Supplemental Materials

Molecular Biology of the Cell

Gutschow et al.

SUPPLEMENTARY MATERIALS

Figure S1. Related to Figure 2. Non time-warped

Non time-warped NF-κB nuclear translocation dynamics. Single cell traces are shown in grey, and the mean traces of the aligned single cell traces are thicker and in red.

Figure S2. Related to Figure 2. Combined NF-κB activation by LPS and TNF fits a linear superposition model.

(A) Parameter determination. We constructed the linear superposition model by representing the fraction of the combined NF-κB output driven by the LPS stimulus as α, and the fraction of the combined output driven by TNF as β. We then calculated a predicted NF-κB dynamic trace for a given TNF-LPS combination as α*(LPS trace) + β*(TNF trace) for α, β = [0:0.01:1]. The α and β values that yielded the smallest cosine distance between the mathematically combined and the experimentally measured outputs occurred at a global minimum (center circle) for all stimulus combinations and concentrations.
(B) Comparing the model predictions to the population average of the experimental data. In each case, the values of α and β are also shown above the traces. The combinations of greatest interest to us were those in which the contributions of LPS and TNF were nearly equivalent. The four combinations that exhibited the greatest interactive effect were: (1) 0.05 μg/mL LPS, 1 ng/mL TNF; (2) 0.05 μg/mL LPS, 10 ng/mL TNF; (3) 0.5 μg/mL LPS, 1 ng/mL TNF; and (4) 5 μg/mL LPS, 1 ng/mL TNF. They are highlighted in purple. Combinations where the number of active cells was too low were not considered further.

Figure S3. Related to Figure 3.
(A) For each of the conditions in Figure 3A (10ng/ml TNF and 0.05 μg/mL LPS alone or in combination) features of the NF-κB single-cell traces were extracted and compared across the three conditions. Significance was calculated using a two-sided independent t-test (* $p<0.05$, ** $p<0.01$, ** $p<0.01$).
(B) For the dual stimulation condition in Figure 3A (10ng/ml TNF and 0.05 μg/mL LPS in combination) based on their position in the scatter plot cells were classified as either TNF-like (cells found on x-axis), LPS-like (cells found on y-axis), or dual responders (in the center of the plot). Features of NF-κB dynamics were extracted and compared across the three groups of cells. Due to the low number of cells

in each group data for each cell is shown along with a summary of the distribution. Significance was calculated using a two-sided independent t-test (* $p<0.05$, ** $p<0.01$, ** $p<0.01$).

Figure S4. Related to Figure 4.

A) Dual stimulation does not induce changes in gene expression for several NF-κB target genes. For each target gene, cells were stimulated with TNF (1ng/ml), LPS (5μg/ml), or both. For each condition, histograms of the square root of mRNA puncta per cell as determined by smFISH are shown. A threshold of expression was set based on the 95^{th} percentile of the unstimulated control and bars are colored green where the signal is above this threshold. Mean of the subpopulation of cells with high transcript expression (green) is shown along with the observed (green) and expected (black) percentage of cells in the population above the threshold. The expected response was calculated using $F_{LPS}+F_{TNF}-F_{LPS}*F_{TNF}$ where F_{LPS} is the fractional response to LPS and F_{TNF} is the fractional response to TNF.

(B) Increased mRNA expression is linked to increased NF-κB dynamics. For *Cxcl5*, cells in each stimulus condition were rank ordered according to the number of mRNA puncta and heatmaps of NF-κB dynamics for all cells are shown. Peaks corresponding to NF-κB nuclear localization are black (see color scale), and were scaled between 0-1.25. The threshold used in Figure 4B is indicated by the red line and was used to separate cells into those with low and high gene expression.

(C) The average NF-κB dynamics of cells with low and high gene expression is distinct. For each condition cells were separated according to the mRNA puncta threshold defined in Figure 4B and the mean NF-κB trace for each subgroup is shown. High expressing cells are indicated by the unbroken line and low expressing cells by the dotted line. Black bars and * indicate the timepoints at which differences between the two subgroups were significant using the Kolmogorov-Smirnov test with a confidence level of $p=0.01$.

Figure S5. Related to Figure 4. Expanded results from the multiplex immunoassay screen.

Data shown represent the measured raw median fluorescence intensity fold change over untreated control cells (averaged over 3 samples, each with 2 technical replicates) six hours after stimulation. Values that are significantly different from control (via Student's T-test) are highlighted in light pink ($p<0.05$) and dark pink ($p<0.01$). The calibrated control values are shown on the right. Values that measured above or below the selected calibration range were reported as either the highest or lowest measured calibration value and are highlighted in grey.

Figure S1

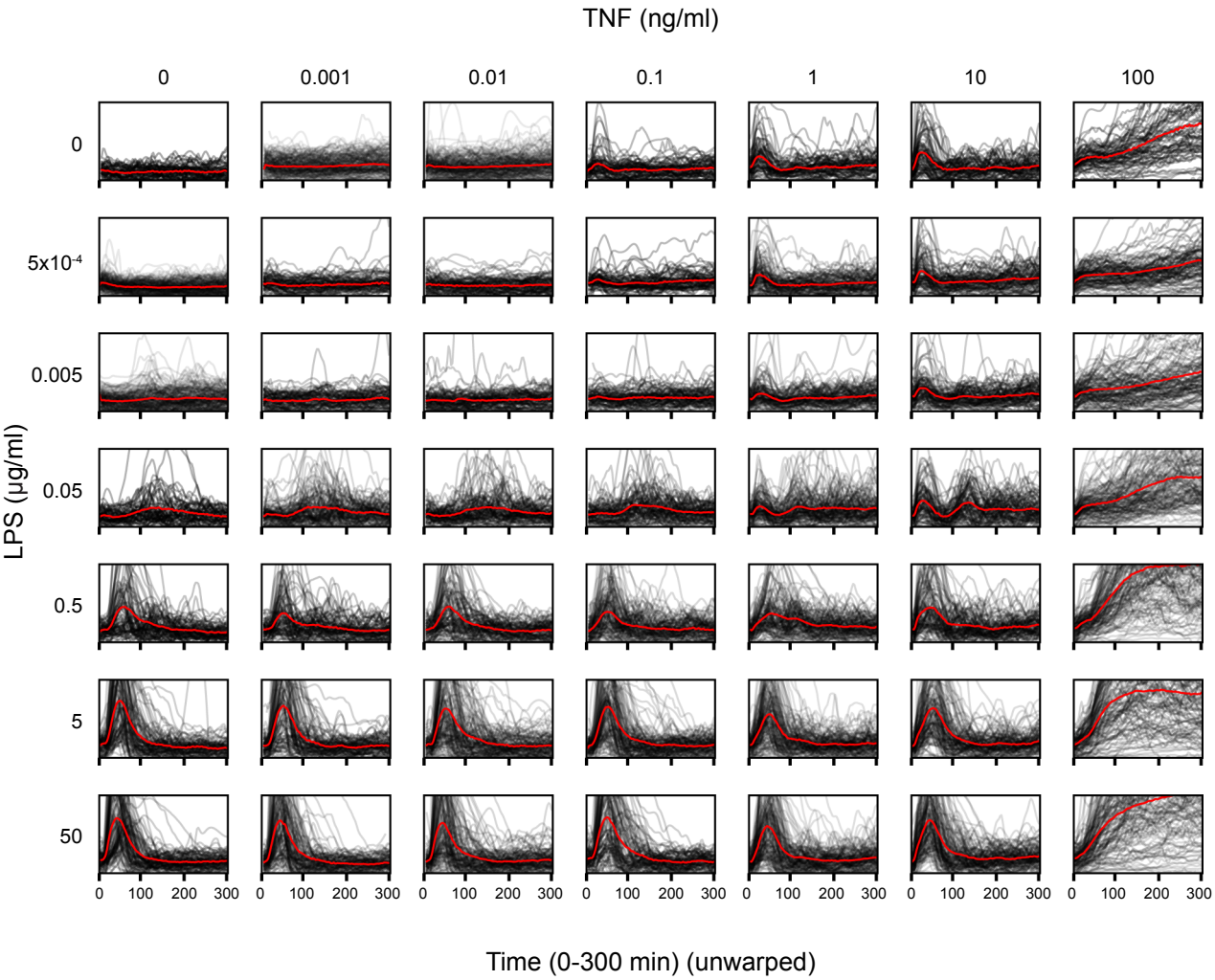
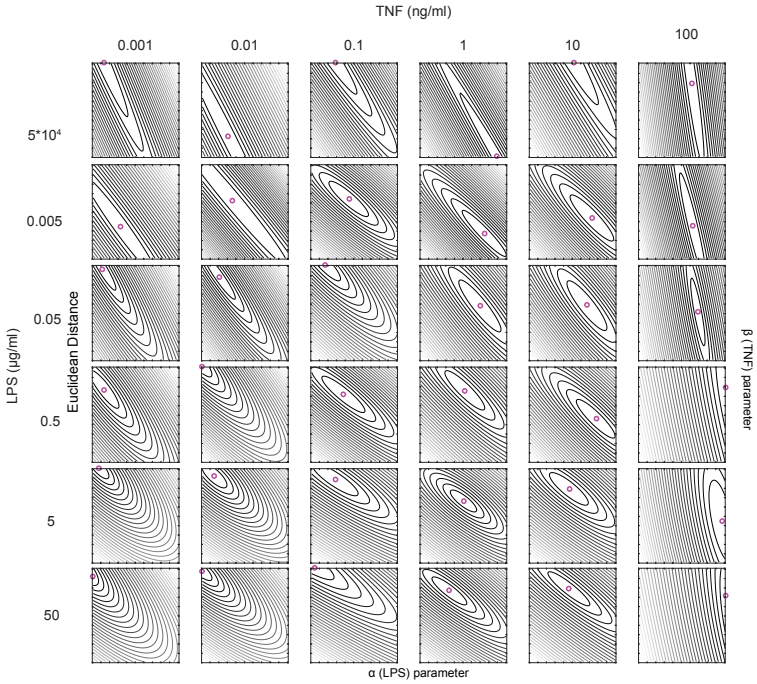

TNF (ng/ml)
0
0.001
0.01
0.1
1
10
100
LPS (µg/ml)
0
5x10-4
0.005
0.05
0.5
5
50
0
100
200
300
Time (0-300 min) (unwarped)

Figure S2

A


TNF (ng/ml)
0.001
0.01
0.1
1
10
100
5*10⁴
0.005
0.05
0.5
5
50
LPS (µg/ml)
Euclidean Distance
β (TNF) parameter
α (LPS) parameter

B

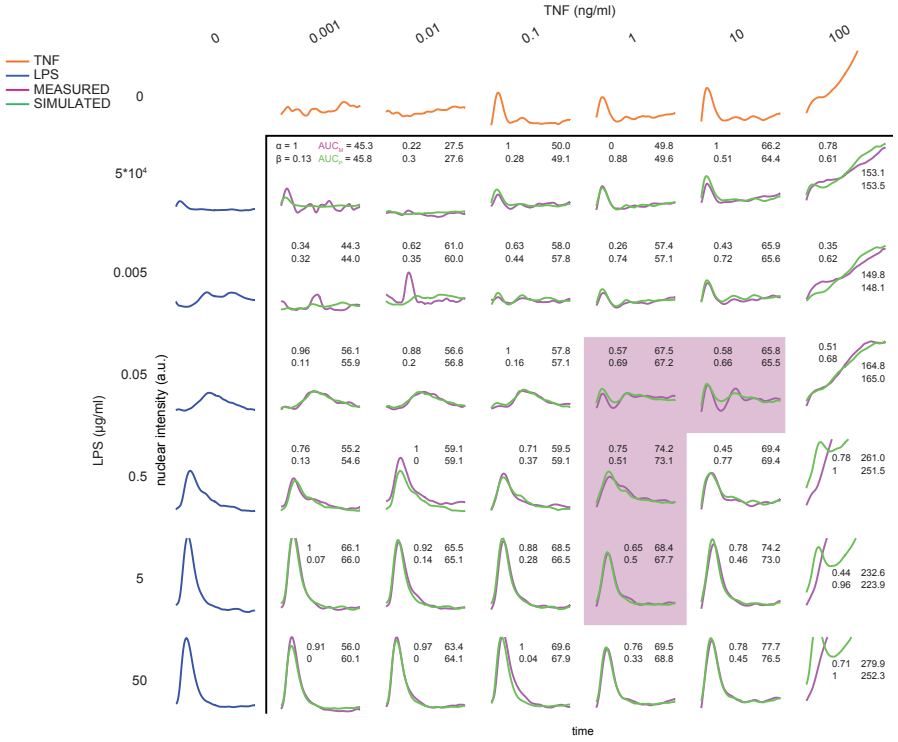

TNF
LPS
MEASURED
SIMULATED
TNF (ng/ml)
0
0.001
0.01
0.1
1
10
100
5*10⁴
0.005
0.05
0.5
5
50
LPS (µg/ml)
nuclear intensity (a.u.)
time

Figure S3

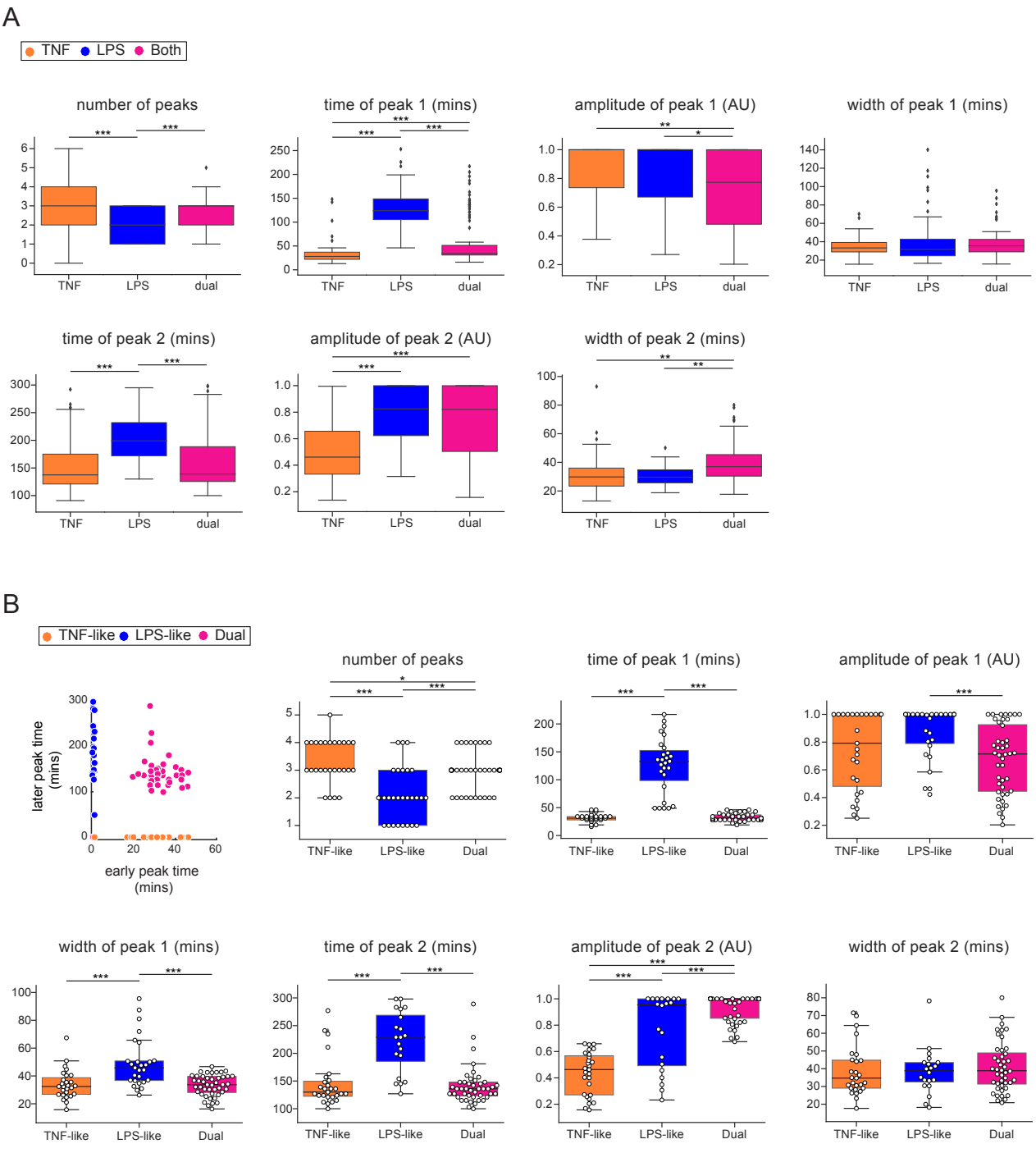

A
TNF
LPS
Both
number of peaks
time of peak 1 (mins)
amplitude of peak 1 (AU)
width of peak 1 (mins)
time of peak 2 (mins)
amplitude of peak 2 (AU)
width of peak 2 (mins)
TNF
LPS
dual
B
TNF-like
LPS-like
Dual
later peak time (mins)
early peak time (mins)
number of peaks
time of peak 1 (mins)
amplitude of peak 1 (AU)
width of peak 1 (mins)
time of peak 2 (mins)
amplitude of peak 2 (AU)
width of peak 2 (mins)

Figure S4

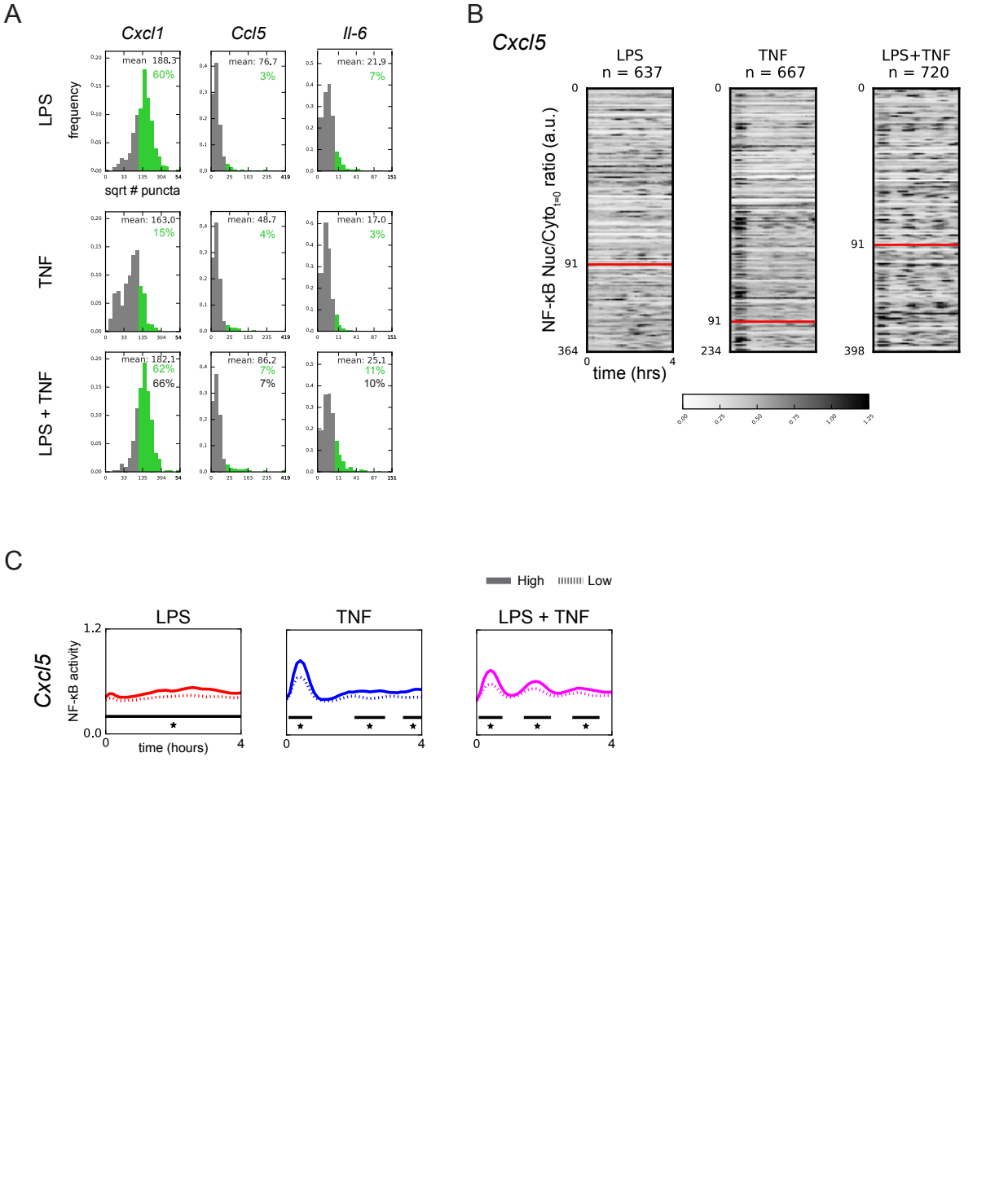

A
Cxcl1
Ccl5
Il-6
LPS
TNF
LPS + TNF
frequency
sqrt # puncta
mean: 188.3
60%
mean: 76.7
3%
mean: 21.9
7%
mean: 163.0
15%
mean: 48.7
4%
mean: 17.0
3%
mean: 182.1
62%
66%
mean: 86.2
7%
7%
mean: 25.1
11%
10%
B
Cxcl5
LPS
n = 637
TNF
n = 667
LPS+TNF
n = 720
NF-κB Nuc/Cyto$_{t=0}$ ratio (a.u.)
time (hrs)
C
Cxcl5
High
Low
LPS
TNF
LPS + TNF
NF-κB activity
time (hours)

Figure S5

	FOLD CHANGE (MEDIAN FLUORESCENCE INTENSITY) (6 HOURS)									CALIBRATED VALUES		
	(ng/ml)			(µg/ml)						(pg/ml)		
	TNF 1	TNF 10	LPS 0.05	LPS 0.5	LPS 5	TNF 10 LPS 0.05	TNF 1 LPS 0.05	TNF 1 LPS 0.5	TNF 1 LPS 5	CTRL	MIN	MAX
TNF / TNFA	365.82	531.79	1.29	1.43	1.34	529.37	356.36	366.53	371.15	0.29	0.29	2585.05
IP10 / CXCL10	24.47	34.34	6.61	15.18	17.55	45.82	37.54	44.37	37.40	3.79	3.79	232.58
RANTES / CCL5	21.91	29.52	4.54	19.78	24.00	33.89	30.27	34.99	34.18	19.87	19.87	702.10
IL6	5.73	9.76	3.33	8.05	9.07	22.48	13.41	28.52	23.25	5.83	5.83	266.73
LIX / CXCL5	2.50	2.27	5.76	11.97	11.22	13.21	13.04	21.89	20.07	18.84	18.84	356.03
MIP2 / CXCL2	2.26	3.50	1.54	2.20	3.34	4.04	3.05	7.58	9.77	2.09	2.09	51.40
GMCSF / CSF2	1.62	2.10	1.01	1.14	2.15	1.83	1.44	3.14	5.80	0.28	0.28	7.60
LIF	2.41	3.23	1.53	2.71	3.59	3.39	2.79	4.76	4.94	1.72	1.72	17.55
KC / CXCL1 / GROA	2.27	2.27	2.46	2.95	2.93	3.05	3.00	3.10	3.22	956.05	956.05	311778.35
CCL3 / MIP1A	2.07	2.69	1.37	2.00	2.11	2.94	2.56	3.45	3.13	0.02	0.02	3.35
MCSF / CSF1	2.15	2.77	1.43	1.73	1.95	2.69	2.24	2.74	2.57	0.08	0.08	0.85
CCL4 / MIP1B	1.80	1.99	1.38	1.76	1.91	2.12	1.91	2.12	2.17	0.44	0.44	0.90
GCSF / CSF3	1.14	1.17	1.09	1.48	1.76	1.53	1.25	2.35	2.71	0.16	0.16	2.41
IL18	1.64	1.83	1.12	1.31	1.40	1.78	1.66	1.93	1.75	1.58	1.58	24.91
TGFB	1.50	1.42	1.50	1.58	1.59	1.62	1.63	1.66	1.60	1.11	1.11	5.88
IL1B	1.35	1.36	1.06	1.32	1.26	1.61	1.40	1.49	1.45	0.03	0.03	0.03
CCL2 / MCP1	1.32	1.43	1.23	1.27	1.30	1.45	1.41	1.46	1.46	1431.40	1431.40	3029.63
IL5	1.36	1.54	1.09	1.14	1.33	1.45	1.21	1.41	1.38	0.92	0.92	2.53
IL1A	1.26	1.34	1.09	1.19	1.21	1.14	1.07	1.20	1.27	0.38	0.38	1.95
IL4	1.22	1.24	1.03	1.14	1.09	1.20	1.16	1.32	1.22	0.27	0.27	0.38
IL17A	1.17	1.25	1.02	1.13	1.09	1.22	1.11	1.19	1.15	0.06	0.06	0.24
IL12P70	1.03	1.26	0.96	1.06	1.02	1.21	1.07	1.24	1.20	0.49	0.48	0.54
IFNG	1.15	1.28	1.01	1.04	1.02	1.09	1.03	1.12	1.05	0.54	0.54	0.62
IL13	1.17	1.16	1.04	1.07	1.09	1.10	1.05	1.12	1.10	1.95	1.95	1.95
IL27	1.19	1.17	1.07	1.09	1.11	1.14	1.13	1.11	1.10	0.05	0.05	0.10
IL22	1.20	1.15	0.98	0.99	1.16	1.18	1.11	1.13	1.16	0.07	0.07	0.27
IL15/IL15R	1.09	1.16	1.01	1.00	1.09	1.07	1.00	1.11	1.14	2.15	2.15	2.15
IL31	1.13	1.13	1.04	1.06	1.03	1.01	0.98	1.10	1.05	5.00	5.00	5.00
CCL7 / MCP3	1.04	1.06	1.06	1.02	1.03	1.05	1.04	1.04	1.04	301.78	301.78	334.08
IL9	1.06	1.17	0.95	1.01	1.01	1.02	1.00	1.04	0.95	9.48	9.46	9.53
IL3	1.09	1.17	1.00	1.08	0.91	1.06	0.97	0.99	0.99	0.09	0.07	0.16
IL2	1.04	1.06	0.97	1.09	0.99	1.00	0.94	1.02	1.02	1.16	1.16	1.16
IL10	1.06	1.09	1.00	0.99	0.97	0.97	0.91	0.98	1.01	3.39	3.39	3.39
IL23	1.15	1.08	1.04	1.06	1.02	0.91	0.98	0.93	0.95	0.38	0.08	1.27
VEGF	1.04	0.94	0.90	0.99	0.92	0.99	0.96	0.88	0.94	0.05	0.05	0.05
IFNA	0.99	0.97	0.93	0.95	0.92	0.86	0.86	0.89	0.91	4.27	4.27	4.27
IL28	0.95	1.03	0.88	0.92	0.90	0.85	0.89	0.95	0.94	37.32	37.32	37.32
CCL11 / EOTAXIN	0.90	0.84	1.05	0.81	0.89	0.89	0.92	0.94	0.97	2.42	1.80	2.60

p < 0.05
p < 0.01

* Measured MFI outside calibration curve