El Amine et al., http://www.jcb.org/cgi/content/full/jcb.201305053/DC1



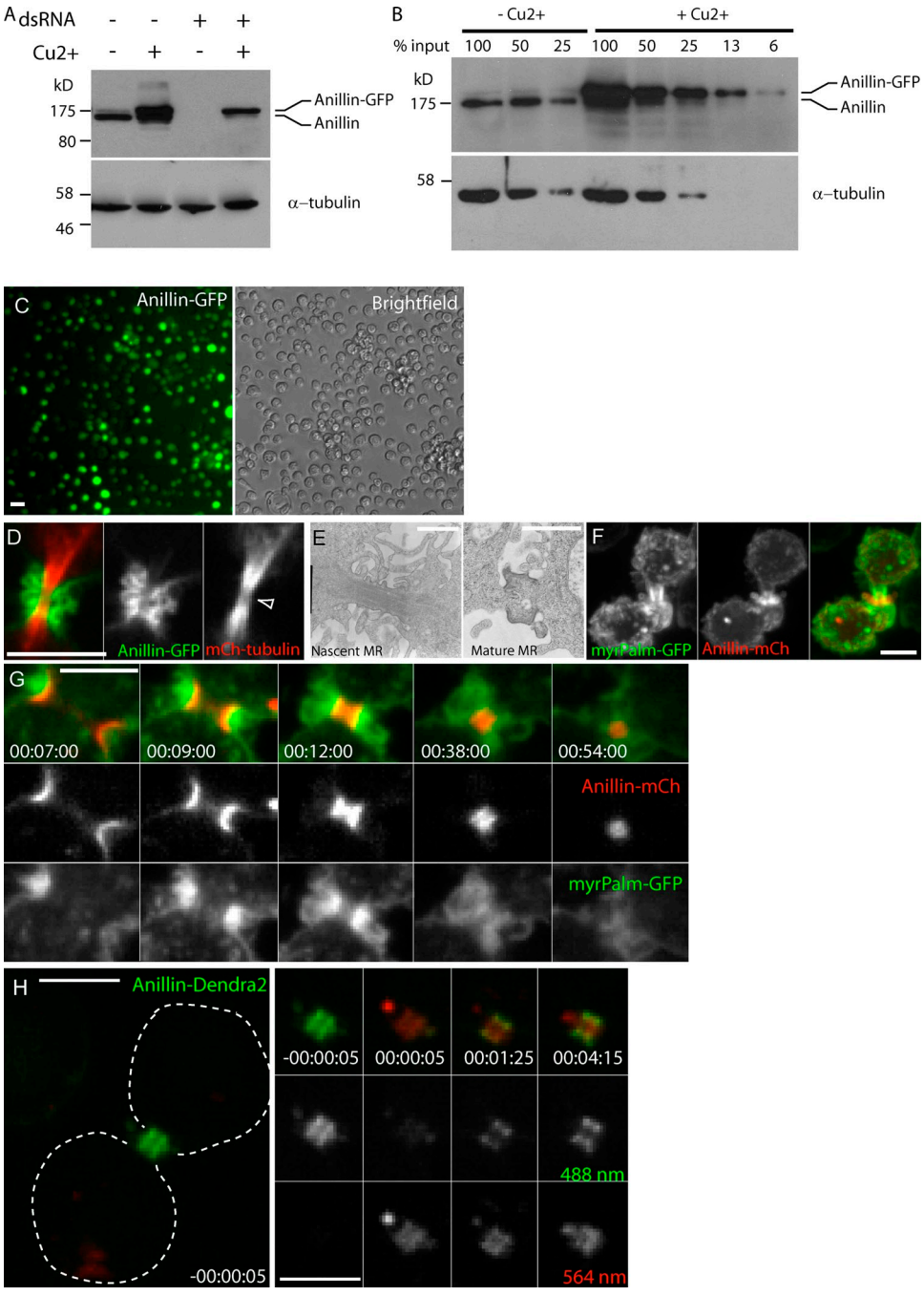



Figure S1. **Anillin-FP expression and localization during MR maturation.** (A) Anti-Anillin immunoblot analysis of cell lysates after 3-d treatment with or without Anillin dsRNAs, with or without $CuSO_4$ induction of Anillin-GFP. The same blot was cut and probed with an anti-tubulin antibody as a loading control. (B, top) Serial dilutions of Anillin-GFP cell lysates with or without $CuSO_4$ induction, probed with anti-Anillin antibodies. (bottom) The same blot was cut and probed with an anti-tubulin antibody as a loading control. From this, it can be estimated that Anillin-GFP is expressed at approximately fourfold higher levels than endogenous Anillin, although we note that this is likely an overestimate in mitotic cells because endogenous Anillin expression is cell cycle regulated, accumulating in G2 (Field and Alberts, 1995), whereas Anillin-GFP expression is not. (C) Fluorescence and bright-field images of Anillin-GFP expression, showing that close to 100% of the cells express Anillin-GFP. (D) Single confocal sections of a nascent MR from a cell expressing Anillin-GFP and mCh-tubulin. Arrowhead marks the midbody region. (E) Transmission electron micrographs of Anillin-GFP–expressing S2 cells. (F and G) Single confocal sections of a nascent MR expressing Anillin-mCh and myrpalm-GFP. (H) Single confocal sections of Anillin-Dendra2–expressing nascent MR before and after photoconversion, imaged using both 488- and 564-nm light. Dotted lines mark the cell boundary, and separated channels of the MR region are shown at the right (63× objective and 2 × 2 camera binning used throughout, except in C, which is a 20× objective). Times are given in hours, minutes, and seconds. Bars: (C, D, and F–H) 5 µm; (E) 1 µm.

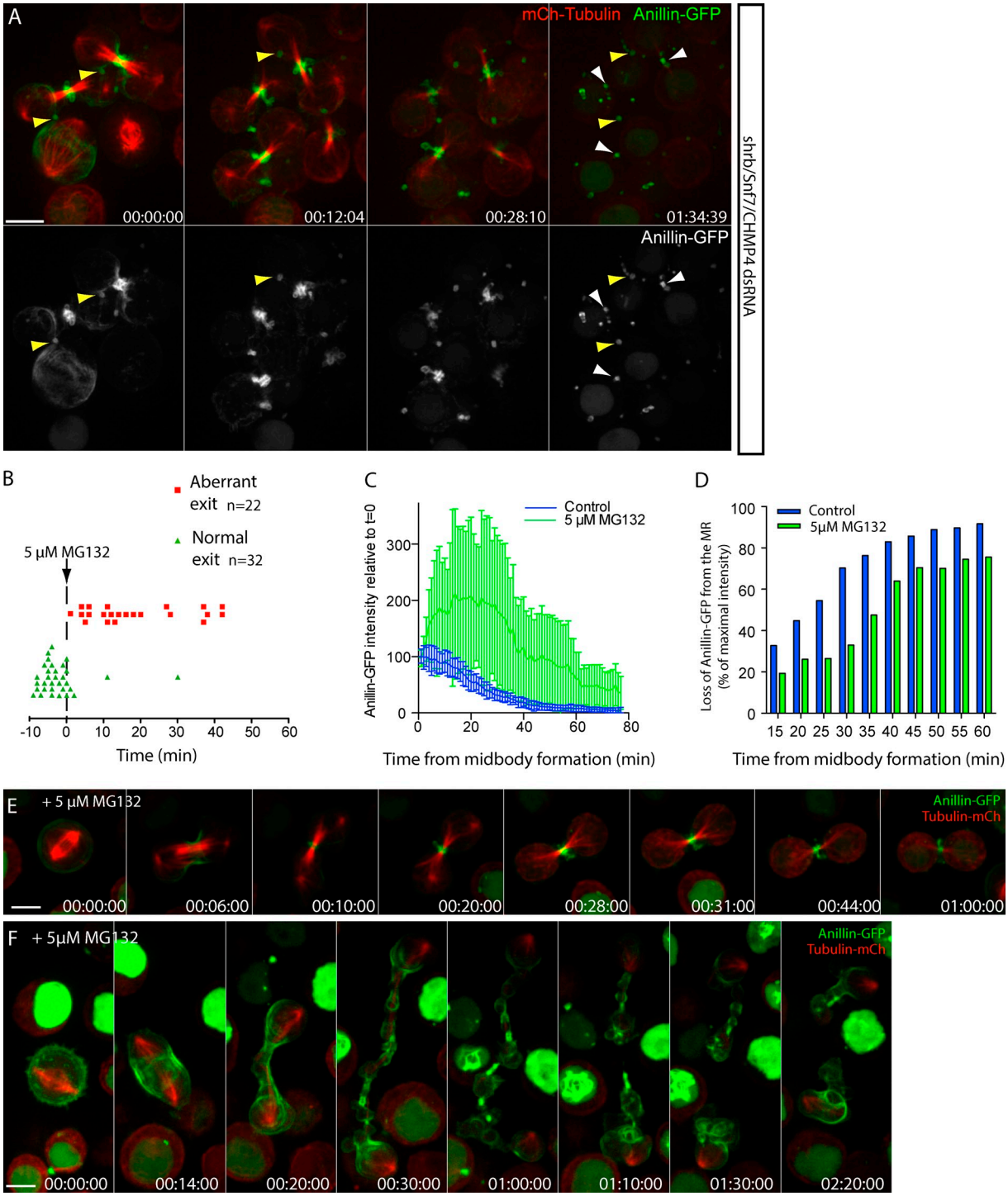



Figure S2. **Additional cells treated with Shrub/CHMP4 dsRNAs or MG132.** (A) Anillin-GFP–expressing S2 cells incubated for 3 d with Shrub (shrb)/CHMP4 dsRNAs. Yellow arrowheads depict MR from a previous division that still connects sister cells. White arrowheads depict shedding from the nascent MRs of the current division. Same cells as shown in Fig. 3 H. (B) Consequences of acute administration of 5 µM MG132 to mitotic cells expressing Anillin-GFP and mCh-tubulin. Points represent individual cells undergoing anaphase at the indicated times relative to MG132 addition. Data are from three independent experiments. (C) Changes in Anillin-GFP intensities at the MR in cells undergoing "normal" exit in the presence or absence of 5 µM MG132, relative to the time of midbody formation (t = 0; mean values ± SD; *n* = 14 and 11, respectively). (D) Anillin-GFP sum intensity values at the MR are plotted at 5-min intervals relative to the maximal sum intensity value for each cell, regardless of when during MR maturation this occurred. Mean values are shown for cells in the presence (*n* = 14) or absence (*n* = 11) of 5 µM MG132. Data are from three independent experiments. (E) Selected frames from a time-lapse sequence of an Anillin-GFP cell in the presence of 5 µM MG132. (F) Example of an Anillin-GFP–expressing cell exhibiting aberrant mitotic exit and cytokinesis in the presence of 5 µM MG132 added just before the metaphase/anaphase transition. Times are given in hours, minutes, and seconds. Bars, 5 µm.

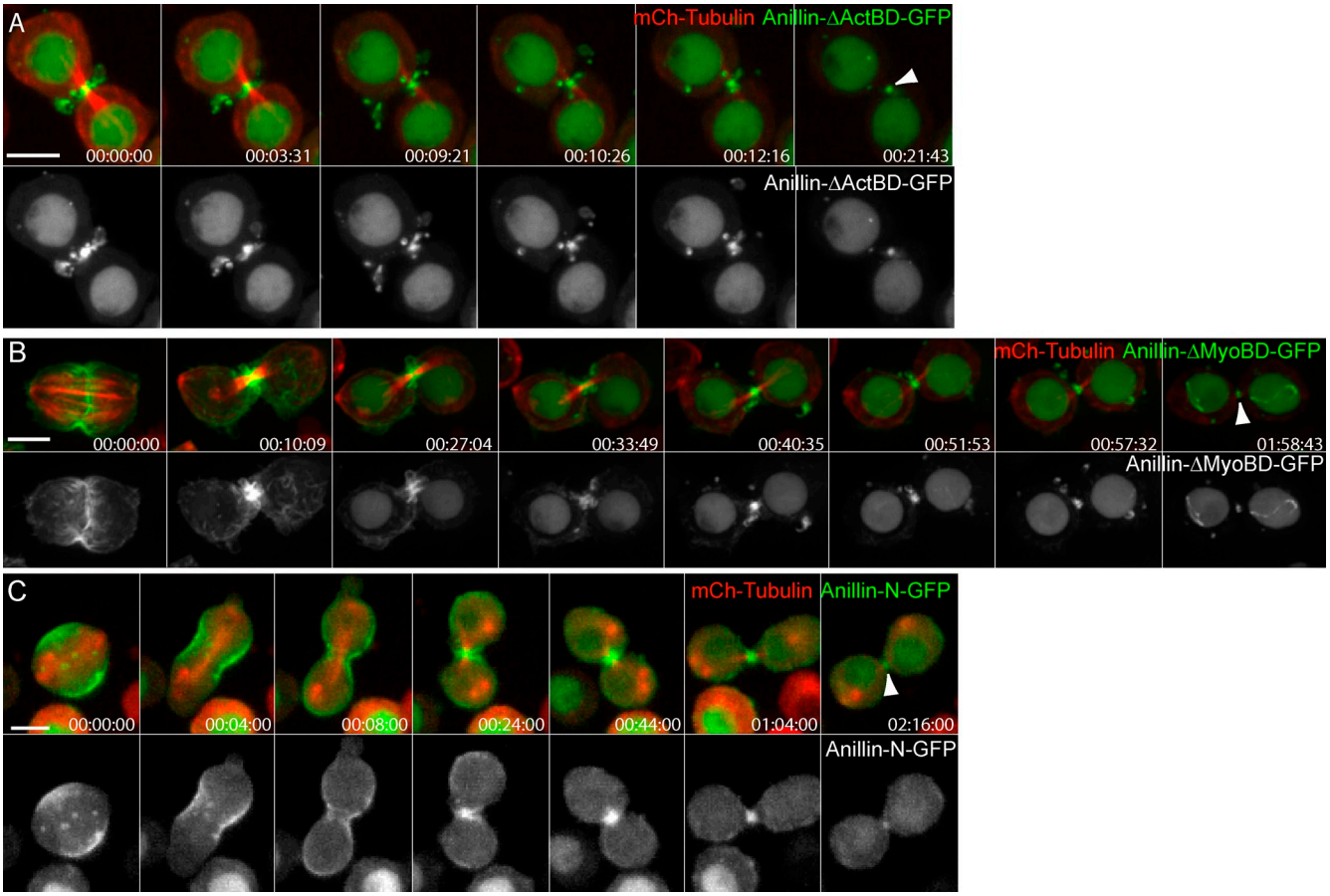



Figure S3. **Behaviors of additional Anillin truncations during MR maturation.** (A) Cell coexpressing Anillin-ΔActBD-GFP and mCh-tubulin. (B) Cell coexpressing Anillin-ΔMyoBD-GFP and mCh-tubulin. (C) Cell coexpressing Anillin-N-GFP and mCh-tubulin. Arrowheads point to the mature MRs. Times are given in hours, minutes, and seconds. Bars, 5 µm.

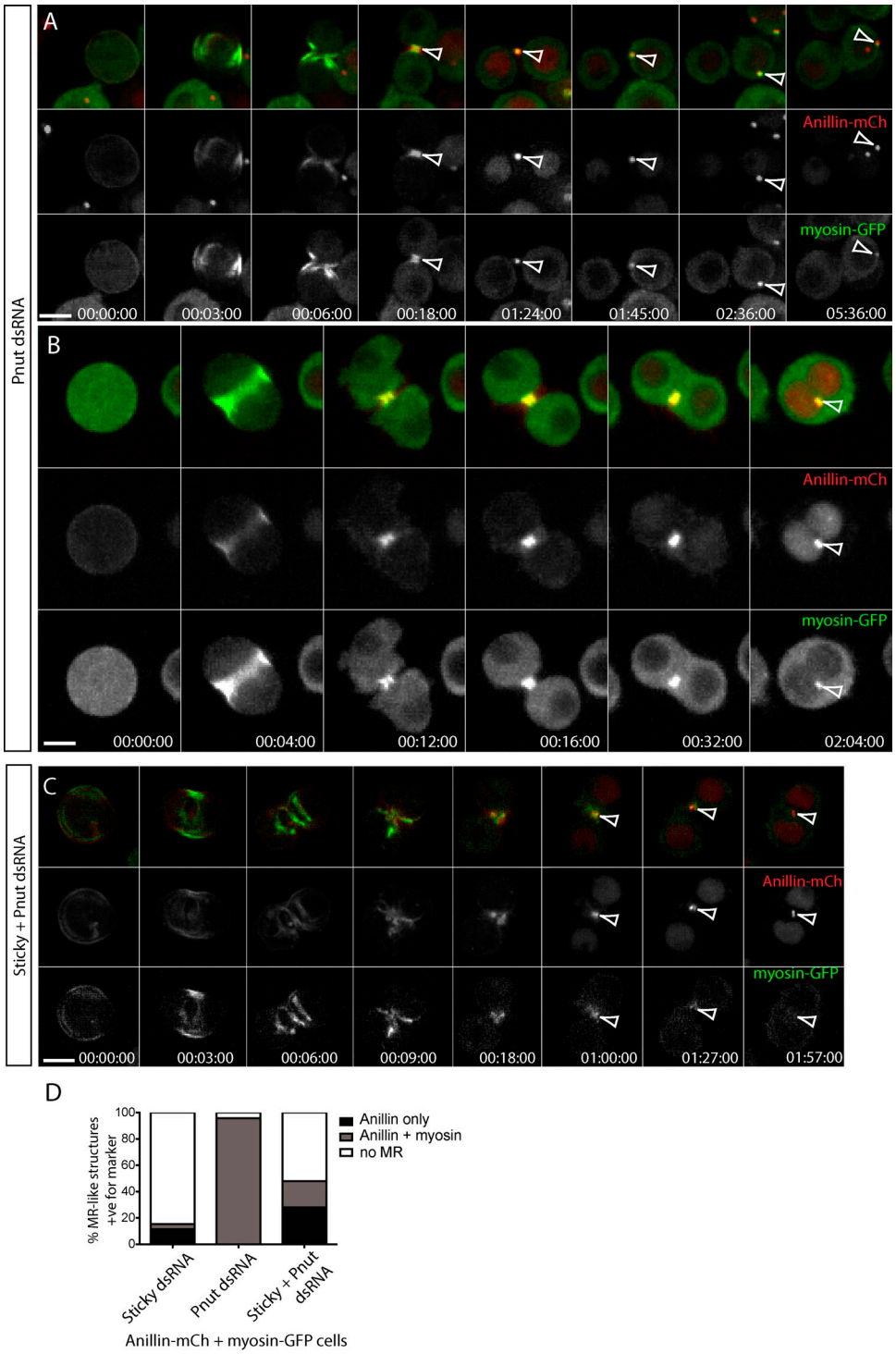



Figure S4. **Codepletion of Sticky and Pnut disrupts MR formation.** (A–C) Time-lapse sequences of cells coexpressing Anillin-mCh and myosin-GFP attempting cytokinesis after incubation with Pnut dsRNA (A and B) or Sticky and Pnut dsRNAs (C). Arrowheads mark the MR structures that result after successful (A) or failed (B and C) division attempts. (D) Quantification from time-lapse records (40× objective and 2 × 2 camera binning) of failed division attempts resulting in the presence or absence of internal MR-like structures positive for Anillin-mCh alone or Anillin-mCh and myosin-GFP after depletion of Sticky ($n$ = 25), Pnut ($n$ = 28), or both ($n$ = 46). Data are from one experiment representative of three repeats. Times are given in hours, minutes, and seconds. Bars, 5 µm.

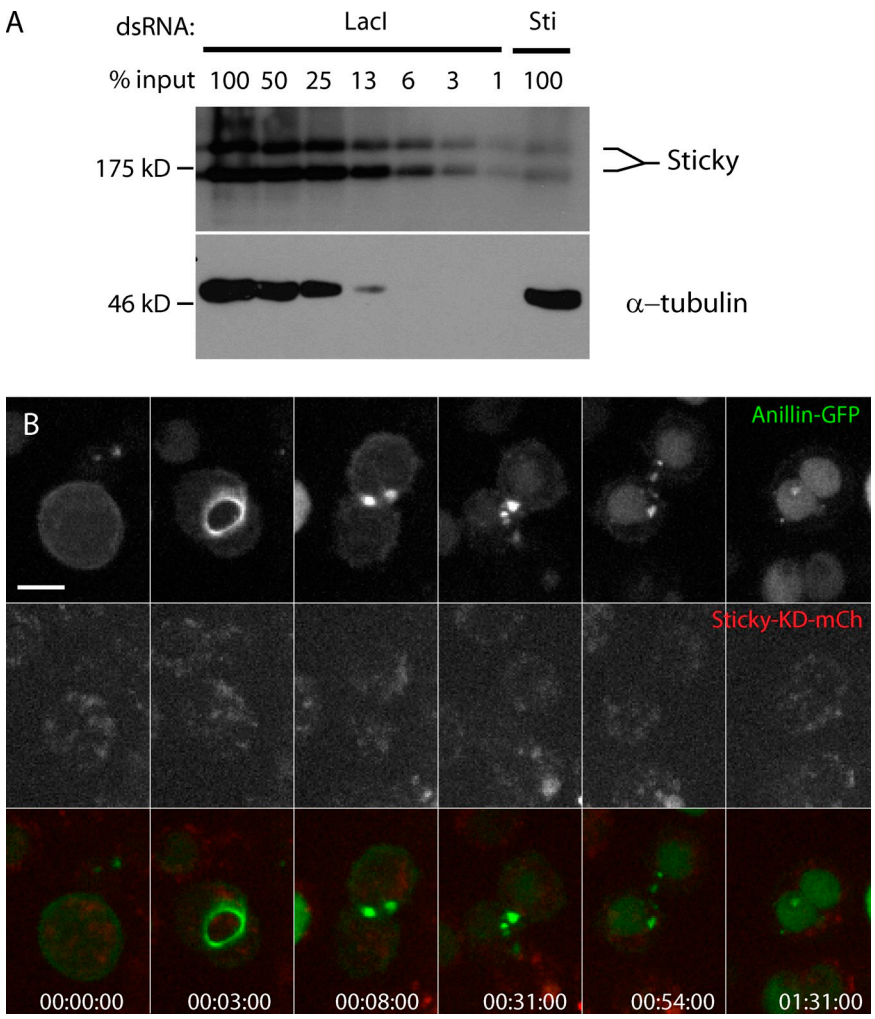
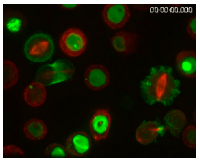
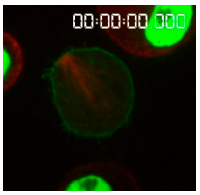


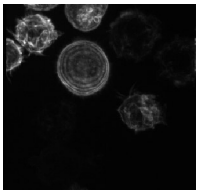

Figure S5. **Sticky immunoblot and example of cell Sticky-KD-mCh failure.** (A) Anti-Sticky immunoblot analysis of S2 cell lysates after a 3-d treatment with LacI or Sticky (Sti) dsRNA. A serial dilution of the LacI lysate demonstrates the extreme sensitivity of detection of the antibody and the efficacy of the RNAi. (bottom) The same blot was cut and probed with an anti-tubulin antibody as a loading control. (B) Example of a cell expressing Anillin-GFP and Sticky-KD-mCh, depleted of endogenous Sticky, which failed to recruit any Sticky-KD-mCh and failed cytokinesis. Times are given in hours, minutes, and seconds. Bar, 5 µm.



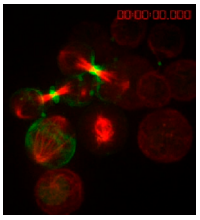
Video 1. **Extrusion of Anillin-GFP from the nascent MR.** *Drosophila* S2 cells stably expressing Anillin-GFP (green) and mCh-tubulin (red). Images were acquired by time-lapse spinning-disc confocal microscopy, with frames taken every 38 s for 1 h and 5 min. A maximum intensity projection of multiple confocal sections is shown. The cell at the top left corresponds to Fig. 1 B. Times are shown in hours, minutes, and seconds.



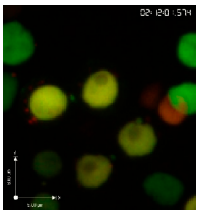

Video 2. **Internalization of Anillin-GFP from the nascent MR.** *Drosophila* S2 cell stably expressing Anillin-GFP (green) and mCh-tubulin (red). Images were acquired by time-lapse spinning-disc confocal microscopy (63×, 1.4 NA objective) with frames taken every 45 s for 2 h. Single confocal sections are shown. Video corresponds to Fig. 1 D. Times are shown in hours, minutes, and seconds.



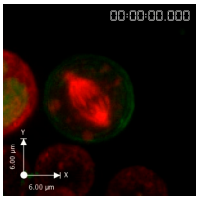
Video 3. **F-actin is not extruded from the nascent MR.** *Drosophila* S2 cell stably expressing LifeAct-GFP (center, green in merged) and Anillin-mCh (left, red in merged). Images were acquired by time-lapse spinning-disc confocal microscopy (63×, 1.4 NA objective) with frames taken every 60 s for 1 h and 35 min. A maximum intensity projection of multiple confocal sections is shown. Video corresponds to the cell in Fig. 2 I. Times are shown in hours, minutes, and seconds.



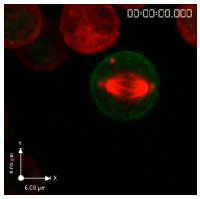

Video 4. **Cells expressing Anillin-GFP undergoing cytokinesis after depletion of Shrub/CHMP4B.** *Drosophila* S2 cells stably expressing Anillin-GFP (left, green in merged) and mCh-tubulin (center, red in merged) treated with Shrub dsRNAs for 4 d. Images were acquired by time-lapse spinning-disc confocal microscopy (100×, 1.4 NA objective) with frames taken every 2 min for 2 h and 45 min. A maximum intensity projection of multiple confocal sections is shown. Video corresponds to Fig. 3 H. Times are shown in hours, minutes, and seconds.



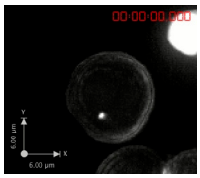
Video 5. **Cell expressing Anillin-ΔN+CD-mCh and Anillin-ΔC-GFP undergoing cytokinesis.** A *Drosophila* S2 cell stably expressing Anillin-ΔN+CD-mCh (center, red in merged) and Anillin-ΔC-GFP (left, green in merged) undergoing cytokinesis. Images were acquired by time-lapse spinning-disc confocal microscopy with frames taken every 60 s for 2 h and 12 min. A maximum intensity projection of multiple confocal sections is shown. Video corresponds to Fig. 4 B. Times are shown in hours, minutes, and seconds.



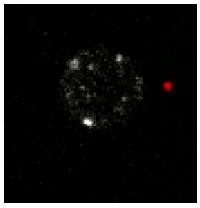

Video 6. **Sticky-depleted cell expressing Anillin-GFP succeeding at cytokinesis.** *Drosophila* S2 cell stably expressing Anillin-GFP (green) and mCh-tubulin (red) after a 48-h incubation with Sticky dsRNA. Images were acquired by time-lapse spinning-disc confocal microscopy with frames taken every 60 s for 1 h and 33 min. A maximum intensity projection of multiple confocal sections is shown. Video corresponds to Fig. 6 A. Times are shown in hours, minutes, and seconds.



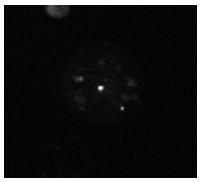
Video 7. **Sticky-depleted cell expressing Anillin-GFP failing cytokinesis.** *Drosophila* S2 cell stably expressing Anillin-GFP (green) and mCh-tubulin (red) after a 48-h incubation with Sticky dsRNA. Images were acquired by time-lapse spinning-disc confocal microscopy with frames taken every 60 s for 2 h and 9 min. A maximum intensity projection of multiple confocal sections is shown. Video corresponds to Fig. 6 B. Times are shown in hours, minutes, and seconds.



Video 8. **Cell expressing Anillin-GFP attempting cytokinesis after Sticky and Pnut codepletion.** *Drosophila* S2 cell expressing Anillin-GFP attempting cytokinesis after Pnut and Sticky codepletion. Images were acquired by time-lapse spinning-disc confocal microscopy with frames taken every 2 min for 2 h. A maximum intensity projection of multiple confocal sections is shown. Video corresponds to Fig. 7 G. Times are shown in hours, minutes, and seconds.



Video 9. **Cell expressing Anillin-GFP and Sticky-mCh undergoing cytokinesis after endogenous Sticky depletion.** Cell expressing Anillin-GFP (left, green in merged) and Sticky-mCh (center, red in merged) incubated with Sticky 3′ UTR dsRNA for 3 d. Images were acquired by time-lapse spinning-disc confocal microscopy with frames taken every 4 min for 3 h and 4 min. A maximum intensity projection of multiple confocal sections is shown. Video corresponds to Fig. 8 C. Times are shown in hours, minutes, and seconds.



Video 10. **Cell expressing Anillin-GFP and Sticky-KD-mCh undergoing cytokinesis after endogenous Sticky depletion.** Cell expressing Anillin-GFP (left, green in merged) and Sticky-KD-mCh (center, red in merged) incubated with Sticky 3′ UTR dsRNA for 3 d. Images were acquired by time-lapse spinning-disc confocal microscopy with frames taken every 60 s for 2 h and 2 min. A maximum intensity projection of multiple confocal sections is shown. Video corresponds to Fig. 8 E. Times are shown in hours, minutes, and seconds.

## Reference

Field, C.M., and B.M. Alberts. 1995. Anillin, a contractile ring protein that cycles from the nucleus to the cell cortex. *J. Cell Biol.* 131:165–178. http://dx.doi.org/10.1083/jcb.131.1.165