Cell

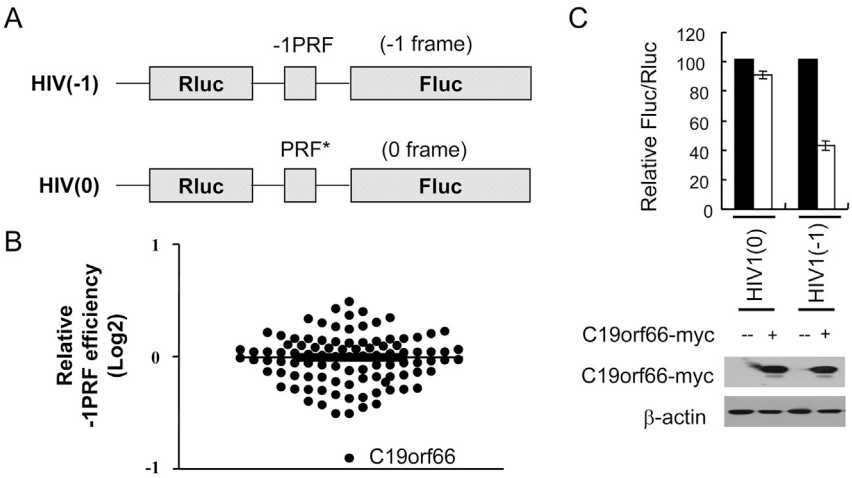

Figure S1. C19orf66 Inhibits the -1PRF of HIV-1, Related to Figure 1

(A) Schematic representation of the dual luciferase reporter carrying the wild-type or mutant −1PRF signal from HIV-1. PRF*, a nucleotide A was inserted into the slippery sequence such that Rluc and Fluc were expressed in the same reading frame. (B) The pHIV(−1) reporter was transfected into 293T cells together with an empty vector or a plasmid expressing an ISG. At 36 h posttransfection, luciferase activities were measured. The Fluc/Rluc ratio reflects the −1PRF efficiency. The relative −1PRF efficiency in the cells transfected with the empty vector was set as 1. (C) The reporter indicated was transfected into 293T cells with or without a plasmid expressing C19orf66. At 36 h posttransfection, luciferase activities were measured. The Fluc/Rluc ratio reflects the −1PRF efficiency. The relative −1PRF efficiency in the absence of C19orf66 was set as 100. Data presented are means ± SD of three independent measurements, representative of three independent experiments.

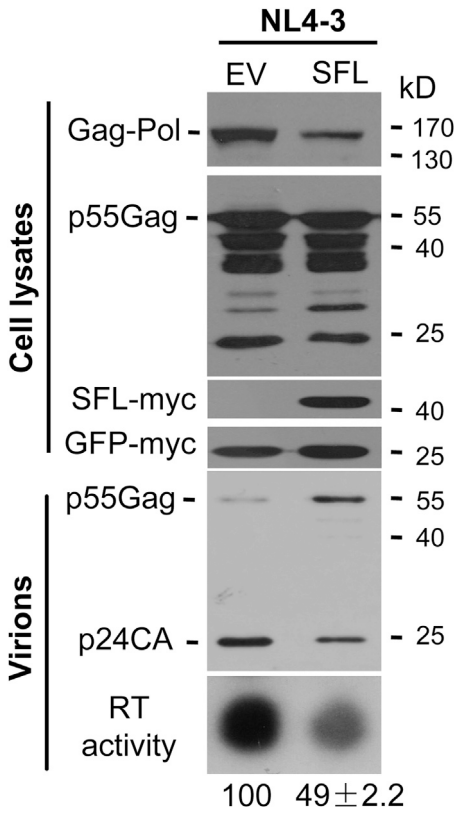

Figure S2. SFL Inhibits Gag-Pol Expression from NL4-3, Related to Figure 1

NL4-3 virus was produced in 293T cells with or without SFL-myc. GFP-myc was included to serve as a control. Cells were lysed and assayed for protein expressions. Virions in the supernatants were purified and analyzed for protein levels and RT activity. The relative RT activities in the virions are indicated. Data presented are means ± SD of three independent experiments. EV, empty vector.

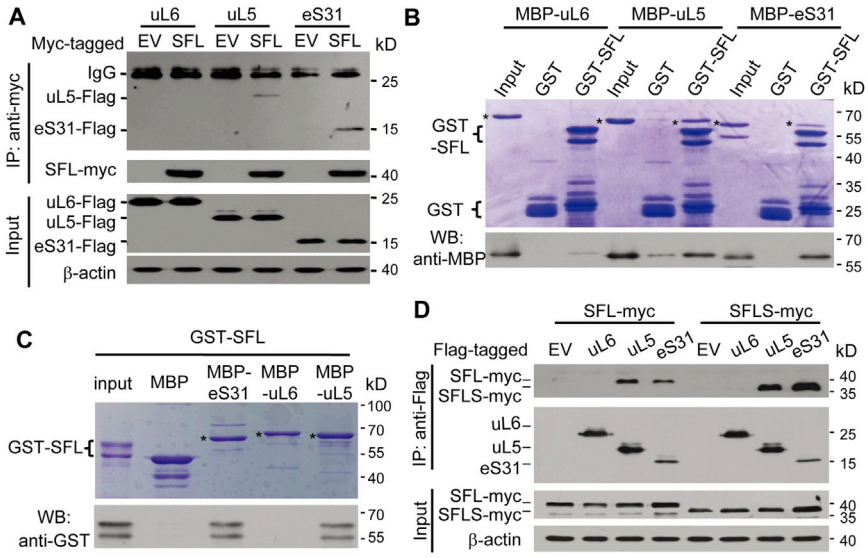

Figure S3. Interactions of SFL-Myc with Selected Ribosomal Proteins, Related to Figure 3

(A) and (D) SFL-myc was co-expressed with the Flag-tagged ribosomal proteins indicated in 293T cells. The cell lysates were immunoprecipitated with anti-myc antibody (A) or anti-Flag antibody (D). The proteins were analyzed by western blotting. (B) and (C) GST-SFL and MBP-tagged ribosomal proteins indicated were expressed in bacteria and partially purified. (B) GST or GST-SFL was immobilized and incubated with MBP proteins. (C) The MBP proteins were immobilized and incubated with GST-SFL. The bound proteins were resolved on SDS-PAGE and detected by Coomassie staining (upper panels) or by western blotting (lower panels). The asterisk denotes MBP fusion proteins.

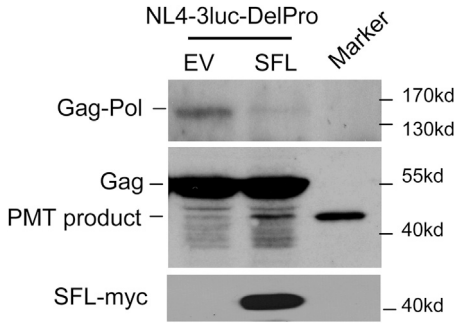

Figure S4. SFL Causes Premature Translation Termination of HIV-1 Gag-Pol, Related to Figure 5

The coding sequence of the protease in NL4-3luc was deleted to generate the reporter NL4-3-DelPro, which was transiently expressed in 293T cells with or without SFL-myc. The proteins were analyzed by western blotting. Marker: A Gag-expressing construct was engineered such that it expresses a protein ending with amino acids NFL, the same as that of the predicted PMT product.

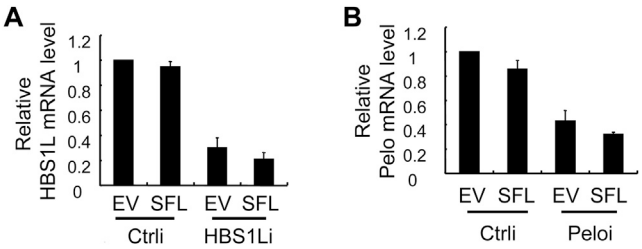

Figure S5. Effectiveness of the shRNAs Targeting HBS1L and Pelo, Related to Figure 6

Levels of the mRNAs in the cells in Figure 6A were measured by RT-qPCR using *gapdh* mRNA as an internal control. Data presented are means ± SD of three independent measurements, representative of three independent experiments.

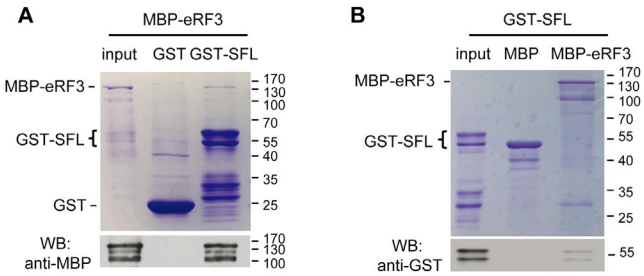

Figure S6. Interactions of Bacterially Expressed SFL with eRF3, Related to Figure 6

GST-SFL and MBP-eRF3 were bacterially expressed and partially purified. (A) GST or GST-SFL was immobilized and incubated with MBP-eRF3. (B) MBP or MBP-eRF3 was immobilized and incubated with GST-SFL. The bound proteins were resolved on SDS-PAGE and detected by Coomassie staining (upper panels) or by western blotting (lower panels).

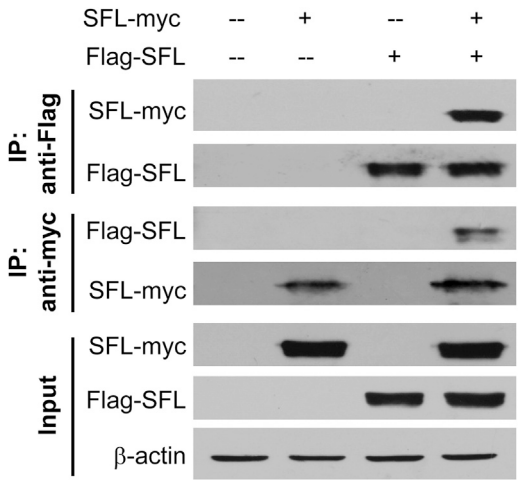

Figure S7. Intermolecular Interactions of SFL, Related to Figure 7

SFL-myc and Flag-SFL were co-expressed in 293T cells. The cell lysates were immunoprecipitated with anti-myc or anti-Flag antibody in the presence of RNaseA. The precipitated proteins were analyzed by western blotting.