

Supplemental Materials

Molecular Biology of the Cell

Clark-Cotton et al.

Figure S1

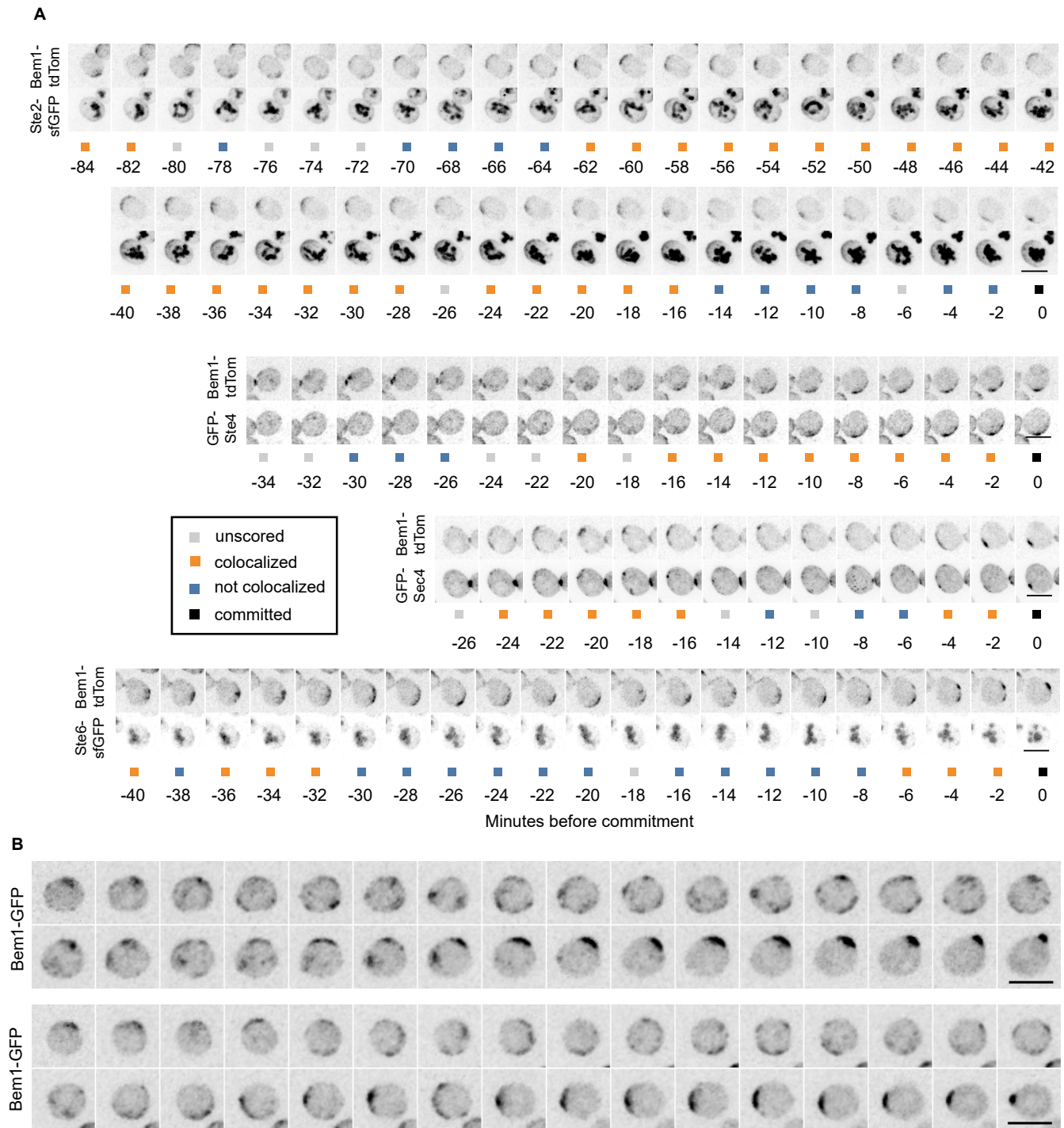


Figure S1. Cell behavior during the indecisive period. (A) Example montages showing time courses during the indecisive period, along with assigned colocalization score. Strains harboring Bem1-tdTomato and either the α -factor receptor Ste2-sfGFP (DLY22243), G β subunit GFP-Ste4 (DLY23354), secretory vesicle marker GFP-Sec4 (DLY13771), or α -factor transporter Ste6-sfGFP (DLY22355) were mixed with wildtype (DLY8156) and imaged. Internal signal in Ste2-sfGFP and Ste6-sfGFP strains is due to sfGFP accumulation in the vacuole following Ste2/Ste6 degradation. Colocalization scores for individual time points are indicated by colored squares. There is an unavoidable level of subjectivity in applying this scoring procedure. (B) Two α -factor treated cells (DLY23016) show indecisive behavior during G1, then polarize and bud. Cells lack the α -factor-degrading Bar1 protease to ensure a stable pheromone concentration throughout the imaging period. Interval between frames: 2 min. Scale bars: 5 μ m.

Figure S2

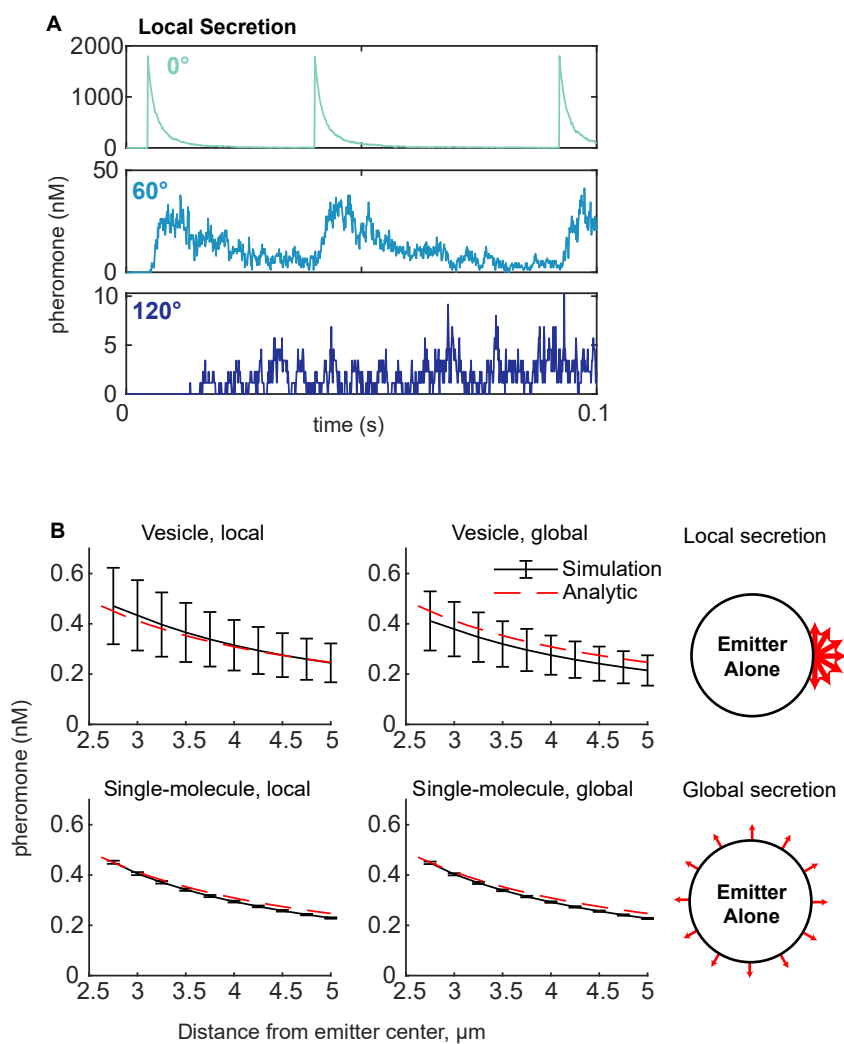


Figure S2. Validation of the pheromone simulations and additional detail. (A) Pheromone concentrations perceived at three different patches in a single simulation as in Figure 3C, but zoomed in to show 0.1 second along the x-axis. (B) Simulations of the emitter alone, comparing concentrations in a spherical 250 nm shell (not a patch) at the indicated distance from the center of the emitter versus the steady-state analytic solution of the diffusion equation under equivalent conditions. Bars show mean \pm s.d., $n = 30$.

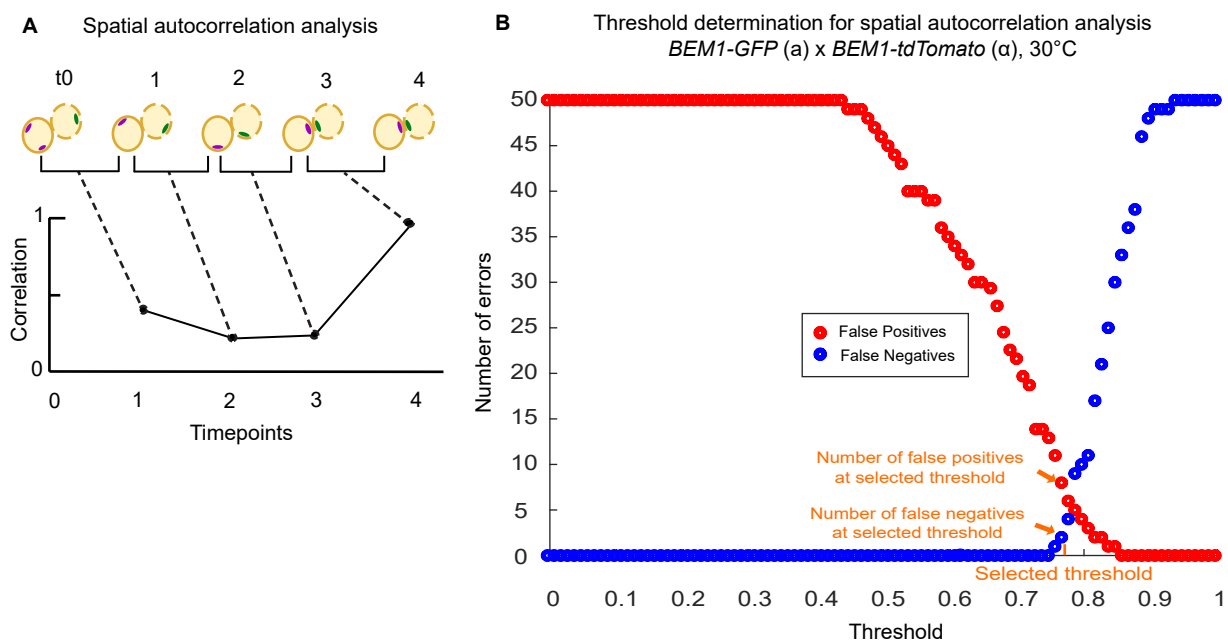
Figure S3

Figure S3. Spatial autocorrelation metric. (A) Cartoon illustrating spatial autocorrelation algorithm to score commitment. The spatial distribution of Bem1 pixel intensities in a cell of interest (magenta clusters) are compared at consecutive time points to yield a normalized correlation measure between 0 (no correlation) and 1 (perfect correlation). Strong and stably oriented polarity sites characteristic of committed cells (3,4) yield a high correlation while weaker, mobile polarity sites characteristic of indecisive cells (0,1,2) yield a low correlation. (B) Threshold determination for scoring commitment. The number of false negatives (in which the spatial autocorrelation trace did not cross the threshold but did commit as scored visually) and false positives (in which the spatial autocorrelation trace crossed the threshold > 4 min before commitment as scored visually) as a function of commitment threshold. A threshold of 0.77 was selected (orange tick). (C,D) Spatial autocorrelation traces for 40 additional wildtype α cells (C) and 10 additional α cells (D) from wildtype by wildtype mixes at 30°C, from the time of the cell's entry into G1 to the time point preceding fusion. (E,F) Spatial autocorrelation traces for 10 wildtype cells mixed with *cdc24-m1 rsr1Δ* (D) or *CDC24^{38A}* (E) cells, from the time of the cell's entry into G1 to the end of the movie. X-axis: Time (min). Y-axis: spatial autocorrelation (yellow line: commitment threshold). Commitment to a partner as determined visually (vertical purple line) or by crossing the threshold (dashed green line).

Figure S3 cont.

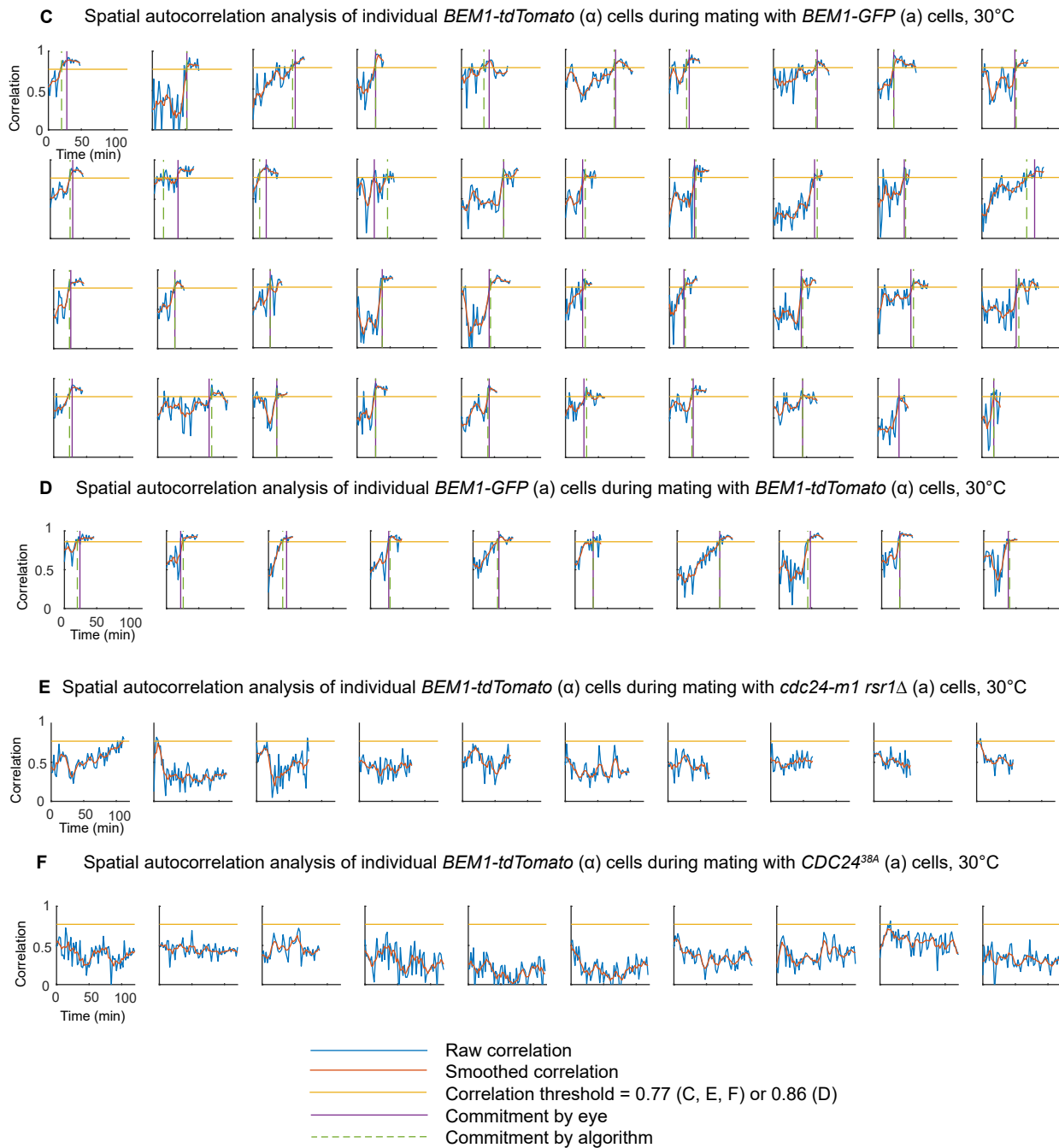


Figure S4

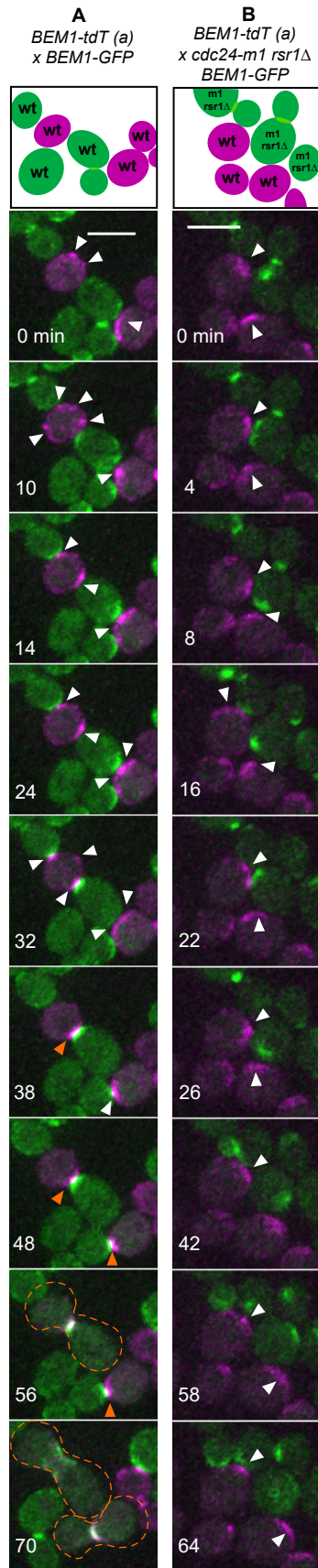


Figure S4. Wildtype cells do not commit to partners with constitutively mobile polarity sites, regardless of mating type. Selected time points from movies of mating mixes. White arrowhead: weak mobile Bem1 cluster characteristic of indecisive cells, focusing on the magenta wildtype cells. Orange arrowheads: stably oriented Bem1 clusters characteristic of committed cells. Dashed outline: fused zygote. (A) MAT α wildtype cells (DLY12943, magenta) were mixed with MAT α wildtype cells (DLY9070, green). Mating type and fluorophore are switched relative to Figure 4A. (B) MAT α wildtype cells (DLY12943, magenta) were mixed with MAT α mutants that form constitutively mobile polarity clusters (*cdc24-m1 rsr1Δ*, DLY23612, green). Mating type switched relative to Figure 4B. Scale bars: 5 μ m.

Figure S5

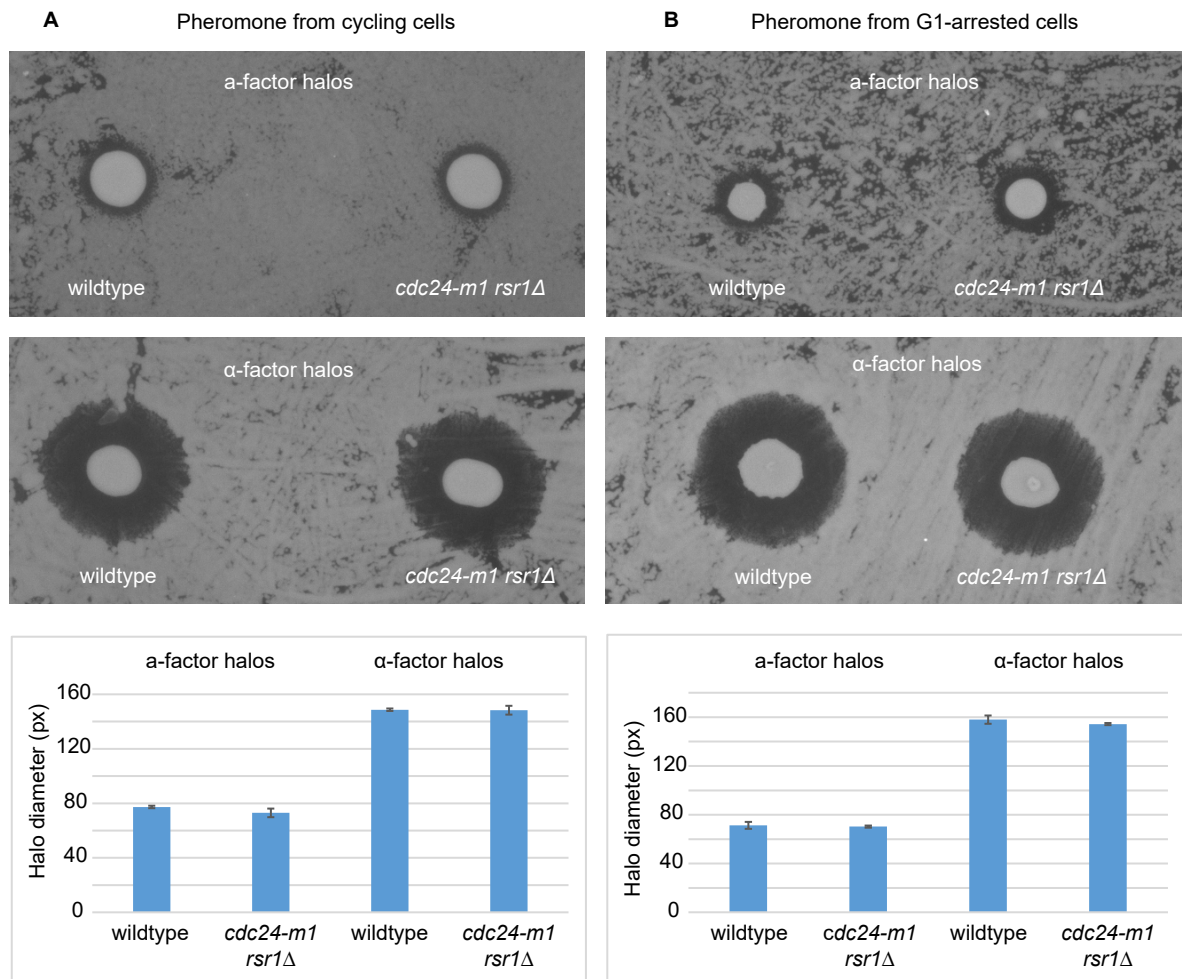


Figure S5. Wildtype and *cdc24-m1 rsr1Δ* mutants secrete similar amounts of pheromones. Halo assays were conducted as described in Methods. (A) Spots of MAT α wildtype (DLY9069) and *cdc24-m1 rsr1Δ* (DLY22797) generate similar a-factor halos, and spots of MAT α wildtype (DLY9070) and *cdc24-m1 rsr1Δ* (DLY23612) generate similar α -factor halos. (B) Spots of MAT α wildtype (DLY20628) and *cdc24-m1 rsr1Δ* (DLY22532) cells arrested in G1 by expression of Ste5-CTM generate similar a-factor halos, and spots of MAT α wildtype (DLY20625) and *cdc24-m1 rsr1Δ* (DLY22533) arrested in G1 by expression of Ste5-CTM generate similar α -factor halos. Quantification: mean \pm SEM halo diameter for n = 3 plates.

Figure S6

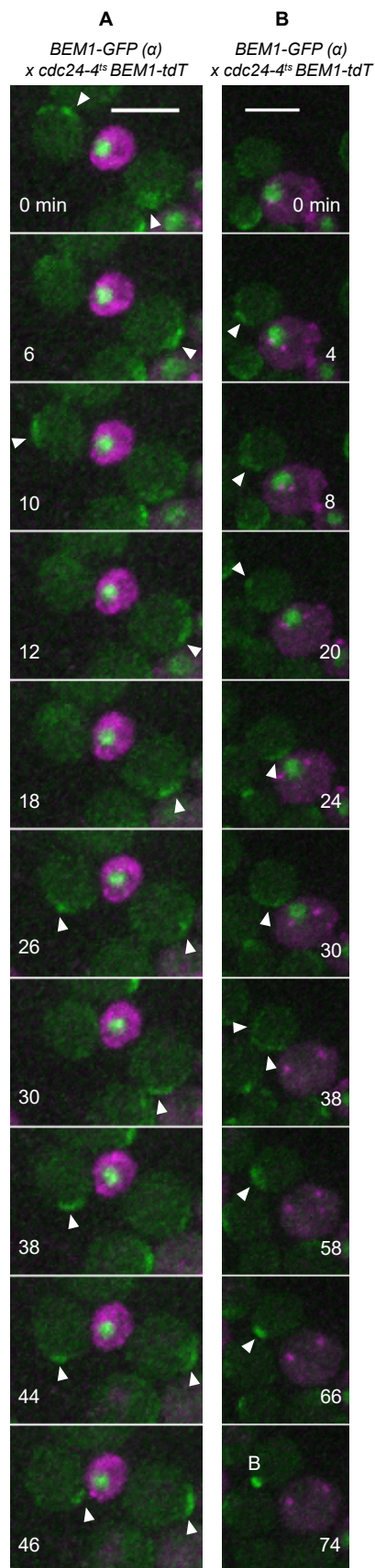


Figure S6. Behavior of *cdc24-4^{ts} ste20ΔCRIB* mutants in mating mixes. Time-lapse movies of mating mixes with wildtype (DLY9070, green) and mutant (DLY23256, magenta with green nuclei) partners grown at 30°C or 24°C, respectively, and shifted to a pre-warmed agarose slab for imaging at 37°C. (A) Montage showing mutant daughter cell that remains arrested in G1 and wildtype partner that remains indecisive. (B) Montage showing mutant mother that exits G1 (Whi5 leaves nucleus at 38 min). While many small mutant daughter cells remained arrested, the larger mothers did not. In all cases, the wildtype partners failed to commit. B: bud. Scale bars: 5 μm.

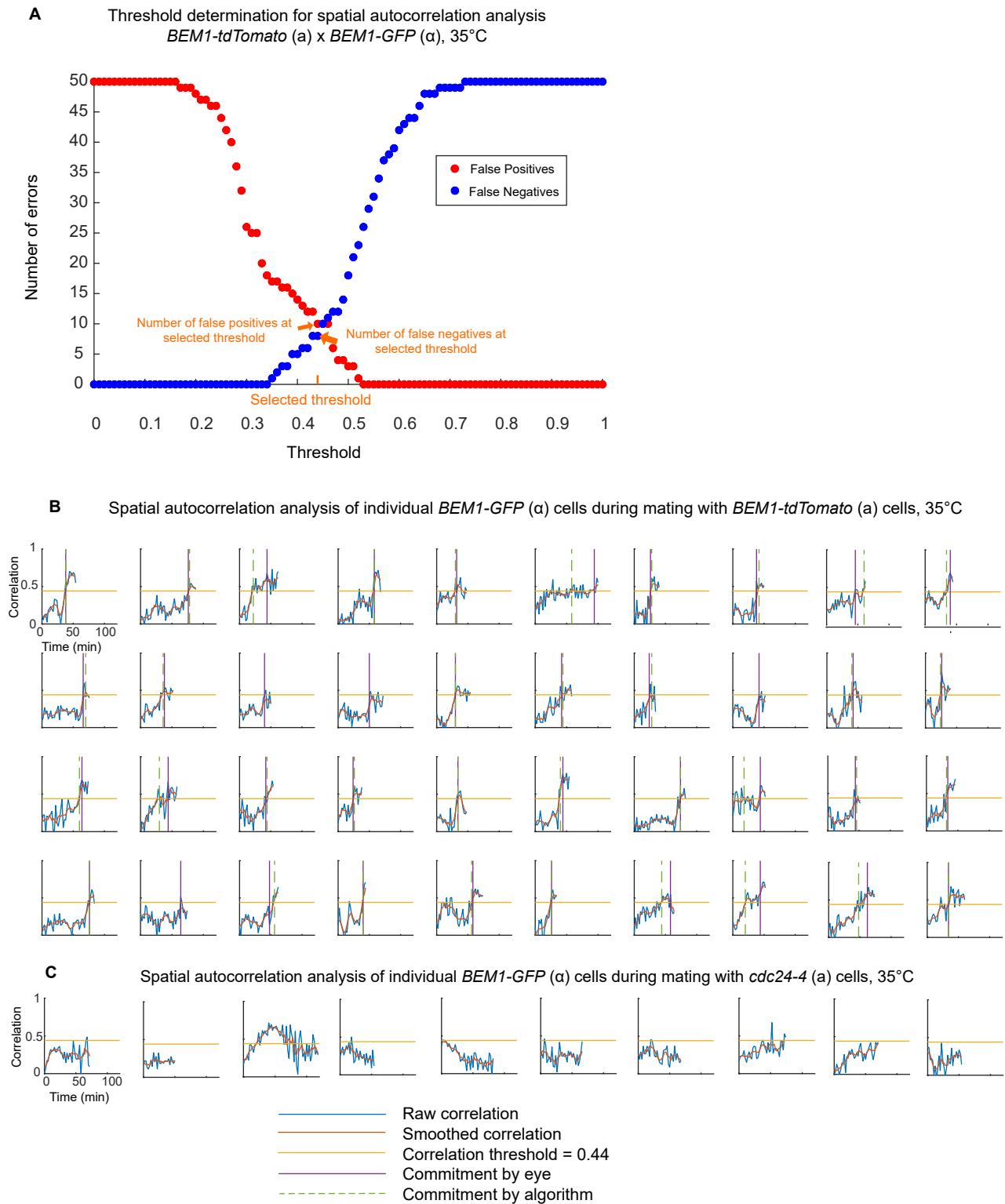
Figure S7

Figure S7. Threshold determination for spatial autocorrelation at 35°C. (A) Threshold determination for scoring commitment. The number of false negatives (in which the spatial autocorrelation trace did not cross the threshold but did commit as scored visually) and false positives (in which the spatial autocorrelation trace crossed the threshold > 4 min before commitment as scored visually) as a function of commitment threshold. A threshold of 0.44 was selected (orange tick). (B) Spatial autocorrelation traces for 40 additional wildtype by wildtype pairs at 35°C, from the time of the cell's entry into G1 to the time point preceding fusion. (C) Spatial autocorrelation traces for 10 wildtype by *cdc24-4* pairs at 35°C, from the time of the cells' entry into G1 until the end of the movie. X-axis: Time (min). Y-axis: spatial autocorrelation (yellow line: commitment threshold). Commitment to a partner as determined visually (vertical purple line) or by crossing the threshold (dashed green line).

Figure S8

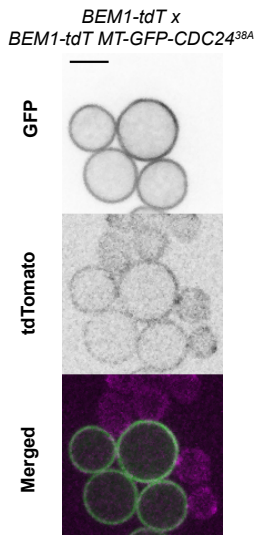


Figure S8. Overexpression of membrane-targeted Cdc24 blocks polarization. Medial plane confocal images of cells induced to express membrane-targeted, phospho-site mutant *GFP-CDC24^{38A}* (*MT-GFP-CDC24^{38A} BEM1-tdTomato*, DLY23351) and mixed with wildtype cells (*BEM1-tdTomato*, DLY12944).