## Supplemental material

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Video 1. WT cells expressing mtGFP were cultured to logarithmic growth in glucose-containing selective complete medium at 25°C, incubated at 37°C for 20 min, and analyzed for 1 h by time-resolved 3D fluorescence microscopy at 37°C. Cells were constantly supplied with fresh glucose-containing rich medium and imaged in 2-min intervals by taking z stacks of differential interference contrast and mtGFP fluorescence. The video shows 3 frames per second, accordingly 10 s of video time equal 1 h of real time. One focal plane of the differential interference contrast is merged with a maximum intensity projection of fluorescence signal.



Video 2. fzo1-1 cells were analyzed as in Video 1.



Video 3. myo2(LQ) cells were analyzed as in Video 1.



Video 4. myo2(LQ) fzo1-1 cells were analyzed as in Video 1.



Video 5. WT cells expressing mtGFP were cultured to logarithmic growth in glucose-containing selective complete medium at  $30^{\circ}C$ . Cells were analyzed for 1 h by time-resolved 3D fluorescence microscopy as in Video 1, with the exception that the temperature was  $30^{\circ}C$ .



Video 6. Ammr1 cells were analyzed as in Video 5.



Video 7. Afzo1 cells were analyzed as in Video 5.



Video 8. Afzo1 Ammr1 cells were analyzed as in Video 5.

Tables S1-S4 are available as Excel files.

Table S1 lists genes and genetic interaction scores from two independent SGA screens of the MATa yeast deletion collection with myo2(LQ).

Table S2 lists genes that negatively interacted with myo2(LQ) in SGA screens.

Table S3 lists yeast strains used in this study.

Table S4 details data pooling and statistics.