Supplemental material

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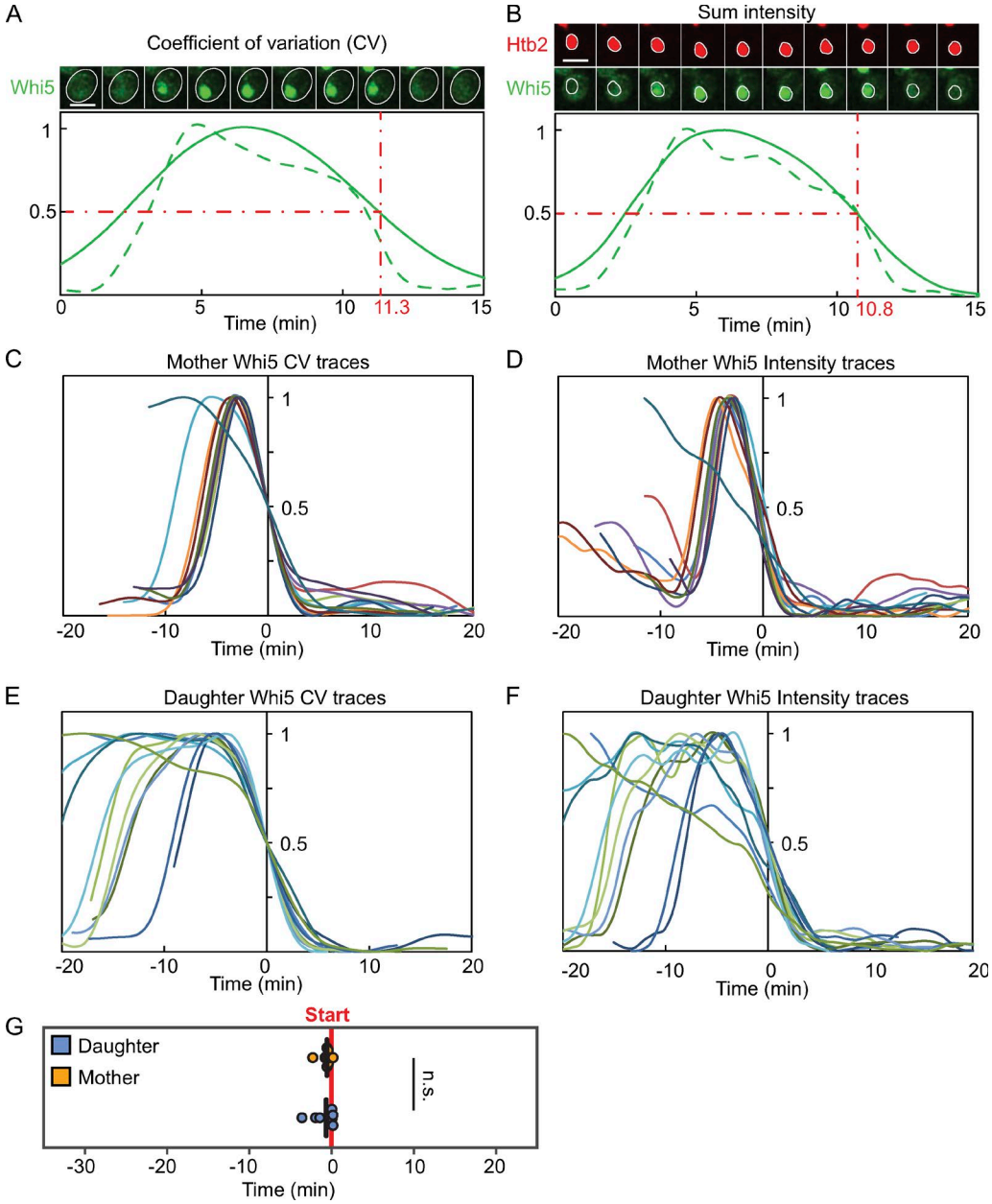

Figure S1. **Determining the time of start. (A)** Tracking Whi5 nuclear localization in a representative cell (DLY15724) using CV of pixel intensity values calculated within a user-defined ellipse. A smoothed fit to the data is used to call the time of 50% CV decrease. **(B)** Tracking Whi5 nuclear localization in the same cell by calculating the sum intensity of Whi5 signal within the nucleus, which was defined by thresholding based on a fluorescent histone protein (Htb2). In A and B, the green dashed lines represent the raw data (CV or sum intensity); the green solid lines represent the spline fits (CV or sum intensity); and the red dashed lines indicate the point at which the signal (CV or sum intensity) has declined to 50% of its maximum. **(C–F)** Comparison of multiple traces by CV and sum intensity in mother cells (C and D) or daughter cells (E and F). Traces of the same color represent the same cell. **(G)** Comparing start times determined by using the sum intensity method relative to the CV method. Negative values indicate the sum intensity method–called start earlier than the that of the CV method. Y-axis represents CV (A, C, and E) or summed intensity (B, D, and F) normalized to the maximum value for each cell. Scale bars, 5 µm.

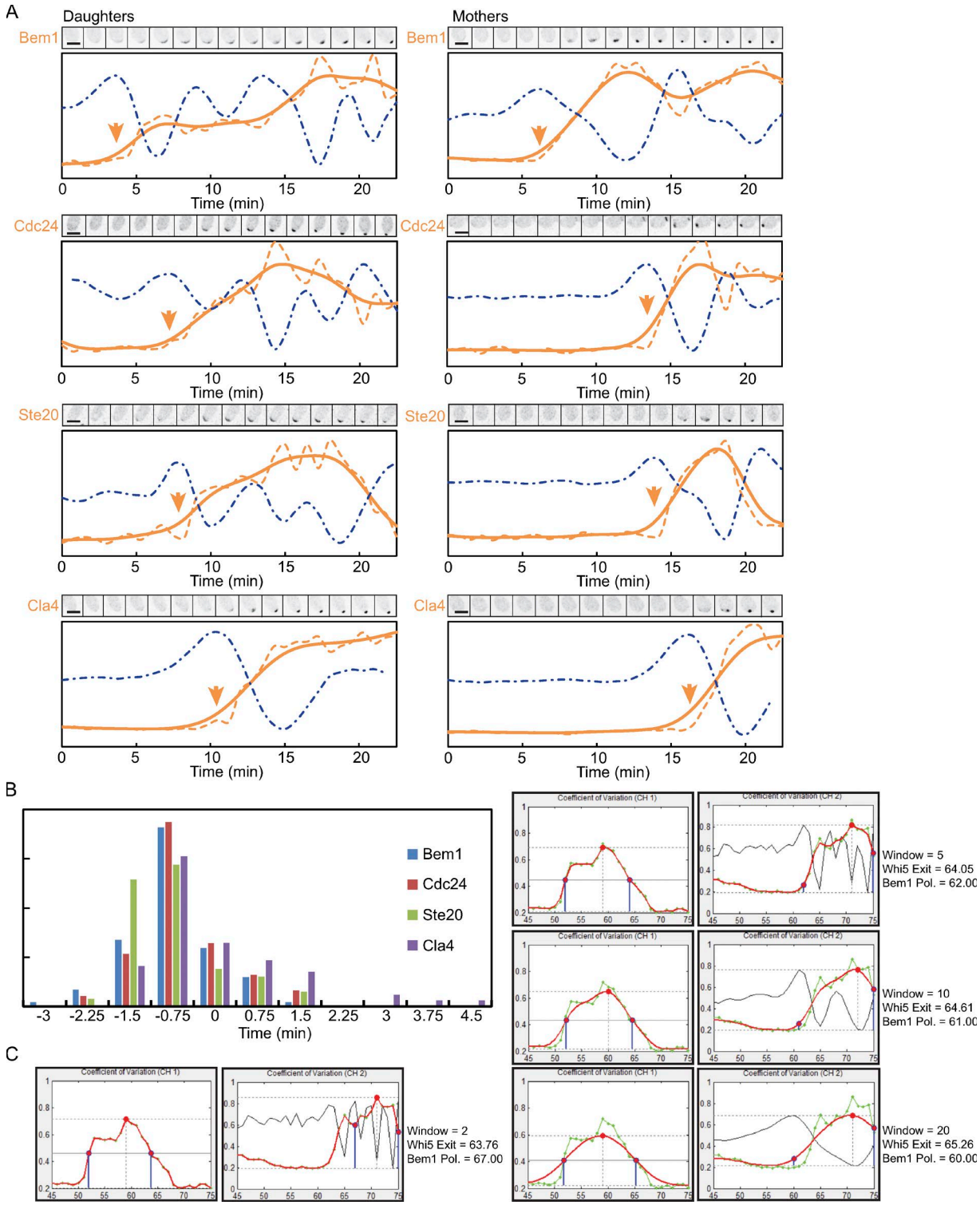

Figure S2. **Determining the time of polarization. (A)** Two representative cells are shown for each probe (Bem1, DLY19682; Cdc24, DLY21642; Ste20, DLY19685; and Cla4, DLY20043). Orange traces show the CV for each cell (dashed lines, raw data; solid lines, spline fits). Blue traces show the second derivative calculated using the CV for each cell. Orange arrows indicate the onset of polarization as determined by the local maximum in the second derivative of the CV for each cell. **(B)** Comparing polarization times determined by using the second derivative of the CV or by visual calling of the first detectable polarity. Negative values indicate that the second derivative method called polarization earlier than the visual method. **(C)** Effect of smoothing parameter (window: larger window = more smoothing) on calling start and polarization times. An illustrative cell is shown with raw (dashed green lines) and smoothed (red lines) spline fits. With insufficient smoothing (window = 2), the maximum of the second derivative of the Bem1 CV calls an obviously incorrect onset time, illustrating the need for smoothing to avoid mis-calling. With sufficient smoothing (window > 5), the timing calls are consistent with polarity onset judged by eye, although there is a general trend such that more smoothing results in calling start time slightly later and polarization onset slightly earlier. Scale bars, 5 µm.

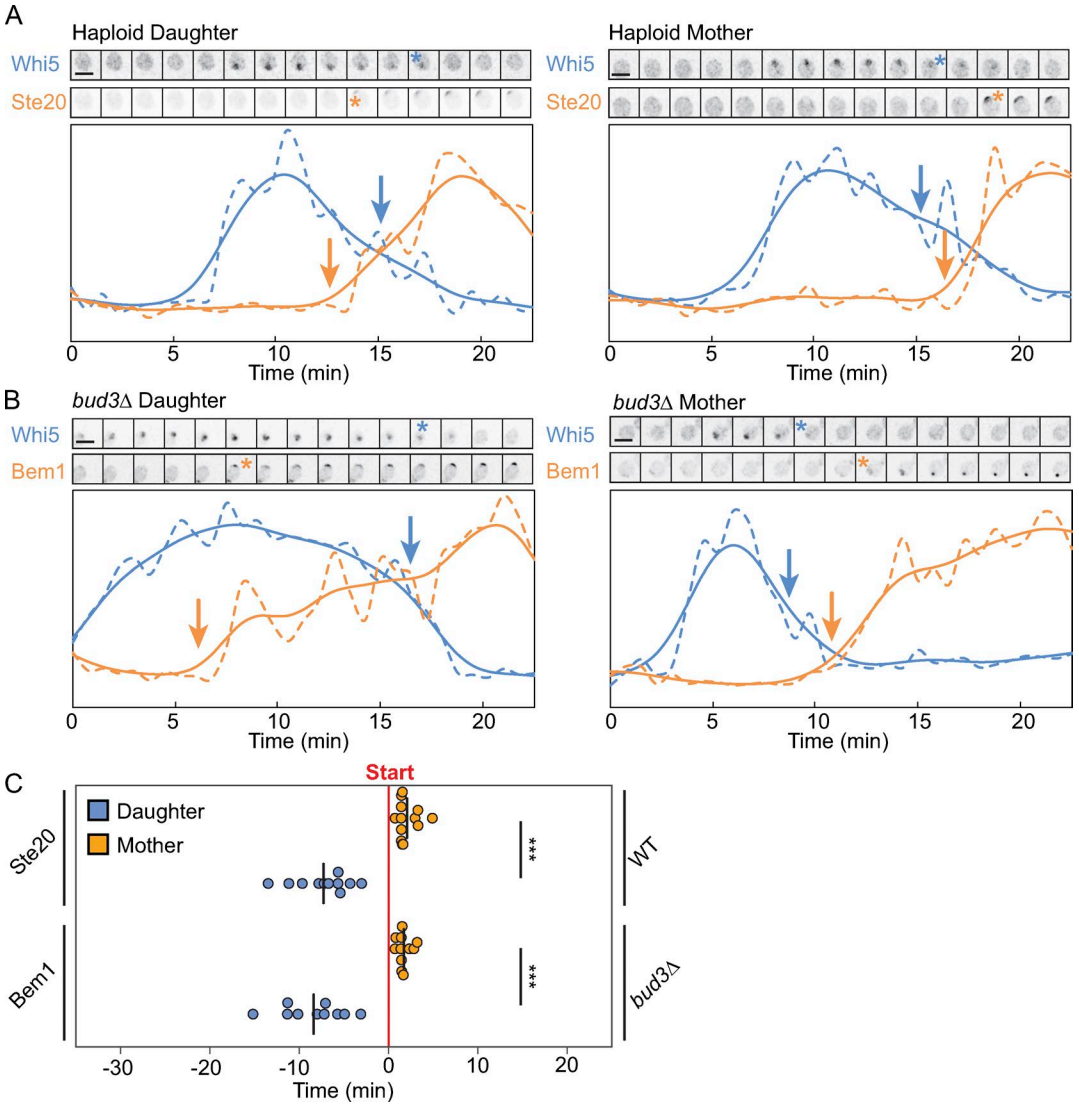

Figure S3. **Timing of polarization in haploid cells. (A)** Ste20 polarizes before start in daughters and after start in mothers. Montages (1.5-min intervals) of representative cells of strain DLY19677. **(B)** Prestart polarization is not due to Bud3. Bem1 polarizes before start in *bud3Δ* daughters and after start in *bud3Δ* mothers. Montages (1.5-min intervals) of representative cells of strain DLY22104. **(C)** Timing of polarization relative to start for the strains in A and B. Scale bars, 5 µm. ***, P < 0.001.

Provided online are two tables in Excel. Table S1 lists the yeast strains used in this study. Table S2 lists the model parameters used in this study.