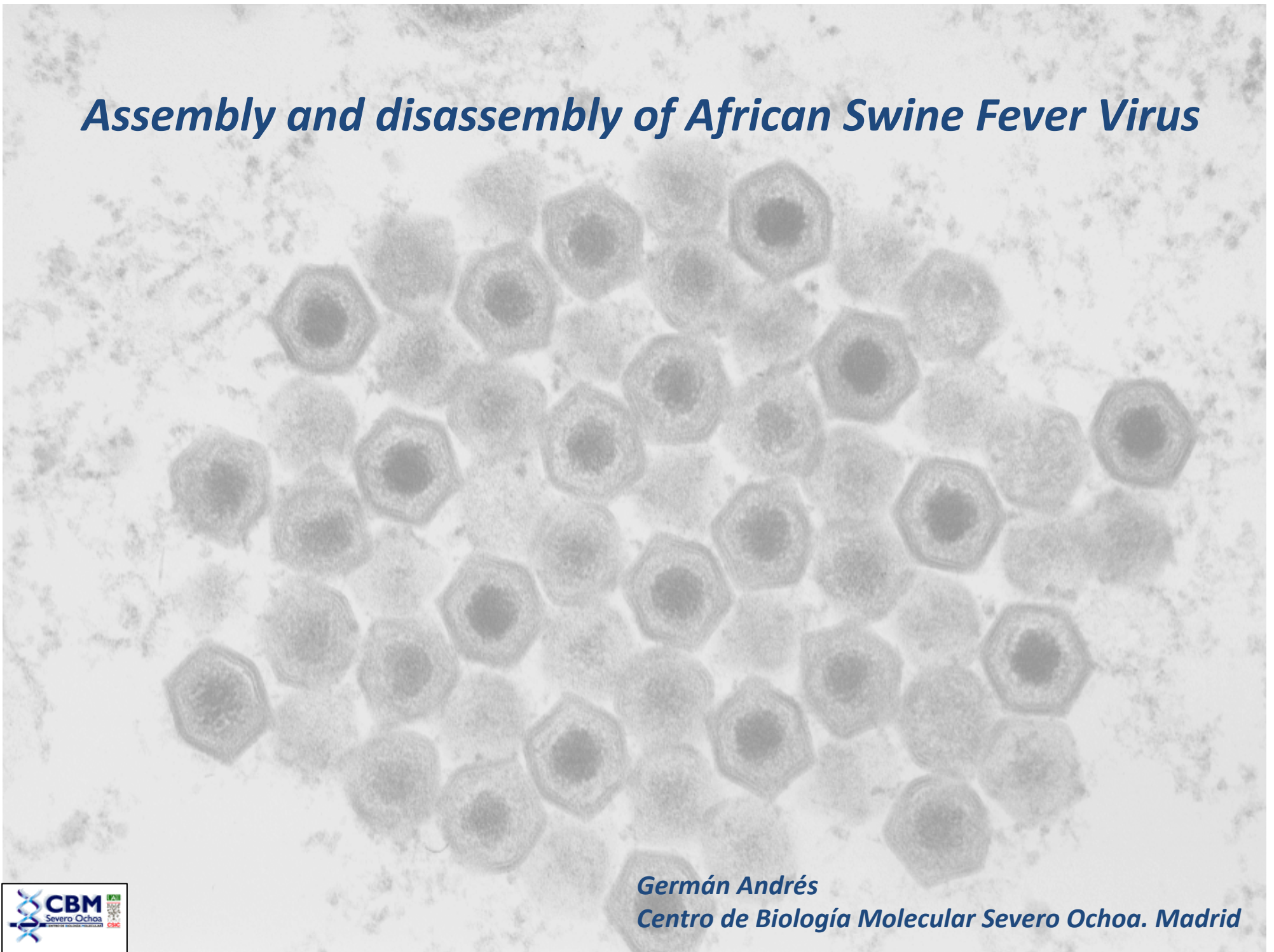


Assembly and disassembly of African Swine Fever Virus



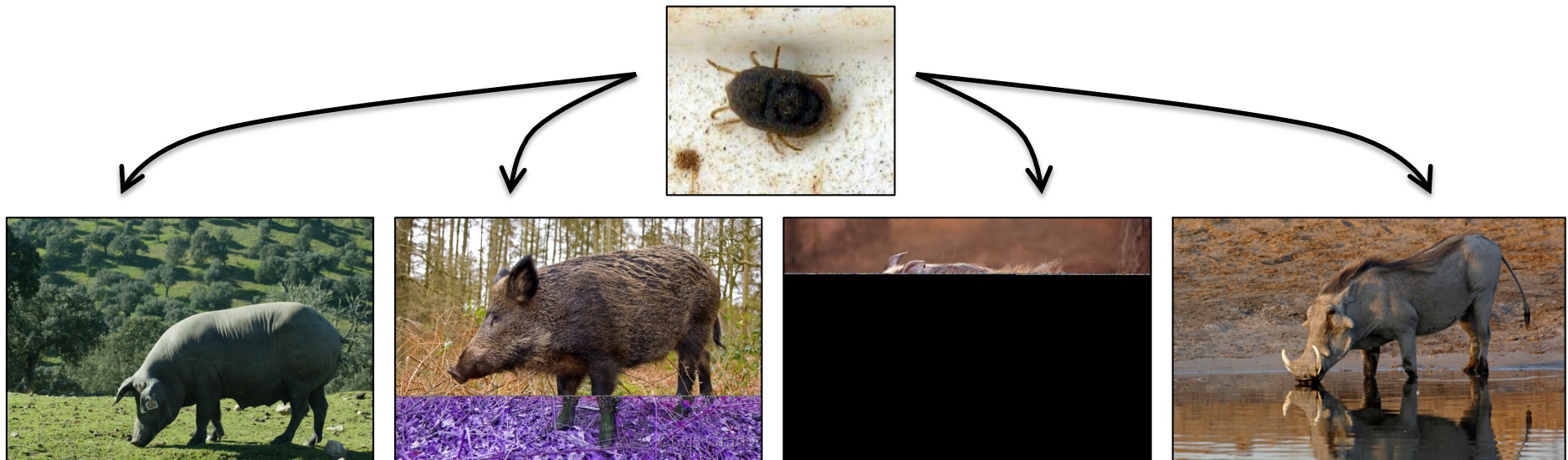
AFRICAN SWINE FEVER

African swine fever (ASF) is a highly lethal hemorrhagic disease of domestic pigs.

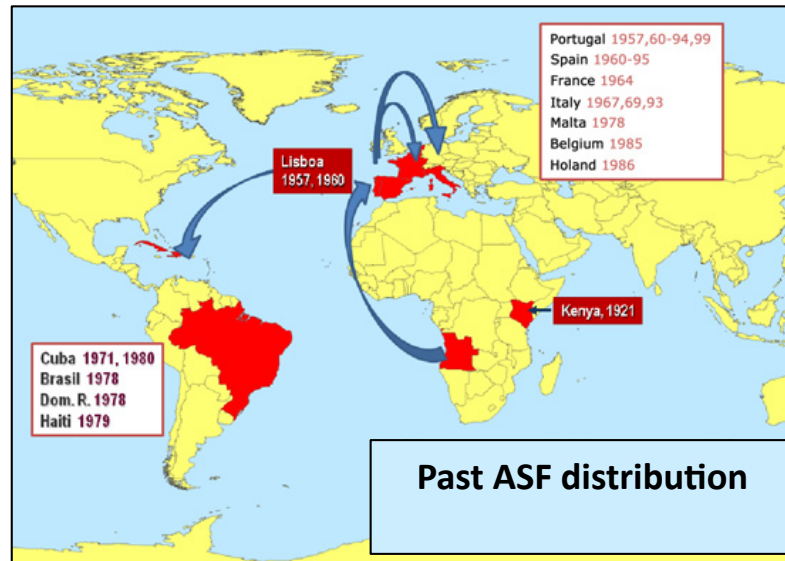
The causative agent, African swine fever virus (ASFV), is a complex large DNA virus.

ASFV also infects warthogs, bushpig and wild boars, with no obvious disease signs, as well as soft ticks, which act as vectors of the disease .

There is no effective treatment or vaccine for ASFV.



AFRICAN SWINE FEVER



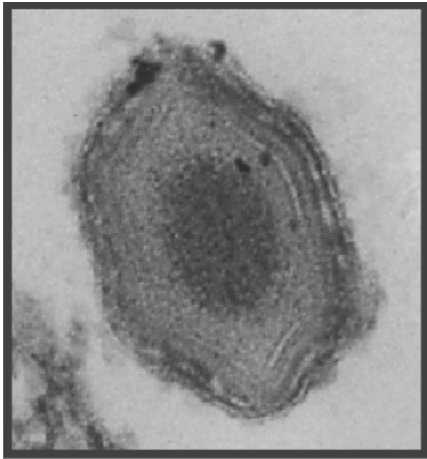
ASF appeared in 1957-1960 in Spain, Portugal and Sardinia. The disease was eradicated from Portugal and Spain in 1995, but remains enzootic in Sardinia.

New ASFV outbreaks (2007-2013) detected in Eastern Europe.

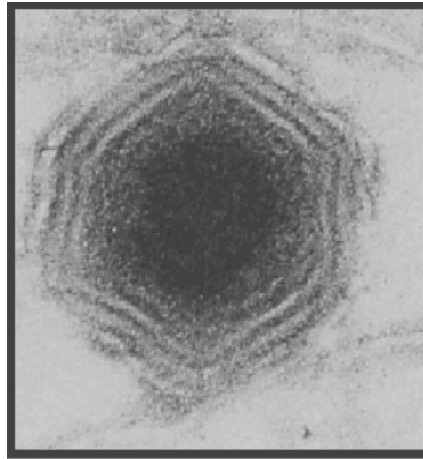
African Swine Fever Virus (ASFV)

- ▶ **Family:** *Asfarviridae* (only member), belongs to superfamily of nucleocytoplasmic large DNA viruses (NCLDV)
- ▶ **Structure:** Large double-enveloped icosahedral virus ($\phi \approx 200$ nm)
Contains > 50 structural proteins
- ▶ **Genome:** dsDNA (170-190 kbp) with terminal cross-links
Encodes ≈ 150 genes
- ▶ **Host:** *Suidae* (pigs)
Ornithodoros (soft ticks)
- ▶ **Cell tropism:** Macrophages and monocytes
- ▶ **Disease:** Haemorrhagic fever leading to death

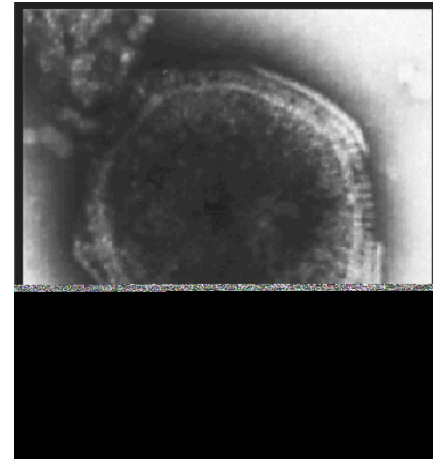
ASFV STRUCTURE



resin-embedded section

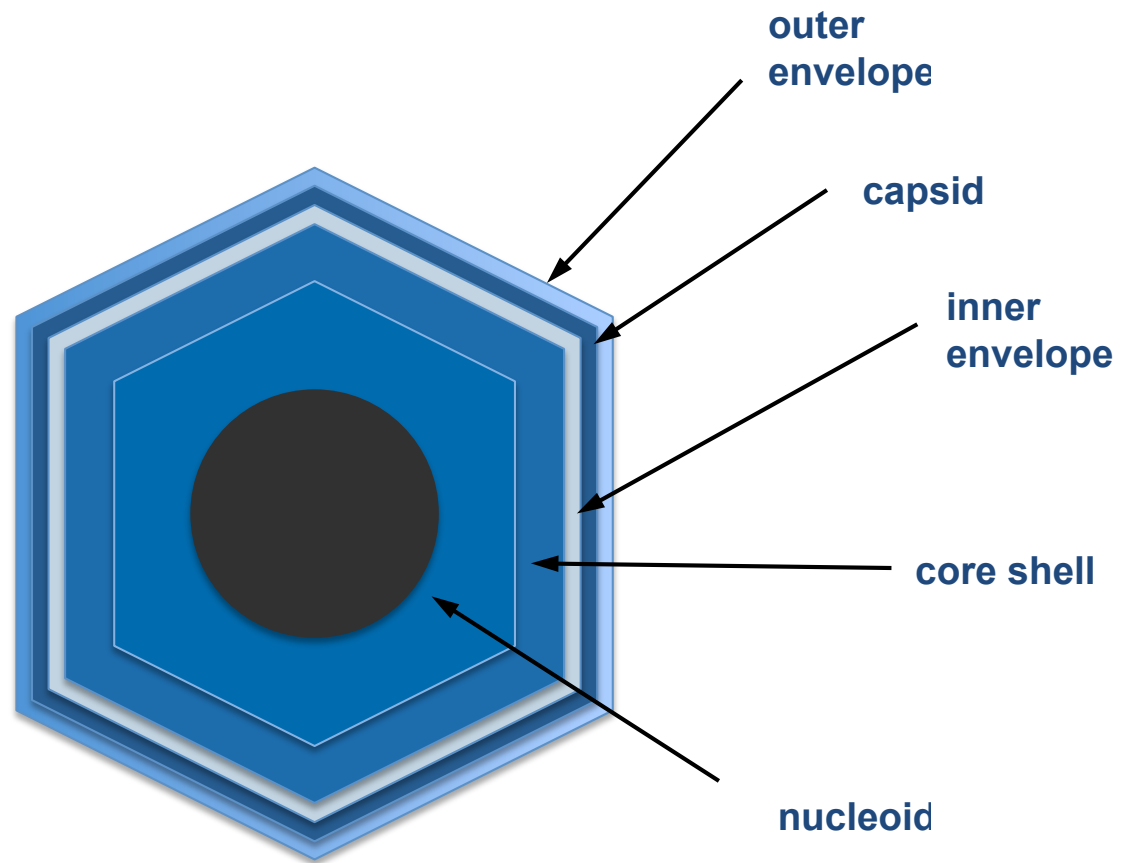


Thawed cryosection

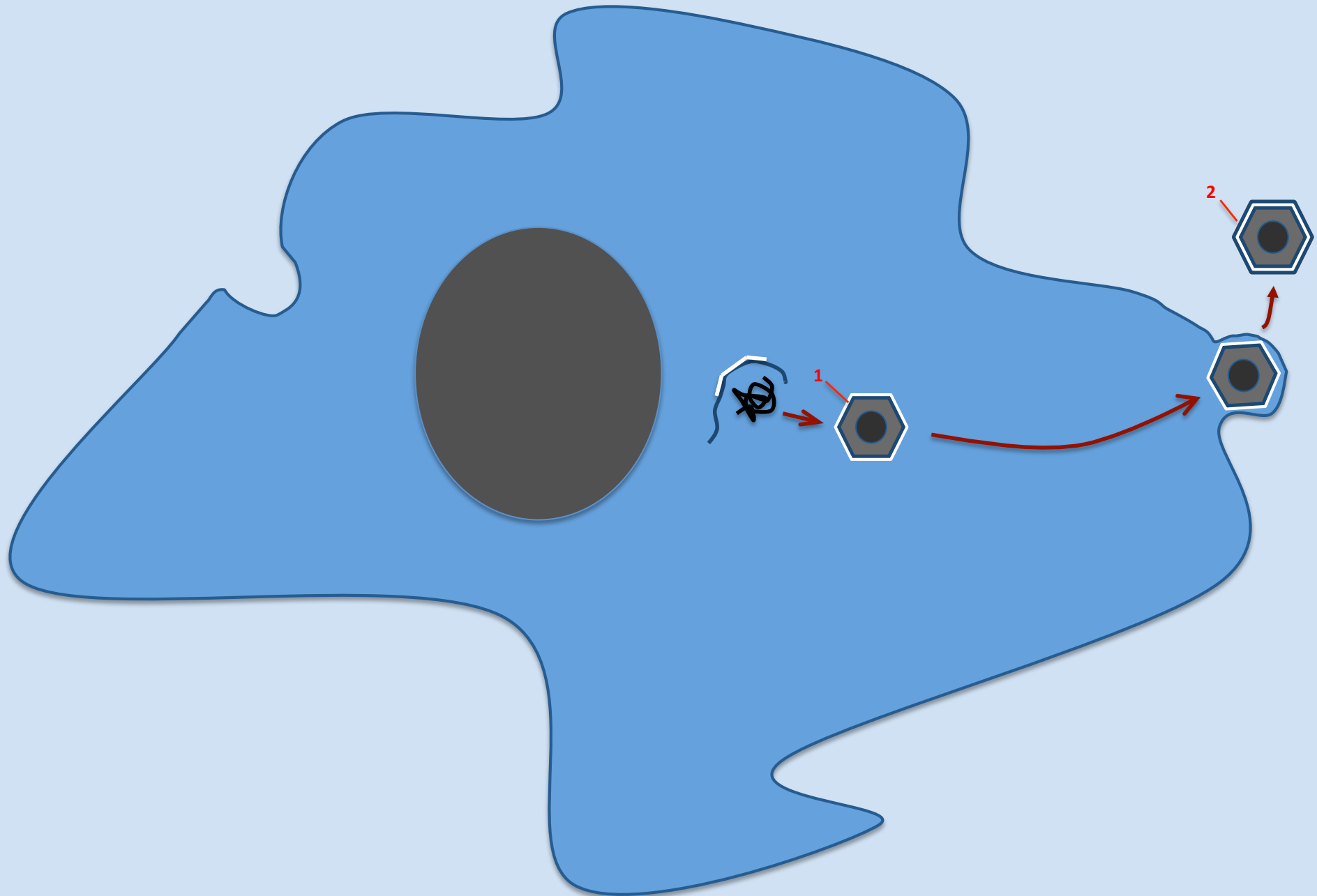


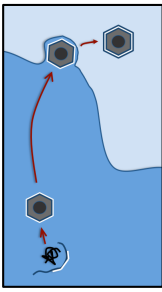
Negative Staining

ASFV STRUCTURE

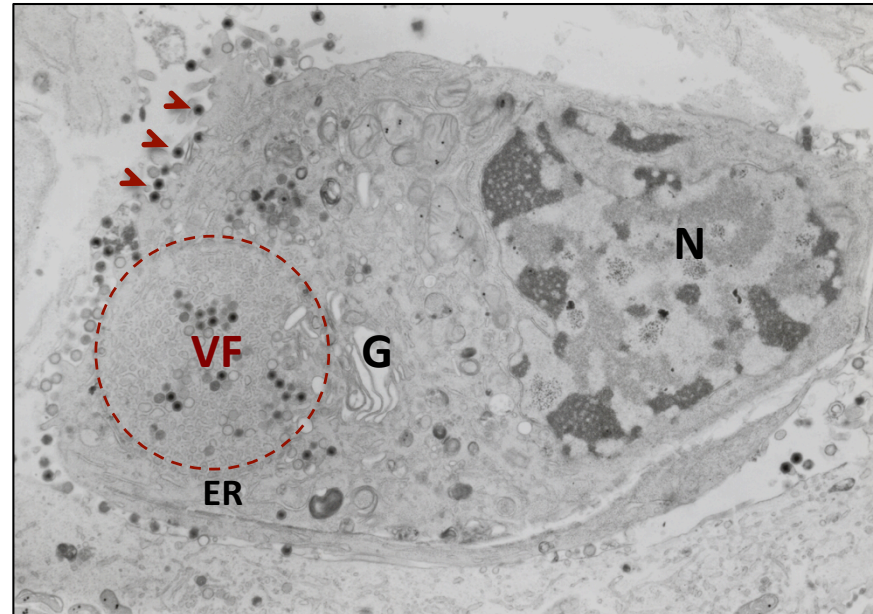
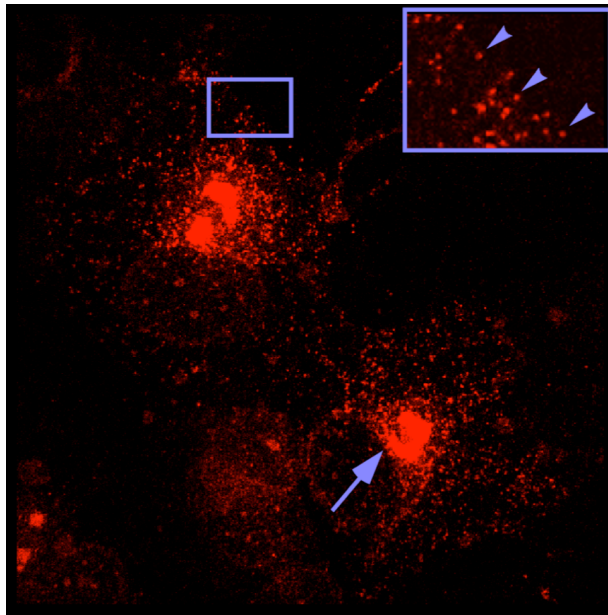


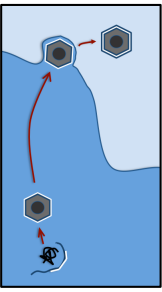
ASFV MORPHOGENESIS



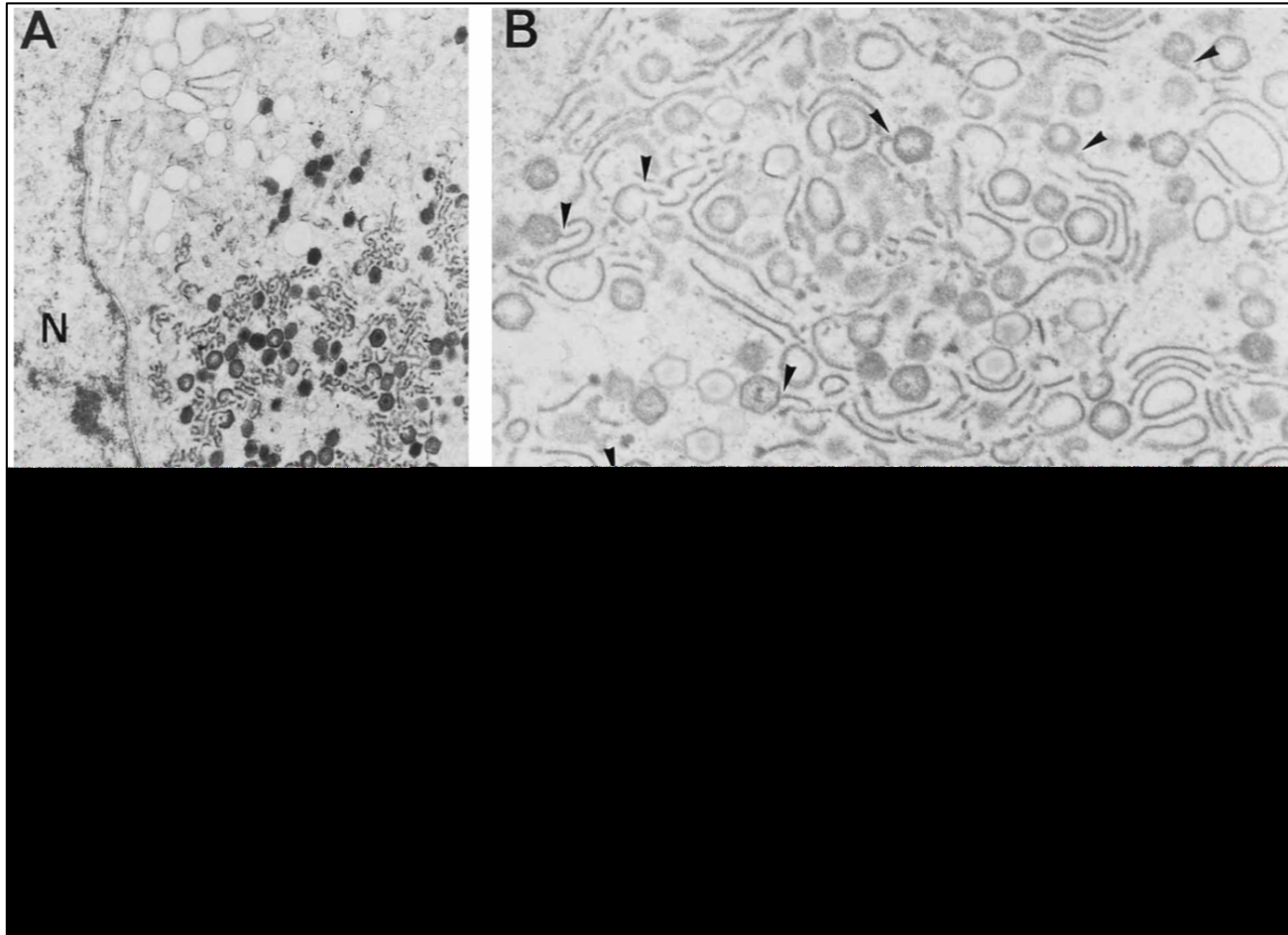


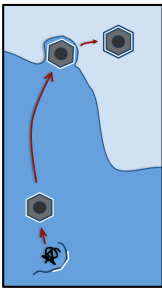
ASFV morphogenesis takes place into cytoplasmic viral factories



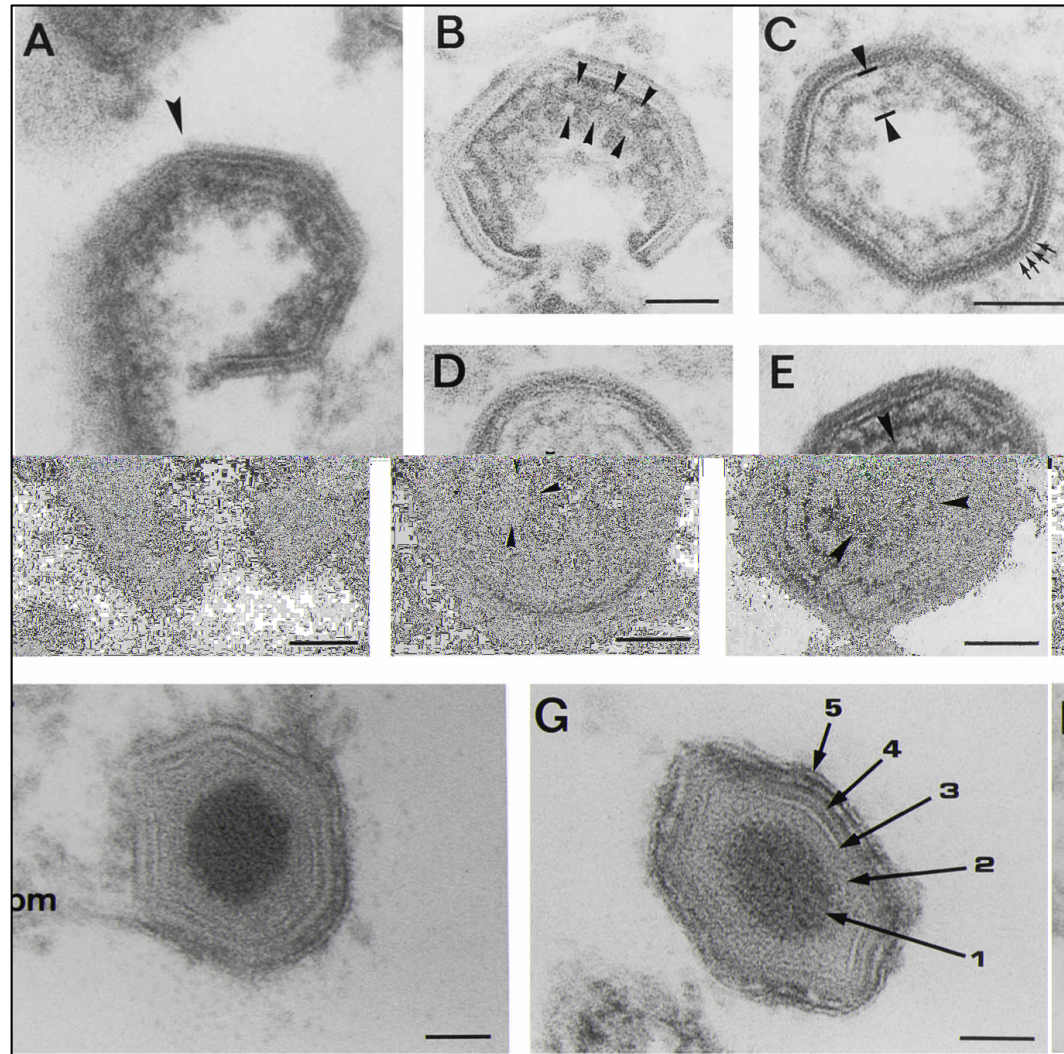


ASFV particles derive from precursor membranes



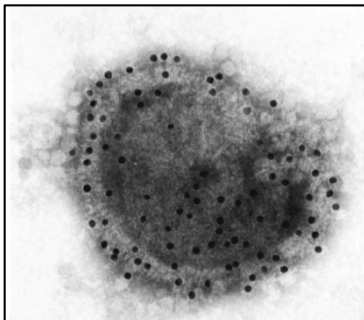
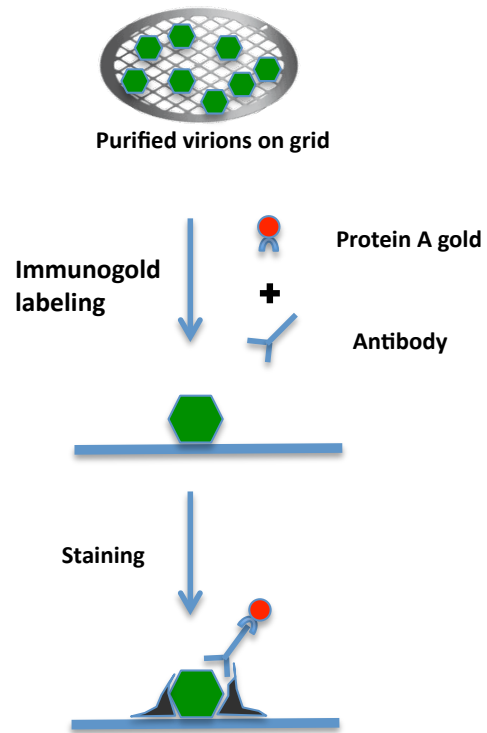


ASFV assembly sequence

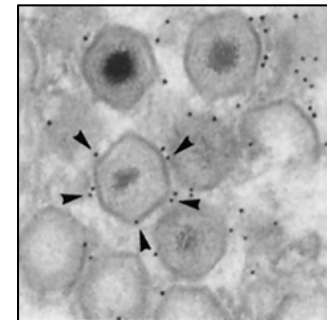
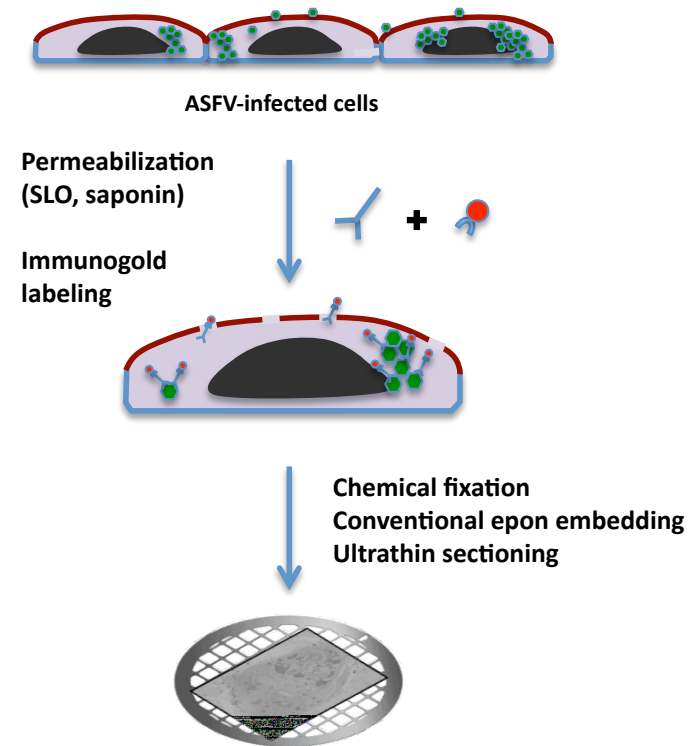


Methods used for immunolocalization of ASFV antigens (I)

Immunogold labeling + Negative staining (NE)

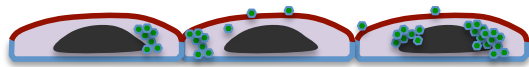


Pre-embedding immunogold labeling (PE)



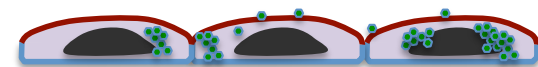
Methods used for immunolocalization of ASFV antigens (II)

Immunogold labeling on resin-embedded sections (FS)

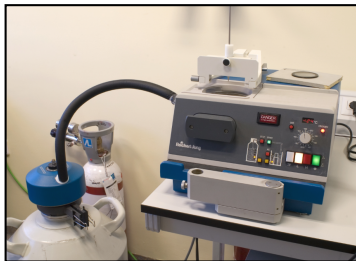


ASFV-infected cells

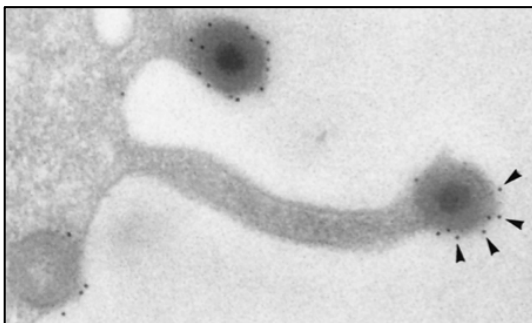
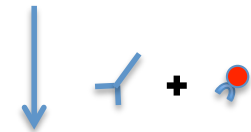
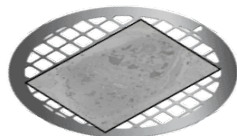
Immunogold labeling on cryosections (CS)



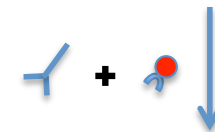
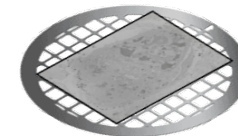
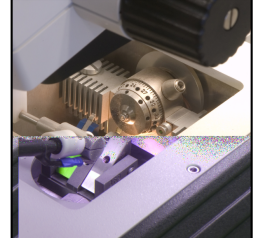
ASFV-infected cells



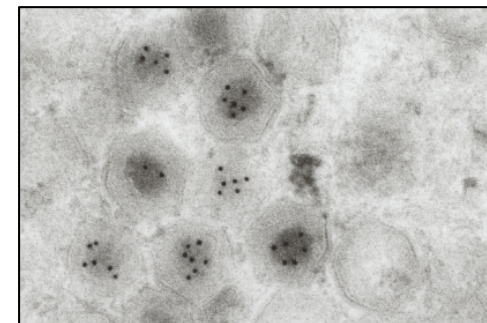
Chemical fixation
Cryoprotection (30% glycerol)
Vitrification (−180 °C)
Freeze-substitution (−90 °C)
Low-temperature embedding
UV-polymerization
Ultrathin sectioning

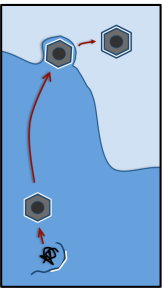


Chemical fixation
Cryoprotection (2.3M sucrose)
Vitrification (−196 °C)
Cryosectioning (−120 °C)
Section retrieval (Methylcellulose/
Sucrose) and thawing

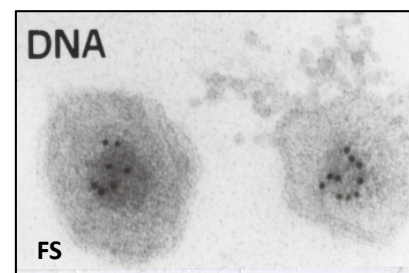
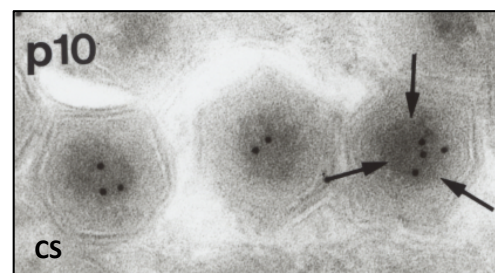
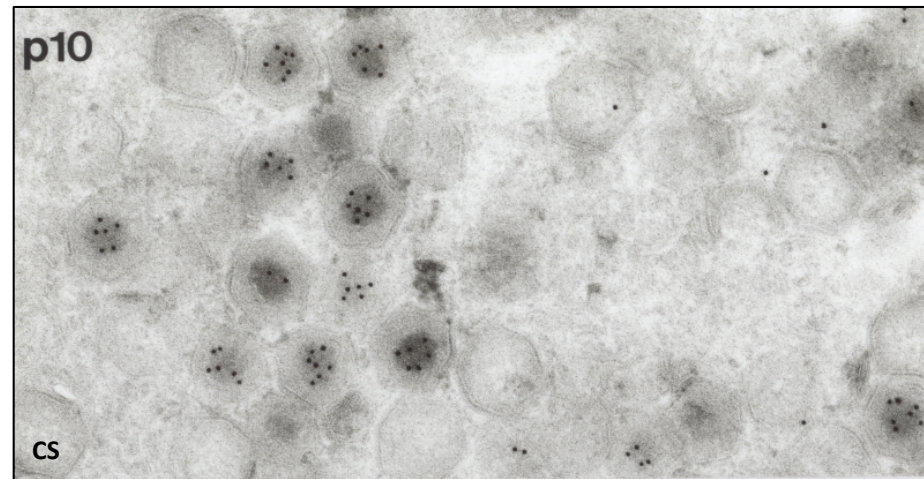


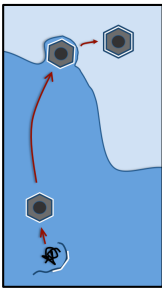
Immunogold
labeling



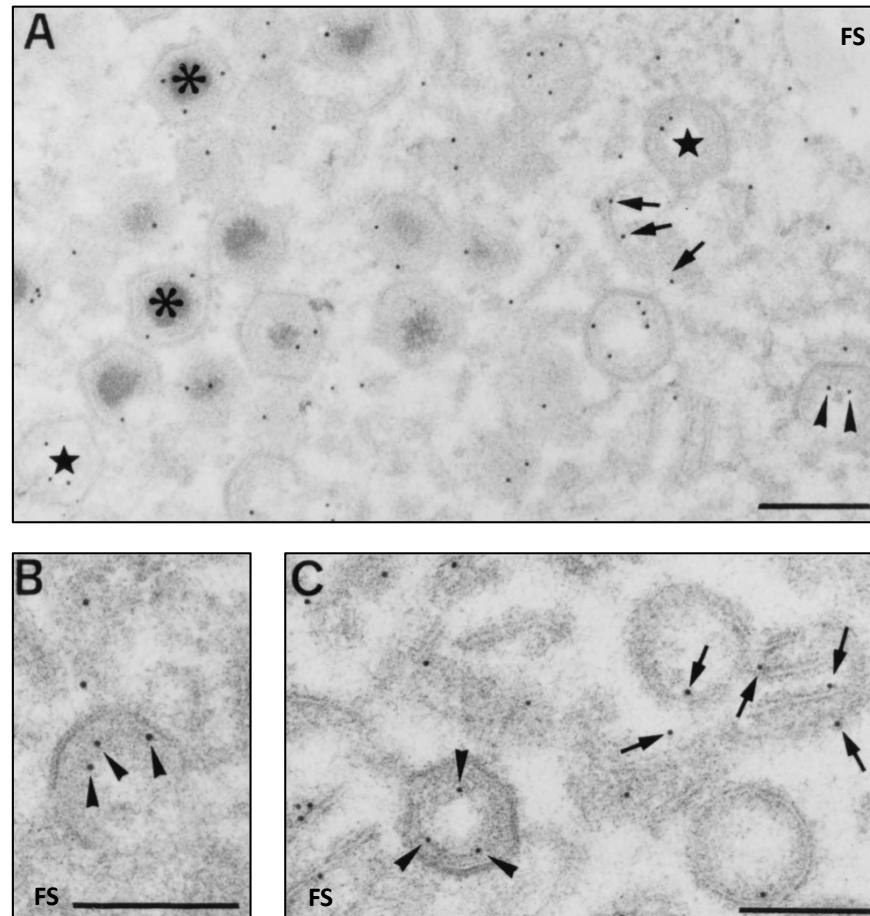


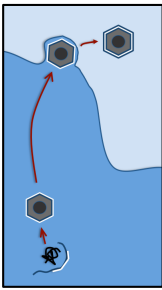
Nucleoid: p10 (DNA-binding protein)



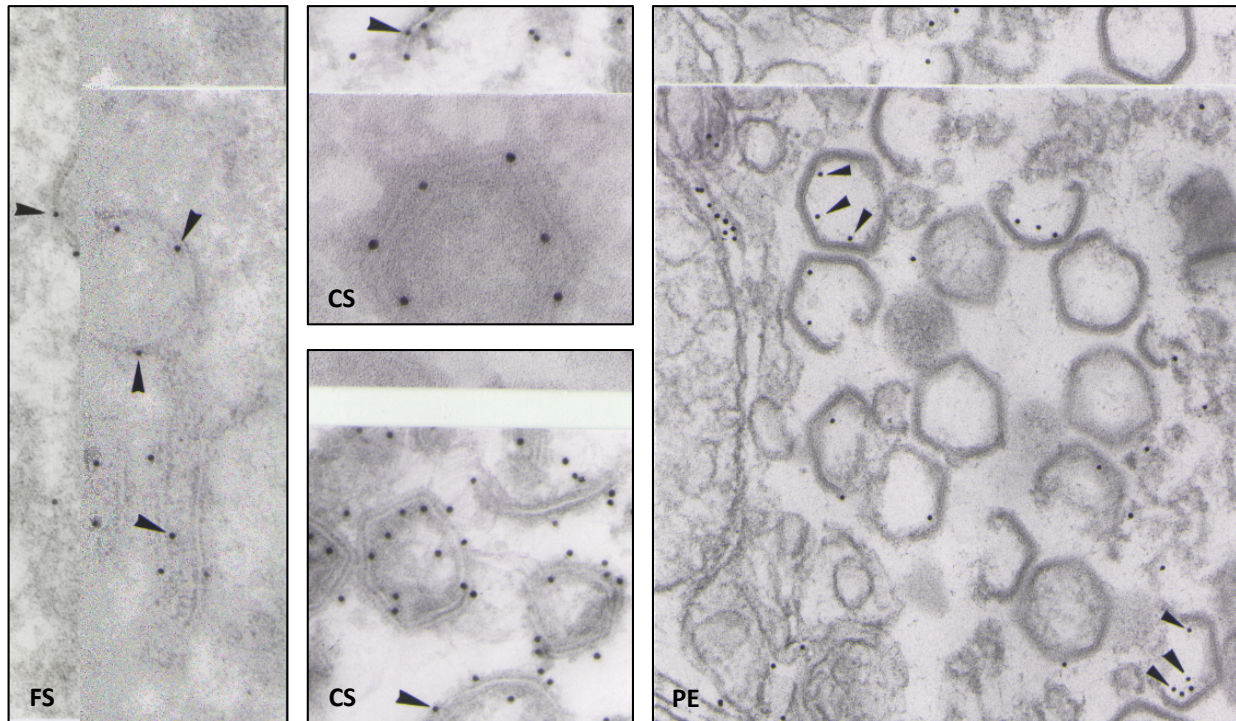


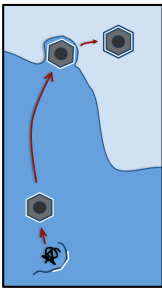
Core shell: polyprotein pp220 (p150+p137+p34+14)



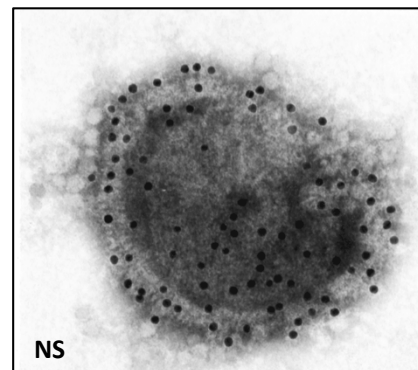
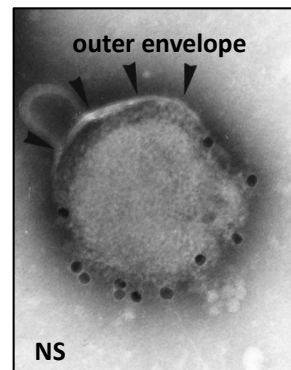
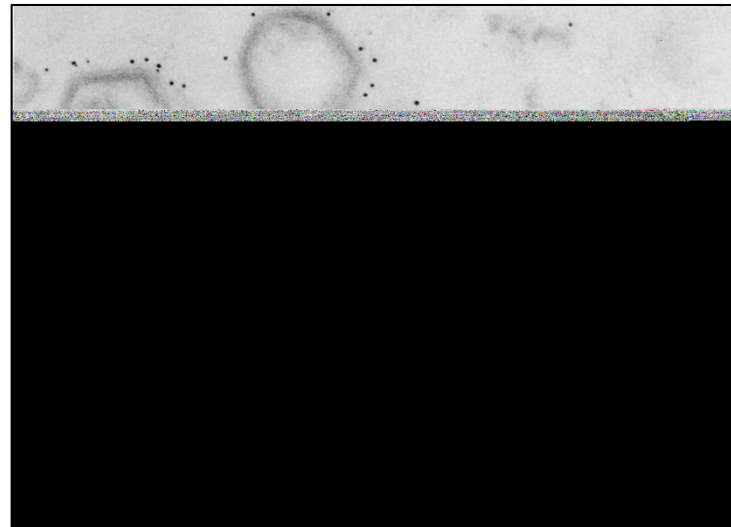


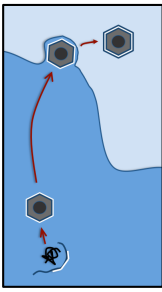
Inner envelope: p17 (transmembrane protein)



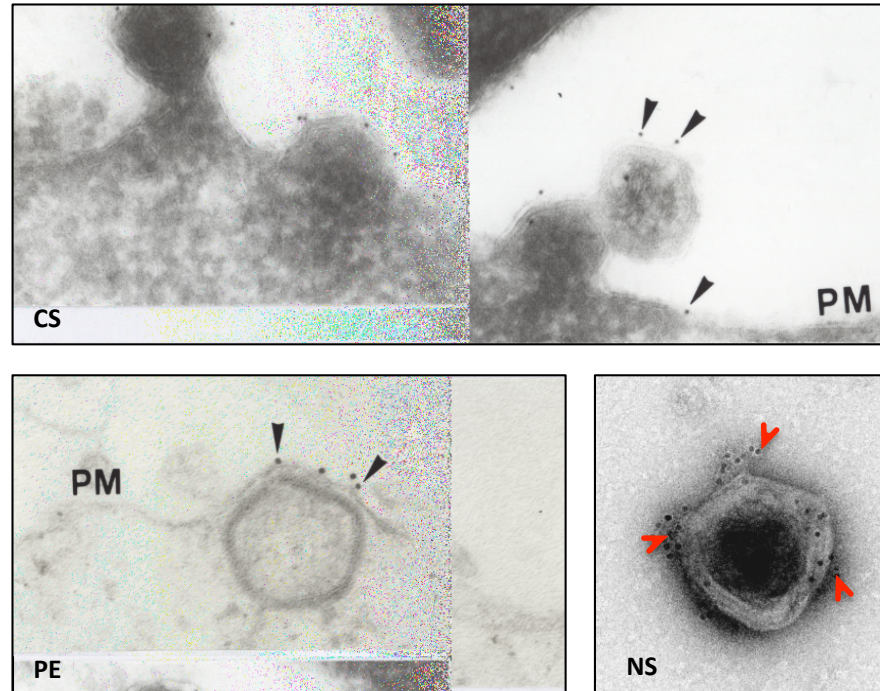


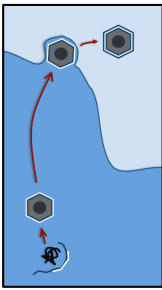
Capsid: p72 (capsomer protein)



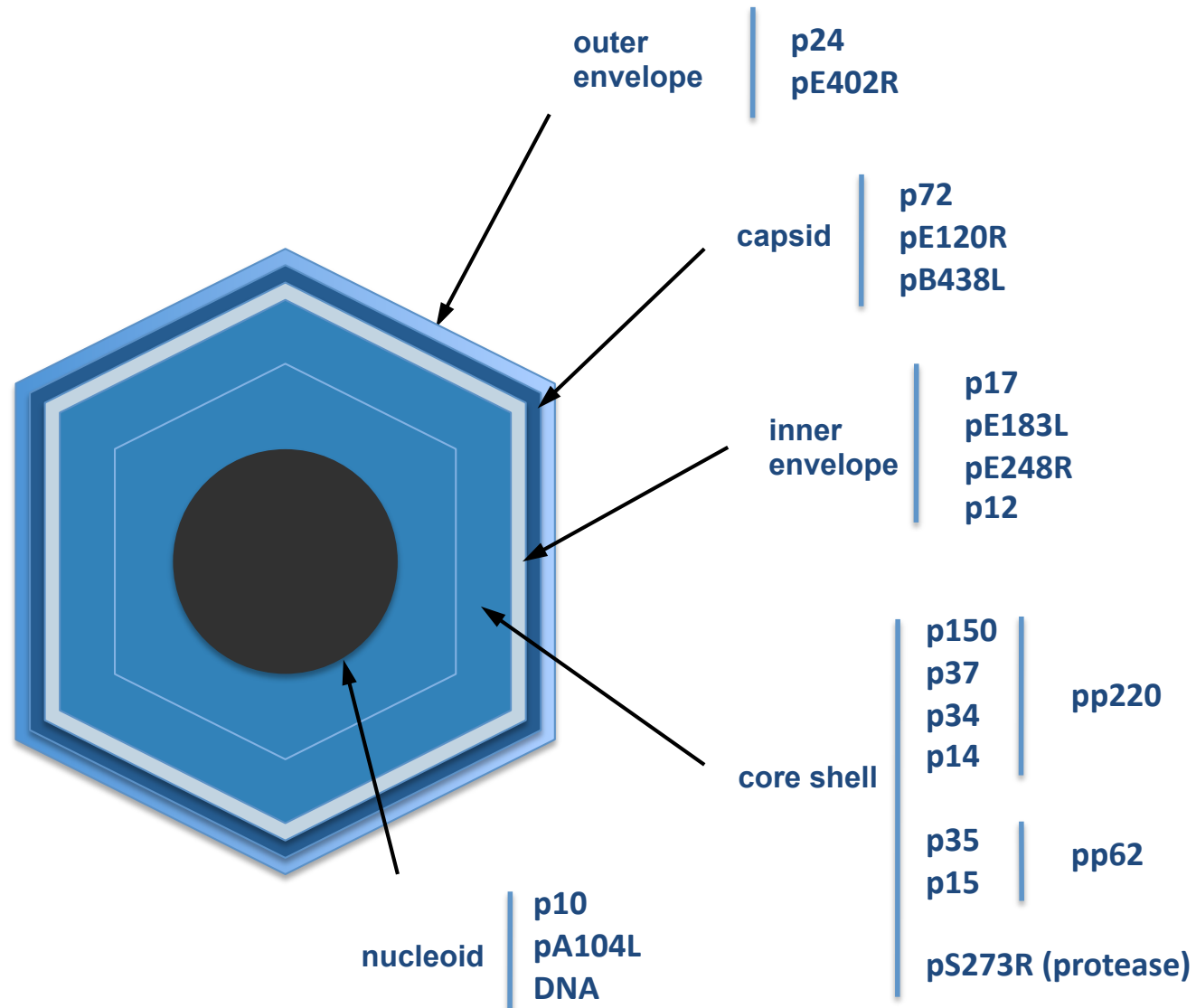


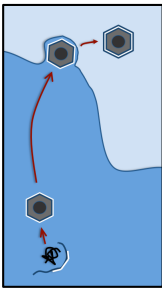
Capsid: p24 (cellular transmembrane protein)



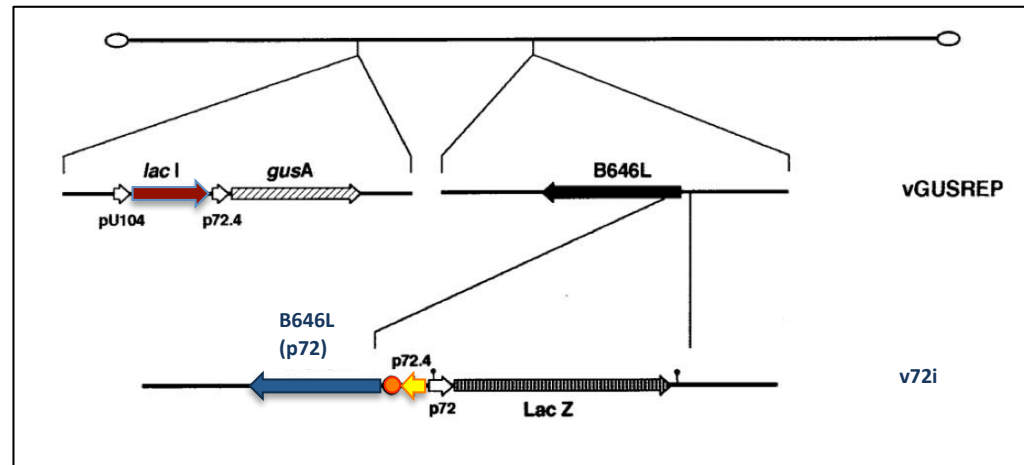


LOCALIZATION OF ASFV STRUCTURAL PROTEINS



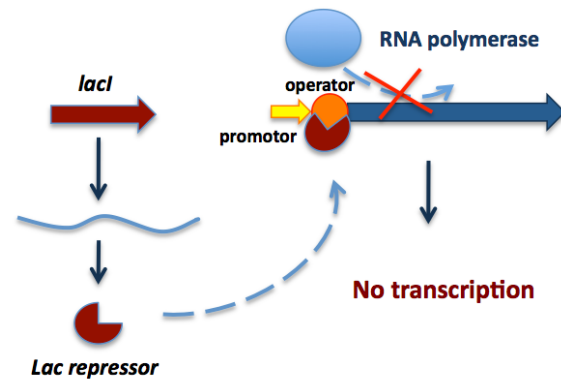


ASFV inducible recombinants: a powerful tool for morphogenetic studies

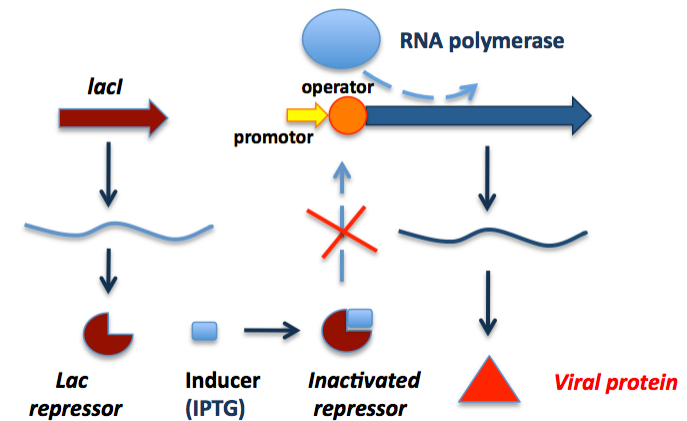


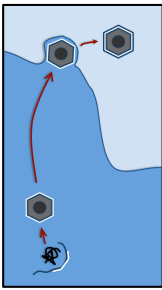
García-Escudero et al. 1998. J. Virol. 72: 3185-95.

- IPTG

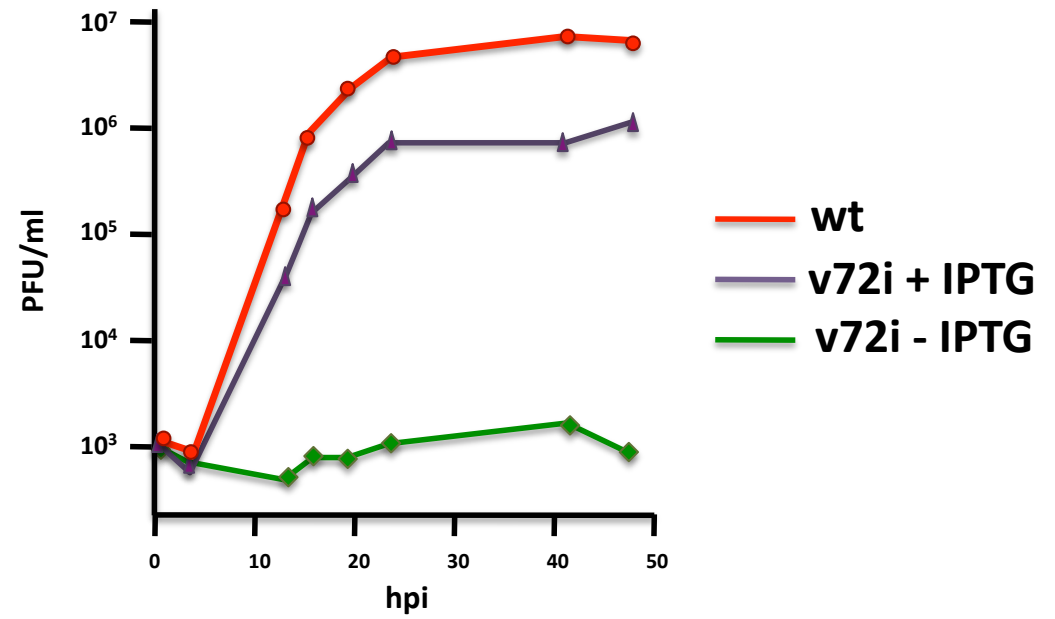
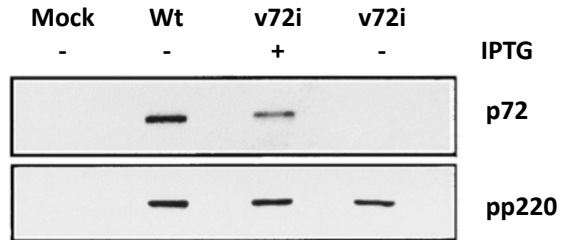


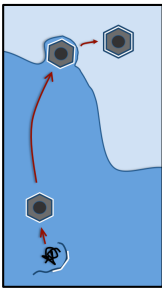
+ IPTG





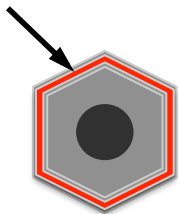
Inducible expression of major capsid protein p72



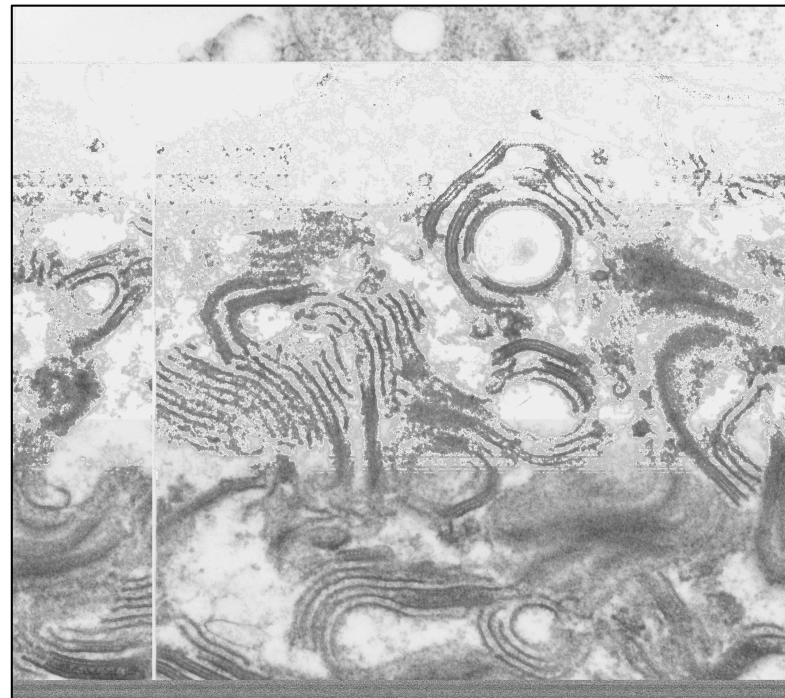


Silencing of capsid protein p72 leads to the accumulation of aberrant double-membrane zipper-like structures

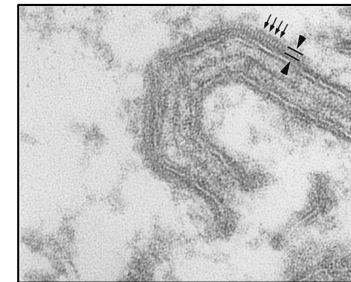
p72 (capsid)

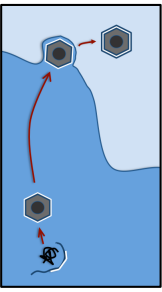


- IPTG (16 hpi)



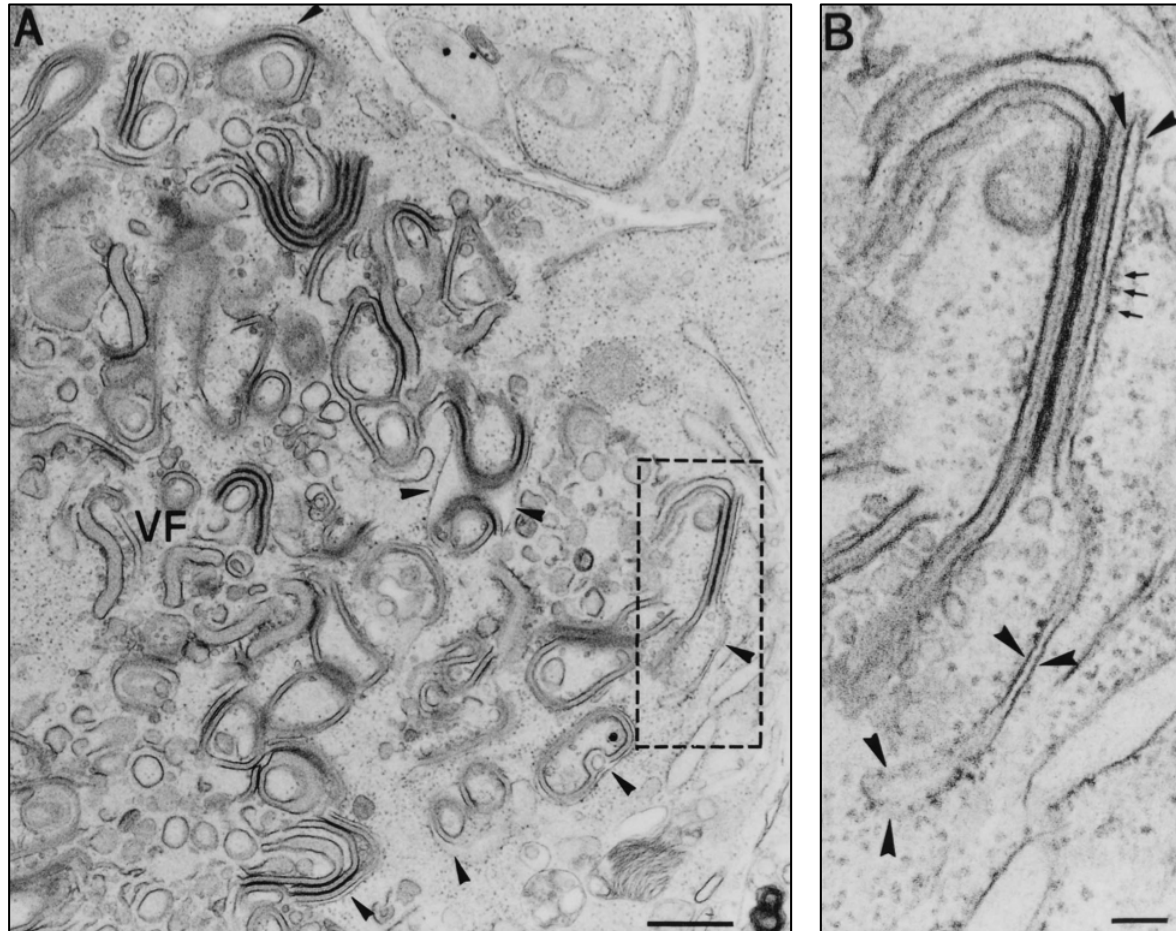
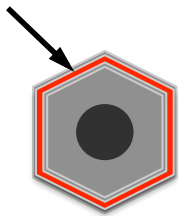
**- IPTG (16 h)
+ IPTG (4h)**

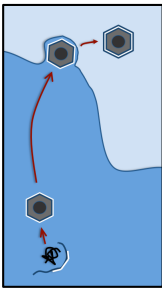




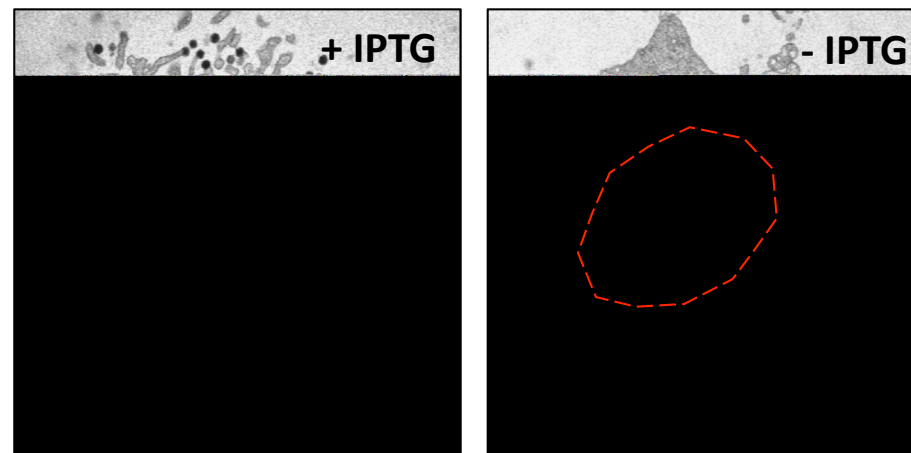
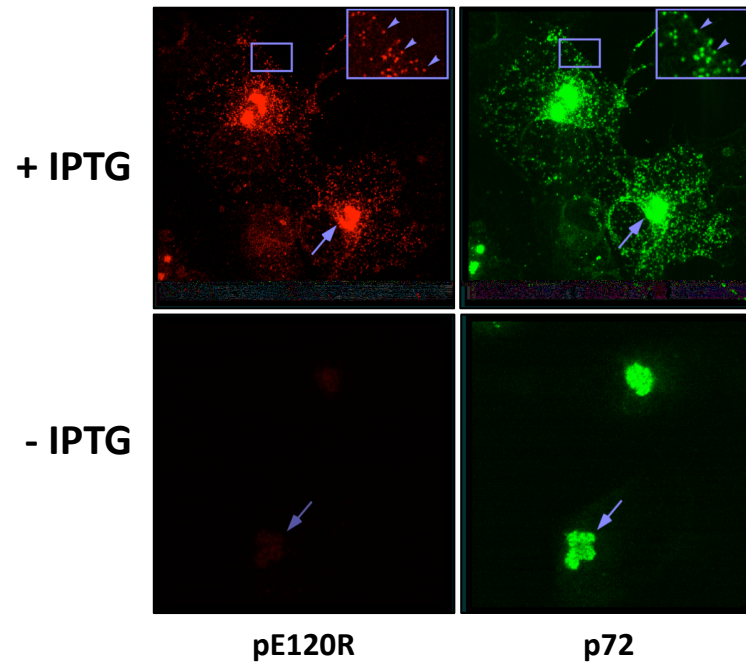
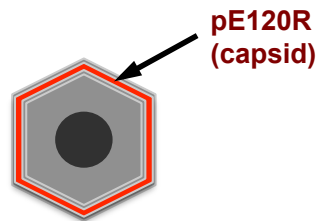
Zipper-like structures associated with ER at the periphery of the viral factories

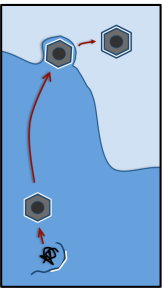
p72 (capsid)





Silencing of minor capsid protein pE120R blocks transport of intracellular virions to the plasma membrane

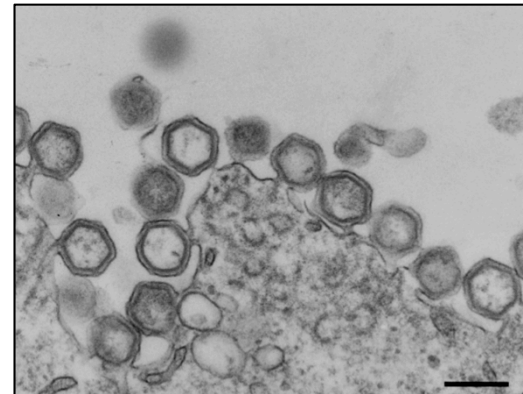
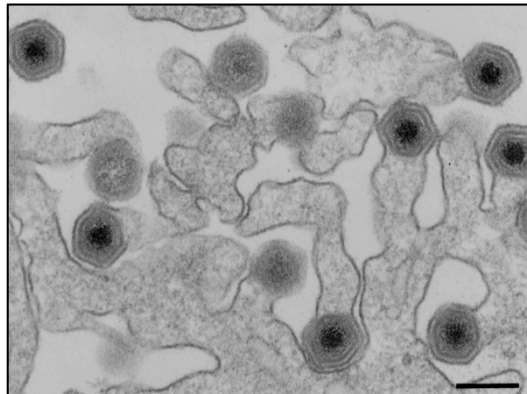
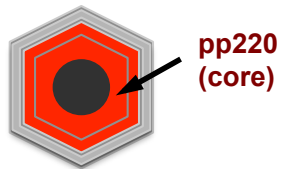
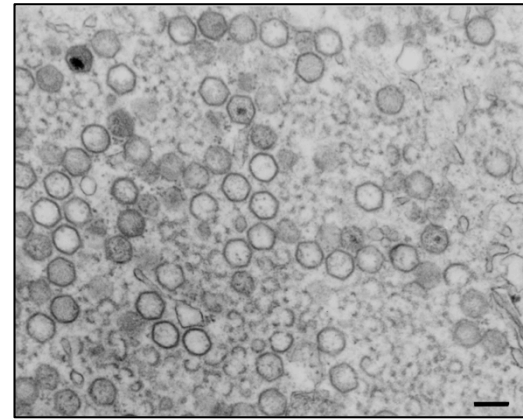
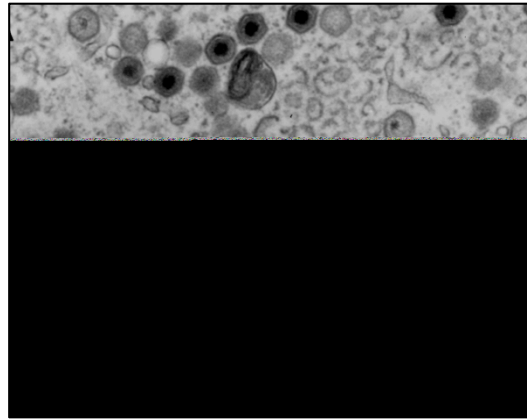


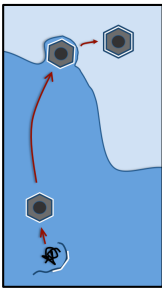


Silencing of core polyprotein pp220 produces empty virus particles

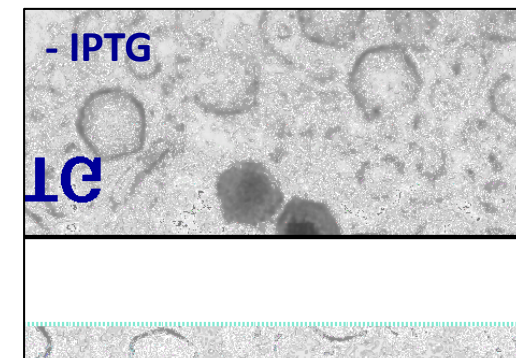
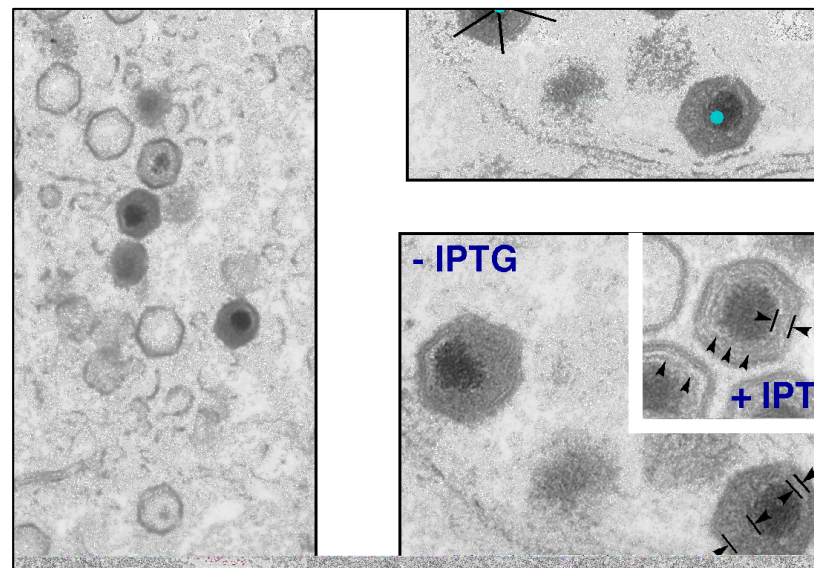
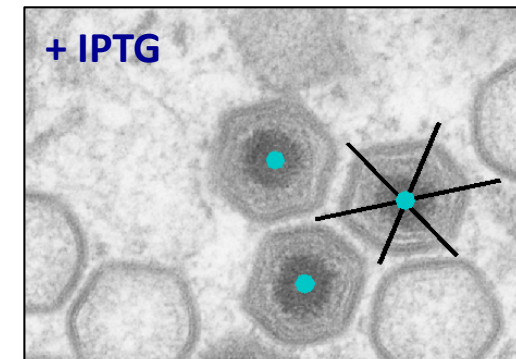
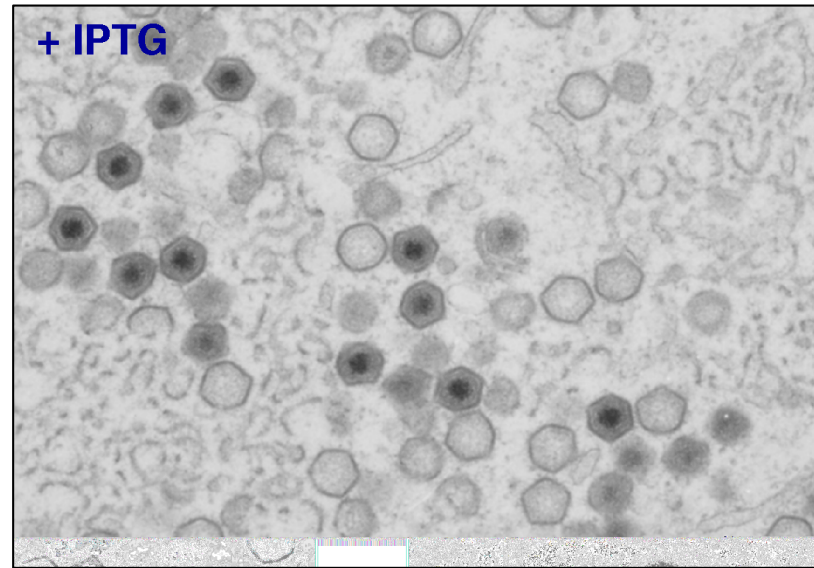
+ IPTG

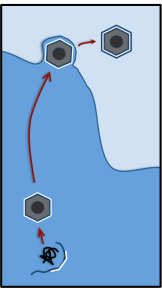
- IPTG





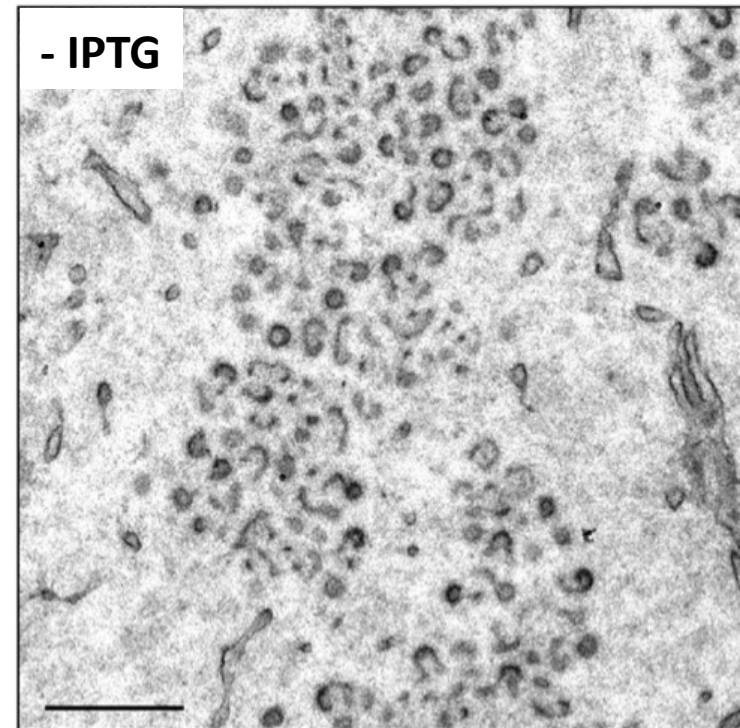
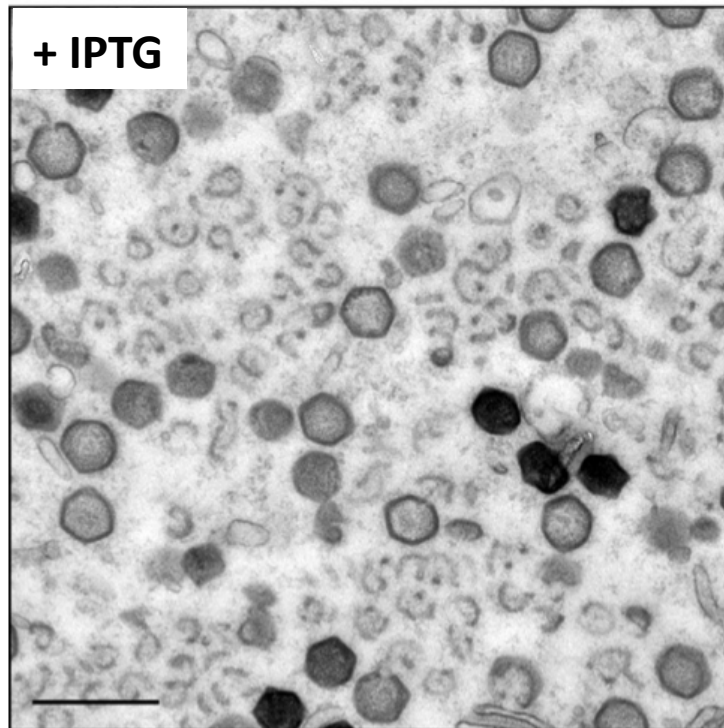
Silencing of core protease pS273R produces virus particles with aberrant cores

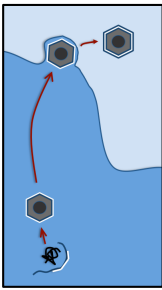




Inner envelope protein p17 is essential for the progression of viral membrane precursors

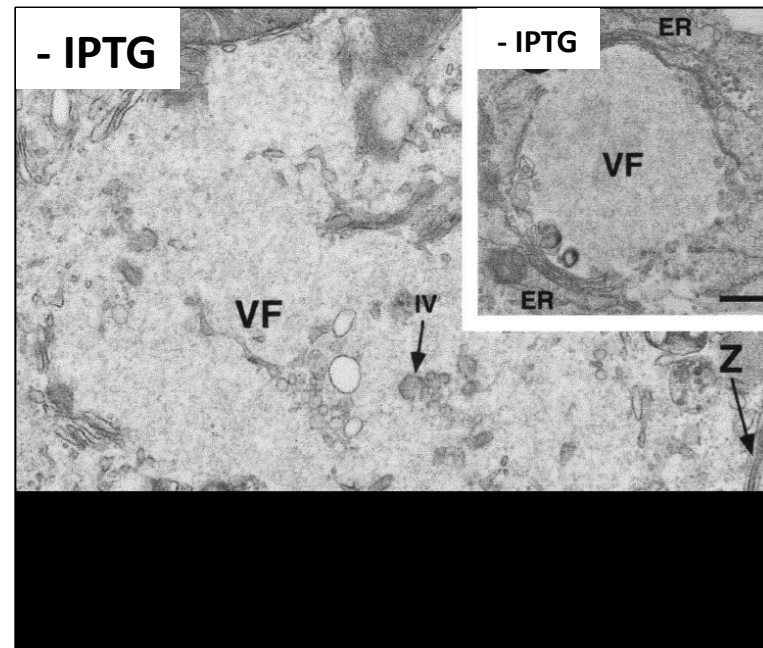
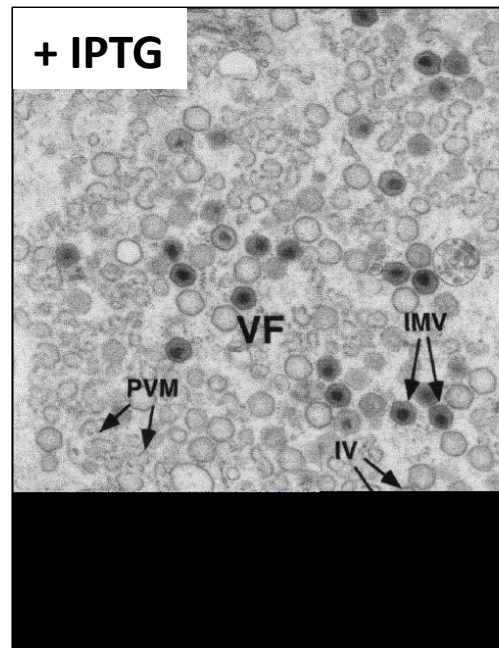
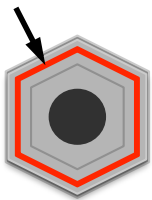
protein 17
(inner
envelope)

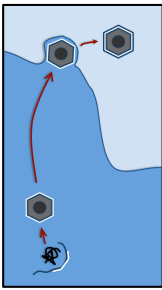




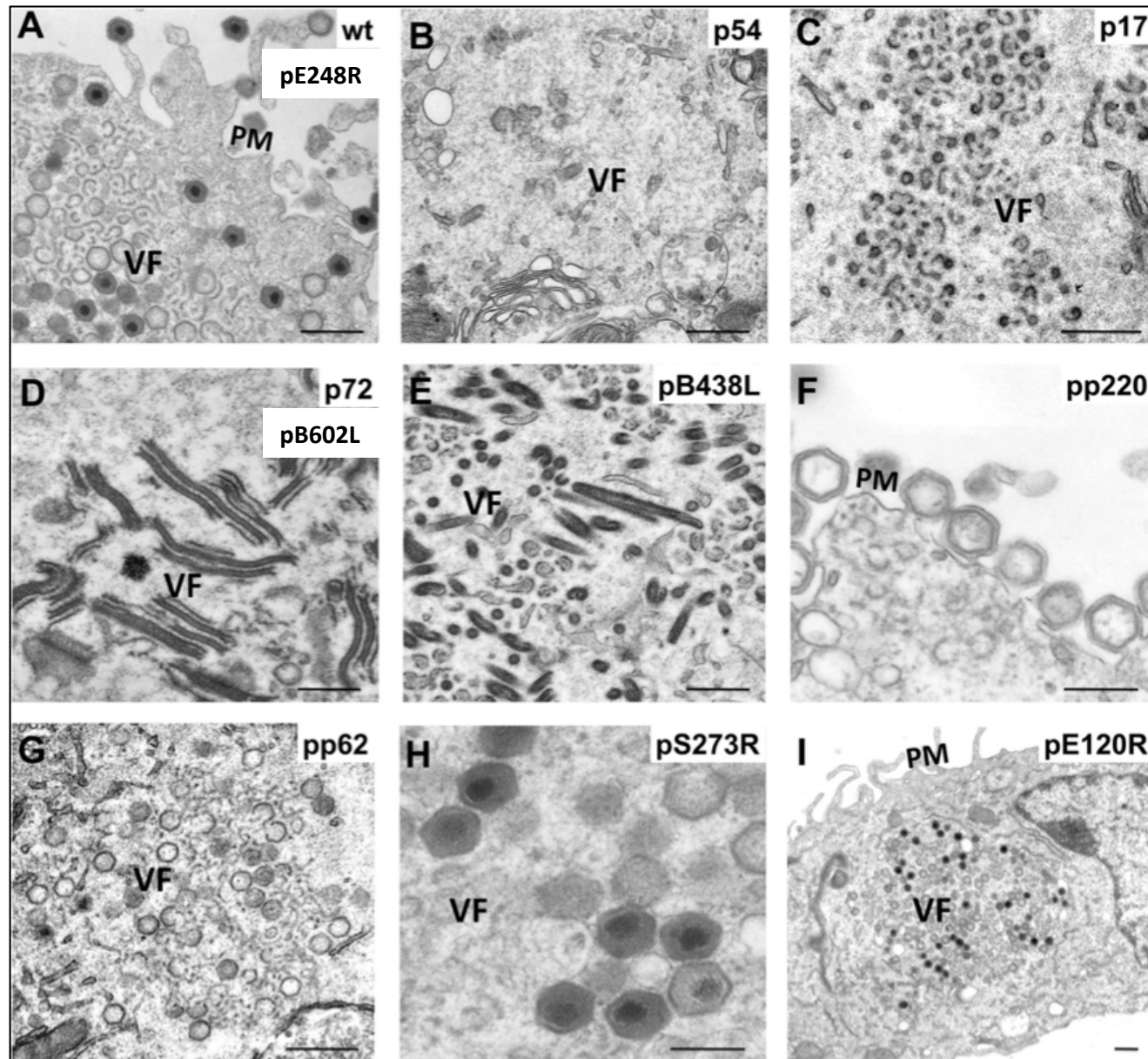
Silencing of transmembrane protein p54 abrogates virus assembly

protein p54
(inner
envelope)

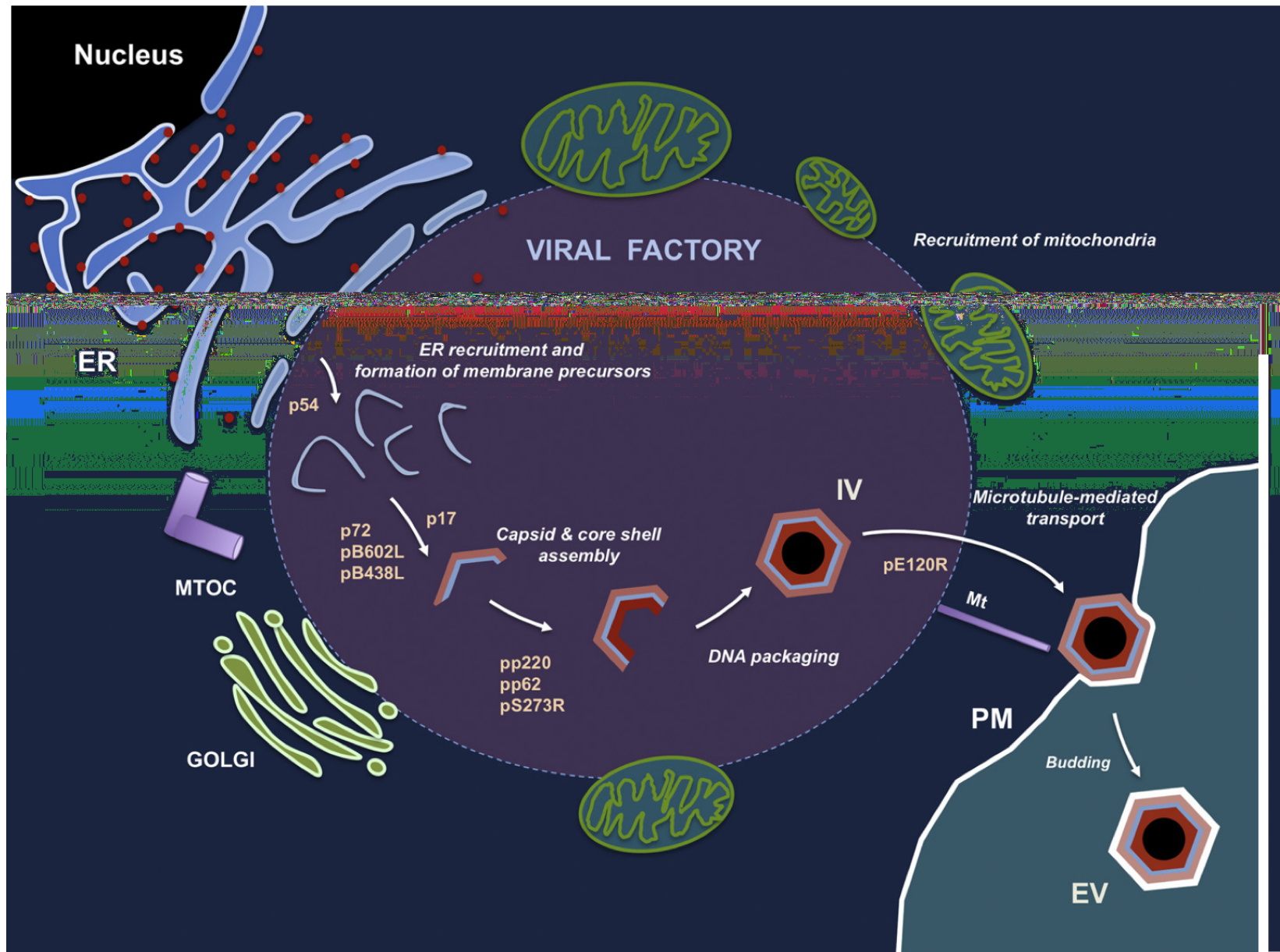




Ultrastructural phenotype of ASFV inducible recombinants



WORKING MODEL FOR ASFV ASSEMBLY



Contributors & Acknowledgments

ASFV morphogenesis

Germán Andrés

Javier M. Rodriguez

Ramón García-Escudero

Alí Alejo

Cristina Suarez

María Luisa Salas

Eladio Viñuela

ASFV entry

Bruno Hernáez

Milagros Guerra

María Luisa Salas

Germán Andrés



Programa Amarouto



Comunidad de Madrid

