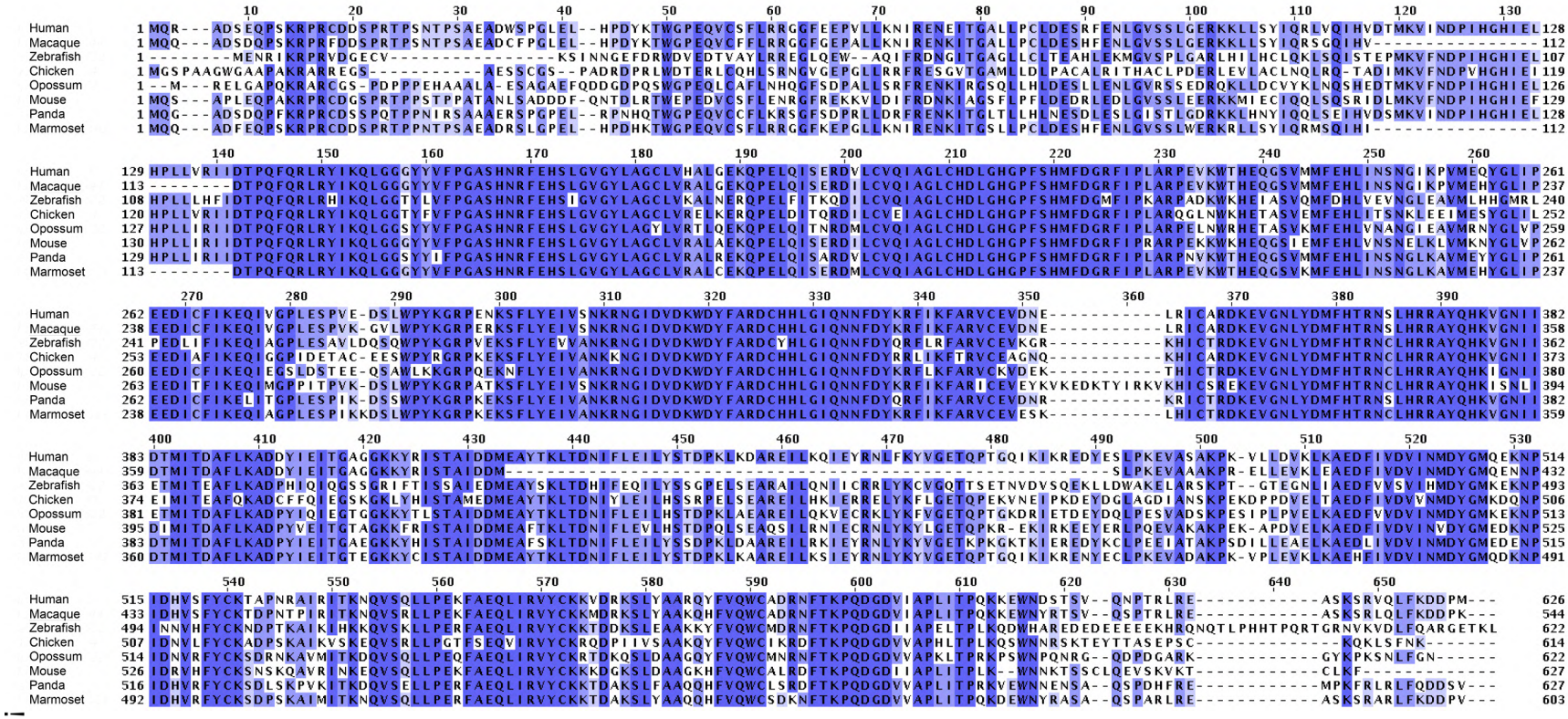
Supplementary Figure 1. A *ClustalW* amino acid sequence alignment of SAMHD1 proteins. Residues were shaded to indicate levels of conservation (dark blue: complete conservation, light blue: partial conservation). The numbers reflect amino acid positions in the alignment and do not correspond directly to any of the individual sequences. Gaps in the alignment are indicated by dashes. GenBank accession numbers for the sequences in the alignment: human, *Homo sapiens* (NM_015474); macaque, *Macaca mulatta* (XP_001097562); zebrafish, *Danio rerio* (NP_001153405); chicken, *Gallus gallus* (NP_001026016); opossum, *Monodelphis domestica* (XP_001381585); mouse, *Mus musculus* (NP_061339); panda, *Ailuropoda melanoleuca* (XP_002915216); marmoset, *Callithrix jacchus* (XP_002747259).

Supplementary Figure 2. The N-terminus of Vpx recruits SAMHD1 to CRL4-DCAF1-Vpx for ubiquitination and downregulation. A. The full-length SAMHD1 was injected into an analytical size exclusion column and separated at a flow rate of 0.8 mL/min. Proteins in peak fractions (1 and 2) were concentrated 10-fold, separated by SDS-PAGE and stained with Coomassie Brilliant Blue (panel G, lane 1 and 2). Mixtures of SAMHD1 and DDB1-DCAF1c were incubated with NusA·Vpx(1-102) (**B**), NusA·Vpx(1-89) (**C**), NusA·Vpx(13-90) (**D**), NusA·Vpx(13-112) (**E**), or NusA·Vpx(1-112) (**F**), and analyzed by analytical size exclusion chromatography as described in A. Only the first peak in each experiment was separated by SDS-PAGE and the results are shown in panel **G**. For reference, the retention volume of SAMHD1 is drawn as a straight vertical line in panels A-F. **H.** *In vitro* ubiquitination of SAMHD1 by $CRL4^{DCAF1c}$ in complex with Vpx(1-102) (left panel), Vpx(1-112) (middle panel), and Vpx(13-112) (right panel). SAMHD1 was incubated with various DDB1–DCAF1c–Vpx complexes in the presence of E1 (UBA1), E2 (UbcH5b), CUL4A–RBX1 and FLAG-tagged ubiquitin at 37°C. Negative control reactions (–) were assembled and carried out without SAMHD1. The reactions were terminated after 10 min (odd numbered lanes) and 30 min (even numbered lanes) by the addition of Laemmli buffer and incubation at 95°C for 5 min. The reaction mixtures were separated by SDS-PAGE, transferred to PVDF, and ubiquitin was revealed with anti-FLAG antibody. The ubiquitinated species seen in the negative control reaction reflect ubiquitynation of the CRL4 subunits and other reaction components. **I.** HEK 293T cells were transiently co-transfected in duplicate with SAMHD1, DCAF1, and wild type or indicated Vpx alanine substitution mutants (N12A/E15A, E15A/E16A, E16A/T17A, E15A/E16A/T17A, and N12A/E15A/E16A/T17A). Cell lysates prepared two days post-transfection were analyzed by immunoblotting with a mixture of DCAF1, SAMHD1 and Vpx antibodies. Asterisk (*) indicates a background protein band that was used as a loading control.



Supplementary Figure 1

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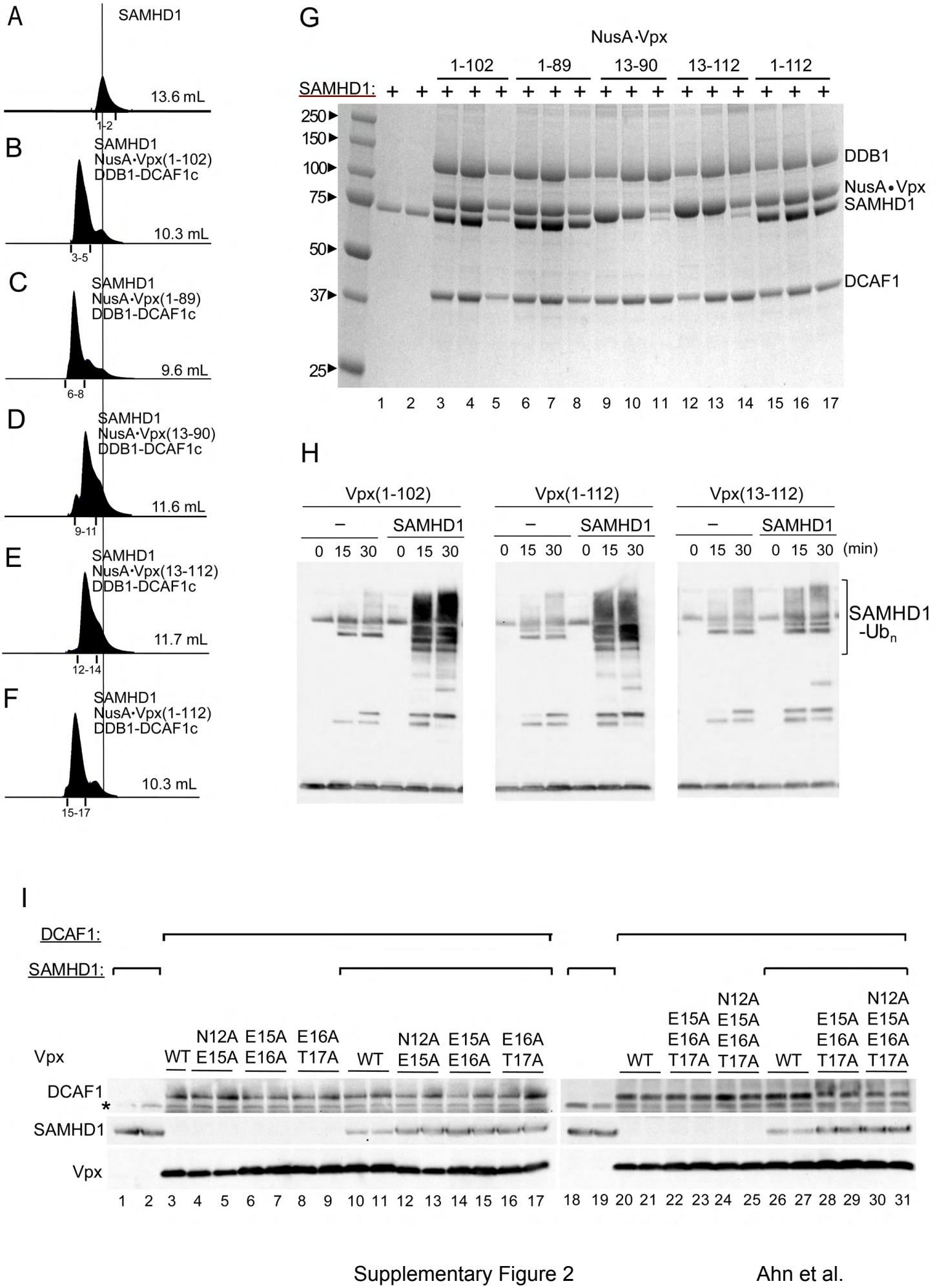

Supplementary Figure 2 Ahn et al.