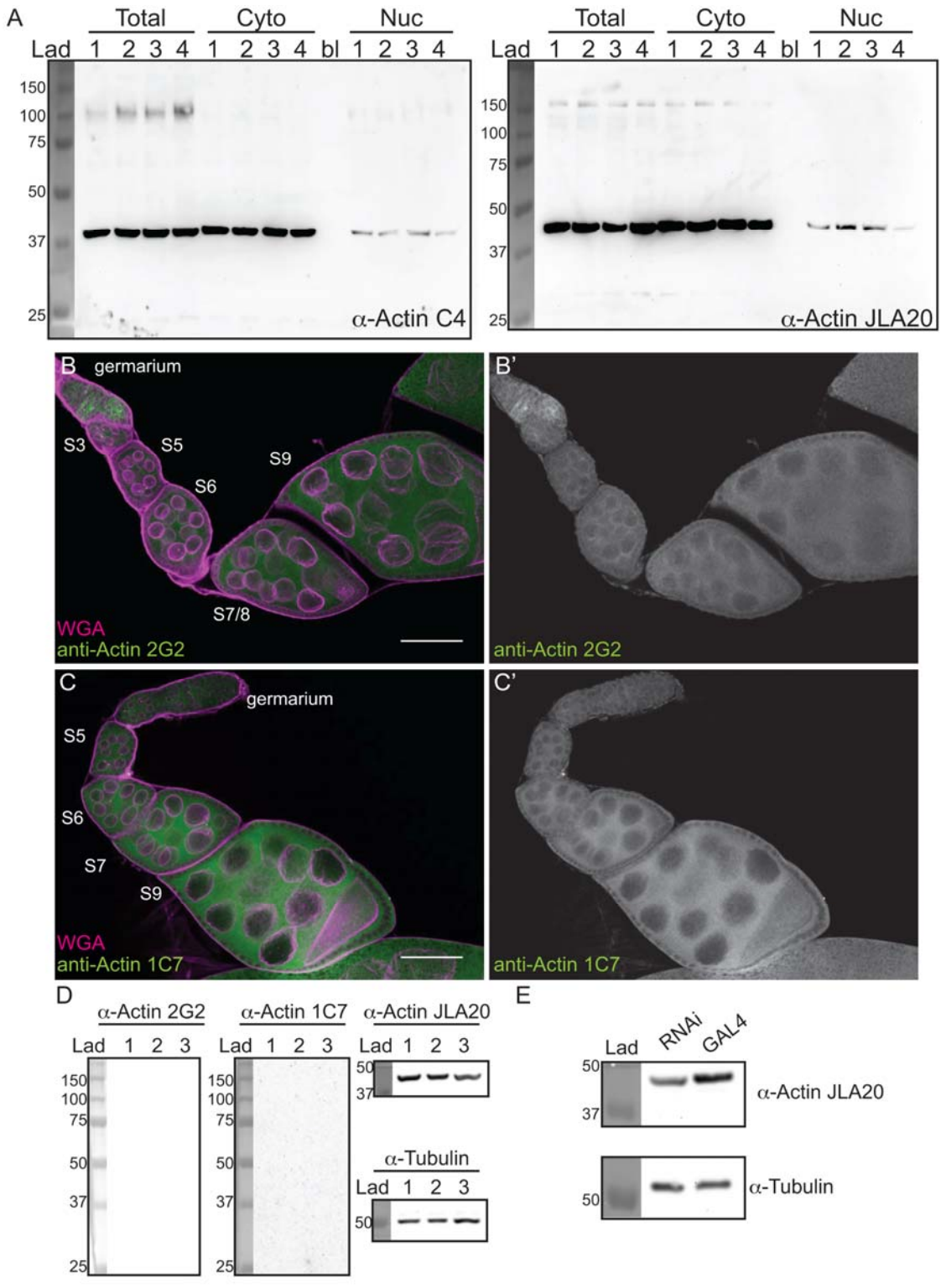
Supplemental Materials

Molecular Biology of the Cell

Kelpsch et al.



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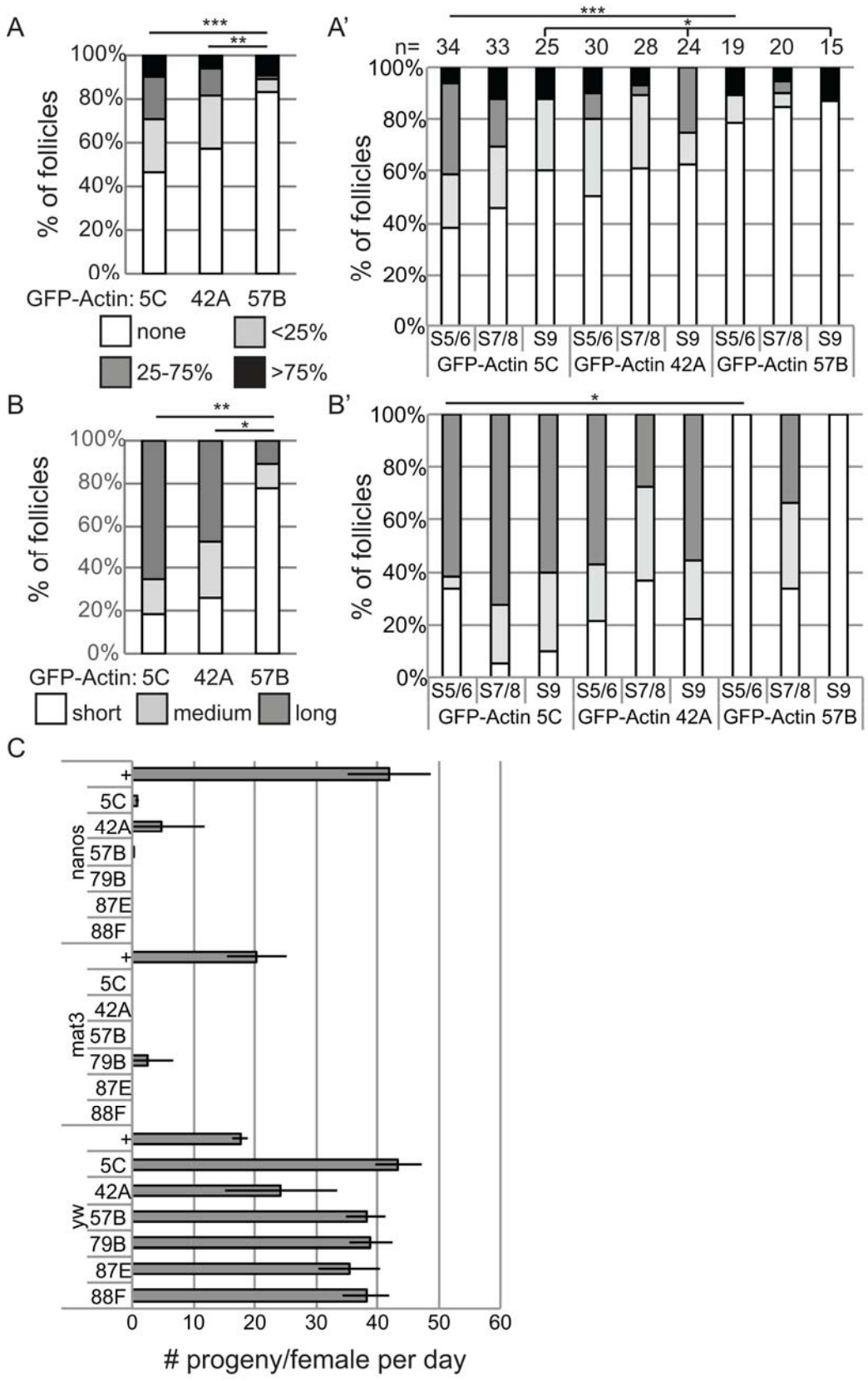
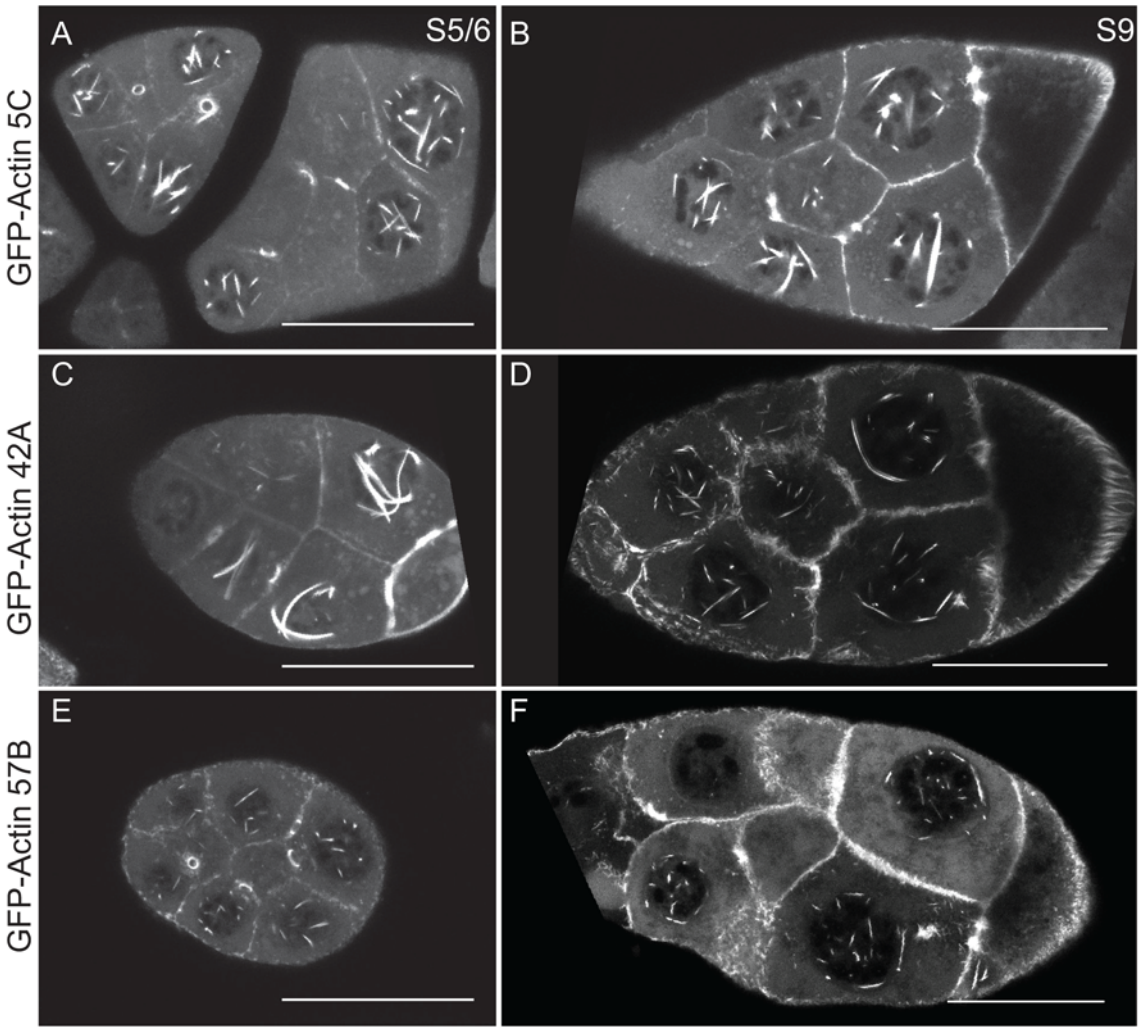
Supplemental Figure 1: Actin antibodies. A. Whole western blots of the subcellular fraction experiment shown in Figure 1E, labeled with either Anti-Actin C4 or Anti-Actin

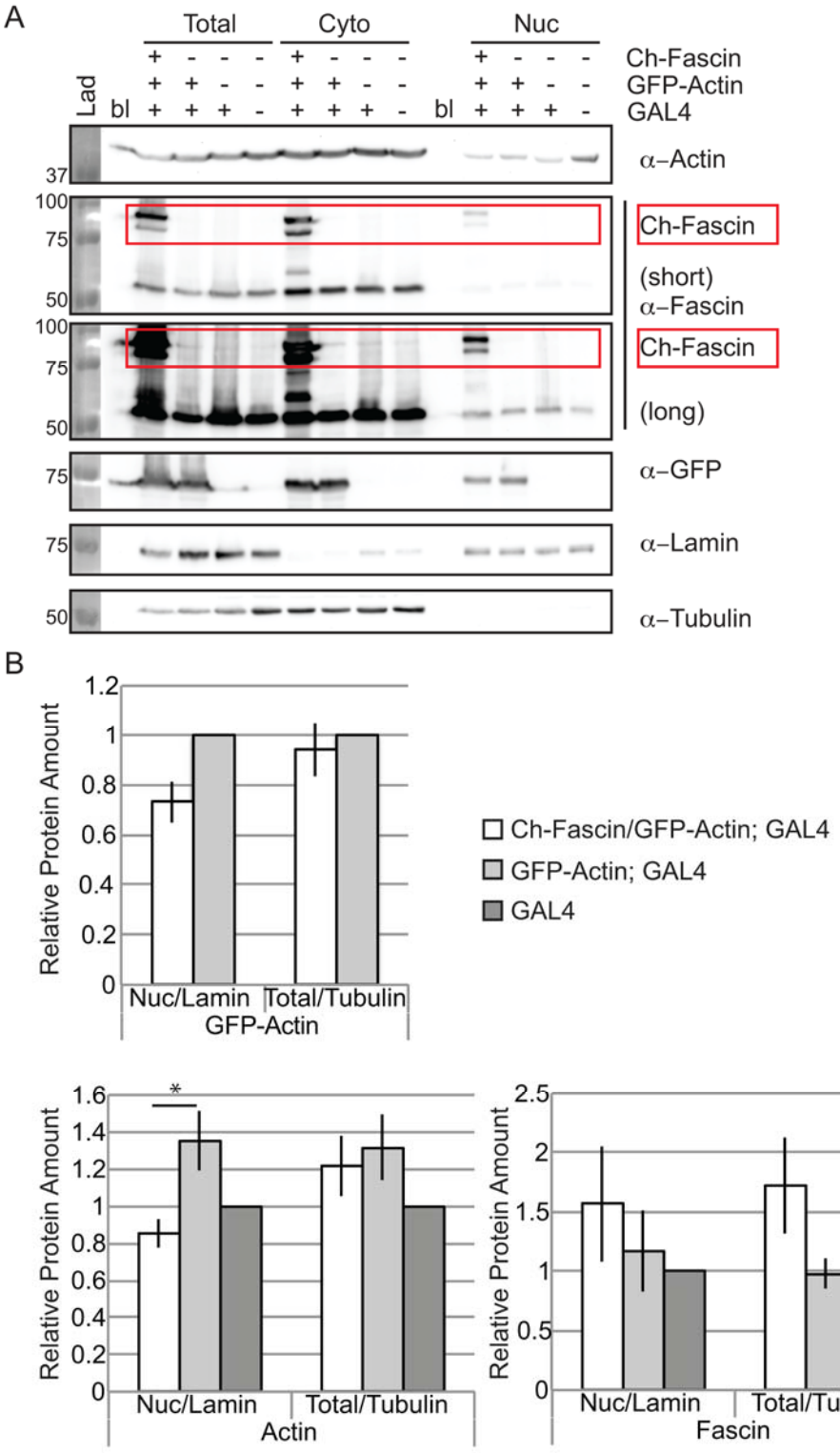
JLA20 (four wild-type samples 1-4; Lad = ladder; bl = blank lane). B-C'. Maximum projections of 2-4 confocal slices of follicles. B-C. Merged images: WGA = magenta and antibody = green. B-C'. Antibody = white. B-B'. Anti-Actin 2G2. C-C'. Anti-Actin 1C7. Scale bars = 50 μm. D. Western blots of three independent *wild-type* whole ovary lysates, labeled 1-3, blotted for anti-Actin 2G2, anti-Actin 1C7, anti-Actin JLA20 (positive actin control), and anti-α-Tubulin (loading control). Neither anti-Actin 2G2 or 1C7 recognize *Drosophila* actin by western blotting. Ladder = Lad with molecular weight markers labeled. E. Western blot of whole ovary lysates from *matGAL4; RNAi actin 5C* (labeled RNAi) and *matGAL4* (labeled GAL4) blotted for actin (JLA20) and α-Tubulin. Ladder = Lad. RNAi knockdown of *actin 5C* in the germline results in a decrease in actin levels.


A
***
**
100%
80%
60%
40%
20%
0%
% of follicles
GFP-Actin: 5C 42A 57B
none
<25%
25-75%
>75%
A'
***
*
n= 34 33 25 30 28 24 19 20 15
100%
80%
60%
40%
20%
0%
% of follicles
S5/6 S7/8 S9 S5/6 S7/8 S9 S5/6 S7/8 S9
GFP-Actin 5C
GFP-Actin 42A
GFP-Actin 57B
B
**
*
100%
80%
60%
40%
20%
0%
% of follicles
GFP-Actin: 5C 42A 57B
short
medium
long
B'
*
100%
80%
60%
40%
20%
0%
% of follicles
S5/6 S7/8 S9 S5/6 S7/8 S9 S5/6 S7/8 S9
GFP-Actin 5C
GFP-Actin 42A
GFP-Actin 57B
C
nanos
mat3
yw
+
5C
42A
57B
79B
87E
88F
0 10 20 30 40 50 60
progeny/female per day

Supplemental Figure 2: Different actins have distinct abilities to induce nuclear actin rods. A-B'. Charts of the percentage of follicles of the indicated stages and genotypes (*GFP-Actin; matGAL4)* from 5-8 day old females that exhibit particular frequencies and lengths of nuclear GFP-Actin rods. A-B. Charts combining all the stages for each GFP-Actin. A'-B'. Charts show the stage specific distribution for each GFP-Actin; n values for both the frequency and the length are indicated across the top of A'. Each follicle within a confocal stack was scored, in a genotypically-blinded manner, for the percentage of nurse cells exhibiting nuclear actin rods (A-A'; 0, ≤25%, 25-75%, or ≥75%) and the length of those rods (B-B'; short ≤ 1/4 of the nuclear diameter, medium ~1/2 diameter, or long ≥ diameter). Actin rod formation is more frequently observed in the earlier stages (S5-8) than in the later stages (S9) for GFP-Actin 5C ($p<0.01$ Fisher's Exact test, A'). Germline expression of GFP-Actin 5C and 42A exhibits a higher frequency of nuclear actin rod formation at all stages compared to GFP-Actin 57B (A-A'). The actin rods induced by both GFP-Actin 5C and 42A tend to be long, while those composed of GFP-Actin 57B are short (B-B'). ***$p<0.001$, **$p<0.01$, and *$p<0.05$ Pearson's Chi-squared test. C. Chart depicting the number of progeny of the indicated genotypes resulting from a female from a 24 hour egg-laying. + = cross to *wild-type*. Germline expression of all six GFP-Actins results in severely reduced fertility or sterility ($p<0.001$ compared to GAL4 [+] only, unpaired t-test with Bonferroni correction).

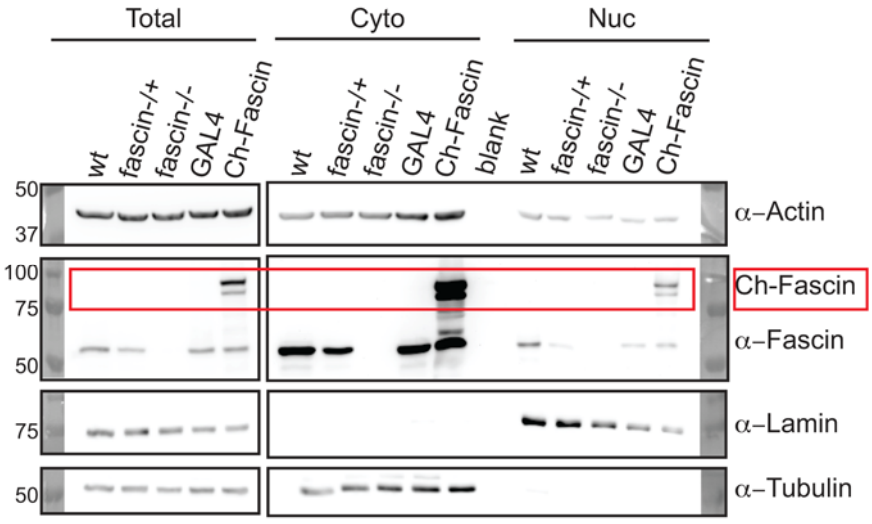


Supplemental Figure 3: Germline expression of GFP-Actin results in nuclear actin rods. A-F. Live imaging of follicles expressing the indicated GFP-Actins in the germline using *matGAL4*. Expression of GFP-Actin 5C (A-B) and 42A (C-D) results in long nuclear actin rods, while expression of GFP-Actin 57B results in short rods (E-F). Scale bar = 50 μm

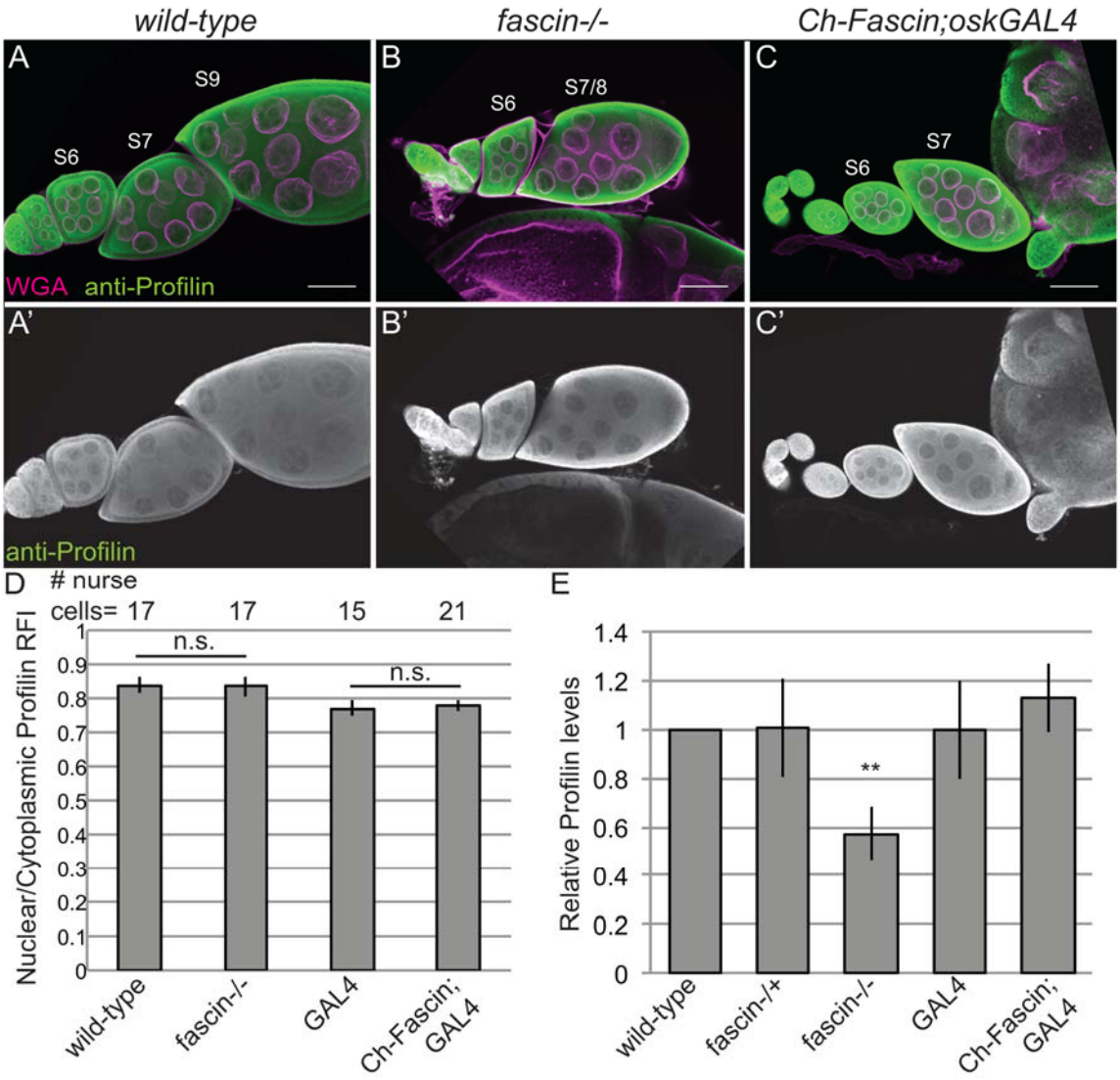


Supplemental Figure 4: Overexpressing Fascin does not increase nuclear actin, GFP-Actin or endogenous Fascin levels. A. Representative western blots of subcellular fractionation samples of whole ovary lysates from the indicated genotypes (total lysate,

cytoplasmic fraction, nuclear fraction) blotted for endogenous actin (JLA20), Fascin (two exposures; the bands representing Ch-Fascin are boxed in red), GFP-Actin, Lamin Dm0 (nuclear marker), and α -Tubulin (cytoplasmic marker). GAL4 = *oskGAL4*; bl = blank lane; Lad = ladder. B. Charts quantifying the relative protein amount of nuclear protein (Actin, endogenous Fascin, or GFP-Actin) to nuclear Lamin and total protein (Actin, Fascin, or GFP-Actin) to total Tubulin from western blots of three subcellular fractionation experiments. For Actin and Fascin values, protein amount was normalized to GAL4 only. For GFP-Actin values, protein amount was normalized to GFP-Actin; GAL4. Error bars = standard error. Overexpression of Fascin does not significantly alter GFP-Actin ($p>0.05$ compared to GFP-Actin alone, unpaired t-test with Welch's correction) or endogenous Fascin levels ($p>0.05$, Ordinary one-way ANOVA). Overexpression of Fascin slightly decreases nuclear actin levels compared to GFP-Actin (*$p=0.04$, Ordinary one-way ANOVA), but is not significantly different from the GAL4 control ($p>0.05$, Ordinary one-way ANOVA).

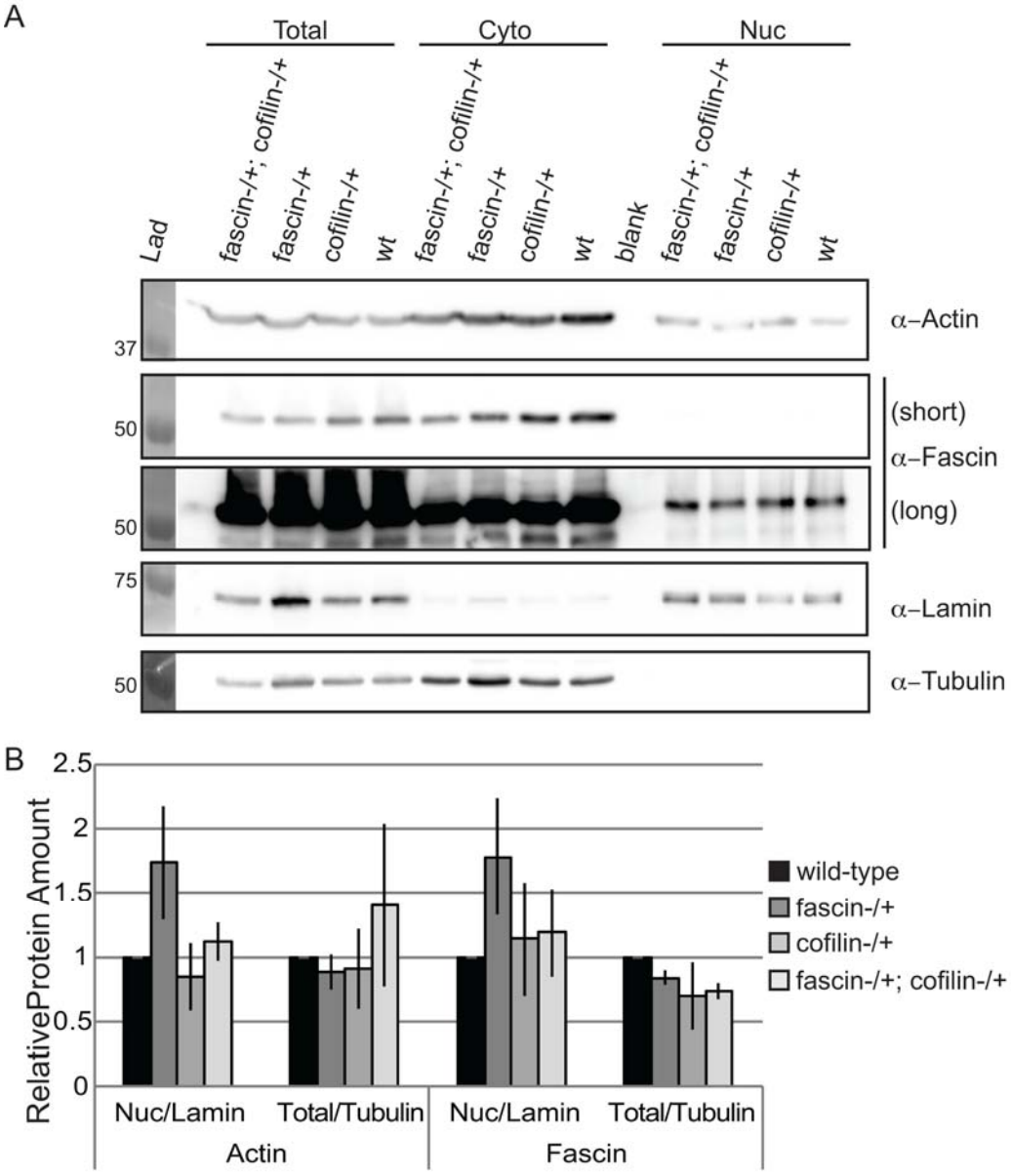


Supplemental Figure 5: Manipulating Fascin does not alter nuclear actin levels. Representative western blots of subcellular fractionation samples of whole ovary lysates of the indicated genotypes (total lysate, cytoplasmic fraction, nuclear fraction) blotted for endogenous actin (JLA20), Fascin (the bands representing Ch-Fascin are boxed in red), Lamin Dm0 (nuclear marker), and α -Tubulin (cytoplasmic marker). *fascin-=fascin*^{sn28}; GAL4 = *oskGAL4*; Ch-Fascin = *UAS Ch-Fascin/+; oskGAL4/+*.



Supplemental Figure 6: Fascin does not affect nuclear Profilin levels. A-C'. Maximum projections of 2-4 confocal slices of S5-9 follicles. A-A'. Wild-type (*yw*). B-B'. *fascin*^{sn28}*/fascin*^{sn28}. C-C'. UAS *mCherry-Fascin; oskGAL4.* A-C. Merged images: WGA = magenta and anti-Profilin (Chi 1J) = green. A'-C'. Anti-Profilin = white. Scale bars = 50 μm. D. Chart depicting the average nuclear to cytoplasmic relative fluorescence intensity of Profilin in nurse cells from S7/8 follicles; the number of nurse cells (n) examined is indicated across the top of the chart. Briefly, the fluorescence intensity along a line traversing from the cytoplasm into the nucleus from 2-3 nurse cells from a minimum of 5 different follicles from each genotype, in a genotypically-blinded manner, was analyzed as described in the Materials and Methods. Error bars = standard error.

Nuclear Profilin appears normal when either Fascin is lost or overexpressed ($p>0.05$, unpaired t-test with Welch's correction). E. Chart quantifying the relative protein amount of Profilin from western blots of whole ovary lysates of the indicated genotypes. Loss of Fascin reduced total Profilin levels compared to *wild-type* ($p<0.01$, unpaired t-test with Welch's correction).



Supplemental Figure 7: Co-reduction of Fascin and Cofilin does not alter total nuclear actin levels. A. Representative western blots of subcellular fractionation samples

of whole ovary lysates from the indicated genotypes (total lysate, cytoplasmic fraction, nuclear fraction) blotted for endogenous actin (JLA20), Fascin, Lamin Dm0 (nuclear marker), and α -Tubulin (cytoplasmic marker). Ladder = lad. B. Chart quantifying the relative protein amount of nuclear protein (Actin and Fascin) to nuclear Lamin and total protein (Actin and Fascin) to total Tubulin from western blots of three subcellular fractionation experiments. Protein amount was normalized to *wild-type*. Error bars = standard error. Nuclear and total actin levels are similar in *fascin-/+; cofilin-/+* compared to *fascin-/+*, *cofilin-/+*, and *wild-type* ($p>0.5$, Bonferroni's multiple comparisons test). Nuclear and total Fascin levels are similar between all four genotypes ($p>0.5$, Ordinary one-way ANOVA).

Supplemental Table 1: Statistical analyses of structured nuclear actin and nuclear actin rod frequency and length. A. Tables of the data represented in Figure 7 quantifying the frequency (none, ≤25%, 25-75%, or ≥75%) and length (short ≤ 1/4 of the nuclear diameter, medium ~1/2 diameter, or long ≥ diameter) of nuclear actin rods in S5/6, S7/8, and S9 of Fascin overexpressing follicles. B. Tables of the data represented in Figure 8 quantifying structured nuclear actin (haze, low, medium, or high) during S5/6, S7/8, and S9 of oogenesis from *wild-type, fascin$^{sn28/sn28}$, GAL4,* and *Ch-Fascin;GAL4* follicles and their corresponding p-values. C. Tables of the data represented in Figure 10 quantifying structured nuclear actin (haze, low, medium, or high) during S5/6, S7/8, and S9 of oogenesis from *fascin^{sn28}/ +*, *cofilin^{tsr1}/+*, and *fascin^{sn28}/ +; cofilin^{tsr1}/+* follicles and their corresponding p-values. D. Tables of the data represented in Supplemental Figure 2 quantifying the frequency (none, ≤25%, 25-75%, or ≥75%) and length (short ≤ 1/4 of the nuclear diameter, medium ~1/2 diameter, or long ≥ 1 diameter) of nuclear actin rods in S5/6, S7/8, and S9 of follicles expressing *GFP-Actin 5C, GFP-Actin 42A,* and *GFP-Actin 57,* and their corresponding p-values.

A. Figure 7.

Frequency												
	S5/6				S7/8				S9			
	None	<25%	25%-75%	>75%	None	<25%	25%-75%	>75%	None	<25%	25%-75%	>75%
Cherry-Fascin/GFP-Actin 5C	13	22	30	17	0	26	21	11	12	10	11	7
GFP-Actin 5C	21	10	5	4	19	13	0	2	13	6	5	1
Pearson's Chi-squared test	p = 0.0001525				$p = 2.67x10^{-10}$				p = 0.206			

Length									
	S5/6			S7/8			S9		
	Short	Medium	Long	Short	Medium	Long	Short	Medium	Long
Cherry-Fascin/GFP-Actin 5C	5	4	60	0	11	47	3	3	23
GFP-Actin 5C	7	4	8	8	1	6	2	6	4
Pearson's Chi-squared test	p = 0.0001785			$p = 2.783x10^{-8}$			p = 0.01066		

B. Figure 8.

Stage analysis of *wild-type*				
	Haze	Low	Medium	High
S5/6	341	110	93	100
S7/8	415	28	37	0
S9	348	12	0	0
Pearson's Chi-squared test	$p < 2.2x10^{-16}$			

Stage analysis of *wild-type*			
p-value	S5/6	S7/8	S9
S5/6		$9.03x10^{-42}$	$1.34x10^{-57}$
S7/8			$4.36x10^{-10}$
S9			

Endogenous nuclear actin quantification												
	S5/6				S7/8				S9			
	Haze	Low	Medium	High	Haze	Low	Medium	High	Haze	Low	Medium	High
wild-type	341	110	93	100	415	28	37	0	348	12	0	0
fascin$^{-/-}$	439	115	44	17	359	13	1	2	267	3	0	0
Pearson's Chi-squared test	$p < 2.2x10^{-16}$				$p = 2.85x10^{-7}$				NA			

Endogenous nuclear actin quantification												
	S5/6				S7/8				S9			
	Haze	Low	Medium	High	Haze	Low	Medium	High	Haze	Low	Medium	High
GAL4	275	137	69	164	338	58	41	28	414	30	6	0
Cherry-Fascin; GAL4	55	296	58	86	234	53	26	47	305	34	6	0
Pearson's Chi-squared test	$p < 2.2x10^{-16}$				p = 0.00268				NA			

Endogenous nuclear actin quantification												
	S5/6				S7/8				S9			
	Haze	Low	Medium	High	Haze	Low	Medium	High	Haze	Low	Medium	High
wild-type	341	110	93	100	415	28	37	0	348	12	0	0
GAL4	275	137	69	164	338	58	41	28	414	30	6	0
Pearson's Chi-squared test	$p < 2.141x10^{-6}$				$p = 4.855x10^{-10}$				NA			

C. Figure 10.

S5/6				
	Haze	Low	Medium	High
fascin$^{-/+}$	462	212	41	140
cofilin$^{-/+}$	628	74	65	148
fascin$^{-/+}$*; cofilin*$^{-/+}$	652	20	51	27
Pearson's Chi-squared test	$p < 2.2x10^{-16}$			

S5/6			
p-value	*fascin*$^{-/+}$	*cofilin*$^{-/+}$	*fascin*$^{-/+}$*; cofilin*$^{-/+}$
fascin$^{-/+}$		$4.20957x10^{-21}$	$2.34808x10^{-63}$
cofilin$^{-/+}$			$1.39979x10^{-23}$
fascin$^{-/+}$*; cofilin*$^{-/+}$			

S7/8				
	Haze	Low	Medium	High
fascin$^{-/+}$	460	47	62	1
cofilin$^{-/+}$	377	54	60	4
fascin$^{-/+}$*; cofilin*$^{-/+}$	480	27	3	0
Pearson's Chi-squared test	$p < 9.599x10^{-15}$			

S7/8			
p-value	*fascin*$^{-/+}$	*cofilin*$^{-/+}$	*fascin*$^{-/+}$*; cofilin*$^{-/+}$
fascin$^{-/+}$		0.1575728	$4.31129x10^{-15}$
cofilin$^{-/+}$			$6.62606x10^{-19}$
fascin$^{-/+}$*; cofilin*$^{-/+}$			

S9				
	Haze	Low	Medium	High
fascin$^{-/+}$	331	27	2	0
cofilin$^{-/+}$	336	30	1	8
fascin$^{-/+}$*; cofilin*$^{-/+}$	329	1	0	0
Pearson's Chi-squared test	$p < 1.858x10^{-7}$			

S9			
p-value	*fascin*$^{-/+}$	*cofilin*$^{-/+}$	*fascin*$^{-/+}$; *cofilin*$^{-/+}$
fascin$^{-/+}$		0.02011352	1.91627×10^{-7}
cofilin$^{-/+}$			4.86282×10^{-9}
fascin$^{-/+}$; *cofilin*$^{-/+}$			

D. Supplemental Figure 2.

Frequency (S5-S9)				
	None	<25%	25%-75%	>75%
GFP-Actin 5C; GAL4	43	22	18	9
GFP-Actin 42A; GAL4	47	20	10	5
GFP-Actin 57B; GAL4	45	3	1	5
Pearson's Chi-squared test	p = 0.000482			

Frequency (S5-S9)			
p-value	*GFP-Actin 5C; GAL4*	*GFP-Actin 42A; GAL4*	*GFP-Actin 57B; GAL4*
GFP-Actin 5C; GAL4		0.3753682	0.00004689257
GFP-Actin 42A; GAL4			0.001512837
GFP-Actin 57B; GAL4			

Length (S5-S9)			
	Short	Medium	Long
GFP-Actin 5C; GAL4	9	8	32
GFP-Actin 42A; GAL4	9	9	16
GFP-Actin 57B; GAL4	7	1	1
Pearson's Chi-squared test	p = 0.005404		

Length (S5-S9)			
p-value	*GFP-Actin 5C; GAL4*	*GFP-Actin 42A; GAL4*	*GFP-Actin 57B; GAL4*
GFP-Actin 5C; GAL4		0.269721252	0.002741905
GFP-Actin 42A; GAL4			0.034147688
GFP-Actin 57B; GAL4			

S5/6 Frequency				
	None	<25%	25%-75%	>75%
GFP-Actin 5C; GAL4	13	7	12	2
GFP-Actin 42A; GAL4	15	9	3	3
GFP-Actin 57B; GAL4	15	2	0	2
Pearson's Chi-squared test	p = 0.01114			

S5/6 Frequency			
p-value	*GFP-Actin 5C; GAL4*	*GFP-Actin 42A; GAL4*	*GFP-Actin 57B; GAL4*
GFP-Actin 5C; GAL4		0.165578606	0.008259774
GFP-Actin 42A; GAL4			0.165578606
GFP-Actin 57B; GAL4			

S7/8 Frequency				
	None	<25%	25%-75%	>75%
GFP-Actin 5C; GAL4	15	8	6	4
GFP-Actin 42A; GAL4	17	8	1	2
GFP-Actin 57B; GAL4	17	1	1	1
Pearson's Chi-squared test	p = 0.07829			

S9 Frequency				
	None	<25%	25%-75%	>75%
GFP-Actin 5C; GAL4	15	7	0	3
GFP-Actin 42A; GAL4	15	3	6	0
GFP-Actin 57B; GAL4	13	0	0	2
Pearson's Chi-squared test	p = 0.004025			

S9 Frequency			
p-value	*GFP-Actin 5C; GAL4*	*GFP-Actin 42A; GAL4*	*GFP-Actin 57B; GAL4*
GFP-Actin 5C; GAL4		0.01675403	0.06322224
GFP-Actin 42A; GAL4			0.01675403
GFP-Actin 57B; GAL4			

S5/6 Length			
	Short	Medium	Long
GFP-Actin 5C; GAL4	7	1	13
GFP-Actin 42A; GAL4	3	3	8
GFP-Actin 57B; GAL4	4	0	0
Pearson's Chi-squared test	p = 0.03143		

S5/6 Length			
p-value	*GFP-Actin 5C; GAL4*	*GFP-Actin 42A; GAL4*	*GFP-Actin 57B; GAL4*
GFP-Actin 5C; GAL4		0.45466065	0.0926087
GFP-Actin 42A; GAL4			0.04901961
GFP-Actin 57B; GAL4			

S7/8 Length			
	Short	Medium	Long
GFP-Actin 5C; GAL4	1	4	13
GFP-Actin 42A; GAL4	4	4	3
GFP-Actin 57B; GAL4	1	1	1
Pearson's Chi-squared test	p = 0.1262		

S9 Length			
	Short	Medium	Long
GFP-Actin 5C; GAL4	1	3	6
GFP-Actin 42A; GAL4	2	2	5
GFP-Actin 57B; GAL4	2	0	0
Pearson's Chi-squared test	p = 0.1113		