

Donovan and Bretscher

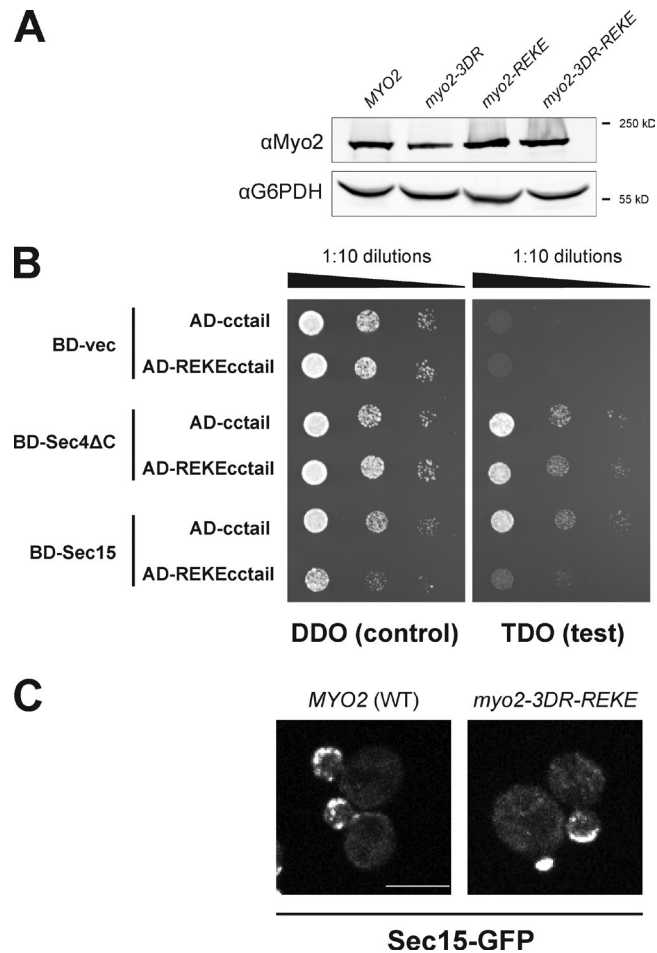


Figure S1. **Charge mutant strains are expressed similarly to wild-type Myo2p and affect Sec15p binding and localization as expected.** (A) Myo2p expression levels in wild-type, *myo2-3DR*, *myo2-REKE*, and *myo2-3DR-REKE* strains. The glucose-6-phosphate dehydrogenase Zwf1p was used as a loading control. (B) Yeast two-hybrid interactions of Myo2p or Myo2-REKEp coiled-coil + tail regions (residues 925–1575) fused to the activation domain (AD), with Sec4ΔCC or Sec15p fused to the binding domain (BD). Sec4p is truncated to remove the C-terminal CXC motif to prevent prenylation. 1:10 serial dilutions were spotted on double-dropout (DDO) SD-Leu-Trp media as a control or triple dropout (TDO) SD-Leu-Trp-His media to test the interaction. Wild-type and Myo2-REKEp cctail constructs can bind to Sec4p, but the Myo2-REKEp cctail is deficient in Sec15p binding. (C) Sec15-GFP localization in wild-type and *myo2-3DR-REKE* strains. Bar, 5 μm.

Table S1. Summary of yeast strains

Strain	Alias	Genotype	Source
ABY1655	BY4741	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	C. Boone ^a
ABY1656	BY4742	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	C. Boone
ABY3587	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR::HIS3</i>	This study
ABY3588	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-REKE::HIS3</i>	This study
ABY3589	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR-REKE::HIS3::LEU2</i>	This study
ABY3590	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR-GFP::URA3</i>	This study
ABY3591	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-REKE-GFP::URA3</i>	This study
ABY3592	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR-REKE-GFP::HIS3::URA3</i>	This study
ABY3410	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 GFP-SEC4::URA3</i>	This study
ABY3597	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR::HIS3 GFP-SEC4::URA3</i>	This study
ABY3598	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-REKE::HIS3 GFP-SEC4::URA3</i>	This study
ABY3599	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR-REKE::HIS3::LEU2 GFP-SEC4::URA3</i>	This study
ABY3593	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 mitoRFP::URA3</i>	This study
ABY3594	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR::HIS3 mitoRFP::URA3</i>	This study
ABY3595	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-REKE::HIS3 mitoRFP::URA3</i>	This study
ABY3596	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR-REKE::HIS3::LEU2 mitoRFP::URA3</i>	This study
ABY3414	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 SEC15-GFP::kanMX6</i>	This study
ABY3702	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR-REKE::HIS3::LEU2 SEC15-GFP::kanMX6</i>	This study
ABY126	NY17	MATα <i>ura3-52 sec6-4</i>	P. Novick ^b
ABY3577	NA	MATa <i>ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 MYO2-2IQ-GFP::URA3</i>	This study
ABY3578	NA	MATa <i>ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 MYO2-4IQ-GFP::URA3</i>	This study
ABY3579	NA	MATa <i>ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 MYO2-6IQ-GFP::URA3</i>	This study
ABY3580	NA	MATa <i>ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 MYO2-8IQ-GFP::URA3</i>	This study
ABY3704	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 GFP-Tub1::URA3</i>	This study
ABY3705	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR::HIS3 GFP-Tub1::URA3</i>	This study
ABY3706	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-REKE::HIS3 GFP-Tub1::URA3</i>	This study
ABY3707	NA	MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR-REKE::HIS3::LEU2 GFP-Tub1::URA3</i>	This study

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