Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/16/eaay6515/DC1

Supplementary Materials for

Nuclear actin regulates inducible transcription by enhancing RNA polymerase II clustering

Mian Wei, Xiaoying Fan, Miao Ding, Ruifeng Li, Shipeng Shao, Yingping Hou, Shaoshuai Meng, Fuchou Tang, Cheng Li, Yujie Sun*

*Corresponding author. Email: sun_yujie@pku.edu.cn

Published 15 April 2020, *Sci. Adv.* **6**, eaay6515 (2020)
DOI: 10.1126/sciadv.aay6515

The PDF file includes:

Figs. S1 to S9
Legends for tables S1 and S2

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/16/eaay6515/DC1)

Tables S1 and S2

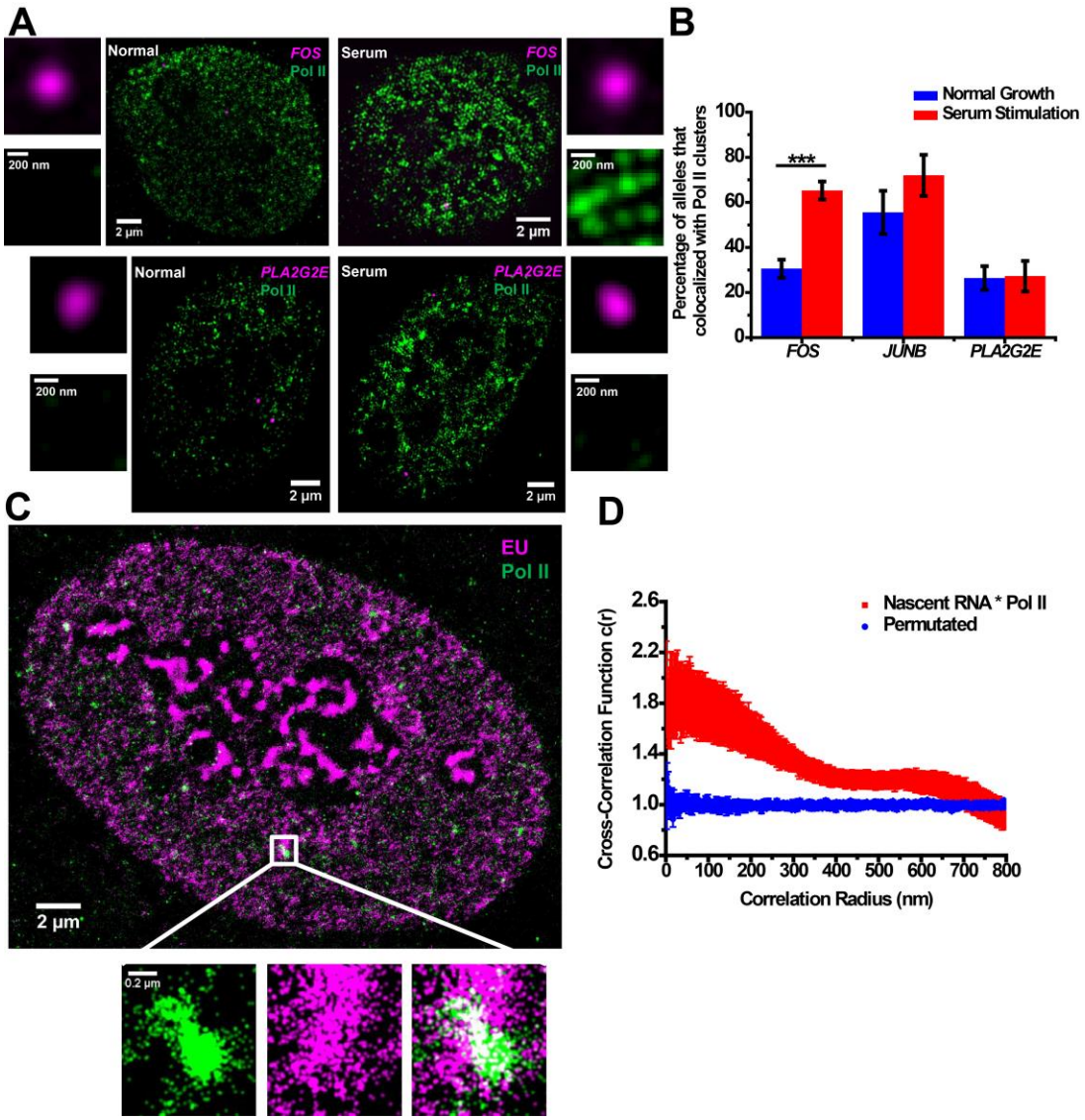

Fig. S1. Specific serum-response genes are localized within Pol II clusters for active transcription upon serum stimulation. (**A**) Representative Structured Illumination Microscopy (SIM) ImmunoFISH images showing Pol II (green) and gene loci (magenta) of *FOS* (top, n = 60 cells under normal-growth condition, n = 65 cells under serum-stimulation condition), *JUNB* (middle, n = 20 cells under normal-growth condition, n = 20 cells under serum-stimulation condition), and *PLA2G2E* (bottom, n = 40 cells under normal-growth condition, n = 26 cells under serum-stimulation condition). Zoom-in images of gene loci and Pol II are shown near the corresponding images of whole nuclei. (**B**) Percentage of alleles that colocalized with Pol II clusters in cells under normal-growth and serum-stimulation conditions. (*FOS*: n = 60 cells under normal-growth condition, n = 65 cells under serum-stimulation condition. *JUNB*: n = 20 cells under normal-growth condition, n = 20 cells under serum-stimulation condition. *PLA2G2E*: n = 40 cells under normal-growth condition, n = 26 cells under serum-stimulation condition). Data are shown as mean ± SD (bootstrapping). Statistical significance was determined by Chi-square test. ***p< 0.001. (**C**) Representative two-color PALM/STORM images showing nascent RNA (magenta) and Pol II (green) (n = 7 cells). Zoom-in images of the selected region are shown below the full-size image. (**D**) Quantification of colocalization by pair cross-correlation analysis. Data are shown as mean ± SEM. (n = 7 cells for original images, n = 7 for pixel-permutated images)

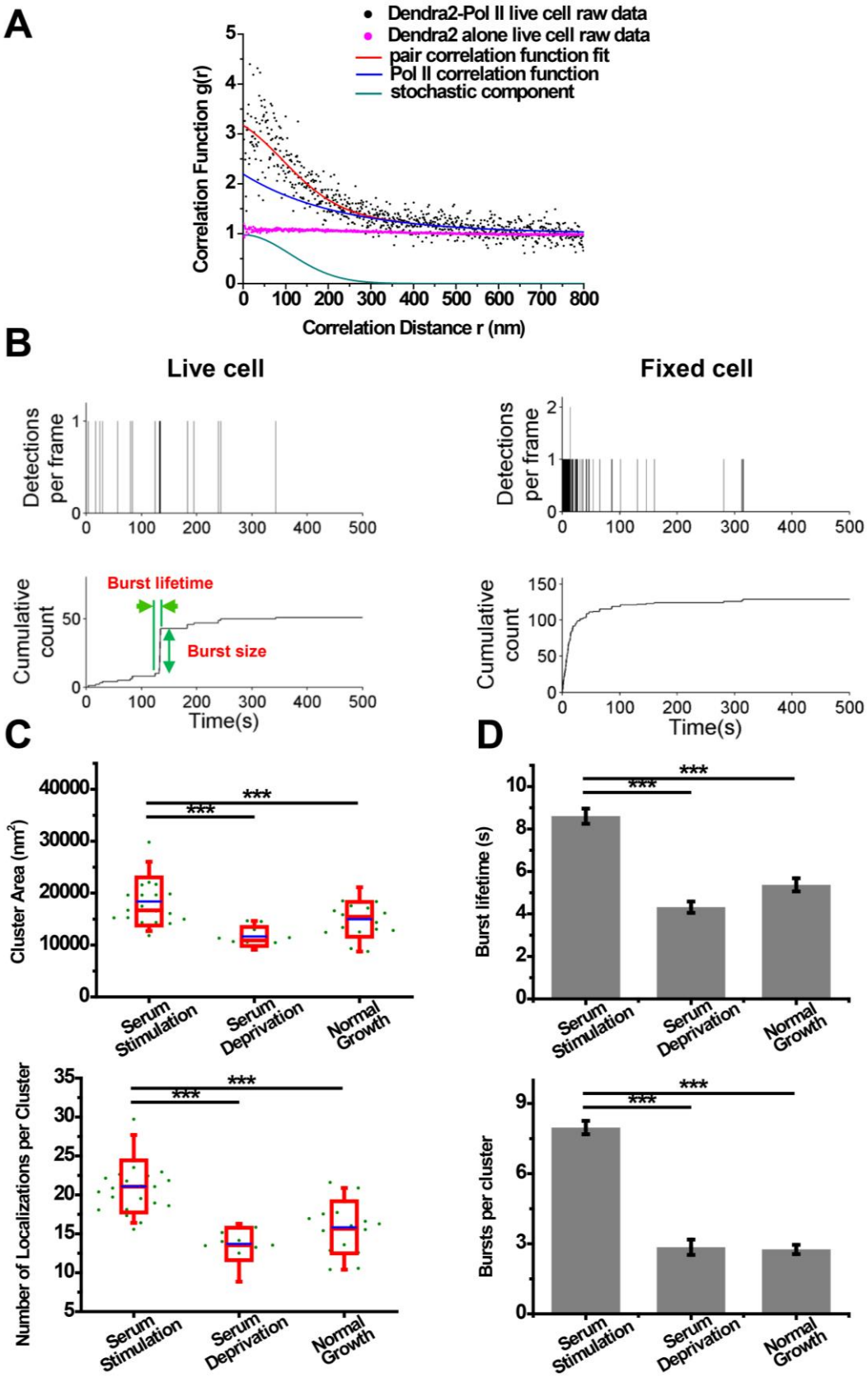
A
Dendra2-Pol II live cell raw data
Dendra2 alone live cell raw data
pair correlation function fit
Pol II correlation function
stochastic component
Correlation Function g(r)
Correlation Distance r (nm)
B
Live cell
Fixed cell
Detections per frame
Cumulative count
Burst lifetime
Burst size
Time(s)
C
D
Cluster Area (nm²)
Number of Localizations per Cluster
Burst lifetime (s)
Bursts per cluster
***
Serum Stimulation
Serum Deprivation
Normal Growth

Fig. S2. Dynamic clustering of Pol II revealed by pair correlation analysis, tcPALM, and SR-Tesseler. (**A**) Representative pair correlation analysis of the U2OS cell line stably expressing Dendra2-Pol II (black dots) and normal U2OS cells transiently expressing Dendra2 (pink dots). The correlation curve of Dendra2-Pol II was fit to a fluctuation model (red line) that separated Pol II correlation function (blue line) from the stochastic component due to blinking of Dendra2 (green line). (**B**) Representative tcPALM profiles of a Pol II cluster in a live cell (left) and a Pol II cluster in a fixed cell (right). (**C**) Cluster area and the number of localizations per cluster under serum-stimulation (n = 23 cells), serum-deprivation (n = 10 cells), and normal-growth conditions (n = 20 cells). Each green dot is the mean for all Pol II clusters from a single nucleus. The blue line is the mean for the whole population of nuclei under the indicated condition. The red box shows the SD around the mean. The red line within the red box is the median. The whiskers show 5% and 95%. (**D**) Burst lifetime and the number of bursts per cluster under serum-stimulation (n = 829 bursts of 104 clusters from 23 cells), serum-deprivation (n = 174 bursts of 61 clusters from 10 cells), and normal-growth conditions (n = 306 bursts of 111 clusters from 20 cells). Data are shown as mean ± SEM. Statistical significance was determined by one-way ANOVA with Tukey–Kramer test. ***$p < 0.001$.

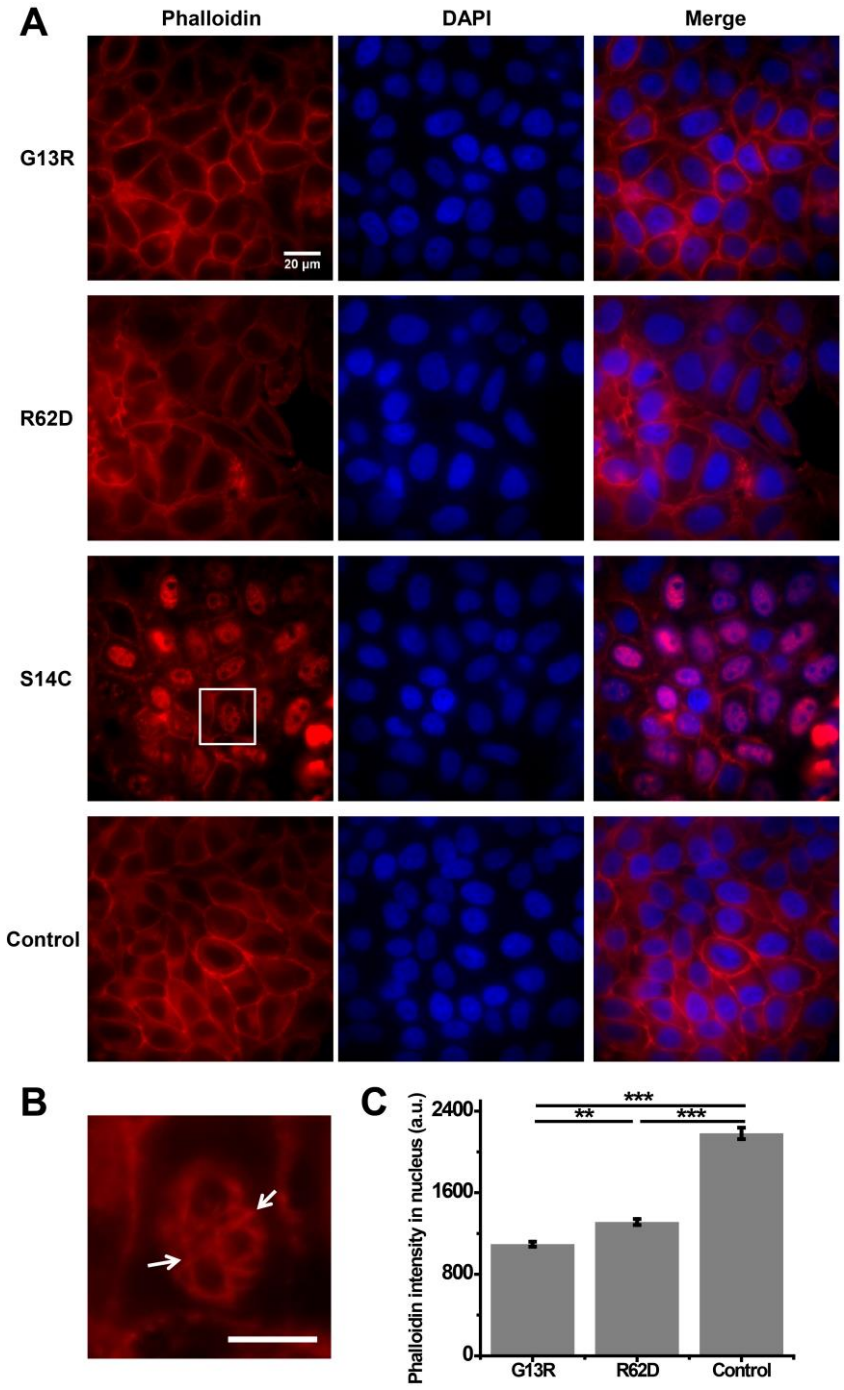

Fig. S3. Validation of actin mutants. (**A**) Representative epifluorescence images showing phalloidin-labeled F-actin (left), DAPI-labeled nuclei (middle), and the merge (right) in cells overexpressing actin mutants and control cells. (**B**) Zoom-in image of the white-box in A (Phalloidin image of cells overexpressing NLS-actin S14C). Arrows indicate actin filaments. Scale bar, 10 µm. (**C**) Phalloidin intensities in the nuclei of cells overexpressing NLS-actin G13R (n = 37 cells), cells overexpressing NLS-actin R62D (n = 42 cells), and control cells (n = 47 cells). Data are shown as mean ± SEM. Statistical significance was determined by one-way ANOVA with Tukey–Kramer test. **p< 0.01 and ***p< 0.001.

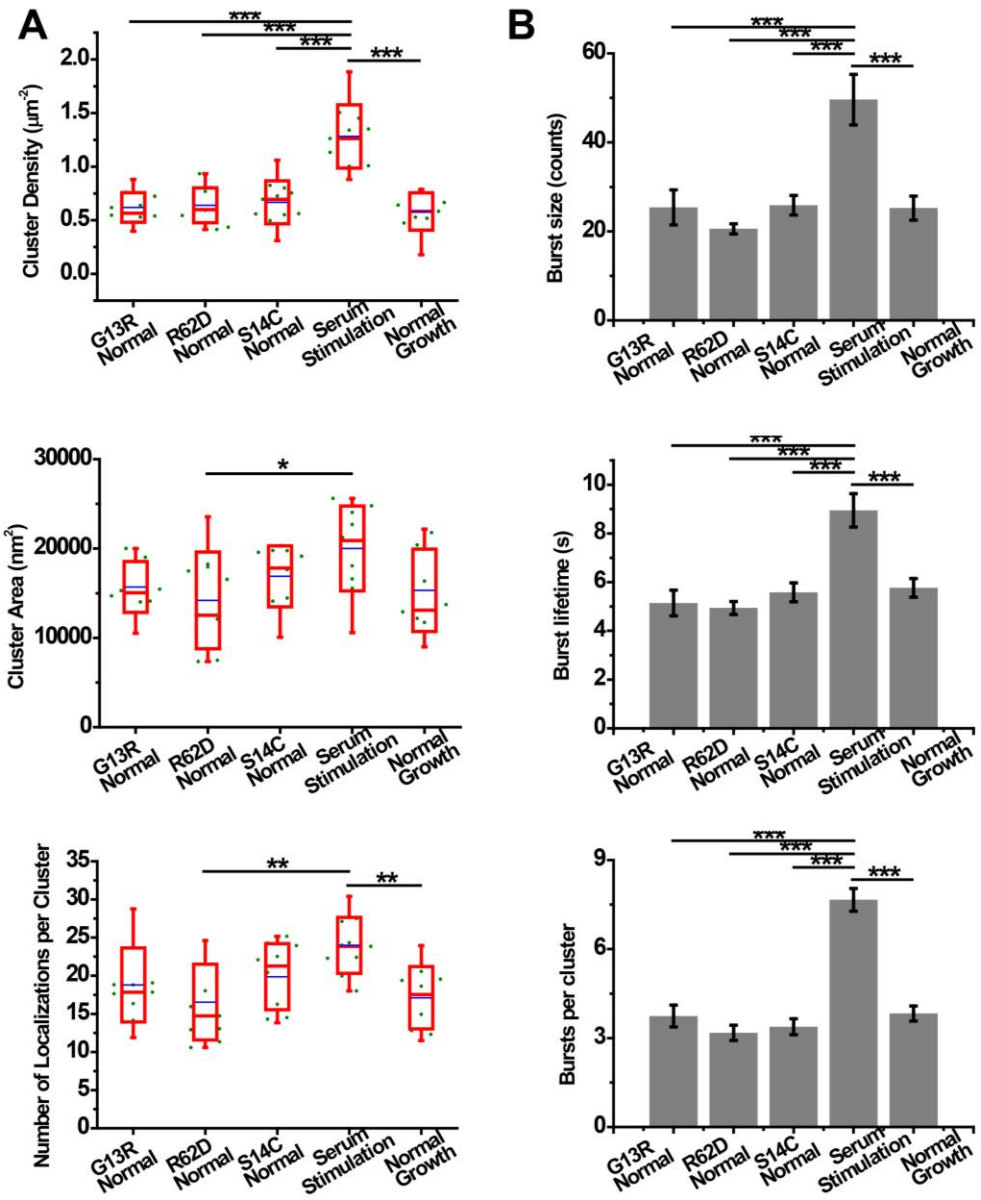

Fig. S4. Overexpression of actin mutants does not affect Pol II clustering under normal-growth condition. (**A**) Cluster density, cluster area, and the number of localizations per cluster in cells overexpressing different actin mutants under normal-growth condition (n = 10, 10, and 11 cells, respectively, from left to right), cells under serum-stimulation condition (n = 10 cells), and cells under normal-growth condition (n = 10 cells). Each green dot is the mean for all Pol II clusters from a single nucleus. The blue line is the mean for the whole population of nuclei under the indicated condition. The red box shows the SD around the mean. The red line within the red box is the median. The whiskers show 5% and 95%. (**B**) Burst size, burst lifetime, and the number of bursts per cluster in cells overexpressing different actin mutants under normal-growth condition (n = 232 bursts of 62 clusters from 10 cells, 216 bursts of 68 clusters from 10 cells, and 240 bursts of 71 clusters from 11 cells, respectively, from left to right), cells under serum-stimulation condition (n = 406 bursts of 53 clusters from 10 cells), and cells under normal-growth condition (n = 287 bursts of 75 clusters from 10 cells). Data are shown as mean ± SEM. Statistical significance was determined by one-way ANOVA with Tukey–Kramer test. *p< 0.05, **p< 0.01, and ***p< 0.001.

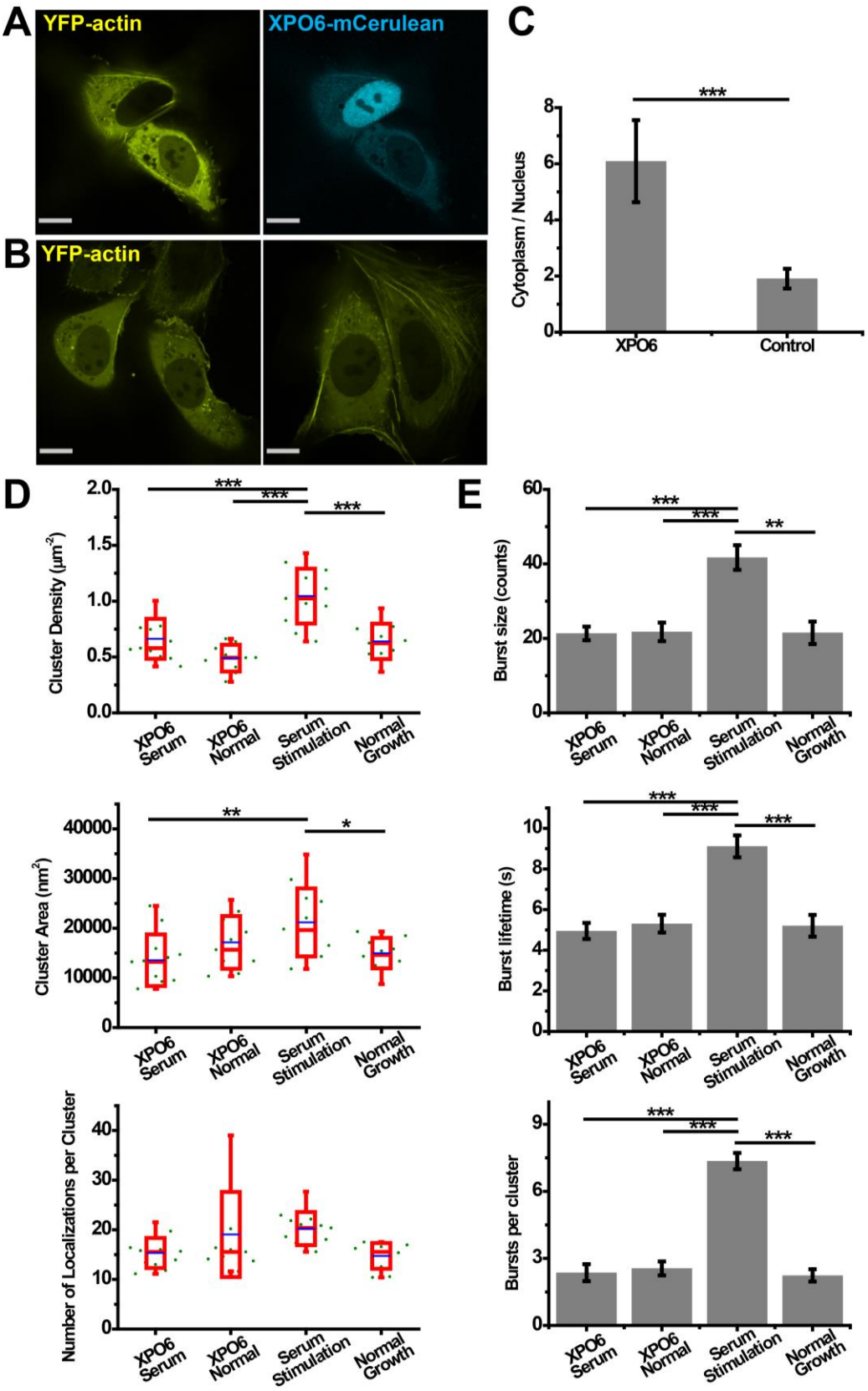
A
YFP-actin
XPO6-mCerulean
B
YFP-actin
C
Cytoplasm / Nucleus
***
XPO6
Control
D
Cluster Density (μm-2)
Cluster Area (nm2)
Number of Localizations per Cluster
XPO6 Serum
XPO6 Normal
Serum Stimulation
Normal Growth
E
Burst size (counts)
Burst lifetime (s)
Bursts per cluster

Fig. S5. Overexpression of XPO6 significantly reduces nuclear actin and abolishes serum-enhanced Pol II clustering. (**A**) Representative spinning-disk confocal images of cells cotransfected with YFP-actin (yellow) and XPO6-mCerulean (cyan). Note that the cell expressing XPO6 has a lower actin level in the nucleus compared to the neighboring cell. (**B**) Representative spinning-disk confocal images of cells transfected with YFP-actin (yellow). (**C**) The ratio of fluorescence in the cytoplasm to that in the nucleus in cells expressing both YFP-actin and XPO6-mCerulean ("XPO6", n = 36 cells) and control cells expressing only YFP-actin ("Control", n = 44 cells). Data are shown as mean ± SD. Statistical significance was determined by two-tailed *t*-test. *p< 0.05, **p< 0.01, and ***p< 0.001. Scale bar is 10 μm in A and B. (**D**) Cluster density, cluster area, and the number of localizations per cluster in cells overexpressing XPO6 under serum-stimulation (n = 12 cells) and normal-growth (n = 10 cells) conditions, cells under serum-stimulation condition (n = 12 cells), and cells under normal-growth condition (n = 10 cells). Each green dot is the mean for all Pol II clusters from a single nucleus. The blue line is the mean for the whole population of nuclei under the indicated condition. The red box shows the SD around the mean. The red line within the red box is the median. The whiskers show 5% and 95%. (**E**) Burst size, burst lifetime, and the number of bursts per cluster in cells overexpressing XPO6 under serum-stimulation (n = 144 bursts of 61 clusters from 12 cells) and normal-growth (n = 125 bursts of 49 clusters from 10 cells) conditions, cells under serum-stimulation condition (n = 397 bursts of 54 clusters from 12 cells), and cells under normal-growth condition (n = 114 bursts of 51 clusters from 10 cells). Data are shown as mean ± SEM. Statistical significance was determined by one-way ANOVA with Tukey–Kramer test. *p< 0.05, **p< 0.01, and ***p< 0.001.

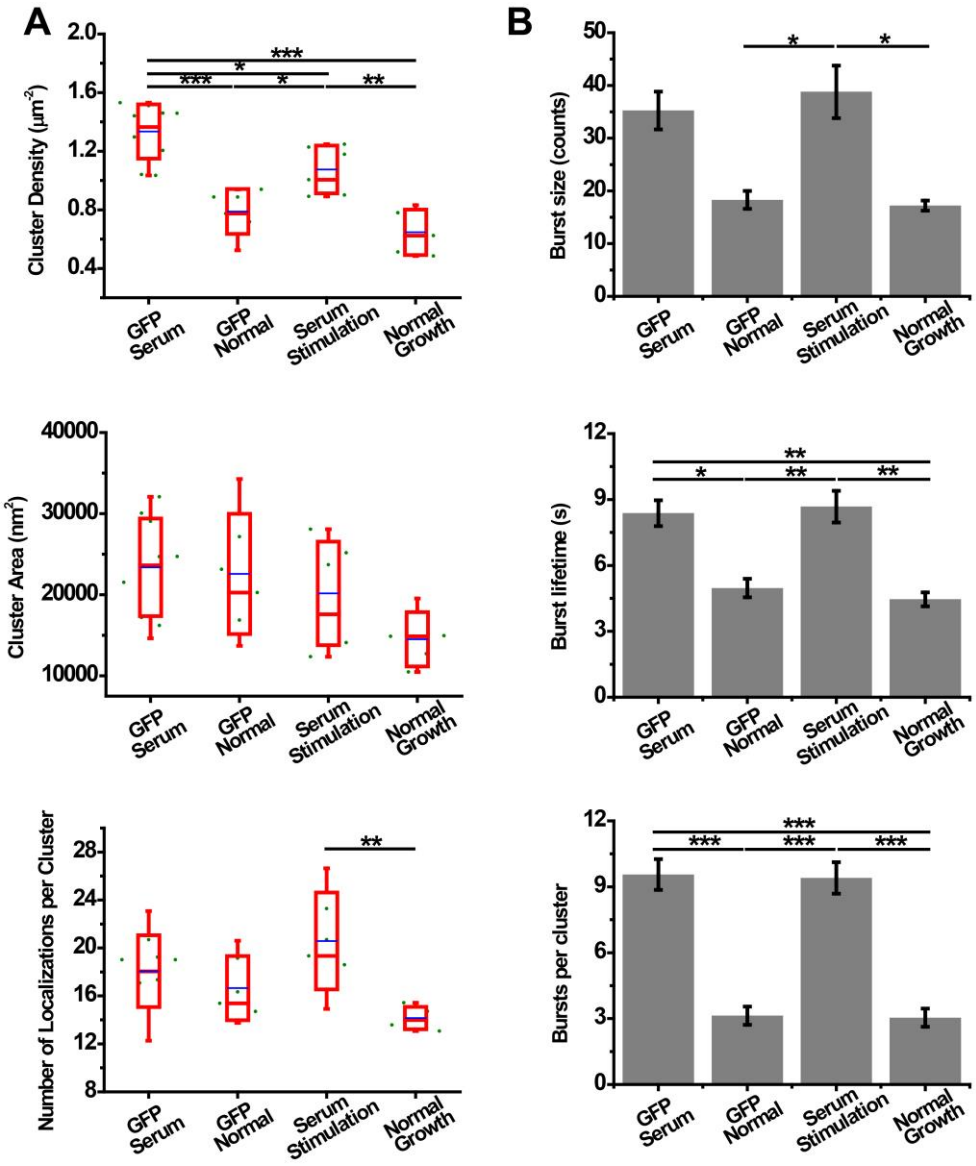

Fig. S6. Transfection procedure does not interfere with the enhanced-level Pol II clustering upon serum stimulation. (**A**) Cluster density, cluster area, and the number of localizations per cluster in cells overexpressing GFP under serum-stimulation (n = 10 cells) and normal-growth (n = 6 cells) conditions, cells under serum-stimulation condition (n = 6 cells), and cells under normal-growth condition (n = 5 cells). Each green dot is the mean for all Pol II clusters from a single nucleus. The blue line is the mean for the whole population of nuclei under the indicated condition. The red box shows the SD around the mean. The red line within the red box is the median. The whiskers show 5% and 95%. (**B**) Burst size, burst lifetime, and the number of bursts per cluster in cells overexpressing GFP under serum-stimulation (n = 325 bursts of 34 clusters from 10 cells) and normal-growth (n = 94 bursts of 30 clusters from 6 cells) conditions, cells under serum-stimulation condition (n = 235 bursts of 25 clusters from 6 cells), and cells under normal-growth condition (n = 76 bursts of 25 clusters from 5 cells). Data are shown as mean ± SEM. Statistical significance was determined by one-way ANOVA with Tukey–Kramer test. *p< 0.05, **p< 0.01, and ***p< 0.001.

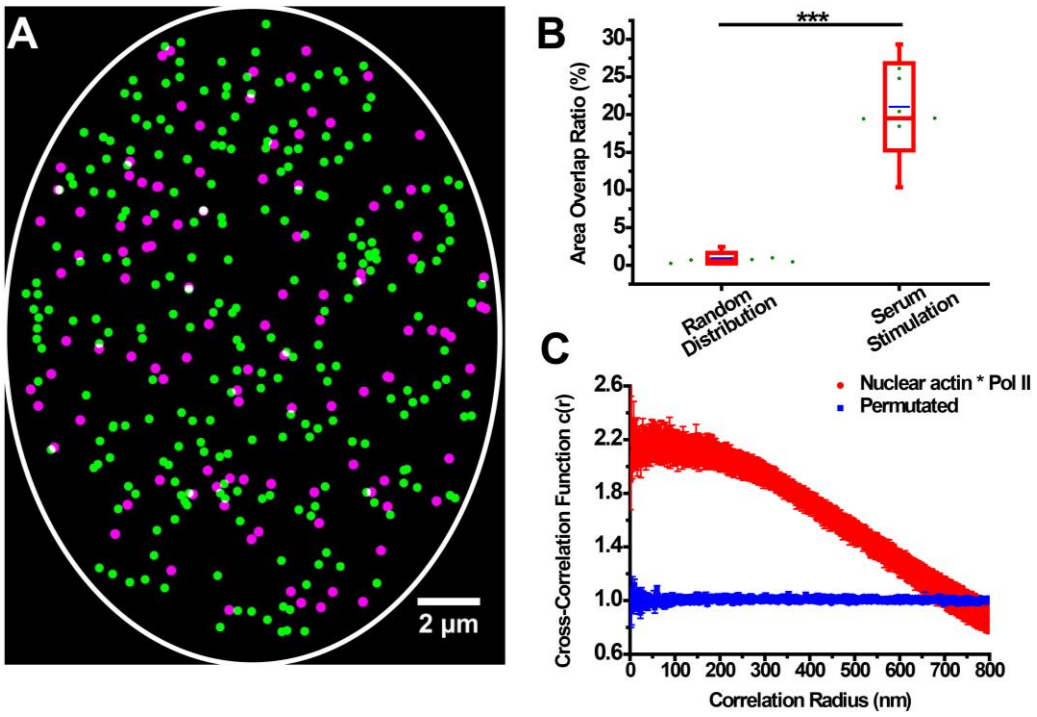

Fig. S7. Colocalization between nuclear actin filaments and Pol II upon serum stimulation is higher than the random level. (**A**) Simulation of randomly distributed clusters in an oval with the same cluster density and cluster area as those in cells upon serum stimulation. A representative simulation image shows nuclear actin clusters (magenta) and Pol II clusters (green). White dash lines delineate the cell nucleus. (**B**) Quantification of colocalization by calculating the area overlap ratio (n = 8 cells). The data of "Serum Stimulation" is the same as that in Fig. 5*B*, left. Statistical significance was determined by pair-sample two-tailed *t*-test. ***$p < 0.001$. (**C**) Quantification of colocalization between nuclear actin filaments and Pol II by pair cross-correlation analysis. Data are shown as mean ± SEM. (n = 7 cells upon serum stimulation for original images, n = 7 for pixel-permutated images)

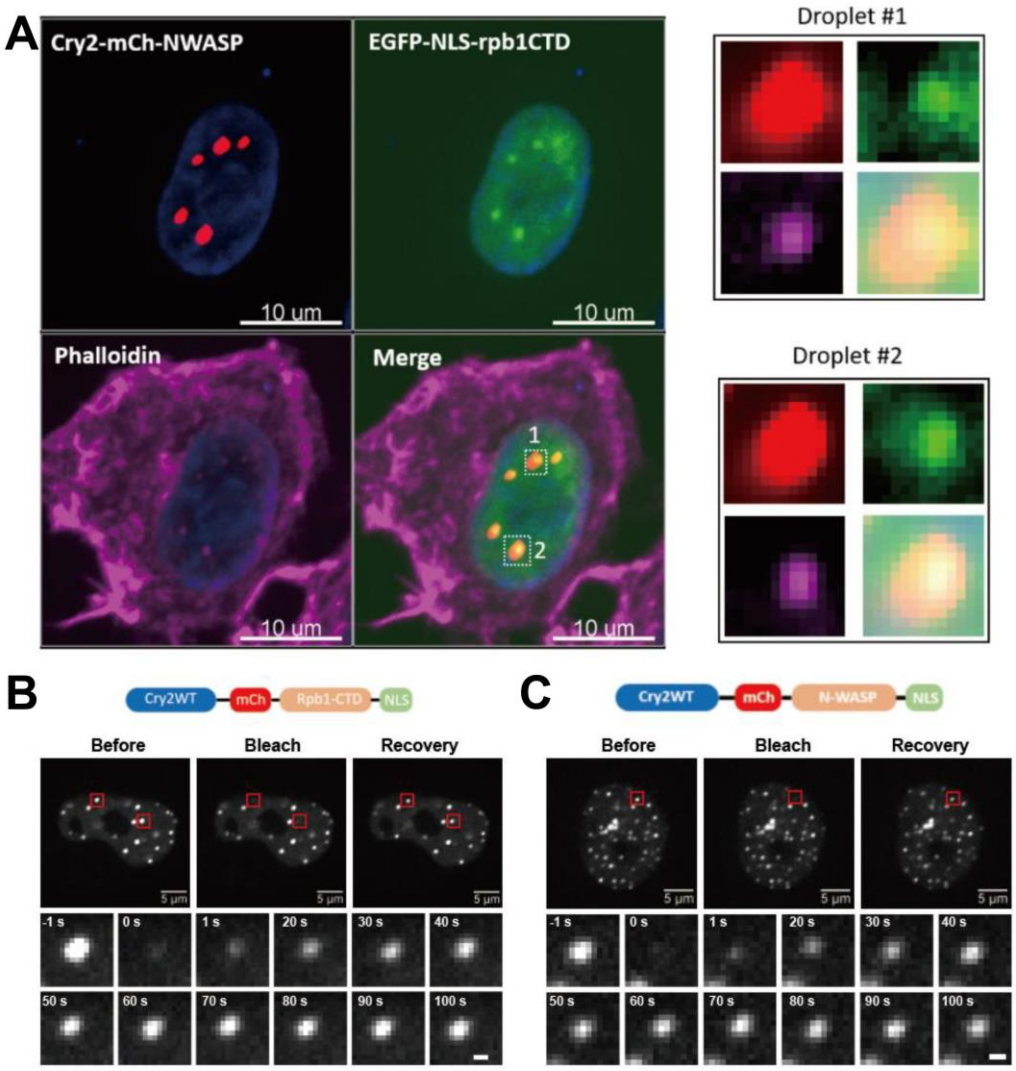

Fig. S8. N-WASP phase-separates with the C-terminal domain (CTD) of RPB1 and nuclear actin. (**A**) Left: representative images show that N-WASP phase-separated droplets incorporate the CTD of RPB1 and nuclear actin. Right: Zoom-in images of two droplets. (**B**) FRAP of CTD Opto-droplets displays liquid property. Top, Whole cell images before and after bleach. Box regions indicate the bleached droplets. Bottom, snapshots of whole droplet FRAP. (**C**) FRAP of N-WASP Opto-droplets displays liquid property. Top, Whole cell images before and after bleach. Box regions indicate the bleached droplets. Bottom, snapshots of whole droplet FRAP. Scale bars, 5 μm (top) and 0.5 μm (bottom) in B and C.

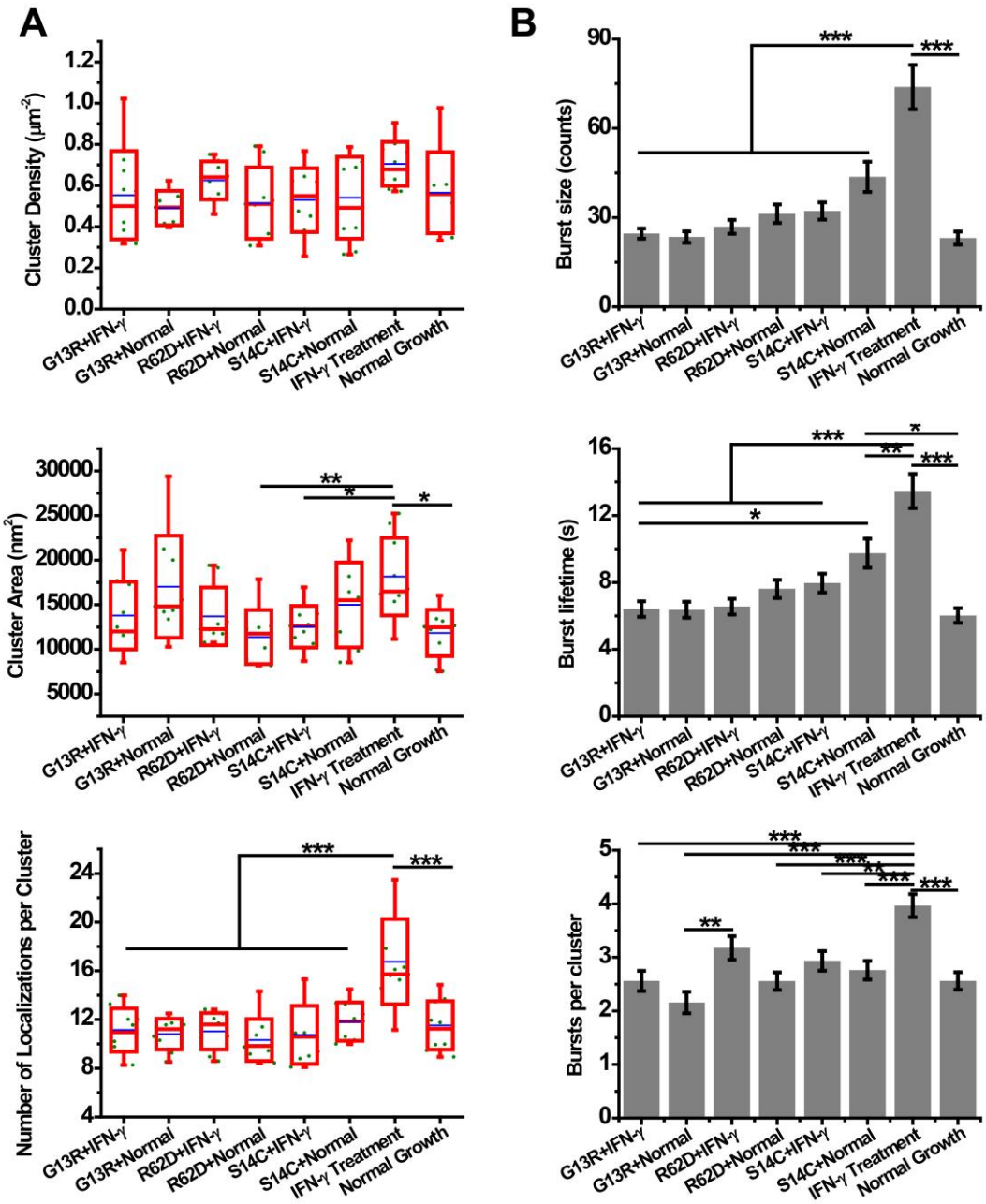

Fig. S9. Overexpression of actin mutants abolishes enhanced-level Pol II clustering upon IFN-γ treatment. (**A**) Cluster density, cluster area, and the number of localizations per cluster in cells overexpressing G13R under IFN-γ treatment and normal-growth conditions (n = 10 and 9 cells, respectively), cells overexpressing R62D under IFN-γ treatment and normal-growth conditions (n = 9 and 9 cells, respectively), cells overexpressing S14C under IFN-γ treatment and normal-growth conditions (n = 9 and 10 cells, respectively), cells under serum-stimulation condition (n = 10 cells), and cells under normal-growth condition (n = 10 cells). Each green dot is the mean for all Pol II clusters from a single nucleus. The blue line is the mean for the whole population of nuclei under the indicated condition. The red box shows the SD around the mean. The red line within the red box is the median. The whiskers show 5% and 95%. (**B**) Burst size, burst lifetime, and the number of bursts per cluster in cells overexpressing G13R under IFN-γ treatment and normal-growth conditions (n = 128 bursts of 50 clusters from 10 cells and n = 97 bursts of 45 clusters from 9 cells, respectively), cells overexpressing R62D under IFN-γ treatment and normal-growth conditions (n = 146 bursts

of 46 clusters from 9 cells and n = 115 bursts of 45 clusters from 9 cells, respectively), cells overexpressing S14C under IFN-γ treatment and normal-growth conditions (n = 132 bursts of 45 clusters from 9 cells and n = 138 bursts of 50 clusters from 10 cells, respectively), cells under serum-stimulation condition (n = 218 bursts of 55 clusters from 10 cells), and cells under normal-growth condition (n = 128 bursts of 50 clusters from 10 cells). Data are shown as mean ± SEM. Statistical significance was determined by one-way ANOVA with Tukey–Kramer test. *p< 0.05, **p< 0.01, and ***p< 0.001.

Supplementary Tables (provided as separate Excel files)

Table S1. Differentially expressed genes and the corresponding functional annotations.

Table S2. Primers for the PCR-based synthesis of FISH probes.