SUPPLEMENTARY MATERIALS AND METHODS

Fly culture and clone generation

Fly stocks were cultured at 25°C on standard food. Clones were generated by Flipase-mediated mitotic recombination on FRT40A or FRT101 chromosome carrying either GFP or RFP as markers (Golic and Lindquist, 1989; Xu and Rubin, 1993). Adult females were fed on abundant yeast diet for 3 days prior to dissection. Ectopic expression of TkvA, Dad or RNAi against *PH4αEFB* or FAK were performed by generating Flip-out Gal4 clones in animals carrying the hs-FLP22 and the AyGAL4 UAS-GFP transgenes (Ito et al., 1997). Flipase expression was induced by heat shocking 3 days-old females at 37.3C for 1 h to generate mutant clones or Flip-out clones. Females are dissected 2 to 4 days after heat shock.

Follicle Staining and mounting

Ovaries from females were dissected directly into fixative 3 to 4 days after Flipase induction and stained following the protocol described in Grammont and Irvine (2001). To avoid fluctuations of the depth of the follicles that are squeezed by the coverslip, each slide contains 15 ovaries, from which S11 to S14 are removed. After dissection of the follicles, most of the PBS is removed and 20µl of the Imaging medium (PBS/Glycerol (25/75) (v/v)) is added before being covered by a 22/32 mm coverslip.

Col IV quantifications

Images were taken with a resolution of 3.2 pixels per µm. Projections were generated with the z sections in which the fibrils are visible. Data quantification for BM fibrils was performed on 50 x 50 µm squares for large follicles (>S7). For younger stages, squares as big as possible. Along the Y axis, squares are positioned in the centre of follicle. Along the X axis, squares are positioned so that it encompasses as much as possible the extremity of the follicles. Objects touching the edges of cropped images were excluded from further analysis. ImageJ (NIH) was used to quantify the number, size and length of the fibrils. Uneven illuminated background was removed by using the 'Rolling Ball Background Subtraction' filter with a radius of 150.0 pixels. In most cases, threshold was done automatically. Analysis particles tool was then used with the following features: Size (pixels):

3-Infinity; Circularity: 0.00-1.00. The angle of the major axis of each object is reported in degrees relative to the X-axis of each image (A-P axis of each follicle) at 0 degrees using Rozeta 2.0 (Pazera-Software).

Electron microscopy (SEM)

Follicles were fixed in 2.5% glutaraldehyde in cacodylate buffer 0.1M pH 7.4, and leave for several days at 4°C. After extensive washing in 0.1 M sodium cacodylate buffer with 0.003M CaCl2 and 0.2M glucose, samples were postfixed in 0,1M sodium cacodylate buffer with 0.003M CaCl2, 0.15M Glucose and 1% OsO4 for 30 minutes at room temperature, briefly rinsed in distilled water and dehydrated through a graded series of ethanol solutions (from 30° to 100°) and two baths of acetone 10 min for each. Then, samples were dessicated in a Critical Point Dryer (CPD) and put in a sputter coater for 3 minutes for covering with gold-palladium before observation on a SEM Hitachi S-800 FEG at an accelerating voltage of 5 KV.

Electron microscopy (TEM)

Ovaries were dissected in a fixative solution (2% glutaraldehyde, 0.5% paraformaldehyde in 0.1M cacodylate buffer) and fixed for at least 4 hours at room temperature. Samples were washed four times in 0.15M cacodylate buffer and left overnight at 4°C. Then, the follicles were incubated for 1 hour in 0.15M cacodylate buffer (pH7.4) containing 1% OsO4, washed for 5 minutes in distilled water and dehydrated through a graded series of ethanol solutions from 30° to 100°, 20 minutes for each bath and two baths of propylene oxide for 15 min each. After substitution and impregnation, follicles were embedded in epoxy resin in flat silicon molds and polymerized at 56°C for 48h. Ultrathin sections were cut parallel or perpendicular to the AP axes of follicles with a UC7 Leica ultramicrotom. Ultrathin sections were contrasted in aqueous uranyl-acetate and lead citrate solutions using a Leica ultrostainer. Sections were observed with a Philips CM12 transmission electron microscope at 120Kv.

Confocal Microscopy

Preparations were examined using confocal microscope (LSM 710 and LSM 700; Carl Zeiss MicroImagin, Inc.) with 40x/NA 1.3 plan-Neofluar and 63x/NA 1.4 plan-Apochromat or 40x/NA A1.1 water immersion lens. Imaging was performed at RT. Images were examined using ImageJ (http://imagej.nih.gov/ij/).

FCs morphometric analysis

Image segmentation and quantification on mid S9 follicles was performed using an ImageJ macro described in Brigaud et al. (2015). Mutant cells are assigned to a row according to the flattening of WT cells. For this study, we generated only small clones (less than 25% of the follicular cells are mutant). Thus, the follicles always have enough WT stretched cells to observe normal flattening and to compare the shape and the position of mutant cells with the WT ones. About 4 rows are required in WT situation to cover the nurse cells compartment.

Figure S1

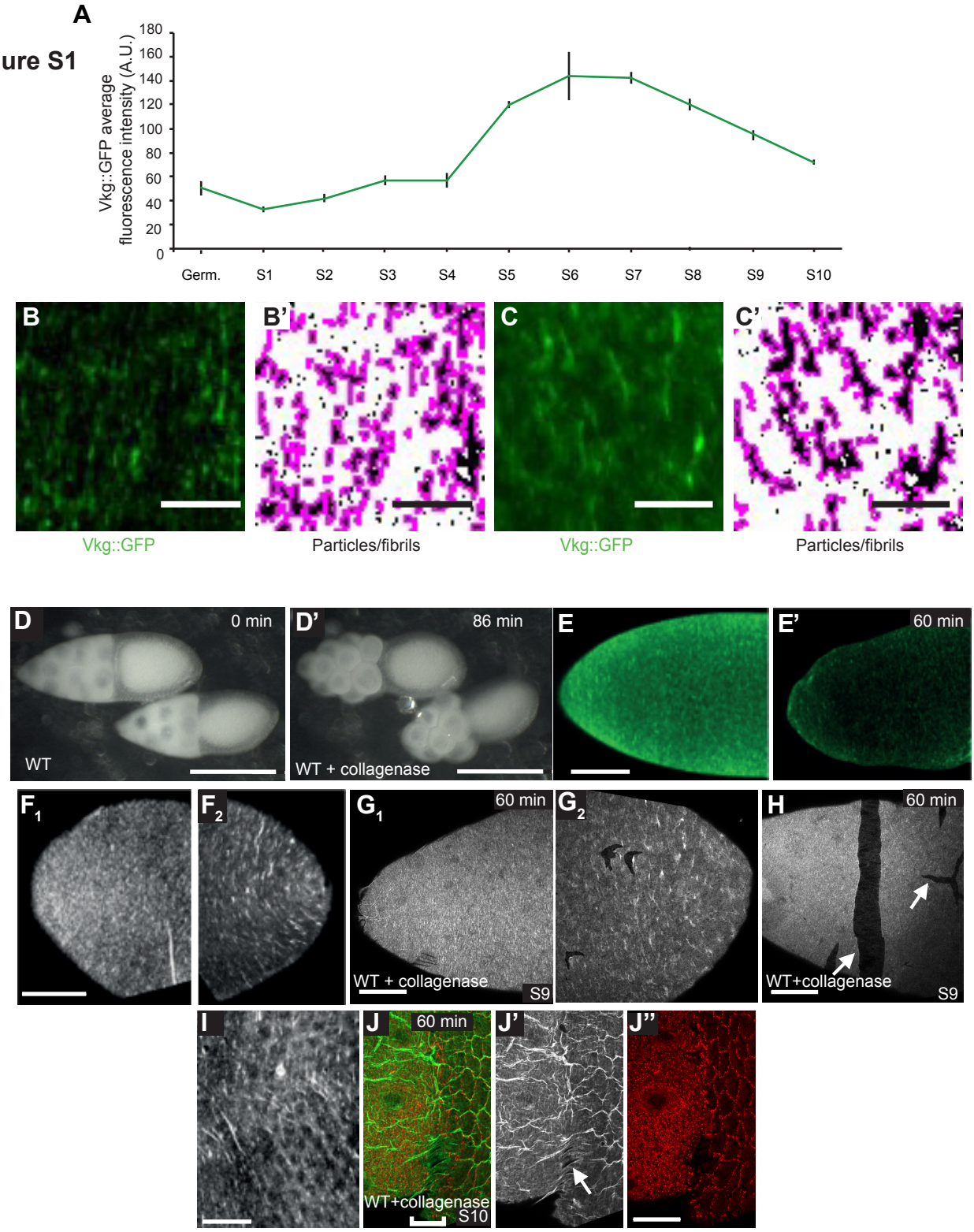


Figure S1: BM fibril-like structures are remodelled during epithelial cell shape changes.

(A) Evolution of the fluorescence intensity of Vkg::GFP from germarium to S10. Error bars indicate s.e.m. **(B, C)** BM of S8 (B) and S10 (C) follicles from a female heterozygous for *vkg*^{G454} before (B, C) and after image treatment (B', C') (see Materials and Methods) to recognize puncta or fibrils used for data quantitation shown in Figure 1 (scale bar for B and C: 10 µm). (D) WT S10 follicles before (D) and after 86 min (D') of collagenase treatment (n=10). Scale bar: 200 µm. (E) Anterior area of a S9 follicle with BM fibrils marked by Vkg::GFP (green) before or after 60 min of collagenase treatment (n=3). **(F)** Late S9 follicle without collagenase treatment with a focus in anterior (F_1) and in posterior (F_2) (n=25). (G) Late S9 follicle with a focus in anterior (G_1) and in posterior (G_2) after 60 min of collagenase treatment (n=5 for each time point). (**H**) Late S9 follicle after 60 min of collagenase treatment (n=8). (**I**) Central area of a S10 follicle without collagenase treatment (n=25) (J) Central area of S10 follicles after 60 min of collagenase treatment (n=7). Adherens junctions are marked by Ecad (red). BM disruption (arrows) and abnormal pattern of adherens junction are observed between StC and the columnar cells (brackets). Scale bar for E-H: 20 µm.

Figure S2

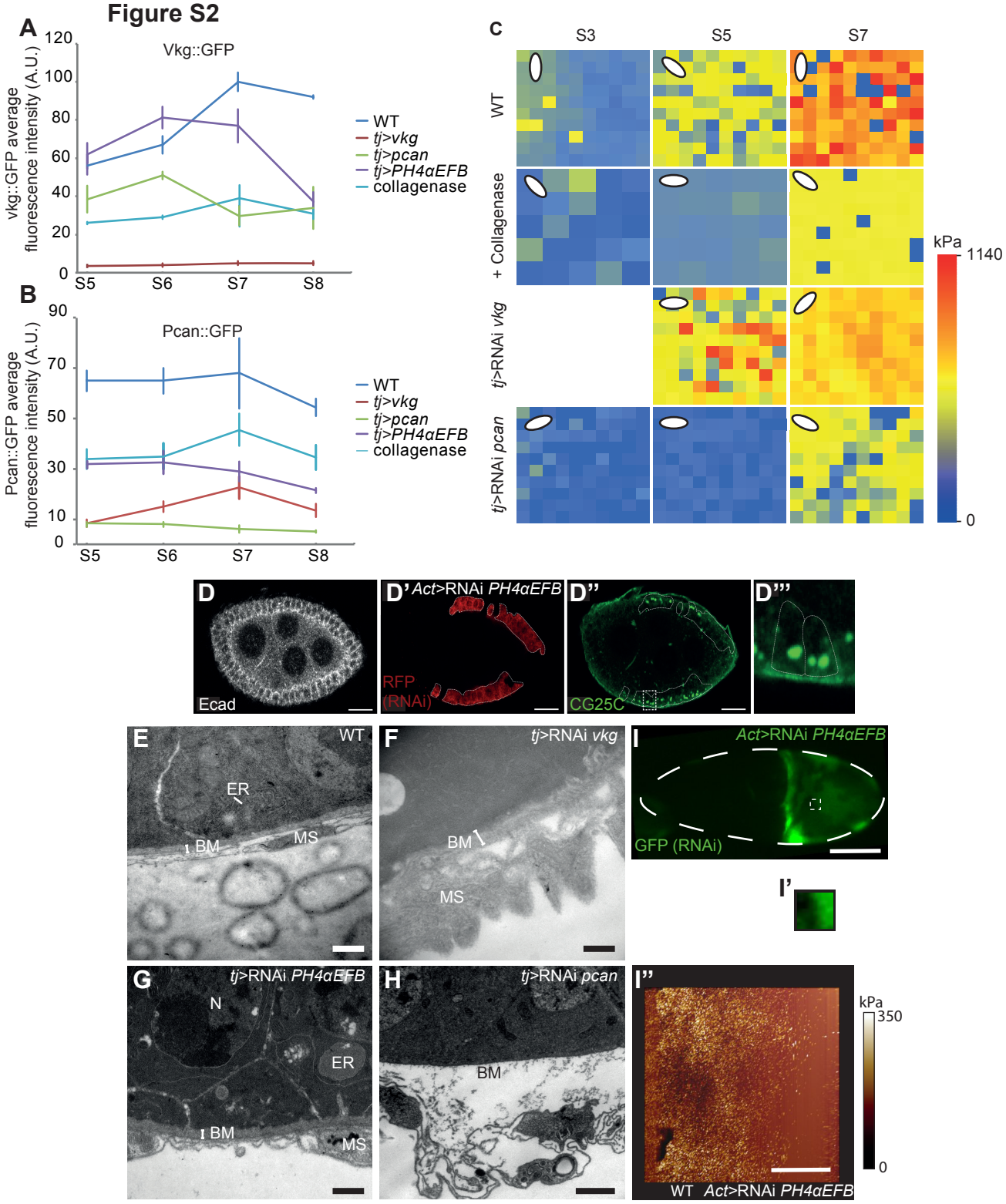


Figure S2. BM stiffness increases during rotation

(A, B) Evolution of Coll IV::GFP (A) and Trol::GFP expression levels from stage 5 to 8 in different genetic conditions. Error bars indicate s.e.m. (C) 10 x 10 matrices of BM stiffness of S3, S5 or S7 follicles with each indentation point spaced 150 nm apart with a specific scale. Follicles are either WT, genetically or chemically impaired for BM structure (n= 5 per condition). The matrices are not oriented regarding the A/P axis. The orientation of the follicles, and therefore of the matrices, is indicated by the ellipsoid drawn in each matrix. (D) S5 follicle with clones of cells expressing RNAi against *PH4αEFB* (red) (n=21). D''' is a magnified view of the box drawn in D'' (n=21). Scale bar: 15 µm (E, F, G, H). Electron microscopy of the edge of a WT S8 follicle (E, n=5), of a S8 follicle expressing RNAi against *vkg* (F, n=4), of a S8 follicle expressing RNAi against *PH4αEFB* (G, n=5) and of a S8 follicle expressing RNAi against *pcan* (H, n=3). ER and MS stand for Endoplasmic Reticulum and Muscular Sheet. Scale bar: 400 nm. (I) S9 follicle with cells expressing RNAi against *PH4αEFB*. I' is a magnified view of the box drawn in I. I'' represents the elastic modulus map of a part of the box drawn in I. Scale bars: 75 µm (I) and 1.5 µm (I'').

Figure S3

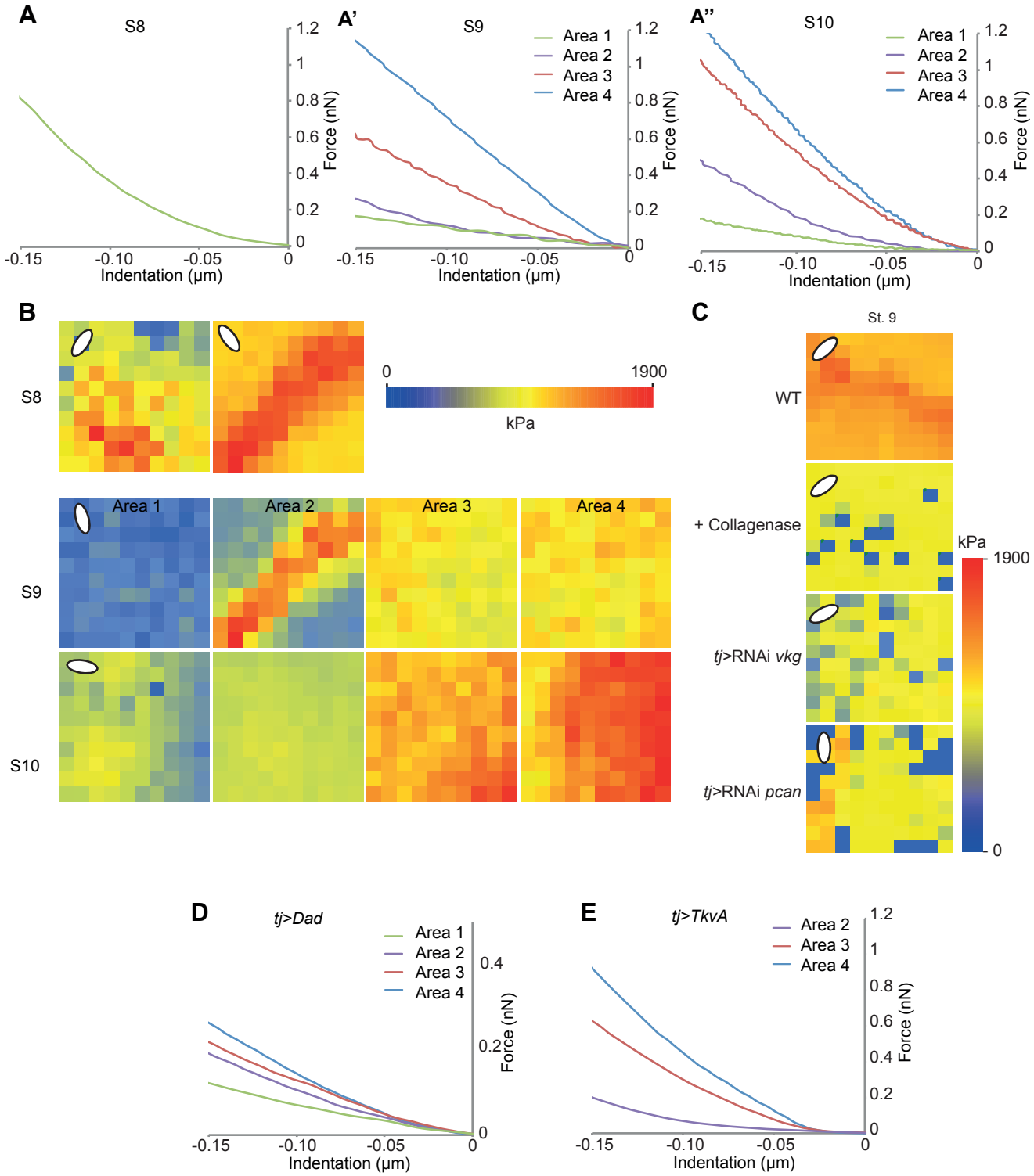


Figure S3. Cell flattening controls locally BM stiffness

(A) Force-indentation curves (A) from BM of a WT S8 (A), S9 (A') and S10 (A'') follicles. 4 areas located along the A/P axis have been probed. (B) 10 x 10 matrices from BM of two WT S8 (two central areas with and without fibrils), a WT S9 and a WT S10 follicles (specific scale). **(C)** 10 x 10 matrices from BM of S9 follicles that are either WT, genetically or chemically impaired for BM structure (specific scale). The orientation of the follicles is given by the ellipsoid drawn in each matrix. (D-E) Force-indentation curves from BM of a S9 follicle expressing Dad (D) or of a S8 follicle expressing TkvA (E).

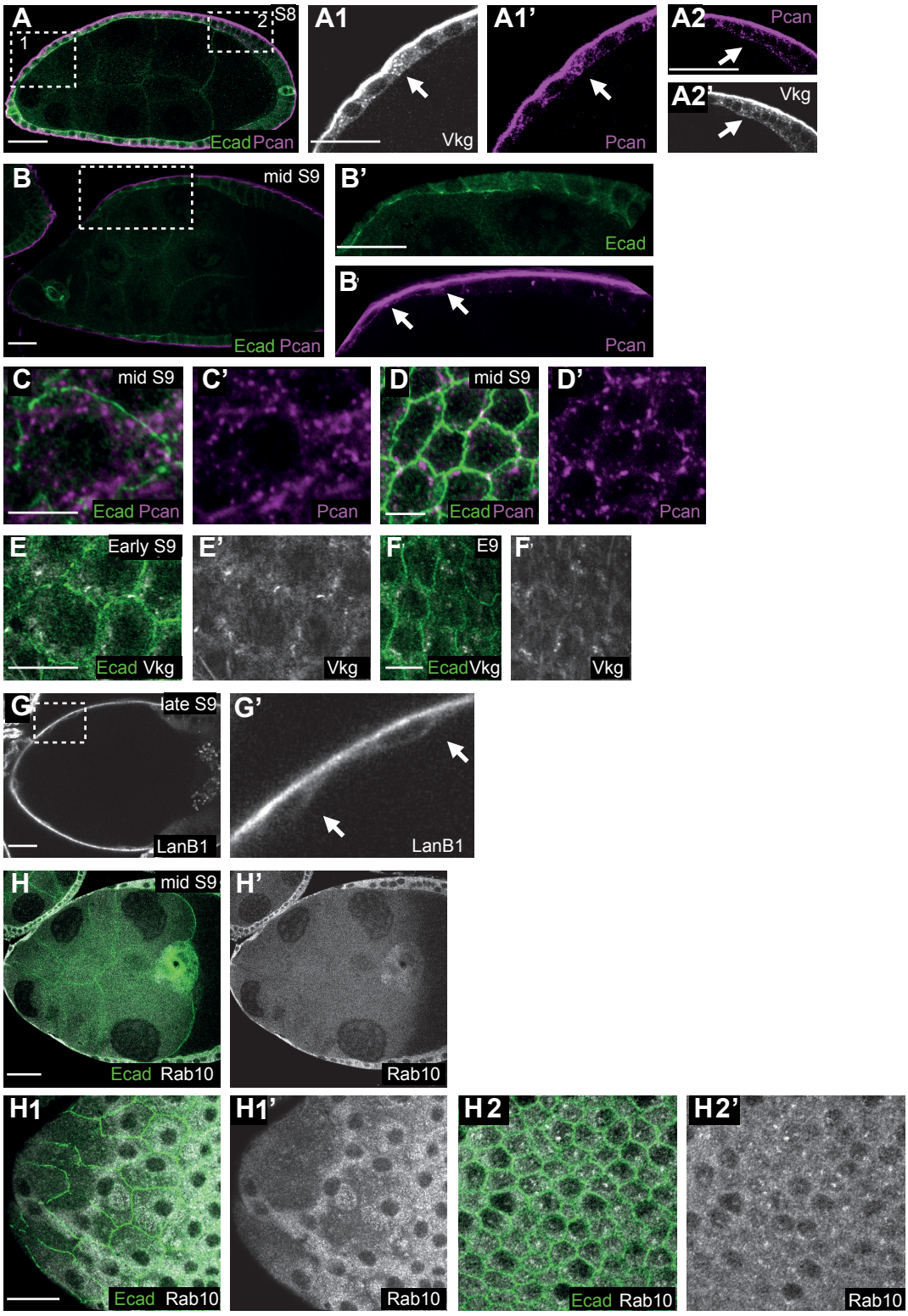


Figure S4: BM protein expression during cell flattening

Arrows indicate punctate pattern of Pcan, Vkg::GFP, lanB1::GFP or Rab10::YFP expression. (**A**) S8 follicle with Pcan and Vkg::GFP expression in the future StC cells and the columnar cells. A_1, A_1', A_2, A_2' are magnified view of the boxes 1 and 2 drawn in A, respectively. (B) Side view of mid S9 follicle with Pcan expression in the flattened and flattening cells. B and B'' are magnified view of the box drawn in B. (**C, D**) Pcan expression in the flattening cells (C) or columnar cells (D) at mid S9 follicle (n=7). (**E, F**) Vkg::GFP expression in the flattening cells (E) or columnar cells (F) in early S9 follicle (n=7). (G) LanB1::GFP expression in flattened cells in late St9. G' is a magnified view of the box drawn in G. (H) Side view (H, H') and top view of Rab10::YFP expression in StC cells (H1) and in columnar cells (H2) at mid S9. Scale bar: 20 µm for A, B, G, H and 10 µm for C-F.

Figure S5

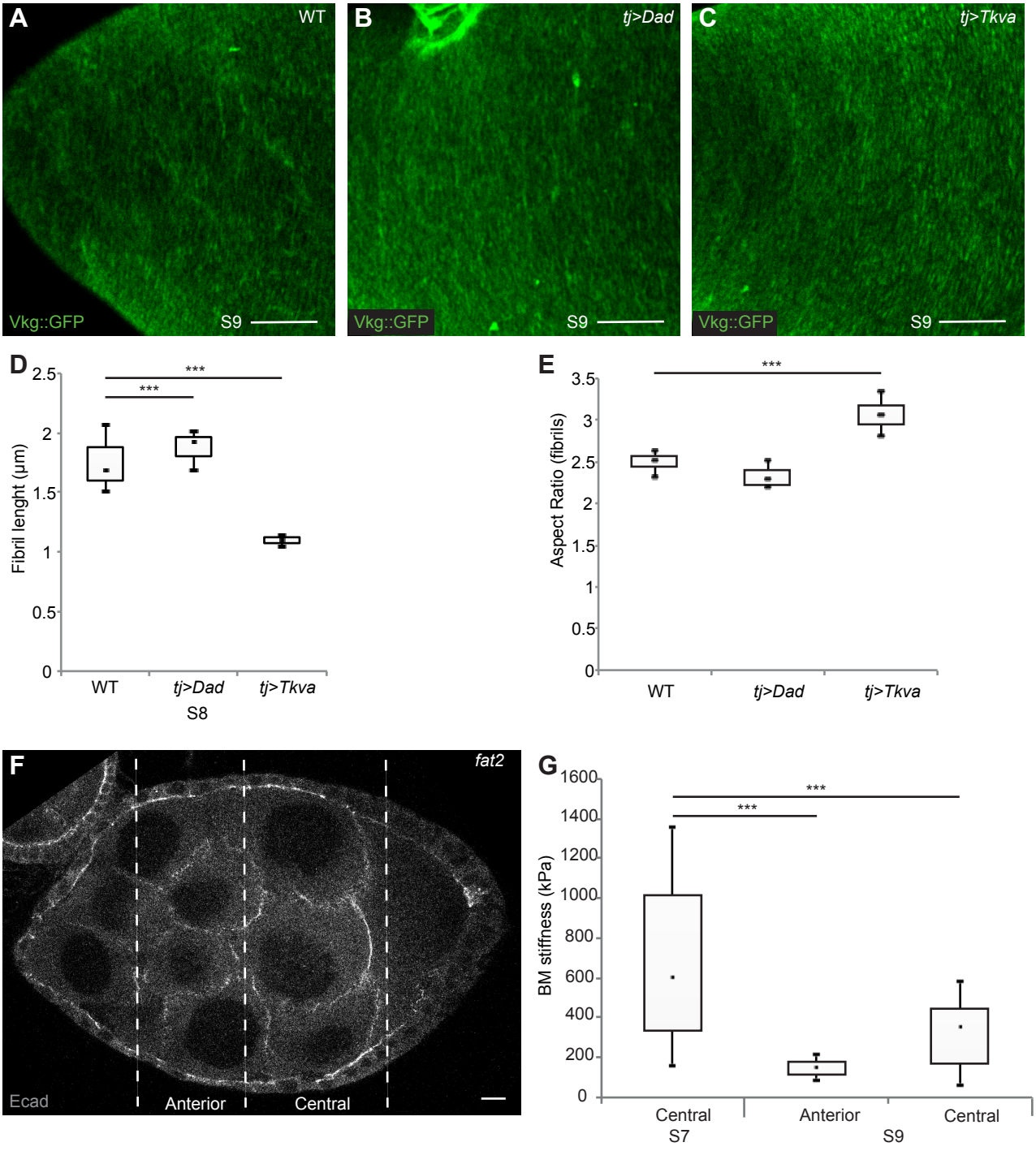


Figure S5: Fibril shape during cell flattening

(A-C) Vkg::GFP expression in WT (A), Dad (B) and TkvA expressing follicles. (**D, E)** Box and whisker plots of fibril length (D) and aspect ratio of the fibrils (E) in WT, Dad or TkvA expressing follicles (n>1000 fibrils per condition). (F) S9 *fat2* follicle. The anterior and central parts that are probed are indicated by a white dotted line. (G) Box and whisker plots of BM stiffness (kPa) of S7 (n=16) and S9 (n=6) follicles, mutant for *fat2*. Scale bar: 10 µm, ***, p<0.005 t-test.

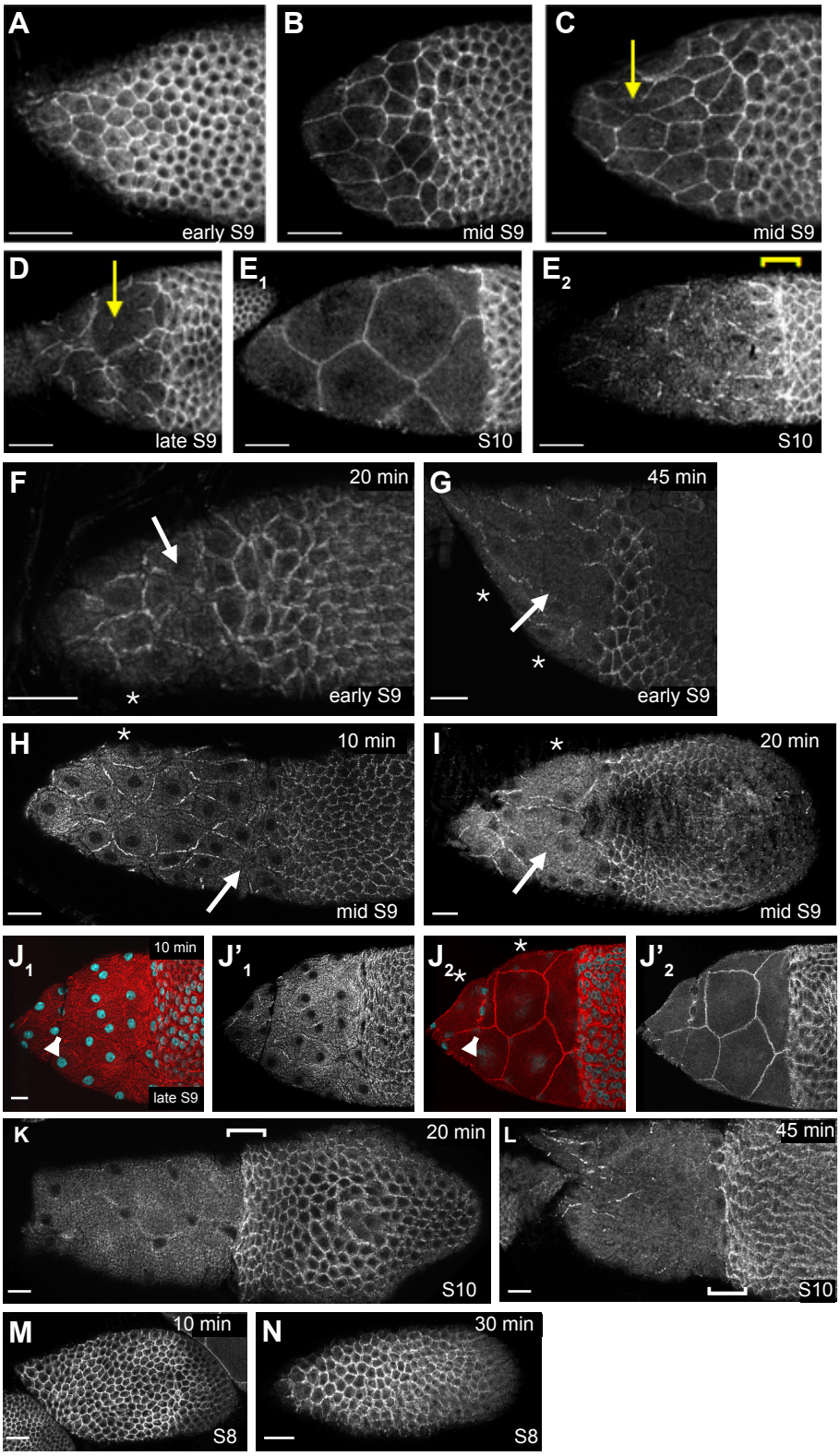
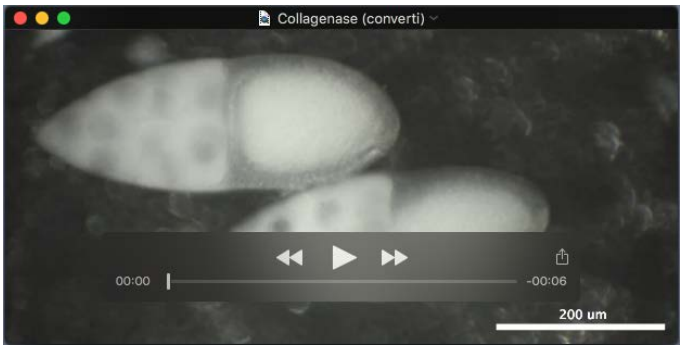
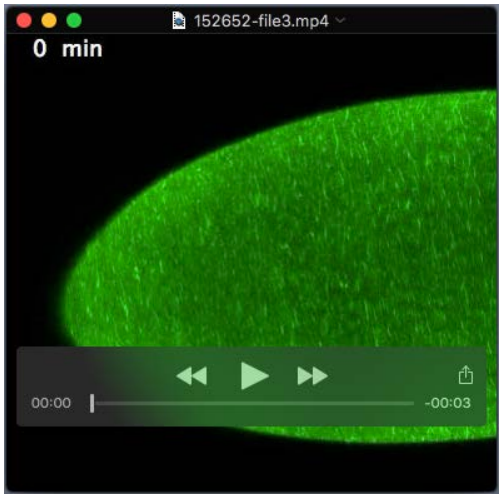


Figure S6: Cell flattening depends on BM structure.

(A-E) Adherens junction remodelling (marked by Ecad, grey or red) of WT follicles without collagenase treatment. The yellow arrows point to the presence of areas without adherens junction between the flattened and the flattening cells. (A) Anterior cells start to elongate. (B) Antero-posterior gradient of cell flattening. (C) Some anterior adherens junctions are disassembled. (D) Most of the adherens junctions are disassembled. (E) S10 follicle at two different z-sections focusing at the nurse cell compartment (E_1) or at the stretched cells (E_2). No epithelium discontinuity between the stretched and the columnar cells is present (yellow bracket). (F-N) Adherens junction remodelling (marked by Ecad, grey or red) of WT follicles after collagenase treatment. Asterisks represent the bulging of the nurse cells. The white arrows point to the presence of areas without adherens junction between the flattened and the flattening cells. (F, G) Follicles after 20 min (F) or 45 min (G) of collagenase treatment (n=8 for each time point). (H, I) Mid S9 follicles after 10 min (H) or 20 min (I) of collagenase treatment (n=5 and 8, respectively). (J) Late S9 follicle after 10 min of collagenase treatment at two different z-sections focusing at the stretched cell nuclei (marked by Hoechst, Cyan) located above the nurse cells (J_1) or at the stretched cell nuclei that are located between the nurse cells (white arrowhead) (J_2) (n=6). (K, L) S10 follicles after 20 min (K) or 45 min (L) of collagenase treatment (n=8 for each time point). Epithelium discontinuity between the stretched and the columnar cells (brackets). (M, N) S8 follicles after 10 min (M) or 30 min (N) of collagenase treatment. No significant changes are observed (n=5 for each time point). Scale bar: 20 μm.



Movie 1: Collagenase treatment induces formation of round follicles.



Movie 2: Coll IV::GFP expression level during collagenase treatment