Lewellyn et al., http://www.jcb.org/cgi/content/full/jcb.201209129/DC1

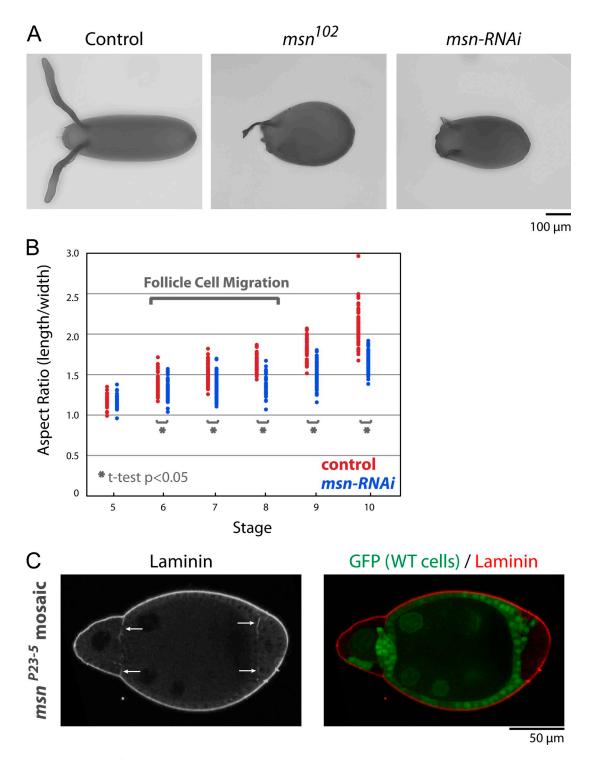


Figure S1. **Misshapen** is **required for egg chamber elongation.** (A) Expression of *msn-RNAi* in the follicle cells produces round eggs, similar to follicle cell mosaics of a published allele, *msn*¹⁰². The wild-type image has been replicated from Horne-Badovinac et al. (2012). (B) Aspect ratios for individual control and *msn-RNAi* egg chambers are plotted for each developmental stage. *msn-RNAi* egg chambers are significantly rounder than controls starting at stage 6. These data are from a single experiment that involved dissection of >12 flies per condition, with *n* = 35–80 egg chambers for each condition at each developmental stage. (C) Transverse optical section of an *msn* mosaic egg chamber that has mutant clones (indicated by lack of GFP) at the anterior and posterior poles, causing wild-type follicle cells to invade the germ cells. Laminin staining shows that invading wild-type cells maintain contact with a BM (arrows). Bar measurements are indicated in the figure.

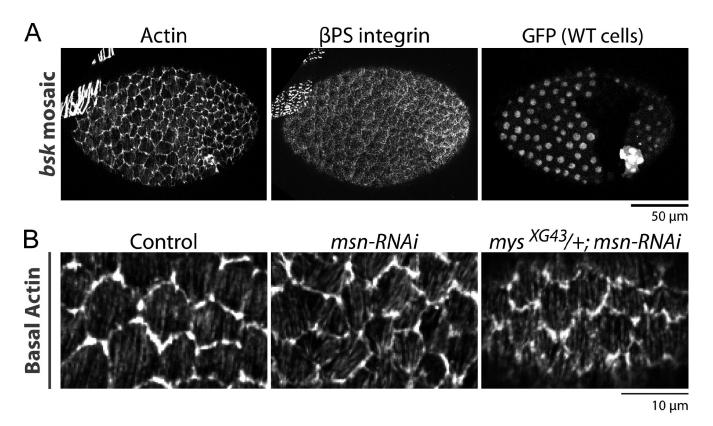
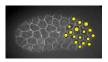


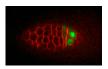
Figure S2. Msn's effect on integrin levels is JNK independent, and reducing integrin levels rescues the basal actin organization defect in msn-RNAi. (A) A basal view of a Df(bsk)^{f(p)147E} mosaic epithelium stained for the β -PS integrin reveals that defects in JNK signaling do not affect integrin levels. (B) Reducing integrin levels in msn-RNAi epithelia restores the planar organization of the basal actin filaments (only 5% show disruptions in basal actin organization compared with 20% in msn-RNAi alone, n = 20 for control and msn-RNAi. n = 22 for mys^{XG43}/+; msn-RNAi).



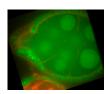
Video 1. **Msn promotes follicle cell migration.** Live imaging of control and *msn-RNAi* egg chambers (Fig. 1 C). Samples were analyzed by time-lapse microscopy using a spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a spinning disk unit (model CSU-10; Yokogawa Corporation of America). Cell membranes are labeled with FM4-64. Frames were taken every 30 s for 22.5 min. Playback rate is 300x real time.



Video 2. A large msn mutant clone blocks global follicle cell migration. msn¹⁷² cells (yellow dots) are stationary, while neighboring wild-type cells attempt to move past them (Fig. 1 D). The msn mosaic egg chamber was analyzed by time-lapse microscopy using a spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a spinning disk unit (model CSU-10; Yokogawa Corporation of America). Cell membranes are labeled with FM4-64. Frames were taken every 30 s for 61 min. Playback rate is 300x real time.



Video 3. *msn-RNAi* mosaic egg chambers exhibit a cell-autonomous defect in follicle cell migration. The cells in an *msn-RNAi* flipout clone (GFP) move very little while neighboring wild-type cells efficiently migrate past them (Fig. 1 F). The mosaic egg chamber was analyzed by time-lapse microscopy using a spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a spinning disk unit (model CSU-10; Yokogawa Corporation of America). Cell membranes are labeled with FM4-64 (red). Frames were taken every 30 s for 32 min. Playback rate is 300× real time.



Video 4. The presence of an msn¹⁷² mutant clone at the anterior leads to invasion of neighboring wild-type cells (GFP) into the germ cell cluster. The time-lapse begins at a transverse section, then goes up to a more basal plane before returning back to the transverse section (Fig. 2, D and E). The mosaic egg chamber was analyzed by time-lapse microscopy using a spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a spinning disk unit (model CSU-10; Yokogawa Corporation of America). Cell membranes are labeled with FM4-64 (red). Frames were taken every 30 s for 90 min. Playback rate is 300x real time.



Video 5. "Tails" form at the back of individual msn-RNAi cells in a small clone (mCD8-GFP, Fig. 5 B). An egg chamber containing a clone of cells expressing UAS-msn-RNAi and UAS-mCD8-GFP was analyzed by time-lapse microscopy using a spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a spinning disk unit (model CSU-10; Yokogawa Corporation of America). Frames were taken every 30 s for 28 min. Playback rate is 300× real time.



Video 6. "Tails" can retract as the msn-RNAi follicle cells migrate forward (mCD8-GFP, Fig. 5 C). An egg chamber containing a clone of cells expressing UAS-msn-RNAi and UAS-mCD8-GFP was analyzed by time-lapse microscopy using a spinning disk confocal microscope (Axiovert 200M; Carl Zeiss) with a spinning disk unit (model CSU-10; Yokogawa Corporation of America). Frames were taken every 30 s for 60 min. Playback rate is 300× real time.

Table S1. Experimental genotypes

Figure	Panel	Genotype		
1	C and D	w ¹¹¹⁸ / +; TubP-GAL80 ^{ts} / +; TubP-GAL4/ + TubP-GAL80 ^{ts} / UAS-msn-RNAi; TubP-GAL4/ +		
1	E	e22c-GAL4, UAS-FLP/ +; msn ¹⁷² , FRT80/ ubi-eGFP, FRT80		
1	F	hsFLP/ +; UAS-msn-RNAi/ +; act5c>>GAL4, UAS-GFP/ +		
2	Α	e22c-GAL4, UAS-FLP/ +; msn ¹⁷² , FRT80/ ubi-eGFP, FRT80 (top, no clones) e22c-GAL4, UAS-FLP/ +; msn ²²³⁻⁵ , FRT80/ ubi-eGFP, FRT80 (bottom, clone)		
2	В	e22c-GAL4, UAS-FLP/ +; msn ¹⁰² , FRT80/ ubi-eGFP, FRT80		
2	С	C306-GAL4/+; UAS-msn-RNAi/+; UAS-mCD8-GFP/+		
2	D and E	e22c-GAL4, UAS-FLP /+; msn ¹⁷² , FRT80 / ubi-eGFP, FRT80		
3	Α	e22c-GAL4, UAS-FLP/ +; msn ¹⁰² , FRT80/ ubi-eGFP, FRT80 (top) e22c-GAL4, UAS-FLP/ +; msn ¹⁷² , FRT80/ ubi-eGFP, FRT80 (bottom)		
3	В	hsFLP/ +;; UAS-HA-myr-msn/ act5c>>GAL4, UAS-GFP		
3	С	w ¹¹¹⁸ / +; tj-Gal4, vkg-GFP/ + (top left) vkg-GFP/ e22c-GAl4, UAS-FLP; msn ¹⁰² , FRT80/ hs-piMyc, FRT80 (top right) tj-Gal4, vkg-GFP/ UAS-msn-RNAi (bottom left) mys ^{XG43} /+; tj-GAL4, vkg-GFP/ UAS-msn-RNAi (bottom right)		
3	D	w ¹¹¹⁸ / +; tj-Gal4/ + mys ^{XG43} / +; tj-GAL4/+ tj-Gal4/ UAS-msn-RNAi mys ^{XG43} / +; tj-GAL4/ UAS-msn-RNAi tj-Gal4/UAS-msn		
4	A and B	e22c-GAL4, UAS-FLP/+; msn-YFP, FRT80/ FRT80		
1	С	w ¹¹¹⁸ (left) e22c-GAL4, UAS-FLP/ +; msn ¹⁰² , FRT80/ ubi-eGFP, FRT80 (right)		
5	Α	w ¹¹¹⁸ / hsFLP;; act5c>>GAL4, UAS-GFP/+ hsFLP/ +; UAS-msn-RNAi/ +; act5c>>GAL4, UAS-GFP/ +		
5	B and C	hsFLP/+; UAS-msn-RNAi/+; act5c>>GAL4, UAS-GFP/ UAS-mCD8-GFP		
5	D	hsFLP/+; UAS-msn-RNAi/+; act5c>>GAL4, UAS-RFP/ talin-GFP		
S1	А	w ¹¹¹⁸ /+; TubP-GAL80*/+; TubP-GAL4/+ e22c-GAL4, UAS-FLP/+; msn ¹⁰² , FRT80/ ubi-eGFP, FRT80 TubP-GAL80*/ UAS-msn-RNAi; TubP-GAL4/+		
S1	В	w ¹¹¹⁸ /+; TubP-GAL80 ^s /+; TubP-GAL4/+ TubP-GAL80 ^s / UAS-msn-RNAi; TubP-GAL4/+		
S1	С	e22c-GAL4, UAS-FLP/+; msn ^{P23.5} , FRT80/ ubi-eGFP, FRT80		
52	Α	y,w, hsFLP/+; Df(2L)flp147E, FRT40A / ubi-GFP, FRT40A (Df removes bsk)		
S2	В	w ¹¹¹⁸ / +; tj-Gal4/ + tj-Gal4/ UAS-msn-RNAi mys ^{XG43} / +; tj-GAL4/ UAS-msn-RNAi		

Table S2. Conditions for transgene expression

	0 1			
Figure	Panel	Temp at which cross was raised	Temp females on yeast	Time females on yeast
		°C	°C	h
	C and D	18	29	68–72
}	C and D	18	25	24–29
51	A and B	18	29	68–72
52	В	18	25	26.5