Supplemental Data

Topologically conserved residues direct heme transport in HRG-1-related proteins

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The supplemental data file contains 7 figures and 2 tables.

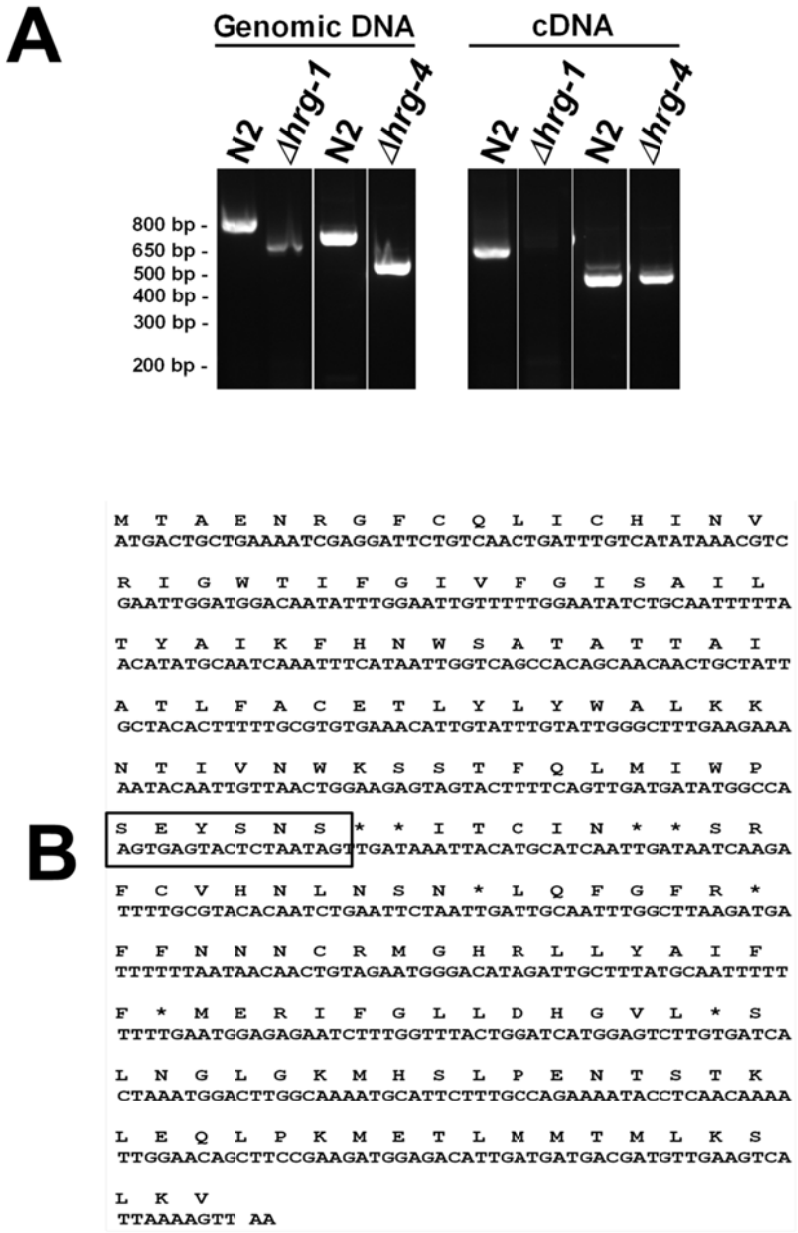

Figure S1. Genotyping of HRG-1-related deletion worms.
(A) Left: PCR analysis of genomic DNA obtained from wild-type N2 worms (lanes 1 and 3) and homozygous mutants. *hrg-1* specific primers were used in lanes 1 and 2; *hrg-4* primers were used for lanes 3 and 4. Right: PCR screening of cDNA synthesized from total RNA isolated from wild-type N2 worms and homozygous mutants. The *hrg-1* primers were used for lanes 1 and 2; *hrg-4* primers were used for lanes 3 and 4. (B) DNA sequence for *Δhrg-4* (tm2994) along with predicted amino acid sequence of the RT-PCR product. The boxed region indicates the new sequences that are added.

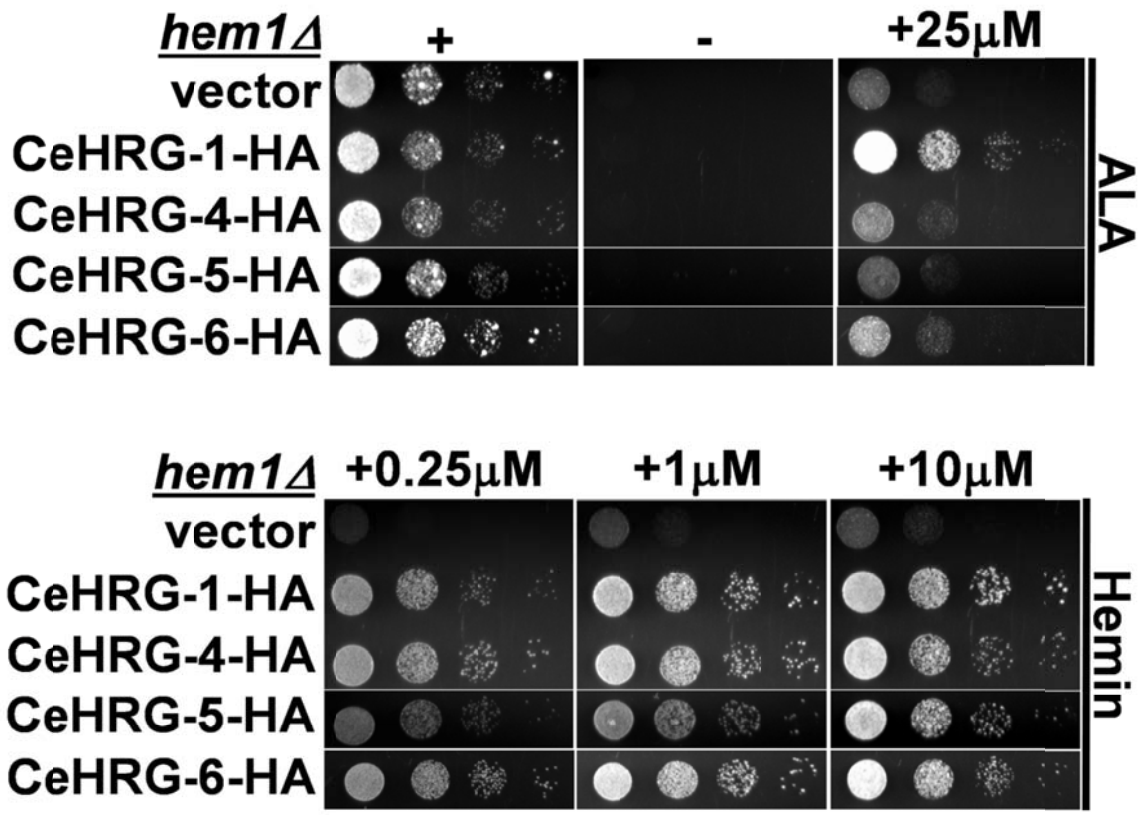

Figure S2. Worm HRG-1 homologs function as heme importers in yeast *hem1*Δ strain.
The *hem1*(6D) strain was transformed with empty vector pYes-DEST52, and c-terminus HA-tagged HRG-1 proteins. Spot growth assay were performed with indicated conditions. Plates were incubated at 30°C for 3 days prior to imaging.

Figure S3. Sequence alignment of CeHRG-1 and CeHRG-4.
Pairwise sequence alignment was performed on CeHRG-1 and CeHRG-4 using EMBOSS Needle. Predicted transmembrane domains were shown as boxed region (TMD 1 - 4). Conserved amino acids shared by CeHRG-1 and CeHRG-4 were shaded in gray. Potential heme interacting motifs (red) and worm HRG-1 specific residues (green) were indicated by asterisks.

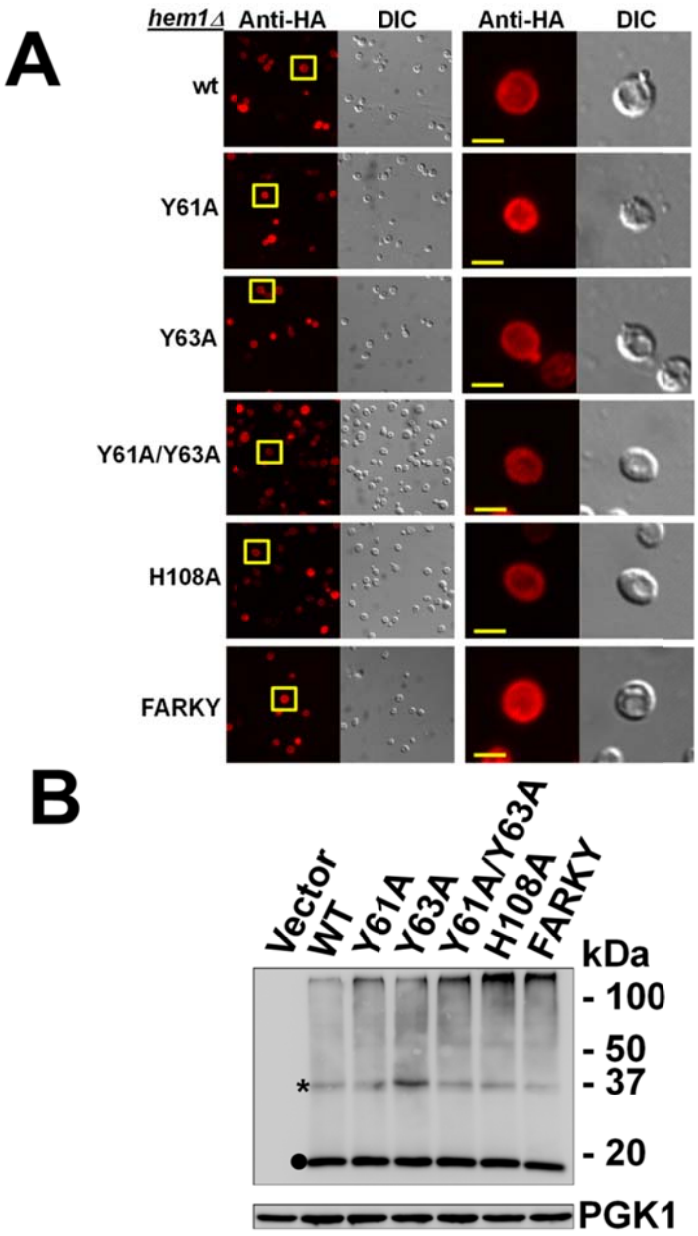

Figure S4. Localization and expression of CeHRG-4-HA and mutants in yeast.
(A) Indirect immunofluorescence microscopy was conducted on *hem1*(6D) strain transformed with empty vector pYes-DEST52, CeHRG-4-HA and mutants. Rabbit anti-HA was used as the primary antibody, and an AlexaFluo568-conjugated goat anti-rabbit antibody was used as the secondary antibody. Boxed regions were enlarged for clarity. Scale bar, 5 μm. (B) Immunoblotting was performed on transformants of CeHRG-4-HA and its mutants in the *hem1*(6D) strain. Predicted molecular mass of CeHRG-4 was detected as indicated by filled circles. Asterisks indicated putative dimers. The endogenous expression level of phosphoglycerate kinase 1 (PGK1) was used as loading control (15 μg total protein / lane).

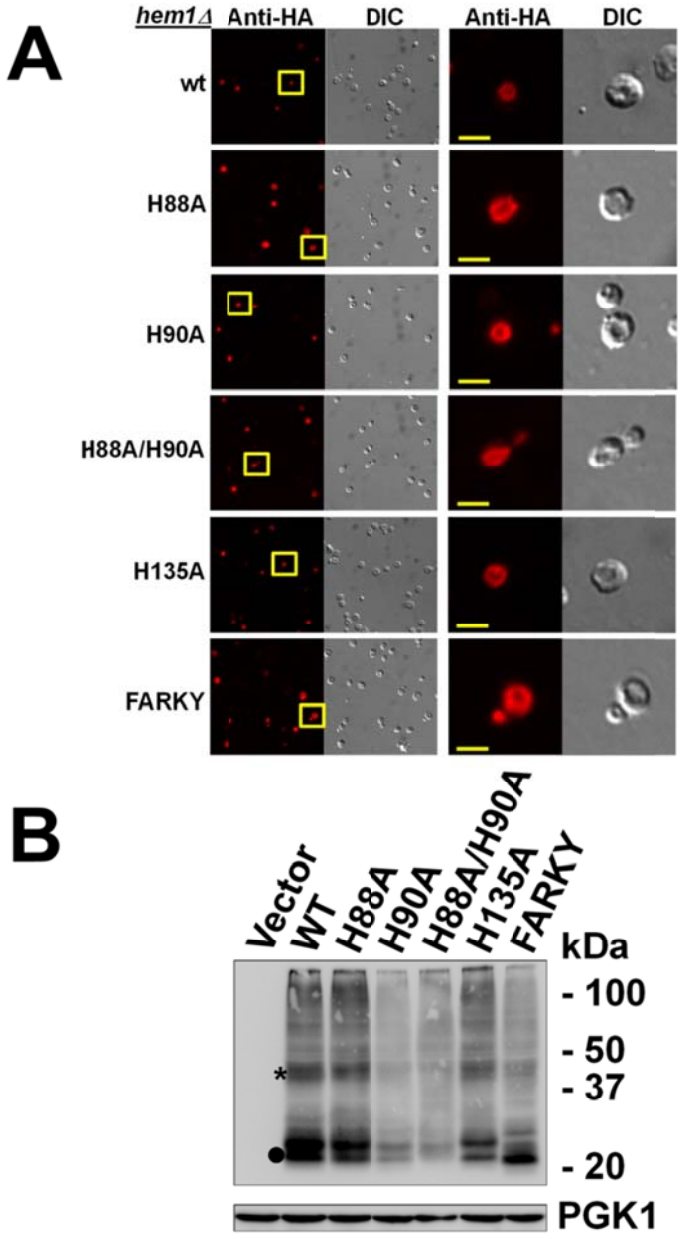

Figure S5. Localization and expression of CeHRG-1-HA and mutants in yeast.
(A) Indirect immunofluorescence microscopy was conducted on *hem1*(6D) strain transformed with empty vector pYes-DEST52, CeHRG-1-HA and mutants. Rabbit anti-HA was used as the primary antibody, and an AlexaFluo568-conjugated goat anti-rabbit antibody was used as the secondary antibody. Boxed regions were enlarged for clarity. Scale bar, 5 μm. (B) Immunoblotting was performed on transformants of CeHRG-1-HA and its mutants in the *hem1*(6D) strain. Predicted molecular mass of CeHRG-1 was detected as indicated by filled circles. Asterisks indicated putative dimers. The endogenous expression level of phosphoglycerate kinase 1 (PGK1) was used as loading control (30 μg total protein / lane).

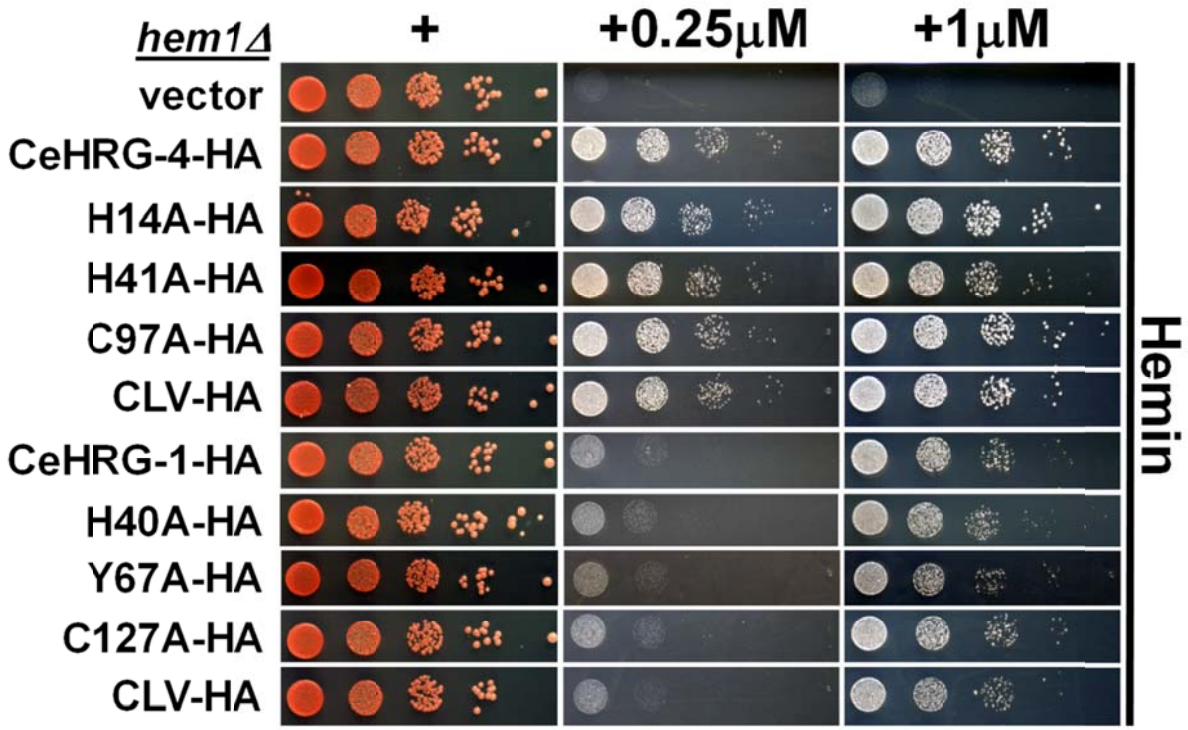

Figure S6. Mutagenesis of worm specific potential heme ligand did not affect HRG-1 function.

The *hem1*Δ(6D) strain transformed with empty vector pYes-DEST52, CeHRG-4-HA, CeHRG-1-HA and alanine substitutions of histidines/tyrosines at the amino-terminus, E1 loop, and a CLV motif in TMD3. Spot growth assay were performed with indicated conditions. Plates were incubated at 30°C for 3 days prior to imaging.

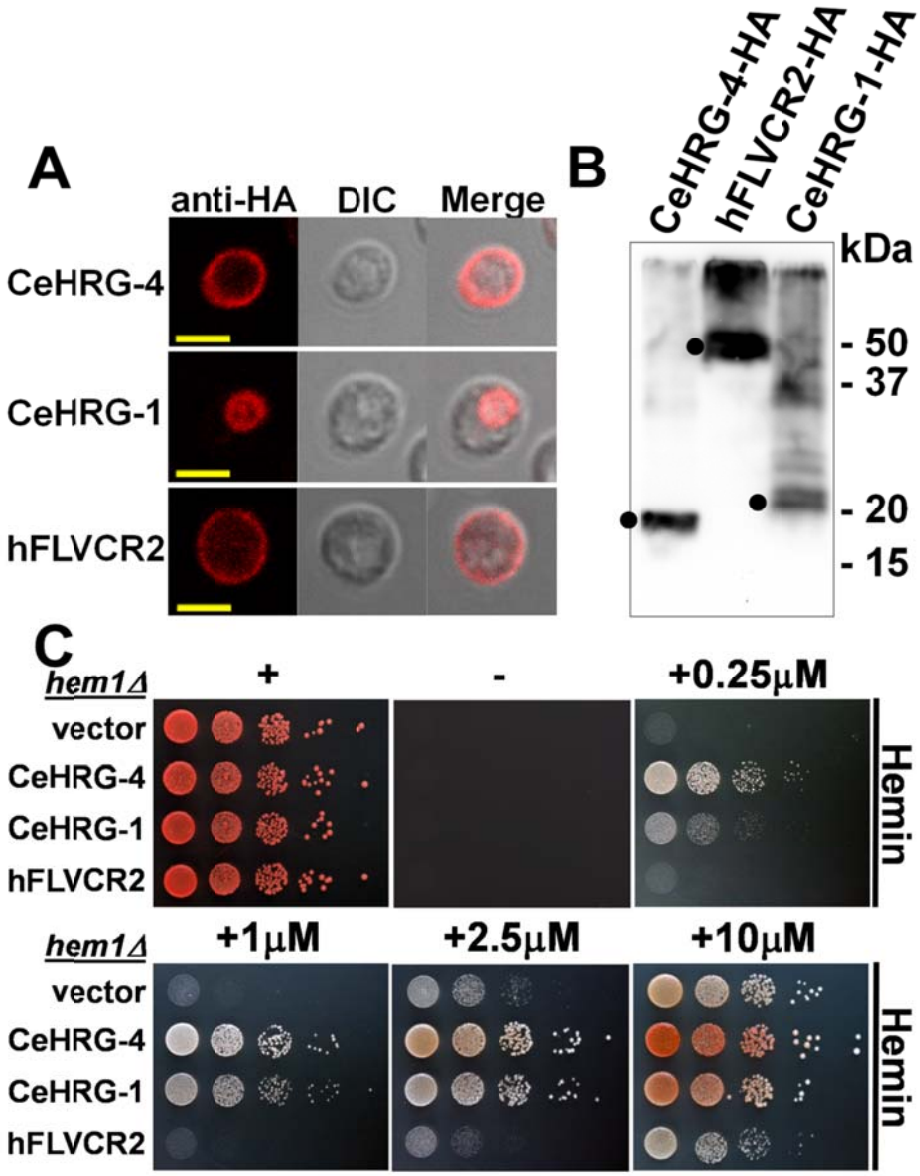

Figure S7. Overexpressing hFLVCR2 did not increase heme uptake in yeast *hem1*Δ strain. (A) Immunofluorescence microscopy results of hFLVCR2 in the *hem1*(6D) strain. Rabbit anti-HA was used as the primary antibody, and an AlexaFluo568-conjugated goat anti-rabbit antibody was used as the secondary antibody. Scale bar, 5 μm. (B) Immunoblotting was performed on transformants overexpressing c-terminus HA epitope tagged CeHRG-4, CeHRG-1 or hFLVCR2 in the *hem1*(6D) strain. Predicted molecular mass were detected as indicated by filled circles (15 μg / lane). (C) The *hem1*Δ(6D) strain transformed with empty vector pYes-DEST52, CeHRG-4, CeHRG-1 and hFLVCR2. Spot growth assay were performed with indicated conditions. Plates were incubated at 30°C for 3 days prior to imaging.

Table S1. Yeast strains used in this study

Strain name	Short genotype	Genetic background	Relevant genotype	Source
DY1457		W303	W303 *MAT_ ura3-52 leu2-3,112 trp1-1 his3-11 ade6 can1-100*	(1)
	*hem1*Δ(6D)	DY1457	*hem1*::*LEU2 trp1-1 his3-11 ura3-52 can1-100*	(1)
YPH499			*MAT*a *ura3-52 lys2-801*(amber) *ade2-101*(ocher) *trp1-63 his3-200 leu2-1*	(3)
OPY102	*hem1*Δ *fre1*Δ *fre2*Δ	YPH499	*hem1*Δ::KanMX *fre1*::*LEU2 fre2*::*HIS3*	(2)
	*hem1*Δ *fre1*Δ *fre2*Δ MET3-FRE1	YPH499	*hem1*Δ::KanMX *fre1*::*LEU2 fre2*::*HIS3 trp1-63*::*TRP1* pMET3-FRE1	This study

Table S2. Oligonucleotides used in this study

Name	Sequence (5’ to 3’)
CeHRG-4H14A	CTGTCAACTGATTTGTGCTATAAACGTTCGAATTGG
CeHRG-4H41A	ACATATGCAATCAAATTTGCTAATTGGTCAGCCACA
CeHRG-4Y61A	GCGTGTGAAACATTGGCTTTGTATTGGGCTTTG
CeHRG-4Y63A	GTGAAACATTGTATTTGGCTTGGGCTTTGAAGAAAAAT
CeHRG-4Y61A/Y63A	GCGTGTGAAACATTGGCTTTGGCTTGGGCTTTGAAGAAAAA
CeHRG-4C97A	CGGACTTCTCGGCGCCCTTGTTTGCTACATTAT
CeHRG-4CLV	GTCTTCTCGGACTTCTCGGCGCCGCTGCTTGCTACATTATTGCAGG
CeHRG-4H108A	TATTGCAGGAATCACTGCTCAGGGTGCAGGAT
CeHRG-4FARKY	ATGGACTTGGCAAAATGCATTCGCTGCCGCAGCAGCCCTCAACAAAATT GGAACAGCTT
CeHRG-1H40A	GCTGTACCTGGTTCGCCAGTCTGAAAGTTCAAATC
CeHRG-1Y67A	GTGTTCGCAATTCAGGCCCAAAATTGGATCGCC
CeHRG-1H88A	GCAACTCTGGTTCTCGCTCTTCACCTGGCCTA
CeHRG-1H90A	CTGGTTCTCCATCTTGCCCTGGCCTATAAGAAAAC
CeHRG-1H88A/H90A	GCAACTCTGGTTCTCGCTCTTGCCCTGGCCTATAAGAAAAC
CeHRG-1C127A	CATCGCAATGGTGTTCGCCCTGGTGGTTGCTGG
CeHRG-1CLV	TTCATTCATCGCAATGGTGTTCGCCGCGGCGGTTGCTGGAATTGAGCATC AG
CeHRG-1H135A	GTTGCTGGAATTGAGGCTCAGACGTTGGATAAG
CeHRG-1FARKY	AATGGAGTGCATTAACCTGGAGAGCTGCCGCAGCAGCCCGAGCATTCTG TGAAGAGTCT
yhHRG-1H56A	GTGGGTTTTGGTTACTGCTGTTATGTACATGCAAG
CeHRG-1_del_f	GGCACAAGGTCCAATAGTTA
CeHRG-1_del_b	AGTCGAGCCATATGGTGCAA
CeHRG-4_del_f	CAATCAAATTTCATAATTGGTCAGC
CeHRG-4_del_b	TTAACTTTTAATGACTTCAACATCGTC

Underlined nucleotides indicated the alanine substitutions.

References

1. Crisp RJ*, et al.* (2003) Inhibition of heme biosynthesis prevents transcription of iron uptake genes in yeast. *J. Biol. Chem.* 278(46):45499-45506.
2. Protchenko O*, et al.* (2008) Role of PUG1 in inducible porphyrin and heme transport in Saccharomyces cerevisiae. *Eukaryot. Cell* 7(5):859-871.
3. Sherman F (1991) Getting started with yeast. *Methods Enzymol.* 194:3-21.