

# **Supplemental Materials**

*Molecular Biology of Cell*

Feng et al.

## ONLINE SUPPLEMENTAL VIDEOS

**Video 1.** Time-lapse analysis of GFP-Mlc1 and Cdc3-mCherry at the bud neck during the cell cycle in cells expressing GFP-Mlc1 as the sole source of Mlc1 (YEF6800) at 25°C (related to Supplemental Figure S1). 3-min interval. Scale bar, 2  $\mu$ m.

**Video 2.** Time-lapse analysis of GFP-Mlc1 and Cdc3-mCherry at the bud neck during the cell cycle in *cdc12-6* cells (YEF6884) at 39°C in the absence (left) or presence (right) of LatA (related to Figure 1, B and D). 2-min interval. Scale bar, 2  $\mu$ m.

**Video 3.** Time-lapse analysis of Myo1-GFP and Cdc3-mCherry at the bud neck during the cell cycle in *cdc12-6* cells (YEF7155) at 39°C in the absence (left) or presence (right) of LatA (related to Figure 2, A and B). 2-min interval. Scale bar, 2  $\mu$ m.

**Video 4.** Time-lapse analysis of GFP-Mlc1 and Cdc3-mCherry at the bud neck during the cell cycle in *cdc12-6 myo1 $\Delta$*  cells (YEF7081) at 39°C in the absence (left) or presence (right) of LatA (related to Figure 2, C and D). 2-min interval. Scale bar, 2  $\mu$ m.

**Video 5.** Time-lapse analysis of GFP-Mlc1 and Cdc3-mCherry at the bud neck during the cell cycle in WT cells (YEF7435) at 25°C in the absence (left) or presence (right) of LatA (related to Figure 3A). 2-min interval. Scale bar, 2  $\mu$ m.

**Video 6.** Time-lapse analysis of GFP-Mlc1 and Cdc3-mCherry at the bud neck during the cell cycle in *bnr1 $\Delta$*  (YEF7200) (left) or *bni1 $\Delta$*  cells (YEF7201) (right) at 25°C (related to Figure 4A). 2-min interval. Scale bar, 2  $\mu$ m.

**Video 7.** Time-lapse analysis of GFP-Mlc1 and Cdc3-mCherry at the bud neck during the cell cycle in *myo1 $\Delta$*  cells (YEF7132) at 25°C in the absence (left) or presence (right) of LatA (related to Figure 5A). 2-min interval. Scale bar, 2  $\mu$ m.

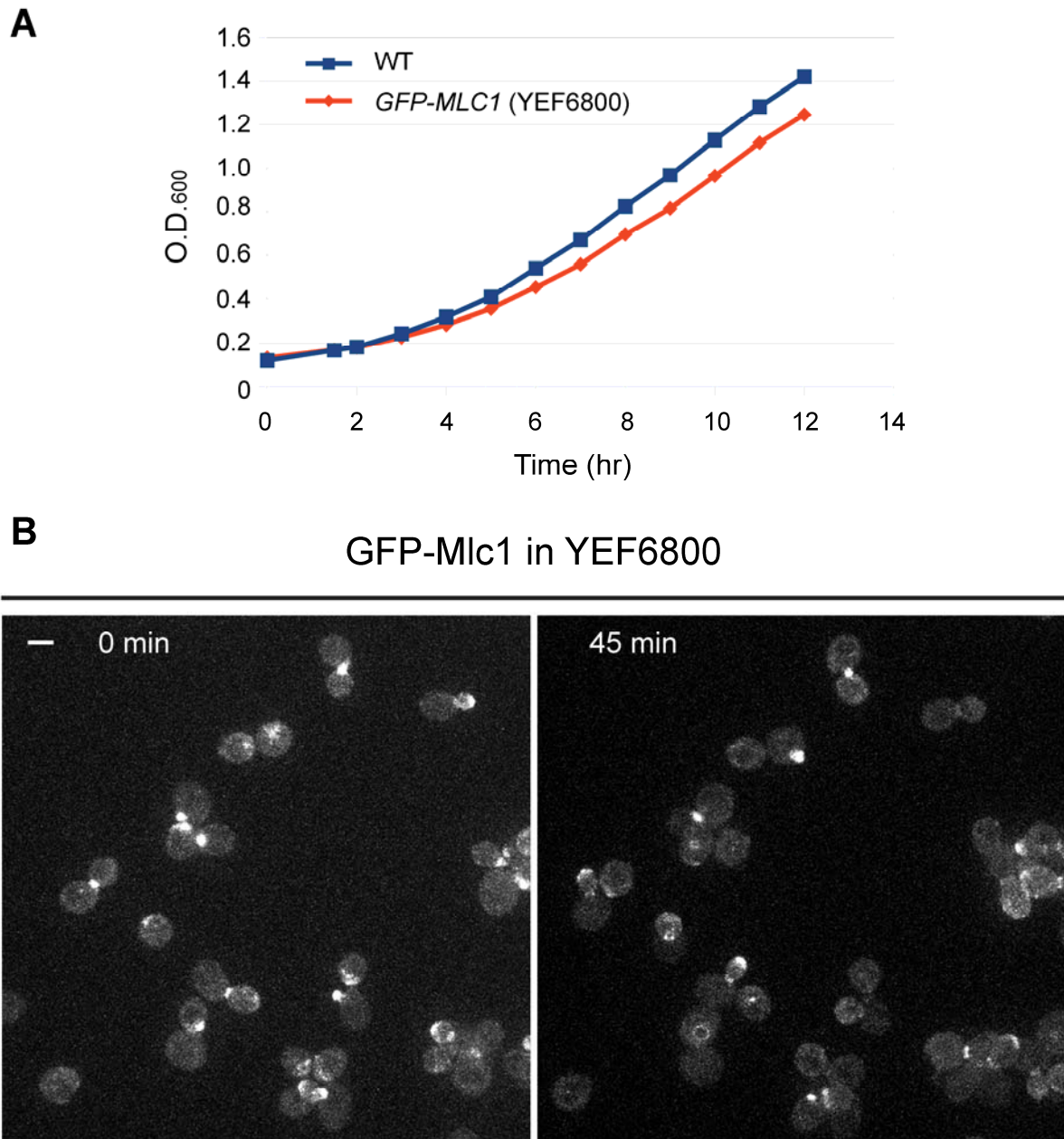
**Video 8.** Time-lapse analysis of GFP-Mlc1 and Cdc3-mCherry at the bud neck during the cell cycle in *bni5 $\Delta$*  cells (YEF7259) at 25°C (Supplemental Figure S2). 2-min interval. Scale bar, 2  $\mu$ m.

**Video 9.** Time-lapse analysis of GFP-Mlc1 and Cdc3-mCherry at the bud neck during the cell cycle in *myo1 $\Delta$  myo2IQ6 $\Delta$  myo4 $\Delta$*  cells (YEF7055) at 25°C in the absence (left) or presence (right) of LatA (related to Figure 6C). 2-min interval. Scale bar, 2  $\mu$ m.

**Video 10.** Time-lapse analyses of GFP-Mlc1-Nterm and Cdc3-mCherry at the bud neck during the cell cycle in WT cells (YEF7208) at 25°C (first column from the left), and of GFP-Mlc1-Cterm and Cdc3-mCherry at the bud neck during the cell cycle in WT (YEF7209) (second column), *myo2IQ6 $\Delta$*  (YEF7245) (third column), and LatA-treated WT (YEF7209) (last column) cells at 25°C (related to Figure 7, B-E). 2-min interval. Scale bar, 2  $\mu$ m.

## SUPPLEMENTAL FIGURE LEGENDS

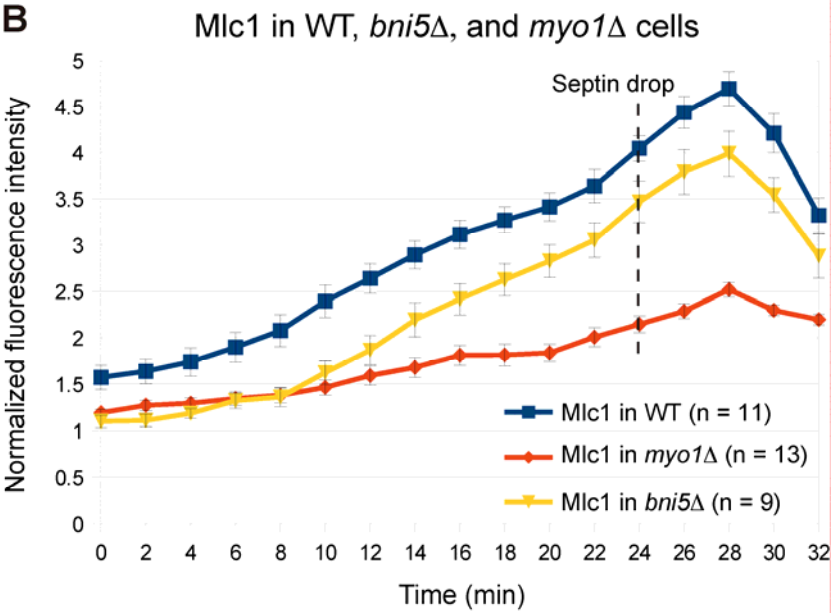
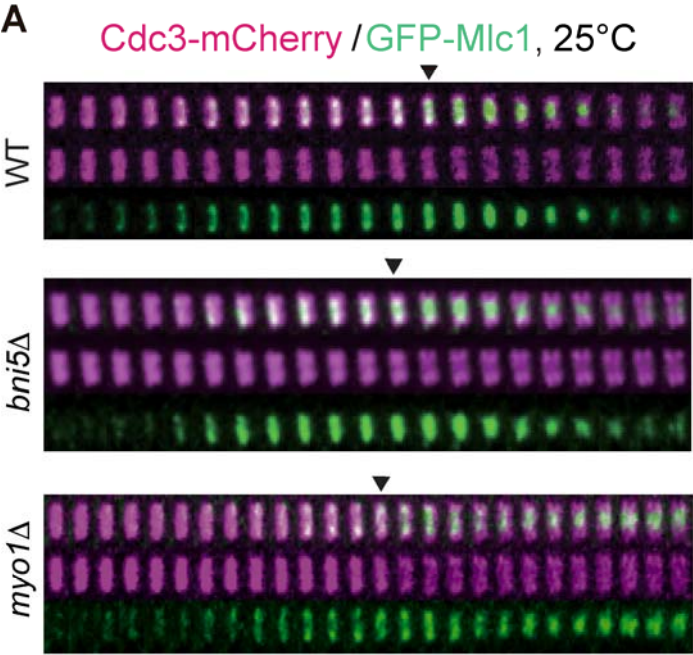
### Feng et al. Supplemental Figure 1



**Supplemental Figure 1.** GFP-Mlc1 can function as the sole source of Mlc1 in the cell. (A) *GFP-MLC1*, in place of the endogenous *MLC1*, does not cause significant defects in cell growth. Wild-type (WT) (YEF5804, *CDC3-mCherry*) and *GFP-MLC1* (YEF6800, *GFP-MLC1*

*CDC3-mCherry*) strains were grown in liquid SC-Leu medium at 25°C and their optical densities at 600 nm (O.D.<sub>600</sub>) were followed over time. In YEF6800, the endogenous *MLC1* was replaced by *GFL-MLC1* (see Materials and Methods for details). (B) Cells expressing GFP-Mlc1 as the sole source of Mlc1 display normal morphology and Mlc1 localization during the cell cycle (see also Supplemental Video S1). Cells of YEF6800 were followed by time-lapse analysis. Two selected frames (0 min and 45 min) of the time-lapse series are shown here. Scale bars, 2 μm.

Feng et al. Supplemental Figure 2



**Supplemental Figure 2.** Bni5 plays a minor role in Mlc1 localization before cytokinesis. (A) Time-lapse analysis of Mlc1 and Cdc3 at the bud neck during the cell cycle in WT (YEF7070; *GFP-MLC1 CDC3-mCherry*), *bni5Δ* (YEF7259; *bni5Δ GFP-MLC1 CDC3-mCherry*) (see also Supplemental Video S8), and *myo1Δ* (YEF7132; *myo1Δ GFP-MLC1 CDC3-mCherry*) cells. (B) A direct comparison of Mlc1 localization profiles at the bud neck of WT, *bni5Δ*, and *myo1Δ* cells. All cells were grown in SC-Leu media at 25°C. Arrows (A) or dashed line (B) indicates the beginning of the septin-intensity drop during the cell cycle.