Identification of Longevity Strains of *Saccharomyces cerevisiae* under High-Glucose Conditions

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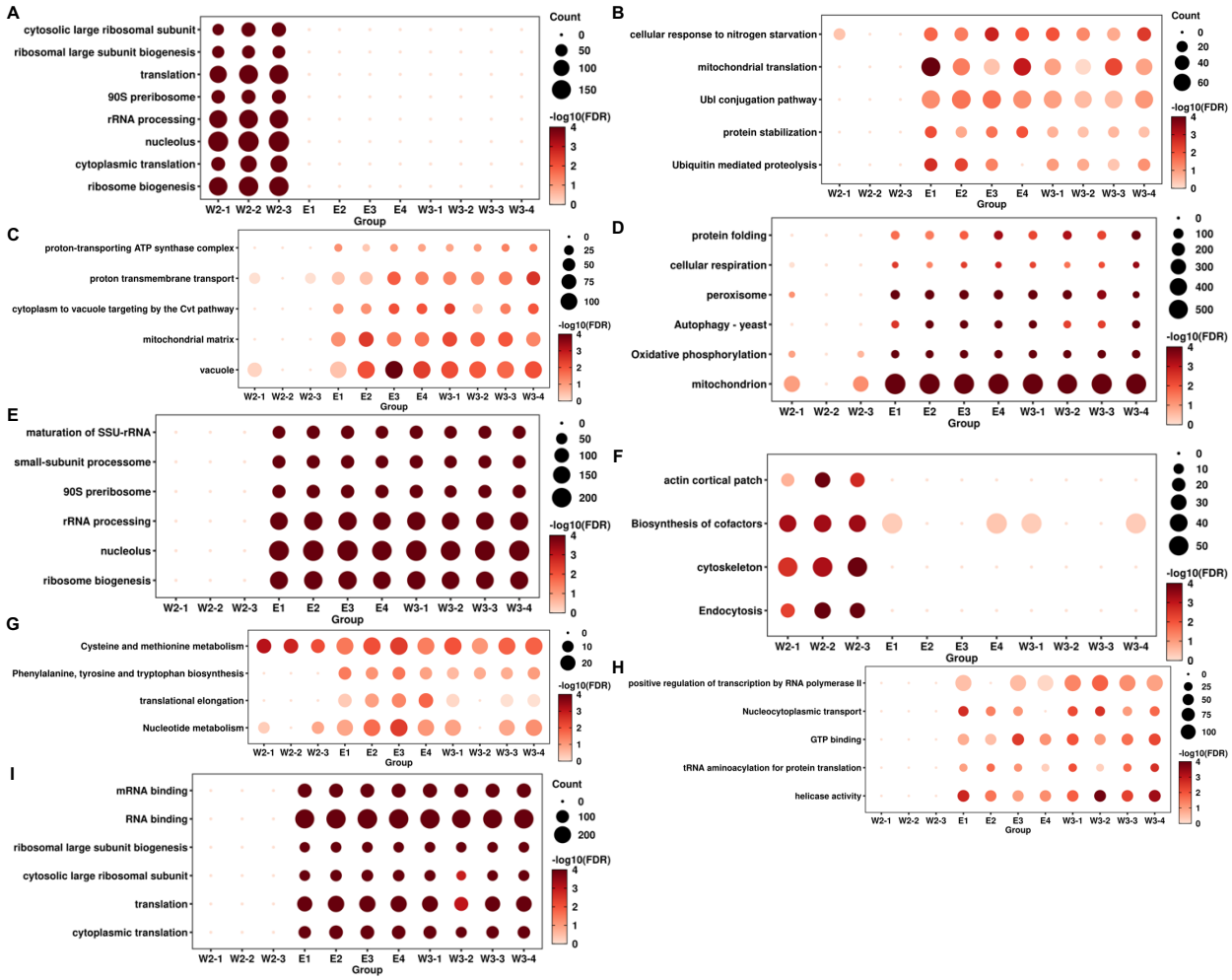
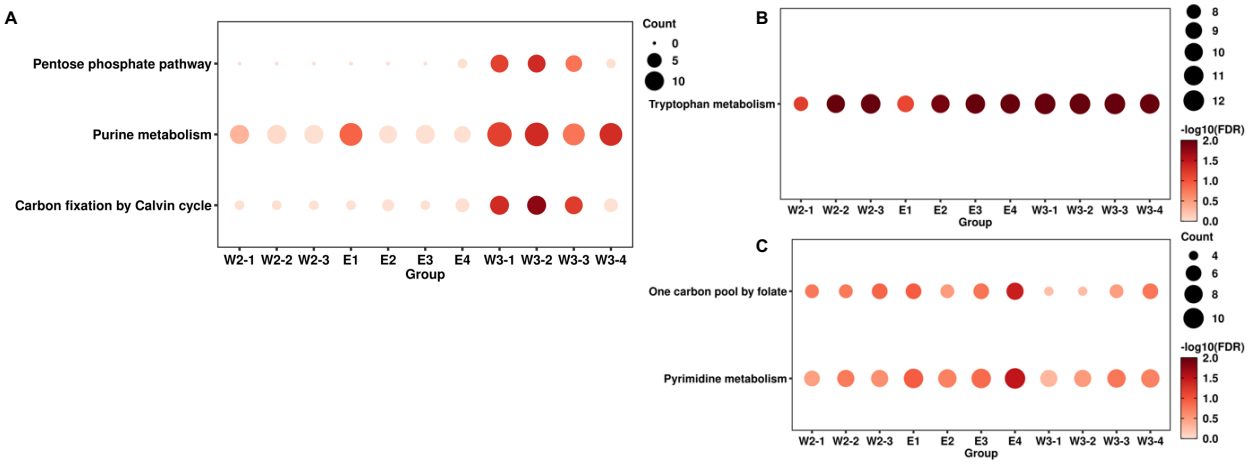
Supplementary Figures

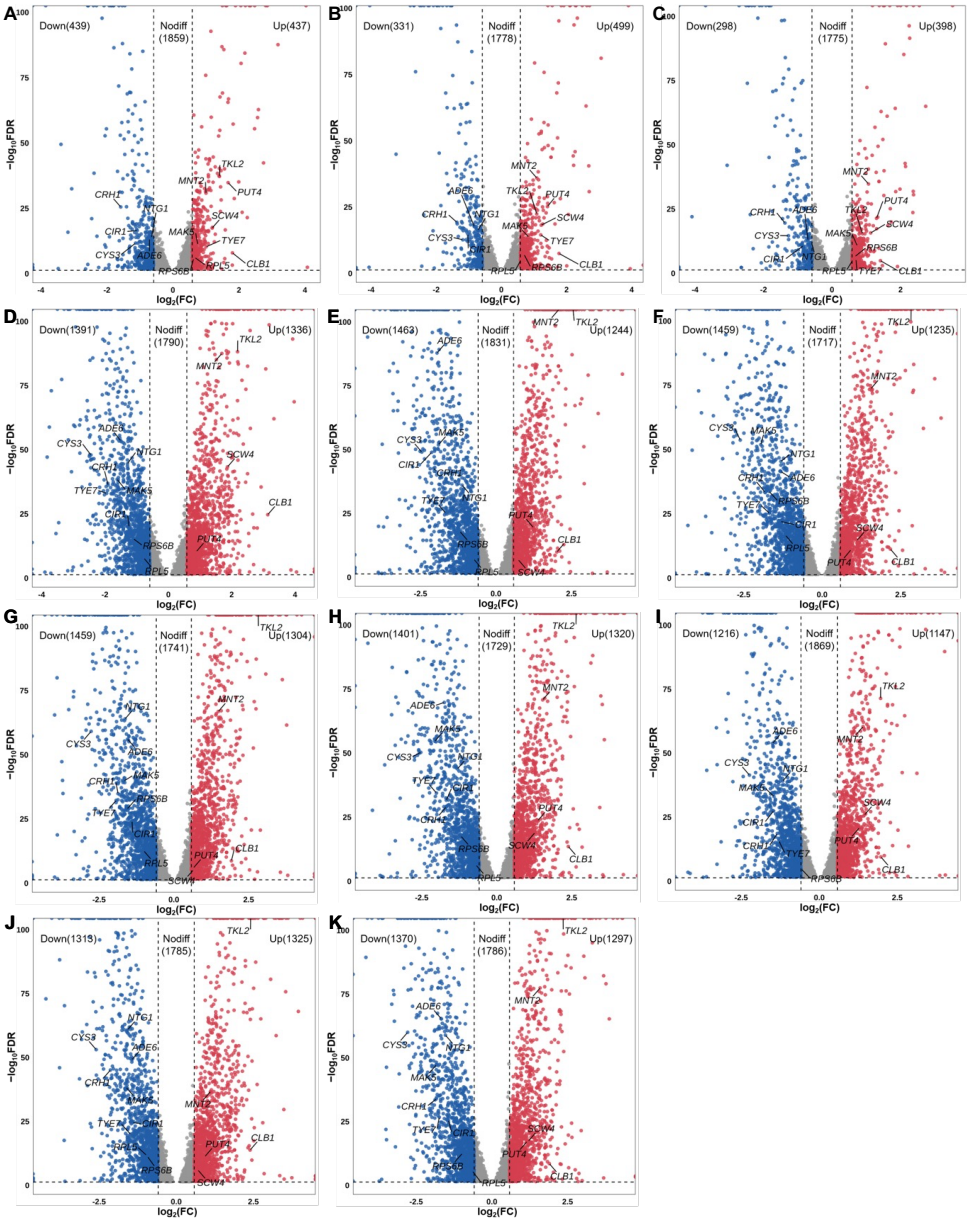


Supplementary Fig. S1. Direction-resolved transcriptomic pathway enrichment across evolved yeast strains. Transcriptomic enrichment analysis was performed using DAVID based on differentially expressed genes (DEGs) identified at adjusted $P < 0.05$. Bubble size represents the number of DEGs mapped to each pathway, and color indicates enrichment significance ($-\log_{10}$ FDR). A: upregulated W2-dominant transcriptional program characterized by ribosome biogenesis, cytoplasmic translation, rRNA processing and nucleolar functions; B: upregulated E-dominant transcriptional program enriched for mitochondrial translation, protein stabilization, ubiquitin-mediated proteolysis and cellular response to nitrogen starvation; C: upregulated W3-dominant transcriptional program highlighting vacuolar functions, mitochondrial matrix organization, proton transmembrane transport and ATP synthase complex assembly; D: upregulated E- and W3-shared transcriptional program enriched for mitochondrial functions, oxidative phosphorylation,

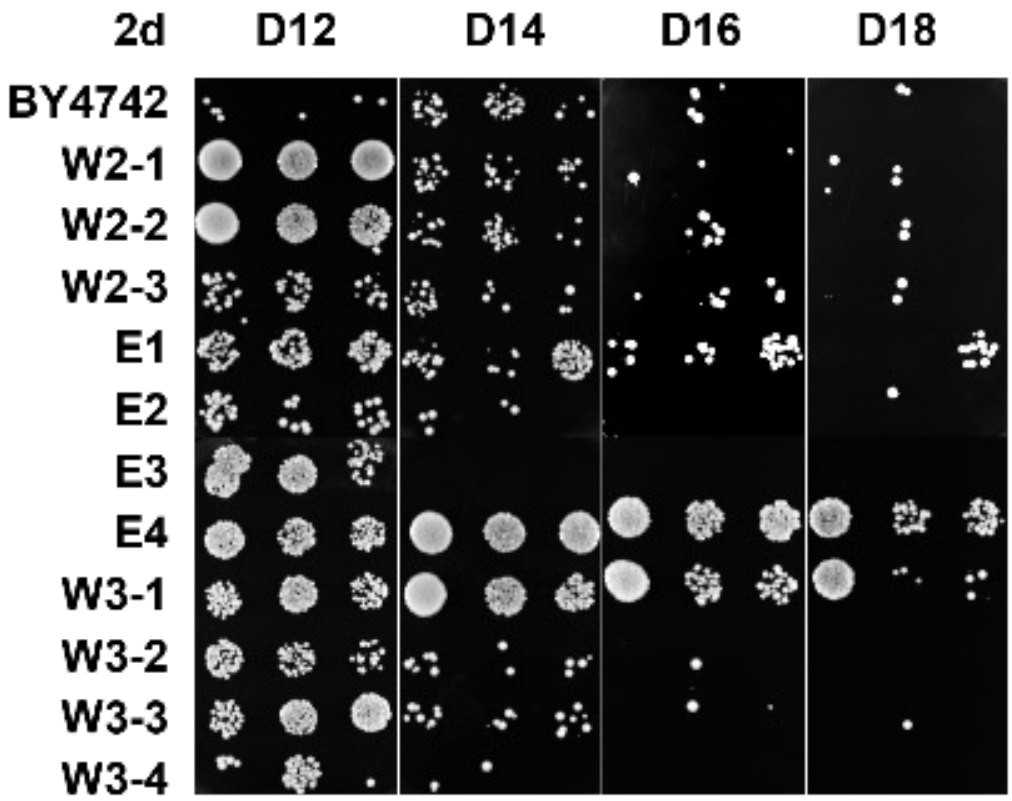
peroxisome, cellular respiration and protein folding; E: downregulated program shared across evolved strains, centered on ribosome biogenesis, nucleolar functions, rRNA processing, small-subunit processome assembly and SSU-rRNA maturation; F: downregulated W2-dominant transcriptional program characterized by endocytosis, cytoskeletal remodeling, cofactor biosynthesis and actin cortical patch organization; G: downregulated E-dominant transcriptional program enriched for nucleotide metabolism, translational elongation, and amino acid biosynthesis including phenylalanine, tyrosine, tryptophan, cysteine and methionine metabolism; H: downregulated W3-dominant transcriptional program highlighting helicase activity, tRNA aminoacylation, GTP binding, nucleocytoplasmic transport and transcriptional regulation by RNA polymerase II; I: downregulated E- and W3-shared transcriptional program enriched for cytoplasmic translation, ribosomal large subunit biogenesis, RNA binding and mRNA binding.



Supplementary Fig. S2. Direction-resolved metabolomic pathway enrichment across evolved yeast strains. Metabolomic enrichment analysis was performed using MetaboAnalyst based on differentially abundant metabolites identified at adjusted $P < 0.05$. Bubble size represents the number of metabolites mapped to each pathway, and color indicates enrichment significance ($-\log_{10}$FDR). A: upregulated W3-dominant metabolic program characterized by pentose phosphate pathway activation and purine metabolism, with additional enrichment of KEGG carbon fixation annotations attributable to overlapping enzymatic reactions rather than autotrophic metabolism; B: downregulated metabolic program shared across evolved strains, centered on tryptophan metabolism; C: downregulated E-dominant metabolic program enriched for pyrimidine metabolism and one-carbon pool by folate.



Supplementary Fig. S3. Transcriptomic volcano plots for all evolved strains. x-axis, $\log_2$FC; y-axis, $-\log_{10}$FDR; dashed lines indicate thresholds of $p < 0.05$ and $|\log_2 FC| > 0.58$. Red dots: significantly upregulated genes; blue dots: significantly downregulated genes; grey dots: non-significant. Labeled genes were consistently differentially expressed across multiple evolved strains. Strains shown in order: W2-1, W2-2, W2-3, E1, E2, E3, E4, W3-1, W3-2, W3-3, W3-4. Three biological replicates per batch.



Supplementary Fig. S4. Spot recovery after 2-day incubation following prolonged culture in extreme high-glucose medium (300 $g \cdot L^{-1}$). Representative replicates are shown. Together with Fig. 6B (1-day incubation), these data reflect the long-term culturability of evolved strains under extreme high-glucose conditions.