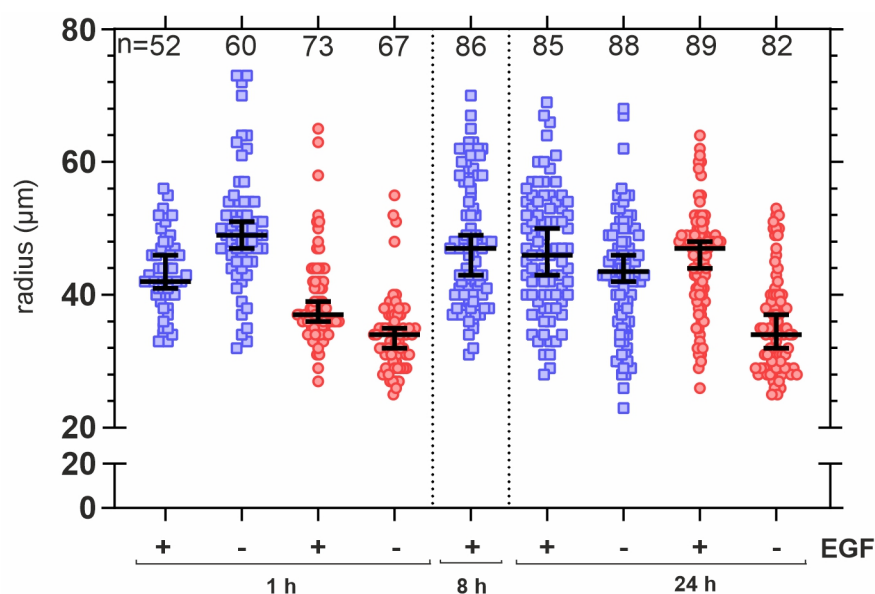
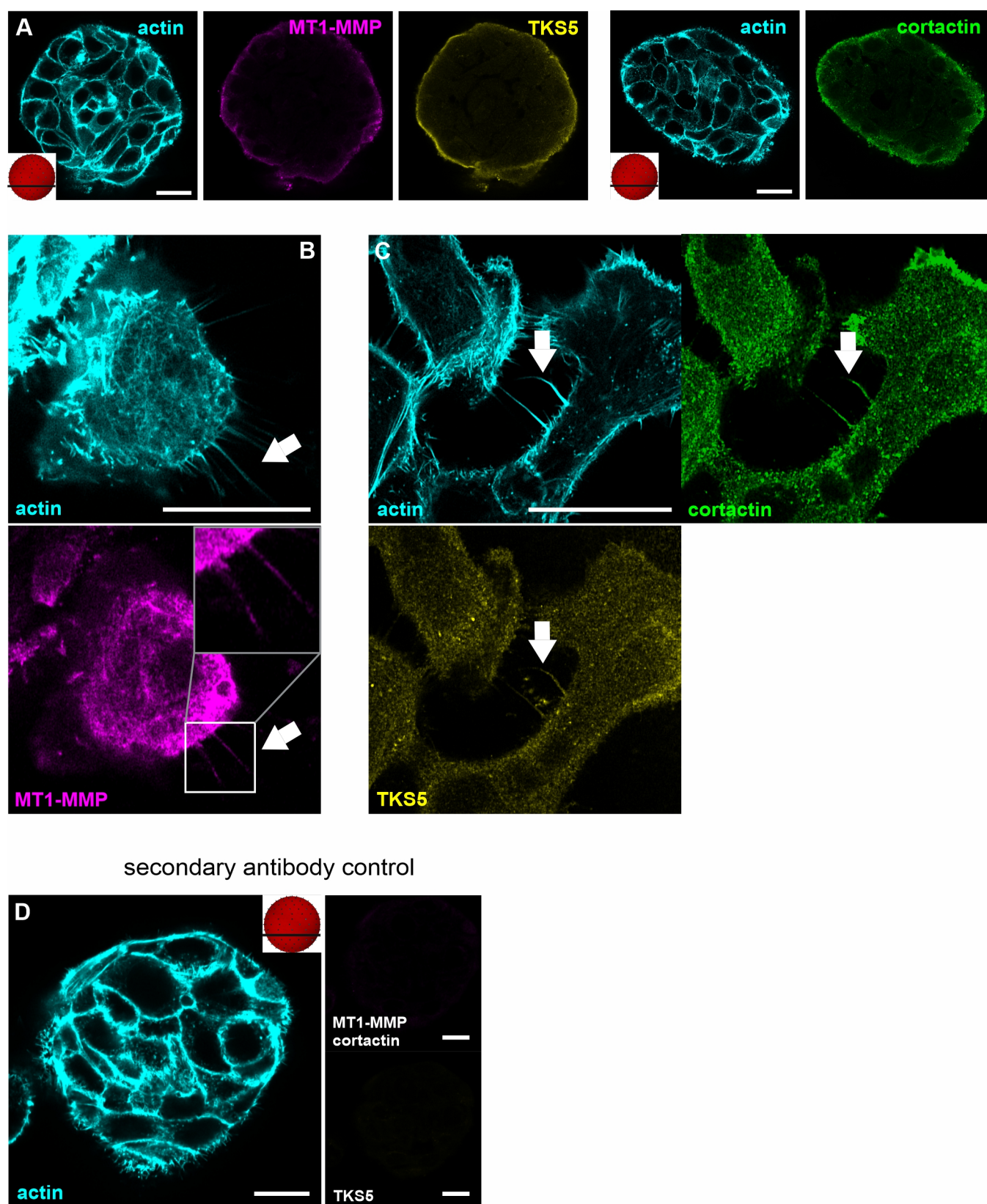


Supplementary Information

# From Microspikes to Stress Fibers: Actin Remodeling in Breast Acini Drives Myosin II-Mediated Basement Membrane Invasion.

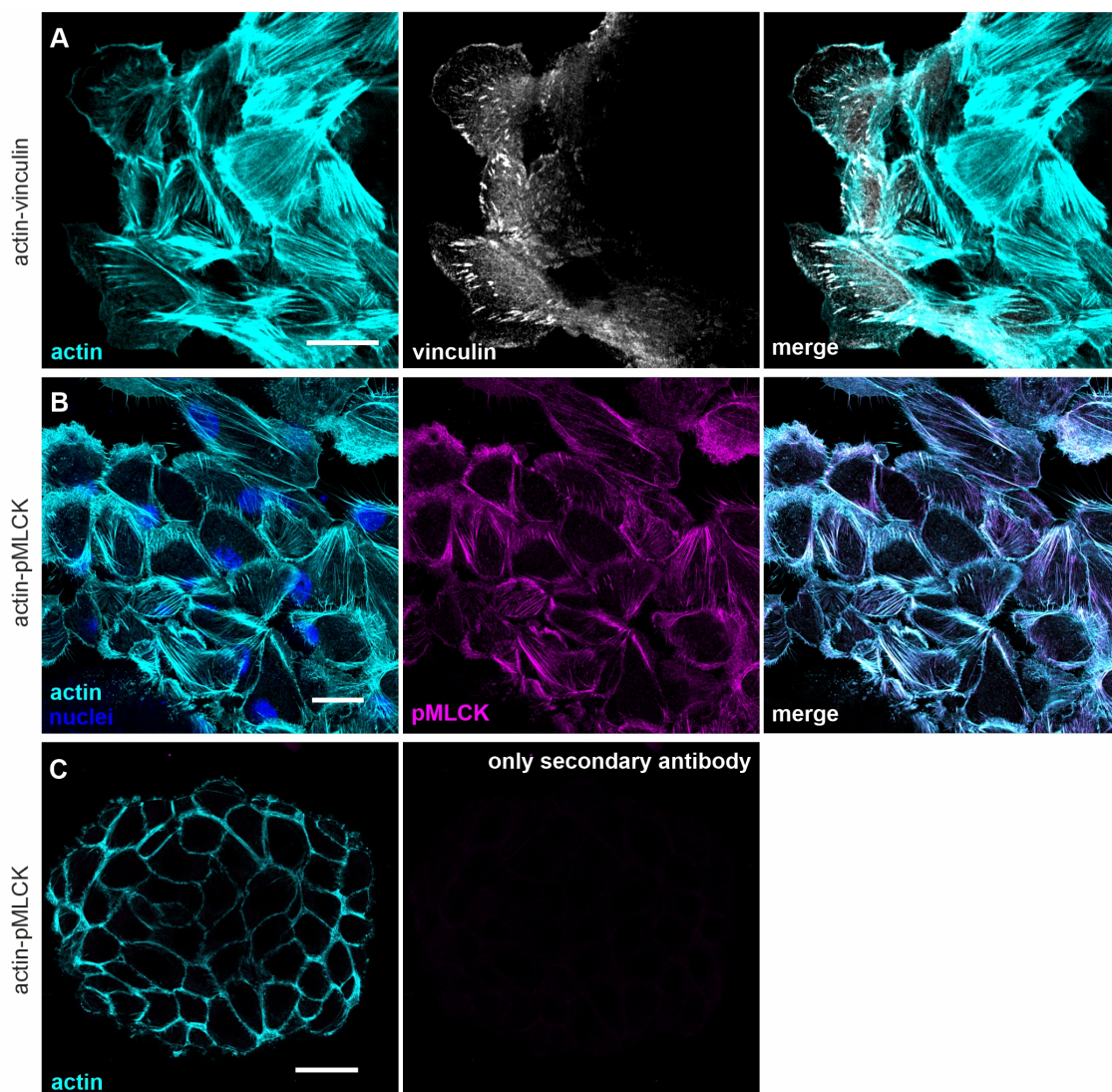


**Figure S1.** The radii of breast acini depend on maturation state and EGF treatment. Equatorial radii for BM perimeter calculation of all acini analyzed for Figures 2B and F. Maturation stages: 11 days old ld-BM acini (blue) and 21 days old hd-BM acini (red) with and without EGF treatment (20 ng/ml). Acini were transferred on a glass substrate and fixed after one, eight and 24 hours cultivation time with assay medium, either with EGF or without.



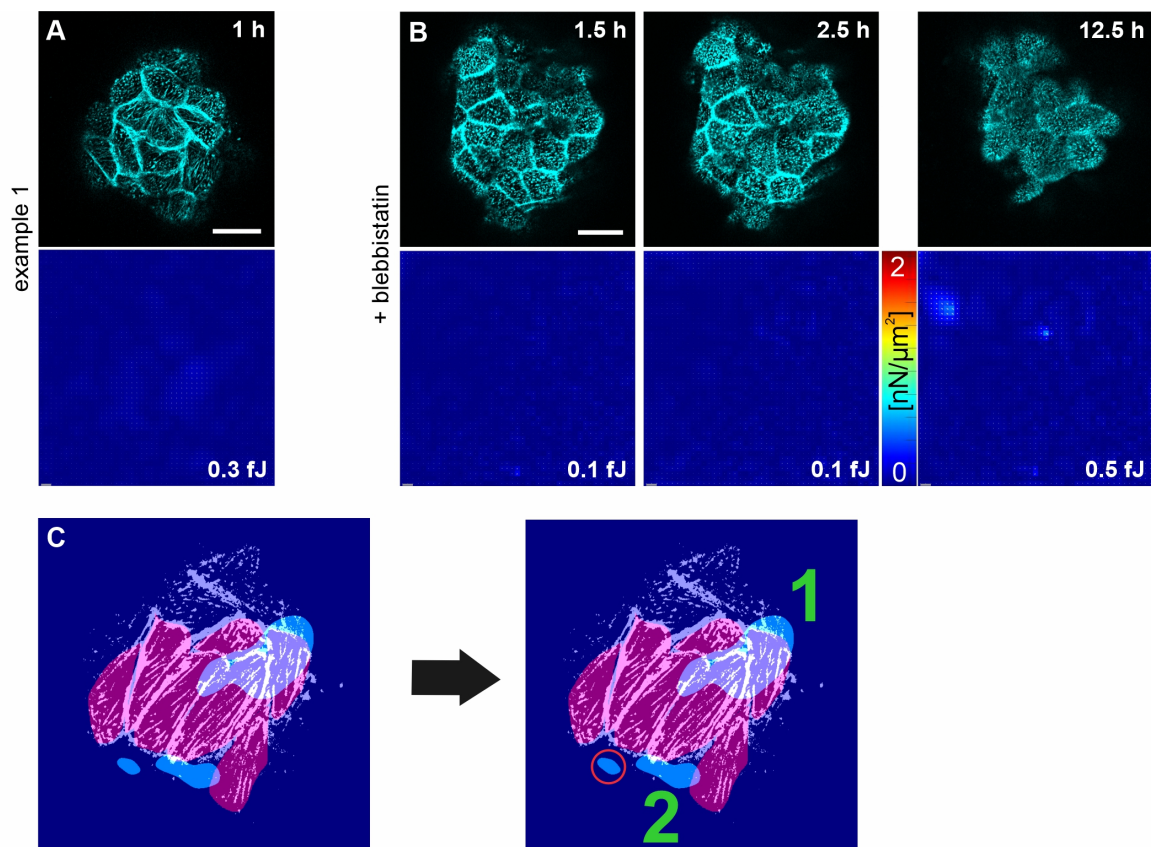
**Figure S2. Invadopodia marker protein localization in single migrating cells and MCF10A acini.** Confocal images of fixed 1d-BM MCF10A acini sections or migrating cells on a planar glass substrate under most invasive conditions (24 h EGF treatment on a glass substrate). **(A)** The actin cortex (cyan) of the basal cell layer co-localizes with the invadopodia marker proteins MT1-MMP (magenta), Tks5 (yellow) and cortactin (green). For merged images see Figure 3A and B. **(B and C)** Single MCF10A cells on a planar substrate showing elongated actin-based filopodia-like cell protrusions positive for

MT1-MMP, TKS5 and cortactin. (D) Secondary antibody control for invadopodia staining shows no unspecific signals of secondary antibodies (cf. Figure 3A and B). Scale bars = 20  $\mu$ m.



**Figure S3. Focal adhesion and pMLCK localization in migrating MCF10-A cells.** (A) Focal adhesions (vinculin) at ends of actin stress fibers in invasive MCF10A cells that left the acinar cell body. Cells are migrating on a planar glass substrate. (B) Representative images of MCF10A cells on a planar glass surface stained for phosphorylated myosin light chain kinase (pMLCK) and co-localization with the actin cytoskeleton. (C) Secondary antibody control for the pMLCK staining, showing no unspecific signal of the secondary antibody (cf. Figure 6G – H). Scale bars = 20  $\mu$ m.





**Figure S4. Traction force microscopy and hot spot segmentation.** Shown are confocal images representing the actin cytoskeleton of basal cells at the BM-ECM interface and the corresponding substrate deformation-related stress fields (A and B). (A) The first image of Example 1 of Figure 7A (after 1 h) with sparse stress fiber formation, low substrate deformation, and accordingly low strain energies. (B) Further time points of Id-BM acini treated with blebbistatin (25 μM) (cf. Figure 7H). (C) Exemplary images illustrate two green numbered hot spots (in light blue; co-localized/covered with a magenta-colored SF-cell). Hot spots (red circle) localized at a distance to the actual acinus contact area were excluded from the analysis. Scale bar = 20 μm.

**Movie S1: Lateral MS formation (Example 1).** Illustration of the formation of a lateral MS by using a living MCF10A/RFP-LifeAct acinus. Confocal images were taken every 65 seconds for 18 minutes. Scale bar = 10 μm.

**Movie S2: Lateral MS formation (Example 2).** Illustration of the formation of lateral MS by using a living MCF10A/RFP-LifeAct acinus. Confocal images were taken every 55 seconds for 2 hours. Scale bar = 10 μm.

**Movie S3: Lateral MS formation (Example 3).** Illustration of the formation of lateral MS by using a living MCF10A/RFP-LifeAct acinus. Confocal images were taken every 55 seconds for 36 minutes. Scale bar = 10 μm.

**Movie S4: Migrating MCF10A Cells.** Visualization of living MCF10A/RFP-LifeAct cells migrating onto glass including the formation of filopodia or filopodia-like structures. Confocal images were taken every 31 seconds for 7 hours. Scale bar = 10 μm.

**Movie S5: Movement of MCF10A acini on a tumor-like substrate (12 kPa).** Illustration of cell movement of MCF10A wild-type cells within the acinar structure. Transmitted light images were taken every 20 minutes for 95 hours. Scale bar = 100 μm.

**Movie S6: Stress fiber formation at the cell-BM-ECM interface (Example 1).** Confocal images of a LifeAct/RFP MCF10A acinus. Scheme 1. depicted in Figure 7A. Images were taken every 7 minutes for 20 hours.

**Movie S7: Stress fiber formation at the cell-BM-ECM interface (Example 2).** Confocal images of a LifeAct/RFP MCF10A acinus. Shown is the complete actin cytoskeleton sequence of Example 2 depicted in Figure 7B. Images were taken every 7 minutes for 20 hours.