ONLINE DATA SUPPLEMENTS

Expanded Methods and Results

Animal handling

In each animal experiment, mice were randomly assigned to each group. Number of animals per group to detect biologically significant effect sizes was calculated using appropriate statistical sample size formula and indicated in the biometrical planning section of the animal license submitted to the governing authority.

All mice were maintained on a 12 hrs light-dark cycle at 22°C with ad libitum access to regular unrestricted diet (Kliba Nafag #3437, Promivi Kliba AG, Kaiseraugst, CH) and water. Mice were killed by cervical dislocation and blood was taken for serum preparation. Organs including tumor, liver, heart, fat pads and GC were weighed, prepared for histology, snap frozen and used for further analysis. Left hind leg was removed for assessment of tibia length.

Echocardiography

C26 mice: Two-dimensional transthoracic echocardiography was performed on conscious mice by using Vevo 2100 (VisualSonics, Ontario, CA) by a single investigator. For assessment of systolic function B-mode parasternal long axis (PSLAX) was performed. Fractional shortening was calculated semi-automatically by supplied tracing software.

MC38 and APC delta 580 mice: A two-dimensional parasternal short axis view and M-Mode tracings of the left ventricle of conscious mice were obtained using a Sonos 5500 echocardiogram with a linear S 15 transducer (15 Hz) (Philips Healthcare, Eindhoven, NL). M-Mode tracings were used to determine left ventricular internal diameter as the largest anteroposterior diameter in either diastole (left ventricular end diastolic diameter (LVEDD)) or systole (left ventricular end systolic diameter (LVESD)). Diameters were averaged from at least three consecutive cardiac cycles. Left ventricular fractional shortening (FS) was calculated as (LVEDD-LVESD)/LVEDD and expressed as a percentage.

Pressure-Volume loop measurement

Mice were anesthetized by intraperitoneal injection of a mixture of medetomidin (0.5 µg/g body weight), fentanyl (0.5 µg/g body weight) and midazolam (5 µg/g body weight). Body temperature was maintained at 37°C using a homoeothermic blanket. Measurements were performed in closed-chest. A 1.2 FR catheter (Scisense Inc., Ontario, CA) was placed into the left ventricle through the carotid artery of the spontaneously breathing mice. Left ventricular volumes were extrapolated from admittance magnitude and admittance phase in real time using the ADVantage PV System (Scisense Inc., Ontario, CA). Pressure and volume data were recorded using a Scisense 404 - 16 Bit Four Channel Recorder with LabScribe2 Software (Scisense Inc., Ontario, CA). Mice were sacrificed after the measurement

Histology

Tissues were collected and immediately stored in 4% paraformaldehyde for 24 h. Paraffin embedded hearts were cut in 1 µm sections and mounted. Sections were stained with hematoxylin and eosin (H&E) or with Masson's trichrome by standard procedures.

Cardiomyocyte area was determined using colored pictures from H&E stained heart cross sections which were captured with a CCD camera (CC-12 camera, Olympus Soft Imaging Solutions, Münster, Germany) under 20x magnification (Olympus BX51, Hamburg, Germany) and the analySIS software (Soft Imaging Systems, Münster, Germany). Analysis was performed in a blinded manner. Only cardiomyocytes with a visible nucleus in the middle of the cell and an unbroken cellular membrane were measured.

High throughput analysis of cardiomyocyte size

Neonatal rat cardiomyocytes were plated onto gelatin (0.1%) coated 384-well plates at 6 x 10^3 cells/well. Three days after seeding cells were treated with conditioned SN for 24 h. Cells were incubated with primary antibody (anti-sarcomeric α-actinin, 1:500 in 5% goat serum; Sigma Aldrich, #A7811) overnight. Next day, cells incubated with secondary antibody (Alexa Fluor®

488 anti-mouse, 1:400 in 5% goat serum; Life Technologies, Darmstadt, Germany) and in parallel with phalloidin-tetramethylrhodamine B isothiocyanate (phalloidin-TRITC, 1:1000 in 5% goat serum; Sigma Aldrich, #P1951) for 1 h and additionally with Hoechst (1:10,000) for 10 min. Imaging was done using an automated BD Pathway 855 Cellular Bioimaging System with a 20x objective (BD, Heidelberg, Germany) in combination with a Hamamatsu ORCA-ER Digital Camera (Hamamatsu Photonics, Herrsching am Ammersee, Germany). 25 fields per well were imaged containing channels for Hoechst (DNA), which was used as the focus channel for every single image, FITC (Fluorescein isothiocyanate; α-actinin) and TRITC (actin). The FITC channel was used to detect the Alexa Fluor® 488 label.

CellProfiler cell image analysis software (Broad Institute, Cambridge, MA, USA) was used to analyze the images. Object selection was based in adaptive intensity and fixed size thresholds for every single object. Object segmentation was optimized to achieve best possible resolution of single objects in dense clusters of cells. Parent objects were segmented in channel 1 (Hoechst) for data analysis. In addition, integrated intensity of objects in channel 1, mean area of child objects in channel 2 (FITC) and mean TRITC-intensity of child objects based on segmentation in channel 2 were measured. Based on the integrated intensity of the Hoechst signal of every single nucleus, nuclei were binned into viable or apoptotic cells. Only viable cells were used for further analysis. According to a threshold that was set manually for the mean TRITC signal, cells were either identified as cardiomyocytes or non-cardiomyocytes. For the cardiomyocyte fraction, each object was measured by its area as measured in channel 2 (FITC). Areas of cardiomyocytes measured in channel 2 (FITC) were displayed as histogram.

Engineered heart tissue (EHT)

EHTs were generated as previously described (Hirt MN. et al., 2012). In brief, ventricular heart cells (the atria were carefully excised) from neonatal Wistar and Lewis rats (postnatal day 0 to 3) were isolated by a fractionated DNase/Trypsin digestion protocol. This procedure was reviewed and approved by the Ethics Commission of the Medical Association of Hamburg (Permit

Number: A6/516). Per EHT 500,000 rat ventricular heart cells, fibrinogen, thrombin and DMEM (2x, to match the volumes of fibrinogen and thrombin and thus ensuring isotonic conditions) were mixed and pipetted into molds, which were obtained by casting 2% agarose (in PBS) around polytetrafluoroethylene spacers in a 24-well culture dish. After polymerization of fibrin (1.5 hours), EHTs were transferable to new cell culture dishes filled with medium. EHTs were maintained in 37 °C, 7% CO_2, and 40% O_2 humidified cell culture incubators throughout experiments. EHT-medium for the first 13 days of culture consisted of DMEM (Biochrom, F0415), 10% horse serum inactivated (Gibco, 26050), 1% Pen/Strep (Gibco, 15140), insulin (10 µg/ml, Sigma-Aldrich, #857653), and aprotinin (33 µg/ml, Sigma-Aldrich, #A1153). From day 13 onwards, three groups each comprising 6 EHTs were formed (matched according to contractile force). One group was further kept in EHT-medium containing 10% horse serum, the second group in serum-free medium, i.e. the above medium without horse serum plus triiodothyronine (T_3, 0.5 ng/ml, European Commission – Joint Research Centre IRMM-469) and hydrocortisone (50 ng/ml, Sigma-Aldrich, #H0888), and the third group in EHT-medium prior to that conditioned on C26 cells for 2 days. The contractile activity of all EHTs was recorded repetitively throughout the culture time by a camera positioned above the incubator equipped with a glass roof. Cell culture dishes remained closed, which allowed repeated measurement under sterile conditions. Contractile force, frequency, and times and velocities of contraction and relaxation were analyzed by customized software (CTMV, Pforzheim, Germany) from these videos.

Triglyceride extraction from tissue

Frozen heart tissues (minimum 70 mg) were lysed in 1.5 ml of chloroform:methanol (2:1) mixture. After centrifugation (30 min, 13,000 rpm, 20°C) 200 µl of 150 mM NaCl were added to 1 ml of the supernatants and were centrifuged again (2000 rpm, 5 min). 200 µl of the lower organic phase were transferred to tubes containing 40 µl chloroform:Triton™ X-100 (1:1) solution. Samples were dried using Speedvac (Christ, Osterode am Harz, Germany) overnight. To

the remaining Triton-lipid solutions were 200 µl of dH_2O added. Samples were mixed by placing on a rotating wheel for 1h at room temperature and stored at -80°C until required for triglyceride (TG) determination. TG level were determined using the Serum TG Determination Kit (Sigma Aldrich, #TR0100) and 20 µl of extracted heart supernatant. TG content (TG-bound glycerol) was determined by subtracting the free glycerol (blank value) from the total glycerol (assay value).

Triglyceride isolation from cells

Neonatal rat cardiomyocytes were seeded at 1.25 x 10^5 cells per well on 6-well plates. Three days after seeding cells were treated with conditioned SN for 24 h.

Cells were harvested in 200 µl of Triton™ X-100 (TX) lysis buffer (150 mM NaCl, 0.05% Triton™ X-100, 10 mM Tris/HCl (pH 8.0), 1x Protease Inhibitor Cocktail), frozen at -80°C for at least 24 h and sonicated after thawing for two cycles (one cycle: 30 sec on and 30 sec off). Cell debris was removed by centrifugation. Protein concentration was determined using the Pierce® BCA Protein Assay Kit (Thermo Scientific, Schwerte, Germany). TG level were determined using the Serum TG Determination Kit (Sigma Aldrich, #TR0100) and 70 µl of extracted cell supernatant. TG content (TG-bound glycerol) was determined by subtracting the free glycerol (blank value) from the total glycerol (assay value).

Palmitate Uptake (Tracer)

Neonatal rat cardiomyocytes were treated and seeded as described for TG isolation from cells. After 30 min starvation in Krebs-Henseleit buffer (KHB) cells were treated with 1 mM BSA palmitate conjugate solution spiked with 0.5 µCi/ml ^3H palmitate in KHB supplemented with 500 µM carnitine and 450 mg/l glucose (based on Seahorse protocol) for 1 h. The aqueous phase of the supernatants was isolated by addition of 2:1 chloroform:methanol. For protein and TG measurements, cells were lysed as described for TG isolation from cells. Disintegrations per minute (dpm) were counted in a scintillation counter.

Transfection of HEK293 A cells with cDNA expression plasmids

1 x 10^5 HEK293 A cells per well were seeded on 6-well plates 24 h prior to transfection and were transfected at 60% confluency using the calcium phosphate method and 2 µg of plasmid DNA. Cells were transfected either with ready-to-use cDNA expression plasmids obtained from Source BioScience (Nottingham, UK) or with pDest-eGFP-N1 plasmid (Addgene, Cambridge, MA, USA). Medium was changed 24 h after transfection and conditioned SN was produced as described before**.**

Transfection of C26 cells with small interfering RNA (siRNA)

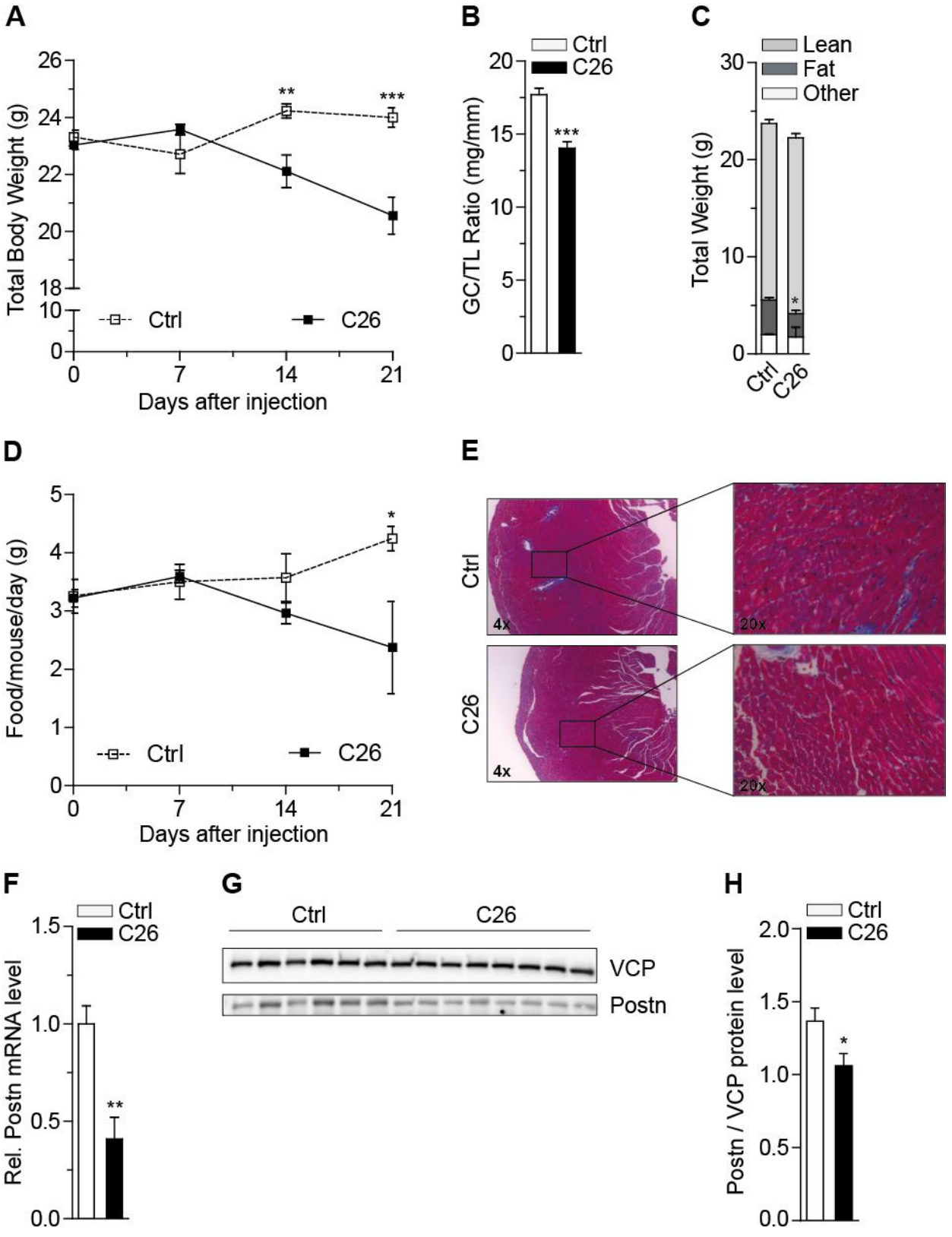
C26 cells were seeded at 1 x 10^5 cells/well on 6-well plates 24 h prior to transfection and were transfected at 60% confluency using HiPerFect Transfection Reagent (Qiagen, Hilden, Germany) and 5 nM of siRNA (Qiagen, Hilden, Germany). Cells were either transfected with a single siRNA or with seven siRNAs at once. An Alexa488 labelled siRNA and AllStars Negative Control (NC) from Qiagen (Hilden, Germany) served as controls. Medium was changed 24 h after transfection and conditioned SN was produced as described before**.**

Immunofluorescence

Cardiomyocytes were seeded at 1.0 x 10^5 - 1.5 x 10^5 cells per well on cover slips in 24-well plates which were coated with 0.1% gelatin. Three days after seeding the cardiomyocytes were treated with conditioned SN for 24 h (neonatal rat cardiomyocytes) or 48 h (adult mouse cardiomyocytes). Cells were stained with the following antibodies: primary antibody: anti-sarcomeric α-actinin (1:200 in 5% goat serum, 4°C, overnight; Sigma Aldrich, #A7811) and secondary antibody: Texas Red® anti-mouse (1:200 in 5% goat serum, 1 h; Vector Labs, Burlingame, CA) together with Hoechst 33258 (1:2000, 30 min; Dianova, Hamburg, Germany). Cover slips with the stained cells were fixated on object slides using FluorSave™ Reagent. Cardiomyocyte area was determined using colored pictures from α-actinin immunostained

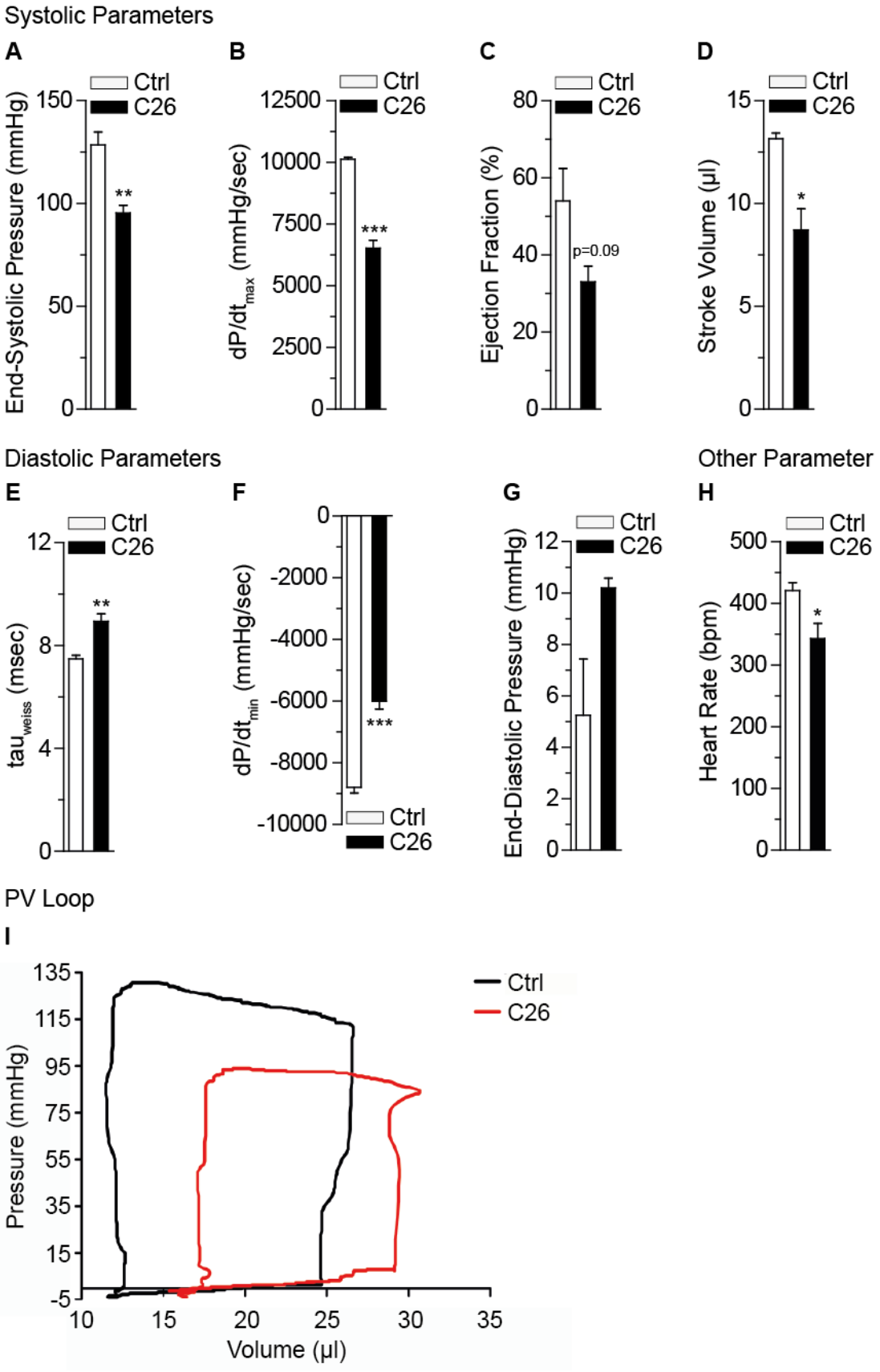
cardiomyocytes which were captured as described for the H&E cross sections. Analysis was performed in a blinded manner. Roughly 100 cells per condition were counted, and the averaged values were used for analysis.

Figure S1


A
Total Body Weight (g)
Days after injection
Ctrl
C26
B
GC/TL Ratio (mg/mm)
C
Total Weight (g)
Lean
Fat
Other
D
Food/mouse/day (g)
E
4x
20x
F
Rel. Postn mRNA level
G
VCP
Postn
H
Postn / VCP protein level

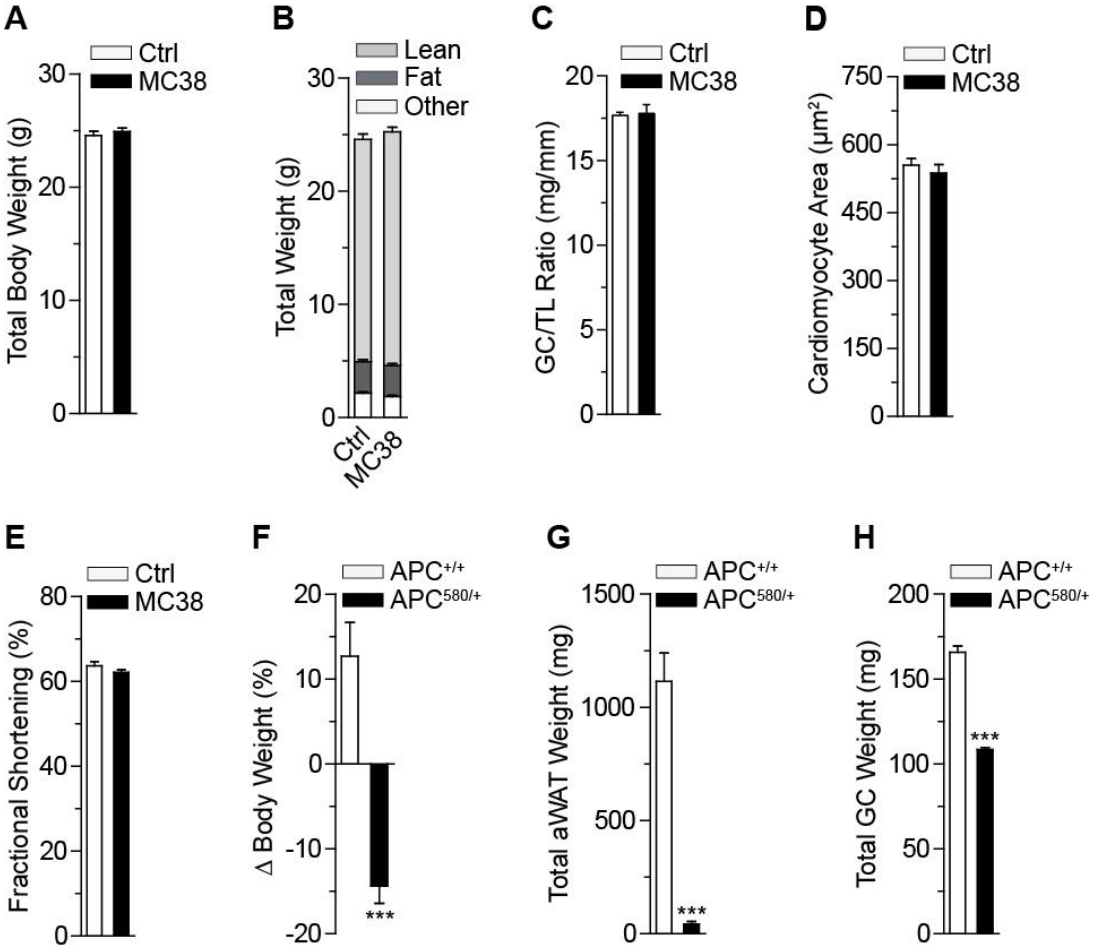
Supplemental Figure S1: (A) Body weight progression of control (Ctrl) and C26 bearing mice (n = 6 animals in Ctrl group, n = 10 animals in C26 group). **(B)** Gastrocnemius (GC) muscle weight/ tibia length (TL) ratio of the same mice at day 21 post PBS (Ctrl) or C26 cell injection. **(C)** Total body fat and total lean mass of the same mice at day 21 post PBS (Ctrl) or C26 cell injection. **(D)** Progression of food consumption for the same mice. **(E)** Representative images of trichrome stained heart cross-sections of a control (Ctrl) and a C26 bearing mouse (C26) at day 21 post PBS (Ctrl) or C26 cell injection (Magnification: 4x and 20x). **(F)** Relative mRNA level of fibrosis marker periostin (Postn) in the hearts of the same mice at day 21 post PBS (Ctrl) or C26 cell injection. **(G)** Immunoblot against periostin (Postn) and valosin-containing protein (VCP; loading control) from heart lysates of the same mice at day 21 post PBS (Ctrl) or C26 cell injection. **(H)** Relative Postn protein levels determined by densitometric analysis of the displayed immunoblot. **(A)** and **(D)** Data are means ± SEM. *indicates significance using 2-way ANOVA, Bonferroni post-test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **(B)**, **(C)**, **(F)** and **(H)** Data are means ± SEM. *indicates significance using Student's t-test with Welch correction, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Figure S2


Systolic Parameters
A
End-Systolic Pressure (mmHg)
150
100
50
0
Ctrl
C26
**
B
dP/dtmax (mmHg/sec)
12500
10000
7500
5000
2500
0
Ctrl
C26
***
C
Ejection Fraction (%)
80
60
40
20
0
Ctrl
C26
p=0.09
D
Stroke Volume (µl)
15
10
5
0
Ctrl
C26
*
Diastolic Parameters
E
tauweiss (msec)
12
8
4
0
Ctrl
C26
**
F
dP/dtmin (mmHg/sec)
0
-2000
-4000
-6000
-8000
-10000
***
Ctrl
C26
Other Parameter
G
End-Diastolic Pressure (mmHg)
12
10
8
6
4
2
0
Ctrl
C26
H
Heart Rate (bpm)
500
400
300
200
100
0
Ctrl
C26
*
PV Loop
I
Pressure (mmHg)
135
115
95
75
55
35
15
-5
10
15
20
25
30
35
Volume (µl)
Ctrl
C26

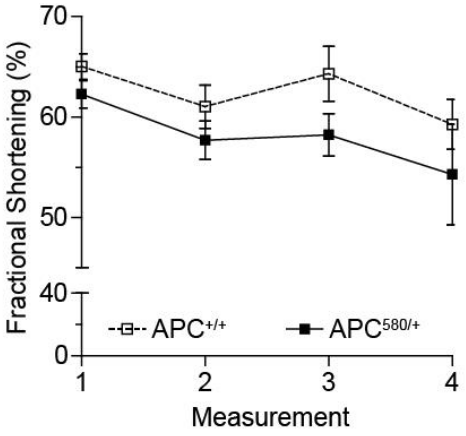
Supplemental Figure S2: Cardiac haemodynamics in control (Ctrl) and C26 bearing mice (C26) assessed by pressure-volume (PV) loops at day 14 (n = 4 animals for Ctrl group, n = 5 animals for C26 group). **(A) - (D)**: Bar graphs showing different parameters for systolic function: **(A)** End-systolic pressure (ESP), **(B)** dP/dt_{max}, **(C)** Ejection fraction (EF) and **(D)** Stroke volume (SV). **(E) - (G)** Bar graphs showing different parameters for diastolic function: **(E)** Tau_{weiss}, **(F)** dP/dt_{min} and **(G)** End-diastolic pressure (EDP). **(H)** Heart rate (HR). **(I)** Representative pressure-volume (PV) loops for a control (Ctrl) and a C26 bearing mouse which were used for the quantifications shown in **(A) - (H)**. **(A) - (H)** Data are means ± SEM. *indicates significance using Student's t-test with Welch correction, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

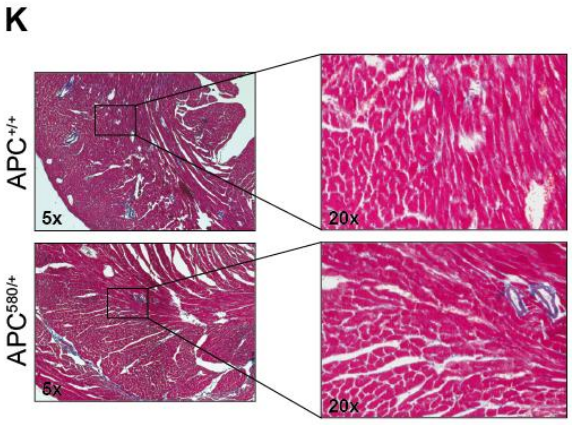
Figure S3

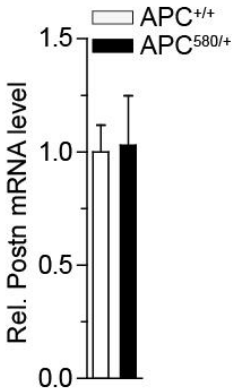


I

Test	APC +/+	APC 580/+
Hb (g/dl)	12.4±1	0.85±0.05
RDW (%)	16.9±0.7	28.2±0.95
MCV (fl)	46.5±0.5	82±2
MCH (pg)	15.5±0.5	22.5±3.5



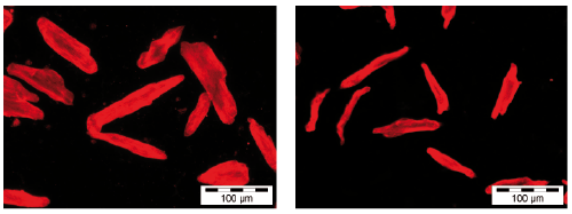




Supplemental Figure S3: (A) Total body weight of control (Ctrl) and MC38 bearing mice (MC38) (n = 8 animals for Ctrl group, n = 10 for MC38 group). Tumor weight was excluded. **(B)** Total lean and body fat mass of the same mice. **(C)** Gastrocnemius (GC) muscle weight/ tibia length (TL) ratio of the same mice. **(D)** Quantification of cardiomyocyte cross-sectional areas for the same mice ($n \geq 100$ cardiomyocytes per heart; n = 4 hearts for Ctrl group, n = 5 hearts for MC38 group). **(E)** Fractional shortening (FS) for the same mice determined by using Sonos 5500. **(F)** Delta body weight change of wild-type ($APC^{+/+}$) and APC delta 580 ($APC^{580/+}$) mice at day of sacrifice (n = 6 animals for $APC^{+/+}$ group. n = 9 animals for $APC^{580/+}$ group). **(G)** Total abdominal white adipose tissue (aWAT) weight of the same mice. **(H)** Total gastrocnemius (GC) muscle weight of the same mice. **(I**) Blood count of anemic markers (hemoglobin (Hb), red cell distribution width (RDW), mean corpuscular/cell volume (MCV) and mean corpuscular/cell hemoglobin (MCH)) in the same mice (Date are means ± SEM; n = 2 per group). **(J)** Progression of fractional shortening (FS) of the same mice determined by using Sonos 5500. **(K)** Representative images of trichrome stained heart cross-sections of a wild-type ($APC^{+/+}$) and APC delta 580 ($APC^{580/+}$) mouse (Magnification: 5x and 20x). **(F)** Relative mRNA level of fibrosis marker periostin (Postn) in the hearts of the same mice. **(A) - (H), (J)** and **(L)** Date are means ± SEM. *indicates significance using Student's t-test with Welch correction, ***$p < 0.001$.

Figure S4

A



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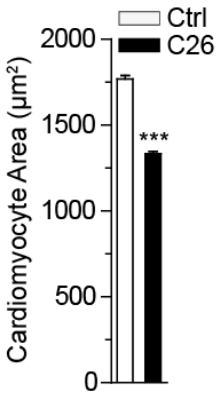
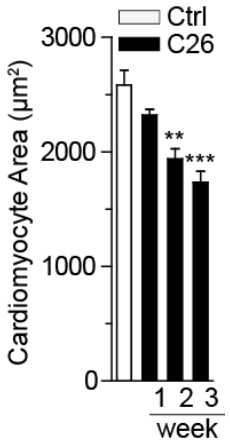


C



D

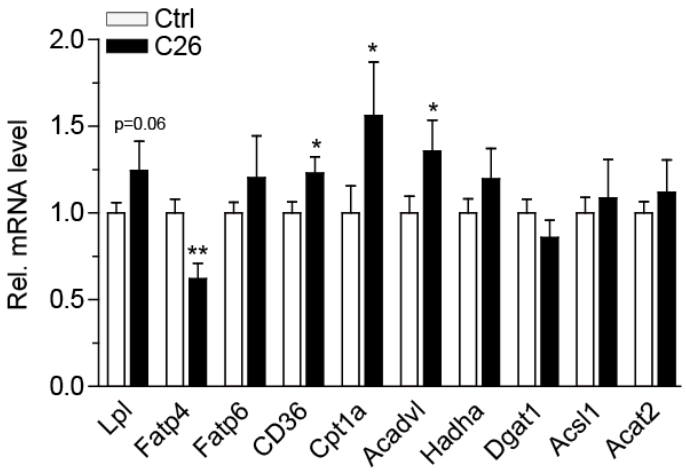
Annotation Cluster 1	Enrichment Score: 8.22						
	Term	Count	PValue	List total	Pop Hits	Pop Total	FDR
GO:0043436	Oxoacid metabolic process	47	$9.1x10^{-10}$	508	479	14,219	$1.6x10^{-6}$
GO:0019752	Carboxylic acid metabolic process	47	$9.1x10^{-10}$	508	479	14,219	$1.6x10^{-6}$
GO:0006082	Organic acid metabolic process	47	$9.6x10^{-10}$	508	480	14,219	$1.7x10^{-6}$
GO:0032787	Monocarboxylic acid metabolic process	33	$2.0x10^{-9}$	508	268	14,219	$3.6x10^{-6}$
GO:0042180	Cellular ketone metabolic process	47	$2.0x10^{-9}$	508	491	14,219	$3.6x10^{-6}$
GO:0006631	Fatty acid metabolic process	25	$4.0x10^{-8}$	508	184	14,219	$6.9x10^{-5}$
GO:0006629	Lipid metabolic process	55	$5.7x10^{-8}$	508	696	14,219	$1.0x10^{-4}$
GO:0044255	Cellular lipid metabolic process	42	$2.3x10^{-7}$	508	482	14,219	$4.0x10^{-4}$

Annotation Cluster 6	Enrichment Score: 1.89						
	Term	Count	PValue	List Total	Pop Hits	Pop Total	FDR
GO:0015718	Monocarboxylic acid transport	6	0.0004	508	19	14,219	0.76
GO:0046942	Carboxylic acid transport	11	0.003	508	99	14,219	4.74
GO:0015849	Organic acid transport	11	0.003	508	100	14,219	5.01
GO:0015908	Fatty acid transport	4	0.01	508	13	14,219	16.00
GO:0015909	Long-chain fatty acid transport	3	0.02	508	7	14,219	34.27
GO:0015804	Neutral amino acid transport	3	0.08	508	13	14,219	75.15
GO:0006865	Amino acid transport	6	0.11	508	71	14,219	86.75
GO:0015837	Amine transport	7	0.11	508	92	14,219	87.12

Annotation Cluster 7	Enrichment Score: 1.85						
	Term	Count	PValue	List Total	Pop Hits	Pop Total	FDR
GO:0010876	Lipid localization	13	0.002	508	128	14,219	3.56
GO:0006869	Lipid transport	12	0.003	508	119	14,219	5.94
GO:0033036	Macromolecule localization	35	0.40	508	902	14,219	99.99

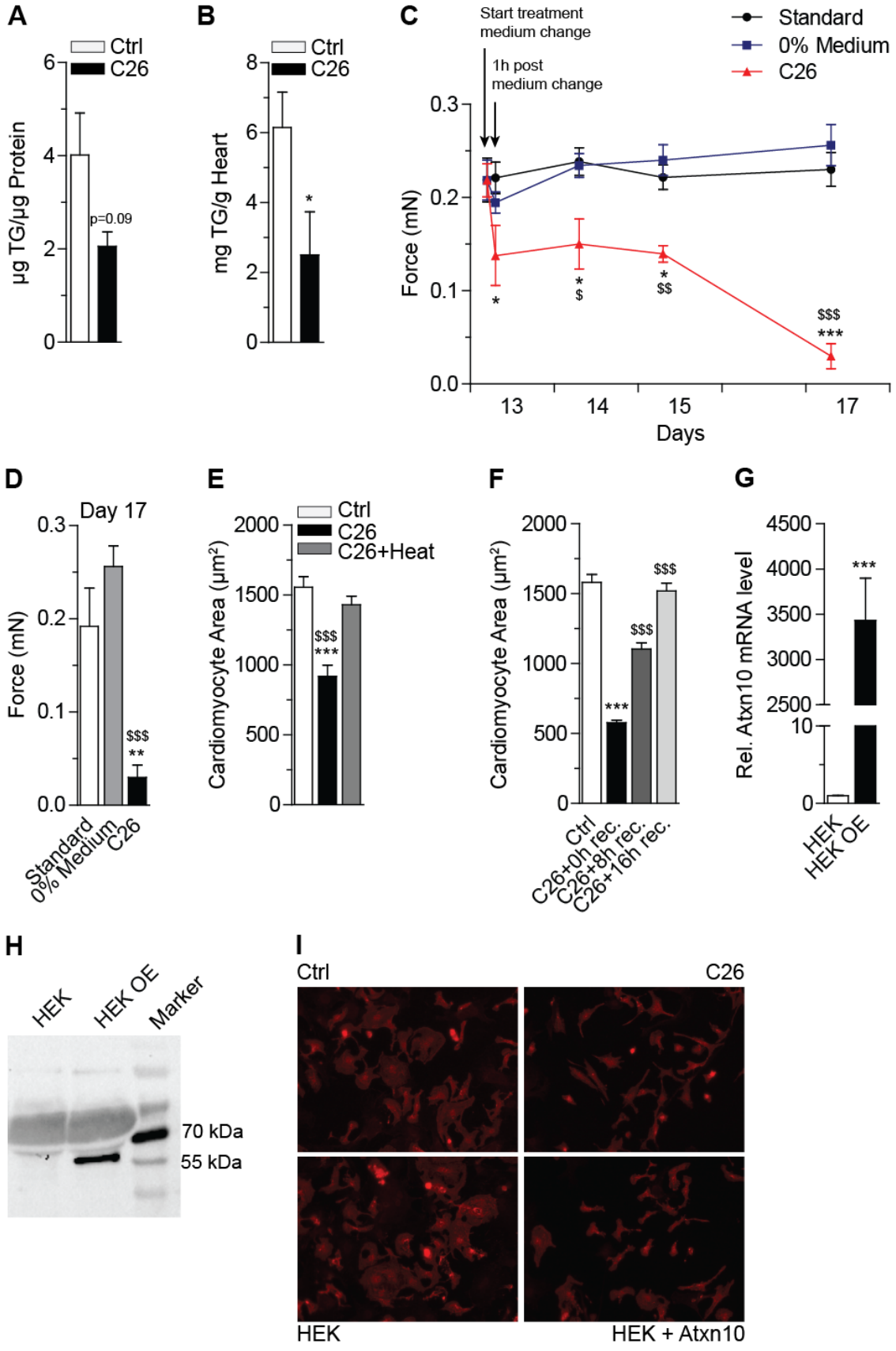
Annotation Cluster 10	Enrichment Score: 1.77						
	Term	Count	PValue	List Total	Pop Hits	Pop Total	FDR
GO:0033559	Unsaturated fatty acid metabolic process	8	0.0002	508	36	14,219	0,42
GO:0006690	Icosanoid metabolic process	7	0,001	508	34	14,219	1,99
GO:0008610	Lipid biosynthetic process	20	0,007	508	285	14,219	11,29
GO:0006633	Fatty acid biosynthetic process	9	0,008	508	81	14,219	13,35
GO:0006692	Prostanoid metabolic process	4	0,01	508	14	14,219	19,40
GO:0006693	Prostaglandin metabolic process	4	0,01	508	14	14,219	19,40
GO:0046456	Icosanoid biosynthetic process	5	0,01	508	27	14,219	22,75
GO:0006636	Unsaturated fatty acid biosynthetic process	5	0,02	508	28	14,219	25,41
GO:0046394	Carboxylic acid biosynthetic process	11	0,03	508	141	14,219	40,85
GO:0016053	Organic acid biosynthetic process	11	0,03	508	141	14,219	40,85
GO:0043450	Alkene biosynthetic process	3	0,12	508	17	14,219	89,66
GO:0019370	Leukotriene biosynthetic process	3	0,12	508	17	14,219	89,66
GO:0006691	Leukotriene metabolic process	3	0,15	508	19	14,219	93,69
GO:0043449	Cellular alkene metabolic process	3	0,17	508	21	14,219	96,28

E



Supplemental Figure S4: (A) Immunofluorescence staining of α-actinin in adult mouse cardiomyocytes cultured in standard medium (Ctrl) or conditioned medium from C26 cells for 48h (Magnification: 20x). **(B)** Quantification of cardiomyocyte area of the experiment shown in **(A)** ($n \geq 100$ cardiomyocytes per condition). **(C)** Size of neonatal rat cardiomyocytes that were cultured in 2% serum from control (Ctrl) or C26 bearing mice (C26) that was collected at day 7, 14 and 21 post PBS (Ctrl) or C26 cell injection. **(D)** Gene ontology enrichment analysis of biological processes (GO-BP) of heart lysates from C26 bearing mice sacrificed at day 14 post PBS (Ctrl) or C26 cell injection (n = 3 animals per group). **(E)** Relative mRNA level of lipoprotein lipase (Lpl), fatty acid transporter 4 (Fatp4), fatty acid transporter 6 (Fatp6), fatty acid translocase (FAT/CD36), carnitine palmitoyltransferase 1a (Cpt1a), acyl-CoA dehydrogenase, very long chain (Acadvl), hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (Hadha), diglyceride acyltransferase 1 (Dgat1), acyl-CoA synthetase long-chain family member 1 (Acsl1) and acetyl-CoA acetyltransferase 2 (Acat2) in hearts from control (Ctrl) and C26 bearing mice mice (C26) at day 21 post PBS (Ctrl) or C26 cell injection (n = 6 animals for Ctrl group, n = 10 animals for C26 group). **(B)** and **(E)** Data are means ± SEM. *indicates significance using Student's t-test with Welch correction, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **(C)** Data are means ± SEM. *indicates significance using 2-way ANOVA, Bonferroni post-test, **$p < 0.01$, ***$p < 0.001$.

Figure S5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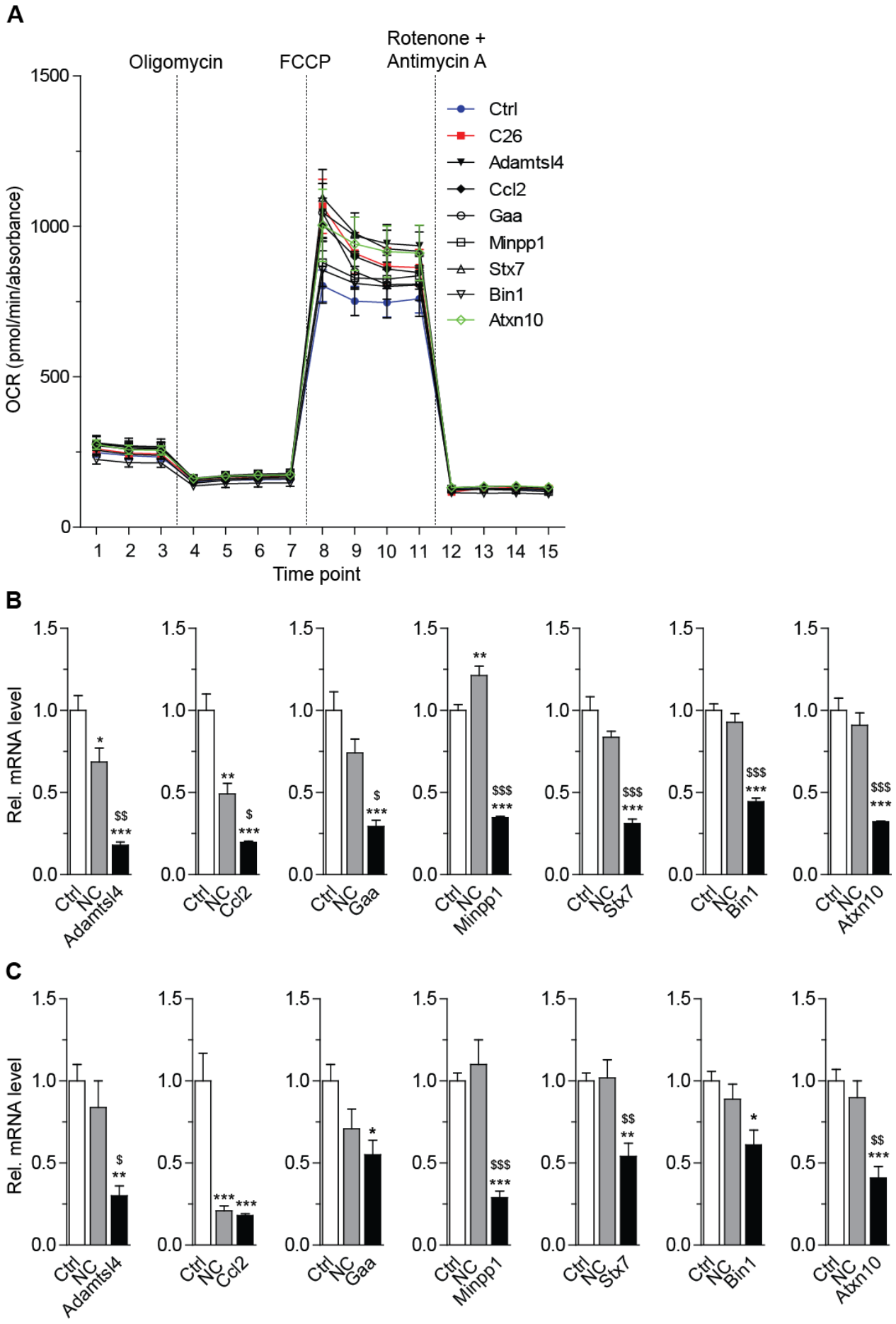
Ctrl
C26
µg TG/µg Protein
p=0.09
B
Ctrl
C26
mg TG/g Heart
C
Start treatment medium change
1h post medium change
Standard
0% Medium
C26
Force (mN)
Days
D
Day 17
Force (mN)
Standard
0% Medium
C26
E
Ctrl
C26
C26+Heat
Cardiomyocyte Area (µm²)
F
Cardiomyocyte Area (µm²)
Ctrl
C26+0h rec.
C26+8h rec.
C26+16h rec.
G
Rel. Atxn10 mRNA level
HEK
HEK OE
H
HEK
HEK OE
Marker
70 kDa
55 kDa
I
Ctrl
C26
HEK
HEK + Atxn10

Supplemental Figure S5: (A) Triglyceride (TG) content of neonatal rat cardiomyocytes cultured in standard medium (Ctrl) or C26 conditioned medium (C26) for 24 h (n = 4 cell culture wells for Ctrl group, n = 4 cell culture wells for C26 group). TG content was normalized to protein content. **(B)** Triglyceride (TG) level in hearts of control (Ctrl) and C26 bearing mice (C26) (n = 5 animals for Ctrl group, n = 6 animals for C26 group). TG content was normalized to frozen dry heart weight. **(C)** and **(D)** Functional impact of conditioned medium from C26 cells on rat engineered heart tissue EHT. **(C)** After 13 days of standard culture 3 groups of each 6 EHTs with the same mean contractile force were formed. EHTs were incubated in standard EHT-medium (Standard), serum-free EHT-medium (0% Medium) and standard EHT after conditioning on C26 cells. Contractile force was recorded after 1 hour, 1 day (day 14 of culture), 2 days (day 15 of culture and 4 days (day 17 of culture) ($n \geq 3$). **(D)** Bar graph showing forces on day 17 of culture of the same EHT experiment (n = 5 cell culture wells for Standard group, n = 6 cell culture wells for 0% Medium group, n = 3 cell culture wells for C26 group). **(E)** Quantification of the size of neonatal rat cardiomyocytes cultured in standard medium (Ctrl), C26 conditioned medium (C26) and C26 conditioned medium that was heated at 95°C for 5 min (C26+heat) for 24 h ($n \geq 100$ per condition). **(F)** Quantification of the size of neonatal rat cardiomyocytes cultured in standard medium (Ctrl) and C26 conditioned medium (C26+0h recovery (rec.)) for 40 h and in C26 conditioned medium for 24 h followed by an exchange to standard medium for additional 8 h (C26+8h rec.) and 16h (C26+16h rec.), respectively ($n \geq 100$ per condition). **(G)** Relative mRNA level of Ataxin-10 (Atxn10) in untreated HEK293A cells (HEK) and HEK293A cells treated with Atxn10 overexpressing cDNA clone (HEK OE) (n = 3 per condition). **(H)** Immunoblot against Atxn10 (55 kDa) from conditioned medium of the same cells shown in **(G)**. Fraction containing Atxn10 was enriched by centrifugal protein enrichment using Nanosep® colums (10K; Pall, Port Washington, NY, USA) **(I)** Immunofluorescence staining of α-actinin in neonatal rat cardiomyocytes cultured in standard medium (Ctrl), conditioned medium from C26 cells or HEK293A cells either untreated or treated with Ataxin-10 (Atxn10) overexpressing cDNA clone for 24h. (Magnification: 20x). **(A)**, **(B)** and **(G)** Data are means ± SEM. *indicates significance

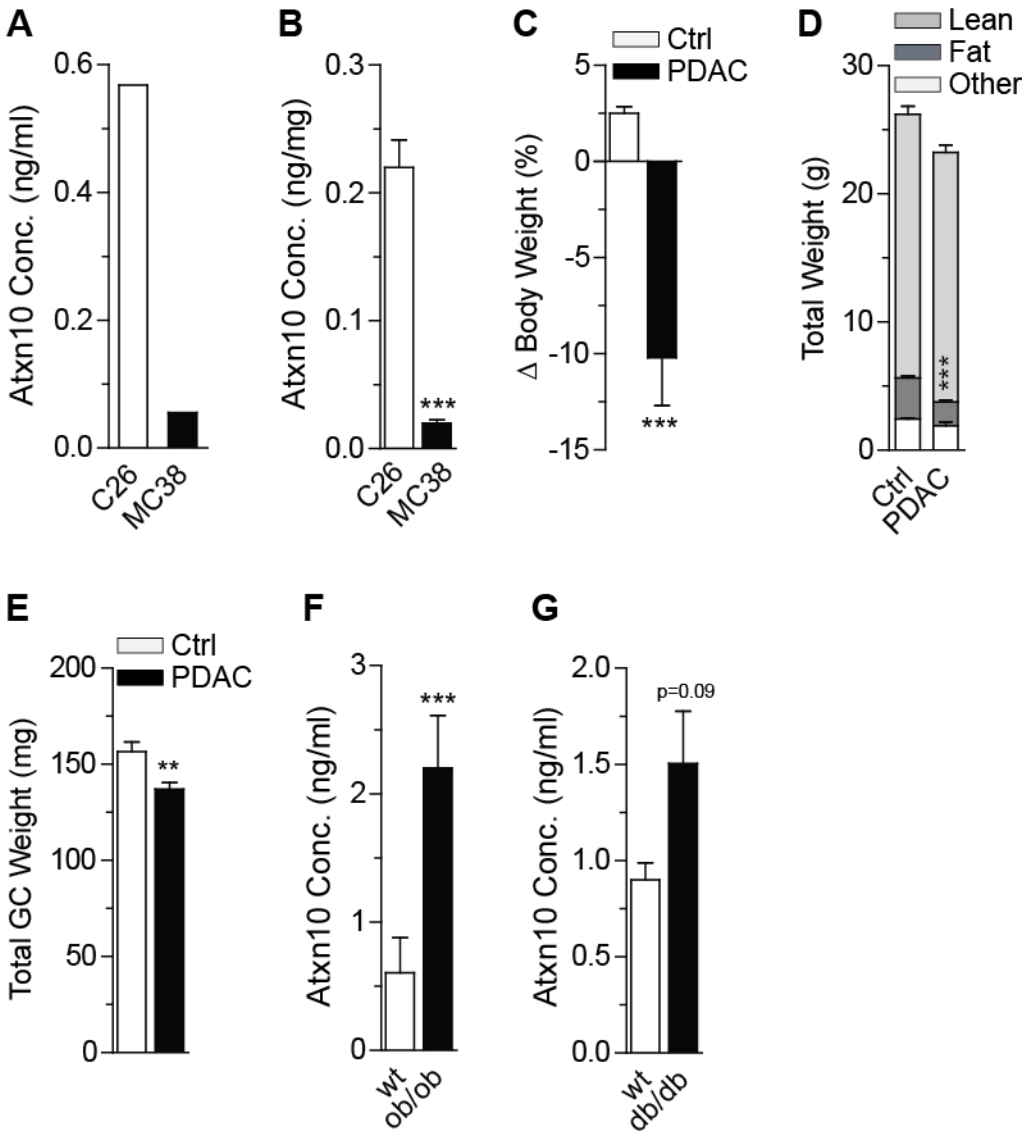
using Student's t-test with Welch correction, *$p < 0.05$, ***$p < 0.001$. **(C)** Data are means ± SEM. *indicates significance using 2-way ANOVA, Bonferroni post-test, compared to standard, *$p < 0.05$, ***$p < 0.001$. $^{\$}$indicates significance using 2-way ANOVA, Bonferroni post-test, compared to 0% medium, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$. **(D)**, **(E)** and **(F)** Data are means ± SEM. *indicates significance using 1-way ANOVA, Bonferroni post-test, compared to standard or Ctrl, **$p < 0.01$, ***$p < 0.001$. $^{\$}$indicates significance using 1-way ANOVA, Bonferroni post-test, compared to C26 or C26+0h rec., $^{\$\$\$}p < 0.001$.

Figure S6


A
Oligomycin
FCCP
Rotenone +
Antimycin A
OCR (pmol/min/absorbance)
Time point
Ctrl
C26
Adamtsl4
Ccl2
Gaa
Minpp1
Stx7
Bin1
Atxn10
B
Rel. mRNA level
Ctrl
NC
Adamtsl4
Ccl2
Gaa
Minpp1
Stx7
Bin1
Atxn10
C
Rel. mRNA level

Supplemental Figure S6: (A) MitoStress test of neonatal rat cardiomyocytes cultured in standard medium (Ctrl) or conditioned medium from C26 cells or HEK293A cells that over-expressed one of the selective "cachexokines" (Adamts like 4 (Adamtsl4), Chemokine ligand 2 (Ccl2), Glucosidase alpha acid (Gaa), Multiple inositol-polyphosphate phosphatase 1 (Minpp1), Syntaxin 7 (Stx7), Bridging integrator 1 (Bin1) and Ataxin-10 (Atxn10)) for 24 h (n = 6 cell culture wells for Bin1 group, n = 7 cell culture wells for Ctrl, Adamtls4 and Minpp1 group, n = 8 cell culture wells for C26, Atxn10, Ccl2, Gaa and Stx7 group). Mitochondrial function is expressed by oxygen consumption rate (OCR; means ± SEM). **(B)** Relative mRNA level of selective "cachexokines" (see **(A)**) of untreated C26 cells and C26 cells that were either transfected with negative control siRNA (NC) or with siRNA directed against the indicated genes (n = 4 per condition). **(C)** Relative mRNA level of selective "cachexokines" (see **(A)**) of untreated C26 cells and C26 cells that were either transfected with negative control siRNA (NC) or with a siRNA mixture directed against all indicated "cachexokines" (n = 4 per condition). **(B)** and **(C)** Data are means ± SEM. *indicates significance using 1-way ANOVA, Bonferroni post-test, compared to Ctrl *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. $^{\$}$indicates significance using 1-way ANOVA, Bonferroni post-test, compared to NC, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$.

Figure S7


A
Atxn10 Conc. (ng/ml)
0.6
0.4
0.2
0.0
C26
MC38
B
Atxn10 Conc. (ng/mg)
0.3
0.2
0.1
0.0
***
C26
MC38
C
Ctrl
PDAC
Δ Body Weight (%)
5
0
-5
-10
-15
***
D
Lean
Fat
Other
Total Weight (g)
30
20
10
0
***
Ctrl
PDAC
E
Ctrl
PDAC
Total GC Weight (mg)
200
150
100
50
0
**
F
Atxn10 Conc. (ng/ml)
3
2
1
0
***
wt
ob/ob
G
Atxn10 Conc. (ng/ml)
2.0
1.5
1.0
0.5
0.0
p=0.09
wt
db/db

Supplemental Figure S7: Ataxin-10 (Atxn10) levels in **(A)** conditioned medium from C26 and MC38 cells (n = 2 per condition), **(B)** in resected tumors from C26 and MC38 mice (n = 3 tumors for MC38 group, n = 5 tumors for C26 group) and **(C)** Delta body weight change of wild-type mice (Ctrl) and mice that underwent orthotopic implantation of pancreatic cancer cells (PDAC) (n = 8 animals for Ctrl group, n = 13 animals for PDAC group). **(D)** Total body fat and lean mass of the same mice. **(E)** Total gastrocnemius (GC) weight of the same mice. **(F)** Ataxin-10 (Atxn10) level in wild-type (Ctrl) and obese (ob/ob) mice (n = 5 animals per group). **(G)** Ataxin-10 (Atxn10) level in wild-type (Ctrl) and diabetic (db/db) mice (n = 5 animals per group). **(A) - (G)** Data are means ± SEM. *indicates significance using Student's t-test with Welch correction, **$p < 0.01$, ***$p < 0.001$.

Supplemental Table Legends

Table 1:

Raw data of secretome analysis of cachexia-inducing C26 cells compared to non-cachexia-inducing MC38 cells (related to Figure 3).

Table 2:

Annotation of all used ready-to-use cDNA overexpression plasmids. All ORF clones are based on the human gene sequence and do not include any tag. cDNA clones were obtained from Source BioScience (Nottingham, UK, USA).

Table 3:

Clinico-pathological data of pancreatic ductal adenocarcinoma (PDAC) patients. Ataxin-10 (Atxn10) serum levels were determined in pre-surgery serum samples of these patients by using Sandwich-ELISA (Fig. 5G). BMI: Body mass index, BW: Body weight, neoTX: neoadjuvant therapy (radio- and/or chemotherapy), pT-, pN-, pM-stage: pathologic (p) tumor staging according to Tumor-Node-Metastasis (TNM) classification, R-status: tumor staging according to residual tumor (R) classification.