DNA, RNA or viral particles: Alphaviruses as vaccine vectors

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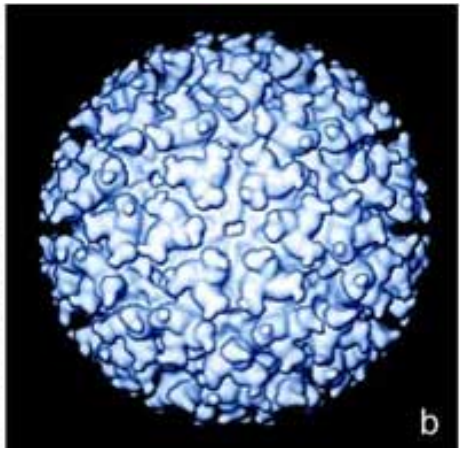
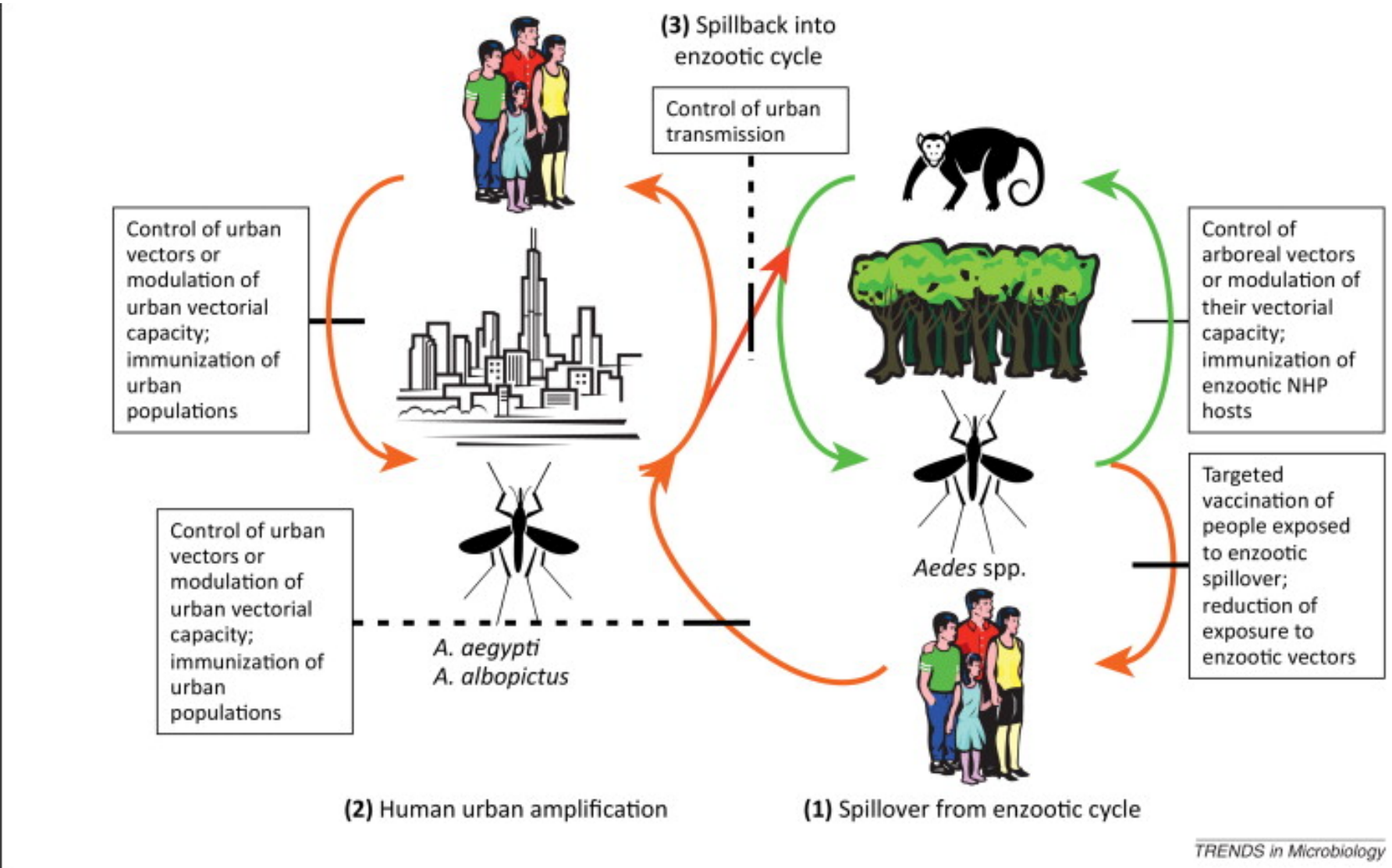



Aedes egypti


Aedes albopictus

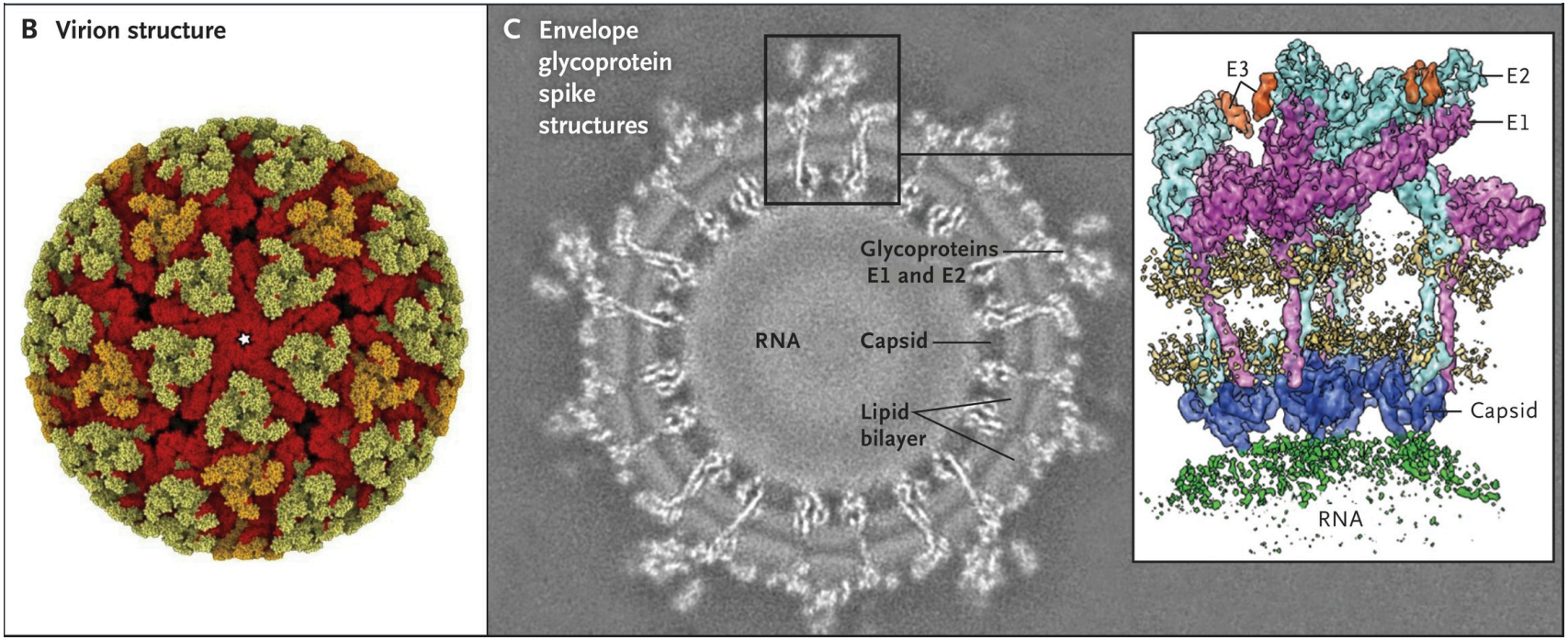
Alphavirus zoonotic cycle



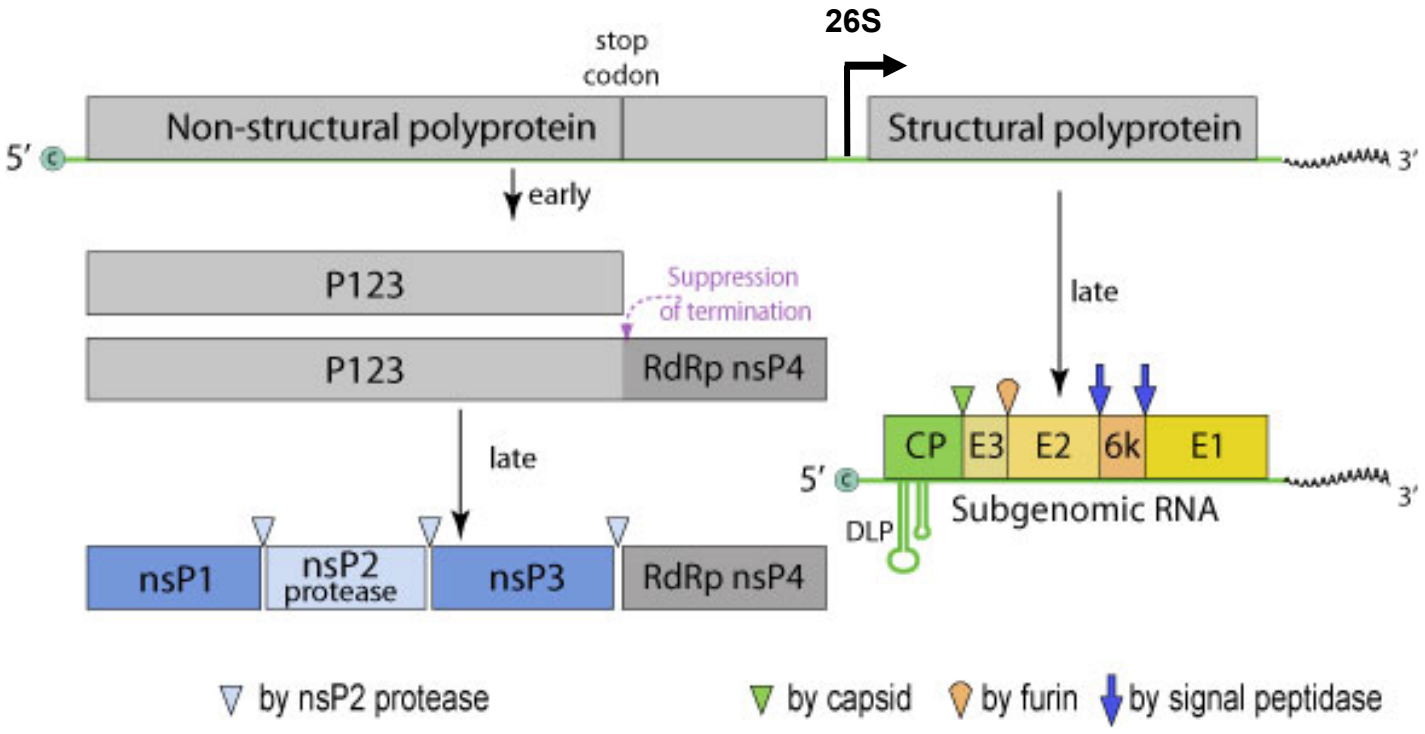
alphaviruses

- Family: *Togaviridae*
- Genus: *Alphavirus*
- +ssRNA genome (~11.5 kb)

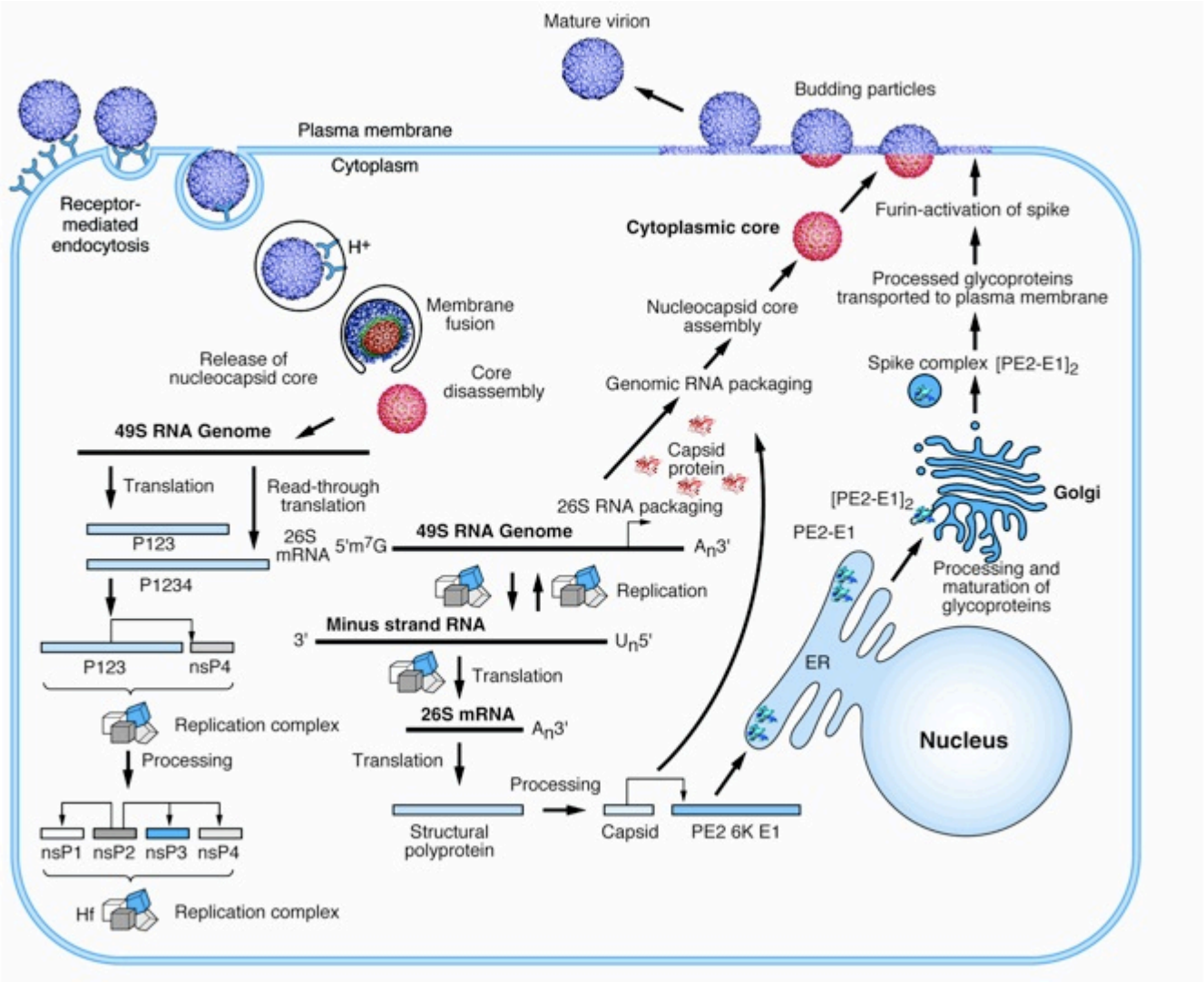
- Enveloped virus, icosahedral capsid
- Receptor mediated endocytosis



alphavirus – genome and proteome

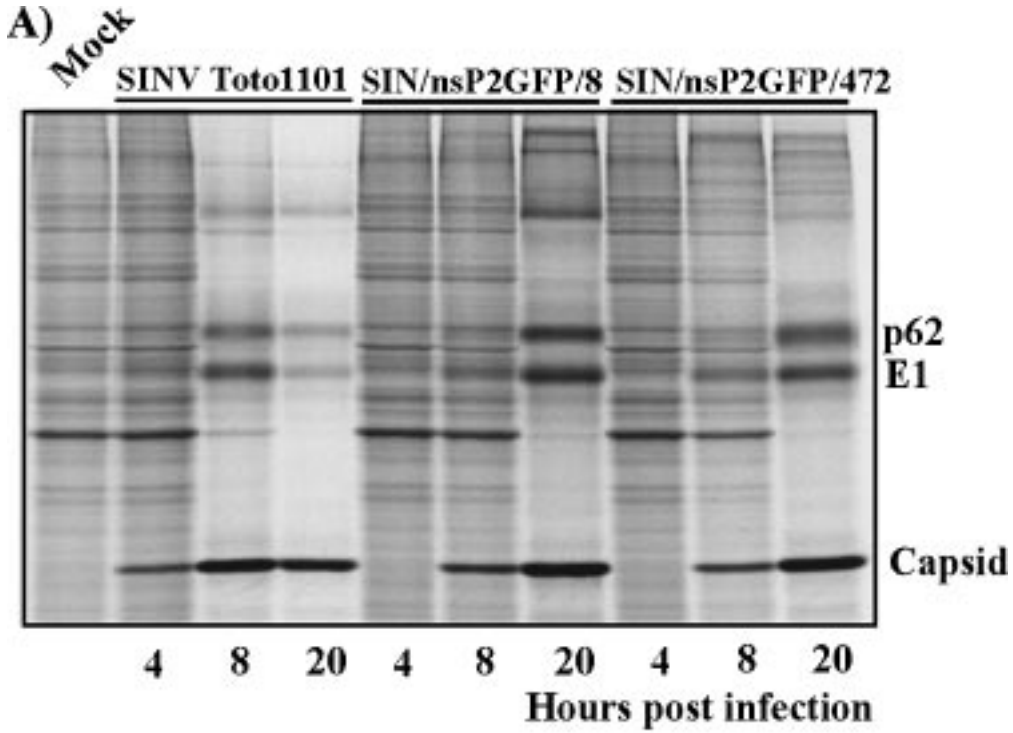


alphavirus life cycle

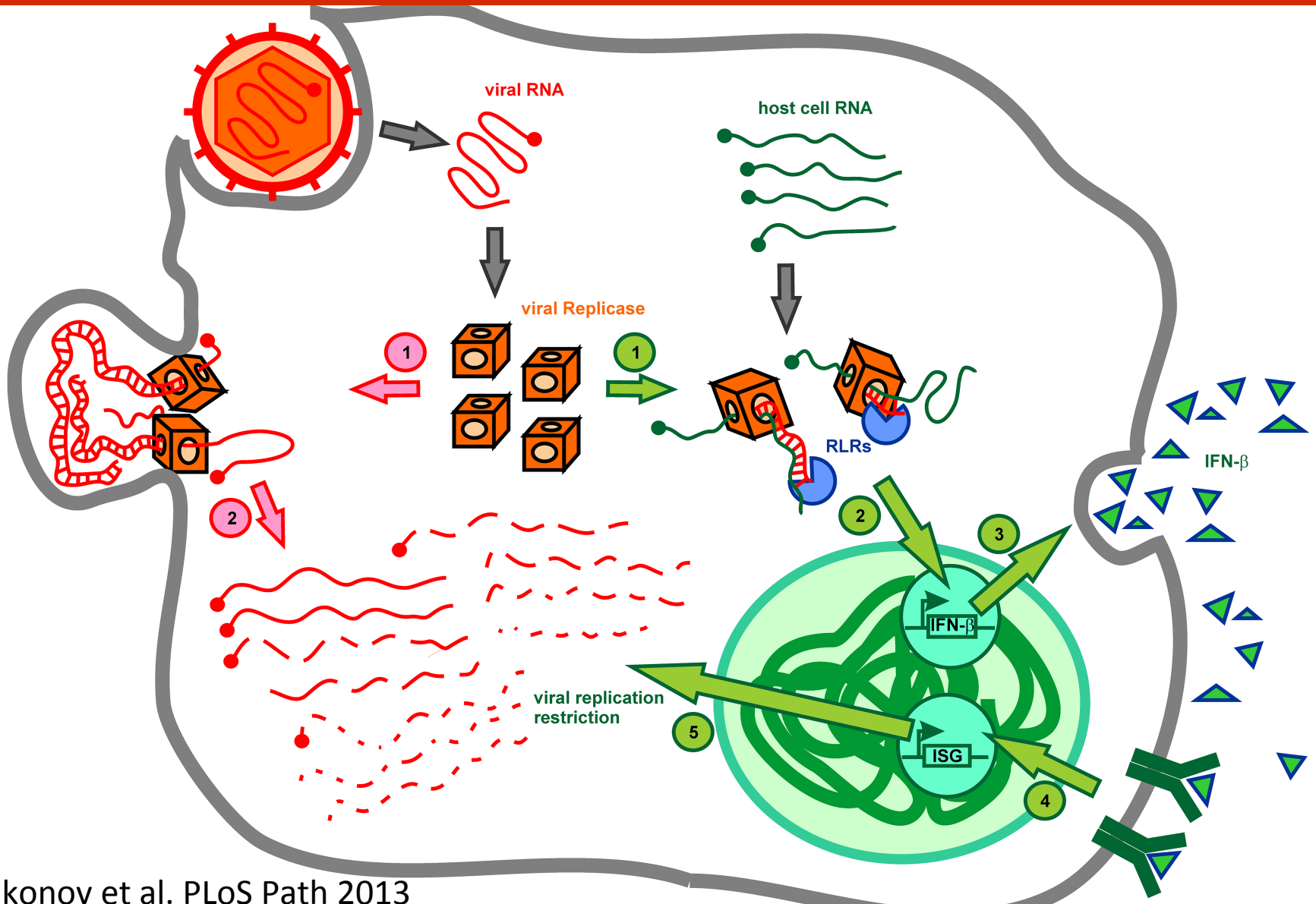


alphavirus –protein expression

- Host cell shutoff
- High level of viral protein expression

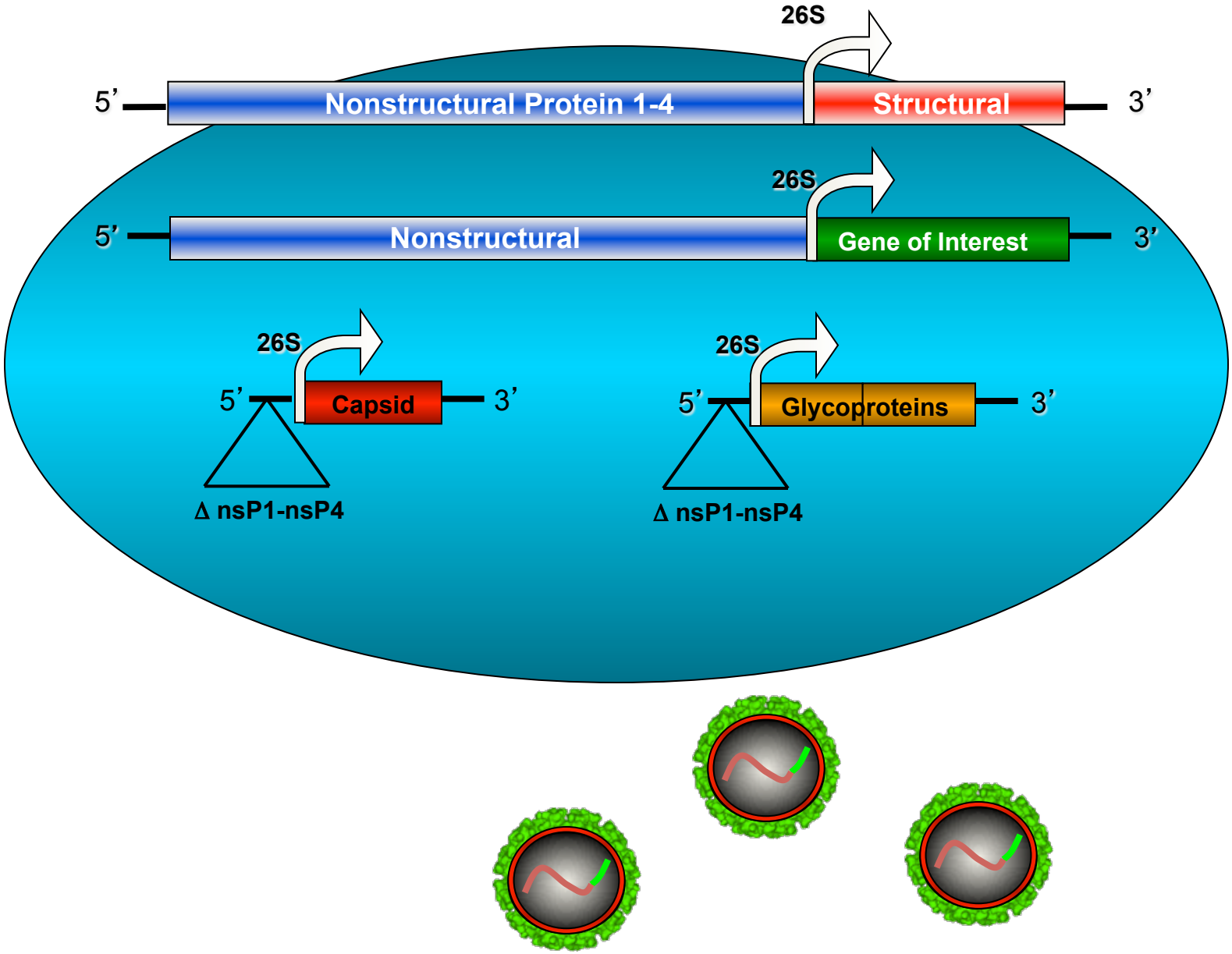


alphavirus – innate immunity

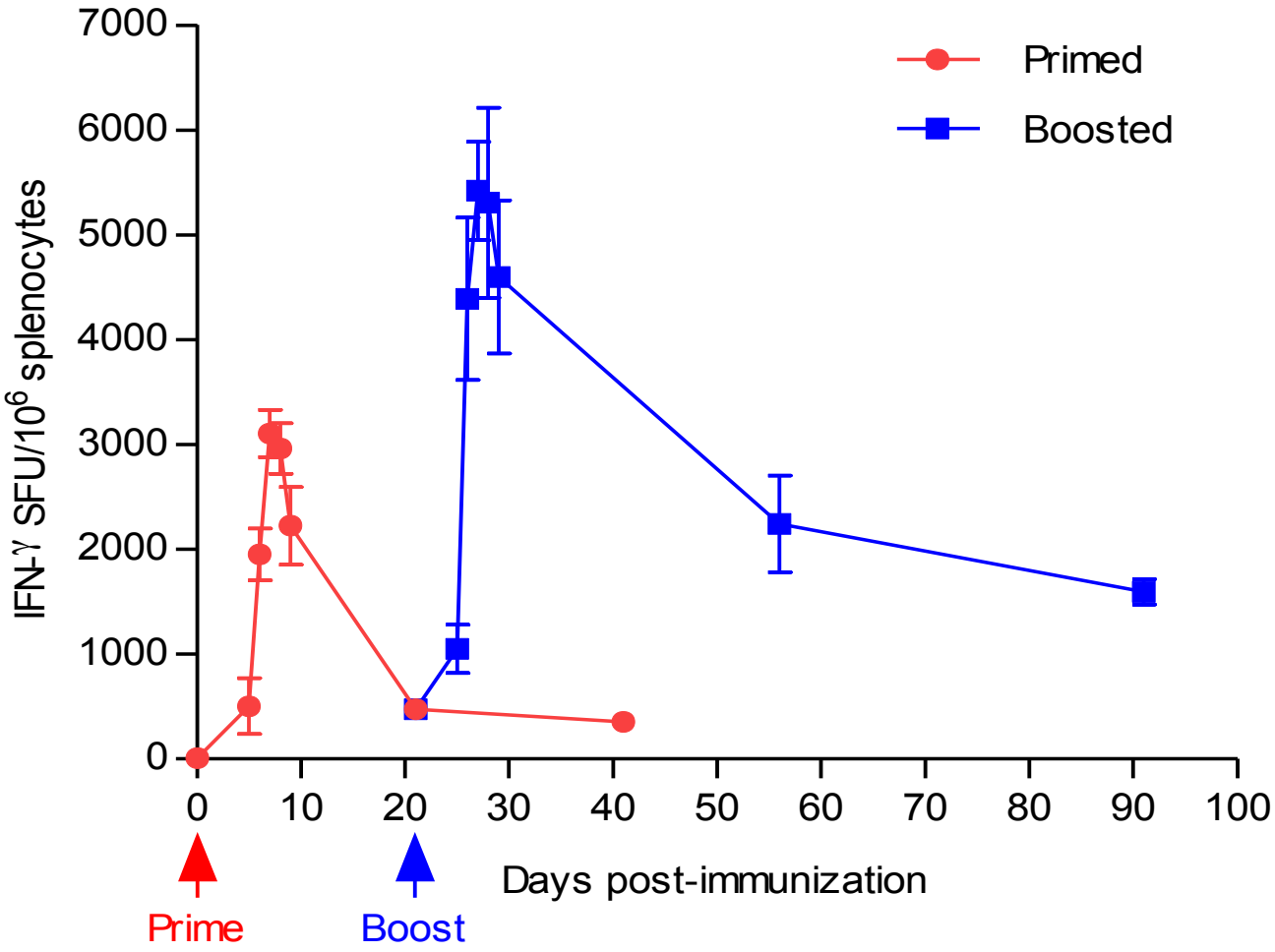


Nikonov et al. PLoS Path 2013

Alphavirus Replicon Particles (VREP)

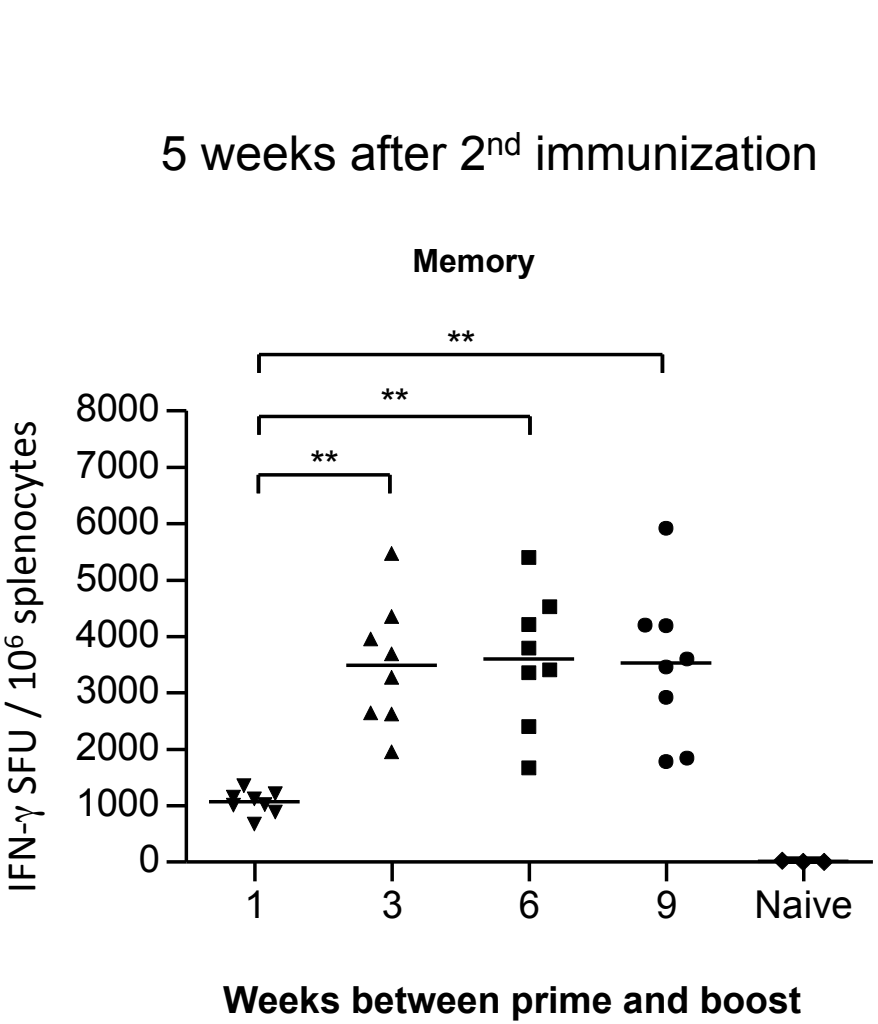


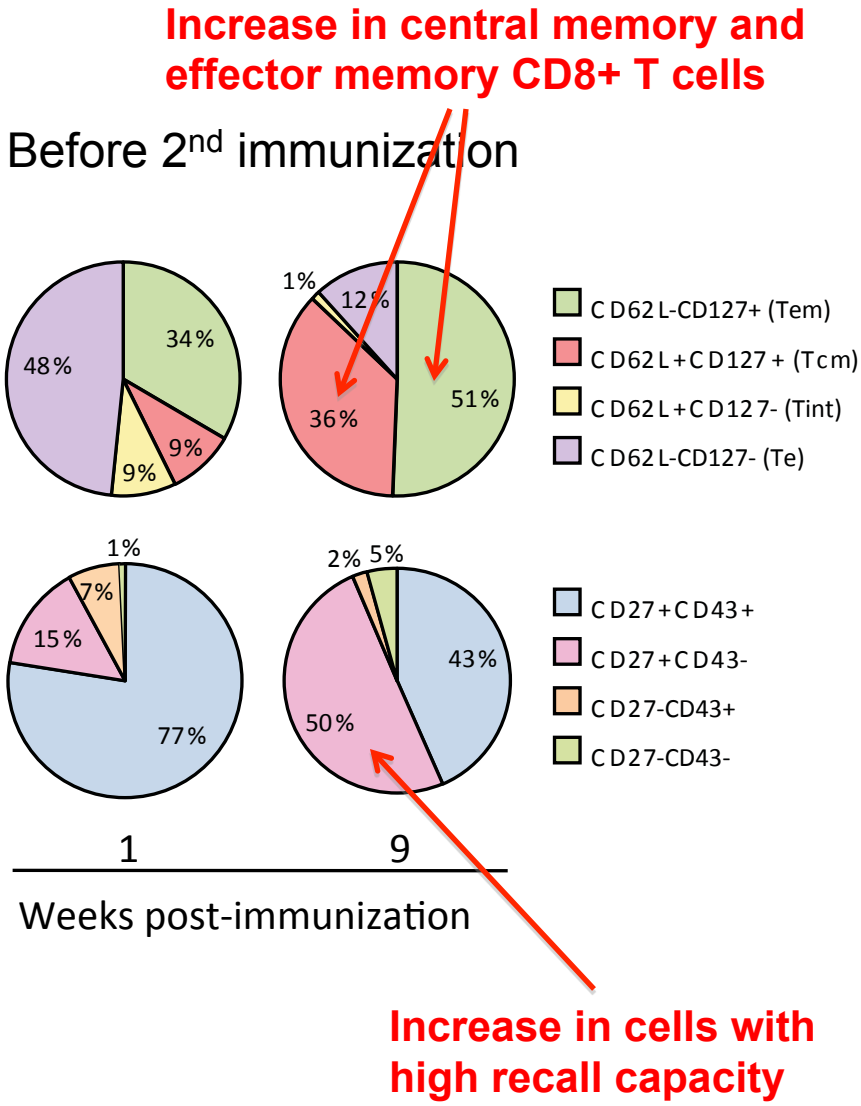
CD8 T cell kinetics



Knudsen et al. (2014). J Virol. 88(21):12438-51

CD8 T cell response qualitatively determines boosting





Knudsen et al. (2014). J Virol. 88(21):12438-51

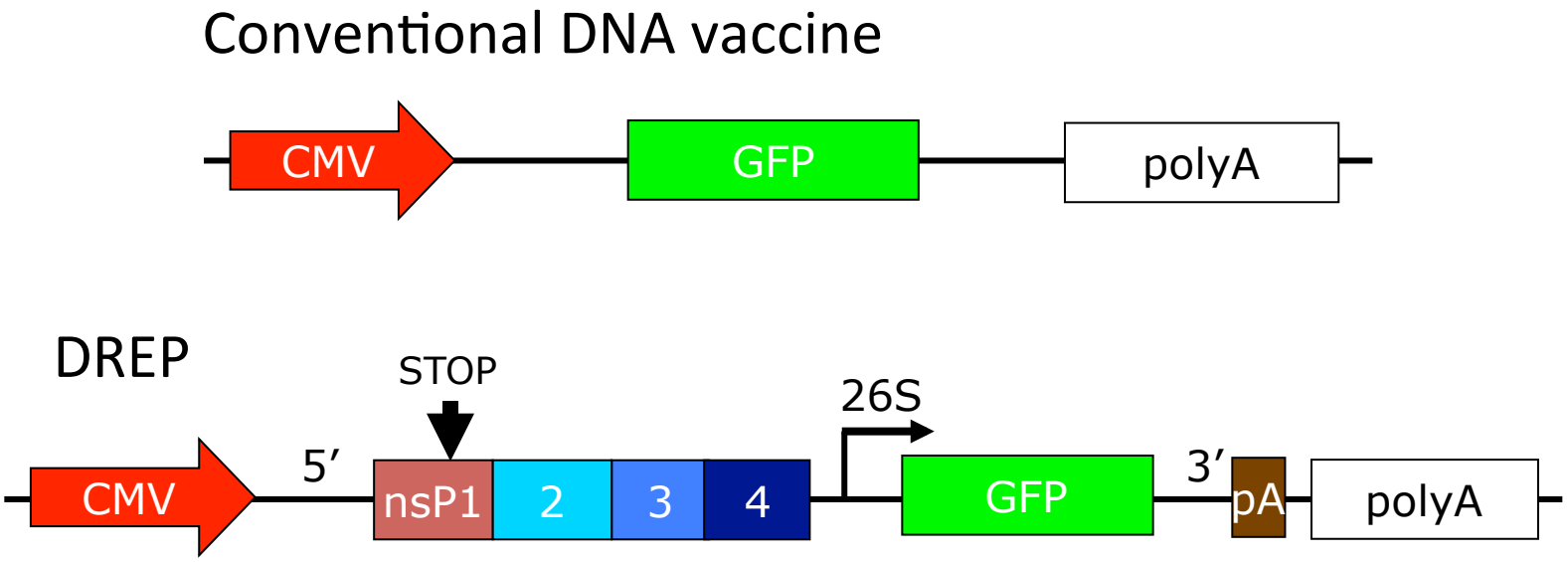
desirable properties of VREP vectors

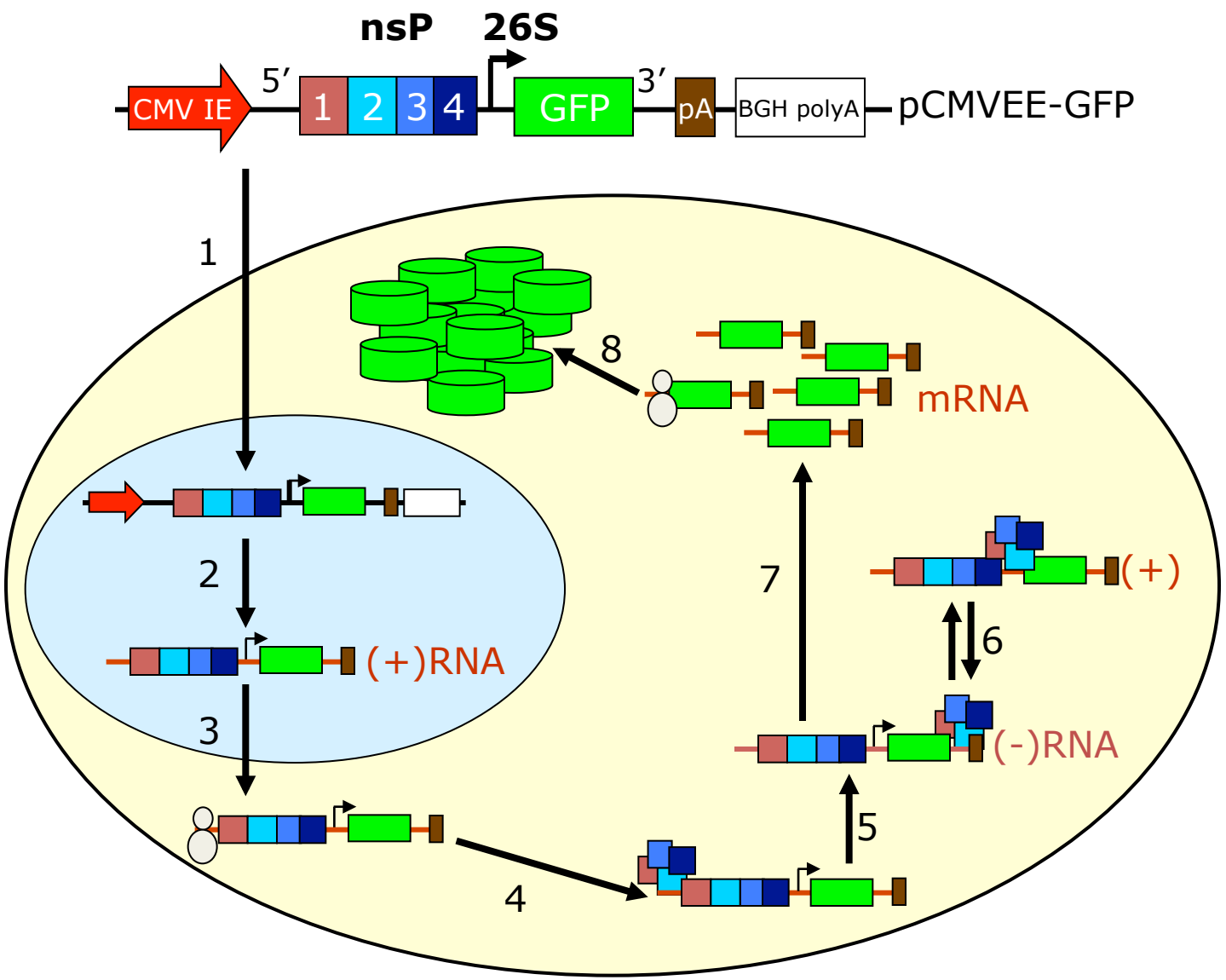
- Efficient delivery
- Delivery to many cell types (also DC)
- Increased antigen expression
- Induction of apotosis → cross presentation
- Activation of Pattern Recognition Receptors (PRR) – TLR3, 7/8, PKR MDA-5
- Strong induction of innate immunity – type I interferons

VREP problems?

- Safety – non-homologous recombination reversion occurs, especially for certain inserts
- Anti-vector immunity
- Production
  - Scale up to industrial scale
  - No stable packaging cell line

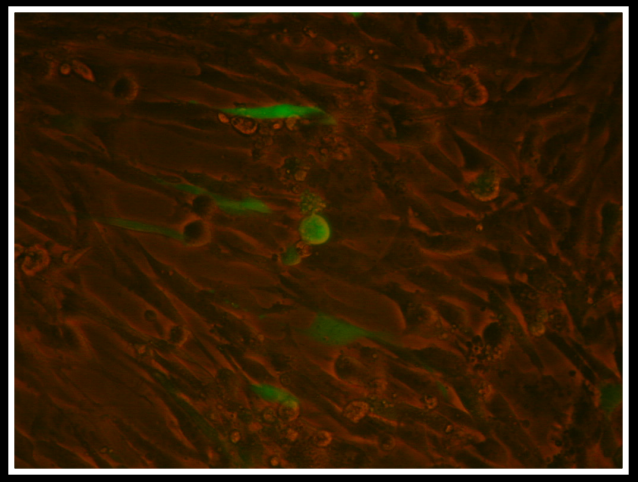
DNA-launched replicons



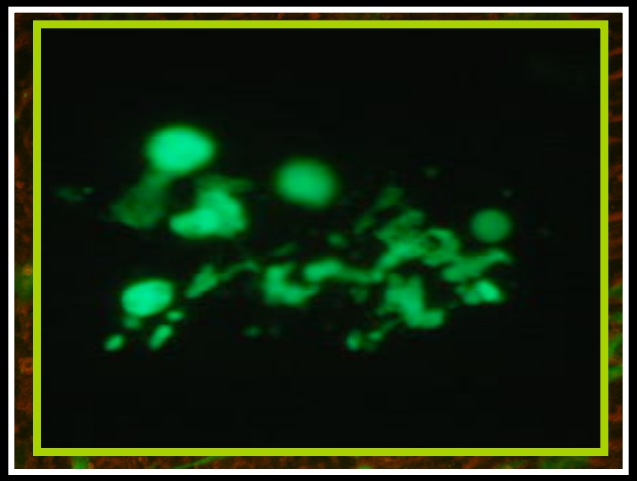


1) Transfection.
2) Transcription.
3) Nuclear export.
4) Translation of replicase (nsP1-4)
5) Negative strand synthesis.
6) Genome replication.
7) Transcription of subgenomic RNA.
8) Translation of subgenomic RNA.

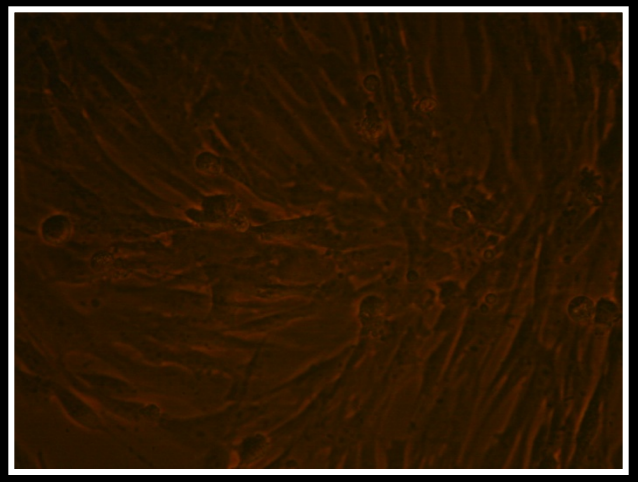
DREP-GFP expression



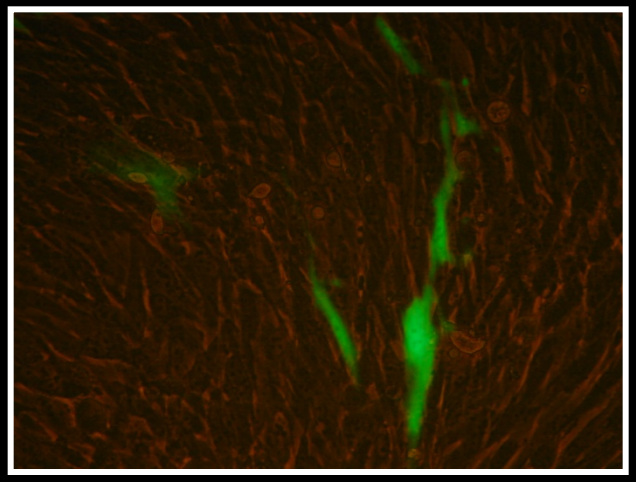
pVAX-GFP-1



DREP-GFP

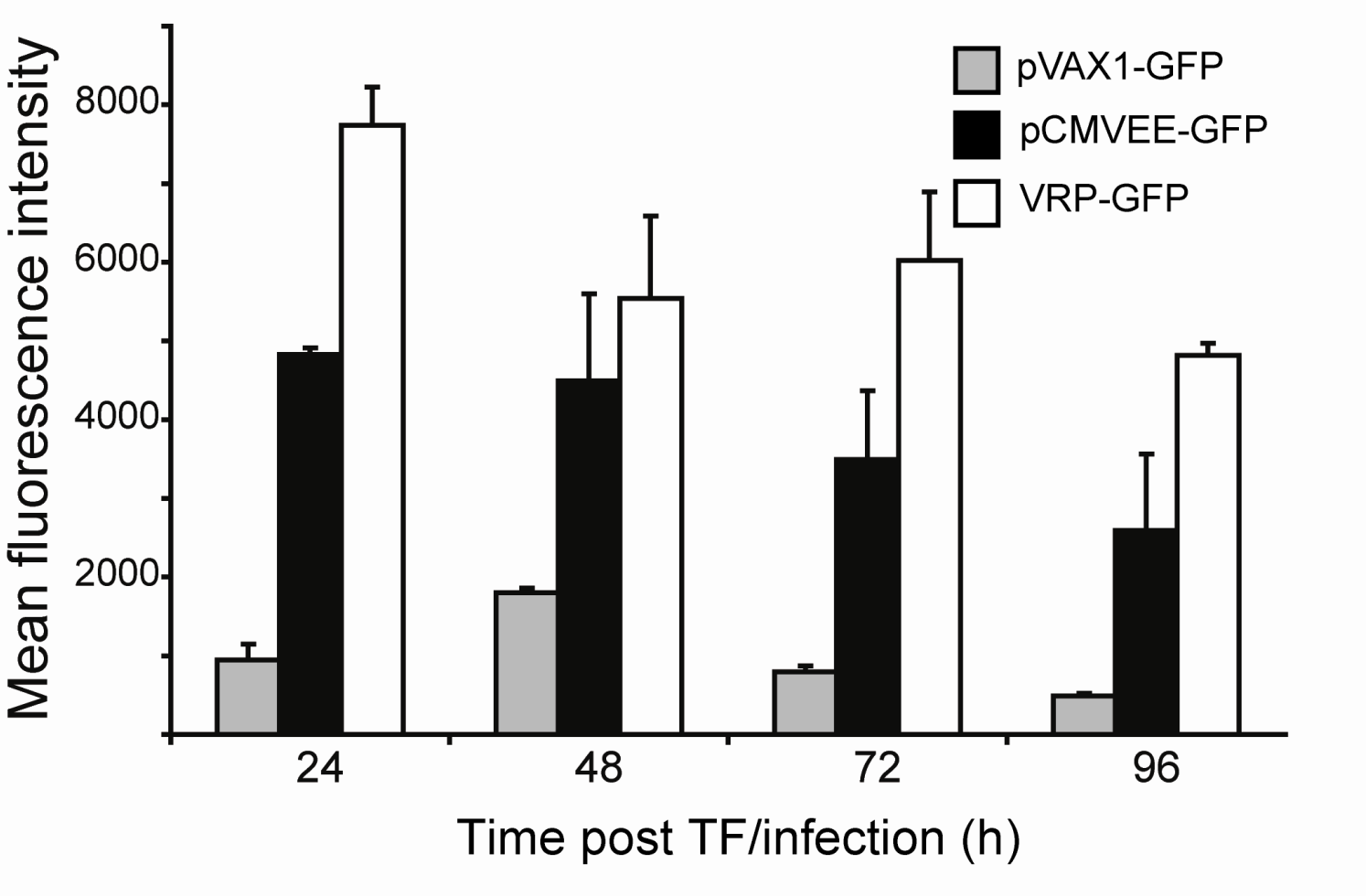


DREPnsP1FS/STOP-GFP



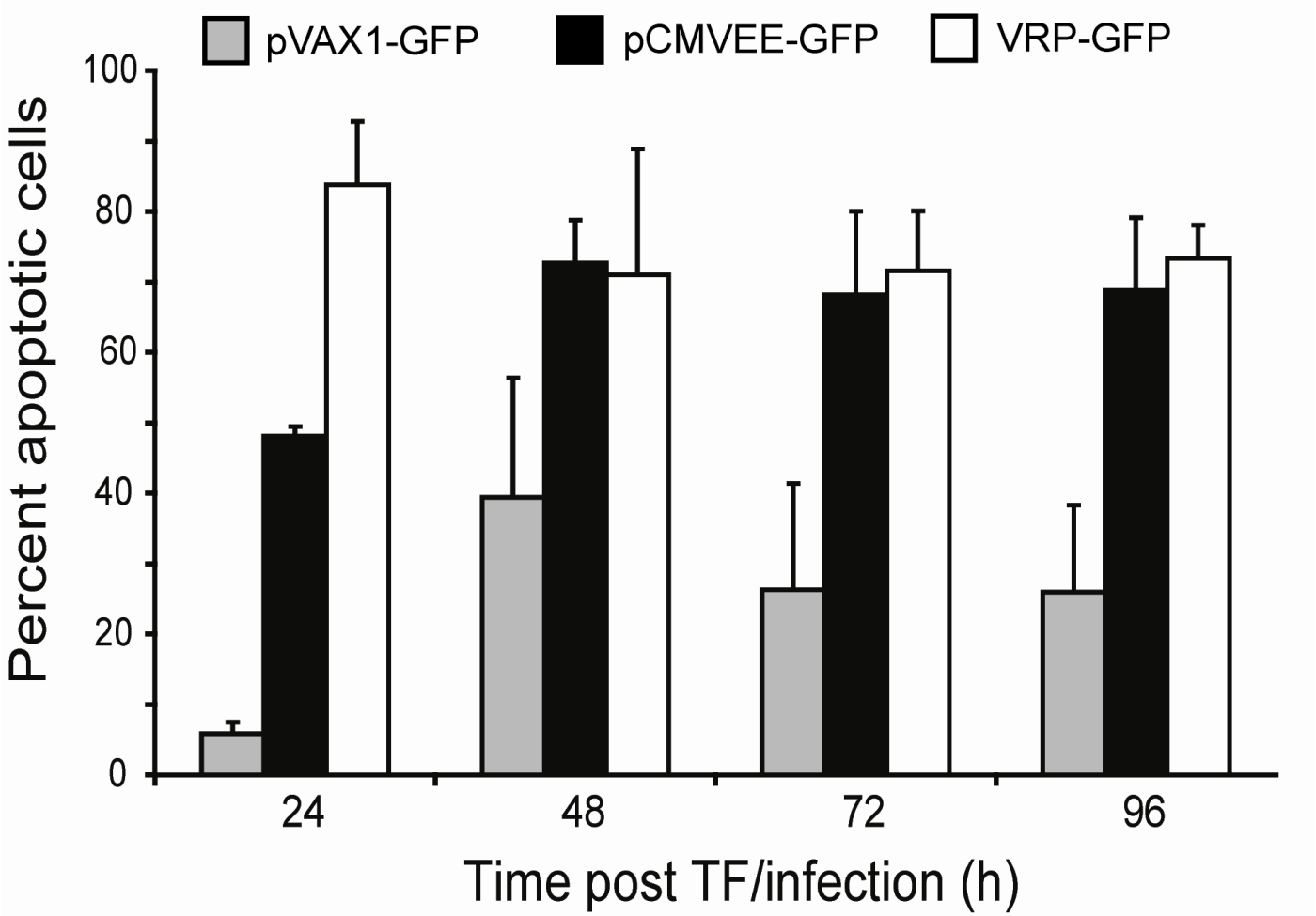
DREP-CMVFLIP-GFP

GFP expression controlled by the 26S promoter is increased



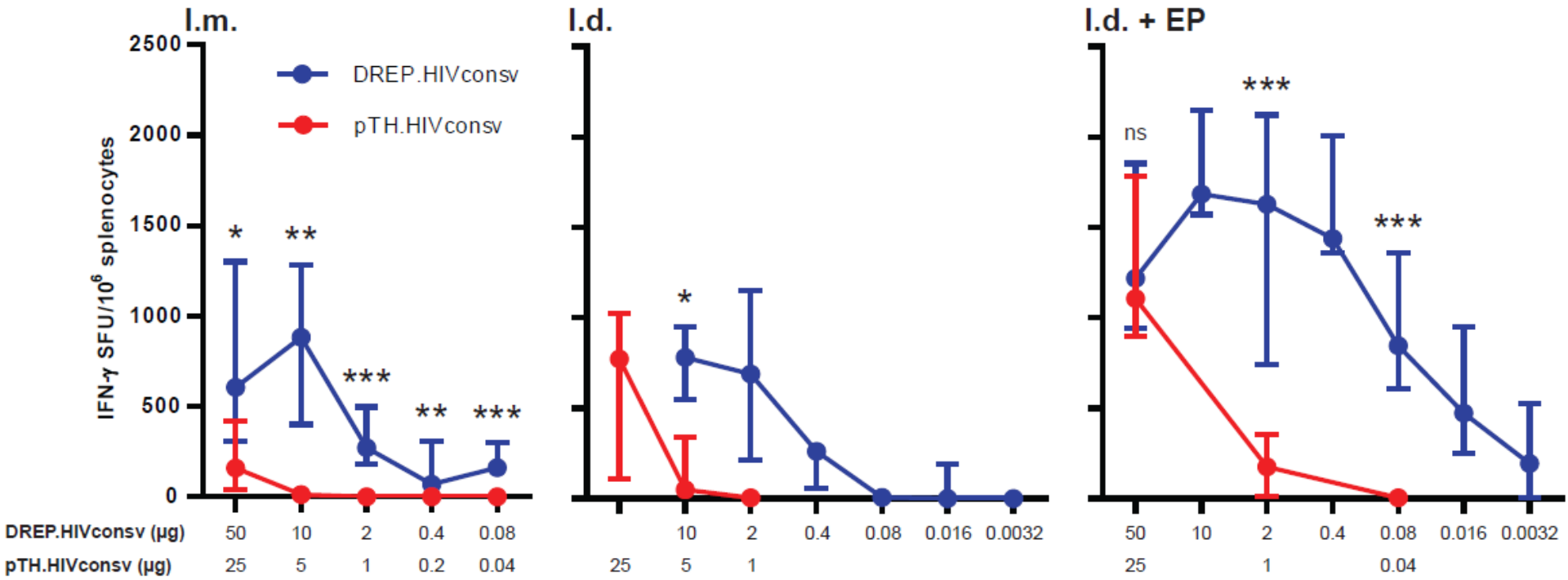
Ljungberg et al. J Virol. 2007;81(24):13412-23

pCMVEE and VRP induce apoptosis



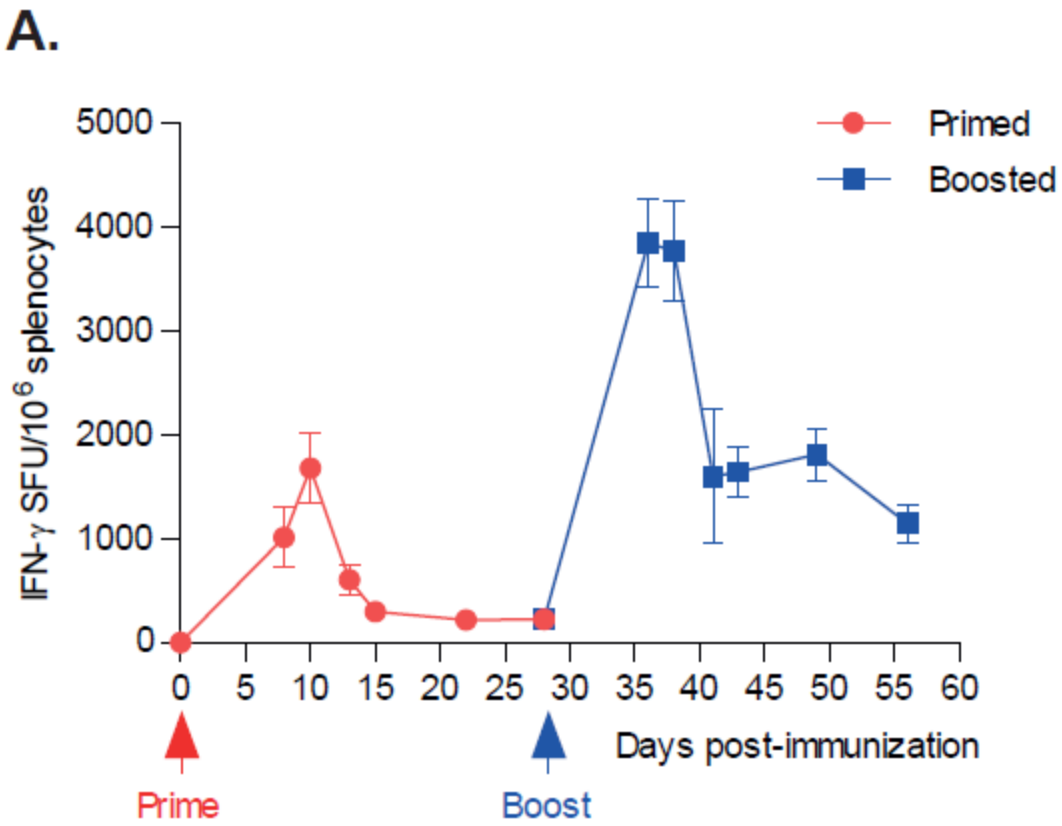
Ljungberg et al. J Virol. 2007;81(24):13412-23

DNA replicon Vs. conventional DNA vaccine



Knudsen et al. J Virol. 2012;86(8):4082-90

CD8+ T cell kinetics



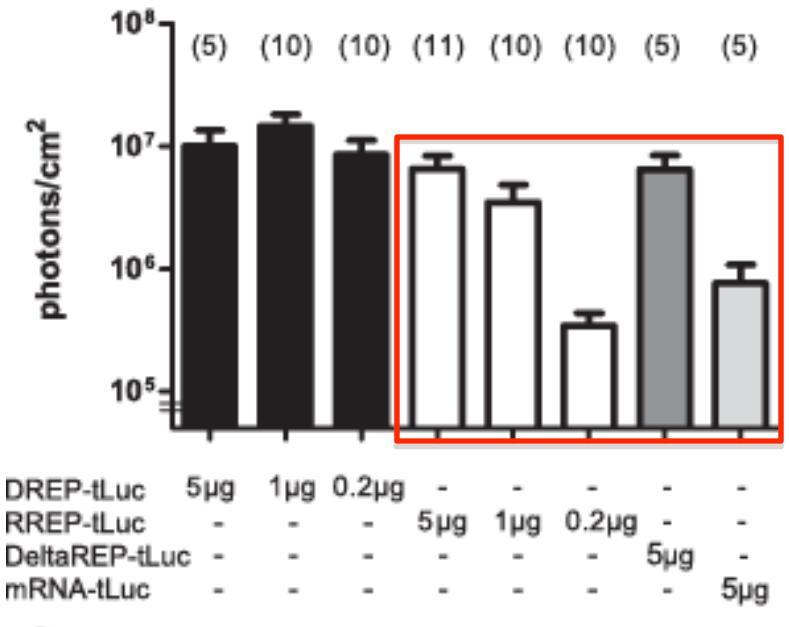
Knudsen et al. (2014). J Virol. 88(21):12438-51

RNA-launched replicons

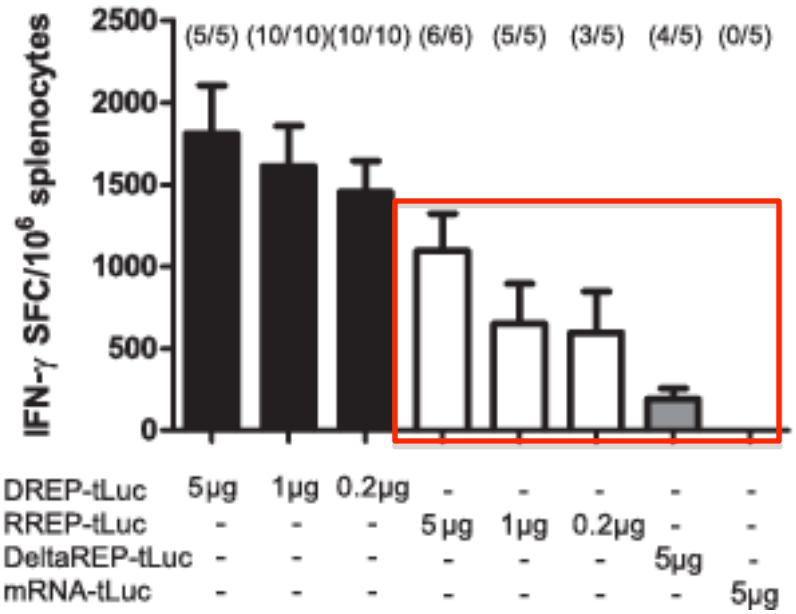
Produced by in vitro transcription from replicon-encoding plasmid from T7 or SP6 promoter

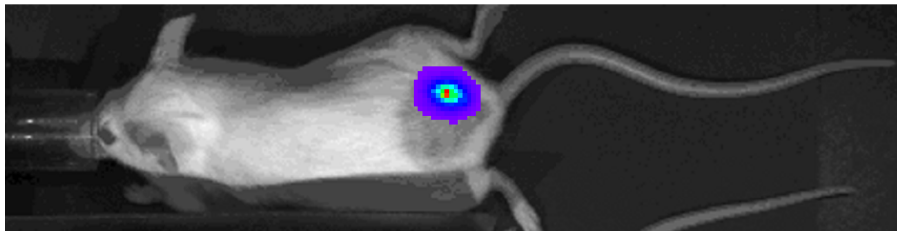
RREP immunization

***in vivo* expression**



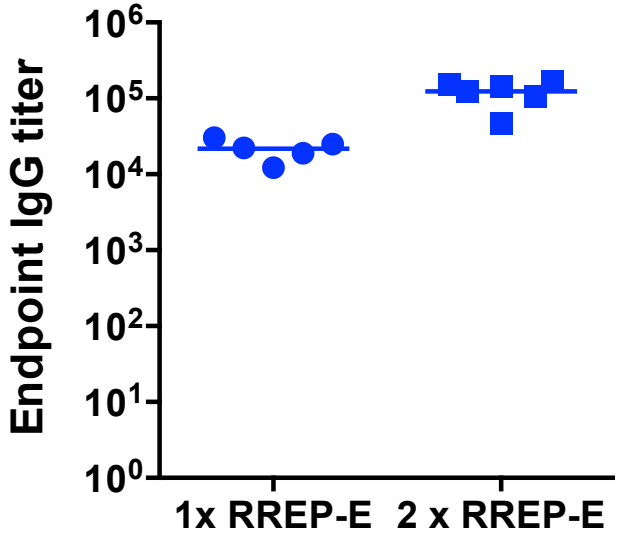
immune response

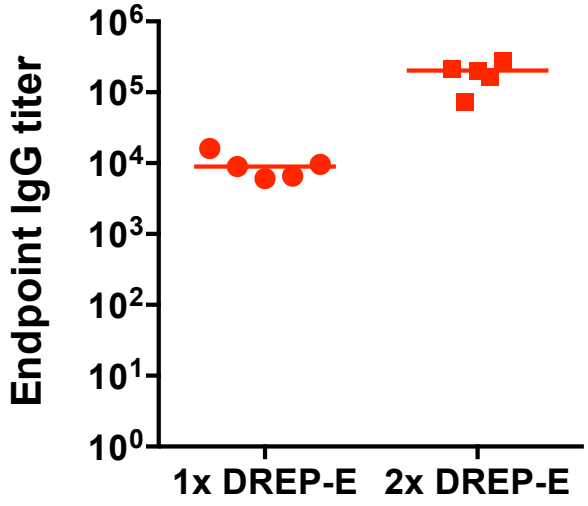




Johansson et al. PLoS One. 2012;7(1):e29732

Compare RREP vs DREP (mice)



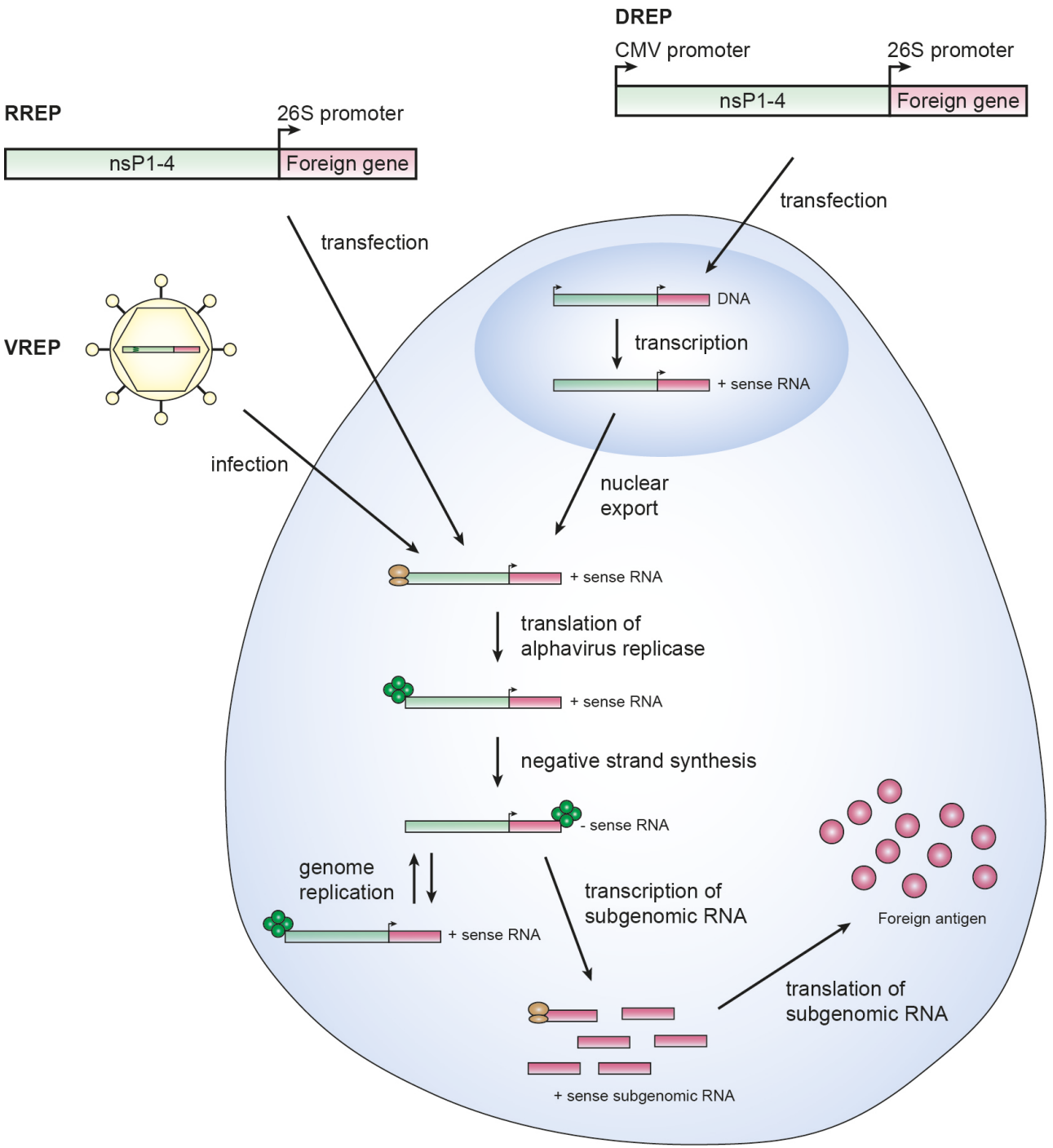


RREP & DREP were given at 10 ug doses

summary

Alphaviruses…

- are +sense ssRNA viruses
- Spread by mosquitoes
- Have epidemic potential
- Strongly promotes innate immunity
- Can be engineered to express foreign proteins
- Can be used as vaccine vectors
  - That can delivered as viral replicon particles, RNA replicons or DNA replicons


DREP
CMV promoter
26S promoter
nsP1-4
Foreign gene
RREP
26S promoter
nsP1-4
Foreign gene
transfection
transfection
VREP
DNA
transcription
+ sense RNA
infection
nuclear
export
+ sense RNA
translation of
alphavirus replicase
+ sense RNA
negative strand synthesis
- sense RNA
genome
replication
+ sense RNA
transcription of
subgenomic RNA
Foreign antigen
translation of
subgenomic RNA
+ sense subgenomic RNA