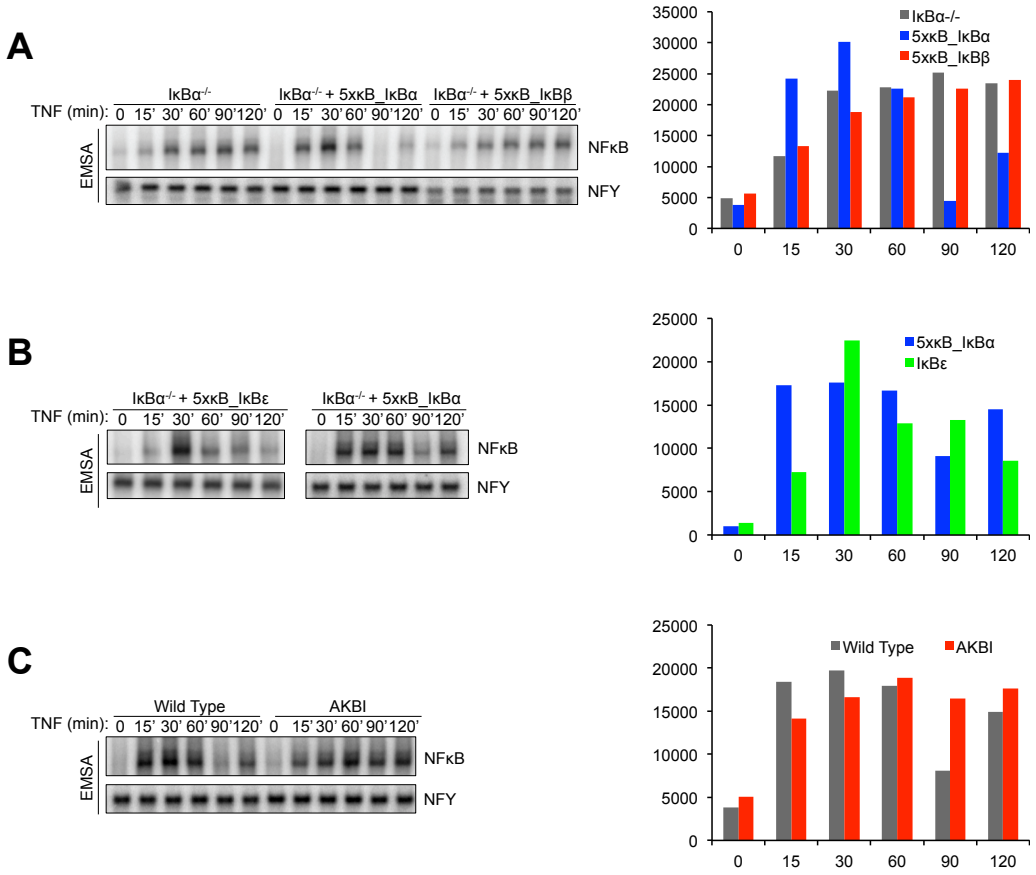
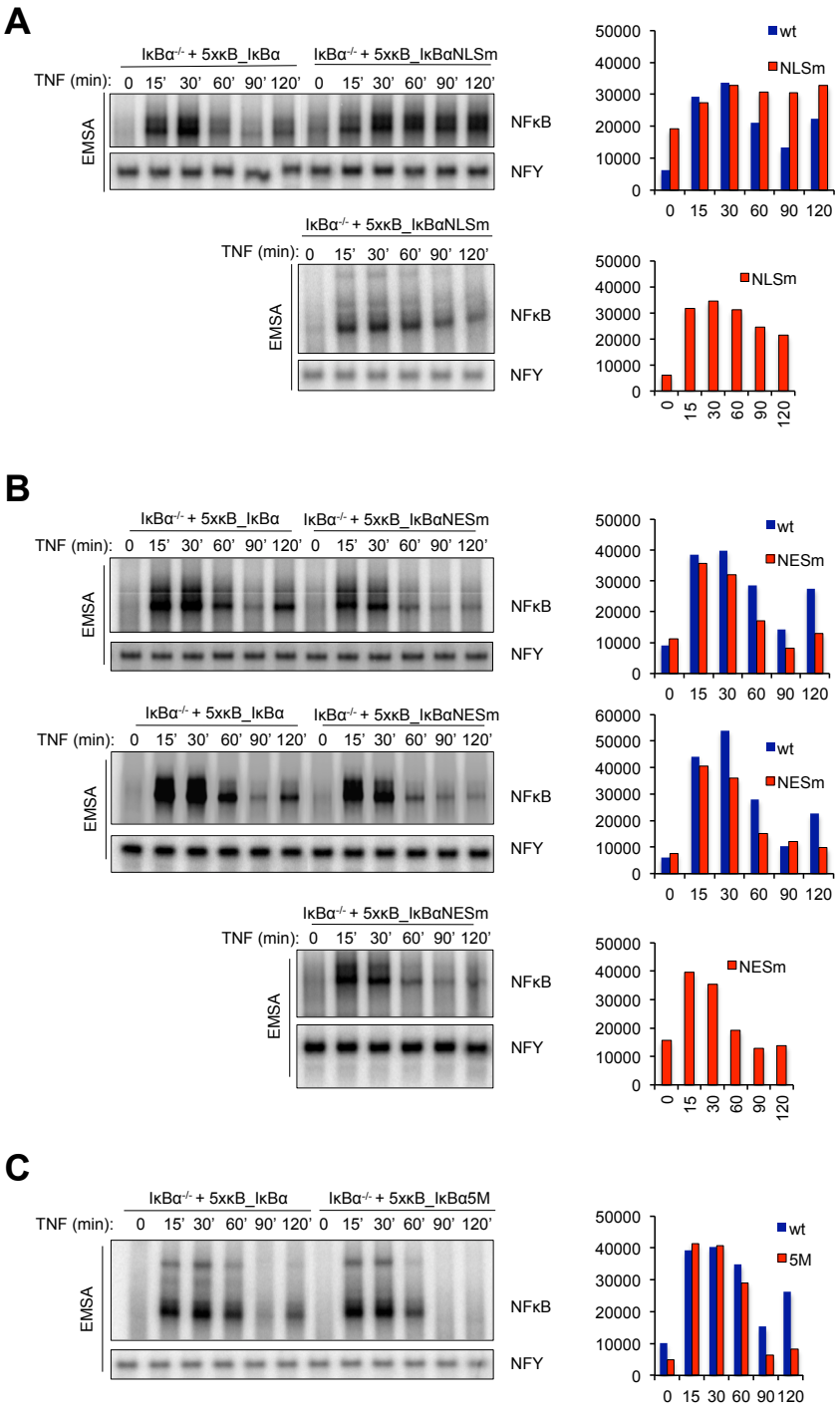
Supplementary Information:

Supplementary Figure 1



Supplementary Figure 1: A) Replicate data of EMSA experiments and quantitation presented in Figure 1B and C showing reproducibility of trends for 5xκB_IκBα and 5xκb_IκBβ reconstitution experiments. **B)** NFκB activity in IκBα-/- cells reconstituted with NFκB inducible IκBε. **C)** Replicate data of EMSA experiments and quantitation presented in Figure 1D showing reproducibility of trends for AKBI experiments. NFκB label indicates p50:p65 heterodimer, in A-C bottom band is NFY control for EMSA.

Supplementary Figure 2



Supplementary Figure 2: A-C Replicate data of EMSA experiments and quantitation presented in Figure 3A, C, & E showing reproducibility of trends for NLSm, NESm, and 5m IκBα constructs, respectively. NFκB label indicates p50:p65 heterodimer bottom band is NFY control.

Supplementary Table 1: Species considered in the kinetic model.

Abbreviation	Component	Compartment
ikk	IKK	cytoplasm
nfkb	NFκB	cytoplasm
nfkbn	NFκB	nuclear
ikbat	IκBα mRNA	cytoplasm
ikba	IκBα	cytoplasm
ikban	IκBα	nuclear
ibkanfkb	IκBαNFκB	cytoplasm
ikbanfkbn	IκBαNFκB	nuclear
ikkikba	IKKIκBα	cytoplasm
ikkikbanfkb	IKKIκBαNFκB	cytoplasm

Supplementary Table 2: Reactions and parameters included in the model. The rightmost column indicates the references used as sources for the parameters. Parameters J, L, and N are given in units of the Boltzman constant K_B times the temperature.

Label	Reaction	Type	Compartment	Value	Units	Ref
a_c_ai	IkBa + IKK => IKKIkBa	Association	Cyt	1.35	μM^{-1} min^{-1}	1
a_c_an	IkBa + NFkB => IkBaNFkB	Association	Cyt	30	μM^{-1} min^{-1}	1
a_c_2ain	IKKIkBa + NFkB => IKKIkBaNFkB	Association	Cyt	30	μM^{-1} min^{-1}	1
a_c_2ani	IkBaNFkB + IKK => IKKIkBaNFkB	Association	Cyt	11.1	μM^{-1} min^{-1}	1
a_n_an	IkBan + NFkBn => IkBaNFkBn	Association	Nuc	30	μM^{-1} min^{-1}	1
d_c_ai	IKKIkBa => IKK + IkBa	Dissociation	Cyt	0.075	min^{-1}	1
d_c_an	IkBaNFkB => IkBa + NFkB	Dissociation	Cyt	0.00006	min^{-1}	2
d_c_2ain	IKKIkBaNFkB => IKKIkBa + NFkB	Dissociation	Cyt	0.00006	min^{-1}	2
d_c_2ani	IKKIkBaNFkB => Ikk + IkBaNFkB	Dissociation	Cyt	0.075	min^{-1}	1
d_n_an	IkBaNFkBn => IkBan + NFkBn	Dissociation	Nuc	0.00006	min^{-1}	2
ex_a	IkBan => IkBa	Export	Nuc->Cyt	0.012	min^{-1}	1
ex_n	NFkBn => NFkB	Export	Nuc->Cyt	0.0048	min^{-1}	1
ex_2an	IkBaNFkBn => IkBaNFkB	Export	Nuc->Cyt	0.828	min^{-1}	1
in_a	IkBa => IkBan	Import	Cyt->Nuc	0.018	min^{-1}	1
in_n	NFkB => NFkBn	Import	Cyt->Nuc	5.4	min^{-1}	1
pd_c_a	IkBa= >	Prot. deg.	Cyt	0.12	min^{-1}	2
pd_c_2ai	IKKIkBa => IKK	Prot. deg.	Cyt	0.0018	min^{-1}	2
pd_c_2an	IkBaNFkB => NFkB	Prot. deg.	Cyt	min^{-1}	min^{-1}	2
pd_c_3ain	IKKIkBaNFkB => IKK + NFkB	Prot. deg.	Cyt	0.36	min^{-1}	2
pd_n_2an	IkBaNFkBn => NFkBn	Prot. deg.	Nuc	0.00006	min^{-1}	2
pd_n_a	IkBan =>	Prot. deg.	Nuc	0.12	min^{-1}	1
ps_c_a	=> IkBa	Prot. synth.	Cyt	0.2448	min^{-1}	1
rd_a	IkBat =>	RNA deg.	Cyt	0.035	min^{-1}	3
rs_an	=> IkBat	(induced by NF-κB) RNA synth.	Nuc->Cyt	0.06	μM^{-1} min^{-1}	3
K_A				0.2	μM	3,4
J				5.8	K_BT	3,4
L				-1.3	K_BT	3,4
N				0.1	K_BT	3,4
rs_a	=> IkBat	(constitutive) RNA synth.	Nuc->Cyt	0.00052	μM^{-1} min^{-1}	3

Supplementary Table 3: Numerical function used to represent IKK activity over time. The function reflects typical IKK activity curves determined by kinase assays ([1, 2]). Activity between time points were determined using linear interpolation.

Time (min)	IKK activity (normalized 0-1)
0	0.01
5	0.6
10	1
15	0.65
20	0.5
25	0.36
30	0.3
60	0.3
360	0.3

The response of the NFκB regulatory module was simulated using the computational ODE-based model described in [3] which was derived from [1] and [2]. Briefly, the model considers the various reactions between complexes comprising combinations of IKK, NFκB and IκBα (Sup. Table 1 and 2). In order to avoid obfuscating the roles of the various regulatory mechanisms involving IκBα, we removed the other IκB family members from the model. The model incorporates production and degradation of IκBα and the corresponding mRNA. Levels of RelA and IKK do not change appreciably in the time scale of the experiments and therefore their abundances are considered fixed parameters. All reactions, except NFκB-dependent induction of IκBα follow mass action kinetics. NFκB-dependent induction of IκBα is represented by the model in [4] (model 3, additive Pol II recruitment, n=5) (Equations 1-3) and occurs with an explicit delay of 15 minutes.

$$\frac{d[mRNA]}{dt} = \mathrm{rs\_an}\frac{1}{1+\frac{1}{F_{REG}}e^{\beta J}} \qquad [1]$$

$$F_{REG} = \frac{2^{-n}\left[\frac{[A]}{K_A}e^{-\beta(L+N)} - \sqrt{\frac{[A]}{K_A}e^{-\beta L}\left(e^{-\beta N}\left(\frac{[A]}{K_A}e^{-\beta(L+N)}-2\right)+4\right)+1}+1\right]^n + 2^{-n}\left[\frac{[A]}{K_A}e^{-\beta(L+N)} + \sqrt{\frac{[A]}{K_A}e^{-\beta L}\left(e^{-\beta N}\left(\frac{[A]}{K_A}e^{-\beta(L+N)}-2\right)+4\right)+1}+1\right]^n}{2^{-n}\left[\frac{[A]}{K_A}e^{-\beta N} - \sqrt{\frac{[A]}{K_A}e^{-\beta N}\left(e^{-\beta N}\left(\frac{[A]}{K_A}e^{-\beta N}-2\right)+4\right)+1}+1\right]^n + 2^{-n}\left[\frac{[A]}{K_A}e^{-\beta N} + \sqrt{\frac{[A]}{K_A}e^{-\beta N}\left(e^{-\beta N}\left(\frac{[A]}{K_A}e^{-\beta N}-2\right)+4\right)+1}+1\right]^n} \qquad [2]$$

$$\beta = \frac{1}{K_B T} \qquad [3]$$

Here, [A] is the concentration of nuclear NFκB and the parameters L, N, J and K_A are as in Supplemental Table 2.

Models were equilibrated with no stimulus until steady state was reached. An IKK multiplier of 0.01 was included during this stage to represent basal activity. As in [3], exposure to TNF was simulated with a numerical function representing IKK activity (Sup. Table 3) determined under conditions similar to the experiments. This function was used as a multiplier for the reaction

terms including IKK-induced degradation of IkB. Time course curves in Figure 2 were generated by applying multipliers to the kinetic parameters corresponding to the reactions in Figure 2A. The multiplier values were:$2^{-3, -2.5, -2, -1.5, -1, -0.5}$ (reactions 1,2,3 and 5) and $2^{-6,-5,-4,-3,-2,-1}$ (reaction 4), reflecting different sensitivities for reaction 4. NFκB time courses are normalized to their peak value. Sensitivity ratios $s_r(t)$ at a particular time t_i is defined as (eq 4):

$$(nucNF\kappa B^{perturbed} - nucNF\kappa B^{unperturbed})/nucNF\kappa B^{unperturbed}, \quad [4]$$

where $nucNF\kappa B^{perturbed/unperturbed}$ are the normalized nuclear concentrations of NFκB at time t_i obtained with a model with/without a multiplicative factor (0.9, 0.5, 0.1) for the indicated kinetic rate parameter (values shown in percent units). The global average sensitivity in figure 2C was calculated as the root mean square of the $s_r(t)$ sampled at 1 minute intervals between 1 and 120 minutes post-stimulation. . The differential equations were solved using the "StiffnessSwitching" method of the NDSolve function in the package Mathematica 8 (Wolfram Research, Champagne, IL).

References:

1. Werner SL, Barken D, Hoffmann A. Stimulus specificity of gene expression programs determined by temporal control of IKK activity. *Science* 2005, 309:1857-1861.
2. Kearns JD, Basak S, Werner SL, Huang CS, Hoffmann A. IkappaBepsilon provides negative feedback to control NF-kappaB oscillations, signaling dynamics, and inflammatory gene expression. *J Cell Biol* 2006, 173:659-664.
3. Mukherjee SP, Behar M, Birnbaum HA, Hoffmann A, Wright PE, Ghosh G. Analysis of the RelA:CBP/p300 interaction reveals its involvement in NF-kappaB-driven transcription. *PLoS Biol* 2013, 11:e1001647.
4. Giorgetti L, Siggers T, Tiana G, Caprara G, Notarbartolo S, Corona T, Pasparakis M, Milani P, Bulyk ML, Natoli G. Noncooperative interactions between transcription factors and clustered DNA binding sites enable graded transcriptional responses to environmental inputs. *Mol Cell* 2010, 37:418-428.