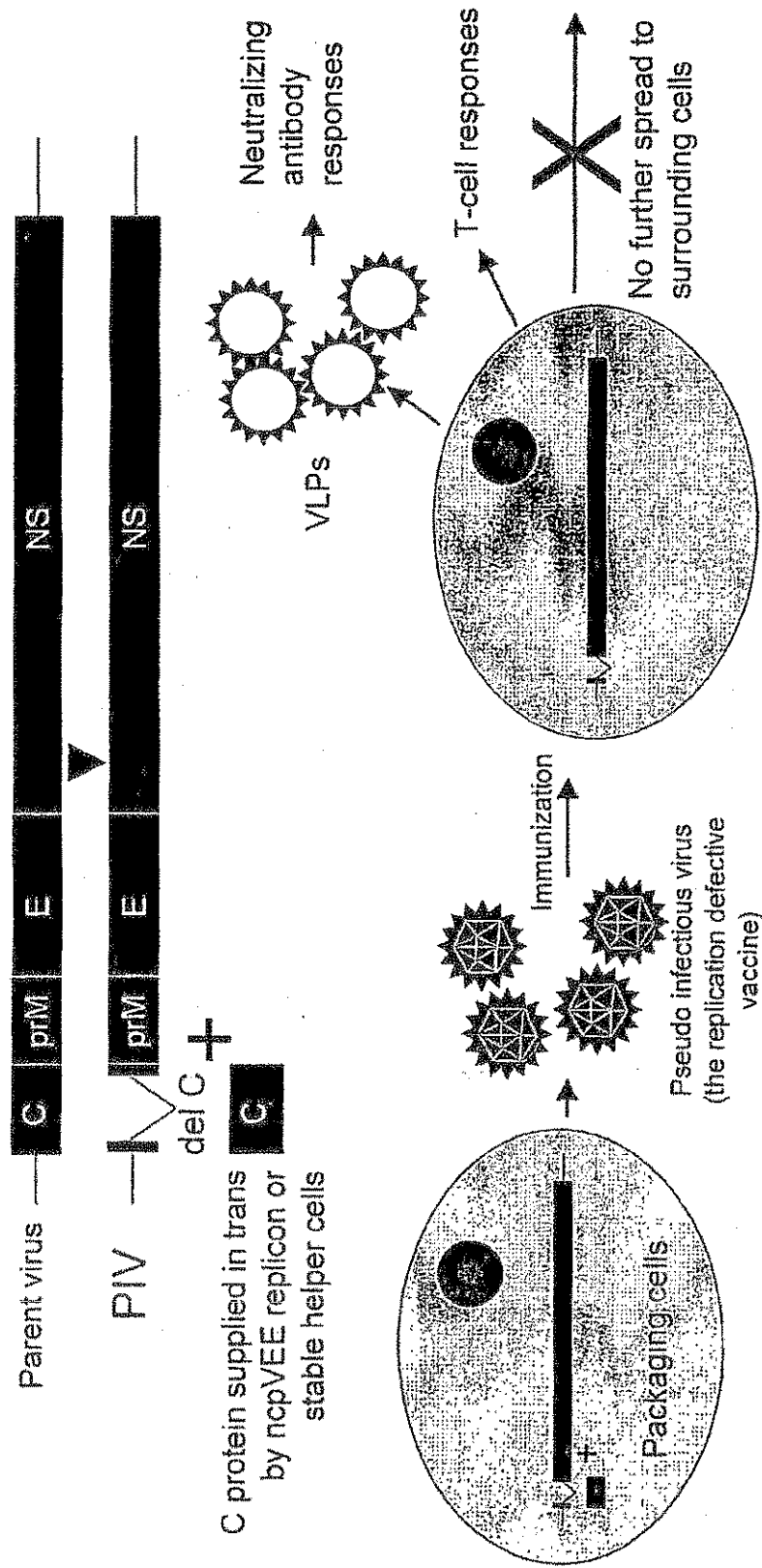
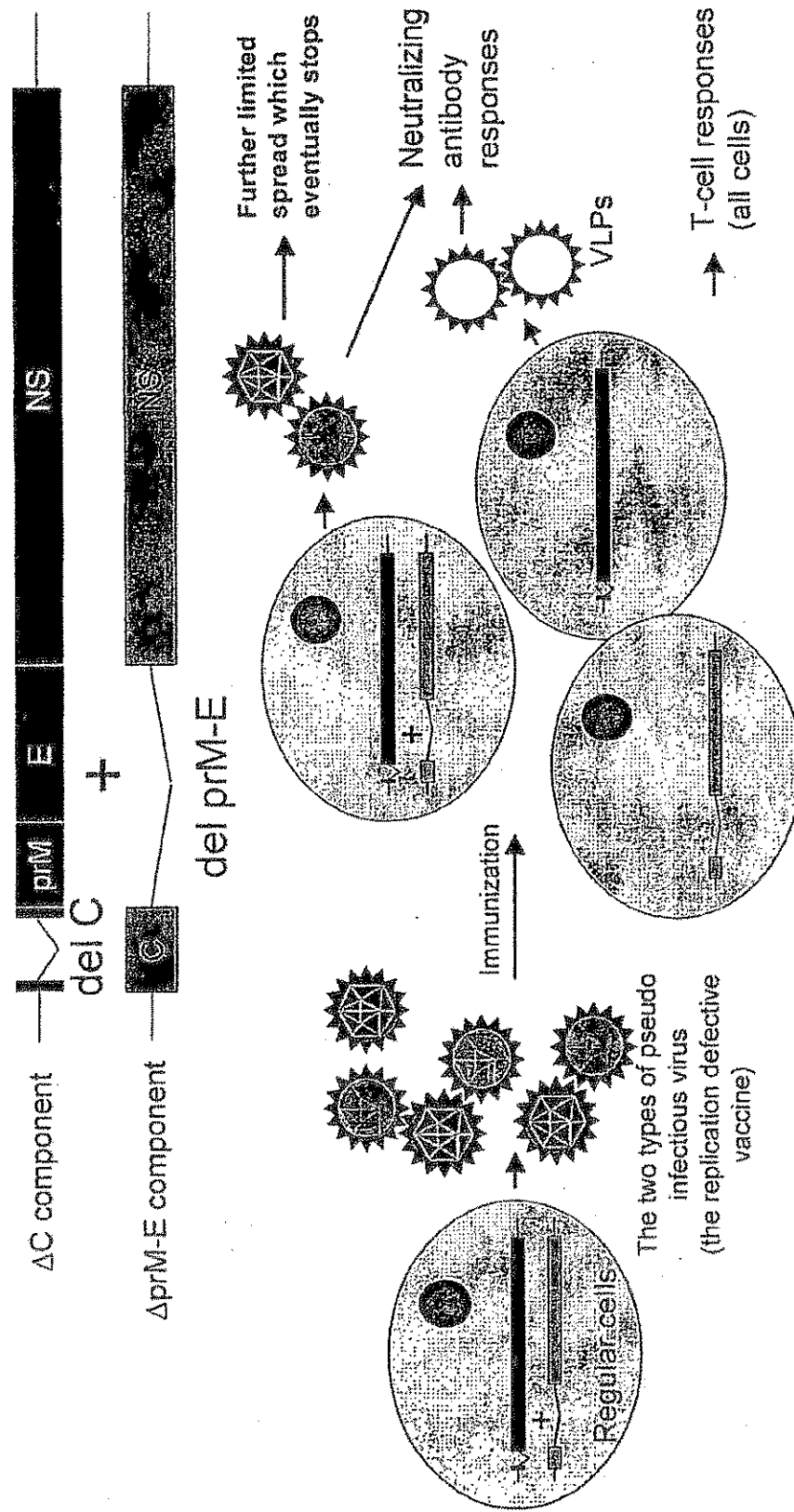


Fig. 1. Principle of single-component PIV (s-PIV; single-round replication in vivo)



For recombinant vaccines: foreign immunogen inserted in place of the ΔC deletion, or elsewhere, e.g. intergenically etc.

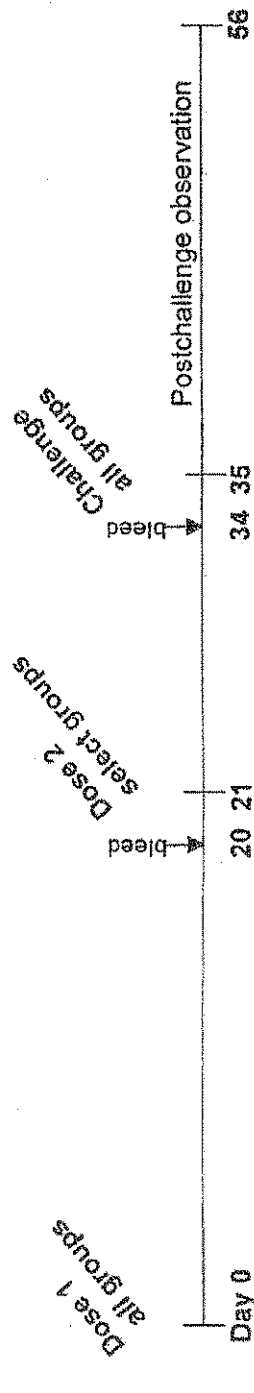
Fig. 2. Principle of two-component PIV (d-PIV; limited spread in vivo)



For recombinant vaccines: foreign immunogen inserted in place of ΔC and/or $\Delta prM-E$, or elsewhere.

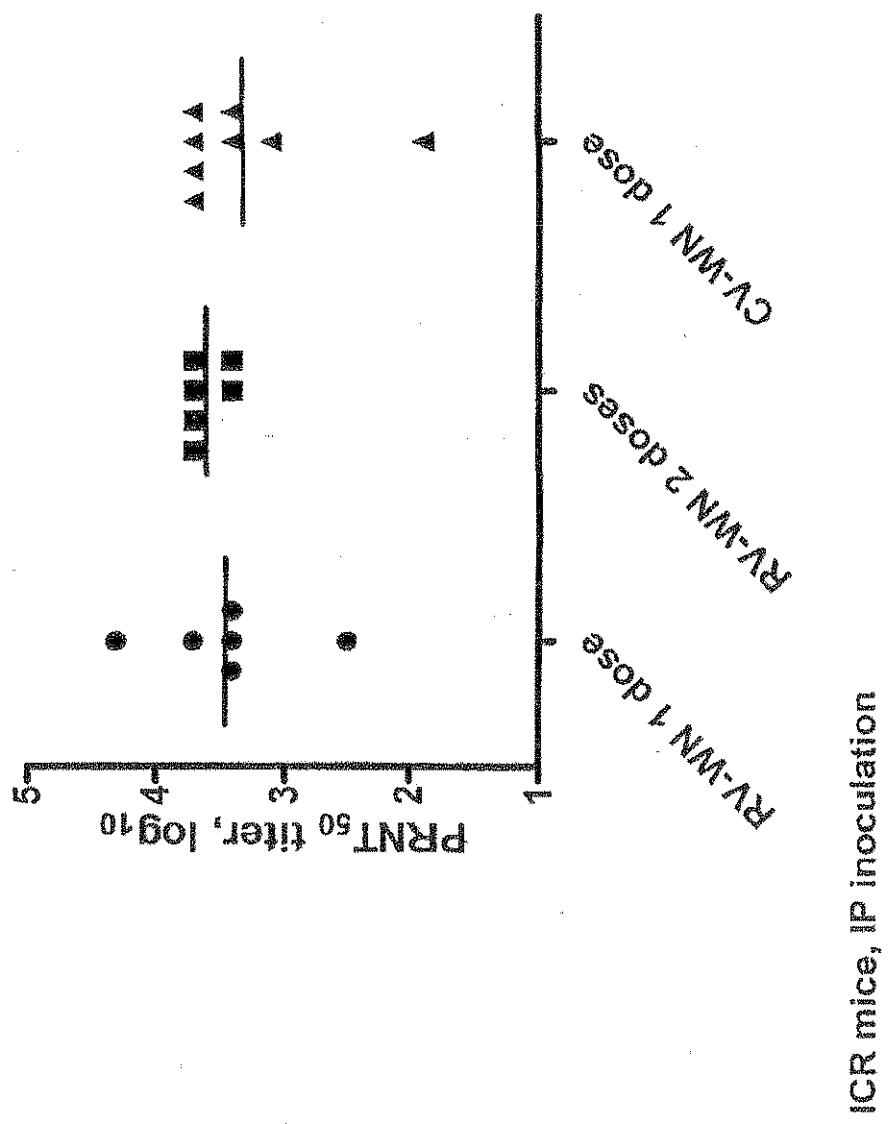
Fig. 3. Immunogenicity/efficacy: general experiment design (mice)

Experiment design in mice



ICR mice for WN and YF groups
C57/BL6 mice for JE groups

Fig. 4. PIV-WN induces uniform humoral immunity in mice



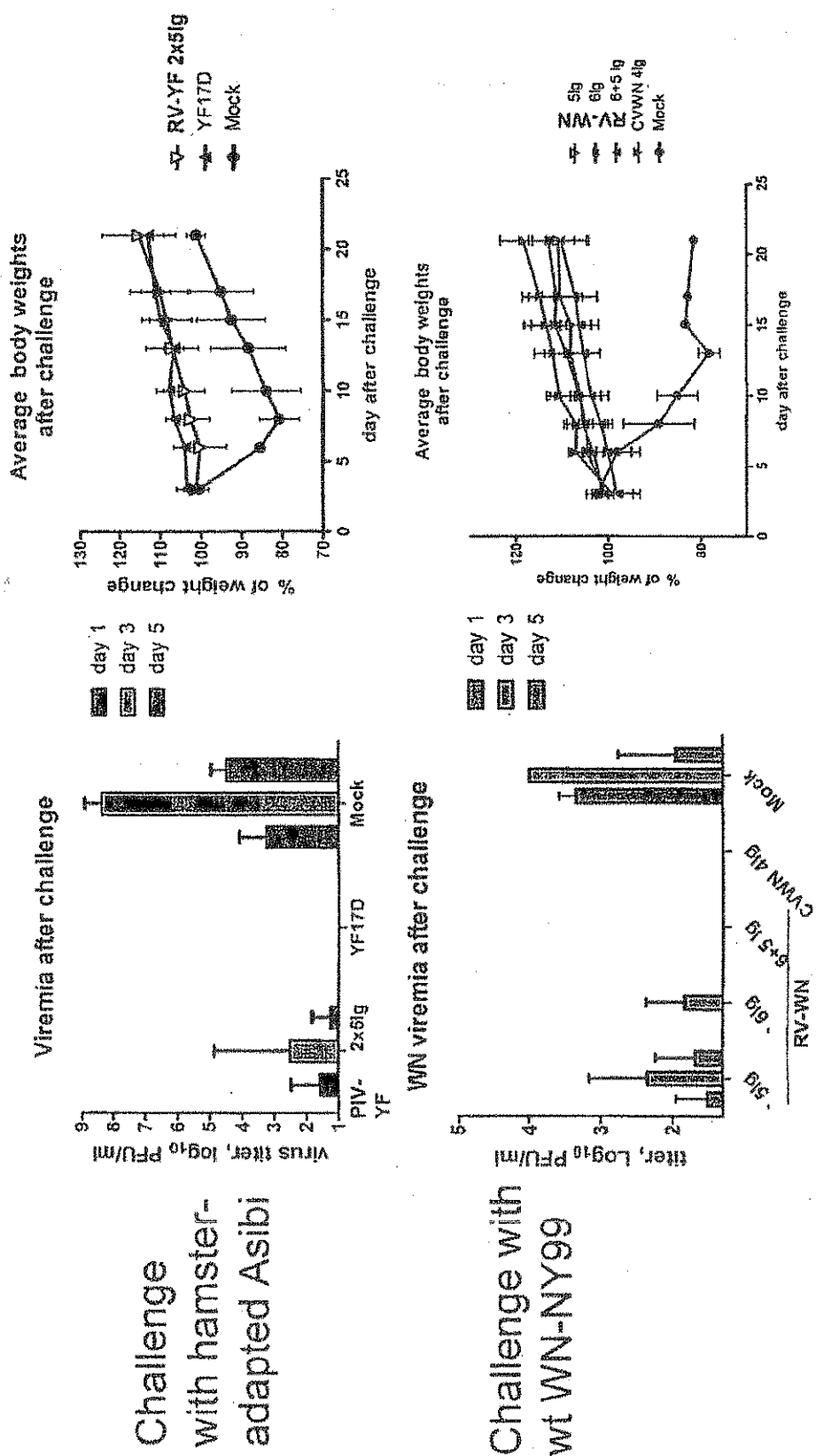
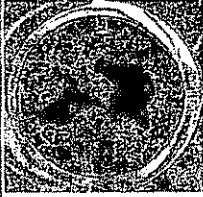
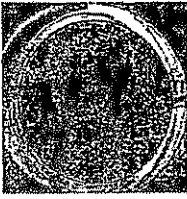
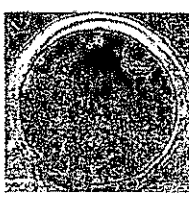
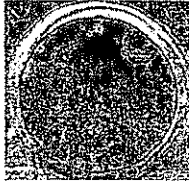
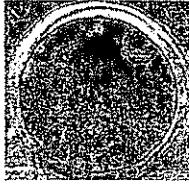


Fig. 6. YF/TBE viruses

prM-E genes	P0 virus titer log ₁₀ PFU/ml	P1 virus titer log ₁₀ PFU/ml	Immuno-staining mHIAF	Immuno-staining αRSSE
TBEV prM-SP (p42)	7.6	7.9		
Hypr WNV prM-SP (p45)	7.1	7.4		
Hypr + dC2 "CΔ3aa" (p59)	5.6	6.5		
LGT/E5 (p43)	7.8	8.1		

NOTES:

- p42, p45, p59, and p43 are designations of plasmids
- plaque morphology for p59-derived chimera was determined in a separate titration experiment (not shown; result of immunofluorescence assay shown)

Fig. 7. PIV-WNV/TBE constructs

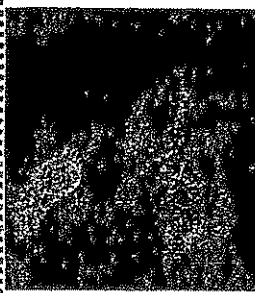
prM-E genes	P0 titer log ₁₀ FFU/ml		P1 titer log ₁₀ FFU/ml		Immuno-staining αRSSE mHIAF
	C helper cells	CprME helper cells	C helper cells	CprME helper cells	
Hypr (TBEV signal) (p39)	7.2	6.7	6.9	77.1	
Hypr (WNV signal) (p40)	6.7	6.0	5.9	66.9	

Fig. 8. Replication kinetics of live YF/TBE and replication defective PIV-WNV/TBE variants in cell substrates

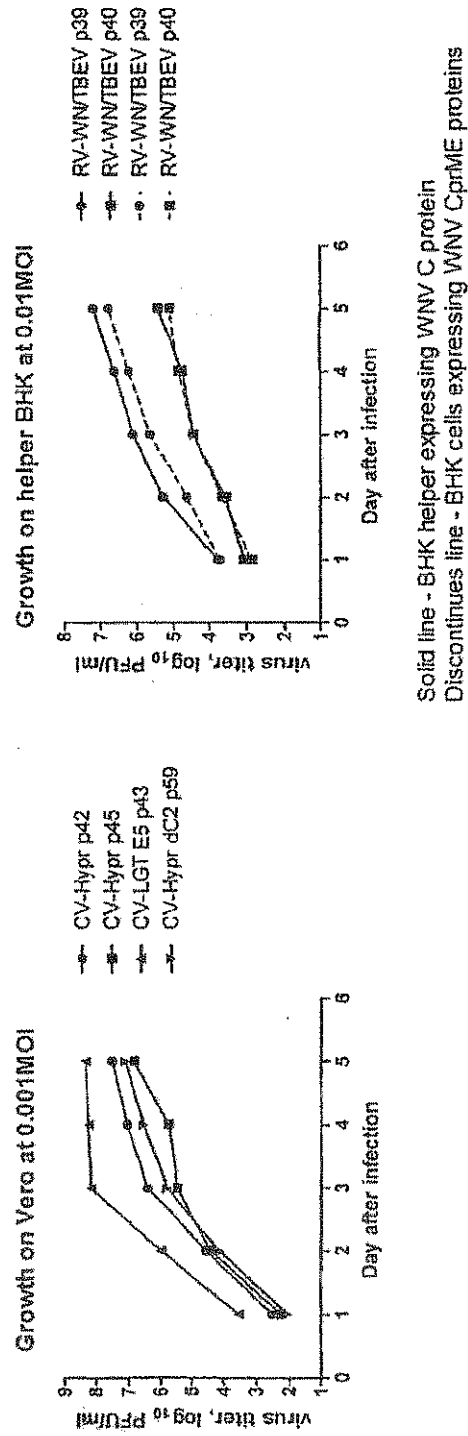


Fig. 9. Survival of mice inoculated IC with PIV-TBE and YF/TBE constructs in the neurovirulence test (3.5 week-old ICR mice)

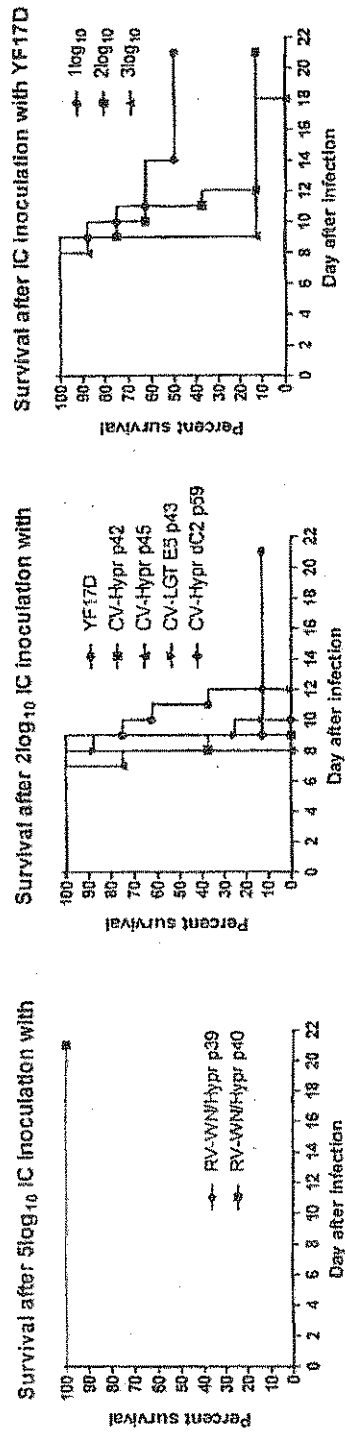


Fig. 10. Survival of mice inoculated IP with PIV-TBE and YF/TBE constructs in a neuroinvasiveness test (3.5 week-old ICR mice)

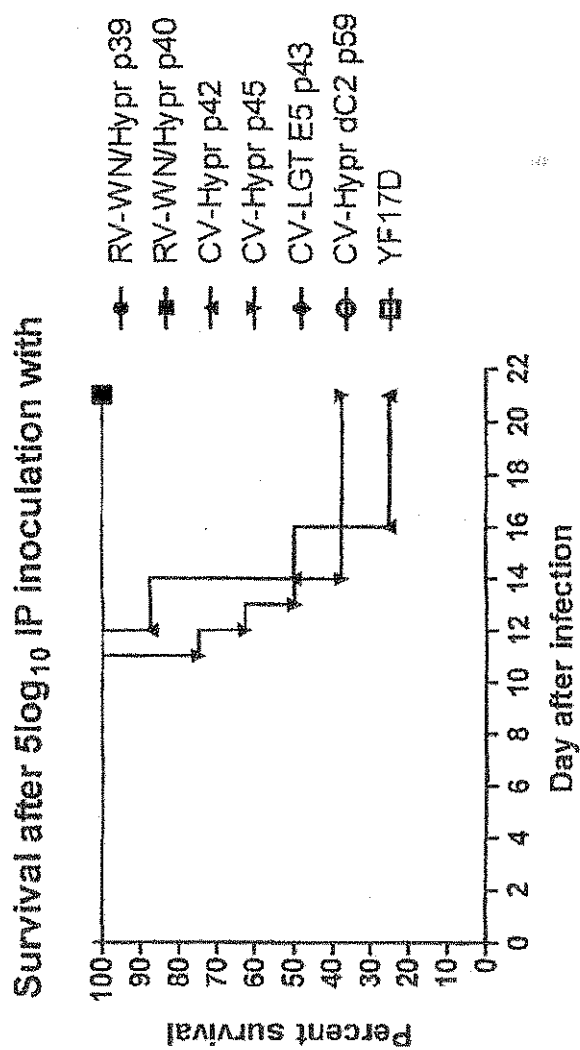


Fig. 11. Post-TBE-challenge morbidity (weight loss); day 9 post-challenge

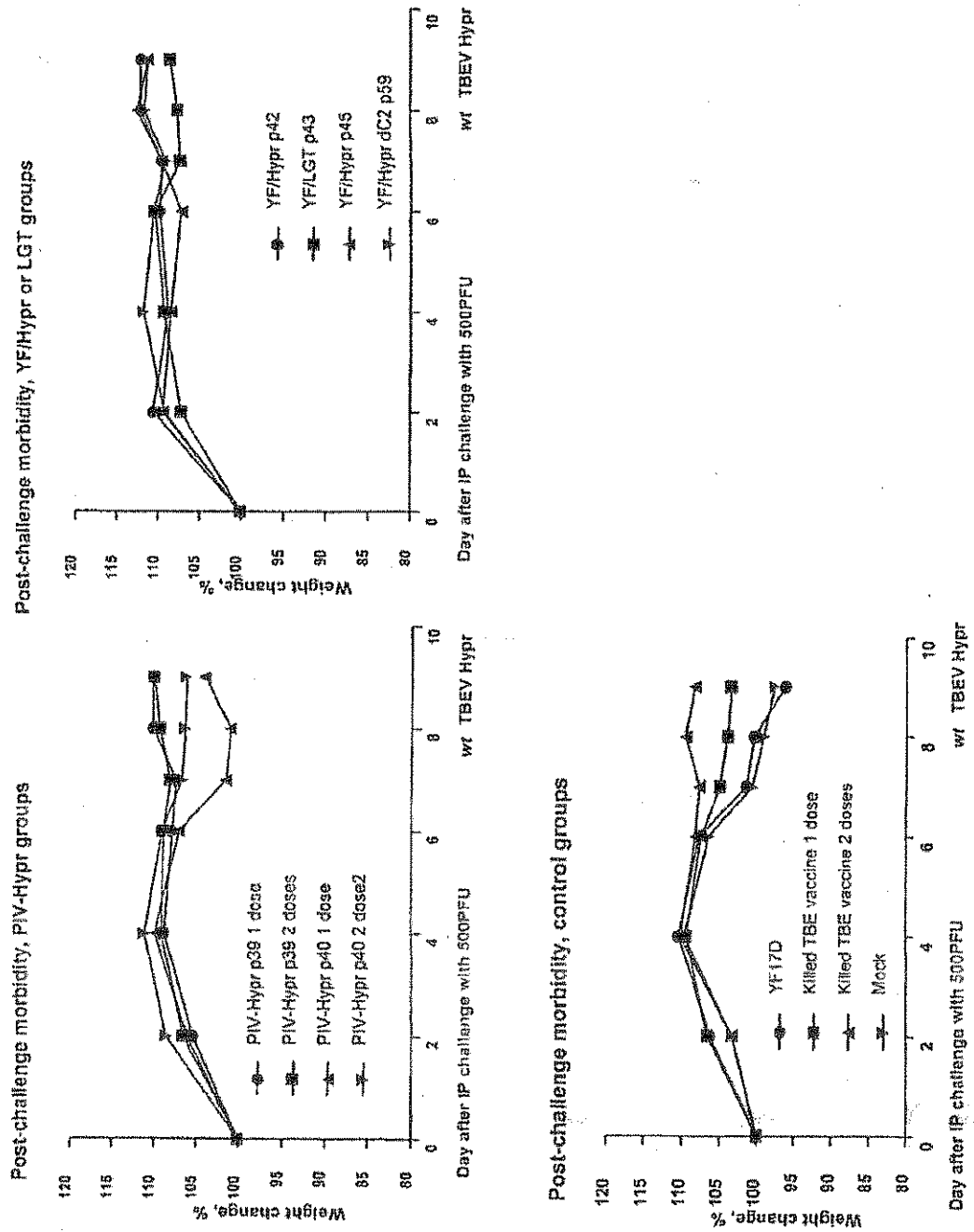


Fig. 12. Examples of PIV constructs expressing foreign antigens (rabies G)

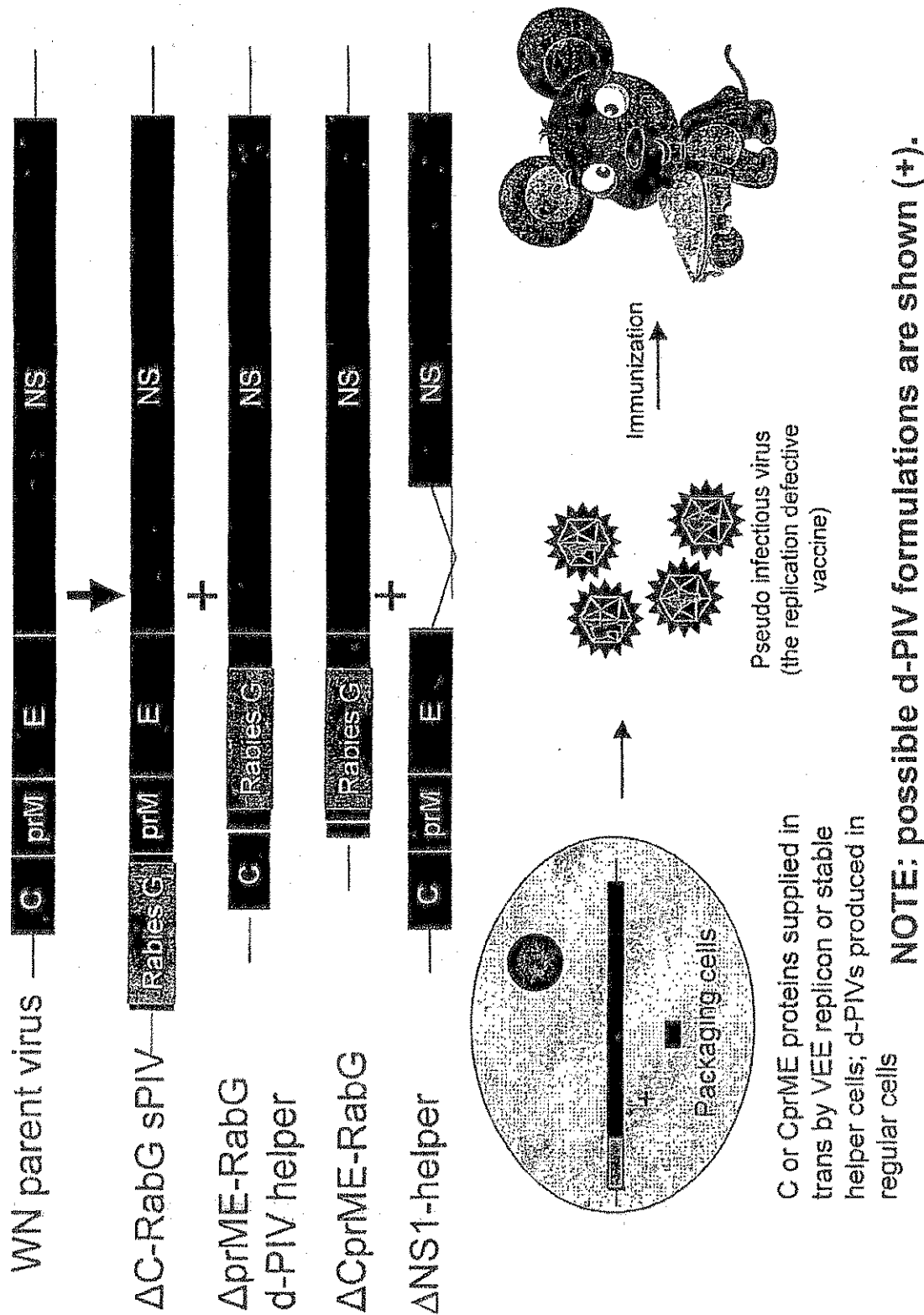
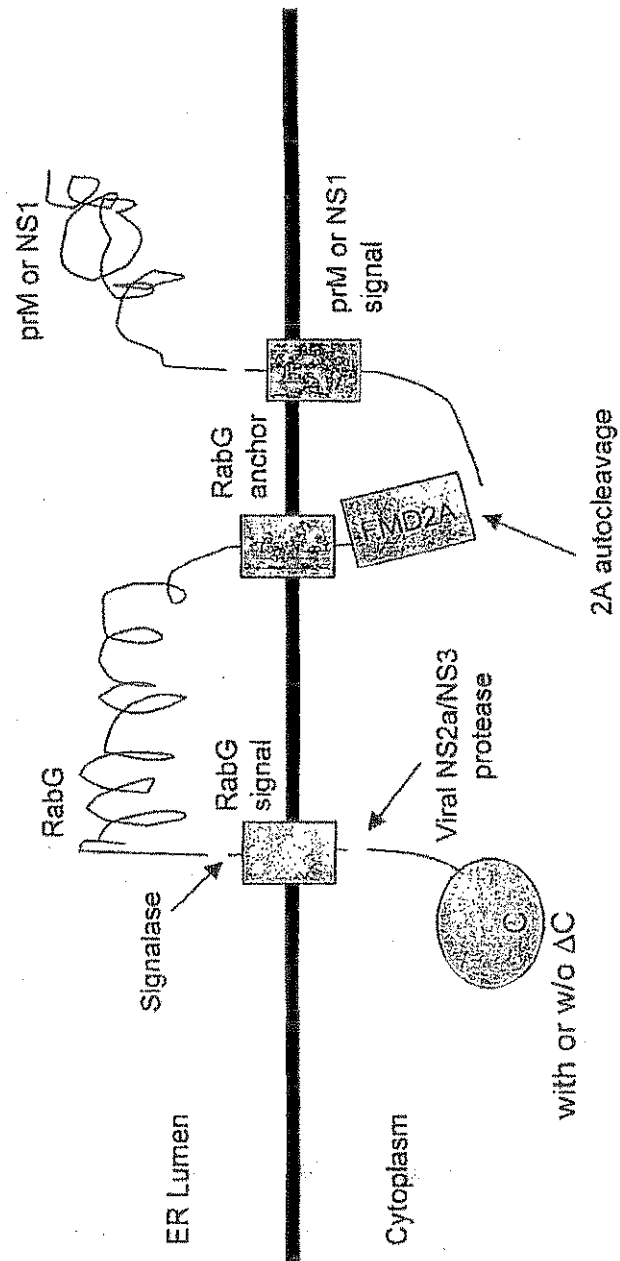


Fig. 13. Schematic representation of insertion designs resulting in viable/expressing constructs (exemplified by rabies G)



α-Rabies G/DAPI

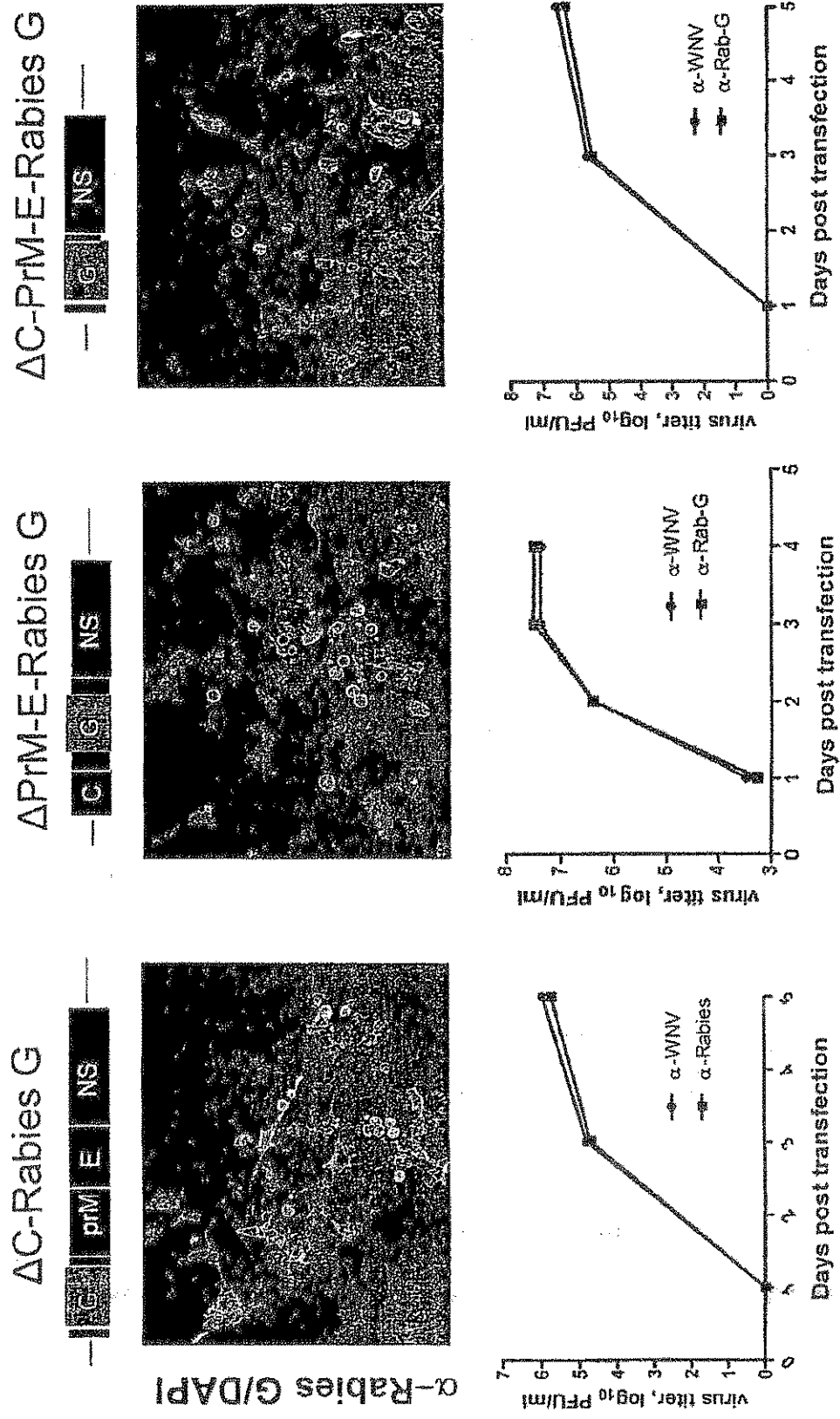
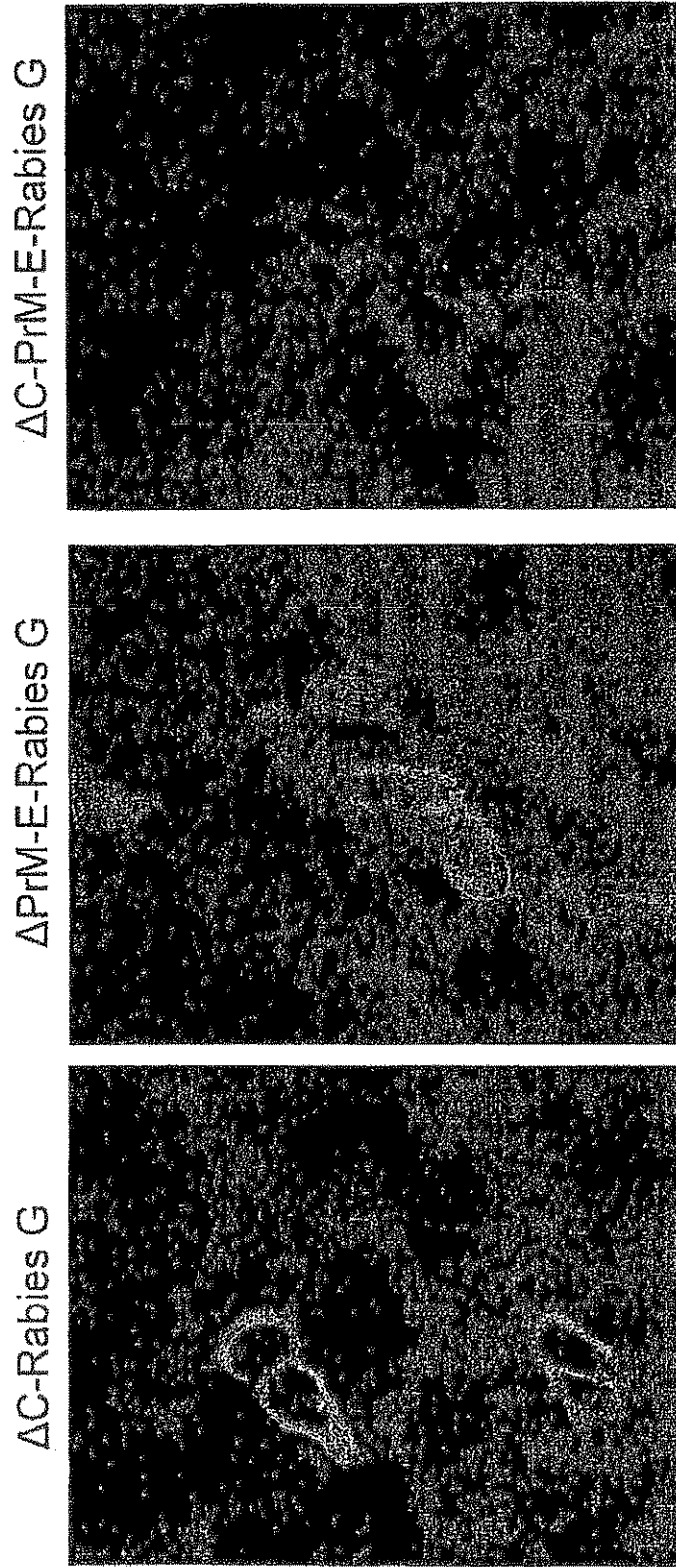
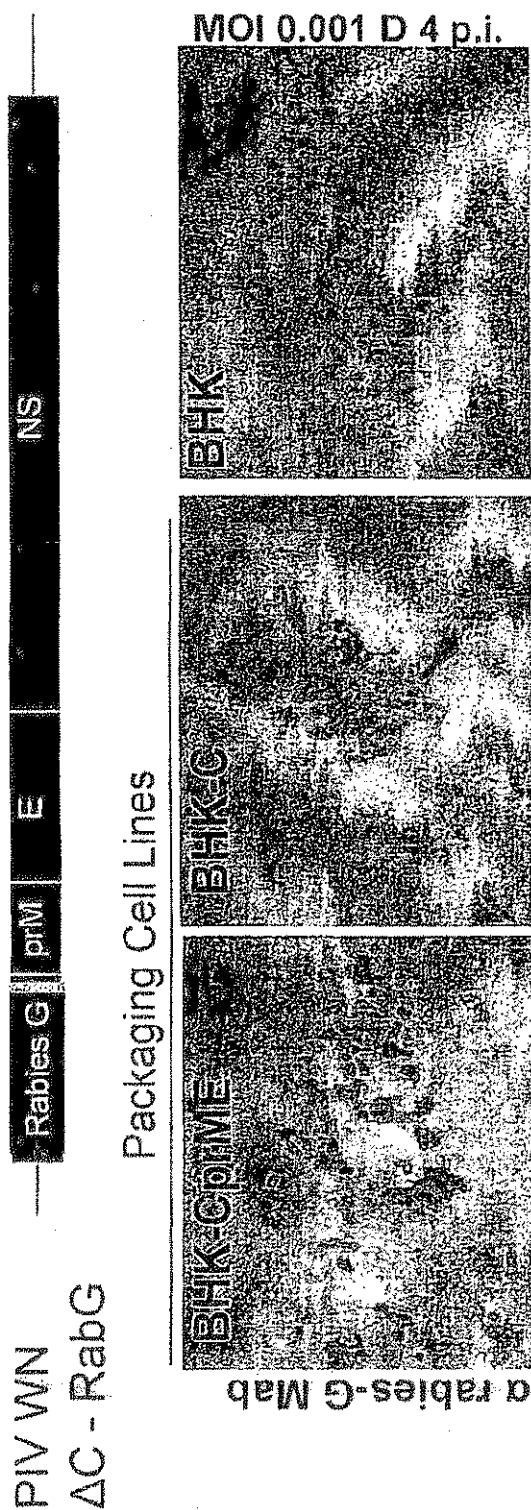


Fig. 15. Efficient expression of RabG on the plasma membrane of Vero cells
 MOI 0.1, day 2 post infection, 4% PFA Fixed non-permeabilized



1Ab (Abcam) Anti Rabies 1:500 2-3hrs RT, 2Ab Anti-Mouse IgG 1:1000 1hr RT
 Images are 40X and exposure time is 40ms

Fig. 16. PIV - Rabies G spread in helper cells, no spread in naïve cells



PIVs spread in helper cells and not naïve cells.
No infectious material in supernatants from BHK cells.

Similarly, VSV G was expressed, and no spread in regular cells (Vero, BHK) was observed and no infectious material detected in the supernatants

Fig. 17. Stability of rabies G protein gene in PIV-WN vectors
 Passages in BHK-C-prM-E helper cells, MOI 0.1; titration in Vero by immunostaining

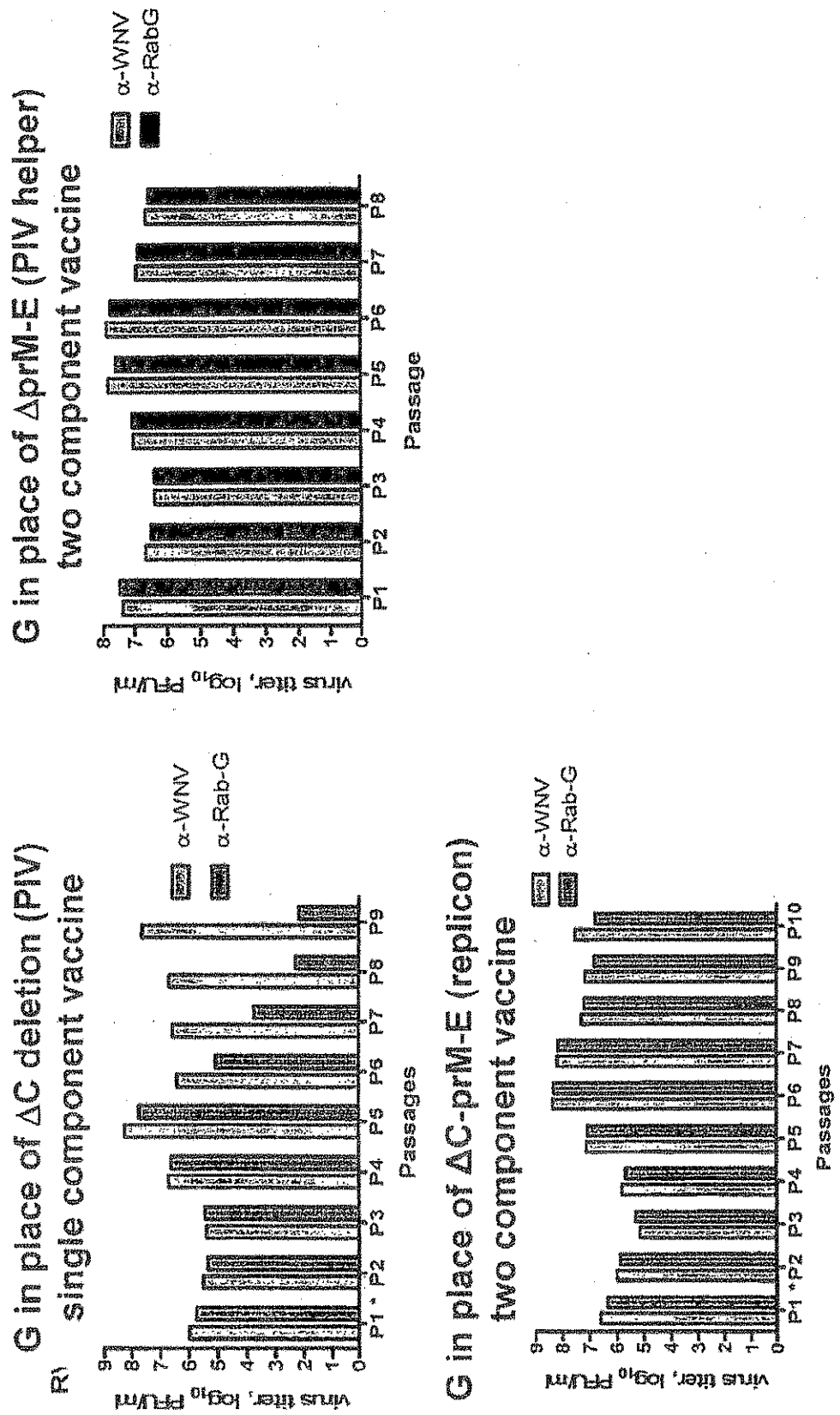
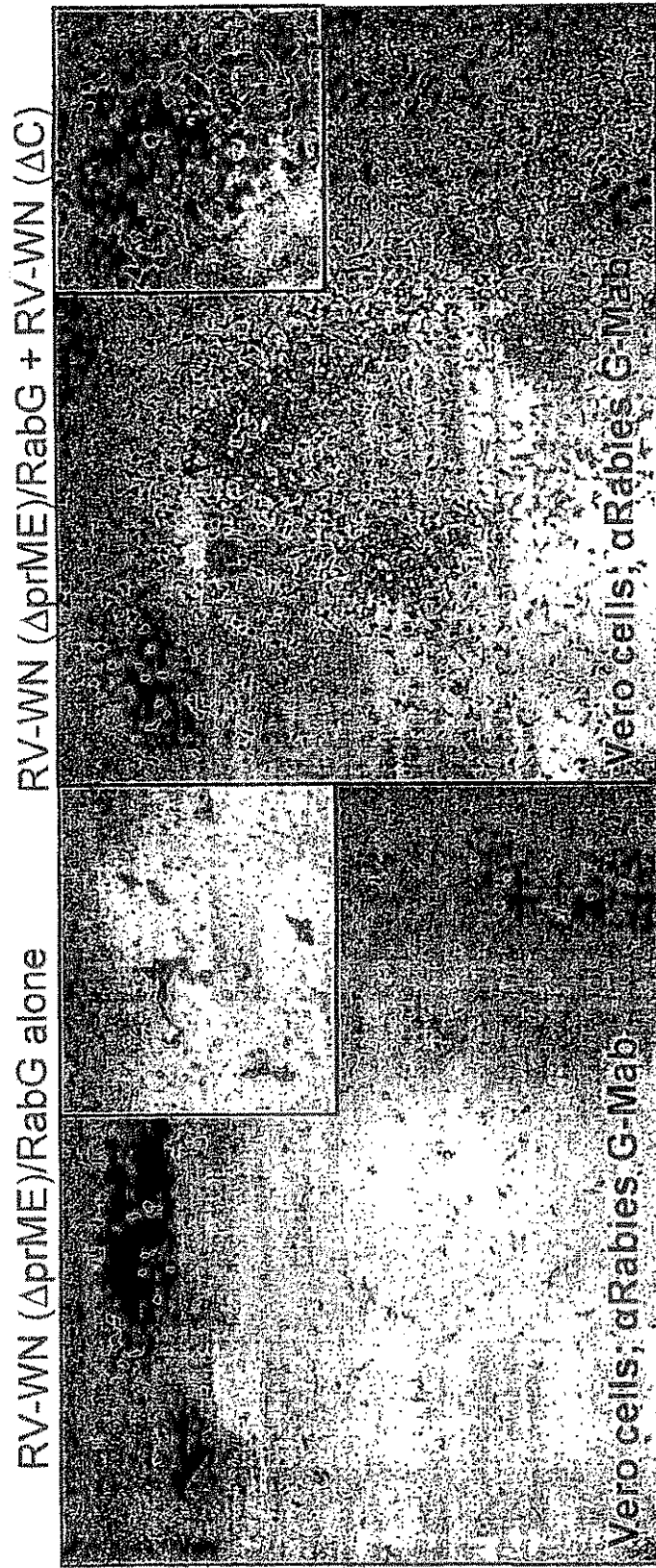


Fig. 18. Comparison of spread in Vero cells of single-component vs. two-component PIV-Rabies variants

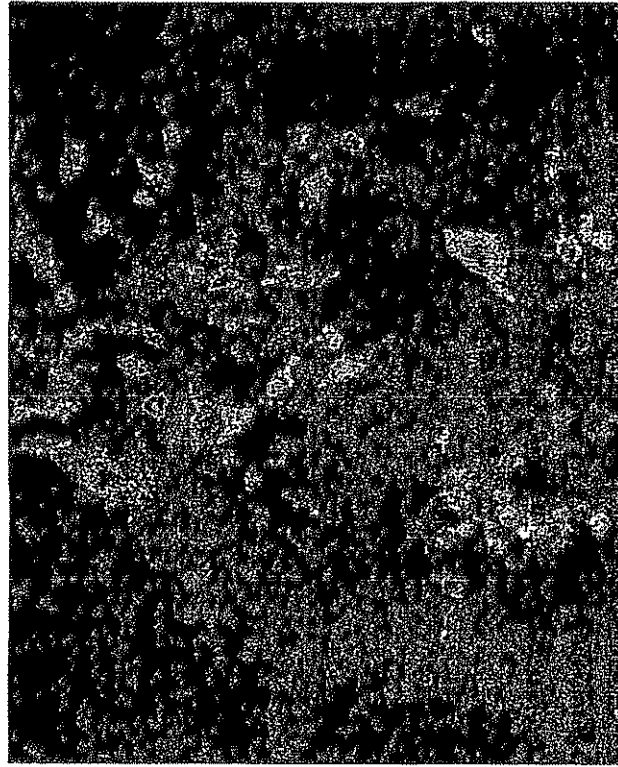
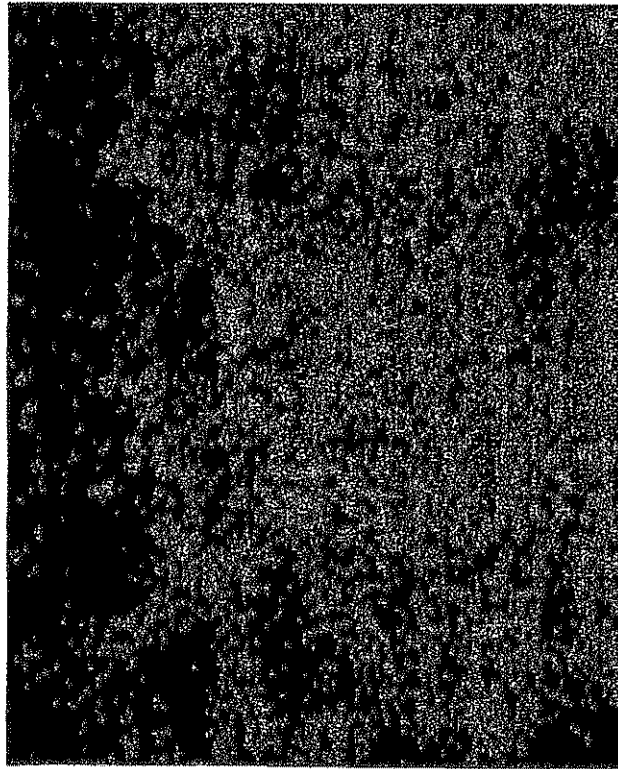


Titer on helper BHK cells: 2.0×10^8
FFU/ml

Titer on naïve Vero cells after
transfection (P0): 2.0×10^8 FFU/ml

Fig. 19. Example of expression of full-length RSV F protein (strain A2): immunostaining of helper cells after transfection

Control BHK helper cells (VN C-prM-E) PIV-WN Δ prME-RSV F, 3 days post-transfection



Cells stained with anti-RSVF Mab, DAPI