Xue and Sokac, http://dx.doi.org/10.1083/jcb.201608025

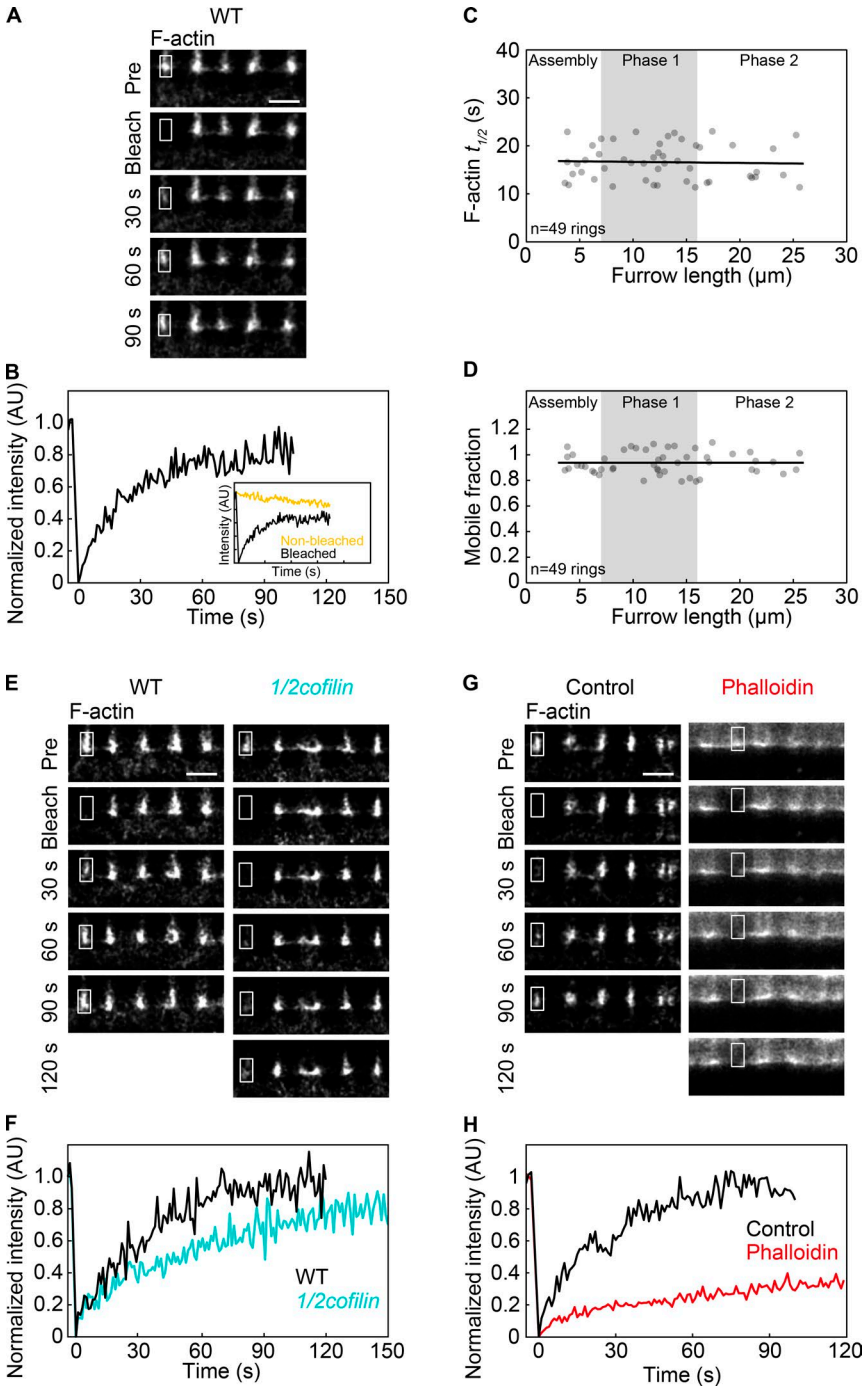


Figure S1. **F-actin disassembly is reduced in *1/2cofilin* and phalloidin-injected embryos.** (A) F-actin (G-actin^{Red}) in a photobleached ring in cross section in a wild-type (WT) embryo. (B) Representative normalized curve from a FRAP experiment in a wild-type embryo. Inset shows raw data from nonbleached (yellow) and bleached (black) rings. (C and D) $t_{1/2}$ (C) and percent mobile fraction (D) of F-actin in rings versus furrow length; each data point represents one FRAP experiment for a ring in cross section. Solid lines are linear fits; n = 49 rings from 30 embryos. Gray shading highlights phase 1 in wild-type embryos. (E and G) F-actin (G-actin^{Red}) in photobleached rings in cross section in wild-type, *1/2cofilin*, DMSO- (control), and phalloidin-injected embryos. (F and H) A representative normalized curve from one FRAP experiment in one wild-type (black in F), *1/2cofilin* (turquoise), control (black in H), or phalloidin-injected embryo (red). The mean ± SE for $n \geq 7$ rings from seven or more embryos per condition is shown in Fig. 4 (F and L). For A, E and G, box is the bleached region of interest; Pre, immediate prebleached frame; bleach, immediate post-bleach frame; seconds after bleaching. Bars, 5 µm. For compiled FRAP results, see Table S1. AU, arbitrary units.

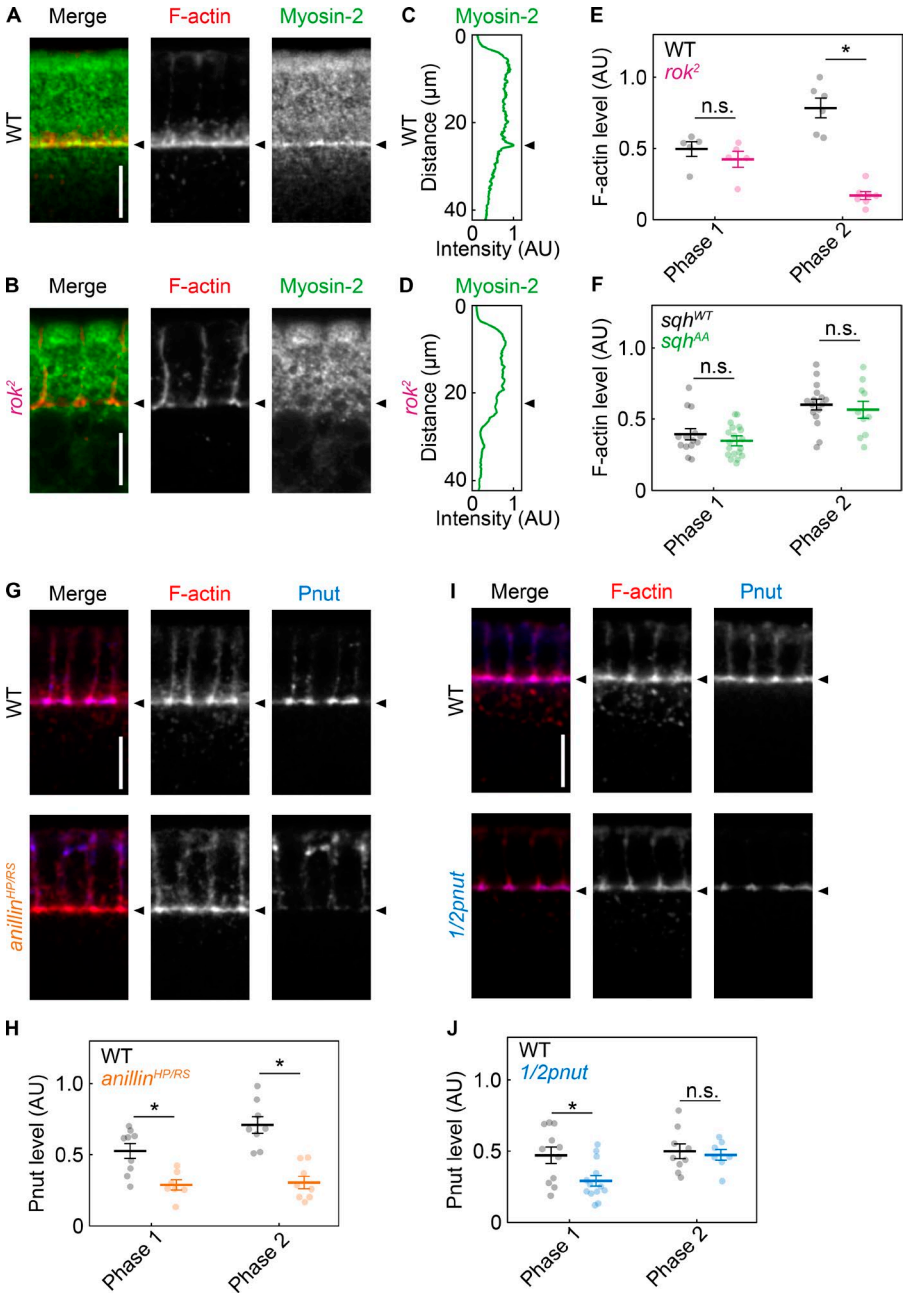


Figure S2. **Ring components are disrupted in *rok*^{2}, *anillin*$^{HP/RS}$ and *1/2pnut* embryos.** (A and B) F-actin (phalloidin; red) and Myosin-2 (green) in rings in cross section in fixed wild-type (WT) and *rok*^{2} embryos. (C and D) Distance from top of the single corresponding wild-type or *rok*^{2} image in A or B versus fluorescence intensity of Myosin-2 (green) in arbitrary units (AU). Result repeated for images from 10 out of 10 wild-type embryos and 9 out of 9 *rok*^{2} embryos. Arrowheads show ring position at furrow tips. (E and F) Fluorescence intensity of F-actin (phalloidin) in arbitrary units (AU) in phase 1 and 2 for fixed *rok*^{2} mutants (pink; E) and *sqh*^{AA} mutants (green; F) compared with wild type (black). (G) F-actin (phalloidin; red) and Pnut (blue) in rings in cross section in fixed wild-type and *anillin*$^{HP/RS}$ embryos. (H) Fluorescence intensity of Pnut in AU in phases 1 and 2 for fixed *anillin*$^{HP/RS}$ mutants (orange) compared with wild ype (black). (I) F-actin (phalloidin; red) and Pnut (blue) in rings in cross section in fixed wild-type and *1/2pnut* embryos. (J) Fluorescence intensity of Pnut AU in phases 1 and 2 for fixed *1/2pnut* embryos (blue) compared with wild type (black). (A, B, G and I) Arrowheads show ring position at furrow tips. Bars, 10 µm. (E, F, H and J) Each data point represents the mean of $n \geq 8$ rings analyzed in one embryo. Small horizontal bars show mean ± SE; *, $P < 0.05$; n.s., not significant.

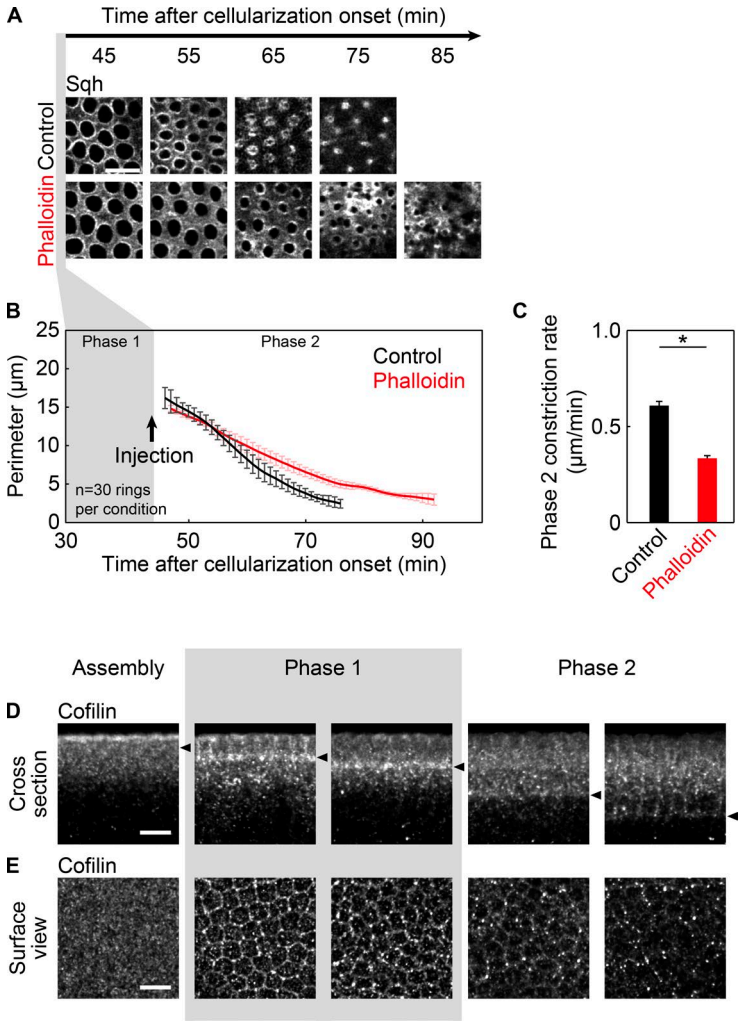
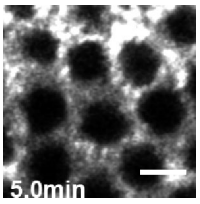


Figure S3. **F-actin disassembly is required for normal ring constriction in phase 2.** (A–C) Live DMSO (control; black) and phalloidin injected (phalloidin; red) embryos. (A) Rings (Sqh-GFP) constricting over time (minutes). (B) Ring perimeter versus time; (C) Constriction rate in phase 2. (D and E) Fixed wild-type embryos. (D) Rings stained for Cofilin in cross section or (E) surface views at furrow lengths of 3, 7, 10, 20 and 27 μm (left to right). Arrowheads show ring position at furrow tips. Bars, 10 μm. (A and B) Gray shading highlights phase 1 in wild-type embryos. (B) Arrow indicates injection time. (B and C) n = 6 embryos per condition, five rings per embryo; mean ± SE. *, P < 0.05.



Video 1. **Ring constriction in a wild-type embryo visualized by injection of G-actin^{Red}.** Time stamp indicates time after cellularization onset. Bar, 10 μm.

Table S1. **Compiled FRAP data**

Genotype or condition	Ring number	Embryo number	$t_{1/2}$	Mobile fraction
			s	%
OreR	49	30	16.6 ± 0.5	87.5 ± 1.1
sqh^{WT}	20	7	17.2 ± 1.2	89.7 ± 3.1
sqh^{AA}	10	4	16.4 ± 2.0	84.2 ± 2.9
rok^{2}	26	12	15.0 ± 0.9	65.8 ± 3.3[a]
1/2cofilin	26	13	26.0 ± 1.3[a]	80.4 ± 2.2[a]
DMSO injected	9	9	17.7 ± 1.6	89.3 ± 3.3
Phalloidin injected	7	7	–	35.8 ± 2.9[a]
anillin$^{HP/RS}$	35	14	16.8 ± 0.6	80.3 ± 1.8[a]
1/2pnut	16	6	17.0 ± 0.8	83.7 ± 2.6

Values are mean ± SE.
[a]$P < 0.05$ when compared with wild type or relevant control, as specified in the text.

Table S2. **Staining conditions for antibodies and probes**

Antibody or probe	Dilution or concentration	Staining condition
Mouse anti-Pnut (DSHB)	1:5	2 h, room temperature
Rabbit anti-Myosin-2 (*Drosophila* Zipper; Sokac and Wieschaus, 2008)	1:1,000	2 h, room temperature
Rabbit anti-Mbs/MYPT1 (Ong et al., 2010)	1:1,000	Overnight, 4°C
Rabbit anti-GFP (Abcam)	1:1,000	Overnight, 4°C
Rabbit anti-Cofilin (Signalway Antibody)	1:500	Overnight, 4°C
Phalloidin–Alexa Fluor 546 (Invitrogen)	5 U/ml	Overnight, 4°C
Goat anti–rabbit Alexa Fluor 488 (Invitrogen)	1:500	1 h, room temperature
Goat anti–mouse Alexa Fluor 488 (Invitrogen)	1:500	1 h, room temperature

References

Ong, S., C. Foote, and C. Tan. 2010. Mutations of DMYPT cause over constriction of contractile rings and ring canals during *Drosophila* germline cyst formation. *Dev. Biol.* 346:161–169. http://dx.doi.org/10.1016/j.ydbio.2010.06.008

Sokac, A.M., and E. Wieschaus. 2008. Local actin-dependent endocytosis is zygotically controlled to initiate *Drosophila* cellularization. *Dev. Cell.* 14:775–786. http://dx.doi.org/10.1016/j.devcel.2008.02.014