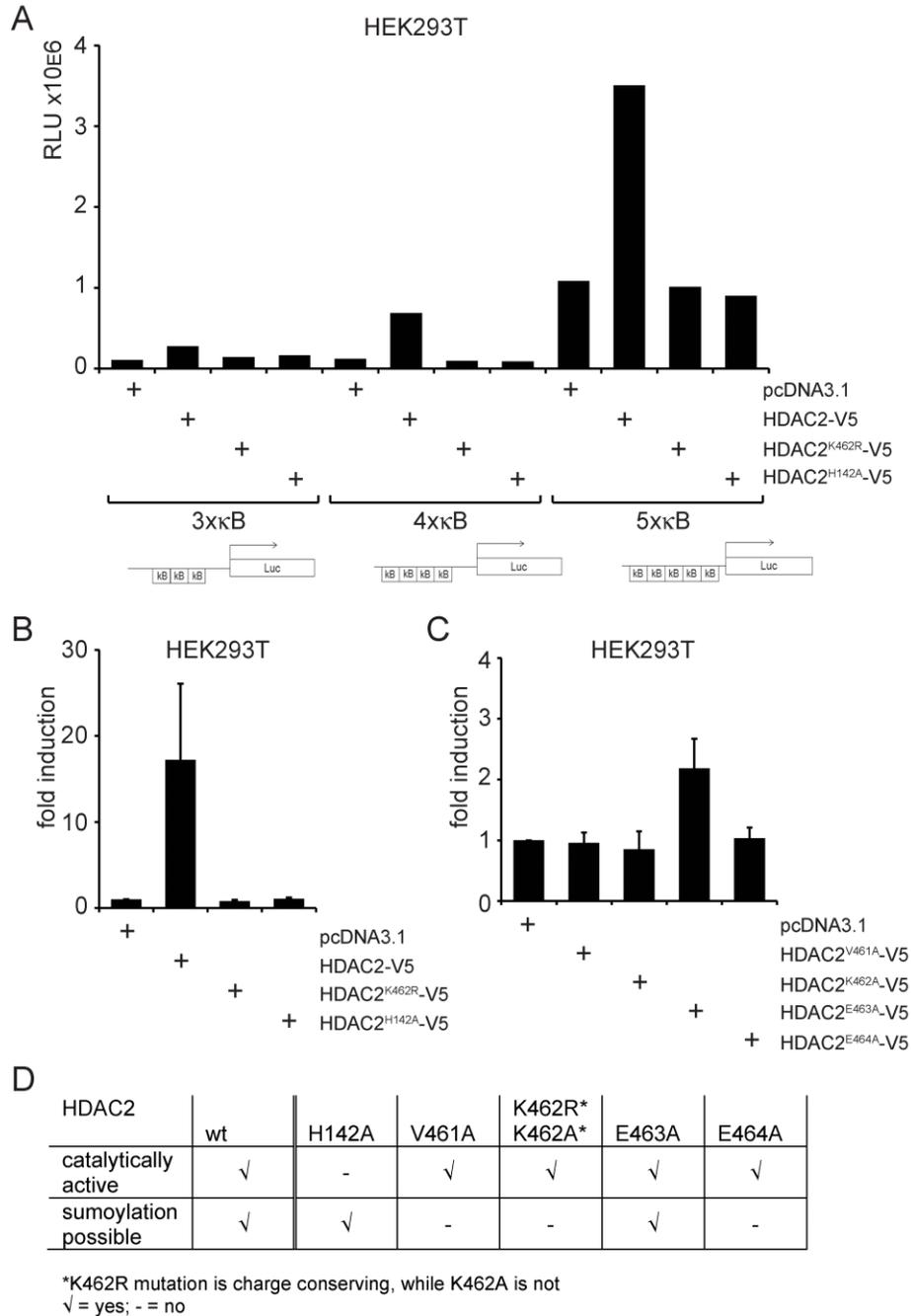


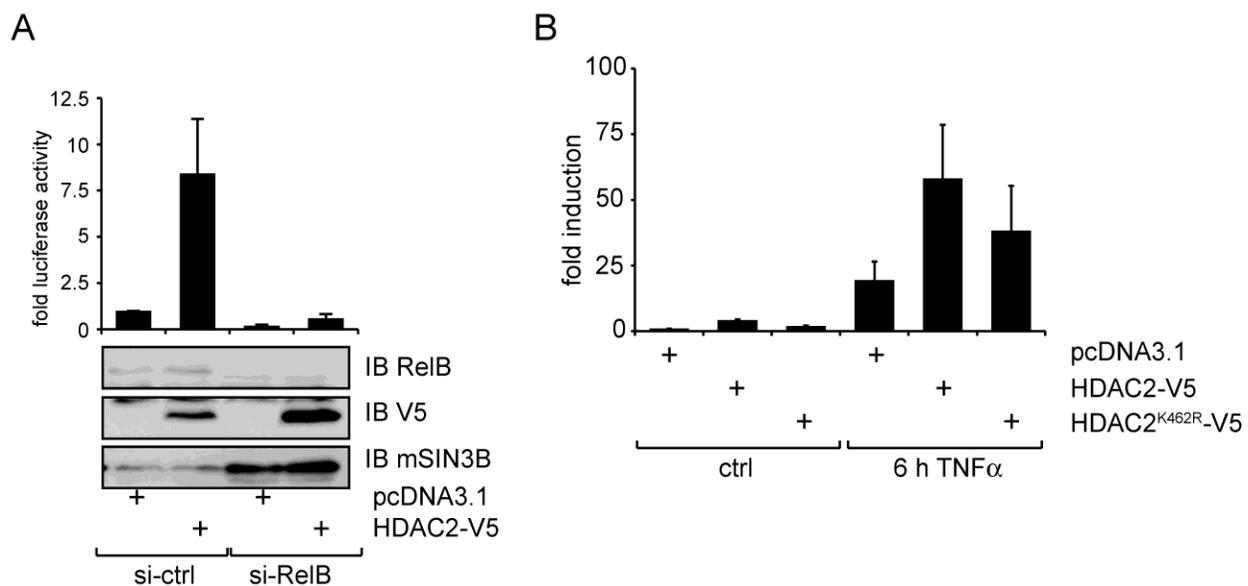
Sumoylation of HDAC2 promotes NF- κ B-dependent gene expression

Supplementary Material

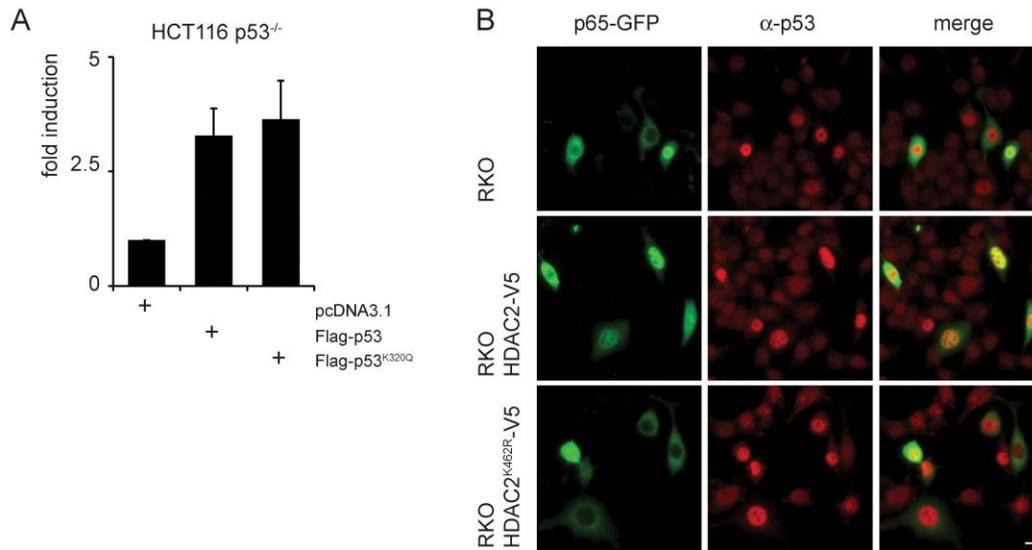


Supplementary Figure S1: (A) Representative diagram of κ B-Luc reporter gene assay in HEK293T cells using reporter constructs with increasing number of κ B consensus sites (3/4/5) with cotransfection of HDAC2 variants or empty vector as in Figure 1A as indicated. HDAC2-V5 cotransfection induces

reporter gene expression in from all reporter gene constructs. Relative induction is highest with 4 \times κ B-Luc. Graph shows relative luciferase units (RLU) derived of luciferase intensity after normalization to activity of cotransfected β -Gal. **(B)** Luciferase assay with 4 \times κ B-Luc in HEK293T cells, as in Figure 1B but with catalytically inactive HDAC2^{H142A} included as control. Assay shows that HDAC2^{H142A} like HDAC2^{K462R} is not affecting reporter gene expression. Bar graph depicts mean \pm sem of n=5 independent experiments. The values for pcDNA 3.1, HDAC2-V5 and HDAC2^{K462R}-V5 are a subset of those shown in Figure 1B. **(C)** Luciferase assay (4 \times κ B-Luc) in HEK293T cells with testing induction of 4 \times κ B-Luc with HDAC2 constructs having point mutations in the Ψ KxE sumoylation consensus motif (VK⁴⁶²EE in HDAC2). Notably, only mutant HDAC2^{E463A} in which the inert “x” position is mutated is able to increase reporter gene activity. Diagram show fold induction of mock transfected control, mean \pm sem of n=3 independent experiments. **(D)** Summary of the HDAC2 mutants used, characterized by us and others. The catalytic activity of each mutant and the ability to become sumoylated is listed.



Supplementary Figure S2: **(A)** RKO cells transfected as in Figure 2A, knockdown of RelB diminishes HDAC2-caused NF- κ B reporter gene induction; n=3. **(B)** RKO cells transfected as in Figure 1C with 4 \times κ B-Luc assay together with HDAC2-V5 and HDAC2^{K462R}-V5. Cells were treated with 20 ng/ml TNF α for 6 h or left untreated for control prior to harvest. n=4. Induction of the reporter shows principally intact NF- κ B signaling in these cells.



Supplementary Figure S3: (A) Luciferase assay with 4xκB-Luc in HCT116 p53^{-/-} cells and reconstitution with either Flag-tagged wild-type p53 or a pseudo-acetylated p53^{K320Q}. n=4. (B) Representative image of RKO, RKO HDAC2-V5 and RKO HDAC2^{K462R}-V5 cells transiently transfected with p65-GFP as in Figure 3A, but with additional staining of endogenous p53. Bar = 10 μm.