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Supplemental Information

A Long Cytoplasmic Loop Governs the Sensitivity of the Anti-viral Host Protein SERINC5 to HIV-1 Nef

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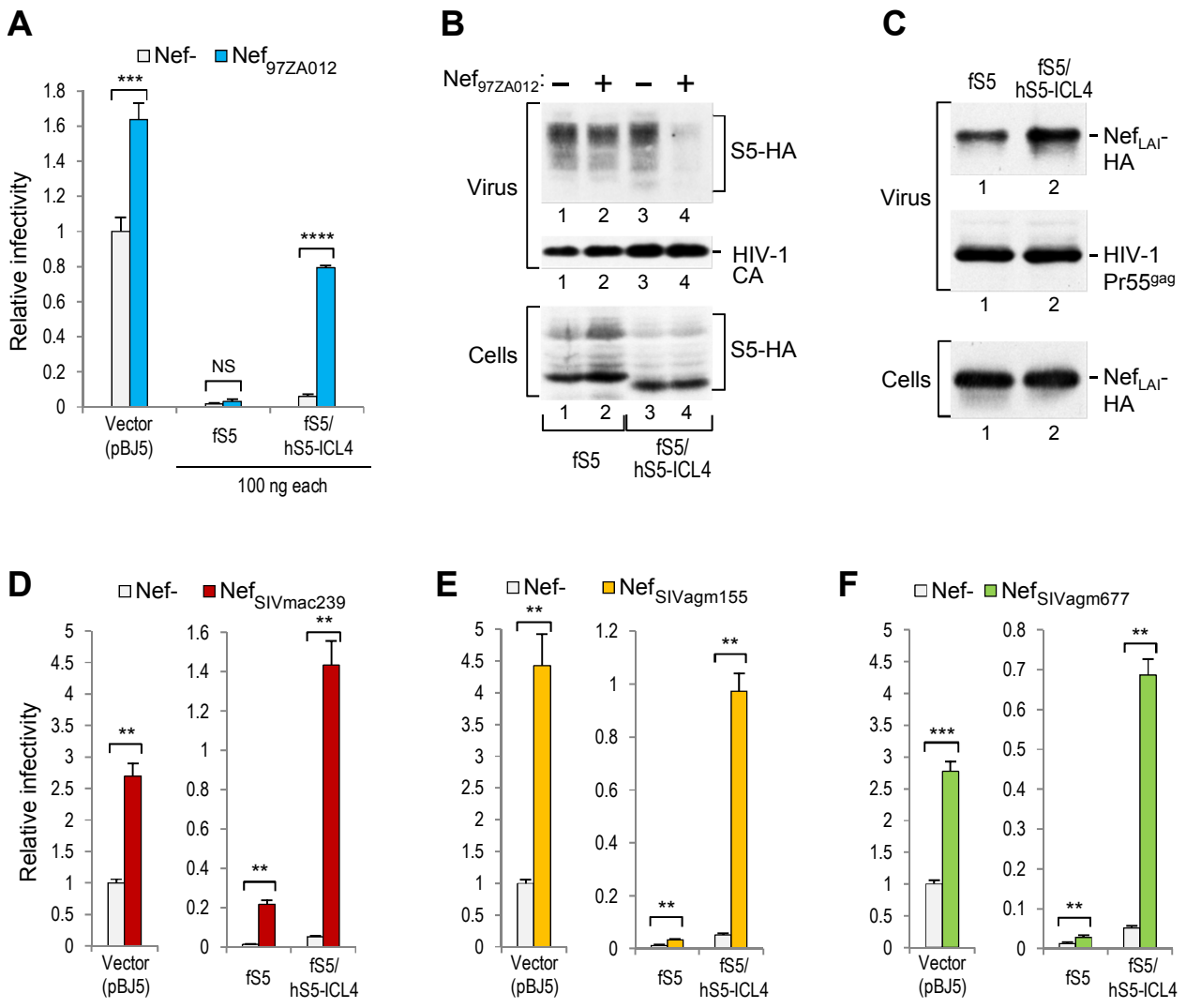

Figure S1 (related to Figure 2). The ICL4 of human SERINC5 confers sensitivity to widely divergent Nefs.

(A) The inhibitory effect of frog SERINC5 on HIV-1 infectivity is resistant to HIV-1 subtype C $Nef_{97ZA012}$ but becomes sensitive in the presence of the ICL4 of human SERINC5.

(B) $Nef_{97ZA012}$ does not affect the incorporation of frog SERINC5 into Nef^- HIV-1 virions but strongly inhibits the incorporation of a chimeric version that harbors the ICL4 of human SERINC5.

(C) A Nef_{LAI} mutant unable to counteract SERINC5 ($LL_{164,165}AA$) is more abundantly incorporated into HIV-1 virions produced in the presence of frog SERINC5 bearing the ICL4 of human SERINC5. The virions lacked protease to prevent the cleavage of Nef.

(D-F) Effects of SIV Nef proteins on the inhibition of HIV-1 infectivity by frog SERINC5 and by a version that harbors the ICL4 of human SERINC5.

The bar graphs represent the mean + SD from 3 biological replicates.

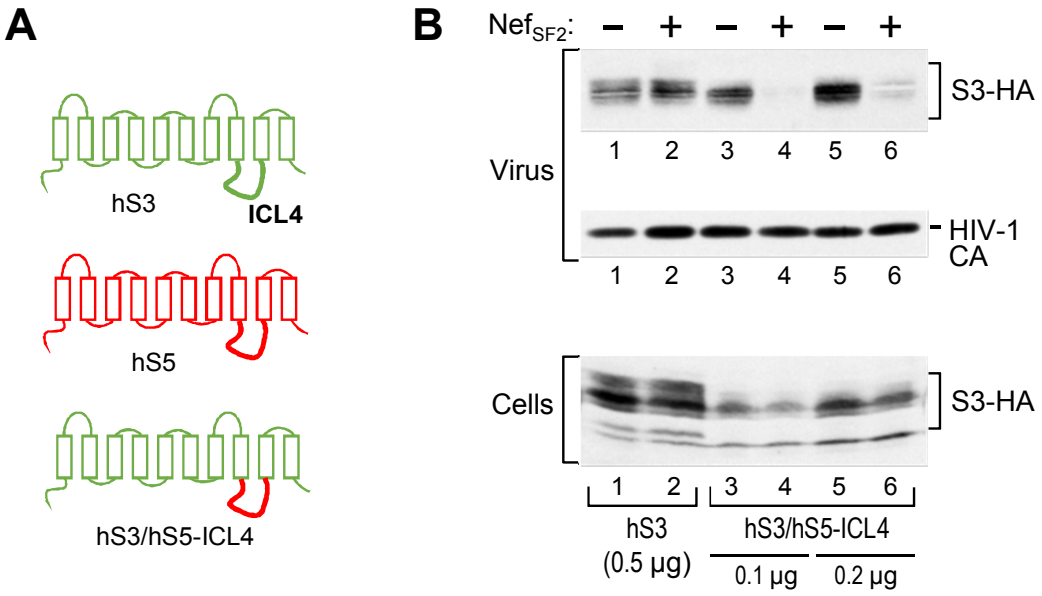

Figure S2 (related to Figure 2). Human SERINC3 acquires sensitivity to Nef$_{SF2}$ in the presence of the ICL4 of human SERINC5.

(A) Schematic illustration of the parental human SERINC proteins and of the chimera examined.

(B) Western blots showing that Nef$_{SF2}$ inhibits the incorporation of human SERINC3 into Nef^{-} HIV-1 virions only upon replacement of its ICL4 by that of human SERINC5.

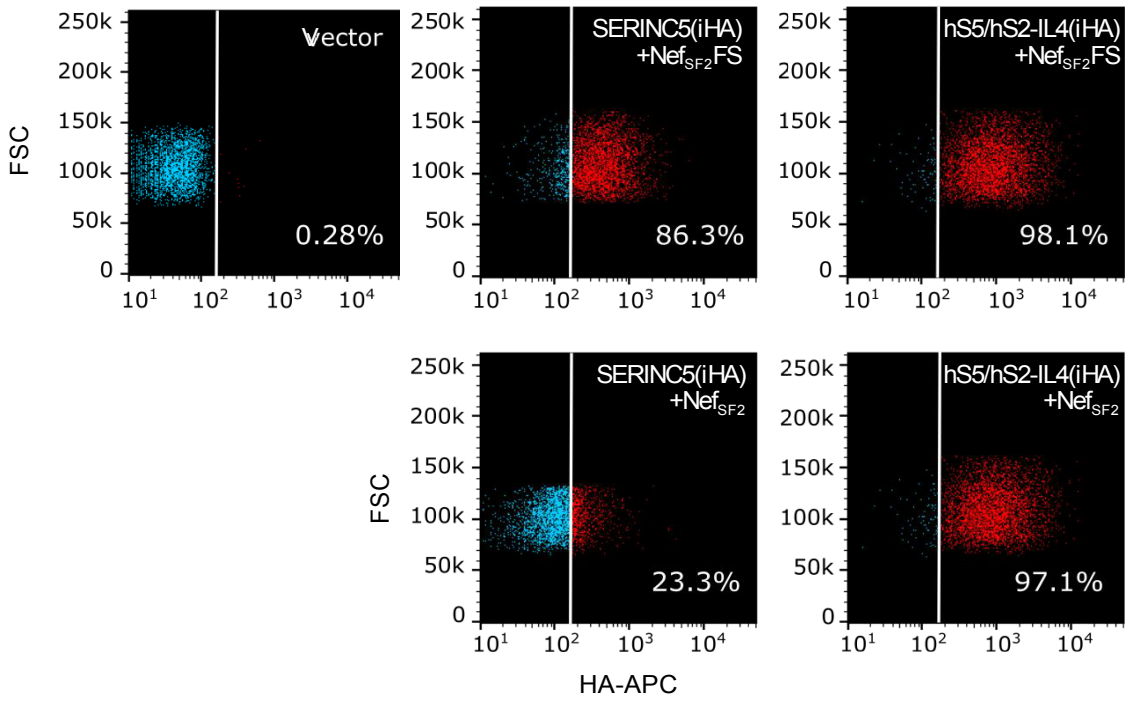

Figure S3 (related to Figure 4). Swapping the ICL4 of human SERINC5 for that of human SERINC2 confers resistance to down-regulation by Nef_{SF2}.
JurkatTAg S5 -/- cells stably expressing SERINC5(iHA) or the S5/hS2-ICL4(iHA) chimera together with Nef_{SF2} were surface-stained with anti-HA antibody and analyzed by flow cytometry. Per cent fractions of cells expressing SERINC5(iHA) or the S5/hS2-ICL4(iHA) chimera on the surface are indicated.

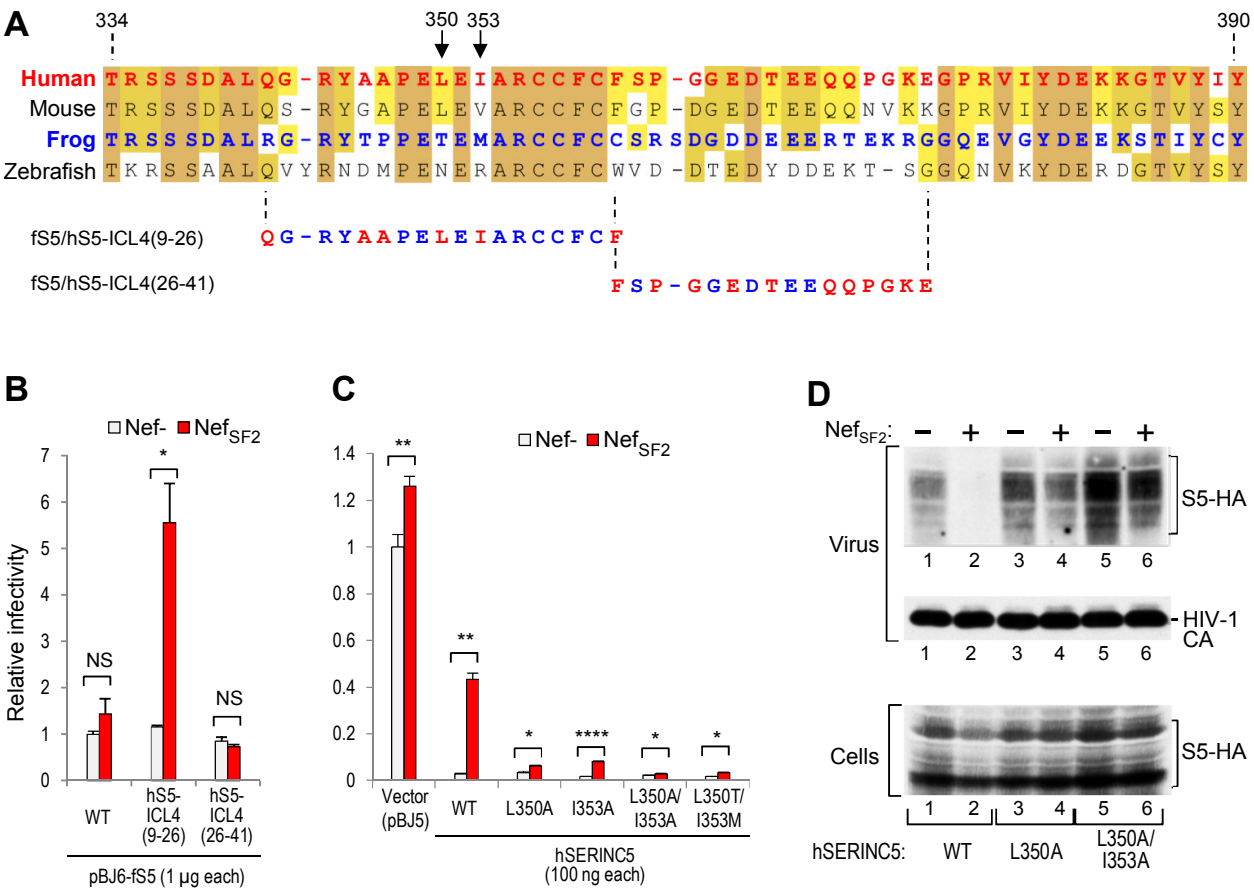

Figure S4 (related to Figures 2 through 4). Point mutations in the ICL4 of human SERINC5 confer resistance to Nef.

(A) Alignment of the ICL4 regions of Nef-sensitive (human, mouse) and Nef-insensitive (frog, zebrafish) SERINC5 proteins, and schematic illustration of intra-ICL4 chimeras examined.

(B) The transfer of human SERINC5 ICL4 residues 9-26, but not of residues 26-41, to the ICL4 of frog SERINC5 increases its sensitivity to Nef$_{SF2}$.

(C) Point mutations targeting small hydrophobic amino acids within the ICL4 of human SERINC5 reduce the sensitivity of its effect on HIV-1 infectivity to Nef$_{SF2}$.

(D) Western blots showing the effects of Nef$_{SF2}$ on the incorporation of WT human SERINC5 and of ICL4 mutants into Nef^{-} HIV-1 virions.

The bar graphs represent the mean + SD from 3 biological replicates.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmids. The *env*- and *nef*-deficient HIV-1 provirus HXB/Env$^-$/Nef$^-$, the pSVIIIenv-based plasmids expressing Env_{HXB2} or Env_{JRFL}, the pBJ5-based expression plasmids for the Nef proteins of HIV-1_{SF2}, HIV-$1_{97ZA012}$, and $SIV_{mac}239$, the *nef*-deficient control plasmid $pNef_{FS}$, and the pBJ5-based expression plasmids for human SERINC3-HA, human SERINC5, and human SERINC5-HA have been described (Dorfman et al., 2002; Helseth et al., 1990; Pizzato et al., 2007; Usami et al., 2015). A pBJ6-based expression plasmid for human SERINC5 was kindly provided by M. Pizzato. The *nef* genes of $SIV_{agm}155$ and $SIV_{agm}677$ were amplified from proviral clones and inserted into pBJ5. The coding sequences for mouse, frog, and zebrafish SERINC5 were amplified from cloned cDNAs purchased from Dharmacon (GenBank: BC062131, XM_002940195, and BC044159). DNAs encoding mutant and chimeric proteins without or with C-terminal HA tags were generated using an overlap extension PCR method (Horton et al., 1989) and inserted into pBJ5 or pBJ6 downstream of a Kozak sequence. The fS5/hS5-ICL4 chimera has residues 342-390 of frog SERINC5 (fS5) replaced by residues 342-389 of human SERINC5 (hS5). The intra-ICL4 chimeras fS5/hS5-ICL4(9-26) and fS5/hS5-ICL4(26-41) have residues 342-359 and 359-375 of frog SERINC5 replaced by the corresponding residues of human SERINC5. The hS5/fS5-ICL4 chimera has residues 342-389 of human SERINC5 replaced by residues 342-390 of frog SERINC5. The hS5/hS2-ICL4 chimera has residues 334-389 of human SERINC5 replaced by residues 343-388 of human SERINC2 (hS2). The hS3/hS5-ICL4 chimera has residues 358-404 of human SERINC3 (hS3) replaced by residues 335-389 of human SERINC5.

Retroviral vectors. The coding sequence for SERINC5(iHA) (Usami et al., 2015) preceded by a Kozak sequence was amplified from pBJ5-SERINC5(iHA) and inserted into the retroviral vector MSCVpuro (Clontech). Additionally, a version of the hS5/hS2-ICL4 chimera that harbors an internal HA tag at the same position as SERINC5(iHA) was cloned into MSCVpuro. The coding sequence for HIV-1 Nef_{SF2} preceded by a Kozak sequence was cloned into MSCVhyg (Clontech), yielding $MSCVhygNef_{SF2}$. A *nef*-deficient version ($MSCVhygNef_{SF2}FS$) was obtained by generating a frameshift at a unique *Xho*I site in *nef*.

Analysis of Nef incorporation. 293T cells were co-transfected with 2 µg of the protease-deficient HIV-1 proviral construct HXB/VPR^+/PR^- (Bukovsky and Gottlinger, 1996), 2 µg of a pBJ5-based plasmid expressing C-terminally HA-tagged Nef_{LAI} with a $LL_{164,165}AA$ mutation (Pizzato et al., 2007), and 2 µg of a pBJ5-based plasmid expressing native frog SERINC5 or a version harboring the ICL4 of human SERINC5 (fS5/hS5-ICL4). Virions released into the medium were pelleted through 20% sucrose cushions, and virus- and cell-associated proteins were detected by western blotting. The antibodies used for western blotting were 183-H12-5C against HIV-1 CA, and HA.11 (BioLegend) against the HA epitope.

Flow cytometry. Ectopic SERINC expression cassettes were introduced into JurkatTAg S5 -/- (1) cells (Usami et al., 2015) by retroviral transduction with

MSCVpuroSERINC5(iHA) or MSCVpurohS5/hS2-ICL4(iHA). Simultaneously, the cells were transduced with the *nef*-deficient control vector MSCVhygNef$_{SF2}$FS or with MSCVhygNef$_{SF2}$. After selection with puromycin and hygromycin, the doubly transduced bulk cultures were surface-stained with anti-HA antibody and APC-conjugated anti-mouse IgG (Jackson ImmunoResearch). Samples were then analyzed on a BD LSR II flow cytometer.

SUPPLEMENTAL REFERENCES

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