Electronic Supplementary Material (ESI) for Molecular BioSystems.

Electronic Supplementary Information (ESI)

"Computational modeling reveals signaling subnetworks with distinct functional roles in the regulation of TNF production"

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1. Supplementary Tables

Table S1. Model reactions and parameters. Parameter names in the table are the same as in the MATLAB code. The numbers in parentheses in the right-most column are the literature references (see the end of this document) for the corresponding parameter values.

IκB and NFκB Cellular Localization Reactions							
#	Reaction	Parameter			Category	Localization	Parameter value source
		Name	Value	Unit			
1	IκBα → IκBαn	in_a	9×10^{-2}	min^{-1}	Import	Undefined	(1)
2	IκBε → IκBεn	in_e	4.5×10^{-2}	min^{-1}	Import	Undefined	(1)
3	IκBαn → IκBα	ex_a	1.2×10^{-2}	min^{-1}	Export	Undefined	(1)
4	IκBεn → IκBε	ex_e	1.2×10^{-2}	min^{-1}	Export	Undefined	(1)
5	NFκBIκBα → NFκBIκBαn	in_2an	0.276	min^{-1}	Import	Undefined	(1)
6	NFκBIκBε → NFκBIκBεn	in_2en	0.138	min^{-1}	Import	Undefined	(1)
7	NFκBIκBαn → NFκBIκBα	ex_2an	0.828	min^{-1}	Export	Undefined	(1)
8	NFκBIκBεn → NFκBIκBε	ex_2en	0.414	min^{-1}	Export	Undefined	(1)
9	NFκB → NFκBn	in_n	5.4	min^{-1}	Import	Undefined	(1)
10	NFκBn → NFκB	ex_n	4.8×10^{-3}	min^{-1}	Export	Undefined	(1)
IκB Protein Degradation Reactions							
11	IκBαn → Ø	pd_n_a	1.2×10^{-2}	min^{-1}	Protein degradation	Nucleus	(1)
12	IκBεn → Ø	pd_n_e	0.18	min^{-1}	Protein degradation	Nucleus	(1)
13	NFκBIκBαn → Ø	pd_n_2an	6×10^{-5}	min^{-1}	Protein degradation	Nucleus	(1)
14	NFκBIκBεn → Ø	pd_n_2en	6×10^{-5}	min^{-1}	Protein degradation	Nucleus	(1)
15	NFκBIκBα → Ø	pd_c_2an	6×10^{-5}	min^{-1}	Protein degradation	Cytoplasm	(1)
16	NFκBIκBε → Ø	pd_c_2en	6×10^{-5}	min^{-1}	Protein degradation	Cytoplasm	(1)
IκB:NFκB Association and Dissociation Reactions							
17	NFκB + IκBα → NFκBIκBα	a_c_an	30	$\mu M^{-1}\ min^{-1}$	Association	Cytoplasm	(1)
18	NFκB + IκBε → NFκBIκBε	a_c_en	30	$\mu M^{-1}\ min^{-1}$	Association	Cytoplasm	(1)
19	NFκBn + IκBαn → NFκBIκBαn	a_n_an	30	$\mu M^{-1}\ min^{-1}$	Association	Nucleus	(1)
20	NFκBn + IκBεn → NFκBIκBεn	a_n_en	30	$\mu M^{-1}\ min^{-1}$	Association	Nucleus	(1)
21	NFκBIκBα → NFκB + IκBα	d_c_an	6×10^{-5}	min^{-1}	Dissociation	Cytoplasm	(1)
22	NFκBIκBε → NFκB + IκBε	d_c_en	6×10^{-5}	min^{-1}	Dissociation	Cytoplasm	(1)
23	NFκBIκBαn → NFκBn + IκBαn	d_n_an	6×10^{-5}	min^{-1}	Dissociation	Nucleus	(1)
24	NFκBIκBεn → NFκBn + IκBεn	d_n_en	6×10^{-5}	min^{-1}	Dissociation	Nucleus	(1)
IKK-mediated IκB Degradation Reactions							
25	IKK + IκBα → IKK	pd_c_2ai	1.8	$\mu M^{-1}\ min^{-1}$	Protein degradation	Cytoplasm	Modified from (1)
26	IKK + IκBε → IKK	pd_c_2ei	0.9	$\mu M^{-1}\ min^{-1}$	Protein degradation	Cytoplasm	Modified from (1)
27	IKK + NFκBIκBαn → IKK + NFκB	pd_c_3ain	1.8	$\mu M^{-1}\ min^{-1}$	Protein degradation	Cytoplasm	Modified from (1)
28	IKK + NFκBIκBεn → IKK + NFκB	pd_c_3ain	0.9	$\mu M^{-1}\ min^{-1}$	Protein degradation	Cytoplasm	Modified from (1)
Volume Ratio and Gene Transcription Function Parameters							
29		cnvr	3	Dimensionless	Cytoplasm to nucleus	Undefined	(2)

					volume ratio		
30		mmVmax	2	μM	Maximum gene transcription rate	Undefined	Assumed
31		mmKm	0.15	μM	Michaelis constant	Undefined	Assumed
32		mmHc	1	Dimensionless	Hill coefficient	Undefined	Assumed
33		a_{rep}	0.1	Dimensionless	Repressor function constant	Undefined	Assumed
34		b_{rep}	0.006	μM	Repressor function constant	Undefined	Assumed
35		k_{rep}	0.006	μM	Inhibition strength	Undefined	Assumed
IκBα mRNA and Protein Synthesis Reactions							
36	NFκBn → NFκBn + pre-IκBαt	prs_an	5×10^{-3}	min^{-1}	NFκB induced pre-mRNA synthesis	Nucleus	Fitted
37	pre-IκBαt → Ø	prd_a	0	min^{-1}	pre-mRNA degradation	Nucleus	Assumed
38	pre-IκBαt + Spliceosome → pre-IκBαt:Spliceosome*	a_n_spa	10	min^{-1}	Spliceosome association	Nucleus	Fitted
39	Ø → pre-IκBαt	prs_a	7×10^{-5}	min^{-1}	pre-mRNA constitutive synthesis	Nucleus	(1)
40	pre-IκBαt: Spliceosome → IκBαt + Spliceosome*	rs_a	10	min^{-1}	Mature mRNA release	Nucleus	Fitted
41	IκBαt → Ø	rd_a	3.5×10^{-2}	min^{-1}	mRNA degradation	Cytoplasm	(1)
42	IκBαt → IκBα	ps_c_a	0.25	min^{-1}	Protein synthesis	Cytoplasm	(1)
43	IκBα → Ø	pd_c_a	0.12	min^{-1}	Protein degradation	Cytoplasm	(1)
IκBε mRNA and Protein Synthesis Reactions							
44	NFκBn → NFκBn + pre-IκBεt	prs_en	6×10^{-3}	min^{-1}	NFκB induced pre-mRNA synthesis	Nucleus	Fitted
45	pre-IκBεt → Ø	prd_e	0	min^{-1}	pre-mRNA degradation	Nucleus	Assumed
46	pre-IκBεt + Spliceosome → pre-IκBεt:Spliceosome*	a_n_spe	10	min^{-1}	Spliceosome association	Nucleus	Fitted
47	Ø → pre-IκBεt	prs_e	1×10^{-6}	min^{-1}	pre-mRNA basal synthesis	Nucleus	(1)
48	pre-IκBεt: Spliceosome → IκBεt + Spliceosome*	rs_e	0.1	min^{-1}	Mature mRNA release	Nucleus	Fitted
49	IκBεt → Ø	rd_e	4×10^{-3}	min^{-1}	mRNA degradation	Cytoplasm	(1)
50	IκBεt → IκBε	ps_c_e	2.5×10^{-2}	min^{-1}	Protein synthesis	Cytoplasm	(1)
51	IκBε → Ø	pd_c_e	0.18	min^{-1}	Protein degradation	Cytoplasm	(1)
A20 mRNA and Protein Synthesis Reactions							
52	NFκBn → NFκBn + pre-A20t	prs_a20n	3×10^{-4}	min^{-1}	NFκB induced pre-mRNA synthesis	Nucleus	Fitted
53	pre-A20t → Ø	prd_a20	0	min^{-1}	pre-mRNA degradation	Nucleus	Assumed
54	pre-A20t + Spliceosome → pre-A20t:Spliceosome*	a_n_spa20	10	min^{-1}	Spliceosome association	Nucleus	Fitted
55	Ø → pre-A20t	prs_a20	0	min^{-1}	pre-mRNA basal synthesis	Nucleus	Modified from (1)
56	pre-A20t: Spliceosome → A20t + Spliceosome*	rs_a20	5×10^{-3}	min^{-1}	Mature mRNA	Nucleus	Fitted

					release		
57	A20t → Ø	rd_a20	3.5×10^{-2}	min^{-1}	mRNA degradation	Cytoplasm	(1)
58	A20t → A20	ps_c_a20	0.2	min^{-1}	Protein synthesis	Cytoplasm	(1)
59	A20 → Ø	pd_c_a20	2.9×10^{-3}	min^{-1}	Protein degradation	Cytoplasm	(1)
TNF mRNA, Protein Synthesis and Export Reactions							
60	NFκBn → NFκBn + pre-TNFt	prs_t	1.6×10^{-2}	min^{-1}	NFκB induced pre-mRNA synthesis	Nucleus	Fitted
61	Pre-TNFt → Ø	prd_t	0	min^{-1}	pre-mRNA degradation	Nucleus	Assumed
62	pre-TNFt + Spliceosome → pre-TNFt:Spliceosome*	a_n_spt	10	min^{-1}	Spliceosome association	Nucleus	Fitted
63	pre-TNFt: Spliceosome → TNFt + Spliceosome*	rs_t	10	min^{-1}	Mature mRNA release	Nucleus	Fitted
64	TNFt → Ø	rd_t	1.4×10^{-2}	min^{-1}	mRNA degradation	Cytoplasm	(3)
65	TNFt → TNF	ps_c_t	1.1	min^{-1}	Protein synthesis	Cytoplasm	Fitted
66	TNF → Ø	pd_c_t	3×10^{-3}	min^{-1}	Protein degradation	Cytoplasm	(2)
67	TNF → TNFe	ex_tnf	1.5×10^{-4}	min^{-1}	Export		(2)
68	TNFe → Ø	pd_e_t	5×10^{-4}	min^{-1}	Protein degradation	Extracellular	(4)
IFN-β mRNA, Protein Synthesis and Export Reactions							
69	pIRF3n → pIRF3n + pre-IFNt	prs_i	1×10^{-2}	min^{-1}	IRF3 induced pre-mRNA synthesis	Nucleus	Fitted
70	pre-IFNt → Ø	prd_ifn	0	min^{-1}	pre-mRNA degradation	Nucleus	Assumed
71	pre-IFNt + Spliceosome → pre-IFNt:Spliceosome*	a_n_spifn	10	min^{-1}	Spliceosome association	Nucleus	Fitted
72	pre-IFNt: Spliceosome → IFNt + Spliceosome*	rs_ifn	10	min^{-1}	Mature mRNA release	Nucleus	Fitted
73	IFNt → Ø	rd_ifn	1.5×10^{-2}	min^{-1}	mRNA degradation	Cytoplasm	Fitted
74	IFNt → IFN	ps_c_ifn	5×10^{-2}	min^{-1}	Protein synthesis	Cytoplasm	Fitted
75	IFN → Ø	pd_c_ifn	1.9×10^{-2}	min^{-1}	Protein degradation	Cytoplasm	Fitted
76	IFN → IFNe	ex_ifn	1×10^{-2}	min^{-1}	Export		Fitted
77	IFNe → Ø	pd_e_ifn	2.5×10^{-3}	min^{-1}	Protein degradation	Extracellular	Fitted
IL-10 mRNA, Protein Synthesis and Export Reactions							
78	pCREBn → pCREBn + pre-IL10t	prs_il	7×10^{-3}	min^{-1}	IRF3 induced pre-mRNA synthesis	Nucleus	Fitted
79	pre-IL10t → Ø	prd_il	0	min^{-1}	pre-mRNA degradation	Nucleus	Assumed
80	pre-IL10t + Spliceosome → pre-IL10t:Spliceosome*	a_n_spil	10	min^{-1}	Spliceosome association	Nucleus	Fitted
81	pre-IL10t: Spliceosome → IL10t + Spliceosome*	rs_il	10	min^{-1}	Mature mRNA release	Nucleus	Fitted
82	IL10t → Ø	rd_il	5×10^{-2}	min^{-1}	mRNA degradation	Cytoplasm	Fitted
83	IL10t → IL10	ps_c_il	7×10^{-2}	min^{-1}	Protein synthesis	Cytoplasm	Fitted
84	IL10 → Ø	pd_c_il	2×10^{-3}	min^{-1}	Protein degradation	Cytoplasm	Fitted
85	IL10 → IL10e	ex_il	1.5×10^{-4}	min^{-1}	Export		Fitted
86	IL10e → Ø	pd_e_il	7×10^{-5}	min^{-1}	Protein degradation	Extracellular	Fitted
DUSP1 mRNA and Protein Synthesis							
87	pATF1n → pATF1n + pre-DUSP1t	prs_du	1×10^{-3}	min^{-1}	ATF induced pre-	Nucleus	Fitted

					mRNA synthesis		
88	pre-DUSP1t → Ø	prd_du	0	min^{-1}	pre-mRNA degradation	Nucleus	Assumed
89	pre-DUSP1t + Spliceosome → pre-DUSP1t:Spliceosome*	a_n_spdu	10	min^{-1}	Spliceosome association	Nucleus	Fitted
90	pre-DUSP1t: Spliceosome → DUSP1t + Spliceosome*	rs_du	10	min^{-1}	Mature mRNA release	Nucleus	Fitted
91	DUSP1t → Ø	rd_du	1×10^{-2}	min^{-1}	mRNA degradation	Cytoplasm	Fitted
92	DUSP1t → DUSP1	ps_c_du	2×10^{-2}	min^{-1}	Protein synthesis	Cytoplasm	Fitted
93	DUSP1 → Ø	pd_c_du	3×10^{-3}	min^{-1}	Protein degradation	Cytoplasm	Fitted
REPressor mRNA and Protein Synthesis							
94	pSTAT3n → pSTAT3n + pre-REPt	prs_re	5×10^{-4}	min^{-1}	ATF induced pre-mRNA synthesis	Nucleus	Fitted
95	pre-REPt → Ø	prd_re	0	min^{-1}	pre-mRNA degradation	Nucleus	Assumed
96	pre-REPt + Spliceosome → pre-REPt:Spliceosome*	a_n_spre	10	min^{-1}	Spliceosome association	Nucleus	Fitted
97	pre-REPt: Spliceosome → REPt + Spliceosome*	rs_re	10	min^{-1}	Mature mRNA release	Nucleus	Fitted
98	REPt → Ø	rd_re	1×10^{-2}	min^{-1}	mRNA degradation	Cytoplasm	Fitted
99	REPt → REP	ps_c_re	1×10^{-3}	min^{-1}	Protein synthesis	Cytoplasm	Fitted
100	REP → Ø	pd_c_re	1×10^{-3}	min^{-1}	Protein degradation	Cytoplasm	Fitted
IKK activation and inactivation reactions							
101	IKKne + TAK1a → TAK1a + IKKa	ka	1	$\mu M^{-1}\ min^{-1}$	Kinase activation	Cytoplasm	Fitted
102	IKKa + A20 → A20 + IKKi	a20ina	10	$\mu M^{-1}\ min^{-1}$	Kinase inactivation	Cytoplasm	Fitted
103	IKKa → IKKi	ki	1×10^{-3}	min^{-1}	Kinase inactivation	Cytoplasm	Modified from (5)
104	IKKi → IKKne	kp	2×10^{-2}	min^{-1}	Kinase inactivation	Cytoplasm	(5)
TAK1 activation and inactivation reactions							
105	TNF:TNFR + TAK1ne → TNF:TNFR + TAK1a	tak1_nat	0	$\mu M^{-1}\ min^{-1}$	Activation	Cytoplasm	Used
106	TRAF6 + TAK1ne → TRAF6 + TAK1a	tak1_nat6	2	$\mu M^{-1}\ min^{-1}$	Activation	Cytoplasm	Fitted
107	TAK1a → TAK1i	tak1_ai	0.2	min^{-1}	Kinase inactivation	Cytoplasm	(1)
108	TAK1i → TAK1ne	tak1_in	5×10^{-3}	min^{-1}	Kinase inactivation	Cytoplasm	Fitted
LPS/TLR4 receptor ligand dynamics							
109	LPS + TLR4 → LPS:TLR4	rl_b_tl	3.2	$\mu M^{-1}\ min^{-1}$	Association	Membrane	(6)
110	LPS:TLR4 → LPS + TLR4	rl_u_tl	5×10^{-2}	min^{-1}	Dissociation	Membrane	(6)
111	LPS:TLR4 → LPS:TLR4int	rl_i_tl	2×10^{-3}	min^{-1}	Import		Fitted
112	LPS:TLR4int → TLR4	rl_r_tl	1×10^{-6}	min^{-1}	Export		Fitted
113	LPS:TLR4int → LPS:TLR4d	rl_d_tl	2×10^{-2}	min^{-1}	Inactivation	Cytoplasm	Fitted
TNF/TNFR receptor ligand dynamics							
114	TNFR + TNF:TNFR + TNFRi = tnfrtot	tnfrtot	0.1	μM	Concentration	Membrane	Assumed
115	TNF:TNFR → TNF + TNFR	rl_u_tt	2×10^{-2}	min^{-1}	Dissociation	Membrane	(1)
116	TNF + TNFR → TNF:TNFR	rl_b_tt	1100	$\mu M^{-1}\ min^{-1}$	Association	Membrane	(1)
117	LPS:TLR4 → LPS:TLR4int	rl_d_tt	1.2×10^{-2}	min^{-1}	Degradation	Cytoplasm	(7)
118	TNF:TNFRi → TNFR	rl_r_tt	1×10^{-2}	min^{-1}	Export	Undefined	(2)
119	TNF:TNFR → TNF:TNFRi	rl_i_tt	4.6×10^{-2}	min^{-1}	Import	Undefined	(7)
120	TNF:TNFR → TNF:TNFRi	rl_i_lt	1.45	min^{-1}	Import (LPS induced)	Undefined	Fitted to data in (8)
IFN-β/IFNAR receptor ligand dynamics							

121	IFNAR + IFN:IFNAR = ifnrtot**	ifnrtot	0.1	μM	Concentration	Membrane	Assumed
122	IFN + IFNAR → IFN:IFNAR	rl_b_ifif	60	μM^{-1} min^{-1}	Association	Membrane	(9)
123	IFN:IFNAR → IFN + IFNAR	rl_u_ifif	0.6	min^{-1}	Dissociation	Membrane	(9)
IL-10/IL10R receptor ligand dynamics							
124	IL-10R + IL10:IL10R = il10rtot**	il10rtot	0.1	μM	Concentration	Membrane	Assumed
125	IL-10 + IL10R → IL10:IL10R	rl_b_ilil	60	μM^{-1} min^{-1}	Association	Membrane	(2)
126	IL-10:IL10R → IL10 + IL10R	rl_u_ilil	6×10^{-3}	min^{-1}	Dissociation	Membrane	(2)
MyD88 reactions							
127	MyD88 + MyD88i = myd88tot**	myd88tot	0.1	μM	Concentration	Cytoplasm	Assumed
128	LPS:TLR4 + MyD88i → LPS:TLR4 + MyD88	pa_c_my	1	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
129	MyD88 → MyD88i	pd_c_my	0.12	min^{-1}	Inactivation	Cytoplasm	Fitted
TRIF reactions							
130	TRIF + TRIFi = triftot**	triftot	0.1	μM	Concentration	Cytoplasm	Assumed
131	LPS:TLR4int + TRIFi → LPS:TLR4int + TRIF	pa_c_tr	2	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
132	TRIF → TRIFi	pd_c_tr	0.185	min^{-1}	Inactivation	Cytoplasm	Fitted
TRAF6 reactions							
133	TRAF6 + TRAF6i = traf6tot**	traf6tot	0.1	μM	Concentration	Cytoplasm	Assumed
134	MyD88 + TRAF6i → MyD88 + TRAF6	a_c_am	1	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
135	TRIF + TRAF6i → TRIF + TRAF6	a_c_at	0.5	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
136	TRAF6 → TRAF6i	d_c_a	1.15	min^{-1}	Inactivation	Cytoplasm	Fitted
TBK1 reactions							
137	TBK1 + TBK1i = tbktot**	tbktot	0.1	μM	Concentration	Cytoplasm	Assumed
138	TRIF + TBK1i → TBK1	a_c_tt	10	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
139	TBK1 → TBK1i	d_c_tt	5×10^{-2}	min^{-1}	Inactivation	Cytoplasm	Fitted
MKK reactions							
140	MKK + MKKi = mkktot**	mkktot	0.1	μM	Concentration	Cytoplasm	Assumed
141	TAK1a + MKKi → TAK1a + MKK	a_c_tt	5	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
142	MKK → MKKi	d_c_tt	3×10^{-2}	min^{-1}	Inactivation	Cytoplasm	Fitted
P38 reactions							
143	P38 + P38i = p38tot**	p38tot	0.1	μM	Concentration	Cytoplasm	Assumed
144	MSK1 + P38i → MSK1 + P38	a_c_mp	10	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
145	P38 → P38i	d_c_mp	8.5×10^{-2}	min^{-1}	Inactivation	Cytoplasm	Fitted
146	DUSP1 + P38 → DUSP1 + P38i	d_c_dp	50	μM^{-1} min^{-1}	Inactivation (Dusp1 induced)	Cytoplasm	Fitted
ERK reactions							
147	ERK + ERKi = erktot**	erktot	0.1	μM	Concentration	Cytoplasm	Assumed
148	MKK + ERKi → MKK + ERK	a_c_me	1.3	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
149	ERK → ERKi	d_c_me	7×10^{-2}	min^{-1}	Inactivation	Cytoplasm	Fitted
MSK1 reactions							
150	MSK1 + MSK1i = msk1tot**	msk1tot	0.1	μM	Concentration	Cytoplasm	Assumed
151	P38 + MSK1i → P38 + MSK1	a_c_pm	95	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
152	MSK1 → MSK1i	d_c_pm	10	min^{-1}	Inactivation	Cytoplasm	Fitted
MSK2 reactions							
153	MSK2 + MSK2i = msk2tot**	msk2tot	0.1	μM	Concentration	Cytoplasm	Assumed
154	ERK + MSK2i → ERK + MSK2	a_c_pm	40	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
155	MSK2 → MSK2i	d_c_pm	0.84	min^{-1}	Inactivation	Cytoplasm	Fitted
JAK1 reactions							
156	JAK1 + JAK1i = jak1tot**	jak1tot	1E-1	μM	Concentration	Cytoplasm	Assumed

157	IFN:IFNAR + JAK1i → JAK1	a_c_ij	100	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
158	JAK1 → JAK1i	d_c_ij	0.1	min^{-1}	Inactivation	Cytoplasm	Fitted
PI3K reactions							
159	PI3K + PI3Ki = pi3ktot**	pi3ktot	0.1	μM	Concentration	Cytoplasm	Assumed
160	JAK1 + PI3Ki → JAK1 + PI3K	a_c_jp	20	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
161	PI3K → PI3Ki	d_c_jp	2	min^{-1}	Inactivation	Cytoplasm	Fitted
GSK3 reactions							
162	GSK3 + GSK3i = gsk3ktot**	gsk3tot	0.1	μM	Concentration	Cytoplasm	Assumed
163	PI3K + GSK3 → PI3K + GSK3i	a_c_pg	1	μM^{-1} min^{-1}	Inactivation	Cytoplasm	Fitted
164	GSK3i → GSK3	d_c_pg	1	min^{-1}	Activation	Cytoplasm	Fitted
JAK1il reactions							
165	JAK1il + JAK1ili = jak3tot**	jak3tot	0.1	μM	Concentration	Cytoplasm	Assumed
166	IL10:IL10R + JAK1ili → JAK1il	a_c_ilj	20	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
167	JAK1il → JAK1ili	d_c_ilj	1	min^{-1}	Inactivation	Cytoplasm	Fitted
IRF3 reactions							
168	IRF3i + pIRF3 + pIRF3n = irf3tot**	irf3tot	0.1	μM	Concentration	Cytoplasm	Assumed
169	TBK1 + IRF3i → TBK1 + pIRF3	a_c_ti	2	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
170	pIRF3 → IRF3i	d_c_i	0.1	min^{-1}	Inactivation	Cytoplasm	Fitted
171	pIRF3 → pIRF3n	in_i	1	min^{-1}	Import	Undefined	Fitted
172	pIRF3n → pIRF3	ex_i	0.1	min^{-1}	Export	Undefined	Fitted
CREB reactions							
173	CREBi + pCREB + pCREBn = crebtot**	crebtot	0.1	μM	Concentration	Cytoplasm	Assumed
174	MSK1 + CREBi → MSK1 + pCREB	a_c_m1c	1	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
175	MSK2 + CREBi → MSK2 + pCREB	a_c_m2c	0	μM^{-1} min^{-1}	Activation	Cytoplasm	Assumed
176	GSK3 + pCREB → GSK3 + CREBi	d_ c_gc	3	μM^{-1} min^{-1}	Inactivation	Cytoplasm	Fitted
177	pCREB → pCREBn	in_c	1	min^{-1}	Import	Undefined	Fitted
178	pCREBn → pCREB	ex_c	0.2	min^{-1}	Export	Undefined	Fitted
179	pCREB → CREBi	d_c_c	2×10^{-2}	min^{-1}	Inactivation	Cytoplasm	Assumed
STAT3 reactions							
180	STAT3i + pSTAT3 + pSTAT3n = stat3tot**	stat3tot	0.1	μM	Concentration	Cytoplasm	Assumed
181	JAK1il + STAT3i → JAK3 + pSTAT3	a_c_js	20	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
182	pSTAT3 → STAT3i	d_c_js	2	min^{-1}	Inactivation	Cytoplasm	Fitted
183	pSTAT3 → pSTAT3n	in_s	1	min^{-1}	Import	Undefined	Fitted
184	pSTAT3n → pSTAT3	ex_s	1	min^{-1}	Export	Undefined	Fitted
ATF reactions							
185	ATF1i + pATF1 + pATF1n = atftot**	atftot	0.1	μM	Concentration	Cytoplasm	Assumed
186	MSK1 + ATFi → MSK1 + pATF	a_c_m1a	82	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
187	MSK2 + ATFi → MSK2 + pATF	a_c_m2a	45	μM^{-1} min^{-1}	Activation	Cytoplasm	Fitted
188	pATF1 → ATF1i	d_ c_ab	1×10^{-2}	min^{-1}	Inactivation	Cytoplasm	Fitted
189	pATF1 → pATF1n	in_a	0.84	min^{-1}	Import	Undefined	Fitted
190	pATF1n → pATF1	ex_a	0.79	min^{-1}	Export	Undefined	Fitted
Miscellaneous parameters							
191		LPS	0.5	nM	Concentration	Extracellular	Used
192		kmuM	3×10^{-4}	Dimensionless	Conversion factor used to convert intracellular concentration to extracellular	Undefined	Derived

					assuming 100K/mL cells with a 10 μm radius		
Functions							
NF-κB induced gene transcription of IκBα, IκBε, and A20				$f(NF\kappa Bn) = mmVmax^* NF\kappa Bn^{mmHc} / (mmKm^{mmHc} + NF\kappa Bn^{mmHc})$			
REP induced inhibition of TNF transcription				$g(REP) = a_{rep} - REP/k_{rep}$ if $REP \leq b_{rep}$, 0 if $REP > b_{rep}$			
NF-κB induced gene transcription of TNF				$h(NF\kappa Bn, REP) = f(NF\kappa Bn) \times g(REP)$			
MyD88 induced activation of TRAF6				$f(MyD88) = mmVmax^* MyD88^{mmHc} / (mmKm^{mmHc} + MyD88^{mmHc})$			
TRIF induced activation of TRAF6				$f(TRIF) = mmVmax^* TRIF^{mmHc} / (mmKm^{mmHc} + TRIF^{mmHc})$			
CREB induced gene transcription of IL-10				$f(pCREBn) = mmVmax^* pCREBn^{mmHc} / (mmKm^{mmHc} + pCREBn^{mmHc})$			
ATF induced gene transcription of DUSP1				$f(pATFn) = mmVmax^* pATFn^{mmHc} / (mmKm^{mmHc} + pATFn^{mmHc})$			
IRF3 induced gene transcription of IFN-β				$f(pIRF3n) = mmVmax^* pIRF3n^{mmHc} / (mmKm^{mmHc} + pIRF3n^{mmHc})$			
LPS induced internalization of TNFR				$f(LPS) = mmVmax^* LPS^{mmHc} / (mmKm^{mmHc} + LPS^{mmHc})$			

Table S1 Legend. The model species appearing in Table S1 are named as follows: X = species located in the cytoplasm; pX = phosphorylated form of the species located in the cytoplasm; Xn = species located in the nucleus; pXn = phosphorylated form of the species located in the nucleus; Xe = species located extracellularly; Xi = inactive form of the species; Xint = extracellular species internalized; Xt = spliced transcript of a species; pre-Xt = unspliced transcript of a species; Xa = active form of a species; Xne = neutral form of a species; X:Y = species X bound to species Y

*In these reactions the species that appears second never changes.
**In these reactions the species on the right-hand-side of the equal sign denotes a conserved quantity.

Table S2. Model parameter numbers and names as they appear in the MATLAB code and in Figs. S5-S8.

Parameter #	Parameter name
1	in_a
2	in_e
3	ex_a
4	ex_e
5	in_2an
6	in_2en
7	ex_2an
8	ex_2en
9	in_n
10	ex_n
11	pd_n_a
12	pd_n_e
13	pd_n_2an
14	pd_n_2en
15	pd_c_2an
16	pd_c_2en
17	a_c_an
18	a_c_en
19	a_n_an
20	a_n_en
21	d_c_an
22	d_c_en
23	d_n_an
24	d_n_en
25	pd_c_2ai
26	pd_c_2ei
27	pd_c_3ain
28	pd_c_3ain
29	cnvr
30	mmVmax
31	mmKm
32	mmHc
33	a_{rep}
34	b_{rep}
35	k_{rep}
36	prs_an
37	prd_a
38	a_n_spa
39	prs_a

40	rs_a
41	rd_a
42	ps_c_a
43	pd_c_a
44	prs_en
45	prd_e
46	a_n_spe
47	prs_e
48	rs_e
49	rd_e
50	ps_c_e
51	pd_c_e
52	prs_a20n
53	prd_a20
54	a_n_spa20
55	prs_a20
56	rs_a20
57	rd_a20
58	ps_c_a20
59	pd_c_a20
60	prs_t
61	prd_t
62	a_n_spt
63	rs_t
64	rd_t
65	ps_c_t
66	pd_c_t
67	ex_tnf
68	pd_e_t
69	prs_i
70	prd_ifn
71	a_n_spifn
72	rs_ifn
73	rd_ifn
74	ps_c_ifn
75	pd_c_ifn
76	ex_ifn
77	pd_e_ifn
78	prs_il
79	prd_il
80	a_n_spil
81	rs_il
82	rd_il
83	ps_c_il
84	pd_c_il

85	ex_il
86	pd_e_il
87	prs_du
88	prd_du
89	a_n_spdu
90	rs_du
91	rd_du
92	ps_c_du
93	pd_c_du
94	prs_re
95	prd_re
96	a_n_spre
97	rs_re
98	rd_re
99	ps_c_re
100	pd_c_re
101	ka
102	a20ina
103	ki
104	kp
105	tak1_nat
106	tak1_nat6
107	tak1_ai
108	tak1_in
109	rl_b_tl
110	rl_u_tl
111	rl_i_tl
112	rl_r_tl
113	rl_d_tl
114	tnfrtot
115	rl_u_tt
116	rl_b_tt
117	rl_d_tt
118	rl_r_tt
119	rl_i_tt
120	rl_i_lt
121	ifnrtot
122	rl_b_ifif
123	rl_u_ifif
124	il10rtot
125	rl_b_ilil
126	rl_u_ilil
127	myd88tot
128	pa_c_my
129	pd_c_my

130	triftot
131	pa_c_tr
132	pd_c_tr
133	traf6tot
134	a_c_am
135	a_c_at
136	d_c_a
137	tbktot
138	a_c_tt
139	d_c_tt
140	mkktot
141	a_c_tt
142	d_c_tt
143	p38tot
144	a_c_mp
145	d_c_mp
146	d_c_dp
147	erktot
148	a_c_me
149	d_c_me
150	msk1tot
151	a_c_pm
152	d_c_pm
153	msk2tot
154	a_c_pm
155	d_c_pm
156	jak1tot
157	a_c_ij
158	d_c_ij
159	pi3ktot
160	a_c_jp
161	d_c_jp
162	gsk3tot
163	a_c_pg
164	d_c_pg
165	jak3tot
166	a_c_ilj
167	d_c_ilj
168	irf3tot
169	a_c_ti
170	d_c_i
171	in_i
172	ex_i
173	crebtot
174	a_c_m1c

175	a_c_m2c
176	d_ c_gc
177	in_c
178	ex_c
179	d_c_c
180	stat3tot
181	a_c_js
182	d_c_js
183	in_s
184	ex_s
185	atftot
186	a_c_m1a
187	a_c_m2a
188	d_ c_ab
189	in_a
190	ex_a
191	LPS
192	kμM

Table S3. Model species numbers and names as they appear in the MATLAB code and in Figs. S5-S8.

Species #	Species name
1	IκBα
2	IκBαn (nuclear)
3	IκBα:NF-κB
4	IκBα:NF-κBn (nuclear)
5	IκBαt (mRNA)
6	IκBε
7	IκBεn (nuclear)
8	IκBε:NF-κB
9	IκBε:NF-κBn (nuclear)
10	IκBεt (mRNA)
11	A20
12	A20t (mRNA)
13	NF-κB
14	NF-κBn (nuclear)
15	IKKa (active)
16	IKKne (neutral)
17	IKKi (inactive)
18	TAK1a (active)
19	TAK1ne (neutral)
20	TAK1i (inactive)
21	prna_a (IκBα unspliced mRNA)
22	srna_a (IκBα spliceosome bound mRNA)
23	prna_e (IκBε unspliced mRNA)
24	srna_e (IκBε spliceosome bound mRNA)
25	prna_a20 (A20 unspliced mRNA)
26	srna_20 (A20 spliceosome bound mRNA)
27	prna_t (TNF unspliced mRNA)
28	srna_t (TNF spliceosome bound mRNA)
29	TNFt (mRNA)
30	TNFi (cytosolic)
31	TNFe (extracellular)
32	prna_i (IFN-β unspliced mRNA)
33	srna_i (IFN-β spliceosome bound mRNA)
34	IFN-βt (mRNA)
35	IFN-βi (cytosolic)
36	IFN-βe (extracellular)
37	prna_il (IL10 unspliced mRNA)
38	srna_il (IL10 spliceosome bound mRNA)
39	IL10t (mRNA)
40	IL10i (cytosolic)

41	IL10e (extracellular)
42	prna_du (DUSP unspliced mRNA)
43	srna_du (DUSP spliceosome bound mRNA)
44	DUSPt (mRNA)
45	DUSP (cytosolic)
46	prna_re (REP unspliced mRNA)
47	srna_re (REP spliceosome bound mRNA)
48	REPt (mRNA)
49	REP (cytosolic)
50	TLR4
51	LPS:TLR4
52	LPS:TLR4i (internalized)
53	LPS:TLR4d (decayed and recycled)
54	TNFR
55	TNF:TNFR
56	IFNR
57	IL10R
58	MyD88
59	TRIF
60	TRAF6
61	TBK1
62	MKK
63	P38a
64	ERKa (active)
65	MSK1
66	MSK2
67	JAK1 (IFN-β bound)
68	PI3K
69	GSK3
70	JAK1il (IL10 bound)
71	pIRF3 (phosphorylated)
72	pIRF3n (nuclear)
73	pCREB (phosphorylated)
74	pCREBn (nuclear)
75	pSTAT3 (phosphorylated)
76	pSTAT3n (nuclear)
77	pATF (phosphorylated)
78	pATFn (nuclear)

2. Supplementary Figures

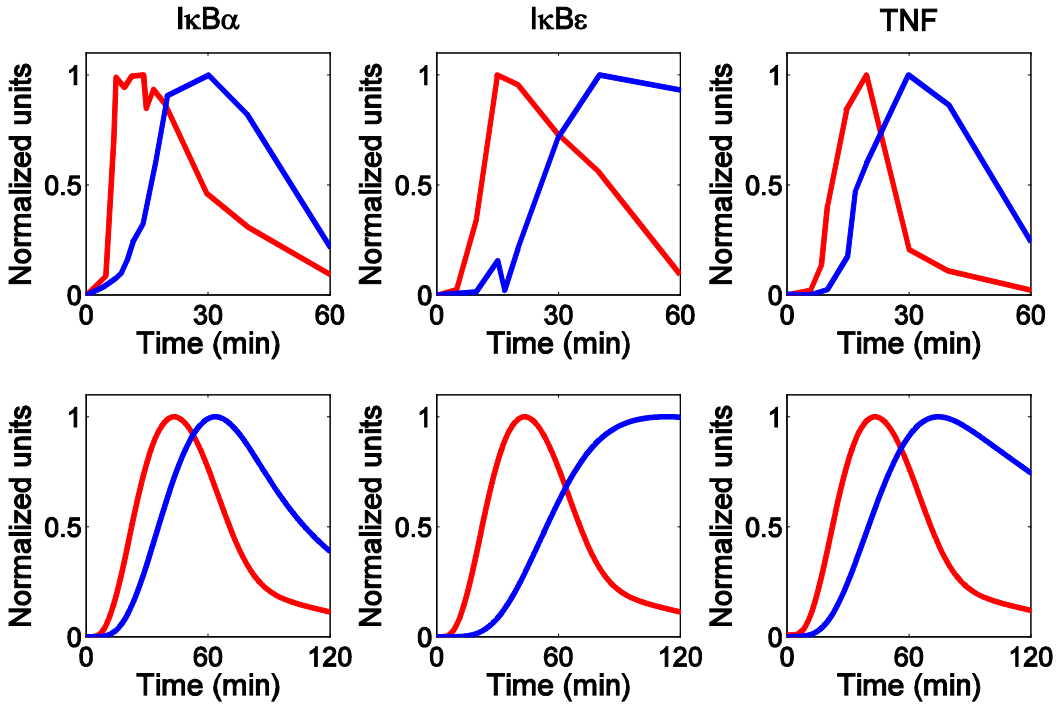

Figure S1. Experimental data used for calibration, and model simulations, of premature and mature mRNA kinetics. Upper panels: unspliced pre-mRNA (red) and spliced mRNA (blue) transcripts for IκBα, IκBε, and TNF measured from bone marrow-derived macrophages challenged with TNF (10 ng/ml) for 1 h (10). Lower panels: simulated trajectories of pre-mRNA (red) and mRNA (blue) of IκBα, IκBε, and TNF after an LPS challenge. The horizontal axes between the upper and lower panel figures differ because the experimental data were obtained after a TNF challenge, whereas simulated data were generated for an LPS challenge. The response to TNF occurs with faster kinetics compared to a response to LPS. Therefore, we expected the simulated species to display slower kinetics under LPS stimulation. In our calibration, we chose to maintain the *relative* timing between the unspliced and spliced transcripts rather than the *absolute* timing.

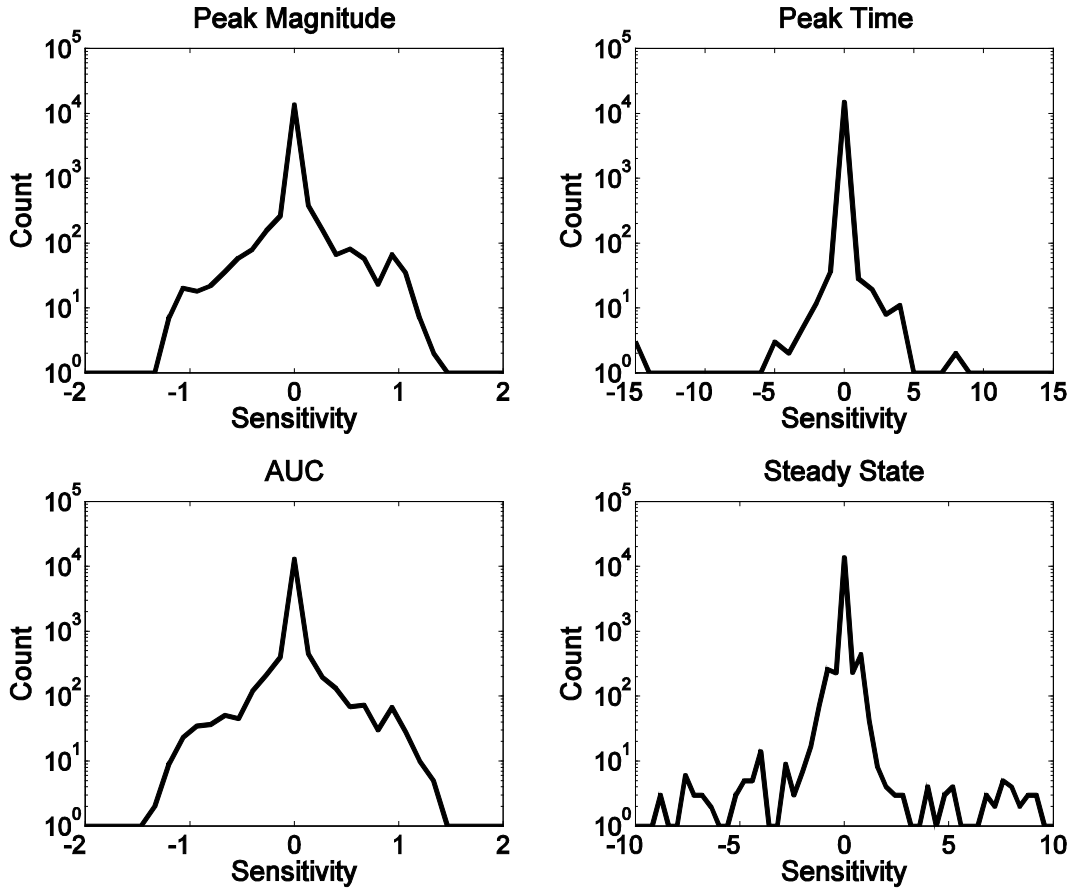

Figure S2. Histograms of sensitivities. We perturbed the value of each parameter in the model by ±1% and evaluated the sensitivity of the trajectory of every model species according to Eq. 5 in the Materials and Methods Section of the main text. Thus, for each feature, we obtained 192 (parameters) × 78 (model variables) = 14,976 sensitivity values, which are plotted in the histograms. The horizontal axis of each subpanel shows sensitivity intervals (indicated by the horizontal axis limits in each subplot) that were divided into 30 evenly spaced bins, while the vertical axis shows the number of sensitivities that fall into each bin. AUC refers to the area under the curve of a biochemical species trajectory. (Note that, for all subplots, the *x*-axis scale is linear, while the *y*-axis scale is logarithmic.)

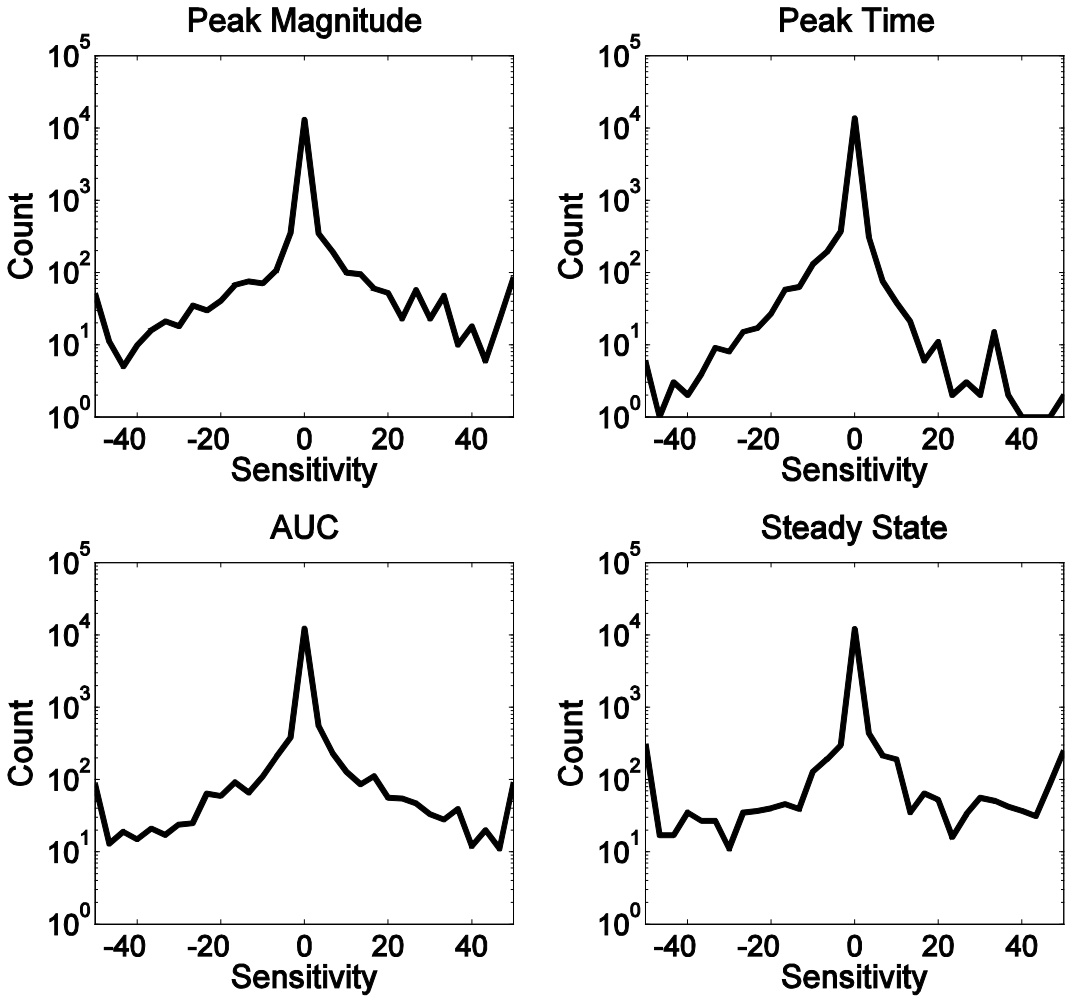

Figure S3. Histograms of sensitivities. We perturbed the value of each parameter in the model by ±50% and evaluated the sensitivity of the trajectory of every model species (see the Materials and Methods Section in the main text). Thus, for each feature, we obtained 192 (parameters) × 78 (model variables) = 14,976 sensitivity values, which are plotted in the histograms. For each subplot, the horizontal axis reflects sensitivity intervals (-50, 50) that were divided into 30 evenly spaced bins, while the vertical axis shows the number of sensitivities that fall into each bin. AUC refers to the area under the curve of a biochemical species trajectory. (Note that, for all subplots, the *x*-axis scale is linear, while the *y*-axis scale is logarithmic.)

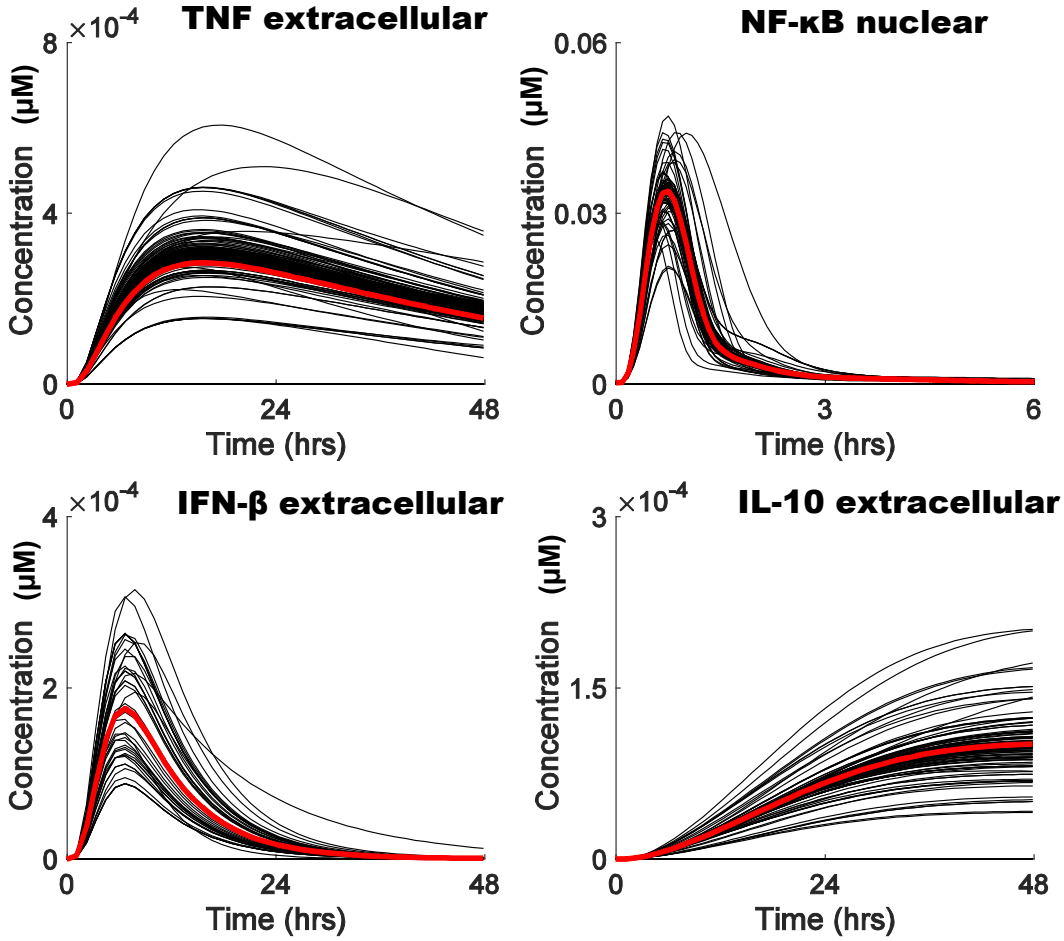

Figure S4. Species trajectories resulting from model simulations using the default parameter set (red trace) or after perturbing the value of each parameter in the model by +50% or -50% (black lines; thus, for each model parameter and each species, we have two black lines: one for the increased parameter and one for the decreased parameter).

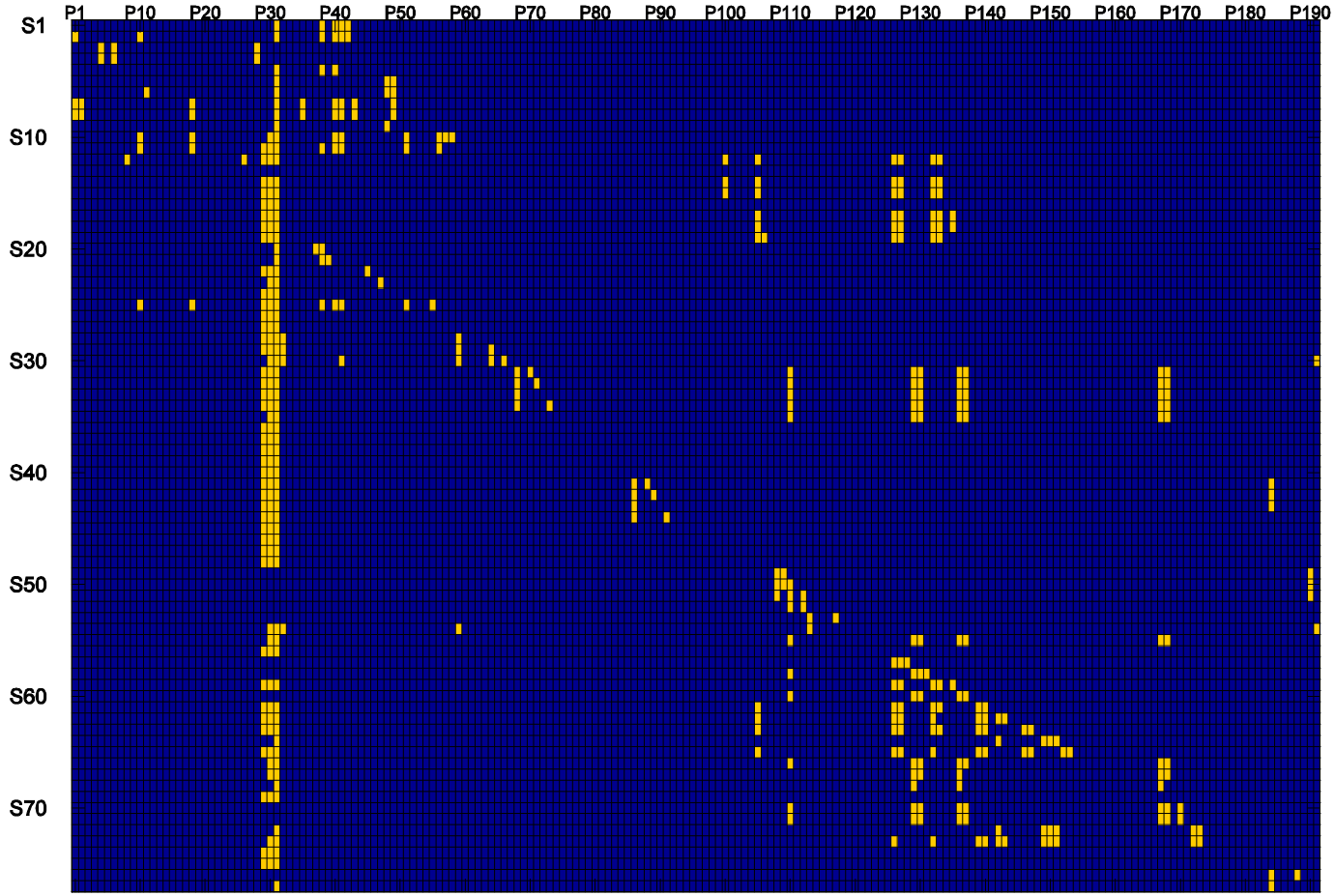

Figure S5. Global sensitivity analysis at 1 h. The *x*-axis shows parameter ordinal numbers (Table S2), whereas the *y*-axis shows model species (Table S3). We ran 50,000 simulations. For each simulation, we generated a random set of parameters using the Latin hypercube sampling scheme, where the value of each parameter in the model was drawn from a uniform distribution with 50% (200%) of the default parameter value as lower (upper) limit. We evaluated the Partial Rank Correlation Coefficient (PRCC) for each variable with respect to each parameter (11). Here, the PRCCs whose value is >0.5 are shown in yellow. The three columns centered on parameter #30 (P30) refer to the parameters controlling gene transcription rates.

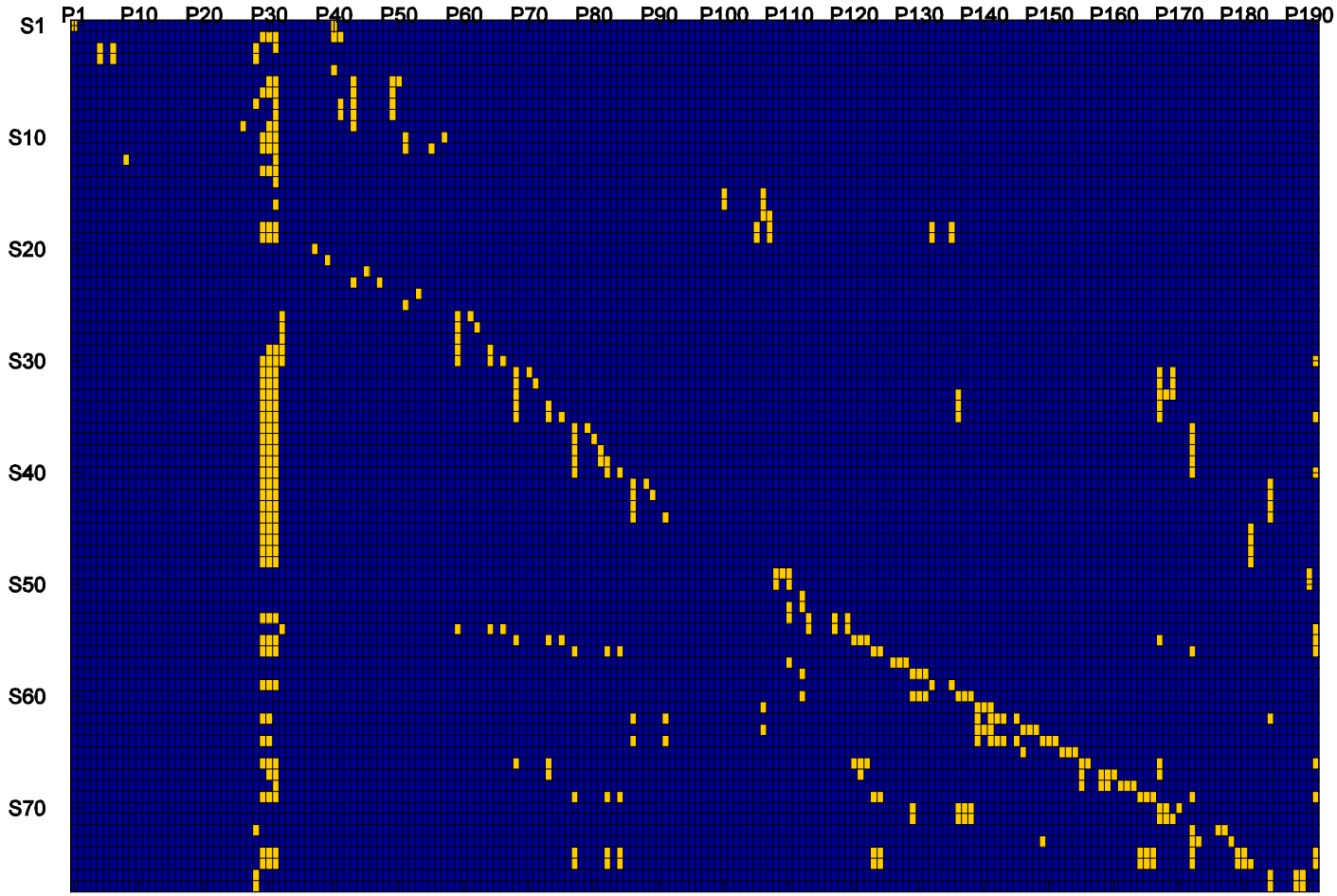

Figure S6. Global sensitivity analysis at 12 h. PRCCs with value >0.5 are shown in yellow.

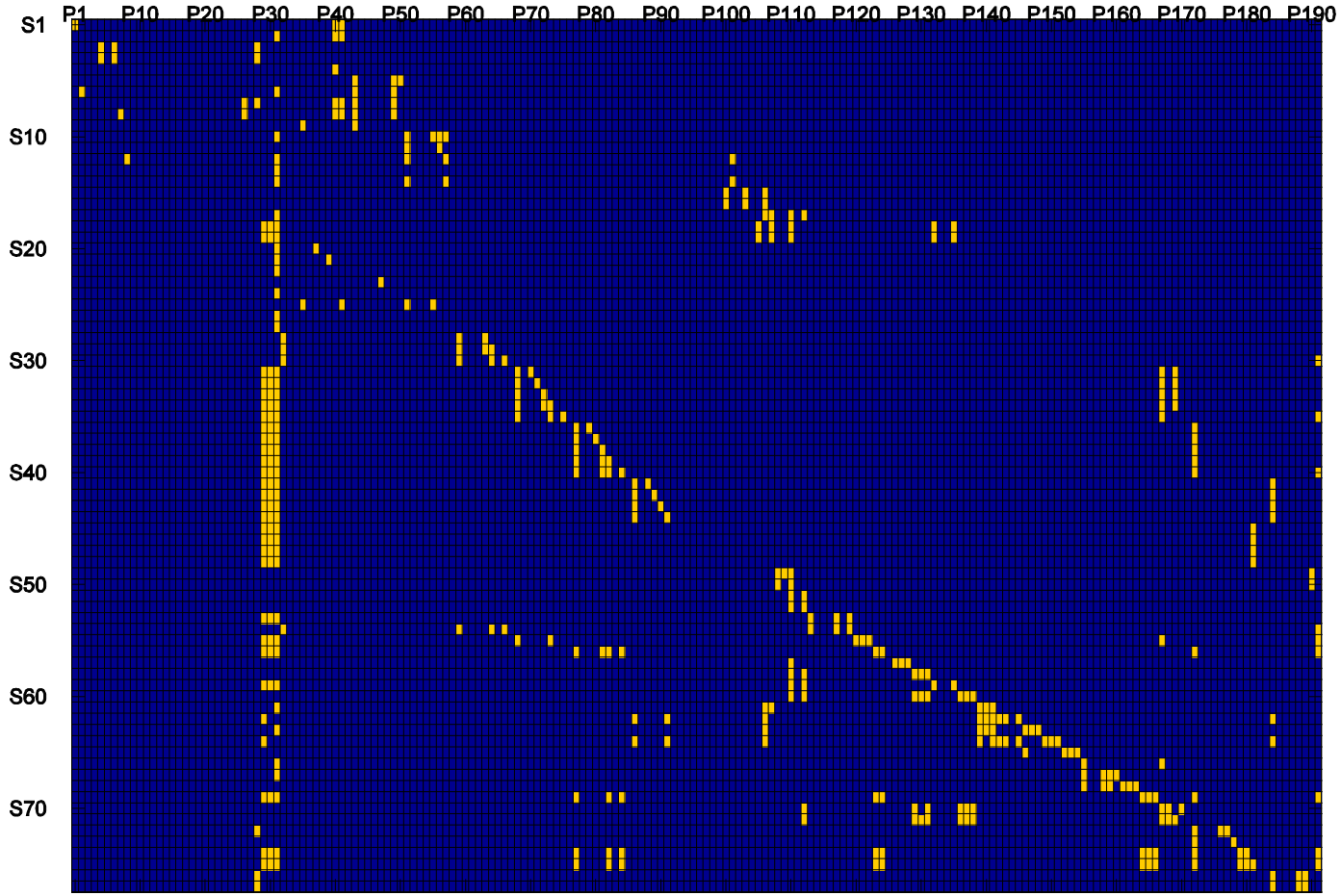

Figure S7. Global sensitivity analysis at 24 h. PRCCs with value >0.5 are shown in yellow.

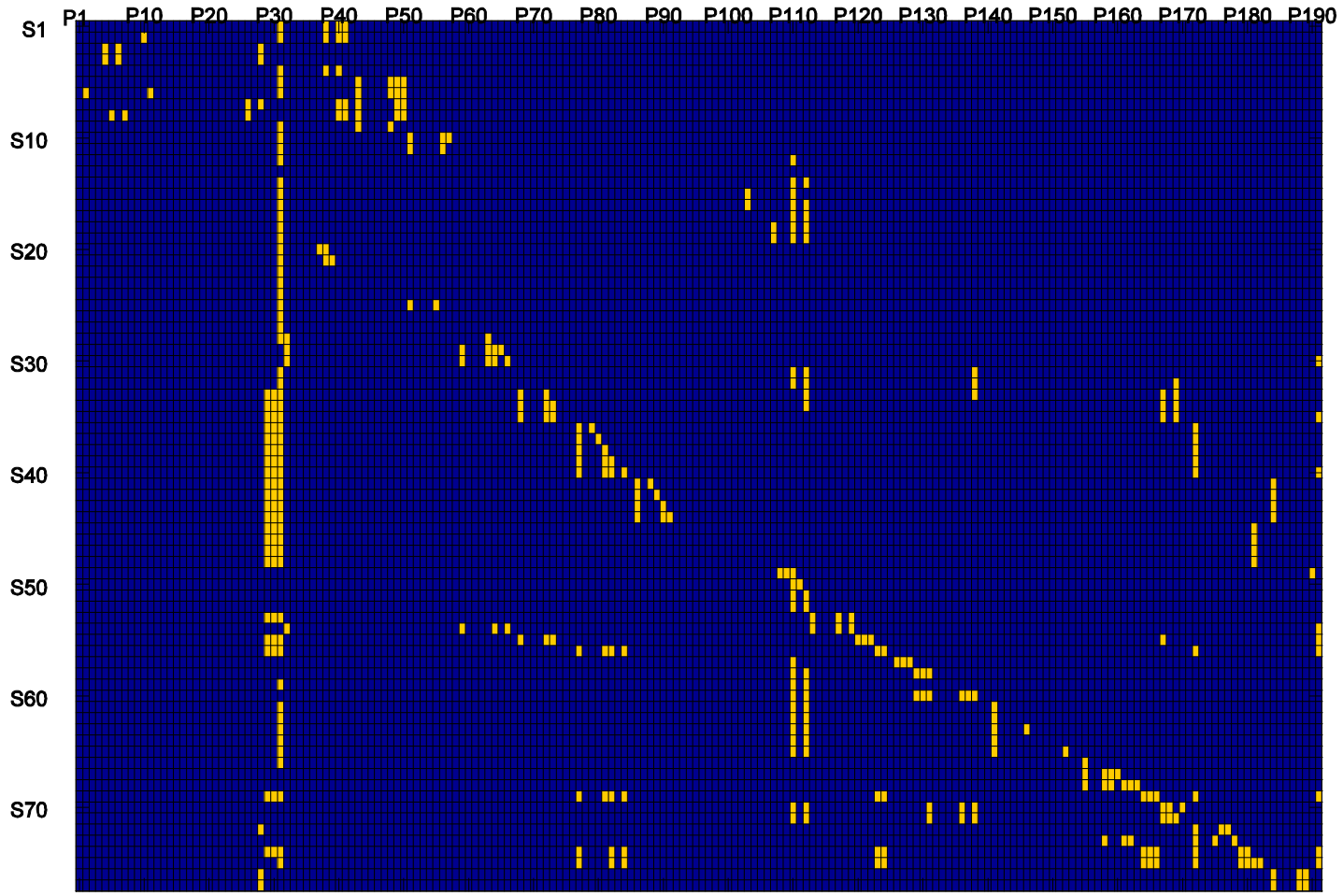

Figure S8. Global sensitivity analysis at 48 h. PRCCs with value >0.5 are shown in yellow.

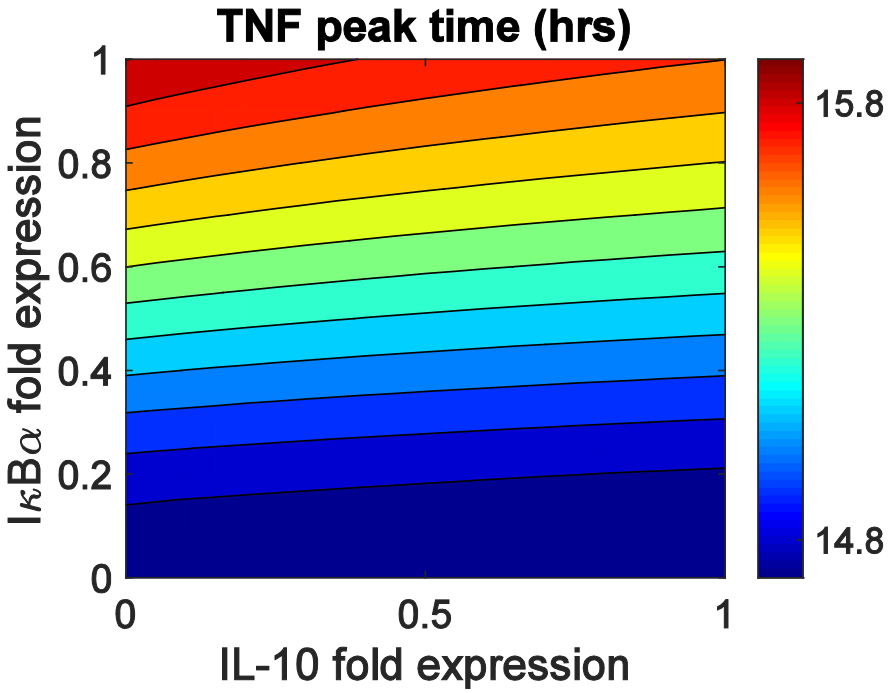

Figure S9. Regulation of the TNF trajectory. We calculated fold expression by dividing the values of the transcription rate parameters for IκBα and IL-10 by their default values. Modulation of IκBα and IL-10 expression does not change the timing of the TNF peak. There is only about 1 h difference in TNF peak time for any combination of the IκBα and IL-10 expression.

3. MATLAB Code Description

Correspondence about modeling- and software-related technical questions to Maurizio Tomaiuolo: mauriziotml@gmail.com

The MATLAB code for the macrophage extra- and intracellular signaling pathway model was developed at the DoD Biotechnology High Performance Computing Software Applications Institute (BHSAI), Fort Detrick, Maryland, to study the long-term interaction between intracellular pathways leading to the production of pro- and anti-inflammatory mediators (TNF, IFN-β, and IL-10). The model simulates the kinetics of the extra- and intracellular species using a set of ordinary differential equations (ODEs). Local sensitivity analysis was implemented to verify the model robustness to local perturbations; this was supplemented with a global sensitivity analysis. The "System requirements" subsection (below) contains the details about the computer system that we used to develop and run the code. The rest of this document provides information about using the code to reproduce the figures in the paper.

System requirements

We used the following software and hardware components:

Software
• Operating System: Windows 7 Enterprise (64-bit operating system)
• MATLAB version 8.3.0.532 (R2014a) (64-bit operating system)

Hardware
• Intel® Core™ i7-3770 CPU @ 3.40 GHz and 8.00 GB RAM
• Disk space: 3-4 GB is recommended for a typical installation

We developed the code in MATLAB R2014a. It includes the following files:

params.txt – file containing the values of all parameters
mmfn.m – returns the value of the activator function
mmrfn.m – returns the value of the repressor function
my_ode_timeout_event.m – stops computation if the ODE solver is taking too long
getPeakTimeAreaSteady.m – computes peak magnitude, peak time, area under the curve, and the steady-state value from a species kinetic trajectory
rescaleTo.m – normalizes a trajectory between two supplied values (min and max)

tnf_il10_model.m – function containing model equations
figure_2.m – function that runs the model to produce Fig. 2
figure_3.m – function that runs the model to produce Fig. 3
figure_4.m – function that runs the model to produce Fig. 4
figure_5.m – function that runs the model to produce Fig. 5
figure_6.m – function that runs the model to produce Fig. 6 (takes a long time to run)
plot_fig_6.m – function that plots Fig. 6 using the output produced by **figure_6.m**
figure_7.m – function that runs the model to produce Fig. 7

figure_s1.m – function that runs the model to produce Fig. S1
runSA.m – function that runs the local sensitivity analysis (1% case)
figure_s2.m – function that plots Fig. S2 using the output produced by **runSA.m**
runSA2X.m – function that runs the local sensitivity analysis (50% case)
figure_s3.m – function that plots Fig. S3 using the output produced by **runSA2X.m**
figure_s4.m – function that plots Fig. S4 using the output produced by **runSA2X.m**
figure_s5_s8.m – function that runs global sensitivity analysis and plots Figs. S5-S8
plot_fig_s9.m – function that plots Fig. S8 using the output produced by **figure_6.m**
run_tnf_il10_model.m – master function that runs the scripts described above

Instructions for downloading and saving the MATLAB files
The files are currently available in the ".txt" format. In order to run them in MATLAB, the file extension needs to be changed to .m, except for the **params.txt** file.

Macrophage signaling pathway model

1. The **INPUT** to the model is the **initial concentration of LPS**. The default value of this parameter is 0.5 nM (corresponding to 10 ng/ml for a molecular weight of 20 kDa). This value is defined as parameter No. 191 in the "param_values.txt" file found in the folder with the code.
   To increase or decrease the input concentration of LPS, increase or decrease the value of the parameter in this file or run the "params(191) = YOUR_VALUE" in the MATLAB command window, and then run the model.
2. To run the model, open **run_tnf_il10_model.m** and click the **"Run"** icon  or hit the F5 key.
3. The simulation will run, and when it is complete, the paper figures will be displayed. Depending on the specific computer configuration, it may take a long time to generate all the figures. **figure_7.m** and **runSA.m** are computationally intensive. If you would like to avoid running the code for a specific figure, simply put a % in front of the function call and MATLAB will ignore that line of code (as shown in the **run_tnf_il10_model.m** file).

Local sensitivity analysis

Simulation: To calculate the local sensitivity values in the vicinity of the default parameter set (the 1% perturbation case), open the file **run_tnf_il10_model.m** and run the **runSA.m** function first and the **figure_s2.m** after that.

Output: Displays Fig. S2 and returns four matrices containing the sensitivity values for all the features selected from the TNF kinetic trace. The matrices are stored in the workspace.
The sensitivity values for the trajectory peak are stored in a matrix labeled **smax**.
The sensitivity values for the area under the curve are stored in a matrix labeled **sarea**.
The sensitivity values for the trajectory peak time are stored in a matrix labeled **stmax**.
The sensitivity values for the steady-state values of the trajectory are stored in a matrix labeled **ssteady**.

Simulation: To calculate the local sensitivity values in the vicinity of the default parameter set (the 50% perturbation case), open the file **run_tnf_il10_model.m** and run the **runSA2x.m** function first and the **figure_s3.m** after that.

Output: Displays Fig. S3 and returns four matrices containing the sensitivity values for all the features selected from the TNF kinetic trace. The matrices are stored in the workspace.
The sensitivity values for the trajectory peak are stored in the matrix labeled **smax2X**.
The sensitivity values for the area under the curve are stored in the matrix labeled **sarea2X**.
The sensitivity values for the trajectory peak time are stored in the matrix labeled **stmax2X**.
The sensitivity values for the steady-state values of the trajectory are stored in the matrix labeled **ssteady2X**.

Global sensitivity analysis

Simulation: To run global sensitivity analysis, open the file **run_tnf_il10_model.m** and run the **figure_s5_s8**. Depending on the specifics of the computer used it may take days to run.

Output: Displays Figs. S5, S6, S7, and S8 of the ESI.

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