

**Supplemental Information**

**Absolute Quantification of Protein  
and mRNA Abundances Demonstrate Variability  
in Gene-Specific Translation Efficiency in Yeast**

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Supplementary Figures and Tables

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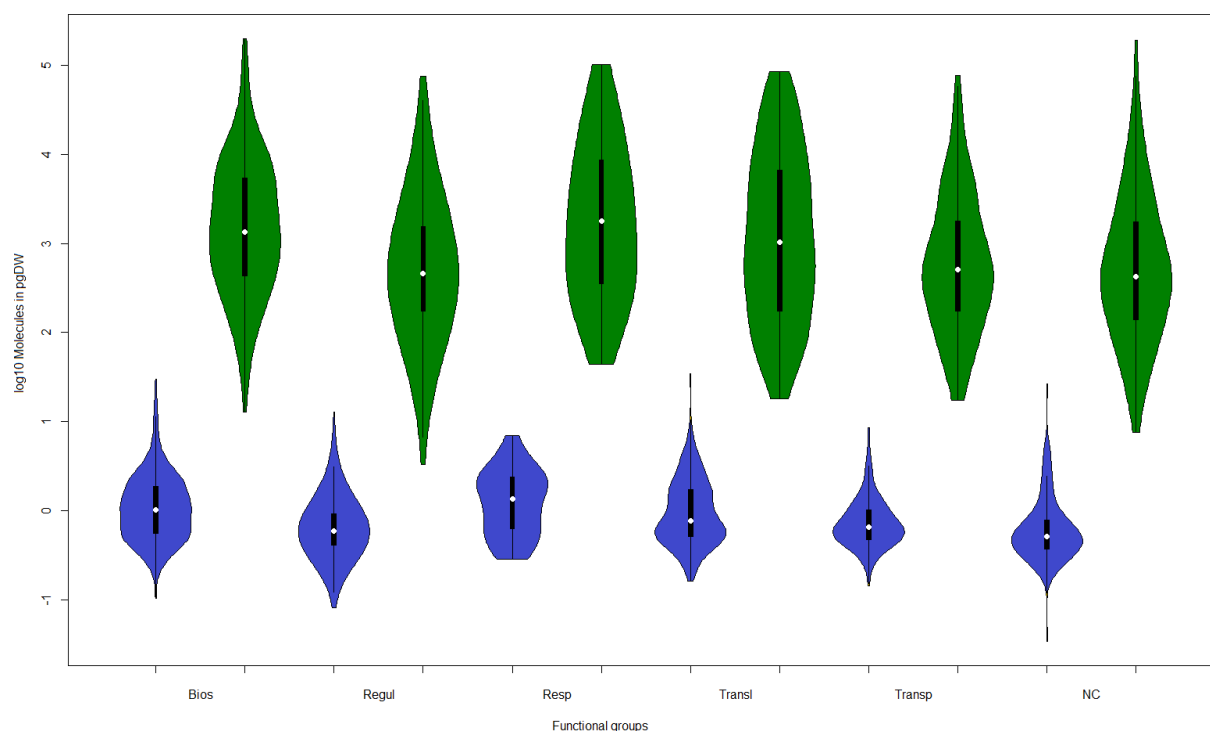
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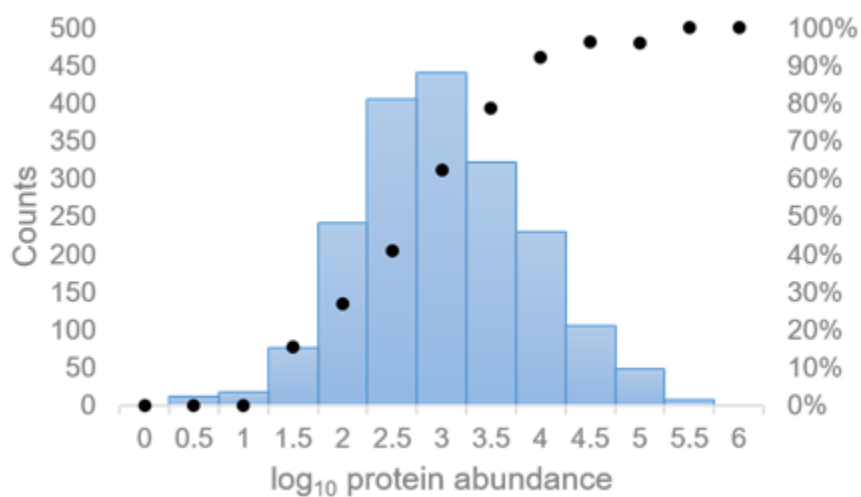
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<sup>6</sup>Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive La Jolla, CA 92093-0412, United States

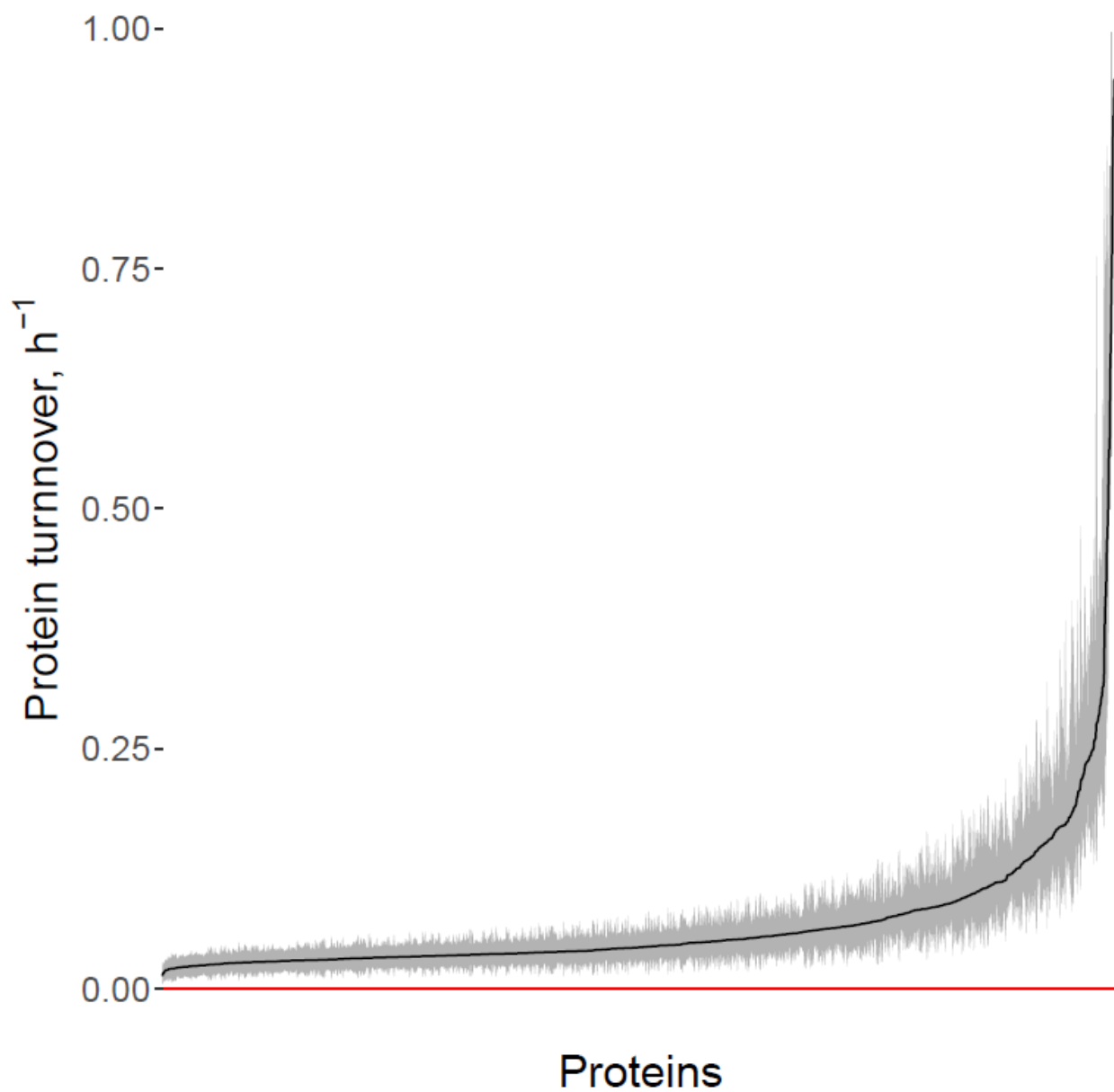
<sup>7</sup>Lead Contact author



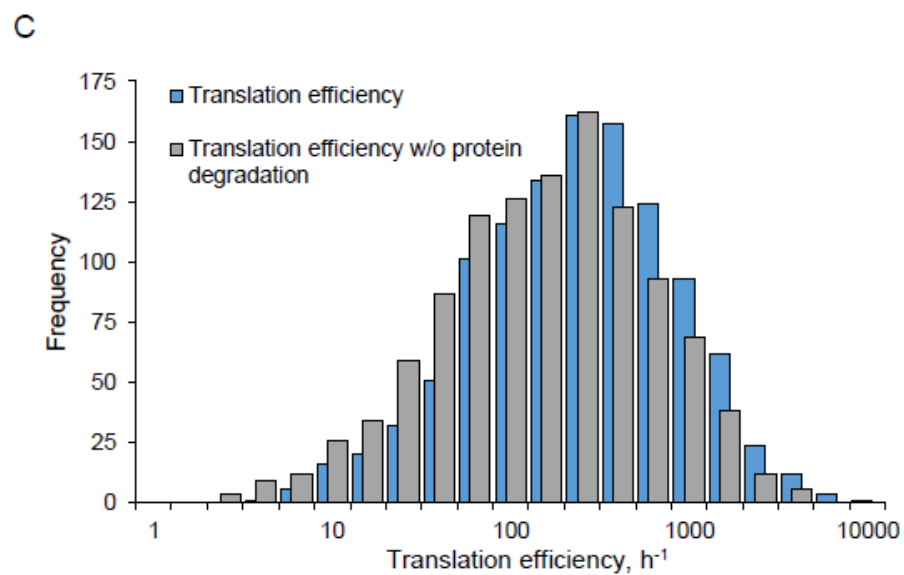
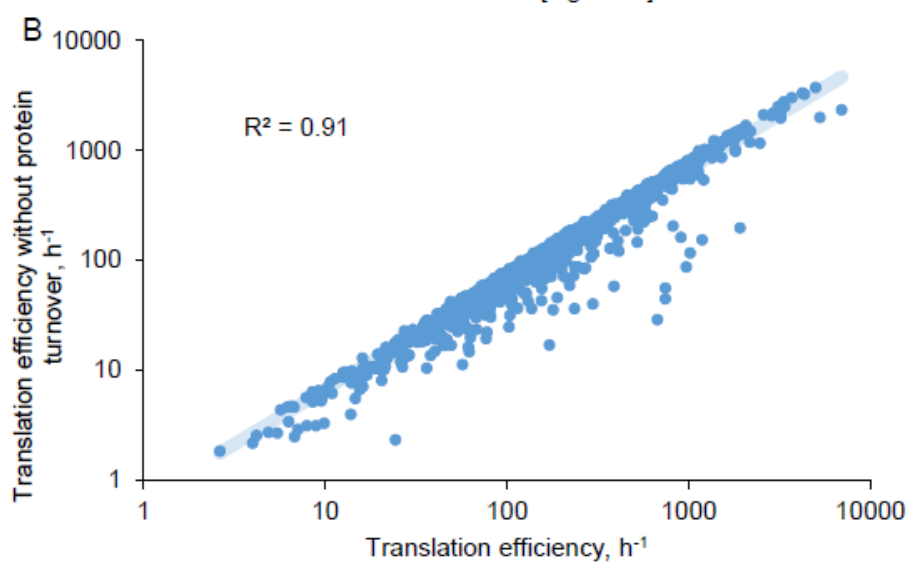
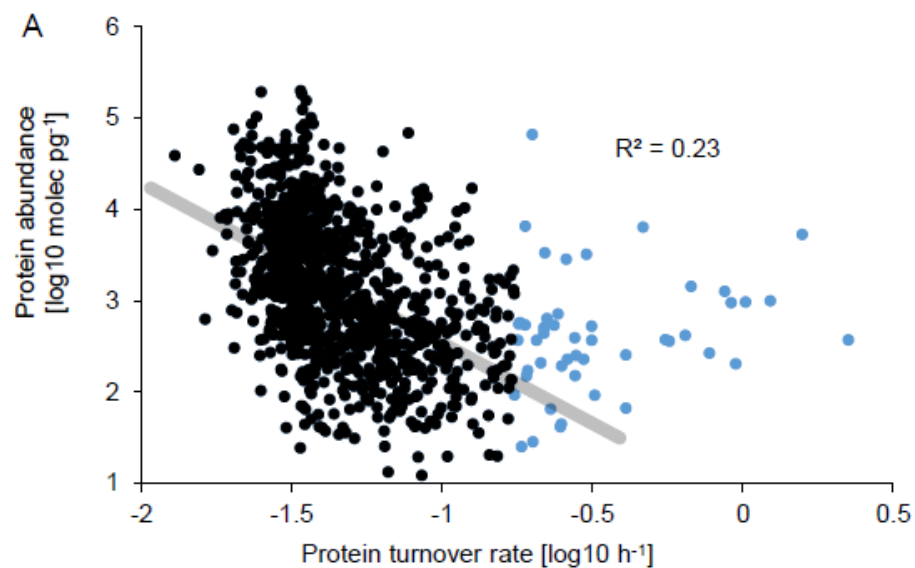
**Figure S1.** Distribution of mRNA (blue) and protein (green) abundances divided into functional groups. Bios – biosynthesis; Regul – regulation; Resp – respiration; Transl – translation; Transp – transporters; NC – not categorized.



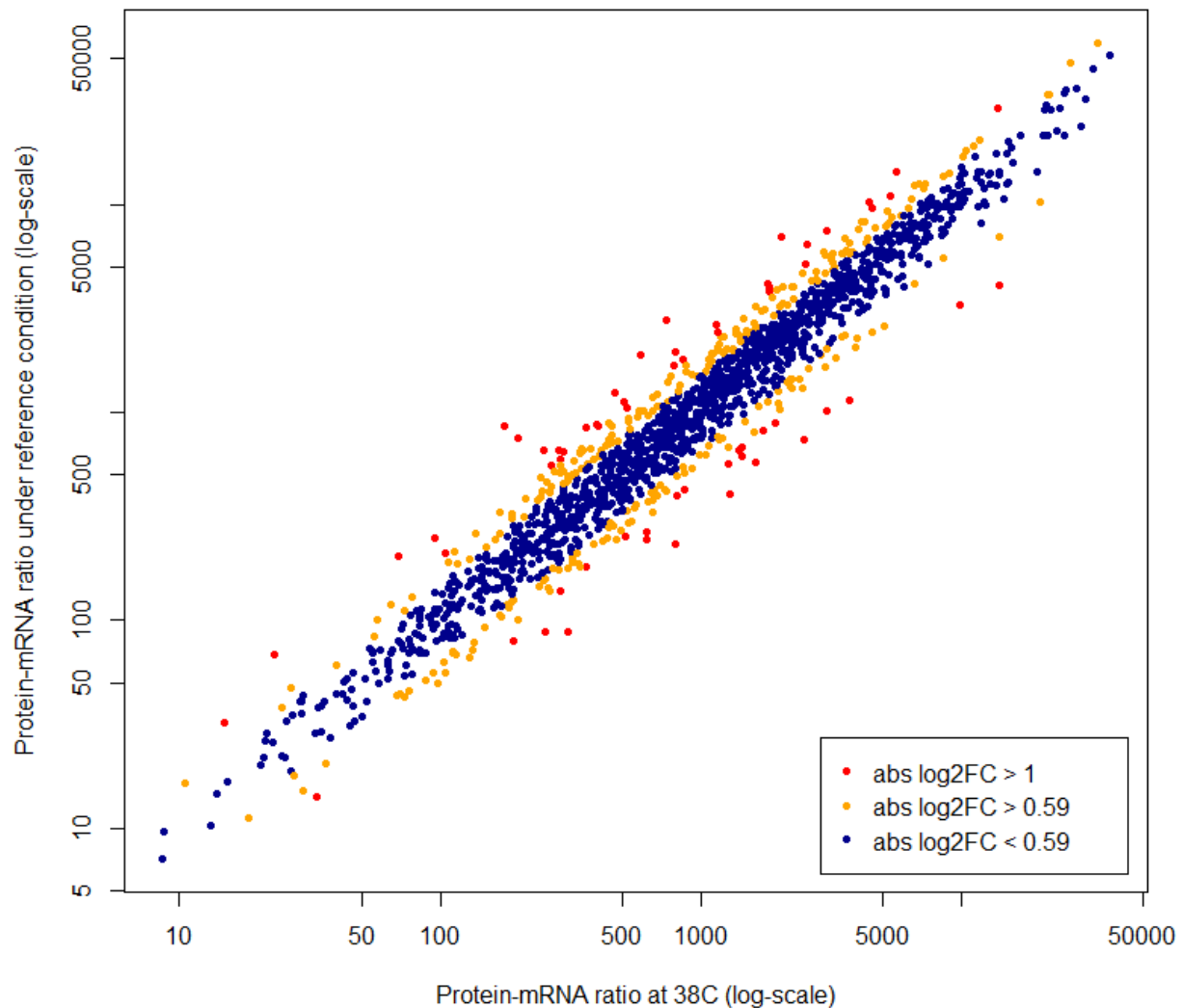
**Figure S2.** Histogram of a protein abundances (blue bars) together with the percentage of proteins which protein turnover was determined for each abundance bar (black dots).



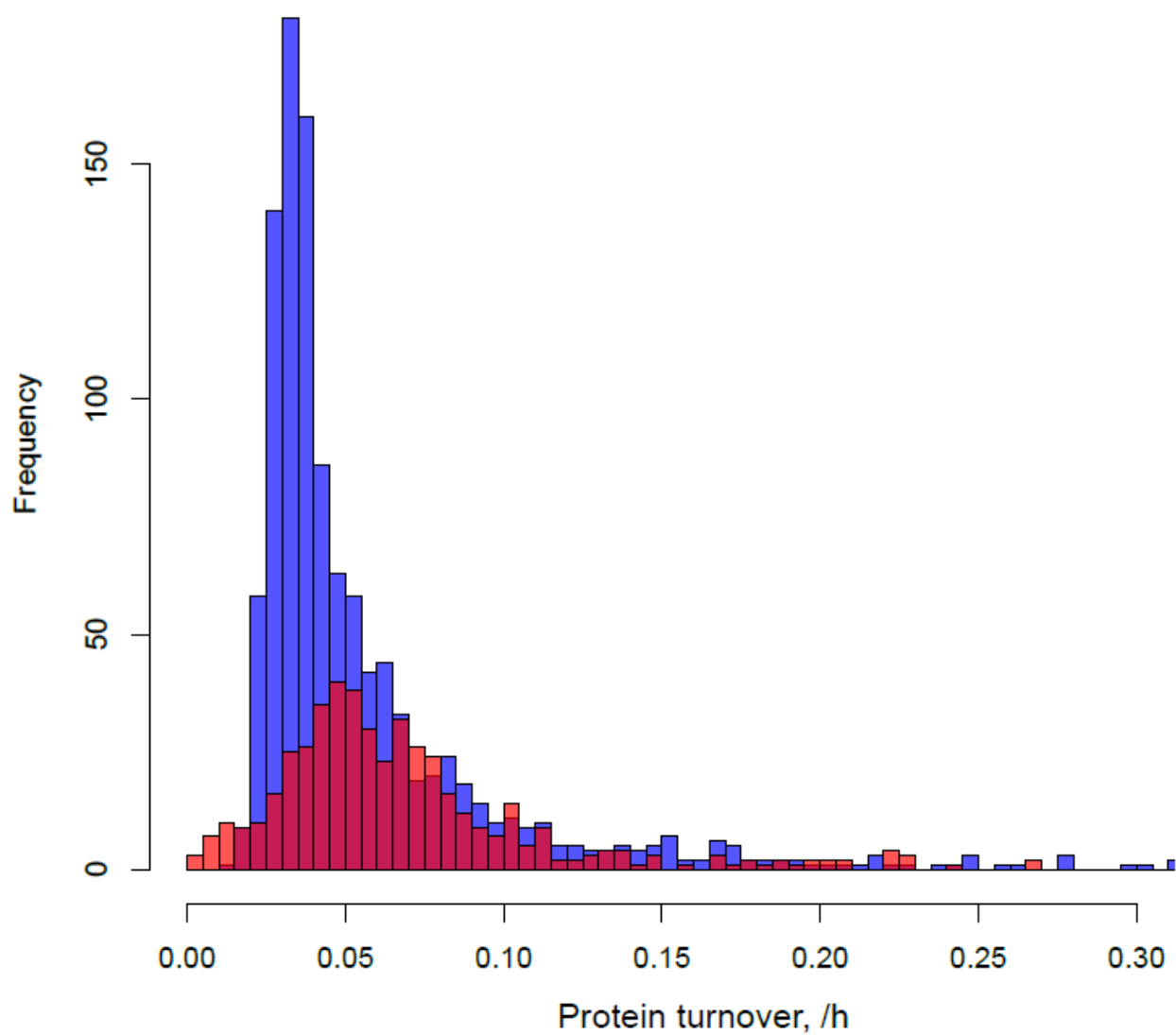
**Figure S3.** Protein turnover rate estimates ( $k_{deg}$ ; black dots) sorted increasingly for all measured proteins for which the regression was statistically significant ( $FDR < 0.01$ ). For each estimate, the 95% confidence interval is shown as a grey line.



**Figure S4.** The impact of protein turnover for calculations of translation efficiency. (A) Moderate correlation between protein turnover and protein abundance was observed. Black circles exclude 5% the highest protein turnover rates. (B) Correlation between translation efficiency and efficiency calculated without protein turnover taken into account. Less than 30% difference was detected for the majority of individual proteins. (C) Histogram of translation efficiencies when protein turnover is or is not taken into account (represented by blue and grey bars, respectively).



**Figure S5.** Correlation of protein-mRNA ratios between the reference and the most extreme condition studied (38°C temperature; both studied in chemostats at  $D = 0.1 \text{ h}^{-1}$ ). Yellow and red circles indicate protein-mRNA ratio differences more than 1.5- and 2-fold between the conditions, respectively.



**Figure S6.** Histogram comparison of the protein turnover data reported in the current study (blue bars) and Helbig et al., 2011 (red bars). Both studies are run under chemostat conditions at  $D = 0.1 \text{ h}^{-1}$ , however, representing aerobic (current study) *versus* anaerobic conditions (Helbig et al. 2011).

**Table S1.** mRNAs selected for the absolute quantification. Log10 FPKM values from RNAseq and log10 measured concentrations are presented.

Gene ID	Gene symbol	Description	log10 FPKM value	log10 Concentration, fmol/mg
YMR169C	ALD3	Cytoplasmic aldehyde dehydrogenase, involved in beta-alanine synthesis; uses NAD <sup>+</sup> as the preferred coenzyme; very similar to Ald2p; expression is induced by stress and repressed by glucose [Source:SGD;Acc:S000004779]	2.635293	-0.78168
YKL166C	TPK3	cAMP-dependent protein kinase catalytic subunit; promotes vegetative growth in response to nutrients via the Ras-cAMP signaling pathway; partially redundant with Tpk1p and Tpk2p; localizes to P-bodies during stationary phase [Source:SGD;Acc:S000001649]	2.995085	-0.35913
YGL248W	PDE1	Low-affinity cyclic AMP phosphodiesterase, controls glucose and intracellular acidification-induced cAMP signaling, target of the cAMP-protein kinase A (PKA) pathway; glucose induces transcription and inhibits translation [Source:SGD;Acc:S000003217]	3.291336	-0.21625
YJL196C	ELO1	Elongase I, medium-chain acyl elongase, catalyzes carboxy-terminal elongation of unsaturated C12-C16 fatty acyl-CoAs to C16-C18 fatty acids [Source:SGD;Acc:S000003732]	3.306934	-0.30395
YMR037C	MSN2	Transcriptional activator related to Msn4p; activated in stress conditions, which results in translocation from the cytoplasm to the nucleus; binds DNA at stress response elements of responsive genes, inducing gene expression [Source:SGD;Acc:S000004640]	3.417923	-0.01738
YPL203W	TPK2	cAMP-dependent protein kinase catalytic subunit; promotes vegetative growth in response to nutrients via the Ras-cAMP signaling pathway; partially redundant with Tpk1p and Tpk3p; localizes to P-bodies during stationary phase [Source:SGD;Acc:S000006124]	3.654339	-0.15492



YCR005C	CIT2	Citrate synthase, catalyzes the condensation of acetyl coenzyme A and oxaloacetate to form citrate, peroxisomal isozyme involved in glyoxylate cycle; expression is controlled by Rtg1p and Rtg2p transcription factors [Source:SGD;Acc:S000000598]	3.728173	-0.00824
YGL205W	POX1	Fatty-acyl coenzyme A oxidase, involved in the fatty acid beta-oxidation pathway; localized to the peroxisomal matrix [Source:SGD;Acc:S000003173]	4.281594	0.252723
YHR183W	GND1	6-phosphogluconate dehydrogenase (decarboxylating), catalyzes an NADPH regenerating reaction in the pentose phosphate pathway; required for growth on D-glucono-delta-lactone and adaptation to oxidative stress [Source:SGD;Acc:S000001226]	4.359378	0.708496
YKL148C	SDH1	Flavoprotein subunit of succinate dehydrogenase (Sdh1p, Sdh2p, Sdh3p, Sdh4p), which couples the oxidation of succinate to the transfer of electrons to ubiquinone as part of the TCA cycle and the mitochondrial respiratory chain [Source:SGD;Acc:S000001631]	4.373571	0.588765
YNR016C	ACC1	Acetyl-CoA carboxylase, biotin containing enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA; required for de novo biosynthesis of long-chain fatty acids [Source:SGD;Acc:S000005299]	4.47165	0.612974
YFR053C	HXK1	Hexokinase isoenzyme 1, a cytosolic protein that catalyzes phosphorylation of glucose during glucose metabolism; expression is highest during growth on non-glucose carbon sources; glucose-induced repression involves the hexokinase Hxk2p [Source:SGD;Acc:S000001949]	4.680155	0.956133
YGL055W	OLE1	Delta(9) fatty acid desaturase, required for monounsaturated fatty acid synthesis and for normal distribution of mitochondria [Source:SGD;Acc:S000003023]	4.816657	0.597592

YHR007C	ERG11	Lanosterol 14-alpha-demethylase; catalyzes the C-14 demethylation of lanosterol to form 4,4"-dimethyl cholesta-8,14,24-triene-3-beta-ol in the ergosterol biosynthesis pathway; member of the cytochrome P450 family; associated and coordinately regula /.../th the P450 reductase Ncp1p [Source:SGD;Acc:S000001049]	5.014566	0.807304
YLR044C	PDC1	Major of three pyruvate decarboxylase isozymes, key enzyme in alcoholic fermentation, decarboxylates pyruvate to acetaldehyde; subject to glucose-, ethanol-, and autoregulation; involved in amino acid catabolism [Source:SGD;Acc:S000004034]	5.031084	1.160538
YKL060C	FBA1	Fructose 1,6-bisphosphate aldolase, required for glycolysis and gluconeogenesis; catalyzes conversion of fructose 1,6 bisphosphate to glyceraldehyde-3-P and dihydroxyacetone-P; locates to mitochondrial outer surface upon oxidative stress [Source:SGD;Acc:S000001543]	5.293373	1.078671
YBR072W	HSP26	Small heat shock protein (sHSP) with chaperone activity; forms hollow, sphere-shaped oligomers that suppress unfolded proteins aggregation; oligomer activation requires heat-induced conformational change; also has mRNA binding activity [Source:SGD;Acc:S000000276]	5.4545	1.715457
YGR192C	TDH3	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3 bis-phosphoglycerate; detected in the cytoplasm and cell wall [Source:SGD;Acc:S000003424]	5.525863	1.766594

**Table S8.** List of transcriptionally regulated fluxes.

Reaction	Corresponding gene(s)
r_0001	DLD1
r_0002	COR1/QCR2,6,7,8,9/RIP1/CYC1,7/OSH7/CYT1
r_0004	COR1/QCR2,6,7,8,9/RIP1/CYC1,7/OSH7/CYT1
r_0042	ARO3,4
r_0061	LEU2
r_0111	ACH1
r_0112	ACS1,2
r_0114	ACS1,2
r_0148	ADK1
r_0167	ADH3,4
r_0175	ALD4,5
r_0178	ALD4,5
r_0203	TRP2/TRP3
r_0214	URA2
r_0226	ATP1-8,14-18,20/OLI1/TIM11
r_0252	YAT1,2
r_0437	COR1/QCR2,6,7,8,9/RIP1/CYC1,7/OSH7/CYT1
r_0438	COX1,2,3,4,5A,5B,6,7,8,9,12,13/CYC1,7
r_0439	COR1/QCR2,6,7,8,9/RIP1/CYC1,7/OSH7/CYT1
r_0451	FUM1
r_0452	FUM1
r_0473	PRO2
r_0505	GCV1,2,3/KGD1,2/LPD1
r_0533	GLK1/HXK1,2
r_0534	GLK1/HXK1,2
r_0535	GLK1/HXK1,2
r_0566	TRP2/TRP3
r_0659	IDP2
r_0662	ICL1
r_0716	DAL7/MLS1
r_0717	DAL7/MLS1
r_0757	INM1/INM2
r_0773	NDI1
r_0831	GCV1,2,3/KGD1,2/LPD1
r_0832	GCV1,2,3/KGD1,2/LPD1
r_0962	CDC19/PYK2
r_1055	TRP5
r_2157	ELO2,3

r_2177	TSC13
r_2330	AYR1
r_2529	EPT1/CPT1
r_2542	GEP4
r_2543	GEP4
r_2544	GEP4
r_2545	GEP4
r_2546	GEP4
r_2547	GEP4
r_2823	LSB6/STT4
r_3130	SAC1
r_3151	INP52,53
r_3217	VAC14/FIG4

**Table S9.** Multivariate regression spline (MARS) analysis to determine the control over total amount of proteins synthesized at optimal environmental conditions in chemostat ( $D = 0.1$  /h). One hundred fifty-two variables were used. Variables included: (i) mRNA abundances; (ii) Secondary structure information (frequency of nucleotide singlets, duplexes and triplexes, normalized to their total length) in sequencing region, 5' UTR and 3' UTR; (iii) Length of a sequencing region, 5' UTR and 3' UTR; (iv) Codon and tRNA adaptation indexes of genes; and (v) Information about co-translational RNA decay.

#	Variable	Level of (additional) control	Accumulated control	Group
1	mRNA	61.280%	61.3%	mRNA abundance
2	CAI	5.709%	67.0%	CAI/tAI (translation elongation)
3	tAI	2.340%	69.3%	CAI/tAI (translation elongation)
4	Gene - TT	1.409%	70.7%	Gene (translation elongation)
5	Gene - GT	1.213%	72.0%	Gene (translation elongation)
6	Gene - A	0.869%	72.8%	Gene (translation elongation)
7	5' UTR - CC	0.733%	73.6%	5' UTR (translation initiation)
8	5' UTR - A	0.723%	74.3%	5' UTR (translation initiation)
9	Gene - AT	0.691%	75.0%	Gene (translation elongation)
10	Gene - GC	0.552%	75.5%	Gene (translation elongation)
11	Gene - GG	0.468%	76.0%	Gene (translation elongation)
12	5' UTR - TA	0.465%	76.5%	5' UTR (translation initiation)
13	Length - Gene	0.378%	76.8%	Gene (translation elongation)

14	Gene - C	0.373%	77.2%	Gene (translation elongation)
15	Gene - CT	0.365%	77.6%	Gene (translation elongation)
16	Gene -T	0.360%	77.9%	Gene (translation elongation)
17	5' UTR - CC	0.326%	78.3%	5' UTR (translation initiation)
18	5' UTR - TG	0.314%	78.6%	5' UTR (translation initiation)
19	5' UTR - GC	0.295%	78.9%	5' UTR (translation initiation)
20	5' UTR - AC	0.293%	79.2%	5' UTR (translation initiation)
21	Length - 3' UTR	0.293%	79.5%	3' UTR
22	Gene - CG	0.287%	79.7%	Gene (translation elongation)
23	5' UTR - GG	0.275%	80.0%	5' UTR (translation initiation)
24	5' UTR -T	0.266%	80.3%	5' UTR (translation initiation)
25	5' UTR - AA	0.235%	80.5%	5' UTR (translation initiation)
26	Gene -TC	0.207%	80.7%	Gene (translation elongation)
27	Gene - TA	0.194%	80.9%	Gene (translation elongation)

**Table S10.** Codon frequencies as the first position after the start codon among 200 the highest protein-mRNA ratios quantified for the yeast *Saccharomyces cerevisiae*

Unique Codon	Codon count
TCT	33
GCT	20
TCC	18
CCA	10
ACT	10
TTG	9
GCC	7
TTT	7
AGC	7
TCA	7
GTT	5
CCT	5
TTA	5

GCA	4
CTA	4
AAG	4
TTC	4
GTC	4
TCG	4
GAG	3
CTT	3
GGT	3
ACC	3
AGT	3
GTA	2
AAA	2
AGA	2
AAT	2
ACA	2
GGA	1
GAC	1
ATT	1
GAT	1
CGT	1
CTG	1
TGT	1
ATC	1