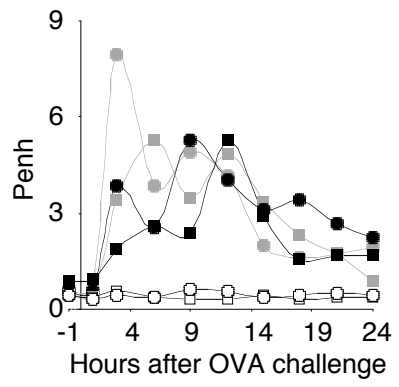
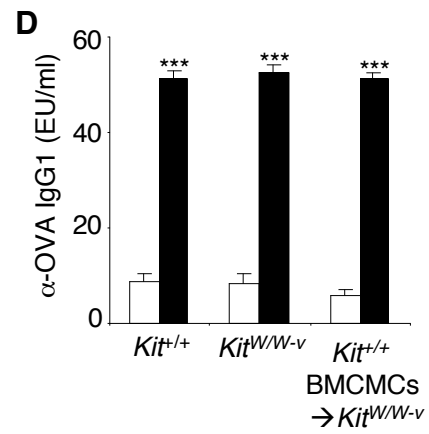
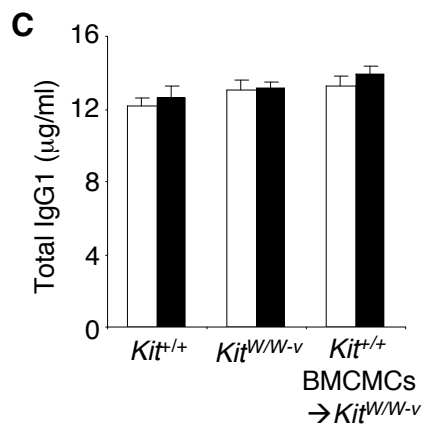
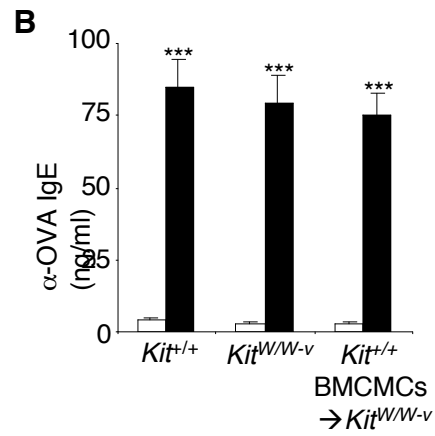
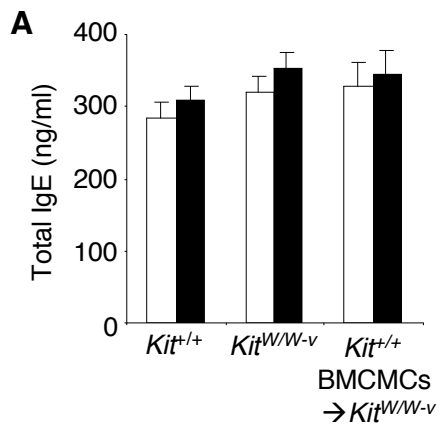


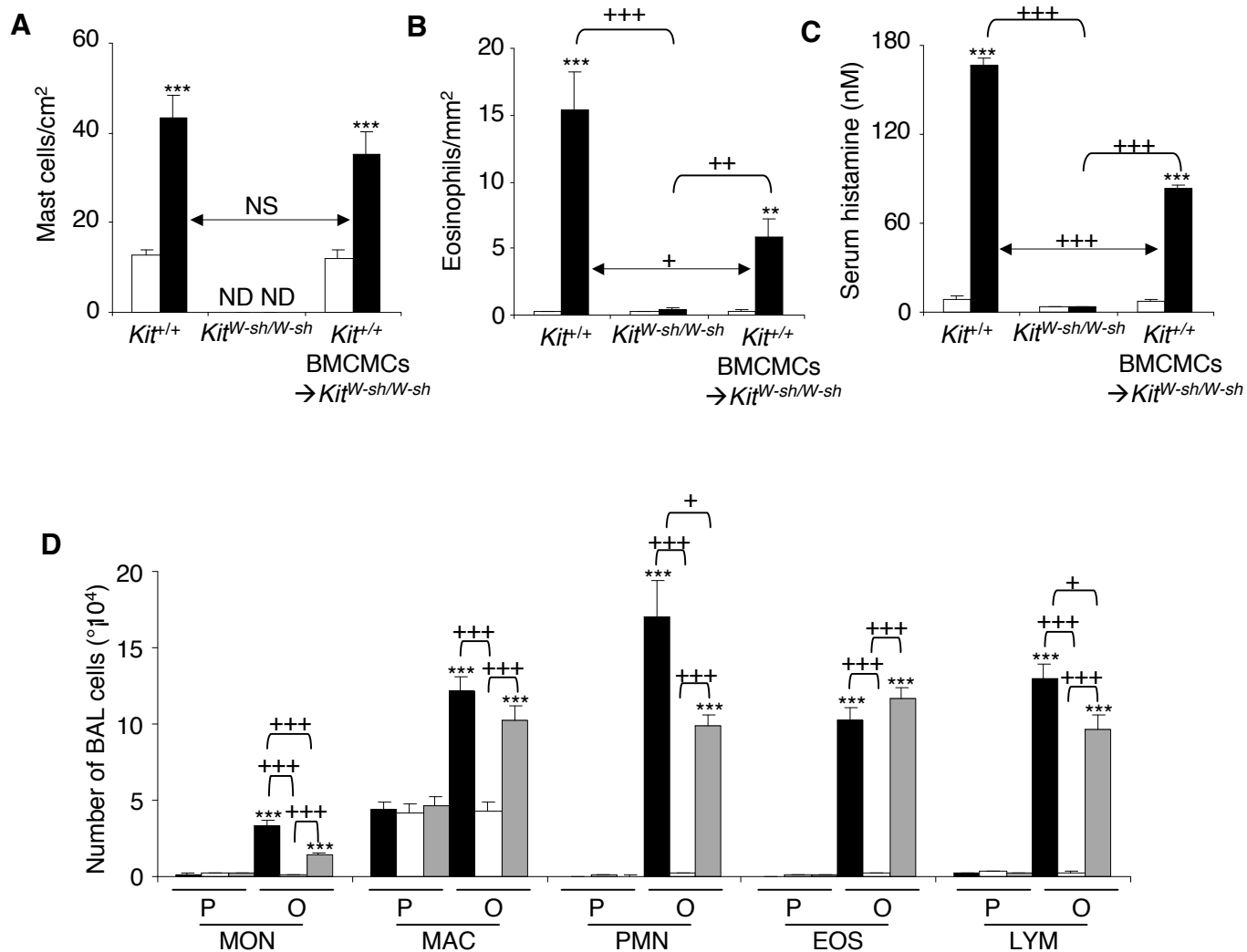
Supplemental Figure 1



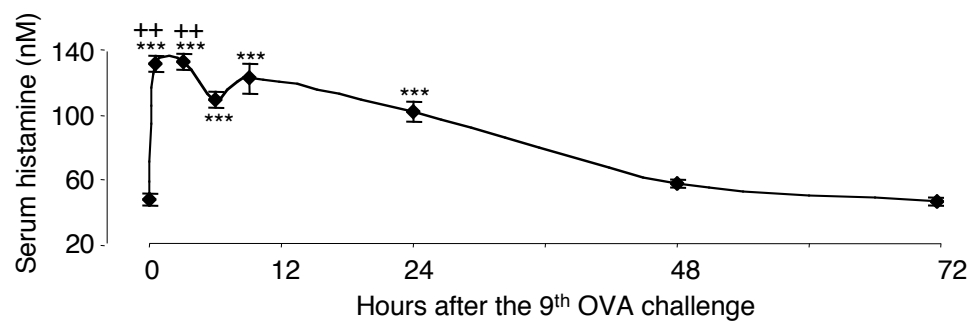
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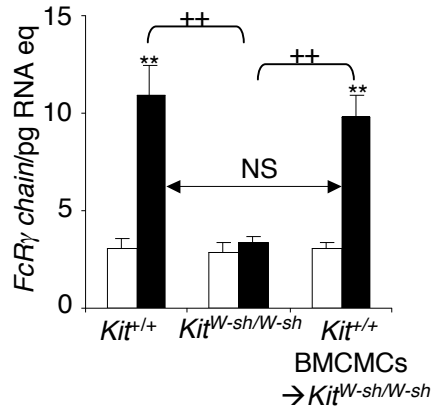
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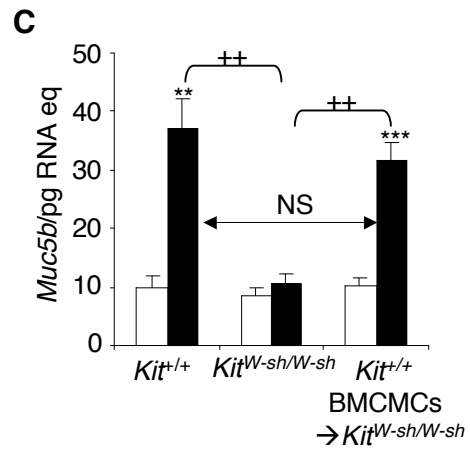
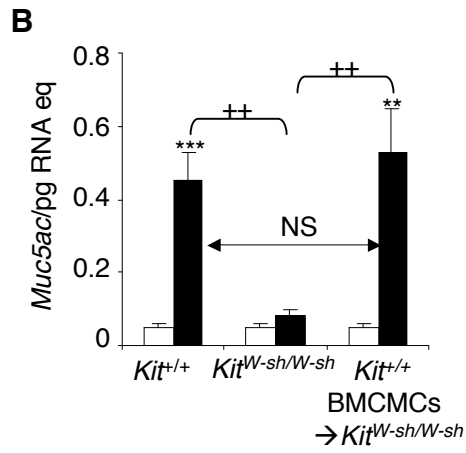
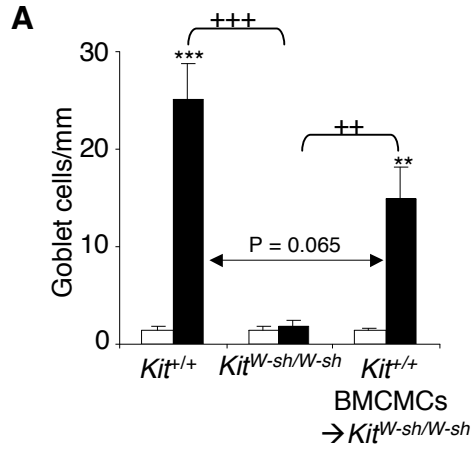
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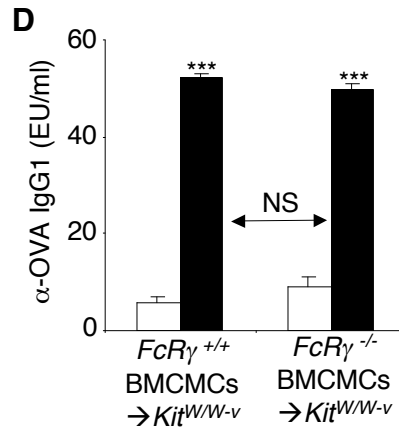
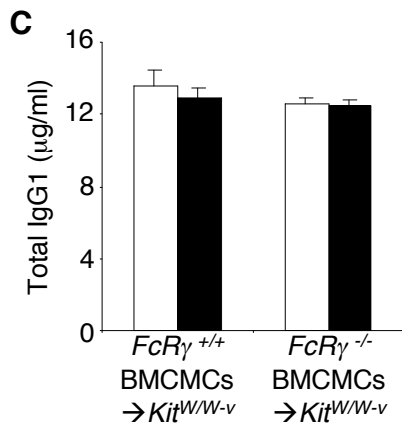
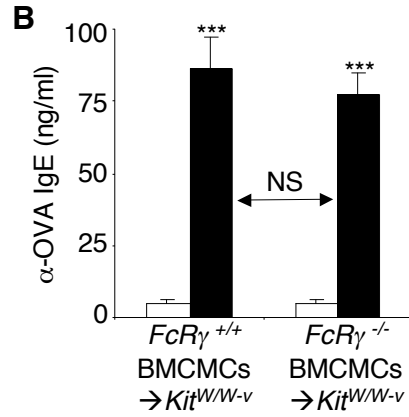
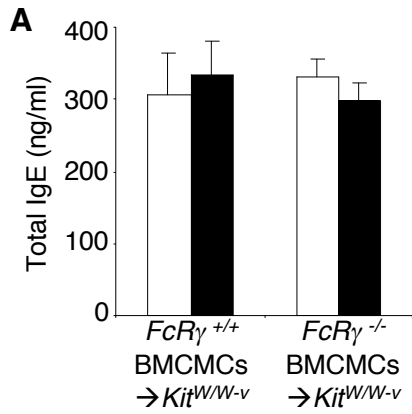
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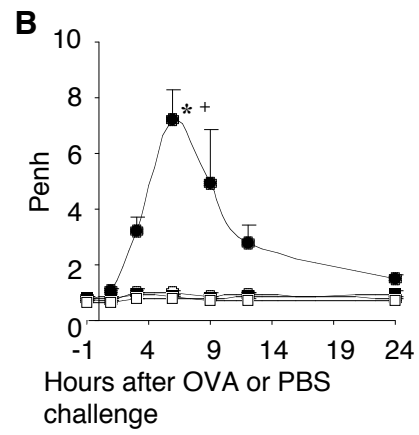
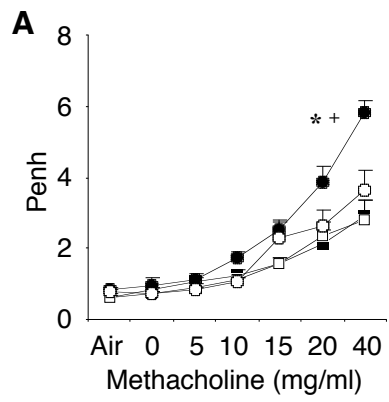
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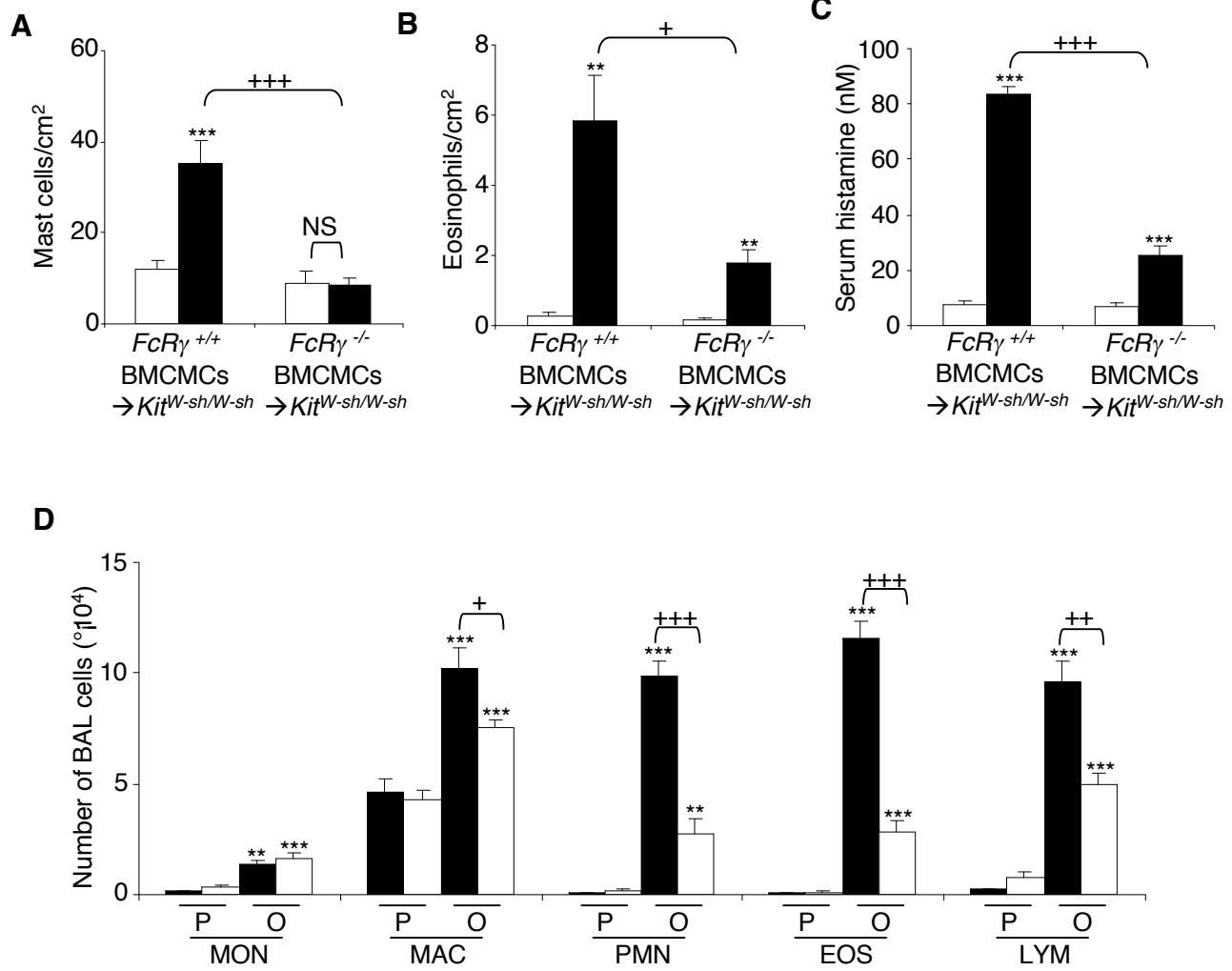
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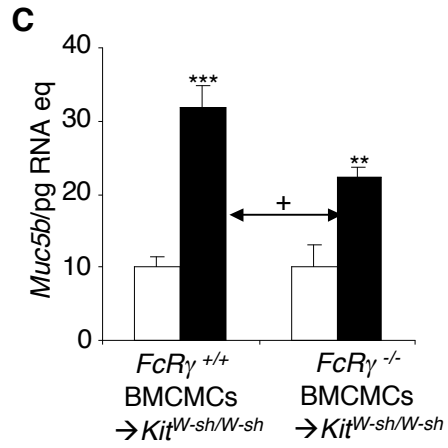
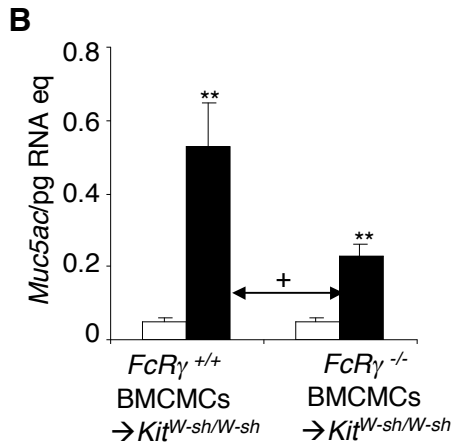
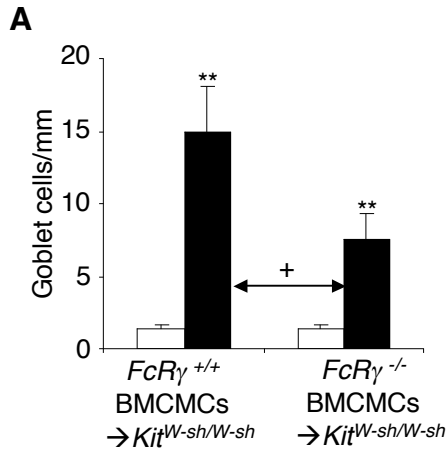
Supplemental Figure 8



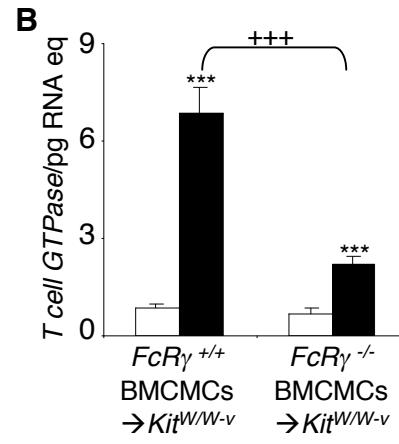
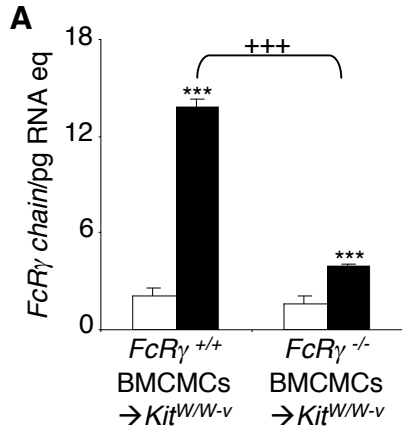
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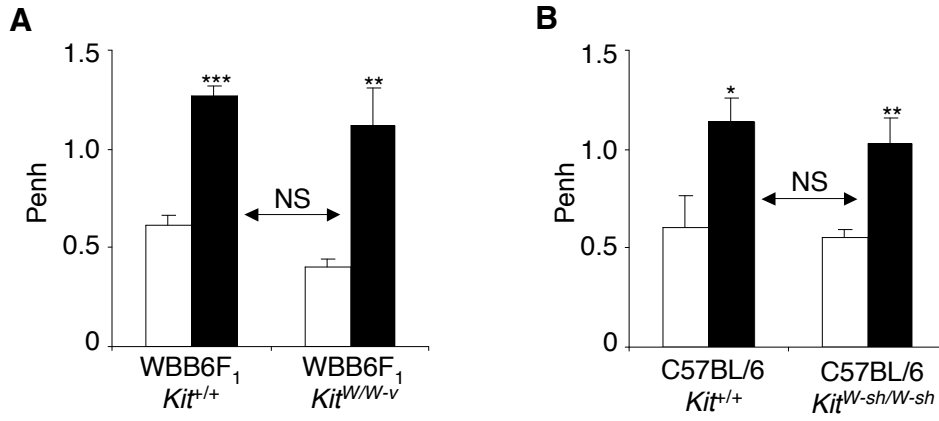
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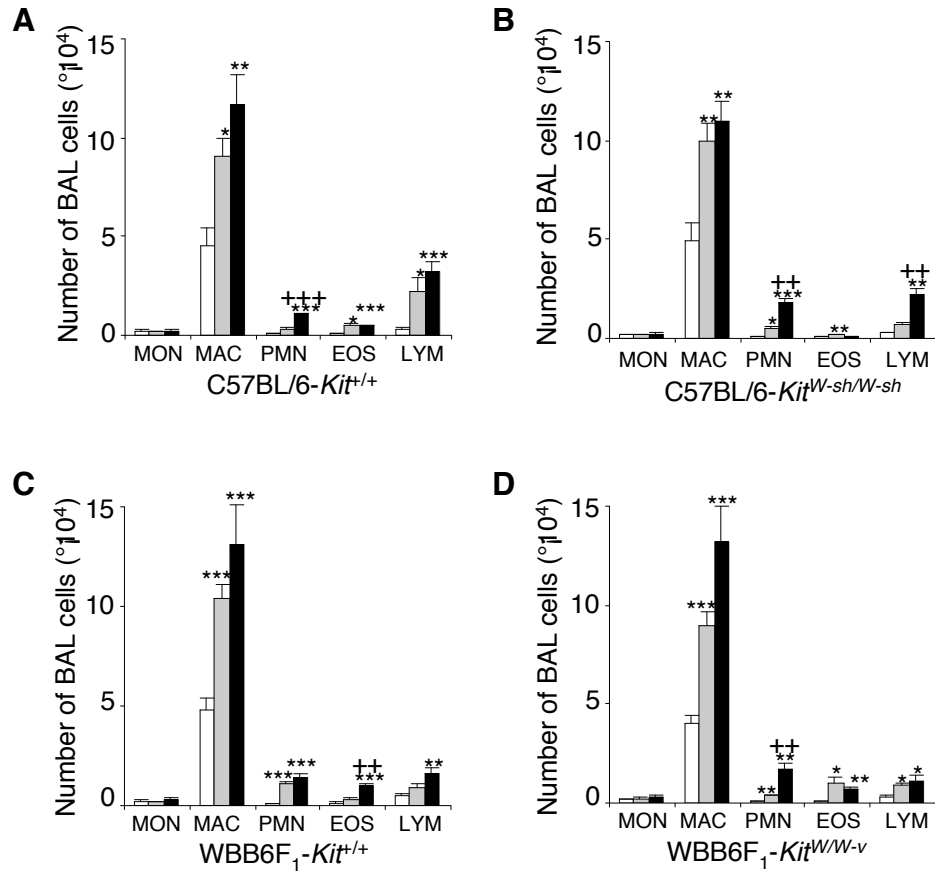
Supplemental Figure 11



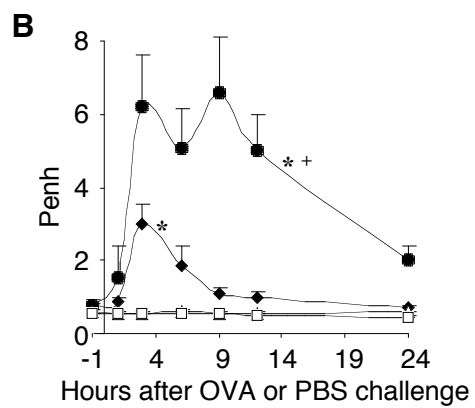
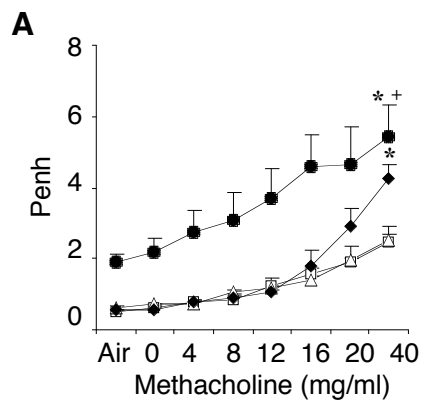
Supplemental Figure 12



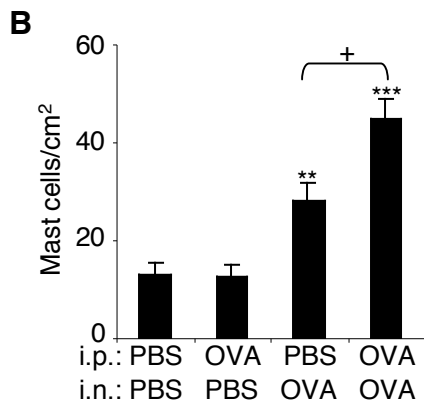
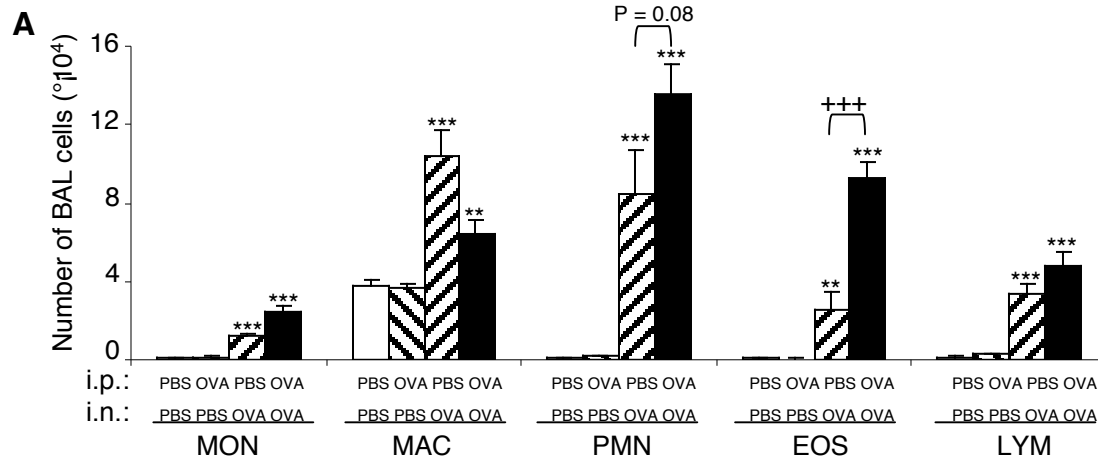
≤ PBS aerosol-treated
 ' C5a aerosol-treated



⊆ Before C5a aerosol exposure
 ′ Three hours after C5a aerosol exposure
 ′ Nine hours after C5a aerosol exposure



Supplemental Figure 15



Supplemental Figure 16

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Supplemental Figure 1

Airway responses following intranasal (*i.n.*) OVA (antigen) or PBS challenge in a model of chronic asthma elicited in wild type (C57BL/6-*Kit*^{+/+}), genetically mast cell-deficient (C57BL/6-*Kit*^{W-sh/W-sh}) and mast cell-engrafted C57BL/6-*Kit*^{W-sh/W-sh} mice. (A) AHR measured by Penh using aerosolized methacholine 24 h after the 8th OVA or PBS challenge. (B) Airway responses (assessed by Penh) to OVA or PBS were measured 1 h before and 1, 3, 6, 9, 12, and 24 h after the 9th OVA or PBS challenge. Data are from OVA *i.p.* sensitized and *i.n.* challenged C57BL/6-*Kit*^{+/+} (●), C57BL/6-*Kit*^{W-sh/W-sh} (○) and C57BL/6-*Kit*^{+/+} BMCMCs→C57BL/6-*Kit*^{W-sh/W-sh} (●) mice, as well as PBS *i.p.* treated and *i.n.* challenged C57BL/6-*Kit*^{+/+} (■), C57BL/6-*Kit*^{W-sh/W-sh} (□) and C57BL/6-*Kit*^{+/+} BMCMCs→C57BL/6-*Kit*^{W-sh/W-sh} (■) mice. * $P < 0.05$ vs. the corresponding PBS controls and ⁺ $P < 0.05$ vs. the OVA-sensitized and challenged *Kit*^{W-sh/W-sh} group, by ANOVA. (A) $n = 8/\text{group}$; (B) $n = 6/\text{group}$.

Supplemental Figure 2

Airway responses of individual mice following OVA challenge. Penh was measured 1 h before and 1, 3, 6, 9, 12, 15, 18, 21, and 24 h after the 9th OVA challenge in OVA-sensitized mice. Data are from two individual WBB6F₁-*Kit*^{+/+} (■ and ●), two WBB6F₁-*Kit*^{W/W-v} (□ and ○) and two WBB6F₁-*Kit*^{+/+} BMCMCs→*Kit*^{W/W-v} (■ and ●) mice.

Supplemental Figure 3

Serum concentrations of total or antigen-specific IgE and IgG1 in WBB6F₁-*Kit*^{+/+}, WBB6F₁-*Kit*^{W/W-v} or WBB6F₁-*Kit*^{+/+} BMCMC→*Kit*^{W/W-v} mice subjected to the chronic asthma model. Blood was sampled 24 h after the 9th OVA or PBS challenge. *** $P <$

0.001 vs. corresponding PBS controls ($n = 10/\text{group}$); NS: not significant ($P > 0.05$).

Open bars: PBS-treated group; solid bars: OVA-sensitized and challenged group.

Supplemental Figure 4

Features of allergic inflammation in this chronic asthma model, assessed in mice on the C57BL/6 background 24 h after the 9th OVA or PBS challenge. (A and B) Numbers of mast cells (A) and eosinophils (B) in the lung. (C) Serum histamine concentration. (A-C) Open bars: PBS-treated group; solid bars: OVA-sensitized/challenged group. ***, $P < 0.001$ vs. corresponding PBS controls ($n = 6$); ++ or +++, $P < 0.01$ or 0.001 vs. group indicated ($n = 5 - 6/\text{group}$); NS: not significant ($P > 0.05$); ND: not detected. (D) Numbers of leukocytes in BAL fluid from the right lungs of C57BL/6-*Kit*^{+/+} (■), C57BL/6-*Kit*^{W-sh/W-sh} (□) and C57BL/6-*Kit*^{+/+} BMCMCs → *Kit*^{W-sh/W-sh} (■) mice following OVA sensitization and challenges (O) or PBS treatment (P). ** or ***, $P < 0.01$ or 0.001 vs. corresponding PBS controls ($n = 5 - 6/\text{group}$); +, ++ or +++, $P < 0.05$, 0.01 or 0.001 vs. group indicated ($n = 5 - 6/\text{group}$); MON: monocytes; MAC: macrophages; PMN: neutrophils; EOS: eosinophils; LYM: lymphocytes.

Supplemental Figure 5

Changes in histamine concentrations in the serum of WBB6F₁-*Kit*^{+/+} mice following OVA sensitization and challenges, measured before (0) and 0.5, 3, 6, 9, 24, 48 or 72 h after the 9th OVA challenge. *** $P < 0.001$ vs. values at time 0; ++ $P < 0.01$ vs. 6 h values; $n = 5/\text{group}$.

Supplemental Figure 6

Lung mRNA levels of the gene encoding the FcR γ chain, analyzed in mice on the C57BL/6 background 24 h after the 9th OVA or PBS challenge. Open bars: PBS *i.p.* treated and *i.n.* challenged group; solid bars: OVA-sensitized and challenged group. ** P

< 0.01 vs. corresponding PBS controls ($n = 4/\text{group}$); $^{+++} P < 0.001$ vs. group indicated ($n = 4/\text{group}$). NS: not significant ($P > 0.05$).

Supplemental Figure 7

Airway goblet cell numbers and mucin gene expression in this chronic asthma model, assessed in mice on the C57BL/6 background 24 h after the 9th OVA or PBS challenge. (A) Numbers of goblet cells along the airway epithelium; (B and C) mRNA levels of genes encoding mucin 5AC and mucin 5B in the lung. Open bars: PBS-treated group; solid bars: OVA-sensitized/ challenged group. ** or *** $P < 0.01$ or 0.001 vs. corresponding PBS controls; $^{++}$ or $^{+++}$, $P < 0.01$ or 0.001 vs. group indicated; (A-C) $n = 5/\text{group}$. NS: not significant ($P > 0.05$).

Supplemental Figure 8

Serum concentrations of total or antigen-specific IgE and IgG1 in $FcR\gamma^{+/+}$ or $FcR\gamma^{-/-}$ BMCMCs \rightarrow Kit^{W/W^y} mice subjected to the chronic asthma model. Blood was sampled 24 h after the 9th OVA or PBS challenge. *** $P < 0.001$ vs. corresponding PBS controls ($n = 10/\text{group}$). NS: not significant ($P > 0.05$). Open bars: PBS-treated group; solid bars: OVA-sensitized and challenged group.

Supplemental Figure 9

Penh responses following intranasal OVA antigen or PBS challenge in C57BL/6- $Kit^{W-sh/W-sh}$ mice which had been engrafted with C57BL/6- $FcR\gamma^{-/-}$ vs. B6- $FcR\gamma^{+/+}$ BMCMCs. (A) Penh responses to aerosolized methacholine 24 h after the 8th OVA or PBS challenge. (B) Penh responses to OVA or PBS measured 1 h before and 1, 3, 6, 9, 12, and 24 h after the 9th OVA or PBS challenge. Data are mean+s.e.m. derived from OVA-sensitized and challenged C57BL/6- $FcR\gamma^{+/+}$ BMCMCs \rightarrow $Kit^{W-sh/W-sh}$ (●) and C57BL/6- $FcR\gamma^{-/-}$ BMCMCs \rightarrow $Kit^{W-sh/W-sh}$ mice (○), and from PBS-treated C57BL/6- $FcR\gamma^{+/+}$

BMCMCs→*Kit*^{W-sh/W-sh} (■) and C57BL/6-*FcRγ*^{-/-} BMCMCs→*Kit*^{W-sh/W-sh} (□) mice. * $P < 0.05$ vs. corresponding PBS controls and ⁺ $P < 0.05$ vs. OVA-sensitized and challenged C57BL/6-*FcRγ*^{-/-} BMCMCs→*Kit*^{W-sh/W-sh} mice by ANOVA; $n = 5/\text{group}$.

Supplemental Figure 10

Features of allergic inflammation in this chronic asthma model, assessed 24 h after the 9th OVA or PBS challenge in C57BL/6-*FcRγ*^{-/-} BMCMCs→*Kit*^{W-sh/W-sh} mice vs. C57BL/6-*FcRγ*^{+/+} BMCMCs→*Kit*^{W-sh/W-sh} mice. Numbers of mast cells (A) and eosinophils (B) in the lung, and concentrations of histamine in the serum (C). (A-C) Open bars: PBS-treated group; solid bars: OVA-sensitized and challenged group. (D) Numbers of leukocytes in BAL fluid derived from the right lungs of C57BL/6-*FcRγ*^{+/+} BMCMCs→*Kit*^{W-sh/W-sh} (solid bars) and C57BL/6-*FcRγ*^{-/-} BMCMCs→*Kit*^{W-sh/W-sh} (open bars) mice. ** or ***, $P < 0.01$ or 0.001 vs. corresponding PBS controls; ⁺, ⁺⁺ or ⁺⁺⁺, $P < 0.05$, 0.01 or 0.001 vs. group indicated ($n = 5/\text{group}$); NS: not significant. P: PBS treatment; O: OVA-sensitization/challenge. MON: monocytes; MAC: macrophages; PMN: neutrophils; EOS: eosinophils; LYM: lymphocytes.

Supplemental Figure 11

Airway goblet cell numbers and mucin gene expression in this chronic asthma model, assessed 24 h after the 9th OVA or PBS challenge in C57BL/6-*FcRγ*^{-/-} BMCMCs→*Kit*^{W-sh/W-sh} mice vs. C57BL/6-*FcRγ*^{+/+} BMCMCs→*Kit*^{W-sh/W-sh} mice. (A) Numbers of goblet cells along the airway epithelium; (B and C) Lung mRNA levels of genes encoding mucin 5AC and mucin 5B. Open bar: PBS-treated group; solid bar: OVA-sensitized and challenged group. ** or ***, $P < 0.01$ or 0.001 vs. corresponding PBS controls; ⁺ $P < 0.05$ vs. group indicated ($n = 5/\text{group}$).

Supplemental Figure 12

Lung mRNA levels of genes encoding (A) the FcR γ chain and (B) T cell-specific GTPase, analyzed 24 h after the 9th OVA or PBS challenge. Open bars: PBS treated group; solid bars: OVA sensitized/challenged group. *** $P < 0.001$ vs. corresponding PBS controls ($n = 6$ /group); +++ $P < 0.001$ vs. group indicated ($n = 6$ /group); NS: not significant ($P > 0.05$). Open bars: PBS-treated group; solid bars: OVA-sensitized and challenged group.

Supplemental Figure 13

Airway responses of mice following exposure to aerosolized PBS or recombinant mouse C5a. Penh was measured 30 min after a 2-min exposure to PBS aerosol or to C5a aerosol generated from 2 μ g C5a/ml PBS solution. Data are from (A) WBB6F₁-*Kit*^{+/+} and WBB6F₁-*Kit*^{W/W^v} mice, or (B) C57BL/6-*Kit*^{+/+} and C57BL/6-*Kit*^{W-sh/W-sh} mice. Open bars: PBS aerosol-treated group; solid bars: C5a-aerosol-treated group. *, ** or *** $P < 0.05$, 0.01 or 0.001 vs. corresponding PBS controls; NS, not significant ($P > 0.05$); $n = 5$ /group.

Supplemental Figure 14

Changes in numbers of BAL cells in mice following exposure to aerosolized recombinant mouse C5a (rmC5a). rmC5a (Cell Sciences, Norwood, MA) was prepared as a 2 μ g/ml solution in PBS from which contaminating LPS was depleted using a Detoxi-Gel AffinityPak Prepacked Column (Pierce Biotechnology, Inc., Rockford, IL). BAL was performed in naïve mice not exposed to aerosol (open bar), or in naïve mice which had been challenged with C5a (1.7×10^{-7} M, in PBS) 3 h (grey bar) or 9 h (solid bar) earlier. Results are shown for (A) C57BL/6-*Kit*^{+/+} and (B) C57BL/6-*Kit*^{W-sh/W-sh} mice and for (C) WBB6F₁-*Kit*^{+/+} and (D) WBB6F₁-*Kit*^{W/W^v} mice. *, **, or *** $P < 0.05$, 0.01 or 0.001 vs. the corresponding baseline values (open bars); ++ or +++ $P < 0.01$ or 0.001 vs. the corresponding 3 h values (grey) bars; $n = 4$ /group. MON: monocytes; MAC: macrophages; PMN: neutrophils; EOS: eosinophils; LYM: lymphocytes. Note: Numbers of BAL leukocytes in mice exposed to aerosolized PBS alone were similar to those in

BAL fluid recovered from naïve mice. For example, levels of cells ($\times 10^4$) 3 or 9 h after PBS challenge were, in naïve C57BL/6-*Kit*^{+/+} mice ($n = 5$): MON: 0.13 ± 0.03 (at 3 h) or 0.07 ± 0.02 (at 9 h); MAC: 4.6 ± 0.6 or 5.0 ± 0.5 ; PMN: 0.05 ± 0.02 or 0.07 ± 0.01 ; EOS: 0.21 ± 0.06 or 0.07 ± 0.02 ; and LYM: 0.23 ± 0.05 or 0.15 ± 0.01 , and, in C57BL/6-*Kit*^{W-sh/W-sh} mice ($n = 5$): MON: 0.16 ± 0.04 (at 3 h) or 0.42 ± 0.14 (at 9 h); MAC: 4.5 ± 0.5 or 4.2 ± 0.7 ; PMN: 0.05 ± 0.02 or 0.06 ± 0.01 ; EOS: 0.11 ± 0.02 or 0.03 ± 0.01 ; LYM: 0.36 ± 0.06 or 0.68 ± 0.18 .

Supplemental Figure 15

Airway responses in WBB6F₁-*Kit*^{+/+} mice subjected to all combinations of 3 *i.p.* injections and 9 *i.n.* challenges with either PBS or OVA. (A) Penh responses to aerosolized methacholine 24 h after the 8th OVA or PBS challenge. (B) Penh responses to OVA or PBS measured 1 h before and 1, 3, 6, 9, 12, and 24 h after the 9th OVA or PBS challenge. Data were derived from OVA *i.p.* sensitized and *i.n.* challenged (solid circles), PBS *i.p.* treated and OVA *i.n.* challenged (solid diamonds), OVA *i.p.* sensitized and PBS *i.n.* challenged (open triangles), and PBS *i.p.* treated and PBS *i.n.* challenged (open rectangles) mice. * $P < 0.05$ vs. PBS *i.p.* treated and PBS *i.n.* challenged mice, + $P < 0.05$ vs. PBS *i.p.* treated and OVA *i.n.* challenged mice, by ANOVA. $n = 6-8$ /group.

Supplemental Figure 16

Features of allergic inflammation in WBB6F₁ mice subjected to all combinations of 3 *i.p.* injections and 9 *i.n.* challenges with either PBS or OVA, measured 24 h after the 9th PBS or OVA challenge. (A) Numbers of BAL cells from the right lungs of PBS *i.p.* treated and *i.n.* challenged (\square), OVA *i.p.* sensitized and PBS *i.n.* challenged (\blacksquare), PBS *i.p.* treated and OVA *i.n.* challenged (\blacksquare), and OVA *i.p.* sensitized and *i.n.* challenged (\blacksquare) mice. MON: monocytes; MAC: macrophages; PMN: neutrophils; EOS: eosinophils; LYM: lymphocytes. (B) Numbers of mast cells in the lungs of the corresponding groups. ** or

*** $P < 0.01$ or 0.001 vs. PBS *i.p.* treated and *i.n.* challenged mice; ⁺, or ⁺⁺⁺, $P < 0.05$ or 0.001 vs. PBS *i.p.* treated and OVA *i.n.* challenged mice, $n = 6 - 8$ /group.

Supplemental Table 1. Primer and probe sequences used for quantitative RT-PCR analysis.

Primer/Probe	Sequence (5' to 3')	GenBank Accession Number
<i>Tgtp</i> -For	ATCTGGATGAAACATTCAATCTCTACAG	NM_011579
<i>Tgtp</i> -Rev	TCAAATCCTGGGCAATGTTTT	
<i>Tgtp</i> -Probe	CAGTGAAGCATCATCCAGCCCAAAGTAAG	
<i>Muc5b</i> -For	AAACGGGCTGCTGTTACTCCT	NM_028801
<i>Muc5b</i> -Rev	CCGCAAGACAGTCGCATTTA	
<i>Muc5b</i> -Probe	TGAAAAAGAGGACTCAAGTACCTGTCAAGTGCA	
<i>Muc5ac</i> -For	CAGGAGAAAGTGATGGAGTCG	AK008656
<i>Muc5ac</i> -Rev	GGGGAAGTAGTCCTTGACCAG	
<i>Muc5ac</i> -Probe	GAGGCTCAGCGGGAAGACCTT	
<i>Fcer1g</i> -For	CAAGATCCAGGTCCGAAAGG	NM_010185
<i>Fcer1g</i> -Rev	GGTGTTCCAGGCCCGTGTAGA	
<i>Fcer1g</i> -Probe	ATAGCCAGCCGTGAGAAAGCAGATGC	

Supplemental Table 2. Mast cell (MC)- and MC FcR γ chain-dependent features of this chronic asthma model in OVA sensitized and challenged mice.

Features	MC dependence ^A		MC FcR γ dependence ^A	
	WBB6F ₁	C57BL/6	WBB6F ₁	C57BL/6
Airway response to antigen	+++	+++	††	†††
AHR to methacholine	+++	+++	†††	†††
Eosinophils in the lung	+++	+	††	††
BAL leukocytes ^B	+ - +++	+ - +++	† - †††	0, † or ††
Increase in number of lung mast cells	+++	+++	†††	†††
Lung hydroxyproline	+++	ND	ND	ND
Increase in serum histamine	++	++	†	††
Increase in airway goblet cells	++	+++	0	††

^A All listed features of the model were observed in *Kit*^{+/+} mice but were absent or only weakly expressed in genetically mast cell-deficient WBB6F₁-*Kit*^{W/W-v} or *Kit*^{W-sh/W-sh} mice. “MC dependence” is defined as the extent to which the expression of that feature in *Kit*^{W/W-v} or *Kit*^{W-sh/W-sh} mice was restored to *Kit*^{+/+} levels in *Kit*^{W/W-v} or *Kit*^{W-sh/W-sh} mice which had undergone long-term engraftment with *Kit*^{+/+} BMCMCs. “MC FcR γ dependence” is defined as the extent to which expression of that feature was stronger in *Kit*^{W/W-v} or *Kit*^{W-sh/W-sh} mice which had undergone long-term engraftment with FcR γ ^{+/+} vs. FcR γ ^{-/-} BMCMCs.

^B Results were different for different types of leukocytes.

+++ Responses in *Kit*^{+/+} BMCMCs→(*Kit*^{W/W-v} or *Kit*^{W-sh/W-sh}) mice not significant (N.S.) vs. those in wild type *Kit*^{+/+} mice and significantly higher than those in MC-deficient *Kit*^{W/W-v} or *Kit*^{W-sh/W-sh} mice.

- ++ Responses in $Kit^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice \geq 50% those in $Kit^{+/+}$ mice and significantly higher than those in MC-deficient $Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$ mice, but significantly lower than those in $Kit^{+/+}$ mice.
- + Responses in $Kit^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice $<$ 50% those in $Kit^{+/+}$ mice and significantly higher than those in MC-deficient $Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$ mice, but significantly lower than those in $Kit^{+/+}$ mice.
- ††† Significant responses (vs. corresponding values in PBS-treated mice) in $FcR\gamma^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice and responses in $FcR\gamma^{-/-}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice N.S. vs. those in corresponding PBS-treated controls.
- †† Significant responses (vs. corresponding values in PBS-treated mice) in $FcR\gamma^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice and responses in $FcR\gamma^{-/-}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice significantly higher than those in corresponding PBS-treated mice but $<$ 50% those in $FcR\gamma^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice.
- † Significant responses (vs. corresponding values in PBS-treated mice) in $FcR\gamma^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice and responses in $FcR\gamma^{-/-}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice significantly higher than those in corresponding PBS-treated mice and significantly lower than those in $FcR\gamma^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice, but $>$ 50% those in $FcR\gamma^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice.
- 0 Significant responses (vs. corresponding values in PBS-treated mice) in $FcR\gamma^{+/+}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice but N.S. vs. those in $FcR\gamma^{-/-}$ BMCMCs \rightarrow ($Kit^{W/W-v}$ or $Kit^{W-sh/W-sh}$) mice.

ND: Not done.