

The signal transducers STAT5 and STAT3 control expression of Id2 and E2-2 during dendritic cell development

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Supplementary information

Figure S1. Sorting strategy for CDPs, pDCs, spleen and liver DC subsets.

CDPs ($\text{lin}^- \text{CD117}^{\text{lo}} \text{CD115}^+ \text{Flt3}^+$) and pDCs ($\text{CD11c}^+ \text{CD11b}^- \text{B220}^+ \text{PDCA-1}^+$) were sorted from BM cells following MACS-mediated depletion of lineage marker- (Gr1, CD11b, CD3, CD19, F4/80 and Ter-119) positive cells, using the indicated strategy. $\text{CD8}\alpha^+$ ($\text{CD11c}^+ \text{B220}^- \text{CD4}^- \text{CD8}\alpha^+$), CD4^+ ($\text{CD11c}^+ \text{B220}^- \text{CD4}^+ \text{CD8}\alpha^-$), and $\text{CD4}^- \text{CD8}\alpha^-$ ($\text{CD11c}^+ \text{B220}^- \text{CD4}^- \text{CD8}\alpha^-$) DCs were isolated from splenocytes following MACS-mediated depletion of lineage marker- (Gr1, CD3, CD19, F4/80 and Ter-119) positive cells. CD103^+ ($\text{CD11c}^+ \text{B220}^- \text{CD103}^+ \text{MHCII}^+$) DCs were isolated from 37%/70% Percoll-enriched liver mononuclear cells following collagenase digestion.

Figure S2. Characterization of hematopoietic-*Stat5*^{Δ/Δ} mice.

(A) Bone marrow cells were isolated from hematopoietic-*Stat5*^{Δ/Δ} and *Stat5*^{+/+} mice. Whole cell lysates were analyzed by immunoblotting, using antibodies specific for total STAT5 or tubulin. Results from 2 independent animals of each genotype are shown. (B) Hematologic parameters and WBC differential counts of hematopoietic-*Stat5*^{Δ/Δ} and *Stat5*^{+/+} mice, determined by automated counting.

Figure S3. Analysis of LN CD103⁺ DCs.

Peripheral (LN) and mesenteric (mLN) lymph nodes were isolated from hematopoietic-*Stat5*^{Δ/Δ} and *Stat5*^{+/+} mice. Single cell suspensions were stained with antibodies for DC surface markers and analyzed by flow cytometry. MHC II and CD103 expression was

analyzed within the CD11c⁺ B220⁻ population, and is shown as a percentage of total LN cells. Data are representative of 2 mice per group.

Figure S4. Characterization of DC marker expression.

(A) Hematopoietic-*Stat5*^{Δ/Δ} and *Stat5*^{+/+} mice were treated by HGT with GM-CSF plasmid or empty pORF vector, as indicated. At d 7, liver mononuclear cells were enriched as described in Figure S1, and CD103 expression was analyzed within the CD11c⁻ or CD11c⁺ B220⁻ populations, by flow cytometry, as shown. Data represent 2-6 mice/group. (B) Surface expression of CD24 and CD11b on CD103⁺ DCs isolated from mice treated by HGT with GM-CSF plasmid or empty pORF vector, as indicated. CD103⁺ DCs were defined as shown in Figure S1. (C) Liver CD103⁺ DCs were purified by FACS as described in Figure S1 and treated with GM-CSF ex vivo. Cells were collected at d 0, d 1 and d 2, as indicated, and analyzed for CD11b expression. Data are representative of 3 independent experiments.

Figure S5. Analysis of the murine *Id2* and *Tcf4* promoter regions. The sequences encoding 2kb immediately 5' of the transcription start sites (arrow) of the murine *Id2* and *Tcf4* genes were identified from the Ensemble database. Putative transcription factor binding sites (underlined) were predicted by analysis with AliBaba and TF search software programs. The location of primers used for H3 ChIPs (Figures 3C, 3D) are shown in orange (sense) and blue (antisense).

Figure S6. Regulation of histone H3 modifications at *Tcf4* and *Id2* promoters.

(A) pDCs and CD11c⁺ CD11b⁺ DCs were isolated from mice that were unmanipulated (no HGT) or from animals following Flt3L or GM-CSF HGT, as indicated. Histone H3

modifications at the *Id2* and *Tcf4* proximal promoters in the vicinity of the STAT-consensus sites were determined by ChIPs using antibodies against H3K9ac, H3K4me3 and H3K27me3. Results were normalized to ChIPs for total H3 and presented as mean \pm SEM. n=10-15. (B) pDCs were purified by FACS from BM cells cultured with Flt3L for 7 d, then stimulated with 5 μ M CpGA for 0, 4, 8 and 16 h. *Id2* mRNA was analyzed by qPCR. (C, D) pDCs and CD11c⁺ CD11b⁺ DCs were isolated by FACS from HGT-treated hematopoietic-*Stat5* ^{Δ/Δ} mice, hematopoietic-*Stat3* ^{Δ/Δ} animals or appropriate controls, as indicated. Histone H3 modifications at the *Tcf4* and *Id2* proximal promoters in the vicinity of the STAT-consensus sites were determined by ChIPs using antibodies against H3K9ac, H3K4me3 and H3K27me3. Results were normalized to ChIPs for total H3 and presented as mean \pm SEM. n=10-15. (E) Whole cell lysates were generated from total BM cells isolated from hematopoietic-*Stat5* ^{Δ/Δ} mice, hematopoietic-*Stat3* ^{Δ/Δ} animals or appropriate controls. Expression of H3K4me3 and total H3 was determined by immunoblotting. (F) Hematopoietic-*Stat5* ^{Δ/Δ} mice, hematopoietic-*Stat3* ^{Δ/Δ} animals or appropriate controls were treated with GM-CSF or Flt3L HGT for 7 d, as indicated. The abundance of H3K4me3 at the *Flt3*, *Gata-1*, *Irf8* and *Gapdh* promoters was analyzed in pDCs and cDCs as described in (C and D).

Figure S7. GM-CSF- and Flt3L-responsive expression of *Id2* and *Tcf4*.

(A, B) CDPs from hematopoietic-*Stat3* ^{Δ/Δ} , hematopoietic-*Stat5* ^{Δ/Δ} mice and respective *Stat* ^{$+/+$} controls were purified and cultured in vitro in the presence of Flt3L or GM-CSF, as indicated. *Id2* and *Tcf4* mRNA was determined by qPCR. (C) Hematopoietic-*Stat5* ^{Δ/Δ} and *Stat5* ^{$+/+$} mice were treated by HGT with GM-CSF or pORF vector, as indicated. After 2 d, *Tcf4* mRNA was measured in CDPs by qPCR.

Figure S8. Characterization of D2SC/1 and D2SC/mFlt3 signaling responses to GM-CSF or Flt3L. (A) Expression of RFP and Flt3 in D2SC/1 or D2SC/mFlt3 cells was analyzed by flow cytometry. (B) *Id2* mRNA abundance was determined in D2SC/1 cells cultured with GM-CSF for 0, 2, 4 and 8 h; *Tcf4* expression was analyzed in D2SC/mFlt3 cells treated with Flt3L for 0, 2, 4 and 8 h. (C, D) STAT1, STAT3 and STAT5 activation was analyzed by immunoblotting to detect tyrosine-phosphorylated isoforms, using whole cell extracts from D2SC/1 and D2SC/mFlt3 cells stimulated with GM-CSF or Flt3L for 0.5, 2, 4 or 8 h, or left untreated, as indicated. IFN- γ , IL-6 or GM-CSF-stimulated whole cell extracts were used as positive controls for phosphorylated STAT1, STAT3 and STAT5, respectively. (E) ChIP assays were performed in D2SC/1 cells treated \pm Flt3L for 1 h, using anti-STAT3 or IgG control antibody. Results are representative of 3 independent experiments.

Figure S9. Role for *Tcf4* in cytokine-driven DC development.

BM cells from *Tcf4*^{+/-} or *Tcf4*^{+/+} mice were cultured with Flt3L for 6 d. pDC and CD11c⁺ CD11b⁺ cDC generation was assessed within the CD11c⁺ population. n=8.

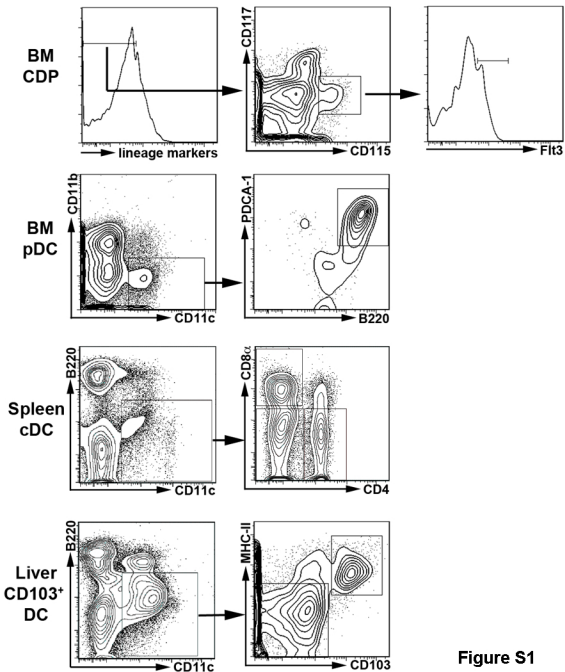
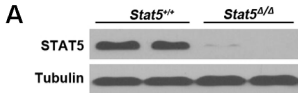


Figure S1



B Hematologic parameters and WBC differential counts of hematopoietic *Stat5^{Δ/Δ}* mice

	<i>Stat5^{+/+}</i>	<i>Stat5^{Δ/Δ}</i>
Hematologic Parameters		
Hemoglobin (g/L)	152.7 ± 6.5	147.3 ± 1.7
Hematocrit (%)	53.8 ± 2.4	50.0 ± 2.4
WBC count (10 ⁹ /L)	3.91 ± 0.73	4.66 ± 0.69
RBC count (10 ¹² /L)	10.9 ± 1.7	9.74 ± 0.94
Platelet count (10 ⁹ /L)	1413 ± 202.5	1233 ± 64.3
WBC Differential Count		
Neutrophil (10 ⁹ /L)	0.92 ± 0.15	1.19 ± 0.20
Monocyte (10 ⁹ /L)	0.07 ± 0.005	0.08 ± 0.03
Basophil (10 ⁹ /L)	0.02 ± 0.004	0.04 ± 0.02
Lymphocyte (10 ⁹ /L)	3.14 ± 0.97	3.32 ± 0.61
Eosinophil (10 ⁹ /L)	0.07 ± 0.02	0.09 ± 0.04

Results represent Mean ± SD of peripheral blood analysis of 4 hematopoietic *Stat5^{Δ/Δ}* mice and their littermate control (*Stat5^{+/+}*).

WBC = white blood cell, RBC = red blood cell.

Figure S2

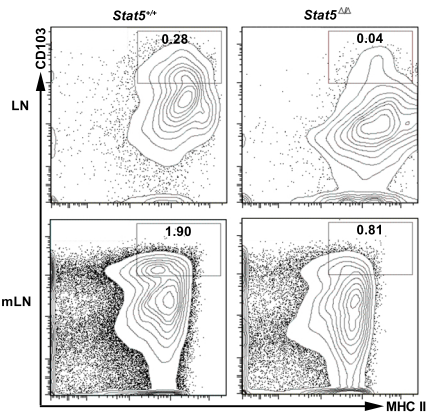


Figure S3

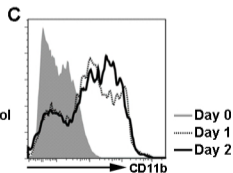
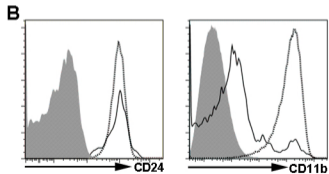
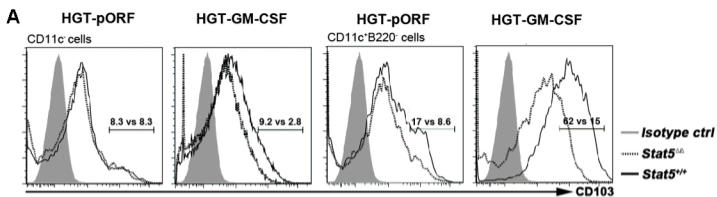


Figure S4

A *Id2* promoter (mouse chr. 12; 25,782,957 - 25,780,957)

TACTGGTAACTCGGCTCTTCACTCAATTAACAGTTCACAATCCCTTAAAATGATCTTTAAATGAATGCAAAAACCTGGGTAGT
 AATGAAATGCAAAAACCTGGGTAGTGTGCCAACAGAACTCGGATTCAGCCGAGCTCGGAGAGCTGGACTTGGCATGGCAGCCAGAGAAAGAAACCGCCAGAA
 ACTTTTACCATAITTAGCAATTTTACTATATACAGGATACAGGAATGGCTTTGGGAGAAAGCCGCTCATGCTGGTFCGCTGCCAGTGTGTGGAGCAGTCCGCT
 GATA-1
 ACAGCCGCATTATCATCCAGCCAGGCGCTTCTGTGGGATTAAGAGCTGGCAGCCGCTGGCCACAGCTGGAGAAATTAATCTTTAAATGGAGAACTGAGATCCGCT
 GATA-1/GATA-2
 AGTACCCGAGAGATCCAAATGTCACAAAGAACTTAATGTAGAAATCCAGAGATGTTGGTTACATTTGGCTATTATTGTAAAATCACTCAGAGTATA
 ACATCATGAACAAAAAGTGTCAAAAACAAAATCTGTTCCGCTGTGGCAGTATGTGACAGAAACACATGACACAAAAGTACATCAAGAGGCTCGAACTGTTG
 GGGGGGGGGGGGACTCAGATAGTAAATCACTTCCAGGCTTAATAACAGAGCGAGTGTGCAATCGAACACTGTGCTGAGTGCAGGAGGGCAATGCAAGCC
 GATA-1
 TTGCTTTCGCGAATTTTCCGCGAGGCGCAGAGTCTTCCAGCTCCAAACTGGAGTGGACGGATTTTACGCGCTCTACATCACCGGACTCTGCCCTCT
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 TCCACACAGAGCTTTGCCCTGGATTCGCACTCTTCTCTTTTATTGCACTCAGAGGATGTGGGGGATTCAGACCAAAAACCCCTTCTTGGGCTTAACATAAA
 GATA-1/GATA-2
 TATTTTITTTCTTTAAATACACAGCAATAATATAGGTAGTCTTCAGCAAAAACAGAAATTCAGAAATTAAGGCAATGGAGTCAAGAGCTCTAACTTTTCAA
 ICSBP
 ATGTGGCTGCATCAGGAAAGGGTGTGAAAGATTCCAAAACCTGTACATGTAACAGAAATTTTCTTTTA AAAACAGCGATAAGCTGTCCAGTCAATAGCTAGGAC
 CACCTACTGACAAAAGAGCTTCCCAAAGAGCTCTAAAGTGTGGAAATGTGACACAGAAATCCAGGATTTGTGCATAATTAATCGCATCACTTTGCCACTACA
 GATA-1
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 CATCTTGTGGGGGTGGGGGAGTTCGGAATGACCCGGGCTCCCTCGGGCTCTCTCGGCTCGAGGAGACCCAGCTGATGTATGTGGTAGGTTCCCCCTCCCAAAATAG
 GTTTCGAGAGAGGCTCCCGCGCTCCCAAGTGTCCAAAGTTCGAAAGCTTCACGCTAAAACCTGTGCTGGAGCTGTATGTGGTAGGTTCCCCCTCCCAAAATAG
 STATA
 CTTTCTTTATCTTAAAGAGGAGCACTCAAATGTGACTTCCGAAAAGCGCGCTTCTGCTGGAGCTGTATGTGGTAGGTTCCCCCTCCCAAAATAG
 GATA-1/GATA-2
 CTTGTGACTTCCCGTCTGTGCGGAGAGAGTGGGGCTGCTGCAAGTCTCGGGGCGGGGTAGAGCTAGCTCCGGGCGACACCCCGGGGAGGGGGCTTCG
 ICSBP
 CAGCTCTGGGAATGGGAATAAGCGGGGACAATTTGCACTACTAGGCCCTGGGCGCCAGCGCACACTGTACTCAATTTGCCACCCAGCTGGGCTCTCG
 CCGCTCTCCACCCCTACAGGCAATGGCTGCGAACCGCGAAGAACCGAGAGCTCATACTCACCAATGGGAGAAATCGGCTGTATGATGGACGGGAGCC
 TTCCACCAATGGCAATTCAGGGATGCCGATTGAGCGGCCAGGGCGAGTGCACATAAAGAGCGCCCGCCGGCTCGCGCTTCATCTGAA

B *Tcf4* promoter (mouse chr. 18; 69,502,146 - 69,504,146)

CACTCAGGAGGCTGAGGCAAGTGGATTTCTGTGTTTCAAAG
 CCAAGCCAGGCTACTAGGCAAGTTCAGGACAGCTGGGGCTCAACAGTAAATCTGTCTCTGAACTGCCCTCTACTCCGAAAGGATAGCTGGAAAGCGGCG
 CATCTCCAAGCCAGAAAAGGAGAGTCTTAGCAGAACTGACCATGTTTGCACCCATAATCTCAGACTTAGACTCTGGAACTGTGAAAAGTGAATGCCCT
 TTGTGCAAGCCACCTGTGCTCTCCCTGTGTTACAGTGGCCAGAGTGGACTAAGACACACATCTGTATCAGATACATAGTCTGGGGGAAATGTGTAGA
 ICSBP ICSBP
 CCAAGGAGCCACCAAGACTCACCCATTACACTAATCTTTTATCTTTAAGCITTACAGTGTCCCTCAAAACACCCCTTTCTAAAGTGTCTCCAAATTTCCA
 ICSBP
 AAGTATAAATGAAAGTACTTATCCCTCTCAGCTTCAGGAAAAAACAAGGACGAAACACCCACATGTGTCCCTTAATTTAAACAGGAGAGGGACTCAC
 ACATTTCCCTTTCTTCTGATCAAAAAGGGGCCAGGAGAAAAACAAGGGGCACTAGACTAGCAGGAGGTTGTTTGCATCCCAAATCTGATCTAATCAGCGATT
 ICSBP GATA-1 PU.1
 CTGGACAGATATTTGCTGCCCTGAAATCAGGAGGGGGAATCTTACAAACCTAAAAGCTAAAAGGGATTTTATGGGATTATCTAATCTAGCCCTGTGAT
 HyoB
 TTTATGGCAAGGTCACCTGGCATGGAGTCTGAGGTCACAGTGTGATTTGGAGGATAGAGCCAAATGGAAATTTTTTCTACGCCAATGCTTGTGATCTTTT
 ATTACAGTGATTTTTTCCCCCCAGAGCTGTGCGAGGGCTTACTCTGAGAACAGGAGATAATGTCTGTGATAATGCATATAAAGAGGTGGCTAAATGTAA
 GATA-1
 AATATTATTTAGGATGAAATGTGGAAATGGGGGAAAAACACTGGAGTCCCTCATGATGTGTGAAATAGCCCAAAATGTCCTCAATTTGCTGTAA
 ICSBP
 ACTCTGAGTAAACATCTCAAACTGTCTAGTGGGCCACGATTTAAATCAGAGATAGAGATAGAGAGCTTCTCACTGCCAGTGGCTTTGGACATGATG
 GATA-2 GATA-1
 GAGGTGCACTCTGTGTCACATGTCTTCAAAGTGGGCTCTGTGCTACTACTATAGAGAGTAGTTTTAGTGGCAAAATCTGTGCTTTTAAAGCAACTGTG
 GGTGTGGGTCAGAGTCTGTGATATTCACTTCTCTGTGGTGTAGGTCAGTCAAGTAAAGGATGTGAATGTACTCTACCTTCAAAGGCCACATCAATATG
 GCCTACATTTGTGAAATCTTAGAACACTGTCTGGATCAGAAATAGGCTTCTGTGTGGGGAGCCGGACACTATTTTTTGGATACGTGAGTTATCTACAAT
 GATA-1
 GCAATGTCTAAGTTAGGGGTATCAGTGAAGTCAAGAAATAGGGATGGAAGCGGTATATTCTATAGTAGTAGCTCACCTGTATGGCTATCATATAG
 E47
 GTATCTCAAAGTCCCAATGCTATTTGTGTGAGGAGAAAAACAGGTTTGAAGAGTAAATGTTGGCCCAAATGGAAATGCTCTGTGTTTGGTCTAAGAGC
 GATA-1/GATA-2
 CATACGGTCTTACATCAATAAAGGTAGACTTTGCACTAACCAAGTCTCACACTCCCGGAGCTTATAAAGAAAGGGGTGGGACCGAGAGGGAGAGGGGA
 GGGACCCCTCTACTTCTCAGGCTCAGGGTCAAGCTTGTGCTTGTGGCTTTCCCAACCCCGCCAAAGCGGCTGAAAATCTGCAATCCCACTGACAGGG
 ACACTGTGCAATTAAGGCCCTCACGGACTCTCCGGGCTCCCGGGGAGGCTTTCACGCTGCAGGAAACCGCTTTTAGTCAAGAGATCAAGGCCCGCA
 GATA-2
 GGGAACGGGGCGCAAGCGGCTCCGAGAGCCAGGACTGAGTGAAGTCCCAACCGCCGGCGGAGCCGCTGTGTTCCCGGAGAGGCTGGCTGCTAGAGCGC
 STATA
 GCCCACTTTGGCGGCTCTTAGCGCTCTGTTTACCCTCTATGCTGCGGTGCTCCCGCCCGCCCTCCCAAGTCAATGCTGTGGAGCAGCCCGGCG
 GATA-2
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Figure S5

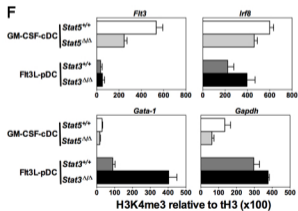
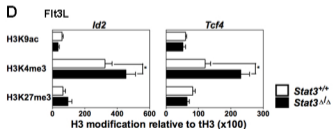
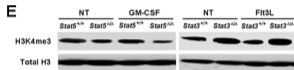
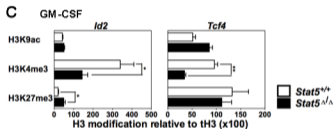
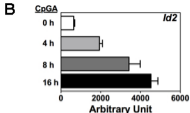
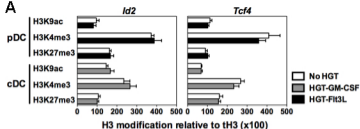
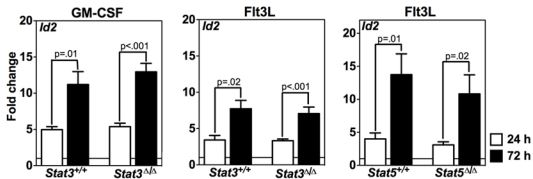
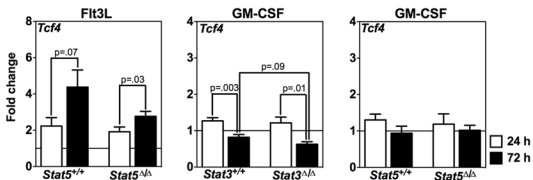
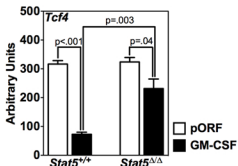
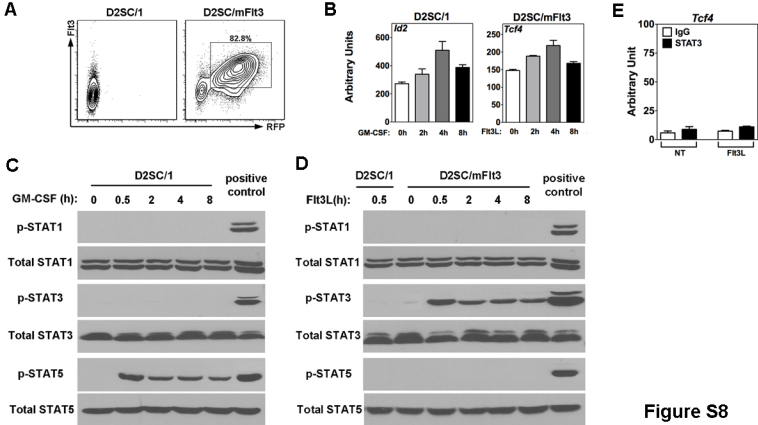


Figure S6

A in vitro**B in vitro****C in vivo****Figure S7**



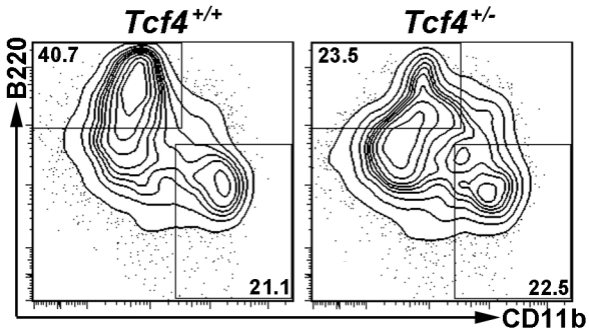


Figure S9

Table S1. Oligonucleotides used in this study.

Real-time PCR primers	
<i>Tcf4</i> sense	AGACCAAGCTCCTGATTCTC
<i>Tcf4</i> antisense	AGGCTCTGAGGACACCTTCT
<i>Id2</i> sense	AAAACAGCCTGTCGGACCAC
<i>Id2</i> antisense	CTGGGCACCAGTTCCTTGAG
<i>Tcf4</i> and <i>Id2</i> promoter cloning primers	
<i>Tcf4</i> sense (XhoI)	ATCTCGAGTGAAGGTGGACTCTG
<i>Tcf4</i> antisense (HindIII)	ACAAGCTTGGCACTGCGGCTTAT
<i>Id2</i> sense (XhoI)	ATCTCGAGTGGCTGCATCTAGGAAG
<i>Id2</i> antisense (HindIII)	GCAAGCTTGCTTTCATGCTGCTCGT
EMSA oligonucleotides	
<i>Tcf4</i> sense	CCCTGGTTCCCGGAGGAGGCG
<i>Tcf4</i> antisense	CGCCTCCTCCGGGAACCAGGG
<i>Id2</i> sense (distal)	CTGAGTTTCCGAGAAGGCCTC
<i>Id2</i> antisense (distal)	GAGGCCTTCTCGGAAACTCAG
<i>Id2</i> sense (proximal)	TGTGACTTCCCAAAGCGCGC
<i>Id2</i> antisense (proximal)	GCGCGCTTTTGGGAAGTCACA
ChIP primers ¹	
<i>Tcf4</i> sense	CTCTTCCAGCTCAGGGTCAC
<i>Tcf4</i> antisense	CTGCTCCACCAGACAATGAC
<i>Id2</i> sense	TGTGCAAACCCCACTAATGA
<i>Id2</i> antisense	CGCTTTTGGGAAGTCACATT

¹ Primer sequences for the ChIP assays performed in Figures 3C and 3D are indicated in Figure S5.