

Upregulated High-Ribo		BY4741		EY0690		Upregulated Low-Ribo		BY4741		EY0690		Upregulated		BY4741		EY0690	
Systematic Name	Name	mRNA	Ribo	mRNA	Ribo	Systematic Name	Name	mRNA	Ribo	mRNA	Ribo	Systematic Name	Name	mRNA	Ribo	mRNA	Ribo
YER067W	RG11	6.7	0.6	4.6	-0.3	YKL217W	JEN1	6.0	-1.6	5.6	-1.5	YLR149C		3.8	-0.8	3.5	-1.2
YLR327C	TMA10	5.9	2.9	6.0	0.9	YFR015C	GSY1	6.0	-1.7	3.7	-2.6	YDR516C	EMI2	3.6	-0.3	2.7	0.1
YCR021C	HSP30	5.9	2.5	6.9	0.8	YDR171W	HSP42	5.8	-2.0	4.7	-1.5	YGR043C	NQM1	3.6	-0.5		
YDR277C	MTH1	5.1	1.3	3.6	-0.7	YFR053C	HXK1	5.5	-1.9	3.5	-1.3	YDL048C	STP4	3.6	-0.4	1.8	-1.5
YGR142W	BTN2	5.0	2.0	5.2	0.6	YEL011W	GLC3	5.5	-1.8	5.0	-2.7	YLR142W	PUT1	3.5	-0.4	3.2	0.1
YHR087W	RTC3	5.0	0.8	4.4	0.7	YGR249W	MGA1	5.1	-1.8	5.4	-2.2	YCL042W	Vnx1	3.2	-0.9	2.4	-1.2
YKR075C		4.6	1.3	4.4	-0.4	YDR342C	HXT6	4.2	-1.4	3.2	-0.1	YNL077W	APJ1	3.1	-0.4	3.2	-0.7
YGR008C	STF2	4.5	1.9	2.9	0.2	YPR160W	GPH1	4.2	-1.8	3.6	-2.0	YBR214W	SDS24	3.1	0.1	2.4	-1.0
YER150W	SPI1	4.2	0.9	4.6	0.2	YGL205W	POX1	4.1	-1.3	2.8	-1.8	YAL054C	ACS1	3.0	-0.1	1.7	1.2
YOL052C-A	DDR2	4.1	1.1	4.5	-0.1	YGR088W	CTT1	3.9	-1.0	4.4	-1.2	YML100W	TSL1	2.9	0.0	2.2	-1.1
YBR072W	HSP26	3.9	1.1	4.9	0.3	YLR258W	GSY2	3.3	-1.1	1.4	-1.1	YCL040W	GLK1	2.9	-0.3	2.1	-1.1
YLL026W	HSP104	3.8	0.7	3.1	0.3	YMR303C	ADH2	3.0	-1.1	0.3	0.7	YOR019W		2.9	-0.7	3.0	-0.2
YHL021C		3.7	1.3	3.7	-0.1	YJL052W	TDH1	2.9	-1.5	2.7	-1.3	YBR169C	Rrp43	2.9	-0.9	3.1	-1.5
YGR243W	FMP43	3.7	2.2	3.0	0.7	YDL021W	GPM2	2.9	-1.6	2.2	0.7	YMR011W	HXT2	2.7	0.0	3.9	0.2
YDR258C	HSP78	3.5	0.3	2.6	-0.2	YDR453C	TSA2	2.8	-1.9	2.9	0.3	YMR250W	Tdp1	2.7	-0.9	2.2	-2.5
YJL144W		3.5	3.2	5.2	-0.4	YOL032W	OPI10	2.8	-1.5	2.7	-2.4	YNL009W	IDP3	2.6	-0.5		
YGL255W	ZRT1	3.3	0.1	-0.9	0.0	YHR092C	HXT4	2.7	-1.5	1.9	-0.2	YPL123C	RNY1	2.6	-0.5	1.6	0.5
YGL037C	PNC1	3.3	0.8	1.7	0.4	YCR091W	KIN82	2.6	-2.1	2.9	-1.9	YPL186C	UIP4	2.6	-0.3		
YMR120C	ADE17	3.3	0.1	-0.9	-0.1	YBR147W	RTC2	2.5	-2.9	3.0	-2.6	YIL101C	XBP1	2.5	-0.4	2.2	-0.5
YMR105C	PGM2	3.2	2.2	3.2	-0.9												
YKL086W	SRX1	3.1	2.3	5.3	0.5												
YML128C	MSC1	3.1	0.4	3.2	-0.2												
YFL014W	HSP12	3.1	0.8	2.6	0.9												
YKL109W	HAP4	3.1	0.7	1.8	-1.0												
YOR173W	DCS2	3.0	0.6	3.3	-1.6												
YMR104C	YPK2	2.9	0.3	2.6	-1.0												
YLR177W		2.8	0.7	2.9	-1.5												
YER103W	SSA4	2.8	1.1	4.9	-0.3												
YPL240C	HSP82	2.8	0.1	2.2	0.1												
YBL078C	ATG8	2.8	0.8	2.2	1.1												
YHR097C		2.7	0.4	2.9	-0.1												
YNR014W		2.7	1.3	2.4	0.1												
YMR280C	CAT8	2.6	3.2	3.5	-0.2												

SupplementaryTable 1 | mRNAs upregulated during glucose starvation have differences in ribosome occupancy. Fold-change in mRNA levels and in ribosome occupancy after 15 minutes of glucose starvation from one measurement of BY4741 and one measurement of EY0690 on total non-polyA selected mRNA. mRNA upregulated, higher ribosome occupancy genes (mRNA>2.5, Ribo>0.09, pink), mRNA upregulated, lower ribosome occupancy genes (mRNA>2.5, Ribo<-1.0, blue), mRNA upregulated (mRNA>2.5, -1.0<Ribo<-0.09, white). mRNA upregulated, higher ribosome occupancy EY0690 (mRNA>2.5, Ribo>-0.5, pink), mRNA upregulated, lower ribosome occupancy EY0690 (mRNA>2.5, Ribo < -1.0, blue), mRNA upregulated (mRNA>2.5, -1<Ribo<-0.5, white) and not upregulated (T<2.5, grey). The cutoff for higher ribosome occupancy was set by the median ribosome occupancy of all genes (BY4741 = 0.09, EY0690 = -0.5). mRNA = log2 mRNA fold change for glucose starvation vs. log-phase glucose-rich. Ribo = log2 ribosome occupancy fold change for glucose starvation vs. log-phase glucose-rich.

Strain Number	Genotype
EY2536	BY4741
EY0690	W303
EY2888	W303, <i>P_{MYO2}-MS2-CP-GFP(3x)::HIS3, DCP2-mRFP1.3::KanMX6</i>
EY2889	EY2888, <i>PUTR_{PGK1(-600-0)}PGK1-MS2-ADH1_3'UTR::LEU2</i>
EY2890	EY2888, <i>PUTR_{PDC1(-898-0)}PDC1-MS2-ADH1_3'UTR::LEU2</i>
EY2891	EY2888, <i>PUTR_{GLC3(-396-0)}GLC3-MS2-ADH1_3'UTR::LEU2</i>
EY2892	EY2888, <i>PUTR_{HXK1(-940-0)}HXK1-MS2-ADH1_3'UTR::LEU2</i>
EY2893	EY2888, <i>PUTR_{HSP26(-604-0)}HSP26-MS2-ADH1_3'UTR::LEU2</i>
EY2894	EY2888, <i>PUTR_{HSP30(-1010-0)}HSP30-MS2-ADH1_3'UTR::LEU2</i>
EY2895	EY2888, <i>PUTR_{PGK1(-600-0)}PGK1-MS2-ADH1_3'UTR::LEU2, PAB1-CFP::URA3</i>
EY2896	EY2888, <i>PUTR_{GLC3(-396-0)}GLC3-MS2-ADH1_3'UTR::LEU2, PAB1-CFP::URA3</i>
EY2897	EY2888, <i>P_{Tet107-lacZ}-MS2-ADH1_3'UTR::LEU2, P_{ERV14-rtTA(S2)}::URA3</i>
EY2898	EY2888, <i>PUTR_{HSP30(-1010-0)}CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2899	EY2888, <i>PUTR_{HSP26(-604-0)}CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2900	EY2888, <i>PUTR_{GLC3(-396-0)}CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2901	EY2888, <i>PUTR_{HXK1(-940-0)}CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2902	EY2888, <i>P_{HSP26(-604--39)}UTR_{GLC3(-32-0)}CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2903	EY2888, <i>P_{GLC3(-396--32)}UTR_{HSP26(-39-0)}CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2904	EY2888, <i>P_{HXK1(-940--105)}UTR_{HSP26(-39-0)}CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2905	EY2888, <i>Chim1PUTR_{HSP26(-694--448)}GLC3(-263-0)CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2906	EY2888, <i>Chim2PUTR_{HSP26(-694--317)}GLC3(-202-0)CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2907	EY2888, <i>Chim3PUTR_{GLC3(-397--245)}HSP26(-407-0)CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2908	EY2888, <i>Chim4PUTR_{GLC3(-397--202)}HSP26(-316-0)CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2909	EY2888, <i>P_{STRE}CFP-MS2-ADH1_3'UTR::LEU2</i>
EY2910	EY2888, <i>P_{STRE+HSE}CFP-MS2-ADH1_3'UTR::LEU2</i>
Open Biosystems	BY4741, <i>HSP30::TAP::HIS3</i>
Open Biosystems	BY4741, <i>HSP26::TAP::HIS3</i>
Open Biosystems	BY4741, <i>GLC3::TAP::HIS3</i>
Open Biosystems	BY4741, <i>GSY1::TAP::HIS3</i>
Open Biosystems	BY4741, <i>RPB3::TAP::HIS3</i>

Supplementary Table 2 | Yeast strains used in this study

Primer	Sequence
<i>ACT1_F</i>	CTGCCGGTATTGACCAAACCT
<i>ACT1_R</i>	CGGTGATTTTCCTTTTGCATT
<i>PGK1_F</i>	GGACAAGCGTGTCTTCATCA
<i>PGK1_R</i>	CGTTTCTTTACCGTTTGGT
<i>PDC1_F</i>	GGCAATACCGTTCAAAGCAG
<i>PDC1_R</i>	CTTACGCCGCTGATGGTTAC
<i>CDC19_F</i>	CACCACCGATGACAAGTACG
<i>CDC19_R</i>	CTTGTCGTCAACGACTTCCA
<i>PGII_F</i>	ACAACCACTTCACCCAAACC
<i>PGII_R</i>	TTGCAAGTAGGCTGGGAATC
<i>HSP30_F</i>	CAATCCGAAACCAAAGAAA
<i>HSP30_R</i>	CGAAATCGCCGTTAGGTAAG
<i>HSP26_F</i>	AAGGCGGCTTAAGAGGCTAC
<i>HSP26_R</i>	ATCATAAAGAGCGCCAGCAT
<i>HSP104_F</i>	CGACGCTGCTAACATCTTGA
<i>HSP104_R</i>	CACTTGGTTCAGCGACTTCA
<i>HSP12_F</i>	CGAAAAAGGCAAGGATAACG
<i>HSP12_R</i>	TTGGTTGGGTCTTCTTCACC
<i>GLC3_F</i>	CAATCCGAAACCAAAGAAA
<i>GLC3_R</i>	CGAAATCGCCGTTAGGTAAG
<i>GSY1_F</i>	GCCGCTGGTGTAGTAGATTT
<i>GSY1_R</i>	GCAGTGATTTGCGACACAGT
<i>HXK1_F</i>	GTACCCAGCTTCCCAAACA
<i>HXK1_R</i>	TTTGTAGCAATGGGACGACA
<i>GPH1_F</i>	TTCCCAAGAAATCAAGTCAA
<i>GPH1_R</i>	TCATAAGCAGCCATGTCATCA
Telomeric_F	CCCAGGTACGAAACGCTAAG
Telomeric_R	ATAAGGTTGTCGACGGTTGC
<i>CFP_F</i>	GGTCACAAGTTCTCTGTTTCTG
<i>CFP_R</i>	CCAAGTCAAAGTAGTAACCAAAG
oTi225	pGATCGTCGGACTGTAGAACTCTGAACCTGTCCGGTGGTCGCCGTATCATT/Sp18/CACT CA/Sp18/CAAGCAGAAGACGGCATAACGATTTTTTTTTTTTTTTTTTTTTVN
oTi9pA	pAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCCGGTGGTCGC/Sp18/CA CTCA/Sp18/TTCAGACGTGTGCTCTTCCGATCTTTTTTTTTTTTTTTTTTTTTVN

Supplementary Table 3 | List of primers used for RT-qPCR, CHIP-qPCR and Ribosome profiling. ‘p’ designates 5’ phosphorylation, ‘Sp18’ designates a hexa-ethyleneglycol spacer, and ‘V’, ‘N’ indicate degenerate nucleotides.