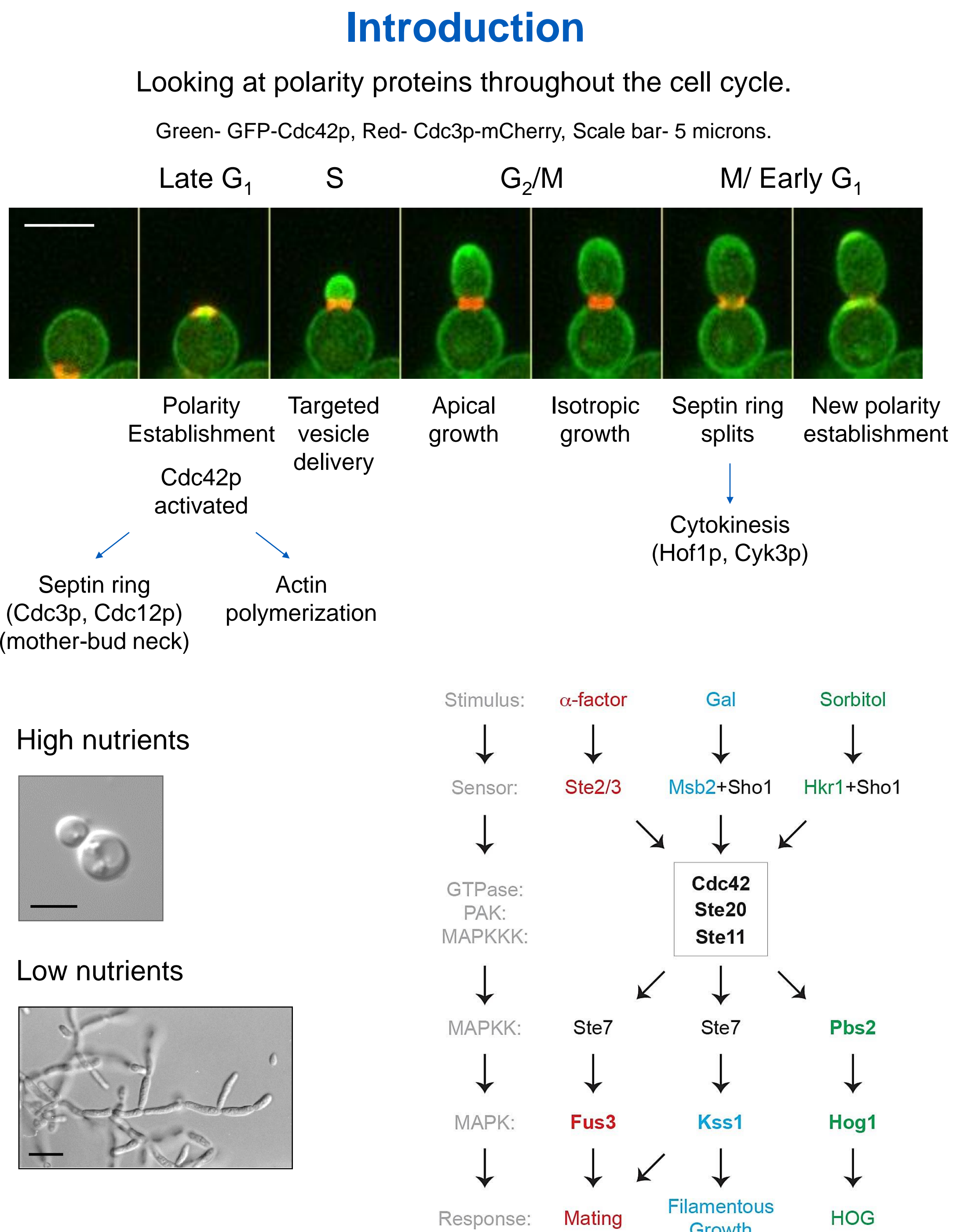


Regulation of Intrinsic Polarity Establishment by a Differentiation-Type MAPK Pathway

Aditi Prabhakar, Jacky Chow, Alan J. Siegel, Beatriz Gonzalez, Sukanya Basu, Heather Dionne, Matthew Vandermeulen and Paul J. Cullen

Department of Biological Sciences, University at Buffalo, NY-14260



fMAPK Pathway Regulates Intrinsic Polarity Establishment Under Low Nutrient Levels

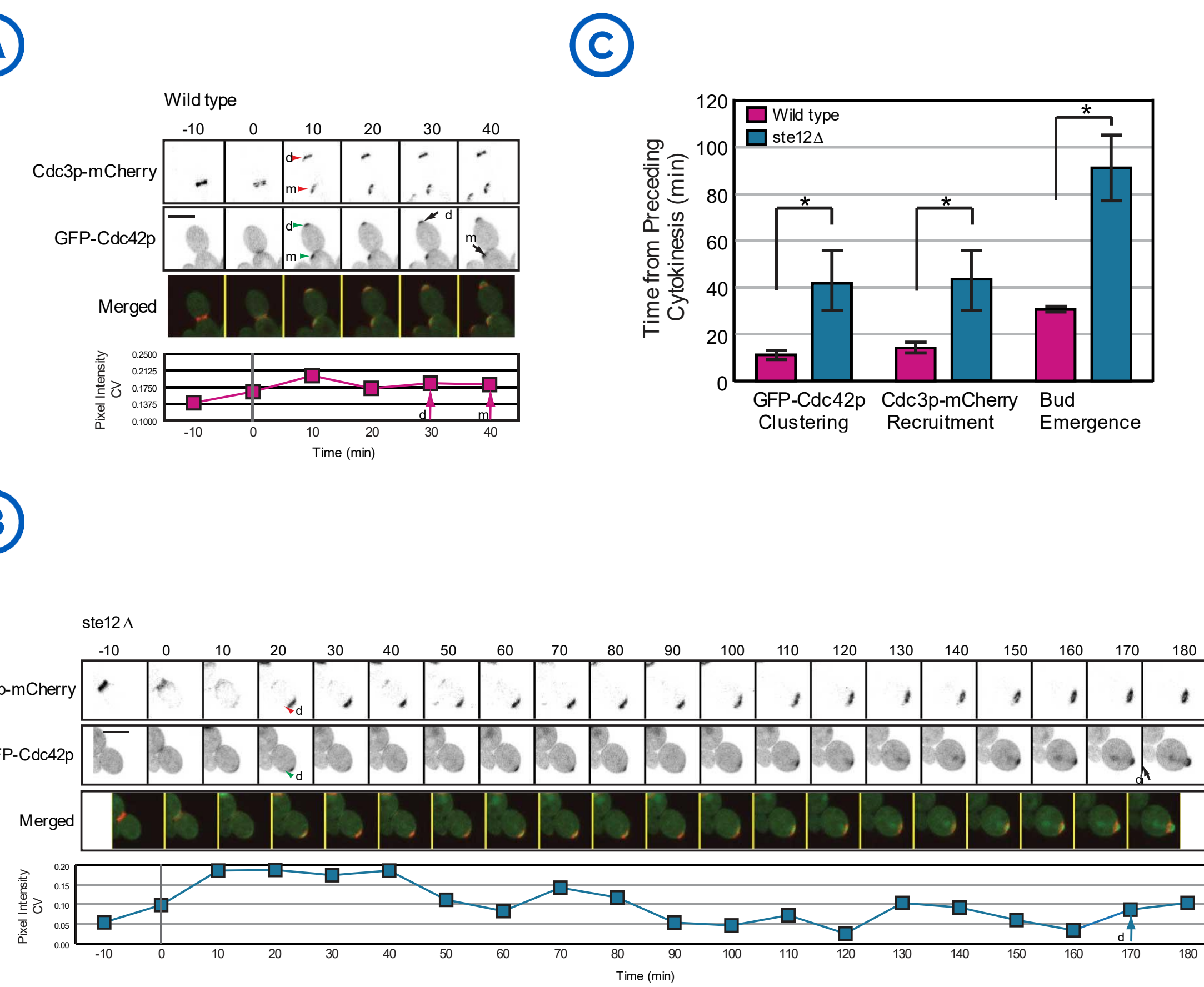


Fig. 1 (A) Wild-type cells establish Cdc42p-dependent polarity which gives rise to new buds. (B) Cells lacking functional fMAPK pathway (*ste12Δ*) showed defects in polarity establishment. (C) Quantitation of the timing of GFP-Cdc42p clustering, Cdc3p-mCherry recruitment and bud emergence in wild-type and *ste12Δ*.

Exploring MAPK Pathways that Share Components for Patterns of Cell Cycle Regulation

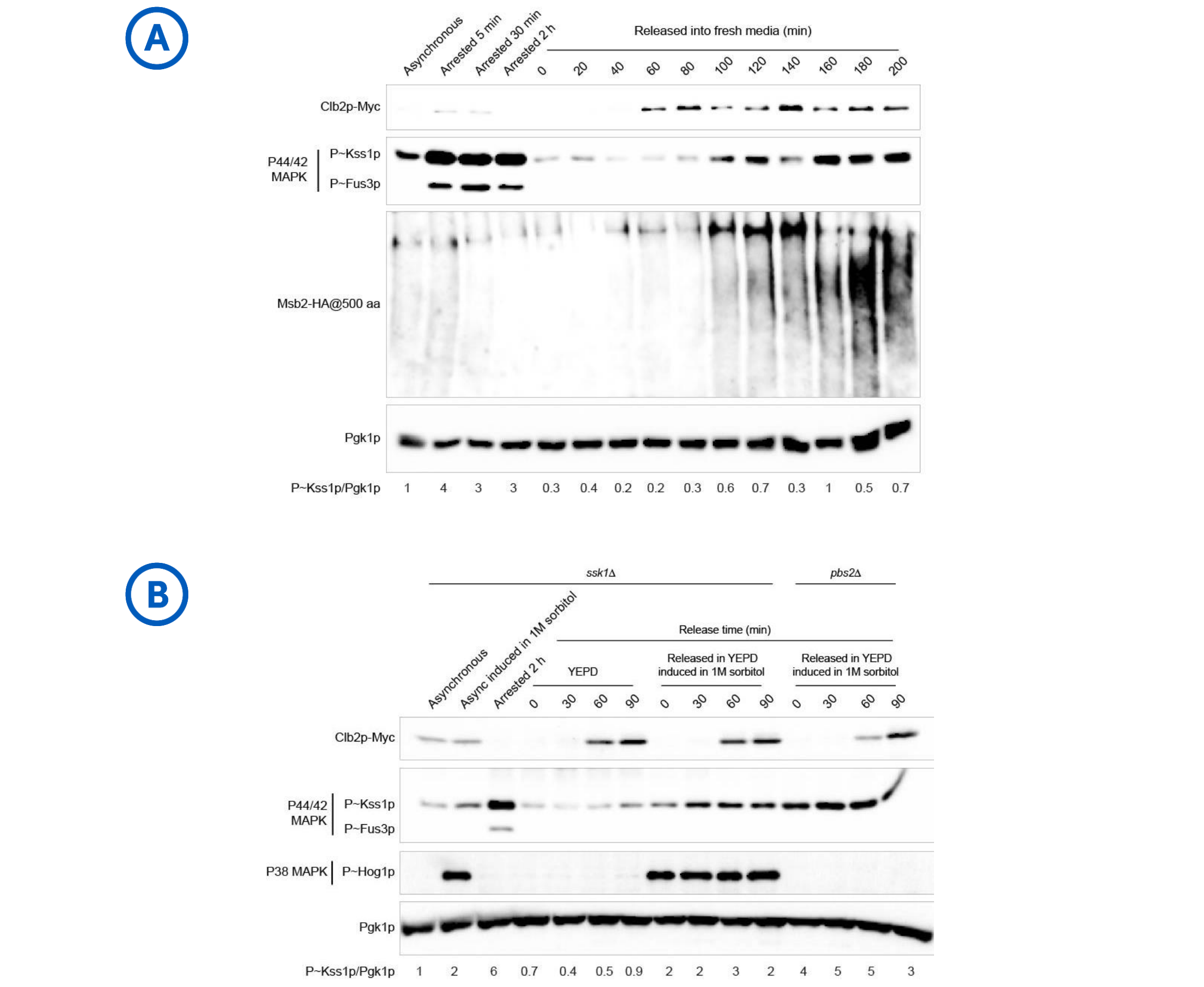


Fig. 2 (A) The fMAPK pathway is cell-cycle regulated. (B) The HOG pathway is not cell-cycle regulated.

Cell-Cycle Regulation of the fMAPK Pathway Is Controlled by Msb2p levels

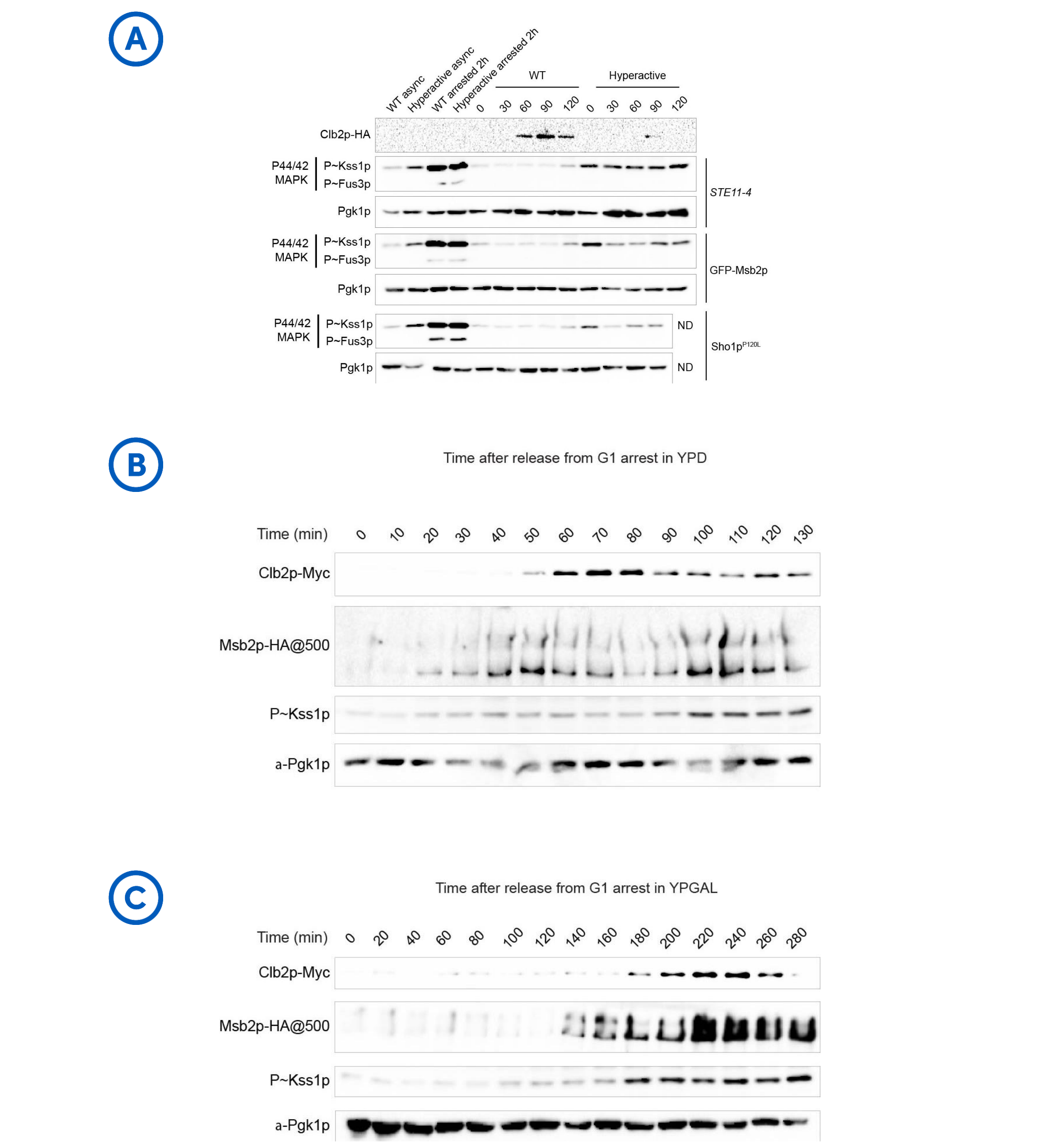


Fig. 3 (A) Effect of hyperactive alleles on the fMAPK pathway activity at early stages in the cell cycle. Msb2p levels fluctuate during the progression of the cell cycle under basal conditions, YEPD (B) and under inducing conditions, YEP-GAL (C).

Visualizing Active MAPK in Single Cells

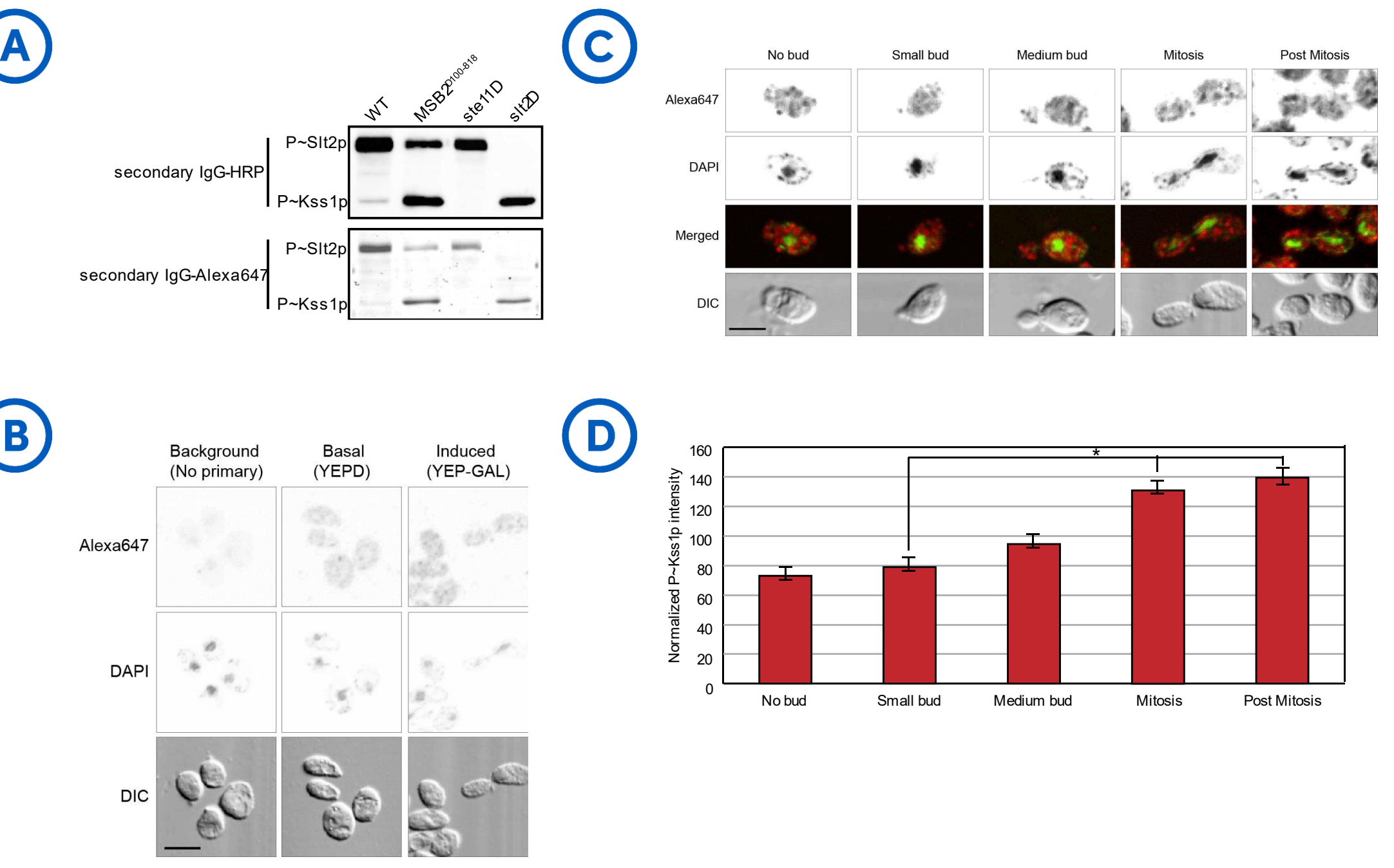


Fig. 4 Monitoring P-Kss1p using indirect immunofluorescence

Sho1p Has a Dynamic Localization Pattern Throughout the Cell Cycle

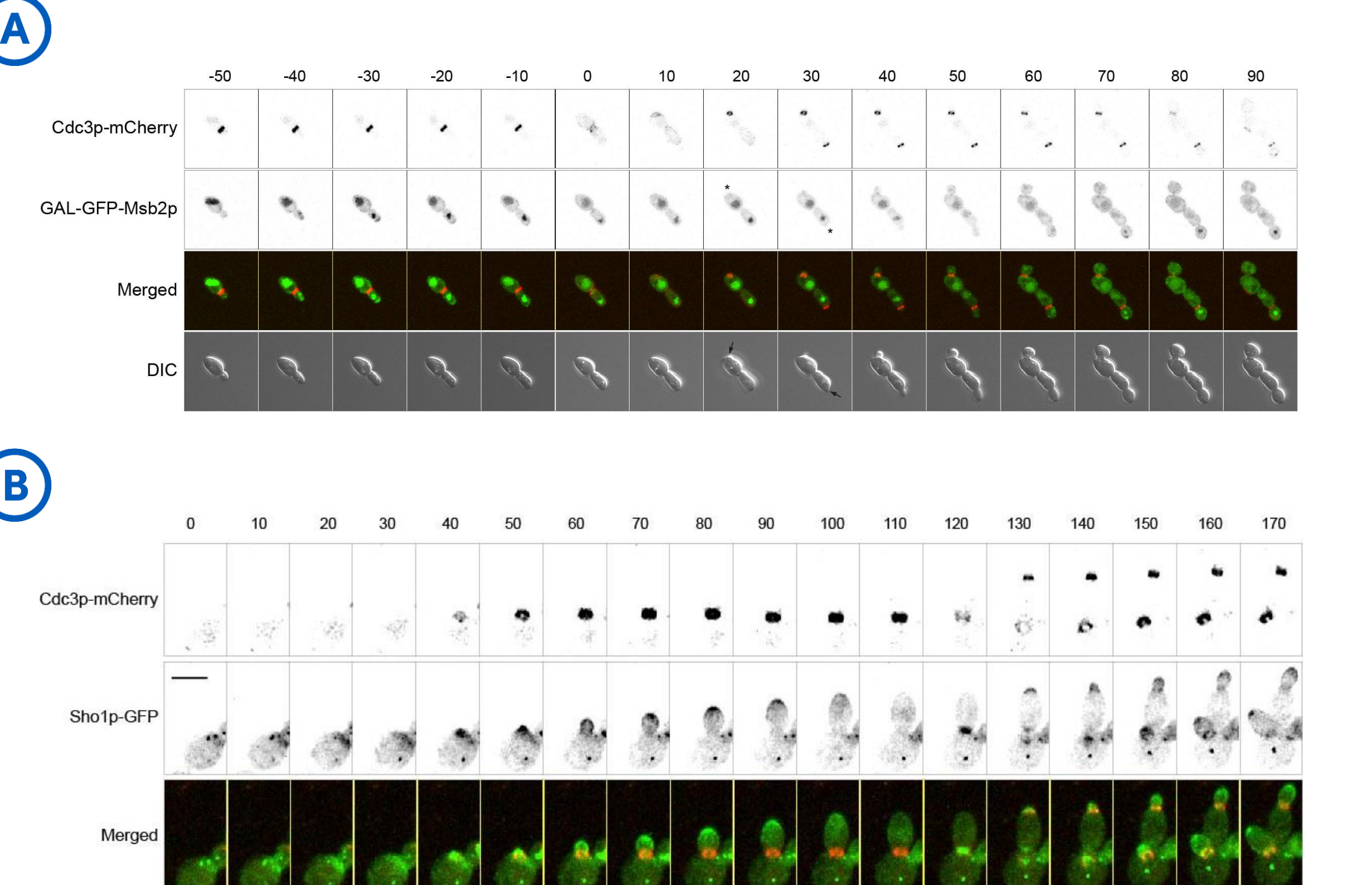


Fig. 5 Time-lapse microscopy for GFP-Msb2p (A) and Sho1p-GFP (B) in nutrient-limiting conditions, S-GAL.

Cells Lacking Functional Septins Show Defects in fMAPK Pathway Signaling

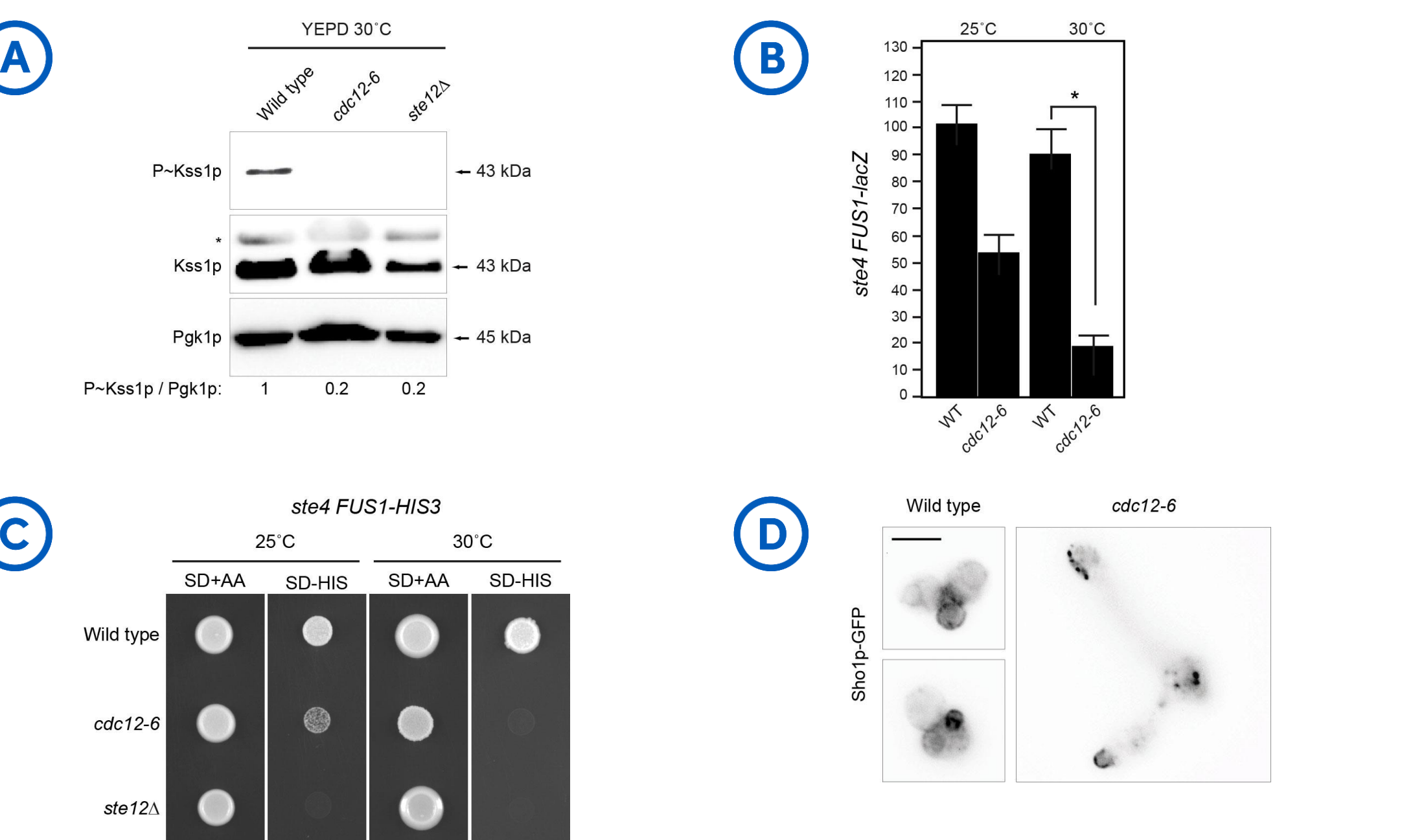


Fig. 6 Measuring fMAPK pathway activity in the indicated strains by immunoblot (A) transcriptional reporter, *FUS1-lacZ* (B) and growth reporter, *FUS1-HIS3* (C). (D) Sho1p-GFP is mislocalized in the *cdc12-6* mutant.

The Cytokinesis Regulatory Protein Hof1p Is Required for fMAPK Pathway Signaling

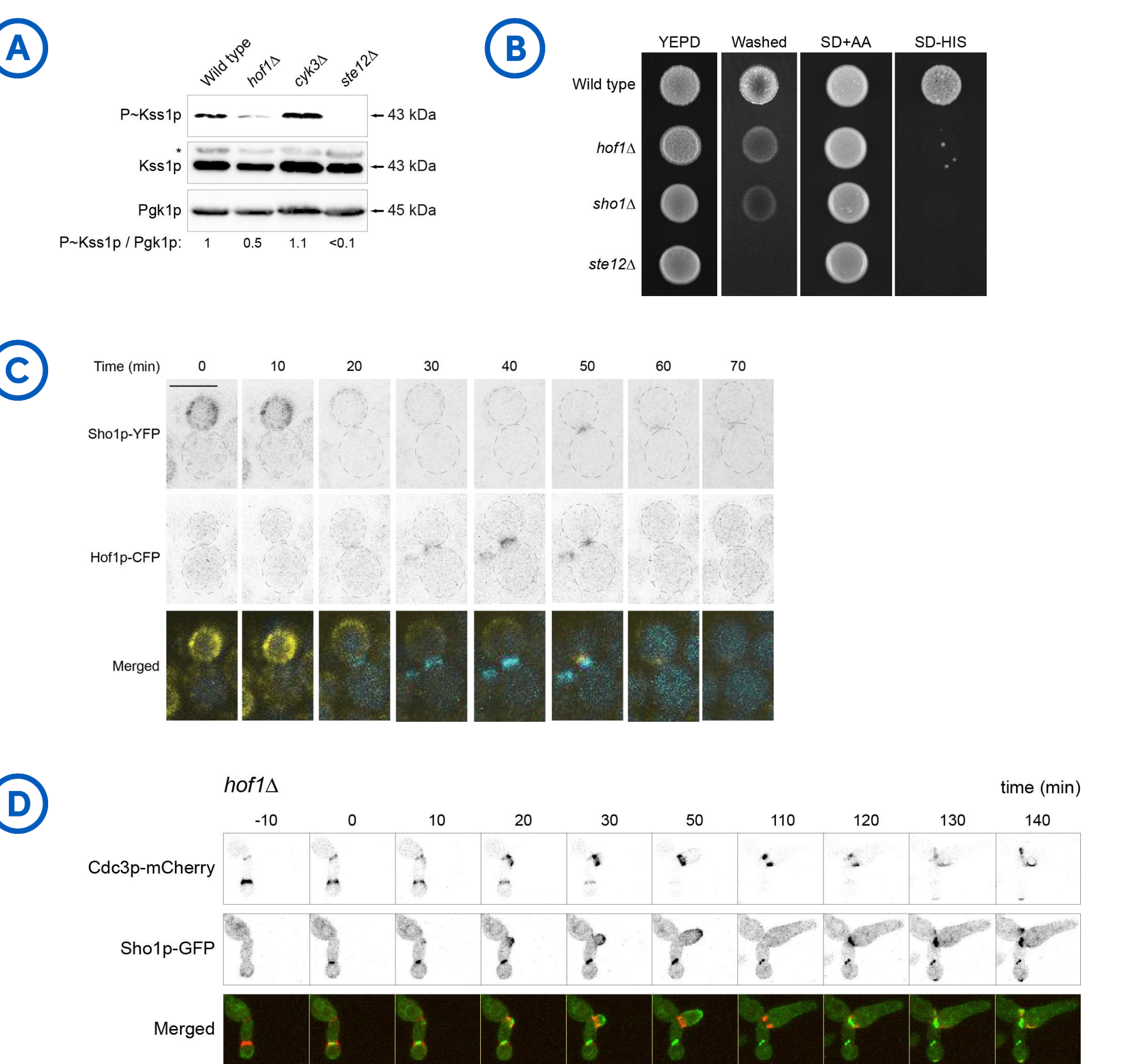


Fig. 7 Measuring fMAPK pathway activity in the indicated strains by immunoblot (A) and growth reporter, *FUS1-HIS3* (B). Sho1p and Hof1p co-localize at the mother-bud neck (C) Sho1p is mislocalized in the *hof1Δ* mutant.

Filamentous Growth Impacts Mother-Bud Neck Integrity

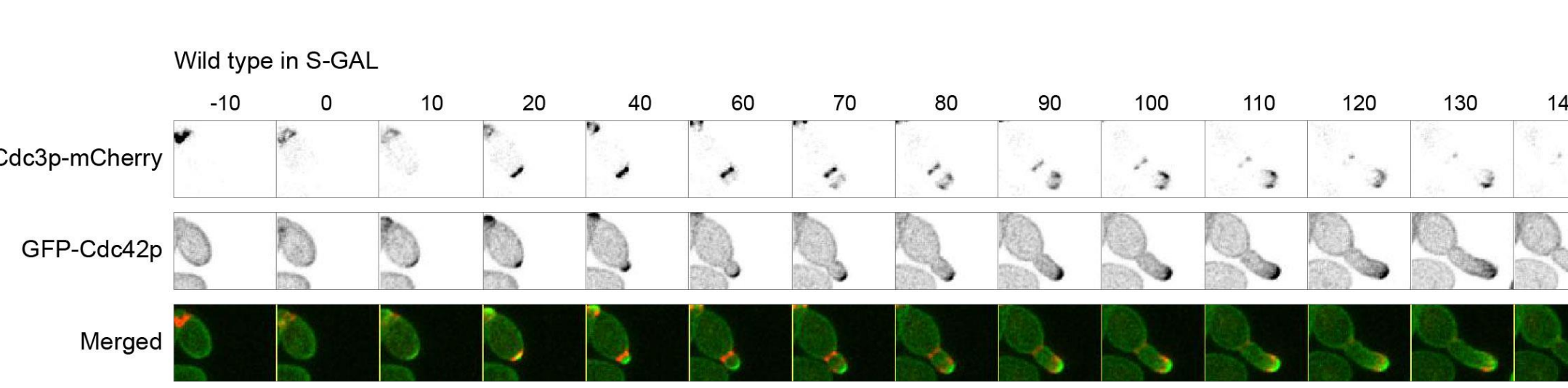


Fig. 8 Cells grown in low-nutrient conditions showed elongated daughter cells due to defects in mother-bud neck integrity.

Conclusions

- The timing of bud emergence was delayed in the fMAPK mutant.
- The fMAPK pathway is cell cycle regulated.
- The HOG pathway that shares components with the fMAPK pathway is not cell cycle regulated.
- Msb2p levels fluctuate throughout the cell cycle and determine fMAPK activation at M/G₁.
- Sho1p co-localizes with Hof1p at the mother-bud neck during cytokinesis.
- Cells lacking cytokinetic factor, Hof1p, or septin integrity show reduced fMAPK activity.
- Wild-type cells undergoing filamentous growth exhibit cytokinesis problems which might make them better suited for the invasive lifestyle.

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