Supplemental information

Zinc finger-IRF composite elements bound by Ikaros/IRF4 complexes function as gene repression in plasma cell

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Supplemental Methods

RNA-seq

Total RNA was prepared from triplicate cell samples by using RNeasy Plus Mini Kit (Qiagen). Each library was prepared using TruSeq RNA sample preparation kit v2 (Illumina). Libraries were clonally amplified on the flow cell and sequenced on an Illumina HiSeq2500 with a 51-mer paired end sequence. Image analysis and base calling were performed using Real Time Analysis (RTA) 1.13. The differential expression analysis was performed by edgeR 3.4.2 (ref.1) with R 3.0.3 (R Core Team; http://www.R-project.org/). By comparing repression values at 0 hour and other time points, genes with more than 10-times fold change and the threshold of P-value for 0.05 with bonferroni correction were selected. Cluster analysis for gene expression patterns was carried using fastcluster 1.1.13 with Pearson correlation distance and average linkage. Differently expressed genes were determined for each state in the cluster against 0-h state.

ChIP assay

ChIP was performed as previously described[14]. Chromatin from stimulated splenic B cells was isolated and sonicated to obtain DNA fragments raging in size from 100 to 500 base pairs. Chromatin fragments were immunoprecipitated with specific antibodies shown in Supplemental Table 4. After reversal of formaldehyde crosslinks, specific DNA sequences were assessed by quantitative real-time PCR by using specific primer sets (Supplemental Table 5).

Quantitative RT-PCR

Total RNA was prepared from triplicate cell samples using RNeasy Plus Mini Kit (Qiagen). cDNA was made with Superscript III reverse transcriptase (Invitrogen). SYBR qPCR Master Mix (Takara-Clontech) and a ABI 9500 Real-Time PCR system (Applied Biosystems) were used for quantitative PCR (primers, Supplemental Table 5).

Complex purification and LC-MS/MS analysis

IRF4 complex purification was carried out using ReCLIP methods[2]. Splenic B cells from B1-8^{hi} mice were stimulated for three days, and were crosslinked with 0.5 mM DTME (Thermo Scientific) and 0.5 mM DSP (Thermo Scientific) at 25 ˚C for 30 min. After removal of crosslink buffer, cells were quenched with 20 mM Tris (pH 7.5) and 5 mM Cystein containing buffer at 25 ˚C for 5 min. Cells were washed with ice-cold PBS, and suspended in RIPA buffer containing proteinase inhibitor cocktail and PhosSTOP

(Roche). The cell lysate was reacted on ice for 10 min, and then sonicated using Bioruptor (Cosmo Bio). After centrifugation, the supernatant was pre-cleared with protein A and G beads (Invitrogen) at 4 ˚C for 1 h, and reacted with conjugated antibodies for immunoprecipitation at 4 ˚C for 2 h. Beads were washed with RIPA buffer for three times, and eluted with elution buffer containing 50 mM Tris (pH 8.0), 0.2 M NaCl, 2% SDS and 125 mM DTT at 37 ˚C for 20 min, then at 70 ˚C for 10 min. Each protein was determined using LTQ OrbiTrap Velos (Thermo Fisher Scientific), and MASCOT search engine (Matrix Science).

Immunoblot analysis and immunoprecipitation

Preparation of protein extracts and procedures of immunoblot analysis have been previously described[3]. Details of primary antibodies are shown in Supplemental Table 4. For immunoprecipitation, whole-cell lysates were prepared using ReCLIP methods, and incubated with specific antibodies followed by immunoblot analysis.

Flow cytometry

Procedures for surface and intracellular staining have been previously described[3]. Antibodies used for staining were shown in Supplemental Table 4. Data were collected with a Cant II or an Aria II (BD Biosciences) and analyzed with FlowJo software (TreeStar).

Retroviral vectors and transduction of naïve B cells

Retroviral vectors used in this study have been described[4-6]. Plasmids encoding short hairpin RNA oligonucleotides were constructed as described[3]. Short hairpin sequences for target genes are shown in Supplemental Table 5. Preparation of virus supernatant and infection to naïve B cells from B1-8^{hi} mice stimulated for 20 h were performed as previously described[3]. After 48 h, transduced cells were sorted on the basis of GFP or RFP expression, and were used for the isolation of RNA.

Stealth RNA *interference*

Stealth RNAi duplexes against Ikaros or Aiolos or Negative control were obtained from ThermoFisher Scientific/Invitrogen. Splenic B cells were transfected as 6 μL of 20 μM Stealth RNAi duplexes using a Nucleofector Kits for Stimulated Mouse B cells (Lonza) according to the manufacturer's protocol.

Luciferase assay

Reporters containing each region were PCR amplified and inserted into pGL4-Luc (Promega) using *Xho* I site and *Hind* III site. Oligo sequences used for insert

amplification are shown in Supplemental Table 5. Motifs contained each reporter constructs were indicated with amplified DNA size in Supplemental Table 2. Effector plasmids of pcDNA3 PU.1 or IRF4 were previously described[7]. Ikaros expression vector was constructed by subcloning of PCR fragments, with sequence encoding an amino-terminal Flag tag, into the pcDNA3. 293T cells were transiently transfected with various combinations of reporter and effector plasmids using FuGENE HD (Roche) according to the manufacturer's instructions. Each transfection was done in duplicate, and the luciferase activity was measured at 24 h after transfection using the dual-luciferase reporter assay kit (Promega) according to the manufacturer's protocol.

Electromobility shift assay and oligonucleotide precipitation assay

293T cells were transfected with pcDNA3 HA-IRF4, Flag-Ikaros, Flag-Batf or MigR Aiolos using FuGENE HD (Roche) according to manufacturer's recommendations. Nuclear extracts were then prepared as previously described[7] and used to prepare protein – DNA complex. EMSA reactions were separated on non-denaturing PAGE and the radioactive signal was imaged from Typhoon FLA 7000 (GE healthcare). Batf expression vector was constructed by subcloning of PCR fragments, with sequence encoding an amino-terminal Flag tag, into the pcDNA3. Aiolos expressing vector was kindly gifted from M. R. Clark (University of Chicago). For oligonucleotide precipitation assay, nuclear extracts were incubated with 1 μg of biotinylated oligonucleotide for 2 h at 4 C followed by another 2 h reaction in the presence of streptavidin-agarose beads (Pierce). After beads were washed with reaction buffer for two times, samples were processed for immunoblot analysis. Oligo sequences used for assay are shown in Supplemental Table 5. Antibodies used for immunoblot were as follows: anti-FLAG (M2)-HRP (SIGMA-ALDRICH; A8592) and anti-IRF4 (M-17; sc-6059).

Statistical analysis

Statistical analysis was performed with Welch two sample *t*-test using the open-source statistical programing environment R.

References in Supplemental methods

1. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*.

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2. Smith AL, Friedman DB, Yu H, Carnahan RH, Reynolds AB. ReCLIP (reversible cross-link immuno-precipitation): an efficient method for interrogation of labile protein complexes. *PLoS One*. 2011;6(1):e16206.

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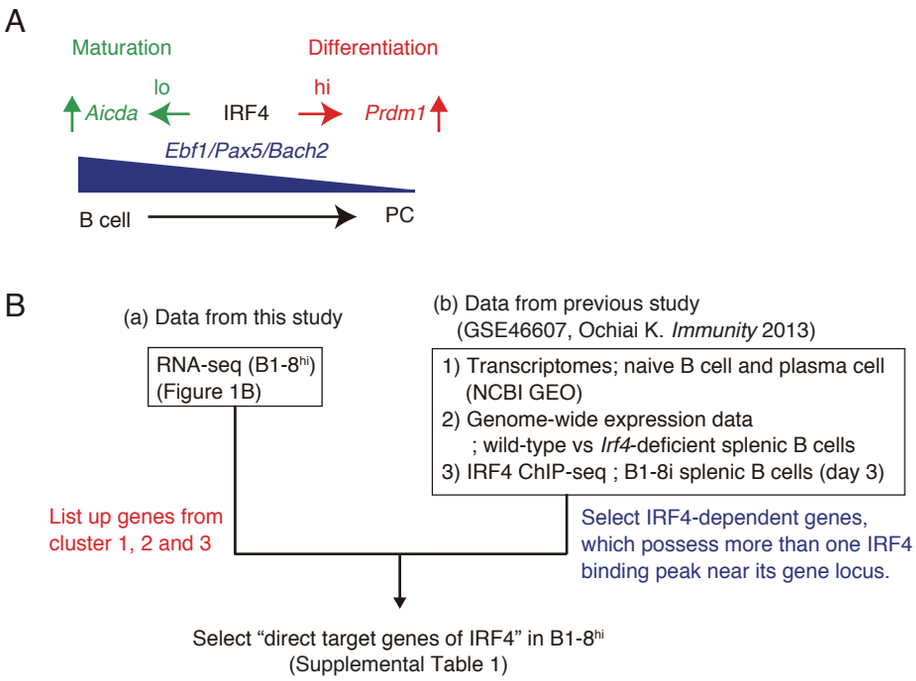
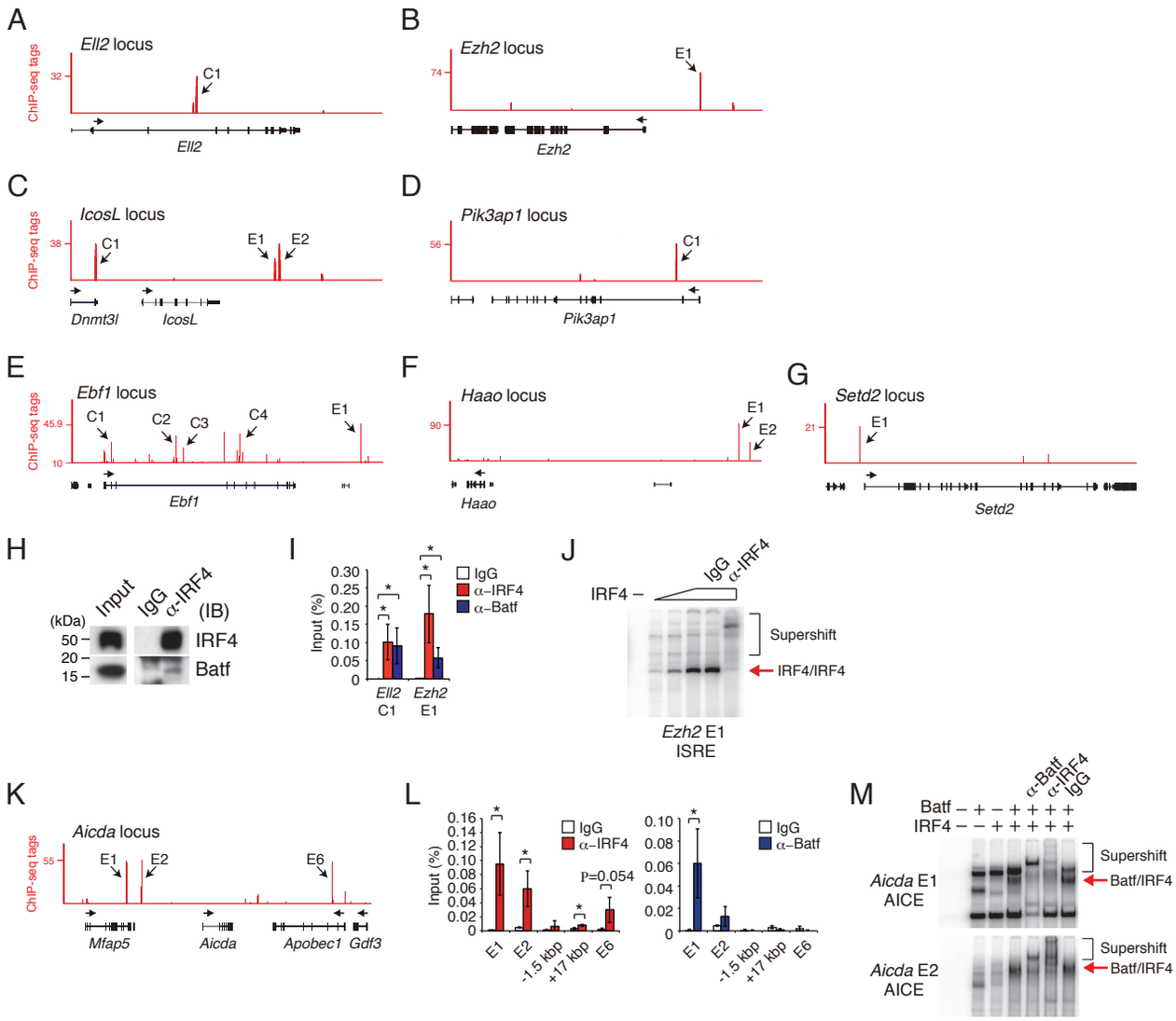
7. Ochiai K, Maienschein-Cline M, Simonetti G, et al. Transcriptional regulation of germinal center B and plasma cell fates by dynamical control of IRF4. *Immunity*. 2013;38(5):918-929.

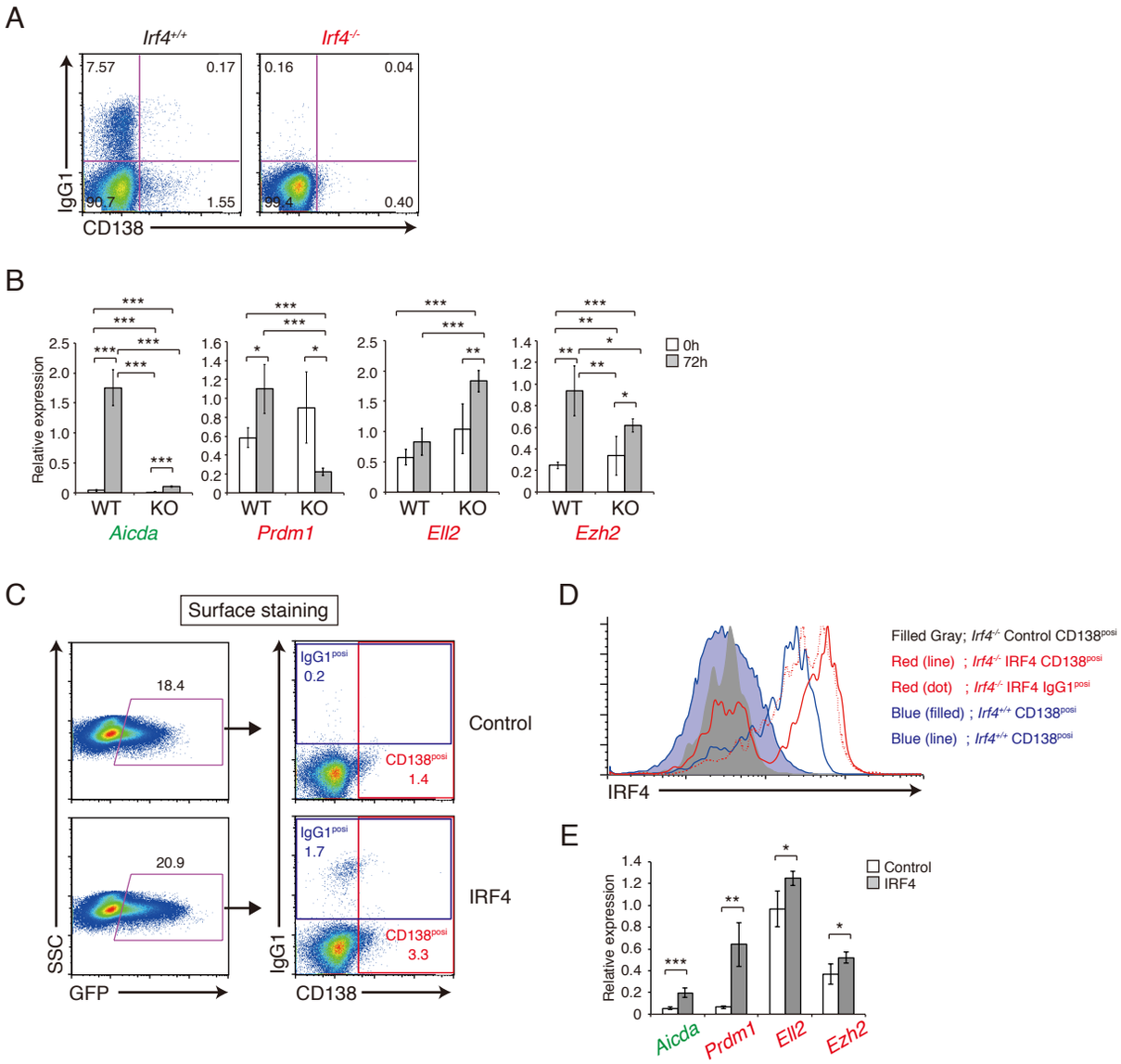


Supplemental Figure 1. Extraction of IRF4 direct target genes in stimulated B1-8^{hi} splenic B cells. (**A**) Schematic representation of function and regulation of key TFs during plasma cell differentiation. (**B**) Schematic of the extraction of IRF4 direct target genes in B1-8^{hi} cells. (a) Genes with differential expression in stimulated B1-8^{hi} cells were identified by RNA-seq and sub-divided into three major clusters as described in Figure 1B. (b) Differentially expressed genes between naive B cells and plasma cells were listed from NCBI GEO database. Among these genes, IRF4-dependent genes were identified by comparing transcripts of wild-type vs *Irf4*-deficient splenic B cells (Ochiai K. *Immunity* 2013). IRF4 direct target genes were further selected based on the possession of IRF4 binding peaks from ChIP-seq data using B1-8i mice splenic B cells (Ochiai K. *Immunity* 2013), and listed in Supplemental Table 1.



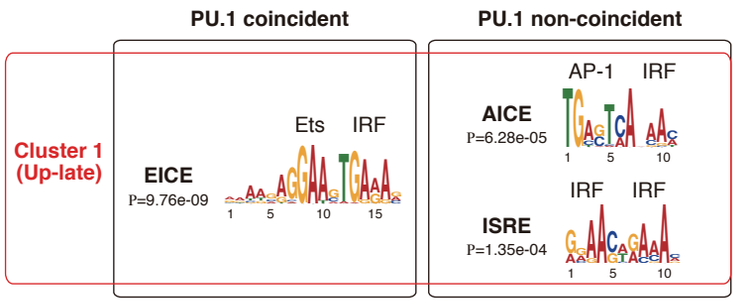
Supplemental Figure 2. Identification of new IRF4 direct target genes.

(**A-G**) IRF4 binding peaks on its target gene loci from previous IRF4 ChIP-seq data (day 3) (Ochiai K. *Immunity* 2013). **A and B**, cluster 1 genes; **C-G**, cluster 3 genes. IRF4 regulatory motifs within each peak are shown in Supplemental Table 2. E, enhancer; C, coding; I, intergenic. (**H**) Immunoblot analysis of IRF4 and Batf after immunoprecipitation (with IgG or α-IRF4) of whole-cell extracts of B1-8^{hi} splenic B cells stimulated for 48 h. Input, 2% of sample before immunoprecipitation. Data is representative of two independent experiments. kDa, kilodalton. (**I**) ChIP-qPCR analysis of IRF4 and Batf binding on indicated regions. (**J**) Binding of IRF4 homodimer to an ISRE motif within *Ezh2* P1 sequence. (**K, L**) IRF4 binding peaks (**K**) and ChIP-qPCR analysis of IRF4 and Batf binding on the *Aicda* locus (**L**). -1.5 kbp or +17 kbp from TSS of *Aicda* were described as negative or positive binding regions of Batf in LPS stimulated B cells (Ise W. *Nature Immunology* 2011). (**M**) Binding of Batf/IRF4 complexes to AICE motifs within *Aicda* E1 and E2 sequences. For (**I**) and (**L**), B1-8^{hi} splenic B cells stimulated for 48 h were used for ChIP, and binding enrichments are presented relative to input DNA. The average and SEM is from three independent experiments. * $P < 0.05$. For (**J**) and (**M**), nuclear extracts were prepared from 293T cells transfected with IRF4 or Batf expressing vectors, and incubated with indicated radio-rabeled oligonucleotide in DNA binding assays. Control, α-IRF4 or α-Batf antibodies were used in supershift assays to confirm each complex.

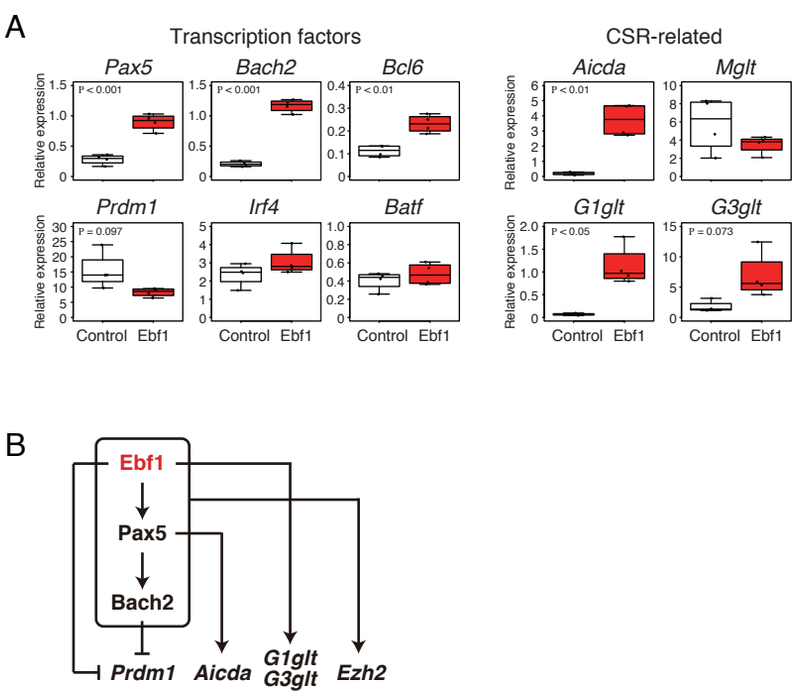


Supplemental Figure 3. The expression of IRF4 activation target genes, *Aicda*, *Prdm1, Ell2* and *Ezh2*, in wild-type vs *Irf4*-deficient splenic B cells.

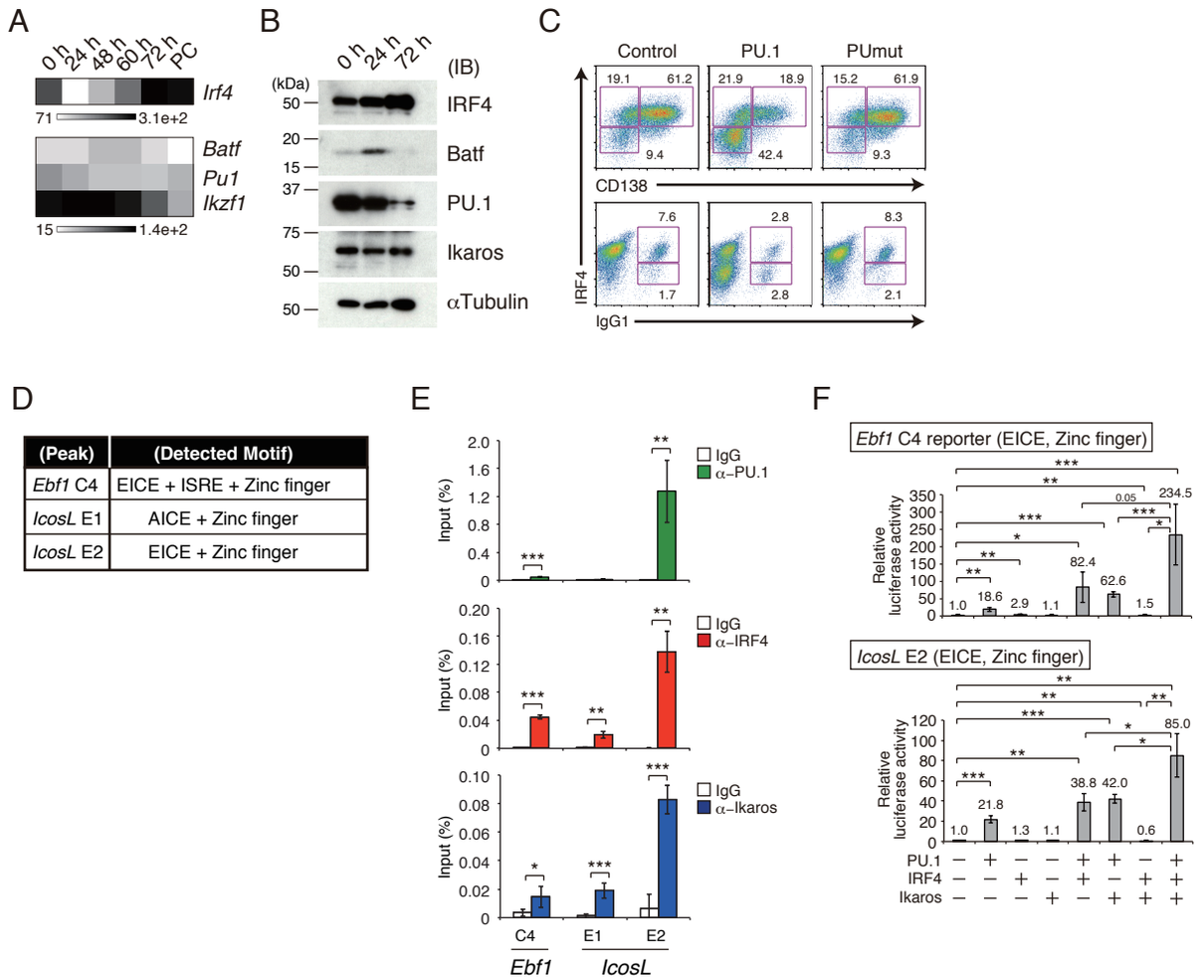
Splenic B cells were purified from wild-type or *Irf4*-deficient mice and stimulated with IL-4 and CD40L for three days. (**A**) Flow cytometry analysis of surface CD138 and IgG1 in wild-type or *Irf4*-deficient splenic B cells. Data is representative of one experiment using four wild-type and five *Irf4*-deficient mice, respectively. (**B**) RT-PCR analysis of transcripts of *Aicda*, *Prdm1*, *Ell2* and *Ezh2* at 0 h and 72 h after stimulation. The average expression and SD is from four wild-type and five *Irf4*-deficient mice. (**C-E**) Reconstitution of IRF4 into *Irf4*-deficient splenic B cells. Splenic B cells from *Irf4*-deficient mice were stimulated with IL-4 and CD40L, and transduced with control retroviral vector or retroviral vector expressing IRF4, sorted on the basis of GFP expression at day 3. Flow cytometry analysis of surface CD138 and IgG1 (**C**), and intracellular staining for IRF4 (**D**). Data are representative of one experiment using four mice. (**E**) RT-PCR analysis of transcripts of indicated genes. For (**B**) and (**E**), results are represented relative to the abundance of transcripts encoding β2-microglobulin (B2m), and the average expression and SD is from indicated mice, respectively. Selected IRF4 target genes are indicated with each colors: red, cluster 1; green, cluster 2 from Supplemental Table 1. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



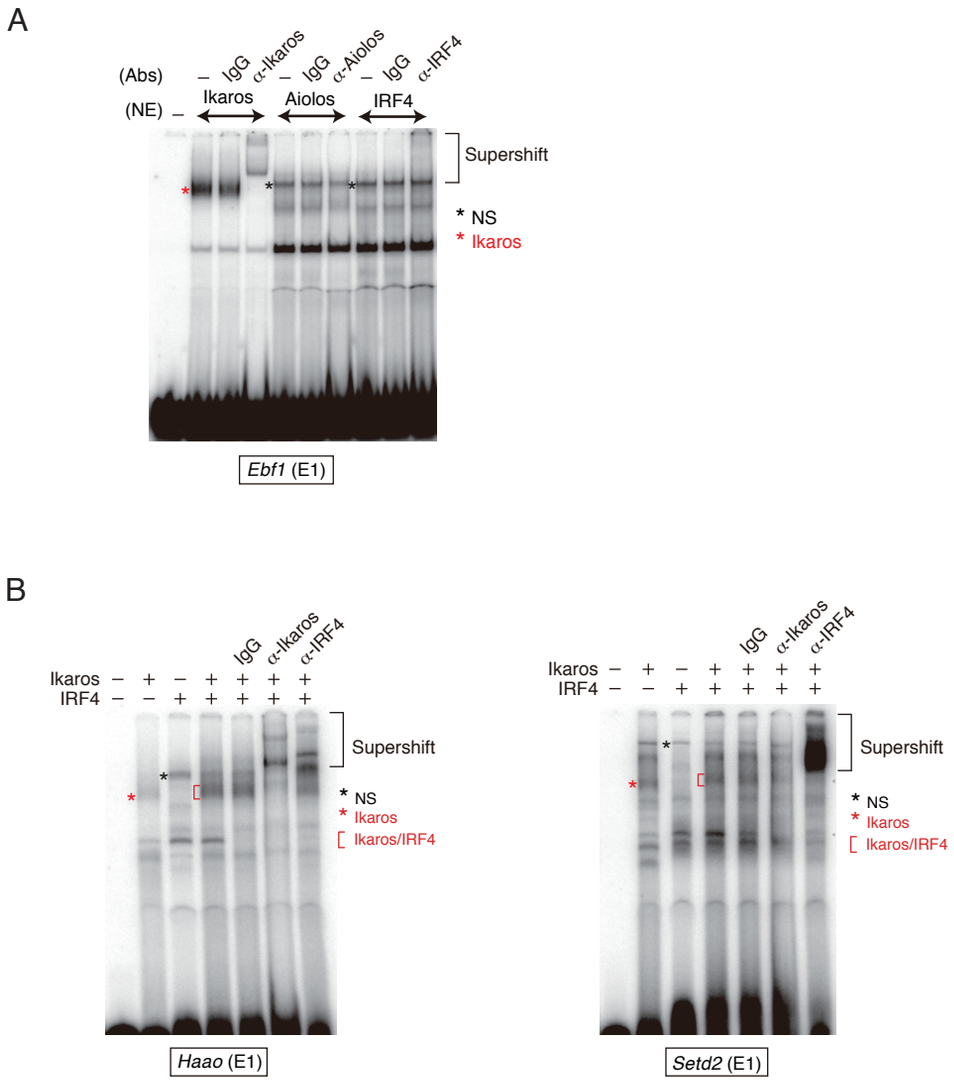
Supplemental Figure 4. Motif analysis of IRF4 binding sequences from culster 1 in Figure 1B.
IRF4 direct targets in B1-8^{hi} splenic B cells were selected using IRF4 ChIP-seq (day 3) in B1-8i splenic B cells (Ochiai K. *Immunity* 2013) as described in Figure 1B. IRF4 binding targets belonging to cluster 1 were further classified into those with and without PU.1 ChIP-seq peaks (PU.1 coincident and non-coincident, respectively). Totally, 47 and 28 sites were extracted for these categories, respectively. These were analyzed with the MEME algorithm to identify over-representated motifs within 100 base pairs in either direction. Results are represented for the enriched motifs. EICE, Ets-IRF composite elements; AICE, AP-1-IRF composite elements; ISRE, interferon sequence response elements.



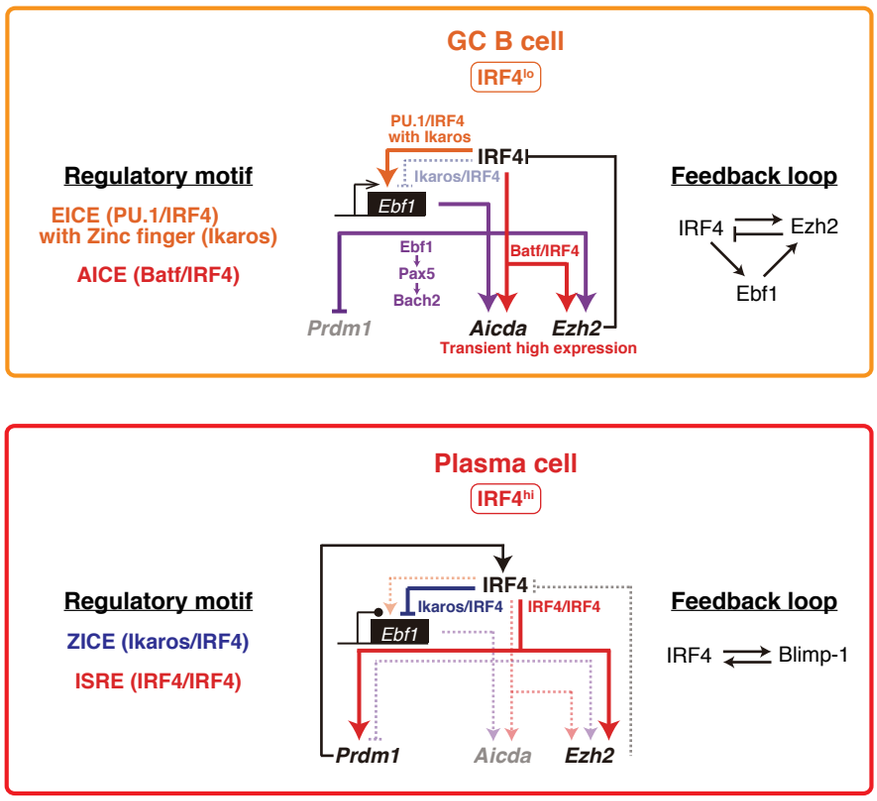
Supplemental Figure 5. The Ebf1-Pax5-Bach2 axis activates CSR-related gene and represses differentiation.
(**A**) RT-PCR analysis of transcripts of indicated genes in Ebf1 transduced cells as described in Figure 3A and B. Results are represented relative to the abundance of transcripts encoding B2m, and shown with box-and-whisker plot. (**B**) Schematic representation of GC-related GRN composed by the Ebf1-Pax5-Bach2 axis. Ebf1 activates *G1glt* and *G3glt* as well as *Pax5* expression. Pax5 is one of activators of *Aicda* and *Bach2*. Bach2 and Ebf1 repress *Prdm1* expression. Ebf1 induces *Ezh2* expression (Figure 3A), indicating that the Ebf1-Pax5-Bach2 axis positively regulates it.



Supplemental Figure 6. Ikaros activates IRF4 target genes in the presence of PU.1.
(**A, B**) Transcripts (**A**) and immunoblot analysis (**B**) of IRF4, Batf, PU.1 and Ikaros at indicated time in stimulated B1-8^{hi} splenic B cells. kDa, kilodalton; IB, immunoblot. (**C**) Flow cytometry analysis of intracellular IRF4, CD138 and IgG1 in B1-8^{hi} splenic B cells transduced with control retroviral vector or its derivative expressing PU.1 or PU.1 DNA binding mutant (PUmut) and then sorted on the basis of GFP expression. Numbers adjacent to outlined areas indicate percent IRF4^{lo}CD138^{nega} cells (top lower), IRF4^{hi}CD138^{nega} cells (top upper left), IRF4^{hi}CD138^{posi} cells (top upper right), IRF4^{lo}IgG1^{posi} cells (bottom lower) or IRF4^{hi}IgG1^{posi} cells (bottom upper). Data is representative of one experiment using three mice. (**D**) Detected motifs within *Ebf1* C4, *IcosL* E1 and E2 regions. The peak positions at gene loci were shown in Supplemental Figure 2E and C, respectively. Regulatory motifs existed in each region were shown in Supplemental Table 2. (**E**) The binding of PU.1 (upper) and IRF4 (middle) and Ikaros (lower) to their regulatory elements at the *Ebf1* and *IcosL* loci in B1-8^{hi} splenic B cells stimulated for 48 h. ChIP assay was performed using control IgG or α-PU.1 or α-IRF4 or α-Ikaros, followed by quantitative PCR analysis. Binding enrichments is presented relative to input DNA. The average enrichment and SEM is from four independent experiments for α-IRF4 and PU.1, and two independent experiments with two technical replicates for α-Ikaros. (**F**) Luciferase assays using *Ebf1* C4 or *IcosL* E2 reporter genes. 293T cells were transiently transfected with indicated reporter and effector plasmids. The amounts of plasmids were as follows: luciferase reporter (1.0 μg), PU.1 (100 ng), IRF4 (100 ng) and Ikaros (100 ng). The reporters used are shown above each panel with containing motifs. The average luciferase activity and SD is from three independent experiments. For (**E**) and (**F**), *; P < 0.05, **; P <0.01, ***; P < 0.001.

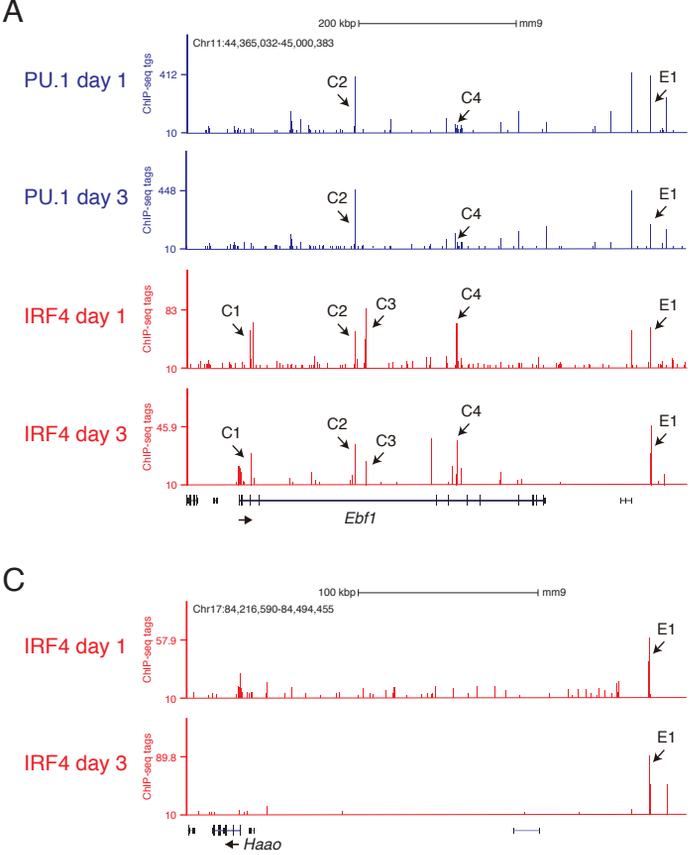


Supplemental Figure 7. Assembly of Ikaros/IRF4 complexes on ZICE motifs.
(**A**) Ikaros but not Aiolos or IRF4 binds the ZICE motif within the *Ebf1* E1 sequence probe. (**B**) Binding of Ikaros/IRF4 complexes to ZICE motifs within *Haao* E1 sequence and *Setd2* E1 sequence probes. For (**A**) and (**B**), nuclear extracts were prepared from 293T cells transfected with Ikaros, Aiolos or IRF4 expressing vector, respectively. Control IgG or α-Ikaros or α-Aiolos or α-IRF4 antibodies were used in supershift assays. Red asterisk, Ikaros specific band; red bracket, Ikaros/IRF4 complexes; black asterisk, non-specific (NS) band.



Supplemental Figure 8. Schematic representation of gene regulatory network by IRF4 and associated TFs in GC B cell and plasma cell.
(Upper) During IRF4^{lo} status in which CSR occurs, IRF4 directly activates the expression of *Aicda* and *Ezh2* by binding to AICE motifs with Batf, which expression is transiently induced upon differentiation stimuli. Considering that Ezh2 maintains low transcripts of *Irf4* expression in GC B cell, there is a negative feedback between IRF4 and Ezh2. Besides the function of Batf/IRF4, PU.1/IRF4 and Ikaros activate *Ebf1* expression by binding to juxtapose motifs, the EICE and the zinc finger motifs, respectively. Ebf1 also promotes the expression of *Aicda* and *Ezh2* by composing the Ebf1-Pax5-Bach2 axis. Activity of the Batf/IRF4 complex and the Ebf1-Pax5-Bach2 axis resulted in transient high expression of *Ezh2*. Thus, there is another feedback between Ebf1 and IRF4. (Lower) Along differentiation, IRF4 protein amount is increased (IRF4^{hi}). In IRF4^{hi} status, IRF4 binds the ISRE as a homodimer for gene activation. *Prdm1* is one of IRF4 target genes which possess an ISRE motif in the regulatory region, and IRF4 and Blimp-1, encoded by *Prdm1*, compose a positive feedback loop to promote PC differentiation. In contrast, PU.1 and Batf reduce their expression followed by decommission of genes which possess EICE and/or AICE motifs. Meanwhile, the Ikaros/IRF4 complex represses *Ebf1* expression by binding to ZICE motifs in the regulatory regions. Thus, IRF4 and partner TFs cooperatively orchestrate GC B cell and PC differentiation. EICE, Ets-IRF composite elements; AICE, AP-1-IRF composite elements; ISRE, interferon sequence response elements; ZICE, zinc finger-IRF composite elements.

A



B

	EICE	AICE	ZICE	ISRE	Zinc finger
C1	–	Yes	–	–	Yes
C2	Yes	–	–	–	Yes
C3	–	–	Yes	Yes	–
C4	Yes	–	–	Yes	Yes
E1	Yes	–	Yes	–	–

C

Supplemental Figure 9. Comparison of IRF4 ChIP-seq peaks on day 1 and day 3.
(**A**) PU.1 and IRF4 ChIP-seq peaks on the *Ebf1* locus. From upper to bottom, PU.1 ChIP-seq peaks on day 1 and day 3, IRF4 ChIP-seq peaks on day 1 and day 3. (**B**) Motifs detected within IRF4 ChIP-seq peaks on the *Ebf1* locus. EICE, Ets-IRF composite elements; AICE, AP-1-IRF composite elements; ISRE, interferon sequence response elements; ZICE, zinc finger-IRF composite elements. (**C**) IRF4 ChIP-seq peaks on the *Haao* locus. For (**A**) and (**C**), IRF4 and PU.1 ChIP-seq data are from a previous report (Ochiai K. *Immunity* 2013). Blue, PU.1 ChIP-seq; red, IRF4 ChIP-seq. E, enhancer; C, coding.

Supplemental Table 1

Selected IRF4 direct target genes in B1-8^{hi} splenic B cells

	Cluster 1		Cluster 2	Cluster 3	
1	*a*	*Lipg*	***Aicda***	*Ablim1*	*Mbp*
2	*A130010J15Rik*	*Mccc2*	*Kirrel*	*Aff3*	*Mgat5*
3	*A4galt*	*Ncapg2*	*Mfap5*	*Ahnak*	*Ncf1*
4	*Acoxl*	*Ncaph*	*Pmepa1*	*Akap13*	*Nfatc1*
5	*Alcam*	*Nek2*		*Akap2*	*Nfkb2*
6	*Aldh1l2*	*Nmral1*		*Arhgap26*	*Notch2*
7	*Alpl*	*Nt5dc3*		*Arhgef18*	*Pecam1*
8	*Amigo2*	*Ntn1*		*Arhgef3*	***Pik3ap1***
9	*Ankrd55*	*Nxph1*		*B3gnt8*	*Pip4k2a*
10	*Arfgap3*	*Nxph3*		*B430306N03Rik*	*Ptk2*
11	*Arsg*	*Olfml3*		*Bank1*	*Rasgef1b*
12	*Aurkb*	*Plxna1*		*Bcl11a*	*Samd9l*
13	*BC030307*	*Pmepa1*		*Camk1d*	*Sbk1*
14	*Bcat1*	*Pomgnt1*		*Car2*	*Sesn3*
15	*Bcl2l1*	***Prdm1***		*Cblb*	***Setd2***
16	*Bcl2l11*	*Prep*		*Ccr1*	*Slamf1*
17	*Bhlha15*	*Prkci*		*Ccr7*	*Slc44a2*
18	*Bub1*	*Prlr*		*Cd38*	*Socs5*
19	*Cdk5rap3*	*Prr5*		*Cd83*	*Sorl1*
20	*Cenpf*	*Pvalb*		*Cd86*	*Stk17b*
21	*Cenpp*	*Rad54l*		*Clec2i*	*Tgfbr2*
22	*Cpeb3*	*Rcbtb2*		*Cxxc5*	*Tlr1*
23	*Cpox*	*Rhobtb1*		*Dusp10*	*Tlr3*
24	*Cth*	*Sar1b*		*Dusp2*	*Tmem2*
25	*Cyb5r3*	*Sec11c*		***Ebf1***	*Tnfaip3*
26	*D10Wsu102e*	*Sec24a*		*Edaradd*	*Tsc22d1*
27	*Dclre1b*	*Sec62*		*Ell3*	*Usp25*
28	*Dtl*	*Sel1l*		*Emr1*	*Vav2*
29	*Edem2*	*Serf1*		*Filip1l*	
30	*Eif2s2*	*Slc16a6*		*Gcnt1*	
31	***Ell2***	*Slc35b1*		*Gns*	
32	*Evi5*	*Socs2*		*Gpr183*	
33	***Ezh2***	*Spata3*		*Gypc*	
34	*Fndc3b*	*Spcs2*		***Haao***	
35	*Glcci1*	*Ssr2*		*Hes1*	
36	*Gpnmb*	*Tg*		*Hhex*	
37	*Gpr160*	*Tnfrsf17*		*Hivep2*	
38	*Gtf2a1*	*Tpx2*		***Icosl***	
39	*Hipk1*	*Tram2*		*Ier5*	
40	*Hmmr*	*Trpc4ap*		*Ifi203*	
41	*Hsp90b1*	*Txndc11*		*Il10ra*	
42	*Hspa13*	*Txndc5*		*Il12a*	
43	*Ica1*	*Ube3a*		*Inpp5f*	
44	*Igf2bp3*	*Vegfa*		*Itpkb*	
45	*Il6st*	*Wipi1*		*Itpr1*	
46	*Ippk*	*Wisp1*		*Klf2*	
47	*Kdelr3*			*Klf6*	
48	*Kif14*			*Lbh*	
49	*Klhdc4*			*Ly86*	
50	*Kntc1*			*Maf*	

Picked up genes in this study are indicated with each colors.

Supplemental Table 2

IRF4 regulatory motifs surrounding IRF4 boundary regions

Cluster 1 & 2

Peak ID	EICE	AICE	ISRE	Distance (EICE/AICE/ISRE)
Ell2 C1	-	AGTGAGTCATAACAG	CACAAGCTAAAAAA	Around 30 bp
Ezh2 E1	-	CATGACTCATACATA	AAGAAAGGGAACTA	Around 20 bp
Aicda E1	-	GCTGAGTCAAAGATG	-	-
Aicda E2	-	TTTGACTCAGAGGAA	-	-
Aicda E6	-	-	-	-

Cluster 3
(PU.1 coincident in day 3 ChIP-seq)

Peak ID	EICE	AICE	Zinc finger	Distance of Zinc finger from EICE/AICE	Motif in Reporter gene
Ebf1 C1	-	AGGAAGTGACTCAGT	-	-	-
Ebf1 C2	GAGGAACTGAAAAT	-	GGGGCAGCAAG	Around 500 bp	-
Ebf1 C4	CAGGAAGAGAAAAT	-	AGGGAAGGGGG	Around 150 bp	EICE, Zinc finger (257 bp)
IcosL E1	-	GAGGAATATGAGACA	GGGGAACACTG	Around 120 bp	-
IcosL E2	CAGGAAATGAAACT	-	AGAGAAGCAGACAG	Around 110 bp	EICE, Zinc finger (175 bp)
Pik3ap1 C1	AGGGAAGTGAGAAA	-	GGGGGAGGATG	Around 260 bp	-

Cluster 3
(PU.1 non-coincident in day 3 ChIP-seq)

Peak ID	EICE	another ISRE	ZICE	Distance of ZICE from EICE/AICE/ISRE	Motif in Reporter gene
Ebf1 E1	GGGGAAGTGAAAGA	-	GGGGAAGTGAAAGA	Overlap with EICE	ZICE/EICE (32 bp)
Ebf1 C3	-	ATAAAAAGGAAACA	TGGGAACCTGAGACA	Around 180 bp (another ISRE)	ZICE (93 bp)
Haao E1	-	CAGAAACAGAAACA	TGGGAATGAGAAAAC	Around 65 bp (another ISRE)	ZICE (32 bp)
		CAGAAACAGAAATT		Around 75 bp (another ISRE)	
Setd2 E1	-	-	TGGGAGACTGAAACC	-	ZICE (95 bp)

(/) ; overlap motifs
(,) ; motifs exist near within the region
DNA size is indicated within the bracket.

Supplemental Table 3

Subgroups of IRF4 downregulation target genes

	ZICE (-) (lack the ZICE)		ZICE (+) (contain the ZICE)
1	*Ablim1*	*Usp25*	*Akap13*
2	*Aff3*	*Vav2*	*Akap2*
3	*Ahnak*		*Arhgap26*
4	*B3gnt8*		*Arhgef18*
5	*Bank1*		*Arhgef3*
6	*Bcl11a*		*B430306N03Rik*
7	*Camk1d*		*Cblb*
8	*Car2*		*Ccr1*
9	*Cd83*		*Ccr7*
10	*Cd86*		*Cd38*
11	*Clec2i*		***Ebf1***
12	*Cxxc5*		*Edaradd*
13	*Dusp10*		*Gcnt1*
14	*Dusp2*		*Gypc*
15	*Ell3*		***Haao***
16	*Emr1*		*Hhex*
17	*Filip1l*		*Itpr1*
18	*Gns*		*Klf2*
19	*Gpr183*		*Maf*
20	*Hes1*		*Mgat5*
21	*Hivep2*		*Rasgef1b*
22	***Icosl***		*Sesn3*
23	*Ier5*		***Setd2***
24	*Ifi203*		*Socs5*
25	*Il10ra*		*Sorl1*
26	*Il12a*		*Tlr3*
27	*Inpp5f*		
28	*Itpkb*		
29	*Klf6*		
30	*Lbh*		
31	*Ly86*		
32	*Mbp*		
33	*Ncf1*		
34	*Nfatc1*		
35	*Nfkb2*		
36	*Notch2*		
37	*Pecam1*		
38	***Pik3ap1***		
39	*Pip4k2a*		
40	*Ptk2*		
41	*Samd9l*		
42	*Sbk1*		
43	*Slamf1*		
44	*Slc44a2*		
45	*Stk17b*		
46	*Tgfbr2*		
47	*Tlr1*		
48	*Tmem2*		
49	*Tnfaip3*		
50	*Tsc22d1*		

Gene with the regulatory region which contains the EICE.

Picked up genes in this study are indicated with blue.

Supplemental Table 4

Antibodies used in this study

Protein	Host	Catalog number	Source	Usage
Goat IgG	-	sc-2028	Santa Cruz	IP, ChIP, FACS
Rabbit IgG	-	sc-2027	Santa Cruz	ChIP, FACS
IRF4 (M-17)	Goat	sc-6059	Santa Cruz	WB, IP, ChIP, FACS, EMSA
PU.1 (T-21)	Rabbit	sc-352	Santa Cruz	WB, ChIP, FACS
BATF	Rabbit	8638	CellSignaling	WB, ChIP, EMSA
Ikaros (H-100)	Rabbit	sc-13039	Santa Cruz	WB, ChIP
Ikaros	Rabbit	ab26083	CellSignaling	EMSA
Aiolos	Rabbit	APR39004_P050	Aviva	WB
Aiolos	Rabbit	ab139408	abcam	EMSA
p300 (N-15)	Rabbit	sc-584	Santa Cruz	WB
Mi-2b	Rabbit	ab72418	abcam	WB
MTA2	Rabbit	ab8106	abcam	WB
Hdac1	Rabbit	ab7028	abcam	WB
Hdac2	Rabbit	ab7029	abcam	WB
αTubulin (B-7)	Mouse	sc-5286	Santa Cruz	WB
Biotin IgG1 (clone; A85-1)	Rat	553441	BD Pharmingen	FACS
CD138 (clone; 281-2)	Rat	561070	BD Pharmingen	FACS
Streptavidin-PerCP	-	554064	BD Pharmingen	FACS

Supplemental Table 5

Oligo sequences used in this study

RT-PCR		
Irf4	Forward	AGATTCCAGGTGACTCTGTG
	Reverse	CTGCCCTGTCAGAGTATTTC
Prdm1	Forward	CCCTCTGAAGAAACAGAATG
	Reverse	GCTTGTGCTGCTAAATCTCT
Batf	Forward	AAGATTAGAACCATGCCTCA
	Reverse	GAGCTGTTTGATCTCTTTGC
Ebf1	Forward	AACTTCTTCCACTTCGTCCT
	Reverse	GAAATCCTGTTCTGTCCGTA
Bcl6	Forward	GCAGTTTAGAGCCCATAAGA
	Reverse	GTACATGAAGTCCAGGAGGA
Pax5	Forward	CGTGTTTGAGAGACAGCAC
	Reverse	AAGTTGGCTTTCATGTCATC
Bach2	Forward	CGCTGTCGAAAGAGGAAGCTGGAC
	Reverse	CCTGGATCTGCTCTGGACTCTGGA
Ezh2	Forward	TTATGGTGGAAGACGAAACT
	Reverse	ATTCTCTGTCACCATGCACT
Ikzf1	Forward	CACTACCTCTGGAGCACAGC
	Reverse	ATAGGGCATGTCTGACAGGCA
Ikzf3	Forward	TGCGGGTTATCCTGCATTAGC
	Reverse	TTGGCATCGAAGCAGTGCCG
Ell2	Forward	ATGTGAAGCTCACCGAGA
	Reverse	TATCTCTTCCCACGTTTGAC
Aicda	Forward	AGGGAGTCAAGAAAGTCACG
	Reverse	CAGGAGGTGGCACTATCTCT
Haao	Forward	CACATTGAAGAGGGTGAGGA
	Reverse	CTGCCGTATAGGCACGTC
Setd2	Forward	ATTTCTACGATCCCGAGCAC
	Reverse	CACTGGTCCTTTGATGAAACC
IgG1glt	Forward	GGCCCTTCCAGATCTTTGAG
	Reverse	GGATCCAGAGTTCCAGGTCACT
IgG3glt	Forward	TGGGCAAGTGGATCTGAACA
	Reverse	CTCAGGGAAGTAGCCTTTGACA
IgMglt	Forward	CTCTGGCCCTGCTTATTGTTG
	Reverse	GAAGACATTTGGGAAGGACTGACT
IgG1postswitch	Forward	CTCTGGCCCTGCTTATTGTTG
	Reverse	GGATCCAGAGTTCCAGGTCACT
IgG3postswitch	Forward	CTCTGGCCCTGCTTATTGTTG
	Reverse	CTCAGGGAAGTAGCCTTTGACA
β2-microglobulin	Forward	AGACTGATACATACGCCTGCA
	Reverse	GCAGGTTCAAATGAATCTTCAG

ChIP-qPCR		
Prdm1 cns9	Forward	AAGCTGCTAAGTGGGAGAGT
	Reverse	GCATAATTTAGCGTTTGGTG
Prdm1 cns1	Forward	CCCTGACAATGTTTGTCCTA
	Reverse	CGGACAAGAAGAGCAAGTTA
Ell2 C1	Forward	GTTTAGACATCAAGCTGCAA
	Reverse	AATACAATTGACCACTGTTGG
Ezh2 E1	Forward	AAGGGTTCCATCACTTCAC
	Reverse	CTCCTCAGGGAGATTAAGGT
IcosL E1	Forward	GACTGCTTCCTCCTGGTC
	Reverse	AGTAACTGGTTGGCTTCTCC
IcosL E2	Forward	GTTCAGACCTCTTAAGAC
	Reverse	GAGTGCAGTAGATGTTGAG
Haao E1	Forward	ACCATCTGGGAAGTGTTAG
	Reverse	TCTGTTTCTGCTTTACTGATTA
Setd2 E1	Forward	CAAAGGAAAGCTCACCAAT
	Reverse	AAGTTCAAGGTCCACCAAA
Ebf1 C1	Forward	AAAGGCAGGAAGTGACTC
	Reverse	CGTTTAACCTTCACTACCAC
Ebf1 C2	Forward	TTGCTGAGAGTCTTGGAAAT
	Reverse	CCGATCAATCCAGTTAAATC
Ebf1 C3	Forward	CTGATGTGGGAAGCAGAC
	Reverse	GGTAGATGTAGACCTATTTGTGTG
Ebf1 C4	Forward	GTTGTCCAGAGCCAAGATAG
	Reverse	CCCAAATGATGCTGAAAG
Ebf1 E1	Forward	GGCACTCTGAATCAGTCATC
	Reverse	AAAGAGAGCACCATGTAGTCA
Aicda E1	Forward	TTAGTGTCAACAACCACAGC
	Reverse	TTCTGCGAAGTCAACTACAG
Aicda E2	Forward	ACTTTCCCTTGGTGATCTTT
	Reverse	ACTGCTTGATGTCAGCATAA
Aicda -1.5 kbp	Forward	GGCCAAAGTAGGGCAAAGG
	Reverse	AGGTGGTGGGTGGACAAGTC
Aicda 17 kbp	Forward	CAGCTGTATTTGTTTGTTCTTTAGTAATTG
	Reverse	CATCCCGAAACACATATACTCACTTT
Aicda E6	Forward	GTGACATAGCCGAGACTAGC
	Reverse	GAGTATCCTGTTTGGGACCT

shRNA		
sh*Ikzf1*		TTTGTGGAATAACAGGTCCTGG
sh*Ikzf3*		CCCAGACGCTCTGAATGACTAC
sh*Batf*		CAGAGGCCTGGACAAGTAT
sh*Ebf1*		GAGCGAGAATCTCCTTCAAGAC
siRNA		
si*Ikzf1*		CCCUAUGACAGUGCCAACUAUGAGA
si*Ikzf3*		GAGAGCUCUCGUCCUGGACAGAUUA

EMSA and oligonucleotide precipitation assay		
Ebf1 E1		ATCTAAAAGGGGAAGTGAAAGATGGATGACTG
Ebf1 E1 IRFmut		ATCTAAAAGGGGAAGTCGTAGATGGATGACTG
Ebf1 E1 Zinc finger/IRFmut		ATCTAAAACATTGAGTCGTAGATGGATGACTG
Haao E1		TCTGAAGTGGGAATGAGAAAACCTTTGTTCGG
Setd2 E1		ATGGTCGTGGGAGACTGAAACCTAGAGCGGGT
Ezh2 E1 (ISRE)		TGAAAGAAAGGGAACTAATC
Aicda E1 (AICE)		GCCAGGGACATCTTTGACTCAGCCCCCGGCTT
Aicda E2 (AICE)		TGGTGATCTTTTGACTCAGAGGAAGCTGGTAC

Oligo sequence used for reporter plasmid construction		
Ebf1 E1	Forward	ATCTAAAAGGGGAAGTGA
	Reverse	CAGTCATCCATCTTTCAC
Ebf1 C3	Forward	GTTGTCCAGAGCCAAGATAG
	Reverse	CCCAAATGATGCTGAAAG
Ebf1 C4	Forward	GACGCCATCCGAAGGATT
	Reverse	GGTAGATGTAGACCTATTTGTGTG
Haao E1	Forward	GGAAATCTGGGAGGAAGA
	Reverse	AGCTATGAACCCTGTCTT
Icosl E2	Forward	GTTCAGACCTCTTAAGAC
	Reverse	GAGTGCAGTAGATGTTGAG
Setd2 E1	Forward	ATGGTCGTGGGAGACTGA
	Reverse	ACCCGCTCTAGGTTTCAG