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Supplementary Materials for

IL-10 induces a STAT3-dependent autoregulatory loop in T_H2 cells that promotes Blimp-1 restriction of cell expansion via antagonism of STAT5 target genes

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Other Supplementary Material for this manuscript includes the following:
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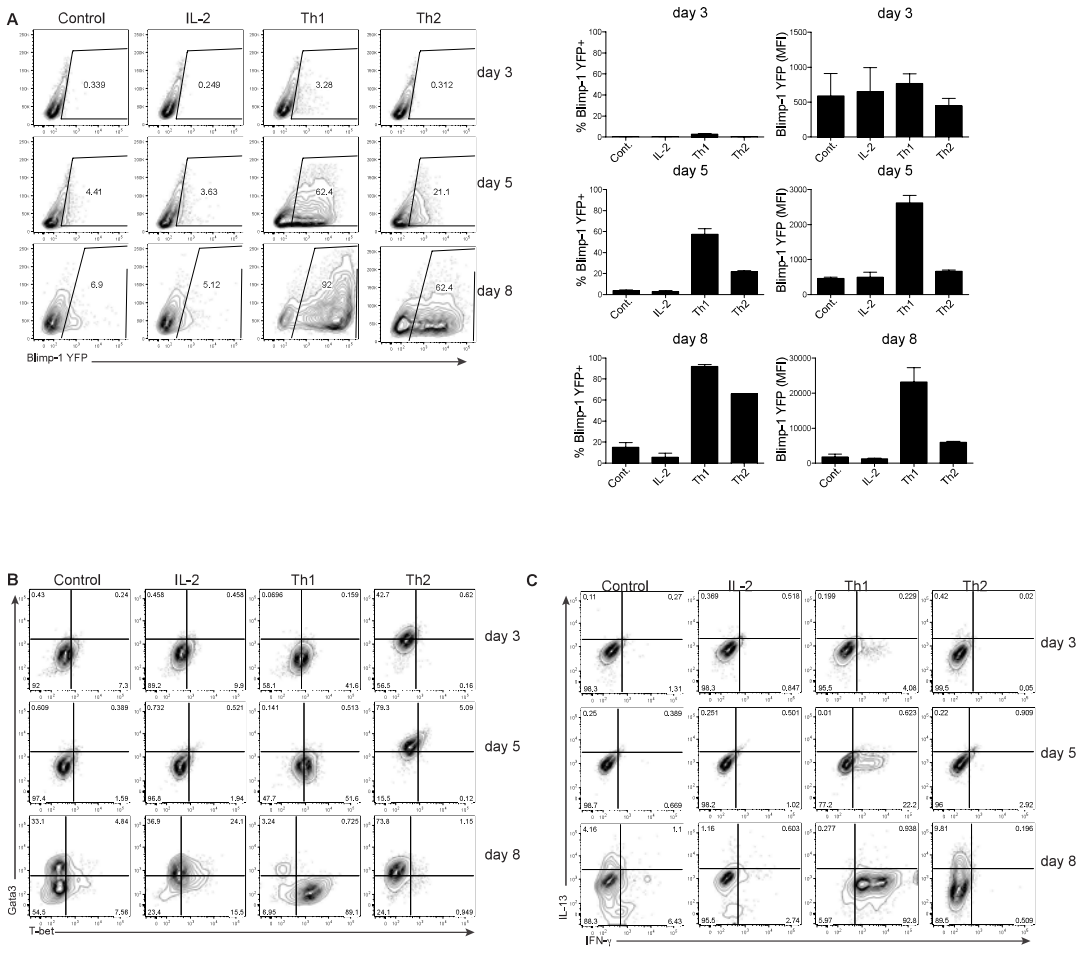
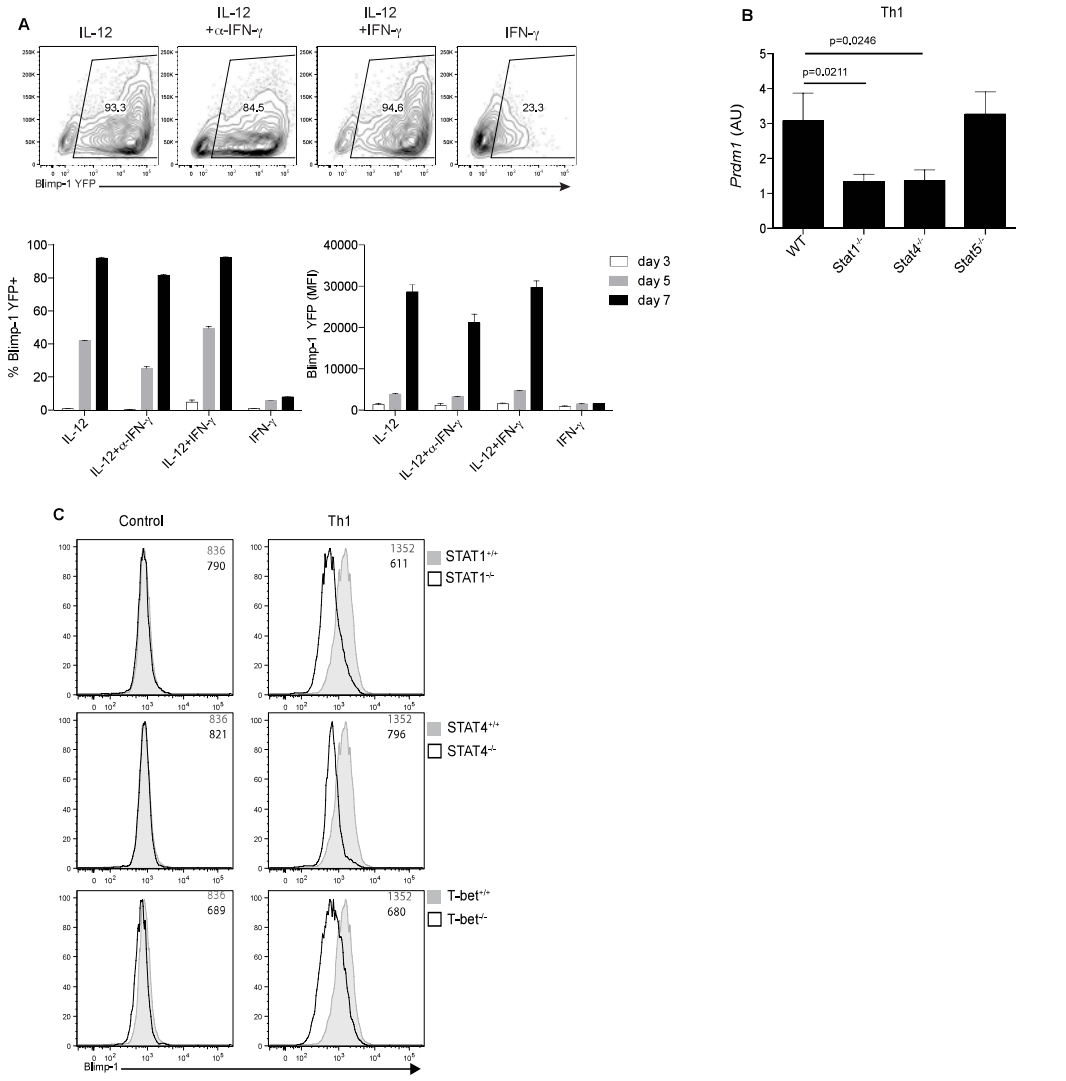
Supplementary Materials

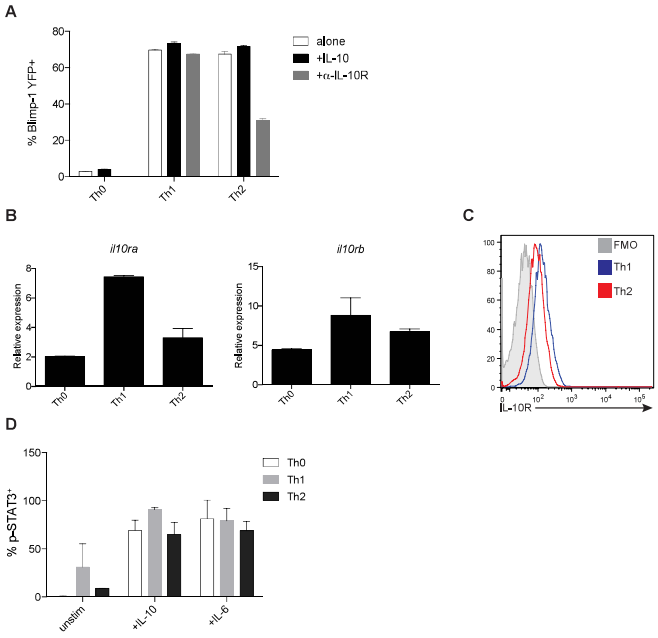


Supplementary Figure 1: Expression of Blimp-1 in T cell subsets in vitro.

A) Expression of Blimp-1 (YFP) in sorted naïve CD4+ from Blimp-1 YFP reporter mice. T cells activated with plate-bound α-CD3 and α-CD28 under the following indicated conditions: Control - anti-IFN-γ, anti-IL-4; IL-2 - anti-IFN-γ, anti-IL-4, IL-2; Th1 - anti-IL-4, IL-12; Th2 - anti-IFN-γ, IL-4. Time course of Blimp-1 expression, percent and mean fluorescence intensity (MFI) shown in graphs on right, data are represented as mean ± SD (n=2) and are representative of three independent experiments. B, C) Expression of GATA3 and T-bet (B) or IL-13 and IFN-γ (C) in cells shown in A.

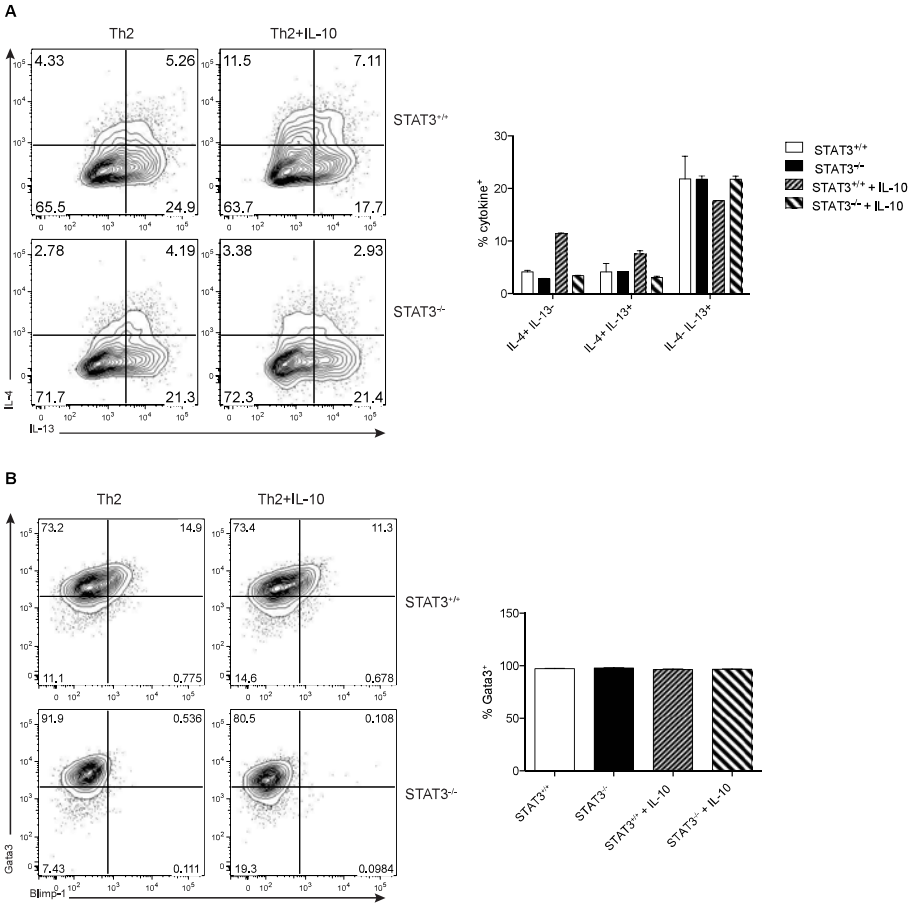


Supplementary Figure 2: The IL-12 – STAT4 – T-bet axis controls Blimp-1 expression in T_H1 cells.
Expression of Blimp-1 (YFP) in sorted naïve CD4+ T cells activated with plate-bound α-CD3 and α-CD28 under previously indicated conditions. In addition, α-IL-4 was added to all conditions, along with the antibodies and cytokines indicated above the figures. A) Expression of YFP at day 8 under indicated conditions, percent YFP+ cells and MFI are depicted below at indicated time points. Data are represented as mean ± SD (n=2) and are representative of two independent experiments. B) Expression of Blimp-1 mRNA measured by qPCR in STAT+/+ or STAT-/- Th1 cells at day 8 (normalized to β-actin) Data are represented as mean ± SD n = 2. C) Expression of Blimp-1 protein measured by intracellular flow cytometry in STAT+/+ or STAT1-/-, STAT4-/- or T-bet-/- Th1 cells at day 7. Geometric MFI of YFP is shown in top right corner of each flow plot. Data are representative of three independent experiments.



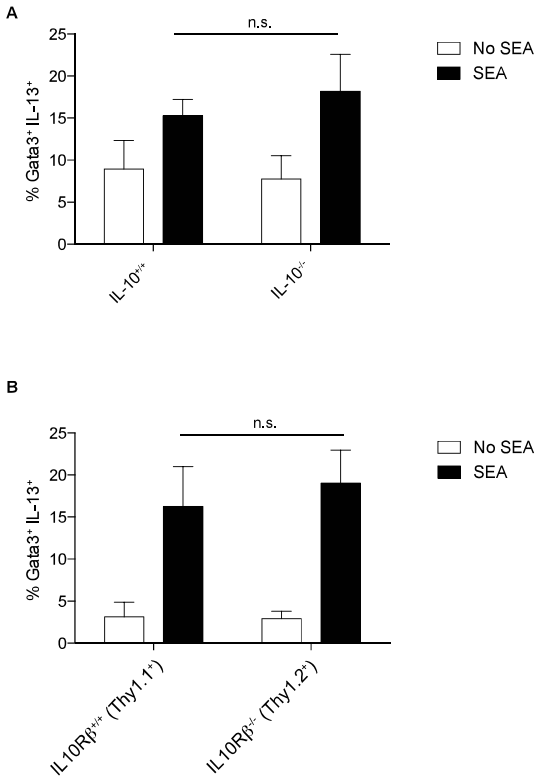
Supplementary Figure 3: IL-10 regulation of T_H1 and T_H2 cells.

A) Expression of YFP in T cells at day 8 under indicated conditions with or without the addition of IL-10 (10 ng/ml) or α-IL-10R (10 ug/ml) throughout the culture. Data are representative of three independent experiments. B) Expression of il10ra and il10rb measured by qPCR in indicated cells cultured for 5 days. Data are representative of three independent experiments. C) Expression of IL-10R by flow cytometry of cells in B. Data are representative of three independent experiments. D) Expression of phospho-STAT3 in indicated T cells stimulated with indicated cytokines for 30 mins after five days of differentiation. Data are representative of three independent experiments.

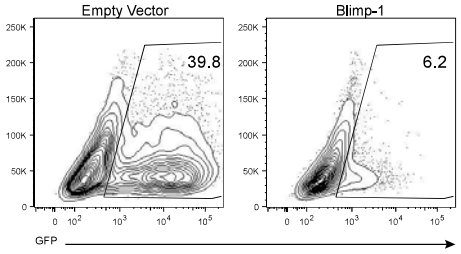


Supplementary Figure 4: T_H2 differentiation is normal in the absence of STAT3.

A) Expression of IL-4 and IL-13 in STAT3+/+ or STAT3-/- Th2 cells cultured with or without exogenous IL-10 for 7 days in vitro. Percentage of cytokine positive cells is graphed at right and represented as mean ± SD n = 2. Data are representative of two independent experiments. B) Expression of GATA3 and Blimp-1 in STAT3+/+ or STAT3-/- Th2 cells cultured with or without exogenous IL-10 for 7 days in vitro. Percentage of GATA3+ positive cells is graphed at right and represented as mean ± SD n = 2. Data are representative of two independent experiments.

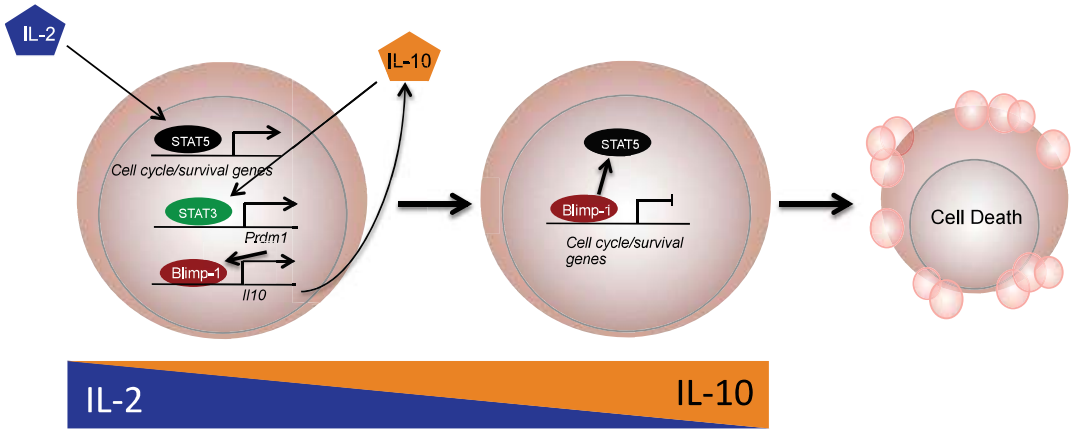


Supplementary Figure 5: The absence of IL-10 signaling on T cells does not affect T_H2 cell differentiation in vivo.
A) Percent of Th2 cells (Gata3+ IL-13+) isolated from IL10+/+ or IL10-/- hosts nine days p.i. with SEA. Cells were re-stimulated in vitro with cognate antigen for 48 hours. Cells additionally stimulated with PMA and ionomycin for 2 hours for IL-13. Quantification of percent Th2 cells from five animals per group. Data are representative of two independent experiments. B) Percent of Th2 cells (Gata3+ IL-13+) isolated from bone marrow chimeras reconstituted with a 50:50 mix of Il10Rβ+/+ (Thy1.1+) or Il10Rβ-/- (Thy1.2+) cells nine days p.i. with SEA and re-stimulated in vitro with cognate antigen for 48 hours. Cells additionally stimulated with PMA and ionomycin for 2 hours for IL-13. Quantification of percent Th2 cells from five animals per group. Data are representative of two independent experiments.



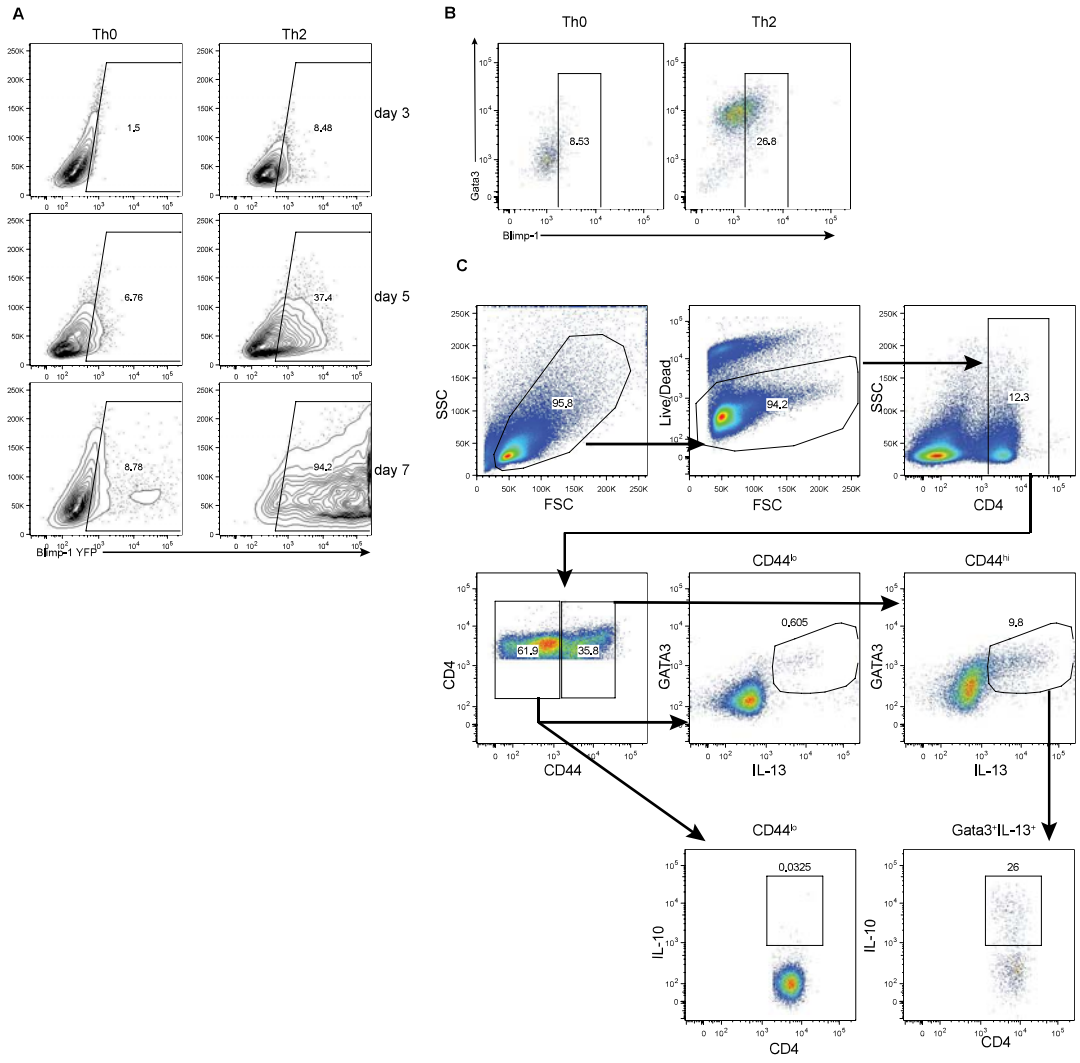
Supplementary Figure 6: Transduction of Blimp-1 GFP-expressing retrovirus.

Retroviral transduction of activated CD4 T cells with control retrovirus or Blimp-1 expressing retrovirus. GFP marks cells transduced with retrovirus. Data are representative of four independent experiments.



Supplementary Figure 7: Model of IL-10–driven Blimp-1 expression via STAT3 in T_H2 cells to promote cell death.

Graphical model of IL-10 mediated Blimp-1 induction and Blimp-1 mediated cell death in Th2 cells.



Supplementary Figure 8: Representative gating strategies.

A) Respresentative gating of Blimp-1 YFP positive cells. B) Representative gating of Blimp-1 staining. C)Representative gating strategy of Th2 effector cells (Gata3^{+} IL-13^{+}) and IL-10 staining in SEA in vivo experiments.

Supplementary Tables

Supplementary Table 1: Flow cytometry antibodies.

Antigen	Clone
CD4	RM4-5
CD44	IM7
CD62L	MEL-14
CD25	PC61.5
Blimp-1	5E7
IL-10	JES5-16E3
IFN-γ	XMG1.2
Thy1.1	OX7
Thy1.2	53-2.1
Gata3	L50-823
IL-13	eBio13A
h-NGFR	ME20.5
IL-10R	1B1.3A
phospho-STAT3	LUVNKLA

Supplementary Table 2: Gene lists for GSEA analysis.

STAT5 Positively Regulated

Ecm1	Ier3	Trat1	AI506816	Puf60	Ywhaz
Ntn4	Itgb3	Grhpr	Myh10	Ydjc	Gm2a
D630039A03Rik	Rgcc	Lss	Fam114a2	Lad1	Bsg
Hist1h4n	Scd2	Hilpda	Pgap2	Fdft1	Nrn1
Slc15a2	Pim1	Tpi1	Nr4a2	Trappc6a	Icos
Gzmb	Atp8b4	Chchd10	Stat4	Ifi30	Atp10d
Hectd2	Gm12250	Slc2a1	Sc5d	Pfkp	Hmgn5
Mgarp	Cpm	Vat1	Dhcr7	P4ha1	Serpinc1
Stc2	4930483K19Rik	Il2ra	Bnip3l	Insig1	Ell2
Aldoc	Ppic	H2-Q6	Gm12070	Zdhhc18	A130040M12Rik
Egln3	Gpr65	Gm5506	Gm6981	Hmgcr	Dbi
Socs2	Serpinb6b	St6gal1	Stard4	Psmb8	Cdv3
Akr1c18	Arsb	Mettl23	Gm5069	Slc39a10	S100a11
Slc16a3	Apobec3	Mt1	Gapdh	Odc1	Galnt6
Zhx2	Sfn	Tesc	Gm5523	Pmvk	Iah1
Cish	Igfbp4	Galk1	Arl2bp	Ttyh3	Mndal
Scd1	Ankrd37	Ncf1	Ccr7	Anxa6	Tmem194b
Gstt1	Syce2	Gbp5	H2-K2	Cndp2	Stag3
Prrg4	Elovl6	Acsl3	Nsdhl	Slc25a1	Atp7a
Gm13546	Hist1h4i	Entpd4	Sc4mol	Ccr4	
Bnip3	Tg	Socs1	Glo1	Esyt1	
Fam71b	Chrm4	Pgk1	Cblb	Lonp1	
Ndrg1	Farp1	Gpi1	Gm6498	Aacs	
Stat5b	Fdps	Kbtbd11	Sqle	Higd1a	
Pdk1	Crabp2	Gm8909	Dhcr24	Cytip	
Rragd	Runx3	Mid1ip1	3000002C10Rik	Fabp5	
Asns	5830417I10Rik	Ldha	Pced1b	Pgd	
Ak4	Mvd	Pfkl	Mpp1	Hk1	
Arhgdig	Gm4070	Pgm2	Mthfd1l	Sigmar1	
Gprin3	Hopx	Notch2	Gbp2	Pgam1	
H2-Q2	Ldlr	Fosl2	Egln1	C2cd5	
Fads2	Trmt1	Ero1l	Itga3	Pcyt2	
Rgs11	Tnfrsf9	Slco4a1	Ap3b1	Rcsd1	
Trib3	Hk2	Acat2	Tkt	Ccnd2	
Vegfa	Mov10	Tmem97	Idi1	Rdh11	
Dnah8	Eif4ebp1	Aldoa	Pim2	Fam162a	
Rnaset2b	Igtp	Prelid2	Acly	Myc	
Thy1	Eno1	Phgdh	Nefh	Slc7a5	
Pdzk1ip1	Irf8	Stat5a	Cyp51	Ctse	
Sirt3	Asah1	Pcgf5	Ctla4	Pmm1	
Ndrg2	Wnt10b	H2-Q10	Sh3bp1	Fam132a	
Tigit	Bmyc	Gsto1	Gcat	Jmjd6	
Gm129	Dapl1	Pkm	Fam213a	Rras2	
Socs3	Nr4a3	Nrp1	Mif	Serpinb6a	
	Stk39	Ccl3	Pop4	Ndufv3	

STAT5 Negatively Regulated				Cell Cycle	
Gm19757	6820431F20Rik	Rasl11b	Cd74	Abl1	Gpr132
Bckdhb	Nnt	A930005H10Rik	Tsc22d1	Atm	Hus1
Ikbke	Ing4	Sesn2	Dsp	Atr	Itgb1
Phyh	Snhg6	Xcl1	Khdrbs3	Aurka	Mad2l1
AA465934	Tmc6	Cd52	Snhg9	Aurkb	Mcm2
Xlr4b	Lpxn	Mxd4	H2-T10	Bcl2	Mcm3
Gm10845	Ptp4a3	Tmem19	Myh7	Birc5	Mcm4
Mmd	Hcst	H2-T9	Napsa	Brca1	Mdm2
Xlr4c	Furin	Ikzf3	Mzb1	Brca2	Mki67
Rab11fip4	Il2	Pdgfb	Zfp383	Casp3	Mre11a
Bach2	Ms4a6c	Npas4	Pik3r3	Ccna1	Msh2
Sesn3	S100a4	Abhd4	Vax2	Ccna2	Myb
Cd5	Rnf149	Ldhb	Ly6d	Ccnb1	Nbn
Tfe3	Aen	Isg20	Fcrl1	Ccnb2	Nek2
Mlf1	Ext1	Icam1	Cib2	Ccnc	Notch2
Pou2af1	Tmem154	Rbl2	Pianp	Ccnd1	Pkd1
Egr2	Rxra	Swap70	Bcar3	Ccnd2	Pmp22
Gypc	Zfp862-ps	Podnl1	Ccl1	Ccnd3	Ppm1d
Hmgn2	Id3	Slc38a6	Kifc3	Ccne1	Rad17
Cd37	Penk	Sap25	Cd5l	Ccnf	Rad21
Pttg1	Rbmx	Mt2	Maf	Cdc20	Rad51
Rtfdc1	Pdcd1	Prkcdbp	Tdgf1	Cdc25a	Rad9a
Fxyd5	Stat1	Lrrc28	Gm5779	Cdc25c	Ran
Tnfrsf14	Hist2h2ac	H2-Oa	Scn1b	Cdc6	Rb1
AI504432	Cdk5rap1	Spib	Ccl22	Cdc7	Rbl1
Cpne8	Ercc1	Twist1	H2-DMb2	Cdk1	Rbl2
Atp6v1d	Rgs2	Tnfsf4	Ms4a1	Cdk2	Sfn
Psap	Ccng1	Snn	Cd79a	Cdk4	Shc1
Axl	Rgs1	Lpar6	Cd19	Cdk5rap1	Skp2
Gimap9	Ncoa7	Fam212b	H2-Eb1	Cdk6	Slfn1
Rgs16	Dcxr	Sectm1a	Rprl3	Cdkn1a	Smc1a
Phf20l1	Psrc1	AI848285	Fcer2a	Cdkn1b	Stag1
Itm2a	H2-Ab1	Angptl2	Rmrp	Cdkn2a	Stmn1
2810428I15Rik	Ubxn11	Spp1	Cyp1a1	Cdkn2b	Terf1
Chst2	Pmaip1	Sfi1	Bcl2l15	Cdkn3	Tfdp1
Dok2	Glipr1	Cd209c	H2-Aa	Chek1	Trp53
Ly6e	Dusp6	Mid1	H2-DMb1	Chek2	Trp63
Zfp36l1	Cdkn1a	Rorc	Ly86	Cks1b	Tsg101
Tnfrsf13c	Litaf	1500011B03Rik	5830428M24Rik	Ddit3	Wee1
Map3k7	Phlda3	Rdh12	Il17f	Dst	
Tmc8	Klhl6	Ramp1	Tnfrsf19	E2f1	
1500012F01Rik	Rnf130	Bcl6	Gm12238	E2f2	
Ypel3	Cd96	Cadm1		E2f3	
Btg2	Hist2h2bb	Cxcr6		E2f4	
	Hist1h4m	Nt5e		Gadd45a	

Supplementary Table 3: ChIP-qPCR custom-designed SYBR primers.

Primer	Fwd (5'-3')	Rev (5'-3')
Negative Control	GGGAACGACACTAACTATCC	GAGCTATATGCCAGGATCTC
SOCS3 TSS	CGAGTGTAGAGTCAGAGTTAG	CCTTTCAGTGCAGAGTAGT
prdm1 +58	TCGTGAACTAGGATGAGAAC	AGAGTCAGCTTTCGGTAAA
prdm1 +55	GTCATGTGAAGCAGTGAAAT	TCTTCGGCTCTTCTTGTATC
prdm1 +26	CAACGAAACTGAACAGTCAA	TGAGTAGATAGATGTGAAAGGG
prdm1 +12	CCTAAAGCTACCTGAGACTG	GGGACTGATCTCATCTTTCA
prdm1 +11	CTATGGCCTCTGTACTTGTG	CAGTGCCTCACTCTGTTATG
prdm1 +10	CTACGGTTTACTGTATCCTTGT	AGATGCTCAGGTTGAGAAAG
prdm1 +9	ACTCAGGATAACTCTTCCATTC	GTGGTTAGATGTGGACTACC
prdm1 TSS	ATACTGACGGTCTGATTCAC	TAGTGTGGGTAAACATGGAG