Jacquemet et al., https://doi.org/10.1083/jcb.201704045

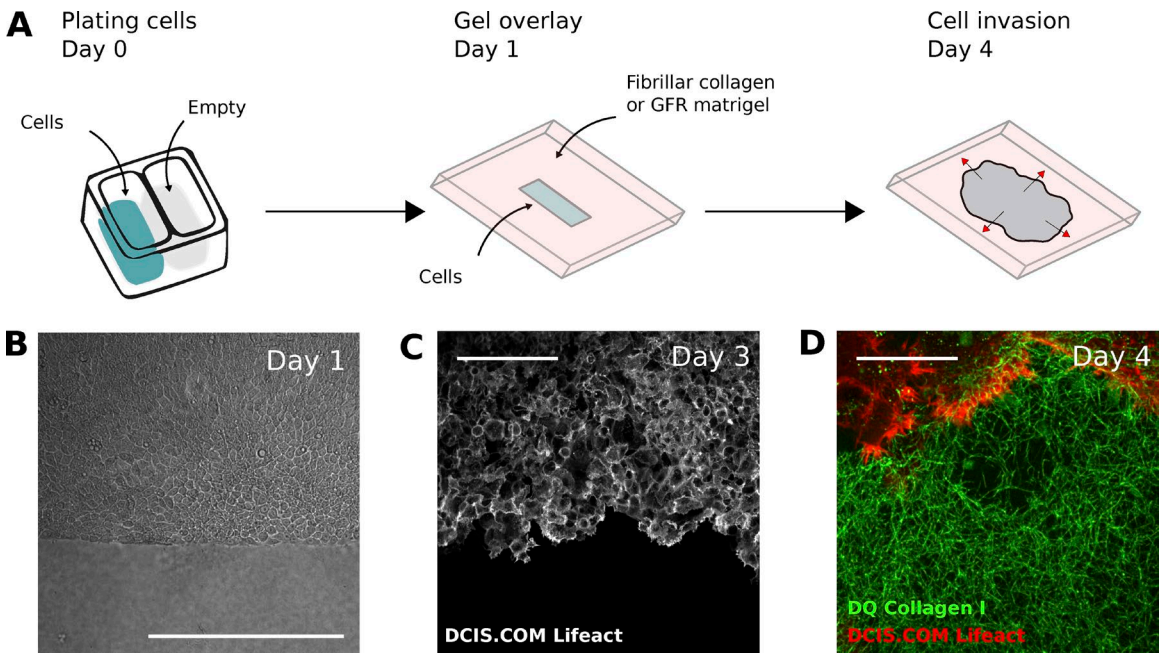


Figure S1. **Circular invasion assay.** (A) Cartoon representing the different steps of the circular invasion protocol used in this study. In brief, cells were seeded in a confined environment using a culture insert. The next day, the insert was removed and a gel composed of either GFR Matrigel or fibrillar collagen I was casted and media added. Cells were then left to invade through the gel for 3 d before fixation or live imaging. (B) Bright-field image of DCIS.COM cells after insert removal and gel overlay (day 1). Bar, 400 µm. (C) DCIS.COM LifeAct cells were left to invade through fibrillar collagen I for 3 d and imaged using an SDC microscope (20×, CMOS camera). Bar, 100 µm. (D) DCIS.COM LifeAct cells were left to invade through fibrillar collagen spiked with 1 µg/ml fluorescently labeled collagen I before gel polymerization (to visualize collagen architecture; DQ collagen) for 3 d and imaged live using an SDC microscope (100×, EMCCD camera). Bar, 25 µm.

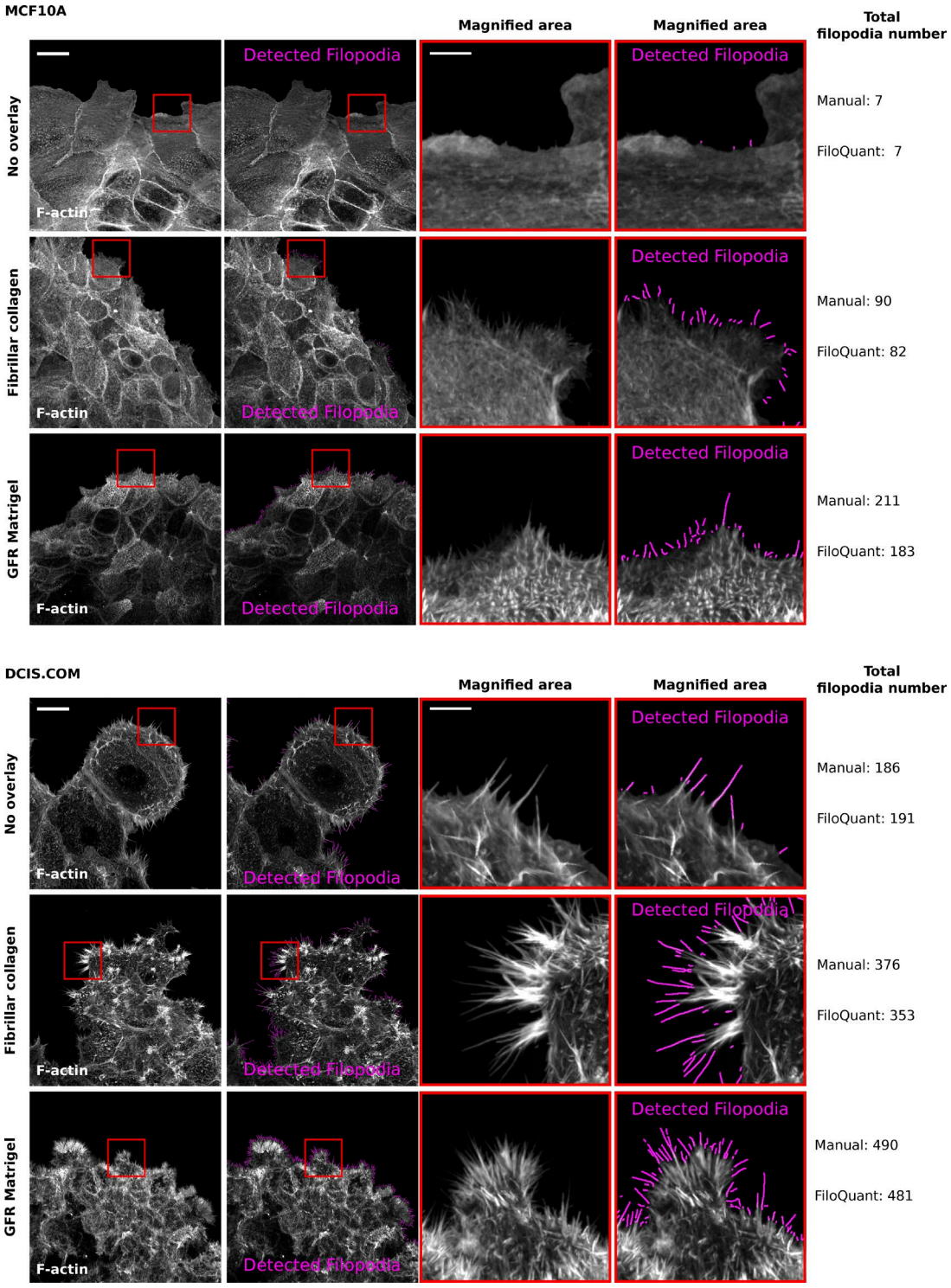


Figure S2. **FiloQuant outputs of the images displayed in Fig. 5.** MCF10A and DCIS.COM cells were plated in circular invasion assays and left to invade through GFR Matrigel, fibrillar collagen I, or media (no overlay) for 3 d. Cells were fixed, stained for actin and DAPI, and imaged using an SDC microscope (100× objective, CMOS camera). For each condition, representative maximal z projection and magnified area (red squares) are displayed. Bars: (main) 20 µm; (inset) 5 µm. Filopodial protrusions detected by FiloQuant are displayed in magenta. The total number of filopodia detected by either FiloQuant or by manual counting is provided for each image.

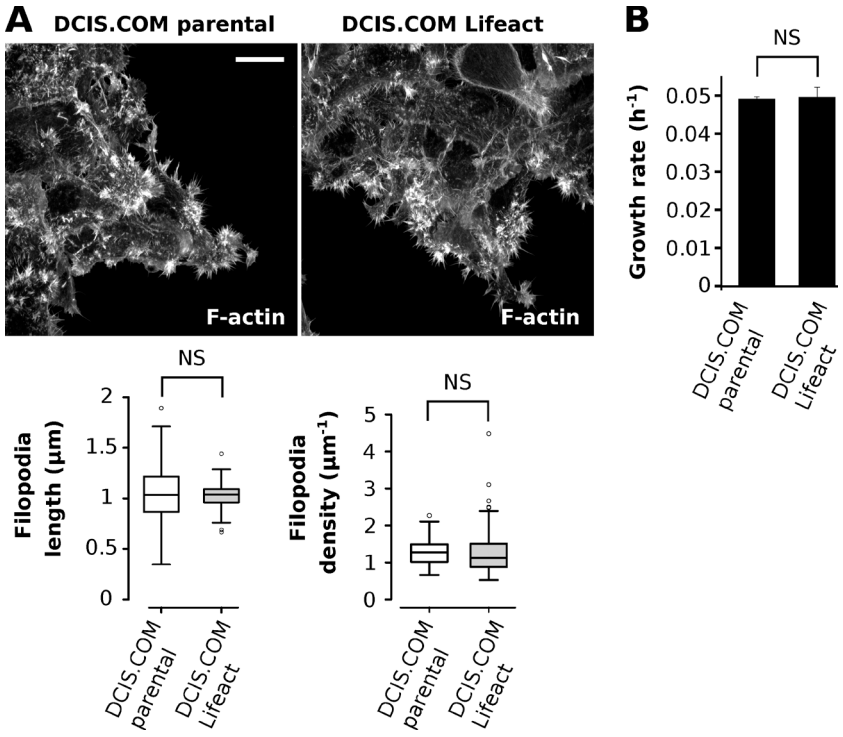


Figure S3. **Generation of a LifeAct DCIS.COM cell line.** (A and B) To analyze filopodia dynamics, DCIS.COM cells stably expressing LifeAct mRFP (DCIS.COM LifeAct) were generated and validated by comparison to parental DCIS.COM cells. Cells were plated in circular invasion assays and left to invade through fibrillar collagen I for 3 d. Cells were then fixed, stained for actin, and imaged using an SDC microscope (100× objective, CMOS camera). Representative maximal z projection (bar, 20 µm), filopodia density, as well as filopodia length, measured using FiloQuant are displayed. Results from three independent experiments are displayed as Tukey box plots (condition, fields of view analyzed: DCIS.COM, 73; DCIS.COM LifeAct, 41; A). In addition, the rate of cell growth was recorded using an IncuCyte ZOOM live-cell microscopy incubator and the confluency method. Results are from three independent experiments and are displayed as Tukey box plots (B). The Tukey box plots represent the median and the 25th and 75th percentiles (interquartile range); points are displayed as outliers (represented by dots) if 1.5 times above or below the interquartile range (represented by whiskers).

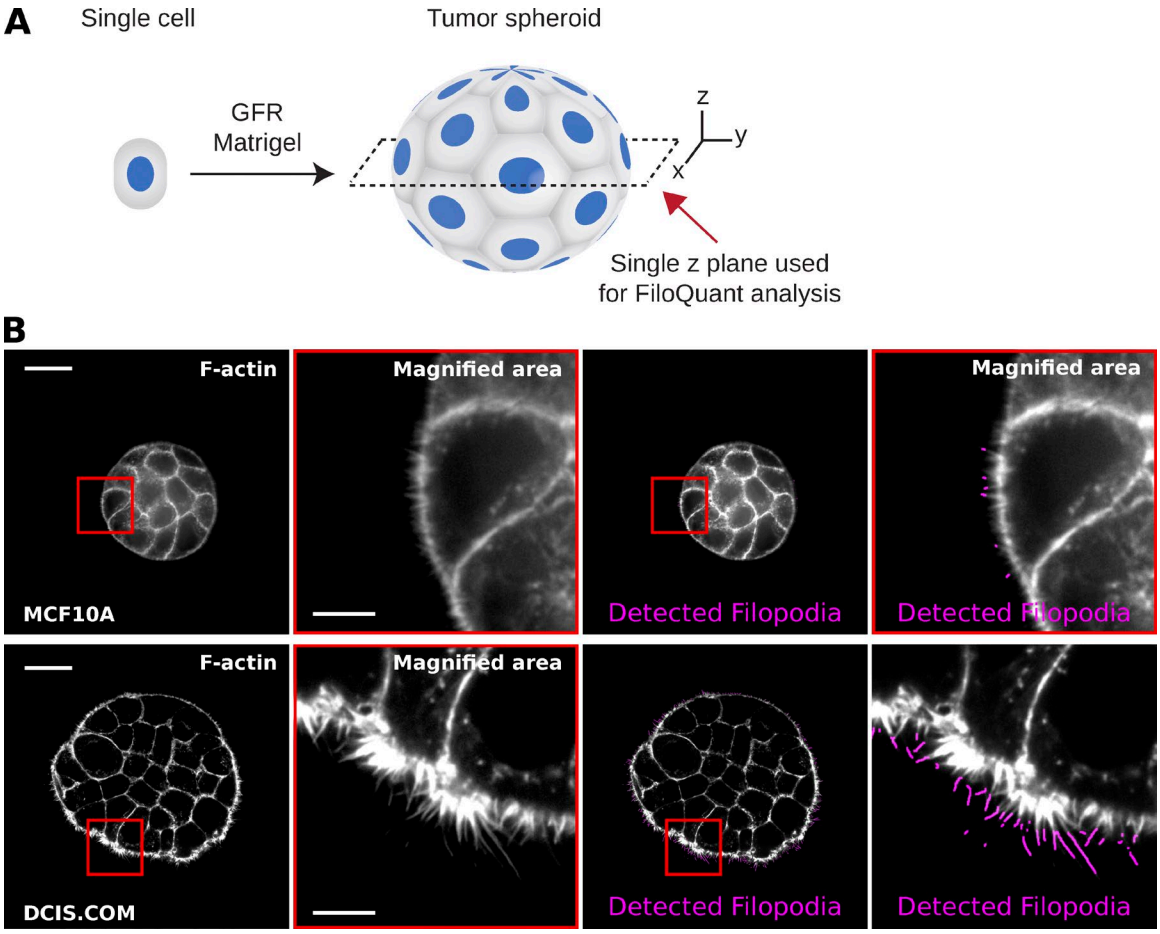
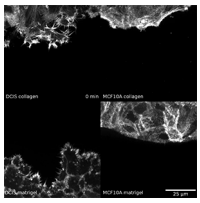
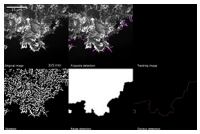


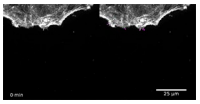
Figure S4. **FiloQuant outputs of the images displayed in Fig. 7.** (A and B) Single MCF10A and DCIS.COM cells were embedded into GFR Matrigel and allowed to form spheroids over 5 d. The spheroids were then fixed and stained for F-actin and imaged on an SDC microscope (100× objective, CMOS camera), and filopodia were detected using FiloQuant (related to Fig. 7). The z plane representing the middle of the tumor spheroid was used for FiloQuant analysis and is highlighted in the illustration in A. For each condition, representative images of the z plane used and magnified area (red squares) are displayed. Bars: (main) 20 µm; (inset) 5 µm. Filopodial protrusions detected by FiloQuant are displayed in magenta (B).



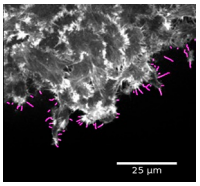
Video 1. **Video depicting MCF10A and DCIS.COM cells invading through fibrillar collagen I or GFR Matrigel.** MCF10A and DCIS.COM cells stably expressing LifeAct-mRFP were plated in circular invasion assays and left to invade through fibrillar collagen I or GFR Matrigel for 3 d before being imaged live on an SDC microscope (100× objective, EMCCD camera) for over 9 h (one picture every 3 min).



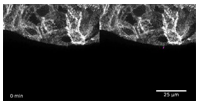
Video 2. **FiloQuant analysis of a movie depicting DCIS.COM cell invasion through fibrillar collagen (circular invasion assay).** The original video (input), in addition to the multiple output files generated by FiloQuant, is displayed. Output files include filopodia detection (magenta), tracking image (filopodia dynamics), skeleton (mask used for filopodia detection), edge detection (defining the filopodia-free cell edge) and contour detection (to measure edge length).



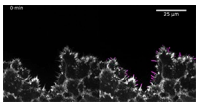
Video 3. **FiloQuant analysis of a video depicting MCF10A cell invasion through fibrillar collagen (circular invasion assay).** The original video (input), in addition to a FiloQuant output of detected filopodia (magenta), is displayed.



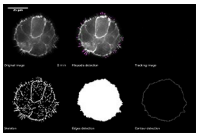
Video 4. **FiloQuant analysis of a video depicting DCIS.COM cell invasion through fibrillar collagen (circular invasion assay).** The original video (input), in addition to a FiloQuant output of detected filopodia (magenta), is displayed.



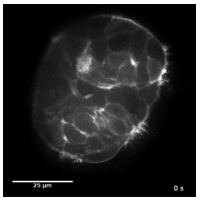
Video 5. **FiloQuant analysis of a movie depicting MCF10A cell invasion through GFR Matrigel (circular invasion assay).** The original movie (input), in addition to a FiloQuant output of detected filopodia (magenta), is displayed.



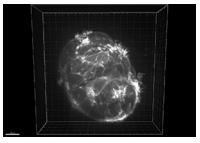
Video 6. **FiloQuant analysis of a video of DCIS.COM cell invasion through GFR Matrigel.** The original video (input), in addition to a FiloQuant output of detected filopodia (magenta), is displayed.



Video 7. **FiloQuant analysis of a video monitoring a single DCIS.COM spheroid in 3D GFR Matrigel.** DCIS.COM cells stably expressing LifeAct-mRFP were plated in 3D GFR Matrigel for 3 d before being imaged live on an SDC microscope (100× objective, EMCCD camera) for over 3 h (one picture every 2 min). The original video (input), in addition to the multiple output files generated by FiloQuant, is displayed. Output files include filopodia detection (magenta), tracking image (filopodia dynamics), skeleton (mask used for filopodia detection), edge detection (defining the filopodia-free cell edge), and contour detection (to measure edge length).



Video 8. **Actin dynamics (single z plane) of a DCIS.COM spheroid growing in the pericardial cavity of a zebrafish embryo.** DCIS.COM LifeAct cells were injected in the pericardial cavity of zebrafish embryos and imaged live using an SDC microscope 24 h postinjection.



Video 9. **Actin dynamics (3D reconstruction) of a DCIS.COM spheroid growing in the pericardial cavity of a zebrafish embryo.** DCIS.COM LifeAct cells were injected in the pericardial cavity of zebrafish embryos and imaged live using an SDC microscope 24 h postinjection. 3D reconstruction of the spheroid was generated using Imaris software.

Provided online is a zip file containing software, the FiloQuant manual, and test images.

Software 1 is a version of FiloQuant designed to analyze a single image already opened in ImageJ. This version of FiloQuant contains step-by-step user validation of the various processing stages to help users achieve optimal settings for filopodia detection.

Software 2 is a version of FiloQuant designed to analyze images within a specified folder. This version of FiloQuant still contains step-by-step user validation of the various processing stages to help users achieve optimal settings for filopodia detection.

Software 3 is a version of FiloQuant designed to automatically analyze images within a specified folder by using the same settings for all images (batch analysis). This version of FiloQuant also allows the analysis of stacks/movies and provides a tracking file as well as a time projection of the detected filopodia when the "stack analysis" option is enabled.