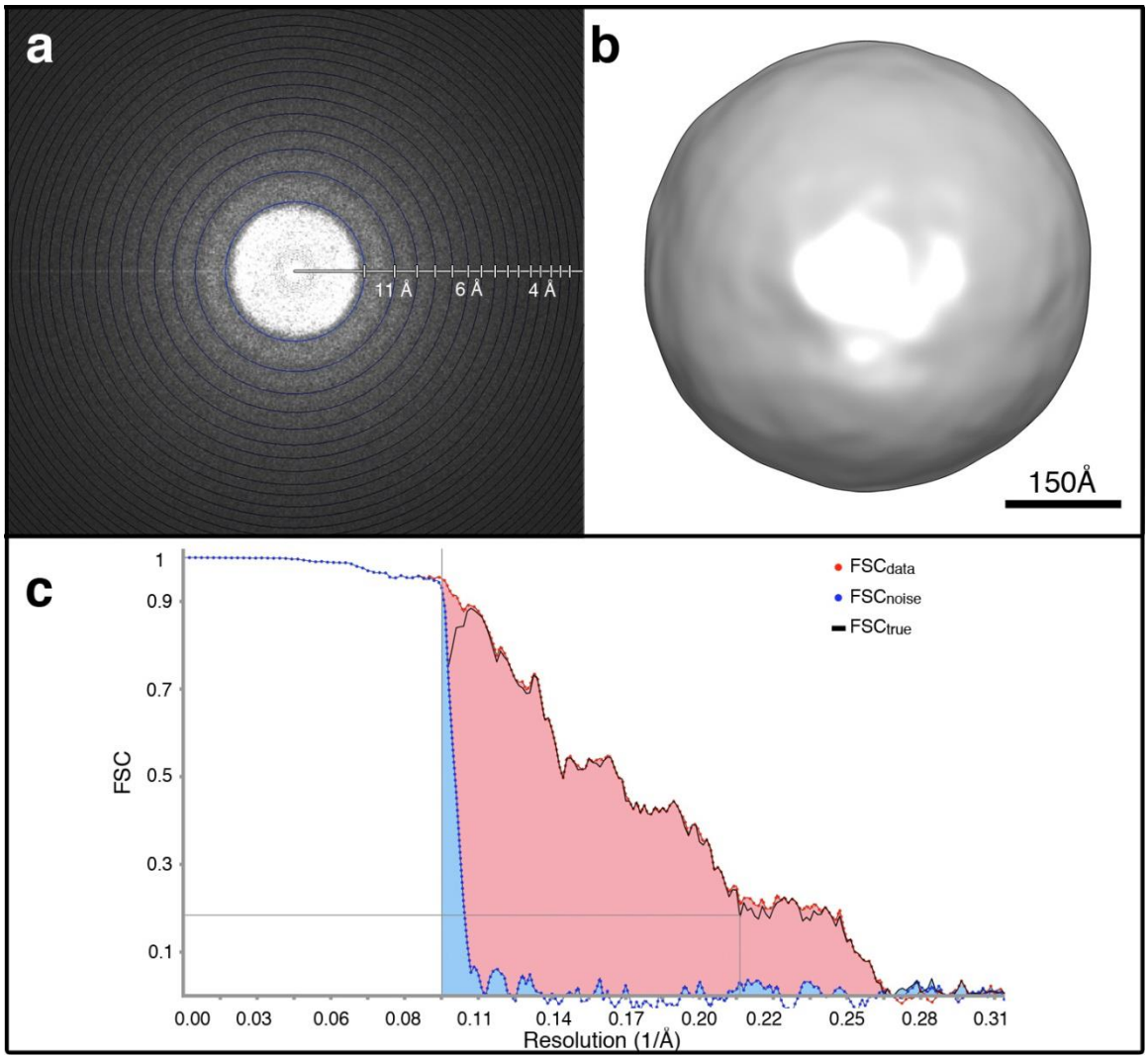
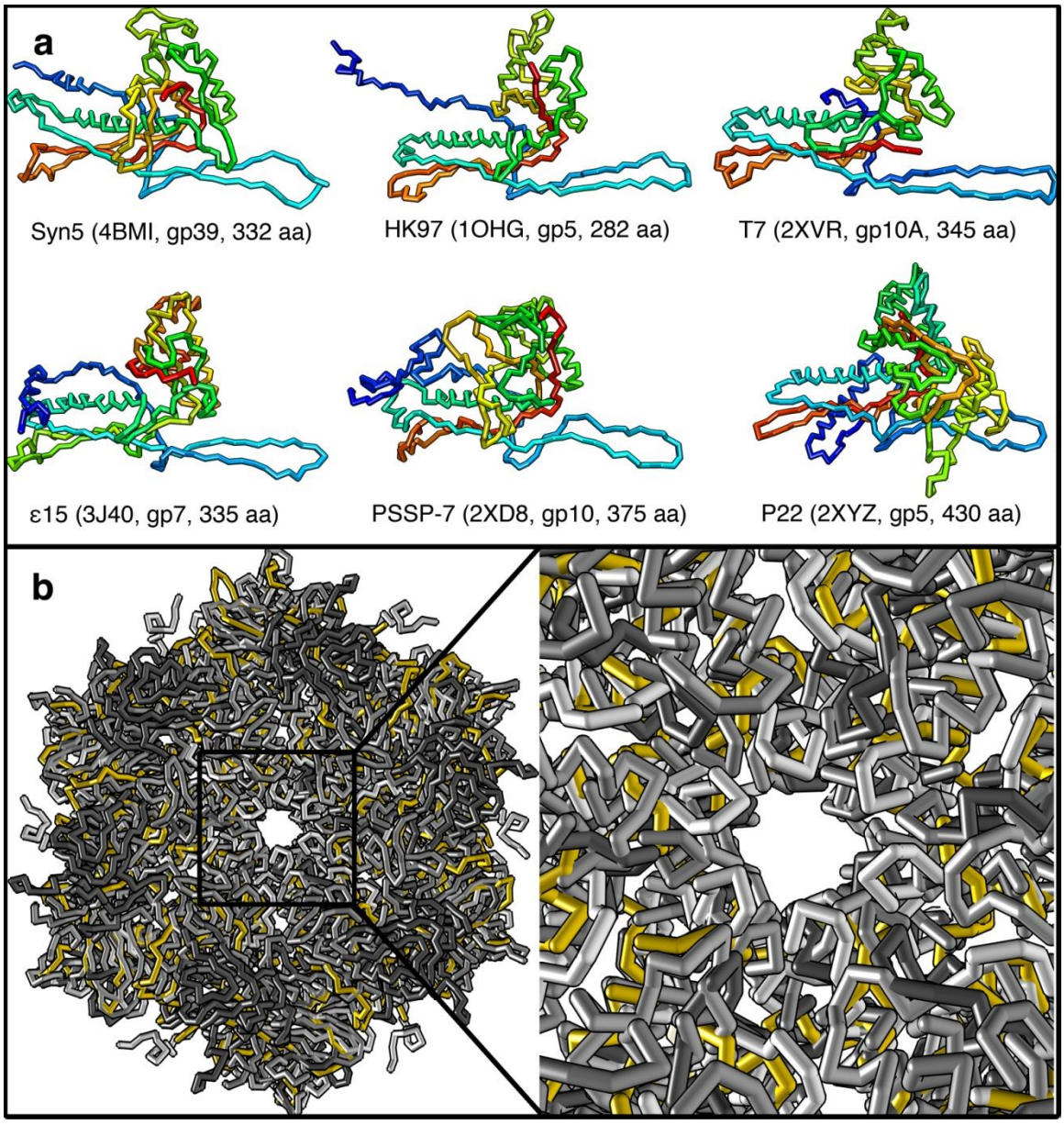
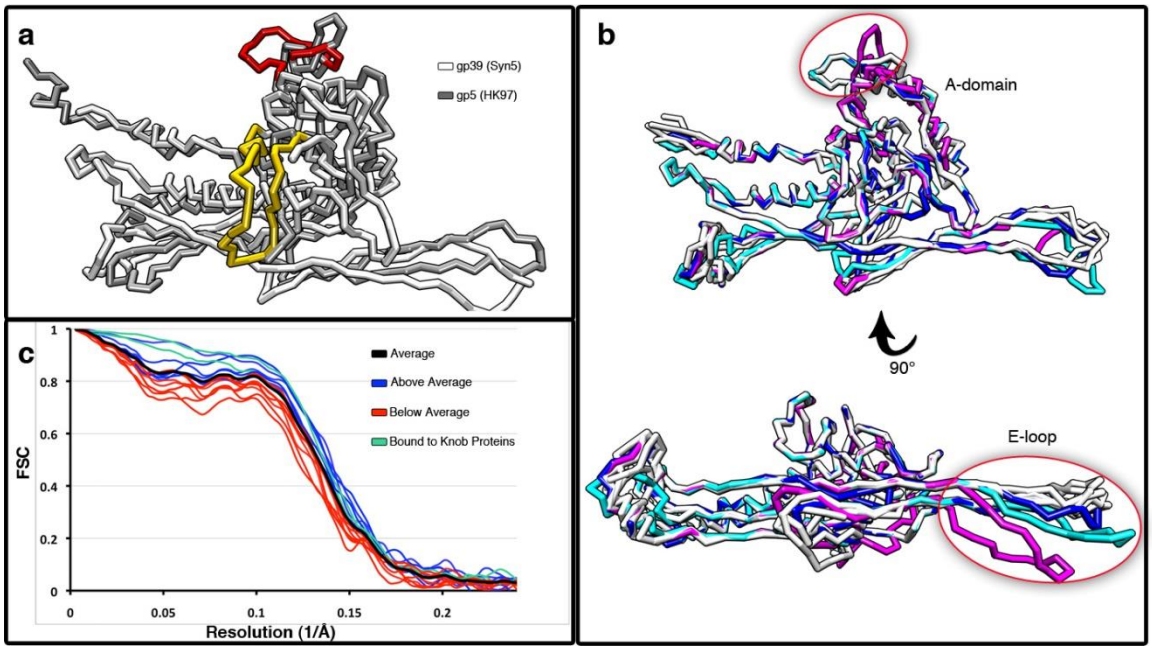


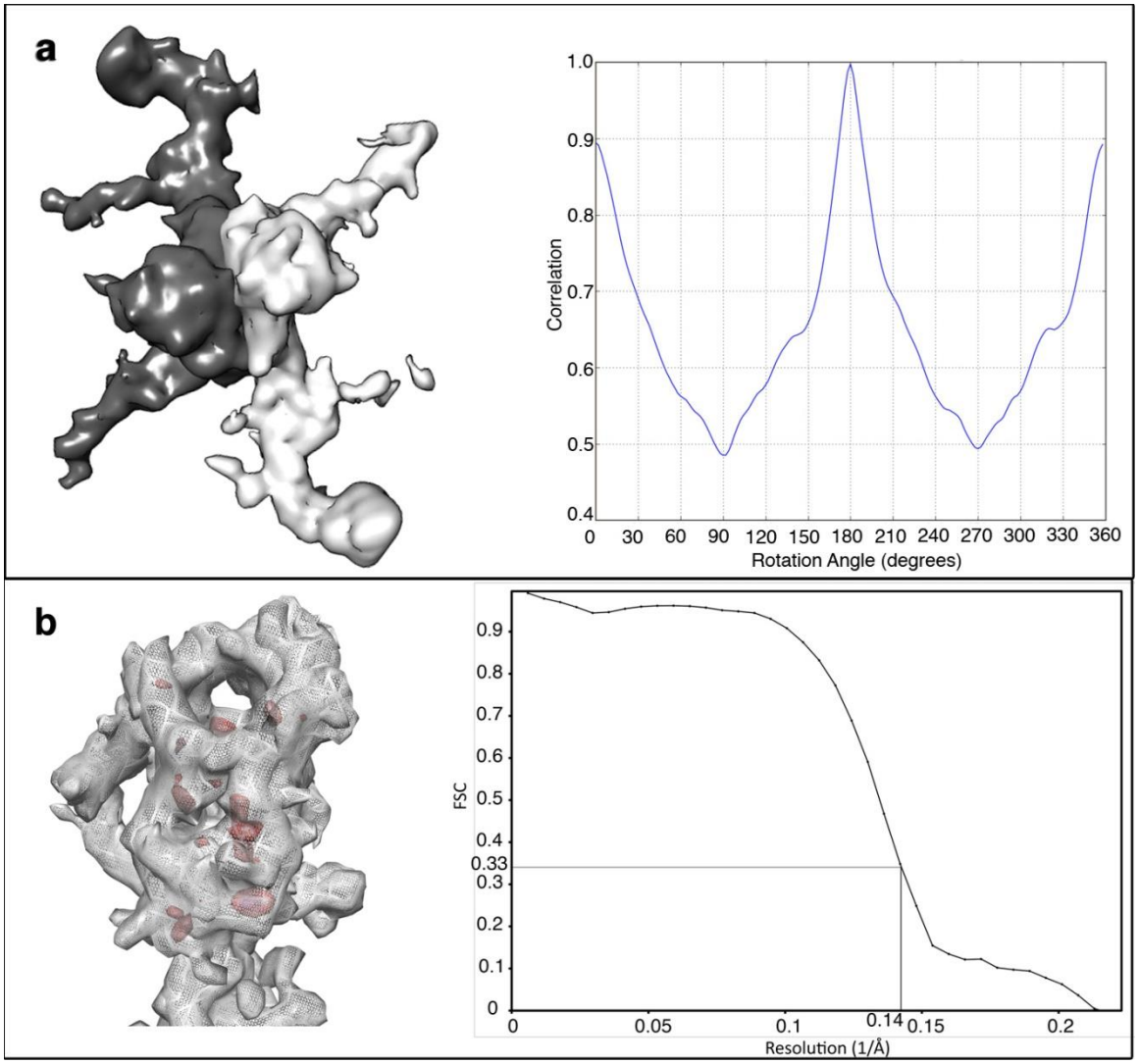
Supplementary Figure 1. Icosahedral reconstruction of Syn5. **(a)** A 2D average power spectra of Syn5 particles observed in a micrograph, showing signal beyond 5 Å. **(b)** The ab initio featureless initial model used for the 3D-reconstruction. **(c)** Results of the high-resolution noise substitution method, as described in the text. Here, the pink dotted curve represents the Fourier Shell Correlation (FSC_{data}) for the map obtained from the data with no noise-substitution, while the blue dotted curve represents the FSC for the map calculated from the noise substituted data (FSC_{noise}). The corrected FSC (FSC_{true}) is shown in solid black line. Here, no significant overfitting was observed (shaded in blue). The difference between the FSC_{data} and FSC_{noise} are shaded in pink, which represent the true features recovered from the data. The resolution of the map is estimated from the FSC_{true} plot to be 4.7 Å at FSC=0.143 cutoff.



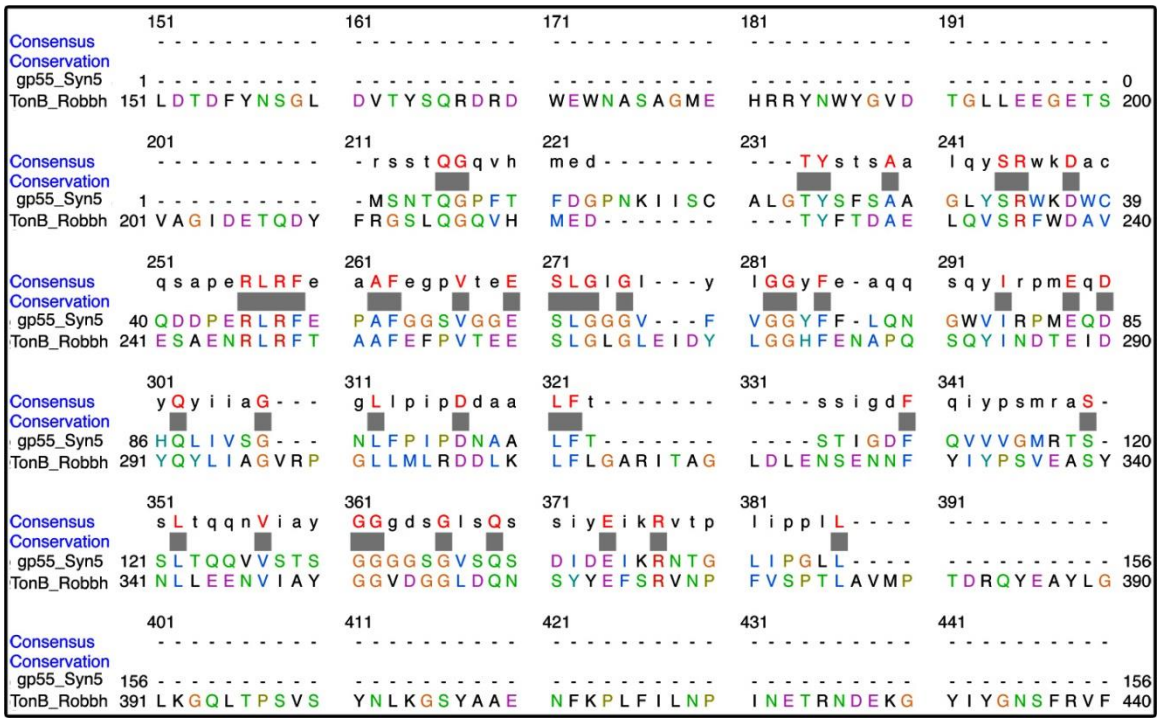
Supplementary Figure 2. Structural homologs of gp39. **(a)** Shows the major capsid proteins with HK-97 like fold, as observed in Syn5 and other phages. The residues are colored blue-red from the N-terminus towards the C-terminus. **(b)** An overlay of the structures of hexameric capsomere in Syn5 (gold) with other known phage structures as mentioned in **(a),** which are shown in shades of grey for clarity. On the right is shown a close up of the six-fold opening at the center of the hexamer, to show the comparatively wider opening observed in Syn5.



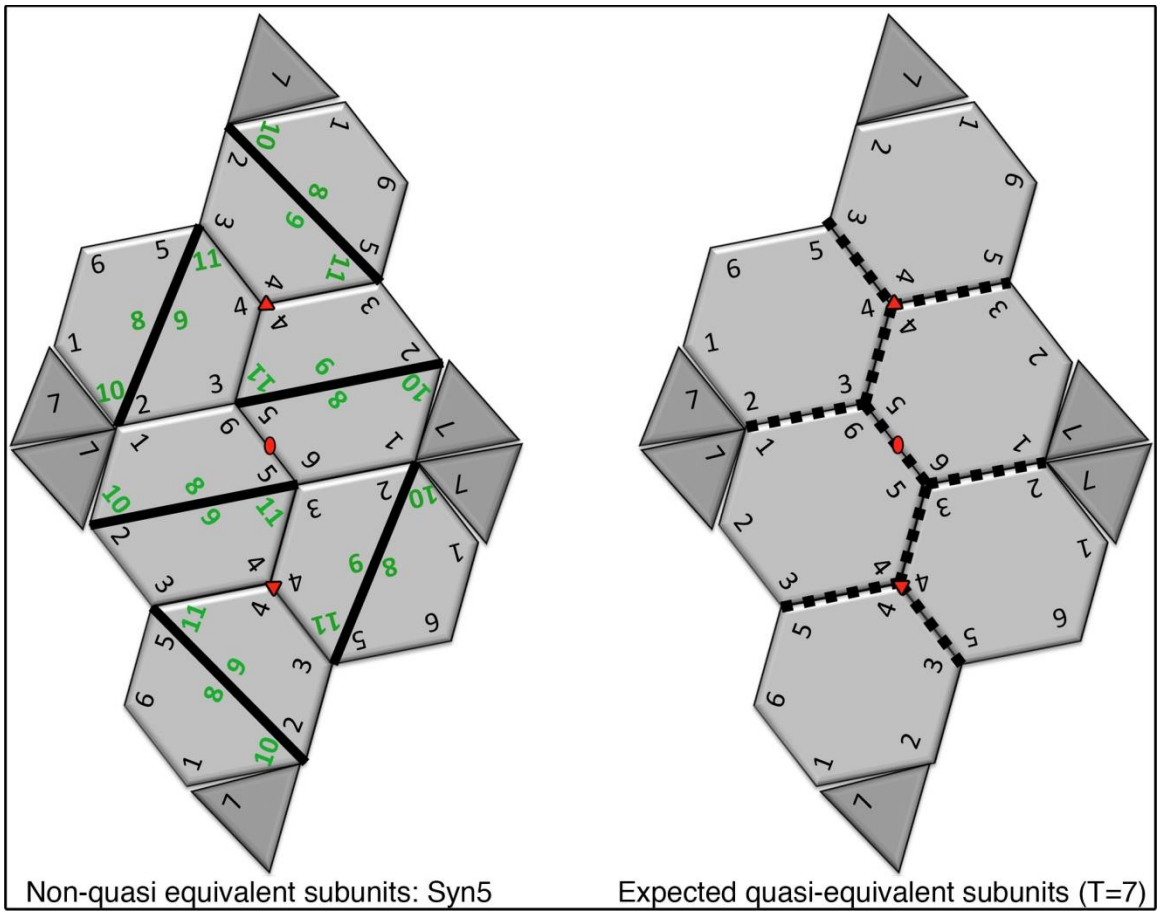
Supplementary Figure 3. Gp39 in an asymmetric unit of Syn5. **(a)** Structural comparison between major capsid proteins, gp39 (Syn5, white) and gp5 (HK97, grey). Two loops (red & yellow) in the A-domain of gp39 show the major differences with respect to that seen in gp5 (HK97, grey). Here, the loop (red) in gp39 which surrounds the opening at the six-fold axis of a hexameric capsomere is tilted by 90° relative to that seen in gp5. Additionally, an extra loop (yellow, ~30 aa) is seen in gp39, which is seen bound to knob-proteins as described in the paper. **(b)** A structural comparison of the seven gp39 models (color scheme from Figure 2b), showing the major conformational variations marked by red ovals. The red oval in the top panel highlights the conformational difference between the A-domain loop forming the pentameric capsomere opening (magenta) versus the hexameric capsomere opening (other colors), where the latter is tilted by 90° compared to the former. While the red-oval in the bottom panel highlights the differences in the E-loops of the seven gp39 subunits. In **(c)** is shown a pairwise Fourier shell correlation (FSC) between the six gp39 subunits (chains A-F) of a hexameric capsomere. Here, each gp39 subunit is compared to the rest of the five subunits of the hexameric capsomere i.e. A:B, A:C, A:D, A:E, A:F etc. The correlations between the subunits less than the average FSC (solid black) are colored red, while, the above average FSC curves are shown in blue. The correlation between the gp39 subunits (chain B:E & C:F) bound to the knob-proteins is shown in green, which are above the average correlation.



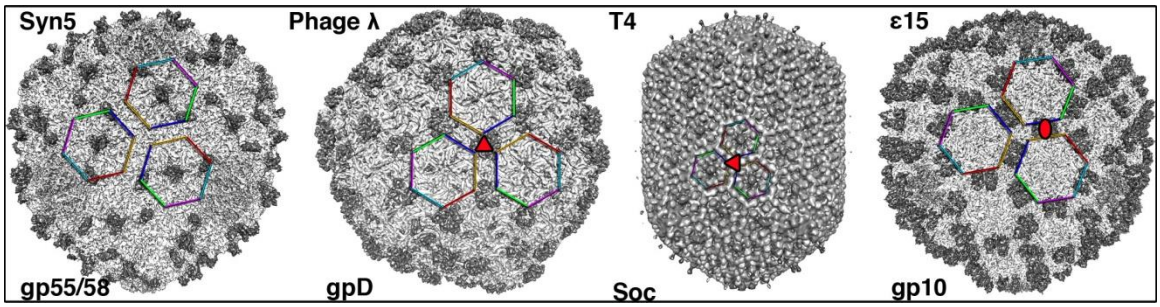
Supplementary Figure 4. Analysis of the knob-like proteins. **(a)** On the left is shown a top view of H density segmentation (smoothened map) showing a dimeric structure, while on the right is seen a rotational symmetry plot of the density, showing two peaks corresponding to a dimer arrangement. **(b)** A superposition of the I/J densities (grey mesh) is shown on the left, where in red is shown a difference map between the two densities. On the right, is shown a Fourier shell correlation between the I/J densities at FSC=0.33.



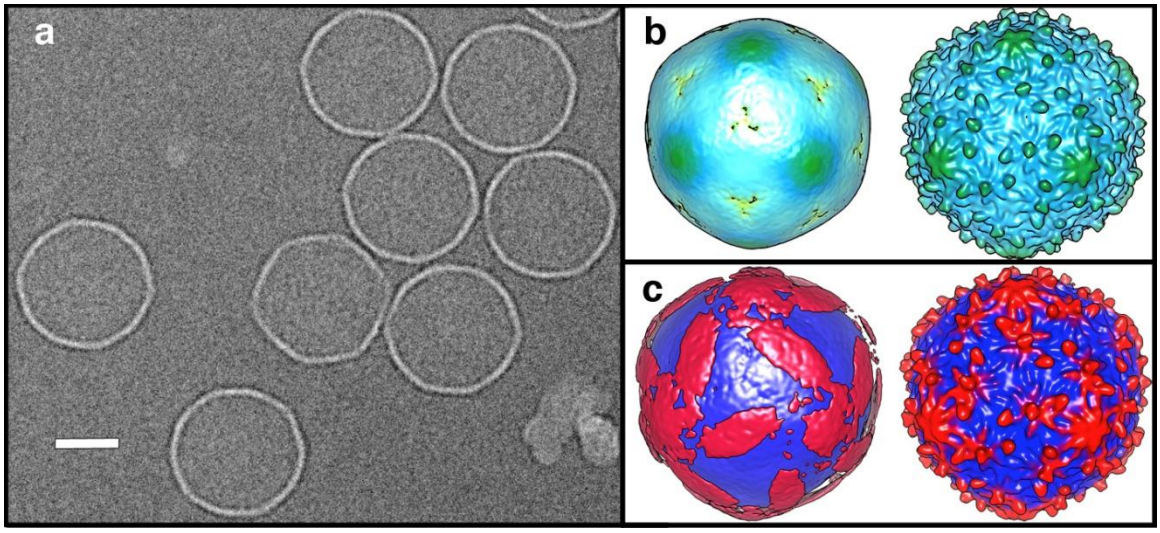
Supplementary Figure 5. Gp55 sequence analysis. A ClustalW multiple sequence analysis of gp55 with the candidates obtained from a BlastP analysis. The residues appear colored based on the ClustalX color scheme for alignment consensus and the height of the grey histograms depict the level of sequence identity from strong to low. The consensus sequence shows the residue type most prevalent at each position in the alignment; highly conserved residues (80% or greater) are capitalized and shown in purple. If several residue types are equally the most prevalent, one is chosen at random to appear in the consensus sequence.



Supplementary Figure 6. Unique arrangement of capsid proteins in Syn5. The light grey hexagons represent hexameric capsomeres, while the dark grey triangle represents a pentameric subunit, together they represent an asymmetric unit of T=7 icosahedral capsid. Two triangular faces consisting of six-asymmetric units are shown, the strict icosahedral 3-fold and 2-fold axes are labeled as per Figure 6. On the left, is shown the arrangement of 11 polypeptide chains in an asymmetric unit of Syn5, where the solid black lines represent the observed diagonal positioning of the knob-like capsid proteins (numbered in green). While on the right side is seen the arrangement of a regular T=7 capsid as seen in other virus structures, where accessory/stabilizing proteins reinforce the expected two/three-fold "inter"-capsomere interaction sites (dotted black lines).



Supplementary Figure 7. Stabilizing protein comparison with other phages. To show the location of stabilizing proteins (dark grey) in syn5 and other phages, three- hexameric capsomeres are marked by hexagons (six-colored sides). In Syn5, the protruding knob-like proteins are observed within a capsomere, unlike in other phages where they are seen at the strict and local two/three-fold symmetry axes.



Supplementary Figure 8. Syn5 procapsid analysis. **(a)** Syn5 procapsid particles observed in vitreous ice in various orientations (Scale bar 300Å). On the left in **(b)** is shown the 3D reconstruction of procapsid at 30 Å resolution from ~700 particles while, on the right is seen the mature capsid map filtered to 30Å. In **(c)** on the left, is shown a difference map (positive:blue & negative:red) calculated from the procapsid and mature capsid maps. On the right is shown the mature capsid map, colored based on the difference (red is the region of major difference).