Supplemental Materials

Molecular Biology of the Cell

Lee et al.

Regulation of spindle-pole body assembly and cytokinesis by the centrin-binding protein Sfi1 in fission yeast

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Supplemental materials

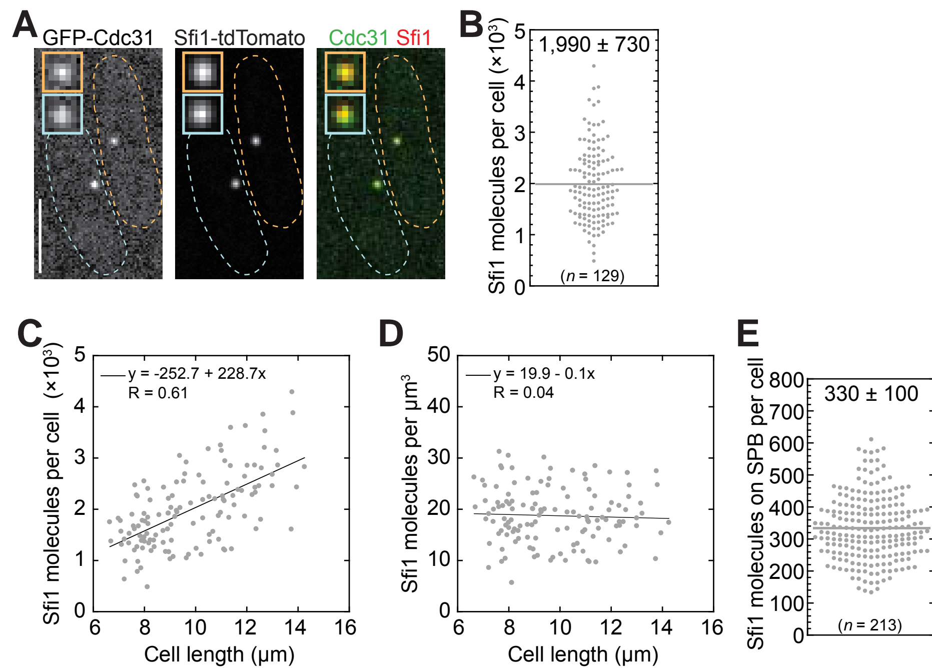
VIDEO LEGENDS

SUPPLEMENTAL VIDEO S1. The first mitotic division of four germinated spores from an *sfi1Δ/sfi1*$^{+}$ tetrad. Germinating spores from a tetrad (strain JW5364) were positioned on a YE5S agar plate using a tetrad microscope and the agar medium was inverted onto a dish for imaging. Pcp1-GFP (green) and mRFP-Atb2 (red) were imaged with 10 min interval for a total time of 3 h 30 min. The maximum intensity projections from 11 slices spaced at 0.6 μm at each time point are merged and shown. Related to Figure 2C. Display rate: 2 frames per second (fps).

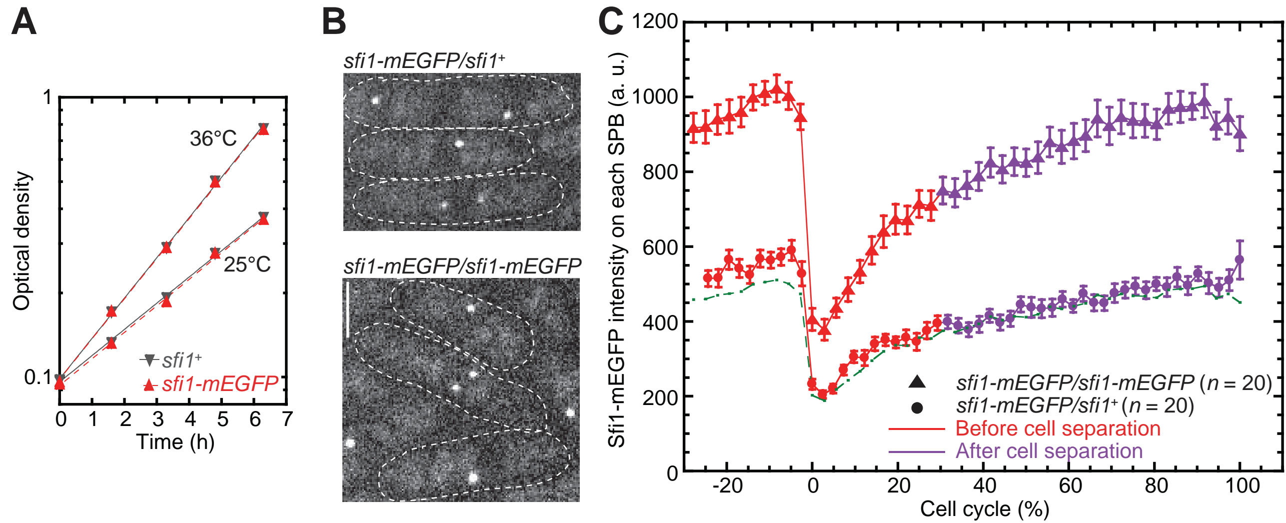
SUPPLEMENTAL VIDEO S2. The second mitotic division of four germinated spores from an *sfi1Δ/sfi1*$^{+}$ tetrad. Germinating spores from a tetrad (strain JW5364) were positioned on a YE5S agar plate using a tetrad microscope, incubated at 25°C for ~12 h, and the agar medium was inverted onto a cover slip for imaging. Pcp1-GFP (green) and mRFP-Atb2 (red) were imaged with 10 min interval for a total time of 6 h 30 min. Cells went out of focus in some time points and those images were not included in the video. The maximum intensity projections from 11 slices spaced at 0.6 μm at each time point are merged and shown. Related to Figure 2D. Display rate: 2 fps.

SUPPLEMENTAL VIDEO S3. Partition of Sfi1-mEGFP and the formation of bipolar spindles in *sfi1*$^{+}$ cells. Sfi1 (green) and Atb2 (red) were imaged with 10 min interval for a total time of 6 h 30 min on a dish covered with a piece of YE5S agar. Strain JW4829 was used. The maximum intensity projections of the fluorescence images from 16 slices spaced at 0.4 μm at each time point are merged with the DIC image taken at the middle of the stack. Related to Figure 6C. Display rate: 2 fps.

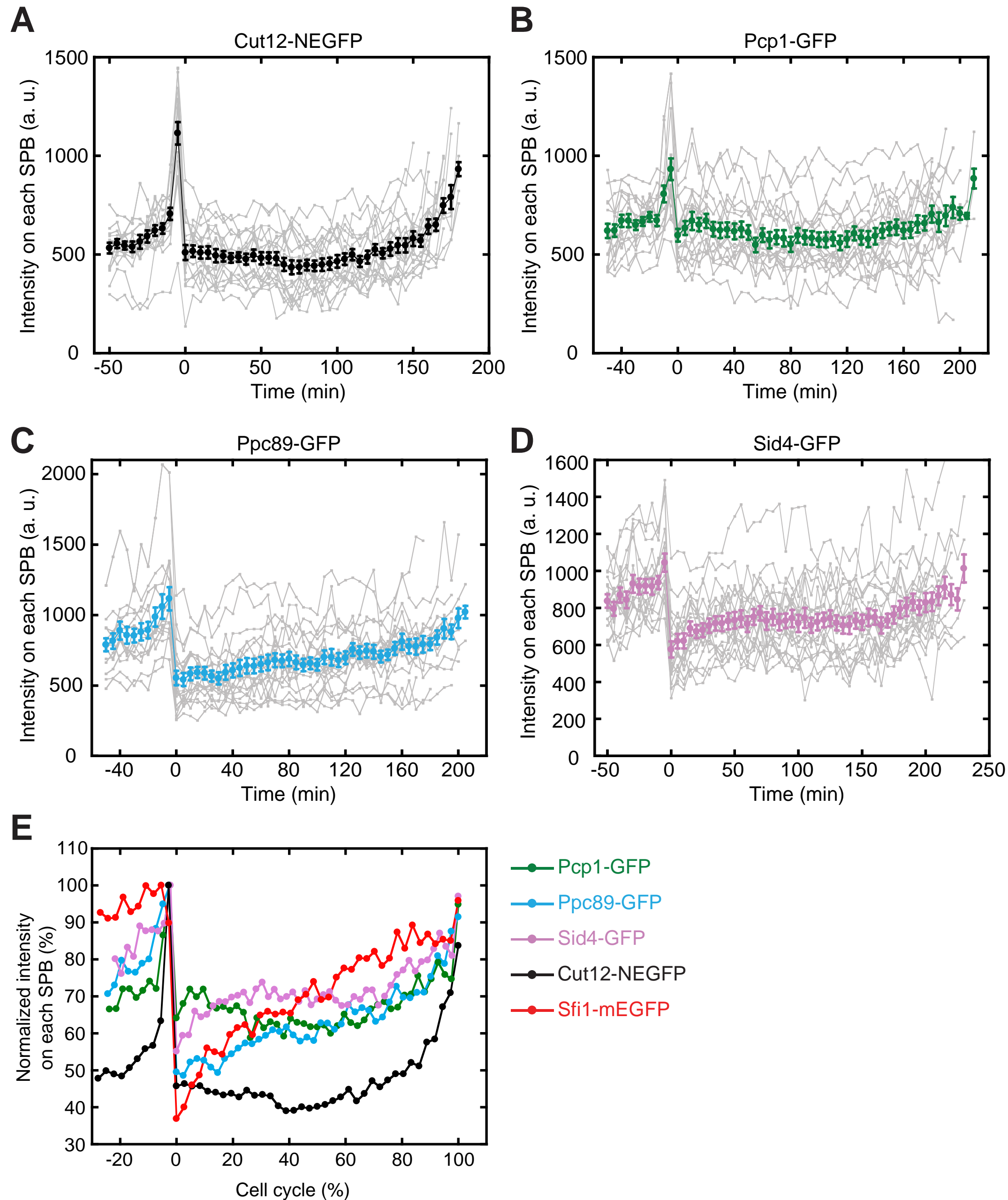
SUPPLEMENTAL VIDEO S4. Unequal partition of Sfi1-M46-mEGFP and the formation of spindles in *sfi1-M46* cells. Sfi1-M46 (green) and Atb2 (red) were imaged with 10 min interval for a total time of 12 h on a dish covered with a piece of YE5S agar. Strain JW4831 was used. The maximum intensity projections of the fluorescence images from 16 slices spaced at 0.4 μm at each time point are merged with the DIC image taken at the middle of the stack. Related to Figure 6D. Display rate: 2 fps.



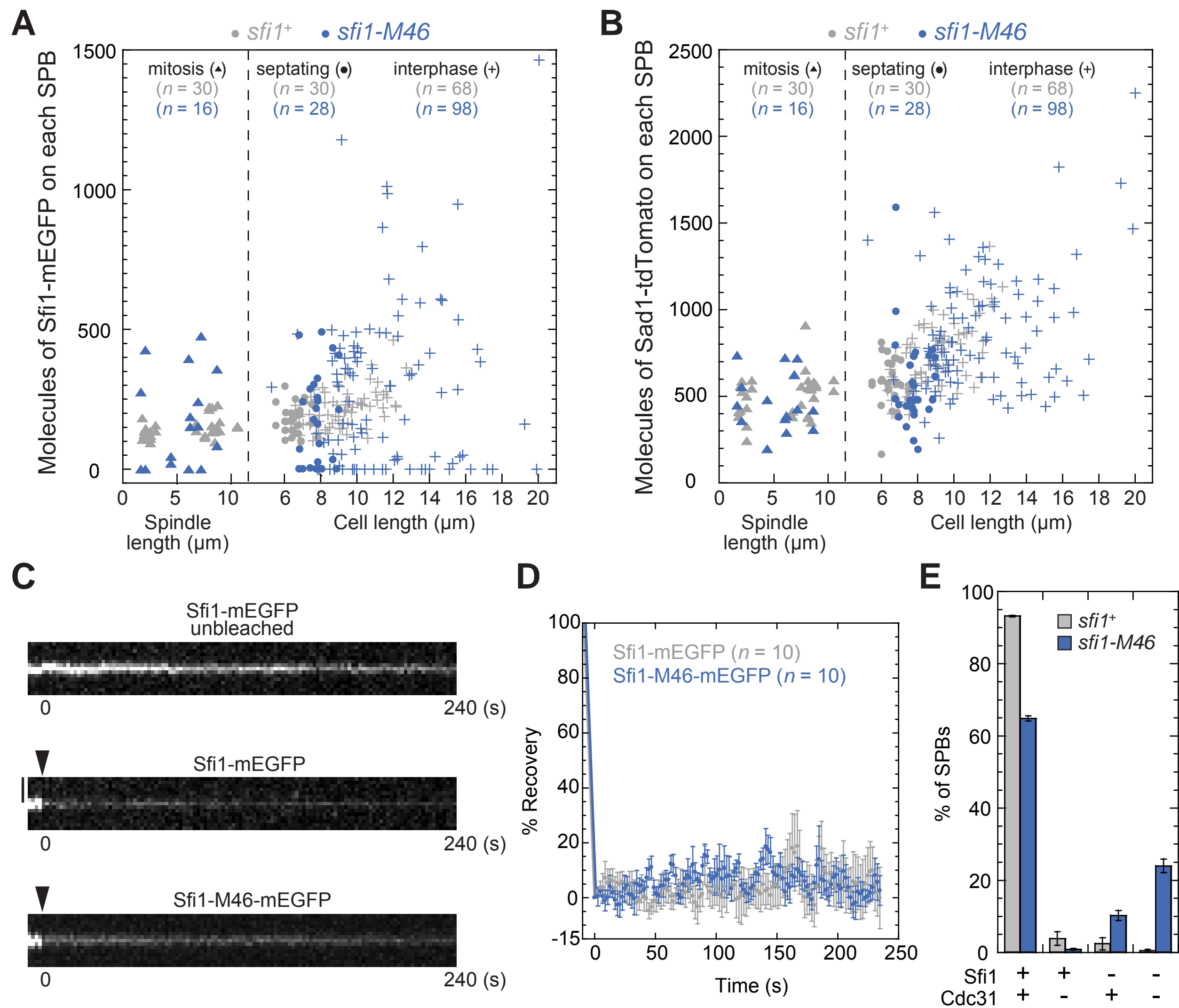
SUPPLEMENTAL FIGURE S1: Sfi1 localization and concentration. (A) Colocalization of Sfi1 and the centrin Cdc31 in the best focal plane (JW4675). The insets show the SPBs magnified 2-fold. Bar, 5 µm. (B) Numbers of Sfi1 molecules in whole cells (JW4361). (C) Correlation between Sfi1 number and cell length. (D) Lack of correlation between Sfi1 concentration and cell length. (E) Numbers of Sfi1 molecules on SPBs (JW4361). The sum was used for each cell that had two SPBs.



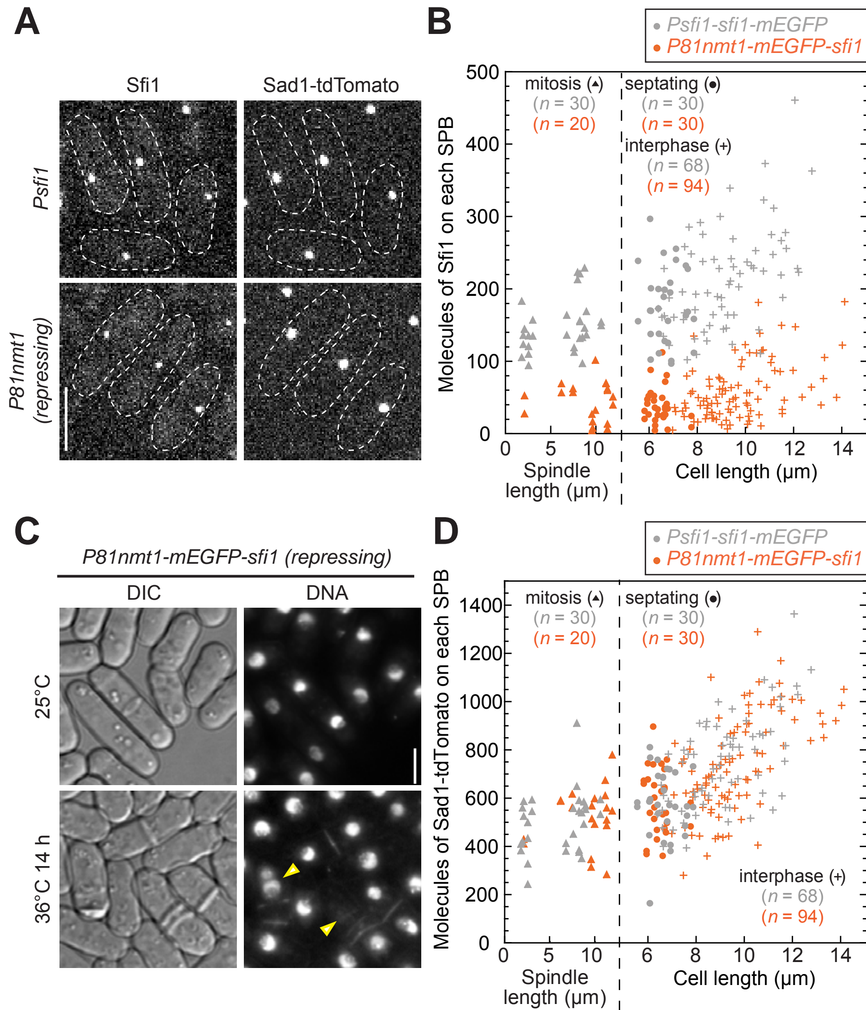
SUPPLEMENTAL FIGURE S2: The functionality of Sfi1-mEGFP. (A) Growth curves of *sfi1*$^+$ (JW729) and *sfi1-mEGFP* (JW4361) cells at 25 and 36°C. Mean optical densities (at 595 nm) from three independent experiments are shown in a semi-log plot. All SDs were < 0.025. (B and C) Micrographs of Sfi1 localization (B) and quantitative assessment of Sfi1 recruitment (C) to SPBs in *sfi1-mEGFP/sfi1*$^+$ (JW5933) and *sfi1-mEGFP/sfi1-mEGFP* (JW5936) cells. In C, mean fluorescence intensities (± SEMs) in arbitrary units (a. u.) of 20 separated SPBs are plotted. Green dashed line shows 50% of the mean intensities in *sfi1-mEGFP/sfi1-mEGFP* cells for comparison. Because the diploid strains have a slightly slower cell cycle than haploids, time is converted to percent cell cycle to aid comparison to Figure 3D. Bar, 5µm.



SUPPLEMENTAL FIGURE S3: Recruitment of various SPB proteins during the cell cycle. (A-D) The fluorescence intensities of Cut12 (A), Pcp1 (B), Ppc89 (C), and Sid4 (D) on each SPB were analyzed in strains IH1711, JW5292, JW4639, and JW4632, respectively. To see the trends clearly, cells that re-entered mitosis at significantly shorter or longer times than usual were not analyzed. Note that *cut12-NEGFP* cells had a relatively short cell-cycle time due to the *ura4*$^{+}$ marker, and *sid4-GFP* cells had a relatively long cell-cycle time, probably due to the tagging of Sid4. Each gray line represents one SPB, and the colored lines represent the means ± SEMs of 20 separated SPBs. Time zero is the time point at which the two SPBs visibly separated. (E) Comparison of the intensities of five proteins on SPBs during the cell cycle. The mean fluorescence intensity at 10 min before SPB separation for each protein was normalized to 100%. Time (min) was converted to percentage of the cell-cycle time for each strain to aid the comparison. a. u., arbitrary units.



SUPPLEMENTAL FIGURE S4: Partitioning and dynamics of Sfi1 and Sad1 in *sfi1*$^{+}$ and *sfi1-M46* cells. (A and B) Numbers of Sfi1 (A) and Sad1 (B) molecules on each SPB in *sfi1*$^{+}$ (JW4841) and *sfi1-M46* (JW4842) cells during the cell cycle in asynchronous cell populations. (C and D) Fluorescence recovery after photobleaching (at time 0) of Sfi1-mEGFP and Sfi1-M46-mEGFP. (C) Kymographs for representative unbleached and bleached SPBs (not corrected for photobleaching during image acquisition). Bar, 1 µm. (D) Quantitative recovery curves. Error bars are SEMs. (E) Percentages of SPBs with Sfi1-tdTomato and GFP-Cdc31 in *sfi1*$^{+}$ (JW4675) and *sfi1-M46* (JW4758) cells. Means ± SDs from two experiments (n > 250 cells for each) are shown.



SUPPLEMENTAL FIGURE S5: Repressing Sfi1 to ~30% of its endogenous level does not significantly affect mitosis. Cells were grown under repressing conditions (YE5S + thiamine) for 48 h at 25ºC or (in C, bottom) at 36ºC for 14 h. (A) Localization of Sfi1 and Sad1 in wt (*Psfi1-sfi1-mEGFP*; JW4841) and *P81nmt1-mEGFP-sfi1* (JW5088) cells. (B) Quantification of Sfi1 on each SPB in wt and *P81nmt1-mEGFP-sfi1* cells. (C) DIC and DNA staining of *P81nmt1-mEGFP-sfi1* cells (JW4615) grown in YE5S + thiamine for 48 h at 25°C or 14 h at 36°C. Arrowheads mark cells with mitotic defects. (D) Quantification of Sad1 on each SPB in wt and *P81nmt1-mEGFP-sfi1* cells. Bars, 5 μm.