Cell Reports

Supplemental Information

Cellular Levels of Signaling Factors Are Sensed by *β-actin* Alleles to Modulate Transcriptional Pulse Intensity

Alon Kalo, Itamar Kanter, Amit Shraga, Jonathan Sheinberger, Hadar Tzemach, Noa Kinor, Robert H. Singer, Timothee Lionnet, and Yaron Shav-Tal

Supplemental Figures

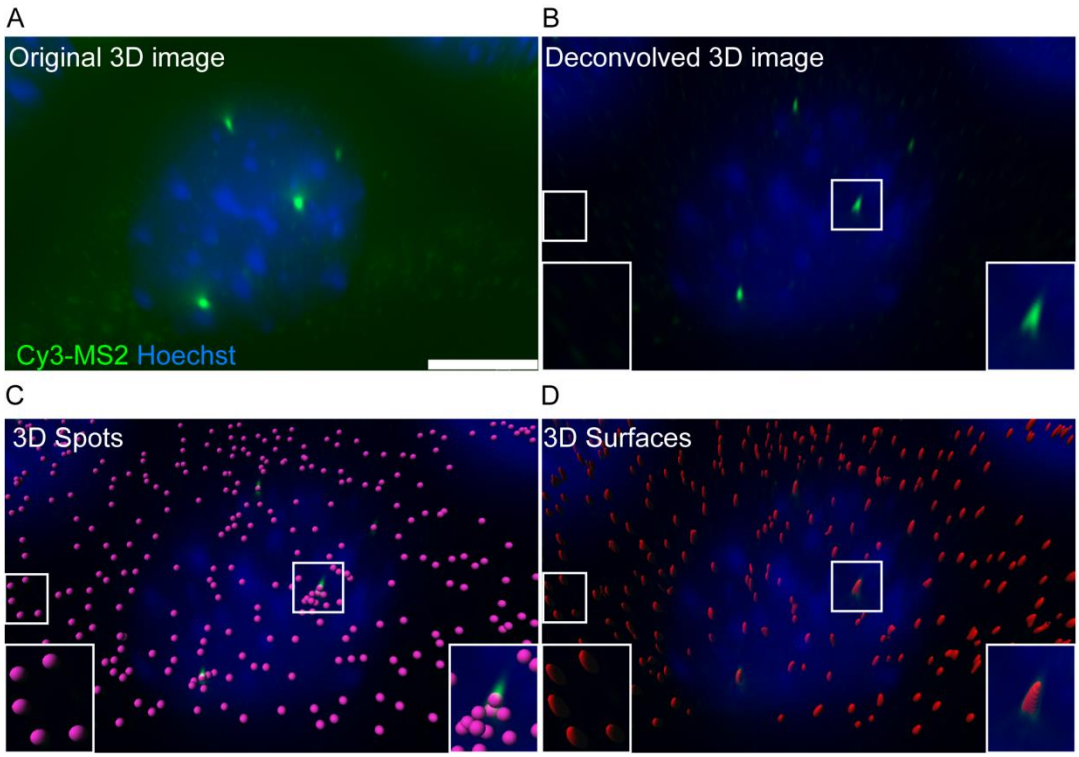

Figure S1, related to Figure 2.
Detection and quantification of β-actin MS2 mRNAs by RNA FISH.
An RNA FISH experiment using the Cy3-labeled probe to the MS2 repeats of the endogenous β-actin mRNAs (green), Hoechst DNA counter stain (blue). (A) 2D representation of an original 3D stack image (0.2 µm steps, 101 planes) of the total volume of a MEF cell exhibiting 4 active alleles acquired in the Cy3 channel (green) and in the Hoechst channel (blue). Scale bar, 5 µm. (B) The image after 3D deconvolution. Allele intensity (inset bottom right) can be compared to single mRNA molecules (inset bottom left). (C) Identification of single mRNAs labeled with pink spots, based on control cells not expressing the MS2 sequence for eliminating background spots from the analysis. (D) Creation of a 3D surface (red) for each object (mRNA) that was identified by the Spot function, generating a volume for each object within the entire 3D cell volume.

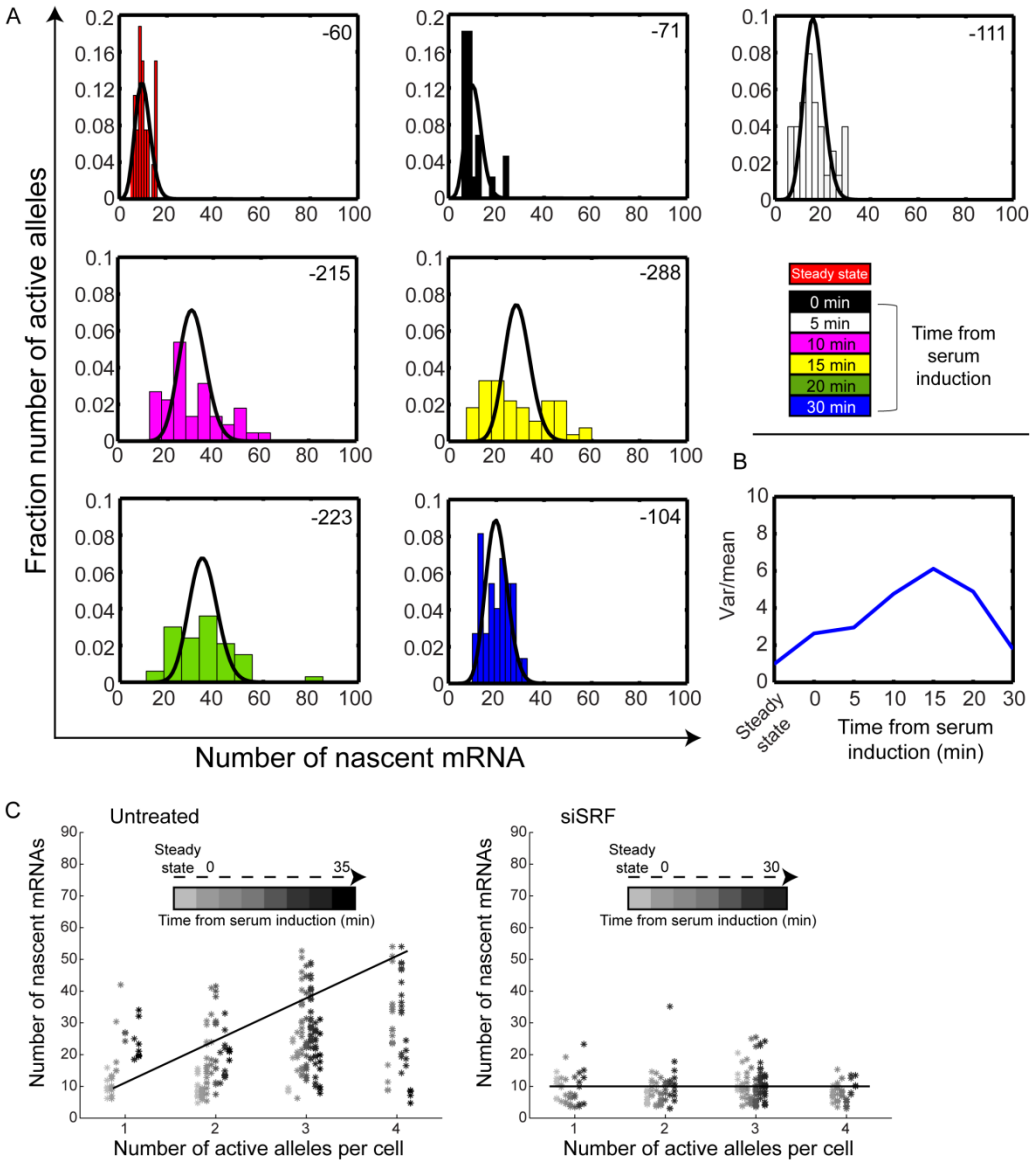

Figure S2, related to Figure 2.

Quantitative analysis of active β-*actin* alleles during serum induction.

(A) Each histogram represents the fraction number of active alleles (y axis) exhibiting different numbers of nascent mRNAs (x axis) for the indicated time points, from the time of serum release (~40 active alleles for each time point). The pooled nascent mRNA numbers originate from the averaged number in figure 2A for each time point of serum induction. Each histogram was fit with a Poisson distribution (black line) (assuming that the time to transcribe is shorter than the duration of the serum induced pulse), and the log-likelihood of the fit is presented inside each plot. (B) Plot representing the variance of nascent mRNA numbers divided by the mean value for each histogram in A. In the case of a Poisson distribution the value of this ratio is expected to be 1 as observed under steady state conditions. We find that when transcription levels reach a peak (between 10-20 minutes of serum release), the amount of nascent transcripts on all analyzed alleles no longer follows a Poisson

distribution but transcripts numbers are more scattered (even when the number of active alleles in the same cell converges to 3-4). These findings point to the existence of more than one "ON" state of β-*actin* promoter activity, accompanied with elevated transcriptional output detected from each allele during the serum response. (C) The accumulation of nascent mRNAs during serum induction correlates with the number of active alleles. The plots represent the number of nascent mRNAs being transcribed (Y axis) sorted to cells exhibiting 1-4 active alleles (X axis), either in untreated cells (left plot) or in SRF siRNA-treated cells (right plot). Each spot represents an active allele. The data are presented from steady state conditions through 0-35 minutes of serum release, and depicted according to different shades of grey. The black line shows the change in the number of nascent transcripts with respect the increasing number of active alleles.

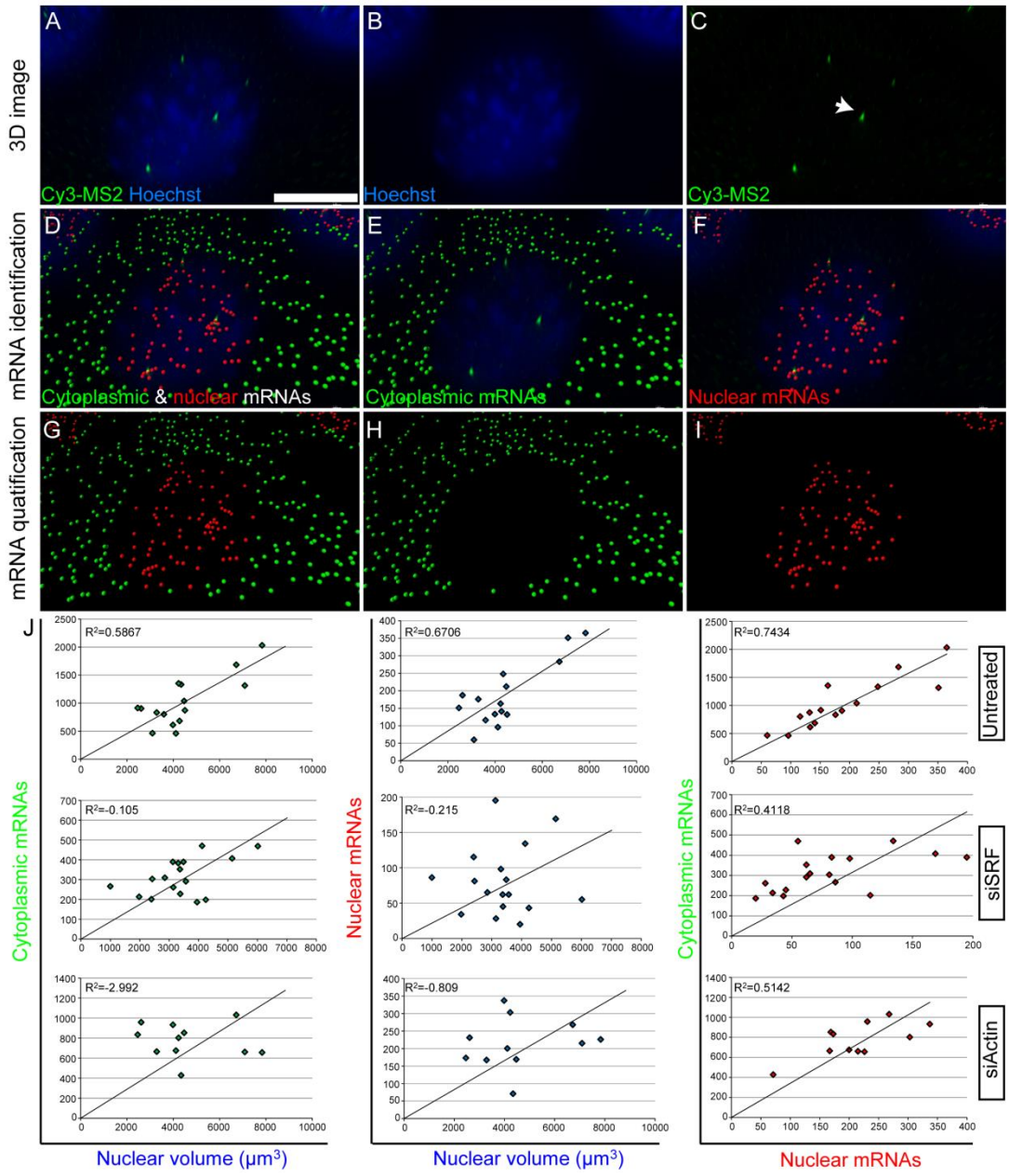

Figure S3, related to Figure 2.

Detection and quantification of cytoplasmic and nuclear β-actin MS2 mRNAs by RNA FISH.

(A) An RNA FISH experiment from figure S1. Scale bar, 5 µm. (B) The image from (A) acquired in the DAPI channel. Hoechst DNA counterstain (blue). (C) The image from (A) acquired in the Cy3 channel (green). (D-F) Identification of cytoplasmic (E - green spots) and nuclear (F - red spots) mRNAs. (G-I) The number of β-actin mRNAs in the nucleus (N) and cytoplasm (C) are counted and the C/N ratio is calculated. (J) Plots representing: (Left) the ratio between cytoplasmic mRNAs and the nuclear volume (green squares); (Center) the ratio between nuclear mRNAs and the nuclear volume (blue squares); and (Right) the C/N ratio (red squares). The rows represent: (Top) untreated cells; (Middle) SRF siRNA-treated cells; and (Bottom) actin siRNA-treated cells. Results were fitted to a linear equation with R square indicated in each plot.

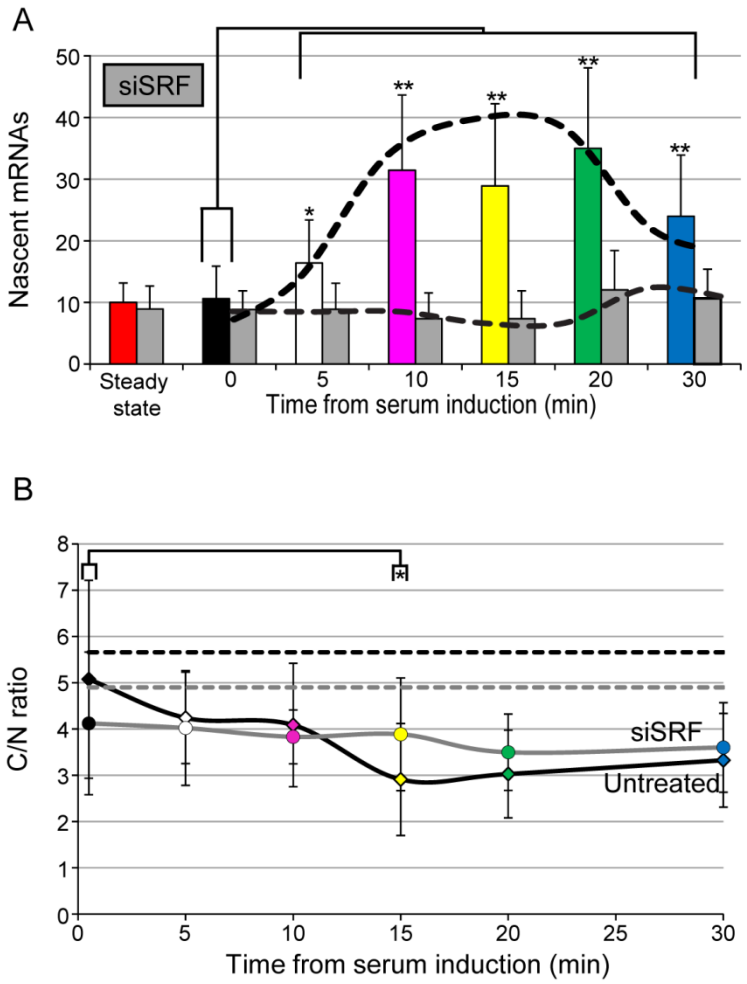

Figure S4, related to Figure 3.
Quantitative analysis of the β-*actin* transcriptional response to serum induction following SRF knockdown.
(A) The number of nascent mRNAs transcribed by the β-actin alleles at different time points after serum induction was counted by quantitative RNA FISH. No significant change in nascent mRNA accumulation was detected for SRF siRNA-treated cells (grey). Black dotted lines denote the change in the number of transcripts for scrambled (scr-siRNA) and SRF siRNA-treated cells during the serum response (30 sites for each time point; error bar = STDEV, ** P value < 0.005, ** <0.0001*). (B) The number of β-actin mRNAs in the nucleus (N) and cytoplasm (C) were counted and the C/N ratio was calculated. Nuclear β-actin mRNA accumulation is observed for 15 min after serum release (decreasing C/N ratio). From 15 min an increase in the C/N ratio is seen due to the reduction in the β-actin transcriptional pulse in conjunction with mRNA nucleo-cytoplasmic export. No significant change in C/N ratio detected for SRF siRNA-treated cells (grey line). The dotted black and grey lines represent the average C/N ratio in scr-siRNA and SRF siRNA-treated cells under steady state conditions (15 cells for each time point; error bar=STDEV, ** P value < 0.005*).

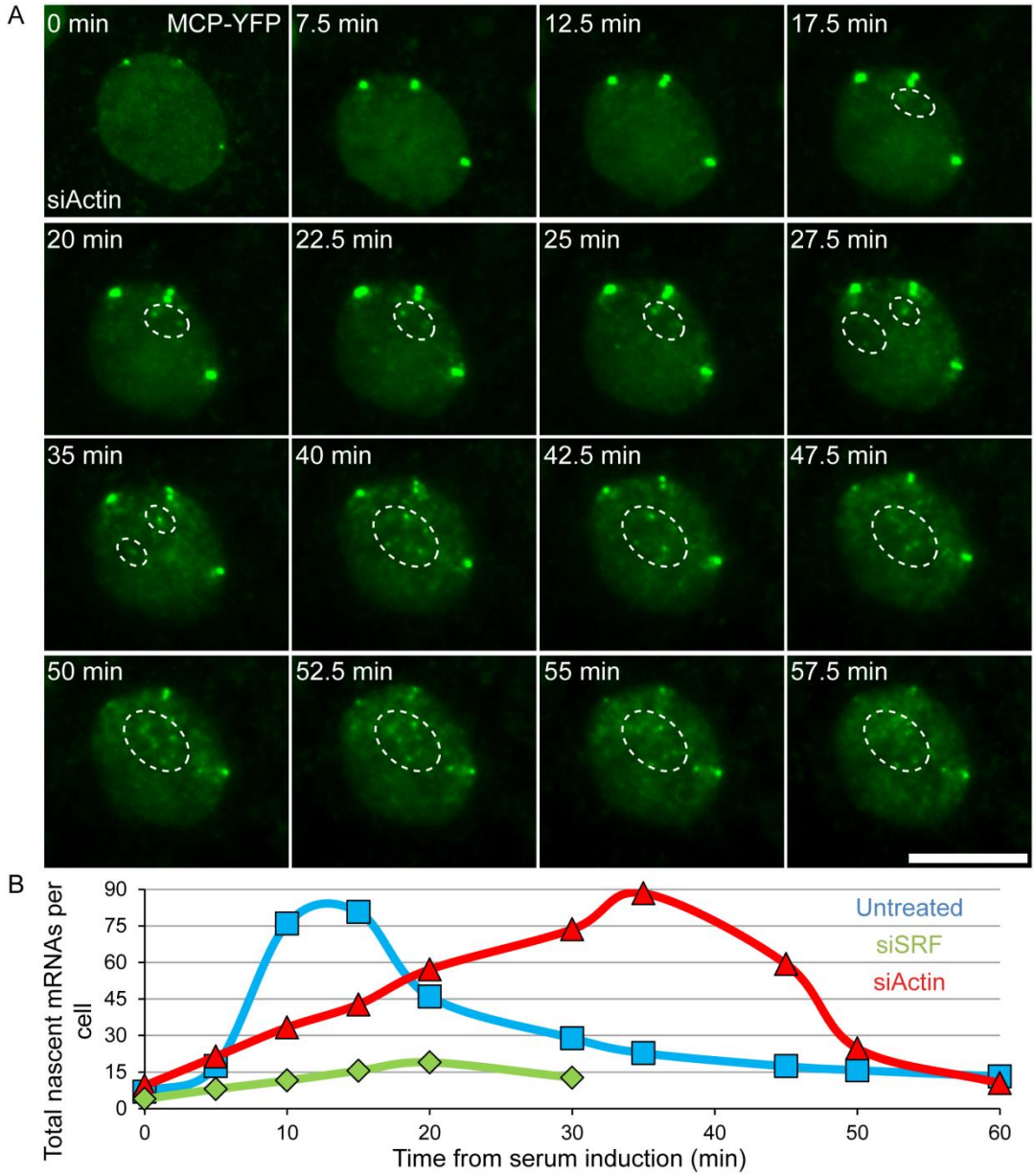

Figure S5, related to Figure 6.
Following the wave of nucleoplasmic β-actin mRNAs after serum induction in actin depleted cells.
(A) Frames from Movie S4 showing the induction of 4 alleles after serum induction. The alleles activate simultaneously and single mRNAs can be seen first in the proximity of the alleles and then moving also within the center of the nucleus (even though the alleles are in the periphery). Some regions containing single mRNAs traveling in the nucleus are marked by dotted white circles, showing the dispersal of the wave over time throughout the whole nucleus. (B) Plot representing the accumulation of the total nascent mRNAs per cell at the indicated time points of serum release for scr-siRNA and siRNA-treated cells to SRF and actin (blue, green and red lines, respectively).

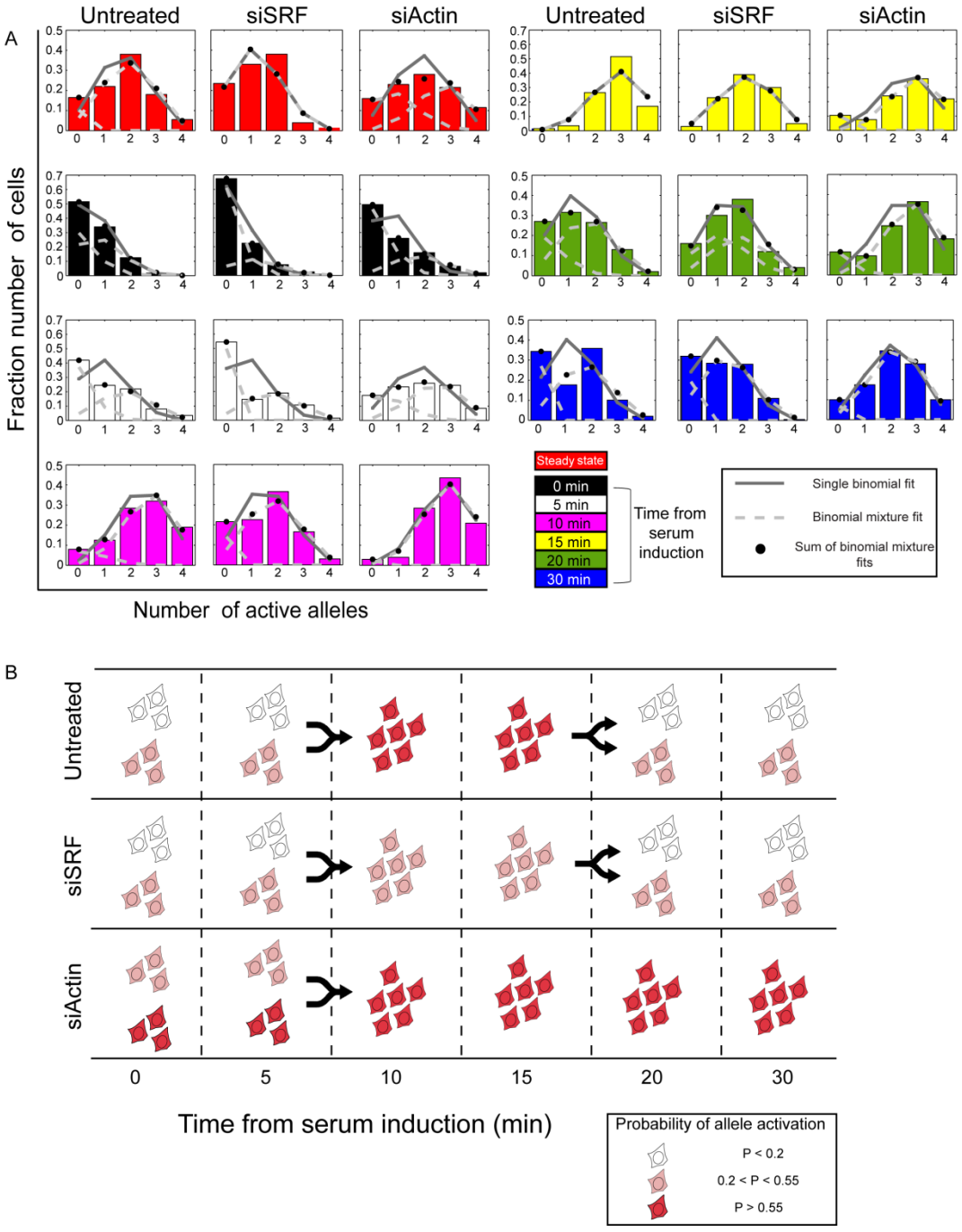

Figure S6, related to Figure 1.

The β-actin serum response reveals sub-populations of cells with different probabilities of allele activation.

(A) Each plot represents the fraction of cells in the population (y axis) exhibiting different numbers of active alleles (x axis) for the indicated time points from serum release (200 cells for each time point) in scr-siRNA and siRNA-treated cells to SRF and actin. Grey solid and dotted lines represent fitting of the data to a binomial fit for either one or two sub-populations (there are 2 dotted lines for 2 populations), respectively. Black dots represent the observed variable which is the sum of the 2 latent variables, in this case each sub population.

Example A - top left plot: The red bars represent the data from untreated cells at steady state conditions, fitted to either 1 (solid grey line) or 2 (dotted grey lines) binomial distributions. The best fit for the data in each plot describes the number of cell populations where a solid line represents one population and each dotted line represents one sub-population within the overall population with a different probability to activate the gene. Once the correct fit is determined the calculated area under the line is used to extract the percentage of cell population while the tendency of the plot is used to estimate the probability (P) of each population to activate a single allele with values between 0-1 (left; $0<P<0.5$ or right; $0.5<P<1$). In this example, fitting the data to 1 binomial distribution resulted in a cell population activating the gene with a probability (P) of 0.45. Fitting the data to 2 binomial distributions resulted in one cell population (90%) and a second cell population (10%) with $P=0.48$ and $P<0.01$, respectively. The black dots represent the sum of the two sub-populations which is considered the observed population.
Example B - second line, left plot: The black bars represent the data from untreated cells after overnight starvation, fitted to either 1 (solid grey line) or 2 (dotted grey lines) binomial distributions. Fitting the data to 1 binomial distribution resulted in a cell population activating the gene with a probability (P) of 0.16. Fitting the data to 2 binomial distributions resulted in one cell population (57%) and a second cell population (43%) with $P=0.22$ and $P=0.07$, respectively. The black dots represent the sum of the two sub-populations which is considered the observed population. Detailed list of probabilities for each treatment are presented in Table S1.
(B) Scheme demonstrating the existence of single or two sub-populations of cells that activate β-actin during the serum response. The color code represents the different probabilities for site activation for each time point from serum release (white = low probability; pink = average probability; red = high probability to activate the gene), in scr-siRNA, SRF siRNA and actin siRNA treated cells.

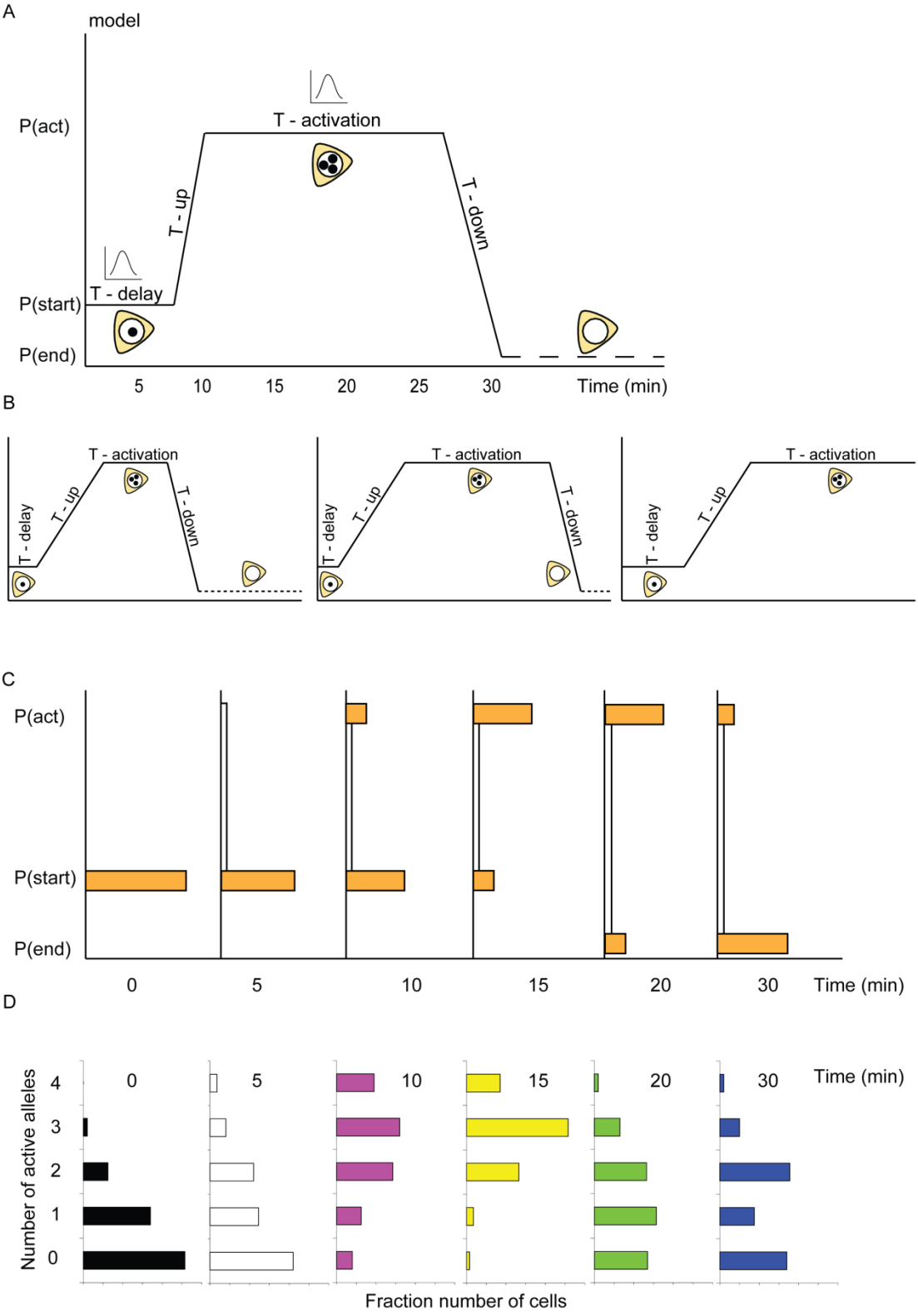
A
model
P(act)
P(start)
P(end)
T - activation
T - up
T - down
T - delay
5
10
15
20
25
30
Time (min)
B
T - activation
T - up
T - down
T - delay
C
P(act)
P(start)
P(end)
0
5
10
15
20
30
Time (min)
D
Number of active alleles
4
3
2
1
0
0
5
10
15
20
30
Time (min)
Fraction number of cells

Figure S7, related to Figure 7.

A mathematical model capturing the transition times from an inactive state to a fully activated allele.

Visual description of the model: (A) We defined the probability to activate a single allele as P_{start} before serum stimulation. After a time period of T_{delay} the activation probability increased to P_{act} during the time of T_{up}. The cell remains at P_{up} to a period of T_{act}. Finally, the activation probability decreases to P_{end} during the time of T_{down}. (B) Example of 3 possible scenarios for allele activation derived from the experimental distributions of T_{act} and T_{delay} obtained from live cell imaging data. (C) Integration of all possible scenarios, as described in B, for each time point of serum release summarized as the distribution of activation probabilities. For instance, at time 0 min all alleles are at P_{start} having the same probability for gene activation. At 5 min, some of the alleles have begun the transition towards P_{act}. (D) The changes in activation probability described in C were used to plot the distribution of the fraction number of cells exhibiting 0-4 active alleles for each time point of serum release. This output of the model was then fit to the actual data of active allele counting from untreated and siRNA-treated cells to SRF and actin.

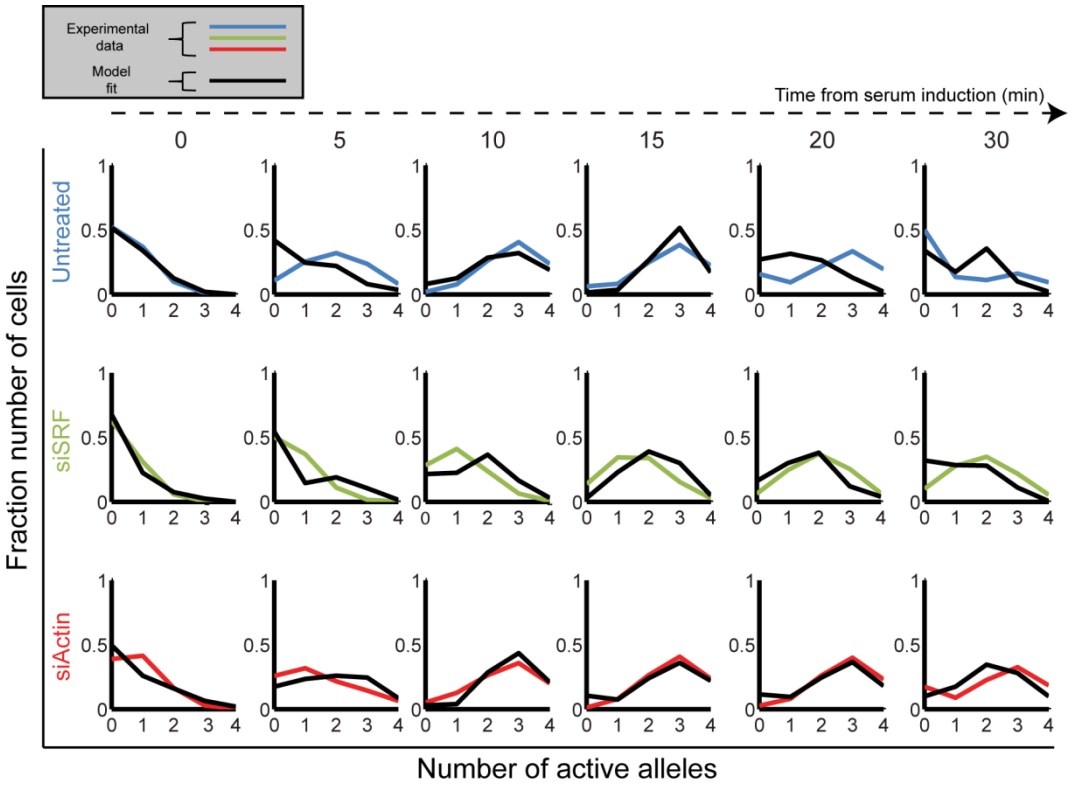

Figure S8, related to Figure 7.
Fitting the mathematical model to experimental data.
Each plot represents the experimental data of the fraction of cells in the population (y axis) exhibiting different numbers of active alleles (x axis), for the indicated time points from serum release (200 cells for each time point) in untreated and siRNA-treated cells to SRF and actin (blue, green and red lines in each plot respectively). The black line in each plot represents modeling results with T_{up} and T_{down} which gave the best fit to the experimental data for untreated and siRNA-treated cells to SRF and actin.

	Single binomial fit			P	Binomial mixture fits					P / %
Treatment / Time from serum induction	Untreated	siSRF	siActin	Untreated		siSRF		siActin		
Steady state	0.43	0.32	0.47	0.48 / 90	0 / 10	0.32 / 100	0 / 0	0.66 / 57	0.24 / 43	
Starvation	0.16	0.11	0.21	0.22 / 57	0.08 / 43	0.3 / 27	0.04 / 73	0.46 / 36	0.08 / 64	
5 min	0.27	0.22	0.46	0.45 / 53	0.06 / 47	0.45 / 50	0 / 50	0.61 / 66	0.17 / 34	
10 min	0.6	0.39	0.69	0.67 / 87	0.14 / 13	0.46 / 86	0 / 14	0.7 / 98	0 / 2	
15 min	0.7	0.53	0.63	0.7 / 100	0 / 0	0.53 / 100	0 / 0	0.7 / 89	0 / 11	
20 min	0.33	0.39	0.6	0.42 / 72	0.09 / 28	0.32 / 95	0.32 / 5	0.68 / 88	0.03 / 12	
30 min	0.32	0.3	0.53	0.44 / 73	0 / 27	0.37 / 81	0 / 19	0.56 / 97	0 / 13	

Table S1, related to Figure 1.

Probability for allele activation for one or two sub-populations.

Probability values (P) extracted for single or mixture binomial fits at the indicated time points of serum release in scr-siRNA, SRF-siRNA and actin-siRNA treated cells. For the mixture binomial fit, the percentage of each latent sub-population originating from the overall observed cell population is shown next to its respective probability value for allele activation.

	P_{start}	P_{act}	P_{end}	T_{delay} (min)	T_{act} (min)	T_{up} (min)	T_{down} (min)
Untreated	0.16	0.7	0	*~N(2,2.1)*	*~N(20.7,73.4)*	*4.6*	*0*
siSRF	0.11	0.53	0	*~N(6.7,2.3)*	*~N(9,15.3)*	*17.3*	*14.8*
siActin	0.21	0.7	0	*~N(5.5,10.3)*	0.5 *~N(22.4,48)* 0.5 *~N(52.8,28.4)*	*1.8*	*4.6*

Table S2, related to Figure 7.
Modeling the experimental data demonstrates different transition times between inactive and active gene states.
The table presents experimental data extracted from both fixed (P_{start}, P_{up} and P_{end}) and live cell imaging (T_{delay} and T_{act}) used in the model for extracting the times in which the gene shifts from inactive to active states (T_{up} and T_{down}). The presented T_{up} and T_{down} gave the best fit for the experimental data obtained from counting active alleles in different time points of serum release (Fig. S8), for untreated and siRNA-treated cells to SRF and actin.

Supplemental Experimental Procedures

Cell culture and transfections

MEF cells (Lionnet et al., 2011) were maintained in high glucose DMEM (Gibco) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT). For transient transfections, cells were transfected with 1-5 μg of plasmid DNA and 40 μg of sheared salmon sperm DNA (Sigma) when using electroporation (Gene Pulser Xcell, Bio-Rad). For lipososmal transfection the Lipofectamin 2000 reagent (Invitrogen) was used. For serum starvation, cells were starved for overnight (19 hrs), and then serum was added to a final concentration of 10% to begin the serum response.

Total RNA purification

Total RNA was isolated using Tri-Reagent (Sigma). DNA-free™ Kit (Ambion) was used to remove genomic DNA contamination. cDNA (1ug RNA) was synthesized using the ReverseAid™ First Strand cDNA Synthesis Kit (Fermentas) with oligo-dT as a primer. Semi-quantitative RT-PCR was performed using an Eppendorf Thermocycler amplification for 19-38 cycles (depending on the saturation level of the genes amplified) using 1 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C; and 72°C for 10 min for final extension. Primers for GAPDH: sense: ACCACAGTCCATGCCATCAC, anti-sense: TCCACCACCCTGTTGCTGTA.
SRF: sense: CACGCTGACAGGGACACAGG, anti-sense: CAGAGGAGACAGGGCCGCTG.
ACTB (β-*actin*): sense: AGCTTCTTTGCAGCTCCTTCGTTGCCGGTC, anti-sense: CATCACAATGCCTGTGGTACGACCAGAGGC.

siRNA knockdowns

MEFS expressing the β-actin-MS2 and MCP-YFP fusion protein were transfected with siRNA for SRF (Cat#10620318, Oligo ID, MSS277298, Invitrogen) or siRNA for β-actin (SASI_Mm02_00311503, Sigma) or a negative scrambled (scr-siRNA) control (Cat#12935-400), using Lipofectamine 2000. mRNA expression levels of SRF and β-actin were examined by semi-quantitative RT-PCR (performed 3 times for scrambled and for each treatment) from total RNA using Tri Reagent (Sigma) 72h after siRNA transfection, and the amplified DNA bands were analyzed by densitometry. Primers used:
SRF forward: CACGCTGACAGGGACACAGG,
SRF reverse: CAGAGGAGACAGGGCCGCTG.
ACTB forward: AGCTTCTTTGCAGCTCCTTCGTTGCCGGTC,
ACTB reverse: CATCACAATGCCTGTGGTACGACCAGAGGC.
Expression levels were normalized to GAPDH mRNA levels using primers:
Forward: ACCACAGTCCATGCCATCAC,
Reverse: TCCACCACCCTGTTGCTGTA.
In experiments using fluorescently labeled siRNAs, the siRNAs were first labeled with Cy5 using the Label IT nucleic acid labeling kit (Mirus, Madison, WI). The fluorescent tag does not interfere with the RNAi process. We concluded from siRNA treatments

combined with immunofluorescence of SRF or actin, that cells receiving the fluorescently-labeled siRNA had a significant reduction in SRF or actin, respectively.

Immunofluorescence

Cells were grown on coverslips, washed with PBS and fixed for 20 min in 4% PFA. Cells were then permeabilized in 0.5% Triton X-100 for 3 min. For actin staining, cells were treated with 70% ethanol for 2 hrs before permeabilization. After blocking, cells were immunostained for 1 hr with a primary antibody, and after subsequent washes the cells were incubated for 1 hr with secondary fluorescent antibodies. Primary antibodies: rabbit anti-SRF (Santa Cruz) and mouse anti-actin (Abcam). Secondary antibodies: Alexa594-labeled goat anti-rabbit IgG and Alexa594-labeled goat anti-mouse (Invitrogen). Phalloidin FITC-labeled (1:1000 with PBS, Sigma) was used for 30 min. Nuclei were counterstained with Hoechst 33342 and coverslips were mounted in mounting medium.

Fluorescence *in situ* hybridization

MEFS expressing β-actin-MS2 were grown on coverslips and fixed for 20 min in 4% paraformaldehyde, and overnight with 70% ethanol at 4°C. The next day cells were washed with 1x PBS and treated for 2.5 min with 0.5% Triton X-100. Cells were washed with 1x PBS and incubated for 10 min in 40% formamide (4% SSC). Cells were hybridized overnight at 37°C in 40% formamide with a specific fluorescently-labeled Cy3 DNA probe (~10 ng probe, 50 mer). The next day, cells were washed twice with 40% formamide for 15 min and then washed for two hours with 1X PBS. Nuclei were counterstained with Hoechst 33342 and coverslips were mounted in mounting medium. The probe for the MS2 binding site was:
CTAGGCAATTAGGTACCTTAGGATCTAATGAACCCGGGAATACTGCAGAC. In some cases, immunofluorescence was performed after the RNA FISH using the standard protocol.

mRNA quantification

3D stacks (0.2 µm steps, 101 planes) of the total volume of the cells were collected from fixed MEF cells. The 3D stacks were deconvolved and the specific signals of mRNAs were identified (Imaris, Bitplane). mRNA identification was performed in comparison to deconvolved stacks from MEF cells not containing the MS2 integration, which therefore served as background levels of nonspecific fluorescence. No mRNAs were identified in control cells. The sum of intensity for each mRNA particle and active alleles was measured in the same cells using Imaris, as previously described (Yunger et al., 2010, 2013). The single mRNA intensities for each cell were pooled and uploaded to a MATLAB script along with the intensity value of the active alleles. The number of nascent mRNAs located at the active allele was estimated by multiple random extractions from the data of different combinations of intensity values (of single mRNAs) reaching the intensity of the site. This value provided the number of mRNAs associated with the transcription unit from the point of the MS2-region and onwards. 40 active alleles were analyzed for each treatment and each time point of serum induction. All quantification and counting experiments were performed 3 times for each treatment and time point of serum release.

Fluorescence microscopy, live-cell imaging and data analysis

Wide-field fluorescence images were obtained using the Cell^R system based on an Olympus IX81 fully motorized inverted microscope (60X PlanApo objective, 1.42 NA) fitted with an Orca-AG CCD camera (Hamamatsu) driven by the Cell^R software. Live-cell imaging was carried out using the Cell^R system with rapid wavelength switching. For time-lapse imaging, cells were plated on glass-bottomed tissue culture plates (MatTek, Ashland, MA) in medium containing 10% FBS at 37°C. The microscope is equipped with an incubator that includes temperature and CO_2 control (Life Imaging Services, Reinach, Switzerland). For long-term imaging, several cell positions were chosen and recorded by a motorized stage (Scan IM, Märzhäuser, Wetzlar-Steindorf, Germany). In these experiments, cells were typically imaged in 3D (13 Z planes per time point) every 1 minute, at 0.3 µm steps for 1 hour. For presentation of the movies, the 4D image sequences were transformed into a time sequence using the maximum projection option or manual selecting using the ImageJ software. 3D analysis was performed with the Imaris. Movie sequences were deconvolved using Huygens.

Correlation coefficients

Correlation between the levels of transcriptional activity were measured at the different time points from serum stimulation. Allele intensity was measured over time by tracking the 3D surface of each active allele for the entire movie using Imaris. Allele intensity was normalized: $I(t)_{site,norm} = \frac{I(t)_{site} - I(t)_{nuc}}{I(t)_{nuc}}$. For frames where the allele was not detectable (ND) we assigned $I(t = ND)_{site,norm} = 0$. We extracted Pearson correlations between all possible pairs of active alleles. In cases where duration of activity for different alleles was not of the same duration we defined the non-overlapping time segments as ND in order to enable correlation between the alleles. Correlation scores of all allele pairs were plotted on a histogram (#bins=10) that was fitted to a normal distribution. Activation duration times for each allele (defined as the time interval from the first to the last frame in which the site was activate), were extracted along with the time of activation of each single allele (defined as the first frame in which the allele was detected). The empirical survival function was used to plot the duration of the activation times in order to characterize the decrease in the number of active alleles during the serum response. The cumulative distribution function (CDF) was used to plot the times of activation in order to illustrate the accumulation of active alleles from the moment of serum stimulation. All measurements were performed at least twice for each treatment.

C/N ratio

Quantification of cytoplasmic and nuclear mRNAs was performed using the 3D stack FISH images acquired in the Cy3 (mRNA detection) and Hoechst channels. 3D stacks of both channels for each cell were uploaded into Imaris and the total cellular mRNAs were identified in the Cy3 channel. In order to quantify the nuclear mRNAs, a specific filter was added for the detection of transcripts according to the mean

intensity of the Hoechst channel. We then quantified the number of cytoplasmic mRNAs by inverting the filter for spotting the transcripts not co-localized with the Hoechst signal. Dividing the number of transcripts in the cytoplasm to their number in the nucleus gave the C/N ratio. All quantifications of cytoplasmic and nuclear mRNAs were performed 3 times using 15 cells in each experiment for each treatment and time point of serum release.

Binomial mixture model

We modeled the activation of the alleles within the cell population with a binomial model. In the model each allele can be activated independently with a certain probability. To expand this description of the behavior of the alleles to a case where there can be more than one type of cell populations with different probabilities of allele activation, we used a "mixture model". This is a probabilistic model for representing the presence of subpopulations within an overall population, with no assignment of members to subpopulations. We modeled allele activation probability, based on the unobserved dataset; in this case, the probability of each sub-population to activate an allele.

The observed samples were $Y_i \in \{0 \ldots N\}$ which record the number of active alleles in the cell. Let $X_i \in \{0,1\}$ be the missing information of the assignment of each cell to the appropriate subpopulation. We define the apriori probability to belong to each subpopulation $P(X_i=0)=P_1$ and $P(X_i=1)=P_2=1-P_1$. The probability of an allele to be active in a cell from type i is λ_i. We wish to maximize the complete likelihood of the data under this model:

$$L(\theta|Y) = P(Y|\theta) = \prod_{t=1}^{n} P(Y_t|\theta) = \prod_{t=1}^{n} \sum_{X_i=0,1} P\left(Y_t, X_{i,t}\middle|\theta\right)$$

Where $\theta = (P_1, \lambda_1, \lambda_2)$ and X_t and Y_t denote the type of cell and the observed data in the t cells, respectively.

Although we know that $P\left(Y_t, X_{i,t}\middle|\theta\right) = P_i \binom{N}{Y_t} \lambda_i^{Y_t} (1-\lambda_i)^{N-Y_t}$, the maximization of $L(\theta|Y)$ to obtain $\hat{\theta}_{ML}$ is difficult.

$\hat{\theta}_{ML}$ can be estimated using an Expectation-Maximization (EM) algorithm. In this algorithm, in the expectation step we defined an auxiliary function $Q(\theta, \theta_0)$ which is the expected value of the log likelihood function of $\mathrm{P}(\mathrm{Y}, \mathrm{X_i}|\,\theta)$, with respect to the conditional distribution of X given the set of observation Y under the current estimate of the parameters θ_0.

$$Q(\theta, \theta_0) = \mathbb{E}_{p(x|y;\theta_0)} log p(x, y|\theta) = \sum_{X_i=0,1} P(x|y;\theta_0) \log P(x, y{:}\,\theta)$$

$$= \sum_{t=1}^{n} \sum_{X_i=0,1} P\left(x_{i,t}\middle|y_t;\theta_0\right) \log P\left(x_{i,t}, y_t{:}\,\theta\right)$$

We now defined $w_{i,t}$ as:

$$w_{i,t} = P(x_t = i|y_t;\theta_0) = \frac{P(y_t|x_t = i;\theta_0) * P(x_t = i)}{P(y_t)}$$
$$= \frac{P_i \binom{N}{Y_t} \lambda_i^{Y_t}(1-\lambda_i)^{N-Y_t}}{\sum_{i=0,1} P_i \binom{N}{Y_t} \lambda_i^{Y_t}(1-\lambda_i)^{N-Y_t}} = \frac{P_i \lambda_i^{Y_t}(1-\lambda_i)^{N-Y_t}}{\sum_{i=0,1} P_i \lambda_i^{Y_t}(1-\lambda_i)^{N-Y_t}}$$

The results of the maximization step are:

$$P_i = \frac{\sum_{t=1}^{n} w_{i,t}}{n}, \lambda_i = \frac{1}{n}\frac{\sum_{t=1}^{n} w_{i,t} * y_t}{\sum_{t=1}^{n} w_{i,t}}$$

We repeated the EM steps until the model parameters estimation (θ) converge.

Mathematical modeling

We modeled the processes of allele activation and inactivation in a cell as a transition between different cell states with different allele activation probabilities to transcribe (Fig. S7A). We assumed that all cells have an activation probability of P_{start} before serum stimulation. After a certain time of T_{delay} (modeled as normally distributed, based on the times to reach activation obtained from live cell imaging data (Fig. 4B & 6B)), the activation probability increased to P_{act} during the time of T_{up}. The cell remains at P_{act} for a period of T_{act} (which we modeled as normally distributed, based on the activation duration times we obtained from the live cell imaging data (Fig. 4C & 6C)). The activation duration times of siRNA actin treated cells were modeled as a mixture of 2 normal distributions. Finally, the activation probability decreased to P_{end} during the time of T_{down}. The parameters for the different probabilities were obtained from the binomial fit model of the quantified active alleles per cell at different time points of serum induction.

The model extracts T_{up} and T_{down} which give the best fit with the number of active alleles counted for each time point of serum release. We integrated the overall possible activation profiles (Fig. S7B), and for each set of parameters (T_{up} and T_{down}) we calculated the distribution of activation probability over the entire possible activation profiles (Fig. S7C). Assuming that the number of active alleles in a cell with a specific activation probability is binomially distributed, we integrated the number of active alleles over the activation probability of the entire population for each time point of serum release to find the distribution of active alleles per cell (Fig. S7D). We searched for a parameter set (T_{up} and T_{down}) which minimizes the Euclidian distance between the model results and the experimental data for each time point of serum release.

Supplemental Movie Legends

Movie S1, related to Figure 1.
The serum induction transcriptional pulse in a cell population.
Cells stably expressing MCP-YFP (green) were serum starved overnight. Serum was added and the activation of the β-actin alleles can be seen over time. Cells were imaged every 2.5 min for 1 hour.

Movie S2, related to Figure 1.
The serum induction transcriptional pulse in a single cell.
Cells stably expressing MCP-YFP (green) were serum starved overnight. Serum was added and the activation of the β-actin alleles can be seen over time in one cell. The cell was imaged every 2.5 min for 1 hour.

Movie S3, related to Figure 4.
β-actin transcription dynamics following SRF knockdown.
Cells stably expressing MCP-YFP (green) treated with Cy5-labeled siRNA to SRF (left cell cytoplasmic magenta dots) for 72 hrs. Cells were released with serum after serum starvation. Compare the activation kinetics between the siRNA-treated (left) and untreated (right) cells. Cells were imaged every 2.5 min, for 1 hour.

Movie S4, related to Figure 4.
β-actin transcription analysis following SRF knockdown.
Cell stably expressing MCP-YFP (green) treated with siRNA to SRF for 72 hrs. Cells were released with serum after serum starvation (left). The 3D surface of each allele was used to measure the sum intensity at each time point (right, blue surfaces). Inner box shows the same cell in 3D together with two neighboring cells (left inset) not transfected with siRNA to SRF, where activation kinetics are more rapid and stronger. Cells were imaged every 1 min, for 1 hour.

Movie S5, related to Figure 6.
β-actin transcription dynamics during serum release after β-actin knockdown.
Cells stably expressing MCP-YFP (green) treated with Cy5-labeled siRNA to actin for 72 hrs. Cells were released with serum after serum starvation and activation of the alleles was followed. The right-hand cell exhibits nuclear accumulation of single transcripts. Cells were imaged every 2.5 min, for 1 hour.

Movie S6, related to Figure 6.
The nucleoplasmic wave of newly synthesized β-actin mRNAs.
The right-hand cell from Movie S5 (scaled 2x) showing single mRNAs in an actin-depleted cell. The mRNAs travel from the activated alleles and diffuse throughout the whole nucleus. Cells were imaged every 2.5 min, for 1 hour.

Supplemental References

Lionnet, T., Czaplinski, K., Darzacq, X., Shav-Tal, Y., Wells, A.L., Chao, J.A., Park, H.Y., de Turris, V., Lopez-Jones, M., and Singer, R.H. (2011). A transgenic mouse for in vivo detection of endogenous labeled mRNA. Nat Methods *8*, 165-170.

Yunger, S., Rosenfeld, L., Garini, Y., and Shav-Tal, Y. (2010). Single-allele analysis of transcription kinetics in living mammalian cells. Nat Methods *7*, 631-633.

Yunger, S., Rosenfeld, L., Garini, Y., and Shav-Tal, Y. (2013). Quantifying the transcriptional output of single alleles in single living mammalian cells. Nat Protoc *8*, 393-408.