Supplementary Materials for

Protein phase separation provides long-term memory of transient spatial stimuli

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This PDF file includes:

Figs. S1 to S5

Supplementary Figure 1

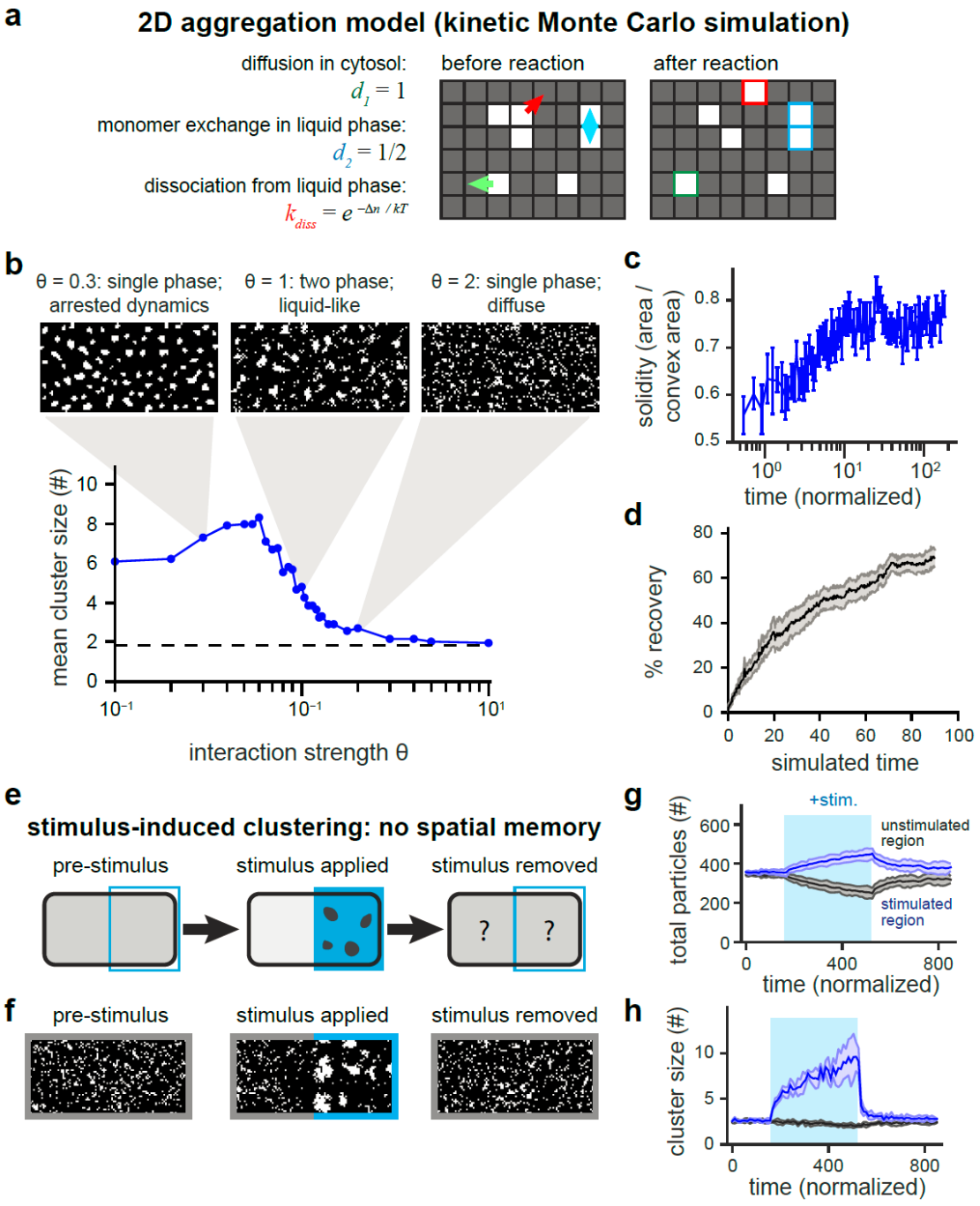

Figure S1, related to Figure 1. Interrogating a simple computational model of phase separation. (**a**) Schematic of different reactions in our Monte Carlo simulation. (**b**) Scan of mean cluster size as a function of the protein-protein association strength θ. Inset images show 2D grid after 10^5 iterations of the kinetic Monte Carlo (~18 time units). (**c**) Solidity of clusters (mean + SD for 10 clusters) shown during droplet formation for the $\theta = 1$ value of interaction strength. A

solidity of 1 would indicate that each aggregate fills its convex hull. (**d**) Simulated FRAP recovery for clusters formed in response to the $\theta = 1$ value of interaction strength. (**e**) Schematic of stimulus application and removal. (**f**) Representative images of 2-D lattice showing local clustering during stimulation, with diffuse subunits before and after stimulation. (**g,h**) Total number of subunits (**g**) and mean cluster size (**h**) in the stimulated (right half of lattice) and unstimulated (left half of lattice) regions before, during and after local stimulation. In every panel, error bars show mean + SD.

Supplementary Figure 2

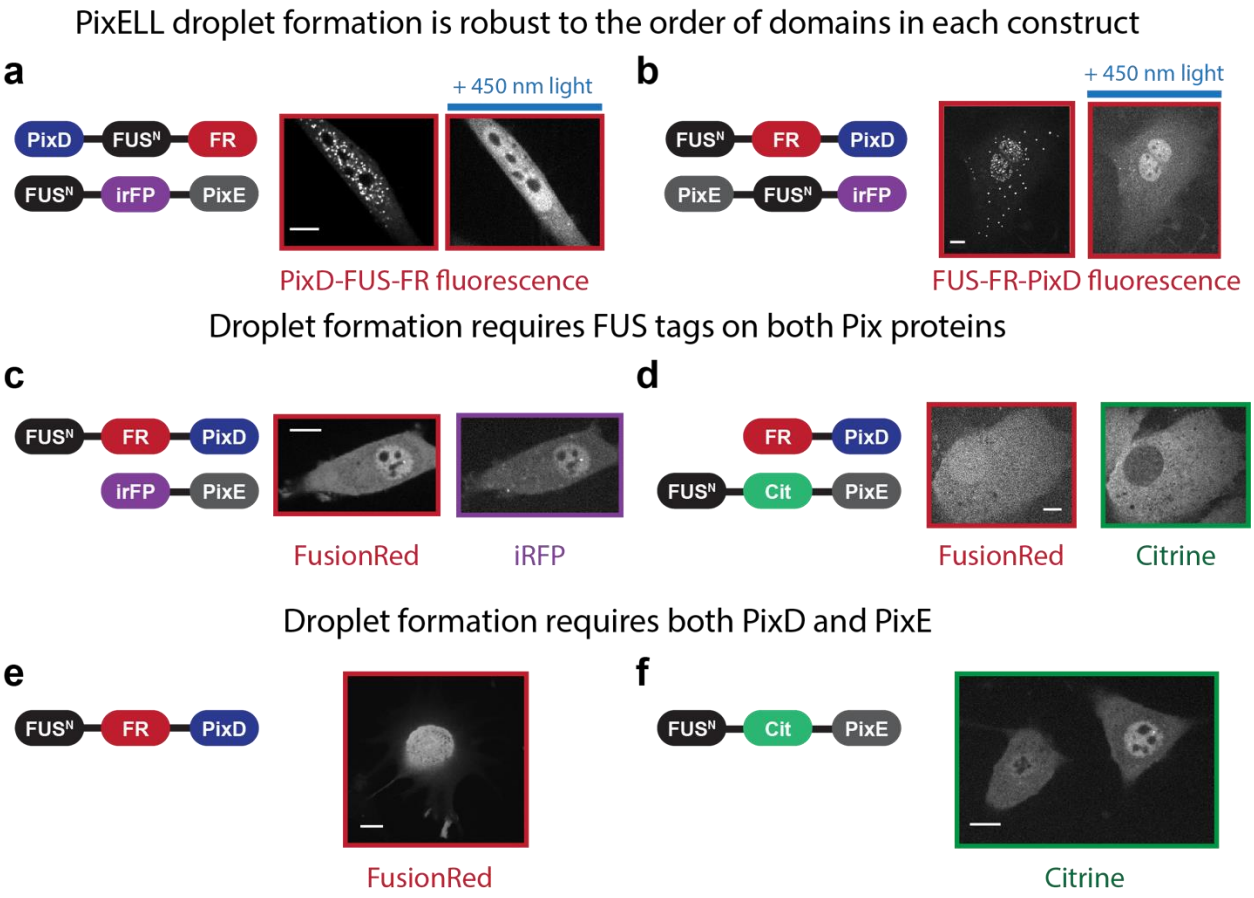

Figure S2, related to Figure 2. Protein domain requirements for functional PixELL expression. All panels show representative still images of cells expressing constructs indicated in cartoon diagrams. In all images scale bars indicate 10µm. (**a,b**) PixD (**a**) and PixE (**b**) still achieve functional, light-switchable clustering when IDRs + fluorescent proteins are fused *via* C-terminal attachment. (**c,d**) Functional light-switchable clustering requires intrinsically disordered protein region (IDR) fusion to *both* PixE (**c**) and PixD (**d**). (**e,f**) Functional light-switchable clustering requires expression of both IDR-tagged PixD and PixE – cells expressing only one component exhibit diffuse localization.

Supplementary Figure 3

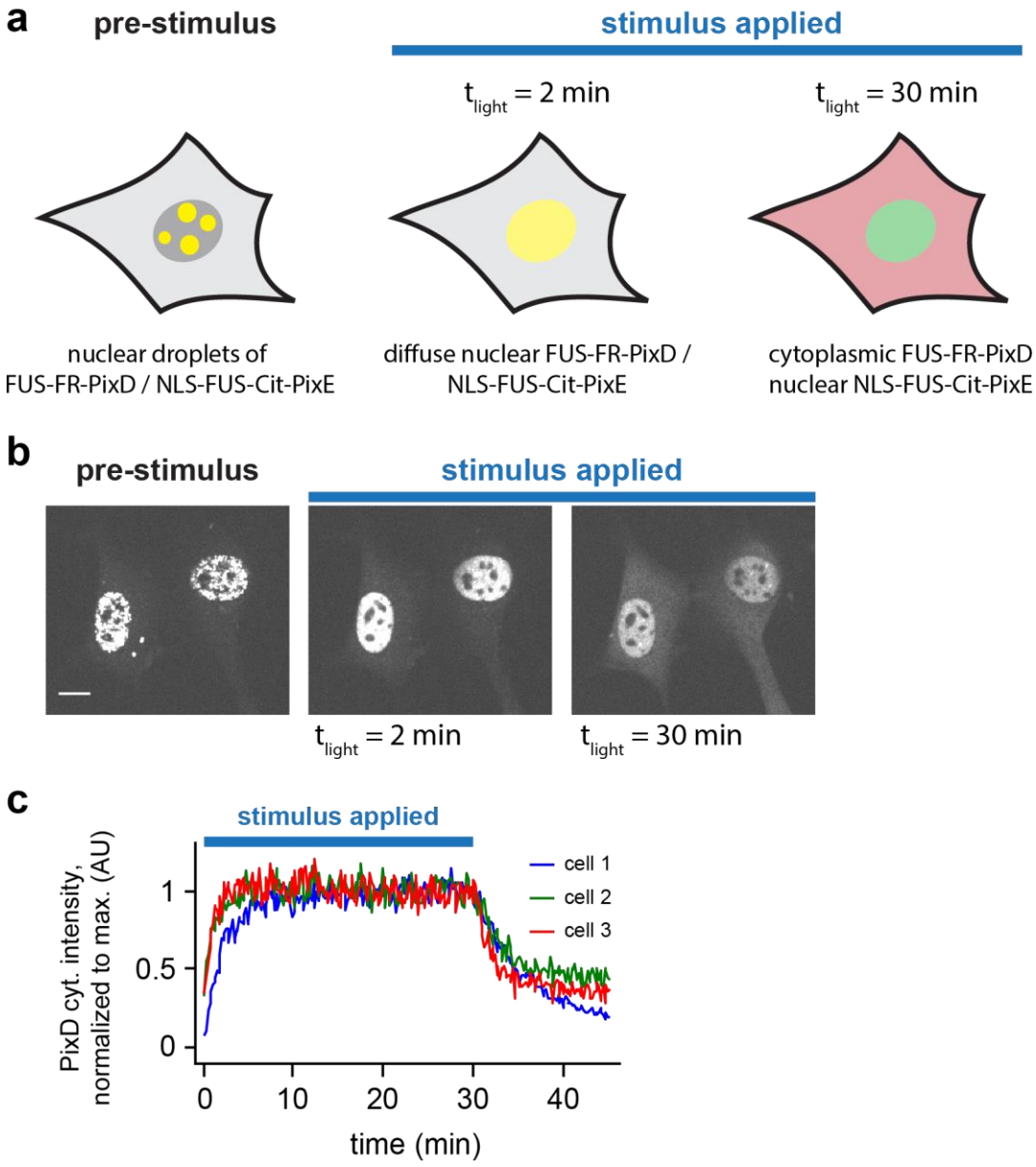

Figure S3, related to Figure 2. Photoswitchable nuclear—cytoplasmic localization using subcellularly-localized Pix proteins. (**a**) Schematic showing nuclear-localized PixELLs formed by FUS^N-FusionRed-PixD and NLS-FUS^N-Citrine-PixE. Droplets would be expected to dissolve shortly after light exposure, and PixD (which lacks an NLS) should be able to subsequently diffuse into the cytoplasm. (**b**) Experimental data of the constructs in **a** showing immediate dissolution of droplets, followed by redistribution of PixD into the cytoplasm. Scale bar indicates 10µm. (**c**) Quantification of the cytoplasmic FUS^N-FusionRed-PixD intensity during the experiment shown in **b** for three independent cells.

Supplementary Figure 4

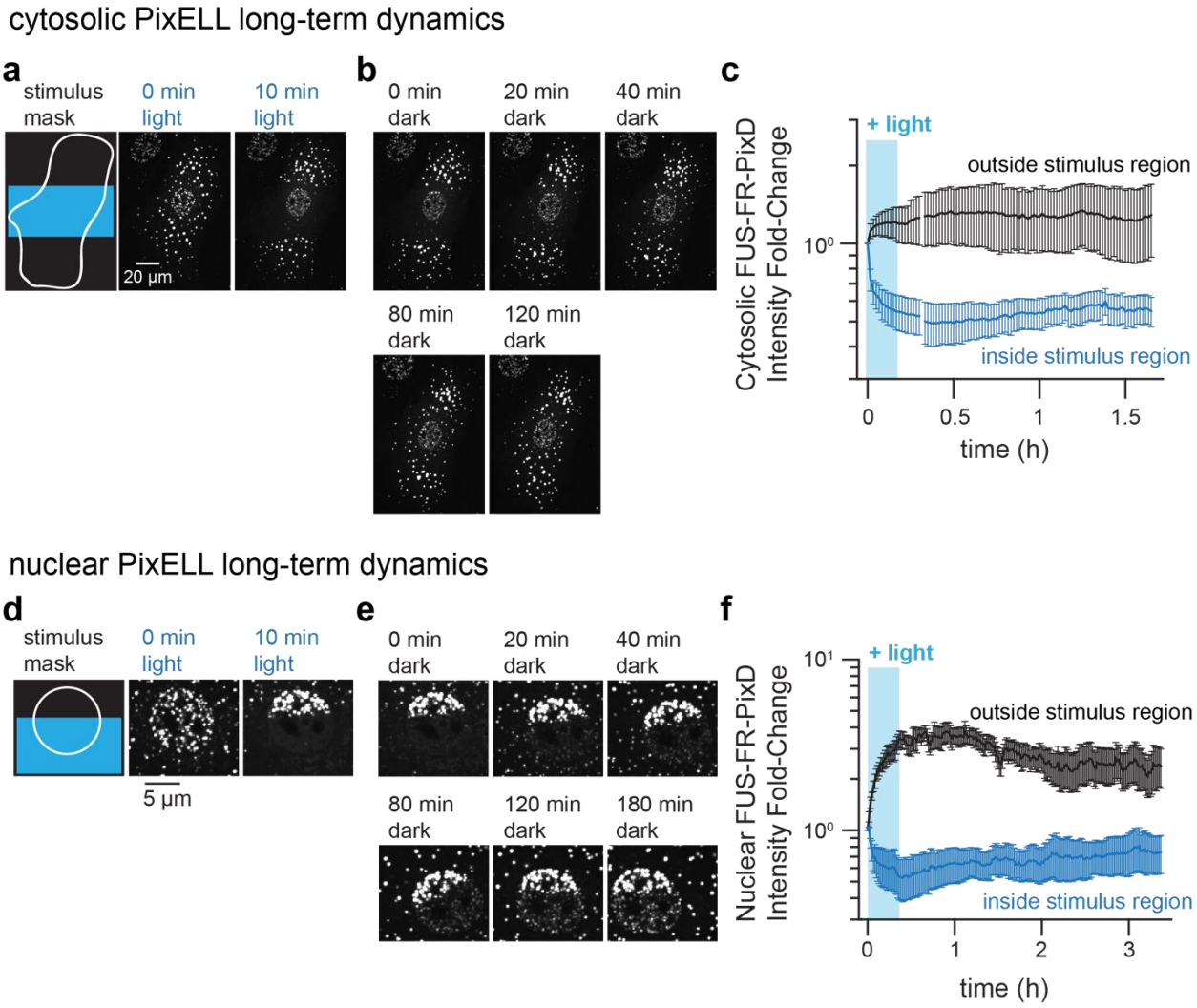

Figure S4, related to Figure 3. Droplet-induced spatial asymmetry is long-lived. (**a**) Schematic and images of local 450nm illumination in the cytosol of NIH 3T3 cells expressing the PixELL system. Fluorescent images of FUS^{N}-FR-PixD are shown both before and during light stimulation. Scale bar indicates 20µm. (**b**) Images of the same NIH-3T3 cell are shown after transient illumination. (**c**) Quantification of Fold Change in cytoplasmic intensity inside and outside stimulation mask region. Mean + SEM for 6 cells are shown. (**d**) Schematic and images of local 450nm illumination in the cytosol of NIH 3T3 cells expressing the PixELL system. Fluorescent images of FUS^{N}-FR-PixD are shown both before and during light stimulation. Scale bar indicates 5µm. (**e**) Images of the same NIH-3T3 cell are shown after transient illumination. (**f**) Quantification of Fold Change in cytoplasmic intensity inside and outside stimulation mask region. Mean + SEM for 5 cells are shown.

Supplementary Figure 5

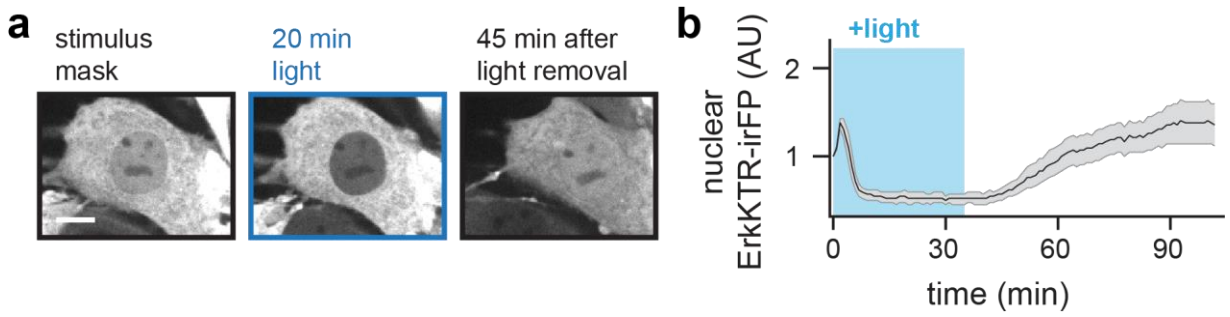

Figure S5: FGFR1-optoDroplet control retraction in NIH-3T3 cells. Related to Figure 6. (a) Images of Erk Kinase Translocation Reporter (KTR) during and after blue light stimulation of FGFR1-optoDroplet. Scale bar indicates 10µm. **(b)** Quantification of nuclear ErkKTR via mean irFP fluorescence during and after blue light stimulation, for optoDroplet-FGFR1. Mean + SEM are shown for N=5 cells.