

Supplementary Information

Light-Inducible Activation of Cell Cycle Progression in *Xenopus* Egg Extracts Under Microfluidic Confinement

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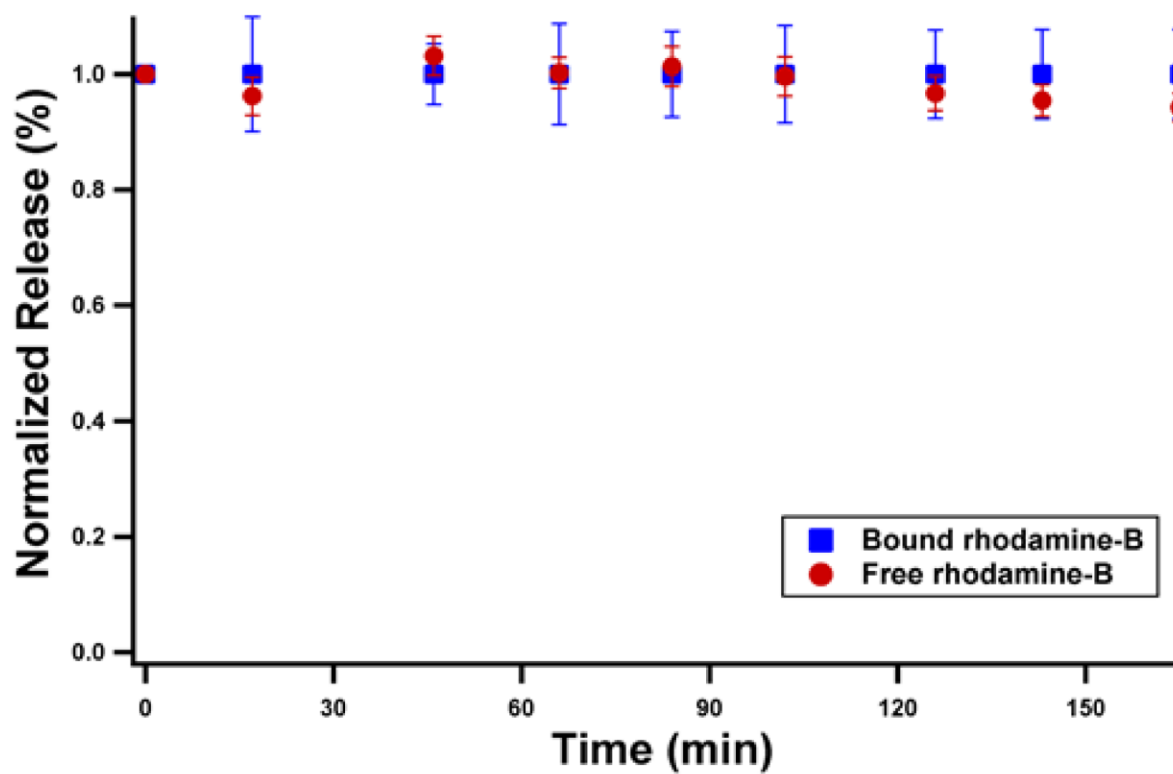
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PEGDA (Mn 3.4k)	
Weight %	Mesh Size (Å)
5%	95
10%	74
20%	43
PEGdiPDA	
Weight %	Mesh Size (Å)
8.20%	80

Table S1. Calculated theoretical mesh sizes PEGDA and PEGdiPDA

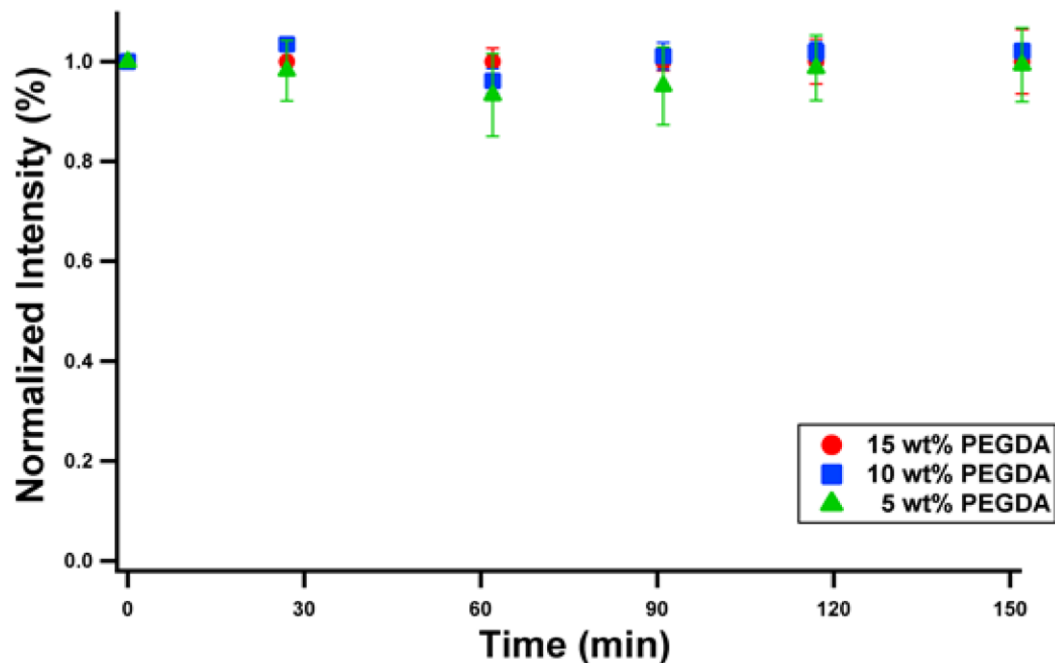


Figure S2. Change in mean fluorescent intensity of GST-GFP-NLS encapsulated within PEGDA posts over time. Intensities are normalized to the intensity of 15% w/w PEGDA (Mn 3.4k). Here, we demonstrate that we may prevent the release of GST-GFP-NLS using PEGDA. Posts of equal size were polymerized on the same microfluidic device from three separate solutions containing equimolar concentrations of GST-GFP-NLS and 15% w/w, 10% w/w, or 5% w/w PEGDA (Mn 3.4k), respectively. Comparison of the theoretical mesh size of our PEGdiPDA, and that of the three experimental solutions, shows that the PEGdiPDA mesh falls within those explicated by our experiments (Table S1). We show that the change in mean fluorescent intensity (ImageJ) of the protein GST-GFP-NLS are equivalent across all concentration values, indicating that PEGDA retains the GST-GFP-NLS protein (Figure S2). A Tukey's one-way ANOVA assuming equal variance rejects the null hypothesis at a significance level of $\alpha = 0.05$ (Minitab 18, n=16).

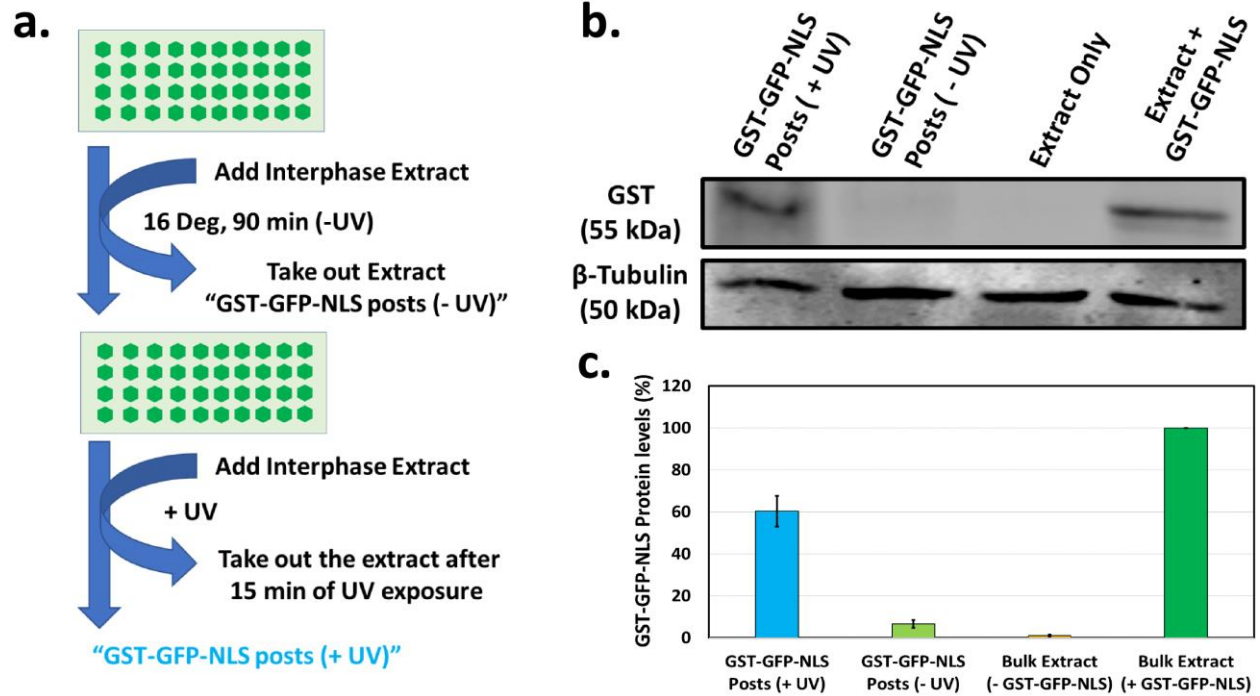


Figure S3. GST-GFP-NLS protein retention and release from hydrogel posts. a) Schematics of the experiment (not drawn to scale). Device containing GST-GFP-NLS-post arrays (in green) were formed as described previously and was filled with interphase extract and incubated at 16 °C for 90 mins without UV exposure. Afterward this incubation period, the interphase extract was collected from the devices leaving the posts intact. We then used the same device and added an equal volume of interphase, degraded the posts by UV exposure, and incubated at 16 °C for 15 mins. After 15 mins incubation period, we collected the interphase extract. b) A represented western blot (probed using GST-antibody and β -Tubulin antibody) of equal volume samples for four different conditions: GST-GFP-NLS Posts (+ UV), GST-GFP-NLS Posts (-UV), Interphase extract only, and Interphase extract containing equivalent concentration of GST-GFP-NLS protein. c) Quantification of GST-GFP-NLS protein levels normalized to β -Tubulin control in four different condition mentioned above. Error bars represent standard deviation. Data are shown from three independent experiments.

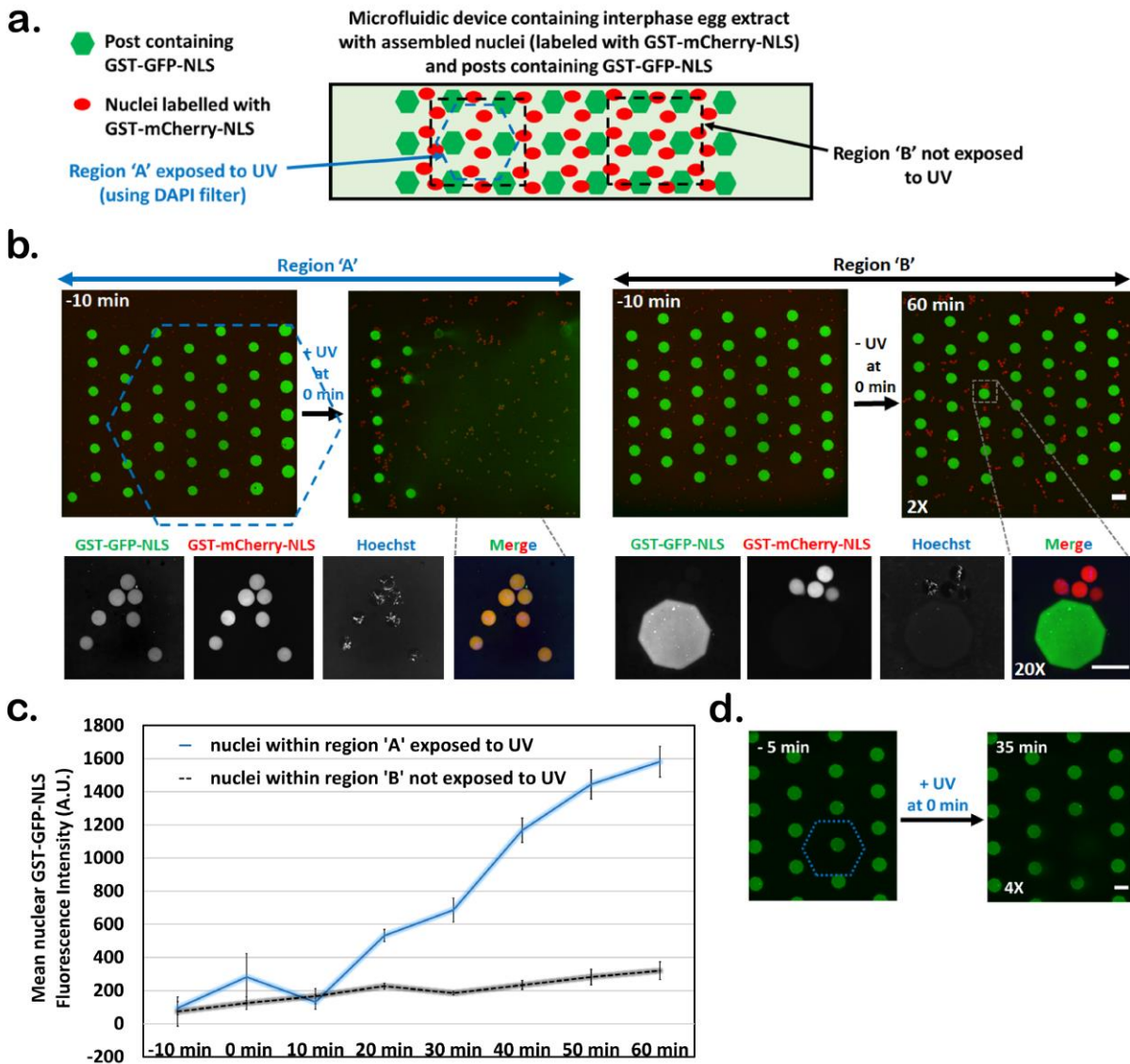


Figure S4. Light-dependent spatial control over the release of GST-GFP-NLS into interphase egg extracts and its nuclear import dynamics. a) Schematics of the experiment (not drawn to scale). A device containing GST-GFP-NLS-post arrays (in green) was formed as described previously and was filled with interphase extract with assembled nuclei labeled with GST-mCherry-NLS (in red). Afterward, within the same device, region 'A' (represented by a dotted blue hexagon) was exposed to UV at 0 min time interval (using the DAPI filter on confocal microscope; exposure time: 2 x 30 sec), whereas region 'B' was not exposed to UV. The centers of region 'A' and 'B' are separated by 15.8 mm. Then zoom-out and zoom-in images were taken on a confocal microscope using a 2X and 20X objectives, respectively. b) Shown are the images of region 'A' before UV exposure at -10 min and after UV exposure at 60 min, and region 'B' at -10 min and at 60 min without UV exposure. Also shown are the zoom-in images of nuclei and post within the region 'A' and 'B' (depicted by dotted grey boxes) and labeled with Hoechst to stain DNA (blue), GST-mCherry-NLS to stain intact nuclei (Red), and GST-GFP-NLS to assess its import by nuclei (Green). c) Nuclei were monitored within region 'A' and 'B' with confocal microscopy and the import dynamics of GST-GFP-NLS was quantified as mean nuclear GST-GFP-NLS fluorescence intensity (background subtracted) over the span of 70 min. Error bars represent standard deviation. n = number of nuclei; n minimum = 3, n maximum = 4. d) Images show the spatial control over post-degradation using our confocal microscope. The dotted blue hexagon represents the region exposed with UV. Images were taken using 4X objective. Data shown is from a single experiment. Scale bar in images from 2X, 4X, and 20X objectives represent 250 μ m, 200 μ m, and 100 μ m, respectively.

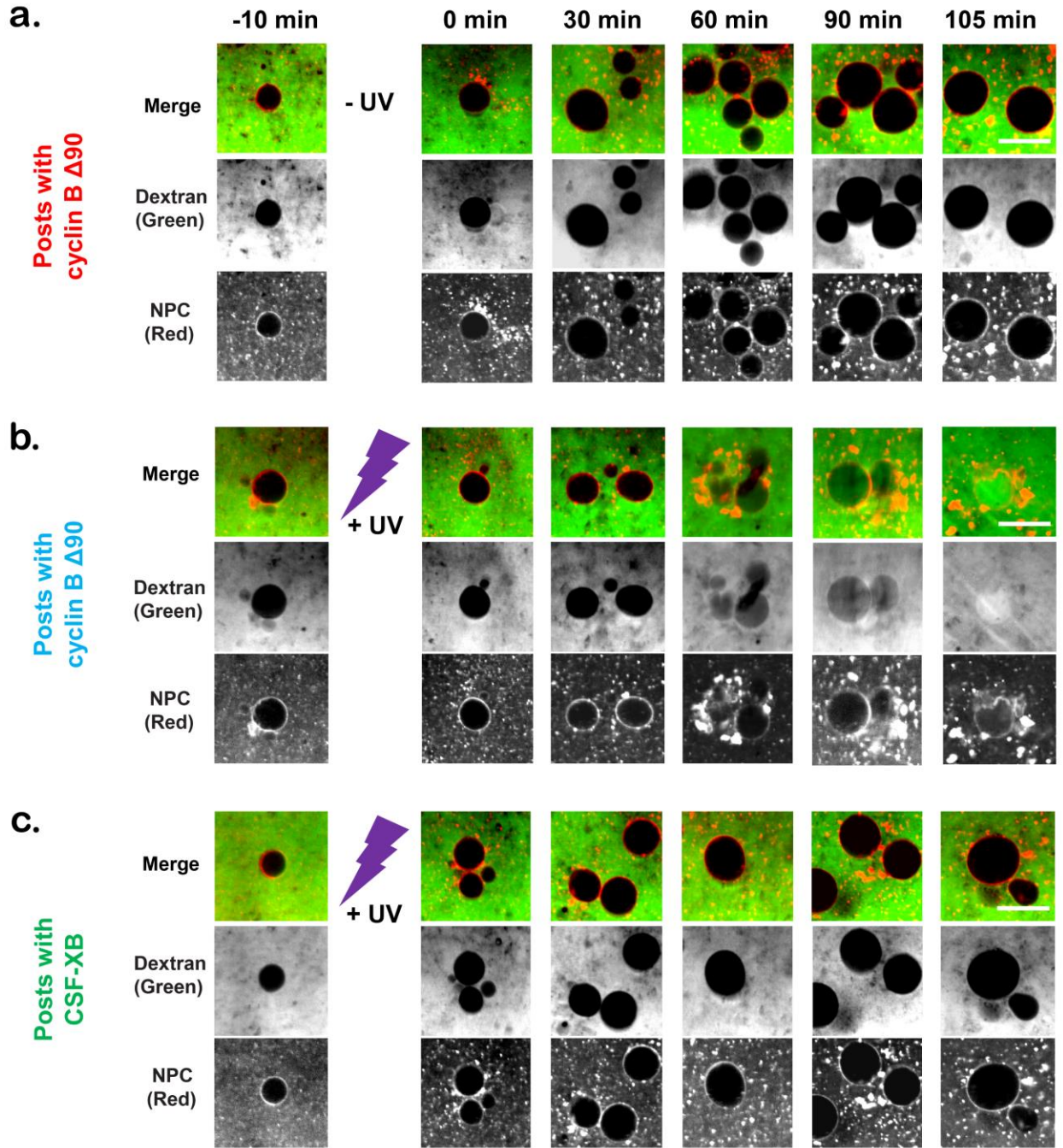


Figure S5. Single channel grayscale representation of the images shown in figure 3.

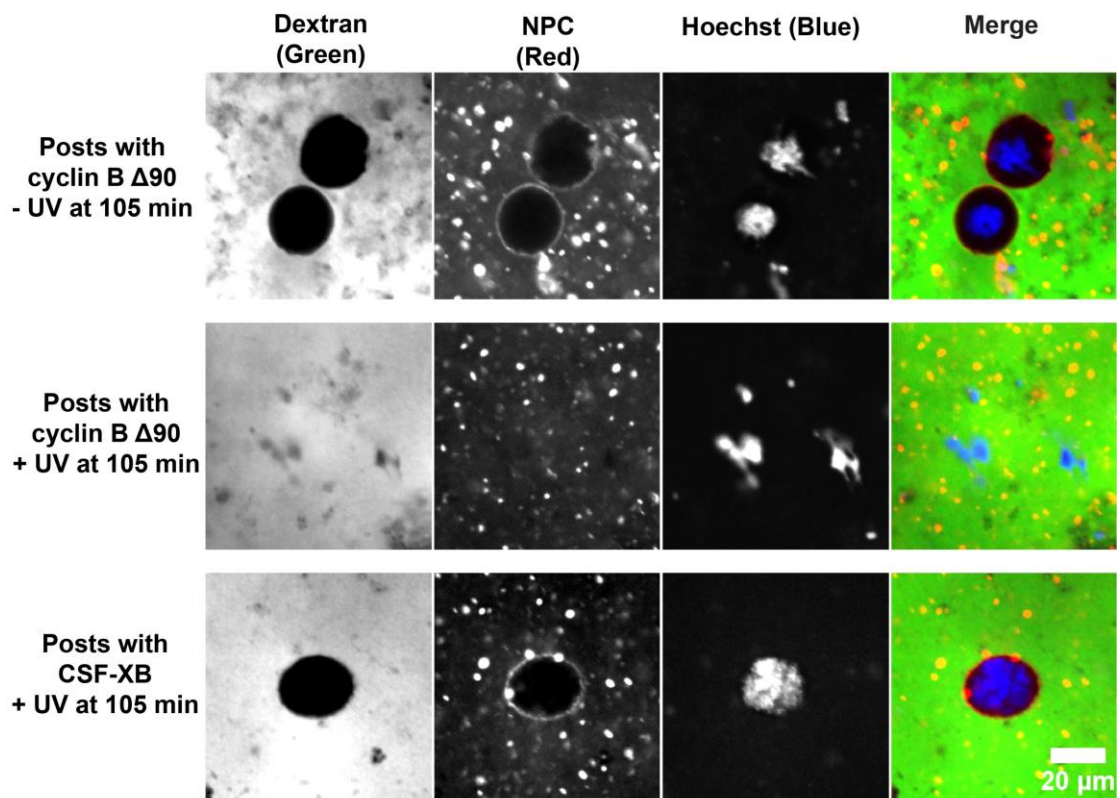


Figure S6. Single channel representation of the images at time point 105 min as shown in figure 3. Hoechst 33342 staining was used to confirm the nuclear envelope breakdown (NEBD) around chromatin following UV exposure in devices containing posts with cyclin B Δ 90 (middle row).

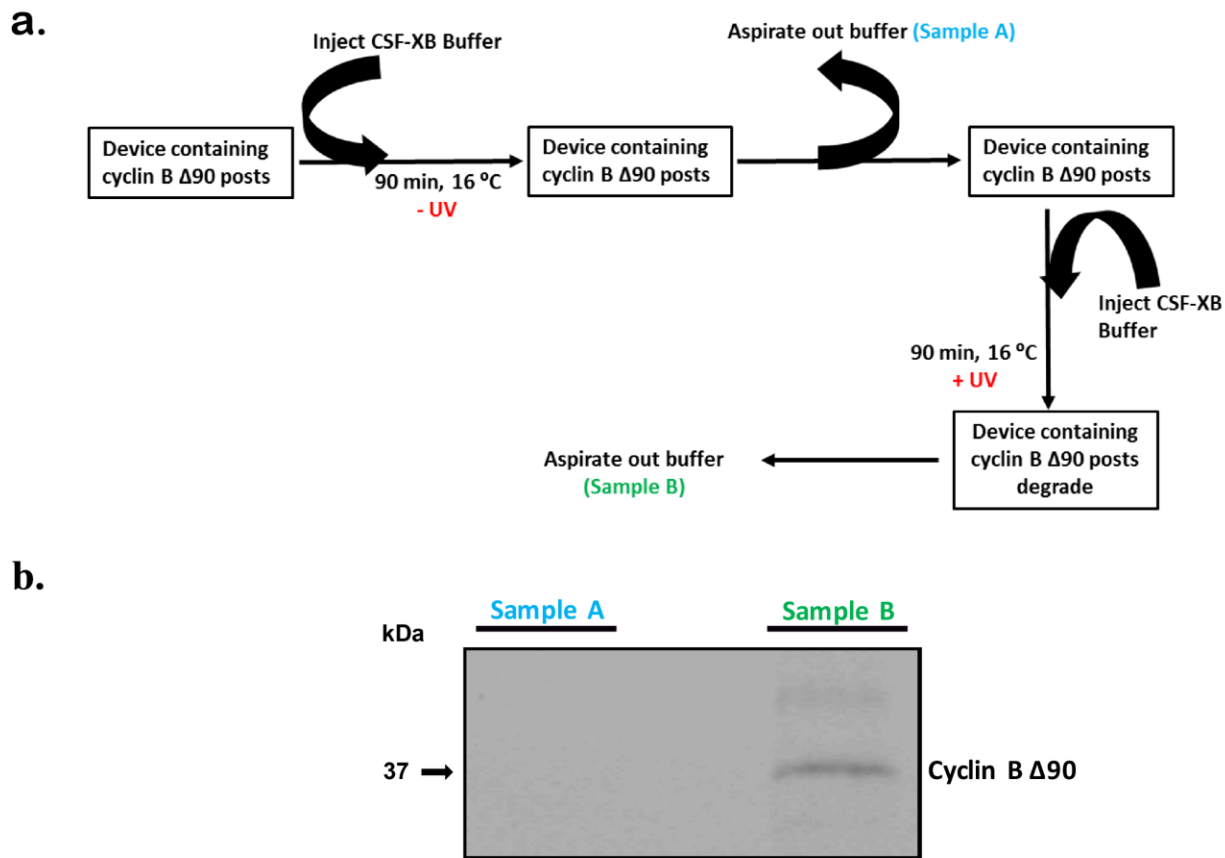


Figure S7. Retention and release of cyclin B $\Delta 90$ protein from hydrogel posts in the absence and presence of UV exposure. a) Schematics of the experiment. Devices containing cyclin B $\Delta 90$ -post arrays were formed as described previously and the intact posts were soaked in CSF-XB buffer (instead of extract) at 16 °C for 90 mins without UV exposure. Afterward this incubation period, the soaking buffer (Sample A; Fig. S5a) was collected from the devices leaving the posts intact. We then used the same device and added an equal volume of CSF-XB buffer, degraded the posts to UV, and incubated at 16 °C for 90 mins. After complete posts degradation and the incubation period, we collected the buffer (Sample B). Note that the schematic is not drawn to scale. b) A western blot of equal volumes of sample A and sample B was probed with cyclin B1 antibody (# AP11096c, Abgent), which showed that there was no detectable leaching of cyclin B $\Delta 90$ protein from intact posts (sample A). However, when the posts were degraded (sample B), we could detect cyclin B $\Delta 90$, suggesting that the cyclin B $\Delta 90$ protein diffuses into the buffer only upon post degradation. The experiment was repeated two times.