SUPPLEMENTARY DATA

SUPPLEMENTARY MATERIALS AND METHODS

Yeast strains, media and growth conditions.
BY4741, MB159-4D and MJ15-9C yeast strains used in this study were described previously (1, 2). Heterozygous diploid RPB10Δ rpbl0Δ (BY4743 MATa/MATa ura3Δ0/ura3Δ0 leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 met15Δ0/MET15 LYS2/lys2Δ0 YOR210w/YOR210w::kanMX) and the deletion mutant upf1Δ (BY4741 MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 YMR080c::kanMX) were obtained from Euroscarf. RPB10Δ rpbl0Δwas transformed with plasmids containing wild type or mutated RPB10 alleles and subjected to meiosis. Generated rpb10Δ haploids expressing RPB10 alleles from the plasmids were subsequently crossed with the rpc128-1007 mutant (MJ15-9C MATa SUP11 ade2-1 ura3-1 lys2-1 leu2-3,112 his3) resulting in rpc128-1007 rpb10Δ[RPB10] or rpc128-1007 rpb10Δ[RPB10 Δ3’154]. The strain Upf1-TAP (MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-52 UPF1 TAP HIS3) kindly supported by A. Dziembowski, was crossed with rbs1Δ (BY4742 MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YDL189w::kanMX4) or Rbs1-Myc (MATa SUP11 ade2-1 ura3-1 lys2-1 leu2-3,112 his3 YDL189w::13Myc::KanMX6) (1) that resulted Upf1-TAP rbs1Δ or Upf1-TAP Rbs1-Myc strains.

The rpc128-1007 upf1Δ strain was obtained by transformation of upf1Δ deletion cassette, which was amplified by PCR from the genomic DNA of BY4741 upf1Δ by using specific Upf1For (5’-AGAAGGAGGAGCAGCAAGAC-3’) and Upf1Rev (5’-CGGCTCATTTACGCGTTGAG-3’) primers. Transformants were selected on YPD medium supplemented with geneticin (200 μg/ml) and the replacement of UPF1 with the kanamycin cassette was confirmed by PCR.

The RBS1-HTP strain was obtained by transformation of the appropriate sequence encoding the HTP tag, which was amplified by PCR using the pBS1539-HIS6-TEV-Protag plasmid as DNA template and specific primers, RBS1HTP_F (5’-CTAGGGATACTGATTCGCTAGATGAAATTGATAAAACATTTAGGAGCCATCACCATCACC-3’) and RBS1HTP_R (5’-TCTATAAACCGTACGTAAATTTCGCTATGTAGTTCACCTCCTACGTAGGCCCATTAGGG-3’). Transformants were selected on SC-ura medium. The presence of RBS1-HTP-encoding sequence was confirmed by western blot method with the PAP antibody.

As required, the YPD medium was supplemented with geneticin (200 μg/ml). Sporulation medium contained 0.05% glucose, 0.1% yeast extract and 1% potassium acetate. LB medium (1% Bacto tryptone, 0.5% yeast extract, 1% NaCl, pH 7.5) was used for growing E. coli strains. As required, the LB medium was supplemented with ampicillin (60 μg/ml). Solid media contained 2% agar. For analysis of the phenotypes, yeast strains grown on SC-ura, SC-leu or SC-ura-leu medium were replica-plated on YPD plates and incubated for 3 days at the desired temperature. Yeast cells were transformed with plasmids by using the lithium acetate method (3).

Plasmids
YEp181-RBSI, called here [RBS1], multicopy plasmid (LEU2, 2μ) containing the RBS1 gene (1). YEp181-RBSI_R3H, called here [RBS1_R3H], the conserved Arg57 aa and His61 aa, located within the R3H domain, were changed to alanine using primers Mut_rev (5’-ATAGAAGAGCCATAATATGAATTTCAGGTC-3’) and Mut_for (5’-CCGGCCCAAATAGCTAGTACCACA-3’). YEp181-RBSI-Myc and YEp181-RBSI R3H-Myc, called here [RBS1-Myc] and [RBS1 R3H-Myc] contain the wild type or mutated version
of RBS1 tagged with Myc epitope at the 3’ termini. They were obtained by transformation of BY4741 rbs1Δ [RBS1] or [RBS1 R3H] with the His3MX6 cassette PCR amplified from pFA6a-13Myc-His3MX6 plasmid using primers RBS1_F2 (5’-TACTGATTCCGTAGATGAATTTGATAAATTAAACATTCGGATCCCCGGGTTAATTAA-3’) and RBS1_R1 (5’-ACTAGAATTCTTATAACCCTTACGTATTTTTTCGCTATGTATAGTTCACTCCGAATTGGCTGCTGGTTAAAC-3’) (4). Deletion of the respective restriction fragments from the YEp181-RBS1 resulted in derivative plasmids, called here [RBS1 ΔC1] and [RBS1 ΔC2], containing deletion of fragments of RBS1 encoding 231-332 aa and 231-457 aa, respectively.

Fragments containing 211 bp upstream and 154 bp, 231 bp or 253 bp downstream of the RPB10 ORF were amplified by PCR the [RPB10] plasmid DNA by using RPBF (5’-GATGGCTACTACACTGGAAG-3’) and RPBR (5’-CCTACAGTATGCAGACAC-3’), 231RpbR (5’-ACTTCCTTTATCGTCTTGAAGAGT-3’) or 253RpbR (5’-TCGCACGATGTAACATCTACA-3’) primers, respectively. PCR products were introduced to pDrive vector (Qiagen), cut by restriction enzyme EcoRI and cloned into EcoRI site of centromeric plasmid pRS316 (URA3 CEN6) resulting in plasmids called here [RPB10 Δ5’Δ3’154], [RPB10 Δ5’Δ3’231] or [RPB10 Δ5’Δ3’253]. Both [RPB10 Δ5’Δ3’] plasmids were constructed in the same way. [RPB10 Δ3’154] plasmid containing 663 bp upstream and 154 bp downstream of the RPB10 ORF was amplified by PCR from the pFL44L-RPB10a plasmid (5) by using MGMF (5’-AGACAGACGATGACGATCG-3’) and RPBR (described above) primers. [RPB10 Δ5’] plasmid containing 211 bp upstream and 647 bp downstream of the RPB10 ORF was amplified by PCR from the pMJ18 plasmid (1) with RPBF (described above) and MGMR (5’-ATTGACCTCCACAGCTG-3’) primers.

HA-epitope sequence fused at N-terminus of RPB10 gene with a 5’ and 3’ regulatory regions, 250 bp and 390 bp, respectively (synthesized by Syngen biotech company) in pEX-A128 vector was cloned to pRS316 in NotI and EcoRI sites, called here [HA-RPB10].

RNA isolation and northern hybridization
Total RNA was isolated by heating and freezing the cells in the presence of SDS and phenol as described previously (6). Samples containing 20 µg of total RNA were denaturated in a glyoxal reaction mixture at 55°C for 1 h (7) and were resolved by electrophoresis in 1.2 % agarose gel in 1×BPTE buffer (10 mM PIPES, 30 mM Bis-Tris, 1 mM EDTA pH 8.0). RNA samples were transferred into a Hybond-N+ membrane (Amersham) with 10×SSC using the TurboBlotter downward capillary transfer system (Schleicher & Schull) and crosslinked by UV radiation (0.14 J/cm²). The blot was prehybridized for 3 h at 65°C in buffer containing 7% SDS, 0.5 M Na₂HPO₄ pH 7.4, 1 mM EDTA, 1% BSA, and hybridized with RPB10, ACT1 and SCR1 probes. DNA probes were amplified by PCR using oligonucleotides listed in the Supplementary Table 1 and were labeled with [α-32P]-dATP by random priming using the HexaLabel DNA labeling kit (Fermentas). Hybridization was carried out overnight at 65°C in the same buffer as prehybridization. Filters were washed three times (15 min each) with 2×SSC, 0.1% SDS at 65°C. Hybridization signals were exposed to a phosphorimager screen. RNA was quantified using the PhosphorImager STORM 820 (Molecular Dynamics). Band intensities were quantified using the MultiGauge v3.0 software (Fujifilm).

Supplementary Table 1. Oligonucleotides for northern hybridization

| Target | Forward | Reverse |
|--------|---------|---------|
| RPB10N | ATCAGCACTCCGATGGCTAC | ACAAGTCTCTTATATCGCAGCA |
| ACT1   | TTCCCATCTATCGTCCGTAAG | GTGGTGAGAAAGAGCTAACC |
cDNA synthesis and reverse transcription-quantitative PCR (RT-qPCR)

100 nanograms of RNA was used for cDNA synthesis using a QuantiTect reverse transcriptase kit (Qiagen). cDNA for each sample was performed according to the manufacturer’s instruction. RT-qPCR reactions contained 1 μL of cDNA template, 300 nM primer pairs and 5 μL of RT PCR Mix SYBR (A&A Biotechnology). Quantitative PCR was performed on a Roche LightCycler 480 System using a 5-min incubation at 95°C, followed by 40 cycles of 30 s at 95°C, 20 s at 60°C, and 20 s at 72°C (with a plate read after each cycle). A melting curve analysis was performed for each sample after PCR amplification to ensure that a single product with the expected melting curve characteristics was obtained. Each sample was loaded in triplicate. Each plate contained cDNA dilutions for the standard curve, a non-reverse transcriptase control, and a no template control. PCR efficiencies were between 90% and 100%. Data were processed in LightCycler 480 Software and then analyzed in Excel (Microsoft). Data are expressed in arbitrary units calculated from standard curve where the highest cDNA concentration was set to 1. The primer sequences are listed in the Supplementary Table 2.

Supplementary Table 2. Oligonucleotides for RT-qPCR

| Target | Forward     | Reverse          |
|--------|-------------|------------------|
| RPB10  | TGTTGGTGACAACTGGGAAA | GATCGACGTGGGGTTAGAATCA |
| PGA1   | ACCACTCGGCTAGGTATCCC | CGTTCAATACGTGGTGCC |
| MSH1   | ACTTGGTCGTTGCCTGCAAGCA | CGTTGGCTTCGTTGCAAGTA |
| SPO16  | ATTTGCAAGCTAAACGAGGA | GGAACCTCAAGGCTTTTCTG |
| CNN1   | AAGCATTGGAATGGGATCCAGG | TCCCTTATGTCGCCTTTG |
| pre-RPL28 | CCATCTCACTGTGAGACGG | CTCAGTTGCAGATGGAAGAG |
| RPL28  | TCACGTCTAGCCGTTGAAAG | ATGTTGACCACCGGTCATAC |
| ACT1   | CATGTTCCCAGGTATGCGGA | GTCAAAAGAGCAGAGATAGA |
| SCR1   | GAGAATTCTGGCGAGGAAACAA | TCTGCCAGGCAAAATTACGA |

Protein extraction and western blot analysis

The protein extraction method was described earlier (8). Protein extracts were separated by 6% or 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis proteins were transferred onto the nitrocellulose membrane (Miliqore), which was then blocked in TBST (10mM Tris, 150 mM NaCl, 0.05% Tween 20) containing 5% fat-free dry milk for 30 min and subsequently incubated with the appropriate antibody: mouse monoclonal antibodies 9E10 anti-Myc (Roche) at a 1:2,000 dilution for 1 h, anti-HA (Covance) at a 1:5,000 dilution for 2h, anti-Pgk1 (Abcam) at a 1:20,000 dilution for 1 h, anti-Nab2 (gift from Torben Heick Jensen) at a 1:1,000,000 dilution for 1 h and rabbit polyclonal antibodies anti-Rbs1 (Gramsh) at a 1:1,000,000 dilution for 1h. Blots with TAP-tagged Upf1 protein were incubated with anti-PAP antibody at a 1:3,000 dilution. PAP is a peroxidase anti-peroxidase antibody which does not necessitate the use of a secondary antibody. Then membrane was incubated for 1 h with a secondary anti-mouse or anti-rabbit antibody coupled to horseradish
peroxidase (DAKO) at a dilution of 1:5,000. The signal on the membrane was visualized by chemiluminescence using the ECL detection kit (Bio-Rad).

**CRAC analysis**

Samples were processed as previously described (PMID: 30718516, PMID: 32585128) with modifications. Cells were lysed in TNMC100 (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% NP-40, 5 mM MgCl₂, 10 mM CaCl₂, 20U of DNase RQ1 and a protease-inhibitor cocktail (1 tablet / 50 mL) with zirconia beads in a 50 mL conical. The cells were lysed with five one-minute pulses, with cooling on ice in between. The supernatant was spun for 20 minutes at 21,000g. The cleared lysate was incubated with the IgG Sepharose for two hours at 4°C, with nutating. Subsequently, the beads were washed two times with TMN600 (50 mM Tris-HCl pH 7.5, 600 mM NaCl, 0.1% NP-40, 1.5 mM MgCl₂) and two times TMN100 (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.1% NP-40, 5 mM MgCl₂). For RNA digestion beads were resuspended in 600 μL TMN100 containing 0.04U of RNase-IT and samples were incubated for 10 minutes at 23 °C 1000 rpm to fragment protein-bound RNA. RNase digestion was slowed down by incubation on ice for 3 min.

The beads were washed three times TMN100 and resuspended in 600 μL TMN100 containing 5 μL HaloTEV (Promega). Protein: RNA complexes were eluted by incubation with HaloTEV for 2h at 18°C with shaking. The supernatant was separated and adjusted for nickel affinity purification with the addition of 400 mg guanidine hydrochloride, 45 μl NaCl (3M) and 7 μL imidazole (1 M) and added to 50 μL of washed nickel beads (Qiagen).

Following 2h incubation, the nickel beads were washed three times with WBI (6.0 M guanidine hydrochloride, 50 mM Tris-HCl pH 7.5, 300 mM NaCl, 0.1% NP-40, 10 mM imidazole, 1.5 mM MgCl₂), three times with C buffer (50mM Tris-HCl pH 7.5, 50 mM NaCl, 1.5 mM MgCl₂, 0.1% IGEPAL CA-630) and twice with 50 mM Bis-Tris pH 6.5 and transferred to a spin column. Subsequent reactions (80 μl total volume for each) were performed in the columns, and afterward washed once with WBI and three times with PNK buffer:

1. Dephosphorylation (70 mM Bis-Tris pH 6.5, 10 MgCl₂, 40 U T4 PNK (NEB), 2 U TSAP (Promega); 37°C 30 min)
2. 3’ linker ligation (70 mM Tris-HCl pH 7.5, MgCl₂, 12.5% PEG8000, 40U T4 RNA Ligase II truncated K227Q, 80U RNasin, 80 pmol preadenylated 3’ miRCat-33 linker (IDT); 16°C overnight).
3. 5’ end phosphorylation and radiolabeling (70 mM Tris-HCl pH 7.5, MgCl₂, 40 U T4 PNK (NEB), 80U RNasin, 40 μCi 32P-γATP; 37°C for 45 min, with addition of 100 nmol of ATP after 30 min).
4. 5’ linker ligation (70 mM Tris-HCl pH 7.5, MgCl₂, 12.5% PEG8000, 40 U T4 RNA ligase I (NEB), 80 U RNasin, linker, 200 pmol 5’ linker, 1 mM ATP; 16°C for 3h and 25°C for 2h).

The beads were washed twice with WBI and three times with C buffer. Protein: RNA complexes were eluted 100 μL of elution buffer (50 mM Tris-HCl pH 7.5, 500 mM NaCl, 0.1% Triton X-100, 6M guanidine hydrochloride and 300 mM imidazole), supplemented 3 μL GlycoBlue and ethanol precipitated overnight (9 volumes of ethanol). RNPs were pelleted at 21000g for 20 minutes, washed twice 80% acetone, rehydrated in 12 μL water. 4 μL 4X NuPAGE sample loading buffer supplemented with 8% β-mercaptoethanol. The sample was denatured by incubation at 65°C for 10 minutes, and run on a 4%–12% Bis-tris NuPAGE gel at 130 V. The protein: RNA complexes were transferred to Hybond-C nitrocellulose membranes with NuPAGE MOPS transfer buffer for 2 h at 100V.

Labelled RNA was detected by autoradiography. The appropriate region was excised from the membrane and treated with 0.2 μg/μL Proteinase K (50 mM Tris-HCl pH 7.5, 50 mM NaCl,
0.1% IGEPAL CA-630, 1% SDS, 5 mM EDTA; 2 hr 55°C with shaking) in a 500 μL reaction. The RNA component was isolated with a standard phenol:chloroform extraction followed by ethanol precipitation with 2 μL of GlycoBlue. The RNA was reverse transcribed using Superscript IV and the miRCat-33 RT oligo (IDT) for 15 min at 50°C in a 20μL reaction. Subsequently 2 μL ExoI (NEB) were added, incubated 30 min at 37°C and heat inactivated 20 min at 80°C. The resulting cDNA was amplified by PCR in 50 μL reactions using Phusion (Thermo) (2 μL template, 19 cycles) PCR reactions were combined, purified and concentrated using AmpureXP beads, and resolved on a 3% Metaphore agarose gel. A region corresponding to 150 to 250 bp was excised from the gel and extracted using the ZumoClean Gel DNA Recovery Kit (Zymo Research). Libraries were measured with Qbit and sequenced using Illumina NextSeq 550 with 150bp single-end reads.

Quantification and statistical analysis of CRAC data

Pre-processing and data alignment
Illumina sequencing data were demultiplexed using in-line barcodes and in this form were submitted to GEO. First quality control step was performed using FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) considering specificity of CRAC data. Raw reads were trimmed with flexbar v3.4.0 (Dodt et al., 2012) with parameters –q TAIL –qf i1.8 –qt 20 to remove bases with QC<20. Subsequently reads were collapsed to remove PCR duplicates using FASTX-collapser v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/) then inline barcodes were removed using pyBarcodeFilter.py script from pyCRAC package v3.0 (9). The 3’ adapter were removed using flexbar v3.4.0 (10) with parameters –as TGGAATTCTCGGGTGCCAAGGC -u 3 –m 17 –n 16 –bt RIGHT.
All datasets were aligned to the yeast genome using Novoalign v2.07.00 (http://www.novocraft.com) with parameter –r random and saved in novo format to calculate classes of bound RNAs and BigWig files for visual inspection in IGV genome browser. Second quality control step was performed using pyReadCounters script (pyCRAC package) which calculates overlaps between aligned cDNAs and yeast genomic features. The 1 nt resolution BigWig files were generated using bamCoverage v3.1.3 script from deepTools package (11). Sam file operations were performed using SAMtools v1.9 (12).
Additionally all datasets were aligned to yeast transcriptome with parameter using STAR aligner v.2.7.3a (13) and only unambiguously mapped reads were used to generate binding profiles.
Downstream analyses were performed using python 3.6.10 Jupiter notebooks, python libraries (pandas v0.19.2, numpy v1.16.0, scipy v1.2.0, matplotlib v2.2.3) and in-house scripts submitted available s an update of gwide toolkit v0.5.27 (https://github.com/tturowski/gwide) (14).

Identifying Rbs1 enriched genes
To compare CRAC data and RNA-seq all reads mapping to mRNAs were normalized to reads per million (RPM) and converted to log2 RPM. For each transcript p-value was calculated using a two-sided T-test. High confidence Rbs1 targets were selected using following criteria: p-value <0.01, ratio between Rbs1 binding and RNA-seq >1.5 and >128 uniquely mapped RPM.

Rbs1 metagene representation
For metagene analysis data were processed independently for each replicate. Reads mapped to each transcript were summed up to 1 and fraction of reads was used further. This excluded risk that the obtained profile is biased by the most abundant transcripts, as each transcript has a value of 1. To combine transcripts of different length, for metagene representation fraction of
reads was binned as follows: 5’UTR 10 bins, CDS 100 bins and 3’UTR 10 bins. Average fraction of reads was plotted for each bin.

**Binding to mature mRNA**
Reads mapped to each transcript were summed up to 1 and fraction of reads was used further. Fraction of reads mapping to each feature was counted. Boxplots present 2nd and 3rd quartile, line marks median and whiskers range between 5th and 95th percentile.

**REFERENCES**

1. Cieśla, M., Makala, E., Płonka, M., Bazan, R., Gewartowski, K., Dziembowski, A. and Boguta, M. (2015) Rbs1, a new protein implicated in RNA polymerase III biogenesis in yeast Saccharomyces cerevisiae. *Mol. Cell. Biol.*, **35**, 1169–1181.

2. Ciesla, M., Skowronek, E. and Boguta, M. (2018) Function of TFIIIC, RNA polymerase III initiation factor, in activation and repression of tRNA gene transcription. *Nucleic Acids Res.*, **46**, 9444–9455.

3. Chen, D.C., Yang, B.C. and Kuo, T.T. (1992) One-step transformation of yeast in stationary phase. *Curr. Genet.*, **21**, 83–84.

4. Longtine, M.S., McKenzie, A., Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P. and Pringle, J.R. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. *Yeast*, **14**, 953–961.

5. Lalo, D., Carles, C., Sentenac, A. and Thuriaux, P. (1993) Interactions between three common subunits of yeast RNA polymerases I and III. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 5524–5528.

6. Schmitt, M.E., Brown, T.A. and Trumpower, B.L. (1990) A rapid and simple method for preparation of RNA from Saccharomyces cerevisiae. *Nucleic Acids Res.*, **18**, 3091–3092.

7. Sambrook, J. and Russell, D.W. (2001) Molecular cloning: a laboratory manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

8. Towpik, J., Graczyk, D., Gajda, A., Lefebvre, O. and Boguta, M. (2008) Derepression of RNA polymerase III transcription by phosphorylation and nuclear export of its negative regulator, Maf1. *J. Biol. Chem.*, **283**, 17168–17174.

9. Webb, S., Hector, R.D., Kudla, G. and Granneman, S. (2014) PAR-CLIP data indicate that Nrd1-Nab3-dependent transcription termination regulates expression of hundreds of protein coding genes in yeast. *Genome Biol.*, **15**, R8.

10. Dodt, M., Roehr, J.T., Ahmed, R. and Dieterich, C. (2012) FLEXBAR-Flexible Barcode and Adapter Processing for Next-Generation Sequencing Platforms. *Biology (Basel)*, **1**, 895–905.

11. Ramírez, F., Ryan, D.P., Grüning, B., Bhardwaj, V., Kilpert, F., Richter, A.S., Heyne, S., Dündar, F. and Manke, T. (2016) deepTools2: a next generation web server for deep-sequencing data analysis. *Nucleic Acids Res.*, **44**, W160–W165.
12. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. and Subgroup, 1000 Genome Project Data Processing (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25, 2078–2079.

13. Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M. and Gingeras, T. R. (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29, 15–21.

14. Turowski, T. W., Leśniewska, E., Delan-Forino, C., Sayou, C., Boguta, M. and Tollervey, D. (2016) Global analysis of transcriptionally engaged yeast RNA polymerase III reveals extended tRNA transcripts. *Genome Res.*, 26, 933–944.
Supplementary Figure S1. Statistical analysis of CRAC data for Rbs1 over all mature mRNAs. (A) Spearman and Pearson correlations between datasets. (B) Scatter plots comparing the Rbs1 RNA binding to RNA-seq and Ribo-seq data. Reads uniquely mapping to mRNAs are presented (log2 RPM). (C) Metagene representation of read density over all mature mRNAs (n=6692). Data were separated into 120 bins: 10 for the 5’ UTR, 100 for the CDS and 10 for the 3’ UTR. Horizontal lines indicate were CDS starts and stops. Metagene analysis performed for mRNA containing at least 100 reads in Rbs1 CRAC data (n=6692).
XP_01757305.1  hypothetical protein PADG_02291 [Paracoccidioides brasiliensis Pb18]

MKO22302.1  R3H domain protein [Aspergillus sp. R937]

QCA11394.1  hypothetical protein BPE2_000988 [Talaromyces marneffii]

QOE15591.1  hypothetical protein PENSITES_0260G0122 [Penicillium stekelii]

KP_00218203.1  hypothetical protein [Uncinorus rewilli 1704]

PSZ19146.1  hypothetical protein AHO_0_04226 [Polystylya hystricis UAM7299]

XP_033404916.1  uncharacterized protein GDIQ_0_05635 [Arthrobacter unfuscatus]

KAF29394.1  hypothetical protein EUHORAGA_7_2564 [Triochodictya spisoria]

KAF193774.1  hypothetical protein EUHORAGA_0F07_04933 [Clathrospora olymae]

SLQ37892.1  hypothetical protein OX1_0944_0100_002965 [Pyricularia oryzae Y34]

TQS37745.1  hypothetical protein GOLMAGNI_17171 [Golovinomyces magnicellulatus]

ZSP02302.1  unnamed protein product [Blumeria graminis f. sp. hordei]

KAF29356.1  hypothetical protein V5G_0_04127 [Pseudogymnoascus sp. VKN F-4520 (FM-2644)]

XP_016645040.1  R3H domain-containing protein [Sarcosporium apiospermum]

XP_01740507.1  R3H domain-containing protein-like protein [Acremonium chrysogenum ATCC 11550]

CCE33401.1  uncharacterized protein CFW1_0_07972 [Claviceps purpurea 20.1]

CJL3027.1  hypothetical protein VRE110_0_0244 [Torundriella bennetiana]

KAF19376.1  hypothetical protein GEOHORAGA_1213834 [Coniochaeta sp. YZ7-11]

XP_014168719.1  R3H domain protein [Grosnania clavigera kw1407]

KAF29356.1  hypothetical protein EUHORAGA_2526619 [Cnemila bistricina]

VNM7180.1  Putative protein of unknown function [Podospora comuta]

XP_008846197.1  hypothetical protein NEUTELDRAFT_14442 [Neurospora tetrasperma PCSC 2508]

KIH88101.1  R3H domain protein [Sporothrix brasiliensis 5150]

XP_030994999.1  hypothetical protein ESZL_0_02626 [Phialocephala curvata]

KKA30932.1  hypothetical protein MIB1OLDRAG_139775 [Microdochium bolleyi]

KKA30932.1  hypothetical protein TDS_0_04644 [Trichosporium venter]

AJV07100.1  Yersp [Serratia marcescens X541418]

GFC5001.1  hypothetical protein Y2OM_0_00314 [Pythium aureum melis]

OVA05589.1  Single-stranded nucleic acid binding R3H domain-like [Pythium aureum melis]

GEO15611.1  R3H domain-containing protein 1-like [Tanacetum cinerariifolium]

GOM87646.1  hypothetical protein CDD15_Pyr002757 [Punica granatum]

XP_00808576.1  uncharacterized protein LOCI10720587 [Phytophthora cactorum]

MBA3130.1  hypothetical protein Taro_0_013800, partial [Colosia scutellata]

XP_02669936.1  R3H domain-containing protein 1 [Dendroboon catenatum]

KKA30932.1  hypothetical protein Taro_0_04402 [Colosia scutellata]

MKM62657.1  Single-stranded nucleic acid binding R3H protein [Zea mays var. marina]

PK46951.1  hypothetical protein AXF42_Ash004637 [Apostasia stolonifera]

XP_00386294.1  R3H domain-containing protein 2 [Vitis vinifera subsp. malaccensis]

XP_02056463.1  uncharacterized protein LOCI10983167 [Isozyme X1 [Aspergillus officinalis]]

XP_02056746.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

OA76127.1  hypothetical protein [Aphididae thomasei]

KAF29356.1  hypothetical protein Taro_0_013800, partial [Colosia scutellata]

XP_002966112.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

GER1529.1  single-stranded nucleic acid binding R3H protein [Striga asiatica]

XP_026114255.1  hypothetical protein LOCI10720593 [Isozyme X1 [Ananas comosus]]

ERH99095.1  hypothetical protein AMTR_0_00110_0012520 [Amorbeula trichopoda]

XP_002966112.1  hypothetical protein LOCI10483164 [Tarentula hakeselesiana]

XP_022966112.1  hypothetical protein LOCI10483164 [Tarentula hakeselesiana]

KAF29356.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

KAF29356.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

KAF29356.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

XP_02056463.1  uncharacterized protein LOCI10983167 [Isozyme X1 [Aspergillus officinalis]]

XP_02056463.1  uncharacterized protein LOCI10983167 [Isozyme X1 [Aspergillus officinalis]]

XO76127.1  hypothetical protein Y2OM_0_013800, partial [Colosia scutellata]

XO76127.1  hypothetical protein Taro_0_013800, partial [Colosia scutellata]

XP_022966112.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

XP_002966112.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

XP_002966112.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

XP_022966112.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

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XP_002966112.1  hypothetical protein EYB26_0_000988 [Talaromyces marneffei]

XP_02056463.1  uncharacterized protein LOCI10983167 [Isozyme X1 [Aspergillus officinalis]]

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Supplementary Figure S2 (seven previous pages). Sequences of proteins comprising R3H-SUZ domain combination. (A) Description of accession numbers of the retrieved sequences. The numbers are color-coded: blue for fungi, green for plants, orange for invertebrates and black for vertebrates. (B) Multiple sequence alignment obtained with Promals3D. Consensus secondary structure and sequence are given in the bottom of each segment. Amino acid number for Rbs1 are given on top of the alignment. For further explanation, see legend of Fig 10. (C) Phylogenetic tree of the analyzed sequences.