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Supplemental Information

A Pathway Switch Directs BAFF Signaling to Distinct NFκB Transcription Factors in Maturing and Proliferating B Cells

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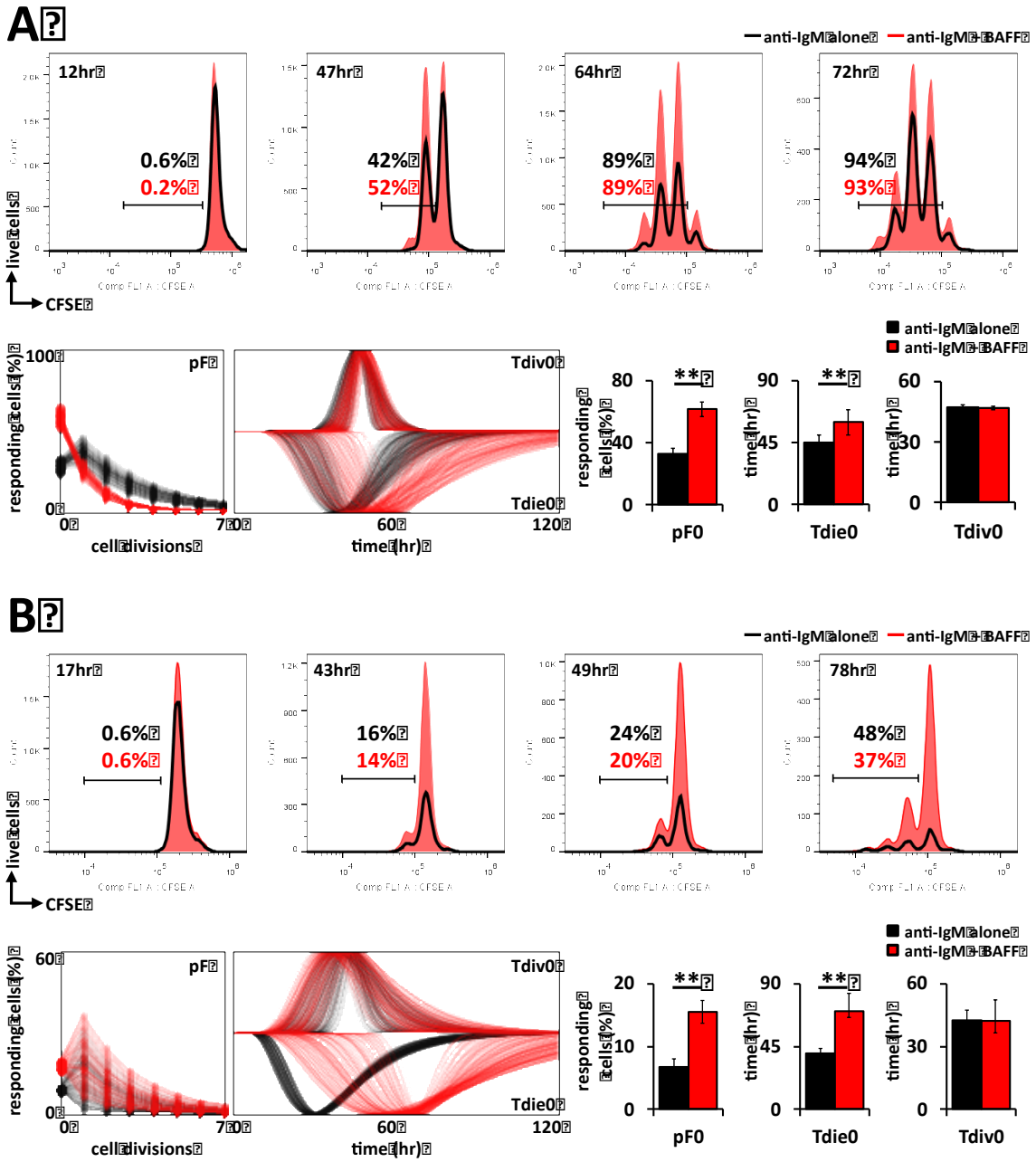


Figure S1. (relates to Figure 1)
Replicate analysis of *wild type* B cell expansion stimulated with anti-IgM and BAFF
(A) *In vitro* proliferation of CFSE labeled wild type B cells and stimulated for three days with 10μg/ml anti-IgM alone (black) or 10μg/ml anti-IgM + 50ng/ml BAFF ligand (red) and analyzed by FACS. Live cell numbers gated by exclusion of 7AADHi population. Gates represent B cells that have undergone at least on cell division. Lower left: Maximum likelihood cell parameters that underlie the population dynamics produced by anti-IgM (black) or anti-IgM + BAFF (red), as derived with FlowMax software tool: the fraction of responding cells at each generation, histograms of the time to division or cell death of the first generation. Lower right: the fraction of generation zero B cells undergoing division (pF0), the average time to division of generation zero (Tdiv0), and average time to death of generation zero (Tdie0) cells.
(B) Same as (A), except using 5μg/ml of anti-IgM (black) or 5μg/ml anti-IgM + 50ng/ml BAFF (red).

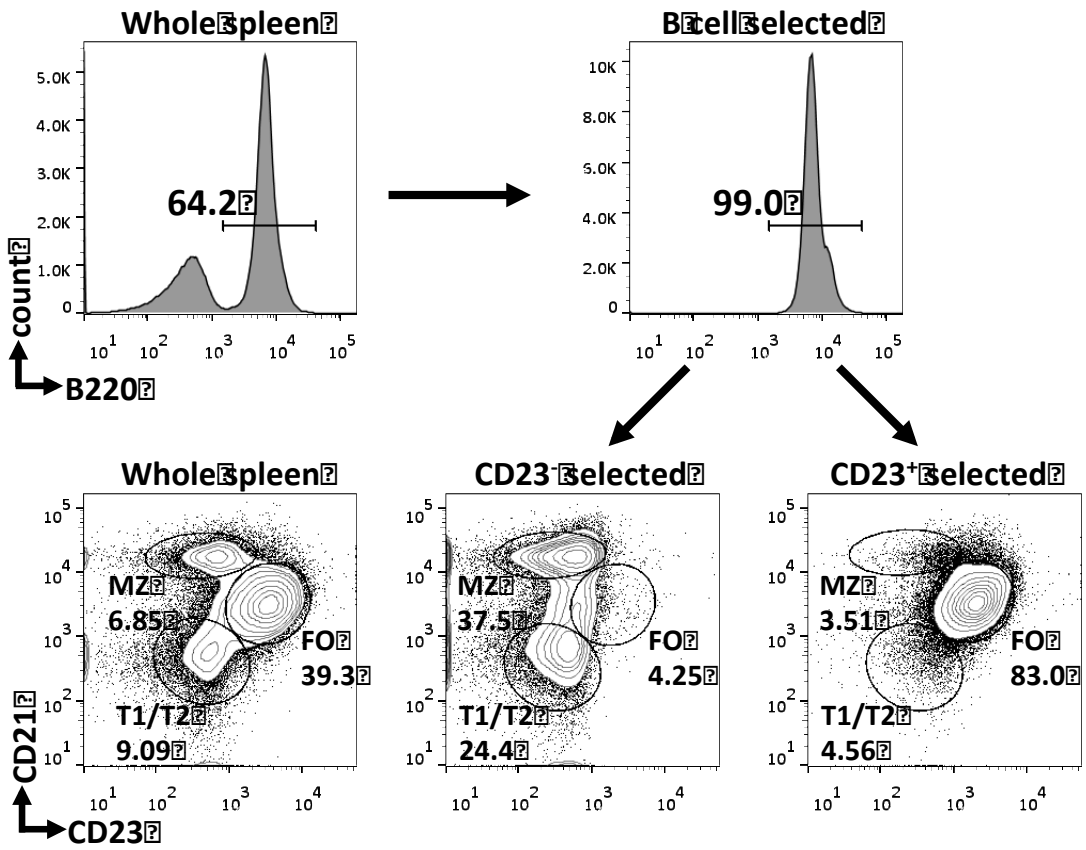


Figure S2. (relates to Figure 3)
Strategy for isolating a homogenous population of follicular mature B cells from spleen. Representative FACS plot of whole spleen B cells before separation (top, left panel) followed by B cell isolation using antibody cocktail consisting of biotin-conjugated monoclonal anti-mouse antibodies against CD43, CD4, CD93, and Ter119 (top, right panel). Resulting B cells are further separated using CD23 MicroBeads. Total splenic B cell populations (bottom, left panel), isolated immature (T1/T2) and marginal zone (MZ) $CD23^-$ population (bottom, middle panel) and purified mature follicular (FO) B cells are shown (bottom, right panel).

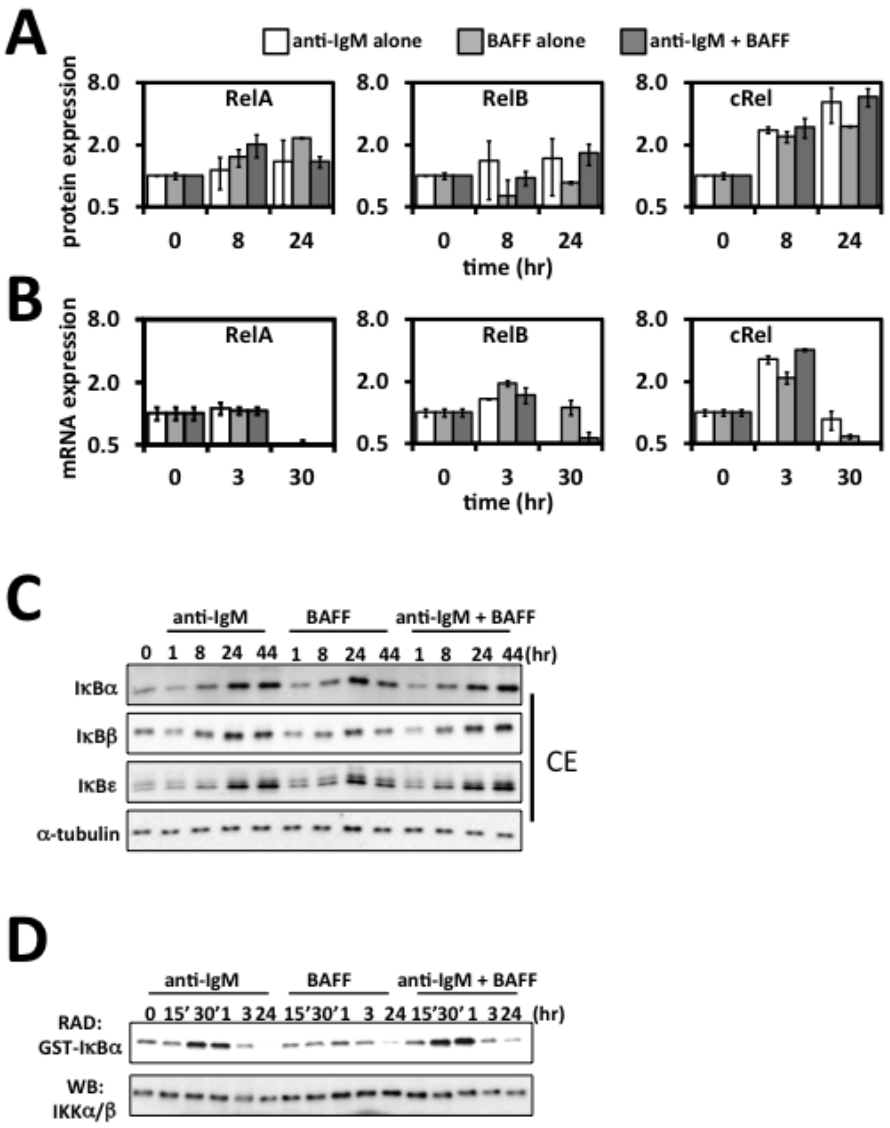


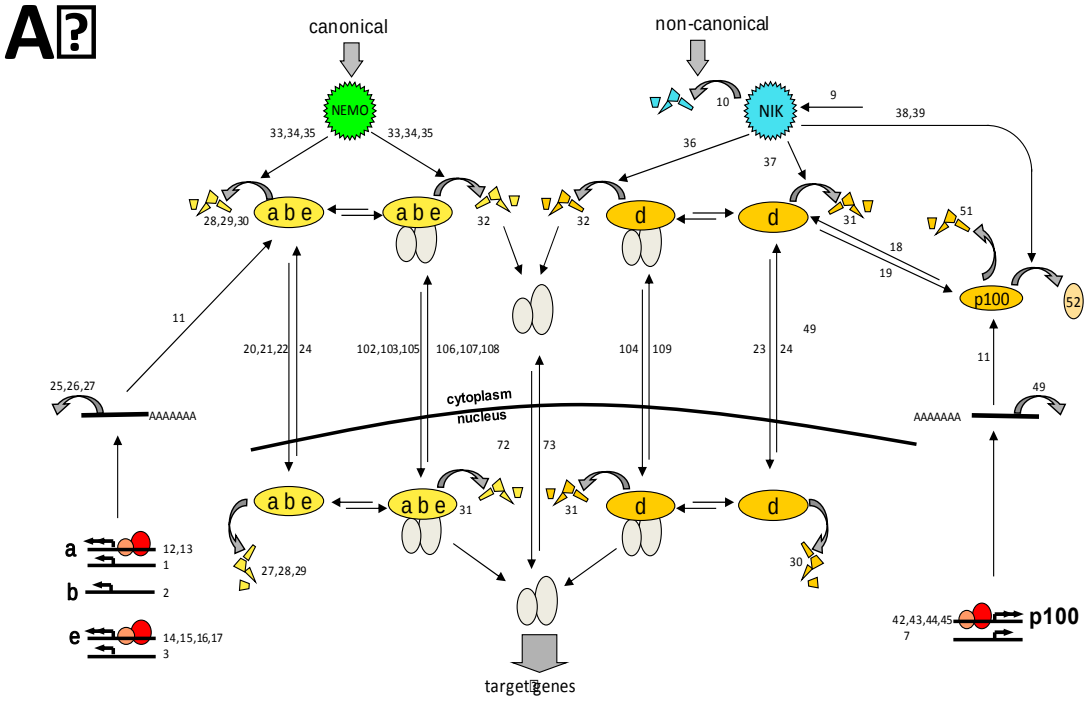
Figure S3. (relates to Figure 2 and 4)
Quantitative experimental data of IKK-NFκB signaling in B-cells used to constrain the parameterization of the mathematical model

(A) Expression profiles of NFκB family proteins in B cells.
Quantification of immunoblots for RelA, RelB, and cRel protein levels in anti-IgM alone, BAFF alone, or anti-IgM + BAFF stimulated *wild type* B cells (N=4) S.D.

(B) Expression profiles of NFκB family mRNAs in B cells. qPCR analysis of RelA, RelB, and cRel mRNA levels in anti-IgM alone, BAFF alone, or anti-IgM + BAFF stimulated *wild type* B cells (N=3) S.D.

(C) BAFF co-stimulation has little effect on BCR-induced canonical NFκB pathway activity. Canonical IκBs protein levels of IκBα, IκBβ, and IκBε in stimulated *wild type* B cells were measured by immunoblot upon stimulation with anti-IgM alone, BAFF alone, or anti-IgM and BAFF co-stimulation as indicated.

(D) NEMO-associated kinase activity was determined in *wild type* B cells in an *in vitro* IP-kinase assay upon stimulation with anti-IgM alone, BAFF alone, or anti-IgM and BAFF co-stimulation as indicated.



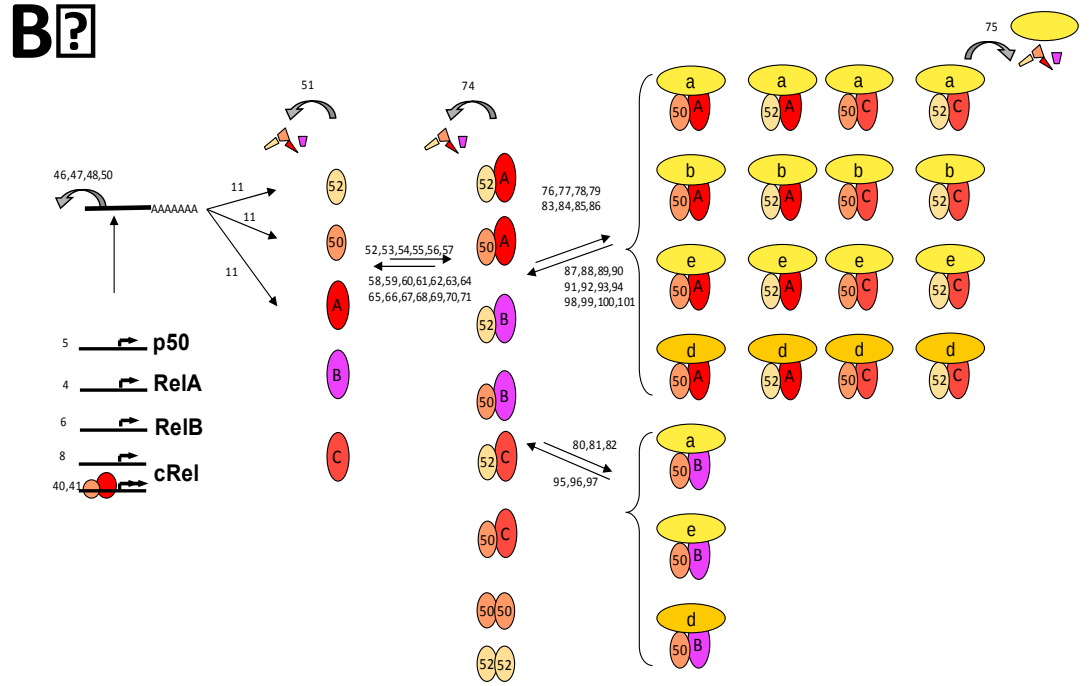


Figure S4. (relates to Figure 4)
Wiring diagram of the NFκB signaling system depicting biochemical reactions described in the mathematical model

(A) A schematic of the IκB network that includes both the canonical pathway through NEMO/IKK2 activity and the non-canonical pathway through NIK/IKK1. Four IκBs interact with a general NFκB dimer (grey) that is then further described in (b). p100 is a substrate for both dimerization to IκBδ and NIK/IKK1-dependent processing to p52.

(B) A schematic of the NFκB network: monomers and dimers as well as IκB-NFκB complexes whose synthesis and degradation is accounted for by the mathematical model.

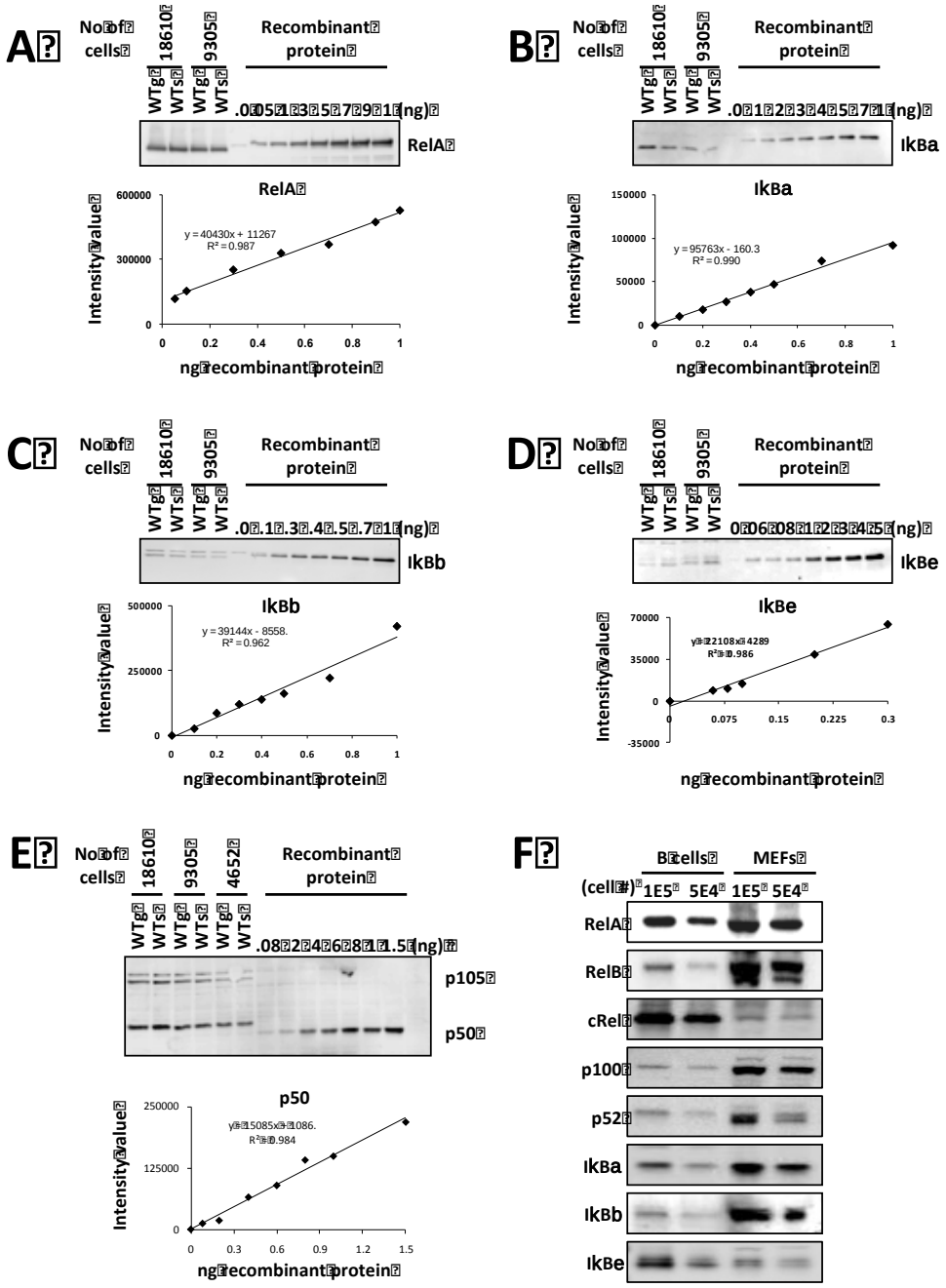


Figure S5. (relates to Figure 4, Table S3)
Determining NFκB and IκB protein levels in MEFs and B-cells
(A-E) Immunoblots of indicated numbers of wild type fibroblast extracts (growing or serum starved) compared with recombinant protein standards diluted in whole cell extracts prepared from mutant fibroblasts deficient in the respective protein. Signal intensities were quantitated by a phosphoimager and a best fit linear standard curve was graphed. Amounts of NFκB protein in MEFS were calculated based on this standard curve.
(F) Immunoblot of wild type B cells and wild type fibroblasts for the basal levels of RelA, RelB, cRel, p100, p52, IκBα, IκBβ, and IκBε. This was used in determining the basal levels of protein abundances in B cells compared to MEFs for the initial parameterization of the B-cell model. Gel images in are representative of three experiments. The quantitated concentrations andc molecule numbers are summarized in Table S3.

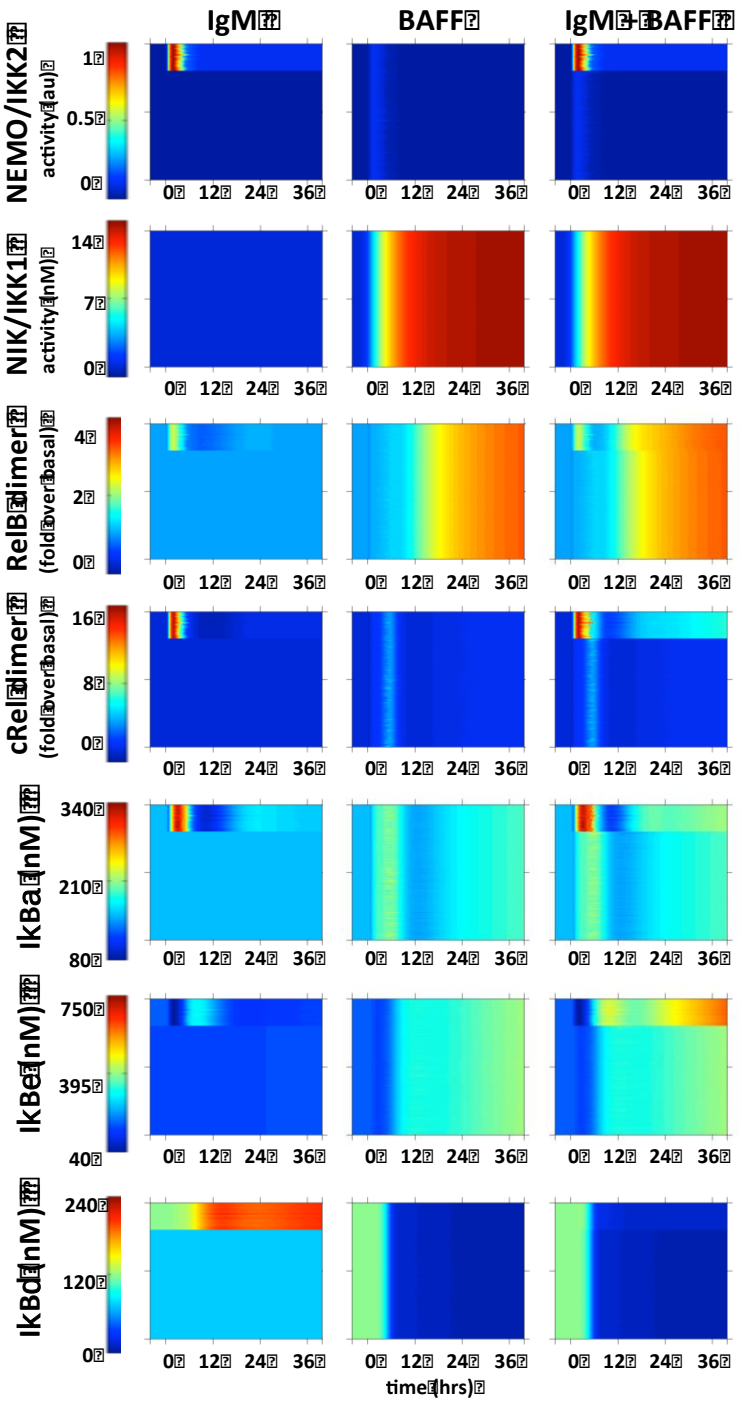


Figure S6. (relates to Figure 4)
Single cell simulation of anti-IgM, BAFF, and co-stimulation of the B cell NFκB model
Timecourses depicting IKK activity (model simulation input) in the top row and calculated timecourses in subsequent rows, specifically nuclear DNA binding activities of RelB and cRel, and abundances of IκBα, IκBε, and IκBδ. Each graph shows the results of simulations of 1000 B cells for each condition (anti-IgM, BAFF, and co-stimulation). Cells vary in whether they activate IKK or not, and when the IKK activity is activated. Individual simulations tracks are shown, as well as the average (dashed line).

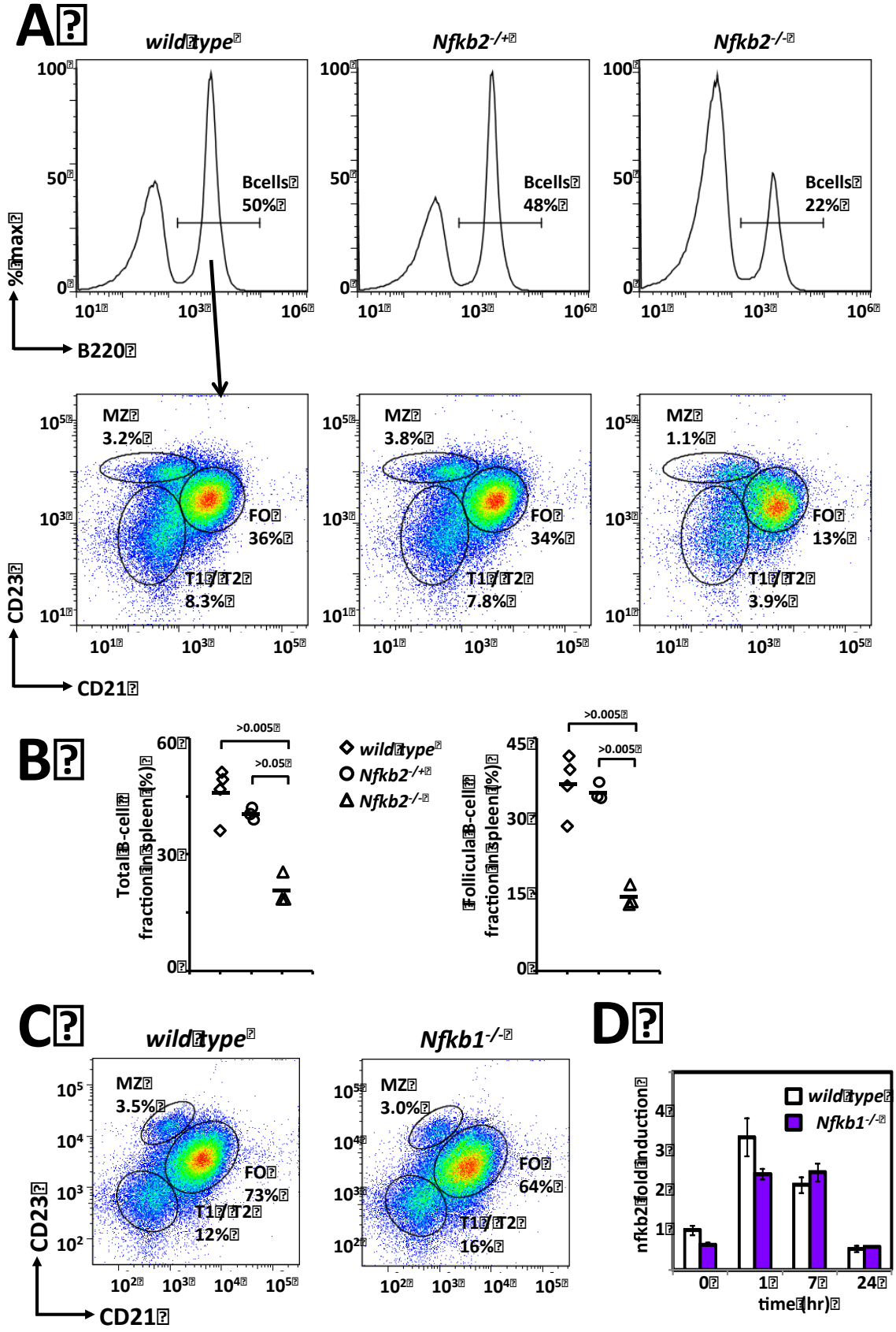

A
wild type
Nfkb2-/+
Nfkb2-/-
Bcells 50%
Bcells 48%
Bcells 22%
%max
B220
MZ 3.2%
FO 36%
T1/T2 8.3%
MZ 3.8%
FO 34%
T1/T2 7.8%
MZ 1.1%
FO 13%
T1/T2 3.9%
CD23
CD21
B
Total B-cell fraction in spleen (%)
Follicular B-cell fraction in spleen (%)
>0.005
>0.05
wild type
Nfkb2-/+
Nfkb2-/-
C
wild type
Nfkb1-/-
MZ 3.5%
FO 73%
T1/T2 12%
MZ 3.0%
FO 64%
T1/T2 16%
CD23
CD21
D
wild type
Nfkb1-/-
nfkb2 fold induction
time (hr)

Figure S7. (relates to Figure 5 and 6)
Normal B cell development in *Nfkb2*$^{+/-}$ and *Nfkb1*$^{-/-}$ mice
(A) B cell development is defective in *Nfkb2*$^{-/-}$ mice, but unaffected in *Nfkb2*$^{+/-}$ mice. Splenocytes were stained with anti-B220, anti-CD21, and anti-CD23. B cells populations are gated as B220^{+} (top row). For cells gated on B220^{+}, marginal zone B cells (CD21^{hi}CD23^{lo}), follicular B cells (CD21^{lo}CD23^{hi}) and transitional 1 and transitional 2 B cells (CD21^{lo}CD23^{lo}) are shown (bottom row). Representative data of 4 mice shown.
(B) The numbers of total and FO B cells obtained from (A) are displayed graphically.
(C) Normal B cell development in *Nfkb1*$^{-/-}$ mice. Splenocytes were stained with anti-B220, anti-CD21, and anti-CD23. B cells populations are gated as B220^{+} (not shown). For cells gated on B220^{+}, marginal zone B cells (CD21^{hi}CD23^{lo}), follicular B cells (CD21^{lo}CD23^{hi}) and transitional 1 and transitional 2 B cells (CD21^{lo}CD23^{lo}) are shown. Representative data of 3 mice shown.
(D) *Nfkb2* gene expression was monitored by qPCR in *wild type* and *nfkb1*$^{-/-}$ B cells stimulated with anti-IgM (n=3).

Table S1. (relates to Figure 4)
Reactions and Parameters
Model parameters and biochemical rate constants. Parameter identifiers (column 4 and supplementary Figure 4) are related to reaction descriptions and reaction rate constants.

Reaction	Location	Parameter No.	Parameter Value	Category	Source
Reaction rates determined by transcriptional programs and cytokine levels					
=>tIκBα (basal)	Nucleus	1	4.8e-3 nM/min	IκB Synth.	Parameter value chosen to fit mRNA and protein Expression profiles as measure by RNase Protection (RPA) and Western blot assays, reformulated from Werner et al. (2008) to fit a Hill function
=>tIκBβ (basal)	Nucleus	2	1.2e-3 nM/min	IκB Synth.	*Refer to #1*
=>tIκBε (basal)	Nucleus	3	1.2e-4 nM/min	IκB Synth.	*Refer to #1*
=>tRelA (basal)	Nucleus	4	3.6e-5 nM/min	NFκB Synth.	*Refer to #1*
=>tp50 (basal)	Nucleus	5	2.9e-5 nM/min	NFκB Synth.	*Refer to #1*
=>tRelB (basal)	Nucleus	6	4.2e-5 nM/min	NFκB Synth.	*Refer to #1*/Fitted
=>tp100 (basal)	Nucleus	7	8e-7 nM/min	NFκB Synth.	*Refer to #1*
=>cRel (basal)	Nucleus	8	3.6e-6 nM/min	NFκB Synth.	*Refer to #1*/Fitted
=> NIK	Cytoplasm	9	4.2e-2 nM/min	NIK Synth.	Set to yield measured abundance in conjunction with #10
NIK =>	Cytoplasm	10	4.6e-2	NIK Deg.	Based on estimated 15-minute half-life/ Fitted
IkB Reactions					
mRNA => mRNA + protein	Nuc - >Cyt	11	12 proteins/ mRNA/min	Translation	Derived from the elongation rate of the ribosome and corrected for the nucleotide spacing between adjacent ribosomes on the same transcript 1800 nt min^{-1} / 150 nt = 12 min^{-1}
=>tIκBα (A50/A52-induced)	Nucleus	12	200 Fold over consitutive	IκB Synth.	*Refer to #1*
Hill K_d (A50/A52-induced)	Nucleus	13	150 nM	IκB Synth.	*Refer to #1*
=>tIκBε (A50/A52-induced, 37 min. delay)	Nucleus	14	25 Fold over consitutive	IκB Synth.	*Refer to #1*
Hill K_d (A50/A52-induced)	Nucleus	15	150 nM	IκB Synth.	*Refer to #1*
=>tIκBε (C50/C52-induced, 37 min. delay)	Nucleus	16	250 Fold over consitutive	IκB Synth.	*Refer to #1*
Hill K_d (C50/C52-induced)	Nucleus	17	150 nM	IκB Synth.	*Refer to #1*
p100 + p100 =>IκBδ	Cyt, Nuc	18	1.2e-2 nM^{-1} min^{-1}	IκB Synth.	Estimated a $K_d$of 10nM
IκBδ => p100 + p100	Cyt, Nuc	19	1.2e-2 min^{-1}	IκB Synth.	*Refer to #19*
IκBα(c) =>IκBα(n)	Cyt ->Nuc	20	6.0e-2 min^{-1}	Transport	Adapted from (Shih et al., 2009)

IκBβ(c) =>IκBβ(n)	Cyt ->Nuc	21	9.0-3 min^{-1}	Transport	(Shih et al., 2009)
IκBε(c) =>IκBε(n)	Cyt ->Nuc	22	4.5e-2 min^{-1}	Transport	(Shih et al., 2009)
IκBδ(c) =>IκBδ(n)	Cyt ->Nuc	23	4.5e-2 min^{-1}	Transport	(Shih et al., 2009)
IκB[α/β/ε/δ](n) =>IκB[α/β/ε/δ](c)	Nuc - >Cyt	24	1.2e-2 min^{-1}	Transport	(Shih et al., 2009)
tIκBα =>	Nucleus	25	2.9e-2 min^{-1}	IκB Deg.	mRNA half-life measurements using actinomycin-D treatment of cells and RPA (unpublished results)
tIκBβ =>	Nucleus	26	2.9e-3 min^{-1}	IκB Deg.	*Refer to #25*
tIκBε =>	Nucleus	27	3.8e-3 min^{-1}	IκB Deg.	*Refer to #25*
IκBα =>	Cyt, Nuc	28	0.12 min^{-1}	IκB Deg.	(Shih et al., 2009)
IκBβ =>	Cyt, Nuc	29	0.12 min^{-1}	IκB Deg.	(Shih et al., 2009)
IκBε =>	Cyt, Nuc	30	1.2e-2 min^{-1}	IκB Deg.	Based on estimated 1 hour half-life
IκBδ =>	Cyt, Nuc	31	3e-3 min^{-1}	IκB Deg.	Based on estimated 4 hour half-life
IκB[α/β/ε/δ]-NFκB =>NFκB	Cyt, Nuc	32	2.4e-4 min^{-1}	IκB Deg.	Based on estimated 48 hour half-life
IκBα => (NEMO-mediated)	Cytoplasm	33	1.4e-3 nM^{-1} min^{-1}	IκB Deg.	Based on measured IκB degradation timecourses given numerical input curves
IκBαNFκB =>NFκB (NEMO-mediated)	Cytoplasm	33	1.4e-3 nM^{-1} min^{-1}	IκB Deg.	
IκBβ => (NEMO-mediated)	Cytoplasm	34	4.5e-4 nM^{-1} min^{-1}	IκB Deg.	*Refer to # 33*
IκBβNFκB =>NFκB (NEMO-mediated)	Cytoplasm	34	4.5e-4 nM^{-1} min^{-1}	IκB Deg.	*Refer to # 33*
IκBε => (NEMO-mediated)	Cytoplasm	35	3.4e-4 nM^{-1} min^{-1}	IκB Deg.	*Refer to # 33*
IκBεNFκB =>NFκB (NEMO-mediated)	Cytoplasm	35	3.4e-4 nM^{-1} min^{-1}	IκB Deg.	*Refer to # 33*
IκBδ => (NIK-mediated)	Cytoplasm	36	0.6 nM^{-1} min^{-1}	IκB Deg.	V_{max} and K_m of NIK-mediated reactions based on protein degradation and estimated NIK abundances.
IκBδNFκB =>NFκB (NIK-mediated)	Cytoplasm	36	0.6 nM^{-1} min^{-1}	IκB Deg.	
IκBδ => (NIK-mediated, K_m)	Cytoplasm	37	100 nM	IκB Deg.	*Refer to #36*
NF-κB reactions					
p100 => p52 (NIK-mediated)	Cytoplasm	38	5.0e-2 nM^{-1} min^{-1}	NFκB Synth.	*Refer to #36*
p100 => p52 (NIK-mediated, p100 K_m)	Cytoplasm	39	10 nM	NFκB Synth.	*Refer to #36*
=>cRel (A50/A52/C50/C52-induced, 1 hr delay)	Nucleus	40	200 Fold over consitutive	NFκB Synth.	*Refer to #1/Fitted*

Hill K_d (A50/A52/C50/C52-induced)	Nucleus	41	150 nM	NFκB Synth.	*Refer to #1/Fitted*
=>tp100 (A50/A52-induced, 4 hr delay)	Nucleus	42	1000 Fold over consitutive	NFκB Synth.	*Refer to #1/Fitted*
Hill K_d (A50/A52-induced)	Nucleus	43	50 nM	NFκB Synth.	*Refer to #1/Fitted*
=>tp100 (C50/C52-induced, 4 hr delay)	Nucleus	44	1500 Fold over consitutive	NFκB Synth.	*Refer to #1/Fitted*
Hill K_d (C50/C52-induced)	Nucleus	45	50 nM	NFκB Synth.	*Refer to #1/Fitted*
tRelA =>	Nucleus	46	2.9e-3 min^{-1}	NFκB Deg.	*Refer to #25*
tp50 =>	Nucleus	47	2.9e-3 min^{-1}	NFκB Deg.	*Refer to #25*
tRelB =>	Nucleus	48	2.9e-3 min^{-1}	NFκB Deg.	*Refer to #25*
tp100 =>	Nucleus	49	9.6e-4 min^{-1}	NFκB Deg.	*Refer to #25*
tcRel=>	Nucleus	50	9.6e-4 min^{-1}	NFκB Deg.	*Refer to #25*
RelA =>	Cyt, Nuc	51	2.3e-2 min^{-1}	NFκB Deg.	Based on estimated 0.5 hour half-life of NF-κB monomers
p50 =>	Cyt, Nuc	51	2.3e-2 min^{-1}	NFκB Deg.	
RelB =>	Cyt, Nuc	51	2.3e-2 min^{-1}	NFκB Deg.	
p100 =>	Cyt, Nuc	51	2.3e-2 min^{-1}	NFκB Deg.	
cRel =>	Cyt, Nuc	51	2.3e-2 min^{-1}	NFκB Deg.	
p52 =>	Cyt, Nuc	51	2.3e-2 min^{-1}	NFκB Deg.	
RelA + p50 => RelAp50	Cyt, Nuc	52	1.9e-3 nM^{-1} min^{-1}	NFκB Synth.	Based on dimerization studies (unpublished results)
RelA + p52 => RelAp52	Cyt, Nuc	52	1.9e-3 nM^{-1} min^{-1}	NFκB Synth.	
RelB + p52 => RelBp52	Cyt, Nuc	53	9.6e-4 nM^{-1} min^{-1}	NFκB Synth.	
RelB + p50 => RelBp50	Cyt, Nuc	53	3e-4 nM^{-1} min^{-1}	NFκB Synth.	
cRel + p50 => cRelp50	Cyt, Nuc	54	9.6e-4 nM^{-1} min^{-1}	NFκB Synth.	
cRel + p52 => cRelp52	Cyt, Nuc	55	1.9e-3 nM^{-1} min^{-1}	NFκB Synth.	
p50 + p50 => p50p50	Cyt, Nuc	56	1.8e-3 nM^{-1} min^{-1}	NFκB Synth.	
p52+ p52 => p52p52	Cyt, Nuc	57	1.8e-3 nM^{-1} min^{-1}	NFκB Synth.	
RelAp50 => RelA + p50	Cyt	58	1.9e-2 min^{-1}	NFκB Synth.	Based on dimerization studies (unpublished results)
RelAp52 => RelA + p52	Cyt	59	3.8e-2min^{-1}	NFκB Synth.	
RelBp52 => RelB + p52	Cyt	60	1.4e-2 min^{-1}	NFκB Synth.	
RelBp50 => RelB + p50	Cyt	61	4.6e-3 min^{-1}	NFκB Synth.	
cRelp50 =>cRel + p50	Cyt	62	1.4e-3 min^{-1}	NFκB Synth.	
cRelp52 =>cRel + p52	Cyt	63	1.4e-3min^{-1}	NFκB Synth.	
RelAp50 => RelA + p50	Nuc	64	1.9e-3 min^{-1}	NFκB Synth.	Estimated 10 fold higher affinity due to DNA binding
RelAp52 => RelA + p52	Nuc	65	3.8e-3min^{-1}	NFκB Synth.	*Refer to #64*

RelBp52 => RelB + p52	Nuc	66	1.4e-3 min^{-1}	NFκB Synth.	*Refer to #64*
RelBp50 => RelB + p50	Nuc	67	4.6e-3 min^{-1}	NFκB Synth.	*Refer to #64*
cRelp50 =>cRel + p50	Nuc	68	1.4e-4 min^{-1}	NFκB Synth.	*Refer to #64*
cRelp52 =>cRel + p52	Nuc	69	1.4e-4min^{-1}	NFκB Synth.	*Refer to #64*
p50p50 => p50 + p50	Cyt, Nuc	70	5.4e-2min^{-1}	NFκB Synth.	Based on dimerization studies (unpublished results)
p52p52 => p52+ p52	Cyt, Nuc	71	5.4e-2min^{-1}	NFκB Synth.	Based on dimerization studies (unpublished results)
RelAp50(c) =>RelAp50(n)	Cyt ->Nuc	72	5.4 min^{-1}	Transport	(Shih et al., 2009)
RelAp52(c) =>RelAp52(n)	Cyt ->Nuc	72	5.4 min^{-1}	Transport	(Shih et al., 2009)
RelBp52(c) =>RelBp52(n)	Cyt ->Nuc	72	5.4 min^{-1}	Transport	(Shih et al., 2009)
RelBp50(c) =>RelBp50(n)	Cyt ->Nuc	72	5.4 min^{-1}	Transport	(Shih et al., 2009)
cRelp50(c) => cRelp50(n)	Cyt ->Nuc	72	5.4 min^{-1}	Transport	(Shih et al., 2009)
cRelp52(c) => cRelp52(n)	Cyt ->Nuc	72	5.4 min^{-1}	Transport	(Shih et al., 2009)
p50p50(c) => p50p50(n)	Cyt ->Nuc	72	5.4 min^{-1}	Transport	(Shih et al., 2009)
p52p52(c) => p52p52(n)	Cyt ->Nuc	72	5.4 min^{-1}	Transport	(Shih et al., 2009)
NFκB(n) =>NFκB(c)	Nuc ->Cyt	73	4.8e-3 min^{-1}	Transport	(Shih et al., 2009)
RelAp50 =>	Cyt, Nuc	74	2.4e-4 min^{-1}	NFκB Deg.	Based on estimated 48 hour half-life
RelAp52 =>	Cyt, Nuc	74	2.4e-4 min^{-1}	NFκB Deg.	
RelBp50 =>	Cyt, Nuc	74	2.4e-4 min^{-1}	NFκB Deg.	
RelBp52 =>	Cyt, Nuc	74	2.4e-4 min^{-1}	NFκB Deg.	
cRelp50 =>	Cyt, Nuc	74	2.4e-4 min^{-1}	NFκB Deg.	
cRelp52 =>	Cyt, Nuc	74	2.4e-4 min^{-1}	NFκB Deg.	
p50p50 =>	Cyt, Nuc	74	2.4e-4 min^{-1}	NFκB Deg.	
p52p52 =>	Cyt, Nuc	74	2.4e-4 min^{-1}	NFκB Deg.	
IκB[α/β/ε/δ]-NFκB =>IκB[α/β/ε/δ]	Cyt, Nuc	75	2.4e-4 min^{-1}	NFκB Deg.	*Refer to #74*
IκB:NF-κB interactions					
IκBα + RelA:p50 =>IκBα:RelA:p50	Cyt, Nuc	76	3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBβ + RelA:p50 =>IκBβ:RelA:p50	Cyt, Nuc	77	2e-4 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBε + RelA:p50 => IκBε:RelA:p50	Cyt, Nuc	78	1.3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBδ + RelA:p50 => IκBδ:RelA:p50	Cyt, Nuc	79	6e-4 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBα + RelA:p52 =>IκBα:RelA:p52	Cyt, Nuc	76	3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBβ + RelA:p52 =>IκBβ:RelA:p52	Cyt, Nuc	77	2e-4 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBε + RelA:p52 => IκBε:RelA:p52	Cyt, Nuc	78	1.3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBδ + RelA:p52 => IκBδ:RelA:p52	Cyt, Nuc	79	6e-4 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*

IκBα + RelB:p50 =>IκBα:RelB:p50	Cyt, Nuc	80	1.3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBε + RelB:p50 => IκBε:RelB:p50	Cyt, Nuc	81	1.3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBδ + RelB:p50 => IκBδ:RelB:p50	Cyt, Nuc	82	6e-4 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBα + cRel:p50 =>IκBα:cRel:p50	Cyt, Nuc	83	3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBβ +cRel:p50 =>IκBβ:cRel:p50	Cyt, Nuc	84	2.1e-4 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBε + cRel:p50 => IκBε:cRel:p50	Cyt, Nuc	85	1.3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBδ + cRel:p50 => IκBδ:cRel:p50	Cyt, Nuc	86	1.98e-2 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBα + cRel:p52 =>IκBα:RelA:p52	Cyt, Nuc	83	3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBβ + cRel:p52 =>IκBβ:cRel:p52	Cyt, Nuc	84	2.1e-4 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBε + cRelp52 => IκBε:cRel:p52	Cyt, Nuc	85	1.3e-3 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBδ + cRel:p52 => IκBδ:cRel:p52	Cyt, Nuc	86	1.98e-2 nM^{-1} min^{-1}	IκB-NFκB interaction	Adapted from *Alves et. al 2013*
IκBα:RelA:p50 =>IκBα + RelA:p50	Cyt, Nuc	87	6e-4 min^{-1}	IκB-NFκB interaction	Fitted (dependent on 76-79)
IκBβ:RelA:p50 =>IκBβ + RelA:p50	Cyt, Nuc	88	1.7e-2 min^{-1}	IκB-NFκB interaction	
IκBε:RelA:p50 =>IκBε + RelA:p50	Cyt, Nuc	89	6e-3 min^{-1}	IκB-NFκB interaction	
IκBδ:RelA:p50 =>IκBδ + RelA:p50	Cyt, Nuc	90	8.4e-4 min^{-1}	IκB-NFκB interaction	
IκBα:RelA:p52 =>IκBα + RelA:p52	Cyt, Nuc	91	6e-4 min^{-1}	IκB-NFκB interaction	Fitted (dependent on 76-79)
IκBβ:RelA:p52 =>IκBβ + RelA:p52	Cyt, Nuc	92	1.7e-2 min^{-1}	IκB-NFκB interaction	
IκBε:RelA:p52 =>IκBε + RelA:p52	Cyt, Nuc	93	6e-3 min^{-1}	IκB-NFκB interaction	
IκBδ:RelA:p52 =>IκBδ + RelA:p52	Cyt, Nuc	94	8.4e-4 min^{-1}	IκB-NFκB interaction	
IκBα:RelB:p50 =>IκBα + RelB:p50	Cyt, Nuc	95	3e-2 min^{-1}	IκB-NFκB interaction	Fitted (dependent on 80 -82)
IκBε:RelB:p50 =>IκBε + RelB:p50	Cyt, Nuc	96	3e-2 min^{-1}	IκB-NFκB interaction	
IκBδ:RelB:p50 =>IκBδ + RelB:p50	Cyt, Nuc	97	8.4e-4 min^{-1}	IκB-NFκB interaction	
IκBα:cRel:p50 =>IκBα + cRel:p50	Cyt, Nuc	98	4.8e-3 min^{-1}	IκB-NFκB interaction	Fitted (dependent on 83-86)

IκBβ:cRel:p50 =>IκBβ +cRel:p50	Cyt, Nuc	99	1.7e-2 min^{-1}	IκB-NFκB interaction	
IκBε:cRel:p50 =>IκBε + cRel:p50	Cyt, Nuc	100	2.7e-5 min^{-1}	IκB-NFκB interaction	
IκBδ:cRel:p50 =>IκBδ + cRel:p50	Cyt, Nuc	101	8.4e-4 min^{-1}	IκB-NFκB interaction	
IκBα:cRel:p52 =>IκBα + cRel:p52	Cyt, Nuc	98	4.8e-3 min^{-1}	IκB-NFκB interaction	Fitted (dependent on 83-86)
IκBβ:cRel:p52 =>IκBβ + cRel:p52	Cyt, Nuc	99	1.7e-2 min^{-1}	IκB-NFκB interaction	
IκBε:cRel:p52 =>IκBε + cRelp52	Cyt, Nuc	100	2.7e-5 min^{-1}	IκB-NFκB interaction	
IκBδ:cRel:p52 =>IκBδ + cRel:p52	Cyt, Nuc	101	8.4e-4 min^{-1}	IκB-NFκB interaction	
IκBα:NFκB(c) =>IκBα:NFκB(n)	Cyt ->Nuc	102	0.28 min^{-1}	Transport	(Shih et al., 2009)
IκBβ:NFκB(c) =>IκBβ:NFκB(n)	Cyt ->Nuc	103	0.028 min^{-1}	Transport	(Shih et al., 2009)
IκBδ:NFκB(c) =>IκBδ:NFκB(n)	Cyt ->Nuc	104	0.028 min^{-1}	Transport	Based on slower import rate of IκBβ:NFκB
IκBε:NFκB(c) =>IκBε:NFκB(n)	Cyt ->Nuc	105	0.14 min^{-1}	Transport	(Shih et al., 2009)
IκBα:NFκB(n) =>IκBα:NFκB(c)	Nuc ->Cyt	106	0.84 min^{-1}	Transport	(Shih et al., 2009)
IκBβ:NFκB(n) =>IκBβ:NFκB(c)	Nuc ->Cyt	107	0.42 min^{-1}	Transport	(Shih et al., 2009)
IκBε:NFκB(n) =>IκBε:NFκB(c)	Nuc ->Cyt	108	0.42 min^{-1}	Transport	(Shih et al., 2009)
IκBδ:NFκB(n) =>IκBδ:NFκB(c)	Nuc ->Cyt	109	0.42 min^{-1}	Transport	(Shih et al., 2009)

Table S2. (relates to Figure 4)
Species Table
Species described in mathematical model (supplementary figure 4).

	Species	Location
1	tIκBα	Nucleus
2	tIκBβ	Nucleus
3	tIκBε	Nucleus
4	tRelA	Nucleus
5	tp50	Nucleus
6	tp100	Nucleus
7	tcRel	Nucleus
8,9	IκBα	Nucleus, Cytoplasm
10,11	IκBβ	Nucleus, Cytoplasm
12,13	IκBε	Nucleus, Cytoplasm
14,15	IκBδ	Nucleus, Cytoplasm
16,17	RelA	Nucleus, Cytoplasm
18,19	RelB	Nucleus, Cytoplasm
20,21	cRel	Nucleus, Cytoplasm
22,23	p50	Nucleus, Cytoplasm
24,25	p100	Nucleus, Cytoplasm
26,27	p52	Nucleus, Cytoplasm
28,29	RelAp50	Nucleus, Cytoplasm
30,31	RelAp52	Nucleus, Cytoplasm
32,33	RelBp50	Nucleus, Cytoplasm
34,35	RelBp52	Nucleus, Cytoplasm
36,37	cRelp50	Nucleus, Cytoplasm
38,39	cRelp52	Nucleus, Cytoplasm
40,41	p50p50	Nucleus, Cytoplasm
42,43	p52p52	Nucleus, Cytoplasm
44,45	IκBα:RelAp50	Nucleus, Cytoplasm
46,47	IκBβ:RelAp50	Nucleus, Cytoplasm
48,49	IκBε:RelAp50	Nucleus, Cytoplasm
50,51	IκBδ:RelAp50	Nucleus, Cytoplasm
52,53	IκBα:RelAp52	Nucleus, Cytoplasm
54,55	IκBβ:RelAp52	Nucleus, Cytoplasm
56,57	IκBε:RelAp52	Nucleus, Cytoplasm
58,59	IκBδ:RelAp52	Nucleus, Cytoplasm
60,61	IκBα:RelBp50	Nucleus, Cytoplasm
62,63	IκBε:RelBp50	Nucleus, Cytoplasm
64,65	IκBδ:RelBp50	Nucleus, Cytoplasm
66,67	IκBα:cRelp50	Nucleus, Cytoplasm
68,69	IκBβ:cRelp50	Nucleus, Cytoplasm
70,71	IκBε:cRelp50	Nucleus, Cytoplasm
72,73	IκBδ:cRelp50	Nucleus, Cytoplasm
74,75	IκBα:cRelp52	Nucleus, Cytoplasm
76,77	IκBβ:cRelp52	Nucleus, Cytoplasm
78,79	IκBε:cRelp52	Nucleus, Cytoplasm
80,81	IκBδ:cRelp52	Nucleus, Cytoplasm
82	NEMO	Cytoplasm
83	NIK	Cytoplasm

Table S3. (relates to Figure S5 and Figure 4)
Abundances of NFκB / IκB proteins in B cells and MEFs
Table indicating the NFκB monomer and IκB protein abundances in B cells and MEFs; second column displays the molecule numbers determined by quantitative immunoblot analyses with recombinant protein standards (Figure S5); third column indicates the cellular concentration based on a 2pl volume; fourth column indicates the concentration in B-cells based on the comparative immunoblotting shown in Figure S5F.

Species	Molecule numbers per Cell in MEFs	Concentration in MEFs (nM)	Concentration in B-cells (nM)
RelA	~480,000	~340	~220
p50	~450,000	~374	~380
IκBα	~400,000	~220	~150
IκBβ	~100,000	~70	~80
IκBε	~25,000	~21	~45