Supporting Information

Li et al. 10.1073/pnas.1404851111

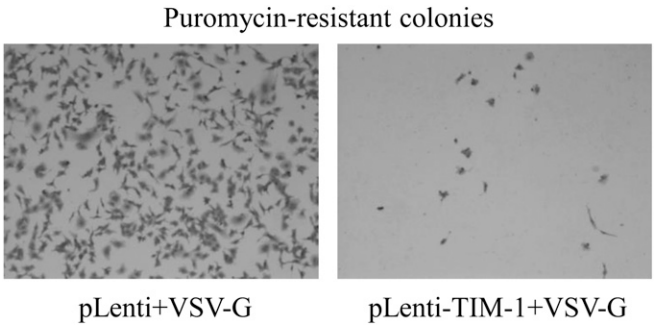


Fig. S1. HIV-1 lentiviral vectors expressing T-cell immunoglobulin (Ig) and mucin domain 1 (TIM-1) exhibit markedly reduced transduction efficiency. Images show the density of puromycin-resistant colonies in HTX cells transduced by VSV-G pseudotyped HIV-1 vector encoding TIM-1 (pLenti–TIM-1) or GFP (pLenti-puro-GFP). Cells were fixed and stained with Coomassie Brilliant Blue.

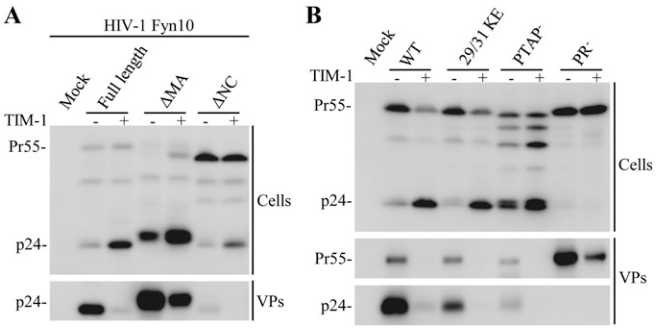


Fig. S2. TIM-1 blocks HIV-1 release regardless of Gag or protease defects. (*A*) HEK293T cells were transfected with HIV-1 Fyn10 mutants with matrix (MA), nucleocapsid (NC), or both deleted, along with TIM-1 expression plasmid or empty vector. Western blotting was performed to assess cell-associated Gag (cells) and cell-free viral particle release (VPs). (*B*) Experimental procedures were the same as described in *A*, except that 293T cells were transfected with plasmids encoding the wild-type pNL4-3 (WT), 29/31KE, PTAP^{-}, or PR^{-}. Positions of the Gag precursor Pr55Gag (Pr55) and the mature CA protein (p24) are indicated.

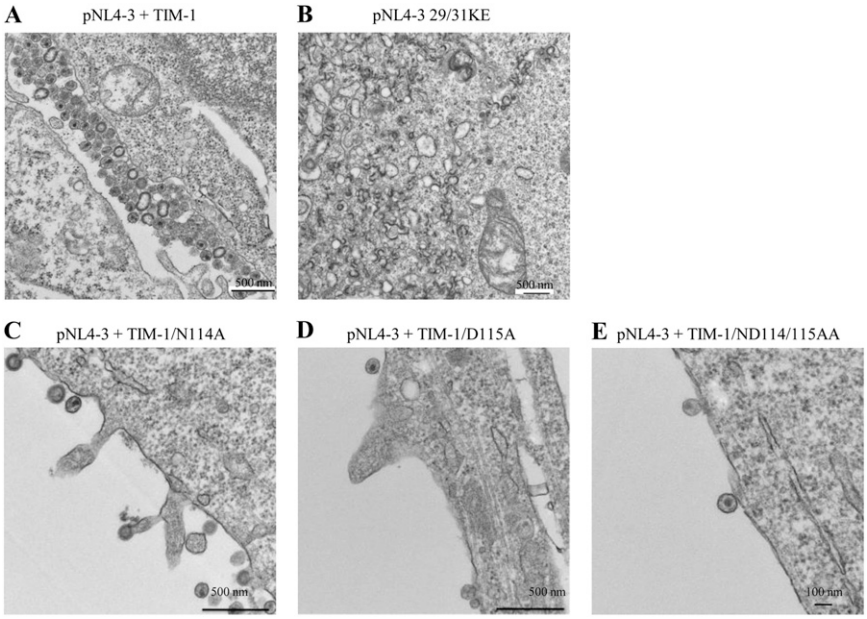


Fig. S3. Expression of WT TIM-1 does not lead to the accumulation of HIV-1 in intracellular vesicles; TIM-1 mutants deficient for phosphatidylserine (PS) binding do not inhibit HIV-1 release. (*A* and *B*) Transmission electron microscopy (TEM) analysis of intracellular HIV-1 virions in 293T cells expressing TIM-1. (*C*–*E*) TEM analysis of 293T cells expressing TIM-1 mutants deficient for PS binding and NL4-3. The experimental procedures were the same as described in Fig. 2*B*, except that TIM-1 mutants or TIM-1 were examined along with the HIV-1 NL4-3 29/31KE mutant, which is known to assemble in multivesicular bodies (MVBs).

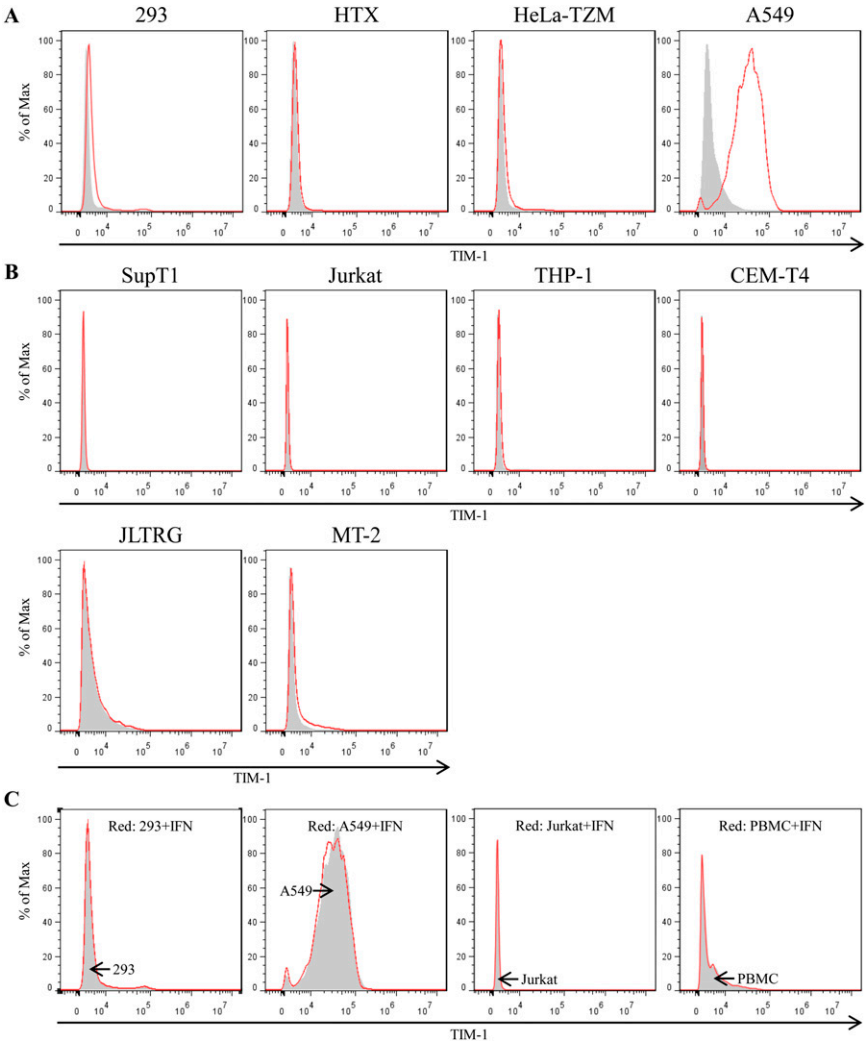


Fig. S4. Examination of the endogenous TIM-1 expression in different cell lines and the effect of IFN treatment on TIM-1 expression. The endogenous TIM-1 expression on the cell surface was determined by flow cytometry in a panel of epithelial cell lines (293, HTX, HeLa-TZM, A549) (*A*), T-cell lines (SupT1, Jurkat, CEM-T4, JLTRG, MT-2), and monocytes (THP-1) (*B*). Gray areas represent the secondary antibody alone controls. Note that a low level of TIM-1 was consistently detected in 293 cells and that A549 cells express a high level of TIM-1. (*C*) HEK293, A549, Jurkat, and peripheral blood mononuclear cells (PBMCs) were treated with 1,000 units of IFN-α2b for 18 h, and TIM-1 expression on the cell surface was determined by flow cytometry. Arrows indicate cells without treatment. In all cases, an anti-hTIM-1 antibody was used as primary antibody for binding.

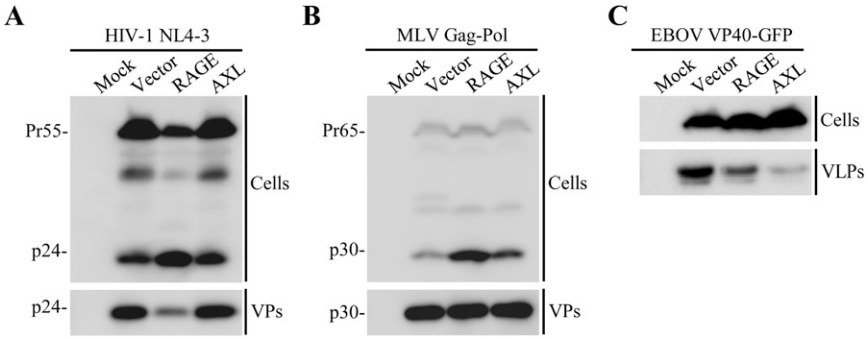


Fig. S5. Effects of additional PS receptors, Axl and RAGE, on HIV-1, murine leukemia virus (MLV), and Ebola virus (EBOV) release. The experimental procedures were as described as in Fig. 5, except that Axl and RAGE were tested. Mock, untransfected 293T cells; Vector, cells were transfected with plasmids encoding HIV-1 NL4-3 provirus (*A*), MLV Gag-Pol (*B*), or EBOV VP40-GFP (*C*) plus an empty expression vector. Positions of the HIV-1 Gag precursor Pr55Gag (Pr55), the MLV Gag precursor Pr65Gag (Pr65), the mature HIV-1 CA protein (p24), and mature MLV CA protein (p30) are indicated.

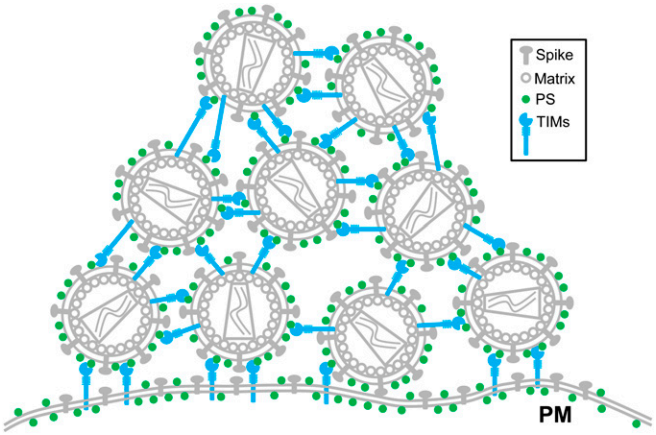
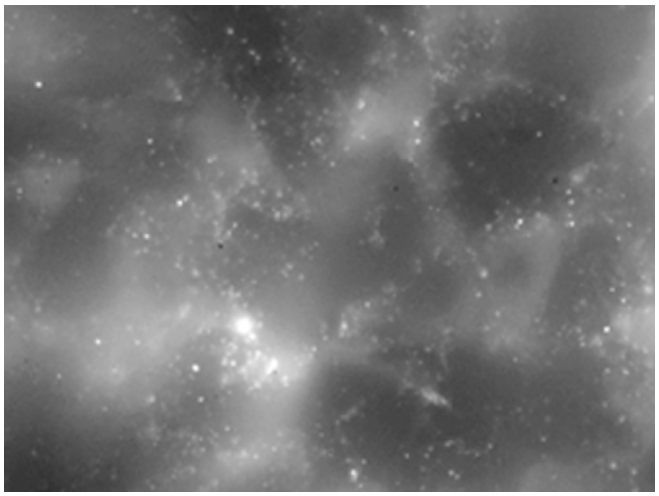
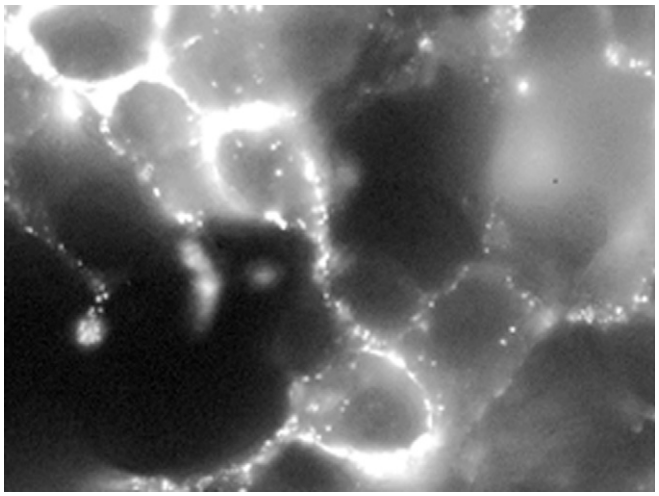


Fig. S6. A proposed model for TIM-mediated inhibition of HIV-1 release. Expression of TIMs in HIV-1 producer cells results in PS flipping onto the outer leaflet of the plasma membrane. HIV-1 acquires PS and TIMs from viral producer cells upon budding. The intimate interaction between TIMs and PS among HIV-1 virions, as well as that between viral producer cells and virions, collectively leads to accumulation of HIV-1 virions on the plasma membrane.



Movie S1. HIV-1 Gag VLP release. HEK293T cells were transfected with plasmids encoding HIV-1 Gag-GFP in the absence of TIM-1. Cells were seeded to glass bottom microwell dishes, and movies were recorded using an Olympus fluorescent microscope (100× Oil).

Movie S1



Movie S2. HIV-1 Gag VLP release in the presence of TIM-1. HEK293T cells were cotransfected with plasmids encoding HIV-1 Gag-GFP and TIM-1. Movies were recorded using an Olympus fluorescent microscope (100× Oil) as described for Movie S1.

Movie S2