Supplementary Information

The myosin V motor requires the Rab GTPases Ypt31/32 for its cellular localization.

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Table S1. Yeast strains and plasmids used in this study

A. Strains

Strain	Alias	Genotype	Source*
NSY468	PJ69-4A	*MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ gal2-ade2 lys2::gal1-his3 met2::gal7-lacZ*	(James *et al.*, 1996)
NSY752	PJ69-4α	*MATα trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ gal2-ade2 lys2::gal1-his3 met2::gal7-lacZ*	(James *et al.*, 1996)
NSY125	DBY1034	*MATa his4-539 lys2-801 ura3-52*	(Jedd *et al.*, 1997)
NSY348	Ypt31Δ/32ts	NSY125 *ypt31Δ::HIS3 ypt32-A141D*	(Jedd *et al.*, 1997)
NSY160		*MATα lys2-801 ura3-52 his4-539*	(Jedd *et al.*, 1995)
NSY161	Ypt1ts	NSY160 *ypt1-A136D*	(Jedd *et al.*, 1995)
NY786	Sec15ts	MATa, *ura3-52 his4-619 sec15-1*	(Gimeno *et al.*, 1995)
NSY1175	LWY2947	*MATa ura3-52 leu2-3,112 his3-Δ200 trp1-901 lys2-801 suc2-Δ9 myo2Δ::TRP1,* p*MYO2*	(Catlett and Weisman, 1998)
NSY1168	LWY3225	LWY2947+pRS413-*MYO2*	(Catlett and Weisman, 1998)
NSY1169	LWY7488	LWY2947+pRS413-*MYO2-Y1415E*	(Pashkova *et al.*, 2006)
NSY1171	LWY7522	LWY2947+pRS413-*MYO2-K1444A*	(Pashkova *et al.*, 2006)
NSY1172	LWY7523	LWY2947+pRS413-*MYO2-L1331S*	(Pashkova *et al.*, 2006)
NSY1173	LWY7524	LWY2947+pRS413-*MYO2-Q1447R*	(Pashkova *et al.*, 2006)
NSY1174	LWY7540	LWY2947+pRS413-*MYO2-L1411S*	(Pashkova *et al.*, 2006)
NSY1268		LWY2947+pRS413-*MYO2*-HA	(Pashkova *et al.*, 2006)
NSY1269		LWY2947+pRS413-*MYO2-Y1415E*-HA	(Pashkova *et al.*, 2006)
		LWY2947+pRS413-*MYO2-Y1415R*	
		LWY2947+pRS413-*MYO2-YPT32*	
		LWY2947+pRS413-*MYO2-Y1415R-YPT32*	

B. Plasmids

Plasmid	Alias	Genotype	Source*
pNS196	pACT2	2μ, *LEU2*, Amp^r	Clontech, CA
pNS1019		pACT2-*MYO2*-GTD	
pNS1024		pACT2-*MYO2-K1444A*-GTD	
pNS1022		pACT2-*MYO2-L1331S*-GTD	
pNS1023		pACT2-*MYO2-L1411S*-GTD	
pNS1021		pACT2-*MYO2-Q1447R*-GTD	
pNS1020		pACT2-*MYO2-Y1415E*-GTD	
pNS459		pACT2-*YPT32-Q72L*	
pNS462		pACT2-*YPT32-Q72L-SS*	
pNS1078		pACT2-*YPT31*	
pNS1079		pACT2-*YPT31-Q72L*	
pNS1080		pACT2-*YPT31-S27N*	
pNS1081		pACT-*YPT31-D129N*	
pNS738		pACT2-*YPT31-SS*	
pNS448	pAS1	2μ, *TRP1*, Amp^r	(Durfee *et al.*, 1993)
pNS657		pAS1-*YPT1*	(Calero and Collins, 2002)
pNS653		pAS1-*YPT6*	(Calero and Collins, 2002)
pNS645		pAS1-*YPT31*	(Calero and Collins, 2002)
pNS647		pAS1-*YPT32*	(Calero and Collins, 2002)
pNS206	pGBDU-C2	2μ, *URA3*, Amp^r	(James *et al.*, 1996)
pNS288		pGBDU-C2-*YPT32-SS*	(Chen *et al.*, 2005)
pNS289		pGBDU-C2-*YPT32-Q72L-SS*	(Chen *et al.*, 2005)
pNS290		pGBDU-C2-*YPT32-S27N-SS*	(Chen *et al.*, 2005)
pNS291		pGBDU-C2-*YPT32-D129N-SS*	

pNS1027		pGBDU-C2-*MYO2*-GTD	
pNS255	pGEX-KG	Amp^r , GST	(Guan and Dixon, 1991)
pNS296		pGEX-KG-*YPT32*	(Jones *et al.*, 2000)
pNS734	pGEX-4T-1-*MYO2*-GTD	Amp^r , GST-*MYO2*-GTD	(Pashkova *et al.*, 2005)
pNS1082	pQE9-*YPT1*	Amp^r , HIS_6-*YPT1*	(Lupashin and Waters, 1997)
pNS619	pTrcHisA-*YPT31*	Amp^r , HIS_6-*YPT31*	
PNS620	pTrcHisA-*YPT32*	Amp^r , HIS_6-*YPT32*	
pNS563	pRS426	2μ, *URA3*	(Christianson *et al.*, 1992)
pNS940		pRS426-*MYO2*	
pNS1085		pRS426-*MYO2-L1411S*	
pNS1086		pRS426-*MYO2-Q1447R*	
pNS1087		pRS426-*MYO2-Y1415E*	
pNS274	Yep24	2μ, *URA3*	(Botstein *et al.*, 1979)
pNS489		Yep24-*YPT1*	(Morozova *et al.*, 2006)
pNS229		Yep24-*YPT31*	(Jones *et al.*, 1999)
pNS1088	pRS413	*CEN*, *HIS3*, Amp^r	(Sikorski and Hieter, 1989)
pNS1070		pRS413-*MYO2*-HA	(Pashkova *et al.*, 2006)
pNS1071		pRS413-*MYO2-Y1415E*-HA	(Pashkova *et al.*, 2006)
		pRS413-*MYO2-Y1415R*	(Pashkova *et al.*, 2006)
		pRS413-*MYO2-YPT32*	
		pRS413-*MYO2-Y1415R-YPT32*	
		GFP-SEC4	(Calero *et al.*, 2003)

*All strains and plasmids, if not marked otherwise, were constructed in this study.

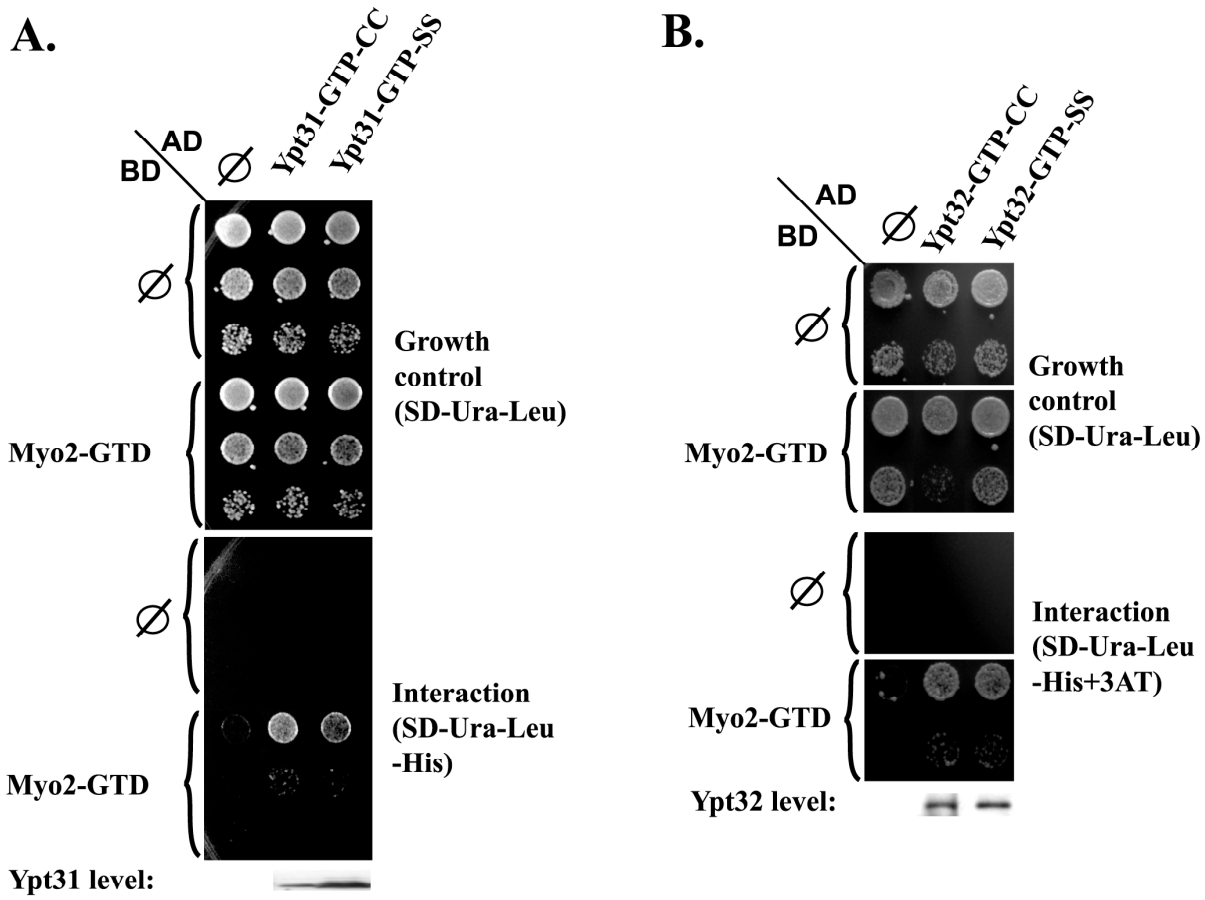

Figure S1: Prenylation of Ypt31/32 is not required for their interaction with Myo2-GTD. **A.** Ypt31 prenylation is not required for its interaction with Myo2-GTD. Replacing the two terminal cysteines (C222, C223) required for prenylation, Ypt31-CC, with serines, Ypt31-SS, does not abolish two-hybid interaction with Myo2-GTD. The experiment was done as described in Figure 1B. **B.** Ypt32 prenylation is not required for the interaction with Myo2-GTD. Replacing the two terminal cysteines (C221, C222) required for prenylation, Ypt32-GTP-CC, with serines, Ypt32-GTP-SS, does not abolish two-hybid interaction with Myo2-GTD. The experiment was done as described in Figure 1C. Results shown in this figure represent at least two experiments.

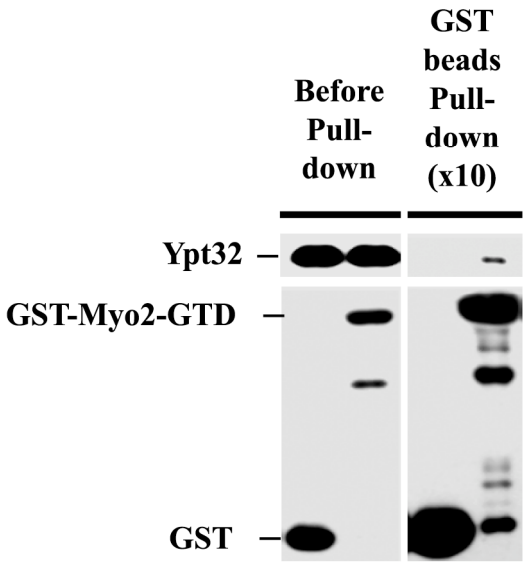

Figure S2. Direct physical interaction of bacterially expressed Ypt32 and Myo2-GTD. Bacterially expressed GST-tagged Myo2-GTD or GST, as a negative control, were bound to glutathione sepharose beads and mixed with purified bacterially expressed Ypt32 protein (in buffer B, see Methods). After incubation of the interaction mix (left), the GST-Myo2-GTD and GST were precipitated (right) and their presence was detected using immuno-blot analysis and anti-GST antibodies (bottom). Co-precipitation of Ypt32 was detected using immuno-blot analysis and anti-Ypt31/32 antibodies (top). Ypt32 co-precipitates with GST-Myo2-GTD, but not with GST. Results shown in this figure represent two independent experiments.

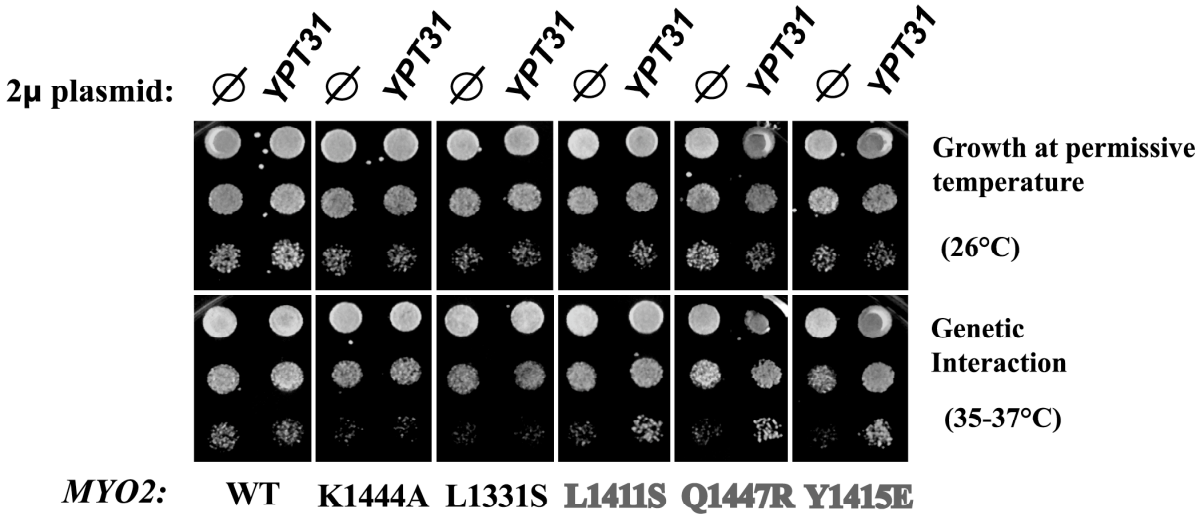

Figure S3. Ypt31 over-expression suppression effect on *myo2* mutant cells is specific to *myo2* alleles compromised in Ypt31-interaction. The experiment was done as explained in Figure 5C legend, except that different *MYO2* alleles were used. For each myo2 mutant strain, the empty vector control (Botstein *et al*.) is shown on the left, and the over-expression of Ypt31 is shown on the right. The growth phenotype of Myo2 alleles that do not disrupt the Ypt31-Myo2-GTD yeast-two-hybrid interaction (Figure 3B) cannot be suppressed by over-expression of Ypt31. In contrast, alleles that compromise the interaction can be suppressed by over-expression of Ypt31. Results shown in this figure represent at least two experiments.

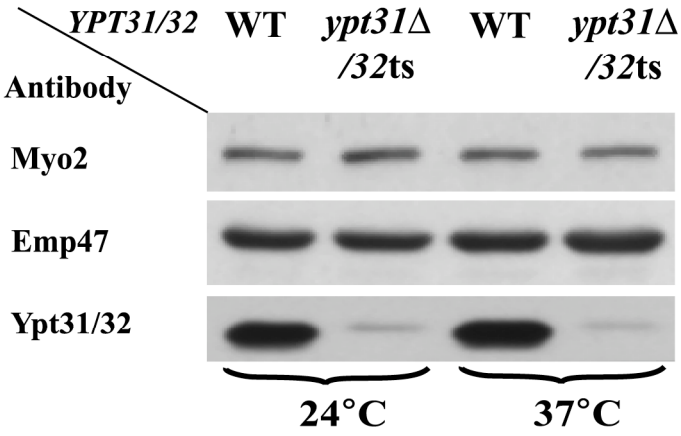

Figure S4. The protein level of Myo2 does not change in *ypt31Δ/32ts* mutant cells. Cells were grown as described in the legend for Figure 6A and cell lysates were prepared as described in Methods. The level of Myo2, EMP47 (as a loading control) and Ypt32 was determined using immuno-blot analysis and specific antibodies. In *ypt31Δ/32ts* mutant cells the level of Myo2 does not change, whereas the level of Ypt32 is reduced.

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