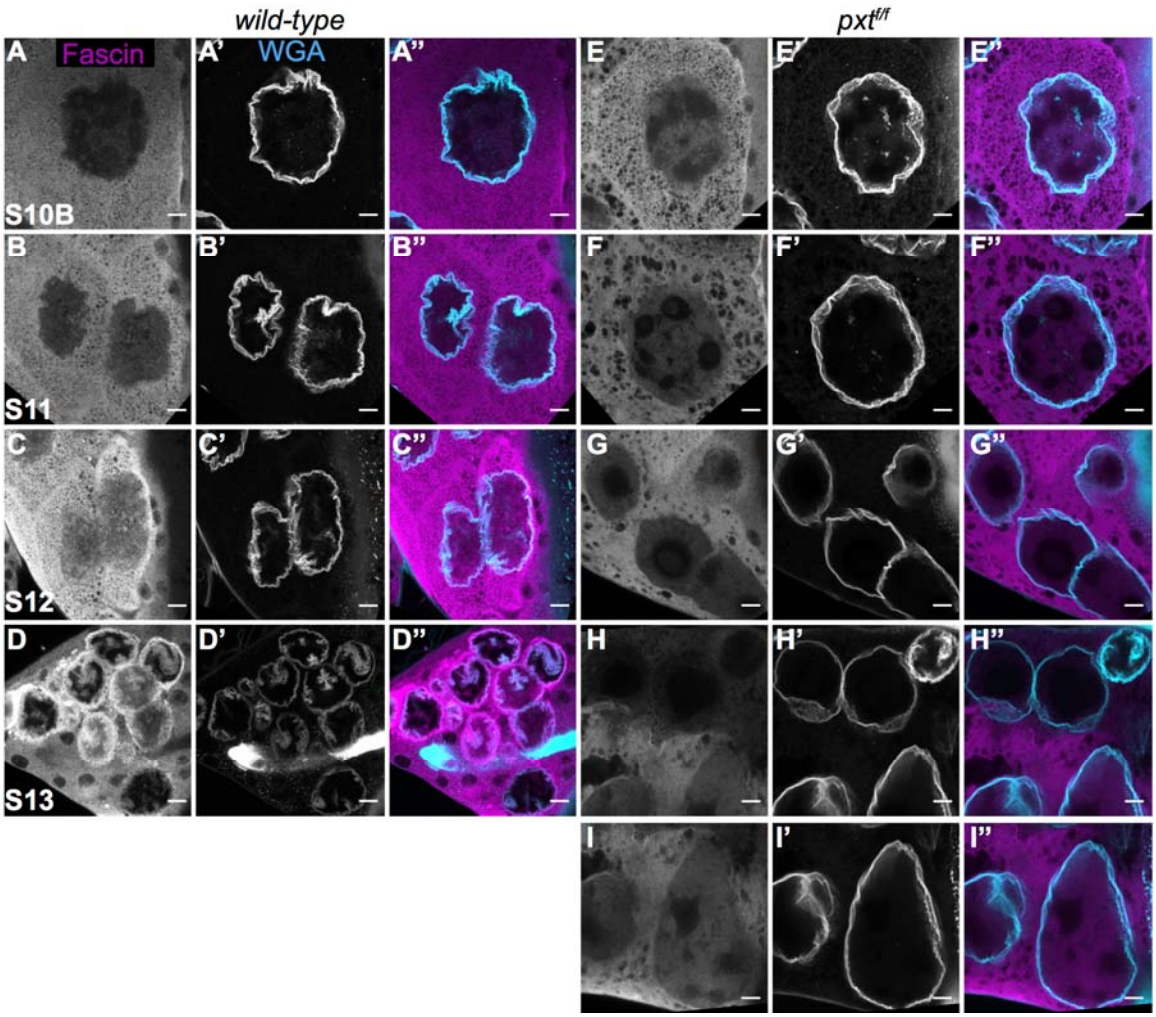
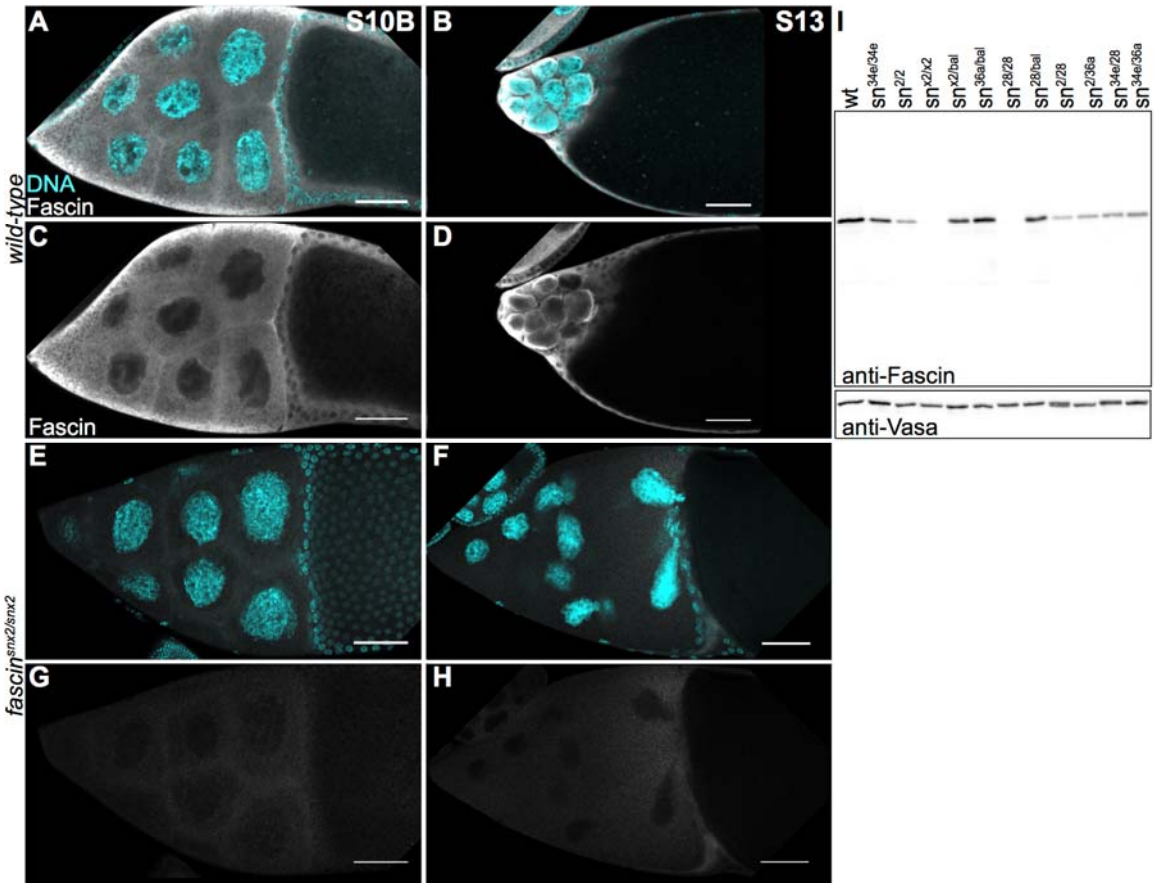
Supplemental Materials

Molecular Biology of the Cell

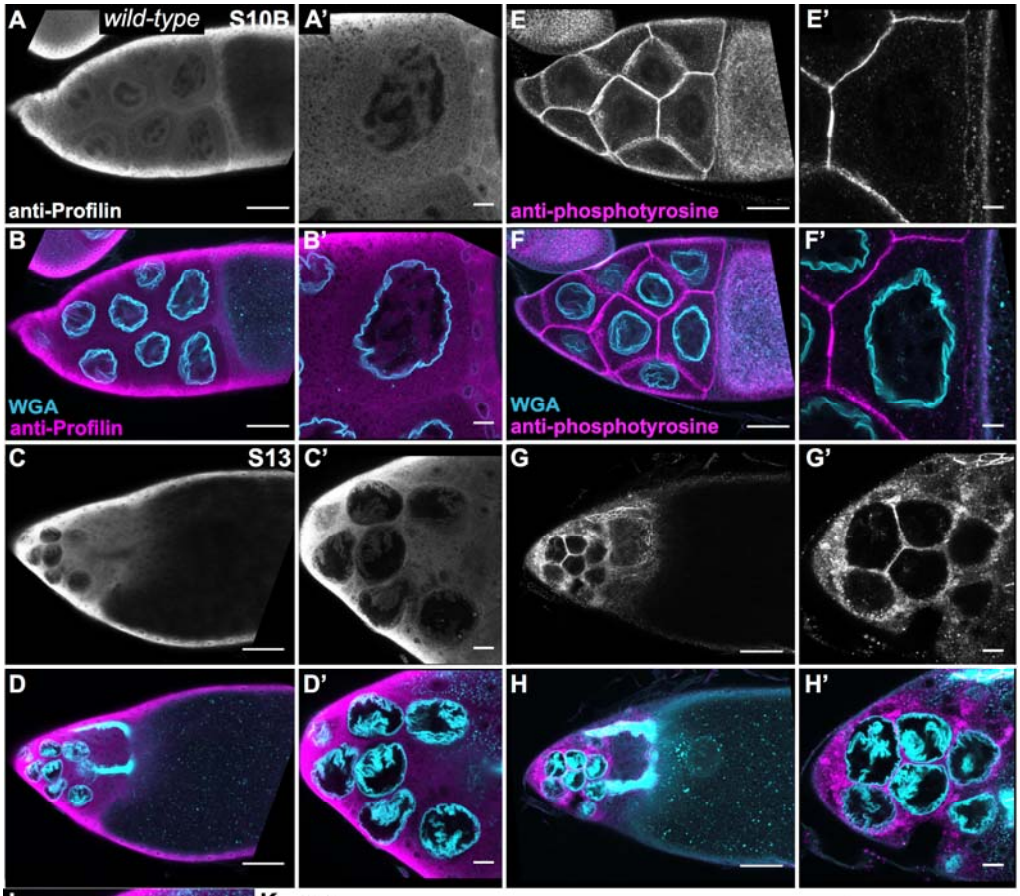
Groen et al.

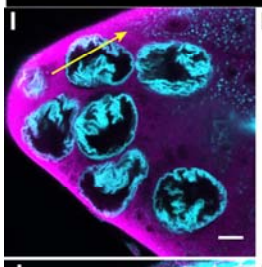


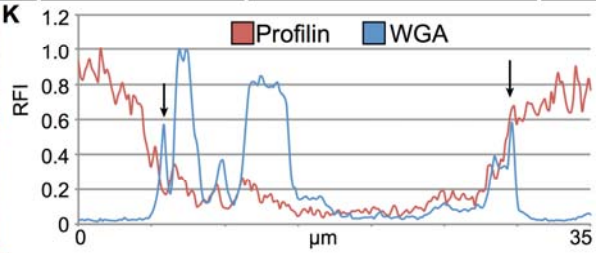
Supplemental Figure 1: Nuclear localization of Fascin is regulated by prostaglandin signaling. A-I''. Maximum projections of 3-5 confocal slices of late stage follicles imaged at 63X; *wild-type* images are of the same follicles shown in Figure 1. A-I. Fascin (white). A'-I'. WGA (wheat germ agglutinin, white). A''-I''. Merged images: Fascin (magenta) and WGA (cyan). A-A", E-E". S10B. B-B", F-F". S11. C-C", G-G". S12. D-D", H-I". S13. A-D". *wild-type*. E-I". $pxt^{f/f}$. In wild type follicles, Fascin, in addition to being cytoplasmic, is relatively low in the nucleus at S10B (A-A") and increases during S11 (B-B") and S12 (C-C") before re-localizing to the nuclear periphery at S13 (D-D"). In *pxt* mutant follicles, Fascin is relatively high in the nucleus at S10B (E-E") and decreases during S11 (F-F") and S12 (G-G"), and then fails to re-localize to the nuclear periphery at S13 (H-I"). Scale bars = 10μm.

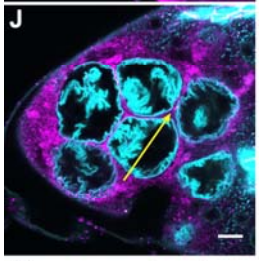


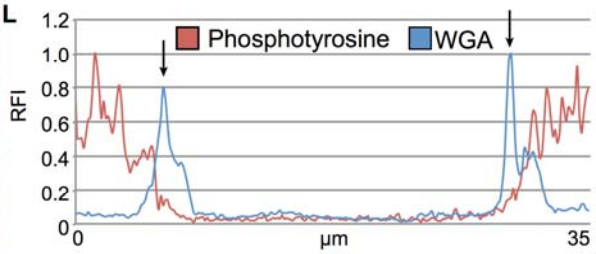
Supplemental Figure 2: Specificity of anti-Fascin sn 7C antibody. A-H. Maximum projections of 3-5 confocal slices of 20X images. A, B, E, F. Merged images: Fascin (white) and DAPI (cyan). C, D, G, H. Fascin (white). A-D: *wild-type*. E-H: $sn^{x2/x2}$ (*fascin*-null mutant). A, C, E, G. S10B. B, D, F, H. S13. Compared to *wild-type* follicles (A-D), the sn 7C antibody shows no detectable signal in the cytoplasm or the nucleus of *fascin*-null follicles at either S10B (E, G) or S13 (F, H). E. Western blot of *wild-type* and *fascin* mutant whole ovary lysates analyzed for Fascin (sn7c) and Vasa (loading control). No Fascin protein band is visible by Western blot in *fascin*-null whole ovary lysates ($sn^{x2/x2}$ and $sn^{28/28}$) and is reduced in all other *fascin* mutant alleles ($sn^{34e/34e}$, $sn^{2/2}$, $sn^{x2/+}$, $sn^{36a/+}$, $sn^{28/+}$, $sn^{2/28}$, $sn^{2/36a}$, $sn^{34e/28}$, and $sn^{34e/36a}$). Scale bars = 50 μm.

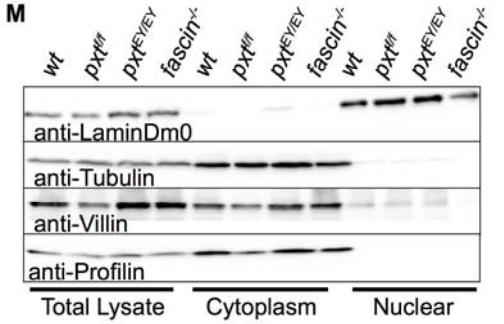

A
wild-type
S10B
A'
E
E'
anti-Profilin
anti-phosphotyrosine
B
B'
F
F'
WGA
anti-Profilin
WGA
anti-phosphotyrosine
C
S13
C'
G
G'
D
D'
H
H'

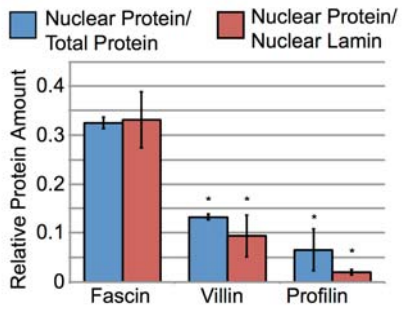

I


K
Profilin
WGA
RFI
1.2
1.0
0.8
0.6
0.4
0.2
0
0
µm
35

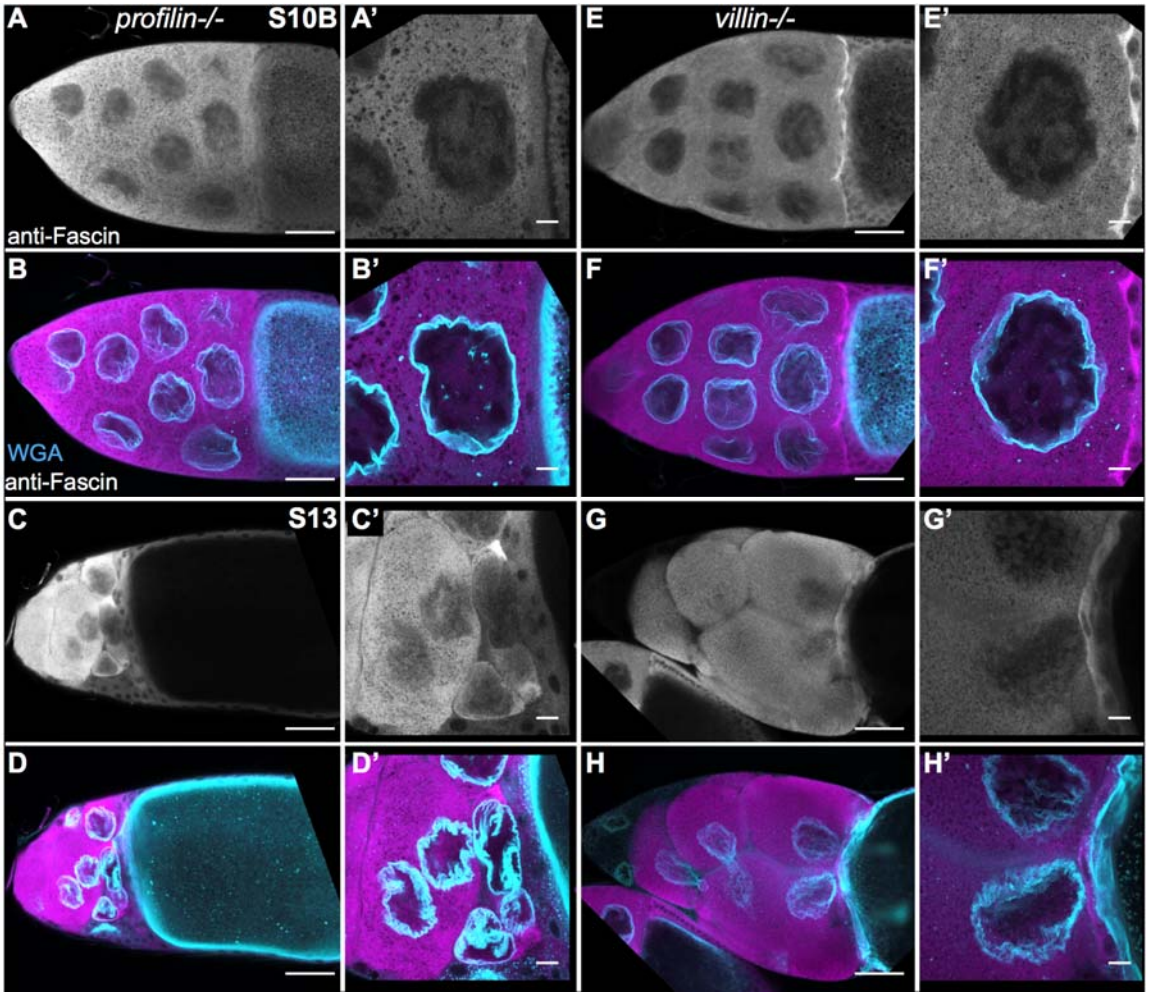

J


L
Phosphotyrosine
WGA
RFI
1.2
1.0
0.8
0.6
0.4
0.2
0
0
µm
35

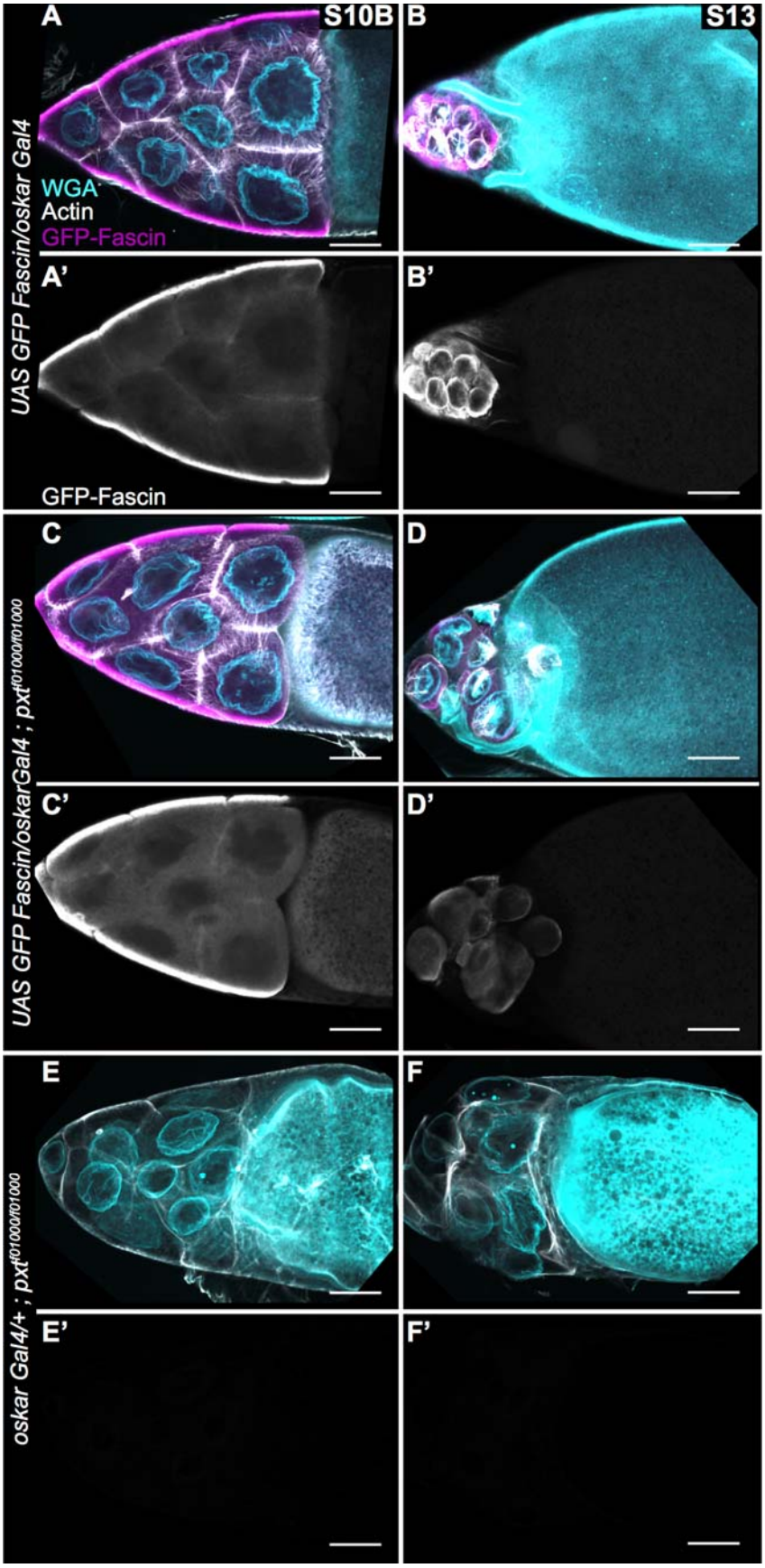

M
wt
pxt^fl/fl
pxt^EY/EY
fascin^-/-
anti-LaminDm0
anti-Tubulin
anti-Villin
anti-Profilin
Total Lysate
Cytoplasm
Nuclear


N
Nuclear Protein/
Total Protein
Nuclear Protein/
Nuclear Lamin
Relative Protein Amount
0.4
0.3
0.2
0.1
0
Fascin
Villin
Profilin

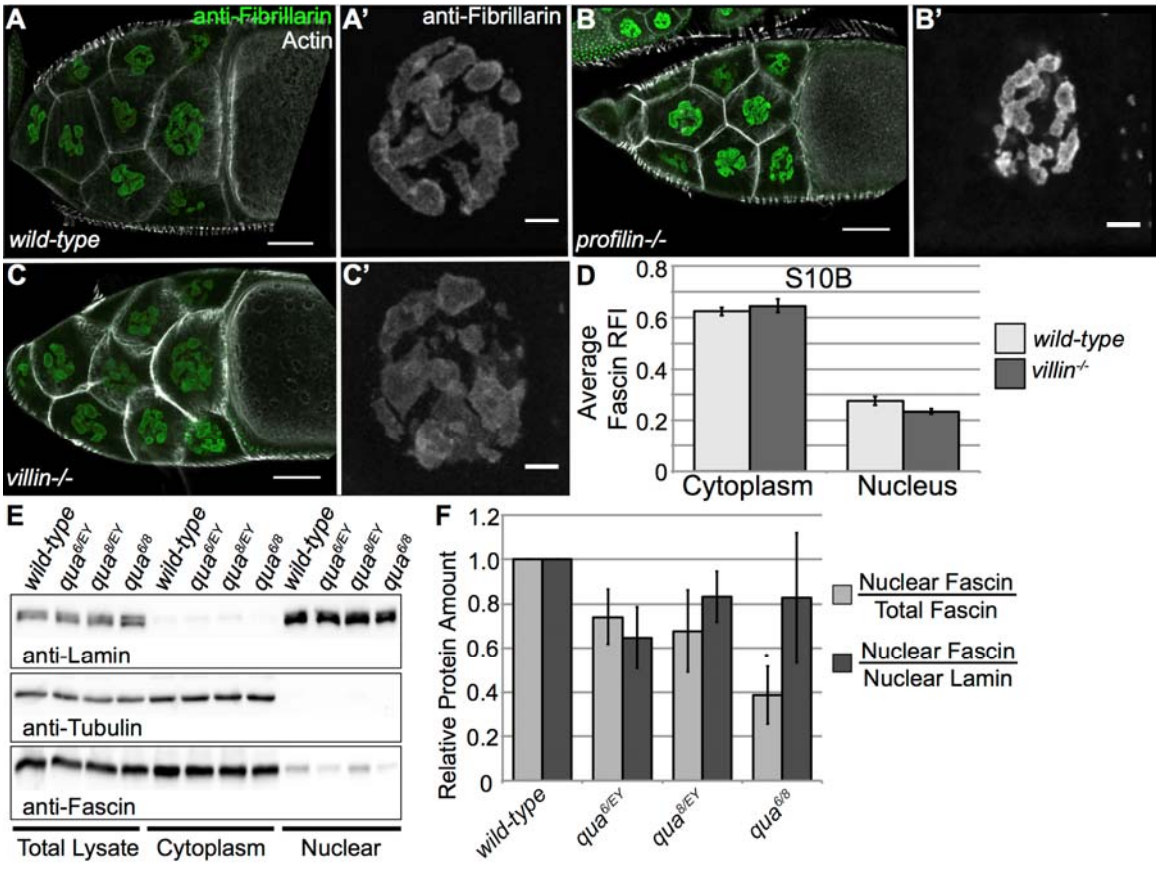
Supplemental Figure 3: Profilin and anti-phosphotyrosine staining show distinct localization patterns. A-J. Maximum projections of 3-5 confocal slices at 20X (A-H) or 63X (A'-H', I, J) of *wild-type* follicles. A, A', C, C'. Profilin (white). B, B', D, D', I. Merged images: Profilin (magenta) and WGA (cyan). Profilin localizes to both the nucleus and the cytoplasm during S10B (A-B'), and remains cytoplasmic during S13 (C-D', I). E, E', G, G'. Phosphotyrosine (white). F, F', H, H', J. Merged images: Phosphotyrosine (magenta) and WGA (cyan). Phosphotyrosine marks the nurse cell membranes in S10B and S13 (E-H', J). K, L. Representative fluorescence intensity plots (minimum of 6 nuclei examined per genotype) of Profilin (K) or Phosphotyrosine (L) (red line) and WGA (blue line) along the yellow arrows in I, J. X-axis = distance. Y-axis = relative fluorescence intensity (RFI), the fluorescence intensity normalized to the brightest point along the plot. Black arrows mark the nuclear envelope (based on WGA peaks). Intensity plot analysis reveals that Profilin is throughout the cytoplasm during S13, as the levels outside the WGA peaks are relatively constant and no sharp peaks of Profilin localization are observed (I, K). Whereas the phosphotyrosine exhibits peak intensity a significant distance from the nucleus (WGA peaks), indicating the localization of the membrane (J, L). M. Representative western blot of subcellular fractionation samples (total lysate, cytoplasmic fraction, nuclear fraction) blotted for Lamin Dm0 (nuclear marker), α-Tubulin (cytoplasmic marker), and Villin and Profilin. N. Quantification of a minimum of three subcellular fractionation assays for Fascin, Villin, and Profilin showing either the ratio of nuclear protein to total protein (blue bars) or the ratio of nuclear protein to nuclear Lamin (red bars). Subcellular fractionation assays reveal that Fascin is higher in the nuclear pellet than either Villin or Profilin. *$p < 0.05$ when compared to Fascin for the corresponding ratio – either nuclear protein to total protein or nuclear protein to nuclear Lamin (paired student's T-Test, unequal variance).



Supplemental Figure 4: Fascin perinuclear localization is disrupted in dumping mutants. A-H'. Maximum projections of 3-5 confocal slices at 20X (A-H) or 63X (A'-H'). A, A', C, C', E, E', G, G'. Fascin (white). B, B', D, D', F, F', H, H'. Merged images: Fascin (magenta) and WGA (cyan). A-D'. Profilin transheterozygous mutant follicles (*chickadee*$^{11/fs(2)neo1}$). E-H' Villin transheterozygous mutant follicles (*quail*$^{6\text{-}396/8\text{-}1062}$). In *profilin* and *villin* mutants, which fail to complete nurse cell dumping due to failure to form cytoplasmic actin bundles, Fascin localization to both the cytoplasm and nucleus appears normal during S10B (A-B', E-F'). However, during S13, Fascin does not localize to the nuclear periphery in follicles that have not completed nurse cell dumping (C-D', G-H'). Scale bars = 50 μm in A-H and 10 μm in A'-H'.


A
S10B
B
S13
WGA
Actin
GFP-Fascin
UAS GFP Fascin/oskar Gal4
A'
B'
GFP-Fascin
C
D
UAS GFP Fascin/oskarGal4 ; pxt^f01000/f01000
C'
D'
E
F
oskar Gal4/+ ; pxt^f01000/f01000
E'
F'

Supplemental Figure 5: Overexpression of GFP-Fascin rescues bundle formation but not perinuclear Fascin localization in *pxt* mutants. A-F' Maximum projections of 3-5 confocal slices at 20X of the indicated genotypes. A-B'. *UAS GFP Fascin/oskarGal4*. C-D'. *UAS GFP Fascin/oskarGal4; pxt$^{f/f}$*. E-F'. *oskarGal4/+ ; pxt$^{f/f}$*. A-F. Merged images: GFP-Fascin (magenta), WGA (cyan), and Phalloidin (white). A'-F'. GFP-Fascin (white). GFP Fascin localizes to the cytoplasm and inside the nucleus during S10B (A, A') and relocalizes to the nuclear periphery during S13 (B, B'). While expression of GFP Fascin rescues bundle formation during S10B (C, C' compared to E, E'), and dumping is largely restored (D, D' compared to F, F'), GFP Fascin does not localize to the nuclear periphery during S13 in *pxt* mutants (D, D'). Scale bars = 50 μm.



Supplemental Figure 6: *Villin* mutants have mildly dispersed nucleoli. A-C'. Maximum projections of 3-5 confocal slices at 20X of the indicated genotypes. A, A'. *wild-type*. B, B'. *chickadee*$^{11/fs(2)neo1}$. C, C'. *quail*$^{6\text{-}396/8\text{-}1062}$. A-C. Merged Images: Fibrillarin (green) and Phalloidin (white). A'-C'. Zoomed in image of posterior nurse cell. Fibrillarin (white). *profilin* mutant follicles have normal nucleolar structure (B-B'), while *villin* mutant follicles have a mild dispersion phenotype (C-C'). Scale bars = 50 μm in A-C and 10 μm in A'-C'. D. Quantification of average relative fluorescence intensity (RFI) from linescans of *wild-type* and *villin* mutant follicles stained with anti-Fascin. Quantification of Fascin fluorescence intensity reveals that *villin* transheterozygous mutants have slightly decreased levels of nuclear Fascin. E. Representative western blot of subcellular fractionation samples (total lysate, cytoplasmic fraction, nuclear fraction) blotted for Lamin Dm0 (nuclear marker), α-Tubulin (cytoplasmic marker) and Fascin. F. Quantification of 3 separate subcellular fractionation assays with *wild-type*, *quail*$^{6\text{-}396/EY03072}$, *quail*$^{8\text{-}1062/EY03072}$, and *quail*$^{6\text{-}396/8\text{-}1062}$ transheterozygous mutants showing the ratios of nuclear Fascin to total Fascin (light gray) and nuclear Fascin to nuclear Lamin (dark gray) and normalized to *wild-type*. Subcellular fractionation assays reveal a mild decrease in the levels of nuclear Fascin in *villin* mutant whole ovaries. *p=0.04 compared to *wild-type* (paired student's T-Test, unequal variance)

Supplemental Table 1. Scoring of nucleolar phenotype by fibrillarin staining separated into S10A and S10B follicles.

Table 1: S10A vs S10B

			Percent Follicles		
Genotype	Stage	# Scored	Interconnected/Tubular	Dispersed	Enlarged/ Fragmented
wild-type	S10A	44	86.4	13.6	0.0
	S10B	42	85.7	14.3	0.0
pxtf/Bal	S10A	26	76.9	19.2	3.8
	S10B	26	92.3	3.8	3.8
pxtEY/Bal	S10A	26	84.6	7.7	7.7
	S10B	32	75.0	0.0	25.0
pxtf/f	S10A	46	41.3	2.2	56.5
	S10B	36	8.3	5.6	86.1
pxtEY/EY	S10A	42	64.3	7.1	28.6
	S10B	54	48.1	5.6	46.3
fascin sn28/Bal	S10A	28	75.0	25.0	0.0
	S10B	20	85.0	10.0	5.0
fascin snx2/Bal	S10A	16	43.8	56.3	0.0
	S10B	24	62.5	37.5	0.0
fascin sn28/sn28	S10A	48	33.3	66.7	0.0
	S10B	38	50.0	50.0	0.0
fascin snx2/snx2	S10A	18	11.1	88.9	0.0
	S10B	32	25.0	75.0	0.0

Supplemental Table 2. Scoring of the nucleolar phenotype by fibrillarin staining separated into anterior and posterior nurse cells.

Table 2: Anterior vs Posterior Nurse Cells

			Percent Follicles		
Genotype	Nurse Cells	# Scored	Interconnected/Tubular	Dispersed	Enlarged/ Fragmented
wild-type	Anterior	43	97.7	2.3	0.0
	Posterior	43	74.4	25.6	0.0
pxtf/Bal	Anterior	26	88.5	7.7	3.8
	Posterior	26	80.8	15.4	3.8
pxtEY/Bal	Anterior	29	69.0	0.0	31.0
	Posterior	29	89.7	6.9	3.4
pxtf/f	Anterior	41	29.3	0.0	70.7
	Posterior	41	24.4	7.3	68.3
pxtEY/EY	Anterior	48	41.7	2.1	56.3
	Posterior	48	68.8	10.4	20.8
fascin sn28/Bal	Anterior	24	91.7	4.2	4.2
	Posterior	24	66.7	33.3	0.0
fascin snx2/Bal	Anterior	20	85.0	15.0	0.0
	Posterior	20	25.0	75.0	0.0
fascin sn28/sn28	Anterior	43	60.5	39.5	0.0
	Posterior	43	20.9	79.1	0.0
fascin snx2/snx2	Anterior	25	36.0	64.0	0.0
	Posterior	25	4.0	96.0	0.0