SUPPLEMENTAL MATERIAL

Ubieta et al., https://doi.org/10.1084/jem.20160514

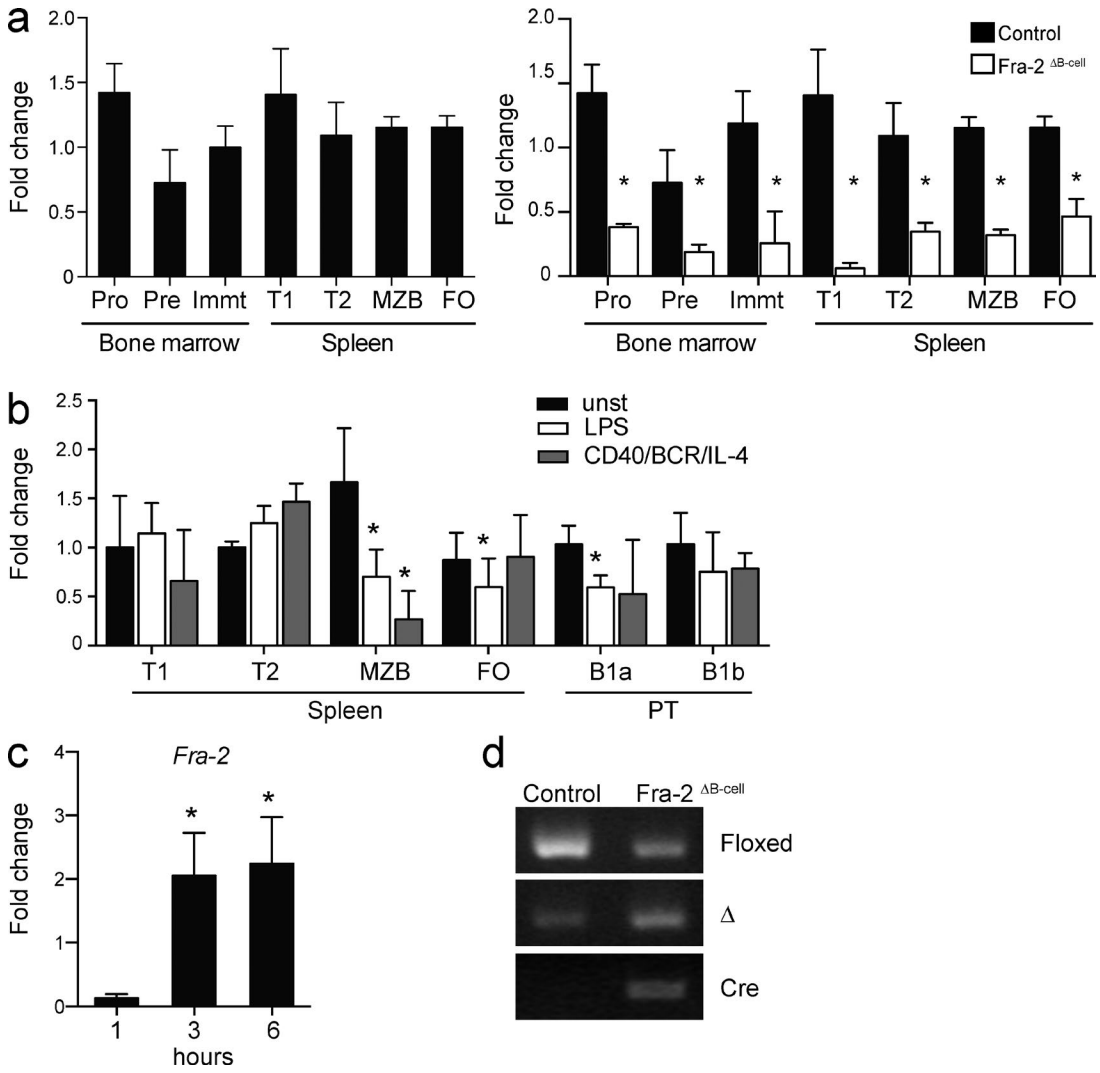

Figure S1. **Expression of *Fra-2* in B cell lineage.** (a) Real-time PCR analyses of *Fra-2* mRNA abundance in bone marrow B cell subsets (pro–, pre–, and immature [immt] B cells) and B cell subsets isolated from spleen (T1, T2, MZB, and FO B cells) of Fra-$2^{\Delta B\ cell}$ and littermate control mice. Relative expression to housekeeping genes (n = 5–6). (b) Real-time PCR analyses of *Fra-2* mRNA levels in unstimulated or in 6 h LPS, LPS+IL4, or CD40/BCR/IL4 stimulated splenic T1, T2, MZB, and FO and peritoneal B1 littermate control B cells. (c) Real-time PCR analyses of *Fra-2* mRNA levels in pro–B cells after 3 and 6 h of IL-7 stimulation (n = 5). Different B cell subsets were sorted by flow cytometry. Relative expression to housekeeping genes. (d) Genotyping PCR of MACS-sorted $B220^+$ cells from bone marrow of WT C57BL/6 mice. Bars represent mean values ± SD. Data show one representative out of three independent experiments. Statistical analyses were performed using Student's t test; *, $P < 0.05$. PT, peritoneal cavity; Unst, unstimulated.

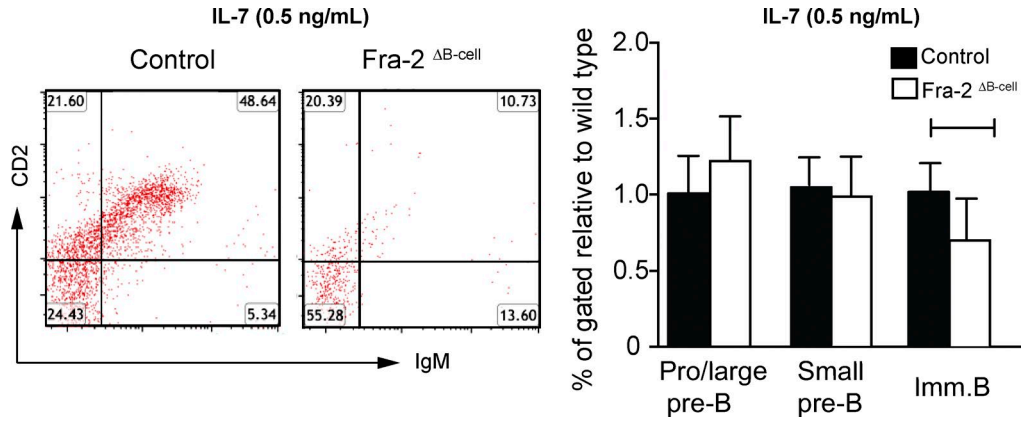

Figure S2. **In vitro IL-7 stimulations of B cells from Fra-2$^{\Delta B\ cell}$ and littermate control mice.** Flow cytometry analysis of pro/large pre–B cells, small pre–B cells, and immature B cells after 5 d of stimulation with IL-7 (0.05 and 5 ng/ml). Bars represent mean values ± SD (n = 5). Data show one representative out of three independent experiments. Statistical analyses were performed using Student's t test.

a

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Back-grounds	STD (Bg STD)	Best Match/Details	Motif File
1		1e-136	-3.143e+02	84.41%	21.13%	13.9bp (126.5bp)	PB0182.1_Srf_2/Jaspar(0.837) More Information | Similar Motifs Found	motif file (matrix)
2		1e-41	-9563e+0l	35.29%	8.66%	42.8bp (110.7bp)	PH0163.1_Six3/Jaspar(0.610) More Information | Similar Motifs Found	motif file (matrix)
3		1e-35	-8.174e+0l	4.71%	0.02%	33.6bp (27.3bp)	PB0098.1_Zfp410_1/Jaspar(0.635) More Information | Similar Motifs Found	motif file (matrix)
4		1e-35	-8.139e+0l	42.35%	14.32%	54.5bp (108.4bp)	TEAD2(TEA)/Py2T-Tead2-ChIP-Seq(GSE55709)/Homer(0.580) More Information | Similar Motifs Found	motif file (matrix)
5		1e-32	-7.418e+0l	45.88%	17.72%	58.7bp (107.9bp)	SD0002.1_at_AC_acceptor/Jaspar(0.677) More Information | Similar Motifs Found	motif file (matrix)
6		1e-29	-6.812e+0l	27.94%	7.35%	48.0bp (111.9bp)	MA0146.2_Zfx/Jaspar(0.632) More Information | Similar Motifs Found	motif file (matrix)
7		1e-27	-6290e+0l	26.76%	7.24%	59.3 bp (110.3bp)	PB0181.1_Spdef_2/Jaspar(0573) More Information | Similar Motifs Found	motif file (matrix)
8		1e-23	-5291e+0l	18.24%	3.93%	62.7bp (113.6bp)	Pitx1(Homeobox)/Chicken-Pitx1-ChIP-Seq(GSE38910)/Homer(0.706) More Information | Similar Motifs Found	motif file (matrix)
9		1e-20	-4.767e+0l	2.65%	0.01%	27.3bp (38.9bp)	MA0130.1_ZNF354C/Jaspar(0.645) More Information | Similar Motifs Found	motif file (matrix)
10		1e-20	-4.767e+0l	2.65%	0.01%	9.4bp (24.0bp)	MA0130.1_ZNF354C/Jaspar(0.696) More Information | Similar Motifs Found	motif file (matrix)
11		1e-20	-4.704e+01	30.29%	11.32%	55.1bp (106.0bp)	PH0037.1_Hdx/Jaspar(0.623) More Information | Similar Motifs Found	motif file (matrix)
12		1e-20	-4.617e+0l	28.82%	10.51%	45.8bp (109.5bp)	ZFX(Zf)/mES-Zfx-ChIP-Seq(GSE11431)/Homer(0.817) More Information | Similar Motifs Found	motif file (matrix)
13		1e-16	-3.739e+0l	21.76%	7.43%	47.2bp (103.3bp)	PH0122.1_Obox2/Jaspar(0.643) More Information | Similar Motifs Found	motif file (matrix)
14		1e-14	-3.352e+0l	7.65%	1.00%	60.7bp (105.4bp)	MA0072.1_RORA_2/Jaspar(0.652) More Information | Similar Motifs Found	motif file (matrix)
15		1e-13	-3.o79e+0l	2.06%	0.01%	31.5bp (28.1bp)	MA0029.1_Mecom/Jaspar(0.686) More Information | Similar Motifs Found	motif file (matrix)
16		1e-12	-2.879e+0l	2.35%	0.03%	57.5bp (33.1bp)	PH0085.1_Irx4/Jaspar(0.679) More Information | Similar Motifs Found	motif file (matrix)
17*		1e-11	-2.759e+0l	1.47%	0.00%	2.4bp (31.0bp)	RUNXl(Runt)/Jurkat-RUNX1-ChIP-Seq (GSE29180)/Homer(0.699) More Information | Similar Motifs Found	motif file (matrix)
18*		1e-11	-2.618e+0l	14.41%	4.69%	61.9bp (103.8bp)	MA0151.1_ARID3A/Jaspar(0.806) More Information | Similar Motifs Found	motif file (matrix)
19*		1e-10	-2.448e+0l	2.65%	0.08%	47.0bp (58.4bp)	PB0147.1_Max_2/Jaspar(0597) More Information | Similar Motifs Found	motif file (matrix)
20*		1e-10	-2.402e+01	7.35%	1.41%	58.5bp (90.2 bp)	PB0119.1_Foxa2_2/Jaspar(0.831) More Information | Similar Motifs Found	motif file (matrix)

Motif 17

RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer
Match rank: 1
Score: 0.70
Offset: -2
Orientation: reverse strand
Alignment: --GGTGGTTTTGAT
NNTGTGGTTT----

PB0035.1_Irf5_1/Jaspar
Match rank: 2
Score: 0.67
Offset: -1
Orientation: reverse strand
Alignment: GGTGGTTTTGAT----
-NTGGTTTCGGTTNNN

PB0034.1_Irf4_1/Jaspar
Match rank: 3
Score: 0.67
Offset: -0
Orientation: reverse strand
Alignment: GGTGGTTTTGAT---
TNTGGTTTCGATACN

b

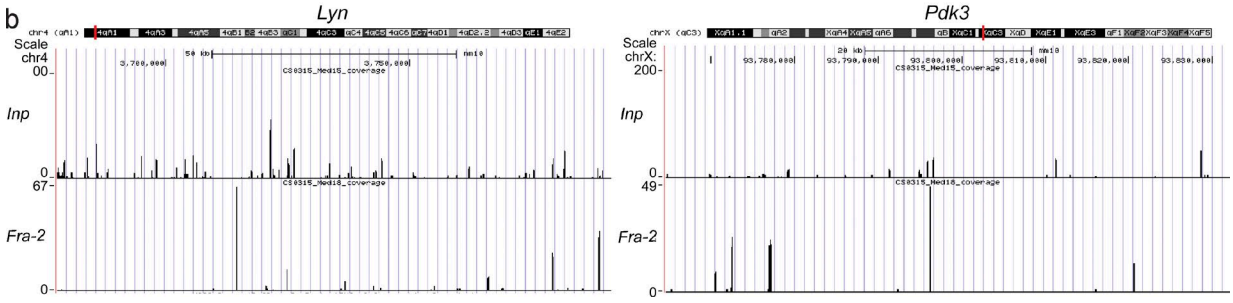

Figure S3. **Fra-2 recognition sequence in B220$^+$ bone marrow B cells.** (a) Consensus Fra-2 recognition sequence in B220$^+$ bone marrow B cells of WT mice identified by ChIP-seq analyses. (b) ChIP-seq analysis of the binding of Fra-2 at *Lyn* and *pdk3* promoters (n = 3) performed in B220$^+$ B cells from control mice.

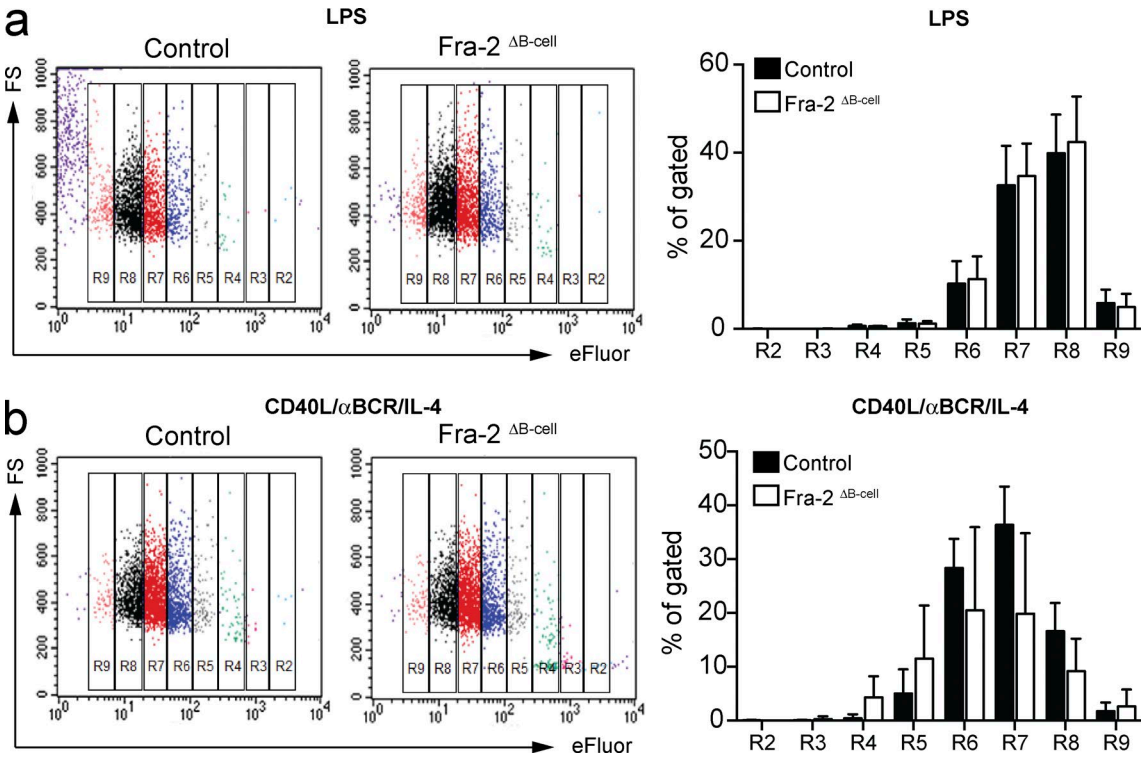

Figure S4. **In vitro LPS or CD40/BCR/IL-4 stimulations of Fra-2$^{\Delta B\ cell}$ and littermate control B cells.** (a and b) Flow cytometry analysis of the proliferation rate in Fra-2$^{\Delta B\ cell}$ and littermate control pro–B cells after 3 d of LPS or CD40/BCR/IL-4 stimulation. Bars represent mean values ± SD (n = 3). Data show one representative out of three independent experiments.

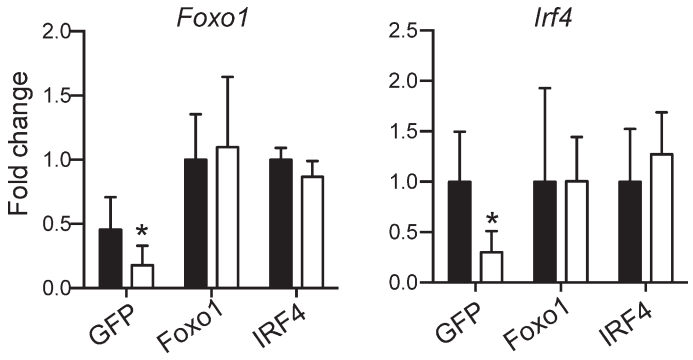

Figure S5. **Rescue expression of B cell genes.** Real-time PCR analysis of *Foxo1* and *Irf4* genes of pro–B cell culture after infection with IRES-GFP, IRES-Foxo1-GFP, and IRES-Irf4-GFP expressing retrovirus and after stimulation with IL-7 for 5 d (n = 5). Bars represent mean values ± SD (n = 5). Data show one representative out of three independent experiments. Statistical analyses were performed using Student's t test; *, P < 0.05.