Supplemental figure legends

S1. Loss of LXR signaling does not impact neutrophil influx. (A) WT mice were treated with 3% thioglycolate for up to 5 h after which peritoneal fluid was collected and the frequency of GR-1^{hi}CD11b^{hi} cells in lavage was assessed to confirm kinetics of neutrophil influx. (B) WT and LXR$\alpha\beta$ null mice were injected i.p. with PBS or 3% thioglycolate. After 3 h, peritoneal fluid was collected and the frequency of GR-1^{hi} CD11b^{hi} cells in lavage was assessed by flow cytometry. FACS plot is representative of 3 mice/group.

S2. LXR signaling influences neutrophil homeostasis. (A) Eye bleed were performed on 6-8 week old WT and LXR$\alpha\beta$ null mice and frequency of neutrophils determined by Hemavet analyzer. Each point represents an individual mouse. (B) Representative FACS plots of Ly6G^{hi} BrdU+ cells gated through CD11b+ in peripheral blood of 6-8 week old WT or LXR$\alpha\beta$ null mice on indicated day post-BrdU (2mg/mL) injection. Frequency of Ly6G^{hi} BrdU+ cells indicated in the plot. Each plot is representative of 4 mice/group.

S3. No detectable inflammatory or neutrophilic cytokines in young LXR$\alpha\beta$ null mice. Serum was collected from (A) 6-8 week old WT and LXR$\alpha\beta$ null mice or (B) 9 month old mice and indicated cytokine concentrations determined by Luminex Analysis. Each point represents an individual mouse. *$p<0.05$

S4. Accumulation of neutrophils requires loss of both LXRα and LXRβ. (A) Spleen and LN counts from 6-8 week old WT and LXR$\alpha\beta$ null mice. (B) An accumulation of neutrophils in LN of LXR$\alpha\beta$ null mice. Frequency of GR-1^{hi}CD11b^{hi} cells in Inguinal, Axillary and Cervical LNs was determined by FACS analysis. (C) Eye bleed were performed on 6-8 week old WT, LXRα and LXRβ null mice, and the frequency of neutrophils determined by Hemavet analyzer. Each point represents an individual mouse. (D,E) Frequency and absolute number of GR-1^{hi}CD11b^{hi} cells in spleens of 6-8 week old WT, LXRα and LXRβ null mice. FACS plots are representative of >10 mice examined.

S5. Accumulation of neutrophils in spleens of young LXRαβ null mice. (A) Representative FACS plots of WT and LXRαβ null spleen cells stained for neutrophil markers GR-1, Ly-6G and MCA771. The frequency of neutrophils indicated in FACs plots and is representative of > than 10 mice per genotype. (B) No evidence for a difference in extramedullary hematopoiesis in spleens of LXRαβ null mice. To determine myelo-erythroid potential, $3x10^4$ spleen cells were plated under standard differentiation conditions. Colonies were scored on day 8.

S6. No evidence for neutrophil activation or generalized cytokine production from T cells. (A) No difference in activation state of neutrophils *ex vivo*. CD11b and CD62L expression pattern was determined on Ly-6G+ cells from spleen of WT and LXRαβ null mice by FACS analysis. (B) Intracellular IL-2 and IFN-γ production from spleen cells stimulated for 5 h with PMA/ionomycinin in the presence of Bref A. Cells were analyzed by flow cytometry. FACS plots are gated through a logical lymphocyte gate and Thy1+. Data presented is representative of 3 mice/group repeated twice. (C) No evidence for pro-inflammatory *IL-1b*, *Cxcl1* and *Cxcl2* gene expression in skin of 6-8 week old LXRαβ null mice. Data is from 5 mice/group.

S7. A modest increase in numbers of tissue macrophage and DCs in young LXRαβ null mice. (A) *Itgam* **(**CD11b), *Emr1* (F4/80) and *CD68* gene expression in liver and spleen of 6-8 week old WT and LXRαβ null mice. (B) Total numbers of neutrophils ($CD11b^{hi}$ $Ly6G^{hi}$), macrophage (F4/80+ MHC class II+) and DCs (CD11c+ MHC class

II+) from spleen of 6-8 week old WT and LXR$\alpha\beta$ null mice. Total cell numbers were computed by multiplying the frequency of subsets by the total splenocyte counts. Data is from 5 mice/group.

S8. Clearance of adoptively transferred neutrophils in bone marrow or spleen. Flow cytometric analysis of bone marrow Ly6G+GFP+ cells in spleen at 18 h (A) or bone marrow at 40 h (B) post-adoptive transfer into WT and LXR$\alpha\beta$ null mice. Frequency of GFP+ cells is indicated in plot. FACS plots are representative of 3 mice.

S9. Phagocytosis of apoptotic neutrophils activates LXR and drives expression of Mertk. *Abca1* expression from thioglycolate-elicited macrophage co-cultured with apoptotic neutrophils for 90 min. LXR ligand GW3965 (1μM for 5h) treated macrophage served as a positive control for upregulation of LXR target genes. (B) WT and LXR$\alpha\beta$ null bone marrow-derived myeloid DCs were treated with LXR ligand GW3965 (1μM) or vehicle for 5h and *Mertk* and *Axl* gene expression determined. *Tyro3* gene expression was not detectable. **p<0.01,***p<0.001

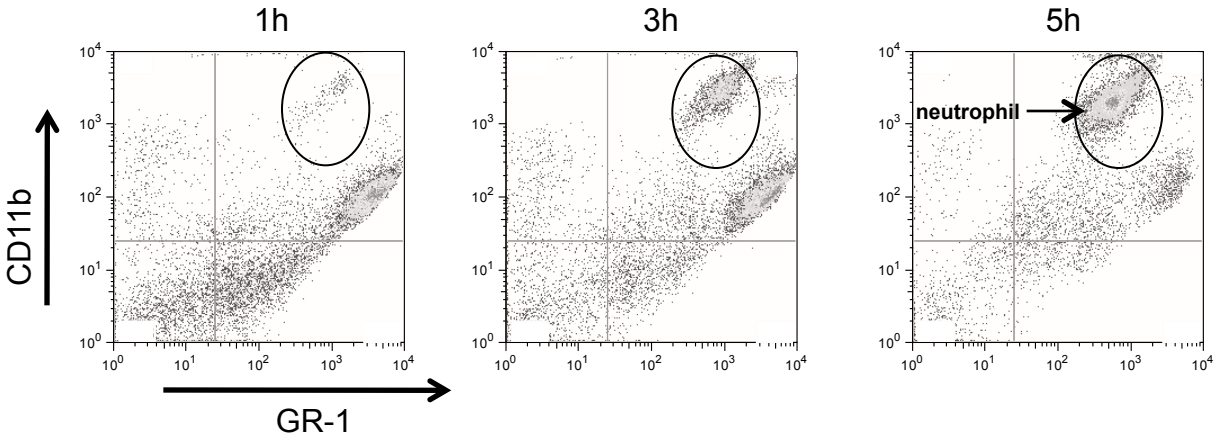
S10. Mertk is required for efficient aged neutrophil clearance. (A,B) Confocal microscopy images and quantification of neutrophil phagocytosis. WT bone marrow-derived macrophage were treated with control IgG or anti-Mer Ab and then co-cultured with aged neutrophils for 90 min. Afterwards, cultures were extensively washed with cold PBS and enzyme free cell dissociation buffer to remove non- and semi-adherent neutrophils. Cells were stained for CD68 expression and Ly6G expression to distinguish

macrophages from neutrophils and phagocytic index determined Data is representative of 3 experiments performed in triplicate.

S11. Altered peripheral neutrophil homeostasis in young Mertk-/- and Gas6-/- mice. (A,B,C) Frequency of spleen and bone marrow GR-1^{hi}CD11b^{hi} cells from 5-6 week old WT, Gas6-/- or Mertk-/- null mice. FACS plots are representative of 4 mice/group.

S12. A model of LXR-mediated control of neutrophil homeostasis. LXR signaling drives expression of Mer promoting the engulfment of apoptotic neutrophils bound to Gas6 protein. Phagocytosis of neutrophils via Mer activates LXRs leading to the further up-regulation of Mer in a positive feedback loop. Phagocytosis of aged neutrophils upregulates Gas6 in an LXR-independent manner. Concomitantly, activation of LXR by apoptotic neutrophils also represses IL-23 gene expression resulting in the attenuation of the IL-17 expression in T cells and downstream G-CSF from bone marrow. In addition, Mer signaling further reinforces the anti-inflammatory program in APC by down modulating inflammatory receptor signaling as described by Lemke and colleagues (44). In combination, the LXR and Mer signaling axis set the IL-23/IL-17 granulopoietic cytokine cascade under non-inflammatory conditions.

A.



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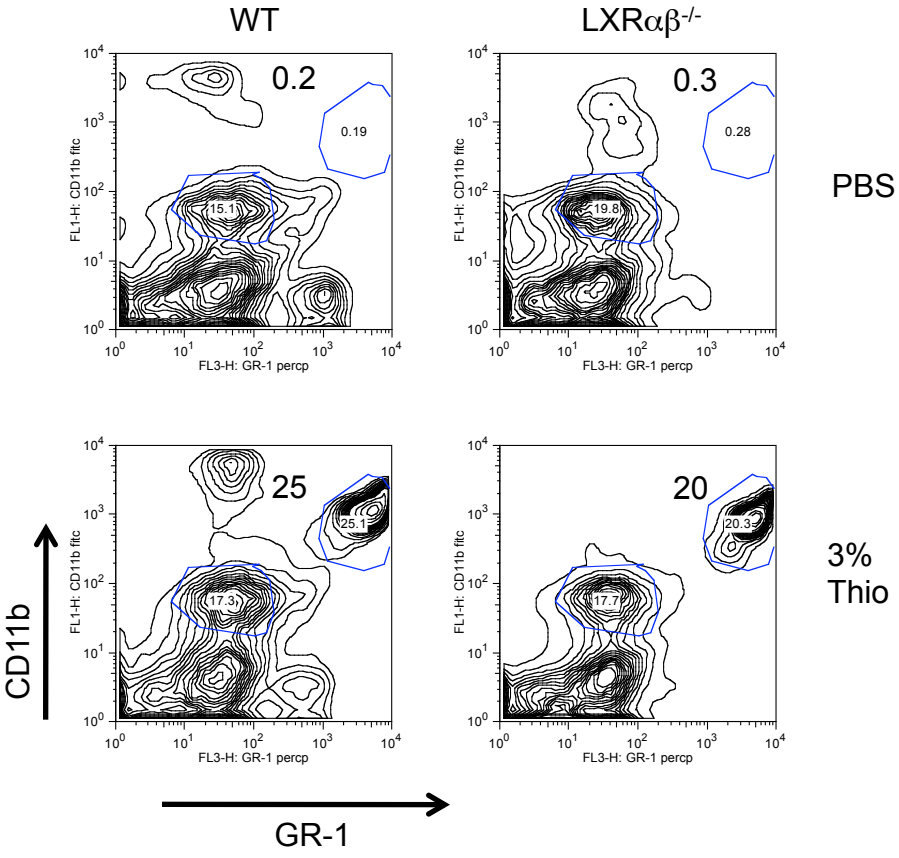
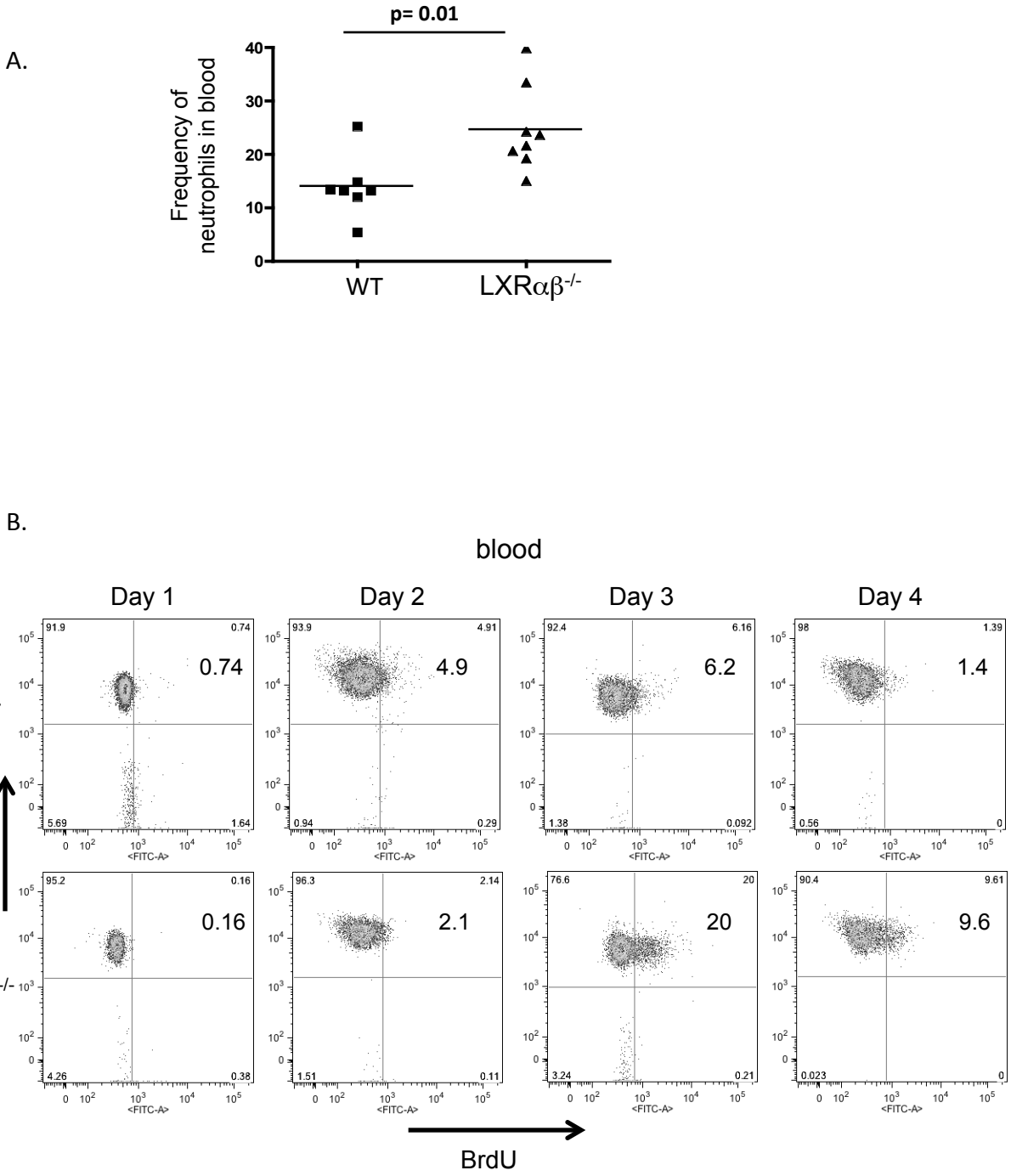
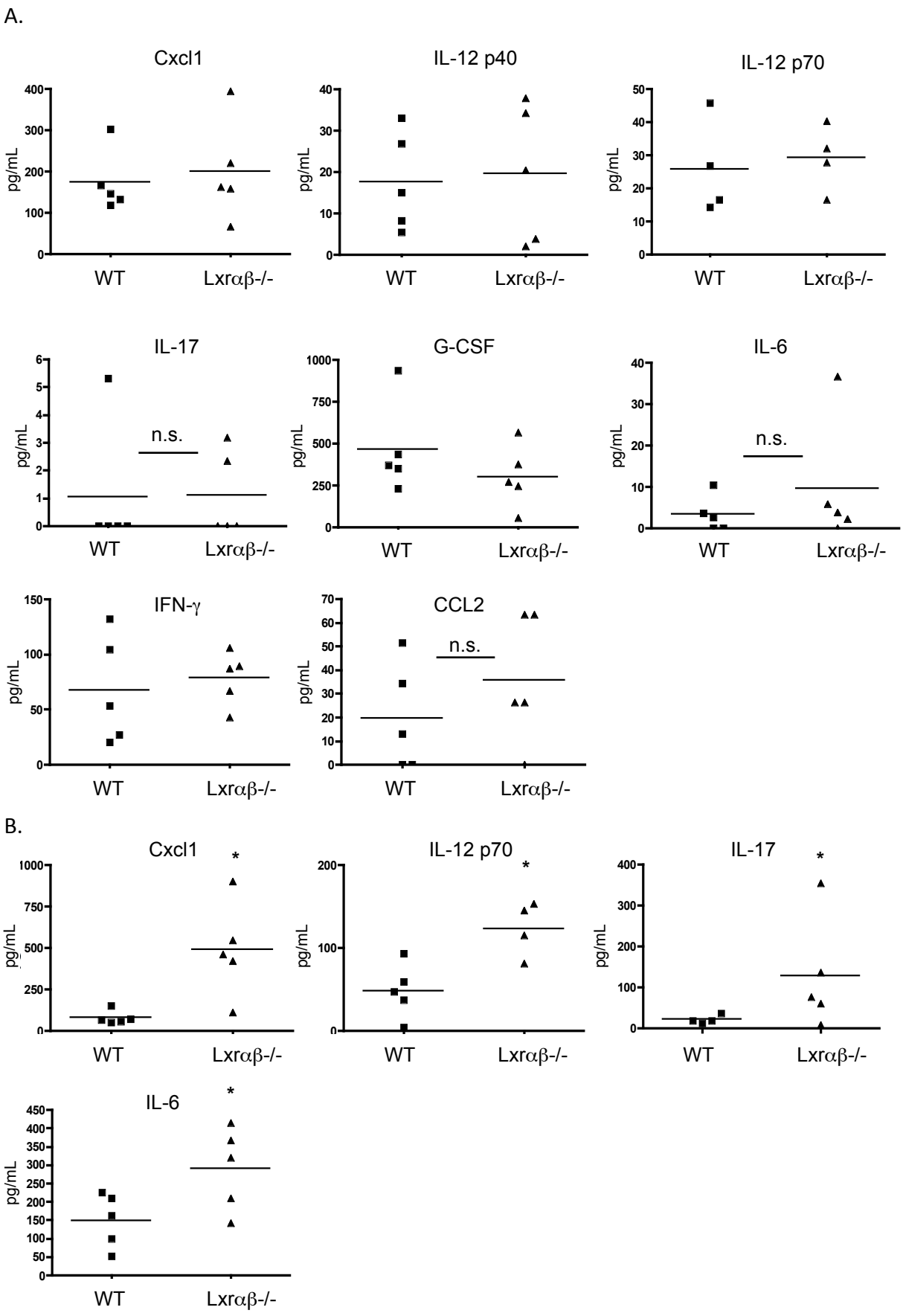


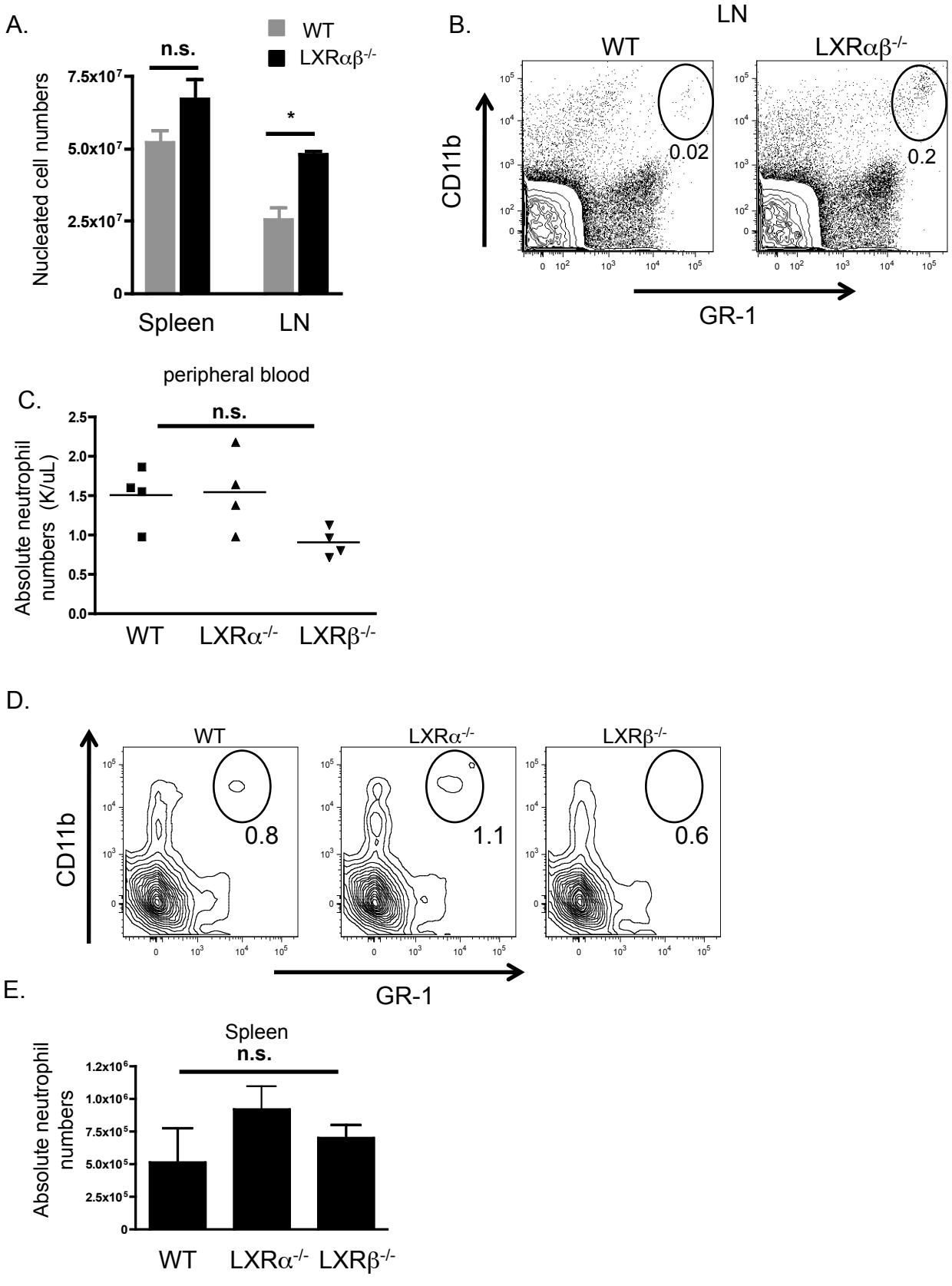
Hong Fig. S1


A.
p= 0.01
Frequency of neutrophils in blood
40
30
20
10
0
WT
LXRαβ-/-
B.
blood
Day 1
Day 2
Day 3
Day 4
WT
LXRαβ-/-
Ly6G
BrdU
0.74
4.9
6.2
1.4
0.16
2.1
20
9.6

Hong Fig. S2

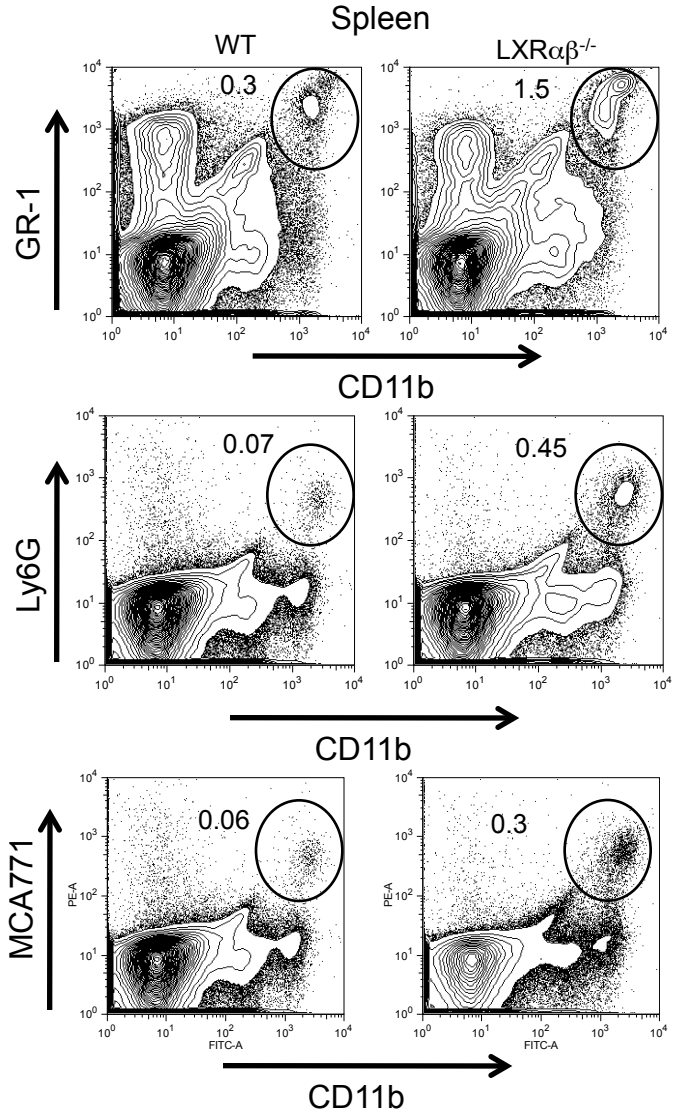

A.
Cxcl1
IL-12 p40
IL-12 p70
IL-17
G-CSF
IL-6
IFN-γ
CCL2
pg/mL
n.s.
WT
Lxrαβ-/-
B.
Cxcl1
IL-12 p70
IL-17
IL-6
*

Hong Fig. S3


A.
WT
LXRαβ-/-
n.s.
*
Nucleated cell numbers
7.5x10^7
5.0x10^7
2.5x10^7
0
Spleen
LN
B.
LN
WT
LXRαβ-/-
CD11b
0.02
0.2
GR-1
C.
peripheral blood
n.s.
Absolute neutrophil numbers (K/uL)
2.5
2.0
1.5
1.0
0.5
0.0
WT
LXRα-/-
LXRβ-/-
D.
WT
LXRα-/-
LXRβ-/-
CD11b
0.8
1.1
0.6
GR-1
E.
Spleen
n.s.
Absolute neutrophil numbers
1.2x10^6
1.0x10^6
7.5x10^5
5.0x10^5
2.5x10^5
0
WT
LXRα-/-
LXRβ-/-

Hong Fig. S4

A.



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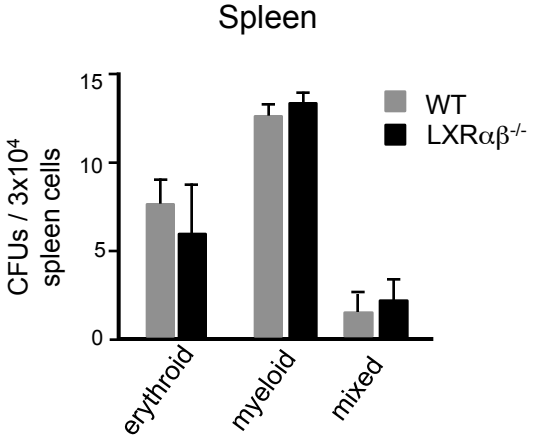
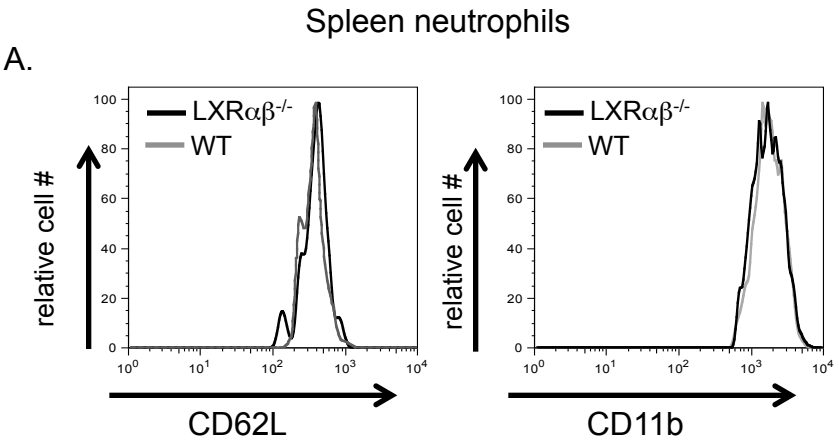
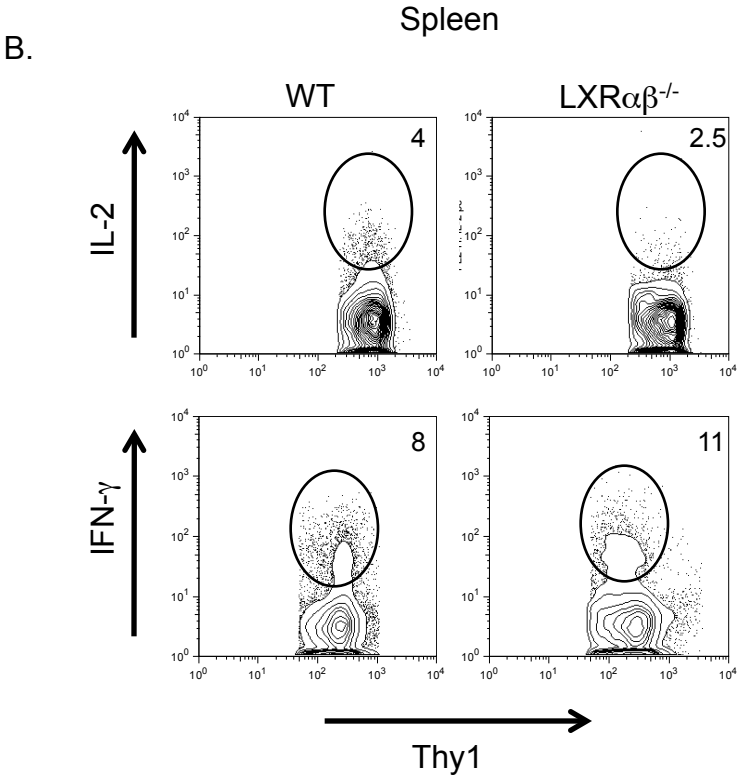
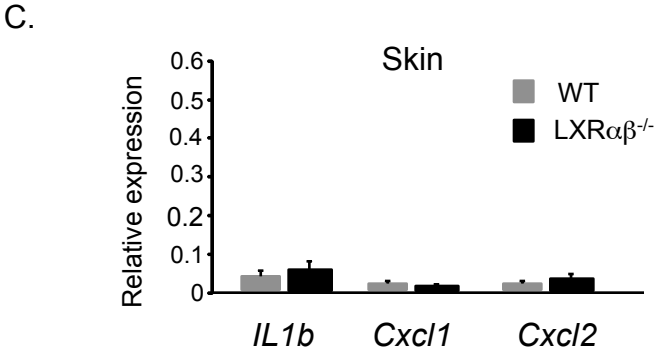


Hong Fig. S5

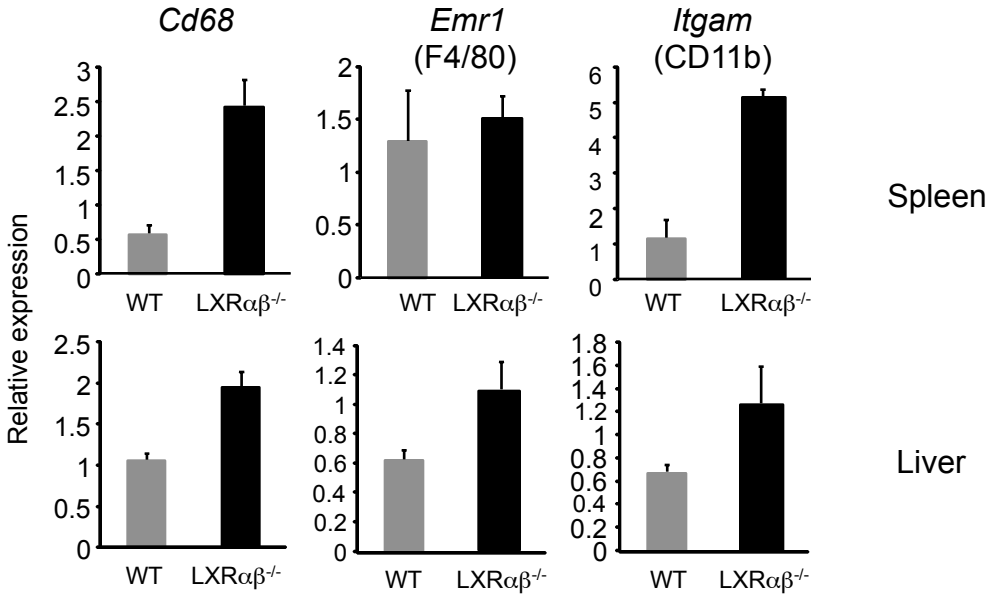

Spleen neutrophils
A.
LXRαβ-/-
WT
relative cell #
CD62L
CD11b


Spleen
B.
WT
LXRαβ-/-
IL-2
IFN-γ
4
2.5
8
11
Thy1


C.
Skin
WT
LXRαβ-/-
Relative expression
IL1b
Cxcl1
Cxcl2

Hong Fig. S6

A.



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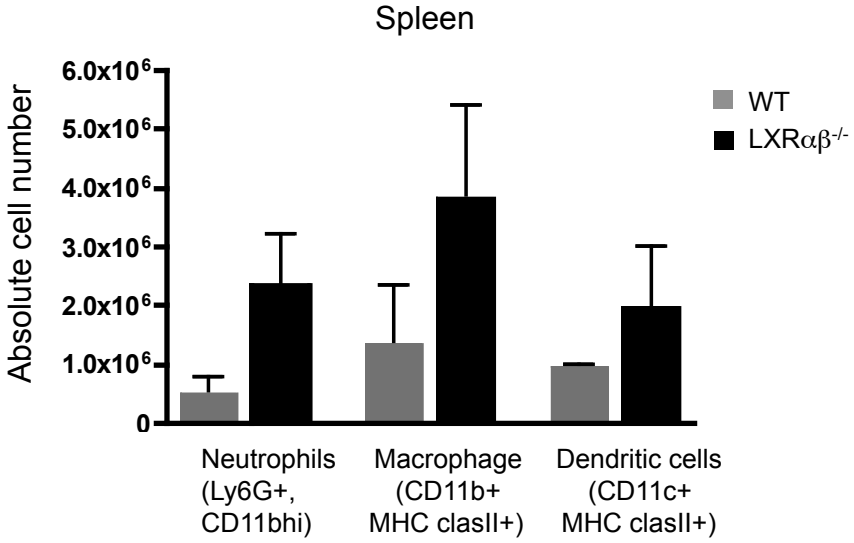
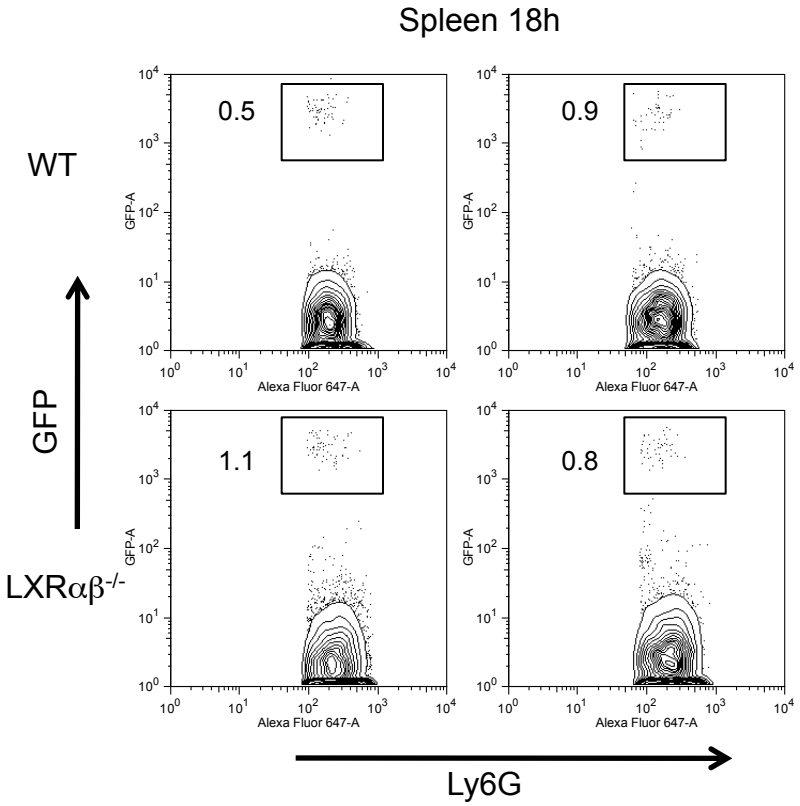


Hong Fig. S7

A.



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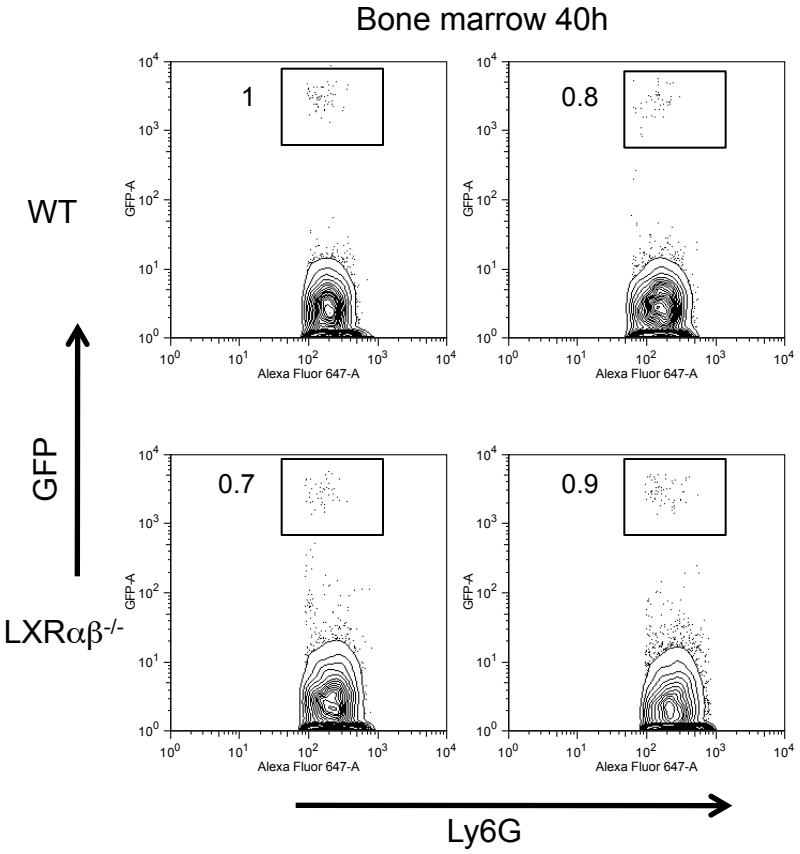
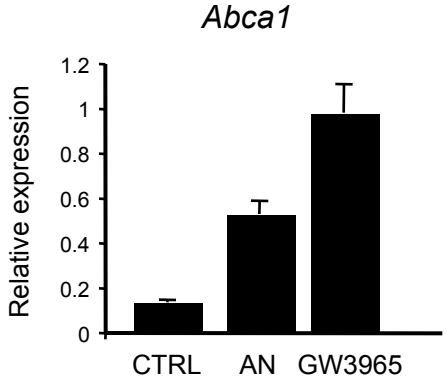


Hong Fig. S8

A.



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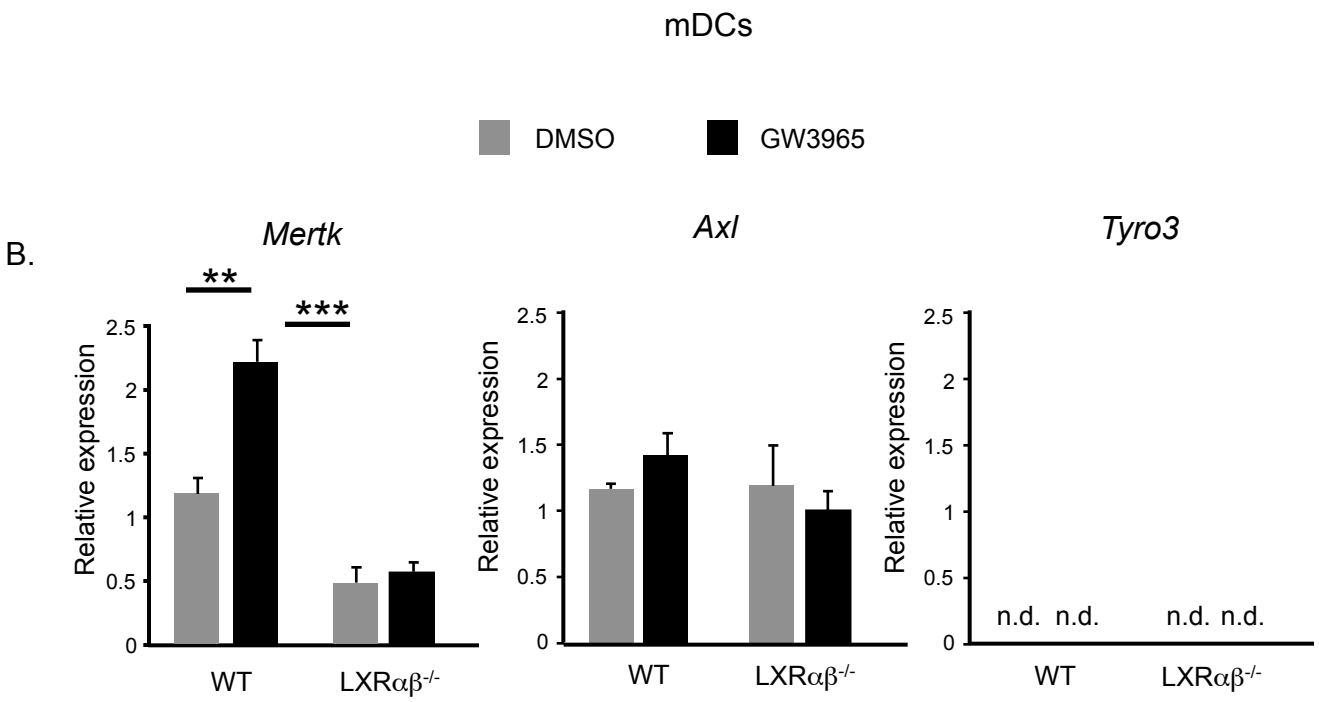
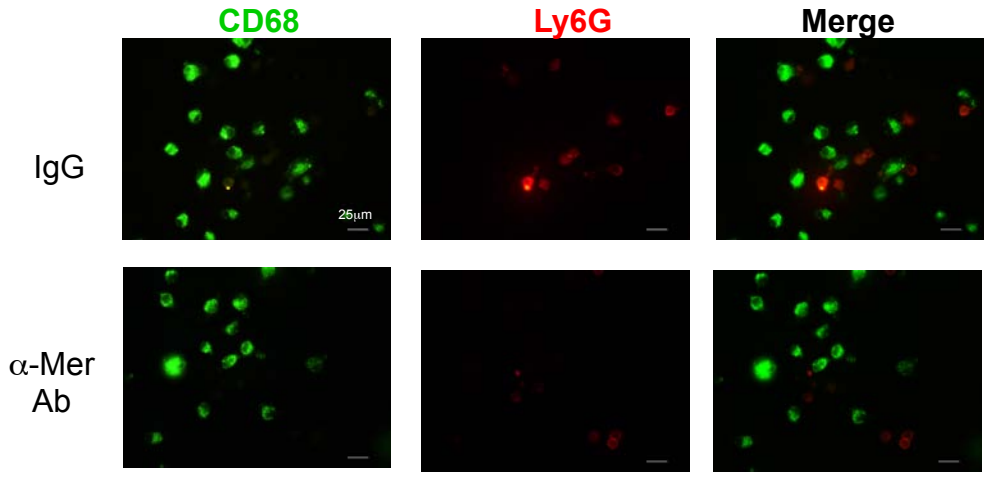


Hong Fig. S9

A.



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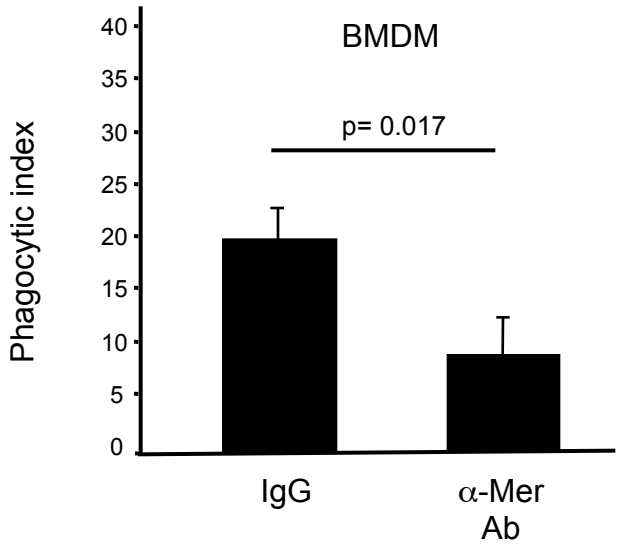
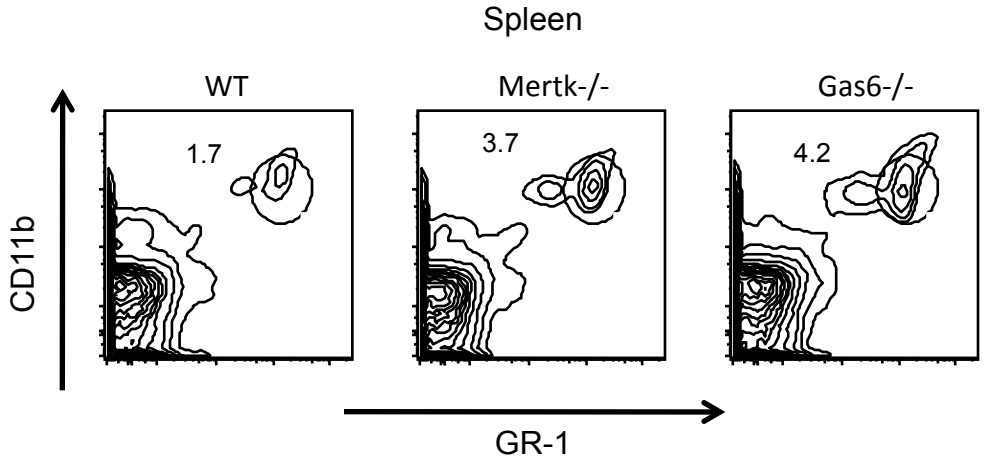


Hong Fig. S10

A.



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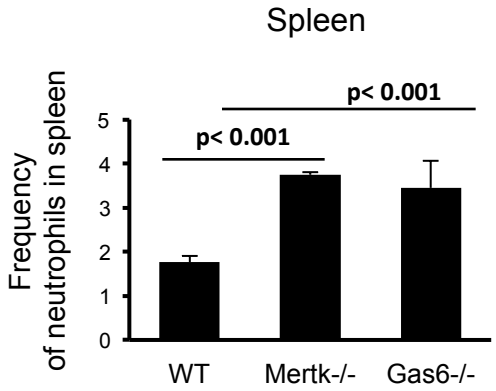
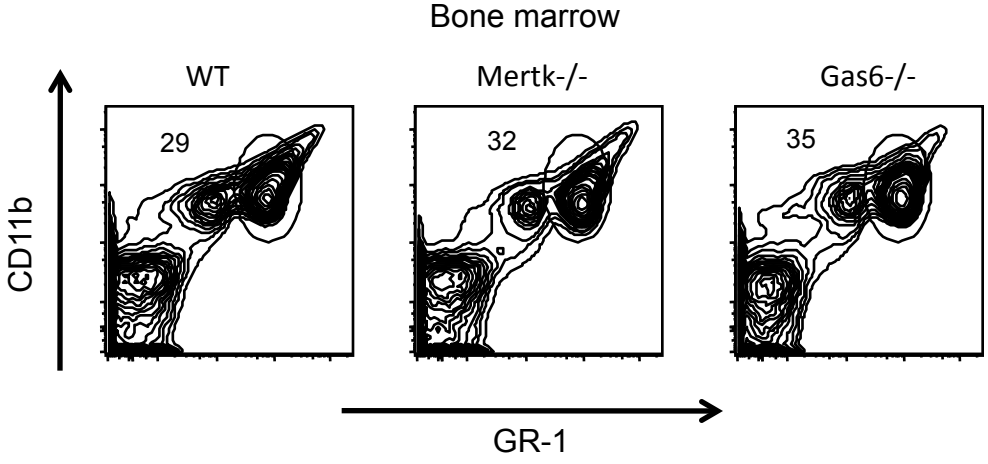
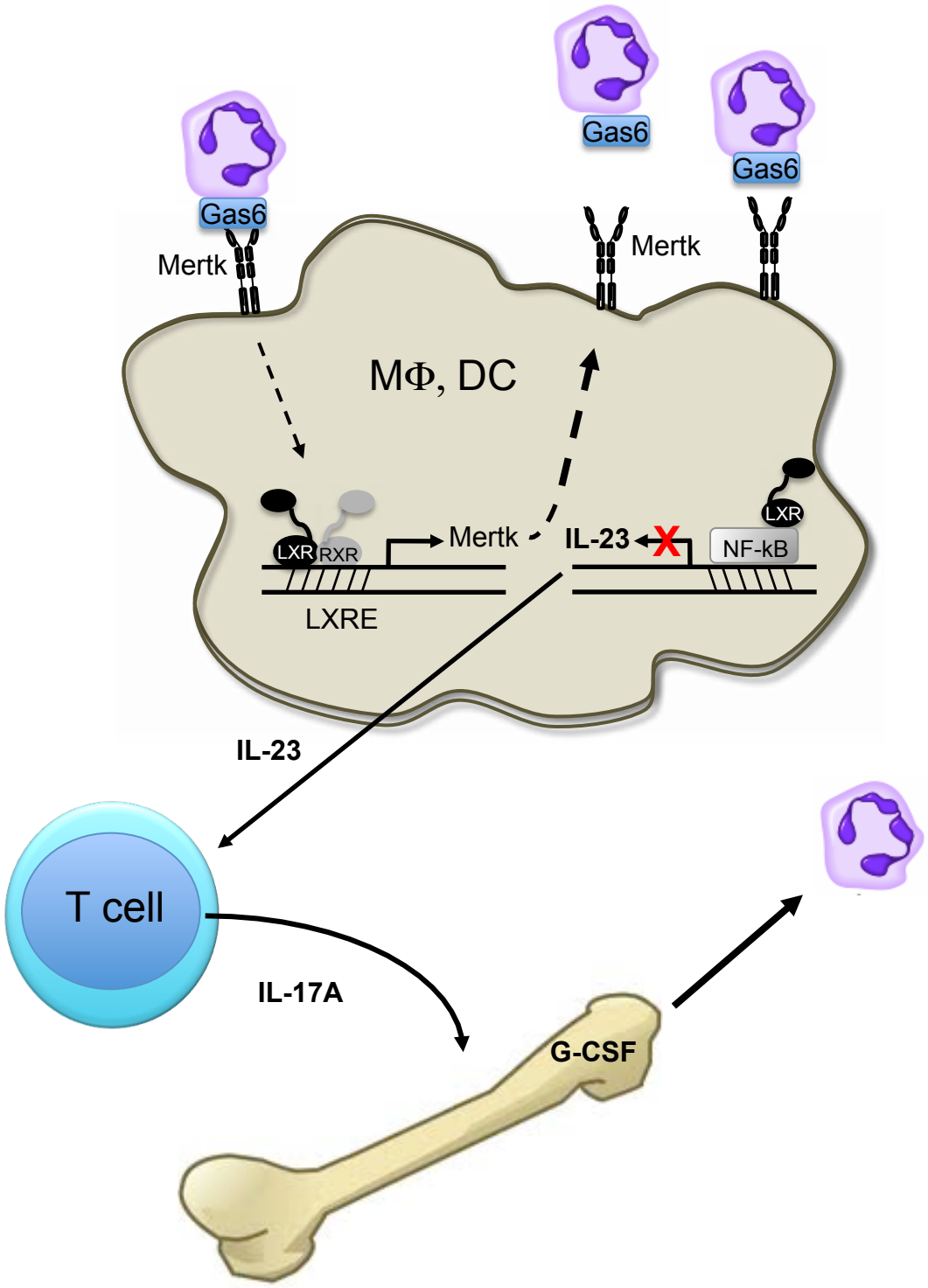


C.



Hong Fig. S11


Gas6
Gas6
Gas6
Mertk
Mertk
MΦ, DC
LXR
RXR
Mertk
IL-23
NF-kB
LXR
LXRE
IL-23
T cell
IL-17A
G-CSF

Hong Fig. S12

Supplemental Table 1.

Primer sequences used in real-time PCR assays.

Gene	Sequence
Rplp0 F	agatgcagcagatccgca
Rplp0 R	gttcttgcccatcagcacc
Abca1 F	ggtttggagatggttatacaatagtt
Abca1 R	cccggaaacgcaagtcc
Axl F	gagattcatacatcgggacctg
Axl R	cacacacggacatgttctca
Itgam F	caatagccagcctcagtgc
Itgam R	gagcccaggggagaagtg
cd68 F	gacctacatcagagcccgagt
cd68 R	cgccatgaatgtccactg
Cxcl1 F	cactgcacccaaaccgaagt
Cxcl1 R	ggacaattttctgaaccaagg
Cxcl2 F	gcccttgagagtggctatga
Cxcl2 R	agtgaactgcgctgtcaatg
Emr1 F	gcatcatggcatacctgttc
Emr1 R	agtctgggaatgggagctaa
Gas6 F	ggatttgctacctacaggctca
Gas6 R	ttaacttcccaggtggtttcc
IL1b F	agaagctgtggcagctacctg
IL1b R	ggaaaagaaggtgctcatgtc
IL12a F	catcgatgagctgatgcagt
IL12a R	cagatagcccatcaccctgt
IL12b F	atcgttttgctggtgtctcc
IL12b R	ggagtccagtccacctctaca
IL23a F	gactcagccaactcctccag
IL23a R	ggcactaagggctcagtcag
Mertk F	gaggactgcttggatgaactgta
Mertk R	aggtgggtcgatccaagg
Tyro3 F	tttcagacaaagggcctagc
Tyro3 R	ataaggcctgagtcggtacg