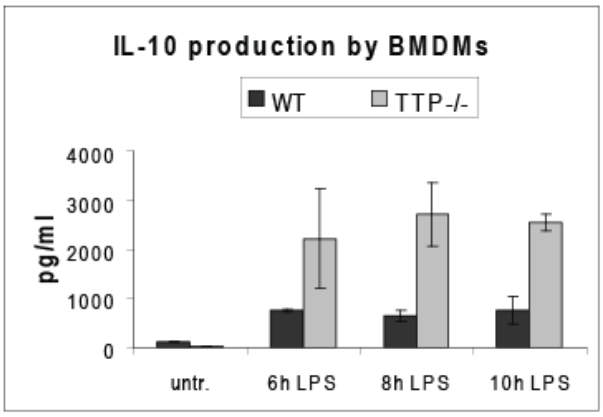
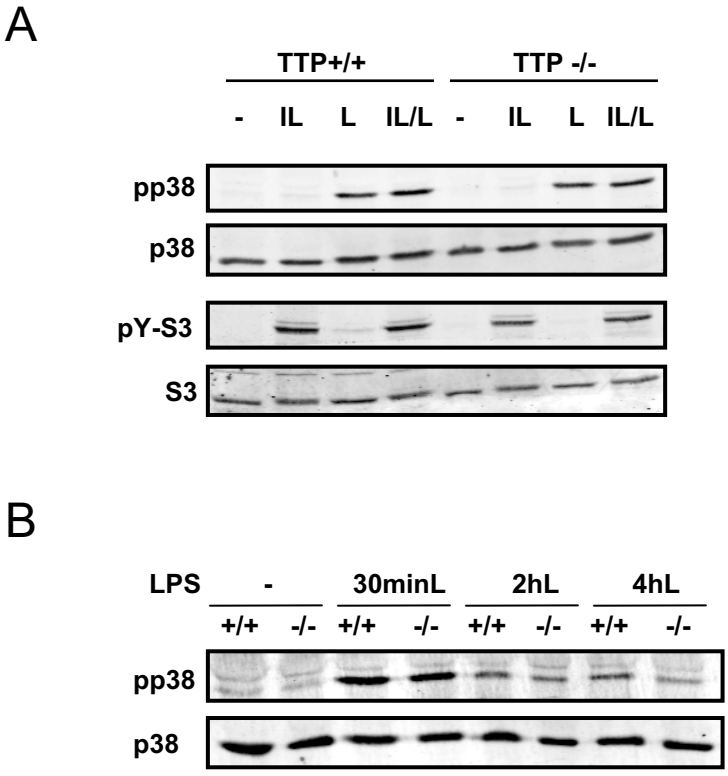
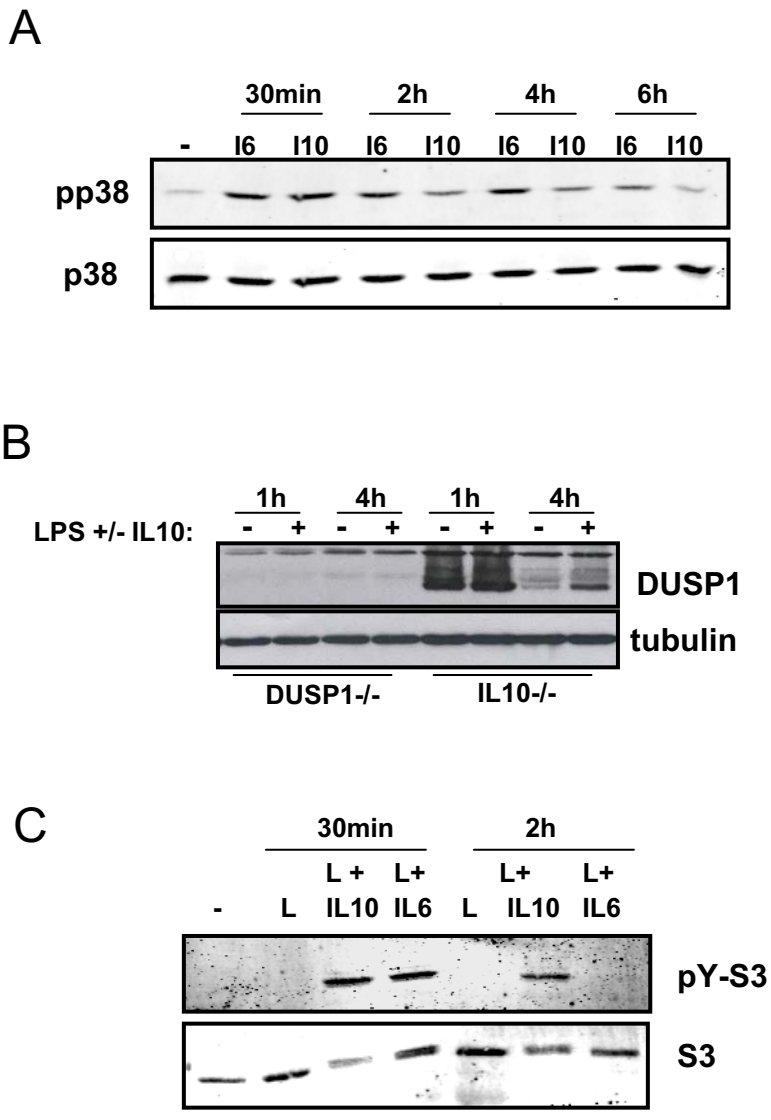


Supplemental Figure S1. TTP-deficient BMDMs produced more IL-10 cytokine after stimulation with LPS. WT and TTP-/- BMDMs were stimulated for 6, 8 or 10 h with LPS. Amounts of IL-10 in supernatants were determined by ELISA.

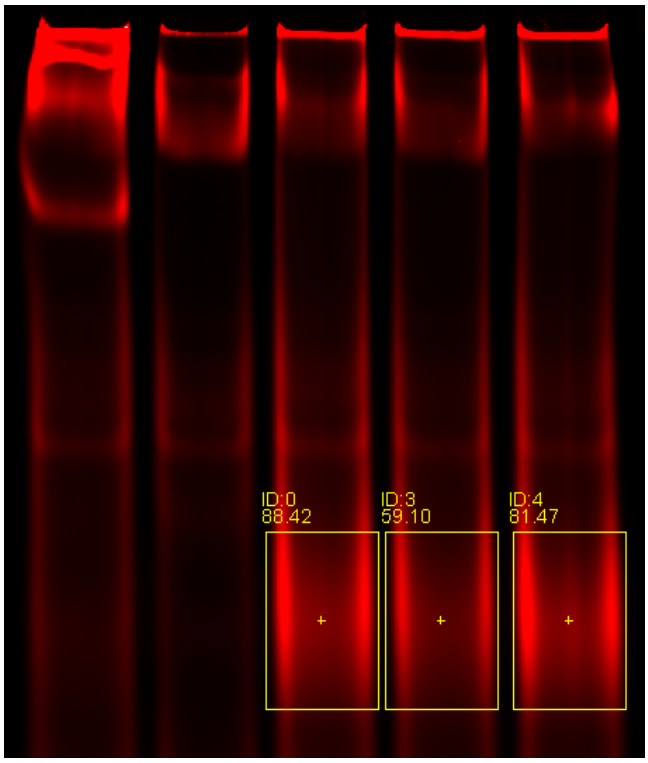


Supplemental Figure S2. p38MAPK and Stat3 activation by LPS or IL-10, respectively, are not affected by inactivation of the TTP gene. *A*, Activation of Stat3 and p38MAPK in LPS-treated $TTP^{+/+}$ and $TTP^{-/-}$ BMDMs. Cells treated for 30 min with IL-10 (IL), LPS (L), or IL-10 + IL-10 (IL/L) were examined for activation of p38MAPK by Western blotting using phospho-p38MAPK antibody (pp38). Membranes were reprobed with p38 antibody for loading control, and phospho-Stat3 antibody (pY-S3) to reveal activation of Stat3. *B*, Activation of p38MAPK in LPS-treated $TTP^{+/+}$ and $TTP^{-/-}$ BMDMs. Whole cell extracts of $TTP^{+/+}$ and $TTP^{-/-}$ BMDMs treated for 30 min, 2 h or 4 h with LPS (L) or left untreated (-) were analyzed for activation of p38MAPK by Western blotting using phospho-p38MAPK antibody (pp38). Membrane was reprobed with p38MAPK antibody (p38) for loading control.

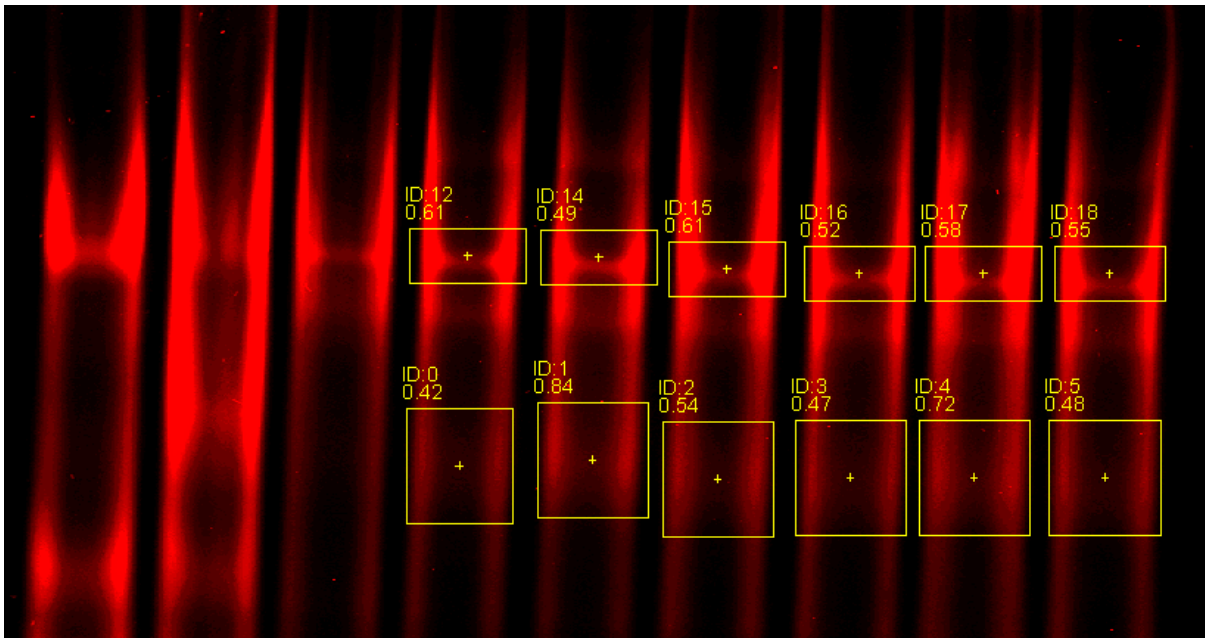


Supplemental Figure S3. IL-10 causes a decrease in p38MAPK phosphorylation, increase in DUSP1 expression, and a prolonged activation of Stat3. *A*, Whole cell extracts of BMDMs treated for 30 min, 2 h, 4 h and 6 h with LPS+IL-6 or LPS+IL-10 were analyzed for activation of p38MAPK by Western blotting using antibody to activated p38MAPK (pp38). Antibody to total p38MAPK (p38) was used for loading control. *B*, Whole cell extracts of DUSP1$^{-/-}$ and IL-10$^{-/-}$ BMDMs treated for 1 h and 4 h with LPS alone or LPS+IL-10 were analyzed for DUSP1 expression by Western blotting. Antibody to tubulin was used for loading control. IL-10$^{-/-}$ cells were used instead of WT BMDMs in order to increase the sensitivity to treatment with exogenous IL-10. C, BMDMs were treated for 30 min and 2 h with LPS alone or in combination with either IL-10 or IL-6. Stat3 activation was determined by Western blotting of whole cell extracts with antibody directed to tyrosine-phosphorylated Stat3 (pY-S3). Antibody to total Stat3 (S3) was used for loading control.

A



B



Supplemental Figure S4. Quantitative analysis of TTP-ARE complexes. The fluorescence signal of the Cy5.5-labeled TNFa ARE within the equally-sized rectangles positioned at the TTP-ARE complexes was measured and quantified (median background setting) using the LI-COR Odyssey system. The signal intensities are depicted. *A*, EMSA from the Fig. 5*B*. Equal amounts of the same extracts used for this EMSA were also used for the TTP Western blot in Fig. 5*A*, thus allowing for TTP loading control. *B*, EMSA from the Fig. 5*E*. The signal of TTP-ARE complexes (lower rectangles) were first normalized to the unspecific signals (upper rectangles) in the corresponding lanes before the relative intensities were calculated for the Fig. 5*E*.