Table S1. Table of strains used in this study.

Strain	Genotype	Source or reference
	Figure 1	
AP240	*ade6-M210 ura4-D18 leu1-32 h-*	Laboratory collection
AP5400	*AP240 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
	Figure 2	
AP5507	*cdr2-EGFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5511	*cdr2-EGFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP5718	*blt1-mEGFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5741	*blt1-mEGFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X- Erg25) ade6-M210 ura4-D18 leu1-32*	This study
	Figure 3	
AP5802	*Pact1-lifeact-GFP:leu1+, rlc1-mcherry:Nat, sid4-mcherry:hphMX6 ade6-M210 ura4-D18 leu1-32*	Laboratory collection
AP5806	*Pact1-lifeact-GFP:leu1+, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF23 (pREP42X- Erg25) ade6-M210 ura4-D18 leu1-32*	This study
	Figure 4 and Figure S5	
AP5595	*mid1-mEGFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6- ura4-D18 leu1-32*	This study
AP5621	*mid1-mEGFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X- Erg25) ade6- ura4-D18 leu1-32*	This study
AP5629	*2mYFP-rng2:ura4+, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6- ura4-D18 leu1-32*	This study
AP5665	*2mYFP-rng2:ura4+, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X- Erg25) ade6-ura4-D18 leu1-32*	This study

AP5598	*GFP-cdc15:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6- ura4-D18 leu1-32*	This study
AP5640	*GFP-cdc15:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X- Erg25) ade6-ura4-D18 leu1-32*	This study
	Figure 5	
AP5601	*cdc12-3YFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5635	*cdc12-3YFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X- Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP6183	*cdc12-3XGFP:kanMX6, rlc1-mcherry:kanMX6, sid4-mcherry:hphMX6 ade6-M210 ura4-D18 leu1-32*	From Bohnert et al., 2013 (KG15568)
AP6193	*cdc12-3XGFP:kanMX6, rlc1-mcherry:kanMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X-Erg25)ade6-M210 ura4-D18 leu1-32*	This study
AP6129	*cdc12-3YFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 cdc25-22 ade6-M210 ura4-D18 leu1-32*	This study
AP6145	*cdc12-3YFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 cdc25-22 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP6166	*cdc12-3YFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 cdc25-22 mid1::ura4^{+} ade6-M210 ura4-D18 leu1-32*	This study
	Figure 6	
AP5802	*Pact1-lifeact-GFP:leu1+, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6-M210 ura4-D18 leu1-32*	Laboratory collection
AP5806	*Pact1-lifeact-GFP:leu1+, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF23 (pREP42X- Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP240	*ade6-M210 ura4-D18 leu1-32 h-*	Laboratory collection
AP5400	*AP240 + pAF12 (pREP3X- Erg25) ade6-M210 ura4-D18 leu1-32*	This study
TP1032	*myo1::kanMX6 h- ade6-M210 leu1-32*	from Riken Institute (FY13570)
AP5894	*myo1::kanMX6 + pAF12 (pREP3X-Erg25) ade6-M210 leu1-32*	This study
	Figure S1	

AP240	*ade6-M210 ura4-D18 leu1-32 h-*	Laboratory collection
AP5400	*AP240 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP5323	*erg6Δ::kanMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5724	*erg6Δ::kanMX6 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP5716	*erg25-ENVY:kanMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5770	*erg25-ENVY:kanMX6, (SPAC1B2.03c)ER-marker-mcherry:natMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5790	*erg25-ENVY:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP5808	*scs2Δ::kanMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5843	*scs2Δ::kanMX6 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP5785	*scs22Δ::kanMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5793	*scs22Δ::kanMX6 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP5837	*scs22Δ::kanMX6, scs2Δ::natMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5847	*scs22Δ::kanMx6, scs2Δ::natMX6 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
	Figure S2	
AP5507	*cdr2-EGFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6-M210 ura4-D18 leu1-32*	This study
AP5511	*cdr2-EGFP:kanMX6, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
AP3788	*cdr2-tagRFP:natMX6 mid1-mEGFP:kanMX6 ade6-M210 ura4-D18 leu1-32*	Laboratory collection
AP6094	*cdr2-tagRFP:natMX6 mid1-mEGFP:kanMX6+ pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
	Figure S3	
AP6184	sid4-GFP:kanMX6, rlc1-mcherry:NatMX6 *ade6-M210 ura4-D18 leu1-32*	This study
AP6155	sid4-GFP:kanMX6, rlc1-mcherry:NatMX6 *+ pAF12 (pREP3X-Erg25) ade6-M210 ura4-D18 leu1-32*	This study
	Figure S4 and S6	

AP5802	*Pact1-lifeact-GFP:leu1+, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 ade6-M210 ura4-D18 leu1-32*	Laboratory collection
AP5806	*Pact1-lifeact-GFP:leu1+, rlc1-mcherry:natMX6, sid4-mcherry:hphMX6 + pAF23 (pREP42X- Erg25) ade6-M210 ura4-D18 leu1-32*	This study

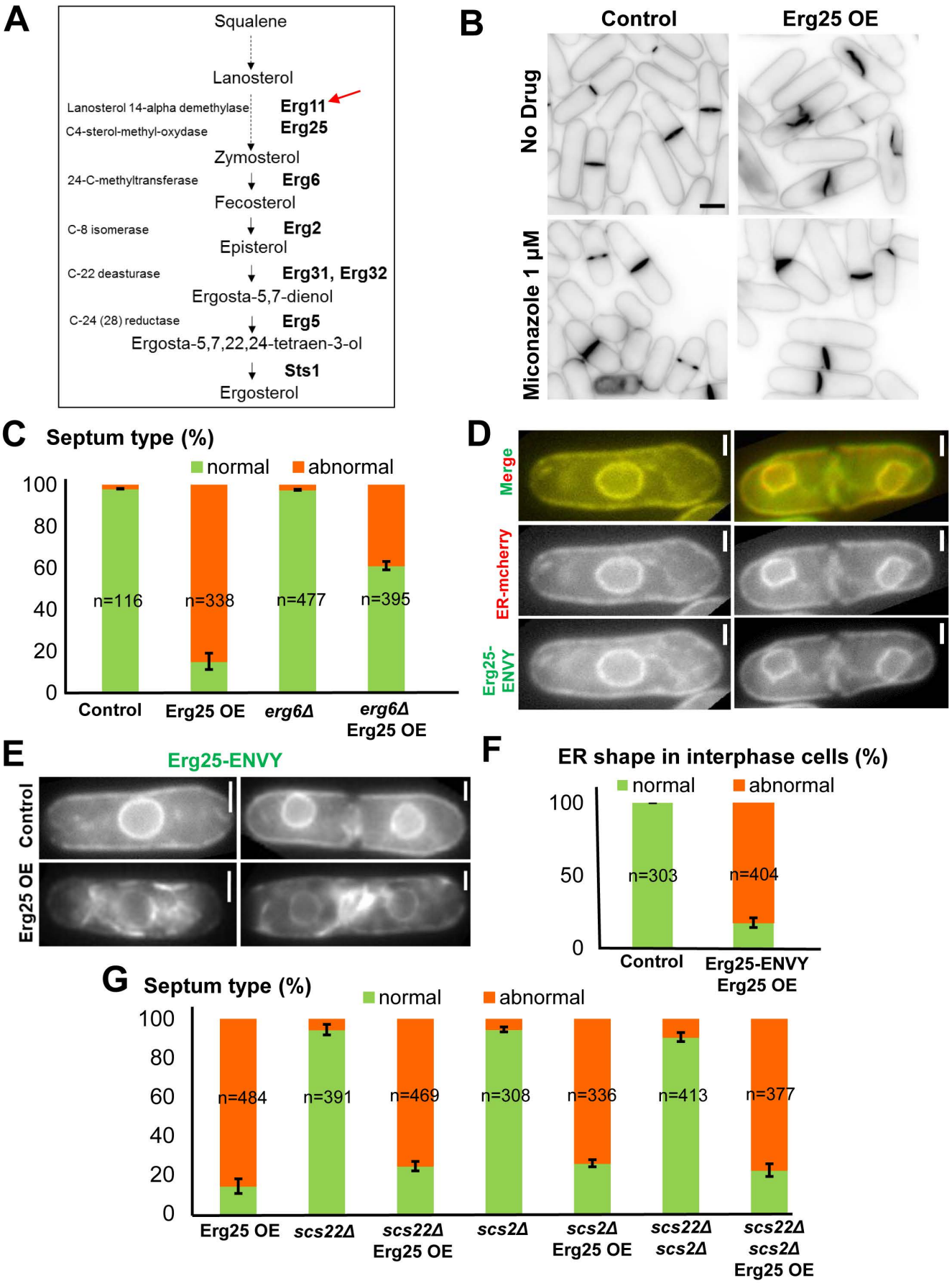


Figure S1. Cytokinetic defects induced by Erg25 OE require an active sterol biosynthetic pathway, while Erg25 affects ER organization with little impact on division plane positioning.

A: Schematic representation of the late stages of ergosterol biosynthetic pathway in *S. pombe* deduced from *S. cerevisiae* (adapted from Iwaki et al., 2008). The red arrow indicates at which level of the pathway miconazole acts. **B:** Calcofluor staining of control and Erg25 OE cells, treated and non-treated with 1µM miconazole. Scale bar, 5µm. **C:** Quantification of septum defects observed by calcofluor staining of control (n=116), Erg25 OE (n=338), *erg6Δ* (n=477) and Erg25 OE *erg6Δ* cells (n=395). Error bars: SD. **D**: Epifluorescence images of Erg25-ENVY and of the ER marker Elo2 fused to mcherry (ER-mcherry). Scale bars: 5µm. **E**: Epifluorescence images of Erg25-ENVY in control (top) and Erg25 OE cells (bottom) in interphase (left) and in late cytokinesis (right). Scale bars: 5µm. **F**: Quantification of ER shape in interphase in control (n=303) and Erg25 OE cells (n=404). Error bars: SD. **G**: Quantification of septum types in Erg25 OE (n=484), *scs22Δ* (n=391), *scs22Δ* Erg25 OE (n=469), *scs2Δ* cells (n=308), *scs2Δ* Erg25 OE (n=336), *scs22Δscs2Δ* (n=413) and *scs22Δscs2Δ* Erg25 OE cells (n=377). Error bars: SD.

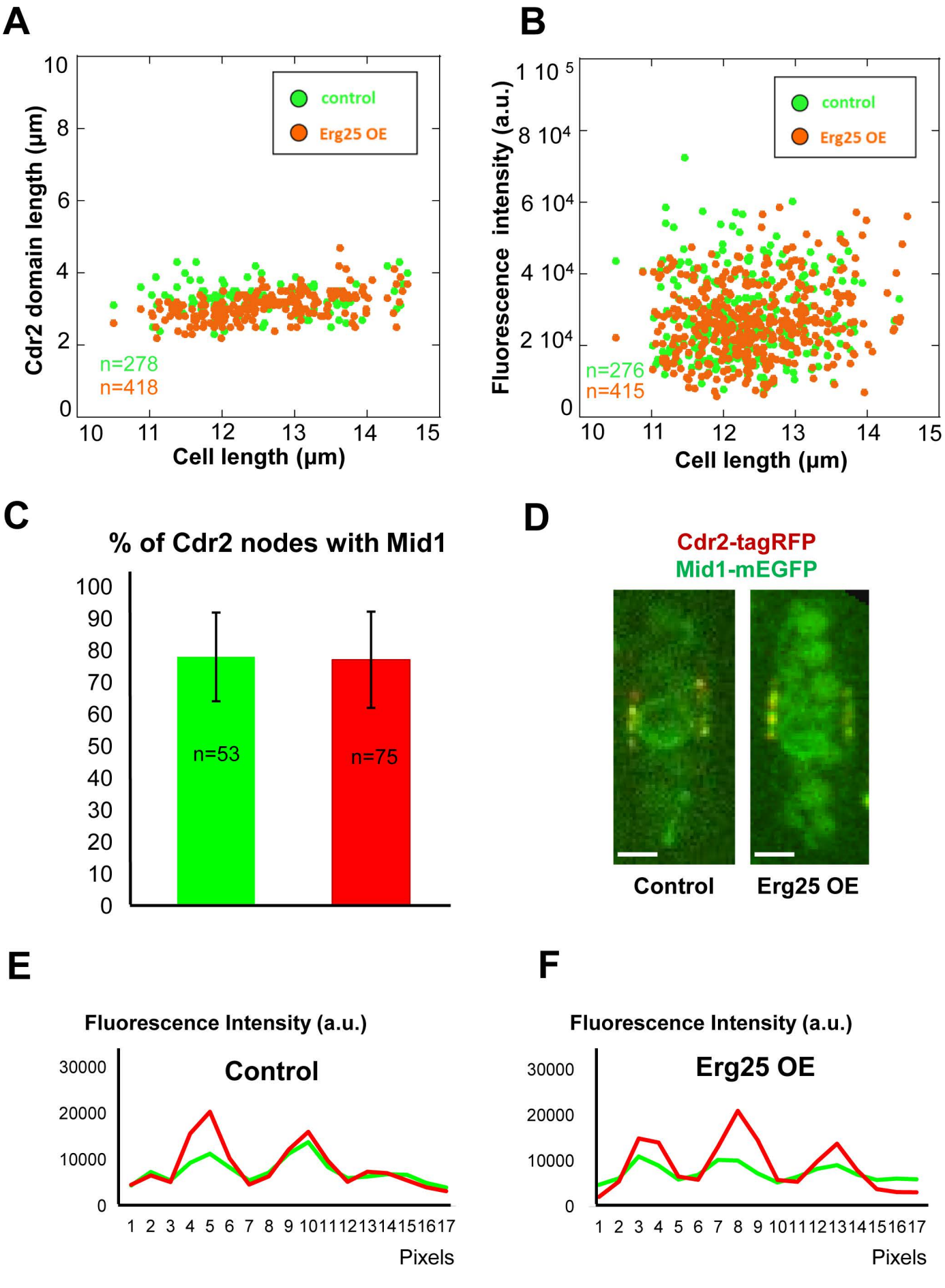
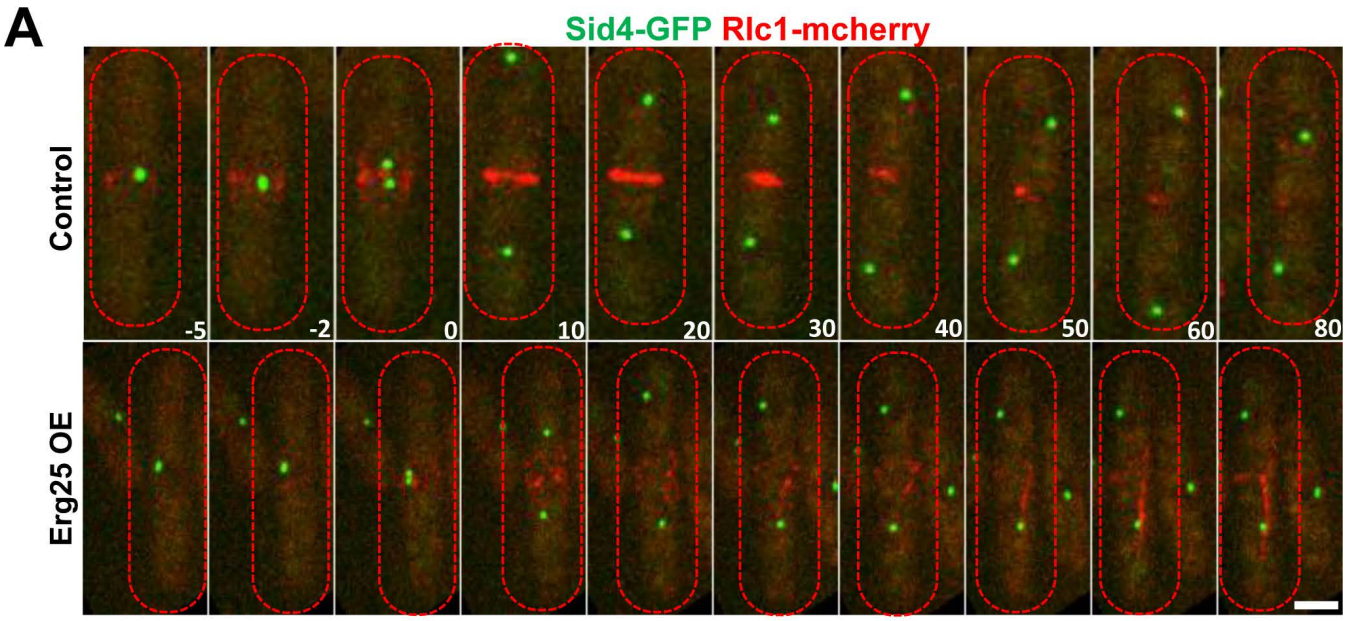
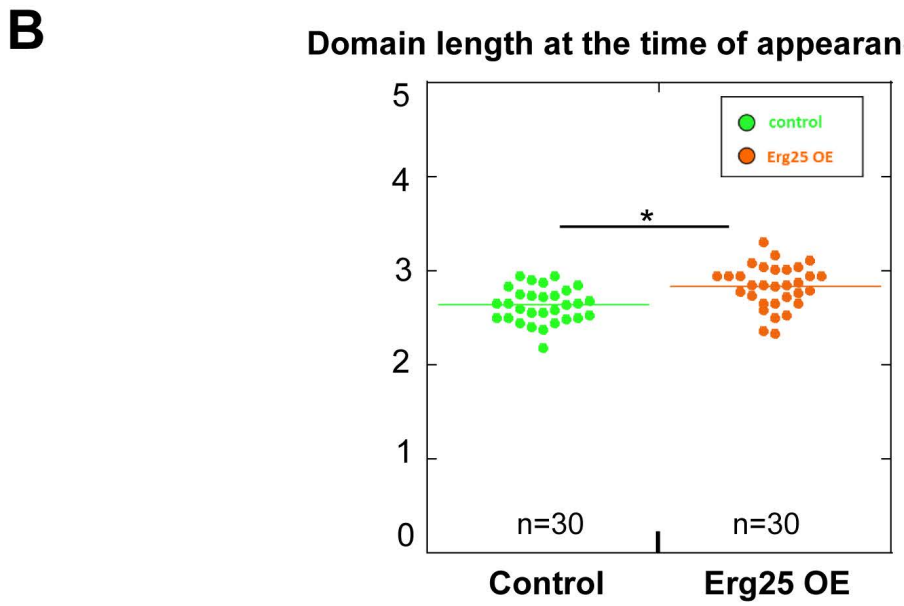


Figure S2. Characterization of Cdr2 and Mid1 domains upon Erg25OE.

A: Length of the Cdr2-EGFP domain relative to cell length in control (n=278) and Erg25 OE cells (n=418). **B:** Integrated fluorescence intensity (a.u.) of Cdr2-EGFP domain relative to cell length in control (n=278) and Erg25 OE cells (n=418). **C:** Percentage of Cdr2-tagRFP nodes containing Mid1-mEGFP in single cells. Error bars: SD (n>50 cells, top right). **D:** Medial planes confocal images of Mid1-mEGFP and Cdr2-tagRFP in control (left) and Erg25 OE cells (right). Scale bar, 5 µm. **E-F**: Linescan analyzing Mid1-mEGFP and Cdr2-tagRFP intensity along the medial cortex (bottom) along the cells shown at the top right.





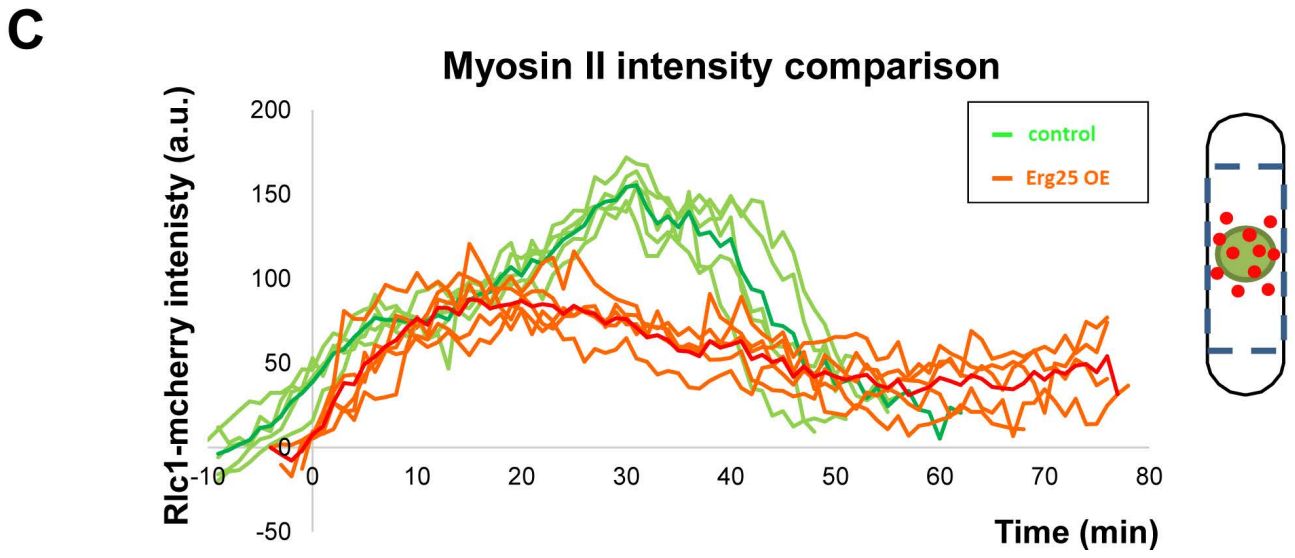


Figure S3. Analysis of myosin II behavior in Erg25 OE cells.

A: Time-lapse analysis of Sid4-GFP and Rlc1-mcherry in control (top) and Erg25 OE cells (bottom). Medial plane confocal images are shown. Time 0 corresponds to mitotic entry. Scale bars: 5 µm. **B:** Measurement of myosin II domain length at the time of its initial recruitment in control and Erg25 OE cells (n=30). **C:** Analysis of myosin II intensity (a.u.) in the central region in control and Erg25 OE cells (n=5). t=0 corresponds to SPB separation.

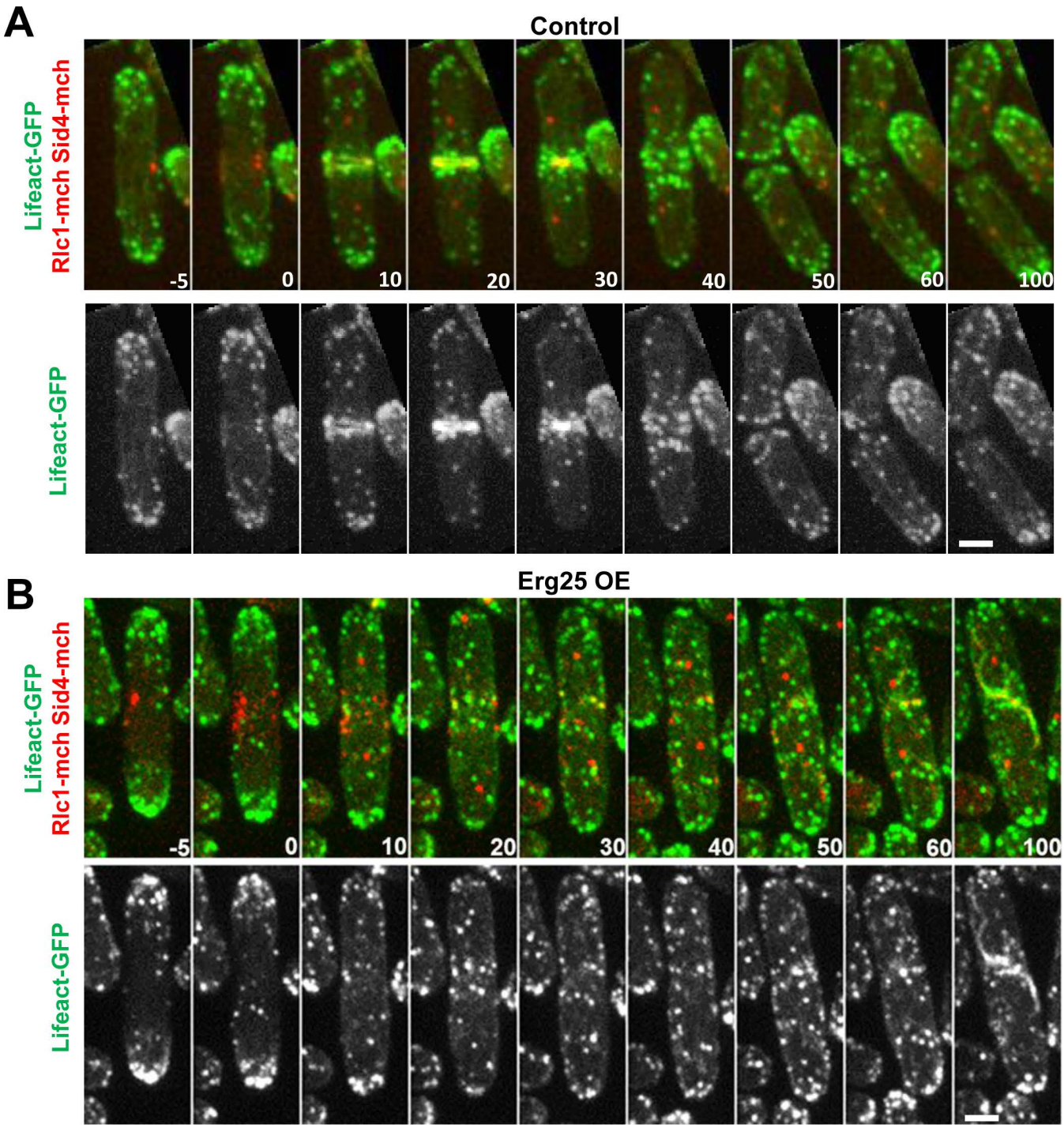


Figure S4: Inhibition of F-actin nucleation from cytokinetic precursor nodes upon Erg25 OE

Time-lapse images of control (**A**) and Erg25 OE cells (**B**) expressing lifeact-GFP, Rlc1-mcherry and Sid4-mcherry. Time 0 corresponds to mitotic entry. Scale bars: 5µm.

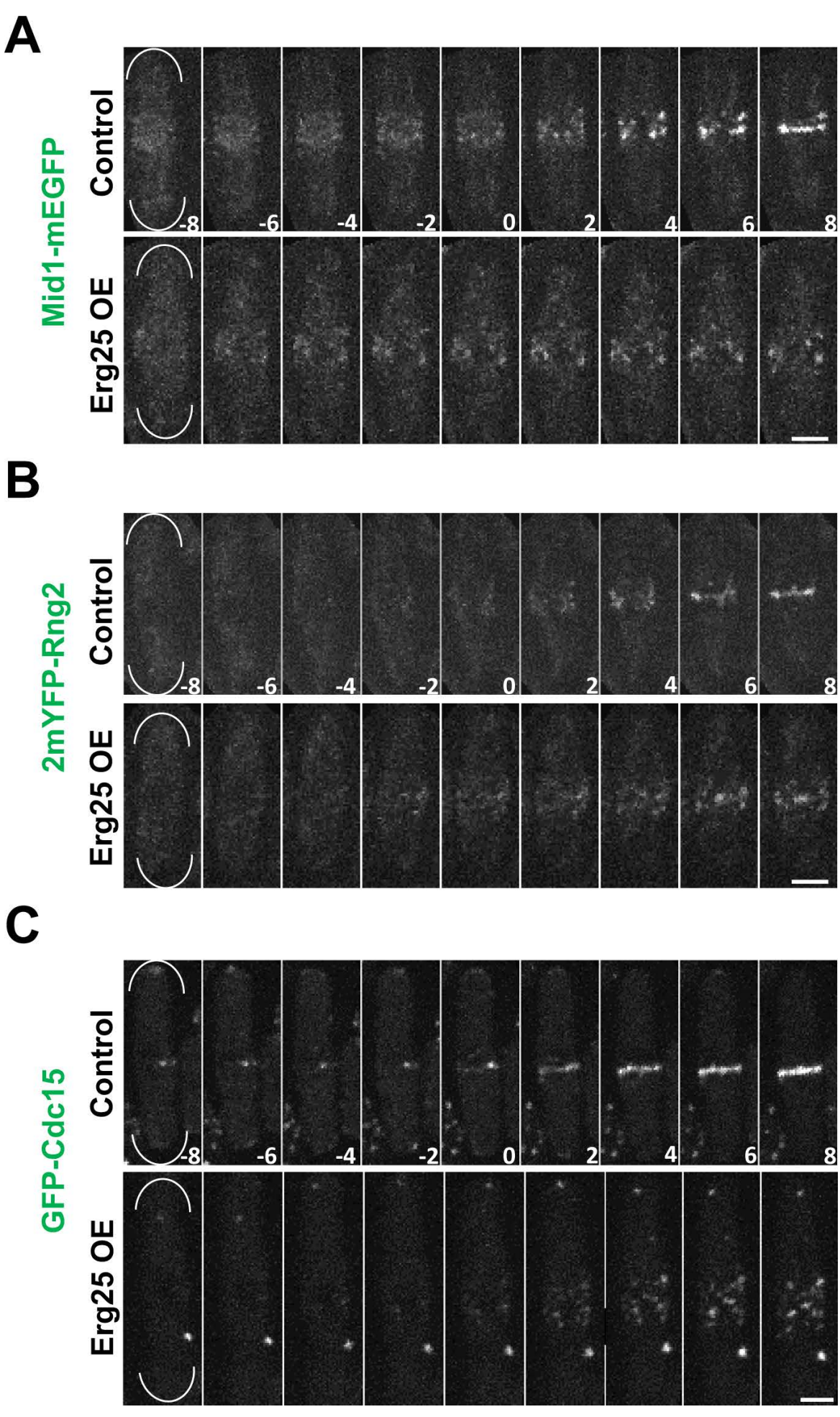


Figure S5. Analysis of Mid1, Rng2 and Cdc15 by time lapse imaging upon Erg25 OE.

Time lapse images of Mid1-mEGFP (**A**), 2mYFP-Rng2 (**B**) and GFP-Cdc15 (**C**) in control (top) and Erg25 OE cells (bottom). Time 0 corresponds to the time of SPB separation. Scale bar, 5 µm.

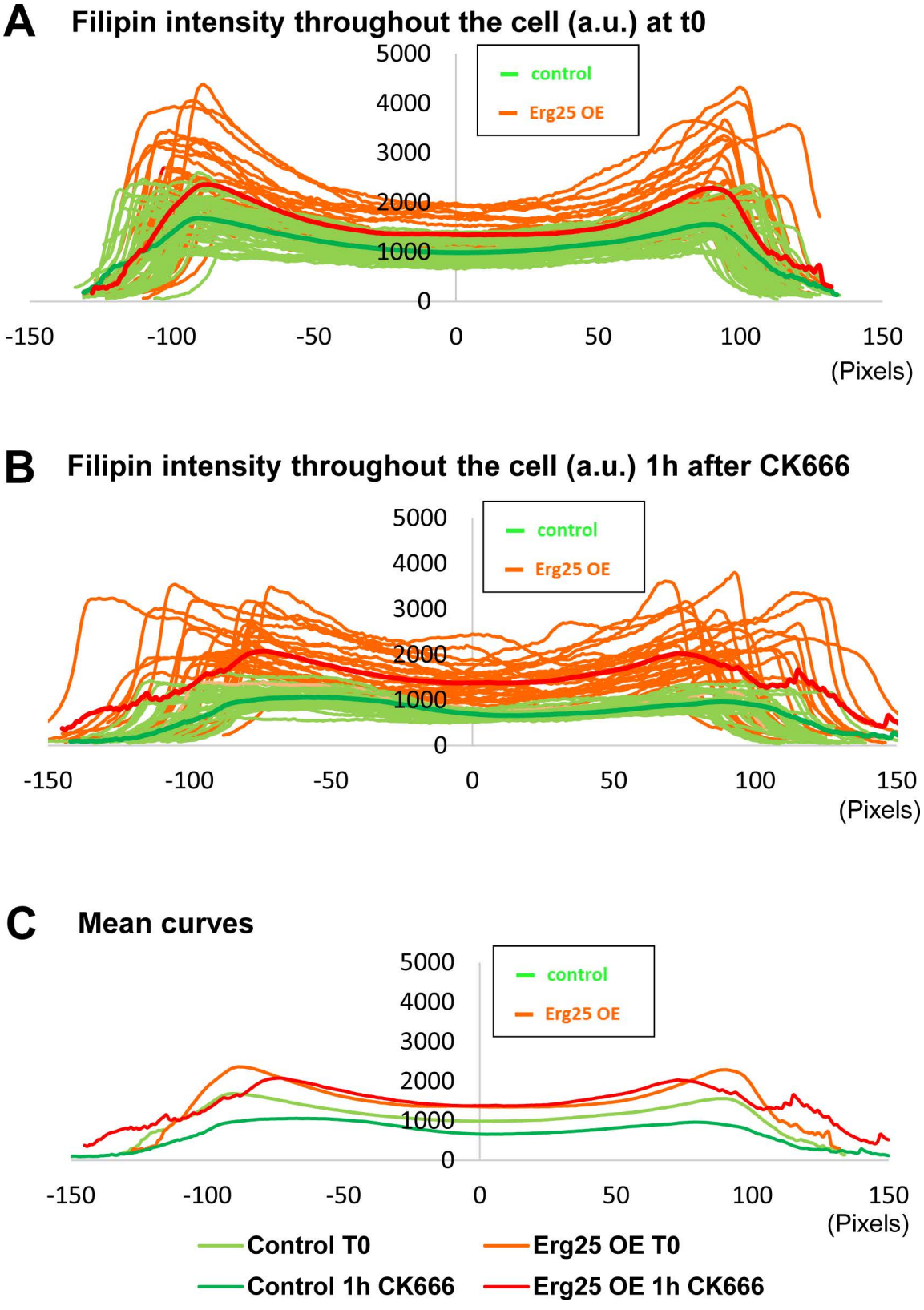
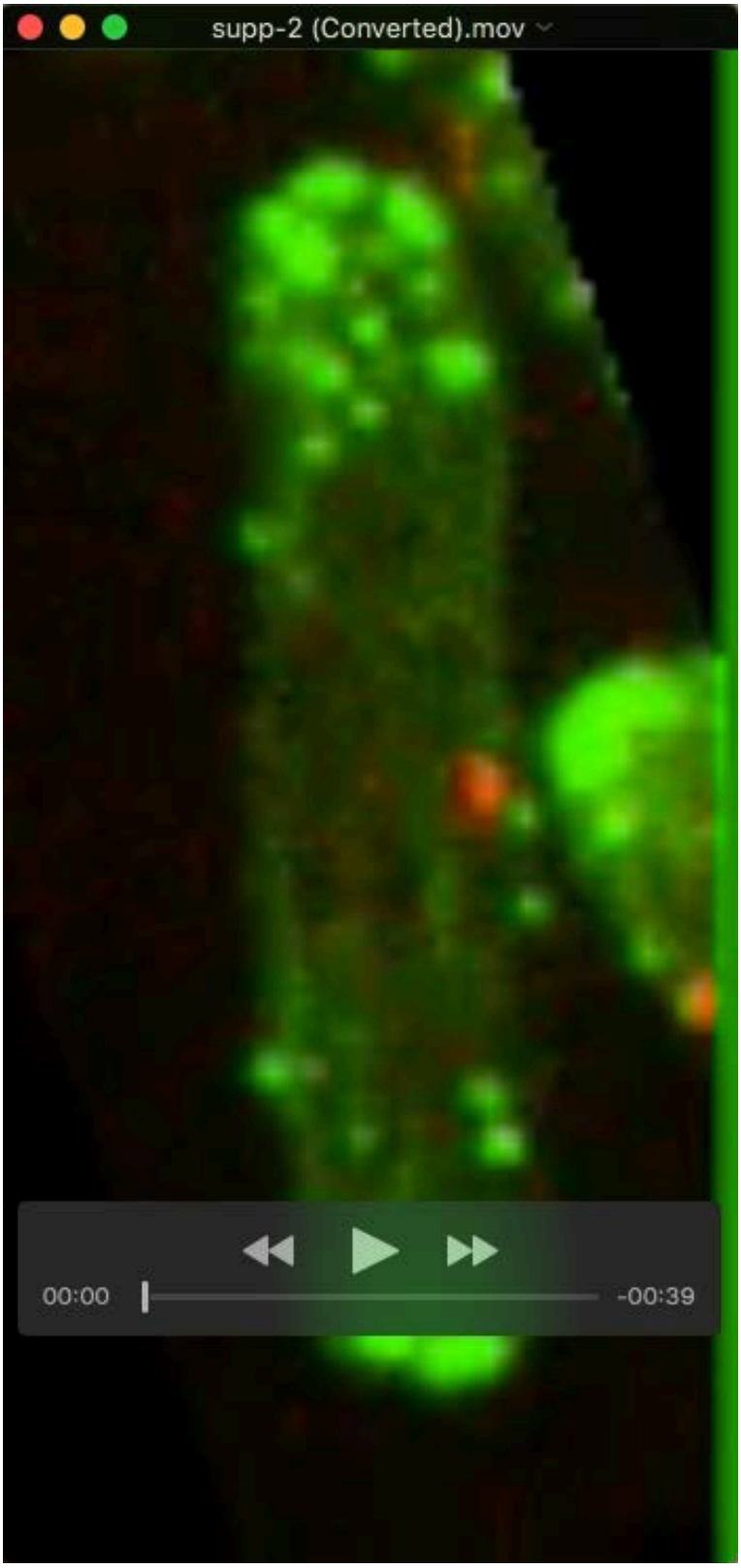
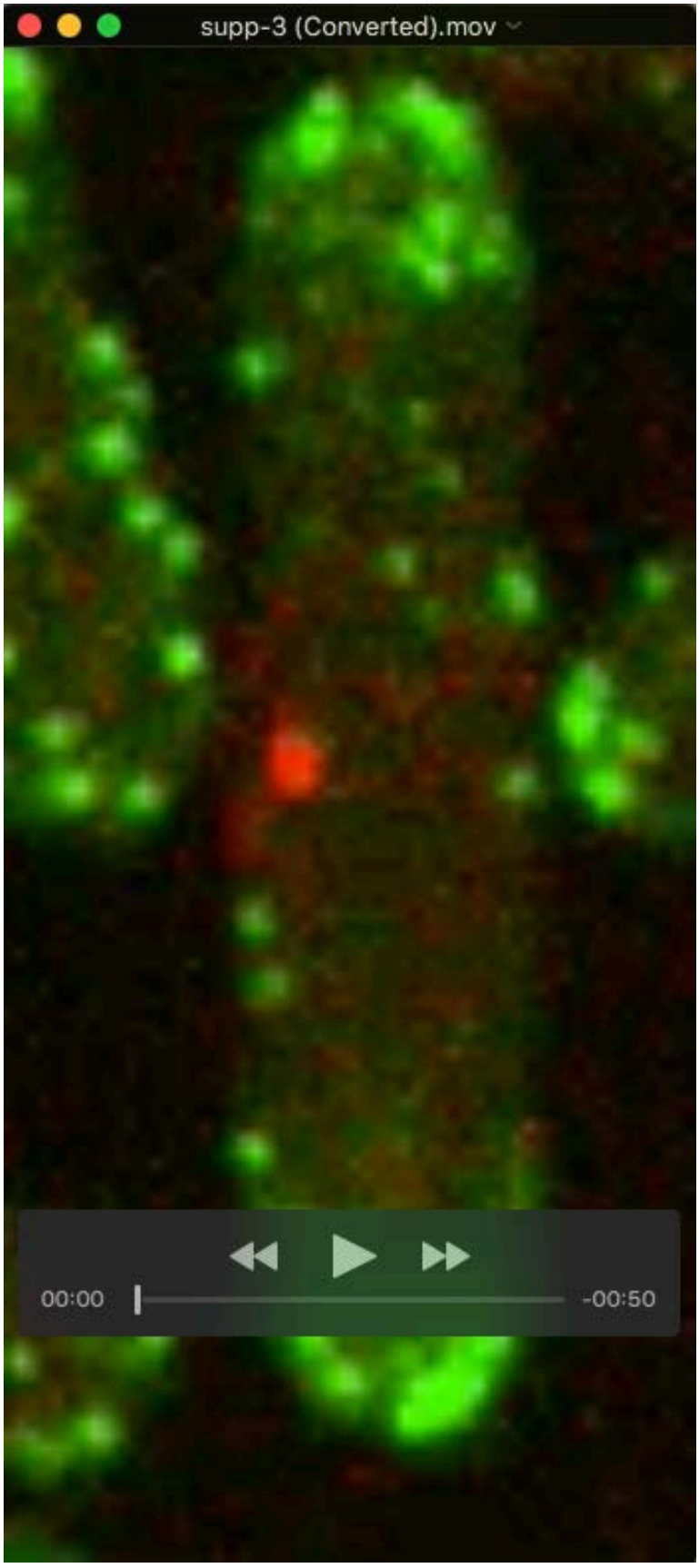


Figure S6. Measurement of Ergosterol levels after treatment with CK666

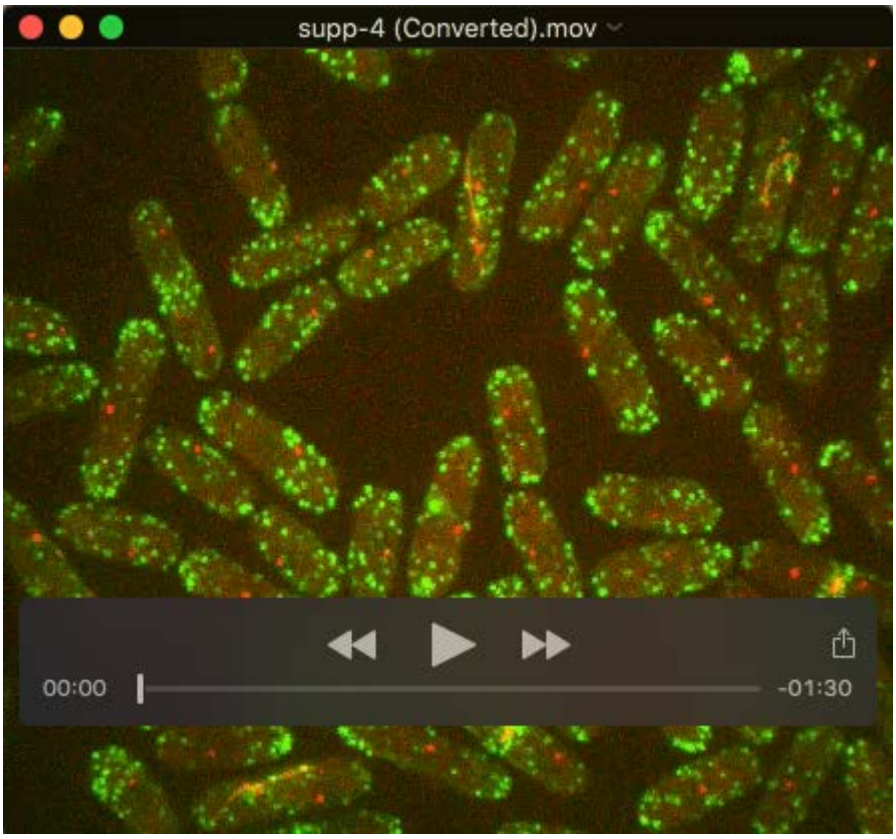
A-B: Filipin intensity expressed in a.u. was measured in control and Erg25 OE cells at time 0 (**A**) and after 1h of CK666 treatment (n=30 cells) (**B**). **C:** Graph showing the average curves of filipin intensity of control and Erg25 OE cells at time 0 and after 1h treatment with CK666.



Movie 1. F-actin dynamic distribution in a representative dividing control cell.



Movie 2. F-actin abnormal organization upon Erg25 OE.



Movie 3. Multiple examples of F-actin aberrant organization upon Erg25 OE in comparison with control cells.