Supporting Information

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SI Materials and Methods

Plasmids. The RT catalytic site mutant (D110E) was generated in pHDV-EGFP by using the Quikchange Site-Directed Mutagenesis Kit (Stratagene) and the forward and reverse primers 5′-AAATCAGTAA CAGTACTGGA GGTGGGCGAT GCATATTTTT C-3′ and 5′-GAAAAATATG CATCGCCCAC CTCCAGTACT GTTACTGATT T-3′, respectively. The p24 capsid (CA) mutant associated with less stable cores (K203A) was made by using the forward and reverse primers 5′-GCGAACCCAG ATTGTAAGAC TATTTTAGCA GCATTGGGAC CAG-3′ and 5′-CTGGTCCCAA TGCTGCTAAA ATAGTCTTAC AATCTGGGTT CGC-3′, respectively. The CA mutant associated with more stable cores (E128A/R132A) was constructed by using the forward and reverse primers 5′-CATAATCCAC CTATCCCAGT AGGAGCAATC TATAAAGCAT GGATAATCCT GGGATTAAAT AAA-3′ and 5′-TTTATTTAAT CCCAGGATTA TCCATGCTTT ATAGATTGCT CCTACTGGGA TAGGTGGATT ATG-3′, respectively.

A3F-venus yellow fluorescent protein (referred to as A3F-YFP) was constructed by modifying A3G-YFP as previously described (1). The catalytic site mutants of A3F (E251Q) and A3G (E259Q) were made by modifying A3F-YFP as previously described (2). A3C-YFP was constructed by amplifying A3C-V5-6xHIS (obtained through the AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, from Drs. B. Matija Peterlin and Yong-Hui Zheng) using the forward and reverse primers 5′-GGCCGGCTAG CATGAATCCA CAGATCAG-3′ and 5′-CCGCCAAGCT TCTGGAGACT CTCCCG-3′, respectively, and was cloned into pcDNA3.1 (Invitrogen). Nonspecific RNA binding peptide fused to YFP (P22-YFP) was constructed by modifying A3G-YFP (1). S15-mCherry (3) was constructed by amplifying mCherry using the forward and reverse primers 5′- GCTAGCATGG GGAGCAGCAA GAGCAAGCCC AAGGACCCCA GCCAGCGCCG GAACAACAAC AACGTGAGCA AGGGCGAG-3′ and 5′-GTTTAAACTT ACTTGTACAG CTCGTCCATG CCG-3′, respectively, and cloned into pcDNA3.1.

1. Friew YN, Boyko V, Hu WS, Pathak VK (2009) Intracellular interactions between APOBEC3G, RNA, and HIV-1 Gag: APOBEC3G multimerization is dependent on its association with RNA. *Retrovirology* 6:56.
2. Mbisa JL, Bu W, Pathak VK (2010) APOBEC3F and APOBEC3G inhibit HIV-1 DNA integration by different mechanisms. *J Virol* 84(10):5250–5259.
3. Campbell EM, Perez O, Melar M, Hope TJ (2007) Labeling HIV-1 virions with two fluorescent proteins allows identification of virions that have productively entered the target cell. *Virology* 360(2):286–293.

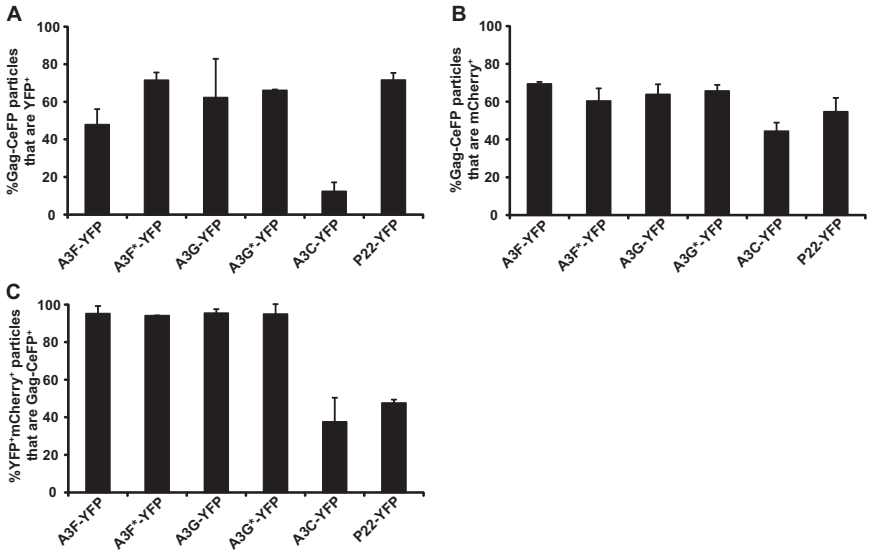

Fig. S1. Labeling efficiency of noninfectious virus-like particles. Gag-CeFP-, YFP-, and S15-mCherry-labeled particles in each image were detected using Localize, and the centers of the spots were used to determine colocalization. (*A*) Percentage of virions (Gag-CeFP) that are YFP$^+$. (*B*) Percentage of virions that are S15-mCherry$^+$. (*C*) Percentage of YFP$^+$mCherry$^+$ particles that are virions. Error bars indicate SD of two independent experiments.

A

Efficiency of infection in HeLa cells

	AVG ± SD	Overall efficiency
P22-YFP particles/cell (0 hrs)	97 ± 16	100% ± 0%
Early RT products/cell (6 hrs)	31 ± 8	34% ± 15%
Late RT products/cell (6 hrs)	30 ± 8	33% ± 15%
GFP-expressing proviruses/cell (48 hrs)	1.8 ± 0.5	1.8% ± 0.4%

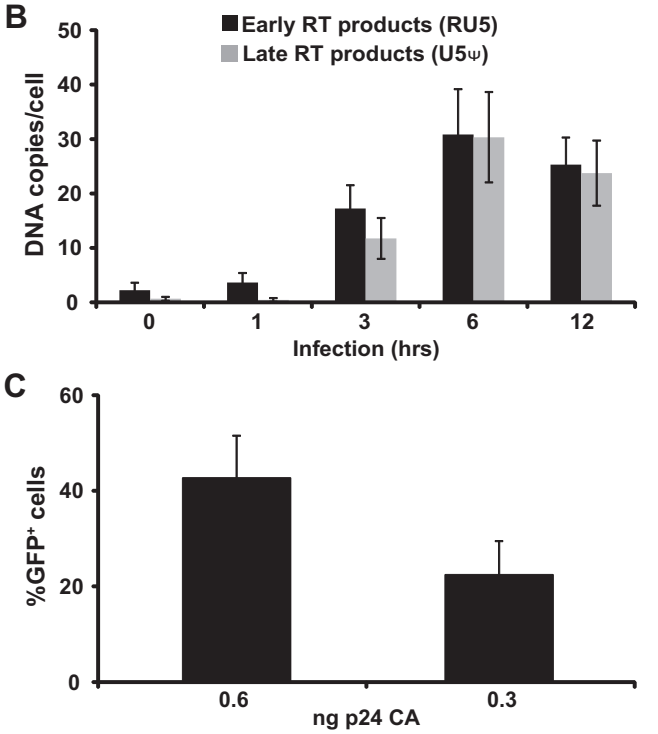

Fig. S2. Multiplicity of infection of HeLa cells. (*A*) HeLa cells were infected in parallel with 2.5 ng p24 CA of P22-YFP–labeled, VSV-G pseudotyped virus. The overall efficiency of the infection was determined by comparing the early (RU5) and late (U5Ψ) RT products at the 6-h time point and GFP-expressing proviruses at the 48-h time point relative to the number of P22-YFP–labeled particles per cell at the 0-h time point (set to 100%). Confocal z-stacks were acquired of the entire cells, and the YFP signals were quantified. (*B*) Viral DNA early (RU5) and late (U5Ψ) products were quantified at 0, 1, 3, 6, and 12 h after infection. Host *CCR5* gene copy numbers were determined for normalization of cell numbers. The time point at which the maximum number of early and late RT products per cell was reached (6 h) is shown in *A*. (*C*) The P22-YFP–labeled virus was titrated onto HeLa cells, and the proportion of GFP^+ cells was determined by FACS 48 h after infection. The number of GFP-expressing proviruses per cell normalized to 2.5 ng p24 CA input is shown in *A*. Error bars indicate SD of four independent experiments.

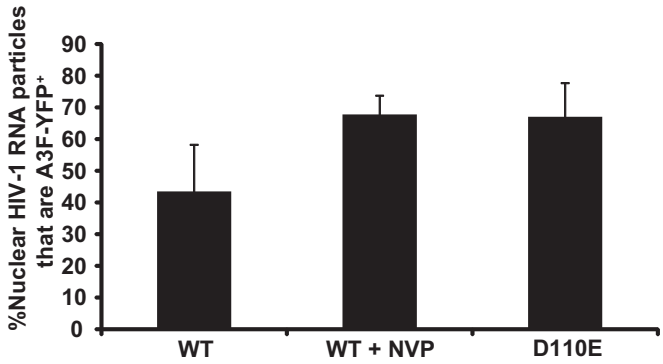

Fig. S3. Nuclear viral RNA signals colocalize with A3F-YFP. Infection of HeLa cells with wild-type A3F-YFP–labeled virus (WT), WT virus in the presence of 5 μM nevirapine (WT + NVP), or RT^{-} virus (D110E). The nuclear envelope was immunostained using an anti-Lamin A/C antibody (followed by an Alexa Fluor 405-labeled secondary antibody), and viral RNA was detected by FISH. Confocal z-stacks were acquired of the entire cells, the YFP and RNA signals in the nuclei were quantified, and the proportion of the RNA signals that colocalized with the YFP signals was determined. There were no significant differences in the proportion of colocalized signals; $P > 0.05$, *t* test.