SUPPLEMENTAL MATERIAL

Ctk1 function is necessary for translation initiation in *Saccharomyces cerevisiae*

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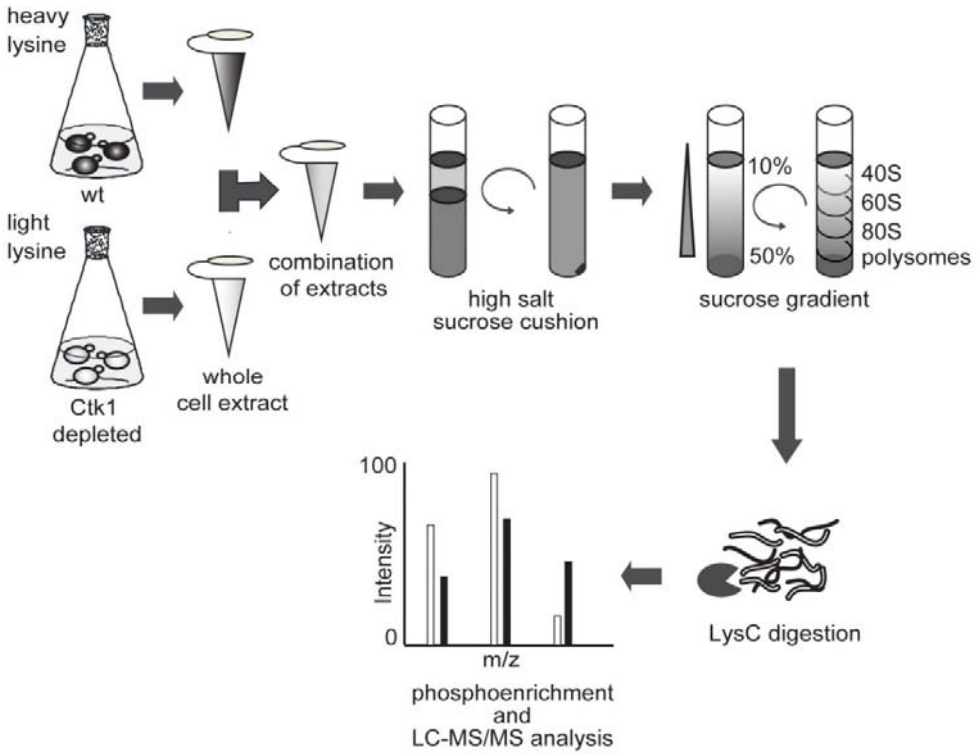

FIG S1 Scheme of the preparation of ribosome enriched samples for SILAC analysis. To compare the phosphorylation status of proteins derived from mock and Ctk1 depleted cells, one culture was grown in media containing light lysine and the other in media containing heavy lysine. After cell lysis, the extracts of the two strains were combined for purification of ribosome and associated proteins, which reduces unspecific variations in the phosphorylation patterns of wt and mutant strain caused by differences in the preparation. 40S, 60S, and 80S ribosomes were prepared by subsequent centrifugation steps with a sucrose density gradient as a final purification step. A high salt wash was omitted in this assay to allow more loosely associated proteins to remain bound to the ribosome. Fractions of the sucrose gradient containing 40S, 60S, and 80S ribosomes were pooled and digested with LysC. The phosphopeptides were enriched and analyzed by LC-MS/MS. The amino acids with a different phosphorylation level were determined and the phosphorylation ratio between mock and Ctk1 depleted samples calculated (also see Materials and Methods).

TABLE S1 Changes in the phosphorylation pattern of proteins upon Ctk1 depletion as determined by SILAC analysis. Samples were prepared as depicted and described in Fig. S1. Replicates are indicated with "Exp".
see xls-file

This data set provides a catalogue of phosphoproteomic changes upon Ctk1 depletion, *i.e.* not obscured by unspecific changes caused by the growth defect of *Δctk1* cells, that might provide a valuable resource for other researcher interested in Ctk1 function.

This data also verifies that ribosomes and translation initiation factors of Ctk1 depleted cells do not exhibit the phosphorylation pattern typical for stressed cells, such as increased eIF2α and decreased Rps6 phosphorylation (1, 2), corroborating that the initiation defect is specific to the loss of Ctk1 function and not to a general stress response triggered by depletion of Ctk1.

TABLE S2 SILAC candidate substrates assessed for *in vitro* phosphorylation by Ctk1. Candidates were chosen based on their known function in ribosome biogenesis or translation. Proteins of the large ribosomal subunit were not considered since *in vitro* Ctk1 only phosphorylates proteins of the small ribosomal subunit (data not shown). Importantly, the reduction in phosphorylated peptides of these proteins should not be caused by a diminished association of these proteins with the ribosome, because we also observed that the phosphorylation of other residues of the same proteins remained unchanged (Supplementary Table 1).

Protein	Function	Site	Phosphorylation compared to wt*
Cbf5	pseudouridine synthase subunit of H/ACA snoRNP	T423	0.55 +/-0.15
Pop1	cleavage of pre-rRNA and tRNA	T524	0.45 +/-0.06
Nop13	nucleolar protein found in preribosomal complexes	S2	0.56 +/-0.17
Nop53	nuleolar protein involved in 60S biogenesis	S31	0.64 +/-0.18
Srp40	nucleolar protein, role in preribosome assembly or transport	S133	0.71 +/-0.15
Nop16	constituent of 66S pre-ribosomal subunits	T152	0.58 +/-0.28
		S176	0.52 +/-0.13
Cic1	copurifies with 66S pre-ribosomal particles	T11	0.70 +/-0.13
		S17	0.63 +/-0.05
		S359	0.66 +/-0.19
		S366	0.77 +/-0.25
Sda1	actin skeleton organization, involved in 60S ribosome biogenesis	S591	0.65 +/-0.15
Jjj1	co-chaperone required for a late step in ribosome biogenesis	S393	0.83 +/-0.08

		T504	0.77 +/-0.07
Rei1	cytoplasmic pre-60S factor	T111	0.64 +/-0.10
Mrs6	regulates the transcription factor Sfp1 controlling genes involved in ribosome biogenesis	S470	0.35 +/-0.09
Ltv1	required for 40S export from nucleus	S281	0.63 +/-0.19
Stm1	required for translation under nutrient stress	S55	0.55 +/-0.07
Rps16a/b	ribosomal protein	S2	0.57 +/-0.01
Rps17a/b	ribosomal protein	S70	0.59 +/-0.05
Rps18a/b	ribosomal protein	S107	0.82 +/-0.12
Rps24a/b	ribosomal protein	S14	0.73 +/-0.13
Rps1a/b	ribosomal protein	S36	0.70 +/-0.05
		S/T245	0.87 +/-0.05
		T254	0.75 +/-0.07
Rps5	ribosomal protein	T4	0.85 +/-0.09
Rps6a/b	ribosomal protein	T163	0.57 +/-0.02
Rps8a/b	ribosomal protein	T132	0.58 +/-0.29
Rps9a/b	ribosomal protein	S9	0.61 +/-0.05

*Error values represent the standard deviation of 2-6 experiments.

SUPPLEMENTAL MATERIALS AND METHODS

SILAC experiments. The labeling of yeast cells with stable isotopes was adapted from (3). Two cultures of 500 ml of liquid YNB medium containing 30 mg/ml lysine or [13C6] lysine were inoculated with *Δlys::KANMX6* or *GAL1::CTK1-TAP Δlys::KANMX6* cells from a stationary YPG culture. Yeasts were grown for 18 h at 30°C until they reached an OD_{600} of 0.5-0.7. Cell pellets were resuspended in 5 ml lysis buffer (20 mM HEPES-KOH pH 7.5, 75 mM KCl, 2.5 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 10 mM NaF, 1 mM $NaVO_4$, 5 mM β-glycerophosphate) and vortexed with glass beads at 4°C for 5 min. The samples were centrifuged at 27,000 rpm (SW40 rotor) at 4°C for 15 min. Equal amounts of A_{280} of the supernatant of *Δlys::KANMX6* and *GAL1::CTK1-TAP Δlys::KANMX6* extracts were diluted 1:1 with lysis buffer containing 1 M KCl, loaded on a 15% sucrose cushion containing 500 mM KCl and centrifuged at 42,000 rpm (Ti45 rotor) for 4 h. The pellet was resuspended in lysis buffer

(as above, but $NaVO_4$ concentration reduced to 200 μM), loaded on a 5-15% sucrose gradient and centrifuged at 32,000 rpm (SW32 rotor) for 2 h. The pooled ribosomal gradient fractions were pelleted by ultracentrifugation (100,000 rpm, TLA110 rotor, 50 min, 4°C) and washed once with lysis buffer. Purified ribosomes were resuspended in 6 M urea, 2 M thiourea, and 10 mM Tris-HCl pH 8 and protein concentrations were determined using Bradford Assay (BioRad).

Protein samples were digested in-solution with Lys-C, and phosphopeptides were enriched by TiO_2 chromatography (4). The resulting phosphopeptide fractions were analyzed by online nanoLC-MS/MS on a high-resolution LTQ-Orbitrap Velos instrument using Higher-energy collisional dissociation (HCD) for high-mass accuracy tandem mass spectrometry (5). Peptides and phosphopeptides were identified by Mascot (www.matrixscience.com) and SILAC pairs were quantified and normalized using the MaxQuant software suite (6).

SUPPLEMENTAL REFERENCES

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