



Supplementary information, Figure S5 UBE2O disrupts the interaction between TRAF6 and MyD88, but not the TRAF6-IRAK1 or MyD88-IRAK4 complex.

(A) HEK293T cells were transfected with Flag-TRAF6, MyD88-Myc and full length (FL) or deleted (D1, D2) UBE2O-Myc expression vectors as indicated. 48 hours after transfection cells were treated with IL-1 β for the indicate minutes and lysed for immunoprecipitation (IP) and immunoblotting (IB) analysis. (B) HEK293T cells stably expressing UBE2O shRNAs (shUBE2O-1+shUBE2O-2) were transfected with Flag-TRAF6 and MyD88-Myc as indicated. 48 hours after transfection cells were treated with IL-1 β for the indicate minutes and lysed for IP and IB analysis. (C) HEK293T cells were transfected with Flag-IRAK1, HA-TRAF6 and UBE2O-Myc as indicated. 48 hours after transfection cells were treated with

IL-1 β for the indicate minutes and lysed for IP and IB analysis. Immunoprecipitates were blotted for IRAK1-associated TRAF6 by using HA antibody. **(D)** HEK293T cells were transfected with Flag-IRAK4, MyD88-Myc and UBE2O-Myc as indicated. 48 hours after transfection cells were treated with IL-1 β for the indicate minutes and lysed for IP and IB analysis. Immunoprecipitates were blotted for IRAK4-associated MyD88 by using Myc antibody.