Supplemental Figure Legends

Supplemental Figure 1. A detached kinetochore is captured by the re-formed spindle and establishes biorientation.

Time-lapse images of an *nda3* cell expressing Nuf2-YFP (kinetochore marker) and CFP-Atb2 (α-tubulin) after recovery from mitotic arrest. Panels show the YFP channel alone (Nuf2), and YFP merged with CFP (merge: YFP and CFP are pseudocolored green and magenta, respectively). Nine serial Z-sections with an interval of 0.4 μm were collected every 20 s, deconvolved, and flattened using a Leica ASMDW microscopy system (Leica Microsystems, Wetzlar, Germany). The kinetochore detached from the spindle is indicated by white arrowheads. Subsequently, the kinetochore was retrieved and remained on the SPB for several minutes (black arrowheads). The numbers indicate the duration in seconds. Bar, 10 μm.

Supplemental Figure 2. Mad2 localizes on mono-oriented kinetochores as well as unattached kinetochores.

Two additional examples of time-lapse analysis of *nda3* cells after recovery from mitotic arrest are shown. See the legend of Figure 1 for details. Mad2-YFP and CFP-Atb2 are pseudocolored green and magenta, respectively. Mad2 localization on mono-oriented kinetochores (i.e. kinetochores remaining at the pole or showing p-movement) was observed reproducibly.

Supplemental Figure 3. Mad2 localizes on mono-oriented kinetochores in *cut9*

mutant cells after spindle re-formation.

(A) The *cut9* mutant cells expressing Mad2-GFP and CFP-Atb2 were incubated at the restrictive temperature for 3 h, and for an additional 1 h in the presence of CBZ. After release from CBZ block, time-lapse images were taken. Eleven serial Z-sections with an interval of 0.4 μm were collected every 20 s, deconvolved, and flattened using a Leica ASMDW microscopy system. Mad2 localized on all three kinetochores in this sample. The kinetochore indicated by white arrows was captured by the spindle at time = 40 s, moved toward the SPB (time = 40–100 s) and remained at the SPB (time = 100–200 s). Mad2 localized on this kinetochore even when the kinetochore appeared to interact with the spindle in a mono-oriented fashion (time = 40–200 s), and suddenly disappeared (time = 220–240 s). The kinetochore indicated by black arrows remained unattached during imaging. The kinetochore indicated by an asterisk, which was located at the SPB from the beginning, appeared to be mono-oriented during observation. Bar, 10 μm.

(B) The intensities of Mad2-YFP on kinetochores indicated by arrows in (A) were measured (left axis). The vertical distances between the spindle and these kinetochores were also calculated using reconstructed three-dimensional images (right axis).

Supplemental Figure 4. Mad2 is retained on unattached kinetochores in anaphase.

Time-lapse images of *cut9* Δ*bub1* cells expressing Mad2-GFP and CFP-Atb2 after the removal of CBZ. Panels show the GFP channel (Mad2), and GFP merged with CFP (merge: GFP and CFP are colored green and magenta, respectively). Stacks of Z-sections with an interval of 0.4 μm were taken every 20 s. Three-dimensional images were flattened after deconvolution. Three-dimensional analysis revealed that the bright Mad2 spot indicated by an arrowhead and the spindle were not located on the same Z-section.

The numbers indicate the duration in seconds. Phase 3 spindle extension started at time = 120 s. The Mad2 spot indicated by an asterisk occurred after the onset of phase 3. These Mad2 spots were never colocalized with the spindle or the SPB, and were presumed to be coincident with kinetochores detached from the spindle. Bar, 10 μm.

Supplemental Figure 5. Δ*bub1* Δ*hos2*, but not Δ*mad3* Δ*hos2*, double deletion impairs kinetochore accumulation of Mad2.

Exponentially growing cells with indicated genotype were fixed in methanol at –80 °C and stained with Hoechst 33342 for visualization of nuclear DNA. Mad2 was visualized by GFP fusion in these cells. In merged panels, Mad2 and DNA are pseudocolored green and magenta, respectively. Arrowheads indicate the bright Mad2 spot localized on the kinetochore, and arrows indicate Mad2 on the spindle/SPBs. Bars, 10 μm.

(A) Cells with Mad2 localizing on kinetochores (arrowheads) were often observed in WT, Δ*bub1,* Δ*hos2*, and *bub1*-K762R Δ*hos2* strains. In contrast, in the Δ*bub1* Δ*hos2* and the *bub1*-Δ28-160 Δ*hos2* double mutant strains, such cells were undetectable, while Mad2 appeared able to be localized on the spindle/SPBs in this strain.

(B) In both Δ*mad3* and Δ*mad3* Δ*hos2* strains, mitotic cells with the Mad2 spot localizing on the kinetochore were observed (arrowheads).

Supplemental Figure 6. Chromosome segregation and mitotic progression in Δ*mad2* and Δ*mad2* Δ*hos2*.

(A) Time-lapse images of Δ*mad2* and Δ*mad2* Δ*hos2* cells are shown. Kinetochores and the SPB were visualized by Mis12-GFP (green) and Pcp1-CFP (magenta), respectively.

Cells were cultured in minimal medium at 26°C, and Stacks of Z-sections with an interval of 0.4 μm were taken every 0.5 min. Poleward movement of kinetochores during anaphase A was impaired in Δ*mad2* Δ*hos2*. The numbers indicate the duration in minutes. The time point at which phase 3 spindle extension began was defined as 0. Bar, 10 μm.

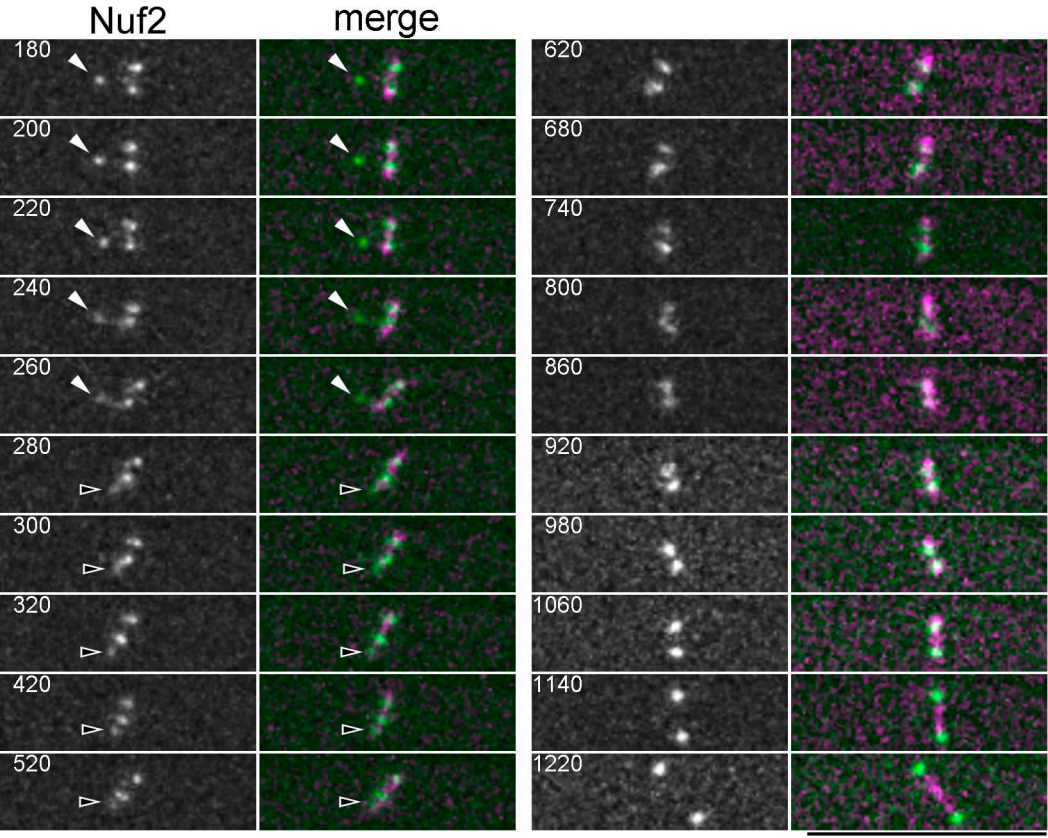
(B) Spindle lengths from multiple movies of exponentially growing the Δ*mad2* and the Δ*mad2* Δ*hos2* mutant cells were analyzed. Cells were cultured in minimal medium at 26°C, and Pcp1-CFP (SPB marker) was used to determine the length. The number of samples examined (n) and average time required for Phase 1 + 2 (average ± S.D.) in each strain are shown. Although metaphase-anaphase transition was accelerated in most cells, some cells showed transient metaphase delay in both strains. This slight delay may have been caused by activation of Mad2-independent SAC pathway(s).

Supplemental Figure 7. Δ*hos2* gene deletion does not significantly impair p-movement of kinetochores in prometaphase.

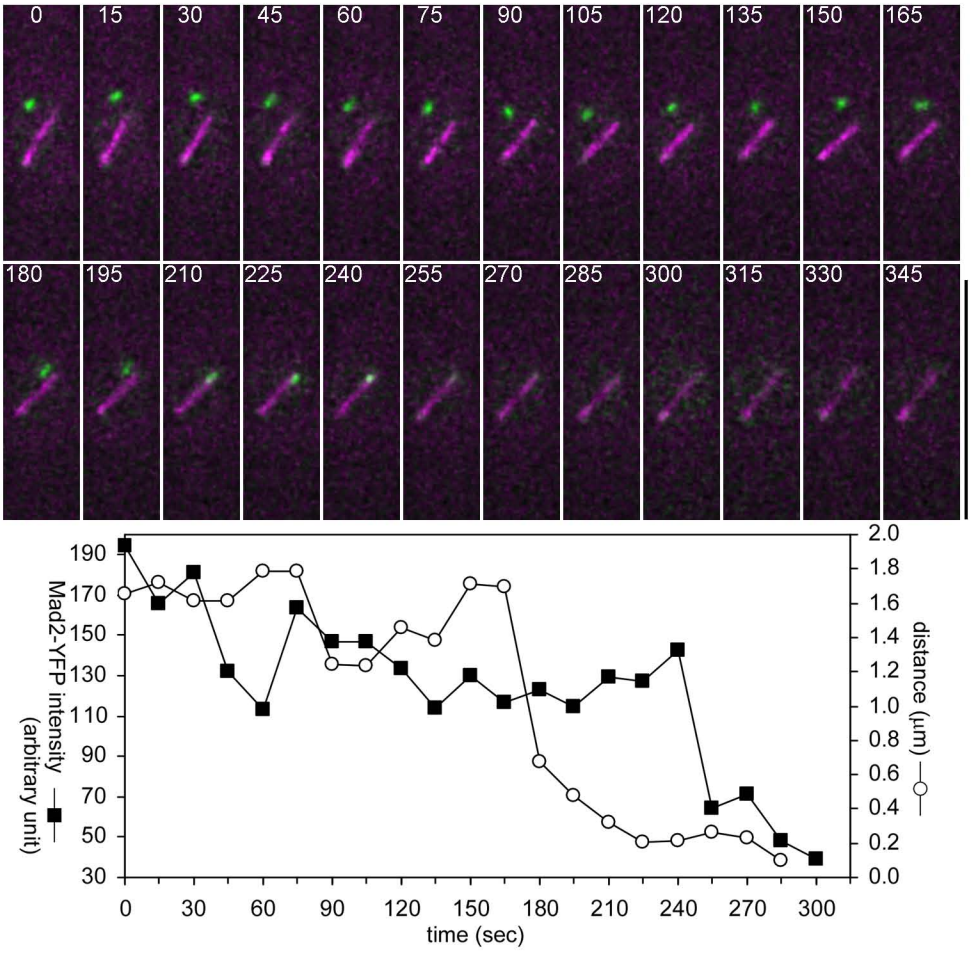
Time-lapse images of an *nda3* Δ*hos2* cell expressing Nuf2-YFP (kinetochore marker) and CFP-Atb2 (α-tubulin) after recovery from mitotic arrest. Panels show the YFP channel alone (Nuf2), and YFP merged with CFP (merge: YFP and CFP are pseudocolored green and magenta, respectively). Stacks of Z-sections with an interval of 0.4 μm were taken every 0.5 min, deconvoluted, and flattened. The kinetochore detached from the spindle is indicated by a closed arrowhead. In 7 of 11 *nda3* Δ*hos2* cells, the detached kinetochore failed to be rescued, whereas the kinetochore was eventually recaptured in the remaining 4 cells, as shown in this figure. The numbers indicate the duration in minutes. The captured kinetochore was delivered to the SPB at a rate slightly slower than that in WT

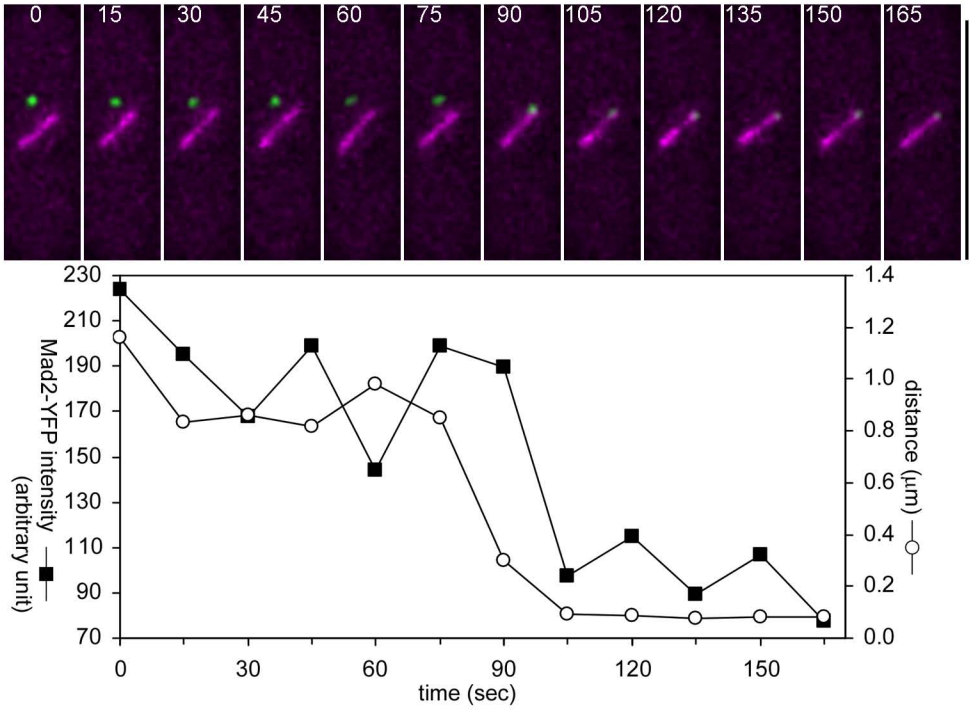
cells (time = 19.0–20.5 min) Bar, 10 μm.

Supplemental Figure 1. Saitoh et al.

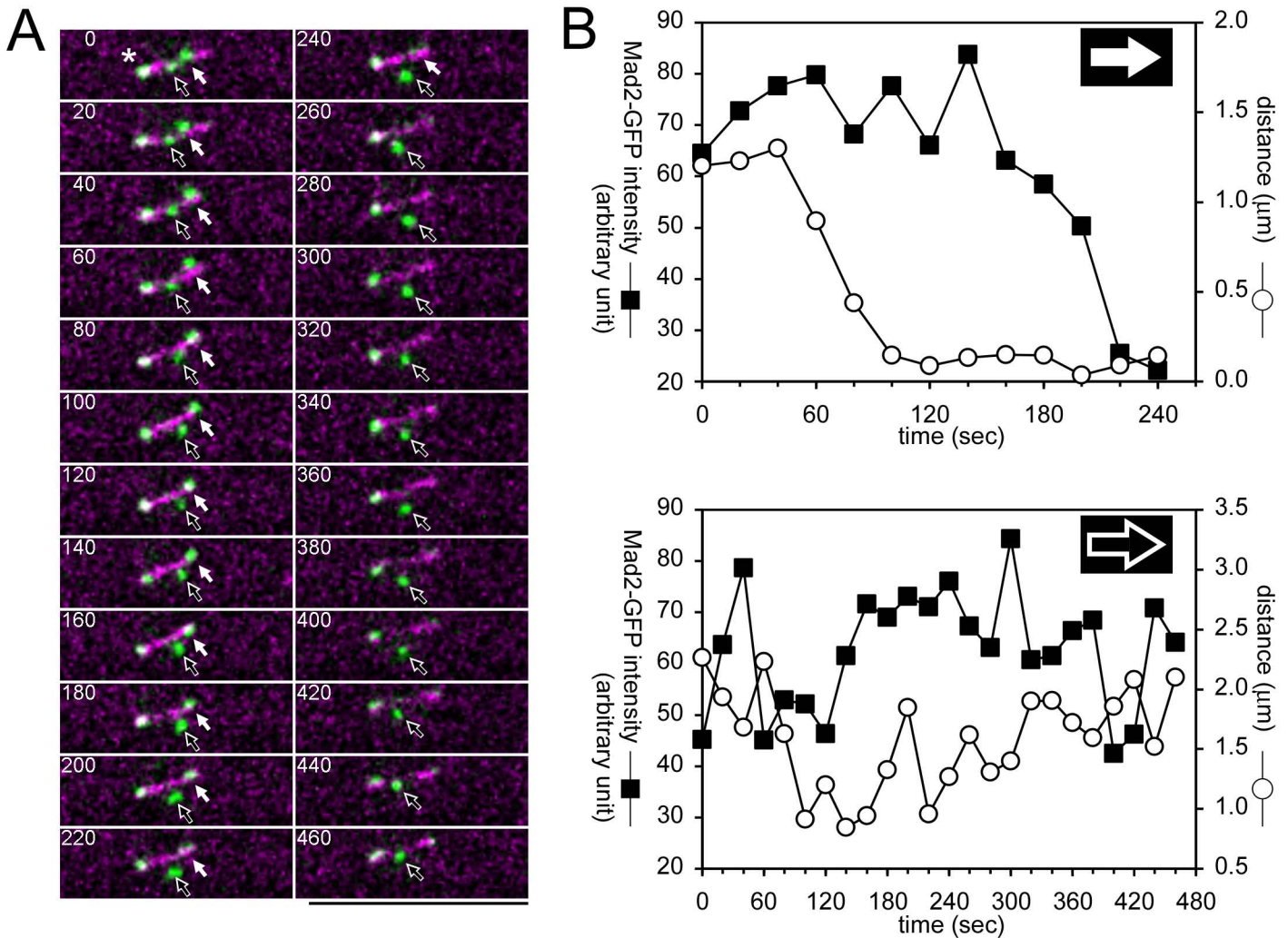


Supplemental Figure 2. Saitoh et al.

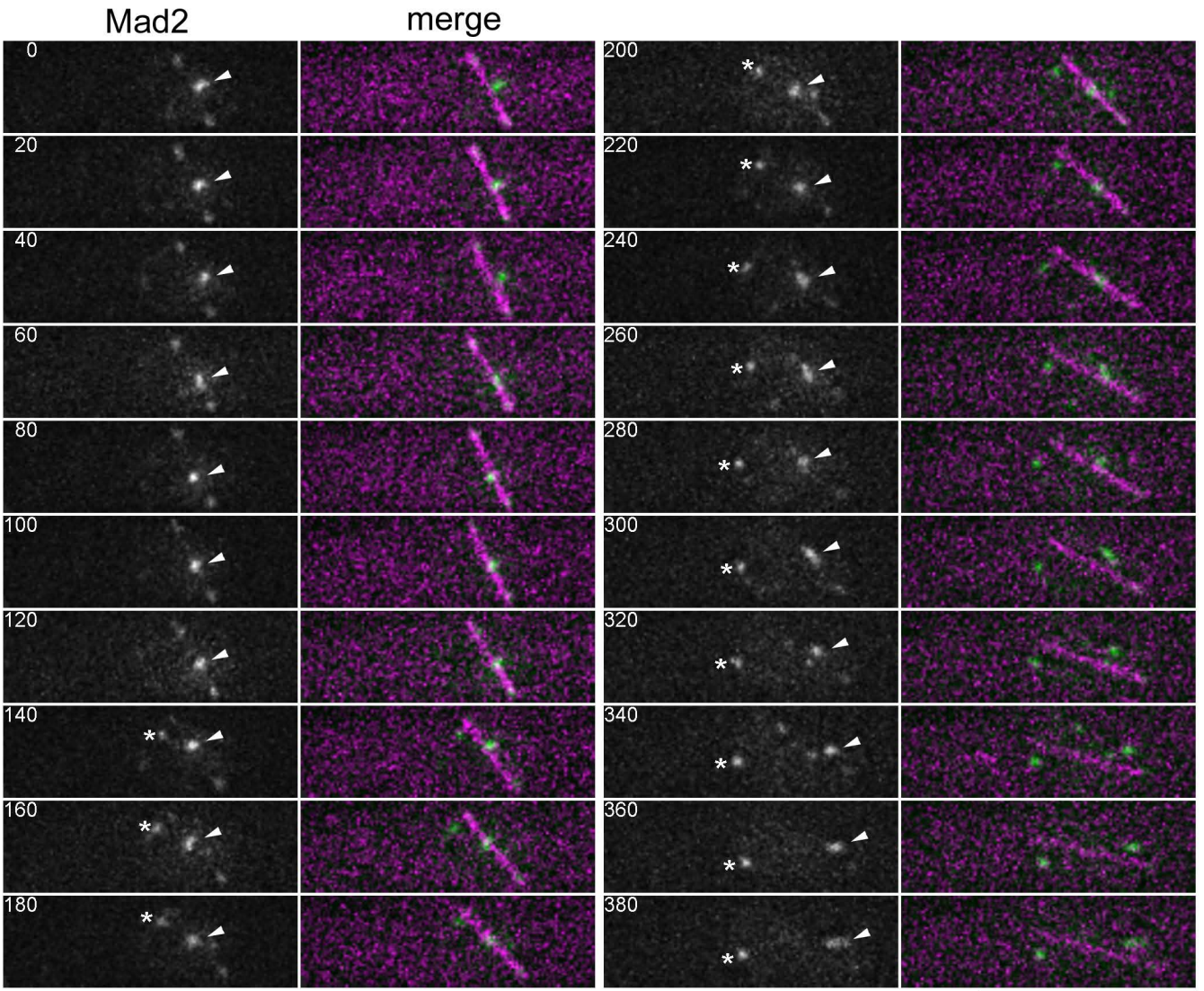




Supplemental Figure 3. Saitoh et al.



Supplemental Figure 4. Saitoh et al.



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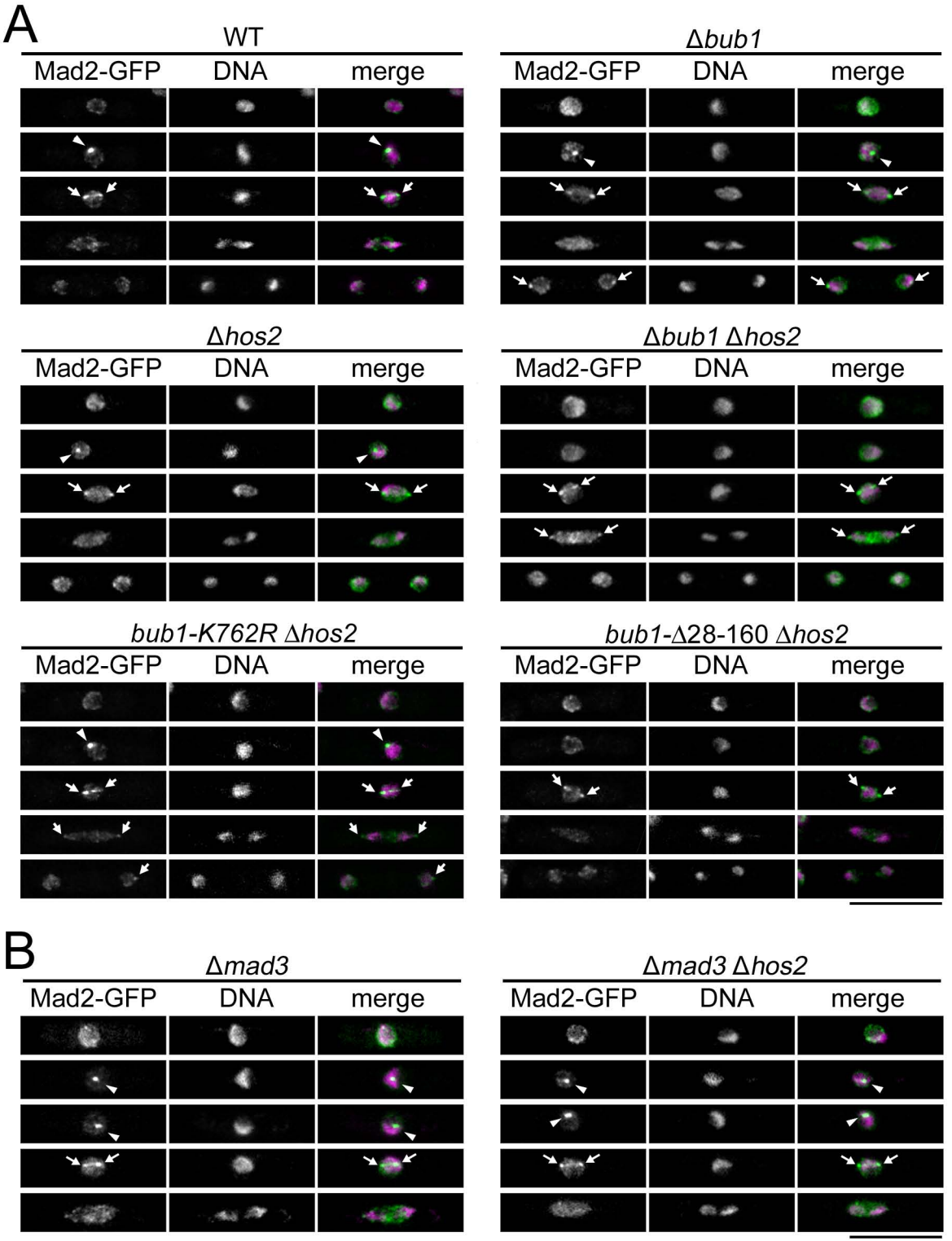
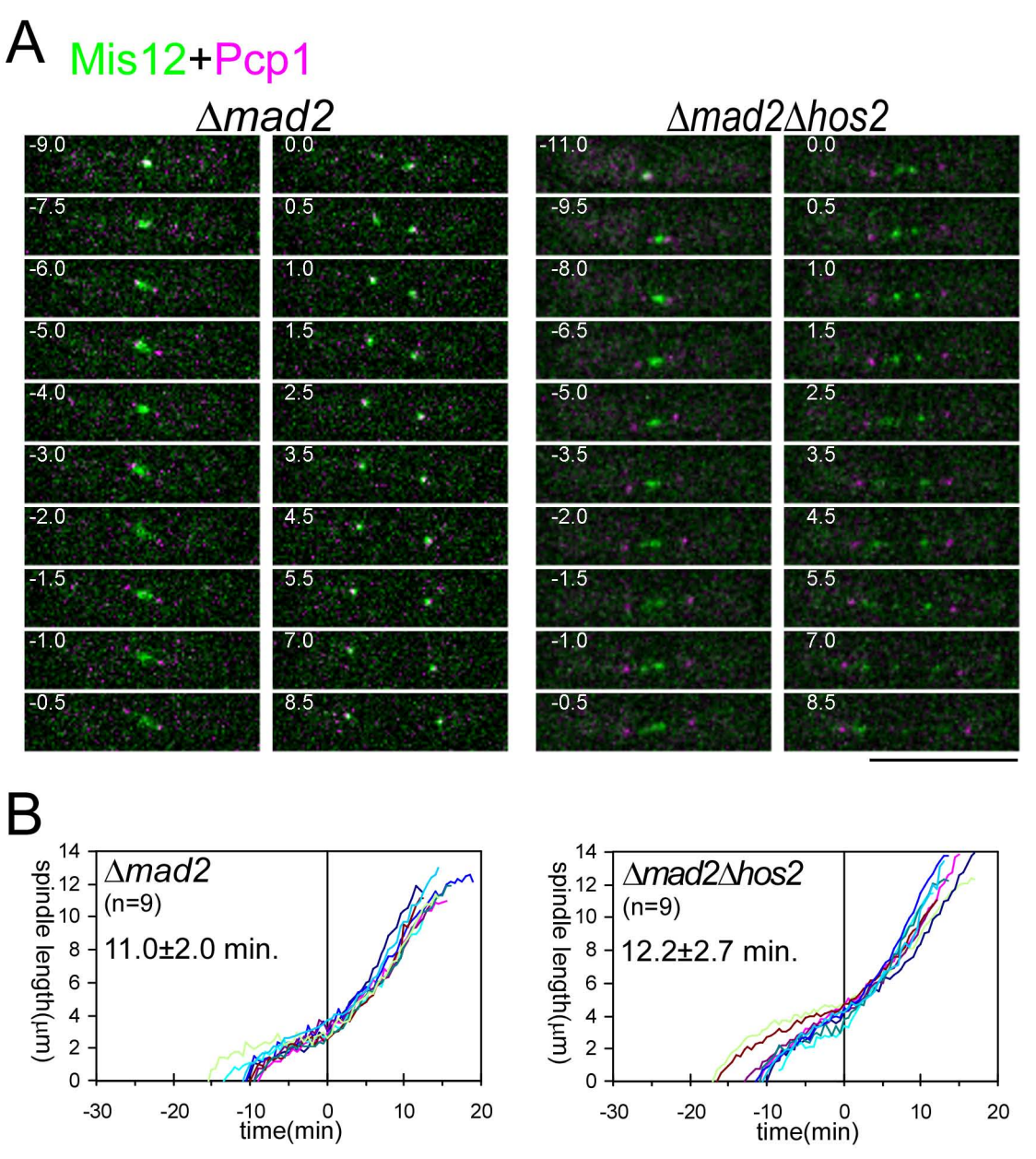


Supplemental Figure 6 Saitoh et al.



Supplemental Figure 7. Saitoh et al.