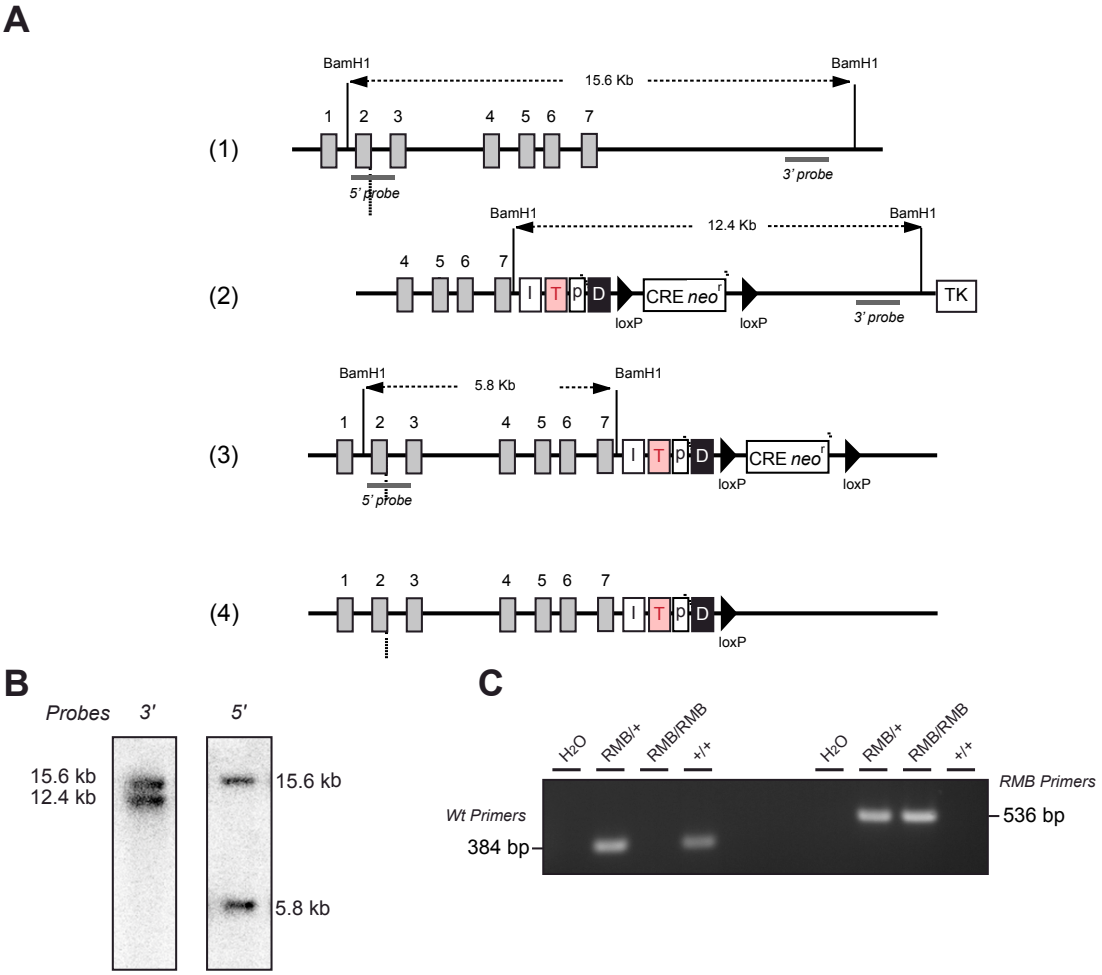
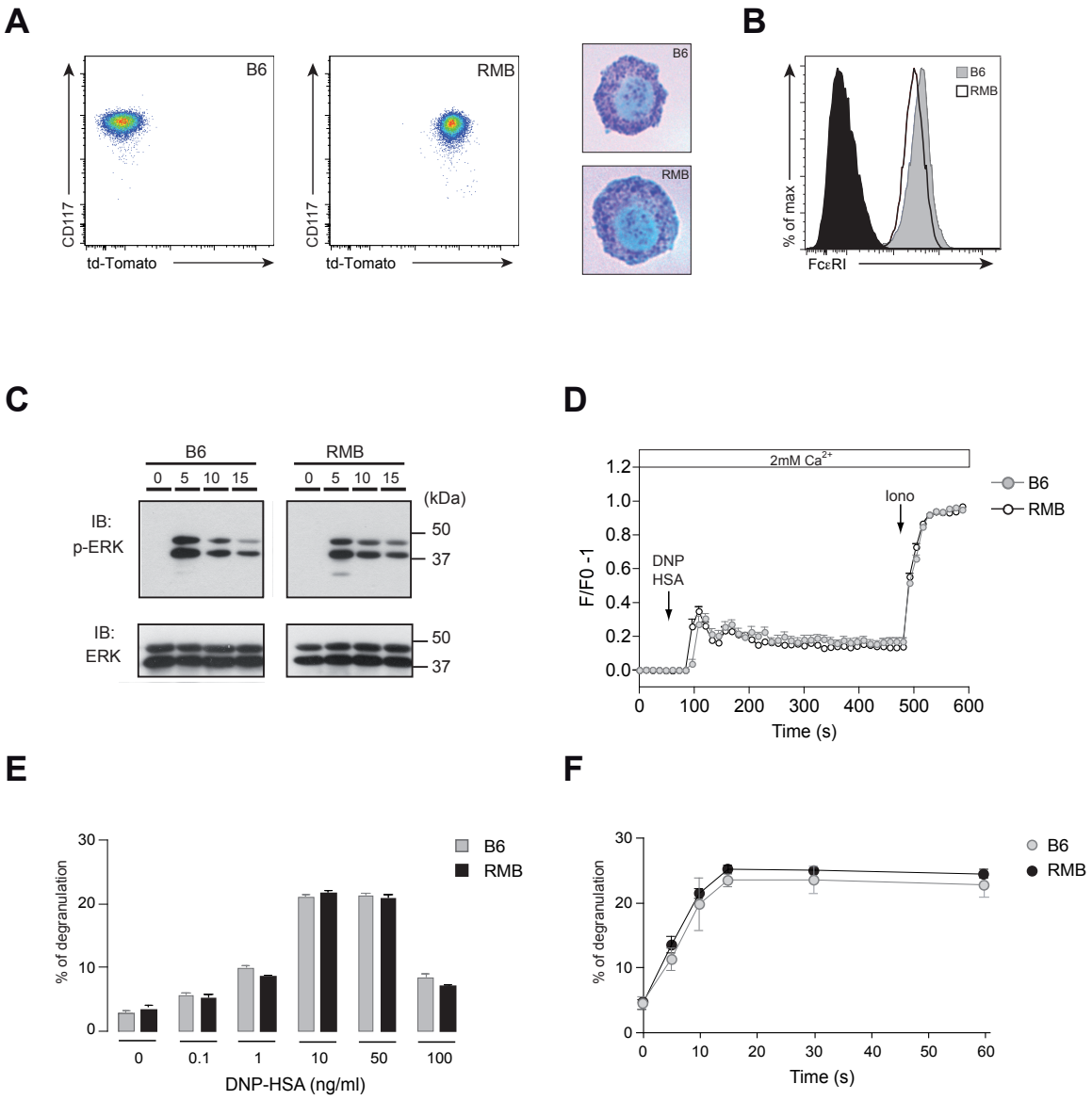
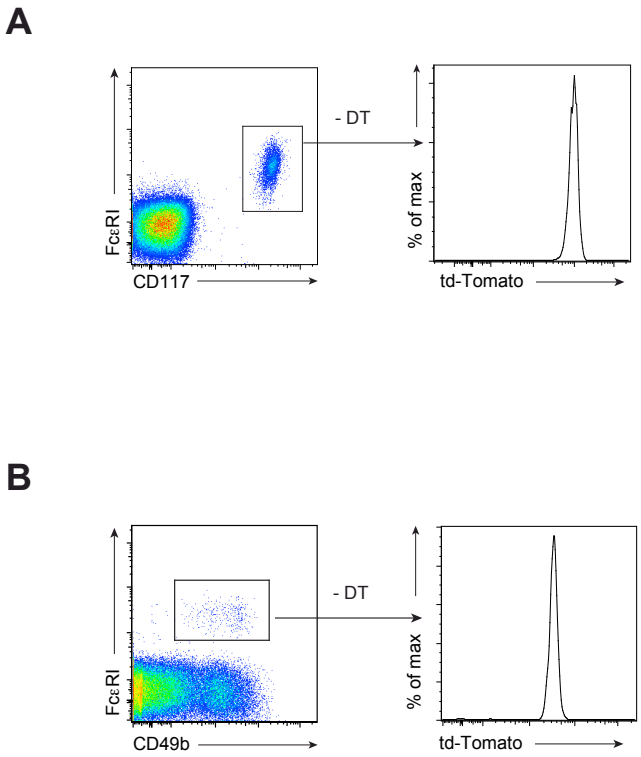
SUPPLEMENTAL RESULTS



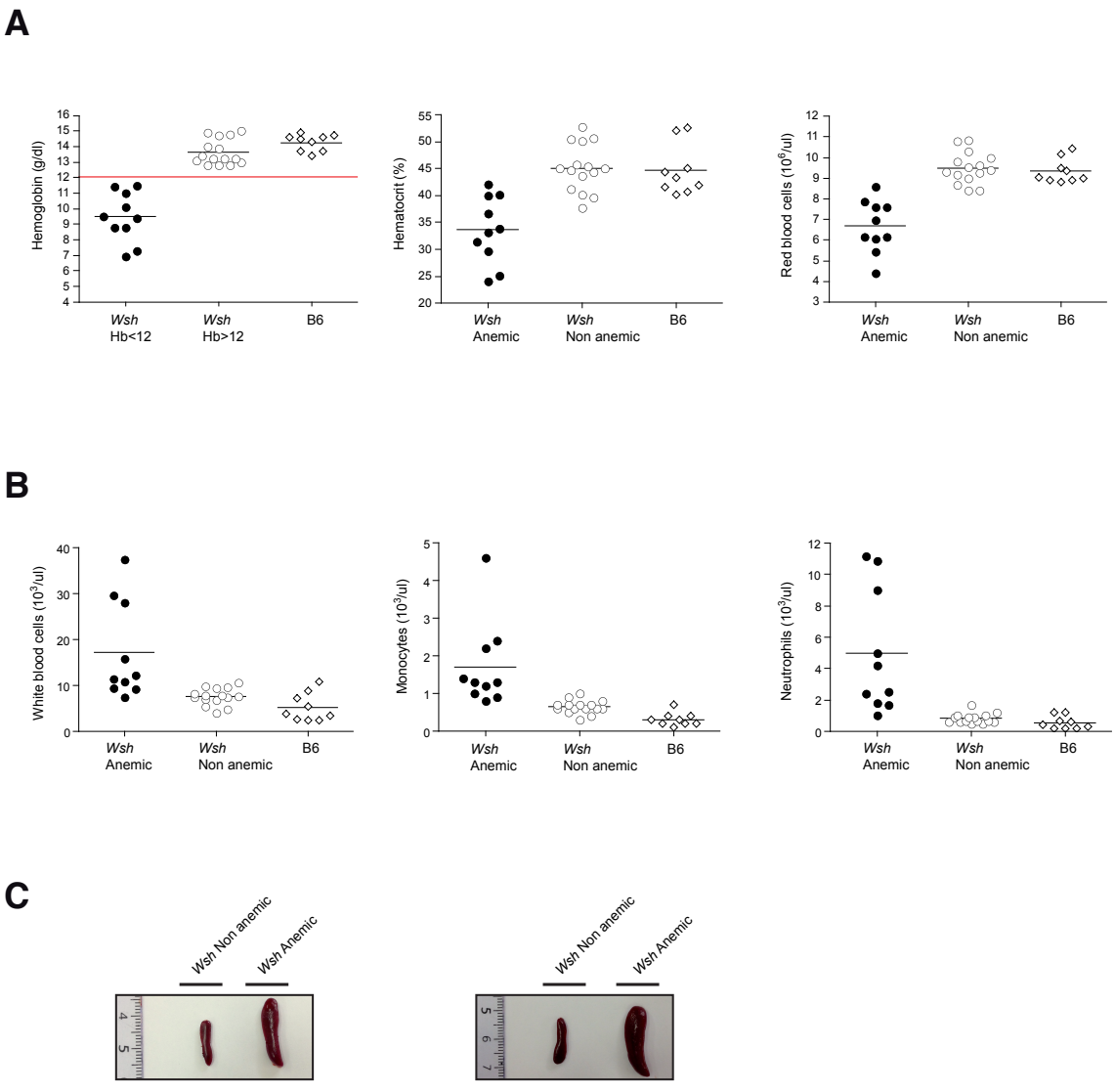
Supplemental Figure 1
Generation and screening of RMB mice.(**A**) Strategy used to generate RMB knock-in mice. (1) Partial restriction map of the wild-type *Ms4a2* gene coding for the FcεRI β chain. Exons are depicted as grey boxes. The 5' and 3' single-copy probes used to verify proper homologous recombination events by Southern blot analysis after BamH1 digestion are shown. (2) Targeting vector used for the introduction of an IRES-tdTomato-2A-hDTR cassette in the 3' untranslated region of the *Ms4a2* gene. This cassette is flanked at its 3' end by an auto-deleting loxP-Cre-NeoR-loxP cassette. TK: thymidine kinase expression cassette abutted onto the 3' end of the right homology arm. I: IRES, T: tdTomato, p: peptide 2A, D: hDTR. (3) Structure of the targeted allele following homologous recombination and prior to Cre-mediated self-excision of the neoR cassette. (4) Structure of the targeted allele after auto-deletion of the Cre-NeoR cassette in male gametes. (**B**) Southern blot analysis of recombinant ES clones. ES cell DNA was digested with BamH1 and hybridized to the 5' and 3' probes shown in (A). (**C**) Genotyping of tail DNA from wild-type and from heterozygous and homozygous RMB mice for the targeted *Ms4a2* allele using a mix of the three primers specified in the Methods section.



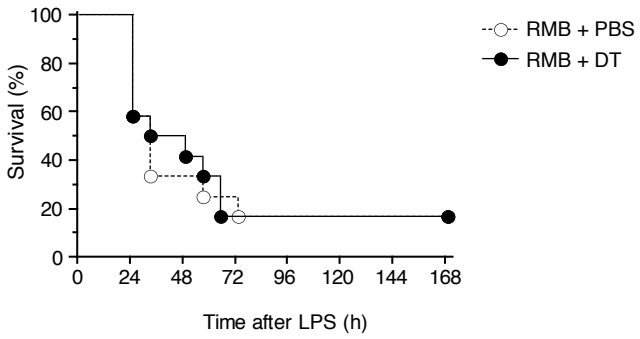
Supplemental Figure 2
Wild-type and RMB mast cells are functionally equivalent. (**A**) CD117 and td-Tomato labeling of BMMCs from RMB and B6 mice. Granules were stained with toluidine blue. (**B**) FcεRI expression in BMMCs from RMB and B6 mice. (**C**) Kinetics of ERK phosphorylation in BMMCs from RMB and B6 mice after FcεRI stimulation. (**D**) FcεRI-induced calcium mobilization in BMMCs from RMB mice (open circles) and B6 mice (gray circles). Ionomycin (Iono) was used to generate the maximum response. (**E** and **F**) DNP-HSA dose response and kinetics of degranulation at 10 ng/ml DNP-HSA of RMB (black bars and black circles) and of B6 (gray bars and gray circles) BMMCs pre-incubated overnight with anti-DNP IgE. Data represent mean ± SEM.



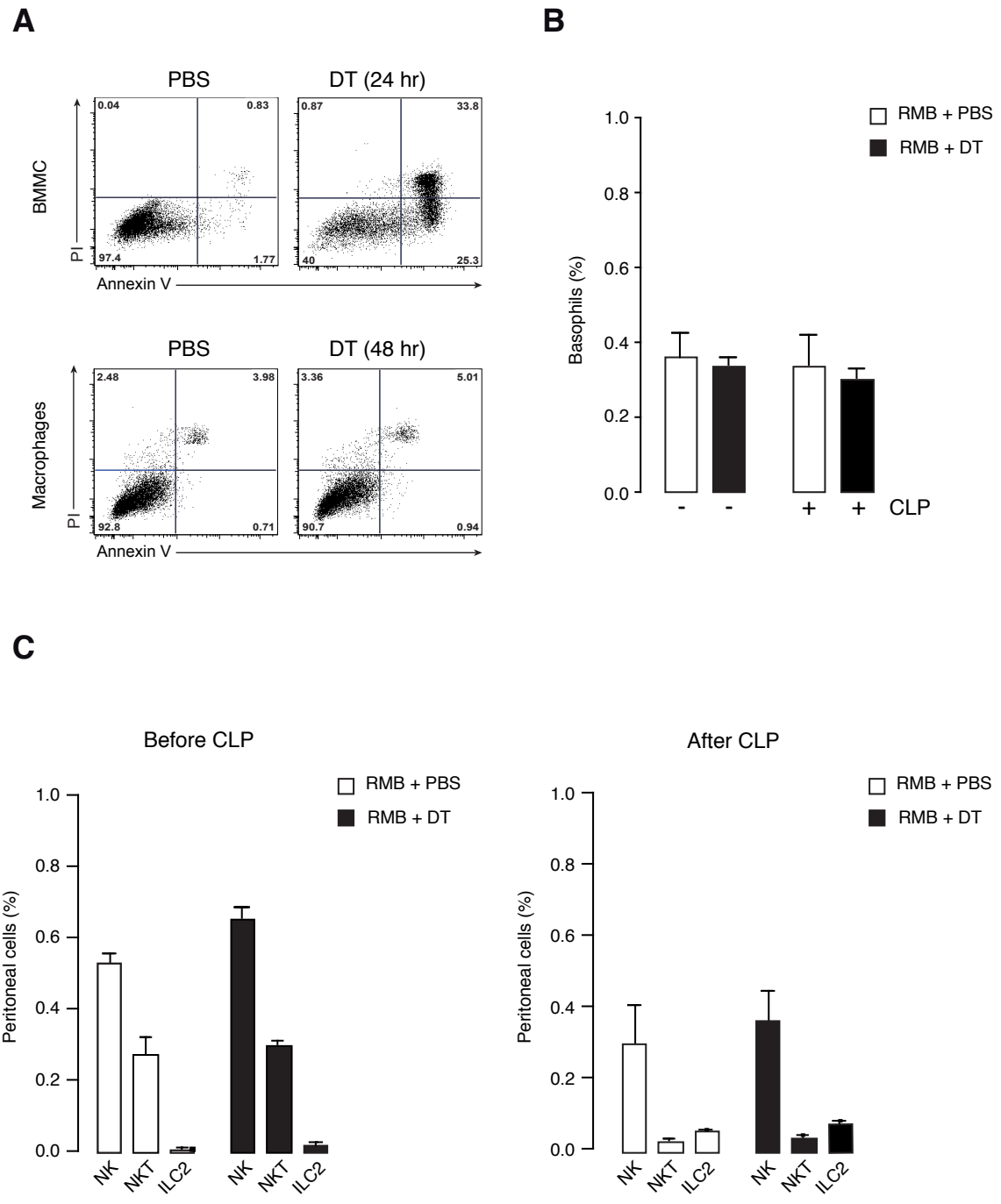
Supplemental Figure 3
FACS characterization of mast cells and basophils from RMB mice. (**A**) td-Tomato fluorescence of the total mast cell population (FcεRI^{+}CD117^{+}) recovered from the peritoneal cavity of RMB mice. (**B**) td-Tomato fluorescence of blood basophils (FcεRI^{+}CD49b^{+}) from RMB mice.



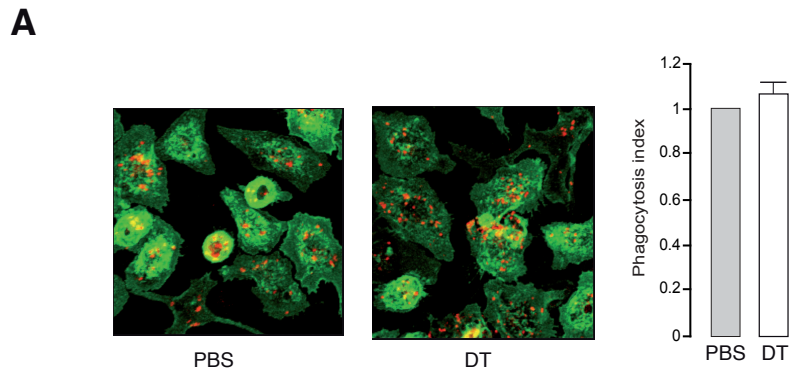
Supplemental Figure 4
About 40% of Kit$^{W\text{-}sh/W\text{-}sh}$ mice exhibit hematopoietic abnormalities (**A**) Blood samples were withdrawn from 25 Kit$^{W\text{-}sh/W\text{-}sh}$ (Wsh) mice (mix of males and females, aged from 7 to 20 weeks) and 9 C57Bl/6 (B6) control mice. Samples were analyzed using a MS9-5 hematological analyzer and mice were separated into two groups based on their level of hemoglobin. Out of 25 Kit$^{W\text{-}sh/W\text{-}sh}$ mice, 10 had a blood hemoglobin level of below 12 g/dl. All B6 mice and the 15 other Kit$^{W\text{-}sh/W\text{-}sh}$ mice had a hemoglobin level of around 14 g/dl. The hematocrit as well as the level of red blood cells were decreased in anemic Kit$^{W\text{-}sh/W\text{-}sh}$ mice. (**B**) The numbers of total white blood cells, monocytes and neutrophils were increased in all anemic Kit$^{W\text{-}sh/W\text{-}sh}$ mice. (**C**) Representative spleen enlargement in two anemic Kit$^{W\text{-}sh/W\text{-}sh}$ mice compared to spleens from two non-anemic Kit$^{W\text{-}sh/W\text{-}sh}$ mice.

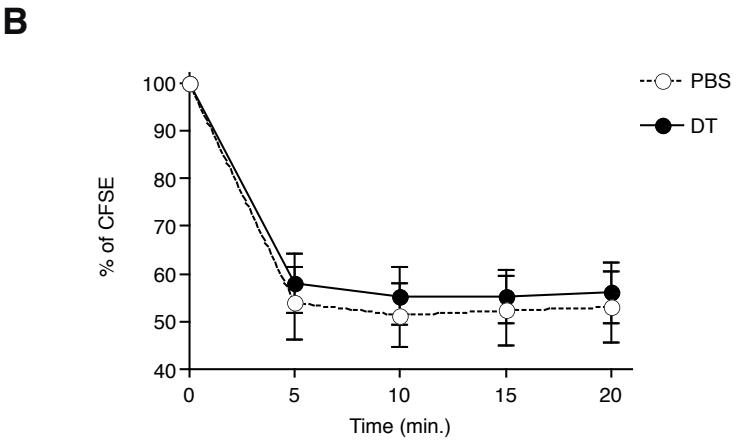


Supplemental Figure 5
Similar LPS induced sepsis survival rate in PBS- and DT-treated RMB mice. Survival after LPS intraperitoneal injection (15 mg/kg) in PBS- (open circles; n = 12) or in DT-treated RMB mice (closed circles; n = 12). Kaplan-Meier curves and the log-rank test were used to analyze mortality rates.



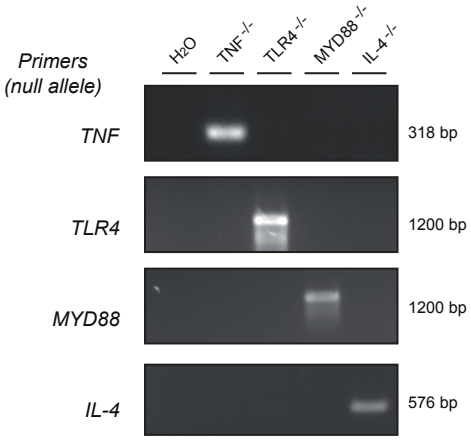
Supplemental Figure 6
Effect of DT treatment on BMMC, basophils and peritoneal cells. (**A**) Representative flow cytometric analysis of DT-induced cell death in BMMC at 24 hr upper panel (no lived mast cells were detectable after 48 hr DT treatment). No macrophage apoptosis were observed after 48 hr of DT treatment assessed by AnnexinV/PI staining in lower panel. (**B**) Blood basophils (% of $CD45^+$ cells) in PBS- and DT-treated RMB mice before and 24 hr after CLP. (**C**) Peritoneal levels of ILC2, NK and NKT cells in PBS- and DT-treated RMB mice before and after CLP. Flow cytometry analysis of peritoneal ILC2, NK and NKT cells (gating strategy in the supplemental methods) represented in percentage of total peritoneal cells 12 days after PBS or DT injected RMB mice before (n=3) or 24 hr after CLP (n=4). Data represent mean ± SEM.





Supplemental Figure 7
Analysis of ex vivo macrophage phagocytosis from PBS- and DT-treated mice. (**A**) Ex-vivo macrophage phagocytosis of pHrodo bacteria (in red): 12 days after PBS or DT injection, resident macrophages were isolated from the peritoneal cavity by adherence to Lab-tek plastic chambers. Phagocytosis by macrophages under both conditions was assessed by incubation with pHrodo bacteria for 15 min. Left and middle panels represent phagocytosis by peritoneal macrophages from PBS- and DT- treated mice, respectively. Histograms in the right panel represent the means of three independent experiments (error bars ± SEM). (**B**) Kinetics of phagosome acidification analysis in peritoneal macrophages from PBS- and DT-treated mice. Fluorescent latex beads were phagocytosed by macrophages and the decrease of CFSE fluorescence was followed in time depend manner. Data represent mean ± SEM.

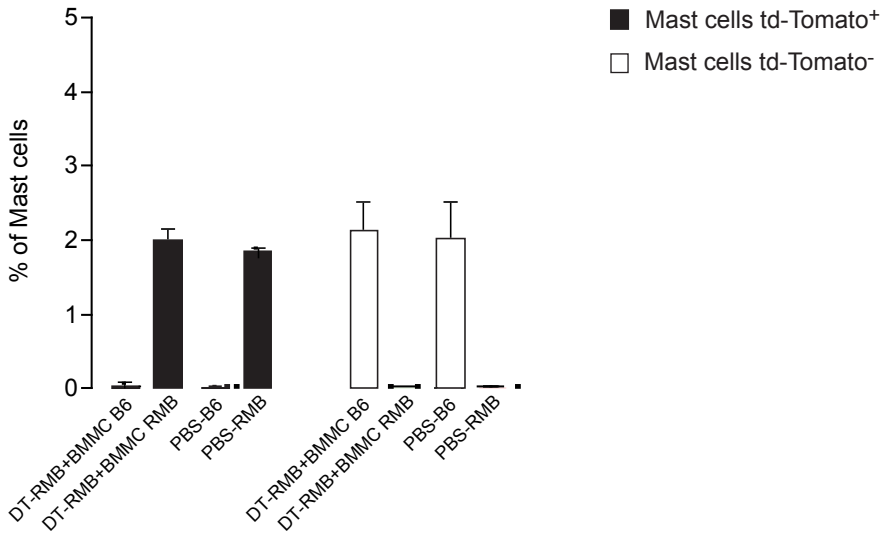
A



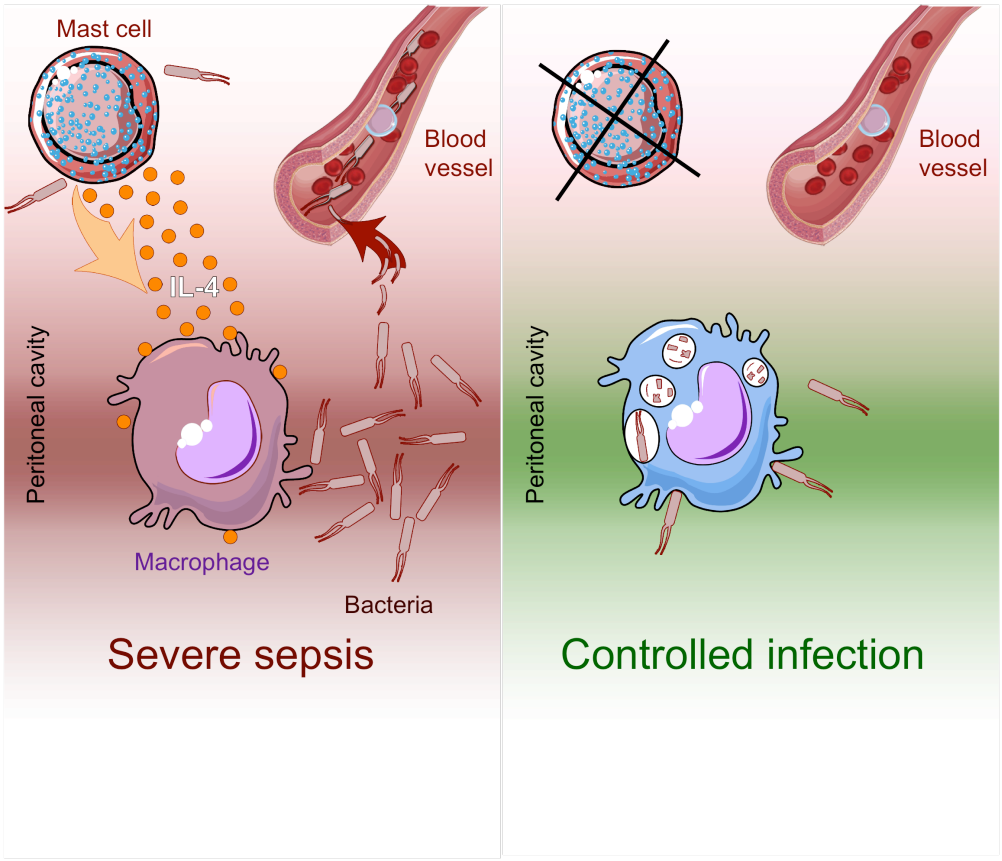
B

Genomic PCR		
Gene Name	Sens Primers	Anti-sens Primers
IL-4 (null allele)	TCAGGACATAGCGTTGGC	GCTGTGAGGACGTTTGGC
TLR4 (null allele)	TGTTGGGTCGTTTGTTCGGATCCGTCG	TGTTGCCCTTCAGTCACAGAGACTCTG
MYD88 (null allele)	ATCGCCTTCTATCGCCTTCTTGACGAG	AGCCTCTACACCCTTCTCTTCTCCACA
TNF (null allele)	CGTTGGCTACCCGTGATATT	TAGCCAGGAGGGAGAACAGA

Supplemental Figure 8
Validation of the specific molecular deficiency in BMMCs (**A**) Mast cells were derived from the bone-marrow of TNF-, TLR4-, MyD88-, or IL-4- deficient mice and cultured for 6 weeks, before DNA extraction. PCR was performed using specific null allele primers (annotated in **B**).



Supplemental Figure 9
BMMC reconstitution in DT-treated RMB mice. $2.5x10^6$ BMMCs from RMB or B6 mice were injected into the peritoneal cavity of RMB mice treated with DT 2 weeks previously. BMMCs repopulation was assessed 8 weeks later by flow cytometry analysis for CD117 and FcεRI double positives cells. The percentage of mast cells was calculated among all $CD45^+$ cells. Data represent mean ± SEM.



Supplemental Figure 10
Mast-cell-derived IL-4 controls macrophage phagocytosis *Left:* in an acute mouse model of sepsis, mast cells, at the beginning of bacterial infection, secrete IL-4, which impairs macrophage phagocytosis.This restrained phagocytosis by resident macrophages leads to bacterial expansion and reduced mouse survival. *Right:* in mast-cell-deficient mice, resident macrophages display effective phagocytosis, allowing better bacterial clearance and positively affecting the outcome of severe sepsis.

SUPPLEMENTAL METHODS

Construction and identification of the RMB mice.

RMB targeting vector. A 8 kb genomic fragment encompassing exons 4 to 7 of the *Ms4a2* gene was isolated from a BAC clone of C57BL/6J origin (clone n° RP23-359014; http://www.lifesciences.sourcebioscience). Using ET recombination, an IRES-tdTomato-2A-hDTR-loxP-Cre-neo^{R}loxP cassette was introduced in the 3' untranslated region of the *Ms4a2*gene, 14 bp downstream of the stop codon. Finally, the targeting construct was abutted to a thymidine kinase expression cassette and linearized with Fse1.

Isolation of recombinant embryonic stem (ES) cell clones. JM8.F6 C57BL/6N ES cells were electroporated with the linearized RMB targeting vector. After selection in G418 and gancyclovir, ES cell clones were screened for proper homologous recombination by Southern blot. When tested on *BamHI*-digested genomic DNA, the 5' single-copy probe used to identify proper recombination events hybridized to a 15.6 kb wild-type fragment and to a 5.8 kb recombinant fragment. When tested on *BamHI*-digested genomic DNA, the 3' single-copy probe used to identify proper recombination events hybridized to a 15.6 kb wild-type fragment and to a 12.4 kb recombinant fragment. A neomycin-specific probe was used to ensure that adventitious non-homologous recombination events had not occurred in the selected ES clones.

Production of knock-in mice. Properly recombined ES cells were injected into FVB blastocysts. Germline transmission led to the self-excision of the loxP-Cre-Neo^{R}-loxP cassette in male germinal cells. RMB mice were identified by PCR of tail DNA. Briefly, tails were digested overnight with proteinase K (100 µg/ml) at 56°C. DNA was precipitated by isopropanol, washed in ethanol and resuspended in TER buffer (Tris-HCl 10 mM, EDTA 1 mM, RNase H 20 µg/ml). PCR were performed using a mixture of the three following primers: sense WT primer: 5'-CAGGTCCCAGATGATCGTCTTTATGAA-3, sense RMB: 5'-CCCAGTGGAAAATCGCTTATATACC-3', and anti-sense WT/RMB: 5'-CAATAAAGCCAACTTATTCCTTGATGAC-3'. After an initial cycle at 95°C for 5 minutes, cycle conditions (35 cycles) were 94°C for 45 sec, 60°C for 45 sec and 72°C

for 45 sec. Finally the reaction was incubated at 72°C for 5 minutes. This trio of primers amplified a 370 bp band in the case of the WT allele and a 536 bp band in the case of the RMB allele.

Ca^{2+} measurement.

Ca^{2+} mobilization was acquired with fluorometric imaging system (Till-photonics), as previously described (Barbet et al., 2008). BMMC were FCS starved and sensitized overnight with DNP-IgE. BMMC were incubated on polylysine pretreated in glass-bottom dishes (MatTek) for 45 min at room temperature to be adherent. BMMC were loaded for 15 min at 37°C and then 15 min at room temperature with pluronic acid and 1 μg/ml Fura 2-AM (both from Invitrogen) in BMMC medium. The cells were then washed with Ringer's solution (in mM: 145 NaCl, 5.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, and 0.1% BSA, pH adjusted to 7.5). Ca^{2+} influx was triggered by adding DNP-HSA (to IgE-sensitized cells). Individual fluorescence values were then analyzed with Origin software to normalize the fluorescence with the first value according to the equation (F/F0)-1, where 'F' is the fluorescence at specific time point and 'F0' is the fluorescence at time 0. The area under the curve was calculated for each cell.

Western-blot on total lysates.

BMMC (2×10^6) were FCS starved and sensitized overnight with anti-DNP IgE before activation for 10 or 30 min with 10 ng/ml DNP-HSA. BMMC were lysed in 100 µl of lysis buffer (62,5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 50 mM DTT) and 20 µl of sample were resolved by 10% SDS-PAGE. After being transferred onto nitrocellulose membrane and blocking in 4% BSA. Membranes were incubated with rabbit anti-phospho ERK (Cell signaling, 1:5,000 dilution) or anti-ERK2 antibody (Santa Cruz, 1:2,000) overnight at 4°C. Donkey anti-rabbit HRP (1:10.000, GE healthcare, Buckinghamshire, UK) was used as a secondary antibody for 1 h at room temperature and labeled bands were detected using ECL western blotting detection system (GE healthcare).

IL-4 secretion assay

IL-4 released from peritoneal mast cells was detected using a Miltenyi Biotech Mouse IL-4 secretion assay. Peritoneal cells were recovered by flushing the peritoneal cavity

of B6 mice with ice-cold PBS. Cells were counted and dispensed at 2 x 10^6 cells per condition in cold medium (RPMI-1640, 10% FCS). Peritoneal cells were incubated with the mouse anti-IL-4 catch reagent for 20 minutes on ice. After a wash step, cells were resuspended in 600 µl of warm medium, alone or supplemented with stimulating agents (live *E. coli* K12 or PMA 2nM/ionomycin 400nM) and incubated at 37°C for 15 min. IL-4 secretion was stopped on ice and washing twice in cold buffer (PBS, 0.5% BSA, 2mM EDTA), and cells incubated with the Mouse IL-4 Detection antibody for 10 minutes on ice. An additional labeling step with a rat anti-mouse CD117 was performed to gate mast cells.

Mast cell degranulation

BMMC were plated at 0.5 × 10^6 cells in 96-well polypropylene plate in BMMC medium in the absence of SCF and incubated overnight with anti-DNP-IgE. Additional anti-DNP-IgE was added the next morning for 2 h. Cells were then washed and stimulated with various concentrations of DNP-HSA for the indicated times in Tyrode's buffer (10 mM HEPES, pH 7.4, 130 mMNaCl, 5 mMKCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, and 0.1% bovine serum albumin (BSA)) and the released β-hexosaminidase was measured directly in supernatants.

Passive systemic anaphylaxis

12 days before experiments, mice were injected twice with either with DT or PBS i.p. An IPTT-300 (Plexx) temperature probe was implanted subcutaneously into the neck of the mouse on Day 10. Mice were sensitized with 20 µg of mouse anti-DNP IgE (H1-ε-26) i.v., 24 h before an i.v. challenge with 1 mg DNP-HSA. Body temperature changes were monitored using a DAS-7007S wireless reader (Plexx) every 10 minutes for 100 minutes.

Flow cytometry

Cells were stained with fluorochrome-conjugated monoclonal antibodies NK1.1 (PK136) (Becton Dickinson), NKp46 (29A1.4), CD90.2 (53-2.1), CD127 (A7R34), Sca-1 (D7), CD25 (PC61.5) (ebioscience), T1/ST2 (Dj8) (MD products). Among tdT^- cells, gating strategy was the following: NK ($CD19^-CD3^-NKp46^+NK1.1^+$), NKT ($CD19^-CD3^+NK1.1^+$), ILC2 ($CD19^-CD3^-\beta TCR^-NK1.1^-CD90.2^+CD127^+CD25^+Sca1^+T1/ST2^+$).

LPS induced sepsis model

12 days after PBS or DT injection, 15 mg/kg of LPS (0111:B4 ; Chondrex) was ip injected. Mouse survival was monitored every 8 h for the first 3 days and then every 12 h until Day 7.

Apoptosis

12 days after PBS or DT injection, peritoneal macrophages were cultured by adherence overnight in RPMI 1% FCS. The next day, cells are washed and adherent cell were cultured in RPMI 10% FCS with PBS or DT (1 μg) for 48 h. Macrophages were stained with FITC Annexin V and PI according to the manufacturer (BD FITC Annexin V apoptosis detection kit I). Briefly, after 2 washes in PBS, cells were resuspended in binding buffer and incubated for 15 min at room temperature in the dark with Annexin V and PI. 400 μl of binding buffer were added and cells were immediately analyzed on LSR Fortessa.

Phagosome acidification assay

3 μm amino latex beads from a 2.5 % aqueous suspension (polysciences) were incubated in 400 μl of sodium hydrogen carbonate 0.1 M buffered at pH 8.5 with 50 μl of a 50 mg/ml CFSE and 50 μl of a 1 mg/ml FluoProbes 647 (Interchim) for 2 h at room temperature with continuous rotation in the dark. Beads were then washed twice in 0.1 M glycine-PBS pH 7.2 buffer once in PBS and resuspended in 1 ml serum free RPMI 20 mM HEPES. Peritoneal macrophages in warm serum-free HEPES RPMI were mixed with 50 μl of resuspended bead, immediately centrifuged for 30 sec at 1000 rpm and incubated for 10 min at 37°C for the pulse phase. Cells were washed 3 times with cold PBS and resuspended in warm RPMI 10% FCS. Cells were incubated at 37°C for kinetics. 50 μl of cells were taken out for each time point, and mixed in 500 μl of cold PBS to stop the reaction and immediately analyzed on a LSR Fortessa.