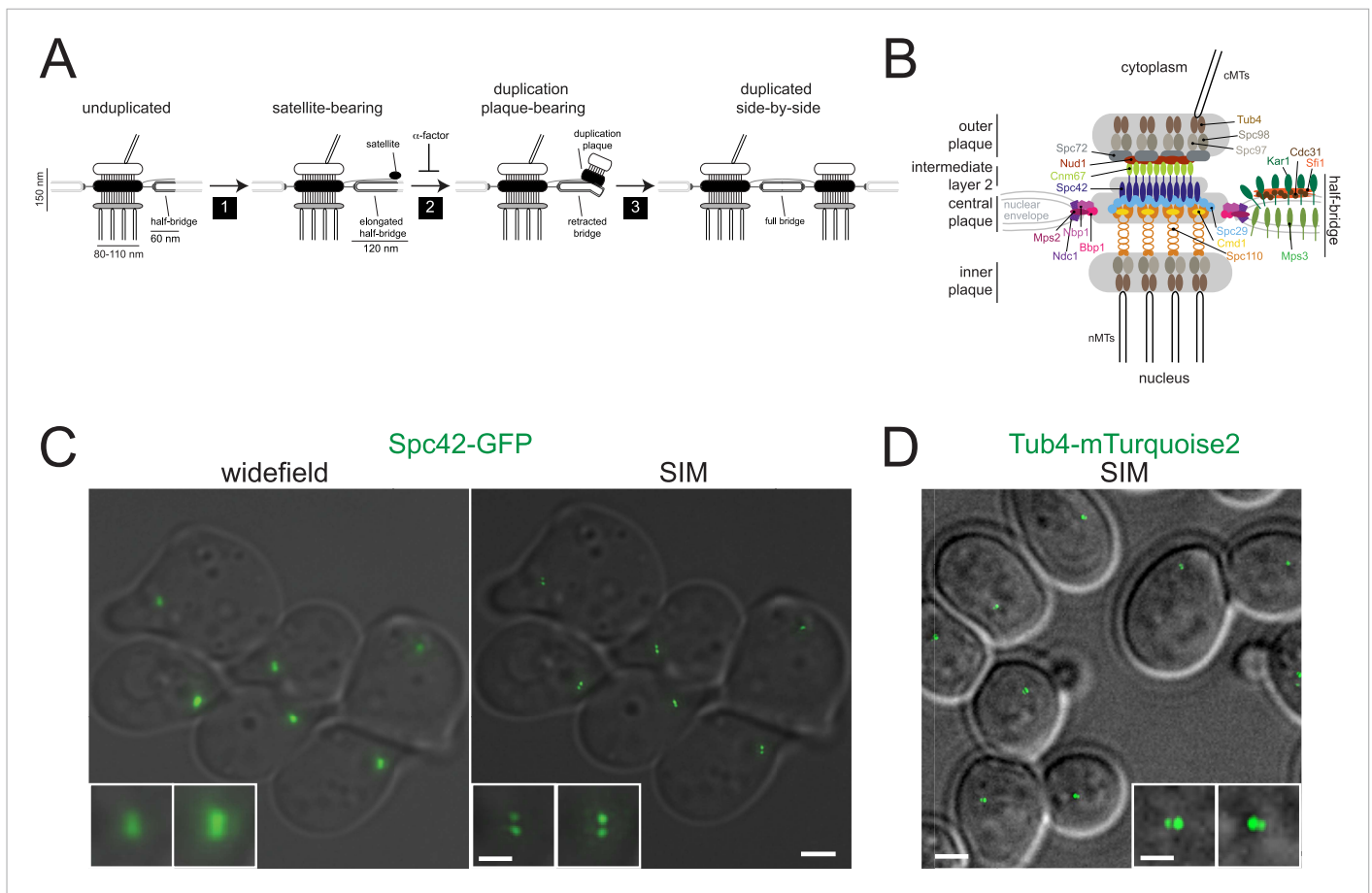


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## Figures and figure supplements

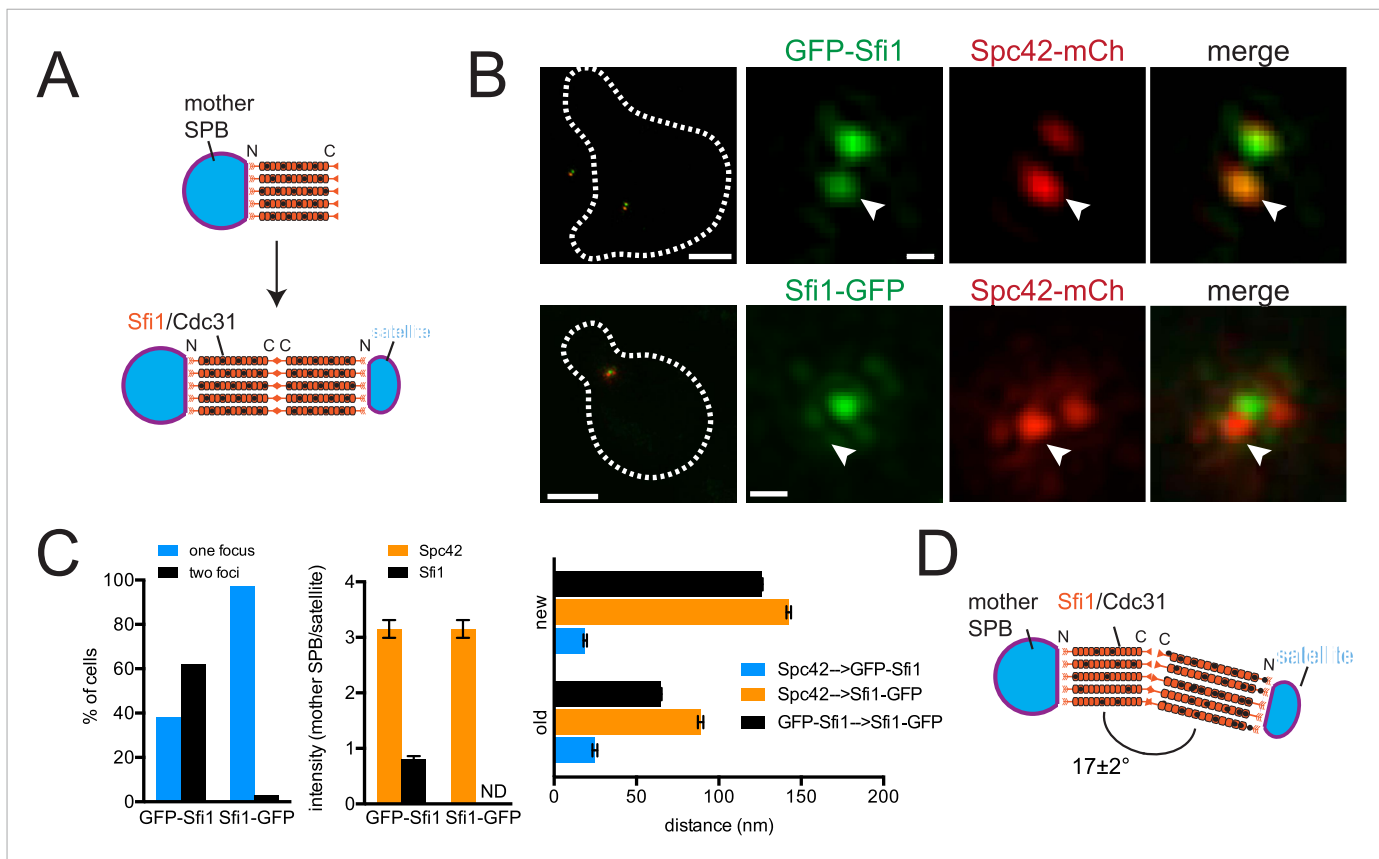
Structured illumination with particle averaging reveals novel roles for yeast centrosome components during duplication

**Shannon Burns, et al.**



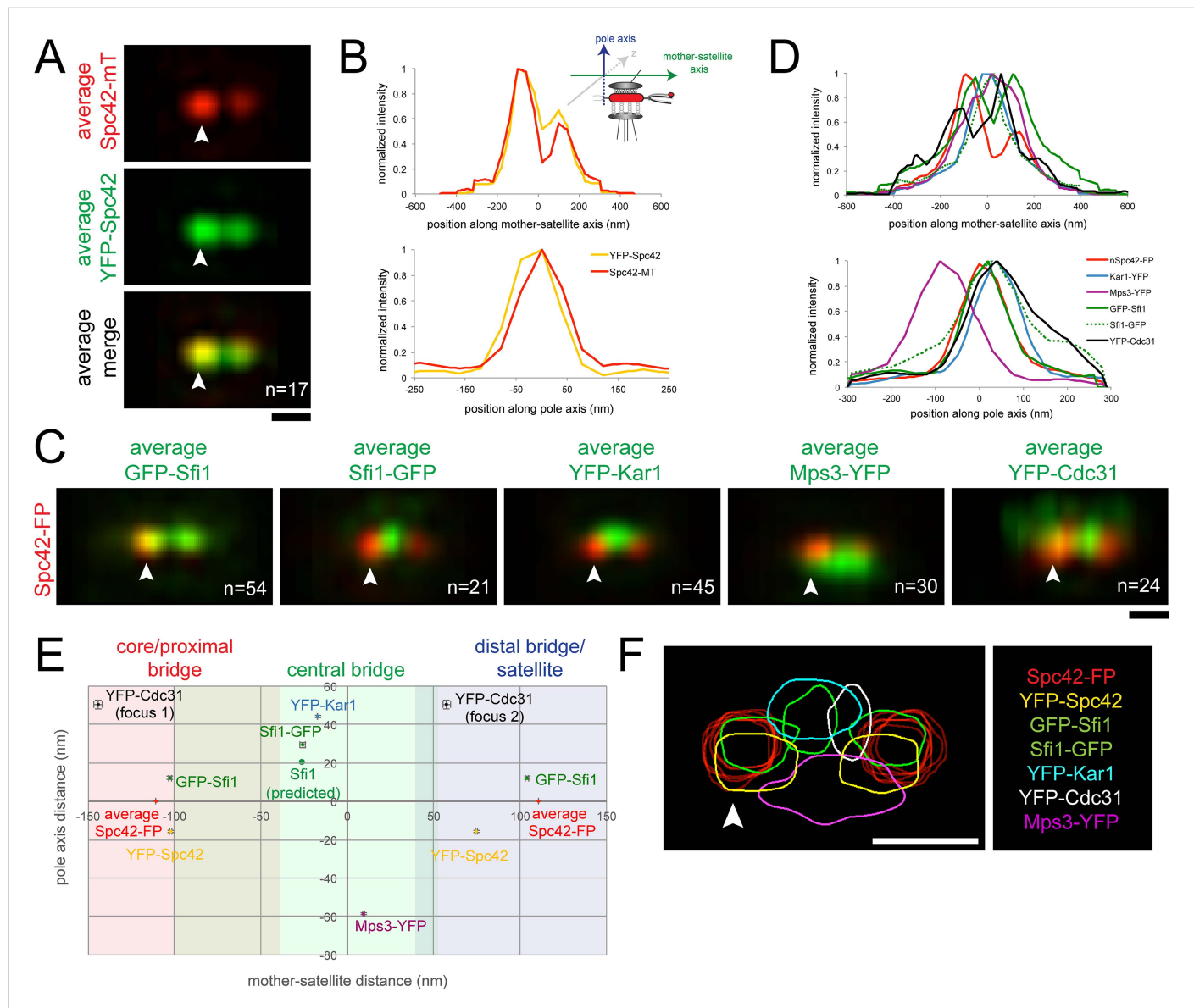
**Figure 1.** Spindle pole body (SPB) sub-structures can be visualized by structured illumination microscopy (SIM). **(A)** Schematic of the SPB duplication pathway deduced from electron microscopy (EM) analysis of wild-type and mutant yeast, including the size of SPB substructures (reviewed in **Adams and Kilmartin, 2000; Jaspersen and Winey, 2004; Winey and Bloom, 2012**). Three steps of SPB duplication: (1) elongation of the half-bridge and deposition of the satellite; (2) maturation of the satellite into a structure known as a duplication plaque and retraction of the bridge; (3) insertion of the duplication plaque into the NE and assembly of nuclear SPB components to create duplicated side-by-side SPBs. Treatment of cells with  $\alpha$ -factor blocks SPB duplication at the satellite-bearing stage. **(B)** Schematic of the SPB showing the locations of all 18 components based on immunoEM, FRET, yeast two-hybrid, biochemical, and genetic data. **(C)** Comparison of widefield and SIM using Spc42-GFP, which is present in the core SPB and satellite in  $\alpha$ -factor arrested cells. Insets show the SPB from top left and bottom right cells. **(D)** SIM of asynchronous Tub4-mTurquoise2 cells. Insets show SPBs from large-budded anaphase cell. Bars, 2  $\mu$ m and 200 nm (inset).

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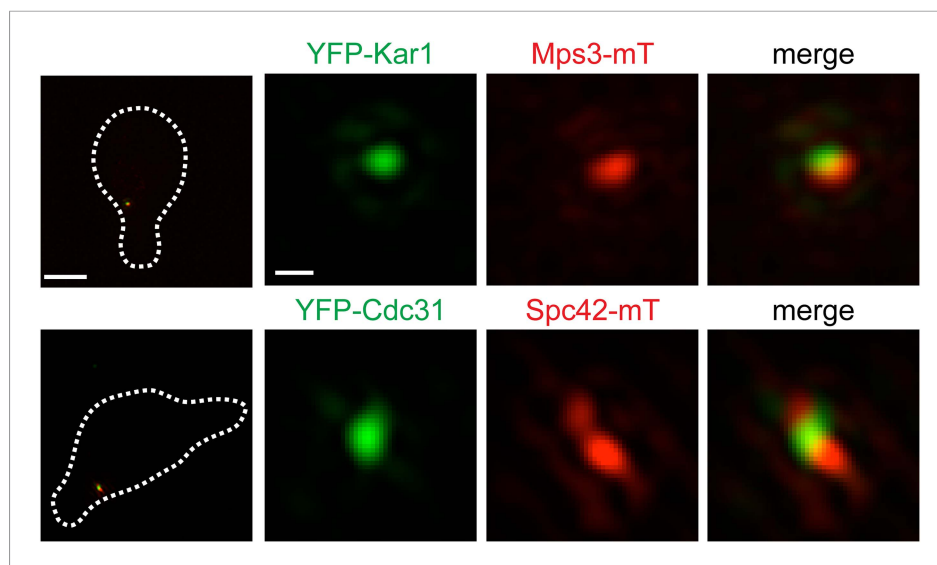
**Figure 2.** Structure of the half-bridge. **(A)** Top-down view of half-bridge showing the mother SPB and the Sfi1-Cdc31 filament extending in a polar fashion along the half-bridge; C-terminal end-to-end association forms an N-terminal end for satellite assembly. **(B)** Cells containing Spc42-mCherry and GFP-Sfi1 (SLJ9741) or Sfi1-GFP (SLJ10040) were  $\alpha$ -factor arrested and imaged by SIM. On the left is a merged image showing the cell outline (dashes). Bar, 2  $\mu$ m. Single channel and merged images of the mother SPB (arrowhead) and satellite. Bar, 200 nm. **(C)** Cells from **B** were quantitated and the percentage of cells containing two Sfi1 foci and the ratio of intensity between the mother SPB/mother proximal signal and satellite/distal signal is shown. ND, not determined. Distance was determined in three dimensions between Spc42-mCherry and GFP-Sfi1 or Spc42-mCherry and Sfi1-GFP on the old and new Sfi1 filament that is proximal and distal to the mother SPB, respectively. The distance between Sfi1 foci was calculated using data in **Figure 3E**. Error bars, standard error of the mean (SEM). **(D)** Modified schematic of bridge from **A**, showing Sfi1 and Cdc31 asymmetry and the bend detected by SIM.

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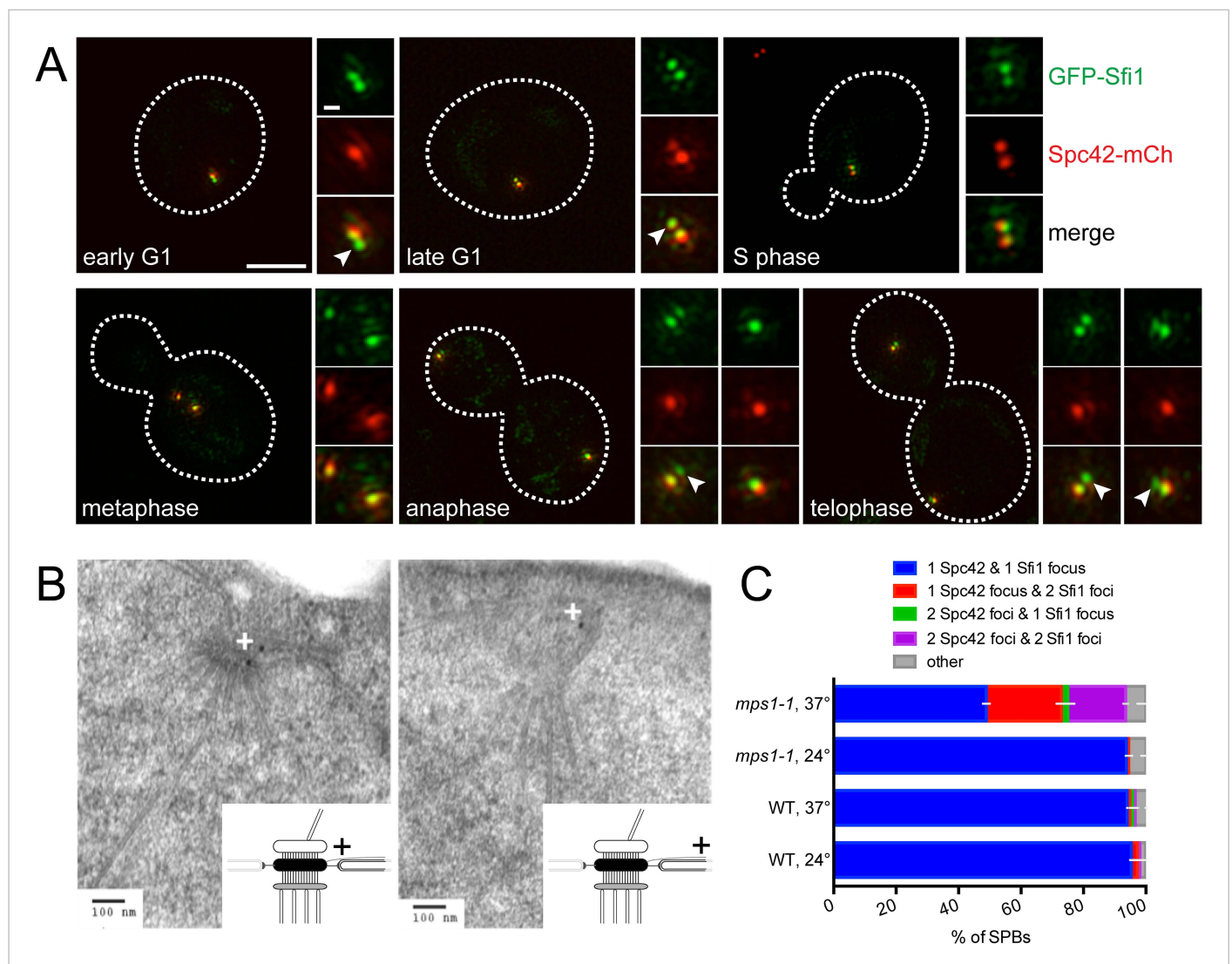
**Figure 3.** SPA-SIM analysis of the extended half-bridge. **(A, B)** YFP-Spc42-mTurquoise2 (SLJ9442) cells were arrested in  $\alpha$ -factor and imaged by SIM. 17 images of the SPB (arrowhead) and satellite were aligned based on mTurquoise2 fluorescence to create the projection view shown in **A**. Bar, 200 nm. In **B**, graphs show the relative fluorescence intensity along the mother-satellite (top) and pole (bottom) axis, in nm, as depicted in the schematic. **(C, D)** SIM images of cells from **Figure 2B** and  $\alpha$ -factor arrested YFP-Cdc31 (SLJ10084; representative image shown in **Figure 3—figure supplement 1**), YFP-Kar1 (SLJ9670) or Mps3-YFP (SLJ9454) strains containing Spc42-mTurquoise2 were aligned and projected. In **C**, Spc42-mTurquoise2 or Spc42-mCherry (red, denoted Spc42-FP) mark the mother SPB (arrowhead) and satellite and localization of the indicated half-bridge component is shown in green. N is indicated. **(D)** Normalized fluorescence intensity of each protein along the mother-satellite and pole axis is plotted. **(E)** To compare positional information between samples, the maximum intensity of GFP-Sfi1, Sfi1-GFP, YFP-Cdc31, YFP-Kar1, Mps3-YFP, and YFP-Spc42 distributions was determined in both axes and plotted using the center position between Spc42-mTurquoise2/mCherry at the mother SPB and satellite as the zero reference position. Localization of Kar1 and Mps3 to opposite sides of the bridge was confirmed by SIM, as shown in **Figure 3—figure supplement 1**. Error bars, SEM. Based on the full-width half-maximum (FWHM) values of Spc42 at the mother (110 nm; -180 to -30 nm), satellite (110 nm; 36 to 184 nm) and Sfi1-GFP (-25.4 nm; -102.4 to 51.6 nm) (**Table 1**), the bridge was divided into core/proximal, central and distal/satellite regions. The predicted position of Sfi1-GFP (Sfi1-predicted) based on the natural curvature of the NE of a nucleus of 1  $\mu$ m is shown. **(F)** Contour map showing the distribution of fluorescent intensity at the extended half-bridge based on all images used in **A** and **C**. The C-terminally tagged Spc42 (Spc42-FP) in each sample is shown in red and other proteins are colored as indicated. Bar, 200 nm.

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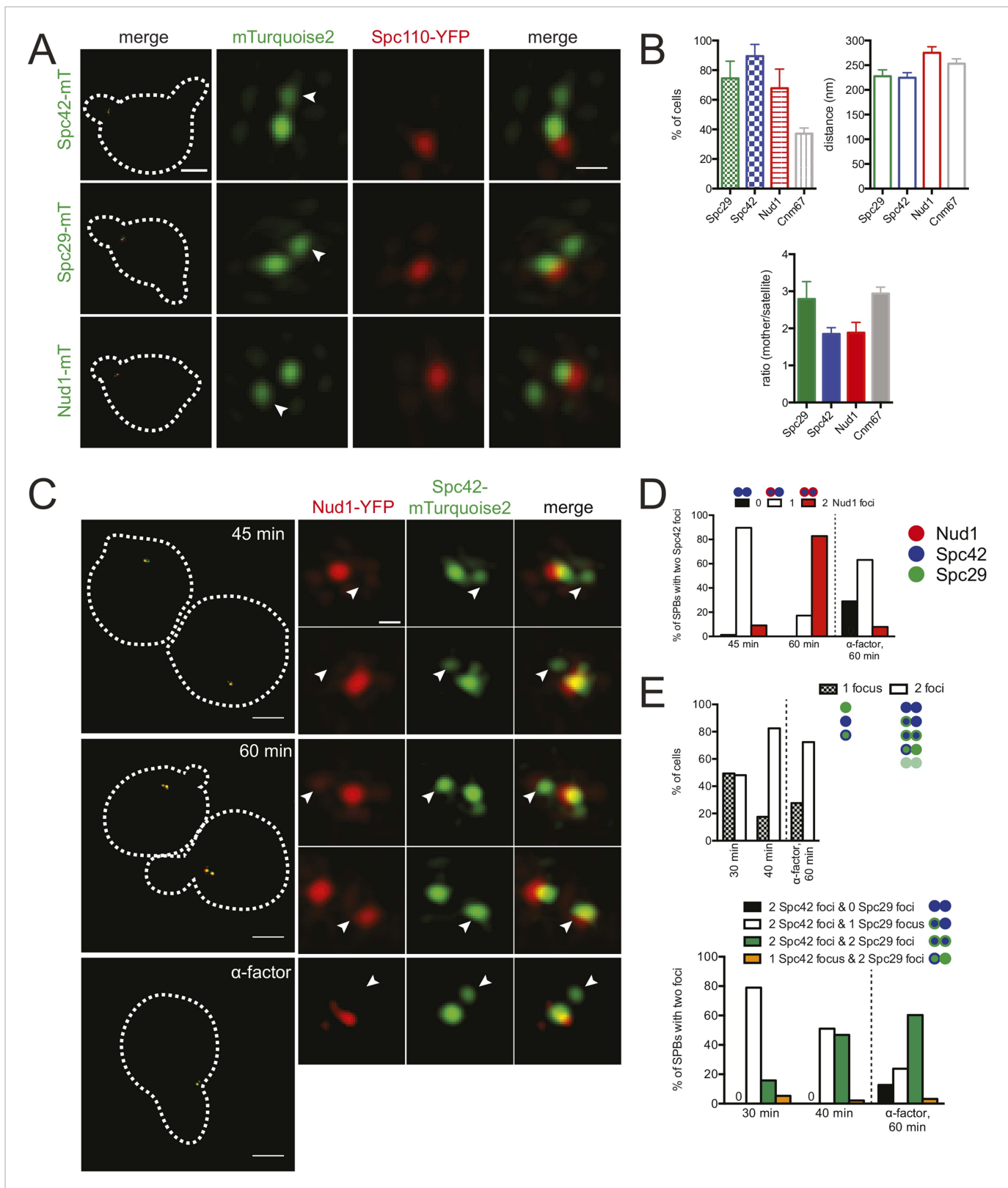
**Figure 3—figure supplement 1.** Mps3 and Kar1 localize to opposite faces of the half-bridge. SIM image  $\alpha$ -factor arrested cells (SLJ1100) containing YFP-Kar1 (green) and Mps3-mTurquoise2 (red) and cells (SLJ10084) containing YFP-Cdc31 (green) Spc42-mTurquoise2 (red). Cell outline is shown with dashed lines. Bar, 2  $\mu$ m. Insets show SPB region. Bar, 200 nm.

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**Figure 4.** Half-bridge elongation is a discrete step in SPB duplication. **(A)** SIM images from asynchronously growing cells (SLJ9741) containing GFP-Sfi1 (green) and Spc42-mCherry (red). A merged image showing the cell outline (dashes) was used together with spindle length to approximate the cell cycle position indicated. Bar, 2  $\mu$ m. The SPB(s) are shown to the right of each cell. Arrowheads in the merged images point to the satellite. Bar, 200 nm. **(B)** GFP-SFI1 *mps1-1* cells (SHJ3829) were grown to log phase at 24°C, shifted to restrictive temperature (37°C, 4 hr) and then prepared for immunoEM with nanogold secondary label. Shown are two representative cells with labeling at the SPB (left) and the distal tip of the elongated half-bridge (right). Some cells also contained labeling closer to the center of the elongated half-bridge (left). +: label representation. **(C)** Wild-type (JA372) and *mps1-1* (JA368) cells containing GFP-Sfi1 and Spc42-mCherry were grown at 24°C or shifted to 37°C for 4 hr then analyzed by SIM. Because *mps1-1* cells arrest in mitosis at the non-permissive temperature (Winey et al., 1991), only large budded cells were examined. The SPBs from early mitotic wild-type cells showed co-localization of GFP-Sfi1 and Spc42-mCherry (95  $\pm$  4%, n = 90, at 24°C or 94  $\pm$  1%, n = 117, at 37°C), with 87  $\pm$  14% (24°C) and 80  $\pm$  17% (37°C) displaying co-localization at both poles. 94  $\pm$  2% (n = 104) of mitotic *mps1-1* cells grown at 24°C showed the same localization as wild-type, with 86  $\pm$  1% of cells exhibiting co-localization at both poles. At 37°C, 49  $\pm$  7% of cells showed co-localization of GFP-Sfi1 and Spc42-mCherry, with the majority of cells displaying a single focus of each (40% of all *mps1-1* cells at 37°C). 24  $\pm$  7% and 19  $\pm$  3% of SPBs (n = 85) contained a single focus of Spc42-mCherry or two Spc42-mCherry, respectively, with two GFP-Sfi1 foci. Error bars, SEM.

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**Figure 5.** Temporal control of satellite assembly. (A, B) Cells containing Spc110-YFP (red) and Spc42-mTurquoise2 (SLJ8980), Spc29-mTurquoise2 (SLJ8820), or Nud1-mTurquoise2 (SLJ9099) (green) were  $\alpha$ -factor arrested for 3 hr and imaged using SIM. The cell is shown on the left with dashes indicating the cell boundary. Bar, 2  $\mu$ m. Single channel and merged images of the mother SPB and satellite (arrowhead). Bar, 200 nm. (B) Cells from A were imaged at 45 min, 60 min, and  $\alpha$ -factor arrest (60 min). (C) Cells from A were imaged at 45 min, 60 min, and  $\alpha$ -factor arrest (60 min). (D) Cells from A were imaged at 45 min, 60 min, and  $\alpha$ -factor arrest (60 min). (E) Cells from A were imaged at 30 min, 40 min, and  $\alpha$ -factor arrest (60 min). Figure 5. continued on next page

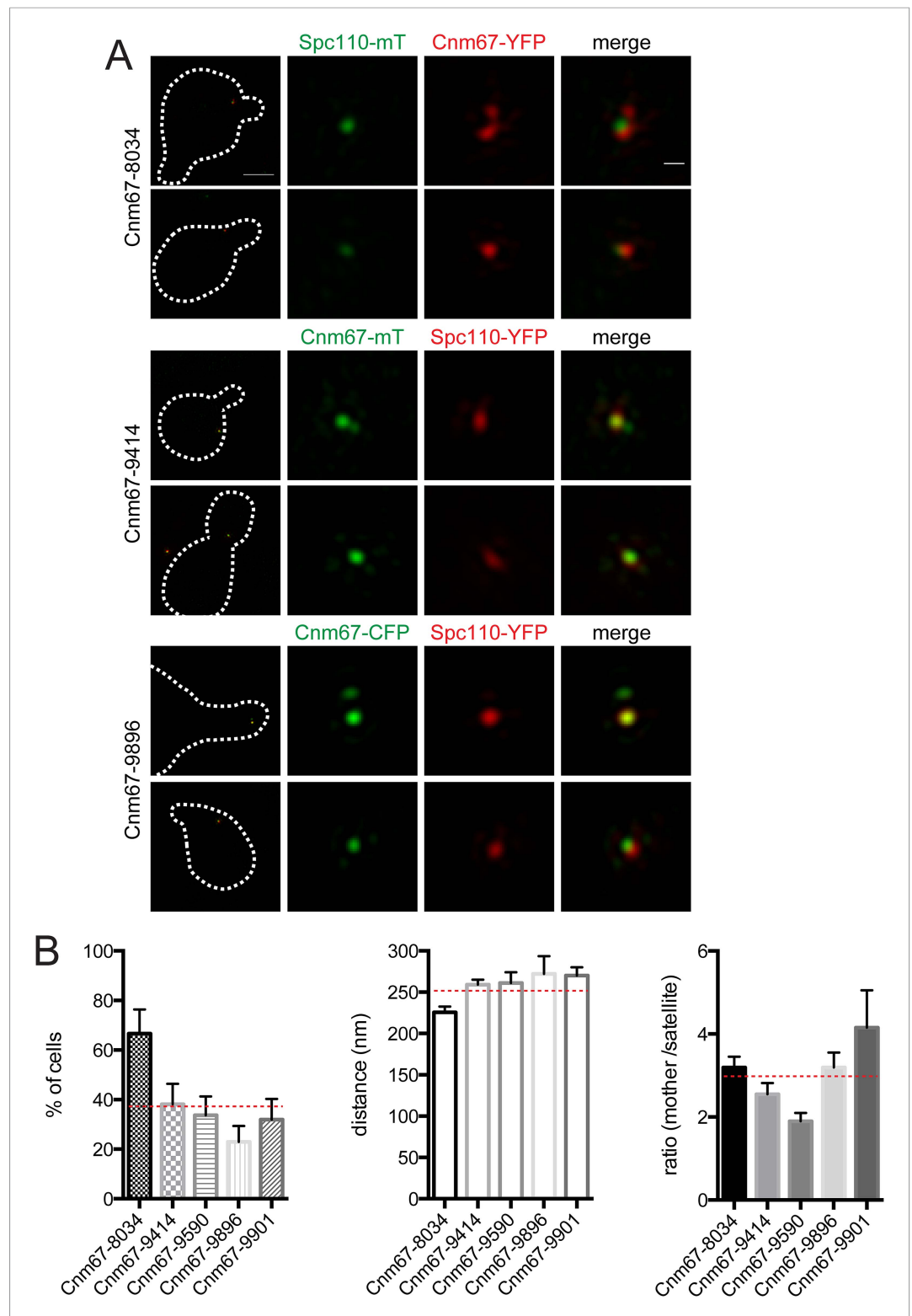


## Figure 5. Continued

quantitated and the percentage of  $\alpha$ -factor arrested cells containing two foci (mother and satellite) is shown, along with the distance from mother to satellite and the ratio of their intensity. The mother SPB overlaps or is adjacent to Spc110-YFP. Data for Cnm67 is an average based on data shown in **Figure 5—figure supplement 1**. Errors, SEM. **(C, D)** A metaphase arrested *MET-CDC20* strain (SLJ9720) containing Spc42-mTurquoise2 (green) and Nud1-YFP (red) was released into the cell cycle using SC-methionine media. Aliquots were taken every 15 min to determine budding index and for analysis of satellite assembly by SIM. **(C)** Images from the 45 and 60 min time points are shown together with cells released into  $\alpha$ -factor for 60 min. The cells are shown on the left with dashes indicating the cell boundary. Bar, 2  $\mu$ m. Single channel and merged images of the SPB and the satellite (arrowhead). Bar, 200 nm. **(D)** Percentage of cells with SPBs that have two foci of Spc42-mTurquoise2 (the pole and the satellite) and 0 (black bar), 1 (white bar) or 2 (red bar) foci of Nud1 is plotted for each time point. The reciprocal plot as well as an experiment in which Nud1 is labeled with mTurquoise2 and Spc42 with YFP is in **Figure 5—figure supplement 2B,C**. A red and blue dot represent Nud1 and Spc42, respectively. **(E)** As in **C**, a *MET-CDC20* strain (SLJ10009) containing Spc29-YFP and Spc42-mTurquoise2 was released and samples were collected every 10 min. The percentage of cells at 30 and 40 min after release from metaphase and the sample released into  $\alpha$ -factor for 60 min with a single focus or two foci of either Spc42-mTurquoise2 and/or Spc29-YFP at the SPB is plotted. A blue and green dot represent Spc42 and Spc29, respectively. At 30 min, approximately 50% of cells have a single unduplicated SPB at the pole but this decreased to 10% by 40 min. Note that none of the cells at 40 min had two foci of just Spc29-YFP. All other combinations were observed and the percentages are shown in the graph below. Reciprocal plots are in **Figure 5—figure supplement 2D**.

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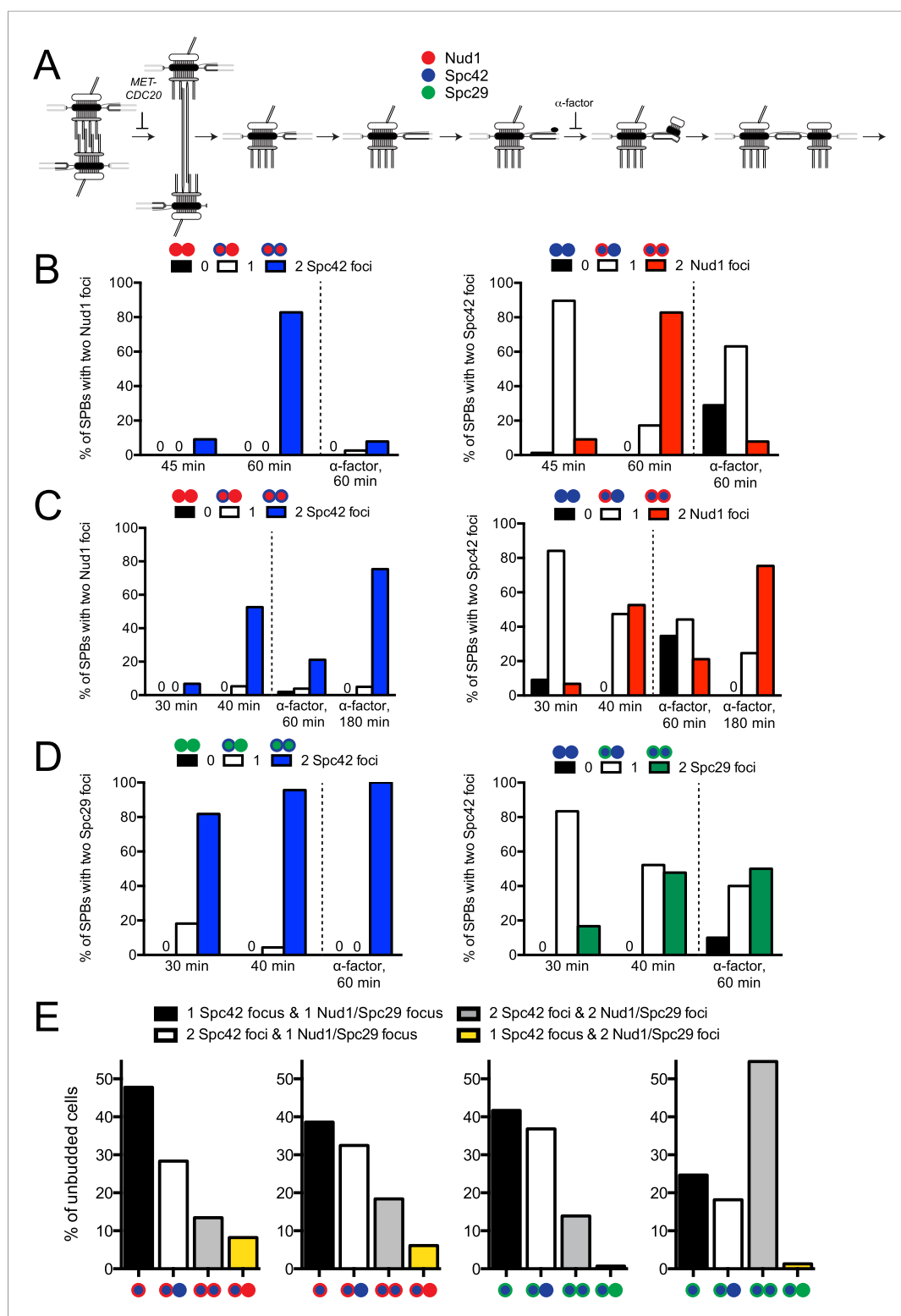
**Figure 5—figure supplement 1.** Cnm67 at the satellite. **(A)** Examples of SIM images from cells containing Cnm67-YFP and Spc110-mTurquoise2 (SLJ8034), Spc110-YFP and Cnm67-mTurquoise2 (SLJ9414) or Cnm67-CFP and Spc110-YFP (SLJ9896) arrested in  $\alpha$ -factor. A merged image showing the cell outline (dashes) is shown on the left. Bar, 2  $\mu$ m. Single channel and merged images of the mother SPB and satellite (arrow). Bar, 200 nm. **(B)** Cells from the Figure 5—figure supplement 1. continued on next page

*Figure 5—figure supplement 1. Continued*

strains in **A** as well as additional isolates of Spc110-YFP Cnm67-mTurquoise2 (SLJ9590 and SLJ9901) were quantitated. The percentage of  $\alpha$ -factor arrested cells containing two foci (mother and satellite) was calculated. The distance and intensity of the satellite focus to the mother SPB focus, which overlaps with Spc110 fluorescence, was also determined for cells containing two foci. Average data from these experiments is plotted in **Figure 5B**. A strain (SLJ9593) derived from the same tetrad as SLJ9590 did not contain cells with two foci of Cnm67-mTurquoise2.

Related to **Figure 5B**.

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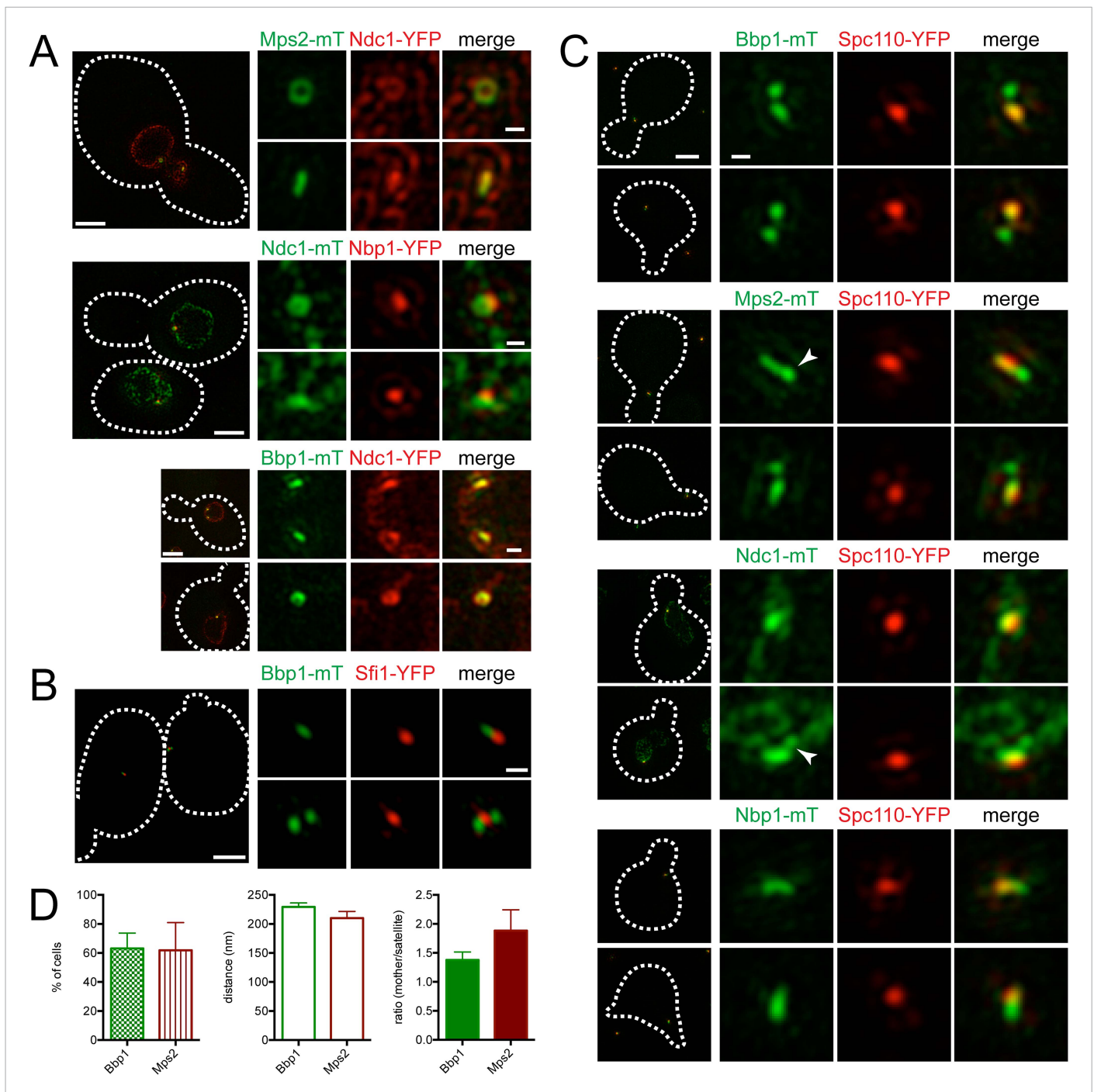


**Figure 5—figure supplement 2.** Temporal control of satellite assembly. **(A)** Schematic showing SPB cycle, including the metaphase arrest following depletion of *CDC20* in *MET-CDC20* strains and the  $\alpha$ -factor arrest point. The color of each satellite component used in the figure is noted. **(B–D)** Metaphase-arrested *MET-CDC20* strains containing Spc42-mTurquoise2 and Nud1-YFP (SLJ9720, **B**), Nud1-mTurquoise2 and Spc42-YFP (SLJ9685, **C**), and Spc42-YFP (SLJ9685, **D**). **(E)** Percentage of unbudded cells with different combinations of foci at 30 min, 40 min, and after  $\alpha$ -factor arrest for 60 min. **Figure 5—figure supplement 2. continued on next page**

*Figure 5—figure supplement 2. Continued*

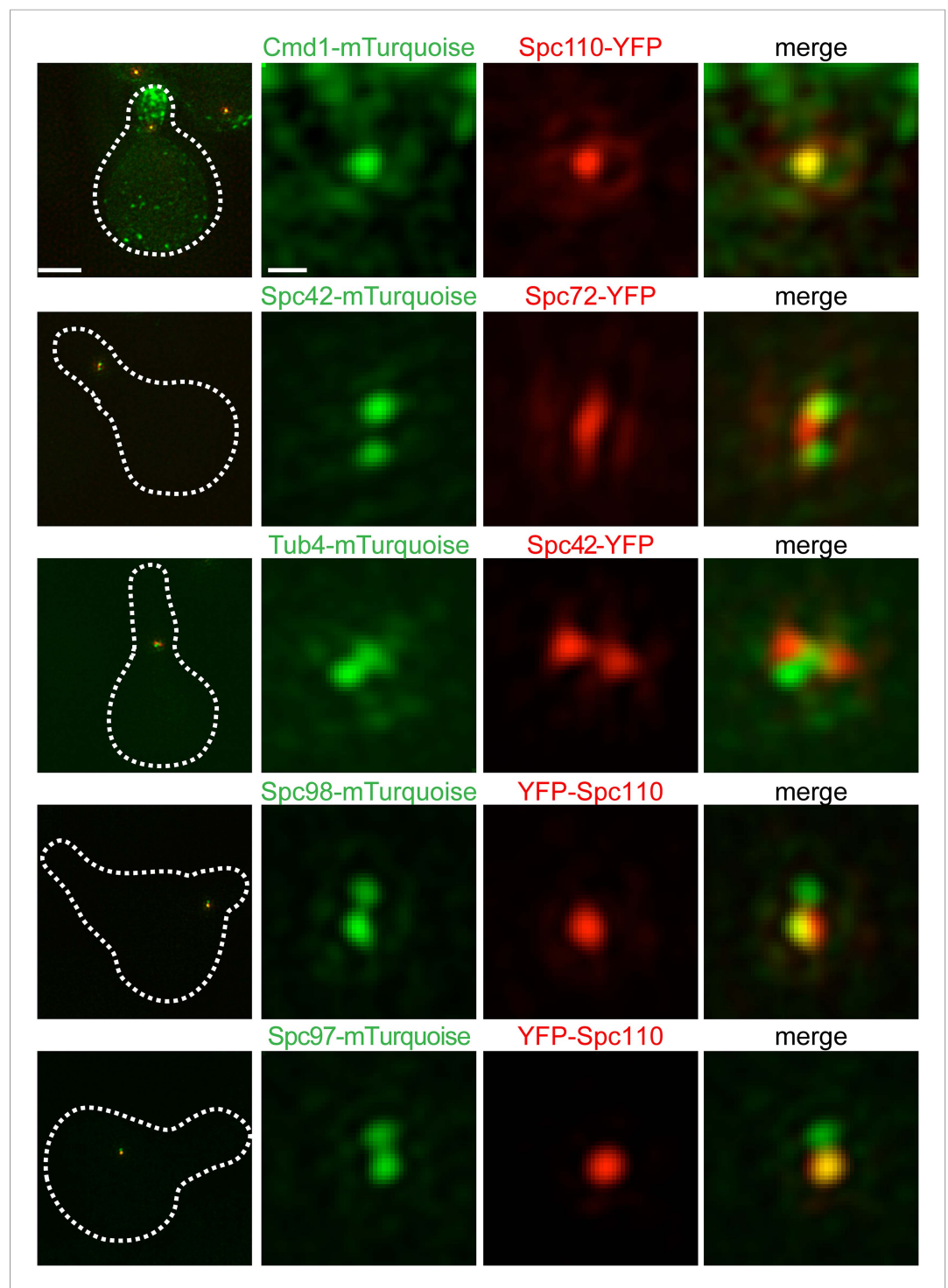
mTurquoise2 and Spc29-YFP (SLJ10009, **D**) were released into the cell cycle using SC-methionine media. Aliquots were taken at the indicated times for analysis of satellite assembly by SIM. Cells were also released into  $\alpha$ -factor for the indicated time. (**B**, **C**) On the left, the percentage of SPBs that contain two foci Nud1 and 0, 1, or 2 overlapping foci Spc42 are plotted. On the right, the percentage of SPBs that contain two foci Spc42 and 0, 1, or 2 overlapping foci Nud1 are plotted. Note, **B** and **C** differ in the fluor; the fact that virtually identical results were obtained in both experiments suggests that mTurquoise2 and YFP have similar maturation rates and can be used to detect the newly forming satellite. (**D**) On the left, the percentage of SPBs that contain two foci Spc29 and 0, 1, or 2 overlapping foci Spc42 are plotted. On the right, the percentage of SPBs that contain two foci Spc42 and 0, 1, or 2 overlapping foci Spc29 are plotted. (**E**) Asynchronously grown cells containing Nud1-mTurquoise2 and Spc42-YFP (SLJ9275, left), Spc42-mTurquoise2 and Nud1-YFP (SLJ9660, center), Spc42-mTurquoise2 and Spc29-YFP (SLJ8082, center right), and Spc29-CFP and Spc42-YFP (DHY47-6B/SLJ10596, right) were imaged by SIM. The percentages of unbudded cells with a single SPB with a single focus of Spc42 and one or two foci of Nud1 or Spc29; or a single SPB with two foci of Spc42 and one or two foci of Nud1 or Spc29 was plotted. A small fraction of cells (2–7%) have other configurations that are not depicted, such as SPBs labeled with only one fluorescent protein. N > 77 in all samples. Related to **Figure 5C–E**.

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**Figure 6.** Localization of SPB pore components to the membrane region and half-bridge/satellite. **(A)** Asynchronously grown Mps2-mTurquoise2/Ndc1-YFP (SLJ8102), Ndc1-mTurquoise2/Nbp1-YFP (SLJ8263), and Bbp1-mTurquoise2/Ndc1-YFP (SLJ9231) were examined for evidence of a pore-like structure formed by Ndc1 and the other protein. **(B)** Location of Bbp1-mTurquoise2 (green) and Sfi1-YFP (red) in asynchronous (SLJ9035) cells. **(C, D)** Cells containing Spc110-YFP (red) and Bbp1-mTurquoise2 (SLJ10019), Mps2-mTurquoise2 (SLJ8084), Ndc1-mTurquoise2 (SLJ10018), or Nbp1-mTurquoise2 (green) were  $\alpha$ -factor arrested and imaged using SIM. In **A–C**, the cell is shown on the left with dashes indicating the cell boundary. Bar, 2  $\mu$ m. Single channel and merged images of the SPB region(s). Bar, 200 nm. In **C**, arrowheads point to the satellite region. **(D)** Bbp1 and Mps2 cells from **C** were quantitated and the percentage of  $\alpha$ -factor arrested cells containing two foci (mother and satellite) is shown, along with the distance from mother to satellite and the ratio of their intensity. Error bars, SEM. The localization of other SPB components is shown in **Figure 6—figure supplement 1**.

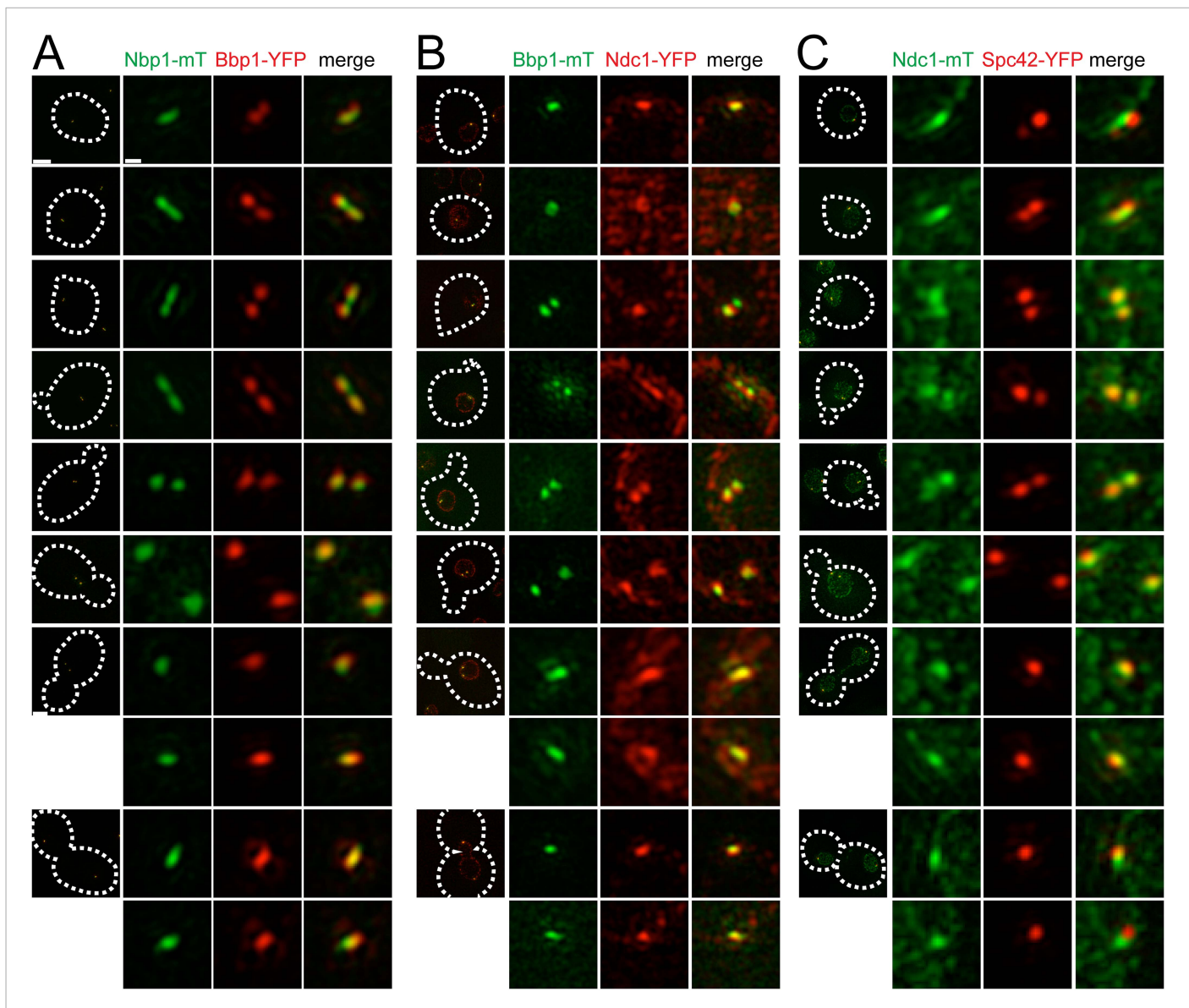
DOI: [10.7554/eLife.08586.012](https://doi.org/10.7554/eLife.08586.012)



**Figure 6—figure supplement 1.** Localization of the  $\gamma$ -tubulin complex and linkers. Cells containing Spc110-YFP (red) and Cmd1-mTurquoise2 (SLJ10004), Spc42-mTurquoise2 (green) and Spc72-YFP (SLJ8631), Tub4-mTurquoise2 (green) and Spc42-YFP (SLJ9384), and Spc97-mTurquoise2 or Spc98-mTurquoise2 (green) and YFP-Spc110 (SLJ9632 or SLJ10041) were  $\alpha$ -factor arrested and imaged using SIM. The cell is shown on the left with dashes indicating the cell boundary. Bar, 2  $\mu$ m. Single channel and merged images of SPB. Note the localization of Spc72-YFP and fraction of Tub4-mTurquoise2 (presumably the pool present at the outer plaque) to the region between Spc42 foci. Bar, 200 nm.

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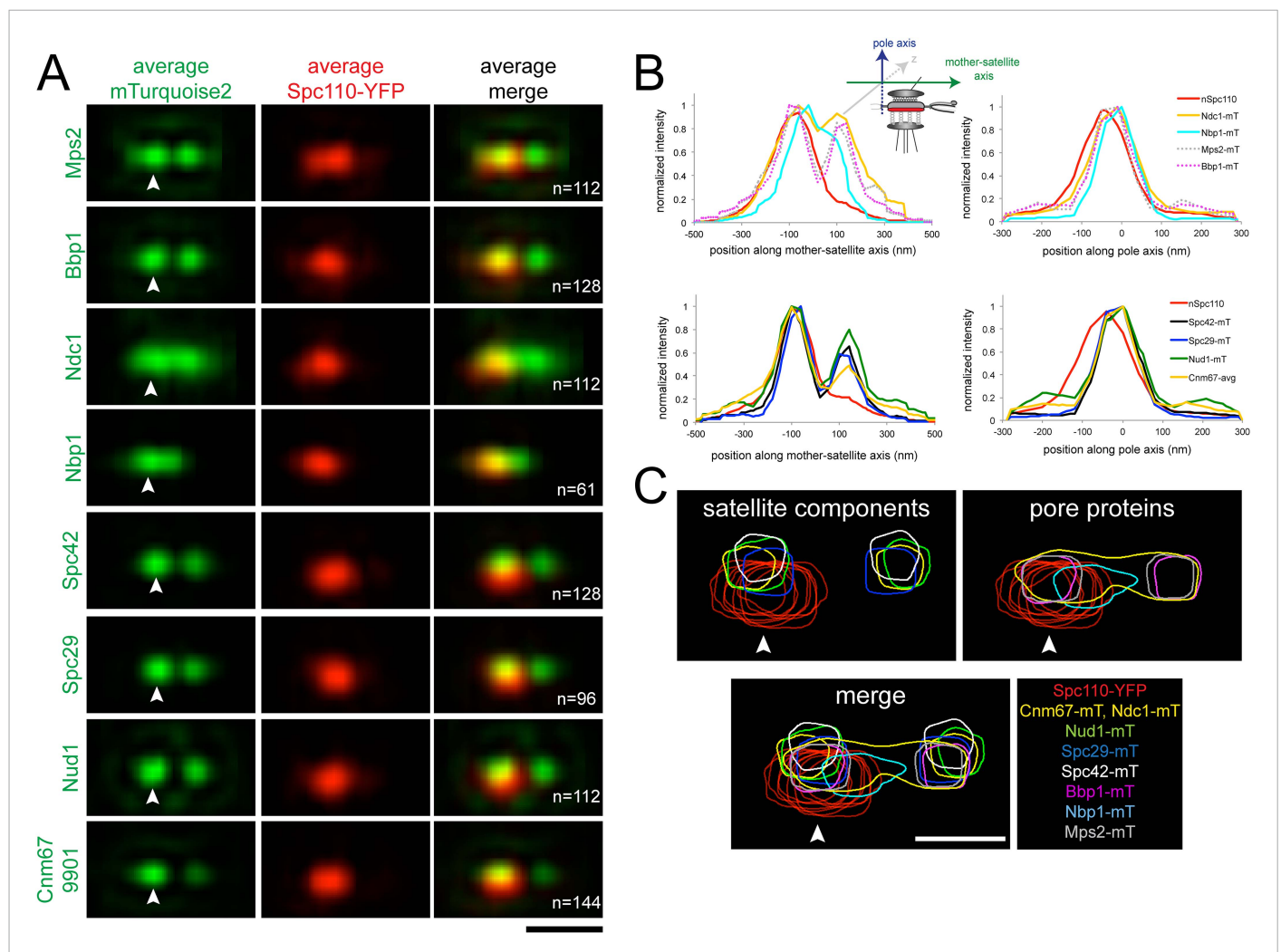




**Figure 7.** Cell cycle analysis of pore membrane component localization. SIM images from asynchronously growing cells containing Nbp1-mTurquoise2 (green) and Bbp1-YFP (red) (SLJ7699, **A**), Bbp1-mTurquoise2 (green) and Ndc1-YFP (red) (SLJ9231, **B**) and Ndc1-mTurquoise2 (green) and Spc42-YFP (red) (SLJ7941, **C**) are arranged based on bud size and distance between SPBs (or satellite structure), which approximates position in the cell cycle. A merged image showing the cell outline (dashes) is shown on the left. Bar, 2  $\mu$ m. The SPB(s) are shown to the right of each cell. Bar, 200 nm.

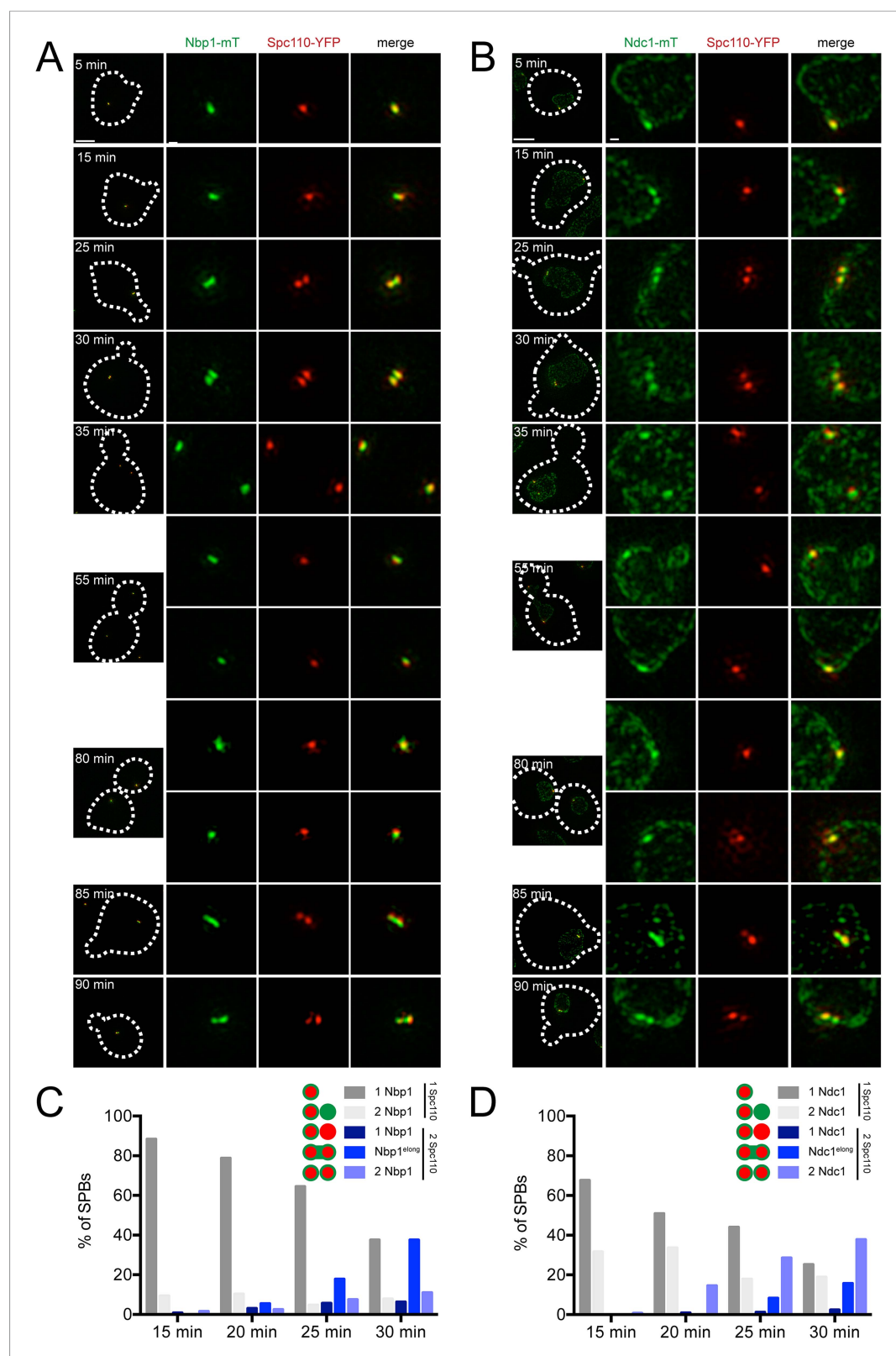
DOI: [10.7554/eLife.08586.014](https://doi.org/10.7554/eLife.08586.014)





**Figure 8.** Early steps of SPB assembly involve both satellite and pore membrane proteins. **(A)** SIM Images from **Figures 5A, 6C** were aligned and average projections generated as in **Figure 3**. The indicated mTurquoise2 protein (green) or Spc110-YFP (red) at the mother SPB (arrowhead) is shown along with N. Bar, 200 nm. **(B)** Normalized fluorescence intensity of each protein in **A** along the mother-satellite and pole axis, as depicted in the schematic, is plotted using the average Spc110-YFP position from all cells. In these plots, as in **Figure 3**, the center of Spc42-mTurquoise2 was used to define the position of the 0 coordinate in the mother-satellite axis and the pole axis. FWHM values for each are listed in **Table 2**. **(C)** Contour maps showing the distribution of fluorescence intensity at the extended half-bridge based images in **A**. Spc110-YFP in each sample is shown in red and other proteins are colored as indicated. Bar, 200 nm.

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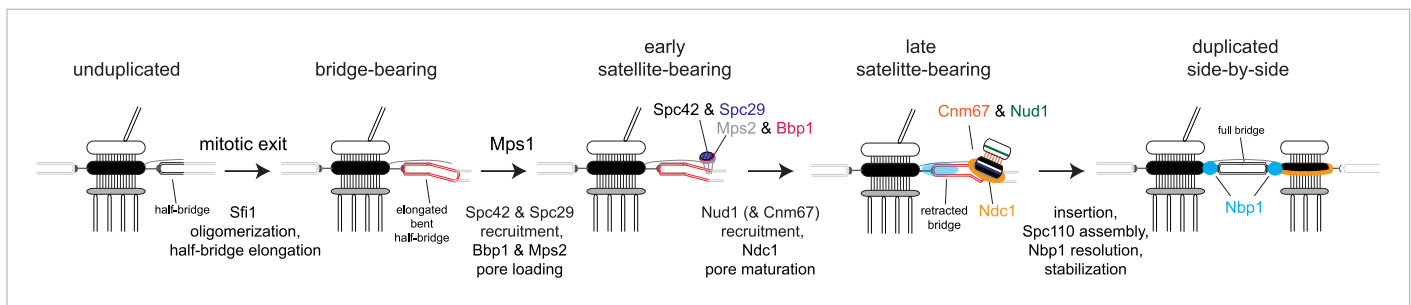


**Figure 9.** Localization of Ndc1 and Nbp1 during SPB insertion. Cells containing Nbp1-mTurquoise2 (**A** and **C**, SLJ10169) or Ndc1-mTurquoise2 (**B** and **D**, SLJ10018) along with Spc110-YFP were released from  $\alpha$ -factor into prewarmed SC-complete media at 30°C. At 5 min time intervals, an aliquot of cells was harvested, fixed and imaged by SIM. (**A**, **B**) Merged images showing the outline (dashes) of a representative cell is on the left. Bar, 2  $\mu$ m. *Figure 9. continued on next page*

*Figure 9. Continued*

The SPB(s) are to the right of each cell. Bar, 200 nm. **(C, D)** Cells at the 15, 20, 25, and 30 min time points were analyzed to determine the percentage of schmooed and small budded cells that have two closely spaced foci of Spc110-YFP and/or Nbp1-mTurquoise2 **(C)** or Ndc1-mTurquoise2 **(D)**. Schematics depict the five configurations of proteins, including the signal that was stretched between mother and daughter SPB.

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**Figure 10.** Model for SPB duplication. Revised model of the early steps in SPB duplication based on results described. In anaphase, Sfi1 oligomerization leads to an elongated half-bridge. Next, Spc42 and Spc29 assemble at the distal cytoplasmic tip of the bridge. At the same time, the pore proteins Bbp1 (magenta) and Mps2 (gray) accumulate at the satellite region. Later, the satellite and membrane-associated region continue to mature through the addition of Nud1 (green), Cnm67 (orange), and Ndc1 (orange ring). Nbp1 (cyan) remains associated primarily with the mother-proximal end of the extended half-bridge until the SPB is inserted into the NE. Note that satellite and pore-associated proteins are also present at the mother SPB.

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