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Supplementary Materials for

A Noisy Paracrine Signal Determines the Cellular NF-κB Response to Lipopolysaccharide

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Published 20 October 2009, *Sci. Signal.* **2**, ra65 (2009)
DOI: 10.1126/scisignal.2000599

This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/2/93/ra65/DC1)

MATLAB files for simulations

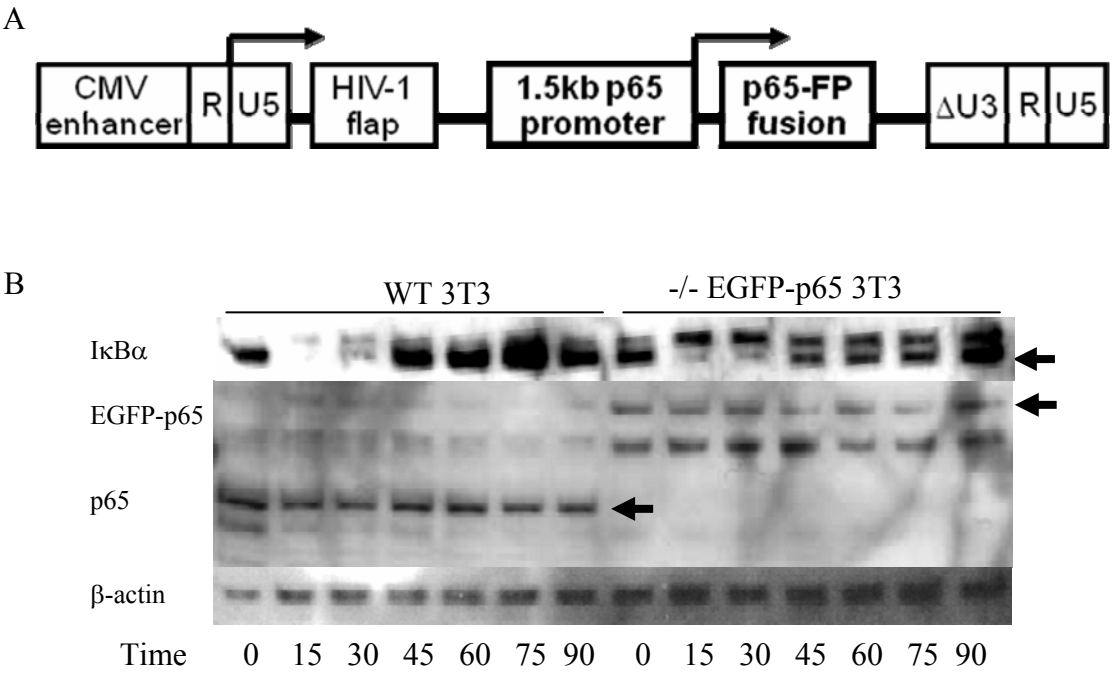

Figure S1. Lentiviral expression construct and activation of NF-κB in cells reconstituted with EGFP-p65. (A) The lentiviral construct created to enable monitoring of endogenous levels of nuclear NF-κB. (**B**) IκBα expression in the EGFP-p65 expressing *relA*$^{-/-}$ 3T3 cells stimulated with TNF-α (10ng/ml). The Western blot shows a time-course profile similar to what is observed in wild-type cells, with initial IκBα degradation and subsequent induction by NF-κB.

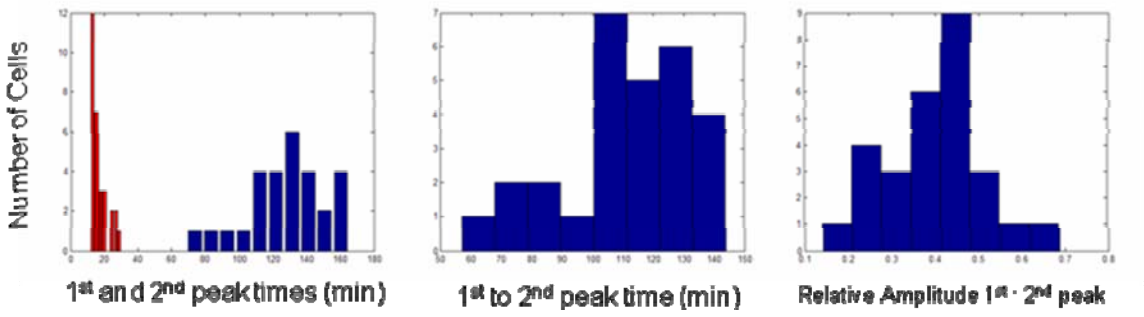

Figure S2: Metrics of variability in the TNF-α response. Peak timing of the first (red) and second peaks (blue), time between first and second peak for individual cells, and relative amplitude of the second peak compared to the first.

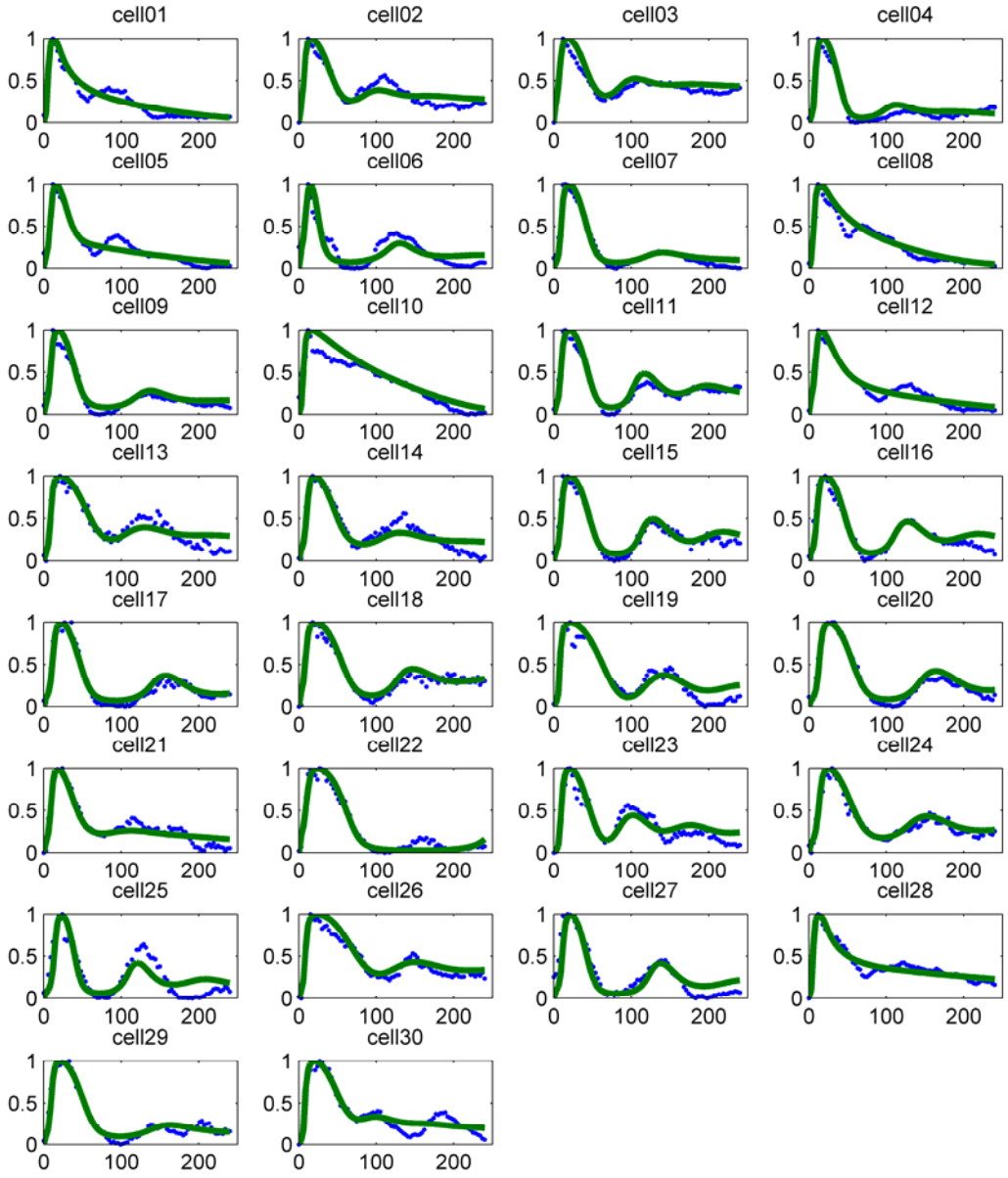

Figure S3. Parameter fitting to single-cell data. Blue points represent experimental data and the green line represents a simulation corresponding to the best fit parameters. Cells 1, 5, 8, 10, 12, 21, 28, and 30 were cells where a parameter set with a good fit could not be found.

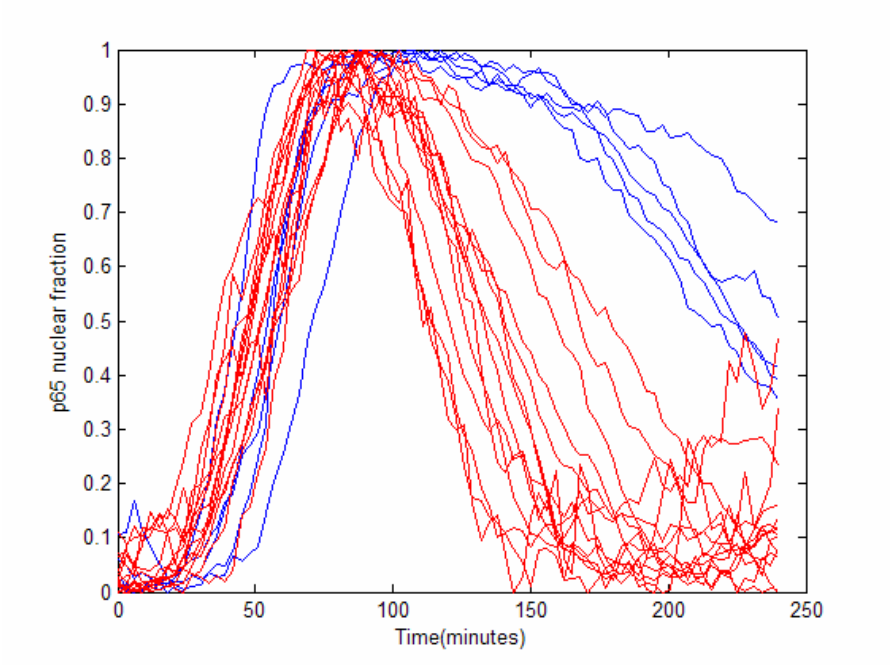

Figure S4: Clonal cells stimulated with LPS. Clonal cells were generated as described in the Materials and Methods and analyzed similar to Figure 2. Response profiles were clustered similar to Figure 2 and colored to represent the major groups. The duration of the first nuclear entry is significantly longer in both the transiently (red) and persistently activated (blue) clonal cells than we observed in our nonclonal cell experiments in Figure 2 (about 120 minutes for the transient response in clonal cells versus 100 minutes in the nonclonal population), possibly a by-product of clonal isolation.

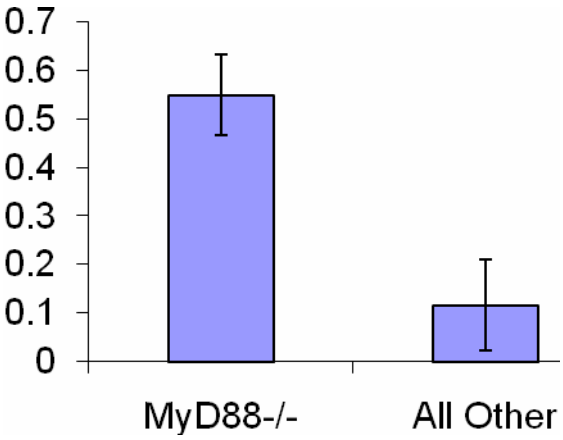

Figure S5: Cells unresponsive to LPS. Fraction of cells in which NF-κB did not localize to the nucleus in experiments with MyD88-/- MEFs (n=131) compared to p65-FP 3T3s and Trif-/- MEFs combined (n=180) in response to stimulation by LPS.

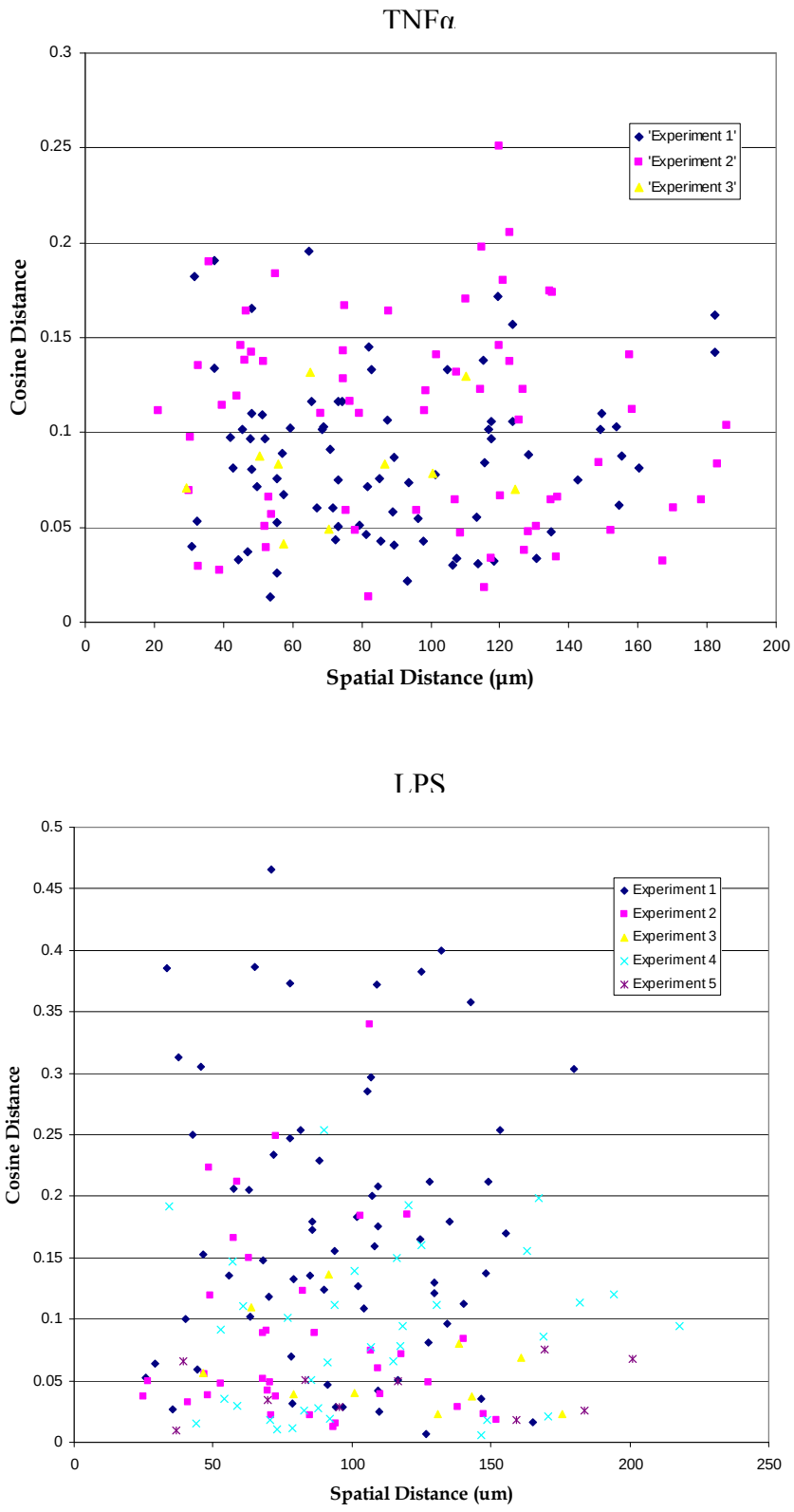

Figure S6: **Lack of correlation between NF-κB dynamics and spatial location.** Cosine distance between single-cell traces and spatial distance between nuclear centers. (**Top**) TNF-α stimulation across 3 experiments. (**Bottom**) LPS stimulation across 5 experiments.

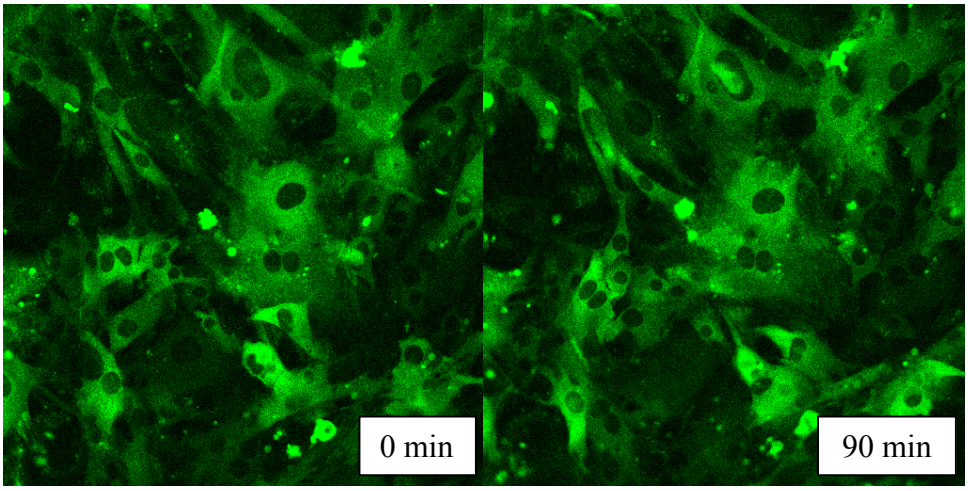

Figure S7: TLR4^{del} MEFs (EGFP-p65) treated with LPS. TLR4 deletion cells do not respond to 5µg/ml LPS (Invivogen). Scale bar corresponds to 25µm.

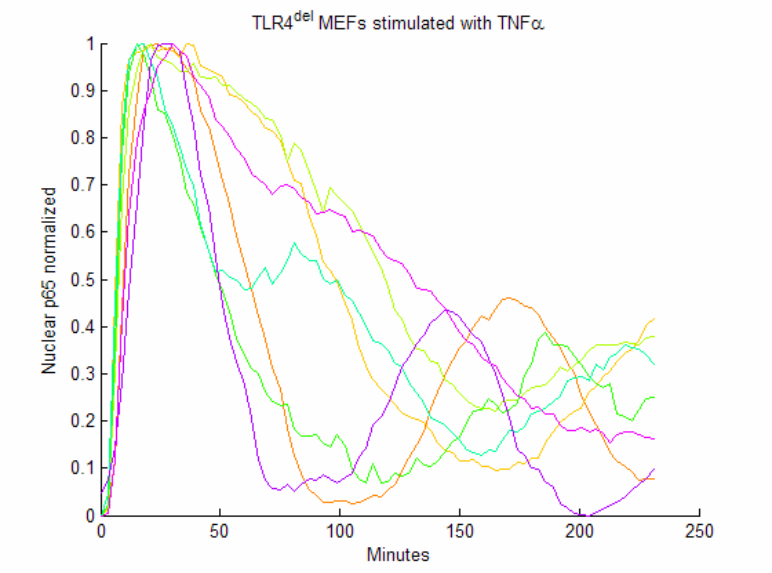

Figure S8: TLR4^{del} MEFs (EGFP-p65) treated with TNF-α. TLR4 deletion mutants treated with TNF-α and analyzed similarly to the 3T3 cell line as shown in Figure 2.

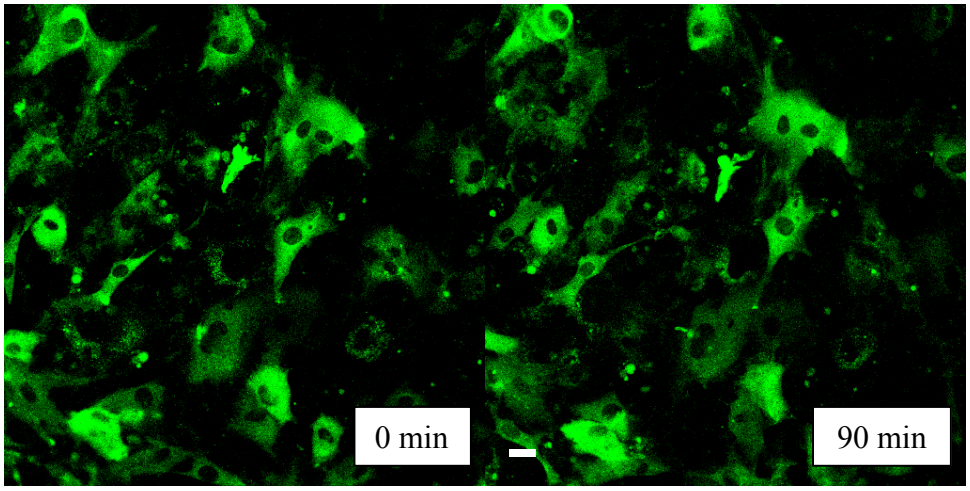

Figure S9: TLR4^{del} MEFs (EGFP-p65) cultured with wild-type MEFs and treated with LPS and soluble TNFR (sTNFRII). Using sTNFRII (8.3 μg/ml) to block TNF-α prevents TLR4 deletion mutants from responding to LPS (5 μg/ml, Invivogen) in the presence of wild-type MEFs. Out of 47 cells observed, 2 showed possible NF-κB activation. Scale bar corresponds to 25μm.

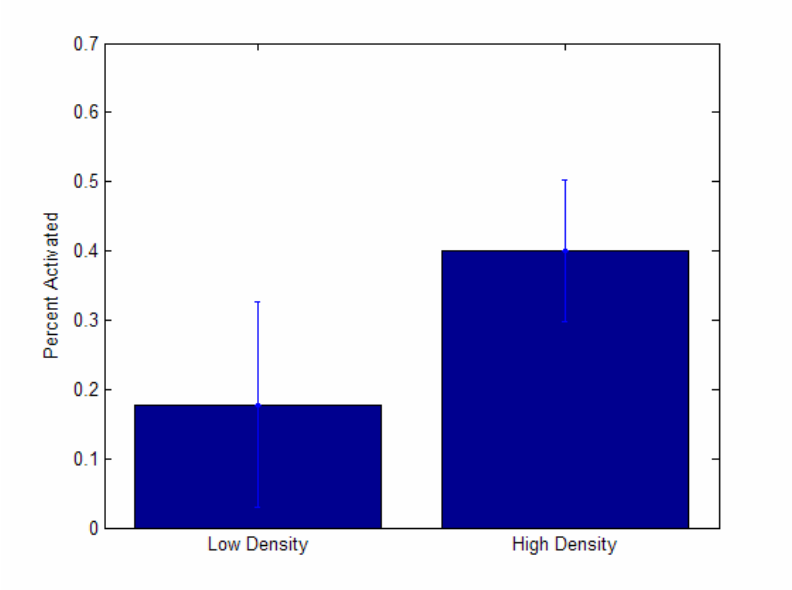

Figure S10: Coculture plating density influences the activation of NF-κB in TLR^{del} cells. TLR4^{del} responses when cultured with wild-type MEFs cultured at different densities. Plating density was increased approximately two-fold with the ratio between genotypes kept at 1:1.

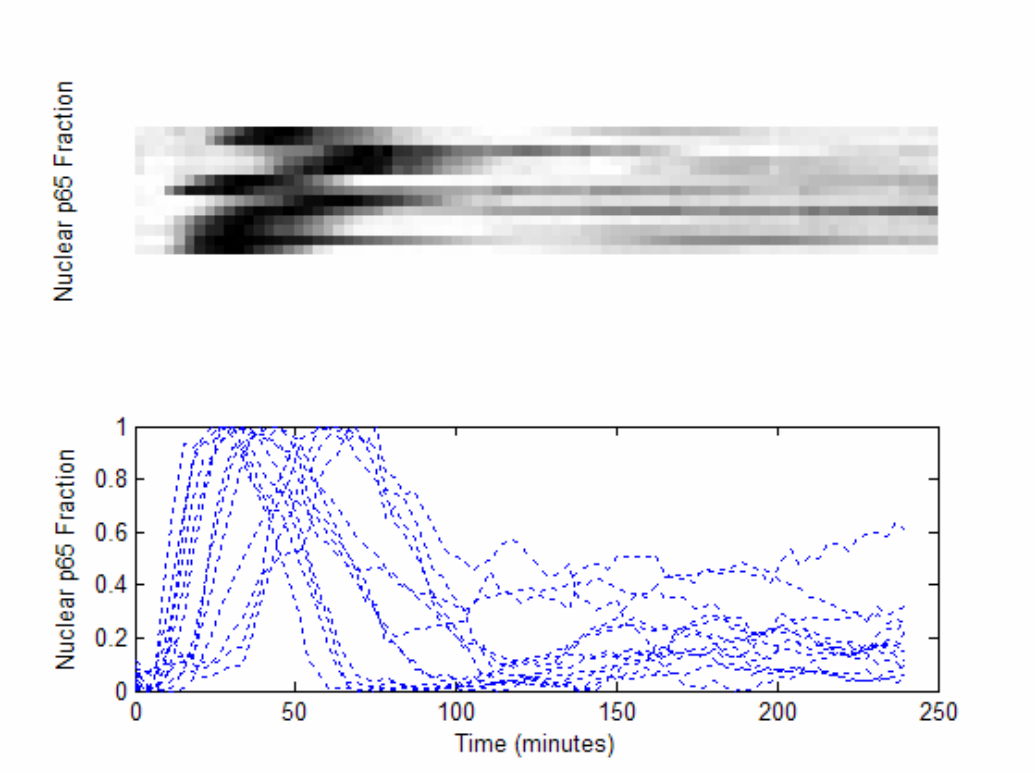

Figure S11: *Trif*$^{-/-}$ MEFs exposed to LPS. Single cell traces of *Trif*$^{-/-}$ MEFs stimulated with LPS (.5ug/mL)

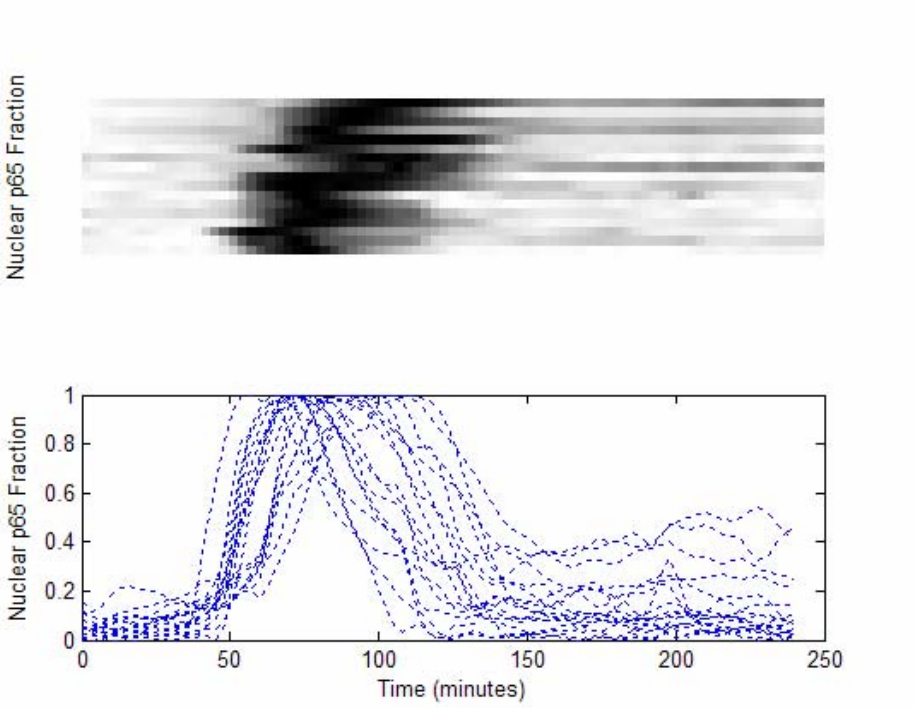

Figure S12: *MyD88*$^{-/-}$ MEFs exposed to LPS. Single cell traces of *MyD88*$^{-/-}$ MEFs stimulated with LPS (.5ug/mL)

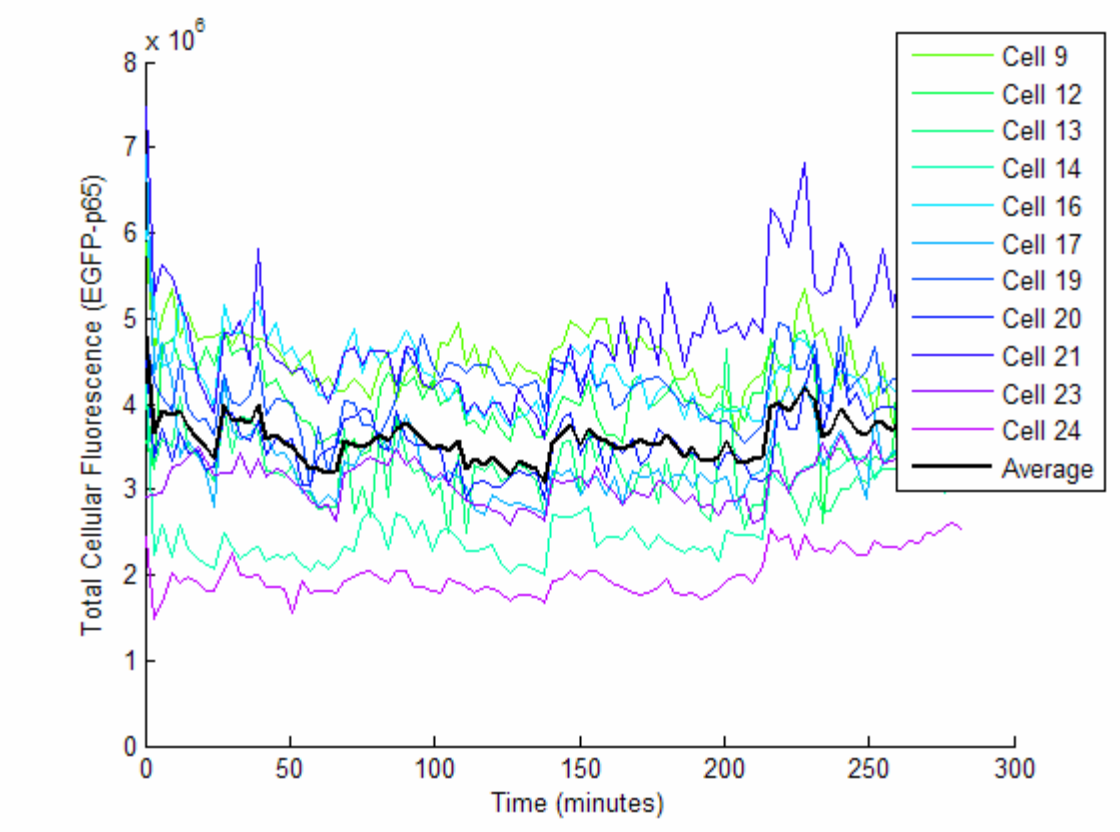

Figure S13: Total cellular fluorescence during a typical experiment. Total fluorescence from a cell was quantified as the sum of nuclear and cytoplasmic fluorescence. Nuclear and cytoplasmic regions were analyzed as described in the Materials and Methods, where cytoplasmic regions applied an additional watershed segmentation step.

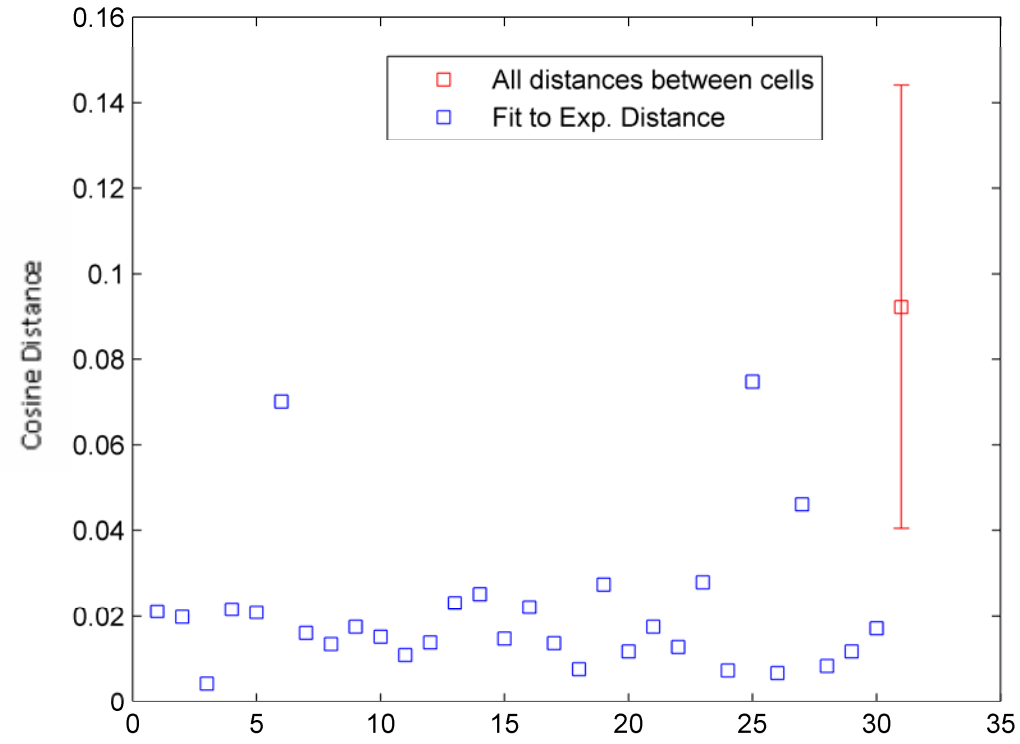

Figure S14: Single-cell fit distances compared to global variation between cells. Each single cell trace was compared to the fitted trace. The red bar shows the distribution of the pairwise cosine distance between all cells in the dataset.

		Gaussian 1			Gaussian 2		
	HL	mean	stdev	fraction	mean	stdev	fraction
IKKO	0.100	0.134	0.006	0.554	0.219	0.014	0.446
tr3	0.017	0.011	0.001	0.575	0.015	0.002	0.425
tr1	0.245	0.299	0.022	0.783	0.662	0.012	0.217
tr2	0.990	1.000	0.086	0.913	2.475	0.059	0.087
tp1	0.018	0.011	0.002	0.502	0.019	0.004	0.498
a7	11.10	15.337	1.227	0.583	25.076	2.742	0.417
r4	1.221	3.159	0.168	0.348	3.663	0.000	0.652
k02	0.0072	0.013	0.001	0.503	0.018	0.001	0.497
r1	0.244	0.428	0.082	0.531	0.724	0.003	0.469
a4	30.00	19.162	3.387	0.543	46.703	12.568	0.457
IkBa_NFkB0	0.083	0.090	0.011	0.507	0.108	0.005	0.493

Table S1. Parameter distributions from single-cell fitting. Parameters of the two Gaussians that describe the variation in single-cell fits to the HL model.