

Figure S1. Relative viral titers were determined by creating 293T producer cell lines with each vector pair, transfecting HIV-1 Env, collecting virus, infecting Hut/CCR5 T cells, and performing flow cytometry to quantify percentages of HSA+ and Thy+ cells. The total titer (HSA+Thy) was set to 100% for each experiment. The data represent the mean \pm sd of at least three independent experiments; n.s.: not significant ($p > 0.05$, one-way ANOVA with Dunnett's post-test).

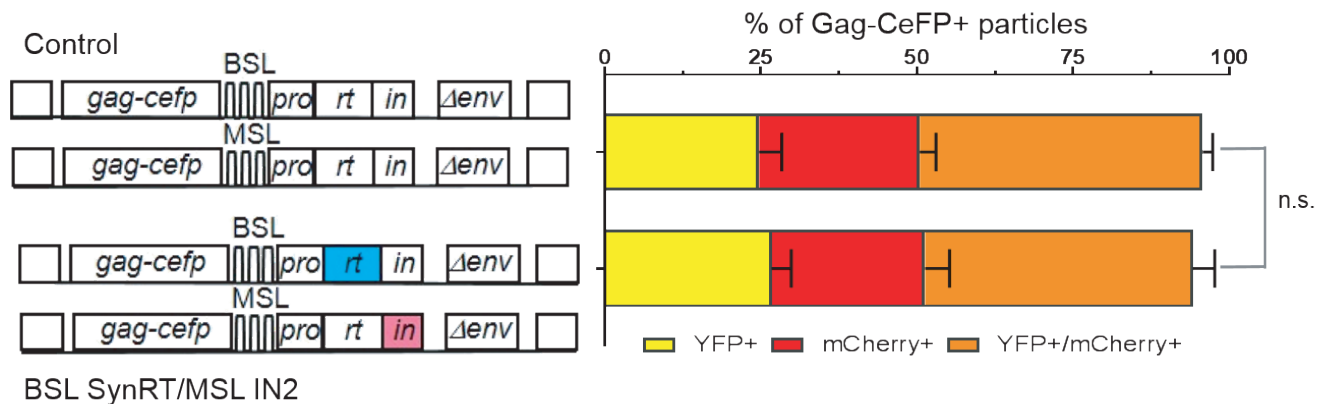


Figure S2. Reduced GFP reconstitution frequencies cannot be attributed to diminished co-packaging. To determine whether recoded *rt* and *in* regions affect copackaging, *pol* genes (with wild-type *rt/in* or recoded *rt/in*) were introduced into single virion analysis constructs that express Gag-CeFP or unlabeled Gag (not shown). Additionally, the constructs encode multiple RNA stem loops that are recognized by the BglG (BSL) or MS2 coat proteins (MSL). The indicated vector pairs were co-transfected into 293T cells, along with the labeling proteins MS2-YFP and Bgl-mCherry, using a 1:1 weight ratio of plasmids encoding labeled and unlabeled Gag. Particles were collected 20 hr post-transfection, centrifuged onto glass slides, and analyzed by fluorescence microscopy. The data represent the mean \pm sd of at least three independent experiments, and at least one thousand particles were analyzed per sample for each experiment; n.s.: not significant ($p > 0.05$, unpaired *t*-test).

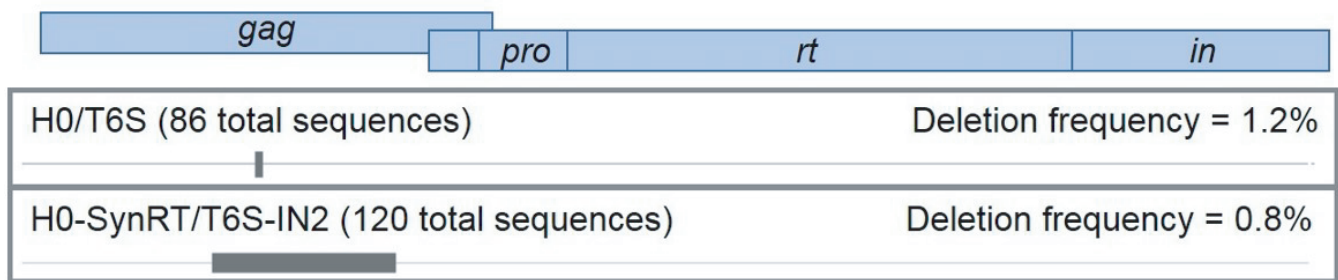


Figure S3. Deletions outside of the *rt* and *in* regions. Single-genome sequences with deletions outside the region in which recombination was blocked (i.e. *rt* and *in*) are shown. The total numbers of sequences analyzed and deletion frequencies (deletions/sequences) are indicated. Deletion frequencies in this region were not significantly different ($p>0.05$, Fisher's exact test).