Supplmentary Materials: Mathematical Modeling and Parameter Estimation of Intracellular Signaling Pathway: Application to LPS-induced NFκB Activation and TNFα Production in Macrophages

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1 States and Parameters

Following four tables containing descriptions of the model states, the parameters, and the model equations. Except those related to Golgiplug™ effects on the NFκB signaling dynamics, the model is adopted from Werner *et al.* (2008); Caldwell *et al.* (2014); Junkin *et al.* (2016). Starting from the initial conditions provided in Table S1, the model is simulated until it reaches an equilibrium, where external stimulus (LPS) is added to predict the signaling dynamics induced by the external stimulus.

Table S1: States and their initial concentrations

States	Description	Initial concentration (μM)
IκBα	cytoplasmic IκBα	0
IκBαn	nuclear IκBα	0
NFκB-IκBα	cytoplasmic NFκB-IκBα	0
NFκB-IκBαn	nuclear NFκB-IκBα	0
IκBα_t	IκBα transcript	0
IκBβ	cytoplasmic IκBβ	0
IκBβn	nuclear IκBβ	0
NFκB-IκBβ	cytoplasmic NFκB-IκBβ	0
NFκB-IκBβn	nuclear NFκB-IκBβ	0
IκBβ_t	IκBβ transcript	0
IκBε	cytoplasmic IκBε	0
IκBεn	nuclear IκBε	0
NFκB-IκBε	cytoplasmic NFκB-IκBε	0
NFκB-IκBεn	nuclear NFκB-IκBε	0
IκBε_t	IκBε transcript	0
NFκB	cytoplasmic NFκB	0
NFκBn	nuclear NFκB	0.125
LPS	extracellular LPS	0
LPS_{pm}	LPS bound to cellular membrane	0
LPS_{en}	internalized LPS	0
TLR4	TLR4 on cellular membrane	0
$TLR4_{en}$	internalized TLR4	0
LPS-TLR4	LPS-TLR4 complex on cellular membrane	0
LPS-$TLR4_{en}$	internalized LPS-TLR4 complex	0
MyD88	inactive MyD88	0.1
MyD88*	activated MyD88	0
TRIF	inactive TRIF	0.1
TRIF*	activated TRIF	0
TRAF6	inactive TRAF6	0.1
TRAF6*	activated TRAF6	0
IKKK	inactive IKKK	0.1
IKKK*	activated IKKK	0
IKK	inactive IKK	0.1
IKK*	activated IKK	0
IKKi	deactivated IKK	0
TNFα_{nas}	TNFα nascent transcript	0
TNFα_t	TNFα transcript	0

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TNFα	intracellular TNFα	0
TNFα_{ext}	extracellular TNFα	0
tnfr	TNFR monomer	0
TNFR	TNFR trimer	0
TNFRtnf	TNFR trimer-TNFα complex	0
C1	activated TNFR-TRAF-TRADD-RIP complex	0
C1$_{\mathrm{off}}$	inactive C1	0
C1tnf	activated C1-TNFα complex	0
C1tnf$_{\mathrm{off}}$	inactive C1-TNFα complex	0
TTR	TRAF-TRADD-RIP complex	8.3×10^{-4}
A20	A20	0
A20$_{\mathrm{t}}$	A20 transcript	0

Table S2: The nominal values of the model parameters

Parameter	Reaction	Value	Unit	Ref.
k_1	IκBα_t synthesis rate	7×10^{-5}	μM min^{-1}	Werner *et al.* (2008)
k_2	IκBβ_t synthesis rate	1×10^{-5}	μM min^{-1}	Werner *et al.* (2008)
k_3	IκBε_t synthesis rate	1×10^{-6}	μM min^{-1}	Werner *et al.* (2008)
k_4	IκBα_t synthesis rate induced by NFκBn	8	min^{-1}	Werner *et al.* (2008)
k_5	IκBβ_t synthesis rate induced by NFκBn	0.02	min^{-1}	Werner *et al.* (2008)
k_6	IκBε_t synthesis rate induced by NFκBn	0.3	min^{-1}	Werner *et al.* (2008)
k_7	Hill coefficient for IκBα_t synthesis induced by NFκBn	3	-	Werner *et al.* (2008)
k_8	Hill coefficient for IκBβ_t synthesis induced by NFκBn	3	-	Werner *et al.* (2008)
k_9	Hill coefficient for IκBε_t synthesis induced by NFκBn	3	-	Werner *et al.* (2008)
k_{10}	IκBα_t degradation rate	0.035	min^{-1}	Werner *et al.* (2008)
k_{11}	IκBβ_t degradation rate	0.003	min^{-1}	Werner *et al.* (2008)
k_{12}	IκBε_t degradation rate	0.004	min^{-1}	Werner *et al.* (2008)
k_{13}	IκBα translation rate	0.25	min^{-1}	Werner *et al.* (2008)
k_{14}	IκBβ translation rate	0.25	min^{-1}	Werner *et al.* (2008)
k_{15}	IκBε translation rate	0.25	min^{-1}	Werner *et al.* (2008)
k_{16}	Rate of IκBα import to nucleus	0.09	min^{-1}	Werner *et al.* (2008)
k_{17}	Rate of IκBβ import to nucleus	0.009	min^{-1}	Werner *et al.* (2008)
k_{18}	Rate of IκBε import to nucleus	0.045	min^{-1}	Werner *et al.* (2008)
k_{19}	Rate of IκBαn export to cytoplasm	0.012	min^{-1}	Werner *et al.* (2008)
k_{20}	Rate of IκBβn export to cytoplasm	0.012	min^{-1}	Werner *et al.* (2008)
k_{21}	Rate of IκBεn export to cytoplasm	0.012	min^{-1}	Werner *et al.* (2008)
k_{22}	Rate of NFκB-IκBα import to nucleus	0.276	min^{-1}	Werner *et al.* (2008)
k_{23}	Rate of NFκB-IκBβ import to nucleus	0.0276	min^{-1}	Werner *et al.* (2008)
k_{24}	Rate of NFκB-IκBε import to nucleus	0.138	min^{-1}	Werner *et al.* (2008)
k_{25}	Rate of NFκB-IκBαn export to cytoplasm	0.828	min^{-1}	Werner *et al.* (2008)
k_{26}	Rate of NFκB-IκBβn export to cytoplasm	0.414	min^{-1}	Werner *et al.* (2008)
k_{27}	Rate of NFκB-IκBεn export to cytoplasm	0.414	min^{-1}	Werner *et al.* (2008)
k_{28}	Rate of NFκB import to nucleus	5.4	min^{-1}	Werner *et al.* (2008)
k_{29}	Rate of NFκBn export to cytoplasm	0.0048	min^{-1}	Werner *et al.* (2008)
k_{30}	Rate of IκBα degradation in cytoplasm	0.12	min^{-1}	Werner *et al.* (2008)
k_{31}	Rate of IκBβ degradation in cytoplasm	0.18	min^{-1}	Werner *et al.* (2008)
k_{32}	Rate of IκBε degradation in cytoplasm	0.18	min^{-1}	Werner *et al.* (2008)
k_{33}	Rate of IκBαn degradation in nucleus	0.12	min^{-1}	Werner *et al.* (2008)
k_{34}	Rate of IκBβn degradation in nucleus	0.18	min^{-1}	Werner *et al.* (2008)
k_{35}	Rate of IκBεn degradation in nucleus	0.18	min^{-1}	Werner *et al.* (2008)
k_{36}	Rate of IκBα degradation in NFκB-IκBα	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{37}	Rate of IκBβ degradation in NFκB-IκBβ	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{38}	Rate of IκBε degradation in NFκB-IκBε	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{39}	Rate of IκBα degradation in NFκB-IκBαn	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{40}	Rate of IκBβ degradation in NFκB-IκBβn	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{41}	Rate of IκBε degradation in NFκB-IκBεn	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{42}	Rate of NFκB-IκBα association in cytoplasm	30	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{43}	Rate of NFκBIκBβ association in cytoplasm	30	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{44}	Rate of NFκB-IκBε association in cytoplasm	30	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{45}	Rate of NFκB-IκBαn association in nucleus	30	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{46}	Rate of NFκB-IκBβn association in nucleus	30	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{47}	Rate of NFκB-IκBεn association in nucleus	30	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{48}	Rate of NFκB-IκBα dissociation in cytoplasm	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{49}	Rate of NFκB-IκBβ dissociation in cytoplasm	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{50}	Rate of NFκB-IκBε dissociation in cytoplasm	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{51}	Rate of NFκB-IκBαn dissociation in nucleus	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{52}	Rate of NFκB-IκBβn dissociation in nucleus	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{53}	Rate of NFκB-IκBεn dissociation in nucleus	6×10^{-5}	min^{-1}	Werner *et al.* (2008)
k_{54}	Rate of IκBα degradation induced by IKK*	0.36	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{55}	Rate of IκBβ degradation induced by IKK*	0.12	μM^{-1}min^{-1}	Werner *et al.* (2008)
k_{56}	Rate of IκBε degradation induced by IKK*	0.18	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{57}	Rate of IKK*-induced IκBα degradation in NFκB-IκBα	0.36	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{58}	Rate of IKK*-induced IκBβ degradation in NFκB-IκBβ	0.12	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{59}	Rate of IKK*-induced IκBε degradation in NFκB-IκBε	0.18	μM^{-1} min^{-1}	Werner *et al.* (2008)
k_{60}	Constitutive A20 transcription rate	2×10^{-6}	μM min^{-1}	Werner *et al.* (2008)
k_{61}	Rate of A20 transcription induced by NFκBn	0.4	min^{-1}	Werner *et al.* (2008)
k_{62}	Hill coefficient for A20 transcription induced by NFκBn	3	-	Werner *et al.* (2008)
k_{63}	A20$_t$ degradation rate	0.035	min^{-1}	Werner *et al.* (2008)
k_{64}	A20 translation rate	0.25	min^{-1}	Werner *et al.* (2008)

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k_{65}	A20 degradation rate	0.0029	min^{-1}	Werner *et al.* (2008)
k_{66}	Shutdown time for NFκBn-induced A20 transcription	120	min	Werner *et al.* (2008)
i_1	Rate of LPS binding to cellular membrane	0.1698	min^{-1}	Caldwell *et al.* (2014)
i_2	LPS_{pm} internalization rate	0.178	min^{-1}	Caldwell *et al.* (2014)
i_3	Rate of LPS_{en} export to cellular membrane	0.261	min^{-1}	Caldwell *et al.* (2014)
i_4	Degradation rate of internalized LPS_{em}	13.4	min^{-1}	Caldwell *et al.* (2014)
i_5	LPS-TLR4 association rate on cellular membrane	0.19	$\mu M^{-1} min^{-1}$	Caldwell *et al.* (2014)
i_6	LPS-TLR4 dissociation rate on cellular memebrane	2.7	min^{-1}	Caldwell *et al.* (2014)
i_7	LPS-$TLR4_{en}$ association rate in endosome	0.19	μM^{-1} min^{-1}	Caldwell *et al.* (2014)
i_8	LPS-$TLR4_{en}$ dissociation rate in endosome	2.7	min^{-1}	Caldwell *et al.* (2014)
i_9	Constitutive TLR4 synthesis rate on cellular membarane	0.0256	μM min^{-1}	Caldwell *et al.* (2014)
i_{10}	Rate of TLR4 degradation on cellular membrane	0.89	min^{-1}	Caldwell *et al.* (2014)
i_{11}	Rate of $TLR4_{en}$ degradation in endosome	2.93	min^{-1}	Caldwell *et al.* (2014)
i_{12}	TLR4 internalization rate	0.134	min^{-1}	Caldwell *et al.* (2014)
i_{13}	Rate of $TLR4_{en}$ export to cellular membrane	3.6	min^{-1}	Caldwell *et al.* (2014)
i_{14}	LPS-TLR4 internalization rate	0.24	min^{-1}	Caldwell *et al.* (2014)
i_{15}	Rate of LPS-$TLR4_{en}$ export to cellular membrane	0.0415	min^{-1}	Caldwell *et al.* (2014)
i_{16}	LPS-TLR4 degradation rate on cellular membrane	14.4	min^{-1}	Caldwell *et al.* (2014)
i_{17}	LPS-$TLR4_{en}$ degradation rate	0.42	min^{-1}	Caldwell *et al.* (2014)
i_{18}	MyD88 activation rate	3.29	$\mu M^{-1} min^{-1}$	Caldwell *et al.* (2014)
i_{19}	Hill coefficient for MyD88 activation	3	-	Caldwell *et al.* (2014); Lin *et al.* (2010)
i_{20}	EC50 term in MyD88 activation	0.058	μM	Caldwell *et al.* (2014)
i_{21}	$MyD88^*$ inactivation rate	0.28	min^{-1}	Caldwell *et al.* (2014)
i_{22}	TRIF activation rate	0.39	$\mu M^{-1} min^{-1}$	Caldwell *et al.* (2014)
i_{23}	$TRIF^*$ inactivation rate	0.012	min^{-1}	Caldwell *et al.* (2014)
i_{24}	$MyD88^*$-induced TRAF6 activation rate	7.47	$\mu M^{-1} min^{-1}$	Caldwell *et al.* (2014)
i_{25}	$TRIF^*$-induced TRAF6 activation rate	3.41	$\mu M^{-1} min^{-1}$	Caldwell *et al.* (2014)
i_{26}	$TRAF6^*$ inactivation rate	0.217	min^{-1}	Caldwell *et al.* (2014)
i_{27}	$TRAF6^*$-induced IKKK activation rate	0.343	$\mu M^{-1} min^{-1}$	Caldwell *et al.* (2014)
i_{28}	Constitutive IKKK activation rate	5×10^{-7}	min^{-1}	Werner *et al.* (2008)
i_{29}	Constitutive $IKKK^*$ inactivation rate	0.25	min^{-1}	Werner *et al.* (2008)
i_{30}	$IKKK^*$-induced IKK activation rate	520	$\mu M^{-1} min^{-1}$	Werner *et al.* (2008)
i_{31}	Constitutive IKK activation rate	5×10^{-5}	min^{-1}	Werner *et al.* (2008)
i_{32}	Constitutive IKK^* inactivation rate ($IKK^* \rightarrow IKK$)	0.02	min^{-1}	Werner *et al.* (2008)
i_{33}	Constitutive IKK inactivation rate ($IKK \rightarrow IKK_i$)	0.15	min^{-1}	Werner *et al.* (2008)
i_{34}	Constitutive IKKi normalization rate ($IKKi \rightarrow IKK$)	0.02	min^{-1}	Werner *et al.* (2008)
a_1	$TNF\alpha_{ext}$ degradation rate	0.0154	min^{-1}	Werner *et al.* (2008)
a_2	tnfr synthesis rate	2×10^{-7}	min^{-1}	Werner *et al.* (2008)
a_3	tnfr degradation rate	0.0058	min^{-1}	Werner *et al.* (2008)
a_4	TNFR formation rate	1×10^{-5}	min^{-1}	Werner *et al.* (2008)
a_5	Rate of TNFR converting to tnfr	0.1	min^{-1}	Werner *et al.* (2008)
a_6	TNFR degradation rate	0.023	min^{-1}	Werner *et al.* (2008)
a_7	Rate of $C1_{off}$ formation	100	μM^{-1} min^{-1}	Werner *et al.* (2008)
a_8	Rate of $C1_{off}$ dissociation	0.1	min^{-1}	Werner *et al.* (2008)
a_9	$C1_{off}$ activation rate	30	min^{-1}	Werner *et al.* (2008)
a_{10}	C1 inactivation rate	2	min^{-1}	Werner *et al.* (2008)
a_{11}	A20-mediated C1 inactivation rate	1000	$\mu M^{-1} min^{-1}$	Werner *et al.* (2008)
a_{12}	C1 dissociation	0.1	min^{-1}	Werner *et al.* (2008)
a_{13}	Rate of $C1_{off}$ internalization	0.023	$\mu M^{-1} min^{-1}$	Werner *et al.* (2008)
a_{14}	Rate of C1 internalization	0.023	min^{-1}	Werner *et al.* (2008)
a_{15}	Rate of TNFRtnf formation from tnfr	1100	$\mu M^{-1} min^{-1}$	Werner *et al.* (2008)
a_{16}	Rate of TNFRtnf formation from TNFR	1100	$\mu M^{-1} min^{-1}$	Werner *et al.* (2008)
a_{17}	Rate of TNFRtnf dissociation	0.021	min^{-1}	Werner *et al.* (2008)
a_{18}	Rate of TNFRtnf degradation	0.023	min^{-1}	Werner *et al.* (2008)
a_{19}	Rate of $C1tnf_{off}$ formation from TNFRtnf and TTR	100	$\mu M^{-1} min^{-1}$	Werner *et al.* (2008)
a_{20}	Rate of $C1tnf_{off}$ dissociation into TNFRtnf and TTR	0.021	min^{-1}	Werner *et al.* (2008)
a_{21}	$C1tnf_{off}$ activation rate	30	min^{-1}	Werner *et al.* (2008)
a_{22}	C1tnf inactivation rate	2	min^{-1}	Werner *et al.* (2008)
a_{23}	A20-mediated C1tnf inactivation rate	1000	μM^{-1} min^{-1}	Werner *et al.* (2008)
a_{24}	Rate of C1tnf dissociation into TNFRtnf and TTR	0.021	min^{-1}	Werner *et al.* (2008)
a_{25}	$C1tnf_{off}$ degradation rate	0.023	min^{-1}	Werner *et al.* (2008)
a_{26}	C1tnf degradation rate	0.023	min^{-1}	Werner *et al.* (2008)
a_{27}	Rate of $C1tnf_{off}$ dissociation into $C1_{off}$ and $TNF\alpha_{\mathbf{ext}}$	0.021	min^{-1}	Werner *et al.* (2008)
a_{28}	Rate of $C1tnf_{off}$ formation from $C1_{off}$ and $TNF\alpha_{\mathbf{ext}}$	1100	$\mu M^{-1} min^{-1}$	Werner *et al.* (2008)

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a_{29}	Rate of C1tnf dissociation into C1 and TNF$\alpha_{\mathbf{ext}}$	0.021	min^{-1}	Werner *et al.* (2008)
a_{30}	Rate of C1tnf formation from C1 and TNF$\alpha_{\mathbf{ext}}$	1100	$\mu M^{-1}\mathrm{min}^{-1}$	Werner *et al.* (2008)
a_{31}	C1-mediated IKKK activation rate	500	$\mu M^{-1}\mathrm{min}^{-1}$	Werner *et al.* (2008)
a_{32}	C1tnf-mediated IKKK activation rate	500	$\mu M^{-1}\mathrm{min}^{-1}$	Werner *et al.* (2008)
v_1	Rate of NFκBn-induced TNFα transcription	1×10^{-5}	$\mu M\mathrm{min}^{-1}$	Caldwell *et al.* (2014)
v_2	Hill coefficient for NFκBn-induced TNFα transcription	2	-	Caldwell *et al.* (2014)
v_3	EC50 constant for NFκBn-induced TNFα transcription	0.65	μM	Caldwell *et al.* (2014)
v_4	Rate of TNFα_{nas} degradation	0.02	min^{-1}	Caldwell *et al.* (2014)
v_5	Rate of TNFα_{t} maturation	0.4	min^{-1}	Caldwell *et al.* (2014)
v_6	Rate of TNFα translation	0.05	min^{-1}	Caldwell *et al.* (2014)
v_7	Rate of TNFα degradation	0.07	min^{-1}	Caldwell *et al.* (2014)
v_8	Rate of TNFα secretion	0.07	min^{-1}	Caldwell *et al.* (2014)
v_9	K_{a0} term for TRIF*-induced TNFα promotion	1×10^{-4}	-	Junkin *et al.* (2016)
v_{10}	K_a term for TRIF*-induced TNFα promotion	0.1	-	Junkin *et al.* (2016)
v_{11}	K_i term for TRIF*-induced TNFα promotion	0.4	-	Junkin *et al.* (2016)
v_{12}	Volume ratio factor between a cell and culture medium	833.3	-	Bagnall *et al.* (2015)
v_{13}	Golgiplug$^{\mathrm{TM}}$ activation time (τ)	90	min	assumed
v_{14}	Rate of A20-induced TRAF6* inactivation	0.01	$\mu M^{-1}\mathrm{min}^{-1}$	assumed
v_{15}	Coefficient for Golgiplug$^{\mathrm{TM}}$-induced translation inhibition	0.5	-	assumed
v_{16}	Denominator constant for Golgiplug$^{\mathrm{TM}}$ translation inhibition	0.2	-	assumed
f_a	Constant for TRIF*-induced TNFα translation promotion	$\frac{TRIF^*+v_9}{TRIF^*+v_{10}}$	-	Junkin *et al.* (2016)
f_i	Constant for TRIF*-induced TNFα translation promotion	$\frac{v_{11}}{TRIF^*+v_{11}}$	-	Junkin *et al.* (2016)
G	Golgiplug$^{\mathrm{TM}}$ inhibition factor	$G=\frac{t}{t+v_{13}}$	-	Assumed
$k_{s_{TNFR},m}$	tnfr synthesis rate altered by Golgiplug$^{\mathrm{TM}}$	$a_2(1-G)$	$\mu M\mathrm{min}^{-1}$	assumed
$k_{s_{TLR4},m}$	TLR4 synthesis rate altered by Golgiplug$^{\mathrm{TM}}$	$i_9(1-G)$	$\mu M\mathrm{min}^{-1}$	assumed
$k_{en_{LPS},m}$	LPS$_{\mathrm{pm}}$ internalization rate altered by Golgiplug$^{\mathrm{TM}}$	$i_2(1-G)$	min^{-1}	assumed
$k_{en_{cp},m}$	LPS-TLR4 complex internalization rate altered by Golgiplug$^{\mathrm{TM}}$	$i_{14}(1-G)$	min^{-1}	assumed
$k_{sec,m}$	TNFα secretion rate altered by Golgiplug$^{\mathrm{TM}}$	$v_8(1-G)$	min^{-1}	assumed

2 Equations when Golgiplug™ is not added

$$\frac{dI\kappa B\alpha}{dt} = k_{13}I\kappa B\alpha_t - k_{16}I\kappa B\alpha + k_{19}I\kappa B\alpha n - k_{30}I\kappa B\alpha - k_{42}NF\kappa B\cdot I\kappa B\alpha + k_{48}NF\kappa B\text{-}I\kappa B\alpha \tag{S1}$$

$$\frac{dI\kappa B\alpha n}{dt} = k_{16}I\kappa B\alpha - k_{19}I\kappa B\alpha n - k_{33}I\kappa B\alpha n - k_{45}NF\kappa Bn\cdot I\kappa B\alpha n + k_{51}NF\kappa B\text{-}I\kappa B\alpha n \tag{S2}$$

$$\begin{aligned}\frac{dNF\kappa B\text{-}I\kappa B\alpha}{dt} = &-k_{22}NF\kappa B\text{-}I\kappa B\alpha + k_{25}NF\kappa B\text{-}I\kappa B\alpha n - k_{36}NF\kappa BI\kappa\text{-}B\alpha + k_{42}NF\kappa B\cdot I\kappa B\alpha\\ &-k_{48}NF\kappa B\text{-}I\kappa B\alpha + k_{55}NF\kappa B\text{-}I\kappa B\alpha\cdot IKK^{*}\end{aligned} \tag{S3}$$

$$\frac{dNF\kappa B\text{-}I\kappa B\alpha n}{dt} = k_{22}NF\kappa B\text{-}I\kappa B\alpha - k_{25}NF\kappa B\text{-}I\kappa B\alpha n - k_{39}NF\kappa BI\kappa\text{-}B\alpha n + k_{42}NF\kappa B\cdot I\kappa B\alpha - k_{51}NF\kappa B\text{-}I\kappa B\alpha n \tag{S4}$$

$$\frac{dI\kappa B\alpha_{\mathrm{t}}}{dt} = k_1 + k_4NF\kappa Bn^{k_7} - k_{10}I\kappa B\alpha_{\mathrm{t}} \tag{S5}$$

$$\frac{dI\kappa B\beta}{dt} = k_{14}I\kappa B\beta_t - k_{17}I\kappa B\beta + k_{20}I\kappa B\beta n - k_{31}I\kappa B\beta - k_{43}NF\kappa B\cdot I\kappa B\beta + k_{49}NF\kappa B\text{-}I\kappa B\beta \tag{S6}$$

$$\frac{dI\kappa B\beta n}{dt} = k_{17}I\kappa B\beta - k_{20}I\kappa B\beta n - k_{34}I\kappa B\beta n - k_{46}NF\kappa Bn\cdot I\kappa B\beta n + k_{52}NF\kappa B\text{-}I\kappa B\beta n \tag{S7}$$

$$\begin{aligned}\frac{dNF\kappa B\text{-}I\kappa B\beta}{dt} = &-k_{23}NF\kappa B\text{-}I\kappa B\beta + k_{26}NF\kappa B\text{-}I\kappa B\beta n - k_{37}NF\kappa BI\kappa\text{-}B\beta + k_{43}NF\kappa B\cdot I\kappa B\beta\\ &-k_{49}NF\kappa B\text{-}I\kappa B\beta + k_{58}NF\kappa B\text{-}I\kappa B\beta\cdot IKK^{*}\end{aligned} \tag{S8}$$

$$\begin{aligned}\frac{dNF\kappa B\text{-}I\kappa B\beta n}{dt} = &-k_{23}NF\kappa B\text{-}I\kappa B\beta + k_{26}NF\kappa B\text{-}I\kappa B\beta n - k_{37}NF\kappa BI\kappa\text{-}B\beta + k_{43}NF\kappa B\cdot I\kappa B\beta\\ &-k_{49}NF\kappa B\text{-}I\kappa B\beta + k_{58}NF\kappa B\text{-}I\kappa B\beta\cdot IKK^{*}\end{aligned} \tag{S9}$$

$$\frac{dI\kappa B\beta_{\mathrm{t}}}{dt} = k_2 + k_5NF\kappa Bn^{k_8} - k_{11}I\kappa B\beta_{\mathrm{t}} \tag{S10}$$

$$\frac{dI\kappa B\varepsilon}{dt} = k_{15}I\kappa B\varepsilon_t - k_{18}I\kappa B\varepsilon + k_{21}I\kappa B\varepsilon n - k_{32}I\kappa B\varepsilon - k_{44}NF\kappa B\cdot I\kappa B\varepsilon + k_{50}NF\kappa B\text{-}I\kappa B\varepsilon \tag{S11}$$

$$\frac{dI\kappa B\varepsilon n}{dt} = k_{18}I\kappa B\varepsilon - k_{21}I\kappa B\varepsilon n - k_{35}I\kappa B\varepsilon n - k_{47}NF\kappa Bn\cdot I\kappa B\varepsilon n + k_{53}NF\kappa B\text{-}I\kappa B\varepsilon n \tag{S12}$$

$$\begin{aligned}\frac{dNF\kappa B\text{-}I\kappa B\varepsilon}{dt} = &-k_{24}NF\kappa B\text{-}I\kappa B\varepsilon + k_{27}NF\kappa B\text{-}I\kappa B\varepsilon n - k_{38}NF\kappa BI\kappa\text{-}B\varepsilon + k_{44}NF\kappa B\cdot I\kappa B\varepsilon\\ &-k_{50}NF\kappa B\text{-}I\kappa B\varepsilon + k_{59}NF\kappa B\text{-}I\kappa B\varepsilon\cdot IKK^{*}\end{aligned} \tag{S13}$$

$$\begin{aligned}\frac{dNF\kappa B\text{-}I\kappa B\varepsilon n}{dt} = &\,k_{24}NF\kappa B\text{-}I\kappa B\varepsilon - k_{27}NF\kappa B\text{-}I\kappa B\varepsilon n - k_{41}NF\kappa BI\kappa\text{-}B\varepsilon n + k_{44}NF\kappa B\cdot I\kappa B\varepsilon\\ &-k_{53}NF\kappa B\text{-}I\kappa B\varepsilon n\end{aligned} \tag{S14}$$

$$\frac{dI\kappa B\varepsilon_{\mathrm{t}}}{dt} = k_3 + k_6NF\kappa Bn^{k_9} - k_{12}I\kappa B\varepsilon_{\mathrm{t}} \tag{S15}$$

$$\begin{aligned}\frac{dNF\kappa B}{dt} = &-k_{28}NF\kappa B + k_{29}NF\kappa Bn + k_{36}NF\kappa B\text{-}I\kappa B\alpha + k_{37}NF\kappa B\text{-}I\kappa B\beta\\ &+k_{38}NF\kappa B\text{-}I\kappa B\varepsilon - k_{42}NF\kappa B\cdot I\kappa B\alpha - k_{43}NF\kappa B\cdot I\kappa B\beta - k_{44}NF\kappa B\cdot I\kappa B\varepsilon\\ &+k_{48}NF\kappa B\text{-}I\kappa B\alpha + k_{49}NF\kappa B\text{-}I\kappa B\beta + k_{50}NF\kappa B\text{-}I\kappa B\varepsilon\\ &+k_{57}NF\kappa B\text{-}I\kappa B\alpha\cdot IKK^{*} + k_{58}NF\kappa B\text{-}I\kappa B\beta\cdot IKK^{*} + k_{59}NF\kappa B\text{-}I\kappa B\varepsilon\cdot IKK^{*}\end{aligned} \tag{S16}$$

$$\begin{aligned}\frac{dNF\kappa Bn}{dt} = &\,k_{28}NF\kappa B - k_{29}NF\kappa Bn + k_{39}NF\kappa B\text{-}I\kappa B\alpha n + k_{40}NF\kappa B\text{-}I\kappa B\beta n\\ &+k_{41}NF\kappa B\text{-}I\kappa B\varepsilon n - k_{45}NF\kappa B\cdot I\kappa B\alpha n - k_{46}NF\kappa Bn\cdot I\kappa B\beta n\\ &-k_{47}NF\kappa B\cdot I\kappa B\varepsilon + k_{51}NF\kappa B\text{-}I\kappa B\alpha n + k_{52}NF\kappa B\text{-}I\kappa B\beta n + k_{53}NF\kappa B\text{-}I\kappa B\varepsilon n\end{aligned} \tag{S17}$$

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$$\frac{dLPS}{dt} = -i_1 LPS \tag{S18}$$
$$\frac{dLPS_{\text{pm}}}{dt} = i_1 LPS - i_2 LPS_{\text{pm}} + i_3 LPS_{\text{en}} - i_5 LPS_{\text{pm}} \cdot TLR4 + i_6 LPS\text{-}TLR4 \tag{S19}$$
$$\frac{dLPS_{\text{en}}}{dt} = i_2 LPS_{\text{pm}} - i_3 LPS_{\text{en}} - i_4 LPS_{\text{en}} - i_7 LPS_{\text{en}} \cdot TLR4_{\text{en}} + i_8 LPS\text{-}TLR4_{\text{en}} \tag{S20}$$
$$\frac{dTLR4}{dt} = -i_5 LPS_{\text{pm}} \cdot TLR4 + i_6 LPS\text{-}TLR4 + i_9 - i_{10} TLR4 - i_{12} TLR4 + i_{13} TLR4_{\text{en}} \tag{S21}$$
$$\frac{dTLR4}{dt} = -i_7 LPS_{\text{en}} \cdot TLR4_{\text{en}} + i_8 LPS\text{-}TLR4_{\text{en}} - i_{11} TLR4_{\text{en}} + i_{12} TLR4 - i_{13} TLR4_{\text{en}} \tag{S22}$$
$$\frac{dLPS\text{-}TLR4}{dt} = i_5 LPS_{\text{pm}} \cdot TLR4 - i_6 LPS\text{-}TLR4 - i_{14} LPS\text{-}TLR4 + i_{15} LPS\text{-}TLR4_{\text{en}} - i_{16} LPS\text{-}TLR4 \tag{S23}$$
$$\frac{dLPS\text{-}TLR4_{en}}{dt} = i_6 LPS_{\text{en}} \cdot TLR4_{\text{en}} - i_8 LPS\text{-}TLR4_{\text{en}} + i_{14} LPS\text{-}TLR4 - i_{15} LPS\text{-}TLR4_{\text{en}} - i_{17} LPS\text{-}TLR4_{\text{en}} \tag{S24}$$
$$\frac{dMyD88}{dt} = -i_{18} \frac{LPS\text{-}TLR4^{i_{19}}}{LPS\text{-}TLR4^{i_{19}} + i_{20}^{i_{19}}} MyD88 + i_{21} MyD88^* \tag{S25}$$
$$\frac{dMyD88^*}{dt} = i_{18} \frac{LPS\text{-}TLR4^{i_{19}}}{LPS\text{-}TLR4^{i_{19}} + i_{20}^{i_{19}}} MyD88 - i_{21} MyD88^* \tag{S26}$$
$$\frac{dTRIF}{dt} = -i_{22} LPS\text{-}TLR4_{\text{en}} TRIF + i_{23} TRIF^* \tag{S27}$$
$$\frac{dTRIF^*}{dt} = i_{22} LPS\text{-}TLR4_{\text{en}} TRIF - i_{23} TRIF^* \tag{S28}$$
$$\frac{dTRAF6}{dt} = -(i_{24} MyD88^* + i_{25} TRIF^*) TRAF6 + i_{26} TRAF6^* + v_{14} A20 \cdot TRAF6^* \tag{S29}$$
$$\frac{dTRAF6^*}{dt} = (i_{24} MyD88^* + i_{25} TRIF^*) TRAF6 + i_{23} TRAF6^* - v_{14} A20 \cdot TRAF6^* \tag{S30}$$
$$\frac{dIKKK}{dt} = -i_{27} TRAF^* \cdot IKKK - i_{28} IKKK + i_{29} IKKK^* - a_{31} IKKK \cdot C1 - a_{32} IKKK \cdot C1tnf \tag{S31}$$
$$\frac{dIKKK^*}{dt} = i_{27} TRAF^* \cdot IKKK + i_{28} IKKK - i_{29} IKKK^* + a_{31} IKKK \cdot C1 + i_{32} IKKK \cdot C1tnf \tag{S32}$$
$$\frac{dIKK}{dt} = -i_{30} IKKK^* \cdot IKK - i_{31} IKK + i_{32} IKK^* - i_{33} IKK + i_{34} IKKi \tag{S33}$$
$$\frac{dIKK^*}{dt} = i_{30} IKKK^* \cdot IKK + i_{31} IKK - i_{32} IKK^* \tag{S34}$$
$$\frac{dIKKi}{dt} = i_{33} IKK - i_{34} IKKi \tag{S35}$$
$$\frac{dTNF\alpha_{\text{nas}}}{dt} = v_1 \frac{NF\kappa Bn^{v_2}}{NF\kappa Bn^{v_2} + v_3^{v_2}} - v_5 \cdot f_a \cdot TNF\alpha_{\text{nas}} \tag{S36}$$
$$\frac{dTNF\alpha_{\text{t}}}{dt} = v_5 \cdot f_a TNF\alpha_{\text{nas}} - v_4 \cdot f_i \cdot TNF\alpha_{\text{t}} \tag{S37}$$
$$\frac{dTNF\alpha}{dt} = v_6 \cdot f_a \cdot TNF\alpha_{\text{t}} - v_7 TNF\alpha - v_8 f_a TNF\alpha \tag{S38}$$
$$\begin{aligned}\frac{dTNF\alpha_{\text{ext}}}{dt} = & -a_1 TNF\alpha_{\text{ext}} - a_{15} tnfr \cdot TNF\alpha_{\text{ext}} - a_{16} TNFR \cdot TNF\alpha_{\text{ext}} + a_{17} TNFRtnf \\ & + v_8 f_a TNF\alpha + a_{27} C1tnf_{\text{off}} - a_{28} C1_{\text{off}} \cdot TNF\alpha_{\text{ext}} + a_{29} C1tnf - a_{30} C1 \cdot TNF\alpha_{\text{ext}}\end{aligned} \tag{S39}$$
$$\frac{dtnfr}{dt} = a_2 - a_3 tnfr - 3a_4 tnfr + 3a_5 TNFR - 3a_{15} tnfr \cdot TNF\alpha_{\text{ext}} \tag{S40}$$

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$$\frac{dTNFR}{dt} = a_4 tnfr - a_5 TNFR - a_6 TNFR - a_7 TNFR \cdot TTR + a_8 C1_{\text{off}} + a_{12} C1 \\ - a_{16} TNFR \cdot TNF\alpha_{\text{ext}} + a_{17} TNFRtnf \tag{S41}$$

$$\frac{dTNFRtnf}{dt} = a_{15} tnfr \cdot TNF\alpha_{\text{ext}} + a_{16} TNFR \cdot TNF\alpha_{\text{ext}} - a_{17} TNFRtnf - a_{19} TNFRtnf \cdot TTR \\ + a_{20} C1tnf_{\text{off}} + a_{24} C1tnf \tag{S42}$$

$$\frac{dC1}{dt} = a_9 C1_{\text{off}} - (a_{10} + a_{11} A20 + a_{14}) C1 + a_{29} C1tnf - a_{30} C1 \cdot TNF\alpha_{\text{ext}} \tag{S43}$$

$$\frac{dC1_{\text{off}}}{dt} = a_7 TNFR \cdot TTR - a_8 C1_{\text{off}} - a_9 C1_{\text{off}} + (a_{10} + a_{11} A20) C1 - a_{12} C1_{\text{off}} + a_{13} C1_{\text{off}} \\ + a_{27} C1tnf_{\text{off}} - a_{28} C1_{\text{off}} \cdot TNF\alpha_{\text{ext}} \tag{S44}$$

$$\frac{dC1tnf}{dt} = a_{21} C1tnf_{\text{off}} - a_{22} C1tnf - a_{23} A20 \cdot C1tnf - a_{24} C1tnf - a_{26} C1tnf - a_{29} C1tnf - a_{30} C1tnf_{\text{off}} \tag{S45}$$

$$\frac{dC1tnf_{\text{off}}}{dt} = \begin{array}{l} a_{19} TNFRtnf \cdot TTR - a_{20} C1tnf_{\text{off}} - a_{21} C1tnf_{\text{off}} + a_{22} C1tnf + a_{23} A20 \cdot - a_{25} C1tnf_{\text{off}} \\ - a_{27} C1tnf_{\text{off}} + a_{28} C1_{\text{off}} \cdot TNF\alpha_{\text{ext}} C1tnf \end{array} \tag{S46}$$

$$\frac{dTTR}{dt} = -a_7 TTR \cdot TNFR + a_8 C1_{\text{off}} - a_{12} C1 - a_{19} TNFRtnf \cdot TTR + a_{20} C1tnf_{\text{off}} + a_{24} C1tnf \tag{S47}$$

$$\frac{dA20}{dt} = k_{64} A20_{\text{t}} - k_{65} A20 \tag{S48}$$

$$\frac{dA20_{\text{t}}}{dt} = k_{60} + k_{61} NF\kappa Bn^{k_{62}} - k_{63} A20_{\text{t}}, \quad \text{if}\, t < k_{66} \quad \text{Or}, \quad k_{60} - k_{63} A20_{\text{t}}, \quad \text{if}\, t \geqslant k_{66} \tag{S49}$$

3 Modified equations when Golgiplug™ is added

$$\frac{dI\kappa B\alpha}{dt} = k_{13}\left(1-\frac{v_{15}}{G+v_{16}}\right)I\kappa B\alpha_t - k_{16}I\kappa B\alpha + k_{19}I\kappa B\alpha n - k_{30}I\kappa B\alpha - k_{42}NF\kappa B\cdot I\kappa B\alpha + k_{48}NF\kappa B\text{-}I\kappa B\alpha \quad \text{(S50)}$$

$$\frac{dI\kappa B\beta}{dt} = k_{14}\left(1-\frac{v_{15}}{G+v_{16}}\right)I\kappa B\beta_t - k_{17}I\kappa B\beta + k_{20}I\kappa B\beta n - k_{31}I\kappa B\beta - k_{43}NF\kappa B\cdot I\kappa B\beta + k_{49}NF\kappa B\text{-}I\kappa B\beta \quad \text{(S51)}$$

$$\frac{dI\kappa B\varepsilon}{dt} = k_{15}\left(1-\frac{v_{15}}{G+v_{16}}\right)I\kappa B\varepsilon_t - k_{18}I\kappa B\varepsilon + k_{21}I\kappa B\varepsilon n - k_{32}I\kappa B\varepsilon - k_{44}NF\kappa B\cdot I\kappa B\varepsilon + k_{50}NF\kappa B\text{-}I\kappa B\varepsilon \quad \text{(S52)}$$

$$\frac{dLPS_{\text{pm}}}{dt} = i_1LPS - k_{en_{LPS},m}LPS_{\text{pm}} + i_3LPS_{\text{en}} - i_5LPS_{\text{pm}}\cdot TLR4 + i_6LPS\text{-}TLR4 \quad \text{(S53)}$$

$$\frac{dLPS_{\text{en}}}{dt} = k_{en_{LPS},m}LPS_{\text{pm}} - i_3LPS_{\text{en}} - i_4LPS_{\text{en}} - i_7LPS_{\text{en}}\cdot TLR4_{\text{en}} + i_8LPS\text{-}TLR4_{\text{en}} \quad \text{(S54)}$$

$$\frac{dTLR4}{dt} = -i_5LPS_{\text{pm}}\cdot TLR4 + i_6LPS\text{-}TLR4 + k_{s_{TLR4},m} - i_{10}TLR4 - i_{12}TLR4 + i_{13}TLR4_{\text{en}} \quad \text{(S55)}$$

$$\frac{dLPS\text{-}TLR4}{dt} = i_5LPS_{\text{pm}}\cdot TLR4 - i_6LPS\text{-}TLR4 - k_{en_{cp},m}LPS\text{-}TLR4 + i_{15}LPS\text{-}TLR4_{\text{en}} - i_{16}LPS\text{-}TLR4 \quad \text{(S56)}$$

$$\frac{dLPS\text{-}TLR4}{dt} = i_6LPS_{\text{en}}\cdot TLR4_{\text{en}} - i_8LPS\text{-}TLR4_{\text{en}} + k_{en_{cp},m}LPS\text{-}TLR4 - i_{15}LPS\text{-}TLR4_{\text{en}} - i_{17}LPS\text{-}TLR4_{\text{en}} \quad \text{(S57)}$$

$$\frac{dTNF\alpha}{dt} = v_6\cdot f_a\cdot TNF\alpha_{\text{t}} - v_7TNF\alpha - k_{sec,m}\cdot f_aTNF\alpha \quad \text{(S58)}$$

$$\frac{dTNF\alpha_{\text{ext}}}{dt} = -a_1TNF\alpha_{\text{ext}} - a_{15}tnfr\cdot TNF\alpha_{\text{ext}} - a_{16}TNFR\cdot TNF\alpha_{\text{ext}} + a_{17}TNFRtnf + k_{sec,m}f_aTNF\alpha + a_{27}C1tnf_{\text{off}} - a_{28}C1_{\text{off}}\cdot TNF\alpha_{\text{ext}} + a_{29}C1tnf - a_{30}C1\cdot TNF\alpha_{\text{ext}} \quad \text{(S59)}$$

$$\frac{dtnfr}{dt} = k_{s_{TNFR},m} - a_3tnfr - 3a_4tnfr + 3a_5TNFR - 3a_{15}tnfr\cdot TNF\alpha_{\text{ext}} \quad \text{(S60)}$$

*Parameters in red are the ones affected by Golgiplug™

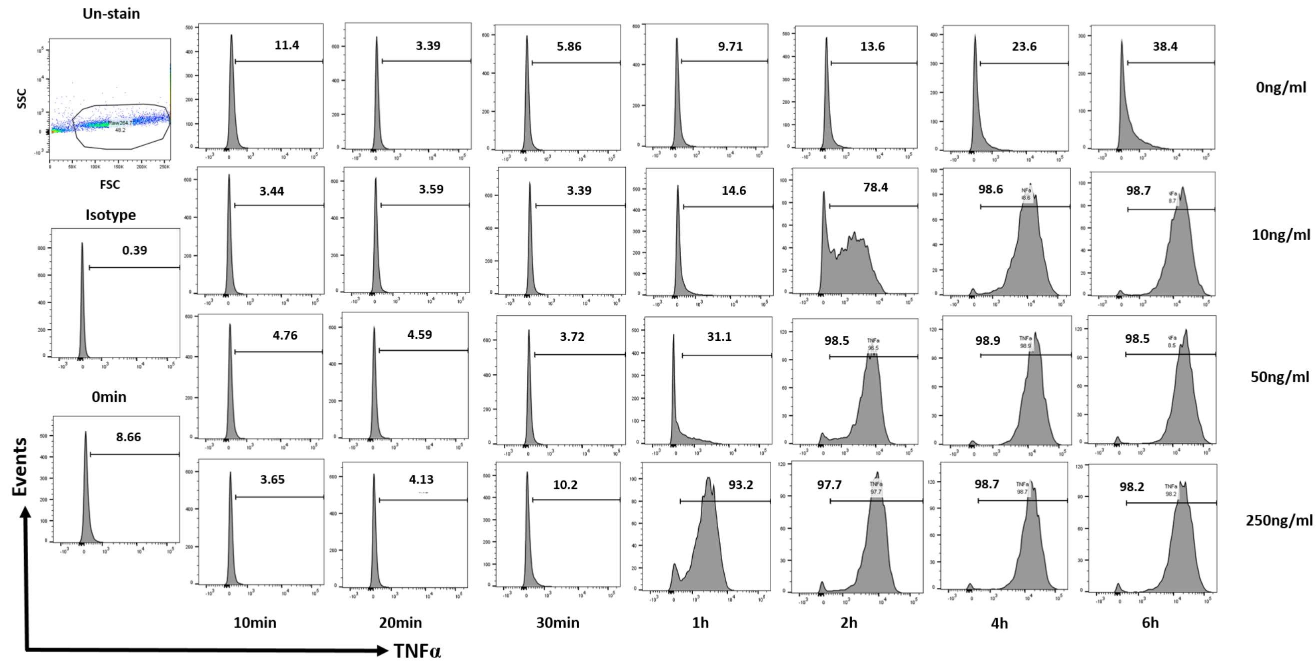

Figure S1: Representative histograms of TNFα production. RAW 264.7 cells were stimulated with different concentrations of LPS along with Golgiplug™, and the production of TNFα were analyzed by flow cytometry.

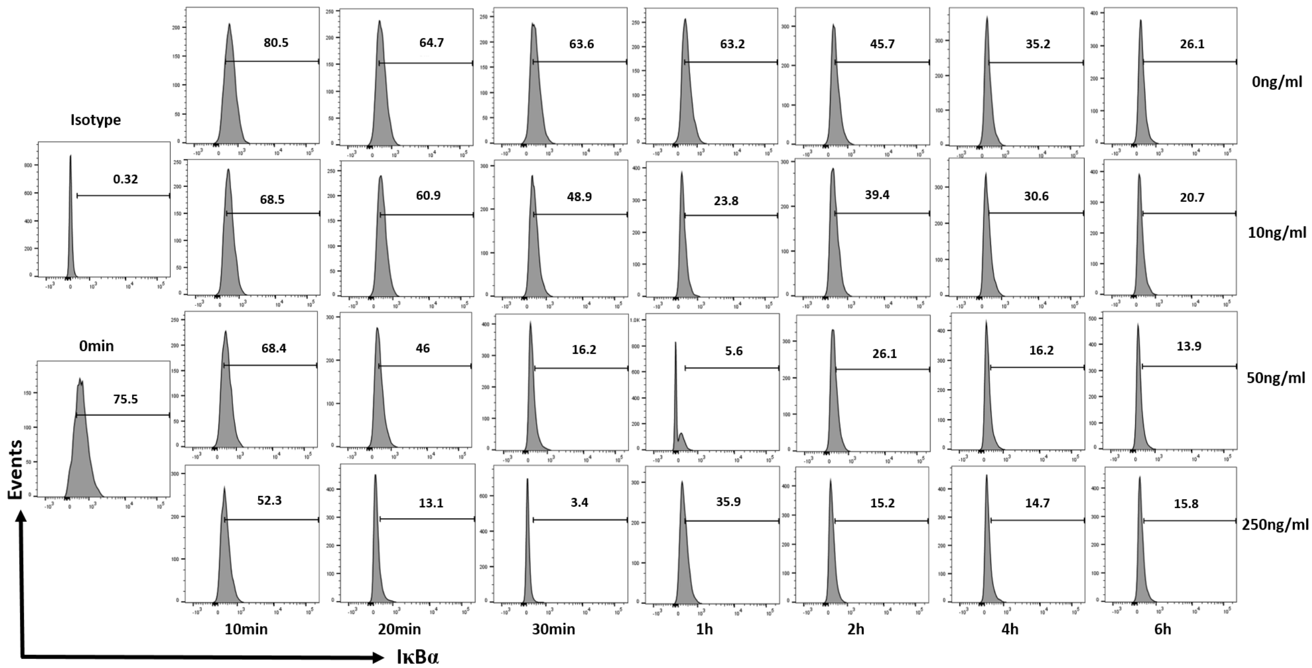

Figure S2: Representative histograms of IκBα. RAW 264.7 cells were stimulated with different concentrations of LPS along with Golgiplug$^{\text{TM}}$, and the intracellular IκBα levels were analyzed by flow cytometry.

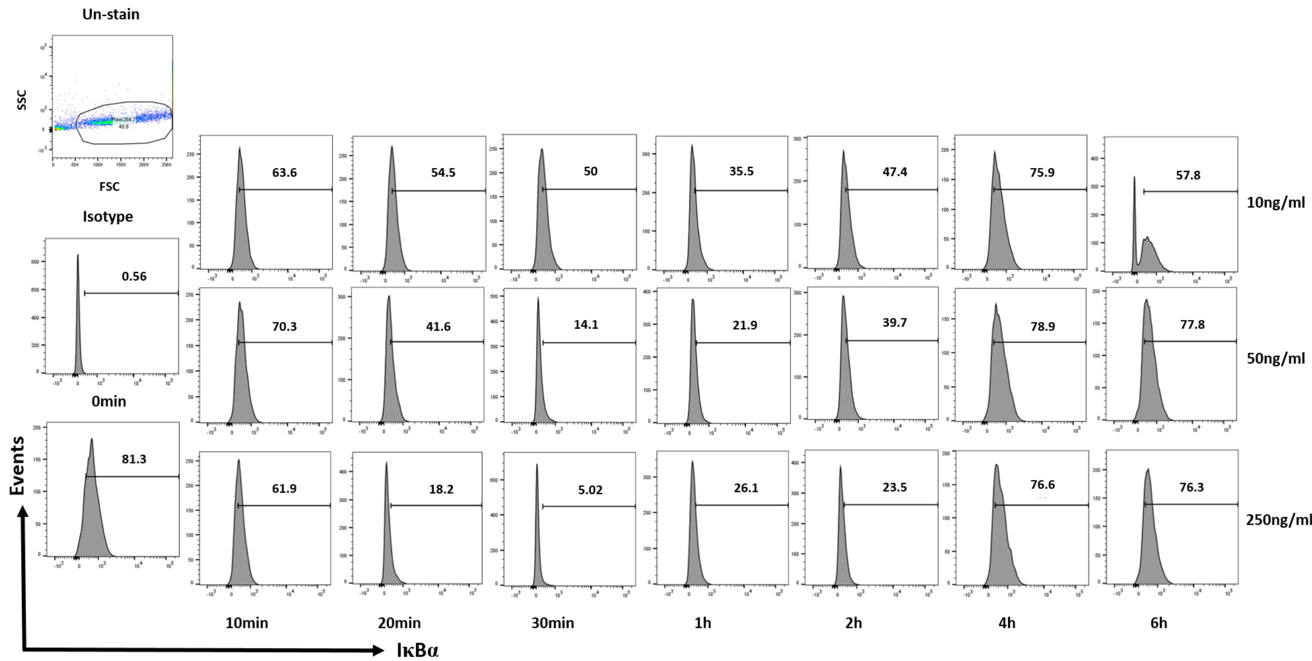

Figure S3: Representative histograms of IκBα. RAW 264.7 cells were stimulated with different concentrations of LPS without the addition of Golgiplug$^{\text{TM}}$, and the intracellular IκBα levels were analyzed by flow cytometry.

References

Werner, S.L.; Kearns, J.D.; Zadorozhnaya, V.; Lynch, C.; O'Dea, E.; Boldin, M.P.; Ma, A.; Baltimore, D.; Hoffmann, A. Encoding NF-κB temporal control in response to TNF: distinct roles for the negative regulators IκBα and A20. *Genes & Development* **2008**, *22*, 2093–2101.

Caldwell, A.B.; Cheng, Z.; Vargas, J.D.; Birnbaum, H.A.; Hoffmann, A. Network dynamics determine the autocrine and paracrine signaling fucntions of TNF. *Genes & Development* **2014**, *28*, 2120–2133.

Junkin, M.; Kaestli, A.J.; Cheng, Z.; Jordi, C.; Albayrak, C.; Hoffmann, A.; Tay, S. High-content quantification of single-cell immune dynamics. *Cell Reports* **2016**, *15*, 411–422.

Cheng, Z.; Taylor, B.; Ourthiague, D.R.; Hoffmann, A. Distinct single-cell signaling characteristics are conferred by the MyD88 and TRIF pathways during TLR4 activation. *Science Signaling* **2015**, *8*, ra69.

Lin, S.C.; Lo, Y.C.; Wu, H. Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. *Nature* **2010**, *465*, 885–890.

Bagnall, J.; Boddington, C.; Boyd, J.; Brignall, R.; Rowe, W.; Jones, N.A.; Schmidt, L.; Spiller, D.G.; White, M.R.; Paszek, P. Quantitative dynamic imaging of immune cell signalling using lentiviral gene transfer. *Integrative Biology* **2015**, *7*, 713–725.