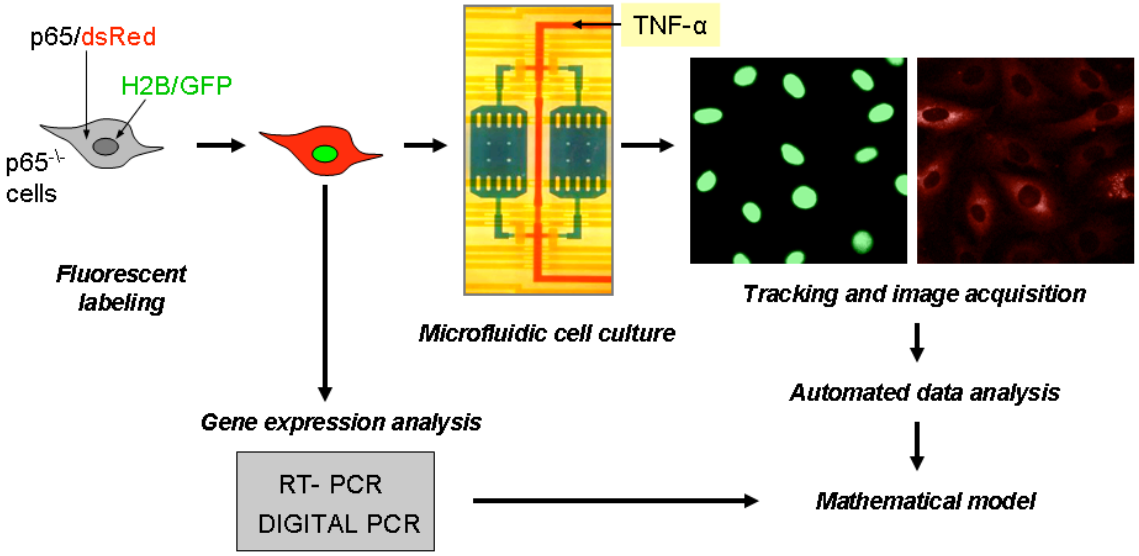

Figure 1: Experimental workflow. p65-knockout mouse fibroblast 3T3 cells were fluorescently labeled with p65-DsRed and with GFP for nuclear tracking. Cells were cultured and stimulated in microfluidic chambers with 10 different concentrations of TNF-α. Time-lapse videos of DsRed and GFP channels were captured and p65 nuclear localization intensity was quantified. Time dependent expression profiles were of 23 genes were also measured using RT-PCR, and absolute mRNA levels were quantified using digital-PCR. The comprehensive set of data obtained from these measurements was used to build a broadly applicable mathematical model of TNF-α induced NF-κB activity and target gene expression.

Microfluidic time lapse experiments	
TNF-α concentration range	0.005 ng/ml - 100 ng/ml
Number of single cells analyzed	20,000
Average time between images	6 minutes
Average experiment duration	8 hours
Fluorescence images processed	30,000

Gene expression measurements	
TNF-α concentration range	0.01 ng/ml - 10 ng/ml
Experiment duration	12 hours
Total conditions tested	63 x (23 genes)
Total RT-PCR reactions	9216
Total digital PCR reactions	9180

Table 1: Various measures of experimental throughput during in this study.

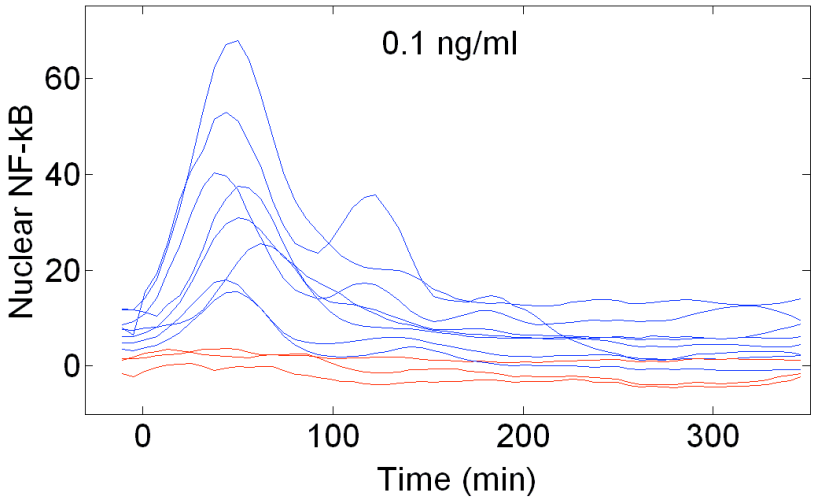

Figure 2: Representative single cell traces measured from a single microfluidic chamber stimulated with 0.1ng/ml TNF-α, showing active (blue) and inactive (red) cells.

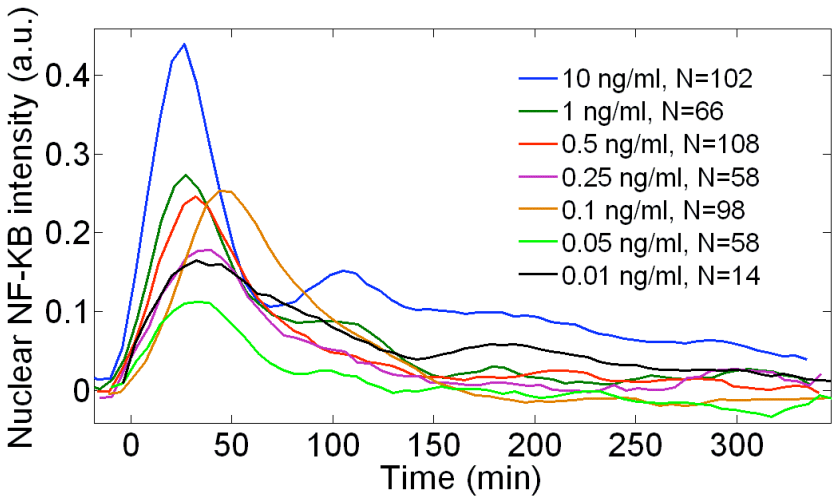

Figure 3: Mean nuclear NF- κB intensity normalized to total cytoplasmic intensity vs. time for different TNF-α doses measured at single culture chambers in a single experiment (only active cells included, N=number of active cells).

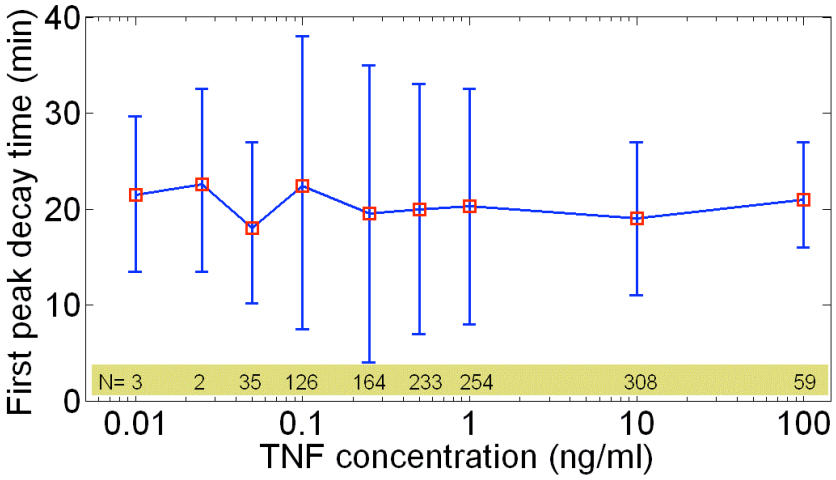

Figure 4: NF-κB first peak decay time vs. TNF concentration (see methods for calculation).

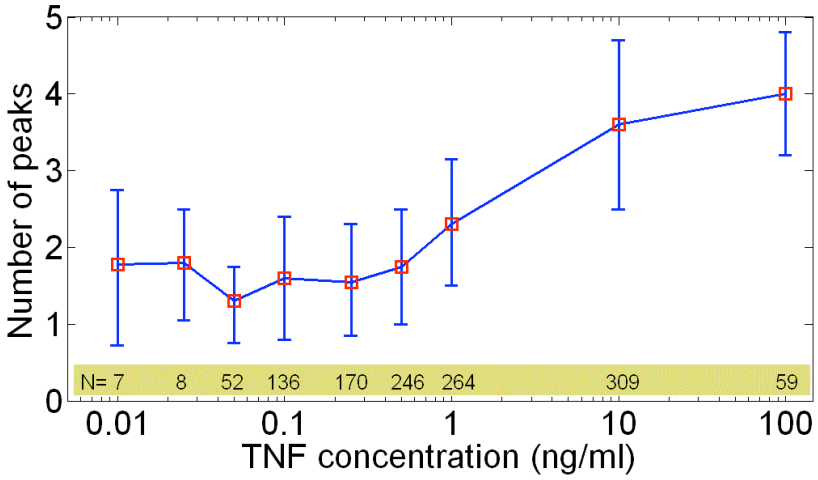

Figure 5 Number of NF-κB nuclear-cytoplasmic oscillation peaks vs. dose in a single experiment (N=number of active cells).

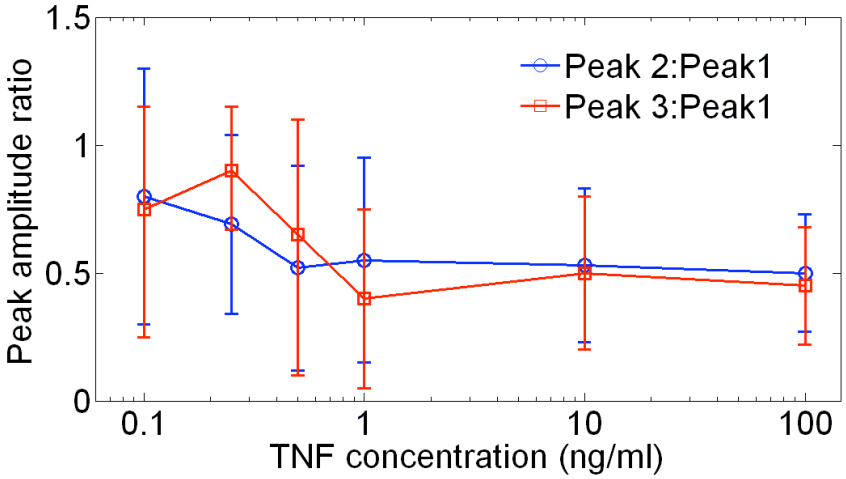

Figure 6: NF- κB peak amplitude ratio vs. TNF concentration.

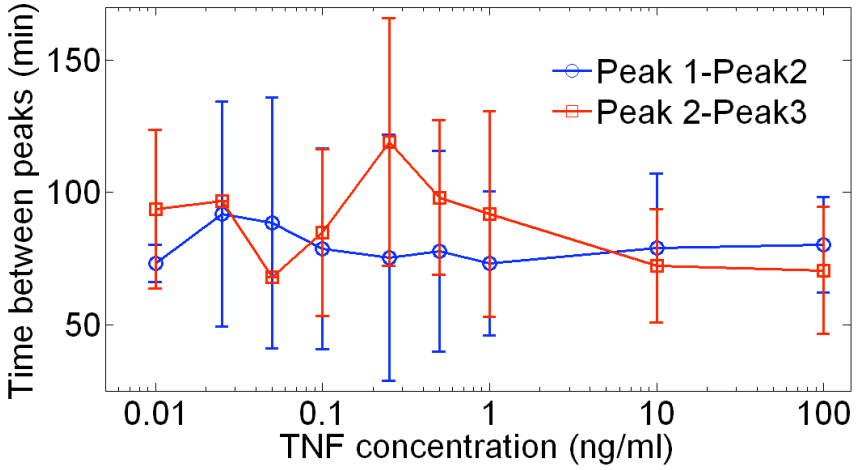

Figure 7: Time between NF-κB peaks vs. TNF concentration

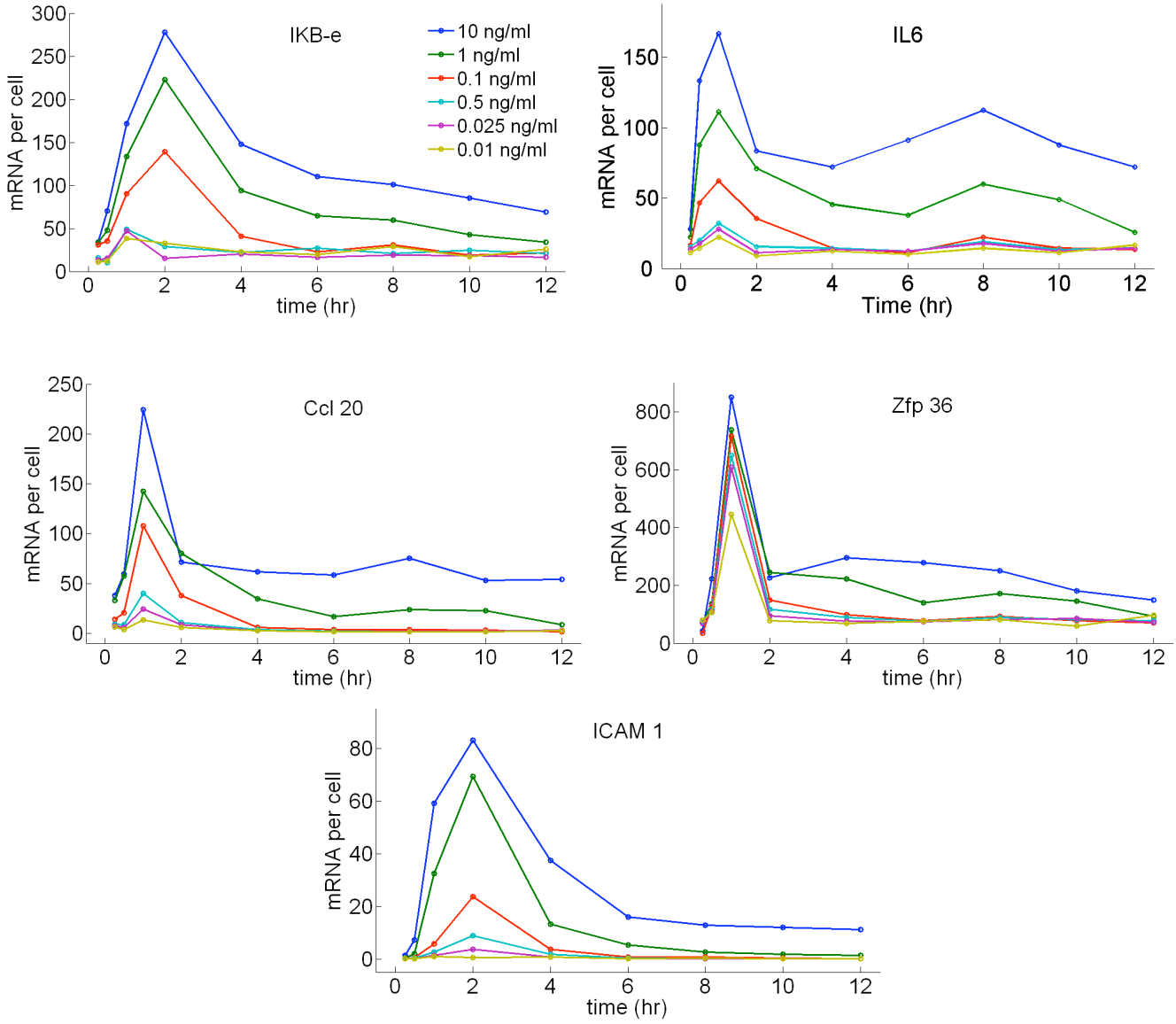

Figure 8: Time dependent gene expression profiles not shown in the manuscript.

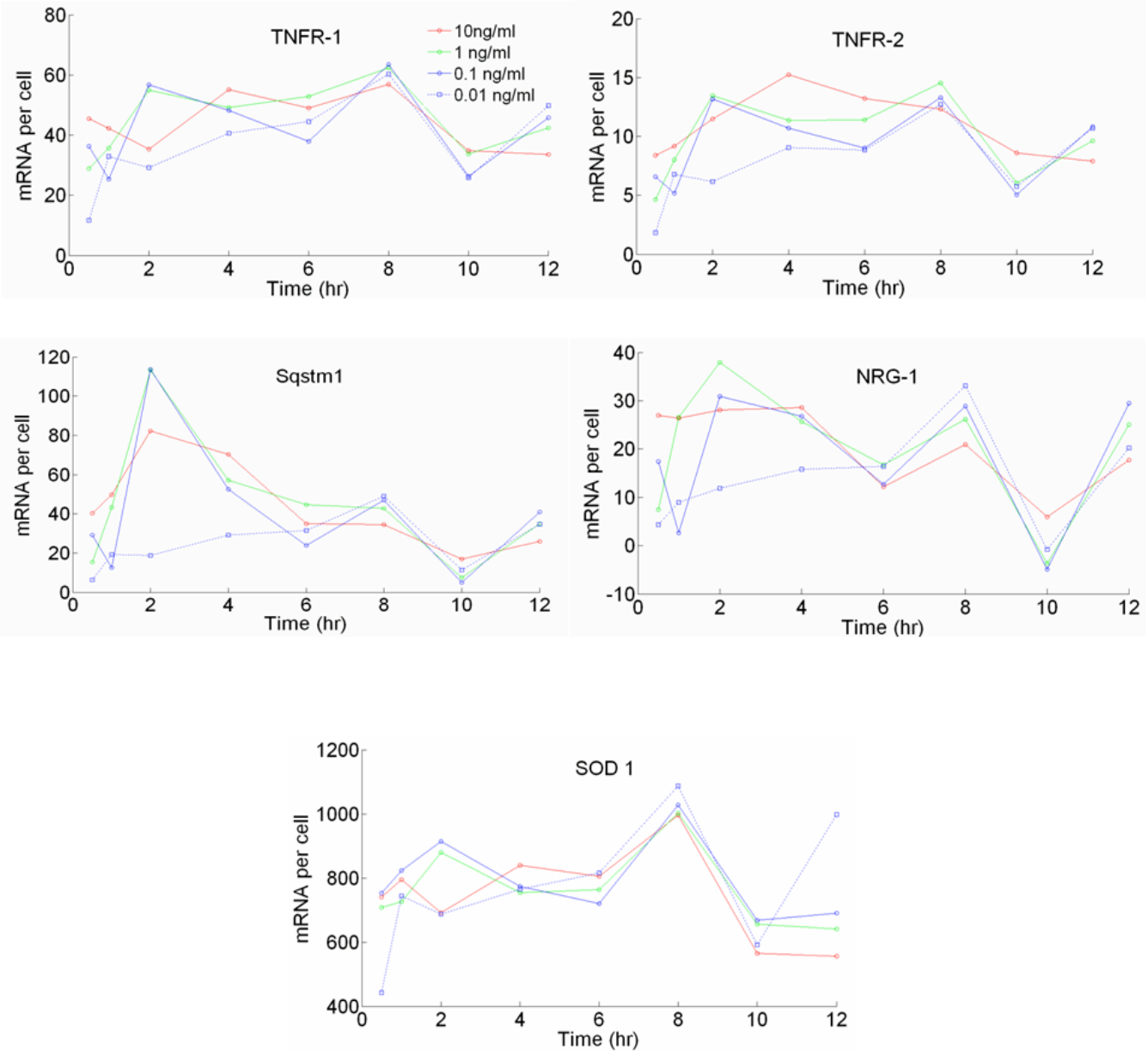

Figure 8 continued: Time dependent gene expression profiles not shown in the manuscript.

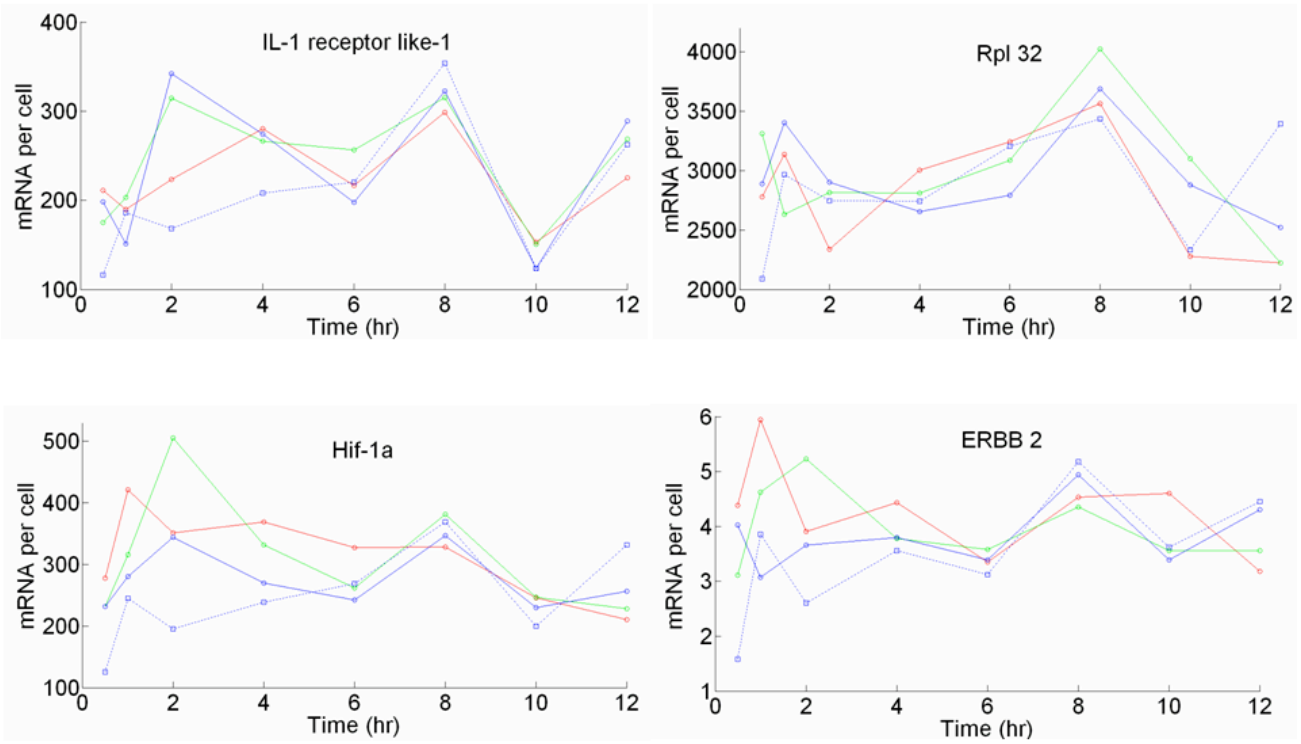

Figure 8 continued: Time dependent gene expression profiles not shown in the manuscript.

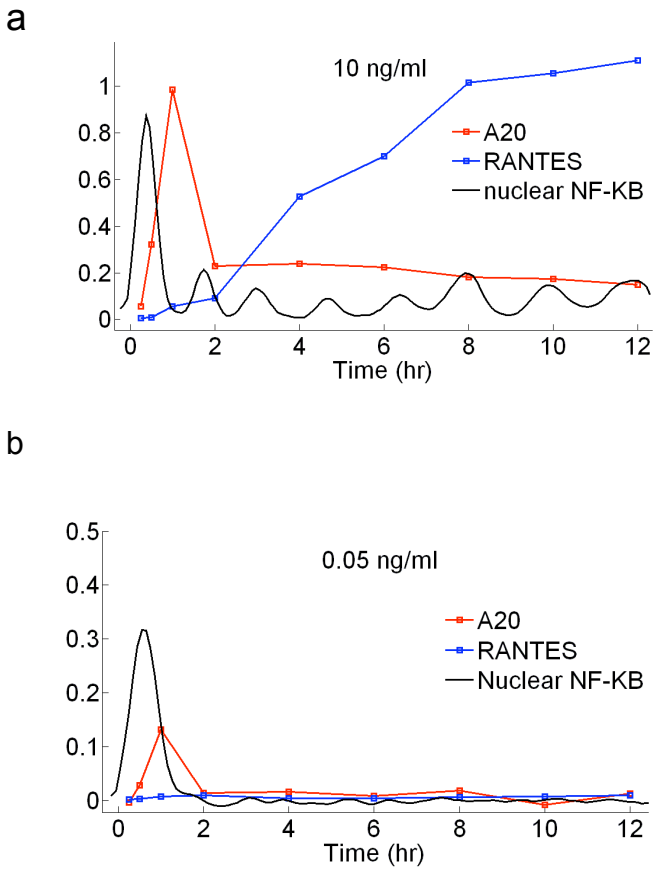

Figure 9 a, Expression profiles of an early (A20) and late gene (RANTES) compared to NF-κB nuclear localization dynamics. All measurements were under 10 ng/ml TNF-α stimulation. Initial NF-κB nuclear translocation results in a burst of mRNA synthesis for both genes, and the late term expression follows persistent NF-κB oscillations. **b,** Expression profiles of an early and late gene compared to NF-κB nuclear localization dynamics under 0.05 ng/ml TNF-α stimulation. The lack of persistent NF-κB oscillations results in reduced early expression (i.e. fewer cells activating), and the late expression is completely diminished.

GAPDH CT values

	0.25 hr	0.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr
10 ng/ml	12.19	11.835	12.8	8.58	8.445	7.85	11.63	12.095	12.04
1 ng/ml	12.96	12.78	12.44	8.57	7.695	8.72	9.82	8.955	10.795
0.1 ng/ml	11.32	12.075	12.425	7.7	7.92	6.5	11.59	8.495	11.465
0.05 ng/ml	12.8	12.52	13.535	8.22	7.24	7.97	12.55	10.105	12.785
0.025 ng/ml	12.78	11.97	13.74	7.255	7.305	7.54	11.665	12.39	11.725
0.01 ng/ml	12.935	12.39	13.57	8.01	7.805	7.94	11.915	11.075	12.355

IKB-α CT values

	0.25 hr	0.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr
10 ng/ml	18.69	16.85	17.23	13.97	14.63	13.52	17.86	18.68	17.93
1 ng/ml	19.60	19.15	16.99	15.12	14.17	15.10	16.13	15.31	17.57
0.1 ng/ml	18.49	17.85	17.19	14.86	15.08	13.12	19.47	14.96	18.97
0.05 ng/ml	19.33	18.91	19.06	15.40	14.13	14.02	20.02	17.01	20.25
0.025 ng/ml	19.66	17.85	19.52	14.00	14.20	14.22	19.08	20.16	18.40
0.01 ng/ml	19.51	18.45	19.34	15.46	14.23	14.60	19.48	18.44	19.70

Table 2 Cycle threshold (CT) values measured during qRT-PCR gene expression experiments for a house keeping gene (GAPDH) and IKB-α. The cells were stimulated with various doses of TNF-α, and were lysed and c-DNA was synthesized at different times after stimulation using Invitrogen Cells Direct One Step qRT-PCR kit and Taq-man primers and probes. Real-time PCR was performed using Fluidigm Biomark system.

Property	Input signal intensity (TNF-α concentration)		
	High (100 - 1 ng/ml)	Mid (1 - 0.05 ng/ml)	Low (0.05 - 0.005 ng/ml)
Cells responding	~100 %	Reducing (90-30 %)	Very few (~5 %)
Response time	Fast (20 min)	Increasing (30-40 min)	Slow (> 50 min)
Response time variation	Very small (~10 min)	Increasing (15-40 min)	Very Large (> 60 min)
Peak intensity	Large (4X)	Reducing (3-2 X)	Low (1X)
Peak intensity variation (compared to mean)	Large (~100%)	Large (~100%)	Large (~100%)
Number of peaks	6-4	1-2	1
Normalized early gene expression	High	High	High
Normalized late gene expression	High	Very low	No expression

Table 3 Response characteristics for high, medium and low input signal intensity levels.

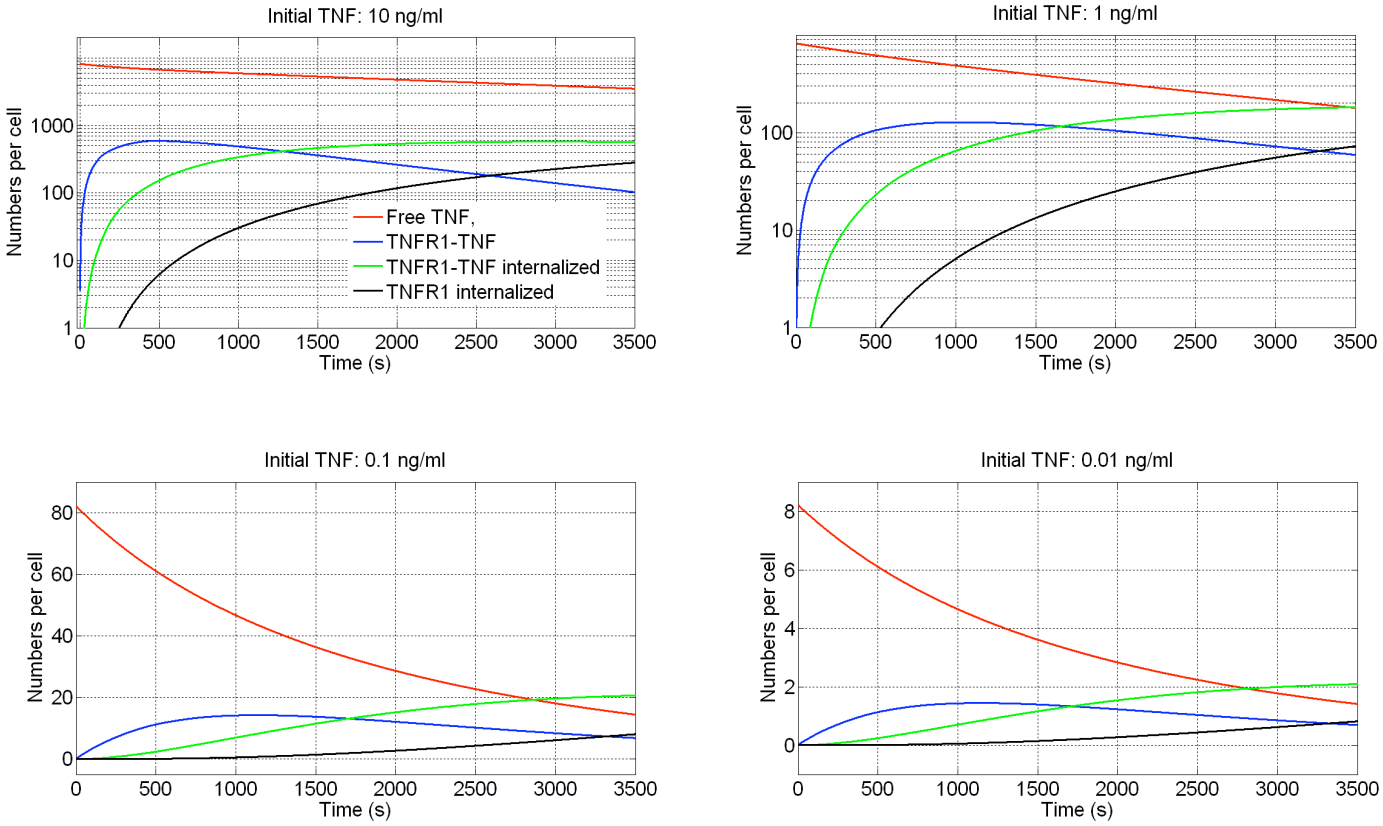

Figure 10: Calculated receptor-binding dynamics in the 35 nanoliter microfluidic chamber. Protein and receptor numbers are in trimers. See Supplementary Mathematical Methods for a discussion of receptor binding calculations.

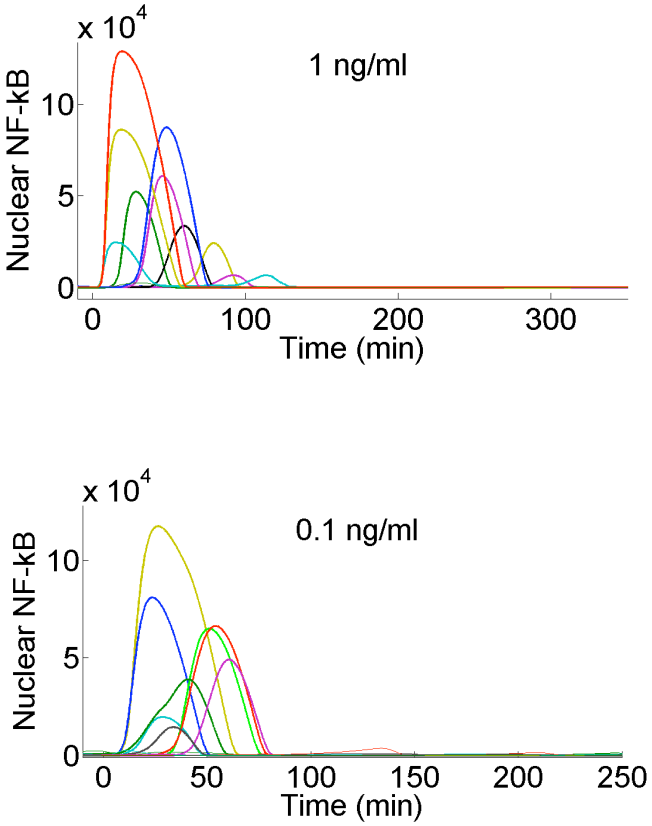

Figure 11: Mid-dose simulations not shown in manuscript Figure 3.

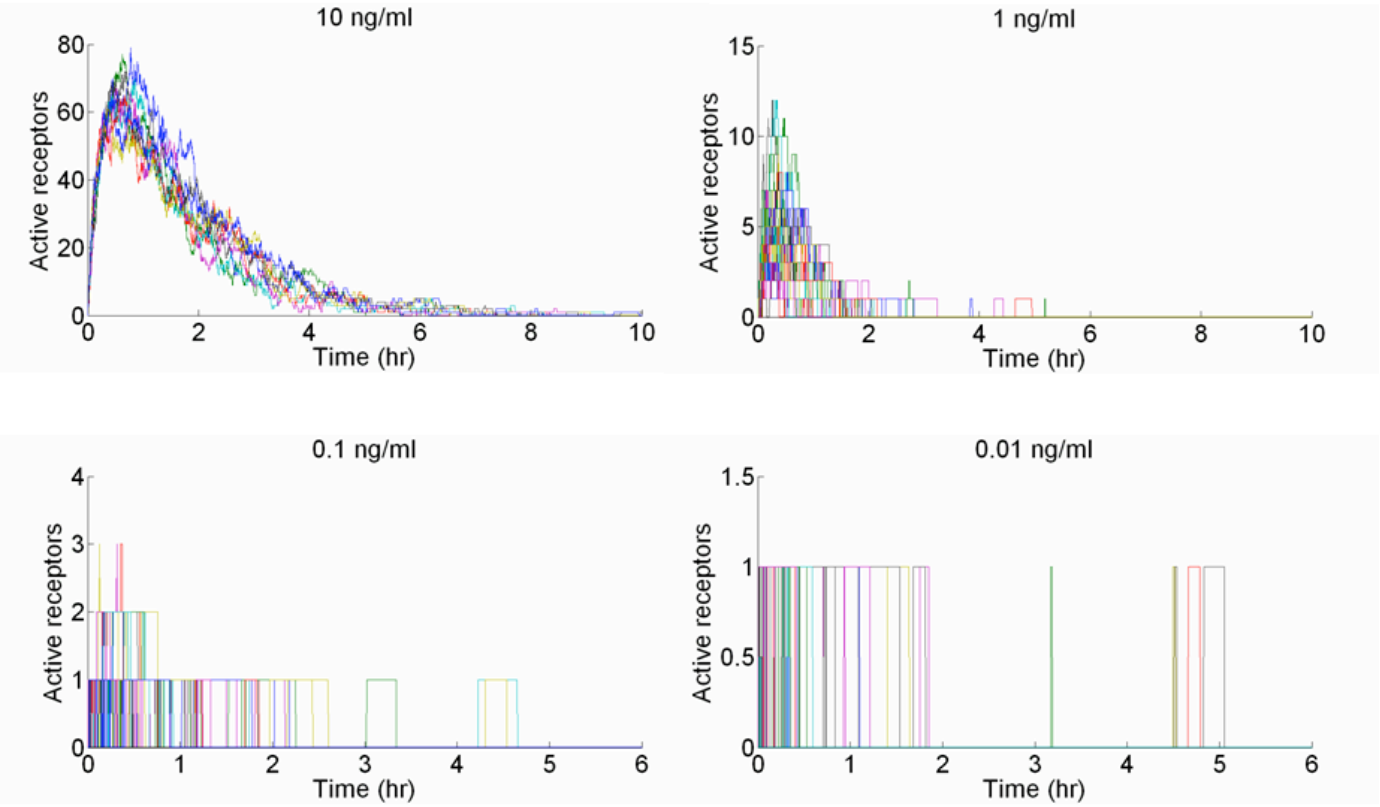

Figure 12: Receptor states calculated during simulations shown in manuscript Figure 3.

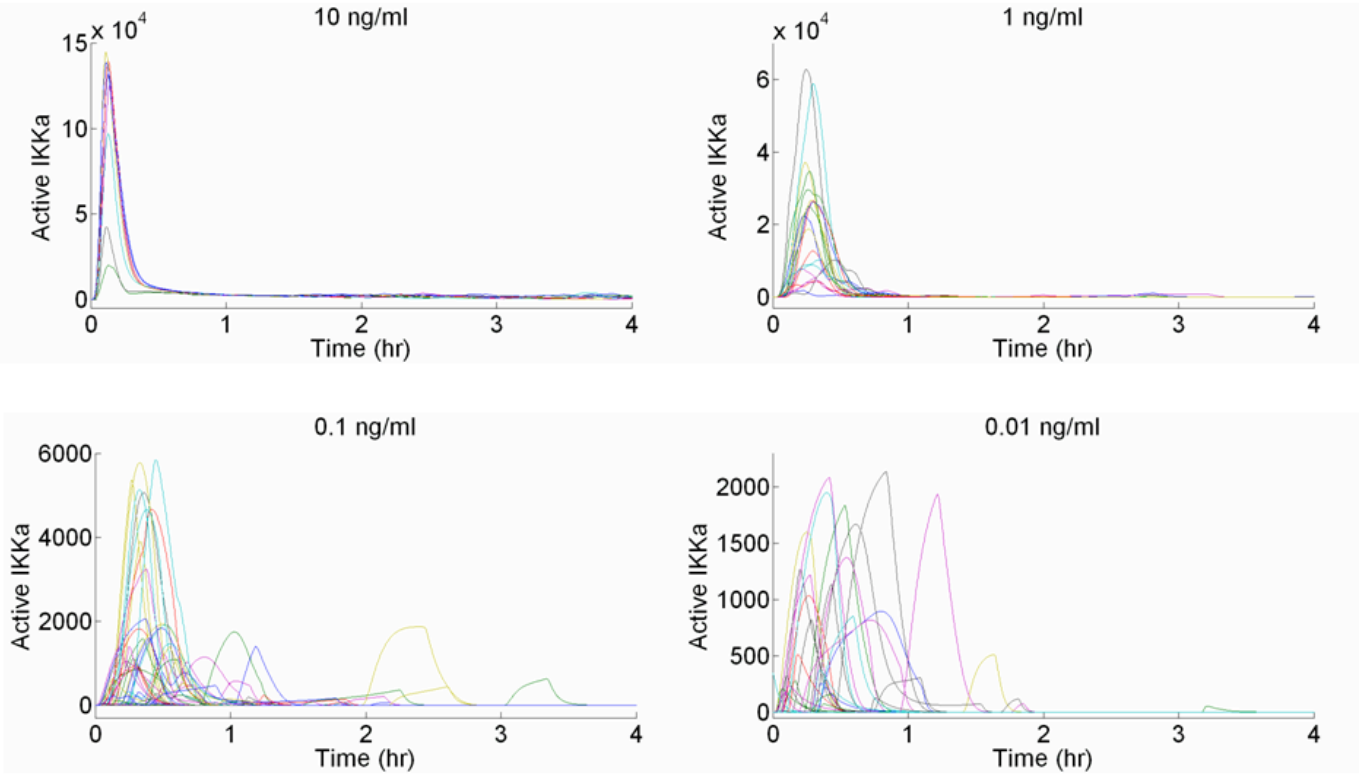

Figure 13: Number of active IKKa from simulations shown in manuscript Figure 3.