Supplemental Materials

Tables S1~S3

Figure S1

Table S1 Yeast strains used in this study

name	genotype	source/reference
W303	*MAT***a**/α *GAL2*/+ *ade2-1*/- *his3-11,15*/- *leu2-3,112*/- *trp1-1*/- *ura3-1*/- *can1-100*/-	Thomas & R. Rothstein*
W303-1A	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	Thomas & R. Rothstein*
X2180-1A	*MAT***a** *SUC2 mal mel gal2 CUP1*	Mortimer & Johnston**
TMSC03	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-4*	This study
TMSC05	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-100*	This study
TMSC07	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 RLG1-HA::CgHIS3*	This study
TMSC09	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 hac1Δ::HIS3*	This study
TYSC335	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 RLG1-protein A::CgHIS3*	This study
TYSC474	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX*/ pTYSC224[*CEN TRP1 ScRLG1*]	This study
TYSC771	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX*/ pTYSC418[2*μ TRP1 CUP1p::HA-ScRLG1*]	This study
TYSC772	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX*/ pTYSC441[2*μ TRP1 CUP1p::HA-KlRLG1*]	This study
TYSC773	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX*/ pTYSC442[2*μ TRP1 CUP1p::HA-SpRLG1*]	This study
TYSC774	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX*/ pAt-R1[2*μ TRP1 CUP1p::HA-AtRLG1*[M74]]	This study

TYSC791	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX hac1Δ::HIS3*/ pTYSC418[2*μ TRP1 CUP1p::HA-ScRLG1*]	This study
TYSC793	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX hac1Δ::HIS3*/ pAt-R1[2*μ TRP1 CUP1p::HA-AtRLG1*[M74]]	This study
TYSC835	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX*/ pAtR1[2*μ TRP1 CUP1p::HA-AtRLG1*[M74]] pTYSC462[2*μ URA3 CUP1p::FLAG*]	This study
TYSC836	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX*/ pAtR1[2*μ TRP1 CUP1p::HA-AtRLG1*[M74]] pTYSC463[2*μ URA3 CUP1p::FLAG-ScRLG1*]	This study
TYSC855	*MAT***a** *GAL2 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rlg1-ΔKpnI::kanMX*/ pTYSC471[2*μ TRP1 CUP1p::HA-AtRLG1*[M54]]	This study
TYSC1130	*MAT***a** *GAL2 ura3-1 leu2-3,112 trp1-1 his3-11,15 ade2-1 can1-100 Δrlg1-ΔKpnI::kanMX4*/pTYSC541[2*μ TRP1 CUP1p::FLAG-AtRLG1*[M74]]	This study
7501879	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 KAP95-TAP::HIS3MX6*	Open Biosystems
BY20597	*Kluyveromyces lactice* wild-type strain	National BioResource Project, Japan
972	*Schizosaccharomyces pombe* h^- wild-type strain	National BioResource Project, Japan

*Thomas, B. J., and Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. Cell *56*, 619–630.

**Mortimer, R. K., and Johnston, J. R. (1986) Genealogy of principal strains of the yeast genetic stock center. Genetics *113*, 35–43.

Table S2 Plamids used in this study

plasmid	type	genotype or characteristics	source or reference
pASZ11	YCp	*CEN ARS ADE2*	Stotz & Linder (1990)*
pRS306	YIp	*URA3*	Sikorski & Hieter (1989)**
pRS314	YCp	*CEN ARS TRP1*	Sikorski & Hieter (1989)**
pRS316	YCp	*CEN ARS URA3*	Sikorski & Hieter (1989)**
pTYSC128	YEp	*2μ CUP1p::HA-MCS TRP1*	Yoshihisa *et al*. (2003)
pTYSC220	YCp	*CEN ARS RLG1 TRP1*	This study
pTYSC224	YCp	*CEN ARS RLG1 URA3*	This study
pTYSC295	YIp	*rlg1Δ::kanMX4*	This study
pTYSC418	YEp	*2μ CUP1p::HA-ScRLG1 TRP1*	This study
pTYSC421	YIp	*hac1Δ::HIS3*	This study
pTYSC441	YEp	*2μ CUP1p::HA-KlRLG1 TRP1*	This study
pTYSC442	YEp	*2μ CUP1p::HA-SpRLG1 TRP1*	This study
pTYSC445	YCp	*CEN ARS HAC1 ADE2*	This study
pTYSC446	YCp	*CEN ARS hac1-m2 ADE2*	This study
pTYSC447	YCp	*CEN ARS hac1-m1 ADE2*	This study
pTYSC461	YEp	*2μ CUP1p::FLAG URA3*	This study
pTYSC462	YEp	*2μ CUP1p::FLAG TRP1*	This study
pTYSC463	YEp	*2μ CUP1p::FLAG-ScRLG] URA3*	This study
pTYSC468	YCp	*CEN ARS hac1-m3 ADE2*	This study
pTYSC469	YCp	*CEN ARS hac1-m4 ADE2*	This study
pTYSC471	YEp	*2μ CUP1p::HA-AtRLG1*[M54] *TRP1*	This study
pTYSC475	YCp	*CEN ARS GAL7p::GFP URA3*	This study
pTYSC476	YCp	*CEN ARS HAC1-GFP-HAC1 URA3*	This study
pTYSC508	YCp	*CEN ARS HAC1-GFP::HAC1 URA3*	This study
pTYSC541	YEp	*2μ CUP1p::FLAG-AtRLG* [M74] *1 TRP1*	This study
pTYSC544	YCp	*CEN ARS HAC1-GFP URA3*	This study
pAt-R1	YEp	*2μ CUP1p::HA-AtRLG1*[M74] *TRP1*	This study
pIVEX WG1.4-Atlig	bacterial	AtRLG1[M74] ORF in pIVEX WG1.4	Englert & Beier (2005)
pHAC1	bacterial	anti-sense of *HAC1* ORF full length under the SP6 promoter in pGEM-4Z	This study
pHAC1-int	bacterial	anti-sense of *HAC1* intron under the SP6 promoter in pGEM-4Z	This study
pACT1	bacterial	anti-sense of *ACT1* full length under the SP6 promoter in pGEM-4Z	This study
pTYE493	bacterial	sense of *HAC1*^{i} cDNA under the T7 promoter in pUC119	This study

* Stotz, A., and Linder, P. (1990). The *ADE2* gene from *Saccharomyces cerevisiae*: sequence

and new vectors. Gene *95*, 91-98.
** Sikorski, R. S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19-27.

Table S3 Primers used in this study

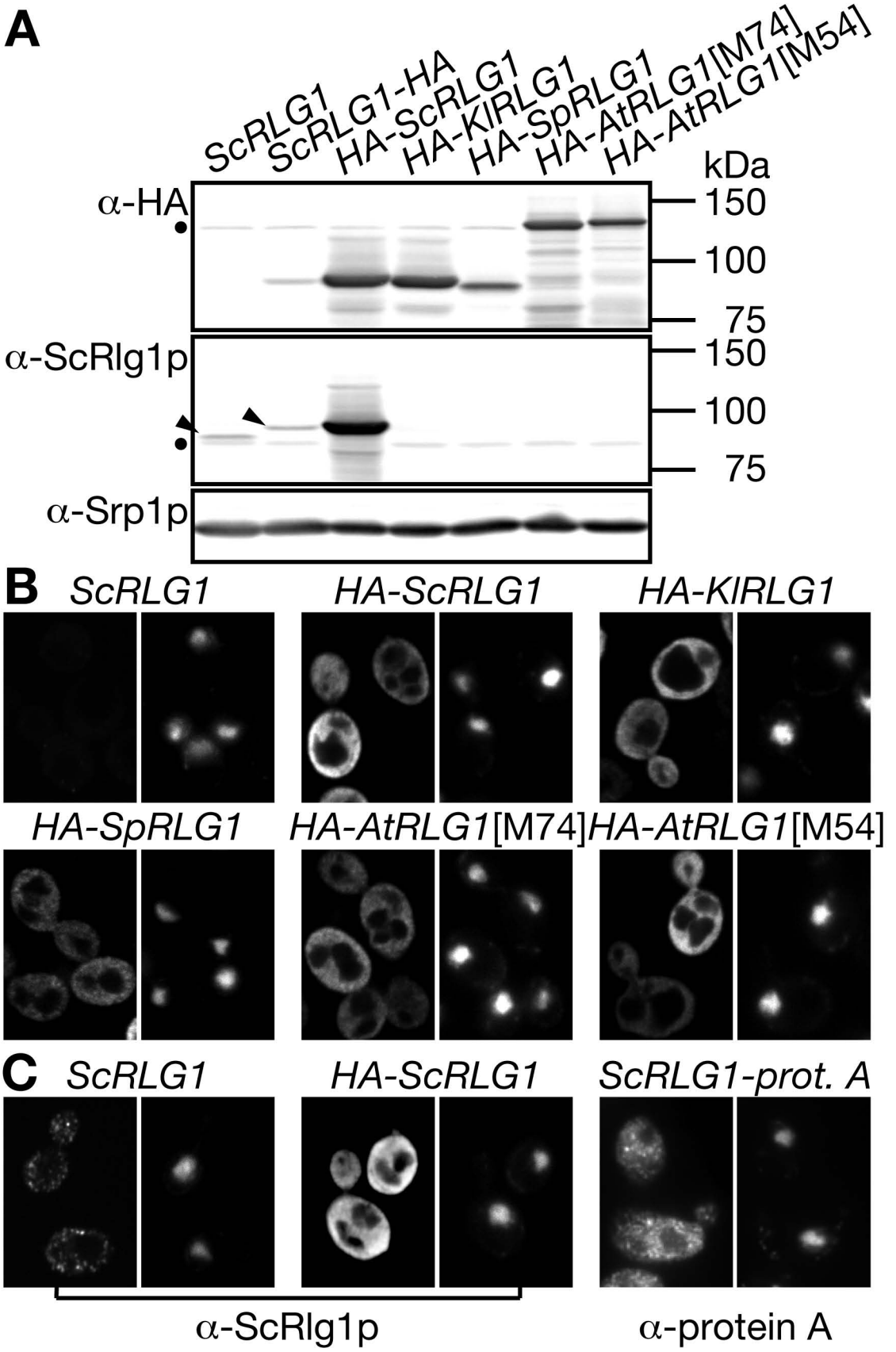
primer	sequence	gene
for cloning of gene fragments		
RLG1 5'	5'-GAAGGATCCATGCCTAGCCCATATGACGG-3'	*ScRLG1* ORF
RLG1 +ter2	5'-CCAGACCGCGGTCTAAAAATTTAAATATAC-3'	
RLG1_long PCR 5'	5'-AAACTCGAGGTTCAAGGAATGCCATGCAC-3'	*ScRLG1* whole gene
RLG1_long PCR 3'	5'-GAGGGATCCGACCGAAGGTGCAGCCACC-3'	
KlRLG1-5	5'-AAAAAAACTCGAGCTATGGCAGAACAAGATGTGAC-3'	*KlRLG1* ORF
KlRLG1-3ter	5'-TTTTGGTACCTTATAAATTAATACAGACTTTCCC-3'	
SpRLG1_5-2	5'-AAAAAAACTCGAGCTATGGTTTTGAATTTTAATAAT-TCAG-3'	*SpRLG1* ORF
SpRLG1_3ter	5'-TTTTGGTACCTAGTAAACGGGTTCCAGC-3'	
AtRLG1_M54-ext	5'-CGGTACCGGCCGCATGCCAAAGAAGCAGAAAAAGAG-AGATCACGCTGAGCAGAAGTGGCAAGTAAAACCGAAGA-TGGATGCTCCATTTGAATC-3'	*AtRLG1*[M54] ORF
AtRLG1_760-741c	5'-GATCCATGGATGCTCCATTTGAATC-3'	
HAC1_long5-2	5'-AAAGGATCCGTTGAAAAATGCTGTGATCGAAC-3'	*HAC1* whole gene
HAC1_long3-2	5'-TTTTGGTACCAGCACGGGAGAAGGAACAG-3'	
HAC1_r1511-491	5'-TCTGGTACCTCATGAAGTGATGAAGAAATC-3'	*HAC1* parts
HAC1_r1927-06	5'-TCTGGTACCTTGAAAAGCTGCCCAACCTAAG-3'	
HAC1_5'-UTR_rv	5'-TTTTTTTACCGGTAGTGGCGGTTGTTGTCGT-3'	
HAC1U_3'-UTR_fw2	5'-AAACCGCGGTTGAACAAGAACACTAGCCCC+3'	
T7-HAC1_f477	5'-AAAGGATCCTAATACGACTCACTATAGGGAGAAGGC-TTTAACTCAGTGTC-3'	*HAC1* mRNA transcription
T7-HAC1_f1121	5'-AAAGGATCCTAATACGACTCACTATAGGGAGAGGCA-GACCCACTCTGCGAC-3'	
for RNase H cleavage		
HAC1_1310-291c	5'-CCATCAGAGAACCACGACTA-3'	
HAC1_1360-41c	5'-TCCAATAACCCTGCATTCTG-3'	
for making mutants		
RLG1 H148Y	5'-ACGTAGACAGGAACTATGCAGAAGCAGGT-3'	*rlg1-100*
RLG1 T180I	5'-TACCCATAATGTCATCGCTGTGGCAGAAT-3'	*rlg1-4*
HAC1_m1-5half_3	5'-TGGGGGAGGAGGAGGTTTCAGAGAACCACGACTAA-3'	*HAC1-m1*
HAC1_m1-3half_5	5'-AACCTCCTCCTCCCCCAAAAGTACCTTCAAAAGCAG-3'	
HAC1_m2-5half_3	5'-AACCTCCTCCTCCCCCATCAGAGAACCACGACTAA-3'	*HAC1-m2*
HAC1_m2-3half_5	5'-TGGGGGAGGAGGAGGTTAAAGTACCTTCAAAAGCAG-3'	
HAC1_m3-5half_3	5'-AACCGGCTCCTCCCCCAGTTATGTTTGACACTGAG-3'	*HAC1-m3*
HAC1_m3-3half_5	5'-ACTGGGGGAGGAGCCGGTTCCTACGACAACAACCGCC-3'	
HAC1_m4-5half_3	5'-TGGGGGAGGAGCCGGTTGTTATGTTTGACACTGAG-3'	*HAC1-m4*
HAC1_m4-3half_5	5'-ACAACCGGCTCCTCCCCCACCTACGACAACAACCGCC-3'	

for RT-PCR		
ACT1 5-25	5'-ATTCTGAGGTTGCTGCTTTGG-3'	*ACT1*
ACT1 1128-06c	5'-GTGGTGAACGATAGATGGAC-3'	
ERO1 1151-70	5'-AACAACATATTGTTGGTAAG-3'	*ERO1*
ERO1 1670-51c	5'-GAAATAGGCTCTCGTGTCTC-3'	
GFP_RT_fw2	5'-GTTTGAAGGTGATACCCTTG-3'	*GFP*
GFP_RT_rv2	5'-TAGTTCATCCATGCCATGTG-3'	
GFPseq_405-24	5'-CATTCTTGGACACAAATTGG-3'	
HAC1 PCR361-81	5'-CTGGCTGACCACGAAGACGC-3'	*HAC1*
HAC1 PCR1080-61c	5'-TTGTCTTCATGAAGTGATGA-3'	
SRP1_1221-40	5'-TTCCAATGCCTCTTCAGGTG-3'	*SRP1*
SRP1_1570-51c	5'-TTTGTGGAGCCATAGTTTCG-3'	

Legend of Supplemental Figure

Figure S1. Rlg1p homologues localized mainly in the cytoplasm.
(**A**) Expression of HA-tagged Rlg1p homologues was monitored by Western Blotting with anti-HA (upper) and anti-ScRlg1p (middle) antibodies. Srp1p was detected as a loading control. ScRlg1-related proteins expressed from the chromosomal loci were marked by arrowheads. Bands marked with dots are unrelated proteins. (**B**) Localization of HA-tagged Rlg1p homologues was visualized by immunofluorescence with an anti-HA antibody. The left panel of a set of microscopic images corresponds to anti-HA staining, and the right panel corresponds to DAPI staining. (**C**) Localization of authentic ScRlg1p (W303-1A, left) and HA-ScRlg1p (HA-ScRLG1, middle) was analyzed with affinity-purified anti-Rlg1p antibodies. In the right set of images, yeast cells with an *ScRLG1-protein A* gene integrated in the chromosomal *ScRLG1* locus were subjected to immunofluorescence with anti-protein A antibodies.

Figure S1


A
ScRLG1
ScRLG1-HA
HA-ScRLG1
HA-KlRLG1
HA-SpRLG1
HA-AtRLG1[M74]
HA-AtRLG1[M54]
kDa
α-HA
150
100
75
α-ScRlg1p
150
100
75
α-Srp1p
B
ScRLG1
HA-ScRLG1
HA-KlRLG1
HA-SpRLG1
HA-AtRLG1[M74]
HA-AtRLG1[M54]
C
ScRLG1
HA-ScRLG1
ScRLG1-prot. A
α-ScRlg1p
α-protein A