Schweizer et al., http://www.jcb.org/cgi/content/full/jcb.201506107/DC1

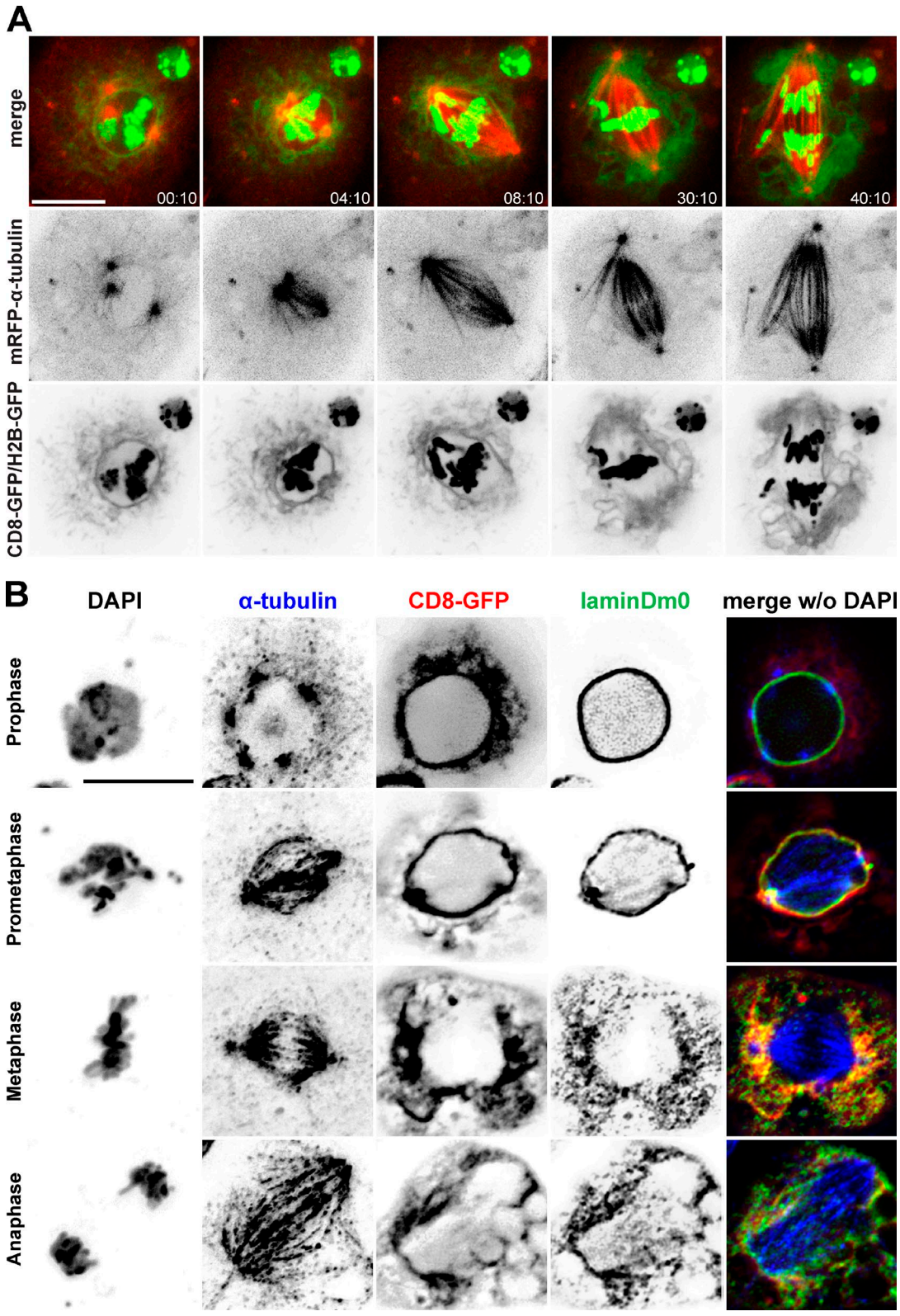


Figure S1. **Spindle envelope integrity decreases over time during mitosis.** (A) Live S2 cell expressing mRFP–α-tubulin, histone H2B-GFP, and CD8-GFP progressing through mitosis. (B) S2 cells expressing CD8-GFP processed for immunofluorescence with anti–α-tubulin and anti-lamin Dm0 (*Drosophila* Lamin B) antibodies. Bars, 10 µm.

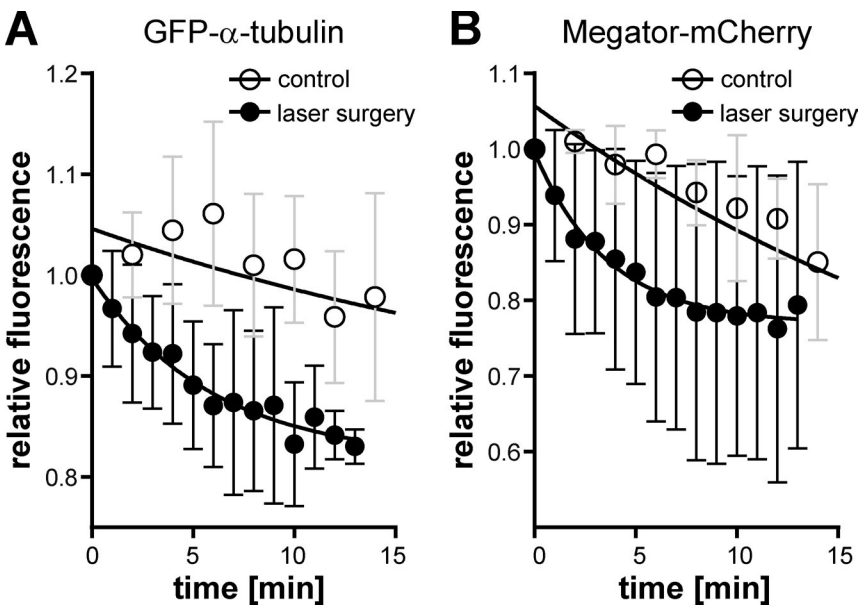


Figure S2. **A spindle envelope confines soluble tubulin and Megator during early mitosis.** Relative fluorescence (fluorescence signal in the nuclear region/fluorescence signal in the cytoplasm) of GFP–α-tubulin (A) and Megator-mCherry (B) in mitotic S2 cells after laser-induced perforation of the spindle envelope. MTs were depolymerized with colchicine before NEB, and laser microsurgery was performed after accumulation of soluble tubulin in the nuclear region. The relative fluorescence 30 s after laser microsurgery was set to 1. Data points represent means obtained from three cells; error bars represent standard deviations. Data are compared with those obtained from control cells at NEB (see Fig. 1 B).

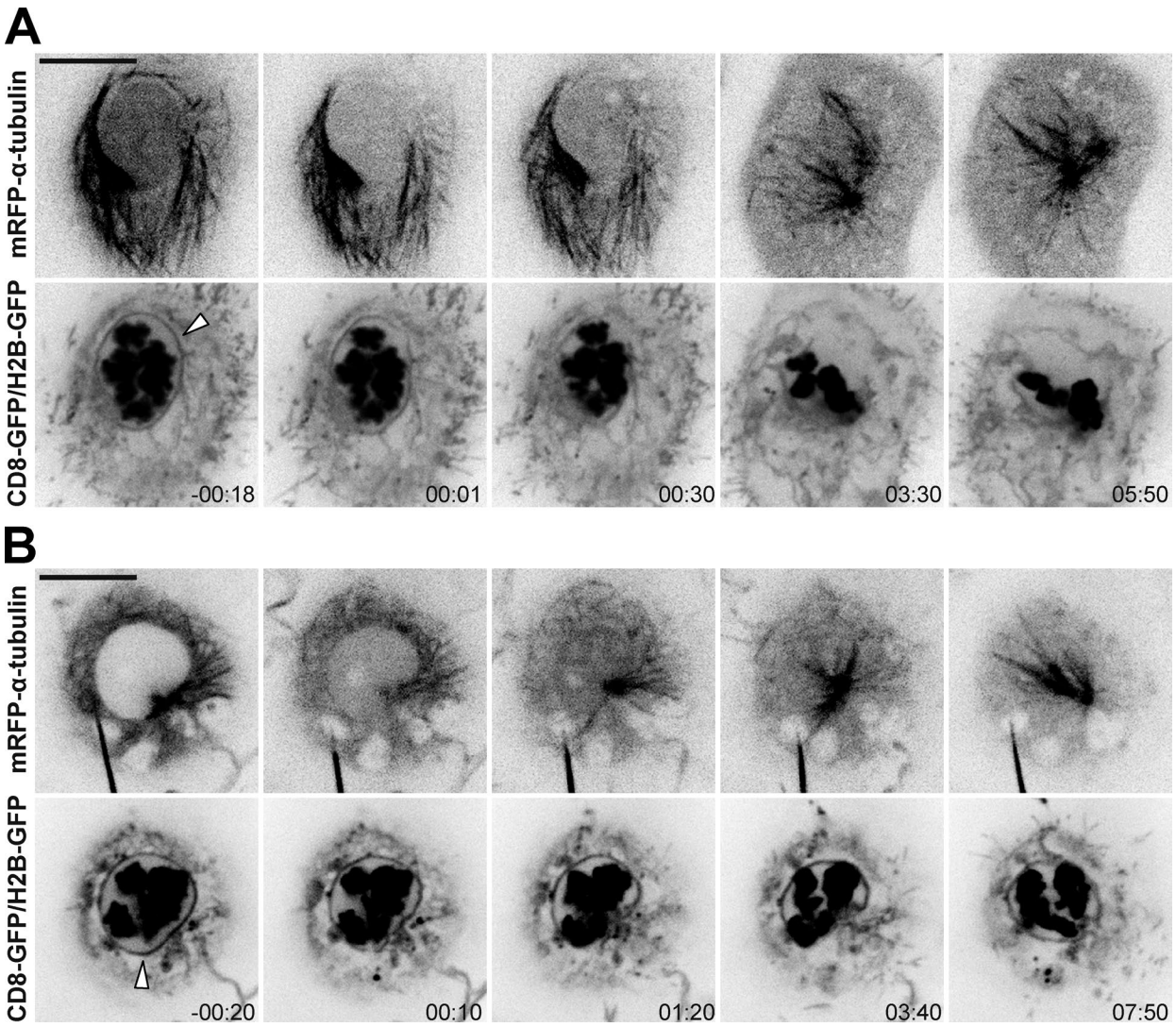
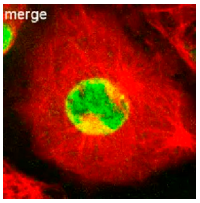
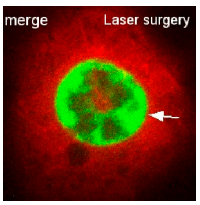


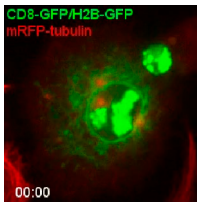
Figure S3. **Visualization of nuclear/spindle envelope disruption by laser microsurgery.** Disruption of the nuclear/spindle envelope in mitotic S2 cells expressing mRFP–α-tubulin, histone H2B-GFP, and CD8-GFP. In 4 out of 6 cells, laser microsurgery permanently disrupted the nuclear/spindle envelope (A), in 2 cells membranes resealed (B). The cut region is marked by the arrowheads. Time is given in minutes:seconds, relative to laser microsurgery. Bar, 10 µm.



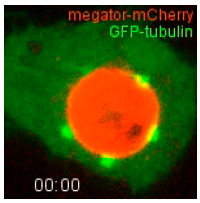
Video 1. **S2 cell expressing Megator-mCherry (red) and GFP–α-tubulin (green).** MTs were depolymerized with 100 µM colchicine before NEB. Images were analyzed by time-lapse confocal microscopy using an inverted microscope (TE2000U; Nikon; 100× 1.4 NA Plan-Apochromat DIC objective lens). Frames were taken every 30 s.



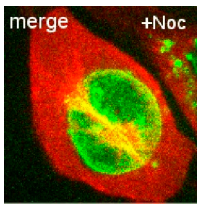
Video 2. **Laser microsurgery in an interphase S2 cell expressing Megator-mCherry (red) and GFP–α-tubulin (green) after MT depolymerization with 100 µM colchicine.** Images were analyzed by time-lapse confocal microscopy using an inverted microscope (TE2000U; Nikon; 100× 1.4 NA Plan-Apochromat DIC objective lens). Frames were taken every 30 s. The arrow indicates the location of laser microsurgery.



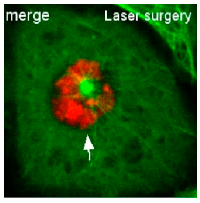
Video 3. **S2 cell expressing mRFP–α-tubulin (red), histone H2B-GFP (green), and CD8-GFP (green) progressing through mitosis.** Images were analyzed by time-lapse confocal microscopy using an inverted microscope (TE2000U; Nikon; 100× 1.4 NA Plan-Apochromat DIC objective lens). Frames were taken every 2 min.



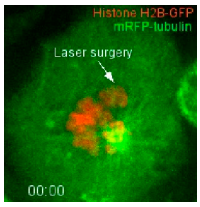
Video 4. **Laser microsurgery in an early prometaphase S2 cell expressing Megator-mCherry (red) and GFP–α-tubulin (green) after MT depolymerization with 100 µM colchicine.** Images were analyzed by time-lapse confocal microscopy using an inverted microscope (TE2000U; Nikon; 100× 1.4 NA Plan-Apochromat DIC objective lens). Frames were taken every 30 s.



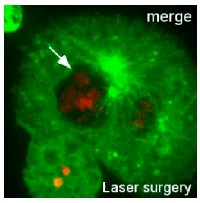
Video 5. **HeLa cell expressing Tpr-GFP (green) and mCherry–α-tubulin (red) after MT depolymerization with 3.3 µM nocodazole before NEB.** Images were analyzed by time-lapse confocal microscopy using an inverted microscope (TE2000U; Nikon; 100× 1.4 NA Plan-Apochromat DIC objective lens). Frames were taken every 30 s.



Video 6. **Laser microsurgery at the NE in a prophase S2 cell expressing mRFP–α-tubulin (green) and histone H2B-GFP (red).** Images were analyzed by time-lapse confocal microscopy using an inverted microscope (TE2000U; Nikon; 100× 1.4 NA Plan-Apochromatic DIC objective lens). Frames were taken every 30 s. The arrow indicates the location of laser microsurgery.



Video 7. **Laser microsurgery at the spindle envelope in an early prometaphase S2 cell expressing mRFP–α-tubulin (green) and histone H2B-GFP (red).** Images were analyzed by time-lapse confocal microscopy using an inverted microscope (TE2000U; Nikon; 100× 1.4 NA Plan-Apochromat DIC objective lens). Frames were taken every 30 s. The arrow indicates the location of laser microsurgery.



Video 8. **Laser microsurgery at the NE in a binucleated prophase S2 cell expressing mRFP–α-tubulin (green) and histone H2B-GFP (red).** Images were analyzed by time-lapse confocal microscopy using an inverted microscope (TE2000U; Nikon; 100× 1.4 NA Plan-Apochromat DIC objective lens). Frames were taken every 30 s. The arrow indicates the location of laser microsurgery.