Supplemental Fig. 2: The C-terminus of TAX1BP1 dictates interaction with TRAF6.
(A) Schematic representations of TAX1BP1 (747 amino acids) and the deletion mutants that were used. Coiled-coils are indicated as CC. Zn, zinc fingers; HA, HA-tag. (B) Effect of different TAX1BP1 deletion mutants on IL-1β induced NF-κB activation. 293-IL1R cells were transfected with a NF-κB luciferase reporter, a RSV-β-galactosidase plasmid, and the indicated HA-TAX1BP1 plasmid. “Control” indicates transfection with empty vector DNA. After 40 hours, IL-1β (10 ng/ml), as indicated, was added for 8 hours, and cells were harvested for luciferase assay. (C) Co-immunoprecipitation of TAX1BP1 and TRAF6. Cells were transfected with FLAG-tagged TRAF6 and HA-tagged full-length (1-747), C-terminal half (388-747), N-terminal half (1-387) TAX1BP1 vectors. 300 μg of cell lysate were immunoprecipitated (IP) with 40 μl of mouse monoclonal anti-HA antibody (αHA), followed by immunoblotting (IB) with rabbit polyclonal anti-FLAG antibody (αFLAG), (upper panel). Transfection efficiency was verified by IB with anti-FLAG (middle panel) and anti-HA (lower panel).