

unbound proteins were removed by three binding buffer washes. Bound GST-CHMP3 and CHMP2A proteins were analyzed by boiling the matrices in 50  $\mu$ l SDS loading buffer followed by Western blotting with anti-GST and anti-CHMP2A antibodies.

**HIV-1 vector release and infectivity.** We seeded 293T cells in 6-well plates at  $5 \times 10^5$  cells per well and co-transfected with pCAG-CHMP3-Myc expression constructs and an HIV-1 vector system (pCMV $\Delta$ R8.2, pWPTS-nlsLacZ, phCMV-VSVG). Briefly, 8  $\mu$ l Lipofectamine 2000 Transfection Reagent (Invitrogen) was combined with 1  $\mu$ g pCMV $\Delta$ R8.2, 1  $\mu$ g pWPTS-nlsLacZ, 0.36  $\mu$ g phCMV-VSVG, and 0.75  $\mu$ g pCAG-CHMP3-Myc plasmid per well according to the manufacturer's instructions. Virions were harvested from the supernatant 24 hours post transfection, and analyzed for infectivity or pelleted through a 20% (w/v) sucrose cushion for western blotting. Cells were lysed in RIPA buffer for western blotting of intracellular proteins and visualized on an Odyssey scanner (LiCor Inc.). Vector titers were determined by transducing HeLa-M cells seeded in 96-well plates at  $5 \times 10^3$  cells per well.

**IST1 partitioning assays.** Transfected HeLa-M cells were harvested, washed in PBS, and resuspended in 50  $\mu$ l lysis buffer (10 mM sodium phosphate, 400 mM KCl, 10% (v/v) glycerol, supplemented with protease inhibitors). Cells were lysed by three freeze thaw cycles, and the soluble supernatant was collected following centrifugation at 13,000  $\times$  g for 30 min at 4  $^{\circ}$ C ("Soluble Fraction"). To prepare the