

Supplementary material

Iron supplementation delays aging and extends cellular lifespan through potentiation of mitochondrial function

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Keywords

Iron; Chronological aging; Cellular lifespan extension; Mitochondria; AMPK;
Saccharomyces cerevisiae

Table S1. List of primers used for RT-PCR in this study.

Primer	Sequence (5' -> 3')	Gene	Primer direction
MA71	GAAGTGTGATGTGCGATGTCC	ACT1	Forward
MA72	TCTTTCTGGAGGAGCAATG	ACT1	Reverse
MA89	TTCTGGTTTAGAAATGGCAC	GDH1	Forward
MA90	TTGACCAAAGATGGCAAG	GDH1	Reverse
MA101	GTTGGTCTCCACCATTTATG	CIT1	Forward
MA102	TGGCAACACCAAACAATAC	CIT1	Reverse
MA144 b	TGGGTGATTTGAACAAAAAG	PYC1	Forward
MA145	GTGCACCAATGTGTAATGG	PYC1	Reverse
MA146	ATGGCTCAGCGTAAGTTTG	CIT2	Forward
MA147	GACACCAGAGTGAGCATCC	CIT2	Reverse
MA148	TGCTTTGGAACCAAGATTC	ACO1	Forward
MA149	TATCGATTCTGTCATCAGGG	ACO1	Reverse
MA152	GATGGAACGGAAGTGCTAC	SDH2	Forward
MA153	AACGGTAGGCTTGCATTAG	SDH2	Reverse
MA202	GACAGGAGATGTTTGACCG	AAT1	Forward
MA203	CGACAATCTACCATCACCTG	AAT1	Reverse
MA204	GGGATGTTCTCCTTTACAGG	AAT2	Forward
MA205	GCACCACTTCATCAATGG	AAT2	Reverse
MA206	TTTGCCAACGAATCTGAC	ACO2	Forward
MA207	TTTGCCTTGATGGTGAAC	ACO2	Reverse
MA210	TCATGATCGCCAATCTATTG	FUM1	Forward
MA211	GGTTCAAAGCGGTGACTAAC	FUM1	Reverse
MA212	TCAAACCAATTGTCCTTAC	GDH2	Forward
MA213	TTTCCAATACGGAAGTGG	GDH2	Reverse
MA214	TATCCGGTCTGGAAATGG	GDH3	Forward
MA215	AATGATGGCAAGGTGTTTG	GDH3	Reverse
MA220	CGGTACCATCTTAGGCAAC	IDH1	Forward
MA221	TCATGGCAGTTGGGTTAG	IDH1	Reverse
MA222	TATTGCCGGTCAAGATAAAG	IDH2	Forward
MA223	GCCAAGTCACCTGTTCTG	IDH2	Reverse
MA224	GTACGAAAGAGGAAACCAAGAG	KGD1	Forward
MA225	TTAATGAATCACGTAGCTGAGC	KGD1	Reverse

MA226	TCAATTCACCACAAACAGC	KGD2	Forward
MA227	CAGTCTTCAAGAAGGTAACAGC	KGD2	Reverse
MA228	CGAAGCTGCTCAATTTCTC	LSC1	Forward
MA229	TTTGGATTCTGCATCAGTTC	LSC1	Reverse
MA230	ATTCCTGGAGGGACTTATCAC	LSC2	Forward
MA231	GATCGCCTCCATTTAATTTG	LSC2	Reverse
MA232	ACCATCACCAACACCTCTG	MAE1	Forward
MA233	TGACCCACTGTAAACATTCG	MAE1	Reverse
MA234	TCGAATTCTTTGCATCTCC	MDH1	Forward
MA235	AGCAACAAAGTTGACACCC	MDH1	Reverse
MA236	TGATGTCCACGATACTCACC	PYC2	Forward
MA237	ATCTGCTGGTGAAGAGACAAC	PYC2	Reverse
MA238	TGATGATGTGAAGACGACC	SDH1	Forward
MA239	AATCCTCTCTTGCATGAGC	SDH1	Reverse
MA242	CCTACAACCAGGATACCGTC	SOD2	Forward
MA243	CATCGAATCTTCTGGATGC	SOD2	Reverse
MA296	TGCTATCGGCGATTGTAC	NDE1	Forward
MA297	CTTCTGAATCGTCTTTGGC	NDE1	Reverse
MA298	GATTTAGGTGCCTTAGCATACC	NDI1	Forward
MA299	CCTTTAATCTCGATCTTGCAG	NDI1	Reverse
MA300	AACCCTTACTTCCCAGGTG	CYT1	Forward
MA301	TTTCTTTCGTCATGTTCCAGG	CYT1	Reverse
MA302	GAGTCAAAGACCCTCAATGG	RIP1	Forward
MA303	GCAGGTCCCTTTCTGATTC	RIP1	Reverse
MA304	TGATTTAGTTCCTGCTCCTG	COX6	Forward
MA305	CATCCTTCAATTCATCCAAG	COX6	Reverse
MA306	AAATCTTCCAATCTTCCAC	COX7	Forward
MA307	CCTTCTTGGCTTTGATAC	COX7	Reverse
MA308	GCCTCCAACAATTACAAGTG	ATP7	Forward
MA309	GTCGTCAACAGTTAATTCATCG	ATP7	Reverse
MA310	TCAAGGCTAACACTAGATTGG	ATP17	Forward
MA311	CTCTTCCGCACCTTTATG	ATP17	Reverse

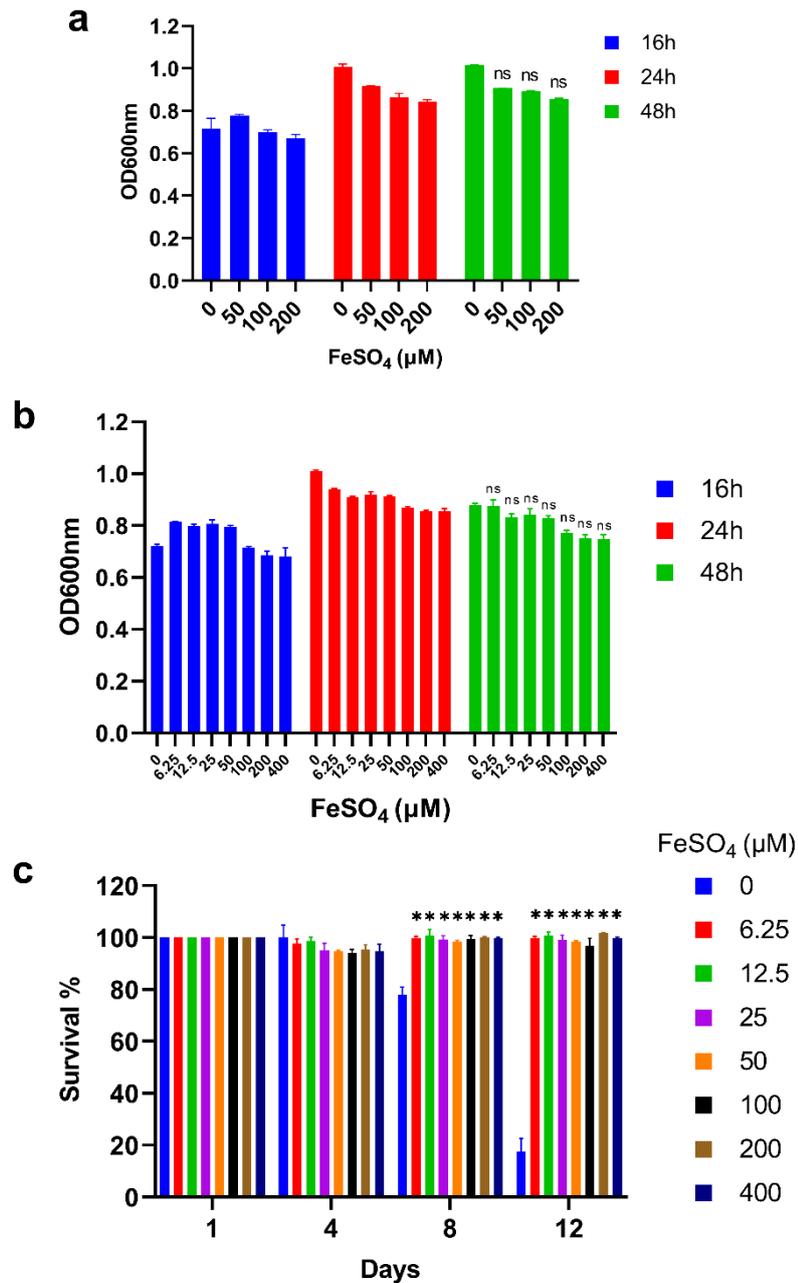


Figure S1. Growth and chronological lifespan analysis of iron supplemented yeast cells. (a) Cell growth analysis of experiments discussed in fig. 1. Cell growth OD600nm was measured at time points 16 h, 24 h, and 48 h using a microplate reader. (b) Growth of cells incubated with different concentrations of FeSO₄. (c) CLS of cells supplemented with different concentrations of FeSO₄. Statistical significance ($*p < 0.05$) was determined by Student's t-test.

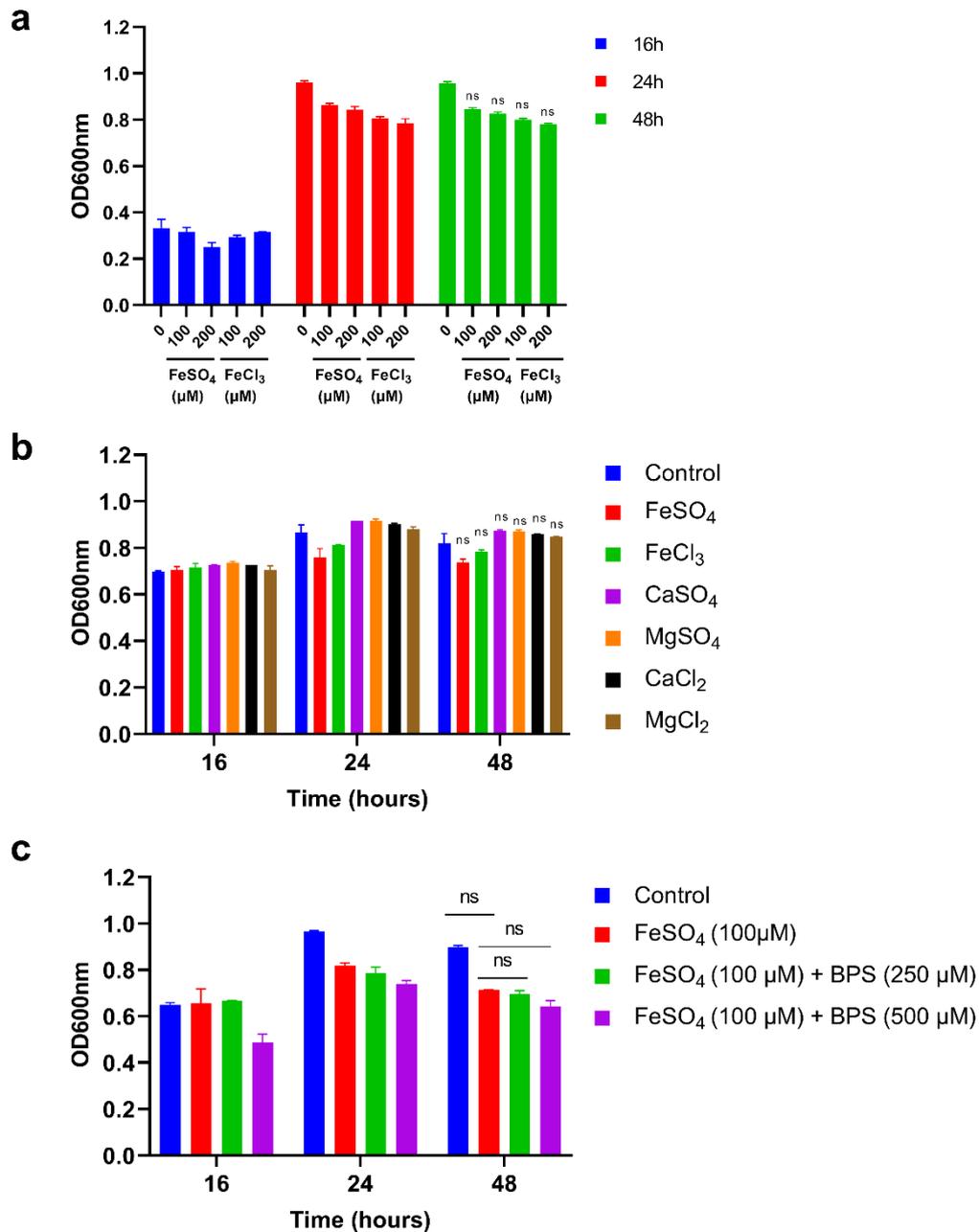


Figure S2. Growth analysis of different iron, sulfate and chloride-containing salts supplemented yeast cells. Cell growth analysis of experiments discussed in fig. 1b, 1c and 1d. Cell growth OD600nm was measured at time points 16 h, 24 h, and 48 h using a microplate reader. (a) Growth of cells incubated with different concentrations of FeSO₄ and FeCl₃. (b) Growth of cells incubated with 100 μM of FeSO₄, FeCl₃, CaSO₄, MgSO₄, CaCl₂, and MgCl₂. (c) Growth of cells incubated with 100 μM of FeSO₄ in the presence of different concentrations of BPS. Statistical significance ($*p < 0.05$) was determined by Student's t-test.

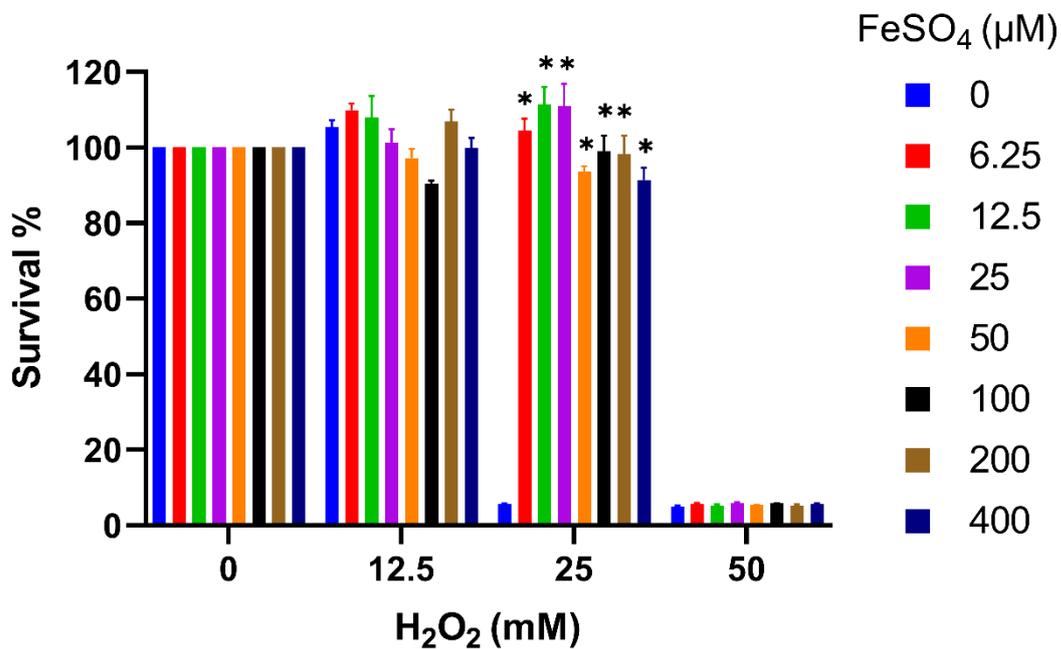


Figure S3. Oxidative stress resistance analysis of iron supplemented yeast cells. The prototrophic yeast strain was incubated with different concentrations of FeSO₄ and grown to stationary phase stage in SD medium for 72 h at 30 °C. After that, cells were diluted to OD_{600nm} ~0.2 in YPD medium with different concentrations of H₂O₂ and grew at 30 °C. Oxidative stress phenotype was analyzed by comparing the cell growth of H₂O₂ treated cells with non-treated control. Statistical significance (**p* < 0.05) was determined by Student's t-test.

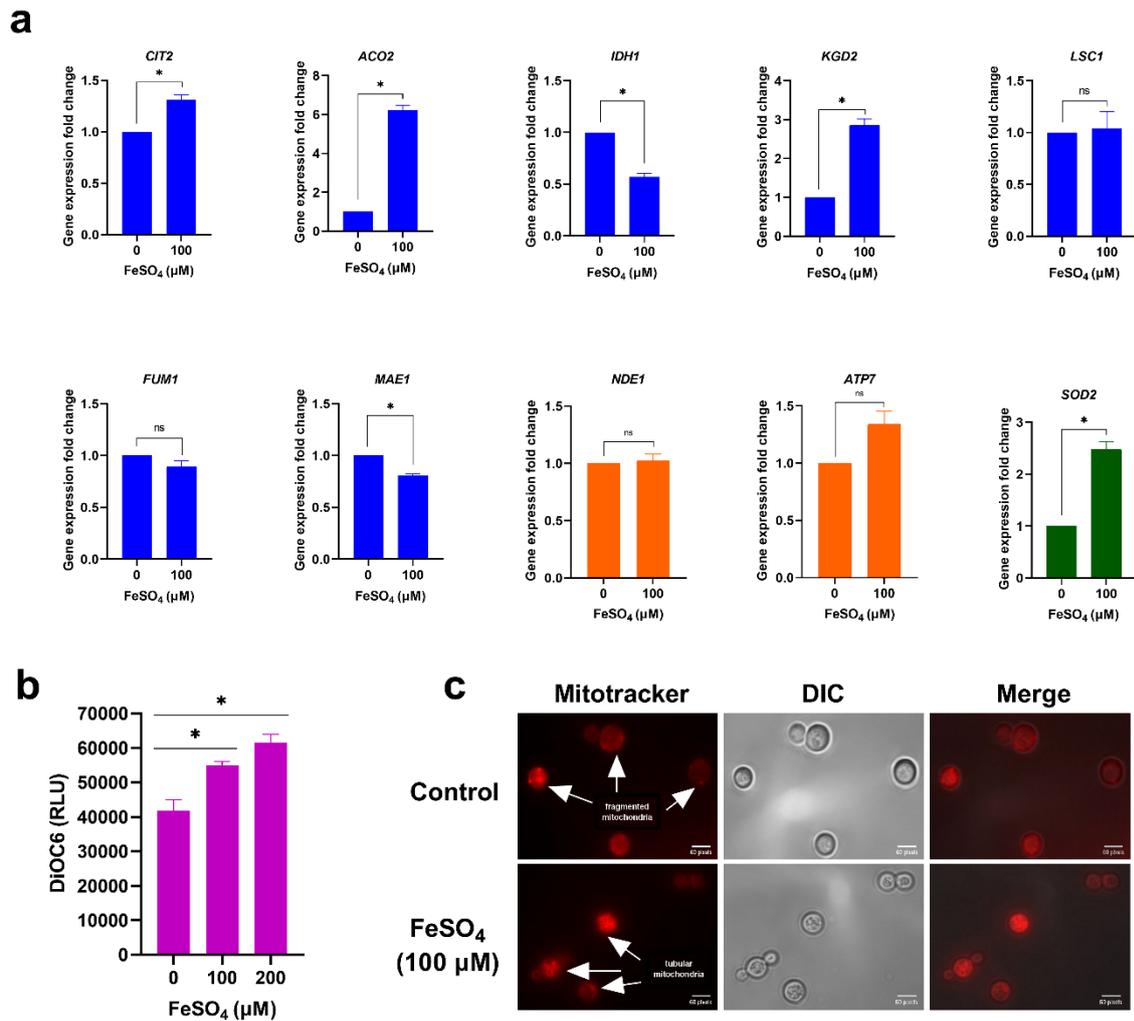


Figure S4. Mitochondrial gene expression, membrane potential and structure analysis of iron supplemented yeast cells. (a) Gene expression analysis of experiments discussed in fig. 3. Quantitative RT-PCR analysis of TCA genes, ETC genes (*NDE1* and *ATP7*), and *SOD2* gene. (b) Determination of mitochondrial membrane potential of cells incubated with indicated concentrations of FeSO₄. The yeast strain was incubated with different concentrations of FeSO₄ and grown to stationary phase stage in SD medium for 72 h at 30 °C. Samples were stained with DiOC6(3) and fluorescence reading (excitation at 482nm, emission at 504nm) were measured by the microplate reader. (c) Mitochondrial structure analysis of control and iron supplemented cells. Cells were stained with MitoTracker™ Deep Red and samples were visualized with fluorescence microscope at 100x magnification. The fluorescence intensity of each sample was normalized with OD600nm. Statistical significance (* $p < 0.05$) was determined by Student's t-test.

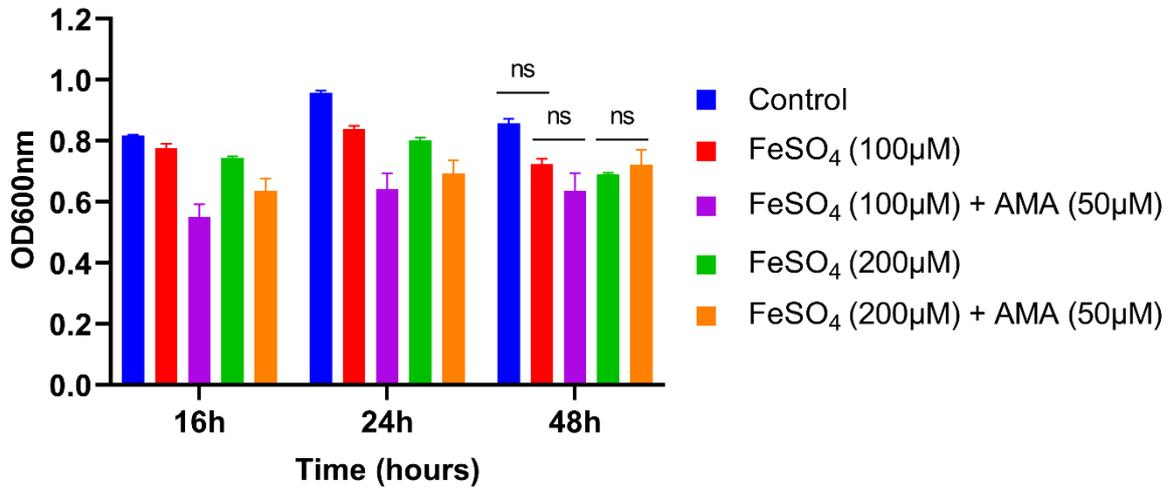


Figure S5. Growth analysis of iron and antimycin A supplemented yeast cells. Cell growth analysis of experiments discussed in fig. 4. Growth of cells (OD600nm) incubated with varying concentrations of FeSO₄ and 50 µM antimycin A (AMA) was measured at time points 16 h, 24 h, and 48 h using a microplate reader. Statistical significance ($*p < 0.05$) was determined by Student's t-test.