

Identification of longevity strains of *Saccharomyces cerevisiae* under high-glucose conditions

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Abstract

Excessive glucose concentrations accelerate cellular aging and impair fermentation robustness in industrial *Saccharomyces cerevisiae*, yet how yeast adapts to persistent glucose excess while maintaining long-term viability is not fully understood. In this study, adaptive laboratory evolution (ALE) was carried out under 300 g L⁻¹ glucose and combined with ethyl methanesulfonate (EMS) mutagenesis to generate multiple independent longevity-adapted lineages of the laboratory haploid strain BY4742. Chronological lifespan, growth kinetics, transcriptomics, and non-targeted metabolomics were integrated to characterize the underlying adaptive changes. The evolved strains showed significantly greater cumulative survival across the chronological lifespan assay, together with improved survival under high-glucose stress and shortened lag phases without major reductions in growth rate. Transcriptomic analysis revealed a conserved ribosome-associated adaptive core shared among lineages. Beyond this common foundation, W2 strains primarily remodeled translational allocation and biosynthetic pathways, E strains emphasized proteostasis, autophagy, membrane remodeling, and mitochondrial functions, and W3 strains showed extensive restructuring of central carbon metabolism, protein folding, and quality control. Despite this transcriptomic divergence, metabolomic remodeling showed clear convergence. Tryptophan metabolism was the most consistent shared signature, accompanied by coordinated alterations in kynurenine, xanthurenic acid, nicotinamide, and indole-3-acetic acid. These findings are consistent with a hierarchical model in which distinct transcriptional trajectories converge on remodeling of tryptophan-kynurenine metabolism and NAD⁺-associated nucleotide and redox networks, offering a systems-level framework for understanding adaptive aging and a basis for engineering robust industrial yeast strains.

Introduction

Saccharomyces cerevisiae is a central model for the study of nutrient sensing, metabolic homeostasis, and cellular lifespan, and it also serves as one of the most widely used industrial microorganisms in food fermentation and bioethanol production (Longo et al. 2012; Liu et al. 2019). In yeast, chronological lifespan (CLS) is defined as the period of cell survival after nutrient exhaustion in stationary phase and is used as a general indicator of cellular robustness under environmental stress (Fabrizio et al. 2001; Longo et al. 2012). In high-cell-density fermentation and in food and beverage production, cell viability during the late stationary phase directly determines product yield and consistency (Liu et al. 2019; Azhar et al. 2017).

Industrial high-cell-density fermentation typically operates with glucose loads above 300 g L⁻¹, which imposes two connected physiological challenges. First, high glucose activates nutrient-sensing pathways such as Ras/PKA and TORC1, which repress stress-resistance programs and accelerate cellular aging (Wei et al. 2008; Zaman et al. 2008; Smets et al. 2010). Second, hyperosmotic pressure and the accumulation of metabolic by-products such as ethanol and acetic acid produce mitochondrial dysfunction, elevated reactive oxygen species (ROS), and proteostatic stress, which further shorten CLS and weaken fermentation performance (Pidcocke et al. 2009; Lam et al. 2014; van Heerden et al. 2014;

Gomar-Alba et al. 2015; Chen et al. 2024). High-glucose conditions therefore represent both a practical bottleneck in industrial microbiology and a tractable experimental system for the study of how cells extend lifespan under persistent nutrient surplus.

Recent work has clarified several molecular routes by which yeast adapts to high-glucose stress. Studies on hyperosmotic tolerance have shown that Hog1-dependent signaling, glycerol accumulation, and remodeling of the cell wall and plasma membrane are central to short-term osmoadaptation (Gomar-Alba et al. 2015; Chen et al. 2024). Longer-term adaptation involves rewiring of central carbon metabolism, changes in mitochondrial activity, and remodeling of translational control mediated by TORC1, Sch9, and Gcn4 (Wei et al. 2008; Steffen et al. 2008; Smets et al. 2010). Autophagy, mitophagy, and one-carbon metabolism have also been identified as conserved longevity-promoting programs that operate during nutrient stress (Sampaio-Marques et al. 2014; Steffen and Dillin 2020; Ducker and Rabinowitz 2021). More recently, remodeling of NAD⁺ biosynthesis and salvage has emerged as an important determinant of yeast chronological aging (Belenky et al. 2007; Verdin 2015; Kalita et al. 2026). However, most previous studies have examined single mutants or short-term glucose exposure, and the way in which multiple parallel adaptive routes assemble into a coherent long-term response under persistent glucose excess is still not well defined.

Adaptive laboratory evolution (ALE) uses long-term directional selection to accumulate adaptive mutations across a population and has become an effective approach for acquiring complex, polygenic phenotypes (Elena and Lenski 2003; Dragosits and Mattanovich 2013; Sandberg et al. 2019). In yeast, ALE has been applied to enhance stress tolerance, substrate utilization, and product biosynthesis (Dragosits and Mattanovich 2013; Caspeta et al. 2014; Gresham et al. 2008; Godara et al. 2019; Hemansi et al. 2022; Salas-Navarrete et al. 2022; Sánchez-Adriá et al. 2023). Conventional ALE is limited by the natural mutation rate and can become trapped at local adaptive optima; chemical mutagenesis such as EMS treatment can expand the accessible genetic variation, although it often introduces phenotypic heterogeneity within the evolved population (Chen et al. 2023). The present study combines both strategies: three rounds of ALE were performed in synthetic defined medium containing 300 g L⁻¹ glucose (SD-300G), and EMS mutagenesis was layered onto the second-round output to generate composite evolutionary trajectories.

CLS served as the primary metric for selecting evolved strains, and growth kinetic parameters were then used to distinguish genuine lifespan extension from apparent longevity produced by reduced proliferation. Transcriptomic and non-targeted metabolomic analyses were then combined to examine the adaptive mechanisms underlying the longevity phenotype at the pathway level. The laboratory haploid strain BY4742 was used as the parental background because it is one of the most extensively characterized yeast strains for chronological aging, has a fully annotated genome, and is compatible with the deletion library and public omics resources needed for pathway-level interpretation. The limitations of this choice, in particular the auxotrophic markers of BY4742, are addressed in the Discussion. Overall, this design was intended to probe the metabolic plasticity of lifespan regulation

under nutrient excess and, at the same time, to yield candidate strains and molecular nodes of possible interest for later work in industrial yeast strains.

Materials and methods

Strains and reagents

The MAT α BY4742 single-gene deletion library and wild-type BY4742 strain were obtained from Dharmacon Yeast Resources (Lafayette, CO, USA). Glucose was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). The glucose assay kit (o-toluidine method) was from Beyotime Biotechnology (Shanghai, China). The ethanol assay kit (micro-method) was from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). The M5 Universal Plus RNA Mini Kit was from Mei5 Biotechnology Co., Ltd. (Beijing, China). All other chemicals were of analytical grade.

Media

YPD liquid medium contained glucose 20 g L⁻¹, peptone 20 g L⁻¹, and yeast extract 10 g L⁻¹. YPD solid medium was prepared as above and supplemented with agar at 20 g L⁻¹. All media were autoclaved at 121°C for 20 min. A glucose stock solution at 600 g L⁻¹ was autoclaved separately at 121°C for 20 min. Synthetic defined (SD) liquid medium was prepared according to Table 1, autoclaved at 121°C for 20 min, and supplemented with sterile glucose solution to a final concentration of 20 g L⁻¹ before use. High-glucose SD liquid medium was prepared according to Table 1, with glucose added after autoclaving to a final concentration of 300 g L⁻¹. This formulation is referred to throughout as SD-300G.

Table 1
Composition of SD medium (per liter).

Reagent	Mass
Yeast nitrogen base	1.7 g
Ammonium sulfate	5 g
Uracil	100 mg
L-histidine	100 mg
L-leucine	300 mg
L-lysine·HCl	150 mg
Adenine	80 mg
L-arginine	40 mg
L-aspartic acid	100 mg
L-glutamic acid	100 mg
L-methionine	80 mg
L-phenylalanine	50 mg
L-serine	400 mg
L-threonine	200 mg
L-tryptophan	200 mg
L-tyrosine	40 mg
L-valine	150 mg

Adaptive laboratory evolution under high-glucose conditions

A cryopreserved BY4742 wild-type stock was revived by streaking onto YPD solid medium and incubated at 30°C for 48 h. A single colony was inoculated into 1 mL of YPD liquid medium and cultured at 30°C with shaking at 220 rpm for 48 h. Cultures were maintained in 1 mL volumes in individual wells of 96-deep-well plates (2 mL well capacity, sealed with breathable film) to allow parallel handling of many independent lineages under identical aeration conditions; this small-volume format has been described previously for high-throughput yeast lifespan and stress-tolerance screening (Wu et al. 2011; Wang and Wu 2024). Each independent lineage was propagated in its own well, and cross-well contamination was monitored by periodic streaking of control wells inoculated with sterile medium. Five microliters of the stationary-phase seed culture was transferred into 1 mL of SD-300G medium and subjected to continuous cultivation for 30 days at 30°C, 220 rpm. Beginning on day 14, samples were collected every

4 days, serially diluted 10-fold, and plated onto fresh YPD solid medium. Colony-forming units (CFUs) were counted after incubation at 30°C for 48 h. At the end of each 30-day cycle, the plate with the highest colony density was selected and four to eight single colonies were picked; preference was given to colonies of larger diameter and well-defined edges in order to enrich for high-viability clones. These isolates were then used as the starting material for the next round, following the same procedure. After three successive rounds of ALE, the strains obtained from each round were designated W1, W2, and W3, respectively.

EMS mutagenesis

Second-round ALE isolates (W2) were revived and grown to mid-log phase. Cells were harvested by centrifugation at $3,000 \times g$ for 5 min at 4°C and washed twice with ice-cold 0.1 M sodium phosphate buffer (pH 7.0). The cell pellet was resuspended in the same buffer containing 4% (v/v) EMS and incubated at 30°C in the dark for 60 min. The reaction was immediately quenched, and cells were transferred into SD-300G medium and subjected to 30 days of stress-driven cultivation and screening under the same conditions used for ALE. The final composite trajectory was BY4742 to W1 to W2 to EMS-derived isolates, and these EMS-derived strains are referred to as E strains.

Seed culture preparation

All strains were revived synchronously. After 48 h of culture at 30°C, single colonies were inoculated into 1 mL of YPD liquid medium and grown at 30°C with shaking at 220 rpm to stationary phase. Cultures were diluted in sterile deionized water to normalize all strains to $OD_{660} = 0.2$. Suspensions were aliquoted into sterile microcentrifuge tubes and stored at 4°C for short-term use.

Chronological lifespan, lag time, and doubling time

CLS was measured using an established microplate-based high-throughput assay (Wu et al. 2011; Wang and Wu 2024). Five microliters of standardized seed culture was inoculated into 1 mL of SD-300G medium and incubated at 30°C with shaking. On days 5 and 10, 5 μ L aliquots were transferred into 96-well plates pre-loaded with 100 μ L YPD. Growth was monitored continuously for 20 h at 30°C with linear shaking at 567 cpm, and OD_{660} was recorded every 5 min. Lag time (λ) and population doubling time were derived from the resulting growth curves using the equations described in the original microplate CLS assay (Wu et al. 2011). Relative viability was defined as the viable cell count on day N for the screened strain divided by the viable cell count for the same strain on day 2 (set to 100%). Eight biological replicates were included per group.

Glucose and ethanol quantification

Glucose concentration in the fermentation supernatant was measured using an o-toluidine colorimetric assay kit. Broth was centrifuged to remove cells, and the supernatant was diluted 20-fold. The reaction was set up according to the manufacturer's instructions, absorbance was measured at 630 nm, and glucose concentration (g L^{-1}) was calculated from a standard curve. Ethanol content was determined using a micromethod assay kit. Fermentation supernatant was diluted 100-fold, and absorbance was measured at 450 nm. Ethanol concentration (mol L^{-1}) was calculated according to the manufacturer's formula. Four biological replicates per group were analyzed for each measurement.

Growth kinetics and total biomass

Five microliters of standardized cell suspension was inoculated into 96-well plates containing 100 μL of YPD, SD, or SD-300G medium. OD_{660} was monitored continuously for 20 h at 30°C, and lag time and doubling time were calculated to assess proliferation kinetics under different medium conditions. For total biomass measurements, strains were cultured in each of the three media and OD_{660} was recorded on days 2 and 3. Wild-type BY4742 served as the reference for biomass accumulation comparisons.

Stress tolerance assay

Strains were pre-cultured and normalized to $\text{OD}_{660} = 0.2$, and then exposed to each of the following stress conditions for 2 h: control (YPD), H_2O_2 (5 mM, 10 mM), NaCl (0.5 M, 1 M), and heat stress at 40°C. After stress treatment, cells were washed, serially diluted 10-fold, and spotted onto YPD plates. CFUs were counted after incubation at 30°C for 48 h.

Transcriptomic analysis

Strains were cultured in SD-300G for 48 h, harvested by centrifugation, and washed in ice-cold PBS. Total RNA was extracted using the M5 Universal Plus RNA Mini Kit, and purity and concentration were checked with a NanoDrop spectrophotometer. Library construction and sequencing were carried out by Novogene (Beijing, China) on an Illumina NovaSeq platform using 150 bp paired-end reads, yielding approximately 6 Gb of clean data and more than 75% uniquely mapped reads per sample. Three biological replicates were sequenced per strain.

Reads were aligned to the *S. cerevisiae* R64-1-1 reference genome using STAR (v2.7.1a) with the two-pass procedure, and gene- and transcript-level abundances were quantified with RSEM (v1.3.3). Differential expression analysis was performed with DESeq2 (Bioconductor) in R (v4.3.1), and the false discovery rate (FDR) was controlled by the Benjamini and Hochberg procedure. Differentially expressed genes (DEGs) were defined as those with $\text{FDR} < 0.05$. A fold-change cutoff was not imposed on the primary DEG list, in order to retain coordinated transcriptional changes of moderate amplitude, which are common in adaptive evolution studies and which underpin pathway-level enrichment. For the representative gene heatmap and for the identification of high-confidence lineage markers, a secondary filter of $|\log_2 \text{fold change}| \geq 1$ was applied on top of $\text{FDR} < 0.05$. Volcano plots showing the global

distribution of differentially expressed genes for all evolved strains are provided in Fig. S3. GO and KEGG enrichment of DEGs was performed with DAVID. Eleven independently evolved strains (W2-1 to W2-3, E1 to E4, and W3-1 to W3-4) and the parental strain BY4742 were analyzed, with three biological replicates each. Shared pathways were defined as those significantly enriched (Benjamini-adjusted $P < 0.05$) across multiple independently evolved strains. Lineage-dominant pathways were defined as those with consistent enrichment within a specific evolutionary lineage and with substantially weaker or no enrichment in the other lineages.

Non-targeted metabolomic analysis

Strains were cultured in SD-300G for 48 h, harvested, and washed in ice-cold PBS. Cells were transferred to grinding tubes containing an equal volume of acid-washed glass beads and 300 μL of pre-cooled extraction solvent (methanol/acetonitrile/water 2:2:1, v/v/v, containing 2 $\mu\text{g mL}^{-1}$ chlorophenylalanine as internal standard; solvent volume was two to three times the cell volume). After bead-beating at 4°C for 2 min, samples were vortexed and centrifuged at 12,000 rpm for 10 min at 4°C. The pellet was re-extracted with an additional 200 μL of solvent under the same conditions. The combined supernatant (approximately 500 μL) was partitioned with chloroform and water at an extract/chloroform/water ratio of 5:4:3, vortexed, and centrifuged as above. The upper aqueous phase was vacuum-concentrated to dryness at room temperature (up to 45°C if needed), reconstituted in 100 μL of methanol/water (3:7, v/v), and centrifuged at 12,000 rpm for 20 min at 4°C before LC/MS injection. A pooled quality control (QC) sample was prepared by mixing 5 μL aliquots from every sample.

LC/MS analysis was performed on a UPLC system (Waters, Milford, MA, USA) coupled to a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Separation was carried out on a SeQuant ZIC-HILIC column (100 $\times$ 2.1 mm, 3.5 μm ; Merck, Darmstadt, Germany) at 45°C with mobile phase A (10 mM ammonium formate in water) and mobile phase B (90% acetonitrile containing 10 mM ammonium formate), using a linear gradient from 10% to 60% A at 0.4 mL min^{-1} (injection volume 1 μL). Detection was performed with a heated electrospray ionization (HESI) source in both positive and negative ion modes under data-dependent acquisition (one full MS scan followed by five MS/MS scans per cycle; normalized collision energies of 15, 30, and 45 with high-purity N_2 as collision gas). Full MS scans covered 67 to 1,000 m/z at a resolution of 70,000 (AGC target 1×10^6 , maximum injection time 100 ms); MS/MS scans were acquired at a resolution of 17,500 (AGC target 5×10^5 , maximum injection time 50 ms). The spray voltage was 3.0 kV, capillary temperature 320°C, and S-lens RF level 50 V.

Raw data were acquired with Xcalibur 3.0 (Thermo Fisher) and processed with Progenesis QI v2.3 (Waters) for peak alignment, peak picking, and deconvolution. Metabolites were annotated by matching against HMDB (<https://www.hmdb.ca>), LIPID MAPS (<https://www.lipidmaps.org>), and METLIN (<https://metlin.scripps.edu>), with retention-time and characteristic-ion confirmation against authentic standards where available. Differentially abundant metabolites were defined as those with $\text{FDR} < 0.05$ and $|\log_2 \text{fold change}| \geq 0.58$ (corresponding to a linear fold change of approximately 1.5). Statistical analysis and pathway enrichment were performed with MetaboAnalyst 6.0. The same set of 11 evolved

strains and BY4742 used in the transcriptomic analysis was analyzed here, with three biological replicates per strain. Shared metabolic pathways were defined as pathways significantly enriched ($\text{FDR} < 0.05$) across multiple independently evolved strains. Lineage-dominant pathways were selected on the basis of consistent enrichment within a specific lineage and substantially weaker enrichment in the remaining lineages.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). For CLS, individual survival curves were summarized by calculating the area under the curve (AUC) from day 2 to day 8 using the trapezoidal method, and AUC values were compared among strains by one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test, with BY4742 serving as the reference. The day-0 value, which was assigned as 100% for graphical normalization but was not experimentally measured, was not included in AUC calculations. Growth kinetic parameters (lag time, doubling time), glucose and ethanol concentrations, and OD₆₆₀-based biomass measurements were analyzed by one-way ANOVA followed by Dunnett's multiple-comparison test. Homogeneity of variances was checked by Levene's test; when the equal-variance assumption was not supported, Welch's ANOVA was used with the Games-Howell post-hoc test. Spot assays under stress conditions were used qualitatively and are shown as representative images; quantitative CFU-based comparisons are reported where relevant. Differences were considered statistically significant at $P < 0.05$. All quantitative data are expressed as mean $\pm$ standard deviation, and the number of biological replicates for each experiment is indicated in the corresponding figure legend.

Results

High-glucose selection enriches for longevity strains

After 30 days of continuous cultivation in 1 mL of SD-300G, serial dilution plating showed that colony counts from third-round ALE isolates (Fig. 1I to L) and from EMS-mutagenized derivatives of the second-round output (Fig. 1E to H) were higher than those from the second-round parental strains (Fig. 1A to D). Each panel (A to L) shows an independent single-colony isolate obtained from the corresponding evolutionary round or EMS treatment, plated at matched dilutions on YPD after 30 days of SD-300G cultivation. These results indicate that both multi-round ALE and the complementary EMS mutagenesis strategy improve cell survival under high-glucose conditions. The higher colony counts of W3 and E strains relative to their W2 parental strains after 30 days are consistent with a progressive accumulation of adaptive changes across successive selection rounds. Cell concentrations of EMS-treated samples and ALE-derived samples were compared only after standardization of the initial seed culture to a common OD₆₆₀, and after normalization of colony counts to matched dilution factors and plating volumes, so that comparisons across the two trajectories are made on equivalent viable-cell input.

The three screening trajectories were designed with a clear rationale. W2 strains represent the baseline evolved output after two rounds of ALE and serve as the reference for subsequent comparisons. W3

strains underwent one additional round of evolution from W2, to test whether further selection pressure could drive deeper adaptive remodeling. E strains were derived from W2 by EMS mutagenesis, to evaluate whether broadening the accessible genetic variation could open adaptive routes that were not reached by the pure evolution trajectory. As shown below, the evolved strains consistently exhibited greater cumulative survival than the wild type in the CLS assay, while displaying divergent patterns in growth kinetics, stress tolerance, and transcriptomic signatures, supporting the multi-trajectory design.

Evolved strains show extended CLS with preserved growth kinetics

Survival curves normalized to each strain's own day-2 value as 100% (Fig. 2A) show that wild-type BY4742 had the shortest chronological survival, with viability falling rapidly and approaching zero by day 8. In contrast, W2, E, and W3 strains all maintained higher survival rates during the early and middle phases of the assay. The consistency of this pattern across lineages, together with the significantly greater AUCs of the evolved strains, indicates that the screening approach reliably enriched for strains with extended cumulative survival under high-glucose stress.

To quantify cumulative survival over the observation period, the area under each individual survival curve was calculated from day 2 to day 8. AUC differed significantly among the 12 strains (one-way ANOVA, $P < 0.000001$). The mean AUC of BY4742 was 121.8, whereas the evolved strains showed substantially higher mean AUCs ranging from 189.5 to 232.2. Dunnett's multiple-comparison test confirmed that all evolved strains had significantly greater AUCs than BY4742 (adjusted $P < 0.0001$ for all comparisons), demonstrating that the evolved strains maintained higher cumulative survival throughout the measured CLS period.

Relative viability on day 2 (Fig. 2B, normalized to BY4742 day 2 = 1) was assessed. The majority of W2, E, and W3 clones showed significantly higher day-2 viability than BY4742 ($P < 1 \times 10^{-6}$), indicating that these lineages already possess markedly enhanced short-term survival capacity at this early time point. The E lineage exhibited noticeable internal heterogeneity: E1 showed significantly lower day-2 viability than E2 to E4 ($P < 1 \times 10^{-6}$), while still remaining significantly above BY4742. This suggests that EMS mutagenesis, while raising the mean population tolerance, can also introduce variants that drive phenotypic divergence within the population. Within-lineage variation was also observed in W3 (for example W3-3 vs. W3-1 and W3-2, $P < 0.01$), indicating that prolonged ALE can likewise generate performance differences among individual isolates. Overall, the improvement in early (day-2) survival was consistently associated with subsequent CLS extension, which suggests that the longevity phenotype is at least partly driven by enhanced early tolerance rather than simply by slowed growth.

Lag time (Fig. 2C) and doubling time (Fig. 2D) on day 2 provide further support for this interpretation. Compared with BY4742, W2, E, and W3 strains adapted substantially faster upon re-inoculation into YPD, with markedly shorter lag times (P mostly $< 1 \times 10^{-6}$), and the doubling times of most evolved strains were not significantly longer than that of BY4742 ($P < 0.05$, except W2-2: $P = 0.18$ and E1: $P = 0.34$). The

combination of shorter lag times with largely unimpaired proliferation rates argues against the alternative interpretation that lifespan extension is a passive consequence of slower growth. This observation is consistent with previous reports that genuine longevity in yeast can be uncoupled from growth-rate trade-offs (Steffen et al. 2008; Alvers et al. 2009; Eisenberg et al. 2009).

Carbon metabolic output during high-glucose fermentation

In the SD-300G fermentation system, both supernatant glucose concentration (Fig. 3A) and ethanol content (Fig. 3B) measured on day 2 varied among strains. One-way ANOVA revealed a significant overall difference in glucose concentration ($P = 1.7 \times 10^{-5}$); however, pairwise comparisons against BY4742 did not reach statistical significance ($P \geq 0.05$ for all strains). This pattern indicates that although group-level variation is present, no individual strain differed significantly from BY4742, a result that likely reflects the limited statistical power associated with $n = 4$ and the within-group variability observed for glucose consumption at 300 g L^{-1} .

For ethanol production, most evolved strains produced significantly more ethanol on day 2 than BY4742 ($P < 0.05$), with the exception of individual clones E1, E2, and W3-3. This indicates that the screening process enhanced glycolytic flux toward ethanol in several strains, which may reflect reprogramming of carbon flux allocation or fermentative metabolic homeostasis. Such reprogramming is relevant to industrial fermentation, where higher ethanol yield translates directly into industrial value.

Growth curves confirm that longevity is not caused by growth inhibition

In rich YPD medium, all strains entered exponential phase and reached steady state within a similar time frame, and the overall shapes of the growth curves were comparable (Fig. 4A). In SD-300G, all strains showed extended lag phases, and most had not reached stationary phase within 20 h (Fig. 4B), indicating that elevated exogenous glucose substantially delays the onset of exponential growth. Taken together with the CLS data, these results show that the advantage of the evolved longevity strains in SD-300G does not come from a faster intrinsic growth rate, but is instead expressed as a shorter lag phase and higher early-phase survival, features that are particularly relevant for maintaining population viability under high-glucose stress.

Biomass accumulation in SD-type media

OD₆₆₀ measurements on days 2 and 3 in YPD, SD, and SD-300G (Fig. 5) revealed substrate-dependent differences in biomass accumulation. In YPD, OD values were high across all groups on both days, with no significant intergroup differences. In SD and SD-300G, the evolved strains generally reached higher mean OD values than BY4742 on both days, with the difference most pronounced under SD-300G conditions (BY4742: 0.314 ± 0.031 vs. W2: 0.425 ± 0.072 , representative comparison), indicating that the screening strategy improved biomass accumulation capacity in SD-type and high-glucose media. E1

showed markedly lower OD values on both days under SD conditions compared with the other strains, reflecting the intra-lineage fitness divergence that EMS mutagenesis can introduce.

Enhanced tolerance to hyperosmotic and extreme high-glucose stress

Spot assays under a panel of abiotic stresses (heat, NaCl at two concentrations, and H₂O₂ at two concentrations; Fig. 6A) revealed divergent tolerance profiles between evolved strains and BY4742, with certain isolates showing clear advantages or disadvantages under specific conditions. For long-term survival under extreme high-glucose conditions (300 g L⁻¹), viability was assessed at two recovery incubation times (1-day incubation shown in Fig. 6B; 2-day incubation shown in Fig. S4). At both time points, the W3 lineage (W3-1 to W3-4) consistently maintained higher culturability than either BY4742 or the W2 lineage. This is consistent with the possibility that the W3 strains have undergone systemic remodeling of carbon metabolic homeostasis or osmotic stress responses, enabling sustained proliferative or recovery capacity under extreme high-glucose selection pressure.

Transcriptomic divergence reveals multiple adaptive routes to longevity

Transcriptomic profiling revealed that lifespan extension under persistent high-glucose stress was not associated with a single conserved transcriptional state. Differential gene expression analysis identified extensive but lineage-specific transcriptional remodeling in all evolved strains relative to BY4742, with each strain displaying a clearly defined set of up- and down-regulated genes (Fig. S3). The evolved strains retained a common adaptive core while diverging into distinct lineage-specific trajectories represented by the W2, E, and W3 populations (Fig. 7).

A compact set of ribosome-associated processes remained consistently enriched across the evolved strains (Fig. 7A), including ribosome biogenesis, nucleolus organization, rRNA processing, 90S preribosome assembly, small-subunit processome formation, and maturation of SSU-rRNA. These pathways collectively indicate extensive remodeling of translational investment and suggest that ribosome-associated adaptation forms a shared foundation of the longevity phenotype. The relationship between ribosome activity and lifespan has been extensively documented in yeast and other model organisms. Reduced ribosomal investment and altered translational control frequently accompany lifespan extension and improved stress resistance through interactions with nutrient-sensing pathways such as TOR, Sch9, and Ras/PKA signaling (Steffen et al. 2008; Alvers et al. 2009; Eisenberg et al. 2009). The conserved enrichment of ribosome-related pathways observed here therefore likely reflects a common adaptive response to prolonged glucose exposure.

Beyond this conserved core, clear lineage-specific transcriptional programs emerged. The W2 lineage preferentially enriched pathways associated with transmembrane transport, one-carbon metabolism, purine metabolism, sulfur amino acid metabolism, and cofactor biosynthesis (Fig. 7B), consistent with a

strategy centered on resource redistribution and biosynthetic optimization. In contrast, E strains preferentially enriched autophagy-related processes, mitochondrial functions, glycerolipid metabolism, nucleotide metabolism, and membrane-associated pathways (Fig. 7C), consistent with enhanced proteostasis and cellular maintenance. The W3 lineage displayed the most extensive remodeling of central metabolism, with enrichment of glycolysis/gluconeogenesis, pyruvate metabolism, citrate cycle activity, protein folding, and nucleocytoplasmic transport (Fig. 7D), indicating deeper reorganization of cellular homeostasis during prolonged adaptation. These lineage-specific signatures recapitulate three well-established longevity-promoting modules in yeast: autophagy- and proteostasis-centered maintenance (Sampaio-Marques et al. 2014), one-carbon metabolism-centered biosynthetic rewiring (Steffen and Dillin 2020; Ducker and Rabinowitz 2021), and nucleotide and central-carbon flux balancing (Lane and Fan 2015), suggesting that each lineage independently engaged a distinct conserved longevity program.

The direction-resolved enrichment analysis further supported this interpretation (Fig. S1). Up-regulated pathways in W2 were dominated by ribosome biogenesis, rRNA processing, and translational machinery, whereas E preferentially activated ubiquitin-mediated proteolysis, mitochondrial functions, and nitrogen-starvation responses. W3 showed stronger enrichment of vacuolar organization, proton transport, and cytoplasm-to-vacuole targeting pathways. These observations indicate that each lineage reinforced a distinct adaptive module rather than converging on a uniform transcriptional solution. Down-regulated pathways revealed a second layer of organization: a shared suppression of ribosome-associated processes was detected across multiple lineages, while route-specific repression involved transport, membrane, cytoskeletal, and biosynthetic functions. The coexistence of lineage-specific activation and selective repression is consistent with lifespan extension being achieved through coordinated resource reallocation rather than through the activation of a single longevity pathway.

Representative DEGs further illustrated these adaptive strategies (Fig. 7E). *MAK5*, *RPL5*, and *RPS6B* reflected remodeling of ribosome biogenesis; *ADE6*, *TKL2*, and *TYE7* highlighted metabolic reprogramming; *PUT4*, *CYS3*, and *NTG1* indicated stress-response adaptation; and *CRH1*, *SCW4*, *MNT2*, *CLB1*, and *CIR1* suggested restructuring of cell-wall integrity (Levin 2005) and cell-cycle regulation. Together, these findings are consistent with a model in which multiple transcriptional trajectories can independently produce a common longevity phenotype under chronic glucose excess, consistent with the convergent-evolution outcomes repeatedly documented in microbial laboratory evolution (Elena and Lenski 2003; Gresham et al. 2008).

Importantly, the evolved strains did not simply exhibit reduced growth. Growth-curve analysis showed that lifespan extension occurred alongside shortened lag phases and largely preserved proliferation rates. Consequently, the observed transcriptomic remodeling is unlikely to represent passive growth inhibition and instead appears to reflect a strategic redistribution of cellular resources from constitutive biosynthesis toward maintenance, repair, and long-term survival.

Metabolic convergence despite transcriptomic divergence

In contrast to the pronounced transcriptomic divergence observed among lineages, metabolomic remodeling showed a substantially higher degree of convergence (Fig. 8), suggesting that distinct transcriptional programs ultimately funnel into a limited number of adaptive metabolic states.

The most consistent shared signal involved tryptophan metabolism (Fig. 8A). Multiple evolved strains showed coordinated alterations in kynurenine, xanthurenic acid, nicotinamide, and indole-3-acetic acid, indicating sustained remodeling of tryptophan catabolism and associated NAD⁺-related metabolic processes. The repeated enrichment of this pathway across independent evolutionary lineages is consistent with tryptophan metabolism representing a major metabolic convergence node associated with lifespan extension. Tryptophan degradation through the kynurenine pathway contributes to the generation of nicotinamide and ultimately influences intracellular NAD⁺ homeostasis (Belenky et al. 2007), linking amino acid metabolism to mitochondrial function, redox balance, and stress responses (Katsyuba et al. 2018; Castro-Portuguez and Sutphin 2020). The consistent remodeling of kynurenine and nicotinamide observed across evolved strains is therefore compatible with coordinated regulation of NAD⁺-associated metabolic networks during adaptation to chronic glucose excess, in agreement with the established role of NAD⁺ in aging and metabolic regulation (Verdin 2015) and with recent evidence that pharmacological modulation of NAD⁺ salvage can extend yeast chronological lifespan (Kalita et al. 2026).

Beyond this shared core, lineage-specific metabolic programs remained evident. E strains preferentially enriched pyrimidine metabolism, one-carbon metabolism, and nucleotide-associated pathways (Fig. 8B), whereas W3 strains showed stronger enrichment of purine metabolism, pentose phosphate pathway activity, and central carbon metabolism, indicating extensive carbon-flux reorganization during adaptation (Fig. 8C). The enrichment of pentose phosphate pathway-associated metabolism in W3 is notable because this pathway contributes to NADPH production and oxidative stress resistance (Ralser et al. 2007). These differences mirror the transcriptomic divergence observed between E and W3 and indicate that alternative adaptive routes can maintain distinct metabolic emphases while converging on a similar longevity phenotype.

Unlike the pronounced E- and W3-dominant metabolic signatures, W2 strains showed only modest lineage-specific metabolic enrichment relative to BY4742. The pathways showing preferential enrichment in W2 were primarily associated with nucleotide turnover and amino-acid utilization, but these signals were generally weaker and less distinct than those observed in E and W3 populations. This pattern is consistent with W2 adaptation relying predominantly on transcriptomic resource reallocation and biosynthetic optimization rather than on extensive downstream metabolic rewiring.

Direction-resolved metabolomic enrichment analysis provided additional support for this interpretation (Fig. S2). Shared up-regulated modules included metabolites associated with tryptophan-derived pathways and nucleotide turnover, whereas route-dominant modules highlighted distinct aspects of carbon utilization, nitrogen allocation, and redox homeostasis. The E lineage showed stronger signatures of pyrimidine and one-carbon remodeling, while W3 preferentially emphasized purine metabolism,

pentose phosphate pathway activity, and carbon-flux reorganization. These route-specific metabolic signatures paralleled the lineage-specific transcriptomic programs identified in Fig. S1.

The representative metabolite heatmap further illustrated coordinated remodeling across multiple metabolic layers (Fig. 8D). In addition to the tryptophan-associated metabolites, significant changes were observed in adenine, hypoxanthine, uracil, hypotaurine, citrulline, and glutamine. Collectively, these metabolites connect lifespan extension to broader reconfiguration of nucleotide metabolism, nitrogen homeostasis, sulfur metabolism, and redox balance.

Integrative view of transcriptomic and metabolomic remodeling

When the transcriptomic and metabolomic signatures were considered together, several concordances between the lineage-specific transcriptional programs and the shared metabolic convergence node became evident. In the W2 lineage, the pathways enriched at the transcriptional level (one-carbon metabolism, purine metabolism, sulfur amino acid metabolism, and cofactor biosynthesis) are all upstream of, or intersect with, the tryptophan-kynurenine-NAD⁺ network: one-carbon and purine metabolism intersect with metabolic processes relevant to NAD⁺ salvage and de novo biosynthesis, while sulfur amino acid metabolism supplies cysteine and glutathione that support redox homeostasis in the same network. In the E lineage, the enrichment of autophagy, mitochondrial function, and nucleotide metabolism at the transcriptional level is consistent with the observed metabolic enrichment of pyrimidine and one-carbon pathways, and both are functionally connected to the maintenance of NAD⁺/NADH pools during proteostatic and mitochondrial stress. In the W3 lineage, the transcriptional enrichment of glycolysis/gluconeogenesis, pyruvate and citrate cycle activity, and protein folding pathways is coherent with the strong metabolic enrichment of purine metabolism and pentose phosphate pathway activity, which together are consistent with altered NADPH and nucleotide precursor availability within broader NAD⁺-associated and redox networks. Thus, although each lineage engages a distinct upstream transcriptional program, these programs plausibly converge on remodeling of nucleotide and NAD⁺-associated metabolism, offering a mechanistic framework linking the divergent transcriptional signatures to the shared metabolomic node. It should be noted, however, that this integrative interpretation is based on pathway-level correspondence between two independent omics datasets and is associative rather than causal; direct testing of gene-metabolite relationships will require targeted perturbation and quantitative flux analysis.

Discussion

This study combined multi-round ALE and EMS mutagenesis under persistent high-glucose conditions with integrative transcriptomic and metabolomic profiling to identify strains of *S. cerevisiae* with extended chronological lifespan and to characterize the adaptive changes underlying this phenotype. Three main observations emerged. First, all three evolved lineages (W2, W3, and E) showed extended CLS relative to the parental BY4742, with significantly greater cumulative survival across the day-2 to

day-8 observation period, together with shortened lag phases and largely preserved proliferation rates. The consistent elevation of survival-curve AUC across all evolved strains further supports the robustness of the longevity-associated phenotype rather than an isolated improvement at a single time point. These results indicate that the longevity phenotype is not a passive consequence of slowed growth. Second, transcriptomic analysis revealed a conserved ribosome-associated adaptive core shared across lineages, on which distinct lineage-specific programs were superimposed: W2 emphasized translational allocation and biosynthetic pathways, E emphasized proteostasis and membrane and mitochondrial functions, and W3 exhibited extensive restructuring of central carbon metabolism and protein quality control. Third, despite this transcriptomic divergence, metabolomic remodeling showed clear convergence, with tryptophan metabolism and associated kynurenine, nicotinamide, and NAD⁺-linked metabolites emerging as the most consistent shared signature. These findings are consistent with a hierarchical model in which multiple transcriptional routes to longevity are possible, but ultimately converge on a common metabolic signature centered on tryptophan-kynurenine metabolism and NAD⁺-associated nucleotide and redox networks.

Comparison with previous work on yeast high-glucose adaptation and aging

Ribosome biogenesis and translational control are established regulators of yeast chronological aging. Depletion of 60S ribosomal subunits extends CLS through Gcn4-dependent transcriptional reprogramming (Steffen et al. 2008), and autophagy-dependent amino-acid homeostasis and spermidine-induced autophagy also extend CLS (Alvers et al. 2009; Eisenberg et al. 2009). The conserved enrichment of ribosome-associated pathways across our evolved strains, together with the E-lineage enrichment of autophagy and mitochondrial functions, is consistent with these earlier findings and suggests that adaptive evolution under high-glucose stress independently converges on the same longevity-promoting programs identified in single-gene studies. The prominence of tryptophan-kynurenine-NAD⁺ metabolism in the shared metabolic signature is likewise consistent with the established role of NAD⁺ in aging and metabolic regulation (Belenky et al. 2007; Verdin 2015; Katsyuba et al. 2018; Castro-Portuguez and Sutphin 2020) and with recent work showing that pharmacological modulation of NAD⁺ salvage can extend yeast chronological lifespan (Kalita et al. 2026). Our results extend this literature by showing that these longevity-associated remodeling events can arise together in a single system as a natural response to chronic glucose excess, and that they can be accessed through several independent evolutionary trajectories.

Choice of BY4742 as parental strain and implications for industrial application

BY4742 is a laboratory haploid strain that carries the auxotrophic markers *his3Δ1*, *leu2Δ0*, *lys2Δ0*, and *ura3Δ0*. It was used here as the parental background for three reasons. First, BY4742 is one of the most extensively characterized yeast strains in the study of chronological aging and stress response, and its

genome, transcriptome, and metabolome are supported by rich public reference resources, which enables reliable pathway-level interpretation of multi-omics data. Second, BY4742 is the background of the widely used systematic gene deletion collection, so any candidate genes identified in this work can be followed up in future studies by direct genetic perturbation without additional strain construction. Third, the use of a haploid background simplifies phenotypic scoring during ALE and EMS-driven screening by eliminating heterozygosity effects that can obscure recessive adaptive mutations.

The use of an auxotrophic laboratory strain also imposes clear limitations. Auxotrophic markers can affect amino acid metabolism, nutrient sensing, mitochondrial function, and chronological lifespan, and can therefore introduce metabolic perturbations that are not present in prototrophic strains such as CEN.PK or in industrial isolates. The SD medium used in this study contains exogenous L-tryptophan, uracil, and other amino acids to complement the auxotrophies of BY4742, and part of the observed remodeling of kynurenine, xanthurenic acid, and nicotinamide may therefore reflect altered uptake and turnover of exogenous tryptophan in addition to endogenous pathway regulation. In addition, industrial yeast strains generally have larger and more heterogeneous genomes and different genetic backgrounds, and the specific molecular events identified here may not translate directly to those strains. Instead, this work should be regarded as a systems-level identification of candidate longevity-promoting modules in a well-characterized background, and future work should test whether the same tryptophan-kynurenine-NAD⁺ module and the same lineage-specific transcriptional programs remain associated with lifespan extension when the corresponding adaptations are introduced into prototrophic and industrial yeast strains.

A hierarchical model of adaptive longevity under glucose excess

The integrated transcriptomic and metabolomic analyses are consistent with a hierarchical framework for adaptive longevity under chronic nutrient surplus (Fig. 9). In this model, a shared ribosome-associated adaptive core forms the first layer of response. Above this common foundation, distinct lineage-specific transcriptional trajectories emerge, generating alternative adaptive strategies represented by the W2, E, and W3 populations. Despite these transcriptional differences, the lineages subsequently converge at the metabolic level, particularly through remodeling of tryptophan-kynurenine metabolism and NAD⁺-associated nucleotide and redox networks. This framework accommodates the coexistence of transcriptomic divergence and metabolic convergence observed throughout the study. Rather than requiring a universal longevity pathway, adaptive evolution appears capable of generating multiple molecular solutions that ultimately stabilize a restricted set of metabolic states favorable for long-term survival. Such a model may help explain why longevity-associated mutations often differ substantially among experimental systems despite producing similar phenotypic outcomes.

Implications for industrial yeast engineering

From an applied perspective, these findings provide a conceptual basis for the engineering of robust industrial yeast strains for food fermentation and bioethanol production. Depending on industrial requirements, alternative adaptive routes emphasizing resource allocation (W2), proteostasis and membrane homeostasis (E), or extensive metabolic restructuring (W3) may be selectively exploited. The identification of tryptophan-kynurenine metabolism and NAD⁺-associated nucleotide and redox networks as a shared metabolic signature further suggests that targeted modulation of this metabolic module, for example through engineering of NAD⁺ biosynthesis and salvage genes or through supplementation with NAD⁺ precursors, is a promising strategy for improving fermentation lifespan and high-cell-density culture performance in baking, brewing, and bioethanol applications. Such strategies would need to be tested in prototrophic and industrial strains before their applicability to production settings can be established.

Limitations and future directions

Several limitations of this study should be noted. First, whole-genome sequencing of the evolved lineages was not performed in the present work, so the specific adaptive mutations underlying the W2, W3, and E phenotypes remain undefined and cannot be distinguished from passenger mutations. Future work will apply whole-genome sequencing to representative isolates from each lineage, together with population-level allele-frequency tracking across ALE rounds, to identify high-confidence adaptive variants and to test whether independent lineages converge on shared mutational targets.

Second, the conclusion that remodeling of the tryptophan-kynurenine-NAD⁺ axis contributes to lifespan extension is based on integrative transcriptomic and metabolomic associations and is not directly supported here by functional perturbation. To move from association toward causality, future studies should include direct quantification of intracellular NAD⁺ and NADH pools by enzymatic or mass spectrometric assay, supplementation experiments with NAD⁺ precursors such as nicotinamide, nicotinic acid, or nicotinamide riboside, and targeted genetic perturbation of key pathway genes including *BNA2*, *BNA4*, *BNA6*, *NPT1*, *NMA1*, and *PNC1*. These experiments would establish whether the axis is a causal driver of the extended CLS phenotype or a downstream marker of a more upstream adaptive state.

Third, RNA-sequencing was not validated by an orthogonal quantitative method in this study. Future work will include qRT-PCR verification of representative lineage markers such as *MAK5*, *RPL5*, *RPS6B*, *ADE6*, *TKL2*, *TYE7*, *PUT4*, *CYS3*, *NTG1*, *CRH1*, *SCW4*, *MNT2*, *CLB1*, and *CIR1*, and, where feasible, deletion or overexpression of the highest-priority candidates in the BY4742 background followed by assessment of CLS, stress resistance, and fermentation performance. Together with the whole-genome sequencing described above, these experiments will provide the mechanistic and functional validation required to distinguish causal from correlative signals in the current dataset.

Fourth, the study was carried out in the auxotrophic laboratory strain BY4742, and the SD medium used here contains exogenous L-tryptophan. As discussed above, the identified adaptive mechanisms should therefore be regarded as candidate modules whose relevance to prototrophic and industrial yeast strains, and to fermentation processes conducted in complex industrial media, will need to be established in subsequent work.

Fifth, the integrative interpretation of transcriptomic and metabolomic signatures presented above is based on pathway-level correspondence between two independent omics datasets and is associative in nature. Direct testing of gene-metabolite relationships through targeted perturbation, isotope tracing, and quantitative flux analysis will be required to establish the causal architecture of the proposed model. Notwithstanding these limitations, the multi-trajectory design, the reproducibility of the CLS extension across independent lineages, and the convergence of independent lineages on a common metabolic node together provide a robust systems-level framework and a defined set of testable hypotheses for future mechanistic work.

Declarations

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Author contributions Y.L.: data curation, formal analysis, investigation, methodology, writing (original draft), writing (review and editing). J.H.: investigation, software. L.W.: investigation, data curation, methodology. Z.W.: conceptualization, funding acquisition, supervision, validation, writing (review and editing). All authors reviewed and approved the final version of the manuscript.

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Data availability The RNA sequencing data generated in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number [to be provided upon acceptance]. The non-targeted metabolomics data have been deposited in MetaboLights under accession number [to be provided upon acceptance]. All other data supporting the findings of this study are available within the article and its supplementary material, or from the corresponding author on reasonable request.

Ethics approval

Not applicable. This study did not involve human participants or animals.

Competing interests

The authors declare that they have no competing interests.

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Figures

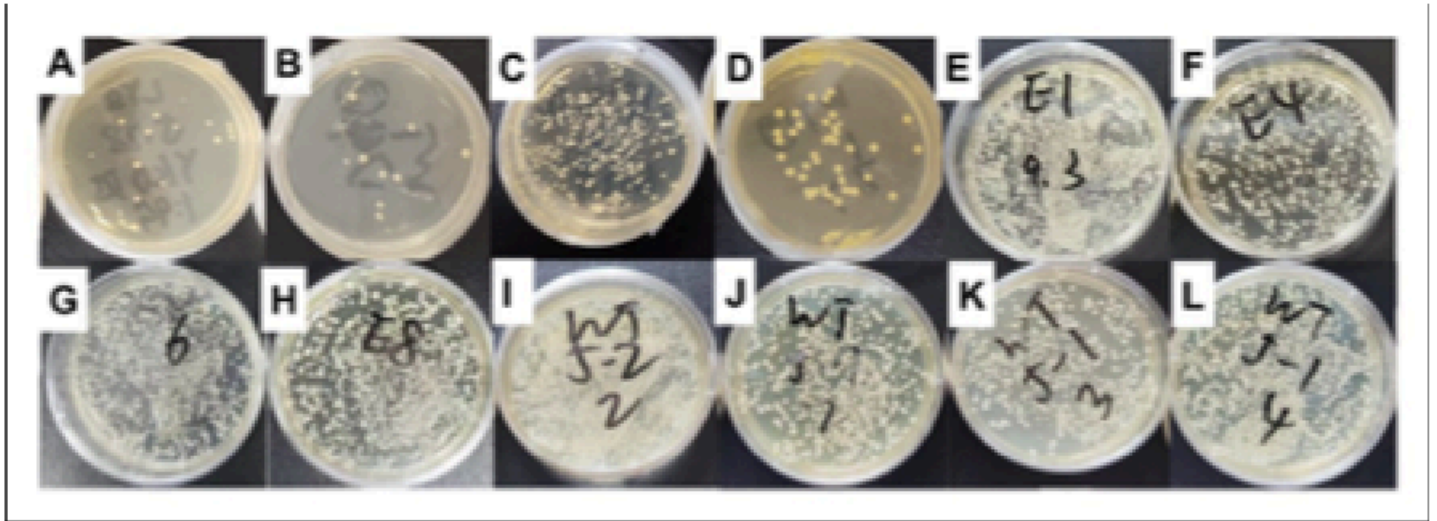


Figure 1

Representative dilution plating after 30 days of culture in SD-300G. (A–D) Representative isolates from second-round ALE (W2); (E–H) representative EMS-mutagenized derivatives of second-round ALE output (E); (I–L) representative isolates from third-round ALE (W3). Each panel shows an independent single-colony isolate from the corresponding evolutionary round or EMS treatment, plated at matched dilutions on YPD after 30 days of continuous SD-300G cultivation. Images are representative results illustrating differences in colony density and morphology.

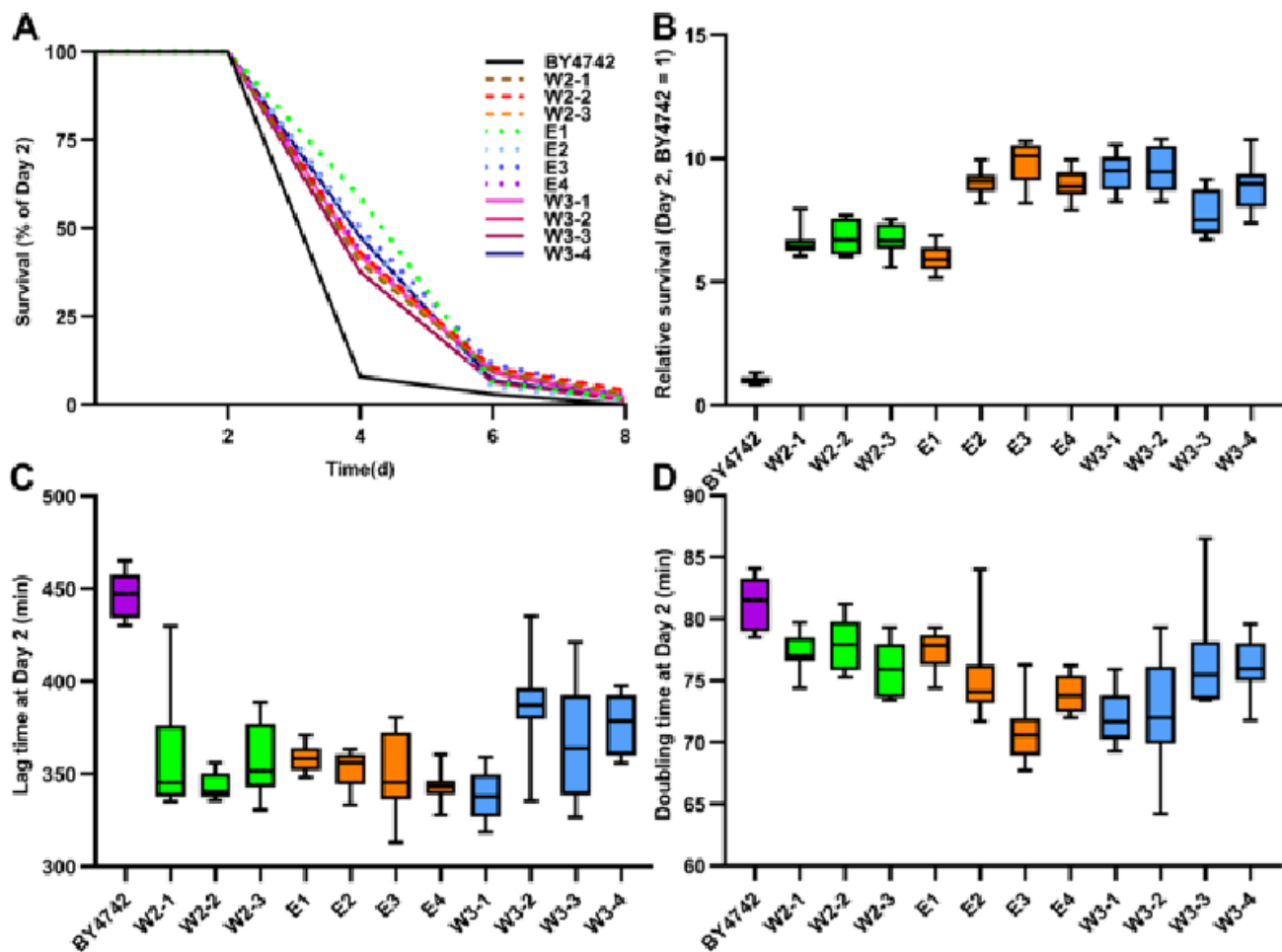


Figure 2

Chronological lifespan and early growth kinetics following culture in SD-300G, assessed by re-inoculation into YPD ($n = 8$). (A) Survival curves over time (normalized to each strain's own day-2 value as 100%); (B) relative viability on day 2 (BY4742 = 1); (C) lag time on day 2; (D) doubling time on day 2. Box plots show min–max range. Colors: purple (BY4742), green (W2), orange (E), blue (W3); same convention throughout.

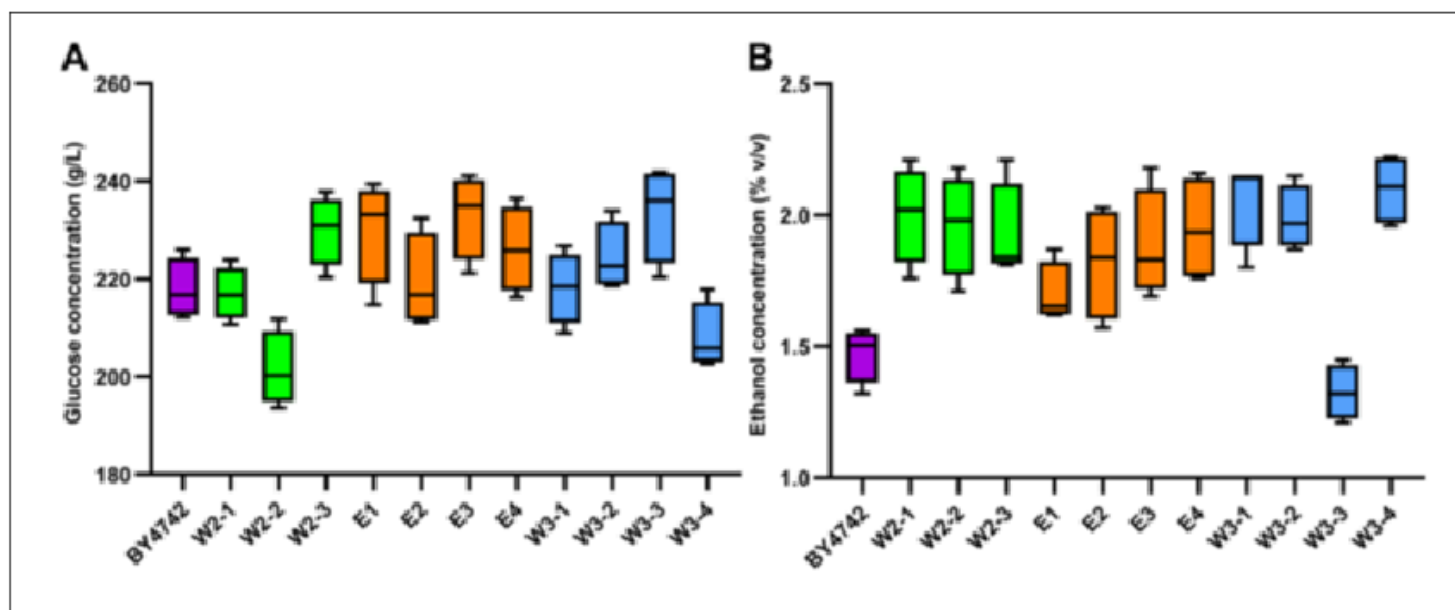


Figure 3

Glucose and ethanol levels on day 2 of fermentation in SD-300G ($n = 4$). (A) Supernatant glucose concentration (g L^{-1}); (B) ethanol content (% v/v).

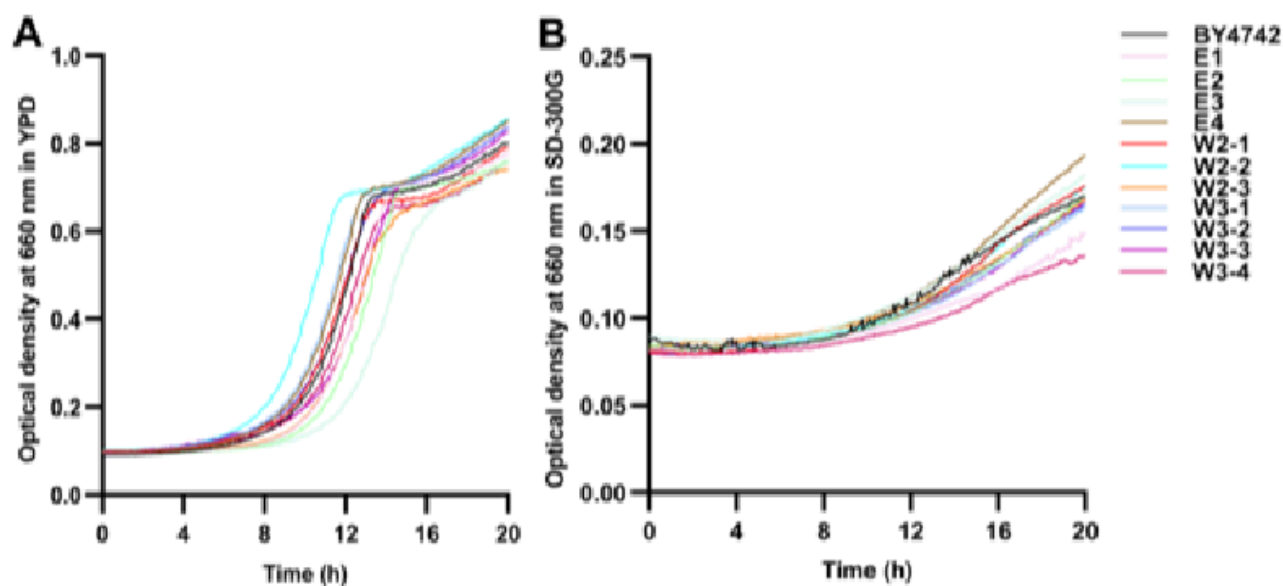


Figure 4

Twenty-hour growth curves in YPD and SD-300G ($n = 4$). (A) YPD (OD_{660} vs. time); (B) SD-300G (OD_{660} vs. time).

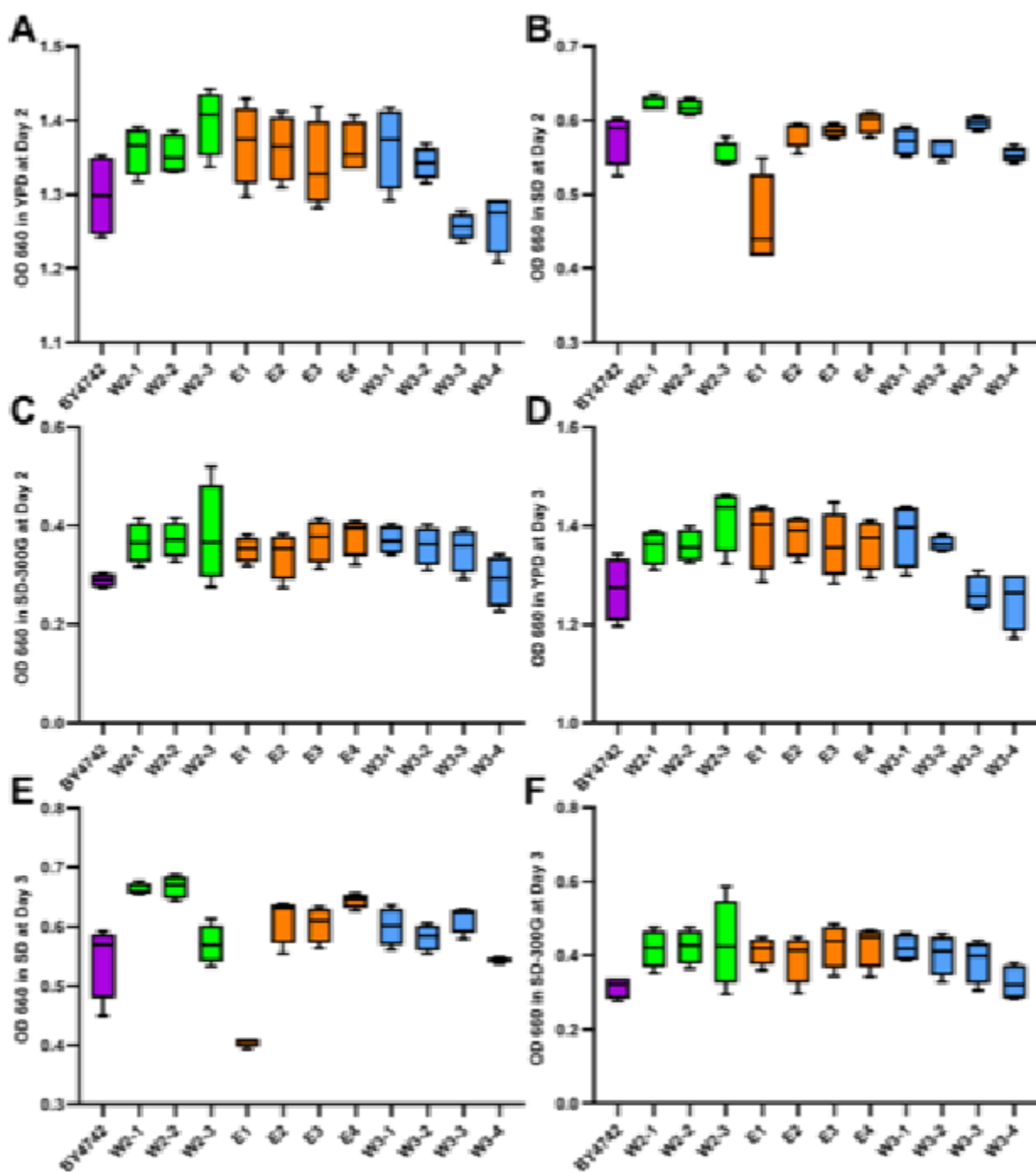


Figure 5

OD₆₆₀ on days 2 and 3 across three media types (YPD, SD, SD-300G; $n = 4$). (A–C) Day-2 OD₆₆₀ in YPD, SD, and SD-300G, respectively; (D–F) day-3 OD₆₆₀ in YPD, SD, and SD-300G, respectively.

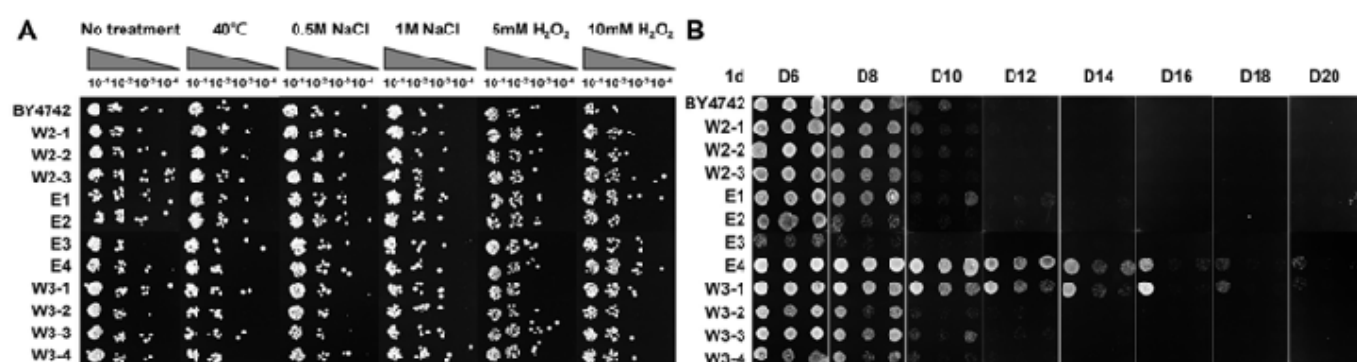


Figure 6

Spot assays showing growth phenotypes of evolved strains under multiple abiotic stresses and extreme high-glucose conditions. (A) Abiotic stress panel (40 °C, 0.5/1.0 M NaCl, 5/10 mM H₂O₂), plated after 10-fold serial dilution (10⁻¹ to 10⁻⁴); (B) spot recovery after prolonged culture in extreme high-glucose medium (300 g L⁻¹), with 1-day post-plating incubation. Representative replicates are shown.

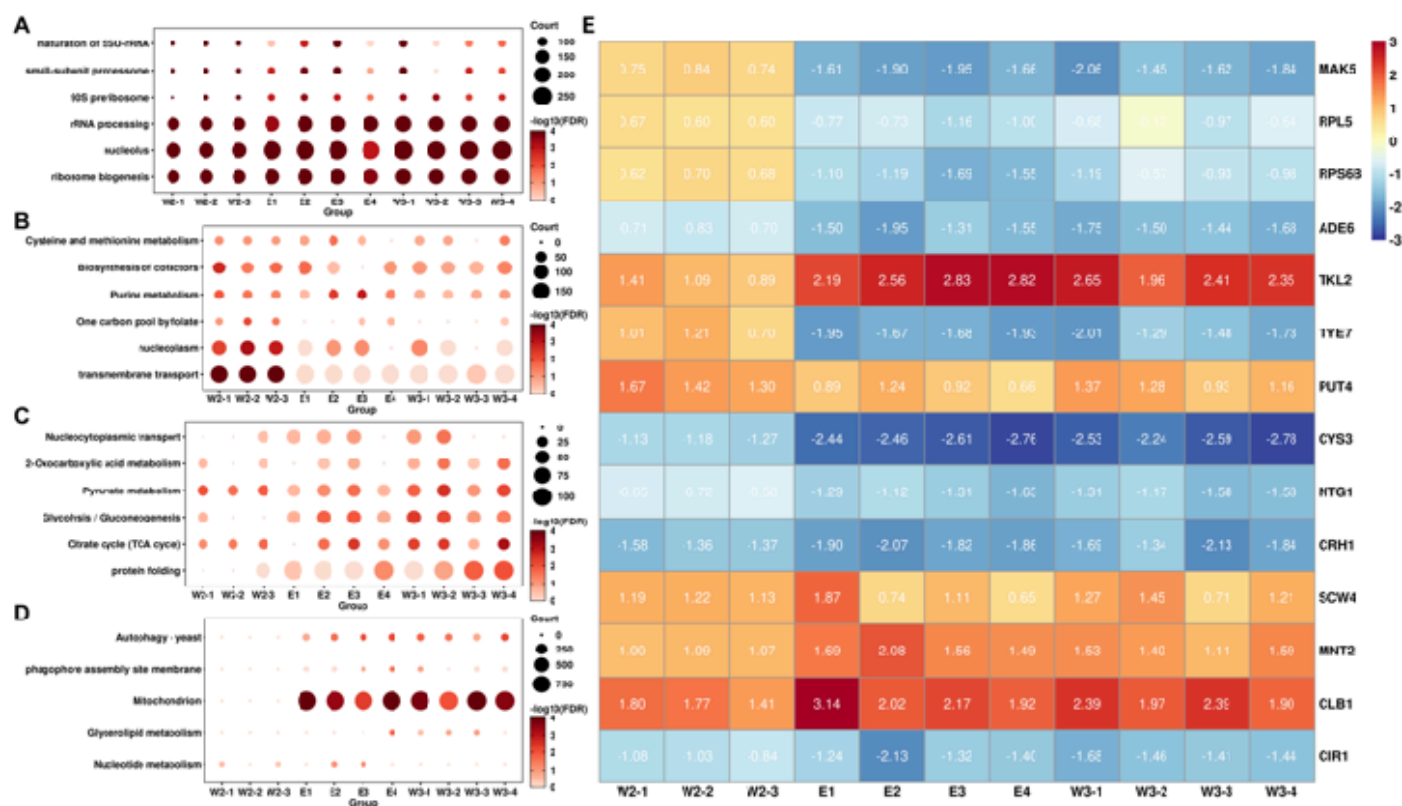


Figure 7

Divergent transcriptomic trajectories underlying lifespan extension in evolved yeast strains. Transcriptomic enrichment analysis was performed using DAVID based on differentially expressed

genes (DEGs) identified at adjusted $P < 0.05$. Bubble size represents the number of DEGs mapped to each pathway, and color indicates enrichment significance ($-\log_{10}$ FDR). (A) Conserved transcriptomic core shared across evolved strains, highlighting ribosome-associated remodeling including ribosome biogenesis, nucleolar functions, and rRNA processing; (B) W2-dominant transcriptional program characterized by resource reallocation, transmembrane transport, purine metabolism, one-carbon metabolism, cofactor biosynthesis, and sulfur amino acid metabolism; (C) E-dominant transcriptional program enriched for proteostasis- and membrane-associated pathways, including autophagy, mitochondrial functions, glycerolipid metabolism, and nucleotide homeostasis; (D) W3-dominant transcriptional program highlighting extensive remodeling of central carbon metabolism, protein folding, nucleocytoplasmic transport, and respiratory-associated processes; (E) heatmap showing representative DEGs associated with translation, metabolism, stress response, cell-wall remodeling, and cell-cycle regulation. Values represent $\log_2$ (fold change) relative to the ancestral strain. For visualization purposes, a value of 0 was assigned to genes that were either not significantly changed or not detected in a given strain.

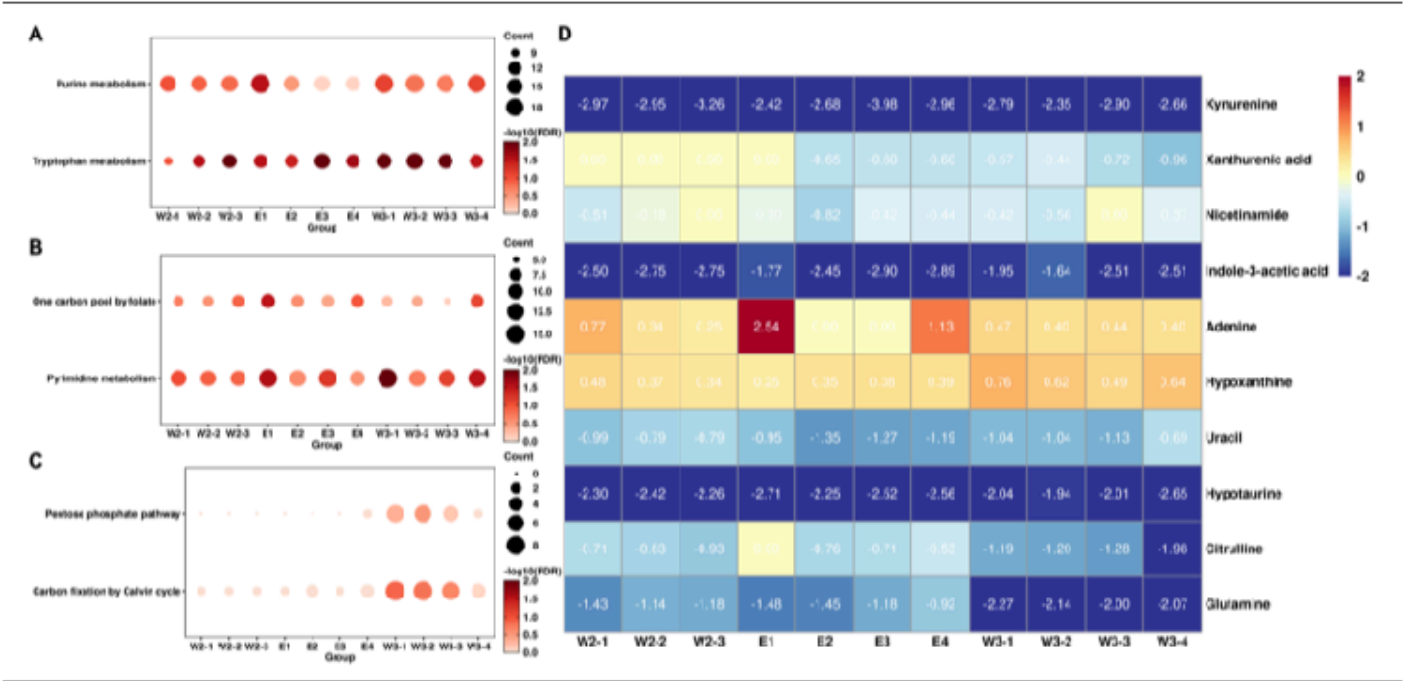


Figure 8

Metabolic convergence and lineage-specific metabolic remodeling in evolved yeast strains. Metabolic pathway enrichment was performed using significantly altered metabolites identified at adjusted $P < 0.05$ and $|\log_2 \text{FC}| \geq 0.58$. Bubble size indicates the number of mapped metabolites and color indicates enrichment significance ($-\log_{10}$ FDR). (A) Shared metabolic core among evolved strains, dominated by tryptophan metabolism and associated amino acid-derived metabolic pathways; (B) E-dominant metabolic program enriched for pyrimidine metabolism, one-carbon metabolism, and nucleotide-associated remodeling; (C) W3-dominant metabolic program characterized by purine metabolism,

pentose phosphate pathway activity, and extensive central carbon metabolism reorganization; (D) heatmap of representative metabolites involved in tryptophan catabolism, nucleotide metabolism, nitrogen metabolism, and redox regulation. Values represent log₂ FC relative to the ancestral strain. Values of 0 indicate metabolites that were either not significantly altered or were below the reliable detection threshold.

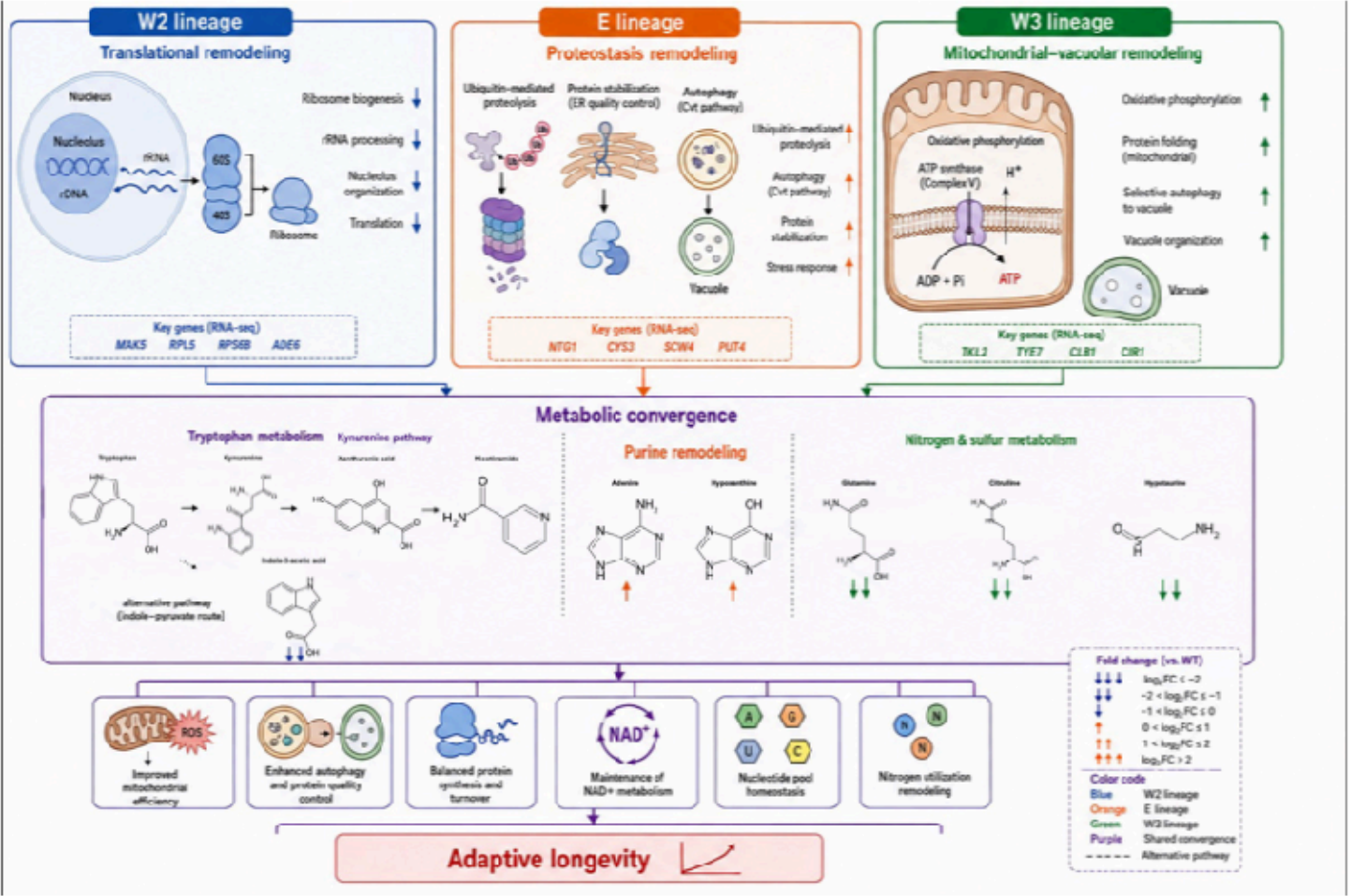


Figure 9

Hierarchical model of longevity adaptation under persistent high-glucose selection. Adaptive evolution under chronic glucose excess generates multiple molecular routes leading to lifespan extension. A conserved ribosome-associated remodeling program forms the shared adaptive foundation across all evolved strains. Above this common layer, distinct transcriptional trajectories emerge: W2 preferentially reallocates translational and biosynthetic resources; E enhances proteostasis and membrane homeostasis; and W3 extensively remodels central carbon metabolism and protein quality control. Despite these divergent transcriptional programs, all lineages converge at the metabolic level, particularly through remodeling of tryptophan-kynurenine metabolism and NAD⁺-associated nucleotide and redox networks. Schematic prepared by the authors.

Supplementary Files

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- [SupportingInformation.pdf](#)