Supplemental Materials

Molecular Biology of the Cell

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LEGENDS FOR SUPPLEMENTAL FIGURES

Supplemental Figure 1. Comparison of C6 glioma cells migrating on laminin coated lines of different width.
(A) Snapshot of C6 cells migrating on laminin micropatterned lines of different sizes. For 3-7 μm lines C6 glioma cells were seeded and imaged in phase contrast. For 20 μm lines, cells were pre-incubated with Hoechst 33342 to label the nuclei (blue). For 100 μm lines, cells were transfected with GFP to label the edge of the cells. (B) Average of mean speed (μm/h) on laminin micropatterned lines of different sizes. Error bars are S.E.M. P-values were calculated using unpaired t-tests.

Supplemental Figure 2. Paxillin-containing adhesion tracking.
Paxillin-containing adhesions were tracked in the tail and at the front. Data extracted from the movie # 6. **(A)** Montage of the overlay of the tracks and the paxillin movie during trail retraction. **(B)** Montage of the overlay of the tracks and the paxillin movie during front advancement. **(C-D)** Adhesion track projections obtained with ImageJ. See also supplemental movies 5-6.

Supplemental Figure 3. Mechanical constraint mediated by an increase in cell density triggers glioma linear migration dependent on laminin.
(A) Schematic of the laminin pattern consisting of a large rectangle (reservoir) joined to strips of widths varying from 10μm (line 1) to 400μm (line 12). **(B)** C6 glioma cells pre-incubated with Hoechst 33342 to label the nuclei were plated on the pattern coated with laminin (500 μg/ml). Cells were imaged for long periods of time (15-20h) (1 image/ 6 min). Images of cells migrating on the reservoir and on the lines. Zooms showing the shape of glioma cells when migrating in the reservoir (a), on a 70μm line (b) and on a 20μm line (c). **(C)** Tracks of the cell bodies in line 10 (130μm width) in 3 different time-window.

Supplemental Figure 4. Behavior of C6 glioma cells on poly-L-lysine coated lines
C6 glioma cells were plated on lines of Poly-L-Lysine of 200 μm width connected to a large reservoir. Cells were imaged after 1h seeding for long periods of time in phase contrast using a 10X objective (1 image/6min). Top: Reconstitution of the entire pattern at the end of the movie (24h). (a-d) zooms of various regions in the reservoirs and lines showing the same perpendicular alignment.

Supplemental Figure 5. Phospho-myosin light chain 2 and GFP-mDia2 localization in C6 glioma cells.
(A) Confocal images of glioma cells migrating on 20μm lines fixed and stained for phospho-myosin light chain 2 (Ser19) (green), Phalloidin (red) and dapi (blue). Bar is 10μm. **(B)** Confocal images of glioma cells seeded on laminin coated glass bottom dish. Panels a-c show a dividing cell with a typical staining of active myosin at the contractile ring (arrow). Panels d-g show a migrating cell with stress fibers decorated with phospho-myosin. **(C)** Confocal images of glioma cells expressing GFP-mDia2 on 3 μm line fixed and stained with Phalloidin (red) and dapi (blue).

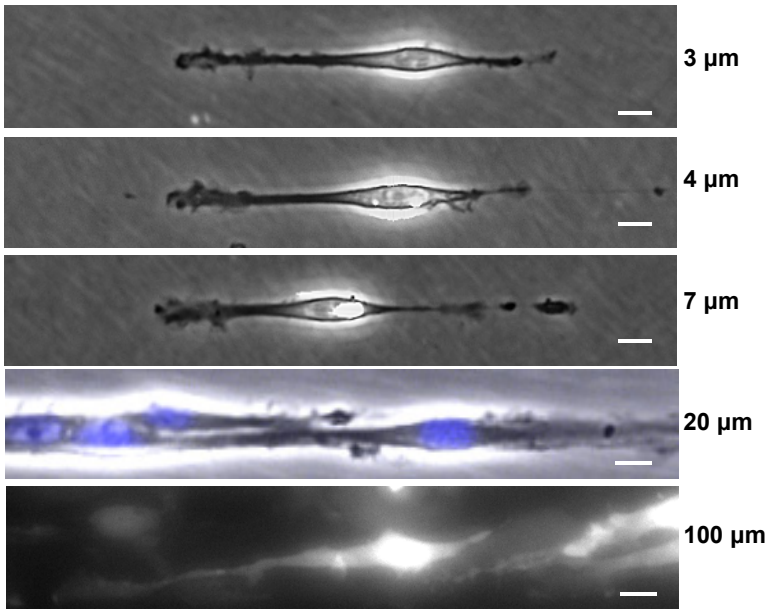
Supplemental Figure 6. Knock down of formins in human GPCs
(A-E) Human Glioma propagating cells were transfected with shRNA targeting mDia1, mDia2 and FHOD3. 3 days later, transfected cells were seeded on 7 μm laminin micropatterned lines

and tested for effective knock down. **(A-C)** Expression of mDia1, mDia2 and FHOD3 in shRNA transfected cells was examined by western blotting with antibodies against mDia1, mDia2 and FHOD3. α-Tubulin was used as a loading control. **(D)** Quantification of the formin expression in the respective knock down in hGPCs corresponding to western blot in A,B,C. **(E)** Average of mean speeds of transfected hGPCs and migrating on 7 μm laminin micropatterned lines reported to the speed of control cells (%). Error bars are S.E.M. P-values were calculated using unpaired t-tests. **(F)** Quantification of the formin expression in the respective knock down in rat C6 glioma cells corresponding to western blot showed in figure 7C-E. **(G)** Mean speeds of C6 glioma cells transfected with shRNAs against mDia1, mDia2 and FHOD3 and migrating on 2D surfaces were measured and sorted in 3 different classes: Immobile cells (mean speed < 10 μm/h), slow cells (10 < mean speed < 20um/h) and fast cells (>20um/h).

Supplemental Figure 7. GFP-FHOD3 overexpressing cells display excessive amounts of longitudinal actin bundles.

C6 glioma cells were transfected with GFP-FHOD3, seeded on laminin coated 2D-subtrates, fixed and stained with phalloidin (red) and Dapi (blue) and imaged using a confocal microscope. **(A)** Typical GFP-FHOD3 transfected cell displaying parallel actin filaments co-stained with GFP-FHOD3. **(B-C)** GFP-FHOD3 overexpressing cells (*) among un-transfected cells display excessive amounts of longitudinal actin bundles. Scale bars = 10 μm.

A



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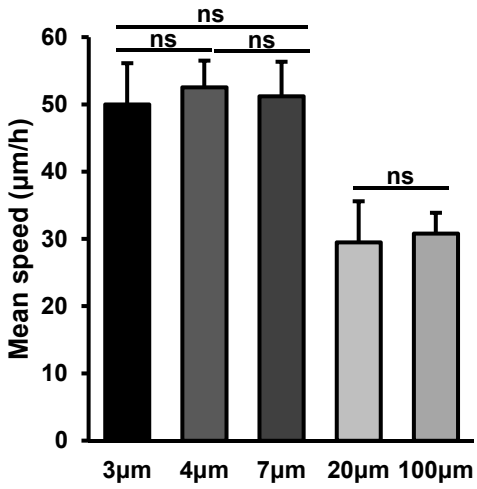
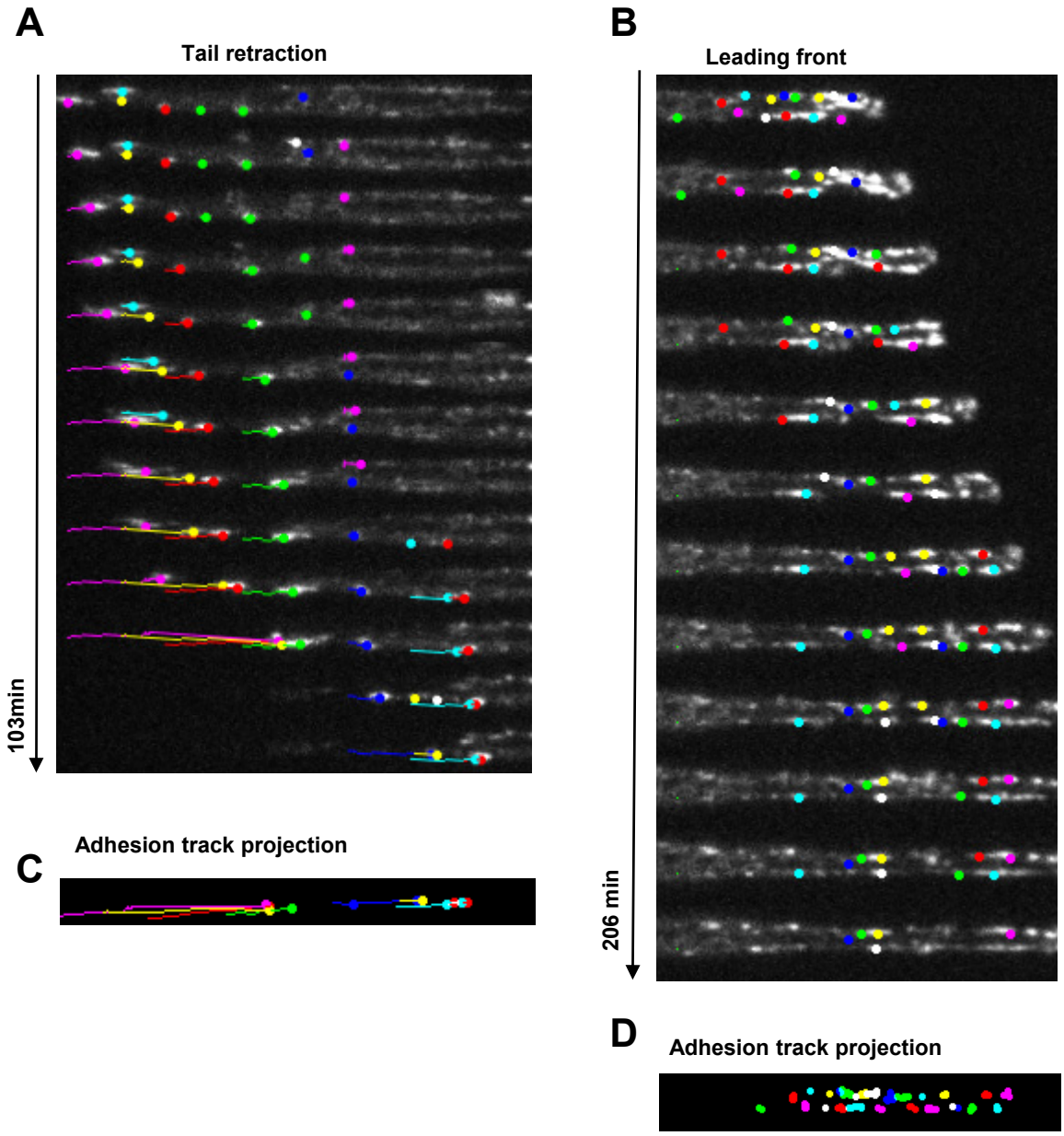


Supplemental Figure 1



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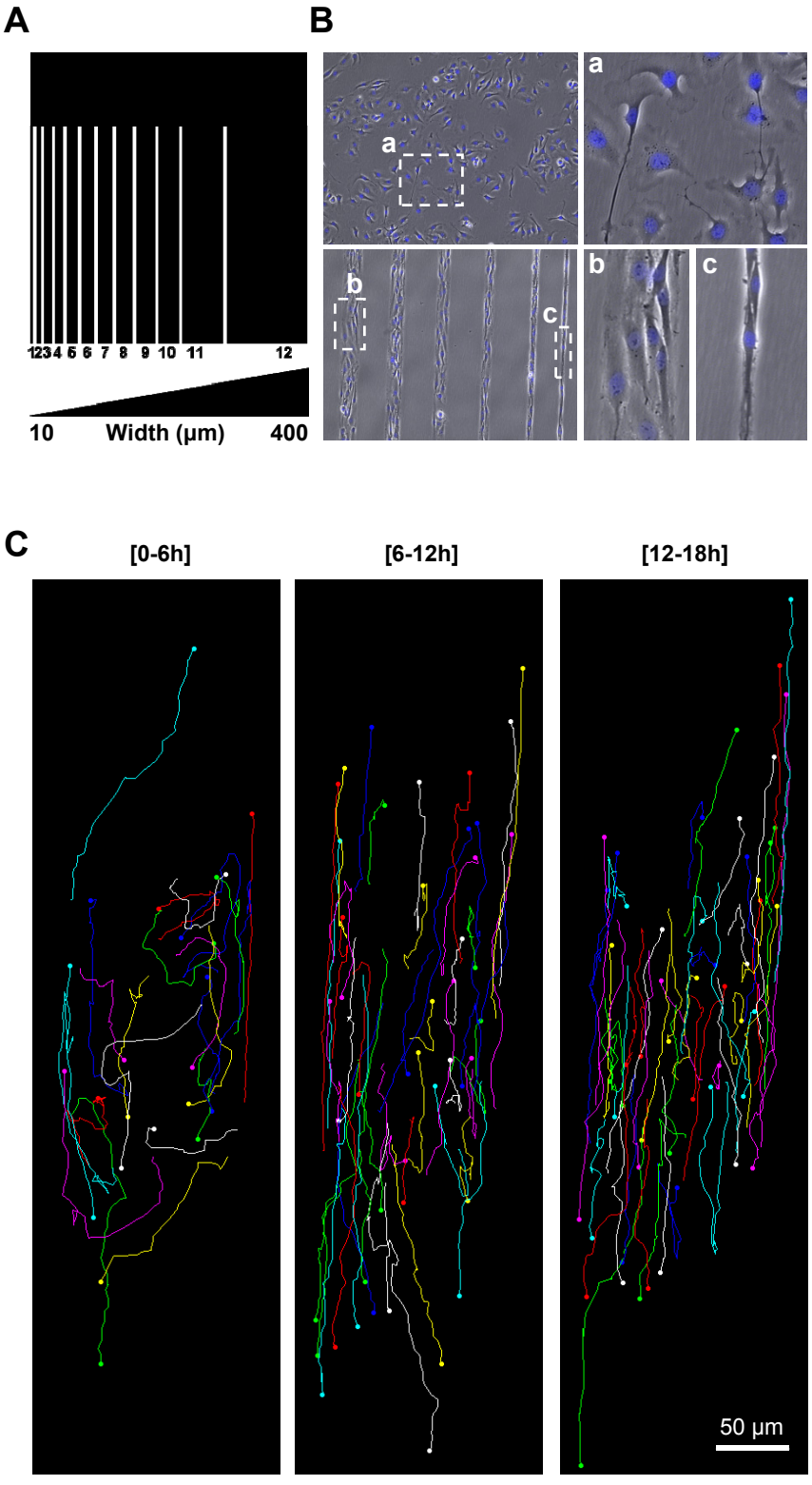

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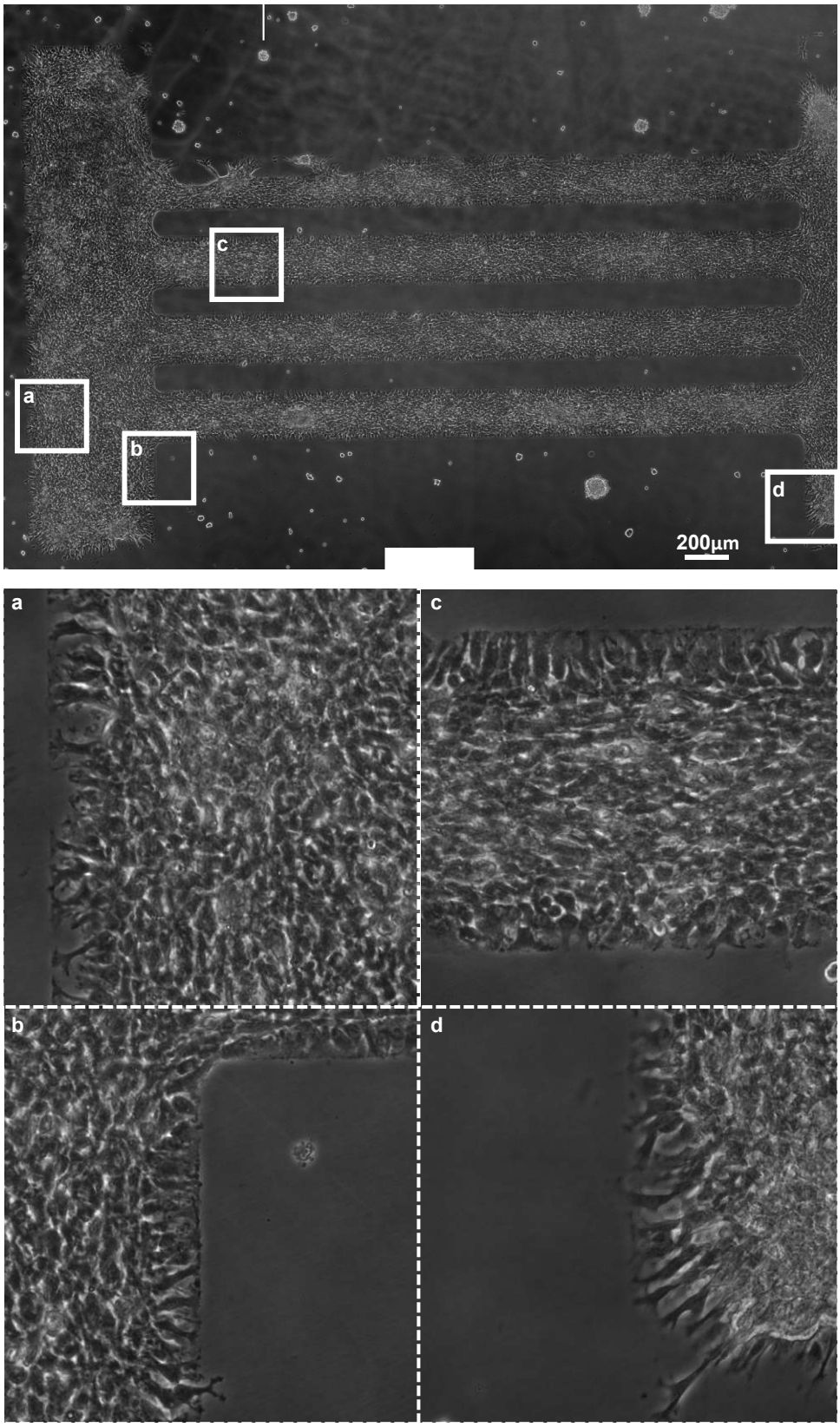

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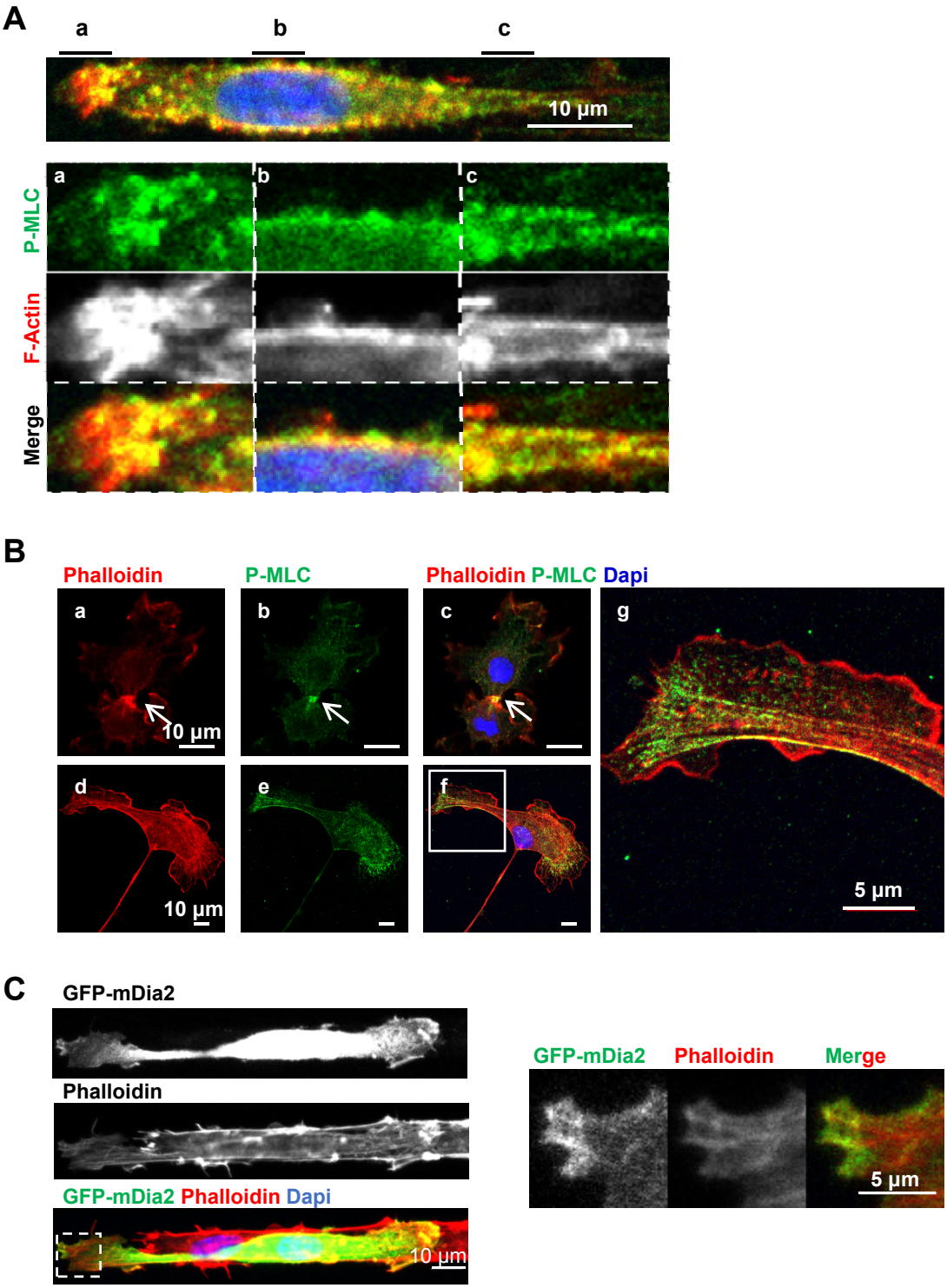

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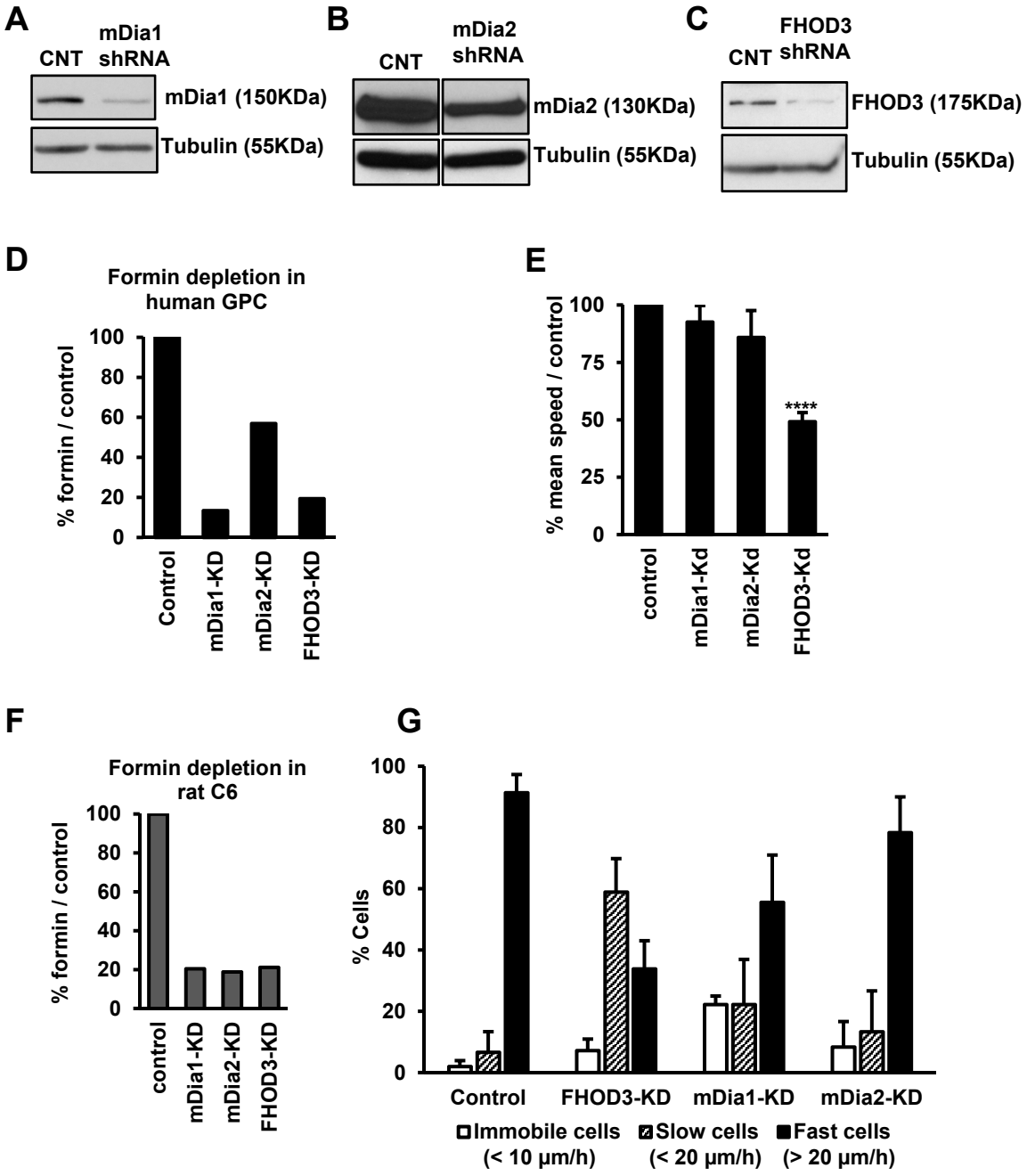
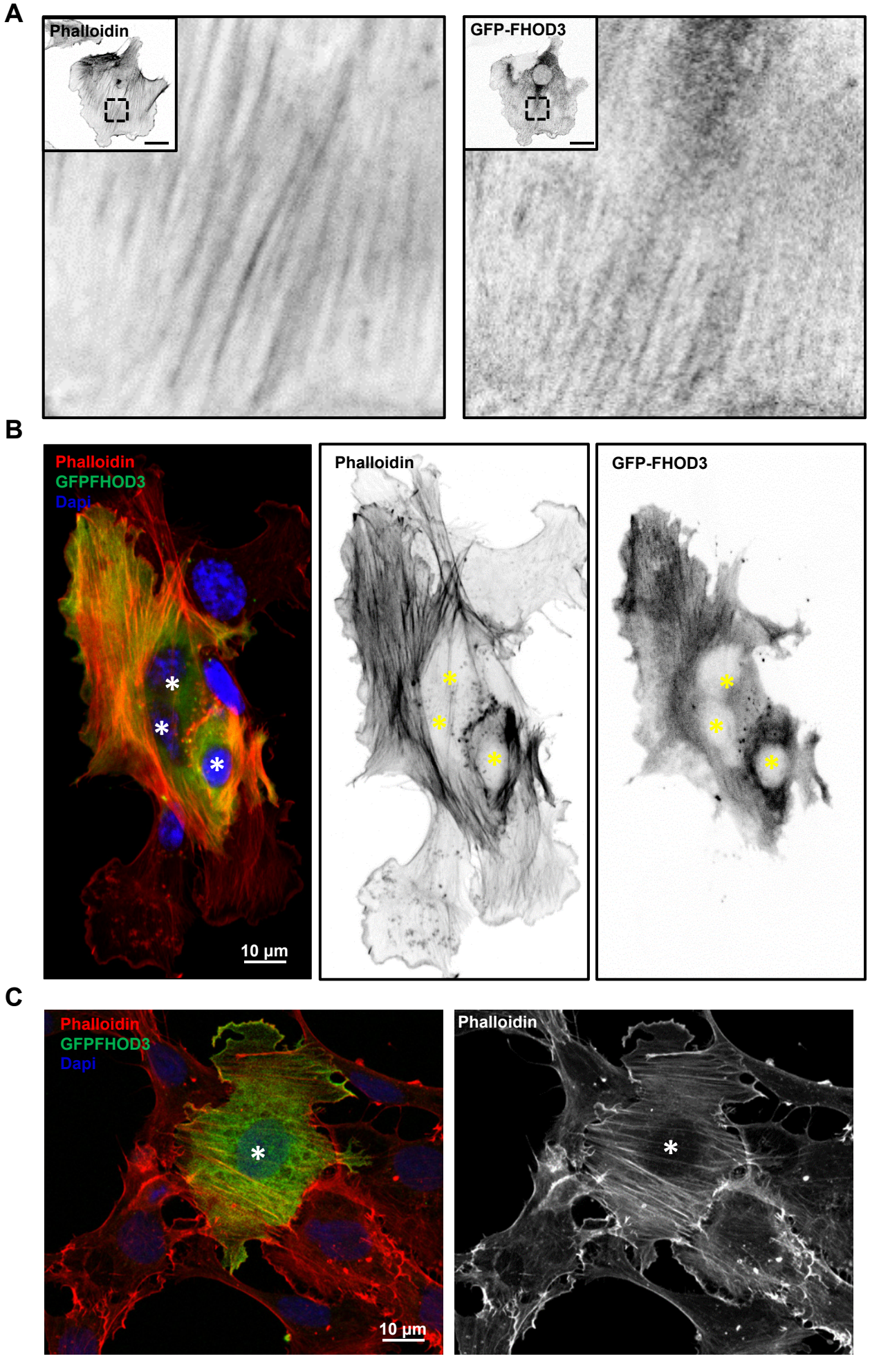


Supplemental Figure 6



Supplemental Figure 7