Supplementary information

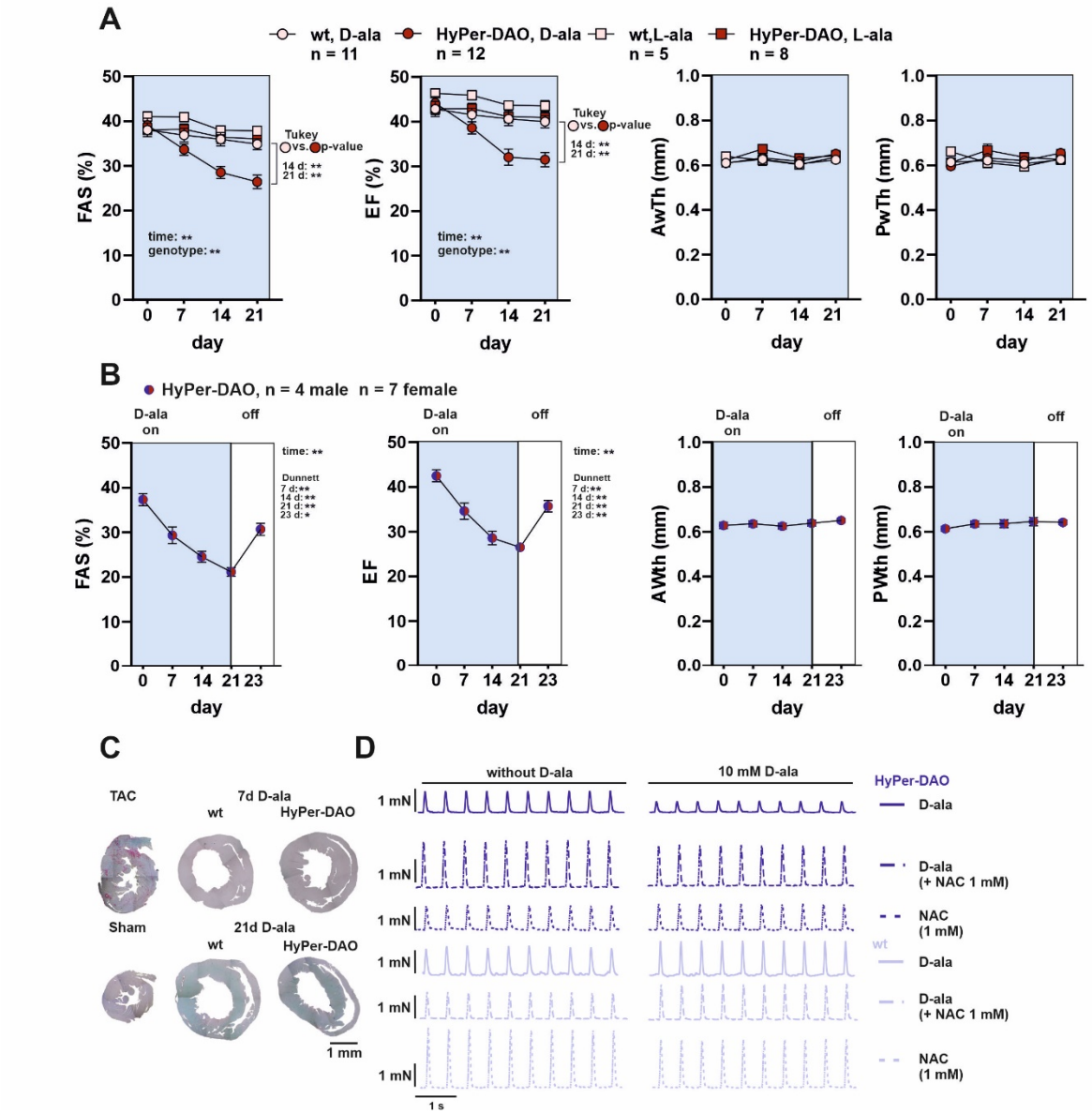
Supplementary Tables

UniProt, accession number	Protein name	STN	p-value
Q8BZF8	phosphoglucomutase-like protein 5	2.2	0.021
Q9CPY7	cytosol aminopeptidase	1.6	0.018
P70404	isocitrate dehydrogenase 3 subunit γ	1.4	0.05
P50431	serine hydroxymethyl-transferase	1.4	0.012
P99029	peroxiredoxin-5	1.2	0.019
Q99JY0	Trifunctional enzyme subunit β	1.1	0.05
P03921	NADH-ubiquinone oxidoreductase chain 5	1.1	0.0023
Q9CZB0	succinate dehydrogenase cytochrome b560 subunit	1.1	0.053

Suppl. Table 1: List of proteins that were identified in the redox proteomics screen to show an increased total reversible oxidation demonstrated by a signal to noise ratio (STN) of > 1 and a p-value of ≤ 0.055 in the hearts of HyPer-DAO mice compared to wild type mice after 7 days of D-ala treatment.

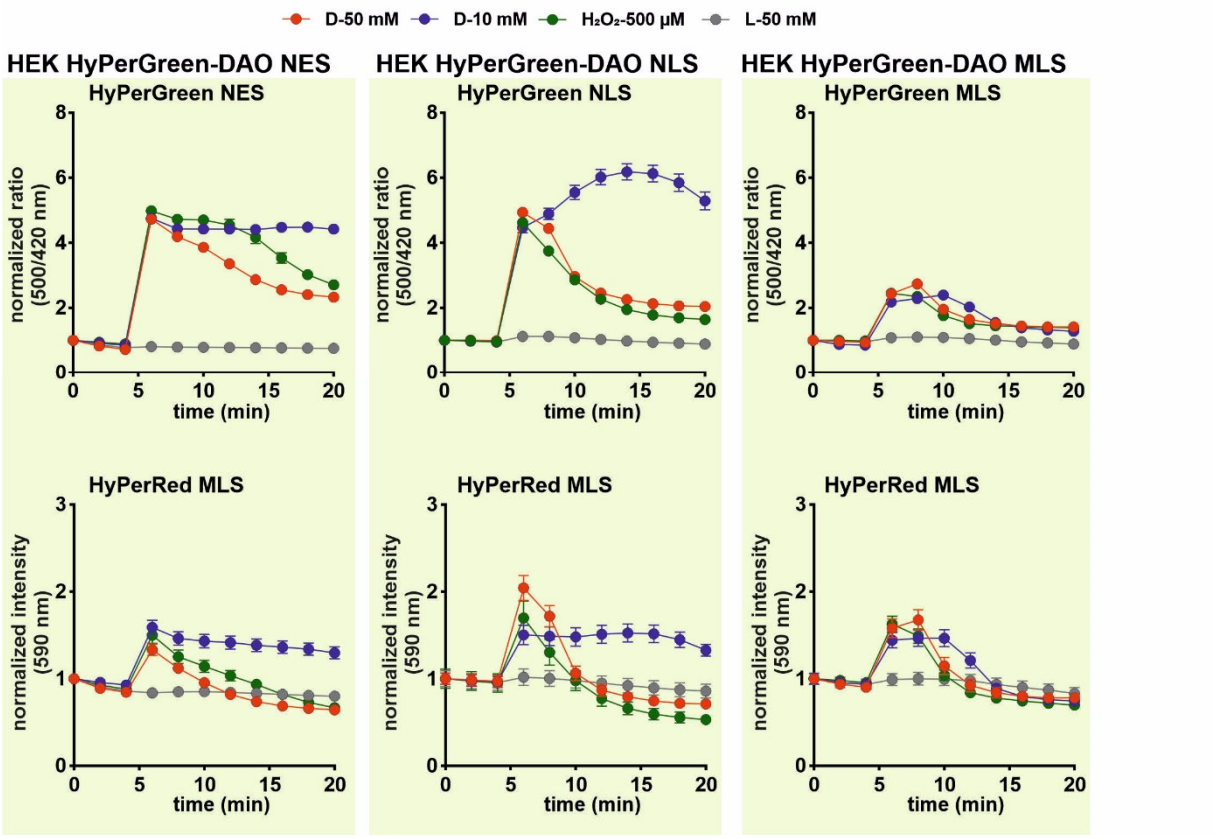
UniProt Accession number	Protein name	STN	p-value
P97449	Aminopeptidase N	-4.0	0.02
P58404	Striatin-4	-3.2	0.011
P08556	GTPase NRas	-2.9	0.036
P46412	Glutathione peroxidase 3	-2.5	0.024
Q9DBB8	Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase	-2.4	0.05
P10493	Nidogen-1	-2.3	0.0065
P16045	Galectin-1	-1.9	0.05
Q8CI70	Leucine-rich repeat-containing protein 20	-1.9	0.021
O55186	CD59A glycoprotein	-1.7	0.05
P05201	Aspartate aminotransferase, cytoplasmic	-1.5	0.019
Q99KB8	Hydroxyacylglutathione hydrolase, mitochondrial	-1.5	0.05
P10518	Delta-aminolevulinic acid dehydratase	-1.5	0.025
Q91WU5	Arsenite methyltransferase	-1.4	0.05
Q9JKS4	LIM domain-binding protein 3	-1.4	0.023
Q8VDK1	Nitrilase homolog 1	-1.3	0.016
P45376	Aldose reductase	-1.2	0.023
P40142	Transketolase	-1.2	0.043
P14152	Malate dehydrogenase, cytoplasmic	-1.1	0.022
P15306	Thrombomodulin	-1.1	0.053

Suppl. Table 2: List of proteins that were identified in the redox proteomics screen to show a decreased total reversible oxidation demonstrated by a signal to noise ratio (STN) of < -1 and a p-value of ≤ 0.055 in the HyPer-DAO mice compared to wild type mice after 7 days of D-ala treatment.



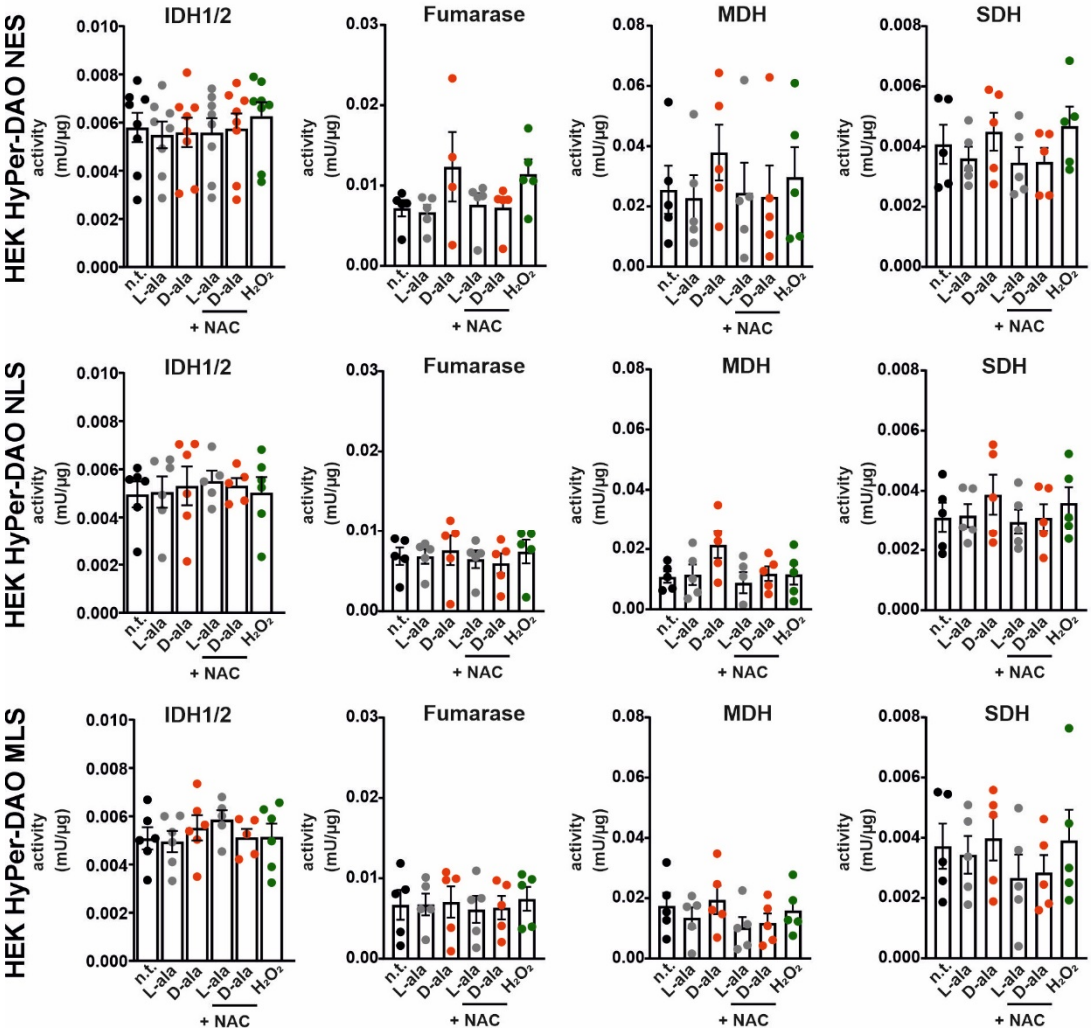
Suppl. Figure 1: Echocardiographic analysis and cardiac fibrosis in HyPer-DAO mice, related to Figure 2

(A and B) Echocardiographic analysis of fractional area shortening (FAS), ejection fraction (EF), anterior wall thickness (AWth) and posterior wall thickness (PWth) in female (in A) and a mixed group of female and male (in B) HyPer-DAO or wild type (wt) mice after treatment with D-ala or L-ala in the drinking water as indicated. **(C)** Representative figures of Sirius red/Fast green stained cardiac slices of HyPer-DAO mice after 7 or 21 days of D-ala treatment and mice after TAC or sham surgery. **(D)** Original tracings of force development measured in heart slices from HyPer-DAO and wt mice after treatment 10 mM D-ala +/- 1 mM NAC. mean ± SEM, *p<0.05, **p<0.01 by one-way ANOVA (B) or two-way ANOVA (A).



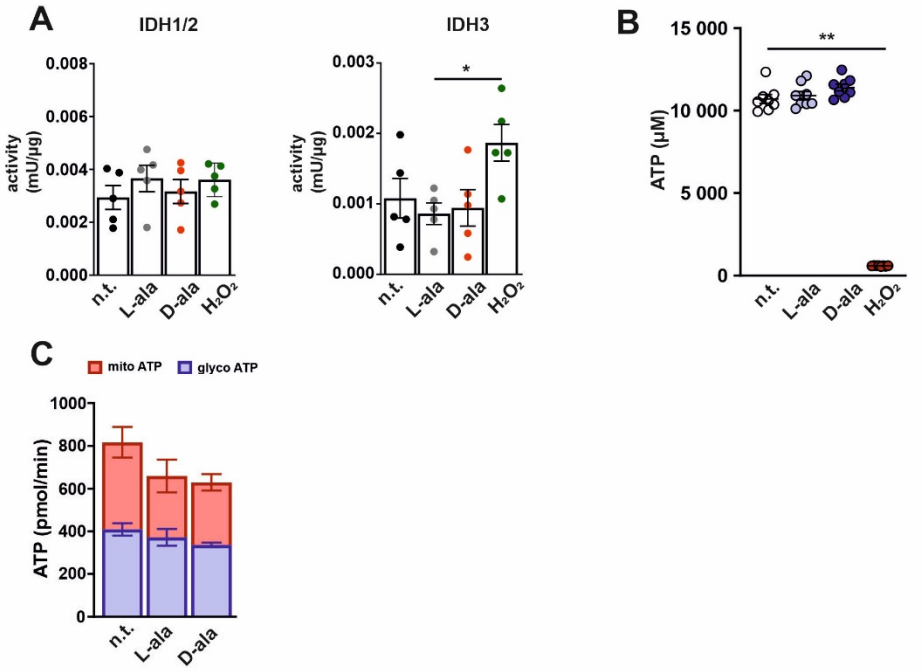
Suppl. Figure 2: Intracellular distribution of H_2O_2 in HEK cells, related to Figure 4A-C

HEK cells overexpressing the green fluorescent HyPer-DAO fusion protein in the cytoplasm (HyPer-DAO NES), the nucleus (HyPer-DAO NLS) and the mitochondrial matrix (HyPer-DAO MLS) were transiently transfected to overexpress the H_2O_2 sensor HyPerRed localized to the mitochondrial matrix (HPerRed MLS). The HyPer Green and HyPerRed fluorescence responses were recorded after stimulation with D-ala, L-ala or H_2O_2. Ratios are normalized to the HyPer ratio prior treatment, 25 cells per condition were analyzed. mean ± SEM.



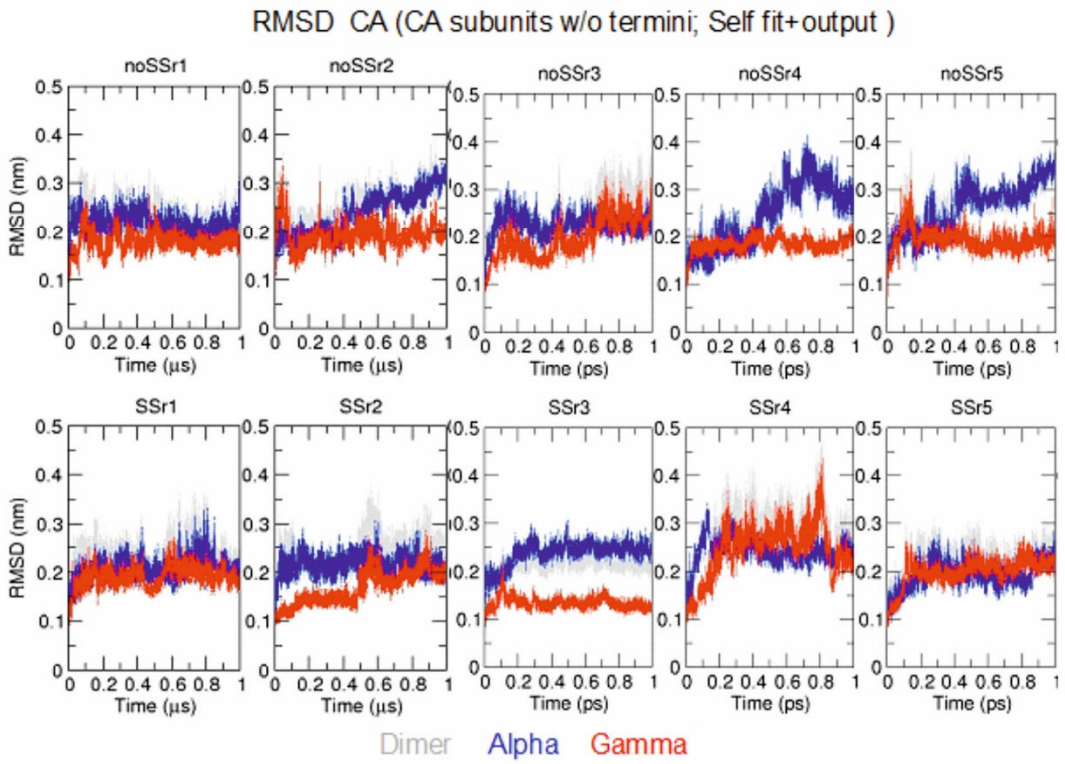
Suppl. Figure 3: TCA cycle enzyme activities in HyPer-DAO overexpressing HEK cells, related to Figure 4 D-G

IDH1/2, fumarase, malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) activities in cell extracts obtained from HEK HyPer-DAO NES, HEK HyPer-DAO NLS and HEK HyPer-DAO MLS either non-treated (n.t.) or after treatment with 50 mM D-ala, 50 mM L-ala ± 8 mM NAC or 500 µM H_2O_2 for 20 min. mean ± SEM.



Suppl. Figure 4: D-ala does not affect IDH1/2 and IDH3 activity nor ATP levels in HEK wild type cells, related to Figure 5

(A) IDH1/2 and IDH3 activity as well as ATP levels **(B)** in cell extracts obtained from HEK wild type (wt) cells after treatment with 50 mM D-ala, 50 mM L-ala or 500 µM H_2O_2 for 20 min. n = 5 independent experiments in A and 8 samples per condition in B. **(C)** Mitochondrial and glycolytic ATP production in HEK wt cells after treatment with 50 mM D-ala or L-ala for 20 min. n = 3 independent experiments. mean ± SEM, *p<0.05, **p<0.01 by one-way ANOVA.



Suppl. Figure 5: Root mean square deviation (RMSD) computed over the Cα-atoms for all MD simulations, related to Figure 6

Curves show results separately for the whole dimer (without disordered termini, grey), the α subunit (blue), and the γ subunit (red).