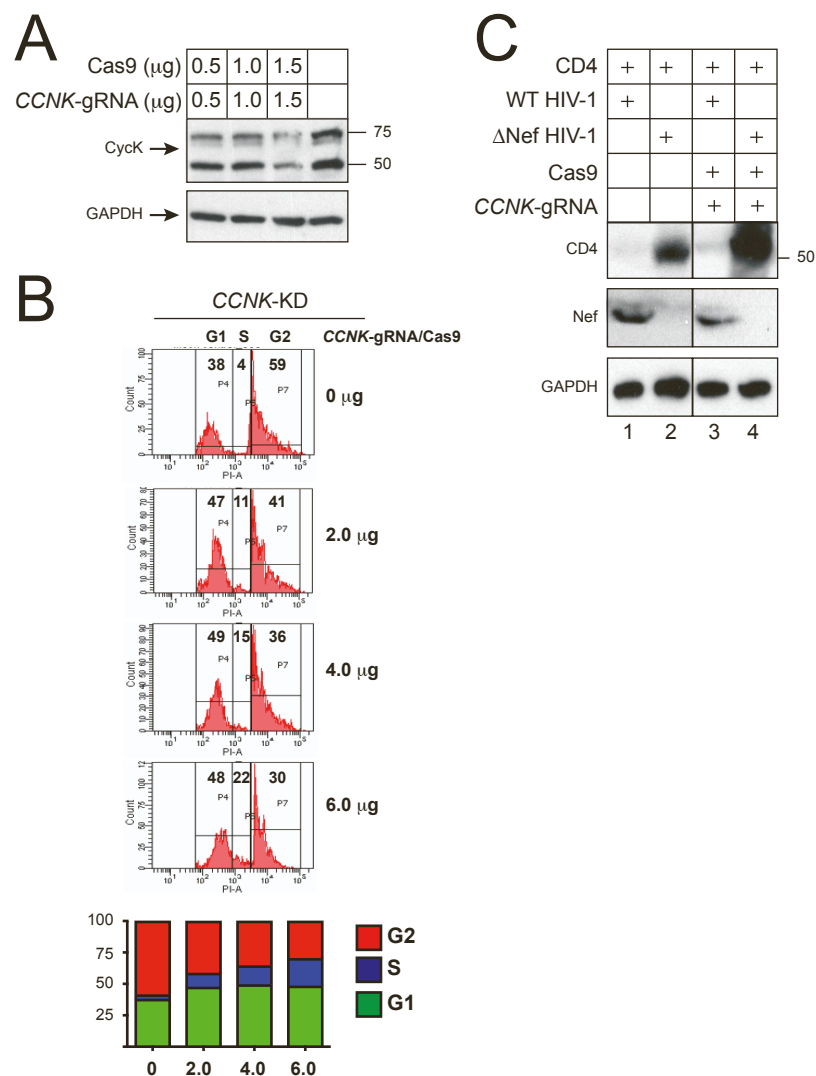


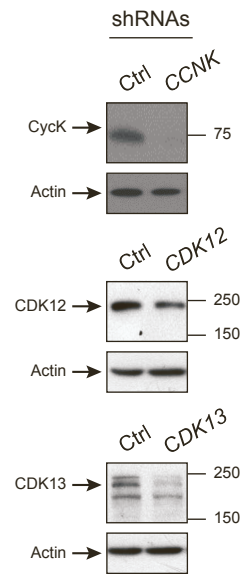
**Supplemental information**

**HIV-1 Nef interacts with the cyclin K/CDK13  
complex to antagonize SERINC5  
for optimal viral infectivity**

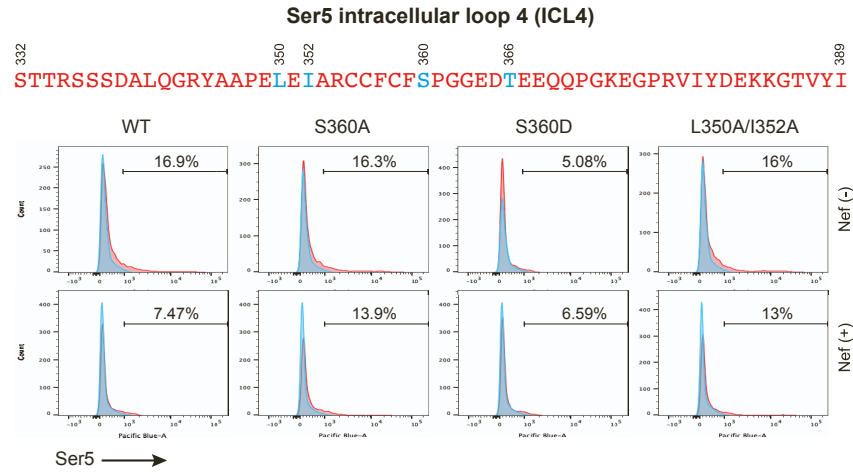
**Qingqing Chai, Sunan Li, Morgan K. Collins, Rongrong Li, Iqbal Ahmad, Silas F. Johnson, Dylan A. Frabutt, Zhichang Yang, Xiaojing Shen, Liangliang Sun, Jian Hu, Judd F. Hultquist, B. Matija Peterlin, and Yong-Hui Zheng**



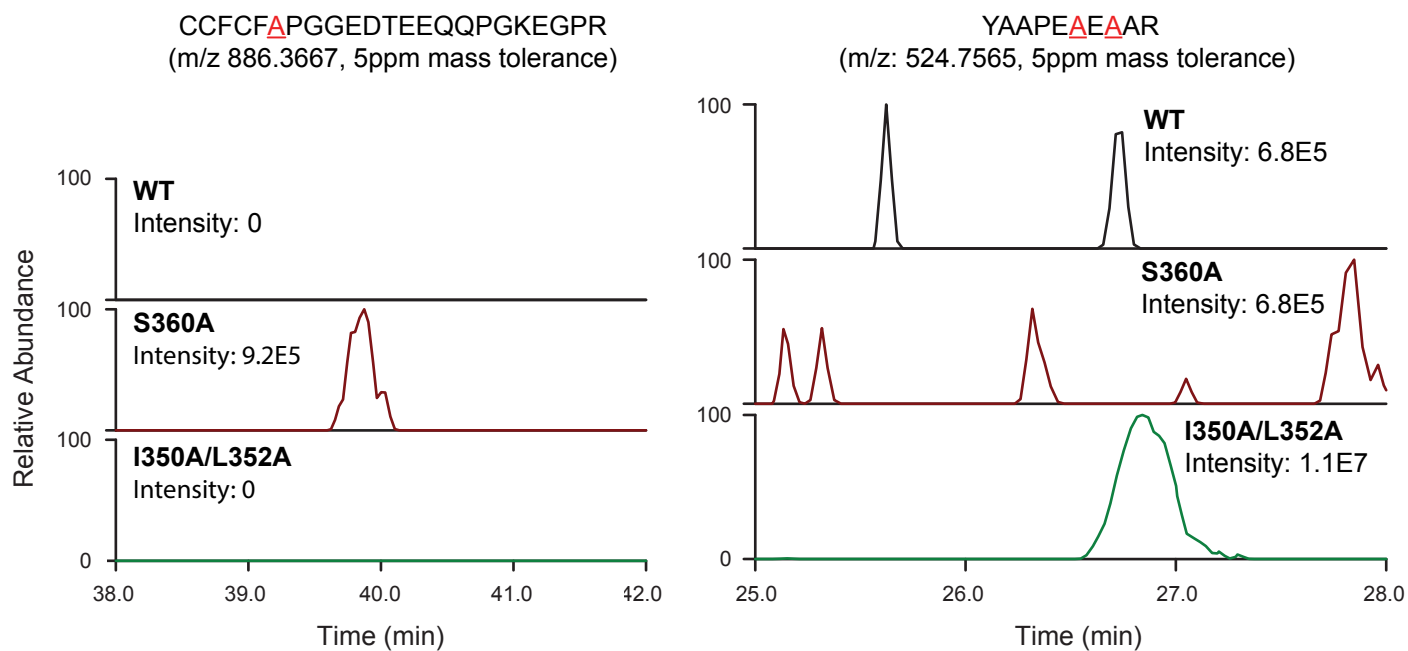
**Figure S1.** Effectiveness of CRISPRi/Cas9. Related to Figure 2. A) HEK293T cells were transfected with increasing amounts of Cas9 and *CCNK*-gRNA expression vectors. CycK expression was detected by WB using an anti-CycK antibody. B) SERINC3/5 knockout Jurkat-TAG cells were transfected with increasing amounts of Cas9/*CCNK*-gRNA expression vectors. After 48 h, cell cycle was analyzed by flow cytometry. Representative individual cell cycle distributions are shown in the histogram. C) CD4 was expressed with WT or  $\Delta\text{Nef}$  HIV-1 in the presence of Cas9 and *CCNK*-gRNA expression vectors in HEK293T cells and detected by WB.



**Figure S2.** Effectiveness of shRNAs. Related to Figure 2. HEK293T cells were transfected with shRNA expression vectors against *CCNK*, *CDK12* or *CDK13*. A scrambled shRNA expression vector was used as control (Ctrl). CycK, CDK12 and CDK13 expression were detected by WB using their specific antibodies.



**Figure S3.** S360 is required for Nef downregulation of SERINC5 in HEK293T cells. Related to Figure 4. SERINC5, SERINC5S360A, SERINC5S360D, and SERINC5L350A/I352A with an internal HA-tag were expressed alone, or with WT or  $\Delta$ Nef HIV-1 in HEK293T cells. Cells were stained with a fluorescent anti-HA antibody, and SERINC5 expression on the cell surface was determined by flow cytometry.



**Figure S4.** Extracted ion chromatogram (EIC) of two ICL4 peptides containing S360A or I350A/L352A mutations. Related to Figure 7. EICs of peptides CCFCFAPGGEDTEEQQPGKEGPR and YAAPEAEAAR detected in samples from CD8-ICL4, CD8-ICL4<sub>S360A</sub>, and CD8-ICL4<sub>I350A/L352A</sub> are presented. The S360A and I350A/L352A mutations are underlined and shown in red.