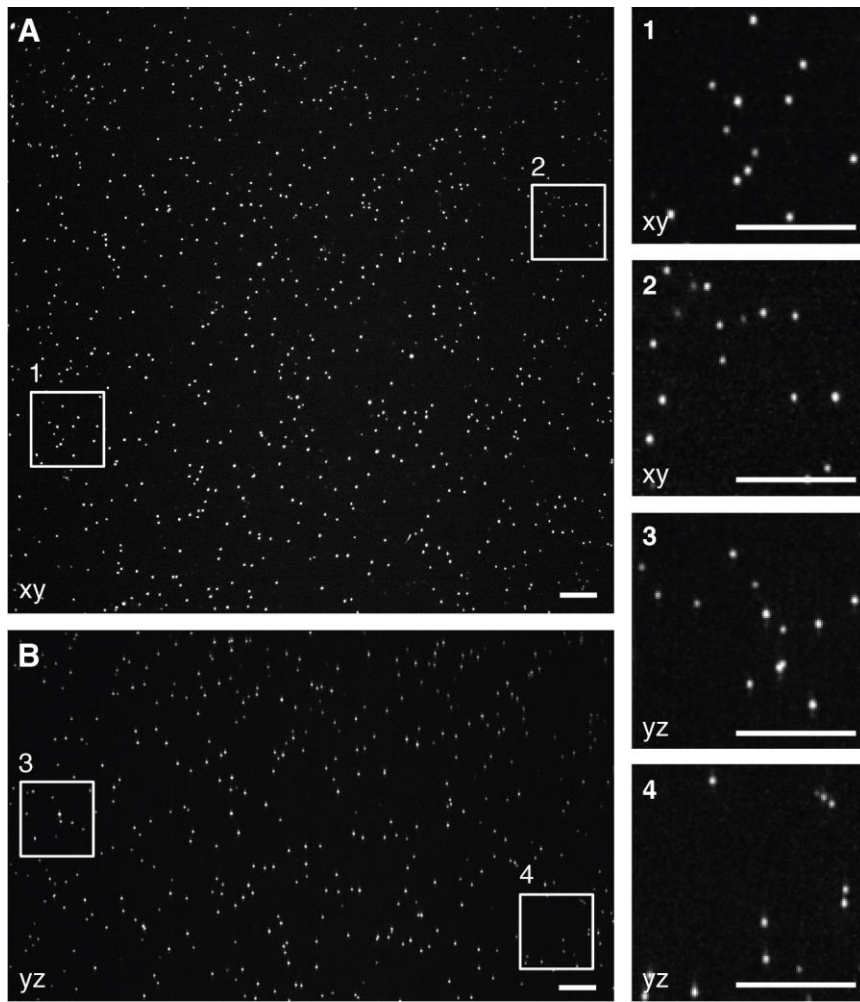
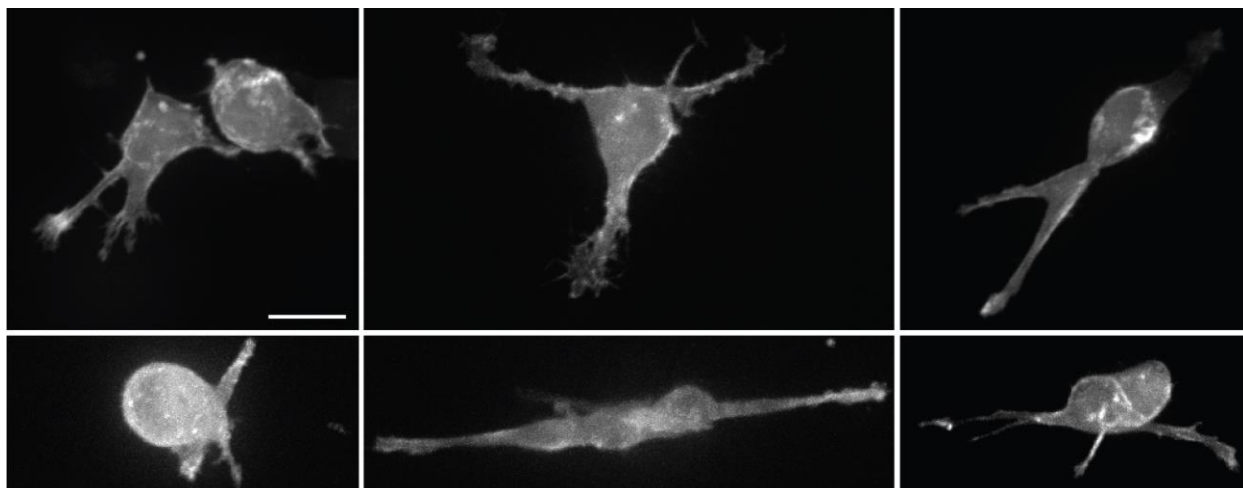


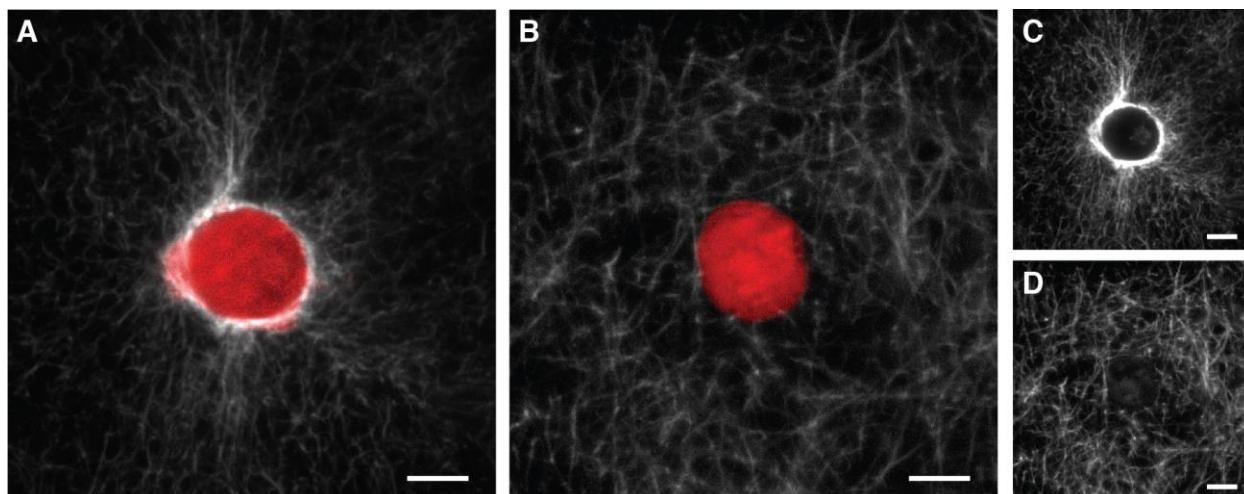
## Supplemental Figures



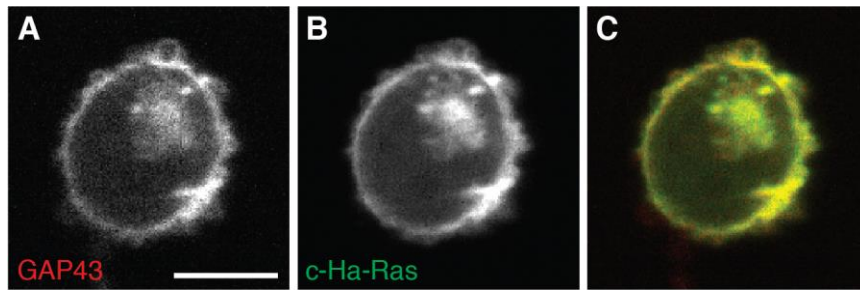
**Figure S1.** Shift invariant imaging of beads. Related to Figure 1. Different views of 100 nm beads over a volume of  $163 \times 163 \times 100 \mu\text{m}^3$  imaged using meSPIM in the normal mode without deconvolution. (A) An xy maximum intensity projection over  $3 \mu\text{m}$ . (B) A yz maximum intensity projection over  $3 \mu\text{m}$ . Right: Magnified views of the boxed regions 1 and 2 in (A) and 3 and 4 in (B). Scale bars:  $10 \mu\text{m}$ .



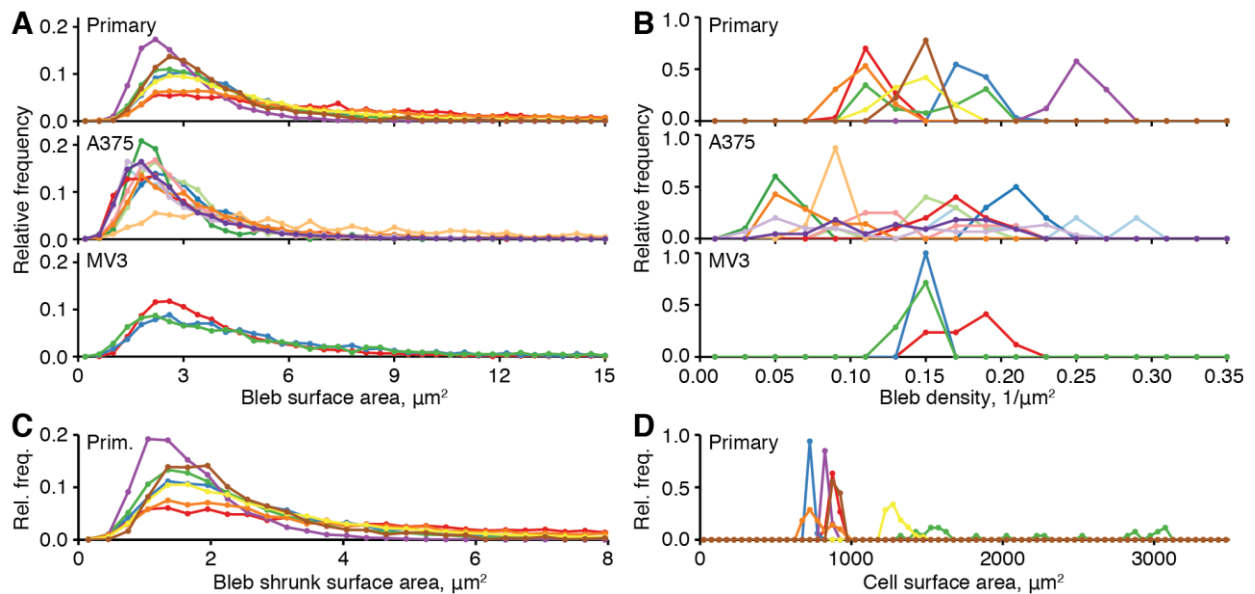
**Figure S2.** Melanoma cells in 3D collagen near a coverslip. Related to Figure 1. Shown are xy maximum intensity projections of cells imaged over multiple days using a spinning disk confocal microscope. Cells are from the same primary melanoma sample as cells shown in Figure 4E. Scale bar: 10  $\mu\text{m}$ .



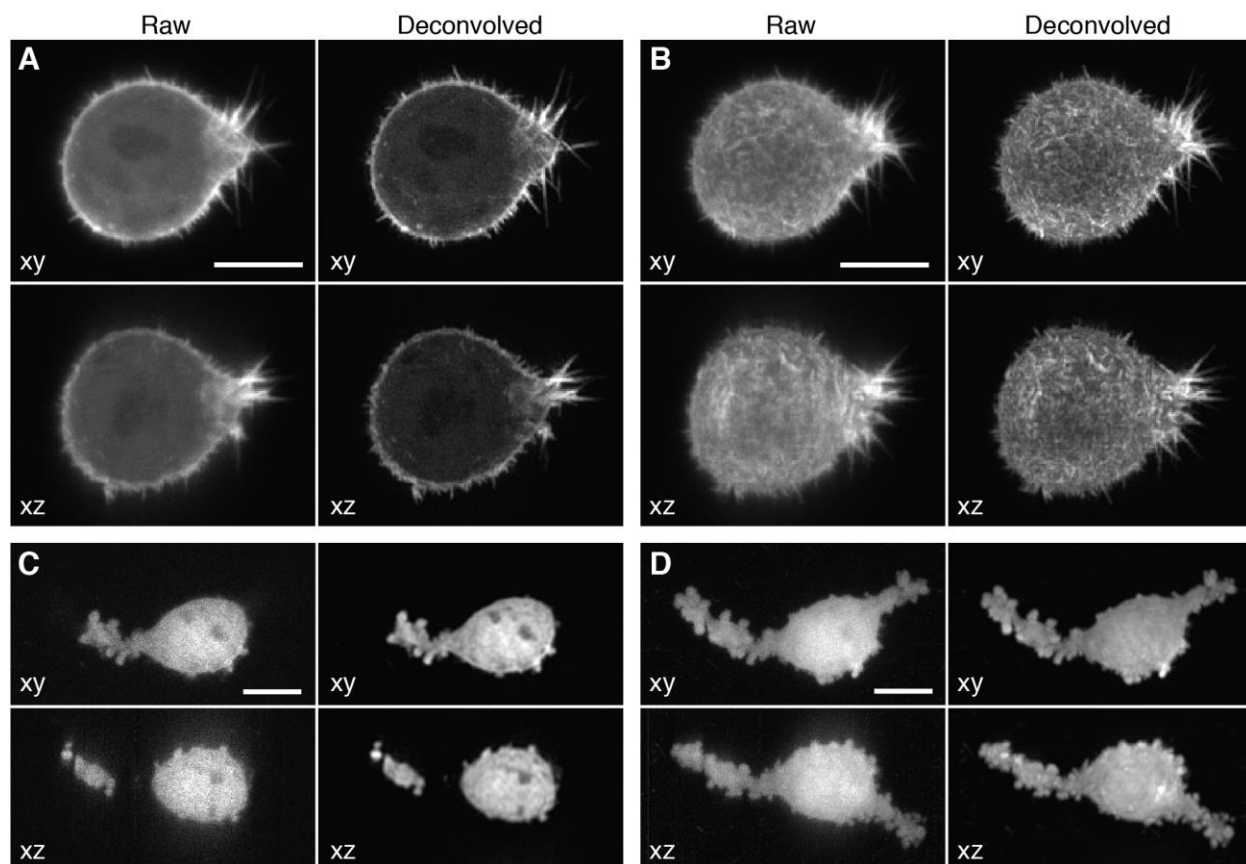
**Figure S3.** Effect of blebbistatin treatment on collagen rearrangement by MV3 cells. Related to Figure 3. All panels show xy maximum intensity projections over 2  $\mu\text{m}$  with collagen labeled by CNA35 peptide shown in white and cells expressing cytosolic mNeonGreen shown in red. (A) An untreated cell, and (B) a cell treated with 20  $\mu\text{M}$  blebbistatin for ~18 hours. (C) and (D) show only the collagen from (A) and (B) respectively to show the gap in the collagen caused by the cell. Scale bars: 10  $\mu\text{m}$ .



**Figure S4.** Comparison of the localization of two different membrane markers in an xy slice of an MV3 cell. Related to Figure 4. (A) td-Tomato fused to the first 60 base pairs of GAP43. (B) GFP fused to the 20-amino acid farnesylation signal from c-Ha-Ras. (C) Overlay of the GAP43 (red) and c-Ha-Ras (green) images. Scale bar: 10  $\mu\text{m}$ .

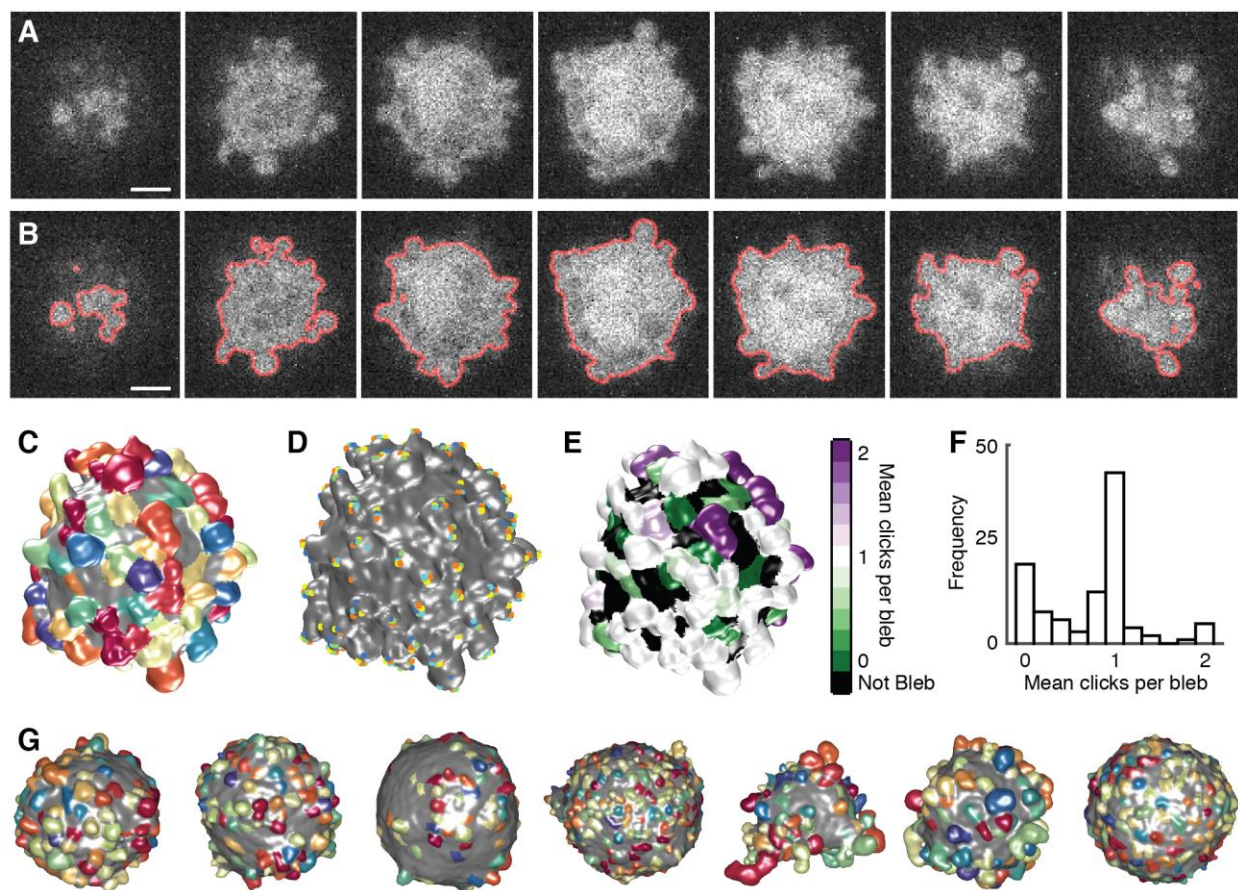


**Figure S5.** Relative frequencies of automated bleb measurements from two different melanoma cell lines (MV3 and A375) and one primary melanoma sample, shown as aggregate histograms for each cell over multiple frames of a timelapse acquisition. Related to Figure 5. The values corresponding to a single cell are indicated with a unique color and each cell is represented by the same color in all panels. (A) Individual bleb surface areas. (B) Bleb densities, defined as the bleb count in each frame divided by the cell surface area in that frame. (C) Shrunk surface areas of blebs (see Figure S8E) on primary cells. The shrunk bleb surface area and bleb surface area distributions are similarly shaped. (D) Surface areas of primary cells.



**Figure S6.** Effect of deconvolution on non-descanned meSPIM images. Related to Figures 1, 2, and 4. Raw and deconvolved images of the GFP-tractin infected, transformed HBEC cell shown in Figure 2E-G as maximum intensity projections over (A) 2 μm and (B) the entire cell volume. Raw and deconvolved images of the melanoma cell shown in Figure 4D as (C) single slices or (D) maximum intensity projections over the entire cell volume. Scale bars: 10 μm.





**Figure S7.** Testing the bleb segmentation workflow. Related to Experimental Procedures. (A) XY maximum intensity projections of the undeconvolved image over consecutive depths of 2  $\mu\text{m}$  from the middle to the front of a cell. (B) The same sequence shown in A with the extracted cell surface overlaid in red. (C) The surface of this cell with automatically segmented blebs colored randomly and regions without a bleb colored gray. (D) Dots indicate the locations of manually identified blebs, and dot colors correspond to the person who identified them. (E) Automatically detected blebs on the cell surface colored by the mean number of times each person clicked on it to indicate the presence of a bleb. White blebs were clicked on once, and hence were likely correctly identified by the algorithm. Purple blebs were clicked on twice and likely correspond to blebs oversegmented by the algorithm, whereas green blebs were not clicked on and likely correspond to undersegmented or not manually identified blebs. (F) A histogram of bleb frequency versus the mean number of times each bleb was clicked on. (G) Automatic segmentation of blebs for seven different cells. For each cell the time point shown was randomly selected. Here, gray indicates not only regions where no bleb was found but also the boundaries between blebs. Scale bars: 10  $\mu\text{m}$ .

## Supplemental Movie Legends

**Movie 1.** Frame 1: An MV3 melanoma cell embedded in collagen. Rotation of a 3D rendering of a  $100 \times 100 \times 100 \mu\text{m}^3$  volume. Related to Figure 2A. Frame 217: An xy maximum intensity projection of a transformed HBEC cell expressing GFP-tractin. Related to Figure 2C. Frame 343: Rotation of a 3D volume rendering of a transformed HBEC spheroid expressing eGFP-Kras<sup>V12</sup>. The image was acquired in descanned mode. Related to Figure 2D.

**Movie 2.** Frame 1: Rotation of the steerable filter result showing collagen fiber locations and MV3 cell expressing GFP. Related to Figure 3A. Frame 201: Rotation of a 3D rendering of the normalized fiber density (averaged over  $2 \mu\text{m}$ ) surrounding a single MV3 cell. Related to Figure 3C.

**Movie 3.** Frame 1: 3D volume rendering of an MV3 cell expressing cyOFP-tractin (green) and cytosolic GFP (red). Related to Figure 4B. Frame 45: A primary melanoma cell expressing GFP. Related to Figure 4C. Frame 91: A primary melanoma cell expressing GFP. Related to Figure 4D. Frame 167: Two primary melanoma cells expressing eGFP-tractin. Related to Figure 4E.

**Movie 4.** Frame 1: Rotation of the bleb segmentation. High-curvature surface structures (blebs) identified by segmentation and region merging with separate blebs shown in different colors. Related to Figures 5B and C. Frame 241: Timelapse of an MV3 cell 3D surface rendering showing mean curvature (left) and automated bleb tracking results (right - colors indicate separate tracks) from a rapid timelapse series (1.2 s per image volume). Related to Figures 5E-H.

**meSPIM Supplemental Information contents (ZIP file). Related to Experimental Procedures.**

1. Microscope components and alignment.
  - a. Autodesk inventor CAD files for each part
  - b. Autodesk inventor assembly file of the entire microscope
  - c. Folder 'CustomPartsDrawings' containing 2D drawings of the custom parts
  - d. Folder 'Mould', containing CAD files and drawings of the Agarose mould
  - e. Folder 'Photomask', containing the file for the fabrication of the photo mask
  - f. Folder 'SampleHolder' containing the CAD files and drawings for the sample holder
  - g. MicroscopeAlignment.doc, a word document describing the initial alignment of the microscope
  - h. MicroscopeCompnnents.doc, a word document containing a parts list of the microscope
2. Matlab code.
  - a. Example workflows written in Matlab for
    - i. Describing and detecting collagen (exampleCollagen3D.m)
    - ii. Segmenting blebs (exampleBleb3D.m)
    - iii. Measuring florescence intensity near the cell surface (exampleSurfaceIntensity3D.m)
  - b. A README text file with an introduction to the examples
  - c. A folder, matlabFunctions, with further matlab code.

Note: To run the supplied code on the provided example data, users will need to download the example data from <https://data.mendeley.com/datasets/> with doi:10.17632/2dnn6n9w9w.1.