

ONLINE REPOSITORY

Figure legends

Fig E1. Levels of PN-specific IgE and IgG₁ in serum from PN-sensitized WT and *Kit^{W-sh/W-sh}* mice. Serum from PN-sensitized C57BL/6J mice (black circles; $n=15$) and MC-deficient *Kit^{W-sh/W-sh}* mice (white circles; $n=13-15$) was collected 24 hours before PN challenge. Levels of PN-specific IgE (A) and PN-specific IgG₁ (B) were measured by ELISA. Data are mean \pm SEM.

Fig E2. Effect of treatment with an anti-Ly6G antibody on blood monocytes, eosinophils and basophils in WT and *Kit^{W-sh/W-sh}* mice. PN-sensitized C57BL/6J mice (WT) and MCdeficient *Kit^{W-sh/W-sh}* mice were treated i.p. with 500 μ g of neutrophil-depleting anti-Ly6G antibody (α -Ly6G; $n=5-8$) or an isotype control (Isotype control; $n=5$) 24 hours before the challenge with PN. Blood was isolated from all groups of mice 1 hour before PN-challenge and blood leukocytes were analyzed by flow cytometry. (A) Gating strategy used to distinguish neutrophils (Gr-1⁺; CD11b⁺; Siglec-F⁻), monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻), eosinophils (SSC^{high}; Siglec-F⁺) and basophils (CD49b⁺; IgE⁺). (B) Percentage of blood monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻). (C) Percentage of blood eosinophils (SSC^{high}; Siglec-F⁺). (D) Percentage of blood basophils (CD49b⁺; IgE⁺). Data are mean + SEM; * or *** = $P < 0.05$ or < 0.001 vs. corresponding isotype control-treated group; # = $P < 0.05$ vs. indicated group (unpaired Student's t test).

Fig E3. Effect of treatment with diphtheria toxin on blood basophils, monocytes, eosinophils and neutrophils in *Mcpt5-Cre*; *iDTR^{fl/+}* mice. *Mcpt5-Cre*; *iDTR^{fl/+}* mice (Cre ; $n=9$) and *Mcpt5-Cre*; *iDTR^{fl/+}* mice (Cre ; $n=5$) mice were treated i.p. with 500 ng of diphtheria toxin once a week starting at the first oral sensitization with PN. Blood was isolated from all groups of mice 1 hour before PN-challenge and blood leukocytes were analyzed by flow cytometry. (A) Percentage of blood basophils (CD49b⁺; IgE⁺). (B) Percentage of blood monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻). (C) Percentage of blood eosinophils (SSC^{high}; Siglec-F⁺). (D) Percentage of blood neutrophils (Gr-1^{high}; CD11b⁺; Siglec-F⁻). Data are mean + SEM. N.S.: not significant ($P > 0.05$ by unpaired Student's t test).

Fig E4. Levels of PN-specific IgE and IgG₁ in serum from DT-treated PN-sensitized *Mcpt5-Cre*; *iDTR⁺* and *Mcpt5-Cre*; *iDTR⁺* mice. Serum from PN-sensitized *Mcpt5-Cre*; *iDTR⁺* mice (black circles; $n=11-13$) and MC-depleted *Mcpt5-Cre*; *iDTR⁺* mice (red circles; $n=5-13$) was collected 24 hours before PN challenge. Levels of PN-specific IgE (A) and PN-specific IgG₁ (B) were measured by ELISA. Data are mean \pm SEM.

Fig E5. Effect of treatment with the basophil-depleting antibody Ba103 on blood monocytes, eosinophils and neutrophils. PN-sensitized C57BL/6J mice were treated i.p. with 50 μ g of a basophil-depleting Ba103 antibody ($n=9$) or an isotype control (Isotype control; $n=7$) 48 hours before PN challenge. Blood was isolated from all groups of mice 1 hour before PN challenge and blood leukocytes were analyzed by flow cytometry. (A) Percentage of blood monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻). (B) Percentage of blood eosinophils (SSC^{high}; Siglec-F⁺). (C) Percentage of blood

neutrophils (Gr-1^{high}; CD11b⁺; Siglec-F⁻). Data are mean + SEM. N.S.: not significant ($P > 0.05$ by unpaired Student's t test).

Fig E6. Effect of treatment with diphtheria toxin on blood monocytes, eosinophils and neutrophils in *Mcpt8^{DTR}* mice. PN-sensitized *Mcpt8^{+/+}* ($n=9$) and *Mcpt8^{DTR/+}* ($n=9$) mice were treated i.p. with 500 μ g of diphtheria toxin 48 hours before PN challenge. Blood was isolated from all groups of mice 1 hour before PN-challenge and blood leukocytes were analyzed by flow cytometry. (A) Percentage of blood monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻). (B) Percentage of blood eosinophils (SSC^{high}; Siglec-F⁺). (C) Percentage of blood neutrophils (Gr-1^{high}; CD11b⁺; Siglec-F⁻). Data are mean + SEM. N.S.: not significant ($P > 0.05$ by unpaired Student's t test).

Fig E7. Levels of PN-specific IgE and IgG₁ in serum from PN-sensitized *Cpa3-Cre⁺;Mcl-1^{+/+}* and *Cpa3-Cre⁺;Mcl-1^{fl/fl}* mice. Serum from PN-sensitized *Cpa3-Cre⁺;Mcl-1^{+/+}* (black circle; $n=6-7$) and MC- and basophil-deficient and *Cpa3-Cre⁺;Mcl-1^{fl/fl}* (grey circle; $n=5-6$) was collected 24 hours before the challenge. Levels of PN-specific IgE (A) and PN-specific IgG₁ (B) were measured by ELISA. Data are mean \pm SEM.

Fig E8. Responses of WT mice, MC-deficient *Kit^{W-sh/W-sh}* mice, and *Kit^{W-sh/W-sh}* mice engrafted with bone marrow-derived cultured MCs in a moderate model of peanut-induced anaphylaxis. C57BL/6J mice (WT), MC-deficient C57BL/6J-*Kit^{W-sh/W-sh}* mice (*Kit^{W-sh/W-sh}*) and C57BL/6J-*Kit^{W-sh/W-sh}* mice engrafted with WT BMCMCs (2×10^6 cells i.p. + 10^7 cells i.v., 12 weeks before the first sensitization with PN) (WT BMCMCs \square *Kit^{W-sh/W-sh}*) were sensitized orally once a week for 4 weeks with 10 mg PN + 10 μ g cholera toxin. Mice were challenged with 2.5 mg PN i.p. two weeks after the last sensitization. (A) PN-induced hypothermia. Crossbones symbol indicates death of one mouse. (B) MCs in the peritoneal lavage fluid or mesentery ("Mesenteric windows") are indicated by arrows in photomicrographs of cells from the peritoneal cavity (May-Grunwald-Giemsa stain) or of the mesenteric windows (Csaba stain). (C-D) Numbers of MCs in the peritoneal lavage fluid (C) and mesenteric windows (D). (E-F) Toluidine blue staining for MCs (E) and MC numbers (F) in sections of forestomach, glandular stomach, lung, spleen and back skin. Data in A, C, D & F are mean \pm SEM or mean + SEM from $n=8-14$ (A) or $n=4-6$ (C, D & F) mice / group. #, ## or ### = $P < 0.05$, 0.01 or 0.001 vs. *Kit^{W-sh/W-sh}* group (in A); *, ** or *** = $P < 0.05$, 0.01 or 0.001 vs. WT group (in A) or for comparisons indicated (in C, D & F); N.S. = not significant ($P > 0.05$) (unpaired Student's t test).

Fig E9. Effect of basophil depletion in a moderate model of peanut-induced anaphylaxis. All mice were sensitized orally once a week for 4 weeks with 10 mg PN + 10 μ g cholera toxin, and challenged with 2.5 mg PN i.p. two weeks after the last sensitization. (A-B) Percentage of blood basophils (CD49b⁺; IgE⁺) in blood samples collected 1 hour before challenge with PN (A) and PN-induced hypothermia (B) in PN-sensitized C57BL/6J mice treated i.p. with 50 μ g of basophil-depleting antibodies Ba103 ($n=7$) or isotype control antibodies ($n=8$) 48 h before PN challenge. (C-D) Percentage of blood basophils (CD49b⁺; IgE⁺) in blood samples collected 1 hour before challenge with PN (C) and PN-induced hypothermia (D) in PN-sensitized *Mcpt8^{+/+}* ($n=6$) and *Mcpt8^{DTR/+}*

($n=7$) mice treated with 500 ng DT 48 h before challenge with PN. $P > 0.05$ by unpaired Student's t test for all time points. Crossbones symbol indicates death of one isotype control antibody-treated mouse.

ACCEPTED MANUSCRIPT

Figure E1

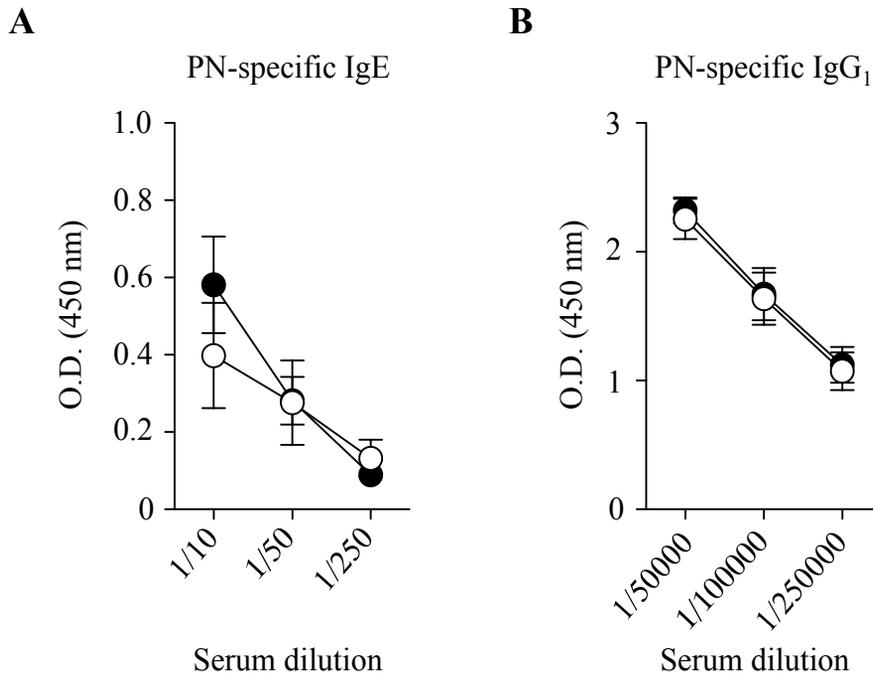


Fig E1. Levels of PN-specific IgE and IgG₁ in serum from PN-sensitized WT and *Kit^{W-sh/W-sh}* mice. Serum from PN-sensitized C57BL/6J mice (black circles; $n=15$) and MC-deficient *Kit^{W-sh/W-sh}* mice (white circles; $n=13-15$) was collected 24 hours before PN challenge. Levels of PN-specific IgE (**A**) and PN-specific IgG₁ (**B**) were measured by ELISA. Data are mean \pm SEM.

Figure E2

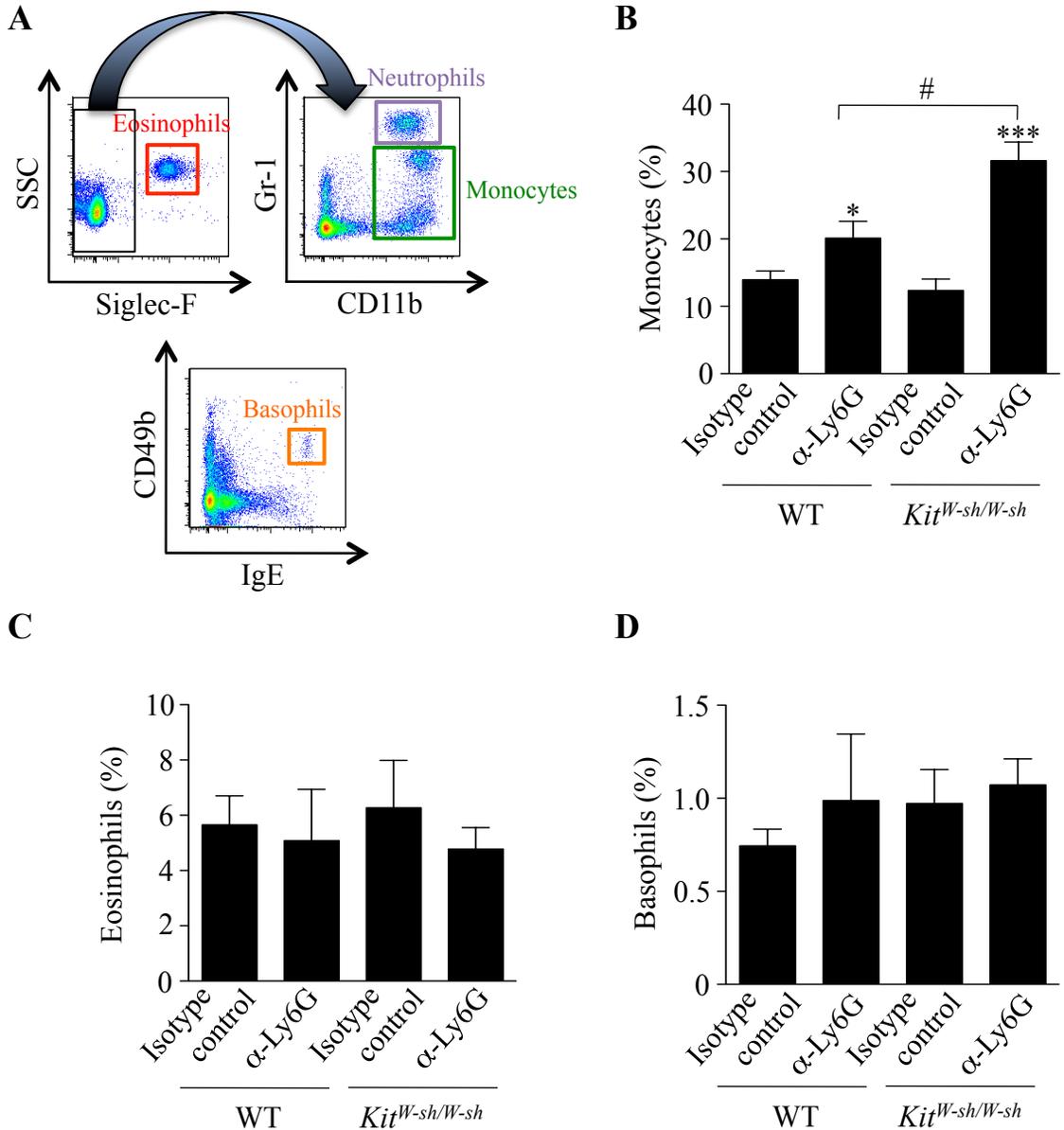


Fig E2. Effect of treatment with an anti-Ly6G antibody on blood monocytes, eosinophils and basophils in WT and *Kit^{W-sh/W-sh}* mice. PN-sensitized C57BL/6J mice (WT) and MC-deficient *Kit^{W-sh/W-sh}* mice were treated i.p. with 500 μ g of neutrophil-depleting anti-Ly6G antibody (α -Ly6G; $n=5-8$) or an isotype control (Isotype control; $n=5$) 24 hours before the challenge with PN. Blood was isolated from all groups of mice 1 hour before PN-challenge and blood leukocytes were analyzed by flow cytometry. (A) Gating strategy used to distinguish neutrophils (Gr-1⁺; CD11b⁺; Siglec-F⁻), monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻), eosinophils (SSC^{high}; Siglec-F⁺) and basophils (CD49b⁺; IgE⁺). (B) Percentage of blood monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻). (C) Percentage of blood eosinophils (SSC^{high}; Siglec-F⁺). (D) Percentage of blood basophils (CD49b⁺; IgE⁺). Data are mean + SEM; * or *** = $P < 0.05$ or < 0.001 vs. corresponding isotype control-treated group; # = $P < 0.05$ vs. indicated group (unpaired Student's t test).

Figure E3

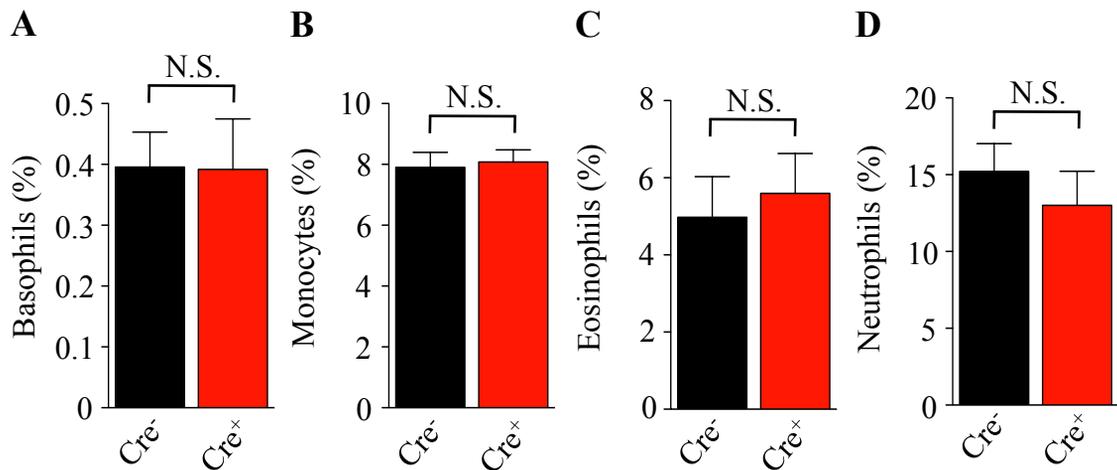


Fig E3. Effect of treatment with diphtheria toxin on blood basophils, monocytes, eosinophils and neutrophils in *Mcpt5-Cre*; *iDTR* mice. *Mcpt5-Cre*⁻; *iDTR*^{fl/+} mice (*Cre*⁻; $n=9$) and *Mcpt5-Cre*⁺; *iDTR*^{fl/+} mice (*Cre*⁺; $n=5$) mice were treated i.p. with 500 ng of diphtheria toxin once a week starting at the first oral sensitization with PN. Blood was isolated from all groups of mice 1 hour before PN-challenge and blood leukocytes were analyzed by flow cytometry. (A) Percentage of blood basophils (CD49b⁺; IgE⁺). (B) Percentage of blood monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻). (C) Percentage of blood eosinophils (SSC^{high}; Siglec-F⁺). (D) Percentage of blood neutrophils (Gr-1^{high}; CD11b⁺; Siglec-F⁻). Data are mean + SEM. N.S.: not significant ($P > 0.05$ by unpaired Student's *t* test).

Figure E4

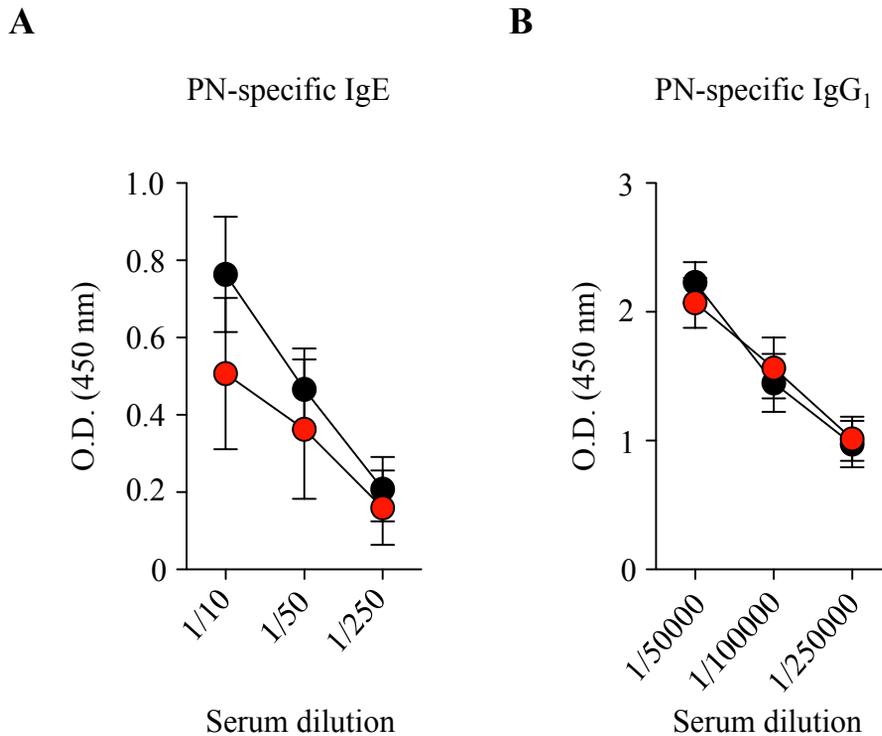


Fig E4. Levels of PN-specific IgE and IgG₁ in serum from DT-treated PN-sensitized *Mcpt5-Cre*; *iDTR*⁺ and *Mcpt5-Cre*⁺; *iDTR*⁺ mice. Serum from PN-sensitized *Mcpt5-Cre*; *iDTR*⁺ mice (black circles; *n*=11-13) and MC-depleted *Mcpt5-Cre*⁺; *iDTR*⁺ mice (red circles; *n*=5-13) was collected 24 hours before PN challenge. Levels of PN-specific IgE (A) and PN-specific IgG₁ (B) were measured by ELISA. Data are mean ± SEM.

Figure E5

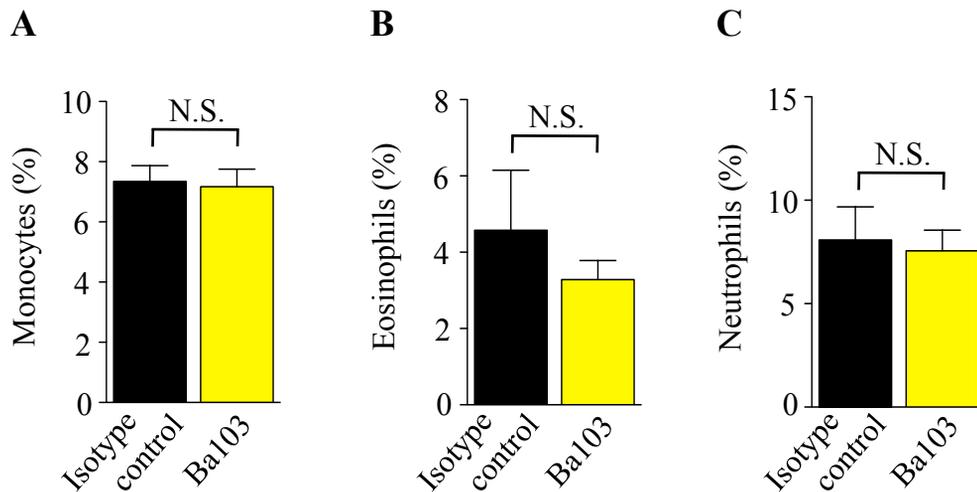


Fig E5. Effect of treatment with the basophil-depleting antibody Ba103 on blood monocytes, eosinophils and neutrophils. PN-sensitized C57BL/6J mice were treated i.p. with 50 μ g of a basophil-depleting Ba103 antibody ($n=9$) or an isotype control (Isotype control; $n=7$) 48 hours before PN challenge. Blood was isolated from all groups of mice 1 hour before PN-challenge and blood leukocytes were analyzed by flow cytometry. **(A)** Percentage of blood monocytes (Gr-1^{low} ; CD11b^+ ; Siglec-F^-). **(B)** Percentage of blood eosinophils (SSC^{high} ; Siglec-F^+). **(C)** Percentage of blood neutrophils ($\text{Gr-1}^{\text{high}}$; CD11b^+ ; Siglec-F^-). Data are mean + SEM. N.S.: not significant ($P > 0.05$ by unpaired Student's t test).

Figure E6

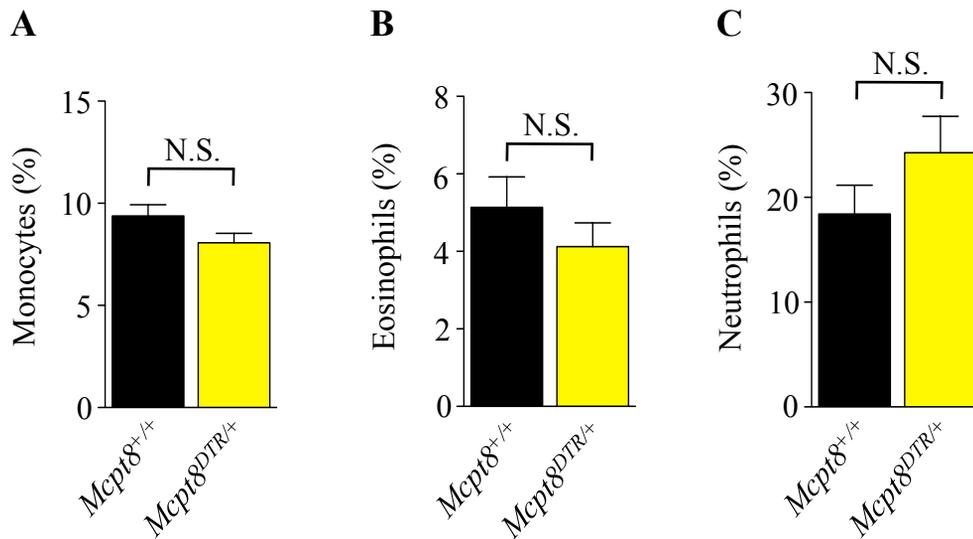


Fig E6. Effect of treatment with diphtheria toxin on blood monocytes, eosinophils and neutrophils in *Mcpt8*^{DTR} mice. PN-sensitized *Mcpt8*^{+/+} ($n=9$) and *Mcpt8*^{DTR/+} ($n=9$) mice were treated i.p. with 500 μ g of diphtheria toxin 48 hours before PN challenge. Blood was isolated from all groups of mice 1 hour before PN-challenge and blood leukocytes were analyzed by flow cytometry. (A) Percentage of blood monocytes (Gr-1^{low}; CD11b⁺; Siglec-F⁻). (B) Percentage of blood eosinophils (SSC^{high}; Siglec-F⁺). (C) Percentage of blood neutrophils (Gr-1^{high}; CD11b⁺; Siglec-F⁻). Data are mean + SEM. N.S.: not significant ($P > 0.05$ by unpaired Student's t test).

Figure E7

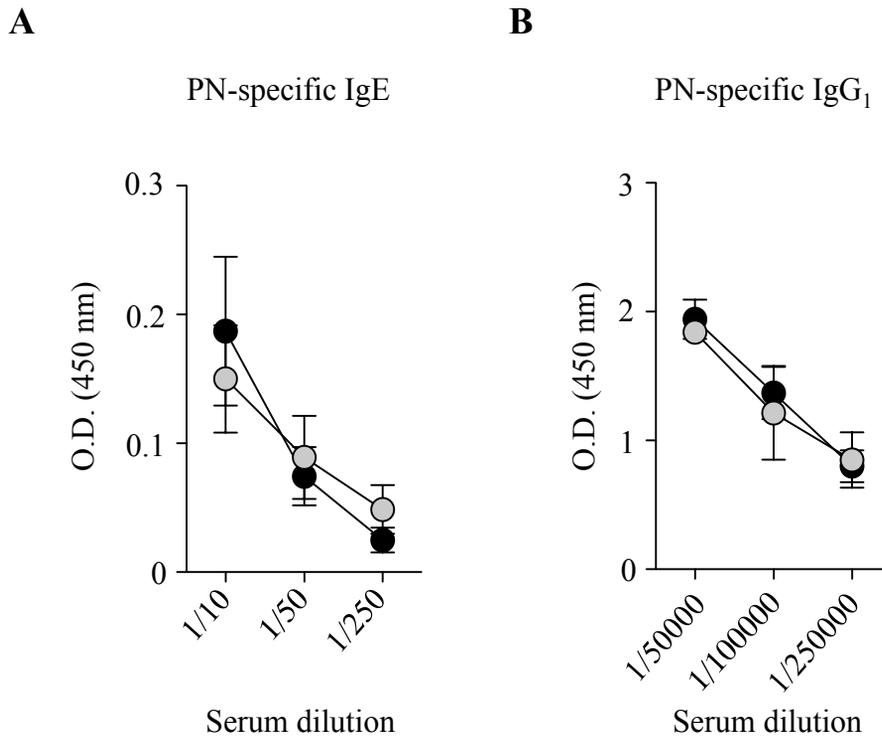
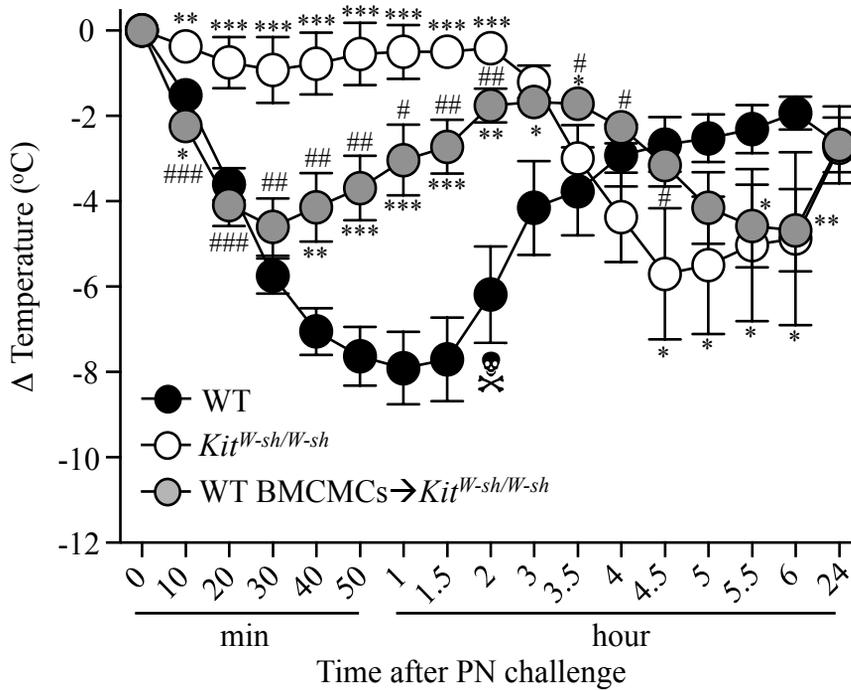


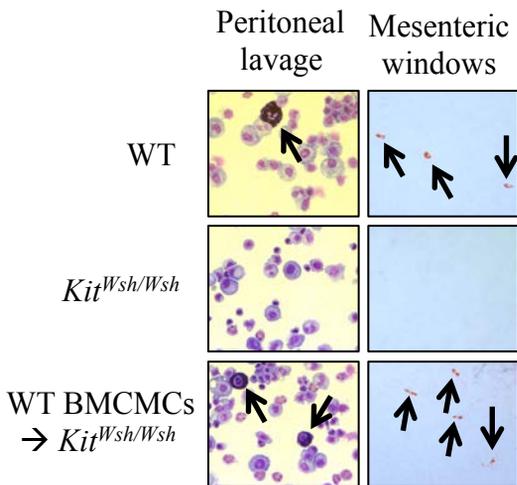
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Figure E8

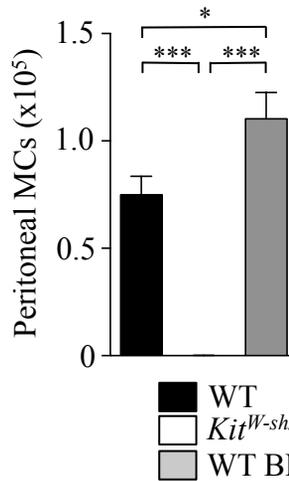
A



B



C



D

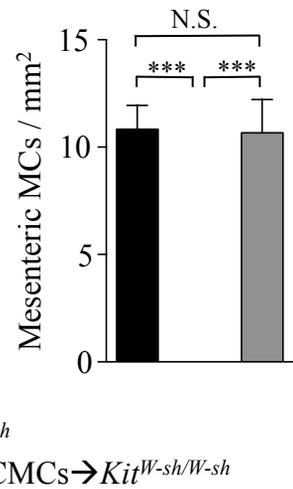
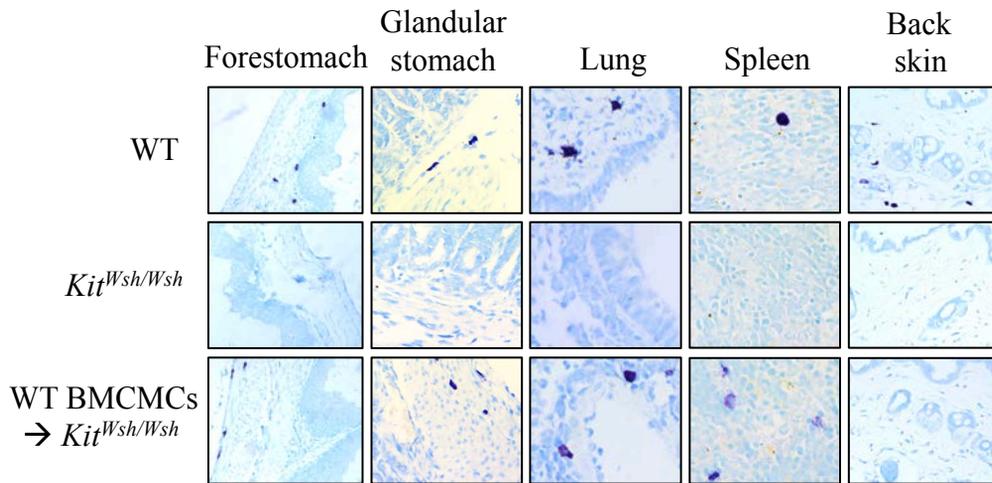


Figure E8, continued.

E



F

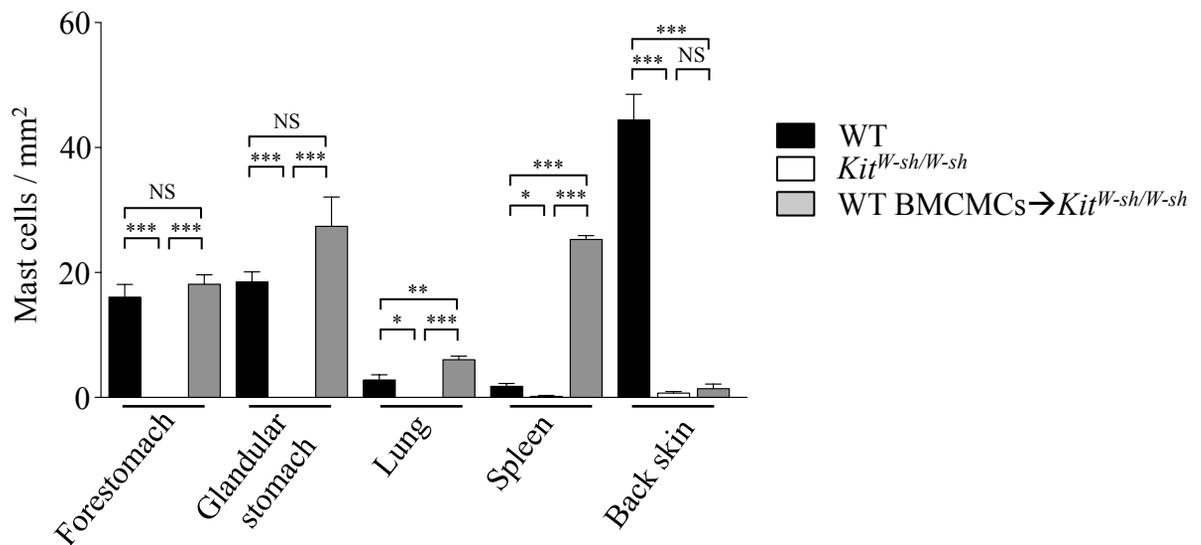


Fig E8. Responses of WT mice, MC-deficient *Kit^{Wsh/Wsh}* mice, and *Kit^{Wsh/Wsh}* mice engrafted with bone marrow-derived cultured MCs in a moderate model of peanut-induced anaphylaxis. C57BL/6J mice (WT), MC-deficient C57BL/6J-*Kit^{Wsh/Wsh}* mice (*Kit^{Wsh/Wsh}*) and C57BL/6J-*Kit^{Wsh/Wsh}* mice engrafted with WT BMCMCs (2×10^6 cells i.p. + 10^7 cells i.v., 12 weeks before the first sensitization with PN) (WT BMCMCs → *Kit^{Wsh/Wsh}*) were sensitized orally once a week for 4 weeks with 10 mg PN + 10 μ g cholera toxin. Mice were challenged with 2.5 mg PN i.p. two weeks after the last sensitization. (A) PN-induced hypothermia. Crossbones symbol indicates death of one mouse. (B) MCs in the peritoneal lavage fluid or mesentery (“Mesenteric windows”) are indicated by arrows in photomicrographs of cells from the peritoneal cavity (May-Grunwald-Giemsa stain) or of the mesenteric windows (Csaba stain). (C-D) Numbers of MCs in the peritoneal lavage fluid (C) and mesenteric windows (D). (E-F) Toluidine blue staining for MCs (E) and MC numbers (F) in sections of forestomach, glandular stomach, lung, spleen and back skin. Data in A, C, D & F are mean \pm SEM or mean + SEM from $n=8-14$ (A) or $n=4-6$ (C, D & F) mice / group. #, ## or ### = $P < 0.05$, 0.01 or 0.001 vs. *Kit^{Wsh/Wsh}* group (in A); *, ** or *** = $P < 0.05$, 0.01 or 0.001 vs. WT group (in A) or for comparisons indicated (in C, D & F); N.S. = not significant ($P > 0.05$) (unpaired Student’s *t* test).

Figure E9

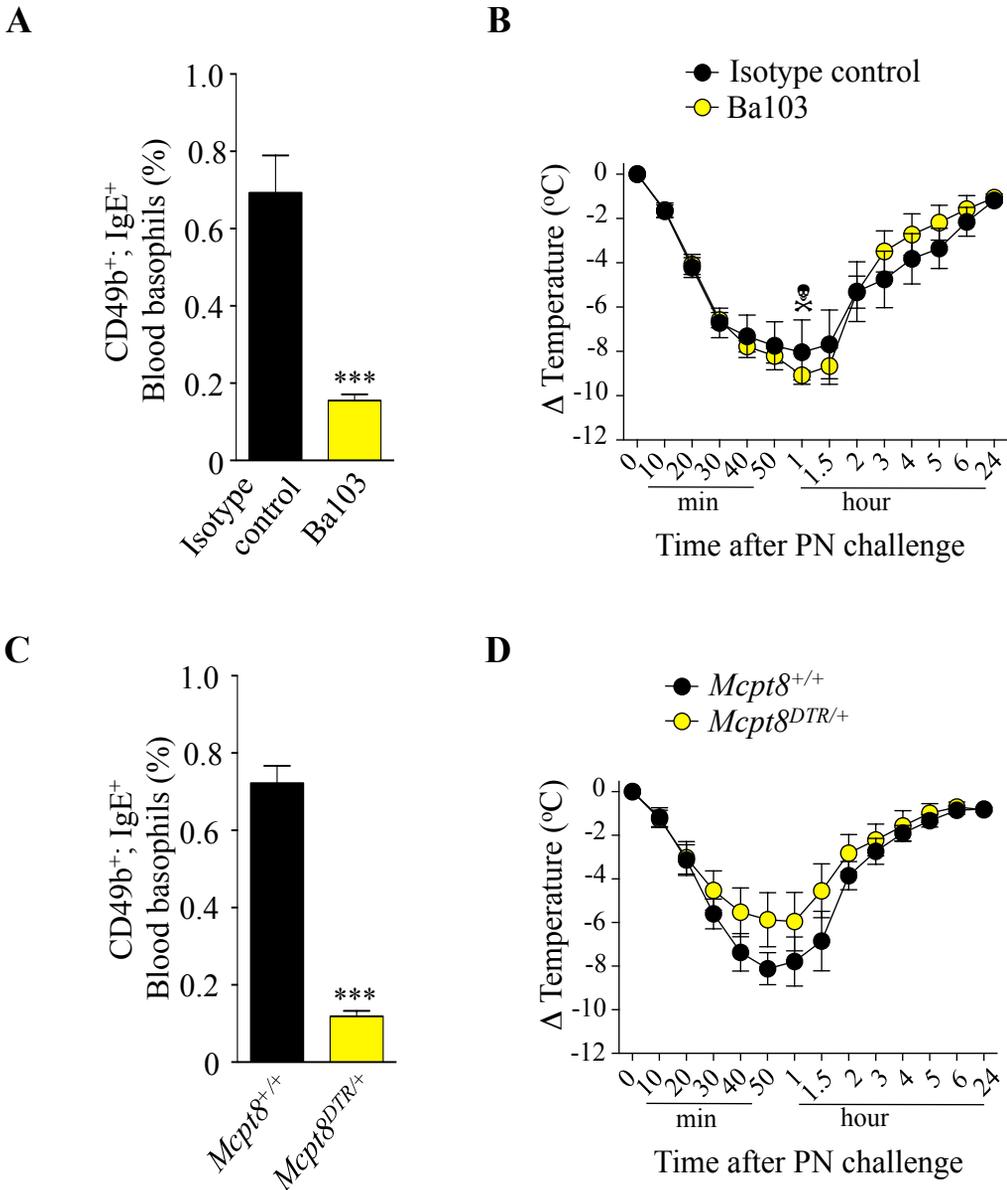


Fig E9. Effect of basophil depletion in a moderate model of peanut-induced anaphylaxis. All mice were sensitized orally once a week for 4 weeks with 10 mg PN + 10 μ g cholera toxin, and challenged with 2.5 mg PN i.p. two weeks after the last sensitization. **(A-B)** Percentage of blood basophils (CD49b⁺; IgE⁺) in blood samples collected 1 hour before challenge with PN **(A)** and PN-induced hypothermia **(B)** in PN-sensitized C57BL/6J mice treated i.p. with 50 μ g of basophil-depleting antibodies Ba103 ($n=7$) or isotype control antibodies ($n=8$) 48 h before PN challenge. **(C-D)** Percentage of blood basophils (CD49b⁺; IgE⁺) in blood samples collected 1 hour before challenge with PN **(C)** and PN-induced hypothermia **(D)** in PN-sensitized *Mcpt8*^{+/+} ($n=6$) and *Mcpt8*^{DTR/+} ($n=7$) mice treated with 500 ng DT 48 h before challenge with PN. $P > 0.05$ by unpaired Student's *t* test for all time points. Crossbones symbol indicates death of one isotype control antibody-treated mouse.