

# Supplemental Materials

*Molecular Biology of the Cell*

Dhani et al.

### Supplementary videos

0h\_depletion

22h\_depletion

25h\_depletion

KT3711 *h<sup>90</sup> ade6-M216 leu1<sup>+</sup>::P<sub>nmt81</sub>-mzt1-S-GFP-2xFLAG P<sub>nda3</sub>-mCherry-atb2-aur1<sup>R</sup>  
mzt1::Hyg<sup>R</sup>*

### Supplementary Figure S1. Examination of the Mzt1 depletion phenotypes.

Cells that are depleted for Mzt1 for 0 hour, 22 hours and 25 hours were filmed for 20 min at 1.5 min interval to record the Mzt1-GFP and mCherry-Atb2 signals. At each timepoint, 15 to 20 images along the Z-axis were taken at 0.4  $\mu$ m intervals to span the whole thickness of the cells and these are Z-projected to produce one flat image per timepoint. For a quantitative analysis, 130 cells (0 h depletion), 214 cells (22 h depletion) and 183 cells (25 h depletion) were observed and classified into categories depicted in the supplementary figure S2. Representative images of one timepoint of 0, 22 and 25 h depleted cells are presented. Corresponding movies for these images are provided as Supplementary movies.

### Supplementary Figure S2.

Cartoon representations of categories of Mzt1 depletion phenotypes used in the quantitative analyses presented in Supplementary figure S1 and Figure 3G.

### Supplementary Figure S3.

Sequence alignment of fission yeast GCP3<sup>Alp6</sup> and human GCP3 by clustalW. Amino acids 118-186, which are predicted to be involved in Mzt1 binding, are indicated in yellow. The human GCP3 residues (551-555), proposed to be at the "hinge" region (Guillet *et al.*, 2011), and corresponding Alp6 residues (499-503) are indicated in red.

### Supplementary videos

0h\_depletion.mov, 22h\_depletion.mov, 25h\_depletion.mov

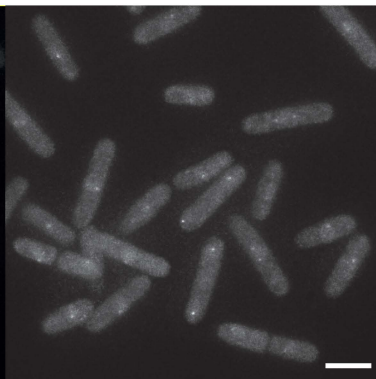
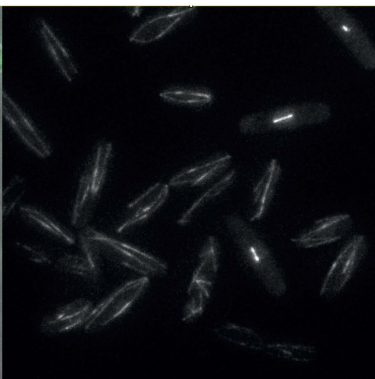
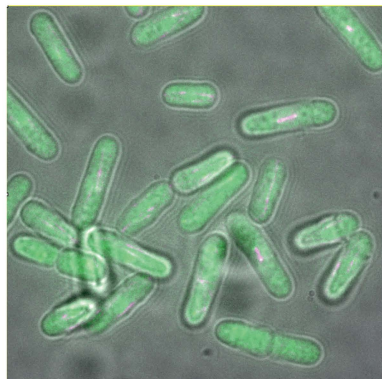
Live cell images of the cells presented in the Supplementary figure S1. GFP tagged Mzt1 was depleted for 0 h, 22 h and 25 h respectively and signals of mCherry tagged  $\alpha$ -tubulin Atb2 was observed at every 90 sec for 20min.

Merged with Brightfield

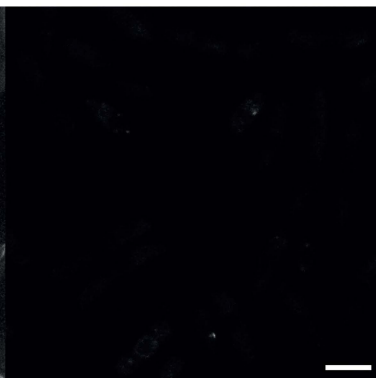
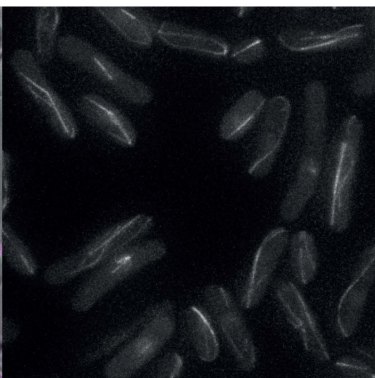
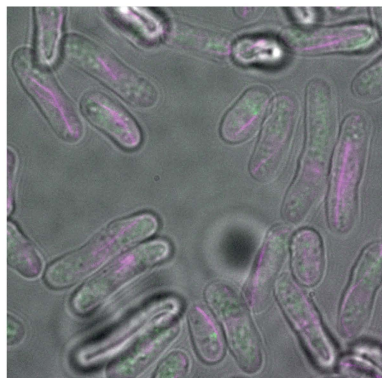
mCherry-Atb2

Mzt1-GFP

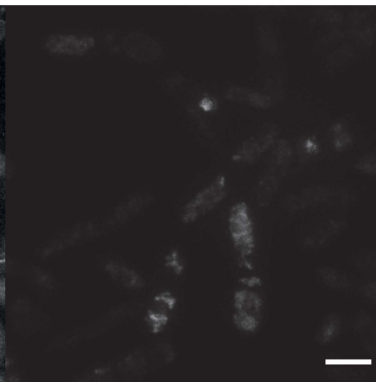
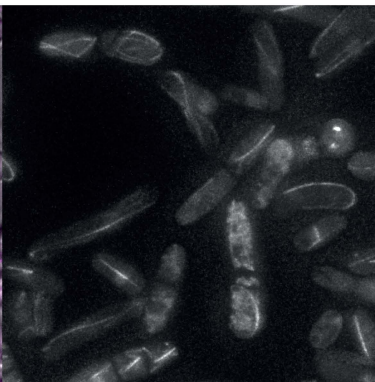
0h



22h

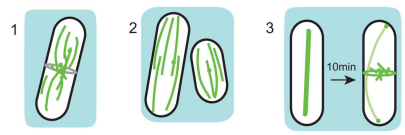


25h

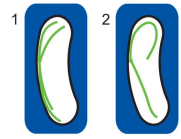


Classification of MT status

Normal interphase



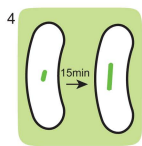
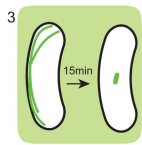
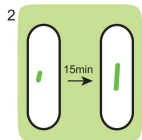
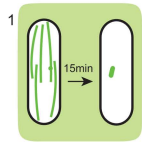
Abnormal interphase



Cytokinesis defect



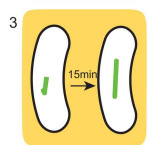
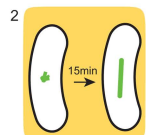
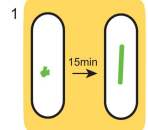
Normal mitosis



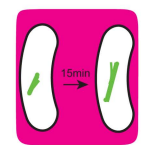
Normal mitosis  
PAA missing



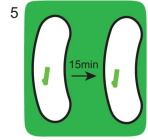
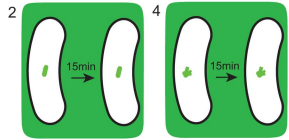
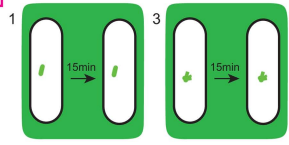
Monopolar spindle  
-> delay  
-> Anaphase



Aberrant spindle shape  
-> Anaphase



Complete arrest in mitosis



Faded mitosis

