SUPPLEMENTAL MATERIAL

Figure S1. CDC20 protein levels in cardiac fibroblasts. CDC20 protein levels in cardiac fibroblasts treated with phenylephrine (PE, 100 μM (A)) or angiotensin II (Ang II, 100 nM, (B)) at different time points (n=4).

Figure S2. Upregulation of CDC20 promoted the phenylephrine (PE)-induced hypertrophic response in cultured cardiomyocytes. (A) Immunoblot analysis of CDC20 in NRCMs infected with Ad-CDC20 or Ad-GFP control (n=3). **(B)** Representative images of double immunostaining (red for α-actinin, blue for DAPI) of NRCMs after 24 hours of PE (100 nM) (left). Scale bar: 50 μm. Quantification of the myocyte surface area (n=3, 150 cells counted per experiment; right) (n=3). **(C)** qPCR analysis of ANP, BNP and β-MHC mRNA levels in NRCMs treated as described in (B) (n=3). **(D)** Immunoblot analysis of AKT, ERK1/2, p38 and GAPDH in NRCMs treated as described in (B) (n=3). * $P < 0.05$, ** $P < 0.01$ vs. control; $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ vs. Ad-control plus PE.

Figure S3. The effect of rAAV9-ZsGreen on mouse organs. (A) Fluorescence detection of rAAV9-ZsGreen (green). **(B)** Western blot showing the expression of CDC20 and β-actin in the heart, liver and lung treated with rAAV9-siCDC20/siZsGreen (n=3). **(C)** Fluorescence detection of rAAV9-ZsGreen (green). **(D)** Western blot showing the expression of CDC20 and β-actin in the heart, liver and lung treated with rAAV9-CDC20/ZsGreen (n=3).

Figure S4. Overexpression of CDC20 aggravates hypertrophy *in vivo*. (A) Wild-type (WT) mice injected with rAAV9-siZsGreen or rAAV9-CDC20 were subjected to sham operation or TAC for 3

weeks. Kaplan–Meier survival curves. **(B)** Echocardiographic assessment of ejection fraction (EF%) and fractional shortening (FS%) (n=6). **(C)** H&E staining of heart sections (upper panel). Scale bar 0.5 cm. The ratios of heart weight to tibia length (HW/TL) and body weight (HW/BW) (lower panels) (n=6). **(D)** Heart cross-sections were stained with TRITC-labeled WGA (upper). Scale bar: 10 μm. Quantification of the relative myocyte cross-sectional area (n=6, 200 cells counted per heart; lower panel). **(E)** Representative images of Masson's Trichrome (upper) and DHE staining (middle) of the ventricular sections. Scale bar: 50 μm. Quantification of the relative fibrotic area and ROS fluorescence intensity (n=6 lower). **(F)** qPCR analysis of ANP, BNP and β-MHC mRNA levels in the heart (n=6). **(J)** Immunoblot analysis of p-AKT, AKT, p-ERK1/2, ERK1/2, NOX4 and GAPDH in heart samples (n=6). * $P < 0.05$, ** $P < 0.01$ vs. sham; $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ vs. the rAAV9-ZsGreen plus TAC group.

Figure S5. The expression of LC3 mRNA in NRCMs. Relative mRNA levels of LC3 in NRCMs infected with Ad-CDC20 or the Ad-GFP control. LC3 mRNA levels were measured using a real-time PCR assay and normalized against GAPDH mRNA (n=3).

Figure S6. Inhibition of autophagy reversed the reduction of cardiomyocyte size after the deletion of CDC20 *in vitro*. (A) Functional link between CDC20 and LC3-dependent autophagy in hypertrophy. C, Representative images of double immunostaining (green for α-actinin) of NRCMs transfected with siRNA-CDC20 or siRNA-control with or without CQ treatment after 24 hours of Ang II stimulation (left). Scale bar 50 μm. Quantification of the myocyte surface area (right) (n=3). **(B)** Representative Western blot analyses of p-AKT, AKT, p-ERK1/2, ERK1/2, NOX4, LC3 I/II and GAPDH in NRCMs infected with siRNA-control or siRNA-CDC20 with or without CQ treatment after 24 hours of Ang II

stimulation. ** $P < 0.01$ vs. saline; $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ vs. saline (n=3).

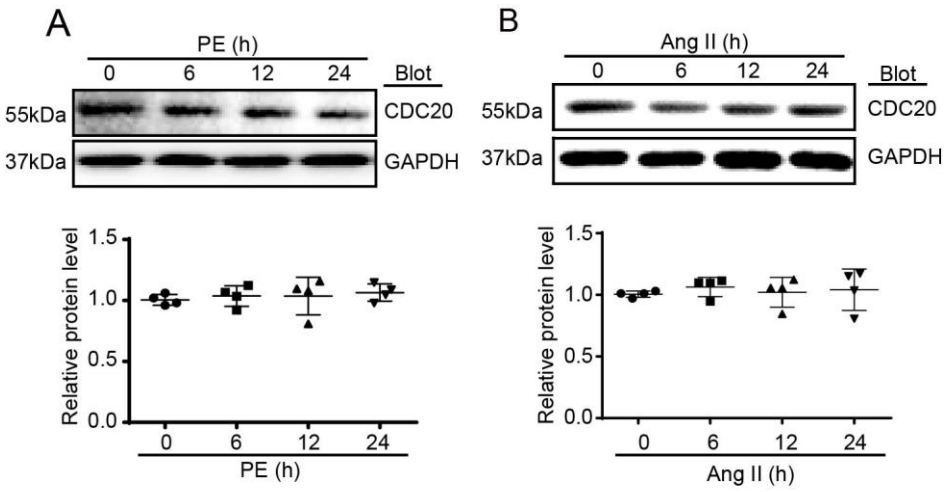
A
PE (h)
0
6
12
24
Blot
55kDa
CDC20
37kDa
GAPDH
Relative protein level
1.5
1.0
0.5
0.0
0
6
12
24
PE (h)
B
Ang II (h)
0
6
12
24
Blot
55kDa
CDC20
37kDa
GAPDH
Relative protein level
1.5
1.0
0.5
0.0
0
6
12
24
Ang II (h)

Figure S1

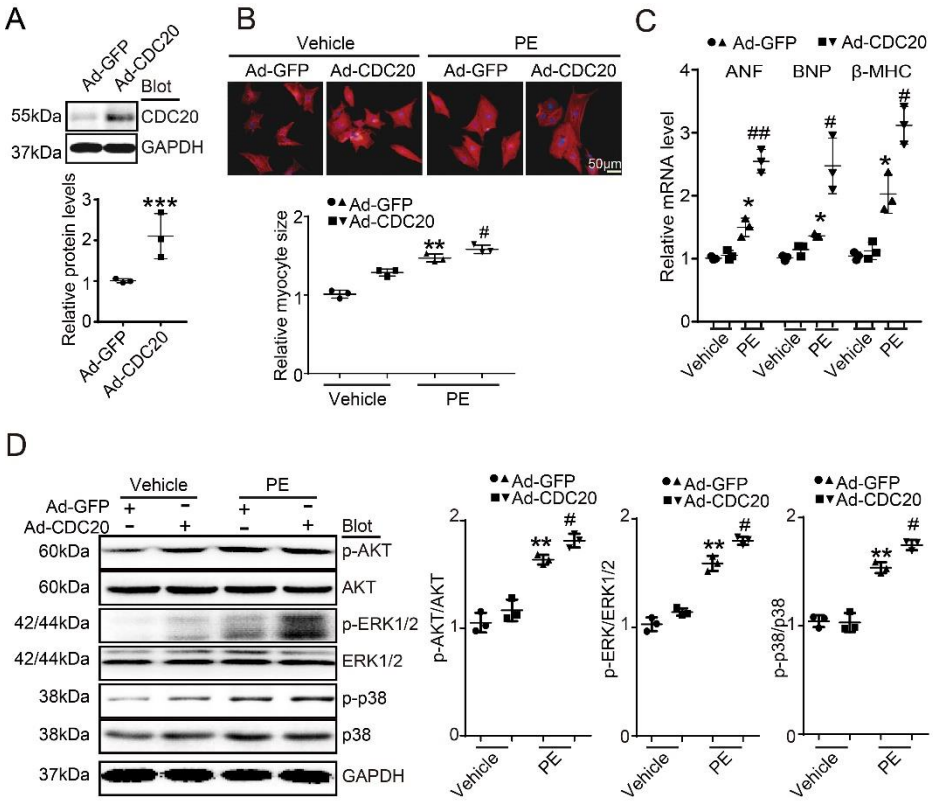
A
Ad-GFP
Ad-CDC20
Blot
55kDa
CDC20
37kDa
GAPDH
Relative protein levels
***
B
Vehicle
PE
Ad-GFP
Ad-CDC20
50μm
Relative myocyte size
C
ANF
BNP
β-MHC
Relative mRNA level
D
60kDa
p-AKT
AKT
42/44kDa
p-ERK1/2
ERK1/2
38kDa
p-p38
p38
p-AKT/AKT
p-ERK/ERK1/2
p-p38/p38

Figure S2

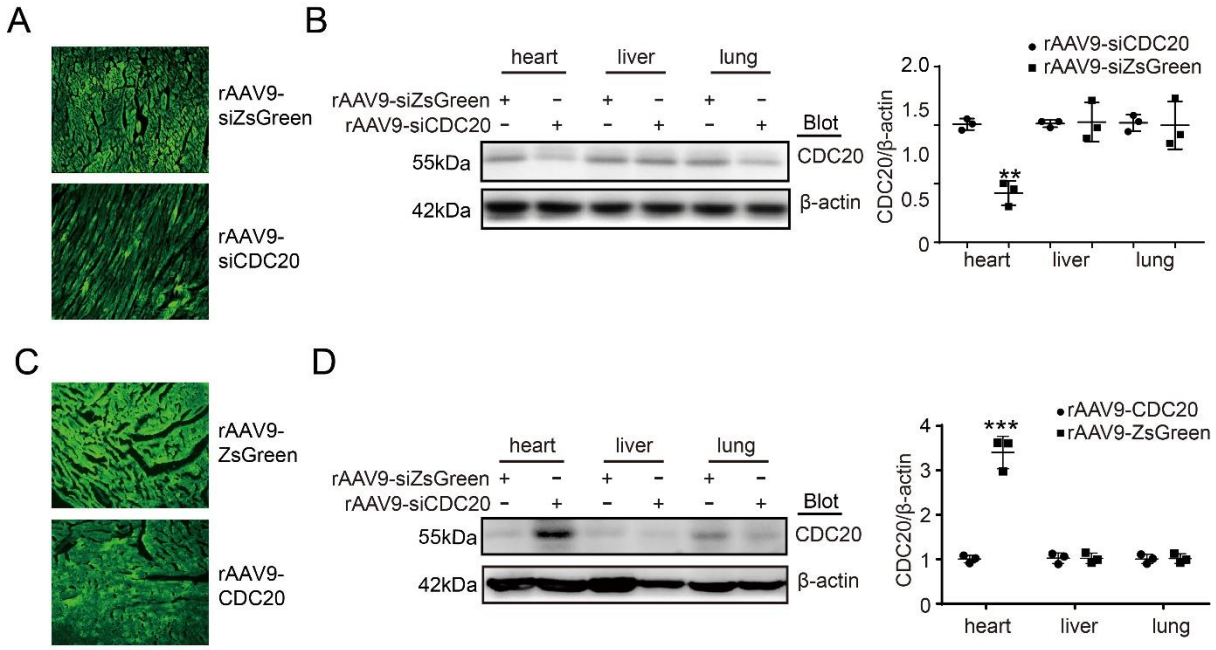
A
rAAV9-siZsGreen
rAAV9-siCDC20
B
heart
liver
lung
rAAV9-siZsGreen + − + − + −
rAAV9-siCDC20 − + − + − +
Blot
55kDa
CDC20
42kDa
β-actin
rAAV9-siCDC20
rAAV9-siZsGreen
CDC20/β-actin
2.0
1.5
1.0
0.5
0
**
heart
liver
lung
C
rAAV9-ZsGreen
rAAV9-CDC20
D
heart
liver
lung
rAAV9-siZsGreen + − + − + −
rAAV9-siCDC20 − + − + − +
Blot
55kDa
CDC20
42kDa
β-actin
rAAV9-CDC20
rAAV9-ZsGreen
CDC20/β-actin
4
3
2
1
0
***
heart
liver
lung

Figure S3

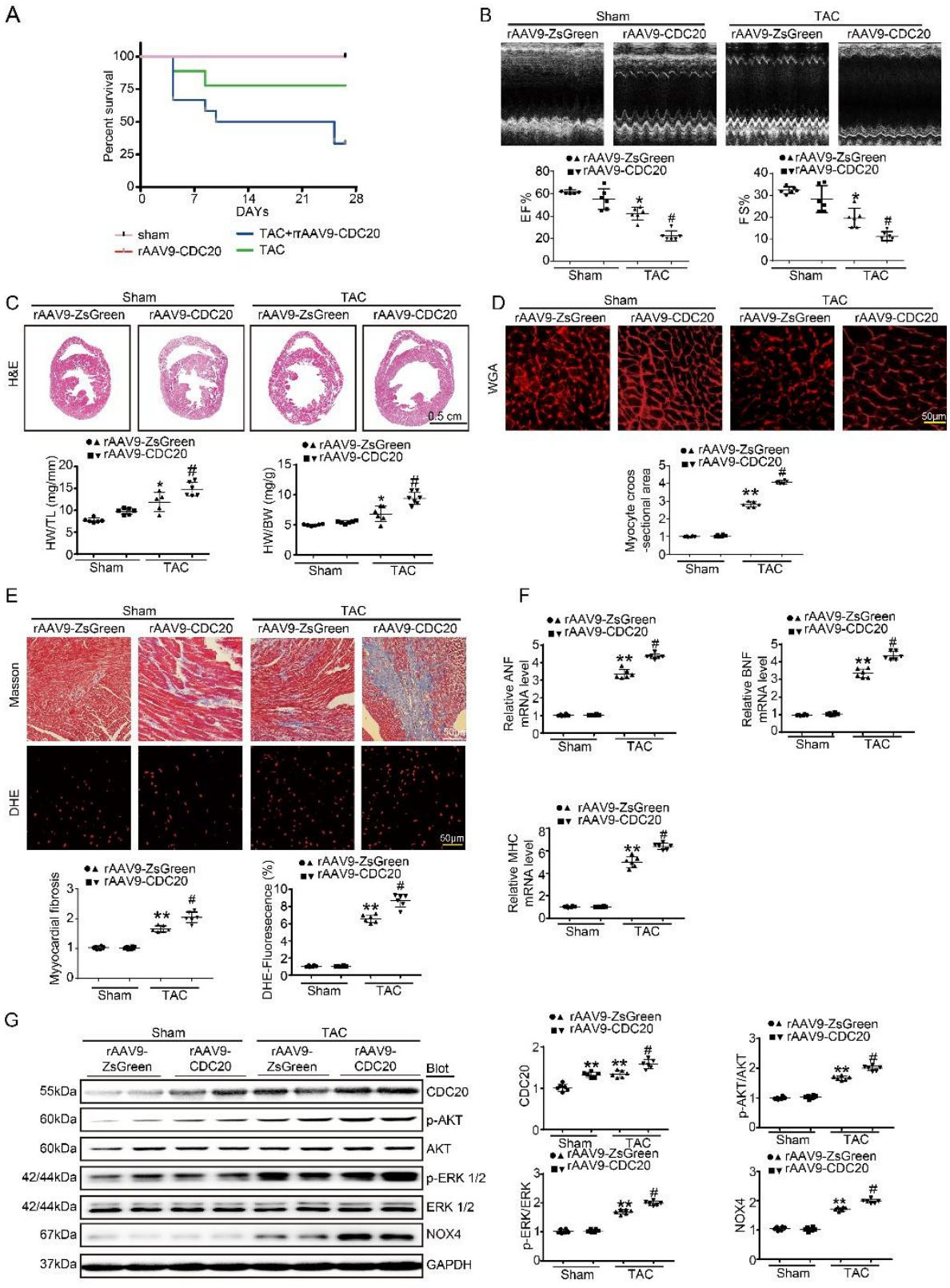
A
Percent survival
DAYs
sham
rAAV9-CDC20
TAC+rAAV9-CDC20
TAC
B
Sham
TAC
rAAV9-ZsGreen
rAAV9-CDC20
EF%
FS%
C
H&E
HW/TL (mg/mm)
HW/BW (mg/g)
0.5 cm
D
WGA
Myocyte croos -sectional area
50μm
E
Masson
DHE
Myocardial fibrosis
DHE-Fluoresecence (%)
F
Relative ANF mRNA level
Relative BNF mRNA level
Relative MHC mRNA level
G
Blot
55kDa
CDC20
60kDa
p-AKT
AKT
42/44kDa
p-ERK 1/2
ERK 1/2
67kDa
NOX4
37kDa
GAPDH
p-AKT/AKT
p-ERK/ERK

Figure S4

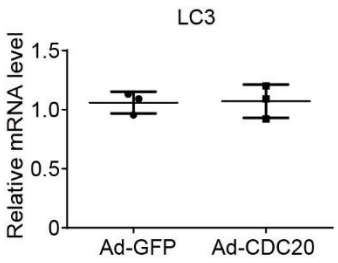

Figure S5

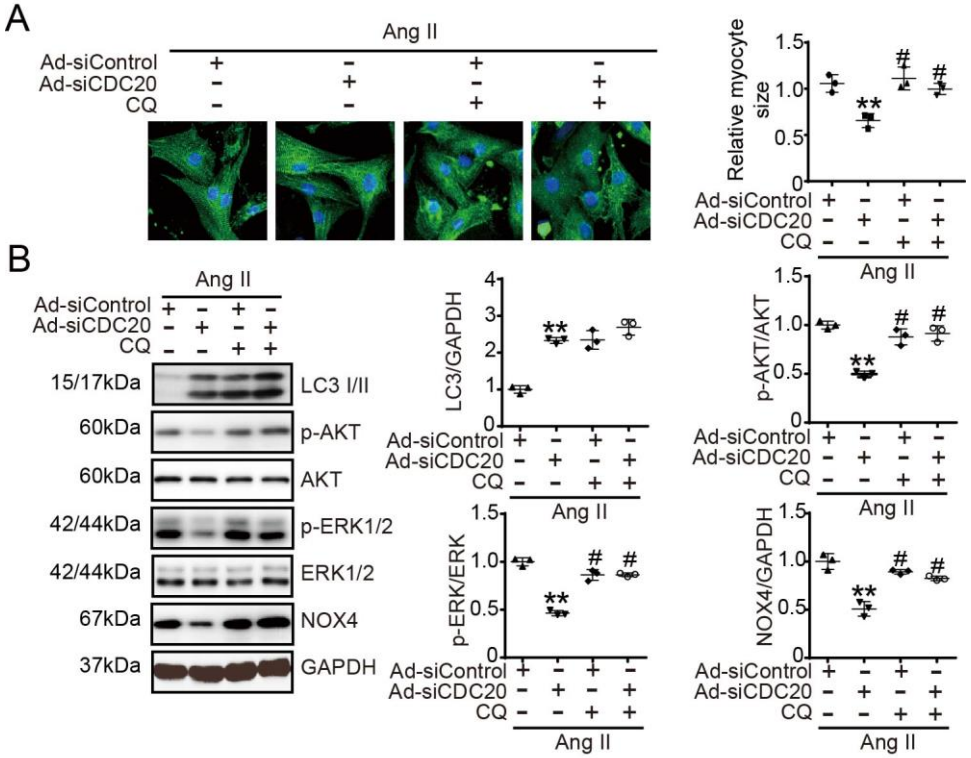
A
Ang II
Ad-siControl
Ad-siCDC20
CQ
Relative myocyte size
B
Ang II
15/17kDa
LC3 I/II
60kDa
p-AKT
60kDa
AKT
42/44kDa
p-ERK1/2
42/44kDa
ERK1/2
67kDa
NOX4
37kDa
GAPDH
LC3/GAPDH
p-AKT/AKT
p-ERK/ERK
NOX4/GAPDH

Figure S6