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Supplemental information

3D mesenchymal cell migration is driven by anterior cellular contraction that generates an extracellular matrix prestrain

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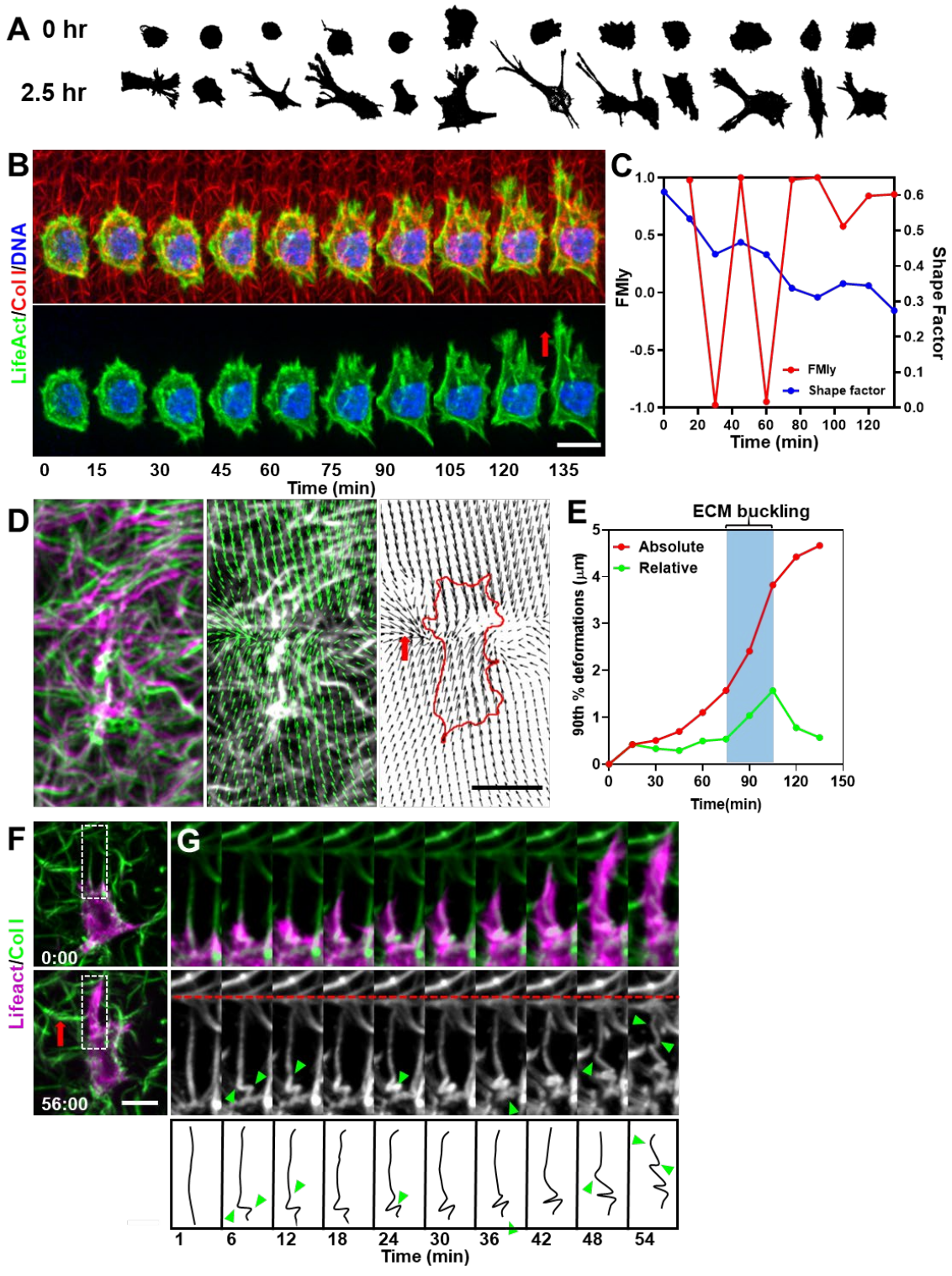

Figure S1. Cell polarization and ECM buckling during 3D cell spreading, Related to figure 1. A) Masked/binarized examples of fibroblast polarity at 0 hr (1st row) and 2.5 hrs (2nd row) after seeding cells in a 3 mg/ml rat tail collagen gel. All cells demonstrate

polarity after 2.5 hrs. B) Fibroblast expressing Tag-GFP2 LifeAct (green) with SiR-DNA (blue) spreading in 3D collagen (red). During the first 2 hrs of spreading, cells often oscillate from side-to-side prior to becoming directionally polarized. C) Graphical plots of forward migration index of the nucleus (FMIy; red) and shape factor of the cell body (blue) of the cell shown in panel B, illustrating the nuclear oscillation prior to cell elongation and polarization. D) Overlay of minute 75 (green) with minute 105 (magenta) images from Figure 1, panels D-F shows the extent of ECM buckling. ECM movement with respect to the ECM (middle) and the cell (right). E) Graphical comparison of the absolute (red) and relative (green) ECM deformations for the cell in Figure 1D-F. Blue area represents min 75-105, when the cell contracts prior to reaching steady-state. F and G) Lightsheet microscopy of a spreading fibroblast (LifeAct: magenta) in 3D collagen (green) at an early (0:00) and later timepoint (56:00 min). Insets (G) of the regions marked by white dashed lines show that as a cell protrudes along a collagen fibril, contractile forces between the tip of the protrusion and the cell body cause the fibrils to kink and buckle (green arrowheads). Red dashed line is a fiduciary mark for reference. Scale bars: B and D; 20 μm, F; 10 μm. Red arrows indicate the direction of cell polarization.

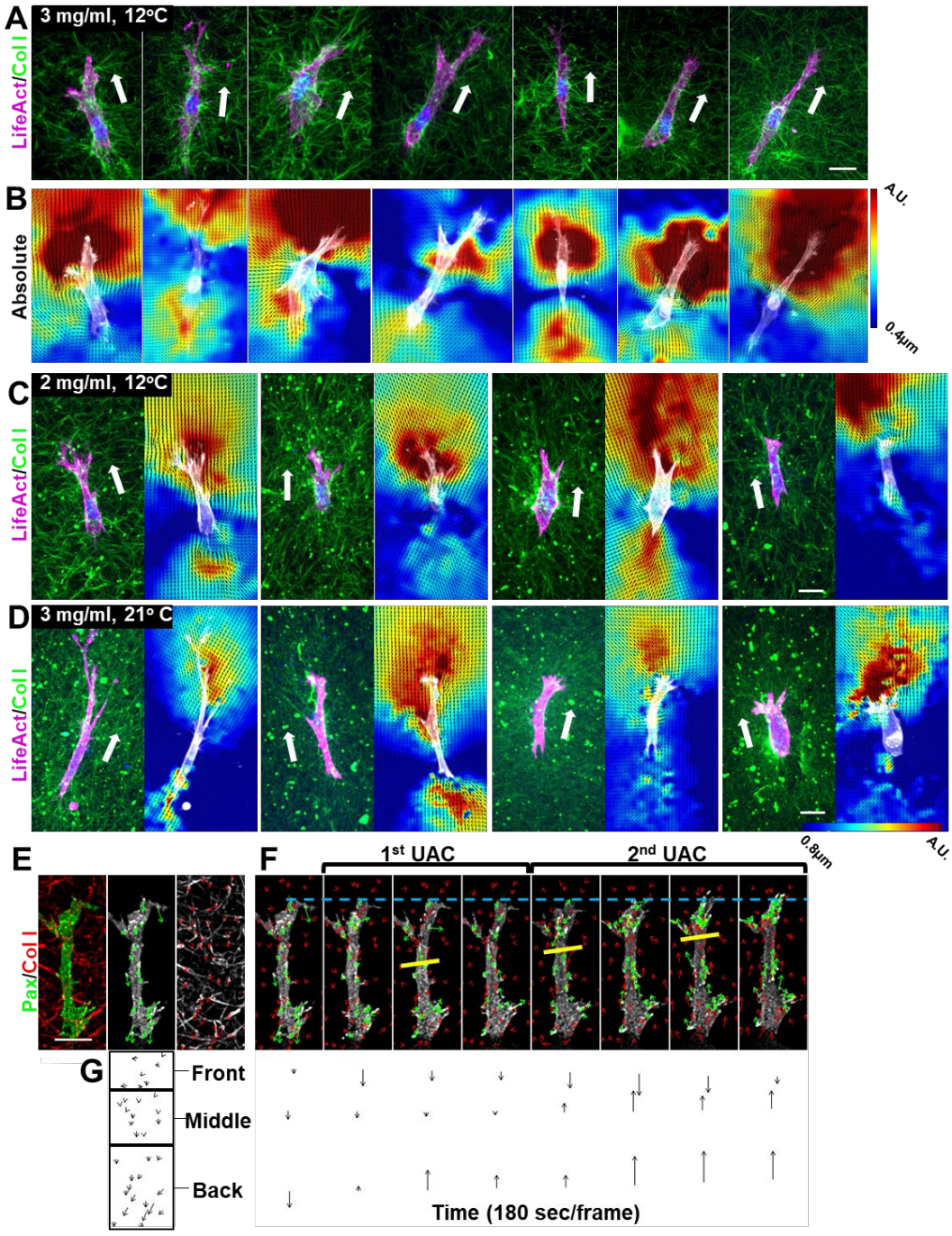

Figure S2. Front/back strain asymmetry in different collagen gels and contraction through adhesions propagates to the ECM and propels the cell forward with a "pinch", Related to figure 2 and 3. A) Seven examples of fibroblasts expressing Tag-GFP2 LifeAct (magenta) and migrating within a 3D collagen ECM (3 mg/ml: green). SiR-DNA highlights the nucleus (blue). All images are maximum intensity

projections (MIP). White arrow indicates the direction of migration. B) PIV imaging of the absolute deformations of the collagen ECM. Heat map scaling was adjusted for each example to illustrate front-to-back strain differences. C and D) Maximum intensity projections (MIP) of multiple fibroblasts in 2 mg/ml collagen gels polymerized at 12^{o} C (C) and 3 mg/ml collagen gels polymerized at 21^{o}C (D). Paired images show Tag-GFP2 LifeAct (magenta), collagen I matrix (green) and the nucleus (blue) on the left and PIV absolute deformations of the matrix on the right. The majority of cells demonstrate a similar matrix prestress during migration. White arrows indicate the approximate direction of cell migration. E and F) Tracking both EYFP-paxillin adhesions (green) collagen (red) demonstrates that cells “pinch” the ECM prior to forming new adhesions at the leading edge. Cyan dashed line indicates initial position of the leading edge. Inward vectors of both the adhesion and ECM move toward a central convergence point (yellow lines in F). G) Three regions of the ECM were tracked and their Y-vectors averaged to demonstrate that the pinch originates from the cell body region and moves forward with each consecutive pinch. Scale bars: A, C and D 20 μm; E 10 μm.

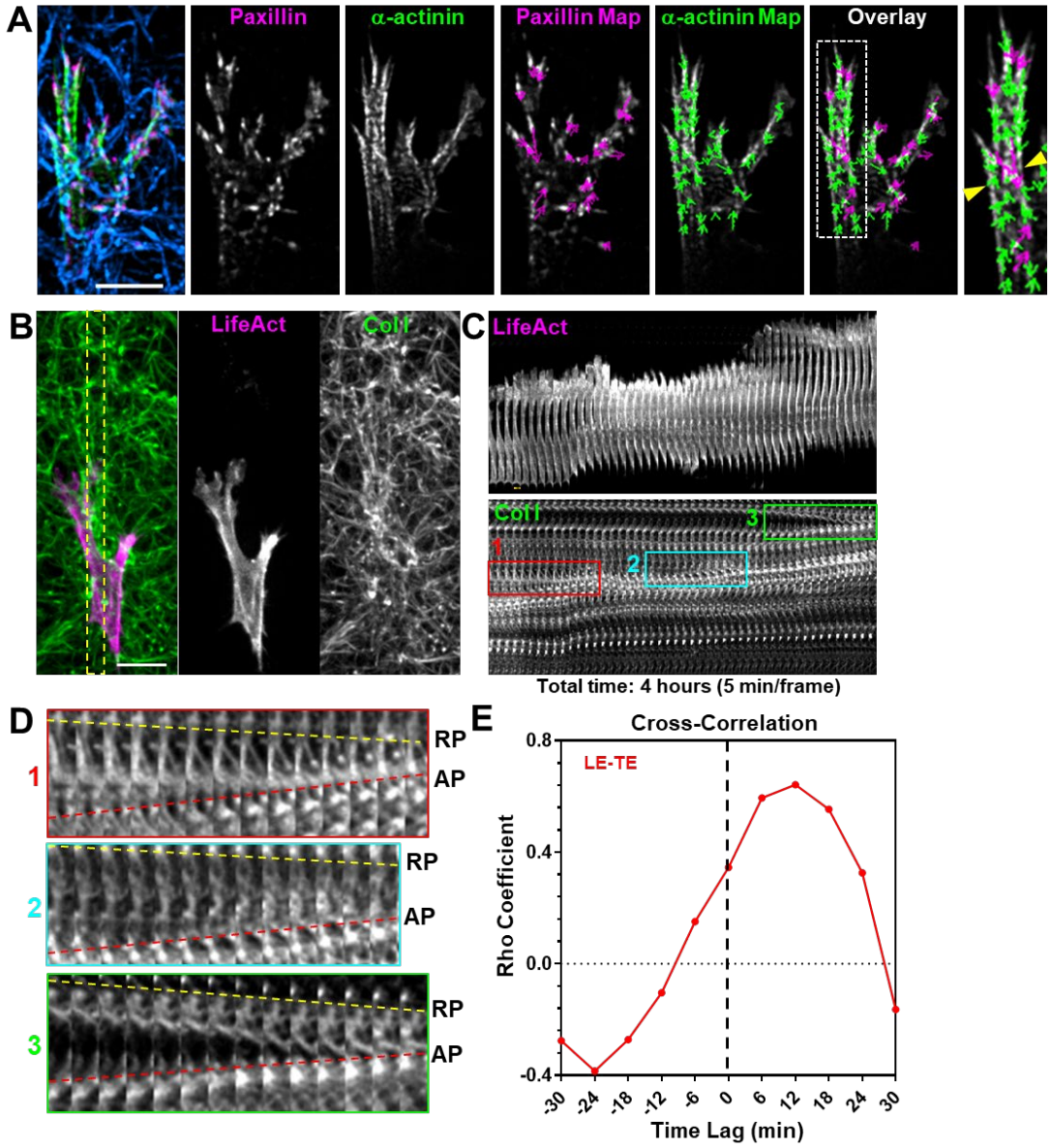

Figure S3. Cytoskeletal contraction in 3D gels and anterior contractions during single cell 3D migration, Related to figure 3. A) Tracking of EGFP-α-actinin (green) and mApple-paxillin (magenta) show similar movements within the cytoskeleton as for the ECM shown in panels C-D below. Overlay and inset of the α-actinin and paxillin maps show movements towards a central region (yellow arrowheads) behind the leading edge. B) MIP image of 3D fibroblast migration showing Tag-GFP2 LifeAct (magenta) and collagen I matrix (green) in 3 mg/ml collagen gels. The yellow dashed box outlines the region for the kymographs shown in panel C. C) A 4 hr kymograph generated from the areas outlined in panel A showing Tag-GFP2 LifeAct (top) and

collagen I matrix (bottom). Three boxes in C (red, cyan and green) indicate areas with an AC. Similar kymographs were generated for each cell analyzed to determine if an AC occurred during cell migration. D) Enlarged regions outlined by boxes in panel B show a small retrograde pull (RP: yellow-dashed line) and anterograde pull (AP: red-dashed) associated with cell protrusion activity. E) Cross-correlation to determine the time lag between leading-to-trailing edge (LE-TE) movement associated with an anterior contraction. Scale bar: A, 10 μm; B, 20 μm.

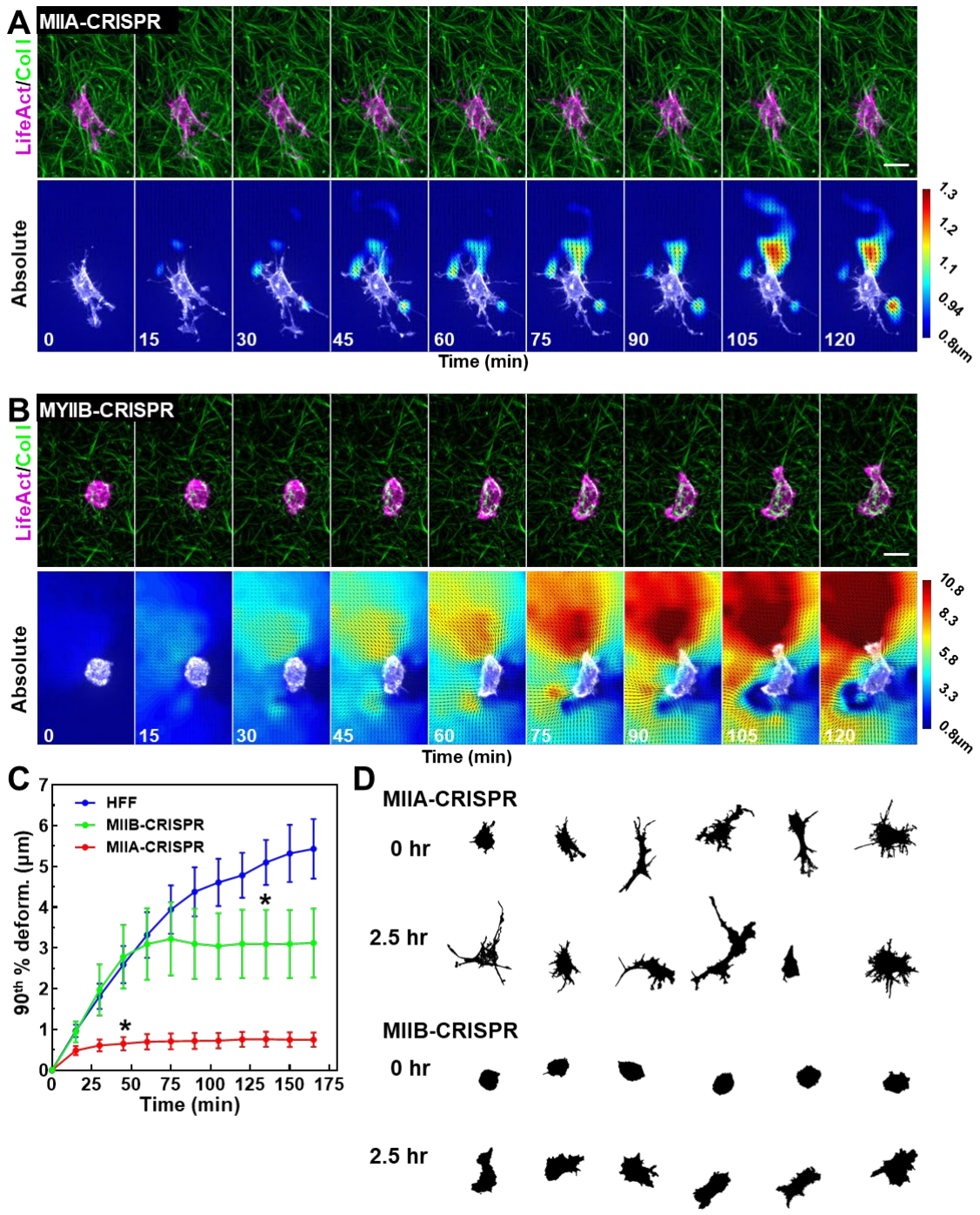

Figure S4. Roles of myosin II isoforms in 3D cell spreading, Related to figure 4. A) Maximum intensity projection (MIP) image of a myosin IIA knockout (MYH9-/-) dermal fibroblast expressing TagGFP2-LifeAct (magenta) imaged immediately after collagen (green) gel polymerization for 2 hrs. Bottom row shows the PIV analysis. Cells fail to

polarize over this time period. B) MIP image of a myosin IIB knockout (MYH10-/-) dermal fibroblast expressing TagGFP2-LifeAct (magenta) imaged immediately after collagen (green) gel polymerization for 2 hrs. Bottom row shows the PIV analysis revealing similar matrix prestrain and strain anisotropy compared to wild type controls (Figure 1D-E). Note the PIV heat map scaling differences between panels A and B. C) Graphical comparison of control fibroblasts versus MIIA (MYH9) and MIIB (MYH9) CRISPR knockout cells. * indicates $P \leq 0.05$ at this timepoint and later compared to control cells ($n \geq 7$). Data represent mean± SEM. D) Masked/binarized examples of MIIA (MYH9) and MIIB (MYH10) knockout fibroblasts at 0 hr (1st row) and 2.5 hrs (2nd row) after seeding cells in a 3 mg/ml rat tail collagen gel. MIIA KO cells spread early and fail to polarize, while MIIB KO cells demonstrate polarity after 2.5 hrs. Scale bar: 20 μm.

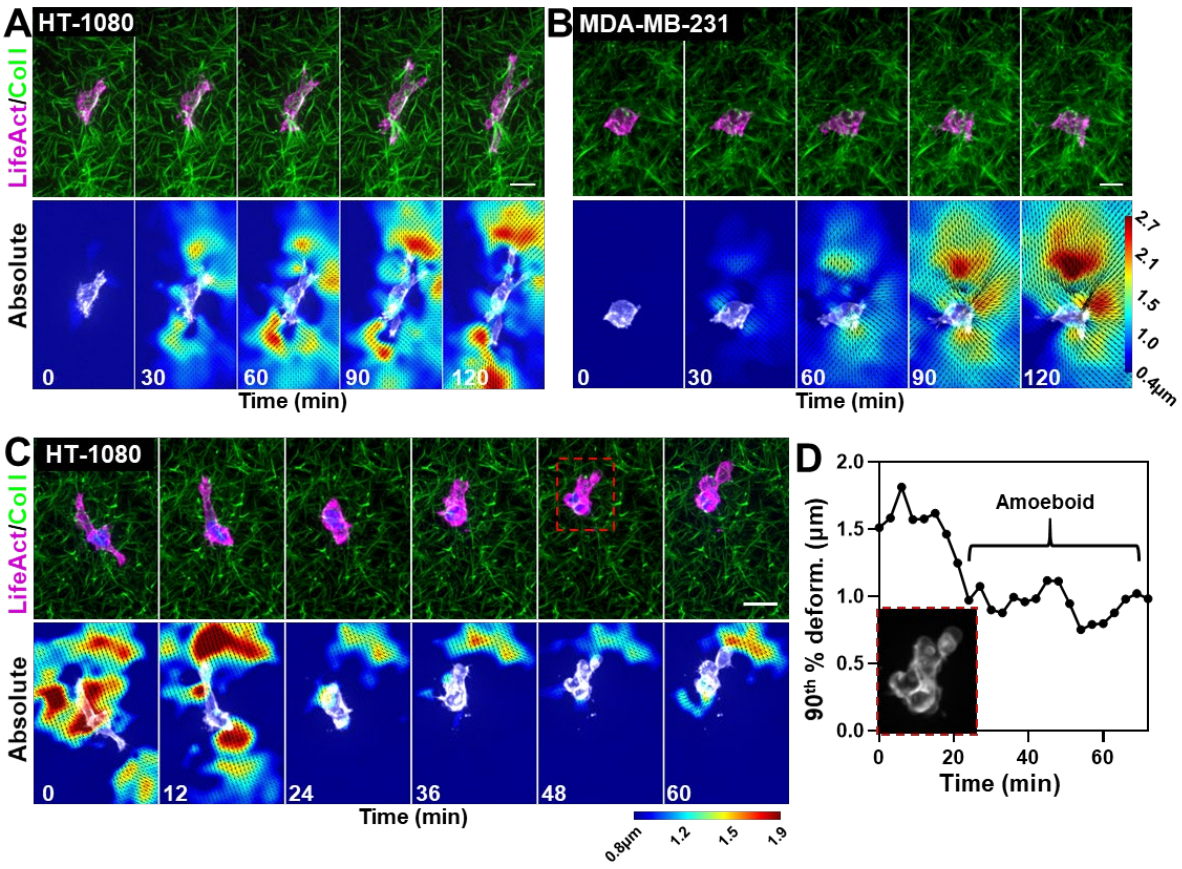

Figure S5. 3D cell spreading of HT-1080 and MDA-MB-231 cells, Related to figure 6. A) Maximum intensity projection (MIP) image of an HT-1080 fibrosarcoma cancer cell expressing TagGFP2-LifeAct (magenta) imaged starting immediately after collagen (green) gel polymerization for 2 hrs. Bottom row shows the PIV analysis. Cell polarizes with anisotropic ECM strain and begins to migrate away rapidly. B) MIP image of an MDA-MB-231 cell expressing TagGFP2-LifeAct (magenta) imaged starting immediately after collagen (green) gel polymerization for 2 hrs. Bottom row shows the PIV analysis and a lack of cell polarity. C and D) MIP time lapse images of HT-1080 cell expressing TagGFP2-LifeAct (magenta) transitioning from mesenchymal to amoeboid migration during 3D migration in a collagen gel (green). PIV analysis (bottom) illustrates the drop in ECM strain accompanying this transition. Dashed box (red) indicates the insert shown in panel C. D) Graphical representation of the 90th percentile ECM deformations over time for the cell in panel C, illustrating the reduction in ECM strain during amoeboid migration. Scale bars: 20 μm.

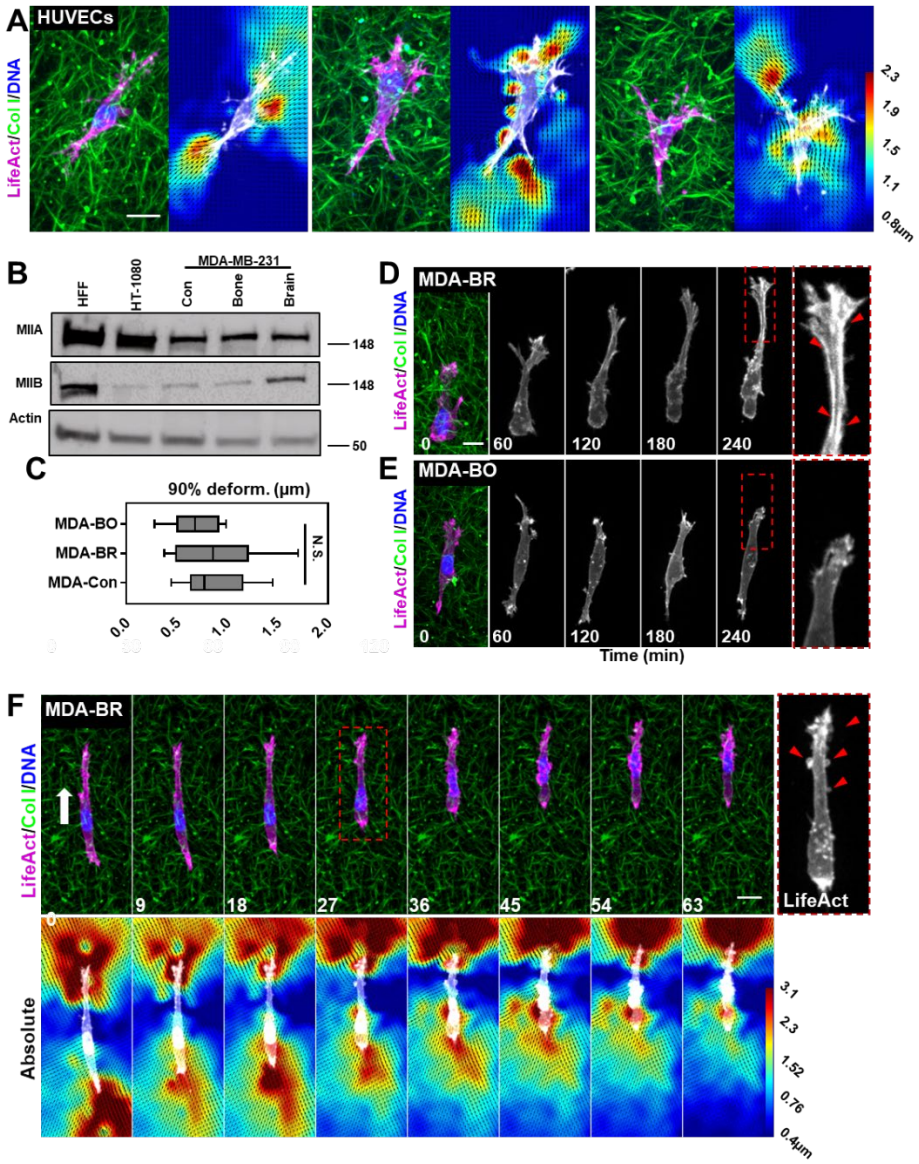

Figure S6. Migration phenotypes of HUVECs MDA-MB-231 cells from secondary tumors, Related to figure 6. A) Three examples of ECM strain distributions in HUVECs in 3 mg/ml collagen gels. HUVECs are expressing TagGFP2-LifeAct (magenta) during 3D migration through 3 mg/ml collagen (green). Images are maximum intensity projections (MIPs), and the nucleus is marked by SiR-DNA (blue). PIV images of matrix deformations are shown to the right. Unlike other cells we observed, HUVECs often demonstrated high ECM deformations closer to the cell body. B) Differing expression of myosin IIA and IIB isoforms in HFF, HT1080, MDA-MD-321 parental (Con) and variants isolated from secondary metastases to bone or brain tissue. C) 90th% ECM

deformations for control (MDA-Con), brain (MDA-BR) and bone (MDA-BO) metastatic MDA-MB-231 cells (n≥14). Data represent mean± SEM. D and E) Time-lapse montage of MDA-MB-231 cells from secondary tumors (metastases) to Brain (D) and Bone (E) expressing TagGFP2-LifeAct (magenta) during 3D migration through 3 mg/ml collagen (green). Images are maximum intensity projections (MIPs), and the nucleus is marked by SiR-DNA (blue). Dashed line boxes (red) in panels C and D indicate the magnified inserts at the right. Note the visible stress fibers (red arrowheads) in only the Brain variant and their directionality. F) Absolute PIV analysis of MDA-brain cells in 3D collagen undergoing directional migration demonstrates matrix prestress similar to HT-1080 cells. Dashed boxed (red) indicates the insert frame to the right showing TAGGFP2-LifeAct and the multiple blebs along the pseudopod. Scale bars: 20 μm.

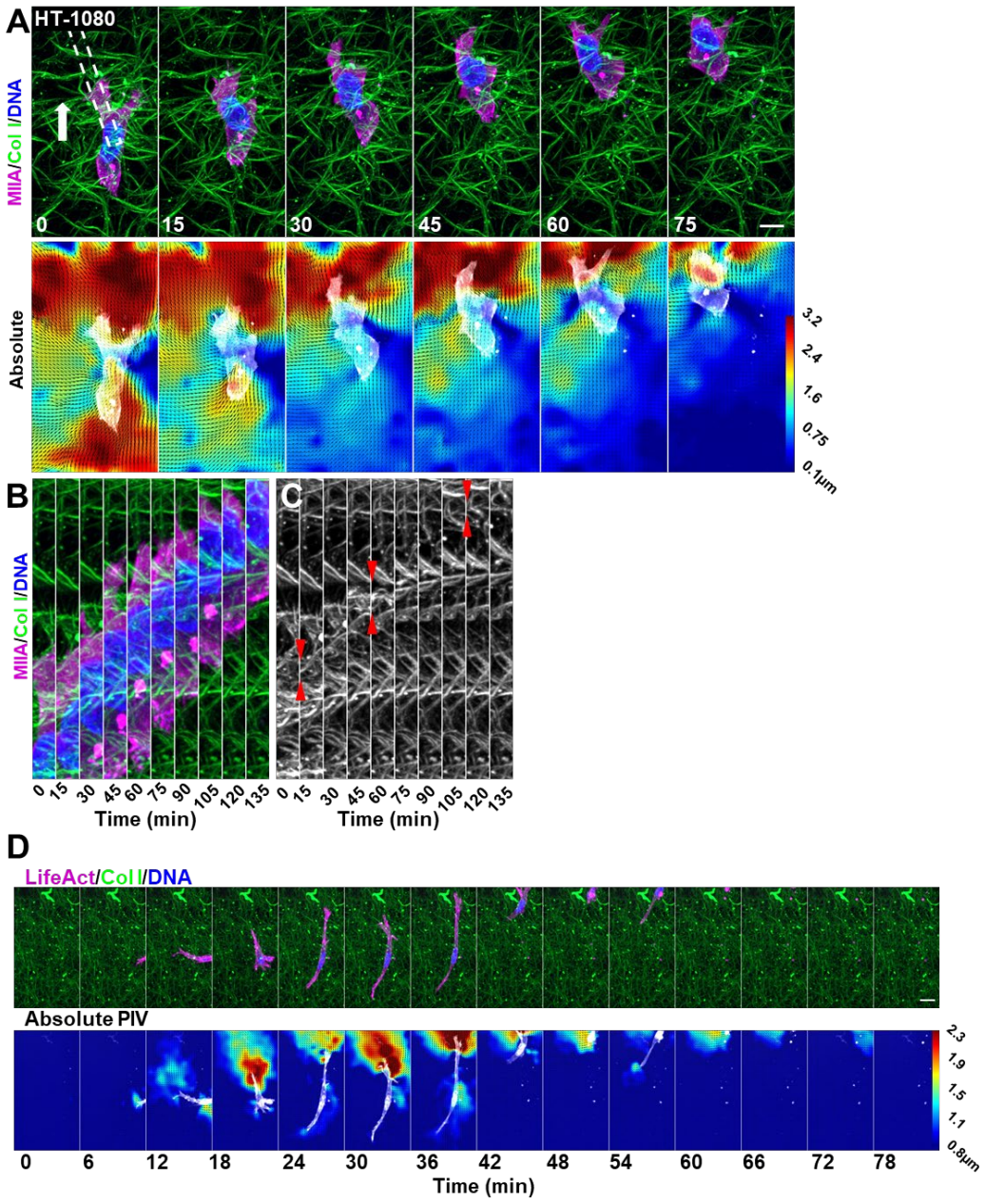

Figure S7: Ablation of ECM prestress and enhanced ACs after MIIA overexpression in HT-1080 cells, Related to figure 7. A) MIP images of an HT-1080 cell overexpressing EGFP-myosin IIA (magenta) while migrating through a 3D collagen matrix (green). SiR-DNA is shown in blue. Dashed white line indicates the kymograph area shown in panels B and C. Three-color kymograph of the cell in panel A (B) and kymograph showing only ECM (C). Red arrowheads indicate the locations of three anterior contractions over this time period. D) Timelapse series of an HT-1080

expressing TagGFP2 LifeAct (magenta) that migrates into and out of a region of 3D collagen (green). The accompanying PIV images show little to no differences in ECM deformation before the cell enters and after the cell leaves the particular region. Scale bars: A, 10 μm, D, 20 μm.