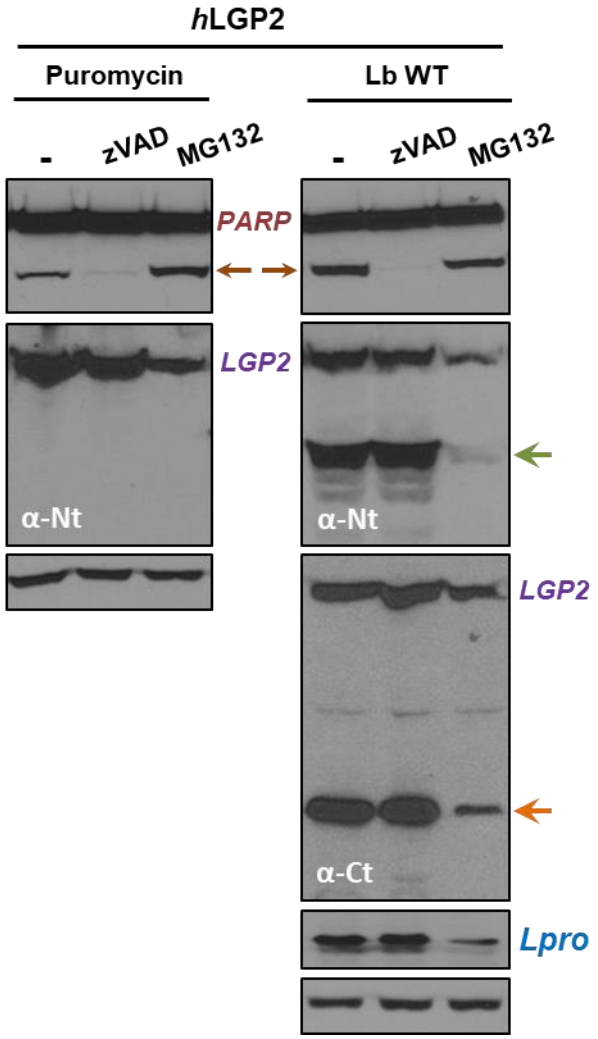


- LGP2 cleavage products associated with Lbpro catalytic activity
- N-terminal (~50 KDa) and C-terminal (~27 KDa) fragments
- Lbpro-dependent degradation of porcine LGP2 was also observed (C-term fragment not detected)

FMDV Lpro and LGP2



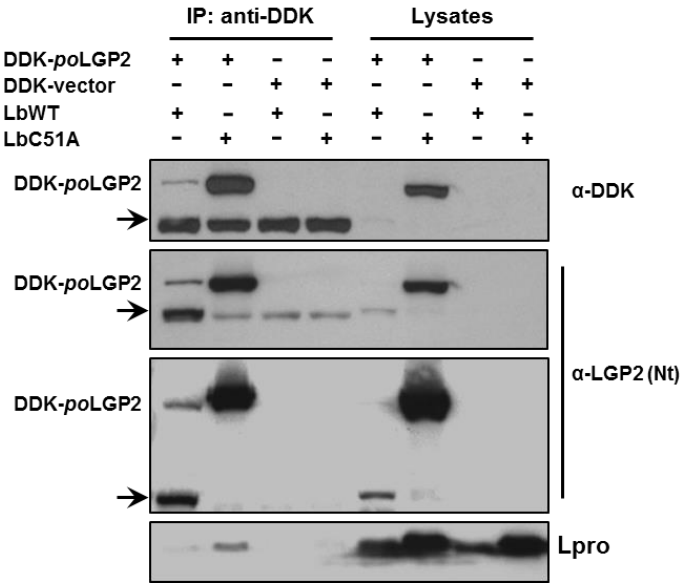


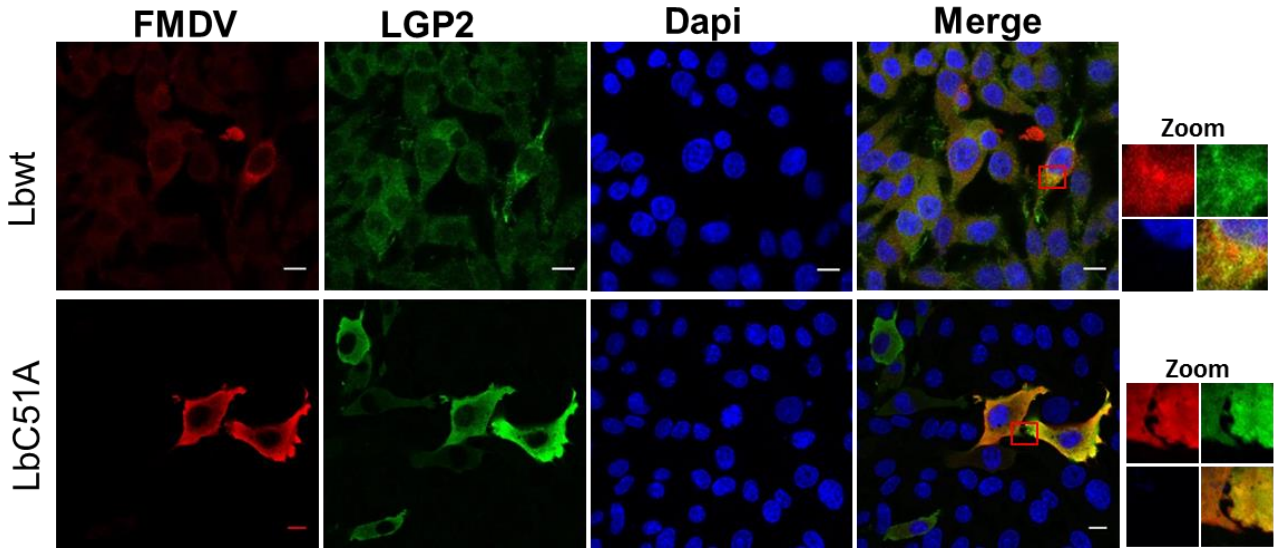


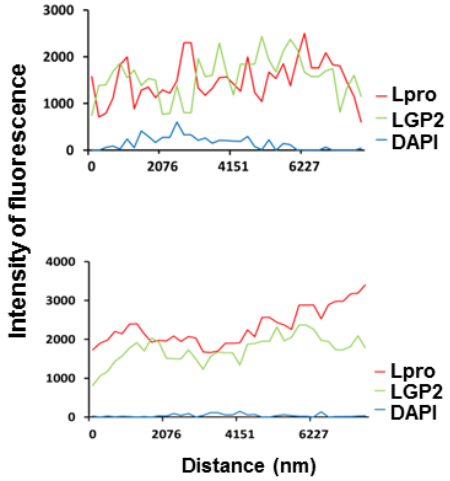
- *LGP2 degradation by Lbpro is progressive and dose-dependent but independent of the caspase and proteasome pathways*

FMDV Lpro interacts with LGP2

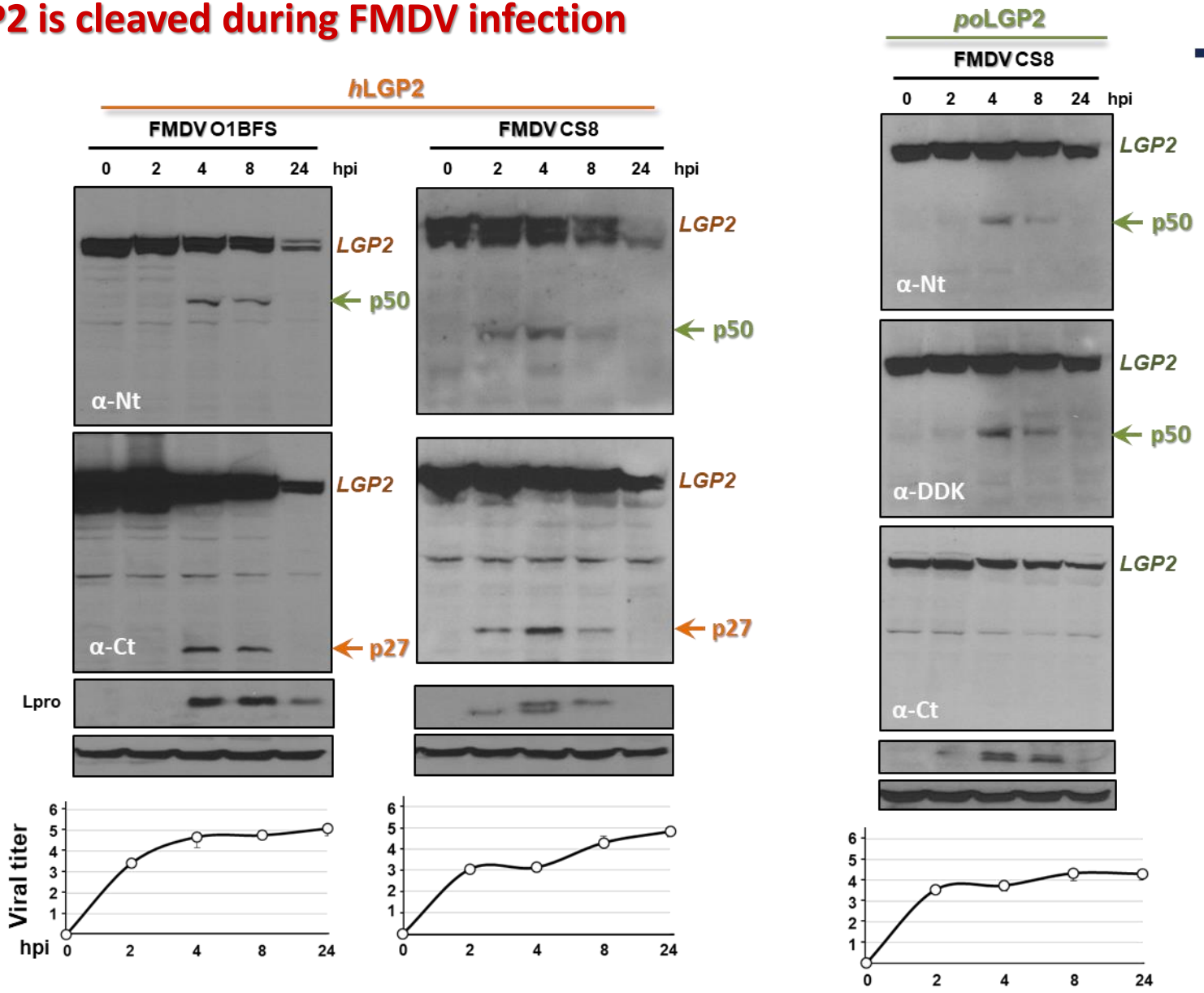
- Lbpro and porcine LGP2 coimmunoprecipitate and colocalize in cells





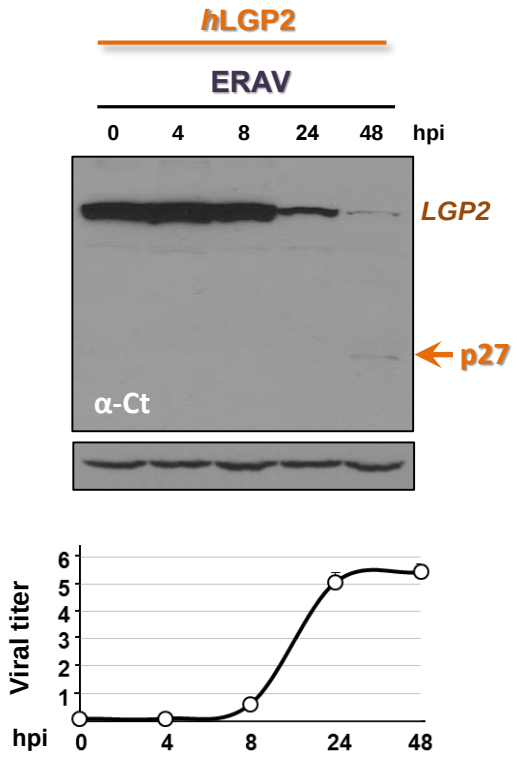


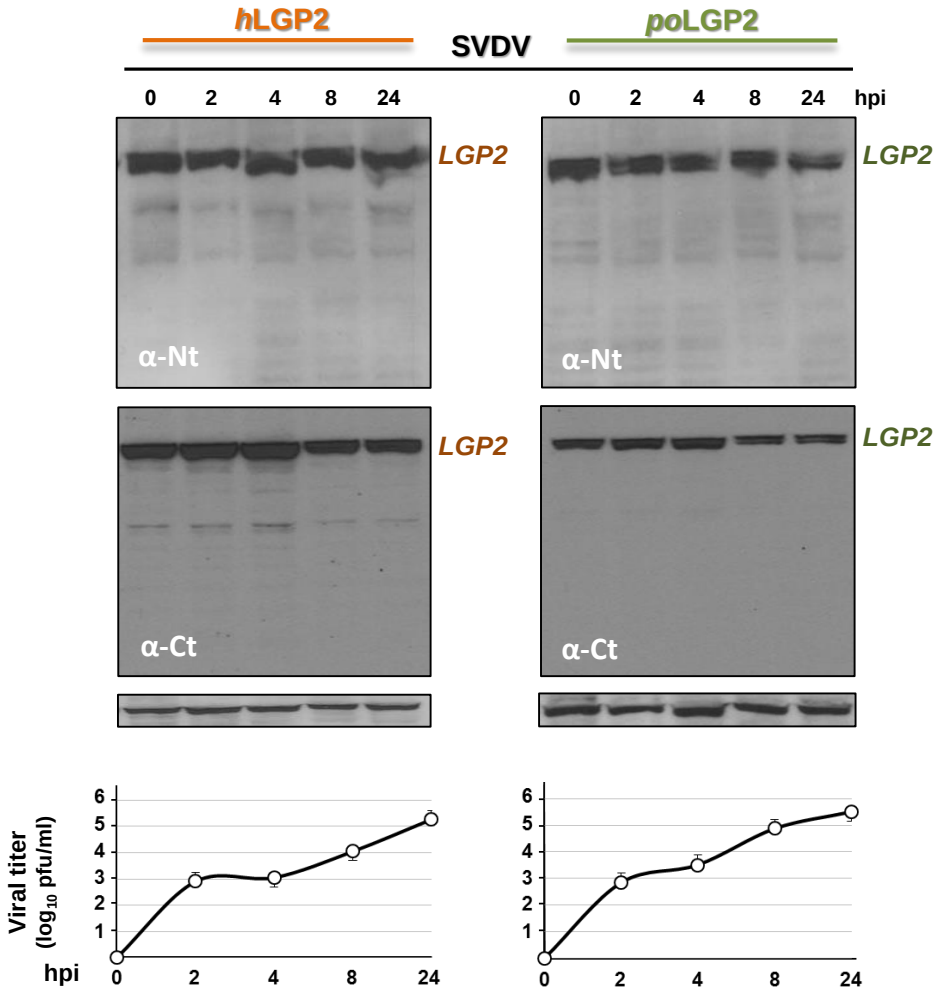
LGP2 is cleaved during FMDV infection



- *Same pattern observed in co-expression of LGP2 and Lbpro*

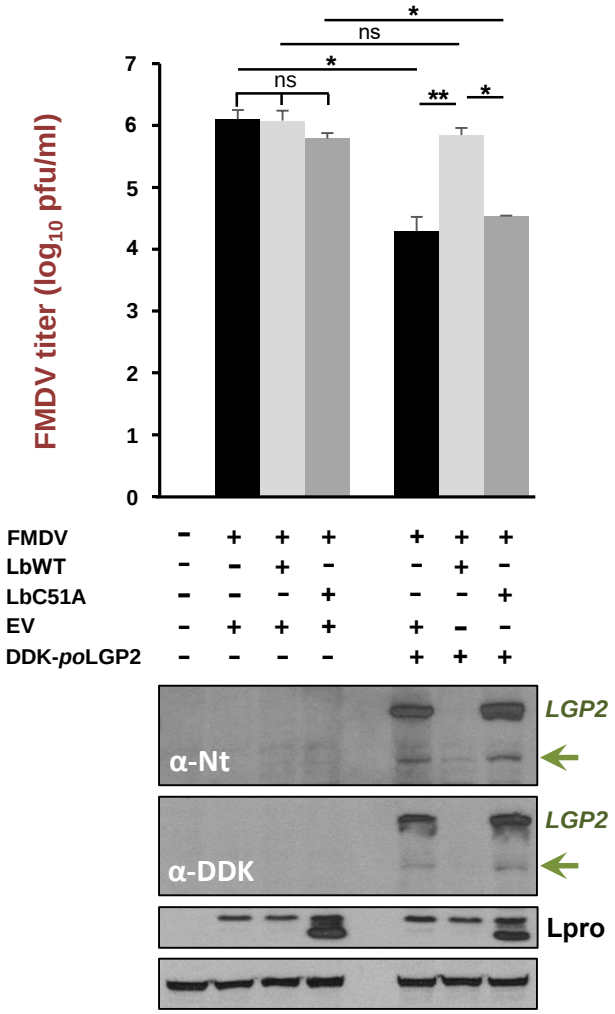
LGP2 is cleaved during ERAV infection

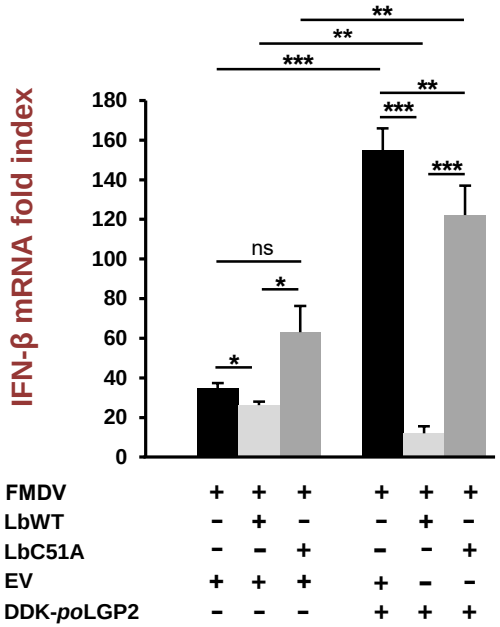




- *No LGP2 cleavage detected during infection with other picornaviruses and swine viruses*

Lbpro subverts the antiviral response induced by LGP2

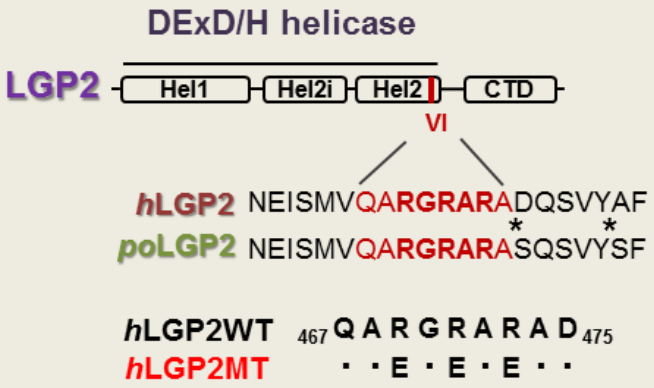


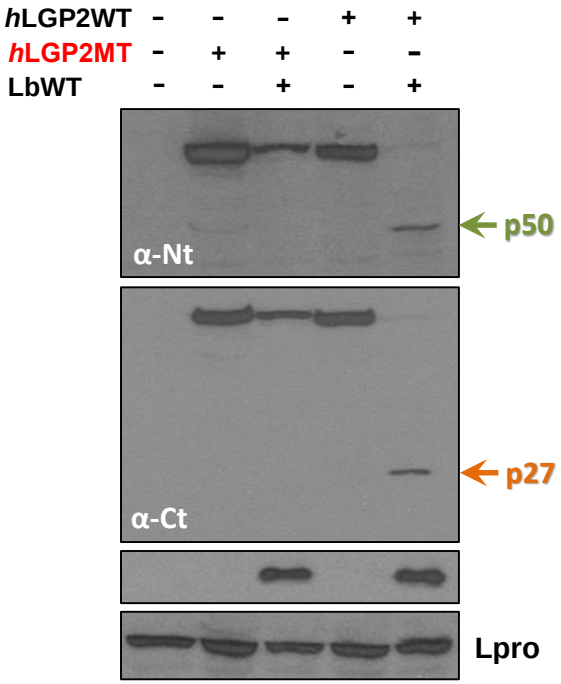


	Control cells	FMDV			
		-	DKK-*po*LGP2		
		-	EV	LbWT	LbC51A
Untreated samples	<15	<15	36.5	<15	41.7
Anti-IFN-α MAb	<15	<15	<15	<15	<15

- ***Lbpro expression induced higher viral titers and lower IFN-β and antiviral activity levels***

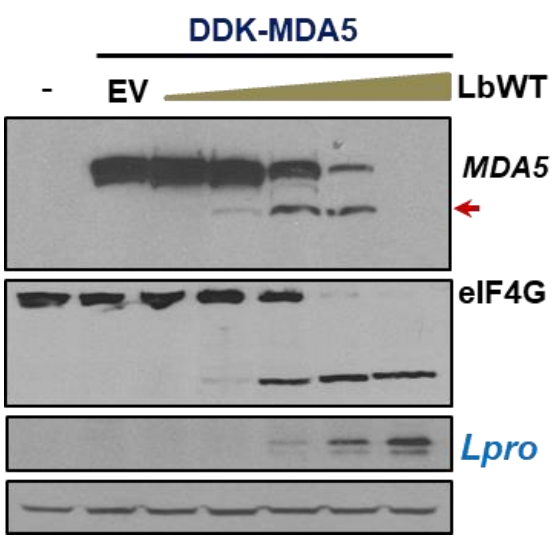
Identifying the Lpro cleavage site on LGP2

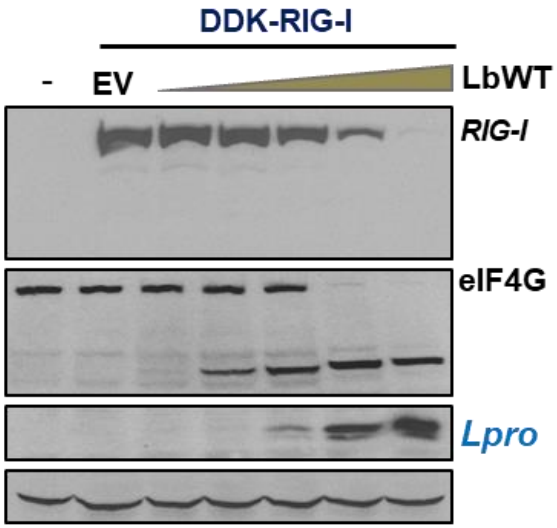




- *The RGRAR sequence in Motif VI resembles cleavage site on other protein targets*
  - *Mutation of that sequence generates an uncleavable LGP2*

Is Lpro targeting other RLRs?





- *No evidence of RIG-I cleavage fragments*
- *MDA5 is degraded in an Lb dose-dependent manner*

CONCLUSIONS

- **The FMDV Leader protease is a powerful weapon for immune evasion**
  - Pleiotropic effect against host defenses
  - Ensuring disruption of the RLR signaling pathway at the early steps of viral RNA recognition

Does Lpro know limits?

Work in progress

- Studies on the interplay of FMDV with effector and adaptor molecules of other PRRs (cGAS/STING, TLR….)

We are facing a reckless enemy but …

Better knowledge of FMDV strategies for immune evasion will hopefully contribute to improvement of disease control

Acknowledgments

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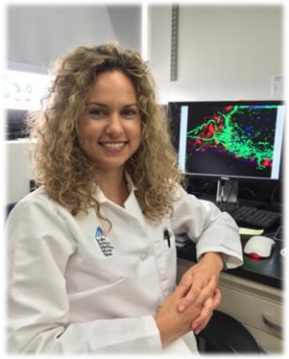

THANKS FOR YOUR ATTENTION