Supplement

Methods

The models were simulated with MATLAB R2013b (The Mathworks, Inc.). The MATCONT Toolbox [1] was used for the bifurcation analysis of the core model. For tracing the Hopf bifurcation points in the 2-parameter bifurcation diagram a script was written in MATLAB R2013b, which determines the Hopf bifurcation points by the eigenvalues of the Jacobian matrix. At Hopf bifurcation points at least one pair of eigenvalues has real parts that are zero and imaginary parts that are non-zero, while all other eigenvalues have negative real parts. If the real parts are sufficiently small (between $-2x10^{-4}$ and $2x10^{-4}$) they were assumed to be zero.

Model reduction

We aim to use the tools of bifurcation analysis to analyse the dynamics of NF-κB signaling. The model published by Kearns *et al.* [2] describes the dynamics of NF-κB activation. However, it consists of 24 variables and 72 parameters (shown in Table S2). Therefore, it is too large for a bifurcation analysis. Moreover, it includes different kinetic rate constants for the degradation of IKK. For our analysis we used the kinetic rate constant for IKK degradation during equilibrium. For reduction of the published model we initially performed a sensitivity analysis to determine processes that influence the dynamics of active NF-κB. The dynamics of active NF-κB was characterised by three different measures, the unstimulated and stimulated steady state concentration of active NF-κB and the characteristic time defined by Llorens *et al.* [3] of the NF-κB dynamics upon stimulation. For the sensitivity analysis every parameter was perturbed separately by +1% and the changes of the three measures were examined individually. Figs S1 and S2 show the sensitivity coefficients for the unstimulated and the stimulated steady state (SC_{stst}) and for the characteristic time (SC_{ct}), respectively. If the sensitivities are sufficiently small (between -0.1 and 0.1), the corresponding parameters are assumed to have no significant influence on the accordant measure.

The sensitivity analysis reveals that several reactions associated to IκBα and IκBβ have an influence on the unstimulated steady state concentration of active NF-κB. In the stimulated system the steady state concentration of active NF-κB is mainly influenced by reactions associated to IκBα, while IκBε-associated reactions have only a very small impact (Fig. S1). Reactions associated with IκBβ and IκBε have a very small or almost no influence on the characteristic time of active NF-κB compared to reactions associated with IκBα (Fig. S2). Therefore, we removed all reactions associated with the IκBβ or IκBε feedback. The influences of IκBβ–associated or IκBε–associated reactions on the unstimulated or stimulated steady state concentration of active NF-κB can be rectified by an adjustment of the total NF-κB and IKK concentrations. This is based on the interaction of the three feedbacks via the IκB isoforms. They are not interacting directly, but via sharing the same pools of NF-κB and IKK. Around 20% of the total NF-κB and 25% of the total IKK is sequestered by IκBβ and IκBε. To account for this, we reduce the total NF-κB concentration by 20% and the total IKK concentration by 25% for the core model, where the IκBβ and IκBε feedbacks are excluded. Additionally, IκBα–associated reactions, for which all three sensitivity coefficients (SC_{ct},

unstimulated and stimulated SC_{stst}) are between -0.1 and 0.1, were eliminated. This is based on our assumption that parameter with absolute sensitivity coefficients below 0.1 do not have a significant influence on the dynamics of active NF-κB. The derived core model includes 8 independent variables and 18 parameters including the total concentrations of NF-κB and IKK.

Model equations of the core model

The derived core model, which is used for the bifurcation analysis, consists of the following ordinary differential equations (ODEs) and parameters (shown in Table S1):

$$NF\kappa B' = r4 * IKK|NF\kappa B|I\kappa B\alpha - a4 * I\kappa B\alpha * NF\kappa B - a4i * IKK|I\kappa B\alpha * NF\kappa B - k1 * NF\kappa B$$

$$NF\kappa B_n{}' = k1 * NF\kappa B - a4n * NF\kappa B_n * I\kappa B\alpha_n$$

$$I\kappa B\alpha_{mRNA}{}' = tr2a + tr4a * NF\kappa B_n{}^2 - tr3a * I\kappa B\alpha_{mRNA}$$

$$I\kappa B\alpha' = -a4 * I\kappa B\alpha * NF\kappa B - a1 * I\kappa B\alpha * IKK + d1 * IKK|I\kappa B\alpha + tr1a * I\kappa B\alpha_{mRNA} - deg1 * I\kappa B\alpha - tp1a * I\kappa B\alpha$$

$$I\kappa B\alpha_n{}' = tp1a * I\kappa B\alpha - a4n * NF\kappa B_n * I\kappa B\alpha_n - deg1n * I\kappa B\alpha_n$$

$$IKK' = r4 * IKK|NF\kappa B|I\kappa B\alpha - a1 * IKK * I\kappa B\alpha + d1 * IKK|I\kappa B\alpha - a7 * IKK * NF\kappa B|I\kappa B\alpha$$

$$IKK|I\kappa B\alpha' = -a4i * IKK|I\kappa B\alpha * NF\kappa B + a1 * I\kappa B\alpha * IKK - d1 * IKK|I\kappa B\alpha$$

$$IKK|NF\kappa B|I\kappa B\alpha' = a4i * IKK|I\kappa B\alpha * NF\kappa B - r4 * IKK|NF\kappa B|I\kappa B\alpha + a7 * IKK * NF\kappa B|I\kappa B\alpha$$

$$NF\kappa B|I\kappa B\alpha_n{}' = a4n * NF\kappa B_n * I\kappa B\alpha_n - k2a * NF\kappa B|I\kappa B\alpha_n$$

$$NF\kappa B|I\kappa B\alpha' = k2a * NF\kappa B|I\kappa B\alpha_n + a4 * I\kappa B\alpha * NF\kappa B - a7 * IKK * NF\kappa B|I\kappa B\alpha$$

Please note that the ODE model consists of only 8 independent variables, since the total concentrations of NF-κB and IKK are defined by the following algebraic equations:

$$total\ NF\kappa B = NF\kappa B + NF\kappa B_n + NF\kappa B|I\kappa B\alpha + NF\kappa B|I\kappa B\alpha_n + IKK|NF\kappa B|I\kappa B\alpha$$

$$total\ IKK = IKK + IKK|I\kappa B\alpha + IKK|NF\kappa B|I\kappa B\alpha$$

Modified core model accounting for variable levels of total IKK

For the modified model, which is used for simulations in Figures 6 and S5 the algebraic equation for total IKK was replaced by the following ODE

$$IKK'_{total} = -k_{trans} IKK_{total} + k_{trans} IKK_{conc}$$

where IKK_{conc} is either 0.00075 μM or 0.80075 μM for the unstimulated or the stimulated system. The parameter k_{trans} is set to 0.0769 min^{-1}.

References

1 **Dhooge A, Govaerts W, Kuznetsov YA.** 2003. MATCONT: A MATLAB package for numerical bifurcation analysis of ODEs. *Acm T Math Software* **29**: 141-64.

2 **Kearns JD, Basak S, Werner SL, Huang CS, et al.** 2006. IkappaBepsilon provides negative feedback to control NF-kappaB oscillations, signaling dynamics, and inflammatory gene expression. *J Cell Biol* **173**: 659-64.

3 **Llorens M, Nuno JC, Rodriguez Y, Melendez-Hevia E, et al.** 1999. Generalization of the theory of transition times in metabolic pathways: a geometrical approach. *Biophys J* **77**: 23-36.

Supplemental Figures and Tables

parameter	value	biological description of the processes
a1	1.35 min^{-1} μM^{-1}	association of IκBα and IKK
d1	0.075 min^{-1}	dissociation of IKK|IκBα
a4	30 min^{-1} μM^{-1}	association of IκBα and NF-κB
a7	11.1 min^{-1} μM^{-1}	association of NF-κB|IκBα and IKK
a4i	30 min^{-1} μM^{-1}	association of IKK|IκBα and NF-κB
deg1	0.12 min^{-1}	degradation of IκBα
deg1n	0.12 min^{-1}	degradation of nuclear IκBα
r4	0.36 min^{-1}	IKK-dependent degradation of IκBα bound to NF-κB
k1	5.4 min^{-1}	import of cytoplasmic NF-κB into nucleus
k2a	0.828 min^{-1}	export of nuclear NF-κB|IκBα into cytoplasm
tp1a	0.018 min^{-1}	import of IκBα into nucleus
a4n	30 min^{-1} μM^{-1}	association of nuclear IκBα and nuclear NF-κB
tr4a	1.386 min^{-1} μM^{-1}	NF-κB-dependent transcription of IκBα mRNA
tr2a	0.0001848 min^{-1} μM	constitutive transcription of IκBα mRNA
tr3a	0.0336 min^{-1}	degradation of IκBα mRNA
tr1a	0.2448 min^{-1}	synthesis of IκBα protein

Table S1: Kinetic parameters of the core model. The total NF-κB concentration is 0.1 μM, the total IKK concentration is 0.00075 μM and 0.80075 μM for the unstimulated and the stimulated system, respectively.

parameter			biological description of processes
k1			import of cytoplasmic NF-κB into nucleus
k01			export of nuclear NF-κB into cytoplasm
related to IκBα	**related to IκBβ**	**related to IκBε**	
a1	a2	a3	association of IκB and IKK
d1	d2	d3	dissociation of IKK|IκB
a4	a5	a6	association of IκB and NF-κB
d4	d5	d6	dissociation of IκB and NF-κB
deg1	deg2	deg3	degradation of IκB
deg4	deg5	deg6	degradation of IκB bound to NF-κB
a7	a8	a9	association of NF-κB|IκB and IKK
d7	d8	d9	dissociation of NF-κB|IκB and IKK
a4i	a5i	a6i	association of IKK|IκB and NF-κB
d4i	d5i	d6i	dissociation of IKK|IκB and NF-κB
a4n	a5n	a6n	association of nuclear IκB and nuclear NF-κB
d4n	d5n	d6n	dissociation of nuclear NF-κB|IκB
r1	r2	r3	IKK-dependent degradation of IκB
r4	r5	r6	IKK-dependent degradation of NF-κB-bound IκB
deg1n	deg2n	deg3n	degradation of nuclear IκB
deg4n	deg5n	deg6n	degradation of nuclear, NF-κB-bound IκB
k2a	k2b	k2e	export of NF-κB|IκB into cytoplasm
tp1a	tp1b	tp1e	import of cytoplasmic IκB into nucleus
tp2a	tp2b	tp2e	export of nuclear IκB into cytoplasm
tr4a	tr4b	tr4e	NF-κB-dependent transcription of IκB mRNA
tr2a	tr2b	tr2e	constitutive transcription of IκB mRNA
tr3a	tr3b	tr3e	degradation of IκB mRNA
tr1a	tr1b	tr1e	synthesis of IκB protein

Table S2: Descriptions of the kinetic parameters of the model published by Kearns *et al.* [2]. Column 1-3 represent the parameter for the three IκB isoforms $\alpha, \beta,$ and ε, respectively. The total NF-κB concentration is 0.125 μM and the total IKK concentration is 0.001 μM and 0.801 μM for the unstimulated and the stimulated system, respectively.

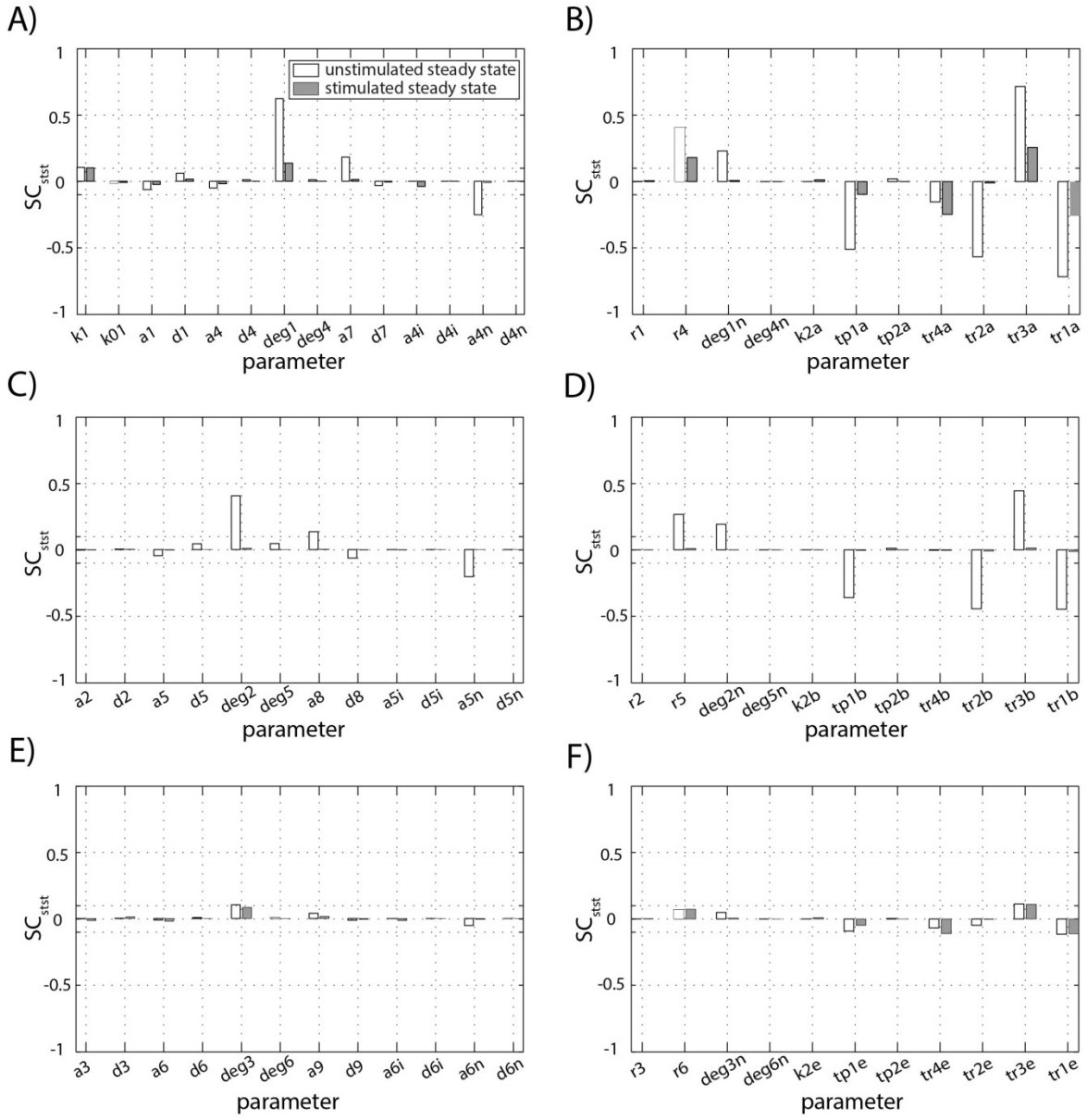

Figure S1: Sensitivity coefficients for the unstimulated (white bars) and stimulated (black bars) steady state concentration of active NF-κB (SC_{stst}). **A, B:** Sensitivity coefficients are shown for all parameters associated to the IκBα feedback including the parameters for the import and export of NF-κB into and out of the nucleus (k1, k01). **C, D:** Sensitivity coefficients are shown for all parameters associated to the IκBβ feedback. **E, F:** Sensitivity coefficients are shown for all parameters associated to the IκBε feedback.

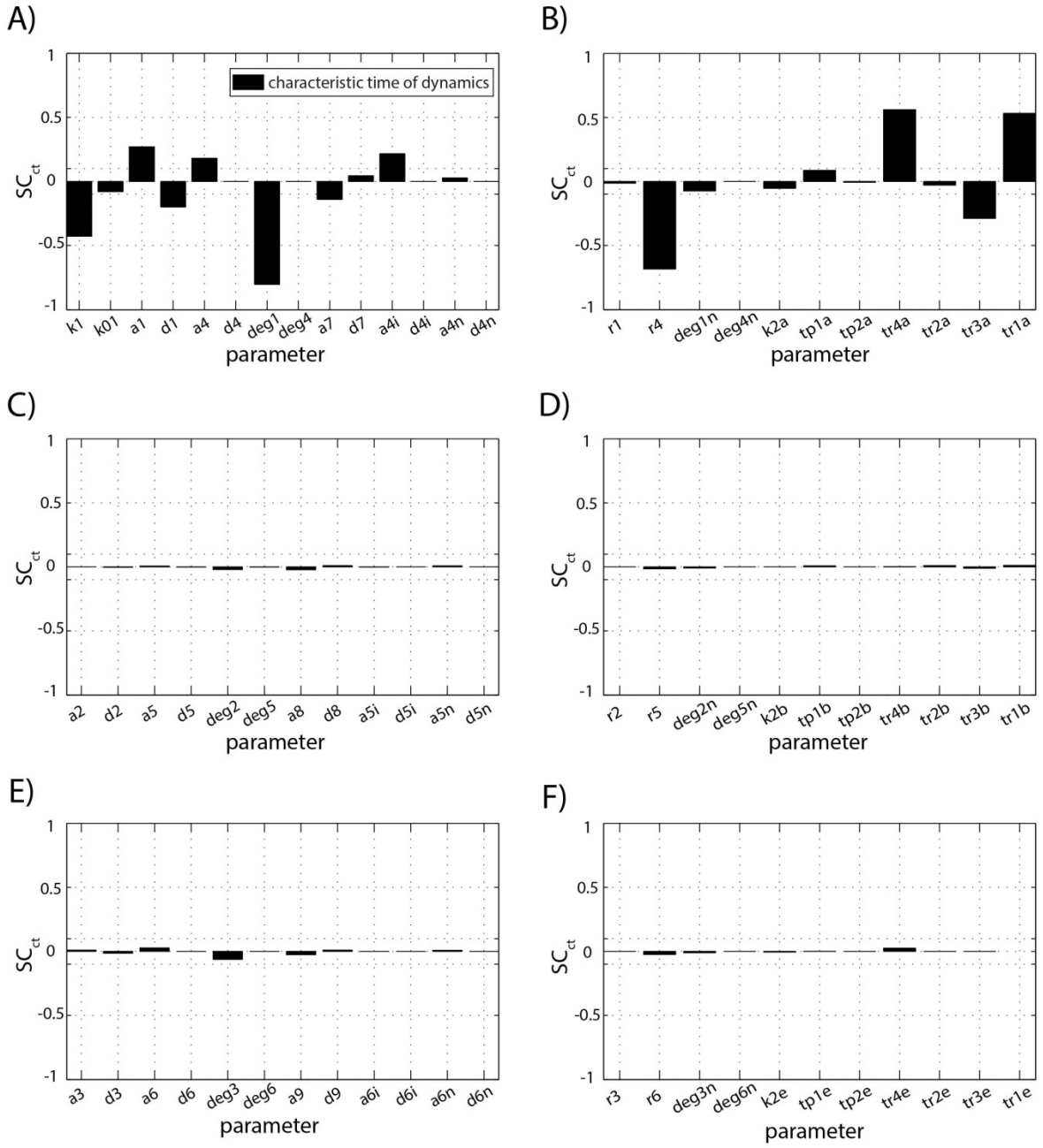

Figure S2: Sensitivity coefficients for the characteristic time of active NF-κB (SC_{ct}). **A, B:** Sensitivity coefficients are shown for all parameters associated to the IκBα feedback including the parameters for the import and export of NF-κB into and out of the nucleus (k1, k01). **C, D:** Sensitivity coefficients are shown for all parameters associated to the IκBβ feedback. **E, F:** Sensitivity coefficients are shown for all parameters associated to the IκBε feedback.

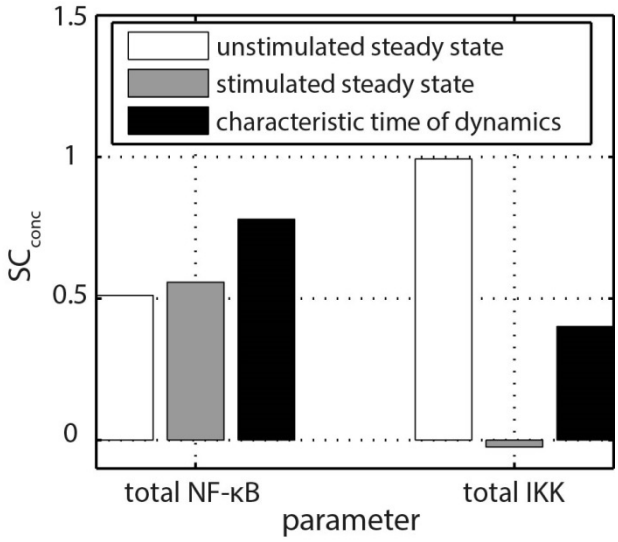

Figure S3: Sensitivity coefficients for the unstimulated (white) and stimulated (grey) steady state concentration of active NF-κB and the characteristic time (black) of active NF-κB are shown for the total concentration of NF-κB and IKK.

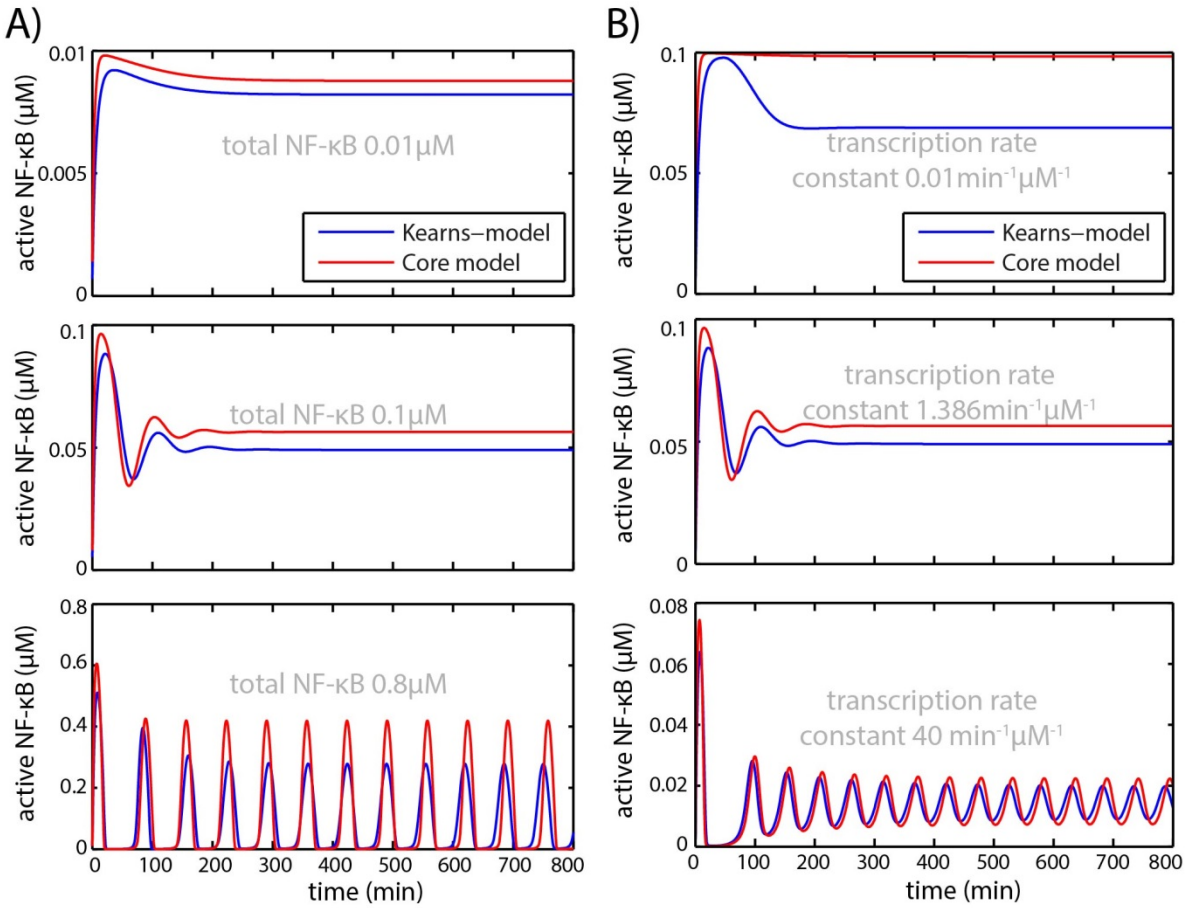

Figure S4: Influence of the total NF-κB concentration (**A**) and the IκBα transcription rate constant (**B**) on the dynamics of active NF-κB in the original Kearns model (blue) and the core model (red). The dynamical behaviour upon stimulation is comparable in both models for variation of the parameter levels. Low and intermediate values of both parameters lead to a monotone increase or damped oscillations evolving to a steady state. High parameter values lead to sustained oscillations.

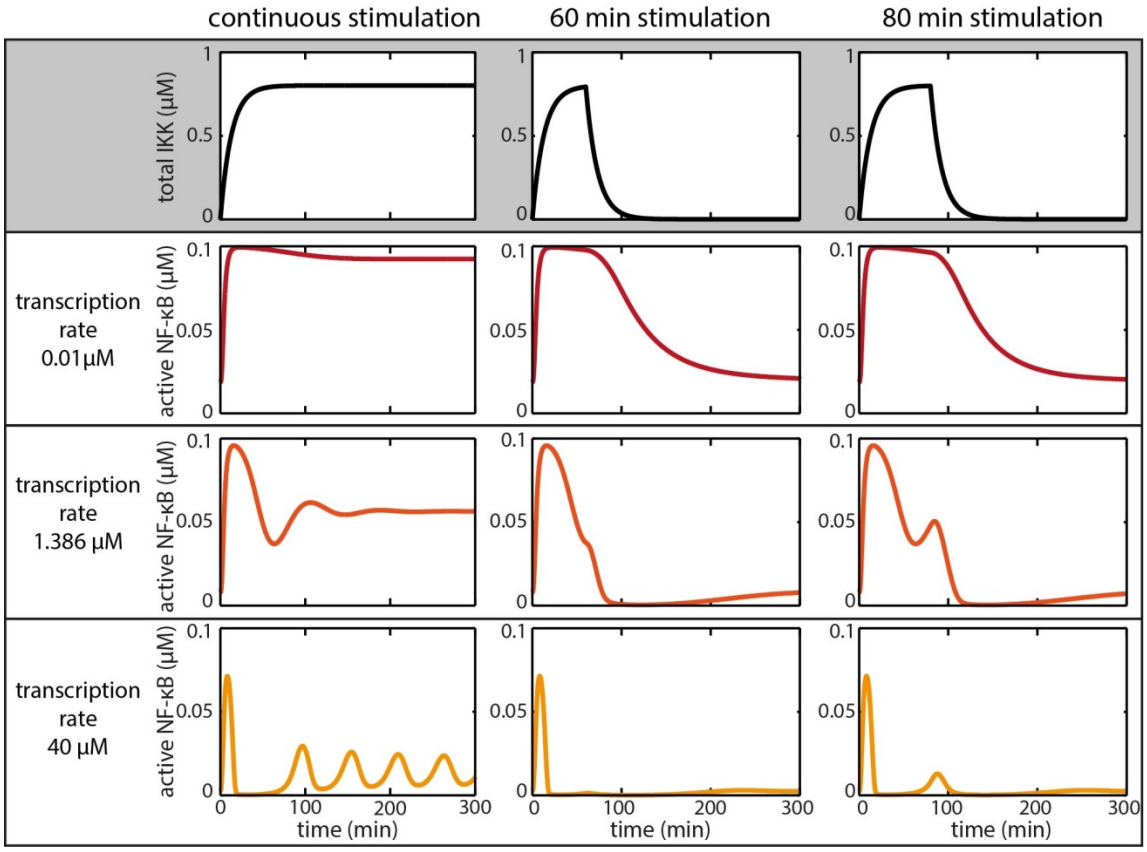

Figure S5: Response of active NF-κB to transient external stimulation for various IκBα transcription rate constants. The core model was modified to allow for a variable total IKK concentration. The left column shows the dynamics upon continuous stimulation with low (dark red), medium (red) and high (orange) transcription rate constants. With increasing transcription rate constant of IκBα, active NF-κB dynamics changes from a monotone increase and damped oscillations to sustained oscillations. The middle and the right column show the dynamics of active NF-κB upon 60 min and 80 min of stimulation for the different IκBα transcription rate constants, respectively.