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Supplemental Information

Natural HIV-1 Nef Polymorphisms Impair SERINC5 Downregulation Activity

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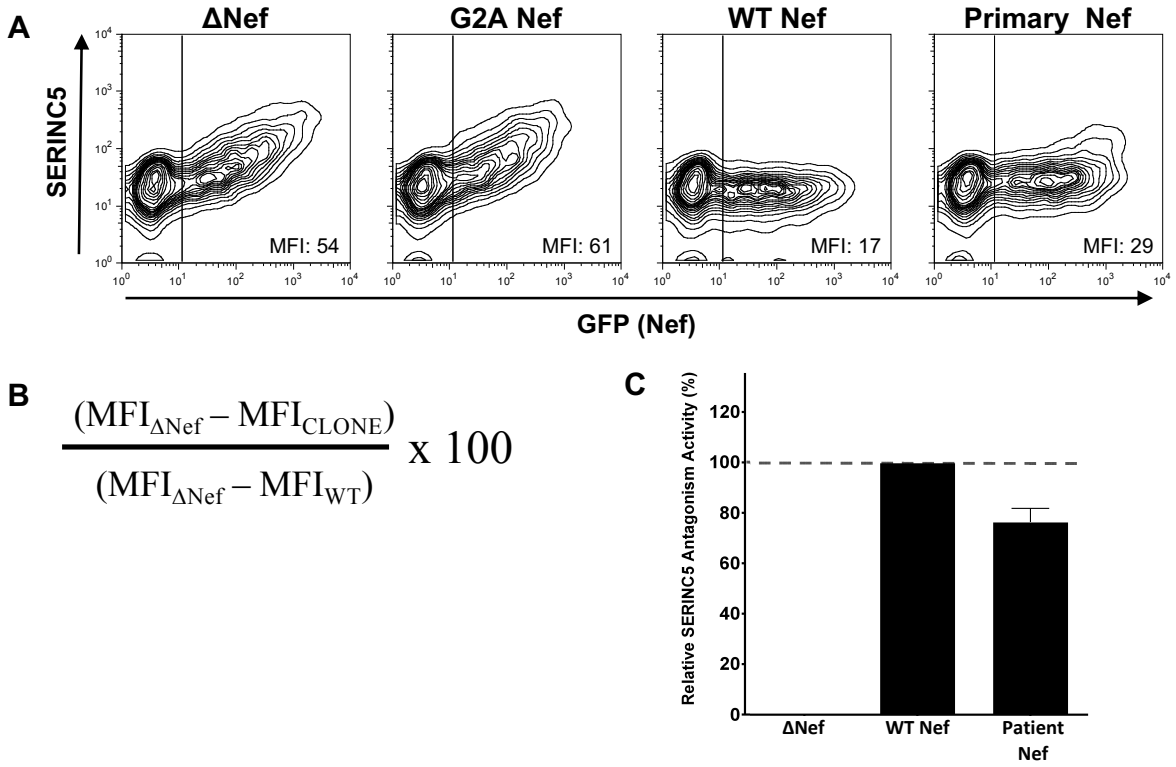

Figure S1. Assay to measure SERINC5 downregulation by primary Nef alleles, Related to Figure 1 and STAR Methods. (A) SERINC5 expression on the cell surface was assessed by flow cytometry following transient expression of Nef and a SERINC5 variant encoding an internal HA epitope tag (SERINC-iHA). Results are shown for two negative controls, empty vector (ΔNef) and Nef G2A mutant, a positive control, WT Nef (SF2 strain), and one representative primary Nef allele. Median fluorescence intensity (MFI) for SERINC5 (y-axis) in the transfected cell population (GFP+, x-axis) are indicated. In repeated experiments, the MFI values obtained using empty vector (ΔNef) and Nef G2A mutant were not discernable. **(B)** SERINC5 MFI values for each primary Nef allele were normalized to those of WT Nef (set to 100%) and G2A Nef (set to 0%) using the indicated formula. **(C)** Normalized SERINC5 downregulation activity (mean ± S.D.) is shown for controls and one representative primary Nef clone, based on three independent experiments.

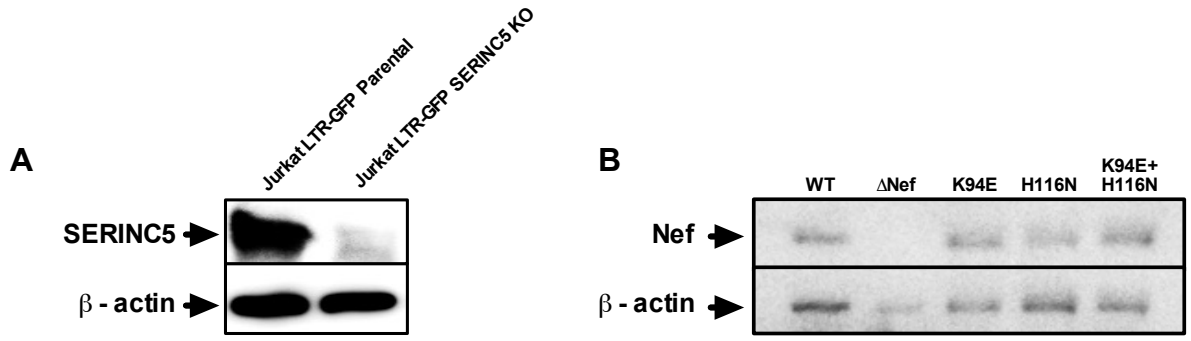

Figure S2. Generation of SERINC5 knockout Jurkat LTR-GFP reporter cells, Related to Figure 3. (A) CRISPR/Cas9 methods were used to disrupt the SERINC5 gene in Jurkat LTR-GFP reporter cells. Single cells were then isolated by FACS sorting and expanded to generate clonal cells lines. Loss of SERINC5 expression was confirmed by Western blot using a rabbit polyclonal anti-SERINC5 antiserum. Results for one SERINC5 KO clone are shown. **(B)** Western blot analysis was used to confirm the presence or absence of Nef in NL4.3-derived viruses following infection of Jurkat LTR-GFP SERINC5 KO cells. Cells were harvested on day 9 of the replication assay shown in Figure 3E.