SUPPLEMENTARY DATA

MATERIALS AND METHODS

Plasmid constructions and site-directed mutagenesis

Table S1 contains brief descriptions of all plasmids employed in this study.

YCpNIP1-Myc-L was constructed by insertion of the SalI-BamHI-digested PCR product amplified from YCpNIP1-His-L (7) using primers TK46 and TK51 back into SalI-BamHI-digested YCpNIP1-His-L. Analogously, YCpNIP1-Δ60, 80, 100, 120 and Δ180-Myc-L were all constructed by insertion of the SalI-BamHI-digested PCR products amplified from YCpNIP1-His-L by using TK46 in combination with primers PS1, TK52, TK53, PS2, and PS3, respectively.

To construct six consecutive 10-Ala c/NIP1 substitutions in plasmids YCpNIP1-693A702-Myc-L to YCpNIP1-743A752-Myc-L, the following two pairs of primers were used for separate PCR amplifications with YCpNIP1-Myc-L as a template: (i) TK46 in combination with TK56, TK58, TK60, TK62, TK64, and TK66, respectively, and (ii) TK74 in combination with TK55, TK57, TK59, TK61, TK63, and TK65, respectively. The corresponding PCR products thus obtained were used in a 1:1 ratio as templates for a second PCR amplification with primers TK46 and TK74. The resulting PCR products were digested with SalI-BamHI and ligated with SalI-BamHI-cleaved YCpNIP1-Myc-L.

YEpNIP1-743A752-Myc-L and YEpNIP1-723A732-Myc-L were constructed by insertion of the BamHI-XbaI fragments of YCpNIP1-743A752-Myc-L and YCpNIP1-723A732-Myc-L, respectively, into BamHI-XbaI-digested YEpNIP1-His-L (7).

pGEX-c/NIP1-571-812 and pGEX-c/NIP1-701-812 expression plasmids were constructed by insertion of the corresponding BamHI-XhoI-digested PCR products amplified from YCpNIP1-His-L using the primer TK1 in combination with primers TK2 and TK3, respectively, into BamHI-XhoI-digested pGEX-5X-3 (Pharmacia). Analogously, pGEX-c/nip1-743A752 and pGEX-c/nip1-723A732 plasmids were constructed by insertion of the corresponding BamHI-XhoI-digested PCR products amplified from YCpNIP1-743A752-Myc-L and YCpNIP1-723A732-Myc-L, respectively, using primers TK2 and TK1.

YCpMJ-MET-NIP1-W was constructed by inserting the 2618-bp BamHI-HindIII fragment from pGAD-NIP1 (16) into YCplac22MET-W (a kind gift of Kim Nasmyth) digested by BamHI-HindIII.

To construct pRSASC1-intron-less-U carrying the *ASC1* gene without an intron, the following two pairs of primers were used for separate PCR amplifications with pRSASC1-U (28) as a template: (i) AJ2 and TK30 and (ii) TK31 and TK45. The PCR products thus obtained were used in a 1:1 ratio as templates for a third PCR amplification with primers AJ2 and TK45. The resulting PCR products were digested with BamHI-XbaI and ligated with BamHI-XbaI-cleaved pRS416 (28).

pFL45s which removes the *SNR24* coding sequence was created by cutting pFL45s/ACTU24 (32) with BamHI and self-ligating the isolated vector fragment.

To construct pGADT7-ASC1-exon1 and pGADT7-ASC1-exon2, the following two pairs of primers were used for separate PCR amplifications with pRSASC1-U as a template: (i) TK4 and TK5 and (ii) TK94 and TK5. The resulting PCR products were digested with BamHI-XhoI and ligated with BamHI-XhoI-cleaved pGADT7 (Clontech).

Two-Hybrid analysis

Two-hybrid analysis of protein-protein interactions between the c/NIP1-CTD and all 33 small subunit ribosomal proteins (RPSs) was conducted using the Matchmaker Two-Hybrid System 3 (Clontech) according to the vendor's instructions using strain AH109. Constructions of all two-hybrid plasmids in the vectors pGADT7 (activation domain hybrid vector) and pGBKT7 (DNA-binding domain hybrid vector) were carried out as follows. The two-hybrid plasmid pGBKT7 RPS1 to RPS32 were used from our previous study (16). The coding sequence of *ASC1* was PCR amplified from a 2.13-kb BamHI-XbaI fragment containing the entire *ASC1* gene locus inserted into pRSASC1-U using primers TK4 and TK5. The obtained PCR product was cleaved with BamHI-SalI and inserted into BamHI-SalI-digested pGBKT7 producing pGBKT7-RPS33/ASC1. To construct pGAD-c/NIP1-571-812 and pGAD-c/NIP1-701-812, the coding sequences of c/NIP1 corresponding to residues 571 to 812 and 701 to 812 were PCR amplified using two pairs of primers in TK1, TK2 and TK1, TK3, respectively, and YCpNIP1-His-L as a template. The PCR product thus obtained was cleaved with BamHI-XhoI and inserted into BamHI-XhoI-digested pGADT7.

Construction of yeast strains

To create HMJ08, H2880 (8) was first transformed with YCpNIP1-His-L to cover for the deletion of *NIP1* that was made in the next step by introducing the SacI-SphI fragment carrying the *nip1Δ::hisG-URA3-hisG* integration cassette from pLV10 (6). The Uracil auxotrophy was regained by growing the cells on SD plates containing 5-fluoro-orotic acid (5-FOA). The resulting strain was subsequently transformed with YCpNIP1-His-U and the Leucine auxotrophy was regained by growing the cells in liquid media containing Leucine and selecting for those that lost the YCpNIP1-His-L plasmid on SD +/- Leucine plates producing HMJ08.

HMJ06 was generated by introducing YCpMJ-MET-NIP1-W into HKN06 (6). The original pNIP1^{+} (*NIP1 URA3*) plasmid was evicted on SD plates containing 5-FOA.

To construct TK01 and ED43 with the chromosomal deletion of *ASC1*, HMJ08 and YBS47 (3), respectively, were introduced with the *asc1Δ::KanMX3* disruption cassette and Geneticin G418 resistant colonies were selected on YPD media containing G418. The *ASC1* disruption was verified by Western blotting. The *asc1Δ::KanMX3* disruption cassette was obtained by PCR form the genomic DNA of AL150 (28) using primers TK32 and TK35.

To produce TK149, TK150 and TK494, HMJ08 was transformed with YCpNIP1-Myc-L, YCpNIP1-743A752-Myc-L and YCpNIP1-Δ60, respectively, and the resident YCpNIP1-His-U plasmid was evicted on SD plates containing 5-FOA.

To generate TK145 and TK142, del'32a9A (3) was first transformed with pRS-a/tif32-box6-His-L and pRS-a/tif32-R731I-His-L (14), respectively, and the original pRSTIF32-His-U (*TIF32 URA3*) plasmid was evicted on SD plates containing 5-FOA. The resulting strains were then transformed to Ura+ with the integrative *GCN2* plasmid pHQ835 (kindly provided by Hongfang Qiu) digested with *Sna*BI. Ura- segregants were obtained by selecting for growth on medium containing 5-FOA, and the resulting TK145 and TK142 were tested for the presence of integrated *GCN2* by testing growth on medium containing 3-aminotriazole (3-AT).

YER27, with the chromosomal deletion of *ASC1*, was made by introducing del'32a9A (3) first with the *asc1Δ::KanMX3* disruption cassette as described above, and subsequently with pFL45s/ACTU24.

Yeast biochemical methods

GST pull-down assays with GST fusions and in vitro-synthesized ^{35}S-labeled polypeptides were conducted as follows. Individual GST-fusion proteins were expressed in *E. coli* (see below), immobilized on glutathione-Sepharose beads and incubated with 10 μl of ^{35}S-labeled potential binding partners at 4 °C for 2 h in binding buffer A (20 mM HEPES [pH 7.5], 75mM KCl, 0,1 mM $MgCl_2$, 0.05% IPEGAL, 1mM DTT, 0.1% milk). The ^{35}S-labeled proteins were synthetized using TNT Quick Coupled Transcription/Translation RRL kit (Promega), according to the manufacturer's instructions. The beads were washed 3 times with 1 ml of phosphate-buffered saline and bound proteins separated by SDS-PAGE. Gels were first stained with Gelcode Blue Stain Reagent (Pierce) and then subjected to autoradiography.

GST-fusion proteins were produced and purified as follows. Transformants of *E. coli* strain BL21-CodonPlus (DE3)-RIL (Stratagene) expressing GST-c/NIP1 fusion proteins were induced at OD_{600} ~ 0.5 for 2.5 h with 1 mM IPTG, harvested and washed once with ice-cold PBS. Following resuspension of the cell pellet from 500 ml culture in 20 mL of lysis buffer L (PBS [pH7.4], 10% glycerol, 2 μg/mL Aprotinin, 2 μg/mL Leupeptin, 2 μg/mL Pepstatin, Complete protease inhibitor cocktail tablets [EDTA-free, Roche diagnostics]), the cells were lysed by sonication on ice. Triton X-100 was added to the resulting cell lysates to final concentration of 1%. The lysate were subsequently clarified by centrifugation at 10,000 g for 10-20 min and incubated with 400 μl of 50% slurry of glutathione-Sepharose 4B beads equilibrated in PBS by gentle rocking for 30 min at 4 °C. The protein-bound beads were then washed three times with 5 ml of PBS.

WCE preparations, polysome profile analysis, 2% HCHO-cross-linking and fractionation of WCEs by high velocity sedimentation followed by Western blot analysis were all carried out as described by (41).

β-galactosidase assays were conducted as described previously (62).

REFERENCES

7. Valášek, L., Nielsen, K.H. and Hinnebusch, A.G. (2002) Direct eIF2-eIF3 contact in the multifactor complex is important for translation initiation in vivo. *EMBO J*, **21**, 5886-5898.
16. Valášek, L., Mathew, A., Shin, B.S., Nielsen, K.H., Szamecz, B. and Hinnebusch, A.G. (2003) The Yeast eIF3 Subunits TIF32/a and NIP1/c and eIF5 Make Critical Connections with the 40S Ribosome in vivo. *Genes Dev*, **17**, 786-799.
28. Gerbasi, V.R., Weaver, C.M., Hill, S., Friedman, D.B. and Link, A.J. (2004) Yeast Asc1p and Mammalian RACK1 Are Functionally Orthologous Core 40S Ribosomal Proteins That Repress Gene Expression. *Molecular and Cellular Biology*, **24**, 8276-8287.
32. Kiss-Laszlo, Z., Henry, Y., Bachellerie, J.P., Caizergues-Ferrer, M. and Kiss, T. (1996) Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell*, **85**, 1077-1088.

8. Nielsen, K.H., Szamecz, B., Valasek, L.J., A., Shin, B.S. and Hinnebusch, A.G. (2004) Functions of eIF3 downstream of 48S assembly impact AUG recognition and GCN4 translational control. *EMBO J.*, **23**, 1166-1177.
6. Valášek, L., Nielsen, K.H., Zhang, F., Fekete, C.A. and Hinnebusch, A.G. (2004) Interactions of Eukaryotic Translation Initiation Factor 3 (eIF3) Subunit NIP1/c with eIF1 and eIF5 Promote Preinitiation Complex Assembly and Regulate Start Codon Selection. *Mol. Cell. Biol.*, **24**, 9437-9455.
3. Szamecz, B., Rutkai, E., Cuchalova, L., Munzarova, V., Herrmannova, A., Nielsen, K.H., Burela, L., Hinnebusch, A.G. and Valášek, L. (2008) eIF3a cooperates with sequences 5' of uORF1 to promote resumption of scanning by post-termination ribosomes for reinitiation on GCN4 mRNA. *Genes Dev*, **22**, 2414-2425.
14. Chiu, W.-L., Wagner, S., Herrmannova, A., Burela, L., Zhang, F., Saini, A.K., Valasek, L. and Hinnebusch, A.G. (2010) The C-Terminal Region of Eukaryotic Translation Initiation Factor 3a (eIF3a) Promotes mRNA Recruitment, Scanning, and, Together with eIF3j and the eIF3b RNA Recognition Motif, Selection of AUG Start Codons. *Mol Cell Biol*, **30**, 4415-4434.
41. Valášek, L., Szamecz, B., Hinnebusch, A.G. and Nielsen, K.H. (2007) In Vivo Stabilization of Preinitiation Complexes by Formaldehyde Cross-Linking. *Methods Enzymol.*, **429**, 163-183.
40. Guex, N. and Peitsch, M.C. (1997) SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis*, **18**, 2714-2723.
56. Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R. *et al.* (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, **23**, 2947-2948.
9. Nielsen, K.H., Valášek, L., Sykes, C., Jivotovskaya, A. and Hinnebusch, A.G. (2006) Interaction of the RNP1 motif in PRT1 with HCR1 promotes 40S binding of eukaryotic initiation factor 3 in yeast. *Mol Cell Biol*, **26**, 2984-2998.
57. Algire, M.A., Maag, D., Savio, P., Acker, M.G., Tarun, S.Z., Jr., Sachs, A.B., Asano, K., Nielsen, K.H., Olsen, D.S., Phan, L. *et al.* (2002) Development and characterization of a reconstituted yeast translation initiation system. *Rna*, **8**, 382-397.
58. Gietz, R.D. and Sugino, A. (1988) New yeast-Escherichia coli shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. *Gene*, **74**, 527-534.
32. Kiss-László, Z., Henry, Y., Bachellerie, J.-P., Caizergues-Ferrer, M. and Kiss, T. (1996) Site-Specific Ribose Methylation of Preribosomal RNA: A Novel Function for Small Nucleolar RNAs. *Cell*, **85**, 1077-1088.
16. Elantak, L., Wagner, S., Herrmannová, A., Karásková, M., Rutkai, E., Lukavsky, P.J. and Valášek, L. (2010) The indispensable N-terminal half of eIF3j co-operates with its structurally conserved binding partner eIF3b-RRM and eIF1A in stringent AUG selection. *J Mol Biol.*, **396**, 1097-1116.
12. Valášek, L., Hašek, J., Nielsen, K.H. and Hinnebusch, A.G. (2001) Dual function of eIF3j/Hcr1p in processing 20 S Pre-rRNA and translation initiation. *J Biol Chem*, **276**, 43351-43360.

60. Asano, K., Vornlocher, H.-P., Richter-Cook, N.J., Merrick, W.C., Hinnebusch, A.G. and Hershey, J.W.B. (1997) Structure of cDNAs encoding human eukaryotic initiation factor 3 subunits: possible roles in RNA binding and macromolecular assembly. *J Biol Chem*, **272**, 27042-27052.
61. Asano, K., Krishnamoorthy, T., Phan, L., Pavitt, G.D. and Hinnebusch, A.G. (1999) Conserved bipartite motifs in yeast eIF5 and eIF2Bε, GTPase-activating and GDP-GTP exchange factors in translation initiation, mediate binding to their common substrate eIF2. *EMBO J*, **18**, 1673-1688.
62. Mueller, P.P. and Hinnebusch, A.G. (1986) Multiple upstream AUG codons mediate translational control of *GCN4*. *Cell*, **45**, 201-207.

FIGURE LEGENDS

Figure S1. (**A**) Structural alignment of the 3D threading model of the c/NIP1-PCI (residues 650-812 in green) with the crystal structure of the PCI of *A. thaliana* CSN7 (yellow) and with the NMR solution structure of the C-terminal winged helix subdomain from the *Mus. musculus* COP9 subunit SGN4 (blue) computed by Swiss-PDBViewer 4.0.1. (40) The alignment of the area of WH subdomains is magnified. The c/NIP1-PCI α-helices 1, 1', 2 and 3 as well as the extra N-terminal α-helices of CSN7 (designated N1 and N2) are indicated. (**B**) Electrostatic potential of the solvent-accessible surface of the c/NIP1-CTD rendered on the molecular surface of its 3D threading model. A gradient of blue to red shows positive to negative charge, respectively, as calculated using Swiss-PDBViewer 4.0.1. (40) tool for analysis of Poisson-Boltzmann electrostatic potential. Positions of *nip1-723A732* and *nip1-743A752* are marked by magenta and sky blue, respectively (left cartoon), a position of helix α7 is shown in red (right cartoon).

Figure S2. (**A**) Western blot analysis of WCEs from the wt *NIP1* and mutant *nip1-743A752* and *nip1-Δ60* strains grown at 37 °C using anti-c/NIP1 (lanes 1 and 2) or anti-Myc (lanes 3 and 4) antibodies. Anti-GCD11 antibodies raised against the γ subunit of eIF2 were used as a loading control. (**B**) To test the expression of the lethal *nip1-723A732* allele, we constructed a derivative of a HMJ06 strain, in which the Myc-tagged c/nip1-723A732 mutant protein was overexpressed from a high copy plasmid on top of the wt His-tagged c/NIP1. The viable *nip1-743A752* mutant allele was used as a control. (**C**) GST fusions of two segments of the c/NIP1-CTD (residues 701-812 [lane 3] or 571-812 [lane 4]) and two 10-ala substitutions 743A752 (lane 5) and 723A732 (lane 6) inserted into the 571-812 fusion, or GST alone (lane 2) were tested for binding to the ^{35}S-labeled fragment of ASC1 corresponding to its exon1. Lane 1 (IN) contains 10% of the input amount.

Figure S3. Amino acid sequence alignment of the c/NIP1-CTD with other species. The amino acid sequence of c/NIP1-CTD from *Saccharomyces cerevisiae* (accession number NP_014040.1) is aligned with its *Zygosaccharomyces rouxii* homolog (accession number XP_002495716.1), *Lachancea thermotolerans* homolog (accession number XP_002552208.1), *Ashbya gossypii* homolog (accession number NP_986323.1), *Schizosaccharomyces pombe* homolog (accession number P_593828.2), *Mus musculus* homolog (accession number NP_666312.1), *Homo*

sapiens homolog (accession number NP_003743.1), *Xenopus laevis* homolog (accession number NP_001129648.1), *Drosophila melanogaster* homolog (accession number NP_611242.1) and *Caenorhabditis elegans* homolog (accession number NP_492638.1). The alignment was conducted with ClustalX version 2.0.12 (56). Identical and similar residues are color coded, a bar diagram shown below indicates the quality of the alignment by plotting a conservation score for each column of the alignment; the higher the bar, the better conservation of a particular column. Numbering refers to the amino acid sequence of *S. cerevisiae* c/NIP1. Mutated regions in *nip1-723A732* and *nip1-743A752* are boxed and marked by magenta and sky blue, respectively.

Table S1 Yeast strains used in this study.

Strain	Genotype	Source or reference
AH109	*MATa trp1-901 leu2-3, -112 ura3-52 his3-200 gal4Δ gal80Δ*	Clontech
H2880	*MATa trp1 leu2-3,-112 ura3-52*	(8)
HMJ08	*MATa, trp1 leu2-3,-112 ura3-52 nip1Δ* YCpNIP1-His-U (*NIP1-His URA3*)	This study
TK149	*MATa trp1 leu2-3,-112 ura3-52, nip1Δ* YCpNIP1-Myc-L (*NIP1-Myc LEU2*)	This study
TK150	*MATa trp1 leu2-3,-112 ura3-52 nip1Δ* YCpNIP1-743A752-Myc-L (*nip1-743A752-Myc LEU2*)	This study
TK494	*MATa trp1 leu2-3,-112 ura3-52 nip1Δ* YCpNIP1-Δ60-Myc-L (*nip1-Δ60-Myc LEU2*)	This study
TK01	*MATa trp1 leu2-3,-112 ura3-52 nip1Δ asc1Δ::KanMX3* (YCpNIP1-His-U) (*NIP1-His URA3*)	This study
HKN06	*MATa trp1 leu2-3,112 ura3-52 gcn2Δ nip1Δ (NIP1 URA3)*	(8)
HMJ06	*MATa trp1 leu2-3,-112 ura3-52 nip1Δ gcn2Δ* YCpMJ-Met-NIP1-W (*MET3-NIP1 TRP1*)	This study
ED43	*MATa trp1 leu2-3,-112 ura3-52 tif32Δ gcn2Δ asc1Δ::KanMX3 URA3::GCN2 ura3* pRSTIF32-His-L (*TIF32-His LEU2*)	This study
del'32a9A	*MATa trp1 leu2-3,-112 ura3-52 tif32Δ gcn2Δ* pRSTIF32-His-U (*TIF32-His URA3*)	(3)

YBS47	*MATa trp1 leu2-3,-112 ura3-52 tif32Δ gcn2Δ URA3::GCN2 ura3* pRSTIF32-His-L (*TIF32-His LEU2*)	(3)
YBS53	*MATa trp1 leu2-3,-112 ura3-52 tif32Δ gcn2Δ URA3::GCN2 ura3* pRStif32-Δ8-His-L (*tif32-Δ8-His LEU2*)	(3)
TK145	*MATa trp1 leu2-3,-112 ura3-52 tif32Δ gcn2Δ URA3::GCN2 ura3* pRStif32-Box6-His-L (*tif32-box6-His LEU2*)	This study
TK142	*MATa trp1 leu2-3,-112 ura3-52 tif32Δ gcn2Δ URA3::GCN2 ura3* pRStif32-R731I-His-L (*tif32-R731I-His LEU2)*	This study
AL150	*MATa/MAT@ his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 asc1Δ::KanMX3/ asc1Δ::KanMX3*	(28)
H428	*MATa leu2-3,-112 ura3-52 hcr1Δ*	(9)
YER27	*MATa trp1 leu2-3,-112 ura3-52 tif32Δ gcn2Δ asc1Δ::KanMX3* pRSTIF32-His-U *(TIF32-HIS, URA3)* pFL45s/ACTU24 (*SNR24, TRP1*)	This study
H503	*MATa leu2-3 112 his4-539 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG*	(57)

Table S2 Plasmids used in this study.

Plasmid	Description	Source or reference
YCplac111	single copy cloning vector, *LEU2*	(58)
YEplac181	high copy cloning vector, *LEU2*	(58)
YCplac22MET-W	single copy cloning vector with conditional *MET3* promoter, *TRP1* plasmid from YCplac22	K. Nasmyth
YCpNIP1-His-U	single copy *NIP1-His*, *URA3* plasmid from YCplac33	(7)
YEpNIP1-His-U	high copy *NIP1-His*, *URA3* plasmid from YEplac195	(7)
YEpNIP1-His-L	high copy *NIP1-His*, *URA3* plasmid from YEplac181	(7)
YEpNIP1-ΔB´-His-U	high copy *nip1-ΔB'-His* [1-570]*, URA3*plasmid from YEplac195	(7)
YCpNIP1-His-L	single copy *NIP1-His*, *LEU2* plasmid from YCplac111	(7)
YCpMJ-MET-NIP1-W	single copy *NIP1* under *MET3* promoter, *TRP1* plasmid from YCplac22	This study
YCpNIP1-Myc-L	single copy *NIP1-Myc*, *LEU2* plasmid from YCplac111	This study
YCpNIP1-Δ60 to Δ180-MYC-L	single copy *NIP1-Myc* truncated by 60, 80, 100, 120 or 180 amino acid residues, *LEU2* plasmid from YCplac11	This study
YCpNIP1-693A702 to YCpNIP1-743A752-Myc-L	single copy *NIP1-Myc* containing 10 Ala substitutions between amino acid residues 693 and 752, *LEU2* plasmid from YCplac111	This study

YEpNIP1-743A752-Myc-L	high copy *NIP1-Myc* containing 10 Ala substitutions between aa 743 and 752, *LEU2* plasmid from YCplac181	This study
YEpNIP1-723A732-Myc-L	high copy *NIP1-Myc* containing 10 Ala substitutions between aa 723 and 732, *LEU2* plasmid from YCplac181	This study
pRS416	low copy cloning vector, *URA3*	(28)
pRSASC1-U	low copy *ASC1*, *URA3* plasmid from pRS416	(28)
pRSASC1-intron-less-U	low copy *ASC1* lacking its intron, *URA3* plasmid from pRS416	This study
pFL45S	high copy cloning vector, *TRP1*	This study
pFL45s/ACTU24	high copy *SNR24*, *TRP1* plasmid from pFL45s	(32)
pRSTIF32-His L	low copy *TIF32-His*, *LEU2* plasmid from pRS315	(7)
pRStif32-Δ8-His-L	low copy *tif32-Δ8-His* [200-964], *LEU2* plasmid from pRS315	(7)
pRS-a/tif32-box6-His-L	low copy *tif32-box6-His*, *LEU2* plasmid from pRS315	(14)
pRS-a/tif32-R731I-His-L	low copy *tif32-R731I-His*, *LEU2* plasmid from pRS315	(14)
YEpLVHCR1-L	high copy *HCR1*, *LEU2* plasmid from YEplac181	(16)
pT7-18S rRNA	full length 18S rDNA under T7 promoter	(12)
pT7-25S rRNA	full length 25S rDNA under T7 promoter	(16)

pT7-18S rRNA-I	domain I of 18S rRNA [24-612] under T7 promoter	(16)
pT7-18S rRNA-II	domain II of 18S rRNA [613-1148] under T7 promoter	(16)
pT7-18S rRNA-III	domain III of 18S rRNA [1148-1798] under T7 promoter	(16)
pKA18	β-globin mRNA under SP6 promoter	(60)
pGEX-5X-3	cloning vector for GST fusions	Pharmacia
pGEX- c/NIP1-571-812	GST-c/NIP1 [571-812] fusion, from pGEX-5X-3	This study
pGEX- c/NIP1-701-812	GST-c/NIP1 [701-812] fusion, from pGEX-5X-3	This study
pGEX-c/nip1-743A752	GST-c/nip1-743A752 fusion, from pGEX-5X-3	This study
pGEX-c/nip1-723A732	GST-c/nip1-723A732 fusion, from pGEX-5X-3	This study
pGBKT7	cloning vector for GAL4 DNA-binding domain fusion, *TRP1*	Clontech
pGADT7	cloning vector for GAL4 activation domain fusion, *LEU2*	Clontech
pGADT7-ASC1-exon1	*ASC1*-exon1 under T7 promoter cloned into pGADT7, *LEU2*	This study
pGADT7-ASC1-exon2	*ASC1*-exon2 under T7 promoter cloned into pGADT7, *LEU2*	This study
pGAD-NIP1	*NIP1* under T7 promoter cloned into pGADT7, *LEU2*	(16)
pGAD-c/NIP1-571-812	*NIP1-571-812* under T7 promoter cloned into pGADT7, *LEU2*	This study
pGAD-c/NIP1-701-812	*NIP1-701-812* under T7 promoter cloned into pGADT7, *LEU2*	This study

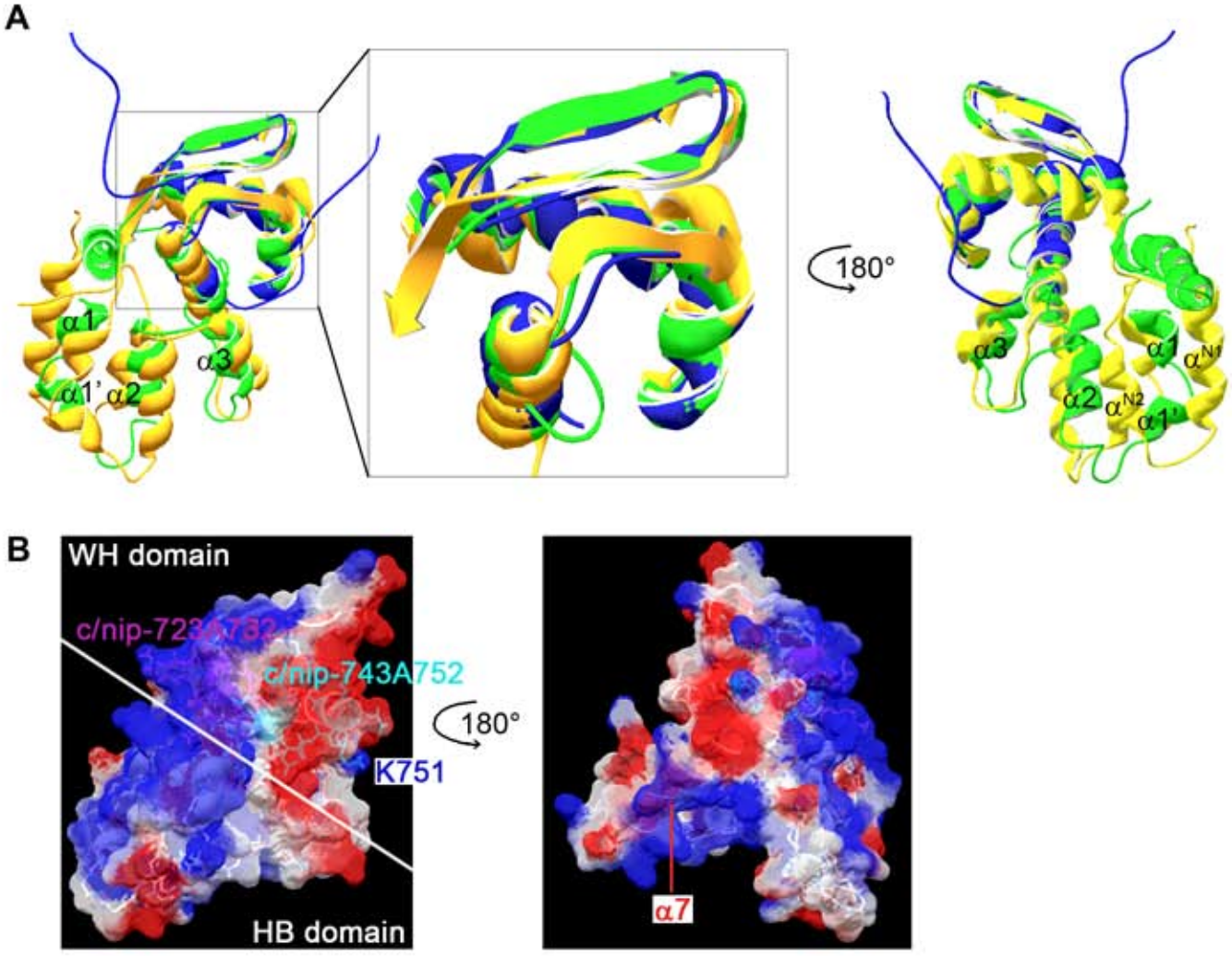
p1780-IMT	high-copy *SUI2, SUI3, GCD11, IMT4*, *URA3* plasmid from YEp24	(61)
p180 (YCp50–GCN4–lacZ)	low copy *URA3* vector containing wild-type *GCN4* leader	(62)

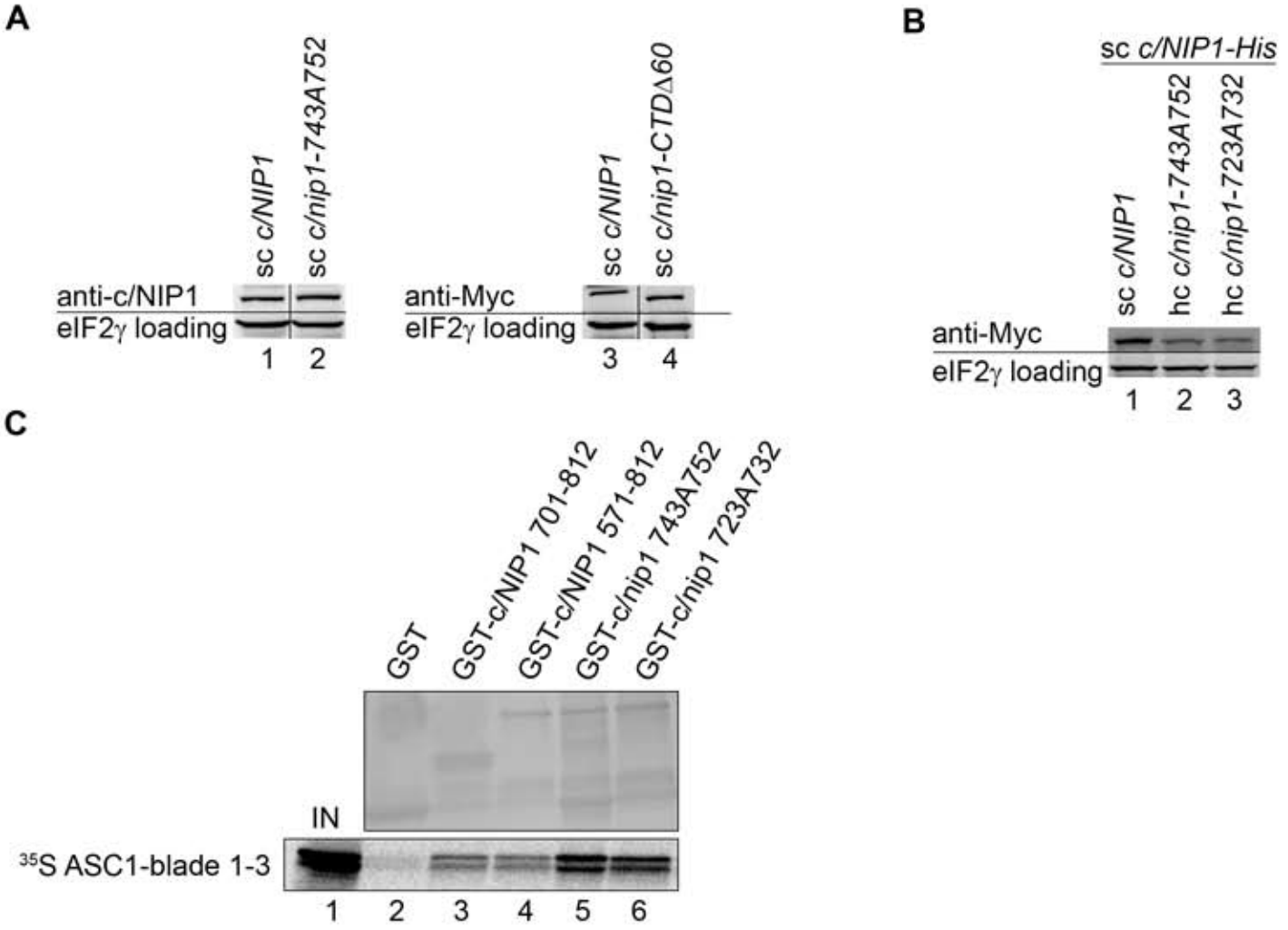
Table S3 List of oligonucleotides used in this study.

Oligonucleotide	Sequence (5' to 3')
TK1	CCCCGTCGACTCGAGACGACGATTTGATGG
TK2	CCCCGGATCCTCGATTCATCCCTACAA
TK3	CCCCGGATCCCTTTATTACCAAACATG
TK4	CCCCGGATCCAAATGGCATCTAACGAA
TK5	CCCCGTCGACTCGAGTTAGTTAGCAGTCAT
TK30	GCTTGGAACTTAAACCAATTCCAAA
TK31	TTTGGAATTGGTTTAAGTTCCAAGCCTTAACCATTTTGTCGTTACCGGCA
TK32	GCCATCTGTAGCCTTATGACTATGT
TK35	ACTGAATTTAATGAACTCCTTGCAG
TK45	ACACAGGAAACAGCTATGAC
AJ2	GATTCACTATAGGGCGAATTGG
TK46	AGACCAGCTCGATTCTGC
TK51	CCCCGGATCCTCACAGGTCCTCCTCTGAGATCAGCTTCTGCTCACGACGATTTGATGGTGGG
PS1	CCCCGGATCCTCACAGGTCCTCCTCTGAGATCAGCTTCTGCTCCACCTTATTTTCTGGAAGATC

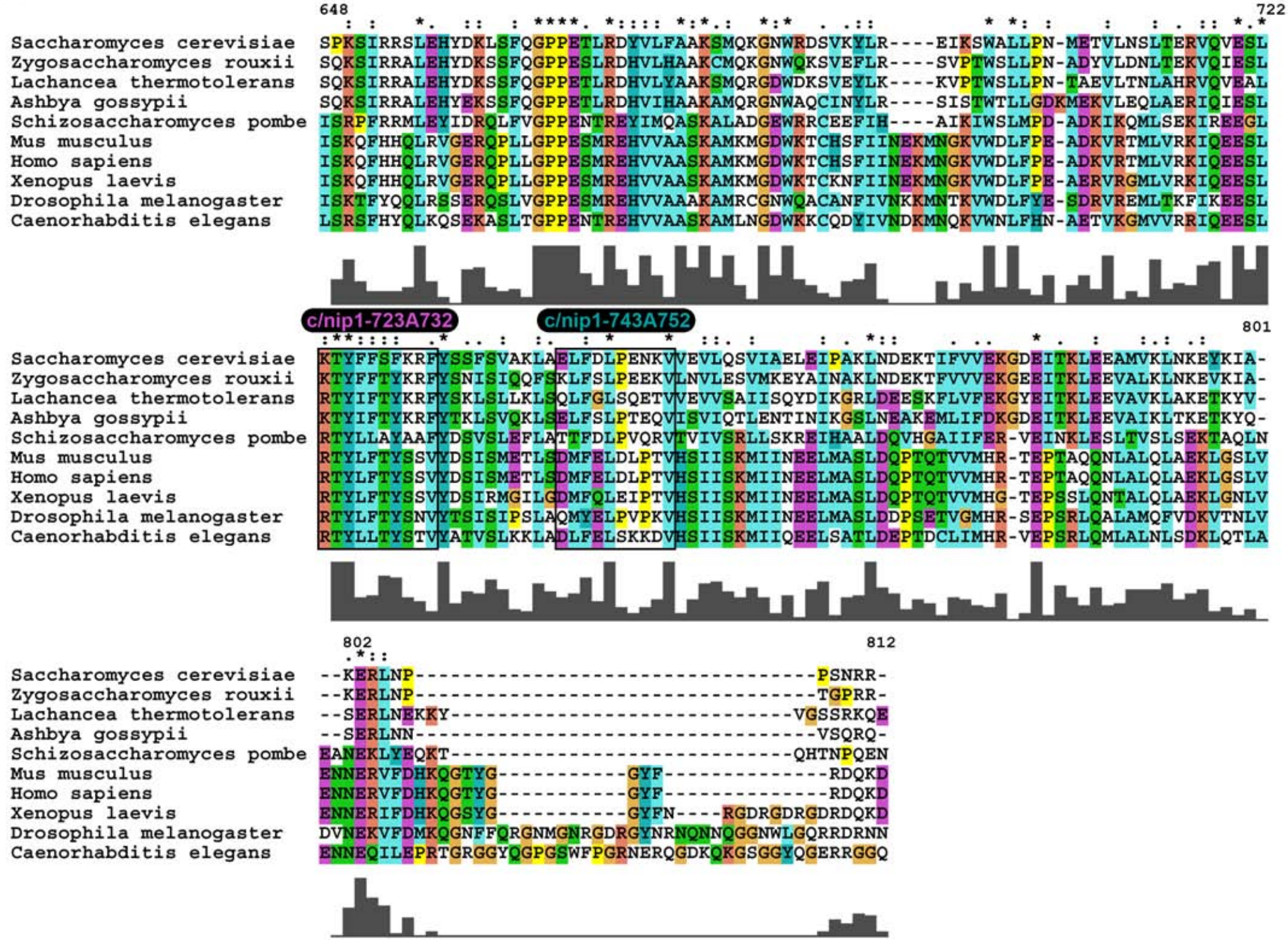
PS2	CCCCGGATCCTCACAGGTCCTCCTCTGAGATCAGCTTCTGCTCGTATTTAACAGAGTCTCTCC
PS3	CCCCGGATCCTCACAGGTCCTCCTCTGAGATCAGCTTCTGCTCTGGAATTTCGATCAATAAGG
TK52	CCCCGGATCCTCACAGGTCCTCCTCTGAGATCAGCTTCTGCTCGAACCTCTTGAAAGAAAAGA
TK53	CCCCGGATCCTCACAGGTCCTCCTCTGAGATCAGCTTCTGCTCACTATTCAACACCGTTTCCA
TK55	TCTGTTGCTAAACTAGCCGAAGCAGCTGCTGCAGCAGCAGCAGCAGCAGCAGAAGTTTTGCAATC TGTTATC
TK56	TTCGGCTAGTTTAGCAACAGA
TK57	TTTTCTTTCAAGAGGTTCTATGCAGCTGCTGCAGCAGCAGCAGCAGCAGCATTATTTGATCTTCCA GAAAAT
TK58	ATAGAACCTCTTGAAAGAAAA
TK59	GTACAAGTTGAATCTTTGAAGGCAGCTGCTGCAGCAGCAGCAGCAGCAGCATCAAGTTTTTCTGT TGCTAAA
TK60	CTTCAAAGATTCAACTTGTAC
TK61	GAAACGGTGTTGAATAGTTTAGCAGCTGCTGCAGCAGCAGCAGCAGCAGCAACTTATTTCTTTTCT TTCAAG
TK62	TAAACTATTCAACACCGTTTC
TK63	ATAAAATCTTGGGCTTTATTAGCAGCTGCTGCAGCAGCAGCAGCAGCAGCAACGGAAAGAGTACA AGTTGAA
TK64	TAATAAAGCCCAAGATTTTAT
TK65	TGGAGAGACTCTGTTAAATACGCAGCTGCTGCAGCAGCAGCAGCAGCAGCACCAAACATGGAAA

	CGGTGTTG
TK66	GTATTTAACAGAGTCTCTCCA
TK74	TTCTTCGGATCCTCACAGGTCCTC
TK94	CCCCGGATCCAAGCTTGGAACTTAAAC


A
α1
α3
α1' α2
180°
α3
α1
α^N1
α2
α^N2
α1'
B
WH domain
c/nip-723A732
c/nip-743A752
K751
HB domain
180°
α7


A
sc c/NIP1
sc c/nip1-743A752
anti-c/NIP1
eIF2γ loading
1 2
sc c/NIP1
sc c/nip1-CTDΔ60
anti-Myc
eIF2γ loading
3 4
B
sc c/NIP1-His
sc c/NIP1
hc c/nip1-743A752
hc c/nip1-723A732
anti-Myc
eIF2γ loading
1 2 3
C
GST
GST-c/NIP1 701-812
GST-c/NIP1 571-812
GST-c/nip1 743A752
GST-c/nip1 723A732
IN
35S ASC1-blade 1-3
1 2 3 4 5 6

A


648
722
Saccharomyces cerevisiae
Zygosaccharomyces rouxii
Lachancea thermotolerans
Ashbya gossypii
Schizosaccharomyces pombe
Mus musculus
Homo sapiens
Xenopus laevis
Drosophila melanogaster
Caenorhabditis elegans
c/nip1-723A732
c/nip1-743A752
801
802
812