Supplementary information for:

THE HIGHLY CONSERVED EUKARYOTIC DRG FACTORS ARE REQUIRED FOR EFFICIENT TRANSLATION IN A MANNER REDUNDANT WITH THE PUTATIVE RNA HELICASE SLH1

Marie-Claire Daugeron[1,2]*, Manoël Prouteau[1,2,#], François Lacroute[1], and Bertrand Séraphin[1,3]*

Addresses: Equipe Labellisée La Ligue, [1] CGM, CNRS FRE3144, 1 Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France; [2] Univ Paris-Sud, Orsay, F-91405; [3] IGMBC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Illkirch F-67400, France; CNRS, UMR7104, Illkirch F-67404, France; Inserm, U964, Illkirch F-67400, France; and Université de Strasbourg, Strasbourg F-67000, France

*: Corresponding authors

Emails: daugeron@cgm.cnrs-gif.fr

seraphin@igbmc.fr

present address: CMU / GEDEV Rue Michel Servet 1211 Genève 4 Suisse

Supplementary Material and Methods

Genetic screen for mutant displaying a negative synthetic interaction with a *Δrbg1Δrbg2* double deletion.

Two *Δrbg1Δrbg2 ura3-1* strains of opposite mating type were constructed (BSY1995 and BSY996). Both strains exhibited wild type growth. BSY1995 was transformed with a plasmid containing the *RBG1* and *URA3* genes (pMCD-F10). The transformed cells grew on 5FOA-plates as well as a wild-type control indicating that plasmid loss occurred and was not disadvantageous. The BSY1995 strain containing pMCD-F10 was grown at 30°C on 2% glucose CSM-Uracil plate for two days at 30°C. Colonies were scraped from the plate and cell dilutions were plated on YPDA ($7x10^7$ cells per plates) for UV mutagenesis (50 J/m^2, ca. 1% survival) and further incubated two days at 30°C after UV mutagenesis. Colonies were then scraped from the plates, resuspended in water and cell dilutions were plated on CSM ($2x10^3$ cells per plate; 40 plates). After two days incubation at 30°C, CSM plates were replica-plated on 5-FOA containing plates (80000 colonies tested). The latter were incubated at 30°C and regularly observed under a binocular to identify clones that either did not grow or only poorly. The corresponding clones were recovered from the master CSM plates and streaked again on CSM and 5-FOA plates to confirm the first observation. They were also transformed with a *TRP1*-plasmid containing the *RBG1* gene or the empty plasmid as a control. Clones able to grow well on 5-FOA plates in the presence of the *RBG1*-containing plasmid but not in the presence of the empty plasmid were selected as candidates. Those were then backcrossed several times to the *Δrbg1Δrbg2* strain of opposite mating type before further analysis. The three independent mutants that we isolated had a strong growth defect in absence of both *RBG1* and *RBG2* but were not synthetically lethal. Standard genetic assays demonstrated that recessive mutation in the same gene had been identified.

As complementation of the slow growth phenotype with genomic libraries led only to the

recovery of *RBG1* or *RBG2* (not shown), we prepared a genomic library from a *Δrbg1Δrbg2* strain. Briefly, genomic DNA from the BSY1996 strain was partially digested with Sau3A, large DNA fragments were gel purified and ligated in a BamHI-linearized pFL38 plasmid (centromeric, *URA3*). After transformation of the ligation mixtures into Electromax $DH5^{E}$ Cells (Invitrogen) bacterial strain, 2 10^6 clones were obtained and pooled. Plasmid DNA was extracted and used to transform the BSY2035 mutant. Fast growing yeast transformants were identified and processed as described previously. In only one case, plasmid rescue and back transformation confirmed that complementation was plasmid linked. This plasmid, pMCD-S0, contained a genomic insert encompassing a portion of *YTA7* gene plus the entire *SLH1* gene. A PstI fragment containing a shorter portion of YTA7 plus *SLHI* was subcloned into PstI-linearized YCplac111 giving pMCD-S4, which was still able to complement BSY2035. Finally, a *Δslh1Δrbg1Δrbg2* strain was constructed and crossed with BSY1996 and BSY2035 for complementation and recessivity tests followed by meiotic segregation analysis. This demonstrated that the mutated locus was indeed *SLH1*.

Plasmid construction

Plasmids encoding the various *RBG1* alleles were constructed as follow.

A DNA fragment containing the *RBG1* gene flanked by BamHI and PstI restriction sites was amplified from genomic DNA using the primer set OBS2834/OBS2837 and cloned into a BamHI-PstI linearized pFL38 plasmid to give **pMCD-F10**.

A DNA fragment containing the open reading frame and 3' region of the *RBG1* gene, flanked by SalI and Sph1 restriction sites was amplified from genomic DNA using the primers set OBS2058/OBS2059 and subcloned into the SalI-SphI restricted pAS24 plasmid that contains *GAL* promoter region and a HA epitope tag (1). Subsequently, an NcoI-SphI fragment (A), containing the *RBG1* ORF with an N terminal HA epitope tag and the *RBG1* 3' region, was

recovered from this plasmid. A DNA fragment (B), containing the putative promoter region of *RBG1* flanked by BamH1 and NcoI restriction sites, was amplified from genomic DNA using the primer set OBS3219/OBS2834. The BamH1-NcoI restricted DNA fragment B and the NcoI-SphI restricted DNA fragment A were cloned into a BamHI-SphI linearized YCplac111 to give **pMCD-F13.**

The plasmids carrying the *rbg1* mutant alleles were obtained by PCR fusion. For example, to generate the *rbg1 GFVSVGKS* allele, two DNA fragments with overlapping regions were amplified from pMCD-F13 using the two primer sets OBS2065/M13rev and OBS2066/M13for. A DNA fragment containing the *rbg1 GFVSVGKS* gene flanked by BamHI-SphI restriction sites was further obtained by PCR fusion using the two previous PCR products as DNA template and the primers set M13rev/M13. This fragment was cloned into a BamHI-SphI linearized YCplac111 plasmid to give **pMCD-F14**. A similar procedure was used to generate the pMCDF15 to pMCD-F20 plasmids. The corresponding primers sets are listed in table S2.

Plasmids encoding the various *SLH1* alleles were constructed as follow.

The plasmid **pMCD-S0** was isolated from a pFL36 plasmid-borne genomic library by complementation of the slow-growth phenotype of the BSY2035 strain and contains an insert of 7,6 Kb encompassing *SLH1* and a portion of *YTA7*. A 6,9Kb PstI restriction fragment encompassing *SLH1* was cloned into a PstI-linearized pMCDV11 plasmid to give **pMCD-S4**. (The plasmid pMCD-V11 was generated by religation of a NarI-EcoRI linearized YCplac 111)

To construct **pMCD-S5,** encoding Slh1 AptGaAAA mutant protein, two DNA fragments with overlapping regions were amplified using pBS1479 (TAP tag) as template with the primers set OBS3872/OBS3873 and pMCD-S4 as template with the primers set OBS3874/OBS3875. The two resulting fragments were then used as DNA template with the primers set

OBS3873/OBS3875 to obtain a DNA fragment containing the 3' part of the *SLH1* ORF followed in frame by the ProtA tag and a part of the 3' end of the *SLH1* gene (from the OBS3873 oligonucleotide). The DNA fragment was transformed into BSY2047 yeast cells together with an EcoRI-SnaBI linearized pMCD-S4 to perform plasmid gap repair and to generate **pMCD-S5**.

For **pMCD-S6**, two DNA fragments with overlapping regions were amplified from pMCD-S4 using the two primer sets OBS3876/OBS3879 and OBS3877/OBS3878. The fragments were used as template for the fusion PCR with the primers set OBS3877/OBS3878. The resulting DNA fragment was transformed into BSY2047 yeast cells together with a SapI-KpnI linearized pMCD-S5 to perform plasmid gap repair and to generate **pMCD-S6**.

Plasmids encoding the various *TMA46 and GIR2* alleles were constructed as follow.

Two DNA fragments with overlapping regions were amplified from genomic DNA using the primers sets OBS262/OBS3467 and OBS263/OBS3298. They were used as templates for PCR fusion using the primers set OBS262/OBS263 to generate a DNA fragment containing the *TMA46* gene with an in frame sequence encoding two HA epitope Tag introduced just after the start codon. The PCR product was first cloned using the Zero Blunt^{R} TOPO^{R} PCR cloning kits from Invitrogen and a NotI-PstI fragment was recovered after digestion of the resulting plasmid. This fragment was then cloned into a NotI-PstI linearized pFL36 plasmid to give **pMCD-T6**. A similar procedure was used to construct **pMCD-G2**, the corresponding primer sets used are indicated in table S3

The plasmids carrying the *tma46* or *gir2* mutant alleles were obtained by the following procedure. For example, to generate the *tma46 ACCH-1* allele, two DNA fragments with overlapping regions were amplified from pMCD-T6 using the two primer sets M13rev/OBS3502 and M13for/OBS3503 and then used as template for the fusion PCR with the primers set M13rev/M13for. The resulting DNA fragment was digested by NotI and PstI

restriction enzymes and cloned into a NotI-PstI linearized YCplac111 plasmid to give **pMCD-T11.** A similar procedure was used to generate the **pMCD-T12 to pMCD-T15** plasmids and the **pMCD-G3** and **pMCD-G4** plasmids. The corresponding primers sets and DNA templates are listed in table S2.

Supplementary Table S1: Yeast strains used in this study

Strains	Notes	Relevant genotypes
BMA64a	*WT* (reference strain)	*MATa ade 2-1 his3-11,15 leu2-3,112 Δtrp1 ura3-1 can1-100*
BMA64α	*WT* (reference strain)	*MATα ade 2-1 his3-11,15 leu2-3,112 Δtrp1 ura3-1 can1-100*
BSY1143	*Δtma46*	*MATa Δtma46::HISMX6*
BSY1261	*Tma46-TAP Rbg1-HA*	*MATa TMA46-TAPtag-TRPKl RBG1-his$_6$-HA$_3$-HISMX6 POP2-his$_6$-VSV$_3$*
BSY1375	*Tma46-TAP*	*MATα TMA46-TAPtag-TRPKl*
BSY1421	*Rbg1-TAP*	*MATα RBG1-TAP-TRPKl*
BSY1586	*Δrbg1Δtma46*	*MATa Δtma46::HISMX6 Δrbg1::HISMX6*
BSY1588	*Δrbg1*	*MATα Δrbg1::HISMX6*
BSY1935	*Δgir2*	*MATa Δgir2::KanMX4*
BSY1966	*Rbg2-TAP*	*MATa RBG2-TAPtag-TRPKl*
BSY1968	*Δtma46Δgir2*	*MAT? Δtma46::HISMX6 Δgir2::KanMX4*
BSY1969	*Δtma46Δrbg2*	*MAT? Δtma46::HISMX6 Δrbg2::KanMX4*
BSY1994	*Δrbg2*	*MATα Δrbg2::KanMX4*
BSY1996	*Δrbg1Δrbg2*	*MATa Δrbg1::HISMX6 Δrbg2::KanMX4*
BSY1998	*Δrbg1Δgir2*	*MATa Δrbg1::HISMX6*
BSY2024	*BSY2031XBSY1996*	*MATa/α Δrbg1::HISMX6/Δrbg1::HISMX6 Δrbg2::KanMX4/Δrbg2::KanMX4 slh1/SLH1*
BSY2025	*BSY2033XBSY1996*	
BSY2026	*BSY2035XBSY1996*	
BSY2031 BSY2033 BSY2035	*Three haploids isolates from the genetic screen*	*MATa Δrbg1::HISMX6 Δrbg2::KanMX4 slh1*
BSY2046	*Δslh1*	*MATa Δslh1::URA3Kl*
BSY2049	*Δrbg1Δrbg2Δslh1*	*MATα Δrbg1::HISMX6 Δrbg2::KanMX4 Δslh1::URA3Kl*
BSY2057	*Δtma46Δgir2Δslh1*	*MATa Δtma46::HISMX6 Δgir2::KanMX4 Δslh1::TRPKl*
BSY2061	*Δrbg1Δgir2Δslh1*	*MATa Δrbg1::HISMX6 Δgir2::KanMX4 Δslh1::URA3Kl*
BSY2094	*Δrbg1Δtma46Δslh1*	*MATa Δtma46::HISMX6 Δrbg1::HISMX6 Δslh1::URA3Kl*
BSY2096	*Δrbg2Δgir2Δslh1*	*MATa Δgir2::KanMX4 Δslh1::URA3Kl*
BSY2098	*Δrbg2Δgir2*	*MATa Δgir2::KanMX4 Δrbg2::KanMX4*
BSY2099	*Slh1-TAP*	*MATa SLH1-TAPtag-TRPKl*
BSY2316	*Slh1ΔRL2*	*MAT? slh1ΔRL2-TAPtag-TRPKl*
CDK33-9A (2)	*cdc33-1*	*MATα ade 2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 Δcdc33::LEU2 YCplac33-cdc33-1*
SS13-3A (3)	*tif1-1*	*MATα ade 2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 Δtif1::HIS3 Δtif2::ADE2 pSSC120 (CEN-LEU2)-tif1-1*
CDK45-6A (2)	*prt1-1*	*MATα ade 2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 prt1-1*

Supplementary Table S2: Plasmids used in this study

Names	Features	References
pMCD-F10	pFL38-CII-*RBG1* (ARS-CEN URA3)	this study
pMCD-F13	YCplac111-*HA-RBG1* (*ARS-CEN LEU2*)	this study
pMCD-F14	YCplac111-*HA-rbg1-GFVSVGKS*	this study
pMCD-F15	YCplac111-*HA-rbg1-GFPSVGKN*	this study
pMCD-F16	YCplac111- *HA-rbg1-GFPSVAKS*	this study
pMCD-F17	YCplac111-*HA-rbg1-GFPSVAAA*	this study
pMCD-F18	YCplac111-*HA-rbg1-DLPGQ*	this study
pMCD-F19	YCplac111-*HA-rbg1-ALPAI*	this study
pMCD-F20	YCplac111-*HA-rbg1-IKIA*	this study
pMCD-F21	YCplac111-*HA-rbg1-ΔTGS*	this study
pMCD-S0	pFL38-library insert 1 (*ARS-CEN URA3*)	this study
pMCD-V11	pBS3122-ΔNarI-EcoRI	this study
pMCD-S4	YCplac111-*SLH1*	this study
pMCD-S5	YCplac111-*SLH1-ProtA*	this study
pMCD-S6	YCplac111-*slh1*-A*ptGaAAA*	this study
pMCD-T6	pFL36-*HA-TMA46*	this study
pMCD-T11	pFL36-*HA-tma46-ACCH1*	this study
pMCD-T12	pFL36-*HA-tma46-CCAH1*	this study
pMCD-T13	pFL36-*HA-tma46-ACCH2*	this study
pMCD-T14	pFL36-*HA-tma46-CCAH2*	this study
pMCD-T15	pFL36-*HA-tma46-ACAH1*	this study
pMCD-G2	pFL36-*HA-GIR2*	this study
pMCD-G3	pFL36-*HA-gir2*-ΔRWD/G1	this study
pMCD-G4	pFL36-*HA-gir2*-ΔDFRP	this study
pFA6a-hisMX6	Disruption cassettes	(4)
pFA6a-kanMX4	Disruption cassettes	(4)
pUC19-URA3$_{Kl}$	Disruption cassettes	This study
pUC19-TRP1$_{Kl}$	Disruption cassettes	This study
pBS1479	TAP-tag	(5)
pU6H3HA	HA-tag	(6)
pAS24	pGAL-HA	(1)
pBS3122	YCplac111-Sap1 mutated	(7)

Supplementary Table S3: Primers sets used for PCR amplification of fragments used for cloning or genomic integration

Notes	Sets of primers	DNA template
PCR fragments used for genomic integration		
Δtma46	OBS256/OBS257	pFA6a-hisMX6
Δrbg1	OBS1111/OBS1112	pFA6a-hisMX6
Δrbg2	OBS1959/OBS1960	pFA6a-kanMX4
Δgir2	OBS2592/OBS2593	pFA6a-kanMX4
Δslh1	OBS3151/OBS3152	Puc19-TRP1Kl/URA3Kl
TMA46-TAP	OBS264/OBS265	pBS1479
RBG1-TAP	OBS1118/OBS1119	pBS1479
RBG2-TAP	OBS1961/OBS1962	pBS1479
SLH1-TAP	OBS3153/OBS3154	pBS1479
slh1ΔRL2-TAP	OBS3886/OBS3154	pBS1479
RBG1-HA	OBS627/OBS628	pU6H3HA
PCR fragments used for plasmid constructions		
RBG1	OBS2834/OBS2837	genomic DNA
HA-RBG1	OBS2058/OBS2059; OBS3219/OBS2834	genomic DNA
HA-rbg1-GFVSVGKS	OBS2065/M13rev; OBS2066/M13for; M13rev/M13for	pMCD-F13
HA-rbg1-GFPSVGKN	OBS2067/M13rev; OBS2068/M13for; M13rev/M13for	pMCD-F13
HA-rbg1-GFPSVAKS	OBS2069/M13rev; OBS2070/M13for; M13rev/M13for	pMCD-F13
HA-rbg1-GFPSVAA	OBS2071/M13rev; OBS2072/M13for; M13rev/M13for	pMCD-F13
HA-rbg1-DPLGQ	OBS2073/M13rev; OBS2074/M13for; M13rev/M13for	pMCD-F13
HA-rbg1-ALPAI	OBS2075/M13rev; OBS2076/M13for; M13rev/M13for	pMCD-F13
HA-rbg1-IKIA	OBS2077/M13rev; OBS2078/M13for; M13rev/M13for	pMCD-F13
HA-rbg1-ΔTGS	OBS2060/M13rev; OBS2061/M13for; M13rev/M13for	pMCD-F13
SLH1-PROTA	OBS3872/OBS3873; OBS3874/OBS3875; OBS3873/OBS3875	PBS1479/pMCD-S4
slh1-AptGaAAA-PROTA	OBS3876/OBS3879; OBS3877/OBS3878; OBS3878/OBS3879	pMCD-S5
HA-TMA46	OBS262/OBS3467; OBS263/OBS3298; OBS262/OBS263	genomic DNA
HA-tma46-ACCH-1	M13rev/OBS3502; M13for/OBS3503; M13rev/M13for	pMCD-T6
HA-tma46-CCAH-1	M13rev/OBS3500; M13for/OBS3501; M13rev/M13for	pMCD-T6
HA-tma46-ACAH-1	M13rev/OBS3504; M13for/OBS3505; M13rev/M13for	pMCD-T6
HA-tma46-ACCH-2	M13rev/OBS3506; M13for/OBS3507; M13rev/M13for	pMCD-T6
HA-tma46-CCAH-2	M13rev/OBS3500; M13for/OBS3501; M13rev/M13for	pMCD-T6
HA-GIR2	OBS3296/OBS2596; OBS3297/OBS2595; OBS2595/OBS2596	genomic DNA
HA-ΔRWD/G1	OBS3883/OBS3880; OBS3882/OBS3881; OBS3880/OBS3881	pMCD-G2
HA-ΔDFRP	OBS3884/OBS3880; OBS3885/OBS3881; OBS3880/OBS3881	pMCD-G2

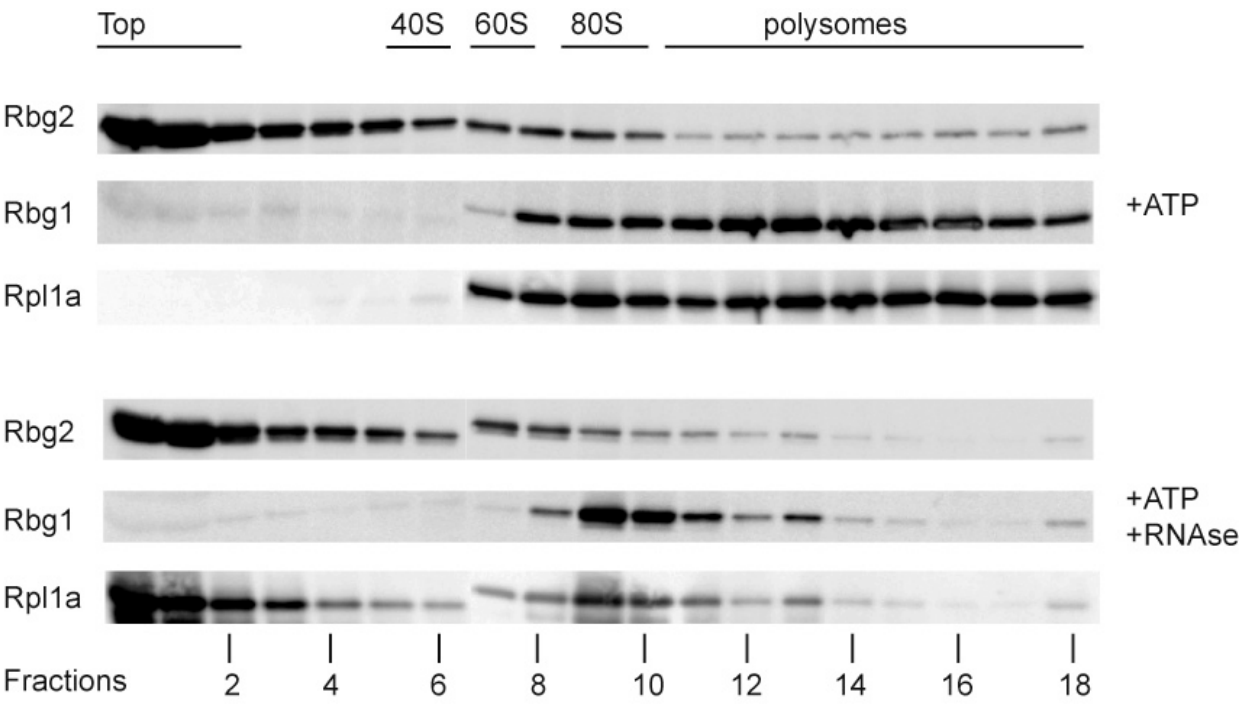
Supplementary Table S4: Oligonucleotides used in this study

Primers	Sequences 5'-3'
M13 for	CGCCAGGGTTTTCCCAGTCACGAC
M13 rev	AGCGGATAACAATTTCACACAGGA
OBS256	GGAGGAAATGAATTGGCACGTAGCTCGTCGAACATGATGAACTGTGCGTACGCTGCAGGTCGAC
OBS257	TTATATATACGGTGGCATATATACAGTTTAGTCTATATGAATTTTAGAATCGATGAATTCGAGCTCG
OBS262	GAAGGGCCGGTAACTGCTCA
OBS263	TTGTCGGGCGGATCCGAGG
OBS264	GGTAGCAACCCCACCTTCGACATCAAAAAAGCCAACTCCGCCACGCTCGCATCCATGGAAAAGAGAAG
OBS265	GTTGGTGTGTGTGCGTGTATTATATATACGGTGGCATATATACAGTTTAGTCTACGACTCACTATAGGG
OBS627	TGAGTCACATTTTGGAAGACGAAGATGTTGTTACCATCTTGAAAAAGCGGATCCCCGGGTTAATTAA
OBS628	CCGATACCCCCTTTTCGAATACAAATGCTTGTATATTCAATTATGAAGAATTCGAGCTCGTTTAAAC
OBS1111	TTTGAAGCTAGATGCAGTAGGTGCAAGCGTAGAGTTGTTGATTGAGCAAACGTACGCTGCAGGTCGAC
OBS1112	CCGATACCCCCTTTTCGAATACAAATGCTTGTATATTCAATTATGAATTATCGATGAATTCGAGCTCG
OBS1118	GGTTGAGTCACATTTTGGAAGACGAAGATGTTGTTACCATCTTGAAAAAGTCCATGGAAAAGAGAAG
OBS1119	CCGATACCCCCTTTTCGAATACAAATGCTTGTATATTCAATTATGAATTATACGACTCACTATAGGG
OBS1959	GTACTAACGAGATACGCATTATCAAAACTAACGTAACAAGCACTACAAACGTACGCTGCAGGTCGAC
OBS1960	CGATGCTGATCTTGTTTTTTCATGTTTCTTTAAAATCTATTCATTTATA TCGATGAATTCGAGCTCG
OBS1961	CTTAAACCACAGGATTGATGATGAAGATGTAGTTTCTTTATTCGCGAAATCCATGGAAAAGAGAAG
OBS1962	CAGTTCTCGATGCTGATCTTGTTTTTTCATGTTTCTTTAAAATCTATTCATACGACTCACTATAGGG
OBS2058	CCCCGTCGACCATATGTCTACTACAGTTGAAAAAATC
OBS2059	CCCCGCATGCTCGAGCCCTCGGTAGCATAACATTTTTA
OBS2060	TGATTCTCGAACAAAAAATAATATAAG
OBS2061	ATATTATTTTTTGTTCGAGAATCACCACATGACTTGCAACAGCTCGTC
OBS2065	GTTTGTCGGGTTCGTGTCGGTGGGGAAATC
OBS2066	GATTTCCCCACCGACACGAACCCGACAAAC
OBS2067	GTCGGTGGGGAAAAATACATTACTGTCC
OBS2068	GGACAGTAATGTATTTTTCCCCACCGAC
OBS2069	GGTTCCCGTCGGTGGCGAAATCTACATTAC
OBS2070	GTAATGTAGATTTCGCCACCGACGGGAACC
OBS2071	GTAATGTAGATTTCGCCACCGACGGGAACC
OBS2072	CTTGGACAGTAATGTAGCTGCCGCCACCGACGGGAACC
OBS2073	GTTGGATTTACCTGGTCAAATCGATGGTGCTAAGG
OBS2074	CCTTAGCACCATCGATTTGACCAGGTAAATCCAAC
OBS2075	CCAAATGTTGGCTTTACCTGCTATTATCGATGGTG
OBS2076	CACCATCGATAATAGCAGGTAAAGCCAACATTTGG
OBS2077	CTATGTGTTAATCAAGATTGCTTCTCTGTC
OBS2078	GACAGAGAAGCAATCTTGATTAACACATAG
OBS2080	GCCGTGGCAAGAACCTGTAACCTG
OBS2081	CTTTACCGAATTCCTAATGCCGTG
OBS2592	AATTTAAGGAAAAGCCCTCCCTCCATATCCAGAAGGCACTTGTTAATACTCGTACGCTGCAGGTCGAC
OBS2593	ATGTCATTATCCATGCAGAAGAAAATACTTAAATACTTAAGTACACAAGTATATCGATGAATTCGAGCTCG
OBS2595	CAGGCATCGTTAGATTGGC
OBS2596	GCCCGTCCTCAGGCTACAAAG
OBS2834	GGCGGATCCCAGTAGTTCTGTTGGTTCT
OBS2837	GGCCTGCAGTCAAGAGGAGATGTGTCAA
OBS3151	ACCATTGGAATTGTGAGAAGTAGATCCGTACCATCAATAGCCGGCTCAAGCACAGGAAACAGCTATGACC
OBS3152	ATTTCCACTGTTATCTTTCACAAAATAATTGTTGTTTTAATTGTGTCTCAGTTGTAAAACGACGGCCAGT
OBS3153	ATTAATGACGCTTTAGGATTACGTTATGATATGGTCCATAAACTAATCTCTTCCATGGAAAAGAGAAG
OBS3154	AGATAGATTTCCACTGTTATCTTTCACAAAATAATTGTTGTTTTAATTGTGTACGACTCACTATAGGG
OBS3219	CCCGAGGCCATGGTTTGCTCAATCAACAACTCTACGC
OBS3296	CCTGACTATGCGGGCTATCCCTATGACGTCCCGGACTATGCAGATTATAAGGAAGAACAGAAGCAGGA
OBS3297	GGGATAGCCCGCATAGTCAGGAACATCGTATGGGTATGCCATAGTATTAACAAGTGCCTTCTGGA
OBS3298	CCTGACTATGCGGGCTATCCCTATGACGTCCCGGACTATGCACCACCAAAAAAGGGAAAACAGGC
OBS3467	GGGATAGCCCGCATAGTCAGGAACATCGTATGGGTATGCCATCTTCGAAATATAACTTTCCTGC
OBS3500	AACAAAGGTGCCAAAGCTAAGTTCTCACATGAC
OBS3501	GTCATGTGAGAACTTAGCTTTGGCACCTTTGTT

OBS3502	CCCAAGTCCATGGTTTGCGCTCTGTTCAAACTG
OBS3503	CAGTTTGAACAGAGCAGCAACCATGGACTTGGG
OBS3504	ACCACCACCGACAAAGTAGCCAAATACTTCATCGAG
OBS3505	CTCGATGAAGTATTTGGCTACTTTGTCGGTGGTGGT
OBS3506	AACGGAGGTGACAAAGCTATGTACAGACACTCC
OBS3507	GGAGTGTCTGTACATAGCTTTGTCACCTCCGTT
OBS3872	TATGATATGGTCCATAAACTAATCTCT GAGCTCAAAACCGCGGCTC
OBS3873	GATAGATTTCCACTGTTATCTTTCACAAAATAATTGTTGTTTTAATTGTGTCTCATCAGGTTGACTTCCCCGCG
OBS3874	AGAGATTAGTTTATGGACCATATCATA
OBS3875	GTTGGCTGTTGAGTATAATGAATTGC
OBS3876	GCACCTACTGGTGCAGCTGCAGCAGACATTGCATTACTG
OBS3877	CAGTAATGCAATGTCTGCTGCAGCTGCACCAGTAGGTGC
OBS3878	GGAGCAAGGGAAGAAGAGC
OBS3879	GATTCTGCTAGTTTGATAAAGT
OBS3880	GCTGGCGAAAGGGGGATGT
OBS3881	GCTTCCGGCTCGTATGTTGT
OBS3882	GAAGCACAAGAACGTGAAAAGAAG
OBS3883	CTTTTCACGTTCTTGTGCTTCTGCATAGTCCGGGACGTCATA
OBS3884	CTCGAGAAGCAGTACGAGCTGTAAATACTTGTGTACTTAAGTATTTA
OBS3885	CAGCTCGTACTGCTTCTCGAG
OBS3886	TGCAAACTATGACATTTTATACACTTTACAATACCAATGAGAATGCGTTTGTTTCCATGGAAAAGAGAAG

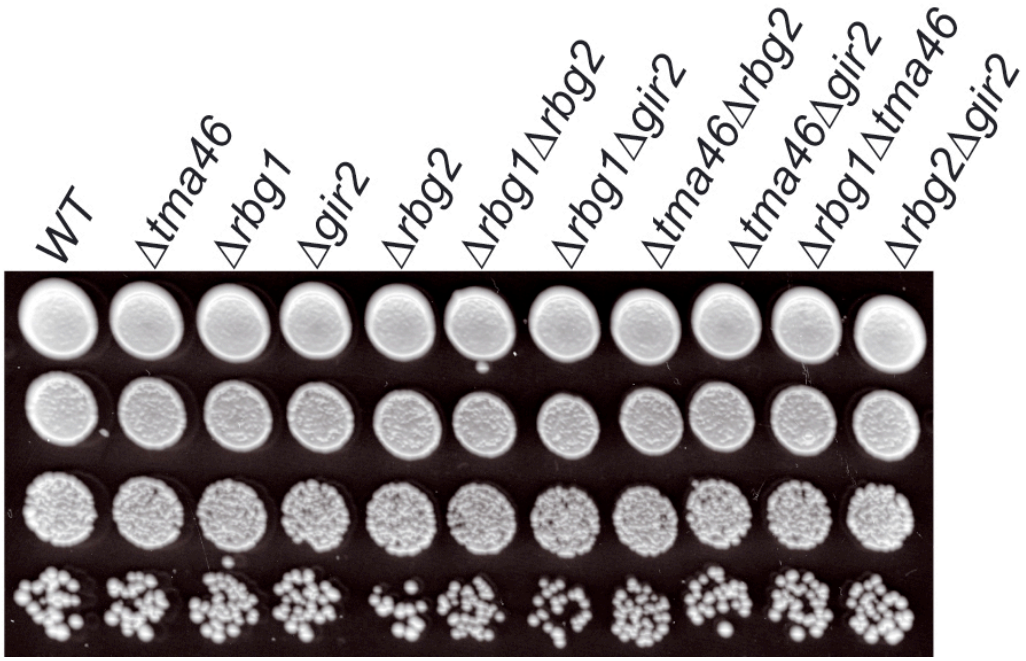
Supplementary Figure S1

Polysomes extracts were prepared from cells expressing both Rbg1 and Rbg2 epitope-tagged proteins from their genomic loci to avoid overproduction. Polysomes were resolved by density sedimentation in 10%-50% sucrose gradient. ATP was included in the extraction buffer at 5mM and in the gradient buffer at 2.5mM. Since gradient contained 2.5mM ATP, UV absorbance trace (254 nm) was not monitored during fractionation. Fractions (numbered) were analyzed by western blotting to detect the TAP and HA tags. The 60S ribosomal protein Rpl1a was detected with specific rabbit polyclonal antibodies and used to position the 60S subunit, 80S ribosomes and polysomes. The 40S was positioned according to previous gradient fractionation. To demonstrate the specificity of the association of factors to polysomes or ribosomes, those were dissociated by treating extracts with RNAse A prior to fractionation on the sucrose gradient.



Supplementary Figure S2

Growth behavior of yeast strains carrying combinations of deletions of *RBG1*, *TMA46, RBG2*, or *GIR2* genes. Serial dilutions of exponential liquid cultures were spotted on YPDA plates and incubated two days at 30°C.



References

1. Schmidt, A., Bickle, M., Beck, T. and Hall, M.N. (1997) The yeast phosphatidylinositol kinase homolog TOR2 activates RHO1 and RHO2 via the exchange factor ROM2. *Cell*, **88**, 531-542.
2. De La Cruz, J., Iost, I., Kressler, D., and Linder, P. (1997) The p20 and Ded1 proteins have antagonistic roles in eIF4E-dependent translation in Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A*, **94**, 5201-5206.
3. Schmid, S.R., and Linder, P. (1991) Translation initiation factor 4A from Saccharomyces cerevisiae: analysis of residues conserved in the D-E-A-D family of RNA helicases. *Mol Cell Biol.* **11**, 3463-3471.
4. Longtine, M.S., McKenzie, A., 3rd, 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. Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M. and Séraphin, B. (1999) A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol*, **17**, 1030-1032.
6. De Antoni, A. and Gallwitz, D. (2000) A novel multi-purpose cassette for repeated integrative epitope tagging of genes in Saccharomyces cerevisiae. *Gene*, **246**, 179-185.
7. Prouteau, M., Daugeron, M.C. and Séraphin, B. (2008) Regulation of ARE transcript 3' end processing by the yeast Cth2 mRNA decay factor. *Embo J.*, **27**, 2966-2976.