Supplement to Materials and Methods

Plasmid constructions

Plasmids listed in Table 1S were constructed by using standard molecular biology methods (1) and amplified *E. coli* TOP10F' (Invitrogen). For PCR ampl**if**ication, chromosomal DNA of X2180-1A strain usually served as template using Taq polymerase (Roche) under common PCR condition unless mentioned for specific condition.

pVANPH2 (2) was the parental vector for addition of various tag sequences to the *RNH2A* gene. The start of the fragment is 605 bp upstream (*Xho*I) of the ATG start site for translation and extends 405 bp downstream (*Ngo*MIV) of the translation termination codon.

pVANPH2-FLAG2

pVANPH2 FLAG2 is the same as pVANPH2 but has the following sequence inserted as shown in bold type upper case and results in production of RNH2Ap with two tandem FLAG tag sequences at the C-terminus of RNH2Ap.

…accctagataattggtaccggt**TAGTCGACTATAAAGACGACGATGACAAGCTTGCCC**
GGTTAGTCGACTATAAAGACGACGATGACAAGCTTGCCCGGTgatcacct

pYESH2HF

pYESH2HF was derived from pVANPH2-FLAG2.

To construct pYESH2HF, PCR amplification generated RNH2A gene containing two successive hemaglutinin tag sequence (2x HA) at 3'-end of the gene by using two primers:

BC915 (5'-CATATGGTACCCCCCACGGTAGAAGC-3', *Nde*I site underlined) and BC916 (5'-GTCGAC**TGCGTAGTCAGGCACATCATACGGATA**ACC **TGCGTAGTCAGGCACATCATACGGATA**ACCCCGGTACCAATTATCTAGGG-3', *Sal*I site underlined and HA sequence in bold). Resulted PCR product was cloned into pCRII-TOPO (Invitrogen). Digestion by *Xba*I (which cuts in the middle of *RNH2A*) and *Sal*I (cuts between the *RNH2A* coding sequence and the FLAG-tag sequence) produced a DNA fragment that was used to replace the corresponding fragment of pVANPH2FLAG2. This replacement resulted in pYESH2HF, a pVANPH2FLAG2-derived expression vector containing the *RNH2A* gene and tandem affinity tags (2xHA-2xFLAG) at 3'-end of the gene. The sequence below is for the C-terminal region of the coding sequence for RNH2Ap with the HA tag shown as bold italic type.

…accctagataattggtaccgg***GGTTATCCGTATGCTGTGCCTGACTACGCAGGTTATCCGT ATGATGTGCCTGACTACGCA*****GTCGACTATAAAGACGACGATGACAAGCTTGC CCGGTTAGTCGACTATAAAGACGACGATGACAAGCTTGCCCGGT**gatcacct

pYESH2HFH and pETH2HFH

To construct pYESH2HFH and pETH2HFH, another affinity tag was attached to RNH2A-2xFLAG-2xHA by using same PCR techniques. For this modification, pYESH2HF served as PCR template and two primers, BC977 (5'-

CCATGGTACCCCCCACGGTAGC 3', *Nco*I site underlined) and BC978 (5'-CTCGAGTTA**ATGATGATGATGATGATGATG**AGCCTTGTCATCGTCGTC-3', *Xho*I site underlined and Hexahistidine sequence in bold) was used to add appropriate restriction sites and hexahistidine sequence (His6) at 3'-end of the gene. The PCR fragment containing RNH2A and triple affinity tags (2xHA-2xFLAG-His6) was cloned into pCRII-TOPO vector. The gene with triple-tag was then cut out from the cloning vector by *Nco*I and *Xho*I digestion and introduced into same restriction sites of pYX242 and pET24a and resulted in pYESH2HFH and pETH2HFH, respectively. The additional sequences are shown below underlined.

…accctagataattggtaccgg***GGTTATCCGTATGCTGTGCCTGACTACGCAGGTTATCCGT ATGATGTGCCTGACTACGCA*****GTCGACTATAAAGACGACGATGACAAGCTTGC CCGGTTAGTCGACTATAAAGACGACGATGACAAGGCTCATCATCATCATCA TCATCATTAACTCGAG**

pET279

To construct pET279, *RNH2B* (*YDR279W*) was amplified by using two primers BC1019 (5'-AGAAGGAGATATCATATGACCGTTTCCAAAATTGGG -3', *Nde*I site underlined) and BC1009 (5'-CTCGAGCTTACGTTTAAAAAATCCATC-3', *Xho*I site underlined) and cloned into pCRII-TOPO. A DNA fragment carrying the *RNH2B* gene was

generated by digestion with *Nde*I and *Xho*I and introduced into pET24a vector digested with *Nde*I and *Xho*I.

pAC154-2

To construct pAC154-2, *RNH2C* (*YDR154C*) was amplified by using two primers BC1010 (5'-CATATGACCAAAGATGCCGTGAAT-3', *Nde*I site underlined) and BC1011 (5'-CTCGAGCTGATTTATGACATCGATGAG-3', *Xho*I site underlined) and cloned into pCRT7/CT-TOPO. The *RNH2C* DNA fragment was produced by digestion using *Nde*I and *Pme*I enzymes and cloned into the *Nde*I and *Eco*RV site of Multicloning Site of pACDuet-1, thereby fusing the C-terminal coding sequence to the 6 His-tag of the pACDuet-1.

pACH2HFH and pACH2HFH154-2

To construct pACH2HFH and pACH2HFH154-2, the fragment containing *RNH2A* and triple affinity tag was cut out from pETH2HFH by using *Nco*I and *Eco*RI and introduced into same restriction sites in Multicloning Site 1 of pACDuet-1 and pAC154-2 to make pACH2HFH and pACH2HFH154-2, respectively.

Site-directed Mutagenesis of *RNH2A*

Site-directed mutagenesis of *RNH2A* was performed by two-step PCR using sets of PCR primers designed to introduce single amino acid change from Asp to Ala in RNH2Ap. A set of primers was used to create each substitution and pETH2HFH DNA was used as

template. Primer sequences used for mutation are as followed; for D39A mutation, BC1052 (Forward primer, 5'-ATGGGTATC**GCT**GAAGCTGGC-3') and BC1053 (Reverse primer, 5'-GCCAGCTTC**AGC**GATACCCATTAT-3'); for D155A mutation, BC1054 (Forward primer, 5'-TATGTG**GCT**ACTGTTGGACCA-3') and BC1055 (Reverse primer, 5'-AACAGT**AGC**CACATACACGTG-3'); and for D183A mutation, BC1056 (Forward primer, 5'-AAGGCA**GCC**TCGCTCTACTGC-3') and BC1057 (Reverse primer, 5'-GAGCGAGGCTGCCTTCTTGGC-3').

DNA for *RNH2A* was amplified by two separate PCR reactions. The first reaction amplified a 5'-fragment of *RNH2A* gene with the 3'-end of those fragments amplified using PCR primers with mutations as shown above. A second set of PCR reactions amplified a 3'-end fragment of the *RNH2A* gene using DNA oligonucleotides for the 5'-end of these fragments with mutant DNA sequences. In these PCR reactions, Vent polymerase (NEB) was used thereby avoiding and additional nucleotides at end of PCR products. The resultant DNA fragments were purified after TAE-agarose electrophoresis and used for a PCR by mixing 1:1 ratio of the pair of DNA fragments. The complete *RNH2A* gene containing Asp → Ala mutation was then generated by PCR using BC977 and BC978 and Taq polymerase. The resultant PCR fragments were cloned into pCRII-TOPO vector. DNA for each mutated *RNH2A* gene was digested with *Nco*I and *Xho*I and cloned into the same restriction sites in pYX242 for expression in *S. cerevisiae.*

Preparation of samples for analysis by SELDI

All proteins used in these experiments were obtained as gel slices following SDS-PAGE stained with Coomassie R-350. “No protein” control gel slices were included into each experiment. Gel slices were processed by destaining and SDS removal with two 0.4 ml washes in 50% methanol, 10% acetic acid; 1 h each and equilibration of the gel slices in 0.4 ml of 0.05 M ammonium bicarbonate, pH 8.0 for 1 h at room temperature followed by incubation at 60 ^{o}C for 30 min in 150 µl of 0.05 M ammonium bicarbonate, pH 8.0, 45 mM DTT. Buffer was removed and gel slices were sequentially treated with 0.5 ml of 50% acetonitrile in 0.05 M ammonium bicarbonate buffer, pH 8.0 for 1 h and 50 µl 100% acetonitrile for 15 min, followed by drying the gel pieces in a Speedvac.

Trypsin (Roche) was dissolved in water to the final concentration of 1 µg/µl. Dried and crushed gel slices were resuspended in 50 µl of 50 mM ammonium bicarbonate buffer, pH 8.0 (freshly prepared) containing 12.5 ng/µl of trypsin and digested for 16 h at 37 ^{o}C. Supernatants were collected and dried in a SpeedVac for 20 min. Peptides were dissolved in 10 µl of H_2O and 3 µl were applied per spot of NP20 ProteinChip® array (Ciphergen Biosystems, Inc) and air dried; 1 µl of 20% CHCA in 50% acetonitrile-0.5%TFA was applied per spot for PBSII (Ciphergen Biosystems, Inc.) analysis. Data were obtained at laser intensity 185, sensitivity 9. SwissProt database was searched using ProFound (3) search engine and peptide masses, excluding peptides resulting from trypsin autodigestion.

Protein identification by LC MS/MS:

Peptides from tryptic digests of gel bands were also analyzed by tandem liquid chromatography/mass spectrometry (LC-MS/MS). Peptides were dissolved in 0.1% TFA and separated by reversed phase chromatography, using Vydac C18 resin (5 micrometer particle, 300 angstrom pore packing), packed into a 75 micrometer I.D. fused silica capillary (PicoFrit, New Objective, Woburn, MA). Peptides were separated at flow rate of 400 nl/min. using a linear gradient from 2-85%B (Buffer A, 5% acetonitrile in water with 0.5% acetic acid and 0.005% TFA; Buffer B, 80% acetonitrile, 10% n-propanol, 10% water, with 0.5% acetic acid, 0.005% TFA). The LC effluent was electrosprayed directly into the sampling orifice of an LCQ DECA mass spectrometer (Thermo Finnigan, San Jose, CA). The LCQ DECA was operated to collect MS/MS spectra in a data dependent manner, with up to three of the most intense ions being subjected to isolation and fragmentation. MS/MS data were analyzed and matched to protein sequences in the NCBI non-redundant database using Mascot (4).

Figure 1S Alignment of RNase H2 subunits from *Saccahromyces* sensu stricto and *Candida albicans*

Alignment of various fungal RNase H2 subunits with identical residues in the *Saccharomyces* sensu stricto species marked by asterisks.

A.
Rnh2Ap

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  S.cerevisiae ~~MVPPTVEA SLESPYTKSY FSPVPSALLE QNDSPIIMGI DEAGRGPVLG PMVYAVAYST QKYQDETIIP NYEFDDSKKL TDPIRRMLFS KIYQDNEELT
   S.paradoxus ~~MVPPTVEA SLESPHTKSY FSPVPTALLE QNVSPVIMGI DEAGRGPVLG PMVYAVAYST QKYQDETIIP NYEFDDSKKL TDPIRRMLFS KMYEDNEELT
    S. bayanus ~~MAPPTVEA SLDSEYTKSY FSPVPPQLLE QNDSPVIMGI DEAGRGPVMG PMVYAVAYST QRYQDETVIP NYEFDDSKKL TDPVRRKLFA KMYQDNEELT
     S.mikatae ~~MIPPTVEA SLGSSHTRSY FSPVPTALLE QNVSPVIMGI DEAGRGPVMG PMVYAVAYST QKYQDENIIP NYEFDDSKKL TDPIRRMLFA KMYENNEELT
S.kudriavzevii ~~MIPPTVEA SLRSPFTKSY FSPVPAALLE QNDSPVIMGI DEAGRGPVMG PMVYAVAYST REYQDDIVIP NYEFDDSKKL TDPVRRKLFA KMYEDNEELT
   S.castellii ~~MLPPTVTD SIGSLHTKTH FSPVPLSVLK GD.SPVILGV DEAGRGPVLG PMVYGISYCT SQYQDEVLIP HYDFDDSKKL TDAVRRDLFH KMYETR.DIE
    S.kluyveri MQELPPTVPS SLDSLHTKTF YSNIPESIVE SN.TPVIIGV DEAGRGPVMG PMVYGISYCT LEYQEAILKP KYAFDDSKKL TDPVRRNLFS KMY.SG.EIG
C.albicans .AISLDWLPPSVTN IEDPFKFASS TYHSEIPQSILQNKTEPIVLGVDEAGRGPVLG PMVYGIAYSL ESFLS.KLQK EYGFADSKVL TDVKREELFK QIEDPDHELH

               101                                                150
                 ***** ** * ******   ***  *****  ******* * * **         ******** **  ******  ***    ** * ********  *********
  S.cerevisiae QIGYATTCIT PLDISRGMSK FPPTRNYNLN EQAHDVTMAL IDGVIKQNVK LSHVYVDTVG PPASYQKKLE QRFPGV.KFT VAKKADSLYC MVSVASVVAK
   S.paradoxus QIGYATTCIT PLDISRGMSK FPPTRNYNLN EQAHDVTMAL IDGVIRQNVK LGHVYVDTVG PPASYQKKLE QRFPDV.KFT VSKKADSLYC MVSVASVVAK
     S.bayanus QIGYATTCIT PLDISRGMSK FPPTRNYNLN EQAHDVTMAL IDGVTKQNVK LDHVYVDTVG PPASYQKKLE QRFPDI.KFT VAKKADSLYC MVSVASVVAK
     S.mikatae QVGYATTCIT PLDISRGMSK FPPTRNYNLN EQAHDVTMAL IEGVIKQNVK LDHVYVDTVG PPASYQKKLE QRFPGI.KFT VAKKADSLYC MVSVASVVAK
S.kudriavzevii QIGYATTCIT PLDISRGMSK FPPTRNYNLN EQAHDVTMAL IDGVTKQNVK LDHVYVDTVG PPTSYQKKLE QRFPDI.KFT VAKKADSLYC MVSVASVVAK
   S.castellii DVGYATTVIT PTDISSGMLR FPPSKNYNLN QQAHDVTMDL IKGVLDRGVQ LSHVYVDTVG PPASYQKKLE DRFPEVGAFT VAKKADSLYC VVSVASVVAK
    S.kluyveri GVGYATTAIT PVDISSGMLR FPPSKNYNLN EQAHDVTMEL IQGVLDRNIA VEHVYIDTVG PPLPYQKKLE QRFPHV.KFT VAKKADSLYC IVSVASVVAK
  C.albicans   KHIGWATTTMTARDISSGMLQSVNGKGAYNLN EQAHDTTINL IKQVLAKGVK ISKIFVDTVG PPVTYQAKLK RFFPEIDVT. VTKKADSIYP IVSTASVVAK

               201                                                250
               **** * * * **  **     ***********  **    *     *   ***** *****    *        * * **    * **            *
  S.cerevisiae VTRDILVESL KRDPDEI...LGSGYPSDPKTVA WLKRNQTSLM GWPANMVRFS WQTCQTLLDD ASKNSIPIKW EEQYMDSRKN AAQKTKQLQL QMVAKPVRRK
   S.paradoxus VTRDILVESL KRNPDEV...LGSGYPSDPKTVT WLKRNQTSLM GWPANMVRFS WQTCQTLLDD TTKDSILIKW EEQYMDSRKN AAQKTKQLQL QMVAKPVRRK
     S.bayanus VTRDILVESL KRDPDEV...LGSGYPSDPKTVA WLRRNQTSLM GWPANMVRFS WQTCQTLLDD ASKDSIIIKW EEQYMDSRKN AAQKTRQLQL QMVGKPARRK
     S.mikatae VTRDILVESL KRNSDEV...LGSGYPSDPKTVA WLKRNQTTLM GWPANMVRFS WQTCQTLLDD PARDSILIKW EEQYMDSRKN AAQKTKQLQL QMVTKPPRRK
S.kudriavzevii VTRDILVESL KRDPDEV...IGSGYPSDPKTVA WLKRNQTSLM GWTTDMVRFS WQTCQTLLDD PTRHSVIIKW EEQYMDSRKN AAQKTRQLQL QMVAKSARRK
   S.castellii VTRDILVESL KRSDDEI...IGSGYPSDPKTVK WLRETQTPLF GWPIEMVRFS WQTCQTLMKD GNNGSIPIEW EEDFINSKKN MAQ....... QWTLDDQNSK
    S.kluyveri VTRDVLLEQL KSYPDEV...LGSGYPSDGKTVS WLHGAKTPLF CWPQQMVRFS WQTCQNII.D KDQDTIPIEW EEDYINSKKN LSS....... QWSF..KGKE
  C.albicans   VTRDTNIKFYNENLPLLQGQKLGSGYPSDPNTSK WLNSNVDPVF GWCYGFIRFS WQTAKDSLVK .NNAA.EVVY EDQGKGVEKG YQD....... ..VFAMIDKK

               301         311
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  S.cerevisiae RLRTLDNWYR
   S.paradoxus KLRTLDNWYQ
     S.bayanus TLRTLDNWYQ
     S.mikatae RLITLDNWYQ
S.kudriavzevii RLRTLDNWYQ
   S.castellii KVITLDSWF
    S.kluyveri KLITLDNWYS T
  C.albicans KDNSKLKRNYFCSSSNVNLL
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B.
Rnh2Bp

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             1                                                  50                                                 100
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S.cerevisiae MTVSNIGGEE ....RLIILP DDY..ETSKT INTFTLPPPS NITSKPRIEL FEN.INGKLYE IRSFQFGKGP SYSHEEDLAN DKYHYTKENH PIKSTFIVNT
 S.paradoxus MTISDIGSEG ....RVIILP DGY..EASKN INTFTLPPPS NITSKHRIEL FED.ISGKLYE IRSFQFGKGP SYSHEEDLAN DKYHYTKENH PIKSTFIVNT
   S.mikatae MTISDIEGEE ....RIIILP DDY..DVSKN INSFTLPPPS NIASKPRIEL FED.TNGKVYE IKSYQFGKGP SYSHEEDLAN DKYHYTKENH PIKSTFIVNT
   S.bayanus MTIAKTGGEE ....RLIILP DEC..DASKI INTFTLPPCS NIESKPLIEL FEN.TDGRLYQ VKSFQFGKGP SYSHNEDLAN DKYHYAKDDH PIKSTFIVNN
S.kudriavzev MSTCDIGGEE ....RLLILP DEC..DASKN INTFTLPPSC NIASKPRLEI FEN.TDGKLYE IKCFQFGKGP SYSEEEDLAN DKYHYTRENN PIKSALMVNT
 S.castellii MTI...NQEE QNTQKLVILP NSLTKNANTS IQVFSLPHPS NITSKPRIAL IQG.IDNQLFQ VHTHSFGRSS AYNYRSDLAN EKYHYTKDSK PVKSTFLVNE
  S.kluyveri MSTSGINKE. ...RRVLVLP STI....DSD LKLIKLPHPS NNLSKEPIII FHH..NGKCYR LNTHTFSKGS QYNQAKDLST EKYHYTNEQN PLKSTFLTNK
  C.albicans       MDKE ...SKVIILP KTN...SSSE FKIINLPNPT NLTTTKSYLLHHNEDSNNQLYE LNIIGGEDDNDGNDDEKTDNGNAGTKNKGQKPKSKLKSENSIKS
             101                                                 150                                                200
                  *     *            * * *           *  **                    **  **   * * *      *   *   *    *   *  *
S.cerevisiae SDPTDGYVFN SSKIHFCSLY DIAFSLIGFY YRN.SVSADE QDYSNSSDTG ENQKSNSKTN EKFLTVRDYH DFLTDNHDKN WENISLSRLK SGLAKVSETI
 S.paradoxus SNPSEGYVFK SSKIYFCSLH DITFSLIGFY YKN.SVTADE QDYANSSDTN DNQSSSSKNH ERFLTVRDYH DLLTDTHDKN WDSISLSCLK SGLTKVSETI
   S.mikatae SDPADGYIFK SSKIHFCCLH DVTFSLIGFY YRN.SISEDE QDYVNSNDTS VNQTSNKKNH ERFLTARDHH DFLTDNHDKN WDNISLSCLR GGLAKISETI
   S.bayanus SNPADGCILN SSKLYFCTLH DVVFSLIGFH YRT.SVAEDE QDYVQPSDTG ENQTANRKDH ERFLTVRDYH DLLTDNHAKN WNHISLRCLE SGLTKISETI
S.kudriavzev SDSADGYIFK SSKIYFCTLY DIAFSLIGFH YKN.SISADE QDYVKSSNTD EEQTAERKEH ERFLTLRDYH DLLTDNHDKN WNNISLGSLK CGLAKISETI
 S.castellii EHREDGYILE SSEFQFTTKY NVVFNLIGFF FKDASEVEQE CDYIKPLVKN E....TIKVD DKFLPLRDYH DLLVDTYDKQ WMNISLKTLE NALSEISEVI
  S.kluyveri SDRMDGYILE SGDFHYSTKY DLCFSLCGAY YSE.NITQSE SDYLNKYTGN .....SVEHD NRFLTVRDFQ DLLIDKHDQQ WSHVSVEALE SALTEISDSI
  C.albicans LIFEPGYVLQ SPKIIISNKF NLSYLLISLF LNI.NQQKSQ QQSQQSSLSS ENLFNDNFKSLEDLKDQLLNEYREDTNNND.. WVLEIPDQLY HQSLINLCDVIT

             201                                                 250                                                300
             ** ** * ** *       *    * **  ** *   *         *    *      ** ** **   * *                              *
S.cerevisiae EEAGDVYYKI TSAMITQFLL GKVSKIVENF PPSIPTLKNA PTEIKQCYKV VMATNLLVSL IPRAAYHNLL TFSPTMDSGC LNPDIKASFI ELENYETTNE
 S.paradoxus EECGDVYYKI TPAMITKYLV GKVSKIVKNF PPSIPTLKNV PTEIEQSYKV VTATSLLISL IPRAAYHNLV TFSPTMASSC TNLDIKASFI DLENYEITKE
   S.mikatae EEAGEVYYKI TPAMITKYLV EKVSKIAENF PPSVPILKHA TTEIEQCYKV VVATNLLVSL IPRAAYHNLV DFSPTMGSYC SDLDIKASFT SLEDYETKNE
   S.bayanus EEAGEIYYKI TPALITRYLV GKVLKITENF PPSFPILKHA PAEIVQCYKV VMATNLLVSL IPKAAYRNLI EFSPSTTDCN FSLEIKASFT NLENYQATNE
S.kudriavzev EEAGDVYYKI TPAMITQYLA GKVAKVIENF PPSIPTLKHA PVEITQCHKV VIAVNLLVSL IPKAAYHNLV EFSPTTTDTF FNLHIKASFT DLEKYETTNE
 S.castellii EEAGDKYYKI TREKITNVLI SRVQKIVENF PKSLPIPIDY PDEIKHCAKV TLACNLLISL IPKMAYEDLI VFE...SSDD AKLNISGLIS KFKKYEDEHA
  S.kluyveri EEAGDTYHKI TIEKVVEWLM RKVSKIVDNF PQTLPMEKNM PADITSYAKI VYSCNLLVSL IPQLVYRALI T.....SKND I.LDIAGAFT KYTEYIETTL
  C.albicans ENIDESFYRF DLSKILHWLN EKVL.ALQKY ILTNDNSILT KLKLELNPS. .MSNNNIEDQ LLNDLGLLYS IDYIFNSYLDDGVNSFLRQKLLEEFKYDFTRV
             301                                                 350
                 *   *    *   **                                  *                *********
S.cerevisiae LQNAERELLM KSAMNVGLN. .......... .........S NGRVSLPVKK .VTKKIVQNKKPKVAIGKGAIDGFFKRK~
 S.paradoxus SQNAEKELLM KSAVNVGLS. .......... .........S NGSVSLSVKK .VTKKVIQSKRPKVAIGKGAIDGFFKRK~
   S.mikatae LKNAEKELLM KSAMNVGLN. .......... .........T NSRASLSIKK .VTKKVVQSKKLKVATGKGAIDGFFKRK~
   S.bayanus LKNVEKELLM KSAMNVGIG. .......... .........T NGG.SVSVKK .VAKKVVLSKKPRVAIGKGAIDGFFKRK~
S.kudriavzev LKNAEKELLM KSATNIGLG. .......... .........S NGSVSVAMKK .VTKKIPQSNRTRVAIGKGAIDGFFKRK~
 S.castellii AAIVEKDILI NAAISVGLG. .......... .........E NSGKRNKVSK .PVIKKKEVIKKKVAIGKGAIDGFFKKGK
  S.kluyveri QEEKEKEILL QAAVKTGLG. .......... .........N SETKKLGVKK .VVISKRIAKVKKITVGKGAIDGFFKKK~
  C.albicans LKHIDDLKIQ QSLIENVLES LKIQQSLIEN VLESNLKSTT NLKSTTNNTKSSSTKKKPATGTTNKKVKRGAIDSFFKKAK
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C.
Rnh2Cp

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            1                                                  50
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S.cerevisiae ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~~~~~~MTKDAV NL.DAYTVSF MPFYTEYQGP TEEF....KDYKFE
 S.paradoxus ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~~~~~~MTKDAV NL.DTYTVNF MPFYTEYEGP TGEF....KDYKFE
   S.mikatae ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~~~~~~MTKD.T TL.DAYALNF MPFYTEYQGP TKEF....KDYKFE
   S.bayanus ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~~~~~~MTKDTG SL.DAYTVNF MPCYTEYEGP TTEF....KDYKFD
S.kudriavzev ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~~~~~~MTKDTT DL.NEYAVSF MPFYTEYQGP TAEL....KDYTFG
 S.castellii ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~M EDCTIE..LT DAPTTYTASF VPLKIRYNGP TTEFVNNFKDNKTPTKDD
  S.kluyveri MLRRDASWSA LSSSTCHRAV RHHCQASGWQ VQRTQRCRGL WTSSKENMNR PEILIEKPLE SLPTSYQAHF VPCKVRYSGT TQEFKNQFQLD

            101                                150
                         **                     *               ******        *     *
S.cerevisiae DTI....... ......YF RGKELK........REKSATPSSSDNTTSNTFSNGAILSGNTITGKIVSVNNYEREGTDRN... ELARLQELIS LIDVINQ
 S.paradoxus DTV....... ......YF RGKELQ........REKLVTTANSD.STTDTFSNGAIFSGNTIIGKVVSVNNYEREGTNRN... ELARLQELIS LIDVINQ
   S.mikatae DTI....... ......YF RGKELQ........REKPATTSNS.NSTNDRLSNGAILSGNAITDRVVSVNNYEREGTNRN... ELARLQELMS LIDLINQ
   S.bayanus DTI....... ......YF RGKELQ........KEKPTSTATHDTATVG.LSNGAILSGNTVTGKIVAVNNYEREGTDRN... ELERLQELIY ITNVINQ
S.kudriavzev DTI....... ......YF RGKELQ........REKLATATACDCMSA.KLSNGAILSGNTINGRIVAVNNYEREGSNRN... ELARLQELIS LTNVINQ
 S.castellii EINNYSTDTT NTTHTTFI RGRCLHGTPVNKYFESASAHVIGKSKQTDNDIENQTY....TVQSTVNSIINYEREGNTQRLTE ELAHLKEFVQ LQNHIHS
  S.kluyveri QEEMQGTRAG KDEYVTYI RGRKIVGKEITA.LEGCLATLV...EETTNPDGNTTW....QPVASLSKLVNYEREGNEGRLEE EMGKFQEFVE LADLIHG
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Supplement to Results

Table 1S. List of yeast strains used in this work

Name	Genotype	Reference
X2180-1A	*MATα SUC2 mal mel gal2 CUP1*	
HIRO29	*MATα ade2 leu2 trp1 ura3 aro2 can1 hom2 sap3 rnh1Δ::URA3*	(2)
HIRO16A	*MATα ura3-52 leu2-3 leu2-112 his1-24 ino1 RNH1 rnh201Δ::kan^r*	(2)
HIRO27AF	*MATα ura3-52 leu2-3 leu2-112 his1-24 ino1 rnh1 rnh201Δ::kan^r*	(2)
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(5)
3638	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydr279Δ::kan^r*	(6)
4141	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ylr154Δ::kan^r*	(6)
279154	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ylr154Δ::kan^r ydr279Δ::URA3*	This work

Table 2S. Sequence of oligonucleotide substrates used in this work

Description	Sequence	Reference
$D_{12}R_4D_{12}$	AATAGAGAAAAGaaaaAAGATGGCAAAG	(7)
$R_{13}D_{27}$	gggaacaaaagcuTGCATGCCTGCAGGTCGACTCTAGAGG	(8)
R_6D_{38}	aaagcuTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTA	(8)
D_{40}	CCTCTAGAGTCGACCTGCAGGCATGCAAGCTTTTGTTCCC	(8)
$D_{12}R_1D_{27}$	GGGAACAAAAGCuTGCATGCCTGCAGGTCGACTCTAGAGG	(8)
D_{28}	CTTTGCCATCTTTTTTCTTTTCTCTATT	(7)
BD2 RNA	acugucaauuauccgcgagc	(9)
BD2-1 DNA	GCTCGCGGATAATTGACAGT	(9)

All sequences are written 5'→3'. Deoxynucleotides are in upper case and ribonucleotides are in lower case.

Results are shown for the identification of proteins by LC/MS/MS (similar data were obtained using SELDI):

Table 3S. Peptides matched to Ydr279p (gi|6320485)
(Total score = 124)

Amino Acid Numbers	Observed (m/z)	Mr (expt)	Mr (calc)	Peptide Sequence	Mascot Peptide Score*
12-23	703.9	1405.79	1405.73	LIILPDDYETSK	59
24-41	662.41	1984.19	1983.08	TINTFTLPPPSNITSKPR	32
280-304	1001.47	3001.39	3000.44	ASFIELENYETTNELQNAERELLMK (M oxidized)	40

Table 4S. Peptides matched to Ylr154p (gi|6323183)
(Total score = 110)

Amino Acid Numbers	Observed (m/z)	Mr (expt)	Mr (calc)	Peptide Sequence	Mascot Peptide Score*
79-87	547.52	1093.03	1092.56	IVSVNNYER	36
98-110	750.03	1498.05	1496.84	LQELISLIDVINQ	74

* Score is –10*Log (P), where P is the probability that the observed match is a random event. Individual ion scores > 44 indicate identity or extensive homology ($p < 0.05$).

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