

eIF5A is an indispensable protein for eukaryotic cells

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File S1_Supplementary Tables

Table S1-S5

Table S1. Yeast strains used in this study

Name	Strain	Genotype	Source
PAY266	WT	BY4741 MATa <i>ura3Δ0 leu2Δ0 his3Δ1 met15Δ0</i>	Euroscarf
PAY716	<i>tif51A-1</i>	BY4741 <i>tif51A-1::kanR</i>	(Li et al. 2011)
PAY717	<i>tif51A-3</i>	BY4741 <i>tif51A-3::kanR</i>	(Li et al. 2011)
PAY1410	<i>tif51BΔ</i>	BY4741 <i>tif51B::his3</i>	This study
PAY1412	<i>tif51A-1 tif51BΔ</i>	BY4741 <i>tif51A-1::kanR tif51B::his3</i>	This study
PAY1414	<i>tif51A-3 tif51BΔ</i>	BY4741 <i>tif51A-3::kanR tif51B::his3</i>	This study
PAY760	<i>rox1Δ</i>	BY4741 <i>rox1::kanMX</i>	Euroscarf
PAY761	<i>mot3Δ</i>	BY4741 <i>mot3::kanMX</i>	Euroscarf
PAY1682	WT <i>ROX1-myc</i>	BY4741 <i>ROX1-13myc-his3MX6</i>	This study
PAY2012	<i>tif51A-1 ROX1-myc</i>	BY4741 <i>tif51A-1::kanR ROX1-13myc-his3MX6</i>	This study
PAY2020	<i>tif51A-3 ROX1-myc</i>	BY4741 <i>tif51A-3::kanR ROX1-13myc-his3MX6</i>	This study
PAY1279	<i>sup1</i>	BY4741 <i>tif51A-1::kanR sup1</i>	This study
PAY1280	<i>sup2</i>	BY4741 <i>tif51A-1::kanR sup2</i>	This study
PAY1729	<i>sup22</i>	BY4741 <i>tif51A-1::kanR sup22</i>	This study
PAY2056	<i>sup23</i>	BY4741 <i>tif51A-1::kanR sup23</i>	This study
PAY1289	<i>sup11</i>	BY4741 <i>tif51A-3::kanR sup11</i>	This study
PAY1293	<i>sup15</i>	BY4741 <i>tif51A-3::kanR sup15</i>	This study
PAY1730	<i>sup25</i>	BY4741 <i>tif51A-3::kanR sup25</i>	This study
PAY2057	<i>sup27</i>	BY4741 <i>tif51A-3::kanR sup27</i>	This study
PAY1762	<i>sup43</i>	BY4741 <i>tif51A-1::kanR tif51B::his3 sup43</i>	This study
PAY1763	<i>sup44</i>	BY4741 <i>tif51A-1::kanR tif51B::his3 sup44</i>	This study
PAY1938	<i>sup45</i>	BY4741 <i>tif51A-1::kanR tif51B::his3 sup45</i>	This study
PAY1939	<i>sup46</i>	BY4741 <i>tif51A-1::kanR tif51B::his3 sup46</i>	This study
PAY1467	<i>sup17</i>	BY4741 <i>tif51A-3::kanR tif51B::his3 sup17</i>	This study
PAY1468	<i>sup18</i>	BY4741 <i>tif51A-3::kanR tif51B::his3 sup18</i>	This study
PAY1732	<i>sup40</i>	BY4741 <i>tif51A-3::kanR tif51B::his3 sup40</i>	This study
PAY1733	<i>sup41</i>	BY4741 <i>tif51A-3::kanR tif51B::his3 sup41</i>	This study
PAY2181	SNP1 <i>ROX1-myc</i>	BY4741 <i>tif51A-1::kanR rox1(W53L)-13myc-his3MX6</i>	This study
PAY2189	SNP2 <i>ROX1-myc</i>	BY4741 <i>tif51A-1::kanR rox1(L73P)-13myc-his3MX6</i>	This study
PAY2201	SNP3 <i>ROX1-myc</i>	BY4741 <i>tif51A-3::kanR rox1(W64C)-13myc-his3MX6</i>	This study
PAY2252	INS1	BY4741 <i>tif51A-3::kanR mot3-714ins17bp</i>	This study

Table S2. Plasmids used in this study

Name	Plasmid description	Source
PA186	pFA6a- <i>HIS3MX6</i>	(Longtine et al. 1998)
PA234	pFA6a-13Myc- <i>HIS3MX6</i>	(Longtine et al. 1998)
PA298	pRS416-TEF- <i>URA3</i>	(Mumberg et al. 1995)
PA317	pRS416-TEF- <i>TIF51A-URA3</i>	This study
PA319	pRS416-TEF- <i>TIF51B-URA3</i>	This study
PA455	pWS158-Cas9- <i>URA3</i>	Dr. Tom Ellis, Addgene plasmid #90517

Table S3. Oligonucleotides used in this study

Primer	Sequence (5'-3')	
Gene expression detection by RT-qPCR and ChIP-qPCR		
ACT1-F	TCGTTCCAATTTACGCTGGTT	RT-qPCR
ACT1-R	CGGCCAAATCGATTCTCAA	RT-qPCR
TIF51-R	TCGACAATCTTACATGGTCT	RT-qPCR/ChIP-qPCR
TIF51A-F	CGATTCTACTTCTGTAGCCA	RT-qPCR
TIF51B-F	CTACACTTTAGTTCCCTTAC	RT-qPCR/ChIP-qPCR
ROX1-F	AGGGCTTACAACCGGAAGAT	RT-qPCR
ROX1-R	GCTGTTGCTCGATTTCTTC	RT-qPCR
MOT3-F	CCCCATCATCAGACCATAAA	RT-qPCR
MOT3-R	CACCAAGGGCATAGAAAATG	RT-qPCR
CYC1-F	TGGCAGACACTCTGGTCAAG	RT-qPCR
CYC1-R	CTTCTTCAACCCACCAAAGG	RT-qPCR
CYC7-F	CAAGAACGTCAAATGGGATG	RT-qPCR
CYC7-R	CTTCTTCAACCCGGCAAAC	RT-qPCR
COX5A-F	ATCCAGATGGGAGAACATGC	RT-qPCR
COX5A-R	AGCTTGCTTTTCAGGCTCAG	RT-qPCR
COX5B-F	GGAGTATTTTTAGGGTTAGG	RT-qPCR
COX5B-R	TCAGATACTCGTCTGATTTT	RT-qPCR
HEM13-F	GCTTTCTTGCCATCCTACTTG	RT-qPCR
HEM13-R	CCTGCCATTGTTGTTCTTCC	RT-qPCR
TIR2-F	GTACTCTGAGGTTGACTTTG	RT-qPCR
TIR2-R	TGGTAGAAGTTCCTAACAAG	RT-qPCR
AUS1-F	GAGAAGTATTGGTTTTGTTC	RT-qPCR
AUS1-R	AAGACATGATATTTCCAGAG	RT-qPCR
TIF51B-p-F	TTCCATTGTTCGTTCGTTG	ChIP-qPCR
TIF51B-p-R	TGAGTTCCGACAACAATGAGTT	ChIP-qPCR
Gene deletion/tagging by PCR		
TIF51B-del-F	CTAGCAAAAAGAAAACATACACCTATTTTCATTCACACACTAAAACCGGATCCCCGGGTAAATTAA	
TIF51B-del-R	CAAAAAAACATCGCGTATTTTGTAAGCTATAGAAATCTAAAATATGAATTCGAGCTCGTTTAAAC	
ROX1-tag-F	AGCAACACTGAGGTCACCGCTAAACTAGCCTAGTTTCTCCGAAACGGATCCCCGGGTAAATTAA	
ROX1-tag-R	ATAGTATAATATAATATAACGGAAAGAAGAAATGGAAAAAAAAAAGAATTTCGAGCTCGTTTAAAC	
Genome editing by CRISPR-Cas9		
gDNA_SNP1_F	GACTTTCTAAAATTATTGGTACGAAG	
gDNA_SNP1_R	AAACCTTCGTACCAATAATTTTAGAA	
donor_SNP1_F	AAGGTGTGGAAATACCCATAATTCAAACATTTCTAAAATTATTGGTACGAAGTTGAAGG	
donor_SNP1_R	GCTAGATTTTCCCAGTGTGCCTTATCTCCGTTGTAAGCCCTTCAACTTCGTACCAATA	
gDNA_SNP2_F	GACTTTAGGAGAACTAGAACATGAA	
gDNA_SNP2_R	AAACTTCATGTTCTAGTTTCTCCTAA	

donor_SNP2_F	GAAGATAAGGCACACTGGGAAAATCTAGCGGAGAAGGAGAAACCAGAACATGAAAGAAAG
donor_SNP2_R	TCTTAGACTTTCTTACCGGCTTGTATTTGTATTCAGGATACTTTCTTTCATGTTCTGGTT
gDNA_SNP3_F	GACTTTGGCACACTGGGAAAATCTAG
gDNA_SNP3_R	AAACCTAGATTTTCCCAGTGTGCCAA
donor_SNP3_F	TGGTACGAAGTGGAAGGGCTTACAACCGGAAGATAAGGCACACTGTGAAAATCTAGCTGA
donor_SNP3_R	GTATTCAGGATACTTCCTTTCATGTTCTAGTTTCTCCTTCTCAGCTAGATTTTCACAGTG
gDNA_insMOT3_F	GACTTTTTCTCACTCAGCCCCAGCCC
gDNA_insMOT3_R	AAACGGGCTGGGGCTGAGTGAGAAAA
donor_insMOT3_F	GCTAACAATGGCAGCAACAACCTCTTCTCACTCAGCCCCAGCCCCTGCCCCCGGTCCACCCCGG
donor_insMOT3_R	GTATTACTATGATGGTGATGGTGATGGGGTGGAACCGGGGGCCGGGGTGGACCGGGGGCAGGGG

Gene amplification for Sanger sequencing

TIF51A_prom_F	CCAAGGCCGGTAAAATTCTG
TIF51A_3'UTR_R	GTGTATGCTTTAGGAGAACG
ROX1_prom_F	CACCTAAGATTCCAAGACCCAA
ROX1_ORF_R	GCTGTTGCTCGATTTCTTC
MOT3_seq_F	CCCACTGCGAATATGCCAAT
MOT3_seq_R	TGGTTAGGTGCTGTCGTAGG

GAP-repair cloning

GAPR 5-TIF51A/B	TACCGGATCAATTCGGGGGATCCGTTTAAACAGGCCTGTTAACATGTCTGACGAAGAACA
GAPR 3-TIF51A	GTGACATAACTAATTACATGATGCGGCCCTCCTGCAGGGCCCTCAGCCGGTAGATATGCG
GAPR 3-TIF51B	CATGATGCGGCCCTCCTGCAGGGCCCTAGCGGCCGCTATCGATCAAAAAATATTTTGA

Table S4. Antibodies used in this study

Primary Antibody	Working dilution	Source	Secondary Antibody	Working dilution	Source
Protein detection by Western blotting					
eIF5A	1:500	Abcam (Ab32407)	α-rabbit	1:10000	Promega
hyp-eIF5A	1:500	Genentech (FabHpu)	α-rabbit	1:10000	Promega
G6PDH	1:15000	Roche (10127671001)	α-rabbit	1:10000	Promega
Chromatin immunoprecipitation (ChIP)					
Rpb1 (8WG16)	5 µL	Invitrogen (MA1-10882)	Dynabeads Pan Mouse IgG		Invitrogen (11041)
Myc	5 µL	Invitrogen (13-2500)	Dynabeads Pan Mouse IgG		Invitrogen (11041)

Table S5. Target genes and promoter binding sequences for Rox1 and Mot3 repressors

Gene	Sequence ¹		Verified regulatory site	Verified gene regulation
Rox1 binding sites				
Consensus	YYHATTGTTCTC			
TIF51B	-315	TCCATTGTTTCGT	-303	(Lowry et al. 1990)
(ANB1)	-284	CCTATTGTTCTC	-272	(Lowry et al. 1990)
	-217	TCCATTGTTCTC	-205	(Lowry et al. 1990)
	-196	CTCATTGTTGTC	-184	(Lowry et al. 1990)
CYC7	-499	GTAATTGT-CTC	-488	(Lowry et al. 1990)
	-126	AGAATaGTTCTC	-114	(Lowry et al. 1990)
COX5A	-98	TTGATTGTTCTT	-86 (←)	(Hodge et al. 1989)
COX5B	-227	TGTATTGTTCGA	-215	(Lowry et al. 1990)
	-84	TCTATTGTTTAA	-72 (←)	(Lowry et al. 1990)
HEM13	-683	GTAATTGTTGCA	-671	Putative
	-660	TGTATTGTTGAA	-648 (←)	Putative
	-593	CGTATTGTTTTT	-581 (←)	Putative
	-474	TCAATTGTTTAG	-462	(Lowry et al. 1990)
	-185	CCCATTGTTCTC	-173	(Lowry et al. 1990)
TIR2	-105	CTCATTGTTCGA	-93 (←)	(Kwast et al. 2002)
AUS1	-500	TCTATTGTTTAG	-488 (←)	(Alimardani et al. 2004)
Mot3 binding sites				
Consensus	TRCCTD			
TIF51B	-299	TGCCTG	-296	(Kastaniotis et al. 2000)
(ANB1)	-119	TGCCTT	-113	Putative
CYC1	-404	TACCTG	-398	Putative
	-259	TGCCTA	-253 (←)	Putative
	-208	TGCCTG	-202 (←)	Putative
	-183	TGCCTG	-177 (←)	(Grishin et al. 1998)
CYC7	-311	TGCCTT	-305	Putative
	-179	TGCCTT	-173 (←)	Putative
COX5A	-270	TGCCTG	-264 (←)	Putative
	-215	TGCCTA	-221	Putative
COX5B	-214	TGCCTT	-208 (←)	(Kastaniotis et al. 2000)
HEM13	-493	TGCCTA	-487 (←)	(Klinkenberg et al. 2005)
	-458	TGCCTT	-452	(Klinkenberg et al. 2005)
	-423	TGCCTA	-417 (←)	(Klinkenberg et al. 2005)
TIR2	-557	TGCCTA	-551	(Abramova et al. 2001)
	-464	TGCCTT	-458 (←)	(Abramova et al. 2001)
	-438	TGCCTA	-432 (←)	(Abramova et al. 2001)
	-400	TACCTT	-394	(Abramova et al. 2001)
	-345	TACCTT	-339	(Abramova et al. 2001)
AUS1	-470	TGCCTA	-464 (←)	(Sullivan et al. 2009)
	-387	TGCCTG	-381	(Sullivan et al. 2009)
	-215	TGCCTT	-209	(Sullivan et al. 2009)
	-209	TGCCTT	-203	(Sullivan et al. 2009)
	-197	TGCCTT	-191 (←)	(Sullivan et al. 2009)
	-75	TGCCTT	-69 (←)	(Sullivan et al. 2009)

¹ Sequence positions are indicated relative to the +1 of the ATG start codon, showing the start position, the sequence itself, and the end position. Bold text indicates the core Rox1 consensus sequence. The arrow (←) indicates that the binding sequence is located on the complementary strand.

Y → C/T; H → A/C/T; R → A/G; D → A/T/G

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