Supplementary Figure Legends

Figure S1

(A) Graph showing the percentage of bulk protein synthesis inhibition in cells treated with 10 μg/mL CHX as compared to cells without CHX. 10 μg/mL CHX is the lowest concentration at which cells did not die over a 60 hour time course. Cells treated with or without CHX were metabolically labeled with 200 μCi/mL ^{35}S-Methionine and protein extracts measured by scintillation count at indicated time points.
(B) Quantitation of NF-κB activity from (A) using ImageQuant following exposure to phosphoimager. The TNF stimulated lane at 15 minutes was set to 100% NF-κB activity and all the points for the CHX timecourse were normalized as a percentage of this.
(C) Quantitation of NF-κB activity from (C). The TNF stimulated lane for each cell line at 15 minutes was set to 100% NF-κB activity and all the points for each respective CHX timecourse were normalized as a percentage of this.

Figure S2

(A) Western blots for IκBα, IκBβ, and IκBε in different cell genotypes (labeled above each lane). The percentage of IκB protein in the *nfκb*$^{-/-}$ cells compared to the amount in wild-type cells was approximated by a dilution series of *iκbα*$^{-/-}$*β*$^{-/-}$*ε*$^{-/-}$ extract with wild-type extract.
(B) Standard curve of Western blot signals for each IκB isoform based on the dilution series in (A). Loss of linearity at the low end indicates background and limits the sensitivity of detection. The line indicates the signal measured for samples derived from *nfκb*$^{-/-}$ cells.
(C) Graph of quantitated IκB protein levels in non-canonical and canonical NF-κB protein knockouts (*relb*$^{-/-}$*nfκb2*$^{-/-}$ and *rela*$^{-/-}$*crel*$^{-/-}$*nfκb1*$^{-/-}$ MEFs) relative to wild type cells as determined in (A) and (B).
(D) RNase protection assay and quantitation showing the respective amounts of IκBα, IκBβ, and IκBε mRNA in wt and *nfκb*$^{-/-}$ cells. Graph of the quantitation relative to wild type cells is shown on the right.

Figure S3

(A) Cytoplasmic extracts of wild-type or *nfκb*$^{-/-}$ cells treated with 1 ng/mL TNF were immunoprecipitated with IKKγ and subject to an *in vitro* kinase assay. Immunoblotting of the kinase assay with IKKα was performed as a loading control.
(B) Western blot for IκBα of protein extracts from TNF (1ng/mL) treated *nfκb*$^{-/-}$ cells.

		association		degradation		NF-κB effect
		IKK + IkB --> IKK-IkB IKK + IkB-NFkB --> IKK-IkB-NFkB		IKK-IkB --> IKK IKK-IkB-NFkB --> IKK + NFkB		
		rate constants $\mu M^{-1}s^{-1}$		rate constants s^{-1}		
IκBα	free	a1	2.25×10^{-2}	r1	1×10^{-3}	1/49
	bound	a4	1.85×10^{-1}	r4	6×10^{-3}	
IκBβ	free	a2	6×10^{-3}	r2	4×10^{-4}	1/40
	bound	a5	4.8×10^{-2}	r5	2×10^{-3}	
IκBε	free	a3	9×10^{-3}	r3	6×10^{-4}	1/42
	bound	a6	7×10^{-2}	r6	3×10^{-3}	

Supplemental Table I. Rate constants for IKK-mediated degradation of IκB proteins

Association rates for IKK and either free or NF-κB bound IκB proteins were unchanged from model version 1.0 as determined in Hoffmann et al where there is a 7-8-fold higher association for IKK to IκB in the presence of NF-κB (Zandi *et al.*). New rate constants governing the IKK-induced degradation of bound IκB proteins were parameter fit after incorporation of the rate constants in Table I using the method described in Hoffmann *et al.* According to Zandi *et al.*, the IKK-mediated catalysis is 5-fold higher for IκB proteins in the presence of NF-κB. We therefore divided r4, r5, and r6 by 5 to generate r1, 2, and r3. The effect of NF-κB sensitizes IκBα to association with and phosphorylation by IKK. The net "NF-κB effect" is thus the ratio of combined association and degradation rate constants in the presence and absence of NF-κB.

Derivation of degradation rate constants for model 1.1.

deg1, 2, and 3: The half-life for free IκBα was determined by CHX treatment of nfkb$^{-/-}$ cells and immunoblotting for IκBα. The time after CHX treatment at which the level of IκB was half was taken as the half-life and converted to a first-order rate constant based on the first order half-life equation. The half-lives for IκBβ and IκBε were approximated based on the quantitations of the amount of protein present in the *nfkb*$^{-/-}$ cells versus wild-type cells (Figure S2B) and converted to first order rate constants. These rate constants are considered IKK-independent because treatment with TNF, which does activate IKK, does not lead to IκB degradation in the *nfκb*$^{-/-}$ cells.

deg4, 5, and 6: In the *ikk*$^{-/-}$ cells, inhibition of protein synthesis with cycloheximide does not lead to NF-κB DNA binding as it does in wild-type cells (Figure 2E) indicating there is no detectable degradation of NF-κB-bound IκB proteins when IKK is not present. To determine a degradation rate constant for the IKK-independent degradation of NF-κB-bound IκB proteins we took the original rate constant used in model 1.0 and lowered it to a percentage of itself until computational simulations imparting an 85% inhibition of all translation parameters gave a level of nuclear NF-κB at 60 hours that was just below the level we can detect via EMSA.

r4, 5, and 6: After alteration of the "deg" parameters, model fitting was performed as described in Hoffmann *et al*. to determine the IKK-induced degradation rate constants of NF-κB-bound IκB proteins.

r1, 2, and 3: According to Zandi *et al*. 1998, the catalytic rate constant for IKK phosphorylation of IκB proteins is 5-fold higher in the presence of NF-κB. We therefore divided the parameter fit rate constants for r4, 5, and 6 by 5 to obtain rate constants for the IKK-induced degradation of free IκB proteins.

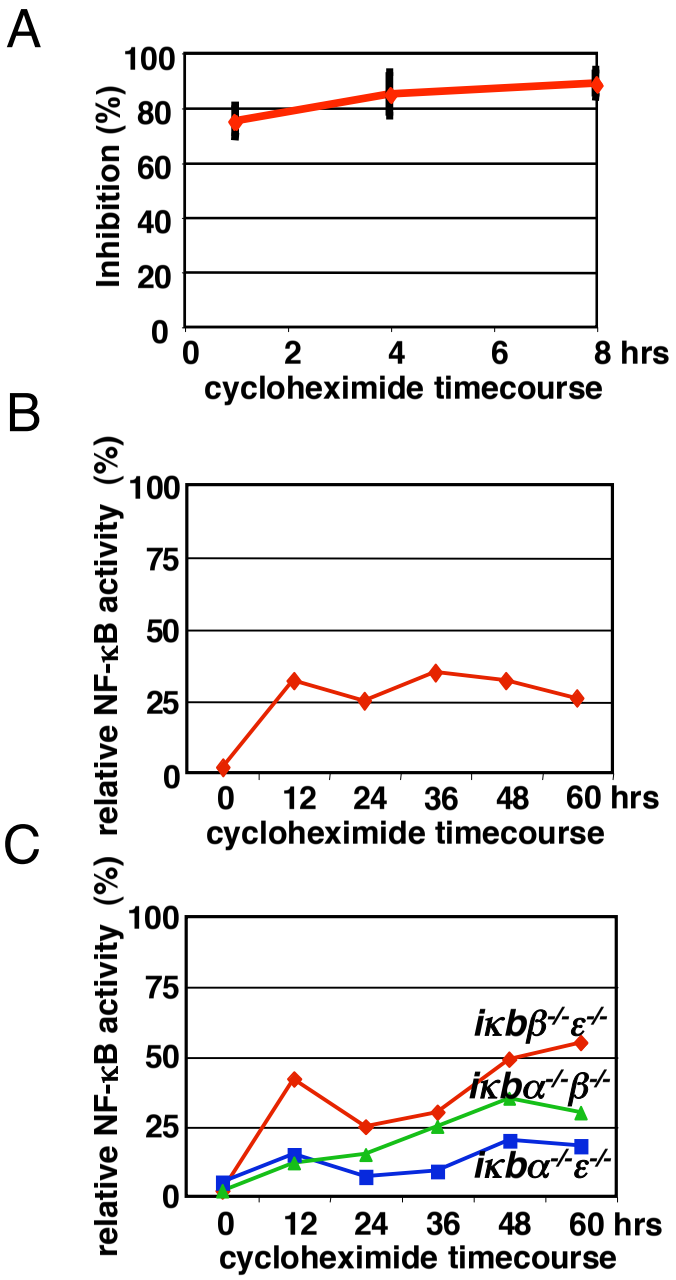
Supplementary Table 2: Reactions and Rate Constants

Parameter	Rate constant	units	Reaction
a1	1.35	$\mu M^{-1}min^{-1}$	IkBa + IKK => IkBaIKK
a2	0.36	$\mu M^{-1}min^{-1}$	IkBb + IKK => IkBbIKK
a3	0.54	$\mu M^{-1}min^{-1}$	IkBe + IKK => IkBeIKK
a4	30	$\mu M^{-1}min^{-1}$	IkBa + NFkB => IkBaNFkB
a4	30	$\mu M^{-1}min^{-1}$	IkBaIKK + NFkB => IkBaIKKNFkB
a4	30	$\mu M^{-1}min^{-1}$	IkBan + NFkBn => IkBaNFkBn
a5	30	$\mu M^{-1}min^{-1}$	IkBb + NFkB => IkBbNFkB
a5	30	$\mu M^{-1}min^{-1}$	IkBbIKK + NFkB => IkBbIKKNFkB
a5	30	$\mu M^{-1}min^{-1}$	IkBbn + NFkBn => IkBbNFkBn
a6	30	$\mu M^{-1}min^{-1}$	IkBe + NFkB => IkBeNFkB
a6	30	$\mu M^{-1}min^{-1}$	IkBeIKK + NFkB => IkBeIKKNFkB
a6	30	$\mu M^{-1}min^{-1}$	IkBen + NFkBn => IkBeNFkBn
a7	11.1	$\mu M^{-1}min^{-1}$	IkBaNFkB + IKK => IkBaNFkB
a8	2.88	$\mu M^{-1}min^{-1}$	IkBbNFkB + IKK => IkBbNFkB
a9	4.2	$\mu M^{-1}min^{-1}$	IkBeNFkB + IKK => IkBeNFkB
d1	0.075	min^{-1}	IkBaIKK => IkBa + IKK
d1	0.075	min^{-1}	IkBaIKKNFkB => IkBaNFkB + IKK
d2	0.105	min^{-1}	IkBbIKK => IkBb + IKK
d2	0.105	min^{-1}	IkBbIKKNFkB => IkBbNFkB + IKK
d3	0.105	min^{-1}	IkBeIKK => IkBe + IKK
d3	0.105	min^{-1}	IkBeIKKNFkB => IkBeNFkB + IKK
d4	0.00006	min^{-1}	IkBaNFkB => IkBa + NFkB
d4	0.00006	min^{-1}	IkBaNFkBn => IkBan + NFkBn
d4	0.00006	min^{-1}	IkBaIKKNFkB => IkBaIKK + NFkB
d5	0.00006	min^{-1}	IkBbNFkB => IkBb + NFkB
d5	0.00006	min^{-1}	IkBbNFkBn => IkBbn + NFkBn
d5	0.00006	min^{-1}	IkBbIKKNFkB => IkBbIKK + NFkB
d6	0.00006	min^{-1}	IkBeNFkB => IkBe + NFkB
d6	0.00006	min^{-1}	IkBeNFkBn => IkBen + NFkBn
d6	0.00006	min^{-1}	IkBeIKKNFkB => IkBeIKK + NFkB
deg1	0.12	min^{-1}	IkBa =>
deg2	0.18	min^{-1}	IkBb =>
deg3	0.18	min^{-1}	IkBe =>
deg1	0.12	min^{-1}	IkBan =>
deg2	0.18	min^{-1}	IkBbn =>
deg3	0.18	min^{-1}	IkBen =>
deg4	0.00006	min^{-1}	IkBaNFkB => NFkB
deg5	0.00006	min^{-1}	IkBbNFkB => NFkB

deg6	0.00006	min^{-1}	IkBeNFkB => NFkB
deg4	0.00006	min^{-1}	IkBaNFkBn => NFkBn
deg5	0.00006	min^{-1}	IkBbNFkBn => NFkBn
deg6	0.00006	min^{-1}	IkBeNFkBn => NFkBn
k01	0.0048	min^{-1}	NFkBn => NFkB
k1_1	5.4	min^{-1}	NFkB => NFkBn
k2_1	0.828	min^{-1}	IkBaNFkBn => IkBaNFkB
k2_2	0.414	min^{-1}	IkBbNFkBn => IkBbNFkB
k2_3	0.414	min^{-1}	IkBeNFkBn => IkBeNFkB
r1	0.072	min^{-1}	IkBaIKK => IKK
r2	0.024	min^{-1}	IkBbIKK => IKK
r3	0.036	min^{-1}	IkBeIKK => IKK
r4	0.36	min^{-1}	IkBaIKKNFkB => IKK + NFkB
r5	0.12	min^{-1}	IkBbIKKNFkB => IKK + NFkB
r6	0.18	min^{-1}	IkBeIKKNFkB => IKK + NFkB
tp1_1	0.018	min^{-1}	IkBa => IkBan
tp1_2	0.018	min^{-1}	IkBb => IkBbn
tp1_3	0.018	min^{-1}	IkBe => IkBen
tp2_1	0.012	min^{-1}	IkBan => IkBa
tp2_2	0.012	min^{-1}	IkBbn => IkBb
tp2_3	0.012	min^{-1}	IkBen => IkBe
tr1_1	0.2448	min^{-1}	IkBat => IkBat + IkBan
tr1_2	0.2448	min^{-1}	IkBbt => IkBbt + IkBbn
tr1_3	0.2448	min^{-1}	IkBet => IkBet + IkBen
tr2_1	1.98	$\mu M^{-1} min^{-1}$	2 NFkB => 2 NFkB + IkBat
tr2a_1	0.0001848	$\mu M\ min^{-1}$	=> IkBat
tr2b_1	0.00004272	$\mu M\ min^{-1}$	=> IkBbt
tr2e_1	0.00003048	$\mu M\ min^{-1}$	=> IkBet
tr3_1	0.0168	min^{-1}	IkBat =>
tr3_2	0.0168	min^{-1}	IkBbt =>
tr3_3	0.0168	min^{-1}	IkBet =>

Supplementary Table 2: Reactions and Rate Constants
The reactions contained in the mathematical model of the NF-κB signaling module version 1.1 are listed with their respective rate constants.

Figure S1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60
40
20
0
Inhibition (%)
0
2
4
6
8 hrs
cycloheximide timecourse
B
100
75
50
25
0
relative NF-κB activity (%)
0
12
24
36
48
60 hrs
cycloheximide timecourse
C
100
75
50
25
0
relative NF-κB activity (%)
iκbβ-/-ε-/-
iκbα-/-β-/-
iκbα-/-ε-/-
0
12
24
36
48
60 hrs
cycloheximide timecourse

Figure S2

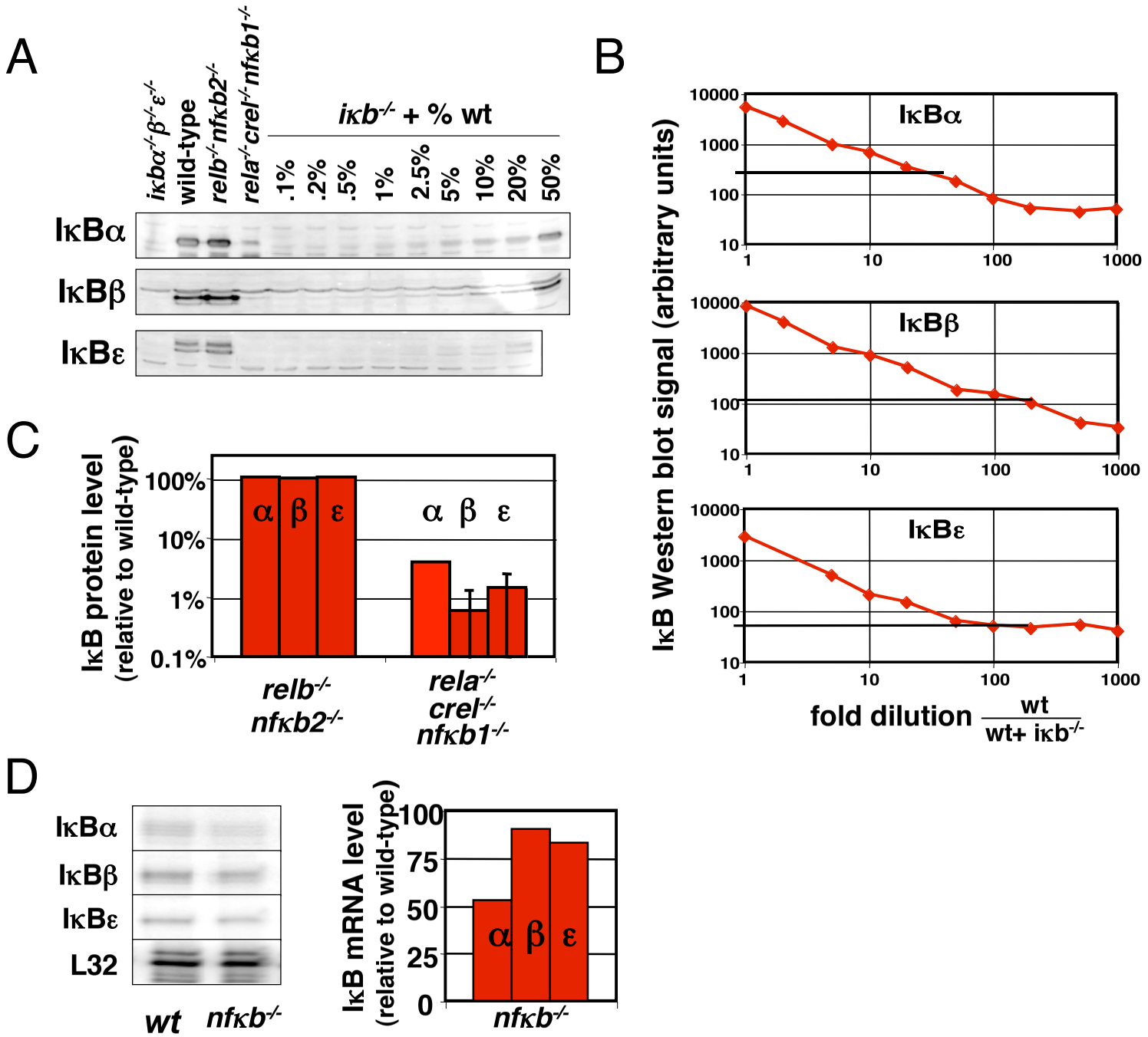
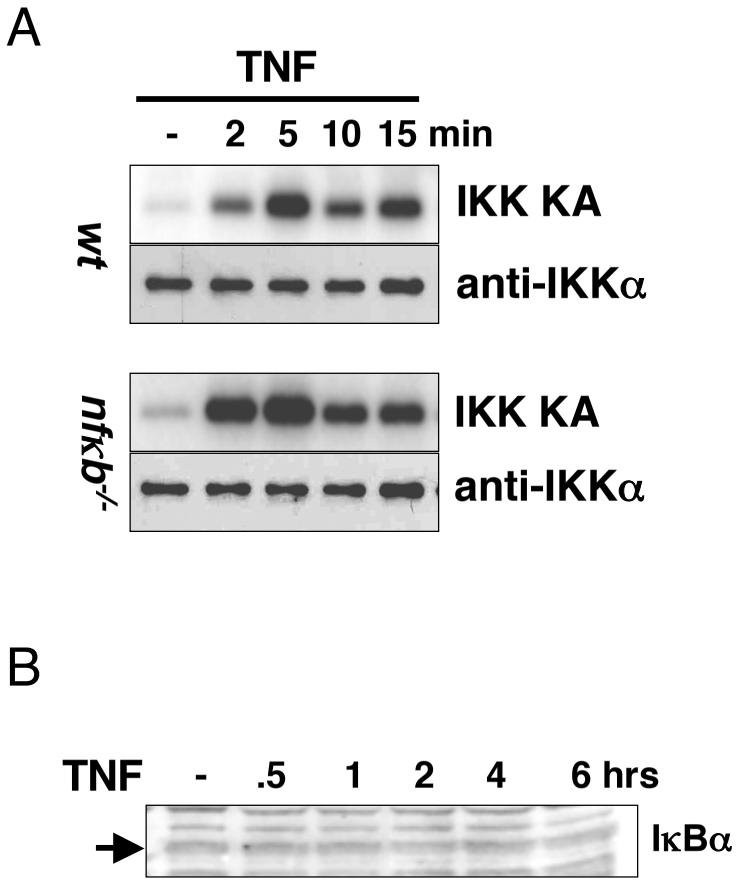

A
iκbα-/-β-/-ε-/-
wild-type
relb-/-nfκb2-/-
rela-/-crel-/-nfκb1-/-
iκb-/- + % wt
.1%
.2%
.5%
1%
2.5%
5%
10%
20%
50%
IκBα
IκBβ
IκBε
B
IκB Western blot signal (arbitrary units)
10000
1000
100
10
1
10
100
1000
IκBα
IκBβ
IκBε
fold dilution wt / wt+ iκb-/-
C
IκB protein level (relative to wild-type)
100%
10%
1%
0.1%
α β ε
α β ε
relb-/- nfκb2-/-
rela-/- crel-/- nfκb1-/-
D
IκBα
IκBβ
IκBε
L32
wt
nfκb-/-
IκB mRNA level (relative to wild-type)
100
75
50
25
0
α β ε
nfκb-/-

Figure S3


A
TNF
- 2 5 10 15 min
wt
IKK KA
anti-IKKα
nfκb-/-
IKK KA
anti-IKKα
B
TNF - .5 1 2 4 6 hrs
IκBα