Supplementary Information

Notch2-dependent classical dendritic cells orchestrate intestinal immunity against attaching and effacing bacterial pathogens

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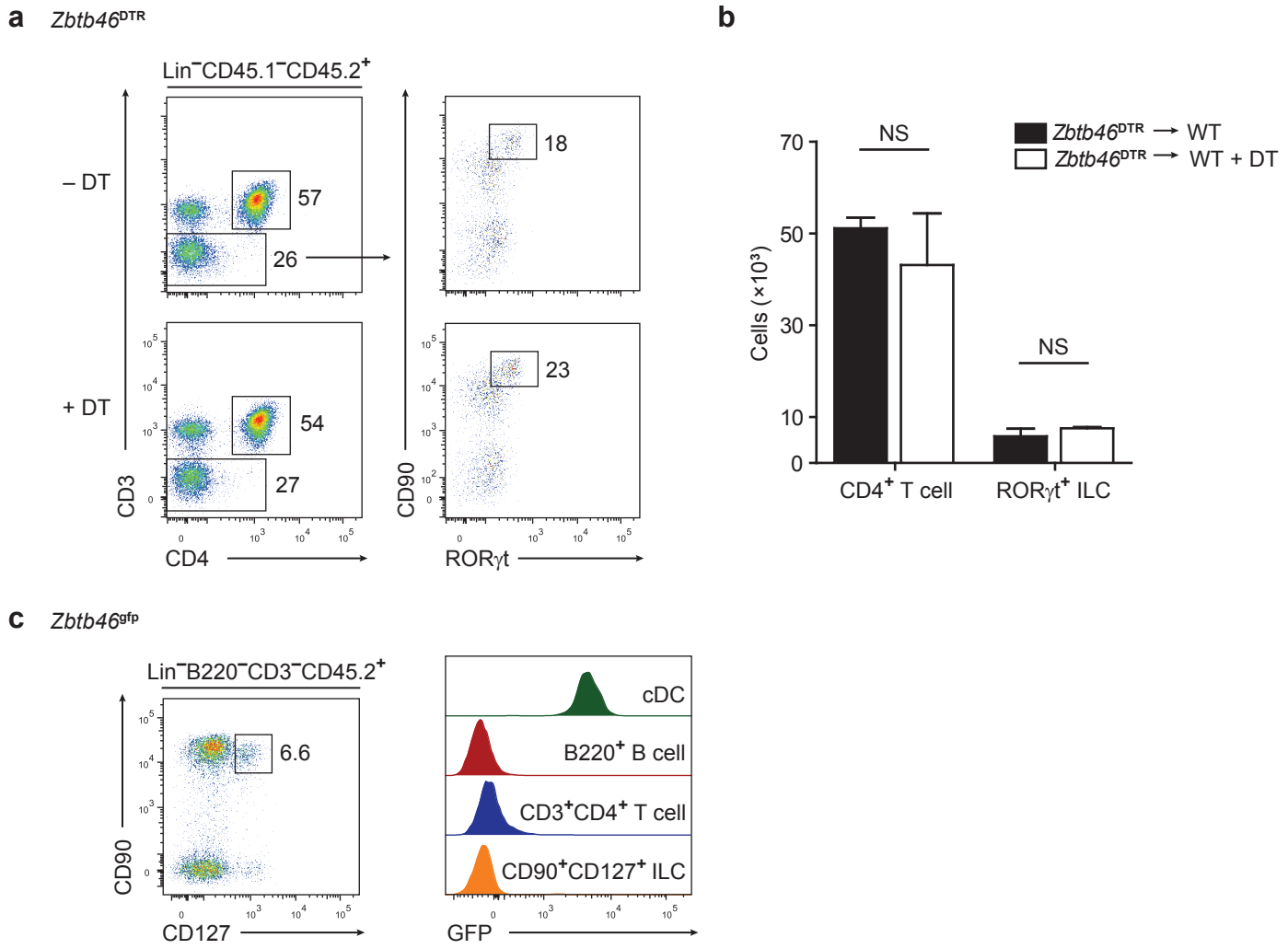
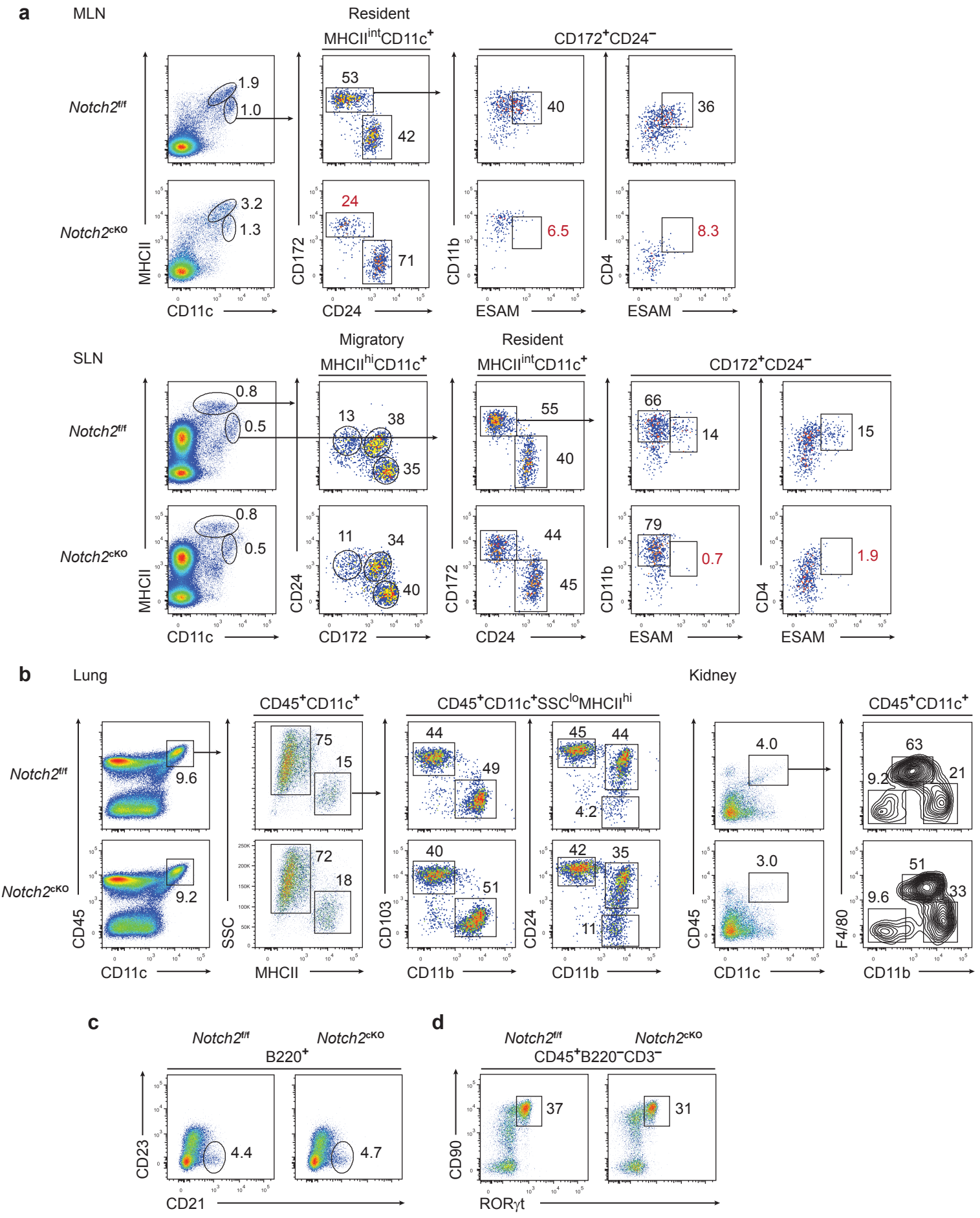
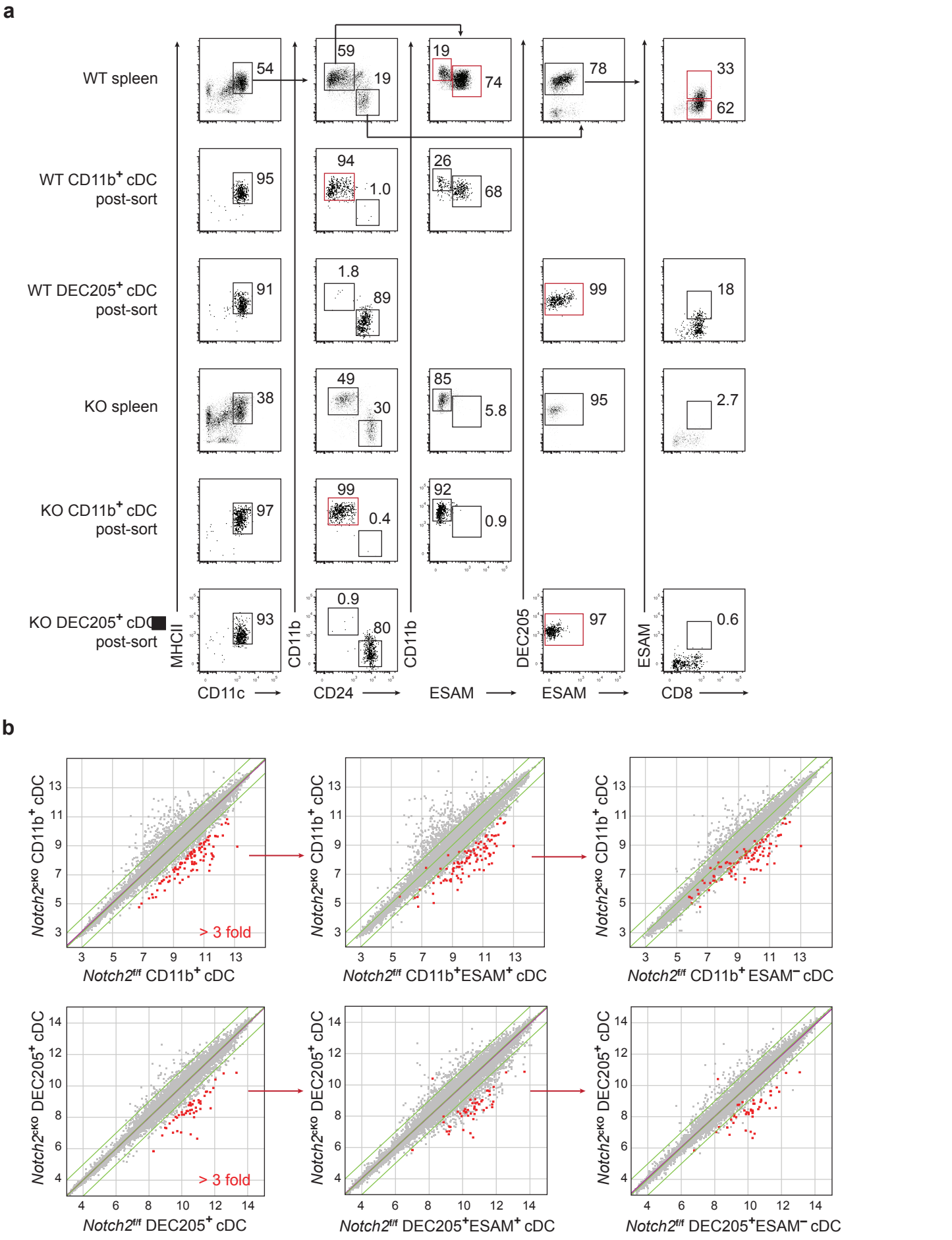


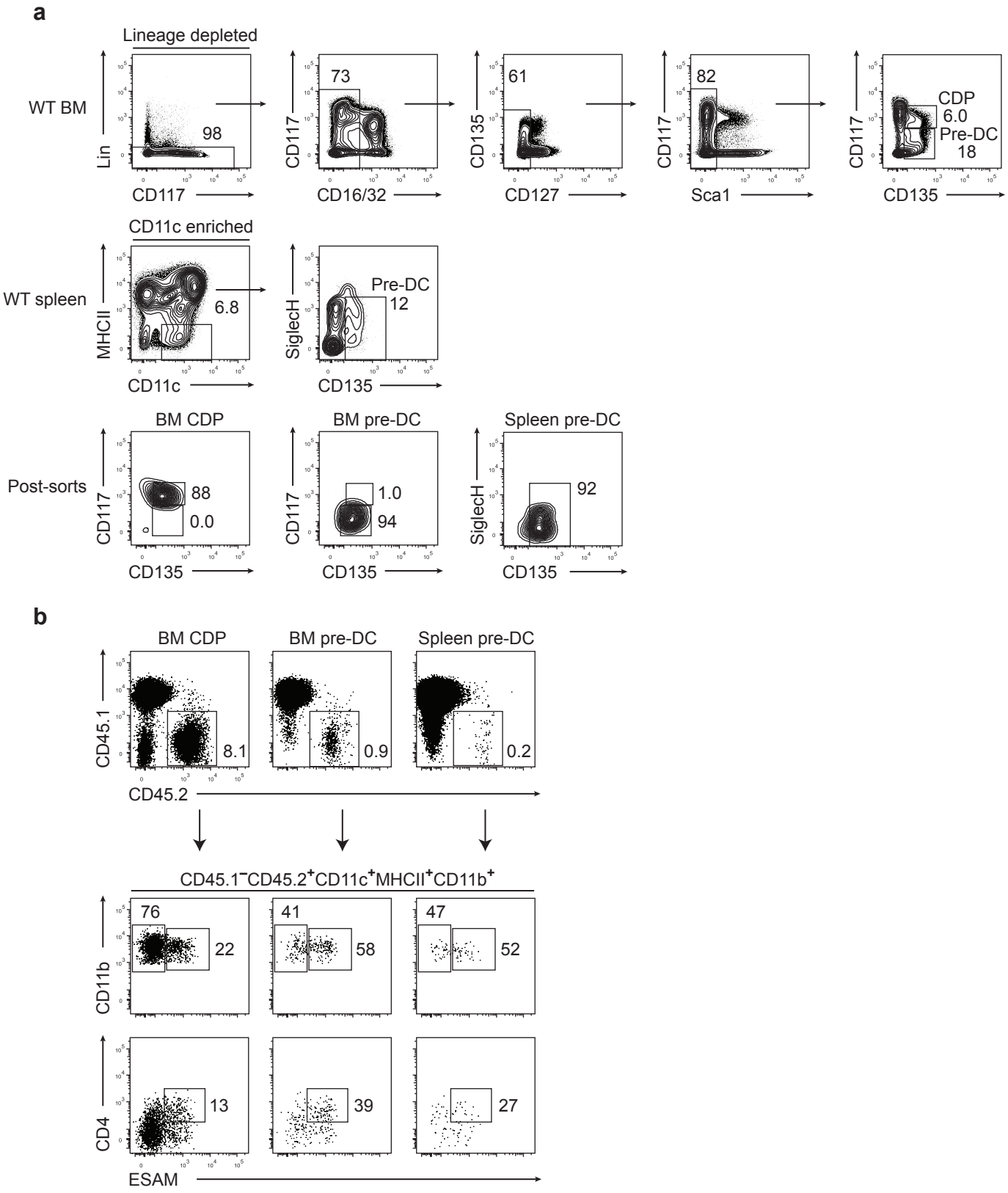
Supplementary Figure 1. *Zbtb46* expression in lamina propria RORγt⁺ ILCs. (**a, b**) *Zbtb46*^{DTR} → WT BM chimeras were treated with 40 ng/g DT on day -3 and day -1 and small intestine lamina propria cells were stained for expression of the indicated markers on day 0. (**a**) Shown are two-color histograms for live cells pre-gated as indicated above the diagram (Lin: Ly6G, CD11b, B220, NK1.1, Gr-1, CD11c). Numbers represent the percentage of cells in the designated gate. (**b**) Quantification of cells (per 1×10^6 lamina propria cells) following DT administration in [**a**]. Data are from two independent experiments (bars, SEM; *n* = 3 mice per group, Student's *t*-test). (**c**) Small intestine lamina propria cells from *Zbtb46*^{gfp} mice were harvested and stained for expression of the indicated markers. Shown is a two-color histogram for live cells pre-gated as indicated above the diagram (left) and a one-color histogram for GFP expression in the indicated cell types (right). Data are representative of four independent experiments (*n* = 8 mice). NS not significant.


a
MLN
Resident
MHCII^intCD11c^+
CD172^+CD24^−
Notch2^f/f
Notch2^cKO
1.9
1.0
3.2
1.3
53
42
24
71
40
36
6.5
8.3
MHCII
CD11c
CD172
CD24
CD11b
ESAM
CD4
ESAM
SLN
Migratory
MHCII^hiCD11c^+
Resident
MHCII^intCD11c^+
CD172^+CD24^−
0.8
0.5
13
38
35
55
40
66
14
15
11
34
40
44
45
79
0.7
1.9
b
Lung
Kidney
CD45^+CD11c^+
CD45^+CD11c^+SSC^loMHCII^hi
CD45^+CD11c^+
9.6
9.2
75
15
72
18
44
49
40
51
45
44
4.2
42
35
11
4.0
3.0
63
21
51
33
CD45
CD11c
SSC
MHCII
CD103
CD11b
CD24
CD11b
CD45
CD11c
F4/80
CD11b
c
Notch2^f/f
Notch2^cKO
B220^+
4.4
4.7
CD23
CD21
d
Notch2^f/f
Notch2^cKO
CD45^+B220^−CD3^−
37
31
CD90
RORγt

Supplementary Figure 2. cDC development in *Notch2*^{cKO} mice. (**a, b**) Cells were harvested from mesenteric or skin-draining lymph nodes (MLN, SLN), lungs or kidneys and stained for expression of the indicated markers. Shown are two-color histograms for live cells pre-gated as indicated above the diagram. Data are representative of three independent experiments (*n* = 6 mice per group). (**c**) Flow cytometry of marginal zone B cells. Splenocytes were stained for expression of the indicated markers. Data are representative of two independent experiments (*n* = 6 mice per group). (**d**) Flow cytometry of small intestine ILCs. Lamina propria cells were stained for expression of the indicated markers. Shown are two-color histograms for live cells pre-gated as indicated above the diagram. Data are representative of two independent experiments (*n* = 4 mice per group).

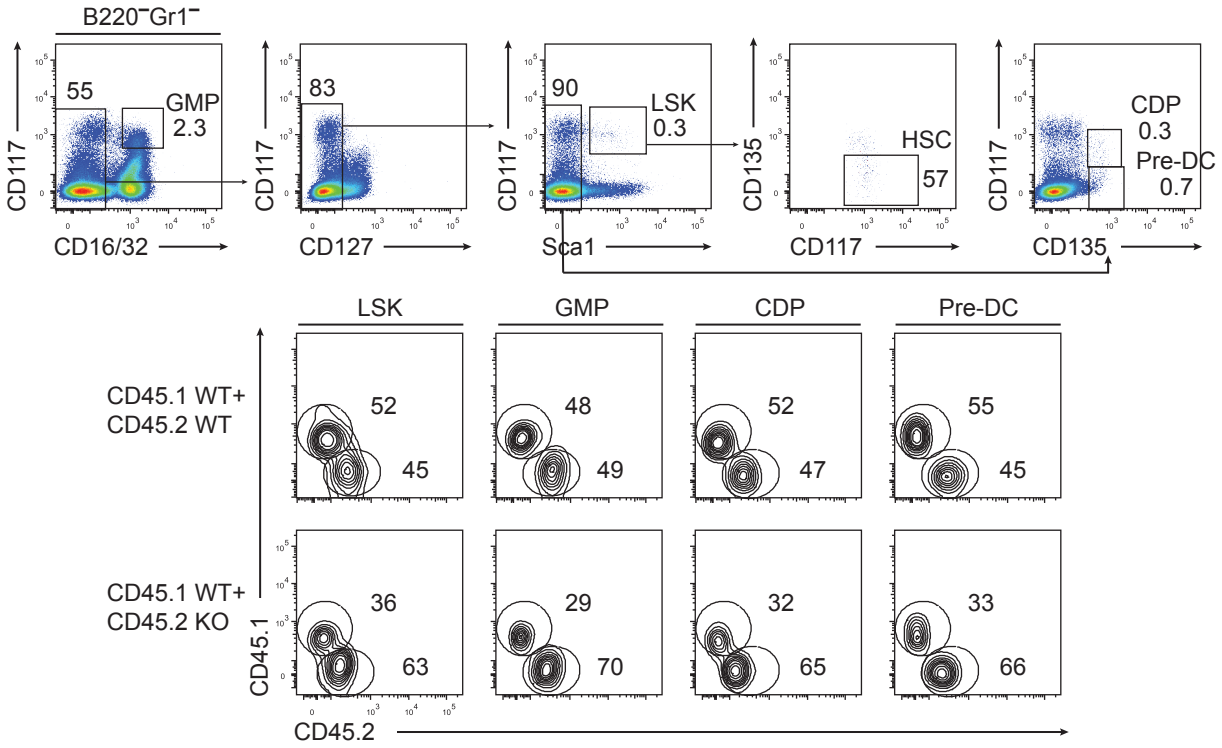

a
WT spleen
WT CD11b+ cDC post-sort
WT DEC205+ cDC post-sort
KO spleen
KO CD11b+ cDC post-sort
KO DEC205+ cDC post-sort
MHCII
CD11c
CD11b
CD24
CD11b
ESAM
DEC205
ESAM
ESAM
CD8
54
59
19
19
74
78
33
62
95
94
1.0
26
68
91
1.8
89
99
18
38
49
30
85
5.8
95
2.7
97
99
0.4
92
0.9
93
0.9
80
97
0.6
b
Notch2cKO CD11b+ cDC
Notch2f/f CD11b+ cDC
> 3 fold
Notch2cKO CD11b+ cDC
Notch2f/f CD11b+ESAM+ cDC
Notch2cKO CD11b+ cDC
Notch2f/f CD11b+ ESAM− cDC
Notch2cKO DEC205+ cDC
Notch2f/f DEC205+ cDC
> 3 fold
Notch2cKO DEC205+ cDC
Notch2f/f DEC205+ESAM+ cDC
Notch2cKO DEC205+ cDC
Notch2f/f DEC205+ESAM− cDC

Supplementary Figure 3. Microarray analysis of *Notch2*^{cKO} splenic cDC subsets. (**a**) Sort strategies for microarray analysis of Notch2$^{f/f}$ (WT) and *Notch2*^{cKO} (KO) splenic cDC subsets. Splenocytes were CD11c-enriched and stained for expression of the indicated markers. Shown are two-color histograms for live cells pre-gated as indicated in the diagram. Red boxes indicate populations sorted for analysis. (**b**) Microarray analysis of sorted cDC subsets from *Notch2*$^{f/f}$ and *Notch2*^{cKO} mice. Genes decreased in expression in *Notch2*^{cKO} CD11b^{+} or DEC205^{+} cDCs relative to *Notch2*$^{f/f}$ cDCs are highlighted in red (left panels) and projected onto M-plots comparing each subset to ESAM^{+} fractions (middle panels) or ESAM^{-} fractions (right panels) within that subset. Data are from two independent experiments (n = 3 biological replicates per cell type).

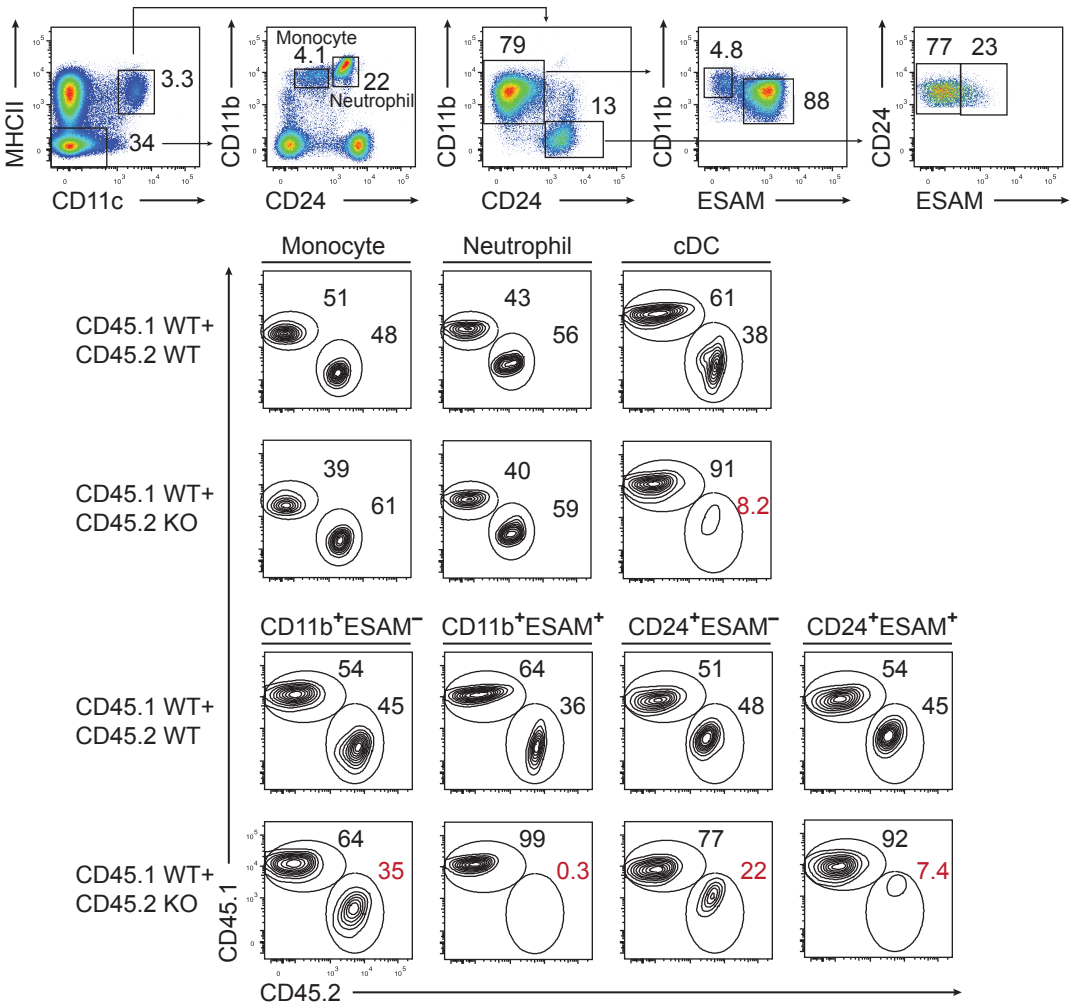


Supplementary Figure 4. Ontogeny of Notch2-dependent cDCs. (**a**) Sort strategies for DC progenitor populations from WT lineage-depleted BM cells or CD11c-enriched splenocytes. Cells were stained for expression of the indicated markers. Shown are two-color histograms for live cells pre-gated as indicated in the diagram (Lin: MHC-II, B220, Gr-1). (**b**) Progeny of sorted cell populations were analyzed 7 days after transfer into sub-lethally-irradiated CD45.1$^+$ mice. Shown are two-color histograms for live cells pre-gated as indicated above the diagram. Data are representative of two independent experiments (*n* = 2-3 biological replicates per cell type transferred).

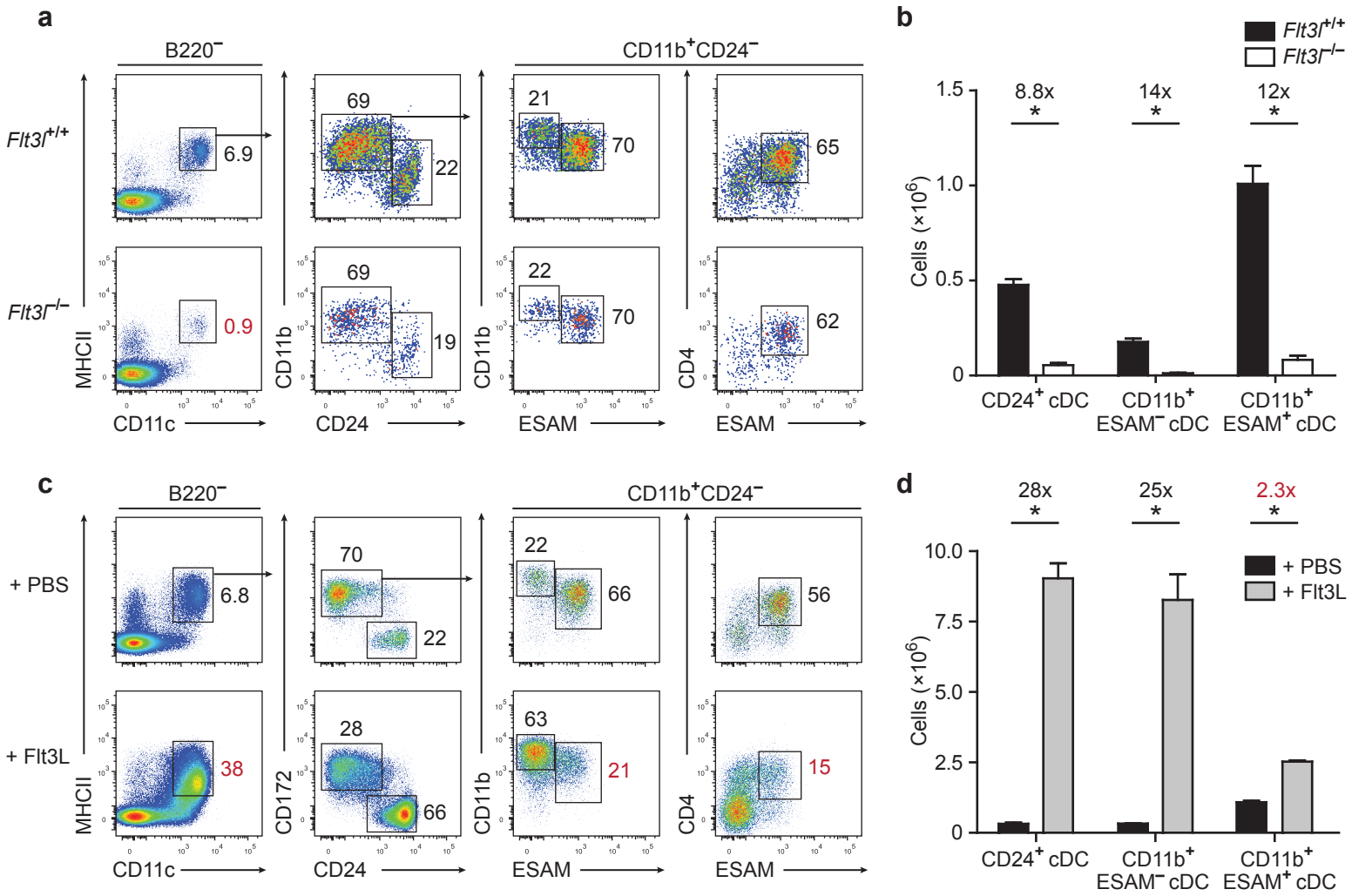
a Bone marrow progenitors



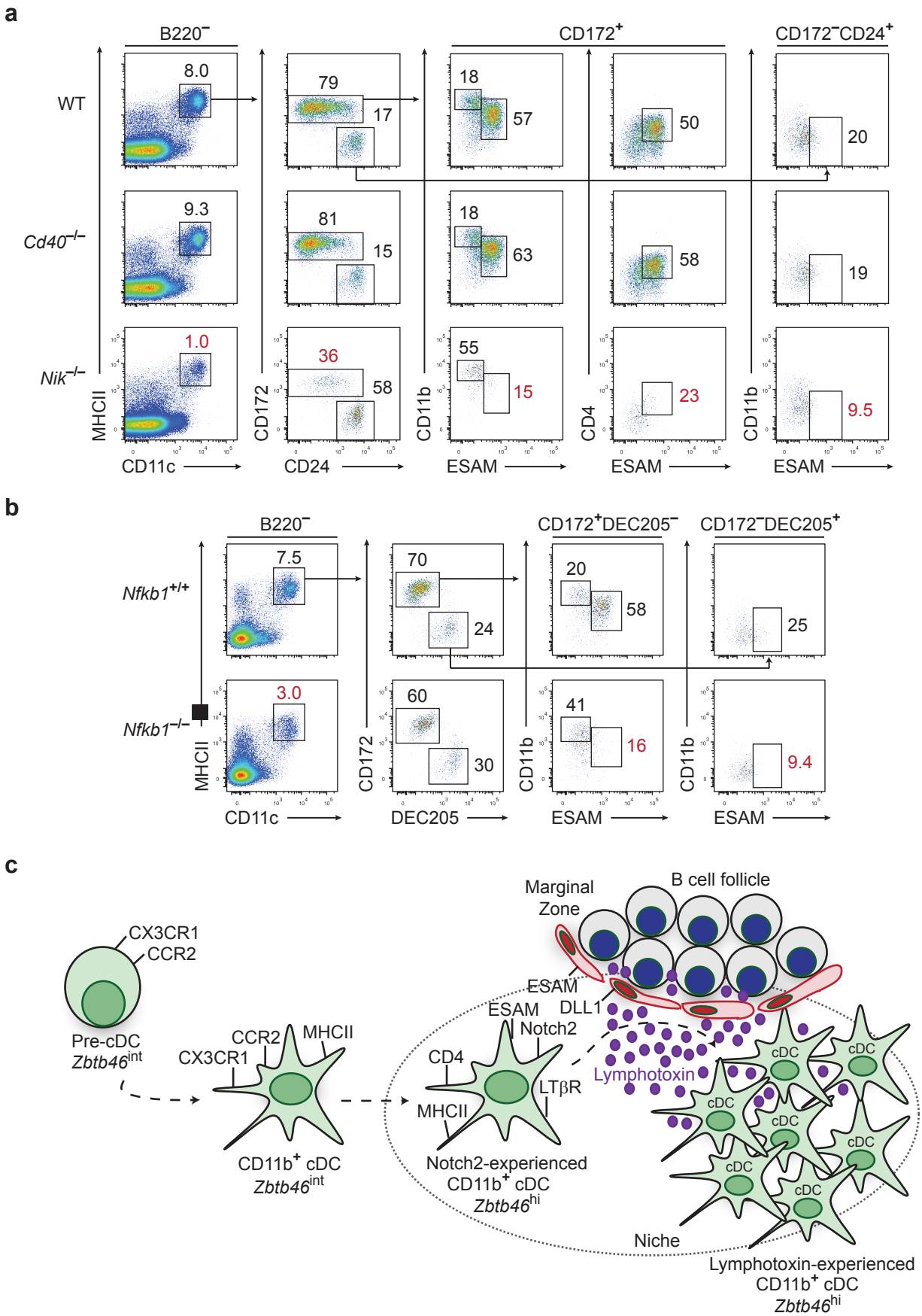
b Splenocytes



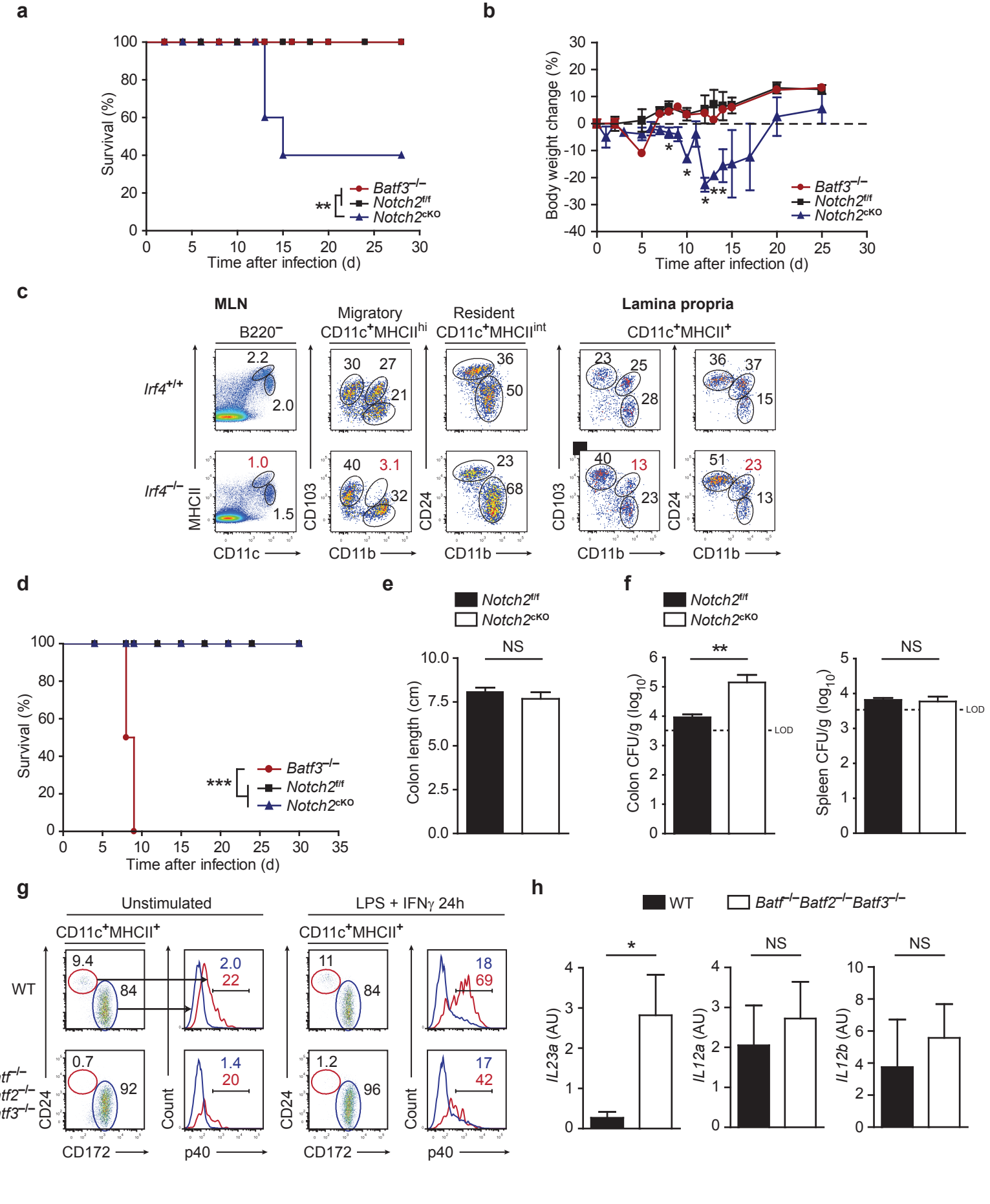
Supplementary Figure 5. FACS analysis of *Notch2*^{vav} mixed BM competitions. (**a**) Gating strategy for analysis of BM progenitors in competitive mixed BM chimeras between WT mice or between WT and *Notch2*^{vav} (KO) mice. Bone marrow cells were harvested 6-8 weeks after transplant and stained for expression of the indicated markers. Shown are two-color histograms for live cells pre-gated as indicated above the diagram. Data are representative of two independent experiments (*n* = 3-5 mice per group). (**b**) Gating strategy for analysis of splenic cell types in competitive mixed BM chimeras from [**a**]. Shown are two-color histograms for live cells pre-gated as indicated above the diagram.



Supplementary Figure 6. Flt3L is necessary but not sufficient for Notch2-dependent cDC development. (**a, b**) Splenocytes were stained for expression of the indicated markers. (**a**) Shown are two-color histograms for live cells pre-gated as indicated above the diagram. (**b**) Quantification of splenic cDC populations from [**a**]. Numbers above bars indicate fold change. Data are from two independent experiments (bars, SEM; *n* = 4 mice per group, Student's *t*-test). (**c, d**) Mice were treated with 10 µg Flt3L i.p. on day -1 and day 0 and splenocytes were harvested on day 7. (**c**) Shown are two-color histograms for live cells pre-gated as indicated above the diagram. (**d**) Quantification of cDC populations from [**c**]. Numbers above bars indicate fold change. Data are from two independent experiments (bars, SEM; *n* = 3 mice per group, Student's *t*-test). * $P < 0.01$, NS not significant.



Supplementary Figure 7. LTβR and NF-κB signaling are required for Notch2-dependent cDC development. (**a**, **b**) Splenocytes were stained for expression of the indicated markers. Shown are two-color histograms for live cells pre-gated as indicated above the diagram. Data are representative of two independent experiments (*n* = 4 mice per group). (**c**) Shown is a schematic illustrating the sequential actions of Notch2 and LTβR signaling in the development of splenic CD11b⁺ cDCs.


a
Survival (%)
Time after infection (d)
Batf3−/−
Notch2f/f
Notch2cKO
**
b
Body weight change (%)
Time after infection (d)
c
MLN
Migratory
Resident
Lamina propria
B220−
CD11c+MHCII^hi
CD11c+MHCII^int
CD11c+MHCII+
Irf4+/+
Irf4−/−
MHCII
CD11c
CD103
CD11b
CD24
d
Survival (%)
Time after infection (d)
***
e
Notch2f/f
Notch2cKO
NS
Colon length (cm)
f
**
Colon CFU/g (log10)
LOD
NS
Spleen CFU/g (log10)
g
Unstimulated
LPS + IFNγ 24h
CD11c+MHCII+
WT
Batf−/−
Batf2−/−
Batf3−/−
CD24
CD172
Count
p40
h
WT
Batf−/−Batf2−/−Batf3−/−
*
NS
IL23a (AU)
IL12a (AU)
IL12b (AU)

Supplementary Figure 8. Notch2-dependent cDCs are required for local IL-23-dependent gut immunity. (**a, b**) Survival (**a**) and weight loss (**b**) of mice orally inoculated with 2×10^9 *C. rodentium*. In these experiments, mice were older than 3 months of age and > 22 g in weight prior to infection. Data are from two independent experiments (bars, SEM; *n* = 5 mice per group, survival: log-rank Mantel-Cox test, weight loss: Student's *t*-test). (**c**) MLN and lamina propria cells were stained for expression of the indicated markers. Shown are two-color histograms for live cells pre-gated as indicated above the diagram. Data are representative of two independent experiments (*n* = 3 mice per group). (**d**) Survival of mice infected with *T. gondii* (100 tachyzoites i.p.). Data are from two independent experiments (*n* = 5-6 mice per group, survival: log-rank Mantel-Cox test). (**e**) Colon lengths 4 days after infection with *C. rodentium*. Data are representative of two independent experiments (bars, SEM; *n* = 5 mice per group, Student's *t*-test). (**f**) *C. rodentium* titers in the spleen and colon from mice infected for 4 days. Data are representative of two independent experiments (bars, SEM; *n* = 3-4 mice per group, Student's *t*-test). (**g**) $CD11c^+$-enriched splenocytes were stimulated e*x vivo* for 24 hours with 1 µg/mL LPS and 50 ng/mL IFN-γ in the presence of brefeldin A. Cells were stained for surface expression of the indicated markers and for intracellular expression of p40. Shown are representative two color histograms for live cells pre-gated as indicated above the diagram. Data are representative of two independent experiments (*n* = 3 mice per group). (**h**) Normalized *Il23a*, *Il12a* and *Il12b* expression values in Flt3L BMDCs treated as in [**g**] determined by qRT-PCR (mRNA/*HPRT*). Data are from two independent experiments (bars, SEM; *n* = 3 mice per group, Student's *t*-test). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS not significant.

a

Comparison of *Notch2*$^{f/f}$ and *Notch2*^{cKO} at day 9

WT/KO		KO/WT	
Gene symbol	Fold change	Gene symbol	Fold change
1810030J14Rik	9.4	*Ccl3*	19.6
Reg3g	8.6	*Cxcl2*	18.4
Mup7	6.9	*Spp1*	10.8
St8sia5	6.6	*Il1b*	9.9
Mup2	6.5	*Il1a*	9.5
Reg3b	6.2	*Gpr50*	7.9
Mup11	6.0	*Arg1*	7.4
Mup16	5.9	*Retnlg*	7.3
Slc26a3	5.8	*Clec4e*	7.3
Mup19	5.8	*Mmp8*	7.2
Apol10a	5.2	*Prg4*	6.9
Cxcl9	4.1	*Irg1*	6.7
Car4	3.9	*Cxcr2*	6.7
Slc37a2	3.8	*Il33*	6.7
Tnfsf10	3.8	*Trim29*	6.4
St3gal4	3.7	*Stfa2l1*	6.4
Iigp1	3.6	*Psca*	6.1
Cyp4f14	3.5	*Chi3l1*	5.8
Ighm	3.5	*Adm*	5.7
Tac1	3.4	*Cyp4f39*	5.7
Ostb	3.4	*Timp1*	5.6
Aadac	3.3	*Trem1*	5.2
Tgtp1	3.1	*Aldh1a3*	5.1
Gm12185	3.1	*Cldn4*	5.0
Slc5a9	3.1	*Lox*	5.0
Gm11437	3.1	*Neto2*	4.8
Gatm	3.0	*Ly6d*	4.7
Krtap13	2.8	*Mmp7*	4.7
Scd1	2.8	*Krtap4-16*	4.7
Hao2	2.8	*Il1f9*	4.7
Ms4a1	2.7	*Gal*	4.7
Ces2e	2.7	*Il1r2*	4.6
Mcpt2	2.7	*Frmpd1*	4.3
Mcpt1	2.5	*Ptgs2*	4.2
Abcg5	2.5	*S100a9*	3.8

b

Comparison of *Notch2*$^{f/f}$ and *Notch2*^{cKO} at day 4

WT/KO		KO/WT	
Gene symbol	Fold change	Gene symbol	Fold change
Reg3g	6.7	*Slc37a2*	8.4
Hsd3b3	6.6	*Sectm1b*	7.1
Reg3b	6.3	*Slc34a2*	6.4
Lrg1	4.8	*Slc2a5*	5.2
Plaur	4.4	*S100g*	4.3
Cxcl5	4.3	*Ifi44*	4.0
Slc7a11	3.7	*Ddx60*	3.7
Prrg4	3.6	*Cyp2d12*	3.6
Car3	3.3	*Apob*	3.3
Cxcl2	3.3	*Eno3*	3.2
Trim29	3.3	*Aqp8*	3.2
Il33	3.2	*Ace2*	3.1
Cldn4	3.2	*Hoxd13*	3.1
Epha2	3.2	*Cyp2f2*	3.1
Slc4a11	3.2	*Cyp2c55*	3.0
Ms4a1	3.2	*Slc6a4*	3.0
Mfsd2a	3.2	*Herc6*	2.9
Trim15	3.1	*Apol10a*	2.9
Bmp8b	3.1	*Slco2a1*	2.9
Dusp1	3.1	*Atp12a*	2.8
Sema7a	3.0	*Fabp6*	2.8
Phlda1	2.9	*Irf7*	2.8
Aldh1a3	2.9	*Krt23*	2.8
Sprr1a	2.9	*Slc15a1*	2.8
Dhrs9	2.9	*Cyp3a13*	2.8
Retn	2.8	*Mep1a*	2.7
Pvr	2.8	*Trim30d*	2.7
Tac1	2.8	*Slc6a19*	2.7
Ccl20	2.5	*Hmgcs2*	2.7
Nos2	2.5	*Fmo4*	2.6
Il1b	2.4	*Dio1*	2.6
Ptgs2	2.3	*Oas3*	2.6
Il22	2.1	*Mov10*	2.6
Ly6d	2.1	*Oas2*	2.5
Cxcr2	2.1	*Id2*	2.5

Supplementary Table 1. Microarray analysis of *Notch2*^{cKO} colons during *C. rodentium* infection. (**a, b**) Microarray analysis of colonic cells in mice infected with *C. rodentium* for 9 days (**a**) or 4 days (**b**). Genes that are increased in expression in *Notch2*$^{f/f}$ (WT) samples relative to *Notch2*^{cKO} (KO) samples are listed with their associated fold change (left). Genes that are increased in KO samples relative to WT samples are listed with their fold change (right). Data are from one independent experiment (n = 2-3 biological replicates per sample).