

SUPPLEMENTAL MATERIAL

Supplemental Figure S1. Cell clustering is enhanced in *cdh1Δ* and *mlc2Δ* mutants. The percentages of cells in clusters of 3 or more were counted (see Materials and Methods) for strains Myo1-GFP (*MYO1-GFP*), GT158 (*MYO1-GFP mlc2Δ*), GT200 (*MYO1-GFP cdh1Δ*), and GT203 (*MYO1-GFP mlc2Δ cdh1Δ*).

Supplemental Figure S2. Myo1 dots persist in *cdh1Δ* cells throughout G1. *MYO1-GFP cdh1Δ* cells (strain GT200) were arrested in mitosis with nocodazole and then released, and images were acquired every 10 min until a new myosin ring and bud formed in the subsequent cell cycle. In 14 of 15 cells observed (93%), Myo1-GFP foci persisted throughout G1 as shown here. Bar, 1 μm.

Supplemental Figure S3. APC activity is required for myosin ring disassembly. Time-lapse microscopy was performed on strains GT074 (*MYO1-GFP pds1Δ clb5Δ SIC1^{10X}*) and GT073 (*MYO1-GFP pds1Δ clb5Δ SIC1^{10X} apc2Δ*), in which the APC is not essential. The *apc2Δ* mutation abolishes all APC activity. Images were captured at 1-min intervals; time point 0' marks the initiation of ring contraction. Bars, 1 μm.

Supplemental Figure S4. Defective Mlc1 ring disassembly in APC mutant cells. Strains (A) GT208 (*MLC1-GFP*) and (B) GT209 (*MLC1-GFP cdh1Δ*) were examined by time-lapse microscopy; time point 0' marks the initiation of ring contraction. Bars, 1 μm.

Supplemental Figure S5. Myo1 and Mlc2 co-localize after ring contraction in *cdh1Δ* cells.

Strain GT132 (*MYO1-Cherry MLC2-GFP cdh1Δ*) was examined by time-lapse microscopy. (A) Myo1-Cherry; (B) Mlc2-GFP; (C) merged fluorescence images; (D) corresponding DIC images. Bar, 1 μm.

Supplemental Figure S6. Myo1 and Iqg1 co-localize after ring contraction in *cdh1Δ* cells.

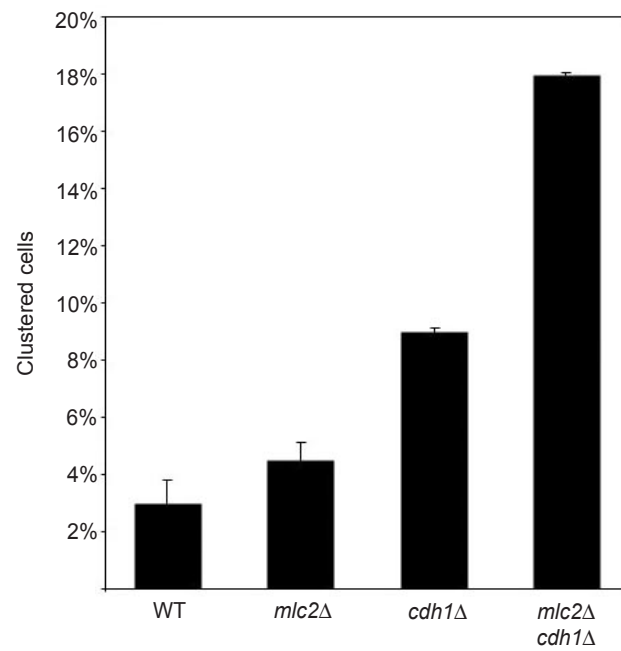
Strain GT133 (*MYO1-Cherry IQG1-GFP cdh1Δ*) was examined by time-lapse microscopy. (A) Myo1-Cherry; (B) Iqg1-GFP; (C) merged fluorescence images; (D) corresponding DIC images. Bar, 1 μm.

Supplemental Figure S7. Myo1 and Mlc1 partially co-localize after ring contraction in *cdh1Δ* cells. Strain GT225 (*MYO1-Cherry GFP-MLC1 cdh1Δ*) was examined by time-lapse microscopy. (A) Myo1-Cherry; (B) GFP-Mlc1; (C) merged fluorescence images; (D) corresponding DIC images. Bar, 1 μm.

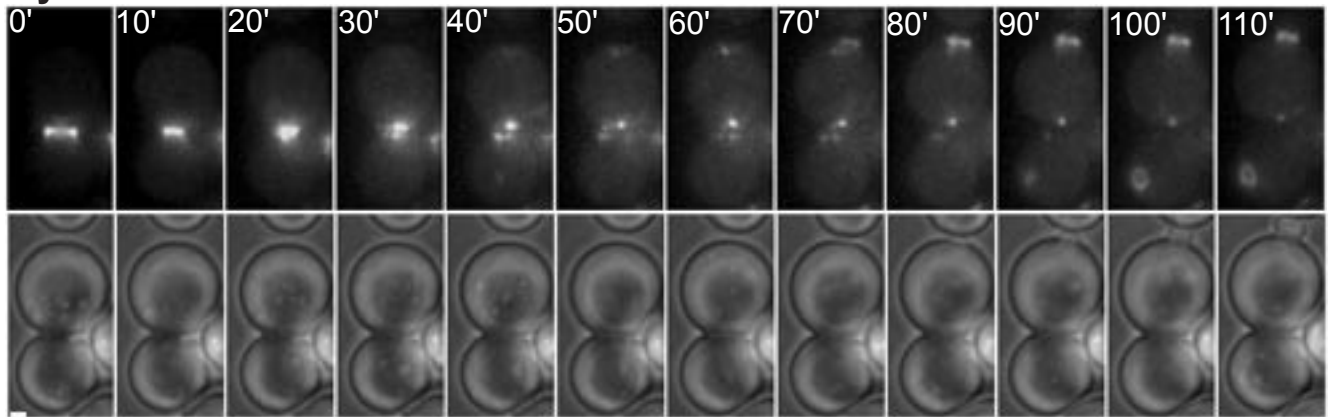
Supplemental Figure S8. Myo1 and Sec2 do not co-localize after ring contraction in *cdh1Δ* cells. Strain GT134 (*MYO1-Cherry SEC2-GFP cdh1Δ*) was examined by time-lapse microscopy. (A) Myo1-Cherry; (B) Sec2-GFP; (C) merged fluorescence images; (D) corresponding DIC images. Bar, 1 μm.

Supplemental Figure S9. Stabilization of the APC substrate Cdc5 does not block actomyosin-ring disassembly. Strain GT108, which expresses Myo1-GFP and the APC-resistant Cdc5Δ5-70, was examined by time-lapse microscopy. Bar, 1 μm.

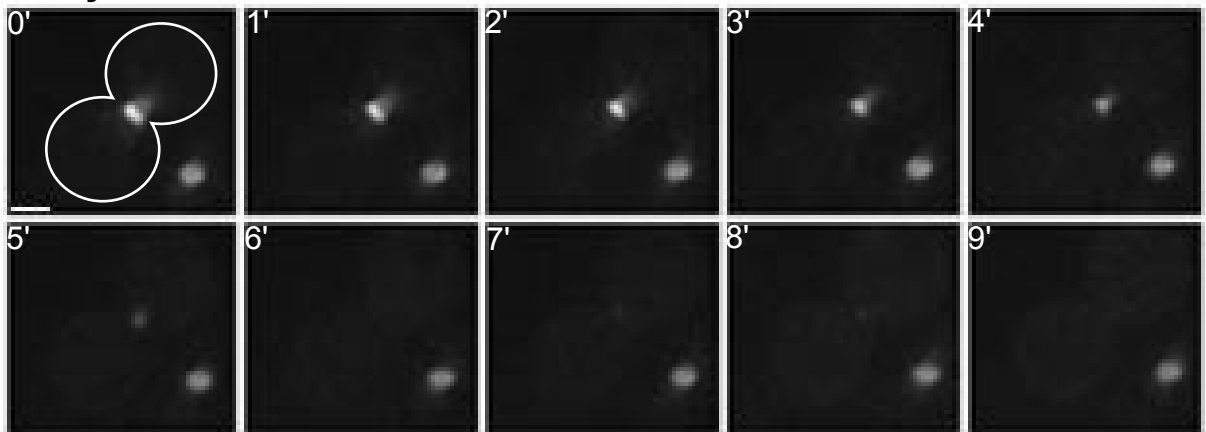
Supplemental Figure S10. Cdk1 inactivation is not sufficient to promote actomyosin-ring disassembly. Strain GT081 (*MYO1-GFP GAL-SIC1 cdh1Δ*) was examined by time-lapse microscopy beginning 3 h after the addition of galactose to induce expression of *SIC1*. A representative cell is shown (see also Table 2). Bar, 1 μm.



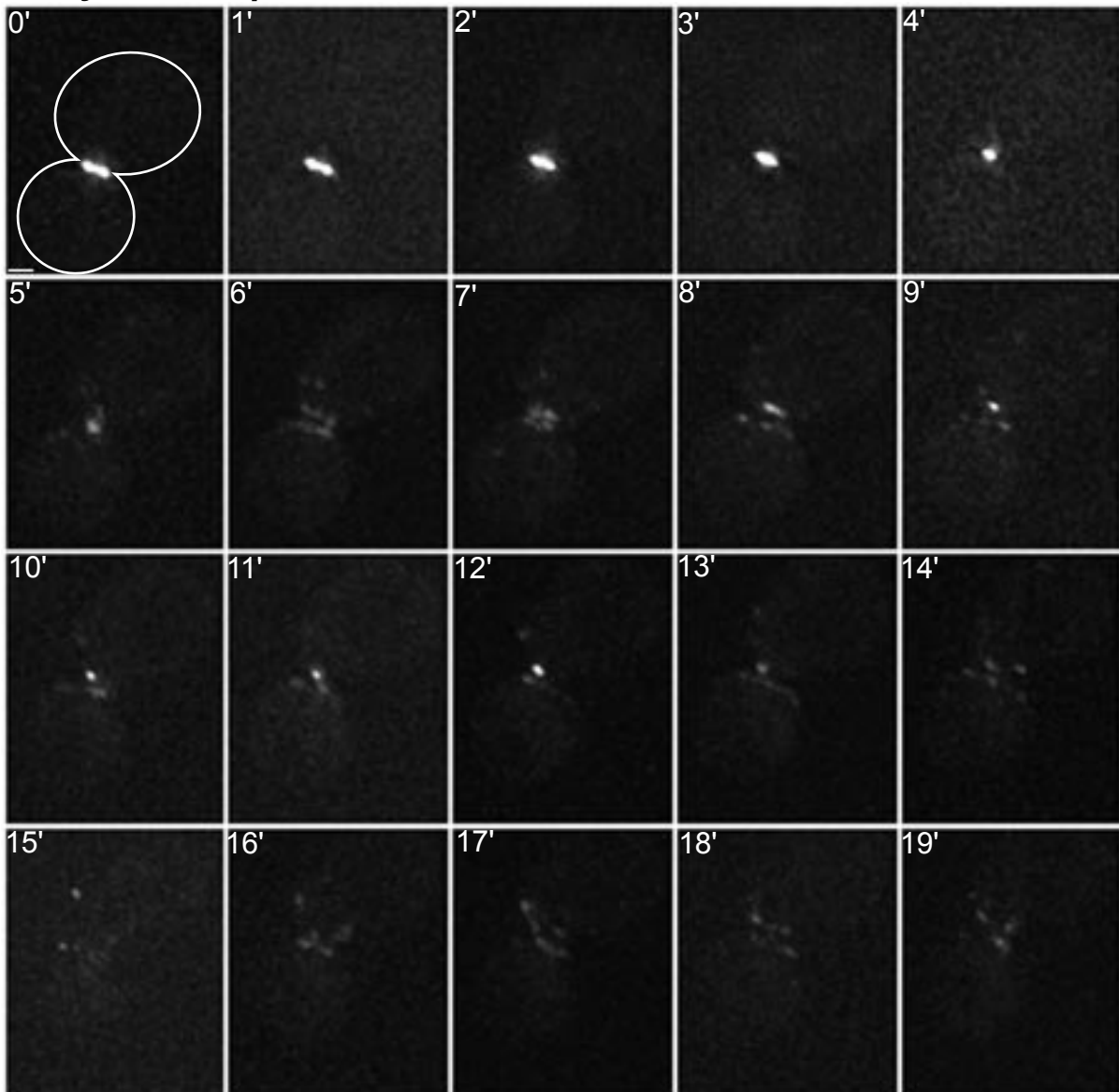
Myo1-GFP *cdh1* Δ



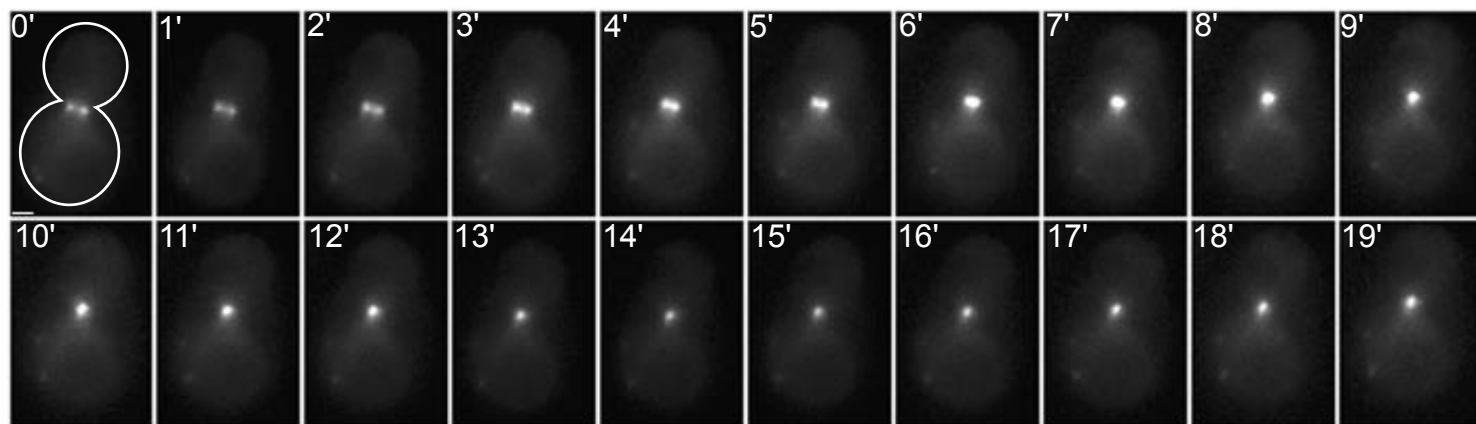
A. Myo1-GFP *APC2*



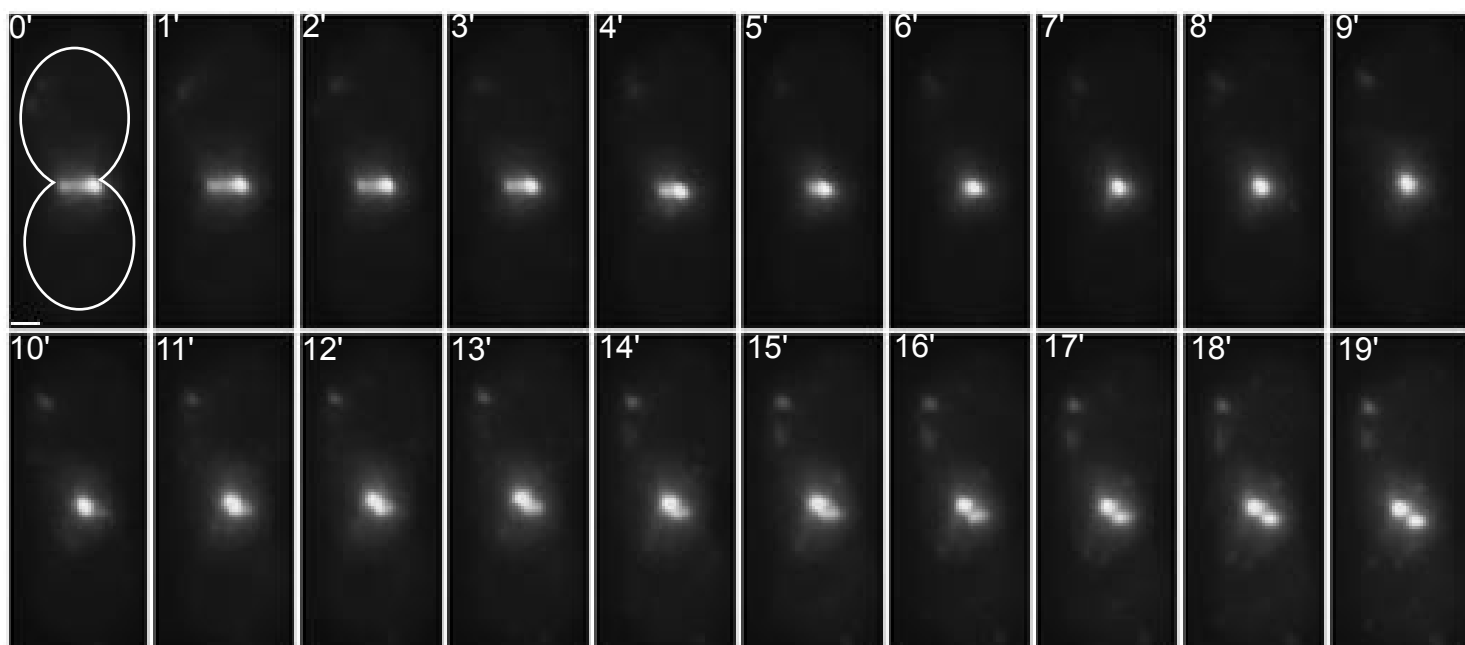
B. Myo1-GFP *apc2* Δ



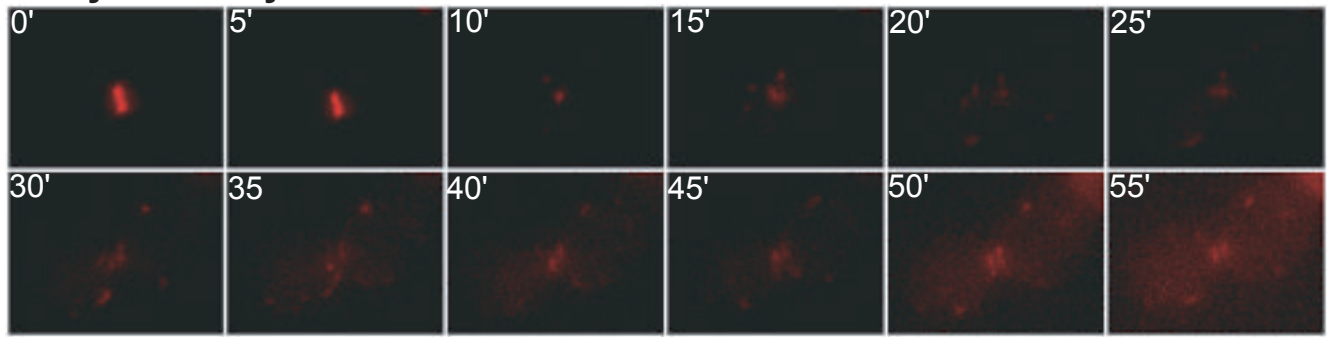
A. GFP-Mlc1



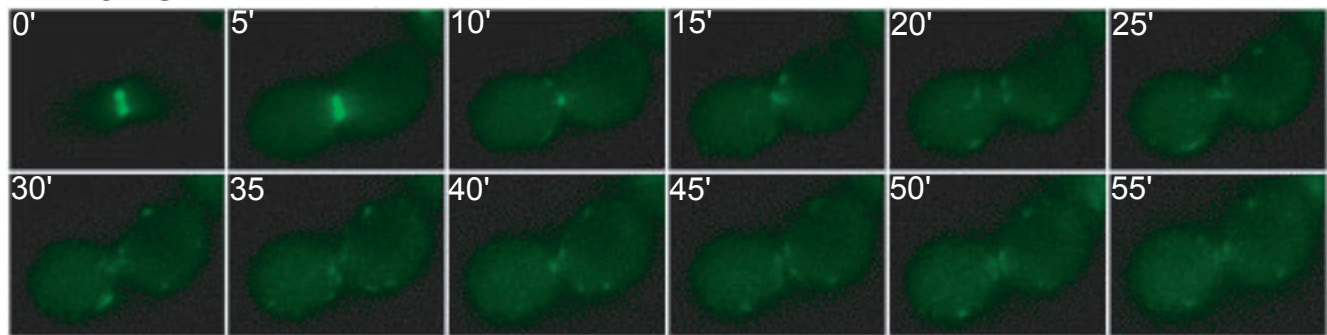
B. GFP-Mlc1 *cdh1* Δ



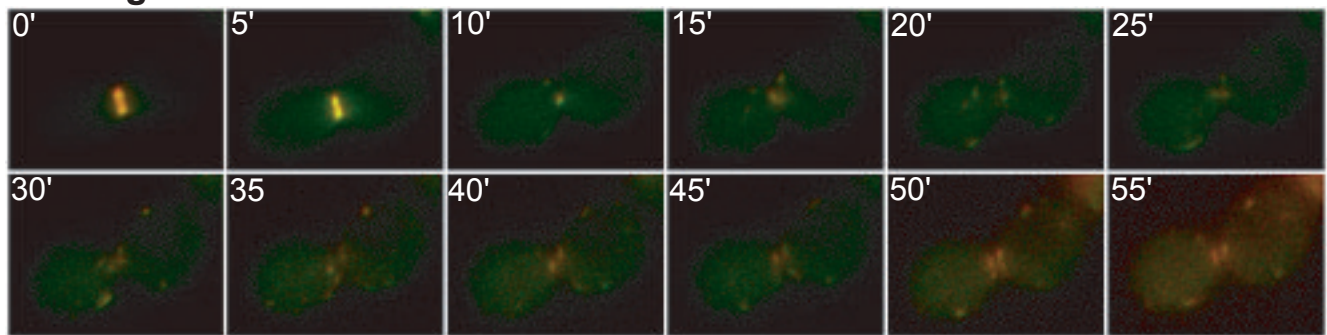
A. Myo1-Cherry



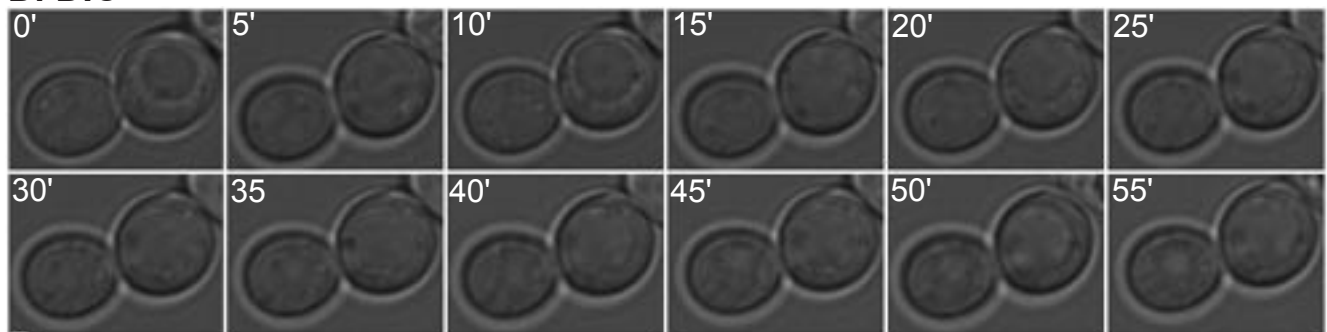
B. Mlc2-GFP



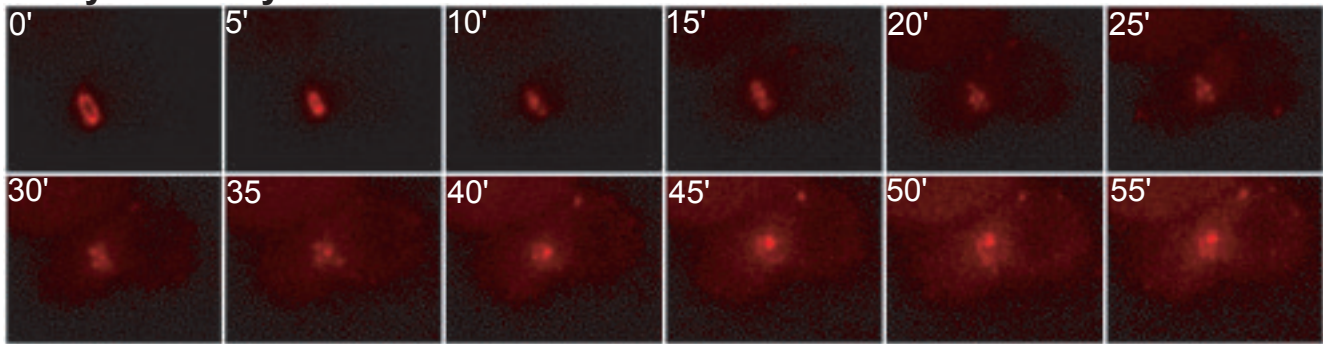
C. merge



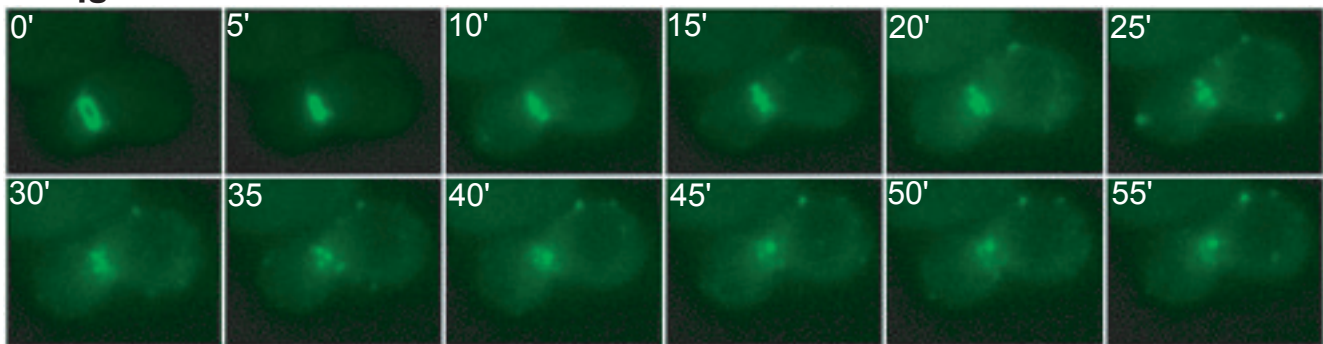
D. DIC



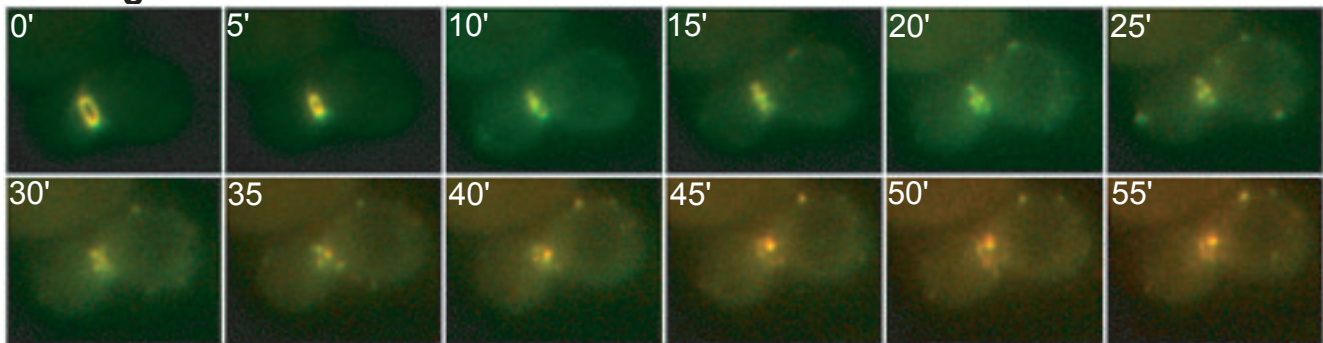
A. Myo1-Cherry



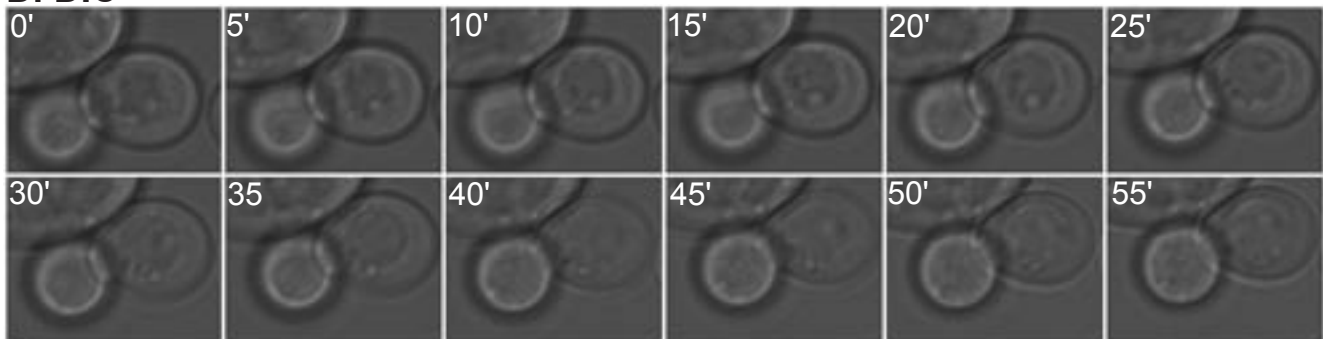
B. Iqg1-GFP



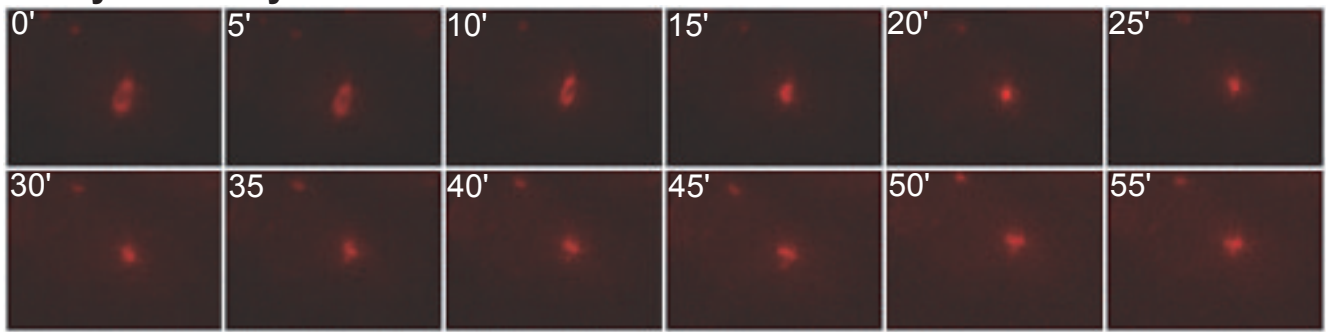
C. merge



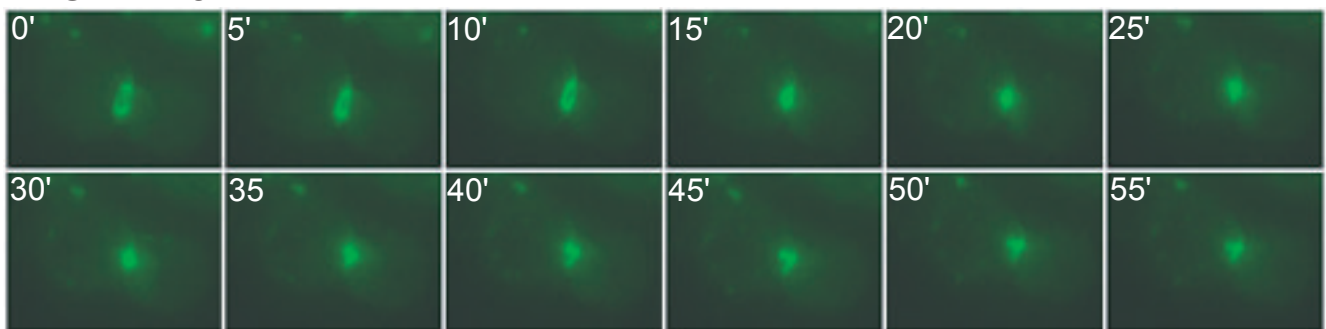
D. DIC



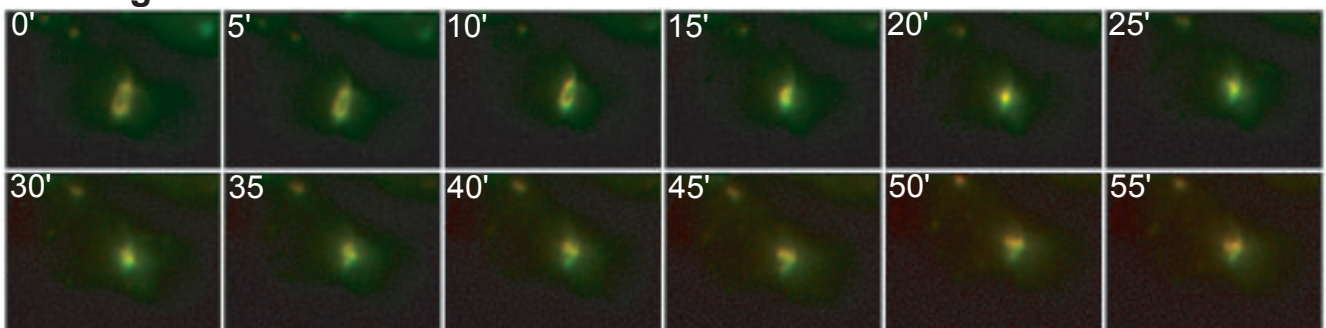
A. Myo1-Cherry



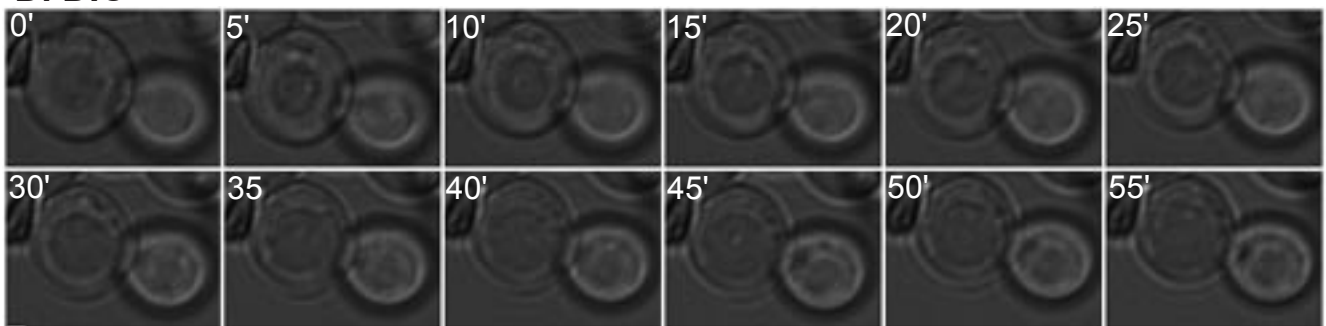
B. GFP-Mlc1



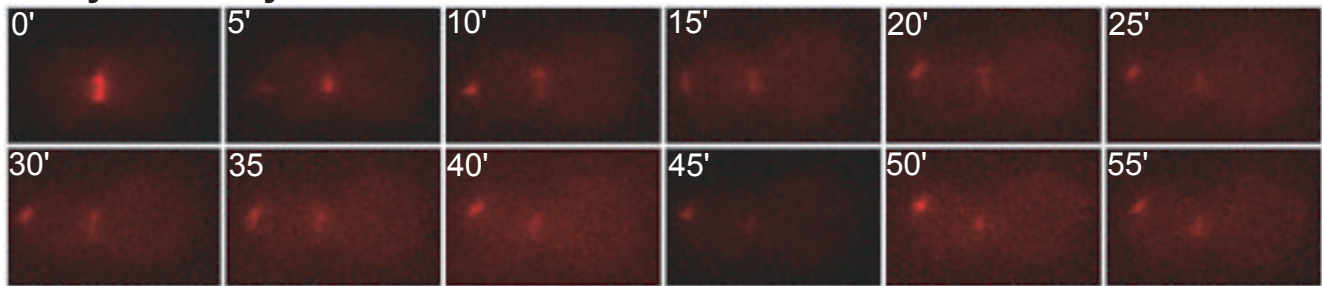
C. merge



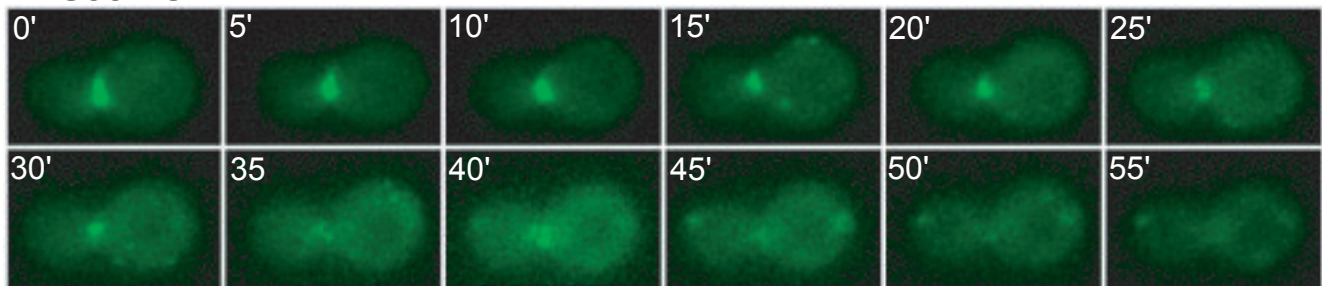
D. DIC



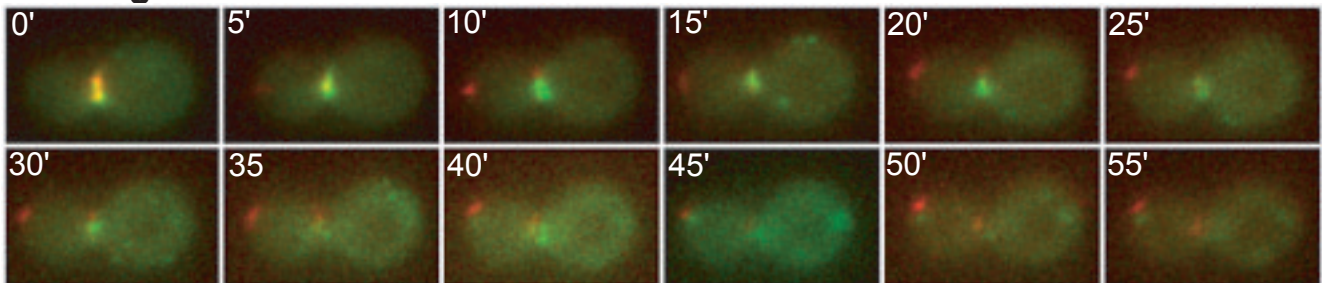
A. Myo1-Cherry



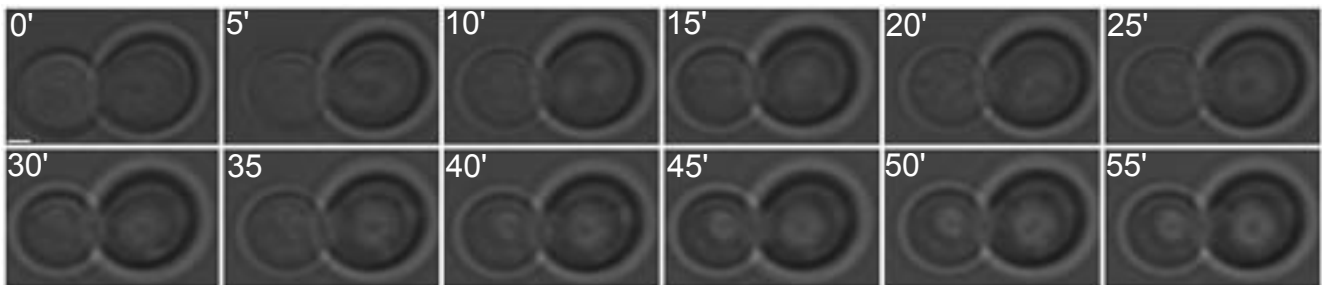
B. Sec2-GFP



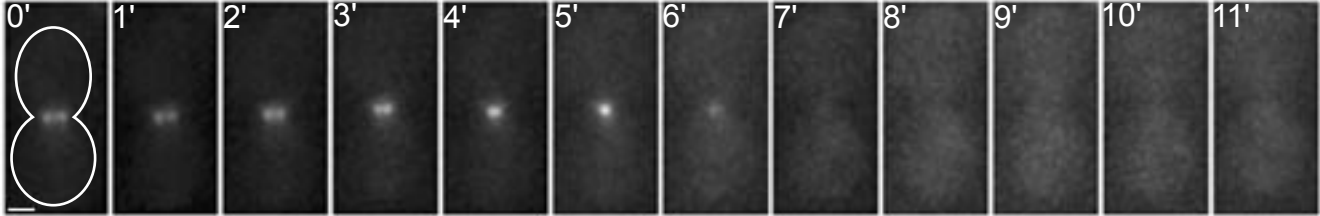
C. merge



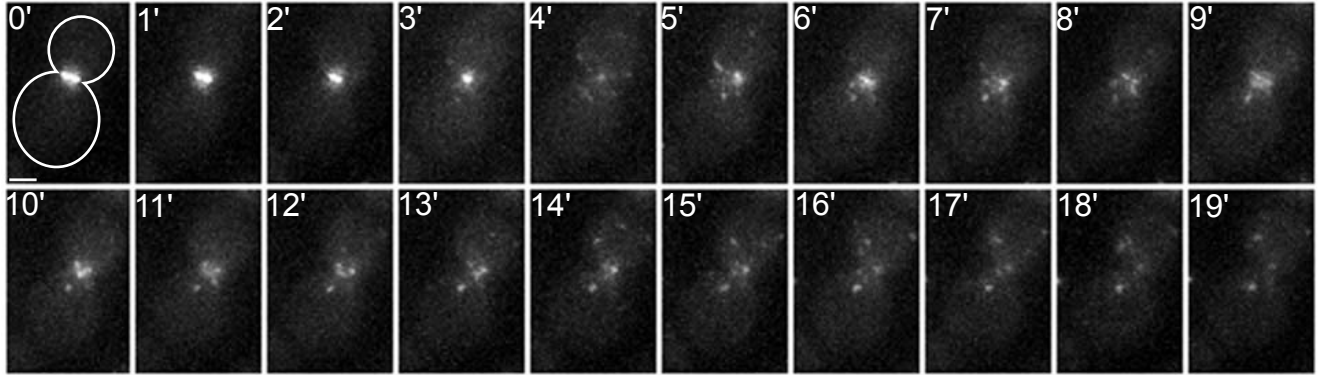
D. DIC



Myo1-GFP *CDC5* Δ 5-70



Myo1-GFP *GAL-SIC1* *cdh1* Δ



Supplemental Table S1. Myo1-Cherry co-localizes with Mlc2-GFP, Iqg1-GFP, and Mlc1-GFP.

| Strain | Genotype | Per cent co-localization | Number of Myo1-Cherry cells |
|--------|-----------------------------------|--------------------------|-----------------------------|
| GT132 | <i>MLC2-GFP MYO1-Cherry cdh1Δ</i> | 100% | 8 |
| GT133 | <i>IQG1-GFP MYO1-Cherry cdh1Δ</i> | 100% | 14 |
| GT225 | <i>GFP-MLC1 MYO1-Cherry cdh1Δ</i> | 73% | 11 |
| GT134 | <i>SEC2-GFP MYO1-Cherry cdh1Δ</i> | 13% | 8 |

This table displays the percentages of GFP and Cherry patches that co-localized for the indicated strains. The numbers of cells scored (based on Myo1-Cherry being visible at ≥ 10 min after the completion of ring contraction) are also shown.