

Supplemental Information

ADAM17 Is a Broad HIV-1 Restriction Factor Targeted by Nef to Restore Env Maturation

Qiuchen Li¹, Sunan Li², Shuvankar Dey¹, Dongjie Sun³, Liangliang Sun⁴, Yong-Hui Zheng^{1*}

¹Department of Microbiology and Immunology, The University of Illinois, Chicago, IL 60612;

²State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, 150069, China;

³Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, China;

⁴Department of Chemistry, Michigan State University, East Lansing, MI 48824.

*Correspondence:

zhengyh@uic.edu (Y.H.Z.)

Bait	IP	MS
Ser5-FLAG	anti-FLAG	A9 A15 A17
Ser5-FLAG Nef-HA	anti-FLAG Nef-HA	A9 A15 A17
Nef-HA	anti-HA	None

Figure S1. Identification of ADAMs as Ser5-binding proteins by IP-MS. FLAG-tagged Ser5 and HA-tagged Nef proteins were expressed alone or together in HEK293T cells and immunoprecipitated using anti-FLAG or anti-HA beads. Bead-associated proteins were identified by mass spectrometry, and the ADAM proteins identified are shown.

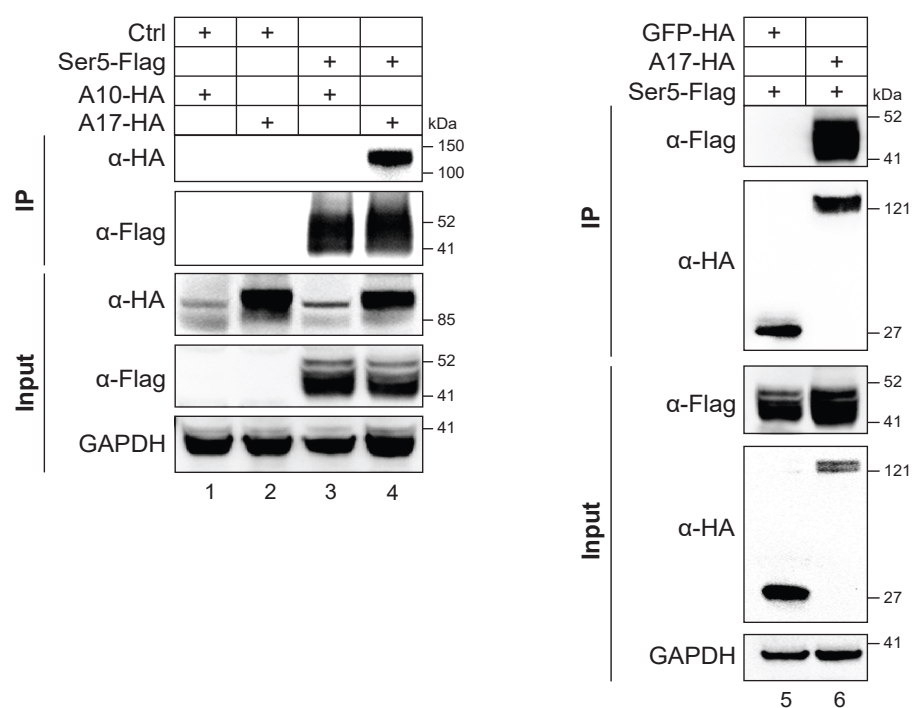


Figure S2. Validation of A17 and Ser5 interaction by immunoprecipitation (IP). Ser5-FLAG was expressed with A10-HA, A17-HA, or GFP-HA in HEK293 cells. Proteins were pulled down with anti-FLAG beads (lanes 1-4) or anti-HA beads (lanes 5-6) and detected by WB. Representative results from 3 biologically independent experiments are shown. Source data are provided as a Source Data file.

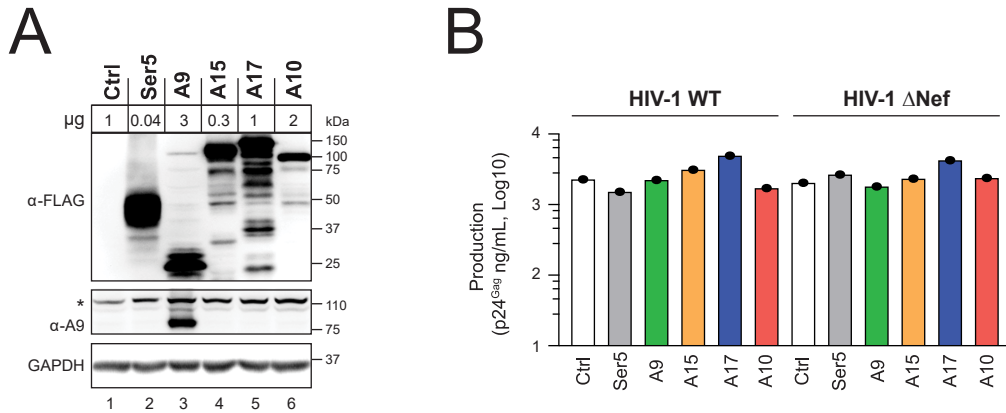


Figure S3. ADAM proteins do not block HIV-1 production. (A) HEK293T cells were transfected with the indicated amounts of FLAG-tagged expression vectors. The expression of these host proteins was determined by an anti-FLAG or anti-A9 antibody. The endogenous A9 band is indicated by a star (*). **(B)** HIV-1 wild-type (WT) and Nef-deficient (Δ Nef) viruses were produced from HEK293T cells in the presence of the indicated host protein expression vector at a ratio as in **A**. Viral production was measured by p24^{Gag} ELISA. Source data are provided as a Source Data file.

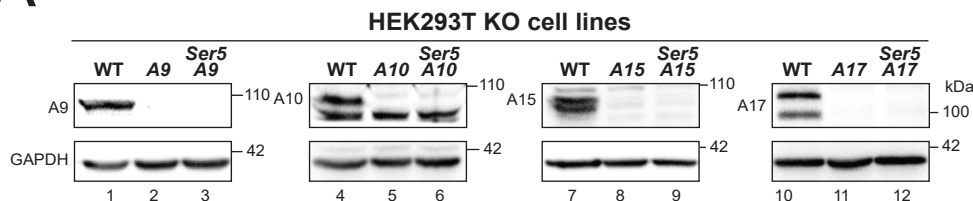
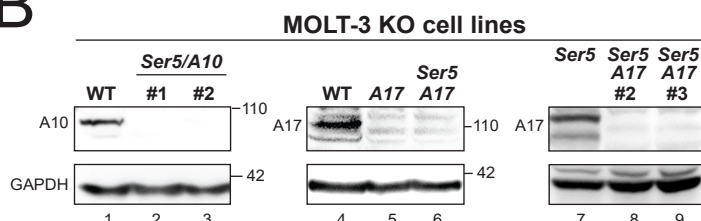
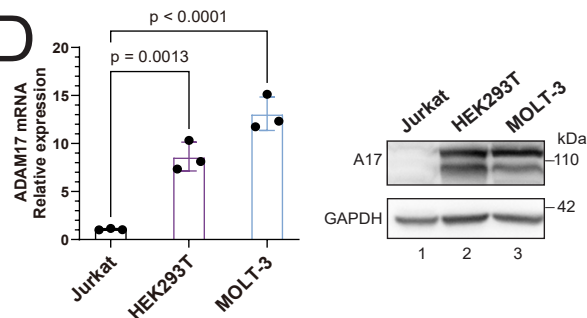
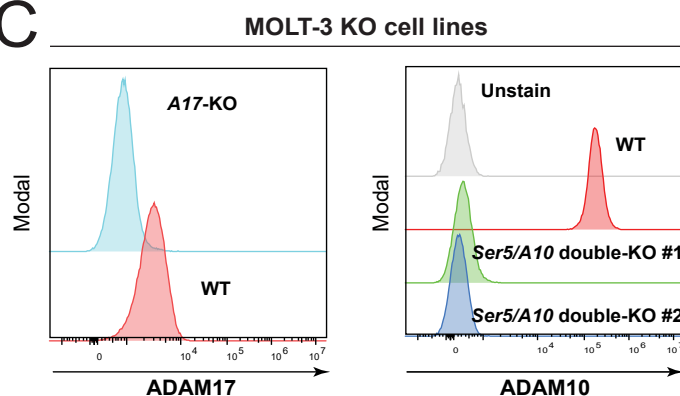
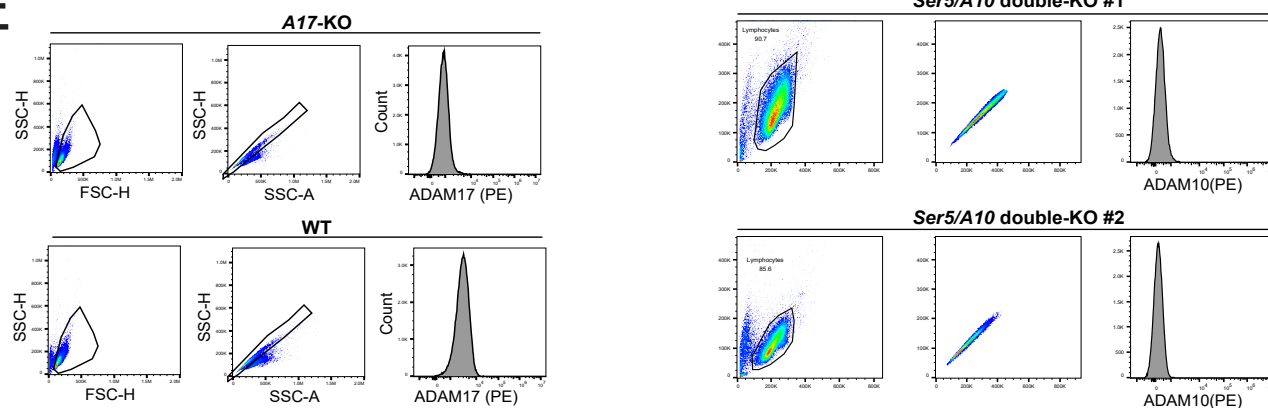
A**B****D****C****E**

Figure S4. Validation of ADAM-KO cell lines by Western blotting (**A,B**) and flow cytometry (**C**). (**D**) Determination of ADAM17 mRNA expression by qRT-PCR (left) ($n=3$, one-way ANOVA, mean $\pm$ SD) and protein expression by WB (right) in Jurkat, HEK293T, and MOLT-3 T cells. (**E**) Representative sequential gating strategies used for the flow cytometry analyses in **C**. Cells were gated based on FSC-H and SSC-H, followed by singlet discrimination, and surface ADAM17 or ADAM10 expression was analyzed by PE fluorescence. Representative gating plots for the indicated WT and KO cells are shown. Representative blots and flow cytometry data are shown. Source data are provided as a Source Data file.

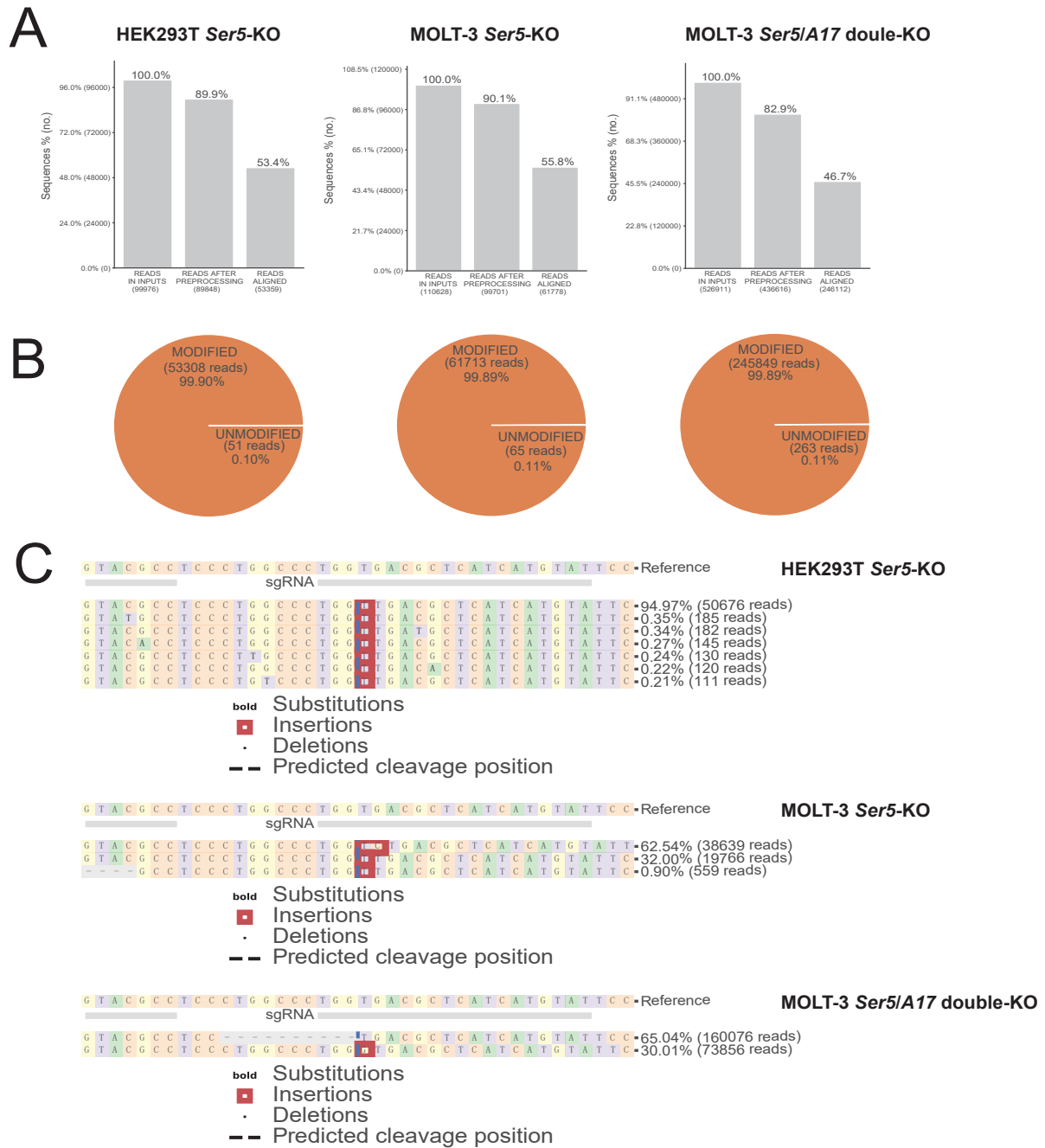


Figure S5. Validation of *Ser5*-KO cell lines by next generation sequencing. (A) The number of reads in input fastqs, after preprocessing, and after alignment to amplicons. **(B)** Alignment and editing frequency of reads as determined by the percentage and number of sequence reads showing unmodified and modified alleles. **(C)** Nucleotide distribution around the sgRNA.

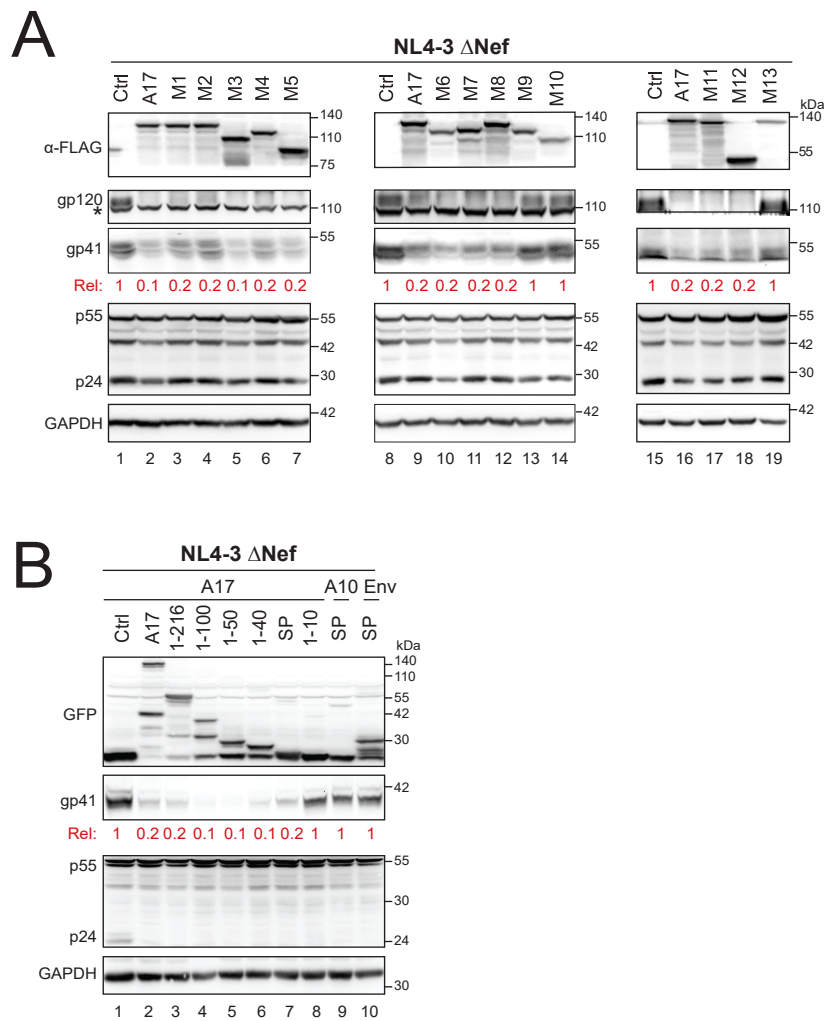


Figure S6. (A) (B) HIV-1 (NL) Δ Nef proviruses were expressed with indicated A17 mutants in HEK293T cells. Protein expression was determined by WB. The gp41 bands were quantified by densitometry and expressed as relative values (Rel) to the control vector (Ctrl), which was set to 1. (*) indicates unspecific bands. Representative data from 3 biologically independent experiments are shown. Source data are provided as a Source Data file.

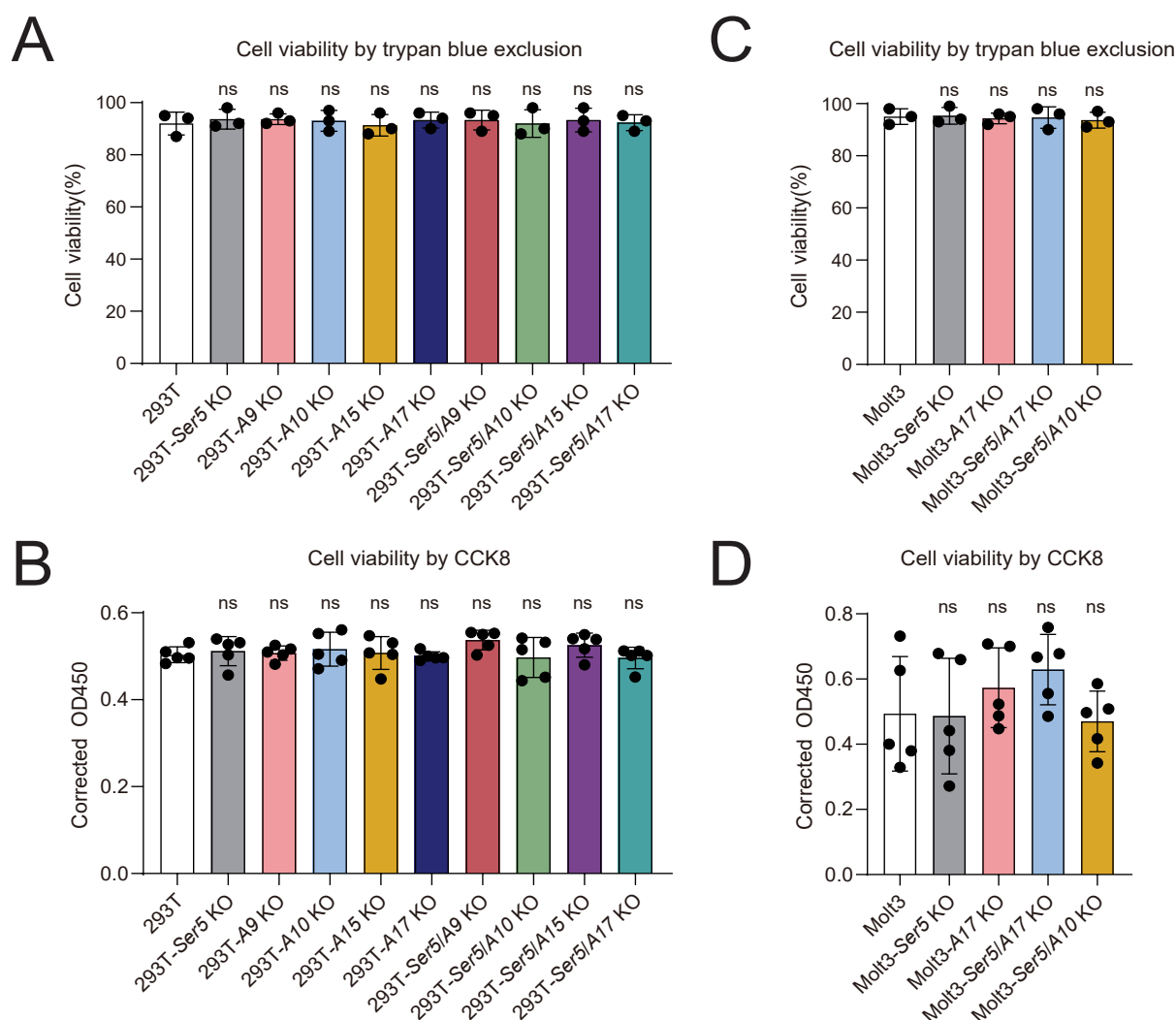


Figure S7. Cell viability of parental and knockout cell lines assessed by trypan blue exclusion and CCK-8 assays. (A, C) Cell viability was determined by trypan blue exclusion 24 h after seeding. (B, D) Cell viability was assessed by the CCK-8 assay 24 h after seeding and is presented as background-subtracted OD450 values. Equal numbers of viable cells were seeded for each cell line. Bars represent mean $\pm$ SD, and dots represent independent experiments. Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple-comparisons test using the corresponding parental cell line as the control. ns, not significant. Source data are provided as a Source Data file.

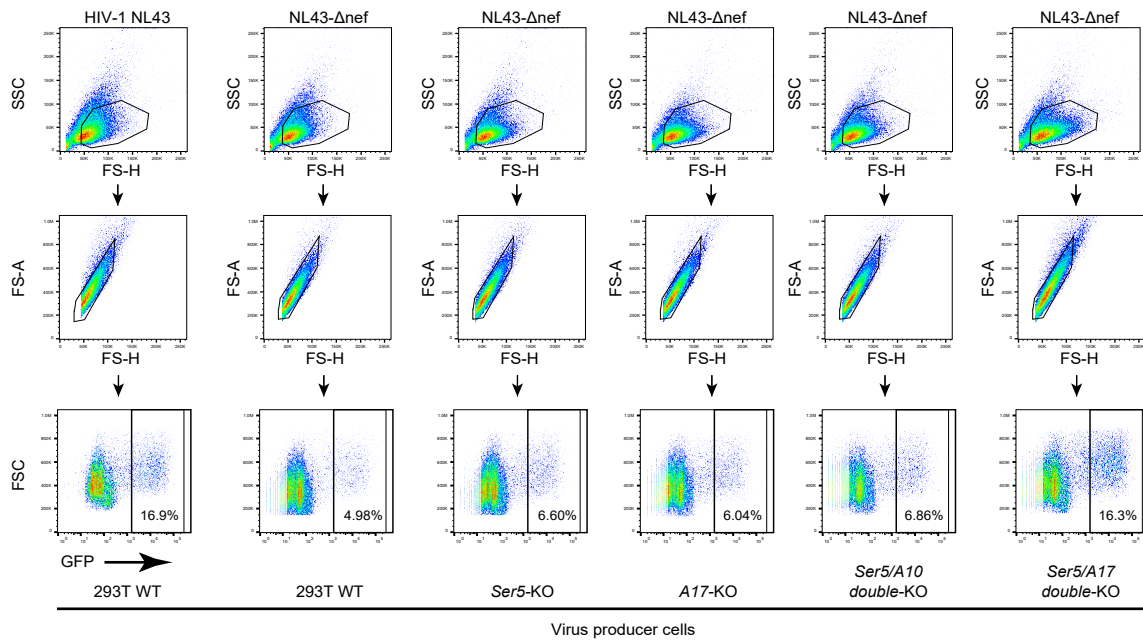


Figure S8. Flow cytometry gating strategies. Representative sequential gating strategy used for the Rev-A3R5-GFP infectivity analysis shown in Fig. 4b. Cells were sequentially gated based on FSC/SSC and singlet discrimination, followed by identification of GFP-positive infected cells. Representative samples for the indicated viruses and producer-cell genotypes are shown.

MOLT-3 Ser5/A17 double-KO

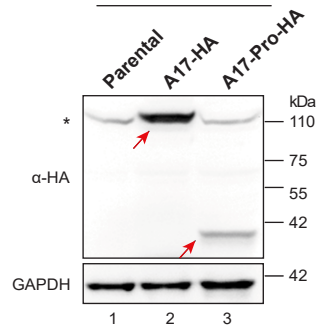


Figure S9. MOLT-3 Ser5/A17 double-KO cells were stably transduced with a lentiviral vector expressing ADAM17 or its prodomain with an HA-tag. Their expression was determined by WB. An unspecific band is indicated by a star (*). Representative data from 3 biologically independent experiments are shown. Source data are provided as a Source Data file.

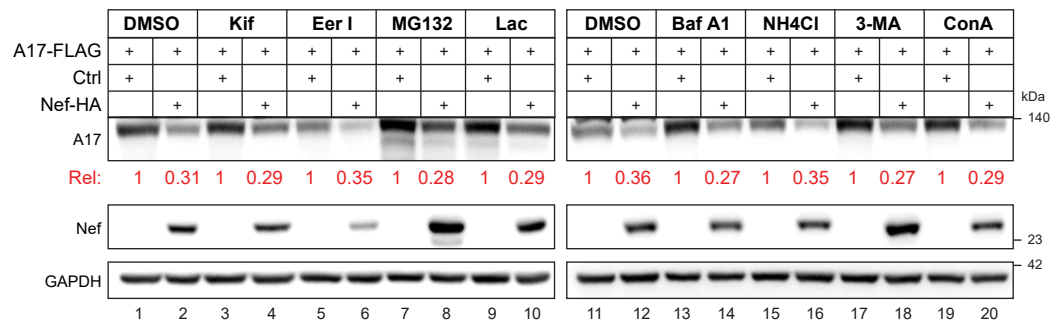


Figure S10. HIV-1 Nef does not trigger ADAM17 degradation. 293T A17-KO cells were tranfested with A17 and Nef expression vectors. After 24 hours, cells were treated with Kifunesine (Kif, 50 μ M), Eeyarestatin I (Eer I, 10 μ M), MG132 (20 μ M), lactacystin (Lac, 20 μ M), Bafilomycin A1 (Baf A1, 100 μ M), NH₄Cl (20 mM), 3-Methyladenine (3-MA, 10 mM), and Concanamycin A (ConA, 20 nM). Protein expression was determined by Western blotting. Representative data from 3 biologically independent experiments are shown. Source data are provided as a Source Data file.

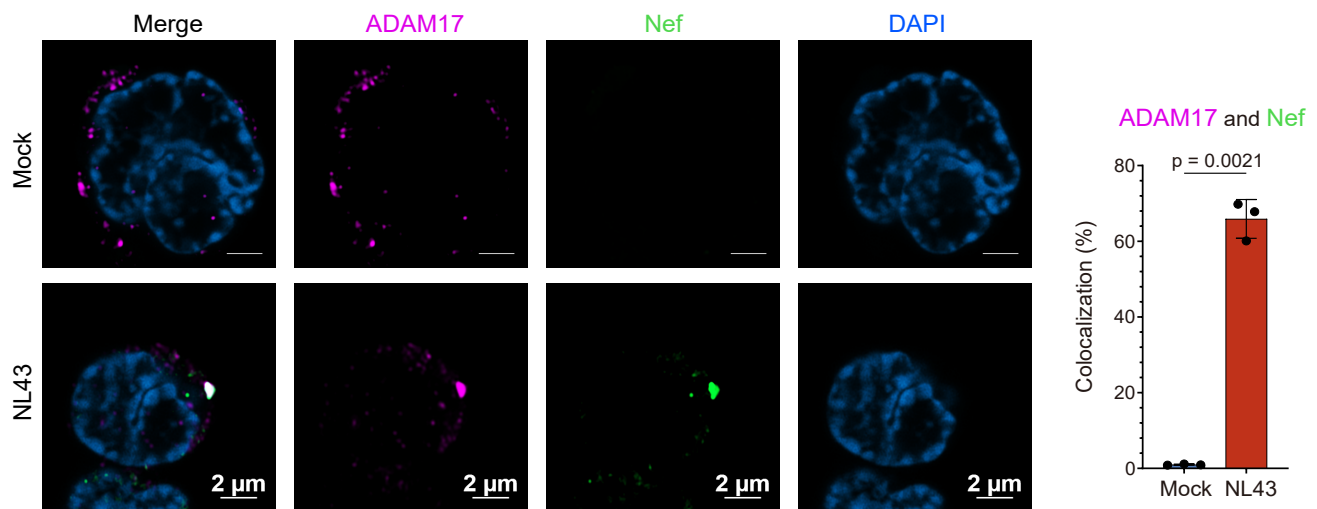


Figure S11. Detection of HIV Nef colocalization with ADAM17 in MOLT-3 cells.

Representative immunofluorescence images of mock-treated or HIV-1 NL4-3-infected MOLT-3 cells at 24 h post-infection. Cells were fixed and stained for ADAM17 (magenta) and HIV-1 Nef (green), with nuclei counterstained by DAPI (blue). Representative images from three independent biological replicates are shown. ADAM17-Nef colocalization was quantified by independently thresholding the ADAM17 and Nef fluorescence channels to generate binary masks, followed by measurement of the overlapping area between the two masks. Colocalization was expressed as the percentage of the total ADAM17-positive area overlapping with Nef-positive regions (mean $\pm$ SD, unpaired two-tailed Welch's t test). Source data are provided as a Source Data file.

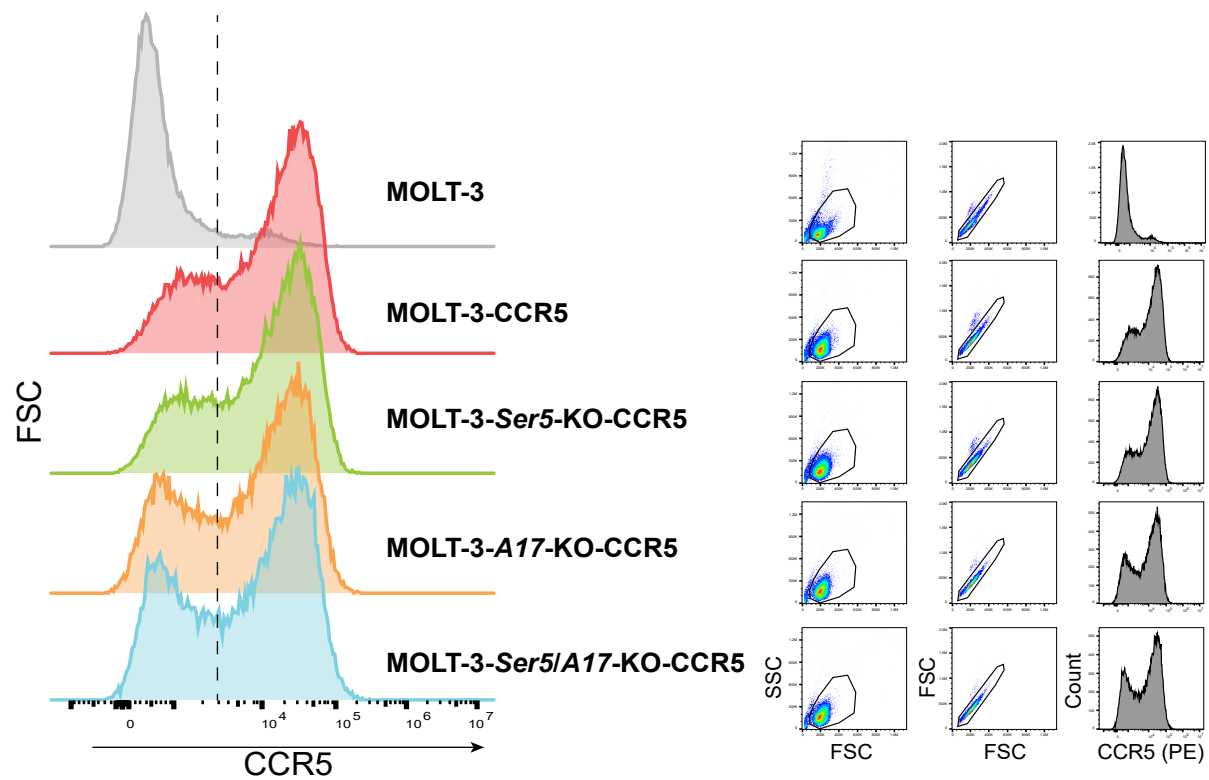


Figure S12. CCR5 expression in MOLT-3 WT and knockout cell lines. Cell-surface CCR5 expression in the indicated MOLT-3 WT and knockout cell lines was analyzed by flow cytometry. Overlaid histograms of CCR5 expression are shown on the left, and the corresponding sequential gating strategies are shown on the right.

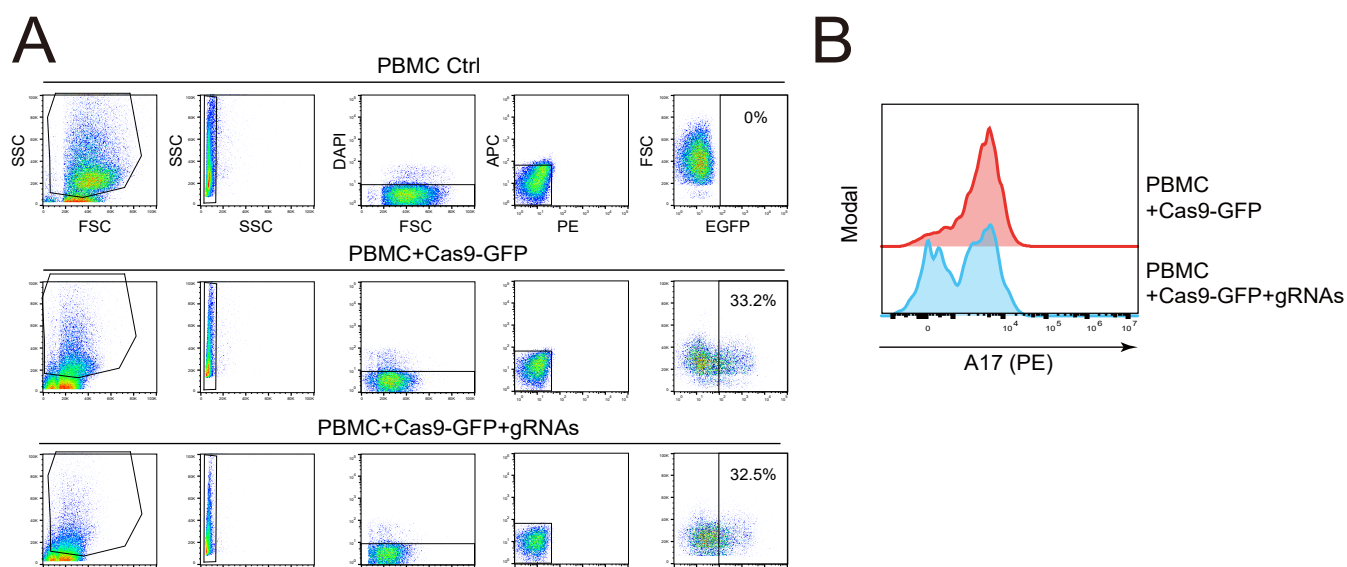


Figure S13. Knockout of *Ser5* and *A17* in human PBMCs by CRISPR. (A) PHA-activated human PBMCs were electroporated with Cas9-EGFP alone or Cas9-EGFP RNPs containing gRNAs targeting *Ser5* and *A17*. At 18 h post-electroporation, EGFP-positive cells were isolated by flow cytometric sorting. Representative sequential gating strategies are shown. (B) Cell-surface ADAM17 expression in the sorted EGFP-positive cells was analyzed by flow cytometry using the gating strategy described in Fig. S4E. Representative histograms from Cas9-EGFP control and *Ser5*/*A17*-targeted cells are shown. Representative data from 3 biologically independent experiments are shown.