**YMTHE, Volume *30***

## Supplemental Information

# miR-486 attenuates cardiac ischemia/reperfusion injury and mediates the beneficial effect of exercise for myocardial protection

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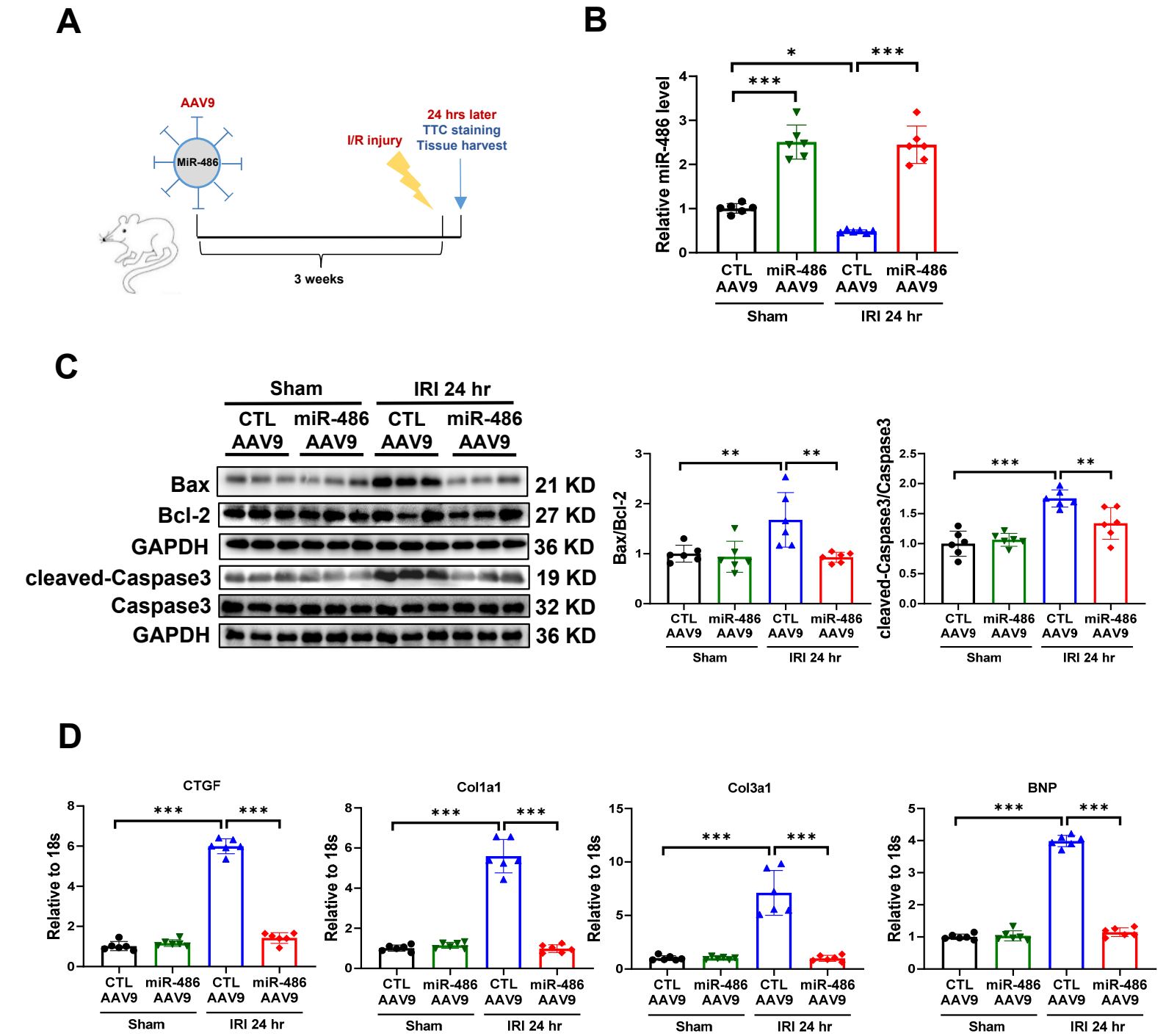
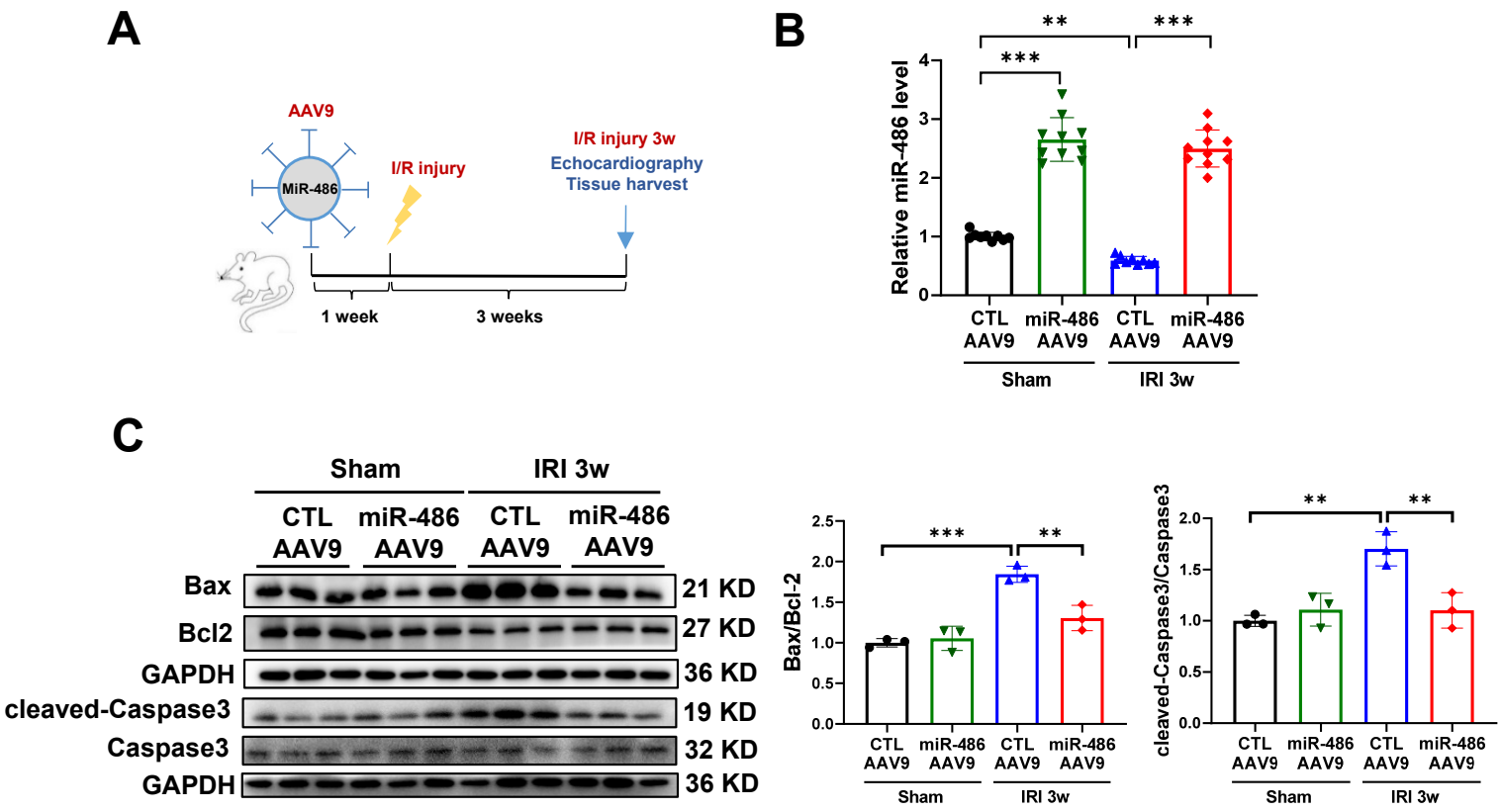
# Supplemental Figures

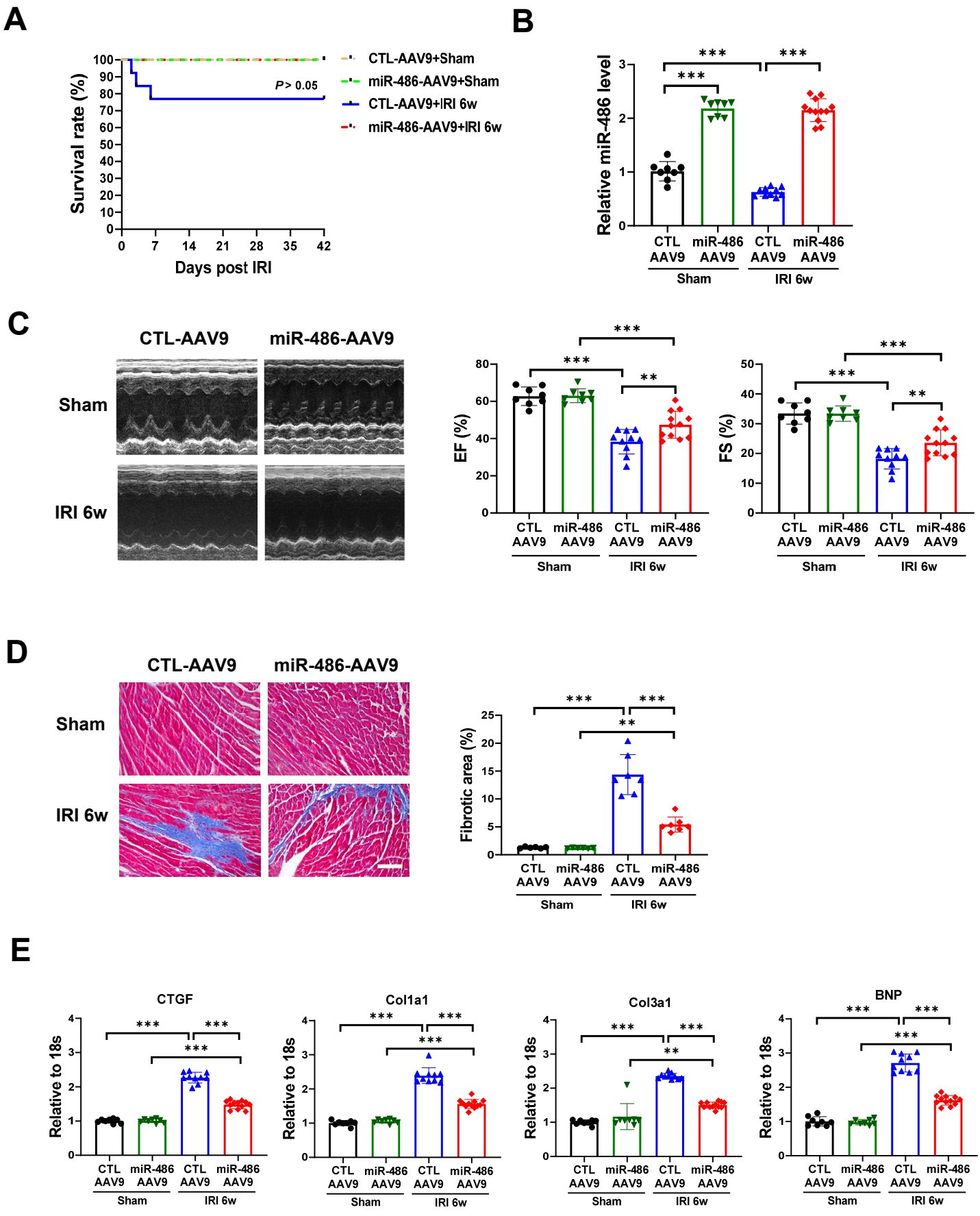
# Figure S1


A
AAV9
MiR-486
I/R injury
24 hrs later
TTC staining
Tissue harvest
3 weeks
B
Relative miR-486 level
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
Sham
IRI 24 hr
C
Sham
IRI 24 hr
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
Bax
Bcl-2
GAPDH
cleaved-Caspase3
Caspase3
GAPDH
21 KD
27 KD
36 KD
19 KD
32 KD
36 KD
Bax/Bcl-2
cleaved-Caspase3/Caspase3
D
CTGF
Col1a1
Col3a1
BNP
Relative to 18s

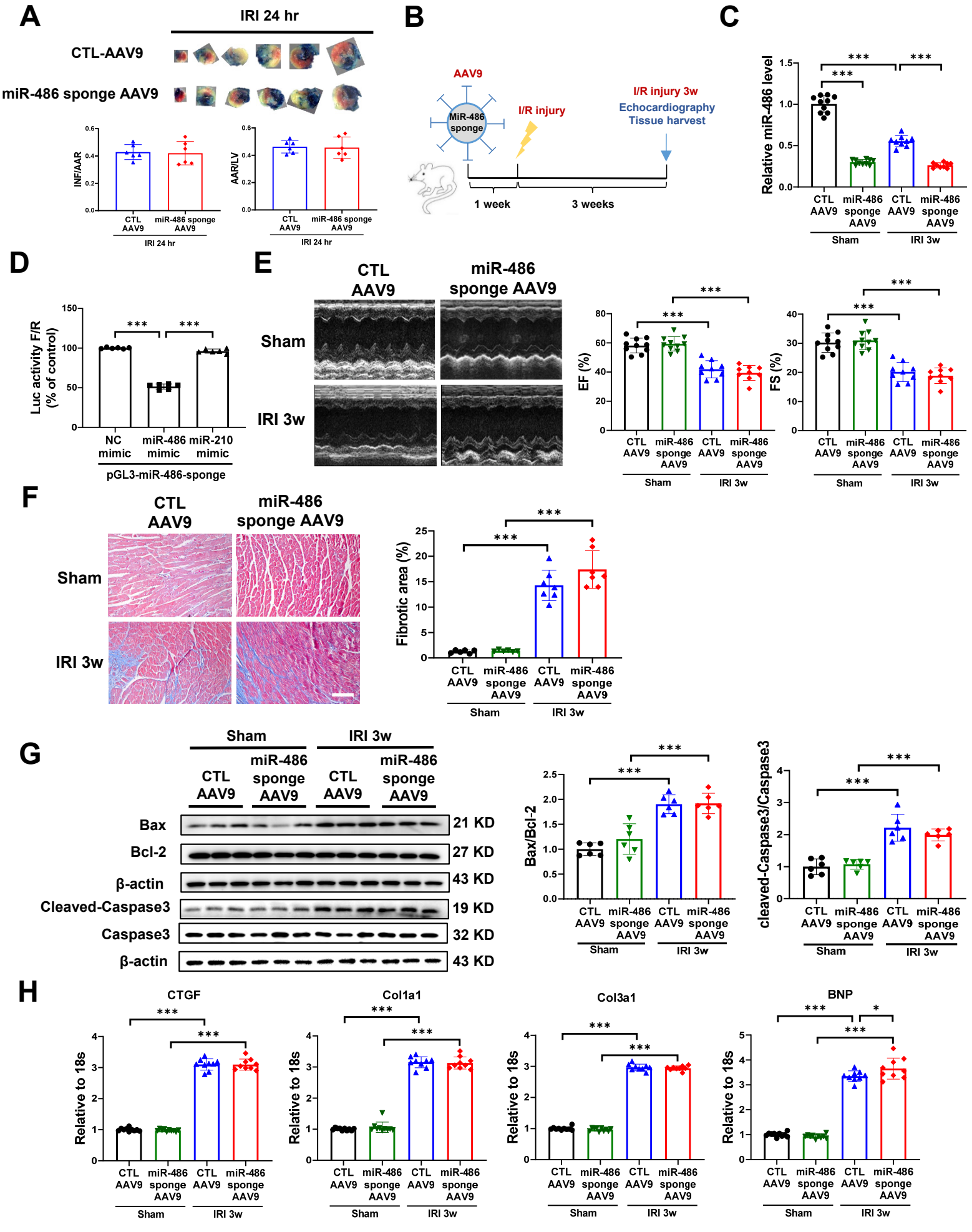
# Figure S2


A
AAV9
MiR-486
I/R injury
I/R injury 3w
Echocardiography
Tissue harvest
1 week
3 weeks
B
Relative miR-486 level
CTL AAV9
miR-486 AAV9
Sham
IRI 3w
C
Sham
IRI 3w
Bax
Bcl2
GAPDH
cleaved-Caspase3
Caspase3
GAPDH
21 KD
27 KD
36 KD
19 KD
32 KD
36 KD
Bax/Bcl-2
cleaved-Caspase3/Caspase3

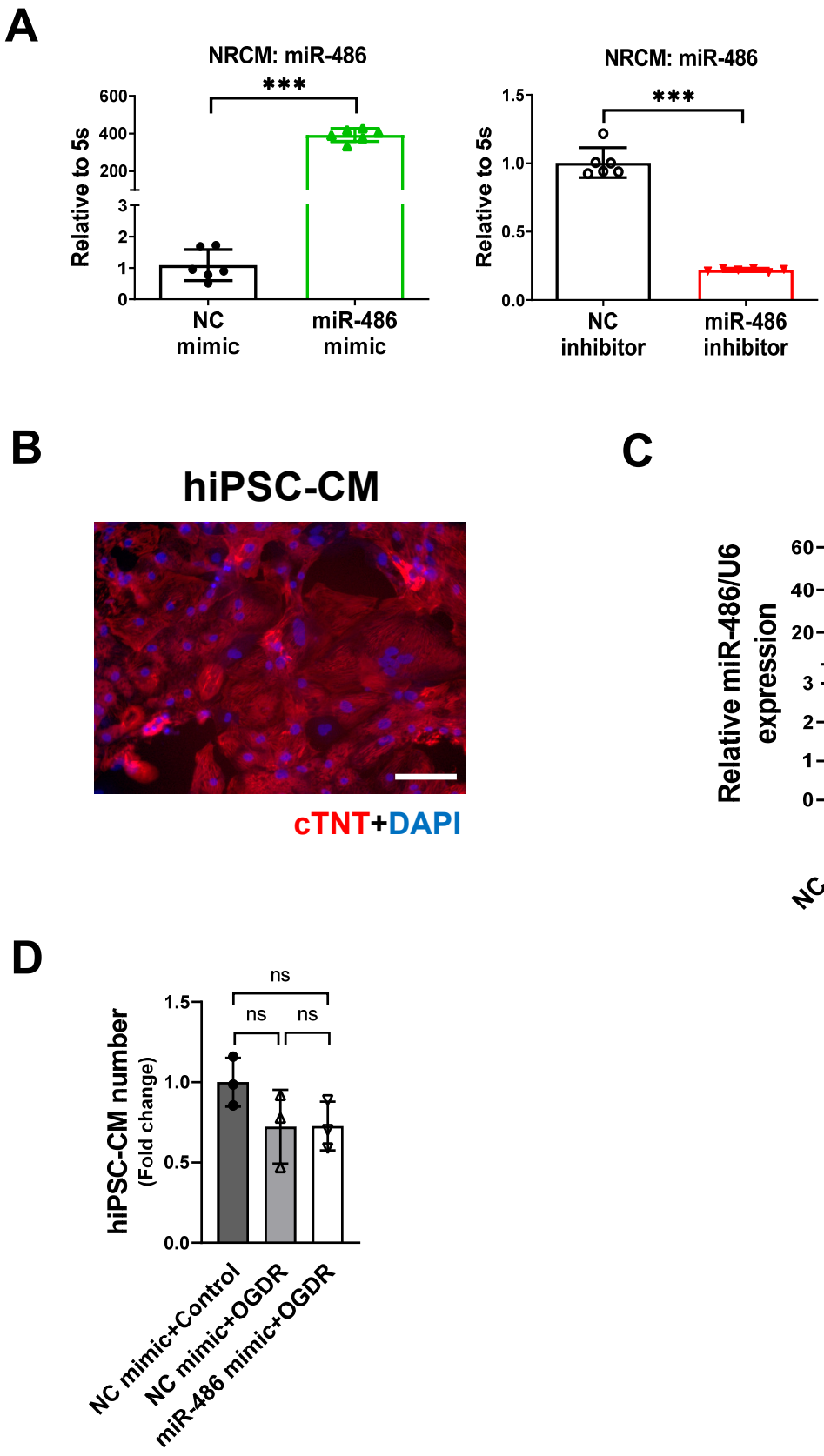
# Figure S3


A
Survival rate (%)
Days post IRI
P> 0.05
CTL-AAV9+Sham
miR-486-AAV9+Sham
CTL-AAV9+IRI 6w
miR-486-AAV9+IRI 6w
B
Relative miR-486 level
CTL AAV9
miR-486 AAV9
Sham
IRI 6w
C
CTL-AAV9
miR-486-AAV9
Sham
IRI 6w
EF (%)
FS (%)
D
Fibrotic area (%)
E
CTGF
Col1a1
Col3a1
BNP
Relative to 18s

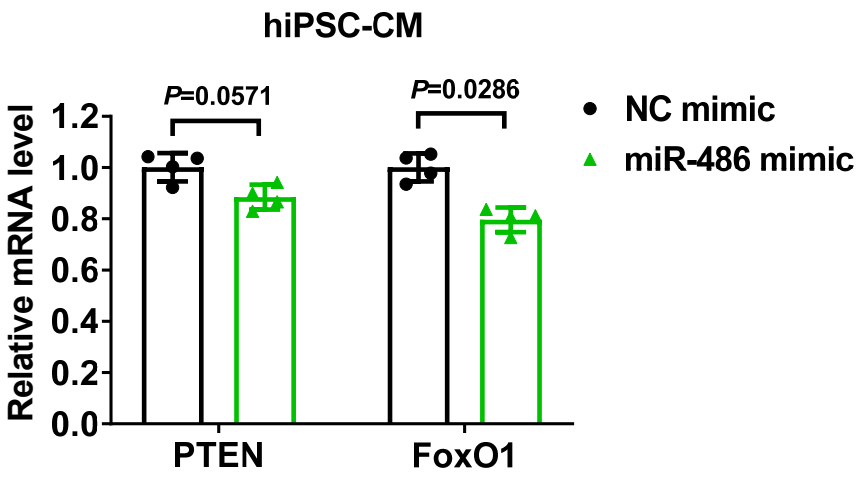
# Figure S4


A
IRI 24 hr
CTL-AAV9
miR-486 sponge AAV9
INF/AAR
AAR/LV
CTL AAV9
miR-486 sponge AAV9
IRI 24 hr
B
AAV9
MiR-486 sponge
I/R injury
I/R injury 3w
Echocardiography
Tissue harvest
1 week
3 weeks
C
Relative miR-486 level
Sham
IRI 3w
D
Luc activity F/R (% of control)
NC mimic
miR-486 mimic
miR-210 mimic
pGL3-miR-486-sponge
E
CTL AAV9
miR-486 sponge AAV9
Sham
IRI 3w
EF (%)
FS (%)
F
Fibrotic area (%)
G
Bax
Bcl-2
β-actin
Cleaved-Caspase3
Caspase3
β-actin
21 KD
27 KD
43 KD
19 KD
32 KD
43 KD
Bax/Bcl-2
cleaved-Caspase3/Caspase3
H
CTGF
Col1a1
Col3a1
BNP
Relative to 18s

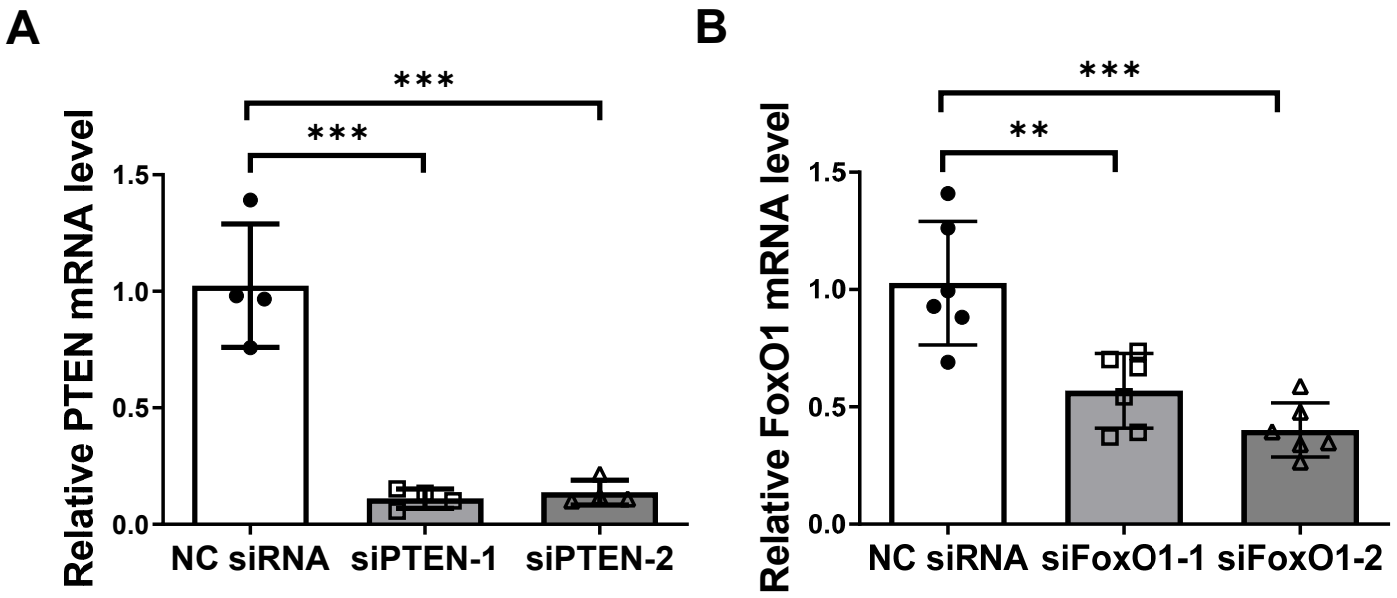
# Figure S5


A
NRCM: miR-486
***
Relative to 5s
600
400
200
3
2
1
0
NC mimic
miR-486 mimic
NRCM: miR-486
***
Relative to 5s
1.5
1.0
0.5
0.0
NC inhibitor
miR-486 inhibitor
B
hiPSC-CM
cTNT+DAPI
C
hiPSC-CM
*
Relative miR-486/U6 expression
60
40
20
3
2
1
0
NC mimic
miR-486 mimic
D
ns
ns
ns
hiPSC-CM number
(Fold change)
1.5
1.0
0.5
0.0
NC mimic+Control
NC mimic+OGDR
miR-486 mimic+OGDR

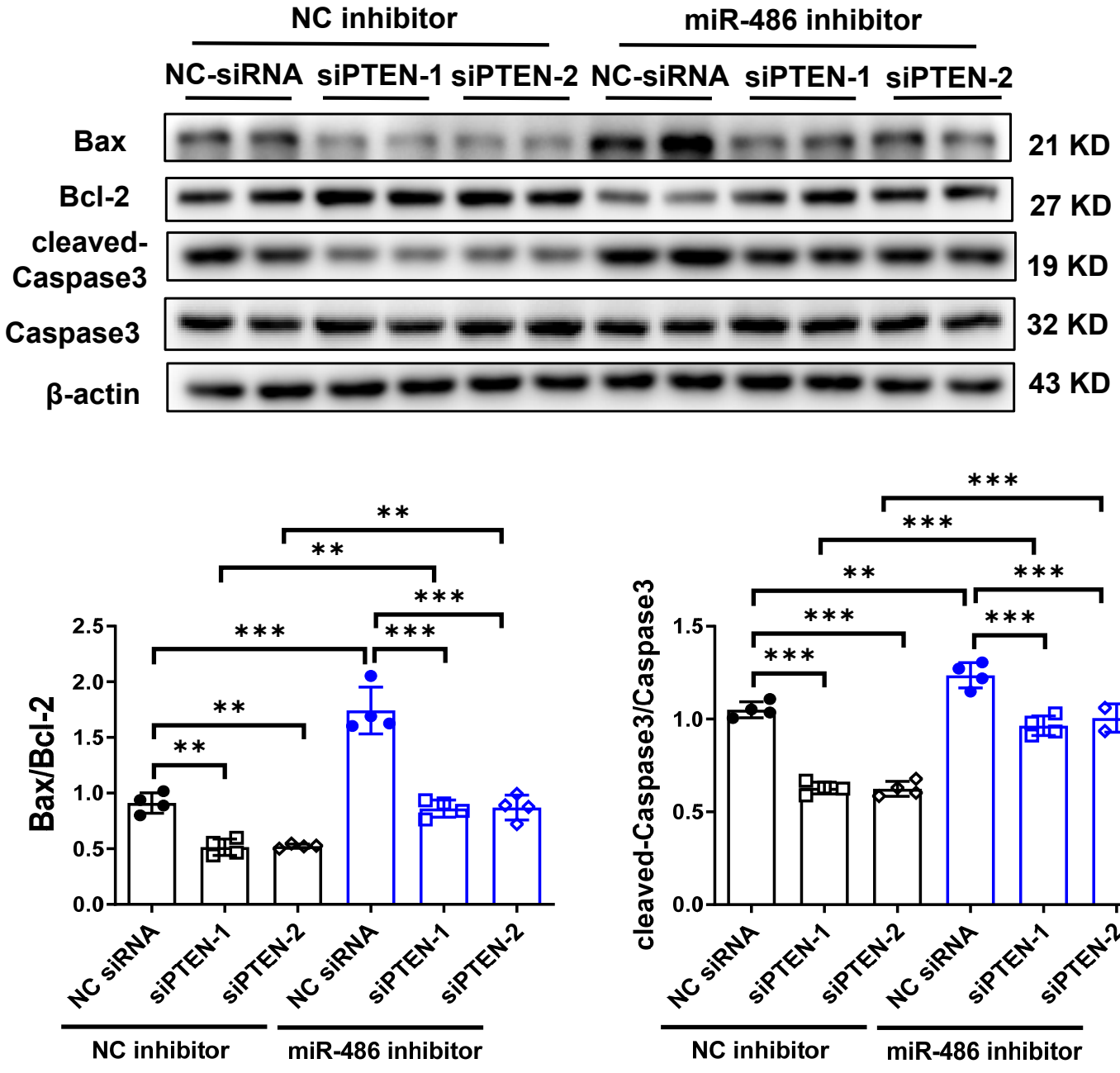
# Figure S6


hiPSC-CM
P=0.0571
P=0.0286
Relative mRNA level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PTEN
FoxO1
NC mimic
miR-486 mimic

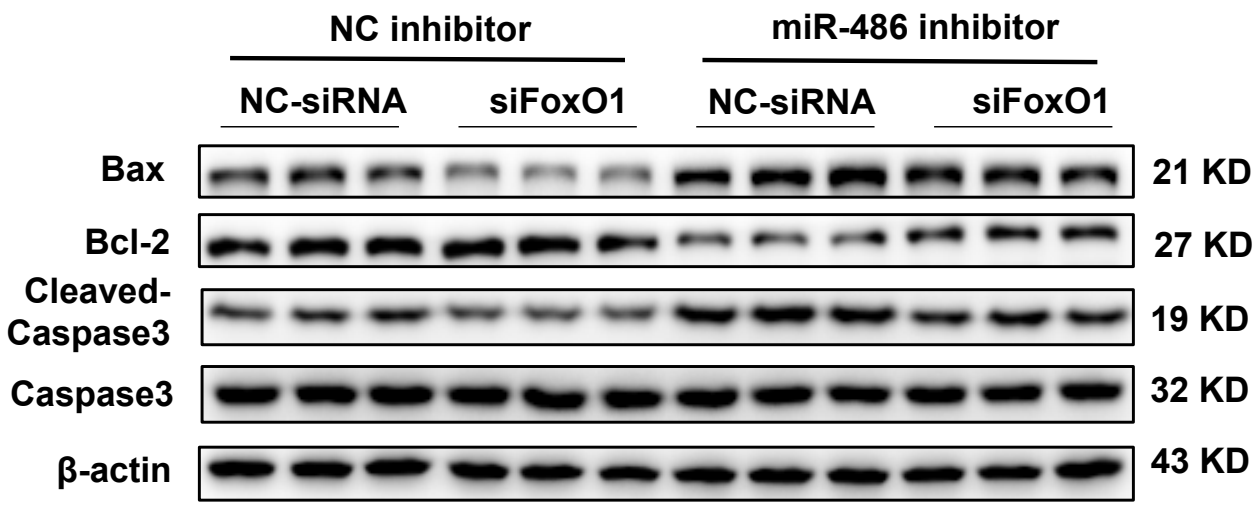
# Figure S7

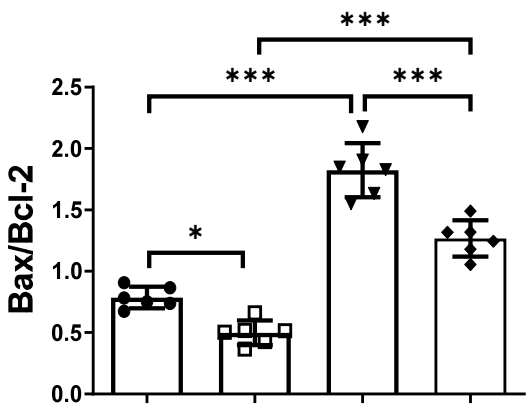

A
***
***
Relative PTEN mRNA level
1.5
1.0
0.5
0.0
NC siRNA
siPTEN-1
siPTEN-2
B
***
**
Relative FoxO1 mRNA level
1.5
1.0
0.5
0.0
NC siRNA
siFoxO1-1
siFoxO1-2

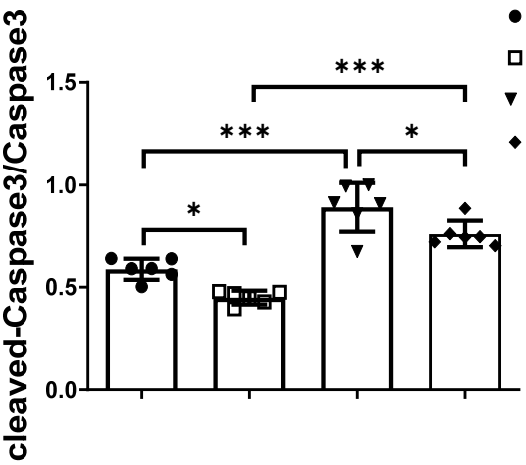
# Figure S8

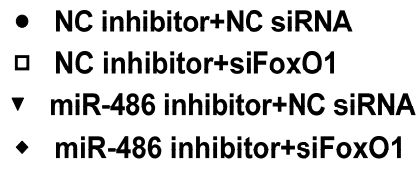

NC inhibitor
miR-486 inhibitor
NC-siRNA
siPTEN-1
siPTEN-2
NC-siRNA
siPTEN-1
siPTEN-2
Bax
Bcl-2
cleaved-Caspase3
Caspase3
β-actin
21 KD
27 KD
19 KD
32 KD
43 KD
Bax/Bcl-2
cleaved-Caspase3/Caspase3
NC siRNA
siPTEN-1
siPTEN-2
NC siRNA
siPTEN-1
siPTEN-2
NC inhibitor
miR-486 inhibitor

# Figure S9

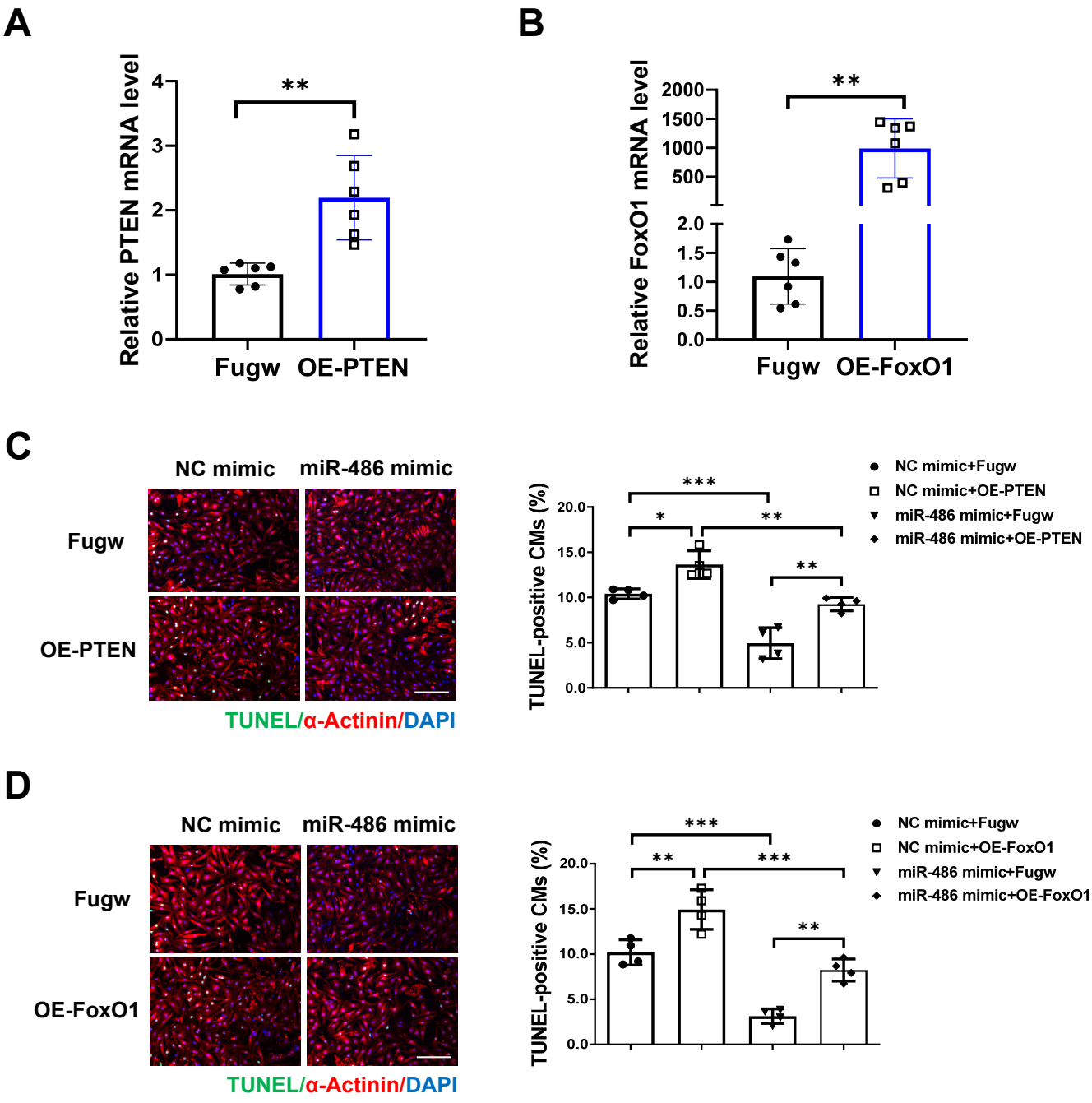

NC inhibitor
miR-486 inhibitor
NC-siRNA
siFoxO1
NC-siRNA
siFoxO1
Bax
Bcl-2
Cleaved-Caspase3
Caspase3
β-actin
21 KD
27 KD
19 KD
32 KD
43 KD


Bax/Bcl-2
2.5
2.0
1.5
1.0
0.5
0.0
***
***
***
*

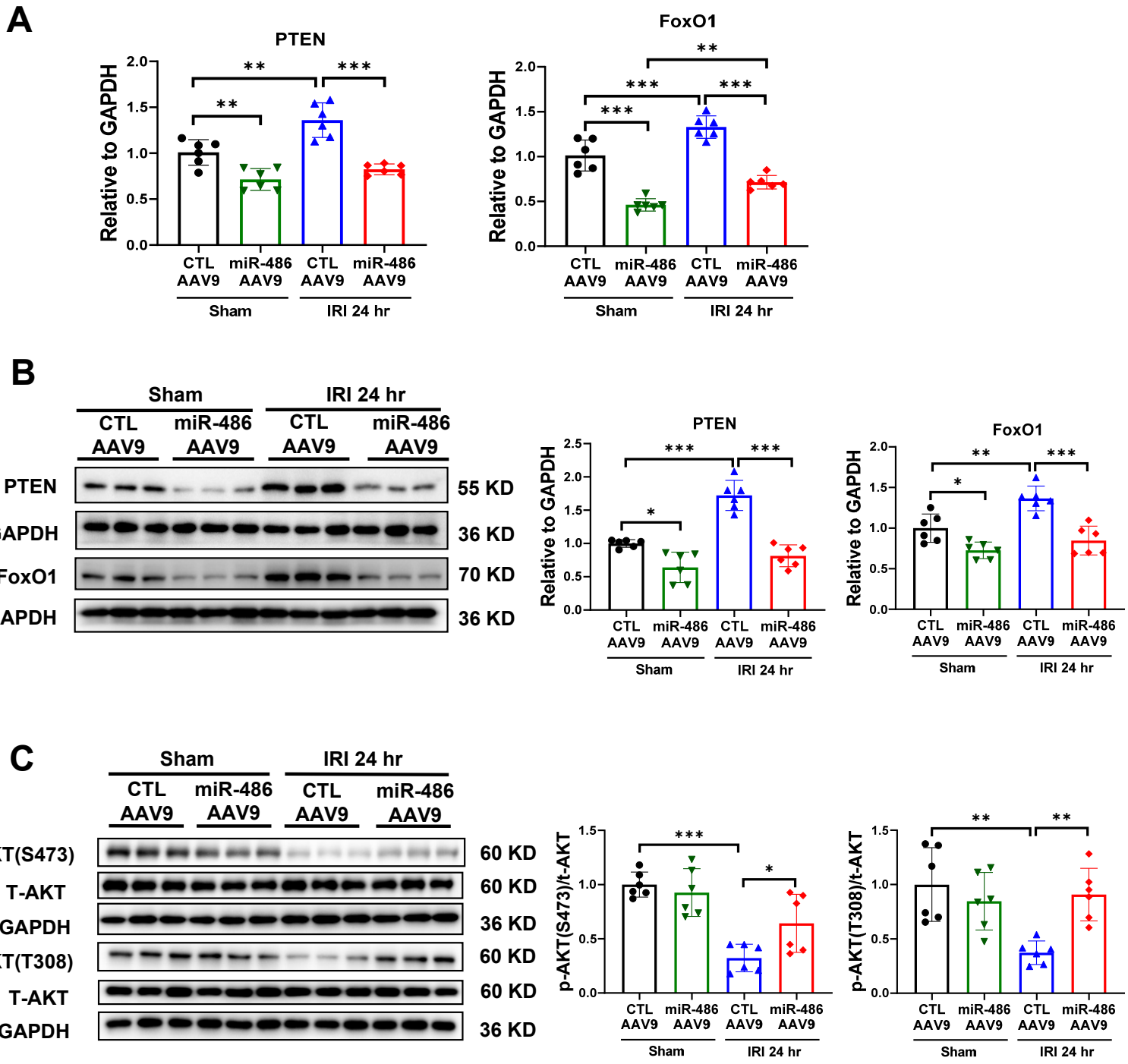

cleaved-Caspase3/Caspase3
1.5
1.0
0.5
0.0
***
***
*
*


NC inhibitor+NC siRNA
NC inhibitor+siFoxO1
miR-486 inhibitor+NC siRNA
miR-486 inhibitor+siFoxO1

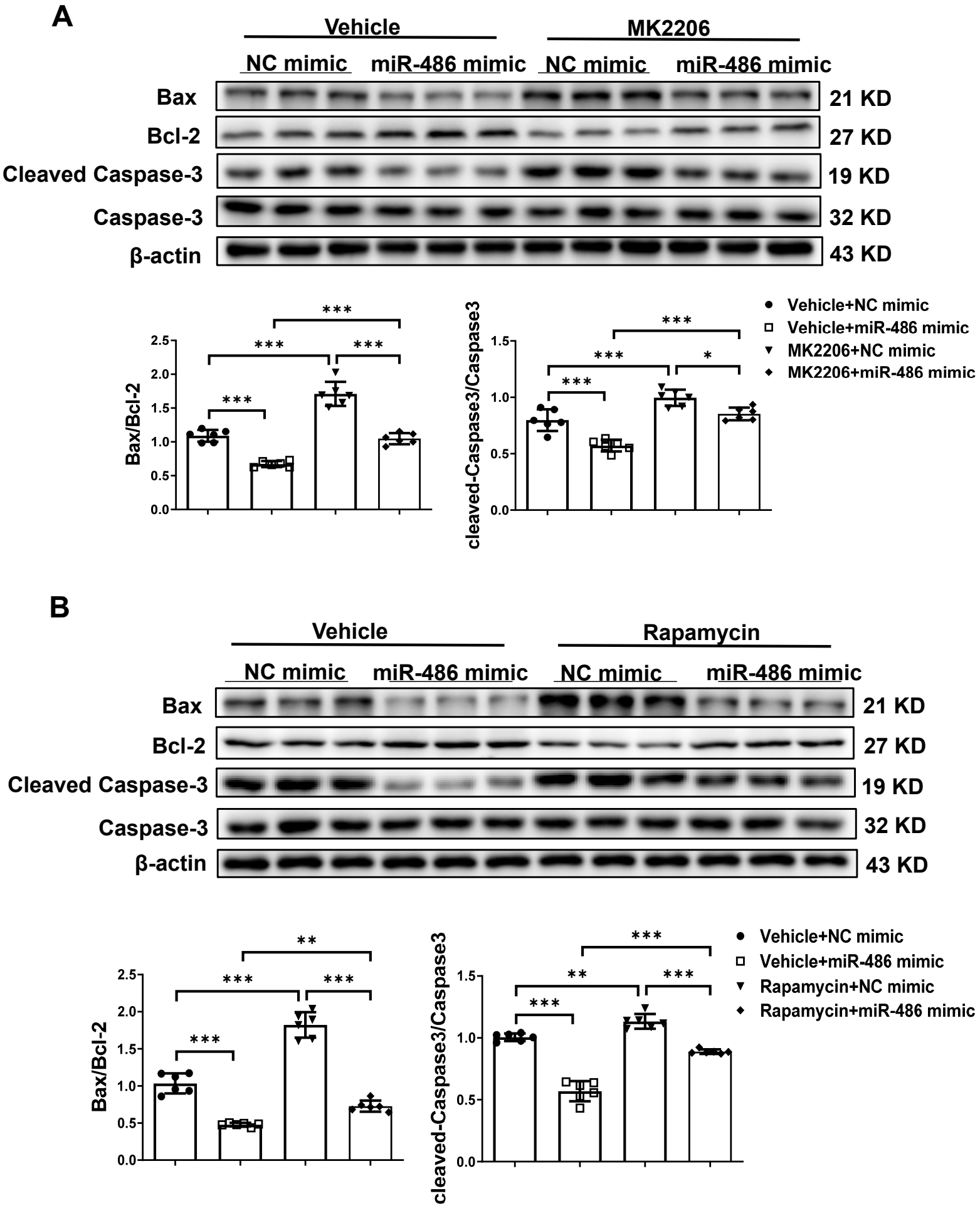
# Figure S10


A
Relative PTEN mRNA level
**
Fugw
OE-PTEN
B
Relative FoxO1 mRNA level
**
Fugw
OE-FoxO1
C
NC mimic
miR-486 mimic
Fugw
OE-PTEN
TUNEL/α-Actinin/DAPI
TUNEL-positive CMs (%)
NC mimic+Fugw
NC mimic+OE-PTEN
miR-486 mimic+Fugw
miR-486 mimic+OE-PTEN
D
NC mimic
miR-486 mimic
Fugw
OE-FoxO1
TUNEL/α-Actinin/DAPI
TUNEL-positive CMs (%)
NC mimic+Fugw
NC mimic+OE-FoxO1
miR-486 mimic+Fugw
miR-486 mimic+OE-FoxO1

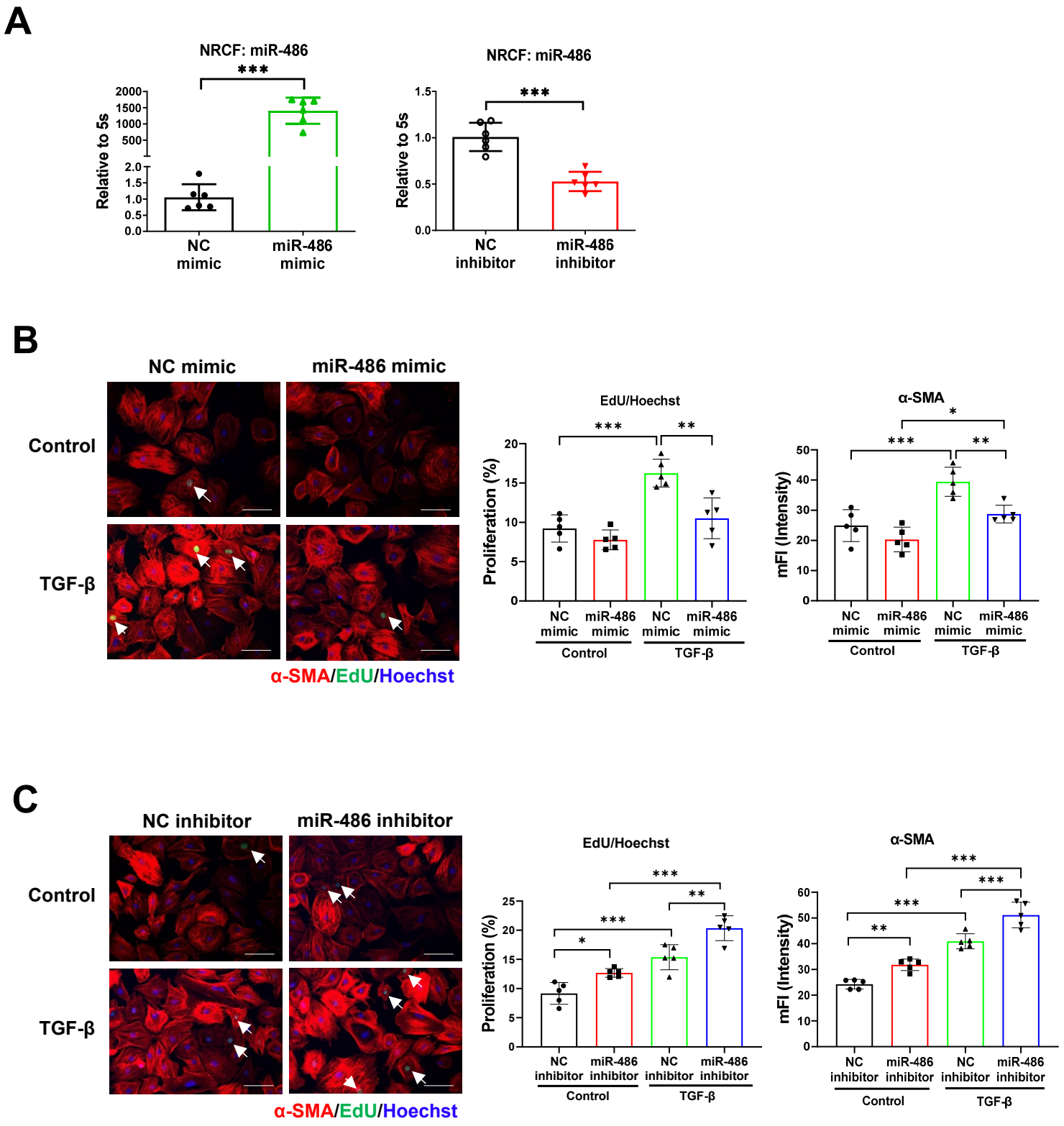
# Figure S11


A
PTEN
Relative to GAPDH
2.0
1.5
1.0
0.5
0.0
**
**
***
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
Sham
IRI 24 hr
FoxO1
Relative to GAPDH
**
***
***
***
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
Sham
IRI 24 hr
B
Sham
IRI 24 hr
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
PTEN
GAPDH
FoxO1
GAPDH
55 KD
36 KD
70 KD
36 KD
PTEN
Relative to GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
***
***
*
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
Sham
IRI 24 hr
FoxO1
Relative to GAPDH
2.0
1.5
1.0
0.5
0.0
**
***
*
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
Sham
IRI 24 hr
C
Sham
IRI 24 hr
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
p-AKT(S473)
T-AKT
GAPDH
p-AKT(T308)
T-AKT
GAPDH
60 KD
60 KD
36 KD
60 KD
60 KD
36 KD
p-AKT(S473)/t-AKT
1.5
1.0
0.5
0.0
***
*
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
Sham
IRI 24 hr
p-AKT(T308)/t-AKT
1.5
1.0
0.5
0.0
**
**
CTL AAV9
miR-486 AAV9
CTL AAV9
miR-486 AAV9
Sham
IRI 24 hr

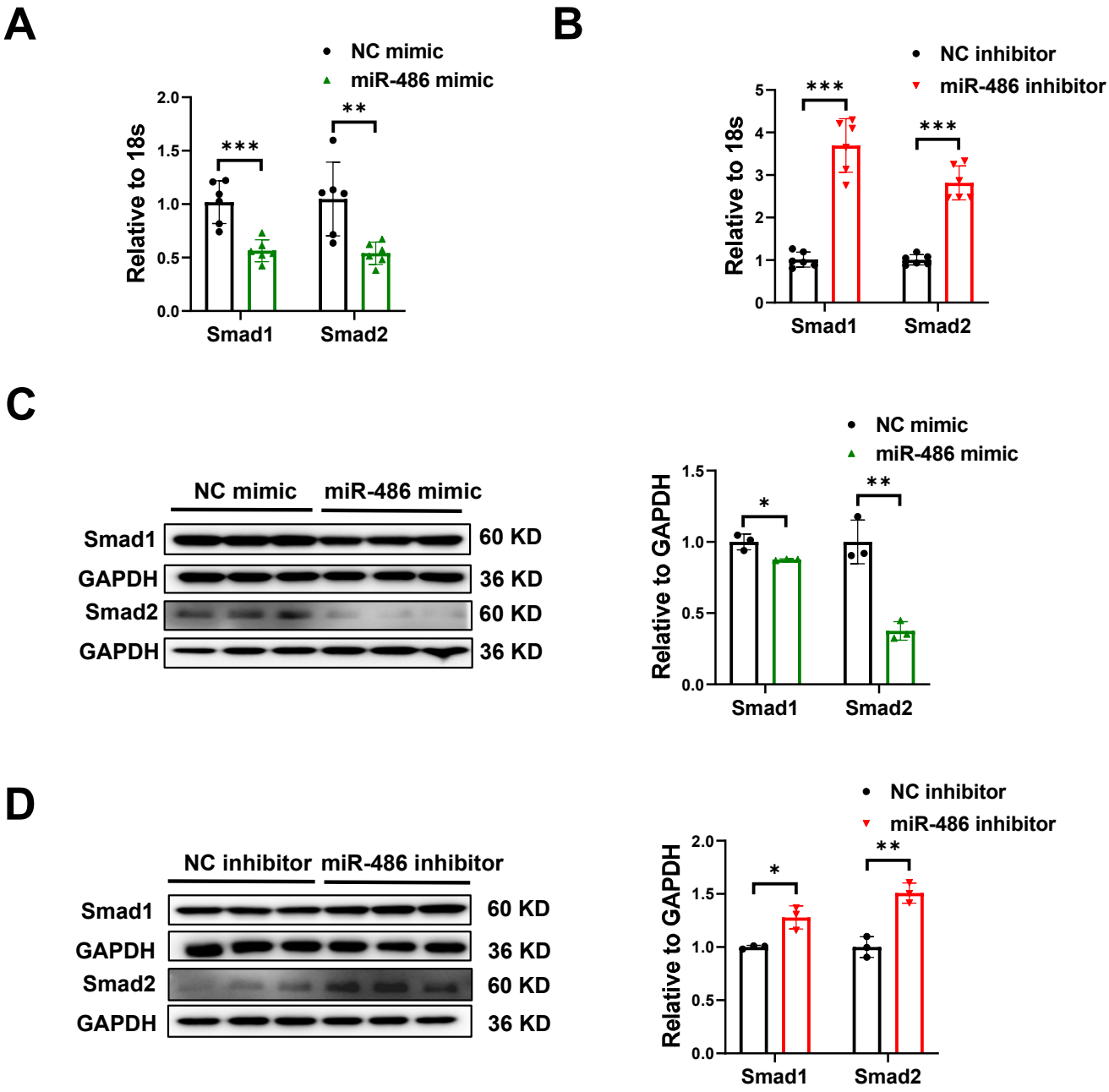
# Figure S12


A
Vehicle
MK2206
NC mimic
miR-486 mimic
NC mimic
miR-486 mimic
Bax
Bcl-2
Cleaved Caspase-3
Caspase-3
β-actin
21 KD
27 KD
19 KD
32 KD
43 KD
Bax/Bcl-2
cleaved-Caspase3/Caspase3
Vehicle+NC mimic
Vehicle+miR-486 mimic
MK2206+NC mimic
MK2206+miR-486 mimic
B
Vehicle
Rapamycin
NC mimic
miR-486 mimic
NC mimic
miR-486 mimic
Bax
Bcl-2
Cleaved Caspase-3
Caspase-3
β-actin
21 KD
27 KD
19 KD
32 KD
43 KD
Bax/Bcl-2
cleaved-Caspase3/Caspase3
Vehicle+NC mimic
Vehicle+miR-486 mimic
Rapamycin+NC mimic
Rapamycin+miR-486 mimic

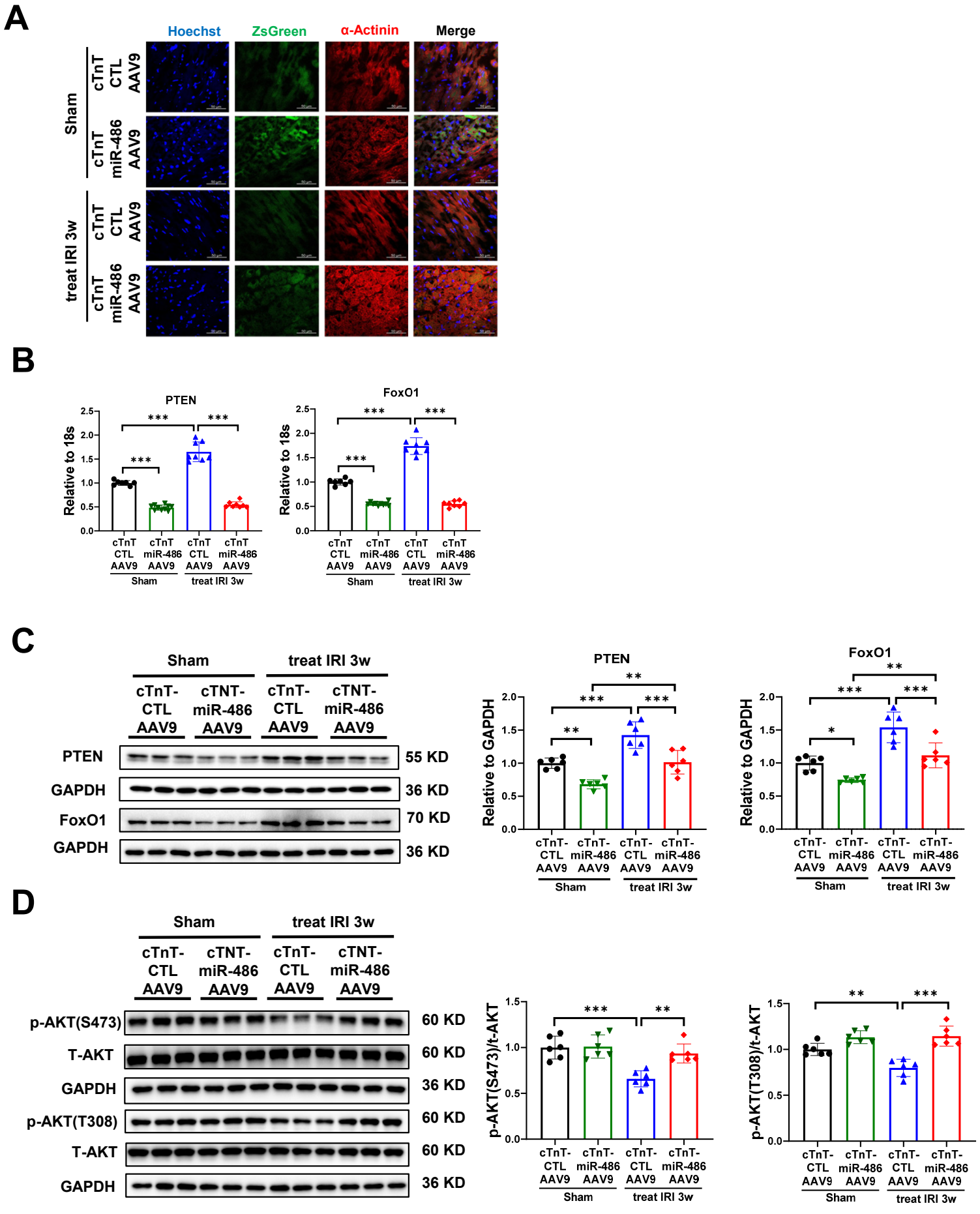
# Figure S13


A
NRCF: miR-486
***
Relative to 5s
2000
1500
1000
500
2.0
1.5
1.0
0.5
0.0
NC mimic
miR-486 mimic
NRCF: miR-486
***
Relative to 5s
1.5
1.0
0.5
0.0
NC inhibitor
miR-486 inhibitor
B
NC mimic
miR-486 mimic
Control
TGF-β
α-SMA/EdU/Hoechst
EdU/Hoechst
***
**
Proliferation (%)
20
15
10
5
0
NC mimic
miR-486 mimic
NC mimic
miR-486 mimic
Control
TGF-β
α-SMA
*
***
**
mFI (Intensity)
50
40
30
20
10
0
NC mimic
miR-486 mimic
NC mimic
miR-486 mimic
Control
TGF-β
C
NC inhibitor
miR-486 inhibitor
Control
TGF-β
α-SMA/EdU/Hoechst
EdU/Hoechst
***
**
*
Proliferation (%)
25
20
15
10
5
0
NC inhibitor
miR-486 inhibitor
NC inhibitor
miR-486 inhibitor
Control
TGF-β
α-SMA
***
***
***
**
mFI (Intensity)
60
50
40
30
20
10
0
NC inhibitor
miR-486 inhibitor
NC inhibitor
miR-486 inhibitor
Control
TGF-β

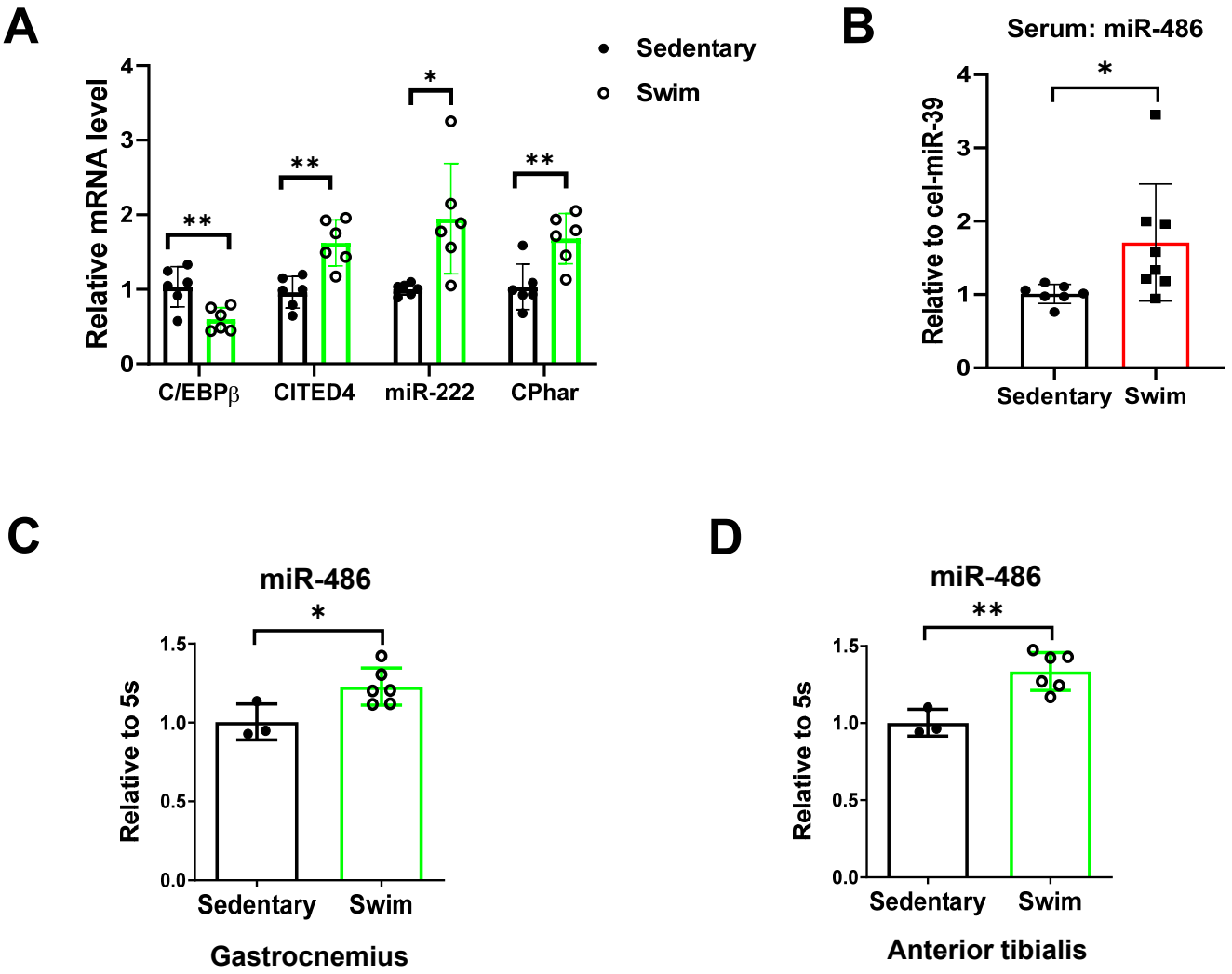
# Figure S14


A
NC mimic
miR-486 mimic
Relative to 18s
2.0
1.5
1.0
0.5
0.0
***
**
Smad1
Smad2
B
NC inhibitor
miR-486 inhibitor
Relative to 18s
5
4
3
2
1
0
***
***
Smad1
Smad2
C
NC mimic
miR-486 mimic
Smad1
GAPDH
Smad2
GAPDH
60 KD
36 KD
60 KD
36 KD
NC mimic
miR-486 mimic
Relative to GAPDH
1.5
1.0
0.5
0.0
*
**
Smad1
Smad2
D
NC inhibitor
miR-486 inhibitor
Smad1
GAPDH
Smad2
GAPDH
60 KD
36 KD
60 KD
36 KD
NC inhibitor
miR-486 inhibitor
Relative to GAPDH
2.0
1.5
1.0
0.5
0.0
*
**
Smad1
Smad2

# Figure S15

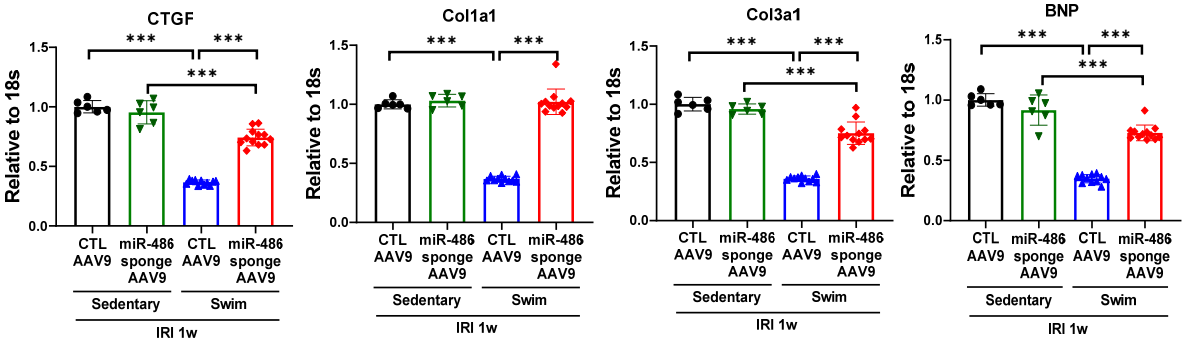

A
Hoechst
ZsGreen
α-Actinin
Merge
Sham
treat IRI 3w
cTnT CTL AAV9
cTnT miR-486 AAV9
B
PTEN
FoxO1
Relative to 18s
cTnT CTL AAV9
cTnT miR-486 AAV9
Sham
treat IRI 3w
C
Sham
treat IRI 3w
cTnT-CTL AAV9
cTNT-miR-486 AAV9
PTEN
GAPDH
FoxO1
55 KD
36 KD
70 KD
Relative to GAPDH
D
p-AKT(S473)
T-AKT
p-AKT(T308)
60 KD
p-AKT(S473)/t-AKT
p-AKT(T308)/t-AKT

# Figure S16

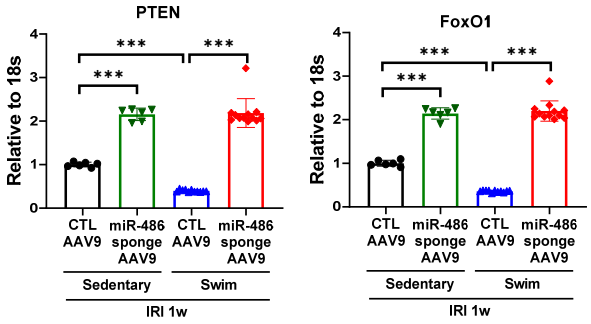

A
Sedentary
Swim
Relative mRNA level
C/EBPβ
CITED4
miR-222
CPhar
B
Serum: miR-486
Relative to cel-miR-39
Sedentary
Swim
C
miR-486
Relative to 5s
Sedentary
Swim
Gastrocnemius
D
miR-486
Relative to 5s
Sedentary
Swim
Anterior tibialis

# Figure S17

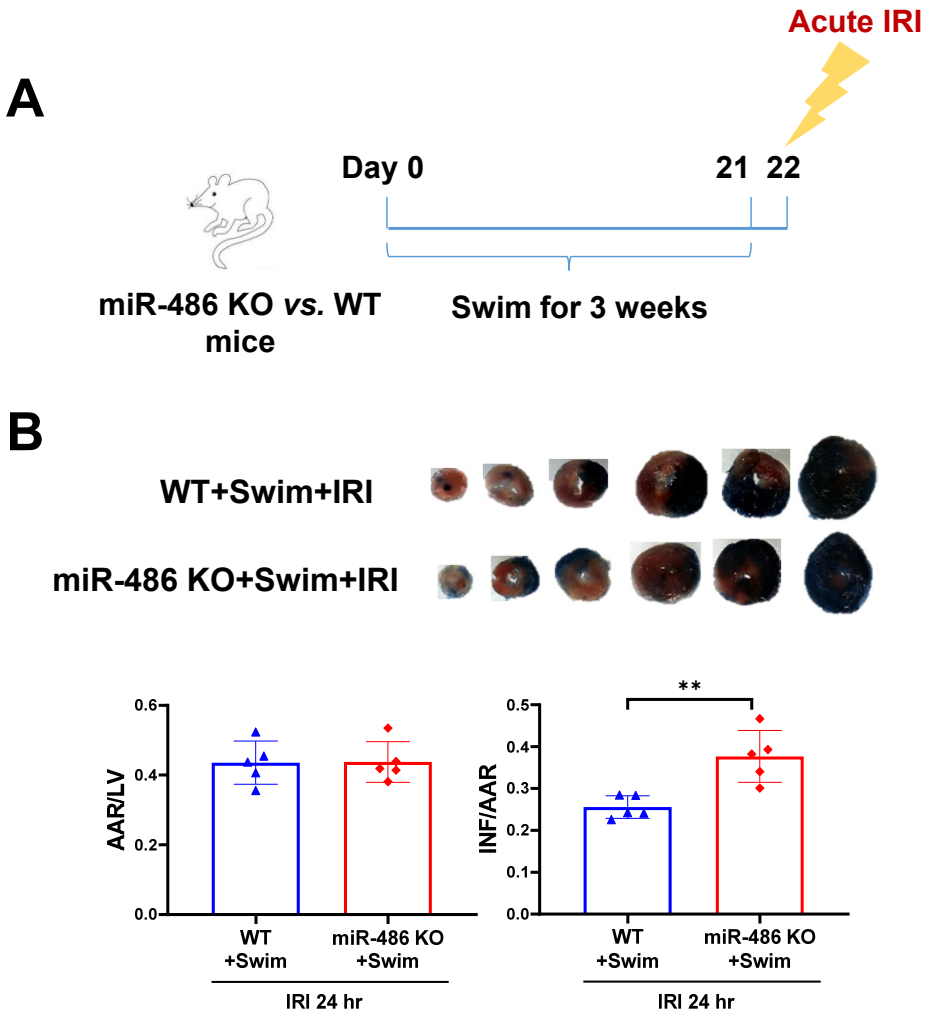
**A**




**B**



# Figure S18


A
Acute IRI
Day 0
21 22
miR-486 KO vs. WT mice
Swim for 3 weeks
B
WT+Swim+IRI
miR-486 KO+Swim+IRI
AAR/LV
0.6
0.4
0.2
0.0
WT +Swim
miR-486 KO +Swim
IRI 24 hr
INF/AAR
0.5
0.4
0.3
0.2
0.1
0.0
**
WT +Swim
miR-486 KO +Swim
IRI 24 hr

**Supplemental Figure Legends**

**Figure S1. Preventive intervention by miR-486-AAV9 in acute cardiac ischemia/reperfusion injury.** (A) Schematic diagram showing that AAV9 expressing miR-486 (miR-486-AAV9) or AAV9 controls (CTL-AAV9) were injected via tail vein, and 3 weeks later mice were subjected to cardiac ischemia/reperfusion (I/R) injury for 24 hrs. (B) RT-PCR for miR-486 expression in mice heart tissues at 24 hrs post cardiac I/R injury (n=6). (C) Western blot for Bax/Bcl-2 ratio and cleaved-Caspase3/Caspase3 ratio in mice heart tissues (n=6). (D) RT-PCR for CTGF, Col1a1, Col3a1, and BNP expressions in mice heart tissues (n=6). Data were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

**Figure S2. Preventive intervention by miR-486-AAV9 in chronic cardiac ischemia/reperfusion injury.** (A) Schematic diagram showing that AAV9 expressing miR-486 (miR-486-AAV9) or AAV9 controls (CTL-AAV9) were injected via tail vein, and 1 week later mice were subjected to cardiac ischemia/reperfusion (I/R) injury for 3 weeks. (B) RT-PCR for miR-486 expression in mice heart tissues at 3 weeks post cardiac I/R injury (n=9-10). (C) Western blot for Bax/Bcl-2 ratio and cleaved-Caspase3/Caspase3 ratio in mice heart tissues (n=3). Data were compared by two-way ANOVA test followed by Tukey post hoc test. **, $P<0.01$; ***, $P<0.001$.

**Figure S3. Long term protective effect of miR-486 overexpression against cardiac ischemia/reperfusion injury and cardiac dysfunction over 6 weeks.** (A) Survival rate of mice injected with miR-486-AAV9 or CTL-AAV9 after 6 weeks of cardiac ischemia/reperfusion (I/R) injury (n=8,8,13,12 before I/R injury, n=8,8,10,12 at 6 weeks post I/R injury). (B) RT-PCR for miR-486 expression in mice heart tissues at 6 weeks post cardiac I/R injury (n=8-12). (C) Echocardiography for left ventricular

ejection fraction (EF, %) and fractional shortening (FS, %) in mice at 6 week after I/R injury (n=8-12). (D) Masson Trichrome staining for cardiac fibrosis in mice heart tissues (n=6-7). Scale bar=100 μm. (E) RT-PCR for CTGF, Col1a1, Col3a1, and BNP expressions in mice heart tissues (n=8-12). Data were compared by two-way ANOVA test followed by Tukey post hoc test. **, $P<0.01$; ***, $P<0.001$.

**Figure S4. Inhibition of miR-486 does not further aggravate cardiac ischemia/reperfusion injury.** (A) The 2,3,5-triphenyltetrazolium chloride (TTC) staining for the infarct size at 24 hrs after cardiac ischemia/reperfusion (I/R) injury as determined by the infarct size/area at risk (INF/AAR) ratio. The area at risk/left ventricle weight (AAR/LV) ratio represents the homogeneity of surgery (n=6). (B) Schematic diagram showing that miR-486 sponge AAV9 or control AAV9 (CTL-AAV9) were injected via tail vein, and 1 week later mice were subjected to cardiac I/R injury for 3 weeks. (C) RT-PCR for miR-486 expression in mice heart tissues at 3 weeks post cardiac I/R injury (n=9-10). (D) Luciferase reporter assays performed in 293T cells co-transfected with negative control (NC mimic), miR-486 mimic or miR-210 mimic and the miR-486 binding site-carrying luciferase reporter plasmids (n=6). (E) Echocardiography for left ventricular ejection fraction (EF, %) and fractional shortening (FS, %) in mice at 3 week after I/R injury (n=9-10). (F) Masson Trichrome staining for cardiac fibrosis in mice heart tissues (n=6-7). Scale bar=100 μm. (G) Western blot for Bax/Bcl-2 ratio and cleaved-Caspase3/Caspase3 ratio in mice heart tissues (n=6). (H) RT-PCR for CTGF, Col1a1, Col3a1, and BNP expressions in mice heart tissues (n=9-10). Data between 2 groups were compared by unpaired two-tailed Student's t-test. Data among 3 groups were compared by one-way ANOVA test. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$; ***, $P<0.001$.

**Figure S5. Transfection of miR-486 mimic or inhibitor in cardiomyocytes *in vitro*.** (A) RT-PCR for miR-486 expression in neonatal rat cardiomyocytes (NRCMs) transfected with miR-486 mimic, inhibitor, or negative controls (NC) (n=6). (B) Representative image of immunofluorescent staining for cardiac Troponin T (cTnT) which ensures the purification of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Scale bar=100 μm. (C) RT-PCR for miR-486 expression in hiPSC-CMs transfected with miR-486 mimic or negative control (n=4). (D) Relative hiPSC-CM numbers after oxygen glucose deprivation/reperfusion (OGDR) and transfection of miR-486 mimic (n=3). Data between 2 groups were compared by unpaired two-tailed Student's t-test. Data among 3 groups were compared by one-way ANOVA test. *, $P<0.05$; ***, $P<0.001$.

**Figure S6. Regulation of PTEN and FoxO1 in human induced pluripotent stem cell-derived cardiomyocytes with miR-486 overexpression.** RT-PCR for PTEN and FoxO1 in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) transfected with miR-486 mimic or negative control (NC mimic) (n=4). Data were compared by unpaired two-tailed Student's t-test.

**Figure S7. Si-RNAs significantly downregulate PTEN or FoxO1 expression in neonatal rat cardiomyocytes.** (A,B) RT-PCR for PTEN (A) or FoxO1 (B) expressions in neonatal rat cardiomyocytes (NRCMs) transfected with siRNAs targeting PTEN or FoxO1, respectively (n=4-6). Data were compared between PTEN or FoxO1 siRNA group and NC siRNA group using unpaired two-tailed Student's t-test. **, $P<0.01$; ***, $P<0.001$.

**Figure S8. Western blot for Bax/Bcl-2 ratio and cleaved-Caspase3/Caspase3 ratio in oxygen glucose deprivation/reperfusion-treated neonatal rat cardiomyocytes transfected with miR-486 inhibitor and PTEN siRNA.** (n=4). Data were compared

by two-way ANOVA test followed by Tukey post hoc test. **, $P<0.01$; ***, $P<0.001$.

**Figure S9. Western blot for Bax/Bcl-2 ratio and cleaved-Caspase3/Caspase3 ratio in oxygen glucose deprivation/reperfusion-treated neonatal rat cardiomyocytes transfected with miR-486 inhibitor and FoxO1 siRNA.** (n=6). Data were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$; ***, $P<0.001$.

**Figure S10. Overexpression of PTEN or FoxO1 attenuates the protective effect of miR-486 mimic against cardiomyocytes apoptosis.** (A,B) RT-PCR for PTEN and FoxO1 in neonatal rat cardiomyocytes (NRCMs) transfected with plasmids expressing PTEN (A) or FoxO1 (B) (n=6). (C,D) TUNEL staining for α-Actinin-labelled NRCMs transfected with miR-486 mimic and plasmids expressing PTEN (C) or FoxO1 (D) in the condition of oxygen glucose deprivation/reperfusion (OGDR) treatment (n=4). Scale bar=100 μm. Data between 2 groups were compared by unpaired two-tailed Student's t-test. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

**Figure S11. Preventive delivery of miR-486-AAV9 regulates PTEN and FoxO1 in mice heart tissues.** (A,B) RT-PCR (A) and Western blot (B) for PTEN and FoxO1 expressions in heart tissues from mice injected with miR-486-AAV9 before cardiac ischemia/reperfusion (I/R) injury. Heart tissues were harvested at 24 hrs post I/R injury (n=6). (C) Western blot for AKT phosphorylation levels in heart tissues from mice injected with miR-486-AAV9 before cardiac I/R injury. Heart tissues were harvested at 24 hrs post I/R injury (n=6). Data were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

**Figure S12. MiR-486 inhibits cardiomyocyte apoptosis through activating AKT and mTOR.** (A,B) Western blot for Bax/Bcl-2 ratio and cleaved-Caspase3/Caspase3 ratio in oxygen glucose deprivation/reperfusion (OGDR)-treated neonatal rat

cardiomyocytes transfected with miR-486 mimic in the presence or absence of AKT inhibitor MK2206 (A) or mTOR inhibitor Rapamycin (B) (n=6). Data were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

**Figure S13. MiR-486 inhibits cardiac fibroblast proliferation and activation.** (A) RT-PCR for miR-486 in neonatal rat cardiac fibroblasts (NRCFs) transfected with miR-486 mimic, inhibitor, or negative controls (NC) (n=6). (B,C) Immunofluorescent staining for α-SMA/EdU in NRCFs transfected with miR-486 mimic (B), inhibitor (C), or NC (n=5). Scale bar=100 μm. Data between 2 groups were compared by unpaired two-tailed Student's t-test. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

**Figure S14. MiR-486 negatively regulates Smad1 and Smad2 in cardiac fibroblasts.** (A,B) RT-PCR for Smad1 and Smad2 in neonatal rat cardiac fibroblasts (NRCFs) transfected with miR-486 mimic (A), inhibitor (B), or negative controls (NC) (n=6). (C,D) Western blot for Smad1 and Smad2 in NRCFs transfected with miR-486 mimic (C), inhibitor (D), or NC (n=3). Data were compared by unpaired two-tailed Student's t-test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

**Figure S15. Therapeutic delivery of cTnT-miR-486 AAV9 regulates PTEN and FoxO1 in mice heart tissues.** (A) Immunofluorescent imaging for co-localization of ZsGreen (indicative of AAV9) and α-Actinin staining in heart tissues from mice injected with cTnT-miR-486 AAV9 or cTnT-control AAV9. Scale bar=50 μm. (B,C) RT-PCR (B, n=7-10) and Western blot (C, n=6) for PTEN and FoxO1 expressions in heart tissues from mice treated with cTnT-miR-486 AAV9 within 30 min post myocardial reperfusion. Heart tissues were harvested at 3 weeks post cardiac ischemia/reperfusion (I/R) injury. (D) Western blot for AKT phosphorylation levels in

heart tissues from mice treated with cTnT-miR-486 AAV9 within 30 min post myocardial reperfusion. Heart tissues were harvested at 3 weeks post cardiac I/R injury (n=6). Data were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

**Figure S16. Expression of miR-486 in a mouse model of swimming exercise.** (A) RT-PCR for the known factors in response to swimming exercise in heart tissues (n=6). (B) RT-PCR for circulating miR-486 levels in the serum from swimmed mice and sedentary mice (n=7-8). (C,D) RT-PCR for miR-486 expression in the gastrocnemius (C) and anterior tibialis (D) from swimmed mice and sedentary mice (n=3-6). Data were compared by unpaired two-tailed Student's t-test. *, $P<0.05$; **, $P<0.01$.

**Figure S17. MiR-486 inhibition regulates fibrosis-associated gene markers and its downstream targets in swimmed mice upon cardiac ischemia/reperfusion injury.** (A,B) RT-PCR for fibrosis-associated gene markers (A) and PTEN and FoxO1 (B) in mice heart tissues (n=6 for sedentary mice, n=11-12 for swimmed mice). Data were compared by two-way ANOVA test followed by Tukey post hoc test. ***, $P<0.001$.

**Figure S18. The beneficial effect of swimming exercise against cardiac ischemia/reperfusion injury is attenuated in miR-486 knockout mice.** (A) Schematic diagram showing that miR-486 knockout (KO) mice and wild type (WT) littermates were subjected to swimming exercise for 3 weeks before cardiac ischemia/reperfusion (I/R) injury for 24 hrs. (B) The 2,3,5-triphenyltetrazolium chloride (TTC) staining for the infarct size at 24 hrs after I/R injury as determined by the infarct size/area at risk (INF/AAR) ratio. The area at risk/left ventricle weight (AAR/LV) ratio represents the homogeneity of surgery (n=5). Data were compared by

unpaired two-tailed Student's t-test. **, $P<0.01$.

**Supplemental Table**

**Table S1. List of primers used for the PCR analysis.**

| Gene | Primer sequence |
|---|---|
| rno_Pten forward | ATTGCCTGTGTGTGGTGA |
| rno_Pten reverse | TCCTCTGGTCCTGGTATGA |
| rno_FoxO1 forward | TGGGGCAACCTGTCGTA |
| rno_FoxO1 reverse | GGGCACACTCTTCACCATC |
| mmu_Pten forward | AGCCCTAACCCCAAGAAC |
| mmu_Pten reverse | ACAAGTCCCGATGAAACCT |
| mmu_FoxO1 forward | GTACAGCGCATAGCACCA |
| mmu_FoxO1 reverse | GCGACAGACAGAGTTCCC |
| hsa_Pten forward | CAGTCAGAGGCGCTATGTGT |
| hsa_Pten reverse | CACCTTTAGCTGGCAGACCA |
| hsa_FoxO1 forward | GGATGTGCATTCTATGGTGTACC |
| hsa_FoxO1 reverse | TTTCGGGATTGCTTATCTCAGAC |
| mmu_C/EBPβ forward | GGGGTTGTTGATGTTTTTGGT |
| mmu_C/EBPβ reverse | TCGAAACGGAAAAGGTTCTCA |
| mmu_CITED4 forward | CCTGGCATACGGCTCCTTC |
| mmu_CITED4 reverse | AGACTGCAGGTGCGTGCTAC |
| mmu_CPhar forward | CATGGATTTCTGGACCTCCTA |
| mmu_CPhar reverse | TTCATGGCTTTACAGCGT |
| rno_Smad1 forward | CGTGTTGGTGGATGGTTT |
| rno_Smad1 reverse | TGTGTCGCCTGGTATTTTC |
| rno_Smad2 forward | GTCAGTGCGATGCTCAAG |
| rno_Smad2 reverse | CTCAAGTGCTGTTTTCGCT |
| mmu_CTGF forward | TAAGACCTGTGGGATGGG |
| mmu_CTGF reverse | GCAGCCAGAAAGCTCAA |
| mmu_Col1a1 forward | TAAGGGTCCCCAATGGTGAGA |
| mmu_Col1a1 reverse | GGGTCCCTCGACTCCTACAT |
| mmu_Col3a1 forward | CTGTAACATGGAAACTGGGGAAA |
| mmu_Col3a1 reverse | CCATAGCTGAACTGAAAACCACC |
| mmu_BNP forward | GAGTCCTTCGGTCTCAAGGC |
| mmu_BNP reverse | TACAGCCCAAACGACTGACG |