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Supplemental Information

Mir30c Is Involved in Diabetic Cardiomyopathy through Regulation of Cardiac Autophagy via BECN1

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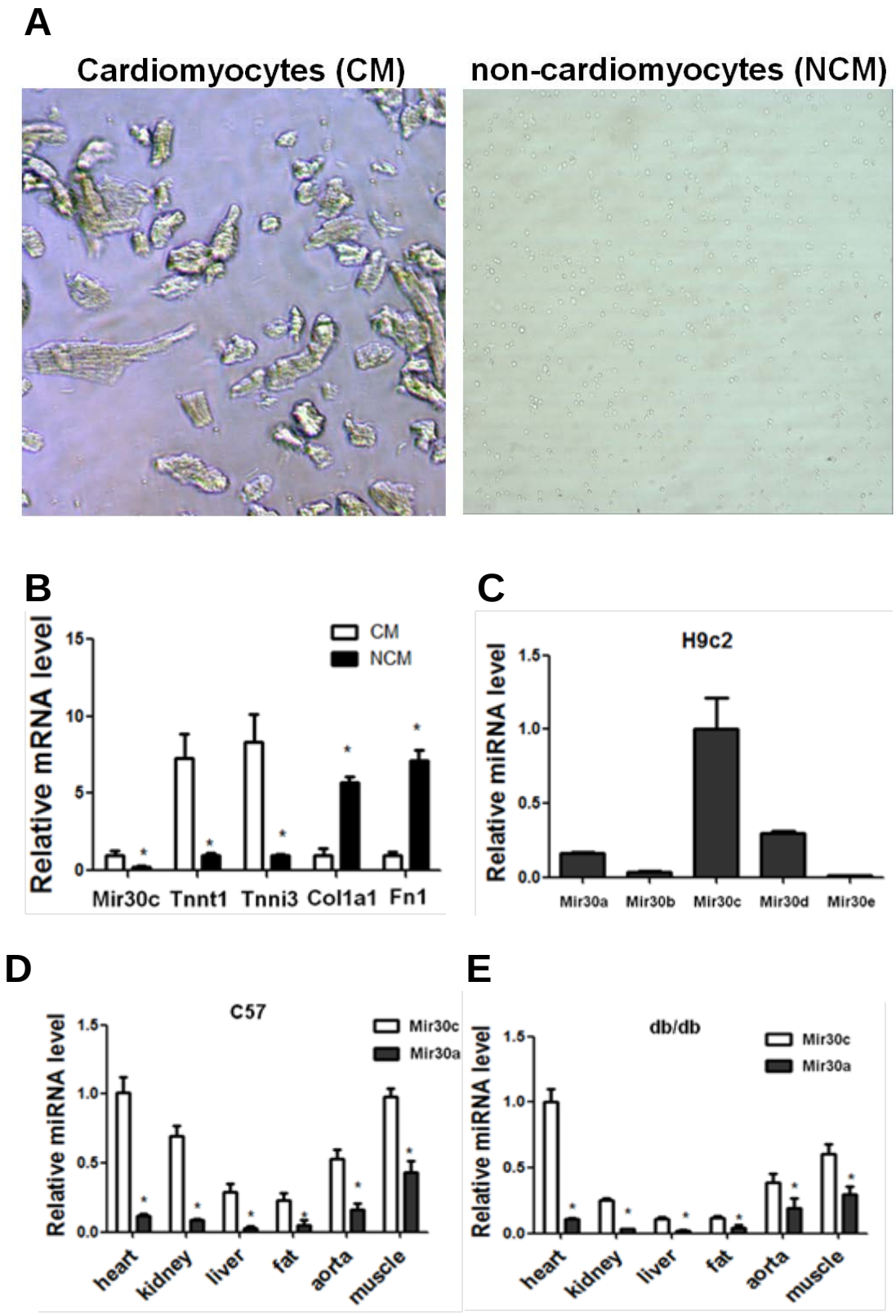
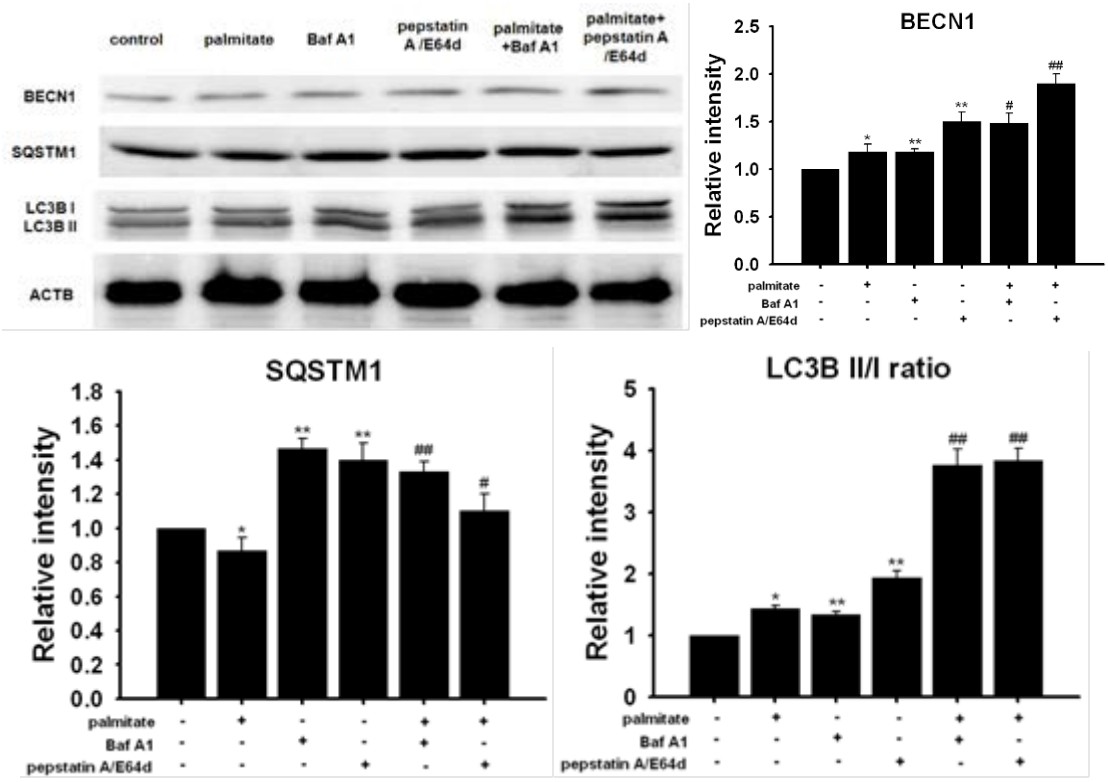


Supplemental Figure 1. Mir30c distribution and expression in various organs. A, Morphology of isolated primary cells. The small circles in the NCM image represent fibroblasts. B to E, Relative gene expression, as determined

by qRT-PCR. B, Relative expression of CM and NCM markers in isolated primary cells. *$p<0.05$ vs. CMs. C, Relative expression of Mir30 family miRNAs in H9c2 cells. D, Relative expression of Mir30a and Mir30c in various organs of C57BL/Ks mice. *$p<0.05$ vs. Mir30c. E, Relative expression of Mir30a and Mir30c in various organs of db/db mice. *$p<0.05$ vs. Mir30c. Data are expressed as mean±SEM, n=8. Data are representative of three independent experiments.

A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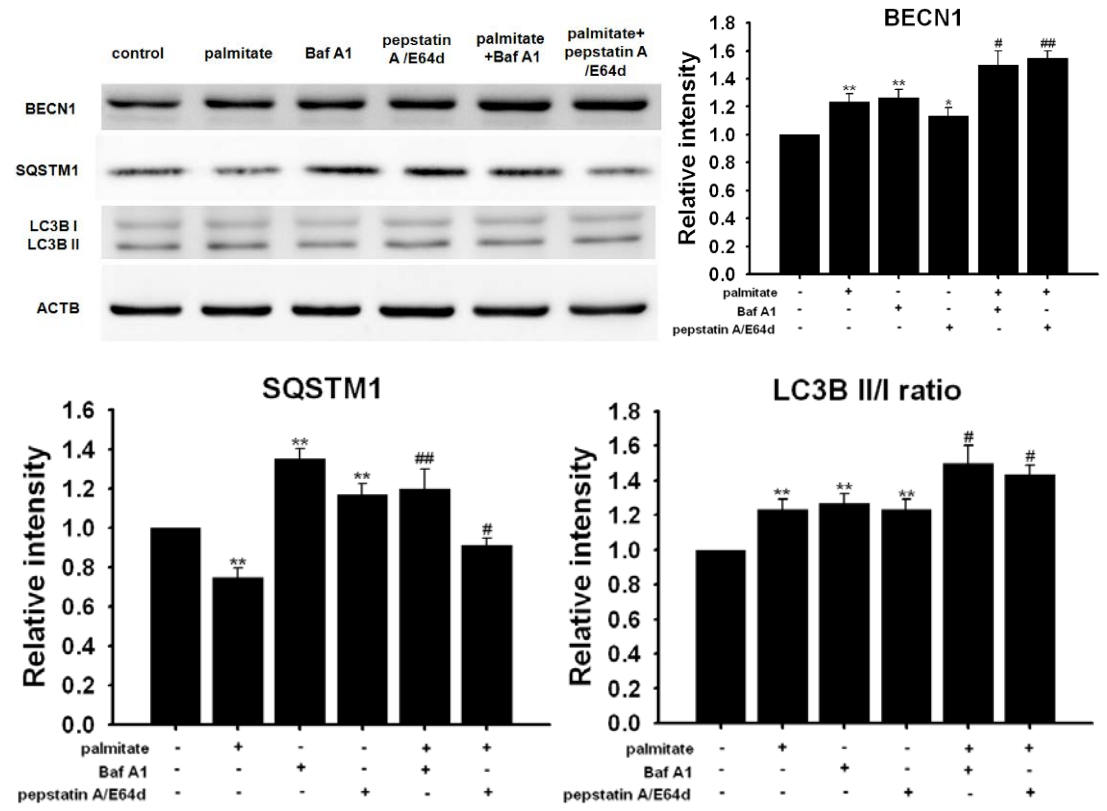
palmitate
Baf A1
pepstatin A /E64d
palmitate +Baf A1
palmitate+ pepstatin A /E64d
BECN1
SQSTM1
LC3B I
LC3B II
ACTB
BECN1
Relative intensity
SQSTM1
LC3B II/I ratio
palmitate
Baf A1
pepstatin A/E64d

B


control
palmitate
Baf A1
pepstatin A /E64d
palmitate +Baf A1
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BECN1
SQSTM1
LC3B I
LC3B II
ACTB
BECN1
Relative intensity
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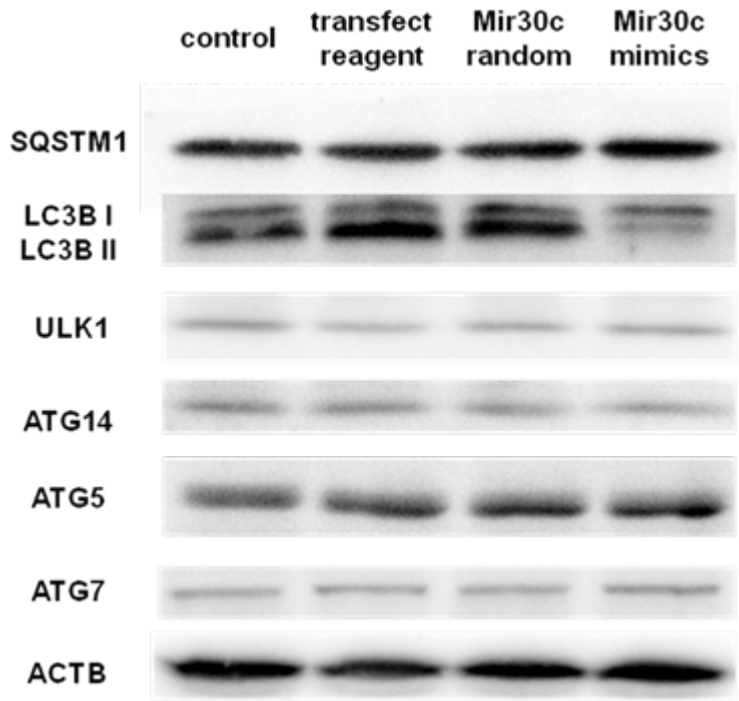
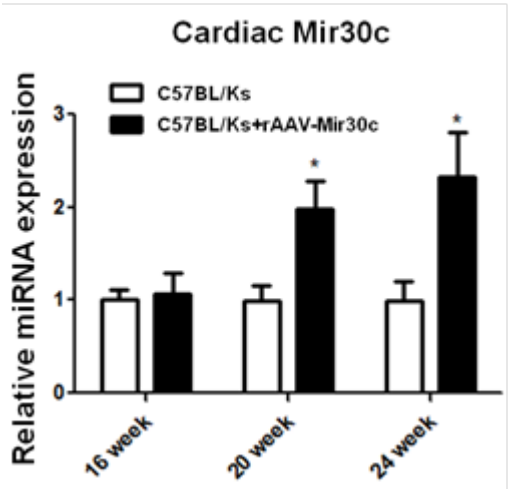
LC3B II/I ratio
palmitate
Baf A1
pepstatin A/E64d

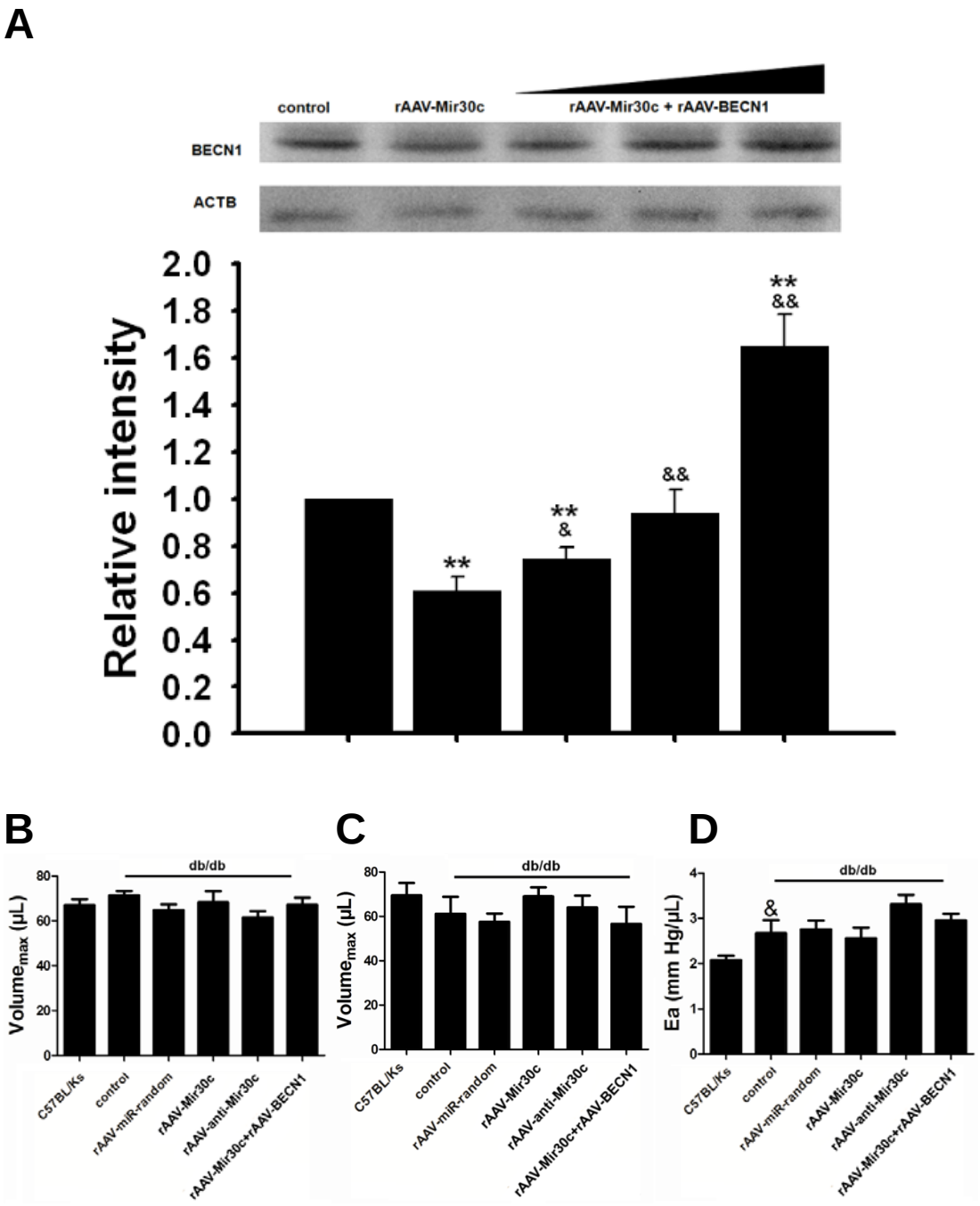
Supplemental Figure 2. High FFA concentrations enhance autophagy in cultured H9c2 cardiac cells. H9c2 cells were analyzed after treatment with palmitate (1 mM) for 48 h. A, Bafilomycin A1 (Baf A1, 10 nM), or pepstatin A (10 μg/mL) and E64d (10 μg/mL) were added 4 h before harvesting. B, Bafilomycin A1 (Baf A1, 10 nM), or pepstatin A (10 μg/mL) and E64d (10 μg/mL) were added 12 h before harvesting. Data are expressed as mean±SEM. *$p<0.05$ vs. control, **$p<0.01$ vs. control, $^{\#}p<0.05$ vs. palmitate, $^{\#\#}p<0.01$ vs. palmitate. Data are representative of three independent experiments.



Supplemental Figure 3. The effect of Mir30c on the abundance of autophagy-associated proteins. Levels of autophagy-associated proteins were detected in H9c2 cells by western blotting. ACTB was used as an internal control. Experimental details can be found in Materials and Methods.

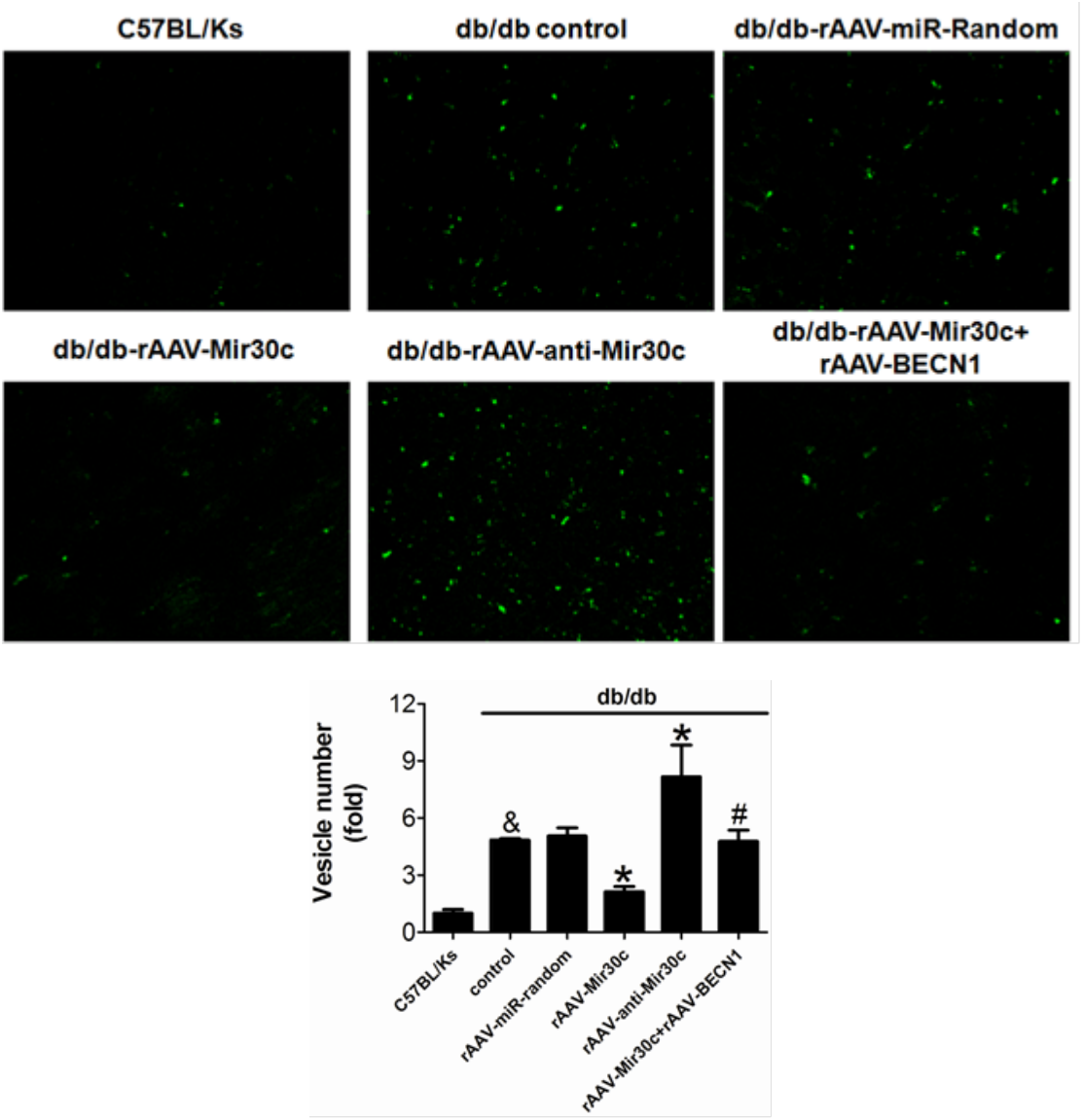


Supplemental Figure 4. The expression of *Mir30c* in C57BL/Ks control mice of different ages. The rAAVs were injected via the tail vein of 16-week-old animals, and cardiac *Mir30c* expression was determined every 4 weeks by qRT-PCR. U6 was used as an internal control. *$p<0.05$ vs. untreated control at the same time point. Data are expressed as means±SEM (n=8). They are representative of three independent experiments.



Supplemental Figure 5. BECN1 rescues cardiac function in db/db mice. A, Three different doses of BECN1 (0.5×10^{11} virion particles, 1×10^{11} virion particles, or 2×10^{11} virion particles) rescued cardiac BECN1 expression in db/db mice, as determined by western blotting. **p<0.01 vs. control, $^{\#}$p<0.05 vs. rAAV-Mir30c, $^{\#\#}$p<0.01 versus rAAV-Mir30c. B, Volume$_{max}$, determined by hemodynamic analyses. C, Volume$_{max}$, assessed by echocardiography. D, Ea,

determined by hemodynamic analyses. $^{\&}p<0.05$ vs. C57BL/Ks. Data are expressed as mean±SEM (n=8). They are representative of three independent experiments.



Supplemental Figure 6. Autophagosome formation in db/db mice. db/db mice were treated with different rAAVs for 4 weeks and injected with pCMV-LC3-GFP via their tail vein 1 week before sacrifice subsequently. GFP-LC3 expression in the heart was analyzed by confocal microscopy (upper panels; 600× magnification). Autophagosomes were quantified by counting GFP-LC3–positive dots and normalizing for cross-sectional area (lower panel). Data are expressed as mean±SEM (n=5). $^{\&}p<0.05$ vs. C57BL/Ks, *$p<0.05$ vs. db/db control, $^{\#}p<0.05$ vs. db/db rAAV-*Mir30c*. Data are representative of three independent experiments.

Supplemental Table 1. Baseline clinical characteristics of controls and patients

Characteristic[a]	Control (n=28)	Diabetes (n=26)	Chronic heart failure (n=22)	Diabetes combined with chronic heart failure (n=15)
Male/female (n/n)	14/14	9/17	14/8	10/5
Age (years)	56.6±1.46	61.6±1.85	58.0±3.44	60.0±3.03
SBP (mmHg)	125±3	147±5*	123±5	138±6#
DBP (mmHg)	77±2	84±4	77±3	82±6
Cr (mmol/L)	69.9±2.3	64.1±4.6	95.1±6.1*	86.1±6.8*
BUN (mmol/L)	5.00±0.25	6.46±0.45*	8.25±0.75*	8.90±1.10*
Fasting glucose (mmol/L)	5.18±0.10	14.2±1.31*	6.17±0.25*	10.0±0.33*#
TG (mmol/L)	1.35±0.17	1.72±0.19	1.19±0.11	1.32±0.17
TC (mmol/L)	4.02±0.13	3.86±0.14	3.86±0.18	4.01±0.33
HDL (mmol/L)	1.10±0.06	0.93±0.05	0.95±0.06	0.80±0.10*
LDL (mmol/L)	2.44±0.12	2.21±0.12	2.49±0.16	2.64±0.31
NT-proBNP (ng/L)	66±10	621±202*	4715±915*	5574±1647*
EF (%)	65.1±1.6	65.6±1.0	36.5±2.6*	35.2±2.6*

[a] BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cr, creatinine; BUN, blood urea nitrogen; TG, Triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, amino-terminal pro-brain natriuretic peptide; EF, ejection fraction. Data are presented as mean±SEM. *$p<0.05$ vs. control; #$p<0.05$ vs. chronic heart failure.

Supplemental Table 2. Hemodynamic variables of C57BL/Ks mice receiving different treatments[a]

Variable[b]	Control (8)	rAAV-miR-Random (8)	rAAV-*Mir30c* (8)	rAAV-anti-*Mir30c* (8)
HR (b.p.m.)	528±54	493±40	505±50	507±39
dP/dt_{max} (mmHg/s)	11,630±542	11,480±633	11,918±385	12,248±703
dP/dt_{min} (mmHg/s)	-10,833±1053	-11,204±726	-11,423±972	-11,504±355
P_{max} (mmHg)	107.8±4.8	107.6±3.4	104.6±3.7	108.4±3.6
P_{es} (mmHg)	94.3±5.6	92.2±1.9	94.8±4.6	94.3±4.6

[a] Data are expressed as mean±SD; numbers in parentheses represent numbers of mice/group; ANOVA test was performed, and $p<0.05$ was considered statistically significant. No significant differences were detected among the groups.

[b] HR, heart rate; P_{max}, maximum pressure; P_{es}, end-systolic pressure.

Supplemental Table 3. Echocardiographic characteristics of LV parameters in C57BL/Ks and db/db mice that received different treatments[a]

Characteristic[b]	**C57BL/Ks (8)**[c]	**db/db**				
		control (8)	**rAAV-miR-Random (8)**	**rAAV-*Mir30c* (8)**	**rAAV-anti-*Mir30c* (8)**	**rAAV-*Mir30c*+ rAAV-BECN1 (8)**
HR (b.p.m.)	502±5	450±13[&]	469±14	461±16	462±18	455±15
LVPWd (mm)	0.79±0.04	0.82±0.05	0.82±0.13	0.84±0.07	1.00±0.06	0.91±0.07
LVPWs (mm)	1.12±0.13	1.13±0.08	1.13±0.10	1.16±0.09	1.19±0.07	1.19±0.14
LVAWd (mm)	0.83±0.03	0.88±0.06	0.89±0.07	0.85±0.07	0.92±0.06	0.90±0.09
LVAWs (mm)	1.18±0.08	1.22±0.08	1.28±0.03	1.26±0.13	1.39±0.16	1.27±0.13
LVIDd (mm)	3.66±0.11	3.77±0.35	3.68±0.20	3.97±0.22	3.84±0.26	3.64±0.42
LVIDs (mm)	2.23±0.07	2.56±0.25[&]	2.49±0.18	2.45±0.12	2.85±0.25	2.48±0.35
LV Vold (μL)	56.72±3.84	61.14±13.29	57.56±7.45	69.04±9.09	64.02±10.74	56.63±15.50
LV Vols (μL)	16.80±1.41	23.88±5.63[&]	22.18±4.03	21.26±2.56	31.15±6.74	22.52±7.74

[a] Data are expressed as mean±SD; numbers in parentheses represent numbers of mice/group; Student's *t* test was performed and $p<0.05$ was considered statistically significant.

[b] HR, heart rate; LVPW, left ventricular posterior wall thickness; LVAW, Left ventricular anterior wall thickness; LVID, left ventricular internal dimension; LV Vol, left ventricular volume; s, end-systole; d, end-diastole.

[c] [&]$p<0.05$ vs. C57BL/Ks.

Supplemental Table 4. Hemodynamic variables of db/db mice receiving different treatments[a]

Variable[b]	Control (8)[c]	rAAV-miR-Random (8)	rAAV-*Mir30c* (8)	rAAV-*Mir30c*+ rAAV-BECN1 3′-UTR WT (8)	rAAV-*Mir30c*+ rAAV-BECN1 3′-UTR mut (8)	rAAV-miR-Random +rAAV-BECN1 3′-UTR WT (8)
HR (b.p.m.)	428±37	415±31	398±44	432±35	397±39	398±25
dP/dt_{max} (mmHg/s)	8302±418	8450±681	9581±696*	9121±326*	7856±546$^{\&}$	6269±422$^{\#}$
dP/dt_{min} (mmHg/s)	-6165±778	-6242±607	-7245±880*	-5922±968	-5195±392$^{\&}$	-5075±380
P_{max} (mmHg)	98.2±5.7	99.9±9.3	99.3±4.2	108.4±3.6$^{\&}$	103.4±5.2	96.8±4.2
P_{es} (mmHg)	85.3±8.7	91.2±9.0	93.9±6.9	88.5±1.8	96.3±9.5	92.4±8.9

[a] Data are expressed as mean±SD; numbers in parentheses represent numbers of mice/group. Student's *t* test was performed.

[b] HR, heart rate; P_{max}, maximum pressure; P_{es}, end-systolic pressure.

[c] *p<0.05 vs. control; $^{\&}$p<0.05 vs. rAAV-*Mir30c*; $^{\#}$p<0.05 vs. rAAV-miR-Random.

Supplemental Table 5. Real-time PCR primers

Gene	Forward	Reverse
Mouse Tnnt1	5′ TCGACCACCTGAATGAAGACC 3′	5′ TTCCTGCAGGTCGAACTTCTC 3′
Mouse Tnni3	5′ TCTATGACCTCCGTGGCAAGT 3′	5′ TCCTCCTTCTTCACCTGCTTG 3′
Mouse Col1a1	5′ CCGAGGTATGCTTGATCT 3′	5′ GACAGTCCAGTTCTTCATTG 3′
Mouse Fn1	5′ GTGTCTATGCTCTCAAGGA 3′	5′ CTAATAGTGATGGTGGTCTCT 3′
Mouse Sp1	5′ AACCTTCCTTGTCTTGGCTGAG 3′	5′ TCAGTTCCCTGGTAGAGAAGGC 3′
Mouse Gapdh	5′ GGTGAAGGTCGGTGTGAACG 3′	5′ CTCGCTCCTGGAAGATGGTG 3′
Rat Becn1	5′ GCCACAAGCATCTCATCTCAAA 3′	5′ AGCACGCCATGTATAGCAAAGA 3′
Rat Gapdh	5′ ACAGCAACAGGGTGGTGGAC 3′	5′ TTTGAGGGTGCAGCGAACTT 3′