Supplemental Figure Legends

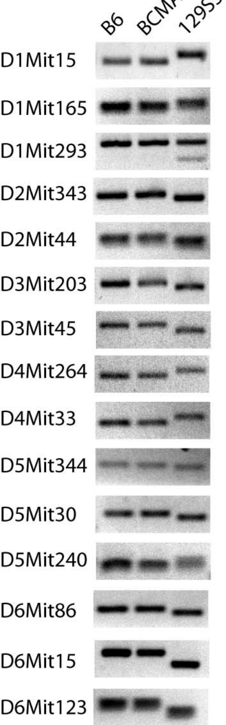
Supplemental Figure 1. Representative markers distributed across chromosomes 1-6 confirm B6 genetic background of BCMA$^{-/-}$ mice. Genotyping was performed using a panel of polymorphic microsatellite markers distributed across the entire genome to confirm B6 genetic background of BCMA$^{-/-}$ mice (Supplemental Table I). The PCR products of all markers were separated on 4% agarose gels. Markers are representative of 5 mice for each genotype.

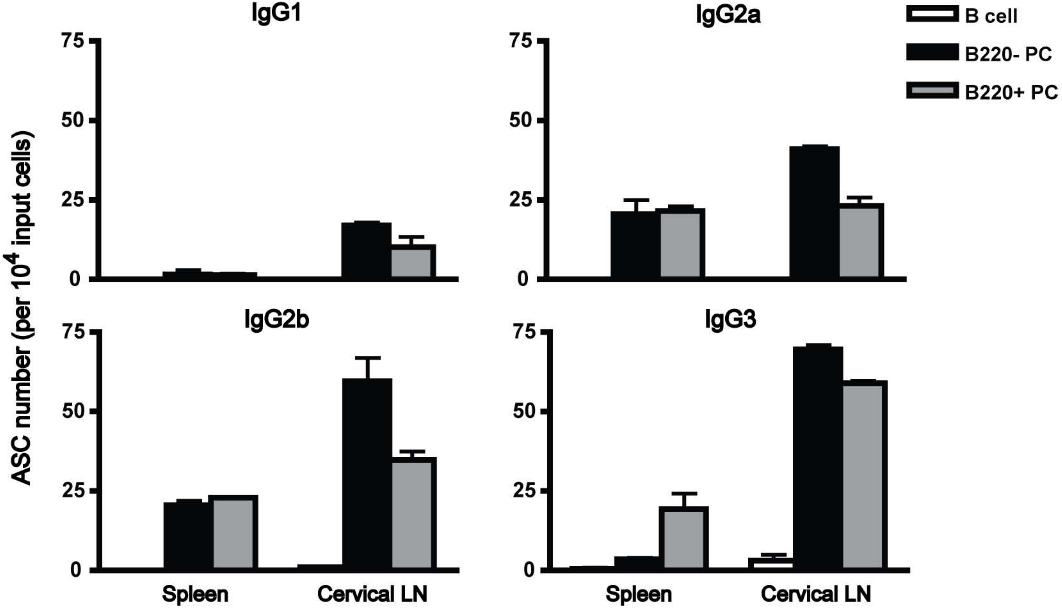
Supplemental Figure 2. IgG-secreting PCs from BCMA$^{-/-}$ *lpr* mice produce mainly IgG$_{2a}$, IgG$_{2b}$, and IgG$_3$. Sort-purified B cells (B220$^+$ CD138$^-$) and PCs (B220$^{+/-}$ CD138$^+$) from spleens and cervical LNs of 4-mo-old BCMA$^{-/-}$ *lpr* mice were analyzed for secretion of the indicated IgG isotype by ELISPOT. Data are expressed and the mean ± SEM from 6 mice.

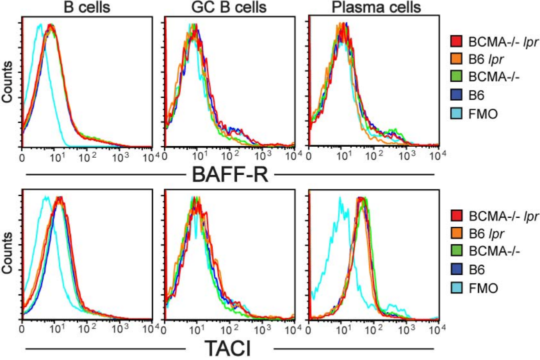
Supplemental Figure 3. Levels of membrane BAFF-R and TACI expressed on B cells, GC B cells, and PCs are equivalent among all mouse strains. Representative FACS plots demonstrate equivalent membrane expression of BAFF-R and TACI on splenic B cells (B220$^+$ CD138$^-$ CD3$^-$), GC B cells (B220$^+$ GL7$^+$ IgD$^-$ CD3$^-$), and total PCs (CD138$^+$ IgD$^-$ CD3$^-$) among all mouse strains. FMO, fluorescence-minus-one control. Data are representative of >6 4-mo old mice for each genotype.

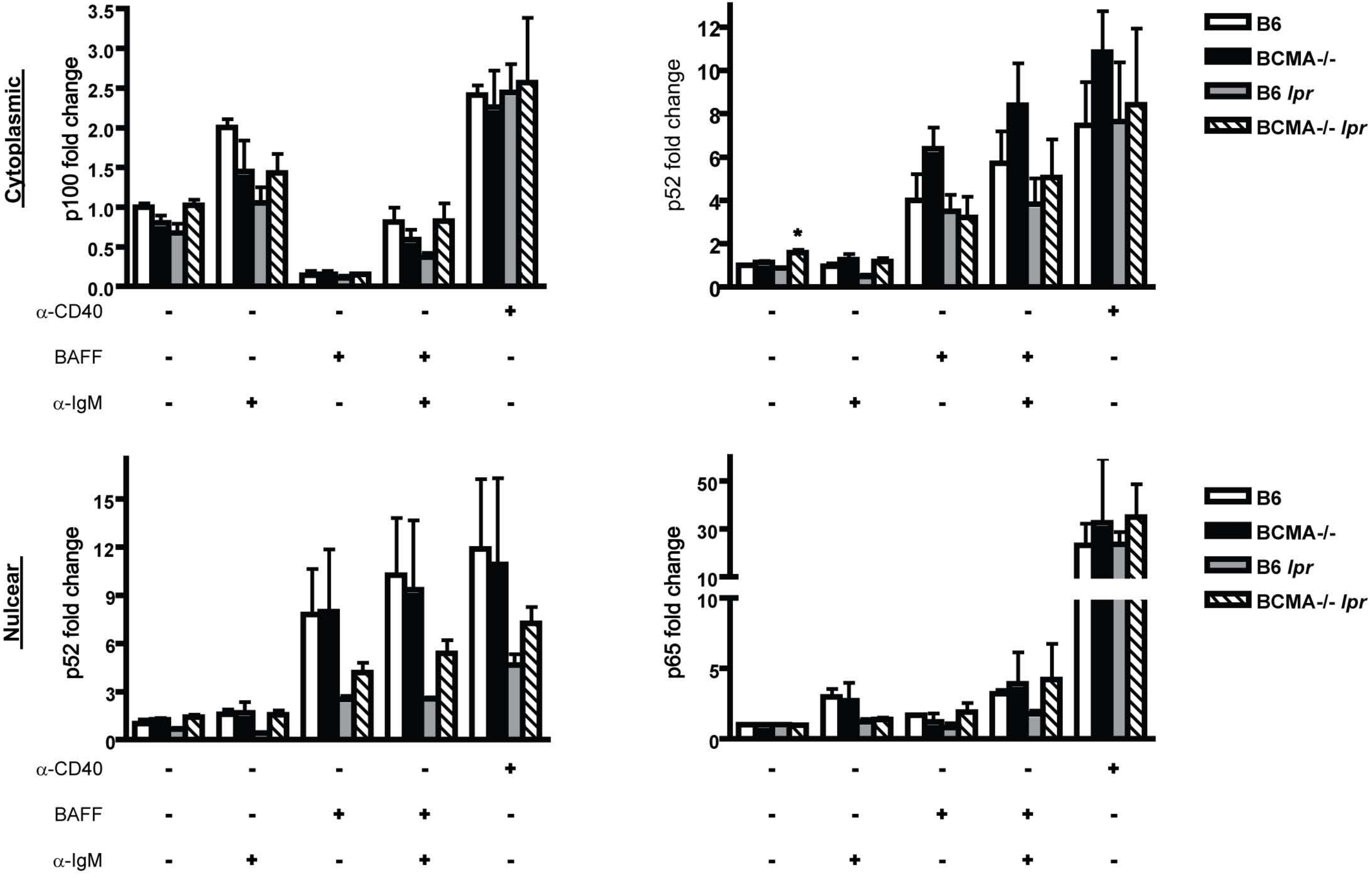
Supplemental Figure 4. Quantified protein levels of NF-κB transcription factors after BCR and BAFF stimulation. Immunoblot analysis of cytoplasmic extracts prepared from 24 hr stimulated cells was performed using antibodies against p100, p52, and β-actin. Nuclear extracts were probed with antibodies recognizing p52, p65, and HDAC1. Densitometry values of p100 and p52 within cytoplasmic extracts relative to β-actin, and p52 within nuclear extracts relative to HDAC1, in each sample were determined. Shown is the mean ± SEM fold change expression of p100 and p52 within stimulated B cells calculated relative to steady-state, nonstimulated levels in B cells from B6 mice. Fold changes of p65 with nuclear extracts of stimulated B cells of each genotype were calculated relative to the steady-state, nonstimulated amount of same genotype. n = 3 4-mo old mice per group from 3 individual experiments.

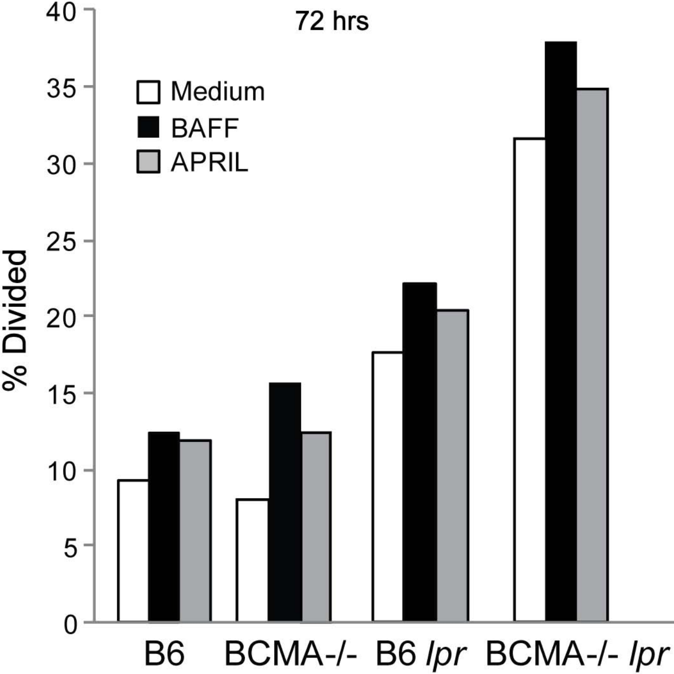
Supplemental Figure 5. B cells from BCMA$^{-/-}$ *lpr* mice proliferate equivalently to BAFF and APRIL stimulation. B cells (B220^{+} CD138^{-} CD3^{-}) isolated from spleens of 4-mo-old mice were labeled with CFSE and cultured 72 hrs in medium ± 0.1 µg/ml murine recombinant BAFF and APRIL. Cells were recovered and the percent divided B cells were determined by flow cytometry. Data are representative of two experiments.


B6
BCMA-/-
129S3
D1Mit15
D1Mit165
D1Mit293
D2Mit343
D2Mit44
D3Mit203
D3Mit45
D4Mit264
D4Mit33
D5Mit344
D5Mit30
D5Mit240
D6Mit86
D6Mit15
D6Mit123


IgG1
IgG2a
IgG2b
IgG3
B cell
B220- PC
B220+ PC
ASC number (per 10^4 input cells)
75
50
25
0
Spleen
Cervical LN


B cells
GC B cells
Plasma cells
Counts
0
10^1
10^2
10^3
10^4
BAFF-R
TACI
BCMA-/- *lpr*
B6 *lpr*
BCMA-/-
B6
FMO


Cytoplasmic
p100 fold change
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
p52 fold change
0
2
4
6
8
10
12
*
B6
BCMA-/-
B6 *lpr*
BCMA-/- *lpr*
α-CD40 - - - - +
BAFF - - + + -
α-IgM - + - + -
Nulcear
p52 fold change
0
3
6
9
12
15
p65 fold change
0
5
10
10
30
50
α-CD40 - - - - +
BAFF - - + + -
α-IgM - + - + -


72 hrs
☐ Medium
■ BAFF
▨ APRIL
% Divided
0
5
10
15
20
25
30
35
40
B6
BCMA-/-
B6 *lpr*
BCMA-/- *lpr*

Microsatellite markers used for genotyping of Nba2 congenic region.

Marker	Mb (NCBI m37)	3' Primer	5' Primer
D1Mit196	150.6	AAAAATGAGGTGCTATTGAAAAGC	TTATGCATCAAACCAAAATCTCA
D1Mit47	154.8	CTGACCTCCACACGACCC	GCTTGGGAAACTGGATGAAA
D1Mit105	162.4	TGGGTATCACCTCCAGAATACA	AGGTATGGTGAAGACAACAAAATG
D1Mit36	171.1	GAGGAATGTAGAGTCCAACCTGG	TGAATAGATTAAGAGCCTGGAAGC
D1Mit166	178.4	GATGAAGTGAAAATGATCCTTGC	TATCTTTTGTGGACTCGGGG
D1Mit209	193.3	TCCATCCATACTCCTGTCTGC	CAAGGACTAGGGCTGTCACTG
D1Mit510	194.2	TTCAGGTTAATTCTACAAACAAGCA	TCAAAATATCATGCTGTATACCATG

Polymorphic microsatellite markers used for genotyping of B6:129 background.

Marker	Mb (NCBI m36)	B6 Product Size (bp)	129S3 Product Size (bp)
D1Mit15	170.1	159	180
D1Mit165	100.0	147	155
D1Mit293	194.6	151	116
D2Mit44	105.1	148	139
D2Mit343	167.9	151	143
D3Mit203	27.1	153	144
D3Mit212	83.4	122	126
D3Mit45	148.0	146	134
D4Mit264	9.3	127	135
D4Mit33	149.0	125	141
D5Mit344	5.3	111	107
D5Mit30	129.8	147	135
D5Mit240	109.3	152	169
D6Mit86	4.4	130	119
D6Mit15	145.6	140	135
D6Mit123	17.5	118	98
D7Mit340	4.2	110	140
D7Mit15	144.0	135	129
D8Mit155	4.9	148	96
D8Mit93	130.1	168	162
D9Mit57	7.6	164	172
D9Mit151	121.3	128	116
D10Mit2	21.2	121	127
D10Mit35	121.6	228	240
D11Mit71	6.8	211	193
D11Mit48	117.9	134	128
D12Mit182	10.8	129	146
D12Nds2	115.3	189	172
D13Mit16	20.3	210	176
D13Mit191	85.3	118	138
D14Mit203	38.9	151	170
D14Mit193	70.2	118	129
D15Mit5	43.2	100	128
D15Mit35	103.3	118	122
D16Mit55	27.5	133	115
D16Mit131	72.3	142	178
D18Mit60	32.6	204	189
D18Mit103	70.3	116	91
D19Mit78	7.6	135	142
D19Mit6	54.2	109	113
DXMit166	47.9	115	127

Supplemental Table II. Absolute numbers of lymphocyte and myeloid cells in spleens and cervical LNs of naïve animals.

Cell number (x10^6)		**B6**	**BCMA$^{-/-}$**	**B6 *lpr***	**BCMA$^{-/-}$ *lpr***
Spleen	Total	61.63±17.52	61.52±23.38	67.30±38.89	288.85±119.46***
	B220$^+$CD138$^-$CD3$^-$ (B cell)	25.35±8.56	24.64±10.54	36.41±6.71	118.40±115.87*
	B220$^+$IgD$^-$GL7$^+$ (GC B cell)	0.15±0.03	0.65±0.15	1.06±0.82	10.26±7.75
	B220$^+$AA4.1$^-$ (Mature B cell)	18.65±4.62	28.22±10.11	15.68±9.78	75.39±35.36*
	B220$^+$AA4.1$^+$CD23$^-$IgM$^+$ (T1 B cell)	0.59±0.03	0.44±0.15	0.67±0.59	0.20±0.08
	B220$^+$AA4.1$^+$CD23$^+$IgM$^+$ (T2 B cell)	1.20±0.22	1.32±0.57	1.34±0.78	0.39±0.23
	B220$^+$AA4.1$^+$CD23$^+$IgM^{low} (T3 B cell)	0.89±0.55	0.74±0.09	0.96±0.39	0.30±0.24
	B220$^+$CD23$^+$CD21^{low} (FO B cell)	20.52±7.72	20.17±7.82	19.34±4.87	39.61±46.08
	B220$^+$CD23$^-$CD21^{hi} (MZ B cell)	1.69±0.63	1.02±0.49	1.97±0.39	1.48±1.52
	CD138$^+$ (PC)	1.40±0.93	2.34±1.05	3.42±2.20	52.82±44.29*
	CD3$^+$CD4$^+$ (CD4 T cell)	9.95±1.44	19.93±4.29	8.22±5.26	45.35±33.34
	CD3$^+$CD4$^+$FoxP3$^+$ (Treg)	3.27±1.12	4.64±1.80	7.04±3.58	4.68±2.41
	CD3$^+$CD8$^+$ (CD8 T cell)	5.13±0.60	8.26±1.22	4.41±1.21	13.19±9.14
	CD3$^+$CD4$^-$CD8$^-$ (DN T cell)	1.99±0.49	2.08±0.94	11.71±7.32	34.89±20.01*
	CD11c$^+$MHCII$^+$ (DC)	0.93±0.24	1.12±0.40	3.05±1.58	10.26±2.45*
	NK1.1$^+$TCR-β$^-$ (NK cell)	1.92±0.02	1.28±0.03	2.62±0.19	2.48±0.65
	F4/80$^+$CD11b$^+$ (Macrophage)	1.05±0.01	0.86±0.01	1.84±0.09	3.89±0.44
Cervical LN	Total	4.55±3.18	4.26±1.96	19.58±14.93	296.50±162.78**
	B220$^+$CD138$^-$CD3$^-$ (B cell)	1.59±1.29	1.84±1.32	14.40±17.39	123.19±82.36*
	B220$^+$IgD$^-$GL7$^+$ (GC B cell)	0.03±0.02	0.06±0.02	0.39±0.25	43.27±15.58**
	CD138$^+$ (PC)	0.14±0.11	0.29±0.24	2.47±1.90	79.75±44.54**
	CD3$^+$CD4$^+$ (CD4 T cell)	1.60±1.09	1.27±0.84	6.10±7.27	93.25±75.18*
	CD3$^+$CD4$^+$FoxP3$^+$ (Treg)	0.08±0.04	0.14±0.07	4.62±5.23	6.85±2.64
	CD3$^+$CD8$^+$ (CD8 T cell)	1.21±0.27	1.24±0.38	3.86±1.98	24.86±13.42**
	CD3$^+$CD4$^-$CD8$^-$ (DN T cell)	0.15±0.25	0.11±0.11	20.58±28.35	84.31±43.30*
	CD11c$^+$MHCII$^+$ (DC)	0.036±0.044	0.027±0.009	0.627±0.602	5.576±4.620
	NK1.1$^+$TCR-β$^-$ (NK cell)	0.021±0.015	0.027±0.019	0.192±0.111	0.649±0.257*
	F4/80$^+$CD11b$^+$ (Macrophage)	0.009±0.007	0.007±0.003	0.095±0.062	0.442±0.097**

Cell numbers shown are expressed as mean ± SEM. n = 3-6 mice per group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to all control strains