Figure S1

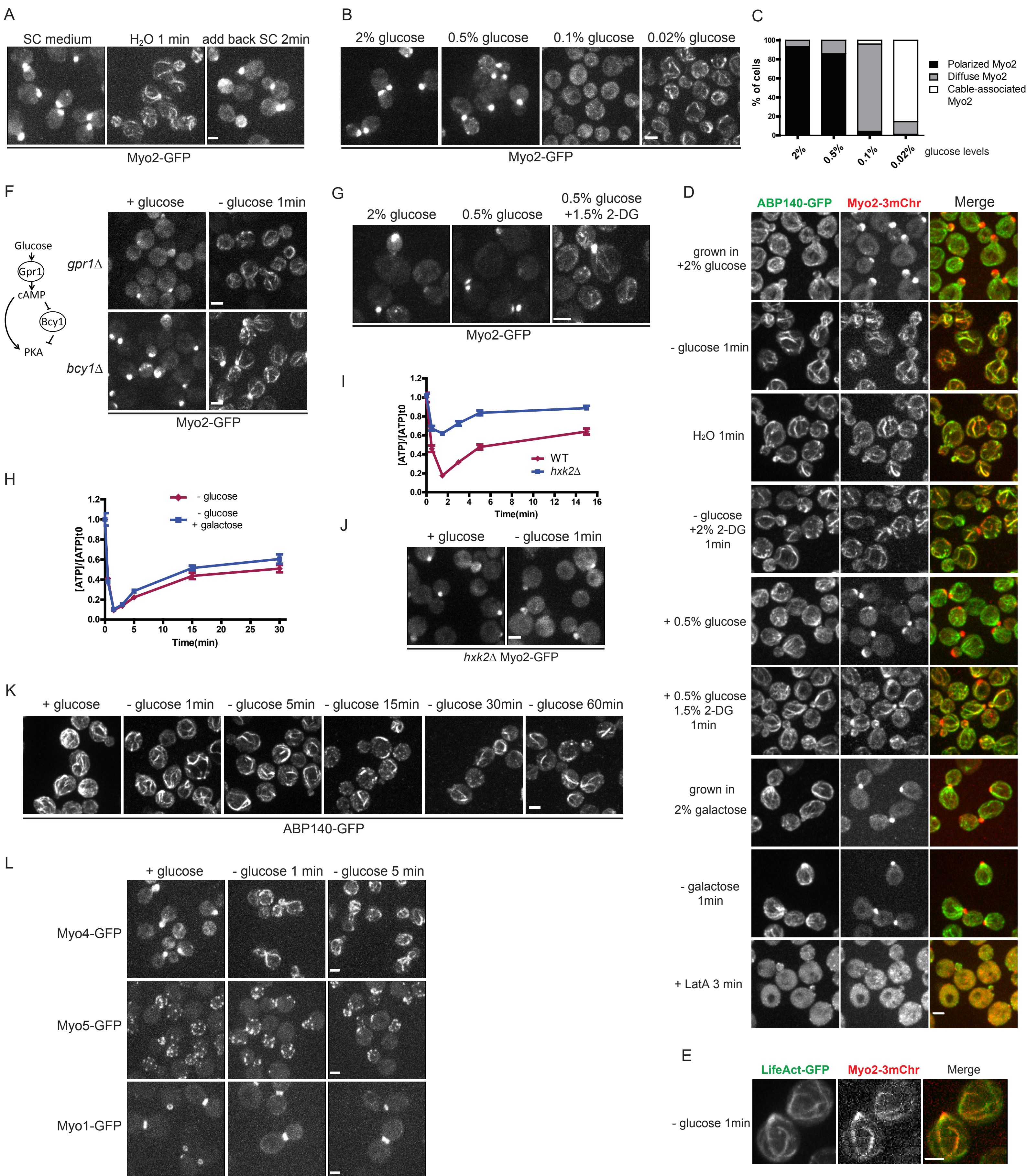


Figure S1. Redistribution of Myo2 to actin cables is regulated by the level of cellular ATP. Related to Figures 1-2.

(A) Cells expressing Myo2-GFP grown to mid-log phase in SC were transferred to fresh SC (left) or water (center), or first transferred into water for 2 min and then transferred back to SC (right).

(B) Cells as in (A) were transferred to fresh SC with 2% glucose (left), 0.5% glucose (center), 0.1% glucose or 0.02% glucose for 1 min.

(C) Quantification of Myo2-GFP localization in (B). At least 200 cells were scored for each condition.

(D) Cells expressing Myo2-3mCherry and Abp140-GFP were transferred to conditions as indicated.

(E) Cells expressing Myo2-3mCherry and LifeAct-GFP were transferred to SC lacking glucose for 1 min.

(F) *bcy1*Δ and *gpr1*Δ cells expressing Myo2-GFP grown in SC were transferred to fresh SC or SC lacking glucose for 1 min.

(G) The glucose analog 2-DG induces Myo2 relocalization even in the presence of glucose. Cells expressing Myo2-GFP grown to mid-log phase in SC were transferred to fresh SC with 2% glucose (left), 0.5% glucose (center), or 0.5% glucose + 1.5% 2-DG (right) for 1 min.

(H) ATP levels of cells after glucose depletion (red) or glucose depletion and galactose addition (blue) were measured at indicated time points. ATP concentrations are normalized to pre-starved cells. Error bars are SD.

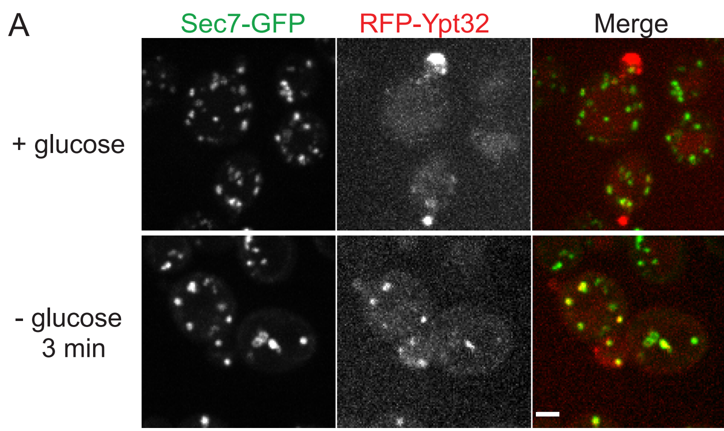
(I) ATP levels in WT and *hxk2*Δ cells were measured before and after glucose depletion at indicated time points. ATP concentrations are normalized to pre-starved cells. Error bars are SD.

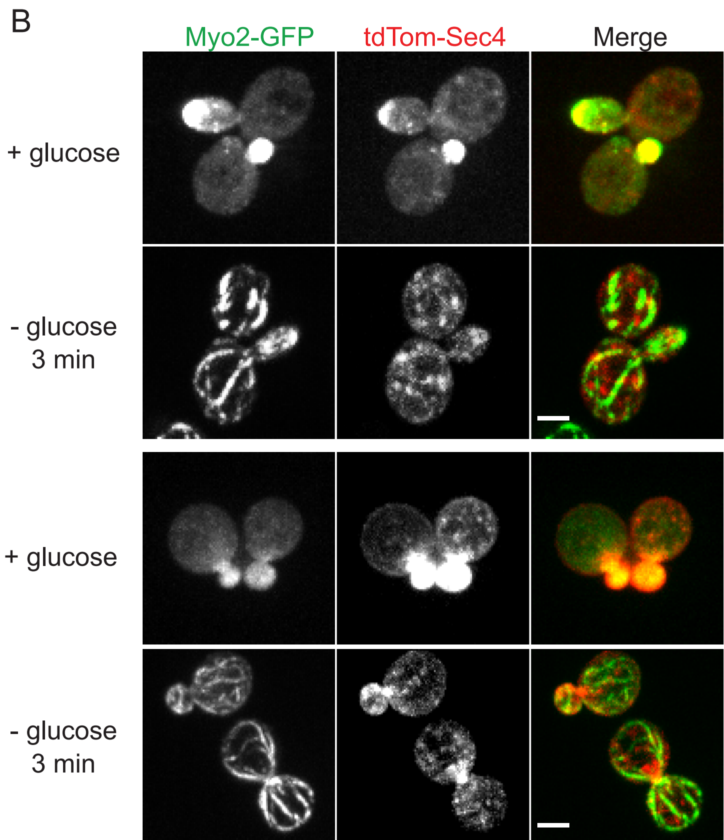
(J) Myo2 is still polarized after glucose depletion in *hxk2*Δ cells. *hxk2*Δ cells expressing Myo2-GFP grown in SC were transferred to fresh SC (left) or SC lacking glucose (right) for 1 min.

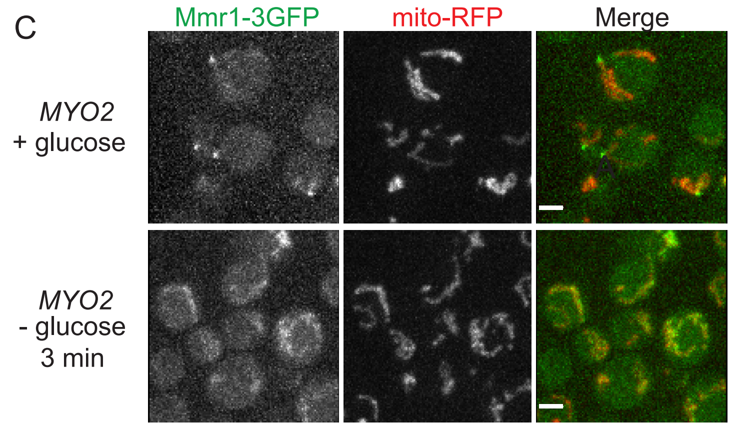
(K) Yeast cells expressing Abp140-GFP were transferred to medium lacking glucose. The presence of actin cables were monitored for 60 min after glucose depletion.

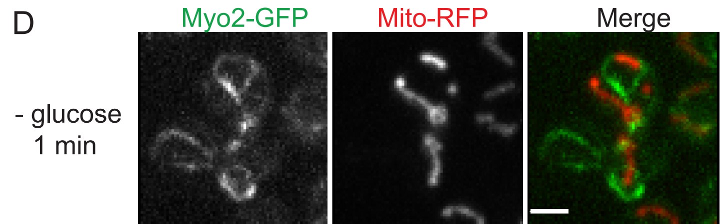
(L) Localization of Myo4-GFP, Myo5-GFP or Myo1-GFP in the presence or absence of glucose. All scale bars, 2 μm.

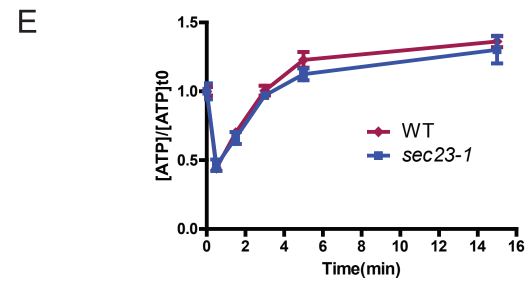
Figure S2











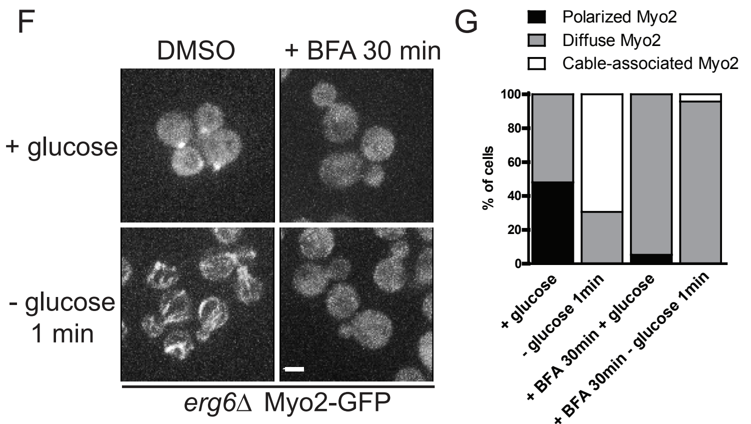


Figure S2. Localization of Myo2 and its interacting proteins in glucose-starved cells. Related to Figure 3.

(A) Cells expressing Sec7-GFP and RFP-Ypt32 were grown to mid-log phase in SC and transferred to fresh SC or SC lacking glucose for 3 min.

(B) WT and *msb3*Δ*msb4*Δ Cells expressing Myo2-GFP and tdTomato-Sec4 were transferred to fresh SC or SC lacking glucose for 3 min.

(C) Cells expressing Mmr1-3GFP and mito-RFP were transferred to fresh SC or SC lacking glucose for 3 min.

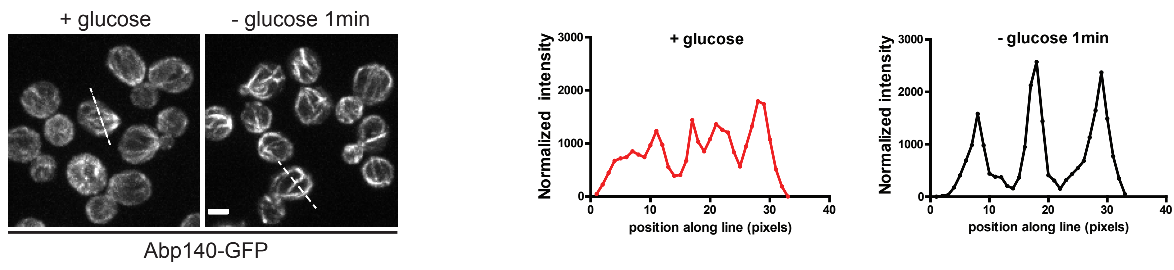
(D) Cells expressing Myo2-GFP and Mito-RFP were transferred to SC lacking glucose for 1 min.

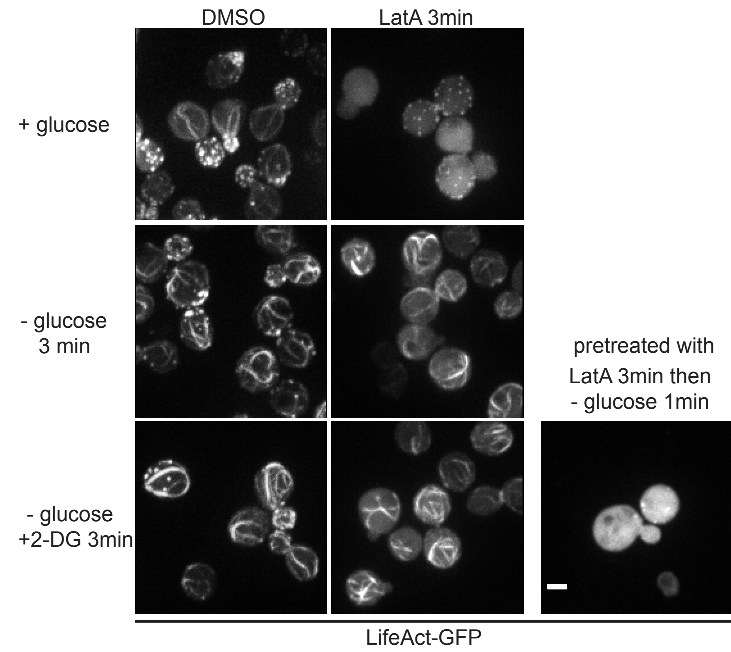
(E) ATP levels change similarly in *sec23-1* and WT cells upon glucose depletion at 35 ^{o}C. ATP levels in WT and *sec23-1* cells at 35 ^{o}C were measured before and after glucose depletion at indicated time points. ATP concentrations are normalized to pre-starved cells. Error bars are SD.

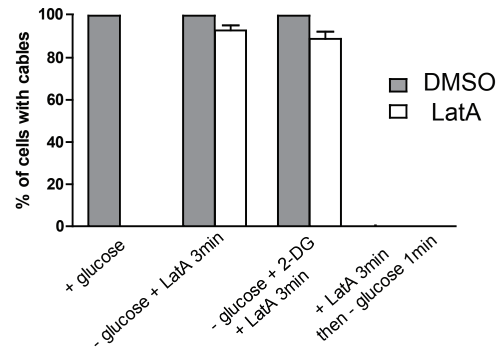
(F) Localization of Myo2-GFP in *erg6*Δ cells pre-incubated with 150 μM brefeldin A (BFA) or DMSO for 30 min and transferred to fresh SC or SC lacking glucose. *erg6*Δ cells have enhanced membrane permeability that allows the uptake of brefeldin A. All scale bars, 2 μm.

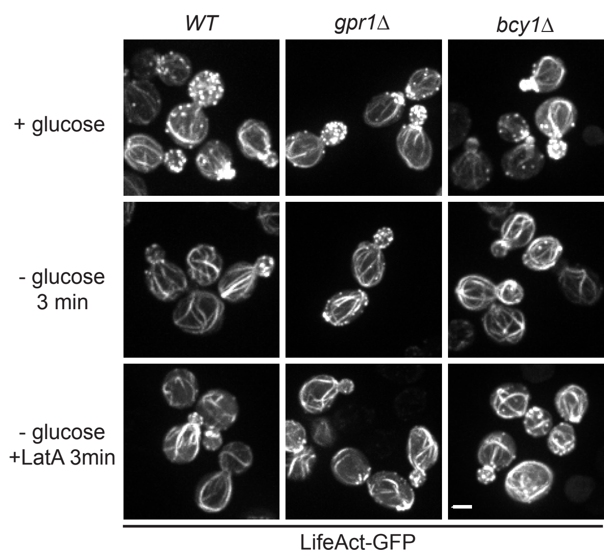
(G) Quantification of Myo2-GFP localization in (F). At least 200 cells were scored for each condition.

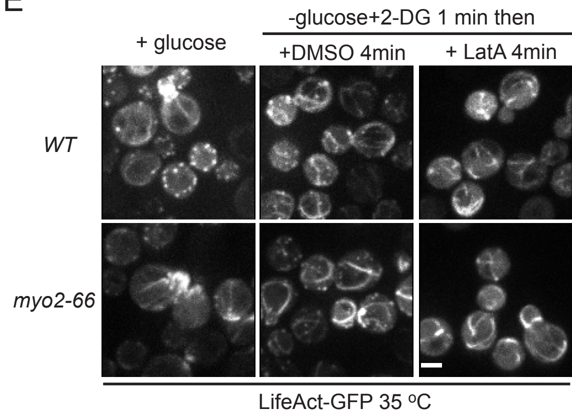
Figure S3

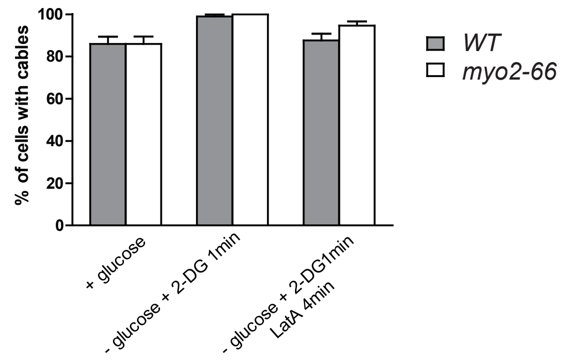


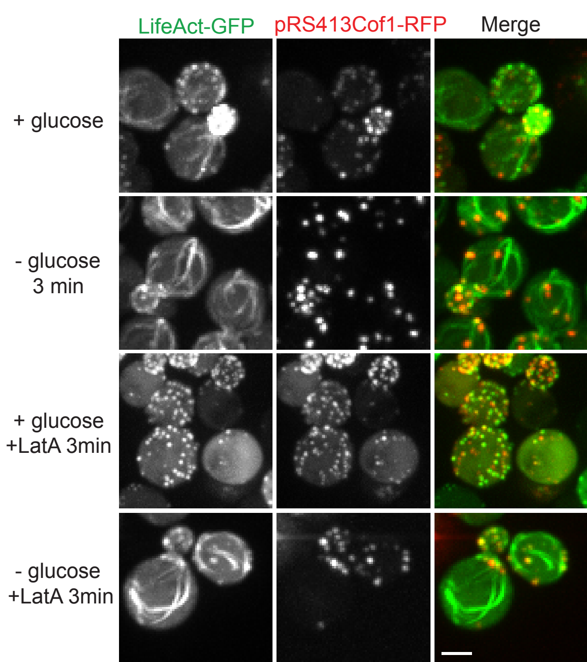












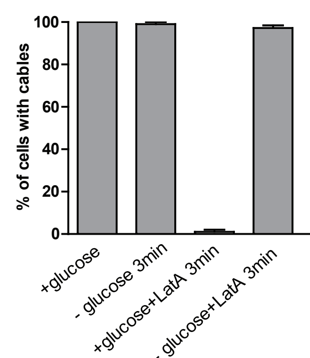


Figure S3. Actin cables are stabilized upon glucose depletion. Related to Figure 4.

(A) Cells expressing Abp140-GFP were transferred to fresh SC or SC lacking glucose for 1 min. Abp140-GFP intensity along the dotted line was plotted in representative cells in both conditions.

(B) Stabilization of actin cables upon glucose depletion. Yeast cells expressing LifeAct-GFP were grown to mid-log phase in SC and transferred to fresh SC, SC lacking glucose or SC with 2-DG but no glucose supplemented with 120 μM LatA or equal volume of DMSO for 3 min.

(C) Percentages of cells with actin cables in (B). At least 200 cells were scored for each condition. Error bars represent SD.

(D) WT, *bcy1*Δ and *gpr1*Δ cells expressing LifeAct-GFP were transferred to fresh SC, SC lacking glucose or SC lacking glucose supplemented with 120 μM LatA for 3 min.

(E) WT and *myo2-66* cells expressing LifeAct-GFP were pre-incubated at 35 ^{o}C for 45 min and transferred to prewarmed SC or medium with 2-DG but no glucose for 1 min, and then treated with LatA or DMSO for 4 min.

(F) Percentages of cells with actin cables in (D). At least 200 cells were scored for each condition. Error bars represent SD.

(G) Cells coexpressing LifeAct-GFP and Cof1-RFP (exogenously expressed on a CEN plasmid driven by Cof1 promoter) were transferred to fresh SC or SC lacking glucose for 1 min.

(H) Percentages of cells with actin cables in (F). At least 200 cells were scored for each condition. Error bars represent SD.

Table S1. Yeast strains

Strain	Genotype	Parent	Source
BY4741	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*		Open Biosystems
BY4742	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*		Open Biosystems
ABY117	*MATa ade1 his6 leu2-3,112 ura3-52 his3-Δ1 myo2-66*		Lab stock
ABY913	*MATa/α ade2-101 ade3 his7 leu2-3,112 trp1-1 ura3-52*		[S1]
ABY971	*MATa/α tpm1-2::LEU2 tpm2Δ::HIS3 his3Δ -200 leu2-3,112 lys2-801 trp1-1 ura3-52*		[S1]
ABY2000	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 bnr1∆::KanR bni1-11::ura3∆::HIS3*	BY4742	Lab stock
ABY3149	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 MYO2-3GFP::URA*	BY4742	[S2]
ABY3643	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 arg4Δ::KanMX MYO2-GFP*	BY4742	this study
ABY3654	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MYO2-3mCherry*	BY4741	this study
ABY3658	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MYO2-3mCherry GFP-SEC4::URA3*	ABY3654	this study
ABY3678	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MYO2-3mCherry pRS416GPD-LifeAct-GFP*	ABY3654	this study
ABY3679	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 arg4Δ::KanMX MYO2-GFP mitoRFP::URA3*	ABY3643	this study
	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 nth1Δ::KanMX	BY4741	Open Biosystems
	MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 gph1Δ::KanMX	BY4741	Open Biosystems
ABY3673	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 gsy1Δ::KanMX gsy2Δ::HIS3*	BY4741	
ABY3680	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 pRS415-GFP-MYO1*	BY4742	this study
ABY3662	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 MYO4-GFP::KanMX*	BY4742	this study
ABY3663	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 MYO5-GFP::KanMX*	BY4742	this study
ISC317	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MMR1-3xGFP::LEU2 MYO2::HIS3 mitoRFP::URA3*	BY4741	[S3]
ISC318	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MMR1-3xGFP::LEU2 myo2-14::HIS3 mitoRFP::URA3*	BY4741	[S3]
ABY3661	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 erg6Δ::KanMX MYO2-GFP::URA3*	BY4741	this study
ABY3652	*MATa ade1 his6 leu2-3,112 ura3-52 his3-Δ1 myo2-66-GFP*	ABY117	this study
ABY3677	*MATa ade1 his6 leu2-3,112 ura3-52 his3-Δ1 myo2-66 pRS416GPD-LifeAct-GFP*	ABY117	this study
ABY3424	*MATα his3-Δ200 ura3-52 leu2-3,112 lys2-801 ade2-101 myo2-13-GFP::URA3*		[S2]
ABY3448	*MATα his4-619 ura3-53 sec23-1 MYO2-GFP::URA3*		[S2]
ABY3449	*MATα his4-619 ura3-53 sec23-1 myo2-13-GFP::URA3*		[S2]
ABY3670	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 pRS416CYC1-tdTomato-Myo2 (aa1-784)*	BY4742	this study
ABY3671	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 pRS416CYC1-tdTomato-Myo2 (aa1-1080)*	BY4742	this study
ABY2439	*MATa ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ABP140-GFP::KanMX*		Lab stock
ABY3674	*MATa/α ade2-101 ade3 his7 leu2-3,112 trp1-1 ura3-52 pRS416GPD-LifeAct-GFP*	ABY913	this study
ABY3675	*MATa/α tpm1-2::LEU2 tpm2Δ::HIS3 his3Δ -200 leu2-3,112 lys2-801 trp1-1 ura3-52 pRS416GPD-LifeAct-GFP*	ABY971	this study
ABY3681	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 bnr1∆::KanR bni1-11::ura3∆::HIS3 pRS416GPD-LifeAct-GFP*	ABY2000	this study

ABY3665	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 ABP1-GFP::KanMX*	BY4742	this study
ABY3660	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 hxk2Δ::KanMX MYO2-GFP::URA3*	BY4741	this study
ABY3682	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 gpr1Δ::KanMX MYO2-GFP::URA3*	BY4741	this study
ABY3683	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 bcy1Δ::HIS3 MYO2-GFP::URA3*	BY4742	this study
ABY3684	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MYO2-3mCherry ABP140-GFP::HIS3*	ABY3654	this study
ABY3685	*MATa/α his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MYO2-3mCherry GFP-SEC4::URA3/SEC4*	ABY3654	this study
ABY3686	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MYO2-3mCherry Sec15-3GFP::HIS3*	ABY3654	this study
ABY3687	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MYO2-3mCherry pRS315YOP1-GFP-YPT32*	ABY3654	this study
ABY2447	*MATa/α ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC7-GFP::KanMX*		Lab stock
ABY3688	*MATa/α ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 SEC7-GFP::KanMX pRS316YOP1-RFP-YPT32*	ABY2447	this study
ABY3689	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 MYO2-3mCherry GFP-SEC4::URA3 sec6-4::HIS3*	ABY3658	this study
ABY3690	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 arg4Δ::KanMX MYO2-GFP pRS415CYC1-tdTomato-Sec4*	ABY3643	this study
ABY3156	*MATa his3 leu2 lys2 trp1 ura3 msb3Δ::HIS3 msb4Δ::TRP1 MYO2-GFP::URA3*		[S2]
ABY3691	*MATa his3 leu2 lys2 trp1 ura3 msb3Δ::HIS3 msb4Δ::TRP1 MYO2-GFP::URA3 pRS415CYC1-toTomato-Sec4*	ABY3156	this study

Table S2. Plasmids

Plasmids	**Marker**	**Source**
pRS306-MYO2-3GFP::URA	URA3 (marker-less after selection on 5'FOA)	[S2]
pRS306-MYO2-GFP::URA	URA3 (marker-less after selection on 5'FOA)	[S2]
pRS306-MYO2-3mCherry::URA	URA3 (marker-less after selection on 5'FOA)	[S2]
pRS306-mitoRFP::URA3	URA3	[S3]
pRS415-GFP-MYO1	LEU2	[S4]
pRS416CYC1-tdTomato-Myo2 (aa1-784)	URA3	this study
pRS416CYC1-tdTomato-Myo2 (aa1-1080)	URA3	this study
pRS416GPD-LifeAct-GFP	URA3	this study
pRS303-Sec15-3GFP	HIS3	Lab stock
pRS316YOP1-RFP-YPT32	URA3	Lab stock
pRS315YOP1-GFP-YPT32	LEU2	Lab stock
pRS303-sec6-4	HIS3	this study
pRS415CYC1-tdTomato-Sec4	LEU2	this study

Supplemental Experimental Procedures

Yeast strains and DNA construction

Yeast trains and plasmids used in this study are listed in Tables S1 and S2. Standard media and techniques for growing yeast and transformation were used [S5]. Gene deletion and chromosomal GFP tagging were performed by standard PCR-mediated techniques [S6]. The

plasmid *pRS306-MYO2-3GFP::URA* has an additional 495 nt region 3' to the *MYO2* gene after the fluorescent tag, thus the URA maker can be looped out by recombination after selection on 5' FOA [S2]. To construct a plasmid expressing a protein fused with tdTomato, the ORF of tdTomato was amplified from pBAD-tdTomato [S7], digested with SpeI/BamHI and ligated into similarly cut pRS413CYC1 [S8]. Then fragments encoding the N-terminal 784 or 1086 amino acids of Myo2 were PCR-amplified from genomic DNA preparations (YeaStar Genomic DNA kit), digested with EcoRI/XhoI and cloned into a similarly cut pRS413CYC1-tdTomato.

The plasmid peGFPN1-LifeAct was kindly provided by Harshad Vishwasrao (Columbia University).To construct a plasmid expressing LifeAct-GFP in yeast, a PCR fragment of Lifeact-GFP was amplified from peGFPN1-LifeAct and inserted into pRS416GPD [S8] with SpeI/HindIII.

Yeast growth and carbon depletion

For glucose depletion, cells were grown in SC medium overnight, diluted next morning in fresh medium and allowed to grow to mid-log phase. Cells were seeded on a 35-mm glass-bottomed dish (MatTek) pretreated with 0.5 mg/ml Concanavanin A (EY laboratories) and quickly transferred into indicated conditions by three washes. For galactose depletion, cells were grown to mid-log phase in SC medium supplemented with 2% galactose instead of glucose and washed into medium lacking any carbon source. Immediately after changing the medium, cells were subjected to fluorescent microscopy.

Cell culture and transfection

HeLa cells (ATCC) were cultured in Dulbecco's modified minimum Eagle's medium (DMEM) (Life technologies) supplemented with 10% fetal bovine/calf serum (FBS) and 2 mM GlutaMAX (Invitrogen). For transfection, cells were grown to about 70% confluence and transfected with Lipofectamin 2000 (Invitrogen) following manufacturer's instructions. Cells were treated and imaged 16 hours post transfection.

Live-cell imaging and FRAP

Micrographs were acquired on a CSU-X spinning disc confocal microscopy system (Intelligent Imaging Innovations) with a DMI 6000B microscope (Leica), 100x1.45 NA objective and a QuantEM EMCCD camera (Photometrics) or an HQII CCD camera (Photometrics) with a 2x magnifying lens.. All images shown are maximum projection of 20 confocal slices taken 0.28 μm apart, unless stated otherwise. Images were analyzed and processed with Slidebook 5.5 software (Intelligent Imaging Innovations). The images were further adjusted in Photoshop (Adobe) to give the clearest presentation of the results and assembled in Illustrator (Adobe).

For live-cell imaging of yeast cells, cells were attached to a glass-bottomed dish coated with Concanavanin A and washed into indicated medium as described above. Imaging at high temperatures was performed in an environmental chamber (Okolab). For imaging of Hela cells, cells were grown on glass-bottomed dishes and washed into prewarmed DMEM without glucose, glutamine, or phenol red (Life technologies) added with 25 mM HEPES (pH=7.4) and other supplements as indicated. Cells were imaged at 37 ^{o}C in the environmental chamber.

FRAP experiment was performed with an argon laser and mosaic digital illumination system (Andor Scientific) as described [S2]. For FRAP of the buds, cells with medium-sized bud were selected and the central plane of the confocal z-section containing the bud was

photobleached for 1000 ms. The recovery of Myo2-GFP fluorescence intensity in the bud was imaged every 2 seconds afterwards. Summed projection of six confocal slices 0.4 μm apart at an exposure of 50 ms was created for analysis. The prebleach GFP intensity of the daughter cells was normalized for comparison between cells. A nearby cell was used for photobleaching correction. Experiments with glucose-depleted cells were always performed within 10 minutes after glucose withdrawal. For FRAP of Myo2-decorated actin cables, cells expressing Myo2 tagged with 3xGFP was used. Within 5 min after glucose depletion, a Myo2-3GFP fiber observed in one confocal plane were selected and a small region across the fiber was photobleached (pixel dwell time 1 μs/px) 5 second after recording and single confocal slice images were taken at an exposure of 150 ms every 0.5 second afterwards.

Measurement of ATP level

ATP concentration was measured according to [S9] to monitor the kinetics of ATP level changes upon glucose depletion. Briefly, yeast cells were grown to exponential phase. About 0.4 O.D. of cells were harvested by centrifugation, with all the supernatants completely removed and quickly resuspended in the indicated medium. At each time point, a fraction of 12.5 μl of cells was added to an equal volume of 10% trichloroacetic acid and vortexed vigorously for 1 min to extract ATP. The mixtures were then neutralized with 1 ml of neutralization buffer (25 mM Tris, pH 9.4 and 2 mM EDTA). For experiments at 35 ^{o}C, the cell pellet was quickly resuspended in SC medium prewarmed to 35 ^{o}C and incubated in a 35 ^{o}C water bath afterwards. For Hela cells, about $2x10^5$ cells were pelleted by centrifugation at 1,000 g for 3 min in an Eppendorf tube. Then after the supernatants were completely removed, the cell pellet was quickly resuspended in indicated medium prewarmed to 37°C and incubated in 37°C water bath afterwards. At each time point, a fraction of 12.5 μl of cells was added to equal volume of 5% trichloroacetic acid, vortexed for 1 min, and then neutralized by 0.5 ml of neutralization buffer. The amount of ATP was measured with the ATP determination kit (Life technologies) by mixing 10 μl of each sample with 100 μl of reaction solution according to the manufacture's instruction. The luminescence was assayed with an EnVision Multimode Plate Reader (PerkinElmer). Relative ATP concentrations are normalized and expressed as the ratios of ATP levels in pre-starved cells.

Yeast cell permeabilization

For permeabilization, mid-log phase yeast cells were first seeded onto Concanavalin A coated glass-bottomed dishes. The cells were then changed to SC medium supplemented with 15 mM ATP and 0.01% digitonin for 5-10 min. A digitonin stock solution is prepared by dissolving 5% (w/v) digitonin in ddH_2O and heated at 95 ^{o}C for 5 min. Immediately after permeabilization, the cells were transferred into indicated medium for subsequent experiments.

FM4-64 staining

Yeast cells were grown in SC to mid-log phase and seeded onto Concanavalin A coated glass-bottomed dishes. The cells were quickly transferred into to fresh SC or SC lacking glucose by three washes. FM4-64 was added to the last wash at the concentration of 32 nM.

Supplemental References

S1. Pruyne, D.W., Schott, D.H., and Bretscher, A. (1998). Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. J Cell Biol 143, 1931-1945.

S2. Donovan, K.W., and Bretscher, A. (2012). Myosin-V is activated by binding secretory cargo and released in coordination with Rab/exocyst function. Dev Cell 23, 769-781.

S3. Chernyakov, I., Santiago-Tirado, F., and Bretscher, A. (2013). Active segregation of yeast mitochondria by Myo2 is essential and mediated by Mmr1 and Ypt11. Curr Biol 23, 1818-1824.

S4. Lord, M., Laves, E., and Pollard, T.D. (2005). Cytokinesis depends on the motor domains of myosin-II in fission yeast but not in budding yeast. Mol Biol Cell 16, 5346-5355.

S5. Sherman, F. (1991). Getting started with yeast. Methods Enzymol 194, 3-21.

S6. Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast 14, 953-961.

S7. Shaner, N.C., Campbell, R.E., Steinbach, P.A., Giepmans, B.N., Palmer, A.E., and Tsien, R.Y. (2004). Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nat Biotechnol 22, 1567-1572.

S8. Mumberg, D., Muller, R., and Funk, M. (1995). Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene 156, 119-122.

S9. Aoh, Q.L., Hung, C.W., and Duncan, M.C. (2013). Energy metabolism regulates clathrin adaptors at the trans-Golgi network and endosomes. Mol Biol Cell 24, 832-847.