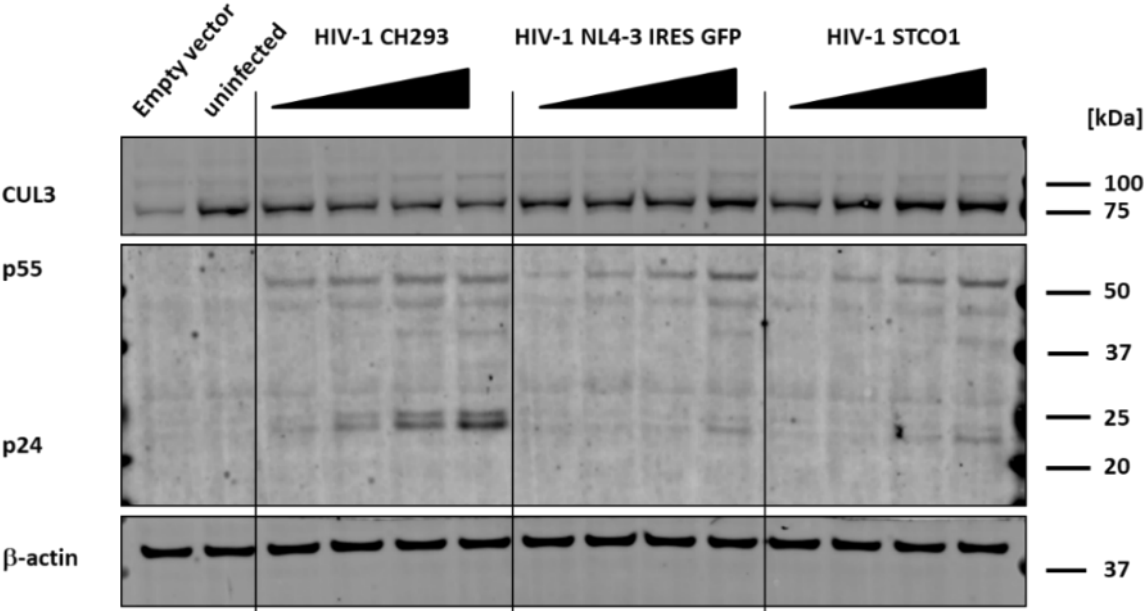
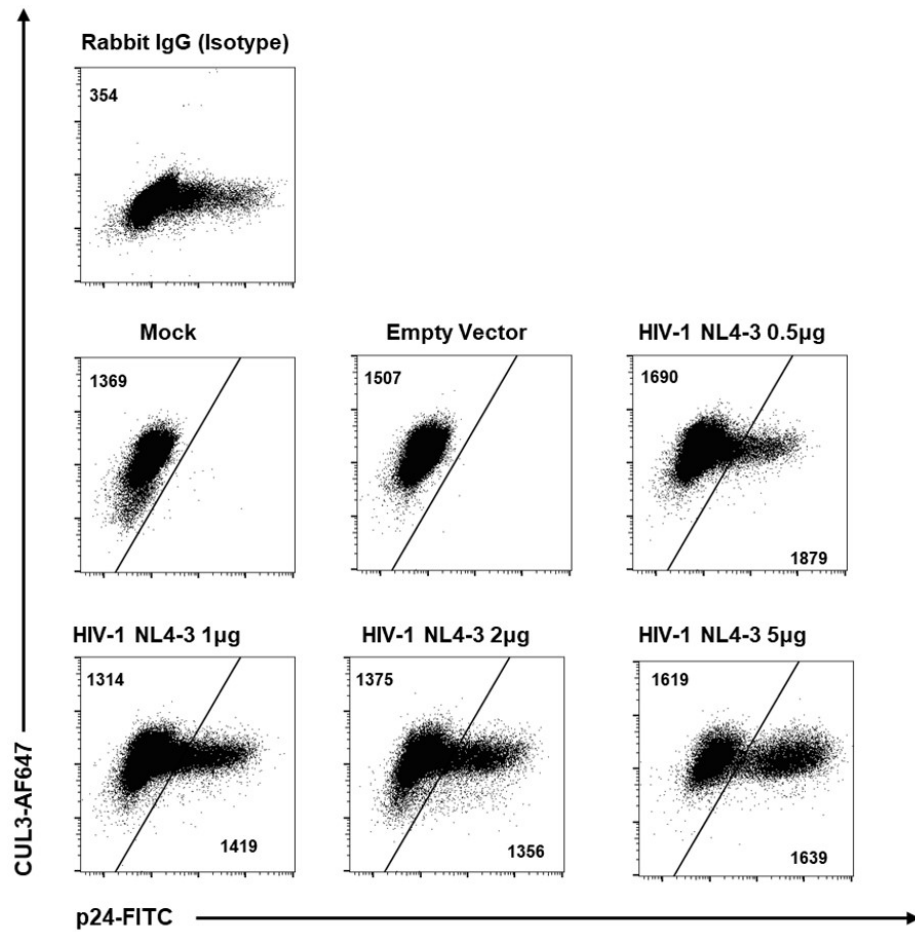
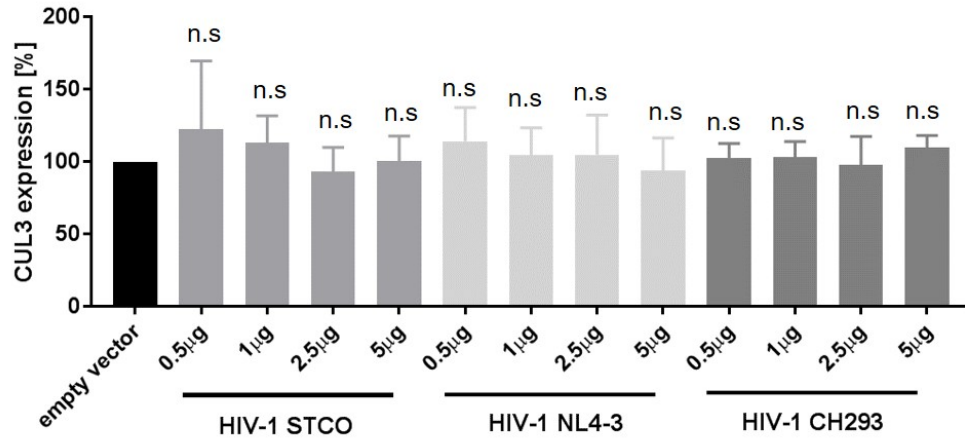


a



b



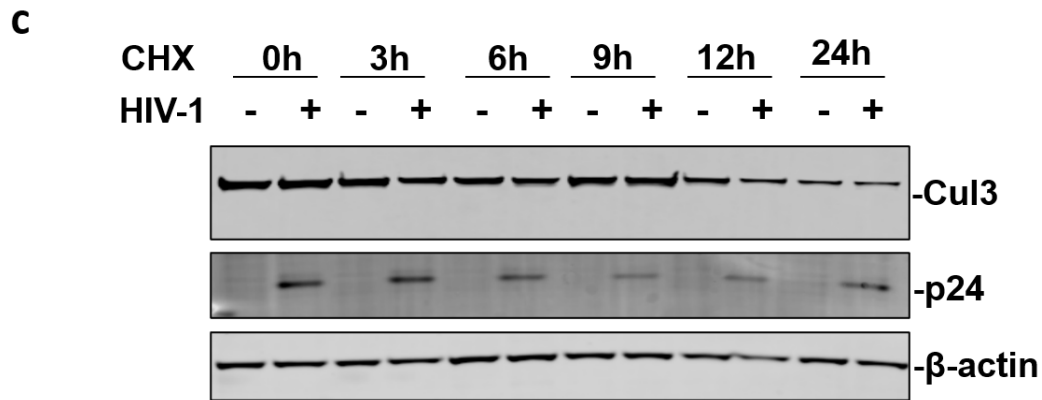
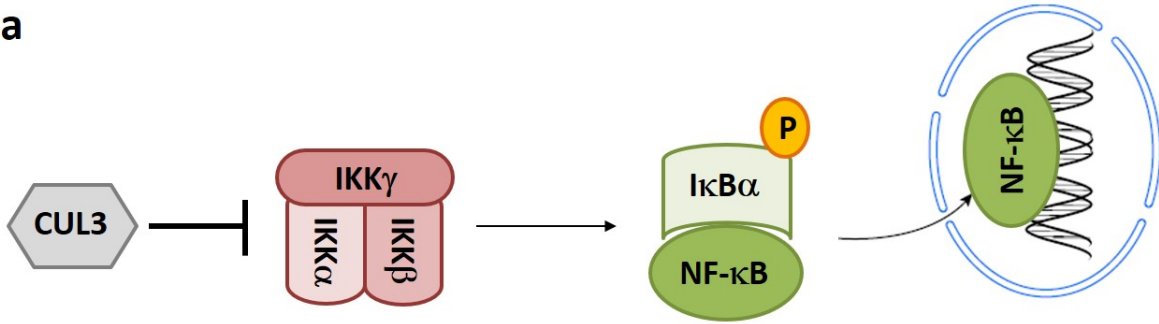


Figure S1. Endogenous Cul3 is not degraded by HIV-1 in transfected HEK293T cells. HEK293T cells were transfected with increasing amounts of the indicated proviral DNA (0.5 μ g, 1.0 μ g, 2.0 μ g, 5.0 μ g). (a) 40 h post transfection, cells were harvested, and Cul3 protein expression was analyzed by western blot using the indicated antibodies (n=3). (b) 40 h post transfection, Cul3 protein expression was monitored by two-color flow cytometry (upper panel) (n=2-5 +/-SD). Dot plots indicating the gating strategy and are shown in the lower panel. Numbers indicate mean fluorescence intensity (MFI). Isotype control consists of pooled cells from all samples. (c) HEK293T cells were transfected with HIV-1 NL4-3 proviral DNA (5.0 μ g). 24 h post transfection, the cells were treated with cycloheximide (CHX; 100 μ g/ml), and then collected at the indicated time point post-treatment. The expression of Cul3, HIV-1 p24 and β -actin was analyzed by western blot using the indicated antibodies.

a



b

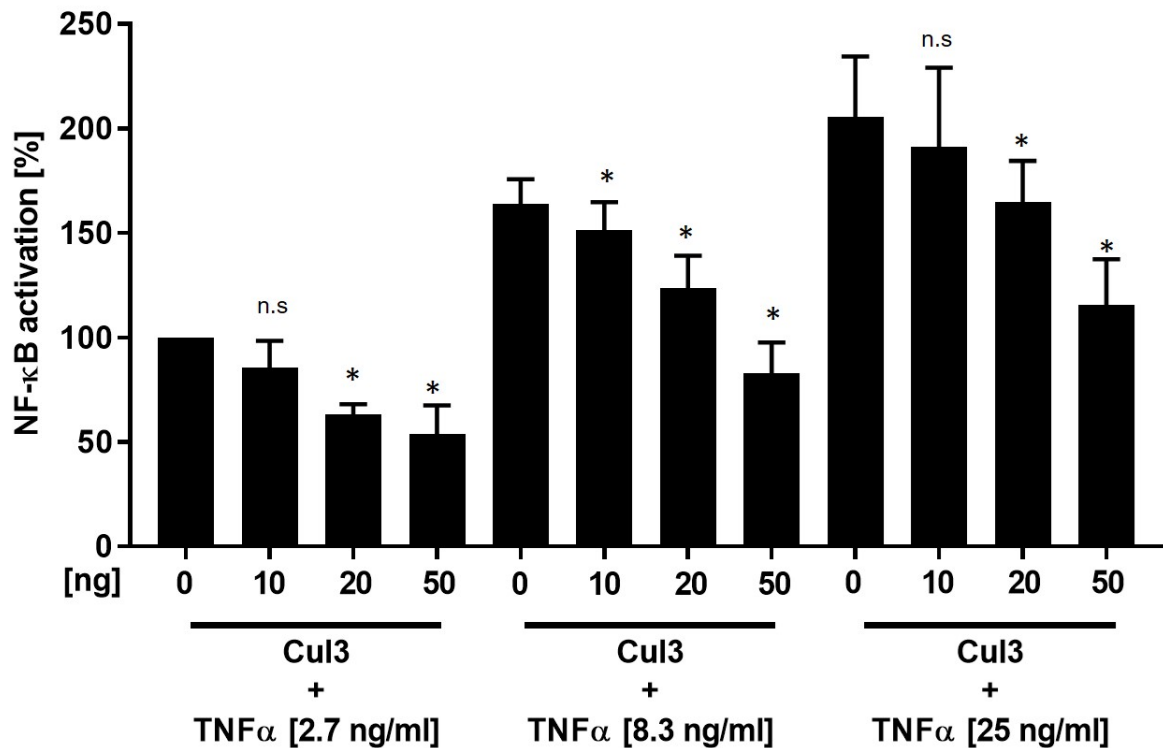


Figure S2. Cul3 overexpression reduces NF-κB activity in transfected HEK293T cells. (a) Cul3 negatively regulates NF-κB activation. (b) HEK293T cells were co-transfected with the indicated amounts of Cul3, 20 ng of an NF-κB-responsive firefly luciferase reporter construct and 5 ng of a Renilla luciferase reporter plasmid. 16 h post transfection, cells were treated with the indicated amounts of human TNFα. 24 h post stimulation, firefly luciferase activity was determined and normalized to the activity of the Renilla luciferase control plasmid. Data is shown as normalized to 0 ng Cul3 and 2.7 ng/mL TNFα (n=2-3 +/- SD).

Supplementary Materials

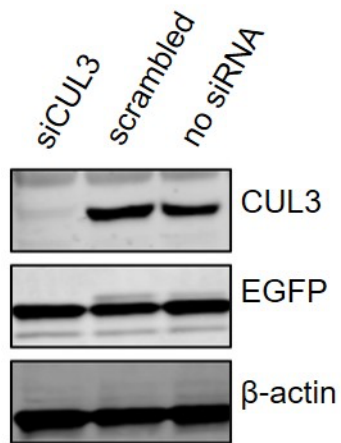


Figure S3. Cul3 depletion does not affect transfection efficiency. HEK293T cells, treated with an siRNA targeting CUL3 (siCUL3), a non-targeting siRNA (scrambled), or untreated (no siRNA) for 48 h, were transfected with the GFP-encoding pEGFP-C1. 24 hours post-transfection, the expression of GFP, CUL3, and β -actin was analyzed by western blot.

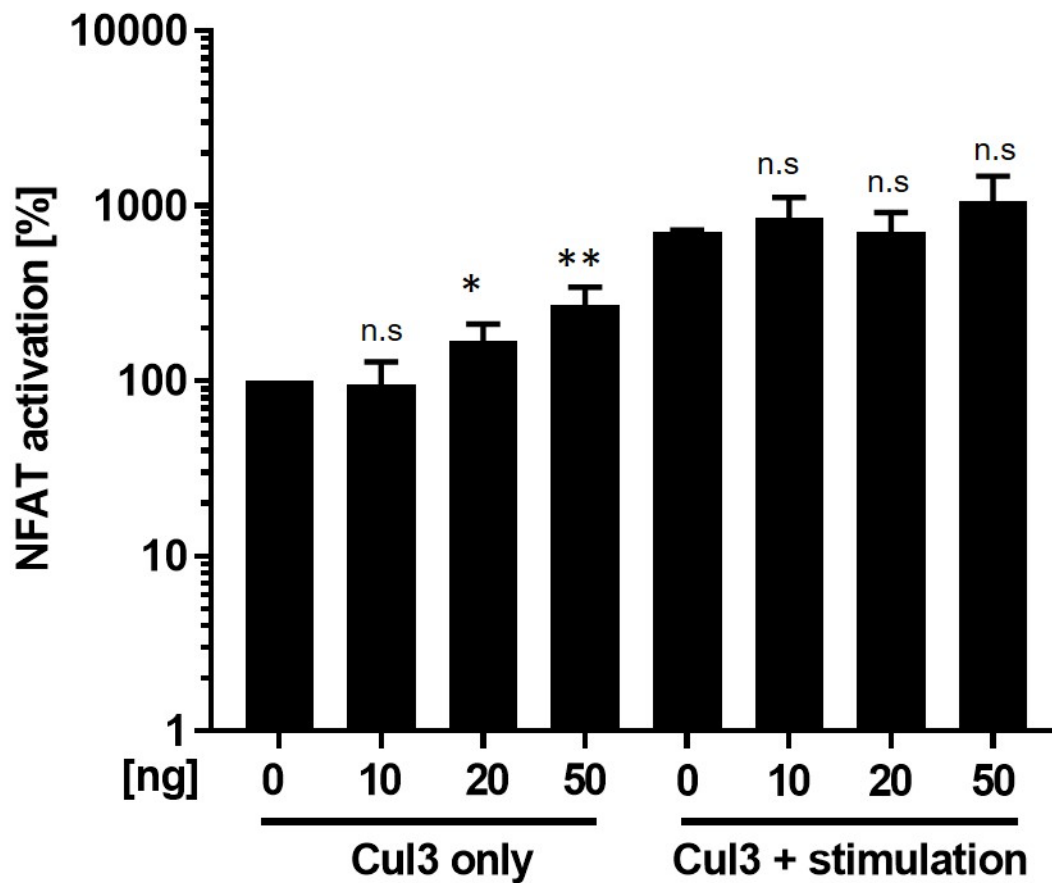


Figure S4. Cul3 overexpression does not reduce NFAT activity in transfected HEK293T cells. HEK293T cells were co-transfected with the indicated amounts of Cul3, 50 ng of an NFAT-responsive firefly luciferase reporter construct and 5 ng of a Renilla luciferase reporter plasmid. 24 h post transfection, cells were treated with human PMA (600 ng/mL) and Ionomycin (1 mM) (“Cul3 + stimulation”) or kept untreated (“Cul3 only”). 17 h post stimulation, firefly luciferase activity was determined and normalized to the activity of the Renilla luciferase control plasmid. Data is shown as normalized to 0 ng Cul3, no treatment (n=3 +/- SD).