SUPPLEMENTARY METHODS.

Generation of RIP140 transgenic mice and analysis of gene expression.

The pCAGGS vector [1] was modified by changing a Xho I site (1726 nucleotides) to a Spe I site and a Hind III site (2275 nucleotides) to a Sal I site. The human RIP140 sequence was inserted at 1726 nucleotides using the Spe I site. The pCAGGS-hRIP vector was then digested using Sal I and the fragment containing the CMV enhancer, beta actin promoter and hRIP cDNA and β-globin polyA sequence was injected into the male pronuclei of a one cell stage blastocyst. Transgenic mice were identified by PCR using primers 5'-CGCCGCCGTCCCCTTCTCCAT-'3 and 5'-TTGTCAACGGCAGTACCTGA-'3. The mice generated had access to food pellets ad libitum and were kept on a 12-hour light and dark cycle, in accordance with Home Office regulations.

Primers used to determine the expression of human RIP140 are as follows: forward primer 5'-ACATTTGGAGGGAGGCTTCAT-3', reverse primer 5'-GCTTTCGTTTCTGCAGTAGGAAGTA-3'. Primers used to detect both mouse and human RIP140 (total RIP140) are as follows: forward primer 5'- ACTCATGGAGAAGAGCTTGG -3', reverse 5'-AATGACTGAAGCAAAGAGGC-3'. All other primer sequences used in this study are available on request.

Quantification of collagen staining (fibrosis) –

One section from each heart was stained with Van Gieson stain, so that collagen fibres were stained red against a pale yellow background. Photomicrographs at x20 magnification were taken over the ventricle only, with 4-6 fields per section. Images were

identified with code numbers and the image analysis was performed without being aware of the identity of the images. Each image was converted to black and white in Adobe Photoshop and was imported into NIH Image software, which was used to calculate the number of black pixels. This was used to calculate the mean percentage area occupied by collagen staining for each section, and finally the mean percentage area per group.

β-oxidation assay – Ventricles from WT and RIP140 transgenic mice (n=3 per group) were assayed for ^3H_2O production from excess [^{3}H]palmitic acid as described[2]. Ventricles were minced and incubated in assay buffer (20mM HEPES, 1mM NaH_2PO_4, 0.4mM $MgSO_4$,1mM $CaCl_2$, 120mM NaCl, 5mM KCl, 5mM glucose) at 37°C for 30 minutes. 3μCi of ^{3}H-palmitic acid was added and the samples were incubated as before.

Histochemical staining for cytochrome c oxidase and succinate dehydrogenase – Hearts were excised and immediately frozen in liquid nitrogen. Frozen sections (10 μm) were mounted onto poly-L-lysine coated glass slides, allowed to dry at room temperature for 30 min then stained for cytochrome c oxidase and succinate dehydrogenase as described previously[3]. Sections were mounted in glycerol gelatin (Sigma). Serial sections were stained independently for either enzyme alone.

MRI and image analysis.

Images were obtained on a 9.4T Varian Inova system (Palo Alto, USA). A 35mm quadrature volume resonator coil was used for these experiments (Rapid Biomedical, Wurzberg, Germany) to both transmit and receive the RF signal.

Cine MRI: Male and female WT and transgenic mice aged 2-4 months (n=20 in total) were scanned under inhalation anaesthesia applied via a nose cone (isoflurane, supplemented with oxygen induced at 4-5% isoflurane and oxygen flow rate of 3L/min and maintained at 1.5% isoflurane with 1L/min oxygen. The body temperature of the animals was maintained at 37^{o}C using warm air heating system controlled via a rectal probe (SA Instruments Inc, New York, USA) ECG was also monitored using 2 needle electrodes placed sub-cutaneously (SA Instruments Inc, New York, USA). The double-gated spoiled gradient echo imaging sequence used to assess contractile dysfunction acquired 10 frames per cardiac cycle, covering the first 75-85% following the R-wave. The sequence parameters consisted of: matrix=128x128, FOV=40x40mm, slice thickness=1mm TE=1.7ms, TRcine=~8ms, FA=20°, Averages=1, TA~20s per slice.

Typically 6-7 contiguous short axis slices were acquired, covering the heart from apex to base. Additional horizontal and vertical long axis slices, relating to 2 and 4 chamber views were also acquired.

Images were analysed using Image J (NIH, Bethesda, Maryland, USA). For each slice of the left ventricle (LV), the delineation of the myocardium/blood boarder was manually drawn with Image J for each cardiac frame in every short axis slice. The largest and smallest LV volumes were chosen to be representative of end diastole and end systole respectively. The difference between end diastolic and end systolic volumes was summed for all slices and expressed as the stroke volume. The ejection fraction was calculated as stroke volume divided by end diastolic volume.

Immunohistochemistry for macrophages.

Paraffin sections of heart tissue from selected samples were dewaxed and incubated with 0.3% hydrogen peroxide in methanol for 30 min. Non-specific staining was blocked by incubation in 1:10 normal rabbit serum/PBS for 30 min, then primary antibody was applied at 4°C overnight. Primary antibody was rat anti-mouse monoclonal F4/80 anti-murine macrophage marker (Serotec), diluted to 1:20 in PBS. Antibody was detected using biotinylated anti-rat IgG (Vector) and Vector Elite ABC peroxidase standard kit.

Transmission Electron Microscopy

Tissue samples measuring 1-2 mm were taken from the right atrium and the left ventricle of hearts from 6 week-old and 14 week-old male mice, both WT and transgenic (n=2 per group). Samples were fixed for 24 hours in 3% v/v glutaraldehyde made up in 0.1M cacodylate buffer pH 7.3, washed in fresh buffer and post-fixed in 1% w/v osmium tetroxide. The samples were dehydrated through a series of alcohols and embedded in Araldite. Ultra thin sections from the blocks were cut on diamond knives, stained in uranyl acetate and lead citrate and examined using a Philips CM10 TEM (FEI, Cambridge, UK).

SUPPLEMENTARY REFERENCES.

1. Niwa H, Yamamura K, Miyazaki J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 1991;**108**:193-199.

2. Cheng L, Ding G, Qin Q, Huang Y, Lewis W, He N *et al.* Cardiomyocyte-restricted peroxisome proliferator-activated receptor-delta deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy. *Nat Med* 2004;**10**:1245-1250.

3. Elson JL, Samuels DC, Johnson MA, Turnbull DM, Chinnery PF. The length of cytochrome c oxidase-negative segments in muscle fibres in patients with mtDNA myopathy. *Neuromuscul Disord* 2002;**12**:858-864

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. A. Design and modification of pCAGGS transgenic vector and cloning strategy for insertion and expression of RIP140. Human RIP140 was inserted into pCAGGS using Spe I and a fragment containing the CMV-enhancer, chicken beta-actin promoter, human RIP140 open reading frame and the sequence corresponding to the rabbit beta-globin poly A tail was digested from the vector using Sal I. **B.** Transgene expression (human RIP140) in hearts from 4-5 month-old mice determined by real-time PCR. n=5 for each line. N.D: Not detected.

Supplementary Figure 2. Body weights of WT and RIP140 transgenic male and female mice at 4 and 8 weeks of age. No differences were seen between the genotypes for each sex at a particular age.

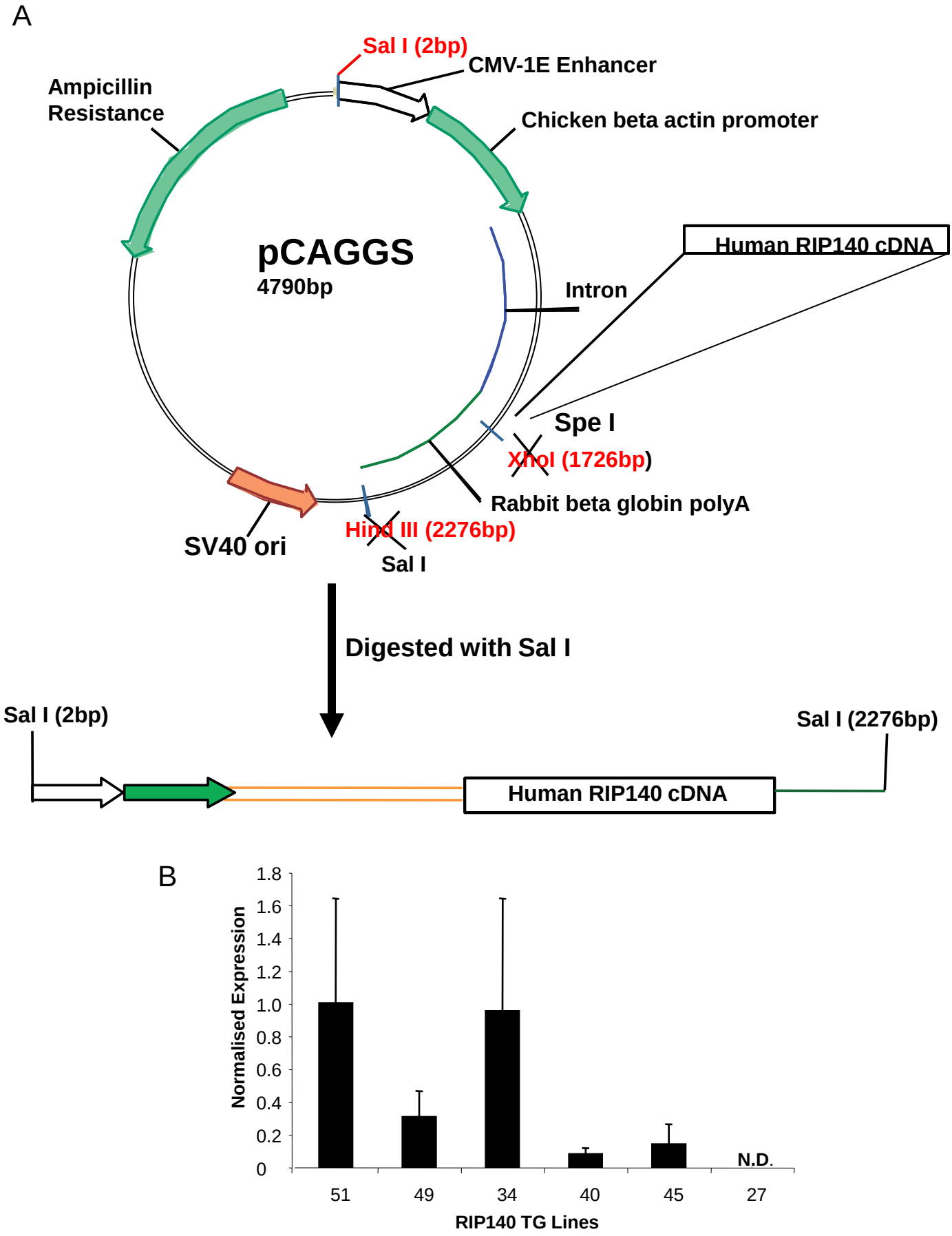
Supplementary Figure 3. Mid-ventricular end diastolic and end systolic cine MRI scans of 4-5 month-old female WT (a,b and e,f) and RIP140 transgenic (TG) (c,d and g,h) mouse hearts with (a-d) short-axis, (e-h) four chamber long-axis view. Data $\pm$ SEM are given for the left ventricle.

Supplementary Figure 4. Histological staining with Masson's trichrome, showing development of fibrosis in the ventricle in hearts from 4-5 month-old RIP140 transgenic mice from lines 27, 34, 40, 45, 49 and 51. Line 27 appeared normal while the other 5 lines had varying degrees of fibrosis. Lines 34 and 51 gave the most consistent results. Size marker = 100 μm.

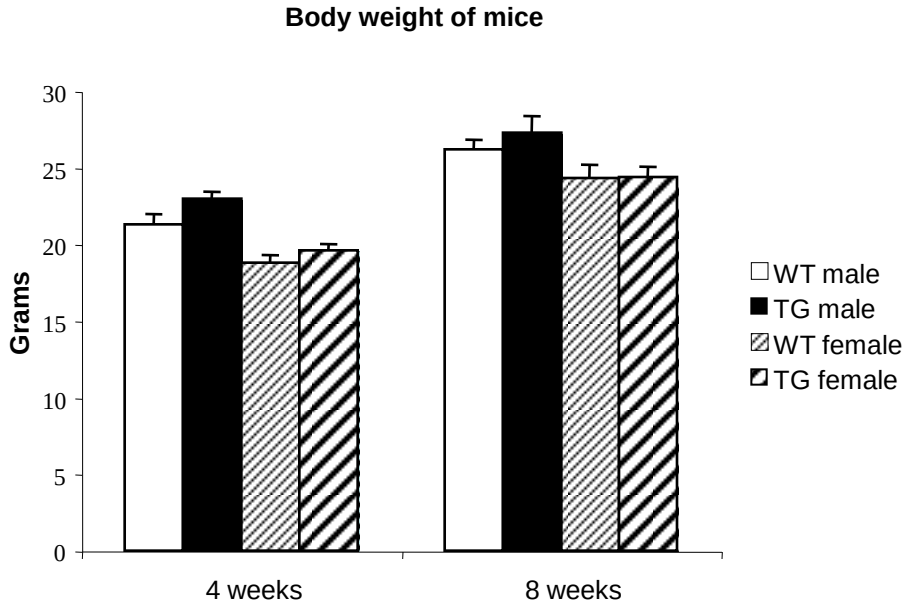
Supplementary Figure 5. Mitochondrial (**A**) and fatty acid oxidation (**B**) gene expression was measured in 4 week WT and TG, 8 week WT and TG female hearts by real-time PCR. For each gene, the levels are normalised to 4 week old WT females.

Supplementary Figure 6. Measurement of ^{3}H-labelled palmitic acid uptake in the ventricles of WT and RIP140 transgenic mice in 8 week-old males (A) and 12 week-old females (B).

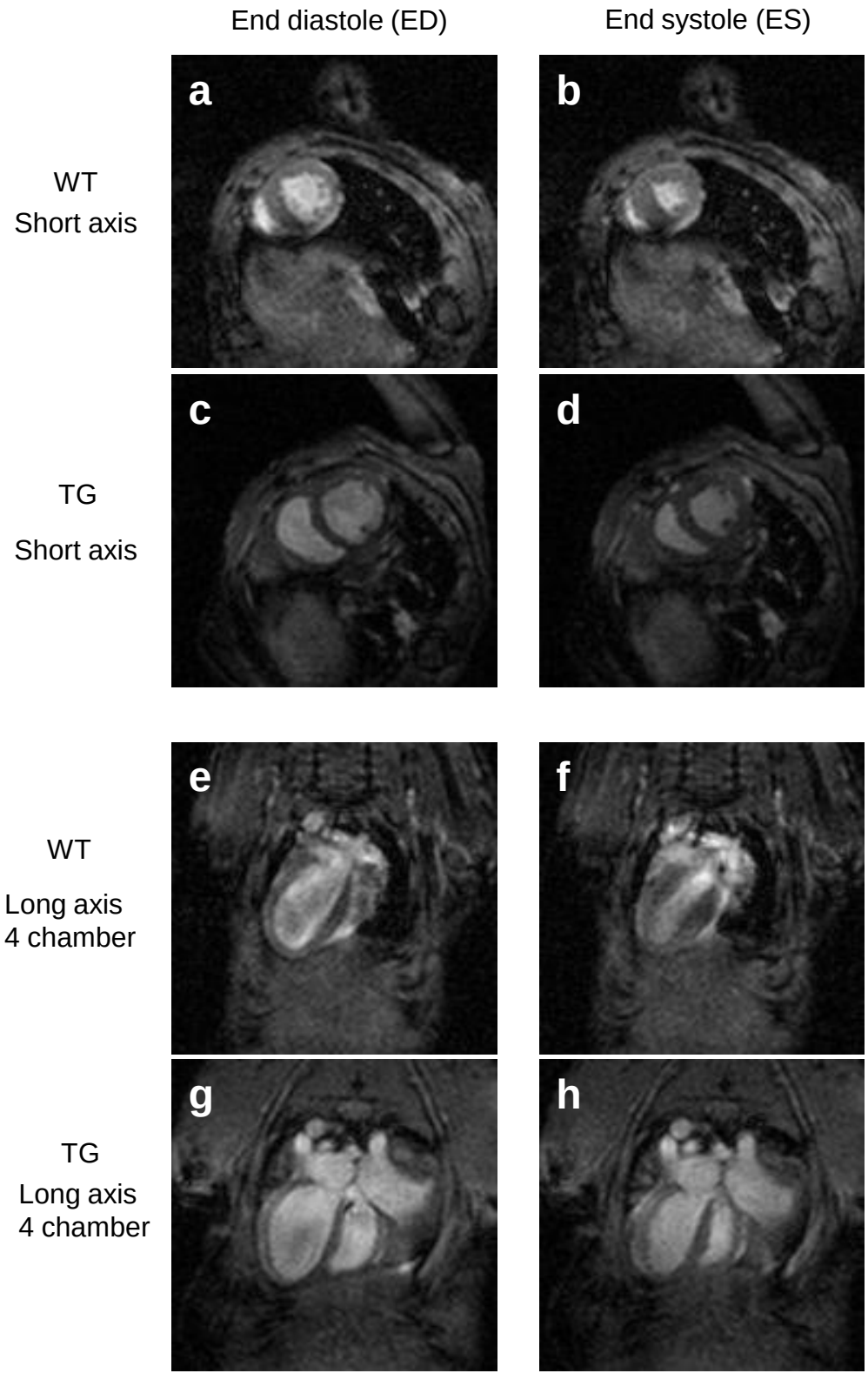
Supplementary Figure 7. A. Cytochrome c oxidase (COX)-succinate dehydrogenase (SDH) staining to identify mitochondrial damage in frozen sections of 1 week-old and 2 week-old RIP140 transgenic mouse hearts. COX-deficient-SDH-positive cells would stain blue, but all cardiomyocytes stain brown indicating that both enzymes are active. A = atrium, V = ventricle, Size marker = 100 μm. **B**. Atrial natriuretic peptide (ANP) expression in RNA extracted from 4 and 8 week-old hearts. *p<0.05, **p<0.01 compared with WT.



Supplementary Figure 1



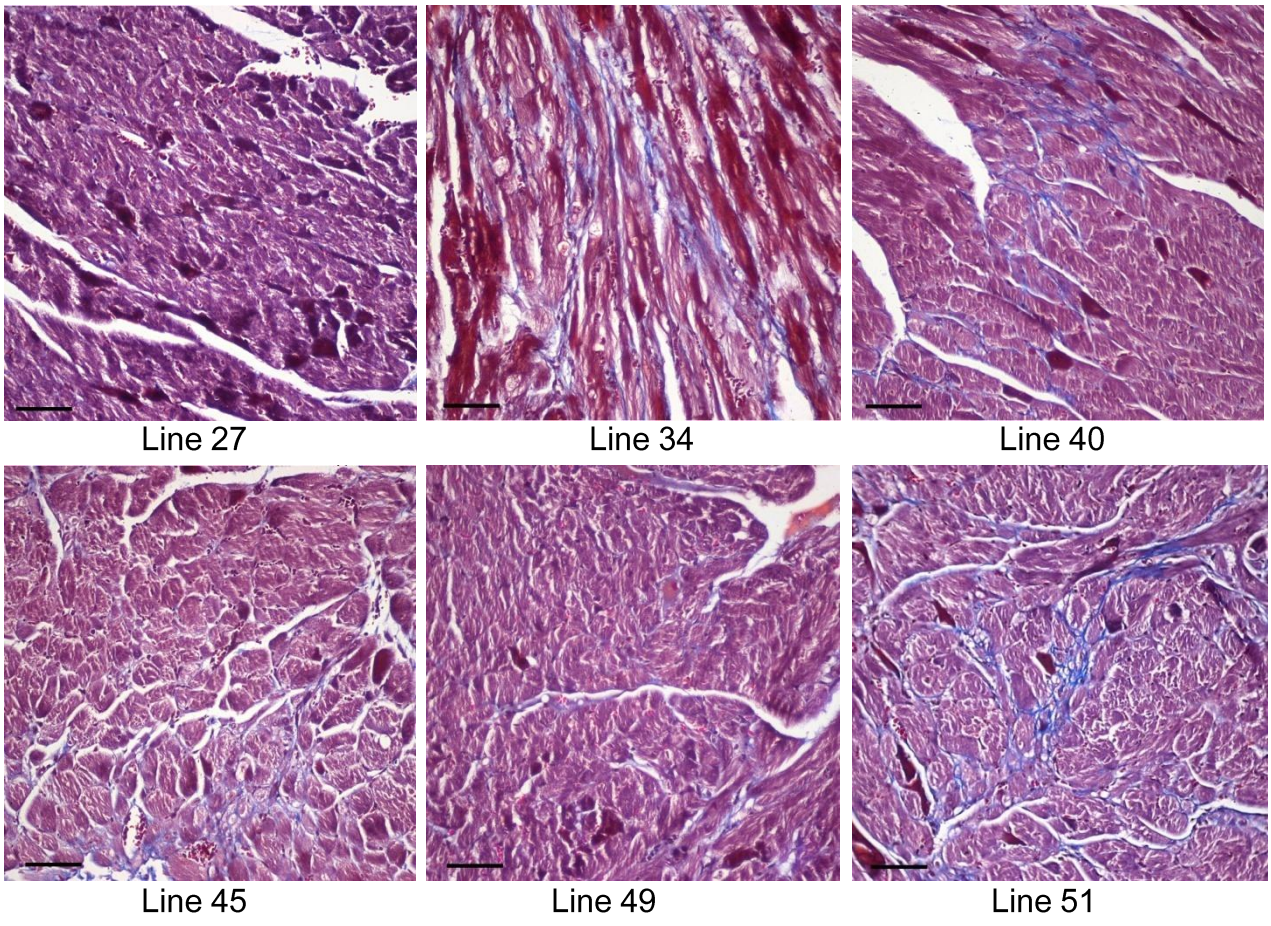
Supplementary Figure 2



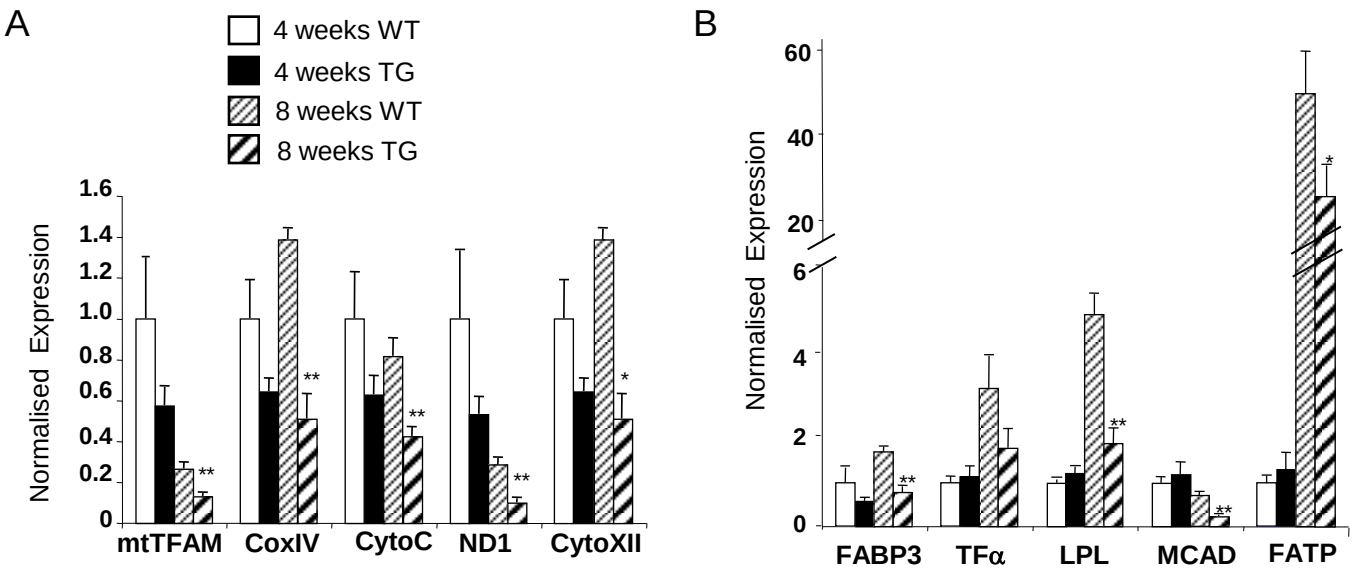
LV volumes:

	ED (μl)	ES (μl)	EF (%)	SV (μl)
WT	44.2 ± 0.6	13.7 ± 0.4	69.1 ± 1.3	30.6 ± 0.9
TG	66.1 ± 7.6	34.5 ± 7.9	49.2 ± 6.4	31.5 ± 0.3

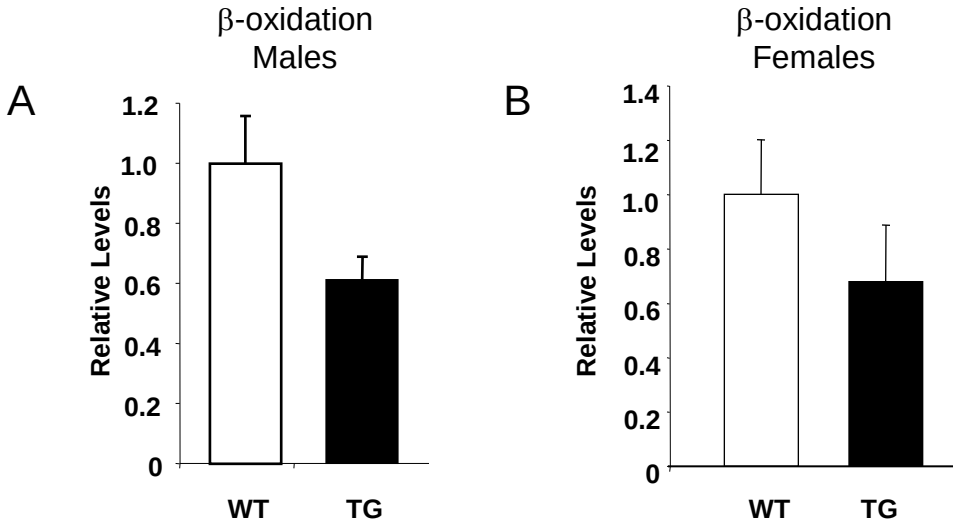
Supplementary Figure 3



Supplementary Figure 4

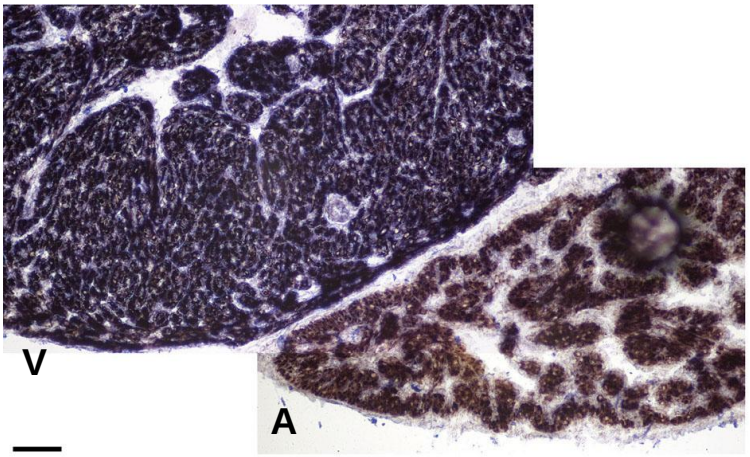


Supplementary Figure 5

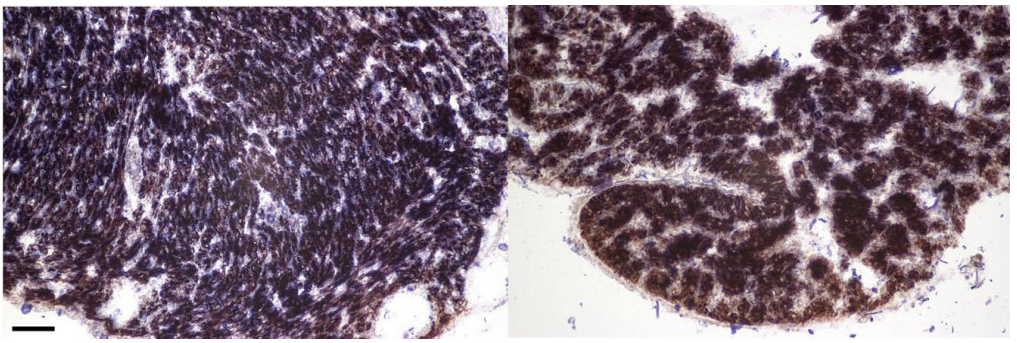


Supplementary Figure 6

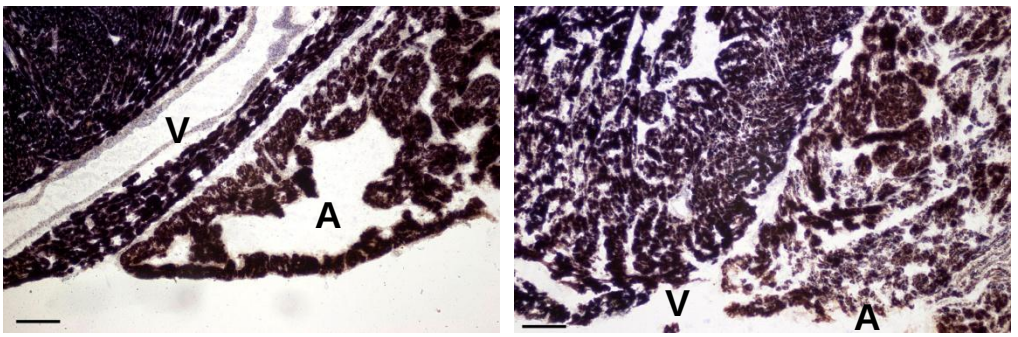
A



1 week-old WT



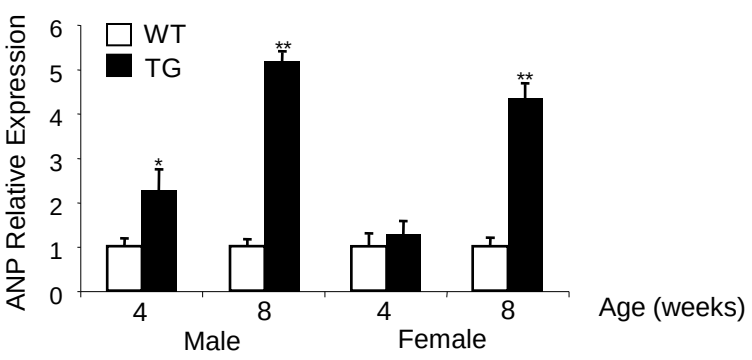
1 week-old TG



2 week-old WT

2 week-old TG

B



Supplementary Figure 7