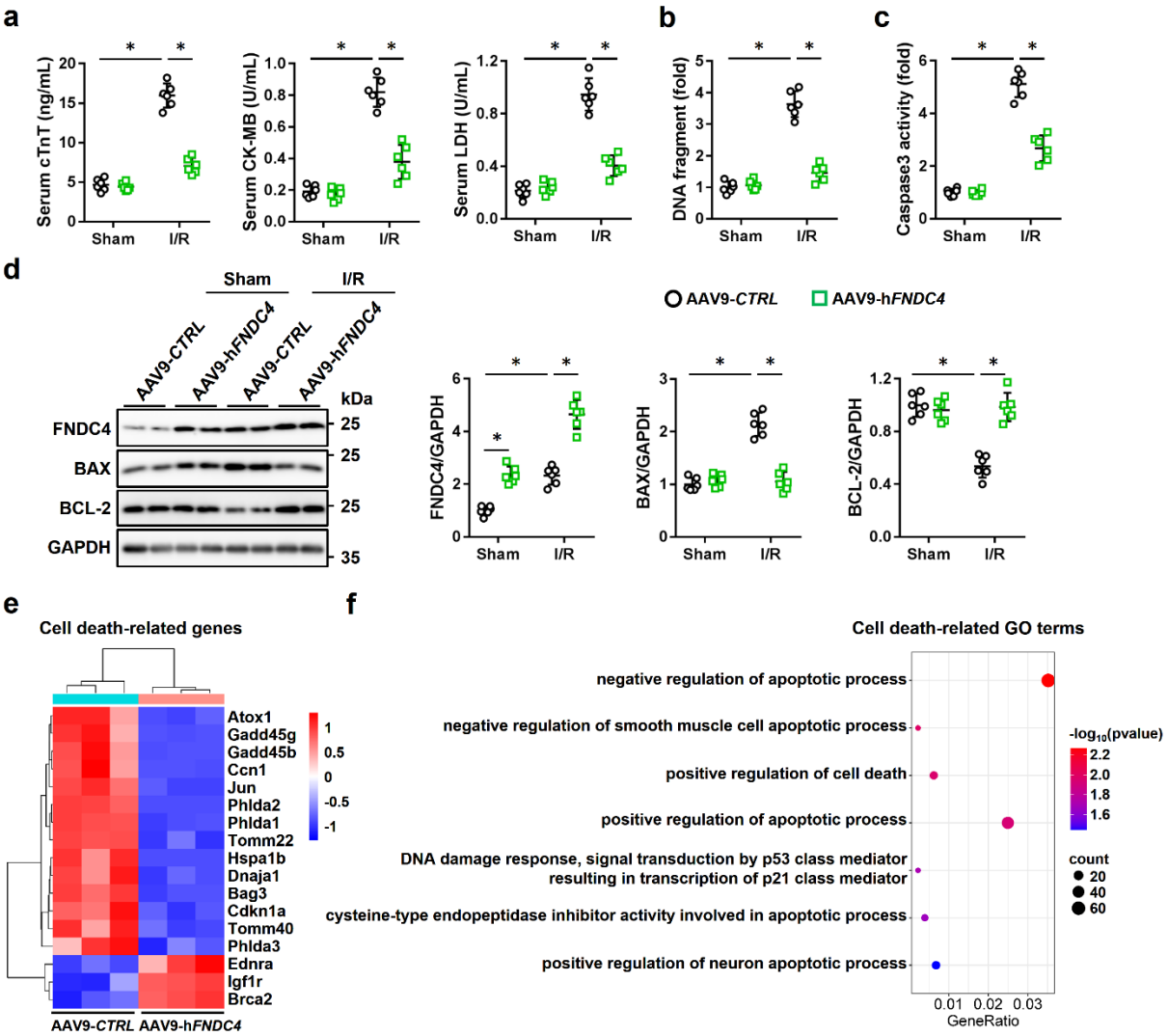
Supplemental Materials

Table S1. List of the primary antibodies used for western blot (WB) and immunofluorescence (IF) staining in this study.

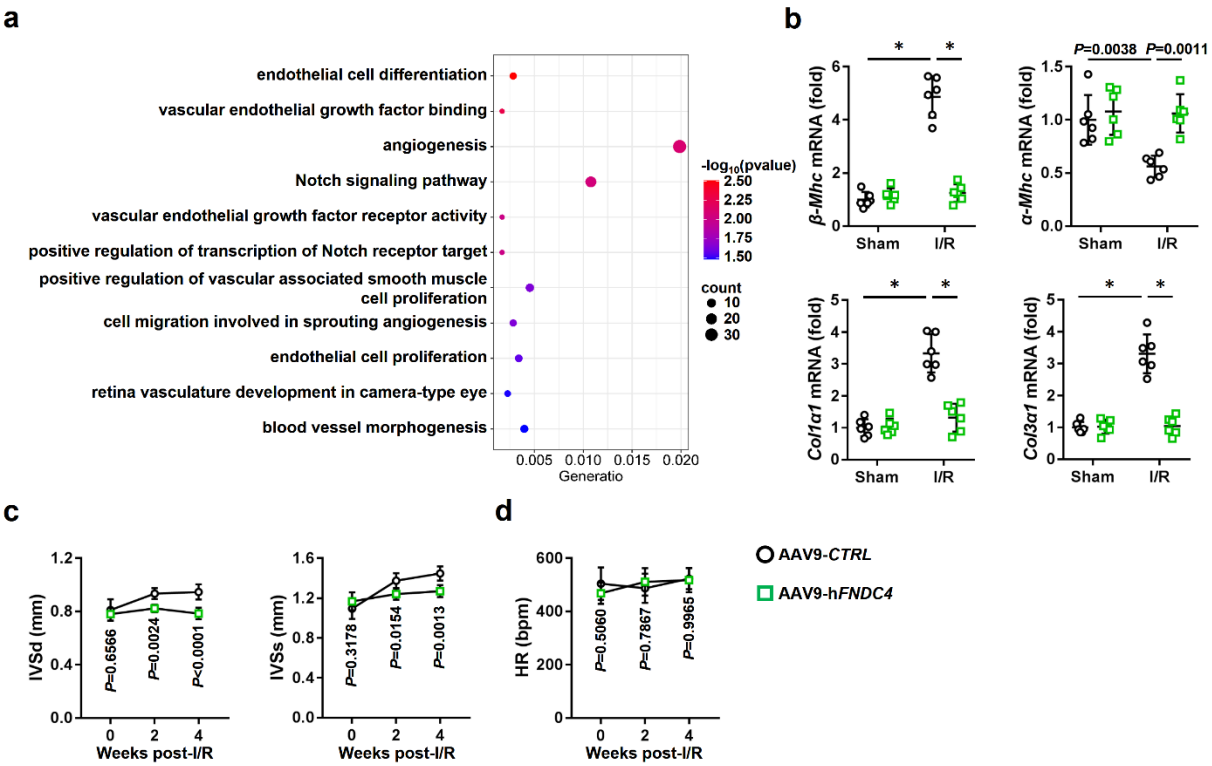
Antibody	Source	Catalog No.	Application (dilution)
FNDC4	OriGene	CF505459	WB (1:500)
GAPDH	CST	2118	WB (1:1000)
CD31	Abcam	ab24590	IF (1:100)
α-SMA	Abcam	ab5694	IF (1:100)
BAX	CST	2772	WB (1:1000)
BCL-2	Abcam	ab196495	WB (1:1000)
HIF1α	CST	36169	WB (1:1000), IF (1:100), IP (1:50)
Hydroxy-HIF1α (Pro564)	CST	3434	WB (1:1000)
Ubiquitin	CST	3936	WB (1:1000)
α-actinin	CST	69758	IF (1:100)

Table S2. Primer sets used for quantitative real-time PCR in this study.

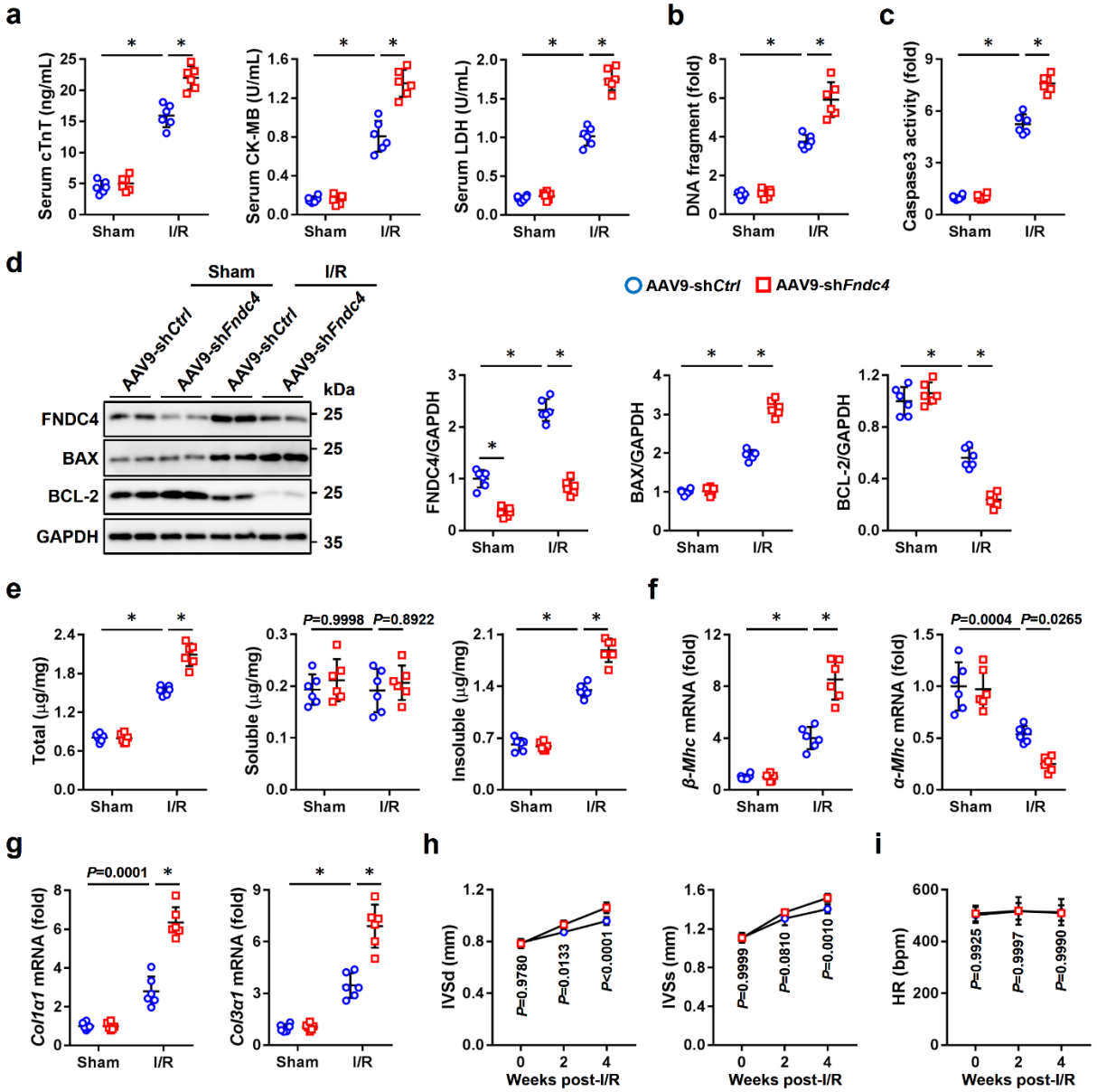
Gene	Species	Primer sequence (5′→3′)	
α-Mhc	Mouse	Forward	GTCCAAGTTCCGCAAGGT
		Reverse	AGGGTCTGCTGGAGAGGTTA
β-Mhc	Mouse	Forward	CCGAGTCCCAGGTCAACAA
		Reverse	CTTCACGGGCACCCTTGGA
Col1α1	Mouse	Forward	AGGCTTCAGTGGTTTGGATG
		Reverse	CACCAACAGCACCATCGTTA
Col3α1	Mouse	Forward	CCCAACCCAGAGATCCCATT
		Reverse	GAAGCACAGGAGCAGGTGTAGA
Fgf1	Rat	Forward	ATGGACACCGAAGGGCTTTT
		Reverse	GAGGCCCACAAACCAGTTCT
Fndc4	Mouse	Forward	TTCCCCATATCTGAGTCCCAC
		Reverse	TGACCGTCACATTCACAGGAG
Gapdh	Mouse	Forward	ACTCCACTCACGGCAAATTC
		Reverse	TCTCCATGGTGGTGAAGACA
Gapdh	Rat	Forward	GACATGCCGCCTGGAGAAAC
		Reverse	AGCCCAGGATGCCCTTTAGT
Hif1α	Mice	Forward	GGTTCCAGCAGACCCAGTTA
		Reverse	AGGCTCCTTGGATGAGCTTT
Slit1	Rat	Forward	GCCGAAAACTCTACTGTCT
		Reverse	GGGCTTCTCCACCTCCTCA
Slit2	Rat	Forward	TGAGGAAGAAGGTCACCAGTCA
		Reverse	GTGAGACCTTTCCCTCGGCA



Supplementary Figure 1. Cardiac-specific FNDC4 overexpression inhibits apoptosis and acute cardiac I/R injury. (a) Mice received a single intravenous injection of AAV9-h*FNDC4* or AAV9-*CTRL*, maintained for 4 weeks and then exposed to I/R surgery. Next, serum levels of cTnT, CK-MB and LDH were measured 4 h after I/R surgery (n=6). **(b-c)** DNA fragments and caspase3 activity in the heart 24 h after I/R surgery (n=6). **(d)** Heart samples with or without FNDC4 overexpression were collected 24 h after I/R surgery and subjected to western blot (n=6). **(e-f)** Heart samples with or without FNDC4 overexpression were collected 24 h after I/R surgery and subjected to unbiased transcriptome analysis. Next, the expressions of cell death-related genes were presented using a heatmap, and cell death-related GO terms were also presented (n=3). Data were presented as the mean ± S.D., and analyzed using one-way ANOVA followed by Tukey post hoc test. *$P < 0.0001$. Source data are provided as a Source Data file.

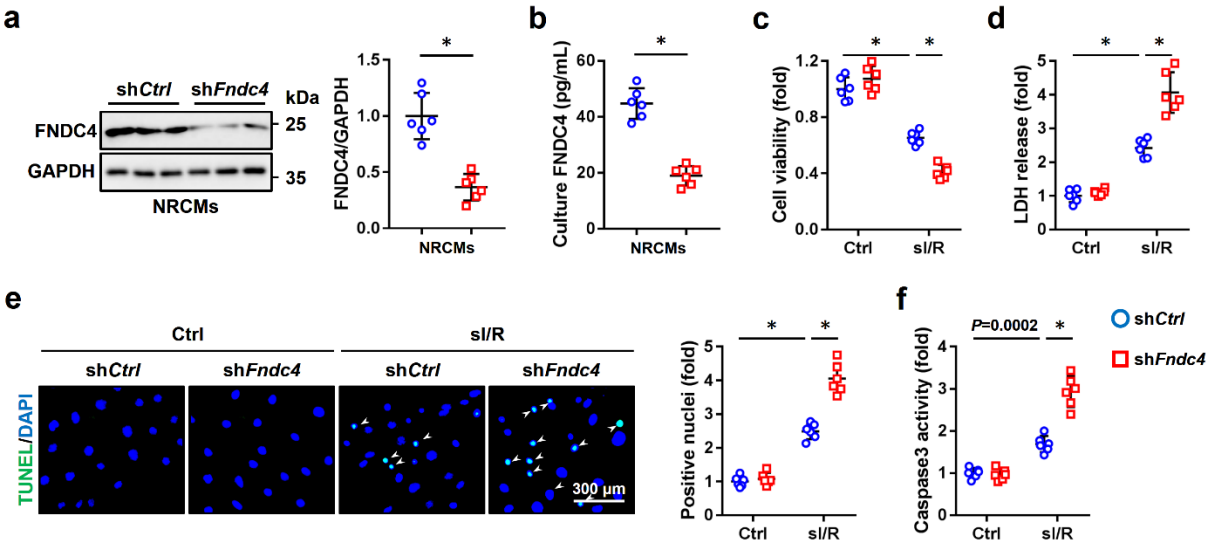


Supplementary Figure 2. Cardiac-specific FNDC4 overexpression attenuates I/R-induced chronic cardiac remodeling and dysfunction. (a) Angiogenesis-related GO terms of the transcriptome analysis (n=3). **(b)** The mRNA levels of *α-Mhc*, *β-Mhc*, *Col1α1* and *Col3α1* in the heart 4 weeks after I/R surgery (n=6). **(c-d)** Cardiac function of FNDC4-overexpressed and control mice was analyzed by transthoracic echocardiography at the indicated time points (n=6). Data were presented as the mean ± S.D., and analyzed using one-way ANOVA followed by Tukey post hoc test. For the analysis in Supplementary Figure 2c-d, repeated measures ANOVA was used. *$P < 0.0001$. Source data are provided as a Source Data file.

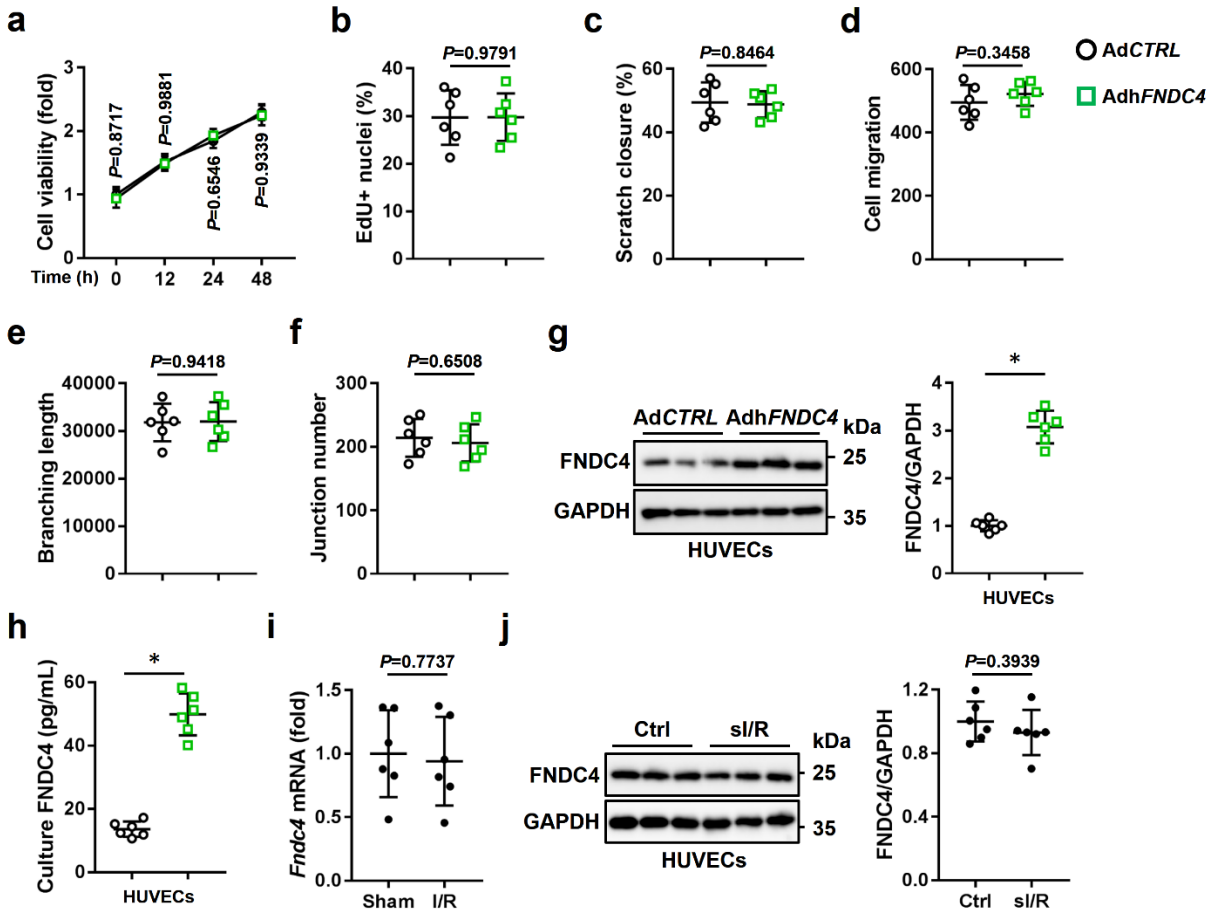


Supplementary Figure 3. Cardiac-specific FNDC4 knockdown exacerbates I/R-induced apoptosis, chronic cardiac remodeling and dysfunction. (a) Mice received a single intravenous injection of AAV9-sh*Fndc4* or AAV9-sh*Ctrl*, maintained for 4 weeks and then exposed to I/R surgery. Next, serum levels of cTnT, CK-MB and LDH were measured 4 h after I/R surgery (n=6). **(b-c)** DNA fragments and caspase3 activity in the heart 24 h after I/R surgery (n=6). **(d)** Heart samples with or without FNDC4 knockdown were collected 24 h after I/R surgery and subjected to western blot (n=6). **(e)** Total, soluble and insoluble collagen content in the heart 4 weeks post-I/R surgery (n=6). **(f-g)** The mRNA levels of *α-Mhc*, *β-Mhc*, *Col1α1* and *Col3α1* in the heart 4 weeks after I/R surgery (n=6). **(h-i)** Cardiac function of FNDC4-silenced and control mice was analyzed by transthoracic echocardiography at the indicated time points (n=6). Data were presented as the mean ± S.D., and analyzed using one-way ANOVA followed by Tukey post hoc test. For the analysis

in Supplementary Figure 3h-i, repeated measures ANOVA was used. *$P < 0.0001$. Source data are provided as a Source Data file.

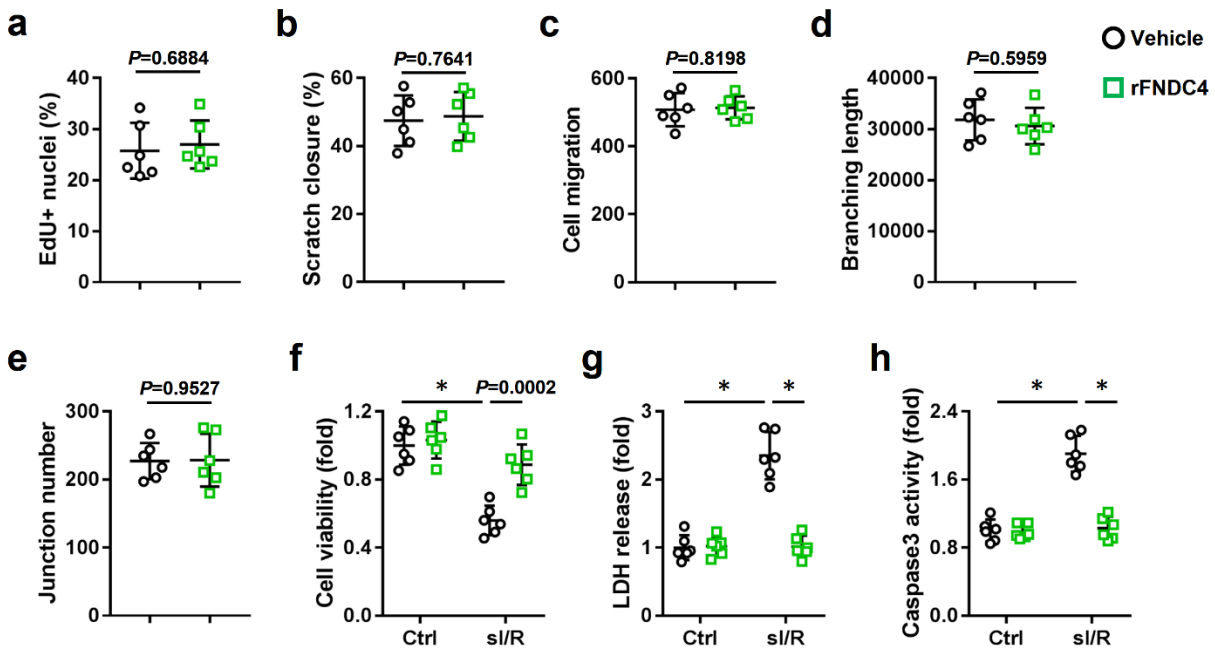


Supplementary Figure 4. FNDC4 knockdown exacerbates sI/R-induced cardiomyocyte injury in vitro. **(a)** NRCMs were infected with sh*Fndc4* or sh*Ctrl* for 4 h and cultured with fresh medium for an additional 24 h. Next, cells were harvested for western blot (n=6). **(b)** FNDC4 level in the medium of NRCMs with or without FNDC4 knockdown (n=6). **(c)** Cell viability was determined using the CCK-8 method (n=6). **(d)** LDH releases were calculated as (LDH level in ischemia medium + LDH in reperfusion medium)/(LDH in ischemia medium + LDH in reperfusion medium + LDH in cell lysate) (n=6). **(e)** Representative TUNEL staining images of cell coverslips and quantitative results. Arrows indicate TUNEL+ nuclei (n=6). **(f)** Caspase3 activity in NRCMs (n=6). Data were presented as the mean ± S.D., and analyzed using one-way ANOVA followed by Tukey post hoc test. For the analysis in Supplementary Figure 4a-b, an unpaired two-tailed Student's *t*-test was used. **P* < 0.0001. Source data are provided as a Source Data file.

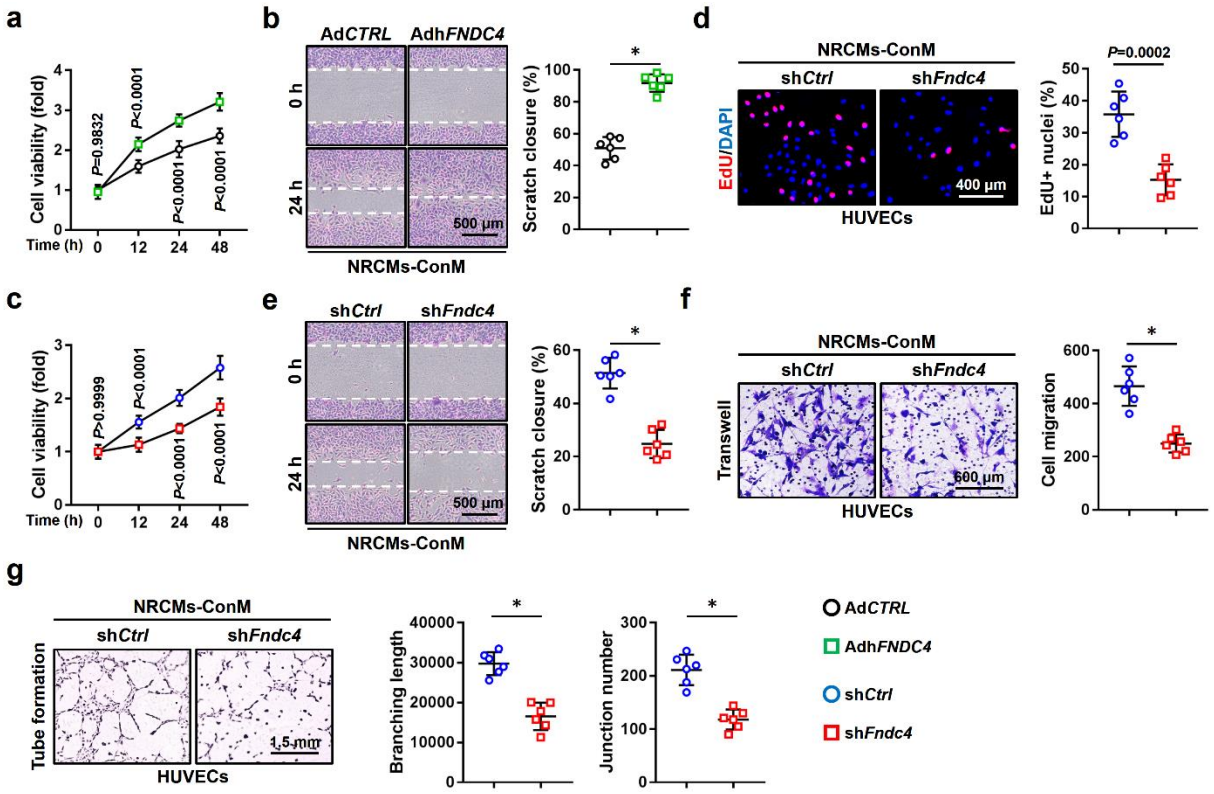


Supplementary Figure 5. FNDC4 overexpression does not directly enhance the angiogenic ability of HUVECs in vitro. (a) HUVECs were infected with Adh*FNDC4* or Ad*CTRL* for 4 h and cultured with fresh medium for an additional 24 h. Next, cells were cultured for the indicated time points, and cell viability was determined using the CCK-8 method (n=6). **(b)** HUVECs were cultured for 24 h, and then EdU+ nuclei were quantified using a commercial kit (n=6). **(c)** HUVECs were seeded in 6-well plates to grow to a monolayer, and were then scraped using a 200 μL sterilized micropipette tip. Next, the wound sizes at 0 h together with 24 h were recorded, and scratch closure (%) was calculated as [(wound at 0 h-wound at 24 h)/wound at 0 h] × 100 (n=6). **(d)** HUVECs were exposed to transwell assay and cultured for 12 h, and cells in the lower chamber were stained with crystal violet to quantify the migrated cells (n=6). **(e-f)** HUVECs were exposed to tube formation assay and cultured for 8 h, and the branching length and junction number were quantified (n=6). **(g)** HUVECs were infected with Adh*FNDC4* or Ad*CTRL* for 4 h and cultured with fresh medium for an additional 24 h. Next, cells were harvested for western blot (n=6). **(h)** FNDC4 level in the medium of HUVECs with or without FNDC4 overexpression (n=6). **(i)** Endothelial cells were separated from sham- or I/R-operated hearts, and the mRNA level of FNDC4 in cardiac endothelial cells was measured by quantitative real-time PCR (n=6). **(j)** HUVECs were exposed to sI/R stimulation, and the cell lysates were prepared for

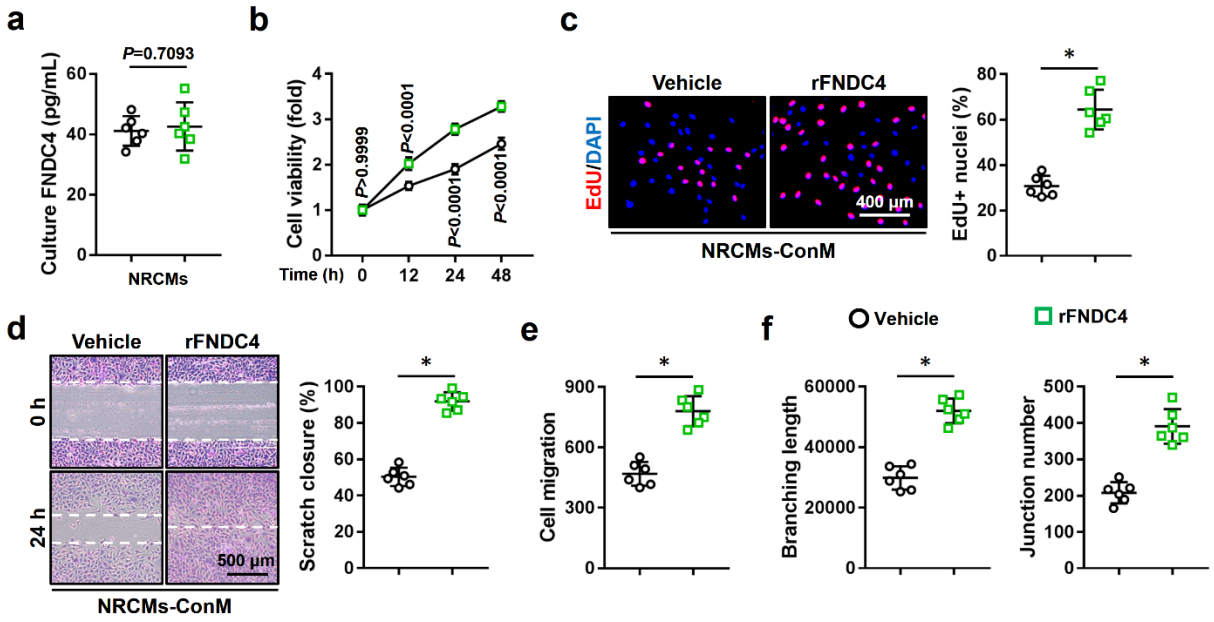
western blot (n=6). Data were presented as the mean ± S.D., and analyzed using an unpaired two-tailed Student′s *t*-test. For the analysis in Supplementary Figure 5a, repeated measures ANOVA was used. *P < 0.0001. Source data are provided as a Source Data file.



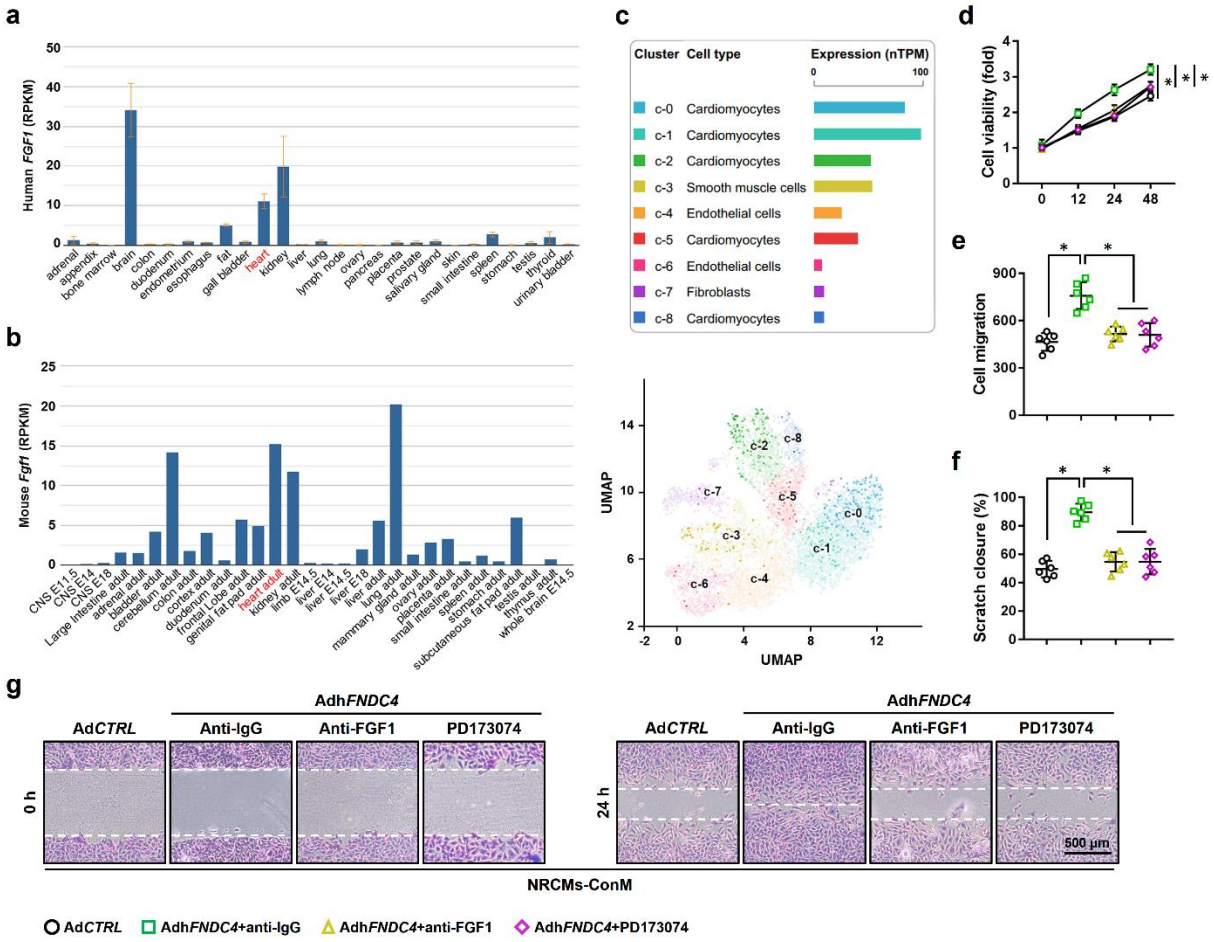
Supplementary Figure 6. Treatment with rFNDC4 prevents sI/R-induced cardiomyocyte injury and apoptosis without affecting the angiogenic ability of HUVECs in vitro. (a) HUVECs were cultured with rFNDC4, and then EdU+ nuclei were quantified using a commercial kit (n=6). **(b)** HUVECs with or without rFNDC4 treatment were exposed to wound scratch assay, and the wound sizes at 0 h together with 24 h were recorded to measure scratch closure (n=6). **(c)** HUVECs with or without rFNDC4 treatment were exposed to transwell assay, and cells in the lower chamber were stained with crystal violet to quantify the migrated cells after 12 h (n=6). **(d-e)** HUVECs with or without rFNDC4 treatment were exposed to tube formation assay, and the branching length and junction number were quantified after 8 h (n=6). **(f)** Cell viability in NRCMs was determined using the CCK-8 method (n=6). **(g)** LDH releases in NRCMs were calculated as (LDH level in ischemia medium + LDH in reperfusion medium)/(LDH in ischemia medium + LDH in reperfusion medium + LDH in cell lysate) (n=6). **(h)** Caspase3 activity in NRCMs (n=6). Data were presented as the mean ± S.D., and analyzed using an unpaired two-tailed Student′s *t*-test. For the analysis in Supplementary Figure 6f-h, one-way ANOVA followed by Tukey post hoc test was used. *$P < 0.0001$. Source data are provided as a Source Data file.



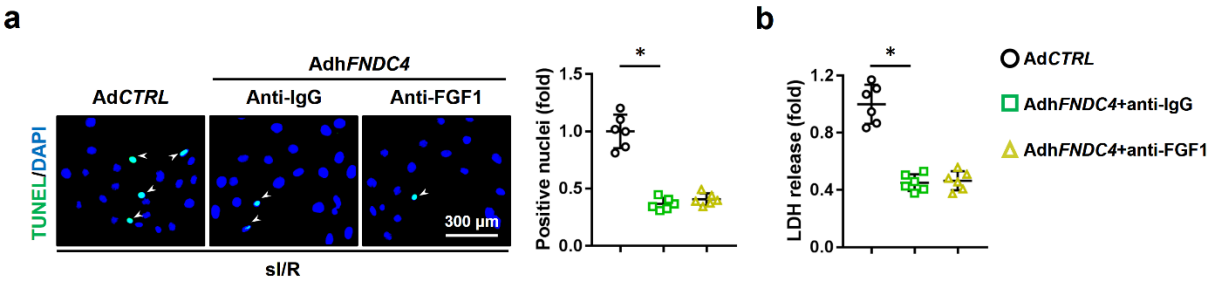
Supplementary Figure 7. The conditioned medium from cardiomyocytes overexpressing FNDC4 enhances endothelial angiogenesis in vitro. (a) HUVECs were cultured with NRCMs-ConM for the indicated time points, and cell viability was determined using the CCK-8 method (n=6). **(b)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to wound scratch assay. Next, the wound sizes at 0 h together with 24 h were recorded to measure scratch closure (n=6). **(c)** HUVECs were cultured with NRCMs-ConM for the indicated time points, and cell viability was determined using the CCK-8 method (n=6). **(d)** HUVECs were cultured with NRCMs-ConM for 24 h, and then EdU+ nuclei were quantified using a commercial kit (n=6). **(e)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to wound scratch assay. Next, the wound sizes at 0 h together with 24 h were recorded to measure scratch closure (n=6). **(f)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to transwell assay. After 12 h, cells in the lower chamber were stained with crystal violet to quantify the migrated cells (n=6). **(g)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to tube formation assay. After 8 h, the branching length and junction number were quantified (n=6). Data were presented as the mean ± S.D., and analyzed using an unpaired two-tailed Student′s *t*-test. For the analysis in Supplementary Figure 7a and c, repeated measures ANOVA was used. *$P < 0.0001$. Source data are provided as a Source Data file.



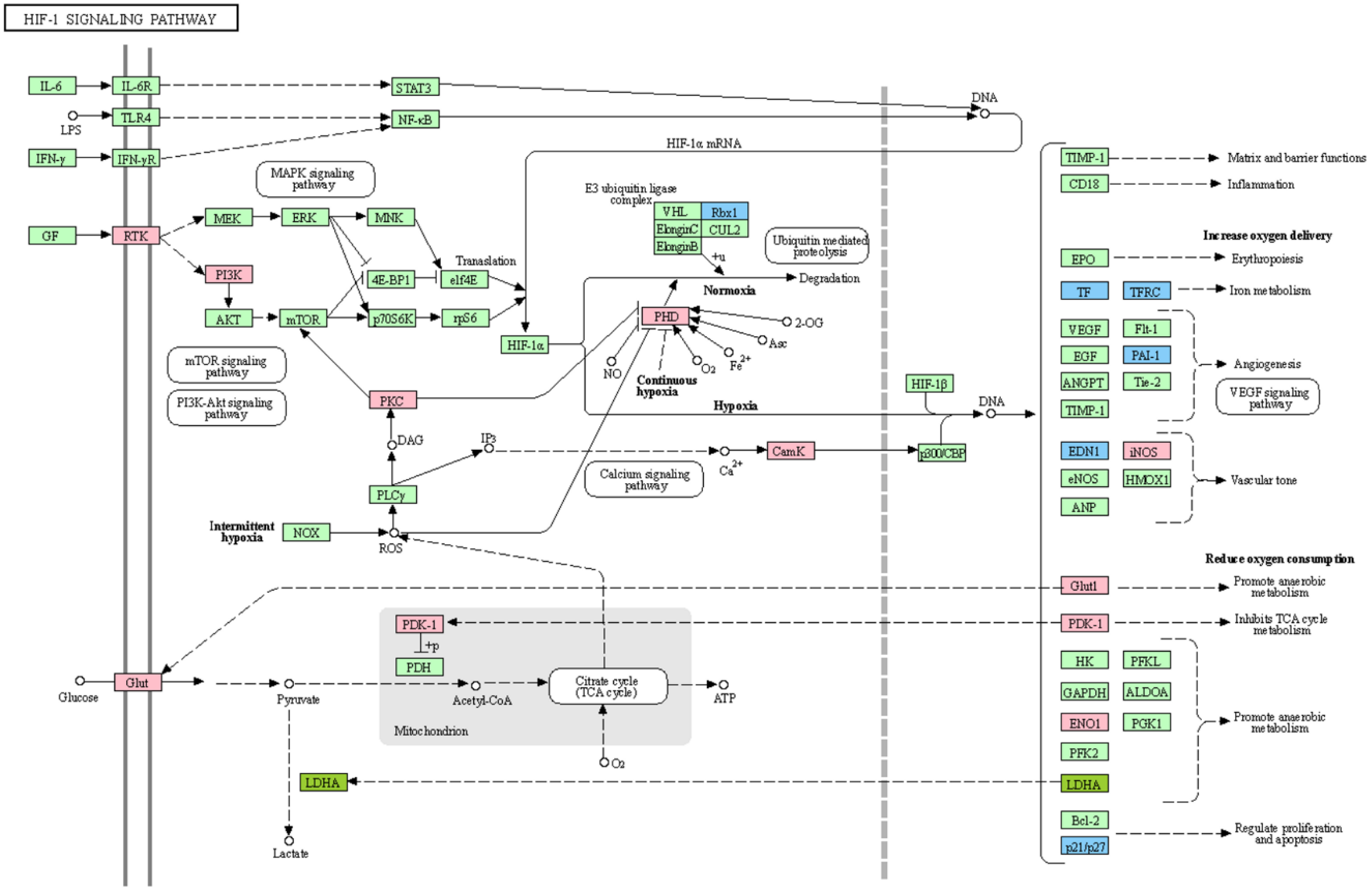
Supplementary Figure 8. The conditioned medium from rFNDC4-treated cardiomyocytes enhances endothelial angiogenesis in vitro. (a) NRCMs were treated with or without rFNDC4, and the conditioned medium was collected for the analysis of FNDC4 (n=6). **(b)** HUVECs were cultured with NRCMs-ConM for the indicated time points, and cell viability was determined using the CCK-8 method (n=6). **(c)** HUVECs were cultured with NRCMs-ConM for 24 h, and then EdU+ nuclei were quantified using a commercial kit (n=6). **(d)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to wound scratch assay. Next, the wound sizes at 0 h together with 24 h were recorded to measure scratch closure (n=6). **(e)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to transwell assay. After 12 h, cells in the lower chamber were stained with crystal violet to quantify the migrated cells (n=6). **(f)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to tube formation assay. After 8 h, the branching length and junction number were quantified (n=6). Data were presented as the mean ± S.D., and analyzed using an unpaired two-tailed Student's *t*-test. For the analysis in Supplementary Figure 8b, repeated measures ANOVA was used. *$P < 0.0001$. Source data are provided as a Source Data file.



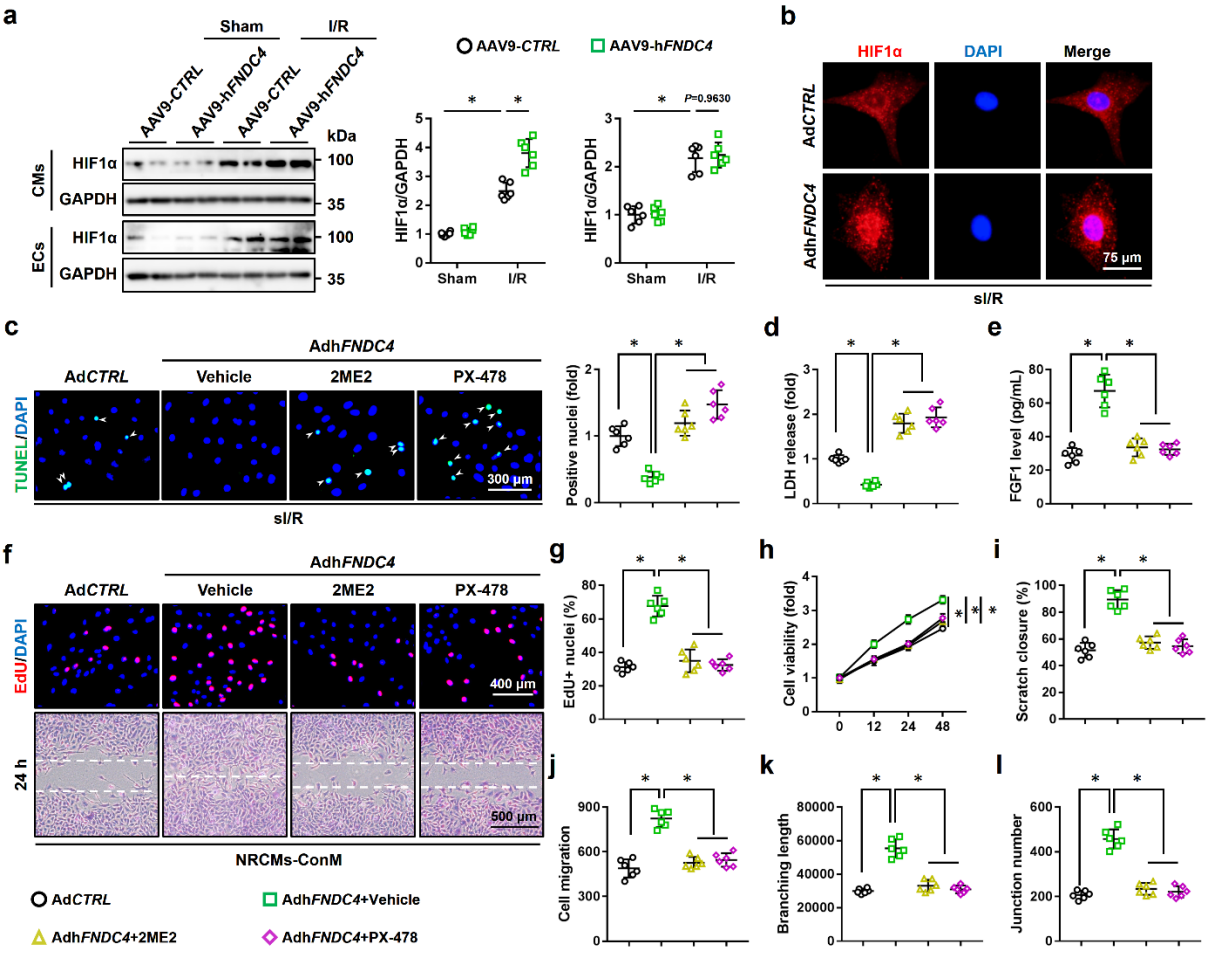
Supplementary Figure 9. FNDC4 promotes angiogenesis of endothelial cells through increasing FGF1 secretion from cardiomyocytes. (a) The expression of human *FGF1* gene in different human tissues was analyzed from HPA RNA-seq normal tissues project (BioProject: PRJEB4337). **(b)** The expression of murine *Fgf1* gene in different mouse tissues was analyzed from Mouse ENCODE transcriptome data project (BioProject: PRJNA66167). **(c)** Single-cell sequencing data of FGF1 in human hearts. **(d)** HUVECs were cultured with NRCMs-ConM in the presence of anti-FGF1 or PD173074 for the indicated time points, and cell viability was determined using the CCK-8 method (n=6). **(e)** HUVECs were cultured with NRCMs-ConM in the presence of anti-FGF1 or PD173074 for 24 h, and then exposed to transwell assay. After 12 h, cells in the lower chamber were stained with crystal violet to quantify the migrated cells (n=6). **(f-g)** HUVECs were cultured with NRCMs-ConM in the presence of anti-FGF1 or PD173074 for 24 h, and then exposed to wound scratch assay. Next, the wound sizes at 0 h together with 24 h were recorded to measure scratch closure (n=6). Data were presented as the mean ± S.D., and analyzed using one-way ANOVA followed by Tukey post hoc test. For the analysis in Supplementary Figure 9d, repeated measures ANOVA was used. *P < 0.0001. Source data are provided as a Source Data file.



Supplementary Figure 10. FGF1 neutralization does not affect the anti-apoptotic role of FNDC4 in sI/R-stimulated NRCMs. (a) NRCMs with or without FNDC4 overexpression were exposed to sI/R stimulation in the presence or absence of anti-FGF1, and TUNEL staining was performed to evaluate cell apoptosis. Arrows indicate TUNEL+ nuclei (n=6). **(b)** LDH releases were calculated as (LDH level in ischemia medium + LDH in reperfusion medium)/(LDH in ischemia medium + LDH in reperfusion medium + LDH in cell lysate) (n=6). Data were presented as the mean ± S.D., and analyzed using one-way ANOVA followed by Tukey post hoc test. **P* < 0.0001. Source data are provided as a Source Data file.

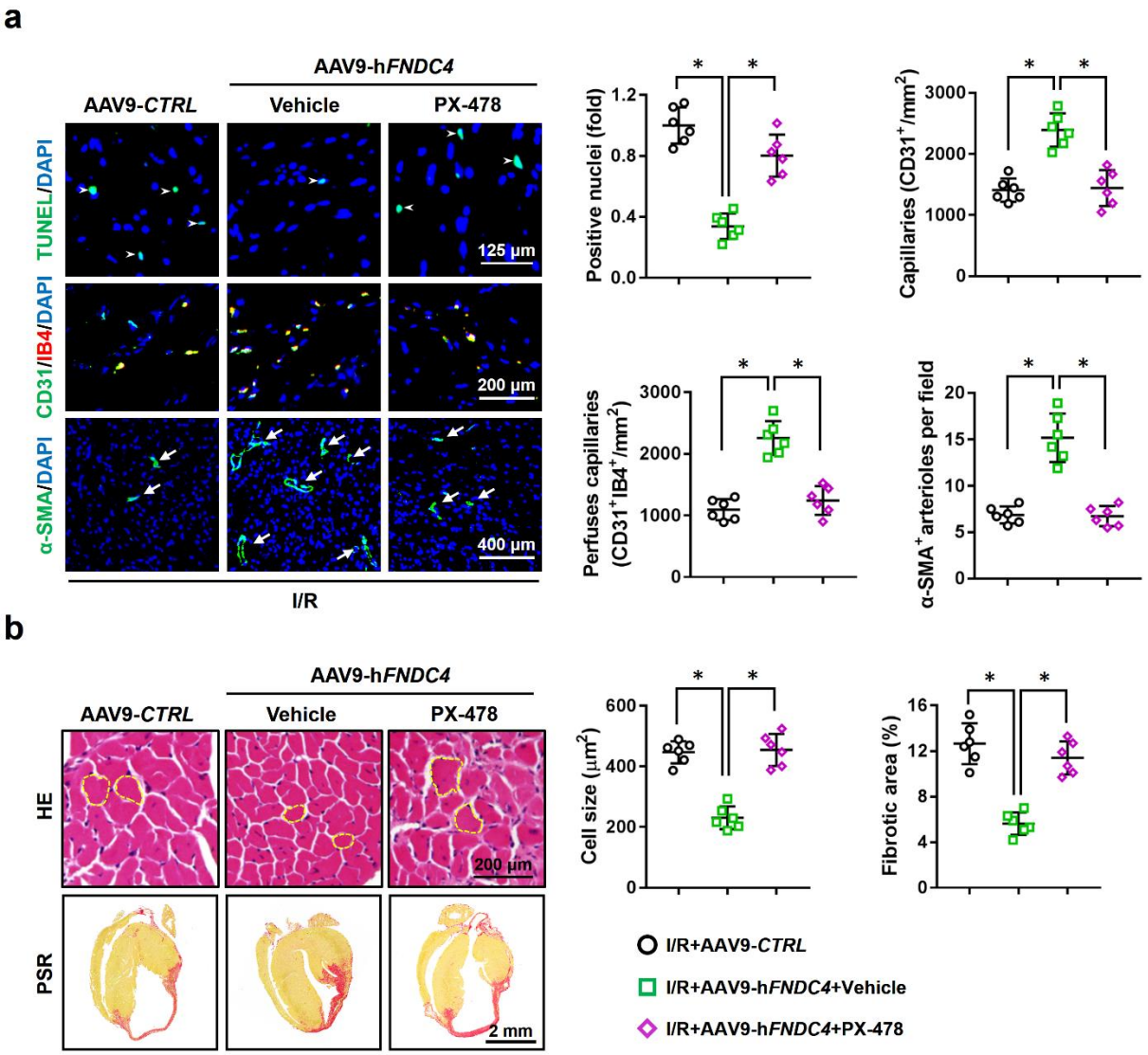


Supplementary Figure 11. The presentation of HIF1 signaling pathway.

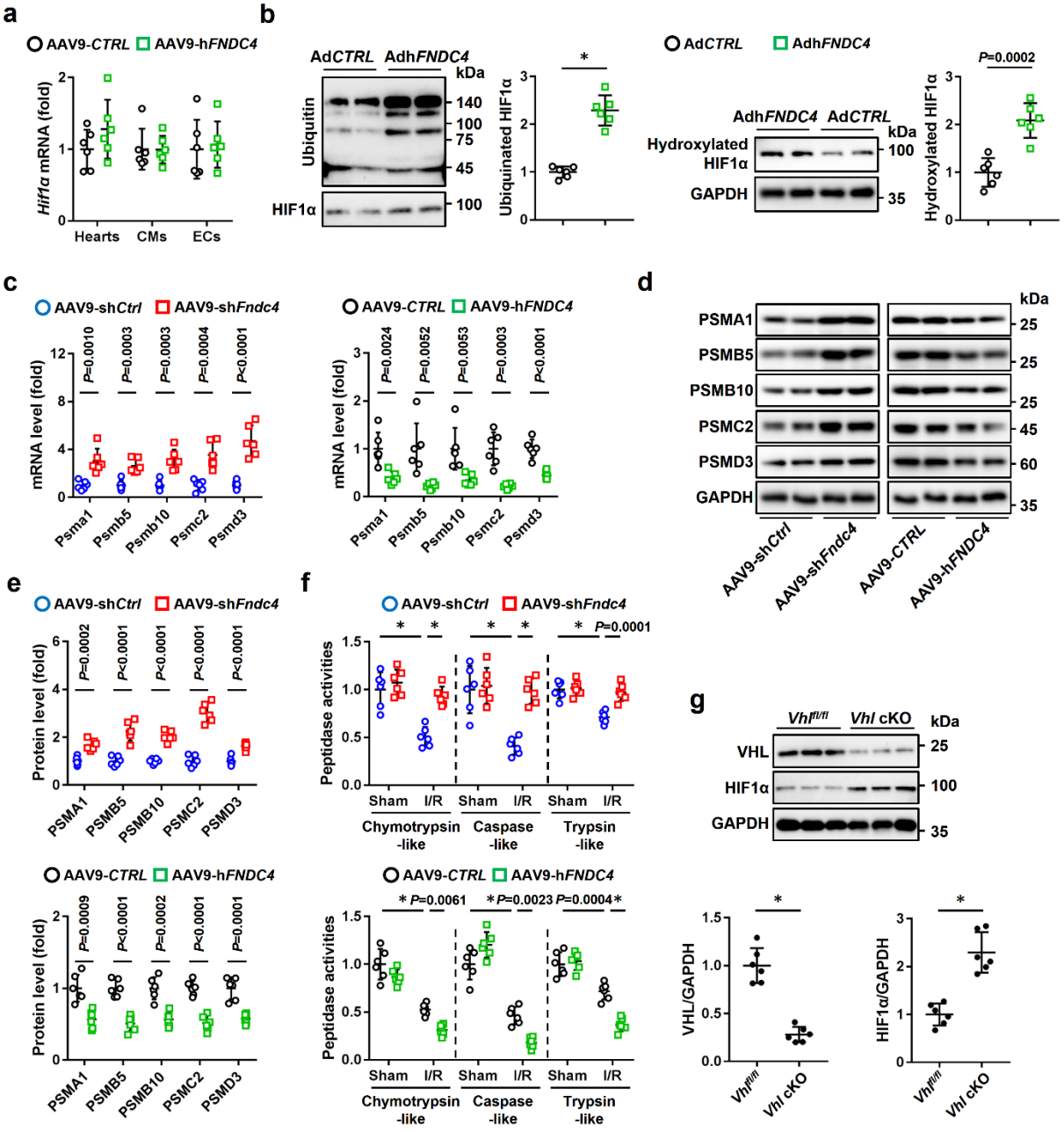


Supplementary Figure 12. FNDC4 ameliorates cardiac I/R injury through activating HIF1α in vitro. **(a)** Cardiomyocytes (CMs) and endothelial cells (ECs) were separated from heart samples, and exposed to western blot (n=6). **(b)** NRCMs with or without FNDC4 overexpression were exposed to sI/R stimulation and subjected to immunofluorescence staining of HIF1α (red) (n=6). **(c)** NRCMs with or without FNDC4 overexpression were exposed to sI/R stimulation in the presence or absence of 2ME2 or PX-478, and TUNEL staining was performed to evaluate cell apoptosis. Arrows indicate TUNEL+ nuclei (n=6). **(d)** LDH releases were calculated as (LDH level in ischemia medium + LDH in reperfusion medium)/(LDH in ischemia medium + LDH in reperfusion medium + LDH in cell lysate) (n=6). **(e)** NRCMs with or without FNDC4 overexpression were exposed to sI/R stimulation in the presence or absence of 2ME2 or PX-478, and the medium was collected for the analysis of FGF1 (n=6). **(f)** HUVECs were cultured with NRCMs-ConM, and then exposed to the measurements of cell proliferation and migration (n=6). **(g)** HUVECs were cultured with NRCMs-ConM for 24 h, and then EdU+ nuclei were quantified using a commercial kit (n=6). **(h)** HUVECs were cultured with NRCMs-ConM for the indicated time points, and cell viability was determined using the CCK-8 method (n=6). **(i)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to wound

scratch assay. Next, the wound sizes at 0 h together with 24 h were recorded to measure scratch closure(n=6). **(j)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to transwell assay. After 12 h, cells in the lower chamber were stained with crystal violet to quantify the migrated cells (n=6). **(k-l)** HUVECs were cultured with NRCMs-ConM for 24 h, and then exposed to tube formation assay. After 8 h, the branching length and junction number were quantified (n=6). Data were presented as the mean ± S.D., and analyzed using one-way ANOVA followed by Tukey post hoc test. For the analysis in Supplementary Figure 12h, repeated measures ANOVA was used. $*P < 0.0001$. Source data are provided as a Source Data file.



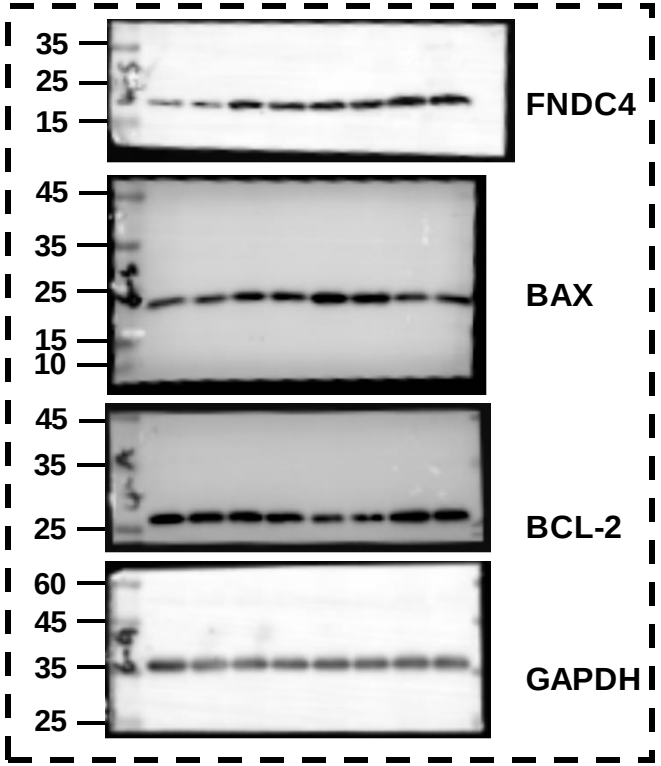
Supplementary Figure 13. FNDC4 ameliorates cardiac I/R injury through activating HIF1α in vivo. **(a)** FNDC4-overexpressed heart samples with or without PX-478 treatment were collected for TUNEL staining (24 h after I/R surgery) and immunofluorescence staining (4 weeks after I/R surgery), and the quantitative results were also provided. Arrows in the upper figures indicate TUNEL+ nuclei, and arrows in the lower figures indicate α-SMA$^+$ arterioles (n=6). **(b)** FNDC4-overexpressed heart samples with or without PX-478 treatment were collected 4 weeks after I/R surgery and subjected to HE and PSR staining, and cell size as well as fibrotic area were quantified. Circles indicate the cross-sectional area of cardiomyocytes (n=6). Data were presented as the mean ± S.D., and analyzed using one-way ANOVA followed by Tukey post hoc test. *P < 0.0001. Source data are provided as a Source Data file.



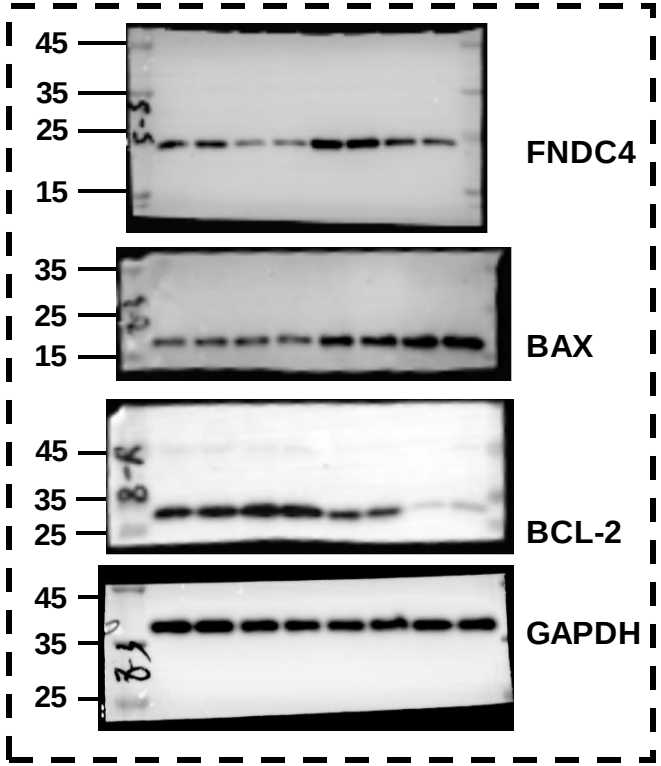
Supplementary Figure 14. FNDC4 elevates HIF1α protein level through suppressing its proteasomal degradation. (a) Cardiomyocytes (CMs) and endothelial cells (ECs) were separated from I/R-stressed heart samples with or without FNDC4 overexpression, and the mRNA levels of *Hif1α* in hearts and cell samples were measured by quantitative real-time PCR (n=6). **(b)** NRCMs were infected with Adh*FNDC4* or Ad*CTRL* for 4 h and cultured with fresh medium for an additional 24 h. Next, cells were harvested for detecting the hydroxylated and ubiquinated HIF1α levels (n=6). **(c)** Mice received a single intravenous injection of AAV9-h*FNDC4* or AAV9-sh*Fndc4*, maintained for 4 weeks and then exposed to I/R surgery, and the hearts were harvested for quantitative real-time PCR (n=6). **(d-e)** The hearts were harvested for western blot (n=6). **(f)** The hearts were harvested for the measurement of proteasomal activity (n=6). **(g)** Heart samples were

harvested from *Vhl* cKO mice and control littermates, and exposed to western blot (n=6). Data were presented as the mean ± S.D., and analyzed using an unpaired two-tailed Student′s *t*-test. For the analysis in Supplementary Figure 14f, one-way ANOVA followed by Tukey post hoc test was used. *$P < 0.0001$. Source data are provided as a Source Data file.

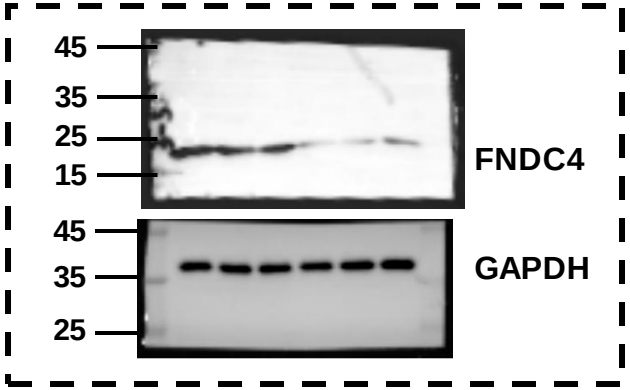
Supplementary Figure 1d



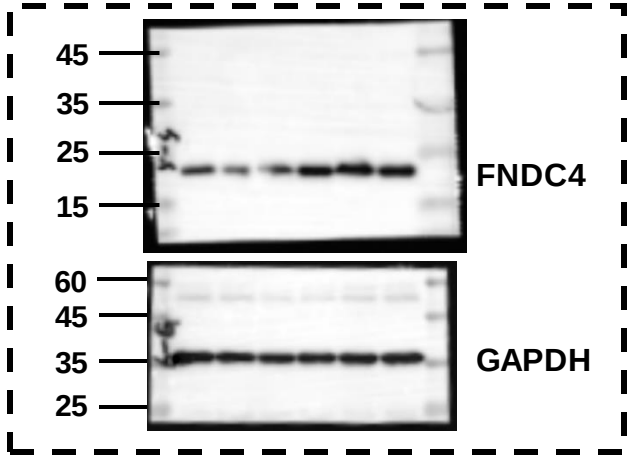
Supplementary Figure 3d



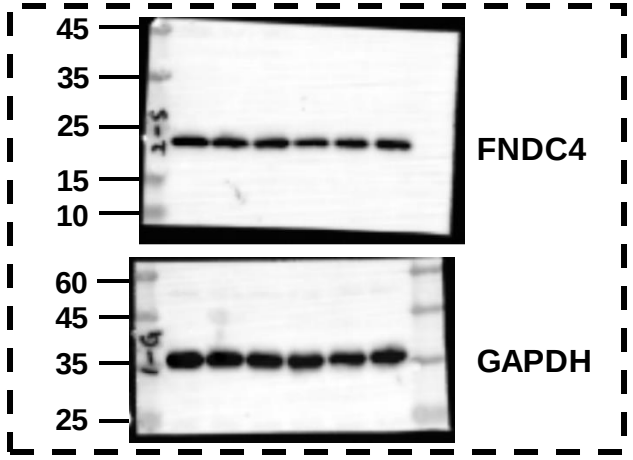
Supplementary Figure 4a



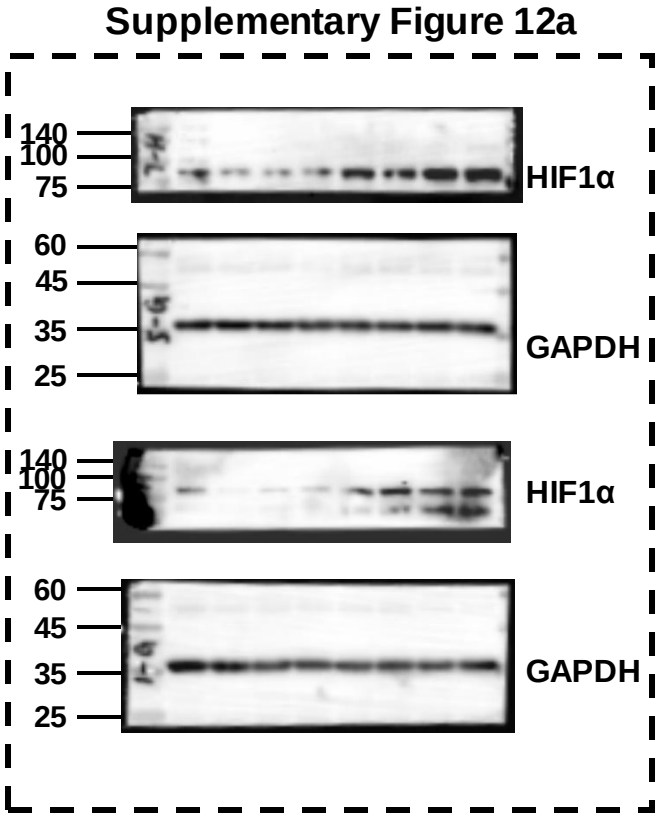
Supplementary Figure 5g



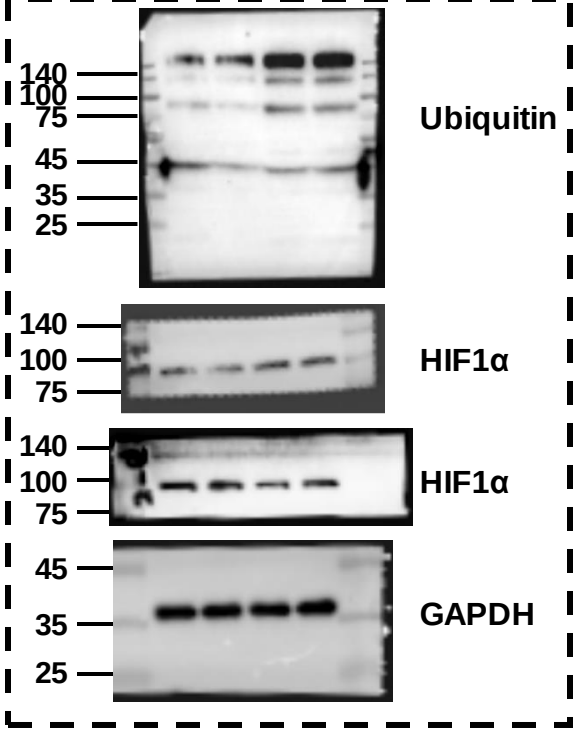
Supplementary Figure 5j



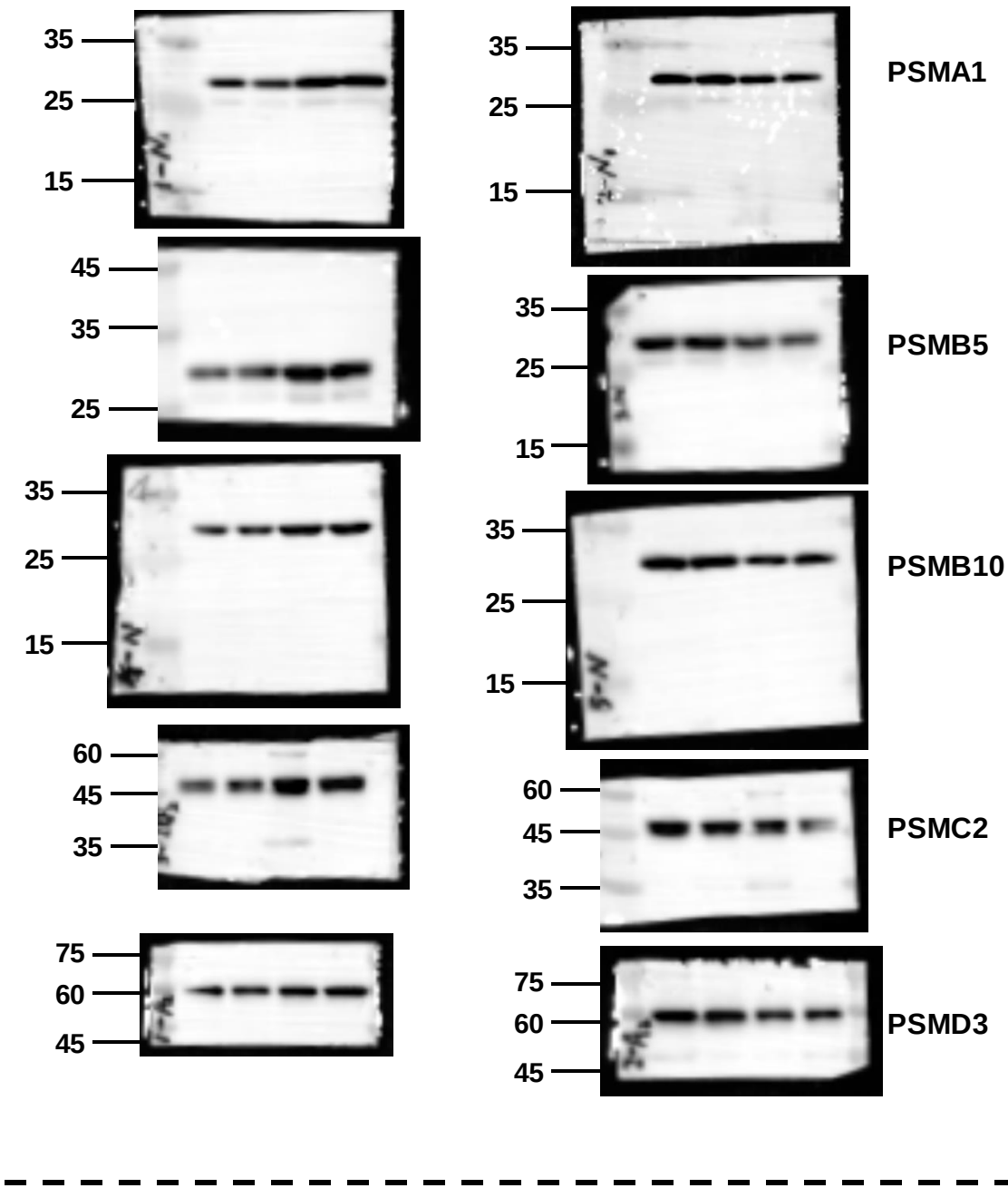
Supplementary Figure 12a



Supplementary Figure 14b



Supplementary Figure 14d



Supplementary Figure 14g