SUPPLEMENTAL MATERIAL

Carotta et al., http://www.jem.org/cgi/content/full/jem.20140425/DC1

Table S1. Cellularity of splenic B cell populations

Mice	Total B cells (×10^7)	Follicular B cells (×10^7)	MZ B cells (×10^6)	Plasmablasts (×10^5)	Plasma cells (×10^5)
+/+	4.1 ± 0.5	3.0 ± 0.3	7.5 ± 0.8	2.1 ± 0.5	1.3 ± 0.2
PU.1cKO	3.1 ± 0.3	2.0 ± 0.2	5.8 ± 0.3	1.4 ± 0.2	1.1 ± 0.1
Irf8$^{-/-}$	5.0 ± 0.6	3.0 ± 0.5	9.4 ± 1.0	2.5 ± 0.3	2.9 ± 0.5**
I*rf8*$^{-/-}$PU.1cKO	3.4 ± 0.2	1.5 ± 0.1*	9.2 ± 0.9	5.3 ± 0.5***	2.2 ± 0.2***
Double cKO					
Cre$^-$ control	3.6 ± 0.7	2.4 ± 0.4	2.1 ± 0.4	0.6 ± 0.1	0.9 ± 0.1
IRF8/PU.1cKO	2.8 ± 0.3	1.9 ± 0.2	2.4 ± 0.6	1.0 ± 0.4	0.6 ± 0.2

Quantitation of the total B cell numbers (B220$^+$CD19$^+$), follicular B cells (B220$^+$IgM^{low}CD23$^+$CD21$^-$), marginal zone B cells (B220$^+$IgM$^+$CD21$^+$CD23$^-$), CD138$^+$BLIMP-1/GFP^{low} plasmablasts, and CD138$^+$BLIMP-1/GFP^{high} plasma cells in the spleen of mice of the indicated genotypes. All strains also carried the BLIMP-1/GFP reporter allele (*Prdm1*$^{gfp/+}$). While CD23 expression was reduced in the absence of PU.1, its expression was sufficient to allow the identification of the splenic B cell subsets. +/+, *n* = 6-10; PU.1cKO (*Spi1*$^{fl/fl}$*Cd19*cre$^+$), *n* = 3-5; *Irf8*$^{-/-}$, *n* = 6-11; *Irf8*$^{-/-}$PU.1cKO (*Irf8*$^{-/-}$*Spi1*$^{fl/fl}$Cd19cre$^+$), *n* = 7-12; Cre$^-$ control (*Irf8*$^{fl/fl}$*Spi1*$^{fl/fl}$), *n* = 5; IRF8/PU.1cKO (*Irf8*$^{fl/fl}$*Spi1*$^{fl/fl}$*Cd19*cre$^+$), *n* = 5. Note that for PU.1cKO, *Irf8*$^{-/-}$, and *Irf8*$^{-/-}$PU.1cKO samples, p-values are compared with +/+ (WT) genotype. *, P < 0.05; **, P < 0.01; ***, P < 0.005. No significant differences were observed between the Cre$^-$ control and IRF8/PU.1cKO experimental genotypes.

Table S2. List of differentially expressed genes during B cell activation

Gene name	Accession number	WT resting	WT activated	Irf8$^{-/-}$PU.1cKO resting	Irf8$^{-/-}$PU.1cKO activated
Receptors and ligands					
Cd93	NM_010740	1.259707398	0.795268362	4.637269009	3.205114922
Slamf7	NM_144539	0.598381596	4.12944978	2.939016275	3.950778747
Emb	NM_010330	−1.789422095	−0.362286564	0.400213495	2.086673454
Cd44	NM_009851	−1.259984499	2.629519278	0.204452809	1.521354779
Cd96	NM_032465	−6.11847247	0.419348986	−4.246774795	−0.186202288
Thy1	NM_009382	−5.11712077	4.040087017	−2.845768871	5.702438599
Cxcr7	NM_007722	1.600983653	−0.165149338	4.235008345	2.804256407
Cd74	NM_001042605	12.89784053	11.22919252	10.49775728	10.5124966
Fcer2a	NM_013517	9.584434217	8.443089282	6.215436937	7.535802918
H2-Aa	NM_010378	9.445776565	8.088121149	7.385963109	7.520062651
CD83	NM_009856	6.133647535	8.08663373	3.638218587	7.297267847
Il21r	NM_021887	5.583468192	4.710625837	3.084167494	4.027923695
Il6ra	NM_010559	3.780972889	2.198529693	−0.345316746	1.08782722
Cd68	NM_001291058.1	1.954141875	1.712217186	−0.108741605	1.191589961
Tnfrsf22	NM_023680	2.111944126	1.801293462	−0.653933359	0.816455751
Ccl22	NM_009137	0.366571765	6.144398545	−1.820611061	6.837005668
Ccl4	NM_013652	−3.128845856	1.079015685	−30.08448567	−0.554496746
Ceacam1	NM_001039185	1.705601355	−1.636935349	1.50094168	−0.869046284
Clcf1	NM_019952	6.418225736	4.453179831	5.37220023	4.518878112
Cxcl16	NM_023158	−1.070319498	−3.445563718	−1.842110709	−3.39826873
Il10	NM_010548	−1.530346034	−5.029849055	−1.927451805	−0.455095958
Il12a	NM_008351	3.415678145	−1.328660243	4.048121358	1.085959402
Il16	NM_010551	4.453805418	2.532329693	3.640468164	2.831112526
Il18	NM_008360	0.101754553	−3.030520014	−0.070822283	−30.08448567
Il24	NM_053095	0.307227245	−2.571951861	−2.516907557	−4.72544163
Il6	NM_031168	−5.122407533	−1.861604808	−4.260315475	−2.405810232

Table S2. List of differentially expressed genes during B cell activation *(Continued)*

Gene name	Accession number	WT resting	WT activated	Irf8$^{-/-}$PU.1cKO resting	Irf8$^{-/-}$PU.1cKO activated
Ccr6	NM_009835	0.958647576	−6.030896893	−3.255526752	−4.72544163
Ccr7	NM_007719	2.737902181	−2.329785807	0.802924113	−0.592867952
Ccr9	NM_009913	−0.734878465	−2.859687919	−2.82111141	−0.868057879
Cd27	NM_001033126	0.246626999	−3.706975321	−0.880916204	−4.139671435
Cd28	NM_007642	−2.94141608	−0.708916781	−3.042064153	2.237950318
Cd97	NM_011925	4.830758198	0.477733974	3.517407445	0.848089255
Csf1r	NM_001037859	−0.471944932	−5.031231019	−1.826000505	−1.151345459
Csf2rb	NM_007780	3.524707713	1.606971207	1.68531542	0.884730736
Csf3r	NM_007782	−1.413157519	−3.708926451	−0.743758396	−2.271471588
ctla-4	NM_009843	−3.798925271	−0.365356508	−3.509239891	0.074494404
Cxcr3	NM_009910	−0.210229647	−4.446588882	0.485211161	−1.409579297
Cxcr4	NM_009911	7.175418465	4.229065678	5.912270355	5.217295604
Tgfbr1	NM_009370	−3.801409403	−1.275817346	−1.337670857	−2.138775228
Tgfbr2	NM_009371	7.521965072	5.459155497	6.265126373	4.99497142
Tnfrsf10b	NM_020275	−5.11712077	−0.600288139	−3.848025714	0.059776106
Tnfrsf12a	NM_013749	0.020175585	−4.446588882	−2.264563194	−3.726826716
Tnfrsf19	NM_013869	0.15326604	−2.223701132	3.460117177	0.092564522
Tnfrsf4	NM_011659	−0.423142364	−2.571370595	−1.943776178	−2.141333604
Tnfrsf8	NM_009401	−4.538629027	−0.176914765	−30.08448567	1.868715623
Lifr	NM_013584	−5.122407533	1.030905435	−30.08448567	−1.823493981
Kit	NM_021099	−0.887282999	−4.030532923	0.823247323	−0.069549722
Il10ra	NM_008348	2.983648554	0.884195095	1.291936324	−0.689931374
Il10rb	NM_008349	3.228071942	0.969021141	1.657541857	0.646258313
Il11ra1	NM_010549	−0.483819304	−2.860893382	−0.736561328	−1.921301329
Il12rb1	NM_008353	−0.590804838	2.005911832	−1.671411669	0.535913856
Il17ra	NM_008359	3.151869412	0.760105123	1.202457581	1.021339483
Il1r2	NM_010555	−3.532158195	0.964269227	−1.943776178	1.008747577
Il2ra	NM_008367	−2.313992829	0.775057852	2.284543771	1.396496332
Il2rg	NM_013563	7.682820252	5.158518055	5.870970058	5.227926165
Il7r	NM_008372	−0.770487498	−2.709177919	4.844085197	1.581068489
Dll1	NM_007865	−0.628747057	−3.708926451	−2.247394311	−1.721294231
Fgf22	NM_023304	−1.220230051	−6.030191133	−1.361579062	−2.131084171
Pdgfa	NM_008808	−3.798925271	1.207414427	−30.08448567	−0.638848245
Itga4	NM_010576	8.487618937	5.176095017	7.463659785	5.326458703
Itgb1	NM_010578	−1.032825354	1.764147397	1.513752424	1.889095819
Il6st	NM_010560	−4.538629027	−1.571055217	−1.5441286	−1.918830647
Igf2r	NM_010515	−6.11847247	−0.278535707	−1.00819623	−0.13612195
Gpr77	NM_176912	0.260370738	−3.223480858	−0.227539425	−1.208111913
Fzd6	NM_008056	−5.122407533	−0.900979005	−0.889563409	−0.171655301
Fzd7	NM_008057	−2.413451923	−0.362968745	−1.385379428	−1.721294231
Flt3	NM_010229	−0.599065431	−3.44620119	−2.035580584	−4.139671435
Emr1	NM_010130	−3.796437698	−0.744085732	−3.834775574	−4.138155498
Gabbr1	NM_019439	2.832177045	0.931990926	0.269960052	0.219824263
Bmpr1a	NM_009758	−3.541904055	−0.905064397	−30.08448567	−1.47858185
Btla	NM_177584	4.699348185	1.903125893	3.177206112	1.370379771
Ccr1	NM_009912	1.170954383	−2.122796107	−3.242729719	−4.141185976
Ccr2	NM_009915	0.100706869	−4.029205995	0.369848433	0.730154358
Wnt10a	NM_009518	−2.948349699	−0.537328915	0.904919733	0.799867169
Wnt10b	NM_011718	−2.52996853	−0.298350269	−1.142845909	1.330402267

Table S2. List of differentially expressed genes during B cell activation *(Continued)*

Gene name	Accession number	WT resting	WT activated	Irf8$^{-/-}$PU.1cKO resting	Irf8$^{-/-}$PU.1cKO activated
Tnfsf11	NM_011613	−4.124727426	−1.507642863	0.262542001	−0.473812113
Tnfsf14	NM_019418	−4.538629027	0.397091791	−2.648069805	0.671843345
Pros1	NM_011173	−5.122407533	0.596087262	−30.08448567	−0.403288748
Il5ra	NM_008370	0.534632553	1.986366378	−1.746336516	1.282700044
Icosl	NM_015790	7.688967201	2.761291979	5.495995967	3.321716625
Bst1	NM_009763	−1.258294245	−5.031231019	1.819218176	−1.339615456
Transcriptional regulators					
Bcl6	NM_009744	5.553898313	3.228418563	2.549263969	2.700817609
Pax5	NM_008782	6.085941034	4.054124764	5.095039628	3.967129908
Mef2c	NM_025282	3.170078357	2.851629576	1.014747275	2.423184534
Aicda	NM_009645	0.424313294	4.900229111	1.02009979	6.30162122
Irf4	NM_013674	2.665080518	3.309192739	3.065980503	3.916905605
Irf8	NM_008320	−1.653956927	−4.445912351	1.401690996	1.691764804
Prdm1	NM_007548	0.249320613	−1.076920418	0.242063646	2.51421476
Nfil3	NM_017373	1.215427345	7.277184258	−1.746336516	6.61385256
Batf	NM_016767	1.736680678	4.227563566	0.592295357	3.921115916
Xbp1	NM_013842	5.362902922	7.550464228	3.975475128	7.928213468
Sox4	NM_009238	3.298776599	0.994961468	6.21833439	4.036668009
Myb	NM_010848	−0.661911003	0.895271078	4.269701519	2.4445918
Lef1	NM_010703	−0.705798151	−1.706554756	2.033009305	0.226689638
Tcf7	NM_009331	−0.796665398	−0.817221896	1.726183352	1.495001707
Bcl11a	NM_016707	5.886693746	5.090083477	3.521758681	4.394233444
Zbtb7b	NM_009565	4.480083801	2.324342559	1.789005511	1.441250397
Lmo2	NM_008505	2.617983624	2.037770877	0.505659429	1.215851788
Zbtb48	NM_133879	0.186154396	−30.08448567	−2.648069805	−4.139671435
Ets2	NM_011809	1.572617921	−2.029430353	1.534798087	−0.590121081
Fos	NM_010234	7.811420229	−2.859084934	6.712035204	−2.550476318
Fosb	NM_008036	9.474180041	−2.031033499	7.655898467	−2.554528674
Foxo1	NM_019739	7.187982505	5.18328744	6.463019978	5.836153283
Gfi1b	NM_008114	−4.119660039	−0.990891958	−1.375598199	0.007113758
Hes1	NM_008235	3.504751816	−0.077774442	2.348176726	0.496589594
Hhex	NM_008245	4.232012987	1.494900069	3.57093457	1.841744654
Maf	NM_001025577	2.739899252	-0.86296144	2.674751812	−0.34884868
Bach2	NM_001109661.1	1.09848062	−0.574700228	1.899343608	−1.268557163
Relb	NM_009046	2.549673006	0.03843273	1.814941414	0.217379014
Jun	NM_010591	8.211433611	5.279818261	7.853651732	5.411019762
Junb	NM_008416	8.460879584	4.509828998	7.157839431	4.620782612
Runx1	NM_009821	3.398674434	0.955886937	2.477684052	1.092492334
notch1	NM_008714	−2.040714989	−1.639406901	−0.513249863	−1.266376878
Suv39h1	NM_011514	−0.166441028	1.835629543	0.444968677	1.800063888
Dnmt3a	NM_007872	−2.663250506	0.41388583	−0.498477477	0.411027823
Stat5a	NM_001164062	−3.126506181	−0.29968653	−1.197453335	−0.592867952
Stat6	NM_009284	4.460731856	2.521703926	3.058217094	2.578015534
Notch2	NM_010928	8.005821984	5.243239854	7.233387082	4.193567525
Jmjd3	NM_001017426	1.532140368	−4.444558452	−2.505014769	−4.72544163
Ezh2	NM_007971	−0.830972816	3.204695415	2.554405343	3.807426406
Rel	NM_009044	1.349068241	−0.569553446	−1.676598775	−1.726082169
Ciita	NM_007575	−0.603195742	1.647704227	0.678087067	0.79393869
Others					
Slpi	NM_011414	−3.110053284	3.119037671	−2.041105027	1.386302031

Table S2. List of differentially expressed genes during B cell activation *(Continued)*

Gene name	Accession number	WT resting	WT activated	Irf8$^{-/-}$PU.1cKO resting	Irf8$^{-/-}$PU.1cKO activated
Mpo	NM_010824	−2.117120552	−4.446588882	5.004929218	0.809018697
Dntt	NM_009345	−30.08448567	−30.08448567	1.503158136	−4.141185976
Blnk	NM_008528	4.620807943	1.898639387	2.478145775	2.22904732
Bcl2l1	NM_009743	−1.421961118	2.430882365	2.512553306	2.406567823
Bcl2a1a	NM_009742	0.346883963	2.735513128	−2.684339465	0.009358924
Bcl2a1d	NM_007536	1.938119932	4.675836749	−0.915440756	1.625532308
Rag1	NM_009019	−0.911483131	−30.08448567	7.590651976	4.427746899
Rag2	NM_009020	−3.128845856	−6.030896893	3.889571637	1.525762216
Bcl3	NM_033601	2.440743948	−0.865785808	−2.370267327	−0.258273337
Angptl4	NM_020581	−4.124727426	−1.570568174	−0.965604351	−1.256551282
Tyro3	NM_019392	−5.122407533	−0.572126884	−30.08448567	0.216687261
Lrp5	NM_008513	−5.122407533	−1.445520682	−1.735747513	−1.47858185
Lrp6	NM_008514	1.262724199	−0.674998757	−0.644424536	−0.05215615
Lrp8	NM_001080926.1	−2.305531897	2.434673345	−1.518680801	2.668635244
Grk5	NM_018869	−0.132093016	−2.330908922	−3.016987084	−2.261061094
Cish	NM_009895	−1.246450229	6.591340484	−0.460176388	6.483513038
Socs1	NM_009896	1.295193094	5.468476969	1.03738715	5.060335006
Socs2	NM_007706	−1.168683431	6.382392528	1.940343218	6.396330407
Socs3	NM_007707	4.078596937	4.763934938	1.94818377	3.795615542

Listed are normalized mean (log_2) reads per kilobase per million (RPKM) of selected genes that are either differentially expressed (>2-fold change, 1% false discovery rate) upon B cell activation or between WT and *Irf8*$^{-/-}$PU.1cKO (*Irf8*$^{-/-}$*Spi1*$^{fl/fl}$ *Cd19*cre^{+}) B cells. Data are the mean of two independent experiments.

Table S3. Primers used for quantitative real-time PCR

Primers	Sequence
Pax5 forward	5′-CCTACCCTATTGTCACAGGCC-3′
Pax5 reverse	5′-CCTCTGTCTGTCTCAGGGGTT-3′
Mef2c forward	5′-AGATCTGACATCCGGTGCAG-3′
Mef2c reverse	5′-TCTTGTTCAGGTTACCAGGTG-3′
Bcl6 forward	5′-GCCGGACACCAGTTTTAAAG-3′
Bcl6 reverse	5′-GGAGGCGATTAAGGTTGAGAAG-3′
Hmbs forward	5′-GACCTGGTTGTTCACTCCCTGAAG-3′
Hmbs reverse	5′-GACAACAGCATCACAAGGGTTTTC-3′

Table S4. Primers used for quantitative ChIP PCR

Primers	Sequence
Pax5 forward	5′-TGTAAGGGGATCCTGGTTGA-3′
Pax5 reverse	5′-GGAACCAGAACTGGGAGGA-3′
Mef2c forward	5′-CACACTCAGCCTGCTCTACT-3′
Mef2c reverse	5′-CGCGAACAATAAAAGGTGTA-3′
Igk3′ forward	5′-TGATCAAGAAGACCCTTTGAGGAAC-3′
Igk3′ reverse	5′-GGTAGGGAGCAGGTGTATGAGGCTT-3′
Bcl6 forward	5′-ATATTCCGCTTTTCCTCCTGCTAA-3′
Bcl6 reverse	5′-CCAGCCACCCTGAGTTTACA-3′
Prdm1 promoter forward	5′-GCATGAGAGGCAGGGCAACA-3′
Prdm1 promoter reverse	5′-CCTGCTAACTGAATACATTCAG-3′
Prdm1 3′ forward	5′-ACCGTTGAAAGACGGTGACT-3′
Prdm1 3′ reverse	5′-AATGGCCCACATCATCTGTG-3′