

**Biophysical Journal, Volume 116**

**Supplemental Information**

**Fold-Change Detection of NF- $\kappa$ B at Target Genes with Different Transcript Outputs**

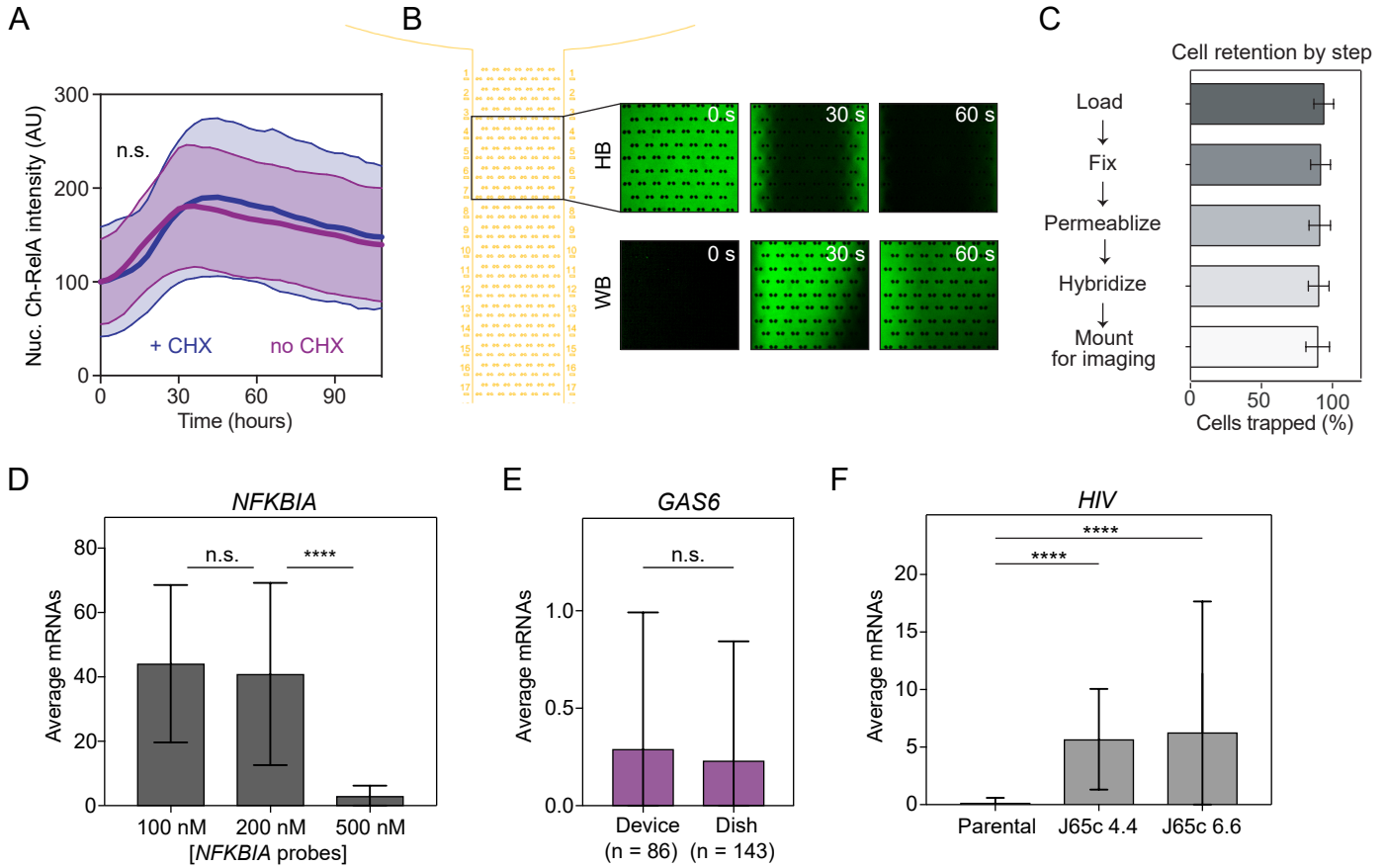
**Victor C. Wong, Shubin Mathew, Ramesh Ramji, Suzanne Gaudet, and Kathryn Miller-Jensen**

**Table S1. List of reactions and parameters in the D2FC model of NF- $\kappa$ B-induced transcription.** For transcriptional reactions:  $h$  = hill function exponent,  $kl$  is the activation coefficient and  $kr$  is the repressive coefficient such that  $1/kl$  is the affinity of NF- $\kappa$ B for the promoter and  $1/kr$  is the affinity of competitor for the promoter. Parameters for which the value was scanned in a figure panel are indicated. Throughout, “NF $\kappa$ B” refers to a RelA-containing NF- $\kappa$ B dimer.

Phenomena and pertinent reactions	Parameter	Parameter value(s)	Source
<b>TNF stimulation</b>			
TNF	$TR$	1/0 = on/off	(1)
TNF + IKK $\alpha$ $\rightarrow$ IKK $\alpha$ (active) + TNF	$ka$	Sampled, dependent on total NF- $\kappa$ B	(2)
<b>Complex formation &amp; dissociation</b>			
Total NF $\kappa$ B	$NF$	Sampled, between 0.04 - 0.4 $\mu$ M	(2)
I $\kappa$ B $\alpha$ + NF $\kappa$ B $\rightarrow$ I $\kappa$ B:NF $\kappa$ B	$ka1a$	0.5 ( $\mu$ M*s) <sup>-1</sup>	(1, 3)
nI $\kappa$ B $\alpha$ + nNF $\kappa$ B $\rightarrow$ nI $\kappa$ B:NF $\kappa$ B	$ka1a$	0.5 ( $\mu$ M*s) <sup>-1</sup>	(1, 3)
I $\kappa$ B $\alpha$ :NF $\kappa$ B $\rightarrow$ I $\kappa$ B $\alpha$ + NF $\kappa$ B	$kd1a$	0.05 s <sup>-1</sup>	(2)
nI $\kappa$ B $\alpha$ :NF $\kappa$ B $\rightarrow$ nI $\kappa$ B $\alpha$ + nNF $\kappa$ B	$kd1a$	0.05 s <sup>-1</sup>	(2)
<b>IKK parameters</b>			
TNF + IKK $\alpha$ $\rightarrow$ IKK $\alpha$ (active) + TNF	$ka$	Sampled, dependent on total NF $\kappa$ B	(2)
IKK $\alpha$ $\rightarrow$ IKK $\alpha$ i	$ki$	0.003 s <sup>-1</sup>	(1)
IKK $\alpha$ i $\rightarrow$ IKK $\alpha$ N	$kp$	0.0006 s <sup>-1</sup>	(1)
* IKK $\alpha$ i $\rightarrow$ IKK $\alpha$ N is sensitive to A20 inhibition rate constant	$kbA20$	0.0018 $\mu$ M	(1)
<b>IKK interactions</b>			
IKK $\alpha$ + I $\kappa$ B $\alpha$ $\rightarrow$ pI $\kappa$ B $\alpha$	$kc1a$	0.074 s <sup>-1</sup>	(1)
IKK $\alpha$ + I $\kappa$ B $\alpha$ :NF $\kappa$ B $\rightarrow$ pI $\kappa$ B $\alpha$ :NF $\kappa$ B	$kc2a$	0.370 s <sup>-1</sup>	(1)
pI $\kappa$ B $\alpha$ $\rightarrow$ degradation	$kt1a$	0.1 s <sup>-1</sup>	(1)
pI $\kappa$ B $\alpha$ :NF $\kappa$ B $\rightarrow$ degradation + NF $\kappa$ B	$kt2a$	0.1 s <sup>-1</sup>	(1)
<b>Transport</b>			
NF $\kappa$ B $\rightarrow$ nNF $\kappa$ B	$ki1$	0.0026 s <sup>-1</sup>	(1)
nNF $\kappa$ B $\rightarrow$ NF $\kappa$ B	$ke1$	0.000052 s <sup>-1</sup>	(1)
nI $\kappa$ B:NF $\kappa$ B $\rightarrow$ I $\kappa$ B:NF $\kappa$ B	$ke2a$	0.01 s <sup>-1</sup>	(1)
I $\kappa$ B $\rightarrow$ nI $\kappa$ B	$ki3a$	0.00067 s <sup>-1</sup>	(1)
nI $\kappa$ B $\rightarrow$ I $\kappa$ B	$ke3a$	0.000335 s <sup>-1</sup>	(1)
<b>I<math>\kappa</math>B protein synthesis and degradation</b>			
nNF $\kappa$ B $\rightarrow$ nNF $\kappa$ B + tI $\kappa$ B $h = 2, kl = k^h, kr = 0; with k = 0.065$	$c1a$	1.4x10 <sup>-7</sup> ( $\mu$ M*s) <sup>-1</sup>	(1)
tI $\kappa$ B $\rightarrow$ tI $\kappa$ B + I $\kappa$ B	$c2a$	0.5 s <sup>-1</sup>	(1)
tI $\kappa$ B $\rightarrow$ Degradation	$c3a$	0.0003 s <sup>-1</sup>	(1)
I $\kappa$ B $\rightarrow$ Degradation	$c4a$	0.0005 s <sup>-1</sup>	(1)
I $\kappa$ B:NF $\kappa$ B $\rightarrow$ NF $\kappa$ B	$c5a$	0.000022 s <sup>-1</sup>	(1)
nI $\kappa$ B:NF $\kappa$ B $\rightarrow$ nNF $\kappa$ B	-	0 s <sup>-1</sup>	(1)
<b>A20 protein synthesis and degradation</b>			
nNF $\kappa$ B + Competitor $\rightarrow$ nNF $\kappa$ B + Competitor + tA20 $h = 3, kl = k^h, kr = k2^h; with k = 0.065, k2 = 0.065$	$c1$	2x10 <sup>-7</sup> ( $\mu$ M*s) <sup>-1</sup>	(2)
tA20 $\rightarrow$ tA20 + A20	$c2$	0.5 s <sup>-1</sup>	(1)
tA20 $\rightarrow$ Degradation	$c3$	0.0004 s <sup>-1</sup>	(2)
A20 $\rightarrow$ Degradation	$c4$	0.0045 s <sup>-1</sup>	(1)
<b>Prototypical inducible target transcript</b>			
nNF $\kappa$ B + Competitor $\rightarrow$ nNF $\kappa$ B + Competitor + tIndTarget $h = 3, kl = k^h, kr = k3^h; with k = 0.065, k3 = 0.0325$ or scanned	$c1t$	2x10 <sup>-7</sup> ( $\mu$ M*s) <sup>-1</sup> or scanned	(2)
tIndTarget $\rightarrow$ Degradation	$c3t$	0.0004 s <sup>-1</sup> or scanned	(2)
<b>Competitor protein synthesis and degradation</b>			
nNF $\kappa$ B + Competitor $\rightarrow$ nNF $\kappa$ B + Competitor + tCompetitor $h = 3, kl = k^h, kr = k4^h; with k = 0.065, k4 = 0.065$ or scanned	$c1a$	1.4x10 <sup>-7</sup> ( $\mu$ M*s) <sup>-1</sup>	(2)
tCompetitor $\rightarrow$ tCompetitor + Competitor	$c2a$	0.5 s <sup>-1</sup>	(2)
tCompetitor $\rightarrow$ Degradation	$c6a$	0.00004 s <sup>-1</sup>	(2)
Competitor $\rightarrow$ Degradation	$c4a$	0.0005 s <sup>-1</sup>	(2)

#### Sources

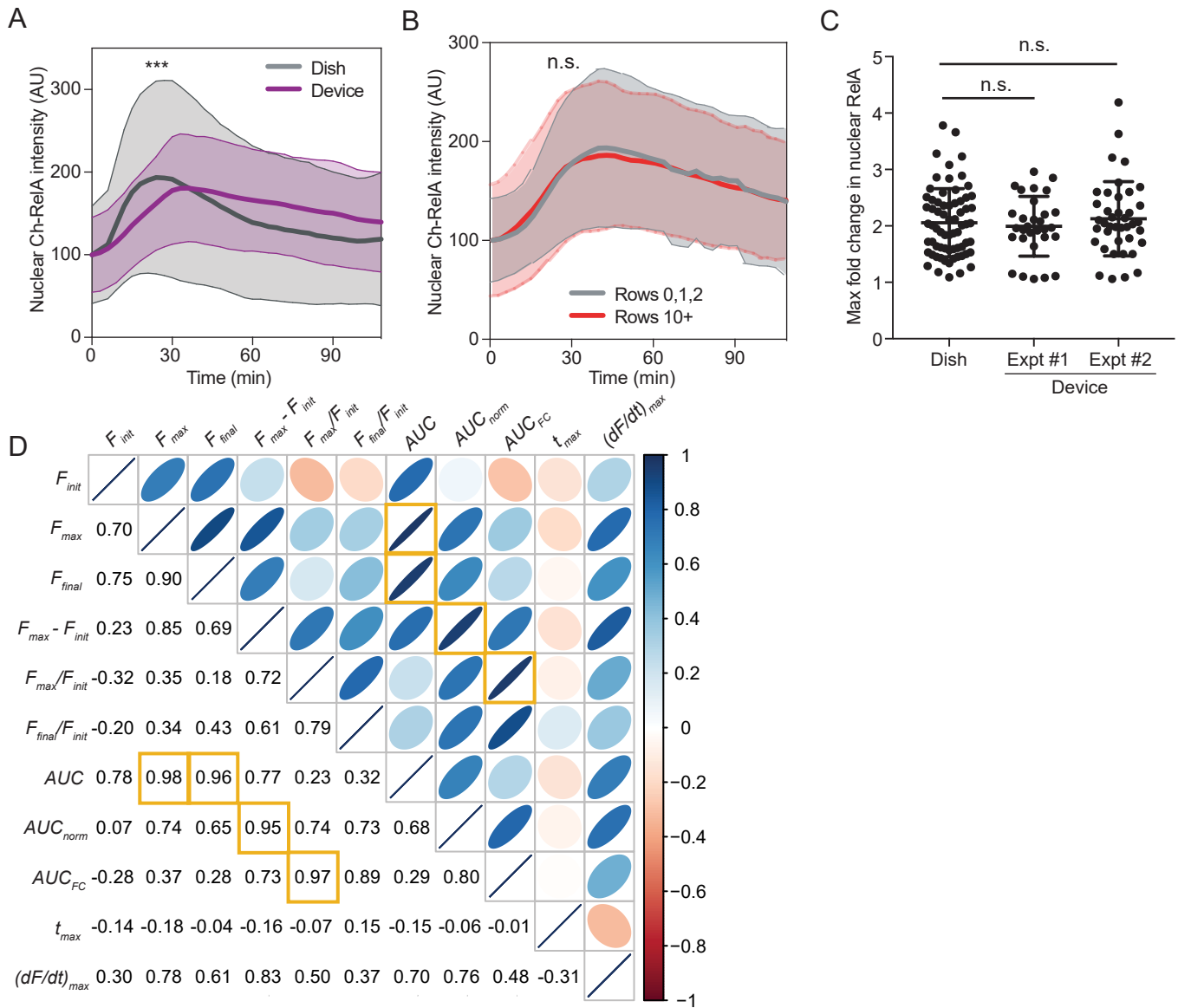
1. L. Ashall *et al.*, Pulsatile stimulation determines timing and specificity of NF-kappaB-dependent transcription. *Science* **324**, 242-246 (2009).
2. R. E. Lee, S. R. Walker, K. Savery, D. A. Frank, S. Gaudet, Fold change of nuclear NF-kappaB determines TNF-induced transcription in single cells. *Mol Cell* **53**, 867-879 (2014).
3. A. Hoffmann, A. Levchenko, M. L. Scott, D. Baltimore, The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. *Science* **298**, 1241-1245 (2002).



**Figure S1 (related to Methods). Optimization of imaging and single-molecule RNA FISH in passive-flow device.** (A) Time courses of nuclear Ch-RelA in J65c cells infected with latent HIV 6.6 collected in the passive-flow device following stimulation with 20 ng/mL TNF with 160 ng/mL CHX (*blue*) and without CHX (*purple*). Data presented as the mean  $\pm$  standard deviation (SD; *shaded*) of individual cell traces (n = 30, no CHX; n = 44, + CHX). No changes in cell viability were observed. Lack of statistical significance of differences in dynamics was determined by comparing distributions of  $t_{max}$  and  $t_{duration}$  of the nuclear RelA intensity peak (n.s.,  $p > 0.05$  by Kolmogorov-Smirnov, or K-S, test). (B) Time-lapses images taken near the outlet of the passive-flow device during buffer exchanges. Buffer containing BSA conjugated with Alexa Fluor 488 is displaced from the passive-flow device channel by hybridization buffer (HB, *top*) within 60 seconds of adding hybridization buffer to the inlet. Similarly, the hybridization buffer is displaced from the channel by wash buffer (WB, *bottom*) containing BSA conjugated with Alexa Fluor 488 within 60 seconds of adding the wash buffer to the inlet. (C) Bar graph quantifying cell retention in the flow device after each step in the smFISH protocol. Fraction of cells remaining trapped after each step was determined by counting the number of trapped cells and dividing it by the total number of traps per device. Data are presented as the mean  $\pm$  SD for three devices. (D) Bar graph of mean *NFKBIA* transcripts for cells stimulated with 20 ng/mL TNF for 1 hour in the device and labelled with increasing *NFKBIA*-specific probes concentrations. Data are presented as the mean  $\pm$  SD of mRNA molecules per cell. Cell numbers: n = 67, 100 nM; n = 62, 200 nM; n = 61, 500 nM. (E) Bar graph of mean *GAS6* transcripts for basal cells in a tissue culture dish and in the passive-flow device. Data are presented as the mean  $\pm$  SD of mRNA molecules per cell. Cell numbers: n = 143, dish; n = 86, device. (F) Bar graph of mean HIV transcripts for HIV-infected cells and parental Jurkat cells (negative control) stimulated with 20 ng/mL TNF for 2 hours. Data are presented as the mean  $\pm$  SD of mRNA molecules per cell. Cell numbers: n = 74, parental; n = 227, J65c 4.4; n = 211, J65c 6.6. Statistics in (D-F) n.s. = not significant, \*\*\*\* $p < 0.0001$ , as determined by K-S, test.

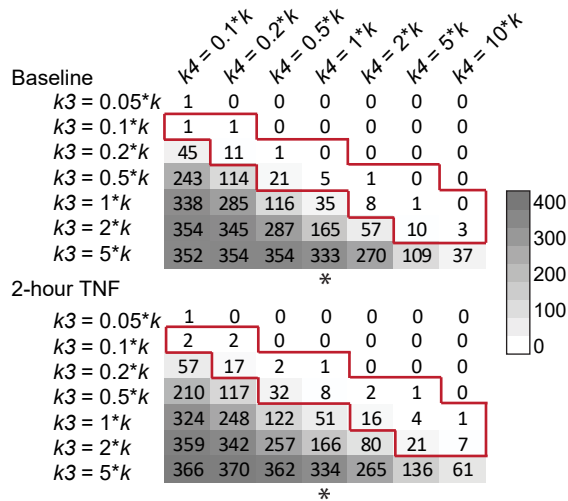




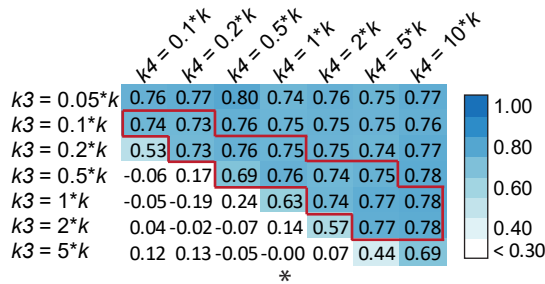


**Figure S3 (related to Fig. 2). Nuclear Ch-RelA translocation dynamics show subtle differences between dish and device but max fold change in nuclear Ch-RelA does not.** (A) Measured time courses of nuclear Ch-RelA from J65c cells collected in the passive-flow device (purple) versus in a tissue culture dish (gray) after 20 ng/mL TNF treatment. Data presented as the mean  $\pm$  SD of individual cell time courses ( $n = 30$ , device;  $n = 68$ , dish). (B) Measured time courses of nuclear Ch-RelA collected in the passive-flow device after 20 ng/mL TNF treatment for cells positioned in the rows 0-2 by the inlet (gray) versus rows 10+ closer to the outlet. Data presented as the mean  $\pm$  SD of individual cell time courses combined from several experiments ( $n = 40$ , gray;  $n = 26$ , red). In A-B, significant differences in dynamics were evaluated by comparing distributions of  $t_{max}$  and  $t_{duration}$  of the nuclear RelA intensity peak between the two data sets (\*\*\*,  $p < 0.001$ ; n.s.,  $p > 0.05$  by K-S test; see Methods for more details). (C) One-dimensional scatter plots of the maximum fold change in nuclear Ch-RelA in individual cells for J65c cells in a tissue culture dish (left) or in the passive-flow device (center, right). Each dot represents an individual cell (Dish,  $n = 68$ , Device, Expt #1  $n = 30$  and Expt #2  $n = 39$ ); bars show the mean  $\pm$  SD. The distributions are not significantly different (n.s.,  $p > 0.05$  by K-S test). (D) Matrix of correlations between RelA signaling features extracted from the time-course data.  $AUC_{norm}$  is the area under the curve for the time course of nuclear Ch-RelA after subtraction of its initial value (time course of  $F - F_{init}$ ). Yellow outlines mark pairwise correlations  $> 0.95$ . Ellipses are shaped and shaded according to the direction and strength of correlation.

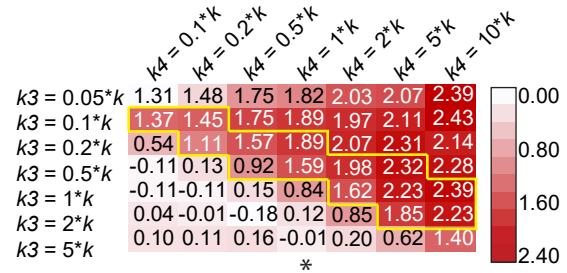
### A Mean transcript number



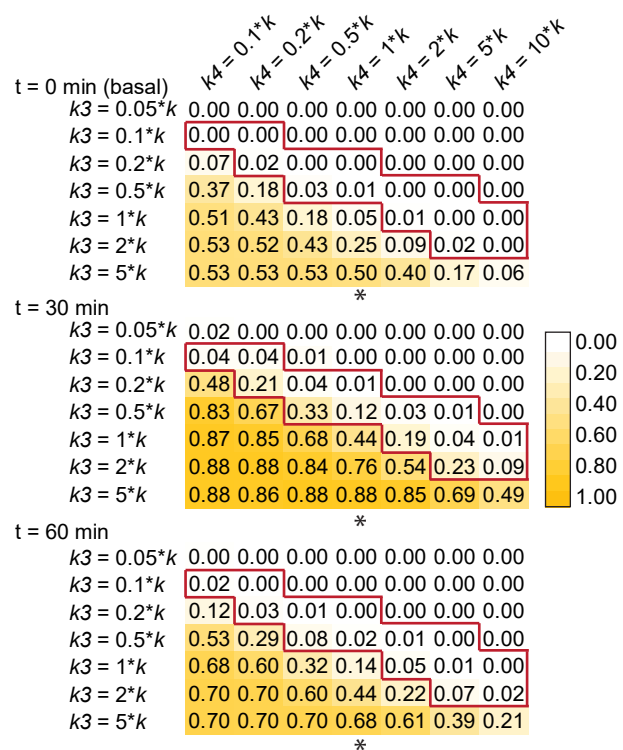
### B $r_p$ - transcript number vs. max nRelA fold change



### C $\text{Log}_{10}(\text{Relative variance})$

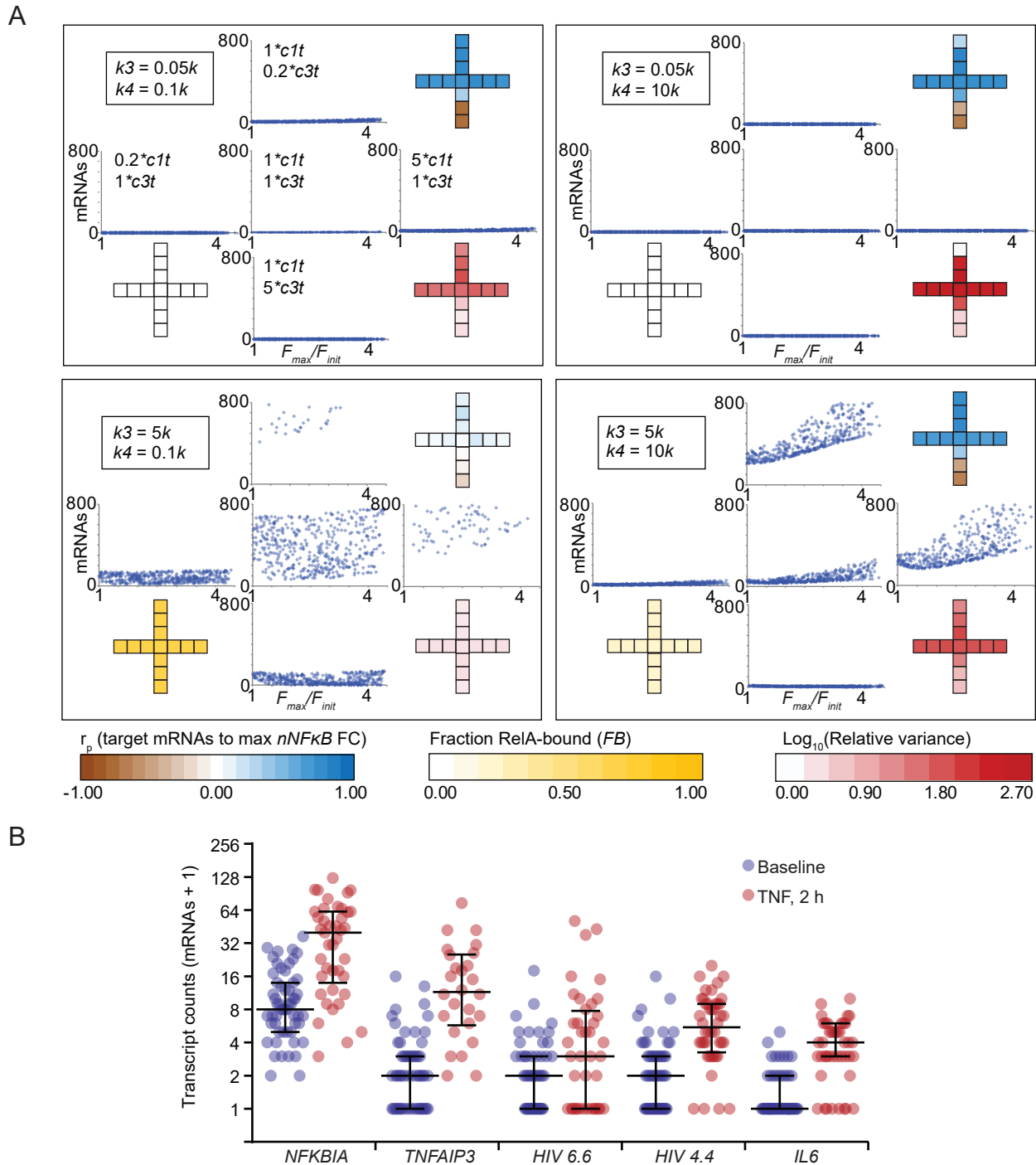


### D Mean 'fraction bound' RelA



**Figure S4 (related to Fig. 4). 2D parameter scan of the competitor affinity for target promoter vs. the parameter controlling competitor abundance predicts decreased RelA binding with decreasing transcript output.** Heatmaps of the outputs of a parameter scan of competitor affinity ( $k_3$ ; columns) and the parameter tuning relative competitor abundance ( $k_4$ ; rows) producing a range of (A) median transcriptional output before and 2 hours after TNF stimulation (gray), (B) the corresponding Pearson correlation coefficients ( $r_p$ ) of transcript abundance at 2 hours post-TNF with maximum fold change in nuclear RelA (blue), (C) the relative variance of transcripts before and two hours after TNF stimulation (calculated as  $\log_{10}(\text{Var}_{t=120 \text{ min}}/\text{Var}_{t=0 \text{ min}})$ ) and (D) mean fraction of RelA bound at the target gene promoter (FB) at 0, 30, and 60 min after TNF stimulation. Both  $k_3$  and  $k_4$  are expressed as a function of affinity of RelA binding to a target promoter ( $k$ ). The range of  $k_3$  and  $k_4$  combinations that correspond to our experimentally observed absolute change in transcript abundance induced by TNF at 2 hours is outlined in red in each heat map. The asterisk marks a column discussed in Fig. 4 ( $k_4 = 1*k$ ).





**Figure S6 (related to Fig. 5). Refining parameter clouds to identify model simulations that reproduce transcript abundance, fold-change detection, and fractional RelA binding patterns observed in experiments. (A)** Parameter spaces were explored for each corner of the parameter range presented in Fig. 5A (fixed  $k3$  and  $k4$  as indicated). Plots depict the simulated values of transcript number versus  $F_{max}/F_{init}$  for nuclear RelA ( $nNF\kappa B$  in the model) for five pairs of fixed  $c1t$  and  $c3t$  values (as marked on the top right panel). Heat map crosses indicate the values for Pearson correlation of mRNAs at  $t = 2$  h to fold change ( $r_p$  with  $FC = F_{max}/F_{init}$ ; brown-to-blue), the fraction RelA bound (FB; white-to-yellow) and the relative variance (calculated as  $\log_{10}(\text{Var}_{t=120 \text{ min}}/\text{Var}_{t=0 \text{ min}})$ ; white-to-red). **(B)** One-dimensional scatter plots showing the median and interquartile range (IQR) of experimental smFISH transcript numbers distributions at baseline (blue) and 2 hours after TNF stimulation (red). The IQRs were then used to identify plausible parameter sets associated with each target gene (Fig. 5B).