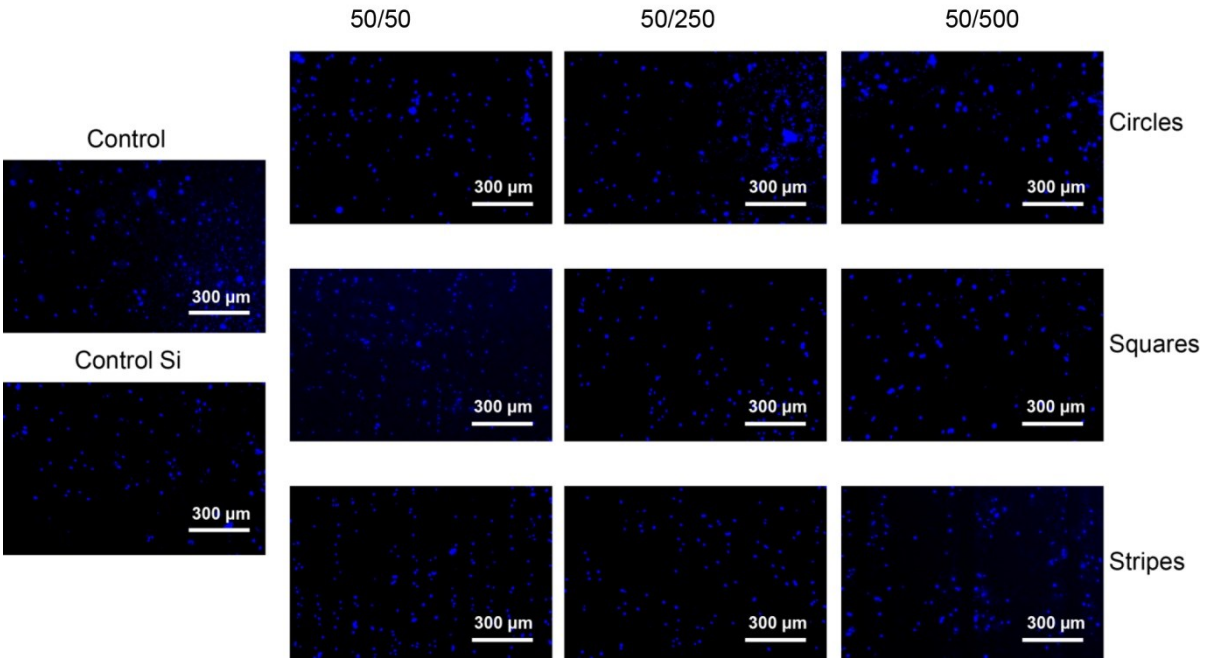
Electronic Supplementary Material (ESI) for Integrative Biology.

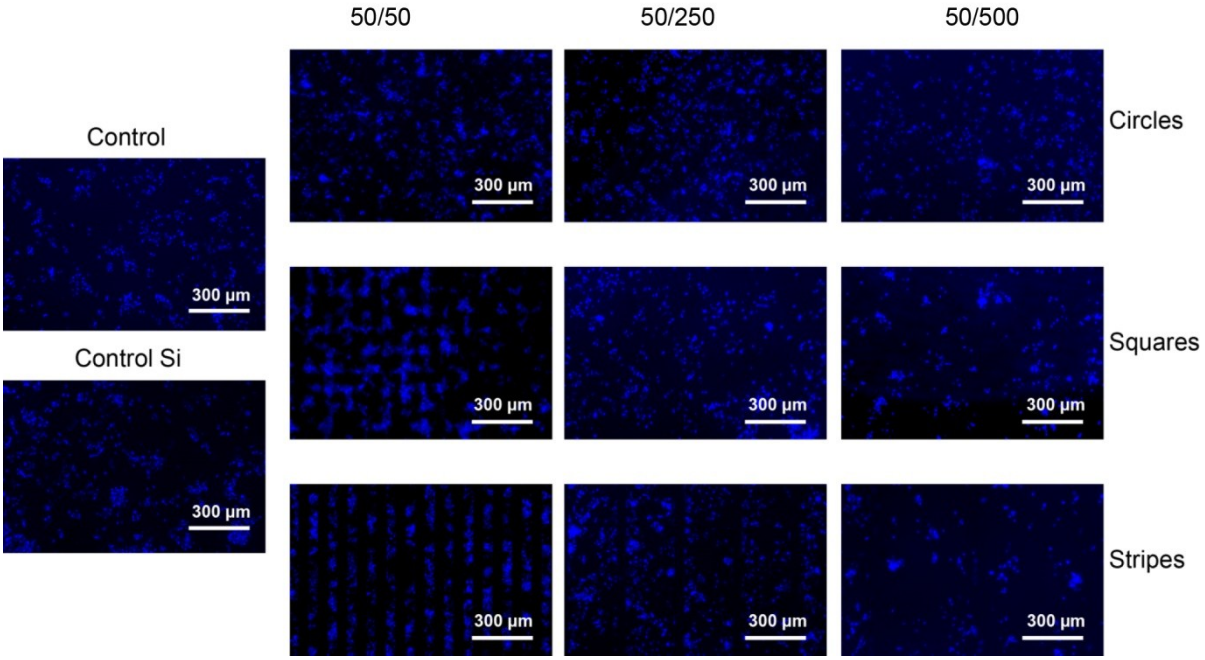
Supporting Information

Title Modulation of collective cell behavior by geometrical constraints

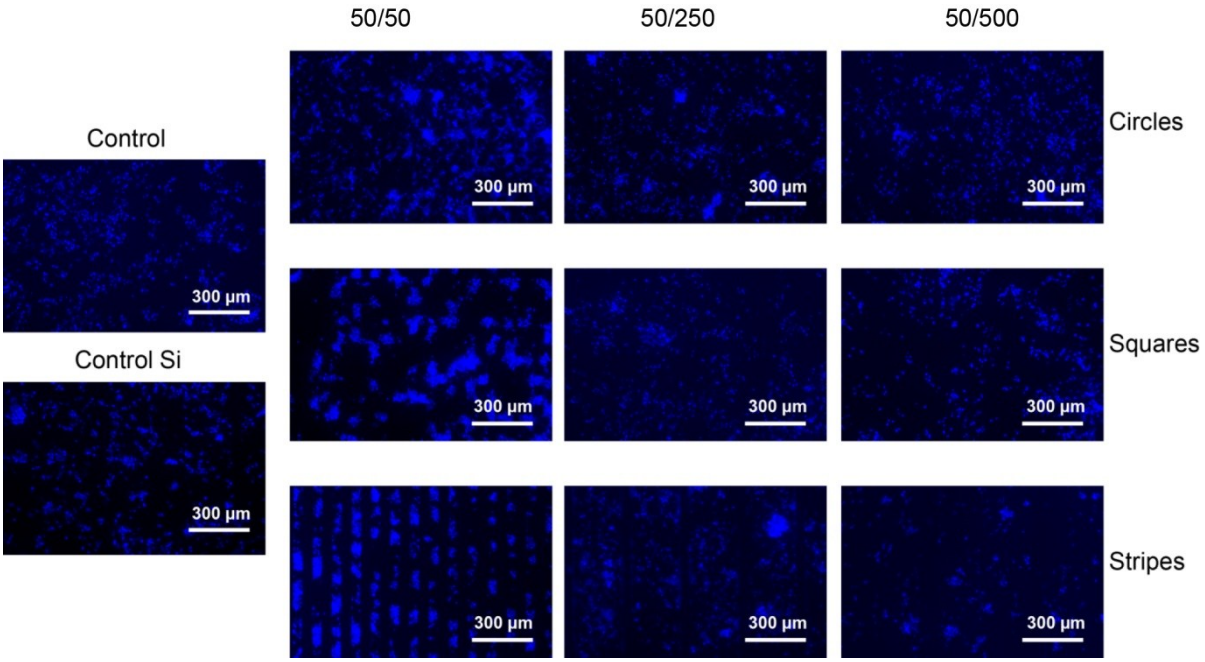
Mariia Lunova, Vitalii Zablotskii, Nora M. Dempsey, Thibaut Devillers, Milan Jirsa, Eva Syková, Šárka Kubinová, Oleg Lunov, and Alexandr Dejneka*



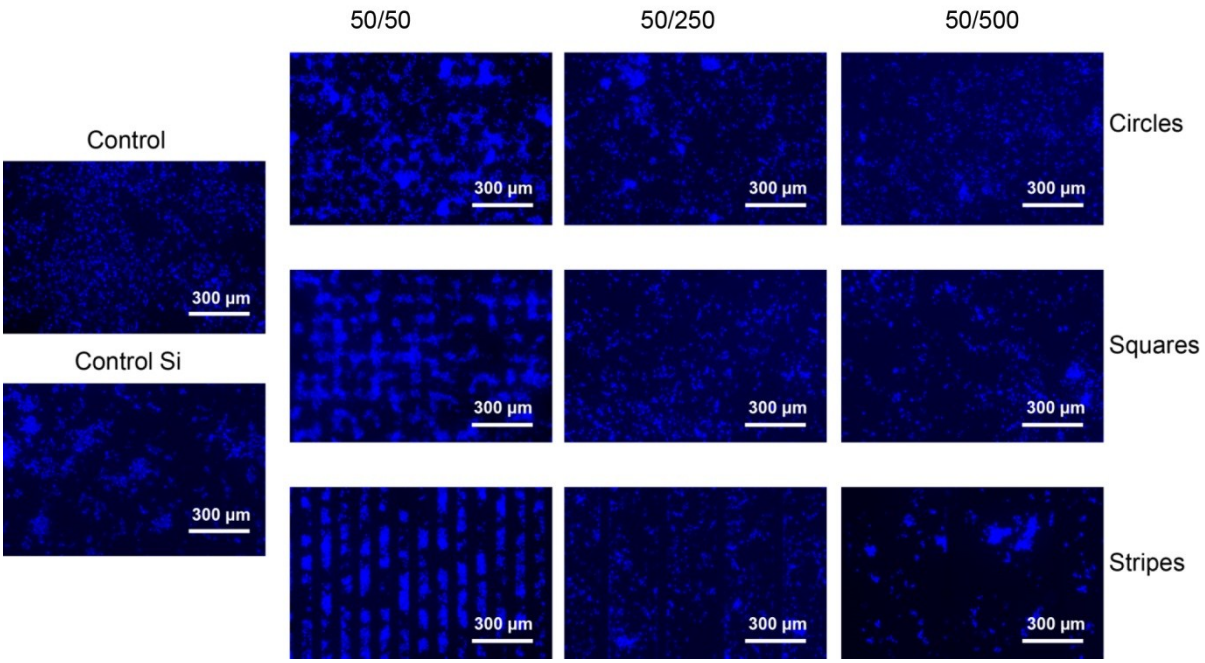
Supplementary Figure S1 HepG2 cells seeded on Si substrates with different geometry having different values of pillar spacing. Cells were imaged with fluorescence microscopy at day 0 after seeding.



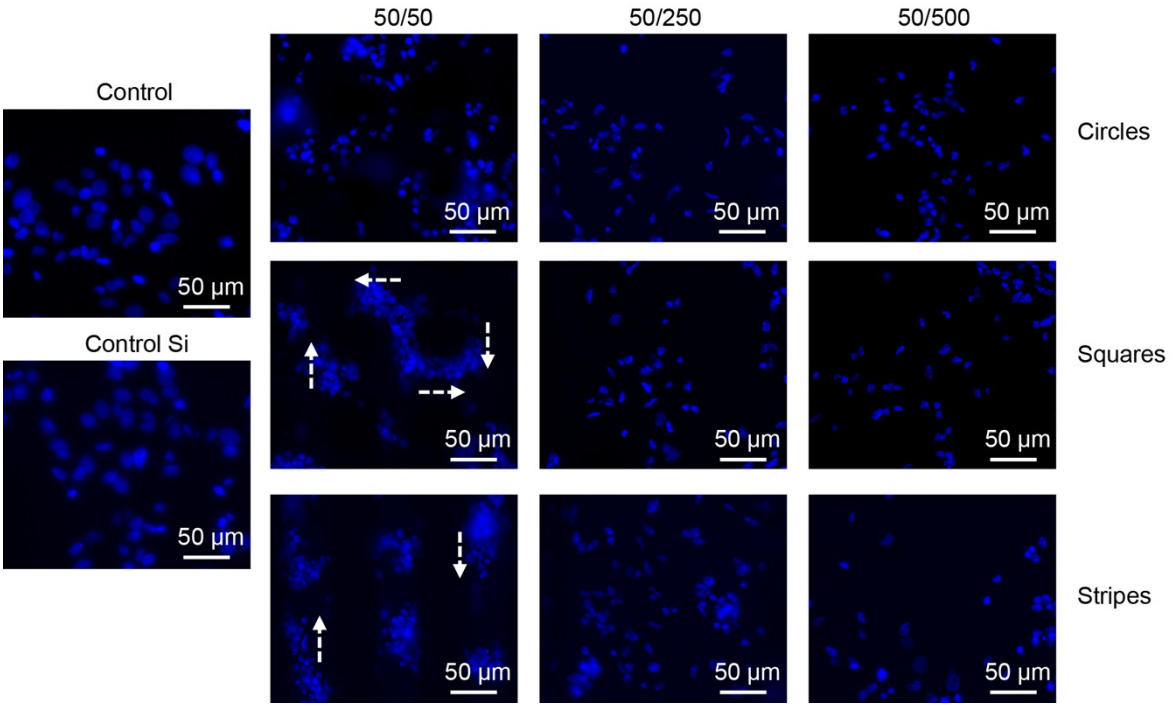
Supplementary Figure S2 HepG2 cells seeded on Si substrates with different geometry having different values of pillar spacing. Cells were imaged with fluorescence microscopy at day 1 after seeding.



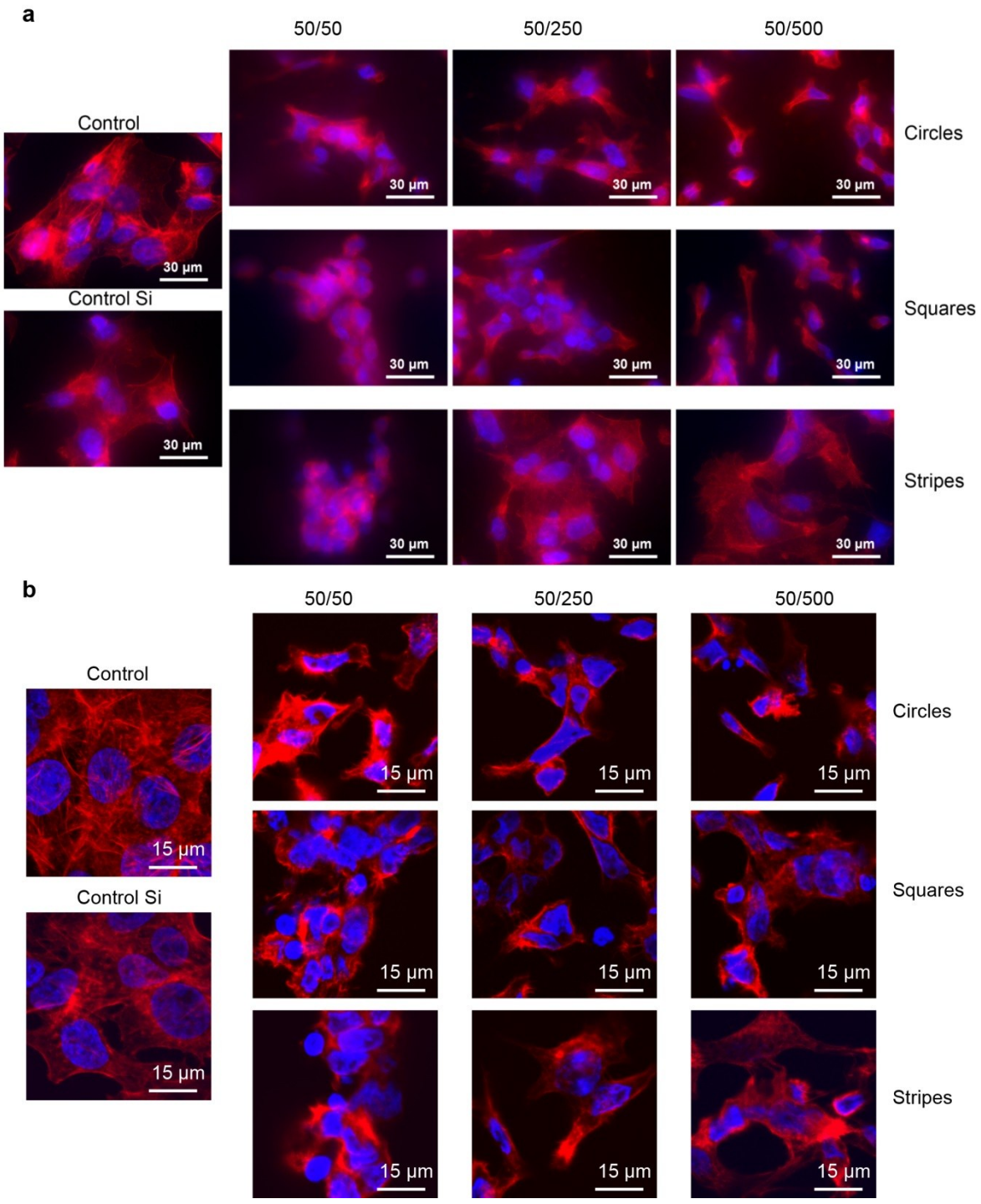
Supplementary Figure S3 HepG2 cells seeded on Si substrates with different geometry having different values of pillar spacing. Cells were imaged with fluorescence microscopy at day 2 after seeding.



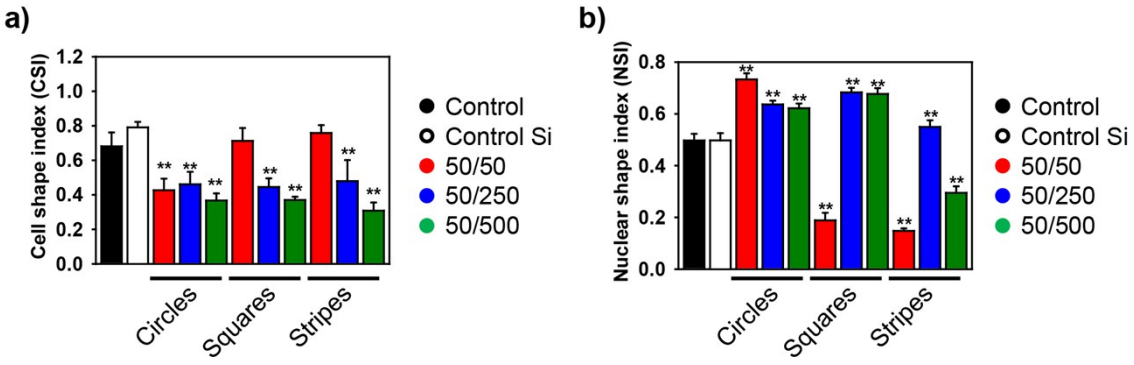
Supplementary Figure S4 HepG2 cells seeded on Si substrates with different geometry having different values of pillar spacing. Cells were imaged with fluorescence microscopy at day 3 after seeding.



Supplementary Figure S5 Representative fluorescent images of cells grown for 3 days on different Si substrates. Cell nuclei were labeled with DAPI.



Supplementary Figure S6 Cell morphology after 3 days growth on Si substrates with different geometries. After growing for 3 days, cells were fixed and stained for F-actin filaments (red dye). Nuclei were counterstained with DAPI (blue dye). Labeled cells were imaged with fluorescence (a) confocal (b) microscopy.



Supplementary Figure S7 Substrate topography imposes modular changes in cell and nucleus size and shape. Cell morphology after 3 days growth on Si substrates with different geometry. Relative deformations of the cell – CSI (a) and nucleus – NSI (b). ImageJ (NIH) software was used for image processing and fluorescent micrograph quantification. Data are presented as mean ± SD, n=15, $^{**}p < 0.01$.

Table S1 Cell doubling time grown on Si substrates with different geometries having different values of pillar spacing.

Sample	Doubling time (h)
Control	47.7 ± 5.5
Control Si	46.6 ± 2.7
CI 50/50	34.9 ± 3.7 **
CI 50/250	51.5 ± 3.5
CI 50/500	47.6 ± 1.9
SQ 50/50	36.3 ± 3.9 **
SQ 50/250	49.9 ± 4.3
SQ 50/500	48.8 ± 1.4
ST 50/50	35.5 ± 3.3 **
ST 50/250	48.4 ± 2.6
ST 50/500	77.2 ± 8.1 **

Cell doubling time was calculated utilizing growth rate kinetics (Fig. 2a). The cell doubling time was calculated using Doubling Time software version 1.0.10 (http://www.doubling-time.com) as described previously in[1].

References

1. Hail, N., Chen, P., Rower, J. & Bushman, L.R. Teriflunomide encourages cytostatic and apoptotic effects in premalignant and malignant cutaneous keratinocytes. *Apoptosis* **15**, 1234-1246 (2010).