SUPPLEMENTAL MATERIAL

Scott et al., http://www.jem.org/cgi/content/full/jem.20151715/DC1

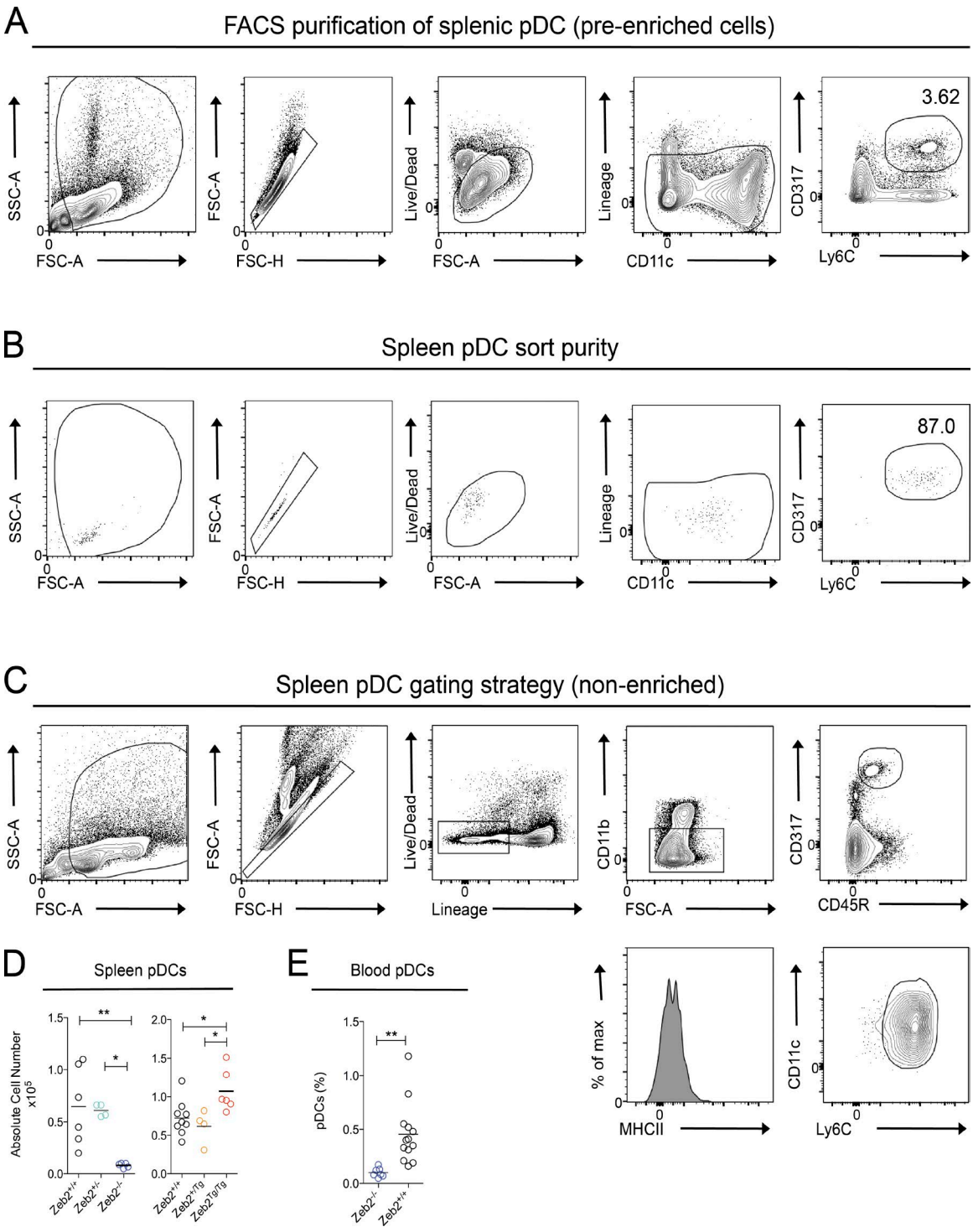

Figure S1. **Splenic pDCs.** (A) Representative FACS plots showing strategy for purification of splenic pDCs. The number represents the proportion of pDCs as a percentage of total cells. Samples were preenriched using streptavidin negative selection beads (Magnisort; eBioscience). (B) Representative FACS plots showing purity of splenic pDCs after sorting. The number represents the proportion of pDCs as a percentage of total cells. (C) Representative FACS plots showing gating strategy and CD11c, Ly6C, and MHCII expression for pDCs in a nonenriched spleen. (D) Absolute cell number of pDCs in the spleen of $Zeb2^{+/+}$, $Zeb2^{+/-}$, $Zeb2^{-/-}$, $Zeb2^{+/Tg}$, and $Zeb2^{Tg/Tg}$ mice. Data are representative of at least two experiments where each dot represents one mouse. One-way ANOVA with Bonferroni posttest was used. (E) Proportion of pDCs in the blood of $Zeb2^{+/+}$ and $Zeb2^{-/-}$ mice. Data are pooled from two experiments where each dot represents one mouse. Two-way Student's *t* test was used. *, $P < 0.05$; **, $P < 0.01$. FSC, forward scatter. SSC, side scatter.

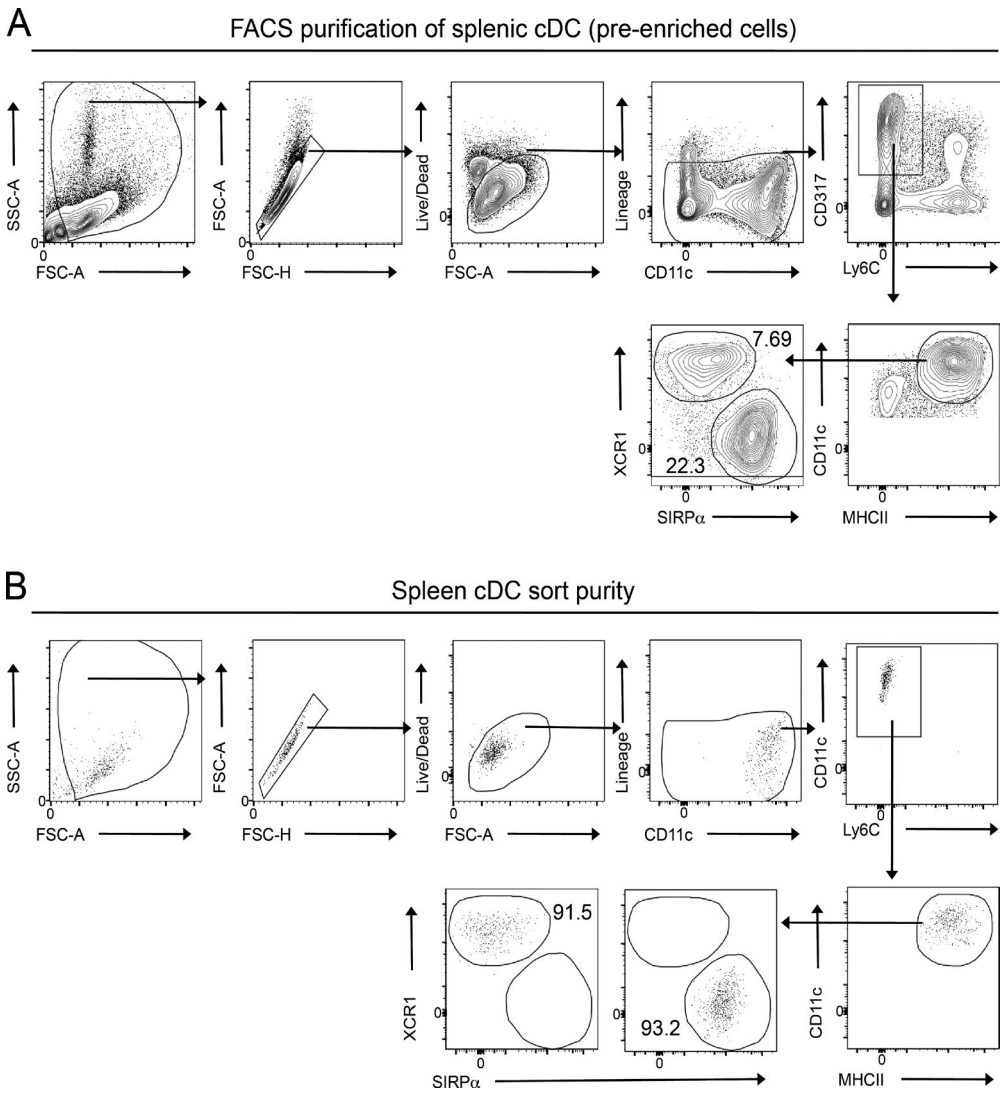

Figure S2. **Splenic cDC sorting strategy and purities.** (A) Representative FACS plots showing strategy for purification of splenic cDC subsets. The numbers represent the proportion of each cDC subset as a percentage of total cells. Samples were preenriched using streptavidin negative selection beads (Magnisort; eBioscience). (B) Representative FACS plots showing purity of splenic cDC subsets after sorting. The numbers represent the proportion of each cDC subset as a percentage of total cells. FSC, forward scatter. SSC, side scatter.

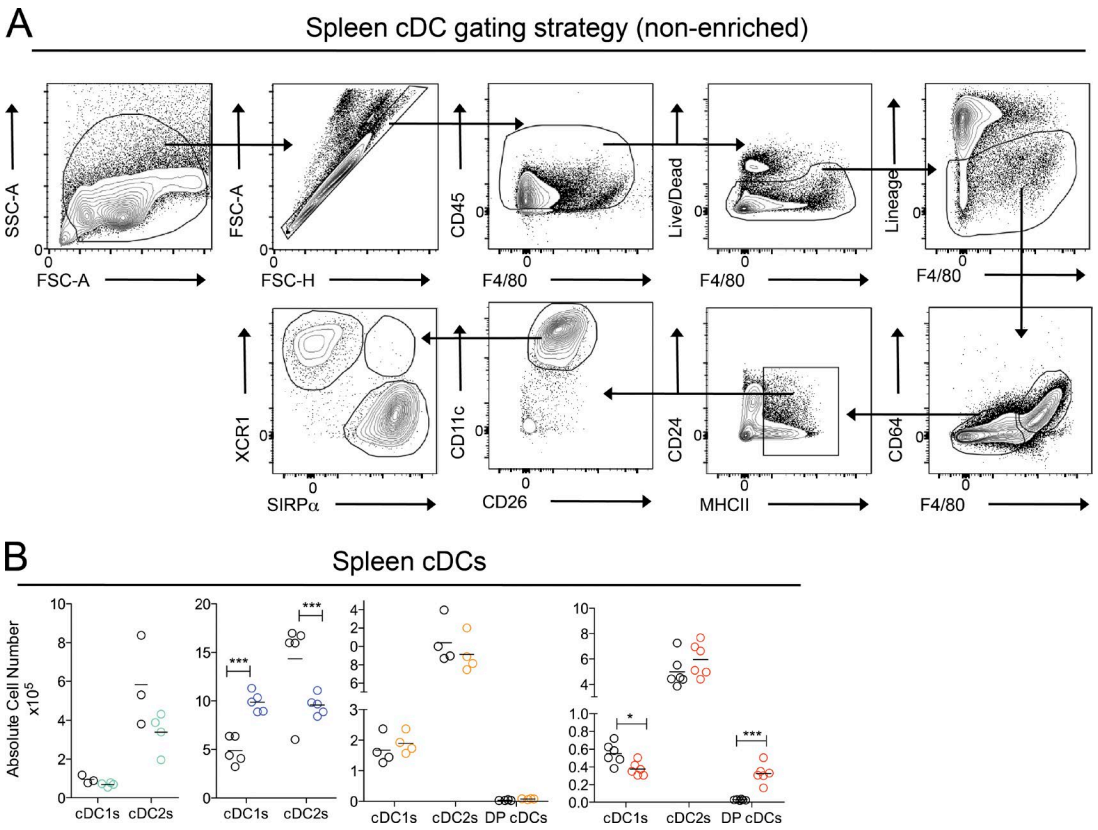

Figure S3. **Splenic cDC gating strategy and absolute numbers.** (A) Representative FACS plots showing gating strategy for cDC1s, cDC2s, and DP cDCs in a nonenriched spleen. This gating strategy was also used for lung, liver, and SI LP. FSC, forward scatter. SSC, side scatter. (B) Absolute cell number of cDC1s, cDC2s, and DP cDCs in the spleen of $Zeb2^{+/+}$, $Zeb2^{+/-}$, $Zeb2^{-/-}$, $Zeb2^{+/Tg}$, and $Zeb2^{Tg/Tg}$ mice. Data are representative of at least two experiments where each dot represents one mouse. *, $P < 0.05$; ***, $P < 0.001$. One-way ANOVA with Bonferroni posttest was used.

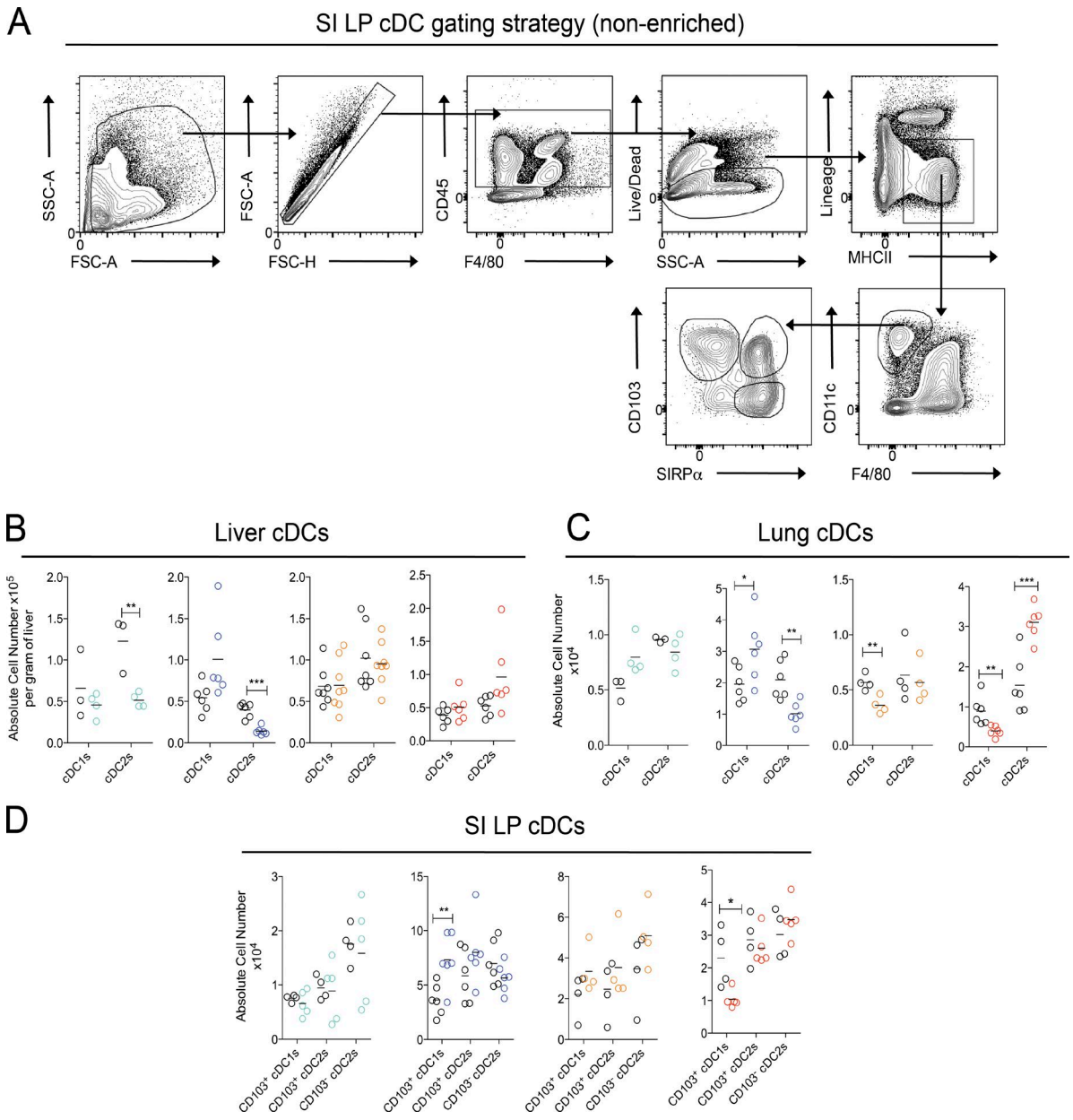

Figure S4. **cDCs in other tissues.** (A) Representative FACS plots showing gating strategy for CD103^{+} cDC1s, CD103^{+} cDC2s, and CD103^{-} cDC2s in the SI LP. FSC, forward scatter. SSC, side scatter. (B–D) Absolute cell number of cDC1s and cDC2s in liver (B), lung (C), and SI LP (D) of Zeb2$^{+/+}$, Zeb2$^{+/-}$, Zeb2$^{-/-}$, Zeb2$^{+/Tg}$, and Zeb2$^{Tg/Tg}$ mice. Data are representative of two experiments where one dot represents one mouse. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. One-way ANOVA with Bonferroni posttest was used.

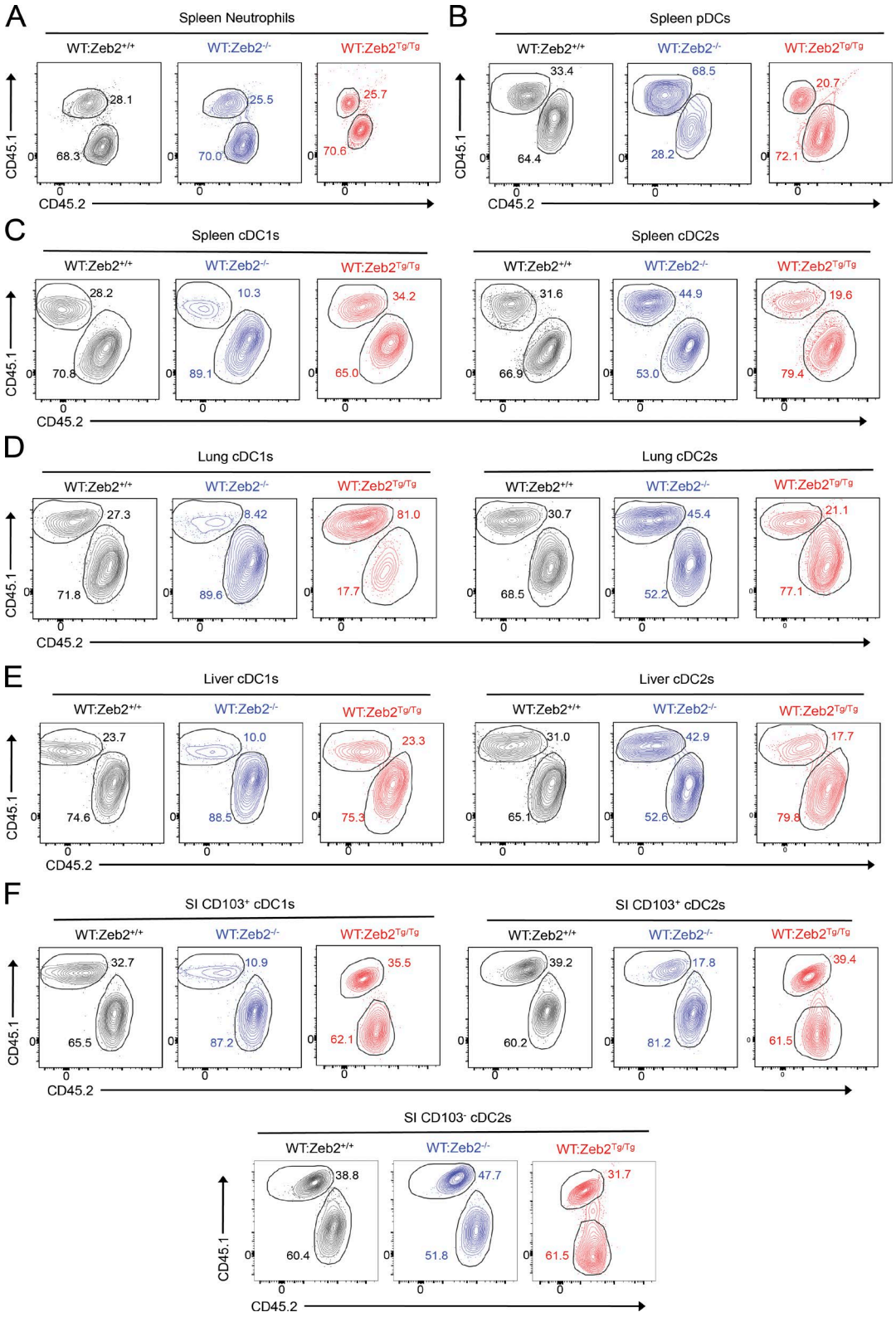

Figure S5. **BM chimeras.** (A–F) Representative FACS plots showing CD45.1 and CD45.2 staining among splenic neutrophils (A), splenic pDCs (B), splenic cDC1s and cDC2s (C), lung cDC1s and cDC2s (D), liver cDC1s and cDC2s (E), and SI LP CD103$^+$ cDC1s, CD103$^+$ cDC2s, and CD103$^-$ cDC2s (F) in WT:Zeb2$^{+/+}$, WT:Zeb2$^{-/-}$, and WT:Zeb2$^{Tg/Tg}$ chimeras. The numbers represent the proportion of each cell type coming from CD45.1 or CD45.2 BM.

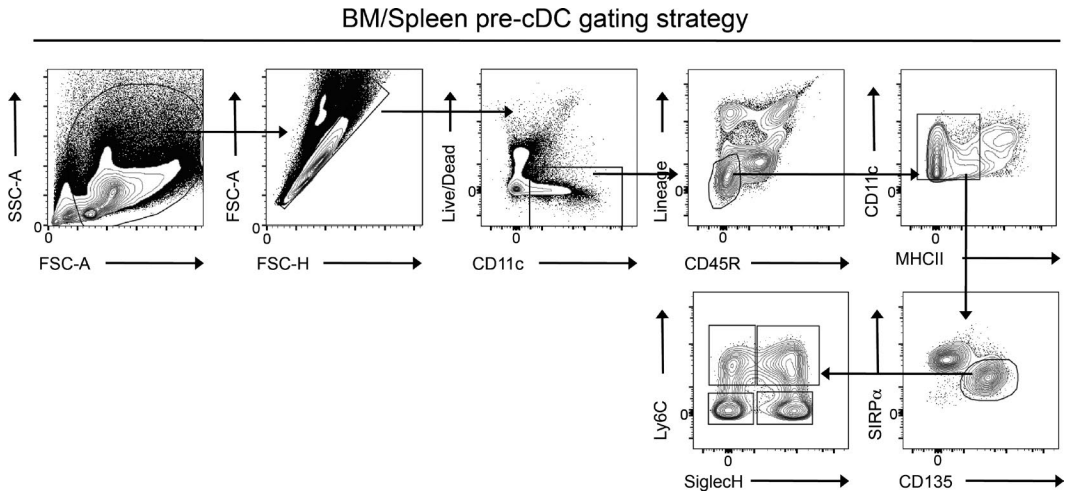

Figure S6. **Pre-cDC gating strategy.** Representative FACS plots showing the gating strategy used to identify pre-cDC subsets in the BM and spleen. Plots shown are from BM. FSC, forward scatter. SSC, side scatter.

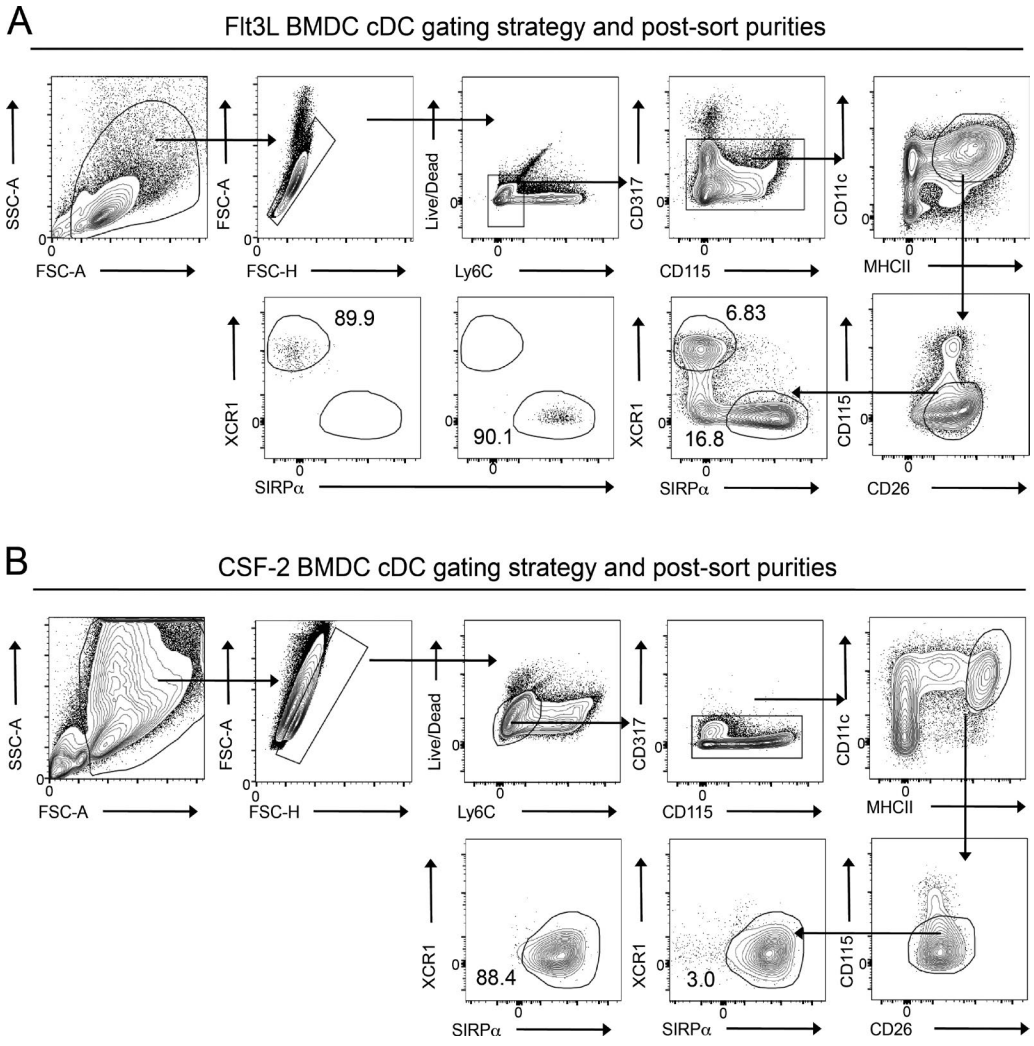

Figure S7. **BMDC gating strategies and sort purities.** (A and B) Representative FACS plots showing gating strategy and postsort purities for WT Flt3L BMDC cultures (A) and WT CSF-2 BMDC cultures (B). The numbers represent the proportion of indicated cells as a percentage of total cells. FSC, forward scatter. SSC, side scatter.

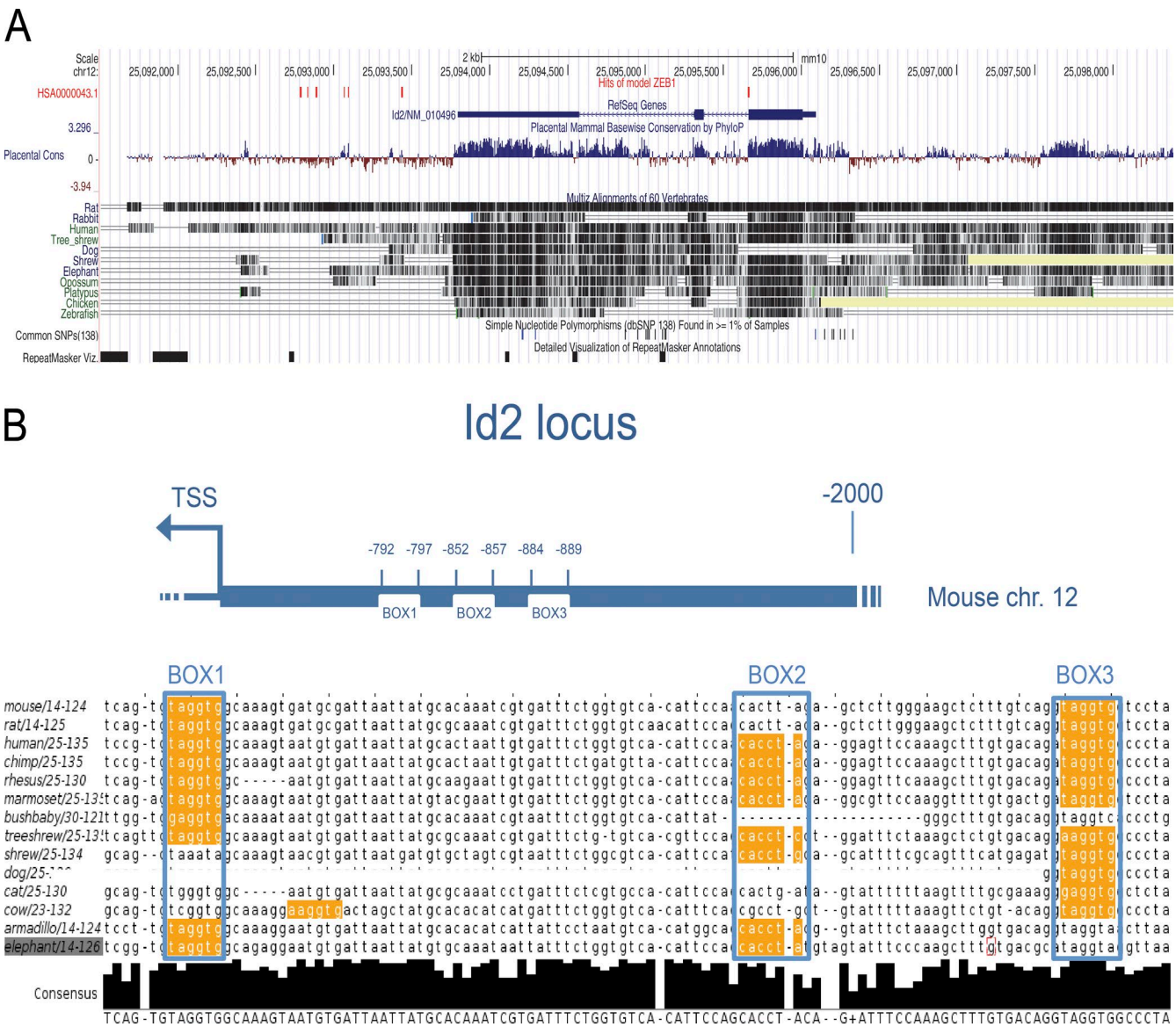

Figure S8. **Predicted Zeb binding sites in Id2 locus.** (A) Physbinder predictions for Zeb1 binding sites in Id2 locus and surrounding regions. Zeb1 was used as a model for Zeb2, as the sequencing binding motifs are conserved between them. The red lines indicate predicted Zeb binding sites. Sequence conservation among other species is also depicted. (B) The top shows a schematic representation of the Id2 promoter region and ConTra-predicted Zeb E box binding sites. The bottom shows sequence conservation of these E boxes across multiple species. TSS, transcription start site. chr., chromosome.

Table S1. **Differentially expressed genes: Zeb2$^{+/+}$ versus Zeb2$^{Tg/Tg}$ cDC1s**

Up-regulated			Down-regulated		
Tgm2	Hcar2	Tgfbi	Cd38	Card10	Slamf6
Clec4a1	Cd302	Ccnb1	Gpr82	Ildr1	Cystm1
Clec7a	Lifr	Neurl2	Pkib	Efcc1	Marveld2
Clec1b	Ifi203	Sh2d1b1	Ccl22	Fas	Homer2
Sirpb1a	Ms4a7	Acp5	Ido1	Cd8b1	Gclc
Cd4	Fam26f	Oas2	Fchsd2	Otud7b	Cxcr3
Adgre1	Hmox1	Esr1	Cnnm1	Lpar3	Cyb561
Oasl2	Slc16a7	Aurkb	Fcrlb	Gramd2	Cdon
Tlr1	Mndal	Cdc25c	Angel1	Elmo3	Esyt3
Wfdc17	Rarg	Aurka	Glce	Asap2	Mmp28
Clec4a2	Cdc25b	Ehf	Sspo	Slc41a2	Notch4
Ifit3	Tespa1	Il1rn	Pik3r3	Mab21l3	Trabd2b
Oas1a	Ctnnd2	Ttk	Sec1	Casr	Aff3
Zeb2	Fam46a	Ckap2l	Slc22a15	Scin	Lsr
Apobec1	Ccl9	Pou2f2	Vps37d	Cd8a	P3h2
Pilrb1	Emb	A630033H20Rik	Nfe2l3	Pdzk1ip1	Nid2
Ifit3b	Pira2	Al504432	Lzts2	Lrrc1	Egfl8
Clec4a4	Gapt	Cenpf	Mtmr4	Myb	Proser2
Sirpa	Slc40a1	Cenpi	Lrrc29	Abcd2	Serpinb6b
Lair1	Lst1	Ifi27l2a	Pacsin1	Pcdh1	Rtn4rl1
Cybb	Tlr7	Gngt2	Tm4sf5	Cldn1	Actn1
Fcer1g	Gpr141	Cenpm	Arc	Hgfac	Gcsam
Ms4a4c	Hpgds	Vegfa	Noxred1	Adgrf5	Dkk3
Cd300ld	Ifitm3	Lag3	Prr13	Ldlrad3	Gm3336
Csf1r	Fut7	Ctsc	Serpinb1a	Gpr157	Ltbp2
Itgam	S100a4	Casp1	Pdcd1	Foxj1	Fcrla
Ddx58	Abhd15	Spic	Dnajc22	Ccr9	Ttc39a
1810011H11Rik	Hacd4	Spc25	Chst11	Rab30	
Gpr162	Ceacam1		Ms4a1	Ankrd55	
Cd300a	Ms4a6b		Anxa6	Fndc4	
Gm12250	Cdr2		Ccndbp1	Arpin	
Nxpe4	Lgmn		Sh3gl3	Mpzl2	
Ncf2	Ube2c		Chrnb1	Frmd5	
Ltb4r1	Cd101		Tlr3	Ptgis	
Cd300lf	Ccl6		Col27a1	Prss57	
Pydc4	Ccnf		Rasip1	Itga3	
Abi3	Sapcd2		Cd200	Gpr33	
Rtp4	Ggt5		Mrvi1	Traf4	
Clec4n	Prc1		Pglyrp1	Sema4c	
Galnt6	Itga9		Acss2	Ffar4	
Sirpb1b	Gm5150		Myzap	Serpine2	
Pilrb2	Prdm1		Ctla4	Inadl	
Ms4a6c	Ear2		Sema4f	Pcsk5	
Plcb1	Klk8		Spint2	D030025P21Rik	
Oas3	C3ar1		Dapk2	2510009E07Rik	
Ifit1	Ifi47		Gucy2c	E330020D12Rik	
Gm9733	Itgad		Prph	Dcstamp	
Slc7a11	Rasgrf2		Kazn	Hepacam2	
Fcgr4	Lpcat2		Dusp18	Arhgef9	
Ifit1bl1	Oas1g		Snn	Lefty1	
Pla2g7	Xaf1		Sept1	Serpinb9	
Ddit4	Pif1		Mmp19	Clec4b2	

Table S2. **Differentially expressed genes: Zeb2$^{+/+}$ versus Zeb2$^{-/-}$ cDC2s**

Up-regulated		Down-regulated	
Plcb1	Lima1	F11r	Ceacam19
1700011B04Rik	Nid1	Nrg2	P2ry2
Plcb4	Pbx1	Wfdc21	Chtf18
Itgad	Tgtp1	Stard10	Cp
Cox6a2	Epcam	Ttll5	Ska1
Ttc39a	Tenm4	Cd101	Fam198b
Sspo	Fcrla	Aff3	Myof
Upp1	E330020D12Rik	Pappa2	Slc1a2
Chac1	Osgin1	Gm3336	2610528A11Rik
Cxcr3	Dact3	Cst3	Cd209b
Lurap1	Snx22	Prg3	Dbn1
Cdh17	Arpin	Car2	Kntc1
Lsr	Zcchc18	Sema4c	BC030867
Cd84	Snn	Gjb2	Slc9a2
Fcer2a	Sox8	G0s2	Clec9a
Prg2	Cystm1	Elovl7	Lyz2
Ecel1	Jag1	Oasl1	Smim5
Pde1b	Apoe	Utf1	Dmwd
Slc22a23	Paqr9	Strip2	Cygb
Cysltr1	Gpr55	Gpr160	Mmp9
Frk	Efnb1	Clec4b2	Dapk1
Cldn1	Gpr157	Slfn1	Gpr83
Tmeff1	Elmo3	Cd69	Ccr2
Hdc	Oasl2	Nid2	Hr
Siglecf	Mnda	C3ar1	Dab2
Rtn4rl1	Hepacam2	Krtcap3	Lyz1
Rnase4	Als2cr12	Ackr3	Zeb2
Gpc1	Ccl24	Cx3cl1	Cx3cr1
Dusp4	Tm4sf5	Rragd	Gp2
Ms4a1	Slc27a2		

Table S3. **Antibodies used for flow cytometry**

Antibody	Clone
Fc Block	2.4G2
CCR2	475301
CD3e	145-2c11
CD4	RM4-5
CD8	53-6.7
CD19	1D3
CD24	M1/69
CD26	H194-112
CD38	90
CD45	30-F11
CD45.1	A20
CD45.2	104
CD45R	RA3-6B2
CD64	X54-5/7.1
CD69	H1.2F3
CD101	Moushi101
CD103	2E7
CD115	AFS98
CD117	2B8
CD11b	M1/70
CD11c	N418
CD127	SB/199
CD135	A2F10
CD161	PK136
CD172a	P84
CD317	120g8
CX3CR1	SA011F11
EpCam	G8.8
F4/80	BM8
IA-IE	M5/114.15.2
IRF4	M-17
IRF8	V3GYWCH
Ly6C	AL-21
Sca1	D7
SiglecF	E50-2440
SiglecH	440c
Ter-119	Ter-119
XCR1	ZET

Table S4. **Primers for RT-qPCR**

Gene	Forward primer	Reverse primer
GAPDH	5′-GCATGGCCTTCCGTGTTC-3′	5′-TGTCATCATACTTGGCAGGTTTCT-3′
Zeb2	5′-GGCAAGGCCTTCAAGTACAA-3′	5′-AAGCGTTTCTTGCAGTTTGG-3′
Id2	5′-TCCTGTCCTTGCAGGCATCTGAAT-3′	5′-AACGTGTTCTCCTGGTGAAATGGC-3′
Batf3	5′-CAGACCCAGAAGGCTGACAAG-3′	5′-CTGCGCAGCACAGAGTTCTC-3′
Nfil3	5′-GAACTCTGCCTTAGCTGAGGT-3′	5′-ATTCCCGTTTTCTCCGACACG-3′
Klf4	5′-CGATGAACTGACCAGGCACTAC-3′	5′-CCTCTTCATGTGTAAGGCAAGGTG-3′
Notch2	5′-CCACCTGCCTGGATAAGATCG-3′	5′-CTGCCCGTTGTTCACACAC-3′
RelB	5′-GAATGTCGTCAGGATCTGC-3′	5′-TGGTGGACTTCTTGTCGTAG-3′
E2-2	5′-CGAAAAGTTCCTCCGGGTTTG-3′	5′-CGTAGCCGGGCTGATTCAT-3′