

Complex of Three PRV Envelope Proteins Mediates Anterograde Transport and Spread



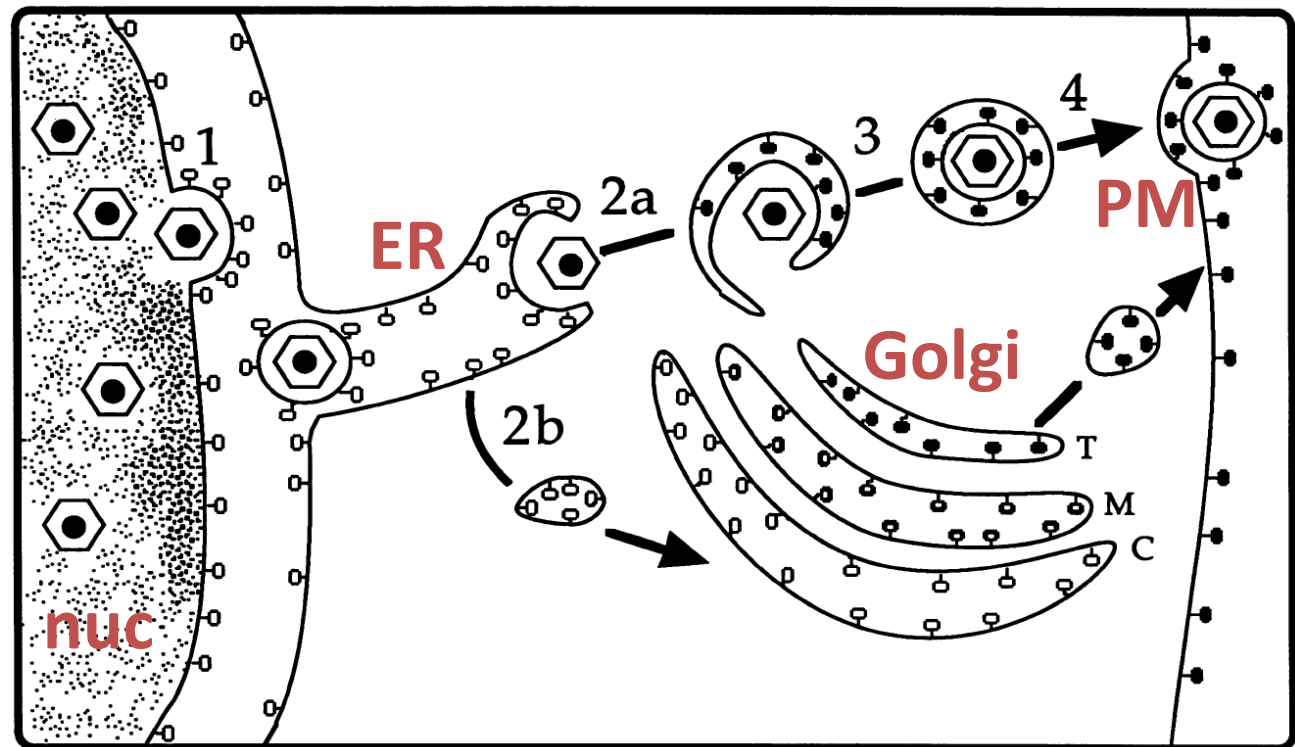
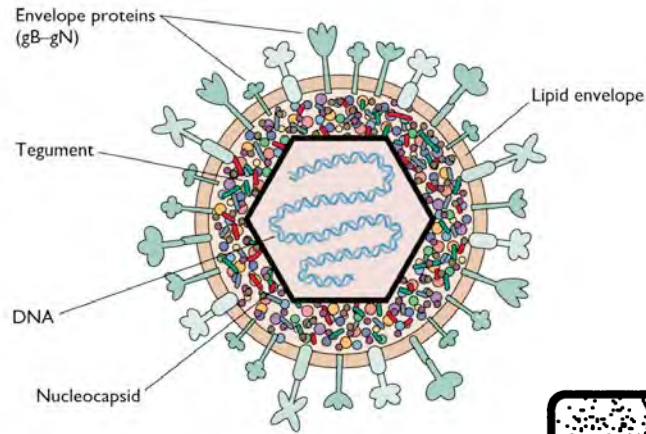
Julian Scherer, PhD

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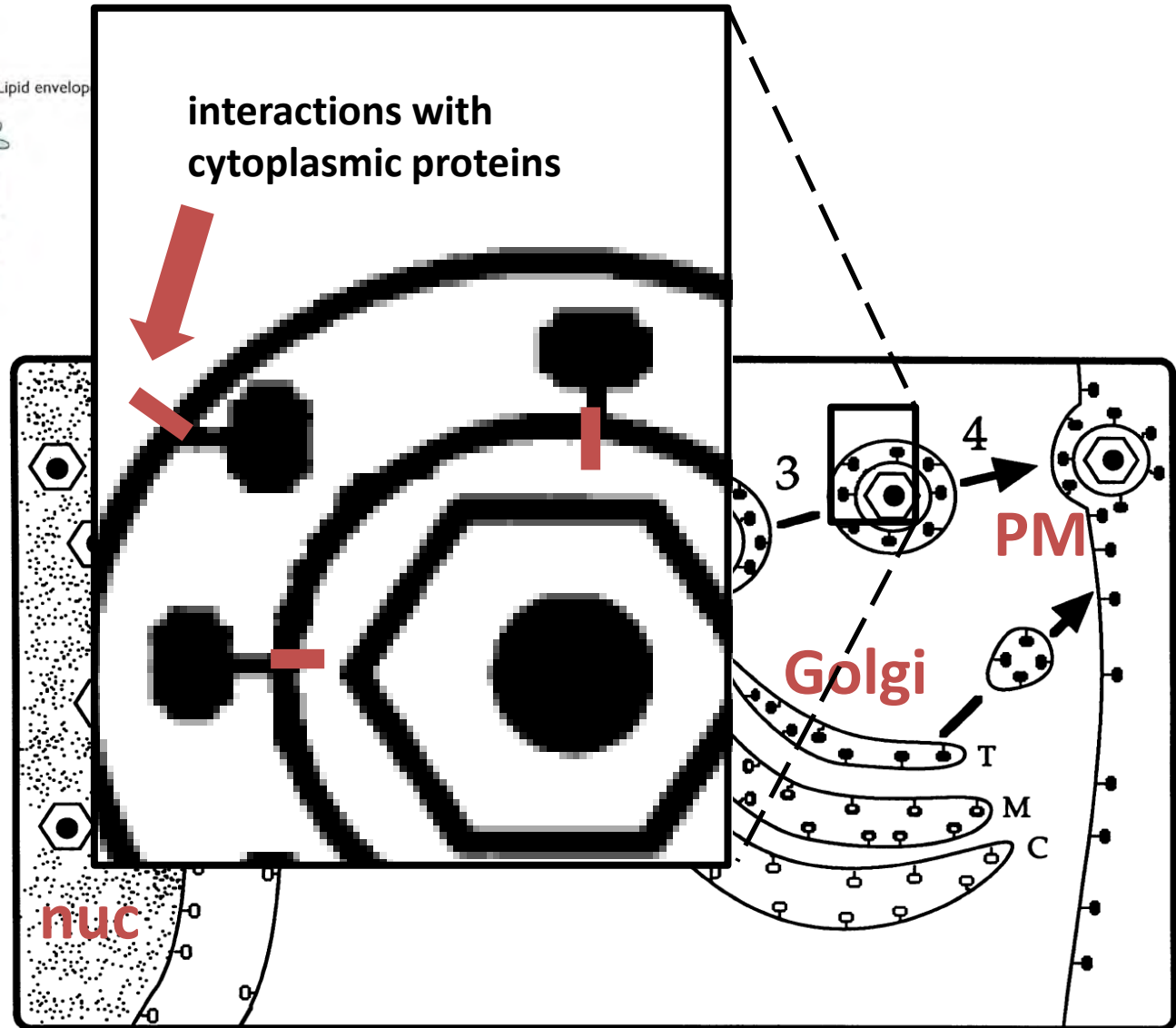
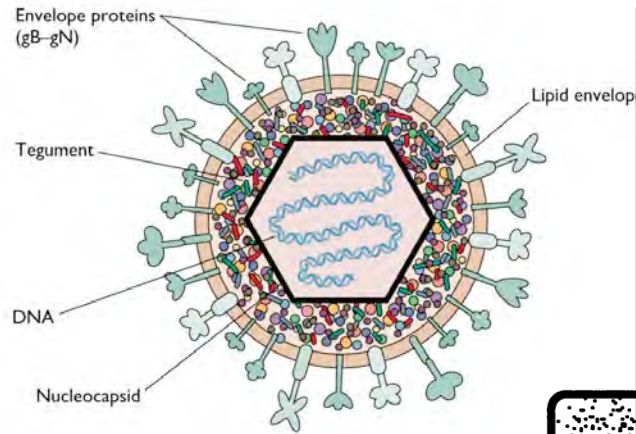


Herpesvirus Egress

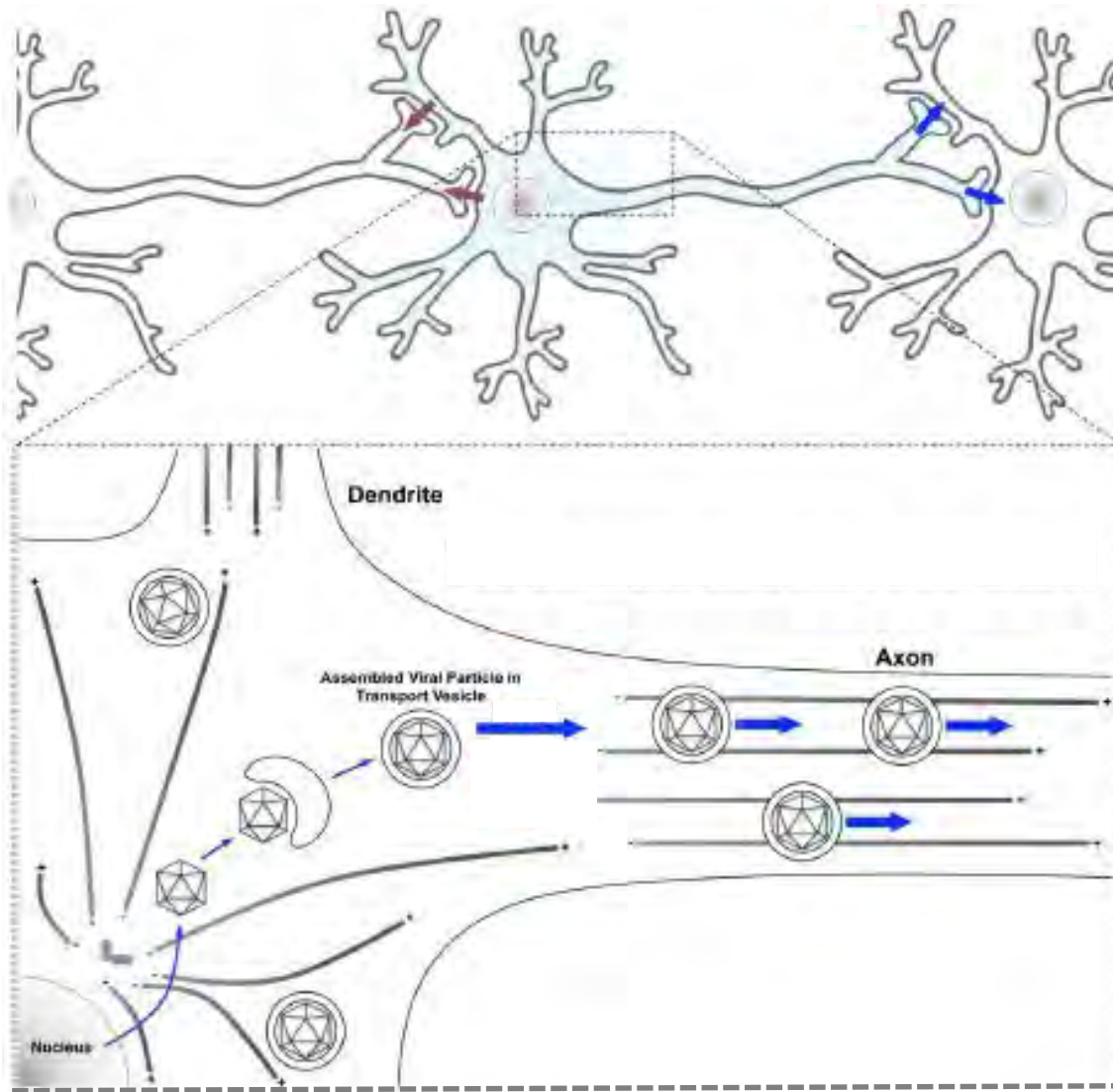




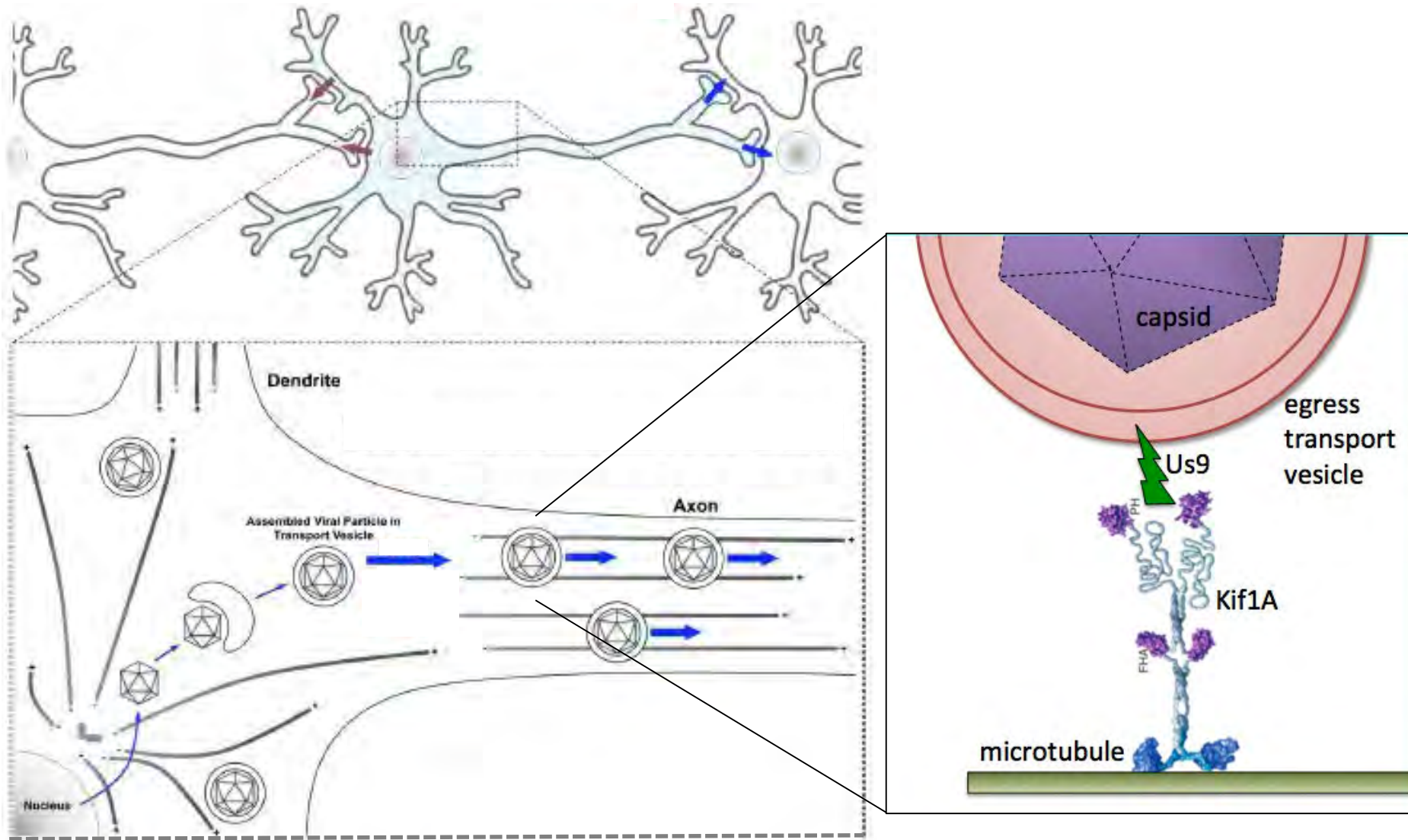
Herpesvirus Egress



Neuronal Herpesvirus Egress

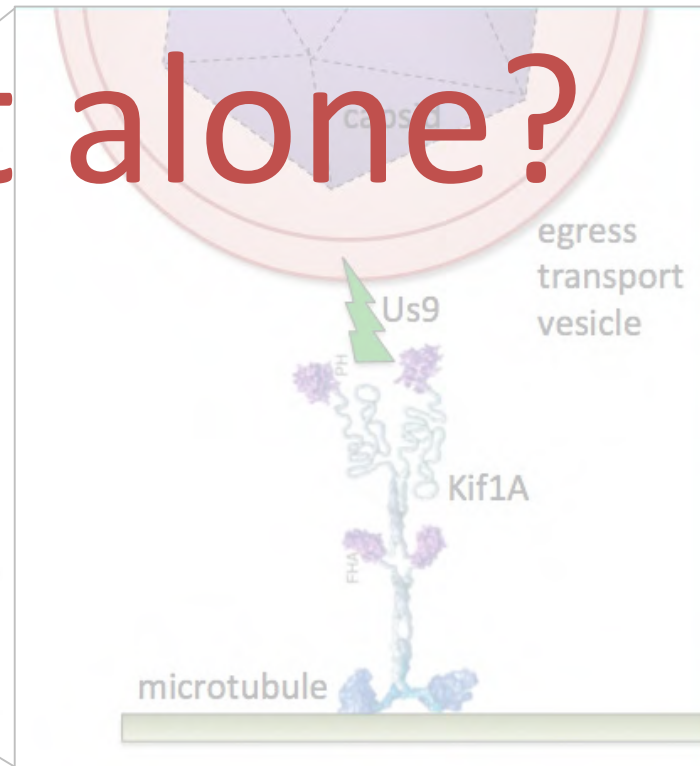
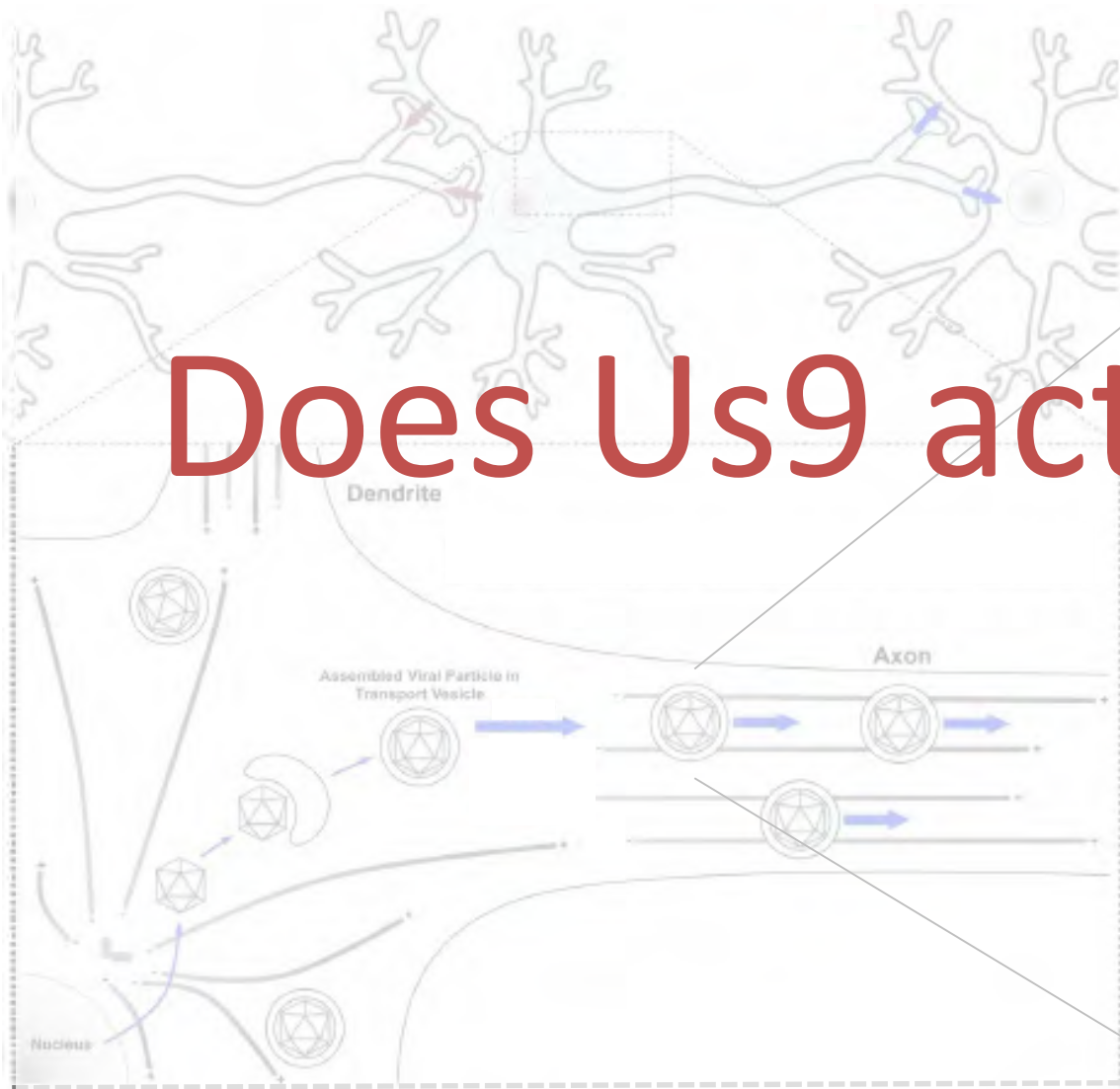


Neuronal Herpesvirus Egress

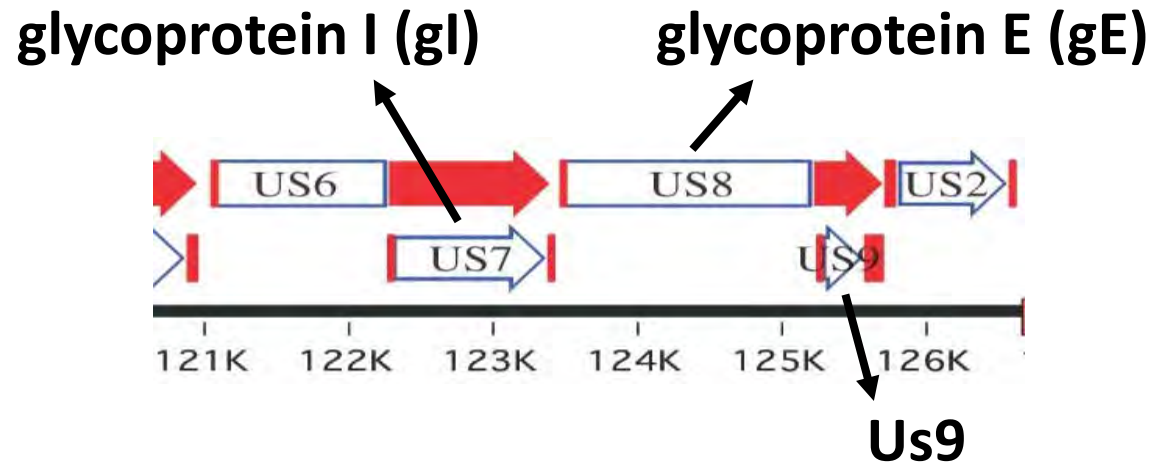




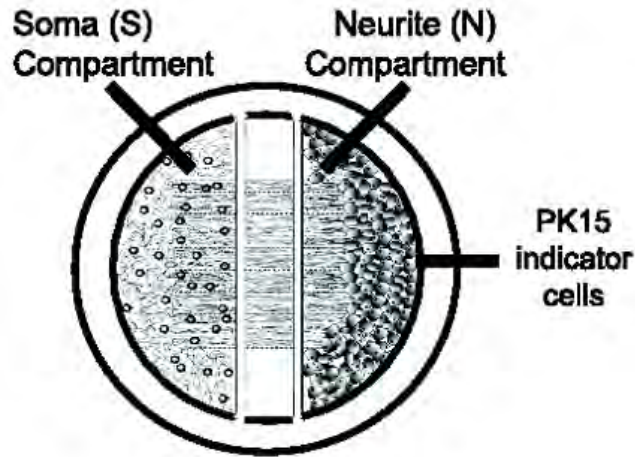
Does Us9 act alone?



Us9 Gene in PRV Genome



Anterograde spread



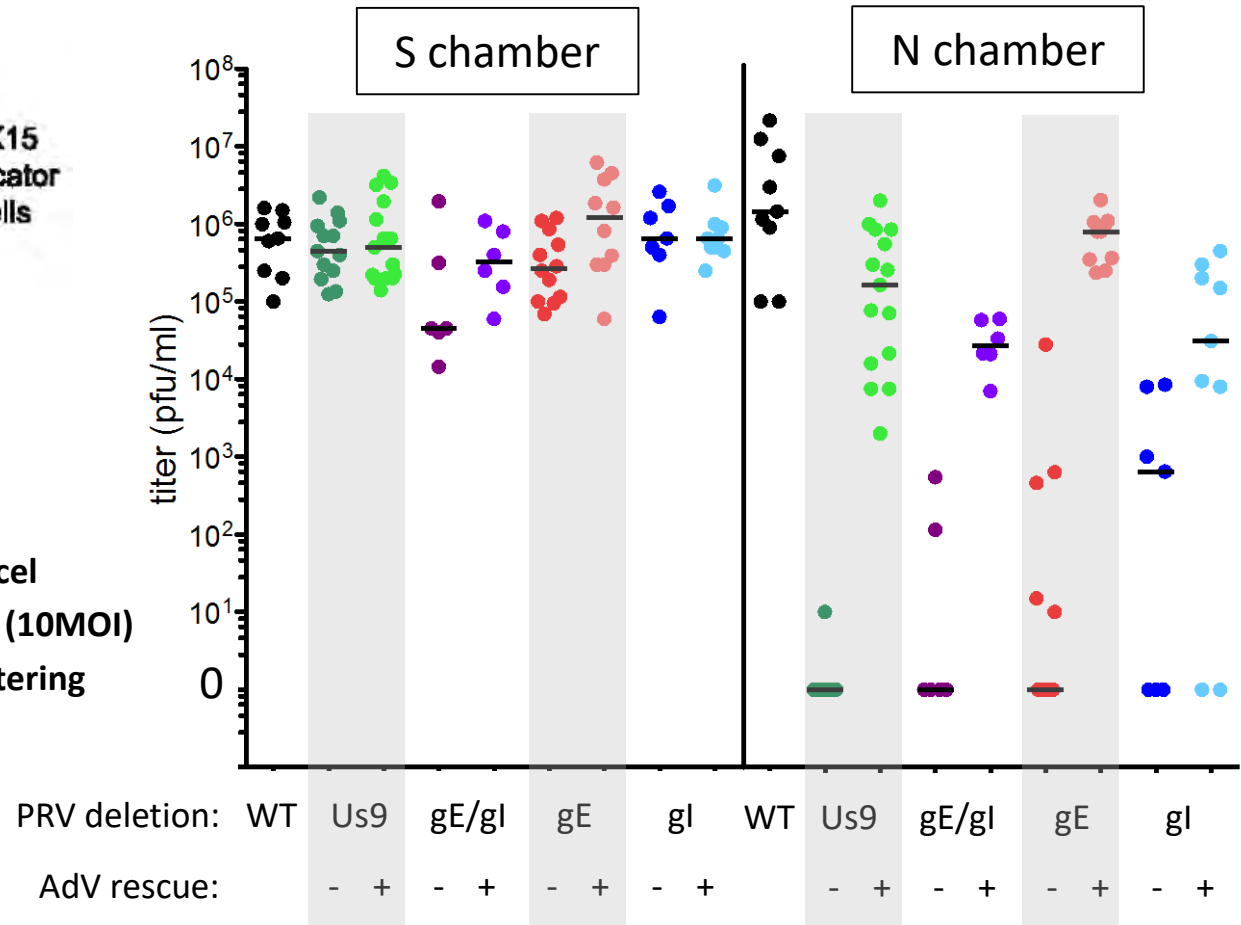
day -14: seed primary SCG neurons

day -3: AdV transduction

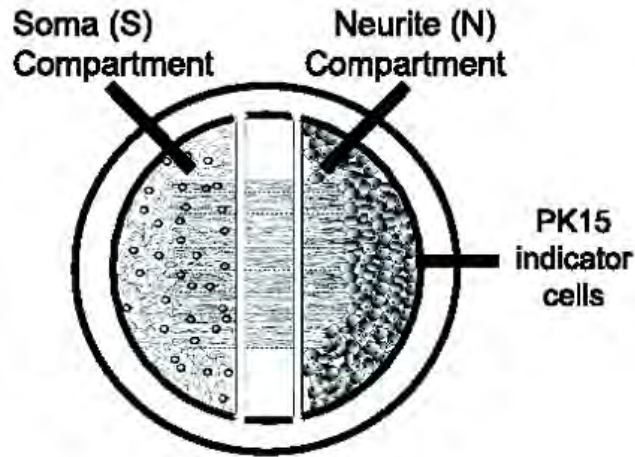
day -1: seed PK15 cells + add methocel

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

24hpi: harvest S & N chamber for titering



Anterograde spread



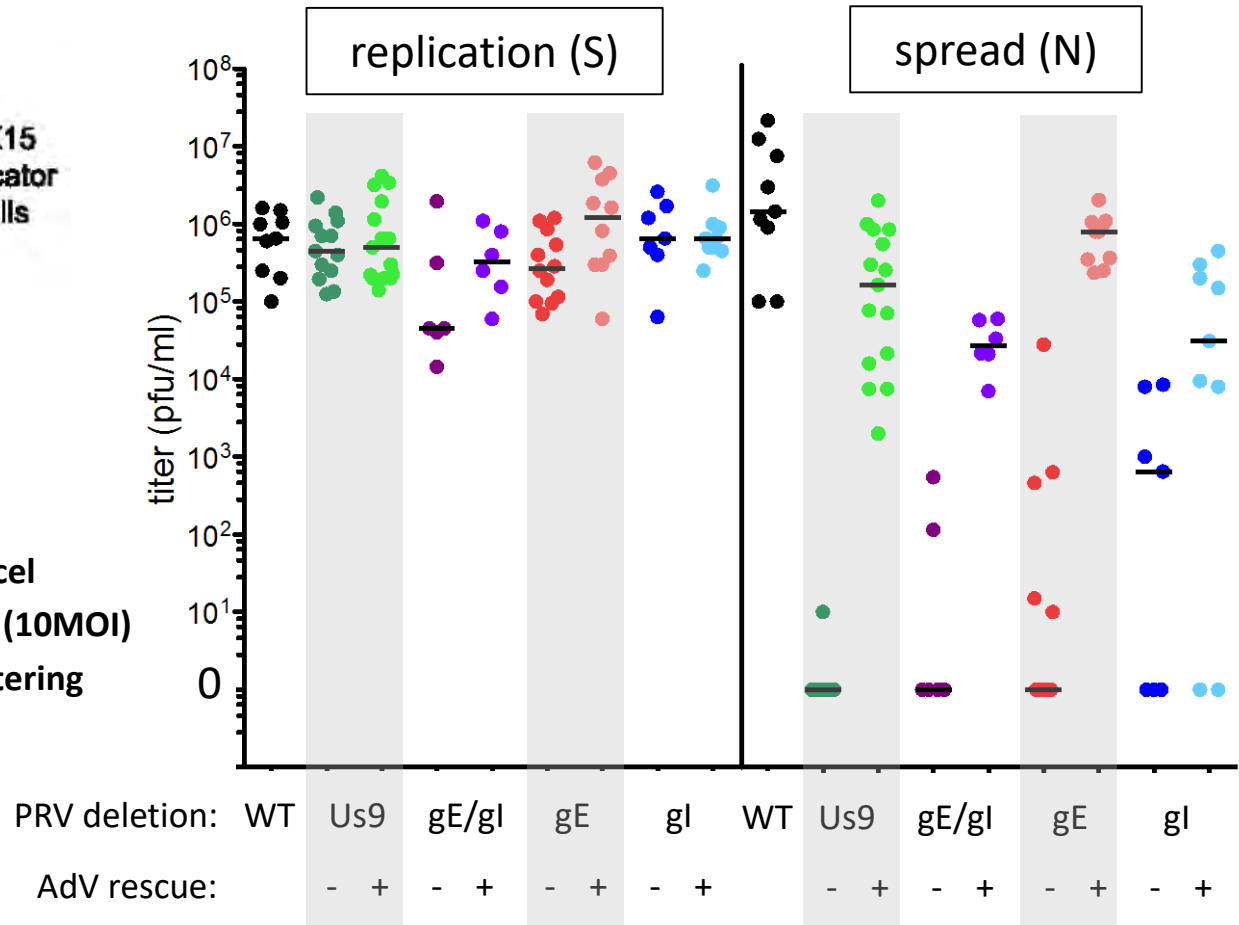
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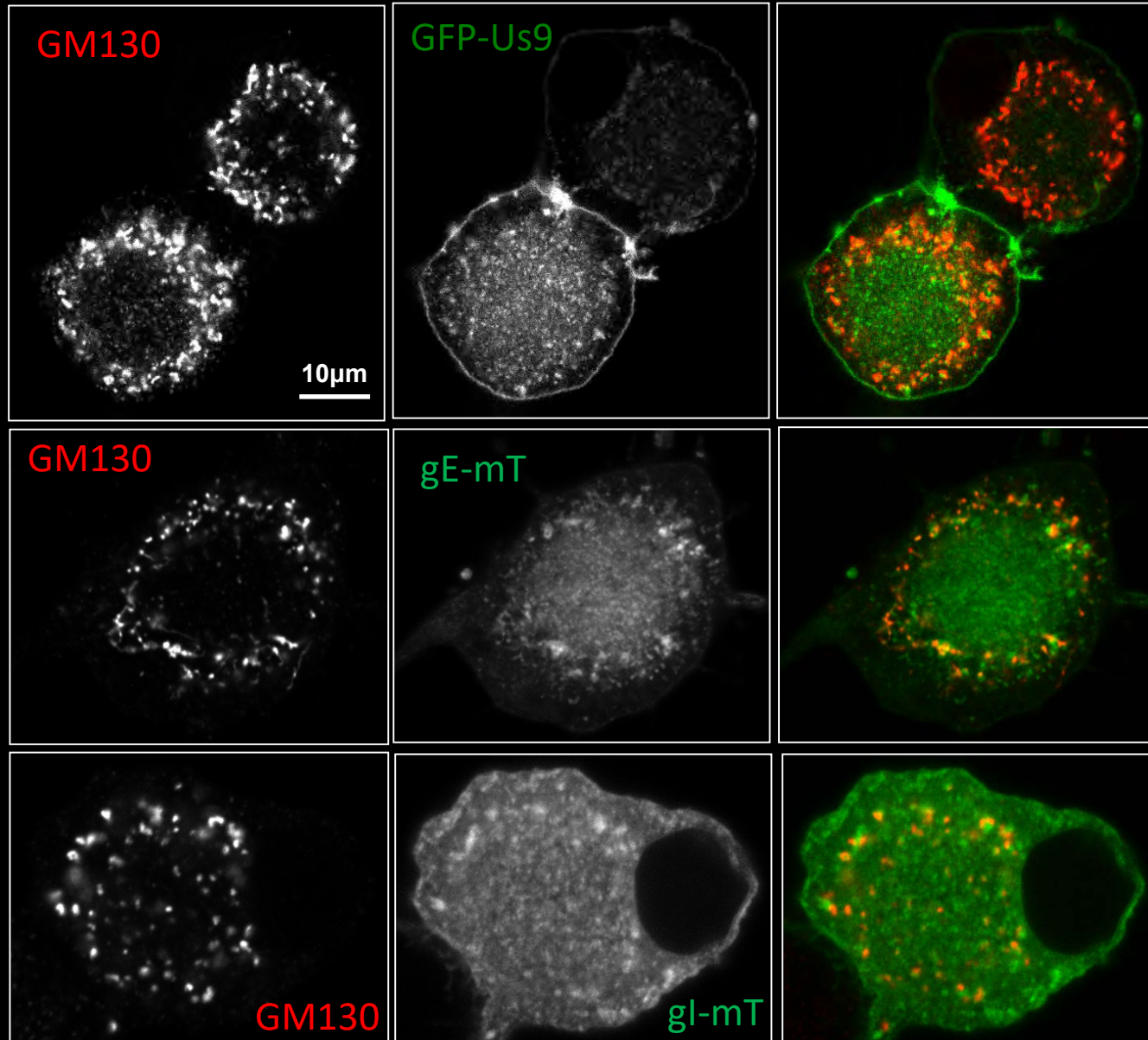
24hpi: harvest S & N chamber for titering





Soma Localization

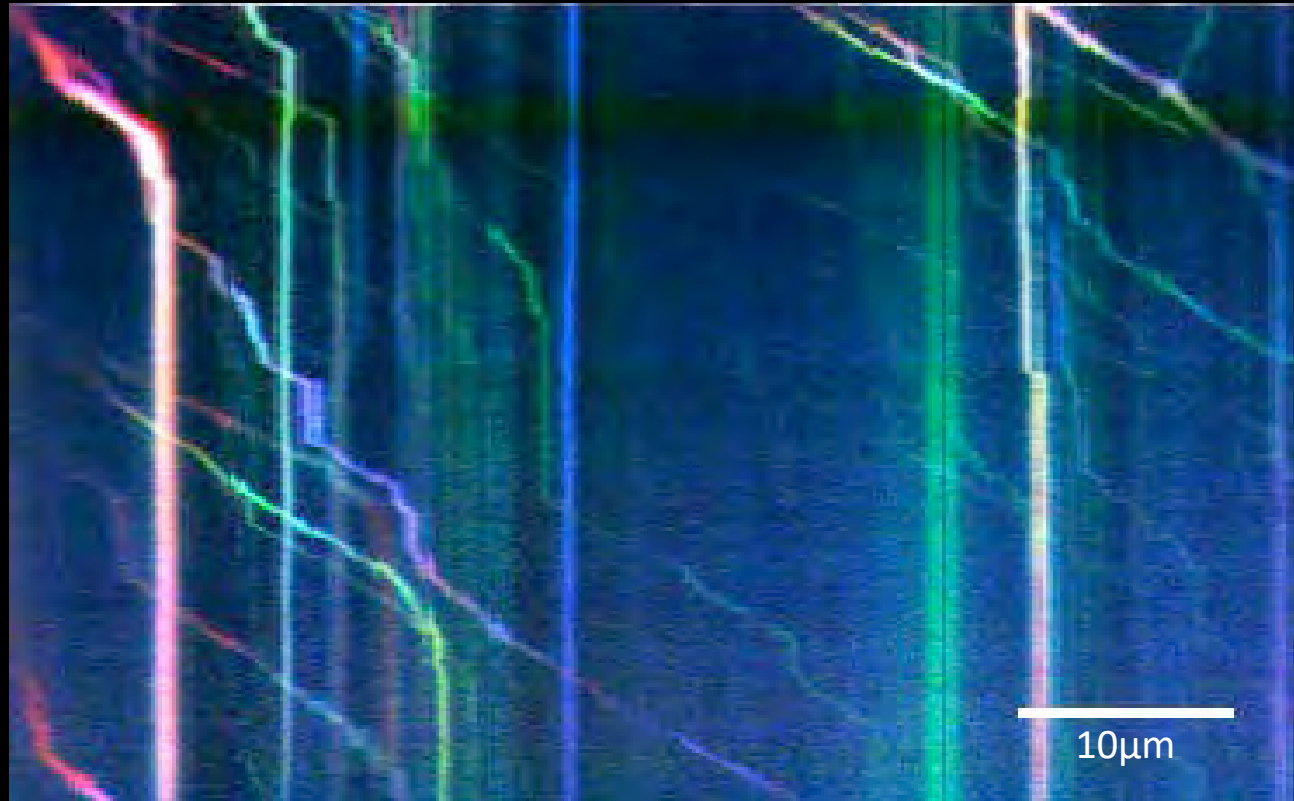
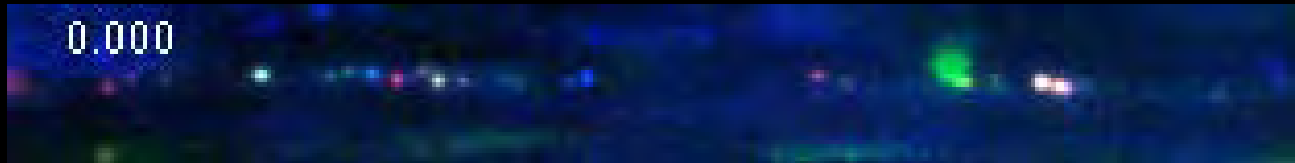
SCG neurons (dissociated), 14DIV
AdV transduction (2days)
confocal imaging of cell soma



Staining and IFM:
Shree Tanneti

Axonal Co-transport

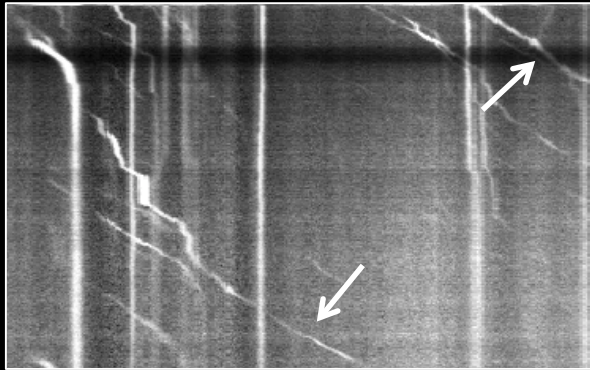
AdV-Us7-mTurquoise2
AdV-Us8-mCherry
AdV-GFP-Us9



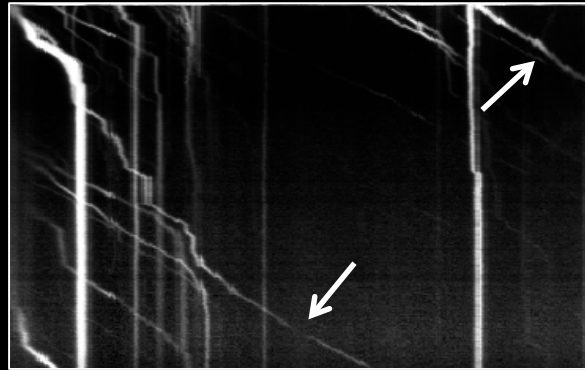
15sec,
at 15fps

Axonal Co-transport

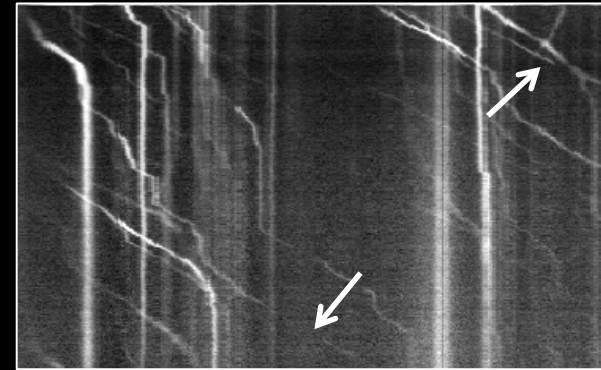
AdV-Us7-mTurquoise2



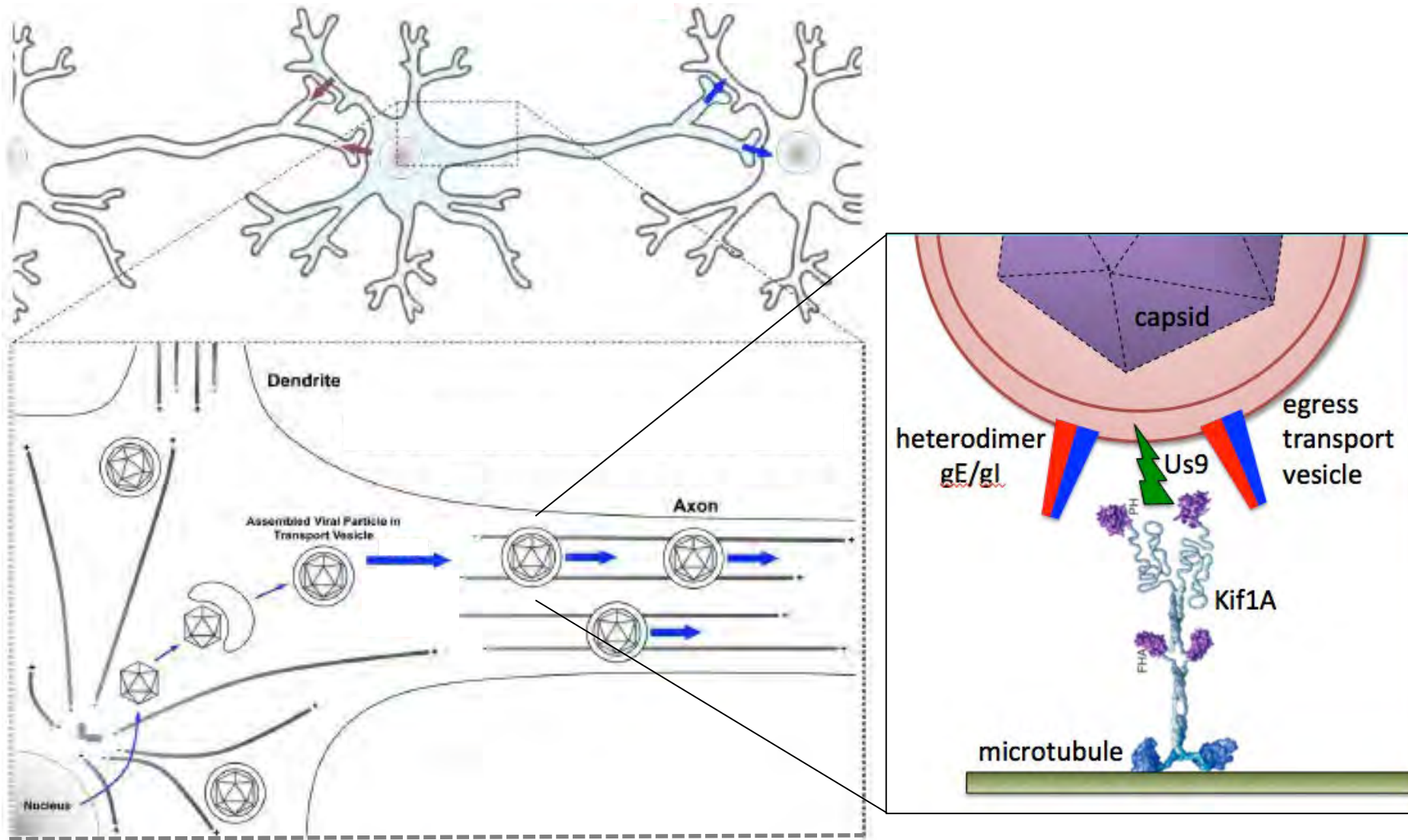
AdV-Us8-mCherry



AdV-GFP-Us9

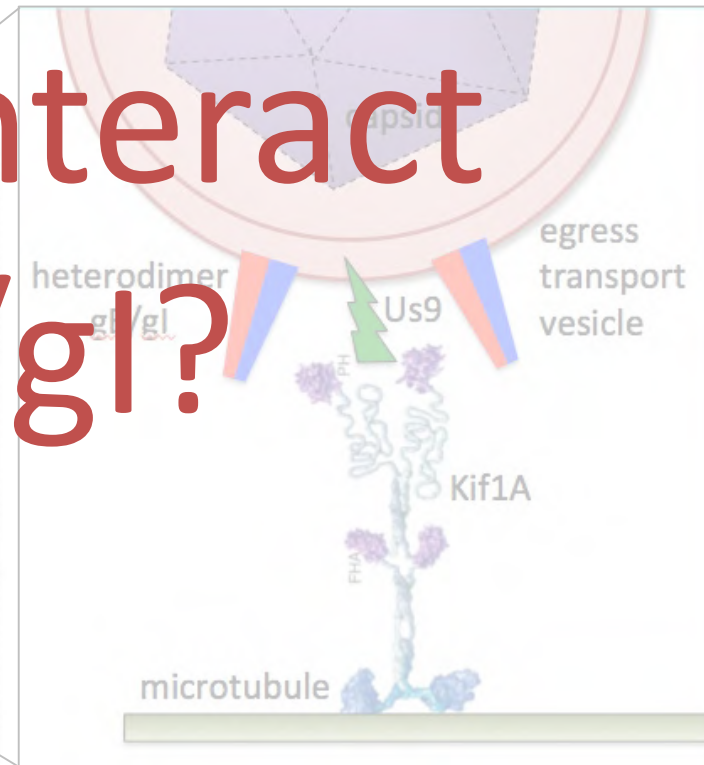
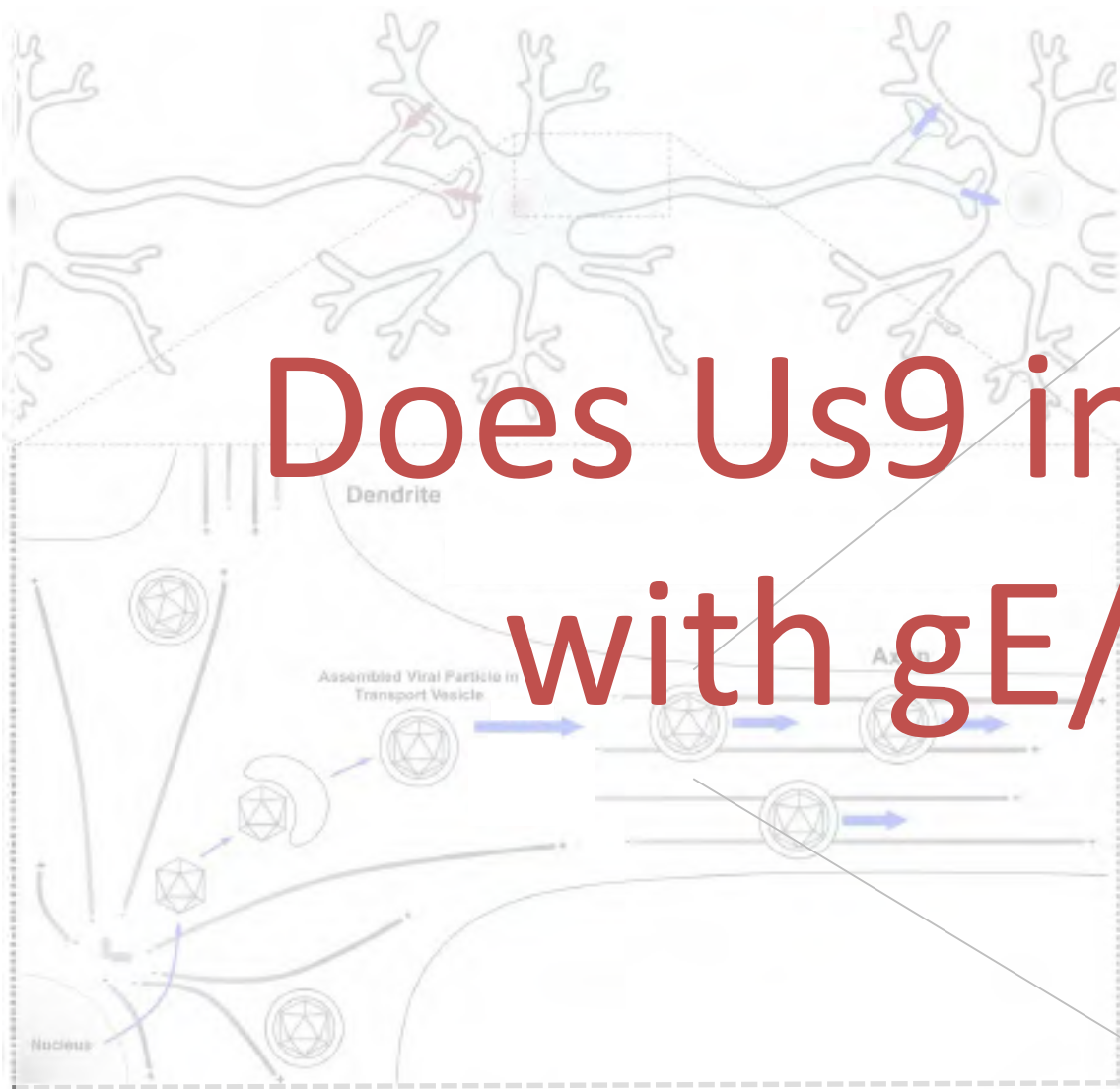


Kinesin Recruitment





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 + gI-Flag
- 4: mock + GFP-Us9
- 5: mock + GFP-Us9 + gE-HA
- 6: mock + GFP-Us9 + gE-HA + gI-Flag

gE-mT-HA
(anti-HA)

gI-mT
(anti-gI)

gI
(anti-gI)

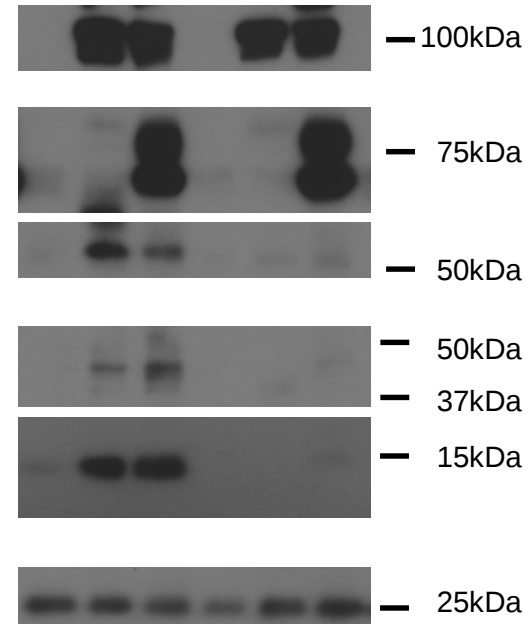
GFP-Us9
(anti-GFP)

Us9
(anti-Us9)

AB LC

anti-HA pellets

1 2 3 4 5 6

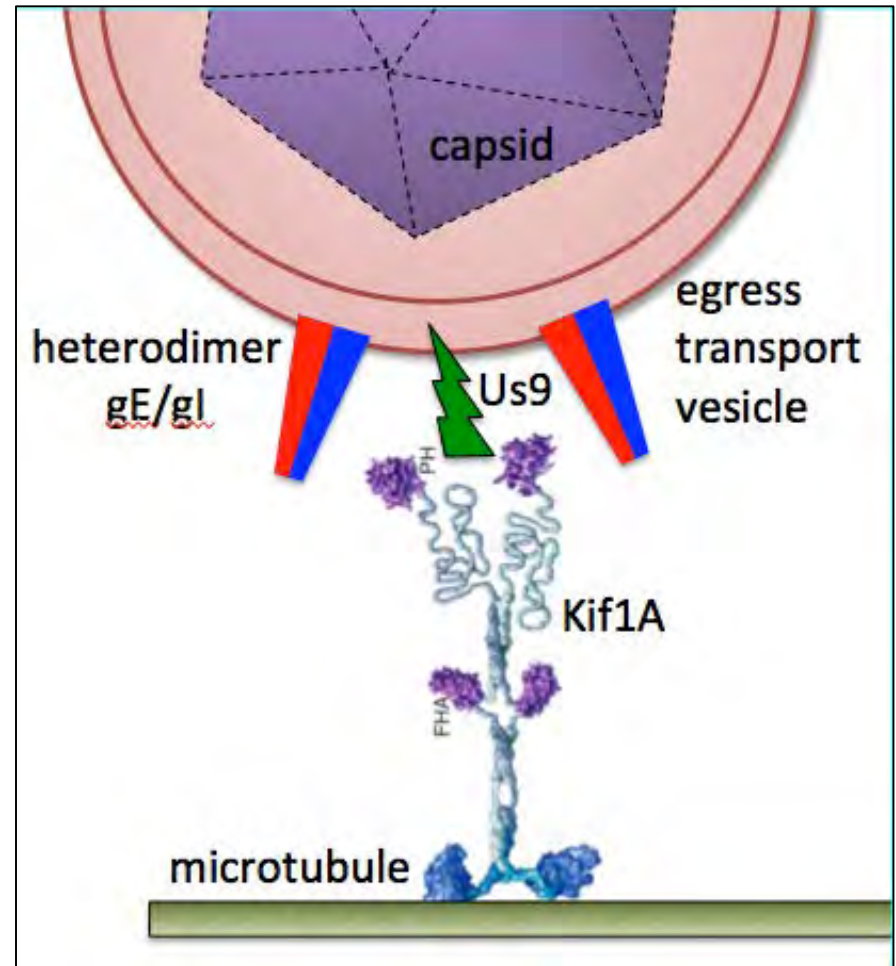


Conclusions

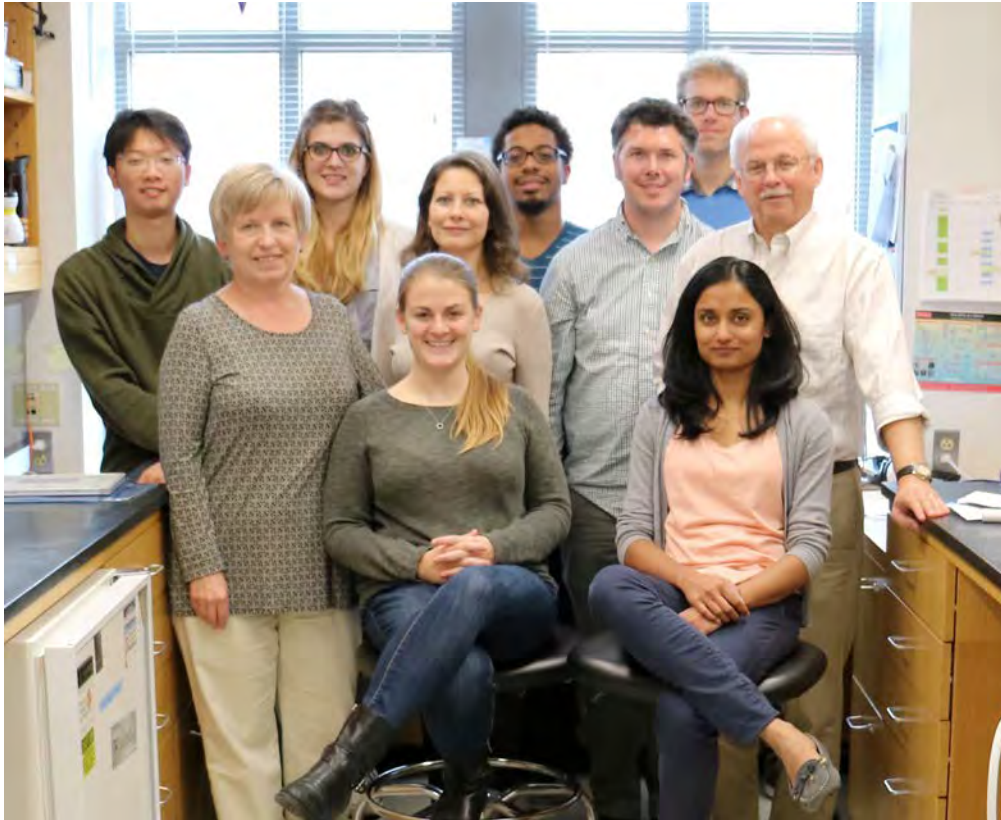
(1) PRV-Us9, -gE, and -gI are required for axonal entry and anterograde spread.

(2) Us9/gE/gI co-transport in non-PRV-infected neuronal cell axons.

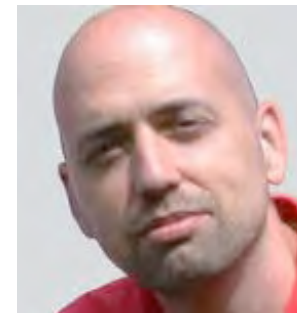
(3) Us9/gE/gI interact and can form a triple complex to recruit Kif1A (kinesin-3).



Thank You!



Zachary Yaffe (now UW)



Michael Vershinin
(University of Utah)



Kathryn Galliers
(Oxford University, UK)



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