
Online Supplemental Material

Supplementary Figure 1: Localization of HIV-1 Gag in early/recycling endosomes

HT*A cells were treated with monensin, nocodazole, or chloroquine, and HIV-1 Gag was detected by immunofluorescence with an antibody against CA(p24) (green). Early and recycling endosomes were labelled with Cy3-transferrin (red). Images correspond to a projection of 3D stacks (47 x 45 μm). Insets show magnifications of the boxed areas stacks (5 x 5 μm).

Supplementary Figure 2: Localization of Gag in late endosomes does not result from endocytosis of released virions.

HT* and HT1080 cells were plated at the same confluency and overnight supernatants from HT* cells were transferred to HT1080 cells, which were fixed at the indicated time and processed for immunofluorescence. Gag: α -CA(p24), green. Rab7-positive vesicles: α -Rab7, red. Panels show a projection of 3D stacks. Only few foci were visible and they did not co-localize with Rab7-positive vesicles. Examination of 3D stacks further revealed that most of them were located on the cell surface (not shown).

Supplementary Figure 3: Gag released after drug treatment is in VLPs rather than in exosomes.

A-supernatants of HT1080 cells were fractionated on 5-20% iodixanol gradients and analyzed with an anti-AP1 μ antibody. Top: fraction 1; bottom: fraction 17. The exosomal fraction revealed by μl is around fraction 5. B-HT* cells were treated with various drugs and the supernatants were collected and analyzed on 5-20% iodixanol gradients. The virions peaked in fractions 12-14 (out of 17), away from the exosomal peak in fraction 5.

Supplemental movies 1 and 2: pTRIP-MS2x24 RNA is actively transported in the cytoplasm of HT* cells. Duration of movie 1: 21 s. Movie 2: a cluster of RNA leaves a compartment of vesicular appearance and reaches the cell periphery (duration: 40 s).

Supplemental Movies 3 to 5: pTRIP-MS2x24 is transported on late endosome labelled by Ti-Vamp in HT* cells. Movies 3 and 4: zooms of the cell shown in Figure 1C, recorded with a high temporal resolution (5 images/second). Movie 5: an entire cell with a low temporal resolution (1 image every 2s). In this last movie, the delay required to switch wavelength induced an artifactual separation of the two colours during movements.
