# Genetic Strategy for Dynamic and Graded Control of Cell Mechanics, Motility, and Matrix Remodeling

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## Supporting Material

## Movie legends

**Movie S1**: U373-MG CA RhoA cells cultured with 100 ng/ml tetracycline on a collagen hydrogel. Scale bar = 50 μm.

**Movie S2**: U373-MG CA RhoA cells cultured without tetracycline on a collagen hydrogel. Scale bar = 50 μm.

**Movie S3**: U373-MG CA RhoA cell cultured without tetracycline in a 3D collagen hydrogel. Scale bar = 15 μm.

**Movie S4**: U373-MG CA RhoA cell cultured with 100 ng/ml tetracycline in a 3D collagen hydrogel. Scale bar = 15 μm.

**Movie S5**: U373-MG CA RhoA cells were cultured initially without tetracycline on a collagen hydrogel and then switched to 100 ng/ml tetracycline at t = 0. Scale bar = 50 μm.

**Movie S6**: U373-MG CA RhoA cells were cultured initially with 100 ng/ml tetracycline on a collagen hydrogel and then switched to media without tetracycline at t = 0. Scale bar = 50 μm.

## Supporting Figures

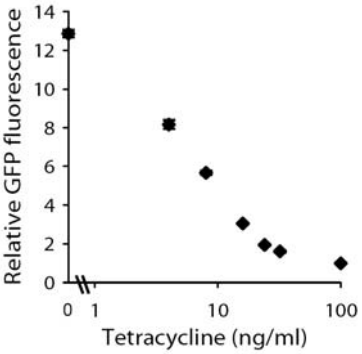


FIGURE S1. Fluorescence of U373-MG GFP cells under varying tetracycline concentrations. Mean ± s.e. (n = 3 samples). All values are significantly different from 100 ng/ml tetracycline ($p < 0.05$ by ANOVA, Tukey post-hoc test).

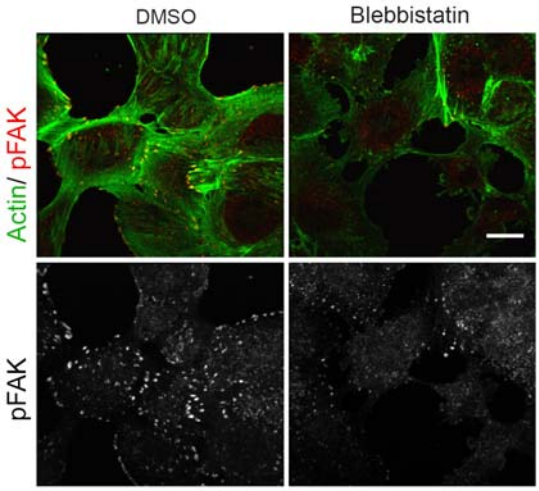


FIGURE S2. Confocal fluorescence micrographs of U373-MG CA RhoA cells cultured without tetracycline and pretreated with 5 μM blebbistatin or DMSO alone before fixation and staining for F-actin (green) and phosphorylated focal adhesion kinase (red and bottom panel alone). Scale bar = 25 μm. The top panel is a projection of z-slices.

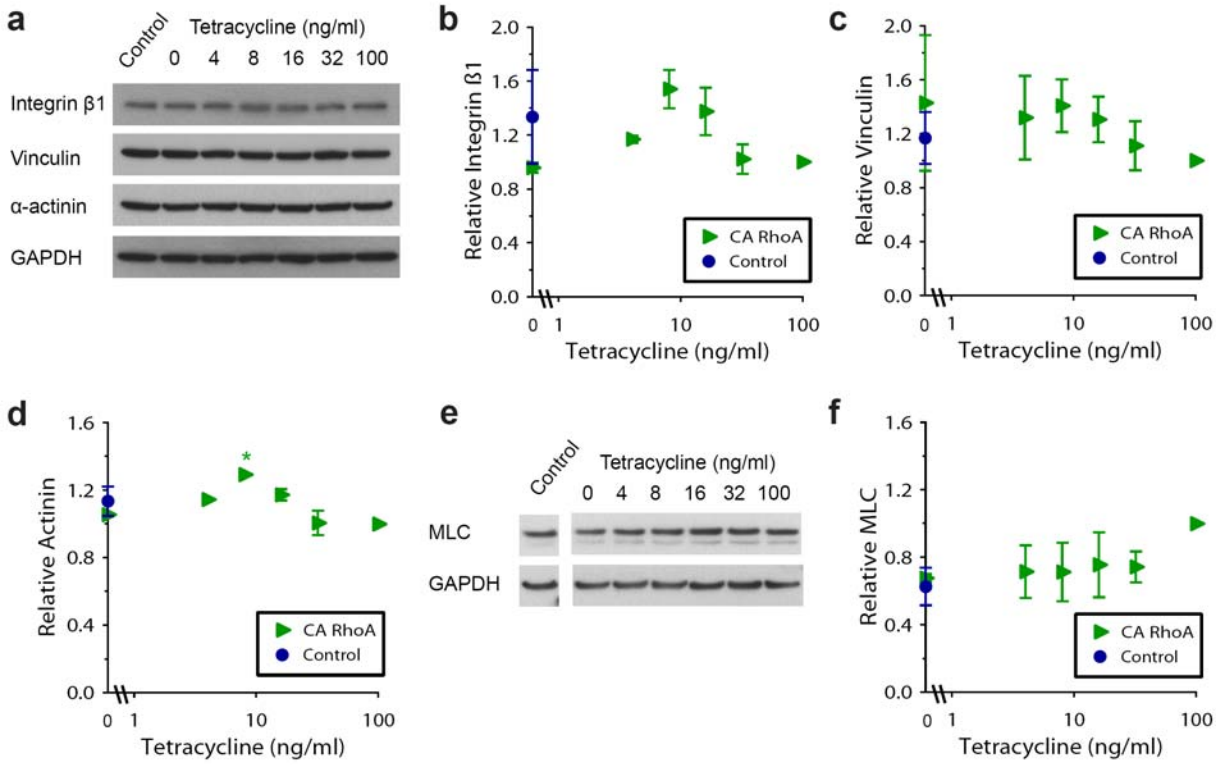


FIGURE S3. Expression levels of cytoskeletal proteins with increasing CA RhoA expression. (a) Western blot showing integrin β1, vinculin, and α-actinin protein levels in U373-MG control cells and CA RhoA cells cultured in varying tetracycline concentrations. Expression levels of integrin β1 (b), vinculin (c), and α-actinin (d) were quantified relative to CA RhoA cells with 100 ng/ml tetracycline. (e, f) Western blot (e) and quantification (f) of myosin light chain protein levels in U373-MG control cells and CA RhoA cells, relative to CA RhoA cells with 100 ng/ml tetracycline. All values are mean ± s.e. (n = 2-3 blots); * indicates $p < 0.05$ compared to 100 ng/ml tetracycline (ANOVA, Tukey post-hoc test).

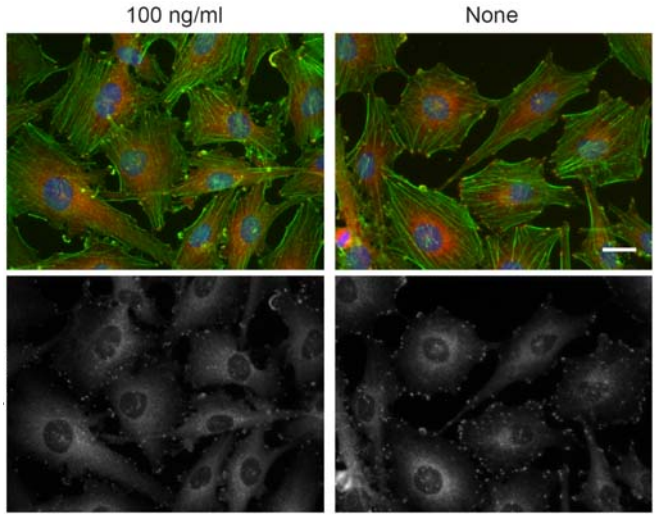


FIGURE S4. Epifluorescence micrographs of U373-MG control cells cultured with or without 100 ng/ml tetracycline before fixation and staining for F-actin (green), vinculin (red and bottom panel alone), and nuclei (blue). Scale bar = 25 μm.

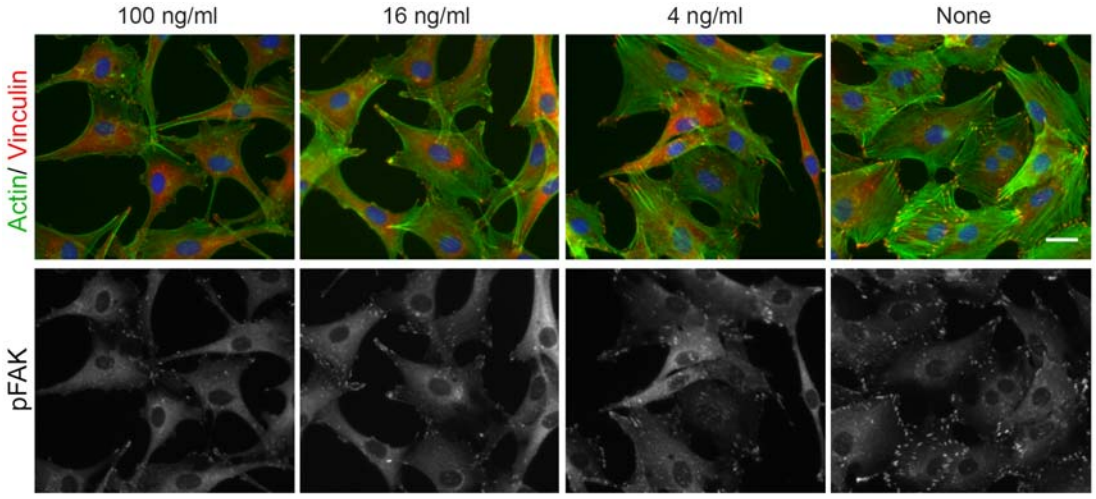


FIGURE S5. Epifluorescence micrographs of U87-MG CA RhoA cells cultured in varying tetracycline concentrations before fixation and staining for F-actin (green), vinculin (red and bottom panel alone), and nuclei (blue). Scale bar = 25 μm.

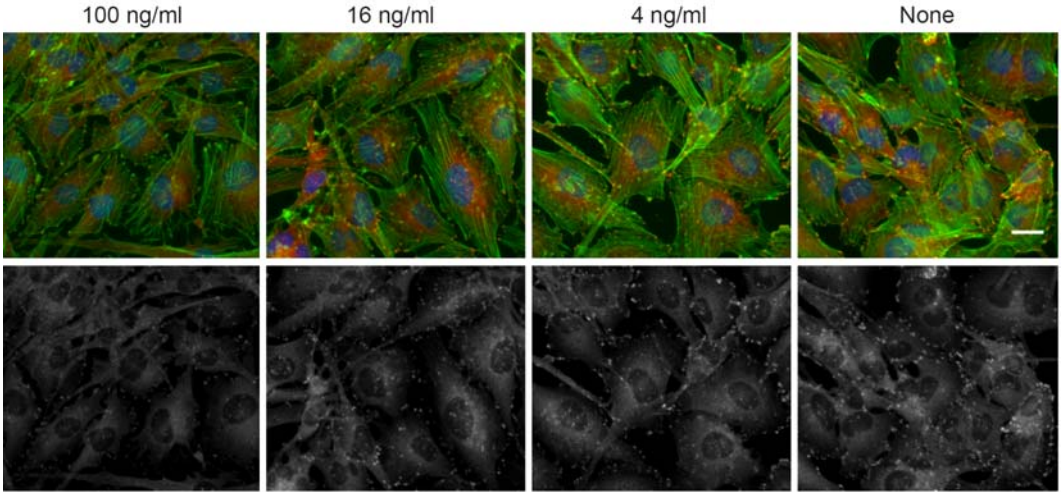


FIGURE S6. Epifluorescence micrographs of U373-MG CA MLCK cells cultured in varying tetracycline concentrations before fixation and staining for F-actin (green), vinculin (red and bottom panel alone), and nuclei (blue). Scale bar = 25 μm.

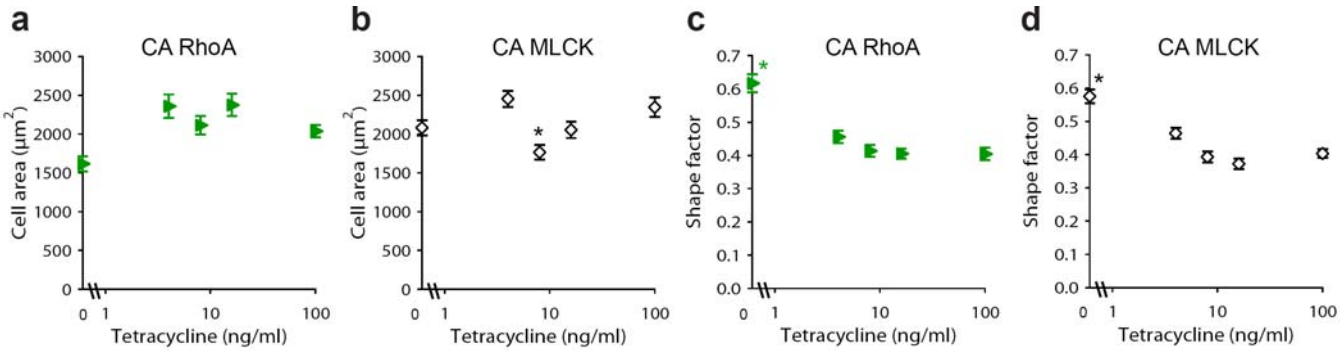


FIGURE S7. U373-MG CA RhoA (a, c) and CA MLCK cells (b, d) were cultured on 8.8 kPa and 12.2 kPa polyacrylamide gels, respectively, and images were used to quantify cell area (a, b) and shape factor as $4*\pi*(\text{area})/(\text{perimeter})^2$ (c, d). Mean ± s.e. (n = 80-105 cells per condition); * indicates $p < 0.05$ compared to 100 ng/ml tetracycline (ANOVA, Tukey post-hoc test).

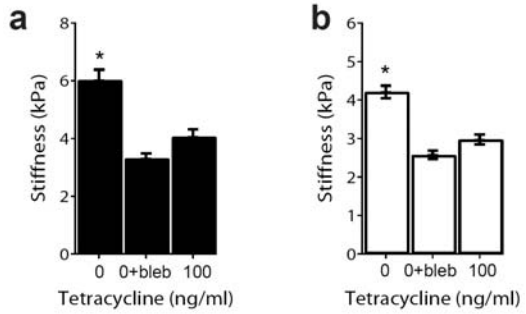


FIGURE S8. Average cortical stiffness of U373-MG CA RhoA (a) and CA MLCK cells (b) cultured without tetracycline and treated with or without 5 μM blebbstatin or cultured with 100 ng/ml tetracycline. Mean ± s.e. (n = 165-195 cells per condition); * indicates $p < 0.05$ compared to 100 ng/ml tetracycline (ANOVA, Tukey post-hoc test).

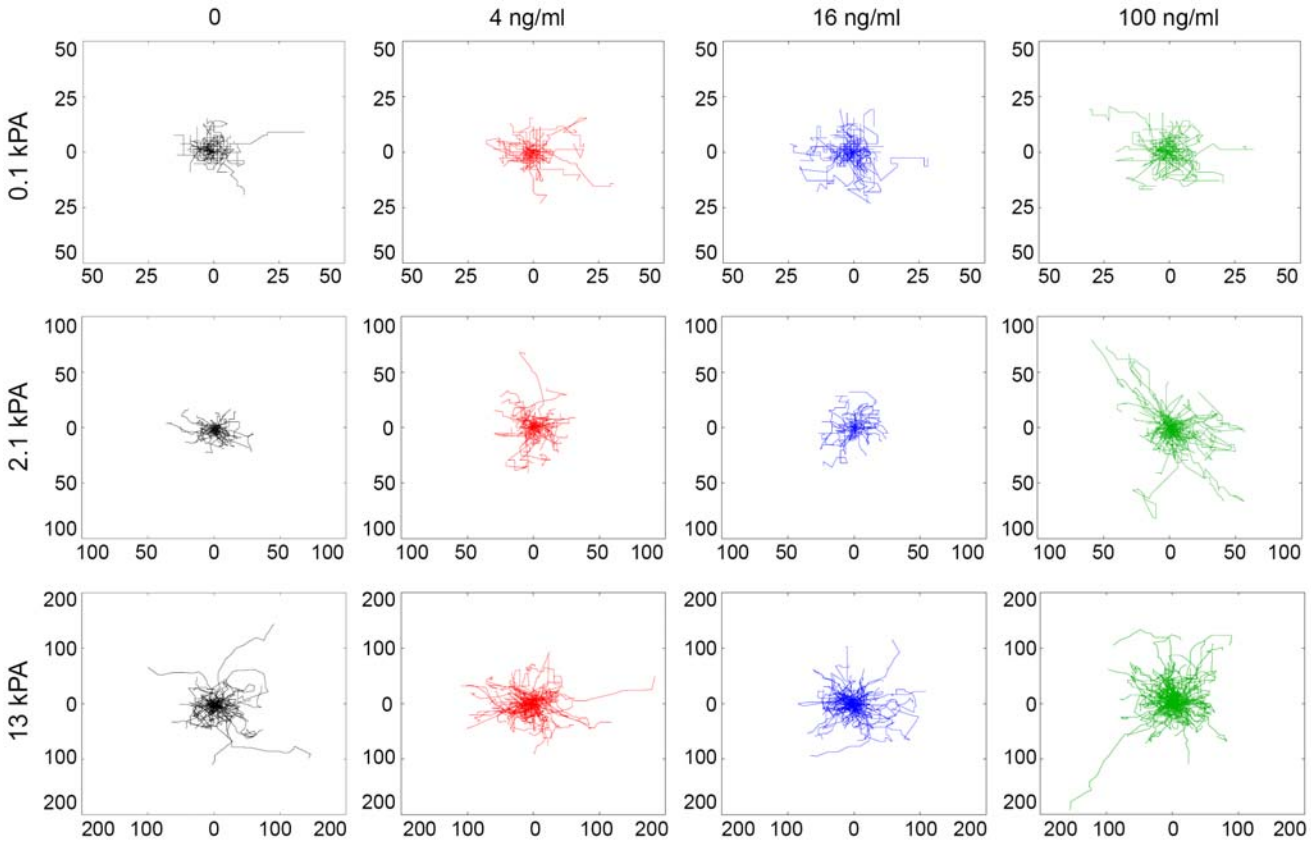


FIGURE S9. Rosette plots depicting single cell trajectories of U373-MG CA RhoA cells on poly-acrylamide gels of defined stiffness. Axes are in μm.

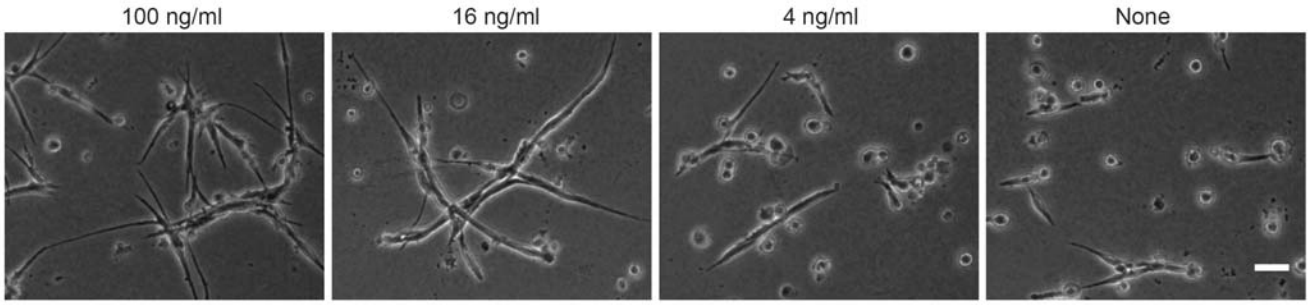


FIGURE S10. Micrographs of U373-MG CA MLCK cells cultured on 1 mg/ml collagen hydrogels in varying tetracycline concentrations. Scale bar = 50 μm.

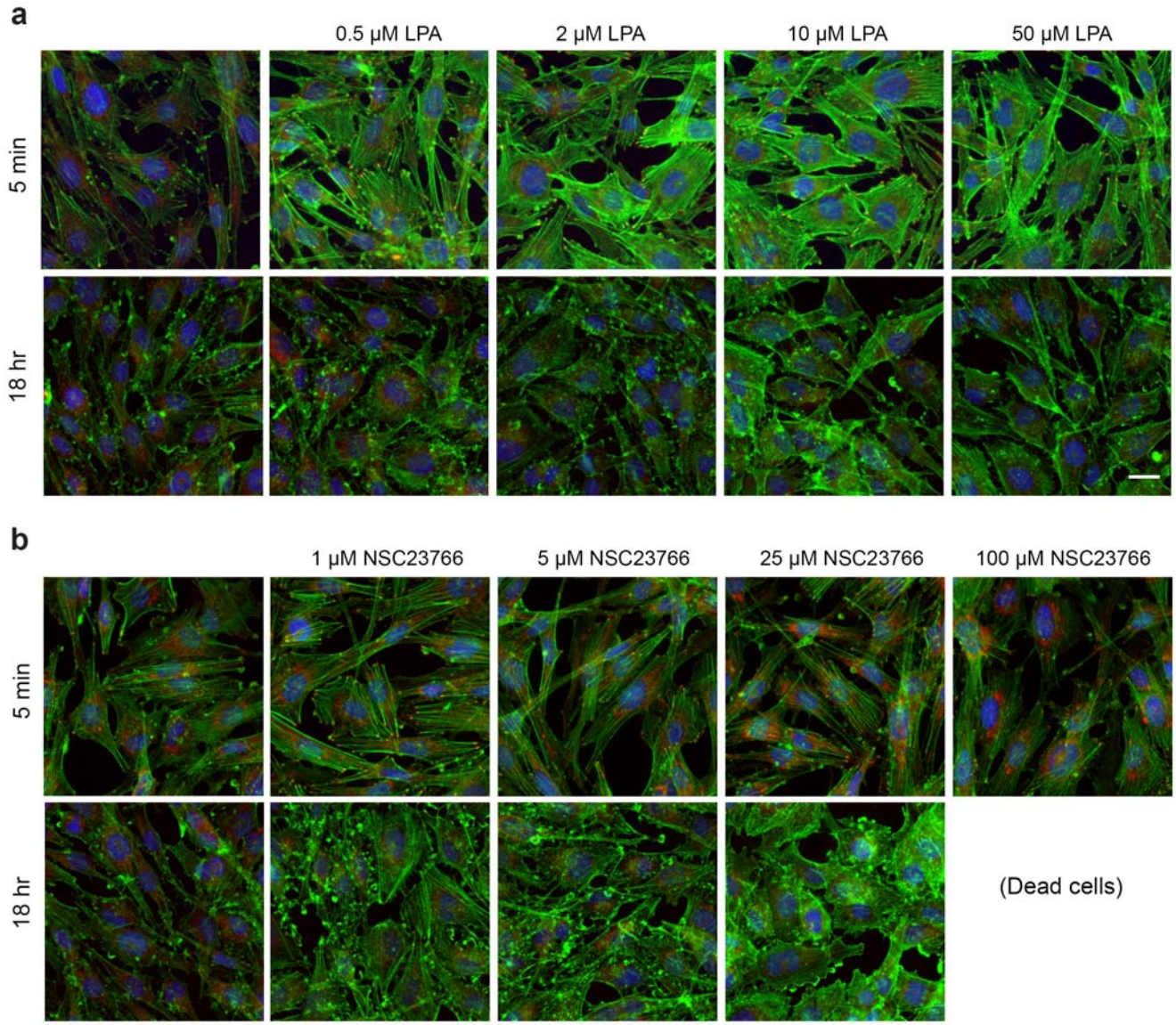


FIGURE S11. Epifluorescence micrographs of U373-MG control cells treated with varying concentrations of LPA (a) or NSC23766 (b) before fixation and staining for F-actin (green), phosphorylated focal adhesion kinase (red), and nuclei (blue). Scale bar = 25 μm.