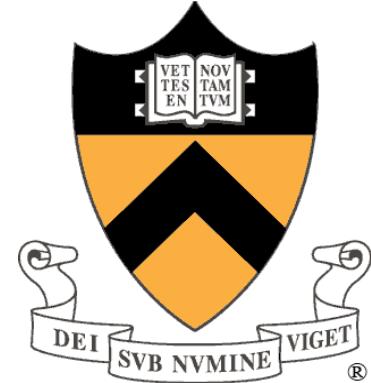


Complex of Three PRV Envelope Proteins Mediates Anterograde Transport and Spread

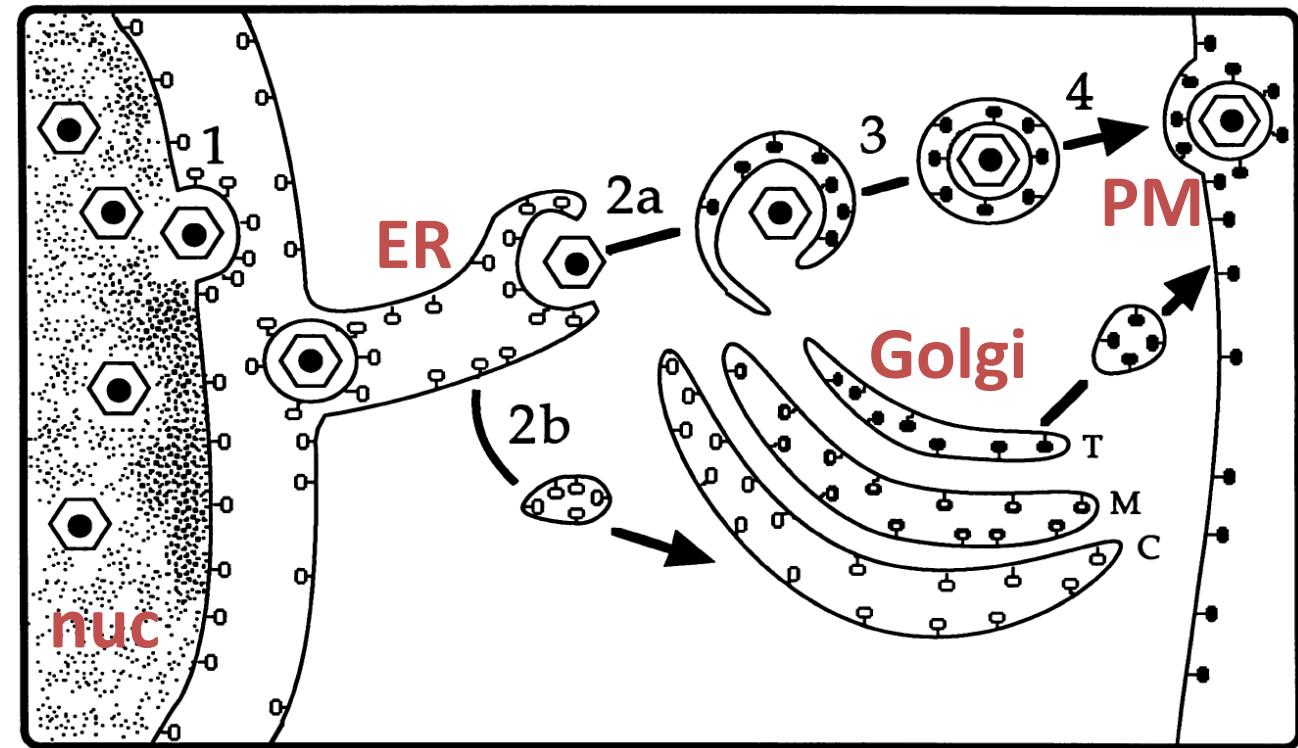
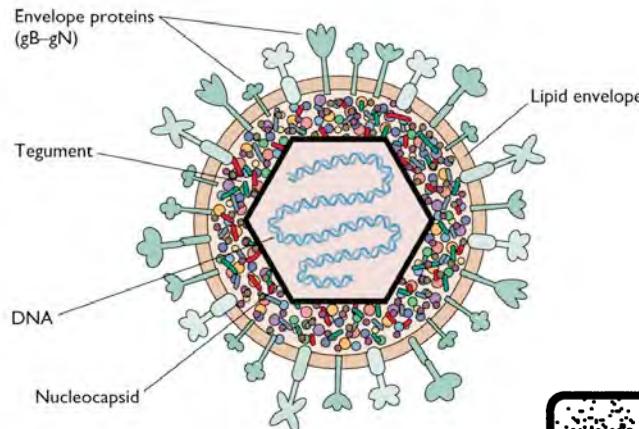


Julian Scherer, PhD

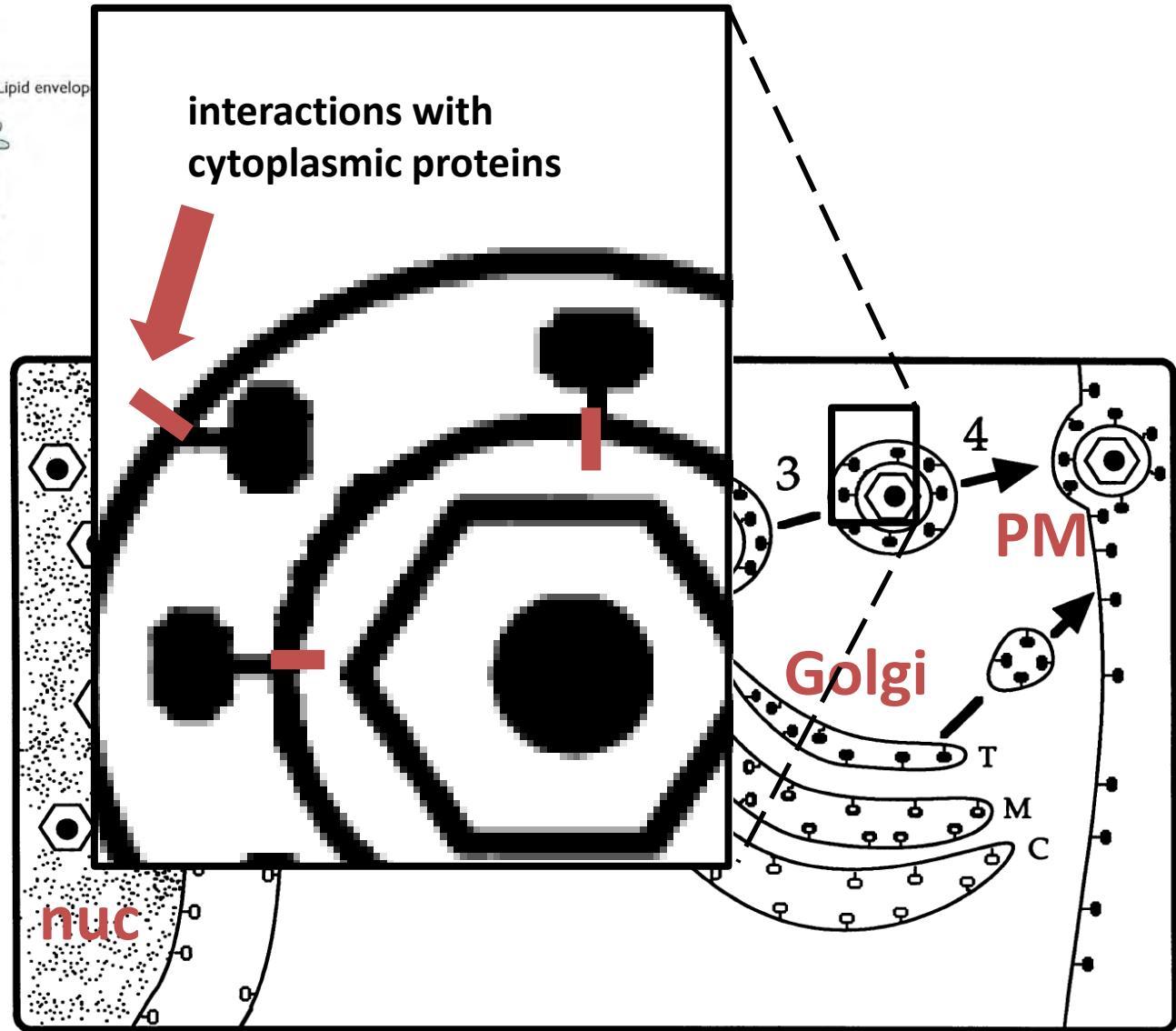
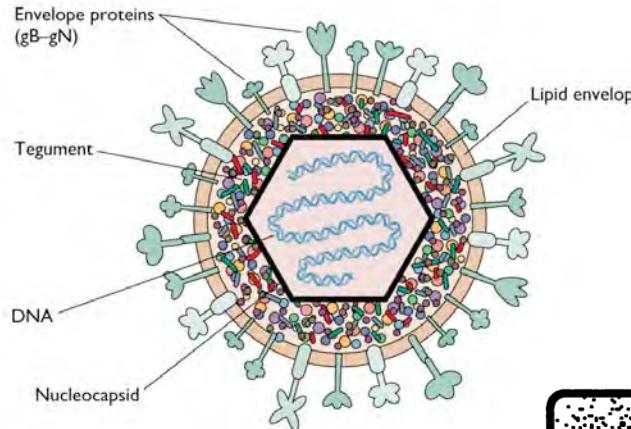
Lynn W. Enquist Lab
Princeton University

Dept. of Molecular Biology & Princeton Neuroscience Institute

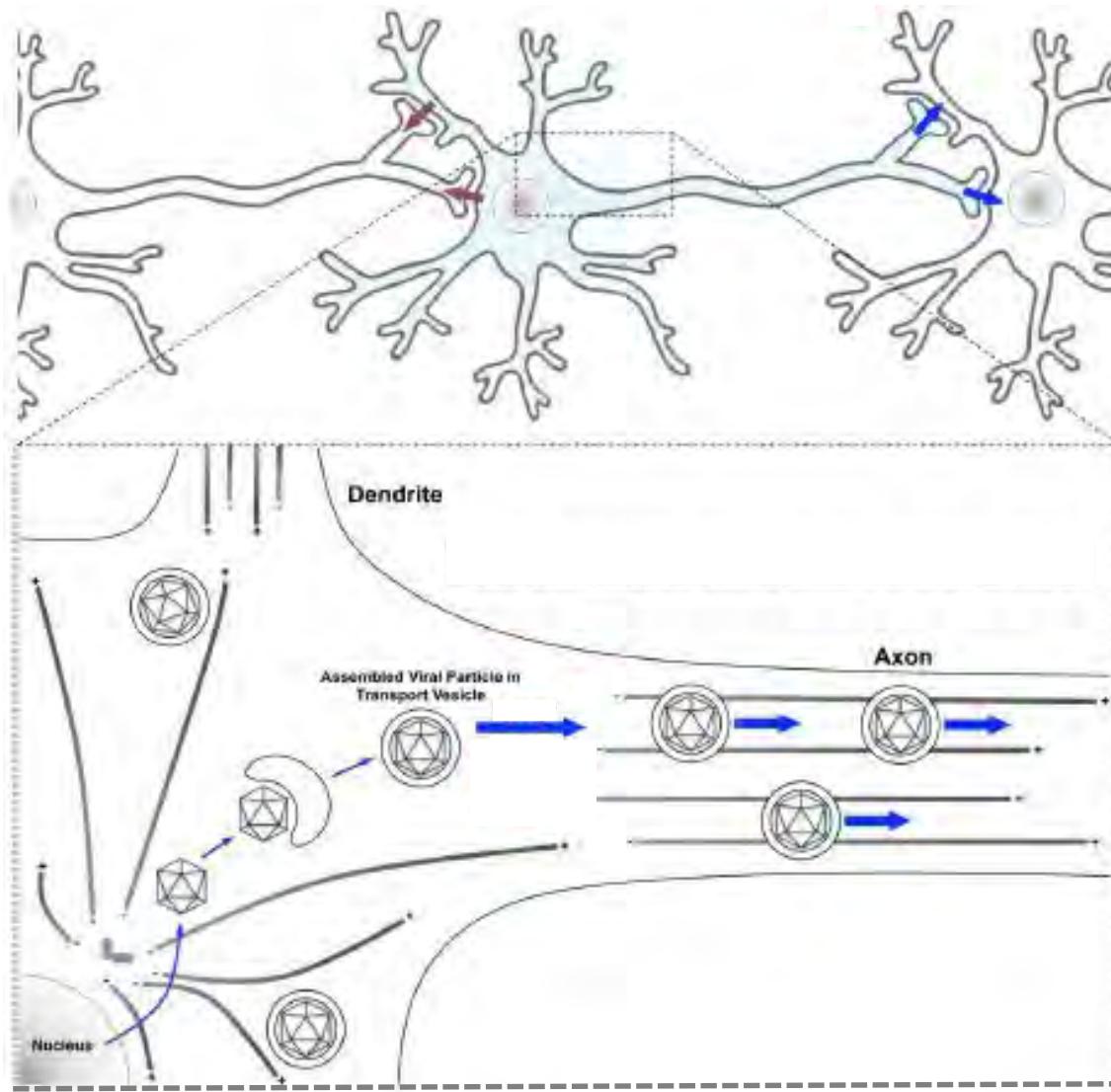
Herpesvirus Egress



Herpesvirus Egress

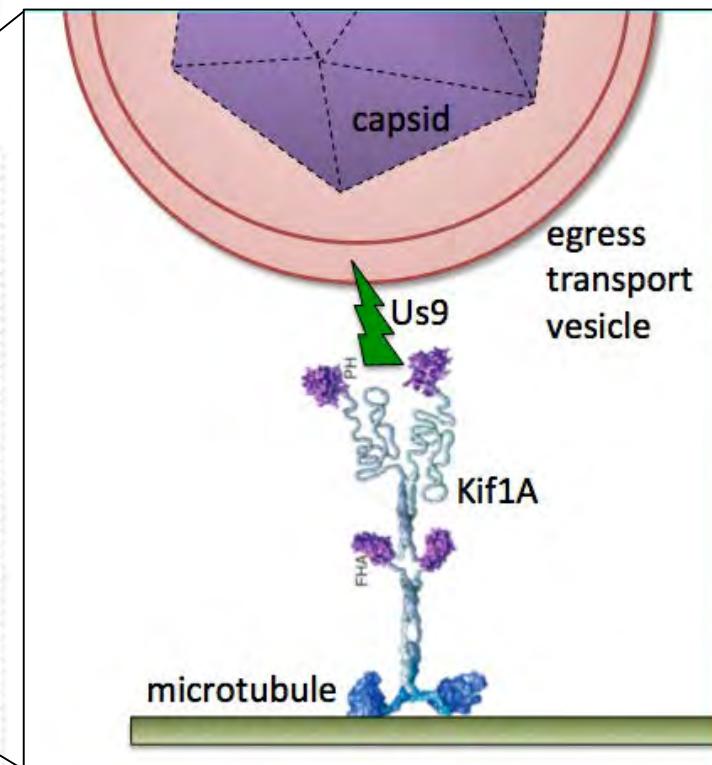
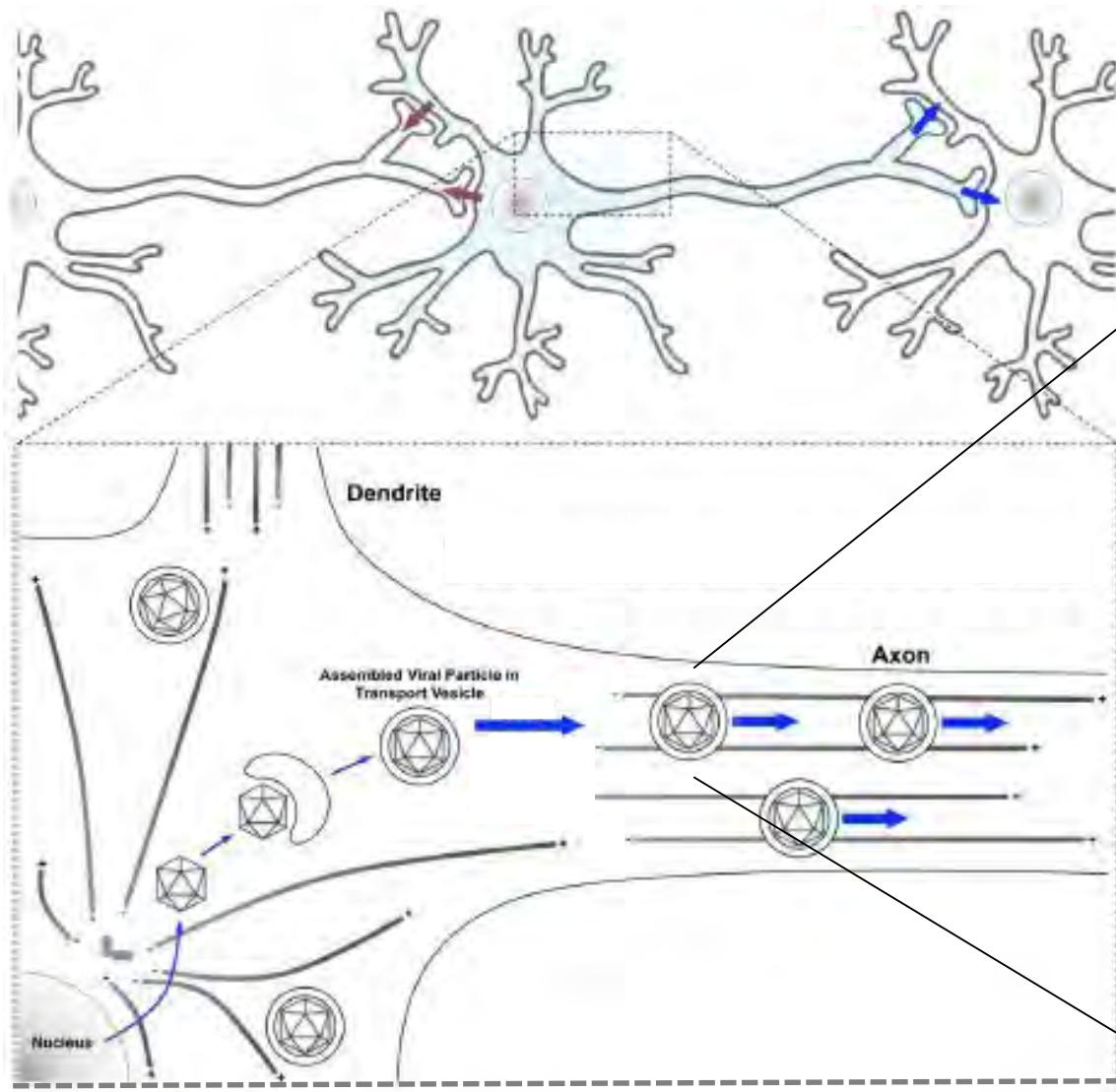


Neuronal Herpesvirus Egress



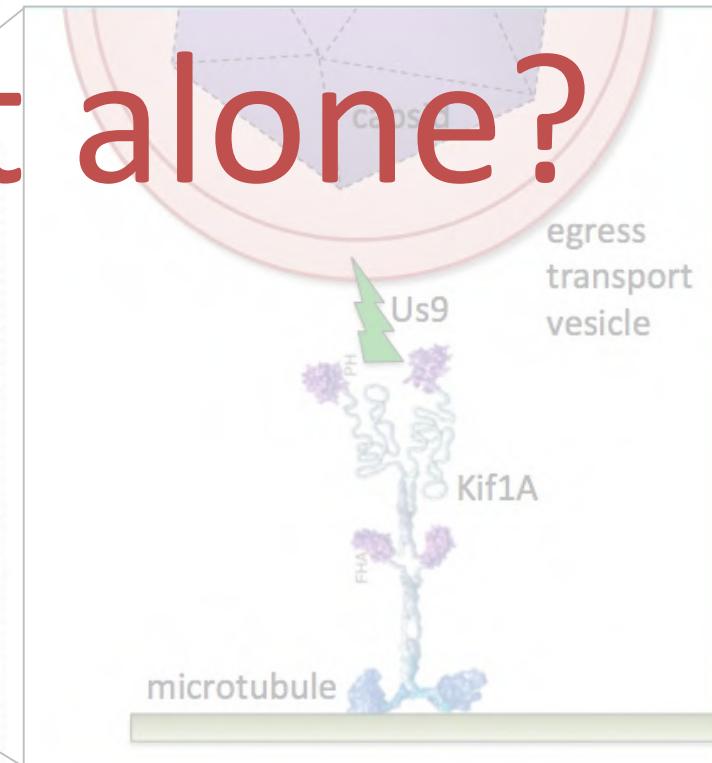
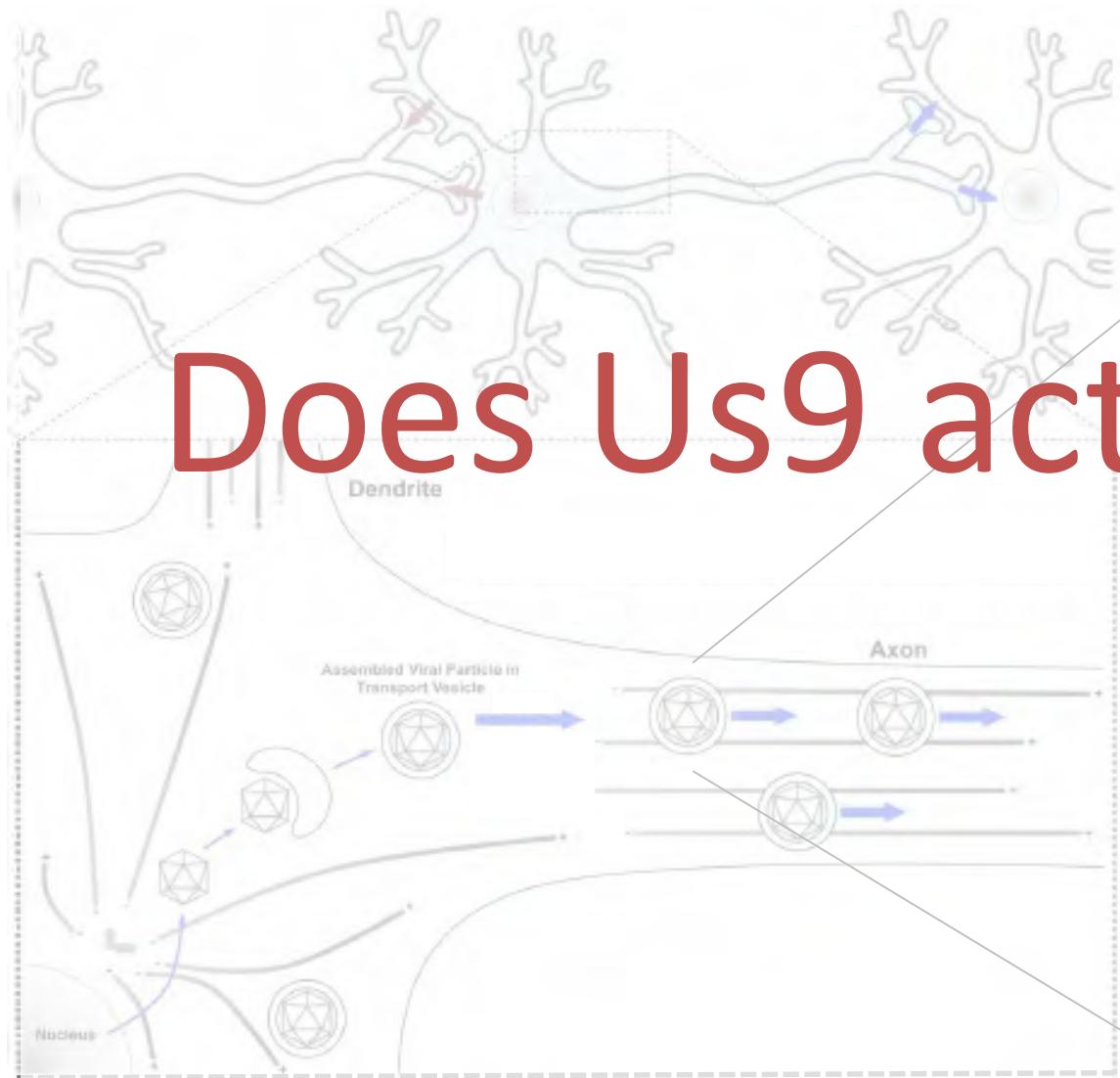
Kramer & Enquist, *Viruses*, 2013

Neuronal Herpesvirus Egress

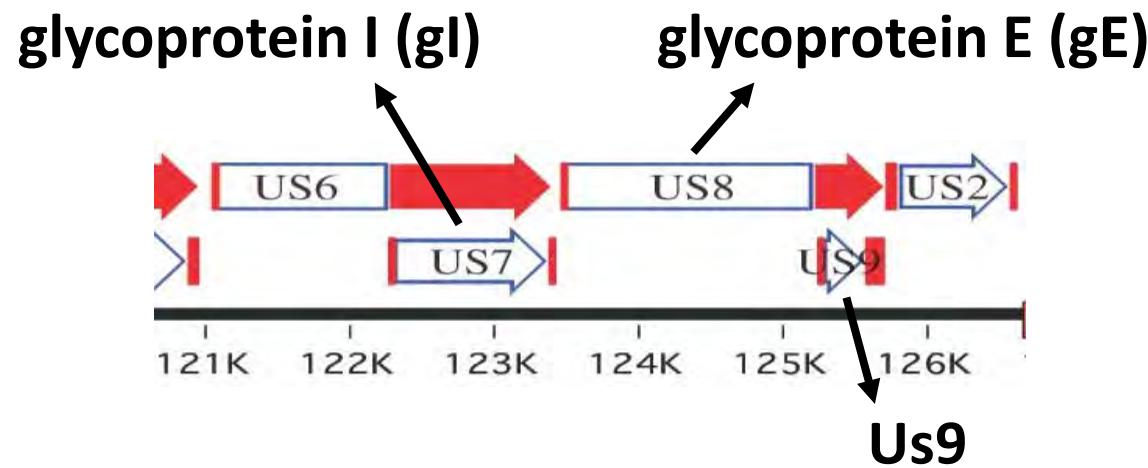




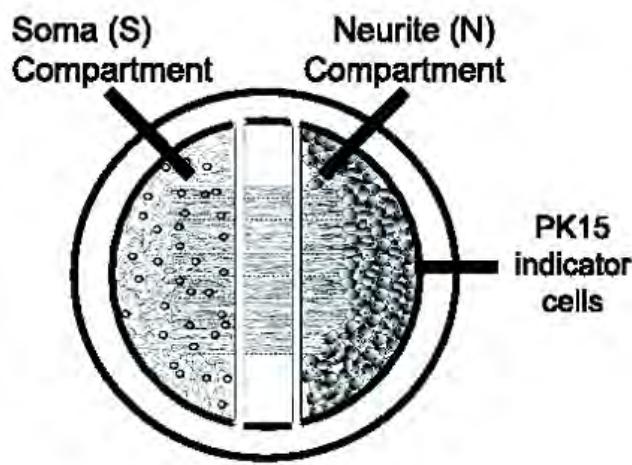
Does Us9 act alone?



Us9 Gene in PRV Genome



Anterograde spread



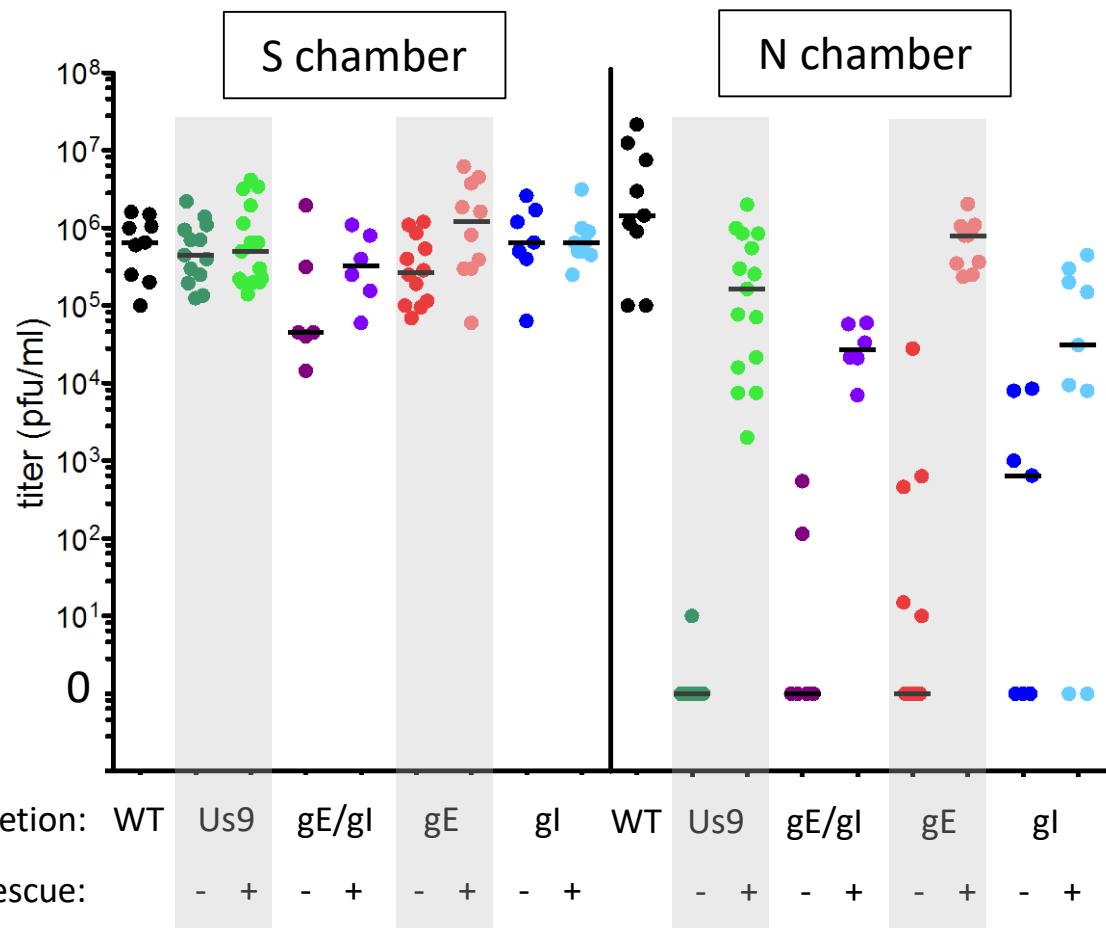
day -14: seed primary SCG neuron

day -3: AdV transduction

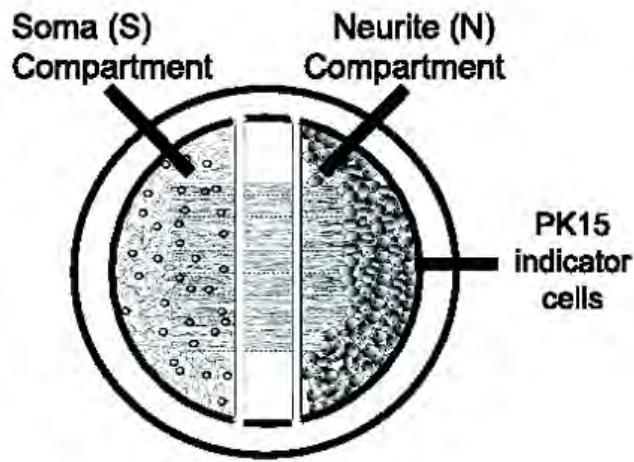
day -1: seed PK15 cells + add methocel

day 0: infect with PRV in S chamber (10MOI)

24hpi: harvest S & N chamber for titering



Anterograde spread



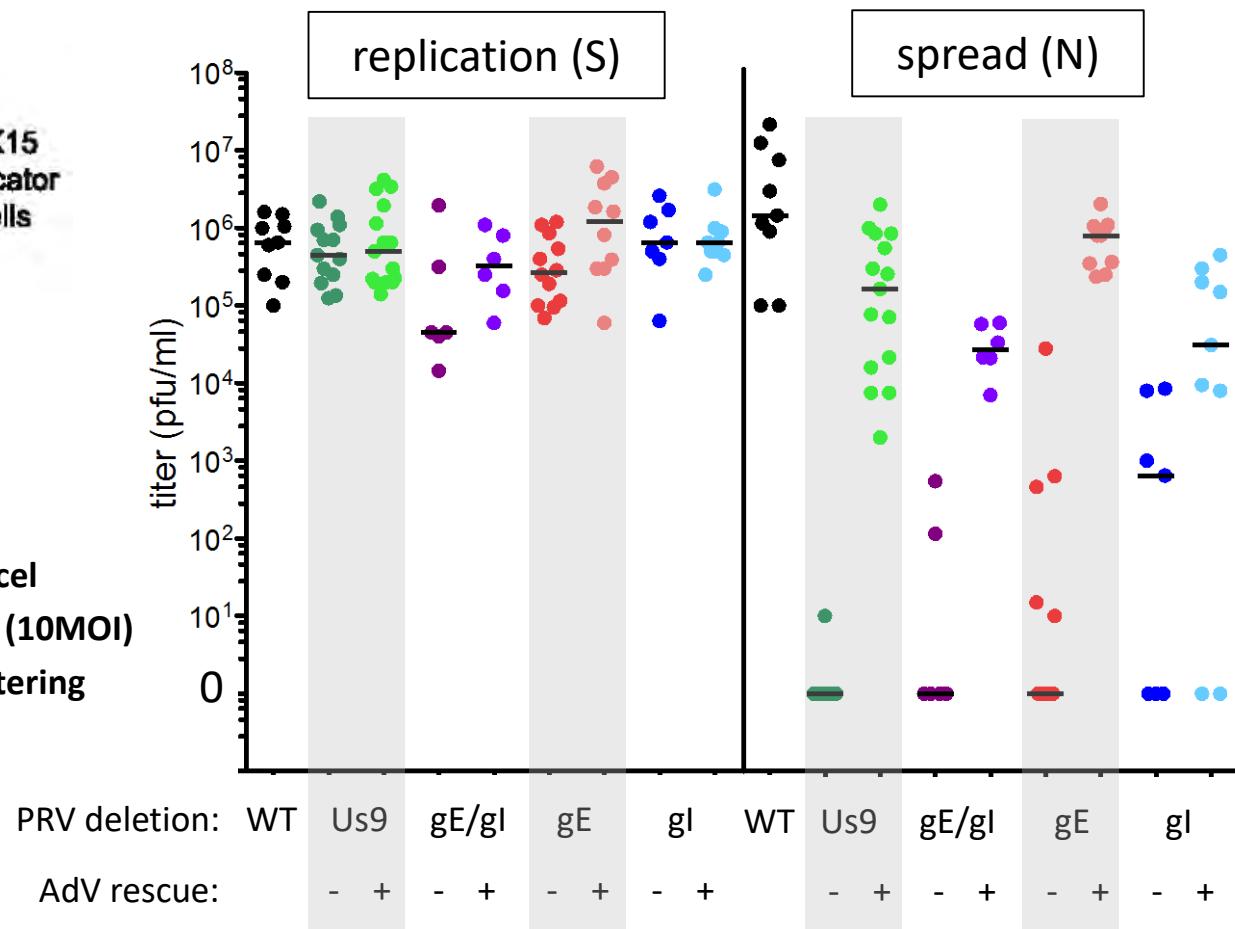
day -14: seed primary SCG neurons

day -3: AdV transduction

day -1: seed PK15 cells + add methocel

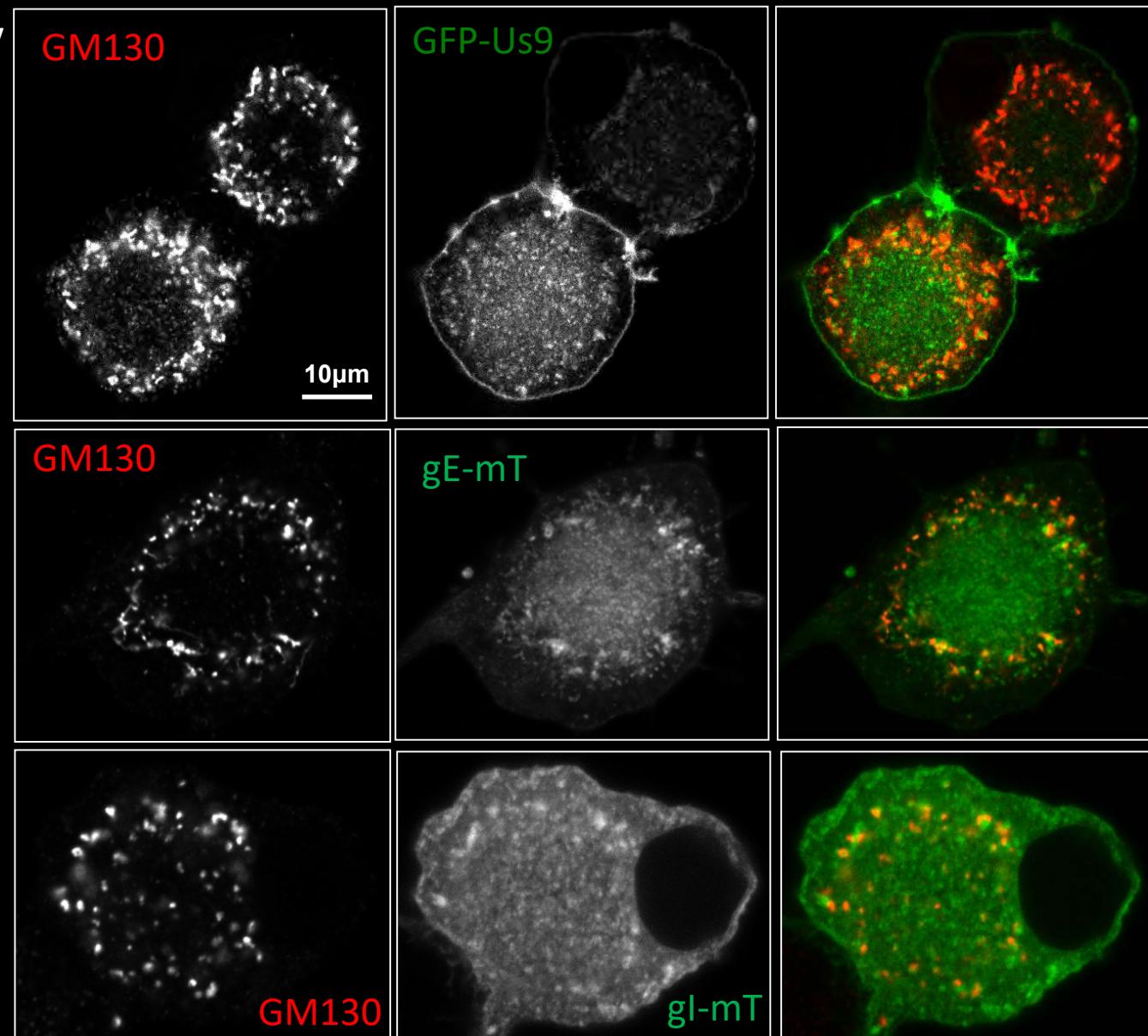
day 0: infect with PRV in S chamber (10MOI)

24hpi: harvest S & N chamber for titering



Soma Localization

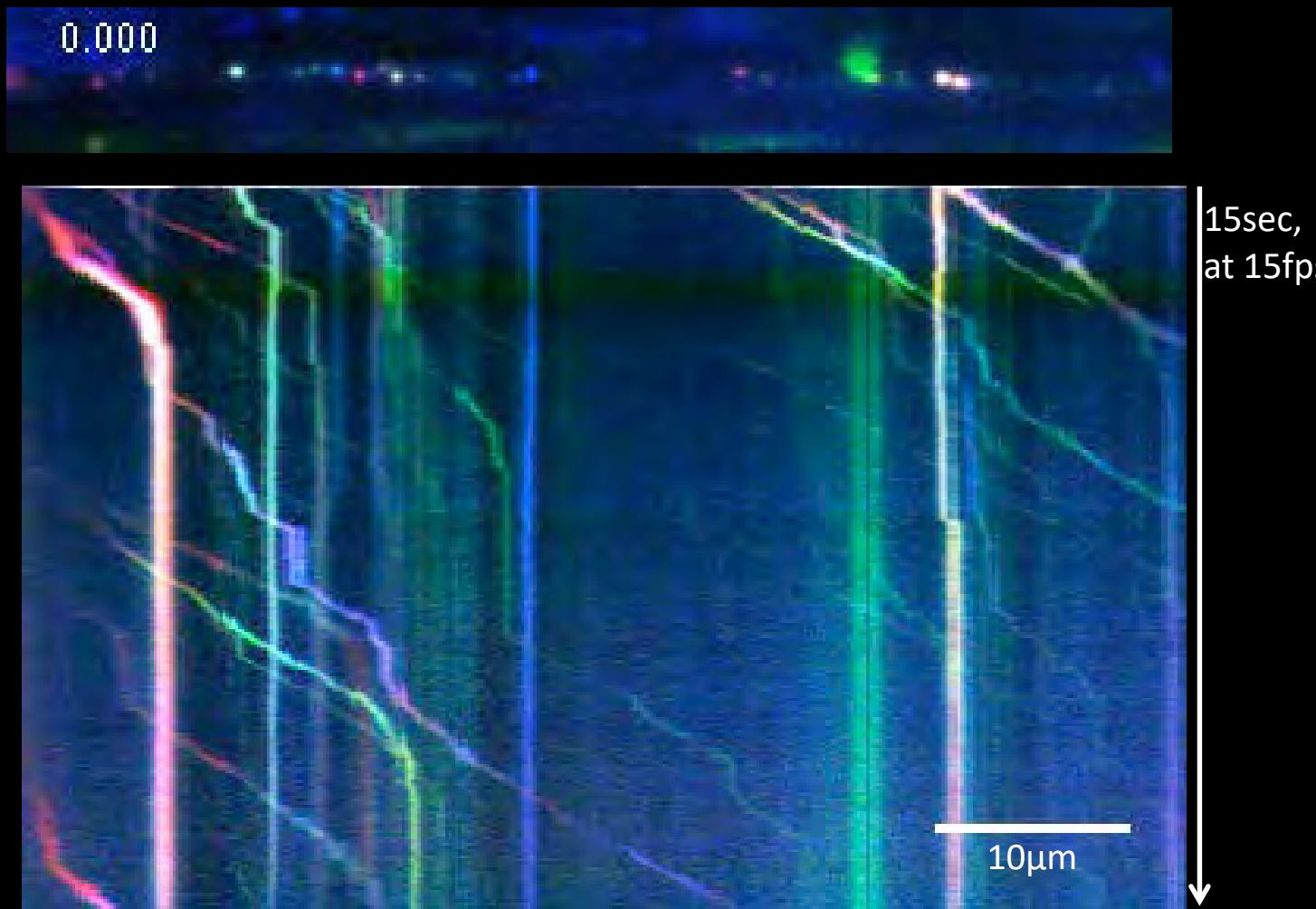
SCG neurons (dissociated), 14DIV
AdV transduction (2days)
confocal imaging of cell soma



Staining and IFM:
Shree Tanneti

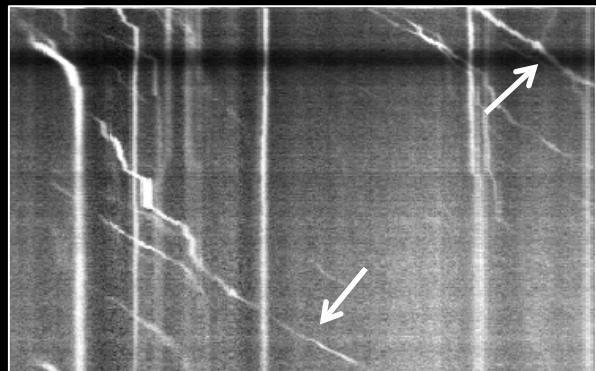
Axonal Co-transport

AdV-Us7-mTurquoise2
AdV-Us8-mCherry
AdV-GFP-Us9

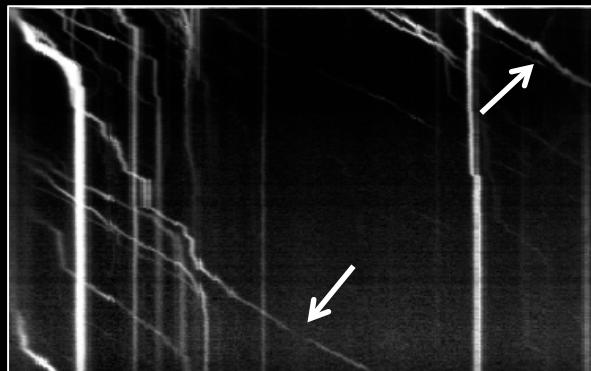


Axonal Co-transport

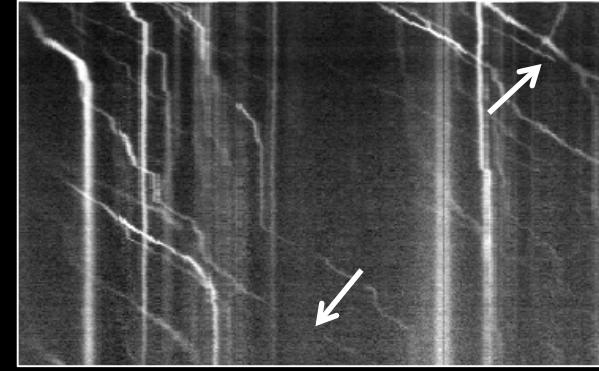
AdV-Us7-mTurquoise2



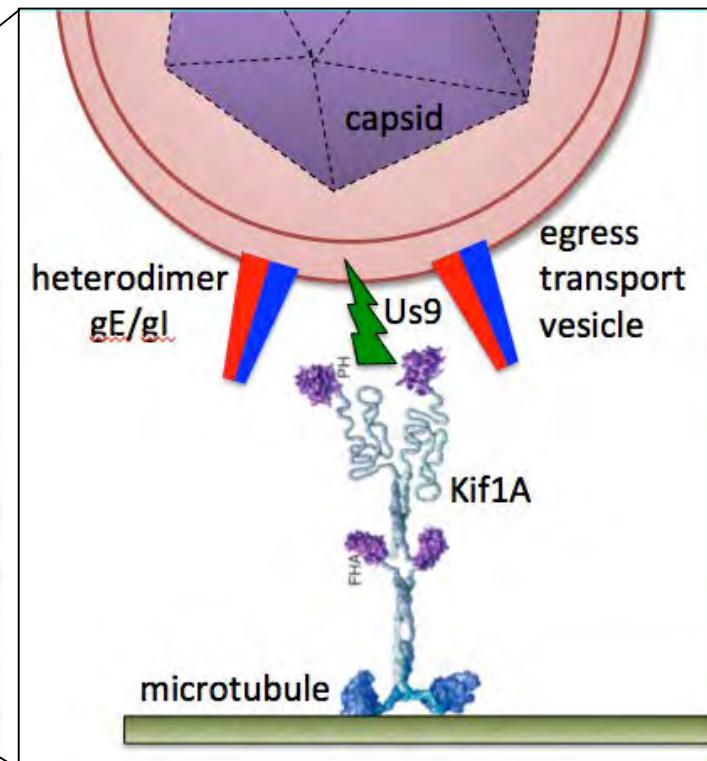
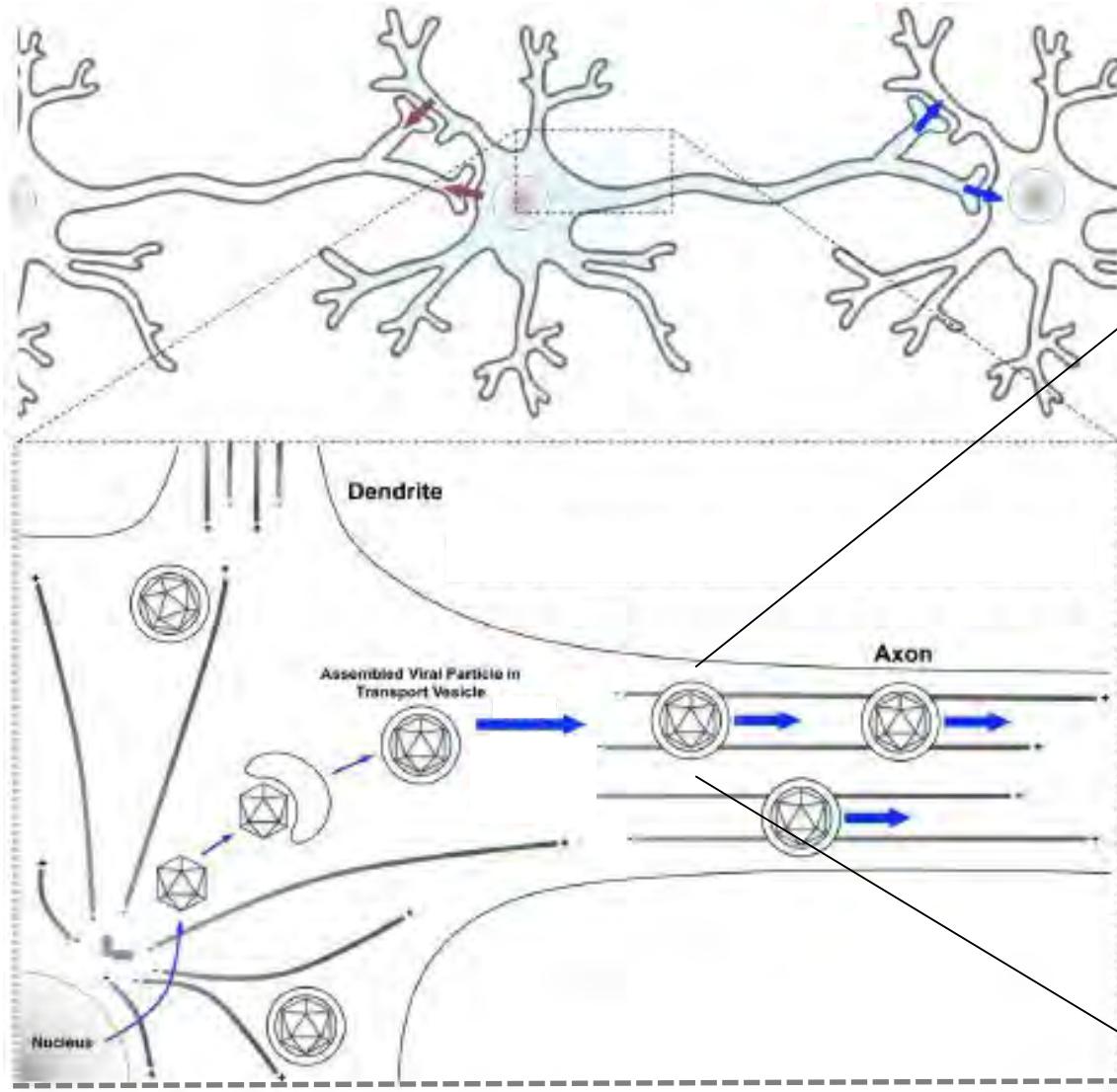
AdV-Us8-mCherry



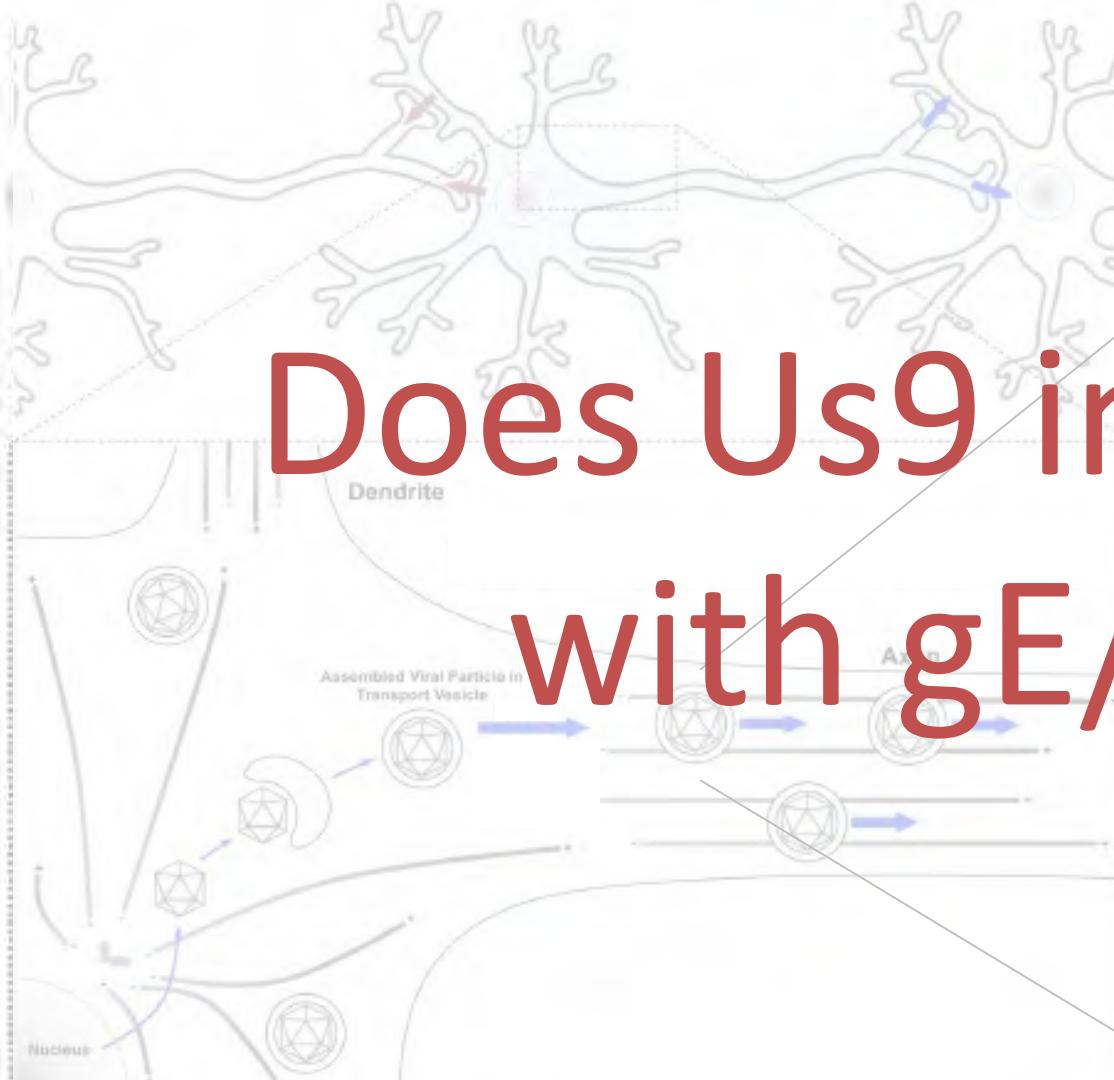
AdV-GFP-Us9



Kinesin Recruitment

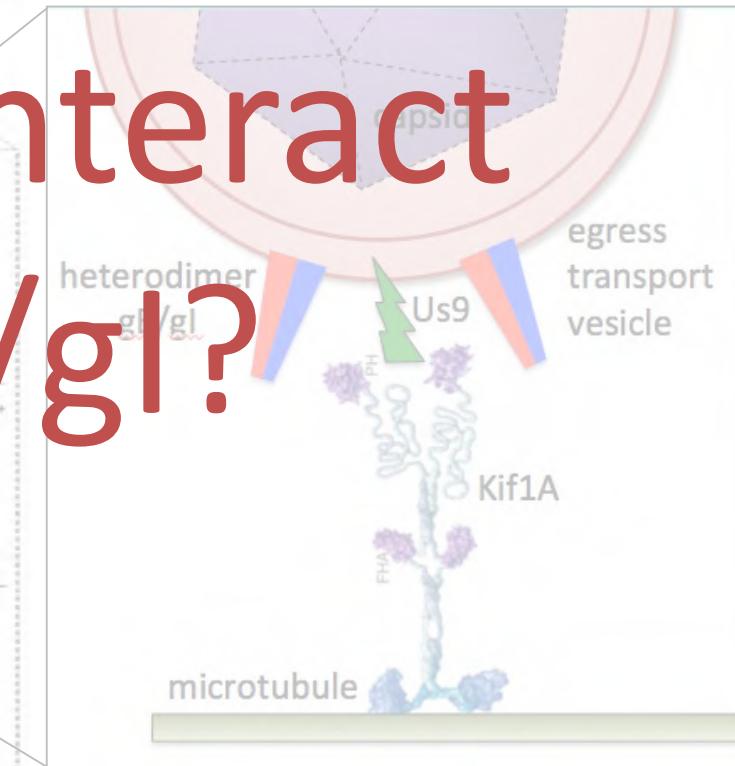


Kramer & Enquist, Viruses, 2013



A grayscale diagram of a neuron with multiple branches. In the center, a dashed box highlights a specific area. Inside this box, a grey circle labeled "Nucleus" contains a pentagonal viral capsid. An arrow points from the nucleus to a "Dendrite". Another arrow points from the dendrite to an "Assembled Viral Particle in Transport Vesicle". This vesicle is shown moving along microtubules towards the "Axon". A final arrow points from the axon to another viral capsid at the "Synapse".

Does Us9 interact with gE/gI?



Triple Complex

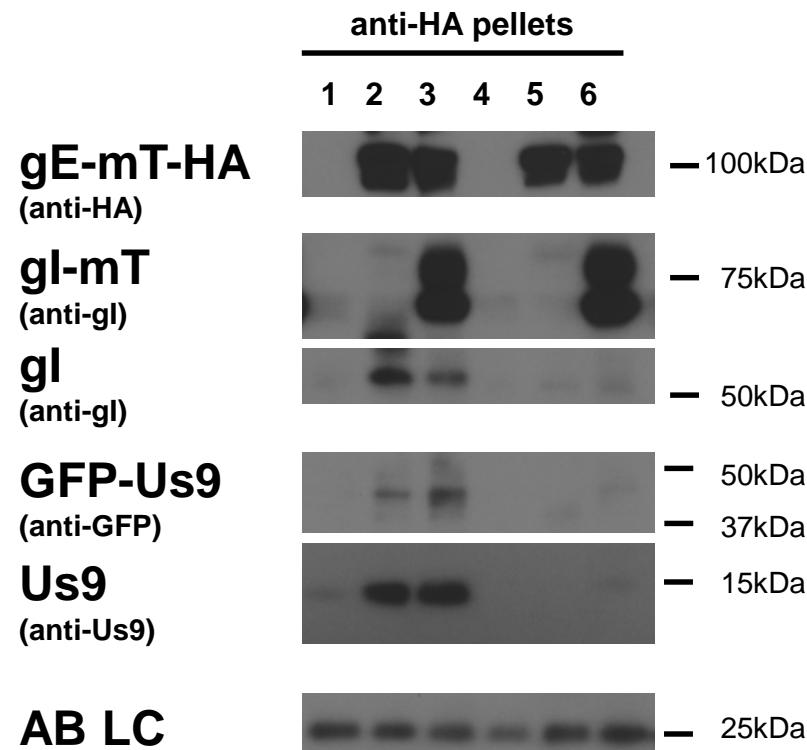
rat2 cells

AdV transduction (2days)

PRV infection (10^6 pfu)

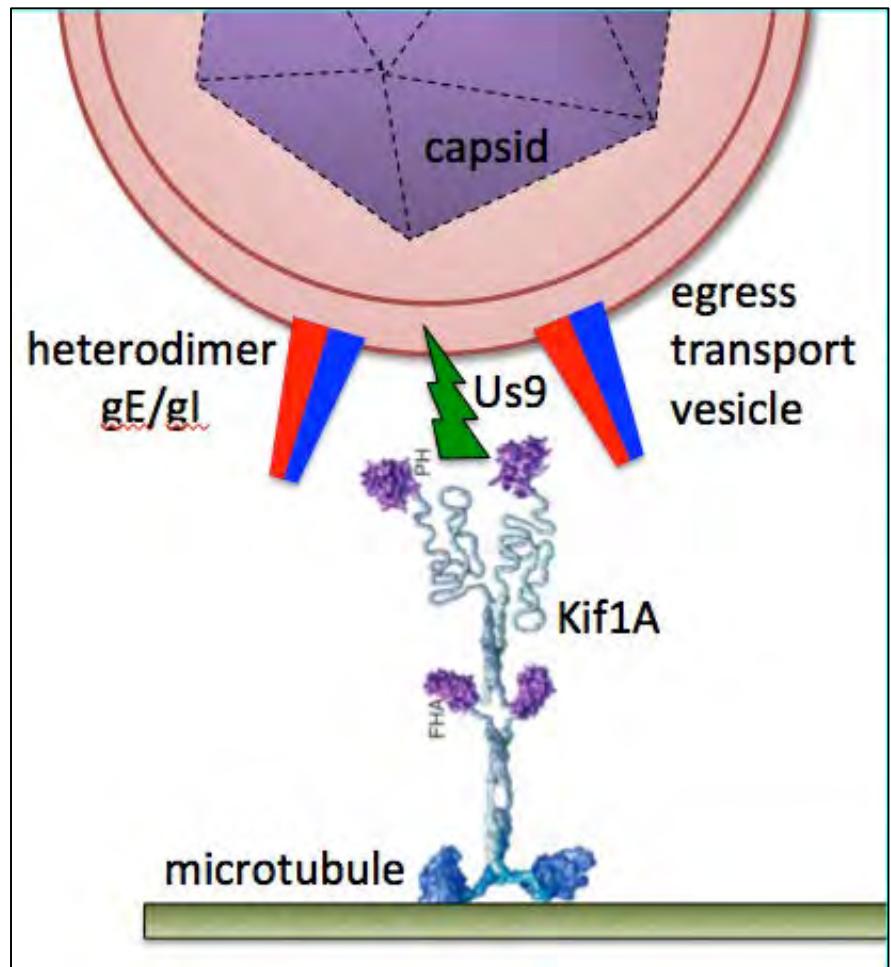
RIPA lysate at 8hpi

- 1: PRV + GFP-Us9
- 2: PRV + GFP-Us9 + gE-HA
- 3: PRV + GFP-Us9 + gE-HA + gl-Flag
- 4: mock + GFP-Us9
- 5: mock + GFP-Us9 + gE-HA
- 6: mock + GFP-Us9 + gE-HA + gl-Flag

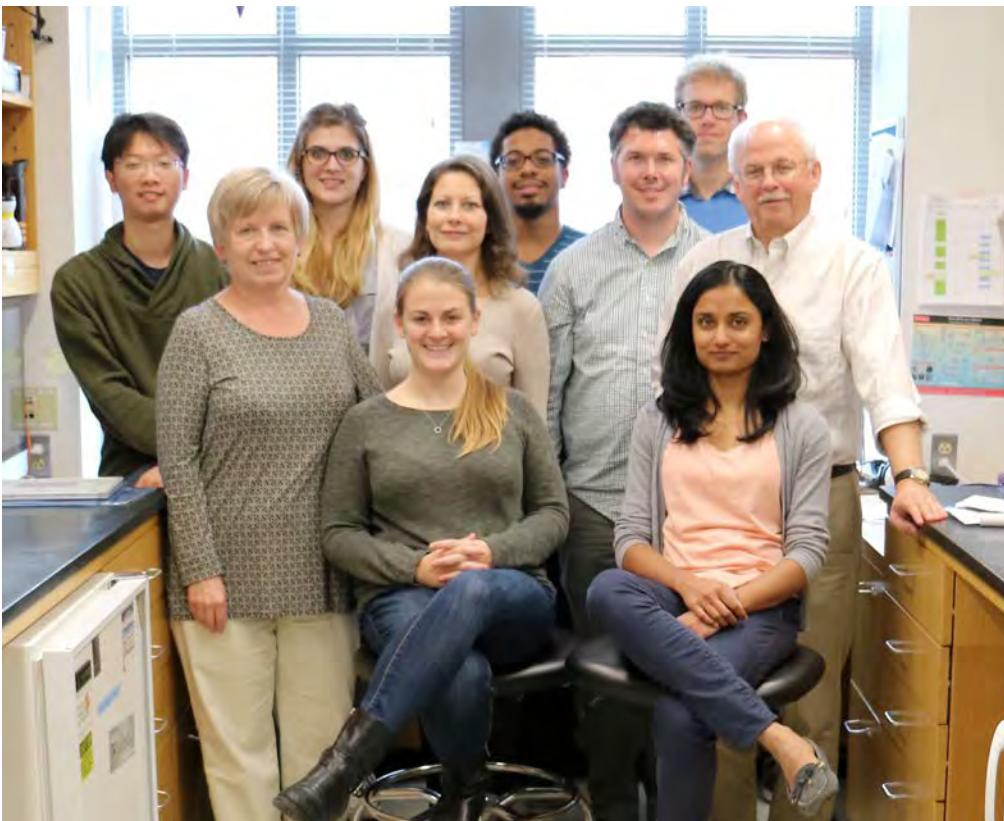


Conclusions

- (1) PRV-Us9, -gE, and -gl are required for axonal entry and anterograde spread.
- (2) Us9/gE/gl co-transport in non-PRV-infected neuronal cell axons.
- (3) Us9/gE/gl interact and can form a triple complex to recruit Kif1A (kinesin-3).



Thank You!



Zachary Yaffe (now UW)



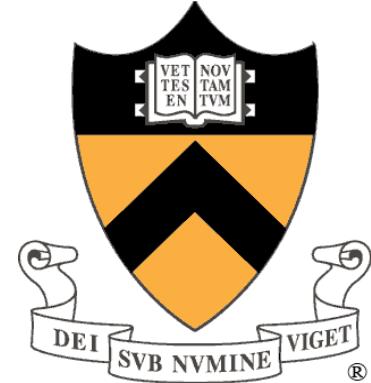
Michael Vershinin
(University of Utah)



Kathryn Galliers
(Oxford University, UK)



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