

Fig 1s. Floxed and knockout alleles of *TPL2*. **(A)** Schematic representation of *TPL2* floxed (Flox) and *TPL2* knockout (T_{PL2}-KO) alleles. Exons are shown as solid purple bars and marked by “Ex” on top. Signals: Neo is a neomycin resistant gene; FLP is flp recombinase; and CRE is cre recombinase. The Flp-mediated excision enables the deletion of the Neo, resulting in a *TPL2* conditional knockout allele or *TPL2* floxed allele. This deletion has been performed *in vivo*, by breeding the recombinant animals with Flp-recombinase expressing deleter mice. The Cre-mediated excision enables the deletion of the exon 3 and 4, resulting in *TPL2* constitutive knockout allele. This deletion has been performed *in vivo*, by breeding the recombined animals with Cre-recombinase expressing delete mice. The position of the primers used for genotyping is shown with arrows. **(B)** Detection of *TPL2* floxed allele with primers F1/R1. The floxed allele produced a 523-bp band, and the wild-type (WT) allele produced a 421-bp band. **(C)** Detection of *TPL2*-KO allele with F2/R2 primers. The *TPL2*-KO allele produced a 3168-bp band. The wild-type (WT) allele is not detectable with this primer pair. (F1: GAGTAACAGCTAGGCAGACAAACAGGTTAGC; R1: AGCTTCCAAGGAACAAGGAGAACATCC; F2: AAACAGCAATCTGGATGTGAGACTAGATGG; R2: AGTTCCAAGCAGAGCAGAAGGAAACG)

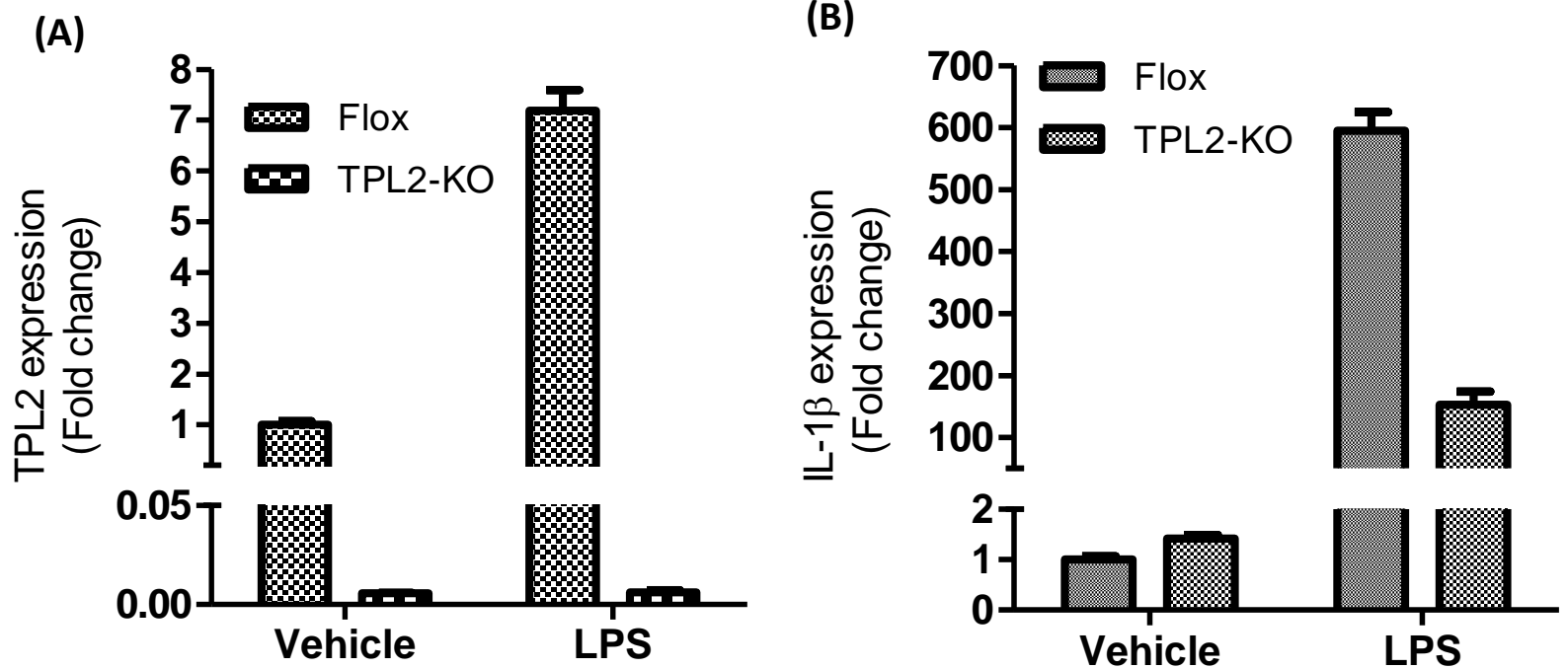


Fig 2s. TPL2 knockout mice (TPL2-KO) display significantly reduced gene synthesis of TPL2 (A) and IL-1β (B). Bone marrow-derived macrophages (BMMP) from TPL2 floxed or knockout mice were treated with LPS (100 ng/ml) for 4 hours. mRNA were extracted and RT-PCR performed.

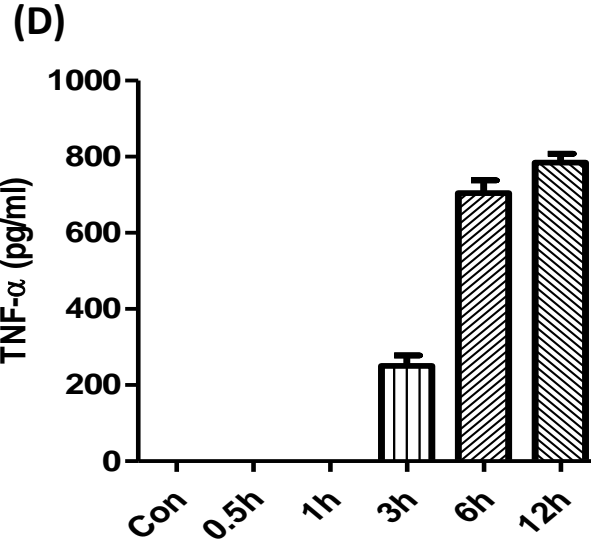
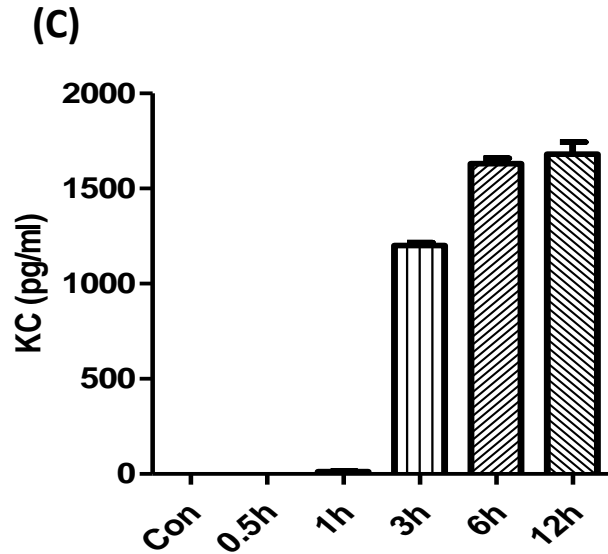
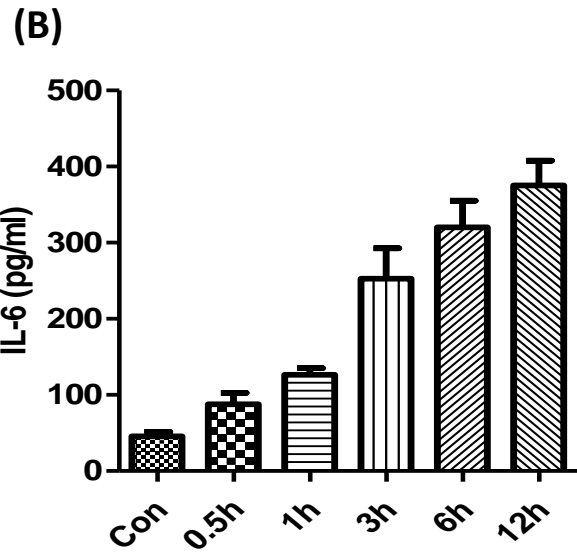
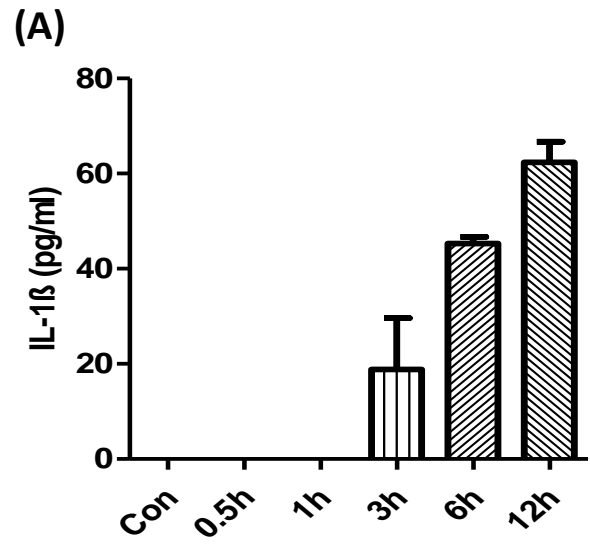


Fig 3s. TcdB induces production of proinflammatory cytokines by bone marrow-derived macrophages (BMMPs). BMMPs derived from wild-type mice were exposed to TcdB for the indicated time, and supernatants were collected for determination of IL-1 β (A), IL-6 (B), KC (C) and TNF- α (D) by ELISA. The results presented were representative of three independent experiments (n=3). Bars stand for means \pm SD.

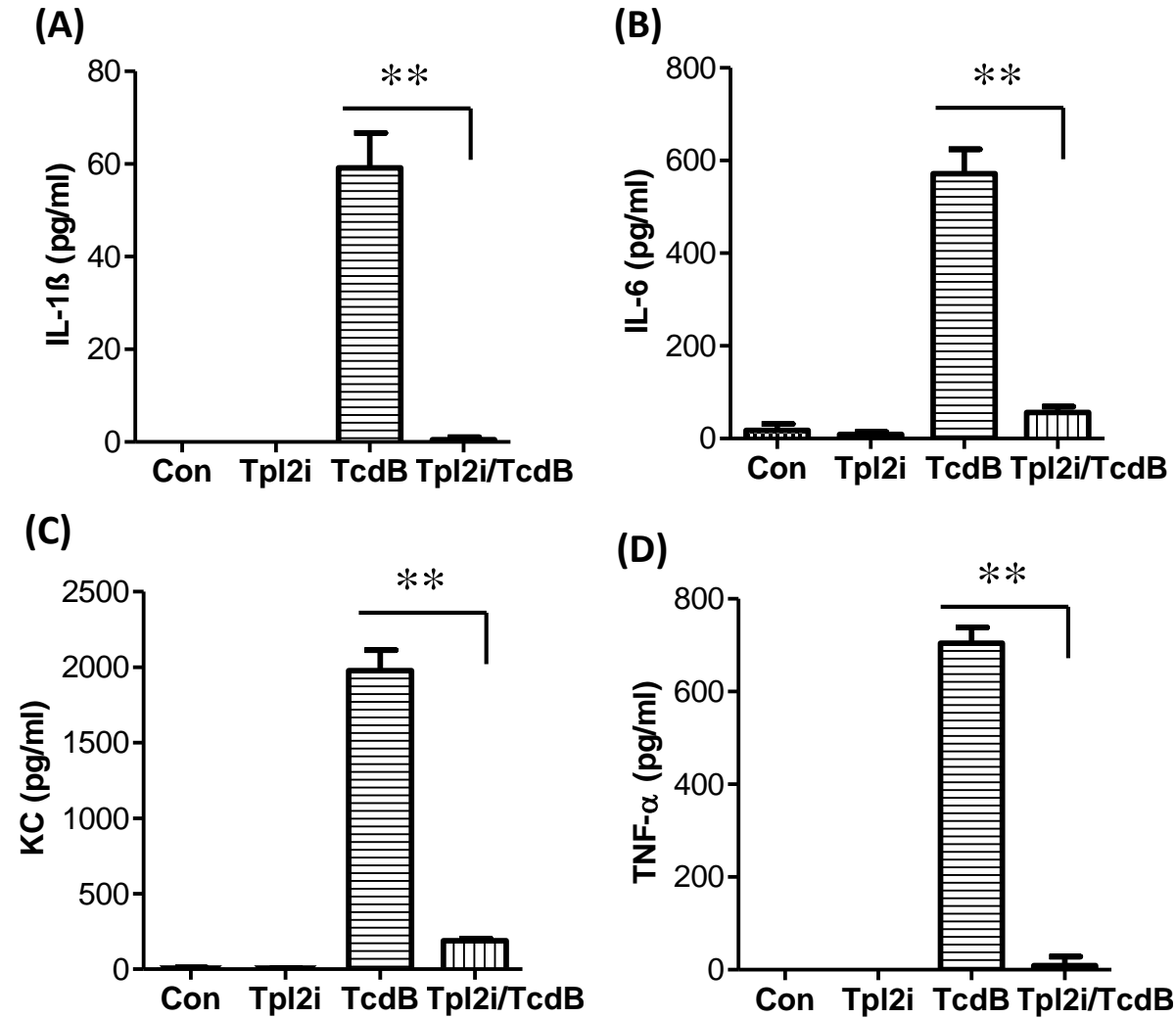


Fig 4s. TPL2 specific inhibitor significantly inhibits production of proinflammatory cytokines/ chemokines by BMMPs. BMMPs derived from wild-type mice were pretreated with TPL2 inhibitor (TPL2i) at 20 μ M for 30 minutes, followed by exposure to TcdB (200ng/ml) 37 $^{\circ}$ C for 3 hours. Controls include cells treated with TPL2i, TcdB, or PBS. Supernatants were collected, and IL-1 β (A), IL-6 (B), KC (C), and TNF- α (D). The results presented were representative of three independent experiments (n=3). Bars stand for means \pm SD. ** $p < 0.01$ between TcdB and TcdB plus TPL2i groups.

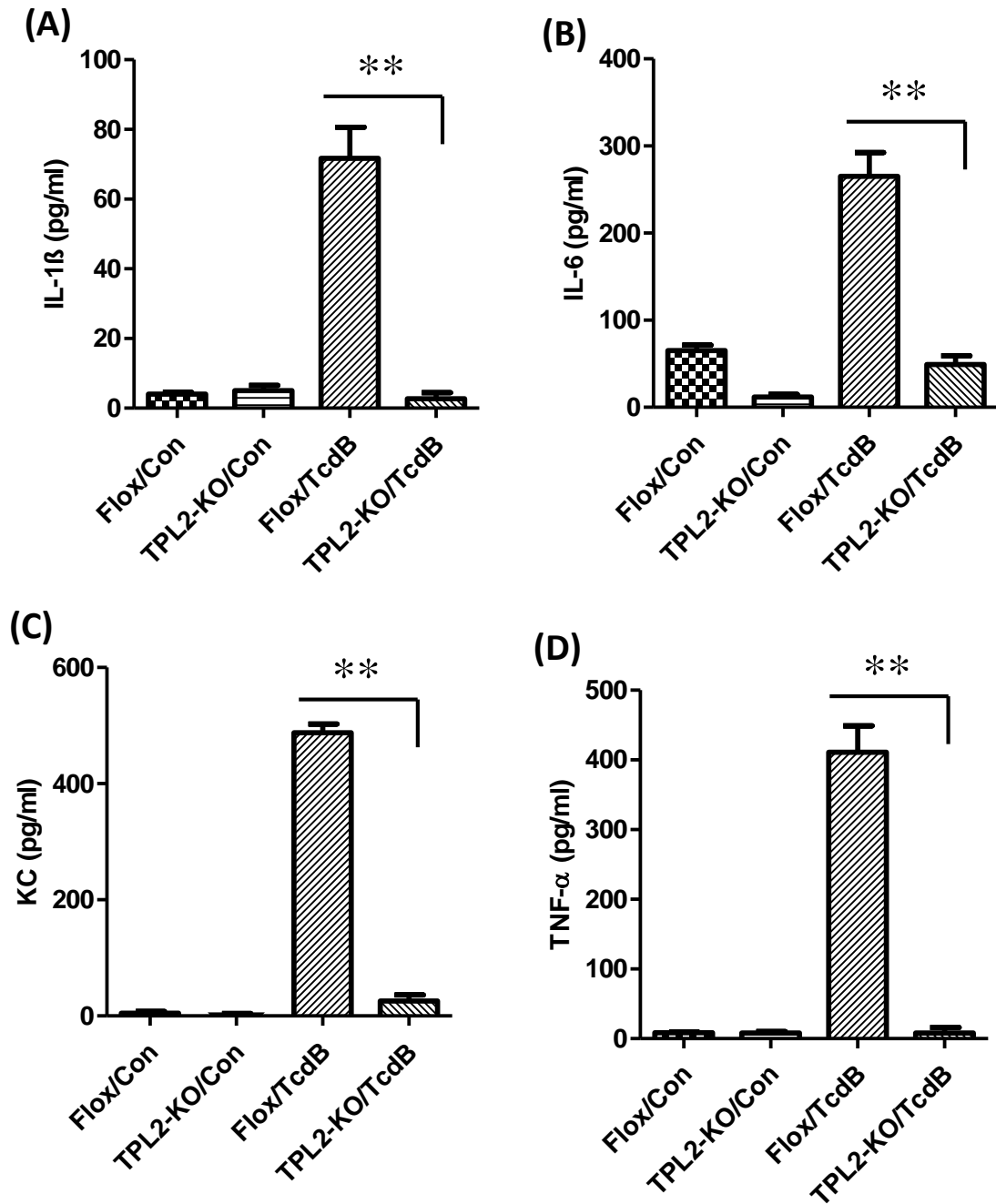
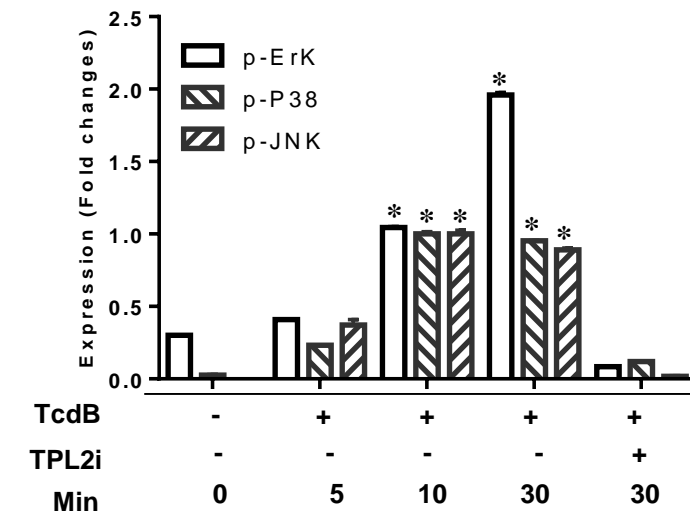
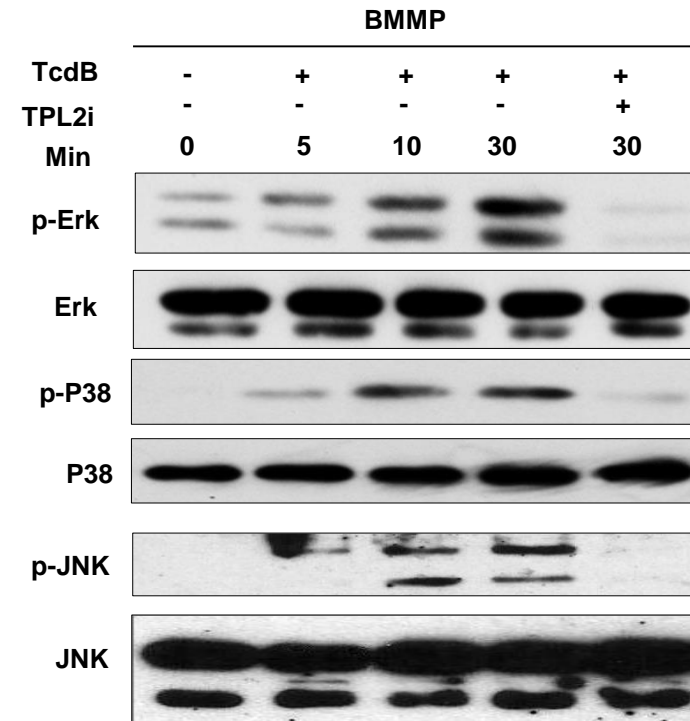


Fig 5s. TPL2 knockout reduces TcdB-induced production of proinflammatory cytokines/chemokines by BMMPs. BMMPs from TPL2 floxed (Flox) or knockout (TPL2-KO) in 24-well plates were exposed to TcdB (200ng/ml) for 6 hours, supernatants were collected and determined for production of IL-1 β , IL-6, KC and TNF- α by ELISAs. The results presented were representative of three independent experiments (n=3). Bars stand for means \pm SD. ** p < 0.01 between Flox/TcdB and TPL2-KO/TcdB groups. **Con:** Control.

Fig 6s. TPL2 inhibition reduces activation of MAPK in BMMPs BMMPs were exposed to TcdB (200 ng/ml) or TcdB (200 ng/ml) plus TPL2i (20 μ m) in 24-well plates at the indicated time. Cells were harvested, lysed and used for determination of activation of Erk, P38 and JNK by Western-blot analysis. [Experiments were repeated 3 times, and representative were shown.](#) [$*P < 0.01$ vs. Control]



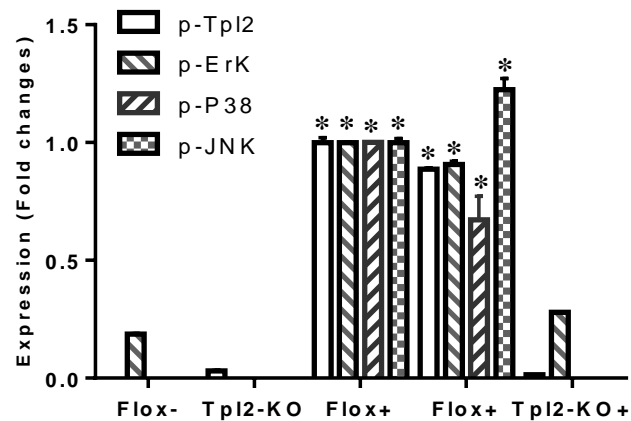
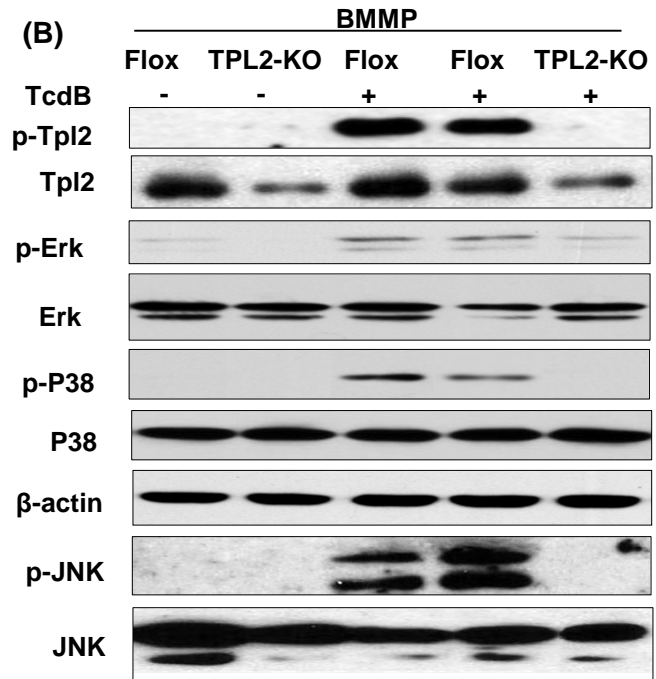


Fig 7s. TPL2 knockout reduced activation of MAPK in BMMPs. BMMPs from TPL2 floxed (Flox) or TPL2 knockout (TPL2-KO) mice were exposed to TcdB (200 ng/ml) in 24-well plates for 30 minutes. Cells or Cecum tissue (C) were harvested, lysed and used for determination of activation of TPL2, Erk, P38 and JNK by Western-blot analysis. Experiments were repeated 3 times, and representative were shown. [$P < 0.01$ vs. Control]

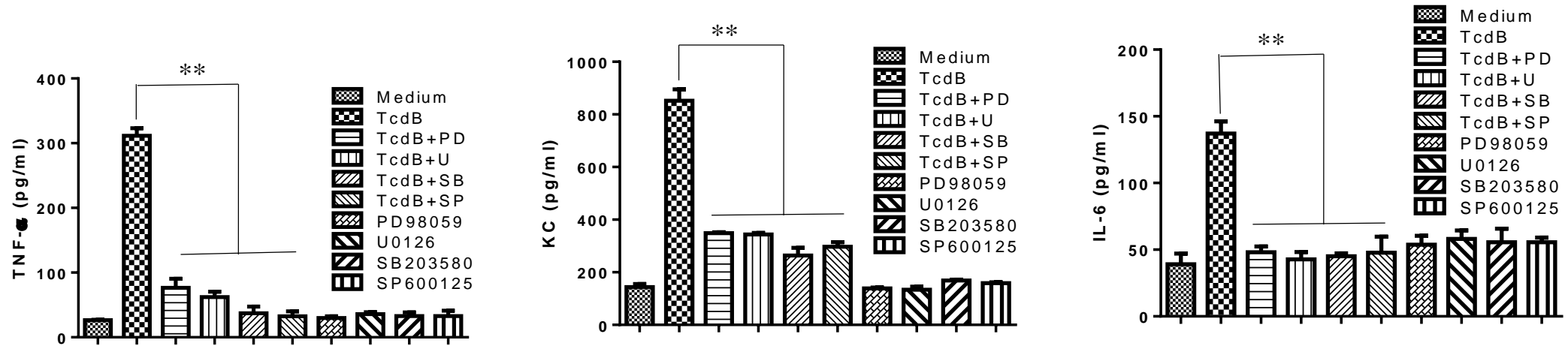


Fig 8s. MAPK specific inhibitors significantly inhibit production of proinflammatory cytokines/ chemokines by BMMPs. BMMPs derived from wild-type mice were pretreated with SB203580 at 10 μ M (inhibitor for p38), PD98059 at 20 μ M (inhibitor for Erk), U0126 at 20 μ M (inhibitor for MERK1/2), or SP600125 at 40 μ M (inhibitor for JNK) at 20 μ M for 30 minutes, followed by exposure to TcdB (200ng/ml) for 5 hours. Controls include cells treated with inhibitors onlt, TcdB, or PBS (medium). Supernatants were collected, and IL-6, KC, and TNF- α . The results presented were representative of three independent experiments (n=3). Bars stand for means \pm SD. ** $p < 0.01$ between TcdB and TcdB plus inhibitors.