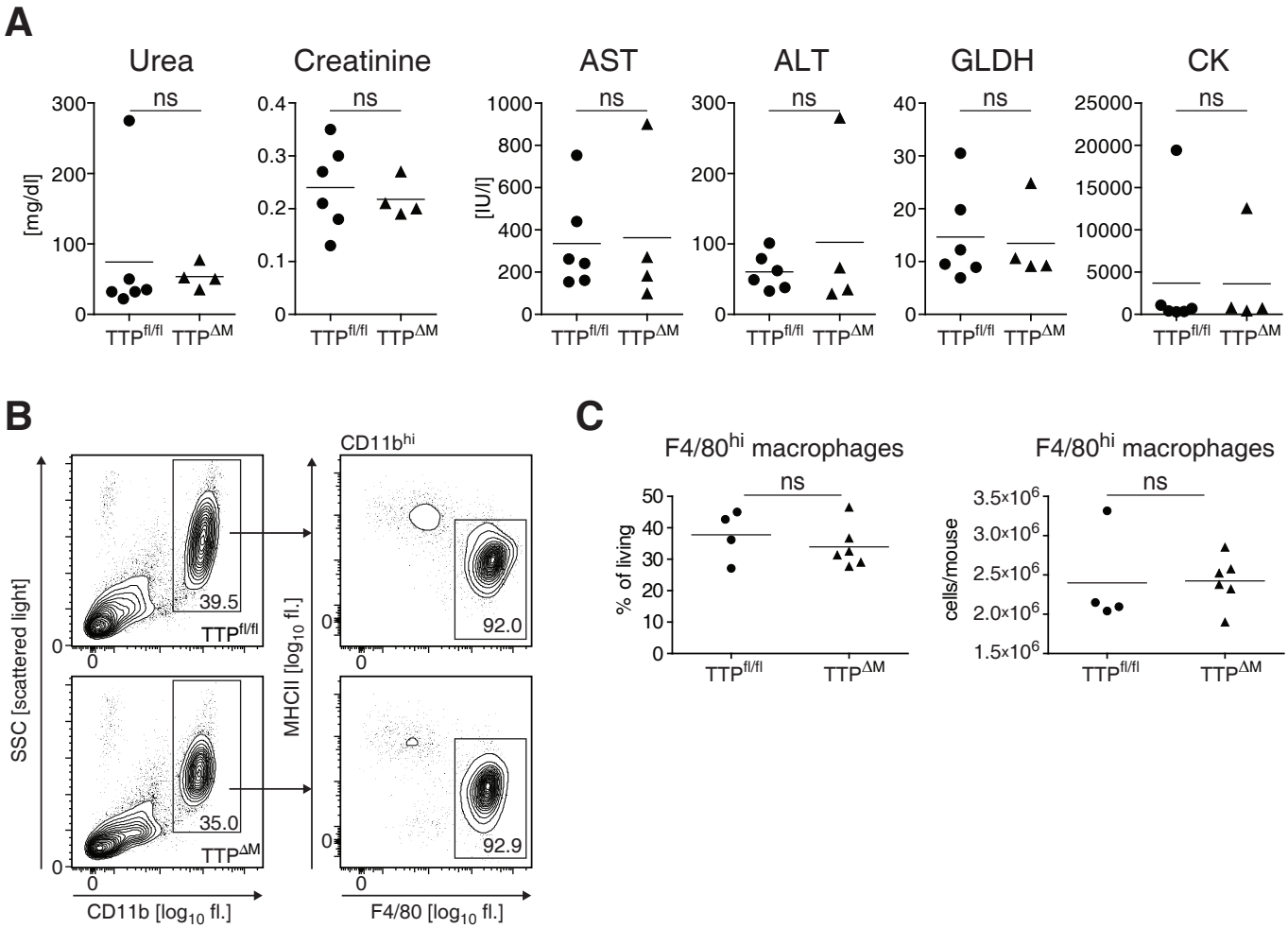
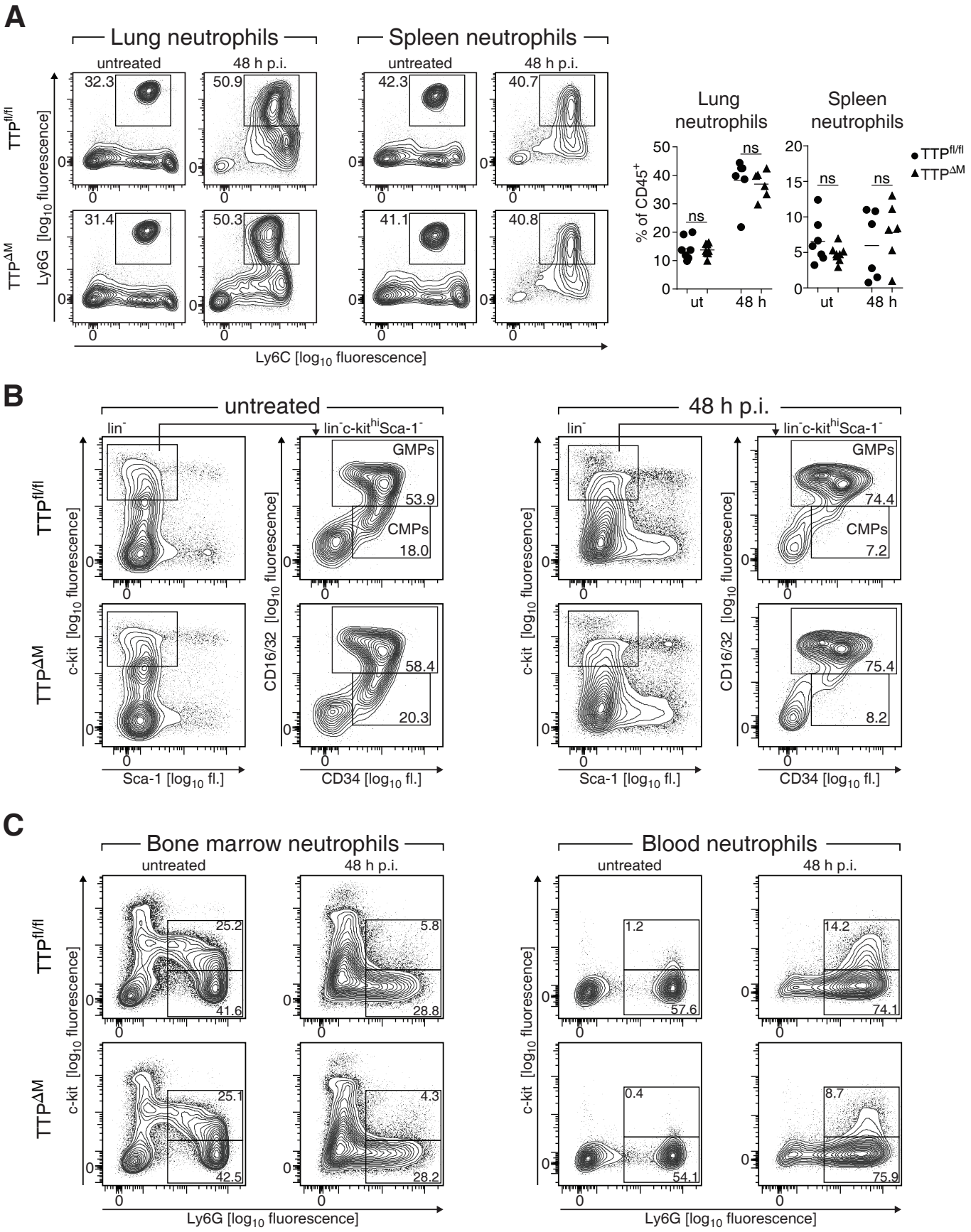
Supplemental Figure 1



Supplemental Figure 1 (related to Figure 2). No effect of TTP on organ injury indicators during infection and on tissue-resident macrophage numbers under homeostatic conditions

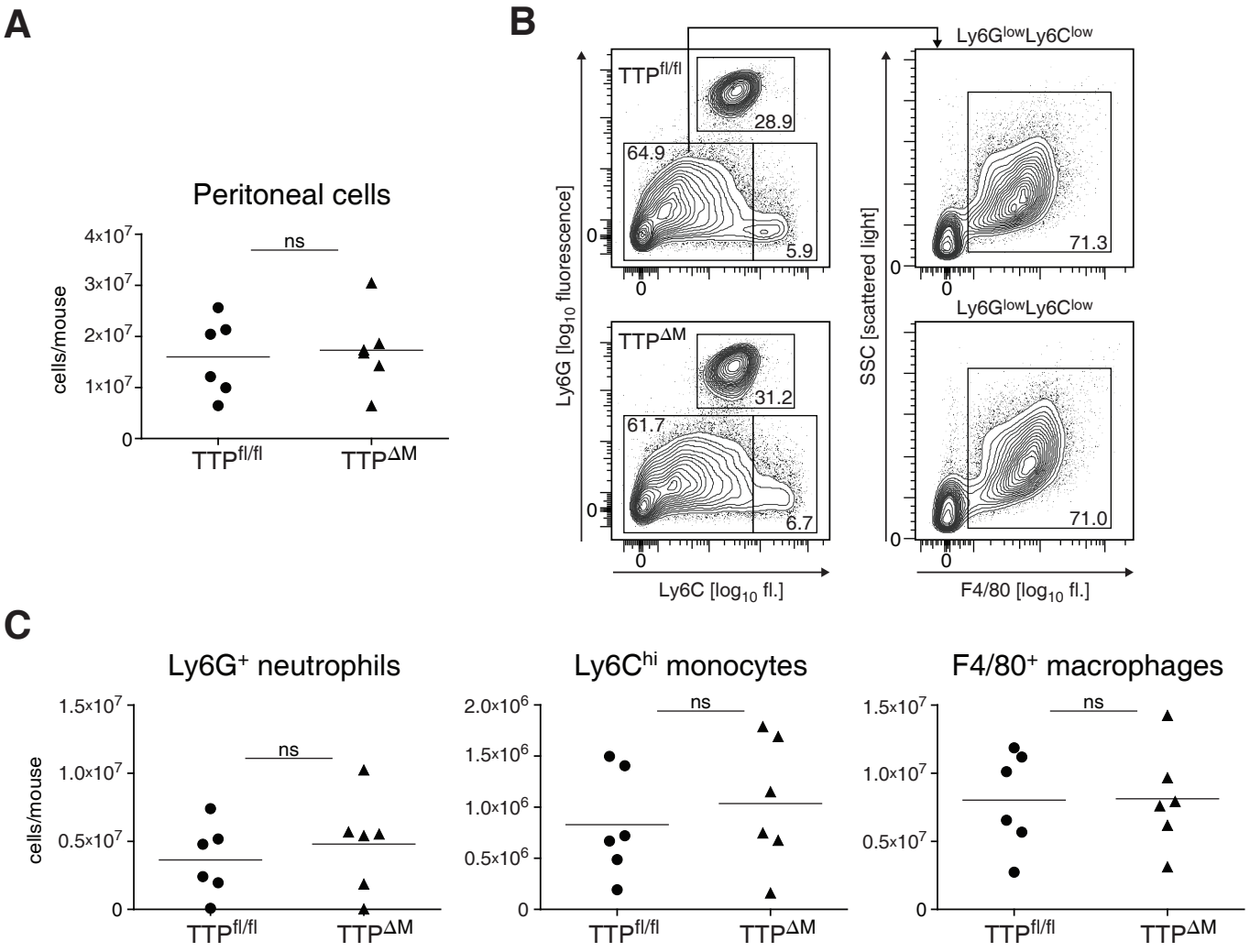
(**A**) Blood serum levels of urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), glutamate dehydrogenase (GLDH) and creatine kinase (CK) of infected mice (5 $TTP^{fl/fl}$ and 4 $TTP^{\Delta M}$) 48 h p.i. (**B**) Representative flow plots of $CD11b^{hi}MHCII^{-}F4/80^{hi}$ peritoneal macrophages from untreated $TTP^{fl/fl}$ and $TTP^{\Delta M}$ mice showing the frequency of $CD11b^{hi}$ (left) and $MHCII^{-}F4/80^{hi}$ cells within the $CD11b^{hi}$ population (right). (**C**) Dot plots of peritoneal macrophages (4 $TTP^{fl/fl}$ and 6 $TTP^{\Delta M}$ mice) analyzed as in (A) shown in percent of all living cells (left) and in absolute numbers (right) per mouse. Horizontal bars: mean. Statistical analysis: unpaired Student's *t* test. ns, not significant.

Supplemental Figure 2



Supplemental Figure 2 (related to Figure 2). Flow cytometry analysis of marginalized neutrophils and progenitor cells in untreated and infected animals.
Animals were left untreated or infected with 3 x 10^8 CFU of S. pyogenes and analyzed by flow cytometry 48 h p.i. (**A**) Representative flow plots (left panels) show marginalized neutrophils of the lung (left) and spleen (right) of untreated and infected animals 48 h p.i. Dot plots (right panels) show percentages of neutrophils of all $CD45^+$ cells of untreated (7 $TTP^{fl/fl}$ and 8 $TTP^{\Delta M}$) and infected (6 $TTP^{fl/fl}$ and 6 $TTP^{\Delta M}$) animals. (**B**) Representative flow plots of bone marrow cells from untreated (left panel) and infected (right panel) animals. Cells were subgated for being negative for lineage markers B220, CD3e, CD11b, Gr-1 and Ter-119, (i.e. lin^-, left plots). Common myeloid progenitors (CMPs) were defined as $lin^-Sca\text{-}1^-c\text{-}kit^{hi}CD16/32^{low}CD34^+$ and granulocyte-monocyte progenitors (GMPs) as $lin^-Sca\text{-}1^-c\text{-}kit^{hi}CD16/32^{hi}CD34^+$. (**C**) Immature and mature neutrophils of the bone marrow (left panels) and the blood (right panels) of untreated or infected animals (48 h p.i.) were analyzed based on the expression of c-kit and Ly6G. Immature neutrophils were defined as $Ly6G^+c\text{-}kit^+$ and mature neutrophils as $Ly6G^+c\text{-}kit^-$. Numbers within the outlined areas in all flow plots show percentages of the parent population. Horizontal bars in (A): the mean. Statistical analysis: unpaired Student's *t* test; ns, not significant.

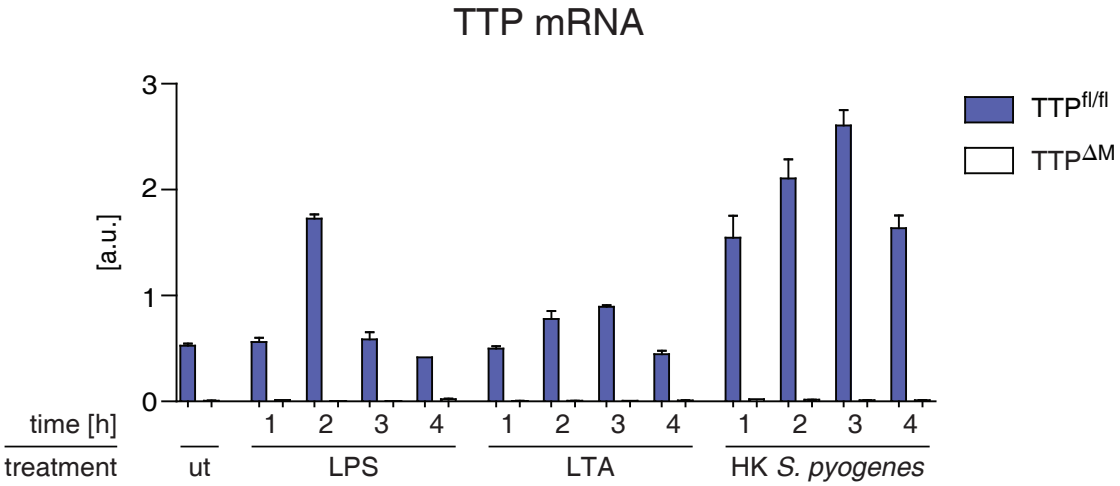
Supplemental Figure 3



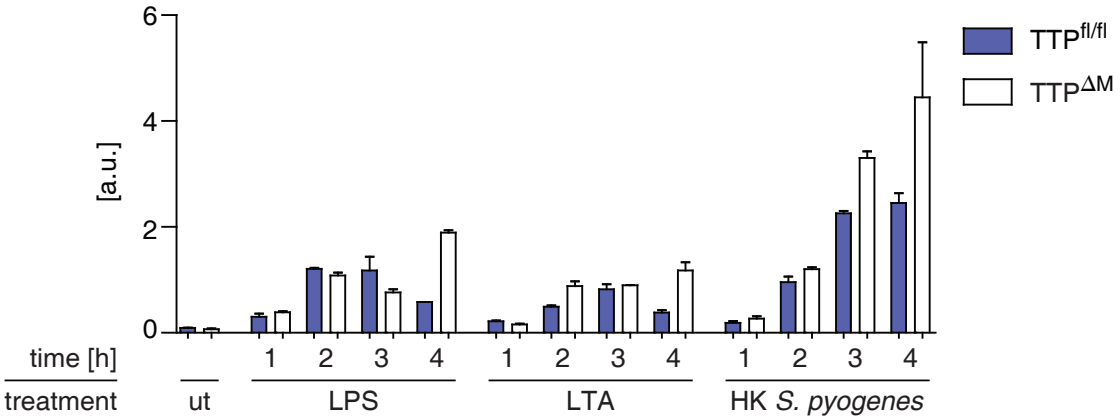
Supplemental Figure 3 (related to Figure 3). Macrophage-driven peritonitis is not regulated by TTP
Analysis of myeloid cells 48 h after inducing peritonitis by injection of HK 2 x 10^8 CFU *S. pyogenes*. (**A**) Dot plot shows total cell counts. (**B**) Representative flow plots demonstrating the $Ly6G^+Ly6C^+$ neutrophil population, the $Ly6G^-Ly6C^{hi}$ inflammatory monocyte population (left panels) and the subgated $F4/80^+$ peritoneal macrophage population. Numbers indicate percentages of parent population in the respective gate. (**C**) Cell counts of the respective populations calculated from percentages of (B) and total cell numbers of (A). Horizontal bars: mean. Statistical analysis: unpaired Student's *t* test. ns, not significant.

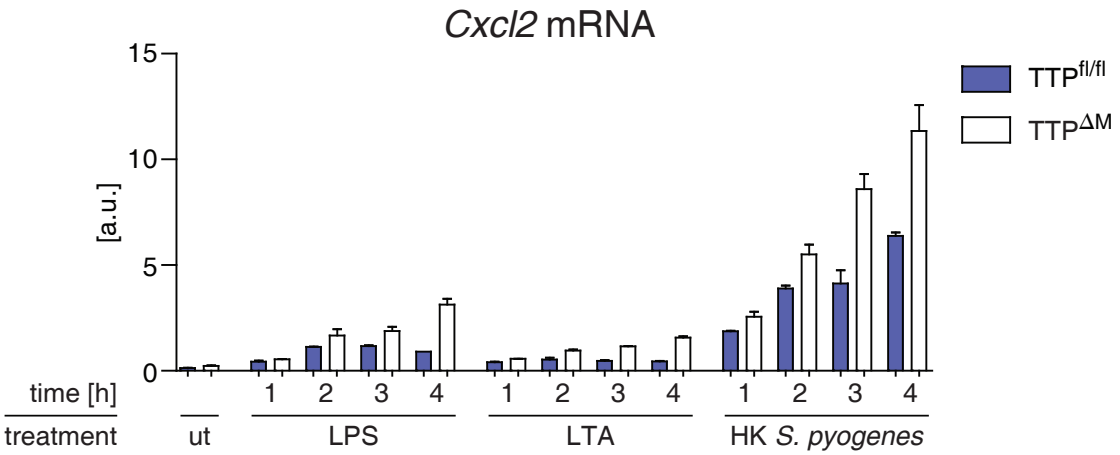
Supplemental Figure 4

A



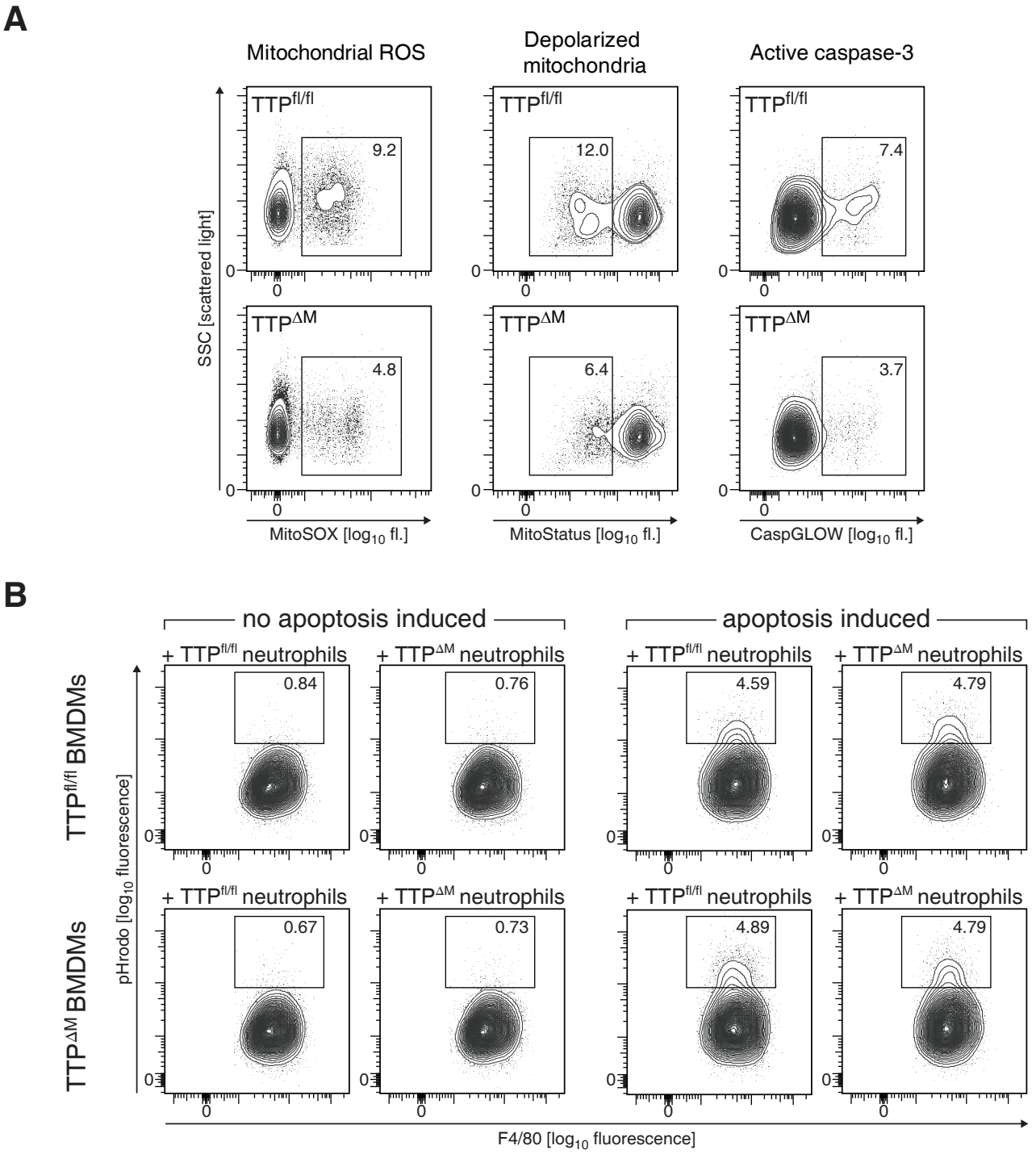
B





Supplemental Figure 4 (related to Figure 4). Immunostimulation of neutrophils increases TTP expression
Purified peritoneal neutrophils were left untreated or stimulated with 10 ng/ml LPS, 5 µg/ml LTA or HK *S. pyogenes* (MOI 1:50). At indicated time points RNA was isolated and expression of mRNAs was assessed by RT-qPCR (normalized to *Rplp0*). (**A**) Bar graph showing increased TTP mRNA expression upon immunostimulation of neutrophils. Note that TTP mRNA was not detectable in $TTP^{\Delta M}$ neutrophils at any time point. (**B**) Bar graphs show kinetics of *Tnf* and *Cxcl2* mRNAs in $TTP^{fl/fl}$ and $TTP^{\Delta M}$ neutrophils. Note that $TTP^{\Delta M}$ neutrophils show consistently higher expression of *Tnf* and *Cxcl2* mRNA at the 4 h time point.

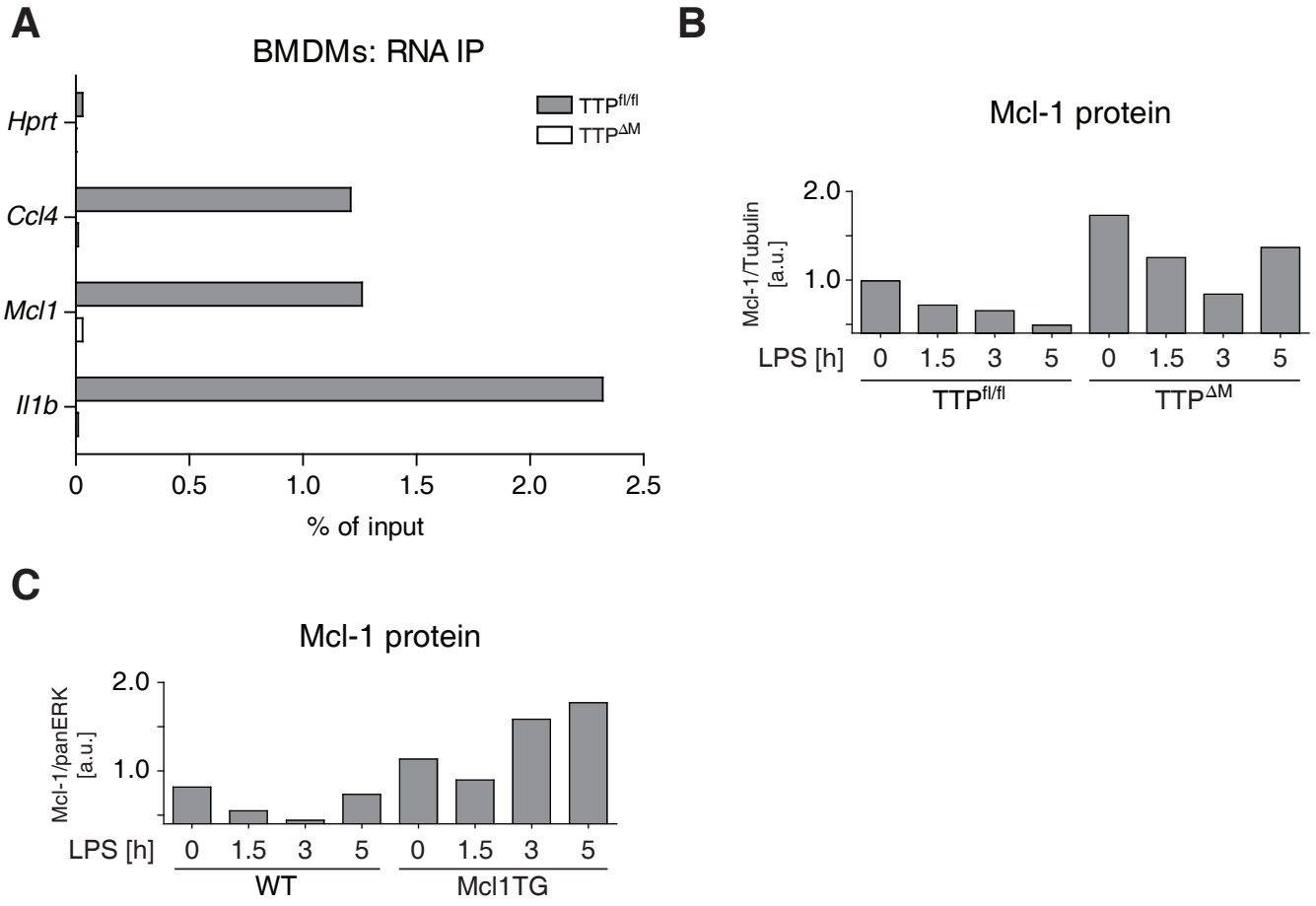
Supplemental Figure 5



Supplemental Figure 5 (related to Figure 5): Analysis of neutrophil apoptosis and phagocytosis of apoptotic neutrophils by macrophages

(**A**) Representative flow plots subgated for $Ly6G^+Ly6C^+$ neutrophils of peritoneal exudate cells stained for mitochondrial superoxide (MitoSOX), polarization of mitochondria (MitoStatus) and active caspase-3 (CaspGLOW). (**B**) Representative flow plots subgated for $F4/80^+$ BMDMs from $TTP^{fl/fl}$ (upper row) or $TTP^{\Delta M}$ (lower row) incubated with purified casein-elicited pHrodo-stained non-apoptotic (left panel) or apoptotic (right panel) neutrophils from $TTP^{fl/fl}$ or $TTP^{\Delta M}$ animals. Numbers within the outlined areas in all flow plots show percentages of the parent population.

Supplemental Figure 6



Supplemental Figure 6 (related to Figure 6). TTP binds to Mcl1 mRNA and regulates Mcl-1 protein expression

(**A**) Binding of TTP to *Mcl1* mRNA assessed by RNA-IP using rabbit TTP antiserum. Bar plot shows immunoprecipitated mRNA from BMDMs stimulated for 3 h with LPS as percentage of input mRNA detected with RT-qPCR. *Ccl4* and *Il1b* mRNAs served as positive controls, *Hprt* mRNA was used as negative control. (**B**) Shows quantification of Western blot in Figure 6D. Mcl-1 protein was normalized to Tubulin as loading control. (**C**) Shows quantification of Western blot in Figure 7A. Mcl-1 protein was normalized to panERK as loading control.

Supplemental Table 3 (related to Methods). Primer sequences used for qPCR analysis
Primer sequences for *Rplp0*, *Cxcl2*, *Hprt*, *Il1b*, *Tnf*, and TTP (gene name Zfp36) are listed in 5' to 3' orientation. Rplp0 expression was used for normalization.

Gene	Sequence (5' -> 3')
Rplp0 fwd	TCCTTCTTCCAGGCTTTGGG
Rplp0 rev	GGACACCCTCCAGAAAGCGA
Cxcl2 fwd	GCCCAGACAGAAGTCATAG
Cxcl2 rev	GTCAGTTAGCCTTGCCTT
Hprt fwd	GCAGTCCCAGCGTCGTGAT
Hprt rev	CAGGCAAGTCTTTCAGTCCTGTC
Il1b fwd	AGATGAAGGGCTGCTTCCAAA
Il1b rev	AATGGGAACGTCACACACCA
Tnf fwd	GATCGGTCCCCAAAGGGATG
Tnf rev	CACTTGGTGGTTTGCTACGAC
TTP fwd	CTCTGCCATCTACGAGAGCC
TTP rev	GATGGAGTCCGAGTTTATGTTCC

Legends for Supplemental Table 1 and 2

Supplemental Table 1 (related to Figure 4). Differential expression analysis of neutrophils from $TTP^{fl/fl}$ and $TTP^{\Delta M}$ mice
Triplicates of purified peritoneal neutrophils from $TTP^{fl/fl}$ and $TTP^{\Delta M}$ were stimulated with 10 ng/ml LPS for 4 h, mRNA was isolated and subjected to deep sequencing. Table shows expression of annotated genes as FPKM (Fragments Per Kilobase of transcript per Million mapped) for each triplicate, the mean values for each genotype and differential expression (as $\log_2$ fold change) and the corresponding p-value; no filter for FPKM values was applied. NA, not available.

Supplemental Table 2 (related to Figure 4). Gene ontology annotation of significantly higher expressed genes in TTP-deficient neutrophils
GO enrichment analysis was performed for genes significantly up-regulated in neutrophils from $TTP^{\Delta M}$ mice as compared to $TTP^{fl/fl}$ controls using DAVID. Table shows GO terms filtered for p-value ≤ 0.05.