Spontaneous NF-κB Activation by Autocrine TNFα Signaling: A Computational Analysis

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Supporting Information

Supplementary Text S1 with Figures S1–S4

1 Parameters and reactions

Table — Notation guide

Symbol	*Description*
$TNFR_{\mathrm{i}}$	inactive TNFR1 receptors
$TNFR_{\mathrm{a}}$	active TNFR1 receptors
$IKKK_{\mathrm{n}}$	neutral form of IKKK
$IKKK_{\mathrm{a}}$	active form of IKKK
IKK_{i}	inactive form of IKK
IKK_{ii}	inactive intermediate form of IKK
IKK_{n}	neutral form of IKK kinase
IKK_{a}	active form of IKK
$A20_{\mathrm{mRNA}}$	A20 transcript
$A20$	cytoplasmic A20
$I\kappa B\alpha_{\mathrm{mRNA}}$	IκBα transcript
$I\kappa B\alpha$	cytoplasmic IκBα
$I\kappa B\alpha_{\mathrm{n}}$	nuclear IκBα
$I\kappa B\alpha_{\mathrm{p}}$	phosphorylated cytoplasmic IκBα
$NF\kappa B$	cytoplasmic NF-κB
$NF\kappa B_{\mathrm{n}}$	nuclear NF-κB
$(NF\kappa B : I\kappa B\alpha)$	cytoplasmic NF-κB:IκBα complexes
$(NF\kappa B_{\mathrm{n}} : I\kappa B\alpha_{\mathrm{n}})$	nuclear NF-κB:IκBα complexes
$(NF\kappa B : I\kappa B\alpha_{\mathrm{p}})$	phosphorylated cytoplasmic IκBα complexed to NF-κB
TNF_{mRNA}	TNFα transcript
TNF	intracellular TNFα
TNF_{ext}	extracellular TNFα
$G^i_{\mathrm{I\kappa B}}$	state of the i^{th} IκBα gene copy, discrete random variable: $G^i_{\mathrm{I\kappa B}} \in \{0, 1\}$
G^i_{A20}	state of the i^{th} A20 gene copy, discrete random variable: $G^i_{\mathrm{A20}} \in \{0, 1\}$
G^i_{TNF}	state of the i^{th} TNFα gene copy, discrete random variable: $G^i_{\mathrm{TNF}} \in \{0, 1\}$
$G_{\mathrm{I\kappa B}}$	$G_{\mathrm{I\kappa B}} \coloneqq \sum_i G^i_{\mathrm{I\kappa B}}$
G_{A20}	$G_{\mathrm{A20}} \coloneqq \sum_i G^i_{\mathrm{A20}}$
G_{TNF}	$G_{\mathrm{TNF}} \coloneqq \sum_i G^i_{\mathrm{TNF}}$

Table — The cell parameters

Parameter	*Symbol*	*Value*	*Remarks*	*References*
$(\text{N:C ratio})^{-1} = \frac{\text{Volume of the cytoplasm}}{\text{Volume of the nucleus}}$	k_{v}	5	—	[2]
Number of IκBα gene copies	N_{I}	2	—	[1]
Number of A20 gene copies	N_{A}	2	—	[1]
Number of TNFα gene copies	N_{T}	2	—	this study
Median number of receptors	M_0	7×10^3	*(see also Figure S1, page 7)*	this study
Mean number of receptors	—	$\simeq 10^4$	mean $= M_0\, \mathrm{e}^{M_1^2/2}, M_1 = \sqrt{0.7}$	this study
Variance of the number of receptors	—	$\simeq 10^8$	variance $= M_0^2\,(\mathrm{e}^{M_1^2} - 1)\,\mathrm{e}^{M_1^2}$	this study
Number of receptors	R	M_0	$R = TNFR_{\text{a}}(t) + TNFR_{\text{i}}(t)$	this study
Number of IKKK molecules	K_{N}	10^5	$K_{\text{N}} = IKKK_{\text{n}}(t) + IKKK_{\text{a}}(t)$	[1]
Number of IKK molecules	K_{NN}	2×10^5	$K_{\text{NN}} = IKK_{\text{n}}(t) + IKK_{\text{a}}(t) + IKK_{\text{i}}(t) + IKK_{\text{ii}}(t)$	[2]
Number of NF-κB molecules	$NF\kappa B_{\text{tot}}$	10^5	$NF\kappa B_{\text{tot}} = NF\kappa B(t) + NF\kappa B_{\text{n}}(t) + (NF\kappa B : I\kappa B\alpha)(t) + (NF\kappa B_{\text{n}} : I\kappa B\alpha_{\text{n}})(t) + (NF\kappa B : I\kappa B\alpha_{\text{p}})(t)$	[2]

(For references, see page 6.)

Table — List of reactions

(See on the next page.)

Reaction	*Rate*	*Coefficients*	*Value*	*References*
		TNFR1 activation and signal transduction cascade		
$TNF_{\mathrm{ext}} \rightarrow \varnothing$	c_{deg}	c_{deg}	$2 \times 10^{-4}\ \mathrm{s}^{-1}$	[1]
$TNFR_{\mathrm{i}} \rightarrow TNFR_{\mathrm{a}}$	$k_{\mathrm{b}} \cdot TNF_{\mathrm{ext}}$	k_{b}	$1.2 \times 10^{-5}\ \mathrm{s}^{-1}(\mathrm{ng/ml})^{-1}$	[1]
$TNFR_{\mathrm{i}} + TNF \rightarrow TNFR_{\mathrm{a}}$	$\dfrac{c_{\mathrm{sec}}}{TNFR_{\mathrm{i}} + c_{\mathrm{b}}}$	c_{sec} c_{b}	$10^{-5}\ \mathrm{s}^{-1}$ 10^{4}	this study this study
$TNFR_{\mathrm{a}} \rightarrow TNFR_{\mathrm{i}}$	k_{f}	k_{f}	$1.2 \times 10^{-3}\ \mathrm{s}^{-1}$	[1]
$IKKK_{\mathrm{n}} \rightarrow IKKK_{\mathrm{a}}$	$\dfrac{k_{\mathrm{a}} \cdot k_{\mathrm{A20}}}{k_{\mathrm{A20}} + A20} \cdot TNFR_{\mathrm{a}}$	k_{a} k_{A20}	$10^{-5}\ \mathrm{s}^{-1}$ 10^{5}	this study [1]
$IKKK_{\mathrm{a}} \rightarrow IKKK_{\mathrm{n}}$	k_{i}	k_{i}	$10^{-2}\ \mathrm{s}^{-1}$	[1]
$IKK_{\mathrm{n}} \rightarrow IKK_{\mathrm{a}}$	$k_1 \cdot IKKK_{\mathrm{a}}^2$	k_1	$6 \times 10^{-10}\ \mathrm{s}^{-1}$	[1]
$IKK_{\mathrm{a}} \rightarrow IKK_{\mathrm{i}}$	$\dfrac{k_3}{k_2} \cdot (k_2 + A20)$	k_2 k_3	$10^{4}\ \mathrm{s}^{-1}$ $2 \times 10^{-3}\ \mathrm{s}^{-1}$	[2] [1]
$IKK_{\mathrm{i}} \rightarrow IKK_{\mathrm{ii}}\ ,\ IKK_{\mathrm{ii}} \rightarrow IKK_{\mathrm{n}}$	k_4	k_4	$10^{-3}\ \mathrm{s}^{-1}$	[1]
		IκBα, A20 and TNFα gene expression		
$(G^i_{\mathrm{A20}} = 0) \rightarrow (G^i_{\mathrm{A20}} = 1)$ $(G^i_{\mathrm{I\kappa B\alpha}} = 0) \rightarrow (G^i_{\mathrm{I\kappa B\alpha}} = 1)$	$q_1 \cdot NF\kappa B_{\mathrm{n}}$	q_1	$4 \times 10^{-7}\ \mathrm{s}^{-1}$	[1]
$(G^i_{\mathrm{A20}} = 1) \rightarrow (G^i_{\mathrm{A20}} = 0)$ $(G^i_{\mathrm{I\kappa B\alpha}} = 1) \rightarrow (G^i_{\mathrm{I\kappa B\alpha}} = 0)$	$q_2 \cdot I\kappa B\alpha_{\mathrm{n}}$	q_2	$10^{-6}\ \mathrm{s}^{-1}$	[2]
$(G^i_{\mathrm{TNF}} = 0) \rightarrow (G^i_{\mathrm{TNF}} = 1)$	$q_{1\mathrm{t}} \cdot NF\kappa B_{\mathrm{n}}$	$q_{1\mathrm{t}}$	$4 \times 10^{-8}\ \mathrm{s}^{-1}$	this study
$(G^i_{\mathrm{TNF}} = 1) \rightarrow (G^i_{\mathrm{TNF}} = 0)$	$q_{2\mathrm{t}} \cdot I\kappa B\alpha_{\mathrm{n}}$	$q_{2\mathrm{t}}$	$10^{-6}\ \mathrm{s}^{-1}$	this study
$(G^i_{\mathrm{TNF}} = 1) \rightarrow (G^i_{\mathrm{TNF}} = 0)$	$q_{2\mathrm{tt}}$	$q_{2\mathrm{tt}}$	$2 \times 10^{-3}\ \mathrm{s}^{-1}$	this study
$\varnothing \rightarrow TNF_{\mathrm{mRNA}}$	$\lambda \cdot G^i_{\mathrm{TNF}}$	λ	*variable*	this study
$\varnothing \rightarrow A20_{\mathrm{mRNA}}$	$c_1 \cdot G^i_{\mathrm{A20}}$	c_1	$0.1\ \mathrm{s}^{-1}$	[2]
$A20_{\mathrm{mRNA}} \rightarrow \varnothing$	c_3	c_3	$7.5 \times 10^{-4}\ \mathrm{s}^{-1}$	[2]
$\varnothing \rightarrow A20$	$c_4 \cdot A20_{\mathrm{mRNA}}$	c_4	$0.5\ \mathrm{s}^{-1}$	[2]
$\varnothing \rightarrow I\kappa B\alpha_{\mathrm{mRNA}}$	$c_1 \cdot G^i_{\mathrm{I\kappa B\alpha}}$	c_1	$0.1\ \mathrm{s}^{-1}$	[2]
$I\kappa B\alpha_{\mathrm{mRNA}} \rightarrow \varnothing$	c_3	c_3	$7.5 \times 10^{-4}\ \mathrm{s}^{-1}$	[2]
$\varnothing \rightarrow I\kappa B\alpha$	$c_4 \cdot I\kappa B\alpha_{\mathrm{mRNA}}$	c_4	$0.5\ \mathrm{s}^{-1}$	[2]
$TNF_{\mathrm{mRNA}} \rightarrow \varnothing$	$c_{3\mathrm{t}}$	$c_{3\mathrm{t}}$	$7.5 \times 10^{-4}\ \mathrm{s}^{-1}$	this study
$\varnothing \rightarrow TNF$	$c_{4t} \cdot TNF_{\mathrm{mRNA}}$	$c_{4\mathrm{t}}$	$0.05\ \mathrm{s}^{-1}$	this study
		Protein interactions and lifetime		
$NF\kappa B + I\kappa B\alpha \rightarrow (NF\kappa B : I\kappa B\alpha)$ $NF\kappa B_{\mathrm{n}} + I\kappa B\alpha_{\mathrm{n}} \rightarrow (NF\kappa B_{\mathrm{n}} : I\kappa B\alpha_{\mathrm{n}})$	a_1 $a_1 \cdot k_{\mathrm{v}}$	a_1 k_{v}	$5 \times 10^{-7}\ \mathrm{s}^{-1}$ 5	[2] [2]
$I\kappa B\alpha \rightarrow I\kappa B\alpha_{\mathrm{p}}$	$a_2 \cdot IKK_{\mathrm{a}}$	a_2	$10^{-7}\ \mathrm{s}^{-1}$	[2]
$(NF\kappa B : I\kappa B\alpha) \rightarrow (NF\kappa B : I\kappa B\alpha_{\mathrm{p}})$	$a_3 \cdot IKK_{\mathrm{a}}$	a_3	$5 \times 10^{-7}\ \mathrm{s}^{-1}$	[2]
$A20 \rightarrow \varnothing$	c_5	c_5	$5 \times 10^{-4}\ \mathrm{s}^{-1}$	[2]
$I\kappa B\alpha_{\mathrm{p}} \rightarrow \varnothing$ $(NF\kappa B : I\kappa B\alpha_{\mathrm{p}}) \rightarrow NF\kappa B$	t_{p}	t_{p}	$10^{-2}\ \mathrm{s}^{-1}$	[2]
$I\kappa B\alpha \rightarrow \varnothing$	$c_{5\mathrm{a}}$	$c_{5\mathrm{a}}$	$10^{-4}\ \mathrm{s}^{-1}$	[2]
$TNF \rightarrow \varnothing$	$c_{\mathrm{sec}} + c_{5\mathrm{t}}$	c_{sec} $c_{5\mathrm{t}}$	$10^{-5}\ \mathrm{s}^{-1}$ $2 \times 10^{-4}\ \mathrm{s}^{-1}$	this study this study
$(NF\kappa B : I\kappa B\alpha) \rightarrow NF\kappa B$	$c_{6\mathrm{a}}$	$c_{6\mathrm{a}}$	$2 \times 10^{-5}\ \mathrm{s}^{-1}$	[2]
		Transport		
$NF\kappa B \rightarrow NF\kappa B_{\mathrm{n}}$	i_1	i_1	$10^{-2}\ \mathrm{s}^{-1}$	[2]
$(NF\kappa B_{\mathrm{n}} : I\kappa B\alpha_{\mathrm{n}}) \rightarrow (NF\kappa B : I\kappa B\alpha)$	$e_{2\mathrm{a}}$	$e_{2\mathrm{a}}$	$5 \times 10^{-2}\ \mathrm{s}^{-1}$	[2]
$I\kappa B\alpha \rightarrow I\kappa B\alpha_{\mathrm{n}}$	$i_{1\mathrm{a}}$	$i_{1\mathrm{a}}$	$2 \times 10^{-3}\ \mathrm{s}^{-1}$	[2]
$I\kappa B\alpha_{\mathrm{n}} \rightarrow I\kappa B\alpha$	$e_{1\mathrm{a}}$	$e_{1\mathrm{a}}$	$5 \times 10^{-3}\ \mathrm{s}^{-1}$	[2]

2 Differential equations

$$\frac{\mathrm{d}}{\mathrm{d}t}TNFR_{\mathrm{a}}(t) = TNF(t)\cdot c_{\mathrm{sec}}\frac{TNFR_{\mathrm{i}}(t)}{TNFR_{\mathrm{i}}(t)+c_{\mathrm{b}}} + k_{\mathrm{b}}\cdot TNF_{\mathrm{ext}}(t)\cdot TNFR_{\mathrm{i}}(t) - k_{\mathrm{f}}\cdot TNFR_{\mathrm{a}}(t) \quad (1)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKKK_{\mathrm{a}}(t) = k_{\mathrm{a}}\cdot\frac{k_{\mathrm{A20}}}{k_{\mathrm{A20}}+A20(t)}\cdot TNFR_{\mathrm{a}}(t)\cdot IKKK_{\mathrm{n}}(t) - k_{\mathrm{i}}\cdot IKKK_{\mathrm{a}}(t) \quad (2)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKK_{\mathrm{a}}(t) = k_1\cdot IKKK_{\mathrm{a}}(t)^2\cdot IKK_{\mathrm{n}}(t) - IKK_{\mathrm{a}}(t)\cdot\frac{k_3}{k_2}(k_2+A20(t)) \quad (3)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKK_{\mathrm{n}}(t) = -k_1\cdot IKKK_{\mathrm{a}}(t)^2\cdot IKK_{\mathrm{n}}(t) + k_4\cdot IKK_{\mathrm{ii}}(t) \quad (4)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKK_{\mathrm{i}}(t) = IKK_{\mathrm{a}}(t)\cdot\frac{k_3}{k_2}(k_2+A20(t)) - k_4\cdot IKK_{\mathrm{i}}(t) \quad (5)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}A20_{\mathrm{mRNA}}(t) = c_1\cdot G_{\mathrm{A20}}(t) - c_3\cdot A20_{\mathrm{mRNA}}(t) \quad (6)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}A20(t) = c_4\cdot A20_{\mathrm{mRNA}}(t) - c_5\cdot A20(t) \quad (7)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha_{\mathrm{mRNA}}(t) = c_1\cdot G_{\mathrm{I\kappa B\alpha}}(t) - c_3\cdot I\kappa B\alpha_{\mathrm{mRNA}}(t) \quad (8)$$

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha(t) = {} & -a_2\cdot IKK_{\mathrm{a}}(t)\cdot I\kappa B\alpha(t) - a_1\cdot I\kappa B\alpha(t)\cdot NF\kappa B(t) + c_4\cdot I\kappa B\alpha_{\mathrm{mRNA}}(t)\\ & -c_{5\mathrm{a}}\cdot I\kappa B\alpha(t) - i_{1\mathrm{a}}\cdot I\kappa B\alpha(t) + e_{1\mathrm{a}}\cdot I\kappa B\alpha_{\mathrm{n}}(t)\end{aligned} \quad (9)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha_{\mathrm{n}}(t) = -a_1\cdot k_{\mathrm{v}}\cdot I\kappa B_{\mathrm{n}}(t)\cdot NF\kappa B_{\mathrm{n}}(t) + i_{1\mathrm{a}}\cdot I\kappa B\alpha(t) - e_{1\mathrm{a}}\cdot I\kappa B\alpha_{\mathrm{n}}(t) \quad (10)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha_{\mathrm{p}}(t) = a_2\cdot IKK_{\mathrm{a}}(t)\cdot I\kappa B\alpha(t) - t_{\mathrm{p}}\cdot I\kappa B\alpha_{\mathrm{p}}(t) \quad (11)$$

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}NF\kappa B(t) = {} & c_{6a}\cdot(NF\kappa B : I\kappa B\alpha)(t) - a_1\cdot NF\kappa B(t)\cdot I\kappa B\alpha(t)\\ & +t_{\mathrm{p}}\cdot(NF\kappa B : I\kappa B\alpha_{\mathrm{p}})(t) - i_1\cdot NF\kappa B(t)\end{aligned} \quad (12)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}NF\kappa B_{\mathrm{n}}(t) = i_1\cdot NF\kappa B(t) - a_1\cdot k_{\mathrm{v}}\cdot I\kappa B\alpha_{\mathrm{n}}(t)\cdot NF\kappa B_{\mathrm{n}}(t) \quad (13)$$

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}(NF\kappa B : I\kappa B\alpha)(t) = {} & a_1\cdot I\kappa B\alpha(t)\cdot NF\kappa B(t) - c_{6\mathrm{a}}\cdot(NF\kappa B : I\kappa B\alpha)(t)\\ & +e_{2\mathrm{a}}\cdot(NF\kappa B_{\mathrm{n}} : I\kappa B\alpha_{\mathrm{n}})(t) - a_3\cdot IKK_{\mathrm{a}}\cdot(NF\kappa B : I\kappa B\alpha)(t)\end{aligned} \quad (14)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}(NF\kappa B : I\kappa B\alpha_{\mathrm{p}})(t) = a_3\cdot IKK_{\mathrm{a}}(t)\cdot(NF\kappa B : I\kappa B\alpha)(t) - t_{\mathrm{p}}\cdot(NF\kappa B : I\kappa B\alpha_{\mathrm{p}})(t) \quad (15)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}TNF_{\mathrm{mRNA}}(t) = \lambda\cdot G_{\mathrm{TNF}}(t) - c_{3\mathrm{t}}\cdot TNF_{\mathrm{mRNA}}(t) \quad (16)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}TNF(t) = c_{4\mathrm{t}}\cdot TNF_{\mathrm{mRNA}}(t) - c_{5\mathrm{t}}\cdot TNF(t) - c_{\mathrm{sec}}\cdot TNF \quad (17)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}TNF_{\mathrm{ext}}(t) = -c_{\mathrm{deg}}\cdot TNF_{\mathrm{ext}} \quad (18)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}G_{\mathrm{A20}}(t) = q_1\cdot NF\kappa B_{\mathrm{n}}(t)\cdot(N_{\mathrm{A}} - G_{\mathrm{A20}}(t)) - q_2\cdot I\kappa B\alpha_{\mathrm{n}}(t)\cdot G_{\mathrm{A20}}(t) \quad (19)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}G_{\mathrm{I\kappa B\alpha}}(t) = q_1\cdot NF\kappa B_{\mathrm{n}}(t)\cdot(N_{\mathrm{I}} - G_{\mathrm{I\kappa B\alpha}}(t)) - q_2\cdot I\kappa B\alpha_{\mathrm{n}}(t)\cdot G_{\mathrm{I\kappa B\alpha}}(t) \quad (20)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}G_{\mathrm{TNF}}(t) = q_{1\mathrm{t}}\cdot NF\kappa B_{\mathrm{n}}(t)\cdot(N_{\mathrm{T}} - G_{\mathrm{TNF}}(t)) - (q_{2\mathrm{tt}} + q_{2\mathrm{t}}\cdot I\kappa B\alpha_{\mathrm{n}}(t))\cdot G_{\mathrm{TNF}}(t) \quad (21)$$

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$$\frac{\mathrm{d}}{\mathrm{d}t}TNFR_{\mathrm{i}}(t) = -TNF(t)\cdot c_{\mathrm{sec}}\frac{TNFR_{\mathrm{i}}(t)}{TNFR_{\mathrm{i}}(t)+c_{\mathrm{b}}} - k_{\mathrm{b}}\cdot TNF_{\mathrm{ext}}(t)\cdot TNFR_{\mathrm{i}}(t) + k_{\mathrm{f}}\cdot TNFR_{\mathrm{a}}(t) \quad (22)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKKK_{\mathrm{n}}(t) = -k_{\mathrm{a}}\cdot\frac{k_{\mathrm{A20}}}{k_{\mathrm{A20}}+A20(t)}\cdot TNFR_{\mathrm{a}}(t)\cdot IKKK_{\mathrm{n}}(t) + k_{\mathrm{i}}\cdot IKKK_{\mathrm{a}}(t) \quad (23)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKK_{\mathrm{ii}}(t) = k_4\cdot IKK_{\mathrm{i}}(t) - k_4\cdot IKK_{\mathrm{ii}}(t) \quad (24)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}(NF\kappa B_{\mathrm{n}} : I\kappa B\alpha_{\mathrm{n}})(t) = a_1\cdot k_{\mathrm{v}}\cdot I\kappa B\alpha_{\mathrm{n}}(t)\cdot NF\kappa B_{\mathrm{n}}(t) - e_{2\mathrm{a}}\cdot(NF\kappa B_{\mathrm{n}} : I\kappa B\alpha_{\mathrm{n}})(t) \quad (25)$$

The last four variables from equations (22–25) can be determined equivalently by the following conservation laws:

$$TNFR_{\mathrm{i}}(t) = R - TNFR_{\mathrm{a}}(t) \quad (26)$$

$$IKKK_{\mathrm{n}}(t) = K_{\mathrm{N}} - IKKK_{\mathrm{a}}(t) \quad (27)$$

$$IKK_{\mathrm{ii}}(t) = K_{\mathrm{NN}} - IKK_{\mathrm{a}}(t) - IKK_{\mathrm{i}}(t) - IKK_{\mathrm{n}}(t) \quad (28)$$

$$(NF\kappa B_{\mathrm{n}} : I\kappa B\alpha_{\mathrm{n}})(t) = NF\kappa B_{\mathrm{tot}} - NF\kappa B(t) - NF\kappa B_{\mathrm{n}}(t) - (NF\kappa B : I\kappa B\alpha)(t) - (NF\kappa B : I\kappa B\alpha_{\mathrm{p}})(t) \quad (29)$$

The complete system (1–25) is however more convenient for further development of the model.

3 Methods and protocols of numerical simulations

Deterministic model

The model was coded both in MATLAB and BIONETGEN. BIONETGEN is a language and software intended for defining and simulating regulatory networks of high combinatorial complexity [3], and is capable of performing deterministic as well as stochastic simulations. BIONETGEN allows for rule-based specification of the model; the rules are then used to build a list of reactions or ODEs which are solved by an integrated CVODE solver or simulated according to the Gillespie direct Stochastic Simulation Algorithm [4]. The model was originally written in MATLAB and then rewritten to BIONETGEN only to enable efficient stochastic simulations. Since the BIONETGEN code results from the exact translation of the original MATLAB code, the number of rules is equal to the number of reactions.

The bifurcation diagrams with TNFα transcription rate λ as the bifurcation parameter (Figs. 3 and 4 in the main text and Supplementary Figs. S2 and S3) were obtained using the MATCONT continuation software. In order to perform the analysis, we used ODEs (1–21) in which variables $TNFR_{\mathrm{i}}$, $IKKK_{\mathrm{n}}$, IKK_{ii} and $(NF\kappa B_{\mathrm{n}} : I\kappa B\alpha_{\mathrm{n}})$ were defined by algebraic equations (26–29).

The simulations of WT and A20$^{-/-}$ cells showed in Figs. 5, 7 and 9 (in the main text) were started from the steady state corresponding to the extracellular TNFα concentration equal zero. A20$^{-/-}$ cells were modeled by setting the number of A20 gene copies equal to zero. Numerical integration was performed using the MATLAB's implementation of the TR-BDF2 method (solver `ode23tb`).

Stochastic model

The stochastic simulations were performed using the Gillespie direct Stochastic Simulation Algorithm [4]. The direct method of stochastic simulation as implemented in BIONETGEN allows for expressing reaction rates through functions of the system state, i.e. current number of molecules of given species. At every time step between consecutive reaction events, reaction propensities calculated using such functions remain constant. This enables exact simulation in accordance with the underlying Chemical Master Equation and at the same time allows for defining propensities not necessarily following the mass action kinetics.

To ensure random initial conditions at time $t = 0$, each stochastic simulation was started at time $t = -300$ h. The population average (Figs. 7 and 9 in the main text) was obtained based on 100 simulations. The fraction of responding cells (Fig. 6 in the main text) for each value of TNFα dose was obtained based on 500 simulations. For the purpose of analysis presented in Fig. 8 (in the main text), cells were considered activated when the fraction of nuclear NF-κB increased above 0.15, which allows for almost maximum expression of IκBα and A20 genes.

4 Experimental protocols

Gene expression analysis was performed on mouse 3T3 fibroblast cells using high-throughput microfluidic qPCR and digital-PCR. Cells grown on regular culture wells at equal density (80% confluence) were stimulated with various doses of TNFα at the beginning of experiments (10, 1, 0.1, 0.05, 0.025 and 0.01 ng/ml). At the end of each experiment ($t = 0.25$, 0.5, 2, 4, 6, 8, 10 and 12 hours), cells were lysed and cDNA was synthesized using standard protocols from Invitrogen (CellsDirect One-Step RT-PCR). Real-time qPCR was performed using the Invitrogen TaqMan probe for TNFα using the 48×48 Dynamic Array microfluidic chips and the Biomark System, both from Fluidigm. Each time and dose condition was repeated four times and the median value was used in Figure 2A (in the main text), resulting in a total of 192 qPCR measurements.

ELISpot experiments were carried out in a 96-well format according to manufacturer's instructions (R&D Systems ELISpot Kit EL410). NIH 3T3 fibroblast and 264.7 RAW macrophage cells were seeded onto plates at $\approx 2 \times 10^5$ cells/ml. The total number of cells added into each well was established via cell counting during seeding. After incubating cells for five hours, the assay was completed. Spots were manually counted from images taken with a Nikon SMZ 1500 microscope, without regard to intensity level of the spot. This allows for quantifying the percentage of cells which have secreted the cytokine over the course of the experiment.

Supplementary references

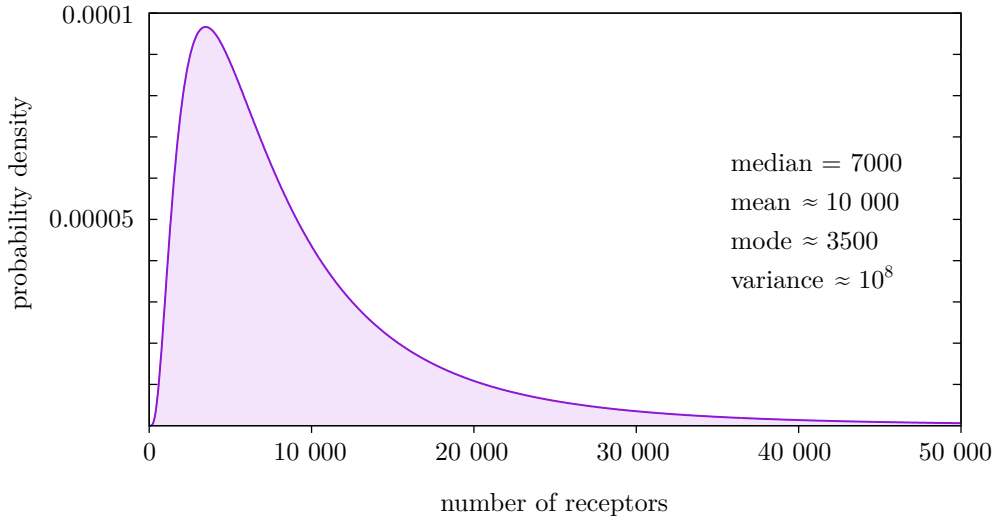
[1] Tay S, Hughey J, Lee T, Lipniacki T, Covert M, Quake M (2010) Single-cell NF-κB dynamics reveal digital activation and analogue information processing. *Nature* **466**:267–271.

[2] Lipniacki T, Puszynski T, Paszek P, Brasier AR, Kimmel M (2007) Single TNFα trimers mediating NF-κB activation: Stochastic robustness of NF-κB signaling. *BMC Bioinformatics* **8**:376.

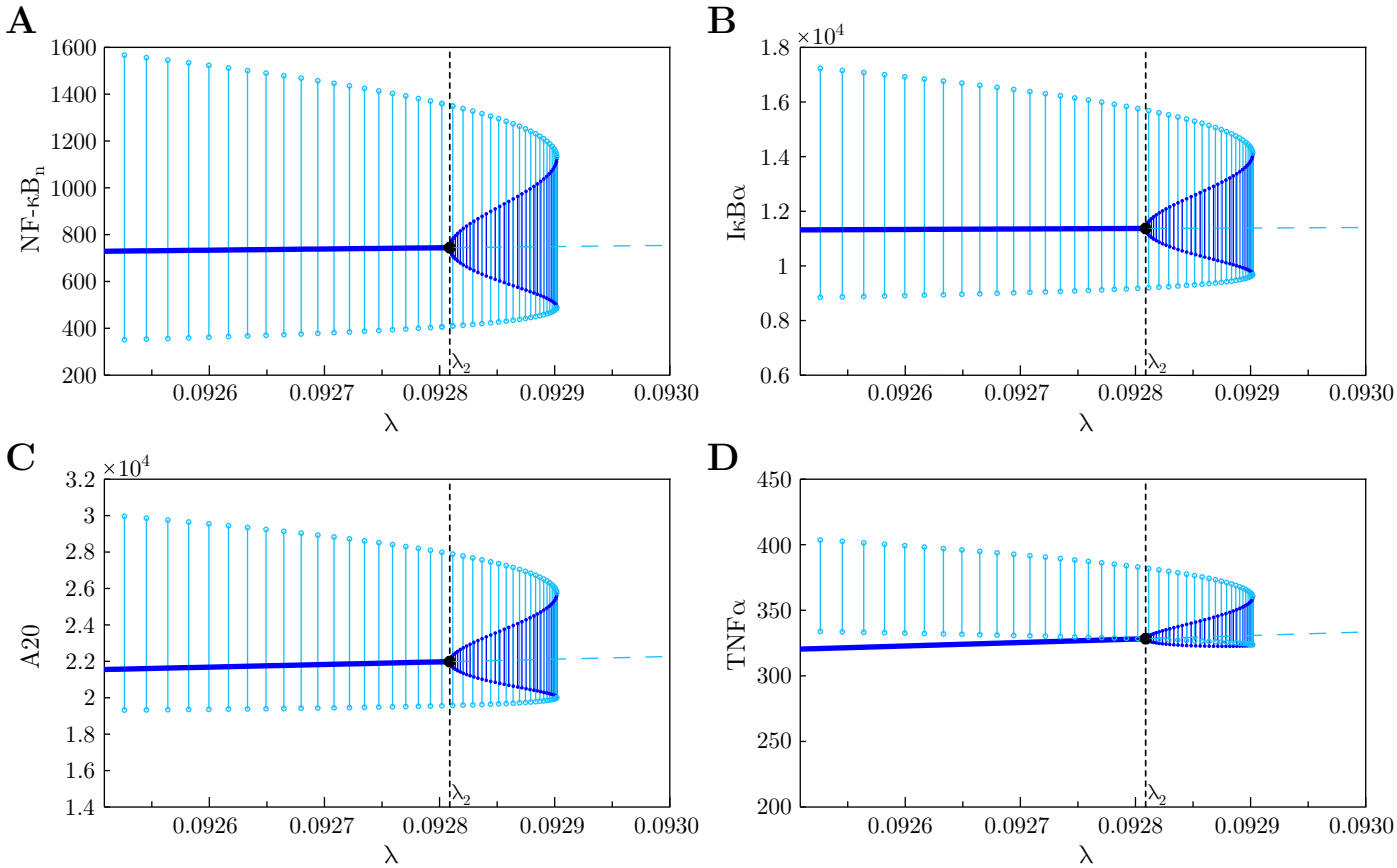
[3] Faeder JR, Blinov ML, Hlavacek WS (2009) Rule-based modeling of biochemical systems with BioNetGen. *Methods Mol. Biol.* **500**:113–167.

[4] Gillespie DT (1977) Exact stochastic simulations of coupled chemical reactions. *J. Phys. Chem.* **81**:2340–2361.

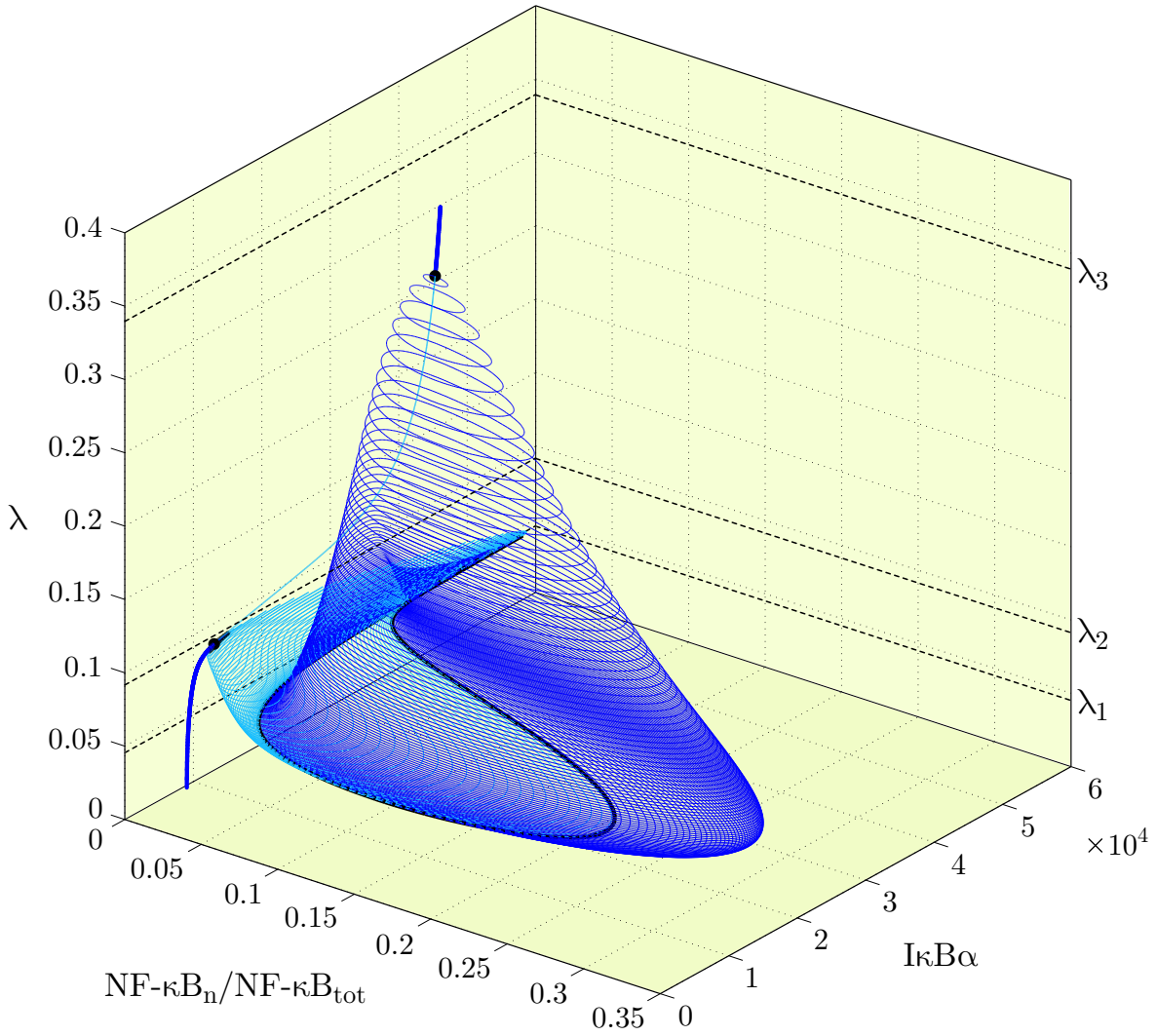
5 Supplementary figures



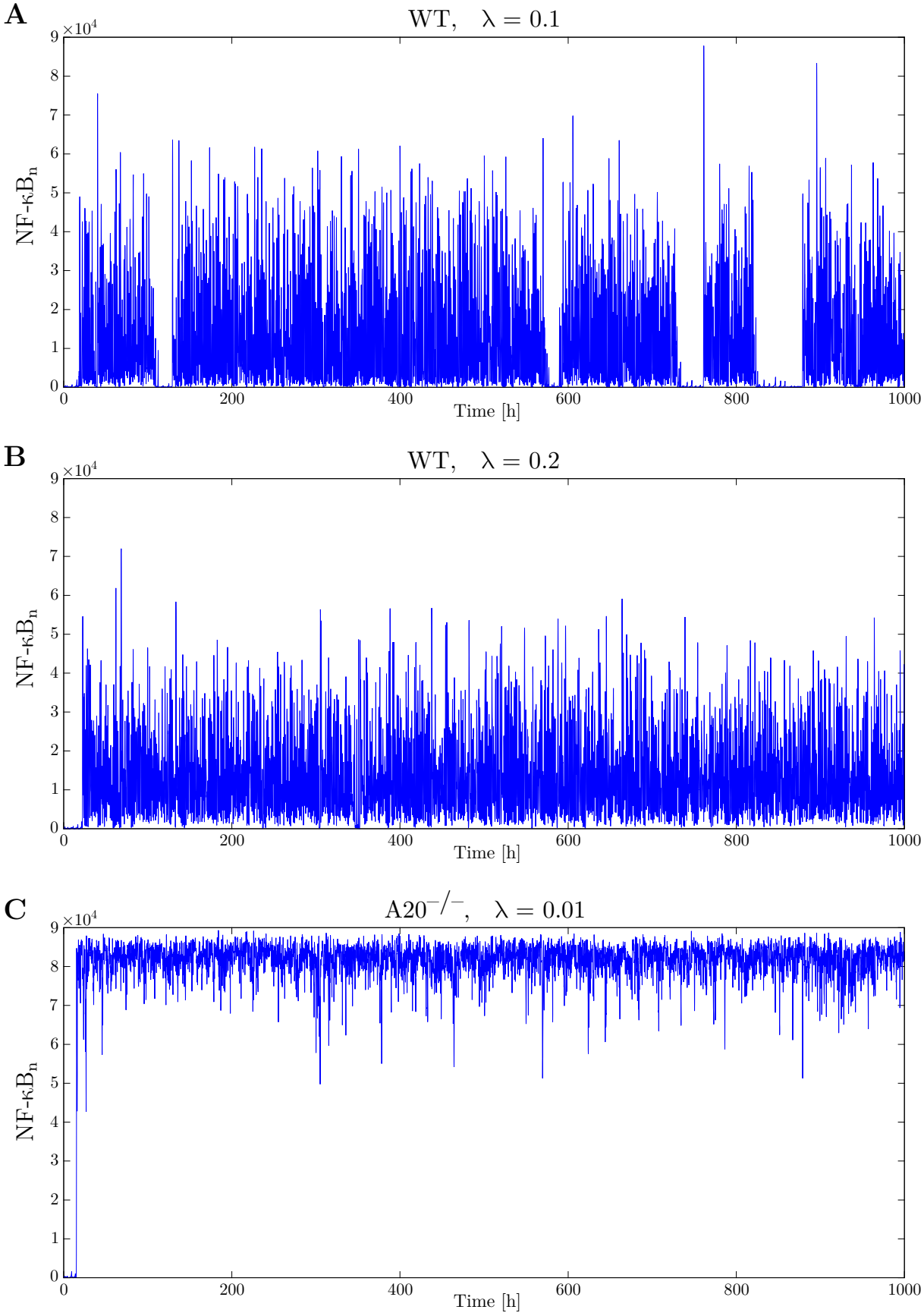
Supplementary Figure S1. The log-normal distribution of the number of TNFR1 receptors.



Supplementary Figure S2. Bifurcation diagrams: close-up of the Hopf bifurcation point in λ_2. Stable steady state – thick dark blue line; unstable steady state – dashed light blue line; stable limit cycle – filled dots connected by vertical dark blue lines; unstable limit cycle – small circles connected by vertical light blue lines.



Supplementary Figure S3. Bifurcation diagram for wild-type cells. Stable recurrent states – dark blue, unstable recurrent states – light blue. Cyclic fold at λ_1 and bifurcation points at λ_2, λ_3 – black.



Supplementary Figure S4. Long run stochastic trajectories for wild type (WT) cells for (A) $\lambda = 0.1$, (B) $\lambda = 0.2$, and (C) for A20-deficient ($A20^{-/-}$) cells for $\lambda = 0.01$.