Supplementary material

USP18 negatively regulates NF-κB signaling by targeting TAK1 and NEMO for deubiquitination through distinct mechanisms

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Figure S1

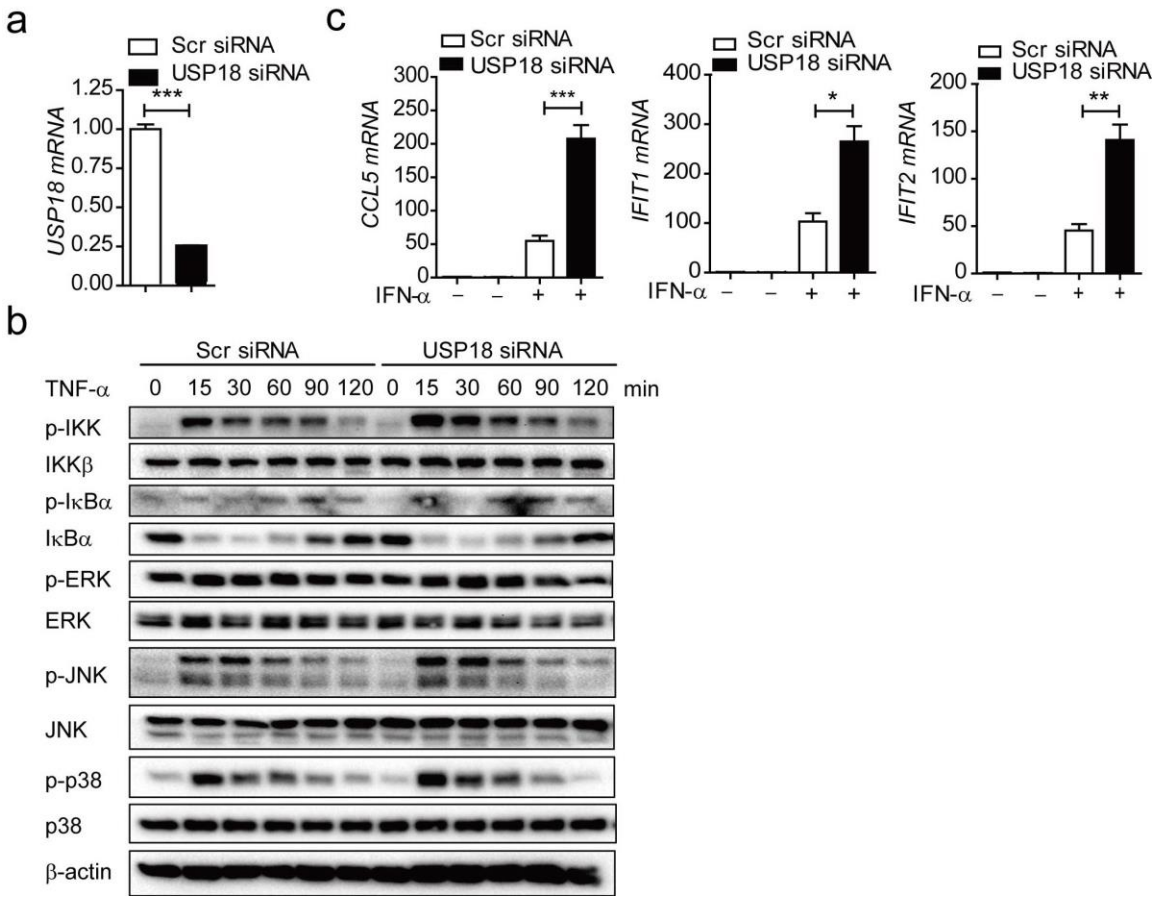

Figure S1 | Knockdown of USP18 enhances activation of NF-κB signaling and expression of interferon-stimulated genes. (a) Knockdown efficiency of USP18-specific siRNA in THP-1 derived macrophages. (b) THP-1 cells transfected with scramble (Scr) siRNA or USP18-specific siRNA, and then treated with TNF-α for the indicated time points. TNFα-induced IKK, IKBα, and MAPK (p38, JNK, and ERK) activation measured by immunoblot analysis with indicated antibodies. (c) USP18-mediated negative regulation of IFN-α induced gene expression. THP-1 derived macrophage cells transfected with Scr siRNA or USP18 siRNA 30 pmol/well for 48 hrs, followed by treatment with IFN-α (10ng/ml) for 6 hrs. Total mRNA harvested after treatment and ISG15, IFIT1, IFIT2 mRNA abundance analyzed by q-PCR. Data in a, c are presented as the means ± SD of three independent experiments. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 versus cells transfected with scrambled siRNA (Student's t-test).

Figure S2

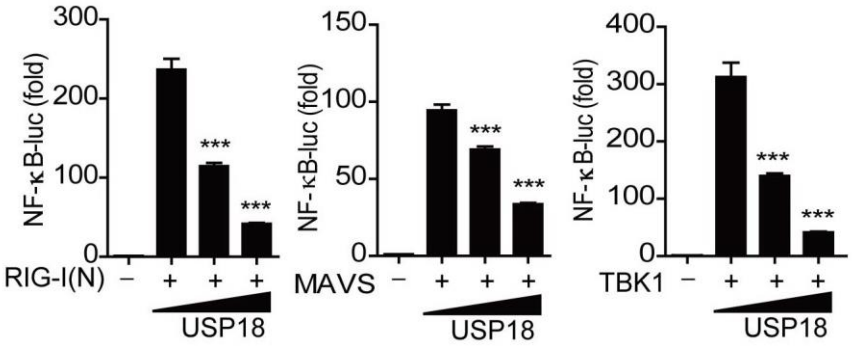

Figure S2 | USP18 inhibits RIG-I (N)-, MAVS-, and TBK1- induced NF-κB activation. Luciferase activity in HEK293T cells transfected with plasmid encoding a luciferase reporter for NF-κB (NF-κB-luc) together with empty vector or plasmids as indicated.

Figure S3

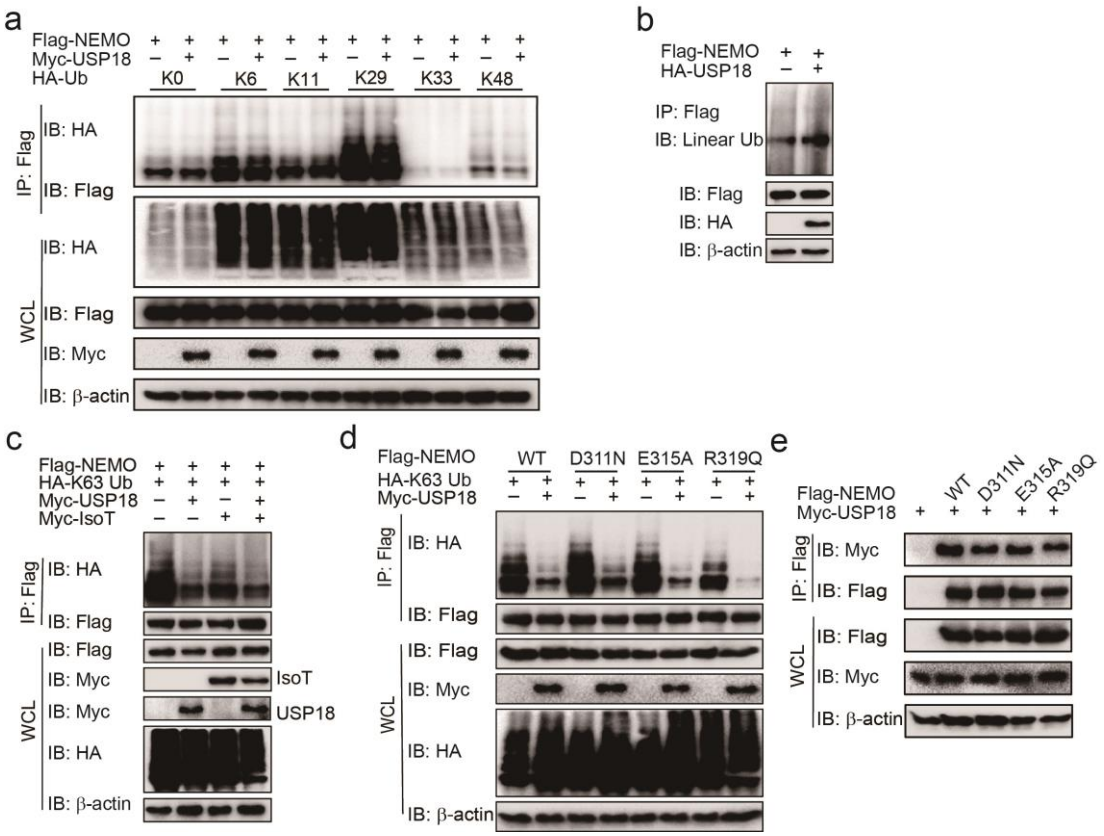

Figure S3 | USP18 blocks covalent conjugated K63-linked polyubiquitin chains of NEMO. (a) Lysates of 293T cells transfected with Flag-NEMO, HA-Ub and Myc-USP18 or EV immunoprecipitated with anti-Flag and immunoblotted with anti-HA. (b) USP18 does not block linear polyubiquitination of NEMO. Lysates of 293T cells transfected with plasmids were immunoprecipitated with anti-Flag and immunoblotted with anti-Linear Ub. (c) Lysates of 293T cells transfected with the indicated plasmids, immunoprecipitated with anti-Flag, and immunoblotted with anti-HA. (d) 293T cells transfected with HA-K63, Flag-NEMO mutants, and Myc-USP18 or EV, then immunoprecipitated with anti-Flag and immunoblotted with anti-HA. (e) Cell lysates from Flag-NEMO mutant- and Myc-USP18-transfected 293T cells immunoprecipitated with anti-Flag and immunoblotted with anti-Myc.

FigureS4

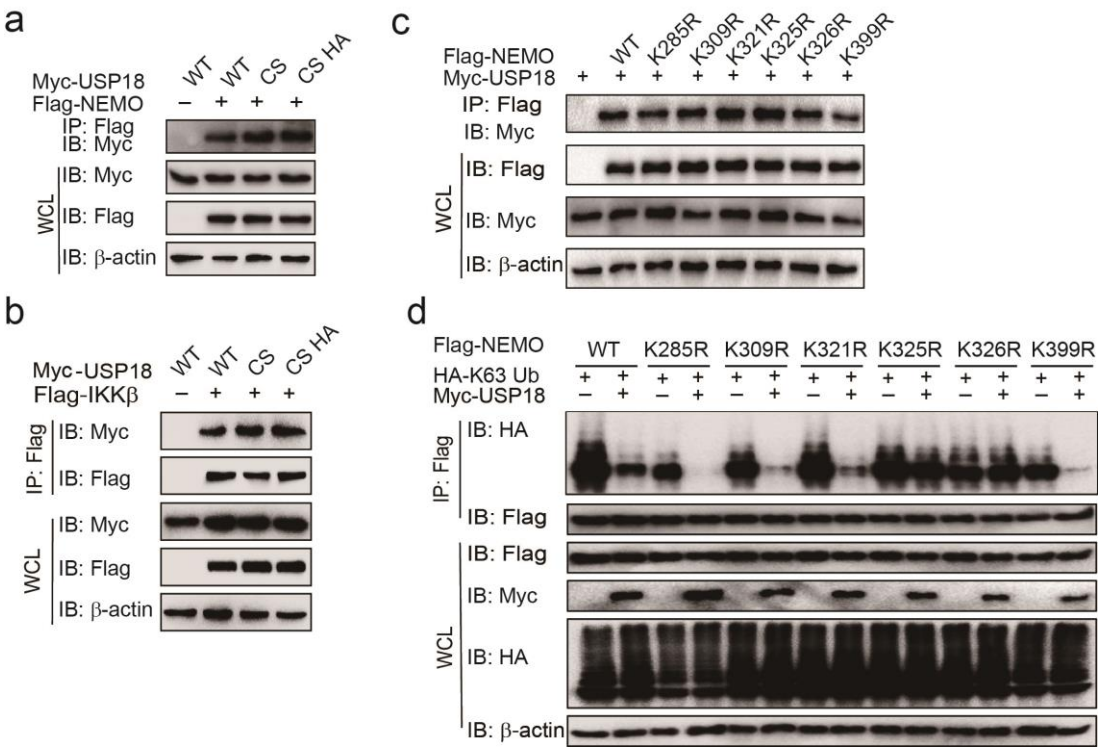

Figure S4 | USP18 blocks K63-linked polyubiquitination of NEMO at Lys 325 and 326 residues. (a-b) Co-immunoprecipitation and immunoblot analyses of 293T cells transfected with Flag-NEMO (a) or IKKβ (b) and Myc-USP18 (WT), Myc-USP18 (C64S), or Myc-USP18 (C64S H318A). (c) 293T cells transfected with Flag-NEMO mutants and Myc-USP18 or EV, immunoprecipitated with anti-Flag, and immunoblotted with anti-Myc. (d) Cell lysates collected from 293T cells transfected with Flag-NEMO mutants and HA-K63 Ub, empty vector (-), or Myc-USP18 were immunoprecipitated with anti-Flag and immunoblotted with anti-HA.

Figure S5

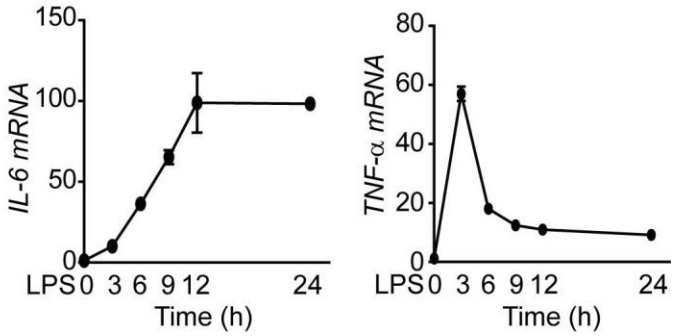

Figure S5 | The expression of proinflammatory cytokines in response to LPS stimulation. Relative IL-6 and TNF-α mRNA abundance in THP-1 cells stimulated with LPS for the indicated time points.