Supplementary Methods

Flow cytometry

We used flow cytometry to identify blood basophils (CD49b$^+$; IgE$^+$), neutrophils (Ly6G$^+$; CD11b$^+$) and monocytes (Ly6G$^-$; CD11b$^+$), as well as spleen neutrophils (Ly6G$^+$; F4/80$^-$) and macrophages (Ly6G$^-$; F4/80$^+$). Briefly, red blood cells were lysed by treatment with pH 7.3 ACK lysis buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$, and 0.1 mM EDTA, pH 8.0) 2 times for 5 min each. Cells were blocked with unconjugated anti–CD16/CD32 antibodies (BioXcell) on ice for 5 min and then stained with combinations of anti-CD49b-APC (DX5), anti-IgE (R35-72), anti-Ly6G (1A8), anti-CD11b (M1/70) and anti-F4/80 (BM8) (all from eBioscience or BioLegend) antibodies on ice for 30 min. Data were acquired using LSRII and Accuri C6 flow cytometers (all from BD Biosciences). Data were analyzed with FlowJo 9.5.3. software (TreeStar). Dead cells (identified by staining with propidium iodide; Gibco; or with LIVE/DEAD aqua amine; Invitrogen) were not included in the analysis.

Peritoneal lavage and differential cell count

To harvest peritoneal cells, 2 ml of ice-cold PBS were injected into the peritoneal cavity 1-4 days (in routine experiments, 3 days) after OVA challenge, and the abdomen was massaged gently for 20 s. Fluid containing peritoneal cells was aspirated and the cells were resuspended in ice-cold PBS. Total cell numbers were measured using a hemocytometer chamber and cells were cytocentrifuged onto glass slides and stained

with May-Grunwald-Giemsa. Differential cell counts on at least 300 cells were obtained using standard morphological criteria.

Depletion of basophils, monocytes/macrophages or neutrophils, and blockade of Fcγ RIV, PAF and histamine

For depletion of basophils, $Mcpt8^{DTR/+}$ basophil-deficient mice and $Mcpt8^{+/+}$ basophil-sufficient littermates received i.p. injections of 500 ng diphtheria toxin (DT; Sigma-Aldrich) 2 days before and one day after the challenge with OVA. The efficiency of depletion was assessed by flow cytometry analysis of blood basophils ($CD49b^{+}$; IgE^{+}) in blood samples collected by retro-orbital bleeding 1 h before OVA challenge.

For neutrophil depletion, mice were injected i.p. with 150 μg of an anti-Gr-1 antibody (RB6-8C5; BioXcell) 40 h before and 24 h after challenge with OVA^{E1}. Control mice were injected with a rat IgG_{2b} isotype control antibody (LTF-2; BioXcell). The efficiency of neutrophil depletion was assessed by flow cytometry analysis of neutrophils in blood samples ($Ly6G^{+}$; $CD11b^{+}$) collected by retro-orbital bleeding 1 h before and 3 days after OVA challenge, as well as in spleen samples ($Ly6G^{+}$; $F4/80^{-}$) collected 3 days after challenge.

For depletion of monocytes/macrophages, mice were injected with clodronate liposomes (150 μg i.p. + 150 μg i.v.; ClodronateLiposomes.com) 24 h before challenge with OVA^{E1}. Control mice were injected in the same manner with the same amount of PBS liposomes. The efficiency of monocyte/macrophage depletion was assessed by flow cytometry analysis of monocytes in blood samples ($Ly6G^{-}$; $CD11b^{+}$) collected by retro-

orbital bleeding 1 h before and 3 days after OVA challenge, as well as in spleen samples ($Ly6G^-$; $F4/80^+$) collected 3 days after challenge.

For blockade of FcγRIV, mice were injected i.v. with 200 µg of an anti-FcγRIV (9E9) antibody 30 min before and 2 days after challenge with OVA[E1]. The hybridoma cell line producing anti-FcγRIV antibodies (9E9) were kindly provided by J. V. Ravetch (Rockefeller University, New York, USA); control mice were injected with polyclonal Armenian hamster isotype control IgG antibodies (BioXcell).

The potential contributions of histamine and PAF in this model were analyzed by specific inhibition of the actions of these mediators following previously described methods[E2], with slight modifications: the H_1-receptor-specific anti-histamine triprolidine was solubilized at 1 mg/ml in sterile saline and 200 µl was injected i.p. into mice 30 min before and 1 day after OVA challenge. The PAF receptor antagonist CV-6209 (Santa Cruz Biotechnology) was diluted at 330 µg/ml in saline and 200 µl were injected i.v. 30 min before and 1 day after OVA challenge.

Evaluation of MCs in the mesenteric windows

Evaluation of MCs in the mesenteric windows was performed as previously described[E3, 4]. Briefly, 4-5 mesenteric windows from approximately the same locations in each mouse were arranged onto slides and fixed for 1 h in Carnoy's solution (3:2:1 v/v/v of ethanol, chloroform, and acetic acid). The preparations were stained with Csaba stain [which contains both safranin (red, identifying "mature" MCs) and alcian blue (blue, identifying "less mature" MCs), which bind to MC cytoplasmic granules][E3, 4].

Histologic analysis

Ear skin (ear pinnae), back skin and spleen specimens were fixed in 10% formalin and embedded in paraffin, and 4-µm sections were stained with 0.1% Toluidine blue for histologic examination and enumeration of MCs. Images were captured with an Olympus BX60 microscope using a Retiga-2000R QImaging camera run by Image-Pro Plus Version 6.3 software (Media Cybernetics).

Measurement of OVA-specific IgG_1 and IgG_{2c} antibodies

Peripheral blood was collected by retro-orbital bleeding 24 h before OVA challenge. Serum OVA-specific IgG_1 and IgG_{2c} antibodies were measured by ELISA. Briefly, 96-well EIA/RIA plates (Costar) were coated with 2 µg/well of OVA in 50 nM carbonate-bicarbonate buffer (pH 9.6; Sigma-Aldrich) overnight at 4°C. Coated plates were blocked with 1% BSA for 2 h at room temperature. Plates were washed three times and diluted serum samples were incubated overnight at 4°C. We diluted samples 1/100,000 for IgG_1 and 1/50 for IgG_{2c}. Plates were then washed three times and incubated with biotinylated anti-mouse IgG_1 (A85-1; BD Pharmingen) or biotinylated anti-mouse $IgG_{2a/c}$ (R19-15; BD Pharmingen) for 1 h at room temperature. Plates were washed five times and incubated with streptavidin HRP (BD Pharmingen) for 30 min at room temperature. Plates were washed three times and incubated with TMB substrate (Sigma) and the reaction was stopped by addition of 2 M H_2SO_4. Absorbance was measured at 450 nm. Anti-OVA IgG_1 (4B4E6; Chondrex) and IgG_{2a} (M12E4D5; Chondrex) were used as standard antibodies (C57BL/6 do not express IgG_{2a} but express IgG_{2c}[E5] [the R19-

15 detection antibody we used in our ELISA recognizes both IgG_{2a} and IgG_{2c}[E6]], we therefore expressed levels of OVA-specific IgG_{2c} as arbitrary units).

Generation and adoptive transfer of bone marrow-derived cultured MCs (BMCMCs)

Female mouse femoral bone marrow cells were cultured in DMEM medium supplemented with 10% fetal calf serum (FCS), penicillin and streptomycin and 20% WEHI-3 cell-conditioned medium (as a source of IL-3) to generate bone marrow-derived cultured MCs (BMCMCs). Cells were cultured for 6 weeks, with medium changed twice a week until >95% were $Fc\varepsilon RI\alpha^{+}KIT^{+}$ (assessed by flow cytometry; data not shown). BMCMCs were transferred by i.p. + i.v. injections (2 x 10^6 cells i.p. + 10^7 cells i.v.) into male $Kit^{W\text{-}sh/W\text{-}sh}$ mice at 4–6 weeks of age. Sensitization with OVA was initiated 12 weeks after transfer of BMCMCs.

Generation of peritoneal cell-derived mast cells (PCMCs)

To generate peritoneal cell-derived MCs (PCMCs)[E7], peritoneal cells from C57BL/6J mice were maintained *in vitro* for 4 weeks in medium containing stem cell factor (SCF; 10 ng/mL; R&D Systems) until MCs represented >95% of the total non-adherent cells, as indicated by May-Grünwald-Giemsa staining.

In vitro *mast cell degranulation assay*

We assessed the ability of serum samples (collected 24 h before the challenge) to sensitize PCMCs to undergo activation in response to OVA *in vitro*, as an *ex vivo* method to quantify the presence and functional activity of OVA-specific IgE[E8]. PCMCs were

incubated overnight with serum from PBS-treated or OVA-sensitized mice diluted 1:10 in the PCMCs' complete culture medium (described above). Cells were washed with PBS and stimulated with 10 ng/ml OVA for 30 min at 37^oC. PCMCs were washed and incubated with avidin-fluorescein isothiocyanate (FITC) (a probe that specifically binds to heparin contained in MC granules) and propidium ionide (PI) for 10 min on ice. The extent of MC degranulation was assessed in live (PI^-) cells by measuring the membrane-bound fraction of granules by flow cytometry, as described previously[E9]. In some experiments, serum samples were incubated with a rat IgG_1 anti-IgE antibody (R35-92; 50 μg/ml; BD Pharmingen) or a rat IgG_1 isotype control antibody (R3-34; 50 μg/ml; BD Pharmingen) for 30 min at 37^oC before incubation with BMCMCs.

OVA-induced model of active anaphylaxis using alum and* B. pertussis *for the sensitization

Mice were sensitized intraperitoneally with 100 μg of endotoxin-free ovalbumin (Endograde OVA ; BioVendor ; < 0.01 EU endotoxin per injection), 300 ng *Bordetella pertussis* toxin (Sigma) and 1 mg Aluminum Hydroxide Gel Adjuvant (alum; InvivoGen) in 200 μL of PBS. Three weeks after the sensitization, mice were challenged i.p. with 500 μg of OVA. Rectal measurements of body temperature were performed immediately before (time 0) and at different time points for up to 2 h after challenge.

IgE-mediated Passive Systemic Anaphylaxis (PSA)

Purified mouse IgE anti-TNP antibodies (clone C38-2, BD Pharmingen) were administered intraperitoneally at a dose of 20 μg in 200 μL of PBS followed by an

intraperitoneal challenge with 500 μg of $TNP_{(21-31)}$-BSA (Santa Cruz) in PBS 24 hours later. Rectal measurements of body temperature were performed immediately before (time 0) and at different time points for up to 2 h after challenge.

Measurement of histamine levels and PAF-AH activity

Histamine levels were measured in plasma collected 20 min after challenge with OVA using a commercially available ELISA kit (Histamine EIA, Beckman Coulter). For measurement of PAF acetylhydrolase (PAF-AH) activity, spleens were collected 20 min after OVA challenge, weighed, and dissociated into single cells in 400 μL of cold buffer (0.1 M Tris-HCl pH 7.2). Cell suspensions were sonicated in ice for 10", centrifuged (20,000 x g for 10 min at 4 ^{o}C) and supernatant was collected for the assay. PAF-AH activity was measured in the supernatant using a commercially available assay according to the manufacturer's instructions (Cayman Chemical). Results were expressed as enzymatic activity/ mg spleen.

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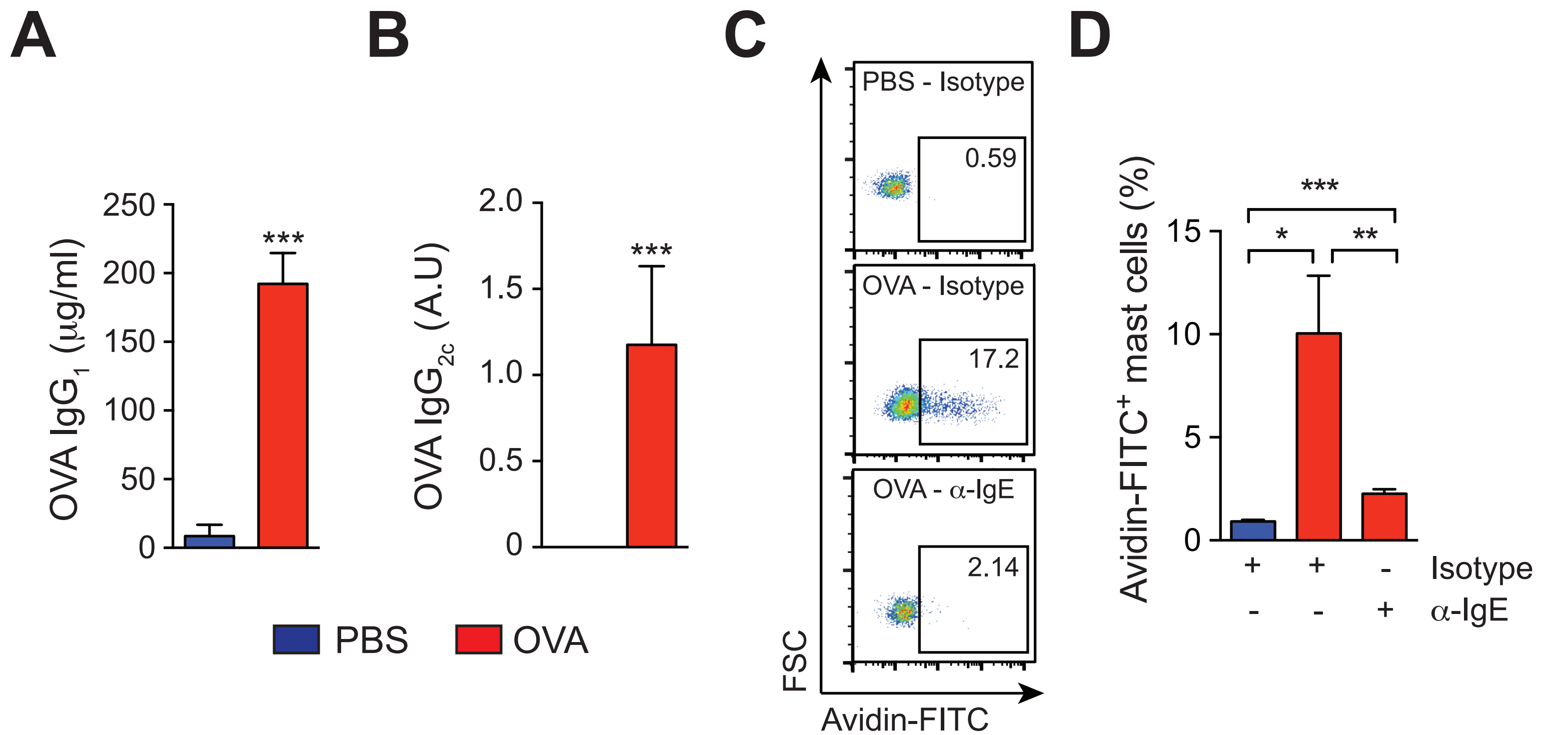

Figure E1. Concentrations of OVA-specific IgG_1 and IgG_{2c}, and functional activity of OVA-specific IgE, in serum from PBS-treated or OVA-sensitized C57BL/6 mice. (**A**, **B**) Concentrations of OVA-specific IgG_1 (**A**) and OVA-specific IgG_{2c} (**B**) in serum samples collected 24 h before challenge in PBS-treated or OVA-sensitized C57BL/6 mice (A.U: Arbitrary Units). (**C**, **D**) Serum from PBS-treated or OVA-sensitized mice was incubated for 30 min with an anti-IgE antibody (to block soluble IgE) or an isotype control antibody, as indicated. Samples were then incubated overnight with peritoneal cell-derived cultured MCs (PCMCs). PCMCs were washed and stimulated for 30 min with OVA. MC degranulation was monitored by FACS analysis using avidin-fluorescein isothiocyanate ('Avidin-FITC') to stain membrane-bound exocytosed granules. Representative FACS profiles (**C**) and percentage of Avidin-FITC$^+$ degranulated cells (**D**) are shown. Data are pooled from serum samples collected from two ('PBS' group, total *n*=5) or three ('OVA' group, total *n*=10) independent experiments. * or *** = *P* < 0.05 or 0.001 *vs.* corresponding control group or indicated group.

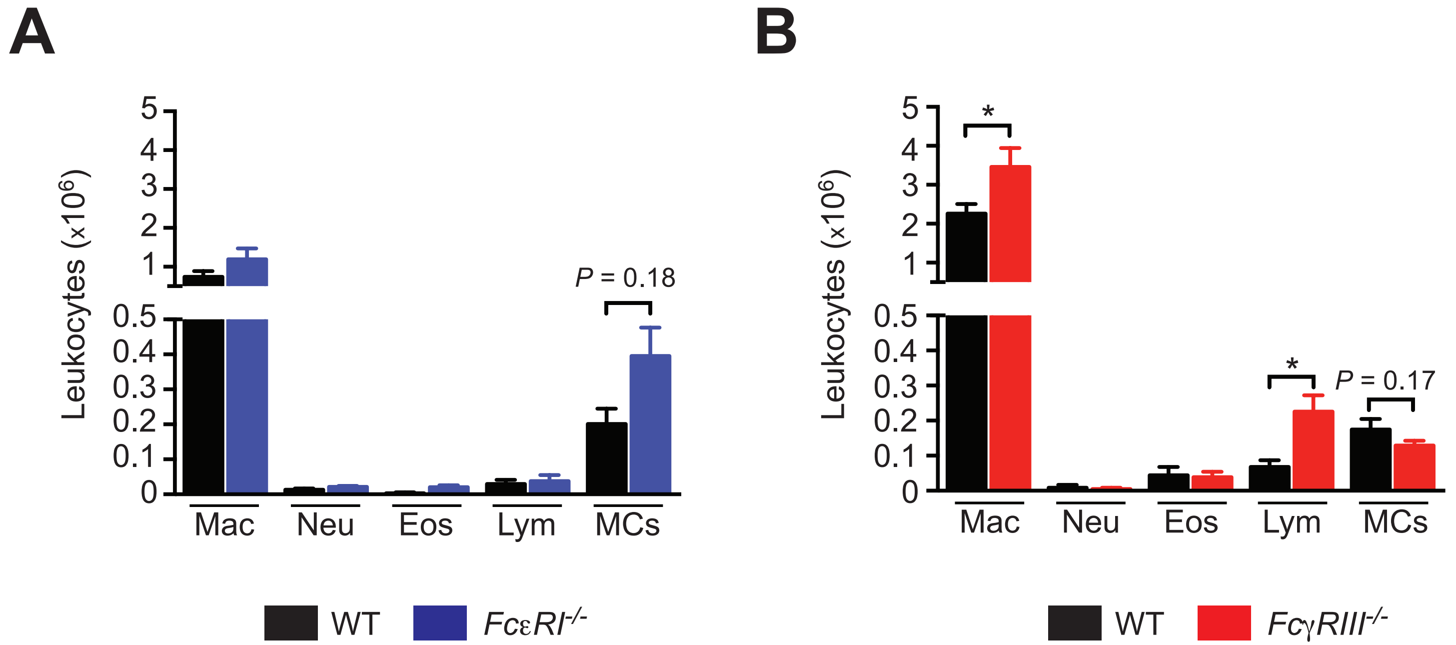

Figure E2. Baseline numbers of intra-peritoneal leukocytes in WT, *FcεRI*$^{-/-}$ and *FcγRIII*$^{-/-}$ mice. Numbers of leukocytes in the peritoneal lavage fluid of naïve WT and *FcεRI*$^{-/-}$ mice (**A**) or naïve WT and *FcγRIII*$^{-/-}$ mice (**B**) (*n*=6/group). * = $P < 0.05$. Mac: macrophages; Eos: eosinophils; Neu: neutrophils; Lym: lymphocytes; MCs: mast cells.

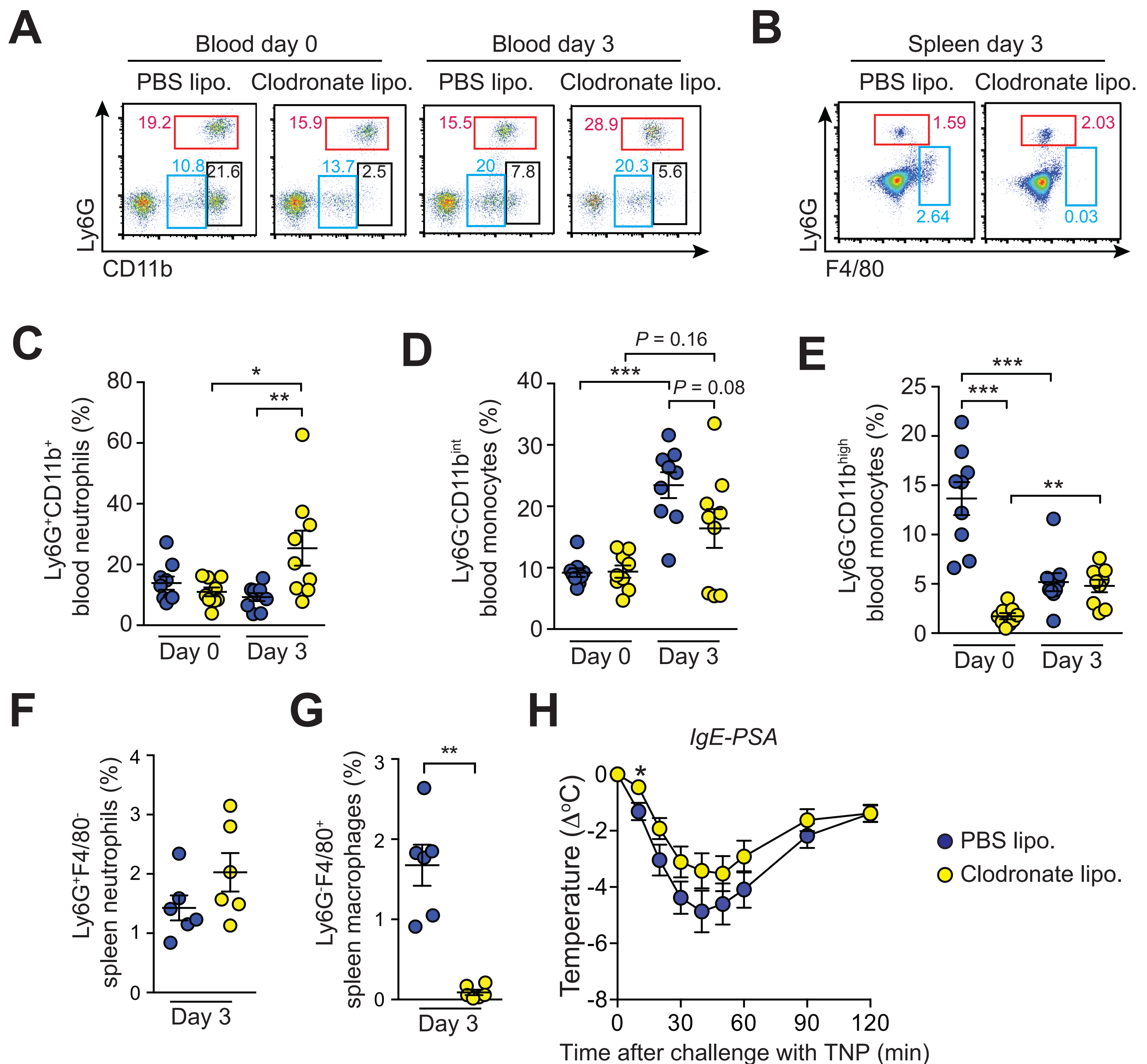

Figure E3. Effect of treatment with clodronate liposomes on macrophages, monocytes, neutrophils and MCs in C57BL/6 mice. OVA-sensitized mice were treated with clodronate liposomes (150 µg i.p. + 150 µg i.v.) or the same amount of PBS liposomes (as a control) 24 h before challenge with OVA. (**A**) Representative FACS profile showing Ly6G$^-$CD11b^{high} monocytes (black rectangles), Ly6G$^-$CD11b^{int} monocytes (blue rectangles) and Ly6G$^+$CD11b$^+$ neutrophils (red rectangles) 1 h before ('Blood day 0') and 3 days after ('Blood day 3') challenge with OVA. (**B**) Representative FACS profile showing Ly6G$^-$F4/80$^+$ macrophages (blue rectangles) and Ly6G$^+$F4/80$^-$ neutrophils (red rectangles) 3 days after challenge with OVA. Numbers in **A** and **B** indicate percentage of each cell population. (**C-G**) Percentage of blood neutrophils (**C**), CD11b intermediate blood monocytes (**D**), CD11b high blood monocytes (**E**), spleen neutrophils (**F**), and spleen macrophages (**G**) at the indicated time-points. (**H**) IgE-mediated PSA in mice treated with clodronate liposomes of PBS liposomes. FACS profiles in **A** and **B** are representative of three and two independent experiments, respectively. Data are pooled from two (total *n*=6-8/group) (**C-E**, **H**) or three (total *n*=9/group) (**F** & **G**) independent experiments. *, ** or *** = $P < 0.05$, 0.01 or 0.001 *vs*. indicated group.

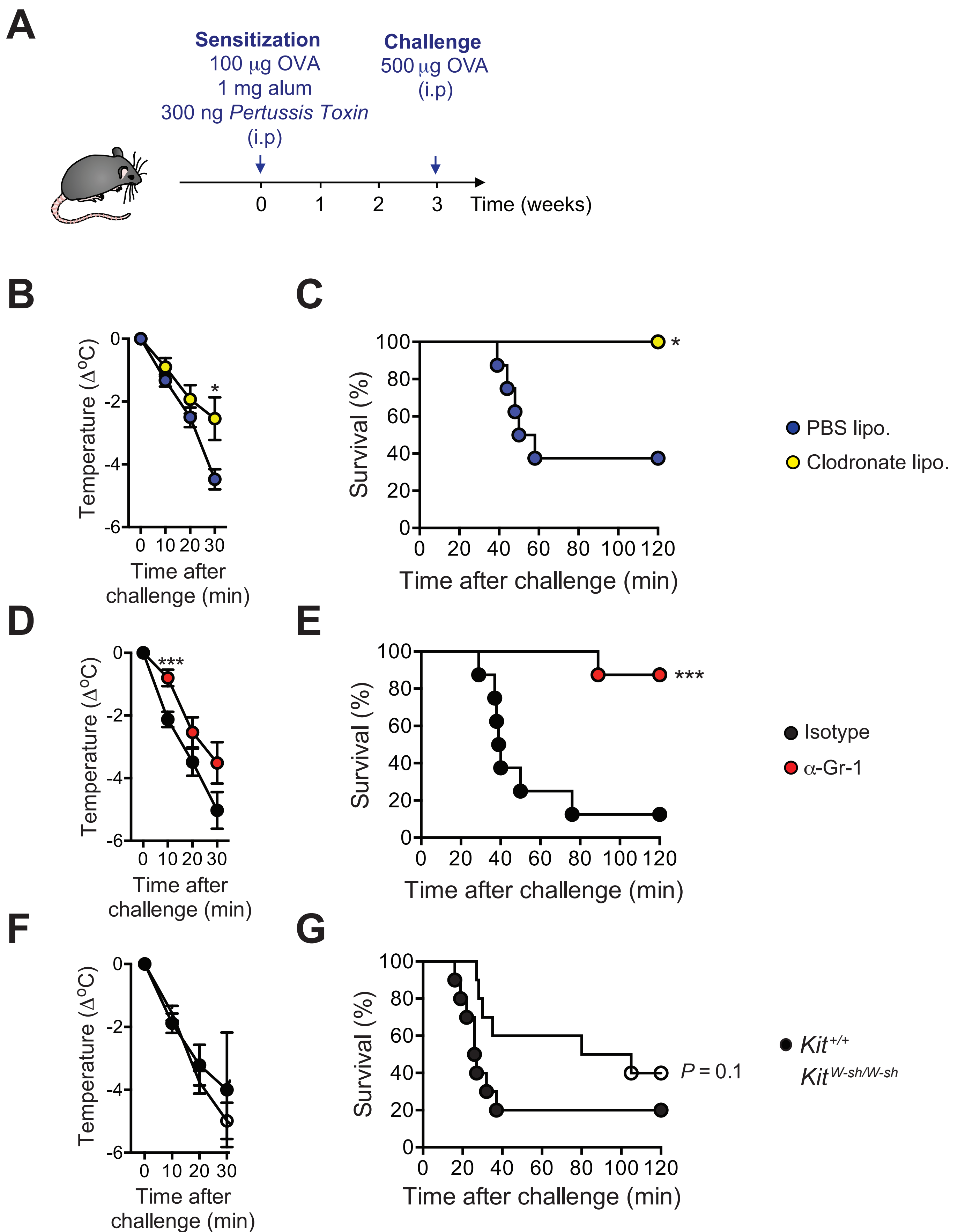

Figure E4. ASA in monocyte/macrophage-depleted mice, neutrophil-depleted mice, and MC-deficient mice sensitized with OVA together with alum and *Bordetella pertussis* toxin. (**A**) Experimental outline. For monocyte/macrophage depletion, OVA-sensitized mice were treated with clodronate liposomes or PBS liposomes (as a control) 24 h before challenge with OVA. For neutrophil depletion, OVA-sensitized mice were treated with an anti-Gr-1 antibody or an isotype control 40 h before challenge with OVA. (**B-G**) OVA-induced hypothermia (**B**, **D**, **F**) and survival (**C**, **E**, **G**) in the indicated group of mice. Data are pooled from two independent experiments (*n*=8-10/group). * or *** = $P < 0.05$ or 0.001 using an unpaired Student's *t* test (**B**, **D**, **F**) or a Mantel-Cox log-rank test (**C**, **E**, **G**).

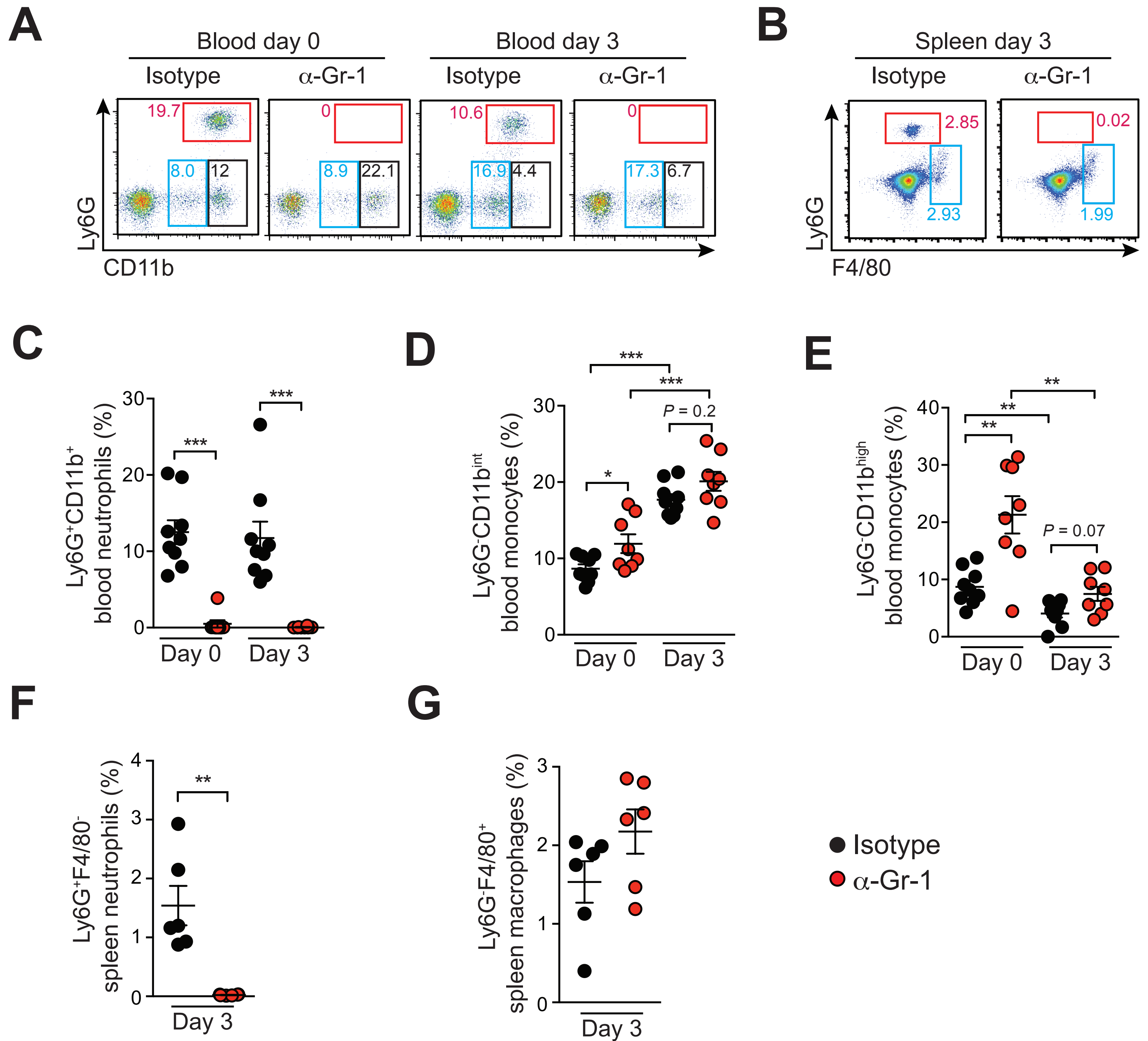

Figure E5. Effect of treatment with an anti-Gr-1 antibody on macrophages, monocytes and neutrophils in C57BL/6 mice. OVA-sensitized mice were treated with an anti-Gr-1 neutrophil-depleting antibody (150 µg) or the same amount of an isotype control antibody 40 h before and 24 h after challenge with OVA. (**A**) Representative FACS profile showing Ly6G$^-$CD11b^{high} monocytes (black rectangles), Ly6G$^-$CD11b^{int} monocytes (blue rectangles) and Ly6G$^+$CD11b$^+$ neutrophils (red rectangles) 1 h before ('Blood day 0') and 3 days after ('Blood day 3') challenge with OVA. (**B**) Representative FACS profile showing Ly6G$^-$F4/80$^+$ macrophages (blue rectangles) and Ly6G$^+$F4/80$^-$ neutrophils (red rectangles) 3 days after challenge with OVA. Numbers in **A** and **B** indicate percentage of each cell population. (**C**-**G**) Percentage of blood neutrophils (**C**), CD11b intermediate blood monocytes (**D**), CD11b high blood monocytes (**E**), spleen neutrophils (**F**), and spleen macrophages (**G**) at the indicated time-point. FACS profiles in **A** and **B** are representative of three and two independent experiments, respectively. Data are pooled from two (total *n*=6/group) (**C**-**E**) or three (total *n*=8-9/group) (**F** & **G**) independent experiments. *, ** or *** = $P < 0.05$, 0.01 or 0.001 *vs*. indicated group.

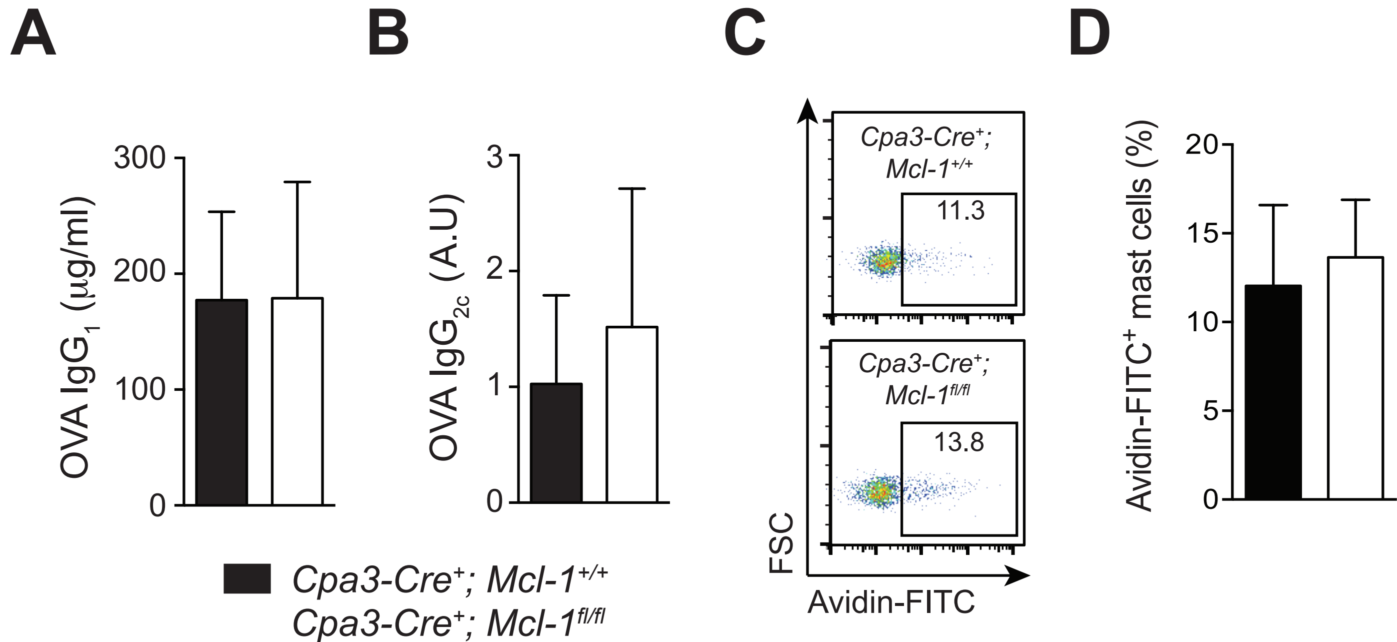

Figure E6. Concentrations of OVA-specific IgG$_1$ and IgG$_{2c}$, and functional activity of OVA-specific IgE, in serum from OVA-sensitized MC- and basophil-deficient *Cpa3-Cre$^+$; Mcl-1$^{fl/fl}$* mice. (**A**, **B**) Concentrations of OVA-specific IgG$_1$ (**A**) and OVA-specific IgG$_{2c}$ (A.U: Arbitrary Units). (**B**) in serum samples collected 24 h before challenge in OVA-sensitized mice. (**C**, **D**) PCMCs were incubated overnight with serum from OVA sensitized *Cpa3-Cre$^+$; Mcl-1$^{fl/fl}$* mice or littermate controls (*Cpa3-Cre$^+$; Mcl-1$^{+/+}$*). PCMCs were washed and stimulated for 30 min with OVA. MC degranulation was monitored by FACS analysis using avidin-fluorescein isothiocyanate ('Avidin-FITC') to stain membrane-bound exocytosed granules. Representative FACS profiles (**C**) and percentage of Avidin-FITC$^+$ degranulated cells (**D**) are shown. Data are pooled from serum samples collected from three independent experiments (total *n*=9-14/group). Differences between groups are not significant (P > 0.05).

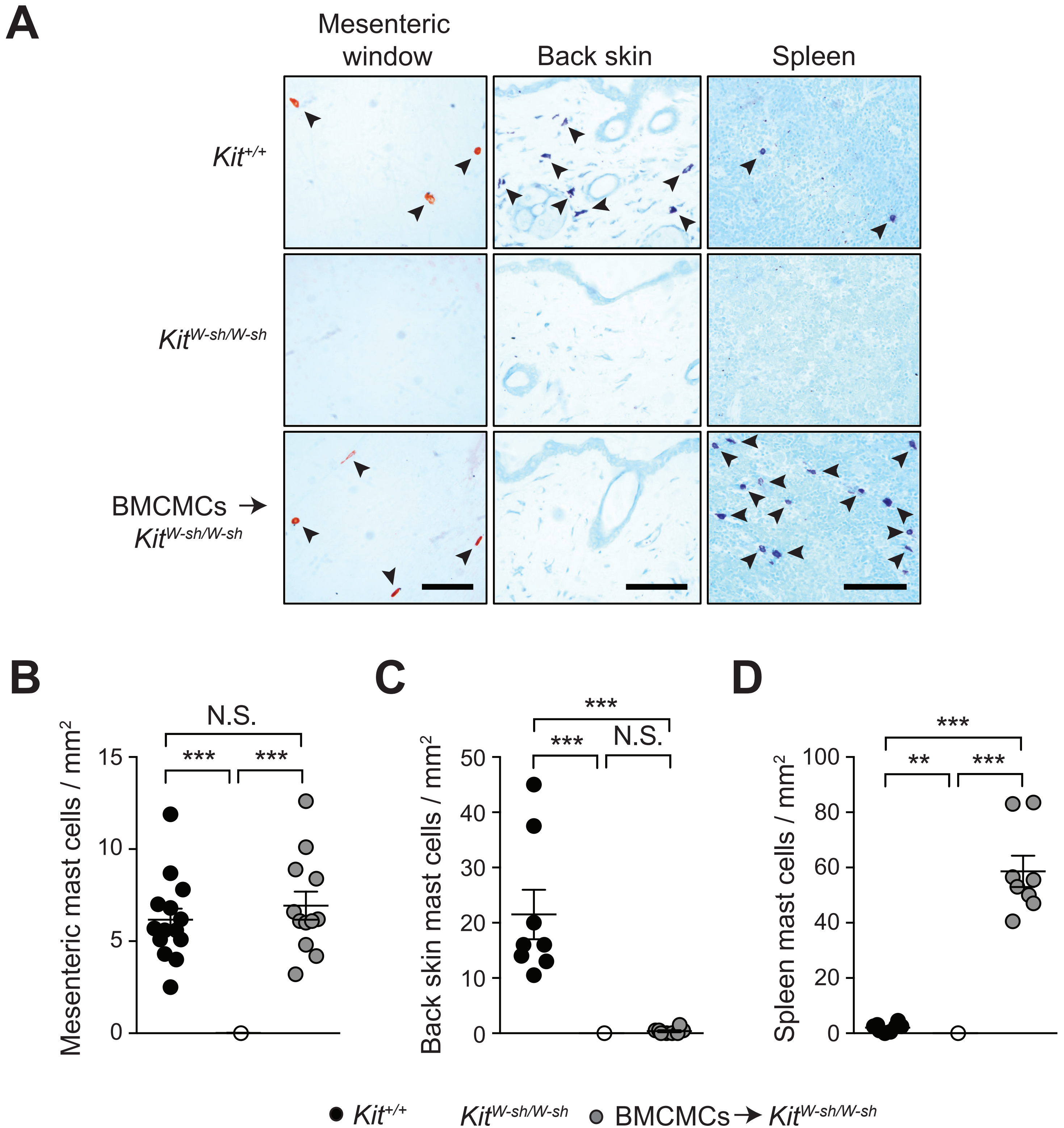

Figure E7. Distribution and numbers of MCs in the skin, spleen and mesenteric windows of Kit+/+ WT mice, MC-deficient *KitW-sh/W-sh* mice, and *KitW-sh/W-sh* mice engrafted with WT bone marrow-derived cultured MCs (BMCMCs). Tissue samples were collected 3 days after challenge with OVA in OVA-sensitized mice. (**A**-**D**) Representative pictures (**A**) and quantification of MCs in the mesentery ('mesenteric window') (**B**), back skin (**C**) and spleen (**D**). MCs in the mesentery ('Csaba' stain), back skin and spleen (Toluidine blue stain) are indicated by arrows in photomicrographs. Pictures in **A** are representative of at least three independent experiments. Data in **B**-**D** are pooled from at least three independent experiments (total *n*=8-14/group). ** or *** = $P < 0.01$ or 0.001 *vs*. indicated group. N.S.: not significant ($P > 0.05$).

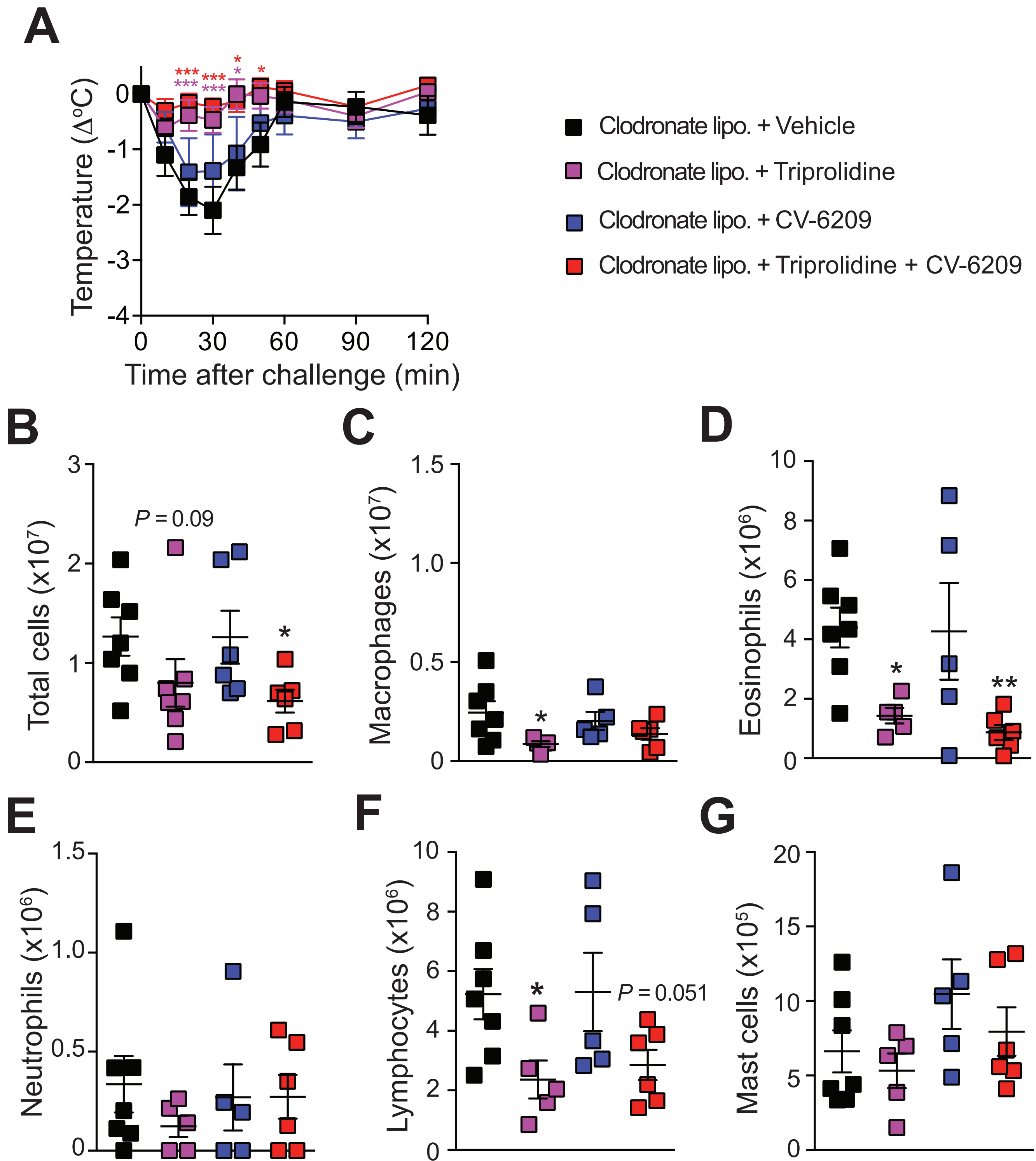

Figure E8. Roles of histamine and PAF in OVA-induced ASA in monocyte/macrophage-depleted mice. OVA-sensitized mice were treated with clodronate liposomes 24 h before challenge with OVA. Mice were then treated with the H1 anti-histamine triprolidine or with the PAF receptor antagonist CV-6209 alone or in combination (or vehicle [saline] as a control) 30 min before and 1 day after challenge with OVA. (**A**) OVA-induced hypothermia. (**B**-**G**) Numbers of leukocytes in the peritoneal lavage fluid 3 days after OVA challenge. Data are pooled from two independent experiments (total *n*=6-8/group). *, ** or *** = $P < 0.05$, 0.01 or 0.001 *vs*. vehicle group.