Supplementary Information

Astrocytes Increase ATP Exocytosis Mediated Calcium Signaling in Response to Microgroove Structures

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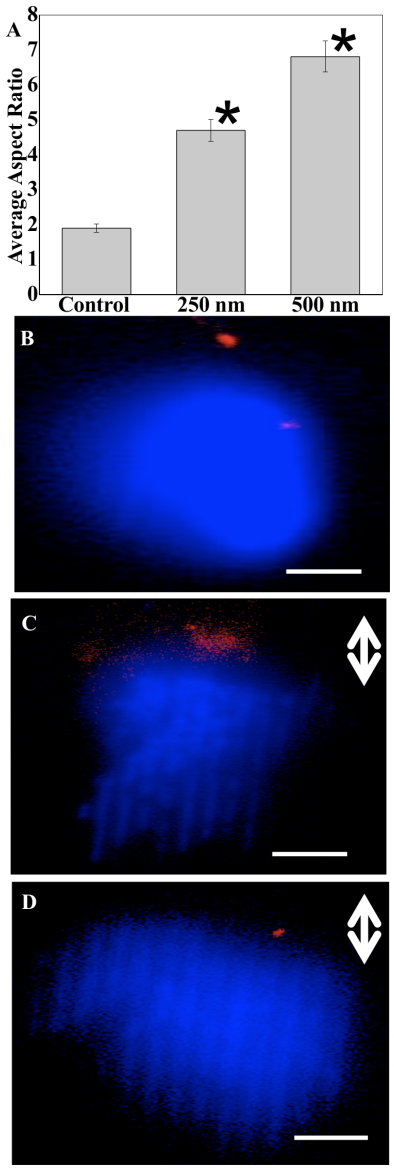


Fig. S1 Quantitative morphological analysis and nucleus centrosome (NC) immunostaining. (A) Cell aspect ratio on control and patterned surfaces. (B-D) NC Polarity and orientation on flat (B) and patterned PDMS (C: 250 nm in depth, and D: 500 nm). Red dot show centrosome. Scale bars: 5 μm, Double-sided arrows show grooves directions. * stands for significant difference from the control.

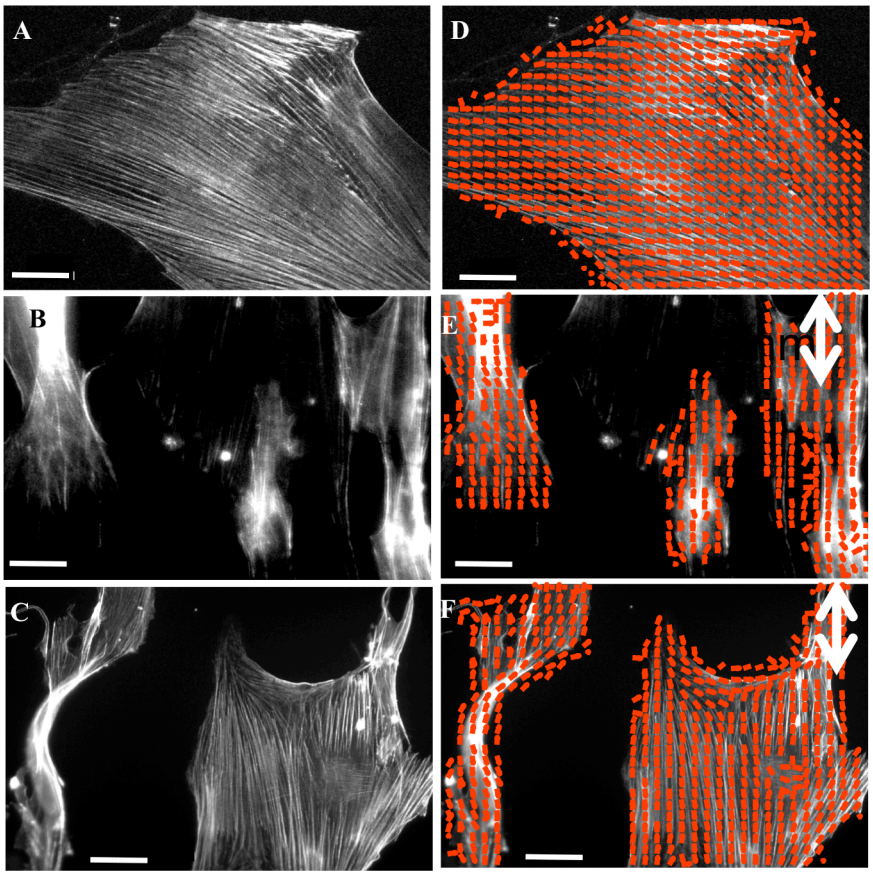


Fig. S2 Actin stress fiber analyses. Overlaid red short lines show the orientation of actin stress fibers along microgroove on flat surface (A, D), 250 nm deep grooves (B, E), and 500 nm deep grooves (C,F). Scale Bars: 20 μm.

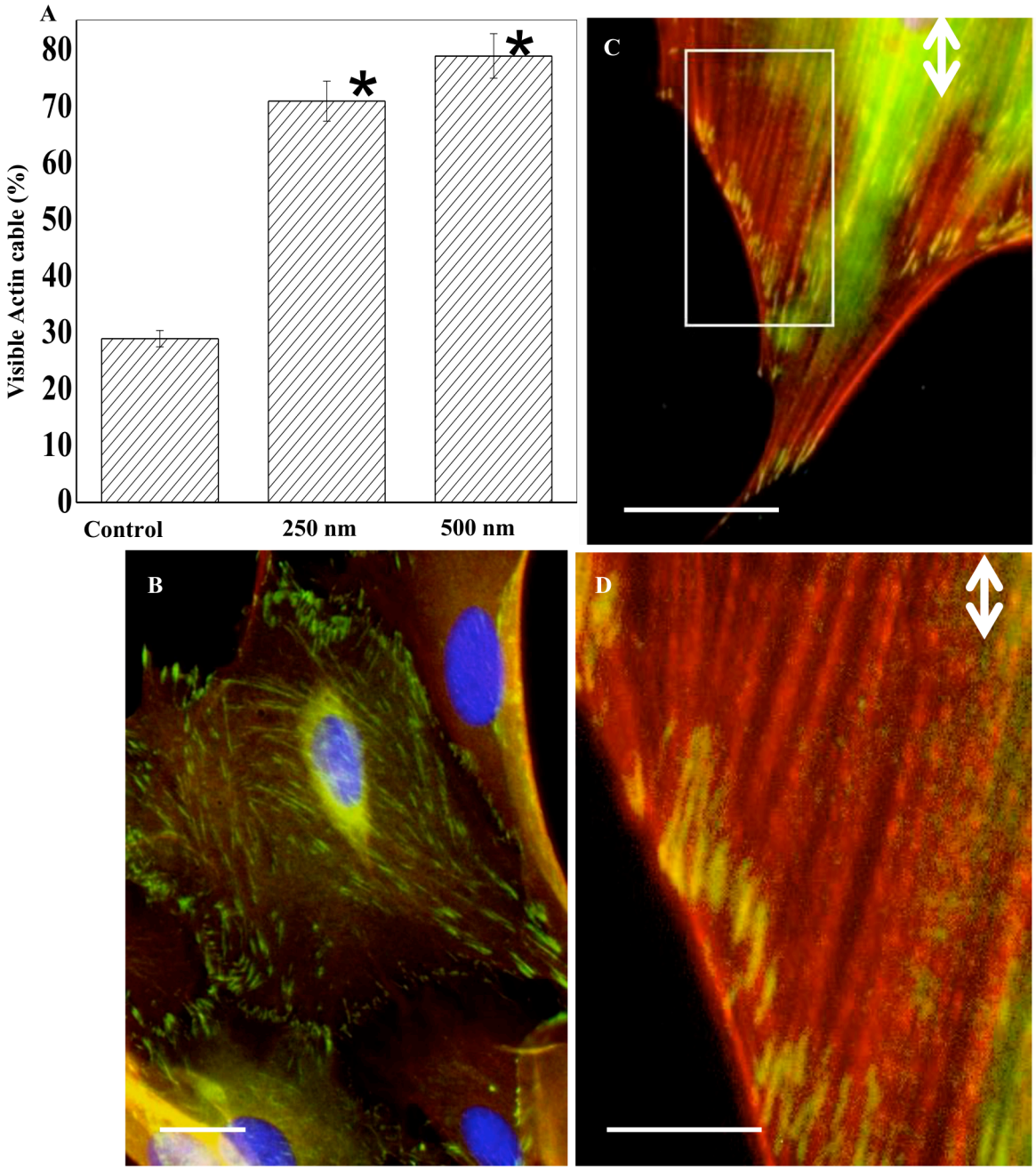
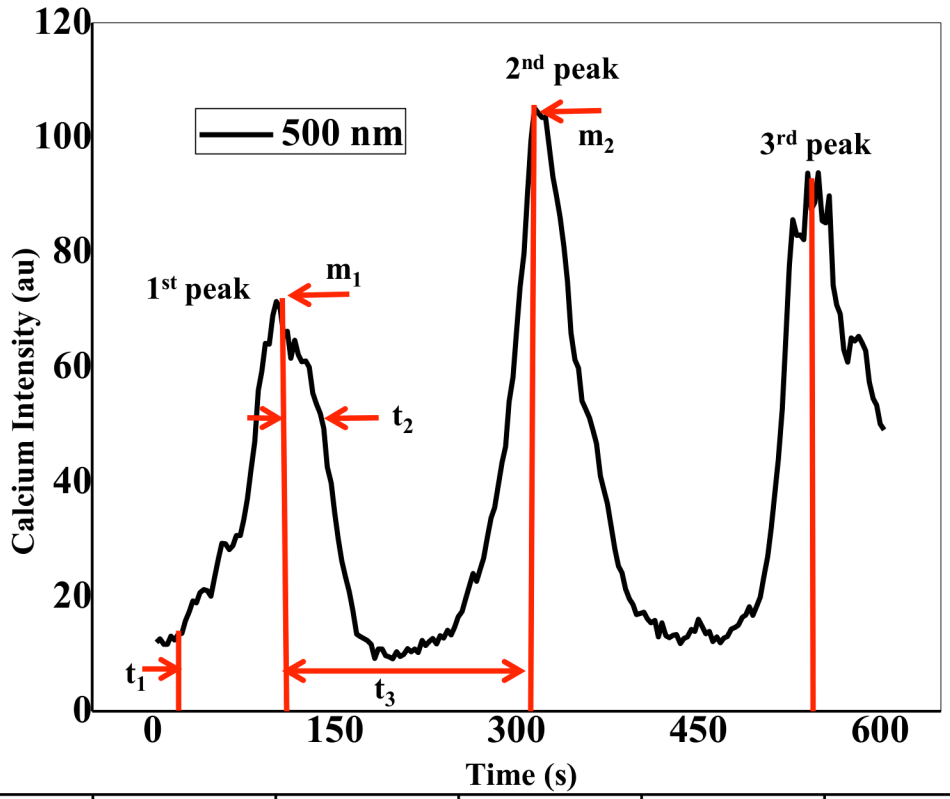


Fig. S3 Qualitative and quantitative analysis of aligned actin filaments and focal adhesion complex (FACs). (A) Percentage of cells showing visible actin cables on micro-/nanopatterns (the number of analyzed cells n= 25). (B-D) Actin (red) – vinculin (green) fluorescence images showing that different from the control group (B), actin cables are highly visible and terminate on vinculin-rich FACs spots (C-D) on pattern surfaces. Scale bars: 20 μm in B & C, and 5 μm in D. Double sided arrows indicate groove directions. * stands for significant difference from the control.

A



B

	m_1 (au)	m_2 (au)	t_1 (s)	t_2 (s)	t_3 (s)
Control	9.6 ± 2.24	9.6 ± 2.24	169 ± 7.65	81 ± 3.59	350 ± 11.23
250 nm	18.4 ± 5.09	33.8 ± 2.81	51 ± 3.48	28 ± 4.52	230 ± 16.90
500 nm	68 ± 1.76	99.4 ± 2.55	114 ± 9.06	58 ± 2.11	126 ± 7.66

Fig. S4 Characteristics of intracellular $[Ca^{2+}]_i$ oscillation of astrocytes on plat an grooved surfaces. (A) The $[Ca^{2+}]_i$ intensity was represented by average fluorescence intensity inside a cell (normalized by baseline level). The analyzed parameters were also defined in the figure: magnitude of 1st peak m_1, magnitude of strongest peak m_2, time to 1st peak t_1, relaxation time of 1st peak t_2, and time interval between two neighboring peaks t_3. (B) Characteristics of intracellular $[Ca^{2+}]$ oscillation on unpatterned and patterned surfaces. Data was shown as mean ± standard deviation.

Supporting movie SM1. Calcium releasing events of the astrocytes immediately after seeded onto unpatterned PDMS. The video was taken at a rate of 3 seconds per frame for a total of 10 minutes.

Supporting movie SM2. Calcium releasing events of the astrocytes immediately after seeded onto 250 nm deep PDMS. The video was taken at a rate of 3 seconds per frame for a total of 10 minutes.

Supporting movie SM3. Calcium releasing events of the astrocytes immediately after seeded onto 500 nm deep PDMS. The video was taken at a rate of 3 seconds per frame for a total of 10 minutes.