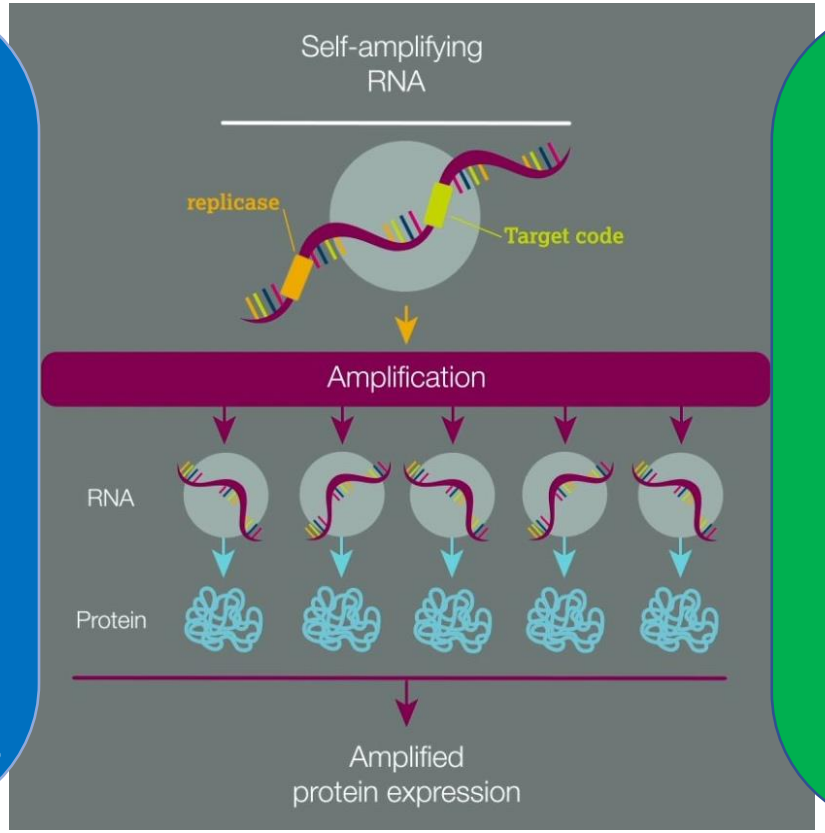
Development of a self-amplifying RNA rotavirus vaccine

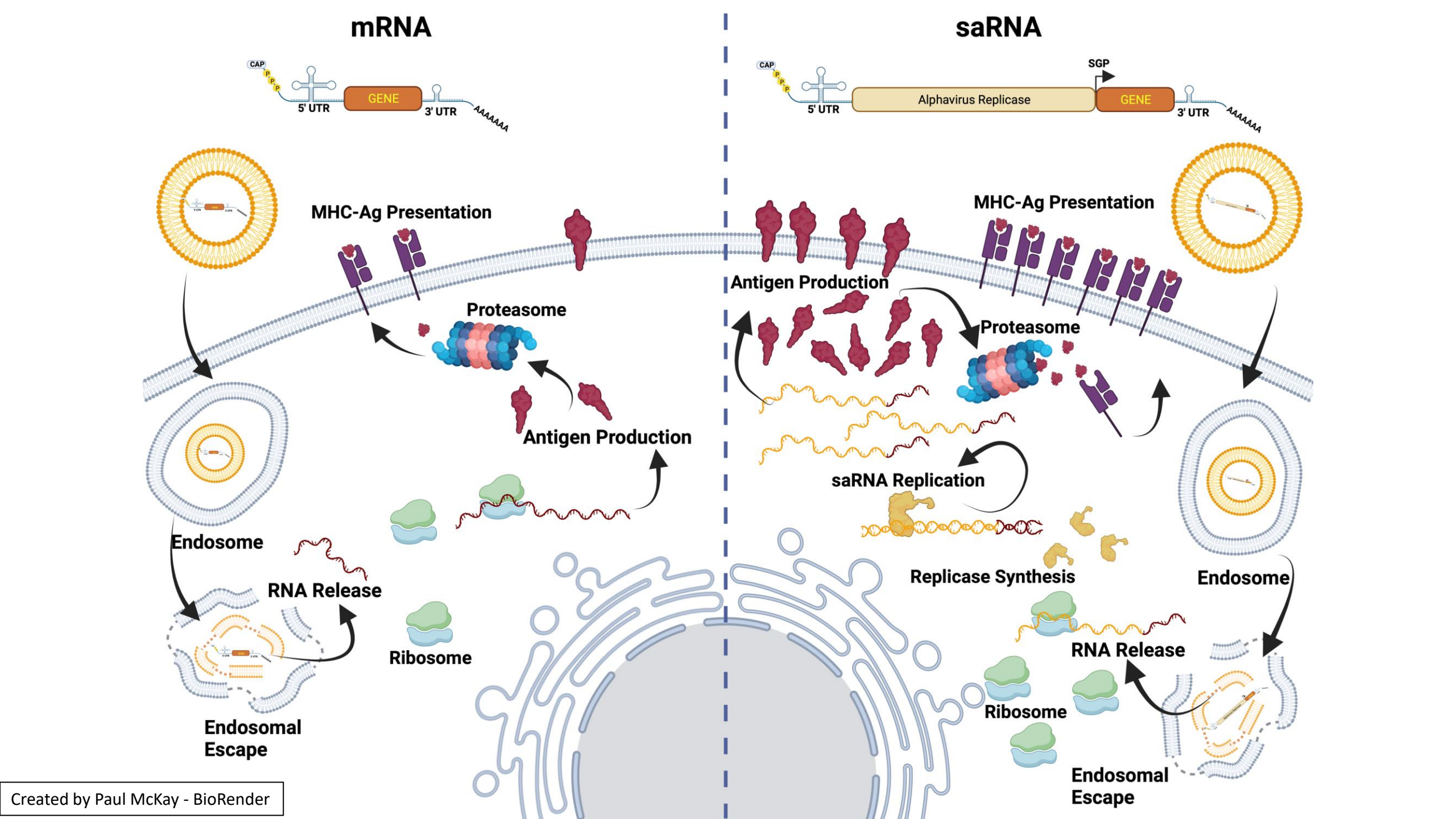
Potential advantages of saRNA

mRNA → **saRNA**

- Simple to produce
- Easy to modify
- Higher dose (25-100ug)
- Higher cost
- Tolerability issues
- Difficult to combine
- Short expression
- moderate cellular responses



- Lower dose (1-10µg)
- Lower cost
- Increased safety
- Ease of combination
- Duration of expression
- Less frequent dosing
- Improved cellular response
- Harder to produce
- Harder to modify



Created by Paul McKay - BioRender

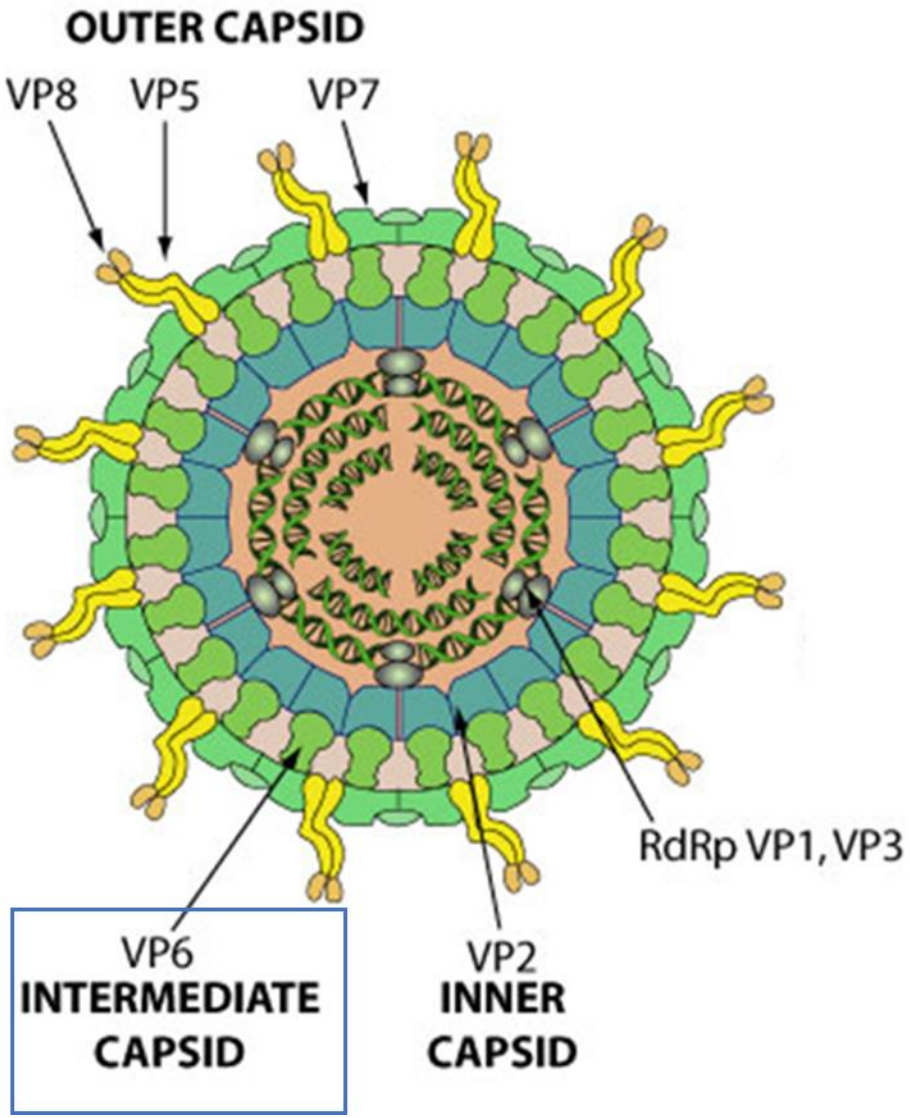
Why make a rotavirus RNA vaccine?

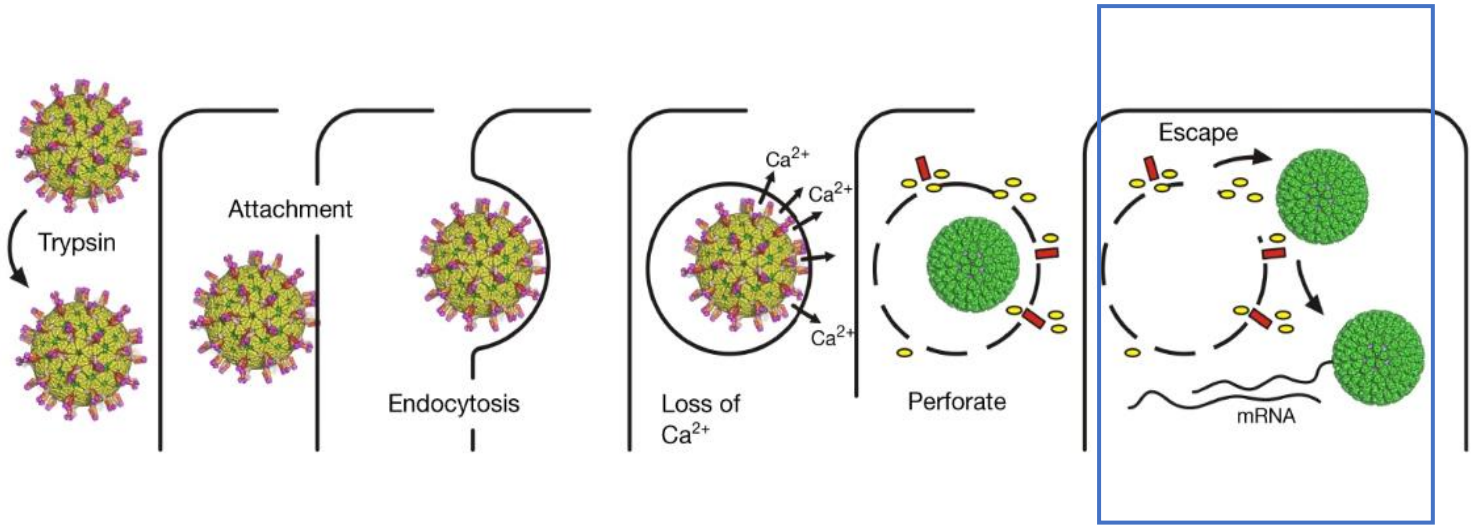
- Diarrhoeal disease is the second leading cause of death in children below the age of 5 years
- There are 4 live attenuated oral vaccines recommended by the WHO for national immunisation
- Lower vaccine effectiveness in low- and middle-income countries (e.g. >95% in Europe but may fall below 50% in sub-Saharan Africa).
- ~130,000 annual rotavirus induced infant deaths.
- Variables affecting vaccine effectiveness:

1. Transplacentally acquired RV-IgG (neutralising antibodies).
2. Environmental enteric dysfunction (EED)—enteropathy of the small intestine, characterised by enteric inflammation.
3. Gut microbiota imbalance.

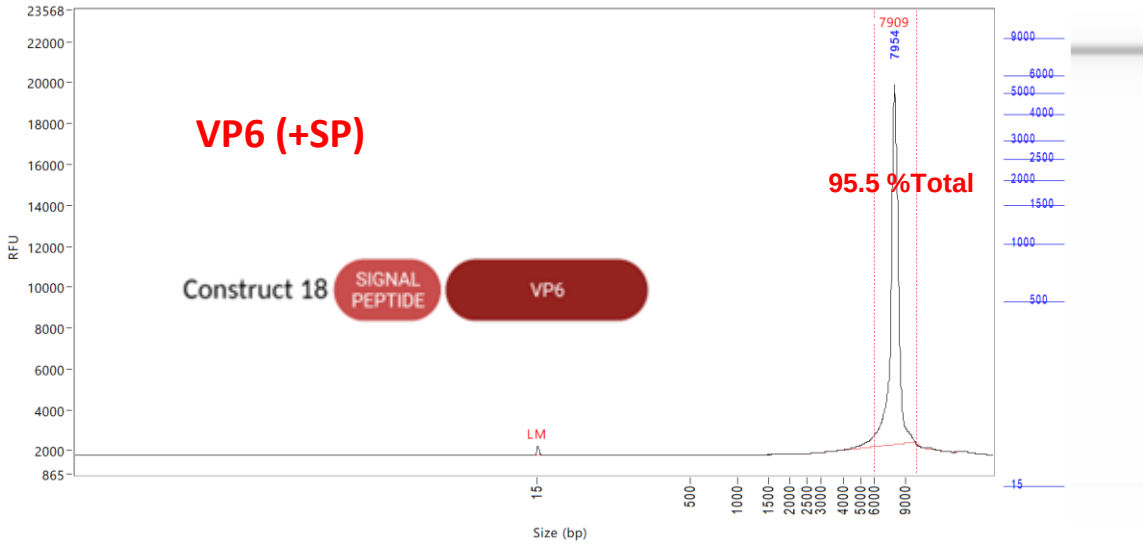
- Potential solution: a (*low cost*) non-replicating parental vaccine that bypasses the intestine and could be used alone or as a boost

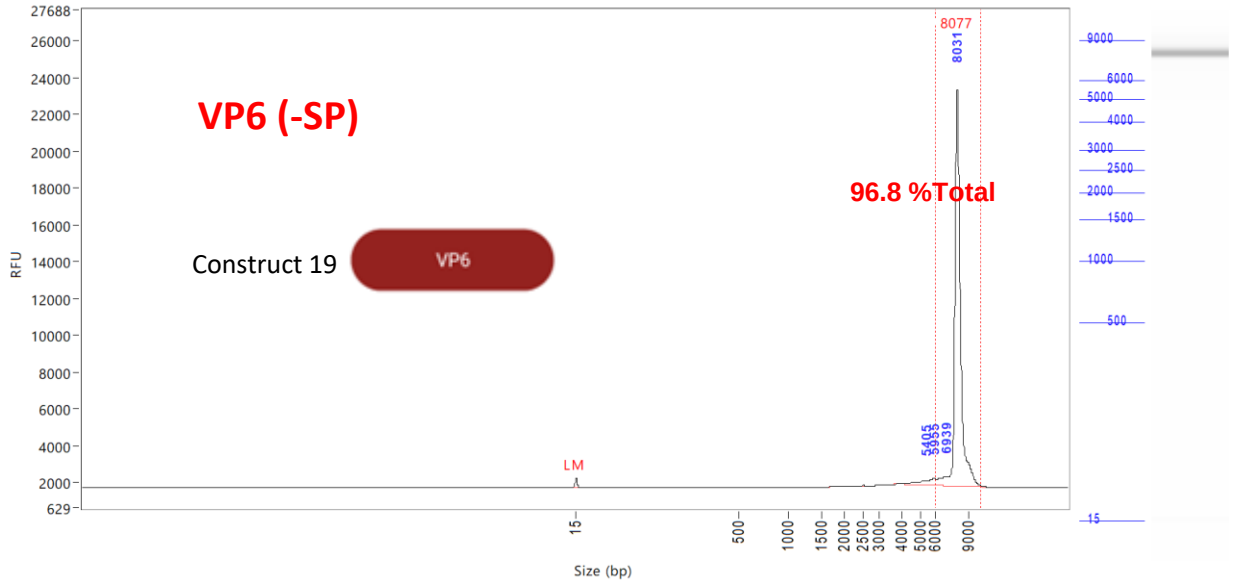
Rotavirus VP6 for developing a universal vaccine



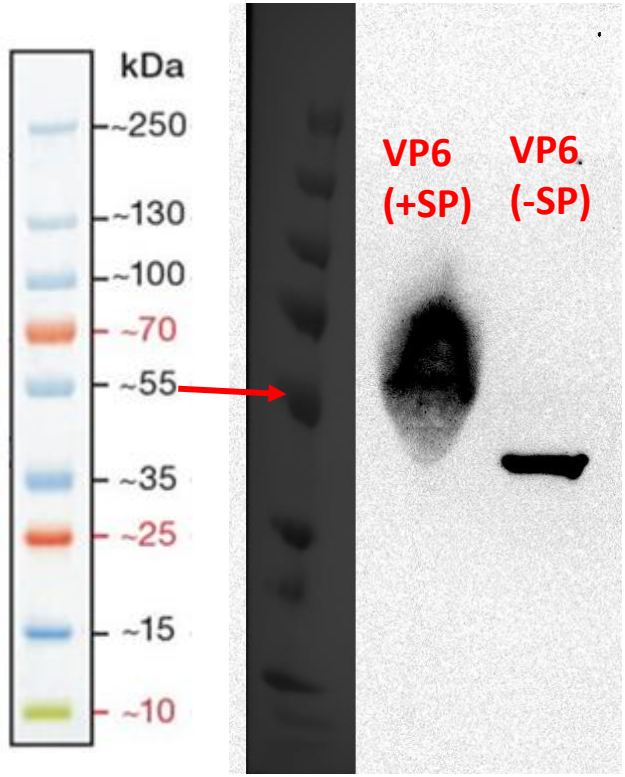


Smear analysis on a fragment analyser

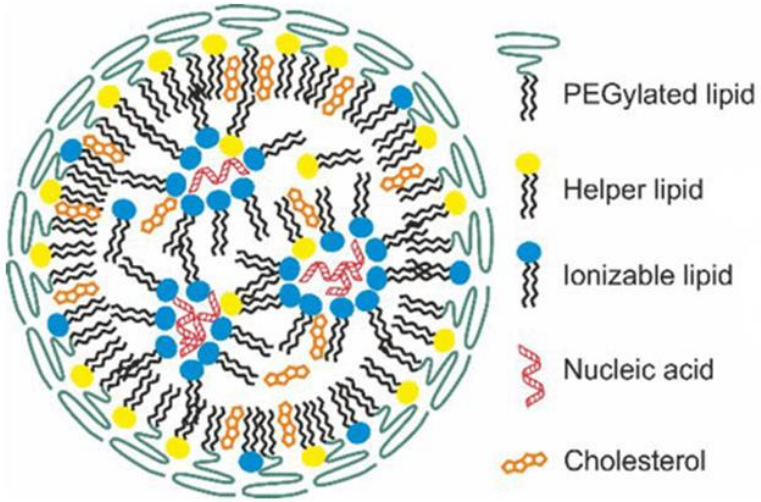


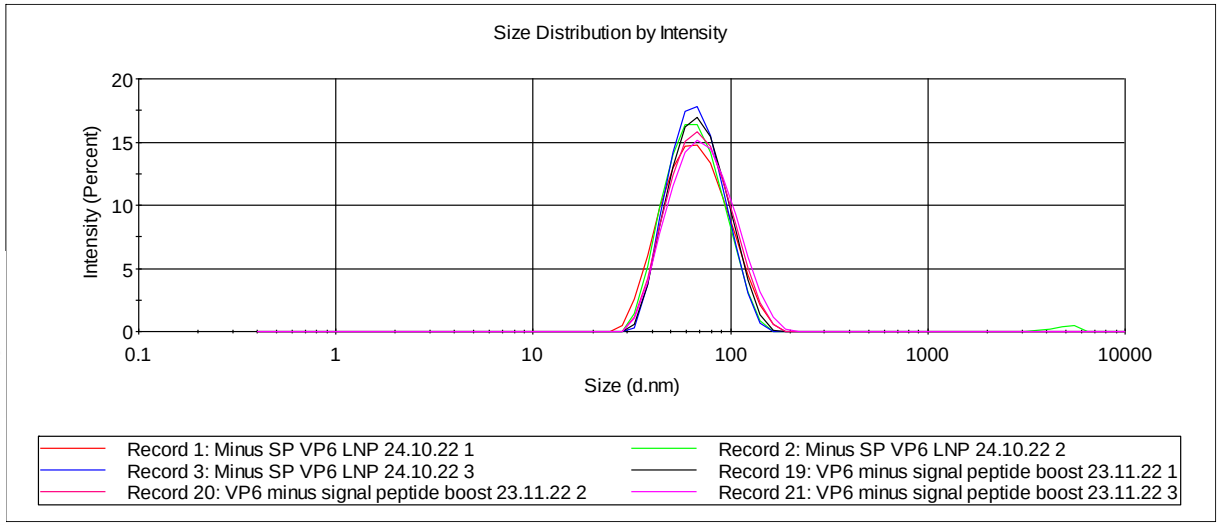


saRNA-VP6 expression confirmed in HEK293T on a Reduced western blot



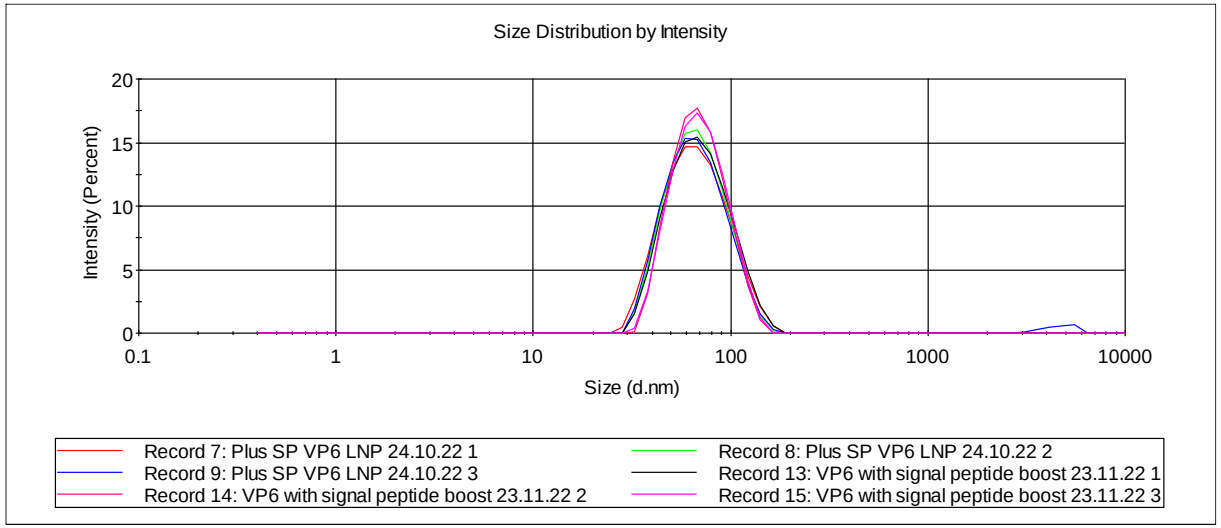
DLS to check LNP size





Prime:
Z-Ave: 63.81 (3 x read ave)
PdI: 0.135 (3 x read ave)

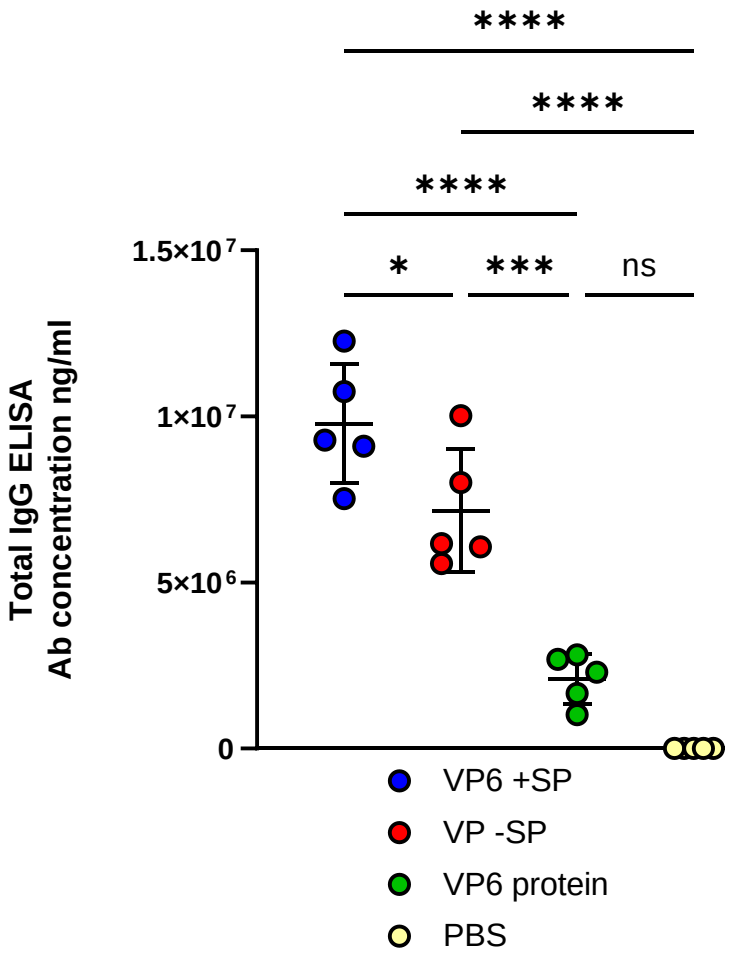
Boost:
Z-Ave: 66.34 (3 x read ave)
PdI: 0.121 (3 x read ave)

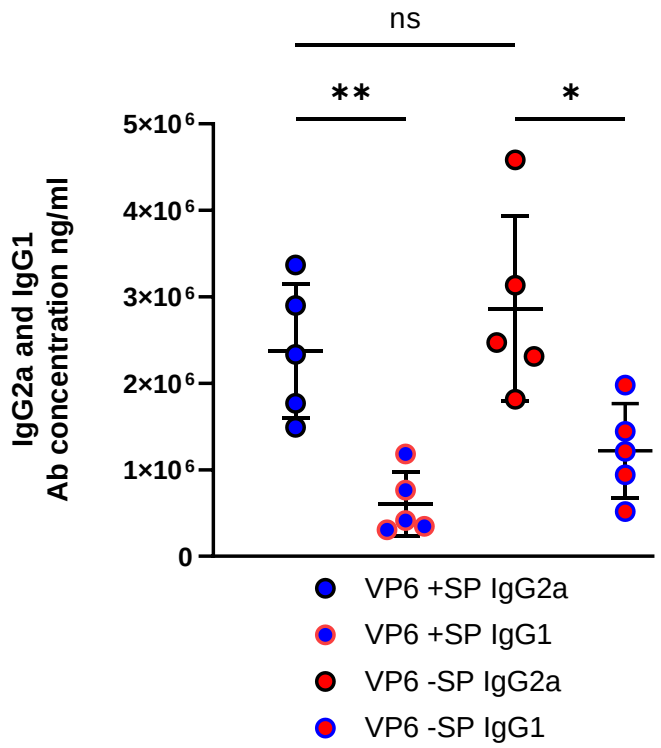


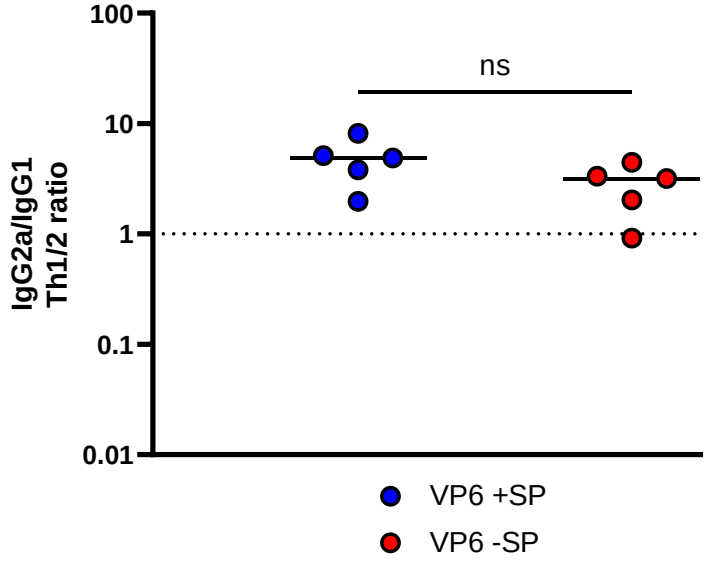
Prime:
Z-Ave: 63.35 (3 x read ave)
PdI: 0.142 (3 x read ave)

Boost:
Z-Ave: 65.22 (3 x read ave)
PdI: 0.104 (3 x read ave)

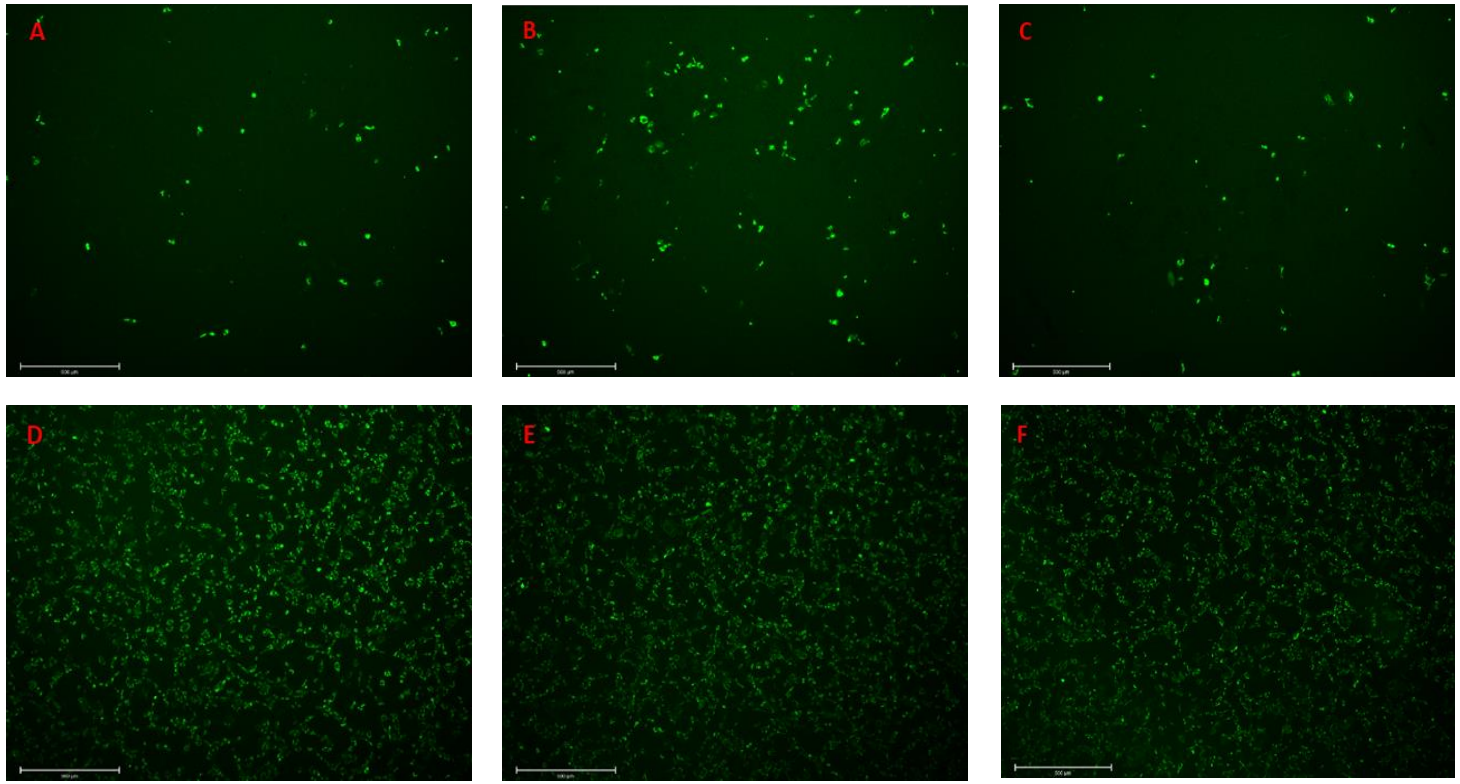
Rotavirus VP6 specific IgG response, 3 weeks post boost



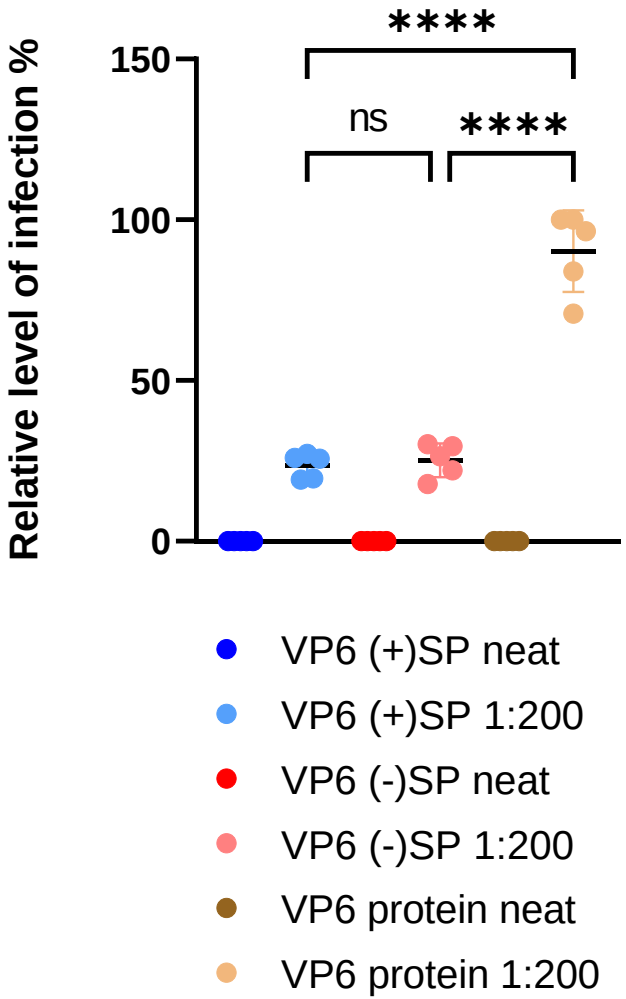




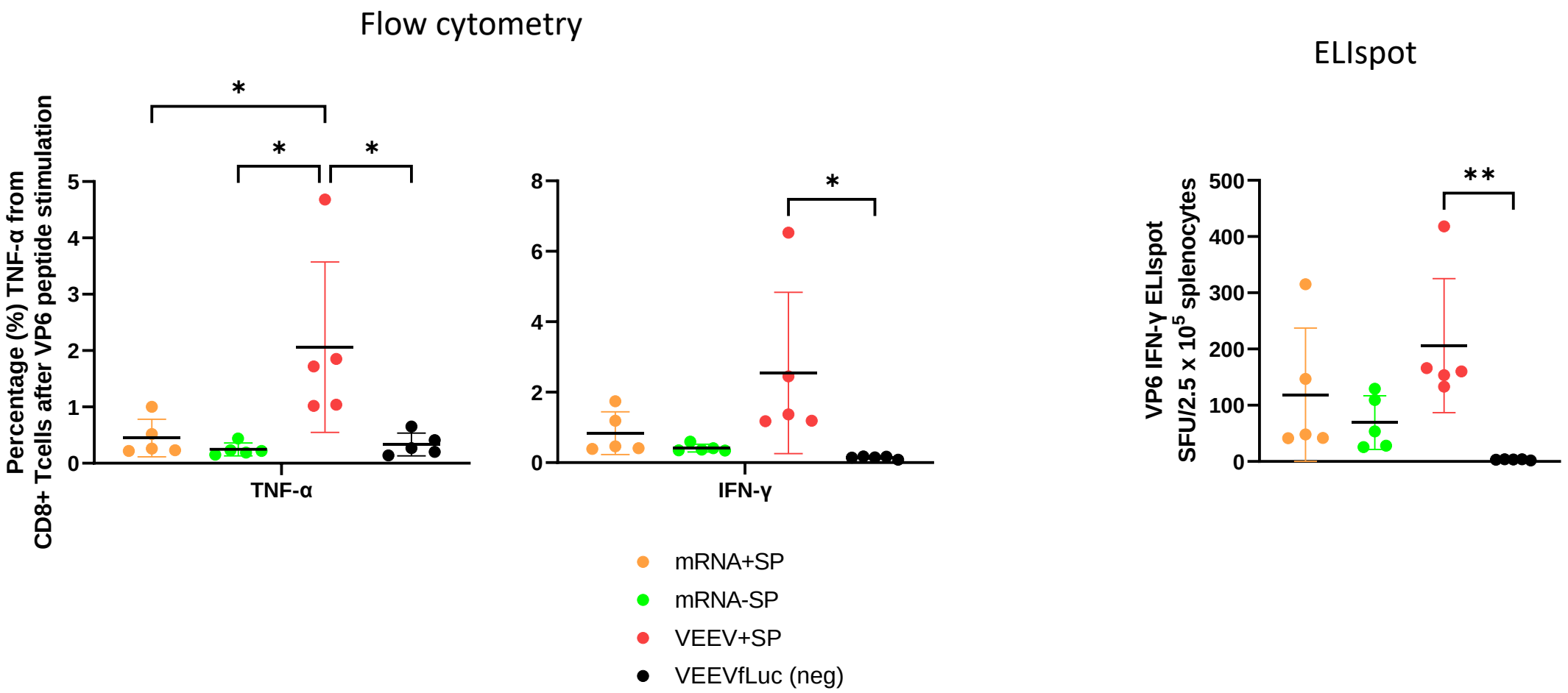
TRIM-21 mediated Intracellular neutralisation



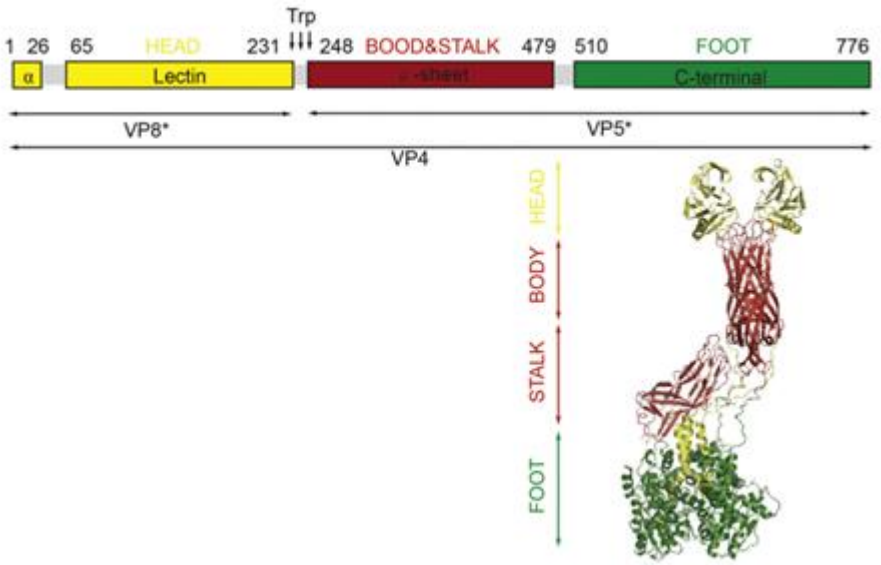
A. VP6 (+)SP
B. VP6 (-)SP
C. VP6 recombinant protein
D. PBS
E. Cell (+) electroporation
F. Cell (-) electroporation

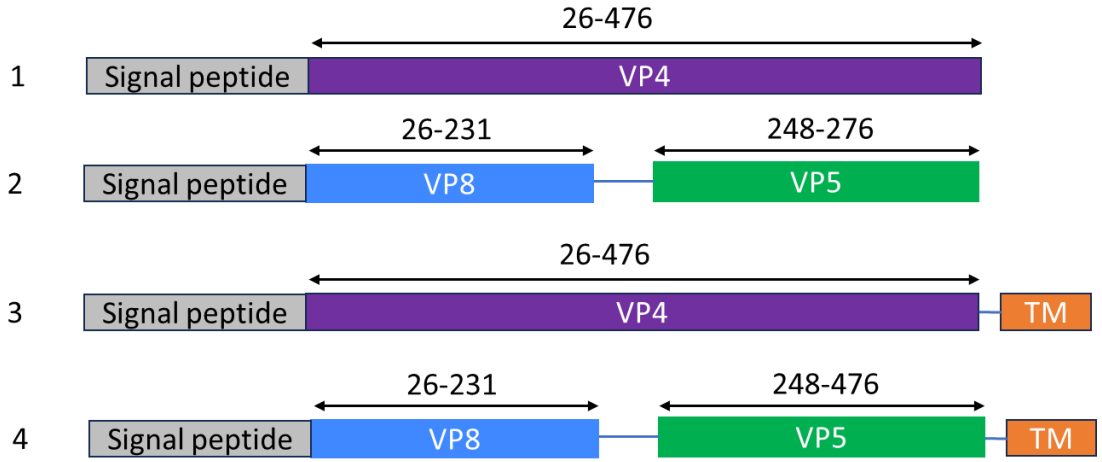


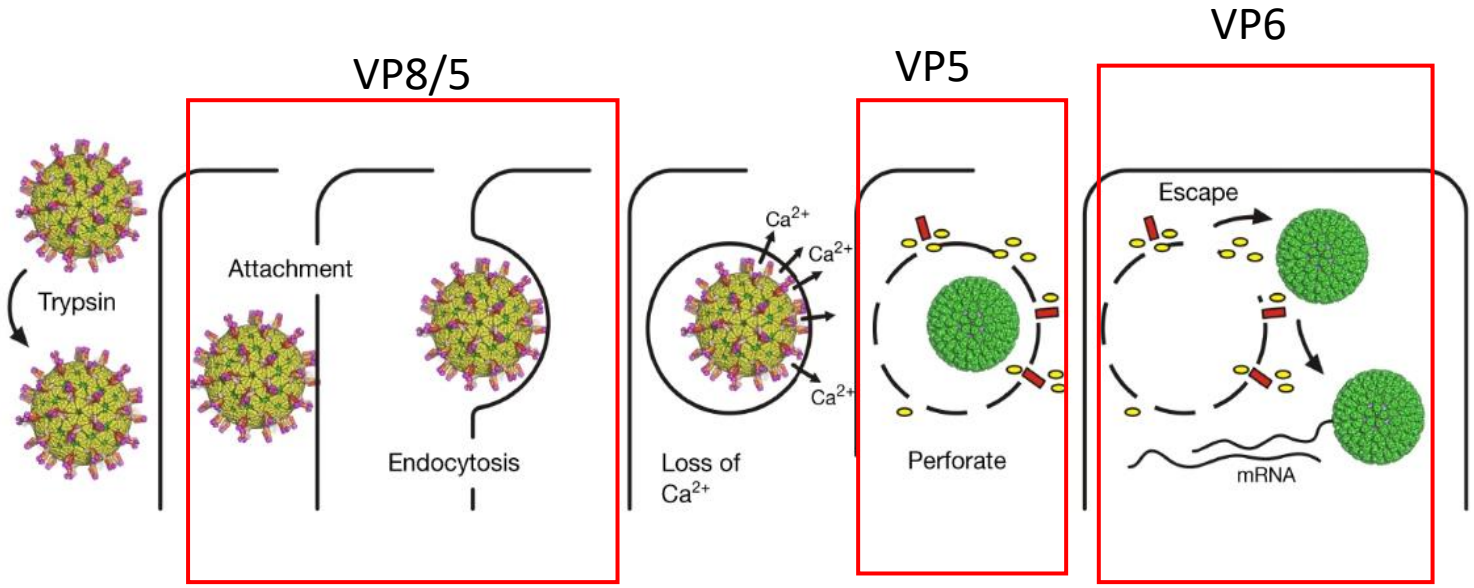
Cellular response from mouse immunogenicity study comparing mRNA with VEEV-saRNA encoding VP6



VP4/6 combination to increase the breadth of protection



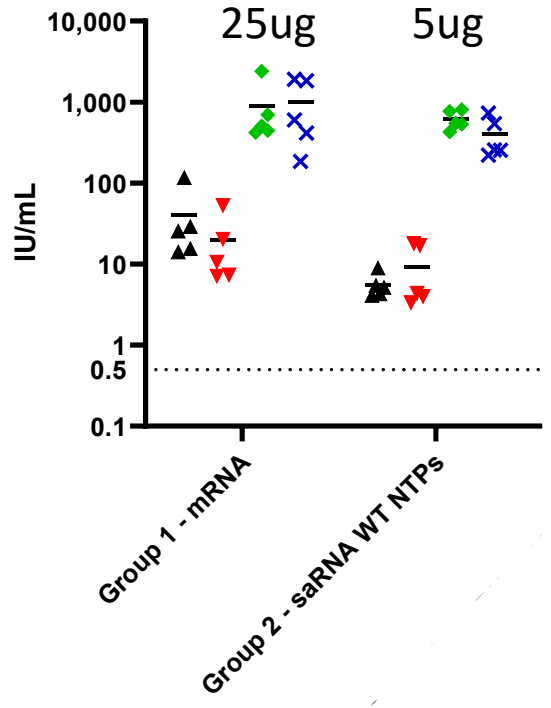




Next step
Pig challenge studies

VP6 vs VP4+6

Preclinical data on rabies saRNA in pigs (Leap)



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centillion
RNA by Design

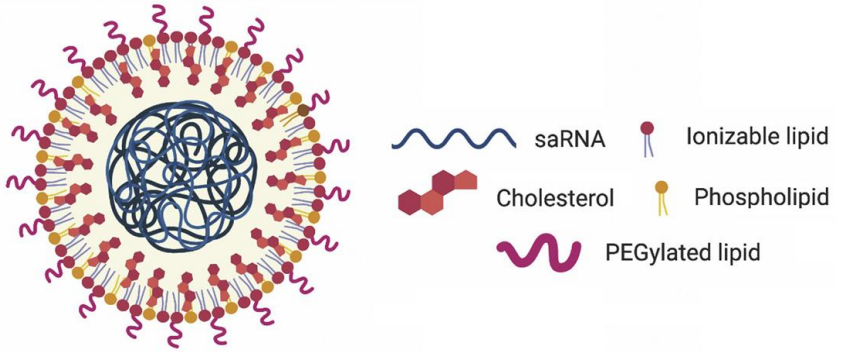
Continuous manufacturing (Flow) able to make up to 50g/day

leap^w

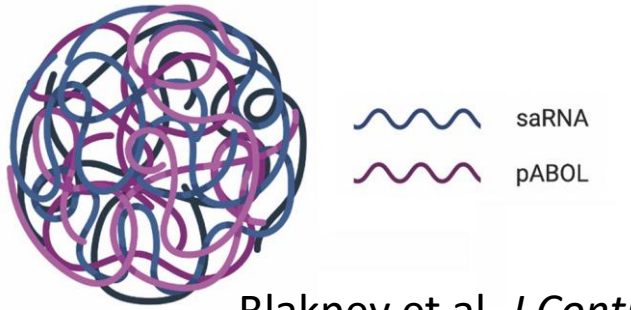
R3 RNA READINESS + RESPONSE

State of the (delivery) art

- Lipid nanoparticles (LNP)
  - ~60-150 nm diameter
  - Slightly negative zeta potential
  - moderate local and distal transfection via IM, ID, SC, IV
  - Possible to lyophilize
  - Inherently immunogenic



- Polyplexes (PP)
  - ~60-150 nm diameter
  - Positive zeta potential
  - Potent local transfection via IM and ID
  - Easy to lyophilize
  - Less-immunogenic

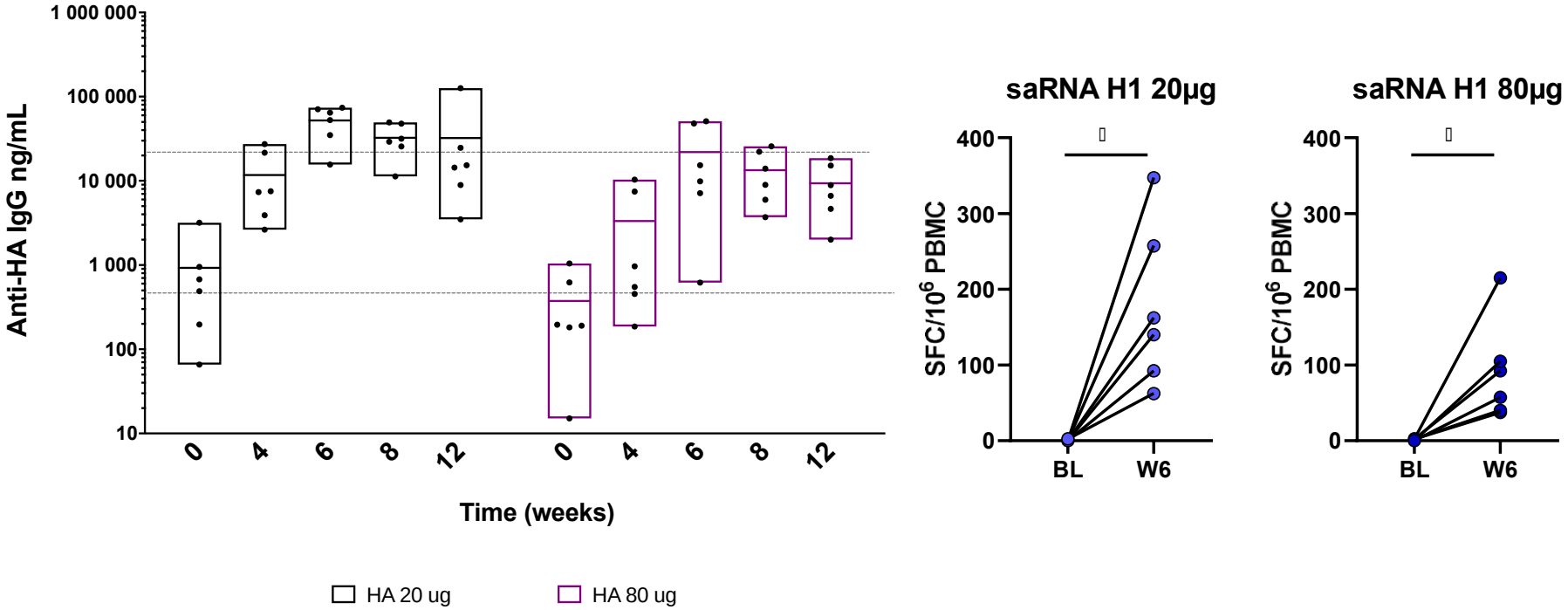


Blakney et al, *J Control Rel* 2021

Lyophilization - Paul.Matejtschuk@mhra.gov.uk

Polyplex scale up - incepta

Do polyplex (pABOL) formulations induce HA-specific antibodies and cellular responses in NHPs?



Summary

- mRNA and saRNA provide different properties and have potential to be used in prime boost combinations
- Low dose vaccines can reduce cost of goods and facilitate combinations
- Early evidence indicate saRNA may provide a platform for a rotavirus vaccine used alone or as a potential augmentation to live attenuated vaccines
- Novel formulations may provide opportunities for cost reduction and simplification of supply chains
- Cost of goods remain the biggest challenge for global adoption of RNA technology

Acknowledgements

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incepta

Future Vaccine Research Hub

EPSRC
Engineering and Physical Sciences Research Council

Department of Health & Social Care

UKaid
from the British people

leap

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CEPI