Lee et al., http://www.jcb.org/cgi/content/full/jcb.201407105/DC1

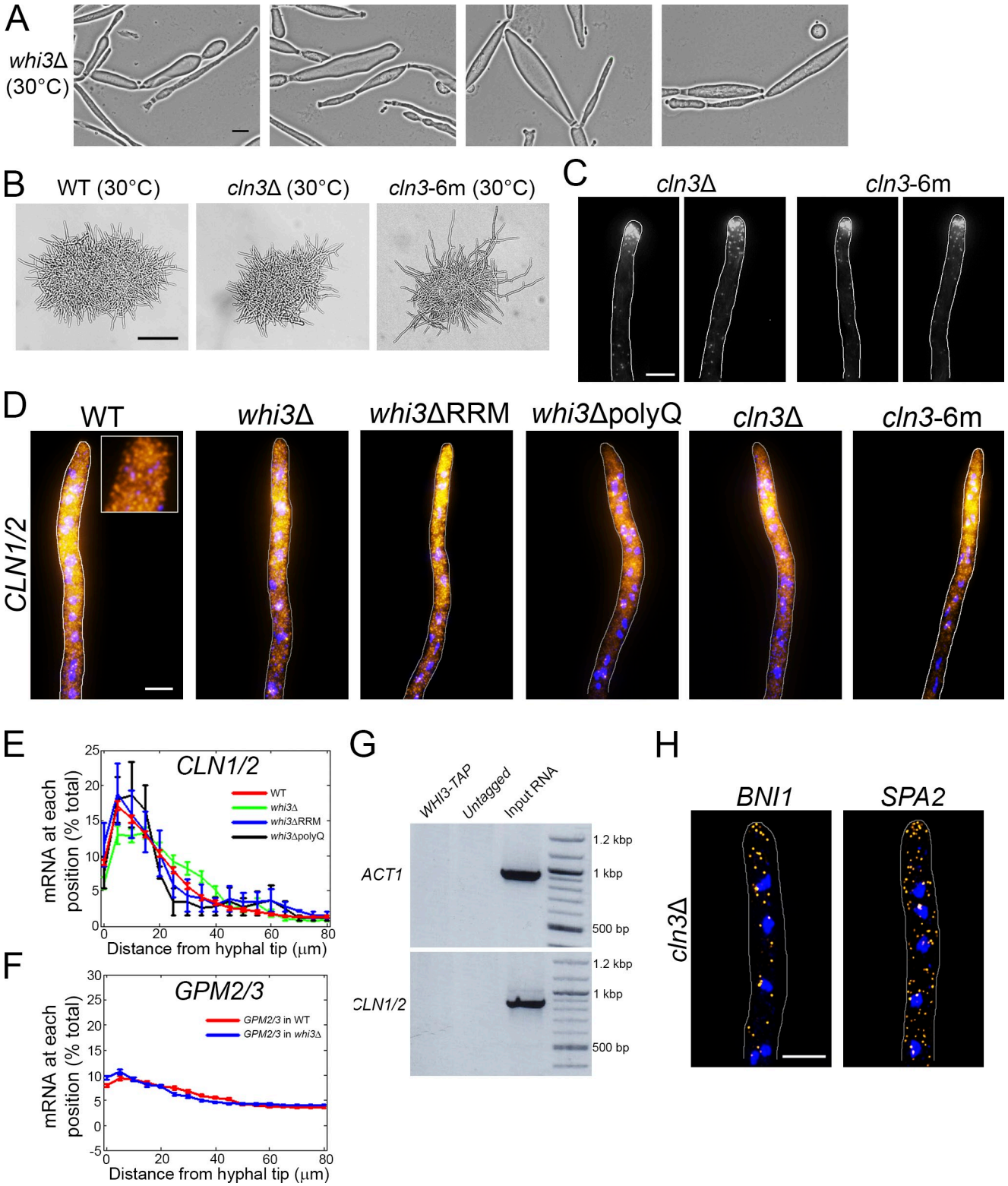


Figure S1. **Whi3-regulated cell polarity does not involve *CLN1/2*, *CLN3*, or *ACT1*.** (A) *whi3*Δ (phase) grown for 30 h at 30°C. (B) Cells grown for 20 h. (C) F-actin is visualized in cells grown for 16 h (30°C). (B, C, and H) Cells are outlined in gray. (D) *CLN1/2* mRNAs are localized. The inset shows a 5× magnification. (E and F) *CLN1/2* or *GPM2/3* mRNAs are counted from each growing tip ($n > 35$). Error bars indicate SEM. (G) mRNAs RIPed with Whi3-TAP using α-TAP antibody are identified after RT-PCR. Untagged Whi3 is used as a control. cDNA was generated from whole RNAs in WCE to test the presence of *ACT1* and *CLN1/2*. (H) *BNI1* or *SPA2* transcripts (yellow) are localized in *cln3*Δ. Blue: DNA. Bars: (A) 5 µm; (B) 200 µm; (C, D, and H) 5 µm.

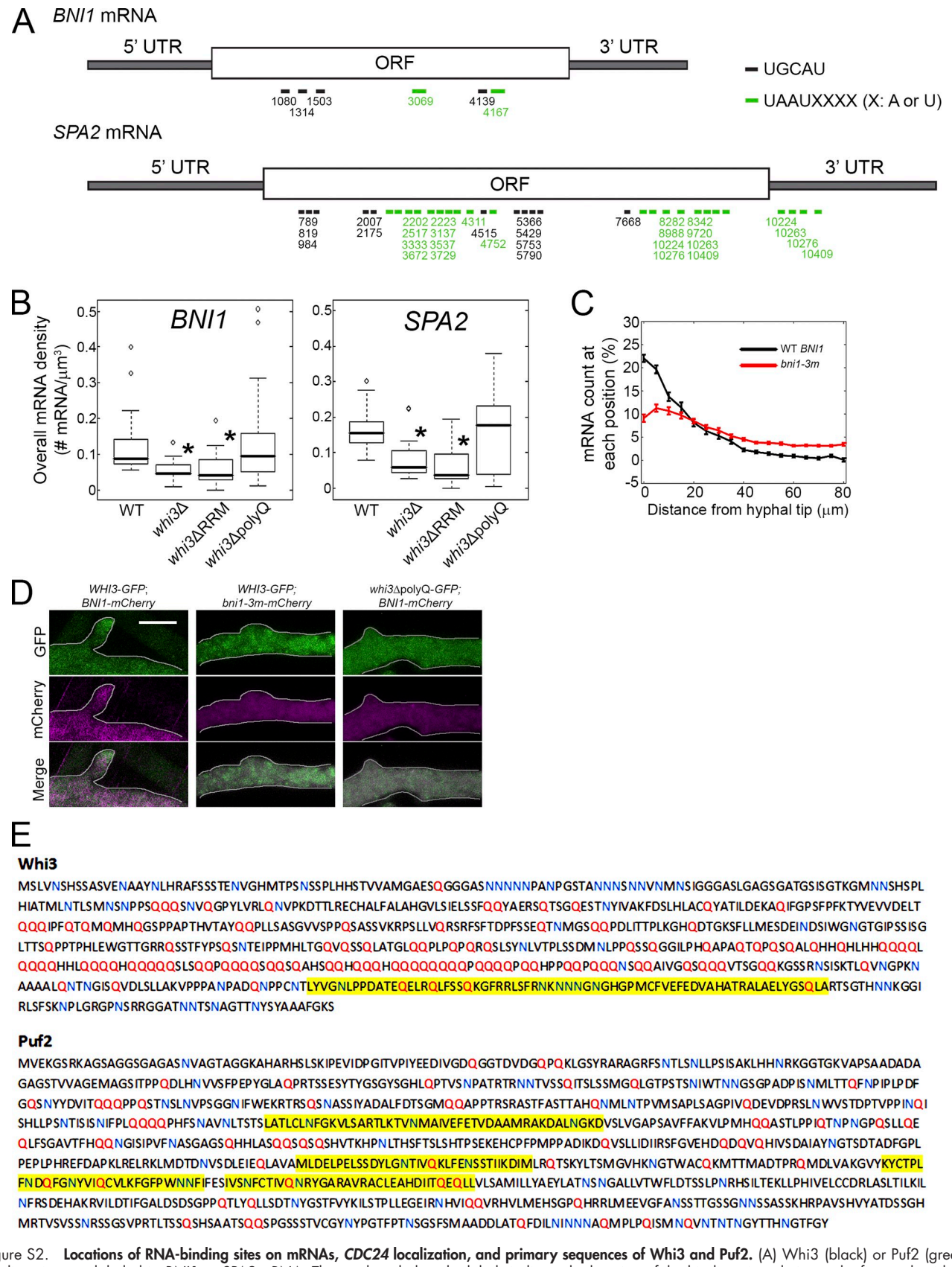


Figure S2. **Locations of RNA-binding sites on mRNAs, *CDC24* localization, and primary sequences of Whi3 and Puf2.** (A) Whi3 (black) or Puf2 (green) binding sites are labeled in *BNI1* or *SPA2* mRNA. The numbers below the labels indicate the location of the binding site relative to the first nucleotide in ORF. (B) Overall *BNI1* or *SPA2* mRNA density in each strain. *, $P < 0.01$ compared to wild type using a KS test. $n > 33$. (C) *BNI1* (or -3m) mRNAs were counted and converted to percentages. Error bars indicate SEM. (D) Whi3 (green) and Bni1 (or -3m, magenta) localization at the nascent branches. Images are z-projected. Cells are outlined in gray. Bar, 5 μm. (E) *A. gossypii* Whi3 and Puf2 sequence. Glutamines (Q) and Asparagine (N) are color-coded as red and blue, respectively. RNA-binding domains are highlighted in yellow. Whi3 contains one RRM 5. Puf2 contains three Pumilios and one RRM 1 (in the order from the N terminus).

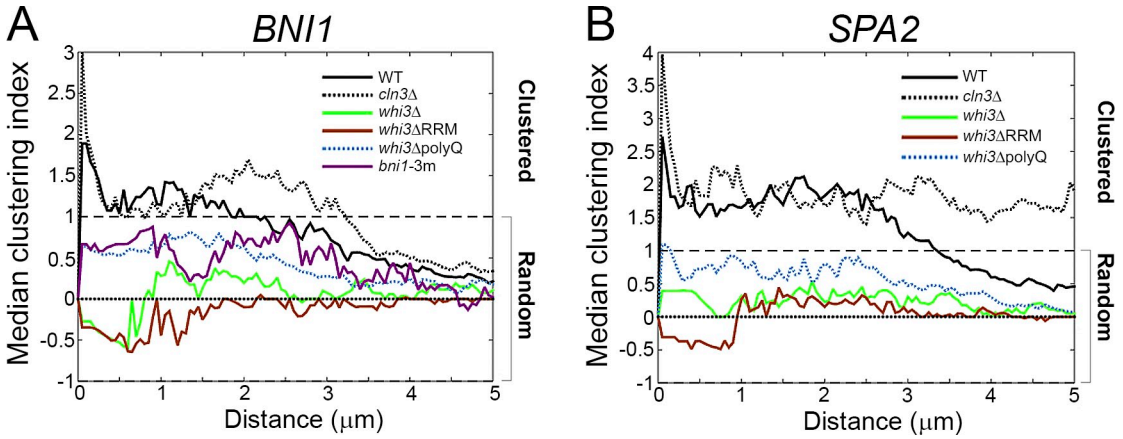
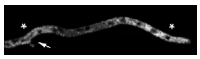
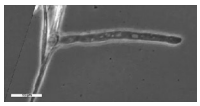


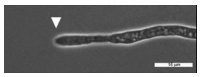
Figure S3. **Spatial pattern analysis of *BNI1* or *SPA2* mRNAs.** (A and B) The median clustering index indicates the median value of Ripley's $H(d)^*$ at distance d across all mRNAs for *BNI1* or *SPA2* ($n > 39$). See Materials and methods.



Video 1. **Concentrated Whi3 on polarization sites.** Whi3-GFP (gray) was imaged in *A. gossypii* using a time-lapse wide-field microscope (AxioImager-M1; Carl Zeiss). Frames were taken every 3 min for 30 min. Asterisks indicate concentrated Whi3. Arrowheads indicate emergence of a new branch.



Video 2. **Isotropic growth of *whi3Δ* at 30°C.** A *whi3Δ* hypha (phase image by AxioImager-M1) grows out from a spore on a microscopic slide with an agarose gel pad. Frames were taken every 10 min for 24 h.



Video 3. **Hyphal growth pauses and restarts in *whi3Δ*.** Phase images were taken every 10 min for 10 h using a time-lapse wide-field microscope (AxioImager-M1; Carl Zeiss). An arrowhead appears when growth pauses.

All MATLAB codes and ImageJ macros are available in a single zip file.