Supplemental Information

Supplemental methods

Flow cytometry and cell sorting

Table S1

Antibody	Color	Clone	Manufacturer	Cat. number
SiglecF	PE	E50-2440	BD Bioscience	552126
Ly6G	PE-Cy7	1A8	BioLegend	127617
CCR3 (CD193)	APC	J073E5	BioLegend	144512
CD11b	PerCP-Cy5.5	M1/70	BioLegend	101228
F4/80	FITC	BM8	eBioscience	11-4801-82
	APC-Fire/750	BM8	BioLegend	123152
CD11c	APC-Cy7	N418	BioLegend	117324
Ly6C	BV711	HK1.4	BioLegend	128037
CD34	eFluor 660	Ram34	eBioscience	50-0341-82
c-Kit	PE-Cy7	2B8	BioLegend	105814
FcγII/IIIR (CD16/32)	APC-Cy7	93	BioLegend	101328
IL-5Rα (CD125)	PE	T21	BD Bioscience	558488
CD3ε	Biotin	145-2c11	BioLegend	100304
CD19	Biotin	6D5	BioLegend	115504
B220	Biotin	RA3-6B2	BioLegend	103204
NK1.1	Biotin	PK136	BioLegend	108704
Gr-1	Biotin	RB6-8C5	BioLegend	108404
Ter-119	Biotin	TER-119	BioLegend	116204
CD127	Biotin	A7R34	BioLegend	135006
Sca1	Biotin	D7	BioLegend	108104
Streptavidin	BV605		BioLegend	405229

BM, spleens and blood were collected and processed using cold PBS with 2% heat-inactivated FBS (Gibco). Red blood cells were lysed using ACK lysis buffer (Lonza) for 5 minutes at room temperature. Cells were counted and stained at $20x10^6$ cells/ml in the presence of 5% 2.4G2 Fc blocking antibody. Zombie Violet (BioLegend) or 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) (Sigma) was used for live/dead discrimination. For all analysis and sorting, doublets were excluded. For mature cell sorting, cells were processed as above. For progenitor cell sorting, cells were processed as above, lineage depleted with biotinylated antibodies and streptavidin MACS beads (Miltenyi), and then surface stained without Fc blockade.

Cytospins

For cytospins, $5x10^4$ cells were resuspended in PBS and spun onto microscopy slides using a Cytospin 3 Cytocentrifuge (Shandon). Slides were air-dried and stained with Diff-Quik (Dade Behring).

Light and electron microscopy

Light microscopy images were acquired using a BX41 microscope (Olympus). For electron microscopy, eosinophils were sorted as above and fixed with 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1M sodium cacodylate buffer, pH7.4, for 1hr at room temperature. After subsequent buffer washes, cells were resuspended in molten 2% agar in 0.1M sodium cacodylate buffer, pH 7.4, and recentrifuged. The samples were then post-fixed in 2.0% osmium tetroxide for 1hr at room temperature. After dehydration through a graded ethanol series, the tissue was infiltrated and embedded in EMbed-812 (Electron Microscopy Sciences). Thin sections were stained with lead citrate and examined with a JEOL 1010 electron microscope fitted with a Hamamatsu digital camera and AMT Advantage image capture software. A total of 75 electron micrographs from two experiments were analyzed.

Phagocytosis assay

Whole BM was isolated as described above and resuspended in Live Cell Imaging Solution (Invitrogen). Cells were incubated with pHrodo Red *E. coli* BioParticles (Invitrogen) for 2hrs at 37°C. Uptake was analyzed by flow cytometry as above.

Immunoblotting

Cells were directly lysed in 2x SDS sample buffer (10% SDS) (BioRad) and boiled for 10min. Cell lysates were clarified, separated by SDS-PAGE and transferred to PVDF (Millipore). Antibodies used were anti-C/EBPα (8178, Cell Signaling Technology), anti-C/EBPε (C-10, Santa Cruz Biotechnology), anti-GATA1 (3535, Cell Signaling Technology), anti-β-actin (A5316, Sigma), anti-rabbit-HRP (NA934V, GE Healthcare), and anti-mouse-HRP (NA931V, GE Healthcare).

Quantitative PCR (qPCR)

RNA was extracted using Trizol (Ambion), followed by cDNA synthesis (SuperScript III kit; Invitrogen). qPCR was performed using TaqMan PCR master mix (Applied Biosystems) on a ViiA 7 system (ABI) and mRNA quantities were relative to *18s*. Primer/probe sets were *18s* (4319413E), *Trib1* (Mm00454875_m1), *Ltf* (Mm00434787_m1), *Epx* (Mm00514768_m1) and were purchased from Life Technologies.

Statistical analysis

Statistical analyses were performed using GraphPad Prism v.7.

Supplemental Figure Legends

Figure S1. ***Gating strategies for BM eosinophils and neutrophils***

A) Representative gating strategy for BM eosinophils and neutrophils. First panel gated on singlet cells. Live cells are identified using DAPI exclusion. Eosinophils are subsequently gated as $CD11b^+$ then $SiglecF^+$ and $CCR3^{+/-}$ (upper panels). Neutrophils are gated as $SiglecF^-$ then $Ly6G^+$ $CD11b^+$ (lower panels). The far-right histogram depicts the strategy for setting the CCR3 gate where neutrophils, which do not express CCR3, are used as a negative staining control. B) Comparison of a tight versus loose CCR3 gate for identifying eosinophils. Dot plots gated on singlet, live, $CD11b^+$ cells and histograms are subsequently gated as $SiglecF^+$ $CCR3^+$. The left panels are set with a tight CCR3 gate using neutrophils as a negative control as in (A). The right panels are set with a loose CCR3 gate. C) Representative gating/sorting strategy for isolating BM GMP and CMP. Left panel gated on singlet cells. CMP/GMP lineage cocktail includes Sca1, CD3ε, CD19, B220, NK1.1, Ter-119, CD127, CD11b, and Gr-1. D) Representative gating/sorting strategy for isolating BM EoP. Left panel gated on singlet cells. EoP Lineage cocktail includes CD3ε, CD19, B220, NK1.1, Ter-119, CD127, and Gr-1.

Figure S2. ***Early Trib1 deletion increases neutrophils and diminishes peripheral eosinophils, but does not affect CMP/GMP.***

A) Representative plots of BM neutrophils from $Trib1^{+/+}$ and $Trib1^{\Delta HSC}$ mice. B) Frequency of live cells and C) absolute numbers of BM neutrophils, n=11-12 mice/group pooled from 4 experiments. D) Representative plots of splenic eosinophils (upper) and neutrophils (lower) from $Trib1^{+/+}$ and $Trib1^{\Delta HSC}$ mice. E) Frequency of live cells and F) absolute numbers of splenic granulocytes, n=11-12 mice/group pooled from 4 experiments. G) Representative plots of blood eosinophils (upper) and neutrophils (lower) from $Trib1^{+/+}$ and $Trib1^{\Delta HSC}$ mice. H) Frequency of live cells and I) absolute numbers of blood granulocytes. Eosinophil plots gated on live, $CD11b^+$ cells, neutrophil plots gated on live, $SiglecF^-$ cells. Frequency n=12-13 mice/group pooled from 3 experiments, absolute numbers n=7 mice/group, representative of 2 experiments. J) Representative plots of BM myeloid progenitors from $Trib1^{+/+}$ or $Trib1^{\Delta HSC}$ mice, gated on live, $lineage^-$ $c\text{-}Kit^+$ cells. K) Frequency of live cells and L) absolute number of CMP and GMP, n=10/group pooled from 4 experiments. ns=not significant, unpaired student's *t* test. Frequencies and error bars are mean±SEM of live cells.

Figure S3. ***Extended phenotypic analysis of Trib1-deficient $Ly6G^+$ eosinophils***

and mixed chimeras from Trib1$^{\Delta HSC}$ mice.

A) Representative histograms of BM eosinophils from Trib1$^{+/+}$ (blue) and Trib1$^{\Delta HSC}$ (red) mice, with Trib1$^{+/+}$ neutrophils (gray solid) as a control. SSC-A: side-scatter area. Representative of 4 experiments. B) Representative histogram of IL-5Rα (CD125) expression on BM eosinophils from Trib1$^{+/+}$ (blue) and Trib1$^{\Delta HSC}$ (red) mice, with Trib1$^{+/+}$ neutrophils (gray solid) and CD11b^{-} cells (black dashed) shown as controls, representative of 2 experiments. C) Representative histograms of CCR3, CXCR2, and CXCR4 staining on BM eosinophils from Trib1$^{+/+}$ (blue) or Trib1$^{\Delta HSC}$ (red) mice with Trib1$^{+/+}$ neutrophils (gray solid) and CXCR4 FMO (dashed black) shown as controls, representative of 1 (CXCR2) or 3 (CCR3, CXCR4) experiments. D) Quantification of CXCR4 MFI on CD11b^{+} CCR3^{+} SiglecF^{+} eosinophils, n=3 mice/group, representative of 3 experiments, ***p=0.0001, unpaired student's *t* test. For all above histograms, eosinophils gated on live, CD11b^{+} SiglecF^{+} CCR3^{+} and neutrophils gated on live, CD11b^{+} Ly6G^{+} SiglecF^{-}. E) Representative cytospins of sorted eosinophils (CD11b^{+} SiglecF^{+} CCR3^{+} F4/80^{+}) at 100x magnification with Diff-quik stain, representative of 3 experiments. F) Scoring of cytospins of sorted CCR3^{+} eosinophils for nuclear lobation, n=5 mice/group from 3 experiments. G) Analysis of mixed BM chimeras, WT competitor BM (CD45.1^{+}, left) and Trib1$^{\Delta HSC}$ BM (CD45.2^{+}, right). Representative histogram of CCR3^{+} eosinophils showing Ly6G expression (WT CD45.1^{+}, blue; Trib1$^{\Delta HSC}$ CD45.2^{+}, red). Representative of 3 experiments. Frequencies and error bars are mean±SEM of live cells.

Figure S4. ***Reduced eosinophils without a concomitant increase in neutrophils in the spleen and blood of Trib1$^{\Delta Eos}$ mice.***

A) qPCR of Trib1$^{+/+}$ and Trib1$^{\Delta Eos}$ d13 cultured eosinophils for *Trib1* expression relative to *18s*, normalized to Trib1$^{+/+}$ eosinophils, representative of 2 experiments. B) Representative plots of splenic eosinophils (upper) and neutrophils (lower) from Trib1$^{+/+}$ and Trib1$^{\Delta Eos}$ mice. C) Frequency of live cells and D) absolute numbers of splenic granulocytes, n=11 mice/group pooled from 4 experiments. E) Representative plots of blood eosinophils (upper) and neutrophils (lower) from Trib1$^{+/+}$ and Trib1$^{\Delta HSC}$ mice. F) Frequency of blood granulocytes, n=9 mice/group pooled from 3 experiments. Eosinophil plots gated on live, CD11b^{+} cells; neutrophil plots gated on live, SiglecF^{-} cells. ****p<0.0001, unpaired student's *t* test. Frequencies and error bars are mean±SEM.

Figure S5. ***Ex vivo IL-5 culture of Trib1-deficient BM***

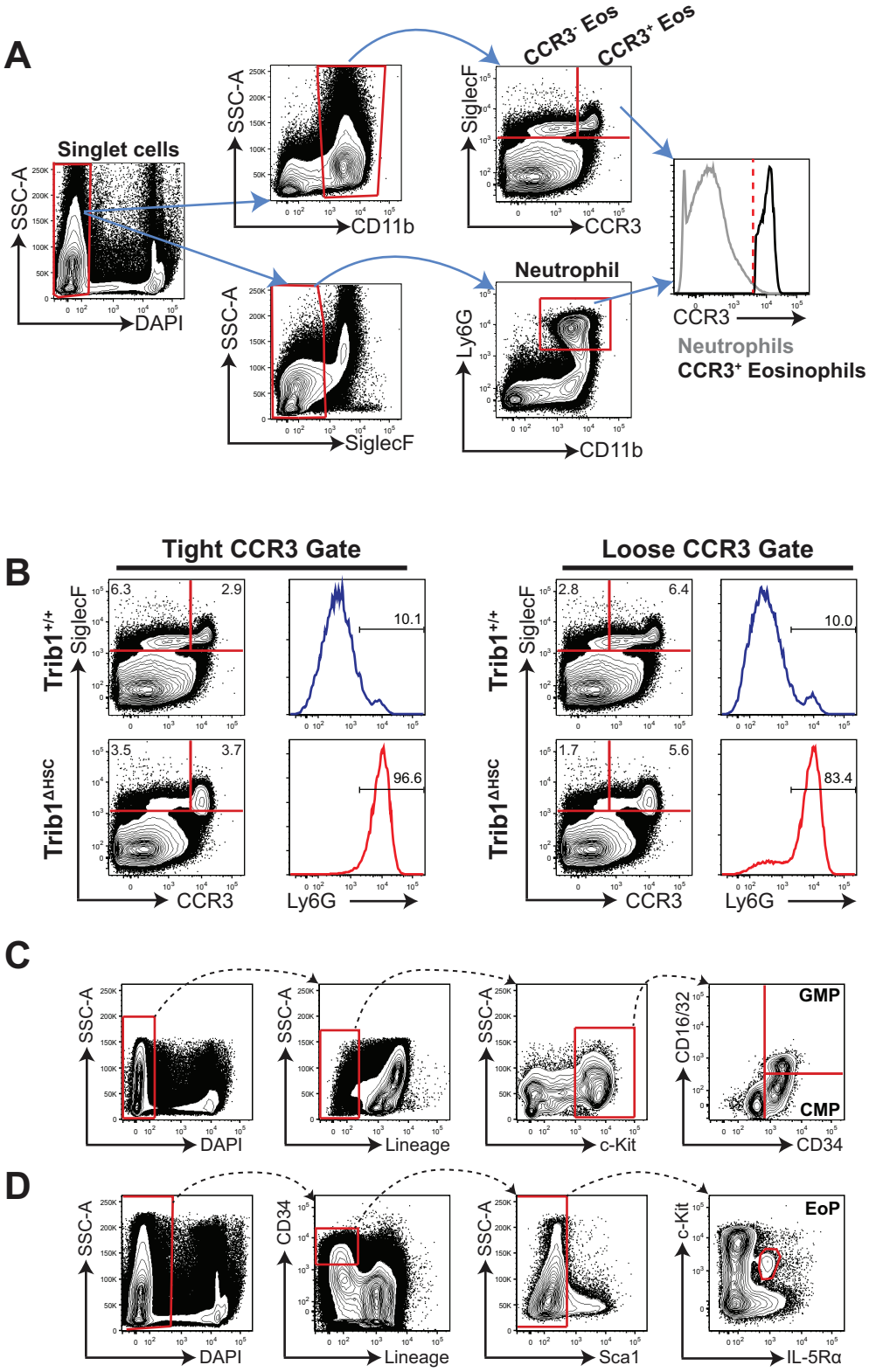
A) Representative plots of d10 IL-5 cultured Trib1$^{+/+}$ and Trib1$^{\Delta HSC}$ whole BM gated on live cells, representative of 4 experiments. B) Representative plots of d10 IL-5 cultured Trib1$^{+/+}$ and Trib1$^{\Delta Eos}$ whole BM gated on live cells, representative of 6 experiments. C) *Trib1* expression during IL-5 *ex*

vivo culture of sorted Trib1$^{+/+}$ or Trib1$^{\Delta HSC}$ GMPs relative to *18s*, normalized to Trib1$^{+/+}$ neutrophils, representative of 3 experiments. ND=not detected. D) Quantification of eosinophil migration using freshly isolated WT BM gated on eosinophils. n=3/group, representative of 3 experiments. Quantification of eosinophil migration starting with 1x10^6 d10-12 IL-5 cultured BM from E) Trib1$^{+/+}$ and Trib1$^{\Delta HSC}$ or F) Trib1$^{+/+}$ and Trib1$^{\Delta Eos}$ mice. n=3/group, representative of 3 experiments. G) Quantification of neutrophil migration using freshly isolated WT BM gated on neutrophils. n=3/group, representative of 3 experiments. H) Quantification of Trib1$^{\Delta HSC}$ neutrophil migration starting with 1x10^6 d11 IL-5 cultured BM from Trib1$^{+/+}$ or Trib1$^{\Delta HSC}$ mice. n=3/group, representative of 3 experiments. Transwell chemotaxis assay performed for 2hrs using KC/CXCL1 (50ng/ml) or eotaxin/CCL11 (500ng/ml). In all panels, eosinophils gated CD11b$^+$ SiglecF$^+$ CCR3$^+$, neutrophils gated CD11b$^+$ Ly6G$^+$ SiglecF$^-$. Frequencies and error bars are mean±SEM of live cells.

Figure S6. ***Peripheral mobilization of Trib1-deficient eosinophils in response to papain.***
Analysis of splenic eosinophils following papain treatment in Trib1$^{\Delta Eos}$ mice. A) Representative plots of splenic eosinophils gated live, CD11b$^+$ cells. B) Frequency of live cells and C) absolute number of CD11b$^+$ CCR3$^+$ SiglecF$^+$ cells in the spleen. D) Representative plots of blood eosinophils gated live, CD11b$^+$ cells. E) Frequency of live cells and F) cells/ml of blood of CCR3$^+$ SiglecF$^+$ cells. n=3/group, representative of 2 experiments. Frequencies and error bars are mean±SEM of live cells. G) Representative gating strategy for identifying lung eosinophils. First left plot gated on singlet, live cells. Alveolar macrophages (AMΦ) identified as CD11b^{lo} SiglecF$^+$. After excluding AMΦ, eosinophils were identified as CD11b$^+$ CD11c$^-$ SiglecF$^+$ SSC^{hi}.

Figure S7. Measurement of GATA1 and C/EBPε expression and characterization of C/EBPα knockdown chimeras.
A) Immunoblot of sorted CCR3$^+$ eosinophils (CCR3$^+$ SiglecF$^+$ F4/80$^+$ CD11b$^+$) probed for GATA1 and C/EBPε, with β-actin as a loading control. Representative of 3 experiments. B) Representative plots of BM cells expressing SiglecF and Ly6G from shRNA-transduced BM after 9 weeks; gated on live, GFP$^+$, CD11b$^+$ cells. Representative of 2 experiments. Frequencies are mean±SEM of GFP$^+$ cells.

Figure S1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Singlet cells
SSC-A
DAPI
SSC-A
CD11b
CCR3⁻ Eos
CCR3⁺ Eos
SiglecF
CCR3
SSC-A
SiglecF
Neutrophil
Ly6G
CD11b
CCR3
Neutrophils
CCR3⁺ Eosinophils
B
Tight CCR3 Gate
Loose CCR3 Gate
Trib1+/+
Trib1ΔHSC
SiglecF
CCR3
Ly6G
6.3
2.9
10.1
3.5
3.7
96.6
2.8
6.4
10.0
1.7
5.6
83.4
C
SSC-A
DAPI
Lineage
c-Kit
CD16/32
CD34
GMP
CMP
D
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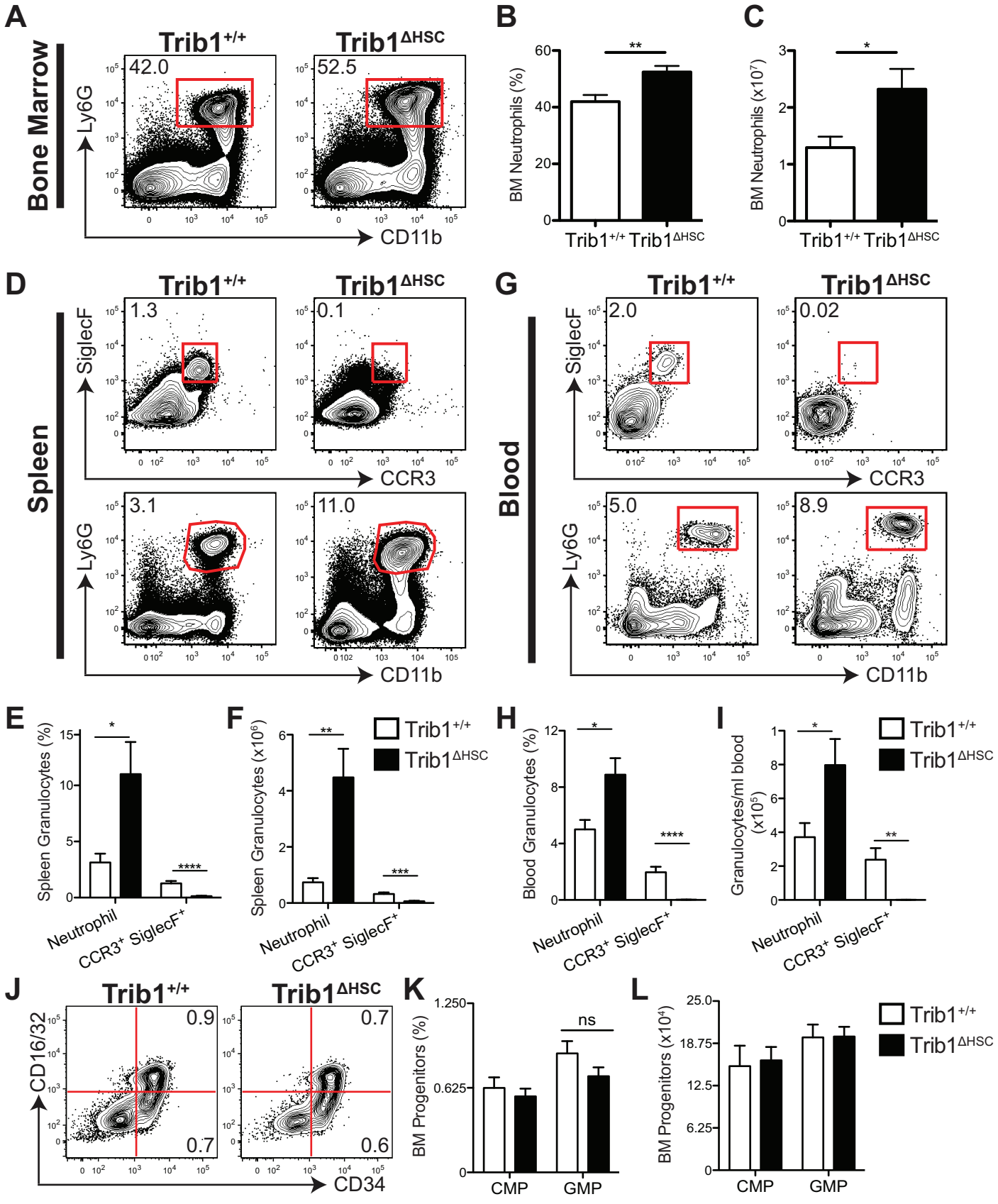
DAPI
CD34
Lineage
SSC-A
Sca1
c-Kit
IL-5Rα
EoP

Figure S2


A
Bone Marrow
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Trib1ΔHSC
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52.5
Ly6G
CD11b
B
BM Neutrophils (%)
Trib1+/+ Trib1ΔHSC
C
BM Neutrophils (x10^7)
Trib1+/+ Trib1ΔHSC
D
Spleen
1.3
0.1
3.1
11.0
SiglecF
CCR3
Ly6G
CD11b
G
Blood
2.0
0.02
5.0
8.9
SiglecF
CCR3
Ly6G
CD11b
E
Spleen Granulocytes (%)
Neutrophil
CCR3+ SiglecF+
F
Spleen Granulocytes (x10^6)
Trib1+/+
Trib1ΔHSC
Neutrophil
CCR3+ SiglecF+
H
Blood Granulocytes (%)
Neutrophil
CCR3+ SiglecF+
I
Granulocytes/ml blood (x10^5)
Trib1+/+
Trib1ΔHSC
Neutrophil
CCR3+ SiglecF+
J
Trib1+/+
Trib1ΔHSC
0.9
0.7
0.7
0.6
CD16/32
CD34
K
BM Progenitors (%)
1.250
0.625
0
ns
CMP
GMP
L
BM Progenitors (x10^4)
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18.75
12.5
6.25
0
Trib1+/+
Trib1ΔHSC
CMP
GMP

Figure S3

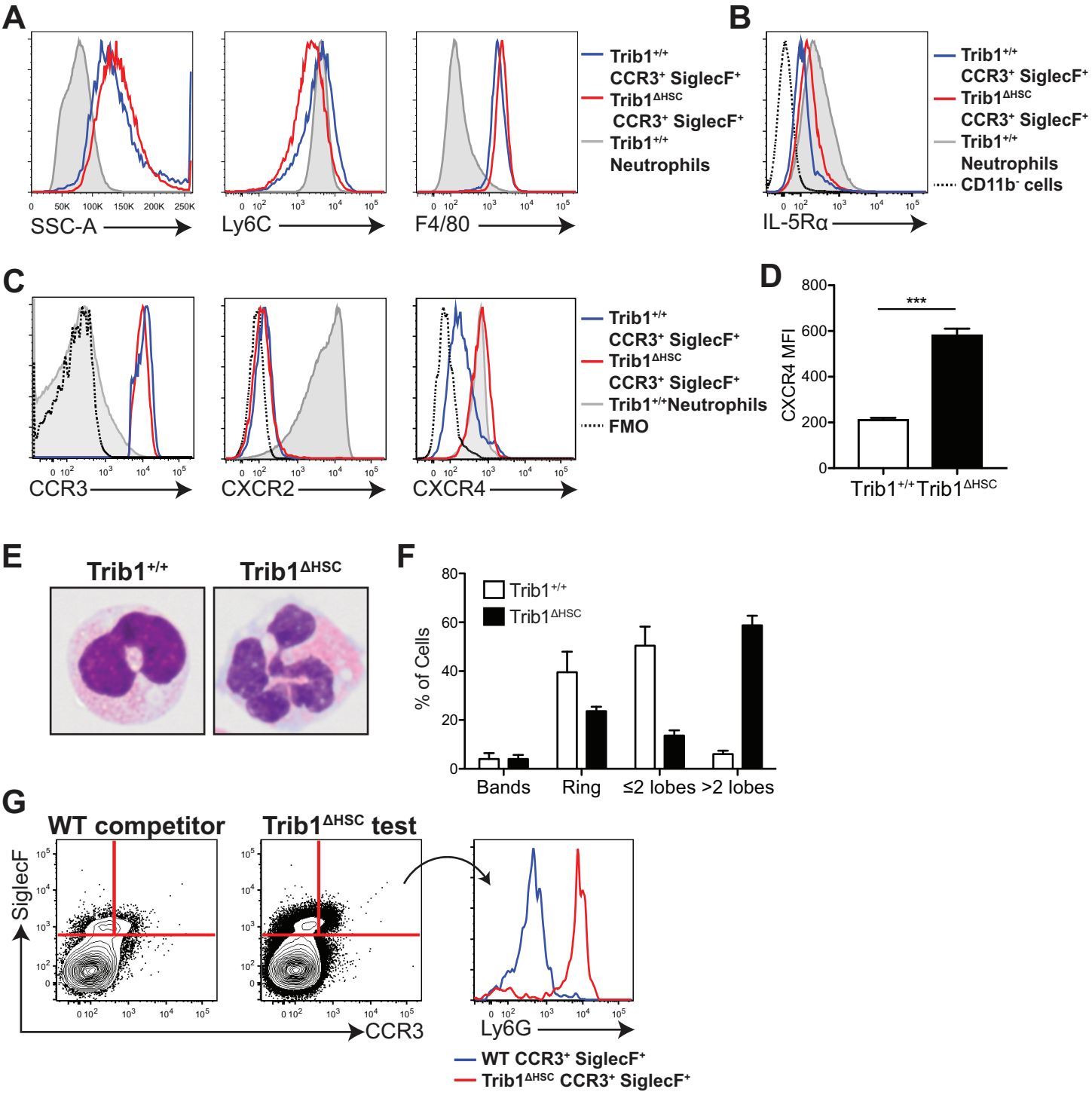

A
SSC-A
Ly6C
F4/80
Trib1+/+ CCR3+ SiglecF+
Trib1ΔHSC CCR3+ SiglecF+
Trib1+/+ Neutrophils
B
IL-5Rα
Trib1+/+ CCR3+ SiglecF+
Trib1ΔHSC CCR3+ SiglecF+
Trib1+/+ Neutrophils
CD11b- cells
C
CCR3
CXCR2
CXCR4
Trib1+/+ CCR3+ SiglecF+
Trib1ΔHSC CCR3+ SiglecF+
Trib1+/+Neutrophils
FMO
D
CXCR4 MFI
***
Trib1+/+ Trib1ΔHSC
E
Trib1+/+
Trib1ΔHSC
F
% of Cells
Trib1+/+
Trib1ΔHSC
Bands
Ring
≤2 lobes
>2 lobes
G
WT competitor
Trib1ΔHSC test
SiglecF
CCR3
Ly6G
WT CCR3+ SiglecF+
Trib1ΔHSC CCR3+ SiglecF+

Figure S4

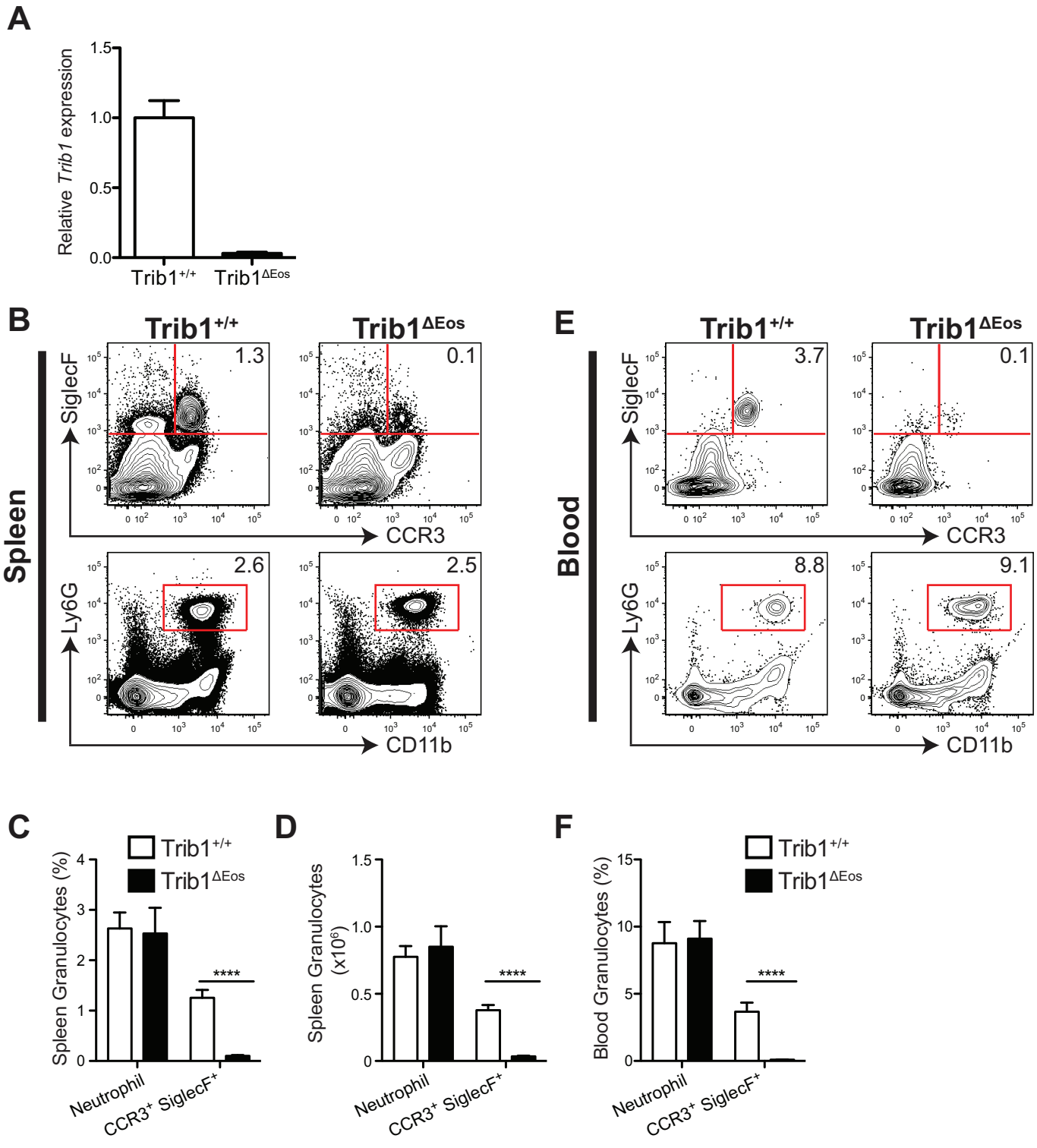
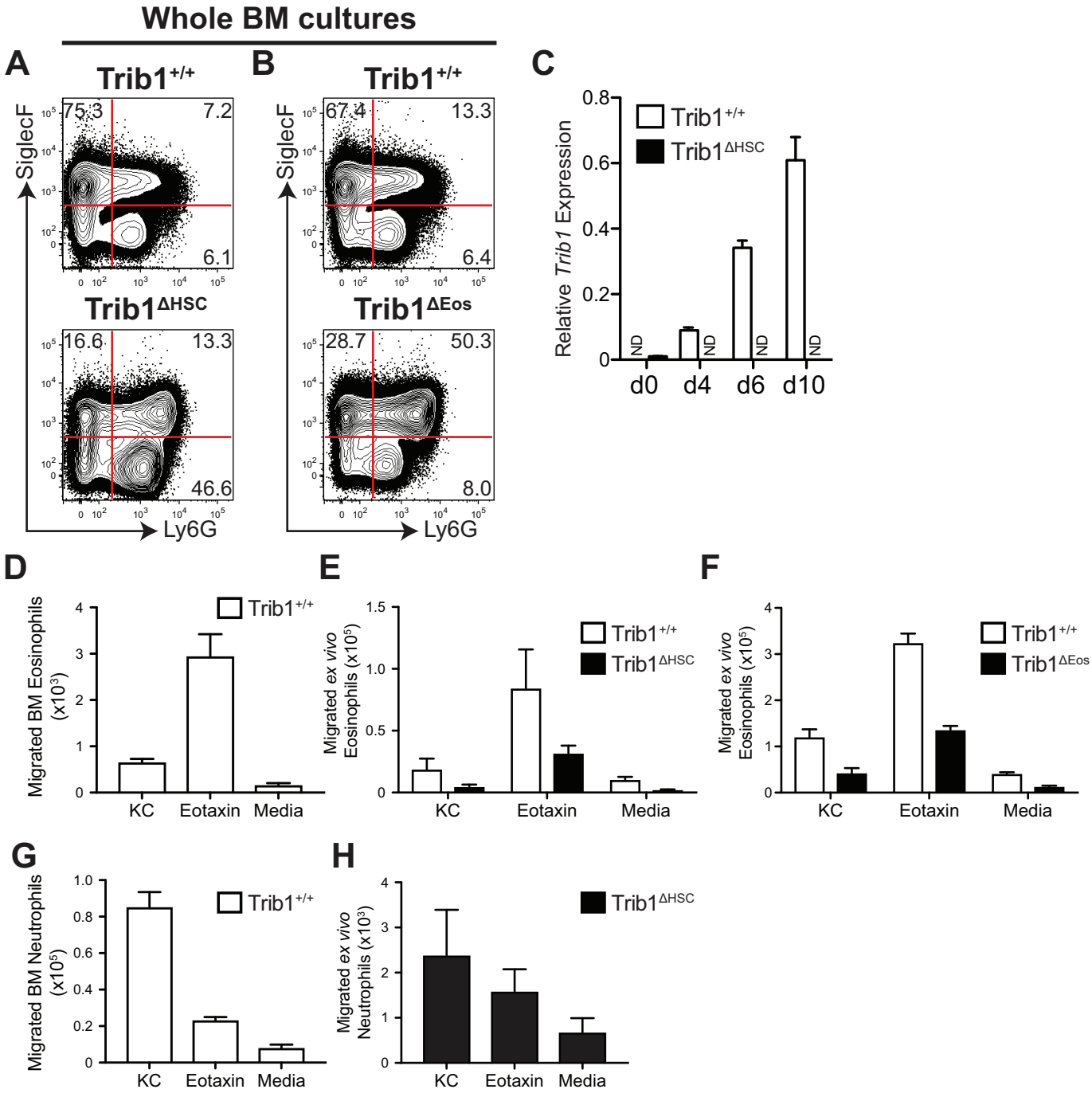

A
Relative Trib1 expression
1.5
1.0
0.5
0.0
Trib1+/+
Trib1ΔEos
B
Trib1+/+
Trib1ΔEos
Spleen
SiglecF
CCR3
1.3
0.1
Ly6G
CD11b
2.6
2.5
E
Blood
3.7
0.1
8.8
9.1
C
Spleen Granulocytes (%)
Neutrophil
CCR3+ SiglecF+
****
D
Spleen Granulocytes (x10^6)
F
Blood Granulocytes (%)

Figure S5


Whole BM cultures
A
Trib1+/+
SiglecF
75.3
7.2
6.1
Trib1ΔHSC
16.6
13.3
46.6
Ly6G
B
Trib1+/+
67.4
13.3
6.4
Trib1ΔEos
28.7
50.3
8.0
Ly6G
C
Relative Trib1 Expression
Trib1+/+
Trib1ΔHSC
ND
d0
d4
d6
d10
D
Migrated BM Eosinophils (x10³)
Trib1+/+
KC
Eotaxin
Media
E
Migrated ex vivo Eosinophils (x10⁵)
Trib1+/+
Trib1ΔHSC
F
Migrated ex vivo Eosinophils (x10⁵)
Trib1+/+
Trib1ΔEos
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Migrated BM Neutrophils (x10⁵)
Trib1+/+
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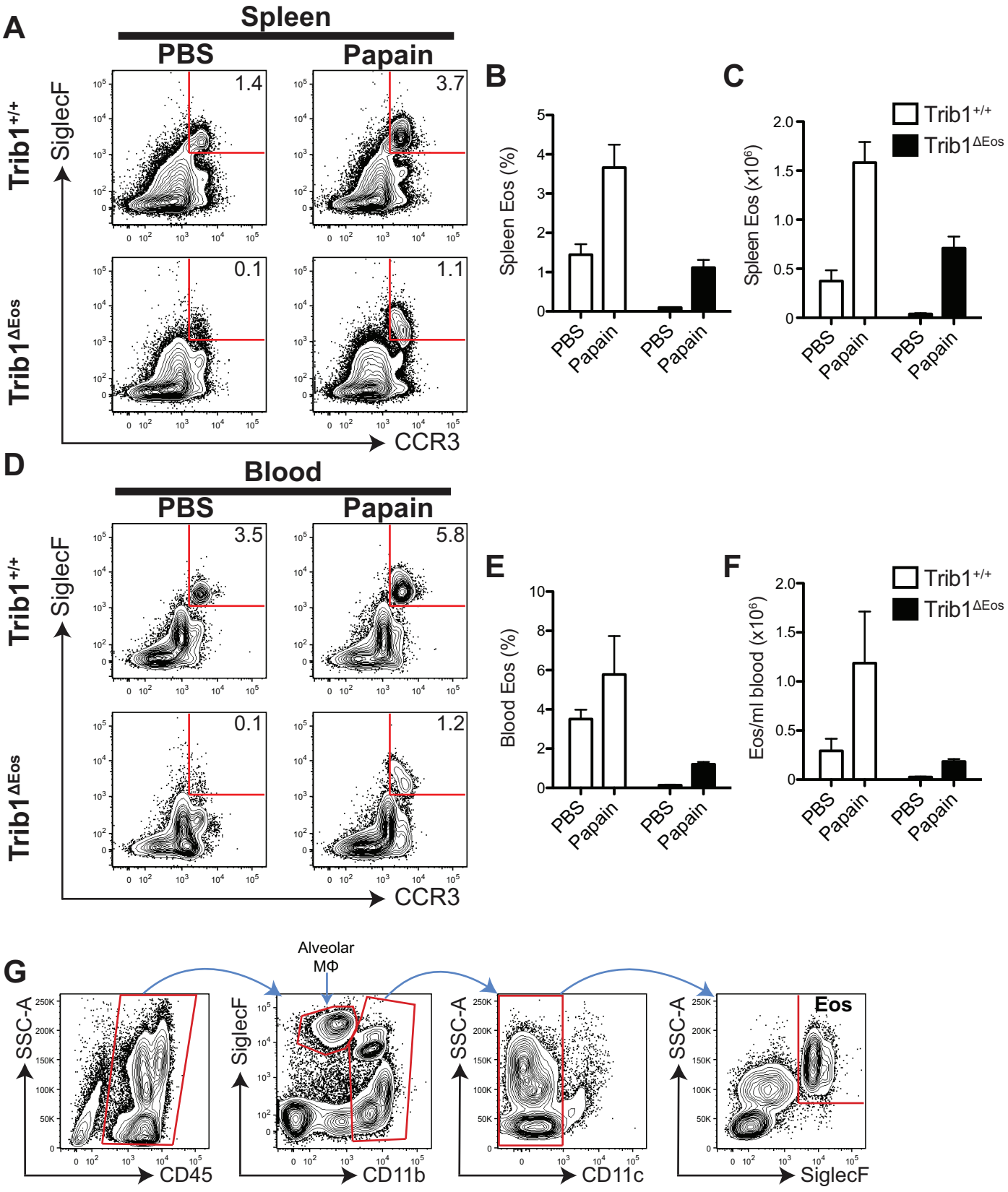
Trib1ΔHSC

Figure S6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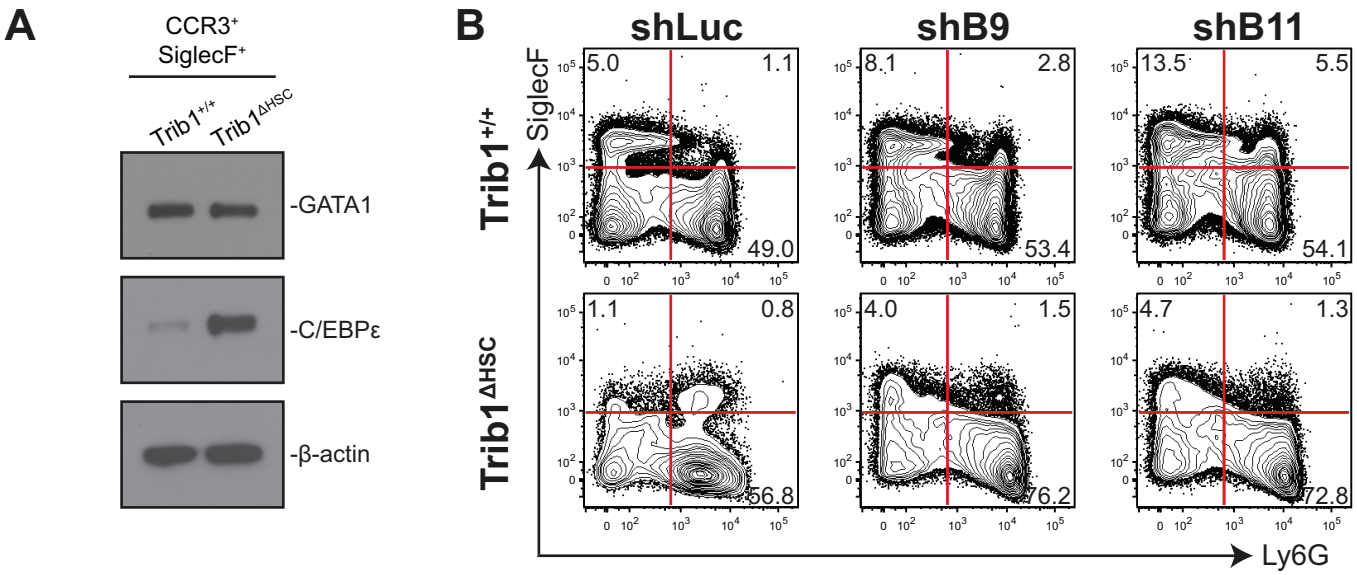
Spleen
PBS
Papain
Trib1+/+
Trib1ΔEos
SiglecF
CCR3
1.4
3.7
0.1
1.1
B
Spleen Eos (%)
C
Spleen Eos (x10^6)
Trib1+/+
Trib1ΔEos
PBS
Papain
D
Blood
3.5
5.8
0.1
1.2
E
Blood Eos (%)
F
Eos/ml blood (x10^6)
G
Alveolar MΦ
SSC-A
CD45
SiglecF
CD11b
CD11c
Eos

Figure S7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CCR3+
SiglecF+
Trib1+/+
Trib1ΔHSC
-GATA1
-C/EBPε
-β-actin
B
shLuc
shB9
shB11
Trib1+/+
Trib1ΔHSC
SiglecF
Ly6G
5.0
1.1
49.0
8.1
2.8
53.4
13.5
5.5
54.1
1.1
0.8
56.8
4.0
1.5
76.2
4.7
1.3
72.8