SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Measurement of mRNA expression levels by quantitative reverse transcriptase PCR (RT-qPCR). Following primer sequences were used for RT-qPCR: β-actin forward, 5'-AGC CAT GTA CGT AGC CAT CCA-3', and reverse, 5'-TCT CCG GAG TCC ATC ACA ATG-3'; FGF21, (fibroblast growth factor) forward, 5'-TCC AAA TCC TGG GTG TCA AA-3', reverse, 5'-CAG CAG CAG TTC TCT GAA GC-3'; ß-Klotho forward, 5'-TGT TCT GCT GCG AGC TGT TAC-3', reverse 5'-TTA TCC CAT ATT GCT TTC CCG TC-3'; CPT1b (Carnitine palmitoyltransferase 1 beta) forward, 5'-GGC ACC TCT TCT GCC TTT AC-3', reverse 5'-TTT GGG TCA AAC ATG CAG AT-3'; PPARalpha (peroxisome proliferator-activated receptor alpha) forward 5'-GTA CCA CTA CGG AGT TCA CGC AT-3', reverse 5'-CGC CGA AAG AAG CCC TTA C-3'; AOX (acyl-coenzyme A oxidase 1) forward 5'-AGA TTG GTA GAA ATT GCT GCA AAA-3', reverse, 5'-ACG CCA CTT CCT TGC TCT TC-3'; MCAD (acyl-coenzyme A dehydrogenase, medium chain) forward, 5'-GAT GCA TCA CCC TCG TGT AAC-3', reverse 5'-AAG CCC TTT TCC CCT GAA-3'; LCAD (acyl-coenzyme A dehydrogenase, long chain) forward, 5'-TTT CCG GGA GAG TGT AAG GA-3', reverse, 5'-ACT TCT CCA GCT TTC TCC CA-3'; PDK4 (pyruvate dehydrogenase kinase, isoenzyme 4) forward, 5'-ATC TAA CAT CAG AAT TAA ACC-3', reverse 5'-GGA ACG TAC ACA ATG TGG ATT G-3'; Ddit3 (DNA damage-inducible transcript 3) forward, 5'- ACC TGA GGA GAG AGA AAC CGG TCC -3', reverse 5'-ACC TCT GCT GGC CCT GGC TC-3'; Grp78 **(**Glucose-regulated protein 78) forward, 5'-CCG TCC CGT GGC ATC AAC CC-3', reverse 5'-GGG CAC CAC AGT GTT CCT CGG-3'; ERdj4 (Endoplasmic Reticulum DnaJ Homolog 4) forward, 5'-TTGC CCC TCC CTC CCC CAA C-3', reverse, 5'-GCC CGA GTT ACA GGG ACC ATA GGC-3'; Xbp1 (X-box binding protein 1) forward, 5'-GGC CCA GTT GTC ACC TCC CC-3', reverse, 5'-CAG CTT GGC TGA TGA GGT CCC C-3'; Chop

(C/EBP homologous protein, mouse) forward, 5’-GTC CAG CTG GGA GCT GGA AG-3’, reverse, 5'-CTG ACT GGA ATC TGG AGA G-3'; Pdi (protein disulfide isomerase, mouse) forward, 5’-CAA GAT CAA GCC CCA CCT GAT-3’, reverse, 5'-AGT TCG CCC CAA CCA GTA CTT-3'; Grp78 **(**Glucose-regulated protein 78/BiP, mouse) forward, 5’-GTT TGC TGA GGA AGA CAA AAA GCT C-3’, reverse, 5'-CAC TTC CAT AGA GTT TGC TGA TAA T-3'; ERdj4 (Endoplasmic Reticulum DnaJ Homolog 4, mouse) forward, 5’- GTGGAGAAGCTGCGTCGGGG-3’, reverse, 5'-TGA GGC AGA CTT TGG CAC ACC T-3'.

Examination of tissue glucose uptake. Tissue specific glucose uptake was determined using [^{3}H]-2-Deoxyglucose (^{3}H-DG). ^{3}H-DG (10 µCi/mouse) was injected i.p. under the conditions described for the glucose tolerance test. After 40 minutes, mice were sacrificed and tissues were excised and rinsed in ice-cold PBS, 1 mM EDTA. Tissue samples were homogenized in 0.5% perchloric acid and centrifuged. The protein pellet was solubilized in 0.3 N NaOH, 0.1% SDS for protein determination. The supernatant was neutralized with KOH and an aliquot was counted to yield total tissue counts (^{3}H-DG and ^{3}H-DG-6-phosphate). A second aliquot was treated with $Ba(OH)_2$ and $ZnSO_4$ to remove ^{3}H-DG-6-phosphate to yield ^{3}H-DG which was counted by liquid scintillation. Specific ^{3}H-DG-6-phosphate accumulation in tissues was calculated as difference between total tissue counts and ^{3}H-DG counts.

Measurement of oleic acid oxidation. FAO in CM homogenates was examined according to the protocol of Hirschey et al (25) and is described in detail in the supplemental material. In brief, tissues from fasted mice were homogenized in chilled STE buffer (0.25M sucrose, 10 mM Tris, 1 mM EDTA) using an Ultra Turrax® (IKA, Staufen, Germany). Homogenates were centrifuged at 420g at 4°C for 10 minutes to pellet nuclei and cell debris. The supernatant was subsequently centrifuged at 15,000g at 4°C for 15 minutes to obtain an enriched mitochondrial fraction. Pellets were washed and suspended in STE buffer. After protein

determination, 20 µl of the suspension was incubated with 400 µl of reaction mix (100mM sucrose, 10mM Tris, 5mM KH2PO4, 0. 2mM EDTA, 0.3% BSA, 80 mM KCl, 1 mM MgCL2, 2 mM Carnitine, 0.1mM Malate, 0.05mM CoA, 2mM ATP, 1mM DTT, 100 mM oleic acid complexed to BSA, 0,1 µCi/reaction ^{14}C oleic acid) for 30 min at 37°C. The reaction was terminated by transferring the reaction mix into fresh tubes containing 200 µl 1 M perchloric acid. For CO_2 trapping, the tube caps were equipped with a piece of filter paper soaked with 20 µl 10 N NaOH. The closed tubes were incubated at 37°C for 1 h. Afterwards, the filter papers were objected to liquid scintillation counting.

TG hydrolase assay. TG hydrolase assays were performed as previously described (14). For the measurement of TG hydrolase activity, cell lysates (1,000xg supernatant) or tissue extracts (10,000xg supernatant) were used. Samples in a total volume of 100 µl buffer A (0.25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol, 20 µg/ml leupeptin, 2 µg/ml antipain, 1 µg/ml pepstatin, pH 7.0) were incubated with 100 µl substrate in a water bath at 37°C for 1 h. The micellar TG substrate contained 330µM triolein, ^{3}H-triolein as tracer, 45µM PC:PI (3:1), and was prepared by sonication (Virsonic 475, Virtis, Gardiner, NJ). The incubation was performed at 37°C for 1 hs with 0.1 ml of substrate and 0.1 ml infranatant. The reaction was terminated by adding 3.25 ml of methanol/chloroform/heptane (10:9:7) and 1 ml of 0.1 ml 0.1 M potassium carbonate, 0.1 M boric acid, pH 10.5. After centrifugation (800 g, 20 minutes, RT) the radioactivity in 1 ml upper phase was determined by liquid scintillation counting.

Mouse echocardiography. Echocardiographic analyses (26) were performed under light isoflurane anaesthesia. Mice were allowed to breathe spontaneously. 2D guided M-mode echoes were obtained from short- and long-axis views at the level of the largest LV-diameter using a VS-VEVO 770 high resolution imaging system (Visualsonics, Toronto, Canada)

equipped with a real-time microvisualization scanhead (30 MHz). LV end-diastolic (LVEDD) and end-systolic (LVESD) dimensions were measured from original tracings by using the leading edge convention of the American Society of Echocardiography. All measurements and calculations were done in triplicates.

SUPPLEMENTARY TABLE

Supplementary Table 1: Transthoracic echocardiography measurements of 20 to 22-week old female CM-Fgf21 transgenic and wt mice (n = 4 -5). Data are presented as mean ± s.d. **P < 0.01 versus wt mice. Abbreviations: HR, heart rate; IVSW, interventricular septal wall thickness; LV, left ventricular; LVEDD, LV enddiastolic diameter; LVESD, LV endsystolic diameter; LVPW, LV posterial wall thickness; LVFS, LV fractional shortening.

Parameters	**WT**	**CM-Fgf21**
Body weight	23.50 ± 1.68	19.35 ± 1.06**
HR (bpm)	429.25 ± 48.07	445.20 ±33.39
IVSW (mm)	0.84 ± 0.09	0.79 ± 0.08
LVEDD (mm)	3.69 ± 0.20	3.58 ± 0.20
LVESD (mm)	1.75 ± 0.14	1.84 ± 0.34
LVPW (mm)	0.81 ± 0.12	$0.66 \pm 0.05^{p=0.06}$
LVFS (%)	52.4 ± 2.4	48.9 ± 7.6
LV Mass (mg)	123.9 ± 28.9	104.9 ± 21.0
LV Mass/BW (mg/g)	5.2 ± 0.9	5.3 ± 1.0

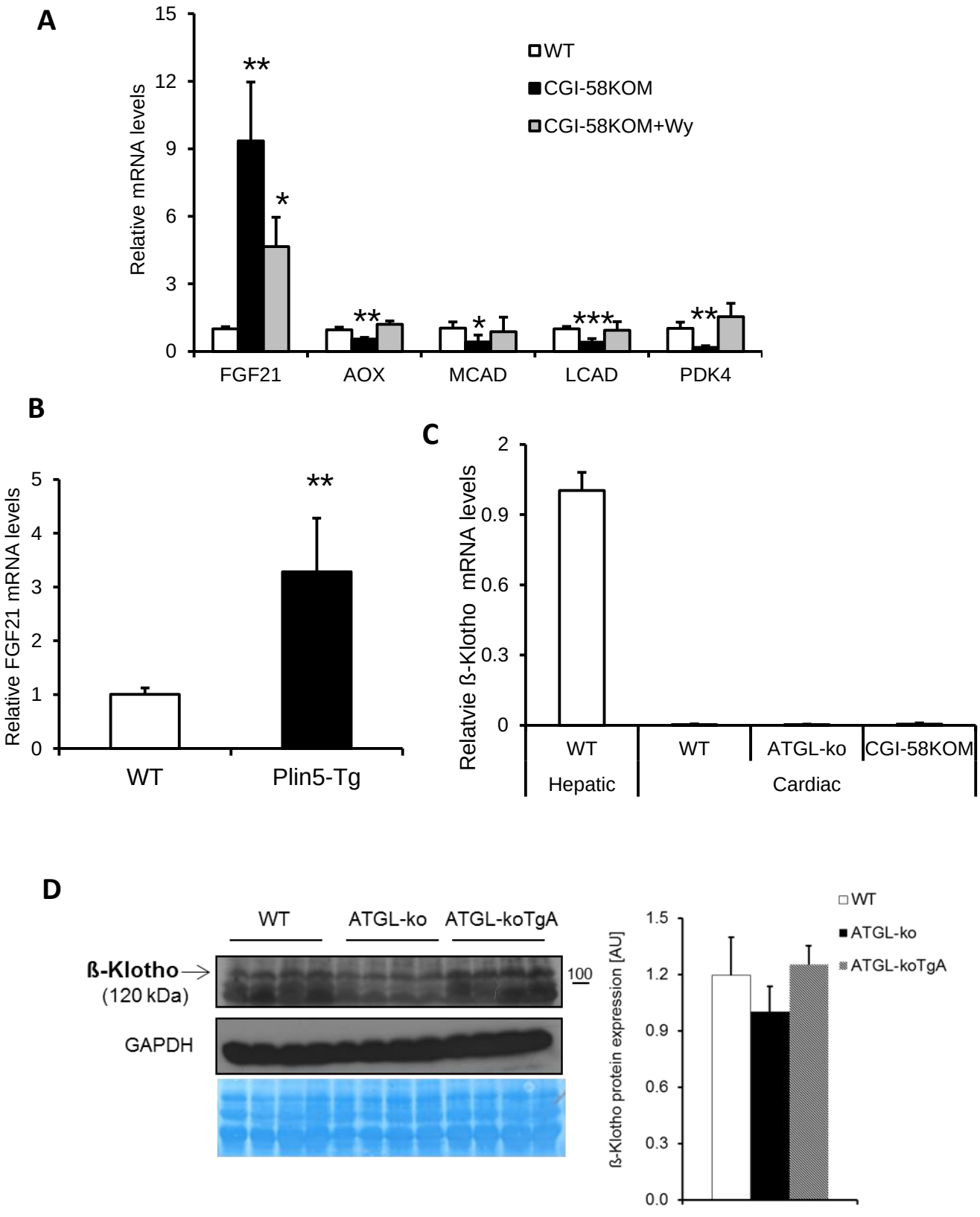
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1. Analysis of PPARα-target gene mRNA expression, Fgf21 and ß-Klotho expression in CM of WT mice and mutant mice exhibiting impaired cardiac TG catabolism. (**A**) Relative cardiac mRNA expression of FGF21 and established PPARα (and β/δ) target genes in 16-week old WT mice lacking the ATGL co-activator CGI-58 in muscle (CGI-58KOM), and CGI-58KOM mice fed with the PPARα agonist Wy14,643 (0.1% wt/wt) for 6 weeks (*n* = 5 - 6). (**B**) Relative FGF21 mRNA expression levels in CM of fasted 16-week old mice overexpressing mouse *Plin5* cDNA exclusively in the heart (*n* = 4). (**C**) ß-Klotho mRNA expression levels were determined by qRT-PCR in hepatic and cardiac tissue derived from fasted WT mice, ATGL-deficient mice (ATGL-ko) and mice lacking the ATGL co-activator CGI-58 in muscle (CGI-58KOM) (*n* = 4 -5).(**D**) ß-Klotho protein levels determined by western blotting using cardiac tissue lysates and a polyclonal antibody raised against ß-Klotho. Equal protein amounts (30 µg) were separated by SDS-PAGE prior blotting. GAPDH served as loading control. Relative intensity of protein signals (lower panel) was determined using the ImageJ software. Data are mean + SD. **P < 0.05*, ***P < 0.01* and ****P < 0.001*.

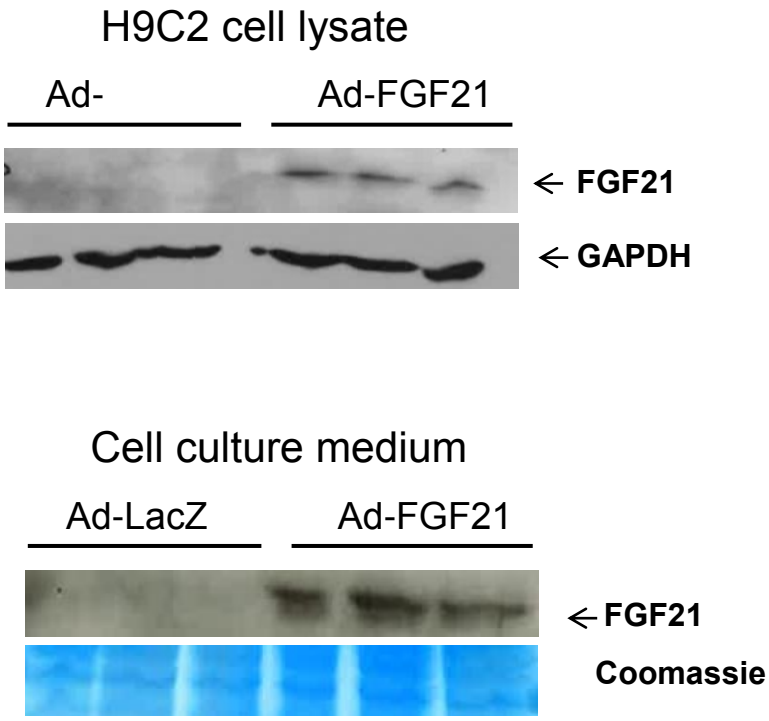
Supplementary Fig. 2. Analyses of FGF21 protein expression in H9C2 cardiomyotubes and in the cell culture medium of cells infected with lacZ or FGF21 expressing adenoviruses. Intracellular FGF21 protein levels and of cell culture medium were determined by western blot analysis using a goat polyclonal antibody raised against human FGF21 peptide. For Western blot analyses, equal protein amounts (50 µg) were separated by SDS-PAGE prior blotting. GAPDH served as loading control and cytosolic marker for intracellular proteins. Proteins in the cell culture medium were precipitated with TCA and equal protein loading was confirmed by coomassie blue staining of the membrane (*n* = 3).

Supplementary Fig. 3. Relative mRNA expression of ER stress marker genes in CM of CM-Ffg21 transgenic mice. Relative mRNA levels of the ER stress markers *Grp78/BiP*, *Chop*, *Pdi* and *Erdj4* in CM of 16-week old fasted WT and CM-Fgf21 transgenic mice. mRNA levels were determined by RT-qPCR of RNA isolated from CM of WT and transgenic mice (*n* = 5 - 6). Data are mean + SD.

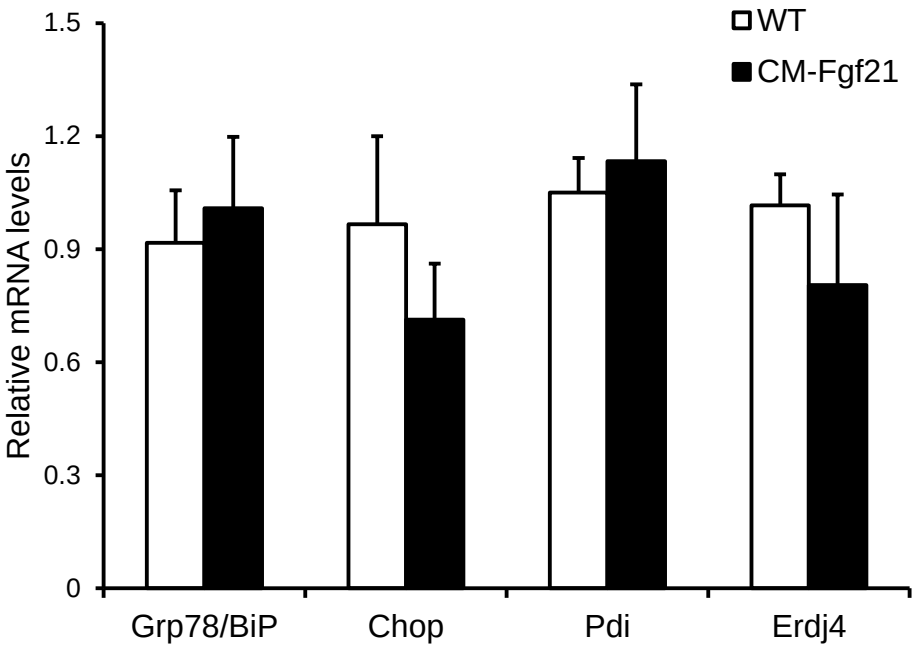
Supplementary Fig. 4. AMPKα and phosphorylated AMPKα protein levels in cardiac tissue of WT and CM-Fgf21 mice. AMPKα and phosphorylated AMPKα protein expression was determined in CM lysates (loading 25 µg protein) of WT and CM-Fgf21 transgenic mice by western blotting. Rabbit monoclonal antibodies specific for AMPKα and phosphorylated AMPKα (Thr172) were applied and GAPD served as loading control.



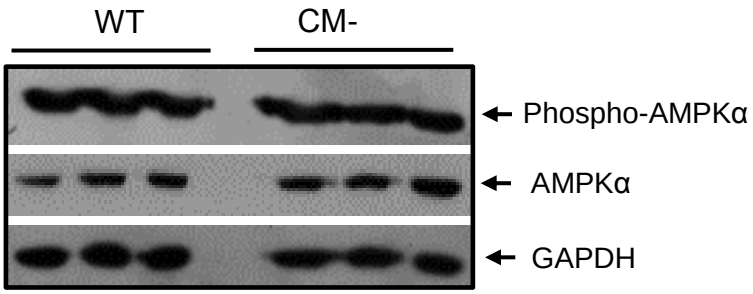
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4