Mason et al., http://www.jcb.org/cgi/content/full/jcb.201603077/DC1

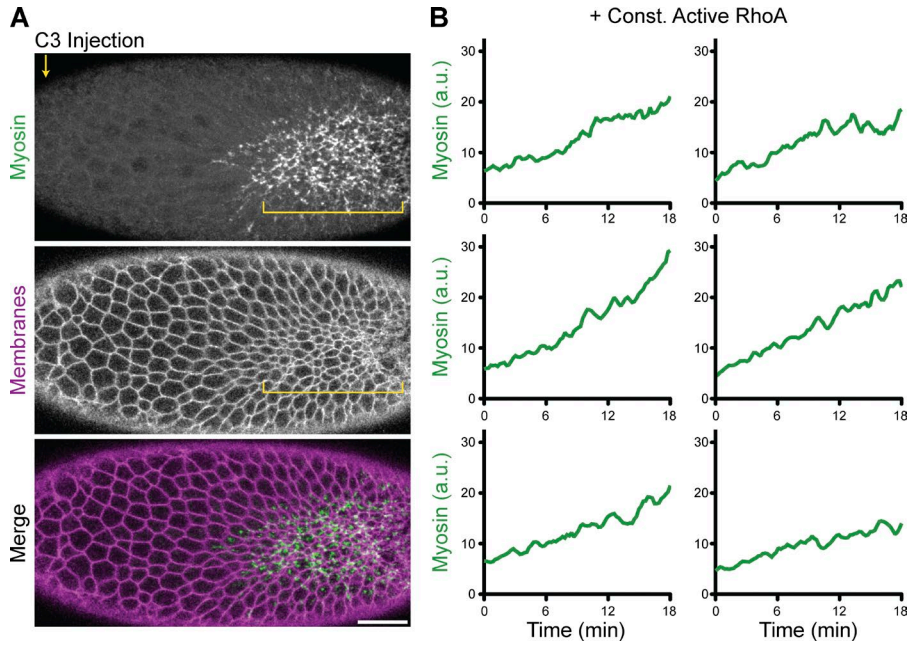


Figure S1. **RhoA activity is required to maintain apical myosin.** (A) Live image of an embryo expressing myosin::GFP and membrane::RFP, and C3 is injected at one pole (arrow) after ventral furrow has started to form (bracket at right), which causes loss of apical myosin. (B) Quantification of myosin accumulation from six individual cells expressing CA-RhoA. Myosin gradually accumulates and lacks discrete pulses. Bar, 20 µm.

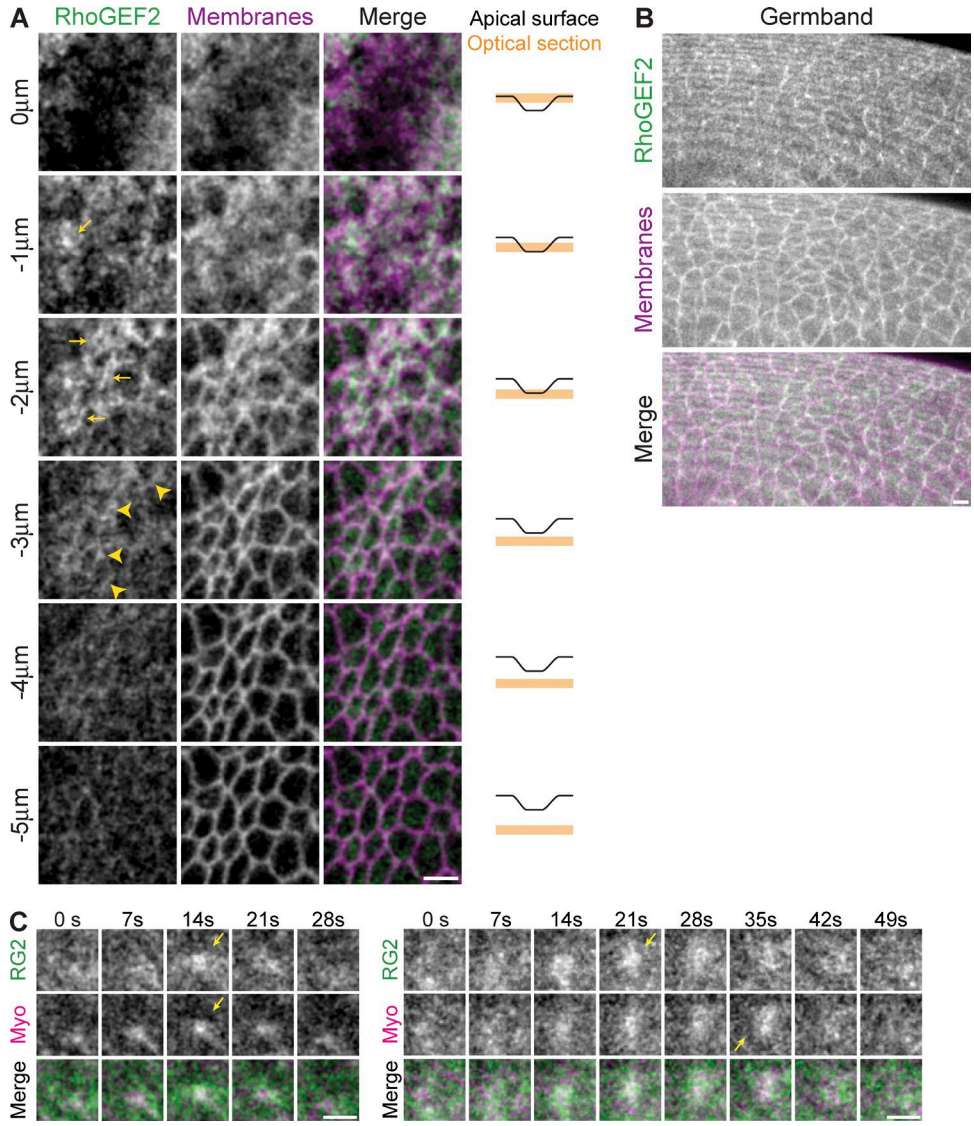


Figure S2. **GFP::RhoGEF2 forms structures across the apical surface, localizes subapically to junctions, and is planar polarized in the lateral (germband) cells.** (A) Sequential z-series from a live embryo expressing GFP::RhoGEF2 and membrane::RFP, which demonstrates that the most apical RhoGEF2 signal is medioapical, and junctional RhoGEF2 is slightly subapical. (B) Live image from embryo expressing GFP::RhoGEF2 and membrane::RFP in the lateral, germband cells. RhoGEF2 is planar polarized. (C) Live images from an embryo expressing GFP::RhoGEF2 and myosin::RFP. RhoGEF2 and myosin pulse behavior is heterogeneous, with pulses co-occurring (arrows at left) or with RhoGEF2 pulses preceding myosin pulses (arrows at right). Bars: (A and B) 5 µm; (C) 2.5 µm.

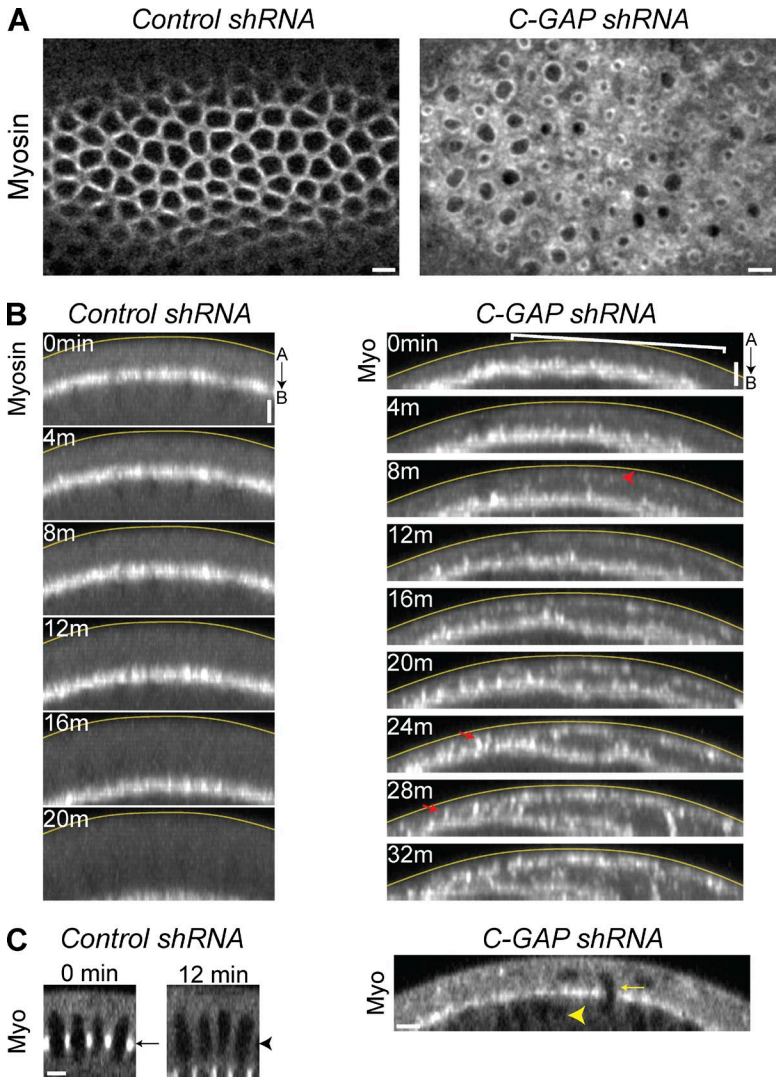


Figure S3. **C-GAP is required for cellularization, which precedes ventral furrow formation.** (A) Surface view from live *Control* and *C-GAP shRNA* embryos expressing myosin::GFP during cellularization. In *control shRNA*, myosin localizes in uniform structures or cables between cells as the cellularization proceeds. In severe *C-GAP shRNA* embryos, cellularization is perturbed as furrow canals ectopically constrict are heterogeneous. (B) Cross section views from live *control* and *C-GAP shRNA* embryos expressing myosin::GFP. Over time, the myosin at the cellularization front moves basally in *control shRNA* embryos. In *C-GAP shRNA* embryos, myosin in the cellularization front does not move basally, and apical myosin (arrowhead) appears in the ventral furrow (white bracket) despite halted cellularization. At later time points, myosin appears in more lateral tissues (arrows). (C) Cross section views from live *control* and *C-GAP shRNA* embryos expressing myosin::GFP. In *control shRNA* embryos, myosin (arrow) moves between the nuclei (arrowhead), and as cellularization proceeds, nuclei remain elongated and apical to basal myosin. In *C-GAP shRNA* embryos, nuclei are squeezed by the basal myosin (arrow) and some nuclei are pushed into the yolk, remaining basal to the cellularization front (arrowhead). Bars, 5 µm.

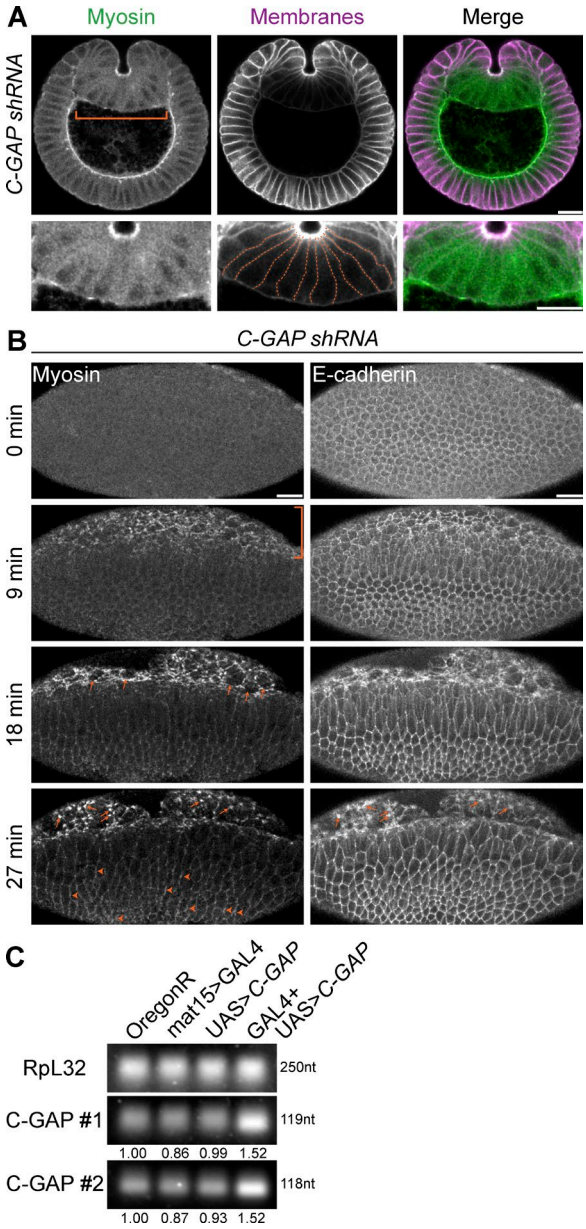
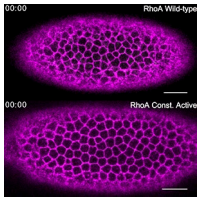
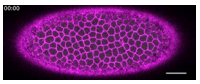


Figure S4. ***C-GAP shRNA* embryos phenotypes appear to be ventral specific.** (A) In *C-GAP shRNA* embryos, ventral furrow cells lose basal myosin (top panel, bracket) during invagination. During ventral furrow formation, loss of basal myosin is thought to be required for basal expansion and tissue invagination, and this is normal in *C-GAP shRNA* embryos (bracket, top). This result, combined with our other data, suggests C-GAP phenotypes are caused by defects specifically within the apical domain of ventral furrow cells. (B) Apical myosin accumulates first in the ventral furrow (bracket, 9 min) in *C-GAP shRNA* embryos. Cells separate as they lose adhesion in the ventral furrow (arrows, 18 min) before robust myosin accumulates in lateral cells. At 27 min, apical domains of ventral furrow cells have collapsed into puncta (arrows), as planar polarized myosin (arrowheads) accumulates and appears unaffected by the loss of C-GAP. (C) Gel of RT-PCR of OregonR (WT), *mat15>GAL4*, *UAS>C-GAP*, and *mat15>C-GAP* OE embryos, demonstrating C-GAP OE embryos express roughly 1.5 times more C-GAP than WT embryos. Bars, 20 µm.

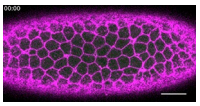


Video 1. **Constitutively active RhoA OE alters myosin dynamics and apical constriction during ventral furrow formation.** Embryos expressing myosin::GFP and membrane::RFP injected at left with mRNA for WT RhoA (top) or CA RhoA (bottom). Note that OE of WT RhoA does not affect tissue invagination. Images were acquired every 13 s, and videos are displayed at 12 frames per second. Bars, 20 µm.

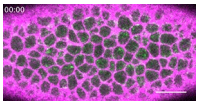
Video 2. **RhoGEF2 and myosin pulsatile dynamics during ventral furrow formation.** Embryo expressing GFP::RhoGEF2 and myosin::RFP, with merge at right. Video shown is corrected for photobleaching. Images acquired every 7 s, and video is displayed at 10 frames per second. Bar, 5 µm.



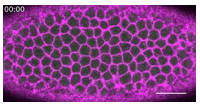
Video 3. **WT ventral furrow formation.** WT embryo expressing myosin::GFP and membrane::RFP. Images were acquired every 6.7 s, and the video is displayed at 15 frames per second. Bar, 20 µm.



Video 4. ***C-GAP shRNA* embryo with mild ventral furrow phenotype.** *C-GAP shRNA* embryo expressing myosin::GFP and membrane::RFP with a mild ventral furrow phenotype. Myosin localization is disrupted and ventral furrow formation is delayed. Images were acquired every 13 s, and the video is displayed at 15 frames per second. Bar, 20 µm.



Video 5. ***C-GAP shRNA* embryo with severe ventral furrow phenotype.** *C-GAP shRNA* embryo expressing myosin::GFP and membrane::RFP with severe phenotype. Myosin localization is disrupted and cells lose adhesion and separate from each other. The ventral furrow never invaginates. Images were acquired every 6 s, and the video is displayed at 15 frames per second. Bar, 20 µm.



Video 6. **C-GAP overexpression embryo.** C-GAP overexpression in embryo expressing myosin::GFP and membrane::RFP. Myosin pulses but there is less persistent myosin. Ultimately, the ventral furrow invaginates, but it is abnormal. Images were acquired every 6.24 s and are displayed at 15 frames per second. Bar, 20 µm.

Table S1. **Rho-family GAP expression and screen during early embryogenesis**

Gene	Expression					shRNA line	Ventral furrow phenotype	Clone ID for probe
	Stage 1 or 2	Stage 3 or 4	Stage 5	Stage 6	Stage 6 ventral furrow			
CdGAPr	+	+				+		LD27836
Conundrum	+	+						LD04957
Cv-c	+							RE02250
Graf	+	+				+		LD28528
OCRL	+	+				+		LD39196
RhoGAP1A	+	+	+	+	+			GH15984
RhoGAP5A	+	+	+			+		SD02309
RhoGAP15B	+	+	+	+	+	+		SD08167
RhoGAP16F						+		SD04011
RhoGAP18B	+	+	+	+	+	+		LD25711
RhoGAP19D	+	+	+	+	+	+		RH60035
RhoGAP54D	+	+	+			+		RE04485
RhoGAP68F	+	+	+			+		LD02491
RhoGAP71E	+	+	+	+	+	+	+	LD04071
RacGAP84C	+	+						AT12815
RhoGAP92B	+	+				+		AT11177
RhoGAP93B	+	+	+	+	+	+		SD01504
RhoGAP100F						+		LP17760
RhoGAP102A	+					+		EP07621
RhoGAPp190	+	+	+	+	+	+		GH17919
Rlip	+							GH01995
Tumbleweed	+	+	+	+		+		RE37229

Outline for screen for Rho GAPs at left. Table shows mRNA expression (indicated by +), determined by in situ hybridization, in the early embryo. We performed shRNA experiments for expressed genes that have TRiP lines. RhoGAP71E/C-GAP exhibited a ventral furrow–specific phenotype.

Provided in a separate Excel file is Table S2, showing genotypes for fly stocks used in this study.