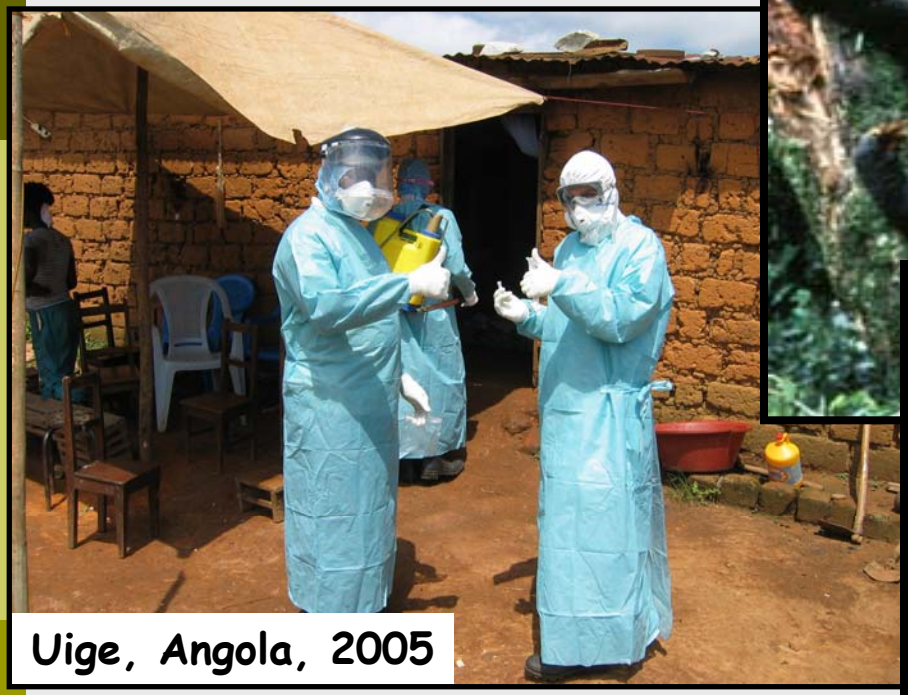
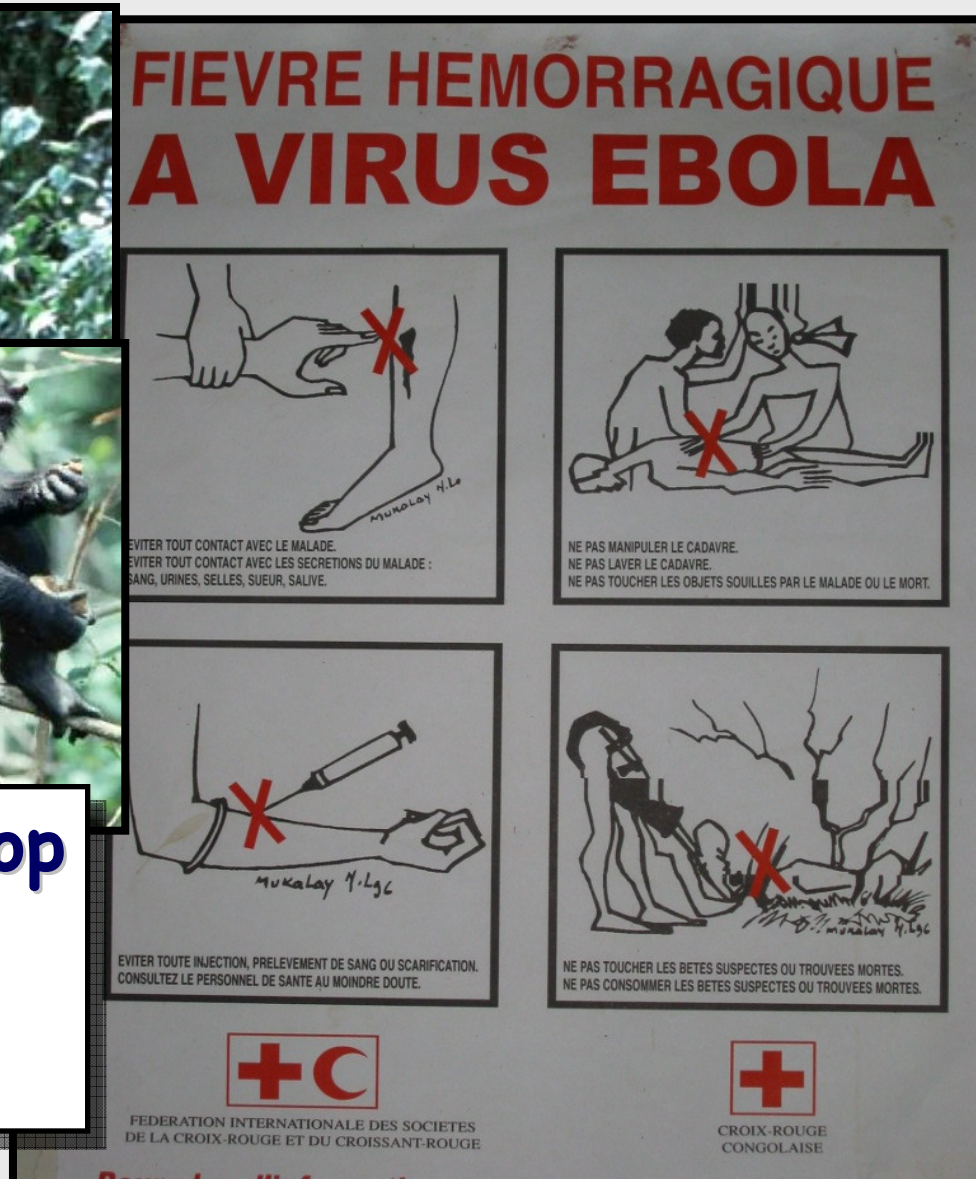
# Introduction to Filoviruses



Uige, Angola, 2005




## Filovirus Animal Model Workshop

Bethesda, Maryland, USA
September 11-12, 2007

# Taxo

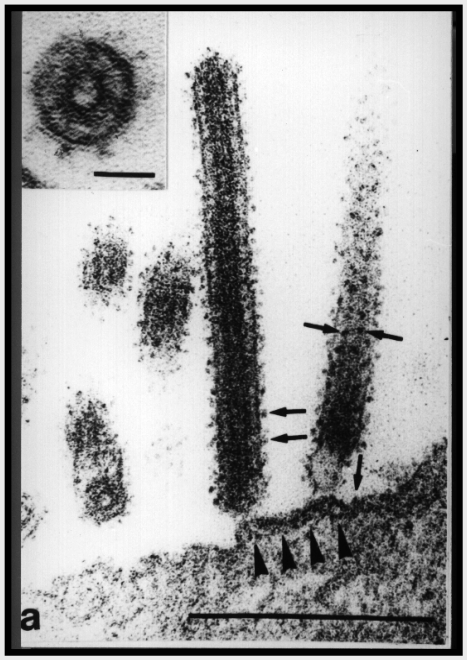
## Public Health Concern

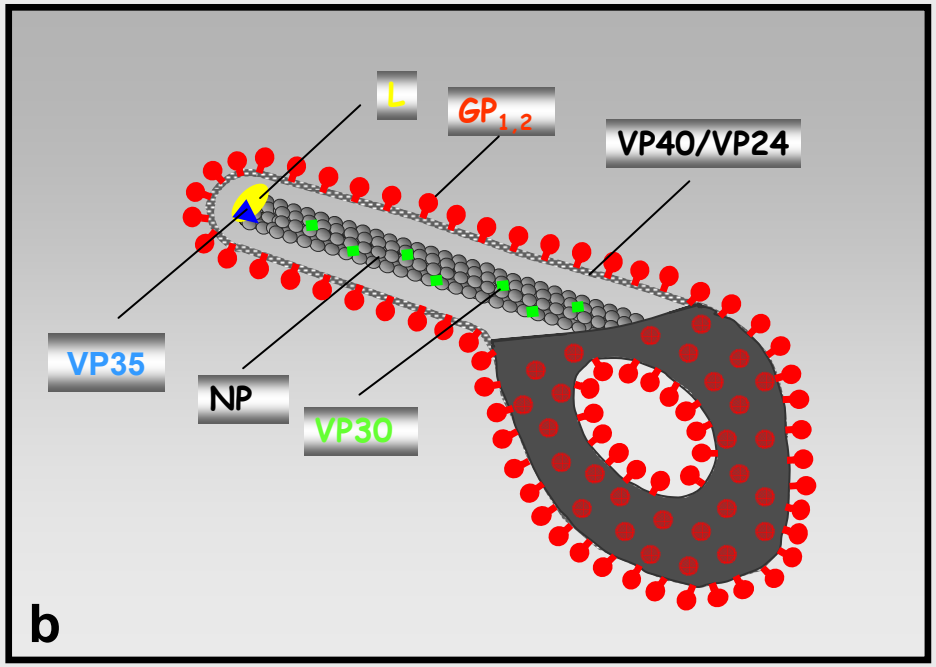
ZEBOV, SEBOV & MARV

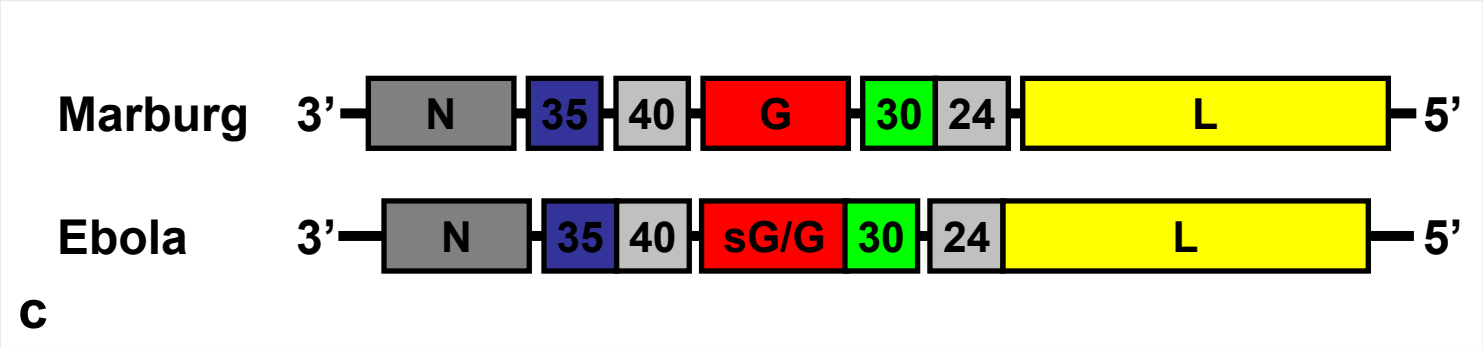
(case fatality ranging from 23-90%)

- *Bornaviridae*
- *Rhabdoviridae*
- *Filoviridae*
  - *Marburgvirus* → *Lake Victoria marburgvirus* (MARV)
  - *Ebolavirus* →
    - *Zaire ebolavirus* (ZEBOV)
    - *Sudan ebolavirus* (SEBOV)
    - *Cote d'Ivoire ebolavirus* (CIEEBOV)
    - *Reston ebolavirus* (REBOV)
- *Paramyxoviridae*

# Particle structure

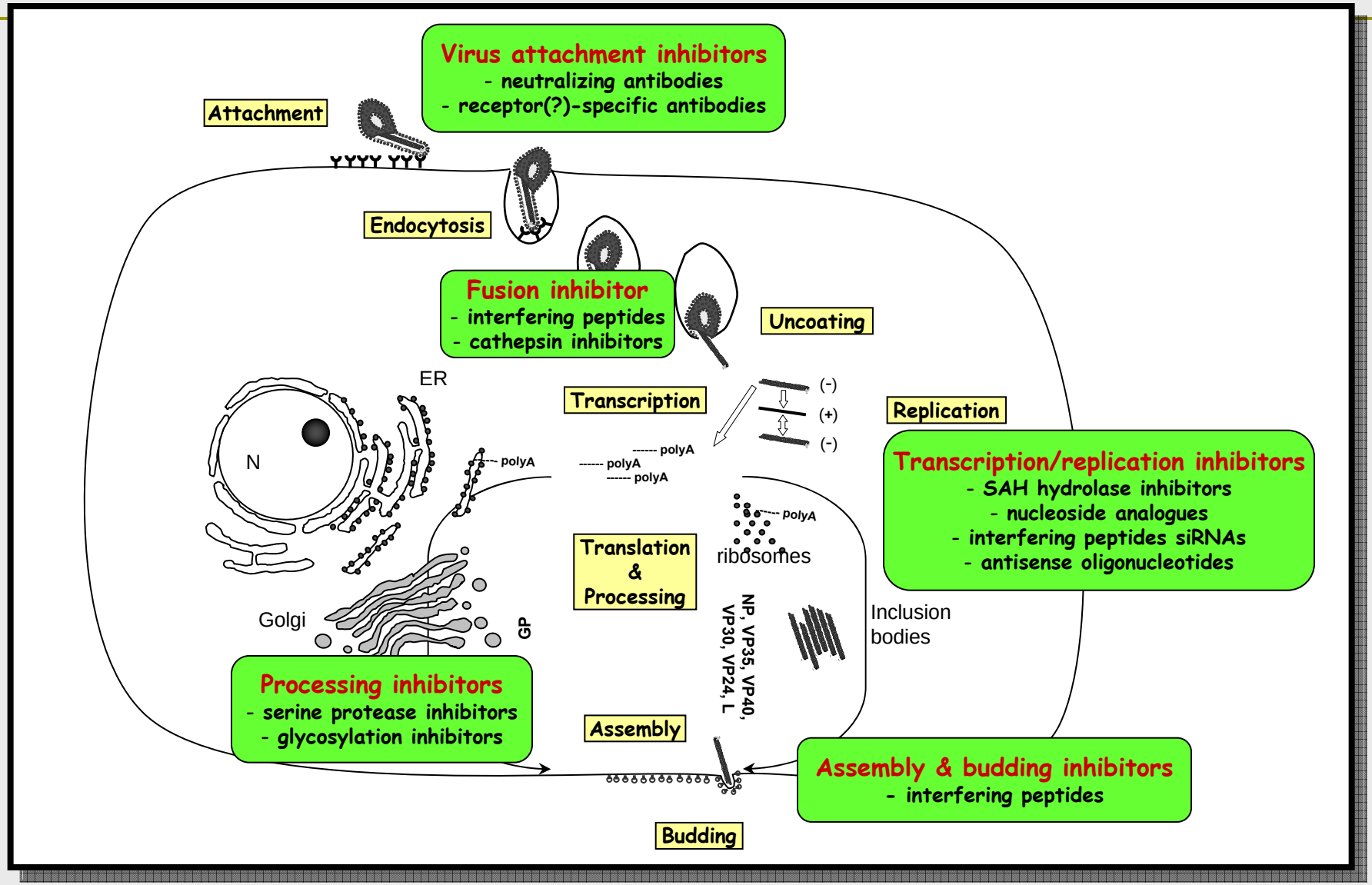


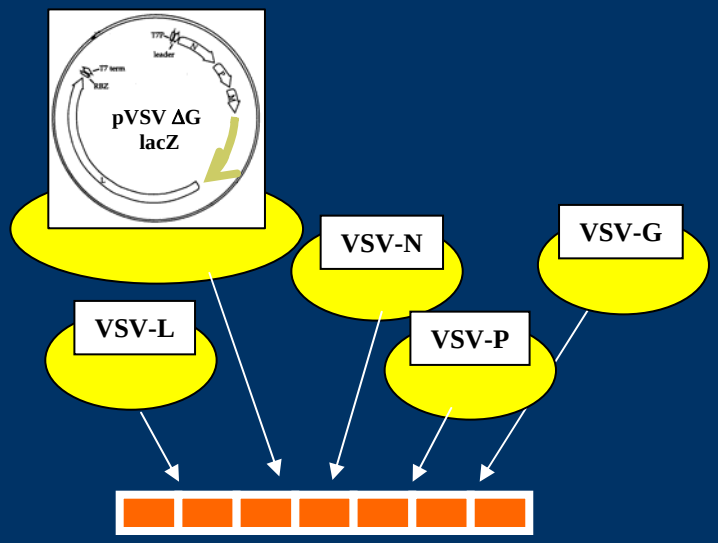



| DESIGNATION | ABBREV. | FUNCTION |
|---|---|---|
| | | |
| nucleoprotein | NP | part of RNP -- encapsidation of genome |
| virion protein 35 | VP35 | part of RNP -- transcriptase/replicase cofactor; interferon antagonist |
| virion protein 30 | VP30 | part of RNP -- transcription activator (EBOV) |
| RNA-dependent RNA polymerase | L | part of RNP; transcriptase & replicase |
| virion protein 40 | VP40 | membrane associated -- matrix protein, particle formation |
| virion protein 24 | VP24 | membrane associated -- interferon antagonist; host adaptation; primary transcription |
| glycoprotein | GP | membrane spanning -- receptor binding, fusion, important immunogen |

# Filovirus life cycle

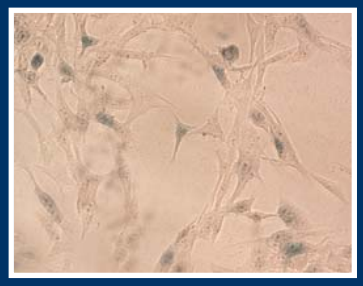


# Powerful tools to study virus entry but need confirmation with live virus system

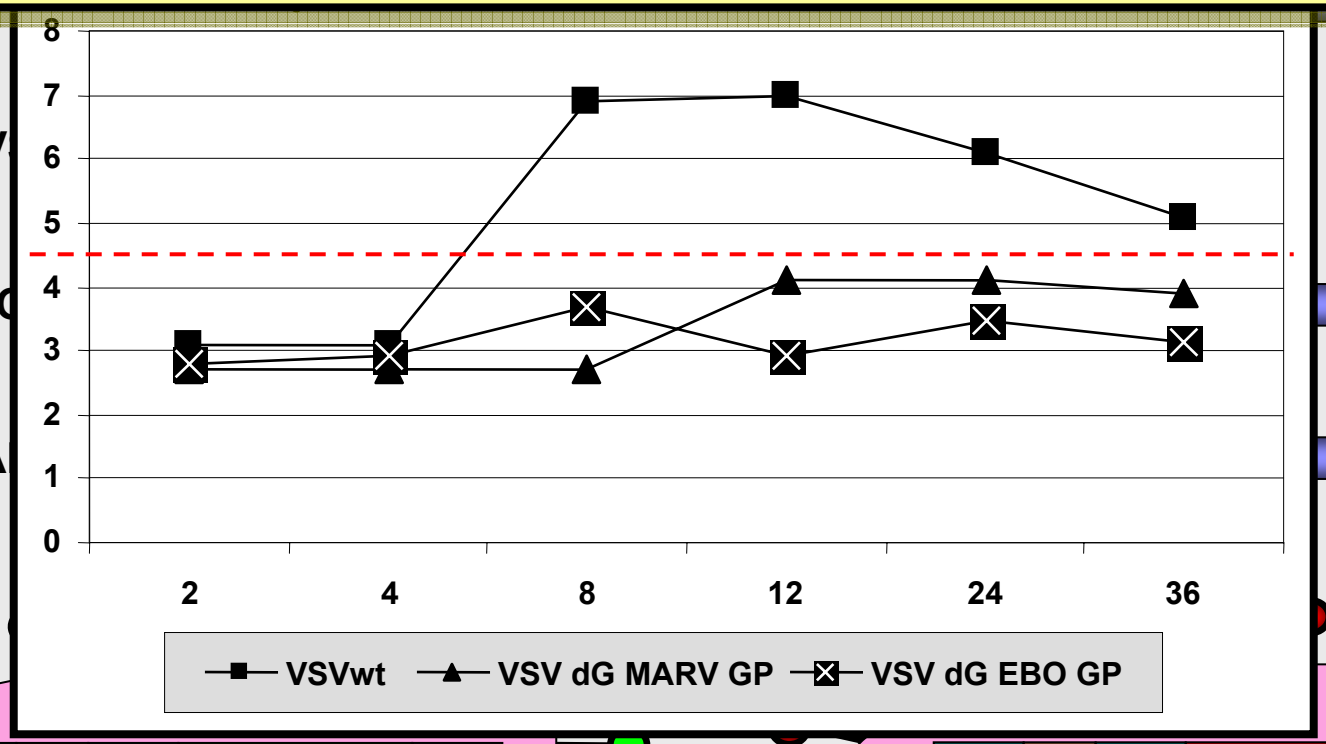


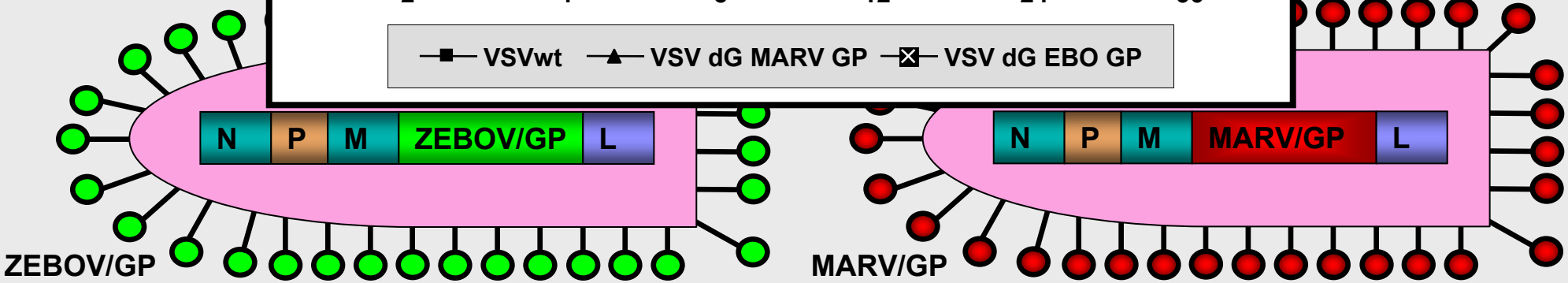

BHK-T7

cell line expressing foreign glycoprotein



# Powerful tools to study virus entry but need confirmation with live virus system; safety needs to be addressed

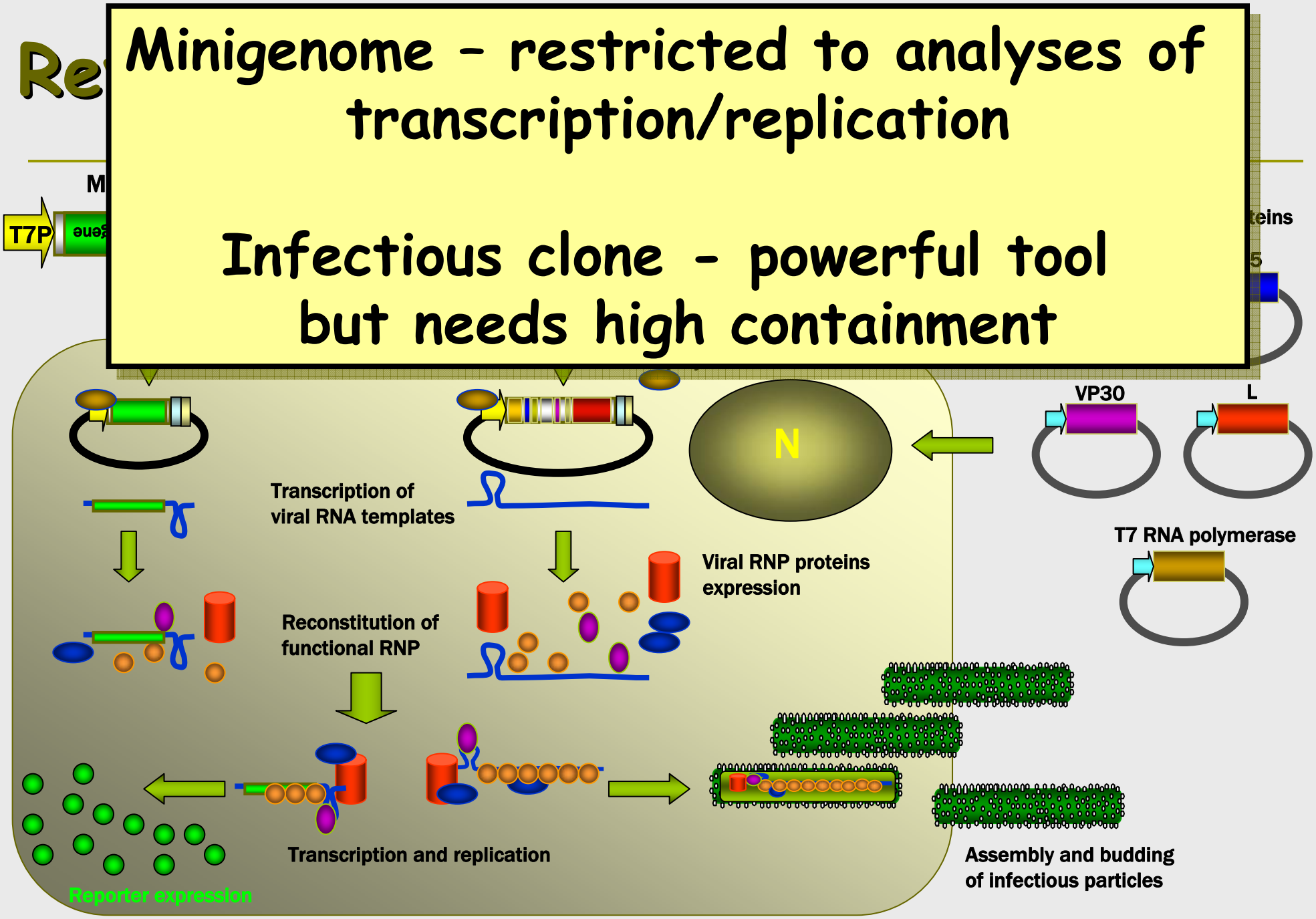


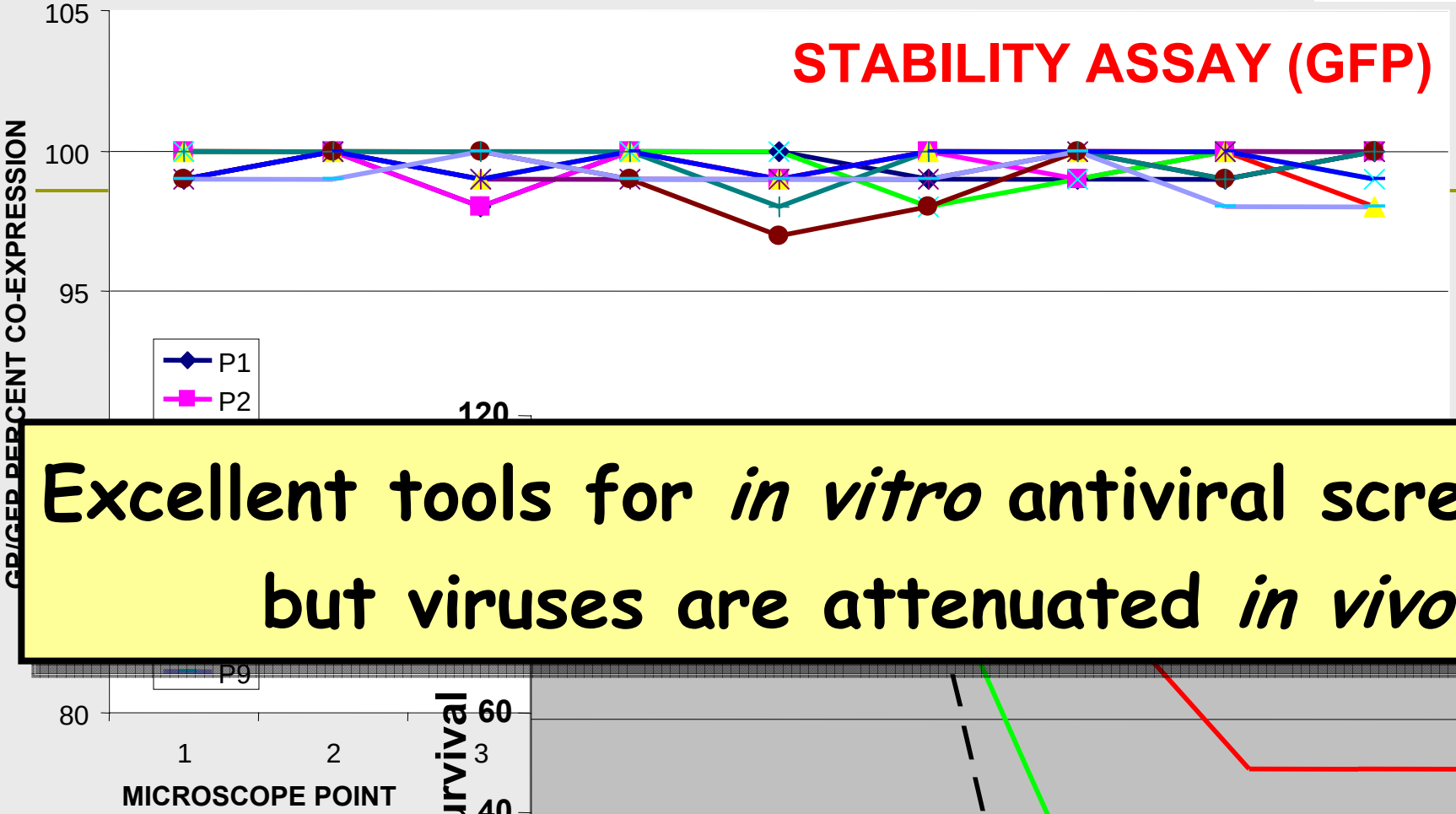

VSV system kindly provided by J. Rose

# Minigenome – restricted to analyses of transcription/replication

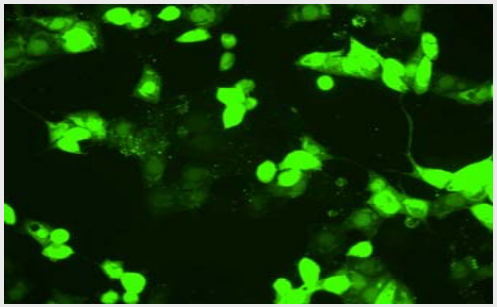
# Infectious clone - powerful tool but needs high containment



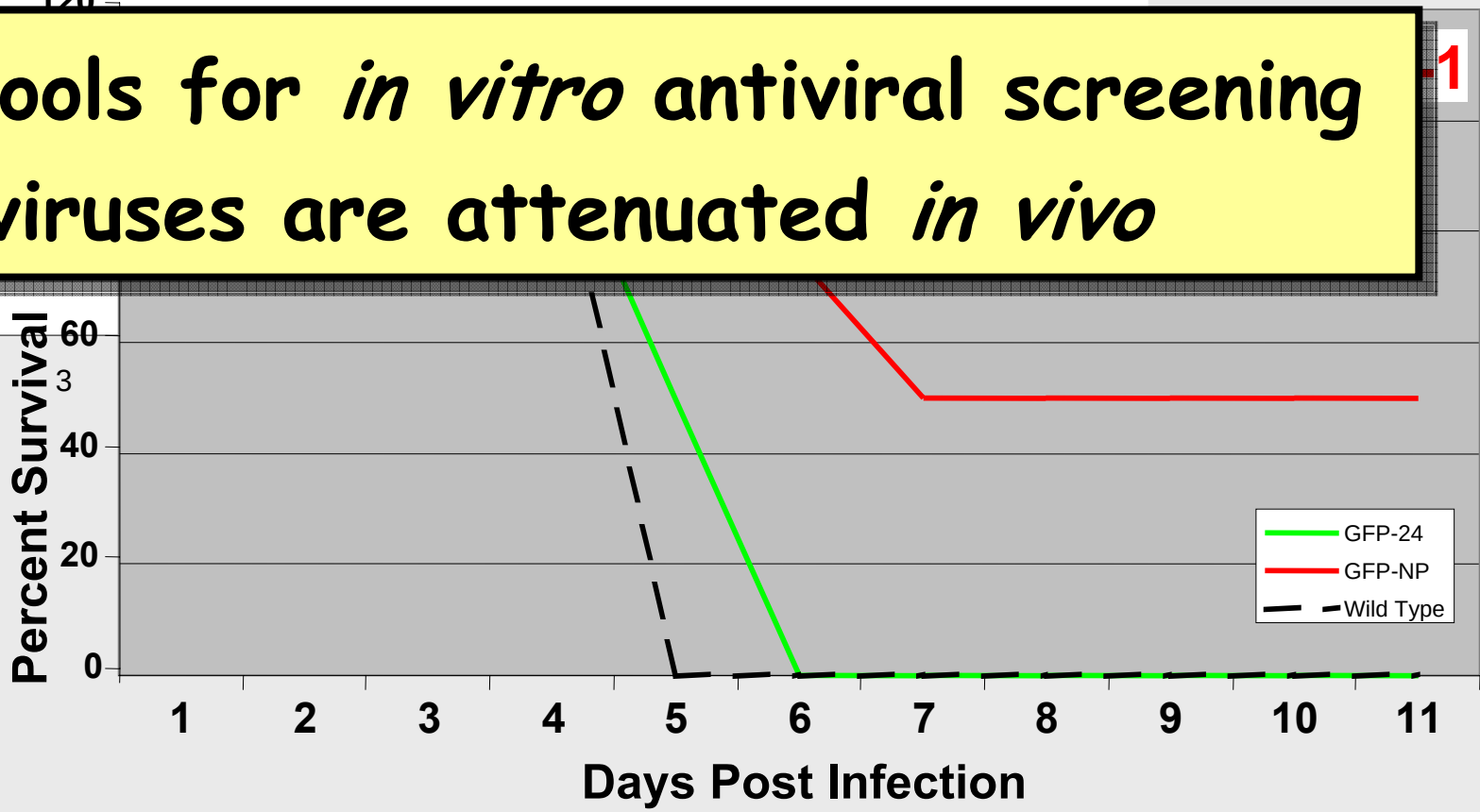
Ebihara et al. JID, 2007



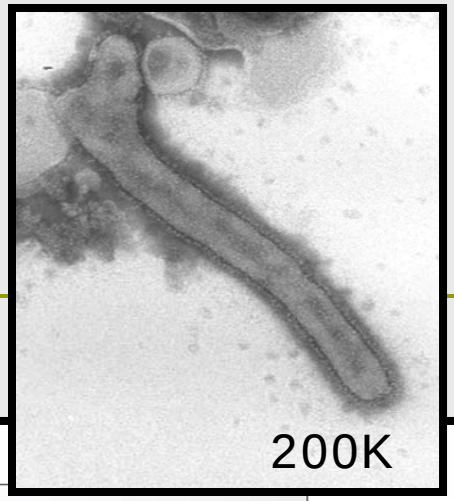

**Excellent tools for *in vitro* antiviral screening but viruses are attenuated *in vivo***

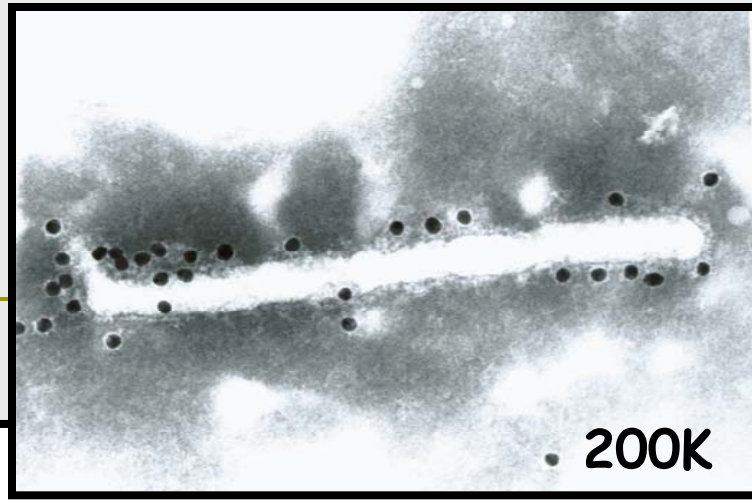


eGFP Ebola Virus



# Infectious VLPs




A

MG
GP
VP24
L
VP30
VP40
NP
VP35

p0

72 hrs

- genome replication

- iVLP morphology
- budding efficiency

p1: naïve VeroE6 cells

- iVLP entry
- minigenome delivery
- RNP functionality

SN

24 hrs

- iVLP entry
- minigenome delivery

**Very promising but usefulness needs to be further demonstrated**

as Hoenen

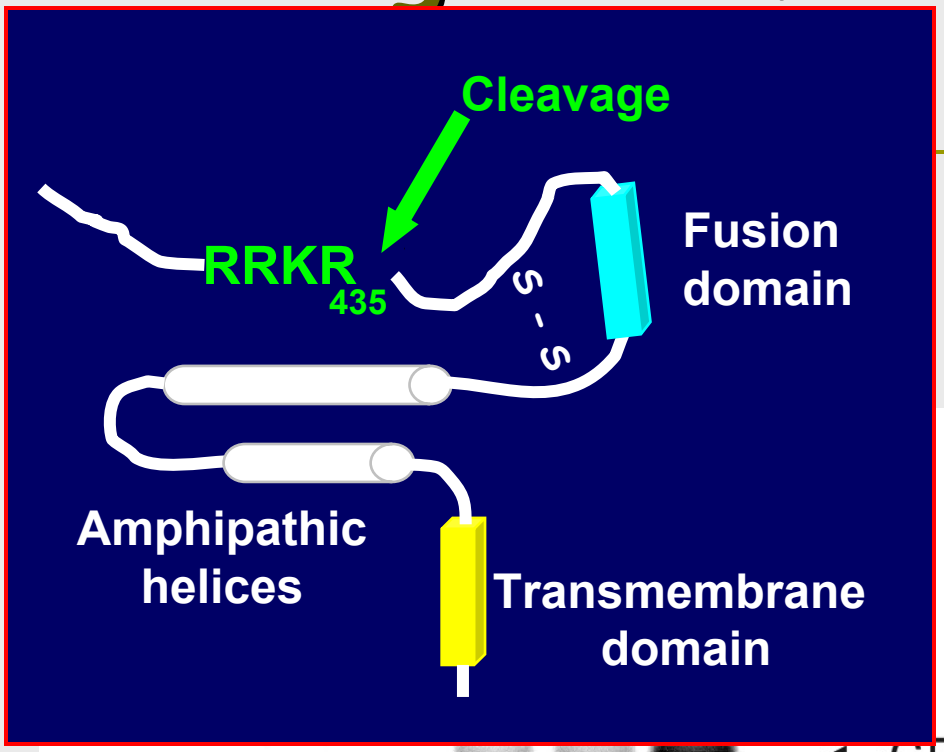
# Glycoprotein expression strategy

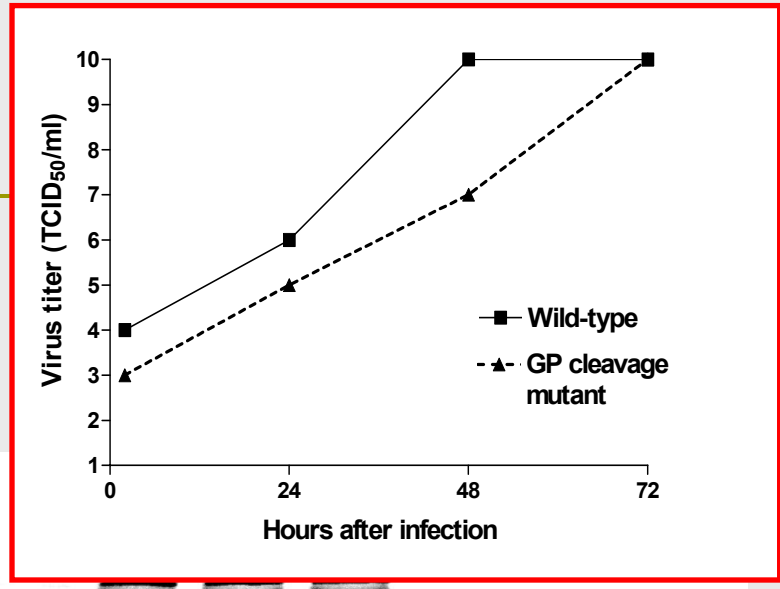
## Features and functions of glycoproteins:

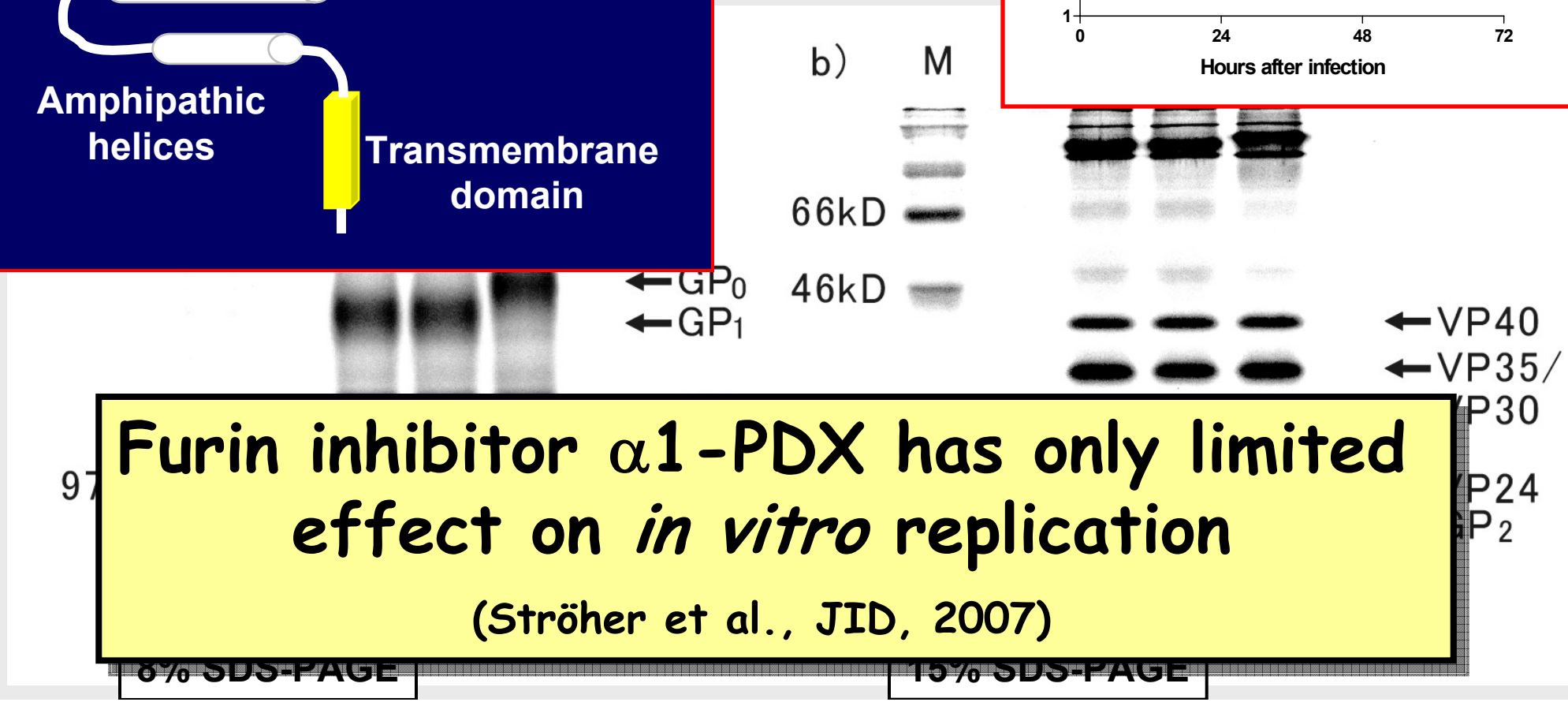
| | |
|---|---|
| $GP_{1,2}$ | $GP_1/GP_2$ heterotrimer; highly N and O-glycosylated and C-mannosylated<br>[receptor binding ($GP_1$) & fusion ($GP_2$); major immunogen] |
| $GP_{1,2\Delta TM}$ | GP1/GP2 heterotrimer; highly N and O-glycosylated<br>[antibody decoy (?) & others] |
| sGP | antiparallel/parallel homodimer; highly N-glycosylated and C-mannosylated<br>[neutrophile inactivation (?), antibody decoy (?) & anti-inflammatory] |
| Δ-peptide | monomer; O-glycosylated<br>[unknown function] |

# Cleavage & infectivity

Neumann et al. J Virol, 2002



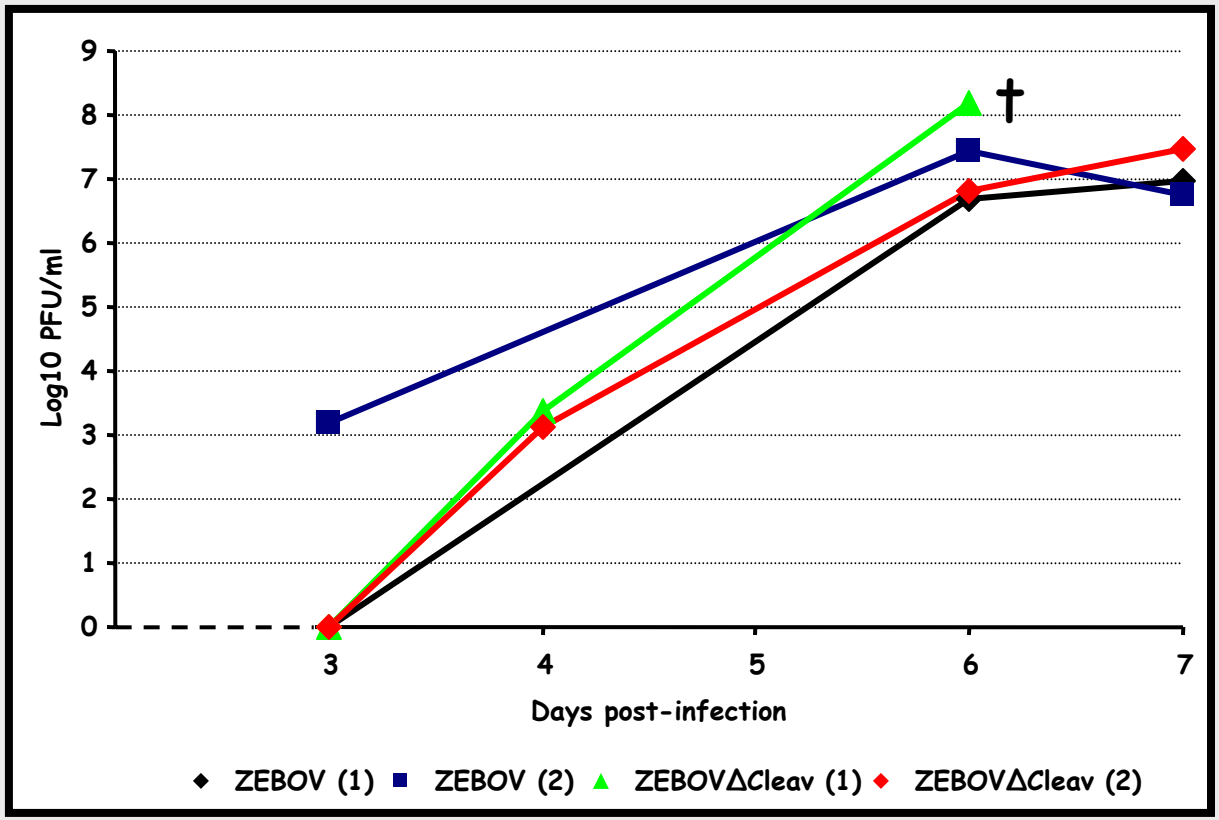




**Furin inhibitor α1-PDX has only limited effect on *in vitro* replication**

(Ströher et al., JID, 2007)

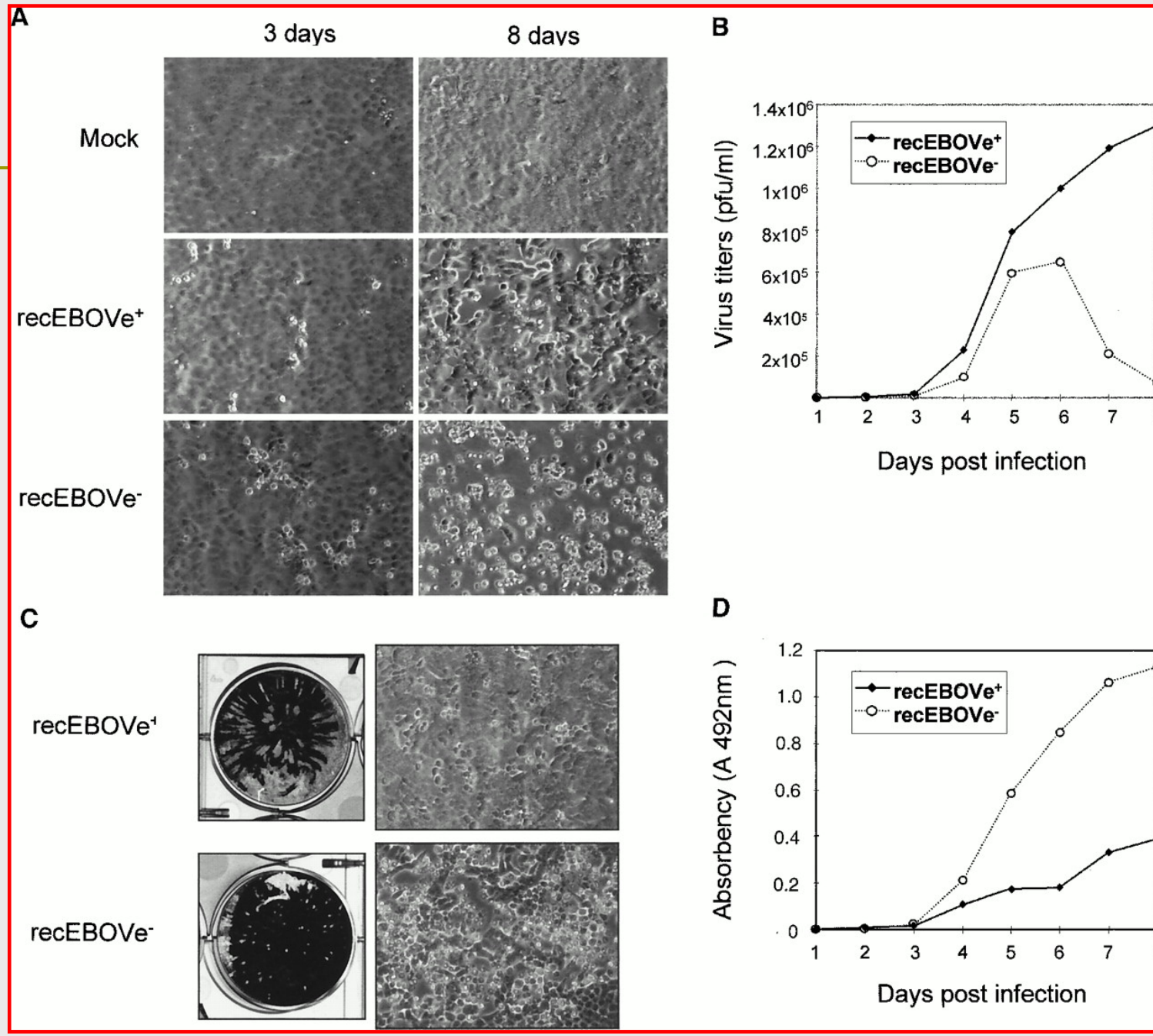
Neumann et al. J Virol, 2007

# Cleavage and pathogenicity



***in vitro*** **and** ***in vivo*** **data indicate that furin cleavage is not important for infectivity and virulence**

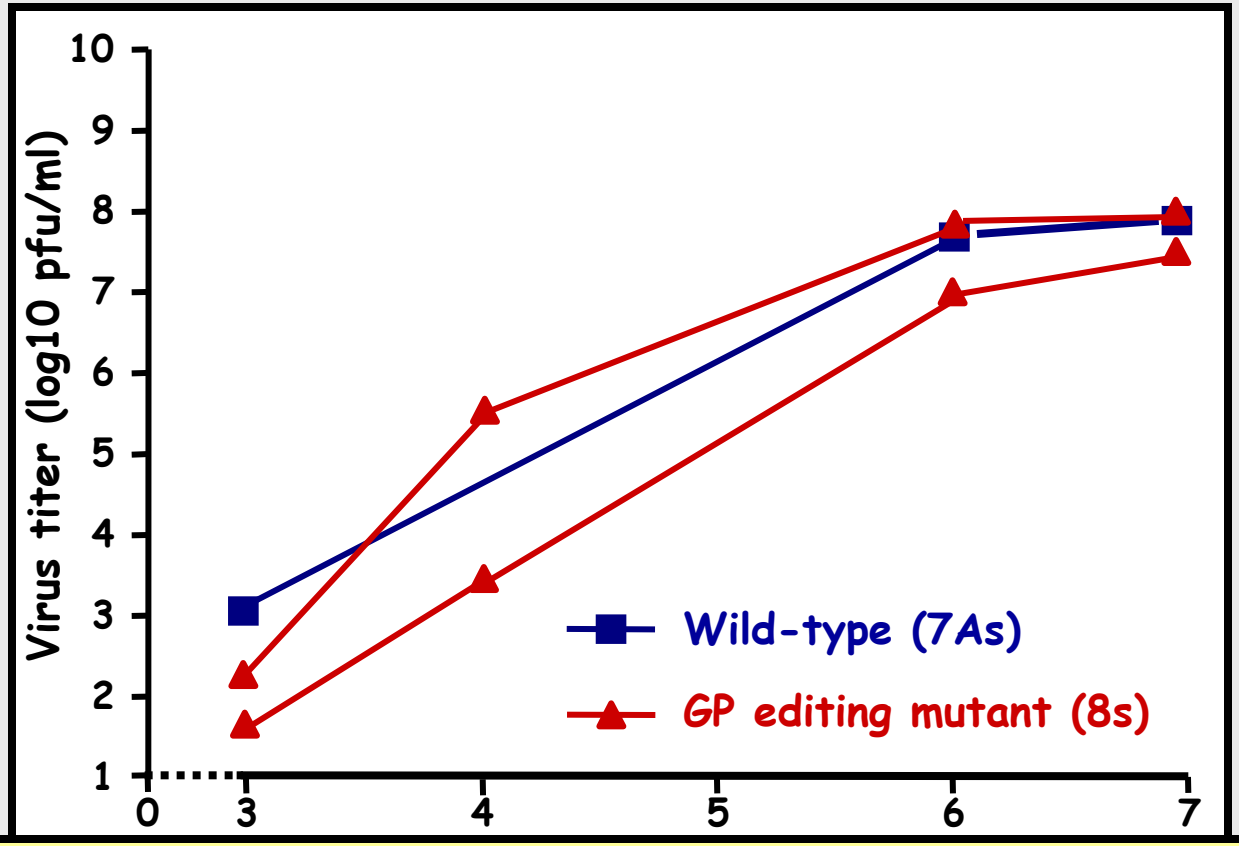
# Effect of editing on infectivity

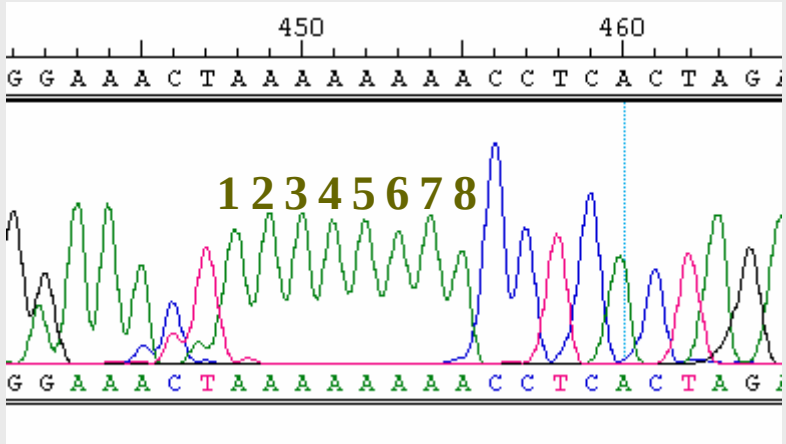



Volchkov *et al.*
Science, 2001

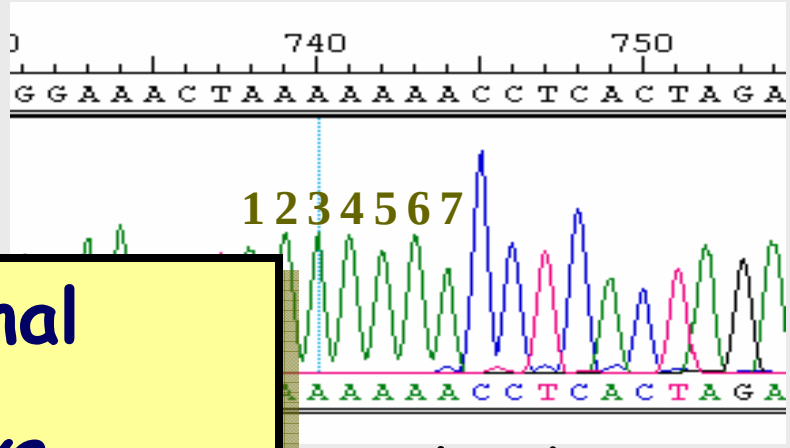
Neumann et al., unpublished

# Ebolavirus editing site mutant




Inoculum (editing mutant, 8xA)




animal 3 days p.i.

**Selective pressure for functional editing site *in vitro* and *in vivo***

# sGP (anti-inflammatory)

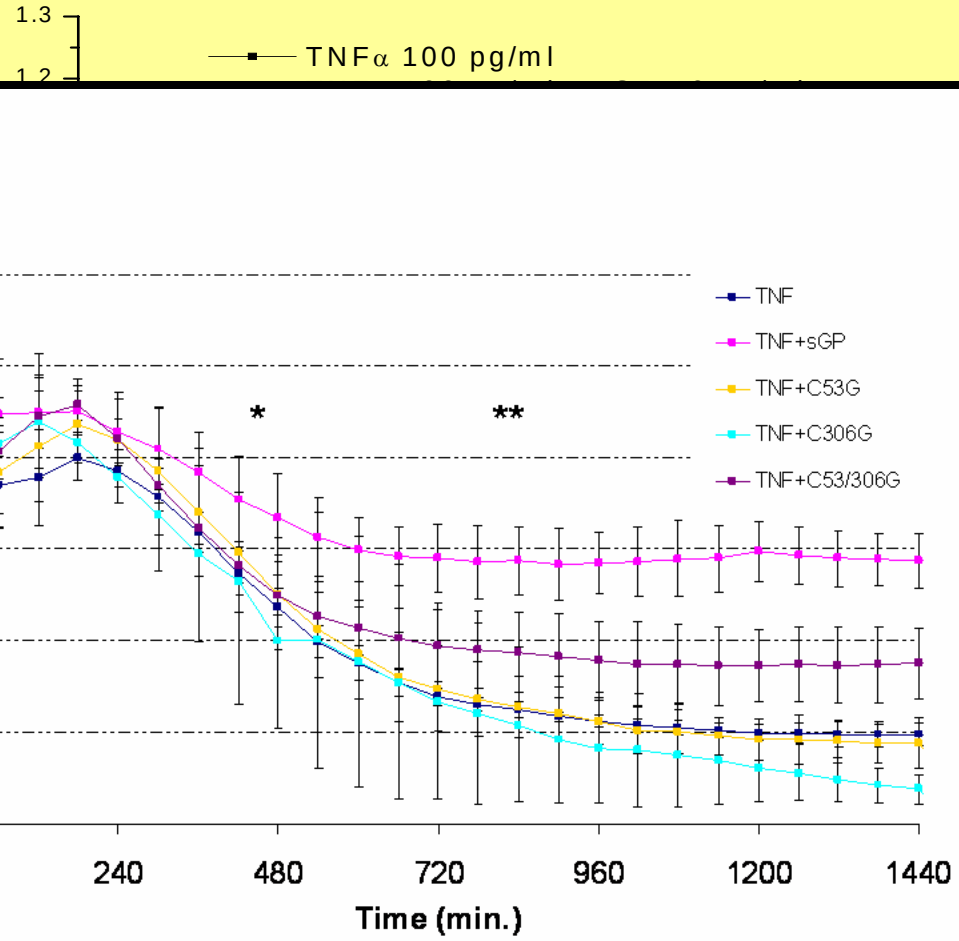
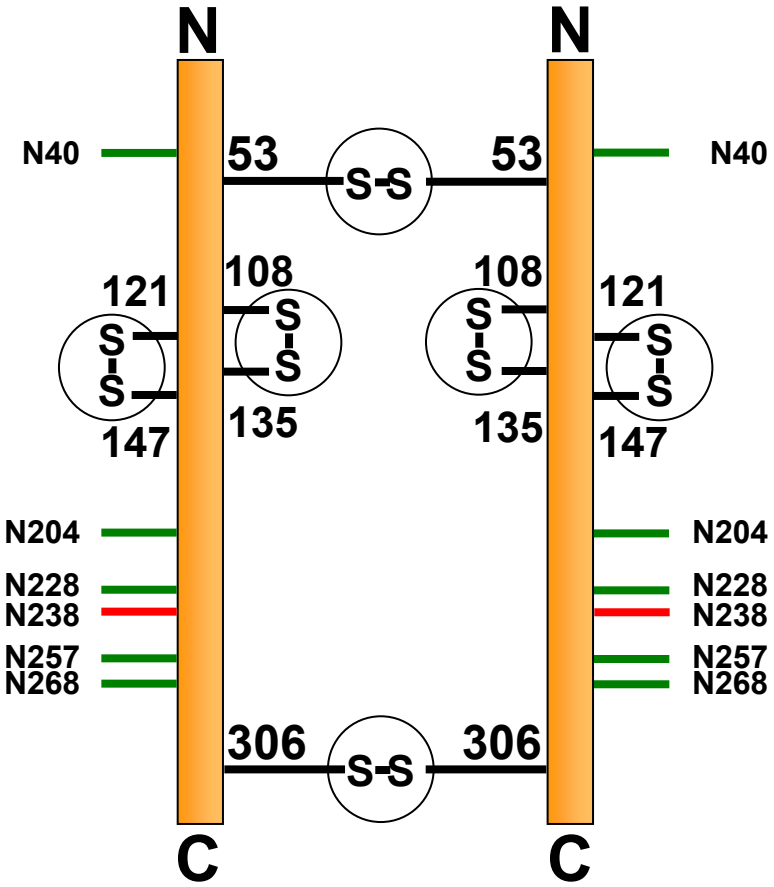
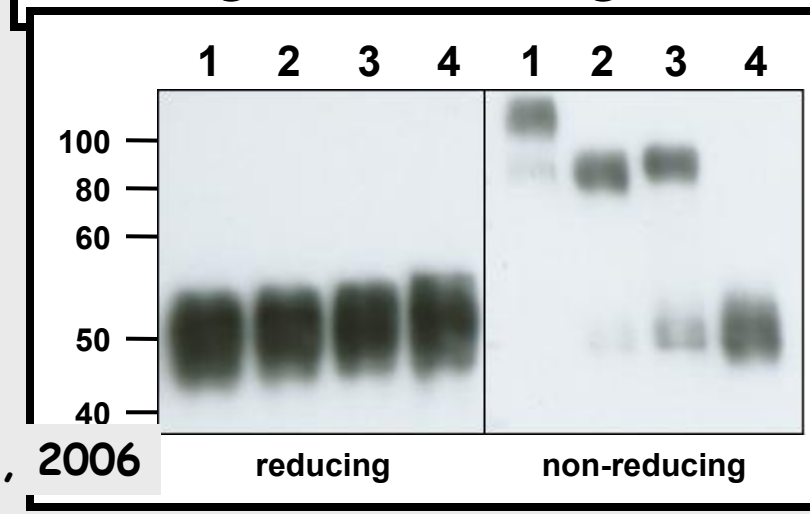


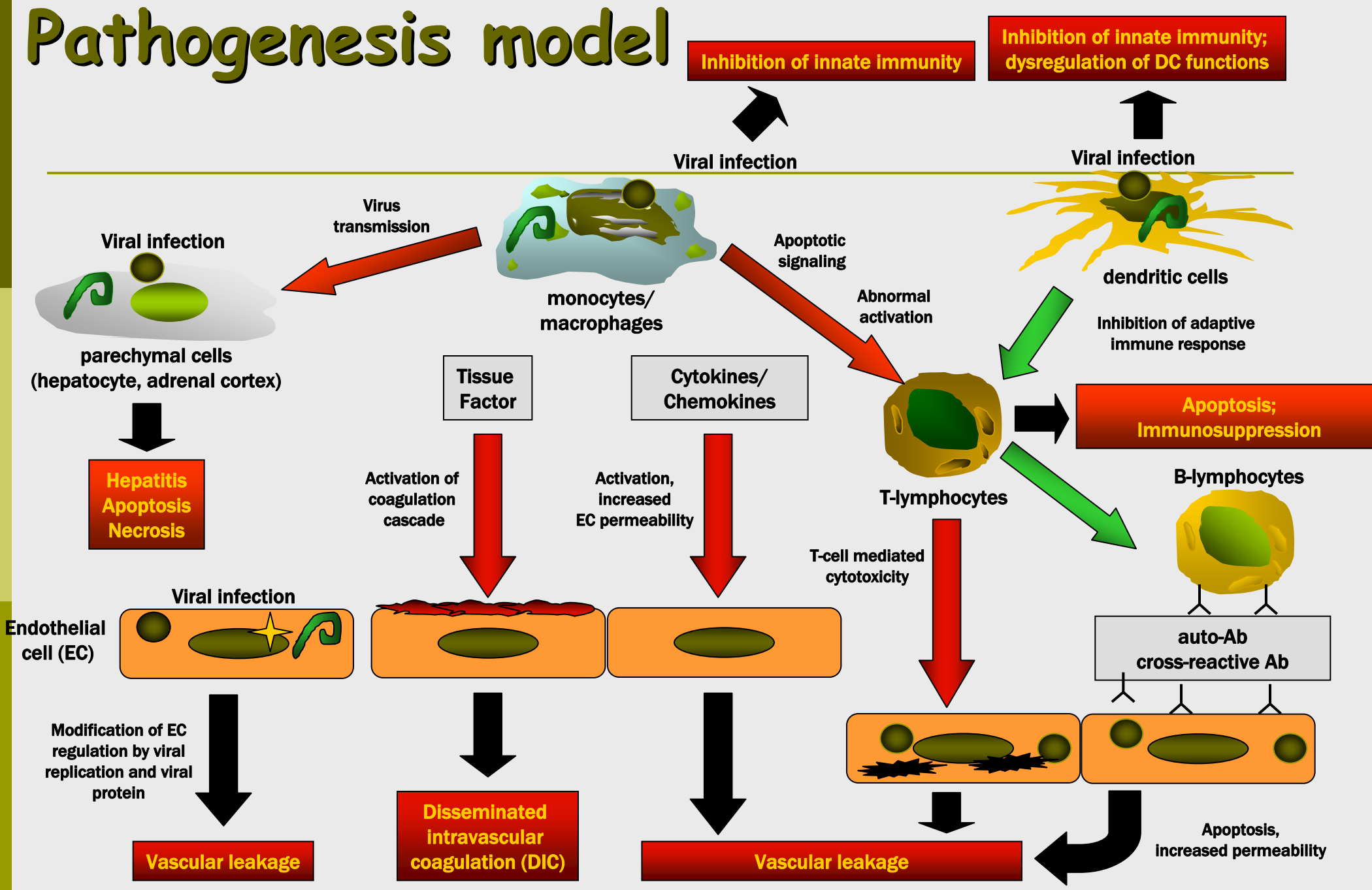

Figure 5.



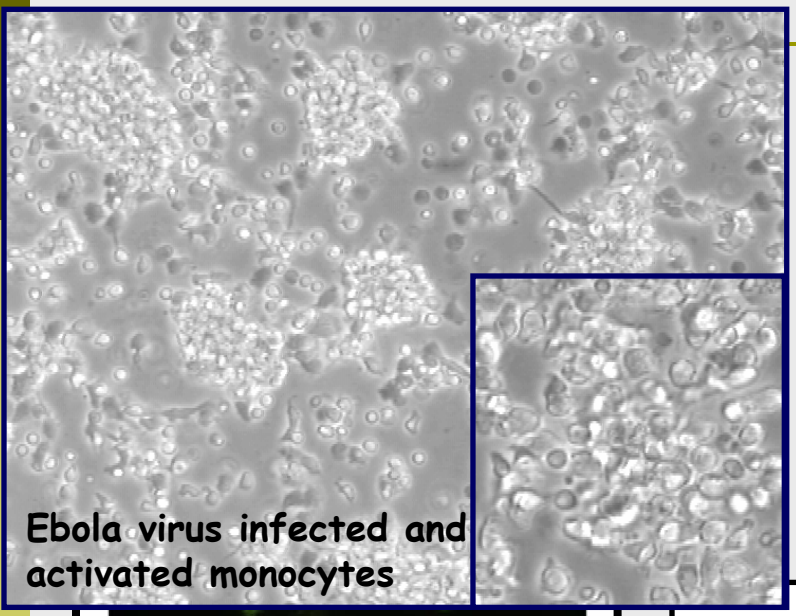



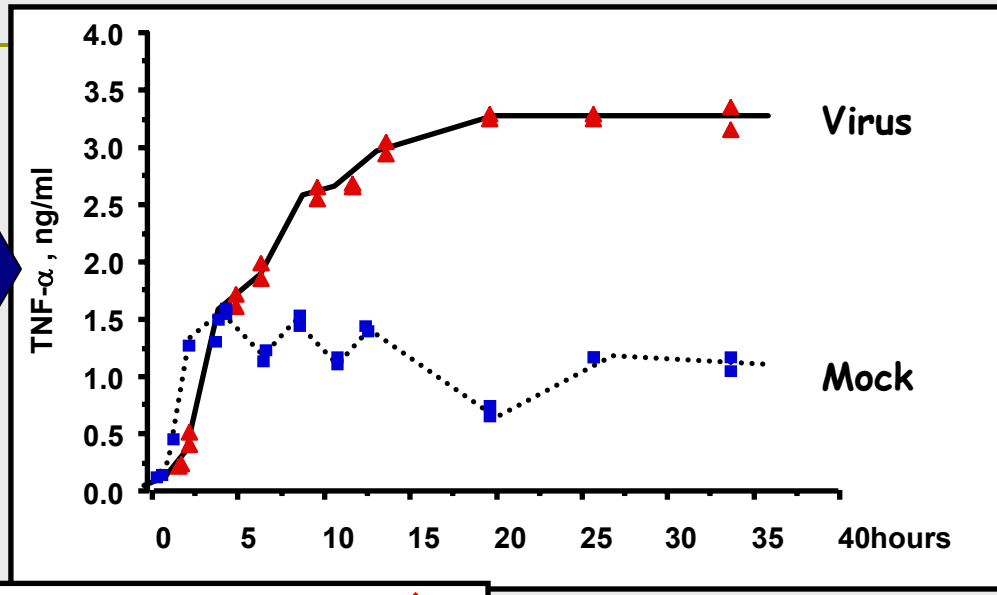

Falzarano et al. Chem Biochem, 2006

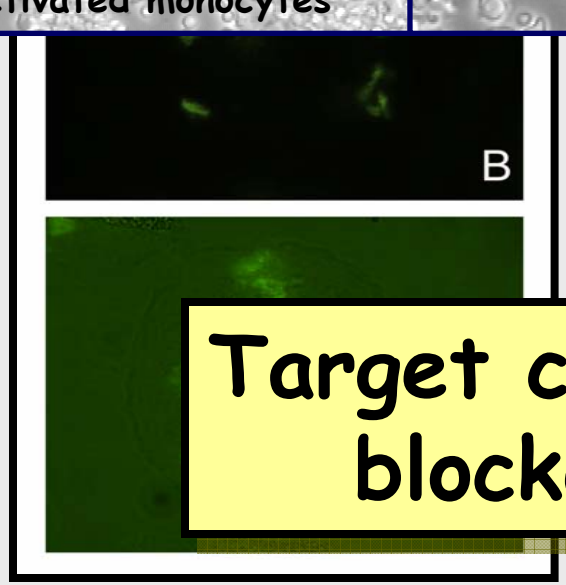
# Pathogenesis model

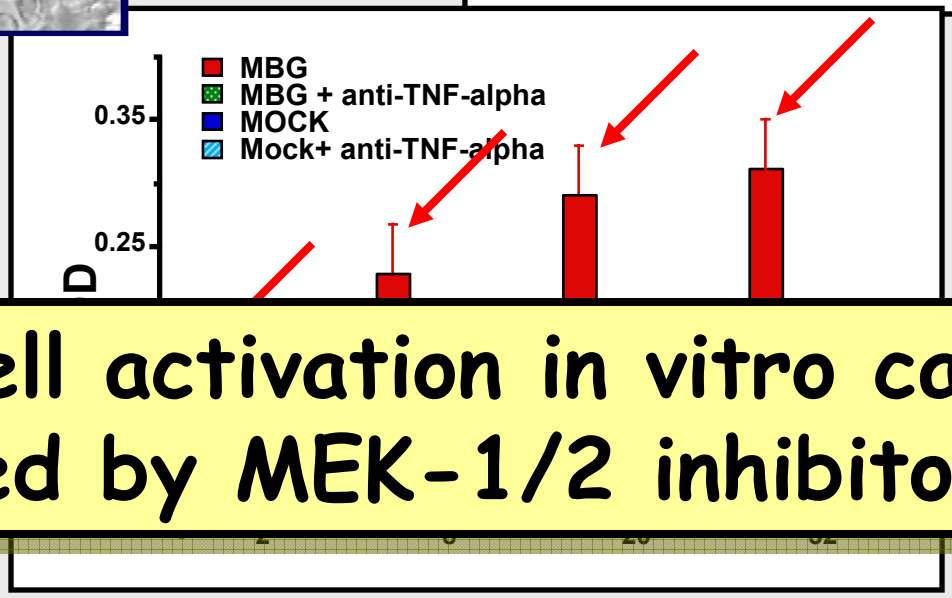


# Target cell activation & permeability



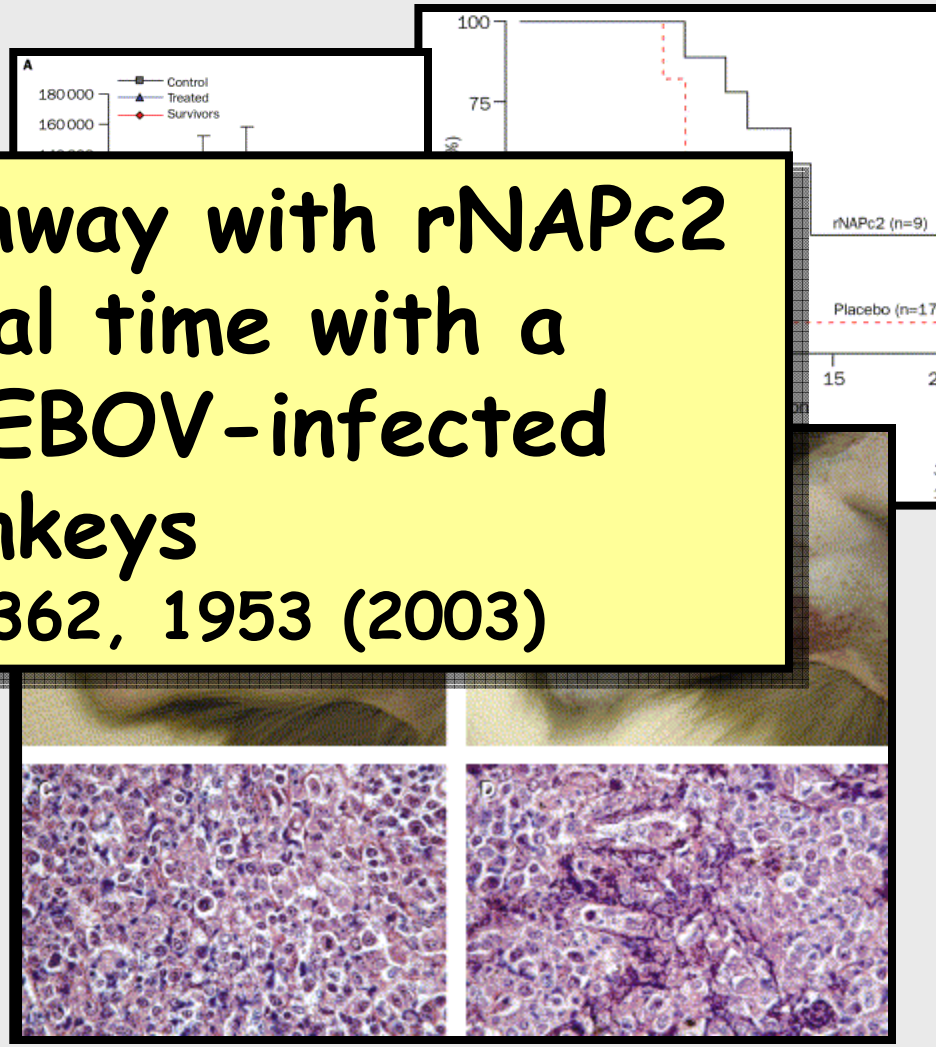




**Target cell activation in vitro can be blocked by MEK-1/2 inhibitors**

# Disseminated Intravascular Coagulation

- Mechanisms trigger DIC:
  - widespread injury to endothelial cells
  - [illegible]
- C[illegible]
  - [illegible]
    anemia resulting from fragmentation of RBCs as they squeeze through narrowed vasculature
  - Hemorrhagic diathesis resulting from consumption of platelets and clotting factors and activation of plasminogen

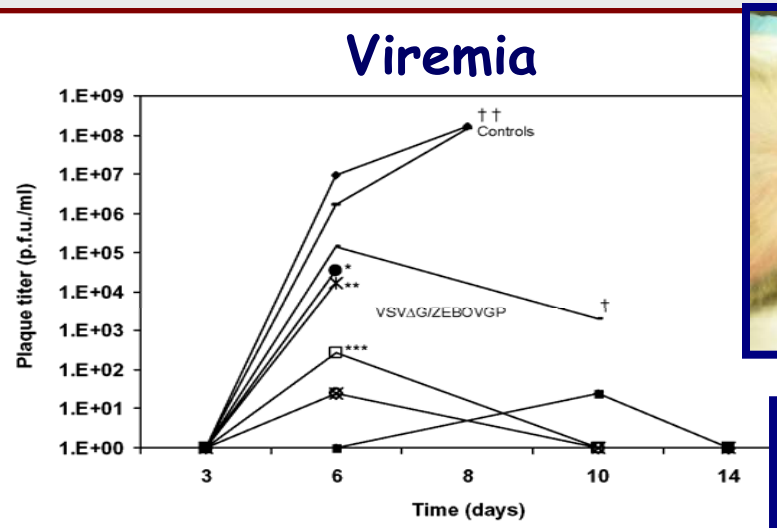


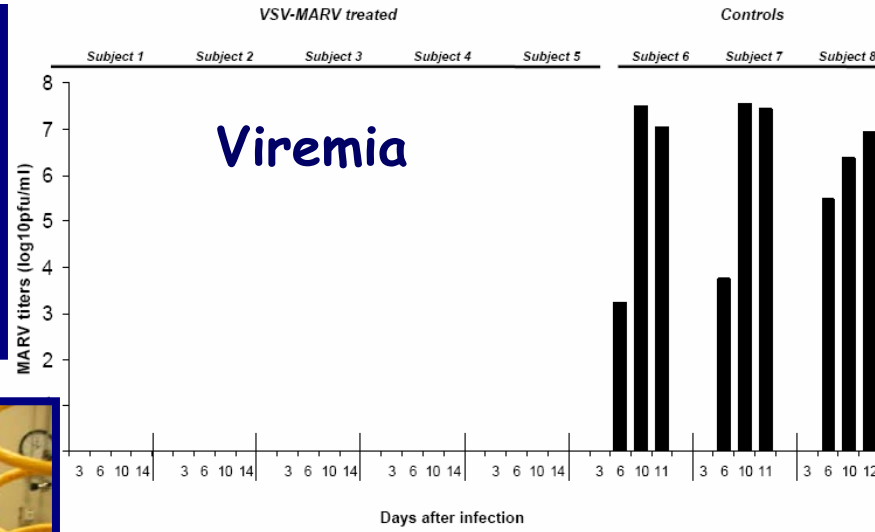

**Blockade of the TF pathway with rNAPc2 prolonged the survival time with a 33% survival rate in EBOV-infected rhesus monkeys**

(Geisbert et al., Lancet 362, 1953 (2003)

# Post-exposure treatment with rVSVs

Challenge: $10^3$ pfu ZEBOV-Kikwit i.m. (left) or MARV-Popp i.m. (right)
Treatment: $10^7$ pfu rVSV-ZEBOV/GP i.m. (left) or rVSV-MARV/GP i.m. (right)





rVSV vectors are likely to infect the same target cells and may establish an antiviral state (viral interference).

rVSV vectors are likely to activate dendritic cells and might prevent their inactivation through MARV and EBOV infection.

Feldmann et al. PLoS Path, 2007

Daddario-DiCaprio et al. Lancet, 2006

# Conclusions

- Tools and systems to investigate antivirals and other therapeutic interventions are established
- Animal models have been developed and characterized
- Effective therapy most likely needs a combined approach targeting distinct mechanisms
- Effective vaccination seems achievable in future (pre- and post-exposure prophylaxis)
- Effective treatment in the field needs on-site laboratory support

S. Jones, J. Strong, U. Ströher, Hideki Ebihara, V. Wahl-Jensen, A. Groseth, D. Falzarano, T. Hoenen, F. Feldmann, A. Grolla, D. Dick, s. Jones

Public Health Agency of Canada (PHAC)

Chemical, Biological and Radionuclear Research and Technology Initiative (CRTI)

National Institutes of Health (NIH)

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Special Pathogens, NML, PHAC

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