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

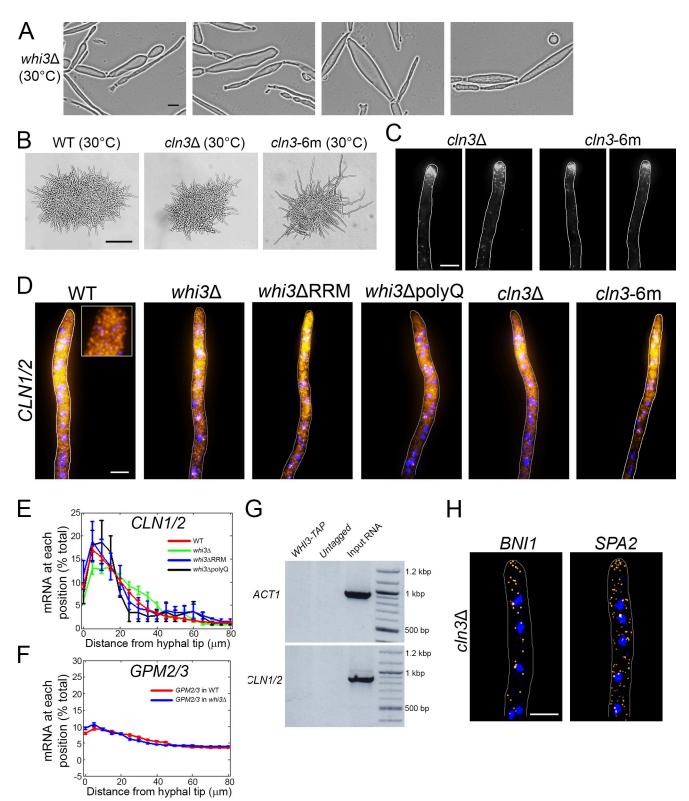
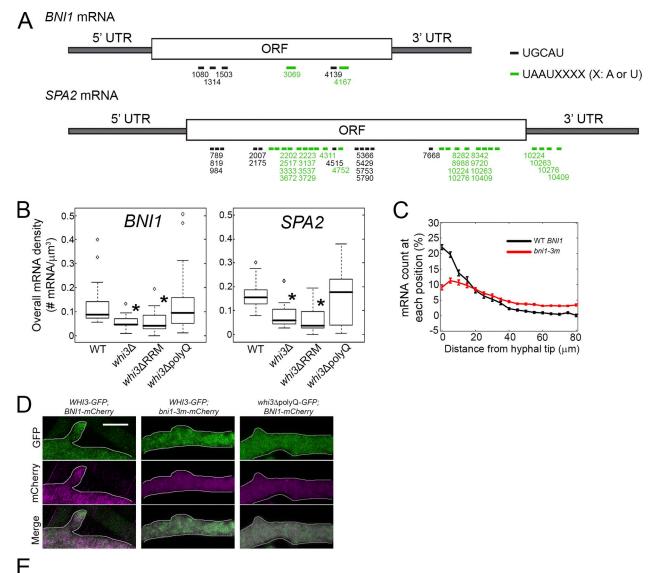


Figure S1. Whi3-regulated cell polarity does not involve CLN1/2, CLN3, or ACT1. (A) whi3 $\Delta$  (phase) grown for 30 h at 30°C. (B) Cells grown for 20 h. (C) F-actin is visualized in cells grown for 16 h (30°C). (B, C, and H) Cells are outlined in gray. (D) CLN1/2 mRNAs are localized. The inset shows 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  $\alpha$ -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) BN11 or SPA2 transcripts (yellow) are localized in cln3 $\Delta$ . Blue: DNA. Bars: (A) 5 µm; (B) 200 µm; (C, D, and H) 5 µm.



## — Whi3

MSLVNSHSSASVENAAYNLHRAFSSSTENVGHMTPSNSSPLHHSTVVAMGAESQGGGASNNNNNPANPGSTANNNSNNVNMNSIGGGASLGAGSGATGSISGTKGMNNSHSPL
HIATMLNTLSMNSNPPSQQQSNVQGPYLVRLQNVPKDTTLRECHALFALAHGVLSIELSSFQQYAERSQTSGQESTNYVAKFDSLHLACQYATILDEKAQIFGPSFPFKTYVEVVDELT
QQQIPFQTQMQMHQGSPPAPTHVTAYQQPLLSASGVVSPPQSASSVKRPSLLVQRSRFSFTDPFSSEQTNMGSQQPDLITTPLKGHQDTGKSFLLMESDEINDSIWGNGTGIPSSISG
LTTSQPPTPHLEWGTTGRRQSSTFYPSQSNTEIPPMHLTGQVQSSQLATGLQQPLPQPQRQSLSYNLVTPLSSDMNLPPQSSQGGILPHQAPAQTQPQSQALQHHQHLHHQQQQL
QQQHHLQQQQHQQQQSLSQQPQQQQSQAHSQQHQQQQQQQQQQQQQQPQQQPQQQPQQQNSQQAIVGQSQQQVTSGQQKGSSRNSISKTLQVNGPKN
AAAALQNTNGISQVDLSLLAKVPPPANPADQNPPCNTLVYGNLPPDATEQELRQLFSSQKGFRRLSFRNKNNNGNGHGPMCFVEFEDVAHATRALAELYGSQLARTSGTHNNKGGI
RLSFSKNPLGRGPNSRRGGATNNTSNAGTTNYSYAAAFGKS

## Puf2

MVEKGSRKAGSAGGSGAGASNVAGTAGGKAHARHSLSKIPEVIDPGITVPIYEEDIVGDQGGTDVDGQPQKLGSYRARAGRFSNTLSNLLPSISAKLHHNRKGGTGKVAPSAADADA
GAGSTVVAGEMAGSITPPQDLHNVVSFPEPYGLAQPRTSSESYTYGSGYSGHLQPTVSNPATRTRNNTVSSQITSLSSMGQLGTPSTSNIWTNNGSGPADPISNMLTTQFNPIPLPDF
GQSNYYDVITQQQPPQSTNSLNVPSGGNIFWEKRTRSQSNASSIYADALFDTSGMQQAPPTRSRASTFASTTAHQNMLNTPVMSAPLSAGPIVQDEVDPRSLNWVSTDPTVPPINQI
SHLLPSNTISISNIFPLQQQQPHFSNAVNLTSTSLATLCLNFGKVLSARTLKTVNMAIVEFETVDAAMRAKDALNGKDVSLVGAPSAVFFAKVLPMHQQASTLPPIQTNPNGPQSLLQE
QLFSGAVTFHQQNGISIPVFNASGAGSQHHLASQQSQSQSHVTKHPNLTHSFTSLSHTPSEKEHCPFPMPPADIKDQVSLLIDIIRSFGVEHDQDQVQHIVSDAIAYNGTSDTADFGPL
PEPLPHREFDAPKLRELRKLMDTDNVSDLEIEQLAVAMLDELPELSSDYLGNTIVQKLFENSSTIIKDIMLRQTSKYLTSMGVHKNGTWACQKMTTMADTPRQMDLVAKGVY
KYCTPL
FNDQFGNYVIQCVLKFGFPWNNFIFESIVSNFCTIVQNRYGARAVRACLEAHDIITQEQLL
VLSAMILLYAEYLATNSNGALLVTWFLDTSSLPNRHSILTEKLLPHIVELCCDRLASLTILKIL
NFRSDEHAKRVILDTIFGALDSDSGPPQTLYQLLSDTNYGSTFVYKILSTPLLEGEIRNHVIQQVRHVLMEHSGPQHRRIMEEVGFANSSTTGSSGNNSSASSKHRPAVSHVYATDSSGH
MRTVSVSSNRSSGSVPRTLTSSQSHSAATSQQSPGSSSTVCGYNYPGTFPTNSGSFSMAADDLATQFDILNINNNAQMPLPQISMNQVNTNTNGYTTHNGTFGY

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).

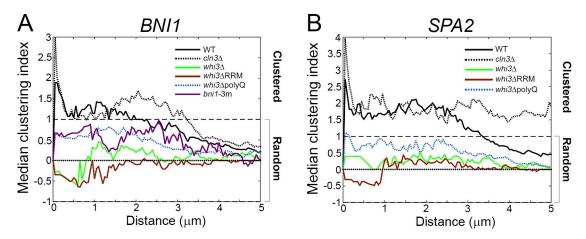


Figure S3. **Spatial pattern analysis of BNI1 or SPA2 mRNAs.** (A and B) The median clustering index indicates the median value of Ripley's  $H(d)^*$  at distance d across all mRNAs for BNI1 or SPA2 (n > 39). See Materials and methods.



Video 1. Concentrated Whi3 on polarization sites. Whi3-GFP (gray) was imaged in A. gossypii using a time-lapse wide-field microscope (Axiolmager-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 whi3** $\Delta$  at 30°C. A whi3 $\Delta$  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 whi3**. Phase images were taken every 10 min for 10 h using a time-lapse wide-field microscope (Axiolmager-M1; Carl Zeiss). An arrowhead appears when growth pauses.

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