

Fig. S1. Spatialtemporal localization of Rho sensor during interphase and mitosis. Live imaging of a syncytial blastoderm embryo expressing the Rho sensor tagged with GFP. Axial position and time are as indicated. Time 0 was defined by the localization of the Rho sensor to new furrows. Scale bar 10 μm.

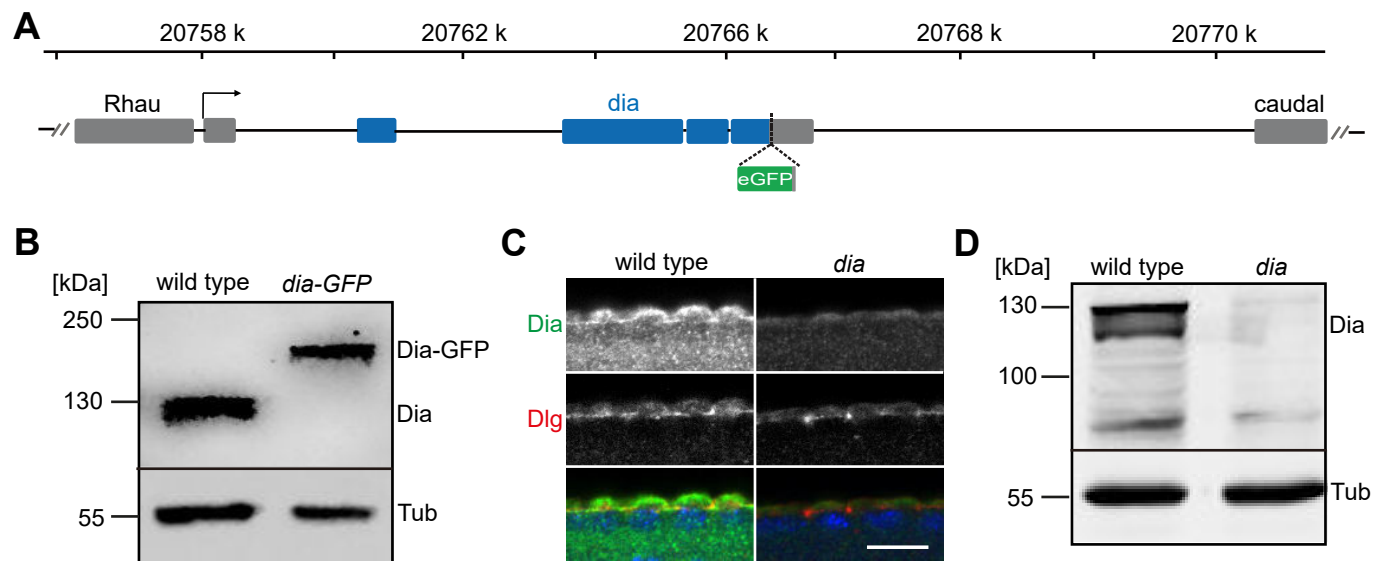


Fig. S2. GFP tagging of Dia. (A) Scheme of the genetic region of *dia* with eGFP insertion. (B) Western blot with wild type and Dia-GFP embryonic lysates stained for Dia (upper blot) and Tubulin as loading control (lower blot). (C) Wild type and *dia* embryos in interphase 13 fixed and stained for Dia (grey/green), Dlg (grey/red) and DNA (blue). Sagittal sections (D) Western blot with wild type and *dia* embryonic lysates stained for Dia (upper blot) and Tubulin as loading control (lower blot). Scale bar 10 μ m.

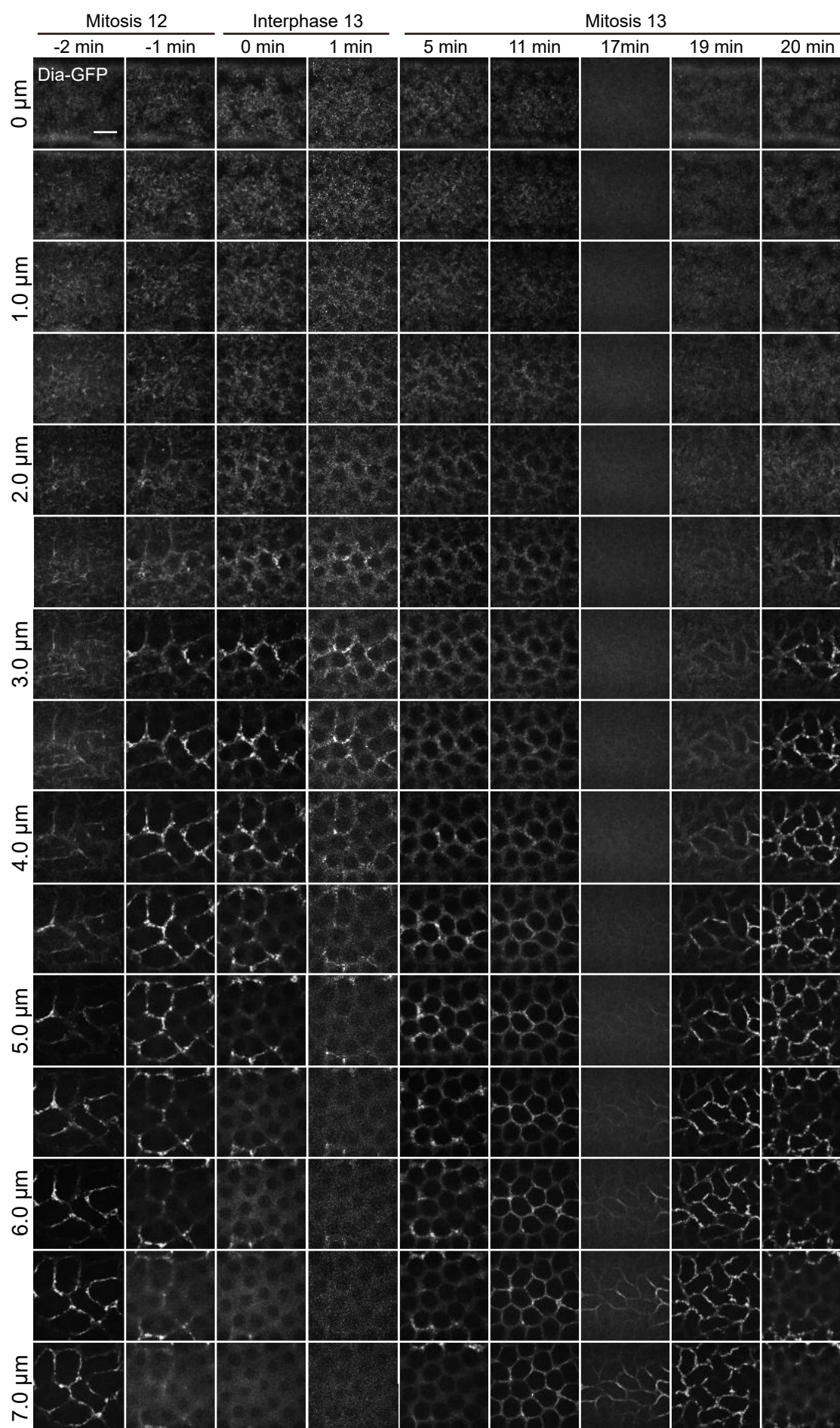


Fig. S3. Dia-GFP is enriched in intercaps and basal tips of metaphase furrow. Images from a movie of a syncytial blastoderm embryo expressing Dia-GFP. Axial position and time are as indicated. Time 0 was defined by the localization of Dia-GFP to new furrows. Scale bar 10 μm .

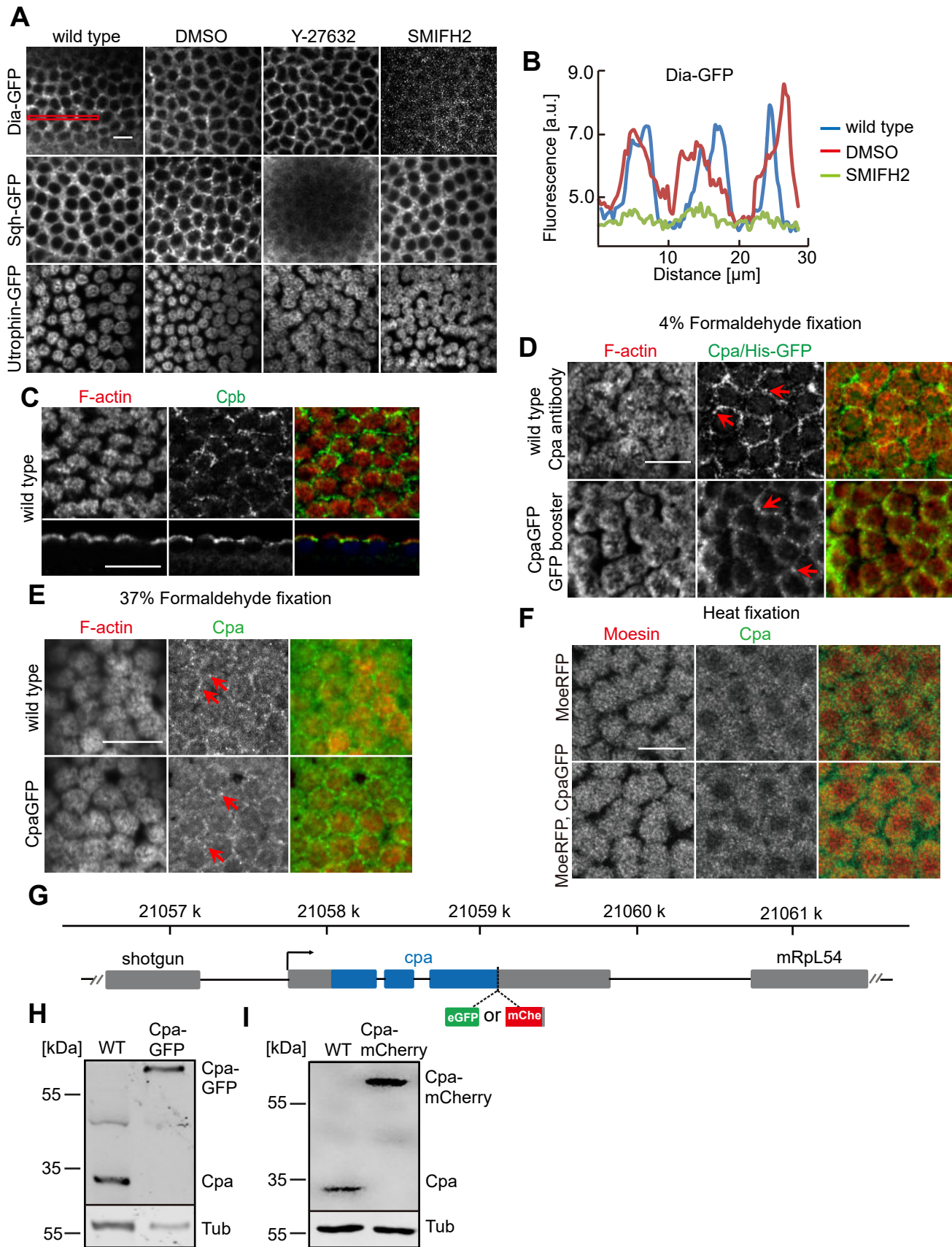


Fig. S4. Injection of inhibitors diminishes fluorescence of Dia-GFP and Sqh-GFP. (A) Embryos expressing indica-ted tagged proteins were injected with the indicated drugs. Images from live embryos during interphase 13. (B) Normali-zed Dia-GFP fluorescence intensity at the intercap domain. (C) Wild type embryo during interphase 13 fixed and stained for F-actin (grey/red), Cpb (grey/green) and DNA (blue). Upper panels shows frontal sections, lower panel sagittal views. (D, E) Histone2Av-GFP and CpaGFP embryos fixed with 4% (D) or 37% (E) formaldehyde and stained for Cpa antibody or GFP antibody as indicated. (F) Embryos expressing indicated tagged proteins were heat-fixed and stained for RFP and Cpa. Arrows in red indicate Cpa punctae. (G) Cpa locus on the second chromosome with eGFP or mCherry insertions as indicated. (H, I) Western blots with wild type and CpaGFP or Cpa-mCherry embryonic lysates probed for Cpa (upper blot) and α -Tubulin as loading control. Scale bars 10 μ m.

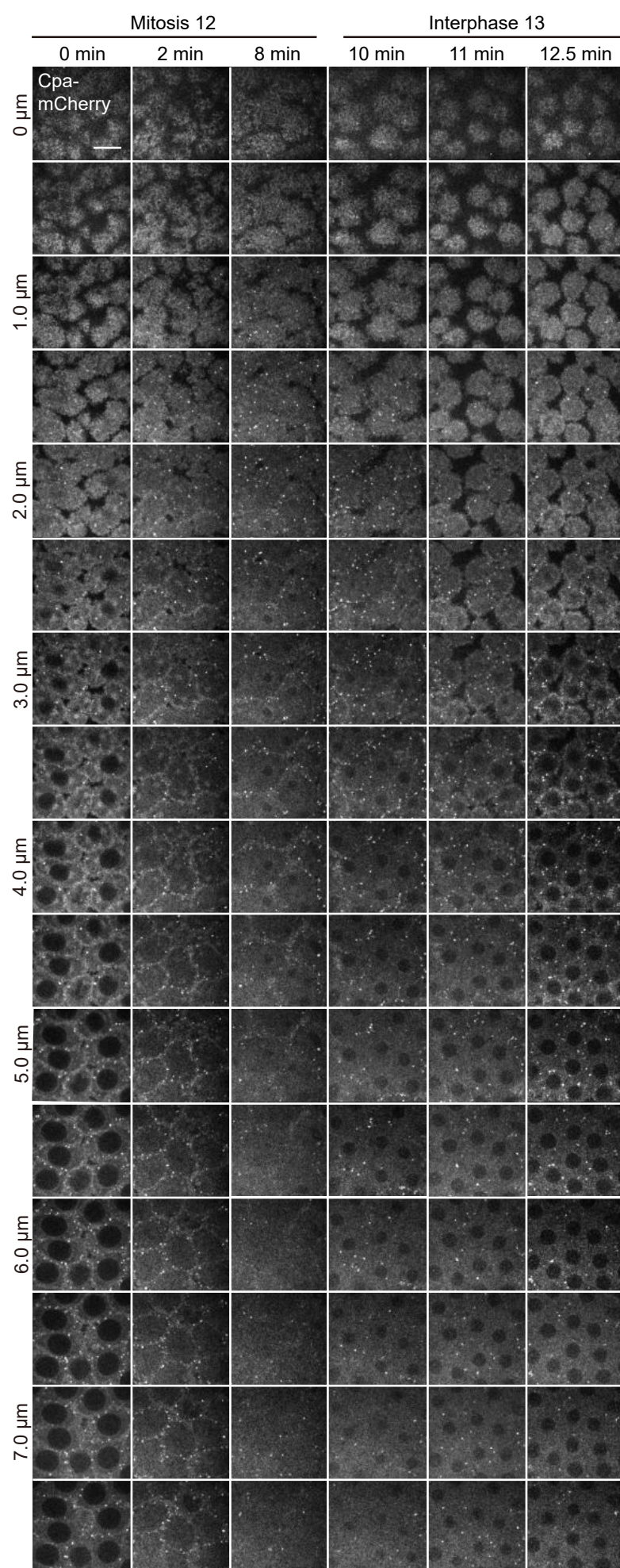


Fig. S5. Dynamics of Cpa-mCherry during mitosis and interphase. Live syncytial blastoderm embryos expressing Cpa-mCherry from mitosis 12 to interphase 13. Axial position and time as indicated. Time 0 was defined by the expansion of the cap region at the beginning of mitosis. Scale bar 10 μm .

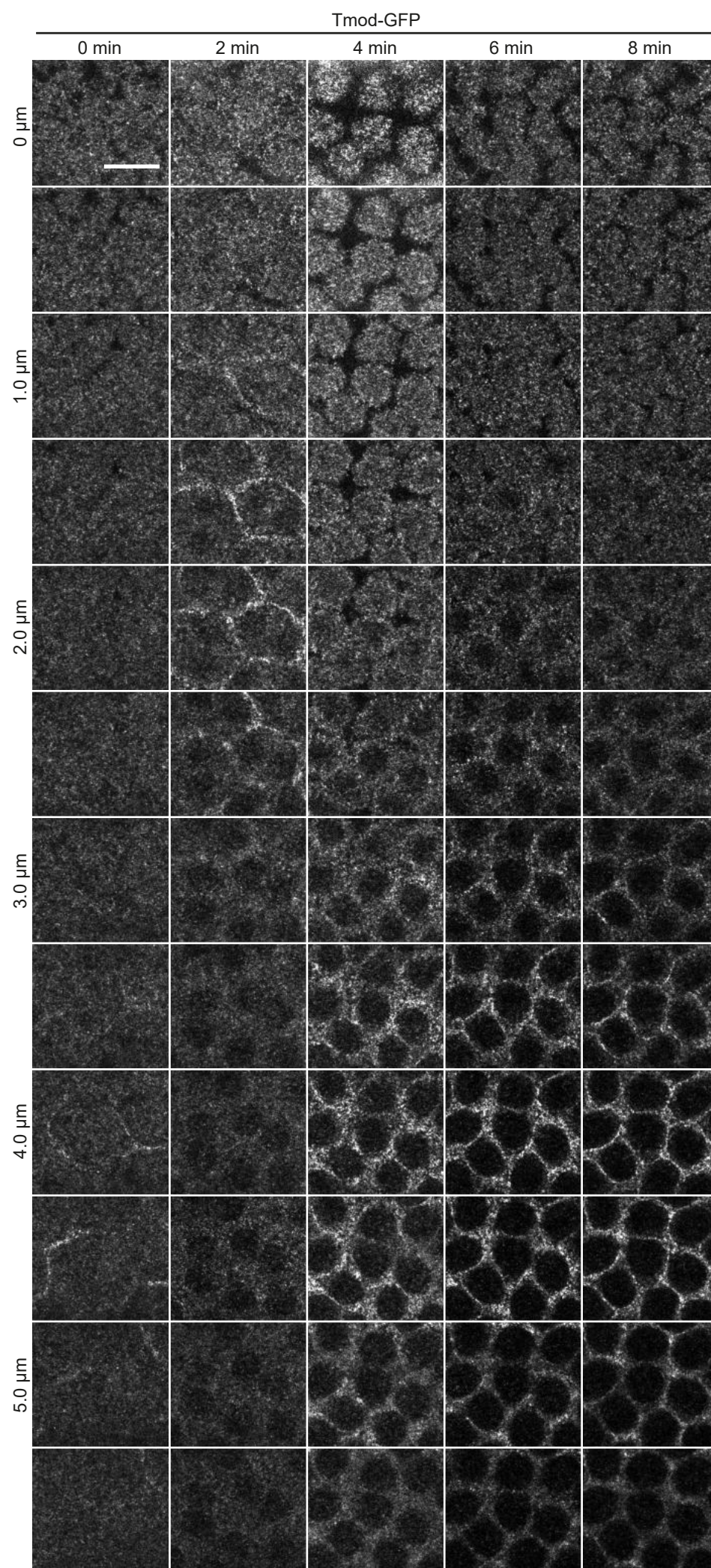


Fig. S6. Dynamics of Tmod-GFP during mitosis and interphase. Live syncytial blasto-derm embryos expressing Tmod-GFP from mitosis 12 to inter-phase 13. Axial position and time as indicated. Time 0 was defined by the expansion of the cap region at the beginning of mitosis. Scale bar 10 μm.

Table S1. Fly stocks

Fly stocks	Reference/Source
<i>Ced-12/ELMO^{c06760}</i>	Drosophila stock center, Bloomington # 17781
<i>dia^{sy5}</i>	Yan et al., 2013
Cpa-GFP	This work
Cpa-mCherry	This work
Dia-GFP	This work
Histone2Av-GFP	Clarkson and Saint, 1999
Moesin-RFP	Großhans lab
MyoII 3xGFP	Pinheiro et al., 2017
Rho sensor	Munjal et al., 2015
Tmod-GFP	Drosophila stock center, Bloomington # 59303
Utrophin-GFP	Rauzi et al., 2010

Table S2. Antibodies, stains and inhibitors

Antibody	Dilution	Reference/Source
mouse anti- α -Tubulin	1:50000 (WB)	B512, Sigma
rabbit anti-Cpa	1:200 (IHC) 1:2000 (WB)	Amândio et al., 2014, this work
rabbit anti-Cpb	1:200	Amândio et al., 2014
Rabbit anti-Dia	1:1000 (IHC)	Großhans et al., 2005
guinea pig anti-Dia	1:5000 (WB)	Großhans et al., 2005
Phalloidin-Alexa647	1:1000	Thermo Fisher (# A12379)
GFP-booster-Atto488	1:500 (IHC)	Chromotek, (# gba488)
DAPI	1:250 (IHC), 0.2 μ g/ml	Thermo Fisher (# D1306)
SMIFH2	2 mM in DMSO	Abcam (# ab218296)
Y-27632	10 mM in water	Sigma (# Y0503)