SUPPLEMENTARY MATERIAL

Regulation of B cell fate commitment and immunoglobulin V_H gene rearrangements by Ikaros

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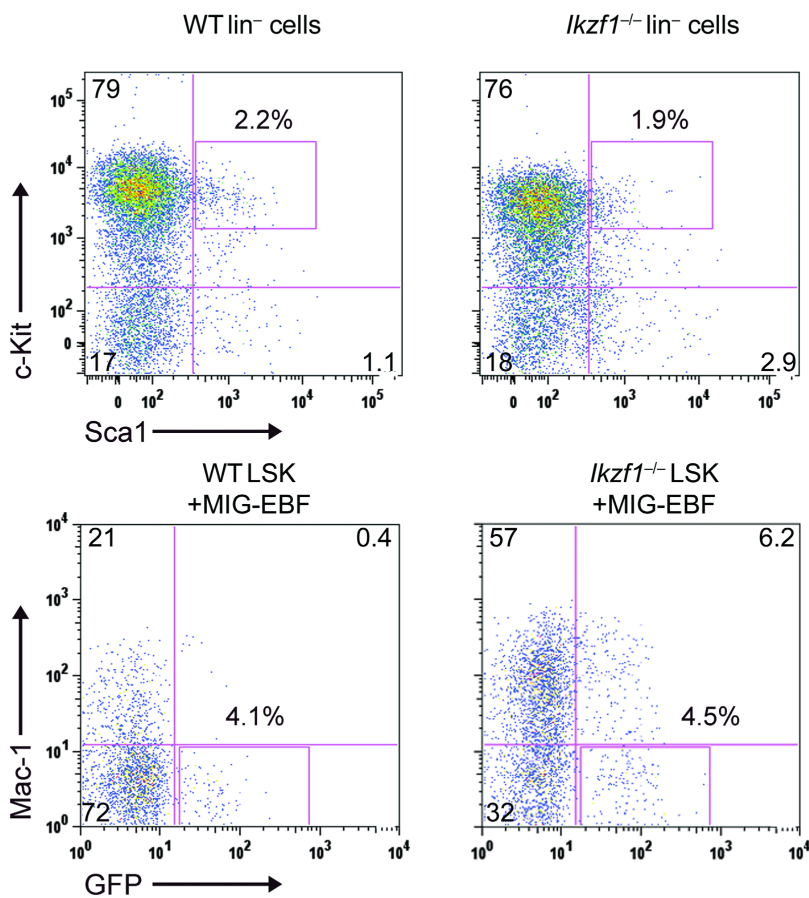
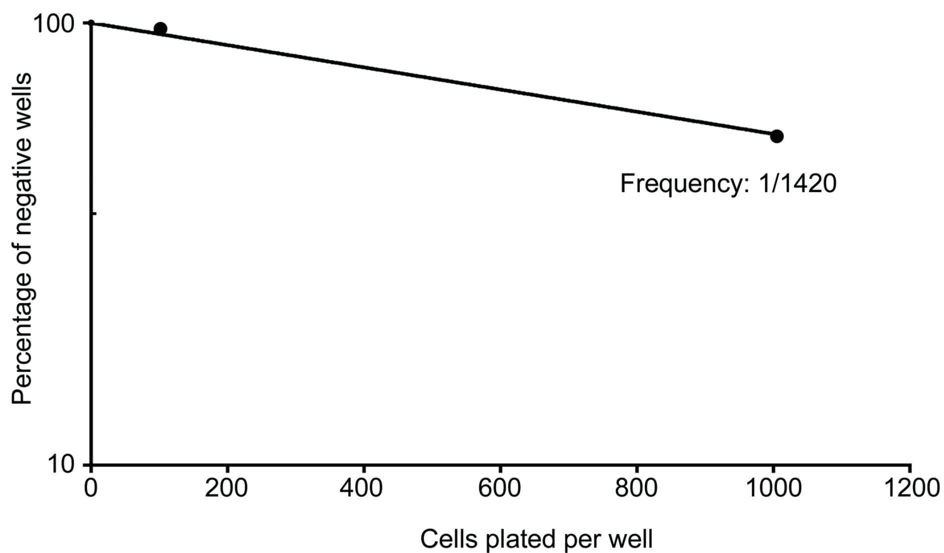
Supplementary Figure 1a-b
Reynaud et al

a



b



Cells plated per well	100	1000
Percentage of $CD19^{+}$ wells	4.2% (4/96)	44% (11/25)

Figure S1. Quantitative analysis of the generation of EBF-rescued *Ikzf1*$^{-/-}$ CD19^{+} cells.

(a) LSK cells were sorted from WT or *Ikzf1*$^{-/-}$ bone marrow mice (top) and directly transduced by spin-infection with MIG-EBF retrovirus. After 72 h of culture, GFP^{+} Mac1^{-} transduced *Ikzf1*$^{-/-}$ cells were sorted directly at the indicated cell numbers onto OP9 stroma cells and cultured in lymphoid conditions (bottom). Cells were harvested after 2 to 4 weeks and analyzed for B lineage (CD19^{+}) precursors. **(b)** The number of EBF-transduced *Ikzf1*$^{-/-}$ LSK cells plated per well is plotted against the percentage of negative wells for growth of CD19^{+} progeny. Solid line represents the best-fit regression used to determine the frequency of generation of CD19^{+} progeny. Data are representative of 2 independent experiments.

Supplementary Figure 2
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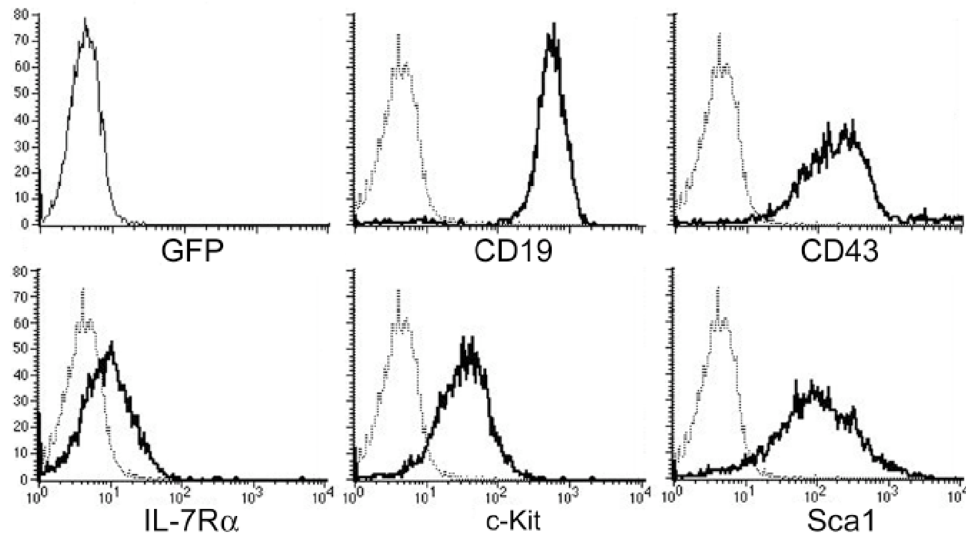

Figure S2. Cell surface phenotype of an EBF-rescued *Ikzf1*$^{-/-}$ clone (type B).

Flow cytometry of an *Ikzf1*$^{-/-}$ CD19^{+} clone (type B) that does not sustain the expression of GFP and the retrovirally encoded *Ebf1* gene. Data are representative of three independently derived clones.

Supplementary Figure 3
Reynaud et al

D_H segments J_H3 segments

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Dsp2.3-----AAGGGATCTACTACTGTG--------TCTACTATGGTTACGAC----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
Dsp2.4-----AAGGGATCTACTACTGTG--------TCTACTATGGTTACGAC----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
Dsp2.5-----AAGGTATCTACTACTGTG--------TCTACTATGGTAACTAC----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
Dsp2.6-----AAGGGATCTACTACTGTG--------CCTACTATGGTTACGAC----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
Dsp2.7-----AAGGGATCTACTACTGTG--------CCTACTATGGTAACTAC----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
Dsp2.8-----AAGGGATCTACTACTGTG--------CCTAGTATGGTAACTAC----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
Dsp2.2-----AAGGGATCTACTACTGTG--------TCTACTATGATTACGAC----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
FL16.1-----AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGCTAC----------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
FL16.2-----AAGGGATCTACTACTGTG--------TTTATTACTACGGCTAC----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
Dq52-------AAGCGGAGCACCACAGTG--------CAACTGGGAC-----------------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
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Spleen cells

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-----------AAGGGATCTACTACTGTG--------TCTATGATGGTTAC-------------------A-------------------------CTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGCAGTAGC-------------CG---------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------CCTACTATAGTAA--------------------AAAG-------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------CCTACTATGGTAACTA-----------------GGTGTTG----------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
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-----------AAGGGATCTACTACTGTG--------TCTACTATGATTACG-----------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTA---------------CCC----------------------TTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------CCTACTATAGTAACTAC----------------GA--------------------TGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGC-------------CT------------------------CTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------CCTACTATAGTAACTAC----------------GATGG-----------------TGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGCAGTAG--------------CCG--------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
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WT CD19⁺ cells

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-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGCTAC----------CACCTGAC-----------------------TACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGCTAC-----------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAG---------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TCTACTATGGTT--------------------CCCCCTTTGAC------------------TACTGGGGCCAAGGGACTCTGG
```

Ikzf1$^{-/-}$ CD19⁺ cells

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-----------AAGGGATCTACTACTGTG--------CCTACTATAGTAAC------------------------------------------CTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTA--------------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG

-----------AAGGGATCTACTACTGTG--------CCTACTATAGTAACTAC---------------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTA--------------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGCTAC-------------------------------------CTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TCTACTATGGTAACTAC-------------------------------------------CTGGTTTGCTTACTGGGGCCAAGGGACTCTGG

-----------AAGGGATCTACTACTGTG--------TCTACTATGGTAACTAC-------------------------------------------CTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TCTACTATGGTAACTAC----------------------------------------------GTTTGCTTACTGGGGCCAAGGGACTCTGG

-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGCTAC----------------------------------------GTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------CCTACTATAGTAACTAC-------------------------------------------CTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTA------------------------------------------------GTTTGCTTACTGGGGCCAAGGGACTCTGG

-----------AAGGGATCTACTACTGTG--------TCTACTATGGTAACTACT--------------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TCTACTATGGTAACTAC----------------------------------------------GTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGC---------------------------------------------TGCTTACTGGGGCCAAGGGACTCTGG

-----------AAGGGATCTACTACTGTG--------TCTACTATGGTAACTAC----------------------------------------------GTTTGCTTACTGGGGCCAAGGGACTCTGG

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-----------AAGGGATCTACTACTGTG--------TCTACTATGATTACGAC---------------------------------------------GGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGCTAC-------------------------------------CTGGTTTGCTTACTGGGGCCAAGGGACTCTGG

-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTA--------------------------------------GCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TCTACTATGGTAAT----------------------------------------------CTGGTTTGCTTACTGGGGCCAAGGGACTCTGG
-----------AAGGGATCTACTACTGTG--------TTTATTACTACGGTAGTAGC------------------------------------------TTTGCTTACTGGGGCCAAGGGACTCTGG
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Figure S3. D-J_H rearrangements in *Ikzf1*$^{-/-}$ pro-B cells are oligoclonal but lack N-region diversity.

DNA sequence analysis of recombined D-J_H regions in *Ikzf1*$^{-/-}$ pro-B cells generated by limiting dilution (see **Supplementary Fig. 1**). D_HJ_H rearrangements were amplified from genomic DNA by PCR. DNA from spleen cells or wild-type pro-B cells were used as controls. Top, germline sequences of 10 D_H segments (GenBank GI:126349412 and GI:23306144).

Supplementary Figure 4
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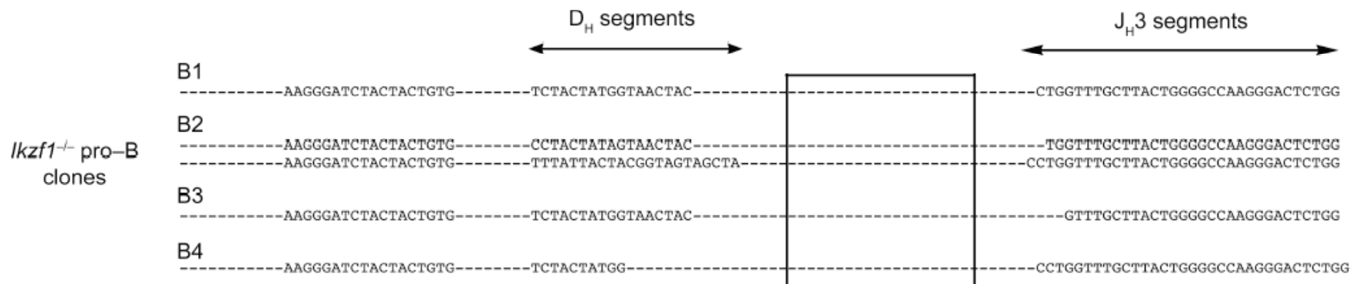

Figure S4. D-J_H rearrangements in *Ikzf1*$^{-/-}$ pro-B clones

DNA sequence analysis of recombined D-J_H regions in *Ikzf1*$^{-/-}$ pro-B clones. Recombined DNA fragments were amplified from genomic DNA by PCR. Note that *Ikzf1*$^{-/-}$ pro-B clones B1, B3, B4 have single D-J_H rearrangements while *Ikzf1*$^{-/-}$ pro-B clone B2 has both alleles rearranged.

Supplementary Figure 5
Reynaud et al

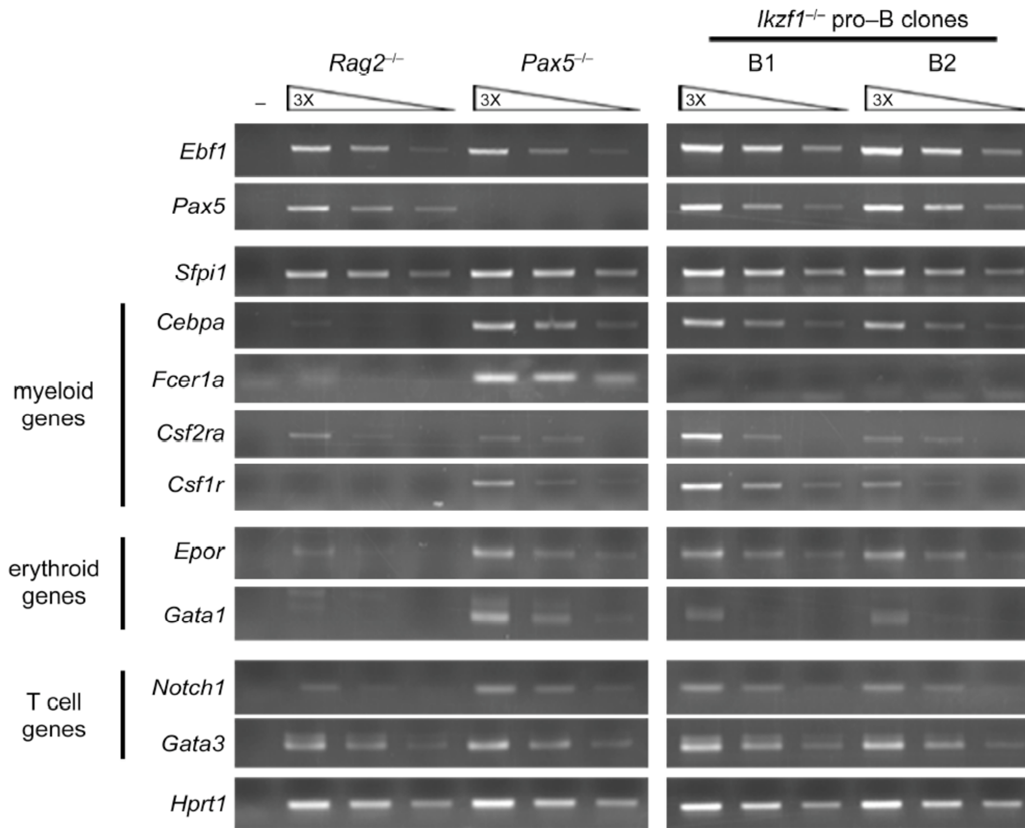

Figure S5. *Ikzf1*$^{-/-}$ pro-B cells mis-express alternate lineage genes.

Analysis of alternate lineage genes in *Ikzf1*$^{-/-}$ pro-B clones. Serial dilutions of cDNA were analyzed by RT-PCR after normalization to *Hprt1*. cDNAs from *Rag2*$^{-/-}$ and *Pax5*$^{-/-}$ pro-B cell lines were used as controls. Data are representative of two independent experiments using 4 independent clones.

Supplementary Figure 6
Reynaud et al

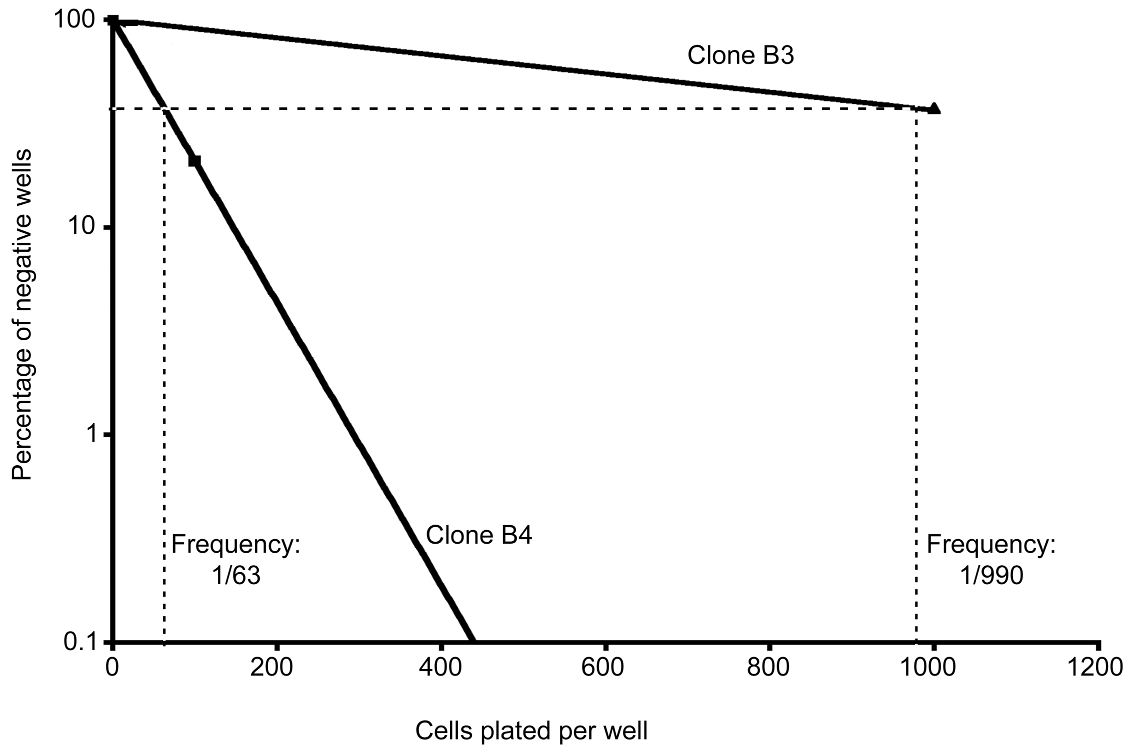

Figure S6. Quantitative assessment of the myeloid potential of *Ikzf1*$^{-/-}$ pro-B clones.

Ikzf1$^{-/-}$ pro-B clones were sorted directly at the indicated cell numbers onto S17 stromal cells in individual wells. Cells were cultured in lymphoid conditions for 3 days and then switched to myeloid conditions. Cells were harvested after 5 to 6 days and analyzed for myeloid (Mac1^{+}) precursors. The number of *Ikzf1*$^{-/-}$ pro-B cells plated per well is plotted against the percentage of negative wells for growth of Mac1^{+} progeny. Solid line represents the best-fit regression and the dashed line intercepts the y-axis at 37%, which indicates the frequency of trans-differentiation. Under these conditions, all the wells plated with 100 *Pax5*$^{-/-}$ pro-B cells scored positive for the generation of Mac-1^{+} progeny (data not shown). Three independent clones were analyzed in this experiment. Data from two clones is shown.

Supplementary Figure 7a-b
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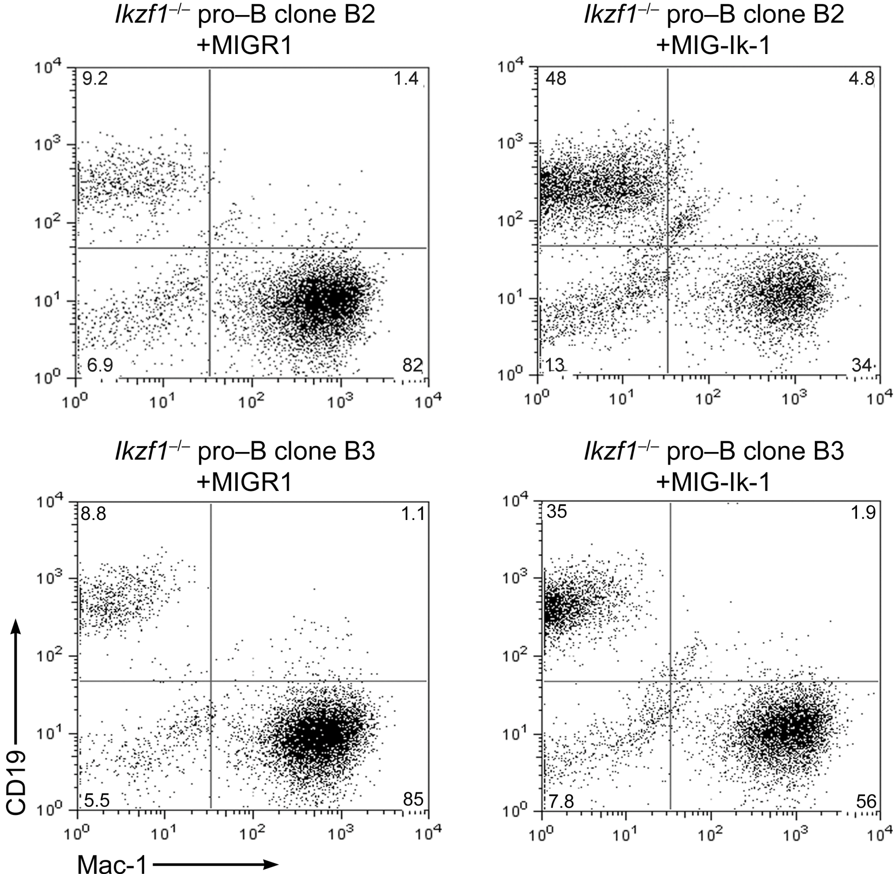
a



b

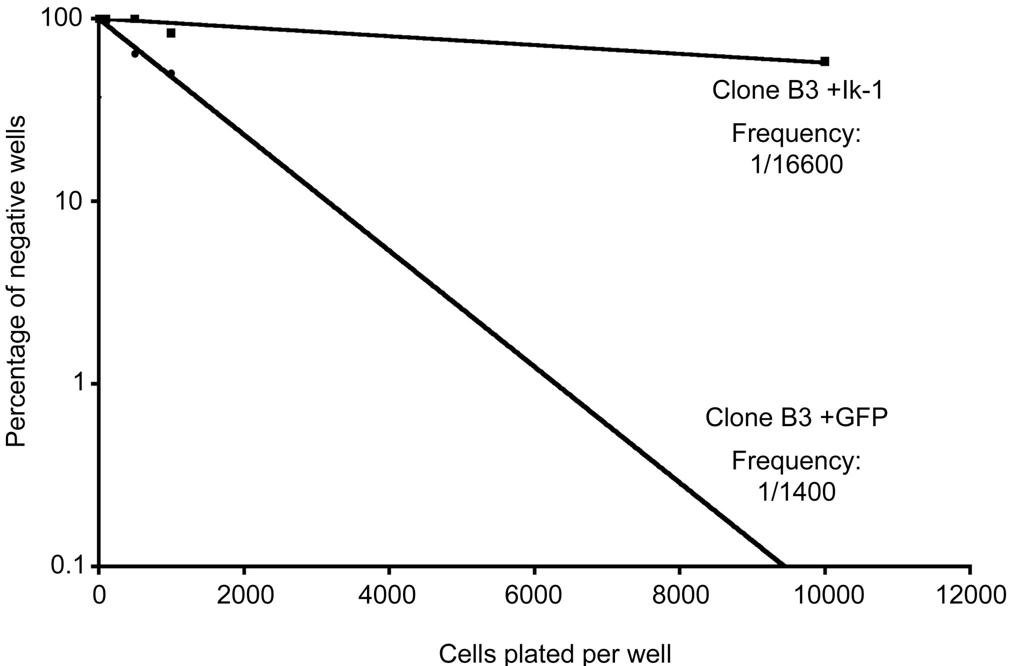


Figure S7. Restoration of Ik-1 expression in *Ikzf1*$^{-/-}$ pro-B cells severely restrained their myeloid potential.

(**a**) *Ikzf1*$^{-/-}$ pro-B cells transduced with MIGR1 or MIG-Ik-1 were cultured as described in **Fig. 2a** and analyzed at day 6 by flow cytometry for the expression of Mac-1 and CD19. **(b)** Transduced *Ikzf1*$^{-/-}$ pro-B cells were sorted directly at the indicated cell numbers onto S17 stromal cells in individual wells and were cultured for 8 days as described in (**a**). The number of transduced *Ikzf1*$^{-/-}$ pro-B cells plated per well is plotted against the percentage of negative wells for growth of Mac1^{+} progeny. Solid line represents the best-fit regression used to determine the frequency of generation of Mac1^{+} progeny. Data are representative of two independent experiments.

Supplementary Figure 8
Reynaud et al

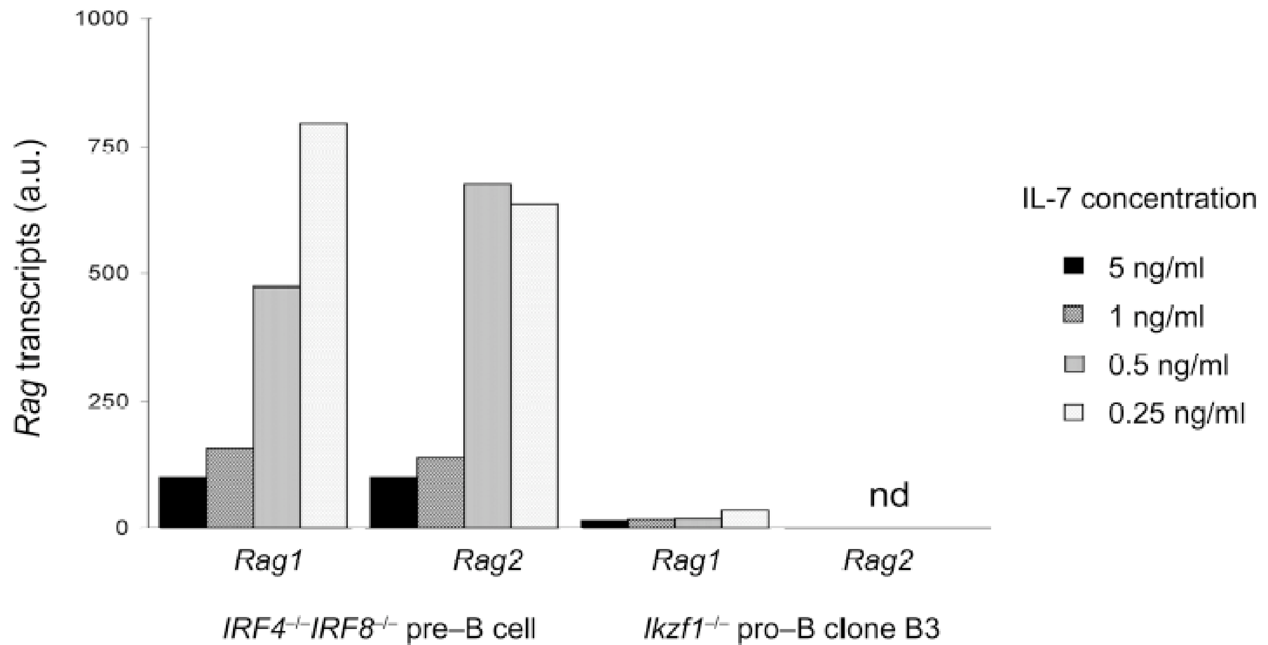


Figure S8. Attenuation of IL-7 signaling does not activate expression of *Rag gene* expression in *Ikzf1*$^{-/-}$ pro-B cells.

Q-PCR analysis of *Rag1* and *Rag2* mRNA expression in *Ikzf1*$^{-/-}$ pro-B or *Irf4*$^{-/-}$*Irf8*$^{-/-}$ pre-B cells cultured in the indicated concentrations of IL-7 (3 days). *Hprt1* transcripts were used for normalization. Data are representative of two independent experiments. n.d. stands for not detectable.

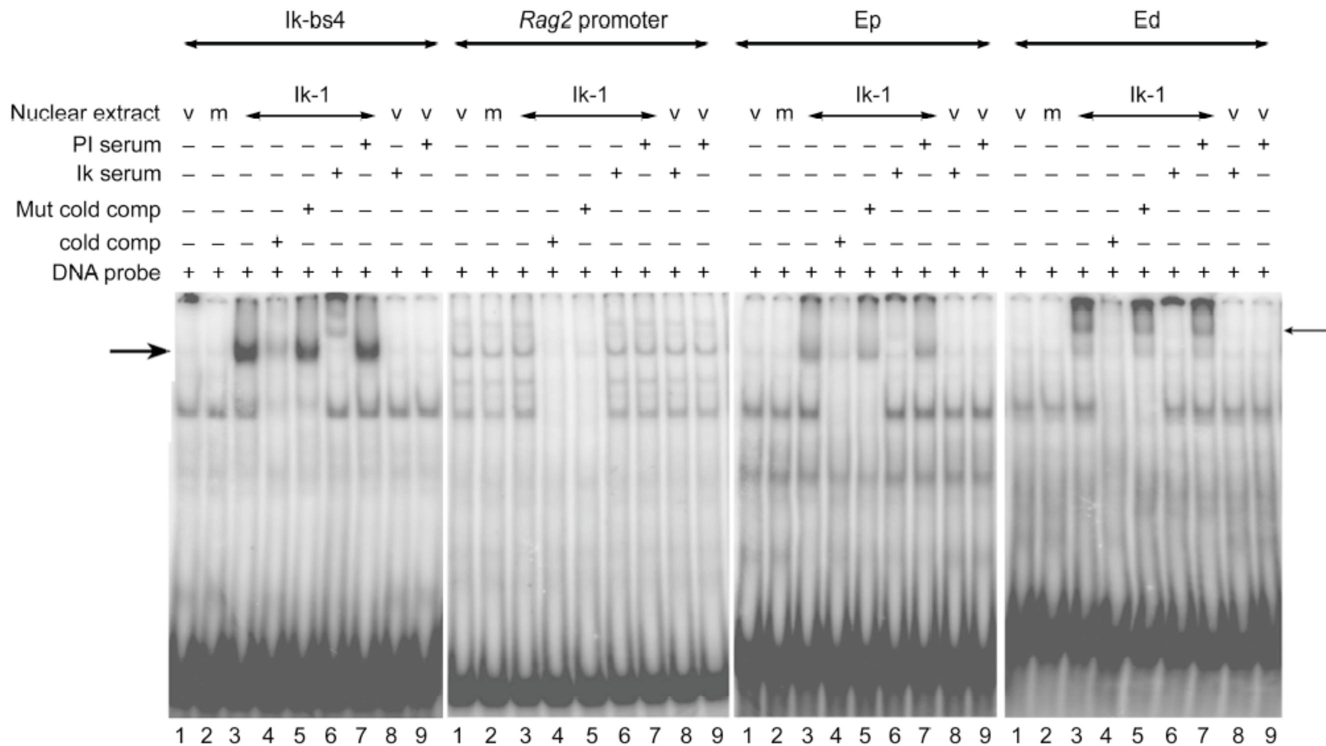


Figure S9. Analysis of Ikaros binding sites in the *Rag* locus by EMSA. EMSAs were performed using nuclear extracts from 293T cells as described in **Fig. 5c**. Control extracts were generated from 293T cells transfected with an empty vector (lane 1) or with Ikaros-159A DNA binding mutant (lane 2). The Ik-bs4 probe contains a high affinity Ikaros binding site and was used as positive control. Other probes spanned putative Ikaros binding sites in *Rag2* promoter as well as Ep and Ed enhancers. Where indicated binding reactions included competitor DNA in 50-fold molar excess or either pre-immune (PI) or anti-Ikaros antiserum[60] (Ik serum). Data are representative of two independent experiments.

Supplementary Figure 10
Reynaud et al

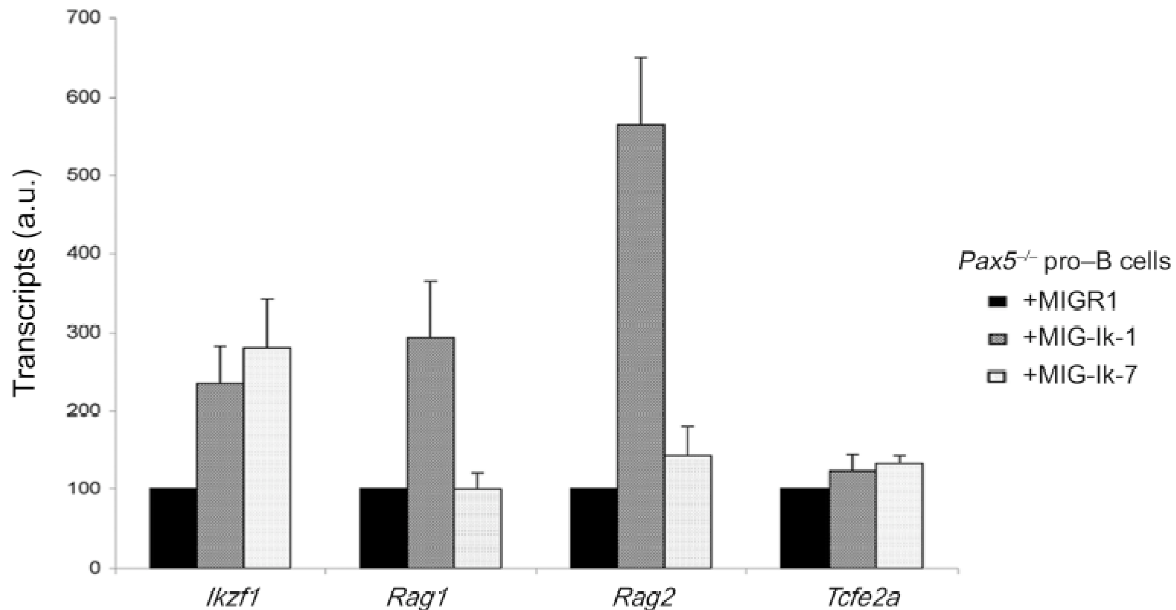


Figure S10. Increased expression of Ikaros in *Pax5*$^{-/-}$ pro-B cells promotes *Rag* gene expression.

Q-PCR analysis of *Ikzf1 (Ikaros)*, *Rag1, Rag2* and *Tcfe2a* (*E2A*) transcripts in *Pax5*$^{-/-}$ pro-B cells transduced with MIGR1, MIG-Ik-1 or MIG-Ik-7 vectors. *Hprt1* transcripts were used for normalization. The relative expression of each gene is expressed as a fold difference in relation to that detected in MIGR1 transduced cells (set to 100). Data are from two independent experiments each involving three samples per condition. Error bars represent the standard deviation from the mean.

Supplementary Figure 11
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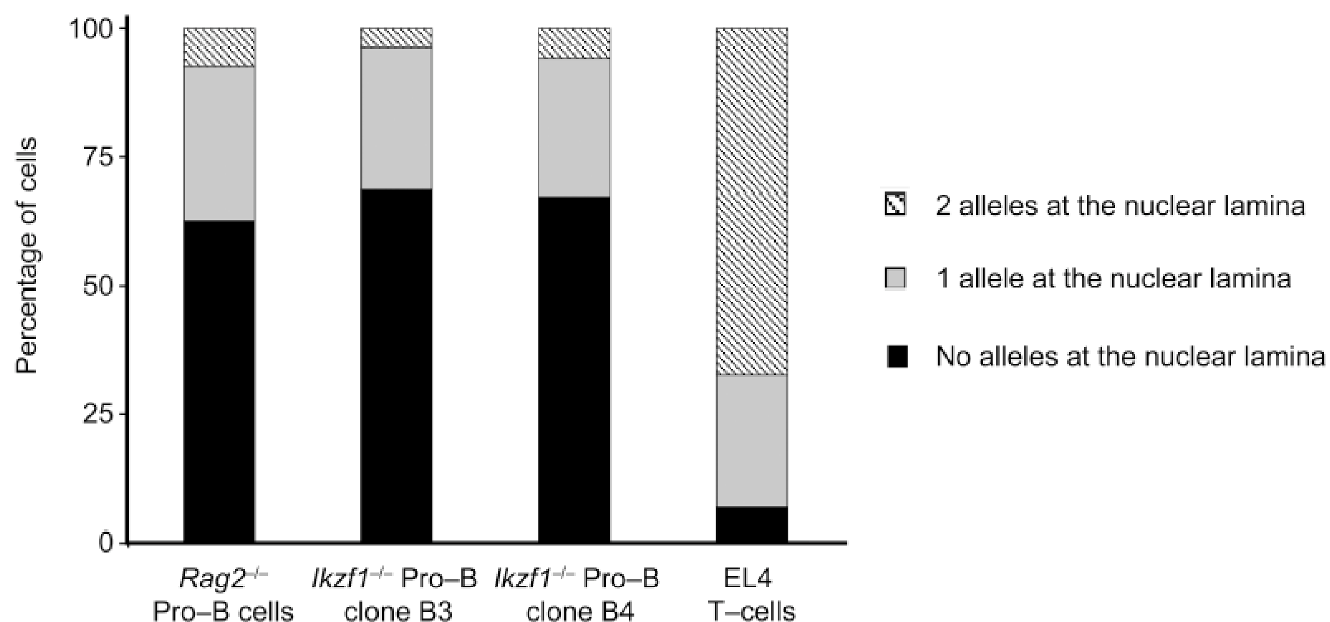

Figure S11. Ikaros is not required for the nuclear repositioning of the *Igh* locus.

Three-dimensional immuno-FISH analysis was performed on the *Igh* locus as described in **Fig. 7**. Signals co-localizing with lamin B1 were scored as peripheral. At least 110 nuclei were scored with each cell type. Graph indicates percentage of cells representing two (stripes), one (grey) or none (black) of their *Igh* alleles at the nuclear lamina.

TABLE 1: Antibodies used in this study

Name	Alternative name	Clone	Fluorochrome	Source
CD19		1d3	PE	Becton Dickinson / Pharmingen
		1d3	APC	Becton Dickinson / Pharmingen
CD11b	Mac-1, Integrin α	M1/70	PE	Becton Dickinson / Pharmingen
CD43	Ly-48, Leukosialin	*s7*	PE	Becton Dickinson / Pharmingen
CD127	IL-7Rα	*SB/199*	PE	Becton Dickinson / Pharmingen
CD117	c-Kit	*2B8*	PE	Becton Dickinson / Pharmingen
		2B8	APC	Becton Dickinson / Pharmingen
Sca1	Ly-6a/e	d7	FITC	Becton Dickinson / Pharmingen
		d7	PE	Becton Dickinson / Pharmingen
CD115	M-CSFR, CSF1R	AFS98	PE	eBioscience

Antibodies used for lineage depletion

Name	Alternative name	Clone	Fluorochrome	Source
CD19		1d3	Biotin	Becton Dickinson / Pharmingen
CD45R	B220	RA3-6B2	Biotin	Becton Dickinson / Pharmingen
IgM		II/43	Biotin	Becton Dickinson / Pharmingen
CD3ε		145-2C11	Biotin	Becton Dickinson / Pharmingen
CD8a	Ly-2	53-6.7	Biotin	Becton Dickinson / Pharmingen
CD11b	Mac-1, Integrin α	M1/70	Biotin	Becton Dickinson / Pharmingen
Gr1	Ly-6C	RB6-8C5	Biotin	Becton Dickinson / Pharmingen
Ter119	Ly-76	TER119	Biotin	Becton Dickinson / Pharmingen

TABLE 2: Primers used for RT-PCR analysis

Gene		Sequence 5'⇨3'
Hprt1	Forward	GTAATGATCAGTCAACGGGGGAC
	Reverse	CCAGCAAGCTTGCAACCTTAACCA
Ikzf1 (Ikaros)	Forward	CACTACCTCTGGAGCACAGC
	Reverse	ATAGGGCATGTCTGACAGGCA
Ikzf3 (Aiolos)	Forward	TGCGGGTTATCCTGCATTAGC
	Reverse	TTGGCATCGAAGCAGTGCCG
endogenous Ebf1	Forward	ACCTTCCTCAATGGCTCAG
	Reverse	TCGTAGCAGAATCCAACCTC
exogenous Ebf1	Forward	ACCTTCCTCAATGGCTCAG
	Reverse	ATTCCTAGCCCATCTGCTG
Pax5	Forward	CGGGTCAGCCATGGTTGTG
	Reverse	GTGCTGTCTCTCAAACACG
Igll1	Forward	CTTGAGGGTCAATGAAGCTCAGAAGA
	Reverse	CTTGGGCTGACCTAGGATTG
VpreB1	Forward	CGTCTGTCCTGCTCATGCTGC
	Reverse	ACGGCACAGTAATACACAGCC
Rag1	Forward	TGCAGACATTCTAGCACTCTGGCC
	Reverse	ACATCTGCCTTCACGTCGATCCGG
Rag2	Forward	CACATCCACAAGCAGGAAGTACAC
	Reverse	TCCCTCGACTATACACCACGTCAA
Dntt (TdT)	Forward	GAAGATGGGAACAACTCGAAGAG
	Reverse	CAGGTGCTGGAACATTCTGGGAG
Csf1r	Forward	GCGATGTGTGAGCAATGGCAGT
	Reverse	AGACCGTTTTGCGTAAGACCTG

Gene		Sequence 5'⇨3'
Ebf1	Forward	CAAGACAAGAACCCTGAAATG
	Reverse	GTAACCTCTGGAAGCCGTAGT
Sfpi1	Forward	CGGATGTGCTTCCCTTATCAAAC
	Reverse	TGACTTTCTTCACCTCGCCTGTC
Cebpa	Forward	GGTGGACAAGAACAGCAACGAGTA
	Reverse	TAGAGATCCAGCGACCCGAAACCA
Fcer1a	Forward	GCATCCAGGATATAGCAGAC
	Reverse	GTGATCTTGTTCTTGCTCCT
Csf2ra	Forward	GAGGTCACAAGGTCAAGGTG
	Reverse	GATTGACAGTGGCAGGCTTC
Epor	Forward	CGGACACATCGAGTTTTGTG
	Reverse	TCATAGGTGCTCCAGGAGGT
Gata1	Forward	ACTTTCCCAGTCCTTTCTTC
	Reverse	CAGGTAGTGACCTGTCCTGT
Notch1	Forward	GGTATGCAAGGAGTCTGAAG
	Reverse	CACACTCATTGATGTCAACC
Gata3	Forward	CTGTCAGACCACCACCAC
	Reverse	CGATTTGCTAGACATCTTCC
Germ-line transcripts		
V_HJ558	Forward	TAACCATGGRATGGAGCTG
	Reverse	TTTCTGACACWCTCAGGATGTG
V_H7183	Forward	CGGTACCAAGAASAMCCTGTWCCTGCAAATGASC
	Reverse	GTCTCTCCGCGCCCCCTGCTGGTCC
V_HQ52	Forward	AGCTATGGTGTACAC
	Reverse	TATGAAAGCTGCATT
Iμ	Forward	ACCTGGGAATGTATGGTTGTGGCTT
	Reverse	ATGCAGATCTCTGTTTTTGCCTCC
μ0	Forward	AACATCTGAGTTTCTGAGGCTTGG
	Reverse	TCATCTGAACCTTCAAGGATGCTC

TABLE 3: Primers used for Q-PCR

Gene		Sequence 5'⇨3'
Hprt1	Forward	CAACAACAAACTTGTCTGGA
	Reverse	ACCTCTCGAAGTGTTGGATA
Ikzf1 (Ikaros)	Forward	GAAGACCTGTGCAAGATAGG
	Reverse	TCCTTCTCATAGTTGGCACT
Rag1	Forward	CTGCAGACATTCTAGCACTC
	Reverse	AACTGAAGCTCAGGGTAGAC
Rag2	Forward	CCTTCAGTGCCAAAATAAGA
	Reverse	TCATAAGTGAGAAGCCTGGT
Tcfe2a (E2A)	Forward	CCAGTCTTTTGCATAACCAT
	Reverse	AGGTCCTTCTTGTCCTCTTC
Csf1r	Forward	TACTGCTGTTGCTGCTCTT
	Reverse	GCTATTGCCTTCGTATCTCT

TABLE 4: Primers used for analyzing *Igh* DNA rearrangements

Gene		Sequence 5'⇨3'
Igh locus	Forward	
	VHJ558-5'	CGAGCTCTCCARCACAGCCTWCATGCARCTCARC
	VHQ52-5'	CGGTACCAGACTGARCATCASCAAGGAC
	VH7183-5'	CGGTACCAAGAASAMCCTGTWCCTGCAAATGASC
	DHL-5'	GGAATTCGMTTTTTGTSAAGGGATCTACTACTGTG
	Reverse	
	JH3-3'	GTCTAGATTCTCACAAGAGTCCGATAGACCCTGG
Acta1 (α-actin)	Forward	GGCATCGTGTTGGATTCTG
	Reverse	CACGAAGGAATAGCCACGC

TABLE 5: Primers used for ChIP analysis

Gene		Sequence 5'⇨3'
γ-satellite	Forward	TATGGCGAGGAAAACTGAAA
	Reverse	TTCACGTCCTAAAGTGTGTAT
Rag1 promoter	Forward	AGCCAGGTGCAGCTGGAGCTGGG
	Reverse	CAACATATGCTGTCTACTCTCTC
Rag2 promoter	Forward	GAAGGAGGTCACAGTCAGTT
	Reverse	CAGGGTAGAGTGTTTGTGTG
Proximal Enhancer *Rag* locus	Forward	CCTAACTGTAGATCGCATCC
	Reverse	AGGTGAATTGAGAACTGGAG
Distal Enhancer *Rag* locus	Forward	GTCACTTGGAAACCACGTGGTTA
	Reverse	TAGTGCACATGCTGCTGCTTATA
Erag *Rag* locus	Forward	TATTCAGGAGGGAATTAAATGAC
	Reverse	GACAGAACCCGAGGGCTTAGCAT
Acta1 (*α-actin*)	Forward	AGAGTCAGAGCAGCAGGTAG
	Reverse	CAAGGCTCAATAGCTTTCTT
Actb (*β-actin*)	Forward	CGCCATGGATGACGATATCG
	Reverse	CGAAGCCGGCTTTGCACATG

TABLE 6: Oligonucleotide probes used for EMSA

Probe		Sequence
Control		
Ik-bs4 (wild type)	Forward	TGACAGGGAATACACATTCCCAAAAGC
	Reverse	GCTTTTGGGAATGTGTATTCCCTGTCA
mutant	Forward	TGACAtttAATACACATTaaaAAAAGC
	Reverse	GCTTTTtttAATGTGTATTaaaTGTCA
Rag1 promoter		
wild type	Forward	TCTGTGGTGTGGGAGAGTGGGAAGGGCAGTGAG
	Reverse	CTCACTGCCCTTCCCACTCTCCCACACCACAGA
mutant	Forward	TCTGTGGTGTtttAGAGTtttAAGGGCAGTGAG
	Reverse	CTCACTGCCCTTaaaACTCTaaaACACCACAGA
Rag2 promoter		
wild type	Forward	GGTCACAGTCAGTTACTCCCGTTACACCAGCACA
	Reverse	TGTGCTGGTGTAACGGGAGTAACTGACTGTGACC
mutant	Forward	GGTCACAGTCAGTTACTaaaGTTACACCAGCACA
	Reverse	TGTGCTGGTGTAACtttAGTAACTGACTGTGACC
Rag proximal enhancer		
Ep (wild type)	Forward	TTTCTACAGCTTCCCTAAGCATTAGCTCCCACTACCCGAG
	Reverse	CTCGGGTAGTGGGAGCTAATGCTTAGGGAAGCTGTAGAAA
mutant	Forward	TTTCTACAGCTTaaaTAAGCATTAGCTaaaACTACCCGAG
	Reverse	CTCGGGTAGTtttAGCTAATGCTTAtttAAGCTGTAGAAA
Rag distal enhancer		
Ed (wild type)	Forward	CGAGAGATGGGAGAAAAATCCCCTCGCCTCCCAGAAGTCC
	Reverse	GGACTTCTGGGAGGCGAGGGGATTTTTCTCCCATCTCTCG
mutant	Forward	CGAGAGATtttAGAAAAATaaaCTCGCCTaaaAGAAGTCC
	Reverse	GGACTTCTtttAGGCGAGtttATTTTTCTaaaATCTCTCG
Rag Erag		
Erag (wild type)	Forward	TGCTCGACTGAAATTCCTCTCCTGCTTGGAAATGGGTAAGAGG
	Reverse	CCTCTTACCCATTTCCAAGCAGGAGAGGAATTTCAGTCGAGCA
mutant 1	Forward	TGCTCGACTGAAATTaaTCTCCTGCTTttAAATGGGTAAGAGG
	Reverse	CCTCTTACCCATTTaaAAGCAGGAGAttAATTTCAGTCGAGCA
mutant 2	Forward	TGCTCGACTGAAATTaaTCTaaTGCTTttAAATtttTAAGAGG
	Reverse	CCTCTTAaaaATTTaaAAGCAttAGAttAATTTCAGTCGAGCA

Ikaros binding motifs are indicated in red

Mutant Ikaros binding motifs are indicated in lower case