Supporting Information

Spatiotemporal patterning of photoresponsive DNA-based hydrogels to tune local cell responses

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1. Supplementary Tables

Supplementary Table 1. Self-complementary DNA sequences (photocleavable or not) used as crosslinker in hydrogel formation process.

Name	Sequence (5'→3')
1	**Acrydite**-AATGCTCGCGCGCGAGCA
4	**Acrydite**-AA//**PC**//TGCTCGCGCGCGAGCA

Supplementary Table 2. Photocleavable single-strand DNA and FAM modified complementary strand.

Name	Sequence (5'→3')
2	**Acrydite**-TTTTT//**PC**//GGCTATAGCACATGGGTAAAACGAC
2'	**FAM**-GTCGTTTTACCCATGTGCTATAGCC

Supplementary Table 3. Photoactivatable hairpin structured DNA, TAMRA modified invading DNA strand and TAMRA modified invading DNA strand with MUC-1 aptamer sequence.

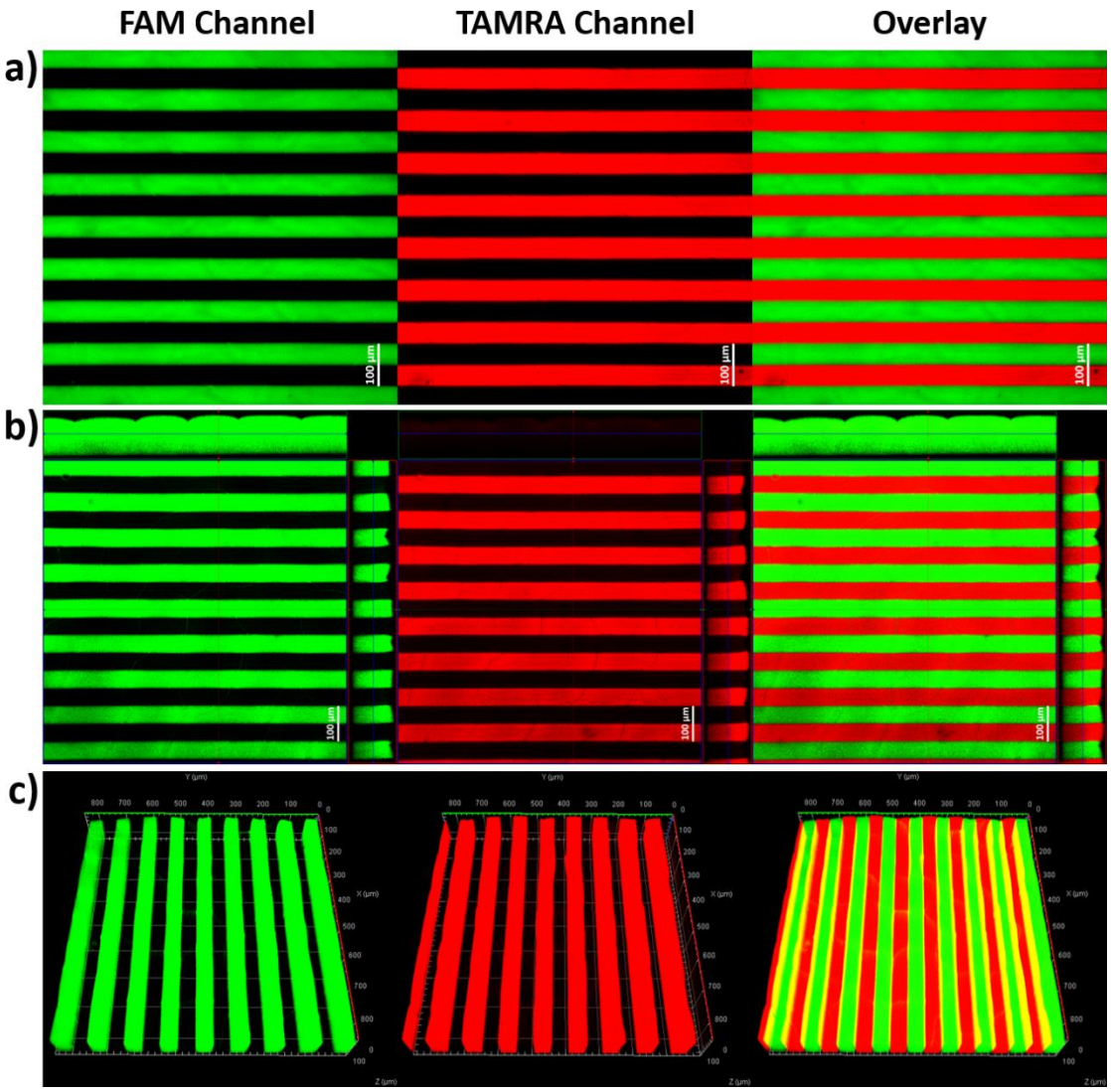
Name	Sequence (5'→3')
3	**Acrydite**-TTTTCCGGAATTCCGCTTCTAGTT//**PC**//GCGGAATTCCGGAAAA
3'	**TAMRA**-AACTAGAAGCGGAATTCCGGAAAA
3"	**TAMRA-** GCAGTTGATCCTTTGGATACCCTGGTTTTTTTTTTAACTAGAAGCG GAATTCCGGAAAA

Note: Underlined is the sequence of MUC-1 aptamer.

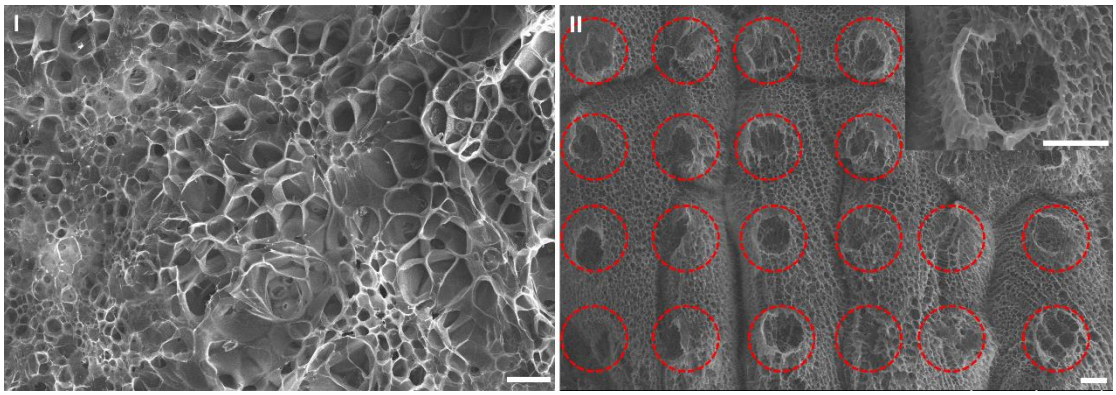
Supplementary Table 4. DNA sequences used in the site-specific swelling process of patterned hydrogels for hydrogel pattern regulation.

Name	Sequence (5'→3')
5	**Acrydite**-TAAGTTCGCTGTGGCACCTGCACG
5'	**Acrydite**-GTACAA//**PC**//CGTGCAGGTGCCACAGCGTGGATC
H1	GATCCGCGCTGTGGCACCTGCACGCACCCACGTGCAGGTGCCACAGCGAA CTTA
H2	TGGGTGCGTGCAGGTGCCACAGCGTAAGTTCGCTGTGGCACCTGCACGTTG TAC

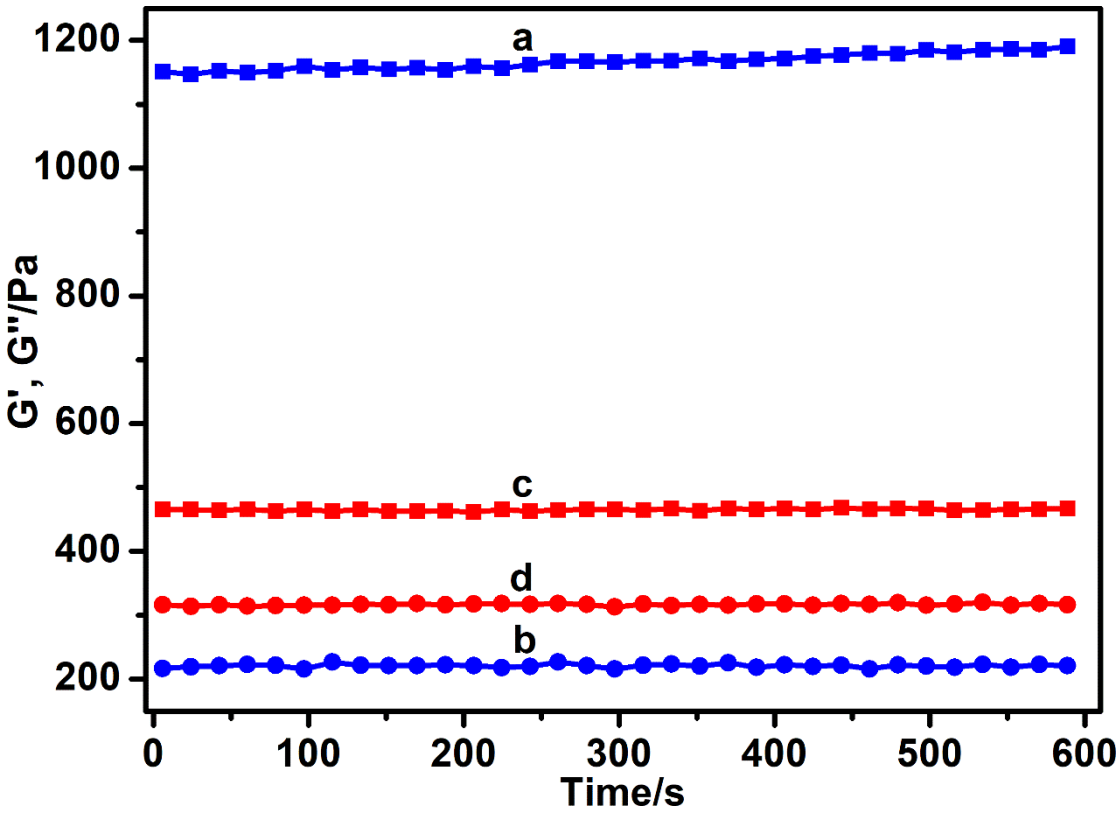
2. Supplementary Figures



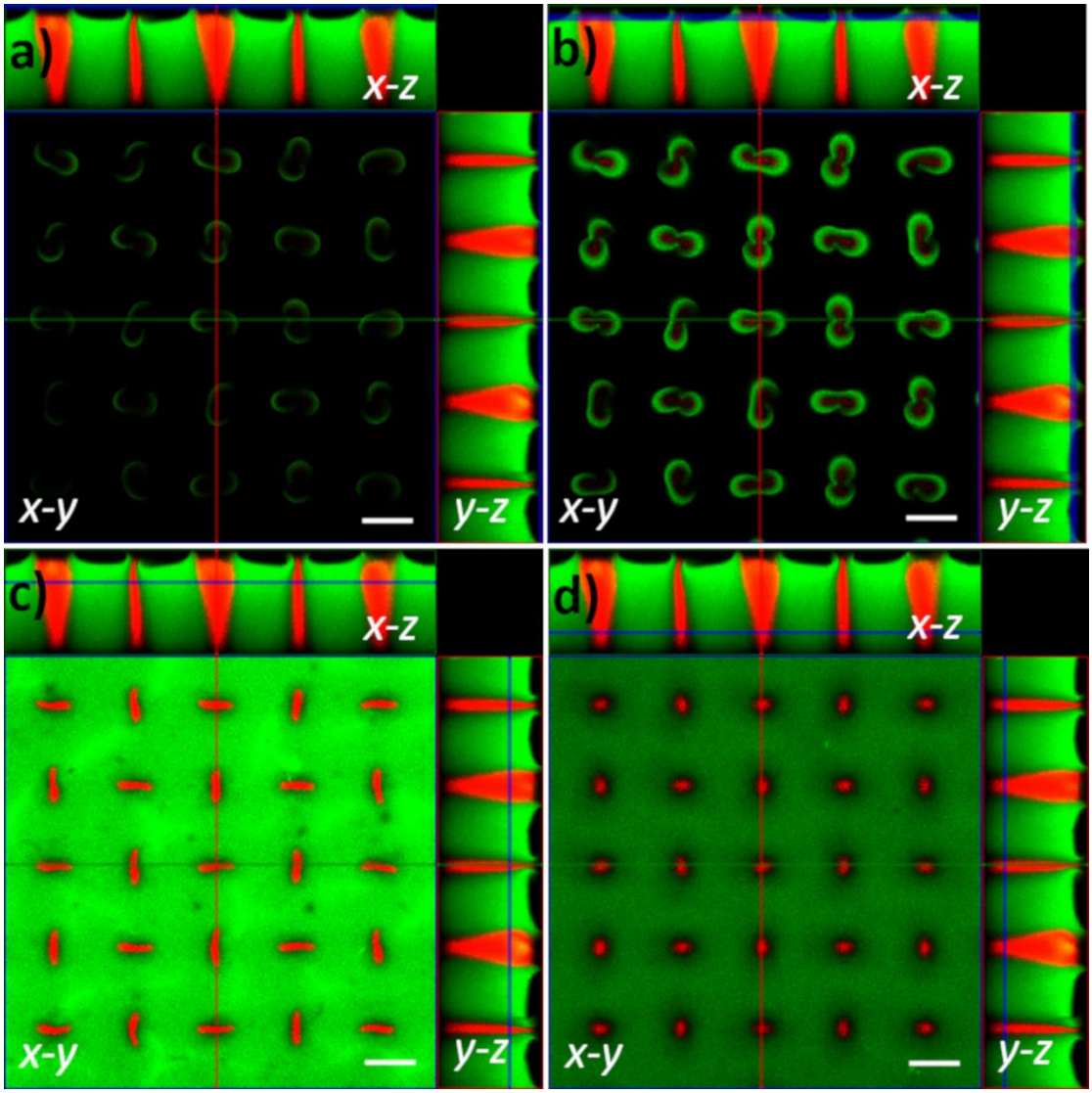
Supplementary Figure 1. Light-induced orthogonal patterning of a hydrogel exhibiting dual functionalities. The polyacrylamide (1)-crosslinked hydrogel is functionalized with the o-nitrobenzylphosphate ester nucleic acids (2) and (3). Exposure of the hydrogel to light yield in the illuminated domains the activated strand (3) and retain in the non-irradiated domains the free nucleic acid strand (2). The dual patterned function allows the selective association of (3')-TAMRA and (2')-FAM to the respective domains. Confocal fluorescence images **a**), orthogonal view **b**), and three-dimensional fluorescence imaging reconstruction **c**) of the dual functional DNA strand patterned hydrogel film by using stripe-like pattern containing photomask. Scale bars are 100 µm. N = 4 independent experiments were performed.



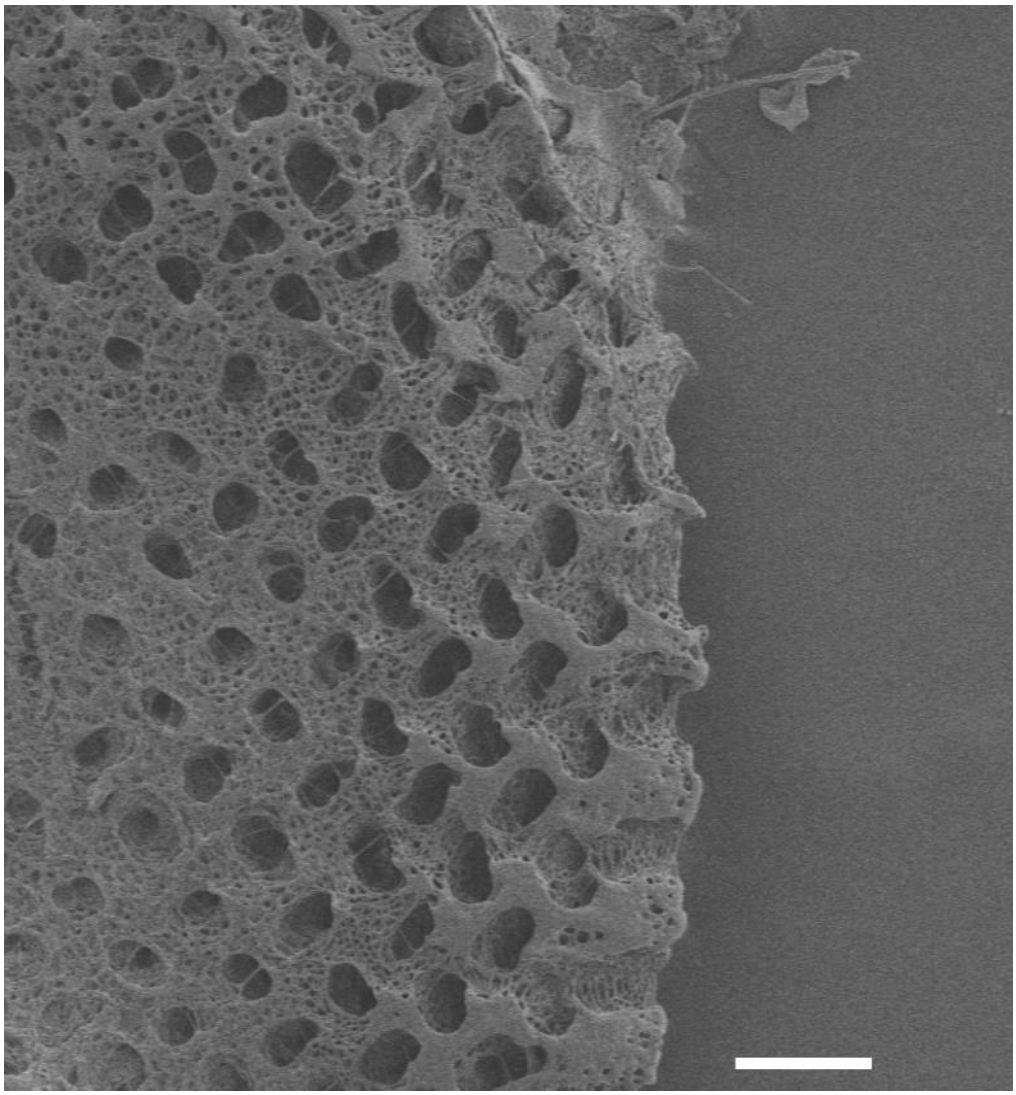
Supplementary Figure 2. SEM image of: Panel I– The parent hydrogel shown in Figure 5a prior to the photopatterning. Panel II– The hydrogel film after photopatterning, $\lambda = 365$ nm, according to Figure 5a. Scale bars correspond to 20 μm. N = 4 independent experiments were performed.



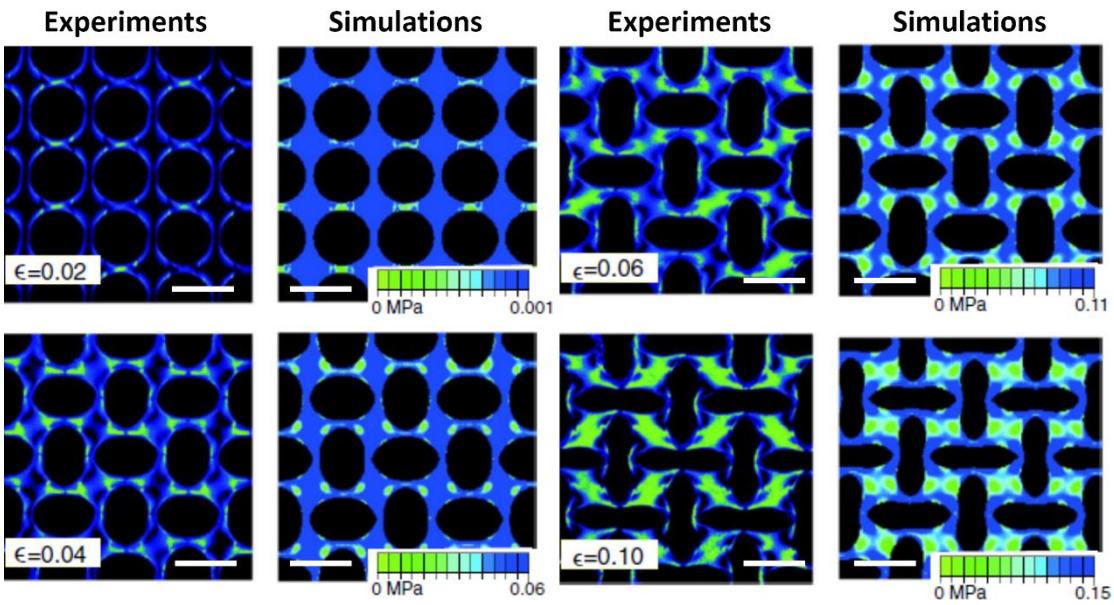
Supplementary Figure 3. Time-dependent sweep rheological measurements of hydrogels crosslinked with photocleavable crosslinker DNA strands (4) without UV irradiation and with 5 min UV irradiation. Curves a and b corresponding to the storage modulus G' and loss modulus G'' before UV irradiation. Curves c and d corresponding to the storage modulus G' and loss modulus G'' after 5 min UV irradiation.



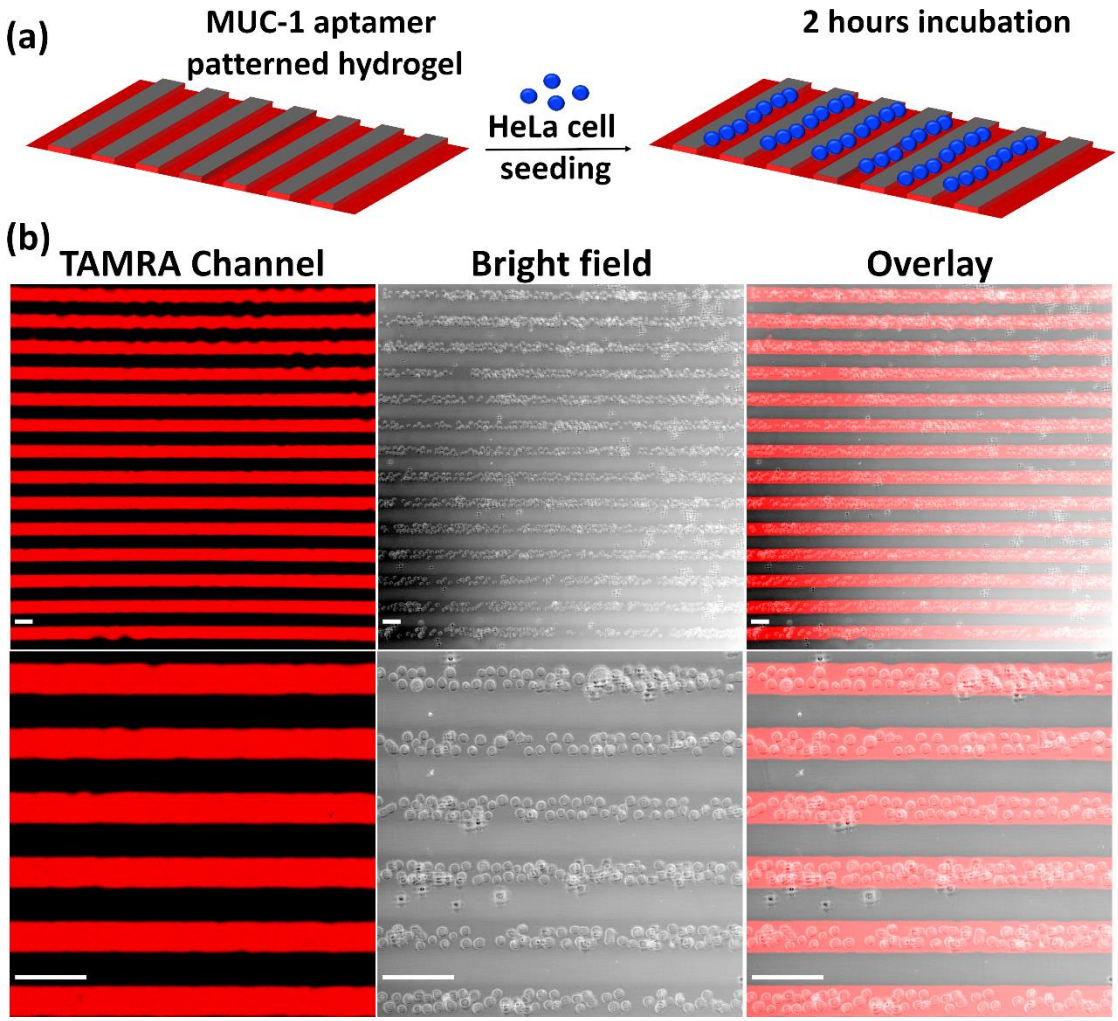
Supplementary Figure 4. Orthogonal views of the patterned DNA based hydrogels after DNA-directed HCR-induced programmable expansion. **a**)→**d**) show different *Z* position imaging from top surface of the hydrogel to the bottom surface of the hydrogels. Scale bars, 50 μm. N = 4 independent experiments were performed.



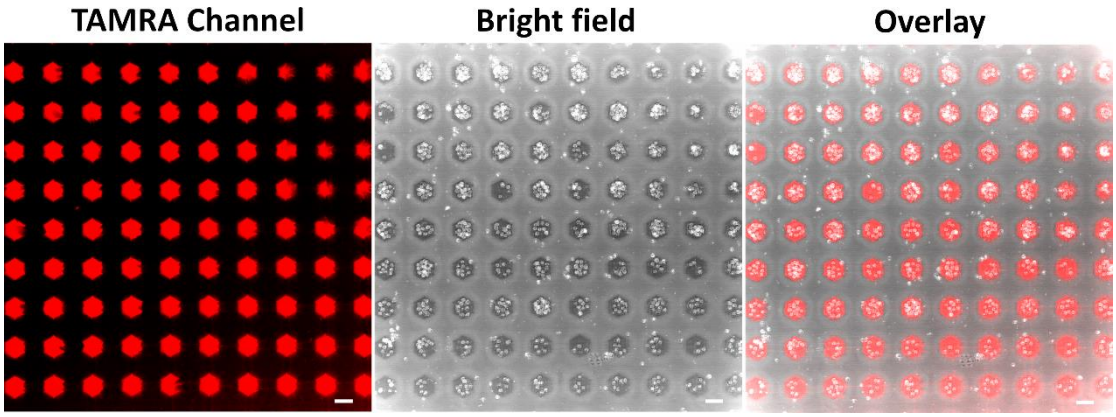
Supplementary Figure 5. SEM image of patterned hydrogels after HCR process with orthogonally arranged ellipsoid shapes. Scar bar is 100 nm. N = 4 independent experiments were performed.



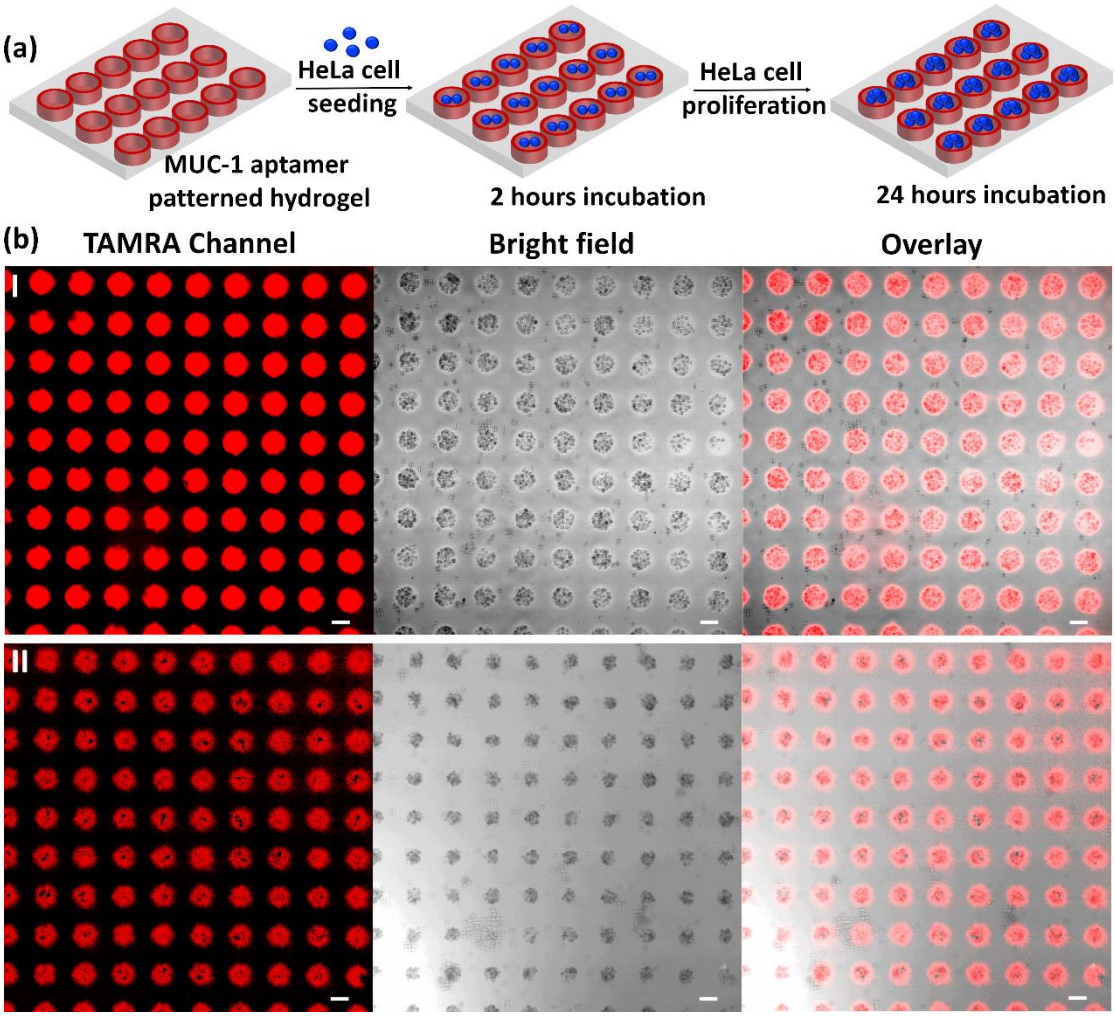
Supplementary Figure 6. Experimental (left) and numerical (right) images of the square lattice at different levels of macroscopic strain: 2%, 4%, 6%, and 10%. The experimental samples were viewed through cross polars and the colors give a qualitative indicator of the regions of localized stress which provide a useful comparison with the calculated principal stress levels. Scale bars correspond to 1 cm. Reproduced with permission from Ref. [1]. Copyright 2007 American Physical Society.



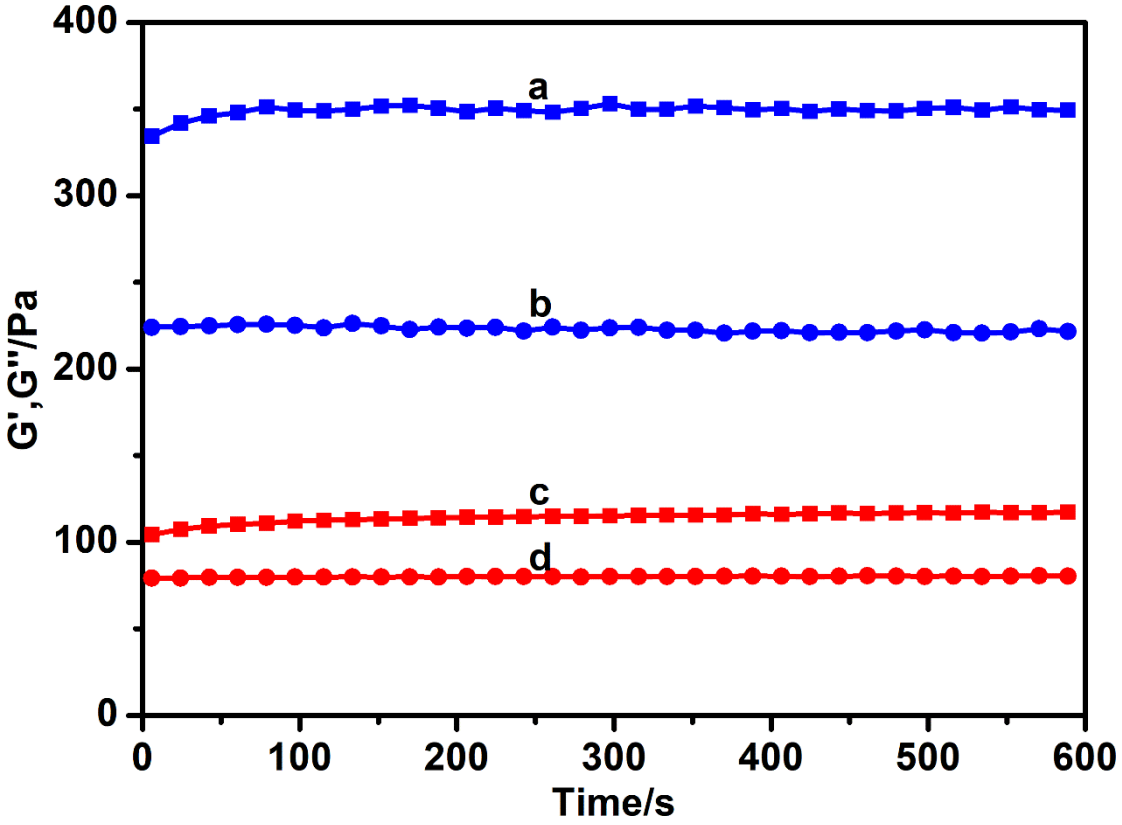
Supplementary Figure 7. (**a**) The patterned population-level control of HeLa cells binding in confined MUC-1 aptamer modified domains is achieved through 2D culture of cells on MUC-1 aptamer patterned hydrogel. (**b**) Fluorescent, brightfield, and overlay images highlight the cells adsorbed onto the confined MUC-1 aptamer patterned strip-like domains. N = 3 independent experiments were performed.



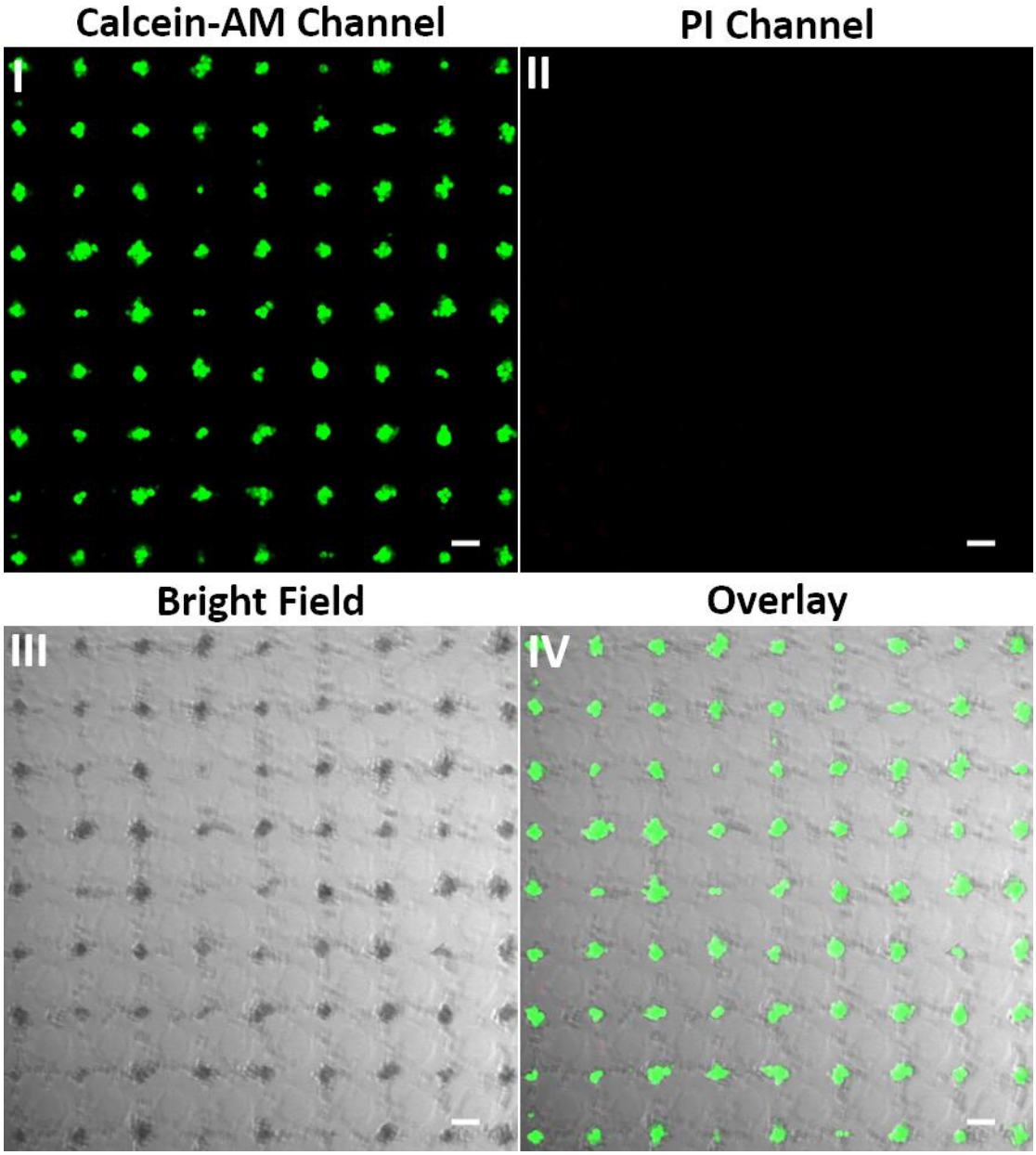
Supplementary Figure 8. Fluorescent, brightfield, and overlay images highlight the cells selectively adsorbed onto the confined MUC-1 aptamer patterned hexagon domains. N = 3 independent experiments were performed.



Supplementary Figure 9. (**a**) The patterned population-level control of HeLa cells binding and proliferation within confined MUC-1 aptamer modified domains is achieved through 2D culture of cells on MUC-1 aptamer patterned hydrogel. (**b**) Fluorescent, brightfield, and overlay images highlight the cells binding and proliferation within the confined MUC-1 aptamer patterned circular domains with 2 hours incubation (panel I) and 24 hours incubation (panel II). Scale bars are 50 µm. N = 3 independent experiments were performed.



Supplementary Figure 10. Time-dependent sweep rheological measurements of hydrogels crosslinked with photocleavable crosslinker DNA strands (4) with 5 min UV irradiation before cell culture and after cell culture. Curves a and b corresponding to the storage modulus G' and loss modulus G'' before cell culture. Curves c and d corresponding to the storage modulus G' and loss modulus G'' after 24 h cell culture.



Supplementary Figure 11. Confocal fluorescence images of proliferated HeLa cells on patterned hydrogel surface. HeLa cells were cultured for 24 hours and then stained with calcein-AM and PI to verify the viability of the cells. Panel I– Fluorescence microcopy image (Calcein-AM channel) of the calcein-AM stained living HeLa cells proliferated in the patterned domains. Panel II– Fluorescence microcopy image (PI channel) of the PI-stained dead HeLa cells in the patterned domains. Panel III–Bright field image of the HeLa cells linked to the confined circular domains modified with MUC-1 aptamer. Panel IV– Overlay of panel I, panel II, and panel III. Scale bars are 50 μm. N = 3 independent experiments were performed.

Reference:

[1] Mullin, T., Deschanel, S., Bertoldi, K., Boyce, M. C. Pattern transformation triggered by deformation, Physical Review Letters, 2007, 99, 084301.