

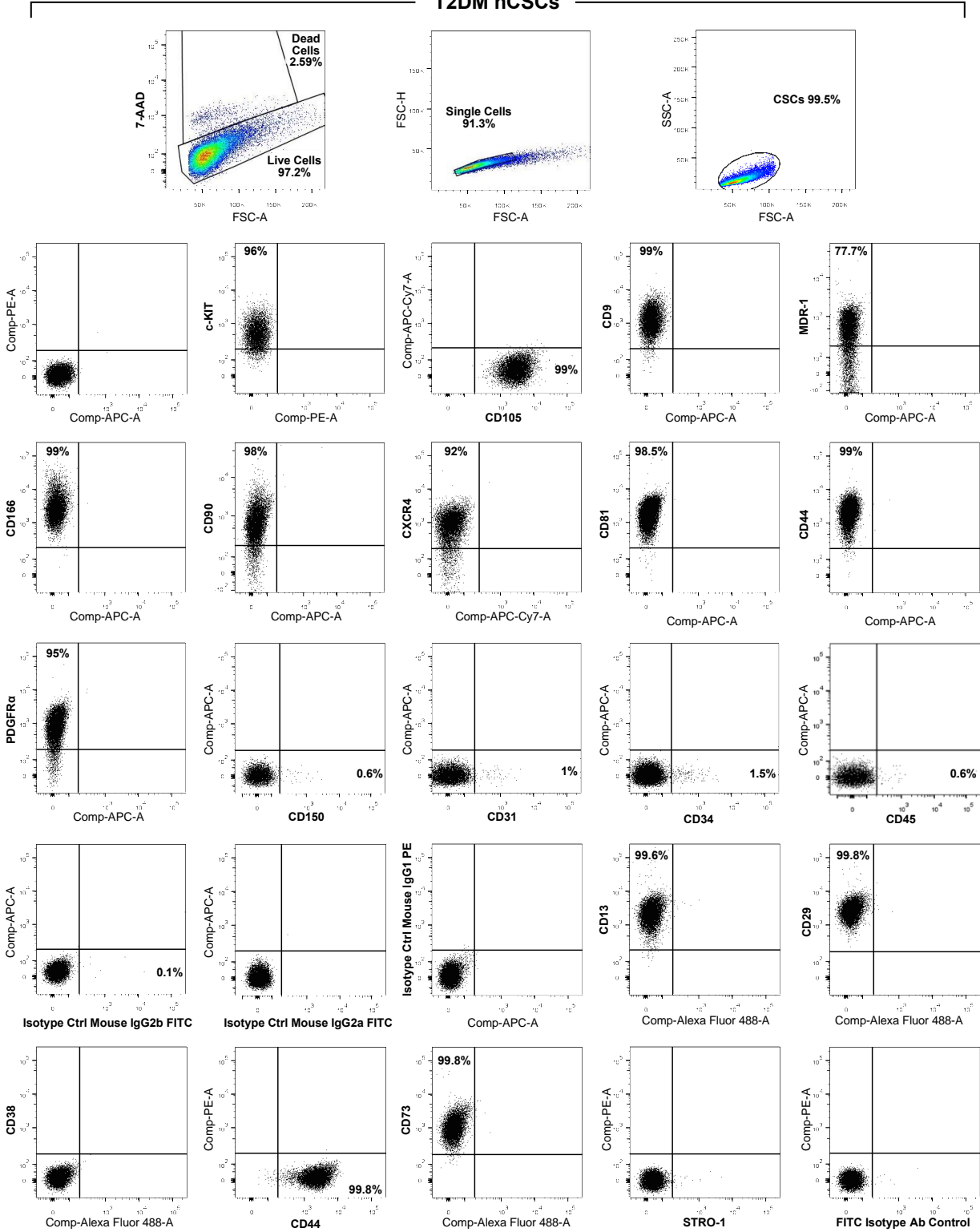
Online supplemental/appendix file

Diabetes-induced Cellular Senescence and Senescence-Associated Secretory Phenotype Impair Cardiac Regeneration and Function Independently of Age

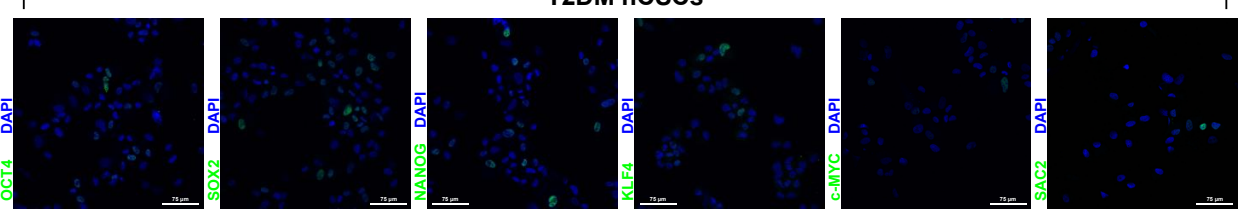
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Supplementary Figure 1

A

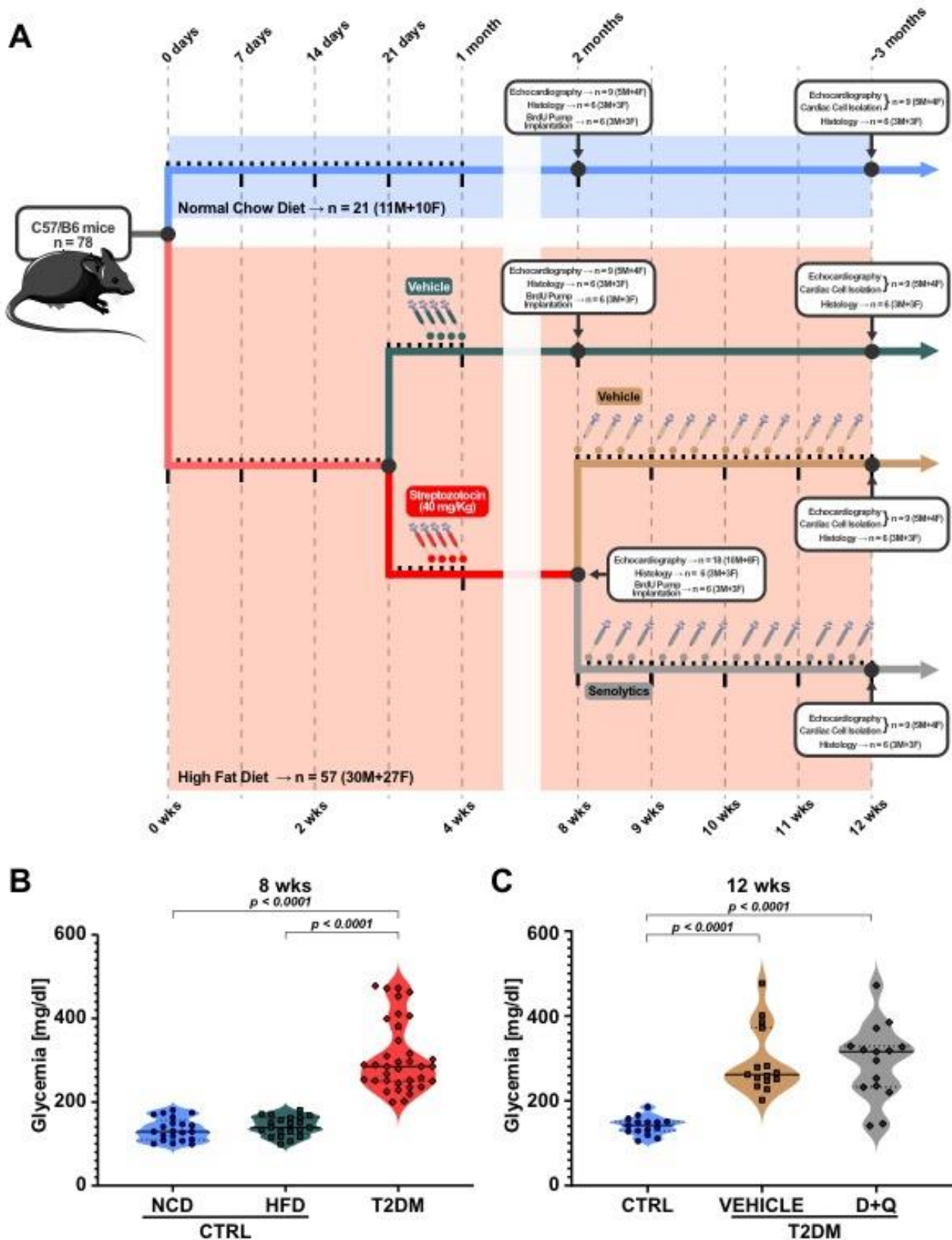


B



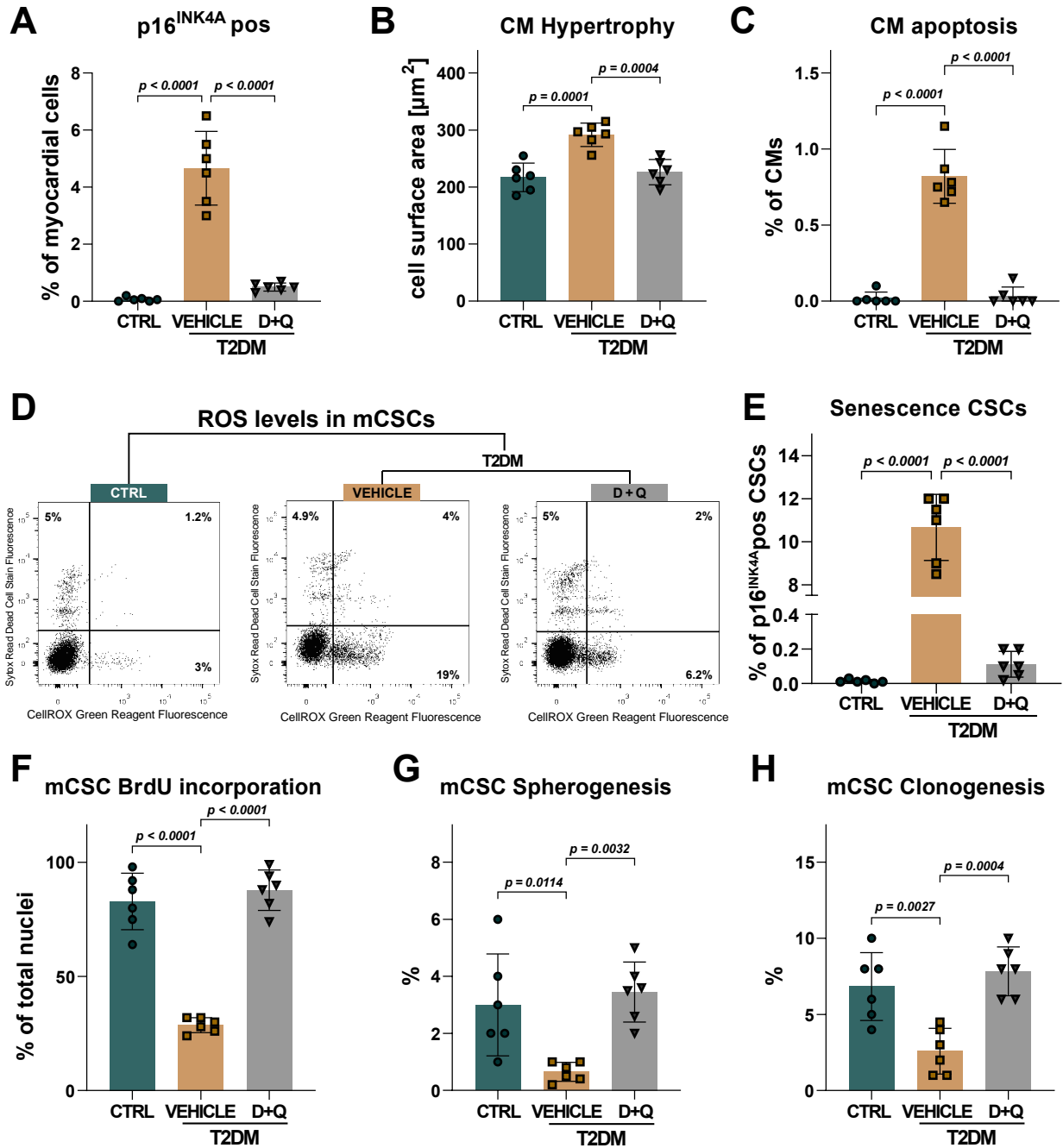
Supplementary Figure 1. (A) Flow cytometry dot plots showing the gating strategy used to evaluate the membrane phenotype of c-kit^{pos}CD45^{neg}CD31^{neg} T2DM-hCSCs and their differential surface marker expression (Representative of n=3 biological replicates). (B) Representative confocal microscopy images from cytopsin preparation of c-kit^{pos}CD45^{neg}CD31^{neg} T2DM-hCSCs expressing the pluripotency markers OCT4, SOX2, NANOG, KLF4, cMYC and Sac. Scale bar=75μm. (OCT4, SOX2, NANOG, KLF4, cMYC, Sac2, green; DAPI, blue; Representative of n=3 number of biological replicates). All data are mean±S.D

Supplementary Figure 2



Supplementary Figure 2. (A) Schematic representation of the *in vivo* study. (B) Violin plots showing the measurements of glycemia respectively at 8 weeks in CTRL (NCD and HFD) and T2DM mice and T2DM (NCD, n=21 ; HFD, n=21; T2DM, n=36), and at 12 weeks in CTRL, Vehicle and D+Q treated mice (CTRL, n=15; Vehicle, n=15; D+Q, n=15).

Supplementary Figure 3



Supplementary Figure 3. (A-C) Bar graphs showing the percentage of myocardial cells $p16^{INK4a}$ positive (A), CM size and hypertrophy (B), apoptotic TdT positive cardiomyocytes (C) in T2DM mice treated with D+Q senolytic combination compared to vehicle treated mice and CTRL mice. (n=6 biological replicates). (D) The ROS levels were assessed by determining CellROX fluorescence intensity by flow cytometry on CSCs obtained from CTRL, vehicle or D+Q-treated T2DM mice (Representative of n=3 biological replicates). (E) Bar graphs showing the percentage of CSCs $p16^{INK4a}$ positive in T2DM mice treated with D+Q senolytic combination compared to vehicle treated mice and CTRL mice. (n=6 biological replicates). (F-H) Bar graphs showing the *in vitro* proliferation potential (F) spherogenesis (G) and clonogenesis (H) of CSCs isolated from vehicle or D+Q-treated T2DM mice compared to CSCs from CTRL mice (n=6 biological replicates). All data are mean \pm S.D

Supplementary Table 1. Characteristics of the Patients Enrolled in the Study

	T2DM Patients n=16	NDM Patients n=12	
Sex, M/F	14/2	11/1	0.54
Age, y	59.4±4.0	59.4±4.1	0.97
Duration of diabetes, y	7.3±4.3	-	
BMI, kg/m ²	27.7±3.1	28.8±3.5	0.39
HbA _{1c} , %	9.1±3.8	-	
FBG, mg/dl	166.4±64.1	92.7±9.3	<0.01
LDL cholesterol, mg/dl	108.6±48.3	118.4±43.1	0.58
HDL cholesterol, mg/dl	41±15.4	47.2±17.0	0.33
TG, mg/dl	168.9±106.5	139.7±67.4	0.38
SBP, mm Hg	133.6±14.7	131.2±19.0	0.75
DBP, mm Hg	79.2±9.1	79.7±11.0	0.91
Three-vessel CHD, n	8	8	0.67
Two-vessel CHD, n	5	4	0.92
Therapy, n			
Statins	16	12	
Aspirin	16	12	
β-Blockers	16	10	0.31
ACEIs/ARBs	16	10	0.29
Nitrates	0	0	

Quantitative data are expressed as mean±SD. Binary data are reported by counts. *P<0.01 vs DM patients. Comparisons of the quantitative data have been made through use of Student's t test for independent samples. The χ^2 test was used to compare binary data. T2DM indicates Type 2 Diabetes Mellitus; NDM non diabetes Mellitus; BMI body mass index; FBG, fasting blood glucose; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; SDP, systolic blood pressure; DBP, diastolic blood pressure; CHD, coronary heart disease; ACEI, angiotensin-converting enzyme-inhibitor; ARB, angiotensin II receptor blocker.

Supplementary Table 2: Antibodies

Antigen	Antibody ID	Company	Application
c-kit		Dako	IF
c-kit		SantaCruz Biotech	IF
p16		SantaCruz Biotech	IF, WB
p53		SantaCruz Biotech	WB
p21		SantaCruz Biotech	WB
p21		Abcam	IF, IHC
8-OH		Origene	IF
3-NT		Thermo Fisher	IF, IHC
4-HNE		Abcam	IF, IHC
cTNI		Abcam	IF
g-H2AX		Cell Signaling	IF
cTNI		Abcam	IF
Actinin		Abcam	IF
WGA		Thermo Fisher	IF
BrdU		Roche	IF
PCM1		AtlasAntibodies	IF
TdT		Invitrogen	IF
Oct-4		SantaCruz Biotech	IF
Nanog		R&D Systems	IF
Klf-4		Invitrogen	IF
Sox-2		SantaCruz Biotech	IF
Sac-2		SantaCruz Biotech	IF
Gapdh		Bioss	WB
c-kit	A3C6E2	Miltenyi Biotec	FC
CD184 (CXCR4)	12G5	Miltenyi Biotec	FC
CD9	SN4 C3-3A2	Miltenyi Biotec	FC
CD44	DB105	Miltenyi Biotec	FC
CD81	5A6	BioLegend	FC
CD90	DG3	Miltenyi Biotec	FC
CD166	3A6	BioLegend	FC
CD105	43A4E1	Miltenyi Biotec	FC
MDR-1	UIC2	BioLegend	FC
PDGFR- α	16A1	BioLegend	FC
CD150	REA151	Miltenyi Biotec	FC
CD31	AC128	Miltenyi Biotec	FC
CD45	REA747	Miltenyi Biotec	FC
CD73	AD2	Miltenyi Biotec	FC
CD38	HIT2	BD Pharmigen	FC
CD29	TS2/16	Miltenyi Biotec	FC
CD44	DB105	Miltenyi Biotec	FC
CD13	REA263	Miltenyi Biotec	FC
STRO-1		Santa Cruz Biotech	FC
CD34	AC136	Miltenyi Biotec	FC

Mouse IgG1 - Isotype control antibody		Miltenyi Biotec	FC
Mouse IgG2a - Isotype control antibody		Miltenyi Biotec	FC
Mouse IgG2b - Isotype control antibody		Miltenyi Biotec	FC
AlexaFluor488 anti-mouse IgG		Invitrogen	FC

FC denotes Flow Cytometry, IF denotes Immunofluorescence, WB denotes Western Blot and IHC denotes Immunoistochemistry

Table 3. RT-PCR Primers**Taqman Primers List**

GENE	SPECIES	ID NUMBER
hGAPDH	Human	Hs02758991_g1
hMEF2C	Human	Hs00231149_m1
hGata4	Human	Hs00171403_m1
hNkx2.5	Human	Hs00231763_m1
hMyh6	Human	Hs01101425_m1
hMyh7	Human	Hs01110632_m1
hTnnt2	Human	Hs00165960_m1
hACTC1	Human	Hs01109515_m1
hCCL2	Human	Hs00234140_m1
hIL-8	Human	Hs00174103_m1
hGM-CSF	Human	Hs00929873_m1
hIGFBP5	Human	Hs00181213_m1
hIL-6	Human	Hs00174131_m1
hPAI-1	Human	Hs00167155_m1
hMMP-3	Human	Hs00968305_m1
hCCL11	Human	Hs00237013_m1
Gapdh	Mouse	Mm99999915_g1
Mef2c	Mouse	Mm01340842_m1
Nkx2.5	Mouse	Mm01309813_s1
Gata4	Mouse	Mm00484689_m1
c-kit	Mouse	Mm00445212_m1
Myh7	Mouse	Mm01319006_g1
Myl2	Mouse	Mm00440384_m1
TnnT2	Mouse	Mm01290256_m1
Actc1	Mouse	Mm01333821_m1

List of mouse gene-specific primer sets for SYBR Green qPCR

GENE	Forward Primer	Reverse Primer
MCP1 (CCL2)	5'-GCTACAAGAGGATCACCAGCAG-3'	5'-GTCTGGACCCATTCCTTCTTGG-3'
IGFBP5	5'-AACGAAAAGAGCTACCGCGA-3'	5'-CCGACAAACTTGGACTGGGT-3'
PAI-1	5'-GGCCATTACTACGACATCCTG-3'	5'-GGTCATGTTGCCTTTCCAGT-3'
GM-CSF	5'-AACCTCCTGGATGACATGCCTG-3'	5'-AAATTGCCCCGTAGACCCTGCT-3'
IL-8	5'-GTGCAGTTTTGCCAAGGAGT-3'	5'-TTATGAATTCTCAGCCCTCTTCAAAAAC TT CTC-3'
CCL11	5'-TGCAGAGCTCCACAGCGCTT-3'	5'-GGGTGAGCCAGCACCTGGGA-3'
IL-6	5'-TGAGAAAAGAGTTGTGCAATGG-3'	5'-GGTACTCCAGAAGACCAGAGG-3'
MMP-3	5'-GTTGGAGAACATGGAGACTTTGT-3'	5'-CAAGTTCATGAGCAGCAACCA-3'
IL1a	5'-AGGGAGTCAACTCATTGGCG-3'	5'-TGGCAGAACTGTAGTCTTCGT-3'
IL1b	5'-TGCCACCTTTTGACAGTGATG-3'	5'-TGATGTGCTGCTGCGAGATT-3'
TNF α	5'-GCAGGTTCTGTCCCTTTCAC-3'	5'-GTCGCGGA TCA TGCTTTCTG-3'

Supplementary Table 4. Echocardiography data

Supplementary Table 4. Echocardiography data for figures 7-8.

	Sex	BW (gr)	LVEDD (mm)	LVESD (mm)	EF (%)	FS (%)	HR (bpm)	E velocity mm/s	A velocity	E/A	E' velocity	E/E'
<u>8 weeks</u>												
NCD	5M+4F	21±0.5	3.84±0.17	2.70±0.13	57.26±3.57	29.63±2.37	449±49	585.56±67.20	307.22±62.4	1.94±0.29	22.28±2.57	26.70±1.75
HFD	5M+4F	25±2*	3.79±0.32	2.53±0.36	62.56±7.21	33.52±5.46	416±28	566.08±79.63	298.63±90.43	2.00±0.46	21.63±3.5	26.39±2.9
T2DM	10M+8F	22±1	3.895±0.34	2.77±0.39	56.28±7.28	29.145±4.84	425±55	550.048±103.56	349.79±116.22	1.72±0.58	15.89±4.45*	36.29±9.41#
<u>12 weeks</u>												
CTRL (HFD)	5M+4F	28±1.5	3.74±0.23	2.49±0.29	62.80±6.20	33.50±4.42	421±42	661.92±180.19	379.55±96.53	1.770±0.31	26.17±4.40	25.42±5.44
T2DM+ Vehicle	5M+4F	23±1.5*	3.99±0.21	2.81±0.25	57.03±4.9	29.59±3.29	427±52	554.80±89.25	359.35±110.34	1.66±0.52	15.68±2.72*	36.34±8.75#
T2DM+ (D+Q)	5M+4F	24±0.5	3.63±0.30	2.38±0.18	64.098±3.42	34.26±2.65	450±47	606.11±136.93	365.24±106.30	1.72±0.37	24.08±4.23	25.61±6.33

BW= body weight

LVEDD= left ventricular-end-diastolic diameter

LVESD= left ventricular-end-systolic diameter

EF= ejection fraction

FS= fractional shortening

HR= heart rate

8 weeks p-values: BW*= p<0.01 vs NCD and T2DM; E' velocity*= p<0.003 vs HFD and NCD; E/E'#= p<0.008 vs HFD and NCD.

8 weeks p-values: BW*= p<0.01 vs NCD and T2DM; E' velocity*= p<0.0002 vs CTRL and T2DM+(D+Q); E/E'#= p<0.008 vs CTRL and T2DM+(D+Q).