HIV-1 Vpr protein directly loads Helicase-like transcription factor (HLTF) onto the CRL4-DCAF1 E3 ubiquitin ligase

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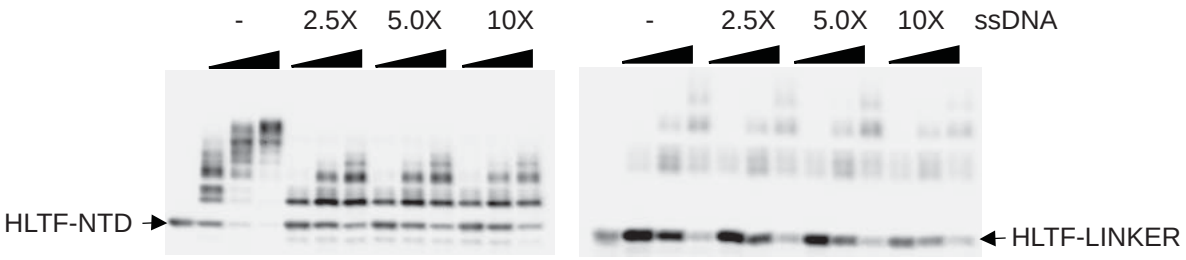
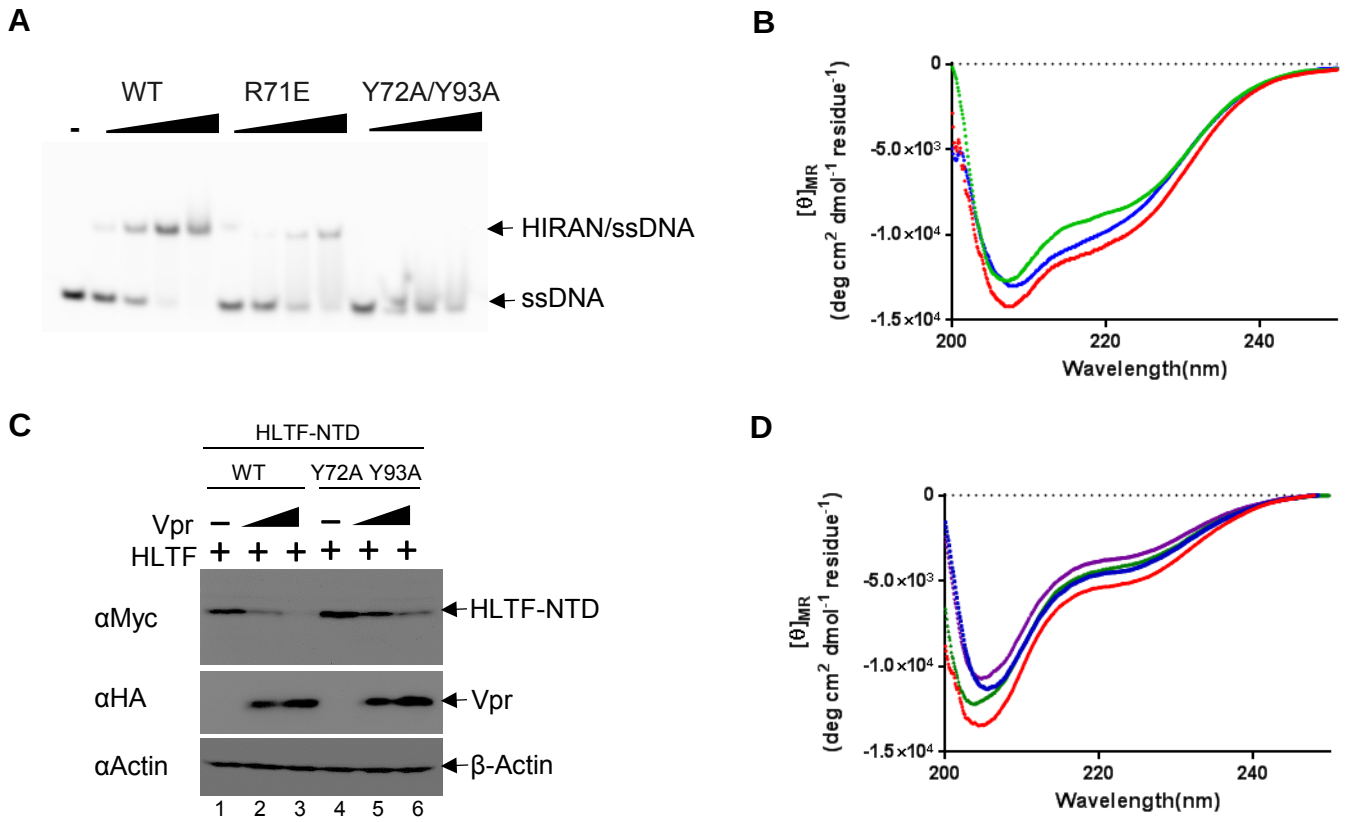
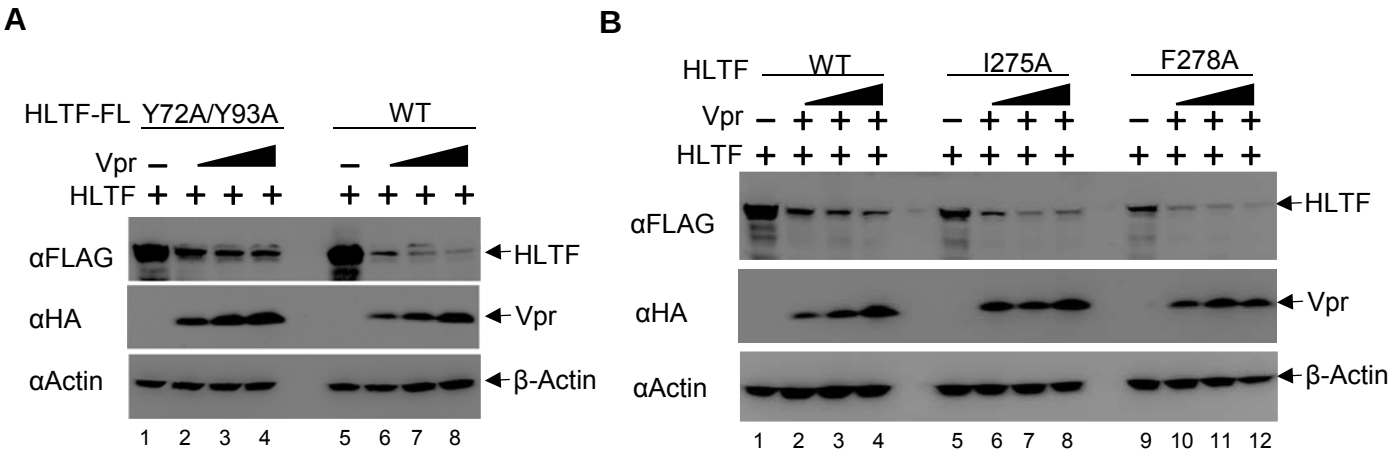
Supplemental Figure 1 - 4



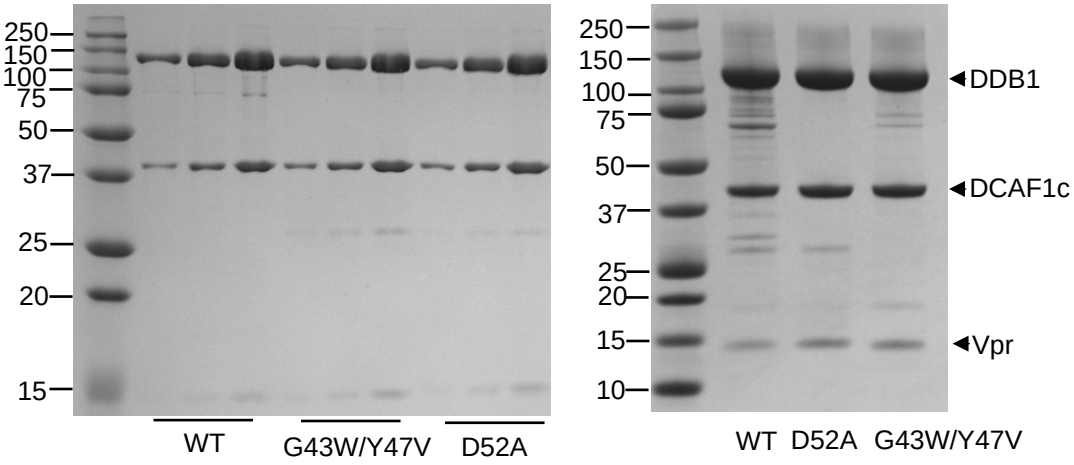
Supplemental Figure 1. *In vitro* ubiquitination assays of HLTF-NTD or LINKER with CRL4-DCAF1c-Vpr. The reaction mixture contained increasing concentrations of single-stranded DNA (ssDNA) at molar ratios of 0, 2.5, 5 and 10-fold over HLTF-NTD or HLTF-LINKER concentration. The assays were repeated three times with equivalent results.



Supplemental Figure 2. *A,* Electrophoretic mobility shift assays of HLTF-HIRAN WT, R71E and Y72A/Y93A binding to single-stranded DNA (ssDNA). The sequence of oligonucleotide used in the assay is 5'-AGCTACCAT GCCTGCCTCAAGAATTCGTAA-3'. The 5'-end of the single-stranded DNA was modified with Cy3. The DNA (1.5 μM) was incubated with increasing concentrations of HLTF-HIRAN (0, 0.15, 0.75, 1.5, and 3.0 μM) in a buffer containing 50 mM TrisHCl, pH 8.0, 1 mM EDTA, 0.5 mM 2-mercaptoethanol, 5% glycerol, and 0.1 mg/mL BSA on ice for 20 min. DNA was detected with Amersham Imager 600RGB (GE Healthcare) after separation with 4-20% PAGE. *B,* Superposition of the circular dichroism (CD) spectra of HLTF-HIRAN WT (red), R71E (green), and Y72A/Y93A (blue). CD spectra of HLTF HIRAN (20 μg/mL) were collected in a buffer containing 5 mM TrisHCl, pH 7.8 and 50 mM NaCl with a Jasco-810 CD spectrophotometer (Easton). Data were collected with a scan rate of 1 nm/sec from 250 to 200 nm at a constant temperature of 12 ˚C and averaged over 10 scans. *C*, A constant amount of plasmid expressing HLTF-NTD WT or Y72A/Y93A mutant was transiently co-transfected into HEK293T cells with increasing amounts of Vpr expression plamid. *D*, Superposition of the CD spectra of HLTF-NTD WT (red), Y72A/Y93A (blue), Y72A/Y93A/I275A (green), and Y72A/Y93A/F278A (purple). The data were collected as described above. CD experiments were repeated twice and the other experiments were repeated three times with equivalent results.



Supplemental Figure 3. *A.* HEK293T cells were co-transfected with a constant amount of full-length (FL) HLTF WT or Y72A/Y93A mutant and DCAF1 and increasing amounts of Vpr expression plasmids. B. A constant amount of plasmid expressing HLTF WT, I275A or F278A mutant was co-transfected into HEK293T cells with a contant amount of DCAF1 and increasing amount of Vpr expression plasmids.



Supplemental Figure 4. SDS-PAGE analysis of DDB1-DCAF1c-Vpr WT or mutants complexes from two independent experiments. Protein complexes were purified by MONO-Q ion exchange column chromatography.