Supplementary Information

The transcription factors of the alternative NF-κB pathway are required for germinal center B-cell development

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Figure S1. Mature GCs are not maintained following combined GC B cell-specific deletion of *relb* and *nfkb2*.

Figure S2. Identification of genes controlled by the alternative NF-κB subunits RELB and NF-κB2 in GC B-cells.

Figure S3. Analysis for BrdU incorporation in GC B cells of *relb/nfkb2*-deleted mice.

Figure S4. GSEA to identify gene signatures that were enriched in GC B cells from Cγ1-Cre vs. *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ1-Cre mice.

Figure S5. Reduced PC frequencies following GC B-cell-specific deletion of the alternative NF-κB subunits RELB and NF-κB2 in GC B-cells.

Figure S6. GC B cell-specific deletion of *nfkb2* does not impair the GC reaction.

Figure S7. Normal splenic plasma cell frequencies following GC B cell-specific deletion of *nfkb2* at day 14 postimmunization.

Figure S8. Normal splenic and bone marrow plasma cell frequencies following GC B cell-specific deletion of *nfkb2* at day 28 postimmunization.

Table S1. Antibodies used in experiments.

Dataset 1. Differentially expressed sequence analysis (DE-SEQ) of RELB/NF-κB2-proficient *vs.* RELB/NF-κB2-deficient GC B-cells at d7 of the GC-reaction: Reduced expression in *relb/nfkb2*-deleted GC B-cells.

Dataset 2. Differentially expressed sequence analysis (DE-SEQ) of RELB/NF-κB2-proficient *vs.* RELB/NF-κB2-deficient GC-B cells at d7 of the GC-reaction: Increased expression in *relb/nfkb2*-deleted GC B-cells.

Dataset 3. GSEA was used to identify gene signatures that were enriched in GC B cells from Cγ1-Cre vs. *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ1-Cre mice.

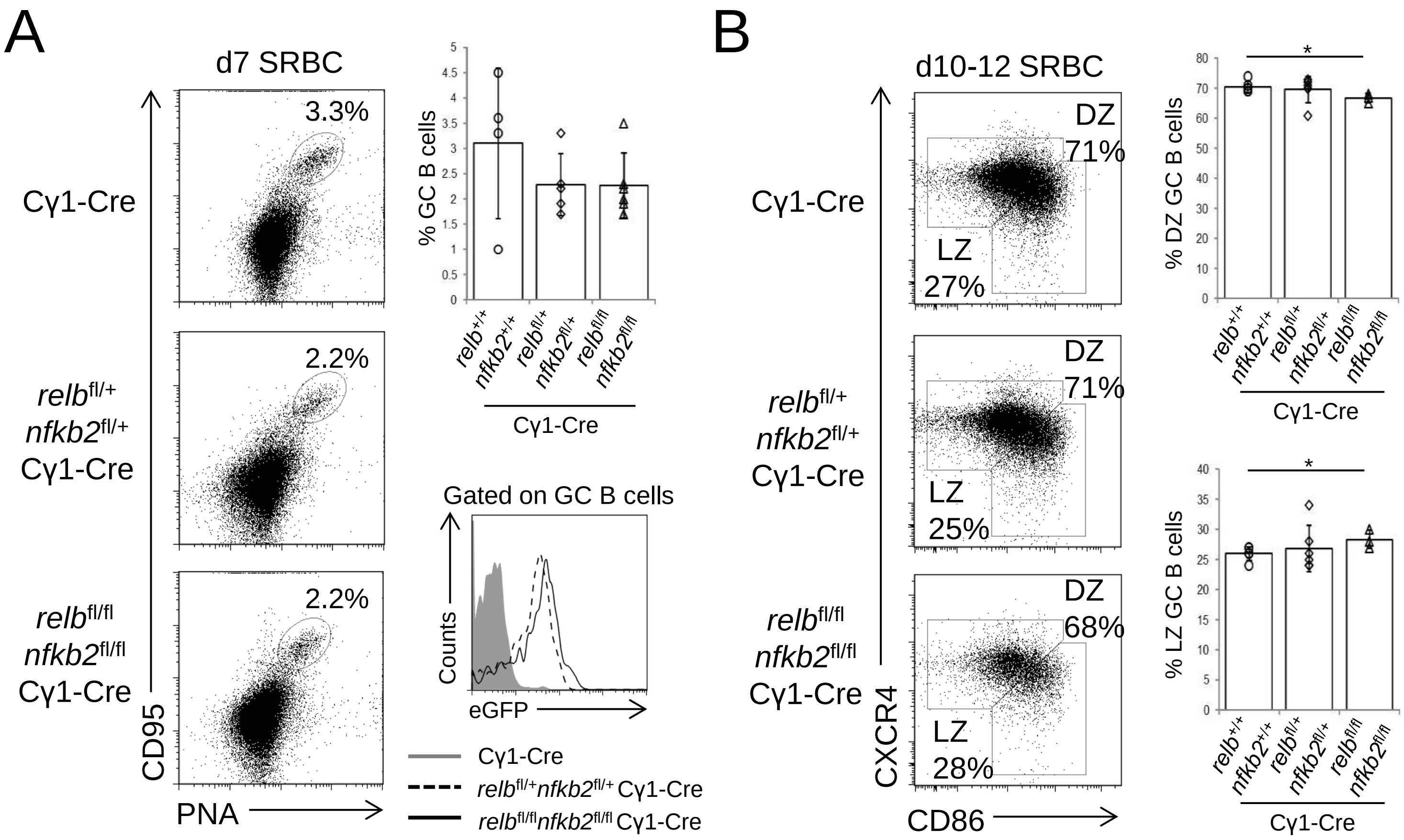
A
d7 SRBC
Cγ1-Cre
3.3%
relbfl/+ nfkb2fl/+ Cγ1-Cre
2.2%
relbfl/fl nfkb2fl/fl Cγ1-Cre
2.2%
CD95
PNA
% GC B cells
relb+/+ nfkb2+/+
relbfl/+ nfkb2fl/+
relbfl/fl nfkb2fl/fl
Cγ1-Cre
Gated on GC B cells
Counts
eGFP
Cγ1-Cre
relbfl/+nfkb2fl/+ Cγ1-Cre
relbfl/flnfkb2fl/fl Cγ1-Cre
B
d10-12 SRBC
Cγ1-Cre
DZ 71%
LZ 27%
relbfl/+ nfkb2fl/+ Cγ1-Cre
DZ 71%
LZ 25%
relbfl/fl nfkb2fl/fl Cγ1-Cre
DZ 68%
LZ 28%
CXCR4
CD86
% DZ GC B cells
*
% LZ GC B cells
*
relb+/+ nfkb2+/+
relbfl/+ nfkb2fl/+
relbfl/fl nfkb2fl/fl
Cγ1-Cre

Fig. S1

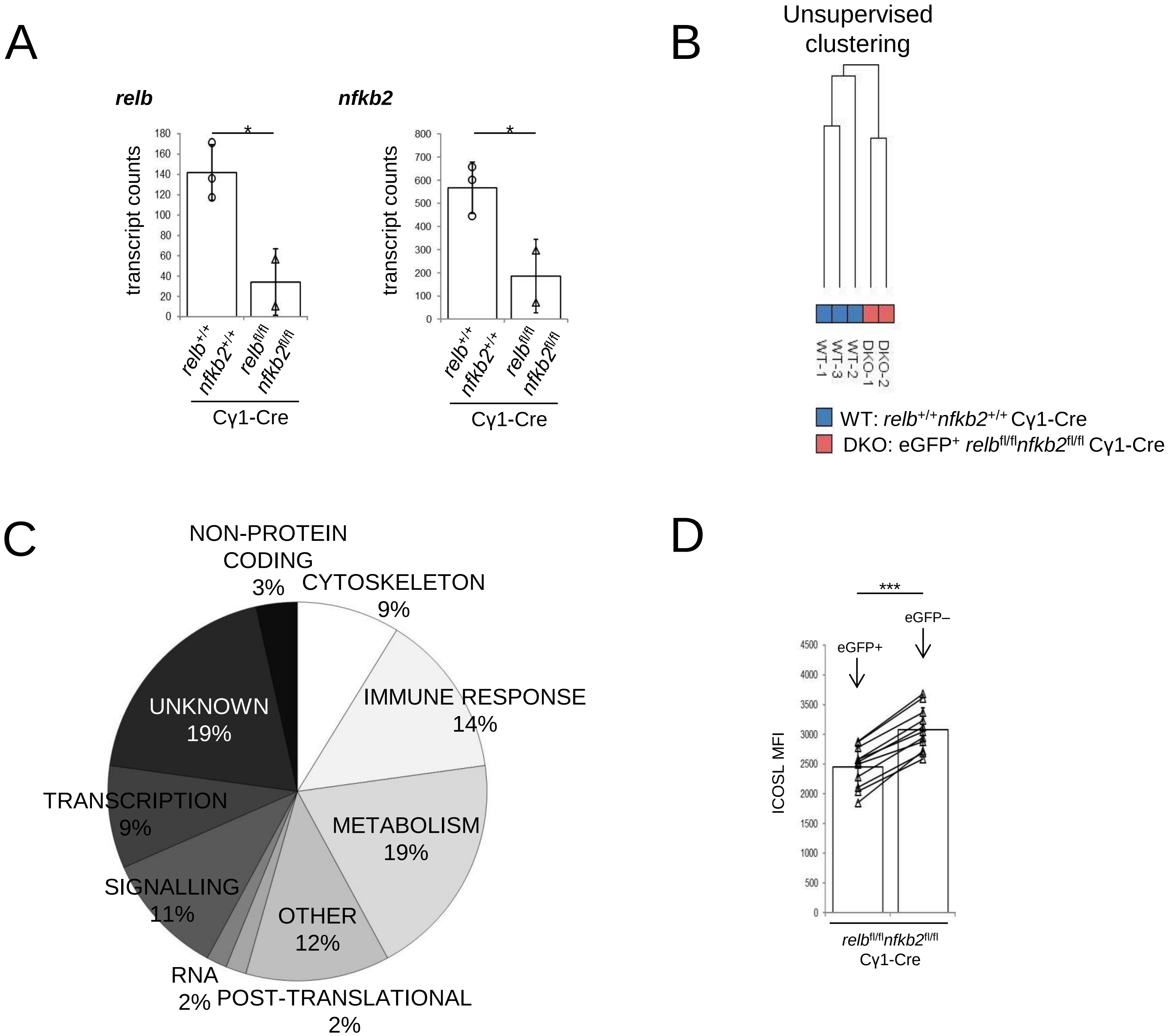

A
relb
nfkb2
transcript counts
180
160
140
120
100
80
60
40
20
0
*
relb+/+ nfkb2+/+
relbfl/fl nfkb2fl/fl
Cγ1-Cre
800
700
600
500
400
300
200
100
0
B
Unsupervised clustering
WT-1
WT-3
WT-2
DKO-1
DKO-2
WT: relb+/+nfkb2+/+ Cγ1-Cre
DKO: eGFP+ relbfl/flnfkb2fl/fl Cγ1-Cre
C
NON-PROTEIN CODING 3%
CYTOSKELETON 9%
IMMUNE RESPONSE 14%
METABOLISM 19%
OTHER 12%
POST-TRANSLATIONAL 2%
RNA 2%
SIGNALLING 11%
TRANSCRIPTION 9%
UNKNOWN 19%
D
***
eGFP+
eGFP–
ICOSL MFI
4500
4000
3500
3000
2500
2000
1500
1000
500
0
relbfl/flnfkb2fl/fl
Cγ1-Cre

Fig. S2

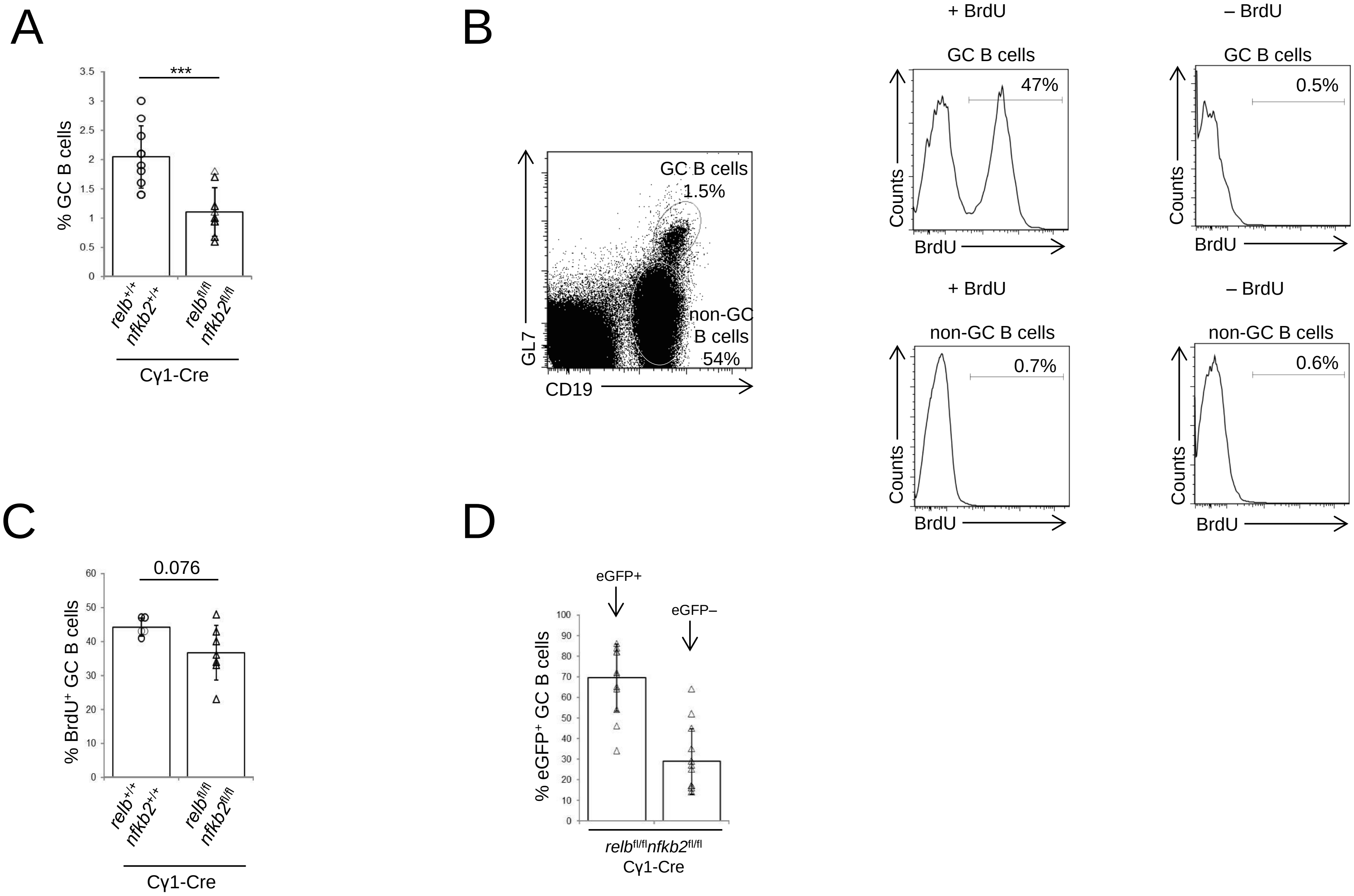

A
***
% GC B cells
3.5
3
2.5
2
1.5
1
0.5
0
relb+/+ nfkb2+/+
relbfl/fl nfkb2fl/fl
Cγ1-Cre
B
GC B cells
1.5%
non-GC B cells
54%
GL7
CD19
+ BrdU
GC B cells
47%
Counts
BrdU
– BrdU
GC B cells
0.5%
Counts
BrdU
+ BrdU
non-GC B cells
0.7%
Counts
BrdU
– BrdU
non-GC B cells
0.6%
Counts
BrdU
C
0.076
% BrdU+ GC B cells
60
50
40
30
20
10
0
relb+/+ nfkb2+/+
relbfl/fl nfkb2fl/fl
Cγ1-Cre
D
eGFP+
eGFP–
% eGFP+ GC B cells
100
90
80
70
60
50
40
30
20
10
0
relbfl/flnfkb2fl/fl
Cγ1-Cre

Fig. S3

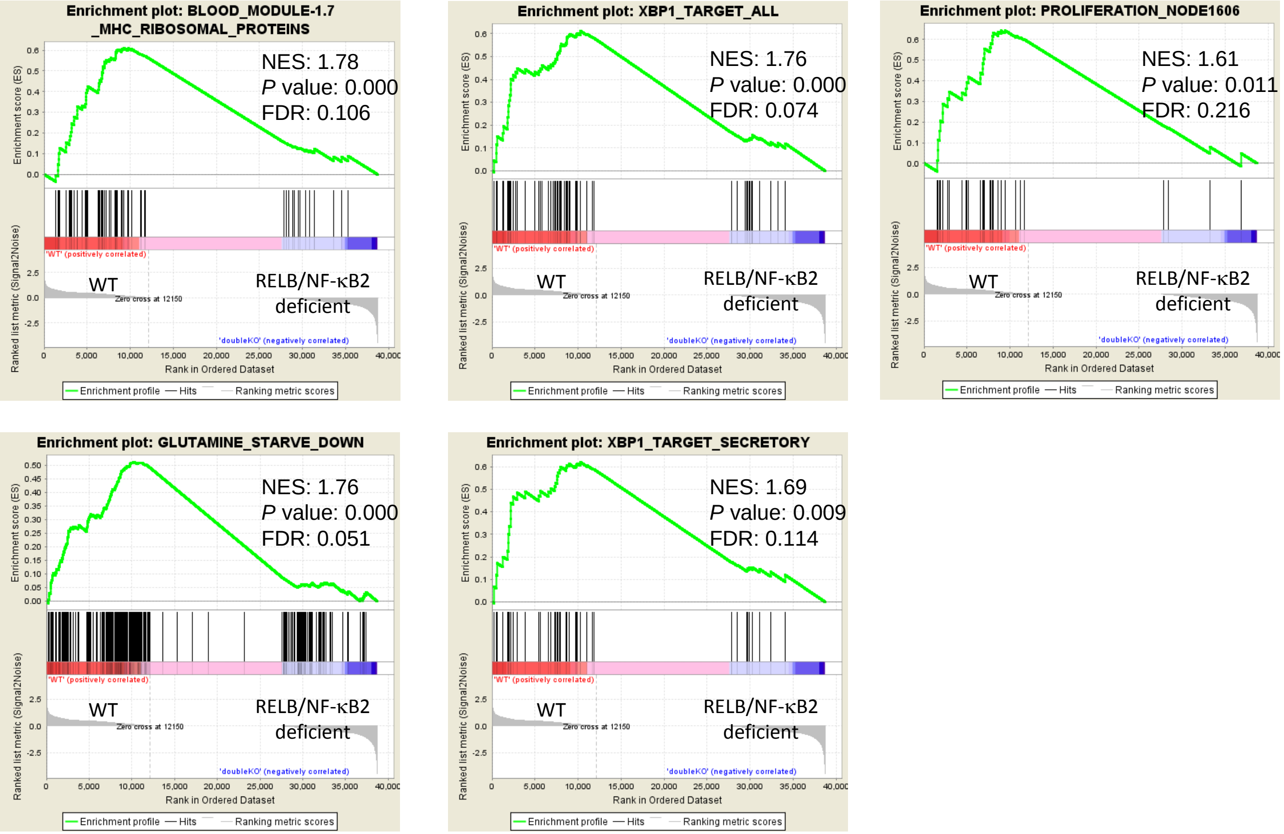

Enrichment plot: BLOOD_MODULE-1.7_MHC_RIBOSOMAL_PROTEINS
NES: 1.78
P value: 0.000
FDR: 0.106
WT
RELB/NF-κB2 deficient
Enrichment plot: XBP1_TARGET_ALL
NES: 1.76
P value: 0.000
FDR: 0.074
WT
RELB/NF-κB2 deficient
Enrichment plot: PROLIFERATION_NODE1606
NES: 1.61
P value: 0.011
FDR: 0.216
WT
RELB/NF-κB2 deficient
Enrichment plot: GLUTAMINE_STARVE_DOWN
NES: 1.76
P value: 0.000
FDR: 0.051
WT
RELB/NF-κB2 deficient
Enrichment plot: XBP1_TARGET_SECRETORY
NES: 1.69
P value: 0.009
FDR: 0.114
WT
RELB/NF-κB2 deficient
Enrichment score (ES)
Ranked list metric (Signal2Noise)
'WT' (positively correlated)
Zero cross at 12150
'doubleKO' (negatively correlated)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Fig. S4

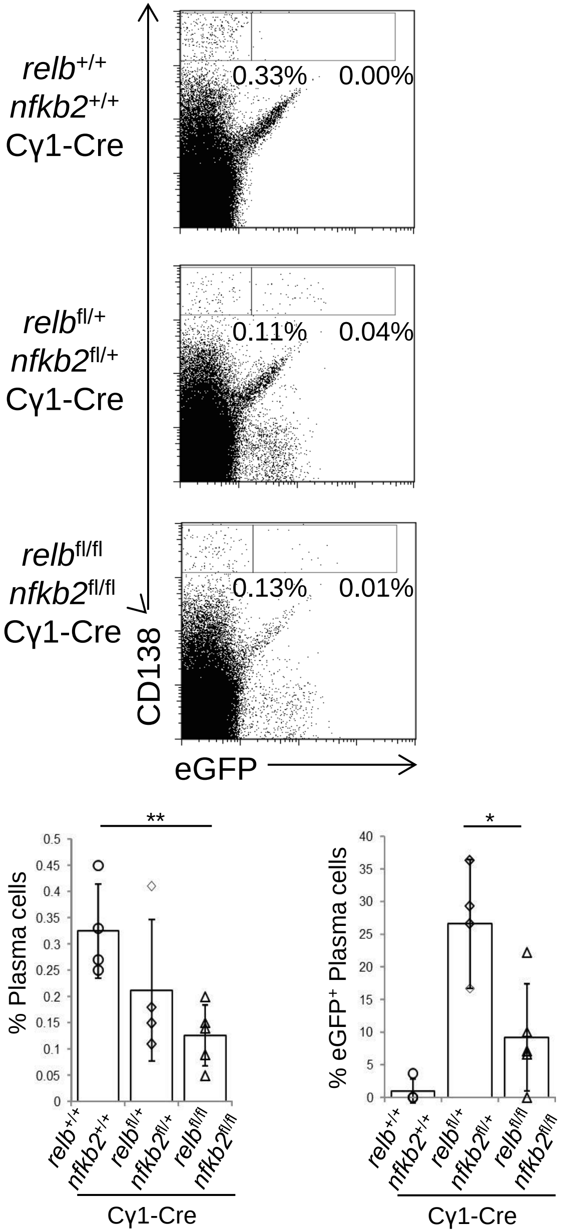
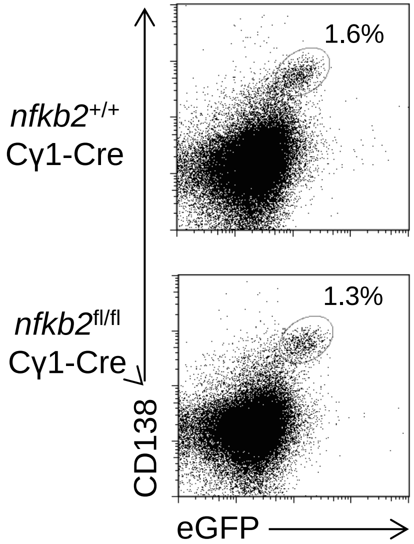
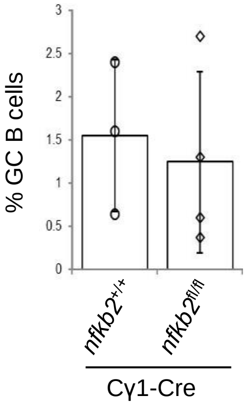

relb+/+
nfkb2+/+
Cγ1-Cre
0.33%
0.00%
relbfl/+
nfkb2fl/+
Cγ1-Cre
0.11%
0.04%
relbfl/fl
nfkb2fl/fl
Cγ1-Cre
0.13%
0.01%
CD138
eGFP
**
% Plasma cells
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
relb+/+ nfkb2+/+
relbfl/+ nfkb2fl/+
relbfl/fl nfkb2fl/fl
Cγ1-Cre
*
% eGFP+ Plasma cells
0
5
10
15
20
25
30
35
40
relb+/+ nfkb2+/+
relbfl/+ nfkb2fl/+
relbfl/fl nfkb2fl/fl
Cγ1-Cre

Fig. S5


nfkb2+/+
Cγ1-Cre
1.6%
*nfkb2*fl/fl
Cγ1-Cre
1.3%
CD138
eGFP


% GC B cells
0
0.5
1
1.5
2
2.5
3
nfkb2+/+
*nfkb2*fl/fl
Cγ1-Cre

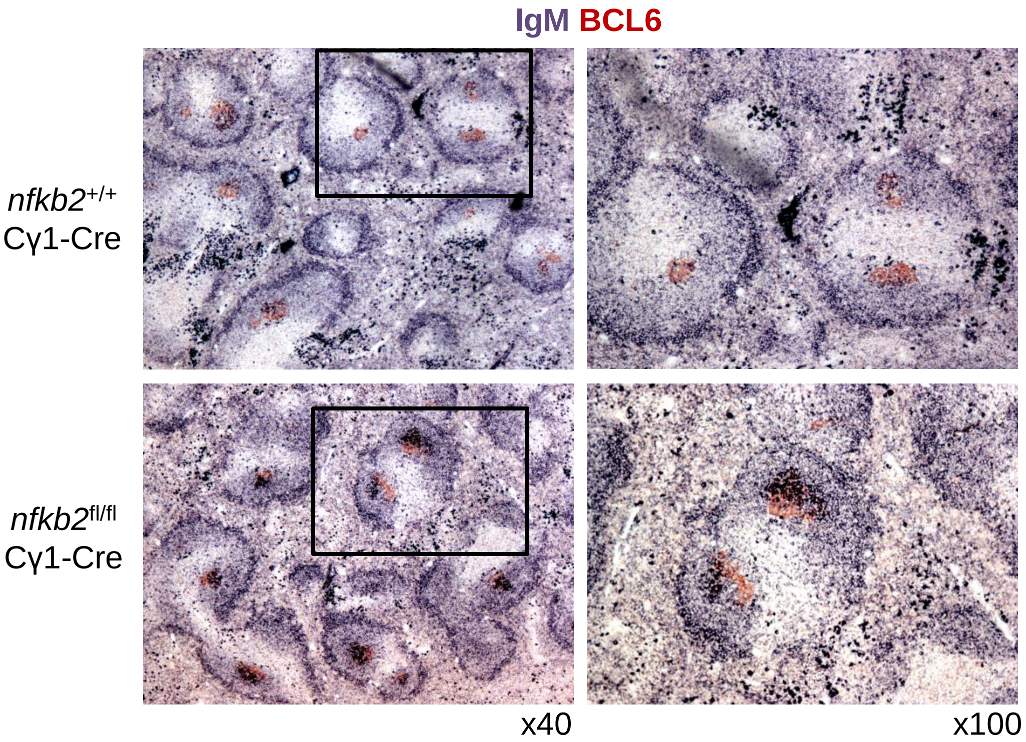

IgM BCL6
nfkb2+/+
Cγ1-Cre
*nfkb2*fl/fl
Cγ1-Cre
x40
x100

Fig. S6

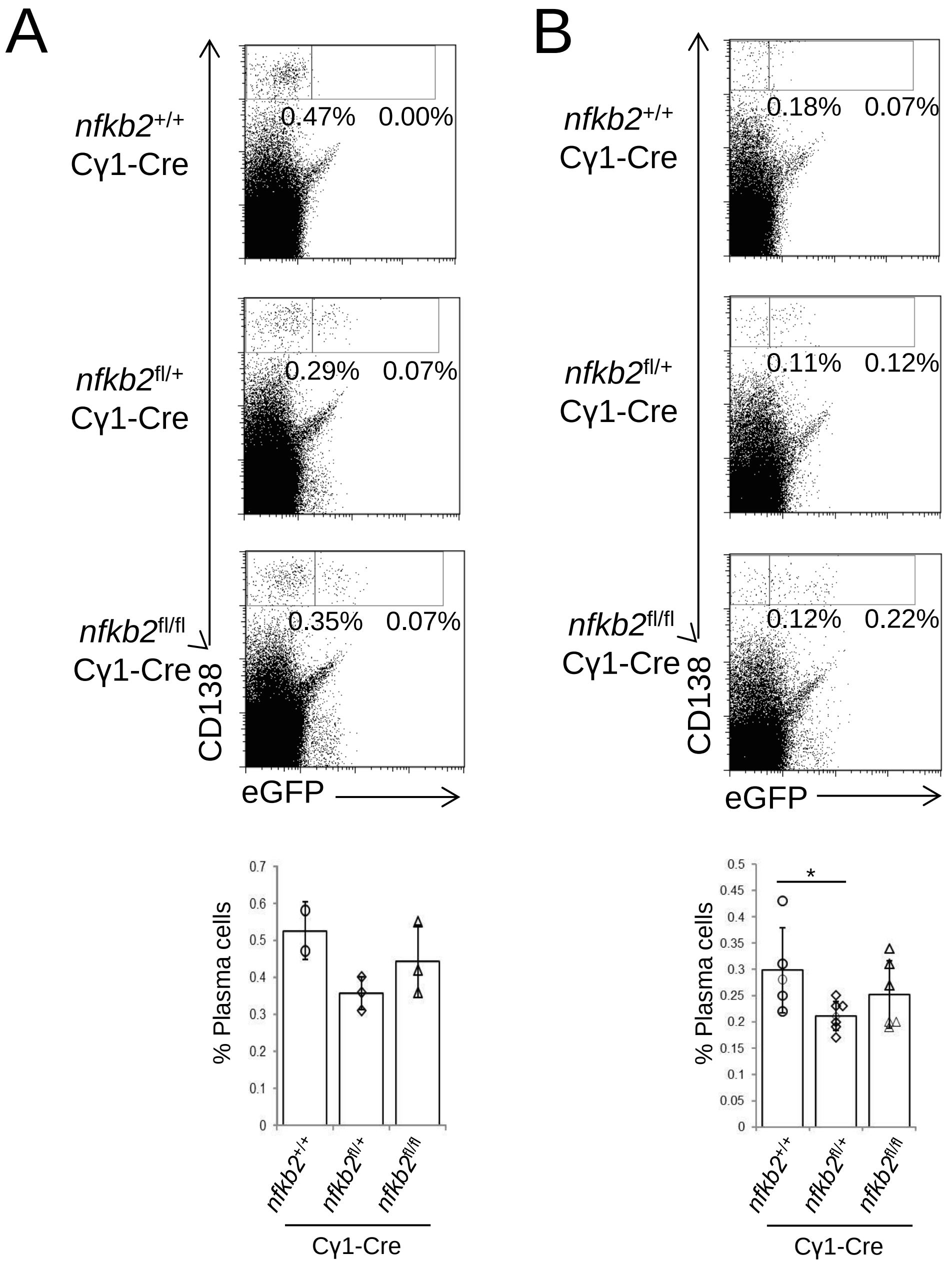

A
nfkb2+/+ Cγ1-Cre
0.47% 0.00%
nfkb2fl/+ Cγ1-Cre
0.29% 0.07%
nfkb2fl/fl Cγ1-Cre
0.35% 0.07%
CD138
eGFP
% Plasma cells
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
nfkb2+/+
nfkb2fl/+
nfkb2fl/fl
Cγ1-Cre
B
nfkb2+/+ Cγ1-Cre
0.18% 0.07%
nfkb2fl/+ Cγ1-Cre
0.11% 0.12%
nfkb2fl/fl Cγ1-Cre
0.12% 0.22%
CD138
eGFP
% Plasma cells
*
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
nfkb2+/+
nfkb2fl/+
nfkb2fl/fl
Cγ1-Cre

Fig. S7

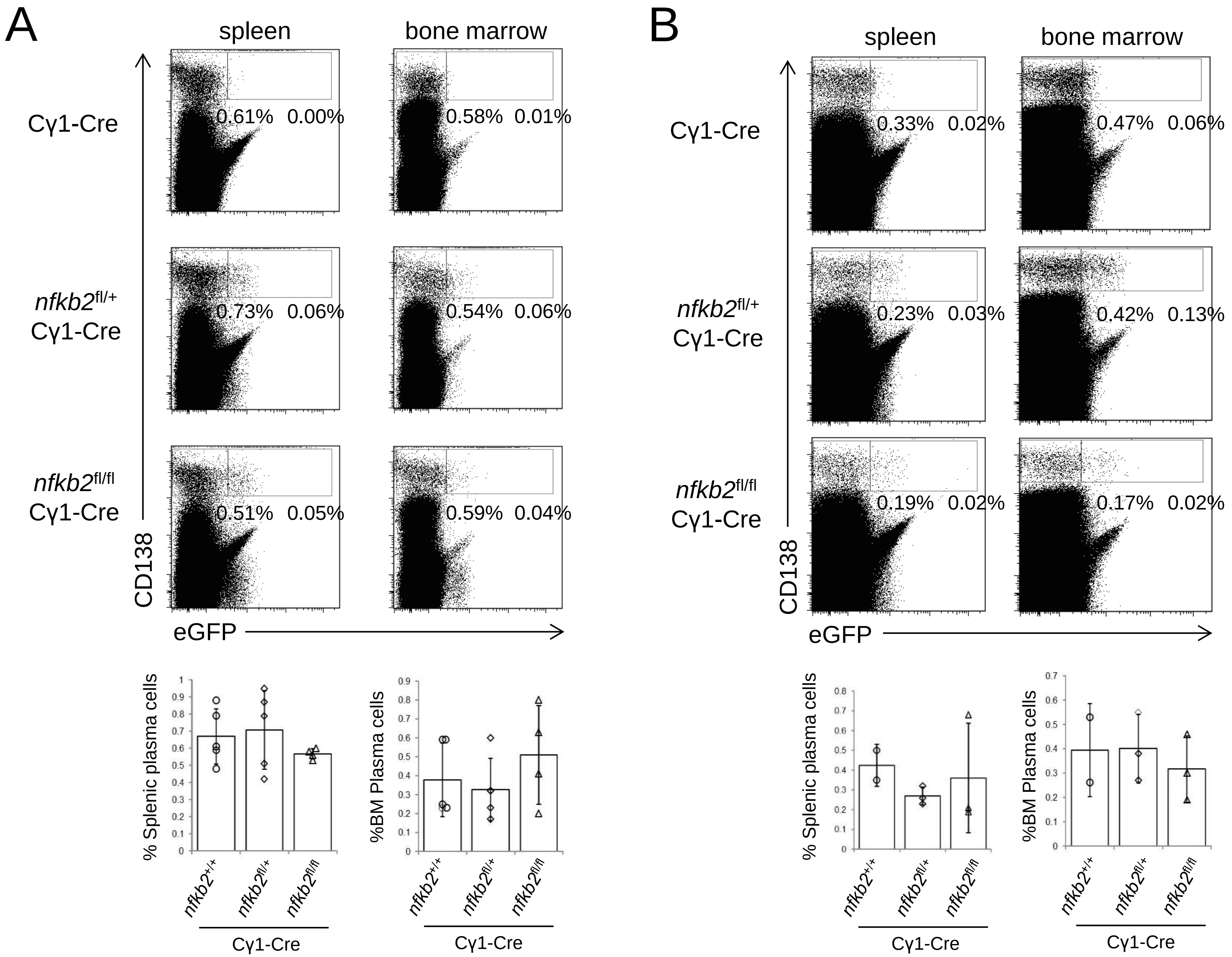

A
spleen
bone marrow
Cγ1-Cre
0.61%
0.00%
0.58%
0.01%
nfkb2$^{fl/+}$ Cγ1-Cre
0.73%
0.06%
0.54%
0.06%
nfkb2$^{fl/fl}$ Cγ1-Cre
0.51%
0.05%
0.59%
0.04%
CD138
eGFP
% Splenic plasma cells
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
%BM Plasma cells
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
nfkb2$^{+/+}$
nfkb2$^{fl/+}$
nfkb2$^{fl/fl}$
Cγ1-Cre
B
spleen
bone marrow
Cγ1-Cre
0.33%
0.02%
0.47%
0.06%
nfkb2$^{fl/+}$ Cγ1-Cre
0.23%
0.03%
0.42%
0.13%
nfkb2$^{fl/fl}$ Cγ1-Cre
0.19%
0.02%
0.17%
0.02%
CD138
eGFP
% Splenic plasma cells
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
%BM Plasma cells
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
nfkb2$^{+/+}$
nfkb2$^{fl/+}$
nfkb2$^{fl/fl}$
Cγ1-Cre

Fig. S8

Figure S1. Mature GCs are not maintained following combined GC B cell-specific deletion of *relb* and *nfkb2*. (**A**) *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ1-Cre mice and the corresponding heterozygous and Cγ1-Cre control mice were analyzed via flow cytometry 7d following immunization with SRBCs for splenic GC B cells. Summary of the frequencies of GC B cells (top right). Each symbol represents a mouse. Expression of eGFP in GC B cells from mice of the indicated genotypes (bottom right). (**B**) *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ1-Cre mice and the corresponding heterozygous and Cγ1-Cre control mice were analyzed via flow cytometry 10-12d following immunization with SRBCs for CD38^{lo}CD95^{hi}CXCR4^{hi}CD86^{lo} DZ B cells and CD38^{lo}CD95^{hi}CXCR4^{lo}CD86^{hi} LZ B cells (left). Summary of frequencies of DZ and LZ B cells (right). Each symbol represents a mouse. Statistical significance was determined by Student's *t* test (*, $P<0.05$). Data are shown as mean ± SD.

Figure S2. Identification of genes controlled by the alternative NF-κB subunits RELB and NF-κB2 in GC B-cells. (**A**) eGFP$^+$ GC B-cells from *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ1-Cre mice and GC B-cells from Cγ1-Cre control mice 7d following immunization with SRBCs were purified via flow cytometric sorting and RNA was subjected to RNA-sequencing (RNA-seq). The mRNA expression of RELB and NF-κB2 is presented as total transcript counts for each gene. Each symbol represents a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (*, $P<0.05$). eGFP$^+$ identifies *relb/nfkb2*-deleted GC B-cells from *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ-Cre mice. (**B**) Unsupervised clustering analysis of transcript counts obtained from RNA-seq of RNA isolated via flow cytometry from eGFP$^+$ GC B-cells from *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ1-Cre mice (red) and GC B-cells from Cγ1-Cre control mice (blue). (**C**) Differentially expressed sequence analysis (DESeq) of RNA from eGFP$^+$ GC B-cells from *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ1-Cre mice and GC B-cells from Cγ1-Cre control mice identified 59 transcripts with less than 2.5-fold increased expression in the combined absence of RELB and NF-κB2 at a significant threshold of $p<0.01$. Genes were assigned to putative functional categories. For the identity of the corresponding genes, fold-change and *p* values, see Table S1. eGFP^{+} identifies *relb/nfkb2*-deleted GC B-cells from *relb*$^{\mathrm{fl/fl}}$*nfkb2*$^{\mathrm{fl/fl}}$Cγ-Cre mice. (**D**) *relb*$^{\mathrm{fl/fl}}$*nfkb2*$^{\mathrm{fl/fl}}$Cγ1-Cre mice were analyzed via flow cytometry 10d following immunization with SRBCs for the expression of ICOSL on CD95$^{\mathrm{hi}}$CD38$^{\mathrm{lo}}$ GC B-cells. Summary of the corresponding MFI in eGFP^{+} and eGFP^{-} GC B-cells.

Fig. S3. Analysis for BrdU incorporation in GC B cells of *relb/nfkb2*-deleted mice. (**A**) Fraction of GC B cells in *relb*$^{\mathrm{fl/fl}}$*nfkb2*$^{\mathrm{fl/fl}}$Cγ1-Cre and Cγ1-Cre mice at day 10 postimmunization with SRBCs. (**B**, left) CD19^{+}GL7$^{\mathrm{hi}}$ gating strategy to identify GC B cells using a single GC B-cell marker (GL7) as described previously[1]. (**B**, right) Examples of BrdU incorporation in GC B cells and non-GC B cells in mice that received BrdU (+BrdU) and mice that did not (–BrdU). (**C**) Summary of fraction of GC B cells incorporating BrdU in *relb*$^{\mathrm{fl/fl}}$*nfkb2*$^{\mathrm{fl/fl}}$Cγ1-Cre and Cγ1-Cre mice at day 10 postimmunization with SRBCs. (**D**) Fraction of eGFP^{+} and eGFP^{-} GC B cells in *relb*$^{\mathrm{fl/fl}}$*nfkb2*$^{\mathrm{fl/fl}}$Cγ1-Cre mice at day 10 postimmunization with SRBCs. (A,C,D) Each symbol represents a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (***, *P*<0.001).

Figure S4. GSEA to identify gene signatures that were enriched in GC B-cells from Cγ1-Cre vs. *relb*$^{\mathrm{fl/fl}}$*nfkb2*$^{\mathrm{fl/fl}}$Cγ1-Cre mice. Comparison of RNA-seq data from eGFP^{+} GC B-cells from *relb*$^{\mathrm{fl/fl}}$*nfkb2*$^{\mathrm{fl/fl}}$Cγ1-Cre mice and GC B-cells from Cγ1-Cre control mice 7d following immunization with SRBCs with a library of normal and pathological lymphoid gene expression signatures identified five signatures enriched in RELB/NF-κB2-proficient vs. deficient GC B-cells.

Figure S5. Reduced PC frequencies following GC B-cell-specific deletion of the alternative NF-κB subunits RELB and NF-κB2 in GC B-cells. (top) *relb*$^{\mathrm{fl/fl}}$*nfkb2*$^{\mathrm{fl/fl}}$Cγ1-Cre mice and the corresponding heterozygous and Cγ1-Cre control mice were analyzed via flow cytometry 14d following immunization with sheep red blood cells (SRBCs) for CD138 and eGFP expression of splenocytes. Numbers beside each gate denote the fractions of CD138^{hi} splenic plasma cells (PCs) in mice of the indicated genotypes. (bottom) Summary of the frequencies of PCs. Each symbol represents a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (*, $P<0.05$; **, $P<0.01$). eGFP identifies *relb/nfkb2*-deleted PCs within the indicated genotypes.

Figure S6. GC B cell-specific deletion of *nfkb2* does not impair the GC reaction. (**A**) *nfkb2*$^{fl/fl}$Cγ1-Cre mice and Cγ1-Cre control mice were analyzed by flow cytometry 14d following immunization with NP-KLH for CD95^{hi}PNA^{hi} splenic GC B cells. Summary of the frequencies of GC B cells (bottom). Each symbol represents a mouse. Data are shown as mean ± standard deviation (SD). (**B**) Spleen sections from the indicated genotypes were analyzed for the expression of BCL6 and IgM via IHC.

Figure S7. Normal splenic plasma cell frequencies following GC B cell-specific deletion of *nfkb2* at day 14 postimmunization. *nfkb2*$^{fl/fl}$Cγ1-Cre mice and the corresponding heterozygous and Cγ1-Cre control mice were analyzed via flow cytometry 14 days following immunization with (**A**) NP-KLH and (**B**) sheep red blood cells (SRBCs) for CD138 and eGFP expression of splenocytes. Numbers beside each gate denote the fractions of CD138^{hi} splenic plasma cells in mice of the indicated genotypes. (**A,B, bottom**) Summary of the frequencies of plasma cells. Each symbol represents a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (*, $P<0.05$). eGFP identifies *relb/nfkb2*-deleted plasma cells within the indicated genotypes.

Figure S8. Normal splenic and bone marrow plasma cell frequencies following GC B cell-specific deletion of *nfkb2* at day 28 postimmunization. *nfkb2*$^{fl/fl}$Cγ1-Cre mice and the corresponding heterozygous and Cγ1-Cre control mice were analyzed via flow cytometry 28 days following immunization with (**A**) NP-KLH and (**B**) sheep red blood cells (SRBCs) for CD138 and eGFP expression of splenocytes. Numbers beside each gate denote the fractions of CD138^{hi} splenic plasma cells in mice of the indicated genotypes. (**A,B, bottom**) Summary of the frequencies of plasma cells. Each symbol represents a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (*, $P<0.05$). eGFP identifies *relb/nfkb2*-deleted plasma cells within the indicated genotypes.

References

(1) Cato, M.H., Chintalapati, S.K., Yau, I.W., Omori, S.A. & Rickert, R.C. Cyclin D3 is selectively required for proliferative expansion of germinal center B cells. *Mol Cell Biol* **31**:127, 2011.

Application	Antibody	Manufacturer
Immunoblotting	rabbit anti-RELB (clone C-19)	Santa Cruz Biotechnology
	rabbit anti-BCL6 (clone N-3)	Santa Cruz Biotechnology
	rabbit anti-RELA (clone C-20)	Santa Cruz Biotechnology
	rabbit anti-c-REL (clone C)	Santa Cruz Biotechnology
	rabbit anti-p100/p52	Cell Signaling Technologies (CST)
	rabbit anti-p105/p50	Cell Signaling Technologies (CST)
	mouse anti-β-actin (clone AC-15)	Sigma
	anti-rabbit IgG	GE Healthcare
	anti-mouse IgG	Thermo Scientific
Flow cytometry	PerCP-conjugated anti-B220 (clone RA3-6B2)	Biolegend
	Alexa Fluor 700-conjugated anti-CD38 (clone 90)	eBioscience
	PE-conjugated anti-CD138 (clone 281-2)	BD Pharmingen
	APC-conjugated anti-CD86 (clone GL1)	eBioscience
	PE-conjugated anti-CD95 (clone Jo2)	BD Pharmingen
	PerCP-eFluor710-conjugated anti-CXCR4 (clone 2B11)	eBioscience
	biotin-conjugated anti-PNA	Vector Laboratories
	streptavidin-APC	BD Pharmingen
	APC-conjugated anti-CD36 (clone HM36)	Biolegend
	PE-CF594-conjugated anti-CD19 (clone 1D3)	BD Horizon
	eFluor® 660-conjugated anti-CD275 (clone HK5.3)	eBioscience
Immunohistochemistry	anti-BCL6 rabbit antibody (clone N-3)	Santa Cruz Biotechnology
	alkaline peroxidase (AP)-conjugated anti-IgM antibody	Southern Biotech
Immunofluorescence	rabbit anti-p100/p52	Cell Signaling Technologies (CST)
	rabbit anti-p105/p50	Cell Signaling Technologies (CST)
	Cy3-conjugated AffiniPure Donkey anti-rabbit IgG	Jackson Immuno-Research Laboratories
	rat anti-BLIMP1(clone 6D3)	Santa Cruz Biotechnology
	goat anti-IRF4 (clone M17)	Santa Cruz Biotechnology
	mouse anti-CD20 (clone Ab-1)	NeoMarkers
	rabbit anti-c-REL (clone C)	Santa Cruz Biotechnology
	FITC-conjugated goat anti-rat IgG	Jackson Immuno-Research Laboratories
	Cy3-conjugated donkey anti-goat IgG	Jackson Immunoresearch Laboratories
	horse anti-mouse-biotin	Vector Laboratories
	Alexa Fluor 350-streptavidin	Molecular Probes
	Cy3-conjugated donkey anti-rabbit IgG	Jackson

Table S1. Antibodies used in the experiments.

DATASET 1

Differentially expressed sequence analysis (DE-SEQ) of RELB/NF-κB2-proficient *vs*. RELB/NF-κB2-deficient germinal center (GC) B cells at day 7 of the GC reaction

Reduced expression in *relb*/*nfkb2*-deleted GC B cells

$p<0.01$

FUNCTIONAL CATEGORY	**TRANSCRIPT**	**FOLD CHANGE**	**p value**
APOPTOSIS	4632434I11Rik	1.76573532	0.004915069
CYTOSKELETON	Kif20b	1.811794953	0.000943751
	Cep290	3.701368327	0.001250872
	Fmn1	6.77867816	0.002083243
	Nebl	3.408183756	0.002700872
	Cenpe	1.861234042	0.0036804
	Pacsin1	1.875424826	0.0037934
	Cenpf	2.162703888	0.004783279
	Lrrcc1	1.816466584	0.006774469
	Smc4	1.616979321	0.008196885
	Rilpl2	2.213476746	0.000904684
	St14	2.286564401	0.001286364
	Pls3	3.639672747	0.009879553
	Cdc42ep4	9.822813676	0.0005552
IMMUNE RESPONSE	Ltb	2.14762194	0.001268232
	Nlrx1	2.046350103	0.000920542
	Oasl2	3.56672381	0.00213694
	Ifit1	3.538140389	0.009510845
	Ifi27	1.745879082	0.005145702
	Ephb2	10.74595395	0.000774539
	Ptpn1	1.548861829	0.005691496
	Igkv1-135	2.012156108	0.000889688
	Ighg3	2.1821529	0.002865123
	Ighg2c	8.537256103	0.004010517
	Igkv3-2	1.940536447	0.004639351
	Igkv6-25	1.692218016	0.006119665
	Iglv1	1.484739556	0.007331529
	Igkv3-4	2.442680718	0.008135836
	Tlr3	3.442985706	0.001035165
	Tox	2.496995356	0.00077797
	Lck	1.962368294	9.93E-05
	Cxcl10	4.015628084	0.00643676
	Rgs3	2.885035238	0.009433817
	Rgs13	3.184725593	1.44E-06
METABOLISM	Aldh2	1.64733719	0.003367119
	Gstt1	8.83364123	1.77E-05
	Gstt3	3.001044657	0.000196394
	Got1	1.704525596	0.000942447
	Slc43a1	2.462379625	0.002191476

	Padi4	3.956754531	0.009296115
	Xpnpep1	1.557546587	0.009497651
	Wbscr17	20.48998638	0.00060861
	St8sia4	1.791879207	0.004161956
	Chst1	6.041301989	0.004207427
	Chst10	2.414915656	0.007955842
	Mocos	5.674386583	0.005944022
	As3mt	2.643569946	1.58E-06
	Pctp	2.491318094	0.002406578
	Osbpl3	2.551941535	0.003505027
	Cers6	2.567999041	0.001065751
	Slc27a4	2.652195026	0.004516612
	Atp10a	1.886788035	0.006078148
	Cpt2	1.825272047	0.009720877
	Cd36	3.252357531	3.74E-10
	Sdhd	1.629279505	0.006638898
	Ndufs6	1.754927683	0.008735515
OTHER	Olfr1444	8.242565212	0.002878901
	Nrep	11.9868501	0.003761633
	Ap5b1	1.857607311	0.005783731
	Tor4a	1.589572866	0.005856674
	Syngr2	1.634926813	0.007306999
	Mea1	1.765582591	0.007840597
	Trp73	5.053304329	0.009116683
	Adam15	5.588725201	0.009544978
	Pdia4	1.68712728	0.00097079
	Hps6	2.547747033	0.005642318
	Arid5a	2.147512473	0.006810885
	Commd4	1.518135904	0.008672118
	Stx11	3.230499654	0.007398995
	Anxa4	2.056819276	0.007722892
	Kcnk5	1.6499379	0.00728454
	Enpp4	8.546181084	7.78E-06
POSTTRANSLATIONAL	Neurl1a	19.24256824	2.73E-06
	Ube2j1	1.631087569	0.006399689
	Cops2	1.658960528	0.002367574
	Dtx1	1.487093742	0.007778813
PROLIFERATION	Cdk5rap3	2.152512764	0.005308663
	Cdk14	2.469387427	0.000235753
RNA	Esf1	1.799916698	0.006586022
	Ssb	1.518742324	0.008669204
	Tceb2	1.880320335	0.001757054
	Gemin7	2.699870842	0.000506399
SIGNALLING	Rhobtb1	4.174352932	0.004952224
	Akt2	1.516925061	0.008288085
	Ptpn14	2.866098295	0.005009919
	Dusp6	1.60799308	0.006220875

	Ptpn13	7.525755869	0.001412894
	Maged1	1.925570109	0.001430889
	Ppm1f	1.784373519	0.001839516
	Evc	4.966383366	0.000100413
	Wls	4.286310822	0.000270604
	Cby1	1.7706718	0.008709796
	Daam1	3.173809654	0.001281587
TRANSCRIPTION	Hoxa7	28.03543672	0.000109421
	Tox2	10.1999987	0.000144835
	Phf11b	1.648989571	0.003212602
	Zfp518a	1.978975539	0.004631411
	Zfp52	1.617881222	0.005142449
	Hmga1-rs1	7.134385975	0.005625708
	Zfp365	2.695290407	0.006027941
	Dnttip2	1.497356727	0.009167669
	Hic1	6.826539202	0.002277476
	Nab2	2.021091041	0.000832352
TRANSPORT	Pttg1ip	1.565319574	0.009825817
NON-PROTEIN CODING	Gm14963	27.12136716	0.000116433
	Rps10-ps2	1.610920132	0.009153303
	Gm12063	7.718110564	0.003664288
UNKNOWN	Gm10800	59.2378127	1.36E-08
	Gm21738	17.17454777	6.78E-07
	Gm10801	24.19518446	3.49E-05
	A930033H14Rik	13.3365832	0.000117682
	Gm10717	18.58433817	0.000145953
	Zfp51	2.030754582	0.000279444
	Sh3bgrl	2.647161401	0.000293758
	R3hdm4	1.634308691	0.000609235
	Ccdc120	5.445959362	0.00172023
	Fam167a	1.801532248	0.003004706
	Endod1	1.787831291	0.004318015
	Ldoc1l	1.755128906	0.004832892
	Fam135a	6.437804933	0.005610792
	7-Sep	1.545761942	0.006904712
	Gm9825	2.794255416	0.006924743
	Paqr7	6.794428153	0.007168583
	4930506M07Rik	3.870257186	0.007304508
N/A	Relb	3.872632342	0.001113217
	Nfkb2	2.93907889	0.001172224

DATASET 2			
Differentially expressed sequence analysis (DE-SEQ) of			
RELB/NF-κB2-proficient *vs*. RELB/NF-κB2-deficient			
germinal center (GC) B cells at day 7 of the GC reaction			
Increased expression in *relb*/*nfkb2*-deleted GC B cells			
***p*<0.01**			
FUNCTIONAL CATEGORY	**TRANSCRIPT**	**FOLD CHANGE**	***p* value**
CYTOSKELETON	Dnm1	28.84816562	1.71E-06
	Tppp	6.031130636	0.000340554
	Ppp1r12b	1.966474662	0.001247336
	Stard9	2.302096874	0.002963074
	Col27a1	3.005588623	0.004863368
	Ttll3	1.789245228	0.005750192
	Zmym6	1.930714227	0.001287388
	Vezt	1.758610136	0.001830602
	Myl6b	2.229711167	0.00494586
IMMUNE RESPONSE	Il18bp	2.182066265	0.001194784
	Iigp1	8.369047288	0.009720592
	Bach2	1.669056135	0.003186453
	Fcgr2b	1.591884604	0.002555638
METABOLISM	Phykpl	1.729897457	0.000264972
	Slc15a2	5.230059989	6.09E-08
	Slc1a5	1.670898557	0.003704039
	Ogt	1.600700579	0.009865998
	Cp	4.031121943	0.002349171
	Ltf	15.26600973	0.000597746
	Mgll	2.645647937	0.001999474
NON-PROTEIN CODING	Gm26789	5.0514664	8.62E-16
	Gm26625	4.09937526	9.00E-10
	Gm26749	4.178514721	3.36E-07
	Gm11932	3.581939544	5.75E-07
	Gm17477	7.342866239	8.25E-07
	Gm4279	4.806875857	9.35E-07
	Gm16153	2.503956747	2.49E-05
	Gm11721	6.337946518	3.94E-05
	BC024582	2.478009337	9.80E-05
	Gm26882	2.868542527	0.000121993
	Gm12543	3.211250557	0.000139233
	Gm10552	2.182269327	0.000171293
	Gm26518	2.857975964	0.00020212
	4632427E13Rik	2.209429133	0.000207424
	Gm16316	3.850261574	0.000445266
	Gm12064	2.967931907	0.00046968
	RP23-308M1.2	2.23360891	0.000497483
	Neat1	2.411691808	0.00066252
	Gm23346	3.2731059	0.000853068

	DLEU2_1	2.533891205	0.000893282
	2610037D02Rik	2.293055741	0.00090429
	Gpr137b-ps	1.911696372	0.000992758
	Gm17388	4.649648388	0.001055935
	Gm13171	5.594264975	0.001063637
	Gm13543	4.955253692	0.001080368
	Gm15478	3.132276391	0.001116323
	Gm15897	4.591754099	0.001322378
	Gm20506	1.999887405	0.001387329
	Gm26981	2.203437115	0.001428371
	Gm17195	3.071474284	0.001583323
	Gm12734	3.19714221	0.001613551
	Gm17414	2.331330014	0.001706457
	Gm11052	2.912597908	0.001772564
	Gm15824	4.182238288	0.001805796
	Gm26857	2.499167522	0.001821849
	RP23-93M1.3	2.766231152	0.002223009
	A730011C13Rik	2.560051398	0.002292352
	Gm15342	2.890674769	0.00242165
	Gm15334	4.256464348	0.002454759
	Gm26531	2.738309967	0.002463093
	Gm26617	2.124240023	0.002632707
	Gm26742	2.227524548	0.002694886
	Gm15765	2.643931266	0.002782114
	0610039K10Rik	11.74433708	0.002876952
	Gm15154	5.406874136	0.002980496
	Gm14052	3.02174149	0.0029832
	Gm20717	3.690336855	0.003063738
	4933426D04Rik	5.357351955	0.003378546
	Gm16023	2.189551554	0.003393034
	RP23-32A8.1	2.028176773	0.003494639
	Gm25820	6.338011442	0.003555737
	Gm20682	3.684991448	0.003602044
	RP23-224I23.1	7.417608209	0.003806439
	Gm12940	2.411520221	0.004024482
	1700099I09Rik	3.275967106	0.004075714
	Gm16305	3.307957241	0.004241203
	Gm19980	1.799601782	0.004747646
	C030005K06Rik	2.168010477	0.005006014
	Gm11805	2.983369408	0.005019858
	1700012B15Rik	1.603357771	0.005495435
	Gm17275	2.359980233	0.005505916
	Gm22304	4.347490289	0.006110111
	Gm25848	2.569071108	0.006148293
	Gm13510	3.983865544	0.006522137

	Rpl31-ps6	4.09498902	0.007240531
	Gm14335	2.63348781	0.007470624
	4933426K07Rik	2.524163198	0.007472611
	E130307A14Rik	1.993156616	0.007532921
	Gm13571	3.331657237	0.007629546
	Gm16091	2.159857865	0.007889815
	Gm13570	3.54909018	0.007968129
	Gm14853	4.61051573	0.007981705
	Mir5120	2.856865965	0.008087406
	Gm26836	2.592935332	0.008418244
	Gm26530	2.401683645	0.008714043
	Gm11655	4.266930256	0.008922059
	4930565N06Rik	2.591519262	0.009337
	Gm15367	6.818256329	0.009380237
	Gm27006	1.966796151	0.009523067
OTHER	Rnf24	2.196605835	0.000528498
	Angpt2	2.900169285	0.001541543
	BC005764	1.919530755	0.001673873
	Lrrc18	2.729916644	0.004545494
	Trpc2	1.614715894	0.006274267
	Efcab12	5.841862587	0.008085481
	H2afy3	3.412721398	0.002619242
	Setbp1	1.540851613	0.009661562
	Snap25	72.08175121	1.51E-07
	Sec61a2	2.938930987	0.001541862
	Hps5	1.627567475	0.002318163
	Kdelr3	5.756452542	0.004405829
	Sec24a	1.738556263	0.005698053
	Eif2ak4	1.640215791	0.004828028
POSTTRANSLATIONAL	Fbxo33	1.91728896	0.000594532
	Trim2	2.819847322	0.001544851
	Usp31	1.834172757	0.005403484
PROLIFERATION	Ccnt2	1.798147479	0.00326556
	Ccnl1	1.723670074	0.006301363
RNA	Rbm4	2.330635342	0.002311844
	Tnrc6c	1.652502454	0.001061403
SIGNALLING	Mtcp1	2.234393199	0.0001191
	Gnas	2.125295649	0.001903867
	Synj1	1.685791402	0.002918681
	Camk2n1	8.271429708	0.003738905
	Prex1	1.941485094	0.004172067
	Supt20	1.647597095	0.007076665
	Pkig	2.299860242	0.007840116
	Arhgap33	1.98592123	0.008402208
	Ppm1e	1.852192892	0.008754716
	Pla2g4c	2.933583458	0.008957931
	Clk4	1.67897501	0.000266637

	Appl2	2.001457941	0.005897816
	Lgr5	1.749665936	0.001810133
TRANSCRIPTION	Zfp142	1.735643654	0.000213949
	Gcfc2	1.849356289	0.000342988
	Zkscan3	1.967805562	0.000917747
	Adnp	3.308903224	0.002100786
	Aff4	1.57044068	0.003686946
	Baz2b	1.581081508	0.004676425
	Zbed6	1.754047601	0.006455156
	Kmt2a	1.777490563	0.000153691
	Ep300	1.617435479	0.000495934
	Mettl8	1.890539374	0.001209602
	Kmt2c	1.902861322	0.006733584
	Brwd1	1.712937581	0.009509292
	Taf5	1.753590873	0.009647337
TRANSPORT	Scn8a	2.985559464	0.001391662
	Atp1a3	8.434327762	0.001444815
	Slc22a23	4.575176512	0.00433535
	Slco4a1	2.310672332	0.009700075
UNKNOWN	4933408B17Rik	5.410317807	0.000242984
	D130062J21Rik	2.427757354	0.00025754
	Armc9	2.149501998	0.000425168
	Ddx26b	1.762250417	0.000529779
	Gm6104	3.31093544	0.000559432
	2610203C22Rik	6.299653481	0.000815857
	A630001G21Rik	1.564847945	0.00257137
	Xkr6	5.22397547	0.002744925
	Fam196b	2.514140993	0.004613704
	Gm17227	5.075807117	0.005449014
	Samd15	2.395885905	0.005787071
	Tanc2	2.119361771	0.005834643
	Gm9754	7.642938157	0.006785963
	Gpr137c	2.393937261	0.007520939

DATASET 3

GSEA was used to identify gene signatures that were enriched in GC B cells from Cγ1-Cre vs. *relb*$^{fl/fl}$*nfkb2*$^{fl/fl}$Cγ1-Cre mice.

GENE SET	FUNCTION	NES	NOM p-val	FDR q-val
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	CELL CYCLE	1.811999	0	0.101812
REACTOME_AUTODEGRADATION_OF_CDH1_BY_CDH1_APC_C	CELL CYCLE	1.643409	0.002915	0.136935
REACTOME_APC_C_CDH1_MEDIATED_DEGRADATION_OF_CDC20_AND_OTHER_APC_C_CDH1_TARGETED_PROTEINS_IN_LATE_MITOSIS_EARLY_G1	CELL CYCLE	1.610237	0.002849	0.161222
REACTOME_SCFSKP2_MEDIATED_DEGRADATION_OF_P27_P21	CELL CYCLE	1.55941	0.005525	0.16866
REACTOME_CHROMOSOME_MAINTENANCE	CELL CYCLE	1.591701	0	0.168939
REACTOME_CDK_MEDIATED_PHOSPHORYLATION_AND_REMOVAL_OF_CDC6	CELL CYCLE	1.572742	0.008876	0.16928
REACTOME_S_PHASE	CELL CYCLE	1.553594	0	0.172814
REACTOME_P53_INDEPENDENT_G1_S_DNA_DAMAGE_CHECKPOINT	CELL CYCLE	1.540152	0.009091	0.17295
REACTOME_CYCLIN_E_ASSOCIATED_EVENTS_DURING_G1_S_TRANSITION_	CELL CYCLE	1.549326	0.003226	0.174812
REACTOME_DEPOSITION_OF_NEW_CENPA_CONTAINING_NUCLEOSOMES_AT_THE_CENTROMERE	CELL CYCLE	1.528166	0.015015	0.176994
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	CELL CYCLE	1.534084	0.006173	0.177012
REACTOME_PACKAGING_OF_TELOMERE_ENDS	CELL CYCLE	1.531013	0.029101	0.177025
REACTOME_ASSEMBLY_OF_THE_PRE_REPLICATIVE_COMPLEX	CELL CYCLE	1.493999	0.002994	0.178501
REACTOME_MITOTIC_G1_G1_S_PHASES	CELL CYCLE	1.483746	0.013043	0.179217
REACTOME_PROCESSIVE_SYNTHESIS_ON_THE_LAGGING_STRAND	CELL CYCLE	1.500619	0.032864	0.179549
REACTOME_CELL_CYCLE	CELL CYCLE	1.491346	0	0.179684
REACTOME_SYNTHESIS_OF_DNA	CELL CYCLE	1.574351	0.00678	0.179956
REACTOME_AUTODEGRADATION_OF_THE_E3_UBIQUITIN_LIGASE_COP1	CELL CYCLE	1.522853	0	0.18023
REACTOME_SCF_BETA_TRCP_MEDIATED_DEGRADATION_OF_EMI1	CELL CYCLE	1.478241	0.026549	0.180952
REACTOME_G1_S_TRANSITION	CELL CYCLE	1.49711	0	0.181338
REACTOME_CDT1_ASSOCIATION_WITH_THE_CDC6_ORC_ORIGIN_COMPLEX	CELL CYCLE	1.473252	0.014925	0.184316

REACTOME_MEIOTIC_SYNAPSIS	CELL CYCLE	1.46446	0.015244	0.19005
REACTOME_P53_DEPENDENT_G1_DNA_DAMAGE_RESPONSE	CELL CYCLE	1.450591	0.01847	0.191076
REACTOME_DNA_STRAND_ELONGATION	CELL CYCLE	1.442521	0.04607	0.199118
REACTOME_CELL_CYCLE_MITOTIC	CELL CYCLE	1.415716	0	0.220079
REACTOME_DNA_REPLICATION	CELL CYCLE	1.416041	0	0.222733
REACTOME_M_G1_TRANSITION	CELL CYCLE	1.419341	0.019293	0.224022
REACTOME_REGULATION_OF_MITOTIC_CELL_CYCLE	CELL CYCLE	1.416288	0.021127	0.225492
REACTOME_TELOMERE_MAINTENANCE	CELL CYCLE	1.724348	0.002933	0.08386
REACTOME_APC_C_CDC20_MEDIATED_DEGRADATION_OF_MITOTIC_PROTEINS	CELL CYCLE	1.593561	0	0.173014
REACTOME_EXTENSION_OF_TELOMERES	CELL CYCLE	1.488889	0.036585	0.179661
REACTOME_TRANSLATION	METABOLISM OF PROTEINS	1.880179	0	0.046584
REACTOME_3_UTR_MEDIATED_TRANSLATIONAL_REGULATION	METABOLISM OF PROTEINS	1.811877	0	0.067875
REACTOME_PEPTIDE_CHAIN_ELONGATION	METABOLISM OF PROTEINS	1.742802	0	0.085568
REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE	METABOLISM OF PROTEINS	1.72942	0	0.089227
REACTOME_CYTOSOLIC_TRNA_AMINOACYLATION	METABOLISM OF PROTEINS	1.693283	0.004926	0.10097
REACTOME_FORMATION_OF_THE_TERNARY_COMPLEX_AND_SUBSEQUENTLY_THE_43S_COMPLEX	METABOLISM OF PROTEINS	1.74659	0.005222	0.112031
REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S	METABOLISM OF PROTEINS	1.747	0	0.139006
REACTOME_METABOLISM_OF_PROTEINS	METABOLISM OF PROTEINS	1.521416	0	0.177564
REACTOME_MITOCHONDRIAL_PROTEIN_IMPORT	METABOLISM OF PROTEINS	1.505901	0.011396	0.187969
REACTOME_AMYLOIDS	METABOLISM OF PROTEINS	1.424508	0.010453	0.219171
REACTOME_TRNA_AMINOACYLATION	METABOLISM OF PROTEINS	1.392996	0.030055	0.238175
KEGG_RIBOSOME	METABOLISM OF PROTEINS	1.754189	0	0.13791
KEGG_PROTEASOME	METABOLISM OF PROTEINS	1.693096	0	0.109451
STRUCTURAL_CONSTITUENT_OF_RIBOSOME	METABOLISM OF PROTEINS	1.725146	0	0.109363
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS_	METABOLISM	1.619906	0	0.153153
REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT	METABOLISM	1.623287	0	0.155189
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT	METABOLISM	1.560152	0.003086	0.173101
REACTOME_PYRIMIDINE_METABOLISM	METABOLISM	1.573597	0.022059	0.174455
REACTOME_CITRIC_ACID_CYCLE_TCA_CYCLE	METABOLISM	1.503968	0.026829	0.182254
REACTOME_REGULATION_OF_ORNITHINE_DECARBOXYLASE_ODC	METABOLISM	1.413184	0.027933	0.220742

REACTOME_DESTABILIZATION_OF_MRNA_BY_AUF1_HNRNP_D0	METABOLISM	1.399007	0.026239	0.23185
KEGG_CITRATE_CYCLE_TCA_CYCLE	METABOLISM	1.729864	0.002618	0.10381
KEGG_PYRIMIDINE_METABOLISM	METABOLISM	1.614906	0.00346	0.201364
SULFOTRANSFERASE_ACTIVITY	METABOLISM	1.698125	0.005168	0.118615
TRANSFERASE_ACTIVITY_TRANSFERRING_SULFUR_CONTAINING_GROUPS	METABOLISM	1.660357	0	0.151372
ELECTRON_CARRIER_ACTIVITY	METABOLISM	1.603336	0	0.239768
REACTOME_RNA_POL_I_PROMOTER_OPENING	GENE EXPRESSION	1.746555	0	0.093359
REACTOME_NONSENSE_MEDIATED_DECAY_ENHANCED_BY_THE_EXON_JUNCTION_COMPLEX	GENE EXPRESSION	1.634397	0	0.142894
REACTOME_ABORTIVE_ELONGATION_OF_HIV1_TRANSCRIPT_IN_THE_ABSENCE_OF_TAT	GENE EXPRESSION	1.563651	0.029851	0.173377
REACTOME_METABOLISM_OF_MRNA	GENE EXPRESSION	1.5039	0	0.178465
REACTOME_RNA_POL_I_TRANSCRIPTION	GENE EXPRESSION	1.48429	0.022801	0.181888
REACTOME_METABOLISM_OF_RNA	GENE EXPRESSION	1.504446	0	0.185749
REACTOME_FORMATION_OF_RNA_POL_II_ELONGATION_COMPLEX_	GENE EXPRESSION	1.463474	0.035616	0.188364
REACTOME_MRNA_SPLICING_MINOR_PATHWAY	GENE EXPRESSION	1.50774	0.00554	0.189572
REACTOME_ELONGATION_ARREST_AND_RECOVERY	GENE EXPRESSION	1.454108	0.039702	0.191917
REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G	OTHER	1.684134	0	0.096182
REACTOME_INFLUENZA_LIFE_CYCLE	OTHER	1.56953	0	0.169132
REACTOME_HOST_INTERACTIONS_OF_HIV_FACTORS	OTHER	1.496161	0.00722	0.179198
REACTOME_RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA2_	OTHER	1.577411	0	0.181002
REACTOME_HIV_INFECTION	OTHER	1.452097	0.009524	0.191694
REACTOME_FORMATION_OF_THE_HIV1_EARLY_ELONGATION_COMPLEX	OTHER	1.435967	0.040302	0.205031
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	OTHER	1.59042	0	0.212275
REACTOME_CROSS_PRESENTATION_OF_SOLUBLE_EXOGENOUS_ANTIGENS_ENDOSOMES	IMMUNE SYSTEM	1.546908	0.008219	0.173392
REACTOME_ANTIGEN_PROCESSING_CROSS_PRESENTATION	IMMUNE SYSTEM	1.540556	0.002817	0.177592
REACTOME_CLASS_I_MHC_MEDIATED_ANTIGEN_PROCESSING_PRESENTATION	IMMUNE SYSTEM	1.461016	0	0.188421
REACTOME_ER_PHAGOSOME_PATHWAY	IMMUNE SYSTEM	1.508333	0.011594	0.193269
REACTOME_ANTIGEN_PROCESSING_UBIQUITINATION_PROTEASOME_DEGRADATION	IMMUNE SYSTEM	1.403658	0.004739	0.229529
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING	SIGNAL TRANSDUCTION	1.698985	0.002793	0.104335
REACTOME_SIGNALING_BY_WNT	SIGNAL TRANSDUCTION	1.482521	0.006623	0.177773
REACTOME_REGULATION_OF_IFNA_SIGNALING	SIGNAL TRANSDUCTION	1.595888	0.016787	0.178083

REACTOME_PKB_MEDIATED_EVENTS	SIGNAL TRANSDUCTION	1.456919	0.037736	0.191353
REACTOME_REGULATION_OF_APOPTOSIS	PROGRAMMED CELL DEATH	1.688258	0	0.099712
REACTOME_APOPTOSIS	PROGRAMMED CELL DEATH	1.591388	0	0.162688
REACTOME_APOPTOTIC_EXECUTION_PHASE	PROGRAMMED CELL DEATH	1.472708	0.017143	0.181795
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	TRAFFICKING	1.679678	0.002786	0.092972
CHEMOKINE_RECEPTOR_BINDING	TRAFFICKING	1.800605	0	0.047045
CHEMOKINE_ACTIVITY	TRAFFICKING	1.81638	0.002577	0.07279