

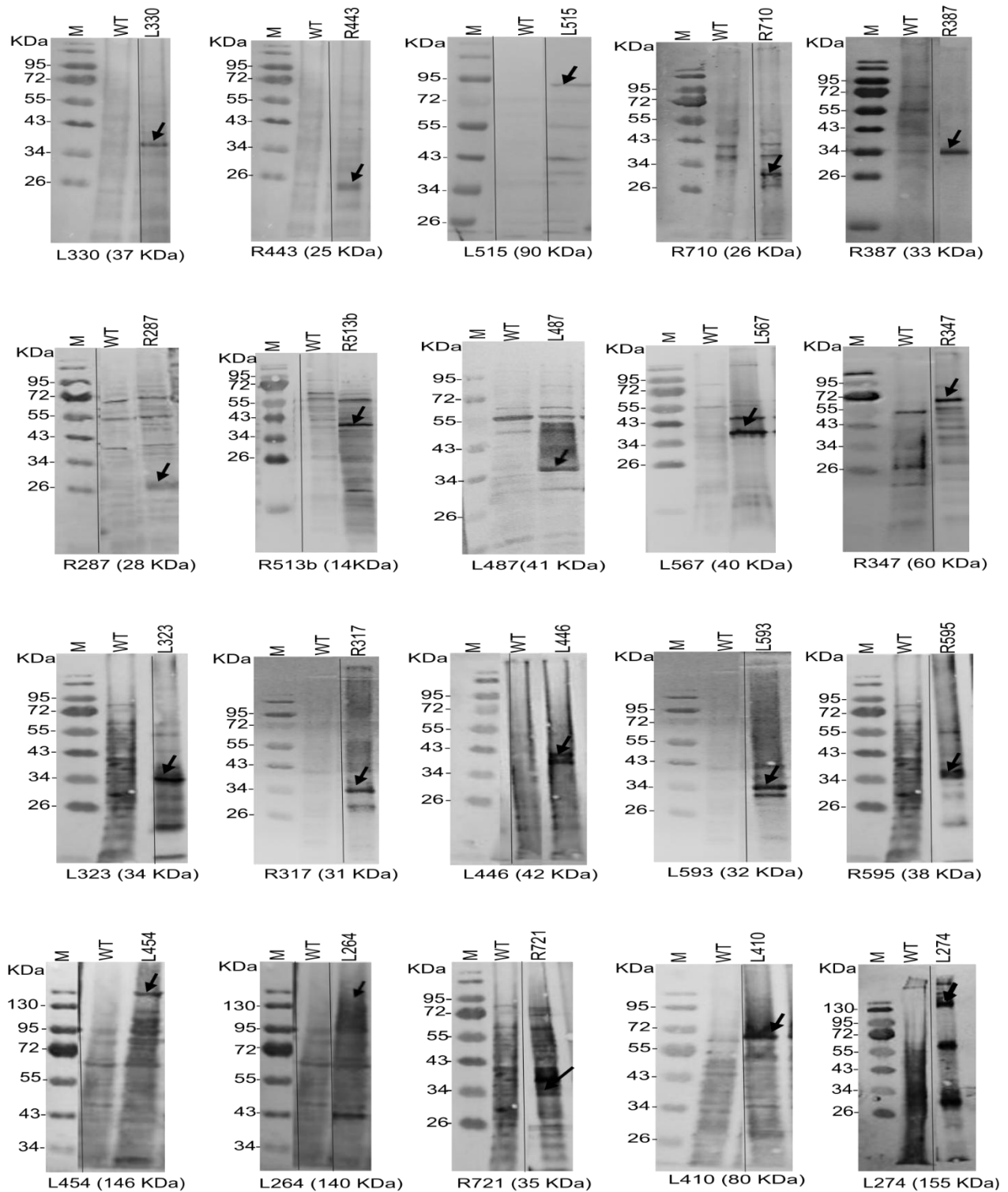
Appendix for: Elucidating the protein interaction network of one of the largest icosahedral capsids in the virosphere

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Appendix Figure S1. Improvement of the clustering by extending the dataset beyond mimivirus Taxon.

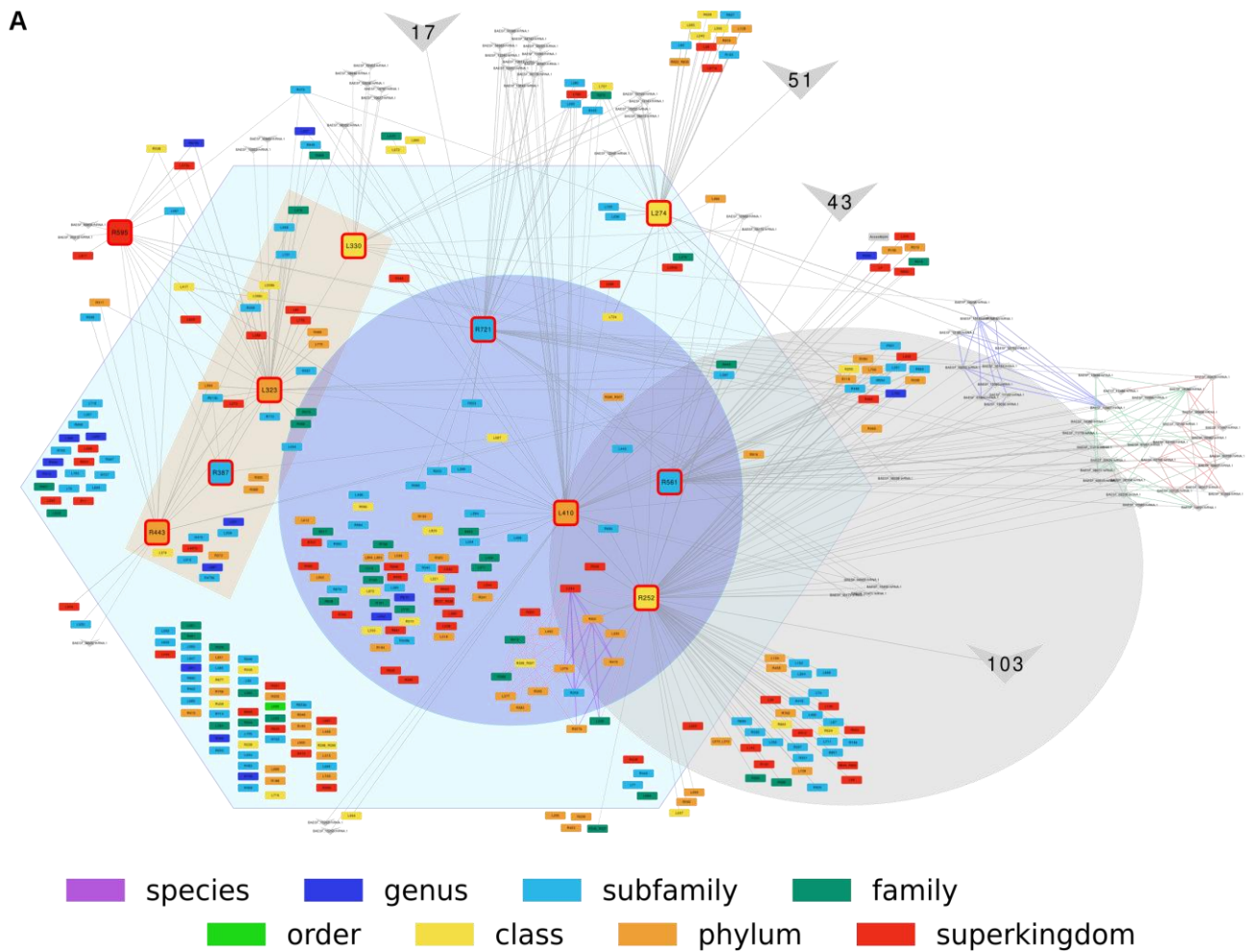
(A) Taxonomic Level of the Last Common Ancestor of the 671 mimivirus clusters (Nomburg et al. (Nomburg et al, 2024) clustering data). (B) Correction of the LCA taxonomy levels obtained after adding 62 giant viruses mostly *Imitervirales*, *Pimascovirales*, *Pandovirales* and some *Asfuvirales*. Taxonomy levels shifts towards the top (resp. bottom) are depicted with green arrows (resp. red arrows). (C) Pie charts indicating the fraction of mimivirus proteins in clusters with AFDB matches (blue), and with PFAM annotations (red) or annotated AFDB homolog, (top) for virion proteins subset, (bottom) total subset. (D) Upset plots showing the sub-level composition of each taxonomy level LCA.



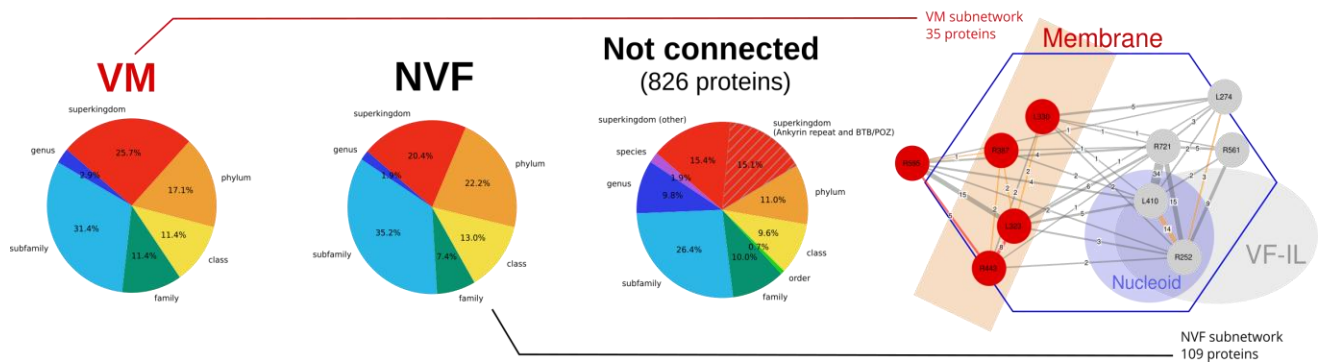
Appendix Figure S2. Western blot of endogenously tagged mimivirus proteins.

Western blot (WB) analyses were performed using anti-HA antibodies to detect the tagged mimivirus proteins (protein bands indicated by black arrows). *Acanthamoeba* cells were infected with mimivirus at MOI = 10 and collected 6 hpi. Wild-type mimivirus infected cells were included as negative controls. M: molecular weight marker. Control lanes corresponding to proteins run on the same gels were re-

used in the corresponding panels. WB1 included WT, L443 and L330; WB2 included WT, L323, R595 and R721; WB3 included WT, R317 and L593; WB4 included WT, L264 and L454. For each blot, the WT control lane runs alongside the corresponding HA-tagged proteins as displayed in the assembled panels (marker + WT + HA-gene).



B Co-IP network subgroups

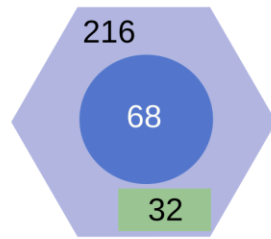


Appendix Figure S3. Conservation analysis of the co-IP network.

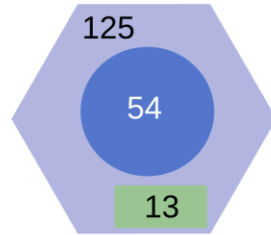
(A) Detailed network showing all connections of the co-IP baits with their connected proteins, as well as direct PPI networks as described in Fig. 3. Taxonomy level of the Last Common ancestor (LCA) is shown as node colors from violet (species) to red (superkingdom). (B) LCA taxonomic level conservation is shown by co-IP subnetworks. Both VM and NVF subnetworks show higher subfamily-level and phylum conservation compared to not-connected proteins.

A

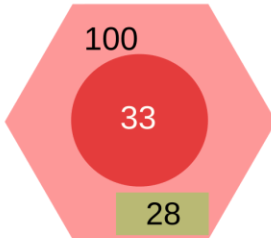
Mimiviridae
Mimivirus reunion
Villalta et al. 2024



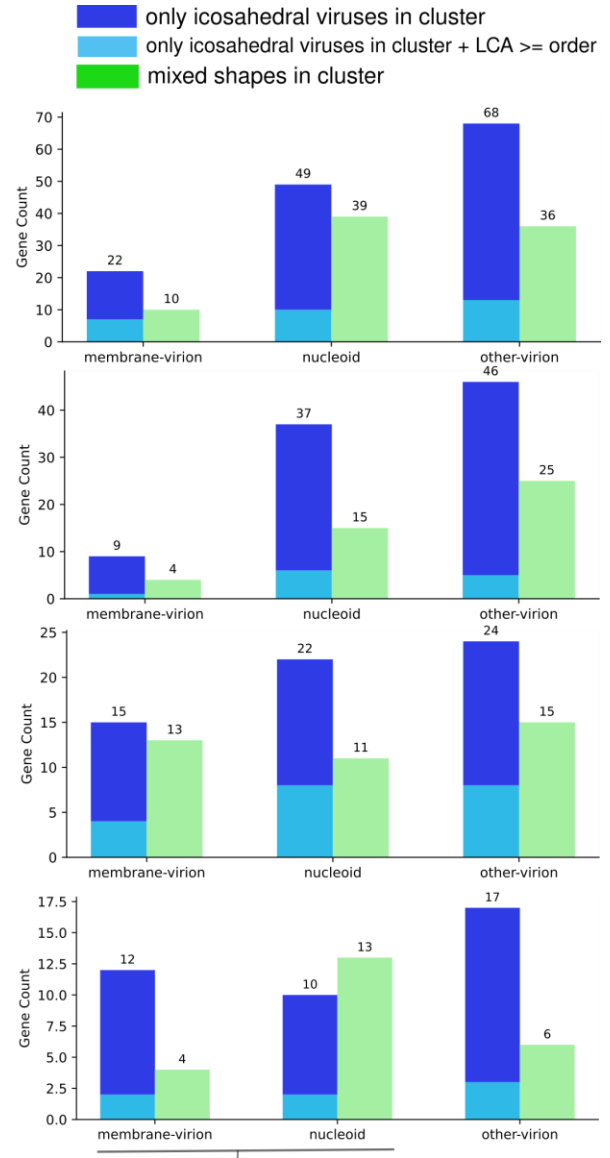
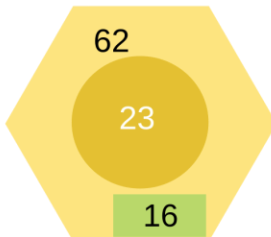
Mimiviridae
Tupanvirus soda lake
Schrader et al. 2020
Abrahão et al. 2018



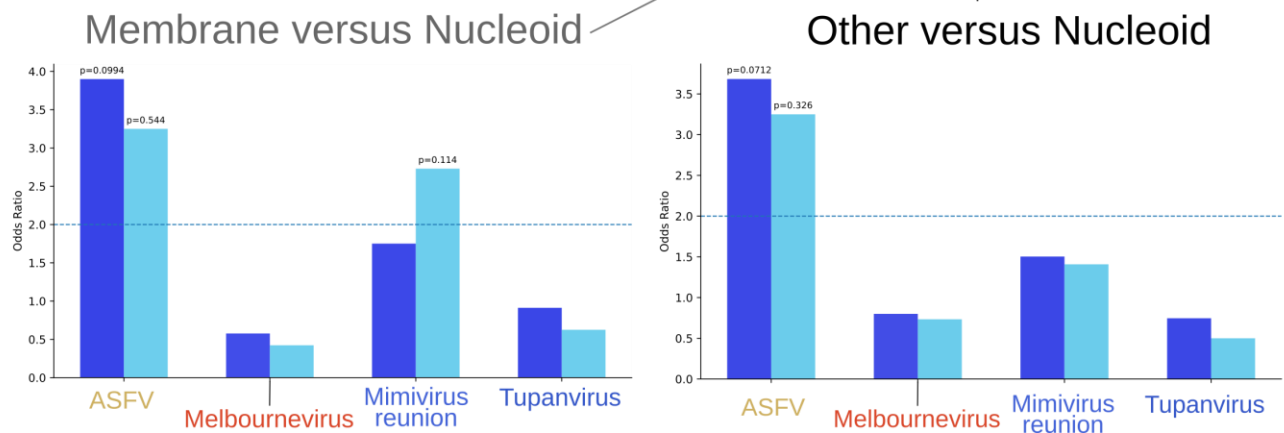
Marseilleviridae
Melbournevirus
Mühlberg et al. 2025



Asfarviridae
African swine fever virus
Alejo et al. 2018



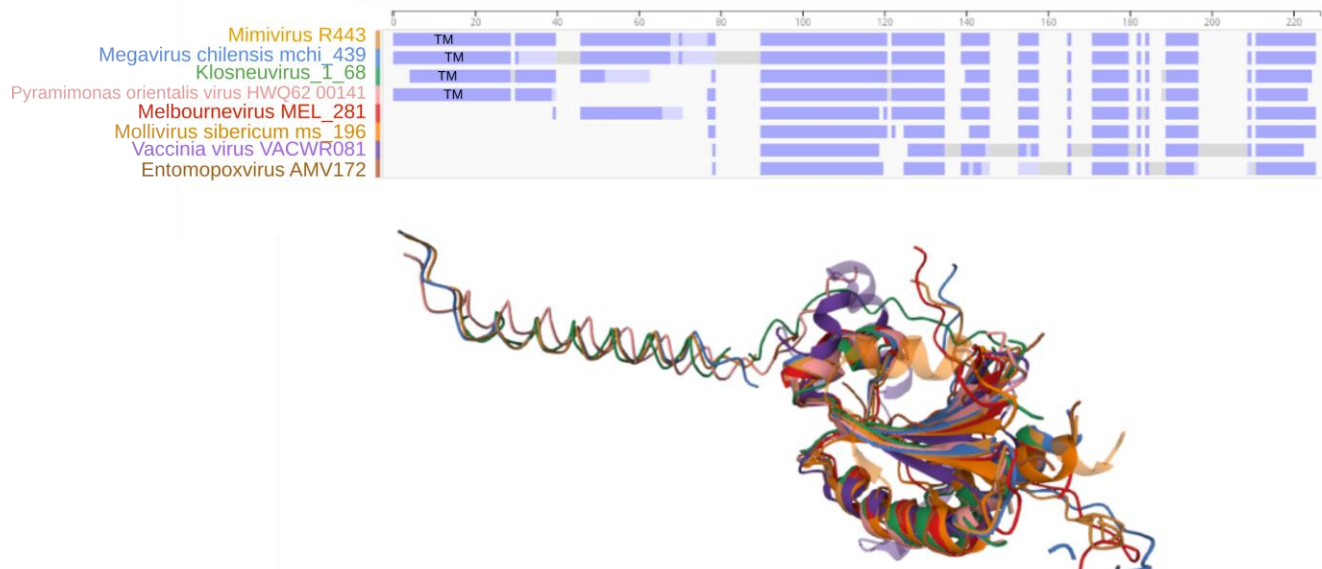
B Icosahedral shape enrichment



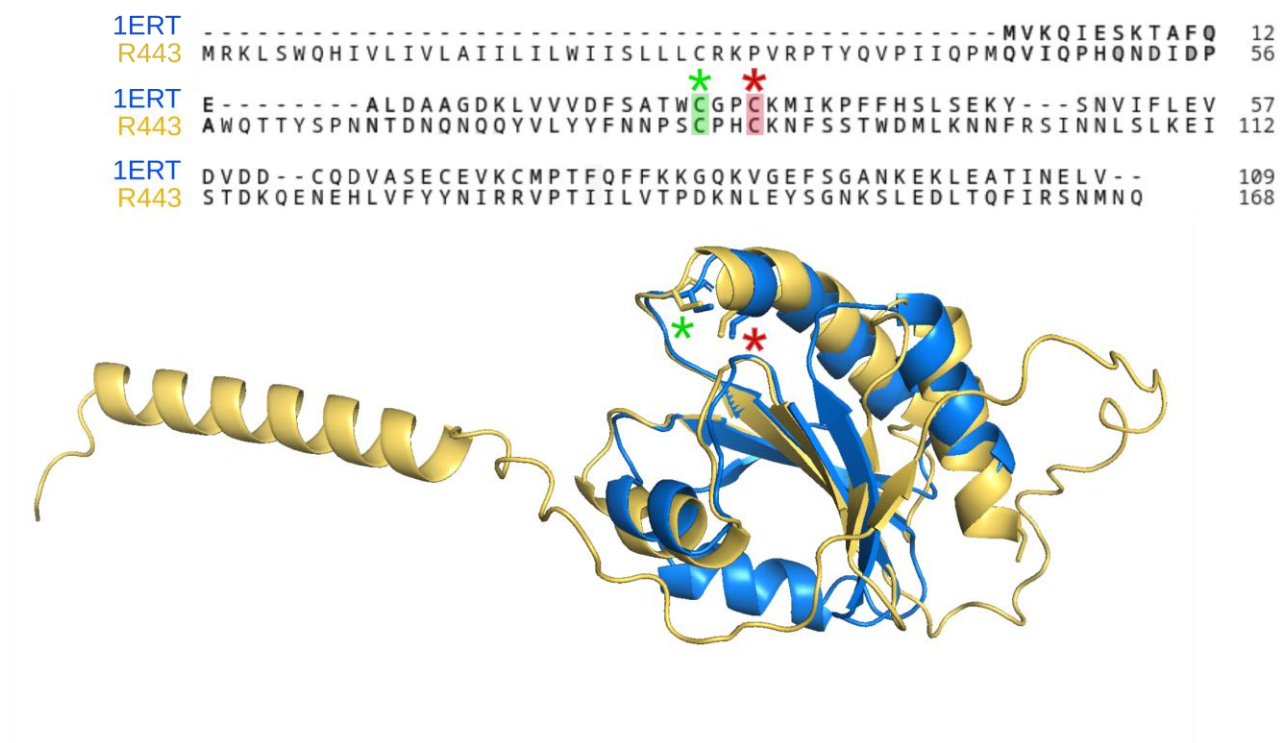
Appendix Figure S4. Icosahedral shape enrichment in different virion regions.

(A) Extension of the analysis of statistical association between viral shape and protein composition across different virion regions using additional proteomic datasets for different icosahedral viruses. For each virus, (left) its family and original publication is recapitulated, (center) total proteins count in the virion, proteins count in the nucleoid and count of predicted membrane proteins is summarized, (right) protein count per virion region and virion shape (Icosahedral only in blue, mixed shape in green). (B) Odds ratio and corresponding P-value using Fisher's exact test are summarized for all 4 icosahedral viruses, by comparing the protein content (left) in the membrane region versus the nucleoid, and (right) in the other region versus the nucleoid region.

A



B R443 vs Human PDB-1ERT



Appendix Figure S5. R443 transmembrane domain and active site.

(A) Structural alignment of selected R443 homologs (Foldmason tool), members of the same cluster with conserved Thioredoxin globular. Transmembrane domain is only conserved in members of the *Imitervirales* order. (B) Alignment of the Human Thioredoxin (RCSB PDB 1ERT) and mimivirus R443. The R443 thioredoxin domain is well conserved and the catalytic CXXC motif is highlighted with green and red stars.

A

ATGCGTAACTATCATGGCAACATATTGTTCTTATTGTTTTGGCTATTATACTAATTTTATGGATTATTAGTCTATTATTATGCAGGA

AACCAGTTCGTCCAACCTTATCAAGTACCCATTATTCAACCAATGCAAGTTATTCAGCCTCATCAAATGATATTGATCCAGCATGGCA

AACAACATATTCTCCAATAAAGCTGATAATCAAAATCAACAATATGTTTTATACTATTTTAATAATCCGAGT **TC**CCACAC**TC**GTAAA

AATTTCTCGTCAACATGGGATATGCTCAAAAATAACTTTAGATCTATCAATAATTTATCATTAAAGGAAATATCAACTGATAAACAAG

AAAATGAGCACTTAGTATTTTATTACAATATTAGAAGAGTACCTACTATTATTCTTGTTACTCCGGACAAAAATCTGAATATTCTGG

AAATAAAAGTCTAGAAGATCTAACTCAATTCATCCGATCTAACATGAATCAATAA

Nucleophilic cysteine Resolving cysteine
C X X C
Active site

B

R443_Wt	CTTATTGTTTTGGCTATTATACTAATTTTATGGATTATTAGTCTATTATTATGCAGGAAA	360
R443_SXXC	CTTATTGTTTTGGCTATTATACTAATTTTATGGATTATTAGTCTATTATTATGCAGGAAA	352
R443_CXXS	CTTATTGTTTTGGCTATTATACTAATTTTATGGATTATTAGTCTATTATTATGCAGGAAA	353

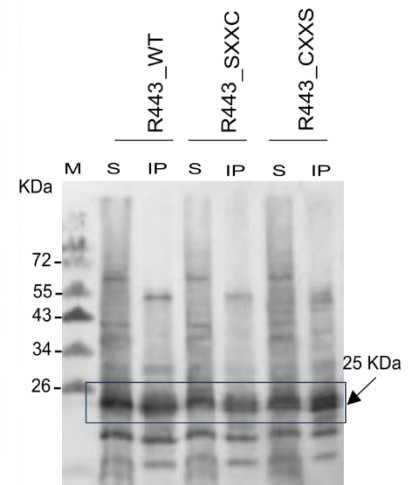
R443_Wt	CCAGTTCGTCCAACCTTATCAAGTACCCATTATTCAACCAATGCAAGTTATTCAGCCTCAT	420
R443_SXXC	CCAGTTCGTCCAACCTTATCAAGTACCCATTATTCAACCAATGCAAGTTATTCAGCCTCAT	412
R443_CXXS	CCAGTTCGTCCAACCTTATCAAGTACCCATTATTCAACCAATGCAAGTTATTCAGCCTCAT	413

R443_Wt	CAAAATGATATTGATCCAGCATGGCAACAACATATTCTCCAATAAAGCTGATAATCAA	480
R443_SXXC	CAAAATGATATTGATCCAGCATGGCAACAACATATTCTCCAATAAAGCTGATAATCAA	472
R443_CXXS	CAAAATGATATTGATCCAGCATGGCAACAACATATTCTCCAATAAAGCTGATAATCAA	473

R443_Wt	AATCAACAATATGTTTTATACTATTTTAAATCCGAGTTGCCACACTGTAAAAATTC	540
R443_SXXC	AATCAACAATATGTTTTATACTATTTTAAATCCGAGTTGCCACACTGTAAAAATTC	532
R443_CXXS	AATCAACAATATGTTTTATACTATTTTAAATCCGAGTTGCCACACTGTAAAAATTC	533

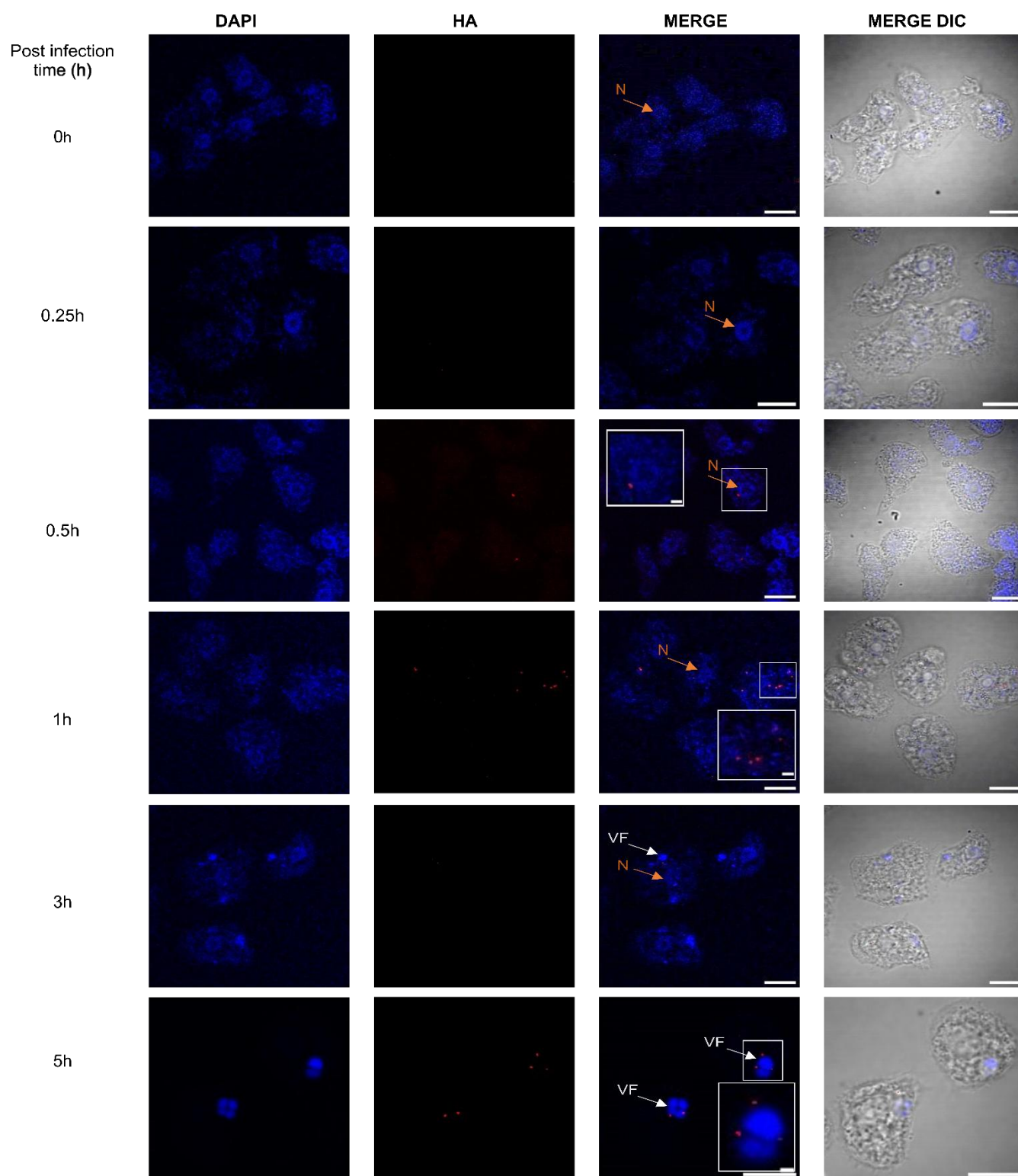
R443_Wt	TCGTCAACATGGGATATGCTCAAAAATAACTTTAGATCTATCAATAATTTATCATTAAAG	600
R443_SXXC	TCGTCAACATGGGATATGCTCAAAAATAACTTTAGATCTATCAATAATTTATCATTAAAG	592
R443_CXXS	TCGTCAACATGGGATATGCTCAAAAATAACTTTAGATCTATCAATAATTTATCATTAAAG	593

R443_Wt	GAAATATCAACTGATAAACAAGAAATGAGCACTTAGTATTTTATTACAATATTAGAAGA	660
R443_SXXC	GAAATATCAACTGATAAACAAGAAATGAGCACTTAGTATTTTATTACAATATTAGAAGA	652
R443_CXXS	GAAATATCAACTGATAAACAAGAAATGAGCACTTAGTATTTTATTACAATATTAGAAGA	653

C

Appendix Figure S6. Cysteine mutagenesis and substrate trapping of R443.

(A) R443 thioredoxin gene sequence and protein active site location. (B) Sequencing confirmation of cysteine mutations. (C) Western blot analysis of R443 substrate-trapping immunoprecipitation using anti-HA antibody. WB is carried out on *A.castellanii* cells at 6hpi (MOI=10). M: marker; S: supernatant; IP: immunoprecipitate.



Appendix Figure S7. Fluorescence observation of mimivirus core release during early infection.

Immunofluorescence of L410 major core protein endogenously tagged with 3xHA at the C-terminal at different stages of infection at a MOI=10 (0, 0.25, 0.5, 1, 3 and 5 hours). Insets

show higher magnification views of representative puncta. N: nucleus; VF: viral factory. VFs were labelled using DAPI. Scale bar: 10 μ m.