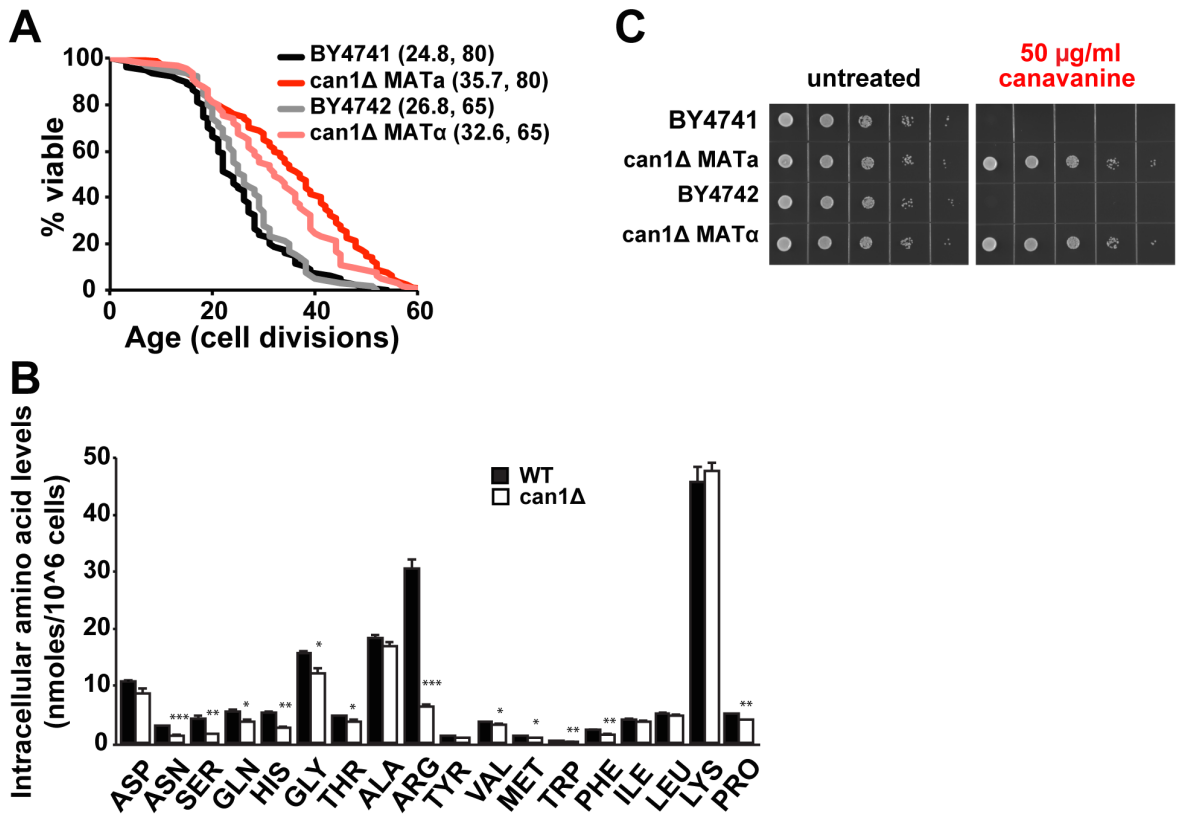
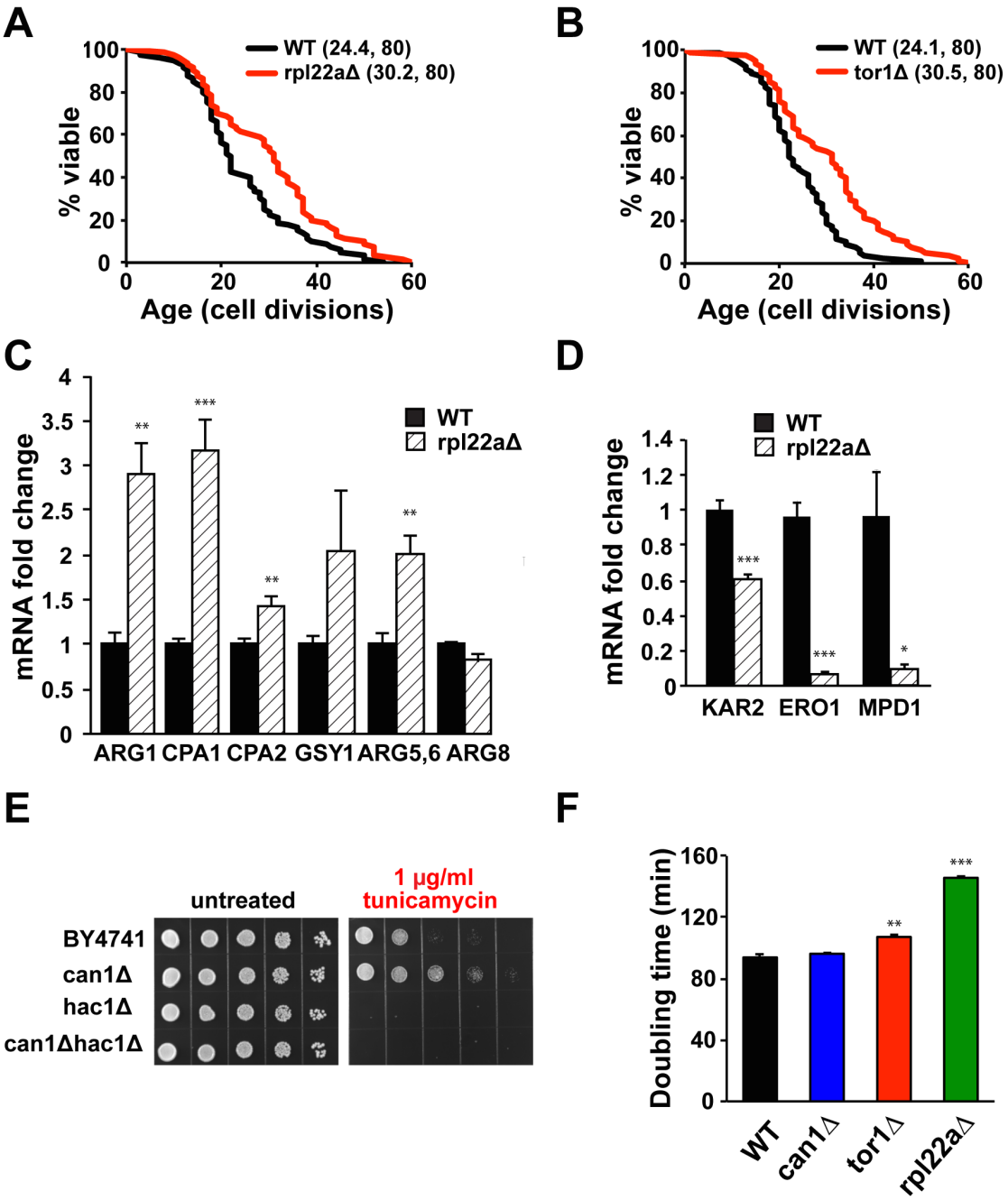
Figure S1



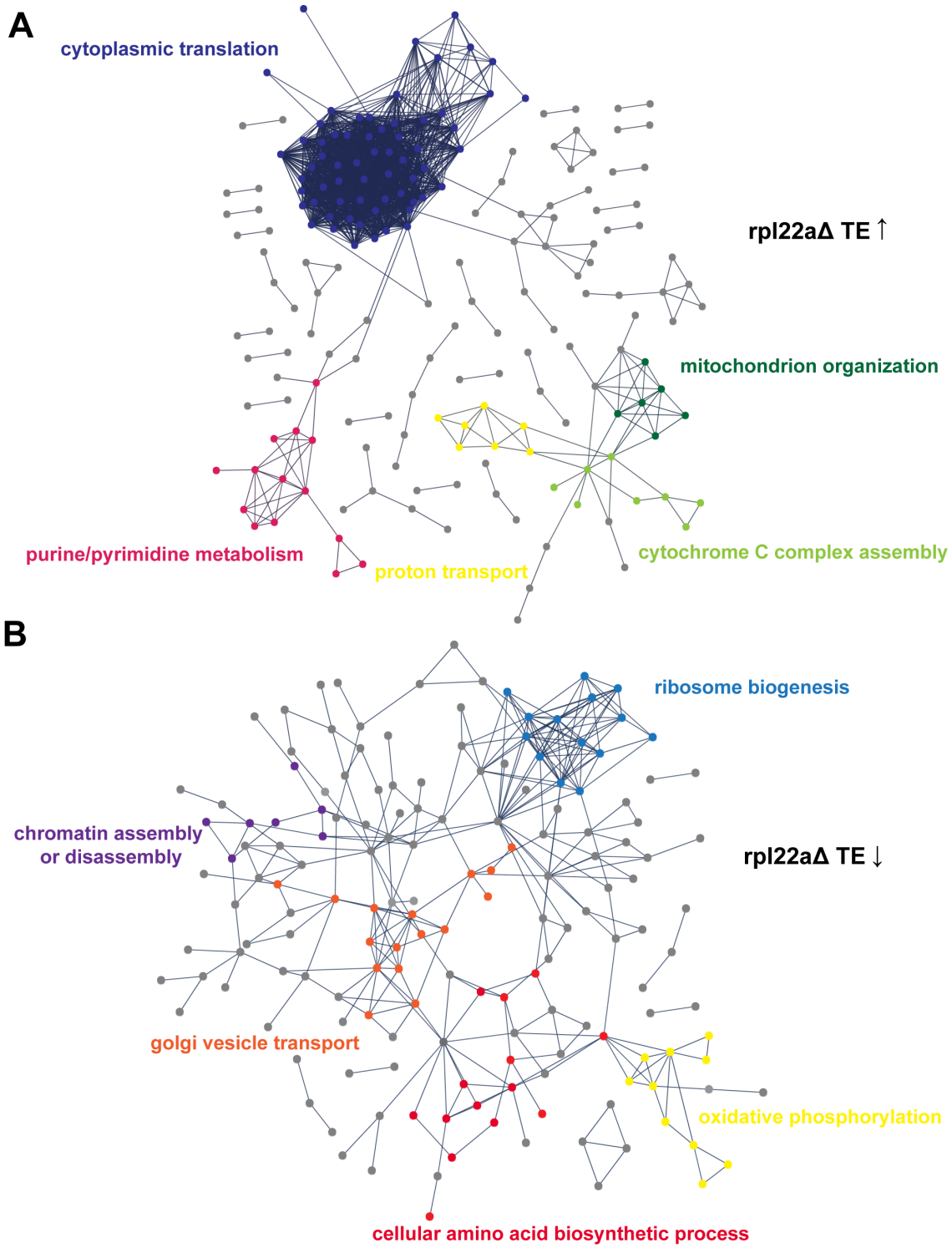
Supplemental Figure S1, related to Figure 1. (A) Replicative lifespan data for *can1Δ* strains from both the MAT**a** and MATα ORF deletion collections. Mean lifespans and the number of cells assayed are shown in parentheses. (B) Intracellular concentration of amino acids. Values are expressed as average concentration in nmoles/10^6 cells ± SEM from three independent experiments; *p<0.05, **p<0.01, ***p<0.001. (C) *can1Δ* strains are resistant to canavanine, a toxic arginine analog. 10× serial dilutions of logarithmically growing cells were spotted on agar plates with indicated concentrations of canavanine and incubated for 48 h at 30°C.

Figure S2



Supplemental Figure S2, related to Figure 3. (A-B) Survival curves for *rpl22aΔ* (A) and *tor1Δ* (B). Mean lifespans and the number of cells assayed are shown in parentheses. (C-D) Relative expression of Gcn4 (C) and Hac1 (D) target genes in *rpl22aΔ* mutant was determined by qPCR. Results are represented as means ± SEM from three independent experiments; *p<0.05, **p<0.01, ***p<0.001. (E) Resistance of *can1Δ, hac1Δ,* and double mutant *can1Δhac1Δ* cells to tunicamycin. 5× serial dilutions of logarithmically growing cells were spotted on agar plates with indicated concentrations of tunicamycin and incubated for 48 h at 30°C. (F) Doubling time for the *can1Δ*, *tor1Δ*, and *rpl22aΔ* deletion mutants was calculated using the Yeast Outgrowth Data Analyzer (YODA) software (Olsen et al., 2010). Results are represented as means ± SEM from three independent experiments; **p<0.01, ***p<0.001.

Figure S3



Supplemental Figure S3, related to Figure 4. Translational regulation of gene expression in *rpl22aΔ* mutant. Genes, whose TE was increased (A) or decreased (B) more than 1.5-fold in *rpl22aΔ* mutant, were visualized using STRING (evidence view, high confidence) (Szklarczyk et al., 2015). Genes without interacting partners were omitted.

Supplemental Table S1, related to Figure 1. Genes whose footprint coverage was changed more than 1.5-fold by *CAN1*, *TOR1* and *RPL22A* deficiency and overrepresented GO categories.

Supplemental Table S2, related to Figure 1. Genes whose mRNA coverage was changed more than 1.5-fold by *CAN1*, *TOR1* and *RPL22A* deficiency.

Supplemental Table S3, related to Figure 4. Genes whose TE was changed more than 1.5-fold by *CAN1*, *TOR1* and *RPL22A* deficiency.

Supplemental Table S4, related to Figure 2. List of qPCR primers used in this study.

Gene name	**Primer sequence**
ADH5	5'-CTGGCTTCACACATGATGGTAC-3' 5'-GATACCTGCACACAAGATTGGG-3'
ARG1	5'-TCATGGCTAATGTAGGGCAAG-3' 5'-AGCTGGGAATAGAATATCCTTGAC-3'
ARG5,6	5'-CATCTATTAAGGCAGGTGCC-3' 5'-GGCTAATTCACCAGCAGCTA-3'
ARG8	5'-GCTAACAAACTGGTTCATTC-3' 5'-GCTTCCGTACCAGAATTACA-3'
CPA1	5'-AGAGGTGCCAATGTAACTG-3' 5'-GACGTTGGAAATTGTAGCTTG-3'
CPA2	5'-GGTCAAGCTGGTGAATTCGA-3' 5'-CAGGGAATGAGAAGTCTGGT-3'
ERO1	5'-TTAAGAACCGCCATTGCCAC-3' 5'-ACGTGATCATTCCTGTCGAC-3'
GSY1	5'-TCTCTCGTCGGAATTCCATC-3' 5'-TGGAAATGGCCAATGATGGCA-3'
KAR2	5'-TAGAGGTGCCGATGATGTAG-3' 5'-CACGTAAGATGGGGTGATTC-3'
MPD1	5'-AAGCTCTCTAGTACGTTCCG-3' 5'-CCATTAACGTGGGAAATCCG-3'
PGK1	5'-AATCGGTGACTCCATCTTCG-3' 5'-GTGTTGGCATCAGCAGAGAA-3'

Supplemental Table S5, related to Experimental Procedures. Yeast strains used in this study.

Strain	**Designation**	**Genotype**
CB001	BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
CB002	*can1Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 can1Δ::KAN*
CB003	*tor1Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tor1Δ::KAN*
CB004	*rpl22aΔ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rpl22aΔ::KAN*
CB005	*rpl20bΔ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rpl20bΔ::KAN*
CB006	*can1Δhac1Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 can1Δ::KAN, hac1Δ::LEU2*
CB007	*hac1Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hac1Δ::KAN*
CB008	BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
CB009	*can1Δ*	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 can1Δ::KAN*

Supplemental Experimental Procedures

Amino Acid Analysis
Yeast cells were grown at 30°C in standard YPD medium until the OD_{600} reached 0.6. The cells were quenched with 0.1% sodium azide and 50 μg/ml cycloheximide, collected by centrifugation, and washed twice with water. Then, the cells were resuspended in 500 μl water and normalized to OD_{600} = 1. The suspension was boiled for 15 min and centrifuged for 3 min at 5,000 rpm to collect the supernatant, representing the metabolite extract. This extract was analyzed by standard PTH-derivatization and HPLC analysis (Heinrikson and Meredith, 1984) at the Texas A&M University Protein Chemistry Facility, to quantify the nmoles of each amino acid present in the extract. These values were normalized for the starting cell density. Results are represented as means ± SEM from three independent experiments and were analyzed for significance using Student's *t* test.

Spot assays
Resistance of strains to canavanine (50 μg/ml), tunicamycin (1 μg/ml), and $CuSO_4$ (1.75 mM) was determined using spot assays. Cells were initially grown in liquid culture without the drugs until OD_{600} = 0.6, and serial dilutions for each strain were spotted on SD agar plates containing indicated concentrations of the drugs. The plates were incubated at 30°C, and images were taken 48 h after plating**.**

Bioinformatics analyses
Ribosomal footprints and mRNA reads were aligned to the *S. cerevisiae* genome from the Saccharomyces Genome Database (SGD, http://www.yeastgenome.org/, release number R64-2-1). Sequence alignment was performed using Bowtie 1.1.2 software (Langmead et al., 2009) allowing two mismatches per read, and rpkm (reads per kilobase per million mapped reads) values were calculated using custom scripts. Custom Perl scripts were used to remove contaminating reads, deal with introns, overlaps and highly homologous sequences. To avoid bias caused by the region with elevated footprint density in the vicinity of the ATG start codon, we disregarded 100 nt from the 5'-end of each gene for the analysis of the differential gene translation. The Ribo-seq experiments were performed at least two times (independent biological replicates). An average rpkm value of biological replicates was calculated for each gene, and the genes with fewer than 10 rpkm were excluded from further analysis. The gene was considered regulated if its rpkm value changed more than 1.5-fold (0.6 in log2 scale). To calculate TE, footprint rpkm values were divided by mRNA rpkm. High-throughput sequence data have been uploaded to GEO database under accession number GSE85198. Gene ontology and pathways enrichment analysis was performed using DAVID database (Huang da et al., 2009).

Quantitative RT-PCR
Yeast cultures were grown to OD_{600} = 0.5 in 500 ml of standard YPD medium, and cells were collected by filtering through 0.45 μm filter (Millipore) with glass holder. Pellets were scraped with spatula, flash frozen in liquid nitrogen and stored at -80°C. Yeast extracts were prepared by cryogrinding the cell paste with BioSpec cryomill. Aliquots of cell lysates were used for total RNA isolation by hot acid phenol extraction. RNA was treated with DNaseI, and 2μg of RNA was used for cDNA synthesis using SuperScript III reverse transcriptase (Thermo Fisher Scientific) with random hexamer primers according to manufacturer's instructions. mRNA expression of the Gcn4 and Hac1 target genes was then analyzed by real-time PCR using KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems) and the CFX-96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories). *PGK1* was used as a reference gene for normalization of mRNA expression between genotypes. Results are represented as means ± SEM from three independent experiments. Statistical significance of the data was determined by calculating p values using Student's *t* test.

Polysome analysis
Polysome analysis was performed as described previously (Labunskyy et al., 2014). Aliquots of cell extracts containing 50 OD_{260} units were loaded on top of sucrose gradients (10-50% wt/wt) in polysome gradient buffer [20 mM TrisHCl (pH 8.0), 140 mM KCl, 5 mM $MgCl_2$, 0.2 g/l cycloheximide, 0.5 mM DTT]. The gradients were sedimented at 35,000 rpm at 4°C in a SW41 Ti rotor (Beckman) for 3 h. Fractions were collected using the Brandel gradient fractionation system and profiles were monitored at 254 nm. The area under the curve was measured using ImageJ software. Student's t-test was used to assess statistical significance.

Gcn4-luciferase assay
Gcn4 expression was analyzed using a dual-luciferase reporter as described (Steffen et al., 2008). Yeast strains were

transformed with pVW31 plasmid containing a *GCN4*-firefly luciferase cDNA fusion under transcriptional control of a 772 bp *GCN4* 5' fragment containing the promoter, upstream open reading frames (uORFs), and other 5' regulatory elements and the *CYC1* transcription terminator. Additionally, pVW31 plasmid contains an independent transcriptional unit with a Renilla luciferase cDNA transcribed from the constitutive *S. cerevisiae PGK1* promoter and terminated with a 3' fragment from *GCY1*. Strains transformed with pVW31 were grown in synthetic glucose minimal medium lacking uracil and containing required amino acids as well as isoleucine, valine and arginine. Arginine starvation was induced by replacing the medium with the synthetic glucose minimal medium described above lacking arginine. The Dual-Luciferase Reporter Assay kit (Promega) and the TD-20/20 Luminometer (Turner Designs) were used to measure luciferase activities. The Firefly luciferase activity was normalized to the Renilla luciferase activity. Results are represented as means ± SEM from three independent experiments. Statistical significance of the data was determined by calculating p values using Student's *t* test.

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