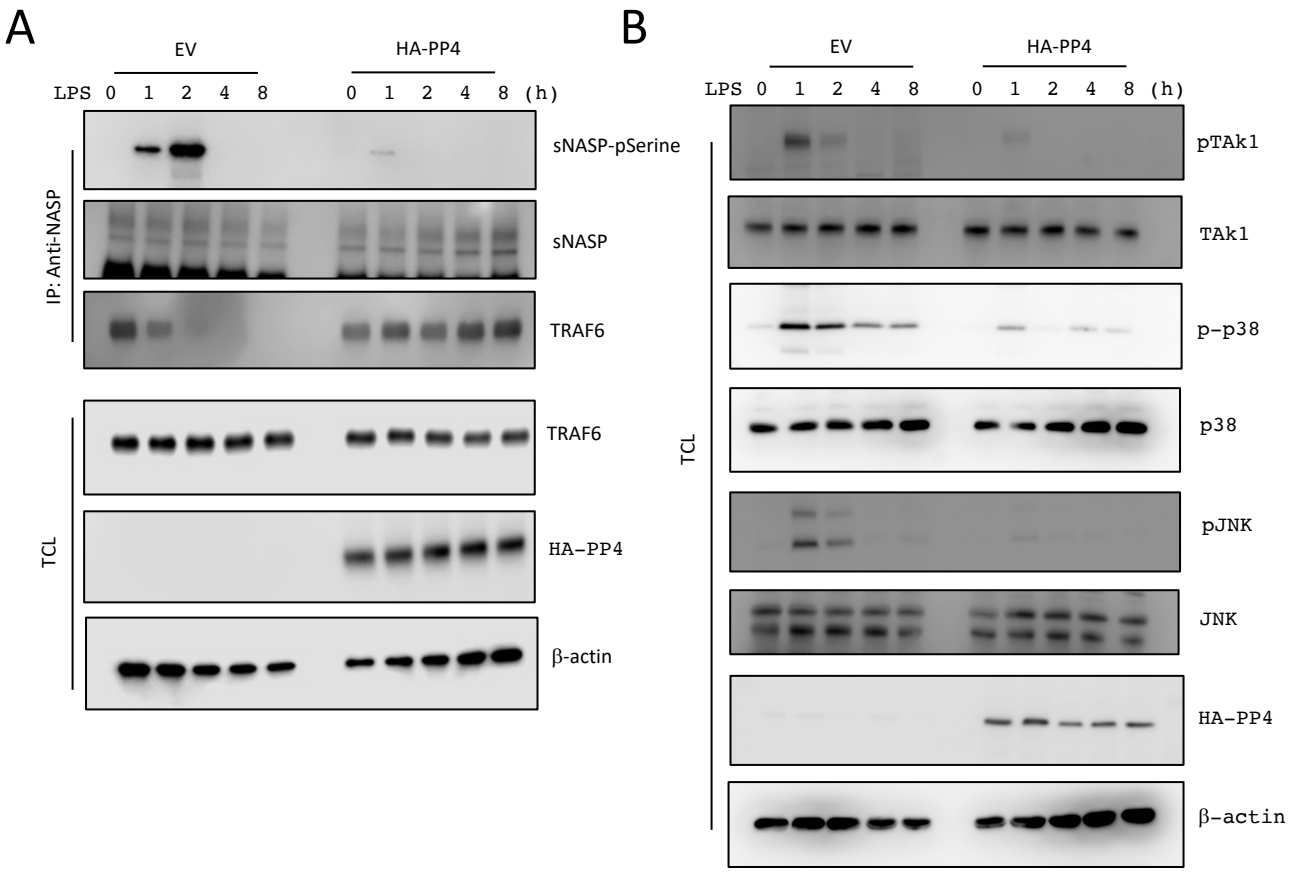

Fig. S1. Overexpression of PP4 de-phosphorylates sNASP and inhibits LPS-mediated TLR4 signaling. (*A*) THP-1 cells were transfected with empty vector (EV) or HA-PP4, followed by IB wit antibodies against pSerine, TRAF6, or sNASP after IP with anti-sNASP. TCL IB was done with anti-TRAF6, anti-HA, or anti-β-actin. (*B*) IB of indicated antibodies in LPS-stimulated THP-1 cells transduced with EV or HA-PP4. Data represent a minimum of three independent experiments.

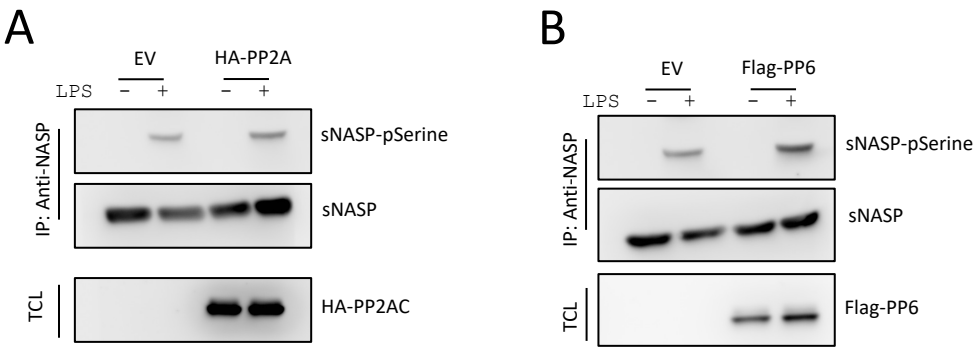

Fig. S2. PP42A and PP6 had no effect on the phosphorylation of sNASP. IP of sNASP (with anti-sNASP) from THP-1 cells was stimulated with LPS in the presence of empty vector (EV), (*A*) HA-tagged PP2A (HA-PP2A) or (*B*) Flag-tagged PP6 (Flag-PP6), and assessed by IB with the indicated antibodies.

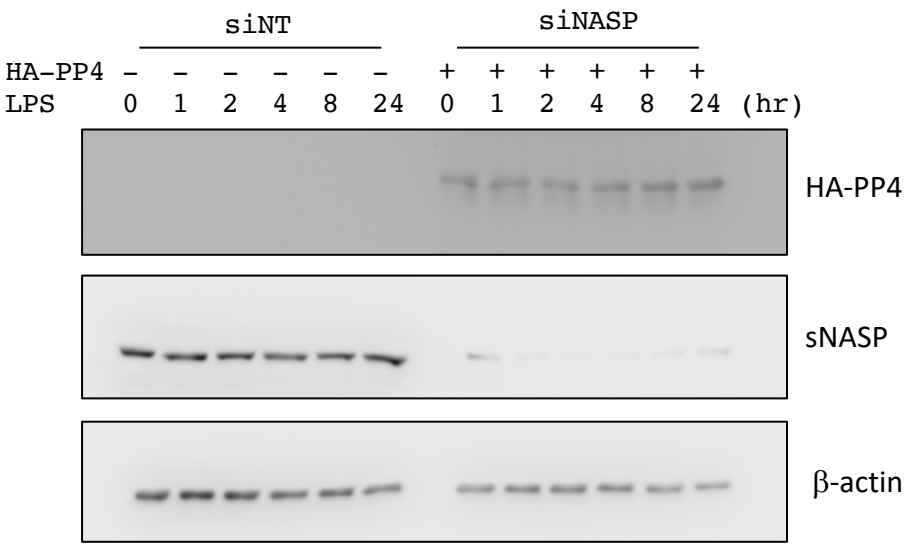

Fig. S3. Overexpression of PP4 and knockdown of sNASP in RAW264.7 cells. RAW264.7 cells were transfected with HA tagged-PP4 (HA-PP4) and siNASP, stimulated with LPS for different time points, and assessed by IB with antibodies against HA, sNASP and β-actin.

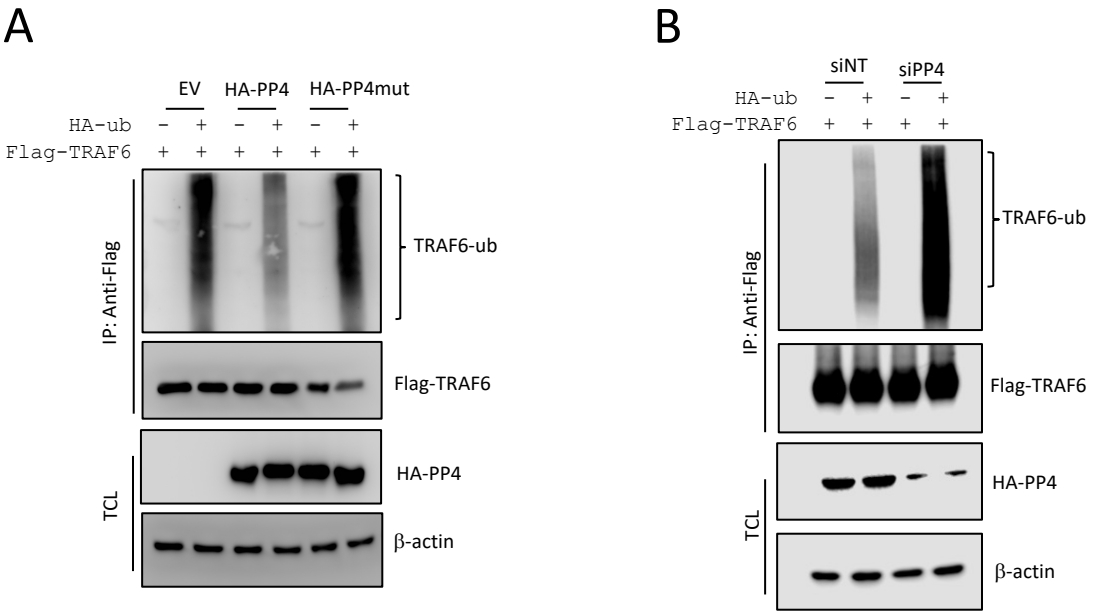

Fig. S4. PP4 negatively regulates TRAF6 ubiquitination. (*A*) Immunoprecipitation (IP) of Flag-TRAF6 (with anti-Flag agarose) from HEK293 cells transfected with empty vector (EV), HA-tagged PP4 WT (HA-PP4) or mutant (HA-PP4mut) in the presence (+) or absence (–) of Flag-tagged TRAF6 (Flag-TRAF6) or HA-tagged ubiquitin (HA-Ub), followed by IB with antibodies against Flag or Ub. TCL IB was done with anti-HA and β-actin. (*B*) IP of Flag-TRAF6 (with anti-Flag agarose) from HEK293 cells transfected with siRNA negative control (siNT) or siPP4 in the presence (+) or absence (–) of Flag-TRAF6 or HA-Ub, followed by IB with antibodies against Flag or Ub. TCL IB was done with anti-HA and β-actin.

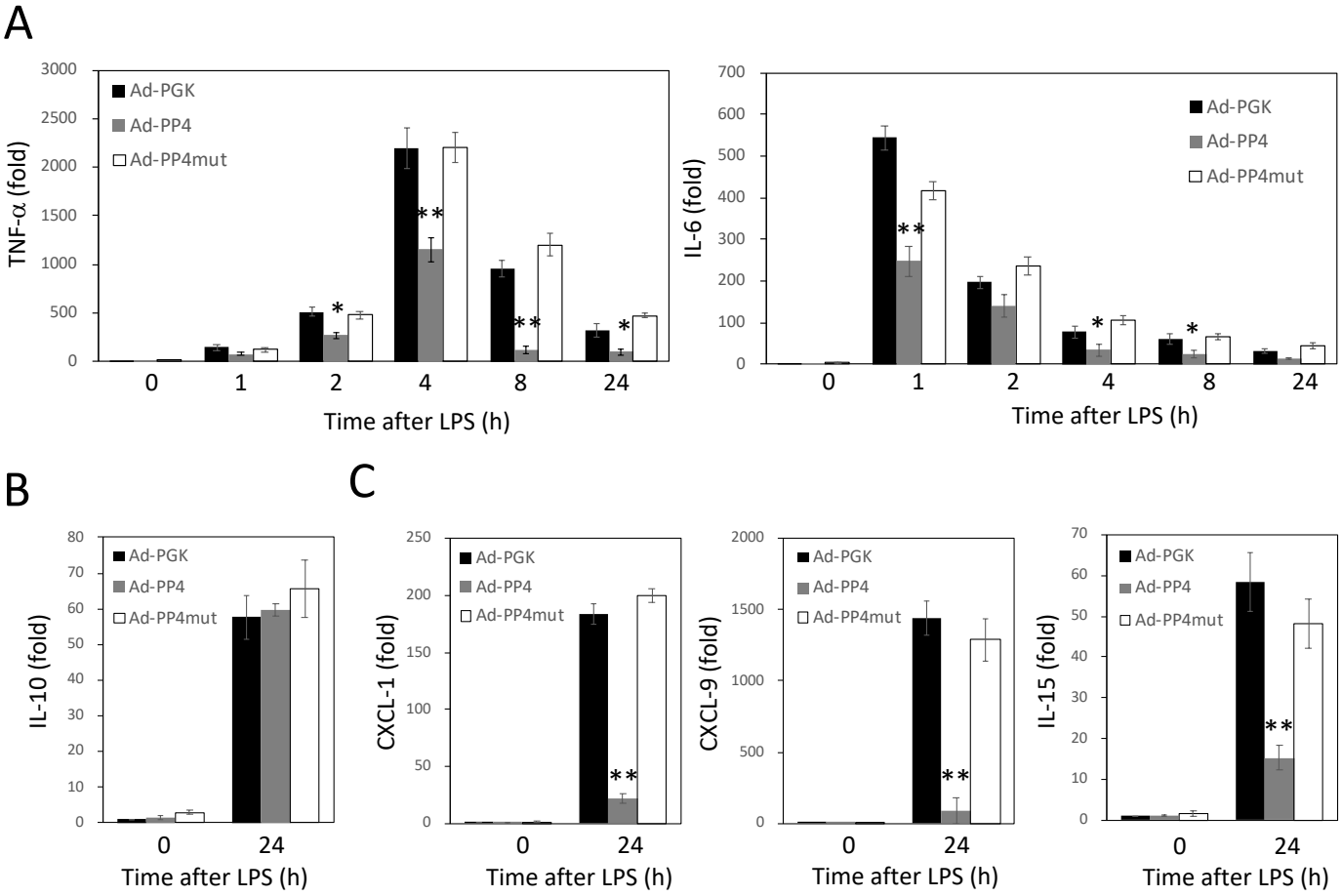

Fig. S5. Primary bone-marrow derived macrophage (BMDM) overexpressing PP4 decreased LPS-induced proinflammatory cytokines and chemokines production. (*A-C*) RNA expression level of TNF-α and IL-6 (*A*), IL-10 (*B*) and CXCL-1, CXCL-9 and IL-15 (*C*) in BMDM cells infected with Ad-PGK, Ad-PP4 or Ad-PP4mut, and stimulated with LPS for different time points. Results were normalized to the expression of ACTB (encoding β-actin) and are presented relative to those of untreated cells. Data are the mean ± SE for each group. **p*< 0.05, ***p*< 0.01 (by one-way ANOVA). Data represent a minimum of three independent experiments.

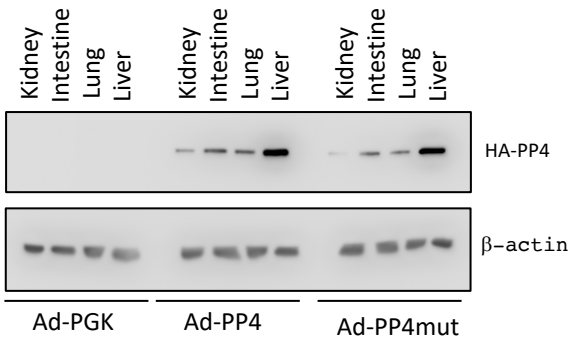

Fig. S6. Adenovirus-mediated overexpression of PP4 in mice. Mice infected with 2.5 x 10^9 pfu of adenoviruses expressing empty vector (Ad-PGK), gene encoding HA-PP4 WT (Ad-PP4) or mutant (Ad-PP4mut). After 4 days, samples of the indicated tissues were harvested and assessed by IB analysis with anti-HA and β-actin .

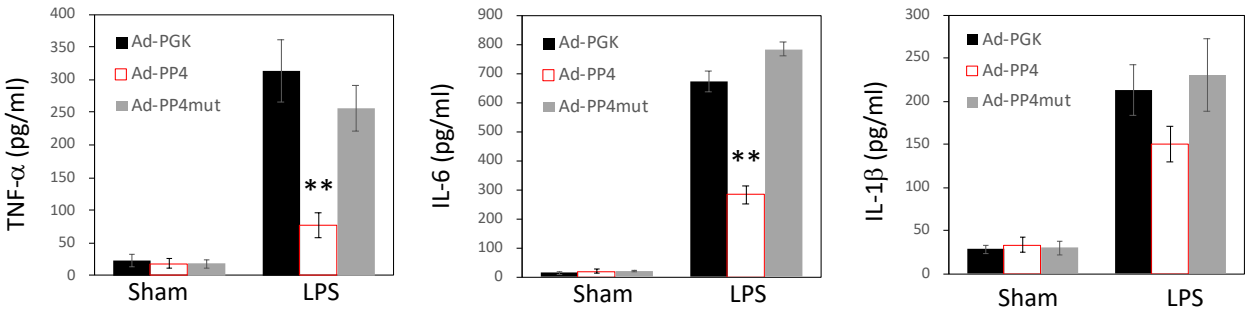

Fig. S7. PP4 suppressed LPS-induced proinflammatory cytokines production in vivo. Serum cytokines in Ad-PGK, Ad-PP4 or Ad-PP4mut infected mice were measured 24 hours after sham or LPS treatment (n=10 per group per experiment). Data are the mean ± SE for each group. *p< 0.05, **p< 0.01 (by one-way ANOVA).