

Supplementary Information

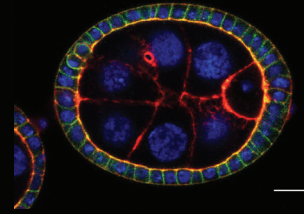
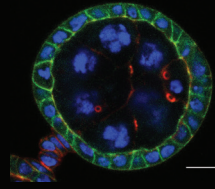
3D Tissue elongation via ECM stiffness-cued junctional remodeling

Chen et. al.

Supplementary Figure 1

a *control^{RNAi}; indy-GFP*

DNA membrane GFP F-actin



stage

4

5

number of cells

184 ± 34 s.d.

413 ± 50 s.d.

aspect ratio

1.17 ± 0.06 s.d.

1.34 ± 0.06 s.d.

volume (μm³)

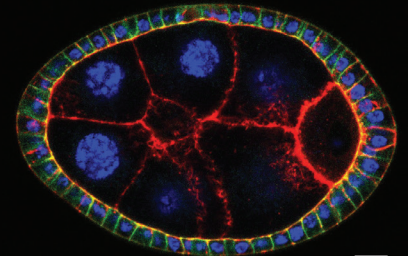
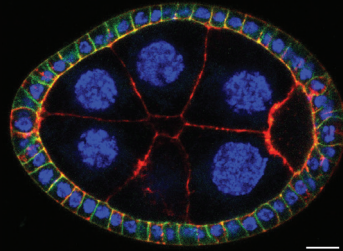
64,252 ± 1,3183 s.d.

107,630 ± 2,1727 s.d.

nurse cell nuclear diameter (μm)

N/A (polytene)

12.95 ± 1.22 s.d.



stage

6A

6B

number of cells

550 ± 44 s.d.

751 ± 66 s.d.

aspect ratio

1.42 ± 0.12 s.d.

1.54 ± 0.12 s.d.

volume (μm³)

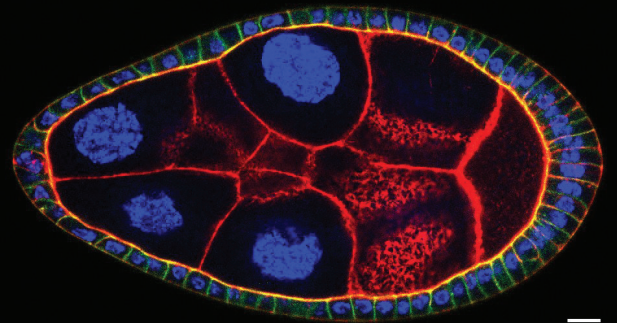
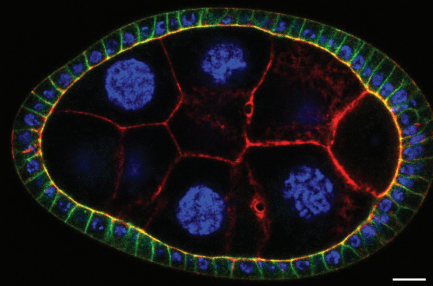
131,636 ± 32,104 s.d.

219,000 ± 8,0991 s.d.

nurse cell nuclear diameter (μm)

14.28 ± 1.95 s.d.

15.59 ± 2.61 s.d.



stage

7

8

number of cells

887 ± 33 s.d.

862 ± 28 s.d.

aspect ratio

1.82 ± 0.09 s.d.

1.91 ± 0.07 s.d.

volume (μm³)

334062 ± 49880 s.d.

552705 ± 91295 s.d.

nurse cell nuclear diameter (μm)

19.86 ± 3.05 s.d.

23.64 ± 1.66 s.d.

10 μm

b

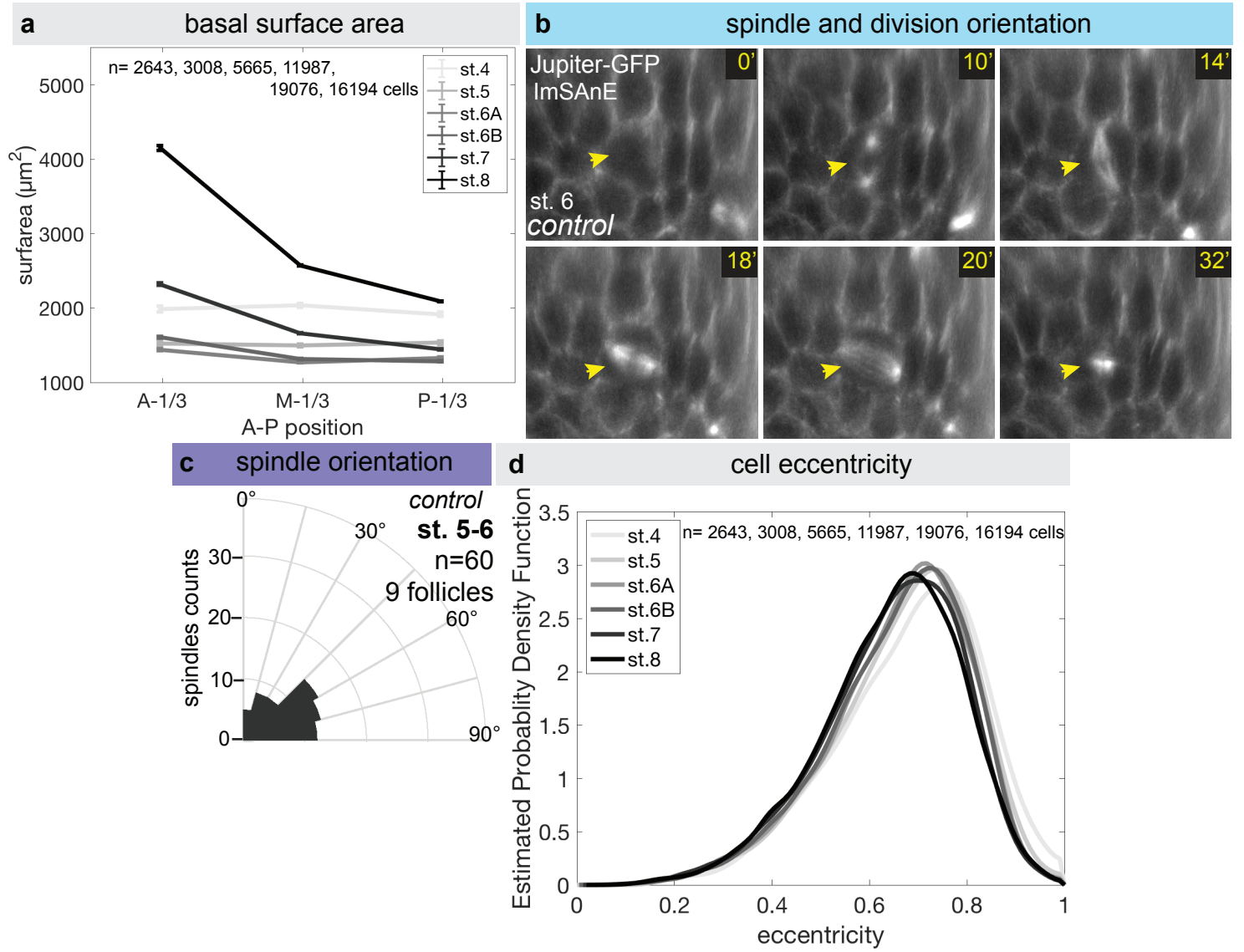
Stage	Duration by Lin&Spradling (h)	Duration by David&Merle (h)	Approx. range of follicle cell number	Approx. range of nurse cell nucleus diameter (μm)
4	6	9.16	125-350	N/A (polytene)
5	5	2.61	350-500	11-14
6A	3	8.45	500-650	14-15
6B			650-850	15-17
7	6	8.69	850+	17-21
8	6	5.21	850+	21+

Supplementary Figure 1| Representative wildtype egg chamber images and morphometrics.

a, Morphometric data for follicles staged by cell number, which generally correlate with classic morphological criteria. Values shown are average \pm standard deviation (s.d.)

b, Table showing durations of stages according to Lin and Spradling (1993) deduced from single ovariole transplants into *ovoD1* female hosts, or David and Merle (1968) deduced from representation of follicle stages in wildtype hosts; Approximate range of follicle cell number and nurse cell nucleus diameter for suggested staging. scale bars, 10 μ m.

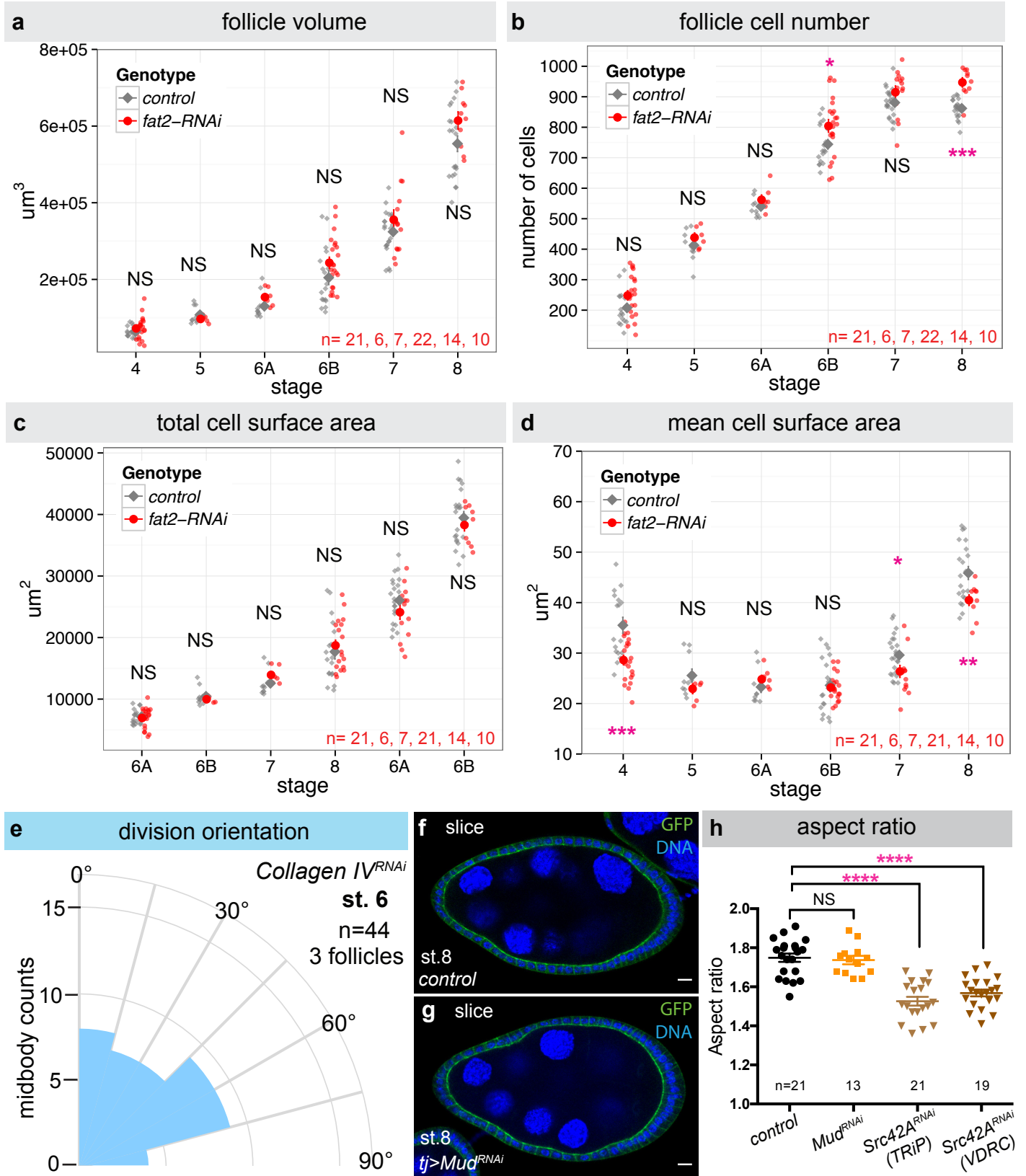
Supplementary Figure 2



Supplementary Figure 2| Additional morphometrics and mitotic spindle orientation.

a, Mean basal surface area in wild type from stage 4-8 along the A-P meridian. (A-1/3: anterior one third; M-1/3: middle one third; P-1/3: posterior one third). **b**, Example from ImSAnE cylinder projection showing mitotic spindle (yellow arrowhead) in control stage 6 follicle rotating from initial latitudinal orientation to final AP-oriented cell division (see also **Supplementary Movie 3**). **c**, Quantitation of mitotic spindle orientations in wild type stage 5-6 follicles (0° = latitudinal; 90° = meridional). **d**, Distribution of cell eccentricities in wild type from stage 4-8. n, biologically independent samples. Error bars, s.e.m. Source data are provided as a Source Data file.

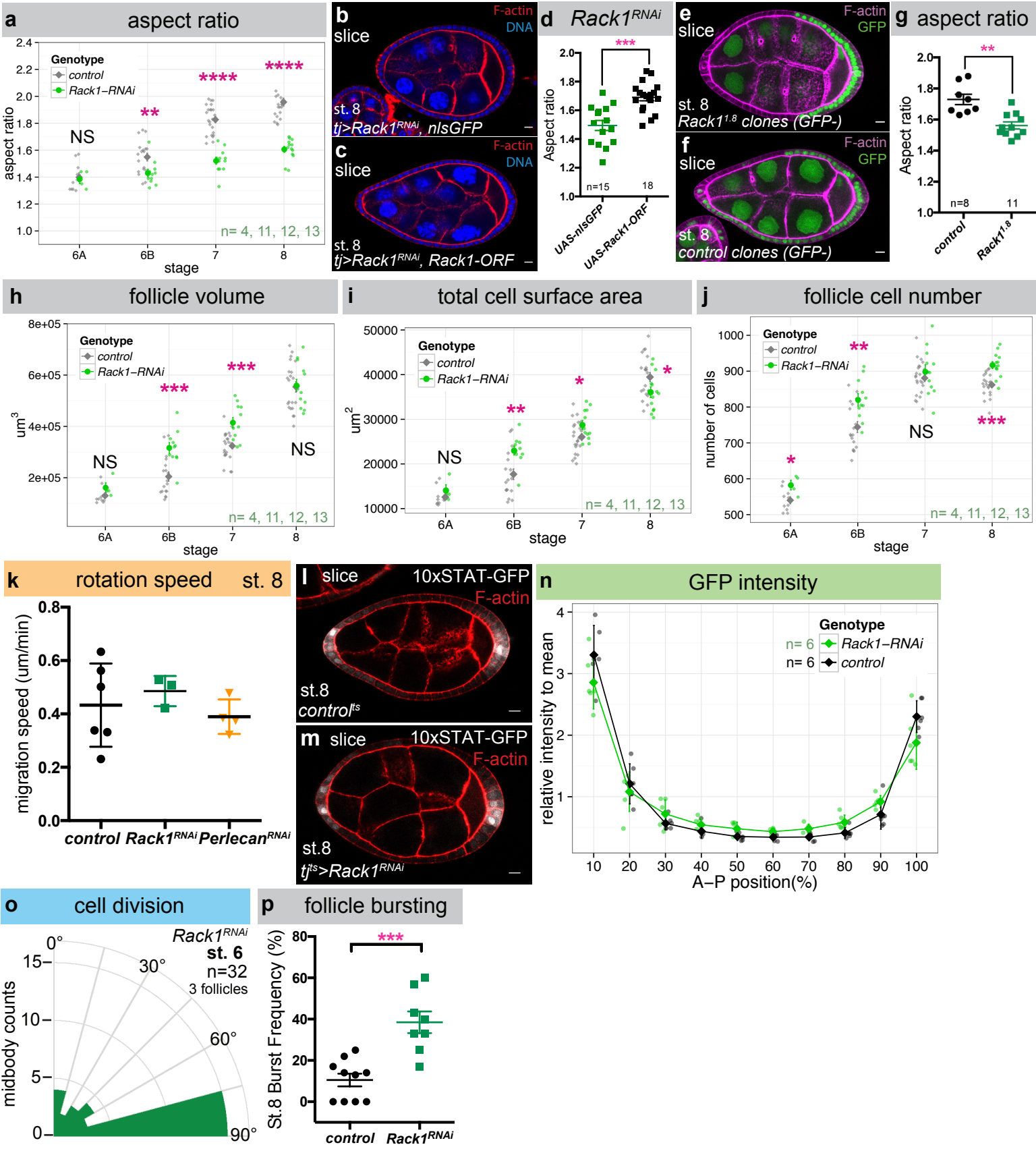
Supplementary Figure 3



Supplementary Figure 3| Additional morphometric analyses of *fat2*, *Collagen IV*, *Mud*, and *Src42A*-depleted follicles.

a-d, Further morphometric data comparing control to *fat2*-depleted follicles with volume (**a**), P-values = 0.28, 0.19, 0.10, 0.27, 0.06; two-sided Welch's t-test, cell number (**b**), P-values = 0.05, 0.26, 0.25, 0.024, 0.15, 2.0e-04; two-sided Welch's t-test, total cell surface area (**c**), P-values = 0.73, 0.38, 0.11, 0.47, 0.17, 0.41; two-sided Welch's t-test, and mean cell surface area (**d**), P-values = 9.1e-04, 0.13, 0.26, 0.77, 0.03, 0.003; two-sided Welch's t-test. **e**, Cell divisions remain ~randomly oriented at stage 6 in *Collagen IV*-depleted follicles. **f-g**, *Mud*-depleted follicles are able to elongate. **h**, Quantitation of follicle elongation in *Mud*-depleted and *Src42A*-depleted follicles stage 8 follicles. P-values = 0.69, <0.0001, <0.0001; two-sided Welch's t-test. n, biologically independent samples. NS, not significant, *P<0.5, **P<0.01, ***P<0.001, ****P<0.0001. scale bars, 10 μ m. Error bars, s.e.m.

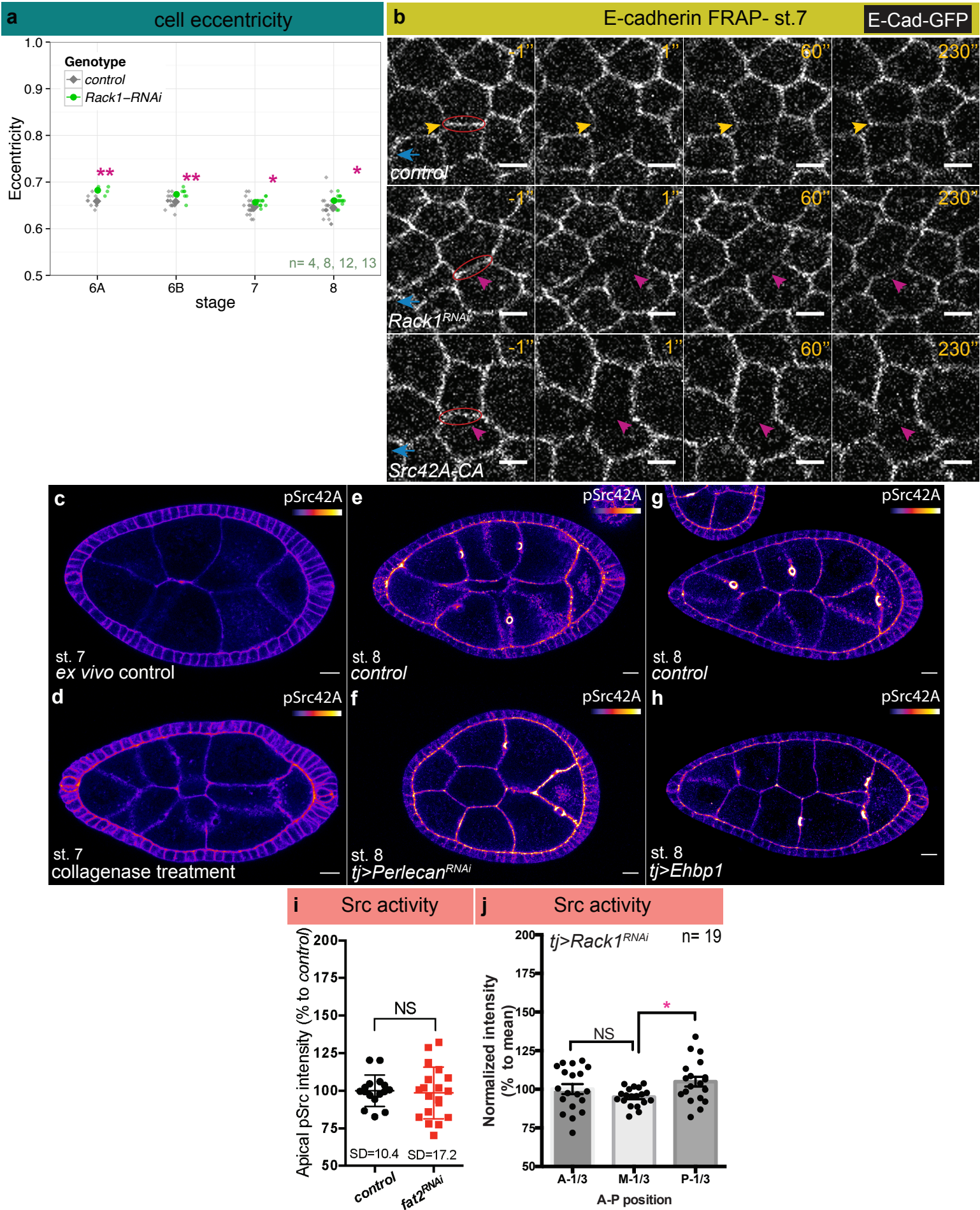
Supplementary Figure 4



Supplementary Figure 4| Morphometrics, follicle rotation speed, STAT activity, cell division orientation, and follicle bursting of *Rack1*-depleted follicle.

a, Elongation of *Rack1*-depleted follicles diverges from control at stage 6B. **b-d**, *Rack1*-depleted round follicle phenotype is rescued by overexpression of the *Rack1*-ORF. P-value = 0.0001; two-sided Welch's t-test. **e-g**, Mitotic clones of a *Rack1* null allele (marked by absence of GFP) perturb elongation. P-value = 0.0013; two-sided Welch's t-test. **h-j**, Further morphometric data comparing control to *Rack1*-depleted follicles follicle with volume (**h**); P-values = 0.21, 5.2e-04, 8.5e-04, 0.86; two-sided Welch's t-test, total cell surface area (**i**); P-values = 0.34, 0.0019, 0.04, 0.04; two-sided Welch's t-test, and follicle cell number (**j**); P-values = 0.04, 0.003, 0.42, 0.0001; two-sided Welch's t-test. **k**, Both *Rack1* and *Perlecan*-depleted follicles undergo rotation (see also **Supplementary Movie 4**). **l-n**, *Rack1*-depleted follicles show normal STAT activity gradients (grey) at poles. **o**, *Rack1*-depleted follicles undergo oriented cell divisions at stage 6 (n=32) similar to control. **p**, Bursting frequency of *Rack1*-depleted follicles under osmotic stress is increased compared to control follicles. n, biologically independent samples. NS, not significant, *P<0.5, **P<0.01, ***P<0.001, ****P<0.0001. scale bars, 10 μ m. except for **n**, Error bars, s.e.m.

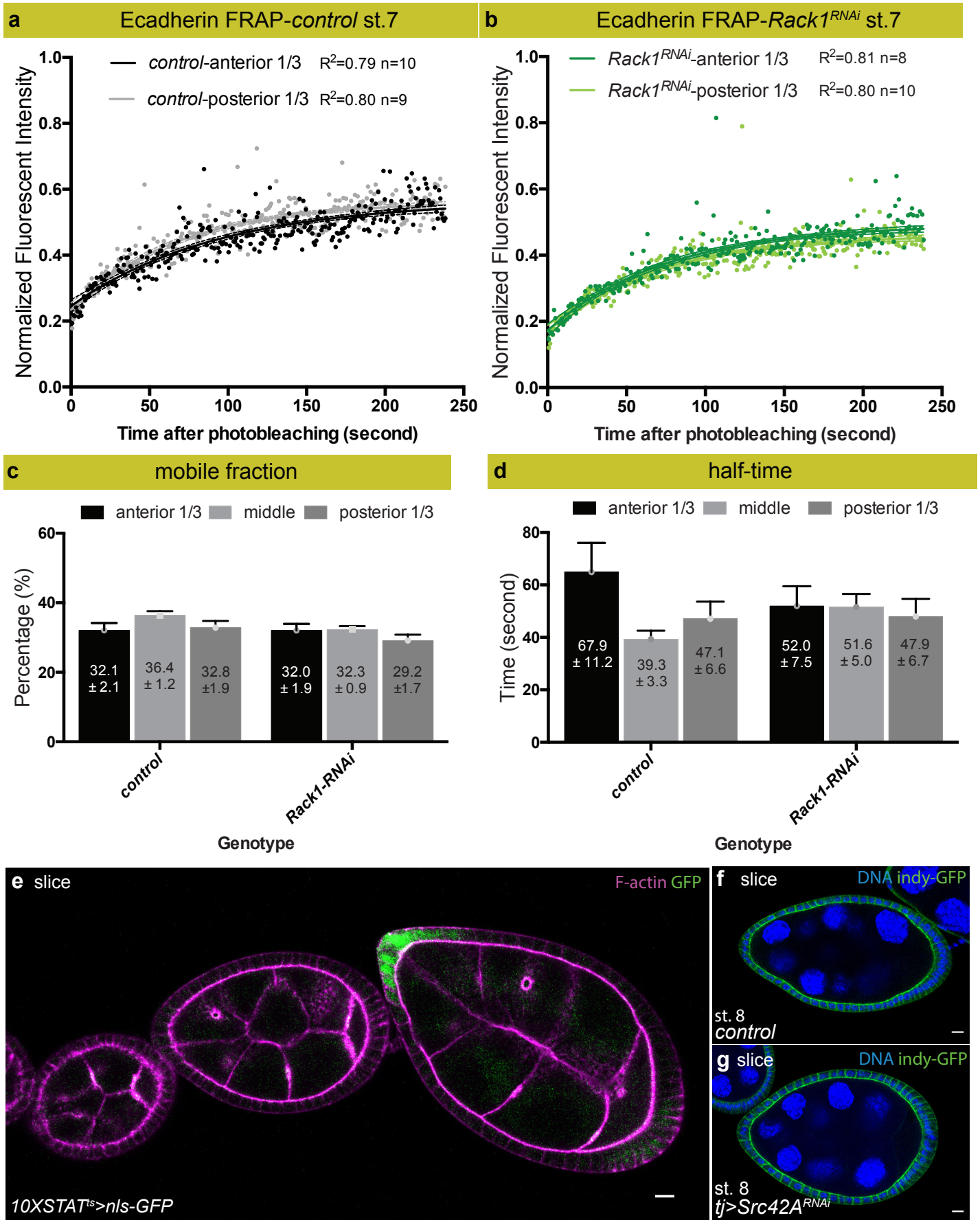
Supplementary Figure 5



Supplementary Figure 5| Additional morphometrics of *Rack1*-depleted follicles, representative FRAP images, and representative images for Src activity measurements.

a, Cell eccentricity is slightly increased when *Rack1* is depleted. P-values = 0.0063, 0.0079, 0.017, and 0.013; two-sided Welch's t-test. **b**, FRAP recovery is not through lateral diffusion. Yellow and magenta arrows mark photobleached junctions; blue arrows indicate direction of follicle rotation. (See also **Supplementary Movie 5**). **c-h**, Representative images from pSrc measurements in Figure 7 on **c-d**, *ex-vivo* culture control and collagenase treatment, **e-f**, control and *Perlecan-RNAi*, **g-h**, control and *UAS-Ehbp1*. **i**, *fat2*-depleted follicles show higher variation of phosphorylated Src at apical junctions compared to wild type. Error bars, standard deviation (SD), n= 16 and 19, P-value s = 0.76; two-sided Welch's t-test. **j**, Quantification of pSrc intensity in stage 8 *Rack1* depleted follicles along the A-P meridian (A-1/3: anterior one-third; M-1/3: middle-one third; P-1/3: posterior one-third). pSrc intensities were normalized to mean pSrc levels of each follicle. Error bars, s.e.m. P-value s = 0.194 and 0.013; Two-sided paired sample t-test. n, biologically independent samples. Scale bars in **b**, 3 μ m. Scale bars in **c-h**, 10 μ m. NS, not significant, *P<0.5, **P<0.01.

Supplementary Figure 6



Supplementary Figure 6| Positional FRAP analyses, 10XSTAT-GAL4 expression pattern, and Src42A-RNAi phenotype.

a-b, FRAP analysis of Ecad-GFP in *control* and *Rack1*-depleted follicles at stage 7, performed at the anterior and posterior one-third. Dots represent mean of normalized intensity after photobleaching and lines are fitted curves with error bars showing 95% confidence intervals. **c**, Mobile fraction of Ecad-GFP is similar along the A-P meridian and in both genotypes. Error bars, 95% confidence intervals. **d**, Recovery half-time is increased at the anterior in *control* compared to the middle (equator); half-time at anterior, middle, and the posterior are similar when *Rack1* is depleted. Error bars show 95% confidence intervals. **e**, *10XSTAT-GAL4* drives transgene expression predominantly in follicle anterior at stage 7-8. **f-g**, *Src42A*-depleted follicles show impaired elongation. n, Sample size. Scale bars, 10 μ m. Source data are provided as a Source Data file.

Supplementary Table 1: Detailed Drosophila genotypes

Figure Panel	Genotype
1	a-g <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
2	a-e <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
3	b-d <i>tj-GAL4/+; mcherry-RNAi/Jupiter-GFP</i>
	f-i <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
4	b-d <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	f-i <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
5	a <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	b <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i>
	c <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	c <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i>
	d <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i>
	e <i>tj-GAL4, tubP-GAL80[ts]/+; mcherry-RNAi/+</i>
	f <i>tj-GAL4, tubP-GAL80[ts]/+; UAS-fzr/+</i>
	f <i>tj-GAL4, tubP-GAL80[ts]/+; mcherry-RNAi/+</i>
	g-h <i>tj-GAL4, tubP-GAL80[ts]/+; mcherry-RNAi/indyGFP</i>
	g-h <i>tj-GAL4, tubP-GAL80[ts]/+; UAS-fzr/+</i>
	g-h <i>tj-GAL4, tubP-GAL80[ts]/+; UAS-fzr/indyGFP</i>
	i-j <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	i-j <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i>
k-l <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i>	
m-o <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>	
m-o <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i>	
6	a <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	b <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i>
	c <i>tj-GAL4, E-cadGFP[ki]/+; mcherry-RNAi/+</i>
	d <i>tj-GAL4, E-cadGFP[ki]/+; Rack1-RNAi/+</i>
	e <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	e <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i>
	f <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	g <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i>
	g <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	h <i>tj-GAL4, E-cadGFP[ki]/+; mcherry-RNAi/+</i>
	h <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i>
	h <i>tj-GAL4, E-cadGFP[ki]/+; Rack1-RNAi/+</i>
	i <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	j <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i>
	k <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
	k <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i>
	l <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>
l <i>tj-GAL4/+; UAS-Src42A-CA/indy-GFP</i>	
m <i>w1118; tj-GAL4, tubP-GAL80[ts]/+; Rack1-RNAi/+</i>	
m <i>tj-GAL4, tubP-GAL80[ts]/Src42A[26-1]; Rack1-RNAi/+</i>	
n-o <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i>	
p-s <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i>	
p-s <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i>	

7	<p>a-c <i>tj-GAL4, E-cadGFP[ki]/+; mcherry-RNAi/+</i> <i>tj-GAL4, E-cadGFP[ki]/+; Rack1-RNAi/+</i> <i>tj-GAL4, E-cadGFP[ki]/+; UAS-<i>Src42A-CA</i>/+</i> <i>tj-GAL4, vkg-GFP/+; mcherry-RNAi/+</i> <i>yw; tj-GAL4/+; Dr/+</i></p> <p>d <i>yw; tj-GAL4/+; UAS-mcherry-Ehbp1/Dr</i> <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i> <i>tj-GAL4/+; Perlecan(trol)-RNAi/indy-GFP</i></p> <p>e-f <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i> <i>tj-GAL4/+; Perlecan(trol)-RNAi/indy-GFP</i> <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p> <p>g <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i> <i>tj-GAL4/+; Perlecan(trol)-RNAi/indy-GFP</i></p> <p>h-i <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p> <p>j <i>w; 10xSTAT-GAL4/+; UAS-nls-GFP/tubP-GAL80[ts]</i></p> <p>k <i>w; 10xSTAT-GAL4/+; Rack1-RNAi/tubP-GAL80[ts]</i> <i>w; 10xSTAT-GAL4/+; mcherry-RNAi/tubP-GAL80[ts]</i></p> <p>l <i>w; 10xSTAT-GAL4/+; Rack1-RNAi/tubP-GAL80[ts]</i> <i>w; 10xSTAT-GAL4/+; UAS-<i>Src42A-CA</i>/tubP-GAL80[ts]</i></p>
S1	<p>a-b <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p>
S2	<p>a <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p> <p>b-c <i>tj-GAL4/+; mcherry-RNAi/Jupiter-GFP</i></p> <p>d <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p>
S3	<p>a-d <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i> <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i></p> <p>e <i>tj-GAL4/vkg-RNAi; cg25c-RNAi/Jupiter-GFP</i></p> <p>f <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p> <p>g <i>tj-GAL4/+; Mud-RNAi/indy-GFP</i> <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p> <p>h <i>tj-GAL4/+; Mud-RNAi/indy-GFP</i> <i>tj-GAL4/+; Src42A-RNAi(TRiP)/indy-GFP</i> <i>tj-GAL4/Src42A-RNAi(VDRC-KK)/; indy-GFP/+</i></p>
	<p>a <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i> <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i></p> <p>b <i>tj-GAL4, tubP-GAL80[ts]/+; Rack1-RNAi/UAS-nlsGFP</i></p> <p>c <i>tj-GAL4, tubP-GAL80[ts]/+; Rack1-RNAi/UAS-Rack1-ORF-3xHA</i></p> <p>d <i>tj-GAL4, tubP-GAL80[ts]/+; Rack1-RNAi/UAS-nlsGFP</i> <i>tj-GAL4, tubP-GAL80[ts]/+; Rack1-RNAi/UAS-Rack1-ORF-3xHA</i></p>

S4	<p>e ubi-eGFP-FRT40/Rack1[1.8]-FRT40A; GR1-GAL4, UAS-FLP/+</p> <p>f ubi-eGFP-FRT40/FRT40A; GR1-GAL4, UAS-FLP/+</p> <p>g ubi-eGFP-FRT40/Rack1[1.8]-FRT40A; GR1-GAL4, UAS-FLP/+</p> <p>ubi-eGFP-FRT40/FRT40A; GR1-GAL4, UAS-FLP/+</p> <p>h-j <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i> <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i> <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p> <p>k <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i> <i>tj-GAL4/+; Perlecan(trol)-RNAi/indy-GFP</i></p> <p>l <i>tj-GAL4, tubP-GAL80[ts]/+; 10XSTAT-GFP/mcherry-RNAi</i></p> <p>m <i>tj-GAL4, tubP-GAL80[ts]/+; 10XSTAT-GFP/Rack1-RNAi</i></p> <p>n <i>tj-GAL4, tubP-GAL80[ts]/+; 10XSTAT-GFP/mcherry-RNAi</i> <i>tj-GAL4, tubP-GAL80[ts]/+; 10XSTAT-GFP/Rack1-RNAi</i></p>
S5	<p>a <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i> <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i> <i>tj-GAL4, E-cadGFP[ki]/+; mcherry-RNAi/+</i></p> <p>b <i>tj-GAL4, E-cadGFP[ki]/+; Rack1-RNAi/+</i> <i>tj-GAL4, E-cadGFP[ki]/+; UAS-<i>Src42A-CA</i>/+</i></p> <p>c-d <i>tj-GAL4, vkg-GFP/+; mcherry-RNAi/+</i></p> <p>e <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p> <p>f <i>tj-GAL4/+; Perlecan(trol)-RNAi/indy-GFP</i></p> <p>g <i>yw; tj-GAL4/+; Dr/+</i></p> <p>h <i>yw; tj-GAL4/+; UAS-mcherry-Ehbp1/Dr</i></p> <p>i <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i> <i>tj-GAL4/fat2-RNAi; indy-GFP/+</i></p> <p>j <i>tj-GAL4/+; Rack1-RNAi/indy-GFP</i></p>
S6	<p>a-d <i>tj-GAL4, E-cadGFP[ki]/+; mcherry-RNAi/+</i> <i>tj-GAL4, E-cadGFP[ki]/+; Rack1-RNAi/+</i></p> <p>e <i>w; 10xSTAT-GAL4/+; UAS-nls-GFP/tubP-GAL80[ts]</i></p> <p>f <i>tj-GAL4/+; mcherry-RNAi/indy-GFP</i></p> <p>g <i>tj-GAL4/+; Src42A-RNAi(TRiP)/indy-GFP</i></p>

Supplementary Table 2 : Antibodies and reagents

Antibody/Reagent	Dilution used	Provider	Catalogue Number
Mouse monoclonal Anti-GFP (3E6)	1:50	ThermoFisher	A11120
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:300	ThermoFisher	A11001
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:300	ThermoFisher	A11008
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	1:300	ThermoFisher	A21244
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	1:300	ThermoFisher	A31571
Phospho-Src Family (Tyr416)	1:500	ThermoFisher	2101
Phospho-Src42A (Tyr400)	1:500	Shigeo Hayashi lab (Shindo, M., et al. 2008)	N/A
FasIII	1:100	Developmental Studies Hybridoma Bank	7G10
Src42A	1:200	Tetsuya Kojima lab (Takahashi, M., et. al. 2005)	N/A
Schneider's Drosophila medium(1x)	N/A	gibco	21720-024
Fetal bovine serum defined insulin	N/A	Hyclone	SH30070.03
Penicillin-Streptomycin (10,000 U/mL)	N/A	sigma	I5500
Phalloidin-TRITC	N/A	gibco	15140
Alexa Fluor 647 Phalloidin	N/A	Sigma	P1951
DAPI	N/A	ThermoFisher	A22287
Bovine Serum Albumin	N/A	Sigma	D9542
			A3912

Supplementary Methods

Complete sequence of the five tandem copies of the Socs36E enhancer fragment for creating *10XSTAT-GAL4* is below:

```
GCGGCCGCGAACCCTCAGAGTGCCTGCGTGTGTGTGTGTGTGTGTGCGTGTGAGTCCG
CTTGTGTATCTGTGAGAGTGAGTGCGAGTACGAGTATCTTTGTATCTGCTGGTTTCGCCT
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CGTATCGCTCTTTTGGGCCACGGAGCTCAGCGAACAACATATATAAAAAAATATACAT
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TGGCATTTGCAAATATTATATAGCAAACAGGGCAACTATTTATTTTGTCTTAGTAATTTATG
CAAACAGCGAGCGGTTTCCAGGGAATTCCAATTACCACAATGTGGTAAACACCTAAGACA
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