

Supplemental Material

Sugar accelerates chronological aging in yeast via ceramides

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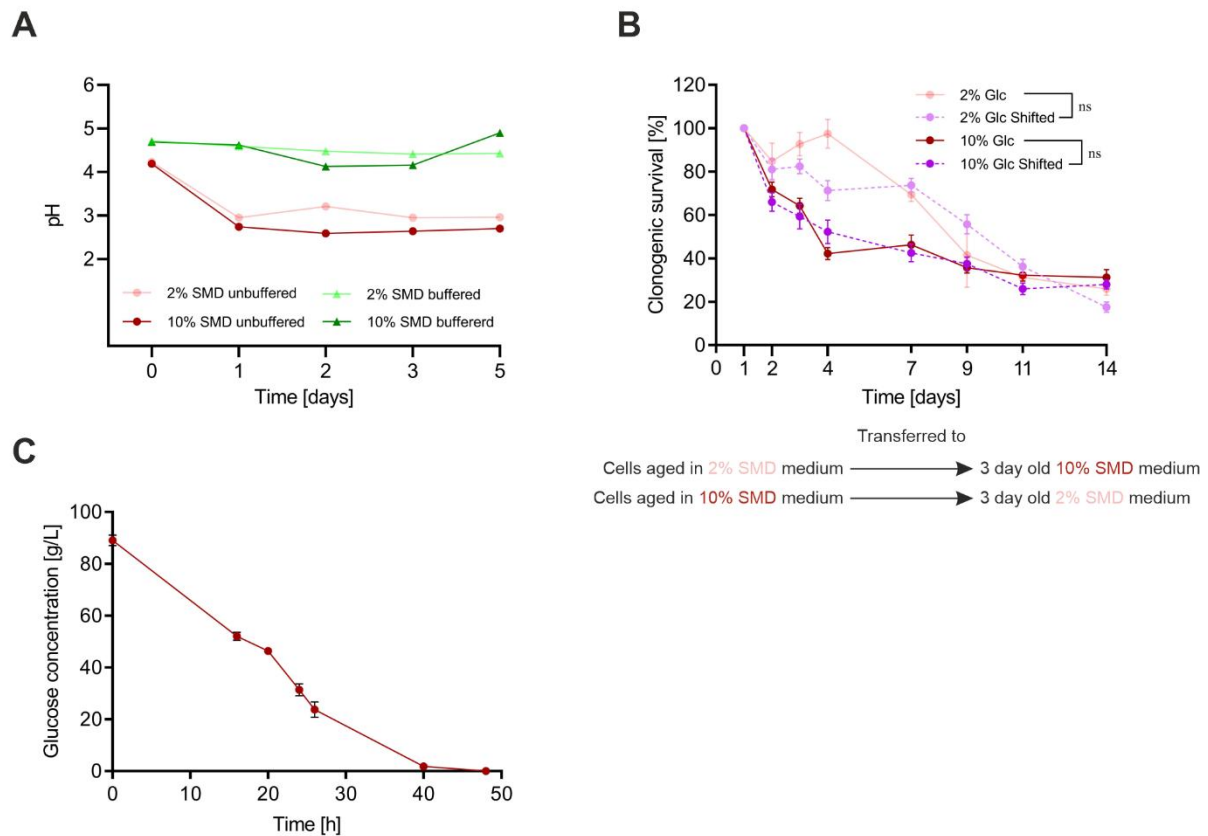
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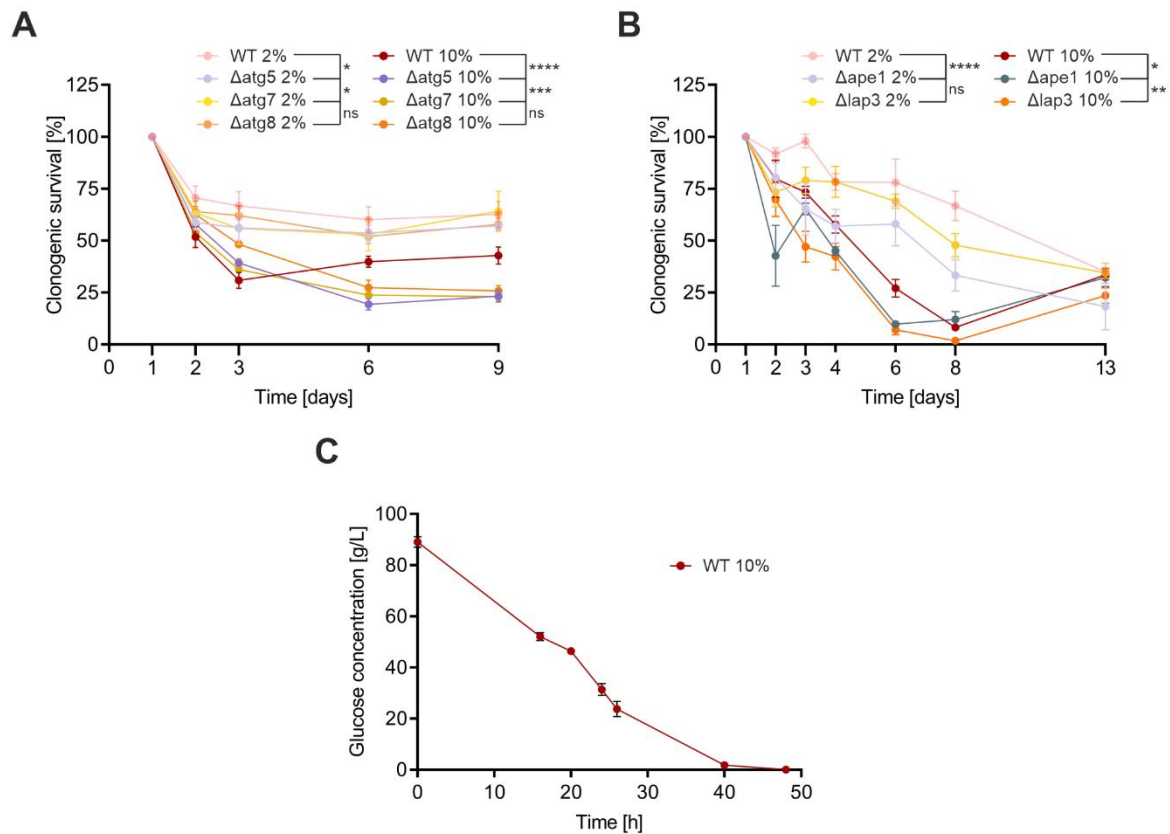
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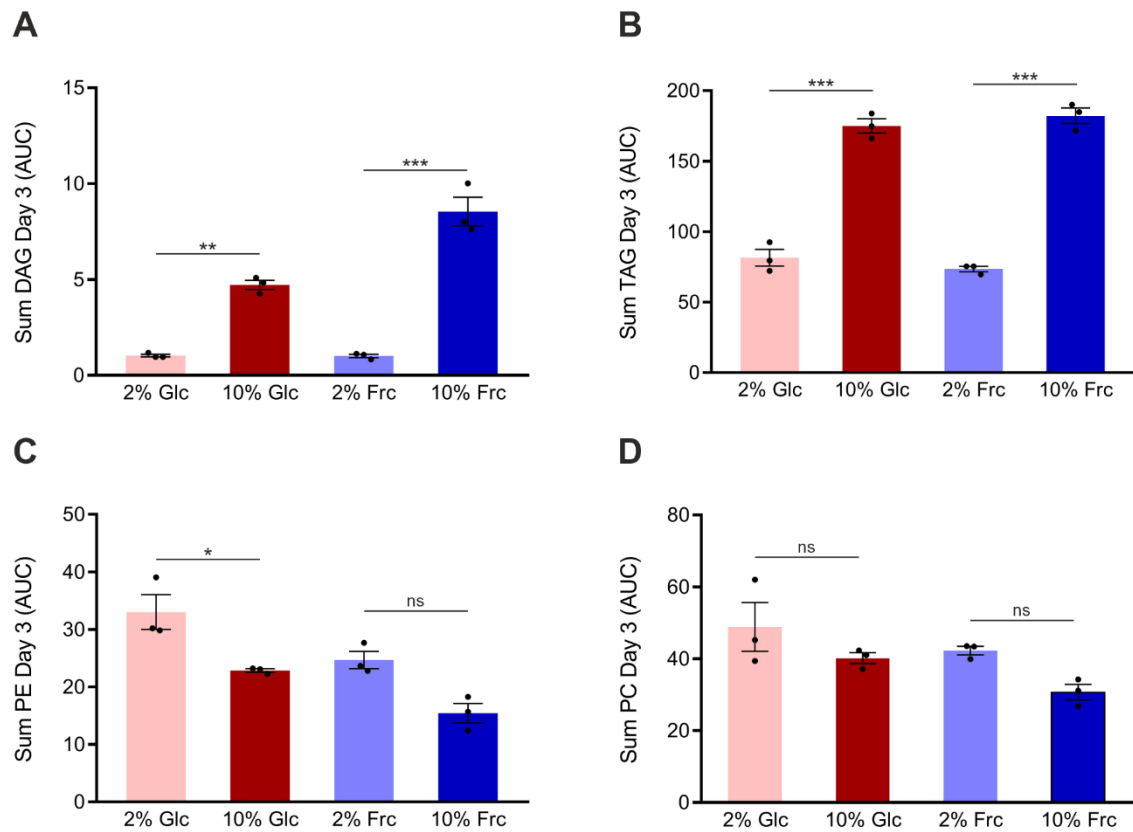
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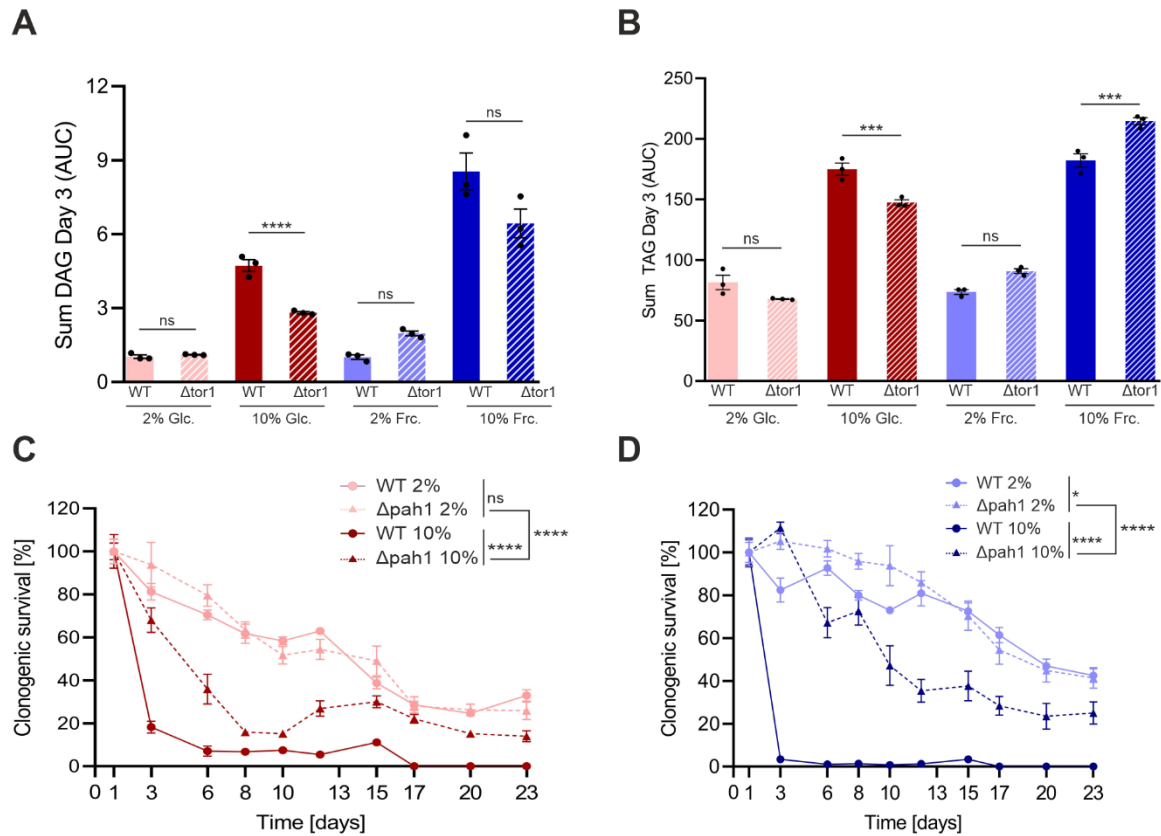
Supplemental FIGURE S1 ● (A) pH stabilization and osmolarity compensation. Overnight cultures of wild type cells (WT) were grown in SMD medium with 2% glucose. The main cultures were grown in buffered (i.e., addition of 25 mM sodium acetate buffer) and non-buffered SMD media over a time course of five days. Osmotic effects were prevented by according amounts of sorbitol supplementation (up to 8% sorbitol to gain same-level osmotic conditions in all different sugar concentrations used). In both media, the buffered samples stayed at a constant value of about 4.5, while in unbuffered conditions the pH dropped to 2.5. (B) A media switch between control and high glucose concentration does not influence survival during aging. Overnight cultures of WT cells grown in minimal complete medium with 2 % glucose, while the main culture was inoculated in minimal media with 10% and 2% glucose. After three days of chronological aging, cultures aged in 2% and 10% SMD were pelleted and the supernatants were exchanged, pellets resuspended and chronological aging resumed. Unshifted samples of both variants served as controls. Data represent the mean $\pm$ S.E.M. of surviving cells of 4 independent experiments. Two-way ANOVA with a Bonferroni post hoc test was performed to determine p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (C) Glucose in 10% SMD is taken up by wt cells within forty hours after inoculation. Overnight cultures of WT cells were grown in minimal complete media with 2% glucose, while the main culture was inoculated in minimal media with 10% glucose. The sugar concentration of the liquid medium was determined during a time course of forty-five hours. Data represent the mean of 3 independent experiments. Error bars indicate S.E.M.



Supplemental FIGURE S2 ● Evaluation of single-gene deletions of autophagy-related genes (A) and of essential genes of the cytoplasm-to-vacuole-targeting (CVT) pathway (B). Wild type (WT) and mutant strains were cultured in 2% and 10% SMD medium, respectively. 25 mM sodium acetate buffer 25mM sodium-acetate buffer was added to avoid pH effects. Osmotic effects were prevented by sorbitol supplementation. The herein showed deletion mutants of autophagy related genes (A) and essential genes of the Cvt pathway (B) do not rescue glucose-mediated cell death. (C) Overnight cultures of BY4741 *Δtor1* mutants were grown in SMD media with 2% glucose. The main culture was inoculated in synthetic media with 10% glucose. The sugar concentration of the liquid medium was determined during a time course of 45 hours. Graphs represent means of 3 independent experiments with error bars indicating $\pm$ S.E.M. Two-way ANOVA with a Bonferroni post hoc test was performed to determine p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplemental FIGURE S3 ● MS analysis of all measured lipid levels of yeast grown under high sugar levels shows alterations in DAG and TAG but not in PE and PC. The lipid content of 2% and 10% SMD/F-cultured wild type cells was analyzed by MS-analysis of 1×10^9 cells. AUCs were normalized to an internal standard. High-sugar cultures showed increased levels of DAG (A) and TAG (B), and showed overall a non-significant trend towards reduction in PE (phosphatidylethanolamine; (C)) and PC (phosphatidylcholine; (D)) content. Graphs represent means of 3 independent experiments with error bars indicating $\pm$ S.E.M. Multiple t test was performed to determine p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplemental FIGURE S4 ● DAG and TAG lipid levels of $\Delta tor1$ mutant strains as well as CLS of $pah1$ mutants cultured in 10% SMD and SMF. (A) DAG and (B) TAG profiles of 1×10^8 wild type (WT), $\Delta vac8$ and $\Delta tor1$ deletion strains cultured in 2% and 10% SMD/ SMF. (C, D) CLS of cells grown in 2% and 10% SMD (A), or SMF (B). WT and mutant strains $\Delta pah1$ were cultured in 2% and 10% SMD (C) or 2% and 10% SMF (D) media supplemented with 25 mM sodium-acetate buffer as well as sorbitol. Survival was determined by PI-staining and subsequent FACS analysis of 30,000 cells at indicated time points. Graphs represent PI-negative cells of means of 3 independent experiments with error bars indicating S.E.M. Two-way ANOVA with a Bonferroni post hoc test (A, B) or Tukey's multiple comparisons test (C, D) was performed to determine p values: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.