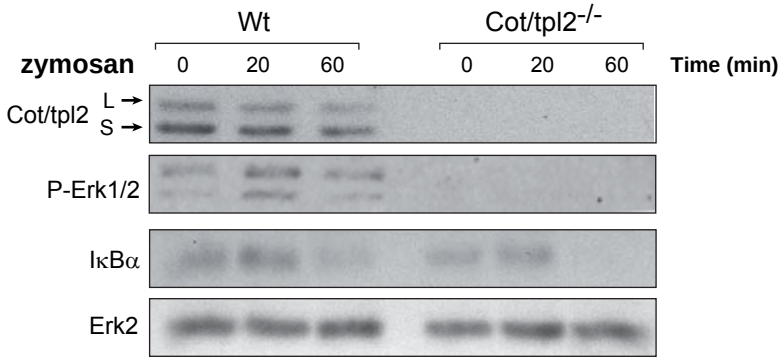
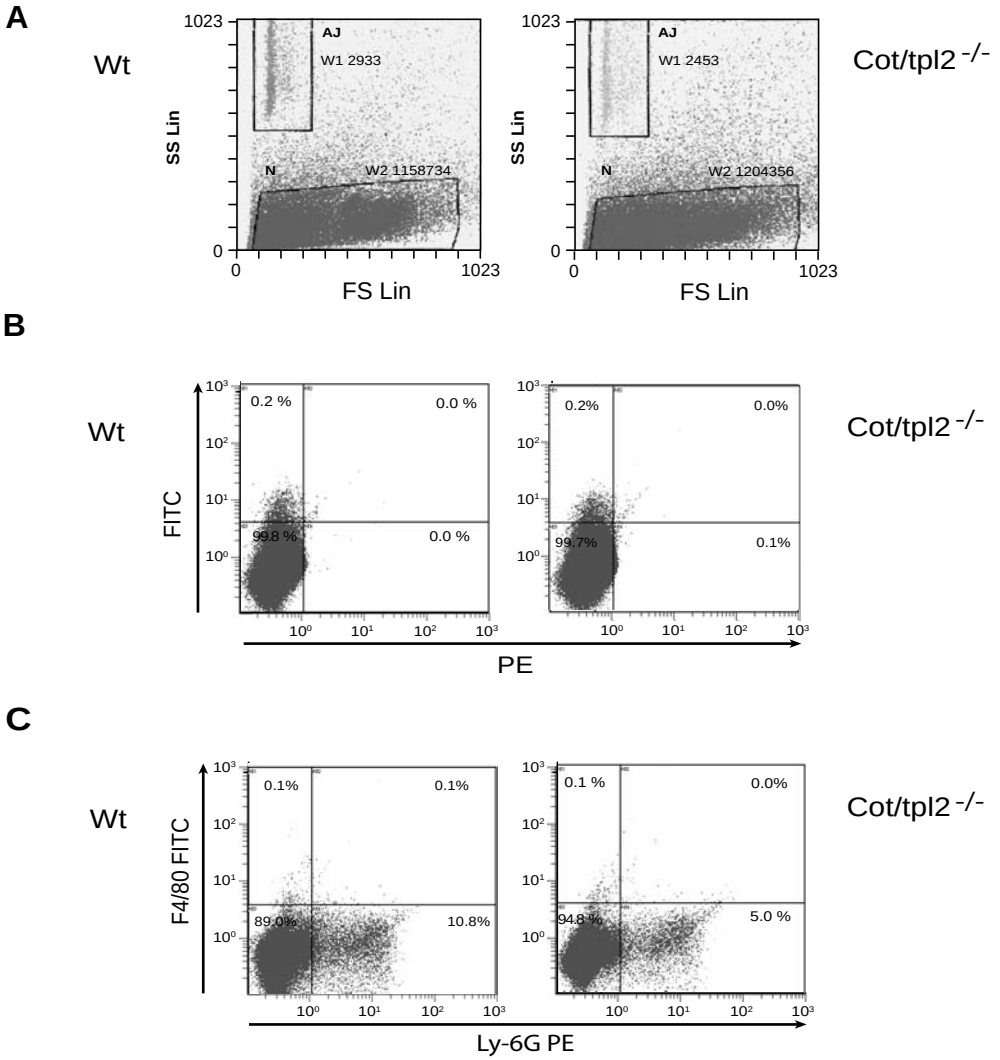
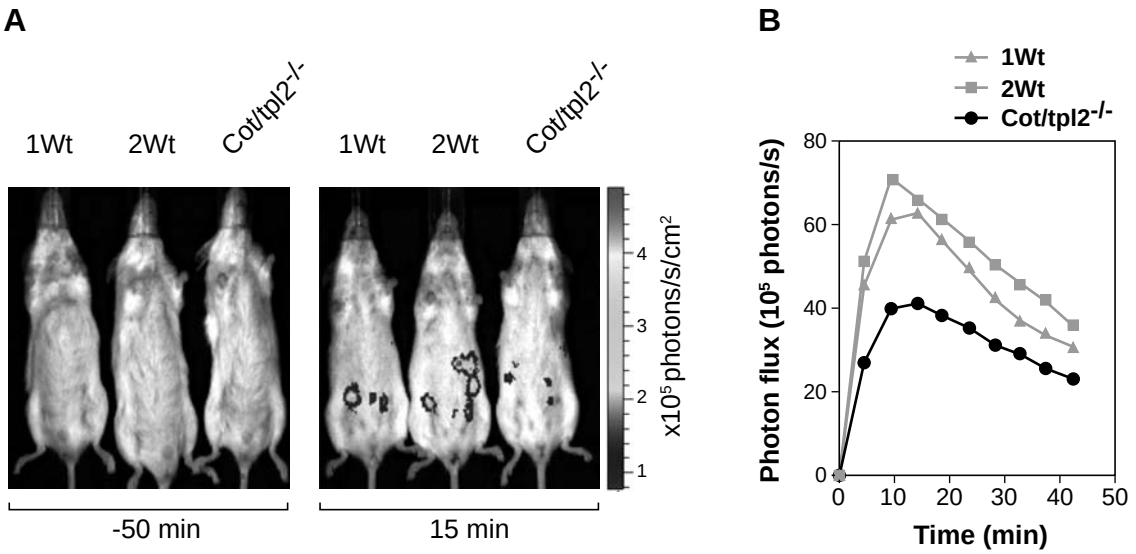


Supplementary Fig. 1. Impaired Erk1/2 phosphorylation in Cot/tpl2 deficient bone marrow derived macrophages by zymosan. Shown here is the incapacity of zymosan to provoke p-Erk1/2 phosphorylation in Cot/tpl2$^{-/-}$ deficient bone marrow derived macrophages (BMDM). Activation of IKKβ is required to activate Cot/tpl2 (Waterfield,et al. 2003, *Mol Cell* 11:685-694), and as shown here in both Wt and Cot/tpl2$^{-/-}$ BMDM IκBα is degraded after zymosan-stimulation, indicating an activation of IKKβ complex. Wt and Cot/tpl2$^{-/-}$ BMDM were stimulated with zymosan (10 µg/ml) for 20 and 60 min. The phosphorylation state of Erk1/2, as well as Cot/tpl2 and IκBα levels were determined by Western Blot analysis. As a control of protein loaded, total Erk2 levels were tested. The antibodies used were anti-Cot/tpl2 and anti-Erk2 (Santa Cruz,Biotechnology) and p-Erk1/2 and IκBα (Cell Signalling). Similar results were obtained in three different experiments.

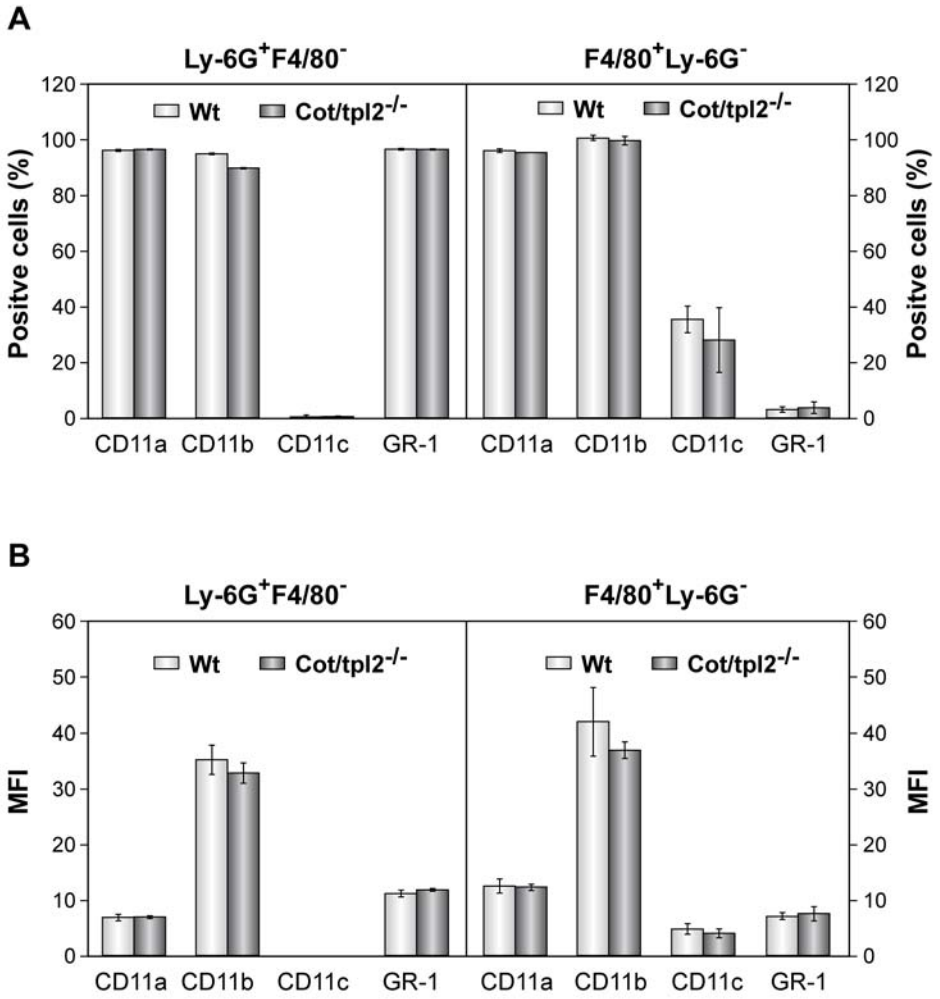


<u>Supplementary Fig. 2</u>. Cot/tpl2 regulates the number of neutrophils (Ly-6G$^+$F4/80$^-$) in zymosan-injected hindpaws. Six hours after the hindpaw intraplantar injection of zymosan (30 μg, 300 μl) in Wt and Cot/tpl2$^{-/-}$ mice intraplantar tissues were digested with collagenase and hyalorunidasase. Isolated cells were resuspended in PBS 0.1% BSA, pretreated with CD16/32 (2.4G2, Fc block, Cultek) and subsequently stained with F4/80 (rat anti-mouse, eBioscience) and Ly-6G (rat anti-mouse, Pharmingen) or their corresponding isotype controls (Pharmingen). The cell samples were resuspended in PBS and analysed by flow cytometry using TruCOUNT tubes (BD), data were examined using the CXP program. *A.* Representative side angle light scatter (SS) signal versus forward angle light scatter (FS) profile of the intraplantar isolated cells. Shown are the AJ gate (microbeads) and W1 events as well as N gate (digested intraplantar tissue) and W2 events. *B.* Representative FACS profile of the isolated intraplantar cells (AJ gate) stained with the isotype controls. *C.* Representative FACS profile of the isolated intraplantar cells (AJ gate) stained for F4/80$^+$ and Ly-6G$^+$. *A-C*. Wt cells, left panel and Cot/tpl2$^{-/-}$ cells, right panel. *A,C.* One representative experiment of the 4 performed is shown.



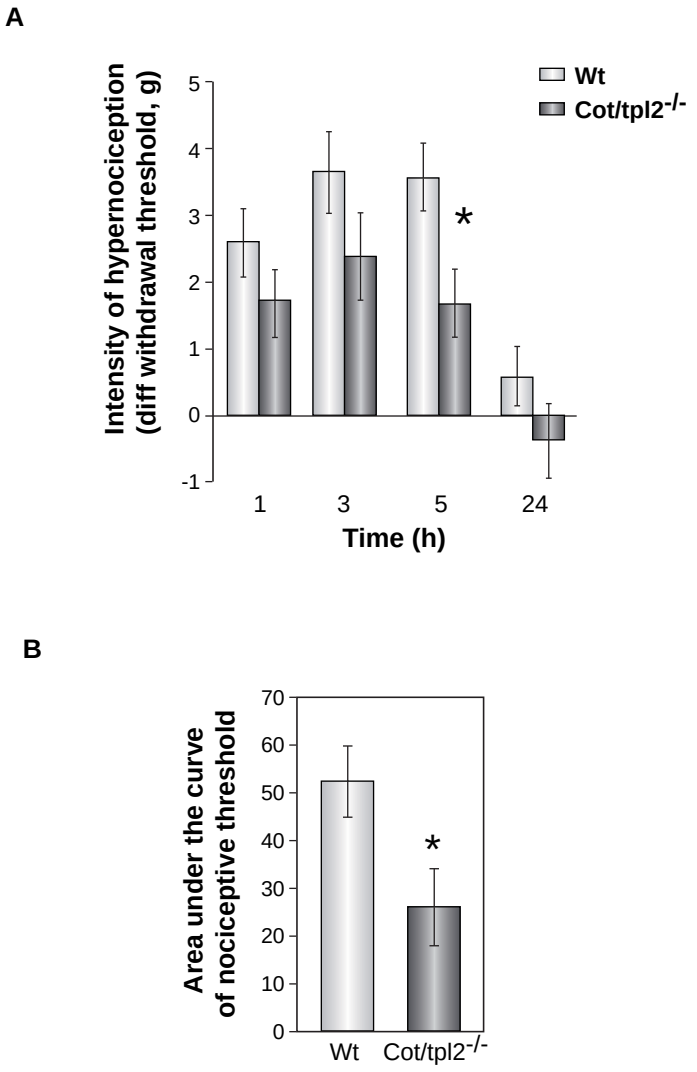
<u>Supplementary Fig. 3.</u> Luminol-mediated bioluminescence in Wt and Cot/tpl2-/- mice 4 h after intraperitoneal injection of zymosan. A. Shown are bioluminescence images of one representative experiment performed with 2 Wt and 1 Cot/tpl2$^{-/-}$ mice 4 h after zymosan-induced peritonitis. The images have been taken before and 15 min after intraperitoneal luminal injection, as previously described (Gross, et al, 2009 Nat Med **15** 455-461). *B*. Representation of the photon influx recorded in *A*. *A,B* One representative experiment of the 4 performed is shown.

Supplementary Fig. 4



Supplementary Fig. 4. Cot/tpl2 does not modulate CD11a, CD11b, CD11c and GR-1 expression on zymosan-activated Ly-6G$^+$F4/80$^-$ and F4/80$^+$Ly6G$^-$ peritoneal cells. *A.*Histograms represent the percentage of Ly-6G$^+$F4/80$^-$ or F4/80$^+$Ly6G$^-$ cells isolated fromthe peritoneum of Wt and Cot/tpl2$^{-/-}$ mice positive for CD11a, CD11b, Cd11c and Gr-1 staining 4 h following 1 mg of zymosan injection. Both Ly-6G$^+$F4/80$^-$ or F4/80$^+$Ly6G$^-$ cells from Wt and Cot/tpl2$^{-/-}$ mice were negative for CD31, CD62L, and CD49 staining. *B.* Quantitative analysis of fluorescence staining for CD11a, CD11b, CD11c, and GR-1 of the double positive Ly-6G$^+$F4/80$^-$ or F4/80$^+$Ly6G$^-$ cells indicated in *A*. *A,B.* Values represent the means fluorescence intensity (MFI) means ± SEM of 6 independent experiments.

Supplemenatry Fig. 5



<u>Supplementary Fig. 5.</u> Cot/tpl2 modulates LPS induced hypernociception. The sensitivity to mechanical stimulus following interplantar application of LPS (8 ng, 25 μl, Sigma L7261) showed an increase following the injection. The baseline withdrawal threshold was 7.1 +/- 0.3 g (n=12; mean +/- SEM). *A*. The Cot/tpl2$^{-/-}$ mice had less pronounced changes in the withdrawal thresholds after the LPS injection in comparison to Wt, this difference being the most pronounced at 5 h (p=0.022, n=6, 2-way ANOVA, Tukey post-hoc). The area under the curve is also shown (lower panel). *B.* The changes in nociceptive threshold and withdrawal latency were calculated for each mouse as the area under the curve versus time (over a 24 h period) and the results are presented as the means +/- SEM (p=0.040, Students T-test).