

Supplementary Figure S1

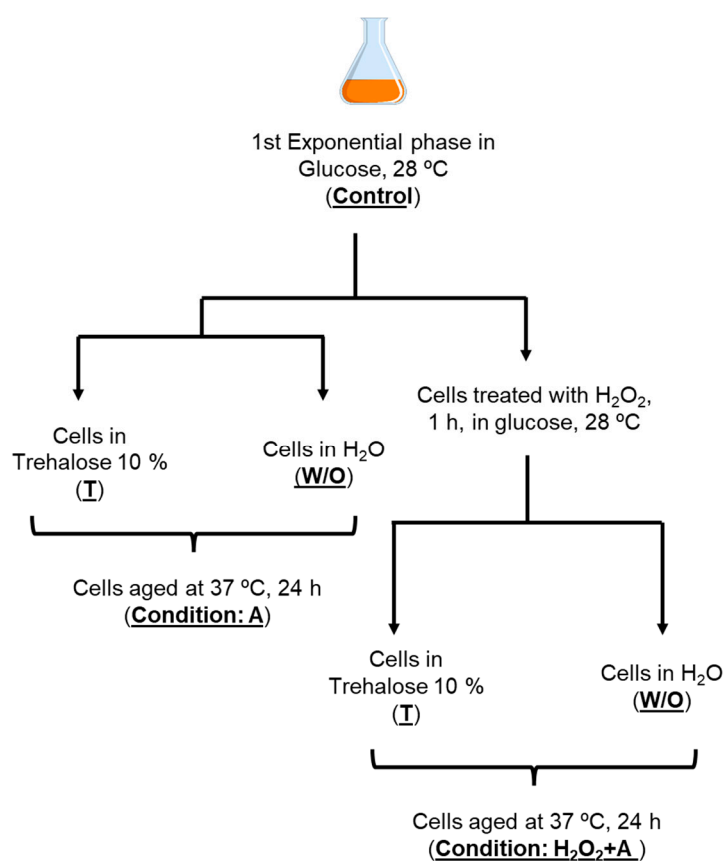


Figure S1. Experimental scheme of treatment with trehalose during chronological aging. Condition A: mid-exponential phase cells (control) were centrifuged at 5000 rpm/5 min, washed twice with sterile distilled water, and resuspended in the same volume of water or trehalose at 10 %. Cells were incubated at 37 °C/160 rpm for 24 h (aged cells). Condition H₂O₂ + A: mid-exponential phase cells (control) were also treated with H₂O₂ at 0.4 mM in glucose, at 28 °C / 160 rpm for 1 h, before chronological aging, as described above.

Supplementary Figure S2

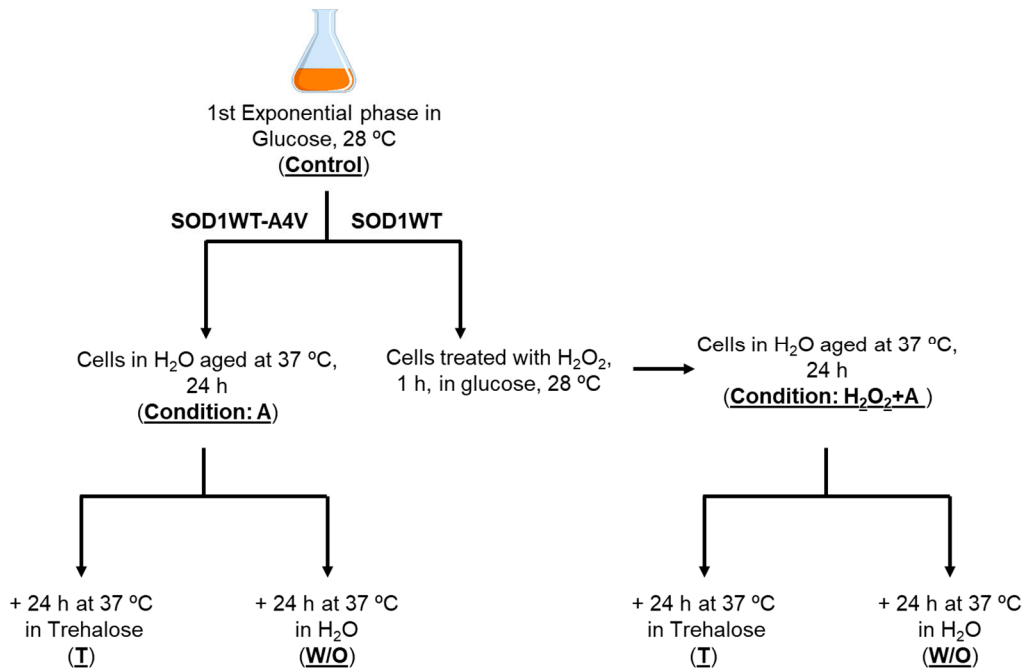


Figure S2. Experimental scheme of treatment with trehalose after chronological aging. SOD1WT-A4V cells: mid-exponential phase cells (control) were centrifuged at 5000 rpm/5 min, washed twice with sterile distilled water, resuspended in the same volume of water, and incubated at 37 °C/160 rpm for 24 h (aged cells). Aged cells were centrifuged at 5000 rpm/5 min, resuspended in the same volume of trehalose at 10 %, and incubated at 37 °C/160 rpm for 24 h. SOD1WT cells: to induce the formation of SOD1WT inclusion, mid-exponential phase cells (control) were treated with H₂O₂ at 0.4 mM in glucose at 28 °C / 160 rpm for 1 h. Treated cells were centrifuged at 5000 rpm/5 min, washed twice with sterile distilled water, resuspended in the same volume of water, and incubated at 37 °C/160 rpm for 24 h (aged cells). Aged cells were centrifuged at 5000 rpm/5 min, resuspended in the same volume of trehalose at 10 %, and incubated at 37 °C/160 rpm for 24 h.

Supplementary Figure S3

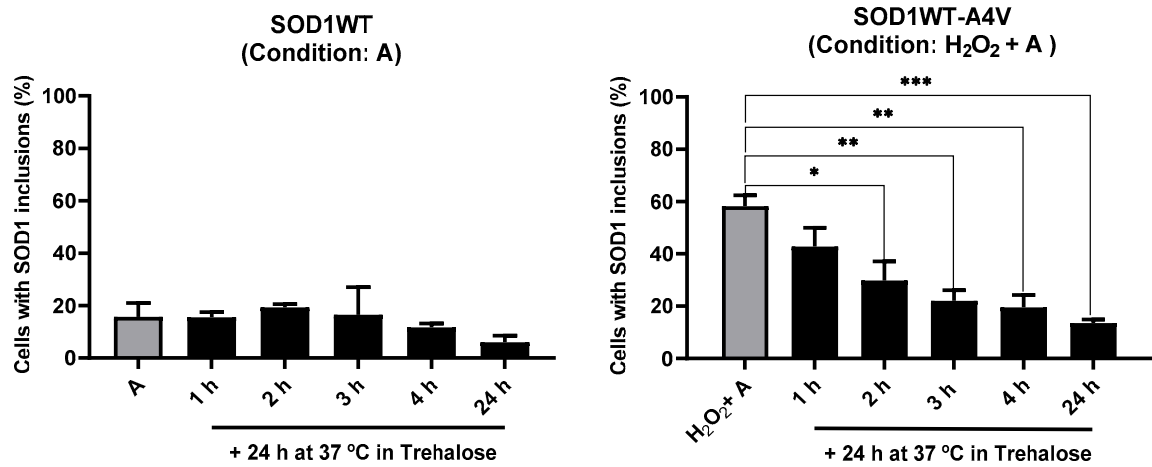


Figure S3. SOD1WT-A4V cells treated with trehalose after oxidative stress showed a decrease in SOD1 inclusions. Quantification of cells with SOD1 inclusion. SOD1WT or SOD1WT-A4V cells were submitted to oxidative stress through incubation in water (gray bar) at 37 °C for 24 hours (A = aging-like condition). For SOD1WT-A4V, cells were previously treated with 0.4 mM of H₂O₂ at 28 °C for 1 hour (H₂O₂ + A condition, gray bar). After aging, cells were reinoculated in trehalose (black bars) or maintained in water (white bars) at 37 °C for 24 hours. Images were taken in the first 4 hours and after 24 hours of incubation. One-way ANOVA with Tukey's test was used for statistical analysis to compare the difference between H₂O₂ + A (SOD1WT-A4V cells- gray bars) or A (SOD1WT cells-gray bars) with + 24 hours at 37 °C in trehalose (significant differences at * p<0.05, ** p< 0.01, *** p< 0.001).

Table S1–Number of viable cells used to obtain the survival rates.

The tables below show the average number of viable cells under each condition for the SOD1WT and SOD1WT-A4V strains. The results are shown as the mean of at least three independent experiments (biological replicates), and at least three plates were used in each experiment (technical replicates).

SOD1WT strain

a) Trehalose before aging.

Condition	Number of viable cells (X 10⁹). Cells that proliferated in solid media and generated a visible colony
Control	154
H ₂ O ₂ + A	74
T	80

b) Trehalose after aging.

Condition	Number of viable cells (X 10⁹). Cells that proliferated in solid media and generated a visible colony
Control	154
A+ H ₂ O ₂	92
W/O 24 h	40
T 24 h	77
W/O 120 h	2
T 120 h	22
W/O 1 week	0
T 1 week	15

SOD1WT-A4V strain.

Condition	Number of viable cells (X 10⁹). Cells that proliferated in solid media and generated a visible colony
Control	233
Aging	108
T	165
W/O (+ 24 h at 37 °C)	69
T (+ 24 h at 37 °C)	112