Supplementary Information

Structural basis for myosin V discrimination between distinct cargoes

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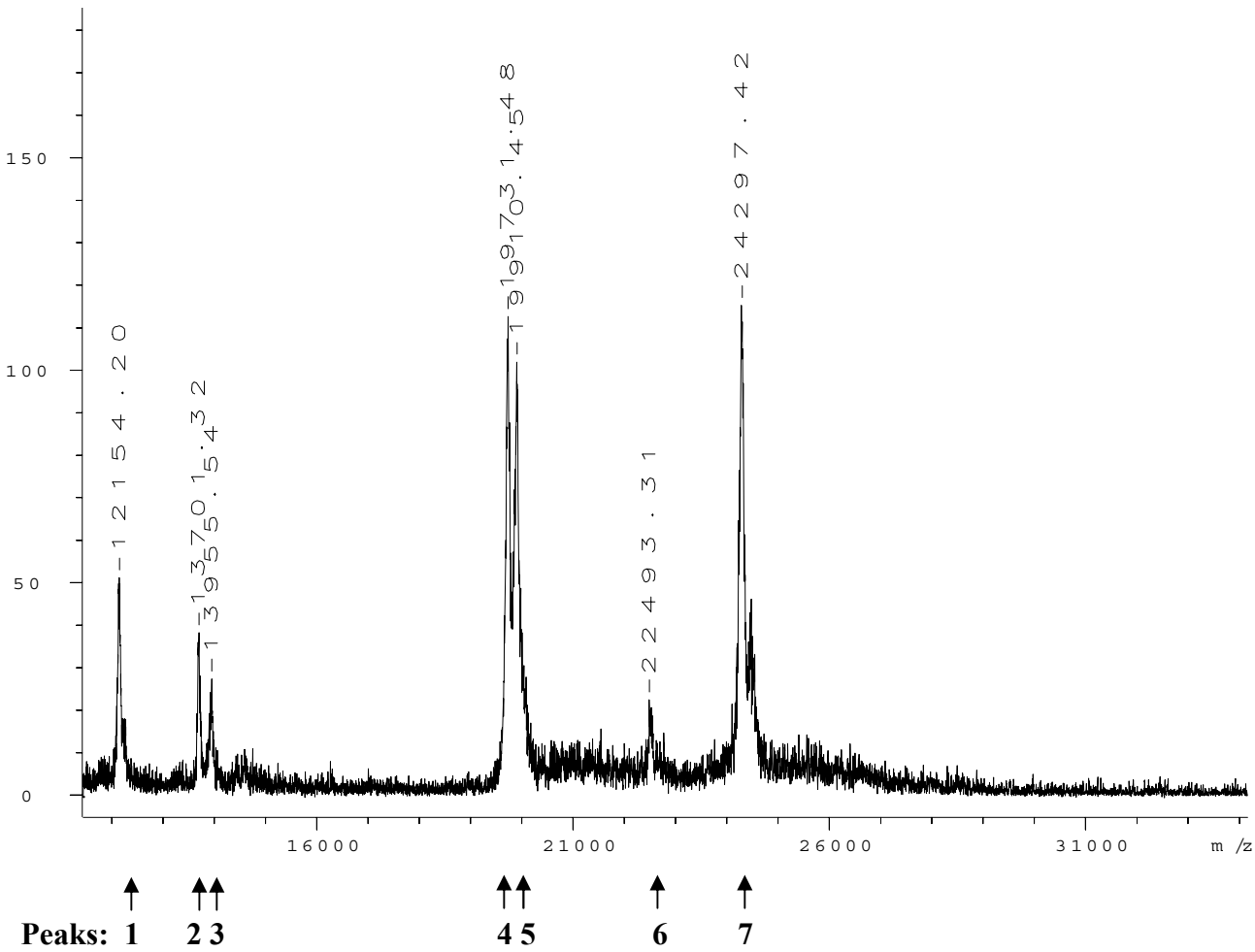
Figure S1. MALDI-TOF mass spectrometry analysis of the crystallized SeMet-Myo2p tail sample.



Amino acid sequence of the Myo2p tail

1131 STPSSGNNHIDSLSVDRENGVNATQINEELYRLLEDTEILNQEITEGLLKGFEVPDAGVA

1191 IQLSKRDVVYPARILIIVLSEMWRFGLTKQSESFLAQVLTTIQKVVTQLKGNDLIPSGVF

1251 WLANVRELYSFVVFALNSILTEETFKNGMTDEEYKEYVSLVTELKDDFEALSYNIYNIWL

1311 KKLQKQLQKKAINAVVISESLPGFSAGETSGFLNKIFANTEEYTMDDILTFFNSIYWCMK

1371 SFHIENEVFHAVVTTLLNYVDAICFNELIMKRNFLSWKRGLQLNYNVTRLEEWCKTHGLT

1431 DGTECLQHLIQTAKLLQVRKYTIEDIDILRGICYSLTPAQLQKLISQYQVADYESPIPQE

1491 ILRYVADIVKKEAALSSSGNDSKGHEHSSSIFITPETGPFTDPFSLIKTRKFDQVEAYIP

1551 AWLSLPSTKRIVDLVAQQVVQDGH

Likely peptides (underlined) that correspond to the peaks from MALDI-TOF MS analysis:

Peak 1 – 12154.20 Da
Peptide - 12193.52 Da (underlined in dashed **black**)

Peak 2 - 13701.32 Da
Peptide - 13700.56 Da (underlined in dashed **pink**)

Peak 3 - 13955.54 Da
Peptide - 13962.92 Da (underlined in **pink**)

Peak 4 - 19731.28 Da
Peptide - 19698.08 Da (underlined in **green**)

Peak 5 - 19910.45 Da
Peptide - 19896.47 Da (underlined in dashed **green**)

Peak 6 - 22493.31 Da
Peptide - 22515.43 Da (underlined in **blue**)

Peak 7 - 24297.42 Da
Peptide - 24283.26 Da (underlined in **black**)

Residues observed in the crystal structure are highlighted in purple.

R1147, K1276, K1345, and K1513 reside within the missing regions, are likely exposed on the surface, and are likely sites of trypsin digestion. Polypeptides derived from these sites likely correspond to peaks 3, 4, 6, and 7. Peaks 1,2, and 5 likely correspond to minor products of partial proteolysis at the surface exposed K1319 and R1449. Note that some of the residues missing from the structure are present in the detected polypeptides, and therefore are likely missing from the structure due to flexibility in the protein. Other residues missing from the structure are likewise missing from the peptides and were likely digested during the mild proteolysis, or subsequent incubation in the crystallization buffer.

Polypeptides with a MW lower than 10 kDa would not be detectable under the conditions used for MALDI-TOF MS sample preparation.

Figure S2. Vacuole and secretory vesicle movement in *myo2* mutants with truncations at the C terminus. (**A**) *myo2*-Δ(1551-1574) is temperature sensitive. (**B**) Quantitative analysis of vacuole inheritance in *myo2* truncation mutants. Data are presented as the percent of budded cells with inherited vacuoles. (**C**) All *myo2* mutants had similar levels of protein as assessed by Western blot analysis using mouse anti-HA tag antibodies. Mouse anti-PGK antibodies (loading control).

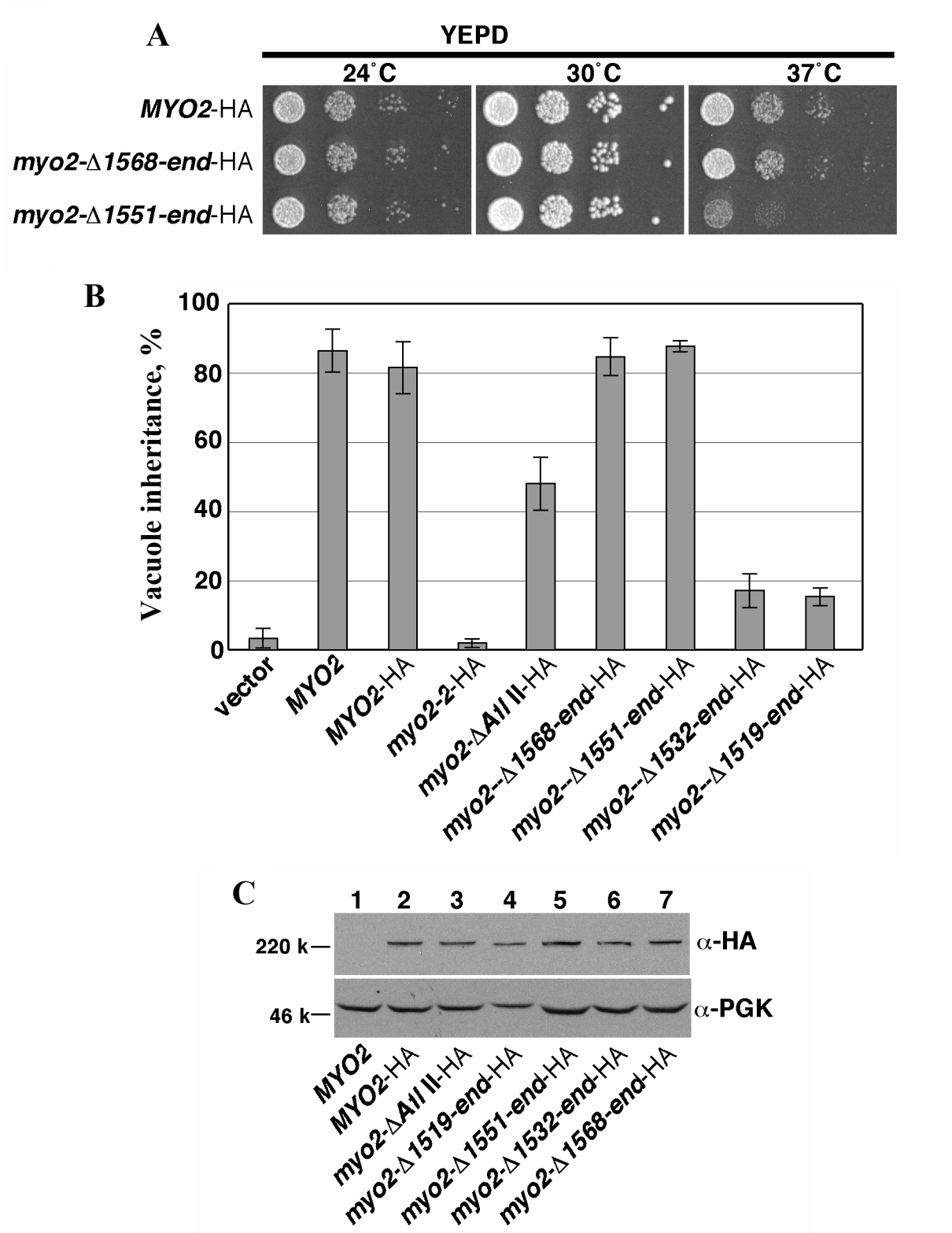
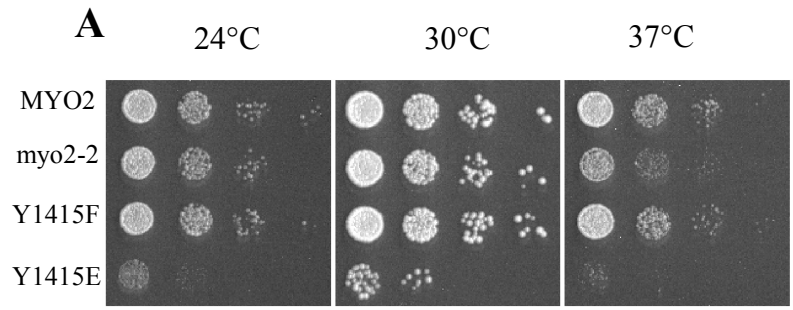
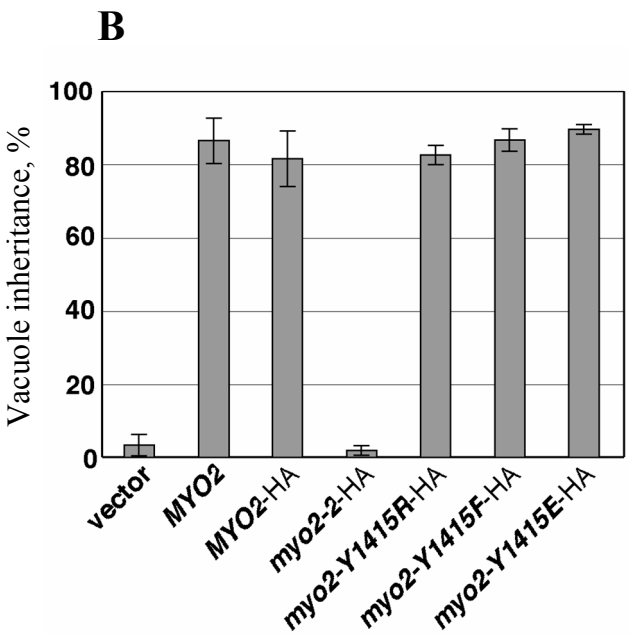


Figure S3. Residue Y1415 is part of a secretory vesicle – binding region (**A**) *myo2-Y1415E* has a severe growth defect at 24°C. (**B**) Quantitative analysis of vacuole inheritance. Data are presented as a percentage of budded cells with inherited vacuoles. (**C**) All *myo2* mutants had similar levels of protein expression as assessed by Western blot analysis using mouse anti-HA tag antibodies. Mouse anti-PGK antibodies (loading control).





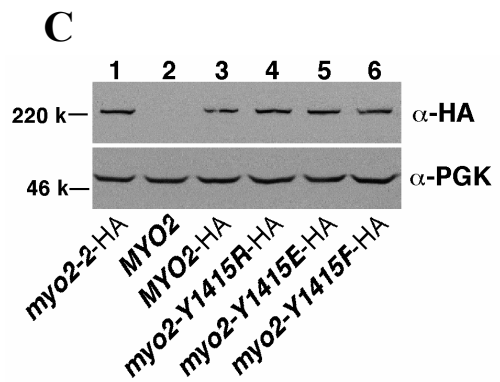
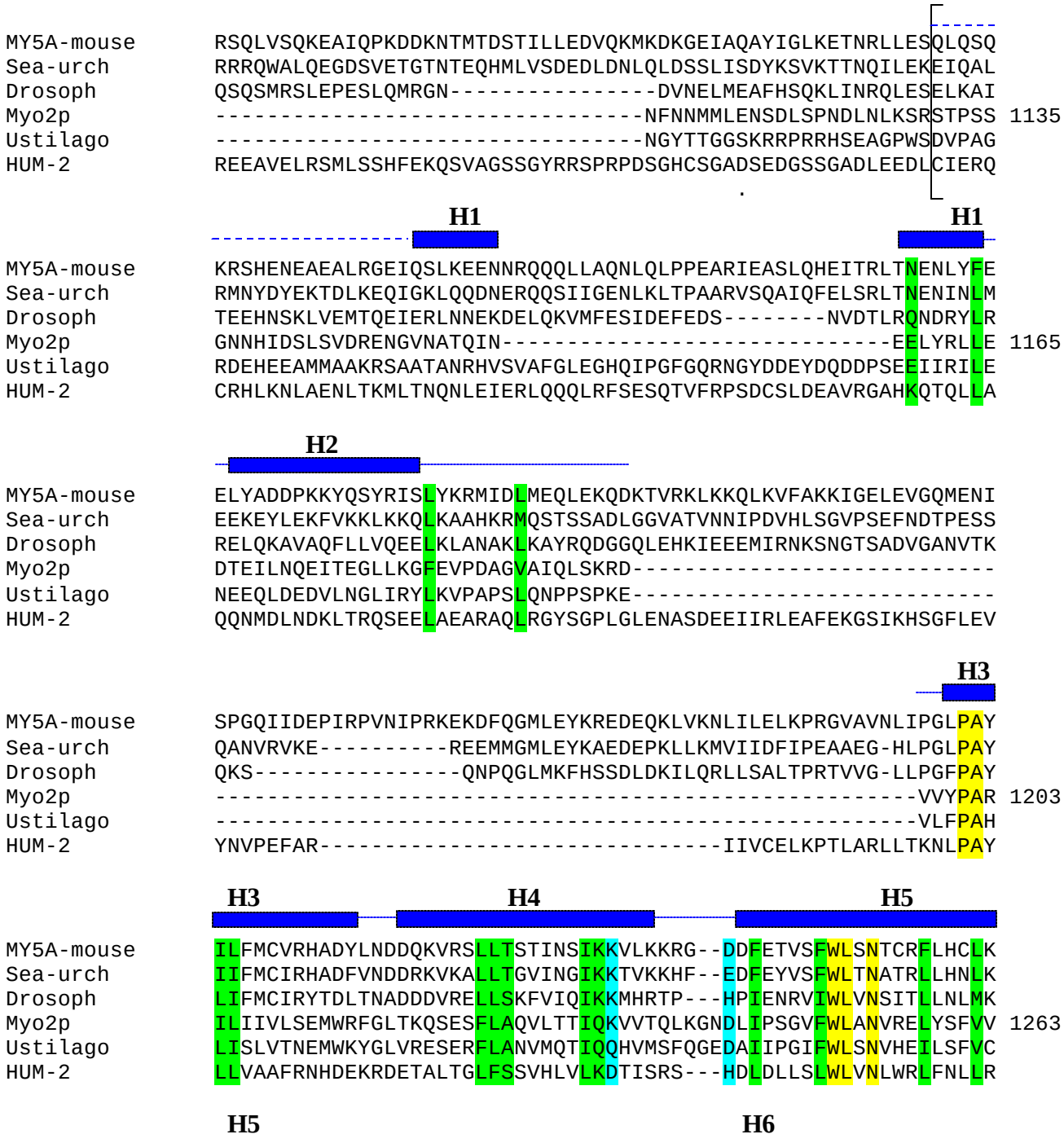


Figure S4. Sequence alignment of the myosin V globular tail domain.

Full-length myosin Vs were aligned using ClustalW. α-helices are shown as rectangles, disordered residues as broken lines, subdomain I colored in blue, and subdomain II colored in red. Identical residues are shaded in yellow, residues with strong similarity in green and with weak similarity in light blue.



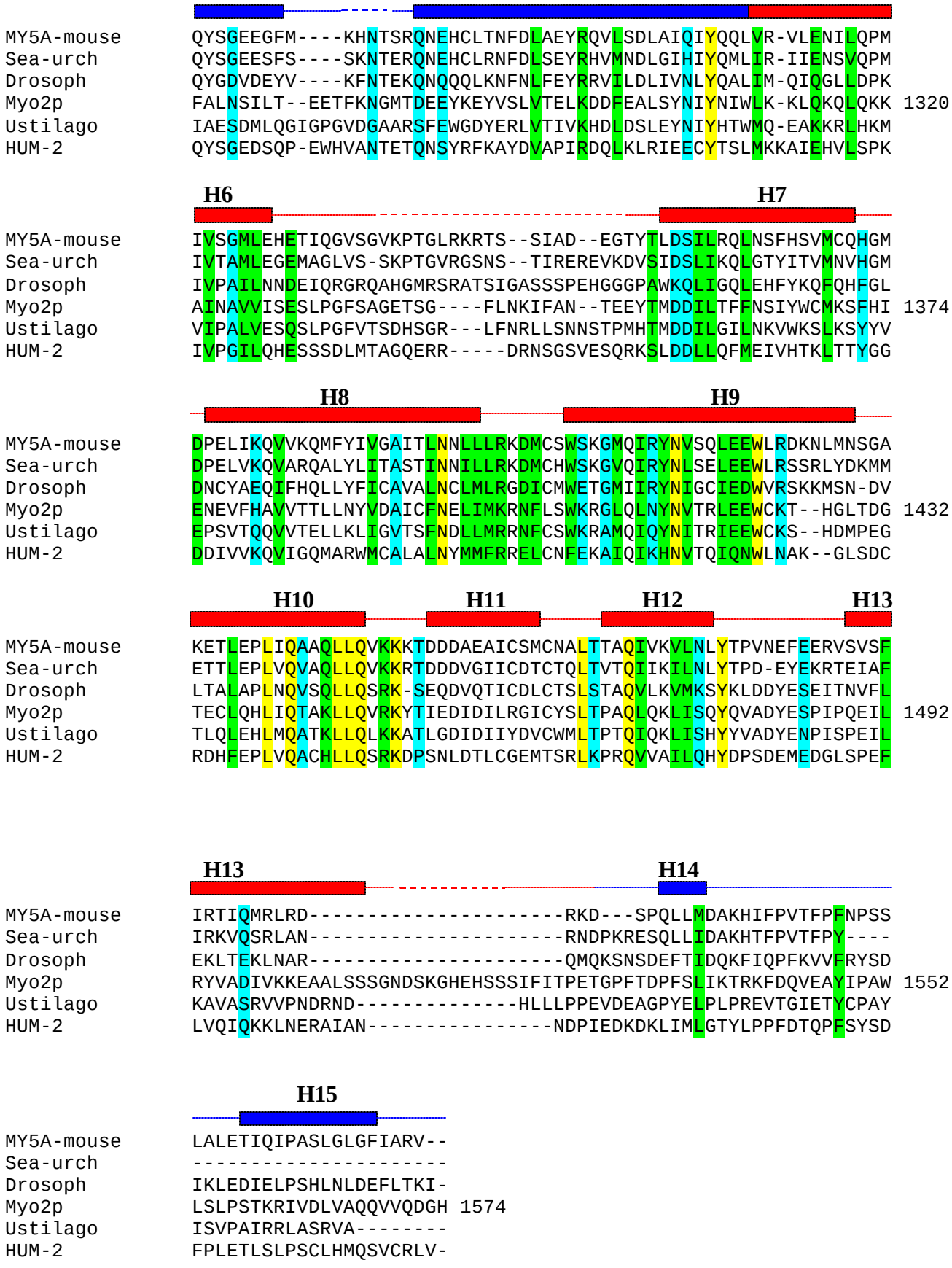

MY5A-mouse QYSGEEGFM----KHNTSRQNEHCLTNFDLAEYRQVLSDLAIQIYQQLVR-VLENILQPM
Sea-urch QYSGEESFS----SKNTERQNEHCLRNFDLSEYRHVMNDLGIHIYQMLIR-IIENSVQPM
Drosoph QYGDVDEYV----KFNTEKQNQQQLKNFNLFEYRRVILDLIVNLYQALIM-QIQGLLDPK
Myo2p FALNSILT--EETFKNGMTDEEYKEYVSLVTELKDDFEALSYNIYNIWLK-KLQKQLQKK 1320
Ustilago IAESDMLQGIGPGVDGAARSFEWGDYERLVTIVKHDLDSLEYNIYHTWMQ-EAKKRLHKM
HUM-2 QYSGEDSQP-EWHVANTETQNSYRFKAYDVAPIRDQLKLRIEECYTSLMKKAIEHVLSPK
H6
H7
MY5A-mouse IVSGMLEHETIQGVSGVKPTGLRKRTS--SIAD--EGTYTLDSILRQLNSFHSVMCQHGM
Sea-urch IVTAMLEGEMAGLVS-SKPTGVRGSNS--TIREREVKDVSIDSLIKQLGTYITVMNVHGM
Drosoph IVPAILNNDEIQRGRQAHGMRSRATSIGASSSPEHGGGPAWKQLIGQLEHFYKQFQHFGL
Myo2p AINAVVISESLPGFSAGETSG----FLNKIFAN--TEEYTMDDILTFFNSIYWCMKSFHI 1374
Ustilago VIPALVESQSLPGFVTSDHSGR---LFNRLLSNNSTPMHTMDDILGILNKVWKSLKSYYV
HUM-2 IVPGILQHESSSDLMTAGQERR-----DRNSGSVESQRKSLDDLLQFMEIVHTKLTTYGG
H8
H9
MY5A-mouse DPELIKQVVKQMFYIVGAITLNNLLLRKDMCSWSKGMQIRYNVSQLEEWLRDKNLMNSGA
Sea-urch DPELVKQVARQALYLITASTINNILLRKDMCHWSKGVQIRYNLSELEEWLRSSRLYDKMM
Drosoph DNCYAEQIFHQLLYFICAVALNCLMLRGDICMWETGMIIRYNIGCIEDWVRSKKMSN-DV
Myo2p ENEVFHAVVTTLLNYVDAICFNELIMKRNFLSWKRGLQLNYNVTRLEEWCKT--HGLTDG 1432
Ustilago EPSVTQQVVTELLKLIGVTSFNDLLMRRNFCSWKRAMQIQYNITRIEEWCKS--HDMPEG
HUM-2 DDIVVKQVIGQMARWMCALALNYMMFRRELCNFEKAIQIKHNVTQIQNWLNAK--GLSDC
H10
H11
H12
H13
MY5A-mouse KETLEPLIQAAQLLQVKKKTDDDAEAICSMCNALTTAQIVKVLNLYTPVNEFEERVSVSF
Sea-urch ETTLEPLVQVAQLLQVKKRTDDDVGIICDTCTQLTVTQIIKILNLYTPD-EYEKRTEIAF
Drosoph LTALAPLNQVSQLLQSRK-SEQDVQTICDLCTSLSTAQVLKVMKSYKLDDYESEITNVFL
Myo2p TECLQHLIQTAKLLQVRKYTIEDIDILRGICYSLTPAQLQKLISQYQVADYESPIPQEIL 1492
Ustilago TLQLEHLMQATKLLQLKKATLGDIDIIYDVCWMLTPTQIQKLISHYYVADYENPISPEIL
HUM-2 RDHFEPLVQACHLLQSRKDPSNLDTLCGEMTSRLKPRQVVAILQHYDPSDEMEDGLSPEF
H13
H14
MY5A-mouse IRTIQMRLRD----------RKD---SPQLLMDAKHIFPVTFPFNPSS
Sea-urch IRKVQSRLAN----------RNDPKRESQLLIDAKHTFPVTFPY----
Drosoph EKLTEKLNAR----------QMQKSNSDEFTIDQKFIQPFKVVFRYSD
Myo2p RYVADIVKKEAALSSSGNDSKGHEHSSSIFITPETGPFTDPFSLIKTRKFDQVEAYIPAW 1552
Ustilago KAVASRVVPNDRND----------HLLLPPEVDEAGPYELPLPREVTGIETYCPAY
HUM-2 LVQIQKKLNERAIAN----------NDPIEDKDKLIMLGTYLPPFDTQPFSYSD
H15
MY5A-mouse LALETIQIPASLGLGFIARV--
Sea-urch ----------
Drosoph IKLEDIELPSHLNLDEFLTKI-
Myo2p LSLPSTKRIVDLVAQQVVQDGH 1574
Ustilago ISVPAIRRLASRVA-------
HUM-2 FPLETLSLPSCLHMQSVCRLV-

Table SI. Functional tests of the *myo2* mutants.

Residues	Secretory vesicle movement /viability	Vacuole movement
WT	+	+
Δ1459-1491	-	+
Δ1519-end	-	-
Δ1532-end	-	-
Δ1551-end	+/-	+
Δ1568-end	+	+
Q1233R	+	-
G1461D	+	+
D1457N	+	+
R1162E	+	+
D1357K	+	+
E1375V	+	+
R1402C	+	+
Y1415R	-	+
K1450I	+	+
W1407F	+	+
T1418A	+	+
E1422A	+	+
K1425A	+	+
Y1415E	+/-	+
S1330A	+	+
N1414S	+	+
K1444A	+/-	+
L1331S	+/-	+
Q1447R	+/-	+
L1411S	+/-	+
L1411R	-	+

"+" - wild-type growth at both 24°C and 37°C;
"+/-" - growth defect at 24°C or/and 37°C;
"-" - no growth or vacuole movement is abolished.

Table SII. Yeast *S. cerevisiae* strains used in this study.

Strain	Genotype	Source
PJ69-4A	*MATa, ura3-52, leu2-3,112, his3-200, trp1-901, gal4D, gal80D, LYS2::GAL1-HIS3, GAL2-ADE2, met::GAL7-lacZ*	(James et al., 1996)
LWY5518	*MATa, ura3-52, leu2-3,112, his3-D200, trp1-D901, lys2-801, suc2-D9, pep4-D1137, myo2-2*	(Catlett et al., 2000)
LWY2949	*MATa, ura3-52, leu2-3,112, his3-D200, trp1-D901, lys2-801, suc2-D9, pep4-D1137, myo2D::TRP1, pMYO2*	(Catlett et al., 2000)

References

Catlett, N.L., Duex, J.E., Tang, F. and Weisman, L.S. (2000) Two distinct regions in a yeast myosin-V tail domain are required for the movement of different cargoes. *J Cell Biol*, **150**, 513-526.

James, P., Halladay, J. and Craig, E.A. (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics*, **144**, 1425-1436.