



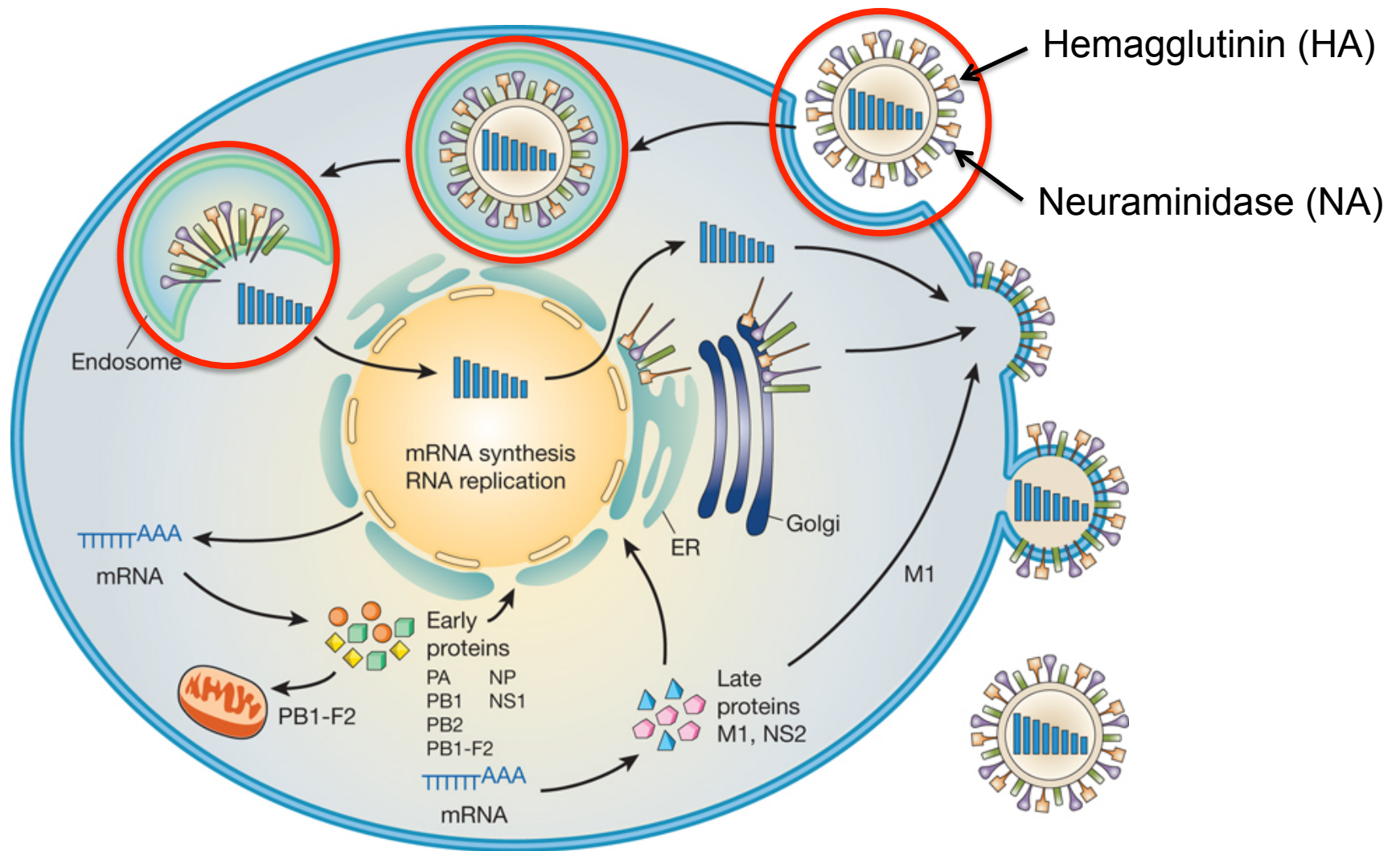
Uncovering host factors for Influenza Virus entry.

Nathan Kipniss

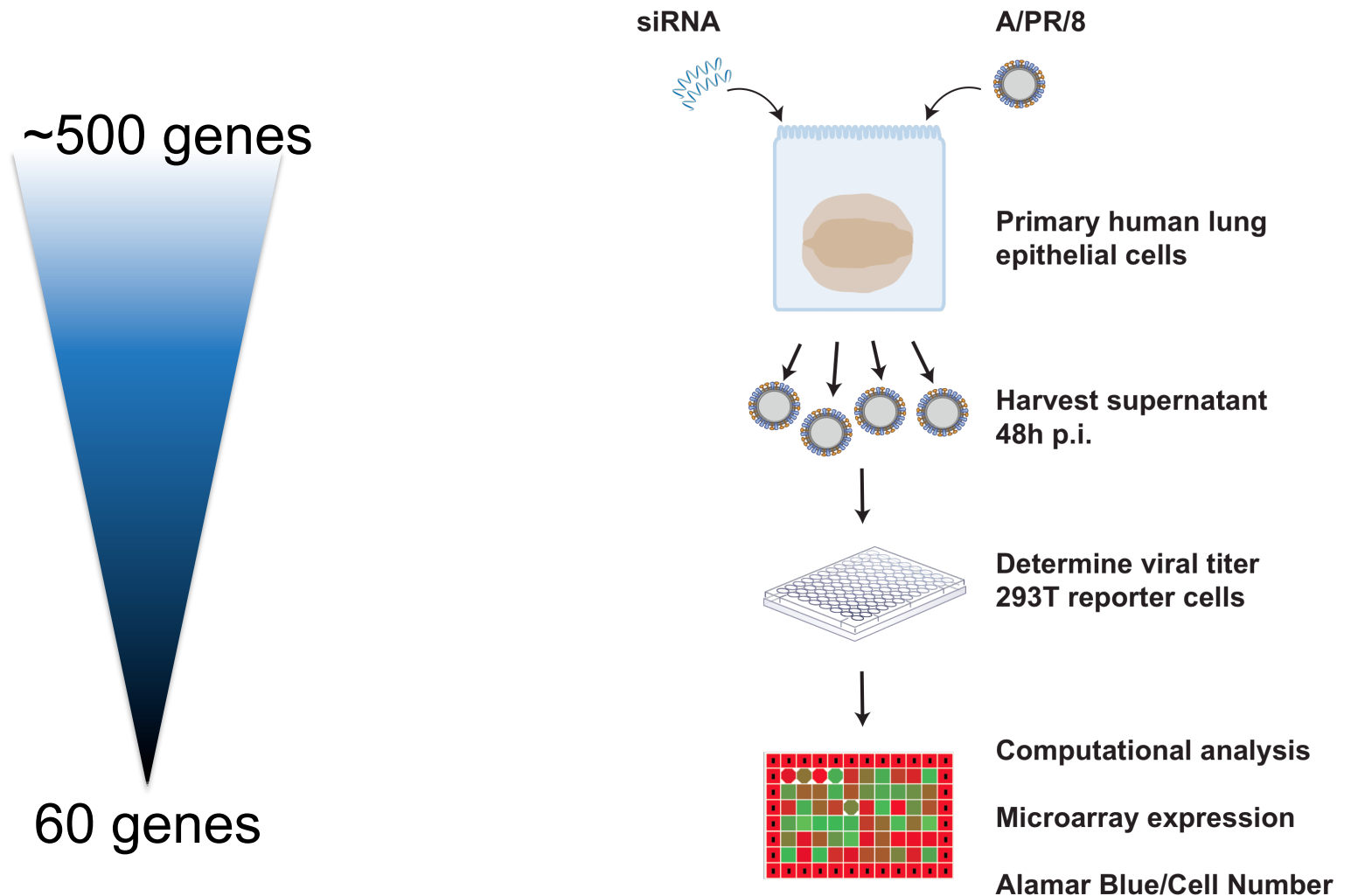
Mentor: Sagi Shapira, PhD

PI: Nir Hacoheh, PhD

Influenza A viral lifecycle



Previous screen identified host factors that regulate influenza trafficking



Hermansky-Pudlak Syndrome



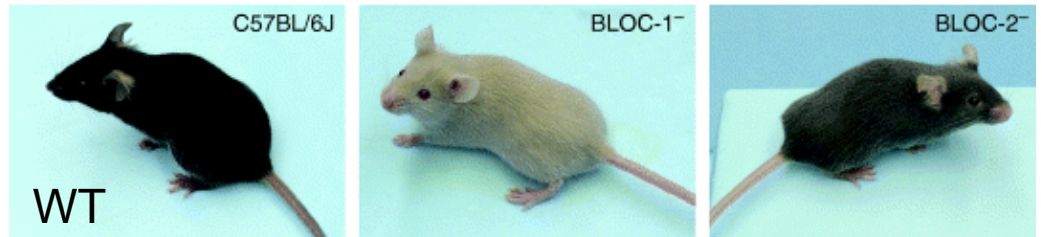
Caused by mutations in genes involved in the Biogenesis of Lysosomal Organelles (BLOC).

Phenotypes

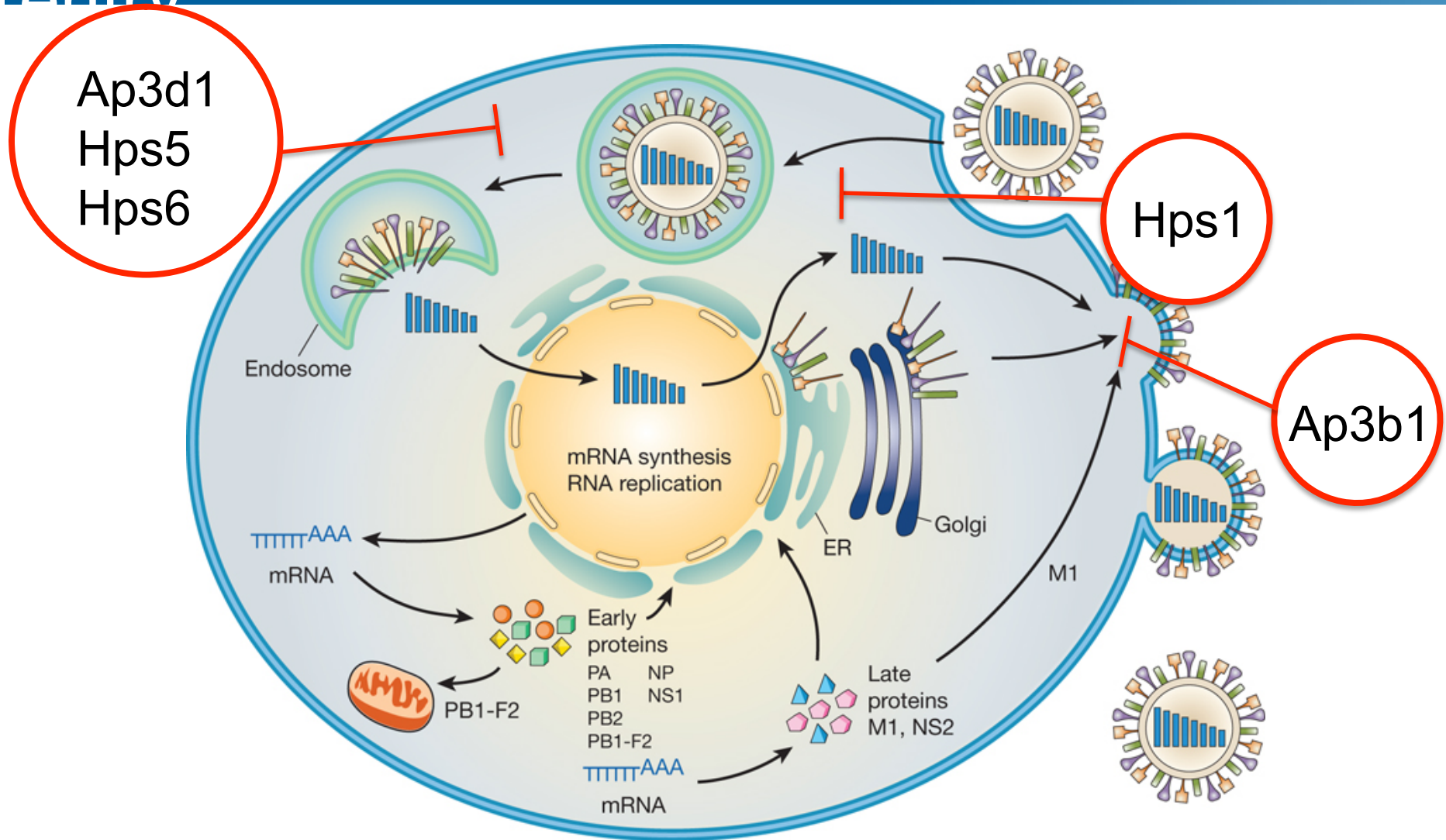
-Albinism

-Immunodeficiency

-Increased rates of viral infections



Genes involved in BLOC complexes identified as restriction factors in influenza entry



Do HPS genes play a role in entry of viruses that utilize other entry pathways?



Virus	Endosomal pH*	Transferrin Receptor 1	α -dystroglycan	Sialic acid	Clathrin dependent	Caveolin dependent	Macropinocytosis	Independent
Influenza	5.3			✓	✓	✓	✓	✓
MACV	<5.5	✓						✓
LASV	4.5		✓					✓
LCMV	5.3-5.5		✓					✓

Key: MACV- Machupo Virus

LASV- Lassa Virus

LCMV- Lymphocytic

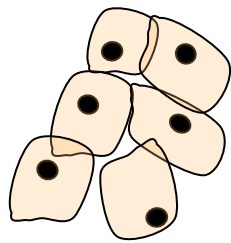
Choriomeningitis Virus

Receptor

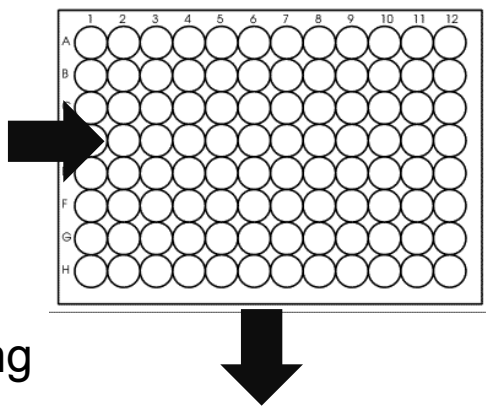
Methods of Endocytosis

* pH required for membrane fusion and viral uncoating.

Pseudovirus Infection Assay



1) A549 human lung epithelial cells are seeded in a 96 well plate.

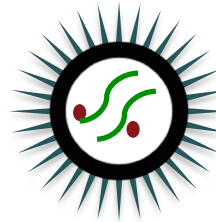


2) siRNA transfection targeting HPS genes.

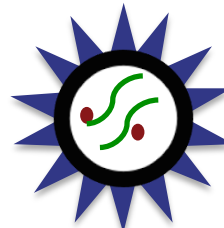
3) Pseudovirus infection 72 hrs. post transfection.



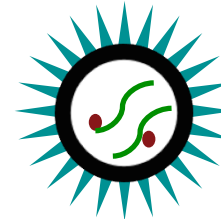
Thai/H5N1



MACV



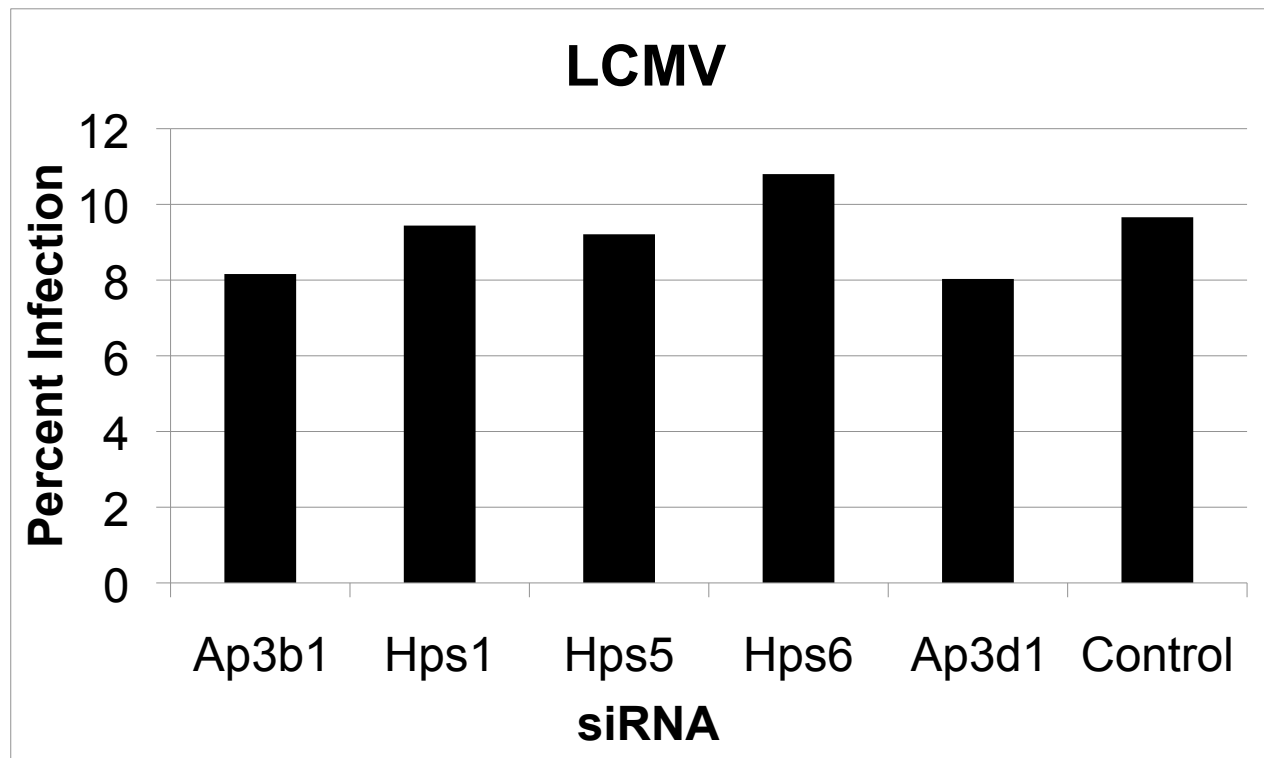
LCMV



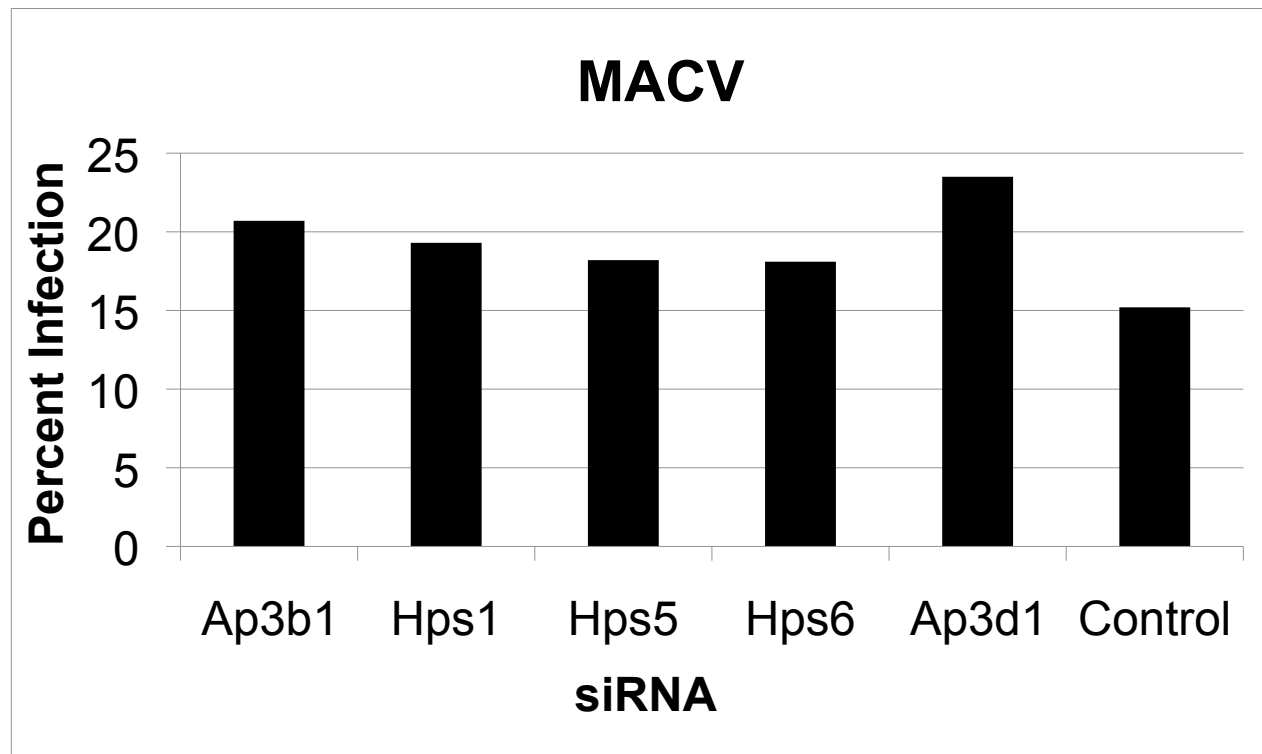
LASV

4) 48 hrs. post infection, harvest and fix cells. Analyze GFP expression using flow cytometry.

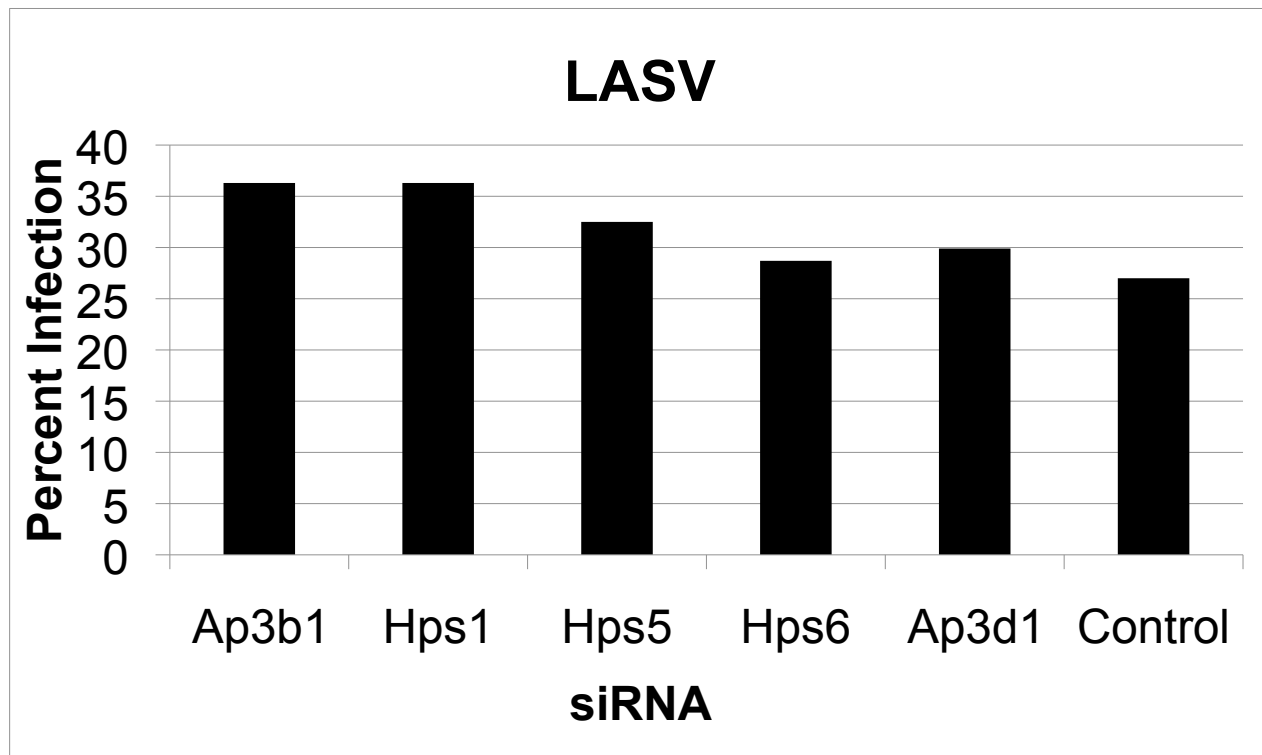
Pseudovirus assay reveals that the HPS genes play no role in LCMV entry.



Pseudovirus assay reveals that the HPS genes play no role in MACV entry.



Pseudovirus assay reveals that the HPS genes play no role in LASV entry.



Summary



- No change in phenotype among arenaviruses.
- Results confirm previous work.
- Eliminating different entry pathways suggests a unique role of HPS genes.

Future Work



- Repeat experiment
- qPCR
- Screen original list of 60 genes
- Examine different viruses
- Tag and isolate endosomes

Acknowledgements



Nir Hacohen

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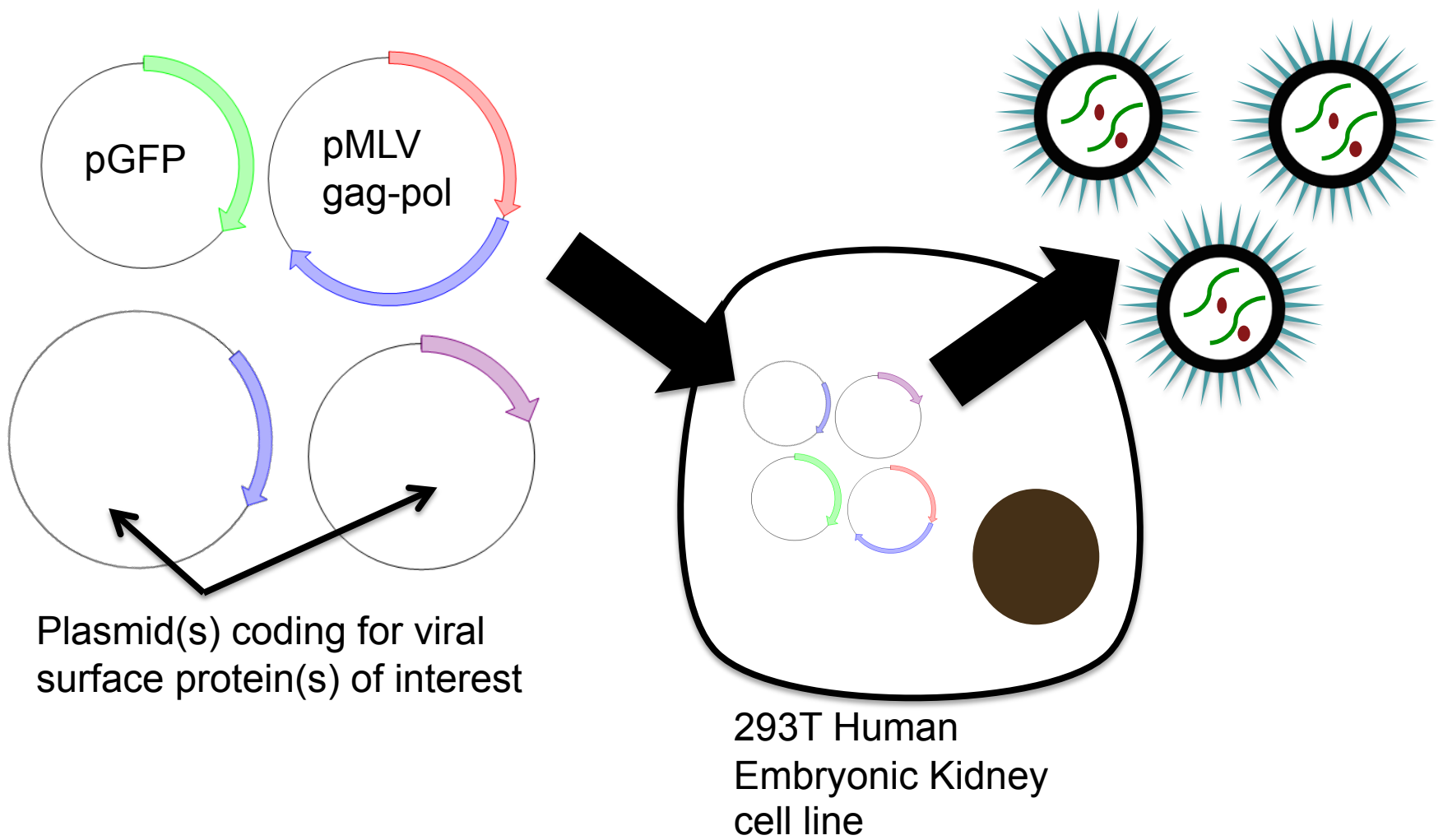
The Broad Institute Summer Research Program in Genomics

Lori Thomae

The Hacohen Laboratory

The Broad Institute

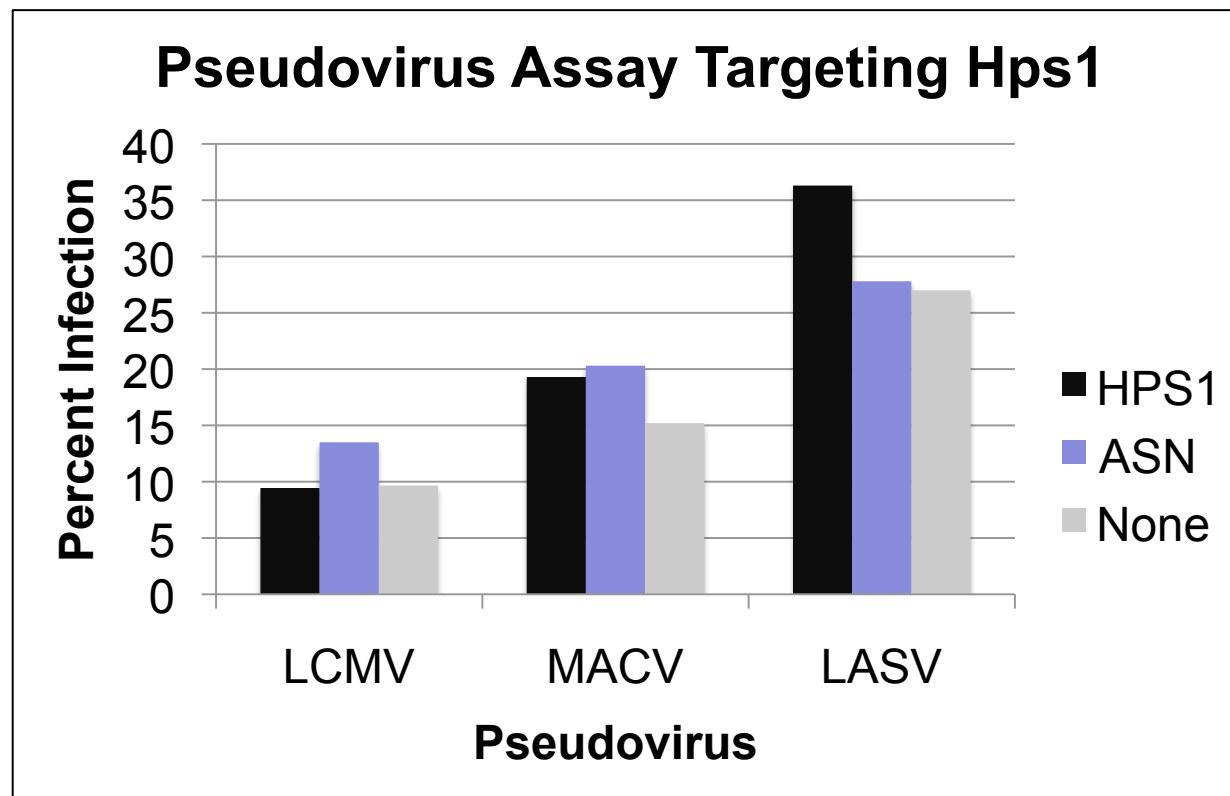
Pseudovirus Production



Plasmid(s) coding for viral surface protein(s) of interest

293T Human Embryonic Kidney cell line

Pseudovirus assay reveals that the HPS genes play no role in Arenavirus entry



Pseudovirus assay reveals that the HPS genes play no role in

