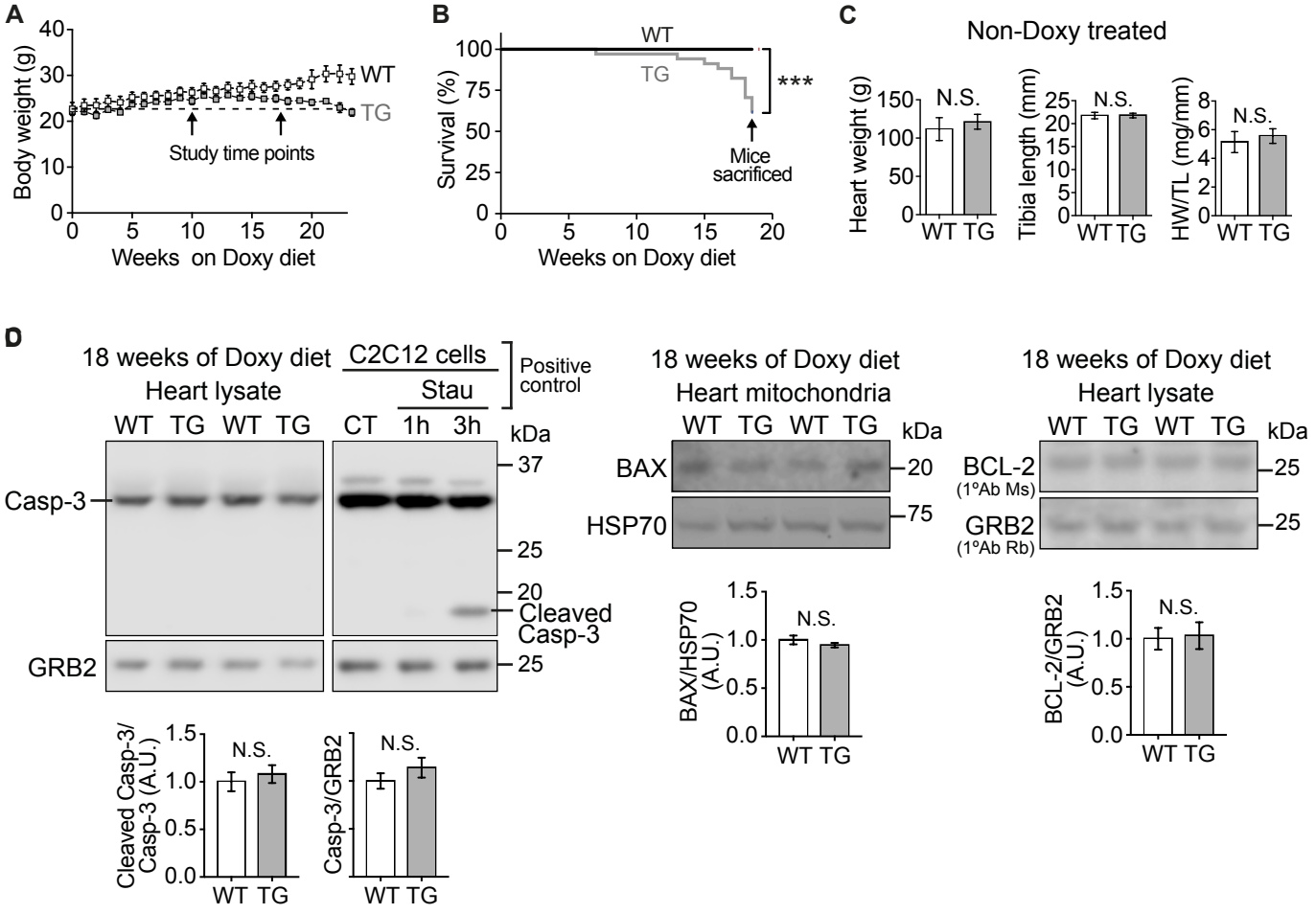
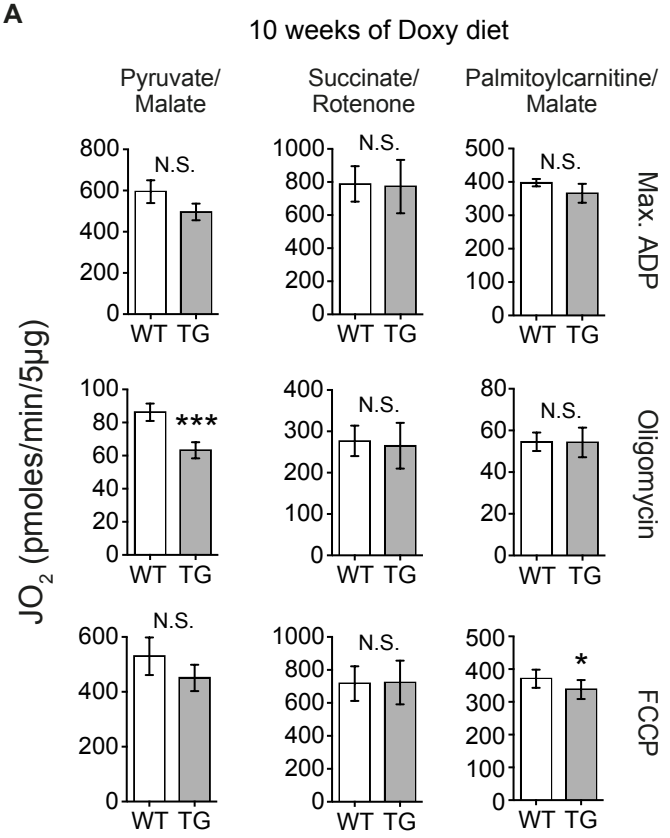
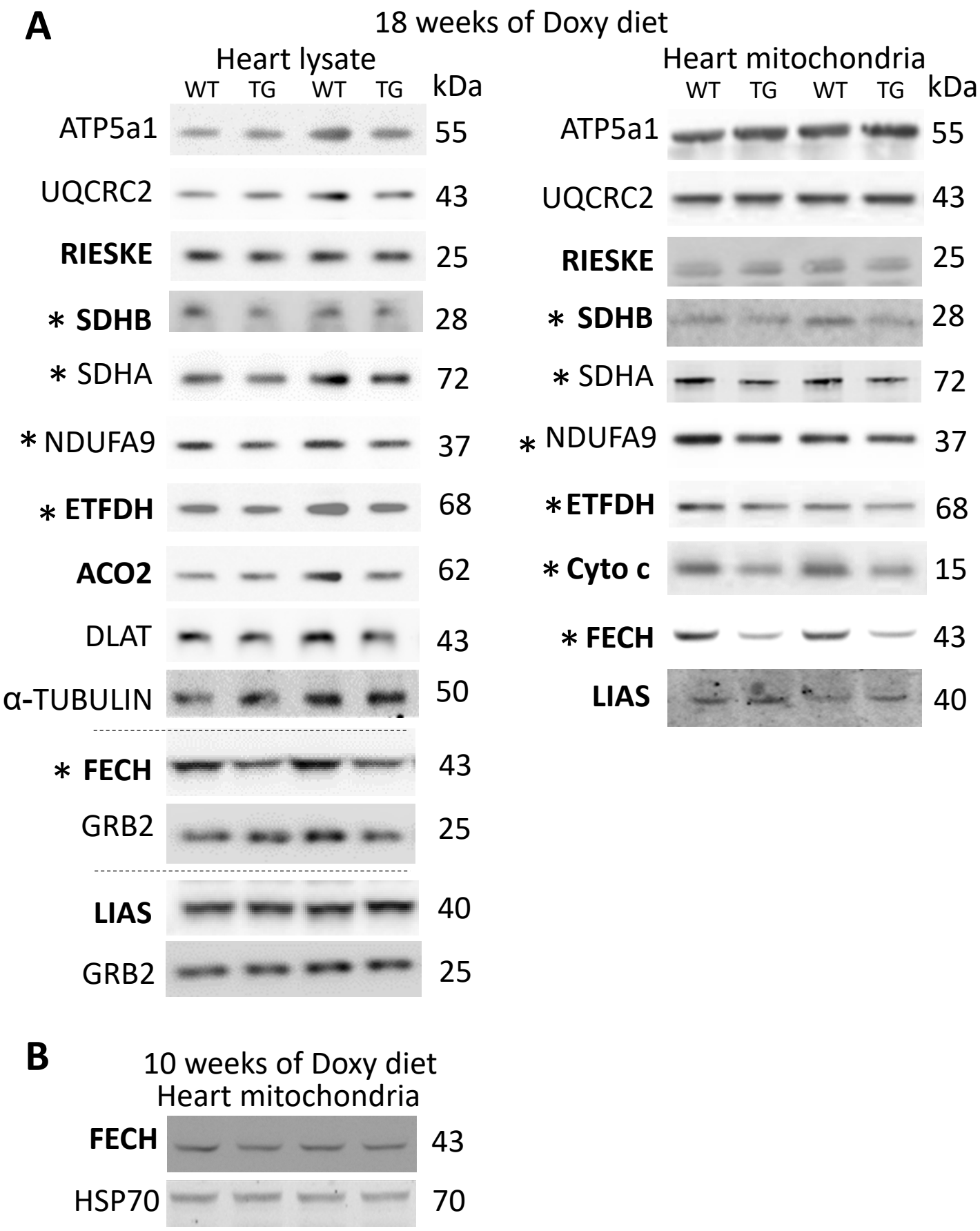
SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL FIGURE 3



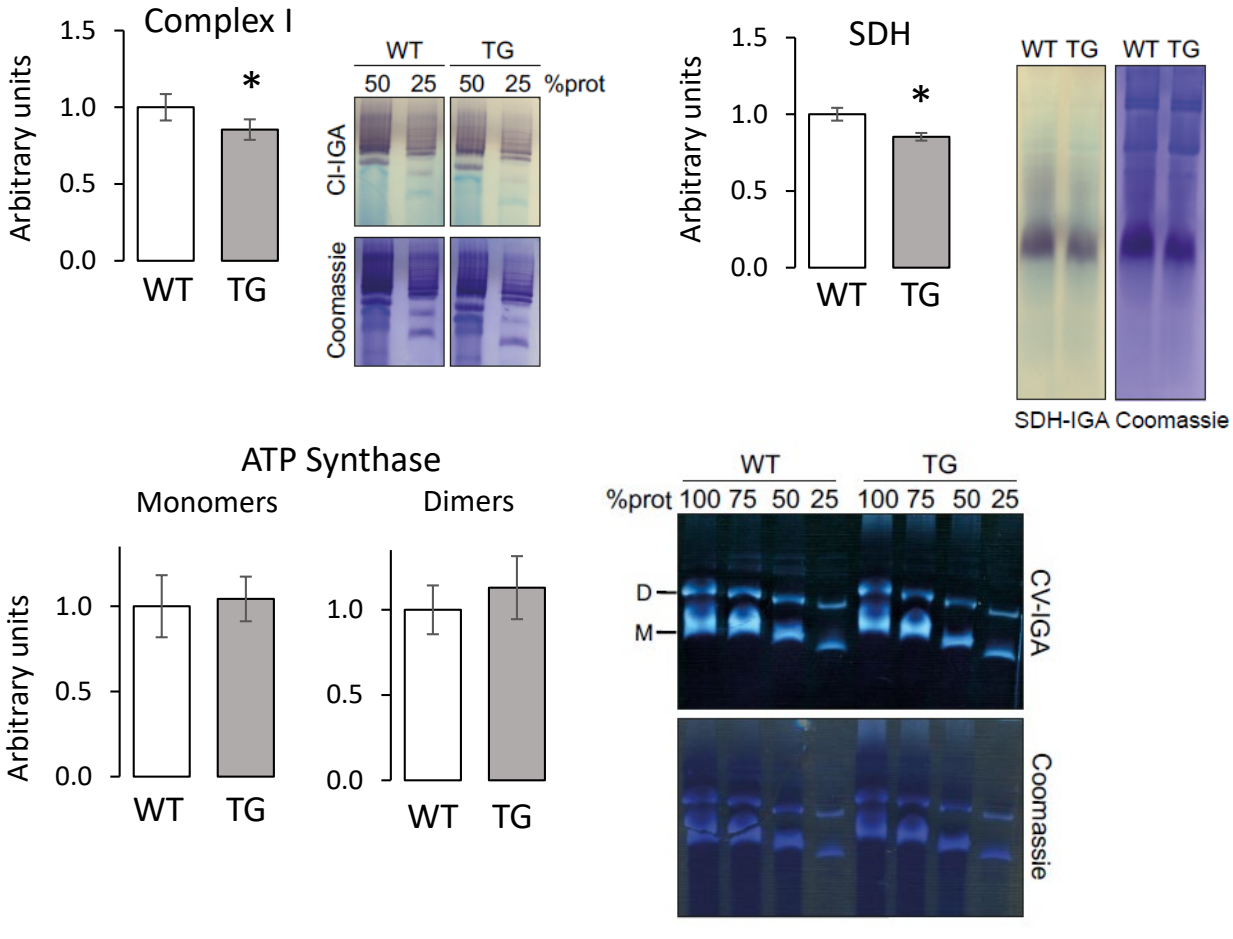
SUPPLEMENTAL FIGURE 3

C

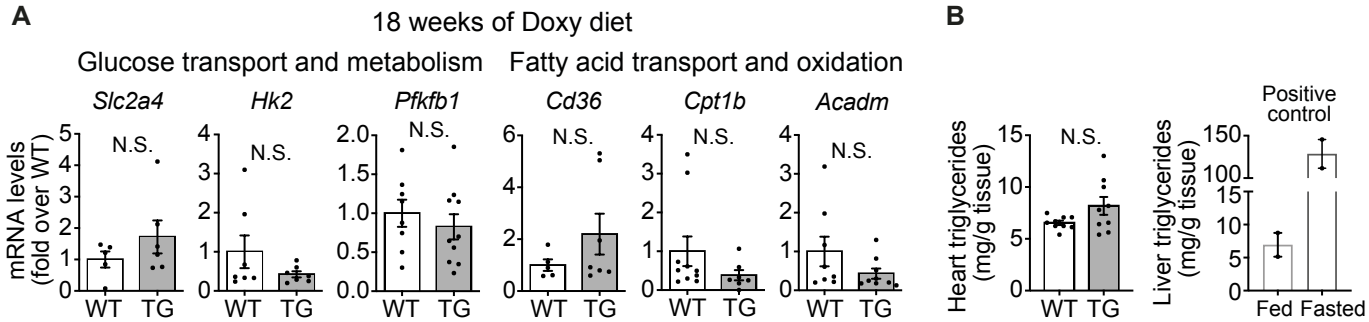
18 wks Doxy	Lysates			Mitochondria		
	WT	TG	P value	WT	TG	P value
ATP5a1	1.00 (0.10)	0.99 (0.06)	0.96	1.00 (0.06)	1.14 (0.03)	0.12
UQCRC2	1.00 (0.09)	1.02 (0.09)	0.88	1.00 (0.06)	0.91 (0.04)	0.20
Rieske	1.00 (0.11)	0.90 (0.03)	0.41	1.00 (0.15)	1.1 (0.10)	0.63
SDHB	1.00 (0.12)	0.70 (0.07)	0.06	1.00 (0.06)	0.86 (0.04)	0.05
SDHA	1.00 (0.03)	0.80 (0.04)	0.01	1.00 (0.05)	0.8 (0.04)	0.03
NDUFA9	1.00 (0.11)	0.72 (0.03)	0.04	1.00 (0.03)	0.82 (0.05)	0.02
ETFDH	1.00 (0.06)	0.82 (0.02)	0.03	1.00 (0.12)	0.71 (0.06)	0.06
Cyto c				1.00 (0.02)	0.6 (0.02)	<0.01
ACO2	1.00 (0.09)	0.84 (0.05)	0.17			
DLAT	1.00 (0.09)	0.81 (0.03)	0.08			
FECH	1.00 (0.12)	0.57 (0.07)	0.03	1.00 (0.08)	0.41 (0.05)	<0.01
LIAS	1.00 (0.09)	0.99 (0.10)	0.96	1.00 (0.08)	1.13 (0.09)	0.45
FECH 10 wks	1.00 (0.05)	0.94 (0.06)	0.43	1.00 (0.10)	0.85 (0.14)	0.43

Quantification of immunoblots shown in panels A and B. Normalization was to ATF5a1. Values: mean (s.e.m.). P values: unpaired t-test, n = 4-6/genotype.

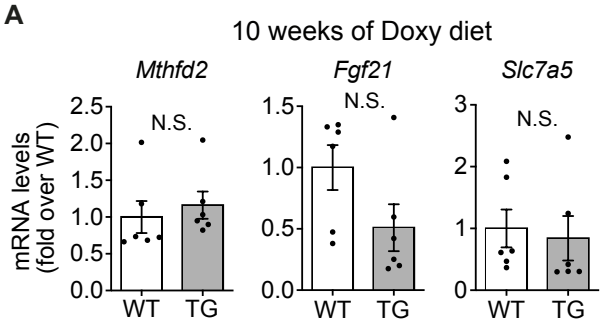
D In-gel Activities



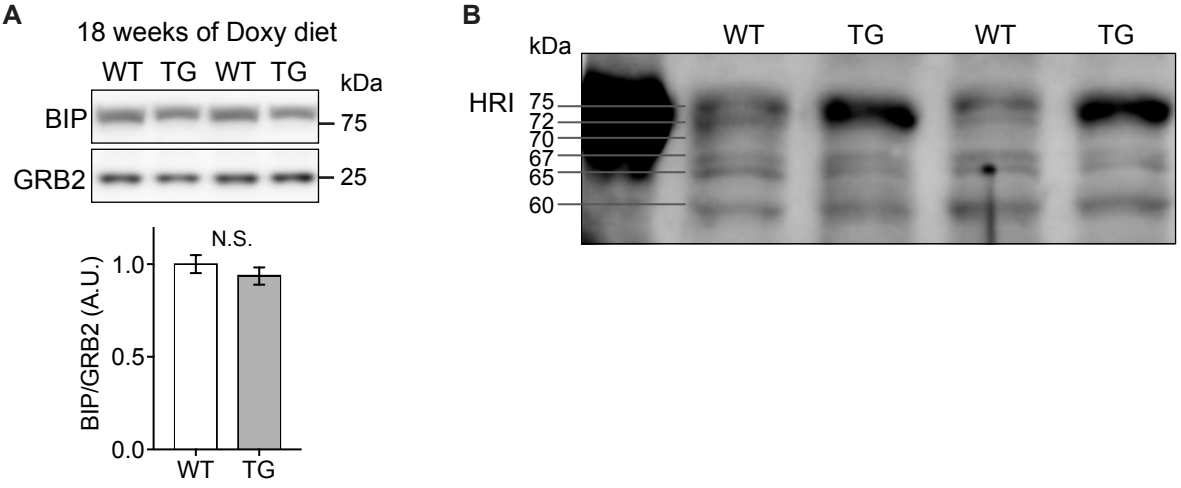
SUPPLEMENTAL FIGURE 4



SUPPLEMENTAL FIGURE 5



SUPPLEMENTAL FIGURE 6



Vasquez-Trincado et al. Supplemental Figure legends:

SFigure 1: Additional characteristics of the TG mouse model

(A) Body weight of WT and TG mice over the course of Doxy feeding. n = 5-12 mice/time point/genotype.

(B) Survival rate, n=29 WT/34 TG mice, ***p<0.001: Curve comparison, Gehan-Breslow-Wilcoxon test.

(C) No difference in heart weight of WT and TG mice not fed Doxy diet; n = 9 WT/4 TG mice.

(D) Representatives immunoblots of caspase-3 (Casp-3), BAX and BCL-2 (GRB2 and HSP70 as a loading controls). Casp-3 and BCL2 were detected in heart lysates (n=4WT/TG). Immunoblot detection of BAX was done in isolated heart mitochondria (n=3WT and 4TG). All samples were from mice fed with Doxy for 18 wks. Averaged cleaved Casp-3/Casp-3, Casp-3/GRB2, BAX/HSP70 and BCL-2/GRB2 values are shown below respective immunoblot. A positive control for Casp-3 antibody was generated using C2C12 cells treated with 1 μM staurosporin (Stau) for 1 and 3 h.

All values are the mean ± s.e.m. Statistical comparison was by unpaired t-test, N.S.: not significant.

SFigure 2: Little effect of FXN depletion on substrate oxidation rates in heart mitochondria from mice on Doxy diet for 10 wks.

Oxygen consumption rate (JO_2) measured in isolated heart mitochondria supplied with pyruvate/malate (10 mM/5 mM), succinate (10 mM + 1 μM rotenone to prevent electron backflow through complex I), palmitoyl-L-carnitine plus malate (20 μM + 1 mM), or octanoyl-L-carnitine plus malate (200 μM+1 mM). Max. ADP, saturating [ADP] (4 mM), was used to evaluate JO_2 reflecting maximal oxidative phosphorylation. Oligomycin was used to evaluated JO_2 that reflects maximal leak-dependent oxidation. The chemical uncoupler, FCCP (1 μM), 2.5 μg/ml) as used to evaluate JO_2 that reflects maximal electron transport chain capacity under the prevailing substrate conditions. All values represent the mean ± s.e.m. Statistical analysis was by unpaired t-test; *p<0.05, n=6/genotype.

Excel file: Mass spectrometry proteomics data set from heart mitochondria isolated from 18-wk Doxy-fed mice

SFigure 3: Selected immunoblot analysis of mitochondrial proteins.

(A) Examples of immunoblots from heart lysates and mitochondria from mice fed with Doxy for 18 wks

(B) Examples of immunoblots from heart mitochondria from mice fed Doxy for 10 wks.

In A and B, bold lettering refers to proteins that contain ISCs, heme or lipoic acid. *: proteins showing significant changes in TG samples (see quantification in **C**)

(C) Quantification of immunoblots from heart lysates and mitochondria from 18-wk Doxy fed mice. Values were normalized to ATP5a1. Values are the mean ± s.e.m. P values: unpaired t-test, n=4/genotype for lysates, n=5/genotype for mitochondria.

(D) In-gel activity for Complex I, SDH and ATP Synthase. Values are the mean ± s.e.m. * p=0.03, paired t-test; Complex I and SDH: n=5/genotype; ATP Synthase: n=3/genotype.

SFigure 4: No change in transcript levels of transcripts of glycolytic and fatty acid handling and metabolism proteins, and triglyceride content in hearts from TG mice fed Doxy diet for 18 weeks.

(A) mRNA values of transcripts involved in fatty acid and glucose oxidation and transport, normalized to *Actb* (β-actin) and expressed relative to WT. Individual points represent values from each mouse. Value = 1, means no change (n=5-10/genotype).

(B) *Left*: Heart triglycerides levels (n=9WT/9TG). *Right*: Liver triglycerides levels from fed and fasted mice, used as a positive control (2 fed/fasted).

All values are the mean ± s.e.m. Statistical comparison was by unpaired t-test, N.S.: not significant.

SFigure 5: No change in ATF4 targets in the heart after 10 wks on Doxy diet

Transcript levels (normalized to *Actb* (β-actin) and expressed relative to WT) of ATF4 target genes in mice fed Doxy diet for 10 weeks. Bars represent the mean ± s.e.m; individual points represent values from each mouse. Value = 1, means no change. Statistical analysis: unpaired t-test, N.S.: not significant, n=6/genotype.

SFigure 6: Absence of endoplasmic reticulum stress in the heart after 18 wks of Doxy diet.

(A) *Upper*: Representative immunoblot of endoplasmic reticulum chaperone protein BIP, and GRB2 (loading control) from heart lysates of mice fed Doxy diet for 18 wks, and quantification (*lower*), means +/- sem. Statistical comparison: unpaired t-test, N.S.: not significant. (n=9 WT/7 TG).

(B) Calibration of immunoblot using anti-HRI antibody.