Supplemental Materials
Molecular Biology of the Cell

Whitfield et al.

Supplementary Table 1. Yeast strains used in this work

Strain ID	Genotype	Source
PJ694a	*MATa trp1-901 leu2Δ-3 ura3Δ-52 his3Δ-200 gal4Δ gal80Δ LYS::GAL1::HIS3 GAL2::ADE-2 met2::GAL7-lacz*	A.Merz (U. Washington)
PJ694α	*MATα trp1Δ-901 leu2Δ-3 ura3Δ-52 his3Δ-200 gal4Δ gal80Δ LYS::GAL1-HIS3 GAL2-ADE-2 met2::GAL7-lacz*	A. Merz
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	GE Dharmacon
MDY122	BY4742 *chs6Δ::NAT*	This study
MDY869	BY4742 *chs6Δ::NAT apl2Δ::KANR*	This study
MDY784	BY4742 *chs6Δ::NAT apl2Δ::HPH*	This study
MDY783	BY4742 *chs6Δ::NAT apm1Δ::KANR*	This study
MDY786	BY4742 *chs6Δ::NAT apm2Δ::KANR*	This study
CTY234	BY4742 *can1Δ::STE2pr::LEU2 lyp1Δ::cyh2*	C. Boone (U. Toronto)
BY4741	*MATa HIS3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	GE Dharmacon
SWY85	BY4741 *apl2Δ::KANR*	This study
SWY89	BY4741 *apm1Δ::KANR*	This study
SWY91	BY4741 *apm2Δ::KANR*	This study
SWY97	BY4741 *mil1Δ::KANR*	This study
HBY577	BY4741 *suc2Δ::GFP-SNC1-SUC2::URA3*	This study
HBY573	BY4741 *apm1Δ::KANR suc2Δ::GFP-SNC1-SUC2::URA3*	This study
HBY574	BY4741 *aps1Δ::KANR suc2Δ::GFP-SNC1-SUC2::URA3*	This study
HBY575	BY4741 *apm2Δ::KANR suc2Δ::GFP-SNC1-SUC2::URA3*	This study
HBY576	BY4741 *apl4Δ::KANR suc2Δ::GFP-SNC1-SUC2::URA3*	This study
HBY571	BY4741 *mil1Δ::KANR suc2Δ::GFP-SNC1-SUC2::URA3*	This study
MDY613	*yap1801Δ::KANR yap1802-3HA::HIS3 suc2Δ::GFP-SNC1-SUC2::URA3*	This study
LC1979	BY4741 *APM4-GFP+::HIS3 CLC1-RFP::NAT*	This study
HBY155	BY4741 *APM4-GFP+::HIS3 CLC1-RFP::NAT sla2Δ::URA3*	
LC1977	BY4741 *APM2-GFP+::HIS3 CLC1-RFP::NAT*	This study
LC1978	BY4741 *APM2-GFP+::HIS3 CLC1-RFP::NAT sla2Δ::URA3*	
CTY301	BY4741 *MIL1-GFP+::HIS3*	This study
CTY564	BY4741 *MIL1-GFP+::HIS3 APL4-3HA::KANR*	This study
CTY574	BY4741 *MIL1-GFP+::HIS3 APL4Δear-3HA::KANR*	This study
CTY265	BY4741 *MIL1-GFP+::HIS3 APM1-3HA::KANR*	This study
CTY661	BY4741 *MIL1-GFP+::HIS3 APM2-3HA::KANR*	This study
MDY1114	BY4741 *APM2-GFP(envy)::HIS3*	This study
MDY1133	BY4741 *APM2-GFP(envy)::HIS3 APM1::tdTomato::NAT*	This study
MDY1136	BY4741 *APM2-GFP(envy)::HIS3 ANP1-mRFP1.5::NAT*	This study
SWY310	BY4741 *APM2-GFP(envy)::HIS3 mil1Δ::KANR*	This study
MDY1153	BY4741 *MIL1-GFP(envy)::HIS3*	This study

MDY1155	BY4741 *MIL1-GFP(envy)::HIS3 apm2Δ::KANR*	This study
CTY157	BY4741 *APM1-3HA::KANR*	This study
HBY567	BY4741 *APM2-3HA::HIS3*	This study
CTY15	BY4741*APM1-GFP+*	This study
CTY416	BY4741 *APM1-GFP+mil1Δ::NAT*	This study

Supplementary Table 2. Plasmids used in this work

Plasmid ID	Description	Source
pCS10	pSNF7-RFP1.5::URA (CEN)	This study
pSec7-dsRed	pSEC7-dsRed::URA3 (CEN)	S. Ferro-Novick (UCSD)
pRS415	pLEU2 (CEN)	Sikorski and Hieter (1989)*
pHFR80	pSNA2pr-SNA2(Y75A)-GFP-DXE::URA (CEN)	P. Morsomme (U. Catholique de Louvain)
pNR36	pHPH (CEN)	This study
pNR32	pAPM1::HPH (CEN)	This study
pMD177	pAPM1(F179A D181S)::HPH (CEN)	This study
pSW50	pAPM1(F179A D181S)-GFP+::HIS3::HPH (CEN)	This study
pMD178	pAPM2(F273A D275S)::HPH (CEN)	This study
pSW48	pAPM1pr-APM2-GFP+::HIS3::HPH (CEN)	This study
pSW60	pAPM1pr-APM2-3HA::HIS3::HPH (CEN)	This study
pNR39	pAPM1pr-APM2^{tyr}-GFP+::HIS3::HPH (CEN)	This study
pLC2632	pMIL1::LEU2 (CEN)	This study
pLC1922	pMIL1-GFP+::HIS3::LEU2 (CEN)	This study
pLC2633	pMIL1(S759A)::LEU2 (CEN)	This study
pLC1912	pMIL1(D817A)::LEU2 (CEN)	This study
pLC1914	pMIL1(H858A)::LEU2 (CEN)	This study
pLC2604	pMIL1(W^{143}QEMP)::LEU2 (CEN)	This study
pLC2646	pMIL1(W^{143}QEMP)-GFP+::HIS3::LEU2 (CEN)	This study
pLC2605	pMIL1(F^{152}NIY)::LEU2 (CEN)	This study
pMD158	pMIL1::HPH (CEN)	This study
pMD159	pMIL1(S759A)::HPH (CEN)	This study
pMD157	pMIL1(W^{143}QEMP)::HPH (CEN)	This study
pGBDU-C2	pGBDU-C2::URA3 (2μ)	P. James (U. Wisconsin)
pCT11	pGBDU-C2-APL4::URA3 (2μ)	This study
pLC2643	pGBD-APM1::URA3 (2μ)	This study
pNR15	pGBDU-C2-APM2::URA3 (2μ)	This study
pNR16	pGBDU-C2-APM2(1-246)::URA3 (2μ)	This study
pNR18	pGBDU-C2-APM2(247-605)::URA3 (2μ)	This study
pNR19	pGBDU-C2-APM2(389-562)::URA3 (2μ)	This study
pGAD-C2	pGAD-C2::LEU2 (2μ)	P. James
pCT15	pGAD-C2-APL4::LEU2 (2μ)	This study
pNR10	pGAD-C2-MIL1::LEU2 (2μ)	This study
pNR11	pGAD-C2-MIL1(1-450)::LEU2 (2μ)	This study
pLC2590	pGAD-MIL1(125-175)::LEU2 (2μ)	This study
pLC2644	pGAD-MIL1(125-175,	This study

pLC2645	W^{143}QEMP>AAEAA)::LEU2 (2μ) pGAD-MIL1(125-175, F^{152}NIY>ANAA)::LEU2 (2μ)	This study

*Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics *122*, 19–27.

Supplementary Figure 1. The tyrosine-binding pockets of Apm1 and Apm2 are highly conserved, and mutation of residues in these pockets does not destabilize the proteins.(A) MUSCLE sequence alignment of Apm1- and Apm2-related sequences, showing conservation of the two tyrosine-binding pockets. Intensity of blue-highlighted residues corresponds to percent identity. Red-highlighted residues interact with the YxxΦsorting signal. (B) Cellular levels of mutant and overexpressed forms of Apm1 and Apm2. The levels of genomically-tagged Apm1-GFP and Apm2-GFP were compared with plasmid-borne GFP-tagged Apm1^{tyr}, and either Apm2^{tyr}-GFP or Apm2^{OE}-GFP expressed from the *APM1* promoter. Samples were resolved by 10% SDS-PAGE on the same gel and detected by immunoblotting. PGK levels are shown as loading control. White gaps between panels indicate cropping of empty lanes from the same blot.

Supplementary Figure 2. Deletion of *MIL1* does not affect Apm1-GFP recruitment or number of Golgi compartments. (A) Localization of Apm1-GFP in wild type or *mil1* mutant strains quantified in a single slice. No significant differences between the means were found (unpaired t-test with wild-type). Error bars represent standard error of the mean (n=3). (B) Number of Sec7-dsRed puncta expressed as mean/cell. No significant differences between the means were found (unpaired t-tests with wild types). Error bars represent standard error of the mean (n=3).

A

S._cerevisiae_Apm 2	251 AKTIIMPISWRTKGIHYAKNEFFLDVIERVQYLMDFEKGVIRKNLIHGEIVCRCYLSGMPKLKISIN-KIL----	320
K._lactis_Apm 2-like	172 LRTMTSAVSWRPKGIHYGKNEFFLDVIEKLEFIMDFEEGVRNNVINGTIICRSYLSGMPQLSIGLN-KLM----	241
P._sorbitophila_Apm 2-like	241 VRTGLSSINWRPKGIFYPKNEIYLDIVELIDFEFDLGRNTIKKNEVKGFCFASCYLSGMPVCRIGFNEEKLSILS	315
S._stipitis_Apm 2-like	233 LRTYSSAINWRQKGIFYSKNEIYIDIIEDCEFYYDLELGVIKRNEVFGTCAVKCYLSGMPVCRIGFNEKQISRIG	307
D._hansenii_Apm 2-like	232 LRATSSAISWRPKGIFYPKNEIFIDIIENCEFLFSLSTNSIKRNEVYGRCLVKCYLSGMPVCKLGFNEKYISGID	306
C._albicans_Apm 2	239 LRTYSSAINWRPKGIFYAKNEIFIDIIEDCEFVYDLGTGVIKCNEIYGTCVVKSYLSGMPVCRLGFNERNLSRI-	312
S._cerevisiae_Apm 1	158 -VALTNSVSWRPEGITHKKNEAFLDVIESINMLMT-QKGQVLRSEIIGDVKVNSKLSGMPDLKLGINDKGI----	226
K._lactis_Apm 1-like	158 -TELTNSVSWRPEGIHYKKNEAFLDVIESINMLMT-QQGQVLRSEILGTVKVRSRLSGMPDLKLGLNDKGI----	226
P._sorbitophila_Apm 1-like	158 -NAVTNSVNWRSEGIMYKKNEAFLDVIESINMLIS-ASGHVLNSEILGKIKIKSHLSGMPDLRLGLNDKGI----	226
D._hansenii_Apm 1-like	159 -NAVTNSVNWRSEGIVYKKNEAFLDVIESINMLIN-AQGQVLNSEILGEVKIKSHLSGMPDLRLGLNDKGI----	227
S._stipitis_Apm 1-like	157 -NAVTNAVSWRKDGIFYKKNEAFLDVVESINMLIN-ASGQVLNSEILGEVKIKSHLSGMPDLRLGLNDKGI----	225
C._albicans_Apm 1	157 -NALTNSVSWRKEGIFYKKNEAFLDVIESINMLIT-ANGQVLNSEILGEIKIKSHLSGMPDLRLGLNDKGI----	225
A._thaliana_Ap1m 1	153 -MAVTNSVSWRSEGLKFKKNEVFLDVIESVNILVN-SNGQIVRSDVVGALKMRTYLSGMPECKLGLNDRIL----	221
A._thaliana_Ap1m 2	153 -MAVTNAVSWRSEGIQYKKNEVFLDVIENVNILVN-SNGQIVRSDVVGALKMRTYLTGMPECKLGLNDRVL----	221
C._elegans_apm-1	149 -MAVTNAVSWRSEGIKYRKNEVFLDVIESVNMLAN-AQGTVLNSEIVGSVKMRVYLTGMPELRLGLNDKVF----	217
C._elegans_unc-101	150 -MAVTNAVSWRSEGIKYRKNEVFLDVIESVNMLAS-ANGTVLQSEIVGSVKMRVYLTGMPELRLGLNDKVL----	218
D._melanogaster_AP-1mu	153 -VAVTNAVSWRSEGIKYRKNEVFLDVIESVNLLAN-ANGNVLRSEIVGAIKMRVYLSGMPELRLGLNDKVL----	221
M._musculus_Ap1m 1	151 -ATVTNAVSWRSEGIKYRKNEVFLDVIEAVNLLVS-ANGNVLRSEIVGSIKMRVFLSGMPELRLGLNDKVL----	219
H._sapiens_Ap1m 1	151 -ATVTNAVSWRSEGIKYKKNEVFLDVIESVNLLVN-ANGNVLRSEIVGSIKMRVFLSGMPELRLGLNDKVL----	219
M._musculus_Ap1m 2	152 --TVTNAVSWRSEGIKYKKNEVFIDVIESVNLLVN-ANGSVLLSEIVGTIKLKVFLSGMPELRLGLNDRVL----	219
H._sapiens_Ap1m 2	152 --TVTNAVSWRSEGIKYKKNEVFIDVIESVNLLVN-ANGSVLLSEIVGTIKLKVFLSGMPELRLGLNDRVL----	219

S._cerevisiae_Apm 2	553 RDK-------LVNIDFEIPYCTCSGLKVEYLKVEEPQLQYQSFPWVRYKTVSDE----------	599
K._lactis_Apm 2-like	445 ENQKEDVDKFALIAMSFEIPYYAVSGLKVEYFKIEEPQLNYQSFPWVRYKTVNDN----------	499
P._sorbitophila_Apm 2-like	611 KQQYKNPSHYNIARATFLLPSLSYSGLKVTYIDVSEPQLKYSCFPWIKYSTESKPHSSTDNSRLSE	676
S._stipitis_Apm 2-like	566 TKQWKIQNFDNDIKLKFSIPMLAYSGLRLNYLSVVEDQLKYSCFPWVRYATESDPHKKTG-KGDNK	630
D._hansenii_Apm 2-like	601 QKSIKFSQDFNHVKCSFNIPMLSYSGLKLTYLKVAEEQMKYTCFPWVRYITESNSDTHSSNKVEDE	666
C._albicans_Apm 2	583 SLKFKSSVFNDDIKVHFKLPMVTYSGLKLSYLSVVEEEQMKYPCFPWVRYLTKSIDHYEPSIALENQ	648
S._cerevisiae_Apm 1	426 ----------VQIKFQIPYFTTSGIQVRYLKINEPKLQYKSYPWVRYITQSGD----------	468
K._lactis_Apm 1-like	395 ----------VQIKFQIPYFTTSGIQVRYLKIEEPKLQYNSYPWVRYITQSGD----------	437
P._sorbitophila_Apm 1-like	385 ----------IKLNFSIPYFTTSGIQVRYLRINEPKLQYQSYPWVRYITQAGD----------	427
D._hansenii_Apm 1-like	386 ----------IKVNFSIPYFTTSGIQVRYLINEPKLQYQSYPWVRYITQAGE----------	428
S._stipitis_Apm 1-like	393 ----------IKVNFSIPYFTTSGIQVRYLINEPKLQYQSYPWVRYITQSGD----------	435
C._albicans_Apm 1	389 ----------IKVNFSIPYFTTSGIQVRYLRINEPKLQYQSYPWVRYITQSGD----------	431
A._thaliana_Ap1m 1	381 ----------IRVKFEIPKFIVSGIQVRYLKIIE-KSGYQALPWVRYITMAGE----------	422
A._thaliana_Ap1m 2	381 ----------IRVKFEIPYFTVSGIQVRYLKIIE-KSGYQALPWVRYITMAGE----------	422
C._elegans_apm-1	379 ----------INVKFEIPYYTTSGLQVRYLKIIE-KSGYQALPWVRYVTQNGD----------	420
C._elegans_unc-101	375 ----------IKVKFEIPYFTTSGIQVRYLKIIE-KSGYQALPWVRYITQNGE----------	416
D._melanogaster_AP-1mu	379 ----------IQVRFEIPYFTTSGIQVRYLKIIE-KSGYQALPWVRYITQNGD----------	420
M._musculus_Ap1m 1	376 ----------ISVKFEIPYFTTSGIQVRYLKIIE-KSGYQALPWVRYITQNGD----------	417
H._sapiens_Ap1m 1	376 ----------ISVKFEIPYFTTSGIQVRYLKIIE-KSGYQALPWVRYITQNGD----------	417
M._musculus_Ap1m 2	376 ----------IGVKFEIPYFTVSGIQVRYMKIIE-KSGYQALPWVRYITQSGD----------	417
H._sapiens_Ap1m 2	376 ----------IGVKFEIPYFTVSGIQVRYMKIIE-KSGYQALPWVRYITQSGD----------	417

B

APM1-GFP apm1Δ+apm1^tyr-GFP APM2-GFP apm2Δ+apm2^tyr-GFP apm2Δ+APM2^OE-GFP

αGFP
-100
-70

αDpm1
-28

A

B