Supplementary information

Cul3-KLHL20 E3 ubiquitin ligase plays a key role in the arms race between HIV-1 Nef and host SERINC5 restriction

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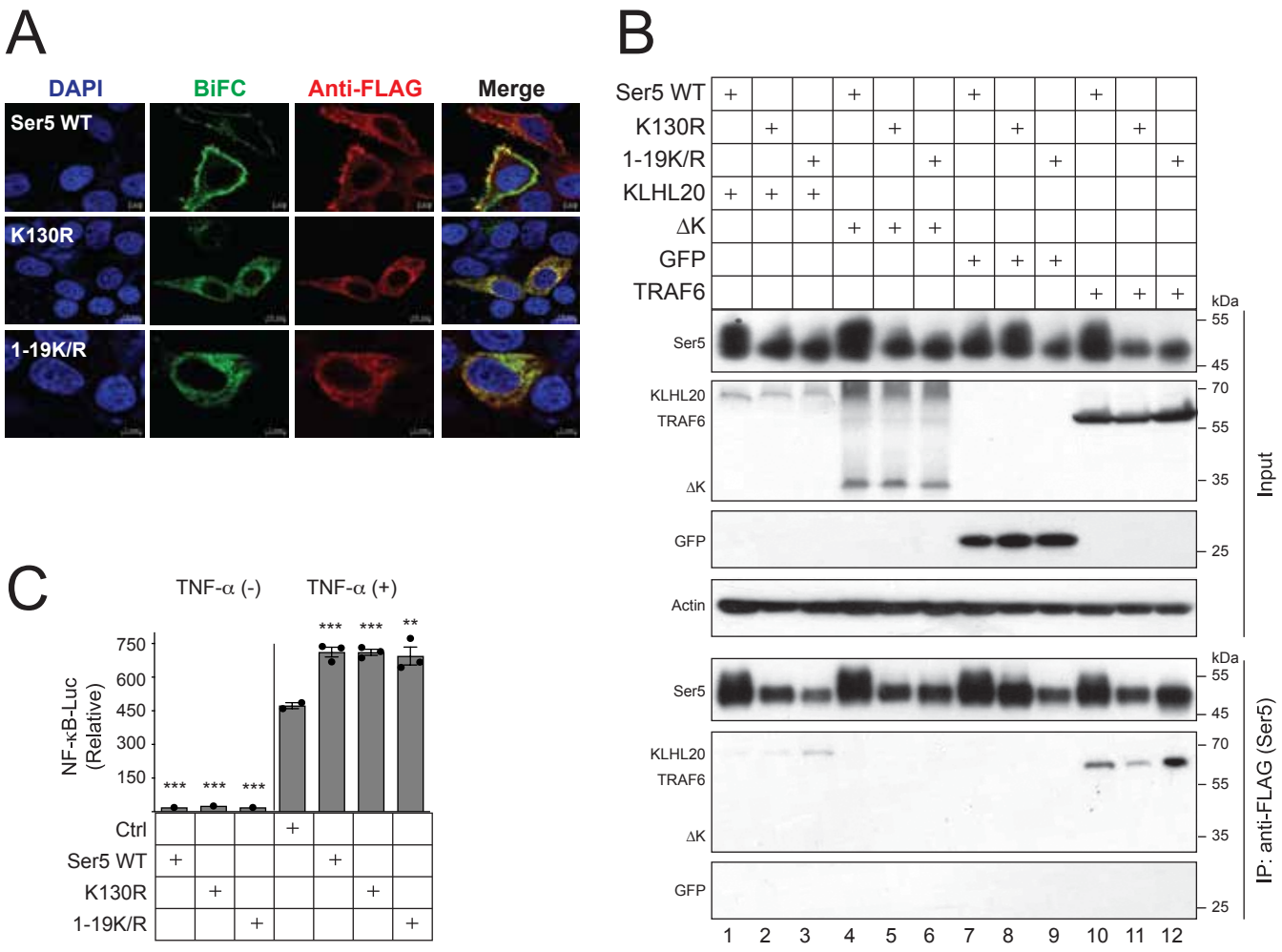
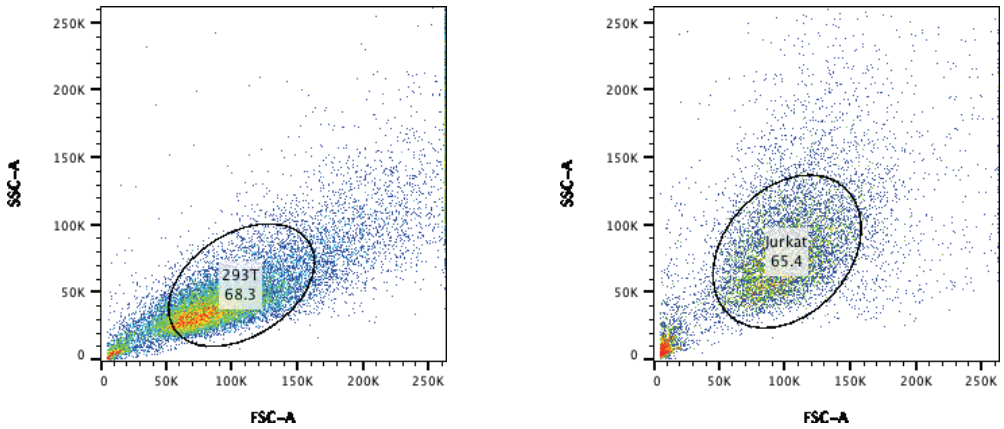


Supplementary Fig. 1. SERINC5 K130R mutant is functional and properly folded.
(A) Ser5-VN/Ser5-VC, K130R-VN/K130R-VC, and 1-19K/R-VN/1-19K/R-VC were expressed in pairs in HeLa cells. After staining with red-fluorescent anti-FLAG for the VN tag and DAPI for nuclei, cells were detected for expression of the green fluorescent BiFC signal and its colocalization with the red-fluorescent signal by confocal microscopy.
(B) FLAG-tagged Ser5 or its K130R and 1-19K/R mutants were expressed with HA-tagged KLHL20, its ΔK mutant, GFP, and TRAF6 in HEK293T cells. Cellular proteins were pulled down by anti-FLAG and analyzed by WB.
(C) Ser5 or its K130R and 1-19K/R mutants were expressed with a NF-κB promoter luciferase (Luc) reporter in HEK293T cells. Cells were treated with TNF-α (10 ng/mL) for 8 h. Forty-eight hours after transfection, the cells were lysed, and luciferase activities were measured. Results were shown as relative values, with that from cells remaining untreated with TNF-α as 1. Experiments were repeated twice, and similar results were obtained.



Supplementary Fig. 2. Gating strategy for HEK293T and Jurkat-TAg cells are shown, which was used to analyze Ser5 surface expression in Fig. 3B and Fig. 4A by flow cytometry.