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Supplementary material

Yeast strains

Strains were grown in rich medium or synthetic medium as described (Guthrie and Fink, 1991).

Table S1. Yeast strains

Strains	Genotype	Source
EY492	*MATα ura3-1 leu2-3,112 trp1-1 his3-11 ade2-1 can1-100 Gal*$^+$	R. Rothstein
EY699	*ura3-1 leu2-3,112 trp1-1 his3-11 ade2-1 can1-100 Gal*$^+$	R. Rothstein
EY700	EY699 *fus3-6::LEU2*	E. Elion
EY957	EY699 *sst1*	E. Elion
EY1775	EY957 *ste5::TRP3*	E. Elion
EYL300	*MATa leu2-3 ura3-52*	R. Li
EYL302	*MATa leu2-3 ura3-52 cdc42-1*	R. Li
EYL427	*bni1::KAN ura3-1 leu2-3,112 trp1-1 his3-11 ade2-100 ade3*	D. Pellman
EYL428	*bni1::KAN ura3-1 leu2-3,112 trp1-1 his3-11 ade2-100 ade3 MATα*	D. Pellman
EYL917	EYL427 *sst1*	B.N. Lee
EYL1096	*sst1::LEU2 FUS3GFP::HIS3 ura3-1 leu3-3,112 trp1-1 his3-11 ade2-1 can1-100*	A. Levchenko
EYL1098	*myo1::KAN ura3-1 leu2-3,112 trp1-1 his3-11 ade2-1 can1-100 Gal*$^+$	R. Li
EYL1730	*bnr1::KAN ura3-1 leu2-3,112 trp1-1 his3-11 ade2-100*	D. Pellman
EYL1735	*tpm1-2::LEU2 tpm2::HIS3 his3Δ-200 leu2-3,112 lys2-801 trp1-1 ura3-52*	A. Bretscher
EYL1736	*tpm2::HIS3 his3Δ-200 leu2-3,112 lys2-801 trp1-1 ura3-52*	A. Bretscher
EYL1748	*bni1::BNI1#1::HIS3 bnr1::KAN ura3-1 leu2-3,112 trp1-1 his3-11 ade2-100*	D. Pellman
EYL1765	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Res Gene
EYL1802	*MATα MYO2::HIS3 his3-Δ200 ura3-52 leu2-3,112 lys2-801 ade2-101 Gal*$^+$	D. Pellman
EYL1807	*MATα myo2-66::HIS3 his3-Δ200 ura3-52 leu2-3,112 lys2-801 ade2-101 Gal*$^+$	D. Pellman
QMY27	*sst1::LEU2 bni1::kanR FUS3GFP::HIS3 ura3-1 leu3-3,112 trp1-1 his3-11 ade2-1* c*an1-100*	This study
QMY458	EYL1802 switched to *MATa*	This study
QMY459	EYL1807 switched to *MATa*	This study

QMY483	*myo3::HIS3 ura3 leu2 trp1 his3 ade2*	R. Li
QMY484	*myo5::TRP1 ura3 leu2 trp1 his3 ade2*	R. Li
QMY485	*myo3::HIS myo5::TRP1 ura3 leu2 trp1 his3 ade2*	R. Li
QMY550	*ura3 leu2 trp1 his3 ade2*	R. Li
QMY551	*rho1-104 ura3 leu2 trp1 his3 ade2*	R. Li
QMY553	*rho1::HIS3 rho1-2 LEU2 ura3 leu2 trp1 his3 ade2*	R. Li
QMY574	*rho3::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
QMY576	*rho5::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	This study
QMY630	*myo2-66::HIS3*	This study
QMY631	*myo4::KAN*	This study
QMY632	*myo2-66::HIS3 myo4::KAN*	This study
5126	*rho4::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Res Gene
7230	*rho2::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Res Gene
22032	*MATa rho5::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Res Gene
	MATα rho5::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	
22277	*MATa rho3::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Res Gene
	MATα rho3::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	

All strains except EY492, EYL428, 22032 and 22277 are *MATa*.

QMY27 was produced by cross of EYL428 with EYL1096. QMY630-632 were created by crossing EYL1685 with EYL1807 (*she1* is *myo4*). QMY574 and QMY576 were made by sporulation of 22277 and 22032.

Plasmid construction

All *BNI1* plasmids were made from a pRS316-based vector (PB1025, former name p182, see Evangelista et al., 1997) containing the *BNI1* coding sequence with 800 base pairs (bp) upstream and 1600 bp downstream sequence. The Rho-binding domain deletion mutant, *bni1ΔRBD* (QMB16, deletes aa 90-343), was made from two PCR amplified fragments of *BNI1* using the following primers: -788~268 fragment: 5′-ACGCGGATCCTTCACCGCTTTCGCACCTACTT-3′(*Bam*HI site at 5′ terminus), 5′-TCCCCGCGGCGATTTTTTATTCAACGGCCT-3′ (*Sac*II site at 3′ terminus) and 1031~2472 fragment: 5′-CTACCGCGGCGAATCCTGGCAGACAAACTT-3′ (*Sac*II site at 5′ terminus), 5′-TGCATGTCTCGAGTTCATACCTCTGGTGCCTTC-3′. The PCR products were cut with *Bam*HI/*Sac*II and *Sac*II/*Xho*I respectively, then performed a 3-way ligation with *Bam*HI/*Xho*I vector fragment of PB1025 to produce QMB16. The *Bam*HI/*Xho*I fragment of QMB16 containing *bni1ΔRBD* mutation was inserted into EBL334 to produce QMB79 (*bni1ΔRBD-GFP)*. The *bni1ΔFH1FH2* mutant (QBM5, deletes

aa 1230-1748) was made by cloning the *BNI1* gene into *Bam*HI-*Eag*I sites of pBR322 to make QMB3. A *BNI1* fragment from the *Xho*I site to the FH1 domain (nucleotides 3572-4791) was PCR amplified with primers: 5′GAACTCGAGCCTA AATTCTTCAG-3′ and 5′-AGATCCGCGGCAGTAGAGAGATCTTCTGCG-3′ (*Sac*II site at 5′ terminus). This PCR product was used to replace the *Xho*I~*Sac*II fragment of *BNI1* gene in QMB3 to make QMB4. Then the *Bam*HI-*Eag*I fragment of QMB4 was cloned into pRS316 to make QMB5. To make *bni1ΔFH1* (QMB21, deletes aa 1230-1328), fragment from *Xho*I site to FH1 domain (3572-4791) was PCR amplified by the same way as for FH domain deletion, but added a *Sph*I site instead of *Sac*II site to the second primers: 5′-GATGCATGCAGTAGAGAGAT CTTCTGCG-3′. Fragment from the end of FH1 to *Sac*II site (5086-6352) were PCR amplified with 5′ primer: 5′-CATGCATGCGCATCGCAAATCAAATCAGCT-3′ (*Sph*I site at 5′ terminus) and 3′ primer: 5′-CTTCCGCGGCTAGATTTTGCGCTT-3′. The first and second PCR products were digested with *Xho*I/*Sph*I and S*ph*I/*Sac*II respectively, then used in a 3-way ligation with the *Xho*I/*Sac*II fragment of QMB3 to make QMB19. The *Bam*HI~*Eag*I fragments of QMB19 was cloned into pRS316 to make QMB21. The same strategy was used to make *bni1ΔFH2* (QMB22, deletes aa 1492-1640), except a different 3′ primer (5′-TGTAGCATGCCCTCACGCC CTCCCAGTCTG-3′) for the first PCR, which amplifies fragment from *Xho*I site to FH2 domain (3572-5577), and a different 5′ primers (5′-CGATGCATGCTCCATTGAGCAGTTAGTTAA-3′) for the second PCR, which amplifies fragment from the end of FH2 domain to *Sac*II site (6021-6352). QMB34 (*bni1ΔCT,* deletes aa 1749-1953) was made by cloning *Bam*HI-*Eag*I fragment of PB1046 (Lee et al., 1999) into pRS315.

Table S2. Plasmids used in this study

Plasmids	Description			Source
QMB5	*bni1ΔFH1FH2*	*CEN*	*URA3*	This study
QMB16	*bni1ΔRBD*	*CEN*	*URA3*	This study
QMB21	*bni1ΔFH1*	*CEN*	*URA3*	This study
QMB22	*bni1ΔFH2*	*CEN*	*URA3*	This study
QMB34	*bni1ΔCT*	*CEN*	*LEU2*	This study
QMB75	*MYO2DN*	*GAL1*	*LEU2*	S. Reck-Peterson
QMB79	*bni1ΔRBD-GFP*	*CEN(ADH1)*	*URA3*	This study
QMB80	*MYO2-HA*	*CEN*	*HIS3*	L. Weisman
EBL95	*FUS1-LACZ*	*2*	*LEU2*	Elion *et al.* (1990)
EBL204	*STE5-CTM*	*CEN(GAL1)*	*LEU2*	Pryciak *et al.* (1998)

EBL284	*STE11-4*	*CEN*	*HIS3*	H. Madhani
EBL332	*BNI1-HA4*	*CEN*	*URA3*	D. Pellman
EBL334	*BNI1-GFP*	*CEN(ADH1)*	*URA3*	D. Pellman
EBL358	*STE5-MYC9*	*CEN*	*URA3*	Mahanty *et al.* (1999)
EBL365	*STE5-MYC9*	*2*	*URA3*	Mahanty *et al.* (1999)
EBL367	*STE5-GFP*	*CEN(CUP1)*	*URA3*	Mahanty *et al.* (1999)
EBL444	*TAgNLS^{K128T}-STE5-M9*	*2*	*URA3*	Mahanty *et al.* (1999)
EBL453	*STE5-MYC9*	*2*	*LEU2*	Mahanty *et al.* (1999)
EBL511	*GFP-STE20*	*CEN*	*URA3*	Leberer *et al.* (1997)
EBL664	*GFP-CDC24*	*CEN(MET)*	*LEU2*	Toenjes *et al.* (1999)
PYEE1102	*FUS3-HA*	*CEN*	*HIS3*	Elion *et al.* (1993)

Supplemental References

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