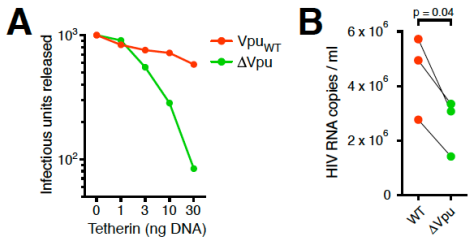

Figure S1 (related to Fig. 2): (A) HIV-Tomato and HIV-GFP ΔVpu plasmids were co-transfected into HEK cells together with increasing amounts of a tetherin expression plasmid and infectious particles in the supernatant were quantified 2 days later by a β-galactosidase assay on the TZM-bl reporter cell line. While WT viral particles are efficiently released even in presence of high amounts of tetherin, HIV-GFPΔVpu particle release is impaired in a tetherin dose-dependent manner. (B) $CD4^+$ T_{CM} generated from PBMCs of three different human donors were infected with either HIV-GFP or HIV-GFP ΔVpu and infectious particles in the supernatant quantified 2 days later.

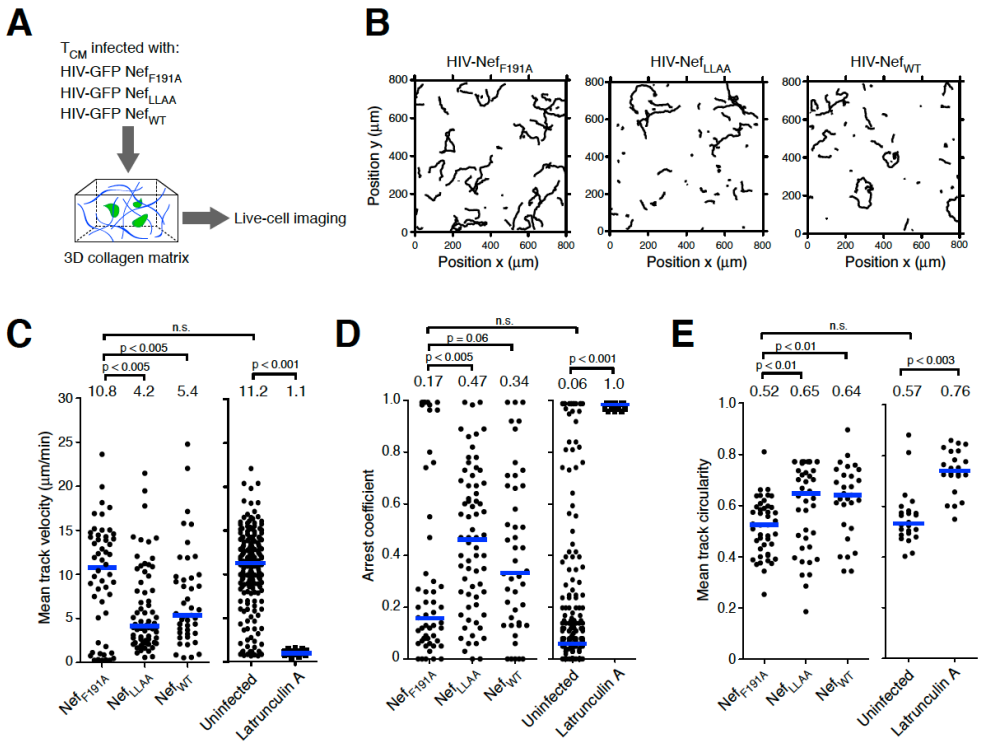

Figure S2 (related to Fig. 5): (A) T cells infected with either HIV-GFP Nef_{WT}, Nef_{F191A} or Nef_{LLAA} were embedded into a collagen gel and immediately recorded by live-cell imaging. (B) Migratory tracks of GFP^+ cells from a 30-minute recording. (C) Mean track velocities, (D) Arrest coefficients, and (E) Mean track circularities of the three populations. Uninfected T cells and uninfected T cells pre-treated with the actin-polymerization inhibitor Latrunculin A were imaged in separate collagen gels and used to provide reference points for all measurements. Numbers above graphs and blue lines represent medians. Data are pooled from 9 individual recordings from 2 independent experiments. n.s. = not significant.

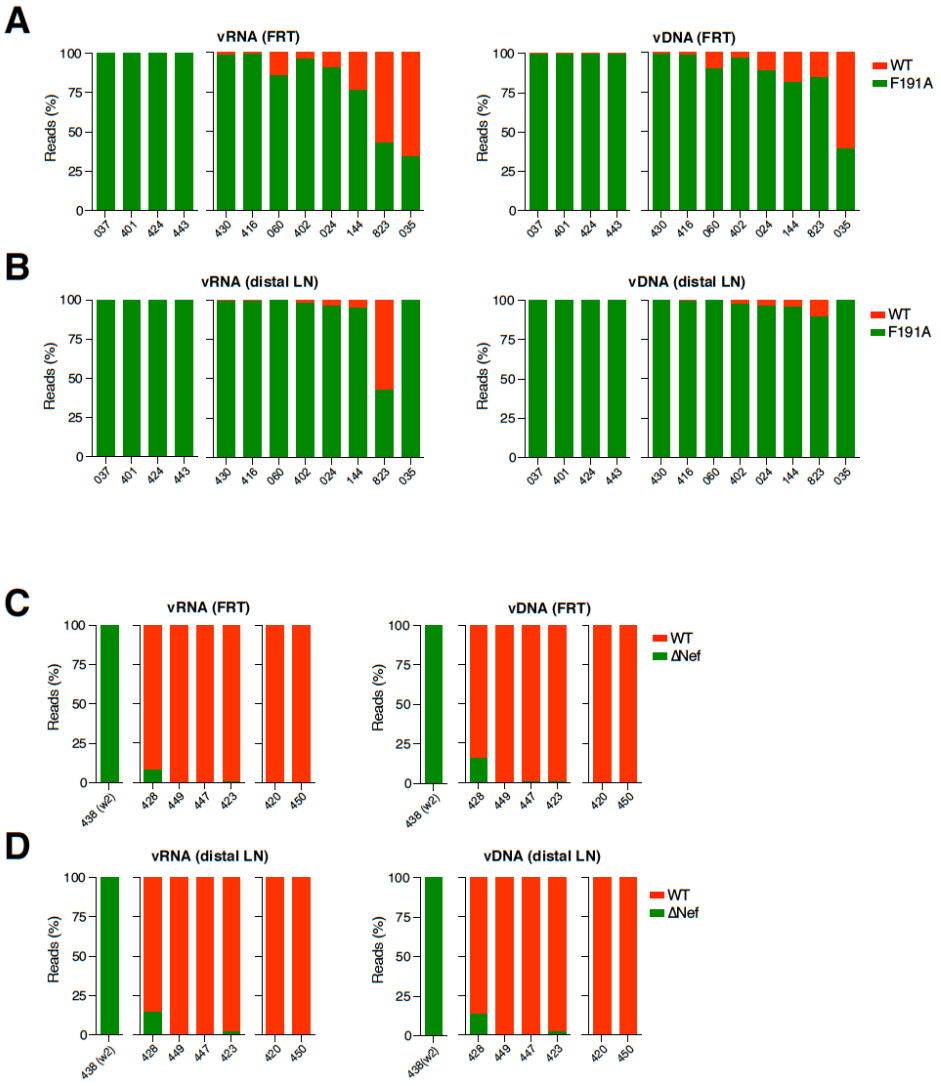

Figure S3 (Related to Fig. 6): (A, B) 10^4 IUs each of HIV Nef_{WT} and HIV Nef_{F191A} were intravaginally inoculated at 50:50 ratio into 12 BLT NSG mice. Two or three weeks later, female reproductive tract (FRT) and lymph nodes (LNs) were harvested from infected animals and processed for NGS analysis (~2 x 10^4 reads/sample) of vRNA (left panels) and vDNA (right panels) in (A) FRT and (B) dLNs to determine the proportions of nef_{WT} (red) and nef_{F191A} (green) sequence. (C, D) Similar as for (A, B), but tissue was harvested 2 to 10 weeks after initial inoculation of HIV Nef_{WT} and HIV ΔNef.

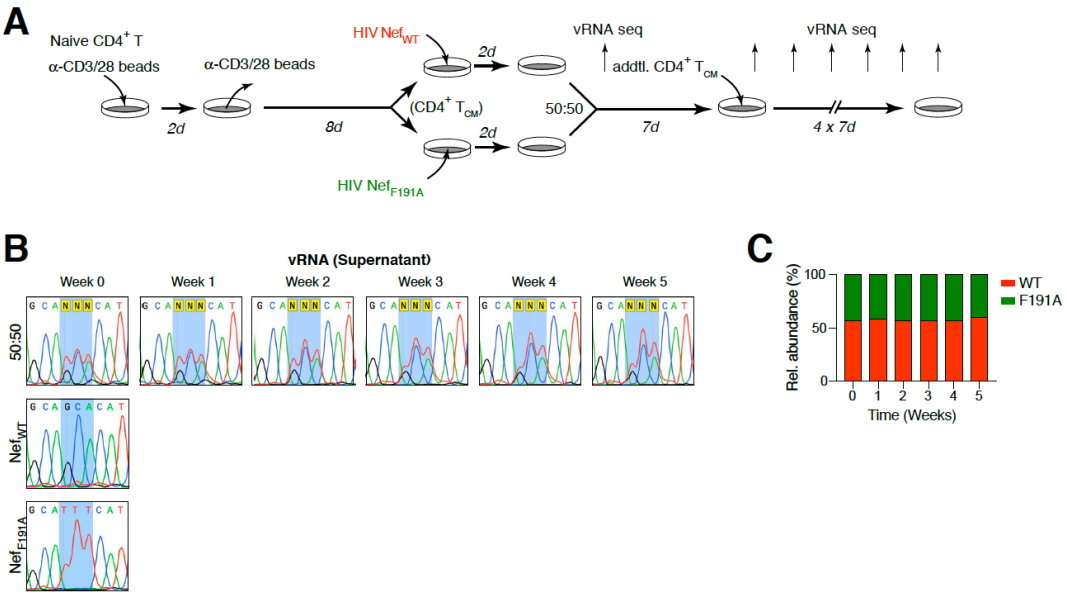

Figure S4 (Related to Fig. 6): (A) Experimental scheme: CD4⁺ T cells purified from PBMCs were activated and then rested for 8 days to produce T_{CM} before being infected with indicated viral clones. 2 days later, infected T_{CM} were mixed at 50:50 ratios and supernatants collected for Sanger sequencing of vRNA 6 hours after cell mixing, and then weekly for a total of 5 weeks. Additional resting T_{CM} were added to the culture every week to compensate for cell losses and to provide new target cells. (B) Sanger chromatograms from supernatants before (bottom rows) and after mixing (top row. (C) Quantification of the proportions of *nef*$_{WT}$ (red) and *nef*F_{191A} (green) sequence over the 5-week period of the culture. This experiment was performed twice with similar results.

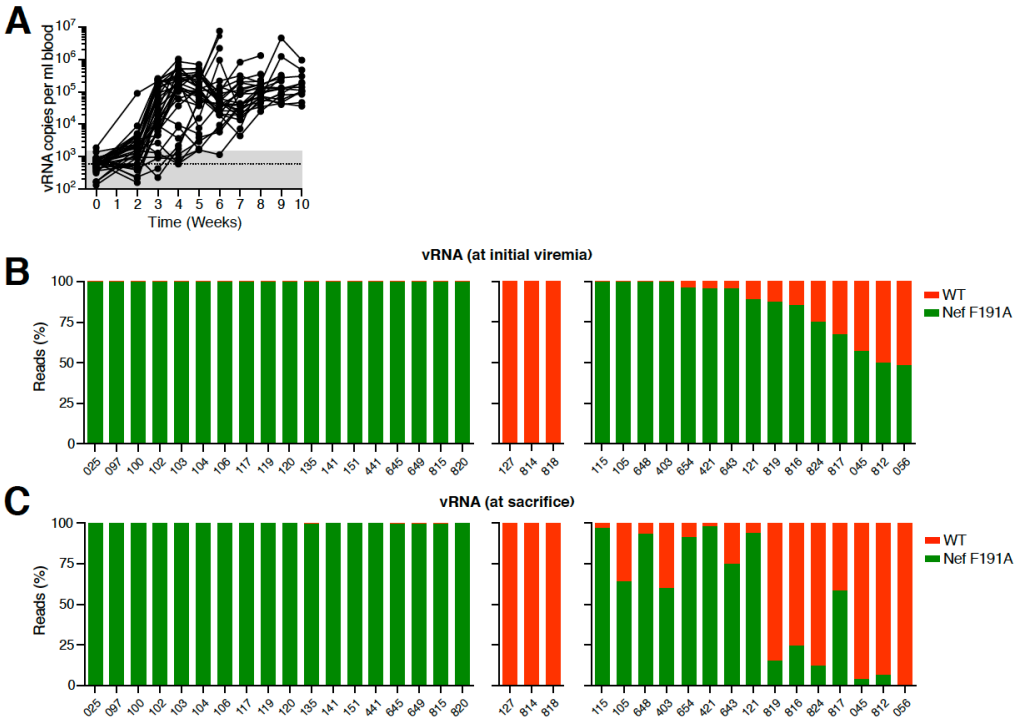

Figure S5 (Related to Fig. 7):
(A) Plasma viremia, as measured by RT-qPCR for HIV *gag*. Dotted line and grey-shaded area indicate mean and range of background signals in three uninfected control animals.
(B, C) Ratio of Next Generation Sequencing reads (~2 x 10^4 reads/sample) for nef_{WT} (red) or nef_{F191A} (green) from vRNA obtained from plasma at the time of initial viremia (B) or at the time of animal sacrifice (C). Numbers at the bottom indicate individual animals. Either HIV nef_{WT} or nef_{F191A} sequence was not detectable in plasma at any time-point in the 18 animals grouped on the left and the 3 animals grouped in the center, respectively. Animals with mixed infections grouped on the right are the same as shown in Fig. 7B.

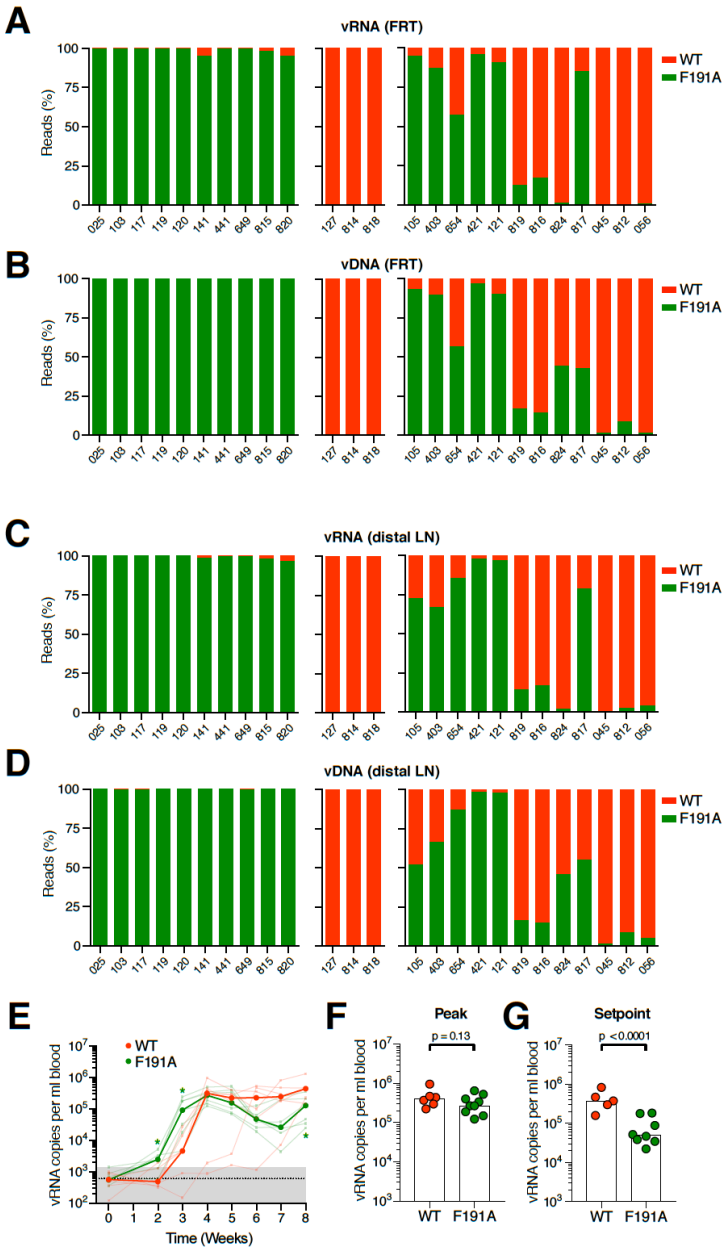

Figure S6 (Related to Fig. 7): (A-D) NGS analysis (~2 x 10^4 reads/sample) of vRNA (A, C) and vDNA (B, D) in FRT (A, B) and dLNs (C, D) to determine the proportions of nef_{WT} (red) and nef_{F191A} (green) sequence. Tissue was harvested between 4 and 10 weeks after initial inoculation of HIV Nef_{WT} and HIV Nef_{F191}. *Note*: From some animals shown in Fig. S5, vRNA or vDNA could not be obtained from tissues. (E) Plasma viremia (median and in individual animals), as measured by RT-qPCR for HIV *gag*, in animals in which either only nef_{WT} or only nef_{F191A} sequence (pooled from experiments shown in Figs. 6 and 7) could be detected at any time-point and from any tissue, and which were thus considered as singly infected. Dotted line and grey-shaded area indicate mean and range of background signals in three uninfected control animals. *$p < 0.05$. (F) Peak viremia levels of animals shown in (E) (G) Viremic set points of animals shown in (E), determined as median of all measurements following peak viremia for each individual animal.