Supplementary materials: Loss of mitochondrial DNA accelerates the early-age decline in stress resistance during yeast replicative aging

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Table S1. Primers used to make and validate the *Δfob1* strain.

Number	Name	Sequence
1	Fob1-F	GGAGAACAATTTAACGATTGTGTGAGTGTGAATTTGTGCTGAG GATAACACGGATCCCCGGGTTAATTAA
2	Fob1-R	TTTTTTTTTCACCTATGGTGACTCCTCCTTTCATTCTATCCTACA TATTAGAATTCGAGCTCGTTTAAAC
3	Fob1-F-test	CGAGGTTTCCAGGAAGAGCT
4	Fob1-R-test	GTGGGGGTGAAGTTTAAATT

Table S2. Experimental stress conditions and treatment protocols. Summary of stress types, solvents, concentrations and exposure durations.

Stress type	**Stress agent**	**Stress conditions**	**Control conditions**	**Reference**
Sugar induced cell death (SICD)	Glucose (Helicon)	1 hour in MQ, then centrifuged and 1 hour in 100 mM	1 hour in MQ, then centrifuged and 1 more hour in fresh	(Valiakhmetov et al., 2019)

		glucose in MQ	MQ	
Acetic acid stress	Acetic acid (Chimmed)	1 hour in 180mM acetic acid in YPD	1 hour in YPD + 5.2 µL MQ	(Sorokin et al., 2014)
Heat Shock	Temperature	1 hour at 45^{o}C in YPD	1 hour on RT in YPD	(Zyrina et al., 2017)
Ethanol stress	Ethanol	18% (v/v), 1 hour in YPD	1 hour in YPD with 93 µL MQ	(Zyrina et al., 2017)
Osmotic stress (NaCl)	NaCl (Helicon)	30 minutes, 2.5M NaCl in YPD	30 minutes, in YPD with 227 µL MQ	(Sorokin et al., 2014)
Oxidative stress	H_2O_2 (Chimmed)	2 hours, 2mM H_2O_2 in PBS	2 hours, in PBS with 1.14 µL MQ	(Kireeva et al., 2021)
Oxidative stress	Menadione (Sigma Aldrich) 100mM	2 hours, 1 mM menadione in YPD	2 hours, in YPD with 5 µL DMSO	(Sorokin et al., 2014)
Surfactant stress	Sodium Dodecyl Sulphate (SDS, Amresco)	2 hours, 0.02% SDS in SC	2 hours, in SC with 1 µL MQ	(Kireeva et al., 2021)
Surfactant stress	Benzalkonium Chloride (BAC, Fluka)	2 hours, 60µM BAC in SC	2 hours, in SC with 1 µL MQ	(Kireeva et al., 2021)

Table S3. Fluorescent microscopy filter settings for imaging of AF488, Propidium iodide and calcofluor white.

In text	Name	Exciter filter, nm	Beam splitter, nm	Barrier filter, nm
AF488	U-MNG2	530-550	570	590
PI	U-MNIBA3	470-495	505	510-550
CW	U-MNU2	360-370	400	420

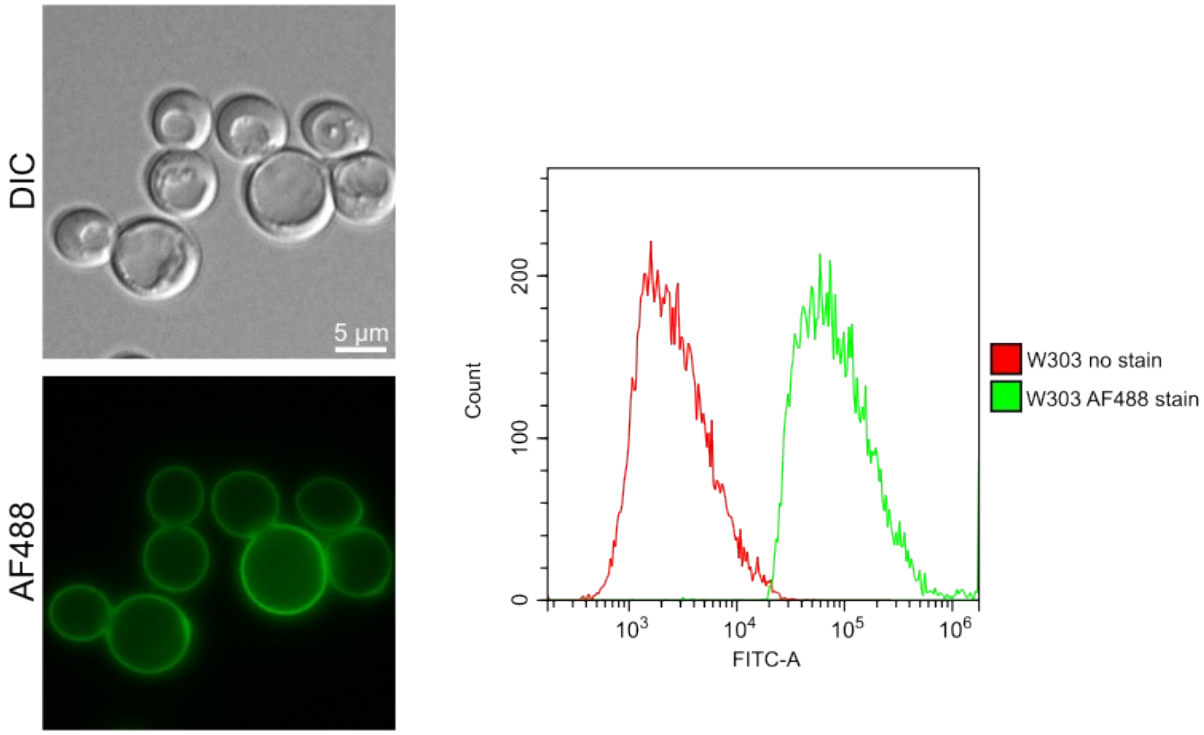

Fig. S1. AF488 binds to the surface of the yeast cells (left panel; differential interference contrast and AF488 fluorescence) and the staining enables distinguishing stained and unstained cells using flow-cytometry (right panel).

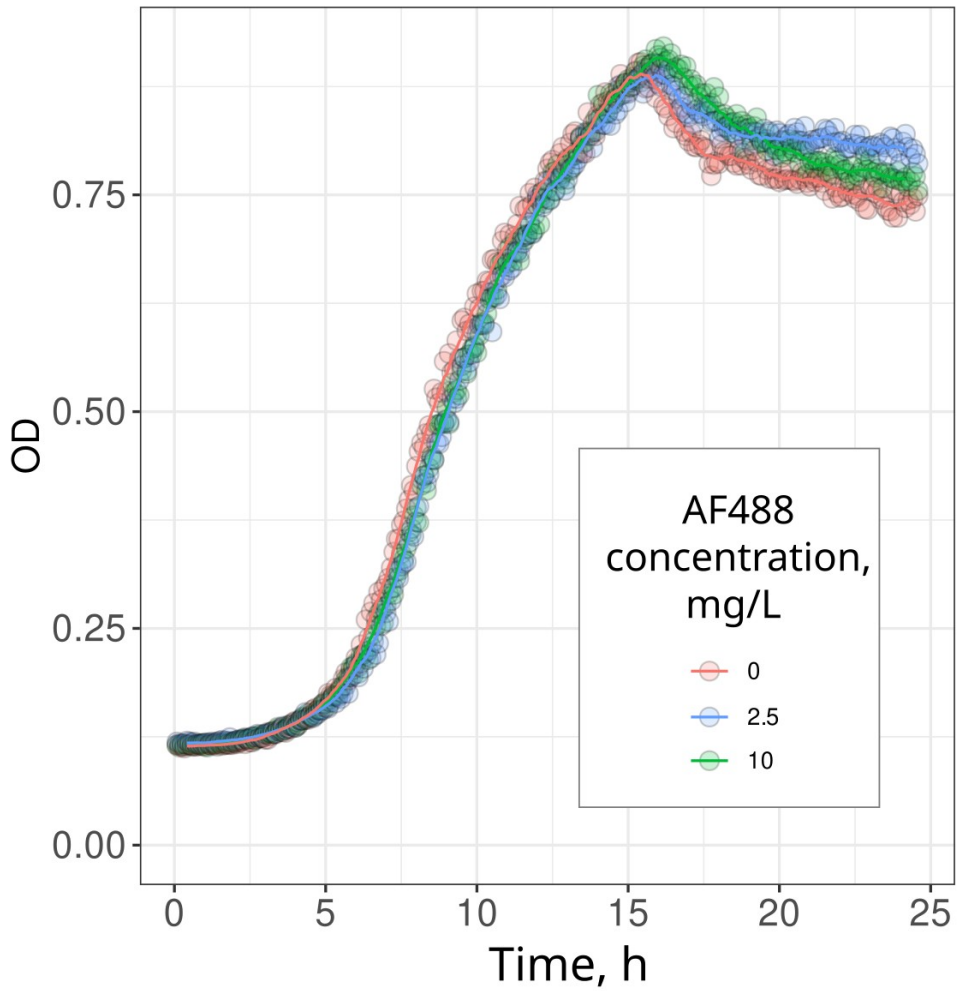

Fig.S2 AF488 does not inhibit yeast growth at concentrations up to 10 mg/L. The figure shows the increase in optical density (OD) of the wild-type yeast suspension in the presence of varying concentrations of the AF488 dye

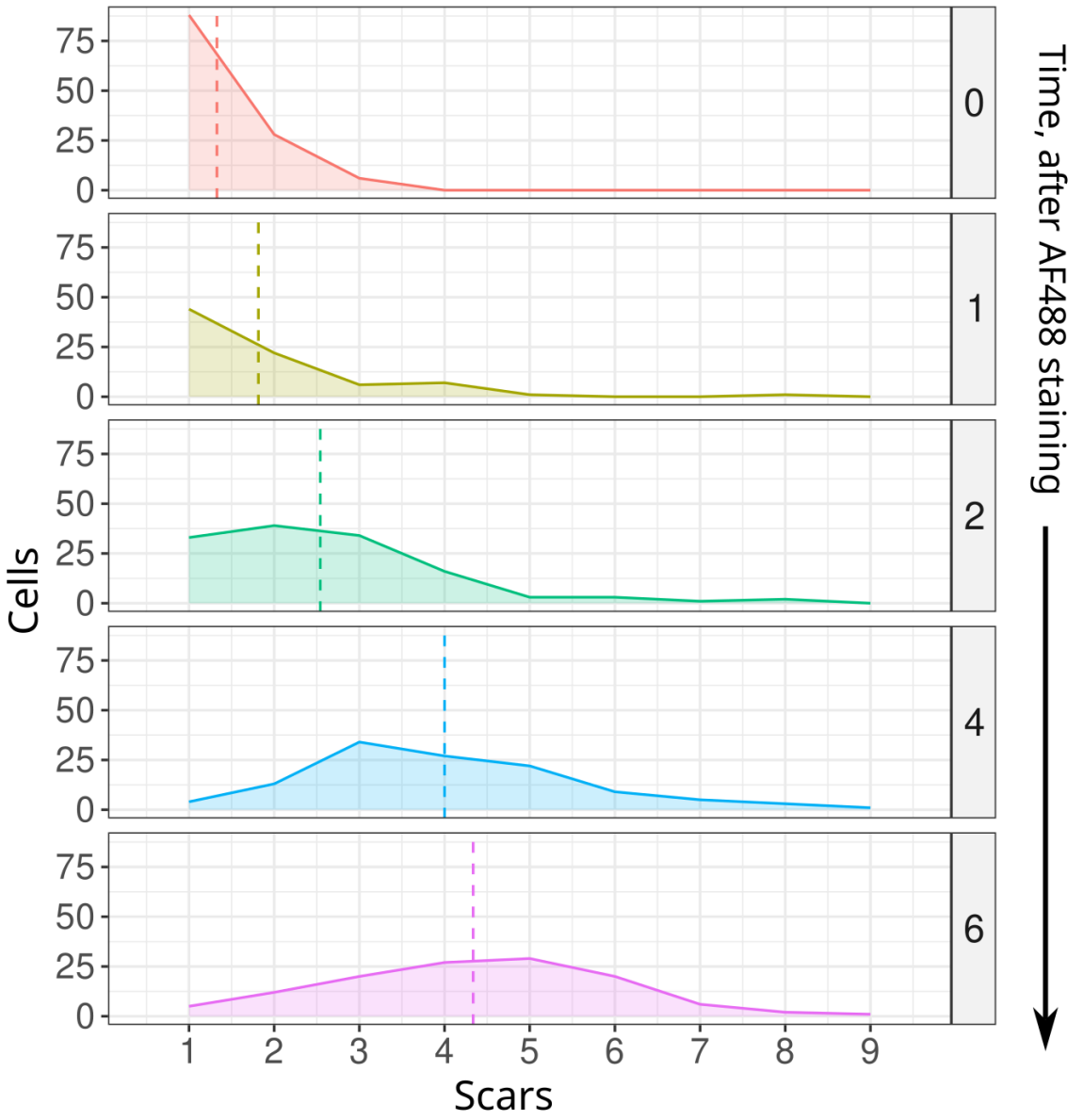

Fig. S3 (related to Fig.2) Distribution of replicative ages in AF488-negative cells (indicated as Time = 0) and AF488-positive cells after incubation for the indicated durations.

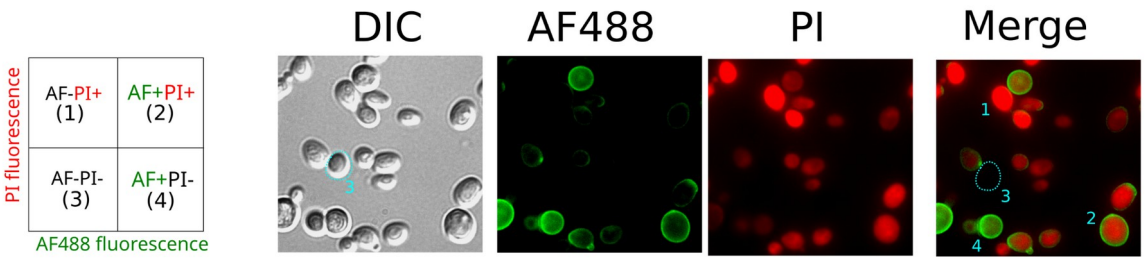

Fig.S4 (Related to Fig. 3) Fluorescence images of yeast cells pulse-chased with AF488 and stained with PI following acetic acid stress (incubation time: 4 hours). The numbers indicate

the four cell types that appear as distinct populations in Figure 3 (see the scheme/legend on the left).

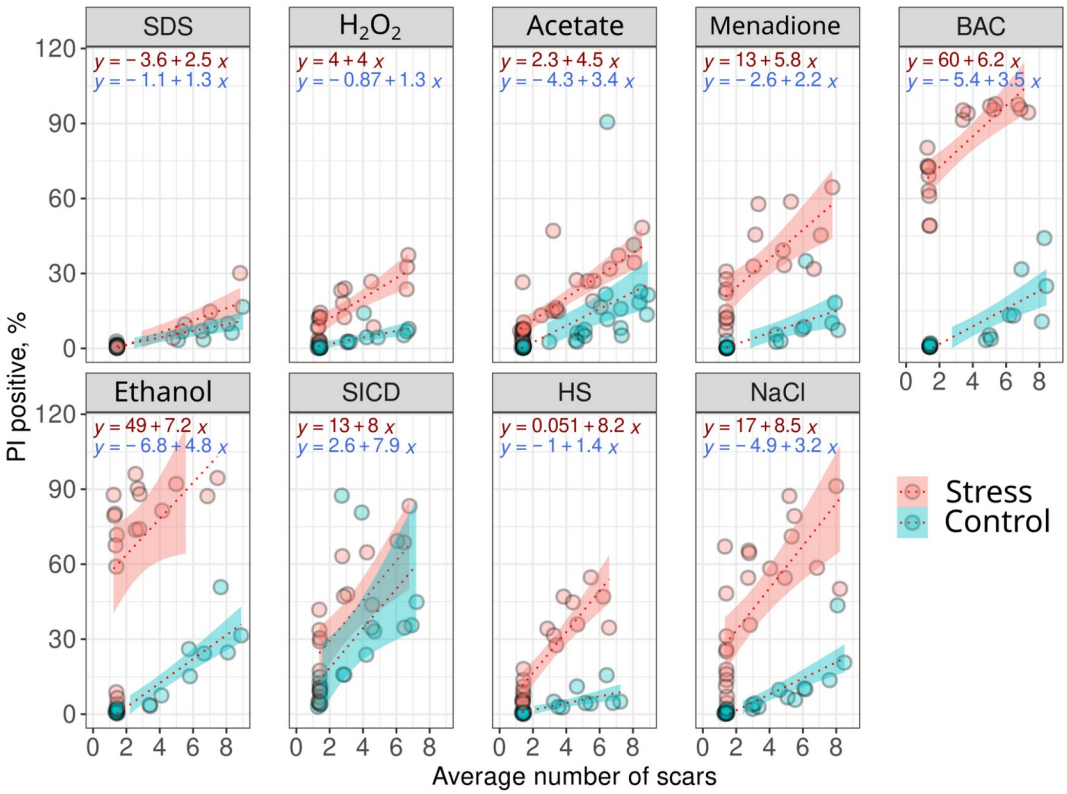

Fig. S5 (related to Fig.4) Age-dependent mortality of *Rho*$^+$ yeast cells under stress and in control conditions. Proportion of dead cells as a function of replicative age. Linear regression coefficients for the stress (red) and control (blue) conditions are shown in the top panel of each facet. Stress conditions and incubation times are provided in Table S2. Linear regression line (red, dashed) with shaded 95% confidence interval is shown for all conditions. Data points represent individual cell cohorts with calculated average age and percentage of propidium positive cells. Note, that Stress data are the same as in Fig. 4.

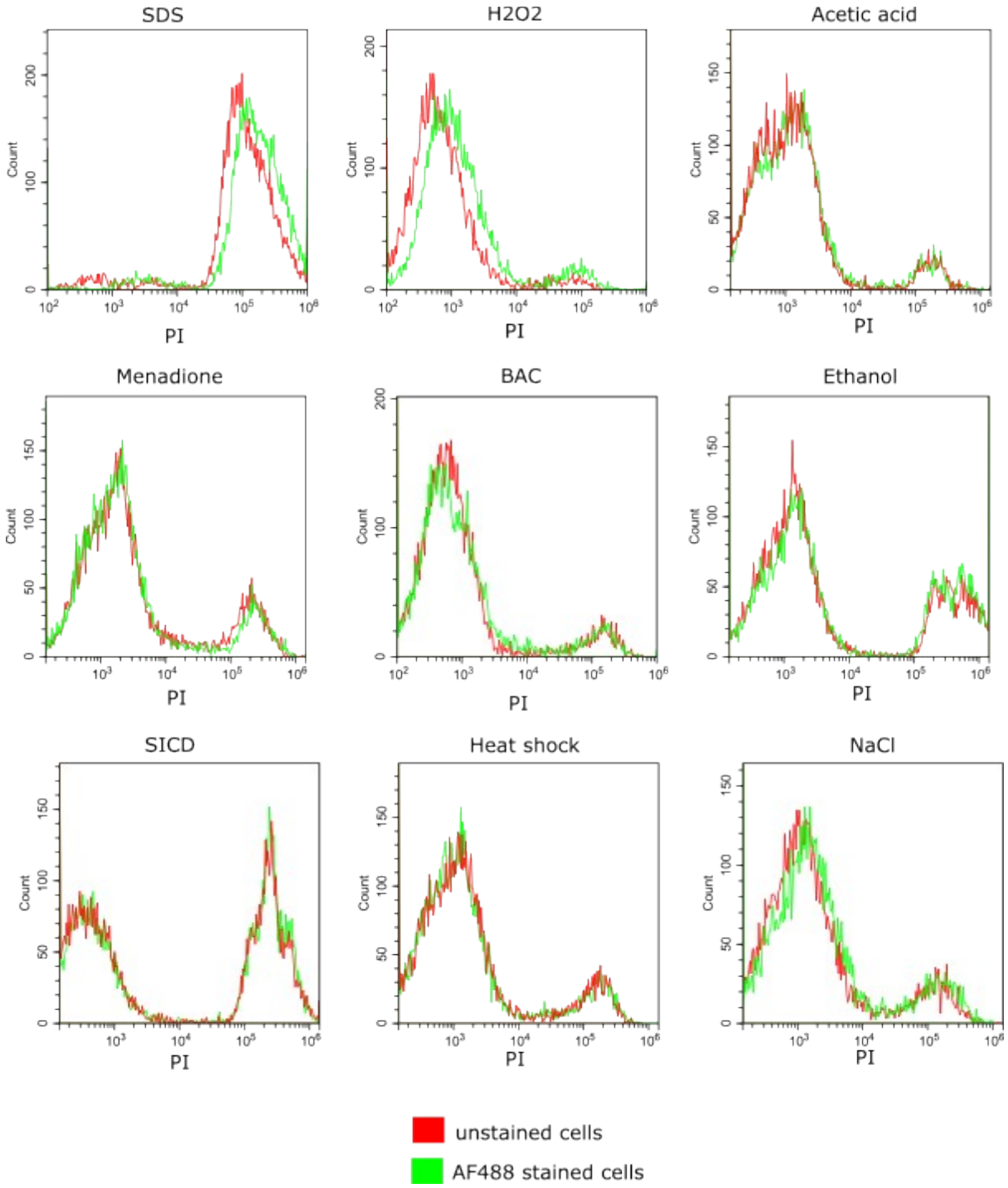

Fig. S6 AF488 staining does not affect yeast sensitivity to stress. AF488-stained and unstained cells were subjected to various stress conditions, followed by propidium iodide (PI) staining and flow cytometric analysis. PI fluorescence distributions are shown for unstained (red) and AF488-stained (green) cells treated with SDS, H_2O_2, acetic acid, menadione, BAC, ethanol, SCID, heat shock, and NaCl (see Table S2 for detailed treatment protocols)

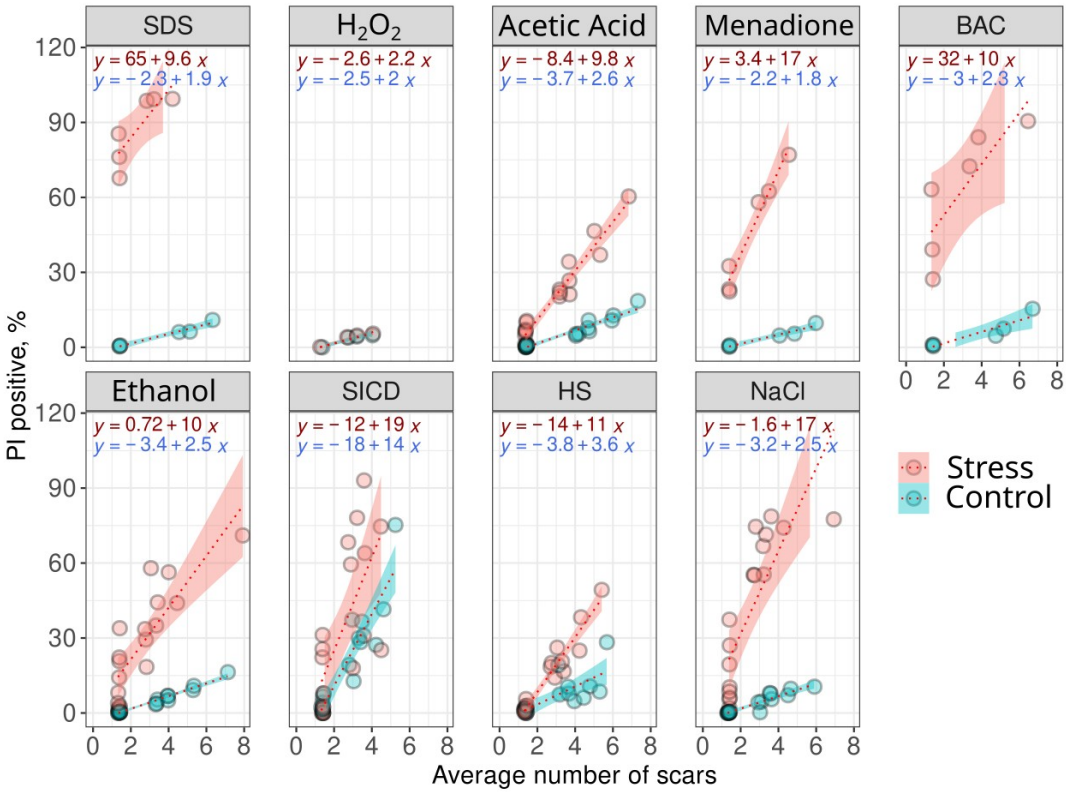

Figure S7 (related to Figure 4). Age-dependent mortality of *Rho*^{0} yeast cells under stress and in control conditions. Proportion of dead cells as a function of replicative age. Linear regression coefficients for the stress (red) and control (blue) conditions are shown in the top panel of each facet. Stress conditions and incubation times are provided in Table S2. Linear regression line (red, dashed) with shaded 95% confidence interval is shown for all conditions. Data points represent individual cell cohorts with calculated average age and percentage of propidium positive cells. Note, that WT control data are the same as in Fig. 4.

References

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