Vasquez et al., http://www.jcb.org/cgi/content/full/jcb.201402004/DC1

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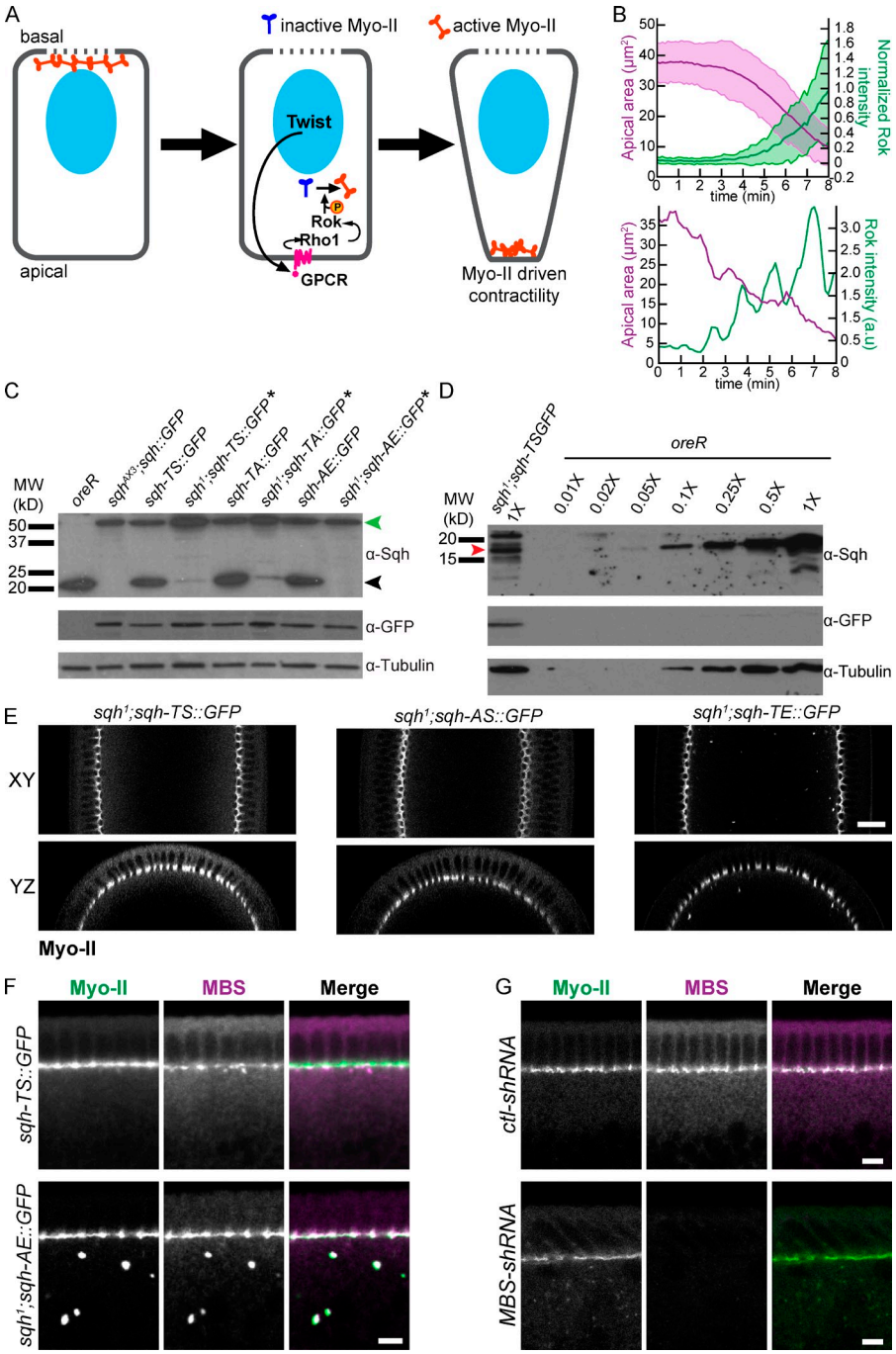


Figure S1. **Characterization of Myo-II phosphomutants.** (A) Schematic of changes in Myo-II localization in the ventral furrow. During a process called cellularization, Myo-II is localized to the basal tips of invaginating plasma membranes, called furrow canals. The invaginating membranes compartmentalize nuclei, forming epithelial cells immediately before gastrulation. At the onset of gastrulation, the Twist transcription factor activates signaling through the small GTPase Rho1 in ventral furrow cells. Rho1 signaling leads to Rok activation, which is thought to phosphorylate and consequently activate Myo-II contractility. (B) Rok intensity increases throughout apical constriction. Mean apical area (magenta) decreases, whereas mean Rok intensity (green) increases (top; $n = 67$ cells; shaded area is ±SD from the mean [solid line]). Plot of apical area (magenta) and Rok intensity (green) for an individual cell (bottom). (C) GFP-tagged *sqh* mutants that prevent (mutated to Alanine) or mimic (mutated to Glutamate) phosphorylation are expressed at the same level as endogenous *sqh*. Western blot of embryo lysates prepared from cellularizing blastoderms probed with α-Sqh, α-GFP, or α-tubulin (loading control). Green arrowhead indicates GFP-tagged Sqh and black arrowhead indicates endogenous Sqh. Germline clones of the *sqh*^{1} allele (indicated by asterisk) were estimated to express one-tenth of the endogenous amount of Sqh of wild-type (OreR) embryos. (D) Endogenous *sqh* expression in *sqh*^{1} germline clone embryos is 10% of endogenous *sqh* expression in OreR embryos. Red arrowhead indicates endogenous Sqh. (E) Representative images of XY semisagittal sections and YZ cross sections for cellularizing live embryos. Bar, 20 µm. (F) Images represent fixed cellularizing embryos expressing *sqh::GFP* (top) or *sqh-AE::GFP* (bottom) and stained for MBS. Note that MBS localizes to basal furrow canals and to small or large cytoplasmic aggregates in *sqh-TS* and *sqh-AE*, respectively. Bar, 5 µm. (G) Representative images of fixed cellularizing *ctl-shRNA* (top) and *MBS-shRNA* (bottom) embryos expressing *sqh::*GFP and stained for MBS. Note that in *ctl-shRNA*, MBS localized at the cellularization front and in the cytoplasm; however, in the MBS knockdown there is little detectable MBS signal. Bars, 5 µm.

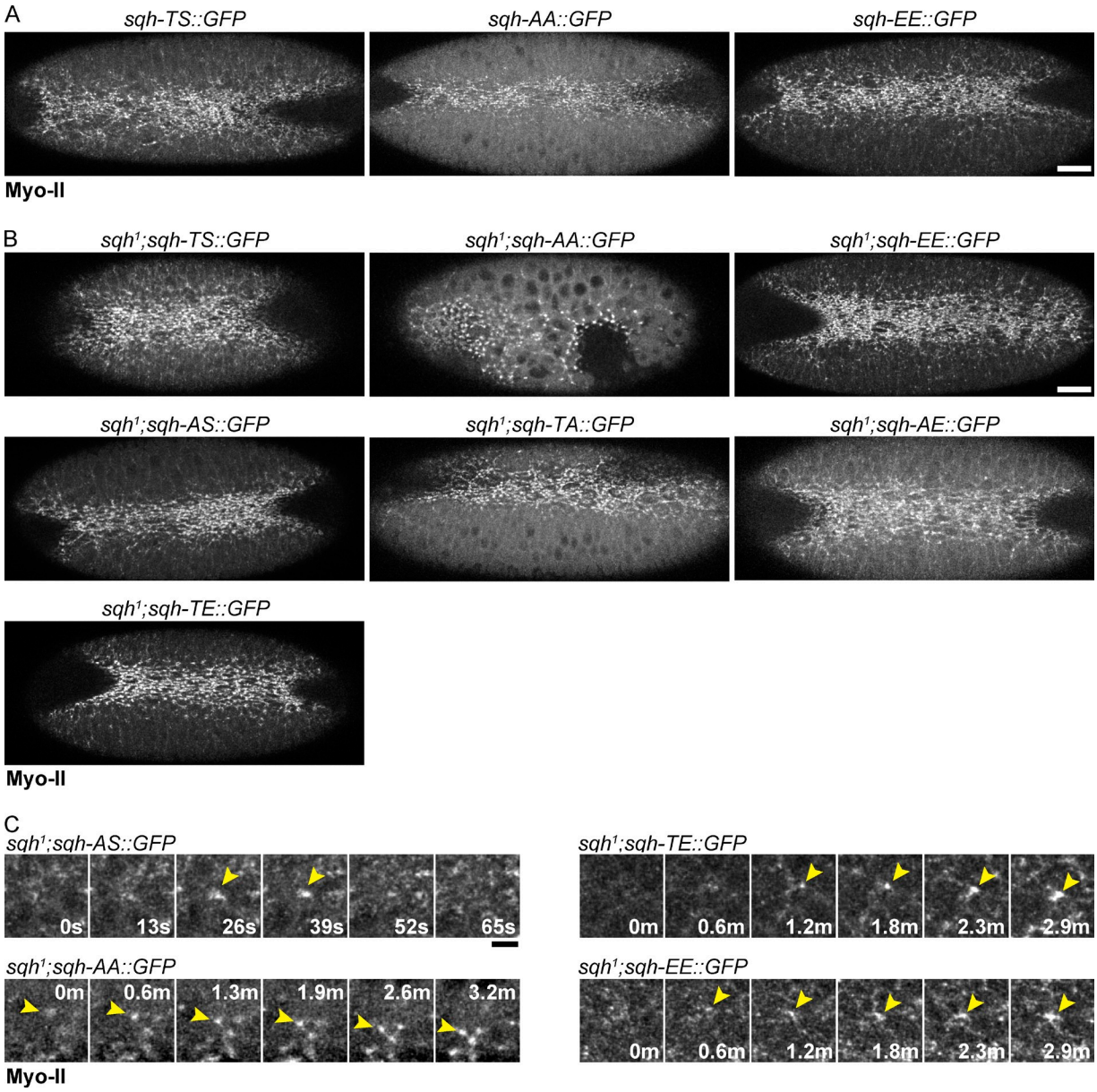


Figure S2. **Ventral furrow phenotypes for *sqh* phosphomutants.** (A) Apical Myo-II localization in *sqh* mutants expressed with wild-type levels of endogenous Sqh. Images represent live embryos expressing the indicated *sqh* allele. Bar, 20 µm. (B) Sqh phosphomutants exhibit apical localization specifically in ventral furrow cells. Images represent live embryos expressing the indicated *sqh* phosphomutant allele expressed in *sqh*^{1} germline clones. Note that *sqh-AA::GFP* localizes to the apical surface of ventral cells, but the tissue fails to form a tissue-wide furrow. Bar, 20 µm. (C) Dynamic phosphorylation of serine-21 is critical for contraction pulses. Time-lapse images show *sqh::GFP* structures in *sqh*^{1} germline clone embryos expressing the indicated allele. Note the shorter time scale for the *sqh-AS* mutant. Mutants that inactivate or activate serine-21 exhibit continuous Myo-II assembly without apparent disassembly/remodeling. Yellow arrowheads indicate instances of Myo-II assembly. Bar, 5 µm.

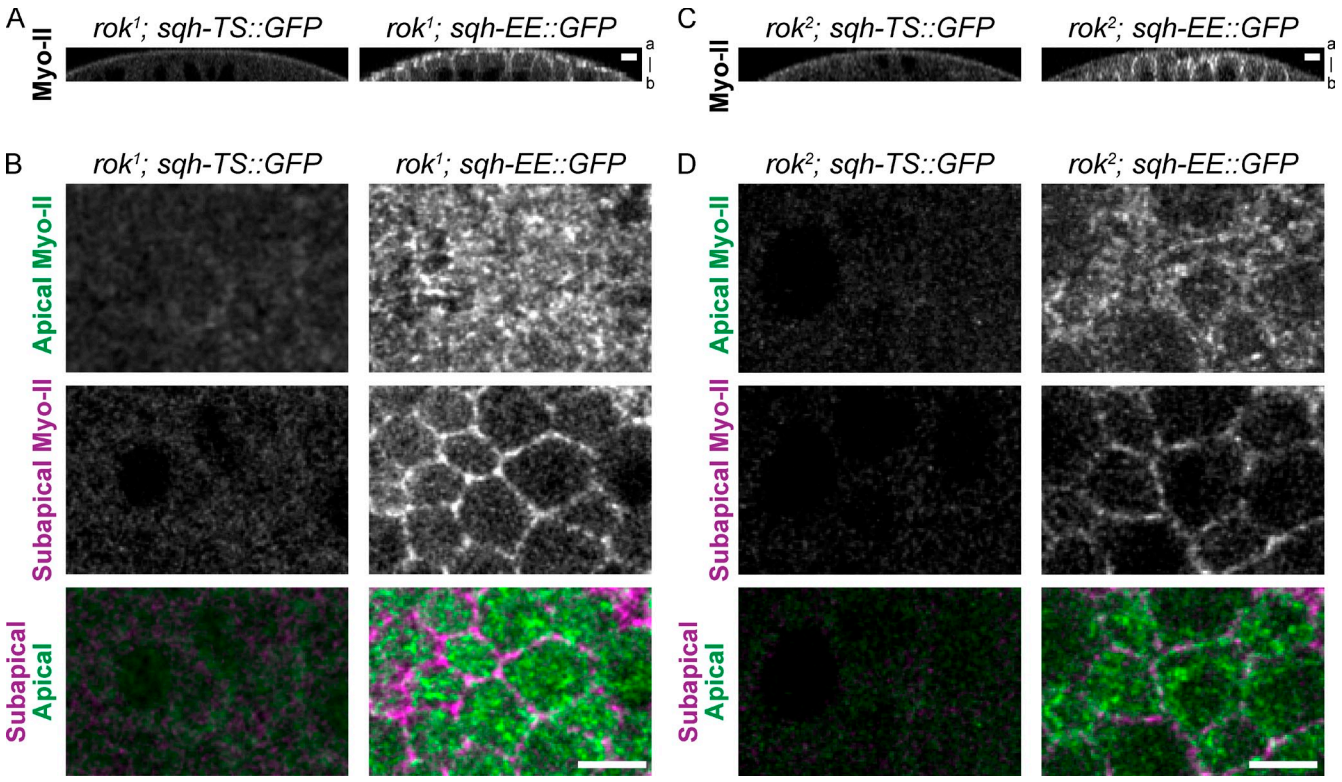


Figure S3. **Phosphomimetic *sqh* fails to suppress ventral furrow defects of *rok* mutants.** (A–D) The *sqh-EE* mutant rescues apical Myo-II localization but not polarized contraction in *rok* germline clones. Images represent endogenous GFP fluorescence from live rok^1 (A and B) and rok^2 (C and D) germline clone embryos expressing either *sqh-TS::GFP* or *sqh-EE::GFP*. Representative images of YZ cross sections (A and C; a, apical; b, basal) or surface views (B and D) are shown. The *rok* mutants disrupt cortical myosin localization, which is partially suppressed by *sqh-EE*. However, *sqh-EE* is present across the entire apical surface in *rok* mutants.

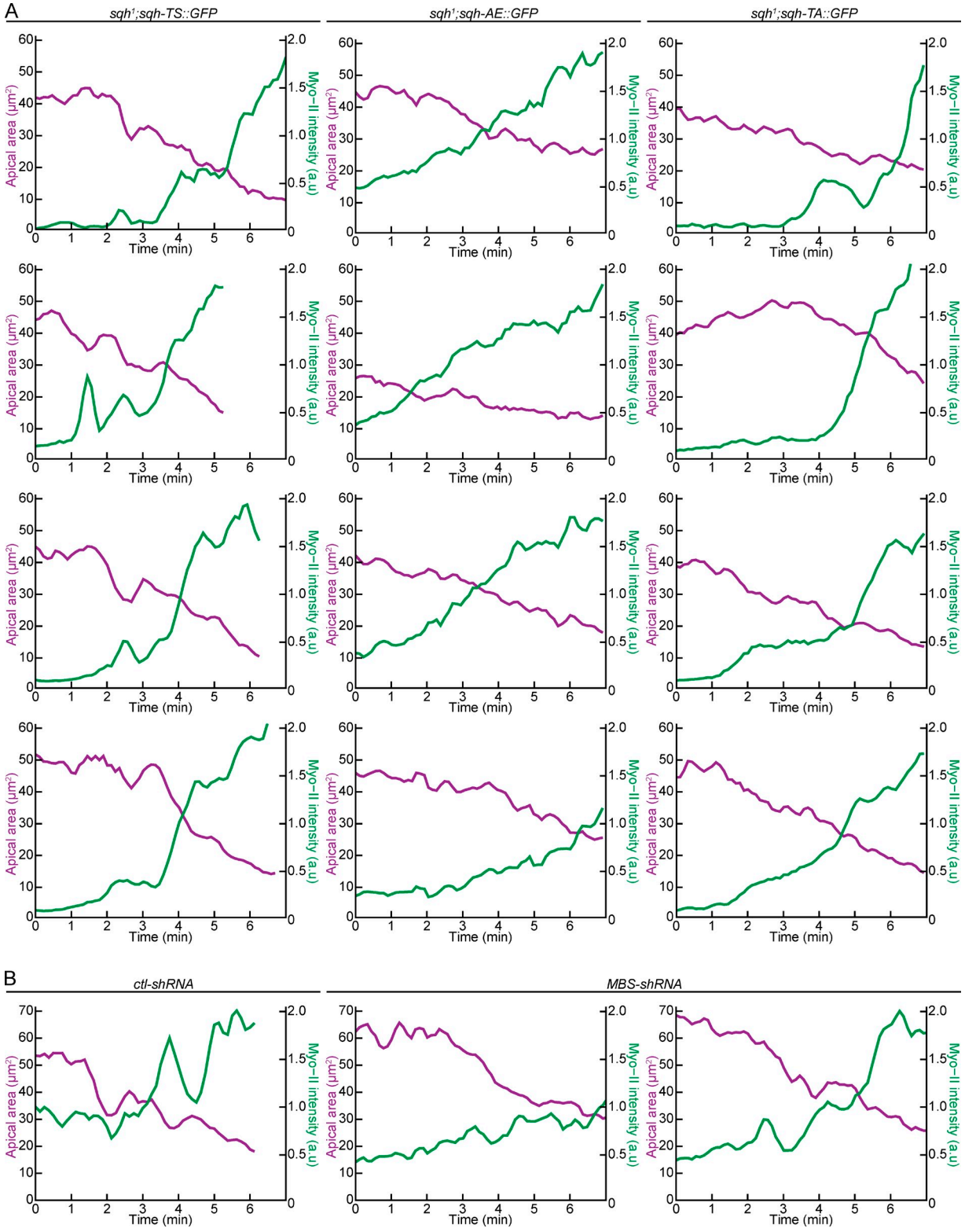


Figure S4. **Phosphomimetic *sqh* mutants and knockdown of MBS result in more continuous Myo-II accumulation and apical constriction.** Examples of individual cell apical constriction in *sqh* phosphomutants (A) and *ctl-shRNA* and *MBS-shRNA* mutants (B). Data plots show apical area and Myo-II intensity as a function of time for representative cells from *sqh*^{1} germline clones expressing the indicated Sqh phosphomutants or embryos expressing the indicated shRNAs. Starting time corresponds to 1–2 min before the onset of mean apical constriction in the corresponding embryo.

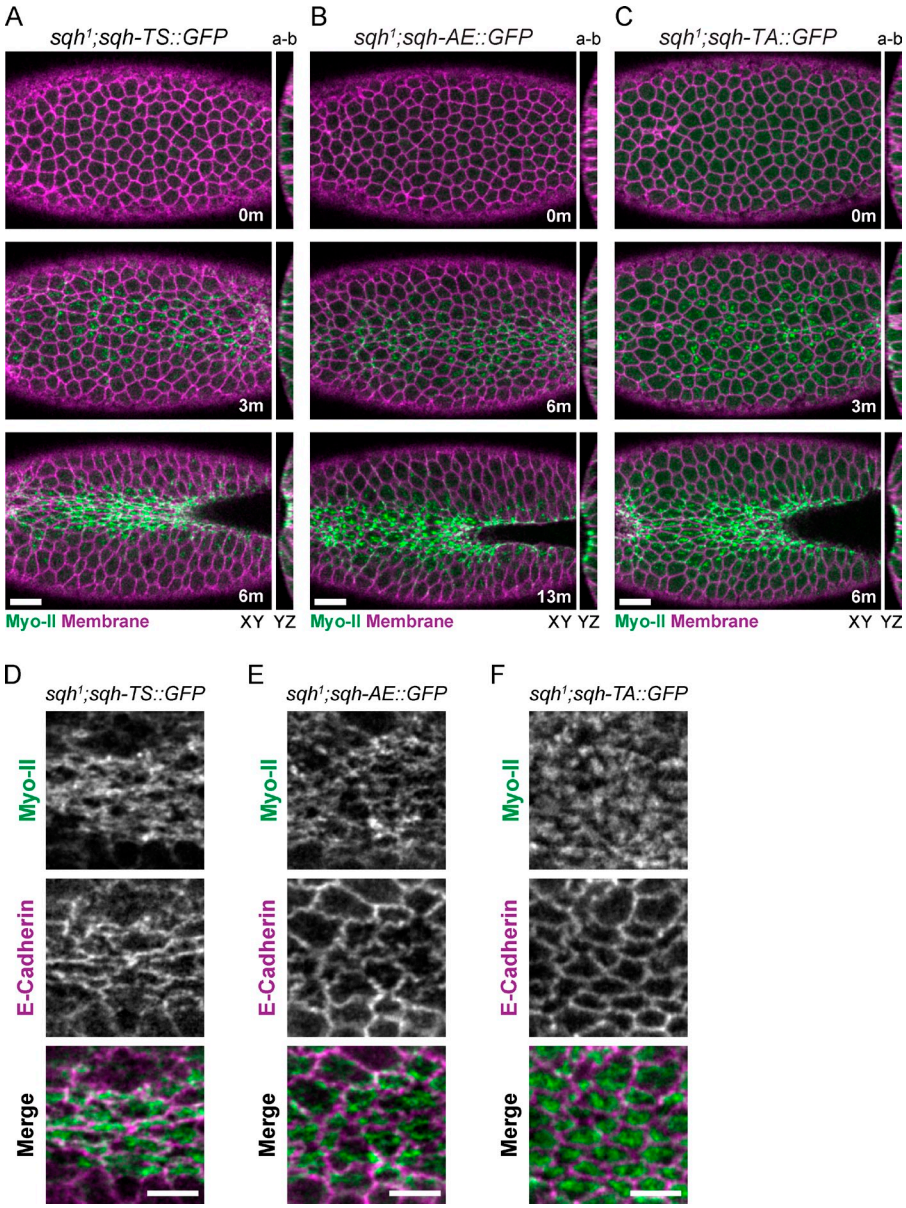
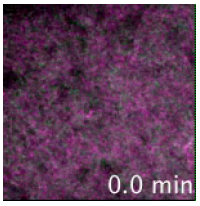
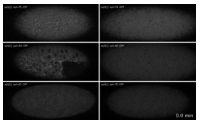


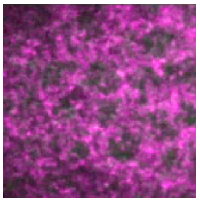
Figure S5. **Tissue invagination and E-cadherin localization in Myo-II phosphomutants.** (A–C) Activating and inactivating *sqh* serine-21 phosphomutants rescue ventral furrow invagination. The *sqh-AE* allele exhibits delayed apical constriction and tissue invagination. Representative images of XY semisagittal sections and YZ cross sections of live embryos from *sqh*^{1} germline clones expressing *sqh-TS::GFP* (A), *sqh-AE::GFP* (B), and *sqh-TA::GFP* (C). Embryos also express Gap43::mCherry as a membrane marker (magenta). a-b indicates apical–basal polarity. Bars, 10 µm. (D–F) Representative images of furrows of fixed *sqh*^{1} germline clone embryos expressing *sqh-TS::GFP* (D), *sqh-AE::GFP* (E), and *sqh-TA::GFP* (F) and stained for E-cadherin. Bars, 5 µm.



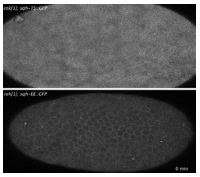
Video 1. **Rok pulses coincide with Myo-II pulses.** Time-lapse images represent maximum intensity Z projections of the apical surface of *rok*^{2} germline clones expressing Venus::Rok (wild type) and Sqh::mCherry. Panels are split and merged images are from the same movie (Rok, green; Myo-II, magenta). Image stacks were acquired on a laser-scanning confocal microscope (LSM 710; Carl Zeiss) at a time interval of 8.1 s per stack.



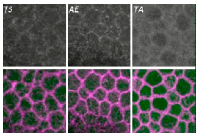
Video 2. **Phenotypes of *sqh* phosphorylation mutants during gastrulation.** Time-lapse images represent ventral furrow formation in *sqh*^{1} germline clone embryos expressing the indicated *sqh::GFP* phosphomutant transgene. Image stacks were acquired on a laser-scanning confocal microscope (LSM 710; Carl Zeiss) at a time interval of ∼4.4 s per stack. Images represent maximum intensity Z-projections of *sqh::GFP* signal.



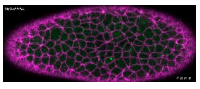
Video 3. **Phosphomimetic *sqh-AE* fails to condense apical F-actin.** Time-lapse images represent maximum intensity Z projections of the apical surface of *sqh*^{1} germline clone embryos expressing Utr::mCherry (labels F-actin) and the indicated *sqh::GFP* alleles. Panels are Utr::mCherry/F-actin channel (top) and merged images (bottom; Myo-II, green; F-actin, magenta). Image stacks were acquired on a laser-scanning confocal microscope (LSM 710; Carl Zeiss) at a time interval of 6.7 to 7 s per stack.



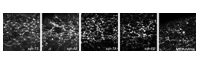
Video 4. ***sqh-EE* mutant suppresses apical Myo-II localization, but not polarized contraction in *rok* mutants.** Time-lapse images represent maximum intensity Z projections of *rok*^{1} germline clone embryos expressing the indicated *sqh::GFP* allele. Images stacks were acquired on a laser-scanning confocal microscope (LSM 710; Carl Zeiss) at a time interval of ∼12.6 s per stack. Note that cells become round and undergo cytokinesis at the end of the movie.



Video 5. **Contractile pulses are inhibited in *sqh* phosphorylation mutants.** Time-lapse images are from *sqh*^{1} germline clone embryos expressing Memb::Cherry and either *sqh-TS::GFP* (*TS*), *sqh-AE::GFP* (*AE*), or *sqh-TA::GFP* (*TA*). Top image for each genotype is *sqh::GFP* signal and bottom image is a merge of *sqh::GFP* (green) and Memb::Cherry (magenta) channels. Image stacks were acquired on a laser-scanning confocal microscope (LSM 710; Carl Zeiss) at a time interval of ∼6 to 6.7 s per stack. Images represent maximum intensity Z projections of apical *sqh::GFP* and Memb::Cherry signals.



Video 6. **Knockdown of MBS inhibits contractile pulses.** Time-lapse images are from a *MBS-shRNA* embryo expressing Memb::Cherry (magenta) and *sqh::GFP* (green). Images represent a maximum intensity Z projection of apical *sqh::GFP* and a single apical slice of Gap43::mCherry. Image stacks were acquired on a laser-scanning confocal microscope (LSM 710; Carl Zeiss) at a time interval of 6.8 s per stack.



Video 7. **Phosphomutant Myo-II and MBS knockdown cause the supracellular Myo-II meshwork to stretch and tear.** Time-lapse images represent maximum intensity Z projections of *sqh*^{1} germline clones expressing the indicated *sqh::GFP* transgene and an embryo expressing the *MBS-shRNA* with *sqh::GFP*. Fluorescent intensity shown is from GFP signal. Image stacks were acquired on a laser-scanning confocal microscope (LSM 710; Carl Zeiss) at time intervals of 4.5–9.0 s per stack.

Table S1. **Fly stocks**

Genotype	Source
OreR	2
sqh^{AX3};P{w^+ sqh::GFP}42	2
ovo^{D2} FRT^{19A}; hsFLP UAS-Venus::Rok/CyO	4, 5
rok^2 FRT^{19A}; mat15 sqh::mCherry^{A11}	1
y w; P{w^+ sqh-TS::GFP}attP1	1
y w; P{w^+ sqh-AA::GFP}attP1	1
y w; P{w^+ sqh-TA::GFP}attP1	1
y w; P{w^+ sqh-AS::GFP}attP1	1
y w; P{w^+ sqh-TE::GFP}attP1	1
y w; P{w^+ sqh-AE::GFP}attP1	1
y w; P{w^+ sqh-EE::GFP}attP1	1
y w; P{w^+ sqh-TS::GFP}attP40	1
y w; P{w^+ sqh-EE::GFP}attP40	1
ovo^{D1} FRT^{101}/Y; hsFLP-38/hsFLP-38	2
sqh^1 FRT^{101}/FM7; P{w^+ sqh-TS::GFP}attP1/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-AA::GFP}attP1/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-TA::GFP}attP1/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-AS::GFP}attP1/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-TE::GFP}attP1/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-AE::GFP}attP1/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-EE::GFP}attP1/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-TS::GFP}attP40/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-TS::GFP}attP1 P{w^+ Gap43::mCherry}attP40/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-TA::GFP}attP1 P{w^+ Gap43::mCherry}attP40/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-AE::GFP}attP1 P{w^+ Gap43::mCherry}attP40/CyO	1
Utr::mCherry	3
sqh^1 FRT^{101}/FM7; P{w^+ sqh-TS::GFP}attP1 Utr::mCherry/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-TA::GFP}attP1 Utr::mCherry/CyO	1
sqh^1 FRT^{101}/FM7; P{w^+ sqh-AE::GFP}attP1 Utr::mCherry/CyO	1
rok^2 FRT^{19A}/FM7; P{w^+ sqh-TS::GFP}attP1	1
rok^2 FRT^{19A}/FM7; P{w^+ sqh-EE::GFP}attP40	1
P{Ubi-GFP::Rok}	6
y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GL00094}attP2 (*ctl-shRNA*)	7
y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GL01207}attP40 (*MBS-shRNA*)	7
y[1]w[1118]; P{w^+ sqh::GFP}42, mat67; P{Gap43:mCherry}, mat15/TM3	1
y[1]w[1118]; P{w^+ sqh::GFP}42; P{Gap43:mCherry}, mat15/TM3	1

sqh-XX = *sqh* promoter and ORF with site-directed mutagenesis at T20 and S21 as noted here. *Gap43* = *sqh* promoter with N-terminal 20 amino acids of rat Gap43 gene, which contains a myristoylation sequence. *Utr* = *sqh* promoter with Utrophin actin-binding domain. Name and mutations: *TS*, *T20,S21*; *TA*, *T20,S21A*; *AS*, *T20A,S21*; *TE*, *T20,S21E*; *AE*, *T20A,S21E*; *EE*, *T20E,S21E*. Sources: 1, this study; 2, Bloomington Drosophila Stock Center; 3, Rauzi et al. (2010), gift from T. Lecuit; 4, Mason et al. (2013); 5, gifts from J. Zallen, S. Simões (Sloan Kettering Institute, New York, NY), and R. Fernandez-Gonzalez (University of Toronto, Toronto, Canada); 6, Bardet et al. (2013), gift from Y. Bellaiche (Institut Curie, Paris, France); 7, gifts from N. Perrimon, L. Perkins, and the Transgenic RNAi Project.

Table S2. **Antibodies used and concentrations**

Antibody	Use	Concentration	Source
Rabbit anti-Zipper	HF and PFA	1:500	Wieschaus Laboratory
Rabbit anti-MBS	PFA	1:500	Tan Laboratory, University of Missouri, Columbia, MO
Rabbit anti–E-Cad2	PFA	1:50	Developmental Studies Hybridoma Bank
Mouse anti-neurotactin	HF	1:100	Developmental Studies Hybridoma Bank
Rabbit anti-Sqh	WB	1:5,000	This study
Mouse anti-GFP	WB	1:1,000	Roche
Mouse anti-tubulin	WB	1:5,000	Sigma-Aldrich

HF, heat fixation; WB; Western blot.