Ingerman et al., http://www.jcb.org/cgi/content/full/jcb.201211069/DC1

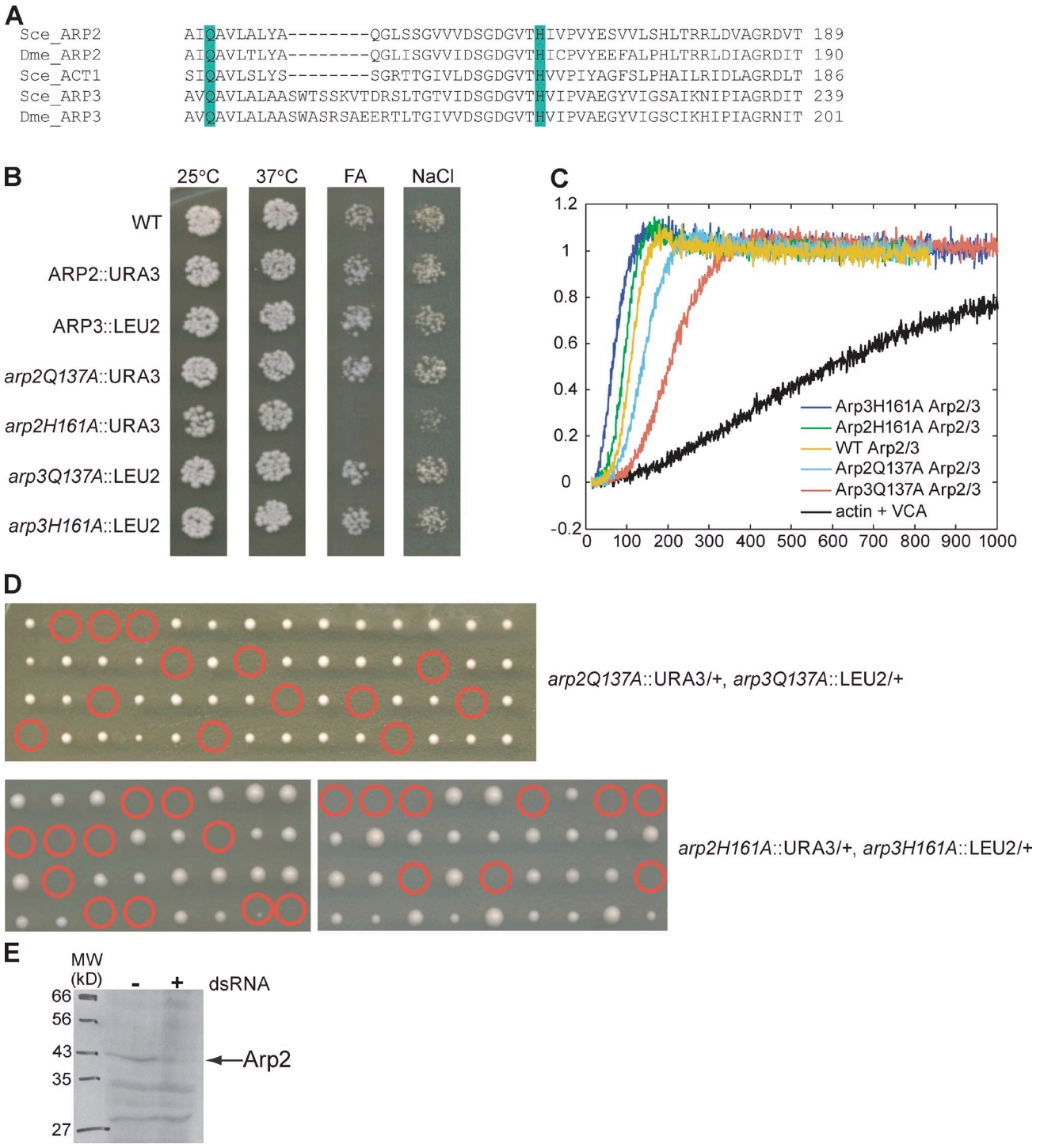


Figure S1. **Yeast and *Drosophila* Arp2/3 mutations and depletions.** (A) Clustal-W alignment of *Saccharomyces* ACT1, ARP2, ARP3, and *Drosophila* ARP2 and ARP3. In ARP2 and ARP3 of yeast and *Drosophila*, we mutated residues analogous to Q137 and H161 of yeast actin to create our ATP hydrolysis mutants. (B) The ATP hydrolysis mutant allele H161A compromises yeast growth. Arp2 ATPase mutant *arp2H161A::URA3* confers a growth defect upon yeast under high stress conditions. FA denotes 3% formamide. NaCl denotes 0.9 M NaCl. (C) The allele *arp2-Q137A::URA3* is synthetically lethal with *arp3-Q137A::LEU2*. The allele *arp2-H161A::URA3* is synthetically lethal with *arp3-H161A::LEU2*. Red circles denote double mutants. (D) Double-stranded RNA directed against the 5′ and 3′ untranslated regions of ARP2 deplete Arp2 protein. Whole-cell lysates from S2 cells treated with dsRNA were resolved by SDS-PAGE, transferred to PVDF membrane, and Western blotted with Arp2 antibodies.

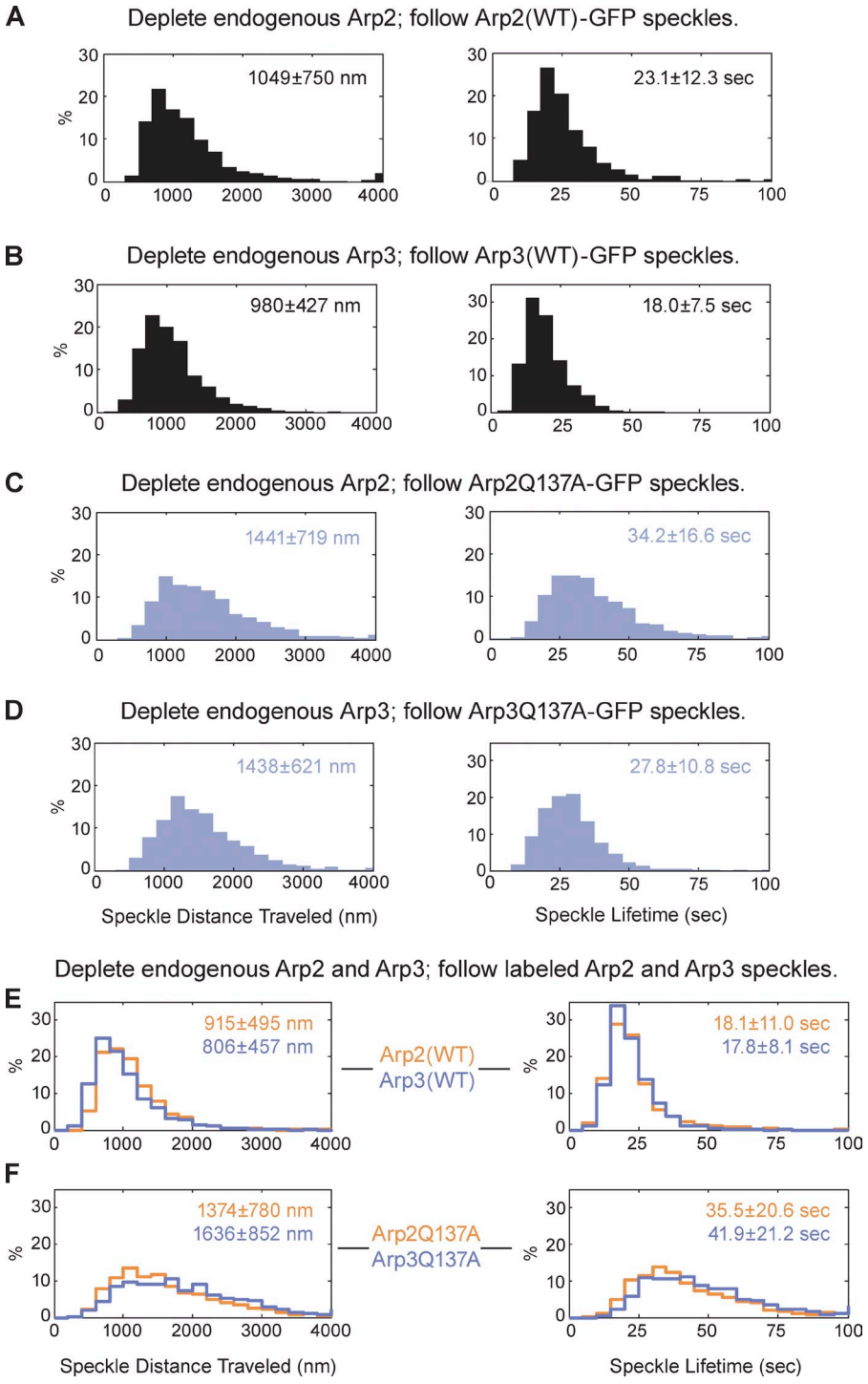


Figure S2. **Histograms of S2 speckle data.** (A–D) Histograms corresponding to data from Fig. 3. (A) Arp2(WT) GFP speckles (25 cells, 676 speckles). (B) Arp3(WT) GFP speckles (70 cells, 853 speckles). (C) Arp2Q137A-GFP speckles (41 cells, 1,398 speckles). (D) Arp3Q137A-GFP speckles (77 cells, 1,600 speckles). (E and F) Histograms corresponding to box-and-whisker plots from Fig. 5. Median values are noted on each histogram. Left column, speckle distance traveled; Right column, speckle lifetime. (E) 67 cells, 788 Arp2 speckles, 1,236 Arp3 speckles. (F) 80 cells, 1,515 Arp2 speckles, 1,904 Arp3 speckles.

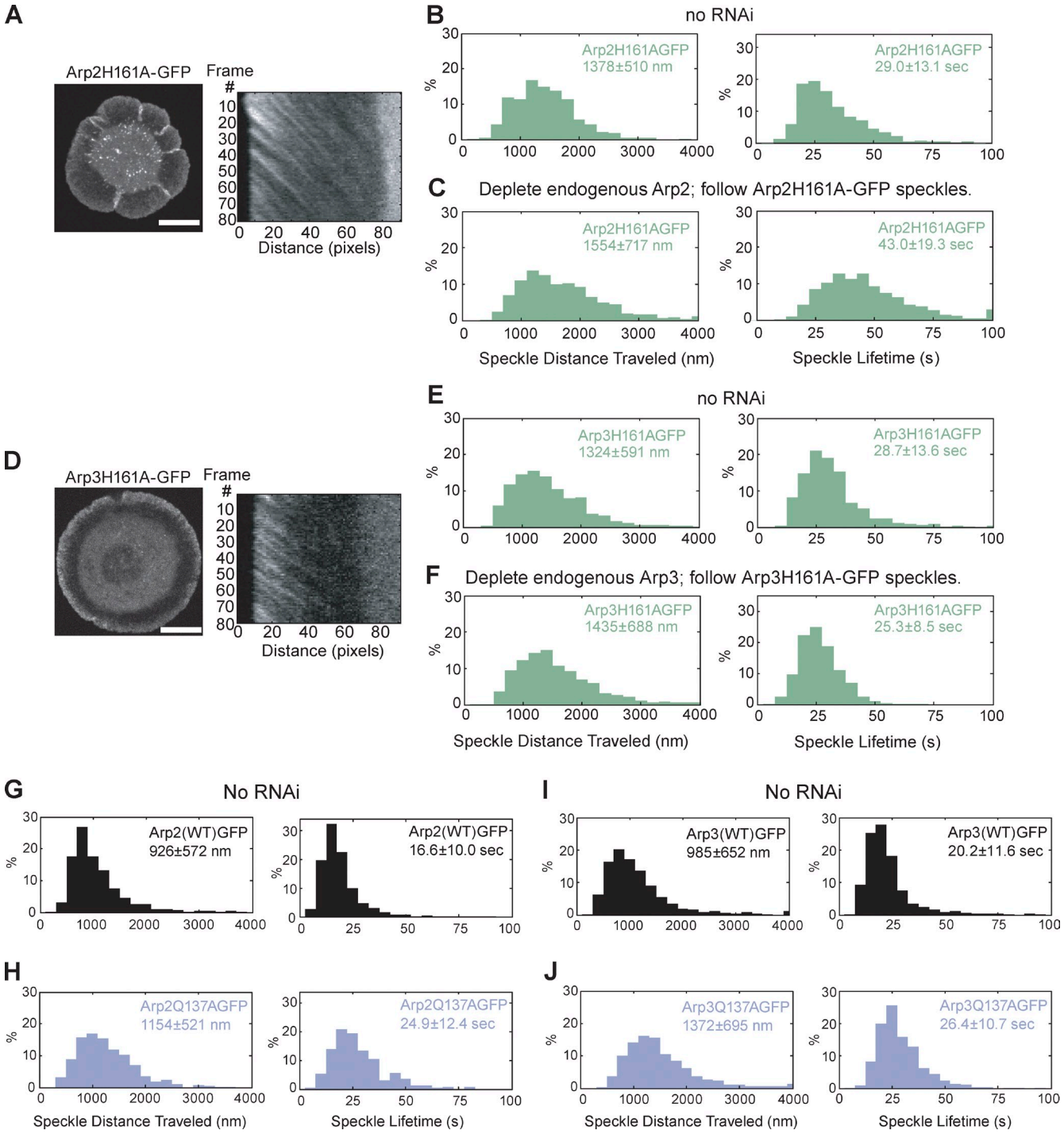


Figure S3. **Another Arp2/3 ATP hydrolysis mutant, H161A, exhibits network disassembly defects.** As in Fig. 3, we created *Drosophila* S2 cell lines stably expressing Arp2H161A-GFP (5) and Arp3H161A-GFP (8) (see Table 1). We imaged stable cell lines in time lapse, created kymographs, and quantified the speckle distances traveled and speckle lifetimes. Endogenous Arp2 (C) and Arp3 (F) were depleted by dsRNA directed against the 5′ and 3′ UTRs, leaving Arp2H161A-GFP (C) and Arp3Arp2H161A-GFP (F) as the sole copy of Arp2 or Arp3, respectively, in the cell. We plotted distributions of the speckle distances traveled and speckle lifetimes. (G–J) These experiments are analogous to those shown in Fig. 3 and in Fig. S2 (A–D), but with no depletion of endogenous Arp2 or Arp3. We created *Drosophila* S2 cell lines stably expressing Arp2(WT)-GFP, Arp2Q137A-GFP, Arp3(WT)-GFP, and Arp3Q137A-GFP (see Table 1.) We imaged these stable cell lines, created kymographs from the movies, and quantified the distances traveled and speckle lifetimes. Median values for speckle distances traveled or speckle lifetimes are noted. Bar, 10 µm. (B) 9 cells, 562 speckles. (C) 32 cells, 1,138 speckles. (E) 26 cells, 723 speckles. (F) 64 cells, 1,470 speckles. (G) 21 cells, 1,329 speckles. (H) 17 cells, 661 speckles. (I) 21 cells, 878 speckles. (J) 29 cells, 1,195 speckles.

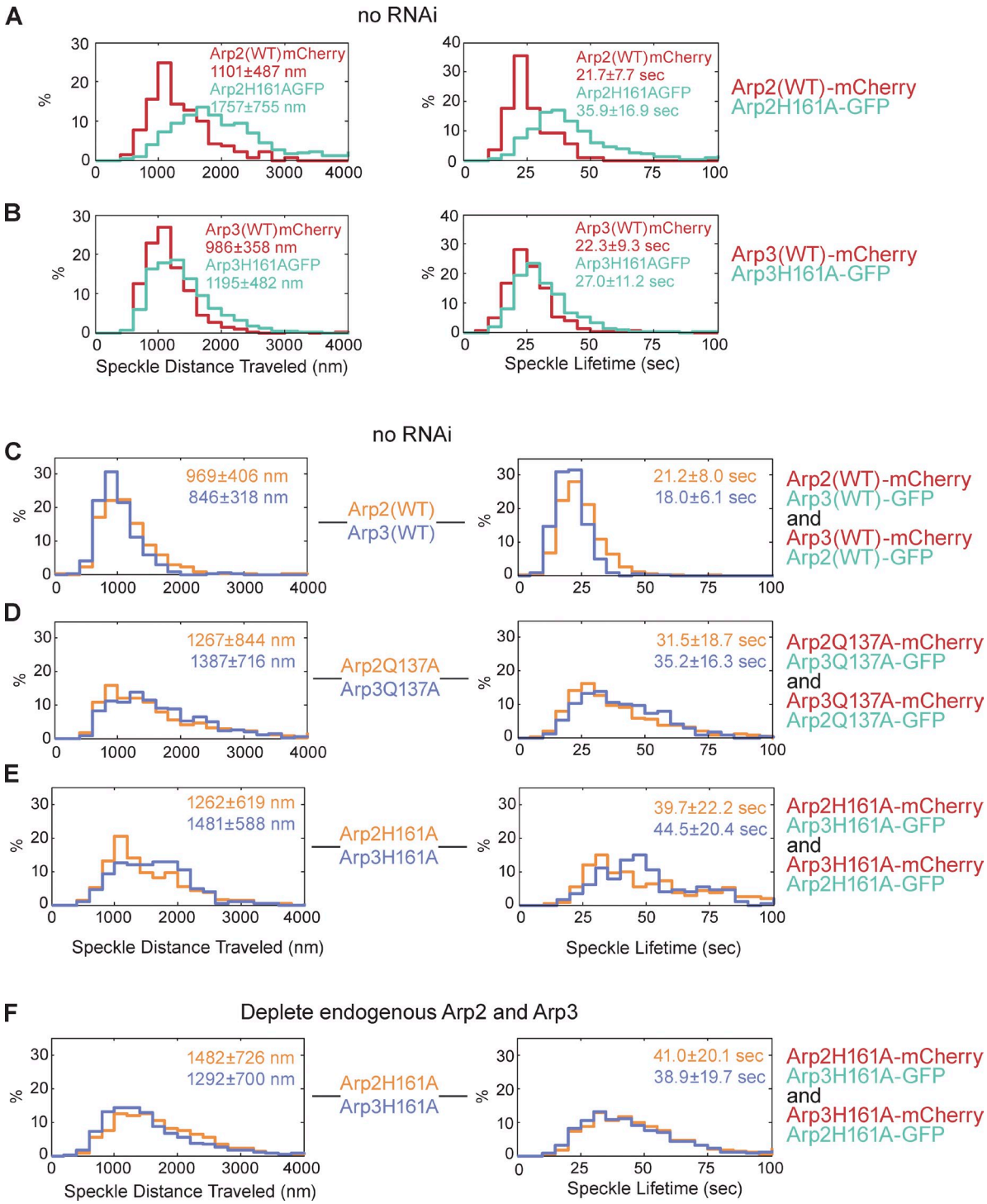


Figure S4. **Histograms of S2 speckle data.** (A and B) Another ATP hydrolysis mutant, H161, produces results similar to those of mutant Q137A. We expressed wild-type mCherry-tagged Arp2 (or Arp3) along with ATP hydrolysis mutant GFP-tagged Arp2 (or Arp3) in the same cells. We imaged stable S2 cell lines (1–5): Arp2H161A-GFP, Arp2(WT)-mCherry; and (2–8): Arp3H161A-GFP, Arp3(WT)-mCherry. As in Fig. 4, we created kymographs showing speckle trajectories, which we used to determine speckle distances traveled and speckle lifetimes. Median values are noted for speckle distance traveled (left column) or speckle lifetime (right column). (C–E) Analogous experiments to those shown in Fig. 5, but with endogenous Arp2 and Arp3 remaining present in the cell. (F) Similar experiment as in Fig. 5, but using ATPase mutant H161A to replace WT Arp2 and Arp3. As seen for Q137A mutants, we observed an increase in speckle lifetimes and distances traveled. (A) 41 cells, 589 GFP speckles, 141 mCherry speckles. (B) 48 cells, 1,723 GFP speckles, 943 mCherry speckles. (C) 18 cells, 1,025 Arp2 speckles, 474 Arp3 speckles. (D) 23 cells, 670 Arp2 speckles, 370 Arp3 speckles. (E) 17 cells, 251 Arp2 speckles, 202 Arp3 speckles. (F) 37 cells, 1,297 Arp2 speckles, 1,867 Arp3 speckles.

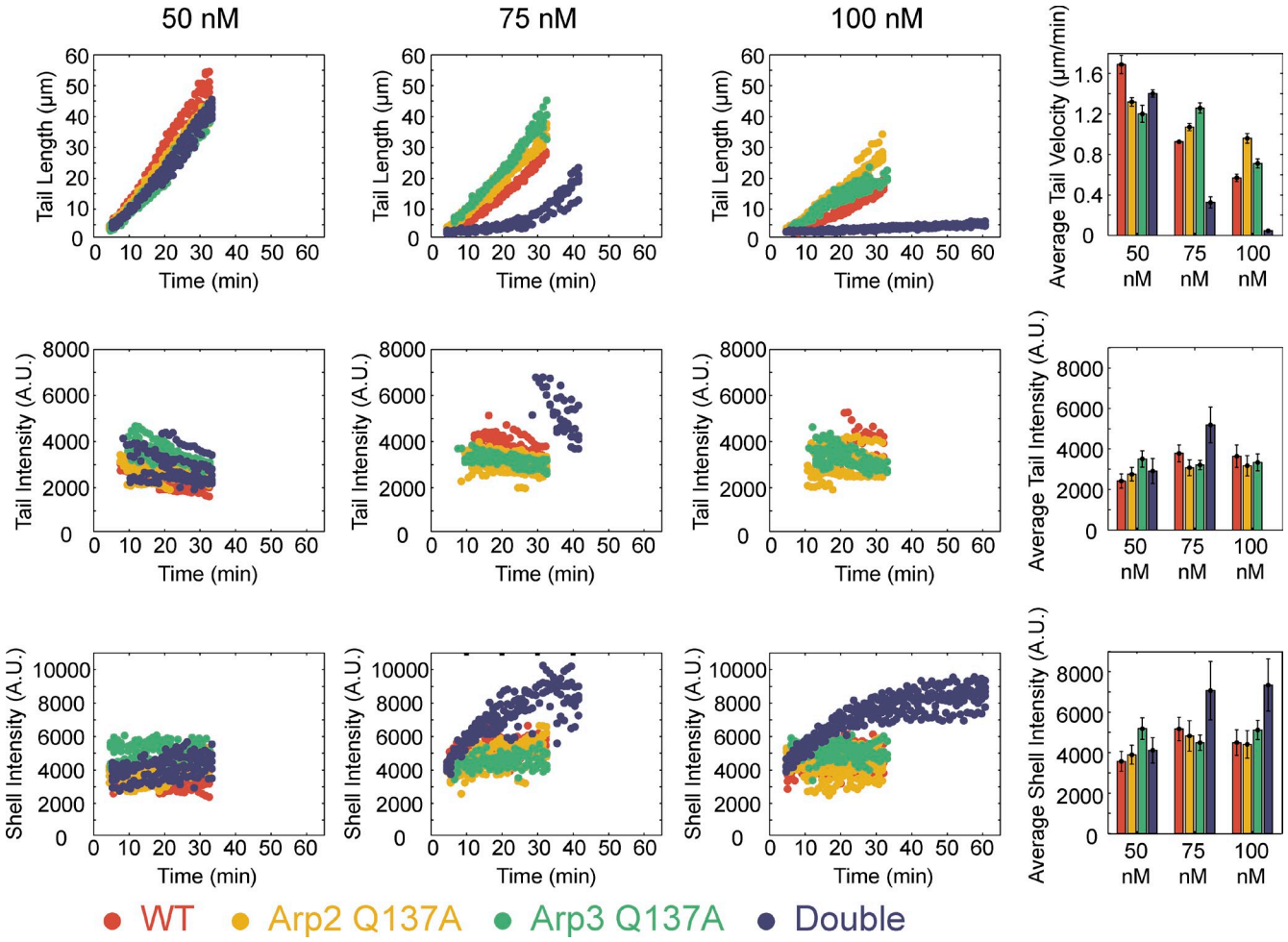
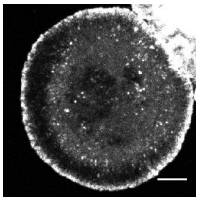
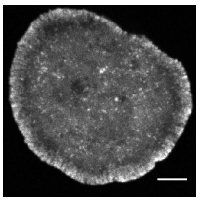


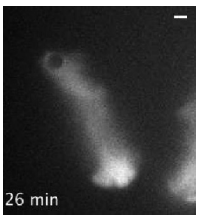
Figure S5. **In vitro actin-based bead motility reactions, under nonrecycling conditions.** As in Fig. 6, we also performed the experiments at 75-nM and 100-nM Arp2/3 concentrations. At 75 nM, actin tails formed more slowly. At 100 nM, shells failed to break symmetry, producing actin tails too short to be measured accurately. Data from a single experiment are presented for each Arp2/3 variant and concentration. Wild-type Arp2/3 (6–9 tails per concentration); Double ATP hydrolysis mutant Arp2/3 (7–8 tails per concentration); Arp2Q137A Arp2/3 (9–13 tails per concentration); Arp3Q137A Arp2/3 (9–10 tails per concentration).



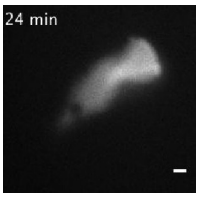
Video 1. **Movement of WT Arp2-GFP speckles in a *Drosophila* S2 cell.** S2 cells were transfected with vector pMT Arp2 GFP. Time-lapse images were taken with a motorized inverted microscope (Eclipse Ti-E; Nikon) equipped with a spinning disk (Yokogawa CSU22; Solamere Technology Group). Frames were taken every 20 s for 3 min. Bar, 5 µm.



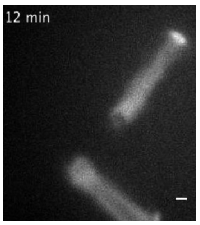
Video 2. **Movement of Arp2Q137A-GFP speckles in a *Drosophila* S2 cell.** S2 cells were transfected with vector pMT Arp2Q137A GFP and imaged as in Video 1.



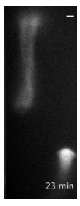
Video 3. **Bead motility with WT Arp2/3 complex, no recycling, Alexa Fluor 488 actin.** Images were taken on an epifluorescence microscope (Eclipse TE2000-E; Nikon). Frames were taken every minute for 40 min. Time is defined as after the reaction was initiated by the addition of 3% Alexa Fluor 488 actin to the protein mixture. Bar, 5 µm.



Video 4. **Bead motility with double ATP hydrolysis mutant Arp2/3 complex, no recycling.** Imaged as in Video 3.



Video 5. **Bead motility and severing of shell built with WT Arp2/3 complex, with recycling.** White arrowhead indicates occurrence of severing event. Imaged as in Video 3.



Video 6. **Bead motility and severing of shell built with double ATP hydrolysis mutant Arp2/3 complex, with recycling.** White arrowhead indicates occurrence of severing event. Imaged as in Video 3.