FMDV- Host interactions in a model of persistently infected bovine cells

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FMDV persistence… a prolonged subclinical infection

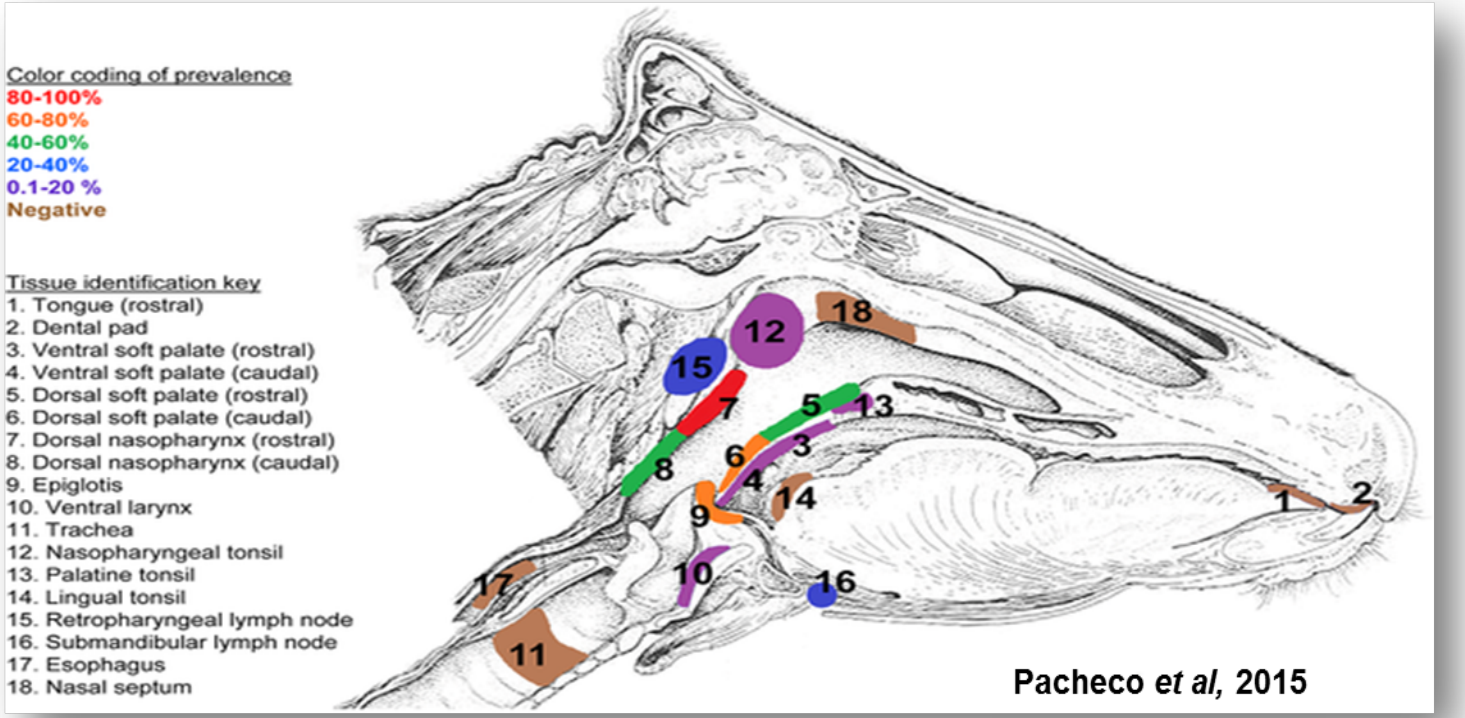
- Live FMDV recovered from pharyngeal samples more than 28 days post infection (Sutmoller *et al*, 1968)
- Up to 50% of ruminant animals become persistently infected after clinical recovery
- Probability of viral persistence decreases with age (84% virus isolated from 1-3 years old cattle)
- No convincing evidence of persistence in pigs
- Persistence can be established irrespectively of adaptive immunity

Duration of FMDV persistence:

- ✓ up to 9 months (small ruminants)
- ✓ up to 3.5 years in cattle
- ✓ up to 5 years in African Buffalo

Transmission to susceptible animals:

- ✓ Reported from Buffalo to cattle
- ✓ Extremely low rate of transmission
- ✓ Remains debatable but…
- ✓ Impediment for FMDV eradication



Anatomical sites of viral persistence:

- ✓ Epithelium of dorsal nasopharynx, Epithelium of dorsal soft palate
- ✓ Germinal centers of lymphoid tissue, palatine tonsils (namely in Buffaloes)

Mechanisms of FMDV persistence?

How is FMDV persistence established?
How is it maintained?

Precise mechanisms remain poorly understood…

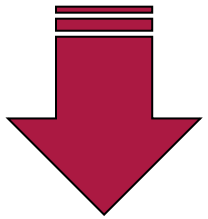
But research works provided clues towards understanding of FMDV persistence…

Mechanisms of FMDV persistence????

- *in Vitro*
  - ✓ Viral factors *(*CPE↓, fitness↑ in naïve cells of same type, fitness ↓ in other cell type and cattle, lack of using integrins as receptors, changes in genes coding for VP1 and NS proteins)
  - ✓ Cell modifications (resistance to superinfection of parental FMDV but not other viruses, cells becomes rounder, less inhibited by contact, grow faster, suppression of antiviral interferons and other cytokines autophagy)

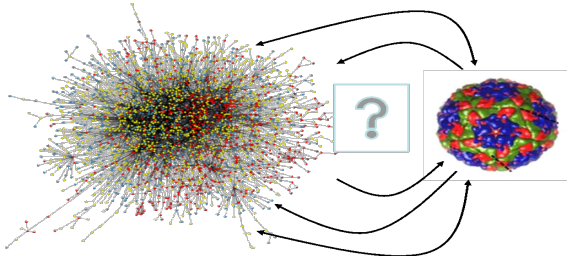
⇒ *Co-adaptation of virus and host cells*

- *in Vivo*
  - ✓ Virus factors (*genetic change leading to escape of neutralizing antibodies, changes in VP2,* persistence is related to viral virulence*)*
  - ✓ Cell modifications (suppression of antiviral host factors during persistent infection, escape of host cellular lysis mechanisms i.e. apoptosis/ necrosis, down-regulation of pro-apoptotic genes expression, over-expression of T regulatory cells related gene)



Host-FMDV interactions facilitate persistence of infectious virus

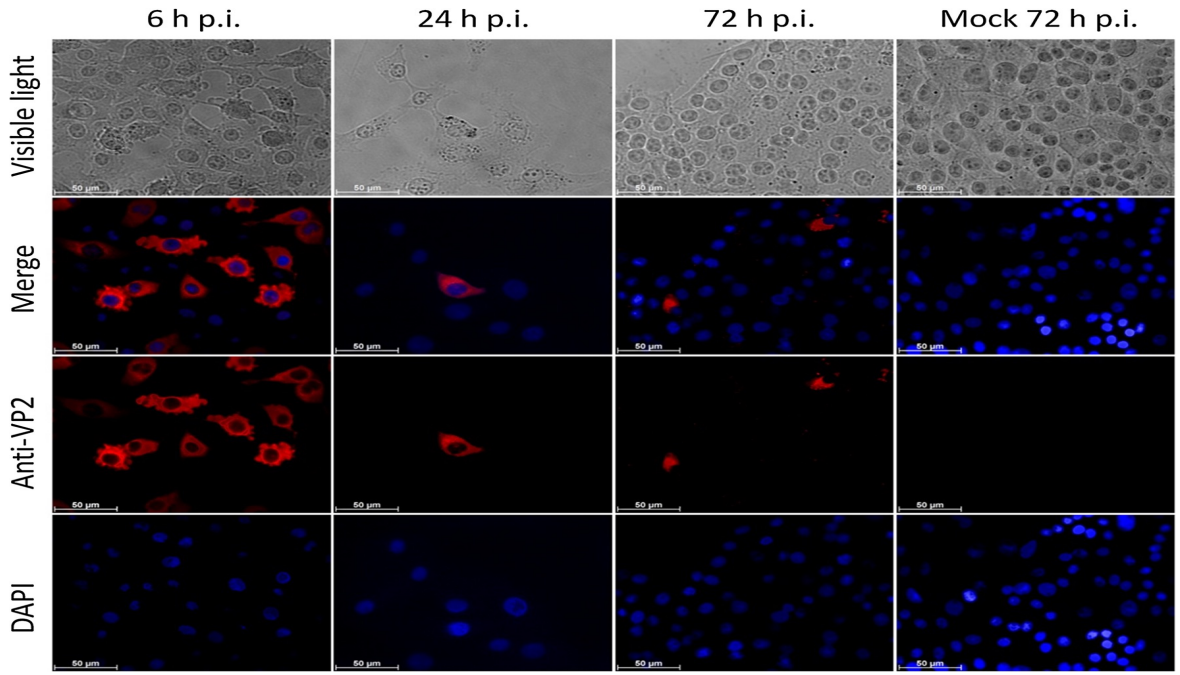
Epithelial bovine cells to study FMDV/Host interaction



- Establishment of persistently infected MDBK cells and collection of persistent viruses
- Persistence assays using primary bovine epithelial cells (SLU/ANSES)
- FMDV / host cell interactome analysis (Y2H)

❑ Establishment of persistently infected MDBK cells and collection of persistent viruses

Establishment of persistently infected MDBK cells (1)

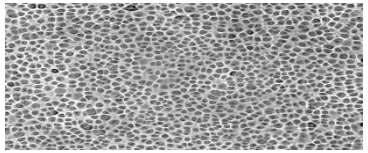


Kopliku *et al*, 2015

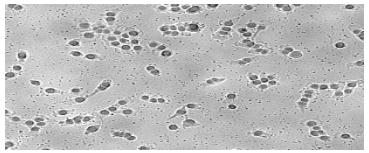
MDBK cells:

- ✓ Epithelial bovine cell line
- ✓ Permissive to FMDV infection
- ✓ Ability to reconstitute cell monolayers after FMDV infection
- ✓ Competent for IFN-I production

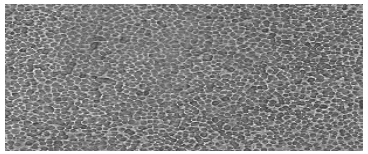
1) Inoculation of MDBK cells with a viral clone of FMDV O (MOI 4)



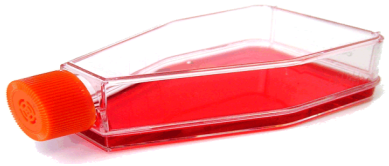
2) Well rinsed 48h p.i.



3) Monolayer of surviving cells



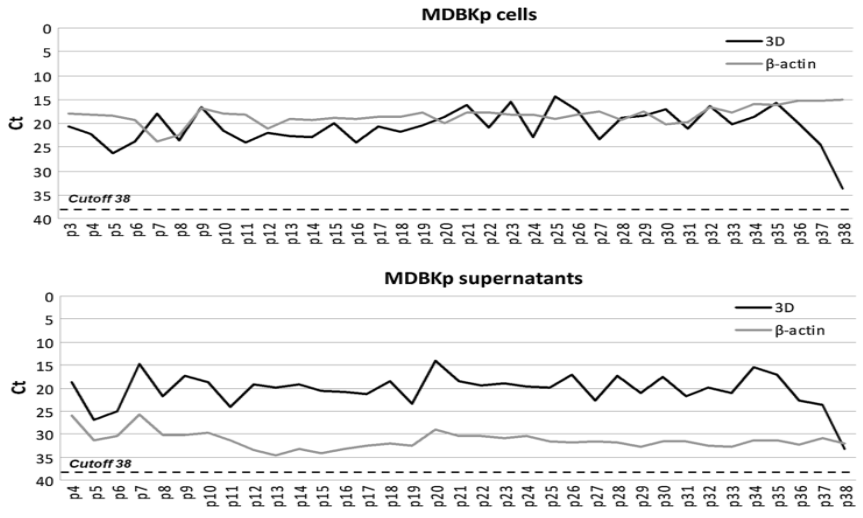
4) Subculturing and sampling



Viral RNA extraction at each passage & RT-PCR targeting 3D coding region

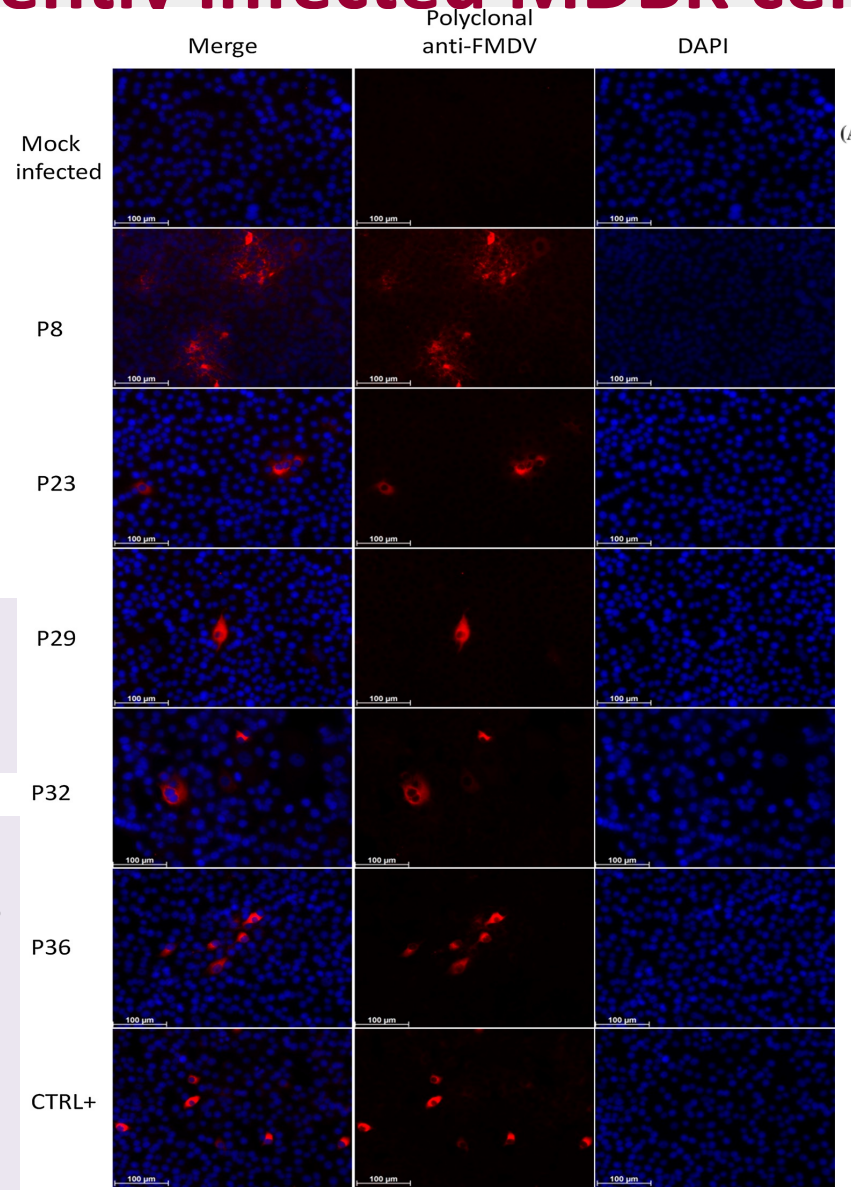
MDBK subcultured during 42 passages p.i
(= 5 months in culture)

Establishment of persistentlv infected MDBK cells (2)

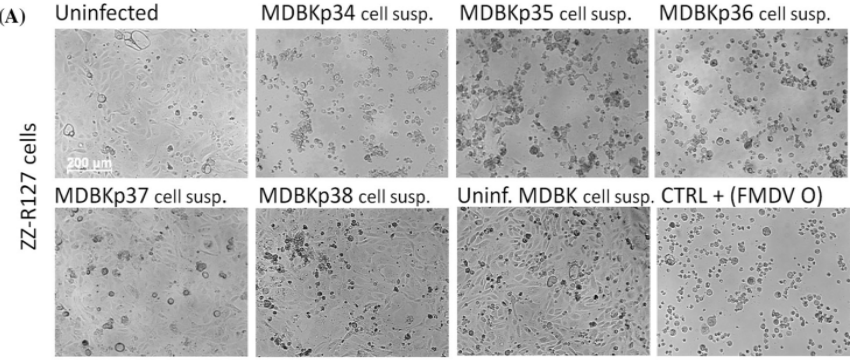


Kopliku *et al,* 2015

- ✓ Viral RNA & viral proteins still detected in highly passaged cells
- ✓ Infectious virus recovered until 36 passages p.i.
- ✓ Duplicate: MDBK subcultured during 25 passages p.i. and viral RNA recovered until 18 passages p.i.

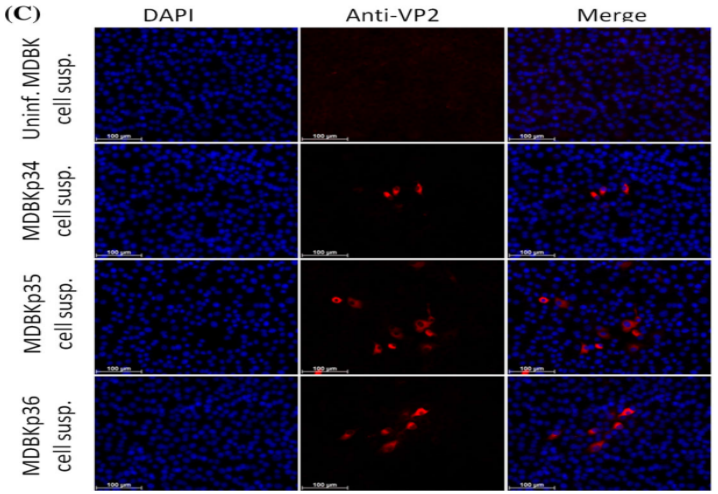


Kopliku *et al,* 2015



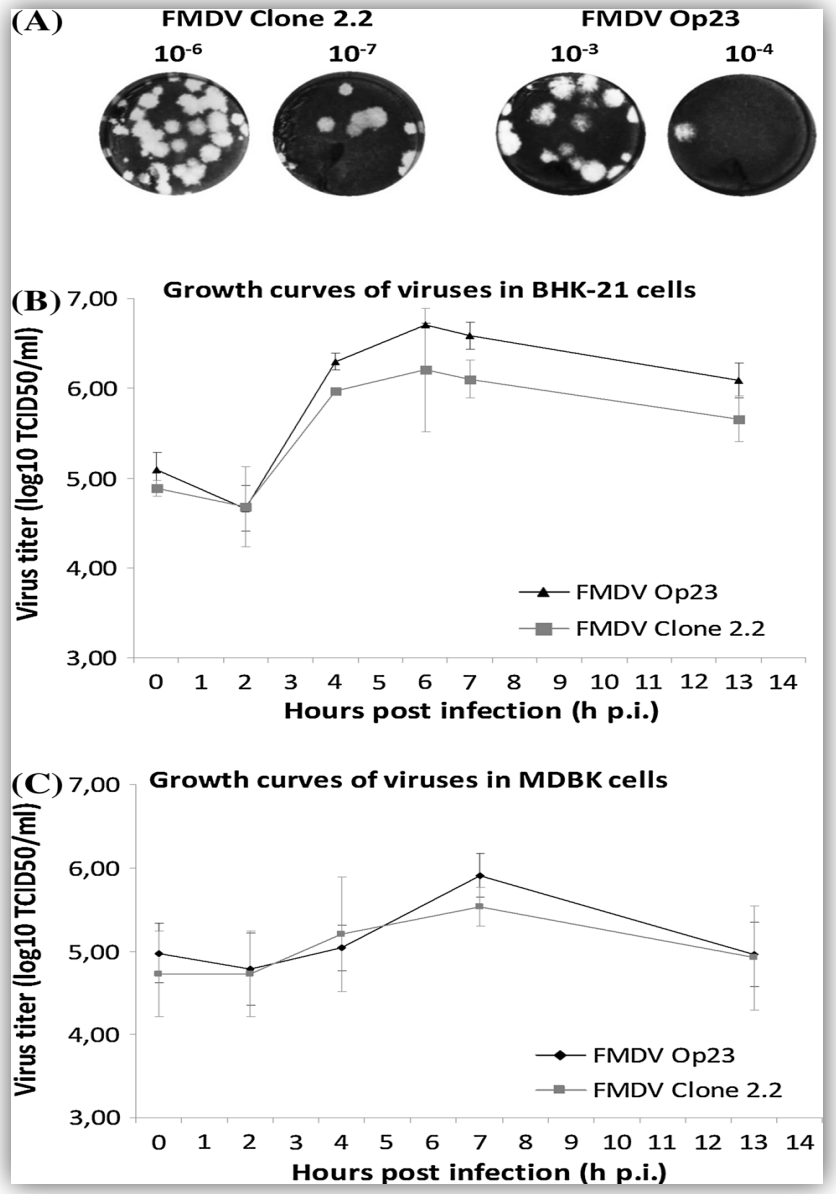
Kopliku *et al,* 2015

MDBK cells (IF detection)

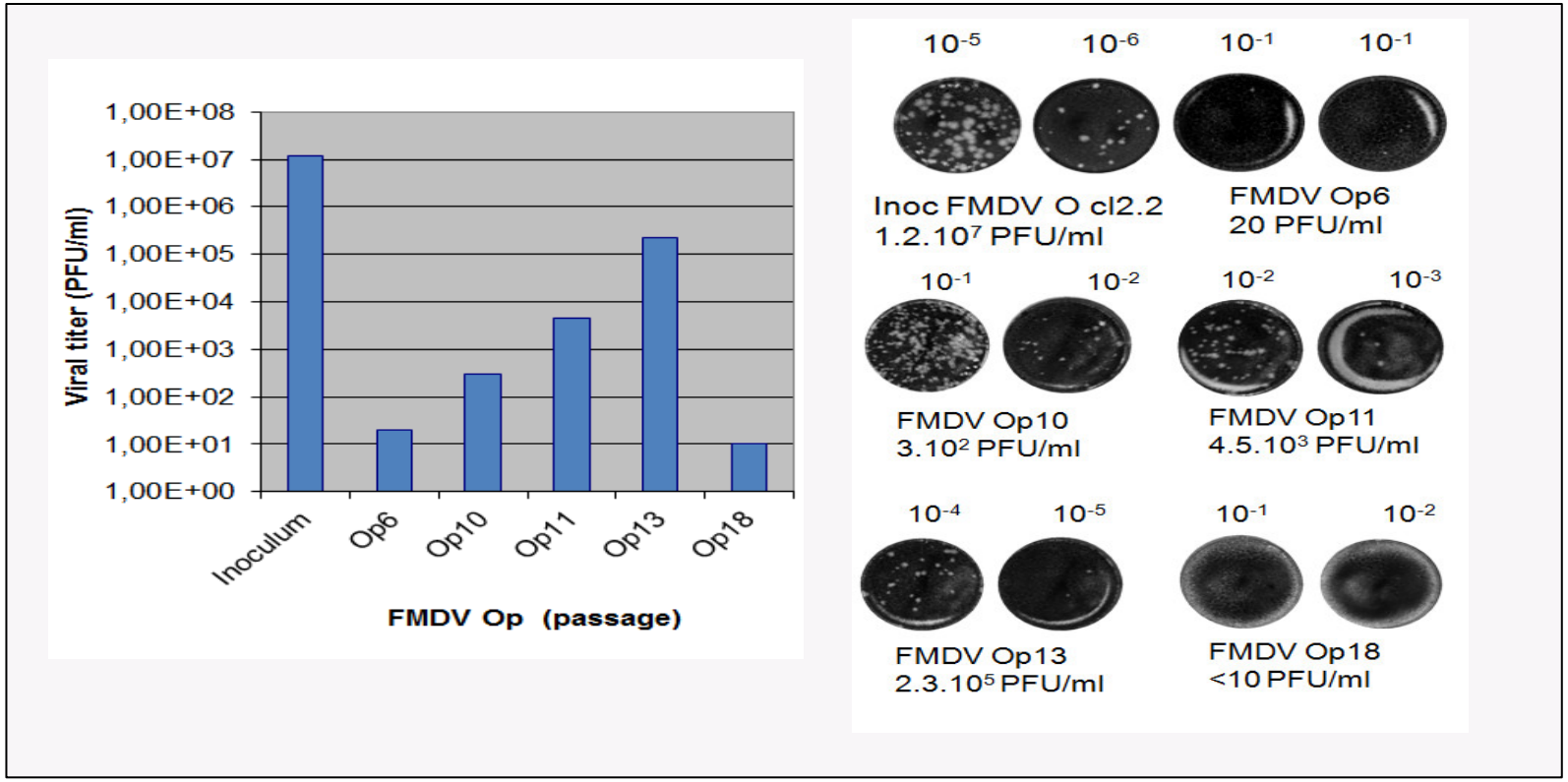


Kopliku *et al,* 2015

Characterization of Persistent viruses recovered from MDBK"p" cells



Kopliku *et al*, 2015

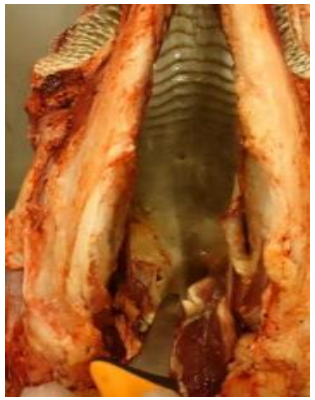


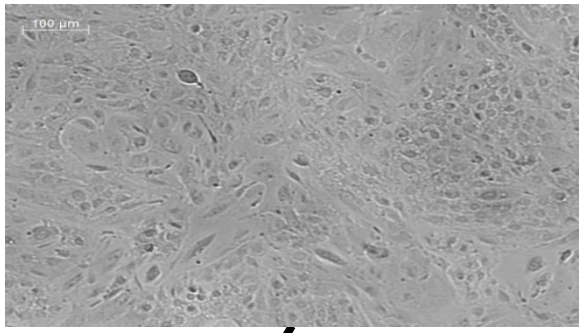
- Similarity of plaque size/shape and growth kinetics between FMDVOp and original inoculum
- Viral production increases along passages
- V50A substitution in the VP1 coding region of FMDVOp23

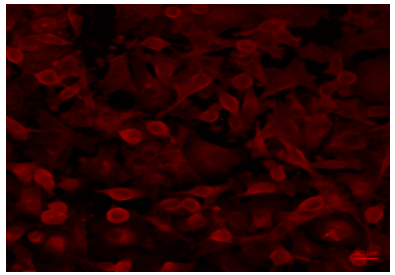
❑ FMDV Persistence assay in primary bovine epithelial cells

Dorsal Soft Palate epithelial cells "DSP"

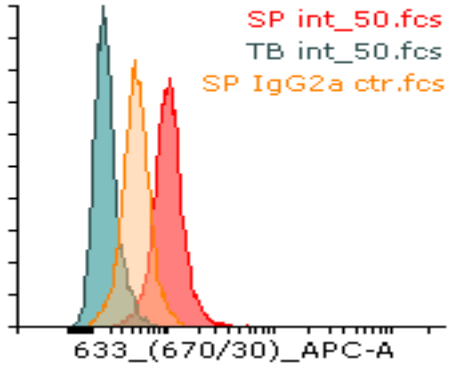
- *purified , propagated and characterized at SLU*



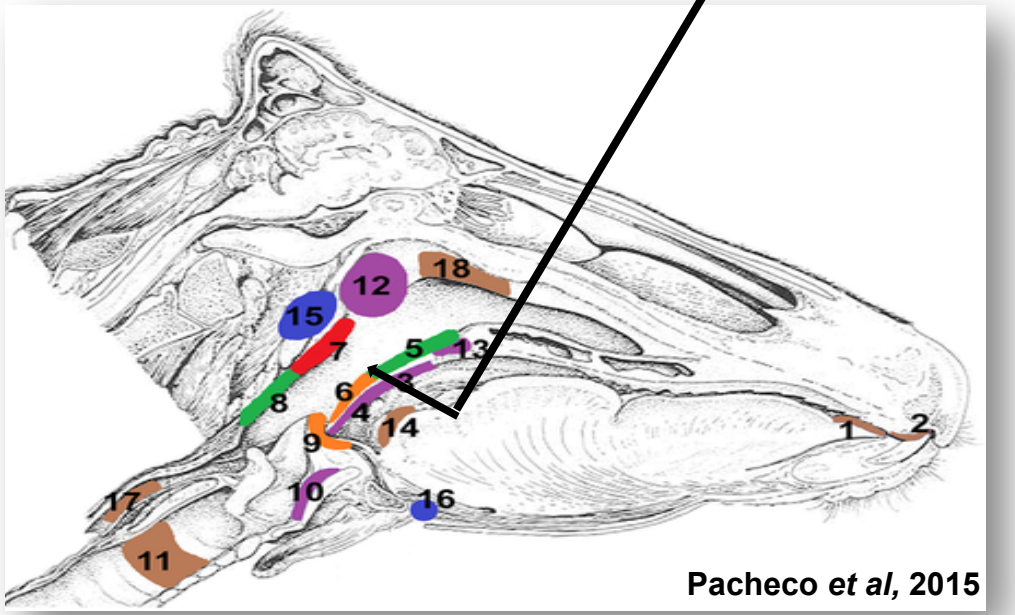




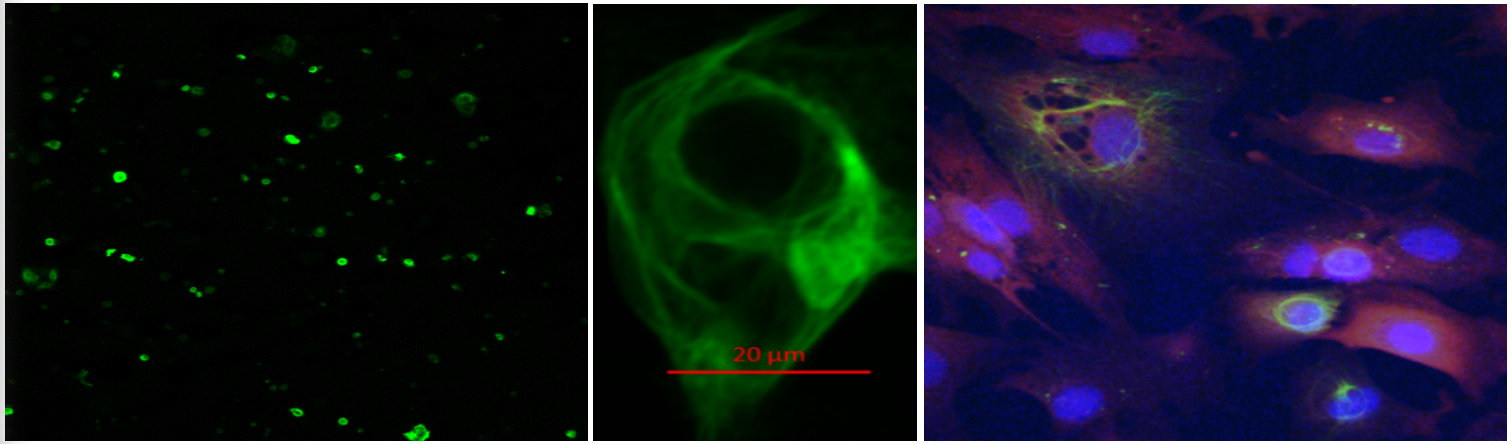
Integrin $\alpha_V\beta_6$



Integrin $\alpha_V\beta_6$, turbinate cells
Isotype control, soft palate cells
Integrin $\alpha_V\beta_6$, soft palate cells



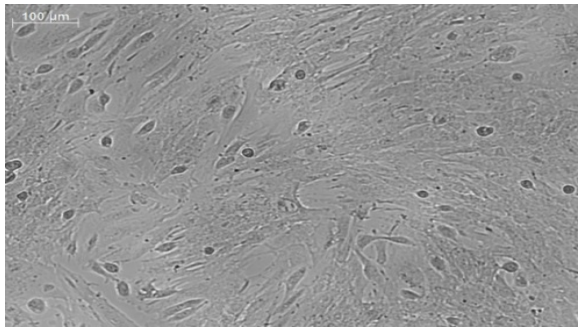
Pacheco *et al*, 2015

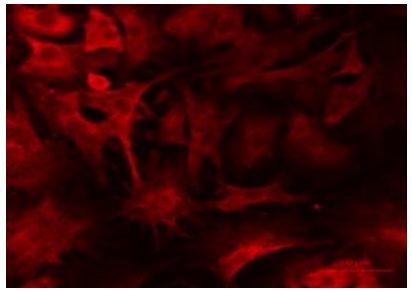


Cytokeratin (4, 5, 6, 8, 10, 13 or 18) staining

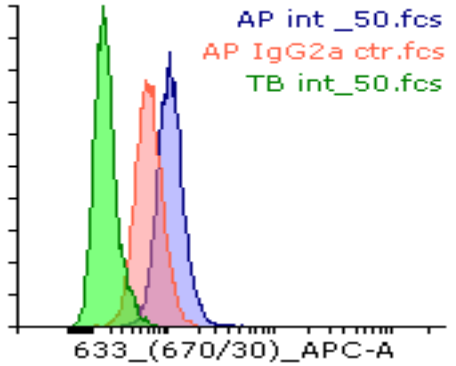
Alveolar pneumocytes, "AP"

- *purified, propagated and characterized at SLU*

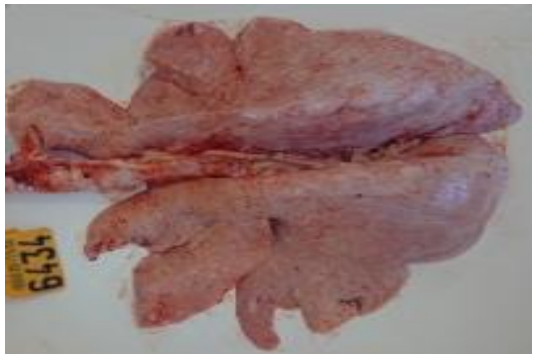


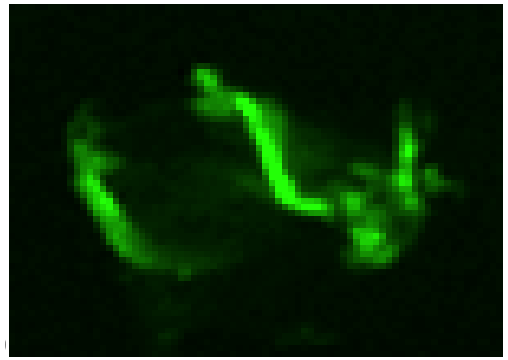


Integrin $\alpha_V\beta_6$

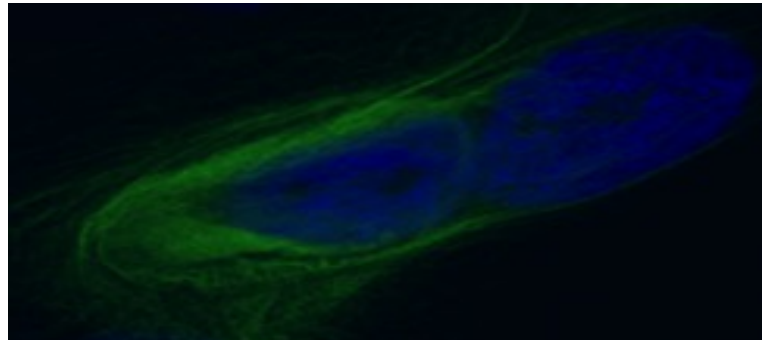


Integrin $\alpha_V\beta_6$, turbinate cells
Isotype control, alveolar pneumocytes
Integrin $\alpha_V\beta_6$, alveolar pneumocytes



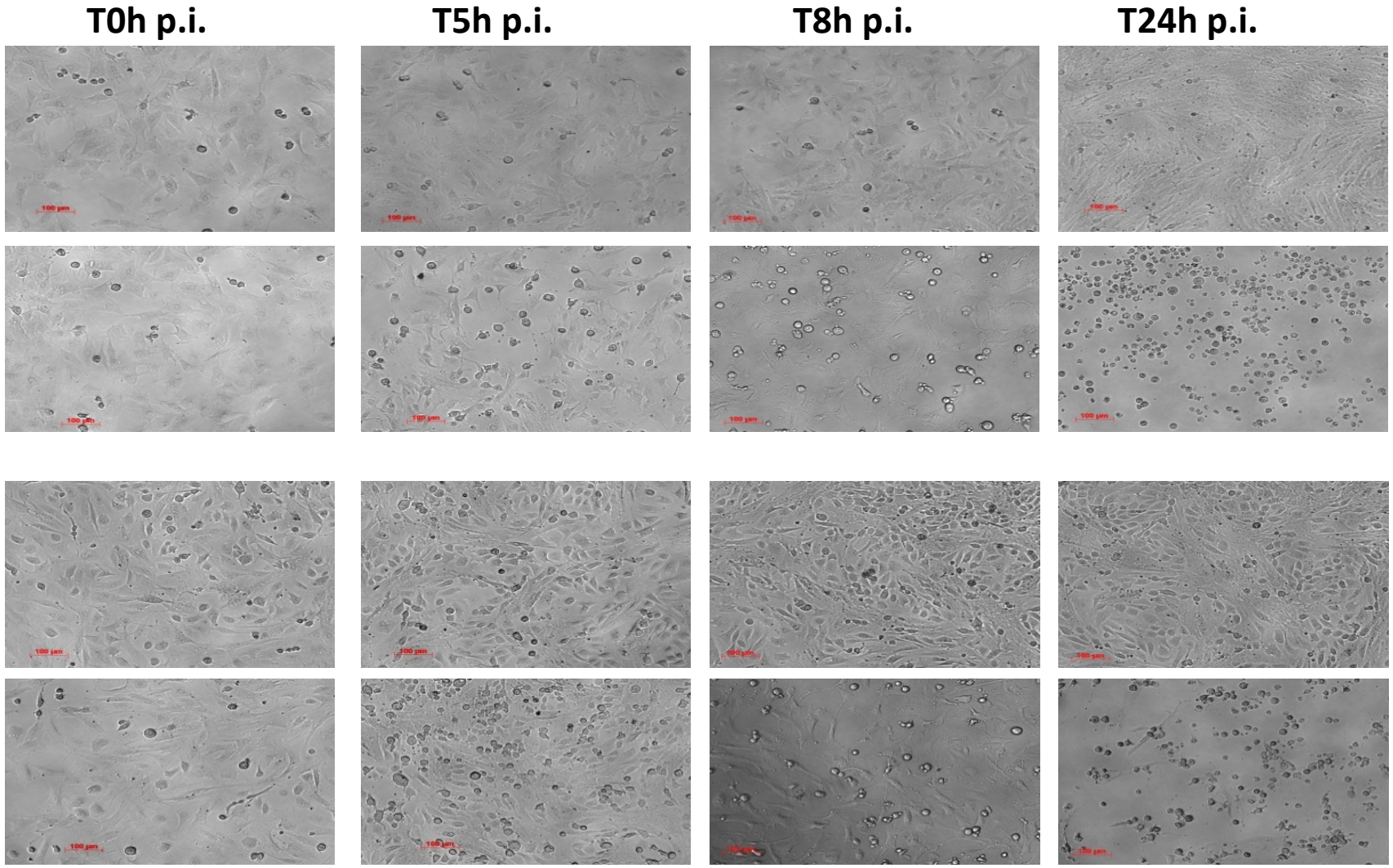


AP type 2 lamellar bodies (CD208) in dividing cells



Cytokeratin (4/5/6/8/10/13/18) in dividing cells.

Inoculation of DSP and AP * (FMDV O/FRA/1/2001)



Complete CPE at 24h p.i., both for DSP and for AP cells

* Batch of cells propagated from the same animal

Subculturing of infected DSP and AP (infectious virus recovery?)

- 48h p.i., 6 then 13 days p.i., surviving cells rinsed and medium refreshed
- DSP cells and AP cells propagated at 13days p.i and 16 days p.i. respectively

DSP and AP P0+1

20 days p.i.: collection of supernatant and inoculation to IBRS-2 cells (i.e. porcine sensitive cells) and DSP

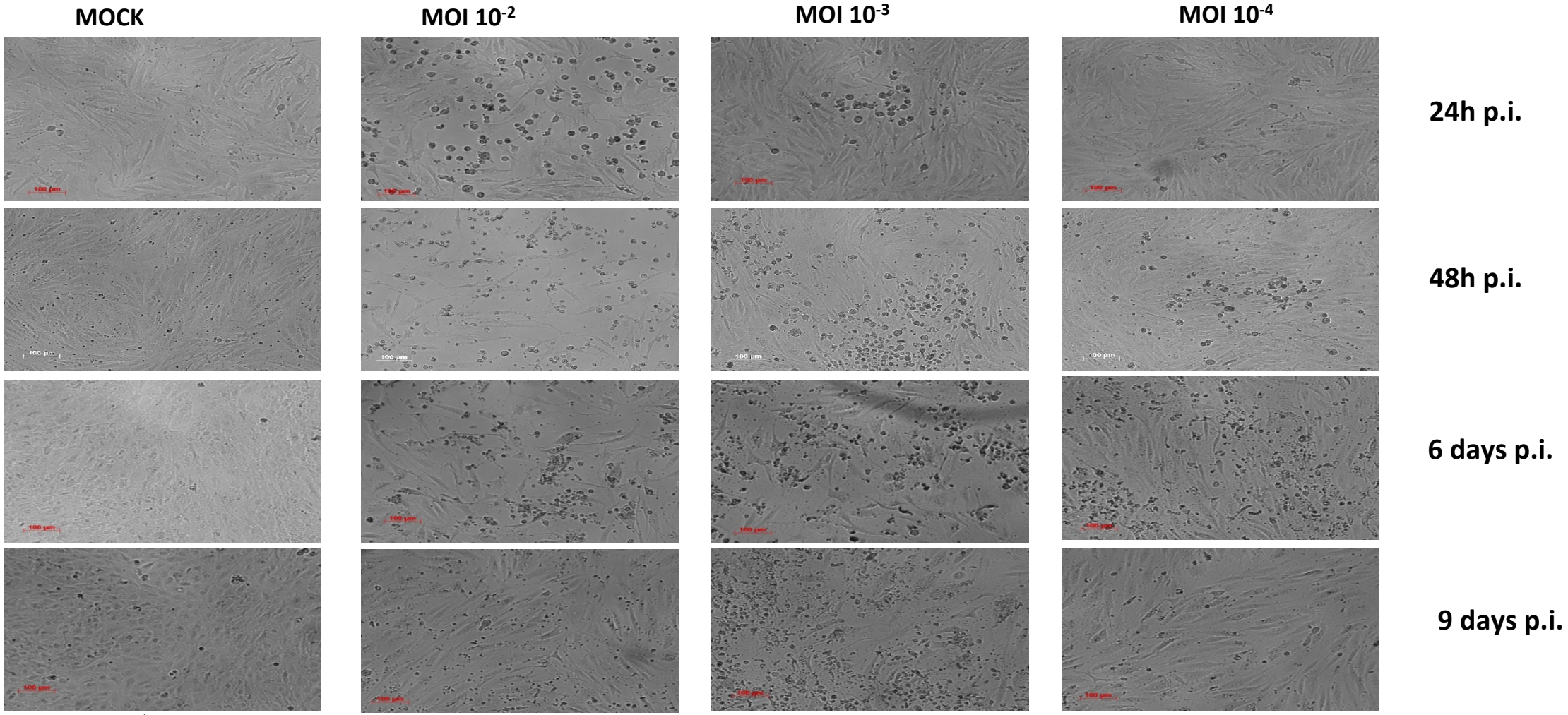
24h p.i.				48h p.i.		
inuculum=	IBRS-2	SP		inuculum=	IBRS-2	SP
AP Mock	no CPE	no CPE		AP Mock	no CPE	no CPE
AP MOI 0.1	no CPE	no CPE		AP MOI 0.1	no CPE	no CPE
SP Mock	no CPE	no CPE		SP Mock	no CPE	no CPE
SP MOI 0.1	50% CPE	25% CPE		SP MOI 0.1	100% CPE	100% CPE

NB: CPE were confirmed by Ag Capture

Infectious FMD virus can be recovered from DSP cells, not from AP cells, 20 days p.i. (one cell passage p.i.).
Results consistent with sites of FMDV persistence *in vivo.*

Ongoing experiment…. persistence assay in DSP cells…(1)

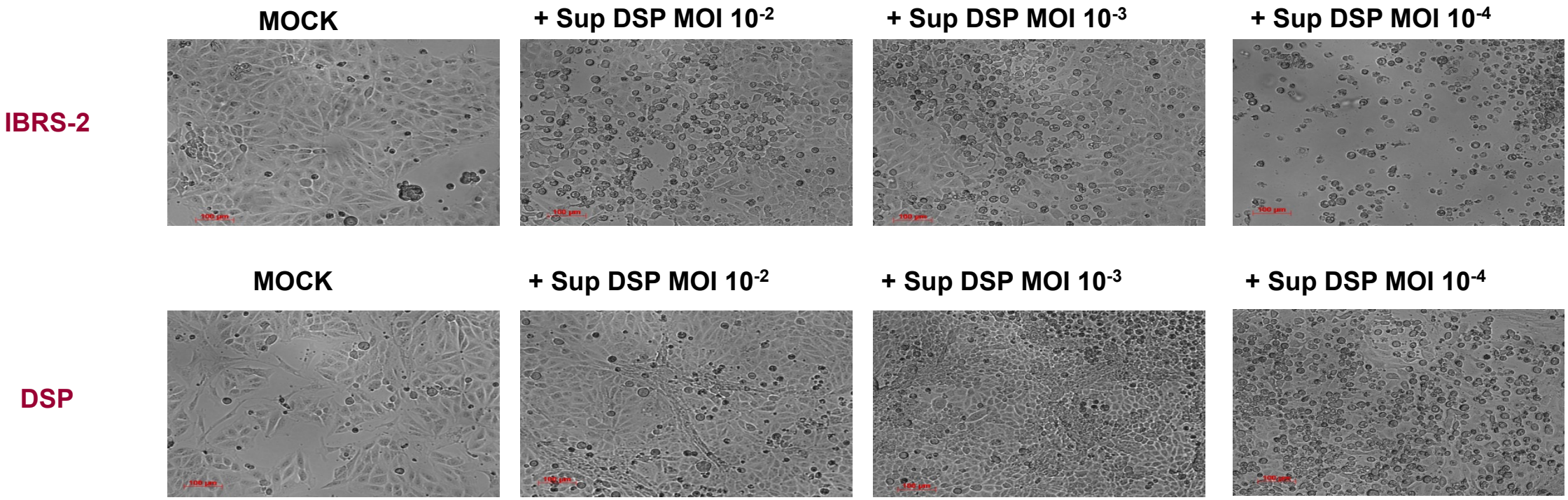
- DSP cells inoculated with MOI 10^{-2} to 10^{-4} (other batch of cells, other animal)



Surviving DSP cells can gradually reconstitute monolayers within 9 days p.i.

Ongoing experiment…. persistence assay in DSP cells…(2)

- IBRS-2 & DSP cells inoculated with 21d p.i. supernatants of inf DSP cells



- Infectious FMD virus can be recovered from supernatants of DSP cells (inoculated with MOI 10^{-2} to 10^{-4}), 21d p.i. (one cell passage)
- Infectious FMD virus can be recovered from supernatants of DSP cells (inoculated with MOI 10^{-2} to 10^{-4}), 65d p.i. (5 cell passages) and 37dpi (one cell passage) respectively

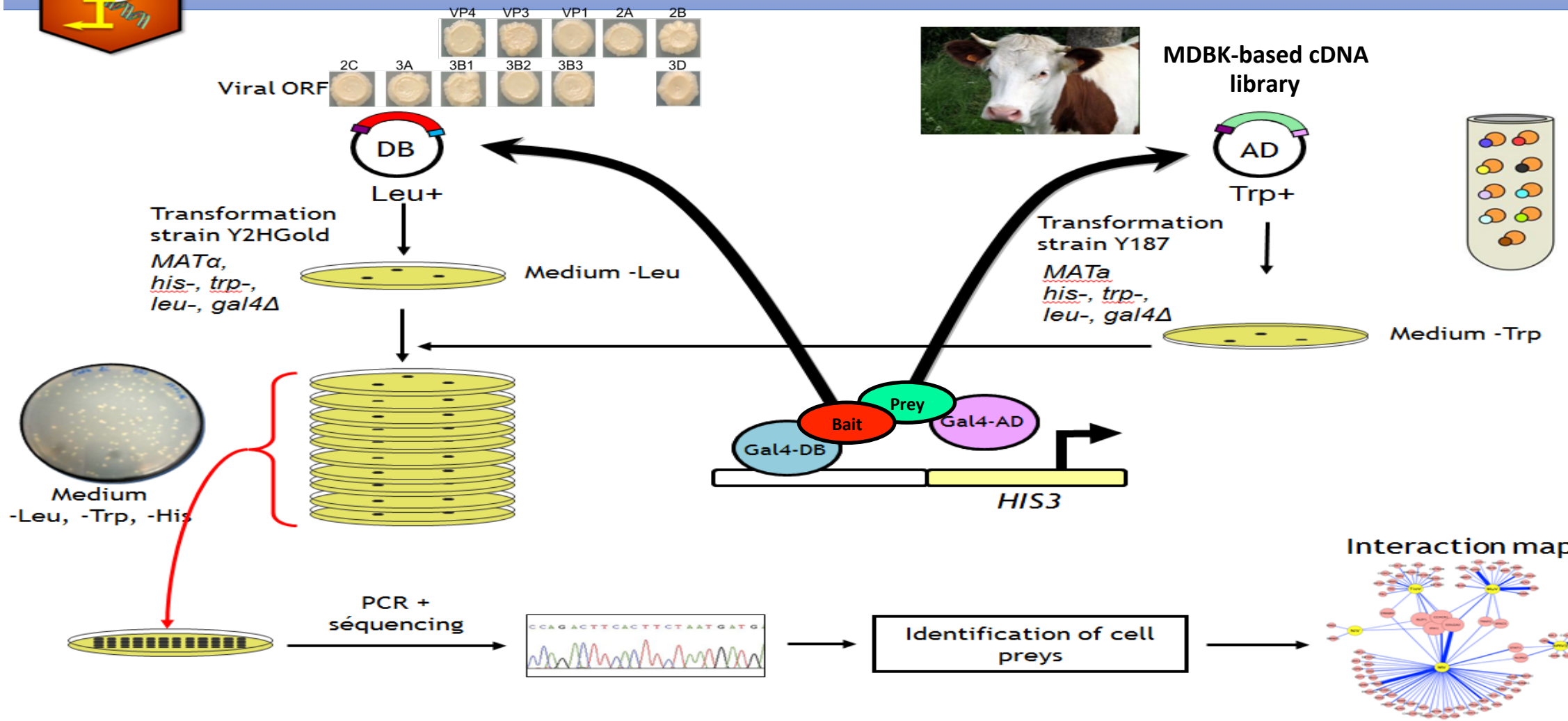
…to be continued

❑ FMDV / host cell interactome analysis (Y2H)

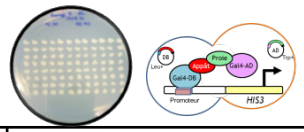
FMDV-host protein interactions analysis by Y2H screening

To better understand the pathogenesis of FMDV by identifying cellular pathways modulated during acute or persistent FMDV infection

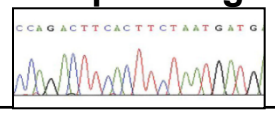
Y2H screening

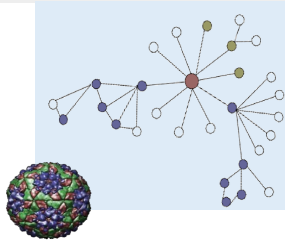


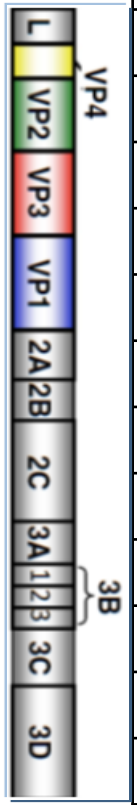
Results of Y2H screening of the MDBK cDNA library



PCR → Sequencing → Identification of cellular preys







	Colonies pricked out	Interactions identified	Candidate host interacting proteins
Lab^{pro}/Lb^{Pro}	0	0	0
VP4	0	0	0
VP3	0	0	0
VP1	0	0	0
2A	0	0	0
2B	2	2	2
2C	**192**	**155**	**7**
3A	2	2	1
3B1	7	0	0
3B2	0	0	0
3B3	2	0	0
3D	**165**	**155**	**8**
Total	**370**	**313**	**18**

18 candidate interactions identified (to be validated),
3 signaling pathways potentially involved (innate immunity, apoptosis and autophagy)

Conclusions & Perspectives

❑ Cellular models of FMDV persistence in bovine epithelial cells

Two complementary cellular models developed to study molecular determinants of FMDV persistence

- ✓ Transcriptomic analysis (GeneChip Bovine array and RT-PCR custom array approach) of persistently, acutely or mock infected cells (MDBK as well DSP cells, potential comparison with AP cells)
- ✓ Characterization (genetic and phenotypic) of the persistent viruses from persistently infected cell lines, full genome sequencing by NGS and identification of mutations potentially involved in persistence.

in collaboration with FLI

❑ Host/Virus protein interactions analysis

18 candidate interactions identified (to be validated, 3 signaling pathways potentially involved)

- ✓ Biochemical and functional validation of candidate interactions, namely using DSP cells
- ✓ Y2H screening completion for genomic region coding for proteases (Labpro, Lbpro and 3C pro)
- ✓ Y2H screening using ORF cloned from persistent viruses (for any mutation characterized)
- ✓ Performing complementary analyses using cDNA library prepared from DSP or AP cells

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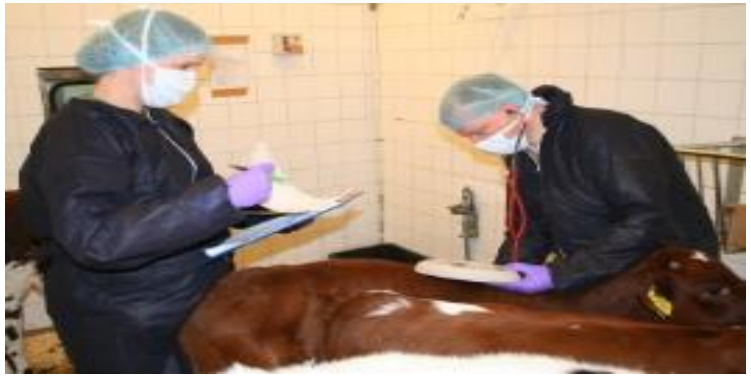

Sara Hägglund & JF Valarcher

Katarina Näslund

Stephan Zientara

And…

Thank you for your attention…

http://www.devenir-veto.com/images