Supplementary Information for

A synthetic gene circuit for imaging-free detection of cell signaling pulses

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This PDF file includes:

Supplementary Sequences

fos 3' UTR

gcagtcagagaaggcaaggcagccggcatccagacgtgccactgcccgagctggtgcattacagagagga
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tubulin 3' UTR

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KTR-Gal4-VP64 amino acid sequence

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Supplemental Figures

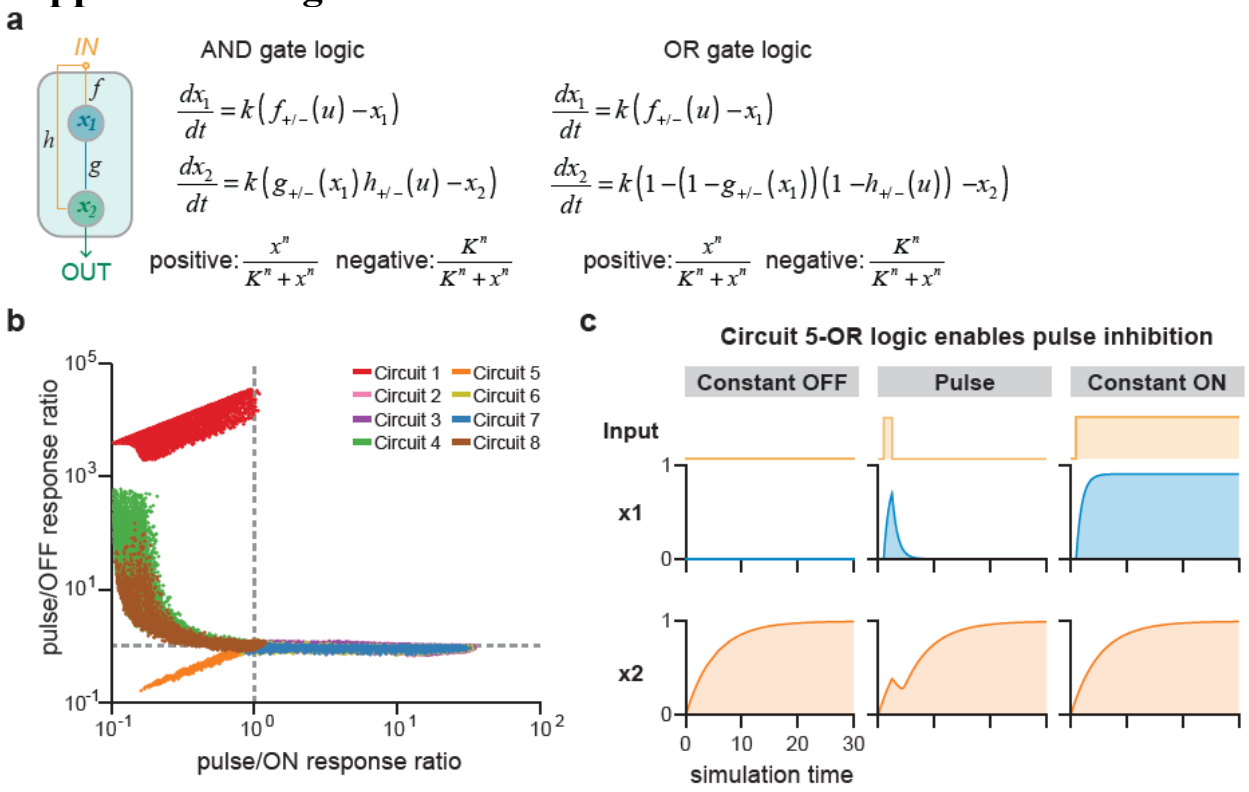


Figure S1. Modeling feed-forward loop motifs and computational screen with OR logic reveals pulse-specific inhibition. (a) Motifs were modeled such that input *u* acts on the intermediate species x_1, which combines with *u* to regulate the final output node x_2. The linkages from one node to the next determine whether *f*, *g* and *h* form positive (activating) or negative (repressing) regulatory links. Each activating/repressing connection is based on a Hill function, with *K* representing the half-maximal concentration of a species *x* for regulation to occur. The parameter *k* represents the timescale of changes for each species. The integration of input and x_1 is either determined using AND logic or OR logic. With AND logic, *g* and *h* are multiplied; thus, output is produced only if the both terms are high. With OR logic, output is produced if either *g* or *h* are non-zero through the term 1 – (1-g)(1-h). **(b)** Plot from computational screen with all 8 circuits (**Figure 1B**) using OR logic. Results of 10,000 simulations per circuit for random choices of parameters. In all cases, the area under the curve of x_2 was calculated, and the pulse:OFF and pulse:ON ratios are plotted for each parameter set. (**c**) Representative time course of the pulse-induced repression circuit, Circuit 5, simulated with either constant OFF (left), a pulsed input (middle) or constant ON (right). The pulsed input causes a transient decrease in GFP expression, but no such decrease is observed in constant-off or constant-on cases. *Related to Figure 1.*

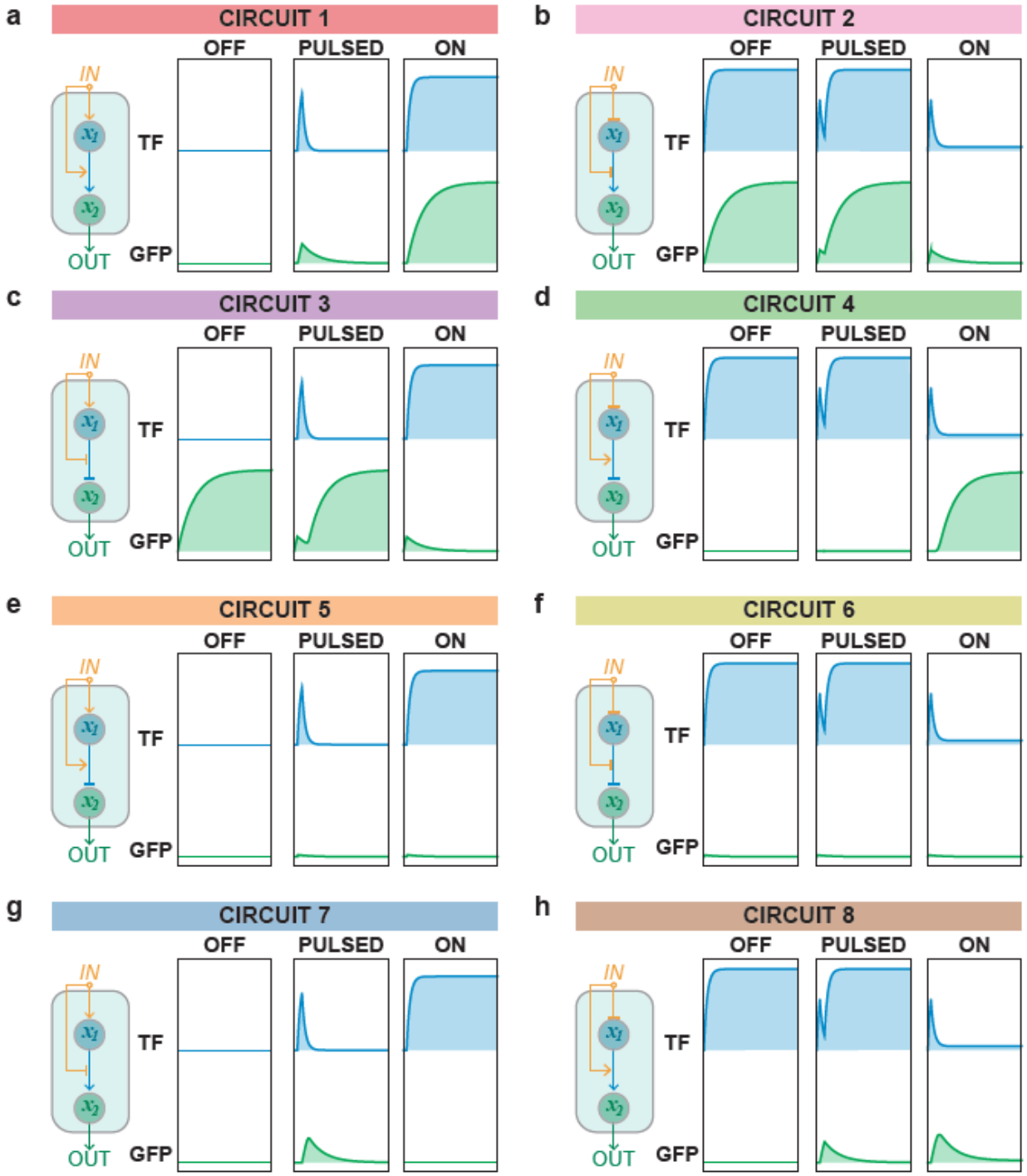


Figure S2. Representative traces of all 8 circuits using AND logic. Network topology diagram and representative time course for cases simulated with constant OFF (left), a pulsed input (middle) or constant ON (right) for coherent FFLs [(**a**) circuit 1, (**b**) circuit 2, (**c**) circuit 3, (**d**) circuit 4] and incoherent FFLs [(**e**) circuit 5, (**f**) circuit 6, (**g**) circuit 7, (**h**) circuit 8]. The pulse case for (**g**) circuit 7 are replicated from **Figure 1E.** *Related to Figure 1.*

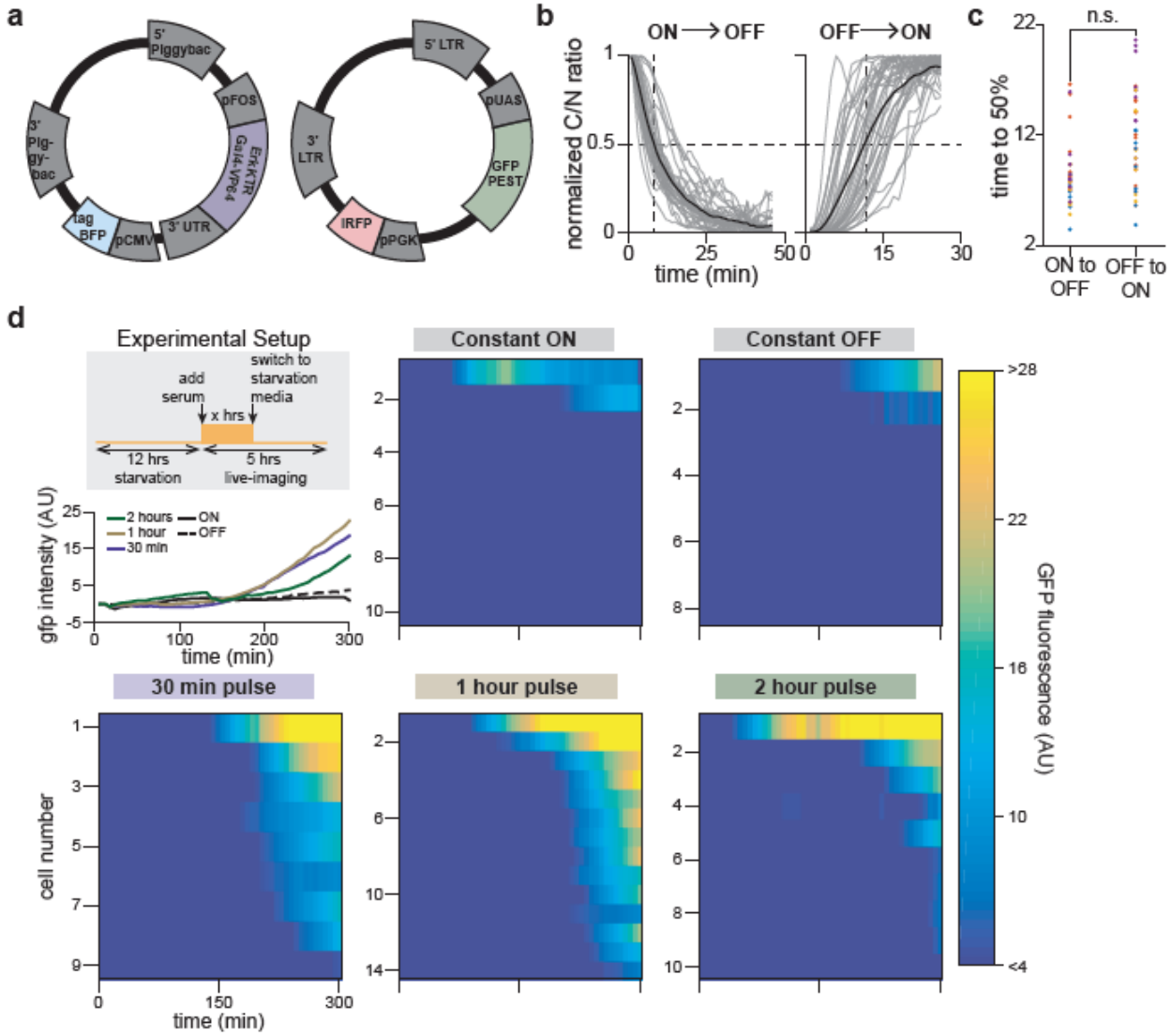


Figure S3. Development and live-imaging of READer system. (**a**) PiggyBAC vector (left) used to express the dynamics-sensitive transcription factor. This construct is co-transfected along with the PiggyBAC transposase enzyme to enable genomic integration. The CMVp-tagBFP is used as a marker to isolate cells that contain the construct. pHR lentivirus vector (right) used to express the UASp-GFP reporter. This construct is co-transfected along with pMD and CMV helper plasmids into Lenti-X 293T cells to generate lentivirus that is then used for genomic integration. The PGKp-iRFP is used to isolate cells that express the construct. **(b)** NIH 3T3s expressing KTR-irfp were plated and then either kept in full media and put in GF-free media before imaging (left, n=36 cells, N= 4 wells) or placed in GF-free media overnight and serum was added immediately prior to imaging (right, n = 38 cells, N= 4 wells). Grey lines show individual cell traces for cytoplasmic/nuclear ratio after min-max normalization and black line represents the mean of all cells. **(c)** Analysis of cells in **b** for time when cells pass 50% threshold. Points represent individual cells (n = ~10 cells) and colors represent different wells (N = 4 wells). Statistics are derived using a two-sided student's t-test where p-values < 0.05 were considered significant. **(d)** NIH3T3 READer cells were plated in glass-bottomed 96 plate wells.

After 12 hours, cells were switched to growth-factor free media for an additional 12 hours. Curves show mean GFP induction as a function of time for cells in 10% serum (constant ON; n = 10 cells), GF-free media (constant OFF; n=8 cells), a 30-minute serum pulse (n = 9 cells), a 1-hour pulse (n=14 cells) or a 2-hour pulse (n=10 cells). Heatmaps show all single cell traces for the specified condition. *Related to Figure 2.*

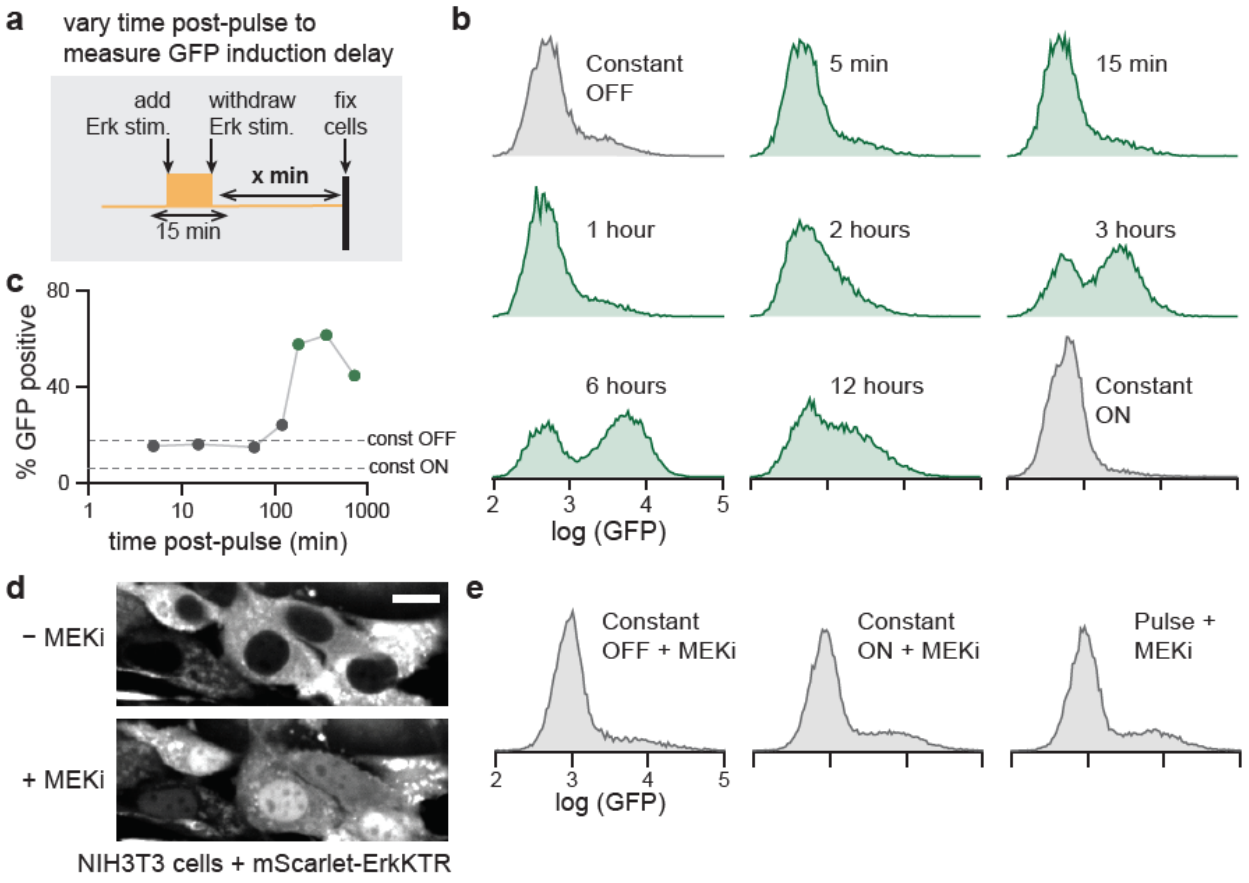


Figure S4. Quantifying the kinetics and specificity of the READer circuit. (**a-b**) To map how wait-time affects READer circuit output, we subjected cells to a 15 min serum pulse, fixed them at various time-points after the end of the pulse (schematic in **a**) and analyzed by flow cytometry for GFP induction (histograms of GFP expression in **b**). (**c**) Quantification of flow cytometry data from single run shown in **b** reveals that GFP levels peak ~3 h after the pulse. Points indicate fraction of GFP-positive cells at each time point, with the fraction of GFP-positive cells under constant stimulation conditions shown as dotted lines. **(d)** To validate the MEK inhibitor we add 1 μM Cobimentinib to NIH 3T3s expressing KTR-mScarlet that are in full media. We see that prior to drug addition, all cells in the field of view exhibit cytoplasmic KTR while after drug addition we have nuclear KTR (Erk is off). Scale bar is 10 μm. **(e)** To test the specificity of the READer system for Erk pulses, we apply our three classic inputs (constant OFF, constant ON and a 1HON/3HOFF pulse) in the presence of MEKi. It is quite clear that none of the cells turn on READer more that in the case of constant OFF and constant ON. *Related to Figure 2.*

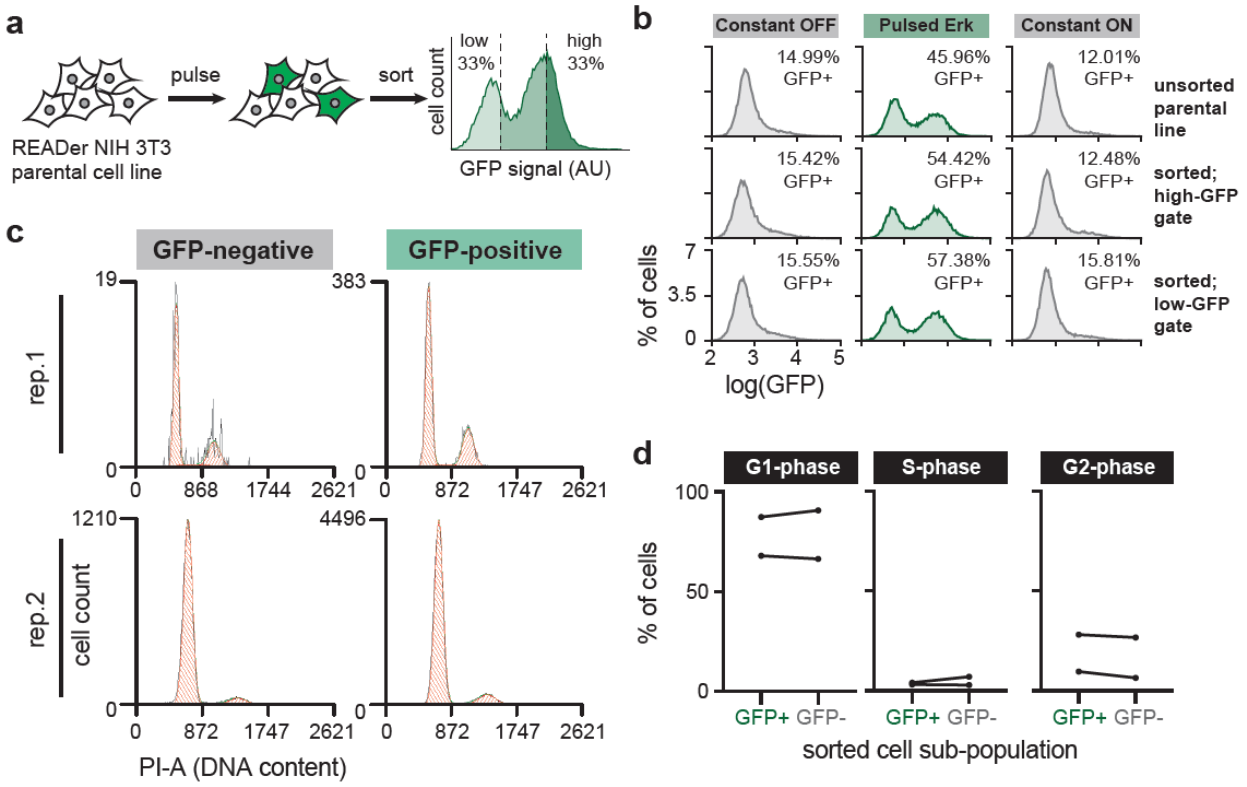


Figure S5. Testing and tuning non-genetic origin of bimodal READer responses. (a) Schematic of experiment to test whether GFP response is heritable. READer cells were subjected to a 20 min stimulus pulse, incubated for 4 h, and GFP-high and GFP-low subpopulations were sorted. After 1 week, constant and pulsatile stimuli were delivered to parental, GFP-high sorted cells, and GFP-low sorted cells to assess their responses. (**b**) Experimental results. Parental READer cells (top row), GFP-high cells (middle row) and GFP-low cells (bottom row) were analyzed. Cells were starved of growth factors overnight and either received GF-free media (left), a 15 min serum pulse followed by incubation for 6 h (middle) or constant 10% serum (right). Flow cytometry plots along with percent GFP positive cells are shown. **(c)** NIH3T3 READer cells were plated and, after 36 hours, switched to GF-free media for 12 hours. Cells were then subjected to a 1 h pulse of 10% serum, incubated for 4 hours, and fixed and stained with propidium iodide (PI) for DNA content analysis of the GFP-high and GFP-low READer subpopulations. Raw PI distributions, indicating cells' DNA content, are shown for the GFP-high and GFP-low subpopulations. Fits of the G1, S and G2 distributions are shown for two biological replicates. (**d**) Quantification of the percentage of cells in the different cell-cycle phases for the two replicates shown in **c**, demonstrating negligible differences between GFP-high and GFP-low subpopulations. Points from the same run of the experiment are connected. *Related to figure 2*.

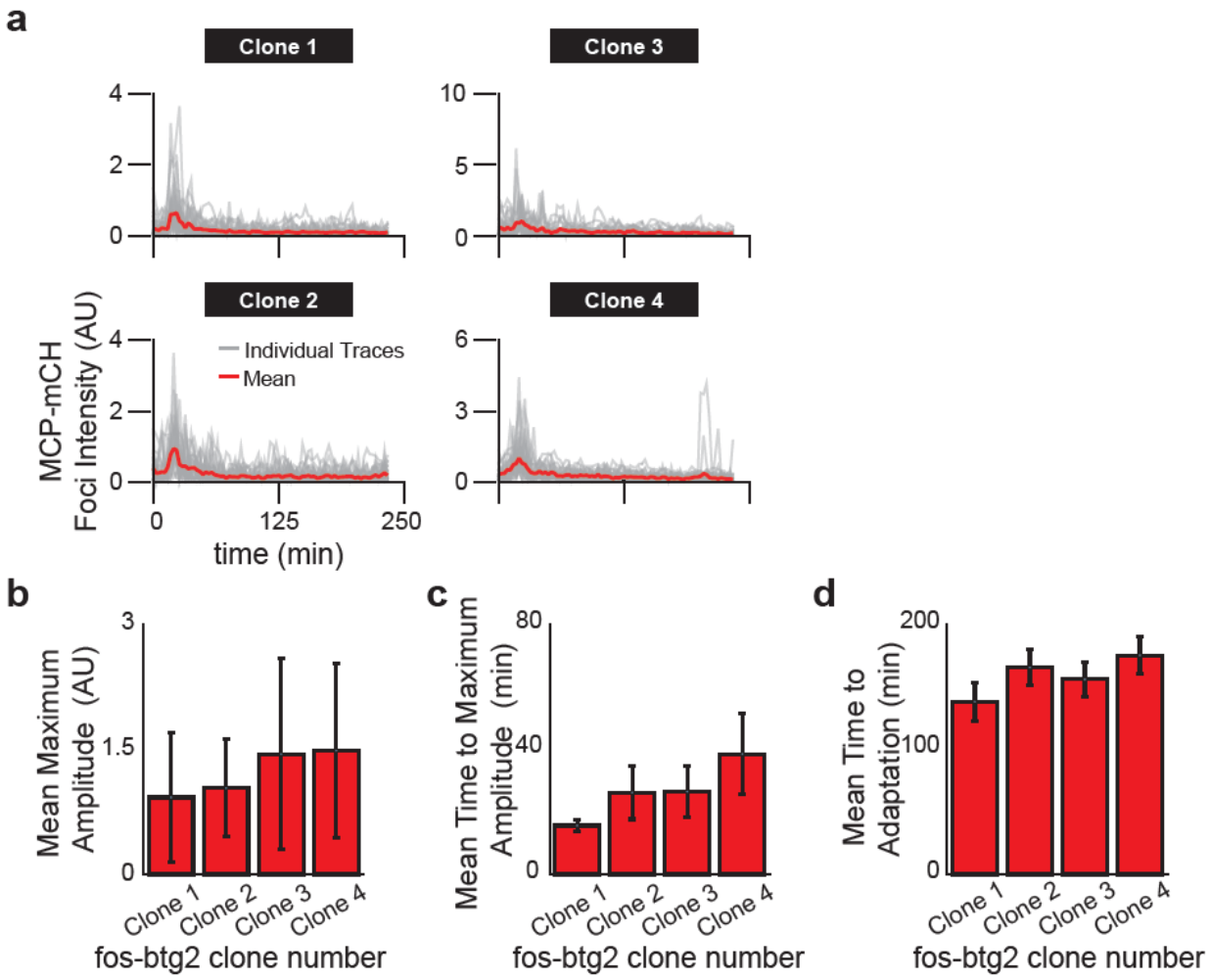
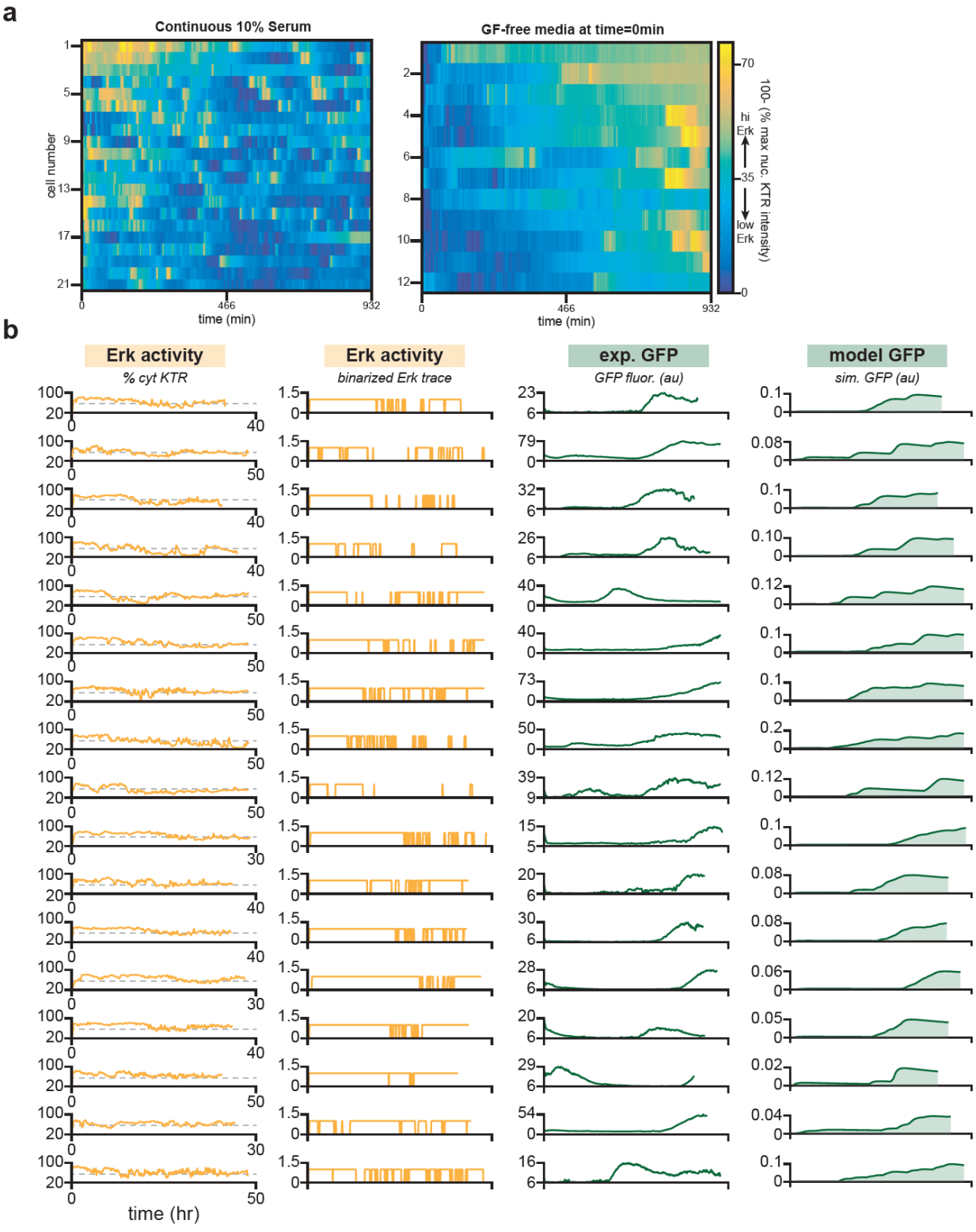


Figure S6. Transcriptional adaptation from the *FOS* promoter in response to constant-on Erk stimuli. We generated NIH3T3 cell lines expressing MCP-mCherry and where the PiggyBAC transposase was used to integrate a synthetic Erk target gene FOSp-YFP-24xMS2-3'*btg2*. Briefly, this construct contains the FOS promoter driving YFP, followed by 24 MS2 stem-loops and the *BTG2* 3' UTR, a long UTR for ensuring high MS2/MCP signal intensity. We then quantified transcriptional foci after treatment with 10% serum for cells in 4 independent clones derived from the parental cell line. (**a**) Quantification of individual foci (grey) and the mean (red) are shown for clone 1 (20 foci, 8 cells), clone 2 (12 foci, 4 cells), clone 3 (13 foci, 6 cells) and clone 4 (21 foci, 7 cells). (**b-d**) Analysis of transcription dynamics for foci shown in **a**. Parameters quantified include the mean maximum amplitude of foci (± S.D.) (in **b**), the delay time to maximum amplitude (in **c**) and the mean time to adaptation defined as the time when signal decays back down to 25% of maximum amplitude (in **d**). All error bars show ± S.E.M., except for **b** which shows ± S.D. *Related to Figure 2.*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Continuous 10% Serum
GF-free media at time=0min
cell number
time (min)
hi Erk
low Erk
100- (% max nuc. KTR intensity)
b
Erk activity
% cyt KTR
Erk activity
binarized Erk trace
exp. GFP
GFP fluor. (au)
model GFP
sim. GFP (au)
time (hr)

Figure S7. READer detects endogenous Erk dynamics in NIH 3T3s. (a) NIH 3T3s expressing Erk-KTR-iRFP were plated in 96-well plate wells and either left in growth media for the entirety of imaging (*left,* n=21 cells) or switched into growth-factor free media at the beginning of imaging (*right,* n=12 cells). Nuclear iRFP fluorescence was quantified and heatmaps show single cell traces for (100 – the nuclear intensity normalized to each cell's maximum nuclear fluorescence). **(b)** Quantification from 17 READer cells expressing KTR-mScarlet stimulated with serum at time 0 and imaged for 34 hours in a single microscopy run. From left to right, columns indicate quantification of (1) % cytoplasmic KTR, (2) "binarized" KTR traces based on 55% cytoplasmic KTR threshold, (3) GFP intensity and (4) simulated GFP response when the binarized KTR-mScarlet trace is used as a model input. Rows represent analyses for the 17 different cells. Cell 1 is the same as shown in **Figure 5C**. *Related to Figure 5.*

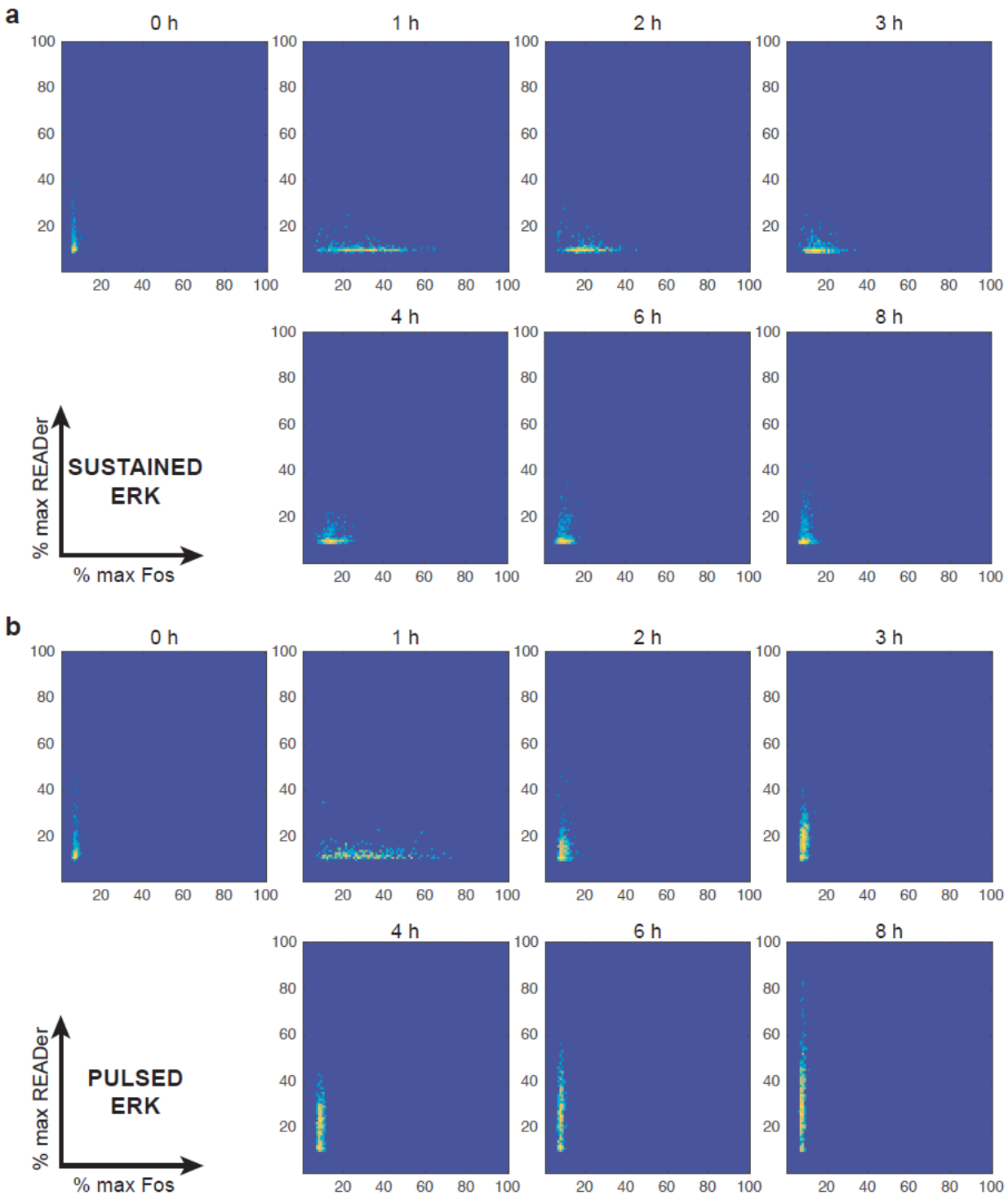

Figure S8. Density plot analysis of Fos-READer immunofluorescence experiment. Quantification of immunofluorescence intensity from fixed cells for Fos and READer for (**a**) sustained serum stimulation or (**b**) a 20-minute pulse of serum. Each cell's intensity is normalized to the maximum Fos or READer GFP intensity for the experiment and then plotted on the Fos-READer plane where Fos is the horizontal axis while READer is the vertical axis.

Each plot represents a different timepoint after the beginning of stimulation. In both stimulation conditions, Fos activation occurs at the 1-hour timepoint. Fos stays high in the sustained input case but goes down rapidly in the pulsed case. Only in the pulsed case is there any activation of READer GFP. *Related to Figure 6.*