Supplementary Information for:

The iron-sulfur protein Rli1 functions in translation termination

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Figure S1

Systematic name	Standard name	Description	Number of independent clones
YLR192C	HCR1	rRNA processing/translation initiation	32
YBL092W	RPL32	protein component of the 60S ribosomal subunit	2*
YBR143C	SUP45	polypeptide release factor eRF1 involved in translation termination	1
YHL015W	RPS20	protein component of the 40S ribosomal subunit	1*
YMR143W	RPS16	protein component of the 40S ribosomal subunit	1*
YDL031W	DBP10	putative ATP dependent helicase of the DEAD box protein family; involved in 60S ribosome biogenesis	1
YLR043C	TRX1	protects cells against both oxidative and reductive stress	1
YOL016C	CMK2	calmodulin-dependent protein kinase	1
YLR044C	PDC1	pyruvate decarboxylase; glucose fermentation	1
YOR047C	STD1	protein involved in control of glucose-regulated gene expression	1
YGR192C	TDH3	glycerinealdehyde-3 phosphate dehydrogenase; glycolysis	1
YOR383C	FIT3	mannoprotein	1
YOR202W	HIS3	histidine biosynthesis	1
YBL036C		alanine racemase activity	1
YDL097C	RPN6	essential, non-ATPase regulatory subunit of the 26S proteasome	1*
YBR085C		function unknown	1
undefined ORF			8

Figure S2

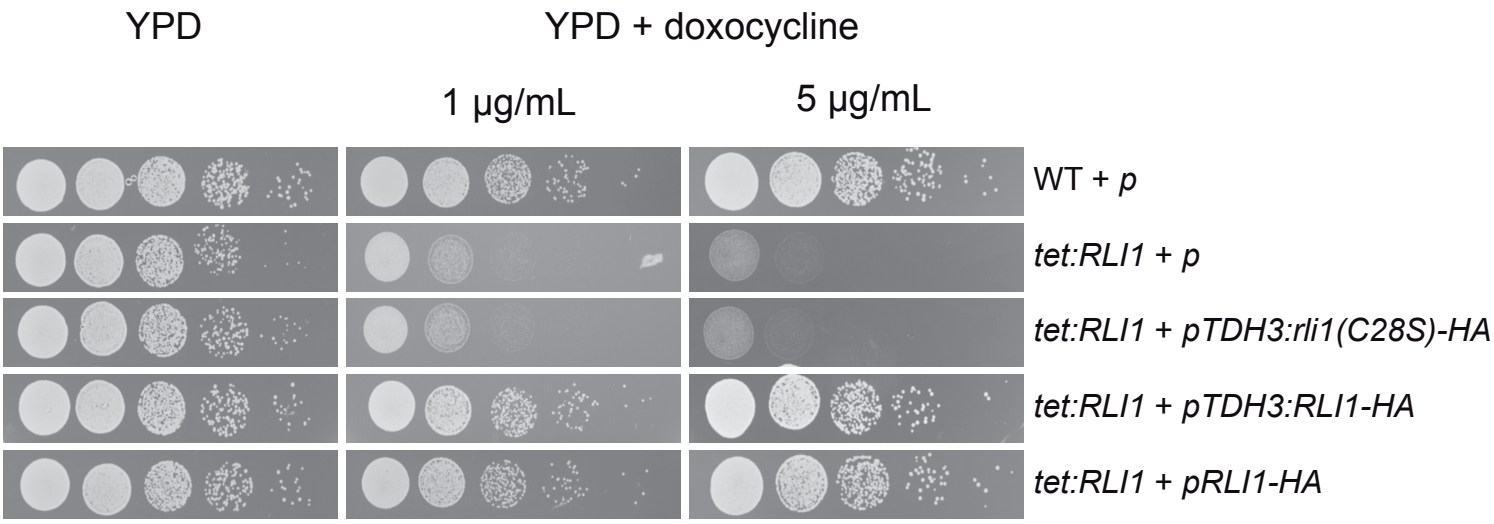
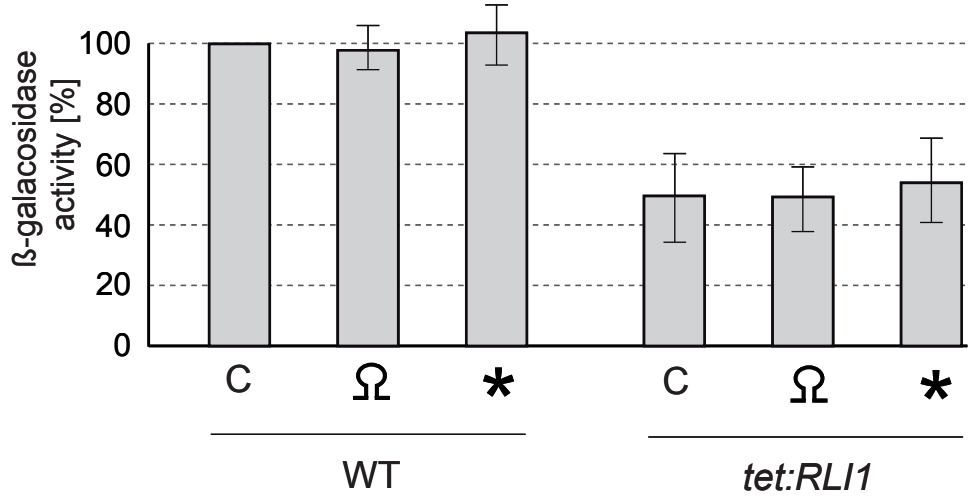

YPD
YPD + doxocycline
1 µg/mL
5 µg/mL
WT + p
tet:RLI1 + p
tet:RLI1 + pTDH3:rli1(C28S)-HA
tet:RLI1 + pTDH3:RLI1-HA
tet:RLI1 + pRLI1-HA

Figure S3

A


ß-galacosidase activity [%]
100
80
60
40
20
0
C
Ω
*
C
Ω
*
WT
tet:RLI1

B

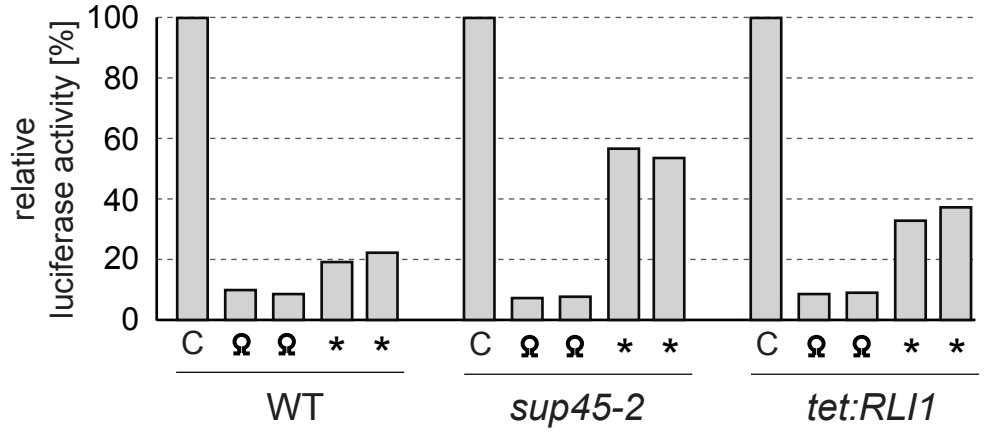
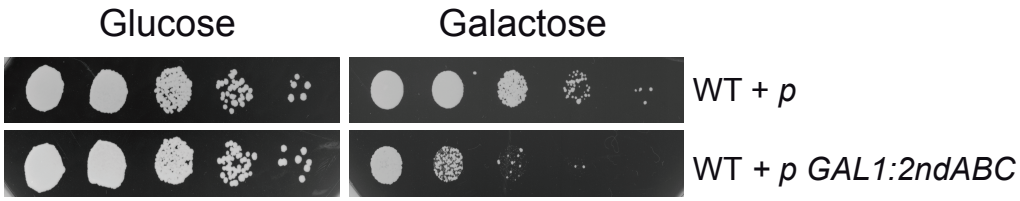

relative luciferase activity [%]
100
80
60
40
20
0
C Ω Ω * *
C Ω Ω * *
C Ω Ω * *
WT
sup45-2
tet:RLI1

Figure S4

A


Glucose
Galactose
WT + p
WT + p GAL1:2ndABC

B

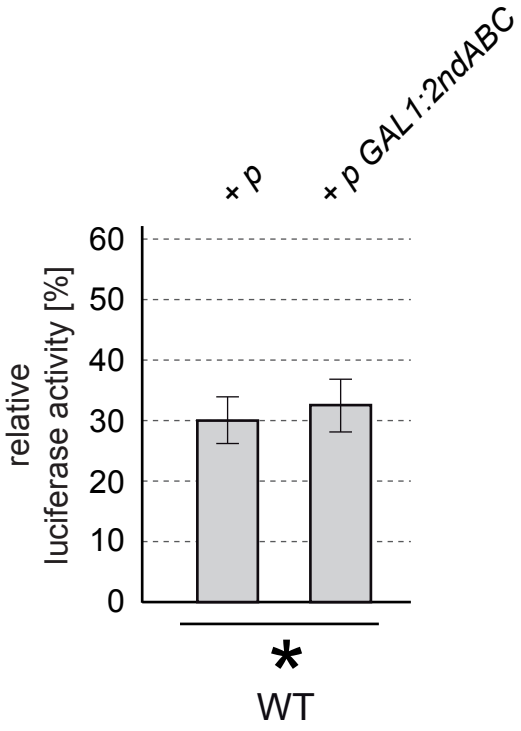

+ p
+ p GAL1:2ndABC
relative luciferase activity [%]
60
50
40
30
20
10
0
*
WT

FIGURE LEGENDS

Fig. S1 | Summary of the results from the yeast-two-hybrid screen including the identified 55 proteins that gave strong yeast-two-hybrid interaction signals with the three reporter genes, *ADE2, HIS3*, and lacZ. * denotes known false-positives in yeast-two-hybrid screens (Hengen, 1997).

Fig. S2 | Downregulation of *RLI1* is lethal, but can be suppressed by a functional copy of HA-tagged *RLI1*. Serial dilutions of the indicated strains and plasmids are shown after growth at 25°C for three days on either full media (YPD) or YPD plates containing the indicated doxocycline concentrations in order to downregulate the *RLI1* expression.

Fig. S3 | Downregulation of *RLI1* leads to defects in translation and to increased read through activity. **A** Comparison of the ß-galatosidase activities in wildtype and downregulated *RLI1* cells (*tet:RLI1*) with three different vectors as depicted in Fig. 4A (C = control, Ω = stem loop, * = stop codon). The ß-galatosidase activity of 1 OD_{600} of wildtype cells (carrying the control vector C) is set to 100%. **B** Comparison of the luciferase activities of wildtype, *sup45-2* and downregulated *RLI1* cells (*tet:RLI1*) from two different examples of the stem loop containing construct (Ω) and the stop codon vector (*). C = control.

Fig. S4 | Overexpression of the 2nd ABC domain is toxic, but does not influence the stop codon recognition. (A) Overexpression of the 2nd ABC domain is toxic to wildtype cells. Wildtype cells carrying either an empty 2µ vector (+ *p*) or a 2µ plasmid encoding the 2nd ABC domain of *RLI1*, driven from the strong *GAL1* promoter were spotted in serial dilutions onto glucose or galactose containing plates and incubated for 2-3 days at 30°C. (B) Overexpression of the 2nd ABC domain does not influence proper stop codon recognition. Readthrough activities for wildtype cells are shown that carry either an empty vector (+ *p*) or a plasmid encoding the second ABC domain of *RLI1*, driven from the strong *GAL1* promoter. (Basal readthrough

activity in galactose containing medium is approximately 30%.) Results of seven independent experiments are shown.

ADDITIONAL METHODS

Construction of GST- and pentaHis- fusion proteins and the *GAL:RLI1* vectors.

To construct GST-fusion proteins of *RLI1*, oligonucleotides corresponding to the 5'-, and 3'-ends or internal sequences were used for PCR with yeast genomic DNA as template and digested with appropriate restriction enzymes corresponding to the terminal sequences (*Bam*H1 or *Sma*I at the 5'-end and *Xho*I at the 3'-end) and subcloned into p GEX6P-3. To insert a polyhistidine tag (His-tag) into the ORF of *SUP45* or *HCR1*, oligonucleotides corresponding to the 5'-, and 3'-ends were amplified from yeast genomic DNA by PCR, introducing terminal *Bam*HI and *Xho*I sites, respectively. The *Bam*HI-*Xho*I fragments of the amplified DNA were subcloned into pET28b (*HCR1*) or pET30a (*SUP45*), respectively. Primer sequences are listed in TABLE S3.

DNA fragments containing either full-length *RLI1* or the C-terminal ABC domain were PCR amplified using the primers listed in TABLE S3 and cloned into pHK907 (pME2795).

Yeast-two-hybrid library screen

A DNA fragment containing either the complete ORF of RLI1 or its different truncations were PCR-amplified using the oligonucleotides listed in table S3 and cloned into pGBKT7 using the Clontech Matchmaker system (Fields und Sternglanz, 1994; James *et al.*, 1996; James, 2001). This construct was used as bait for a two-hybrid screen of a yeast cDNA library fused to the *GAL4* activation domain in pGAD GH (kindly provided by Prof. D. Gallwitz (Max Planck Institute for Biophysical Chemistry Göttingen)). Purified DNA from the libraries was transformed into yeast strain AH109 carrying the bait plasmid pGBKT7-RLI. Transformants were plated onto the triple dropout plates (SD/-trp/-leu/-his). Transformants that grew on the triple dropout plates were rescreened under more stringent conditions on SD/-trp/-leu/-ade/-his dropout plates to test for *ADE2* expression and on X-gal- filter assays to test for lacZ expression. Plasmids were isolated from yeast transformants that were his+, ade+, and blue on X-gal and the inserts adjacent to the GAL4 domain were sequenced.

Co-immunoprecipitation and protein purification

Cells were grown at 25°C to a density of OD_{600} 0.5-0.8 in the appropriate SD medium. If indicated a temperature shift to 37°C was done before 100-200 OD_{600} of the cells were collected, washed once in ddH_2O and resuspended in 600 µl of PBSMKT-buffer (137 mM NaCl, 5.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.5 mM $MgCl_2$, 0.05-0.5% (v/v) Triton X-100). Yeast cells were transfered to microcentrifuge tubes, one cell volume of glass beads and protease inhibitor cocktail (Sigma-Aldrich (Nr. P8215) and Roche Applied Science

(Nr. 11873580001)) were added, and the cells were lysed by vigorous vortexing for 20 sec and 4.5°m/s using FastPrep®-24 (MP Biomedicals). After spinning for 5 min at 16.000 x g, the clarified supernatants were split into two equal portions (+/- addition of RNase A) and incubated for 2-4 h at 4°C with gentle agitation either with 10 µl of Protein G sepharose beads (Amersham Biosciences) or with 10 µl IgG sepharose beads (Amersham Biosciences), conjugated to HA-specific antibodies. In the RNase containing samples the enzyme was added to the PBSMKT-buffer to a final concentration of 200 µg/ml. The beads were washed five times with 1 ml PBSMKT-buffer by repeated low speed centrifugation (600 x g). Samples of the lysates, the final washes and the eluates were mixed with 2 x SDS sample loading buffer and separated on 10-15% (v/v) SDS-PAGEs.

GST-pulldowns

Rosetta II (DE3) cells were transformed with pGEX6P3-Rli1.and either pET30a-Sup45 or pET28b-Hcr1. pGEX6P-3 alone or GST-PNC1 (pGEXTT) co-expressend with either His-Sup45p or His-Hcr1 served as negative controls. Overexpression was induced by 0.2 mM IPTG. Cells were grown at 16°C for 20 h, harvested and resuspended (50 mM Tris-HCl, pH 7.5, 200 mM NaCl, 2 mM 2-mercaptoethanol, 5% (v/v) glycerol, 0.2 mM PMSF, protease inhibitor mix). After cell lysis and centrifugation steps, the supernatant was diluted 1:1 with 50 mM Tris-HCl, pH 7.5, 2 mM 2-mercaptoethanol, 5% (v/v) glycerol, 0.2 mM PMSF, 2 mM $MgCl_2$, 2 mM ATP and incubated with 100 µl of GSH-sepharose beads for 2 h at 4°C. After several washing steps (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 2 mM 2-mercaptoethanol, 5% (v/v) glycerol, 0.2 mM PMSF), beads were analysed on SDS page and by immunostaining. For immunostaining, a penta-His antibody (Quiagen) was employed as first antibody, followed by an alkaline-phophatase conjugated secondary antibody (Dianova) and visualization of the His-tagged protein by alkaline phosphatase assay.

SUPPLEMENTARY TABLES

TABLE S1. Yeast strains

Number	Name	Genotype	Source
HKY35	FY86	*MATa ura3-52 leu2Δ1 trp1Δ63*	Winston *et al.*, 1995
HKY36	FY23	*MATα ura3-52 leu2Δ1 his3Δ200*	Winston *et al.*, 1995
HKY130	CSY550	*MATa ura3-52 leu2Δ1 trp1Δ63 rat8-2*	Snay-Hodge *et al.*, 1998
HKY446	MT552/8a	*MATα ura3-1 his5-2 ade2-1 can1-100 sup45-2*	Stansfield *et al.*, 1997
HKY473		*MATa ura3 leu2Δ1 trp1Δ63 ade2-1 lys sup45-2*	Gross *et al.*, 2007
HKY486	SC0117	*MATa ade2 arg4 leu2-3,112 trp1-289 ura3-52 SUP45:TAP:k.l.URA3*	Euroscarf
HKY487	SC0134	*MATa ade2 arg4 leu2-3,112 trp1-289 ura3-52 SUP35:TAP:k.l.URA3*	Euroscarf
HKY552	21-33G-D373	*MATα pheA10 ade2-144,717 his7-1(UAA) lys9-A21(UAA) trp1-289(UAG) ura3-52 leu2-3,112 sup35-21*	Cosson *et al.*, 2002
HKY940		*MATa ade2-1 his3-11,15 leu2-3,112 ura3-1 can1-100 kanMX:TetO7:rli1*	Kispal *et al.*, 2005
HKY955		*MATa ade2-1 ura3-1 trp1 can1-100 kanMX:TetO7:rli1 sup45-2*	this study
HKY999		*MATα ade2 his leu2-3,112 ura3 trp kanMX:TetO7:rli1 sup35-21*	this study

TABLE S2. Plasmids

Number	Name	Features	Source
pHK86	pRS314	*CEN TRP1*	Christianson *et al.*, 1992
pHK88	pRS316	*CEN URA3*	Christianson *et al.*, 1992
pHK102	pRS424	*2μ TRP1*	Christianson *et al.*, 1992
pHK104	pRS426	*2μ URA3*	Christianson *et al.*, 1992
pHK606	pAC 1798	*CEN LEU2 lacZ-(HIV-1 stemloop)-luc*	Stahl *et al.*, 1995
pHK607	pACTMV	*CEN LEU2 lacZ-(UAG)-luc*	Stahl *et al.*, 1995
pHK608	pACTQ	*CEN LEU2 lacZ-luc*	Stahl *et al.*, 1995
pHK886		*CEN URA3 RLI1-HA*	Kispal *et al.*, 2005
pHK888		*CEN URA3 TDH3:RLI1-HA*	Kispal *et al.*, 2005
pHK891		*CEN URA3 TDH3:rli1(C28S)-HA*	Kispal *et al.*, 2005
pHK903		*2μ URA3 GAL1:RLI1*	this study
pHK904		*2μ URA3 GAL1:RLI1-2ndABC*	this study
pHK907	p426GAL1	*2μ URA3 GAL1*	Mumberg *et al*., 1994

TABLE S3: Oligonucleotides

Construct	**Restriction site**	**Oligonucleotide sequence (5' – 3') forward / reverse**
pGADT7_RLI1/ pGBKT7_RLI1	*Nde*I, *Xho*I	ACGACAGACCCATATGAGTGATAAAAACAGTCG/ CATACTCGAGTTAAATACCGGTGTTATCCAAGAAA
pGADT7_SUP45 / pGBKT7_SUP45	*Nde*I, *Xho*I	GGAATTCATATGGATAACGAGGTTGAAAAAAATAT/ ACCGCTCGAGAATGAAATCATAGTCGGATCCTT
pGADT7_DBP10	*Nde*I, *Xho*I	GGAATTCATATGGCAGGCGTGCAGAAA/ ACCGCTCGAGCTAAAATTTACGCTTTTTGGAAGG
pME2795_RLI1-2ndABC domain (pHK904)	*Sma*I, *Xho*I	TTCCCCGGGAATGACTGAAGCTTTACAATTTAGAATAGC/ CATACTCGAGTTAAATACCGGTGTTATCCAAGAAA
pGBKT7_RLI1-2ndABC domain / pGEX6P-1_RLI-2ndABC domain	*Nde*I, *Xho*I	GGAATTCATATGACTGAAGCTTTACAATTTAGAATAGC/ CATACTCGAGTTAAATACCGGTGTTATCCAAGAAA
pGBKT7_HCR1/ pET22b_HCR1	*Nde*I, *Xho*I	GGGAATTCCATATGTCTTGGGACGACGAAGCTA/ GCCCTCGAGTTACATAAAGTCGTCATCACCAAGTTC
pET30a_SUP45	*Bam*HI, *Xho*I	ACGCGGATCCATGGATAACGAGGTTGAAAAAAATAT/ ACCGCTCGAGAATGAAATCATAGTCGGATCCTT

ADDITIONAL REFERENCES

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