Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/5/11/eaaw3631/DC1

Supplementary Materials for

Hierarchical assembly governs TRIM5α recognition of HIV-1 and retroviral capsids

Katarzyna A. Skorupka, Marcin D. Roganowicz, Devin E. Christensen, Yueping Wan,
Owen Pornillos*, Barbie K. Ganser-Pornillos*

*Corresponding author. Email: bpornillos@virginia.edu (B.K.G.-P.); opornillos@virginia.edu (O.P.)

Published 27 November 2019, *Sci. Adv.* **5**, eaaw3631 (2019)
DOI: 10.1126/sciadv.aaw3631

This PDF file includes:

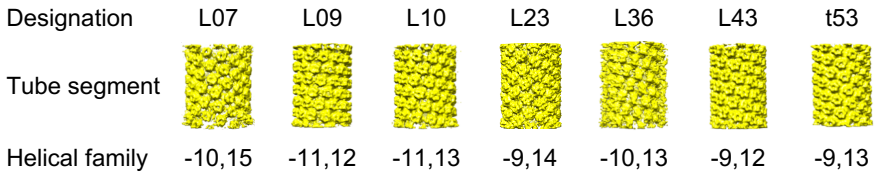

Fig. S1. Gallery of TRIM5α-coated HIV-1 CA tubes analyzed in this study. Tube segments refer to the initial reconstruction of the capsid lattice.

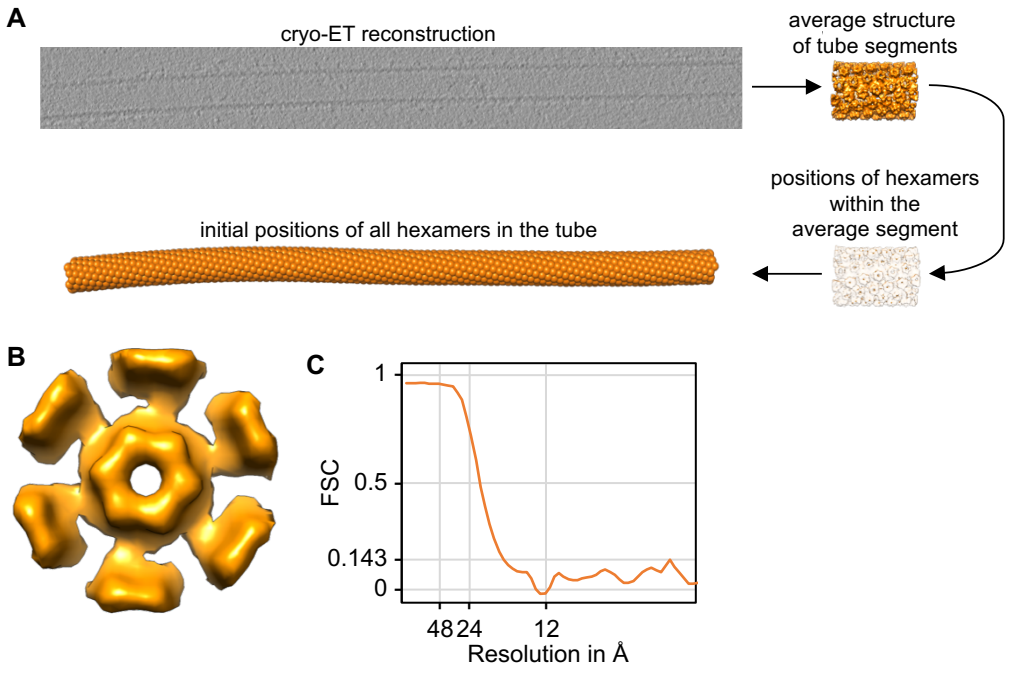

Fig. S2. Subtomogram averaging of the HIV-1 CA hexamer. (**A**) Workflow of initial lattice mapping and identification of hexamer positions within an individual tube. (**B**) Average structure of the HIV-1 CA hexamer from 7 tubes. (**C**) Fourier shell correlation (FSC) plot between even/odd half-maps. FSCs were calculated within a soft-edged sphere of 35 px radius.

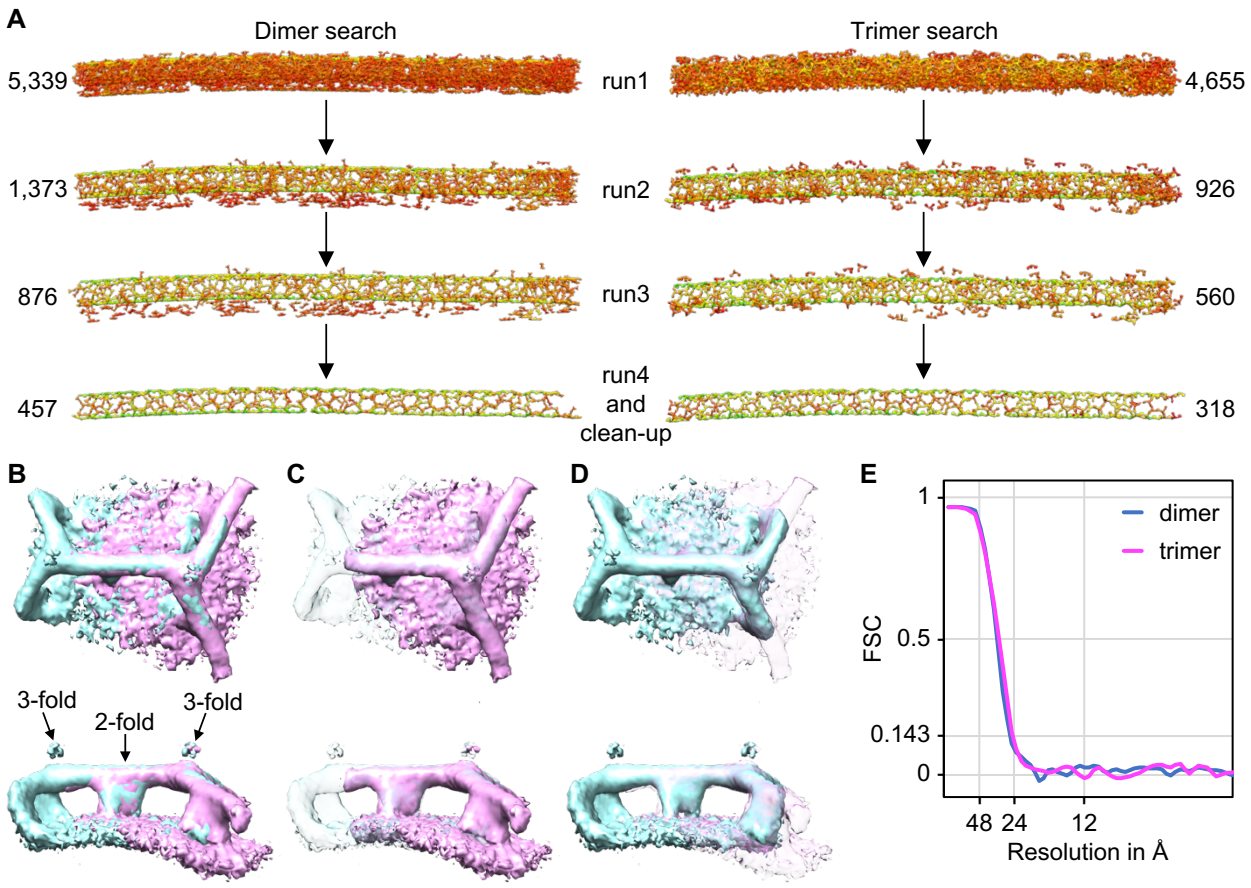

Fig. S3. Subtomogram averaging of the TRIM5α dimer and trimer. (**A**) Workflow of lattice mapping for the dimer (left) and trimer (right), illustrated with a representative tube. In this particular case, the initial mesh was used to extract 11,885 sub-volumes that oversampled the TRIM lattice by 26× for the dimer and 37× for the trimer. (**B**) Orthogonal views of isosurface representations, with the trimer map in magenta and dimer map in cyan. (**C-D**), Same views with the dimer (**C**) or trimer (**D**) rendered translucent. (**E**) Fourier shell correlation (FSC) plots between even/odd half-maps. FSCs were calculated within a soft-edged sphere of 35 px radius.

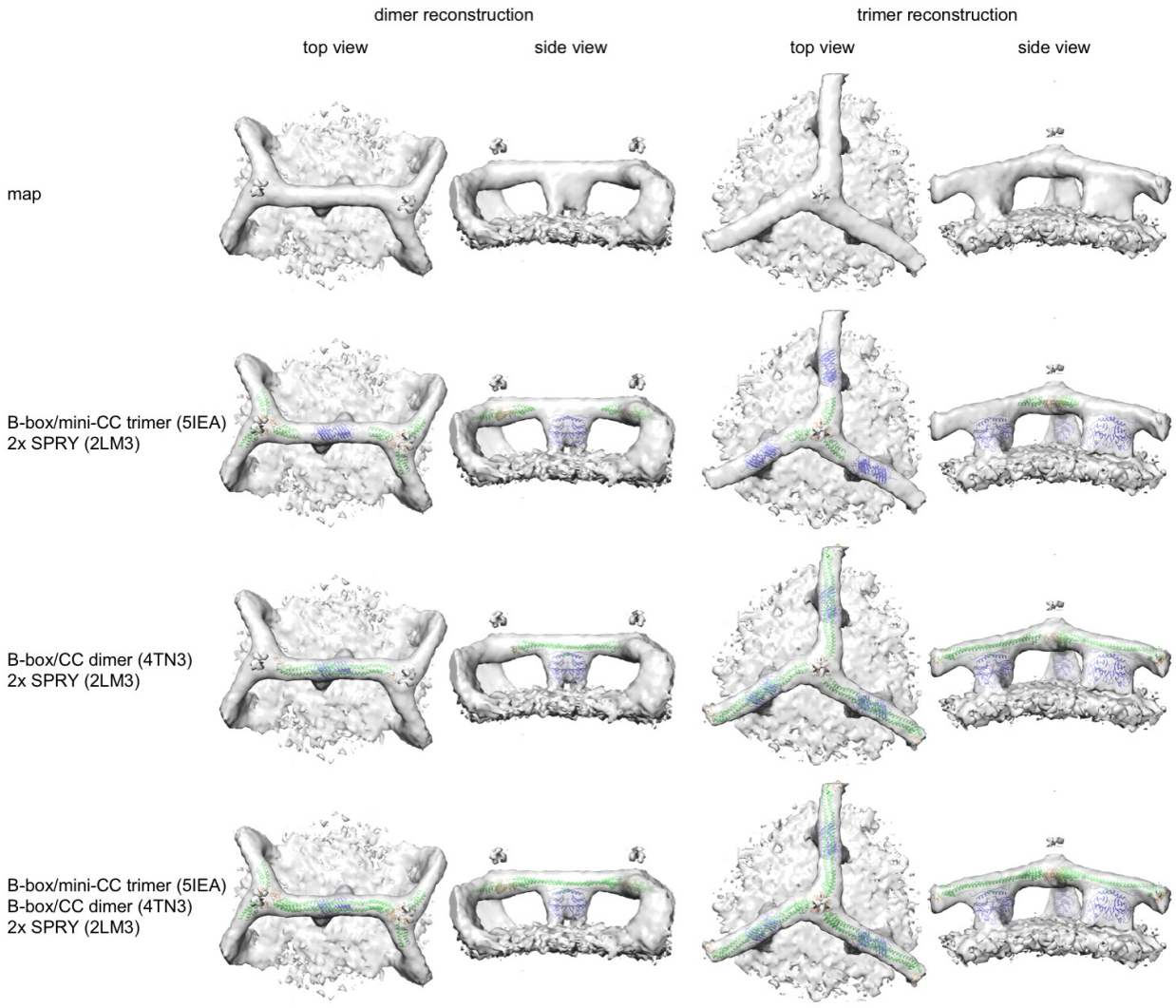

Fig. S4. Molecular fitting of the TRIM5α dimer and trimer. Subtomogram averaged maps are shown with rigid-body docked PDB models of the B-box 2/coiled-coil dimer (PDB code 4TN3) (*6*), B-box 2/mini-coiled-coil trimer (PDB code 5EIA) (*8*), and two SPRY domains (PDB code 2LM3) (*18*). Docking of the coiled-coil/SPRY segment was guided by a previously published model (*17*).

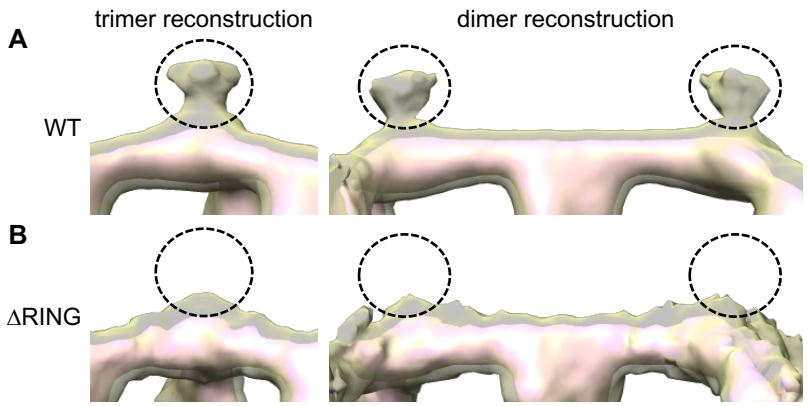

Fig. S5. Identification of the RING domain. (**A**) The trimer (left) and dimer (right) reconstructions, shown in two contour levels. Densities ascribed to the RING domain are evident at lower contour levels and encircled. Note that in the dimer, these densities do not coincide with the applied symmetry axis, and so are clearly not symmetry averaging artifacts. (**B**) Trimer (left) and dimer (right) reconstructions from complexes made of TRIM5α lacking the RING domain (ΔRING), in equivalent contour levels as in (**A**).

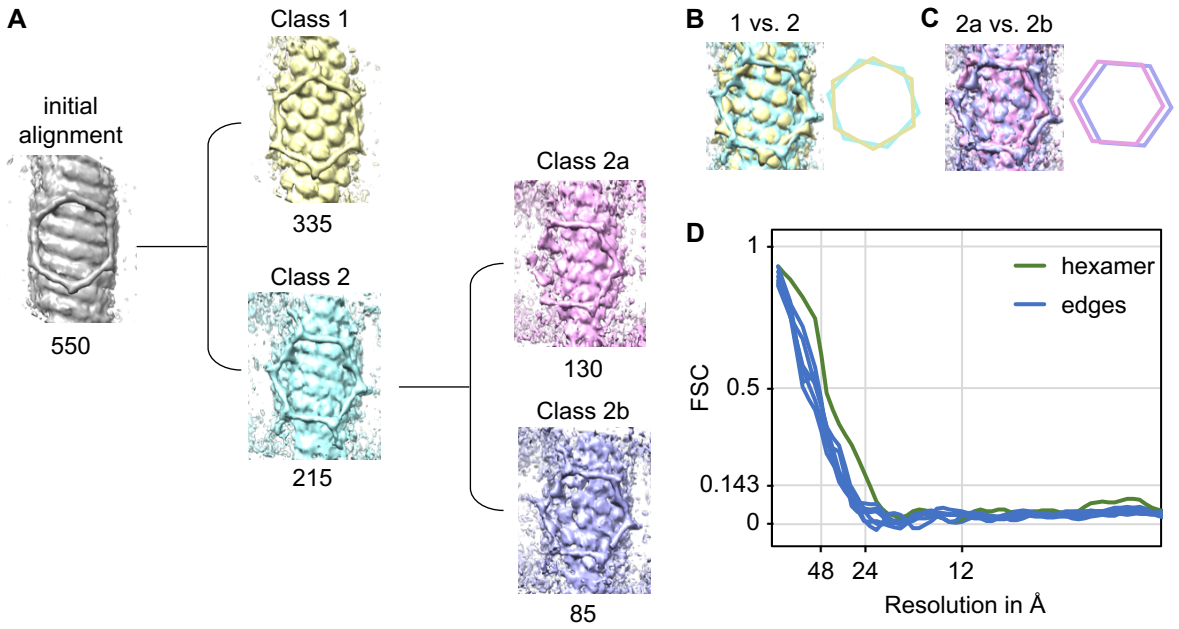

Fig. S6. Subtomogram averaging of the TRIM5α/CA complex. (**A**) Classification workflow, with the number of particles (sub-volumes) used for map calculations indicated below each reconstruction. (**B**) Comparison of Class 1 and Class 2 reconstructions indicate that the TRIM hexagons differ by rotation relative to the long axis of the tube. (**C**) Comparison of Class 2a and Class 2b reconstructions indicate that the TRIM hexagons differ in terms of translation (slippage) relative to the capsid lattice. (**D**) Fourier shell correlation (FSC) plots between the final Class 1 reconstruction and the CA hexamer reconstruction (green curve) and each of the hexagon edges and the TRIM dimer reconstruction (blue curves). Map 'resolution' values were estimated at the 0.143 cut-off.

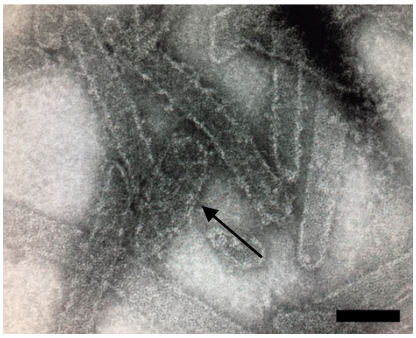

Fig. S7. De novo assembly of TRIM5α cages around capsid-like particles in the presence of inositol hexakisphosphate. Purified TRIM5α dimers (final concentration, 2 μM) were added to pre-formed HIV-1 CA assemblies in the presence of 10 μM IP6 and incubated at 37 °C for 2 h. The resulting complexes were visualized by negative stain EM. Stable complexes were not formed in the absence of IP6. Arrow points to a cone-shaped particle. Scale bar, 100 nm.