



Supplementary information, Figure S1 UBE2O interacts with and inhibits TRAF6-induced NF- κ B reporter activity.

(A) HEK293T cells were transiently transfected with UBE2O-Myc and Flag-TRAFs expression vectors and analyzed by immunoprecipitation (IP) and/or immunoblotting (IB) as indicated. Anti-c-Myc agarose affinity gel was used to immunoprecipitate UBE2O. Flag antibody was used for detection of UBE2O-associated TRAFs. TCL: total cell lysate. (B) HEK293T cells were transfected with the indicated UBE2O vectors and analyzed by immunoprecipitation (IP) and/or immunoblotting (IB) to detect UBE2O-associated

endogenous TRAF6. **(C)** and **(D)** HEK293T cells were co-transfected with NF- κ B reporter and Flag-TRAFs (1, 2, 3, 4, 5, 6) in the absence or presence of UBE2O. Luciferase activity was measured after 36 hours transfection. For all the luciferase assays, *LacZ* expression plasmid was co-transfected as internal reference. Each experiment above was performed in triplicate. * indicates $p < 0.05$. **(E)**, **(F)** and **(G)** Cell lysates from Figure 1D, Figure 1E and Figure 1G were used to analyze expression of Flag-TRAF6, UBE2O-Myc and Flag-TLR4. Actin was served as internal reference.