26th SEACFMD National Coordinators Meeting

Investigation of innate immune evasion by foot-and-mouth disease virus and its application

Dr. Zixiang Zhu

State Key Laboratory for Animal Disease Control and Prevention, National Reference Laboratory for Foot-and-mouth disease, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China

LANZHOU VETERINARY RESEARCH INSTITUTE,
CHINESE ACADEMY OF AGRICULTURAL SCIENCES

FMDV: Background

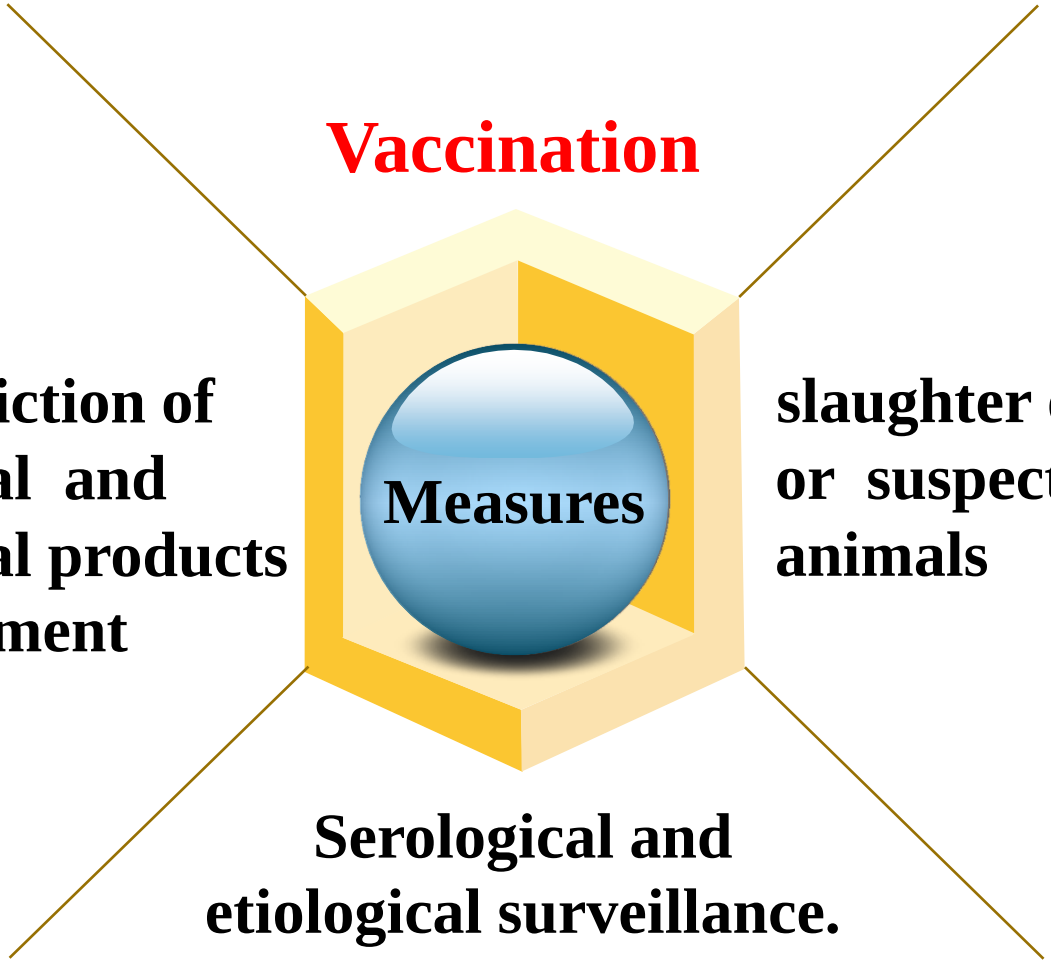
- **Foot and mouth disease (FMD) is a severe, highly contagious viral disease of livestock that has a significant economic impact.**
- **FMD is endemic in several parts of Asia and in most of Africa and the Middle East. In Latin America, the majority of countries apply zoning and are recognised as FMD-free, either with or without vaccination.**

2001 UK FMD

2010 Japan FMD

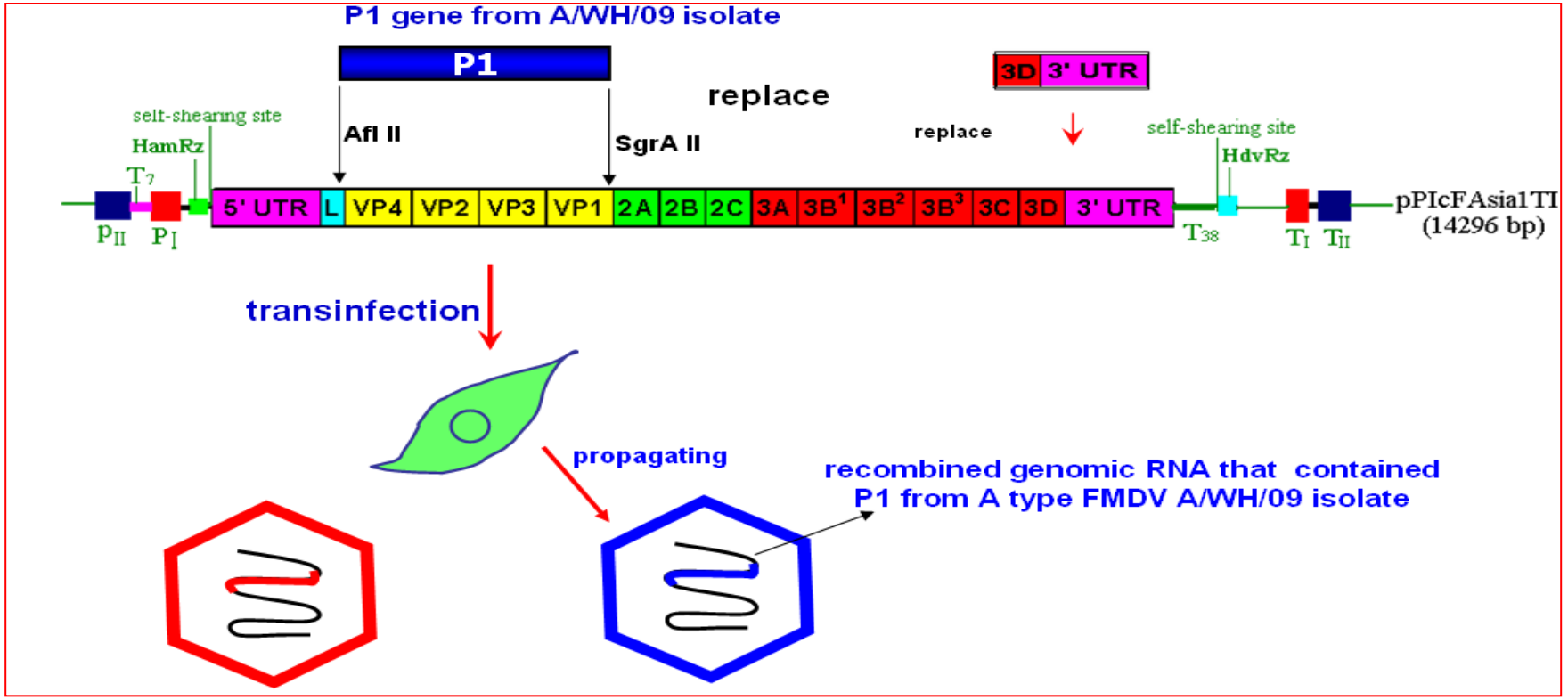
2017 Jordan FMD

Outlines of prevention and control of FMD



Compulsively inoculated with vaccine combined with slaughtering in China

Deletion or modification of the immunosuppressive sites or domains in viral proteins is a prominent strategy to develop FMDV vaccine strain



Wildtype FMDV → **Recombinant FMDV** **Improvement of the vaccine efficacy**

FMD Etiology and Immunity Team

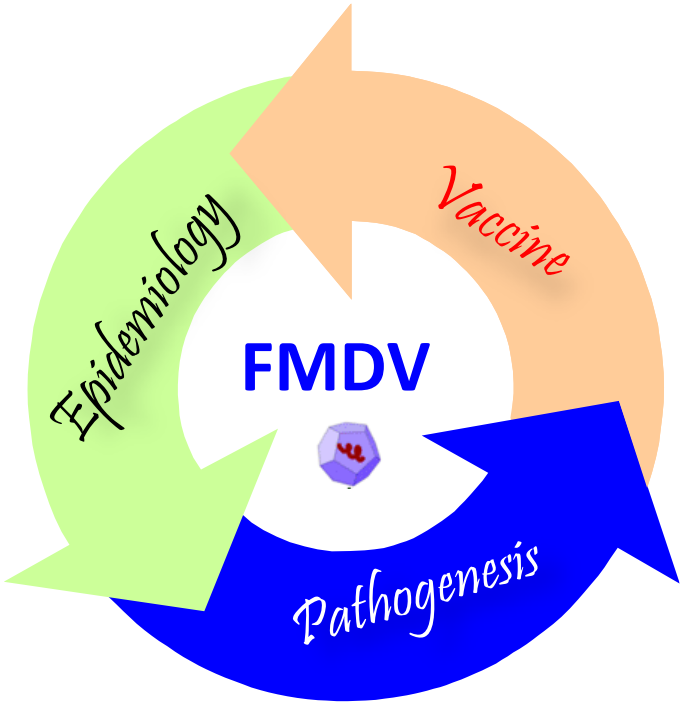
- **Epidemiology**
  - FMD virus (FMDV) Isolation
  - FMDV evolution and distribution study
  - Identifying risk factors for FMD
- **Pathogenic mechanism**
  - Host tropism of FMDV
  - The virulence and antigenic variation of FMDV
  - The suppressive role of FMDV on host immune system
- **Establishment of vaccine development platform**
  - Vaccine development
  - Vaccine process development and manufacture

FMD vaccines have been developed:

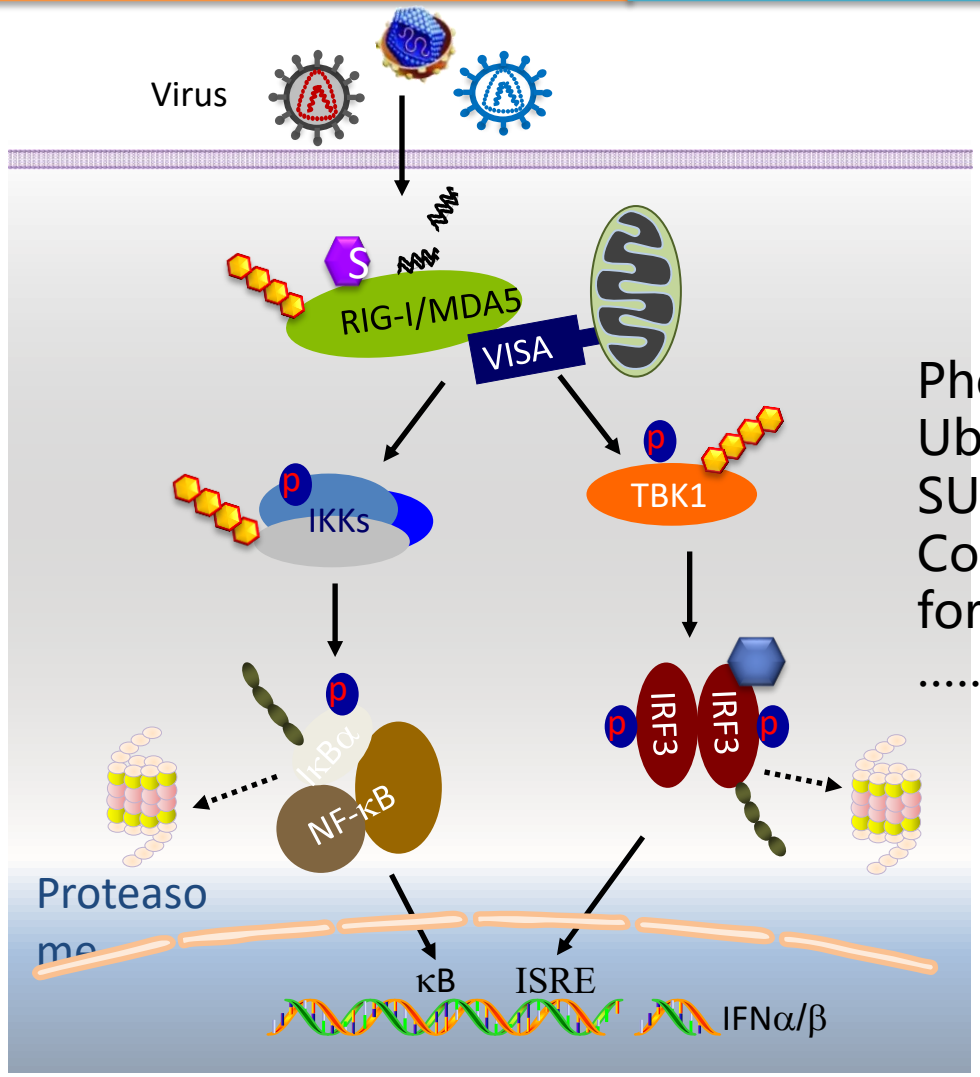
I. FMD O/May-98 inactivated vaccine
II. FMD O-Asia1-A trivalent inactivated vaccine
III. FMD DNA vaccine

Two of the developed Vaccines were recommended by WOAH/FAO for FMD control.

Prof. Dr.
Zixiang Zhu
Email:
zhuzixiang@caas.cn



Innate immune pathways are critical for induction of host antiviral response during viral infection



Phosphorylation
Ubiquitination
SUMOylation
Complex formation
.....

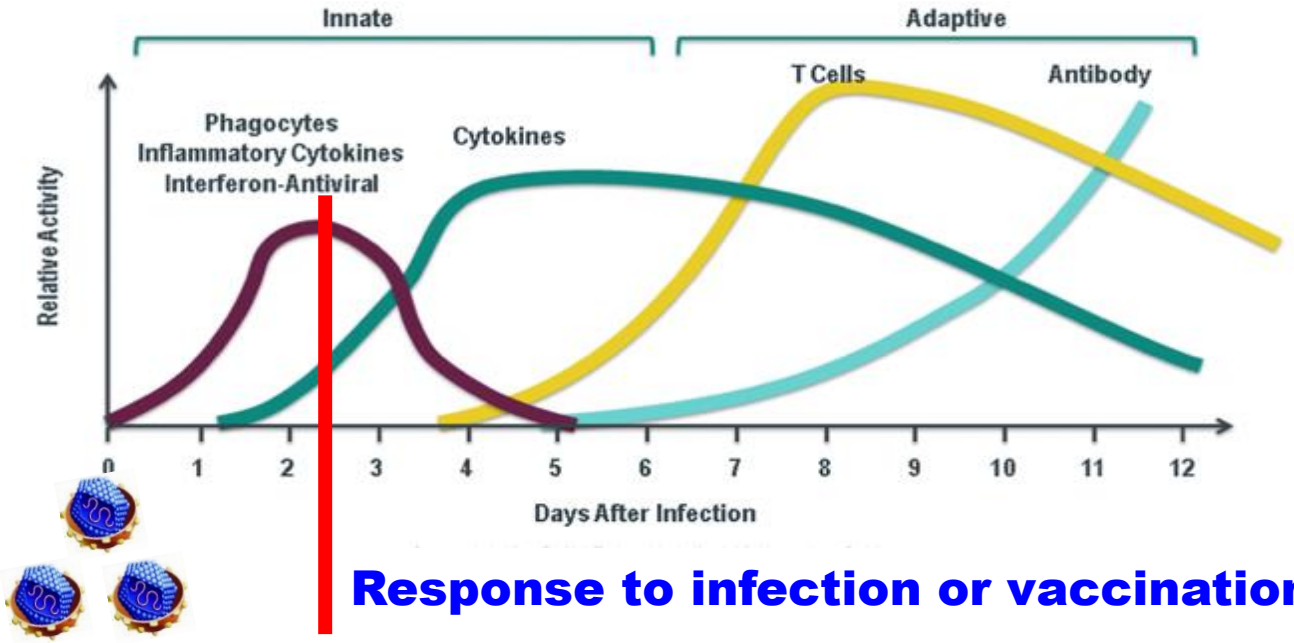
Host/Viral proteins

Virus infection triggers IFN production

K63-linked Ub
K48-linked Ub
ISG15
phosphorylation
SUMOylation

The mechanisms used by FMDV to antagonize host innate immune response are complicated

Innate immune response is critical for initiation of adaptive immune response



Response to infection or vaccination

Infection of FMDV

IFN-α/β

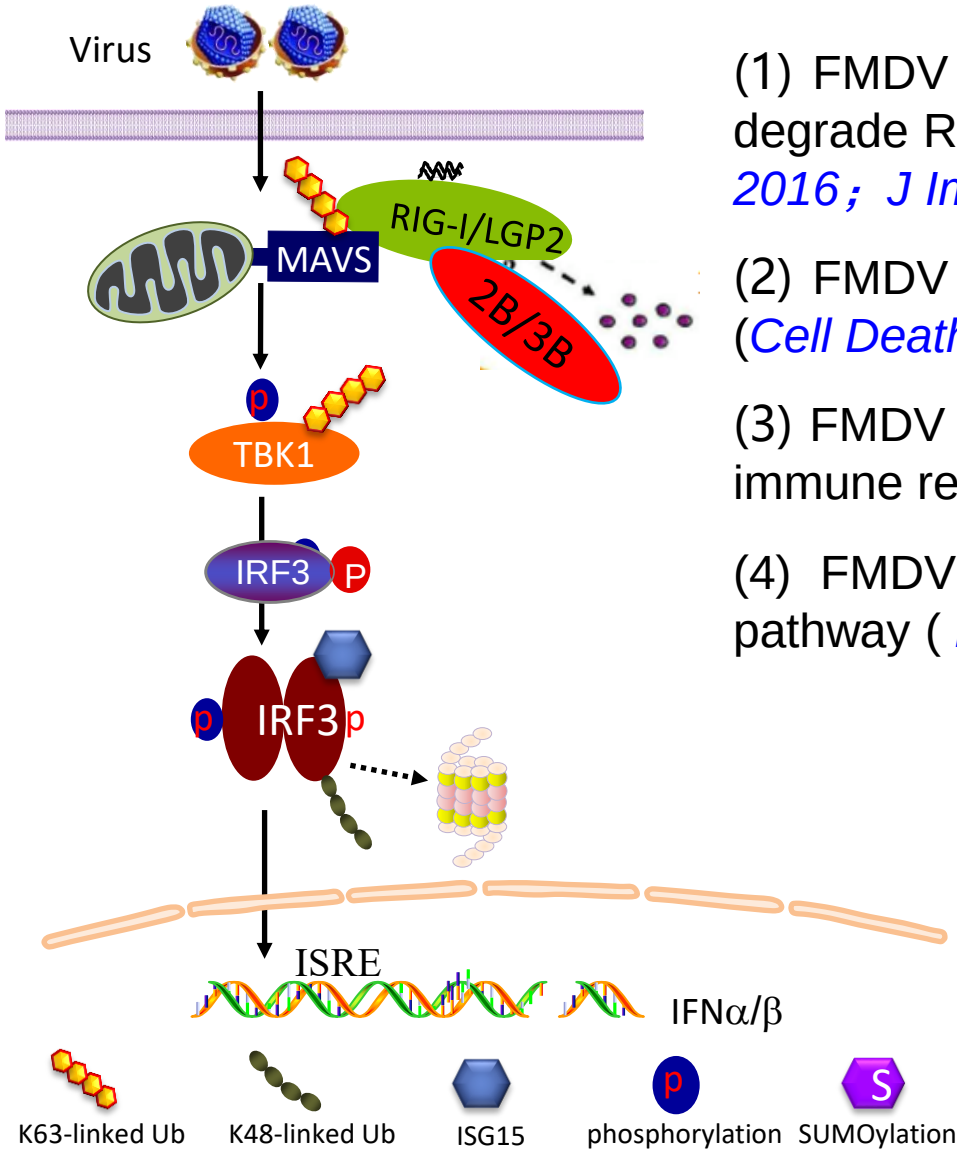
DC

Immune cells

Clarification of the antagonistic mechanisms will provide insights and direction for FMD control

Adaptive immune response

1. Regulation of pattern-recognition receptors (PRRs) by FMDV

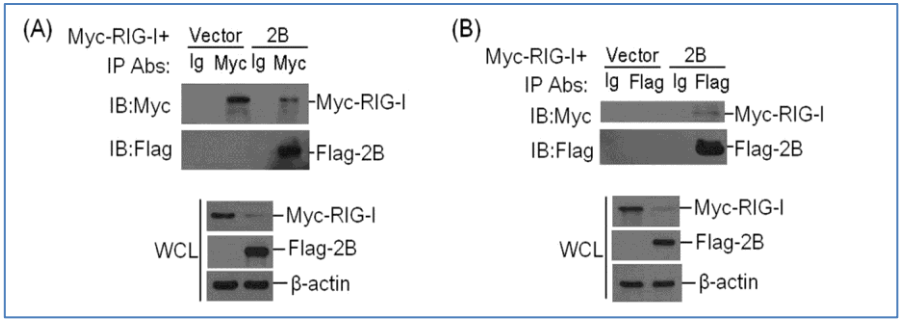


(1) FMDV 2B and 3B proteins interact with RIG-I to degrade RIG-I and promote FMDV replication (*J Virol 2016；J Immunol 2020*).

(2) FMDV 2B interacts with LGP2 to degrade LGP2 (*Cell Death Dis 2017*).

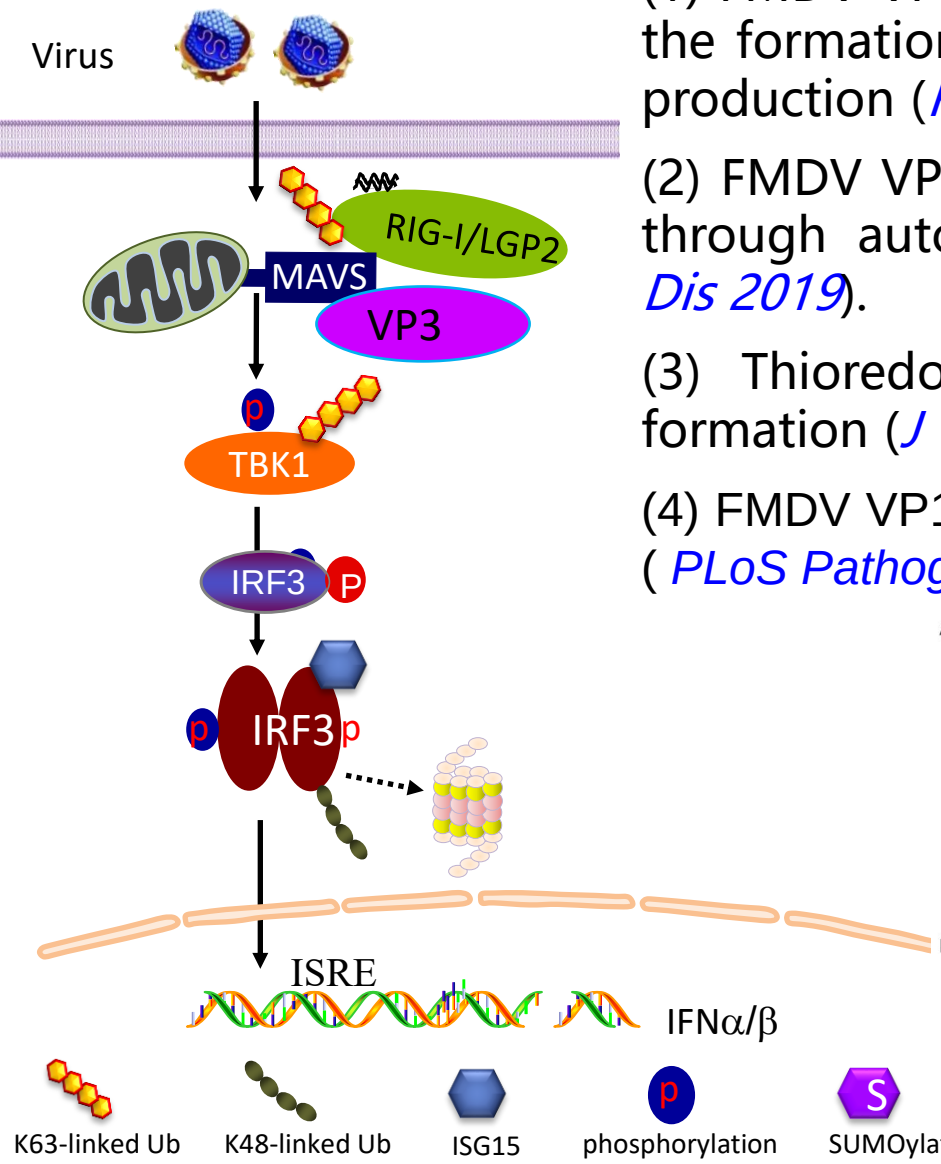
(3) FMDV 2B and 2C degrade NOD2 to block innate immune response (*J Virol 2019*).

(4) FMDV 2C degrades cGAS through autophagy pathway (*PLoS Pathog 2023*)



2B interacts and degrades RIG-I

2. Regulation of adaptor protein MAVS by FMDV

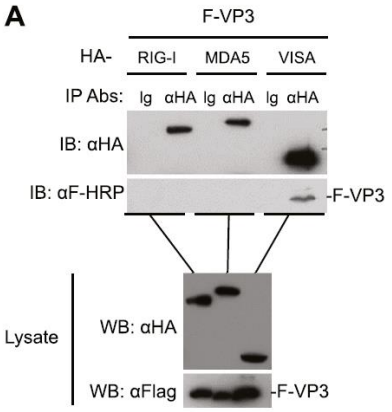


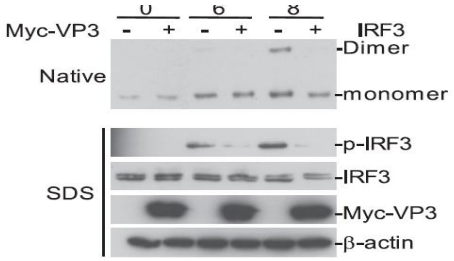
(1) FMDV VP3 interacts with MAVS and interferes with the formation of MAVS complex, blocking type I IFNs production (*FASEB J 2016*).

(2) FMDV VP0 interacts with PCBP2 to degrade MAVS through autophagy-dependent pathway (*Cell Death Dis 2019*).

(3) Thioredoxin 2 (TRX2) disrupts MAVS complex formation (*J Virol 2020).*

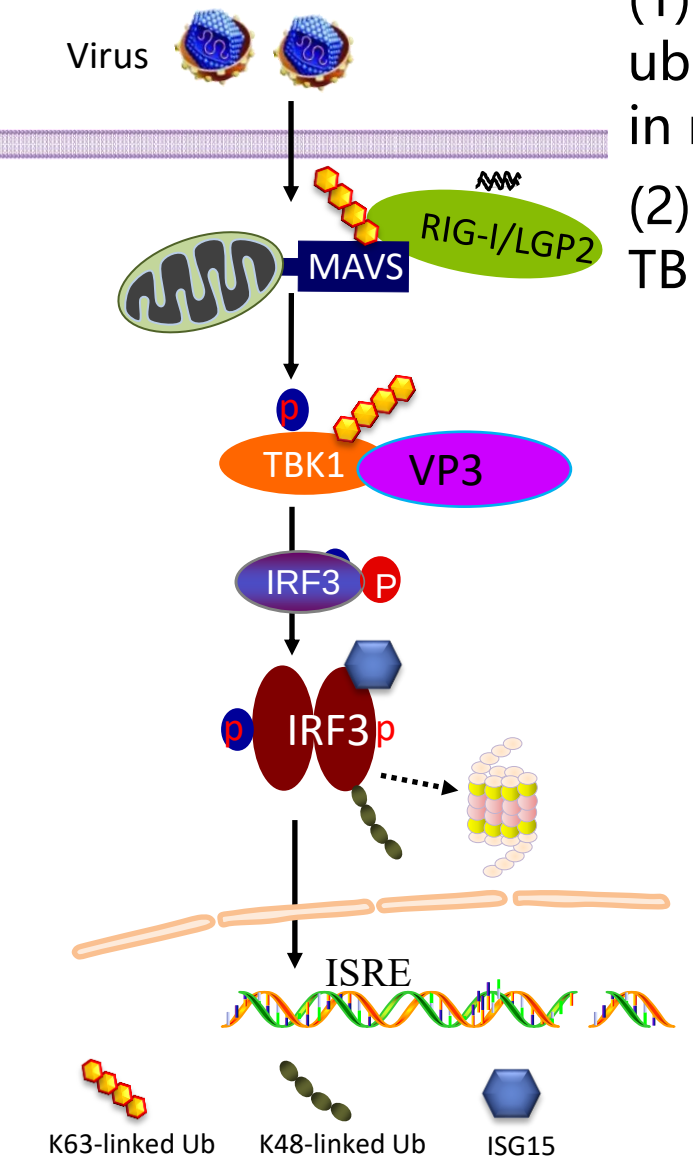
(4) FMDV VP1 interacts with IRF3 to block IFNs production (*PLoS Pathog 2021*)





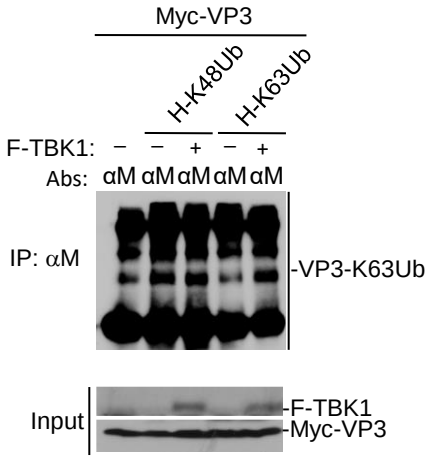
FMDV VP3 interacts with MAVS and blocks IRF3 dimerization.

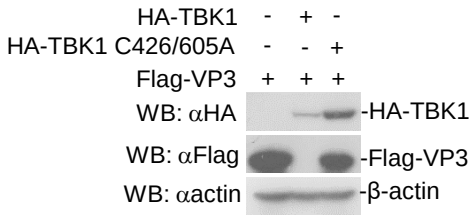
3. Regulation of adaptor protein TBK1 by FMDV



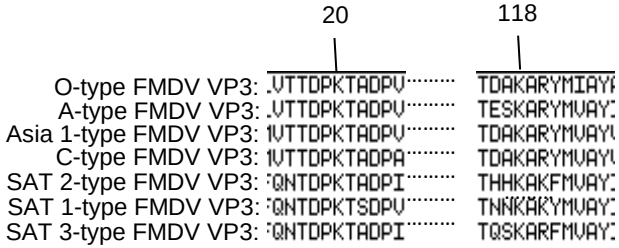
(1) TBK1 is a novel E3 ubiquitin ligase, and E2 ubiquitin-conjugating enzyme UbcH5c is involved in regulation of its ubiquitination function.

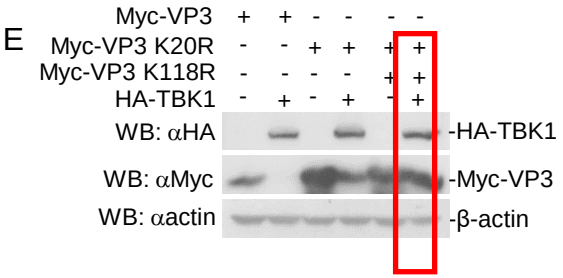
(2) The E3 ubiquitin ligase activity is essential for TBK1-mediated degradation of VP3 (*J Virol 2019*).



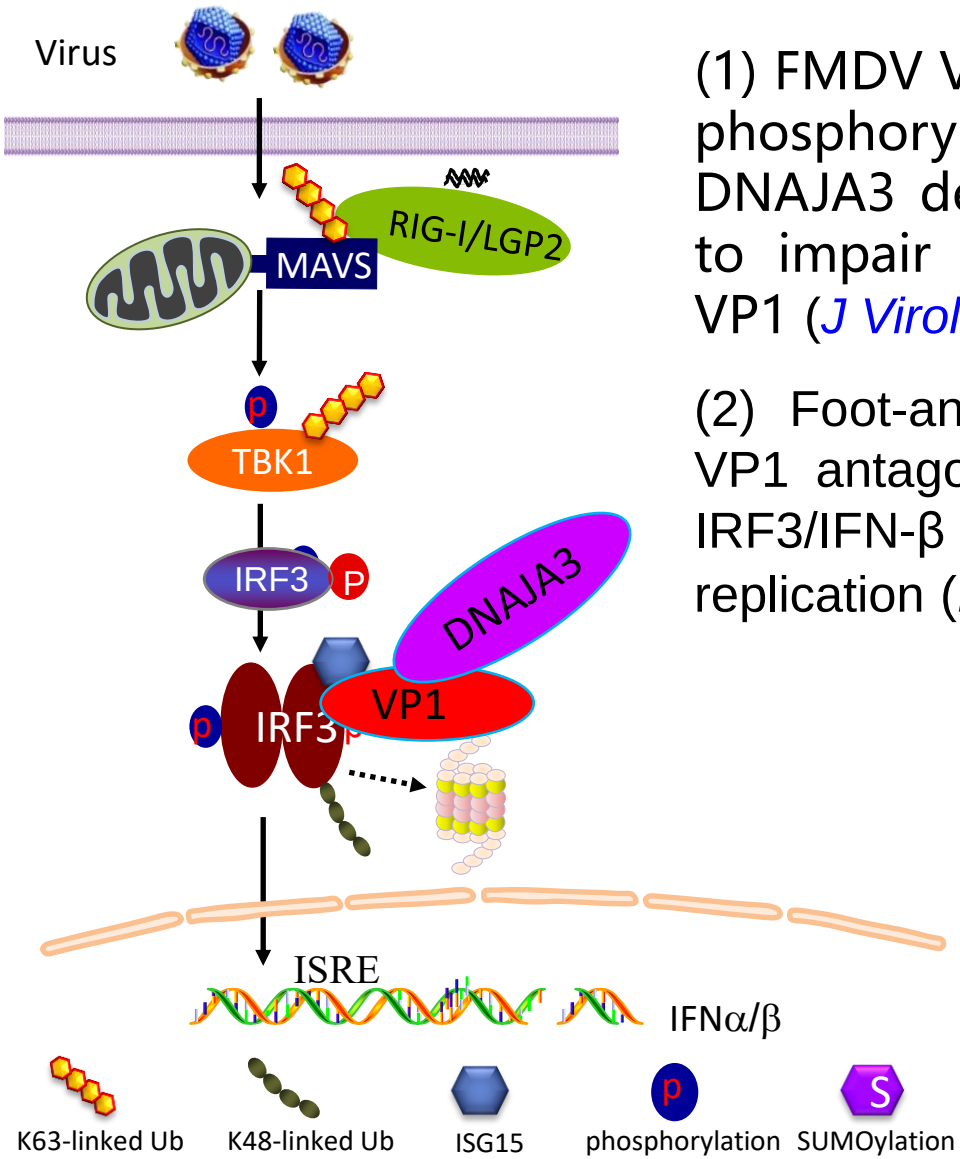


TBK1C426/605A and VP3K118R are the critical sites.



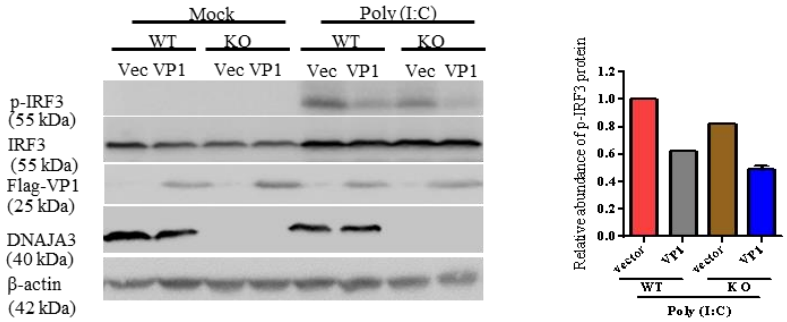


4. Regulation of transcription factor IRF3 by FMDV



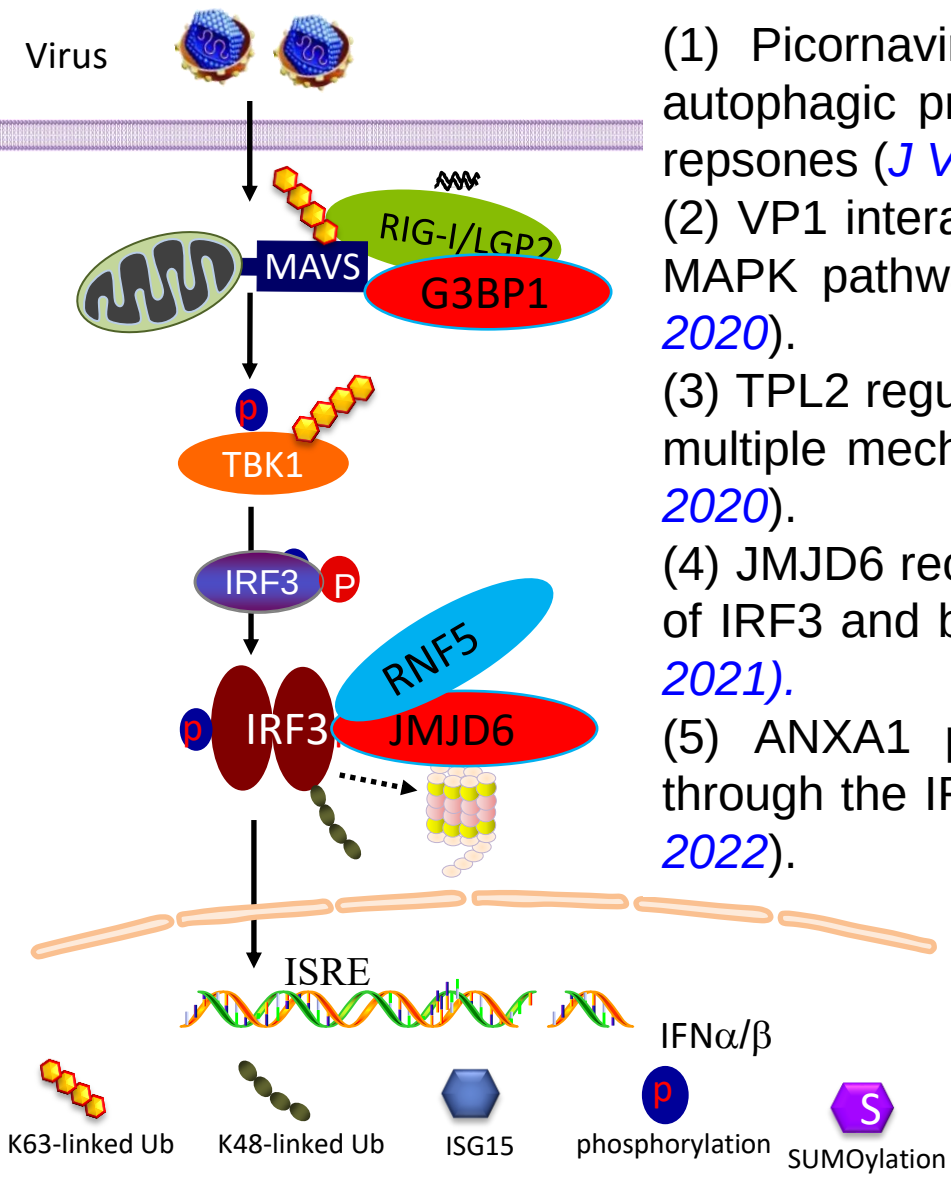
(1) FMDV VP1 interacts with IRF3 and inhibits its phosphorylation and nuclear translocation. Host DNAJA3 degrades VP1 through the autophagy to impair this antagonistic effect induced by VP1 (*J Virol 2019，Cover Story*).

(2) Foot-and-mouth disease virus capsid protein VP1 antagonizes TPL2-mediated activation of the IRF3/IFN-β signaling pathway to facilitate the virus replication (*Frontiers Immunol，2021*).



Deletion of DNAJA3 enhanced VP1-induced antagonistic effect.

5. Regulation of antiviral response by host proteins



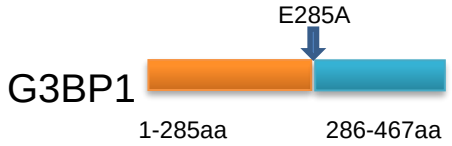
(1) Picornavirus 3A protein degrades G3BP1 through autophagic protein LRRC25 which impairs host antiviral repsones (*J Virol 2020*).

(2) VP1 interacts with RPSA to maintain the activation of MAPK pathway and promote FMDV replication (*J Virol 2020*).

(3) TPL2 regulates host innate immune response through multiple mechanisms (*J Virol, 2020； Frontiers Immunol, 2020*).

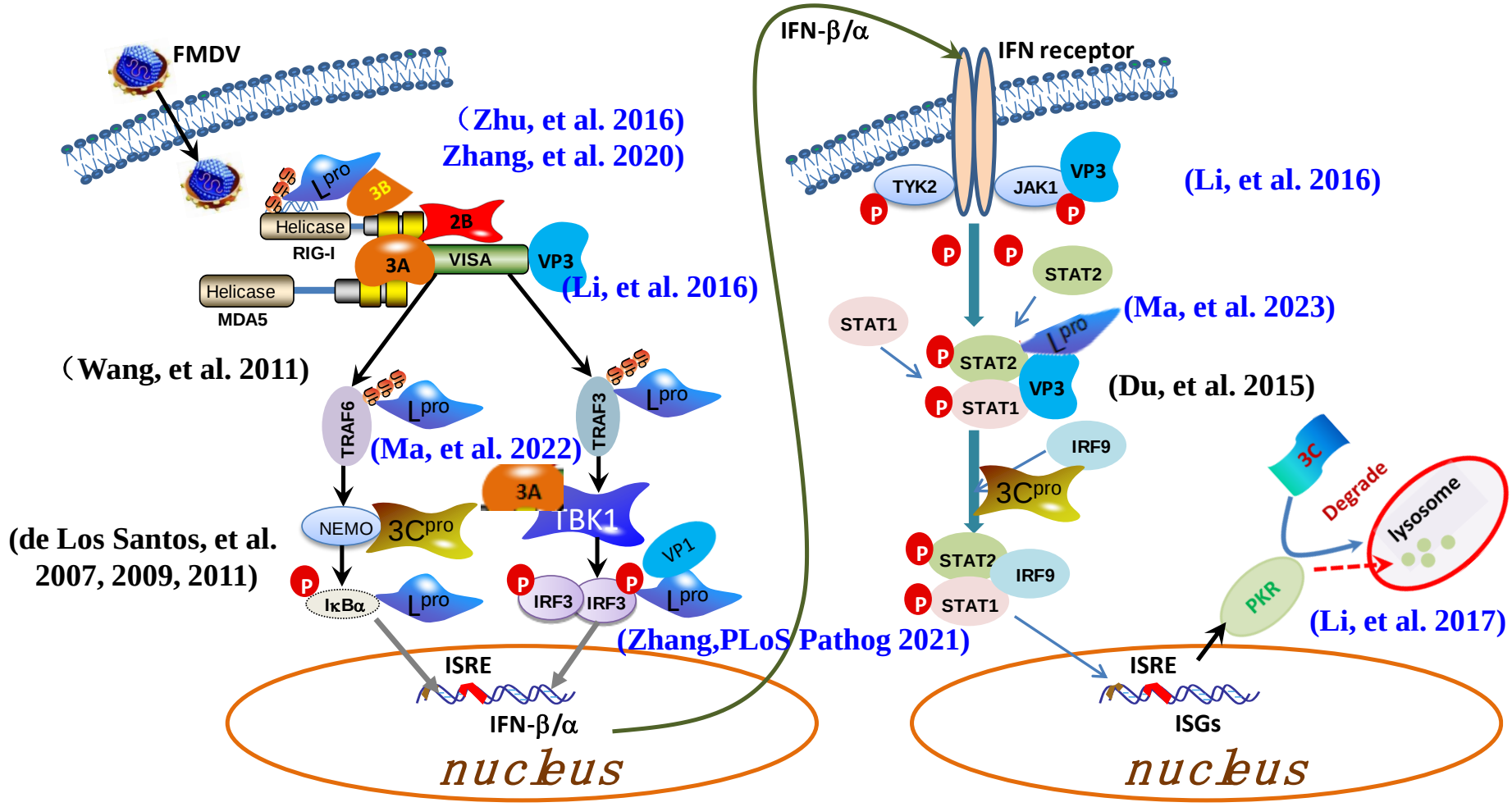
(4) JMJD6 recruits RNF5 to induce the K48 ubiquitination of IRF3 and blocks host antiviral response *(Plos Pathog, 2021).*

(5) ANXA1 promotes FMDV-induced IFNs production through the IRF3 axis at MAVS and TBK1 levels (*J Virol, 2022*).



The 286-467aa of G3BP1 interacts with RIG-I Helicase domain.

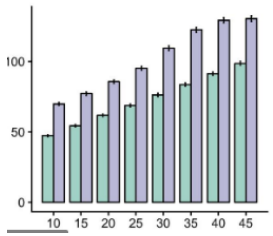
6. Schematic representation of the antagonistic mechanisms used by FMDV



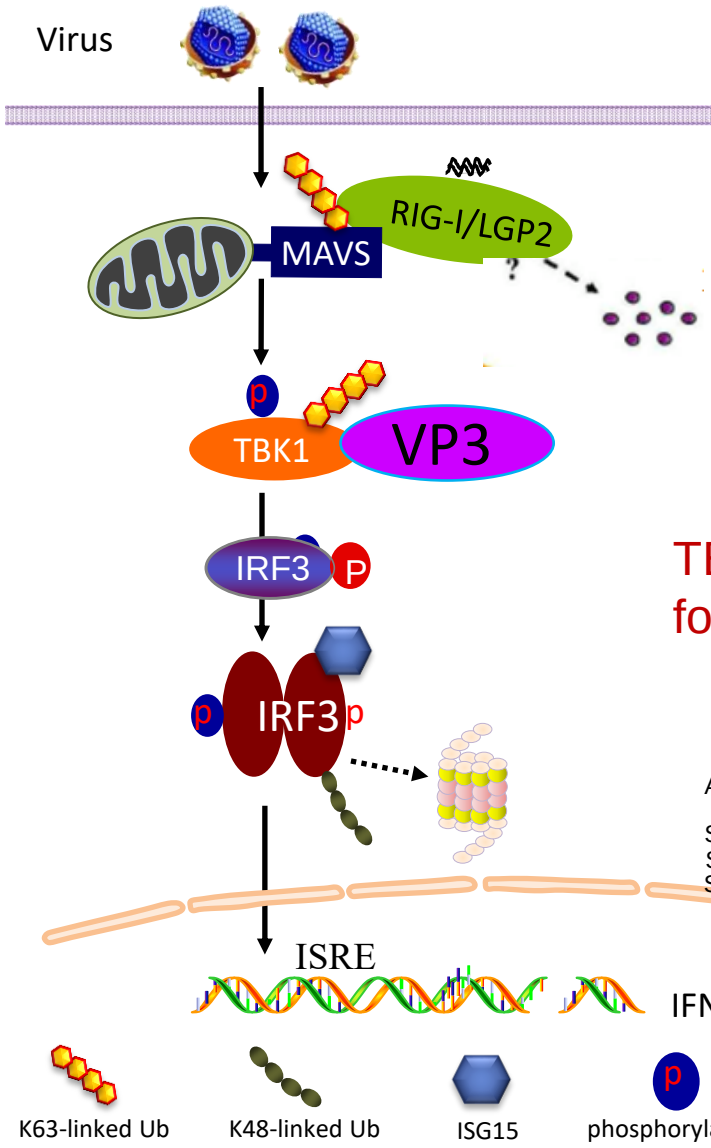
The multiple mechanisms of immune suppression used by FMDV.

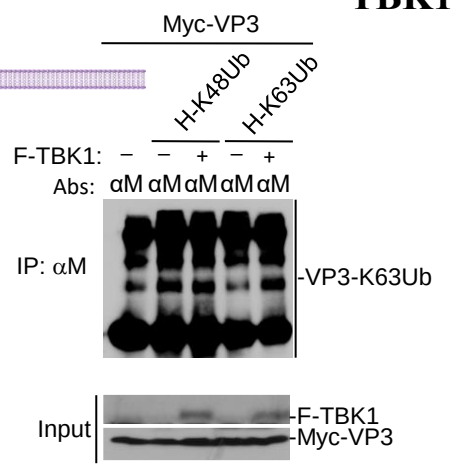
Application

Improvement of vaccine production, safety and efficacy



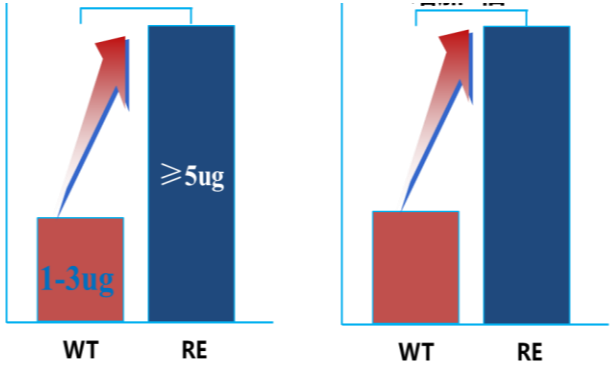
1. TBK1 degrades VP3 which decreases the antigen level during vaccine production



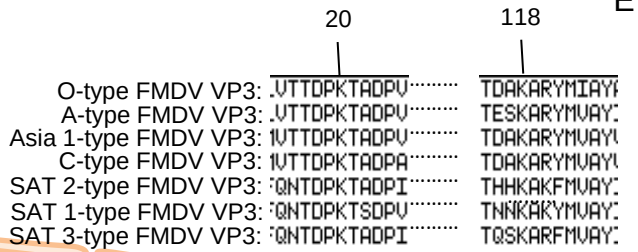


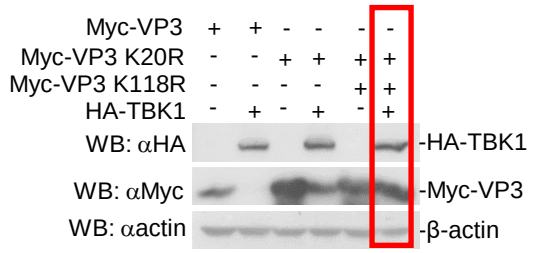
TBK1 catalyzes the K48 ubiquitination of VP3.

Increase of antigen production



TBK1C426/605A and VP3K118R are the critical sites for degradation of VP3.

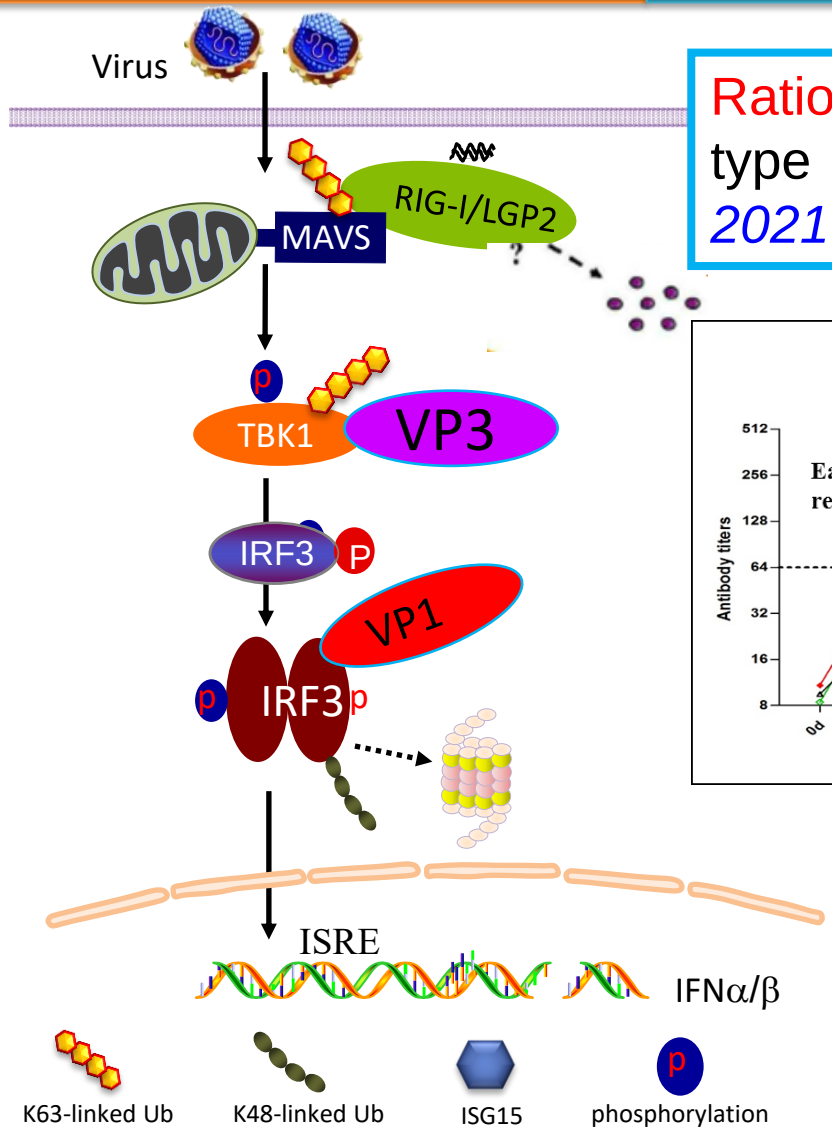




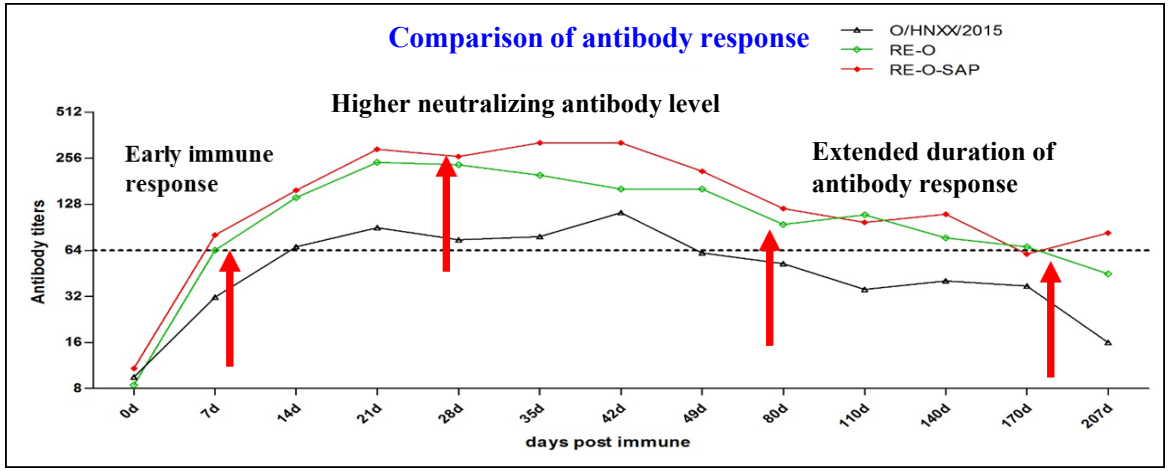
Improvement of FMDV antigen by targeting TBK1 (Patent, ZL201910401904.8, 2020)

Knockout of TBK1 in BHK-21 cells, and introduction of VP3K118R mutation in FMDV .

2. Deletion or modification of the immunosuppressive sites in structural proteins to improve the efficacy of FMDV vaccine



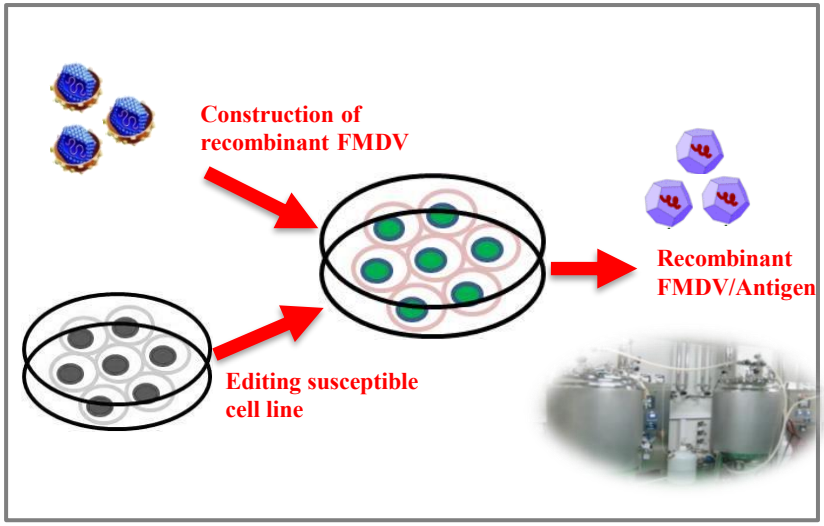
Rationale：FMDV VP1, VP3 and VP0 inhibit type I IFN production（*J Virol. 2019； J Virol. 2021； Cell Death Dis. 2019*）

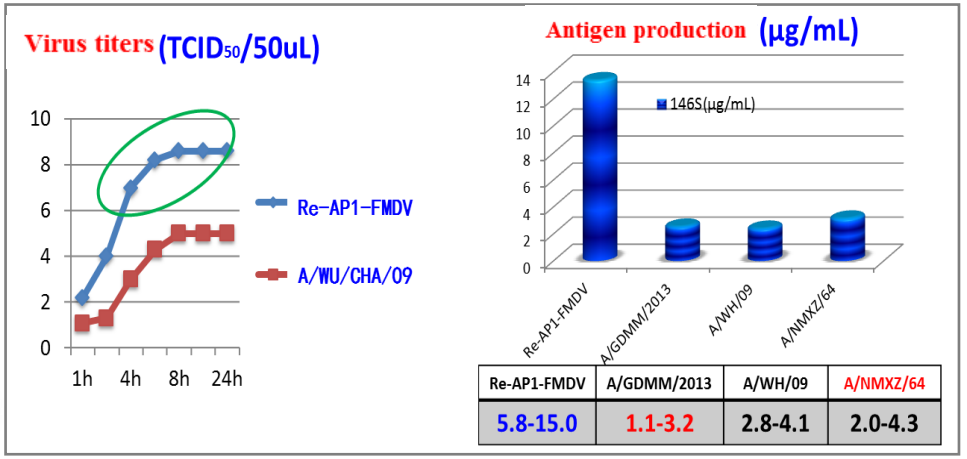


Mutation of the critical sites in FMDV proteins involved in suppression of host innate immune response accelerate, increase as well as extend antibody response in vaccinated animals.

3. Improvement of vaccine performance based on the mechanisms used by FMDV

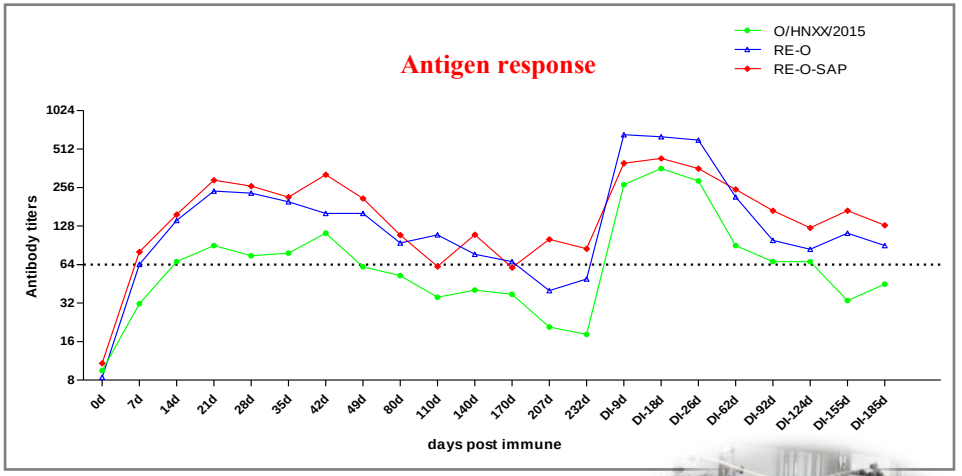
Producing high-quality vaccines with the performance of high production rates, increased immune efficacy, and improved safety. (Editing virus and cells)





Re-AP1-FMDV	A/GDMM/2013	A/WH/09	A/NMXZ/64
5.8-15.0	1.1-3.2	2.8-4.1	2.0-4.3

performance	Wildtype vaccine	Modified vaccine
Antigen production	1-3μg/mL	3.89~15.0μg/mL
Start of immune response	5-7 d	2-3d
Protection duration	4-6 month	8-12 month
Stability	4℃	√ improved
Cross-protection	No	√ improved
Immunosuppressive activity	Yes	√ improved
Marker	+/-	√ included



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Wen Dang (LVRI)

Weijun Cao (LVRI)

Ye Jin (LVRI)

Yanmin Li (SMU)

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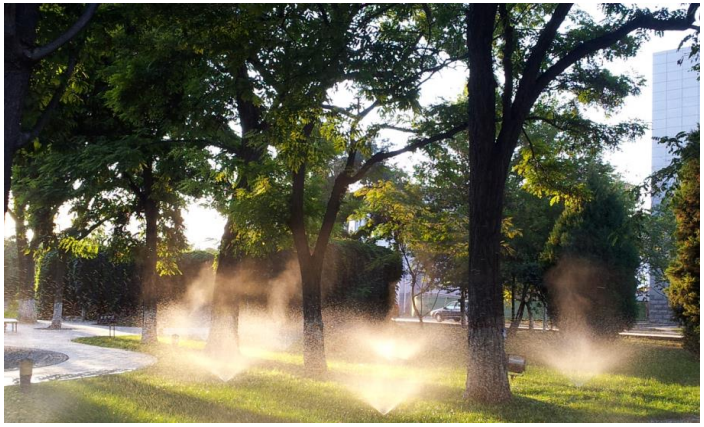


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