

Antibodies	Clones	Supplier	Cat. No
c-kit(CD117)	2B8	BD Pharmingen	553355
Sca-1(Ly6-A/E)	D7	BD Pharmingen	558162
c-kit(CD117)	2B8	BioLegend	105826
c-kit(CD117)	2B8	BioLegend	105808
Sca-1(Ly6-A/E)	D7	BioLegend	122514
Mouse Lineage Antibody Cocktail	145-2C11, M1/70, RA3-6B2, TER-119, RB6-8C5	BD Pharmingen	558074/ 561301
Biotin Mouse Lineage Panel	145-2C11, M1/70, RA3-6B2, TER-119, RB6-8C5	BD Pharmingen	559971
CD48	HM48-1	BioLegend	103432
CD150	TC15-12F 12.2	BioLegend	115922
CD34	RAM34	eBioscience	11-0341
CD16/CD32	93	eBioscience	48-0161
CD3e	145-2C11	BioLegend	100330
CD19	1D3	eBioscience	11-0193
CD19	1D3	BD Pharmingen	557399
B220	RA3-6B2	eBioscience	11-0452
Gr1(Ly-6G/C)	RB6-8C5	eBioscience	48-5931
CD11b	M1/70	BD Pharmingen	552850
CD127	SB/199	BD Pharmingen	564175
CD106/VCAM-1	429	BD Pharmingen	553332
CD51	RMV-7	eBioscience	13-0512
CD31	MEC 13.3	BD Pharmingen	553373
CD31	390	eBioscience	17-0311
CD135	A2F10	BioLegend	135315
IgM	RMM-1	BioLegend	406515

IgD	11-26c.2a	BioLegend	405712
CD45.1	A20	BioLegend	110726
CD45.1	A20	BD Pharmingen	553776
CD45.2	104	BD Pharmingen	558702
CD144/VE-cadherin	BV13	BioLegend	138005
CD144/VE-cadherin	BV13	eBioscience	53-1441
CD45	30-F11	eBioscience	13-0451
TER-119	TER-119	eBioscience	13-5921
CD61	2C9.G3	eBioscience	11-0611
Ki-67	SolA15	eBioscience	11-5698

1

2 **Supplementary Table 1: Antibodies used for flow cytometry analysis.**

3 **Supplementary Figures**

4 **Supplementary Figure 1.** Fluorescent microscopy image showing the distribution of Del-1 in the
5 BM. VE-cadherin staining is used for endothelial cells (scale bar, 100 μ m).

6

7 **Supplementary Figure 2.** G-CSF levels in the plasma of *Edil3*^{-/-} (n=10 mice) and *Edil3*^{+/+} mice
8 (n= 9 mice). Mann-Whitney U test.

9

10 **Supplementary Figure 3. Analysis of lymphoid lineage populations in the BM of *Edil3*^{-/-} and**
11 ***Edil3*^{+/+} mice.** (A) Representative flow cytometry plots and number of (B) B220⁺CD19⁻IgM⁻ pre-
12 pro B, (C) B220⁺CD19⁺IgM^{hi}IgD⁻ transitional (D) B220⁺CD19⁺IgM^{int} immature and (E)
13 B220⁺CD19⁺IgD⁺ mature B cells in the BM of 10 week-old *Edil3*^{-/-} (n=8 mice) and *Edil3*^{+/+} mice
14 (n=12 mice). (F) Number of common lymphoid progenitors (CLP;
15 Lin⁻Sca1^{low}cKit^{low}Flt3⁺IL7R α ⁺) in the BM of 10 week-old *Edil3*^{-/-} and *Edil3*^{+/+} mice (n= 5 mice
16 per group). Data presented as mean \pm SEM. Mann-Whitney U test.

17

18 **Supplementary Figure 4. Hematopoietic progenitors in Del-1 deficiency.** (A) Representative
19 flow cytometry plots, (B) LSK, (C) LT-HSC, (D) ST-HSC and (E) MPP in the BM of *Edil3*^{-/-} and
20 *Edil3*^{+/+} mice (n=10 mice per group). (F) Representative flow cytometry plots and (G) percentage
21 of Flt3⁻CD34⁺ myeloid biased MPPs in *Edil3*^{-/-} (n= 8 mice) and *Edil3*^{+/+} mice (n=9 mice). (H)
22 *Cebpe*, *Csf1r*, *Csf2ra*, *Irf8* expression in CD48⁻ LSK cells from the BM of *Edil3*^{-/-} and *Edil3*^{+/+}
23 mice (n=4 mice per group). The mRNA expression was normalized against 18s. Data presented
24 as mean \pm SEM. Mann-Whitney U test, **P*<0.05, ***P*<0.01.

25

26 **Supplementary Figure 5. Circulating and splenic hematopoietic and myeloid progenitor**
27 **cells under steady-state conditions.** (A) LSK and (B) LK cell numbers in the peripheral blood of
28 *Edil3*^{-/-} and *Edil3*^{+/+} mice (n=7-9 mice per group). (C) Number of CFC in the peripheral blood of
29 *Edil3*^{-/-} and *Edil3*^{+/+} mice (n=6-7 mice per group). (D) Numbers and (E) percentages of LSK cells
30 in the spleen of *Edil3*^{-/-} and *Edil3*^{+/+} mice (n=8 mice per group) and (F) numbers and (G)
31 percentages of LK cells in the spleen of *Edil3*^{-/-} and *Edil3*^{+/+} mice (n=8 mice per group). Data
32 presented as mean±SEM. Mann-Whitney U test, **P*<0.05, ***P*<0.01.

33

34 **Supplementary Figure 6. Differentiation assay of MPPs and CMPs.** (A) MPPs from WT mice
35 were cultured with Del-1-Fc or Fc-control (500 ng/ml each) in cell suspension cultures and
36 analysis was performed after 48h. Percentage of GMPs is shown (n=5 cultures). (B) CMPs from
37 WT mice were cultured with Del-1-Fc or Fc-control (500 ng/ml each) in cell suspension cultures
38 and analysis was performed after 48h. Percentage of GMPs is shown (n=5 cultures). Mann-
39 Whitney U test.

40

41 **Supplementary Figure 7. Del-1 does not affect early homing into the BM.** Short-term
42 migration assay of hematopoietic progenitors. Accumulation of CFSE⁺ LSK in the BM of
43 recipient *Edil3*^{-/-} (n=6 mice) or *Edil3*^{+/+} mice (n=5 mice) at 3h after adoptive transfer of CFSE-
44 labelled Lin⁻ cells from WT mice. Cell accumulation in the BM is expressed as the percentage of
45 CFSE⁺ LSK in total LSK. Data presented as mean±SEM. Mann-Whitney U test.

46

47 **Supplementary Figure 8. β3 integrin mediates the interaction of hematopoietic progenitors**
48 **with Del-1.** (A) Lethally irradiated *Edil3*^{+/+} or *Edil3*^{-/-} mice were transplanted with CD45⁺ BM
49 cells derived from *Itgb3*^{-/-} mice. Numbers of (B) LSK cells, (C) CMPs, (D) GMPs and (E)
50 Gr1^{hi}CD11b⁺, (F) Gr1^{int}CD11b⁺ myeloid cells in the BM of recipient *Edil3*^{+/+} or *Edil3*^{-/-} mice 6

51 weeks post-transplantation (n= 10 mice per group). Data presented as mean±SEM. Mann-
52 Whitney U test was used.

53

54 **Supplementary Figure 9** (Complementary to Figure 8). G-CSF levels in the plasma of *Edil3*^{-/-}
55 and *Edil3*^{+/+} mice at 24h following the second injection of LPS (n=6 mice per group). Mann-
56 Whitney U test.

57

58 **Supplementary Figure 10. Circulating and splenic hematopoietic and myeloid progenitor**
59 **cells after LPS administration.** (Complementary to Figure 8). (A) LSK and (B) LK cell
60 numbers in the peripheral blood of *Edil3*^{-/-} and *Edil3*^{+/+} mice after 72h following the second
61 injection of LPS (n=4-5 mice per group). (C) Numbers of LSK and (D) numbers of LK cells in
62 the spleen of *Edil3*^{-/-} and *Edil3*^{+/+} mice after 72h following the second injection of LPS (n=9
63 mice per group). Data presented as mean±SEM. Mann-Whitney U test.

64

65 **Supplementary Figure 11. Role of Del-1 in hematopoietic progenitor response to G-CSF**
66 **administration.** (Complementary to Figure 9). (A) Total cellularity, (B) Gr1^{hi}CD11b⁺ and (C)
67 Gr1^{int}CD11b⁺ myeloid cell numbers in the BM of *Edil3*^{-/-} (n=14 mice) and *Edil3*^{+/+} mice (n=10
68 mice) 24h after a single G-CSF administration. (D) LSK, (E) LT-HSC, (F) ST-HSC, (G) MPP
69 and (H) CMP cell numbers in the BM of *Edil3*^{-/-} (n=6 mice) and *Edil3*^{+/+} mice (n=5 mice) 24h
70 after a single G-CSF administration. (I) Representative flow cytometry plots and (J) cell cycle
71 analysis in LT-HSC derived from *Edil3*^{-/-} (n=5 mice) and *Edil3*^{+/+} mice (n=7) after three
72 injections of G-CSF. Data presented as mean±SEM. Mann Whitney U test, **P*<0.05, ***P*<0.01,
73 ****P*<0.001.

74

75 **Supplementary Figure 12. Del-1 promotes myelopoiesis in response to G-CSF**
76 **administration.** (Complementary to Figure 9). *Edil3*^{-/-} and *Edil3*^{+/+} mice were injected daily

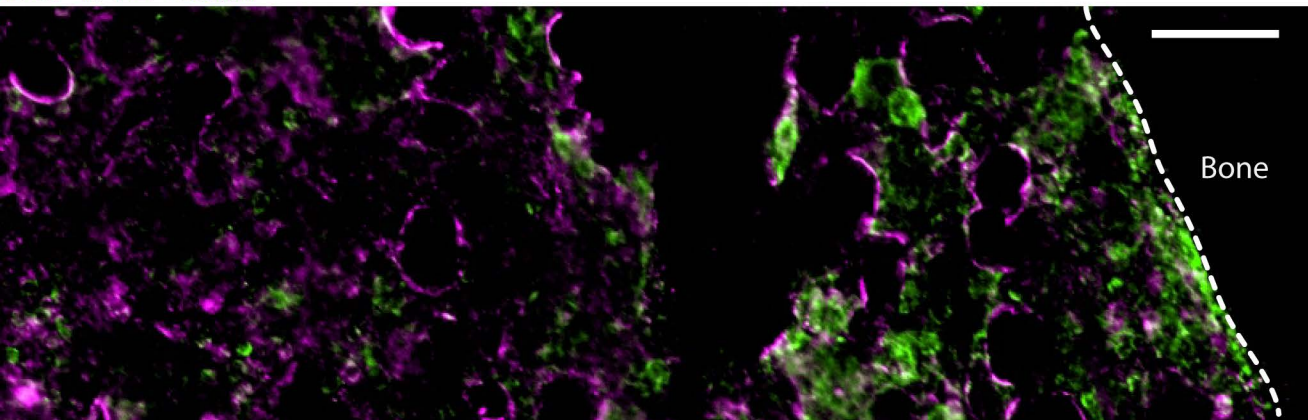
77 with G-CSF (n=6 mice per group) or PBS (n=5 mice per group) for 6 days. (A) The percentage of
78 GMPs was assessed by flow cytometry. The percentage of GMPs in the BM after 6 daily
79 injections of G-CSF was expressed relative to the percentage of GMPs in the BM after 6 daily
80 injections of PBS (% G-CSF/ % PBS). (B) The percentage of LSK cells was assessed by flow
81 cytometry. The percentage of LSKs in the BM after 6 daily injections of G-CSF was expressed
82 relative to the percentage of LSKs in the BM after 6 daily injections of PBS (%G-CSF/ %PBS).
83 Data presented as mean±SEM. Mann Whitney U test, ** $P<0.01$.

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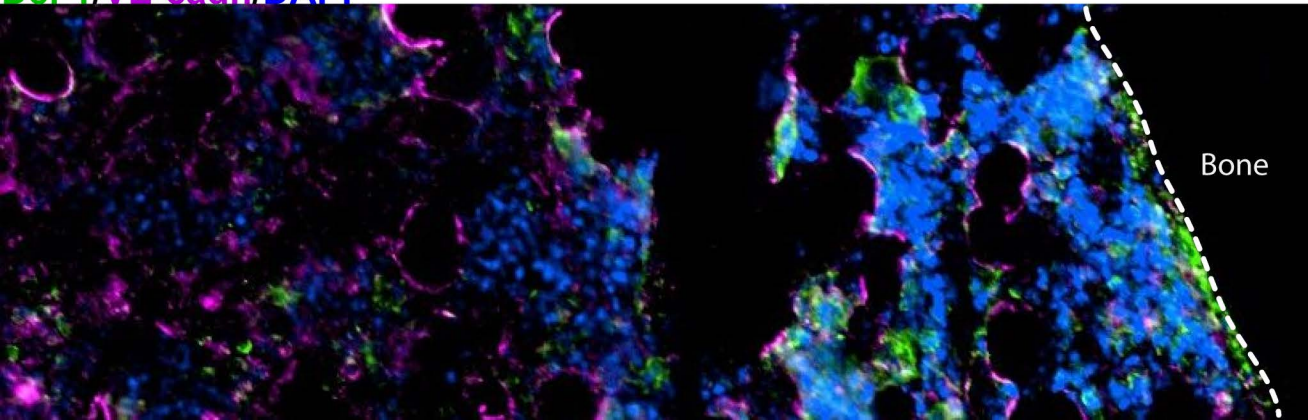
85 **Supplementary Figure 13.** (Complementary to Figure 9). (A) *Cxcl12* (B) *Angpt1* and (C) *Kitl*
86 mRNA levels in the BM of *Edil3^{+/+}* and *Edil3^{-/-}* mice on day 6 of the G-CSF administration
87 protocol. Mice treated with PBS were used as control (Ctrl) (n=6-7 mice per group). Data
88 presented as mean±SEM. One-way ANOVA followed by Holm Sidak's multiple comparison test,
89 * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

Supplementary Figure 1

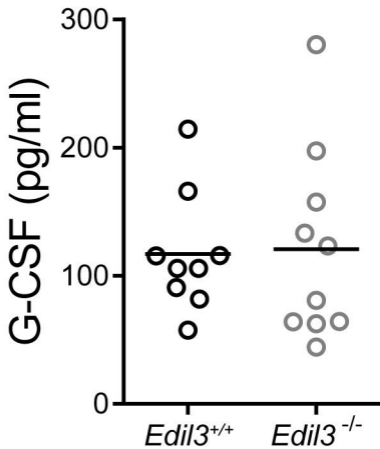
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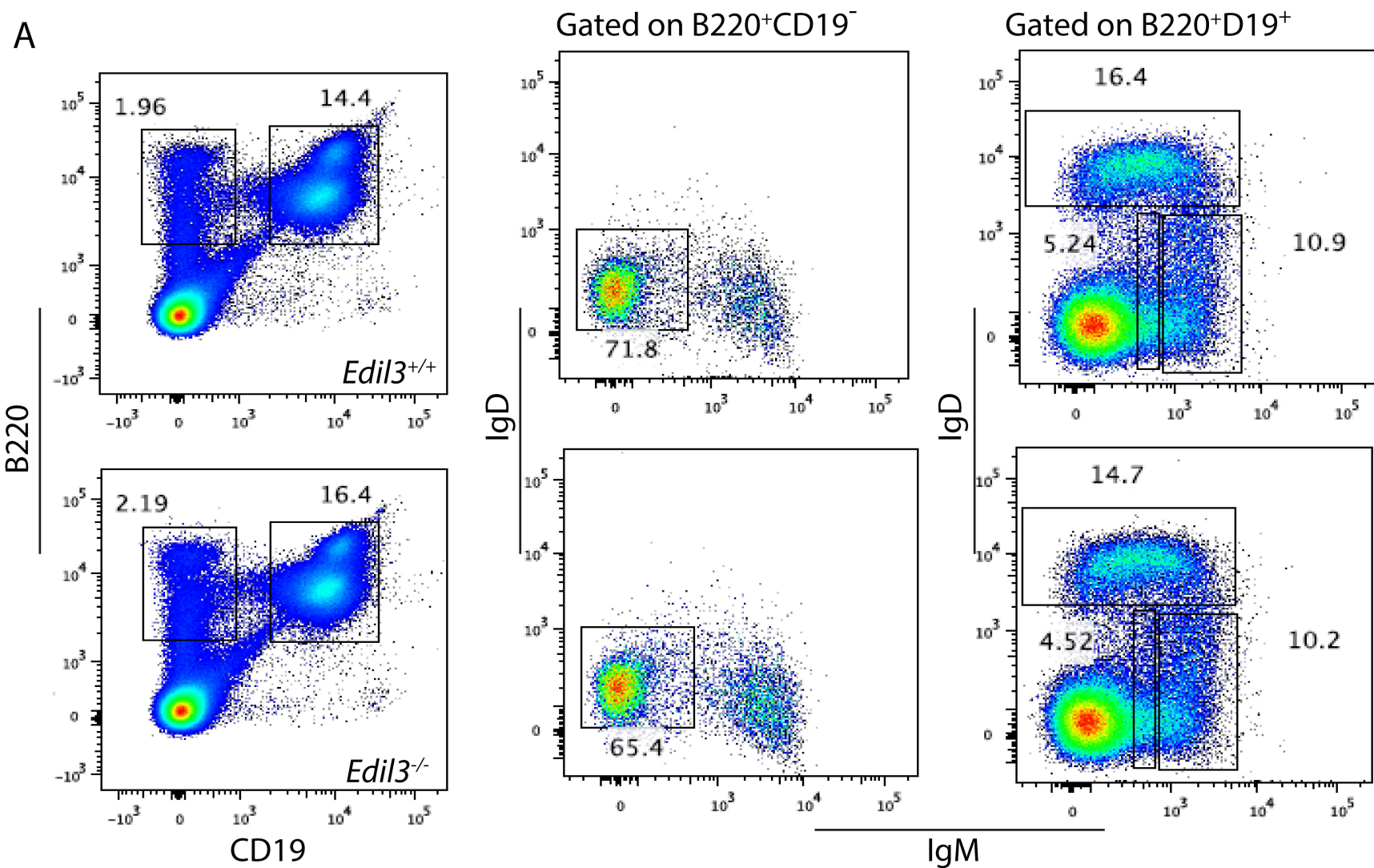
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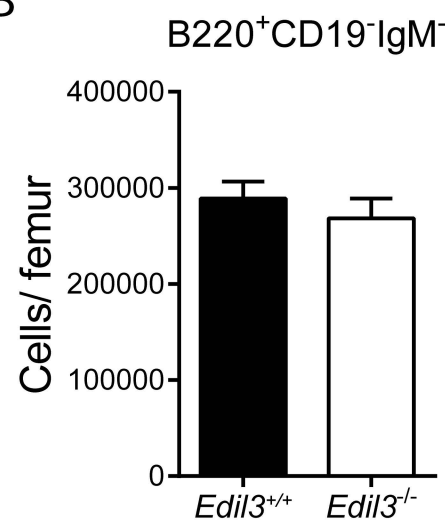
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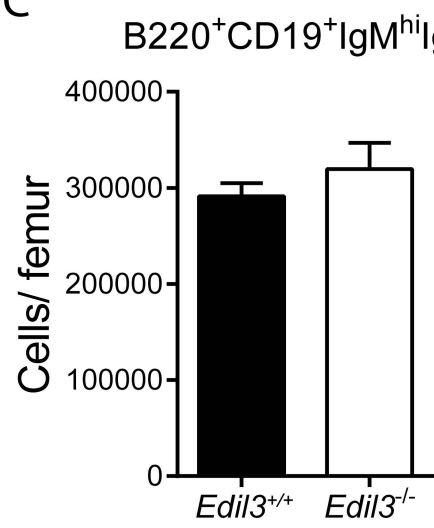
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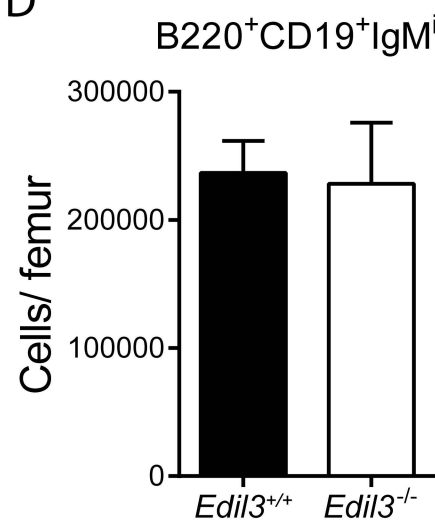
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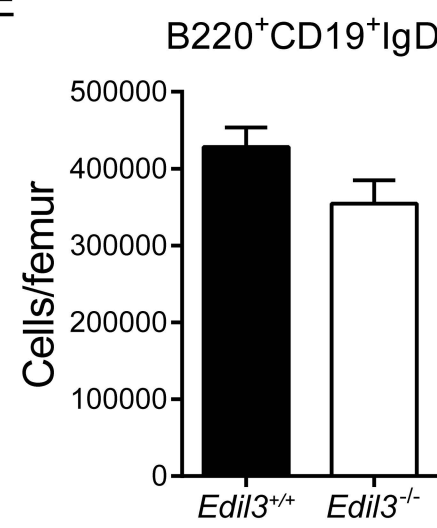
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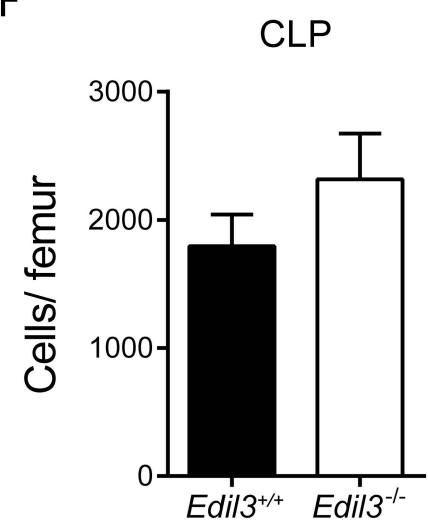
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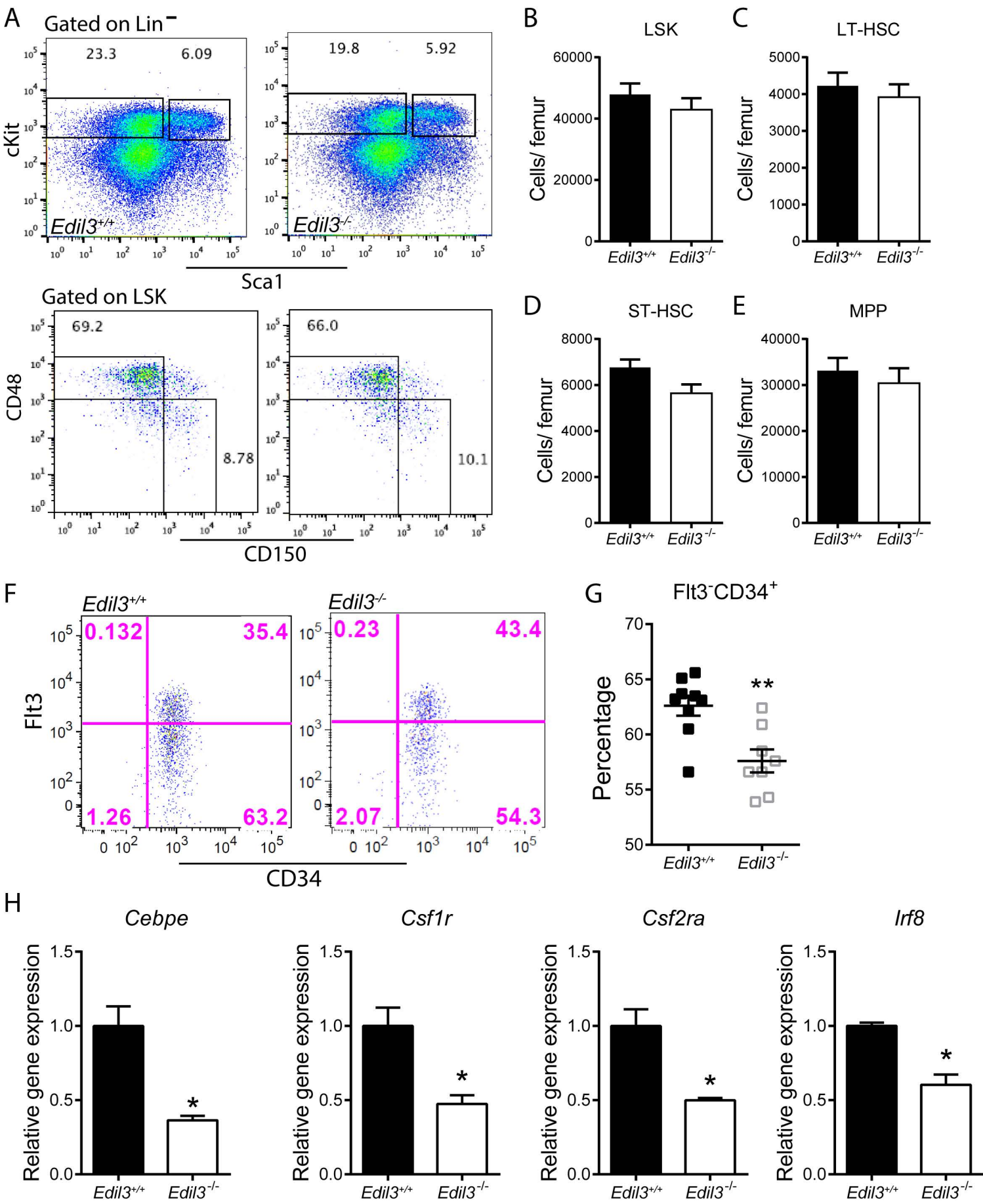
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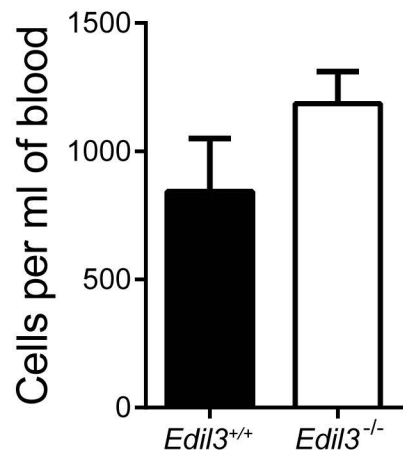
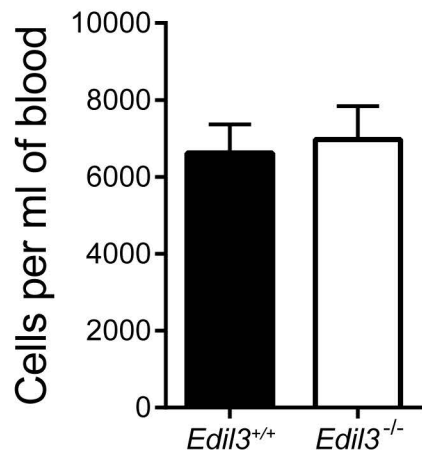
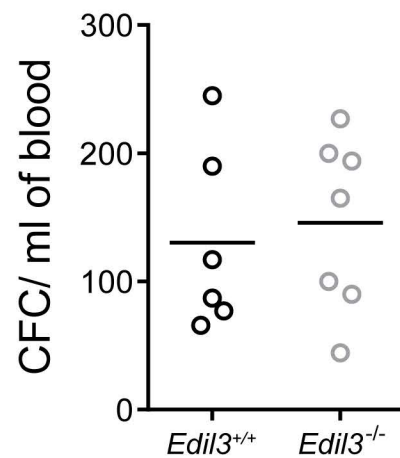
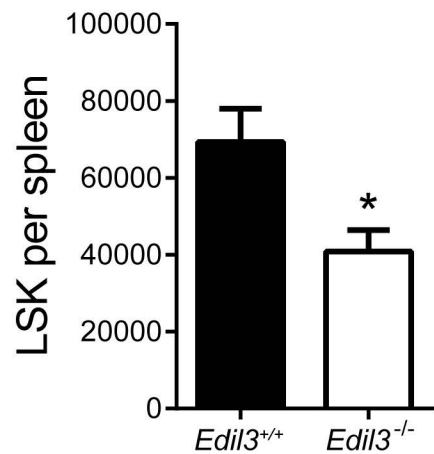
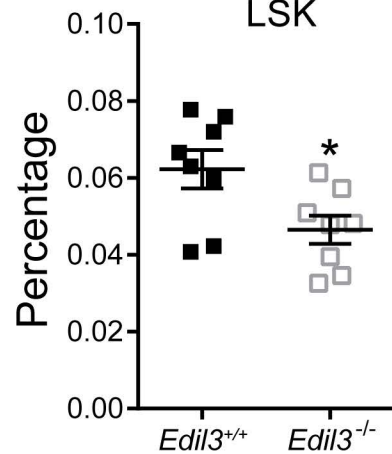
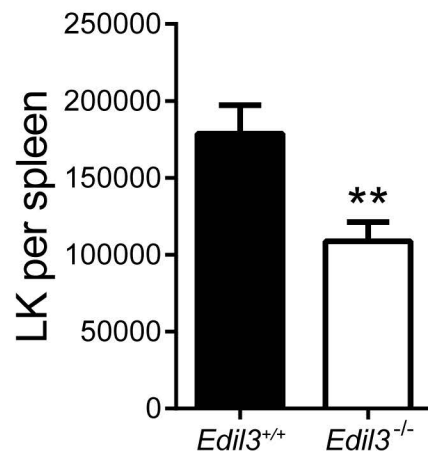
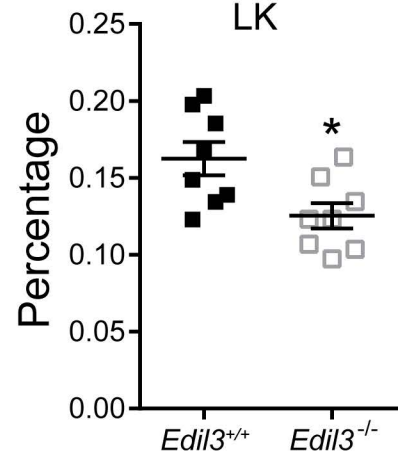
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Supplementary Figure 4

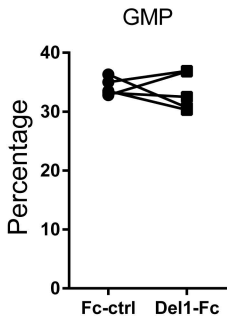
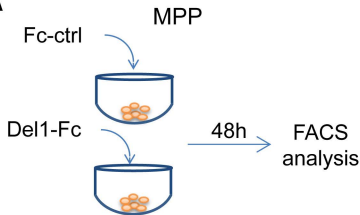


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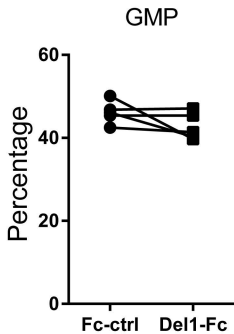
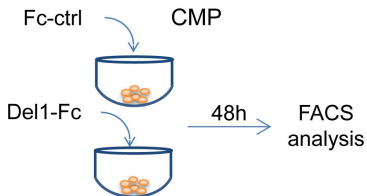
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Supplementary Figure 6

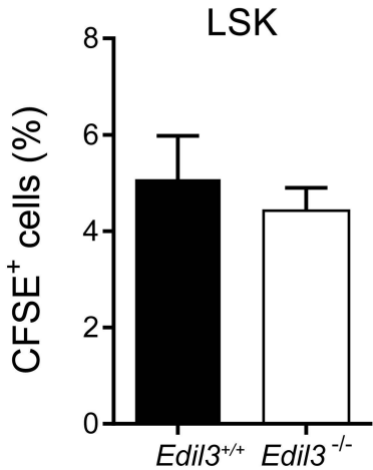
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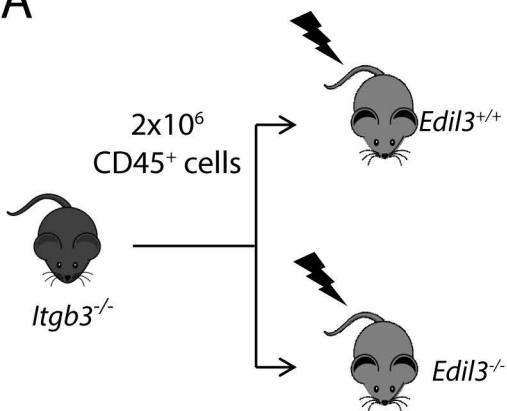
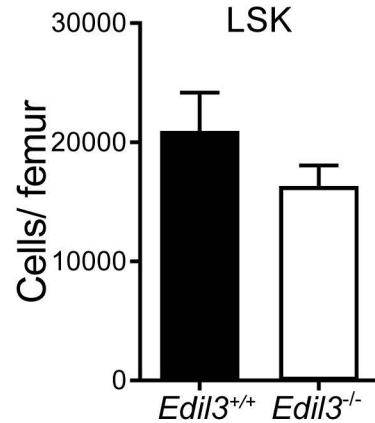
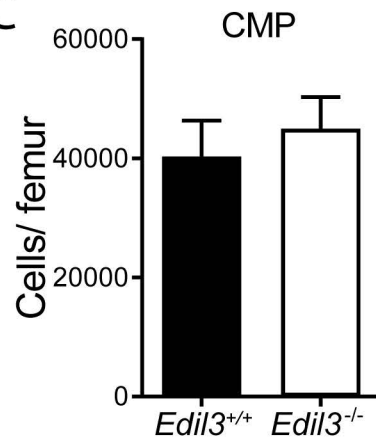
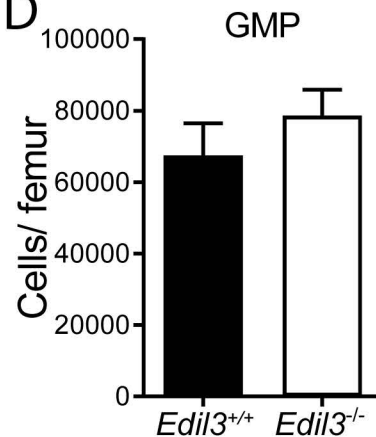
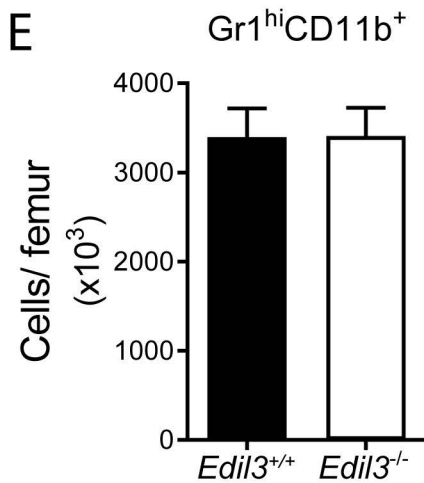
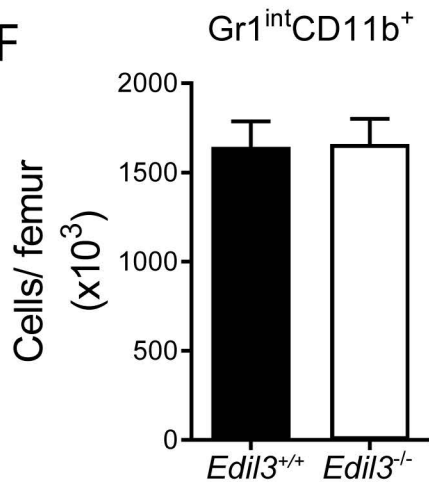
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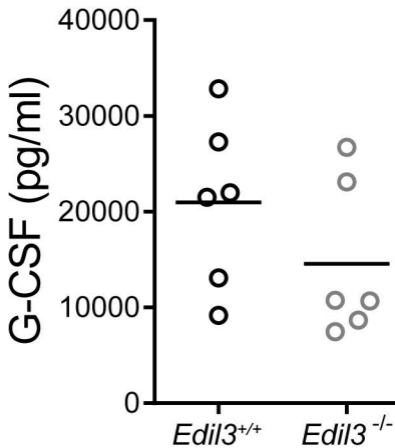
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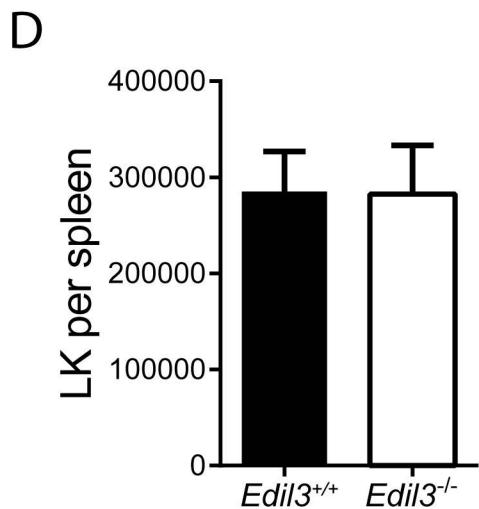
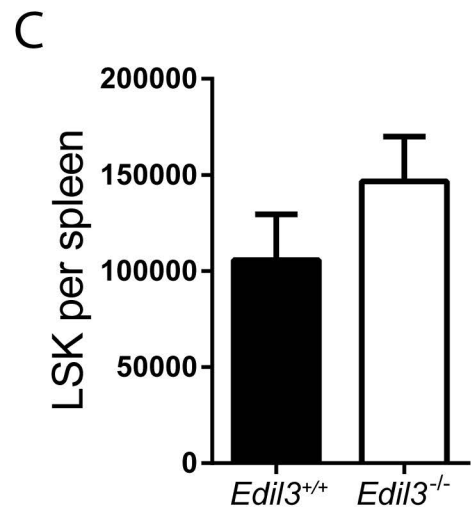
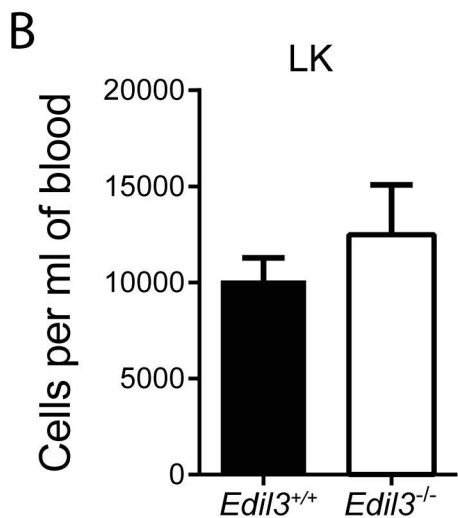
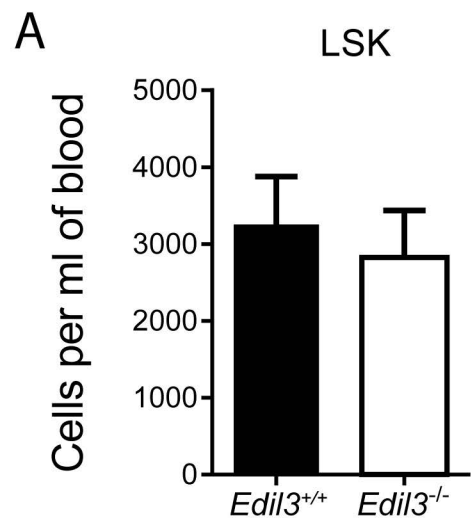


Supplementary Figure 8

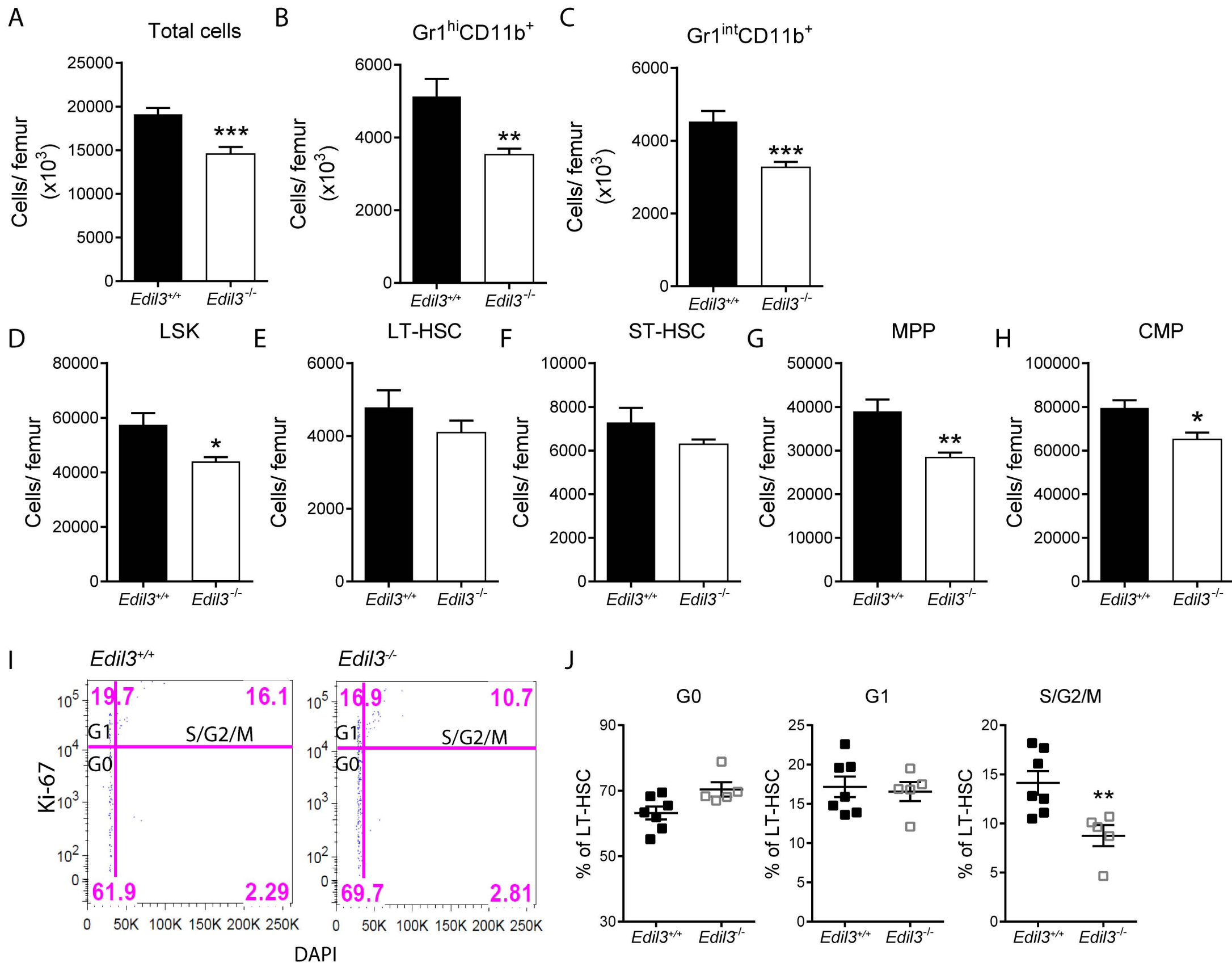
A**B****C****D****E****F**

Supplementary Figure 9



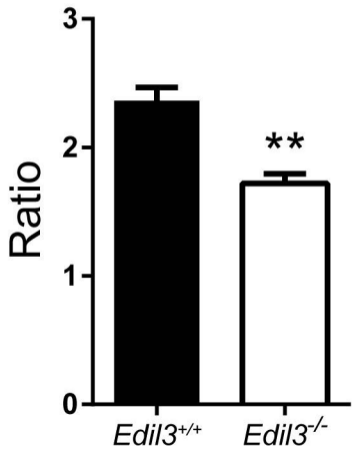


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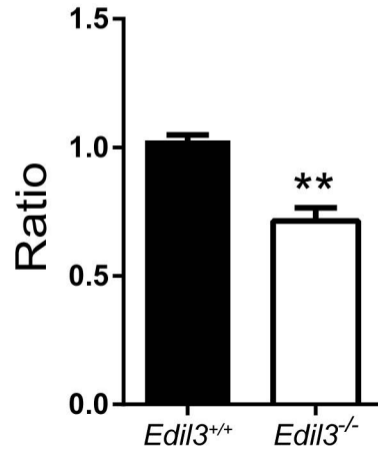


Supplementary Figure 12

A **GMP** **B**



B **LSK**



Supplementary Figure 13

