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Supplementary Materials for

The Deubiquitinase A20 Mediates Feedback Inhibition of Interleukin-17 Receptor Signaling

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Published 4 June 2013, *Sci. Signal.* **6**, ra44 (2013)
DOI: 10.1126/scisignal.2003699

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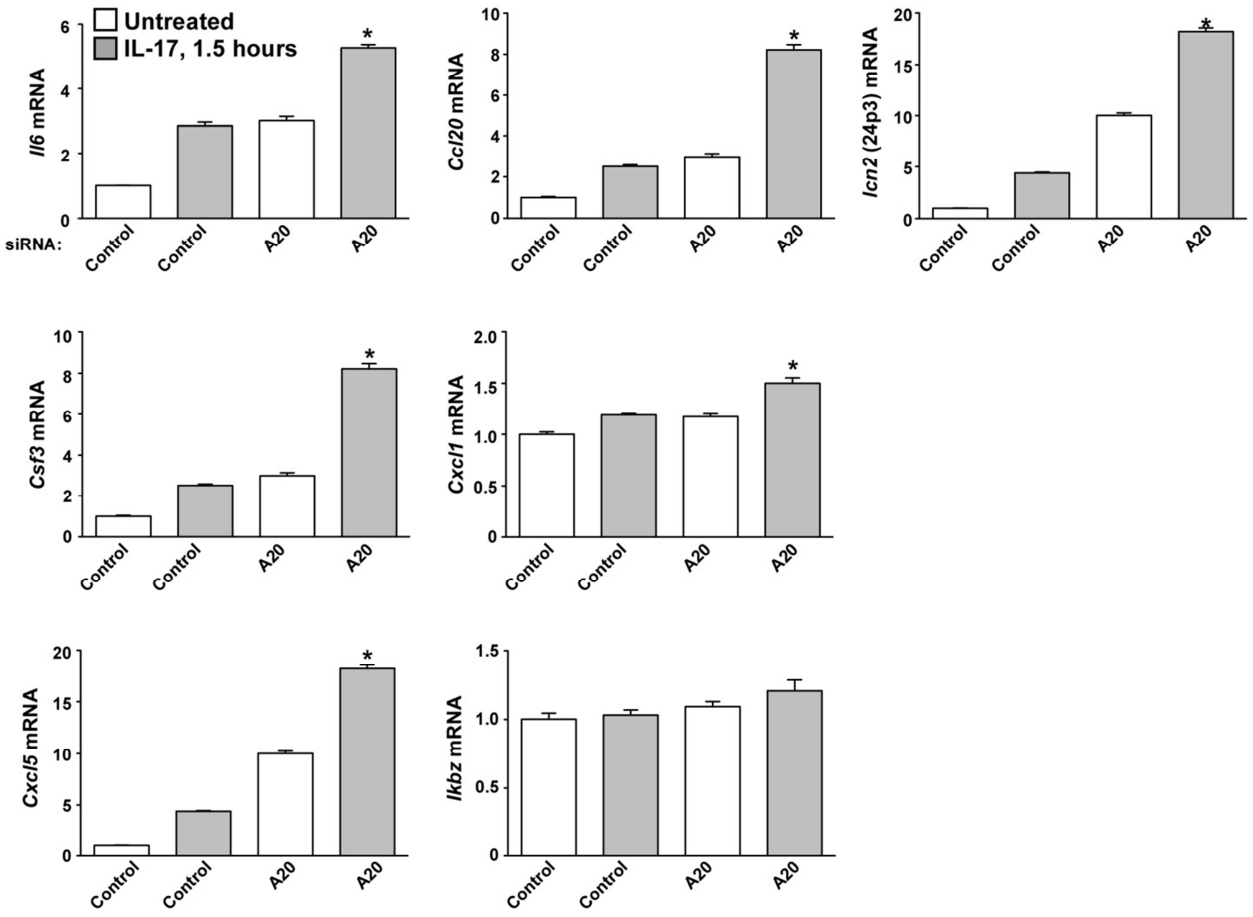

Fig. S1. Knockdown of A20 enhances the expression of most IL-17 target genes. ST2 cells transfected with the indicated siRNAs were left untreated or were treated with IL-17 for 1.5 hours. Lysates were analyzed for the expression of the indicated genes by qPCR. Graphs show the fold-increase + SD in the abundance of the indicated mRNAs relative to their abundance in untreated cells transfected with control siRNA. **P* <0.05 by ANOVA and post-hoc Tukey's test; n = 2 experiments.

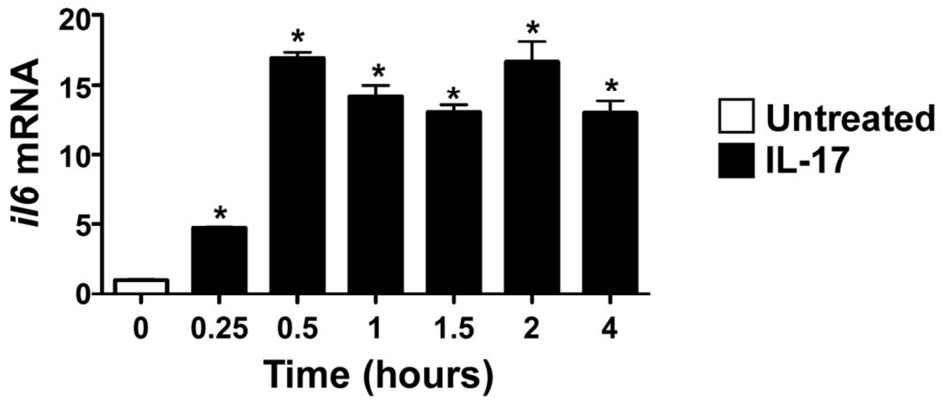

Fig. S2. Kinetics of IL-17–dependent IL-6 production. ST2 cells were treated with IL-17 for the indicated times and the fold-change in *Il6* mRNA abundance compared to that of the cells at the zero time point was assessed by qPCR in triplicate samples. **P* < 0.05 by ANOVA and post-hoc Tukey's test compared to the zero time point; n = 3 experiments.

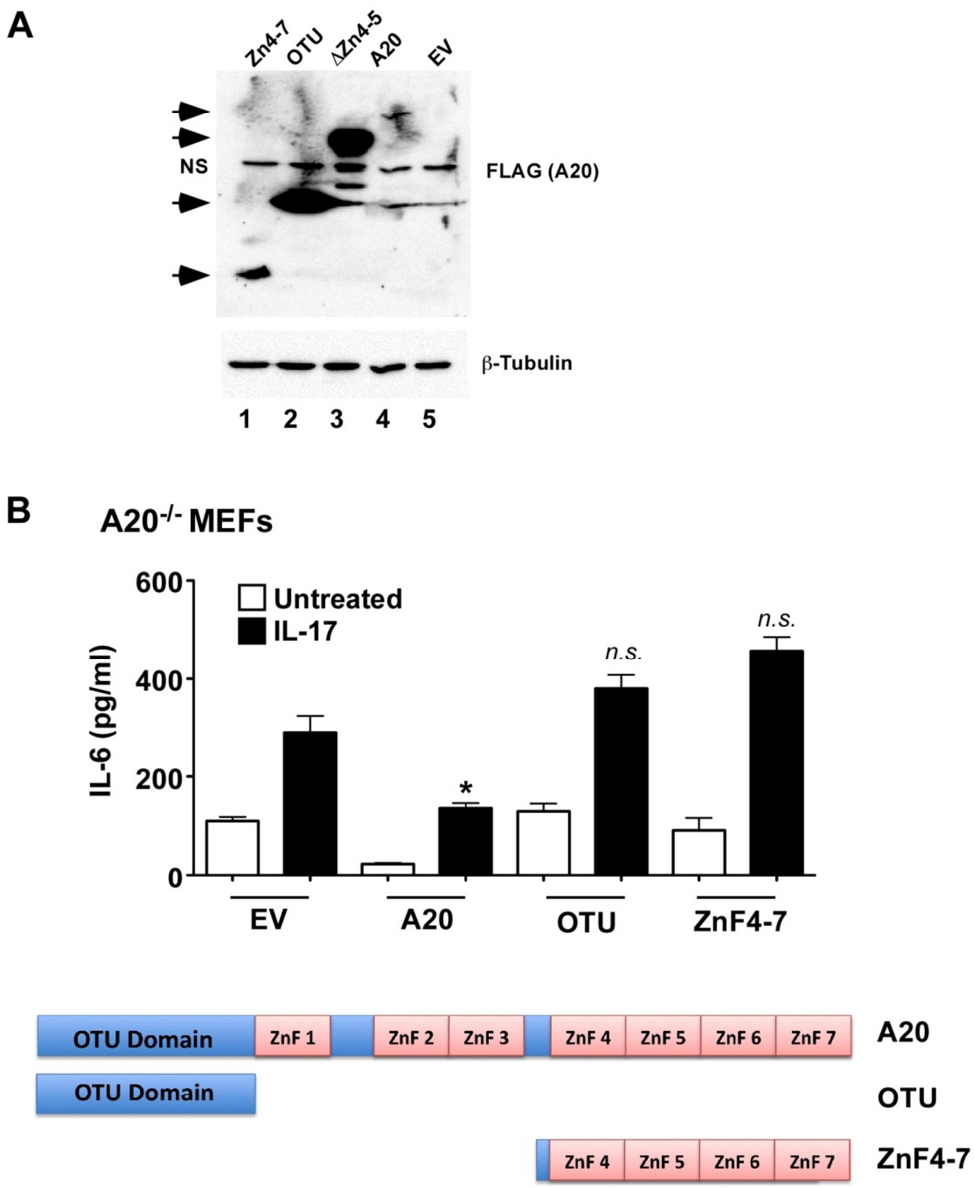

Fig. S3. ZnF4 to ZnF7 of A20 are insufficient to suppress IL-17–mediated signaling. (**A**) Expression of A20 mutants. Lysates from $A20^{-/-}$ cells transfected with empty vector (EV), plasmid encoding wild-type A20, or plasmids encoding the indicated A20 mutants were analyzed by Western blotting with anti-FLAG antibody to detect A20 (top) or with antibody against β-tubulin (bottom), which was a loading control. Arrows indicate the migration of individual A20 mutants. NS, nonspecific band. (**B**) $A20^{-/-}$ MEFs were transfected with EV, plasmid encoding wild-type A20, or plasmids encoding the indicated A20 mutants, were treated with IL-17 for 24 hours, and then IL-6 in the culture medium was measured by ELISA from triplicate samples. **P* < 0.05 by ANOVA and post-hoc Tukey's test. n = 2 experiments.

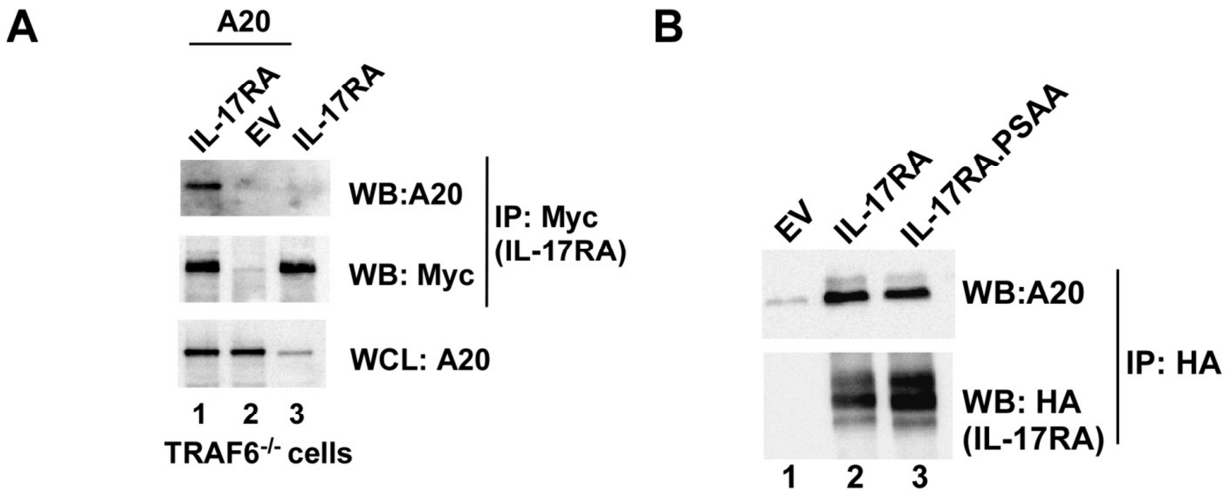

Fig. S4. TRAF6 and TRAF3 are not required for the association between A20 and IL-17RA. (**A**) A20 binds to IL-17RA in a TRAF6-independent manner. TRAF6$^{-/-}$ MEFs were cotransfected with plasmid encoding A20 and with plasmid encoding Myc-tagged IL-17RA or empty vector (EV). Lysates were subjected to immunoprecipitation (IP) with antibody against Myc and were analyzed by Western blotting (WB) with antibody against A20 (top) or Myc (bottom). WCLs before immunoprecipitation were analyzed by Western blotting with antibody against A20. Data are representative of three experiments. (**B**) A20 does not bind to the TRAF consensus site of the CBAD. HEK 293T cells were cotransfected with plasmid encoding IL-17RA or IL-17RA.PSAA (both tagged at the C-terminus with HA) together with plasmid encoding A20. Anti-HA antibody was used to pull down IL-17RA, and immunoprecipitates were analyzed by Western blotting with antibody against A20 (top) or HA (bottom) to detect IL-17RA. Data are representative of two experiments.