Looking at pathogenesis in AFM: the virus and the cells

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Why are cellular membranes interesting for pathogenesis and host dissemination of a non-enveloped virus?

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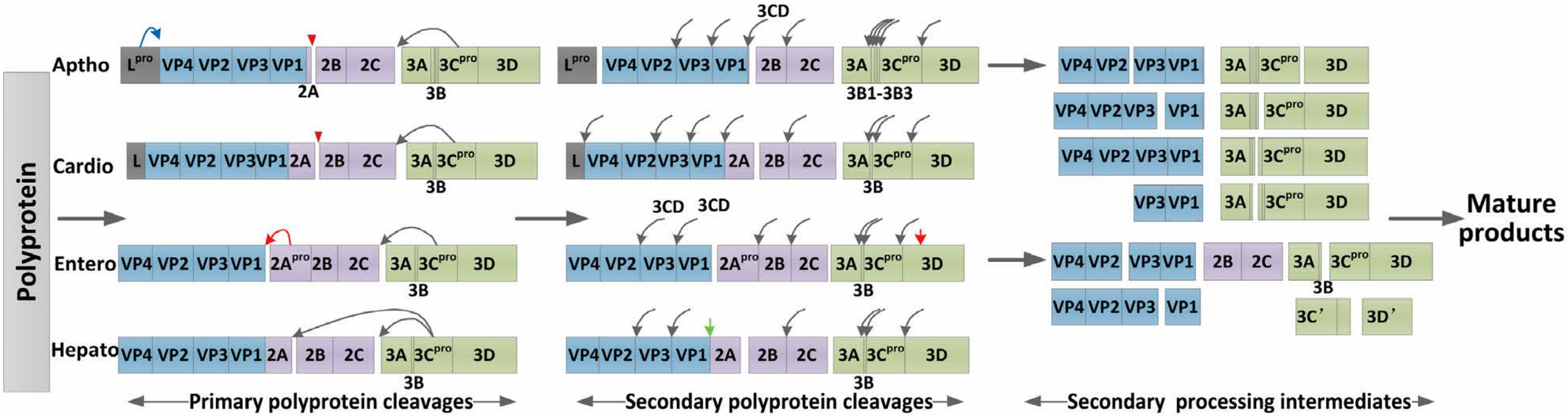
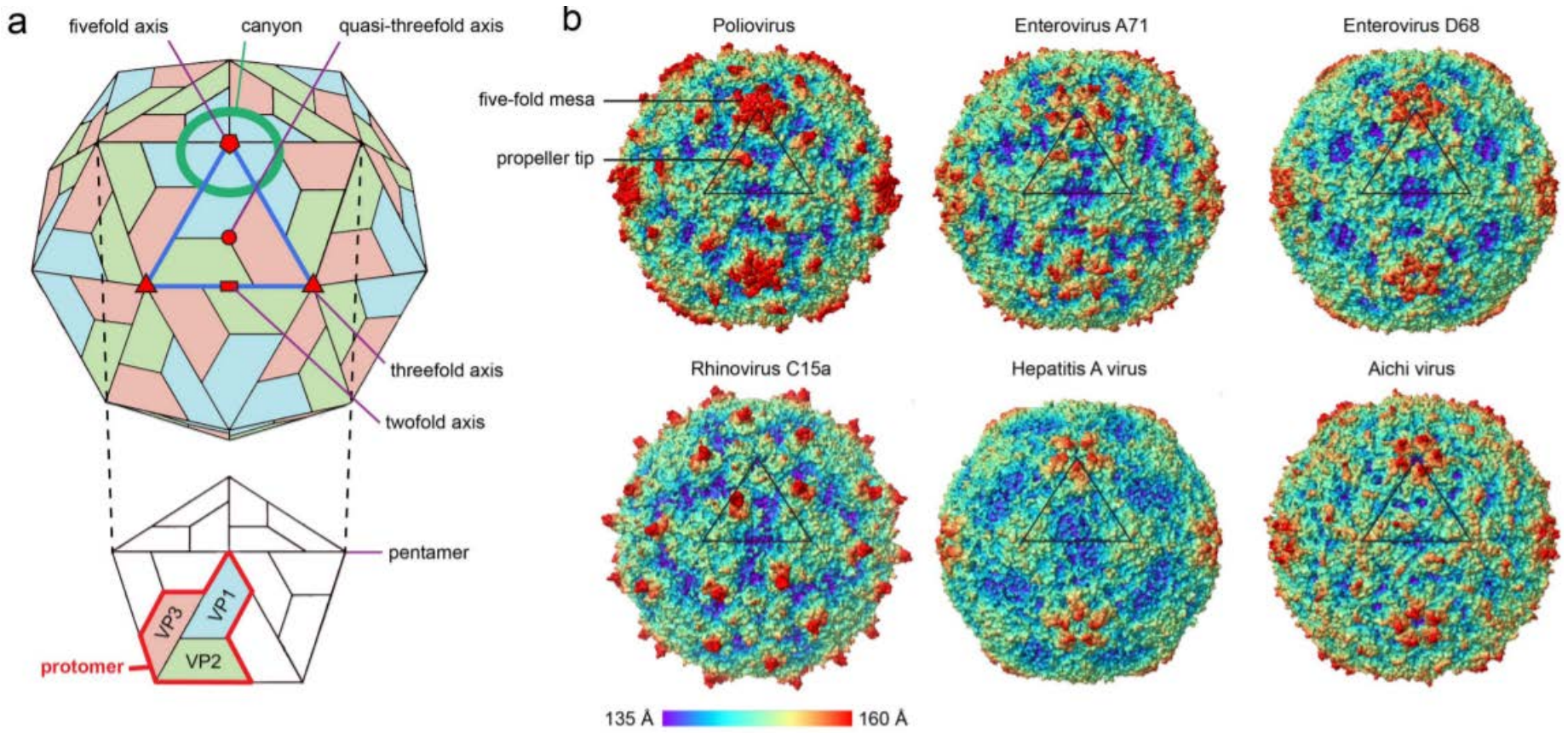
Picornaviruses have remarkably conserved genome organization



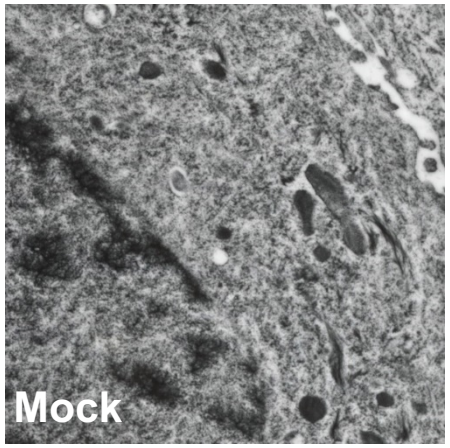
Viruses **2016**, *8*(3), 82; https://doi.org/10.3390/v8030082

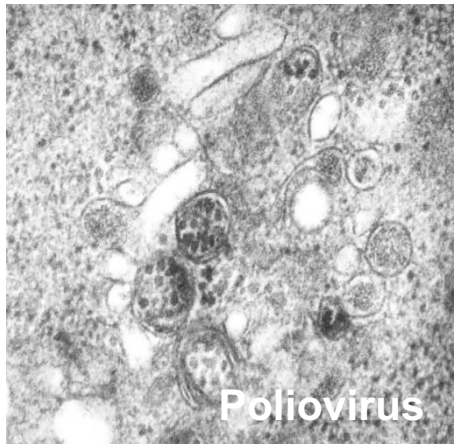
Picornaviruses are traditionally classified as non-enveloped viruses with similar capsid structures

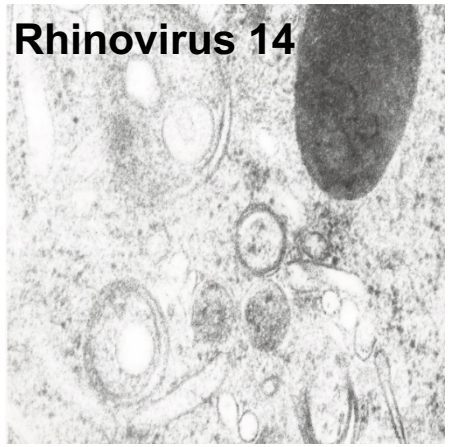


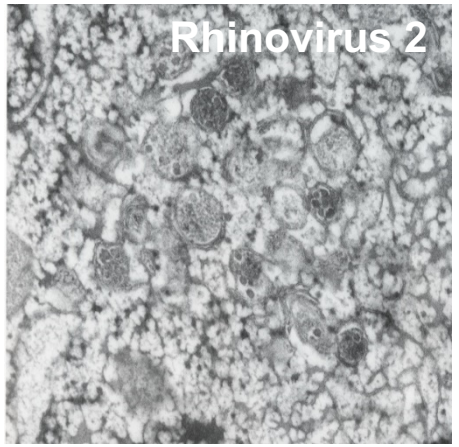
Nat Rev Microbiol. 2018 Jun;16(6):368-381. doi: 10.1038/s41579-018-0005-4.

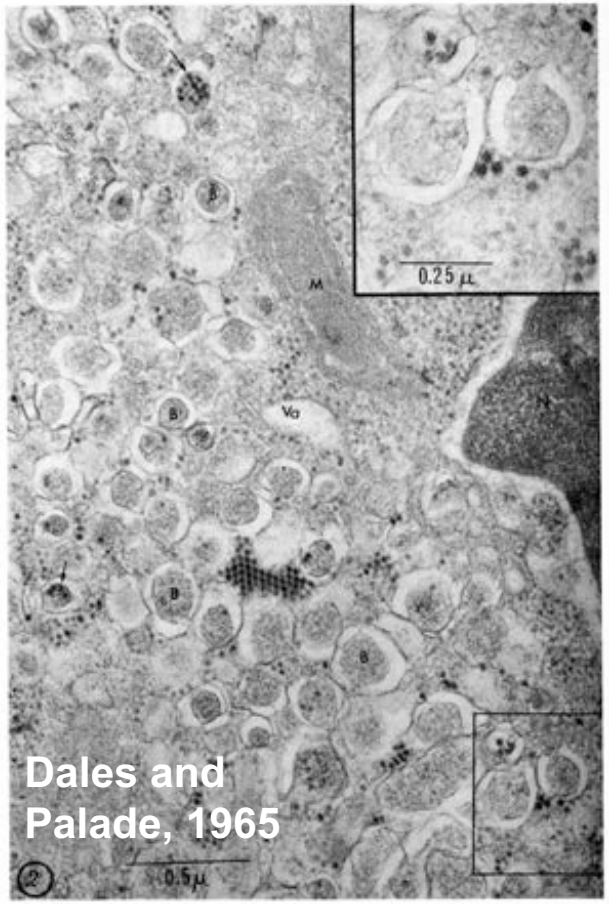
Double-membraned vesicles have long been known to proliferate in picornavirus-infected cells but it was never clear why.



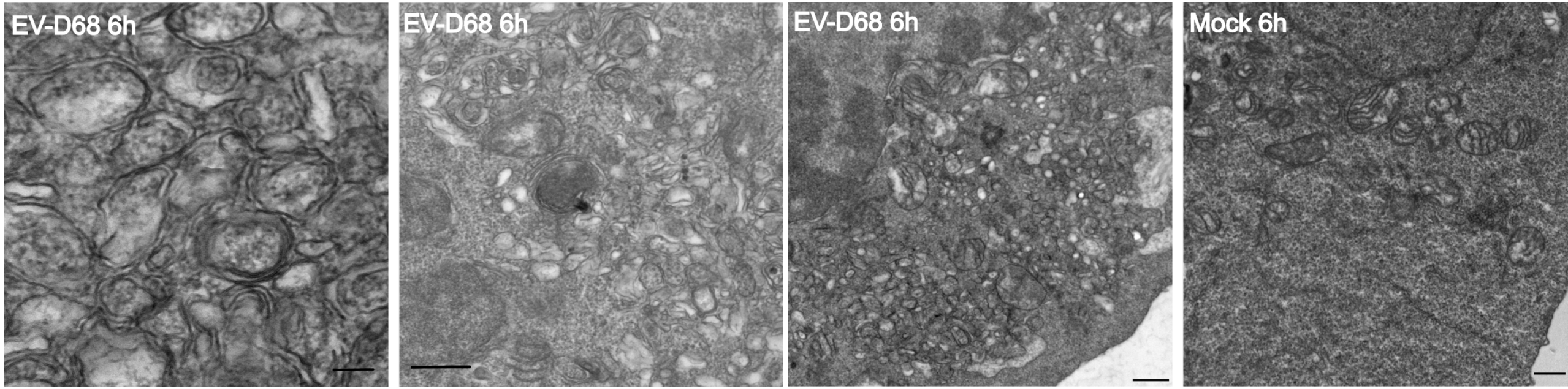








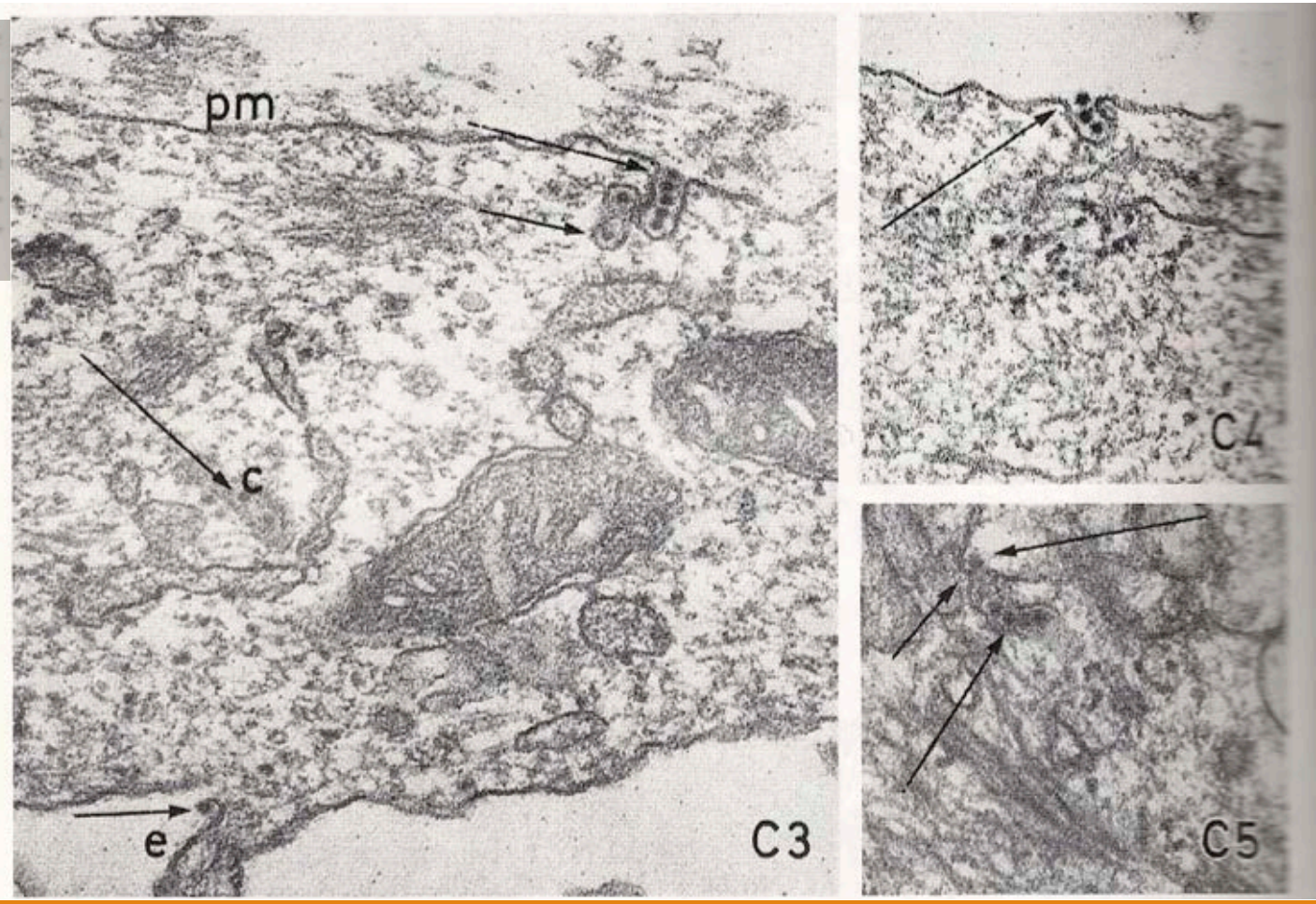
Autophagosomes are also observed during infection with EVD-68



Vesicle-based release has been proposed for picornaviruses for many years

Fig. 102 C. Accumulation of progeny virions within cisternae of endoplasmic reticulum and release of virions from cisternae by fusion with the plasma membrane

Electron micrographs *(1)* and *(2)* show progeny polio-virions within cisternae of endoplasmic reticulum at various depths in the cytoplasm of infected HeLa cells, 8 h.p.i. *(1)* and of infected human chorion cells, 20 h.p.i. *(2)*. Electron micrographs *(3–5)* illustrate the release of progeny virions from cisternae *(c)* by fusion with the plasma membrane *(pm)* in poliovirus infected human chorion cells, 20 h.p.i. *(3)* and infected HeLa cells, 8 h.p.i. *(4)* and 12 h.p.i. *(5)*. – Figures from Dunnebacke *et al.* 1969 [J. Virol. *4*, 511, 512 (1969)]



But the topology of double-membraned vesicles didn't make sense for release of naked virions

Thelma Dunnebacke &
Robley Williams
J.Virol *4*, 511-512 (1969)

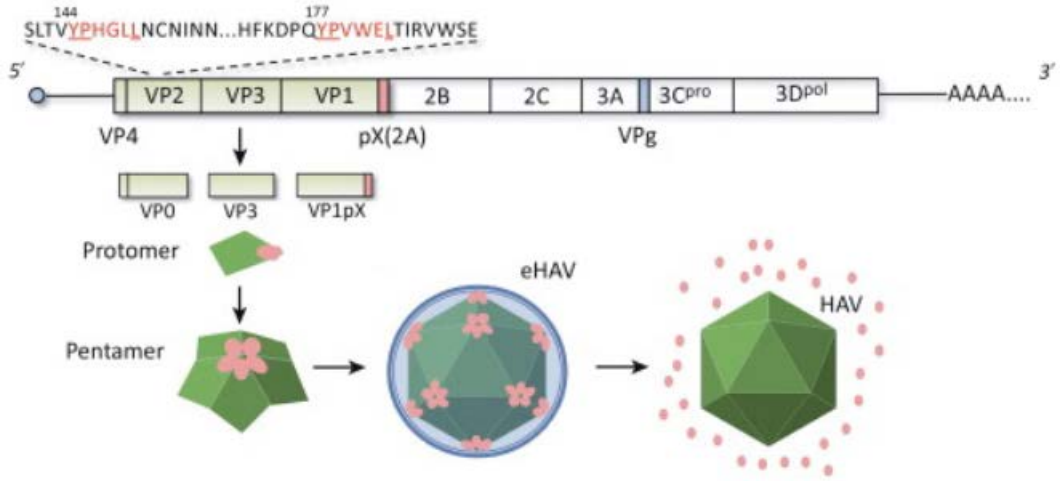
eHAV and the discovery of enveloped forms of picornaviruses

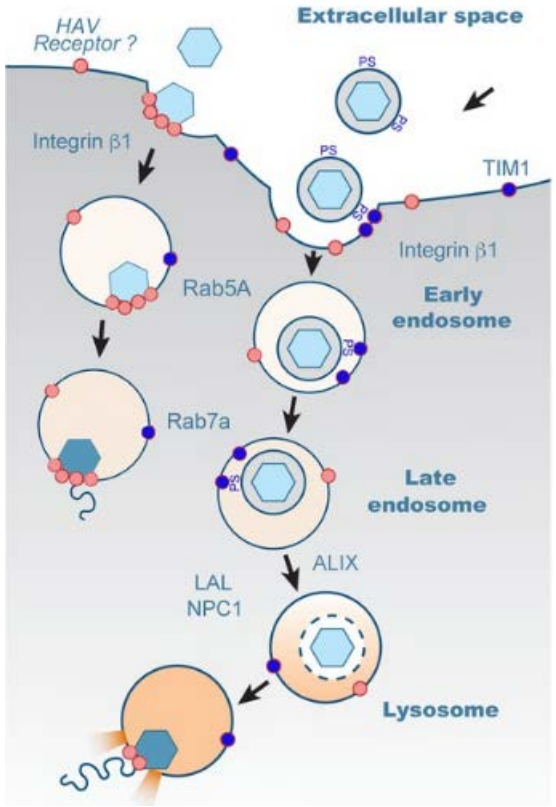
LETTER

doi:10.1038/nature12029

A pathogenic picornavirus acquires an envelope by hijacking cellular membranes

Zongdi Feng[1], Lucinda Hensley[1], Kevin L. McKnight[1], Fengyu Hu[1], Victoria Madden[2], LiFang Ping[1], Sook-Hyang Jeong[3], Christopher Walker[4], Robert E. Lanford[5] & Stanley M. Lemon[1,6,7]





The enveloped form of coxsackievirus is derived from the autophagy pathway

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PLOS | PATHOGENS

Coxsackievirus B Exits the Host Cell in Shed Microvesicles Displaying Autophagosomal Markers

Scott M. Robinson[1☯¶], Ginger Tsueng[1☯¶], Jon Sin[2☯¶], Vrushali Mangale[1], Shahad Rahawi[1], Laura L. McIntyre[1], Wesley Williams[1], Nelson Kha[1], Casey Cruz[3], Bryan M. Hancock[3], David P. Nguyen[1], M. Richard Sayen[2], Brett J. Hilton[1], Kelly S. Doran[3], Anca M. Segall[3], Roland Wolkowicz[1], Christopher T. Cornell[4¤], J. Lindsay Whitton[4], Roberta A. Gottlieb[2], Ralph Feuer[1]*

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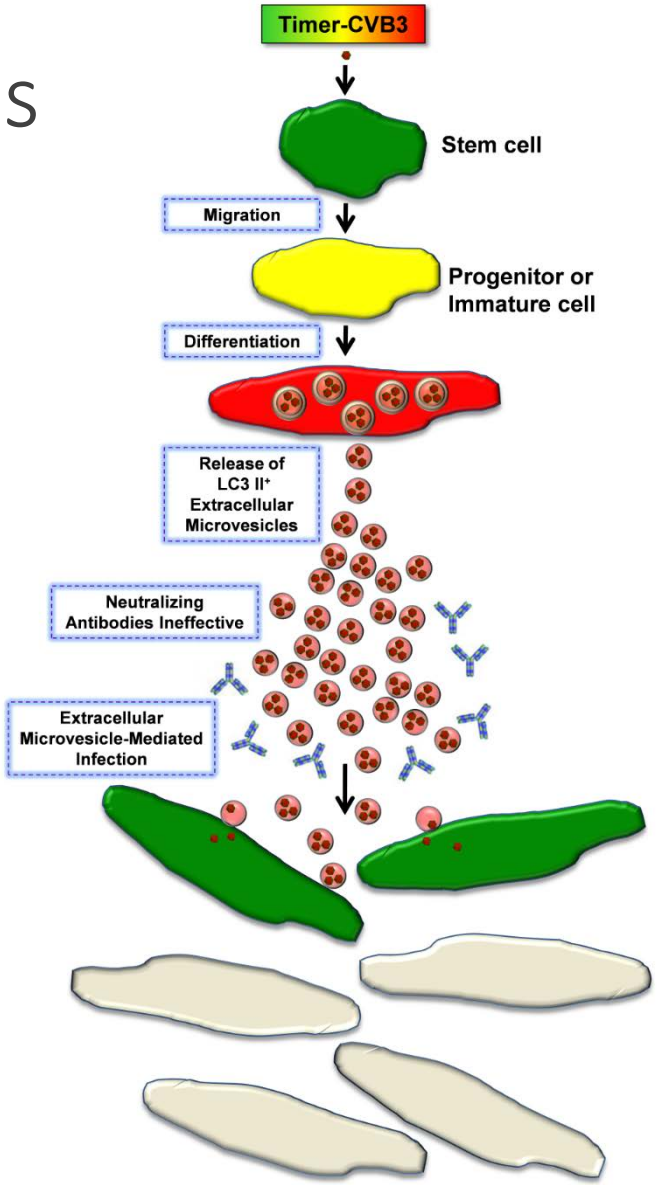
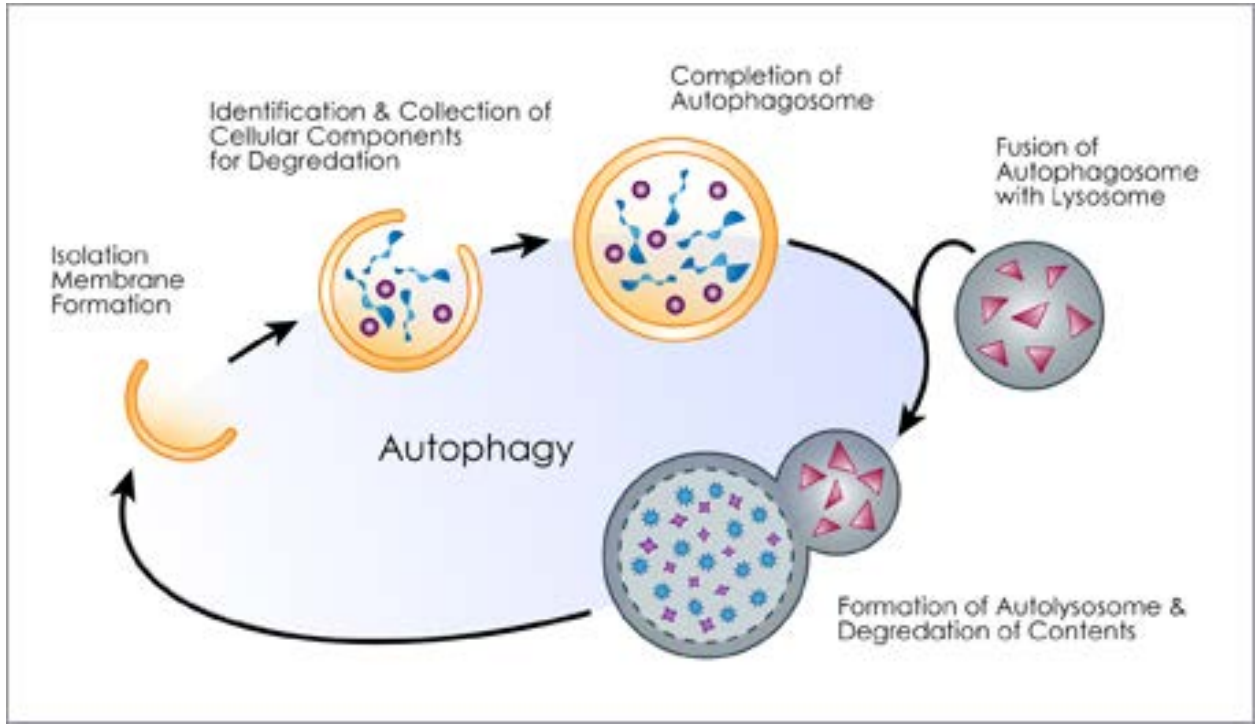
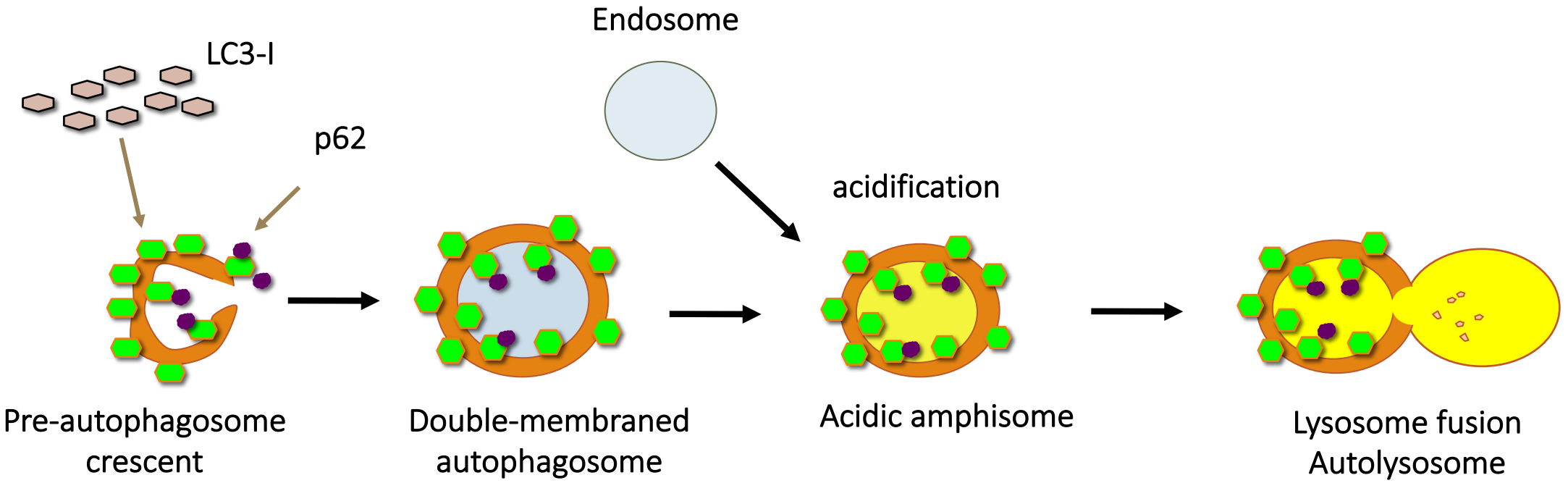


What is autophagy?

- "Self Eating," cells literally digest themselves
- Important part of cellular homeostasis, organelle turnover.
- Critical in development, esp. transition from womb to postnatal feeding in mammals.
- Stress response, especially to starvation.
- Diseases: Neurodegenerative (Huntingtons)
  Digestive (Crohn's)
  Cancer
  Anti- and pro- microbial

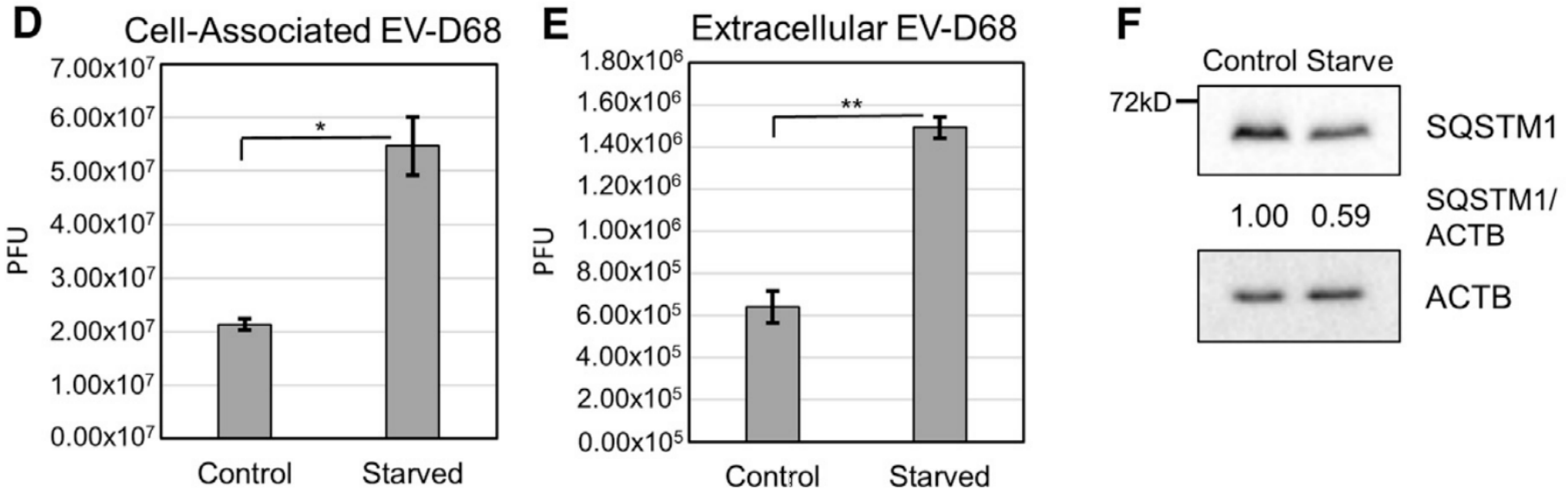


Membranes of the autophagic pathway

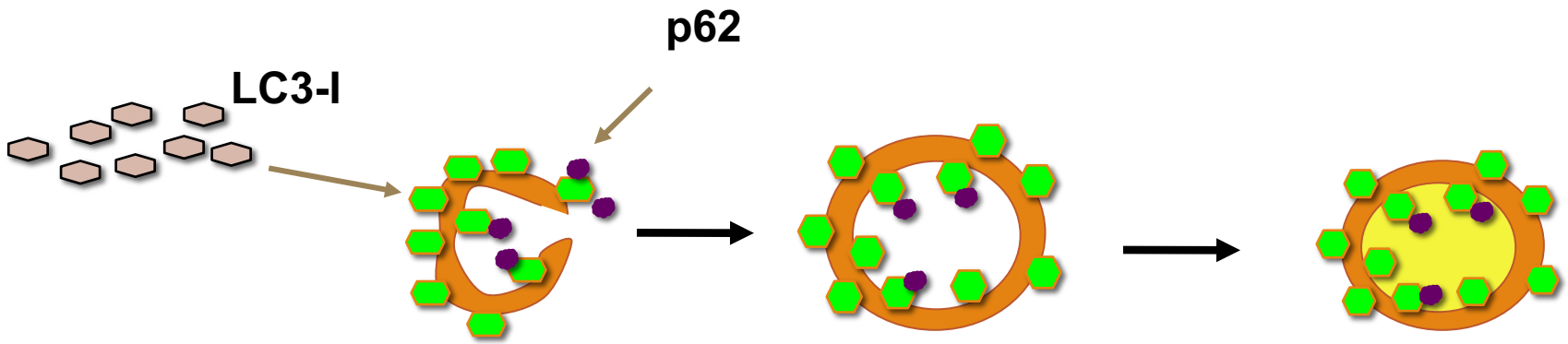


Does this represent an anti-viral response to degrade virions?

Autophagy promotes replication of EV-D68



Is infection activating autophagic degradation?



Is autolysosome activity required
for infectious virus replication?

Is an anti-viral factor being degraded?

If virus is inside autophagosomes, then
how does the virus survive the autolysosome?

Lysosome fusion
Autolysosome

Autophagosome-lysosome fusion is regulated by SNARE proteins

Syntaxin17

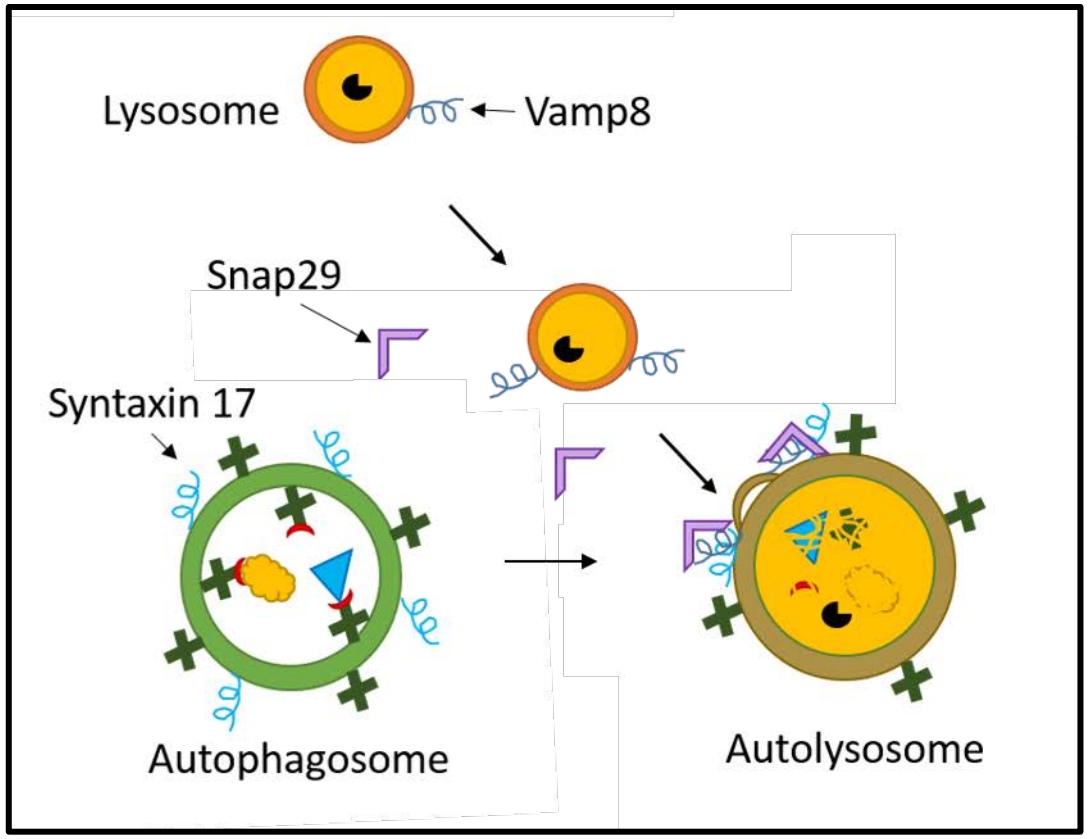
- Autophagosomal SNARE
- May also play role in ER to Golgi transport

Vamp8

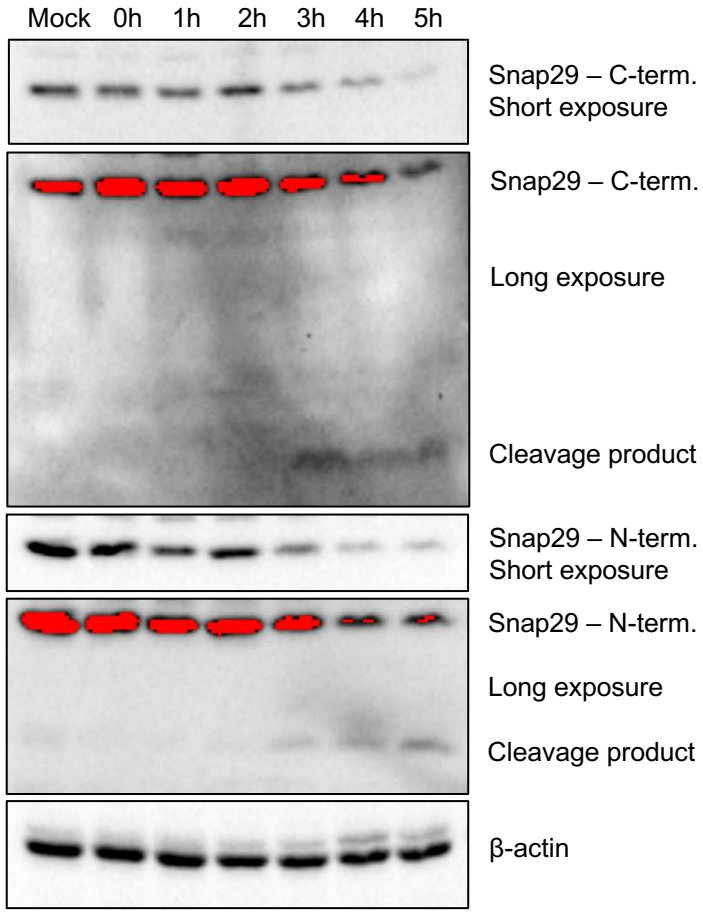
- Lysosomal SNARE

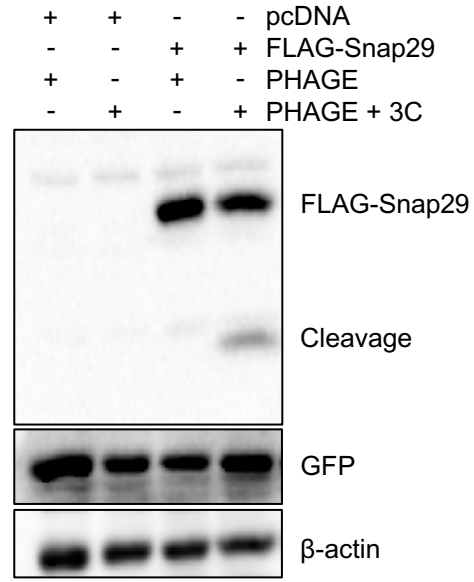
Snap29

- Regulator of autophagosome and lysosome membrane fusion



Snap29 is cleaved by the EV-D68 3C protease



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MSAYPKSYNP FDDDGEDEGA RPAPWRDARD 30
LPDGPDAPAD RQQYLRQEVL RRAEATAAST 60
SRSLALMYES EKVGVASSEE LARQRGVLER 90
TEKMVDKMDQ DLKISQKHIN SIKSVFGGLV 120
NYFKSKPVET PPEQNGTLTS QPNNRLKEAI 150
STSKEQEAKY QASHPNLRKL DDTDPVPRGA 180
GSAMSTDAYP KNPHLRAYHQ KIDSNLDELS 210
MGLGRLKDIA LGMQTEIEEQ DDILDRLTTK 240
VDKLDVNIKS TERKVRQL
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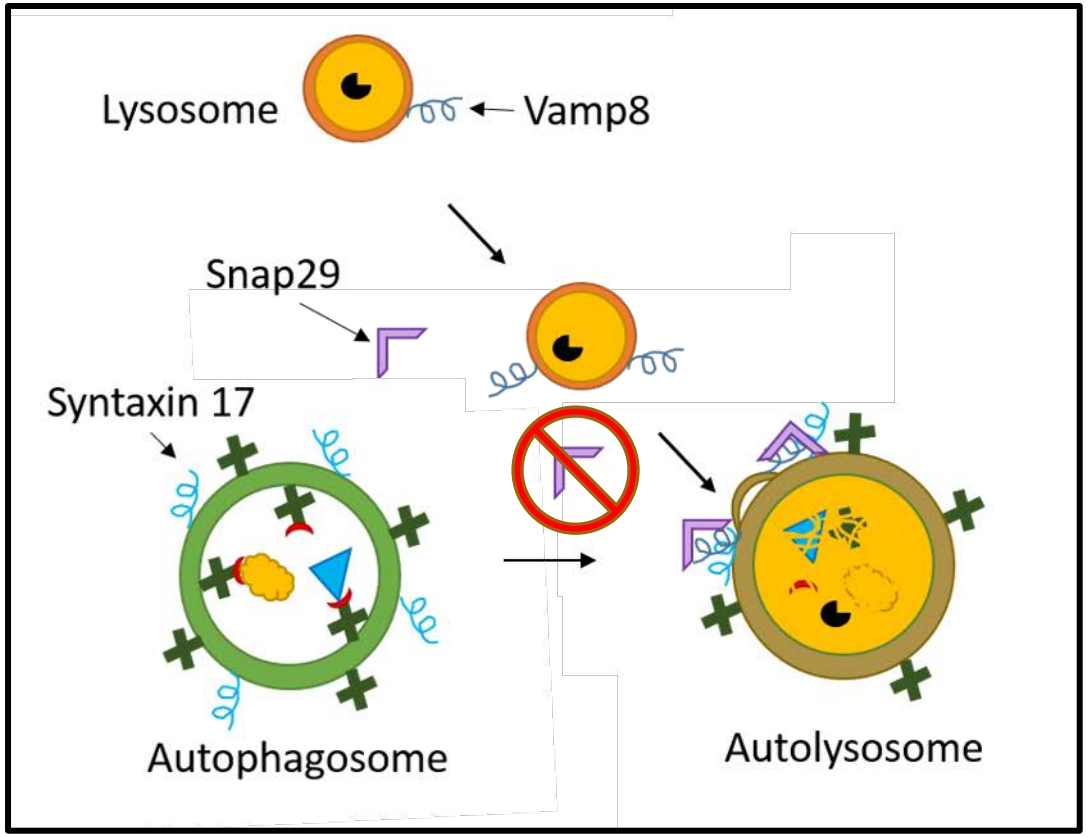
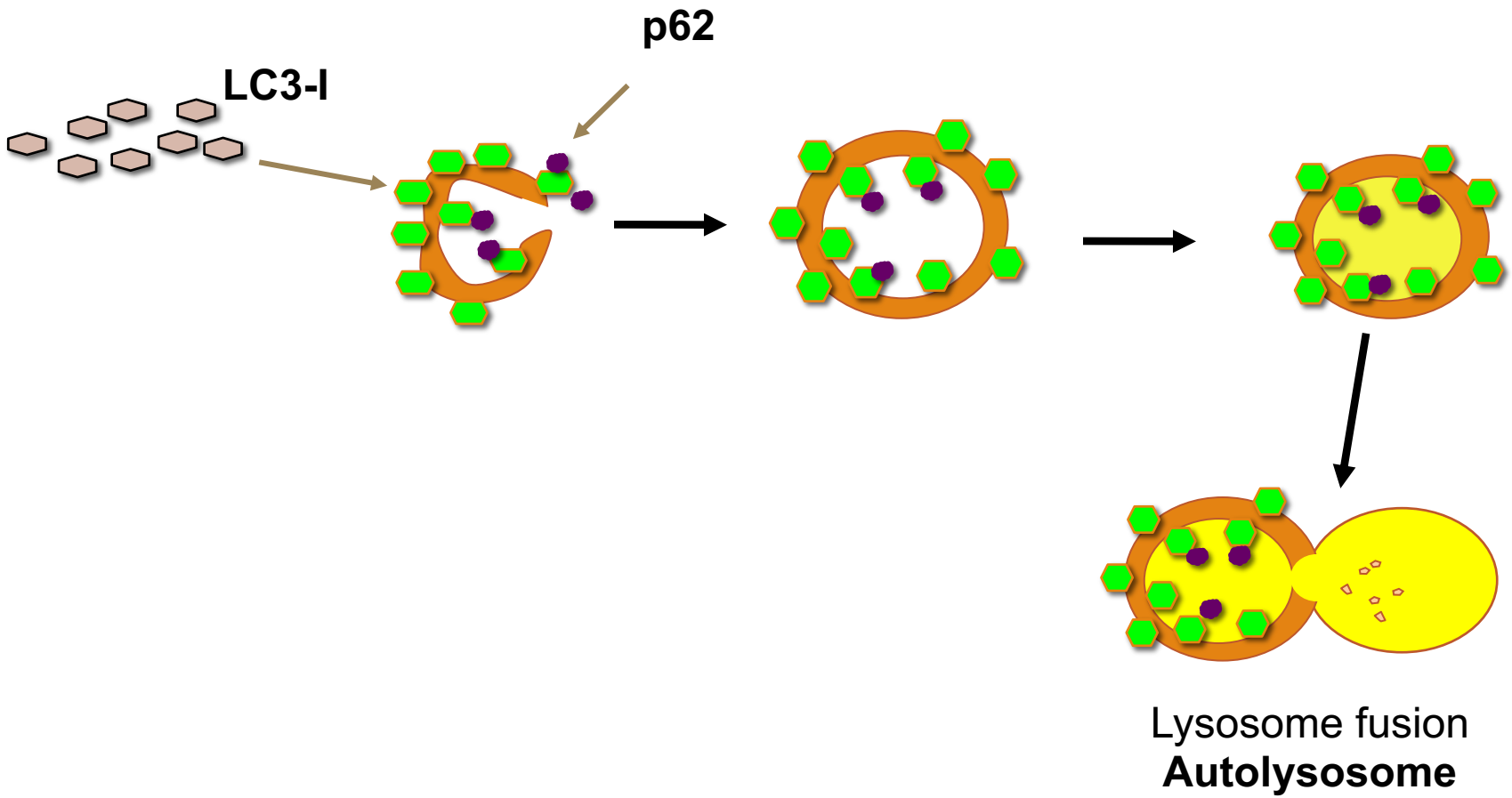
Corona *et al,* Cell Rep 22 (12), 3304-3314

Separating the two halves of SNAP29 inhibits progression of the pathway

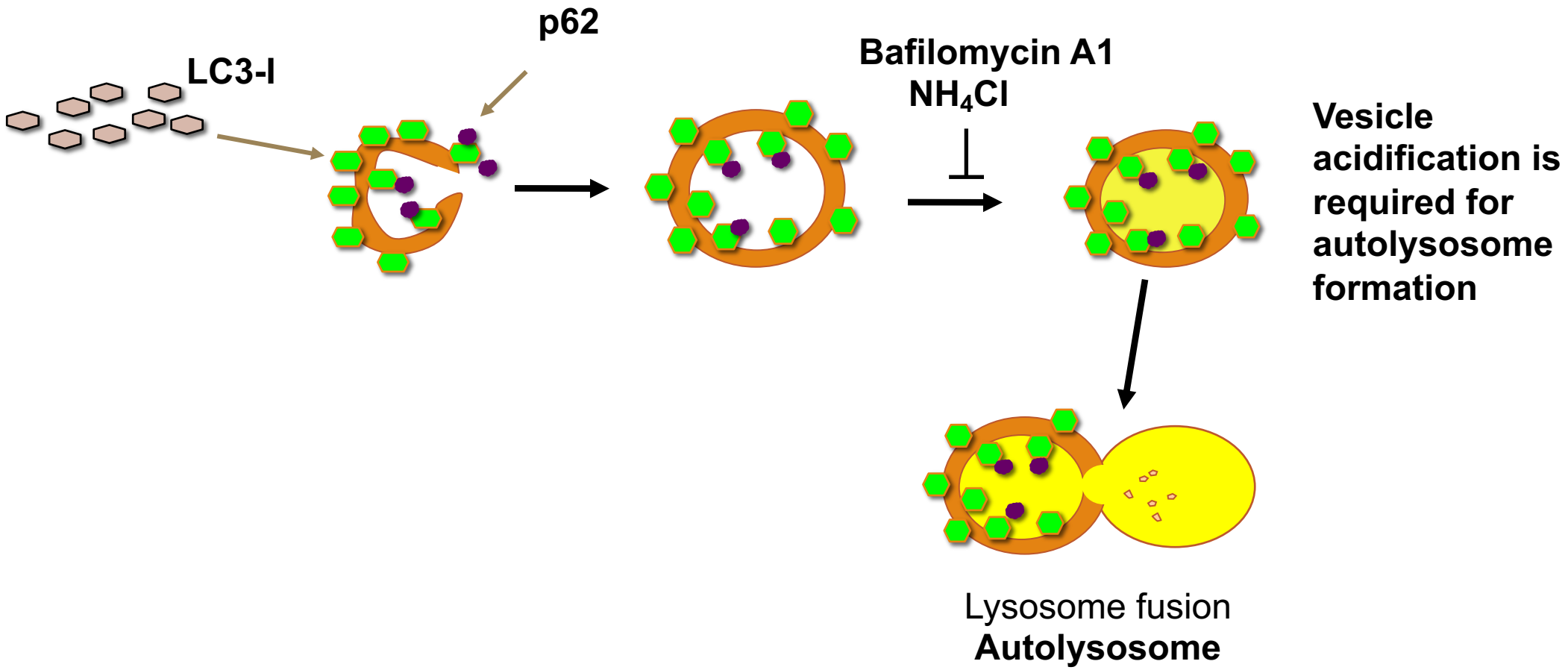


Why is the virus activating the autophagic pathway?

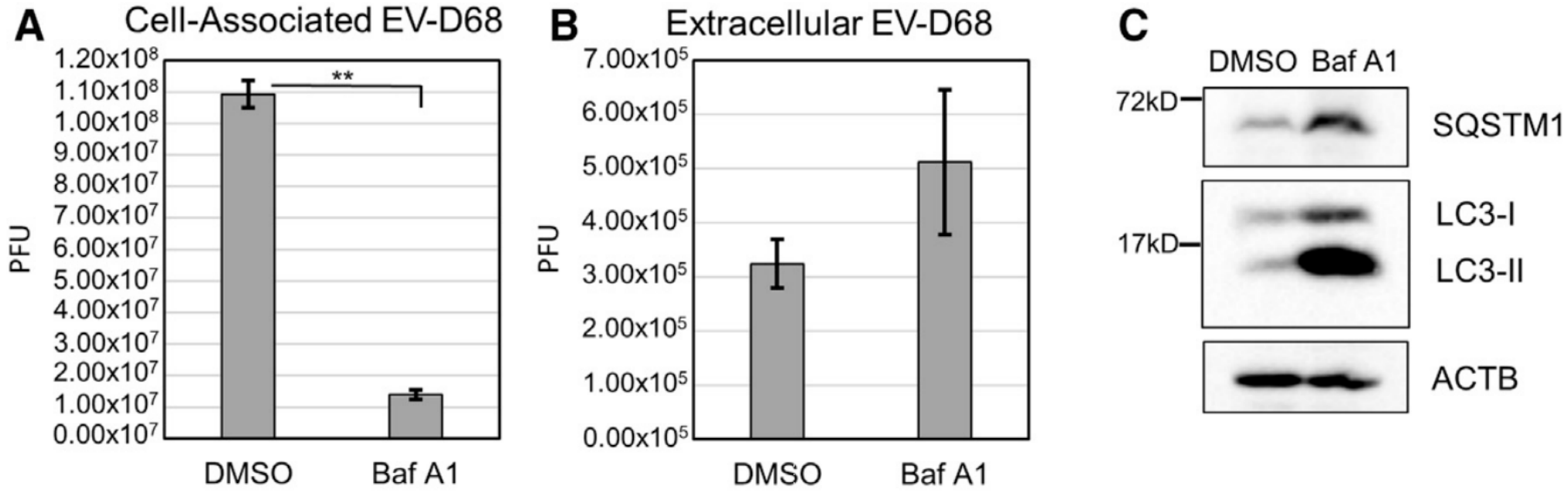


It's not the Lysosome

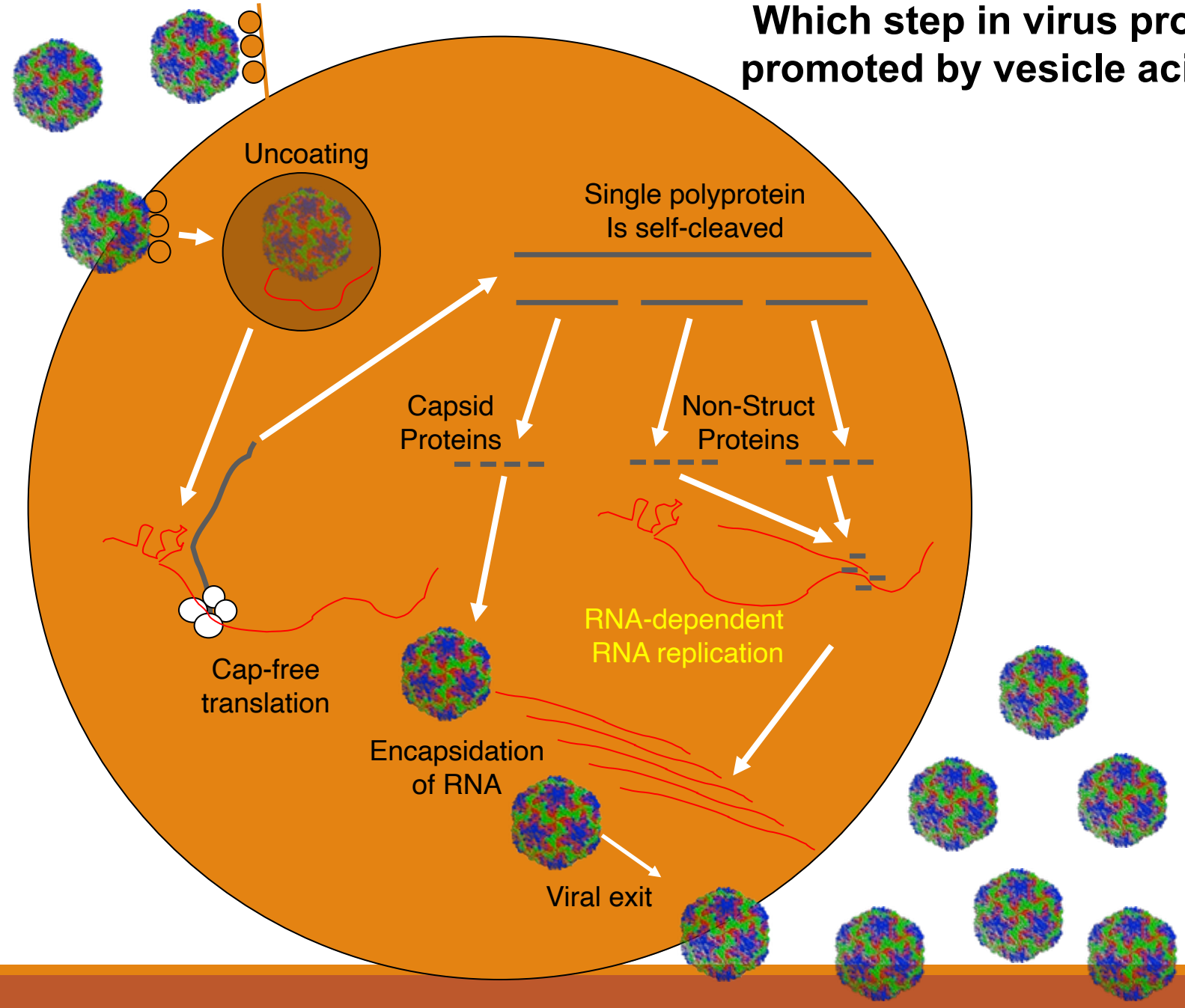
Why is the virus activating the autophagic pathway?



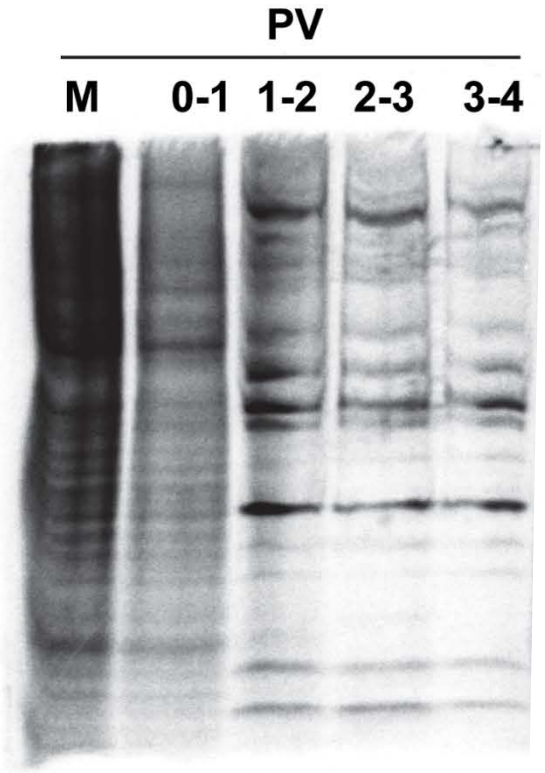
Infectious virus production is reduced following inhibition of vesicle acidification



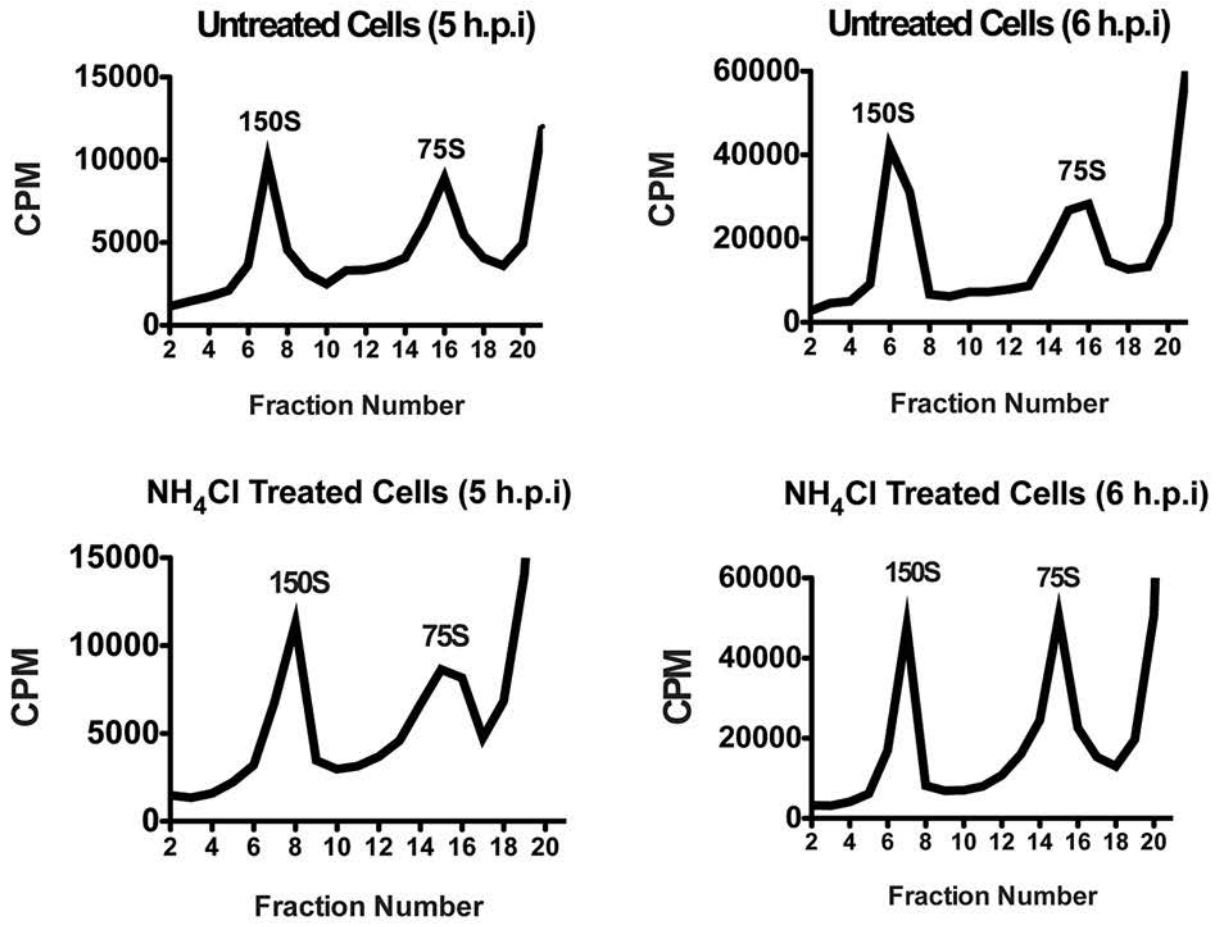
Which step in virus production is promoted by vesicle acidification?



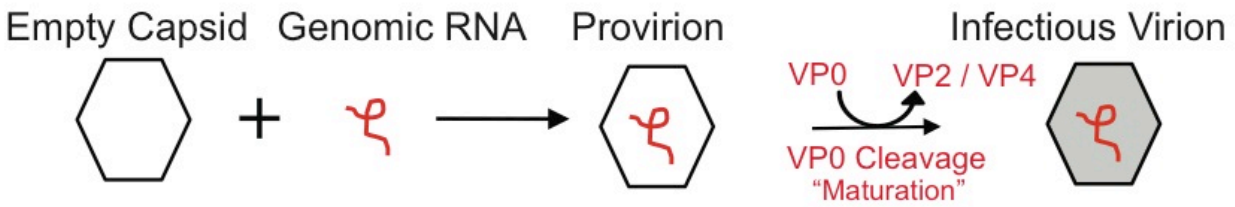
Which step in virus production is promoted by vesicle acidification?



NH_4Cl treatment does not have a major effect on overall levels of empty and non-infectious PV capsids

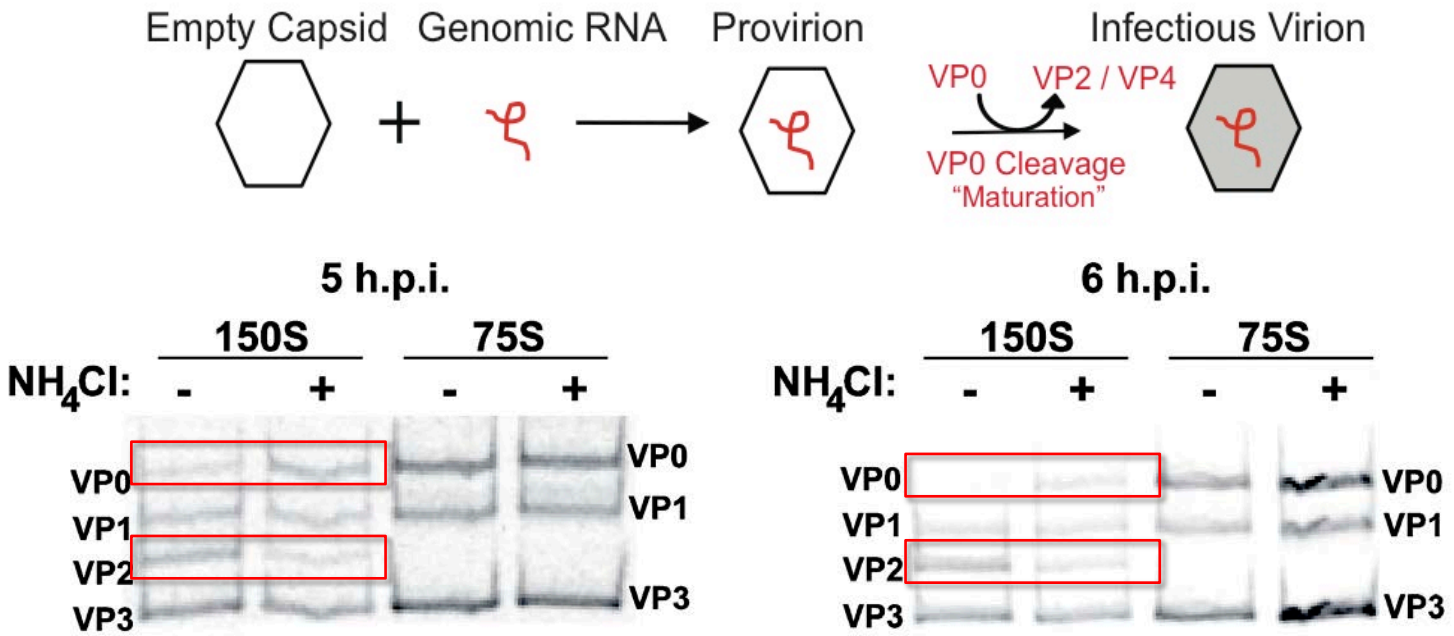


Sucrose gradients can be used to separate empty and genome-containing capsids



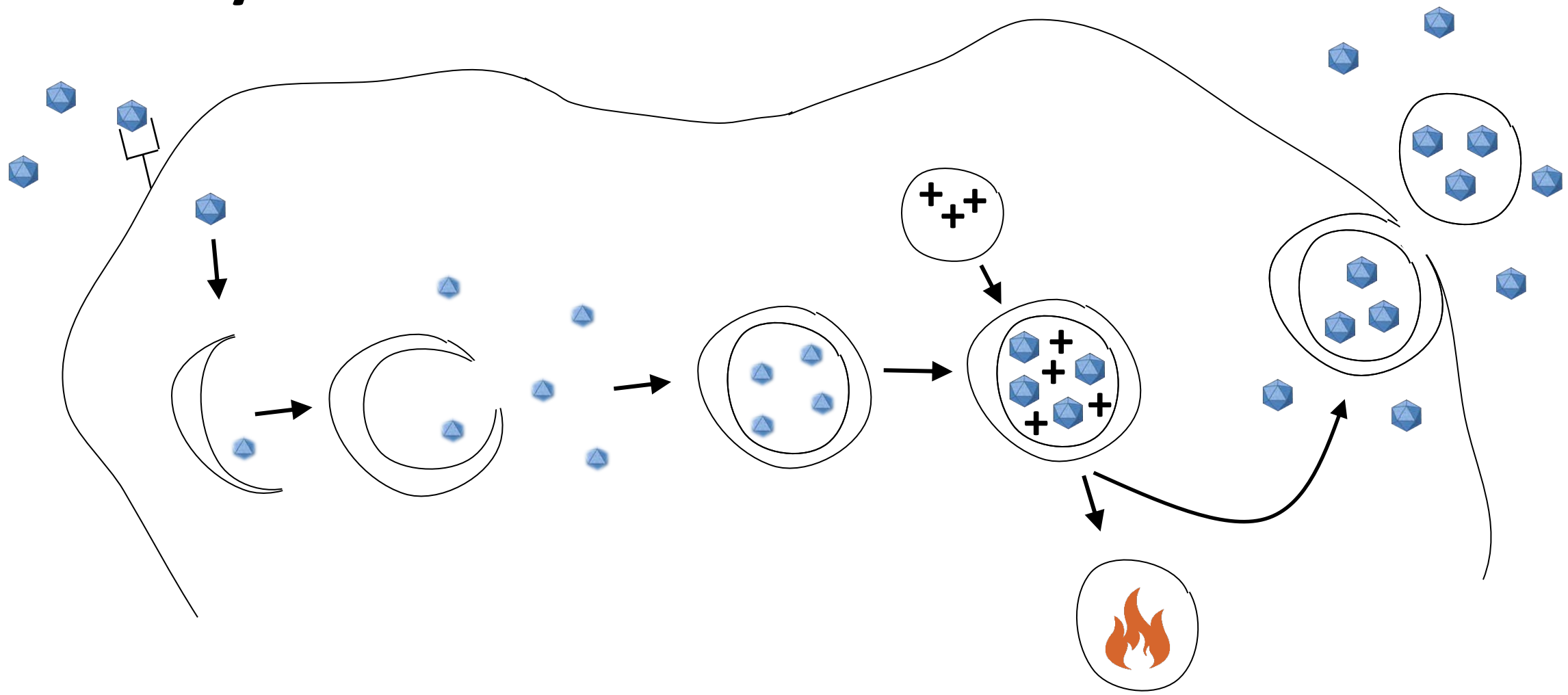
	Capsid Proteins	Sedimentation Velocity	Genome
Empty Capsid	VP0, VP1, VP3	75S	No
Provirion	VP0, VP1, VP3	150S	Yes
Infectious Virion	VP1, VP2, VP3, VP4	150S	Yes

NH_4Cl treatment inhibits the VP0 maturation cleavage required to generate infectious virus.



Acidic vesicles promote capsid maturation, the very last step in generating infectious poliovirus.

Summary



Summary

- Picornaviruses, including EV-D68, can be released in membranous vesicles.
- These vesicles are believed to be for cell-to-cell transmission within a host.
- They often have multiple virions and display phosphotidylserine.
- For many enteroviruses these membranes are derived from the autophagy pathway.
- The viruses appear to rewire the autophagy pathway to promote non-canonical secretion of virus-filled vesicles.

Acknowledgements

Milwaukee

Kate Klein

Alexsia Richards

Geoff Riddell

Jamaria Martins

Sarah Timmler

Baltimore

Abby McGillivray

Angel Corona Velazquez

Holly Saulsbery

Margaret Gerardi

Zhiqiang Hu

Sohha Ariannejad

Katelyn Miller

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Enterovirus D68 and neurotropism

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