Supplementary Figure S1

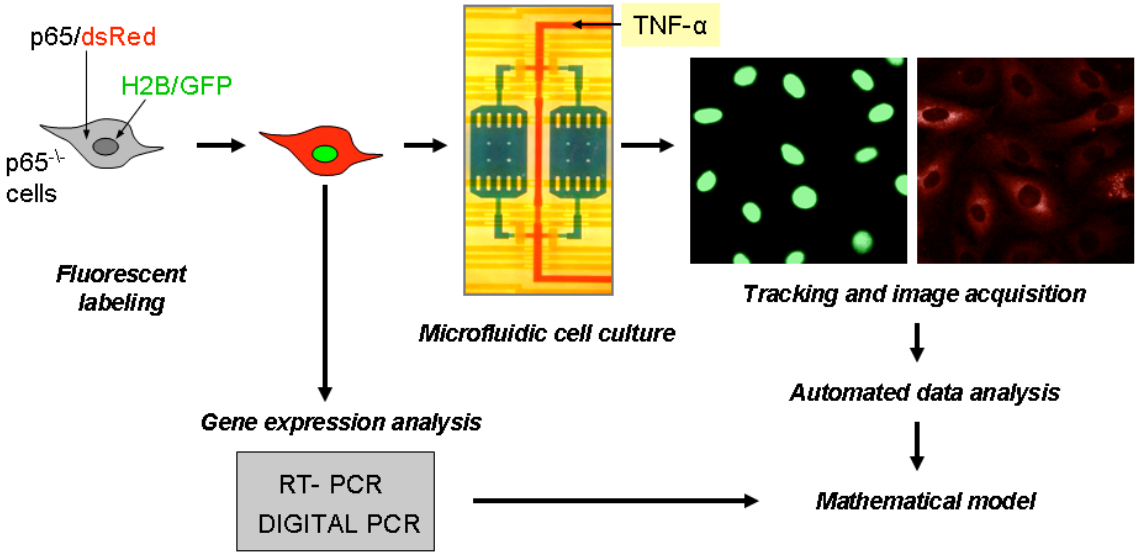

Figure S1: Experimental workflow. p65 deficient mouse fibroblast cells were fluorescently labeled with p65/DsRed and with GFP for nuclear tracking. Cells were cultured and stimulated microfluidic chambers with 10 different concentrations of TNF-α. Time-lapse videos of DsRed and GFP channels were captured and p65 nuclear localization intensity was quantified. Time dependent expression profiles were of 24 genes were also measured using RT-PCR, and mRNA levels were quantified using digital PCR.

Supplementary Table 1

Microfluidic cell culture	
TNF concentration range	0.005 ng/ml - 100 ng/ml
Total stimulated cells	5177
Total cells responded	3000
Average time between images	6 minutes
Average experiment duration	8 hours
Responding cells tracked	1250
Fluorescence images processed	8000

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

Table S1: Various measures of experimental throughput used in this study.

Supplementary Figure S2

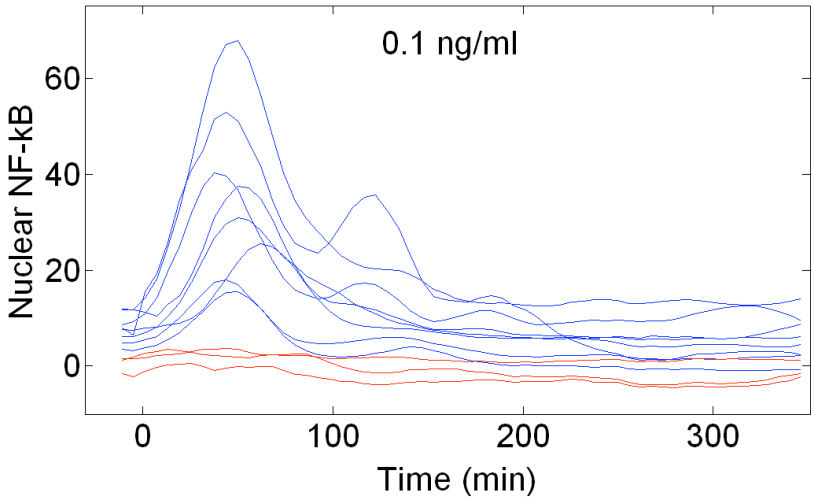

Figure S2: Representative single cell traces measured from a single microfluidic chamber stimulated with 0.1ng/ml TNF-α, showing active (blue) and inactive (red) cells.

Supplementary Figure S3

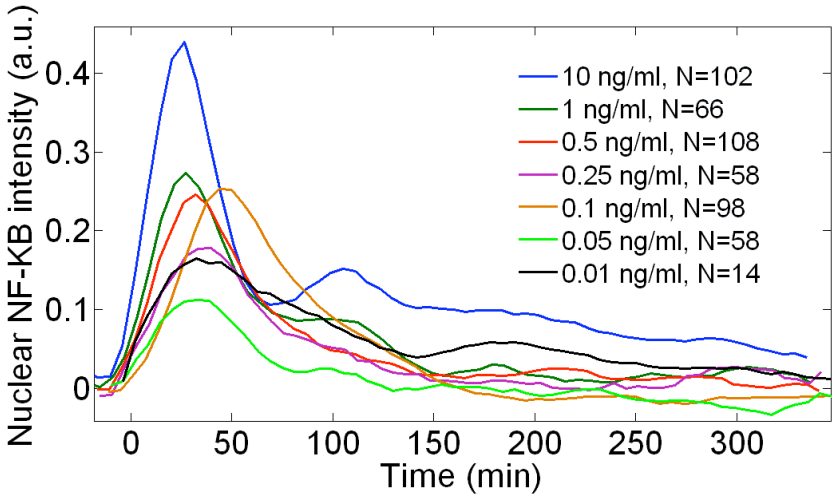

Figure S3: Mean nuclear NF-KB intensity normalized to total cytoplasmic intensity vs. time for different TNF-α doses measured at single culture chambers in a single experiment (only active cells included, N=number of active cells).

Supplementary Figure S4

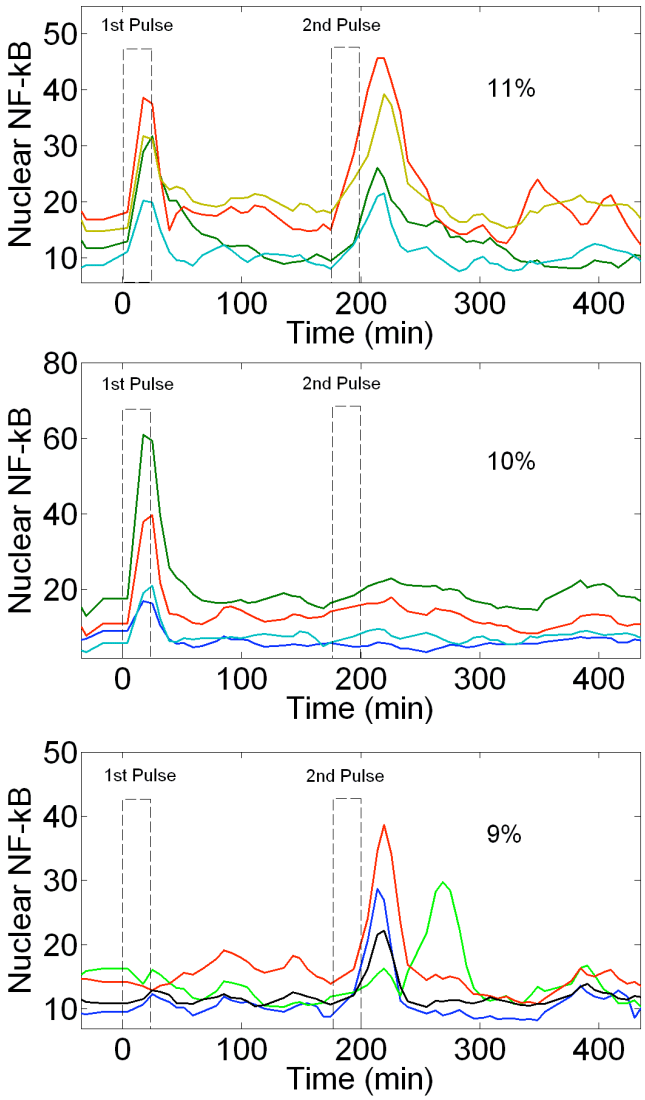

Figure S4: Representative single cell traces for low dose, short-pulsed stimulation experiments. Cells were stimulated with two consecutive 20 minute pulses of 0.1 ng/ml TNF-a. The pulses were separated by 170 minutes. 11% of the cells in the chamber respond to both pulses (top), while 10 % respond to only the first pulse (middle) and 9% respond only to the second pulse (bottom). The existence of cells responding to only one of the pulses indicate that NF-kB activation is partly governed by a stochastic process, while the high percentage of cell responding to both pulses indicate high sensitivity to TNF-a in this subpopulation.

Supplementary Figure S5

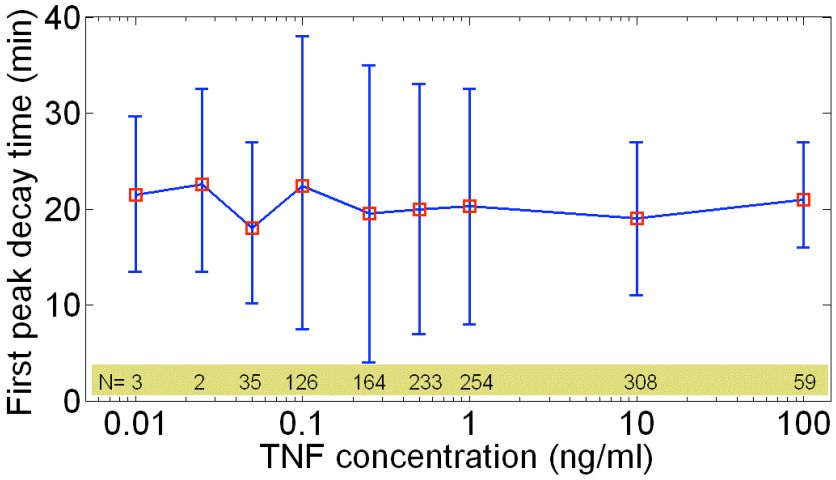

Figure S5: First peak decay time (FWHM) vs. TNF concentration

Supplementary Figure S6

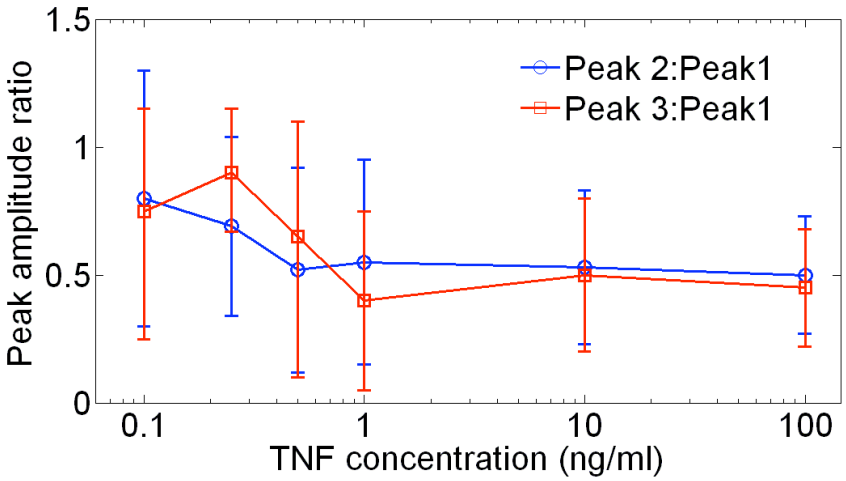

Figure S6: NF-kB peak amplitude ratio vs. TNF concentration.

Supplementary Figure S7

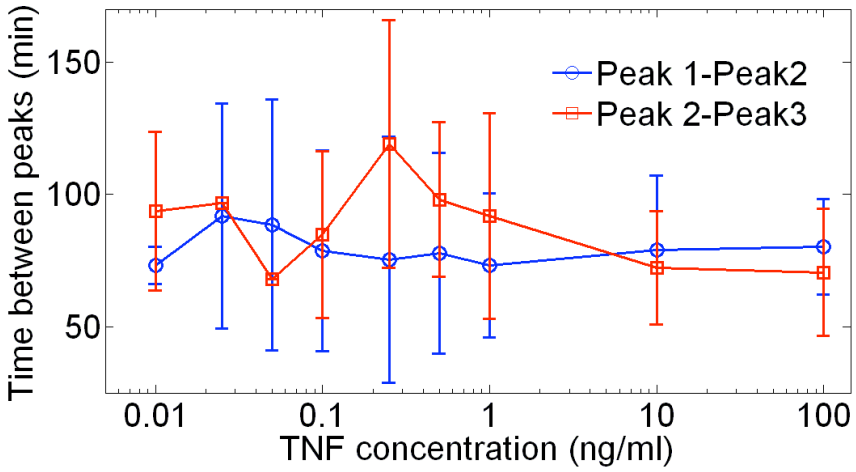

Figure S7: Time between peaks vs. TNF concentration

Supplementary Figure S8

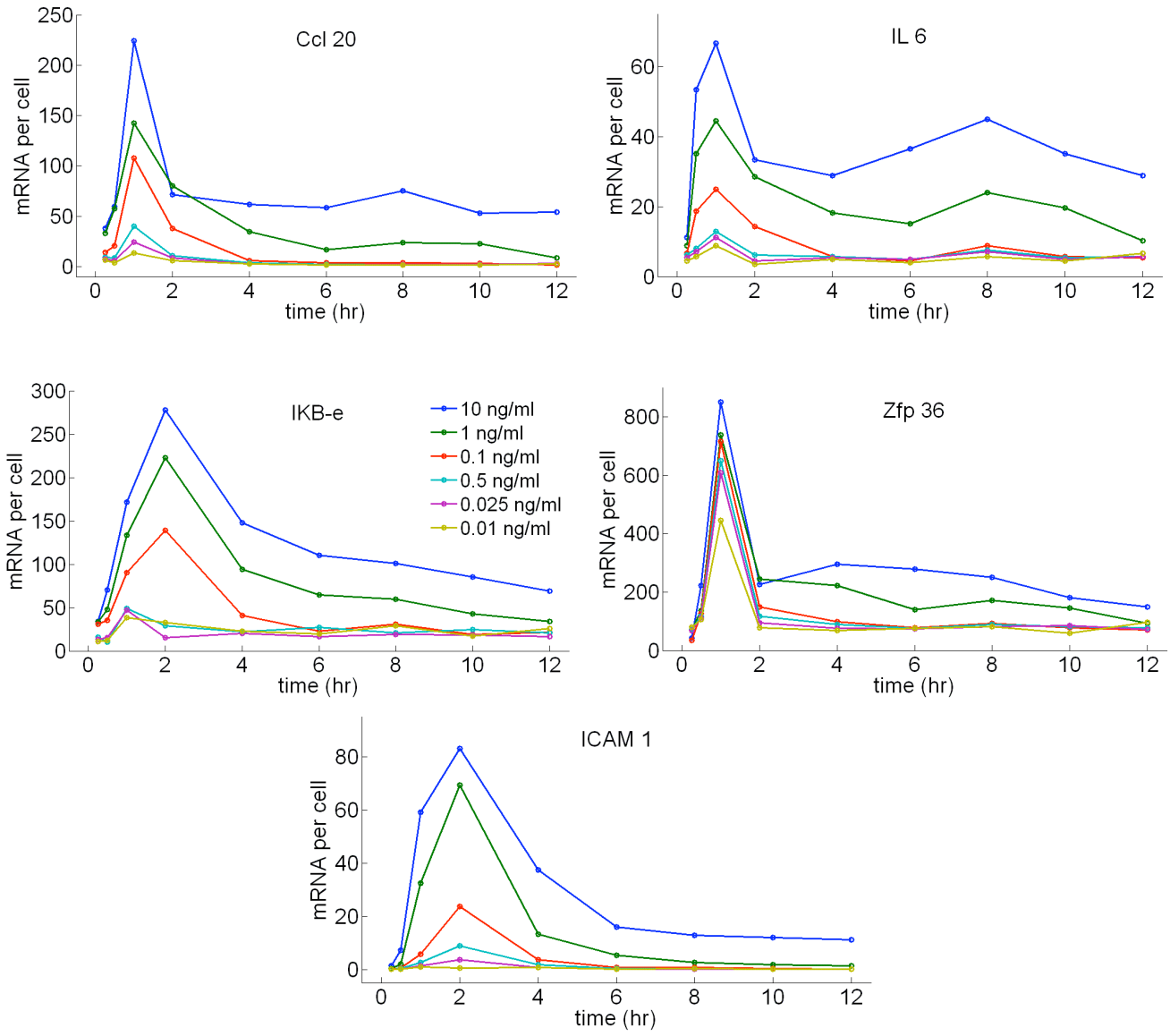

Figure S8: Other time dependent gene expression profiles.

Supplementary Figure S8, continued

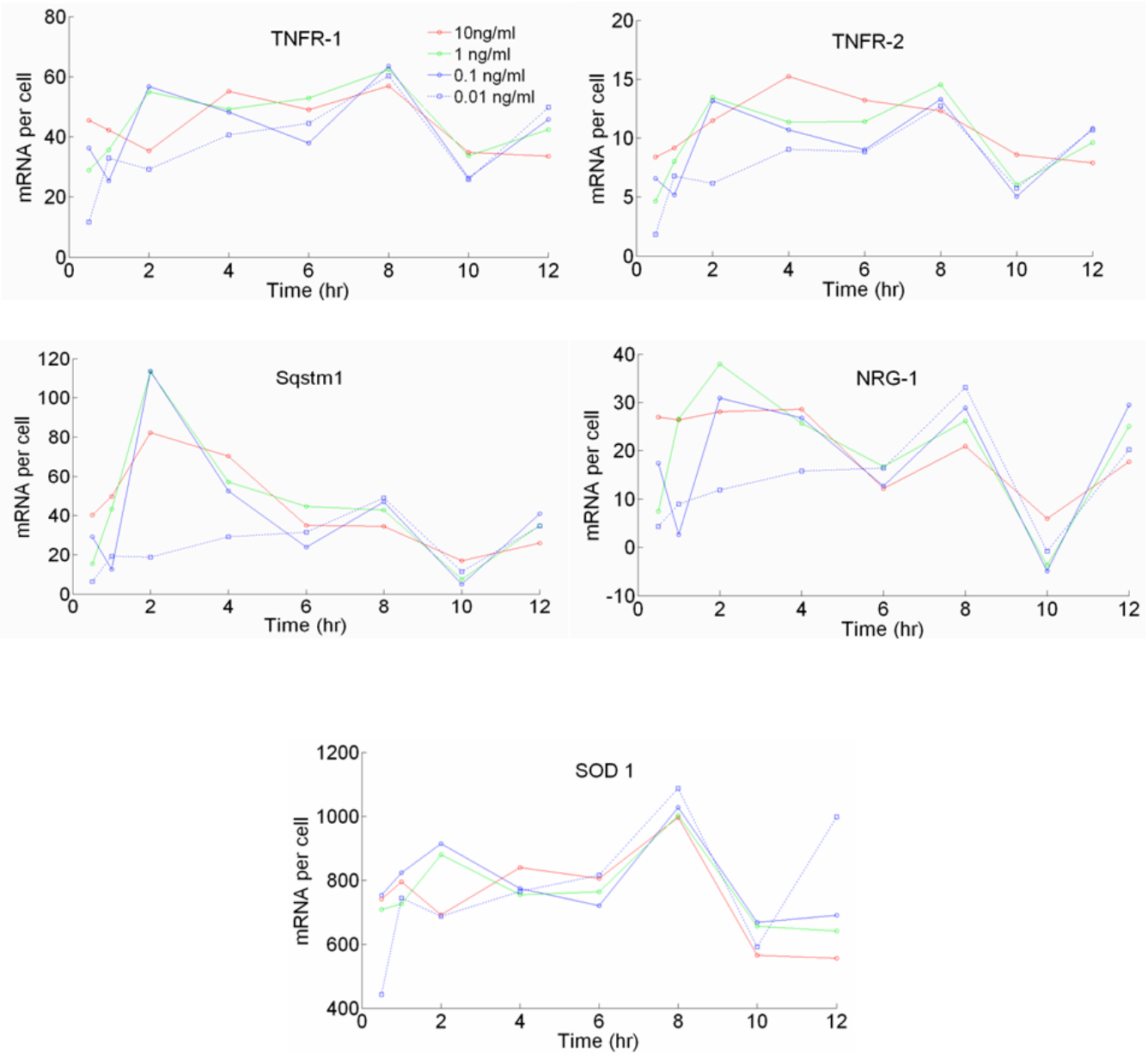

Figure S8: Other time dependent gene expression profiles.

Supplementary Figure S8, continued

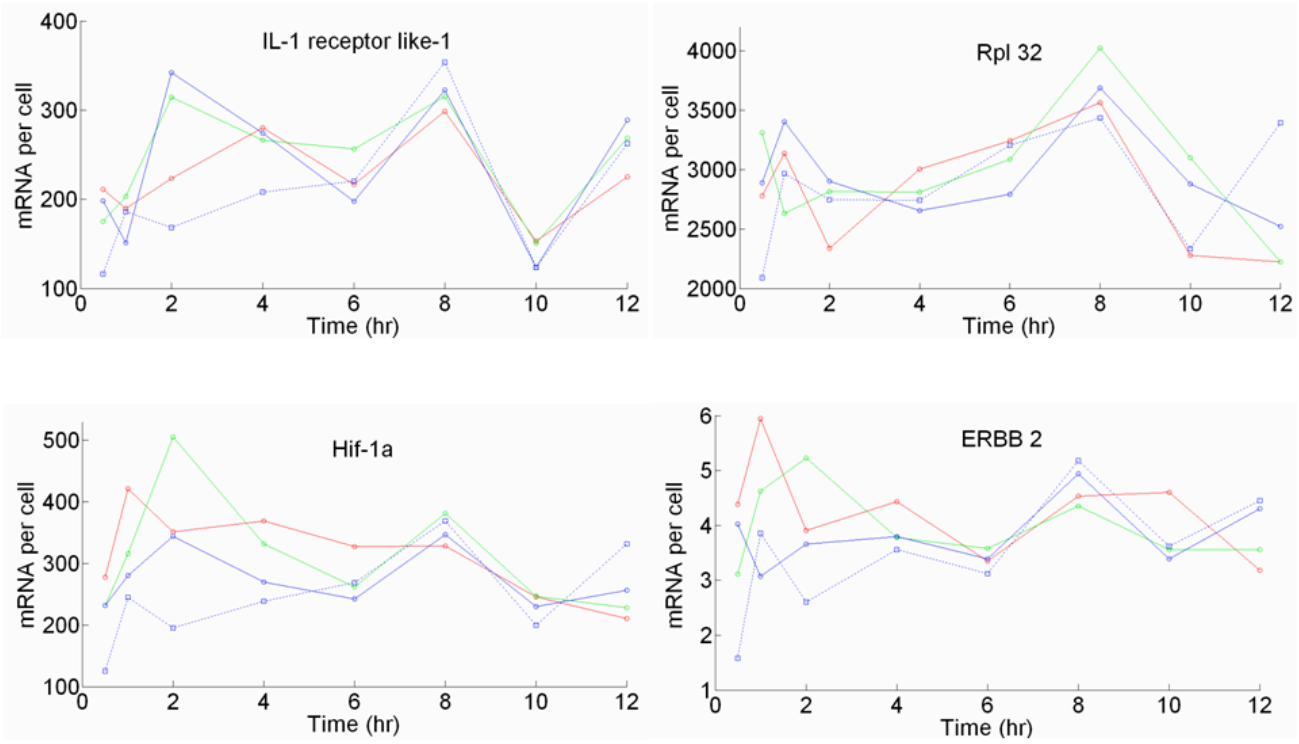

Figure S8: Other time dependent gene expression profiles.

Supplementary Table 2

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 S2 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.

Supplementary Table 3

Property	Input signal intensity (TNF-α concentration)		
	High (100 - 1 ng/ml)	Mid (1 - 0.05ng/ml)	Low (0.05 - 0.005ng/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)
Response intensity	Large (4X)	Reducing (3-2 X)	Low (1X)
Intensity variation	Large (~100%)	Large (~100%)	Large (~100%)
Number of NF-KB peaks	6-4	1-2	1
Early gene expression	High	High	High
Late gene expression	High	Low	No expression

Table S3 Response characteristics for high, medium and low input signal intensity levels were summarized.

Supplementary Figure S9

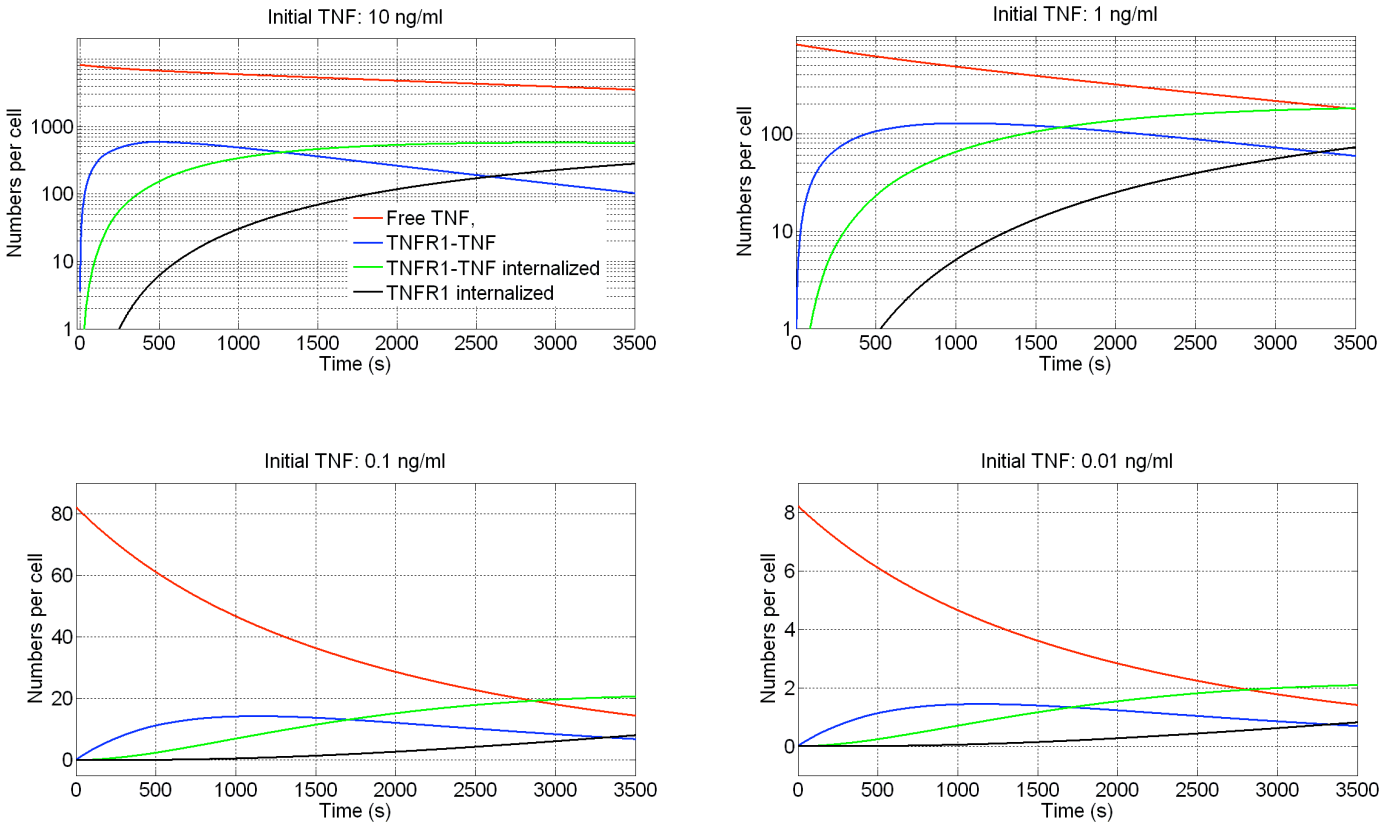

Figure S9: Calculated receptor binding dynamics in 35 the nanoliter microfluidic chamber. Protein and receptor numbers are in trimers. See Supplementary Mathematical Methods for a discussion of receptor binding calculations.

Supplementary Figure S10

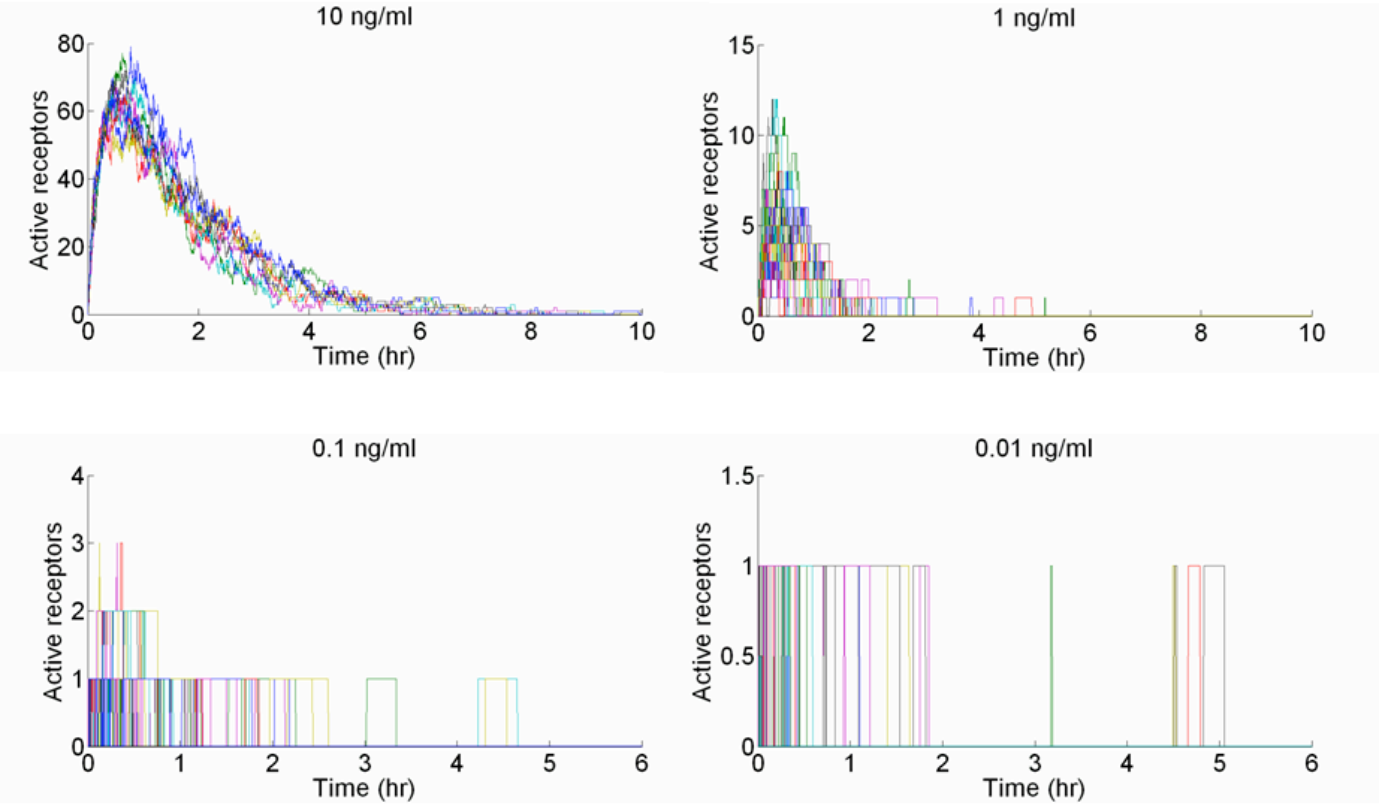

Figure S10: Receptor states calculated during simulations shown in Figure 4.

Supplementary Figure S11

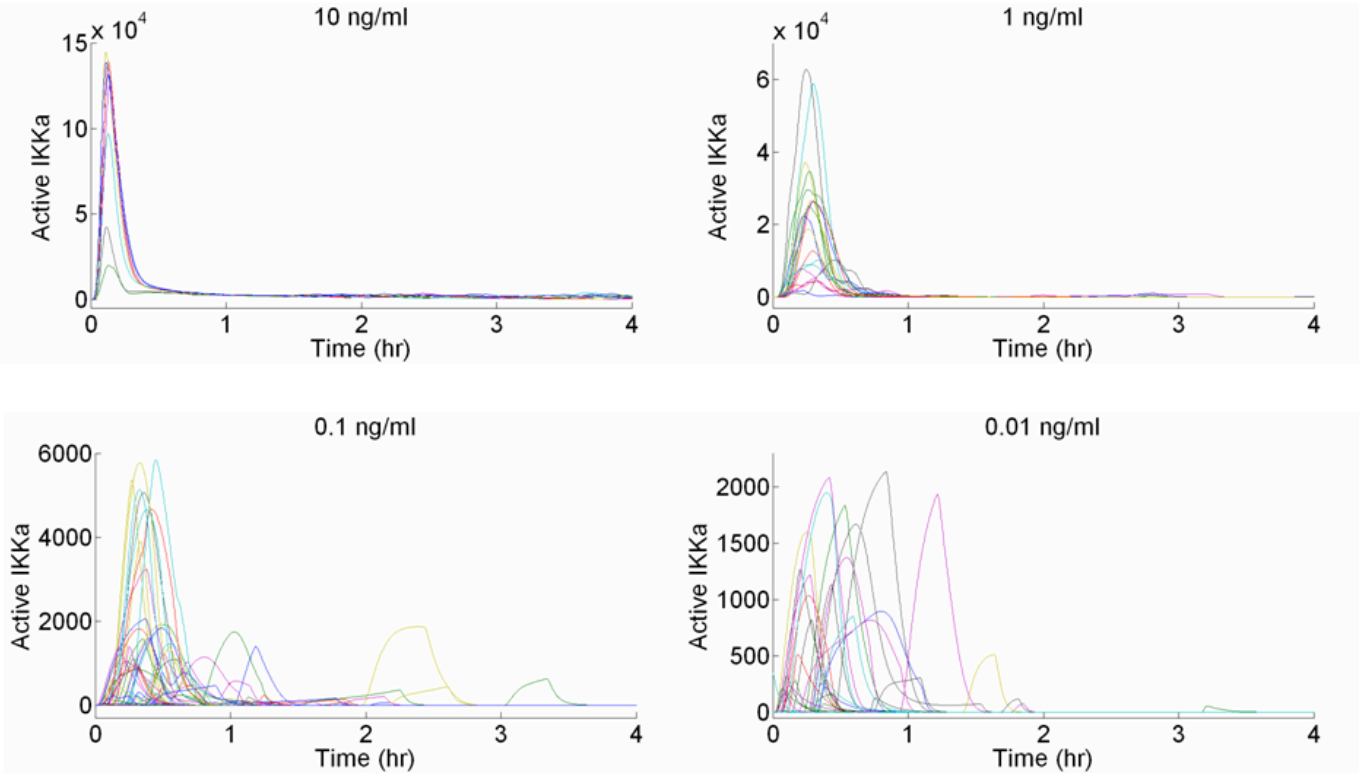

Figure S11: Number of active IKKa from simulations shown in Figure 4.

Supplementary Figure S12

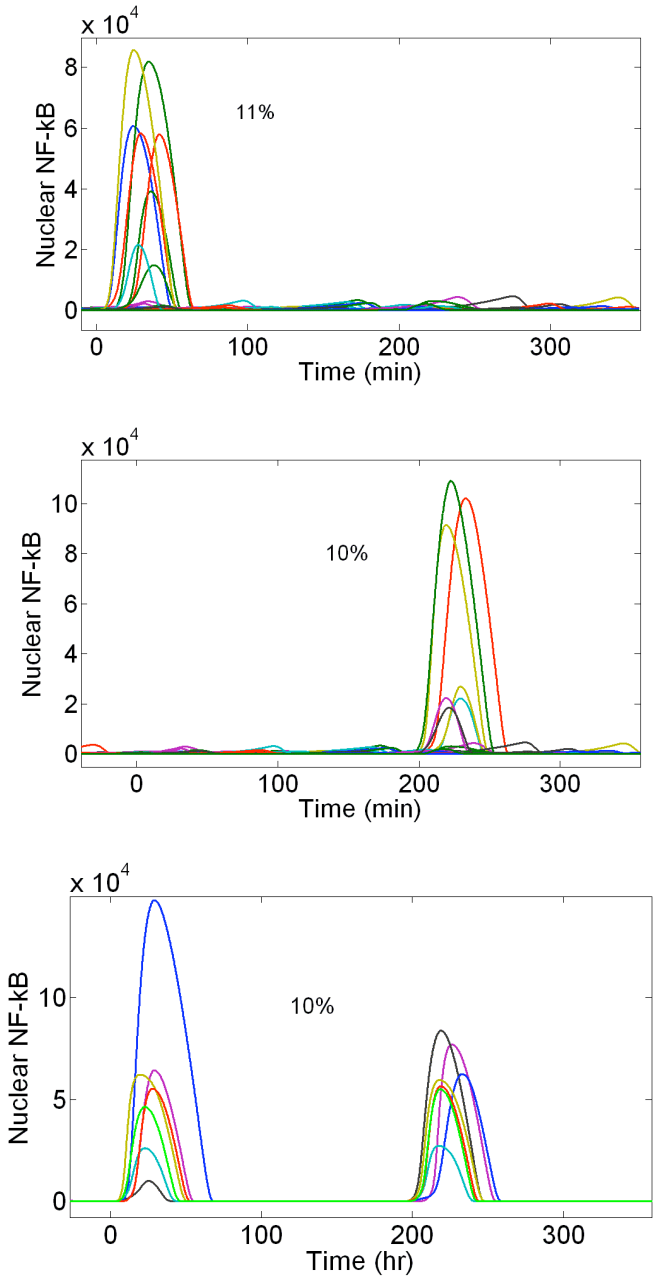

Figure S12: Simulated single cell traces for low dose short-pulsed stimulation, similar to experiments shown in Figure S2. Cells were stimulated with two consecutive 20 minute pulses of 0.1 ng/ml TNF-α. The pulses were separated by 170 minutes. 11% of the cells in the chamber respond to both pulses (top), while 10 % respond to only the first pulse (middle) and 10% respond only to the second pulse (bottom).