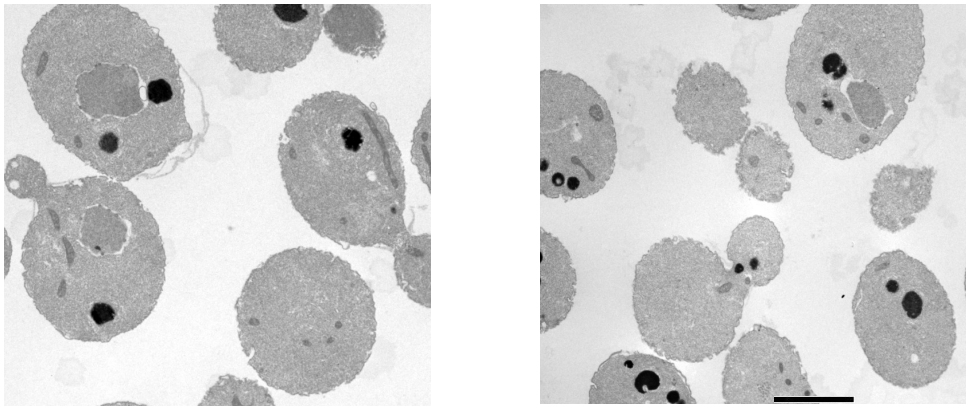
A.

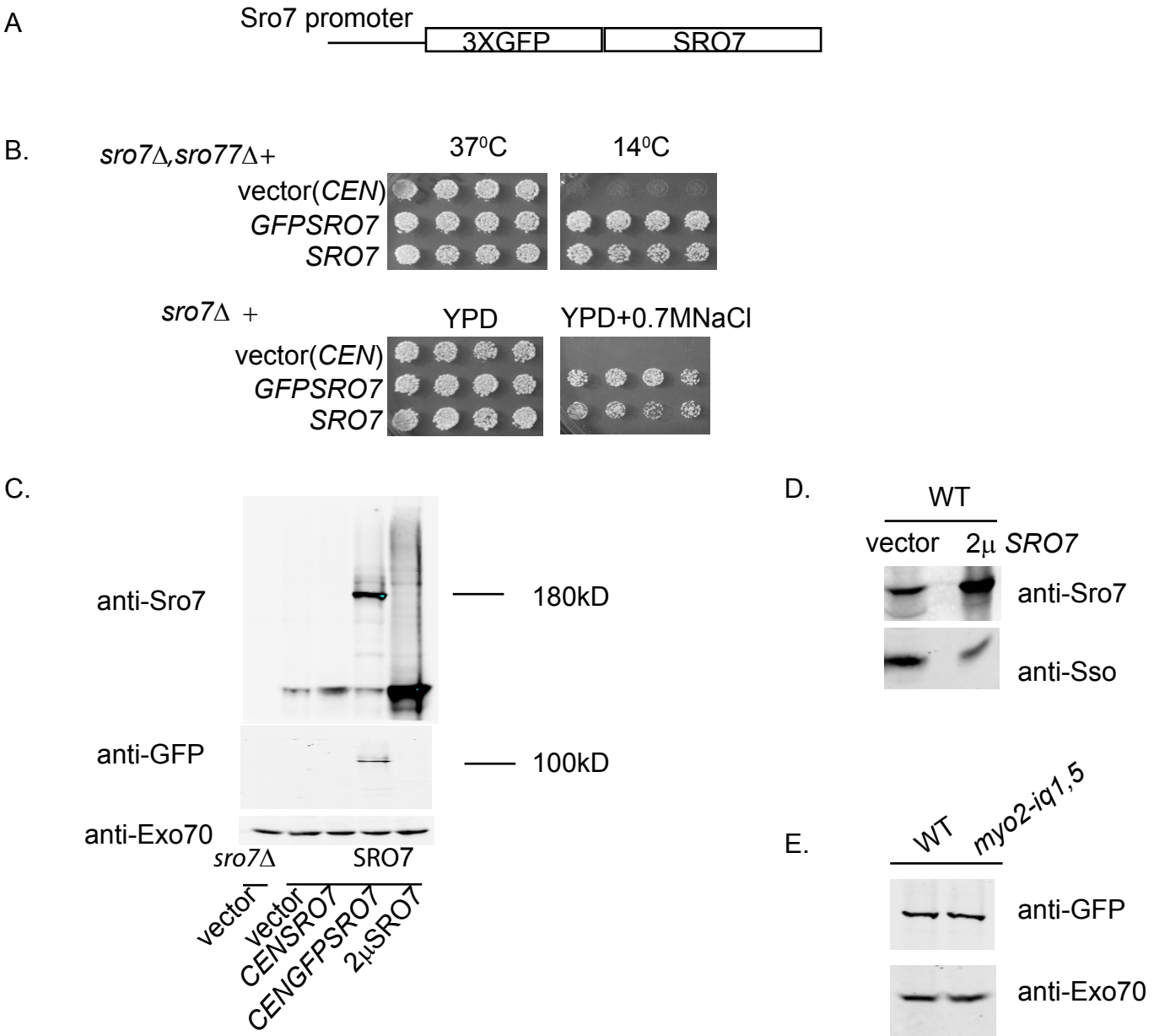
WT

YPD 32°C

YPD 25°C

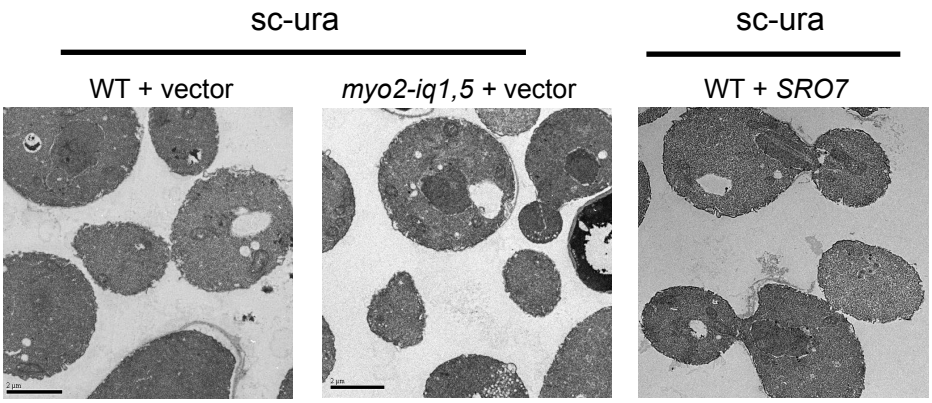


Supplemental Figure 1. Control cells for electron microscopy seen in Figure 2C. Wild type and *myo2-iq1,5* cells were grown overnight at 32°C and then shifted to 25°C for 8 hours before fixing and processing for electron microscopy. Bar 1 micron.

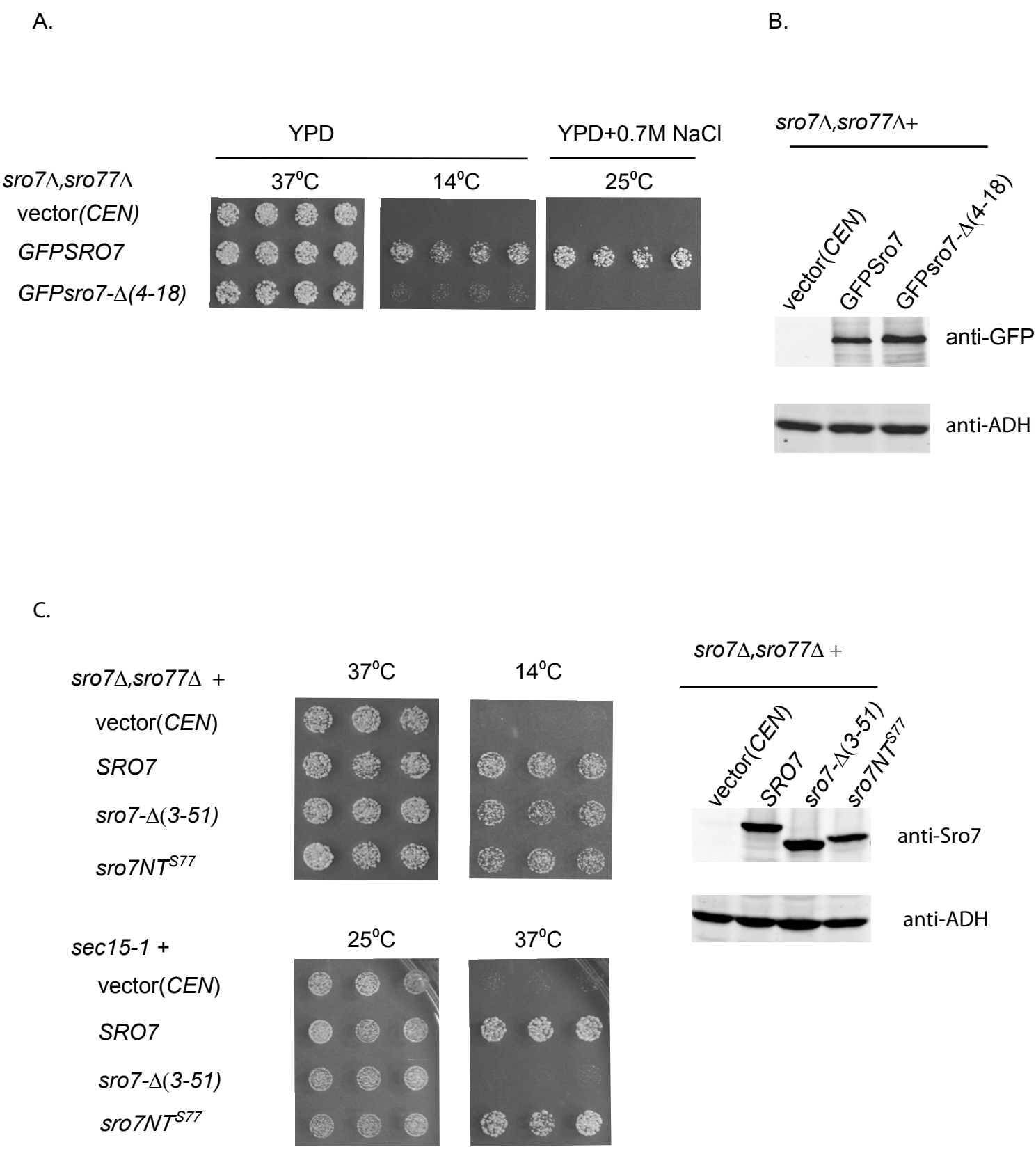


Supplemental Figure 2. N-terminally tagged GFP-Sro7 is functional. (A) A schematic of Sro7 tagged with 3 tandem copies of GFP at the N-terminus. (B)*sro7*Δ,*sro77*Δ and *sro7*Δ mutant cells were transformed with *GFP*SRO7 (*CEN*), SRO7 (*CEN*) or an empty vector. Individual transformants were picked into microtiter wells and transferred onto SC-ura plates at the indicated temperatures and salt concentrations.(C) Glass bead lysates and Western blot analysis of sro7Δ and wild type strains expressing GFP-Sro7 (*CEN*), Sro7 (*CEN*) or Sro7 (2μm) using affinity purified anti-Sro7, anti-GFP and control anti-Exo70 antibodies. (D) Glass bead lysates of wild type cells expressing *SRO7*(2μ) or vector only using anti Sro7 and control anti-Sso antibodies show Sro7 is expressed 9.5 times above endogenous levels.Glass bead lysates of WT and *myo2-iq1,5* mutant cells expressing GFP-Sro7 (*CEN*) were subjected to Western blot analysis using anti-GFP and control affinity purified anti-Exo70 antibodies.(E) Wild type, *myo2-iq1* and *myo2-iq1,5* were transformed with GFP-SRO7(CEN). Transformants analysed for GFP-Sro7 fluorescence at sites of polarized growth showed polarized Sro7 fluorescence for *MYO2 and myo2-iq1.*

A.



Supplemental Figure 3. Control cells for electron microscopy seen in Figure 5C. Wild type and *myo2-iq1,5* mutant cells expressing Sro7 behind a tetracycline repressible promoter (*CEN*) were transformed with *GFP-SEC4* (*CEN*). Cells were grown in selective media in the presence of doxycycline and then shifted to media lacking doxycycline to induce Sro7 expression for 8 hours at 25^{0}C.



Supplemental Figure 4. (A) Mutant *sro7*Δ,*sro77*Δ cells were transformed with full length and the N-terminal deletion GFP construct. Transformants were picked into microtiter wells and transferred onto plates at the indicated temperatures and salt concentrations. (B) Glass bead lysates and Western blot analysis of *sro7*Δ,*sro77*Δ cells expressing GFP-Sro7 and GFP-sro7-Δ(4-18) were conducted using anti-GFP antibody and anti-ADH antibody as a control. (C) Mutant *sro7*Δ,*sro77*Δ and *sec15-1* cells were transformed with a (*CEN*) plasmid expressing Sro7, sro7-Δ(3-51) and Sro7NT^{S77}.Transformants were picked into microtiter plates and transferred onto plates at the indicated temperatures. Glass bead lysates and Western blot analysis of *sro7*Δ,*sro77*Δ cells expressing Sro7, sro7-Δ(3-51) and Sro7NT^{S77} were done using anti-Sro7 antibody and anti-ADH antibody as a control.

Supplemental Table I: Yeast strains used in paper

Strain	Genotype	Reference
BY2	*ura3-52*	P.B.collection
BY40	*ura3-52*; *sec8-9*	P.B.collection
BY44	*ura3-52*; *sec15-1*	P.B.collection
BY100	*LEU2*::*sec4P-48*; *sec4Δ*::*HIS3*; *his3-Δ200*; *ura3-52*	P.B.collection
BY706	*sro7Δ*::*LEU2*; *ura3-52*; *leu2-3,112*; *his3-Δ200*	P.B.collection
BY704	*SRO7*; *ura3-52*; *leu2-3,112*; *his3-Δ200*	P.B.collection
BY855	*sro7Δ*::*LEU2*; *sro77Δ*::*HIS3*; *ura3-52*; *leu2-3,112*; *his3-Δ200*	P.B.collection
BY1101	*sro7Δ*::*LEU2*; *sro77Δ*::*HIS3*; *ura3-52*; *his3-Δ200*; *leu2-3,112*;*ADH1*p-protASro7 (2μ, *URA3*)	P.B.collection
BY1291	*sro7Δ*::*LEU2*; *sro77Δ*::*HIS3*; *ura3-52*; *his3-Δ200*; *leu2-3,112*; *ADH1*p-protA (2μ, *URA3*)	P.B.collection
BY1660	*MYO2*::kanR; *ura3-52*; *leu2-3,112*	This study
BY1661	*myo2-iq1*::kanR; *ura3-52*; *leu2-3,112*	This study
BY1662	*myo2-iq5*::kanR; *ura3-52*; *leu2-3,112*	This study
BY1663	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*	This study
BY1673	*myo2-iq4*::kanR; *ura3-52*; *leu2-3,112*	This study
BY1674	*myo2-iq6*kanR; *ura3-52*; *leu2-3,112*	This study
BY1746	*MYO2*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*	This study
BY1748	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*	This study
BY1839	*ura3-52*; *leu2-3,112*; *GFP-SRO7*(pB1564)	This study
BY1866	*ura3-52*; *sec6-4*; *GFP-SRO7*(pB1564)	This study
BY1872	*ura3-52*; *leu2-3,112*; *myo2-66*; *GFP-SRO7*(pB1564)	This study
BY1901	*ura3-52*; *leu2-3,112*; *GFP-SEC4*(pB1571); *RFP-SEC2*(pB1566)	This study
BY1908	*ura3-52*; *leu2-3,112*; *GFP-SRO7*(pB1763); *RFP-SEC2*(pB1566)	This study
BY1951	*ura3-52*; *sec2-41*; *GFP-SRO7*(pB1564)	This study
BY1991	*MYO2*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-SRO7*(pB1564)	This study
BY1992	*MYO2*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-SEC4*(pB1571)	This study
BY1993	*MYO2*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-MLC1*(pB1544)	This study
BY1994	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-SRO7*(pB1564)	This study
BY1995	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-SEC4*(pB1571)	This study
BY1996	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-MLC1*(pB1544)	This study
BY2026	*myo2-iq3*::kanR; *ura3-52*; *leu2-3,112*	This study

BY2034	*MYO2*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-SEC4*(pB1571); pCM189 (pB1105)	This study
BY2035	*MYO2*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-SEC4*(pB1571); *SRO7*-pCM189 (pB1107)	This study
BY2036	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-SEC4* (pB1571); pCM189 (pB1105)	This study
BY2037	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *GFP-SEC4* (pB1571); *SRO7*-pCM189 (pB1107)	This study
BY2197	*myo2-iq2*::kanR; *ura3-52*; *leu2-3,112*	This study
BY2250	Mata/α *MYO2*/*MYO2*::*myo2-Δ6IQ*natR; *ura3-52*/*ura3-52*; *leu2-3,112* /*leu2-3,112*	This study
BY2315	*MYO2*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; pCM189 (pB1105)	This study
BY2316	*MYO2*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *SRO7*-pCM189 (pB1107)	This study
BY2318	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; pCM189 (pB1105)	This study
BY2319	*myo2-iq1,5*::kanR; *ura3-52*; *leu2-3,112*; *his3-Δ200*; *SRO7*-pCM189 (pB1107)	This study
BY2368	*Gal*$^+$ *ura3-52*; *leu2-3,112*; *his3-Δ200*; *SRO7* in pNB527 (pB1850)	This study
BY2369	*Gal*$^+$ *ura3-52*; *leu2-3,112*; *his3-Δ200*; *SEC15* in pNB527 (pB1851)	This study
BY2370	*Gal*$^+$ *sec2-41*; *leu2-3,112; SRO7 in pNB527 (pB1850)*	This study
BY2371	*Gal*$^+$ *sec9-4; leu2-3,112*; *SRO7* in pNB527 (pB1850)	This study
BY2375	*Gal*$^+$ *ura3-52*; *leu2-3,112*; *his3-Δ200*; pNB527(pB24)	This study
BY2376	*Gal*$^+$ *sec2-41*; *leu2-3,112*; pNB527(pB24)	This study
BY2377	*Gal*$^+$ *sec9-4*; *leu2-3,112*; pNB527(pB24)	This study
BY2378	*Gal*$^+$ *sec4-8*; *leu2-3,112*; pNB527(pB24)	This study
BY2379	*Gal*$^+$ *sec2-41*; *leu2-3,112*; *SEC15* in pNB527(pB1851)	This study
BY2380	*Gal*$^+$ *sec9-4*; *leu2-3,112*; *SEC15* in pNB527(pB1851)	This study
BY2381	*Gal*$^+$ *sec4-8*; *leu2-3,112*; *SRO7* in pNB527(pB1850)	This study
BY2382	*Gal*$^+$ *sec4-8*; *leu2-3,112*; *SEC15* in pNB527(pB1851)	This study
BY2583	*ura3-52*; pB1946 (*GFP-SRO7Δ4-18*)	This study

Supplemental Table II. Plasmids used in this study

Strain	Host	Description
pB497	DH5α	*SRO7* in pRS426 (2μ, *URA3*)
pB694	DH5α	*MYO2* in YCp50(*CEN*,*URA3*) L.Weisman
pB695	DH5α	*MYO2* in pRS413(2μ, *HIS3*) as a ClaI fragment L. Weisman
pB741	DH5α	*SRO7* in pRS313 (*CEN, HIS3*)
pB1105	DH5α	pCM189 (*CEN*,*URA3*)
pB1107	DH5α	*SRO7* in pCM189 (*CEN*,*URA3*) as BamHI-NotI subclone
pB1176	DH5α	*MYO2-Δ6IQ* in pRS172 T.Davis
pB1240	BL21	*MLC1* in pGEX4T1 as a BamHI-SalI subclone
pB1211	BL21	*SEC9* in pGEX4T1 as BamHI-XhoI subclone
pB1214	BL21	*MYO2IQ* in pGEX4T1 as BamHI-Sal subclone
pB1242	DH5α	*2XGFP* L.Shenkman
pB1486	DH5α	*MYO2* in pRS306 (*URA3,*integrative) as Sal1-Not1(Cla1)fragment
pB1487	DH5α	*myo2-iq1* in pB1486 (*MYO2*, *URA3*, integrative) as EcoRI-BamHI
pB1488	DH5α	*myo2-iq5* in pB1486 (*MYO2*, *URA3*, integrative) as EcoRI-BamHI
pB1489	DH5α	*myo2-iq1,iq5* in pB1486 (*MYO2*, *URA3*, integrative) as EcoRI-BamHI
pB1490	DH5α	*myo2-iq2* in pB1486 (*MYO2*, *URA3*, integrative) as EcoRI-BamHI
pB1491	DH5α	*myo2-iq3* in pB1486 (*MYO2*, *URA3*, integrative) as EcoRI-BamHI
pB1492	DH5α	*myo2-iq4* in pB1486 (*MYO2*, *URA3*, integrative) as EcoRI-BamHI
pB1493	DH5α	*myo2-iq6* in pB1486 (*MYO2*, *URA3*, integrative) as EcoRI-BamHI
pB1544	DH5α	*GFP-MLC1* in pUG34 (*CEN*, *HIS*) E.Bi
pB1564	DH5α	*3XGFP-SRO7* in pRS316 (*CEN*, *URA3*)
pB1561	DH5α	*SRO7*(Δ3-52) in pCM189 (*URA*, *CEN*)
pB1566	DH5α	*RFP-SEC2* in pRS316 (*CEN*, *URA3*) R. Collins
pB1571	DH5α	*GFP-SEC4* in pRS315 (*CEN*, *LEU2*) R. Collins
pB1763	DH5α	*3XGFP-SRO7* in pRS315 (*CEN*, *LEU2*)
pB1816	DH5α	*SEC15* in pCM189 (*CEN*, *URA3*) as BamHI-Not I

pB1850	DH5α	*SRO7* in pNB527 (*GAL*, *LEU2*, integrative) as BamHI-HindIII
pB1851	DH5α	*SEC15* in pNB527 (*GAL*, *LEU2*, integrative) as BamHI-HindIII
pB1946	DH5α	*3XGFP-SRO7* (Δ4-18) in pRS316 (*CEN*, *URA3*)
pB1947	DH5α	*SRO7* (Δ4-18) in pCM189 as BamHI-Not I
pB1976	DH5a	Myo2-IQ (782-990) in pGEX6P-1 as BamHI-SalI
pB1980	DH5a	Myo2-IQ1,5 (782-990) in pGEX6P-1 as BamHI-SalI