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Supporting Information

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HIPK1 Inhibition Protects against Pathological Cardiac Hypertrophy by Inhibiting the CREB-C/EBPβ Axis

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Supplementary Materials for

HIPK1 inhibition protects against pathological cardiac hypertrophy by inhibiting the CREB-C/EBPβ axis

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Supplementary Figures

Figure S1

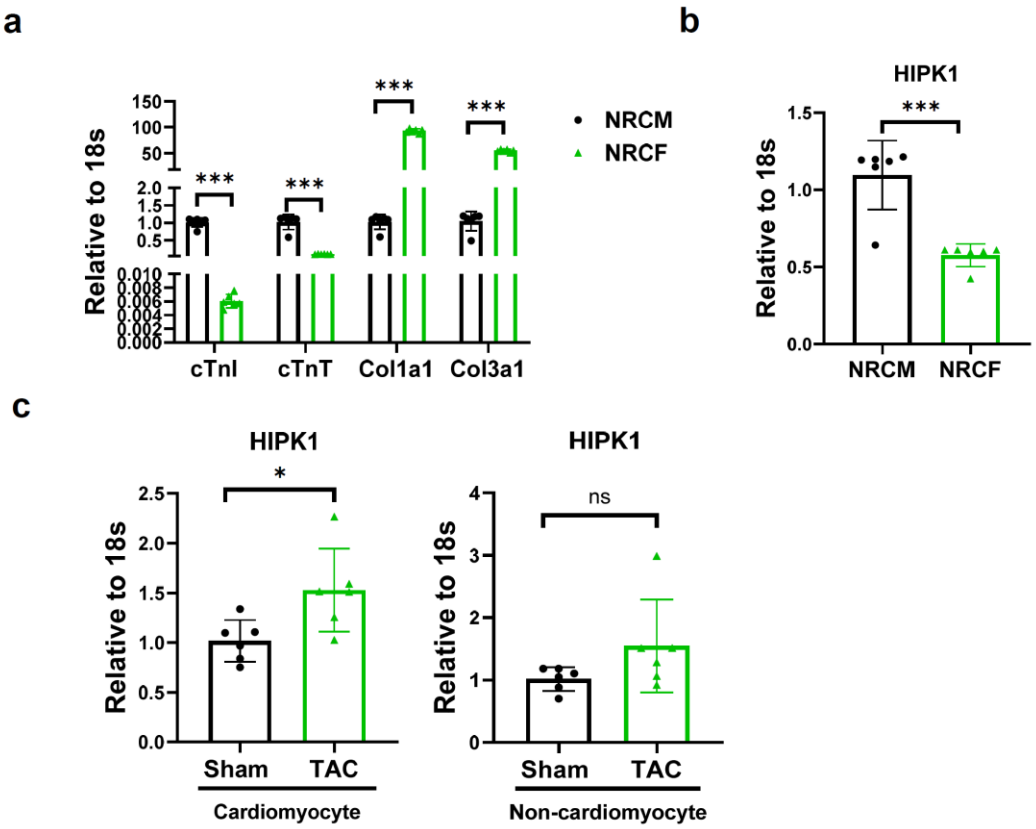

Figure S1. HIPK1 expression in primary cardiomyocytes and non-cardiomyocytes isolated from neonatal rat or adult mice hearts. a and b, qRT-PCR for cTnI, cTnT, Col1a1, Col3a1 **(a)** and HIPK1 **(b)** in isolated neonatal rat cardiomyocytes (NRCM) or fibroblasts (NRCF) (n=6). **c,** qRT-PCR for HIPK1 in isolated adult mouse cardiomyocytes *versus* non-cardiomyocytes (majorly fibroblasts) from hearts post sham operation or transaortic constriction (TAC)-induced pathological cardiac hypertrophy (n=6). Data between 2 groups were compared by independent-sample two-tailed Student's t-test. *, $P<0.05$; ***, $P<0.001$; ns, not significant.

Figure S2

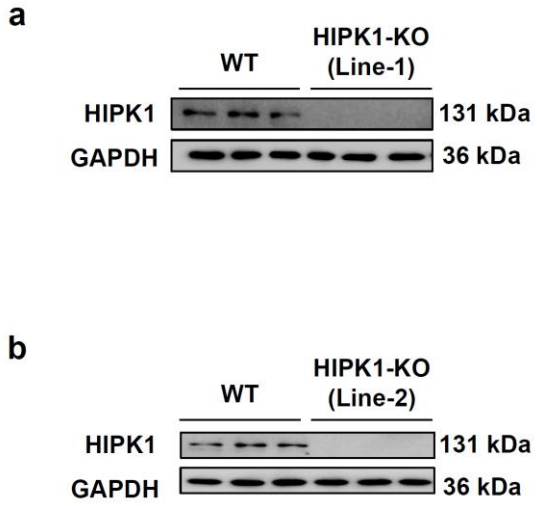

Figure S2. HIPK1 knockout (KO) mice have ablated HIPK1 expression in heart tissues. a and b, Western blot for HIPK1 in heart tissues to confirm HIPK1 ablation in HIPK1 KO mice (Line-1) **(a)** and HIPK1 KO mice (Line-2) **(b)** (n=3).

Figure S3

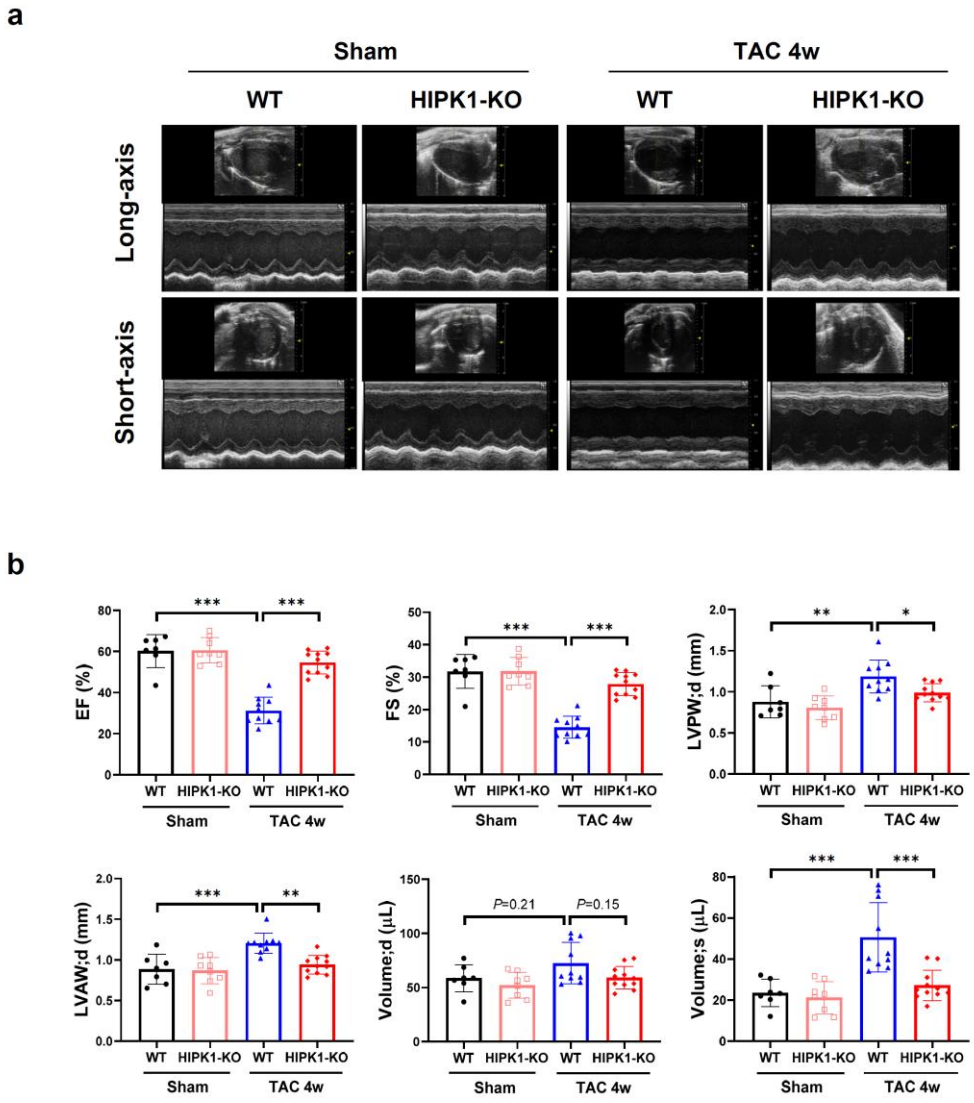

Figure S3. HIPK1 knockout (Line-1) mice have improved cardiac function at 4 weeks after transaortic constriction (TAC) surgery. a, Echocardiography for left ventricle (LV) systolic function of mice with representative images showing the long-axis view and the corresponding short-axis view of LV at the papillary muscle level. **b,** Statistical analysis of LV ejection fraction (EF) and fractional shortening (FS), end-diastolic LV posterior wall thickness (LVPW;d) and anterior wall thickness (LVAW;d), and end-diastolic LV volume (Volume;d) and end-systolic LV volume (Volume;s) according to measurements of three consecutive cardiac cycles from the long-axis view of LV at the papillary muscle level (n=7-11). 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 S4

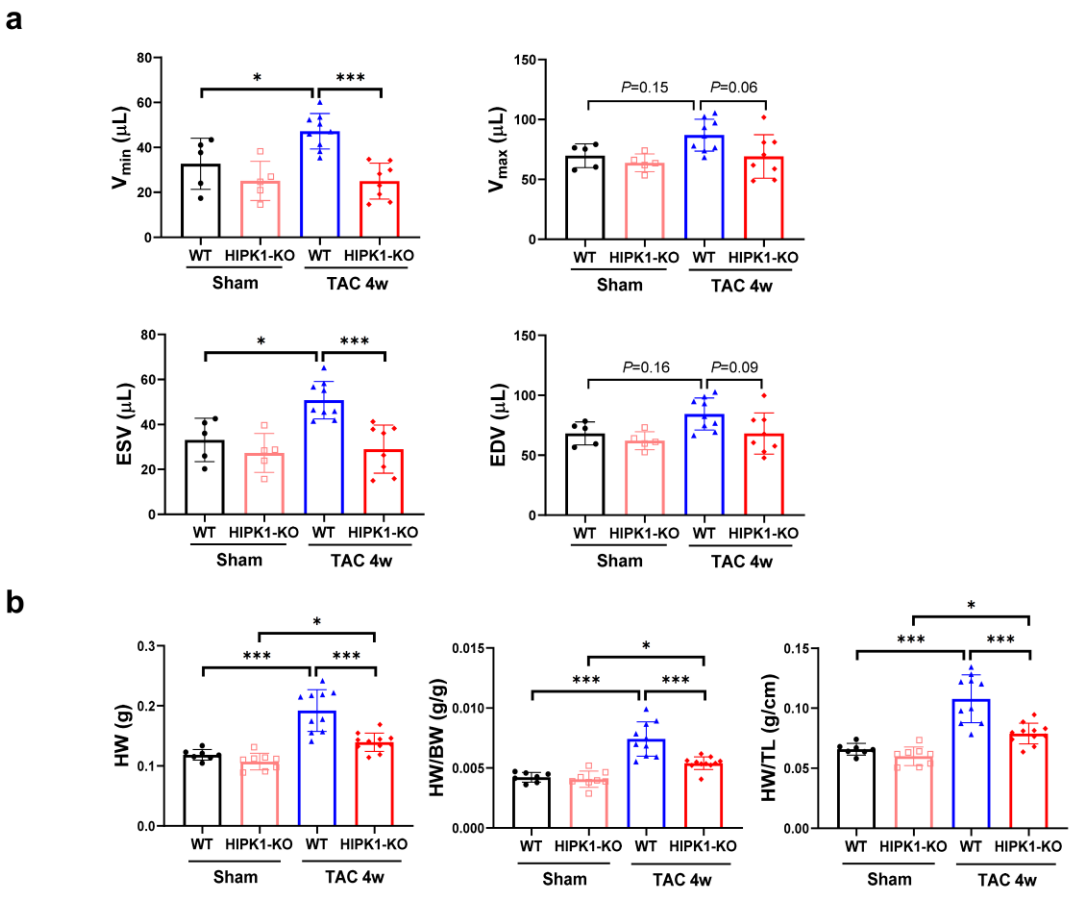

Figure S4. HIPK1 knockout (Line-1) mice have reduced left ventricle volume and heart weight at 4 weeks after transaortic constriction (TAC) surgery. a, Hemodynamic quantification of maximum and minimum left ventricle volume (max of volume, V_{max}; min of volume, V_{min}), end-systolic volume (ESV), and end-diastolic volume (EDV) (n=5-9). **b,** Heart weight (HW), heart weight/body weight (HW/BW) ratio, and heart weight/tibia length (HW/TL) ratio of mice (n=7-11). 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

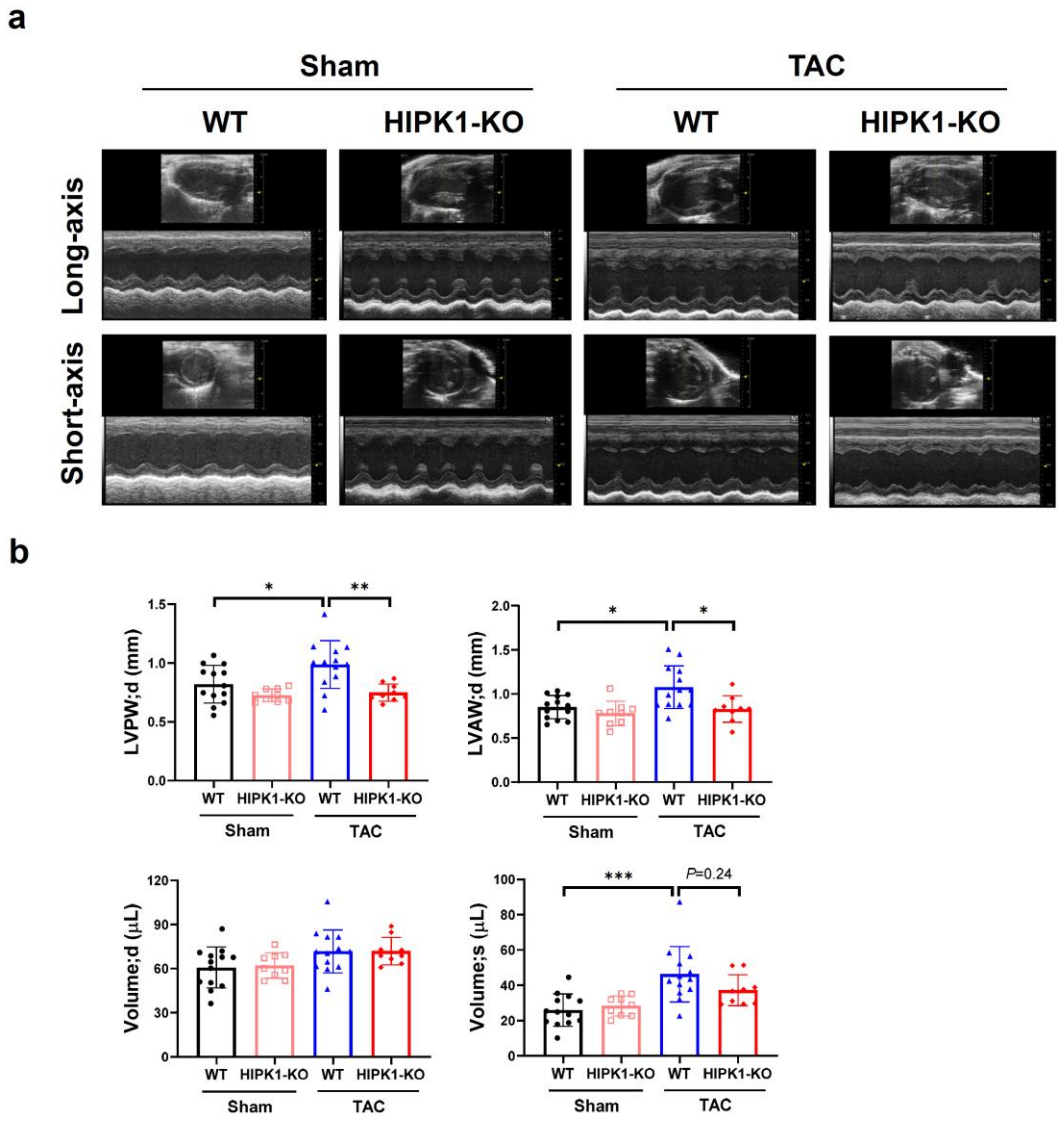

Figure S5. Echocardiography for left ventricle (LV) cardiac function of HIPK1 knockout (Line-1) mice at 8 weeks after transaortic constriction (TAC) surgery. a, Representative images showing the long-axis view and the corresponding short-axis view of left ventricle (LV) at the papillary muscle level. **b,** Statistical analysis of end-diastolic LV posterior wall thickness (LVPW;d) and anterior wall thickness (LVAW;d), and end-diastolic LV volume (Volume;d) and end-systolic LV volume (Volume;s) according to measurements of three consecutive cardiac cycles from the long-axis view of LV at the papillary muscle level (n=13 for WT mice, n=9 for HIPK1 KO mice). 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 S6

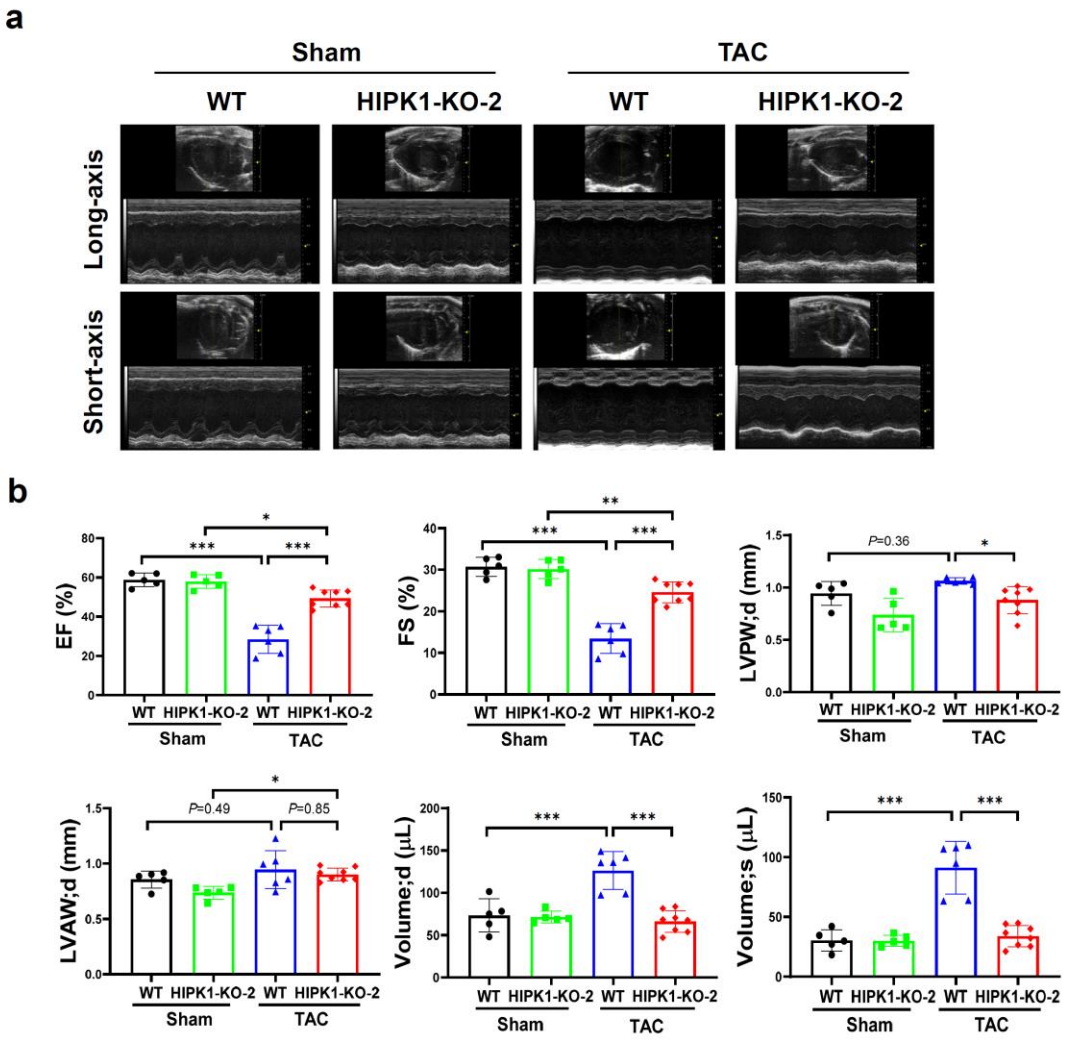

Figure S6. Echocardiography for left ventricle (LV) cardiac function of HIPK1 knockout (Line-2) mice at 6 weeks after transaortic constriction (TAC) surgery. a, Representative images showing the long-axis view and the corresponding short-axis view of left ventricle (LV) at the papillary muscle level. **b,** Statistical analysis of LV ejection fraction (EF) and fractional shortening (FS), end-diastolic LV posterior wall thickness (LVPW;d) and anterior wall thickness (LVAW;d), and end-diastolic LV volume (Volume;d) and end-systolic LV volume (Volume;s) according to measurements of three consecutive cardiac cycles from the long-axis view of LV at the papillary muscle level (n=5-8). 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 S7

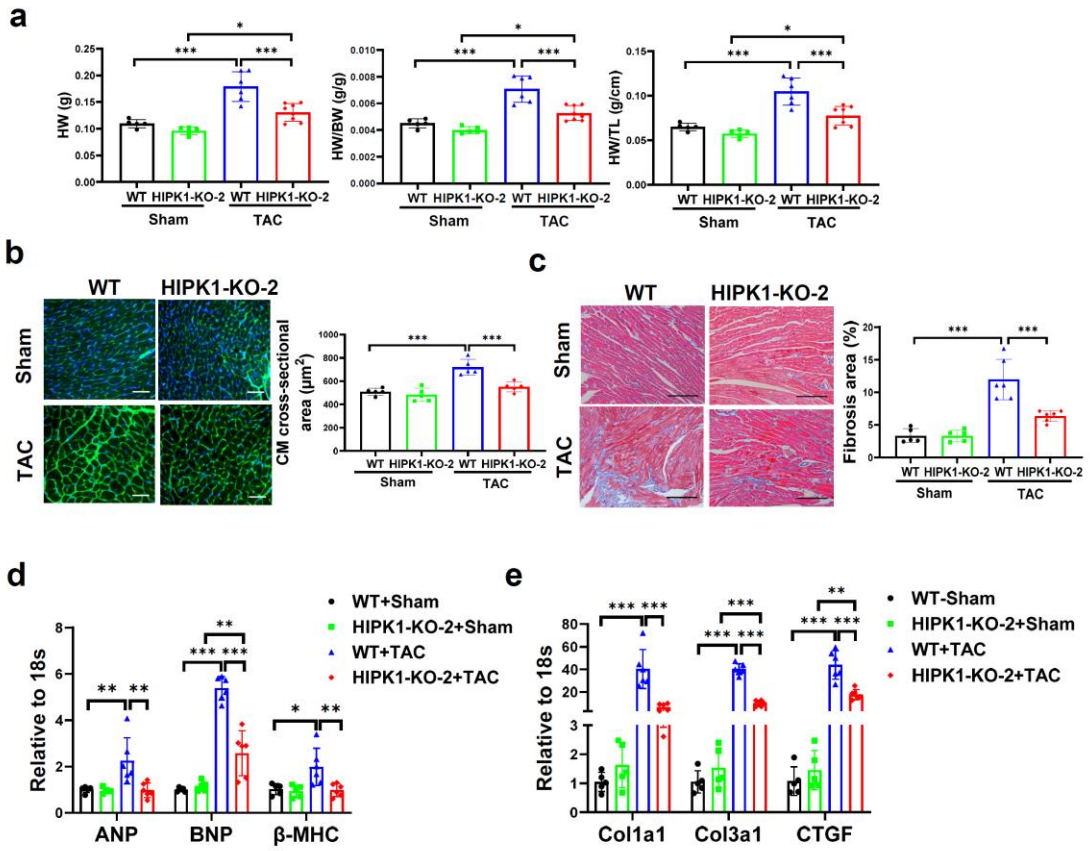

Figure S7. HIPK1 knockout mice (Line-2) are resistant to transaortic constriction (TAC)-induced pathological cardiac hypertrophy. a, Heart weight (HW), heart weight/body weight (HW/BW) ratio, and heart weight/tibia length (HW/TL) ratio of mice (n=5-8). **b,** Wheat germ agglutinin (WGA) staining for cardiomyocyte (CM) cross-sectional area (n=5). Scale bar=50 μm. **c,** Masson staining for cardiac fibrosis area (n=5-6). Scale bar=100 μm. **d and e,** qRT-PCR for ANP, BNP, and β-MHC **(d)** and Col1a1, Col3a1, and CTGF **(e)** in mice heart tissues after sham or TAC surgery (n=5-6). 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 S8

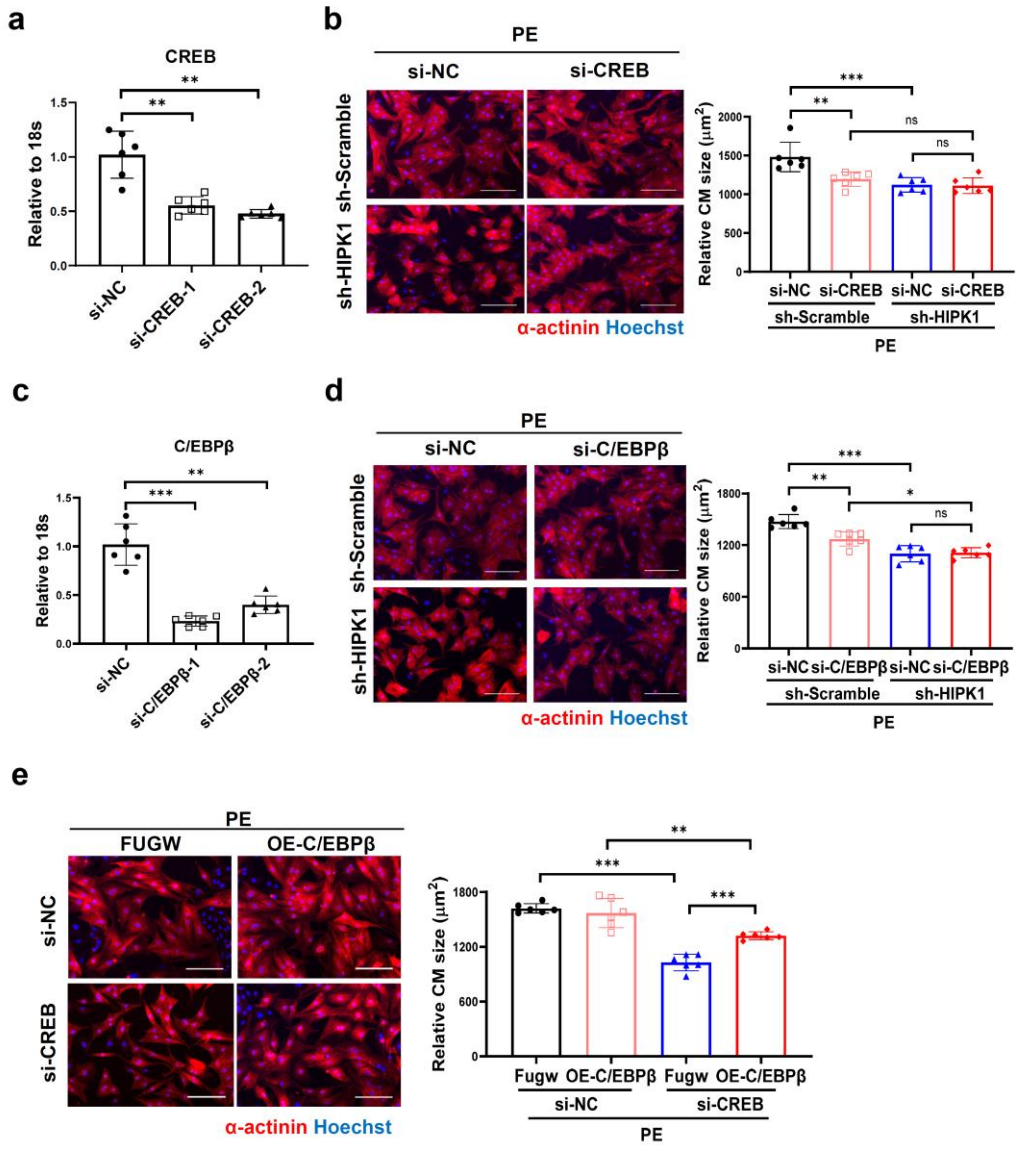

Figure S8. Function-rescue experiments between HIPK1 and CREB-C/EBPβ axis in neonatal rat cardiomyocytes (NRCMs) under phenylephrine (PE) stress. a and c, qRT-PCR for CREB **(a)** and C/EBPβ **(c)** in NRCMs transfected with CREB siRNA, C/EBPβ siRNA, or negative control, respectively (n=6). **b and d,** Immunofluorescent staining for α-actinin in NRCMs treated with sh-HIPK1 lentivirus and CREB siRNA **(b)** or C/EBPβ siRNA **(d)** (n=6). Scale bar=100 μm. **e,** Immunofluorescent staining for α-actinin in NRCMs treated with CREB siRNA and OE-C/EBPβ plasmid (n=6). Scale bar=100 μm. 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. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant.

Figure S9

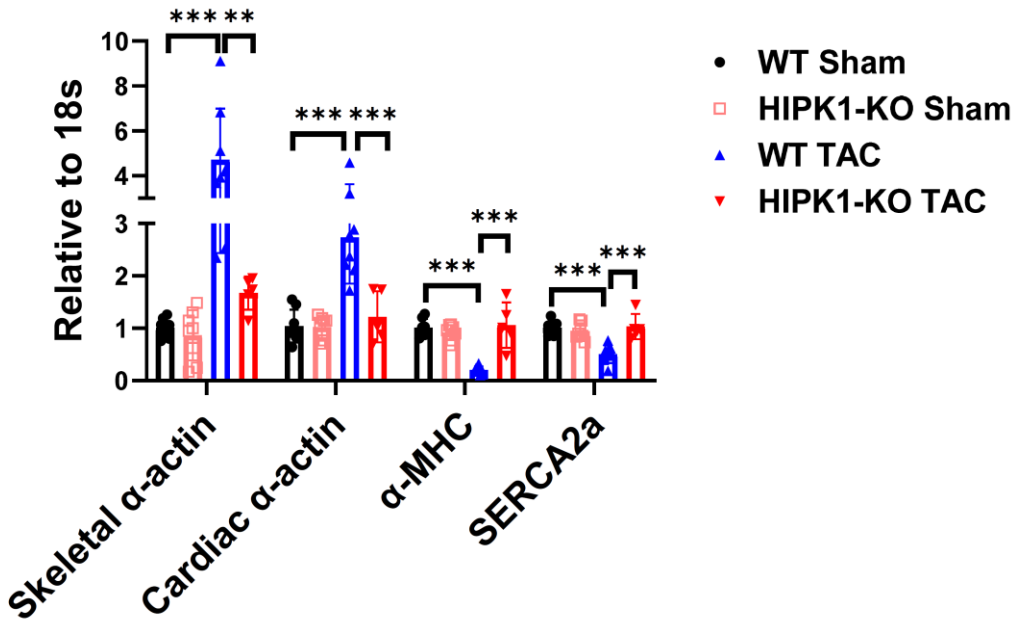

Figure S9. Suppressing HIPK1 reverses the genetic program changes associated with pathological cardiac hypertrophy. qRT-PCR for skeletal α-actin, cardiac α-actin, α-MHC, and SERCA2a in heart tissues from WT or HIPK1 knockout mice after sham or transaortic constriction (TAC) surgery (n=5-8). Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. **, $P<0.01$; ***, $P<0.001$.

Figure S10

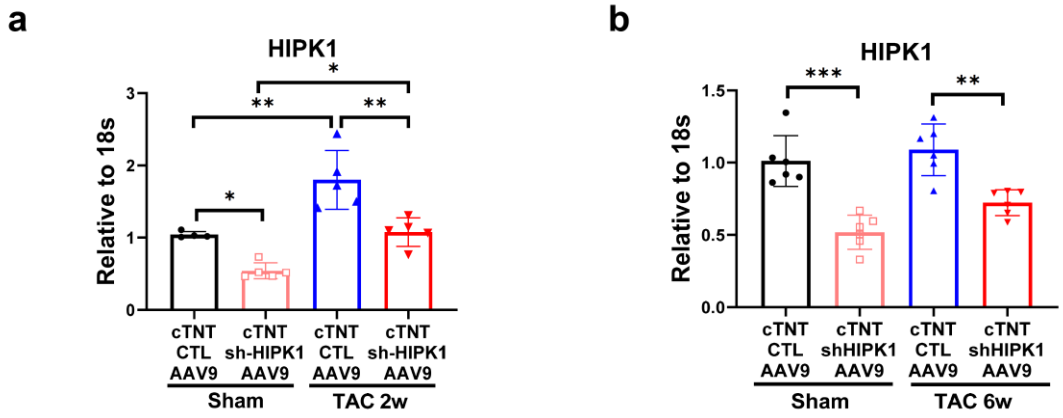

Figure S10. cTnT-shHIPK1-AAV9 injections significantly reduce HIPK1 level in mice hearts. Mice were treated via tail vein injections of cTnT promoter-mediated knockdown of HIPK1 AAV9 (cTnT-shHIPK1-AAV9) or control AAV9 vectors (cTnT-CTL-AAV9) and subsequently followed 1 week later by transaortic constriction (TAC) surgery to induce pathological cardiac hypertrophy. **a and b,** qRT-PCR was used to determine HIPK1 mRNA level in mice heart tissues at 2 weeks **(a**, n=4-5**)** and 6 weeks **(b**, n=6**)** after sham or TAC surgery, respectively. 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

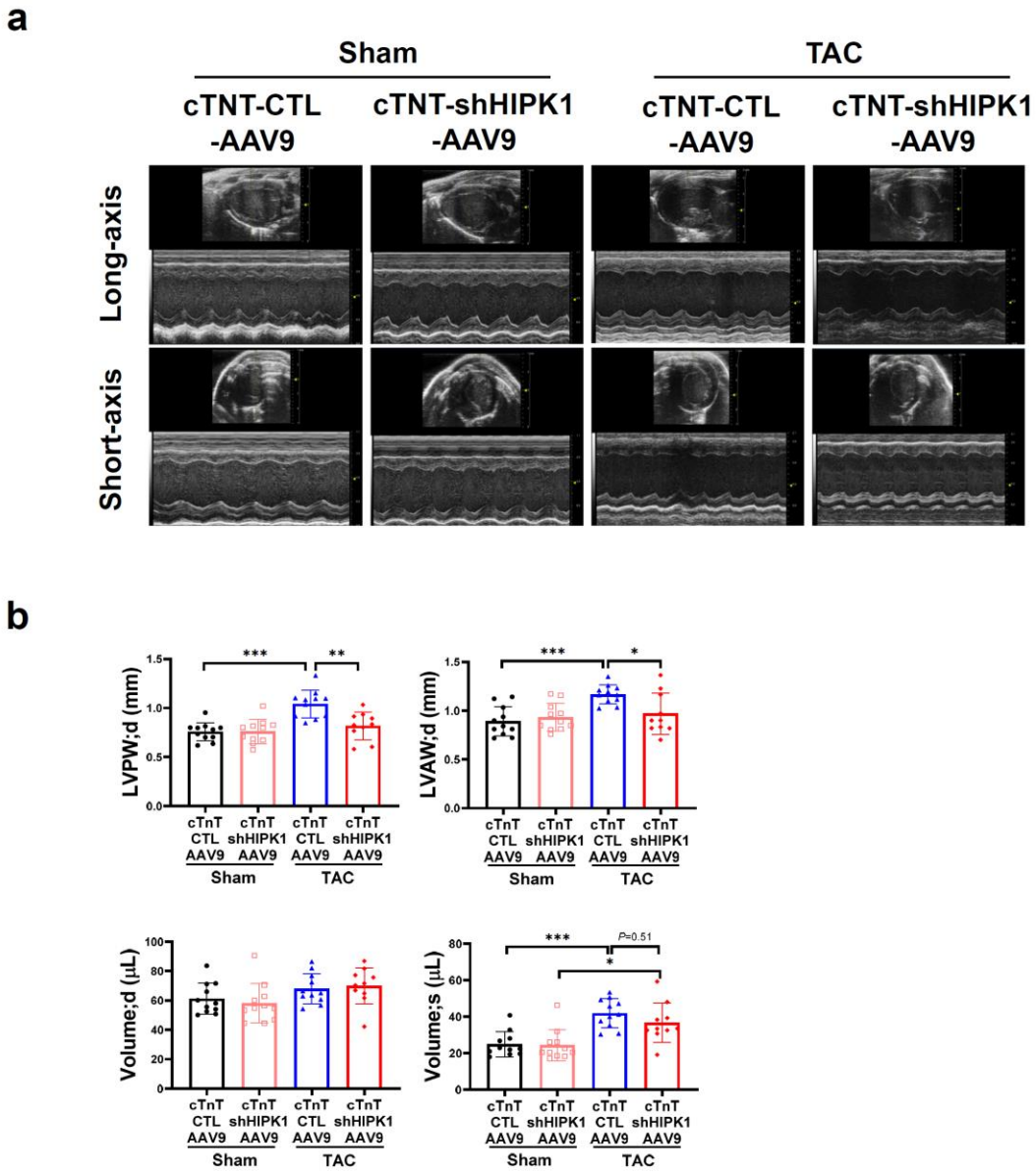

Figure S11. Echocardiography for left ventricle (LV) cardiac function of cTnT-shHIPK1-AAV9 injected mice at 6 weeks after transaortic constriction (TAC) surgery. a, Representative images showing the long-axis view and the corresponding short-axis view of left ventricle (LV) at the papillary muscle level. **b,** Statistical analysis of end-diastolic LV posterior wall thickness (LVPW;d) and anterior wall thickness (LVAW;d), and end-diastolic LV volume (Volume;d) and end-systolic LV volume (Volume;s) according to measurements of three consecutive cardiac cycles from the long-axis view of LV at the papillary muscle level (n=10-11). 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 S12

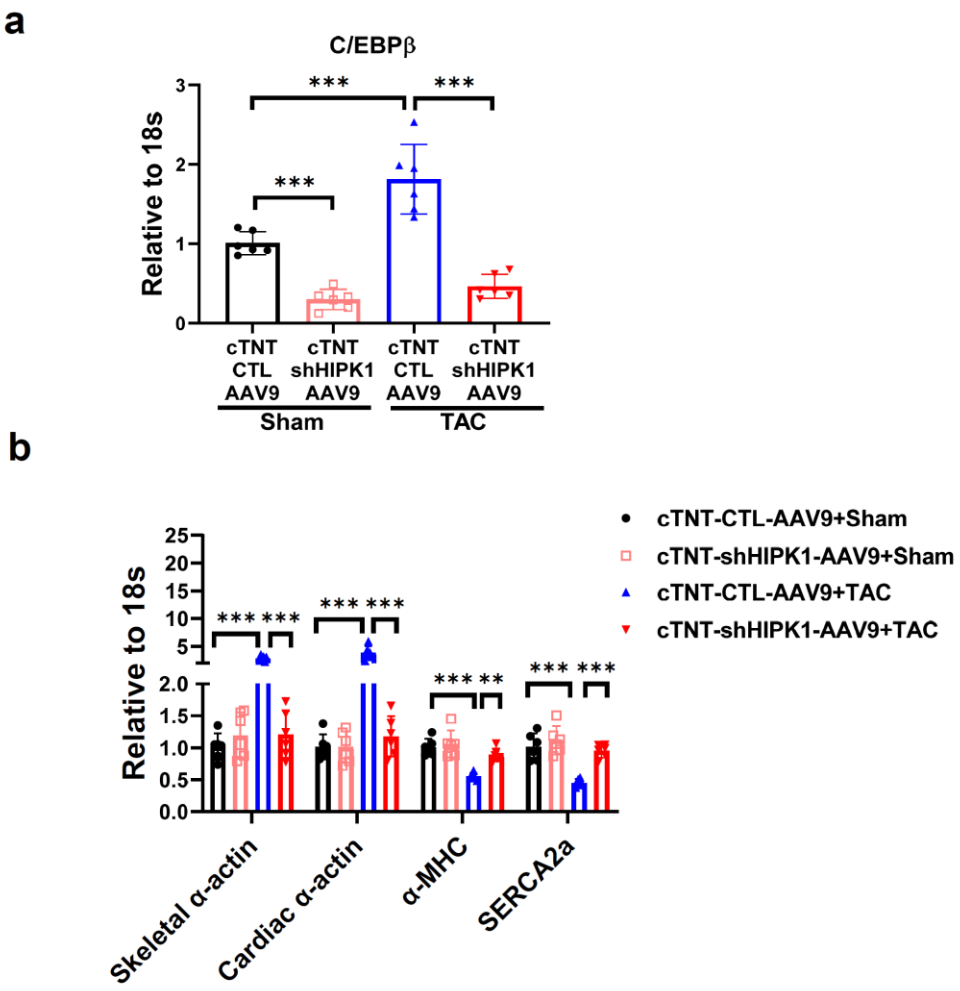

Figure S12. Knockdown of HIPK1 reduces C/EBPβ and its downstream hypertrophic response genes in transaortic constriction (TAC)-induced pathological cardiac hypertrophy. a and b, qRT-PCR for C/EBPβ **(a)** and skeletal α-actin, cardiac α-actin, α-MHC, and SERCA2a **(b)** in heart tissues from sham- or TAC-operated mice injected with cTnT promoter-mediated knockdown of HIPK1 AAV9 (cTnT-shHIPK1-AAV9) or control AAV9 vectors (cTnT-CTL-AAV9) (n=6). Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. **, $P<0.01$; ***, $P<0.001$.

Figure S13

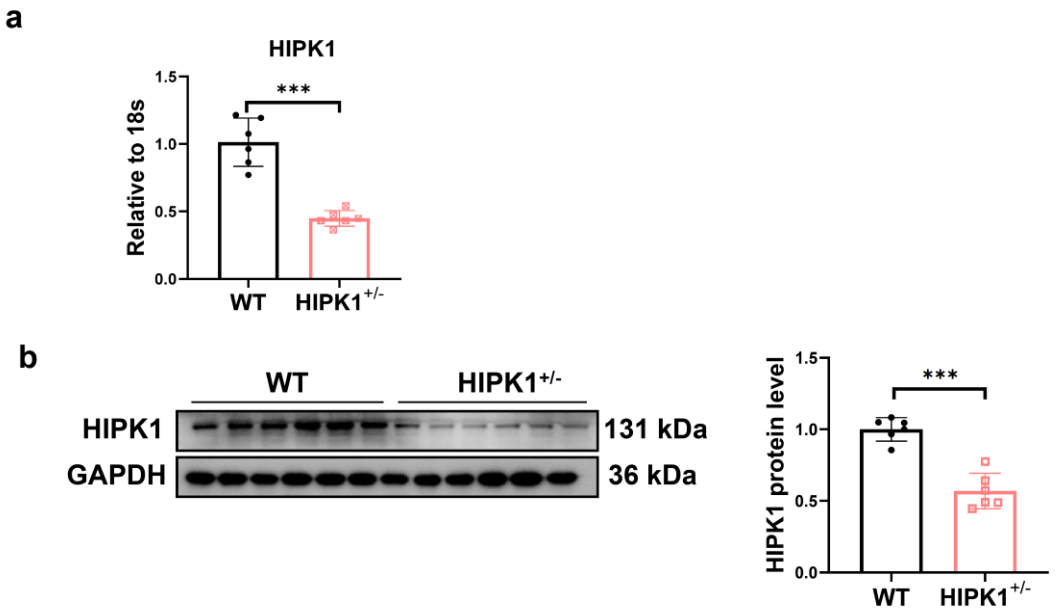

Figure S13. Mice with hetero-knockout of HIPK1 (HIPK1$^{+/-}$) have reduced HIPK1 expression in heart tissues. a and b, qRT-PCR **(a)** and Western blot **(b)** for HIPK1 in heart tissues from HIPK1$^{+/-}$ mice and wild type (WT) mice (n=6). Data between 2 groups were compared by independent-sample two-tailed Student's t-test. ***, P<0.001.

Figure S14

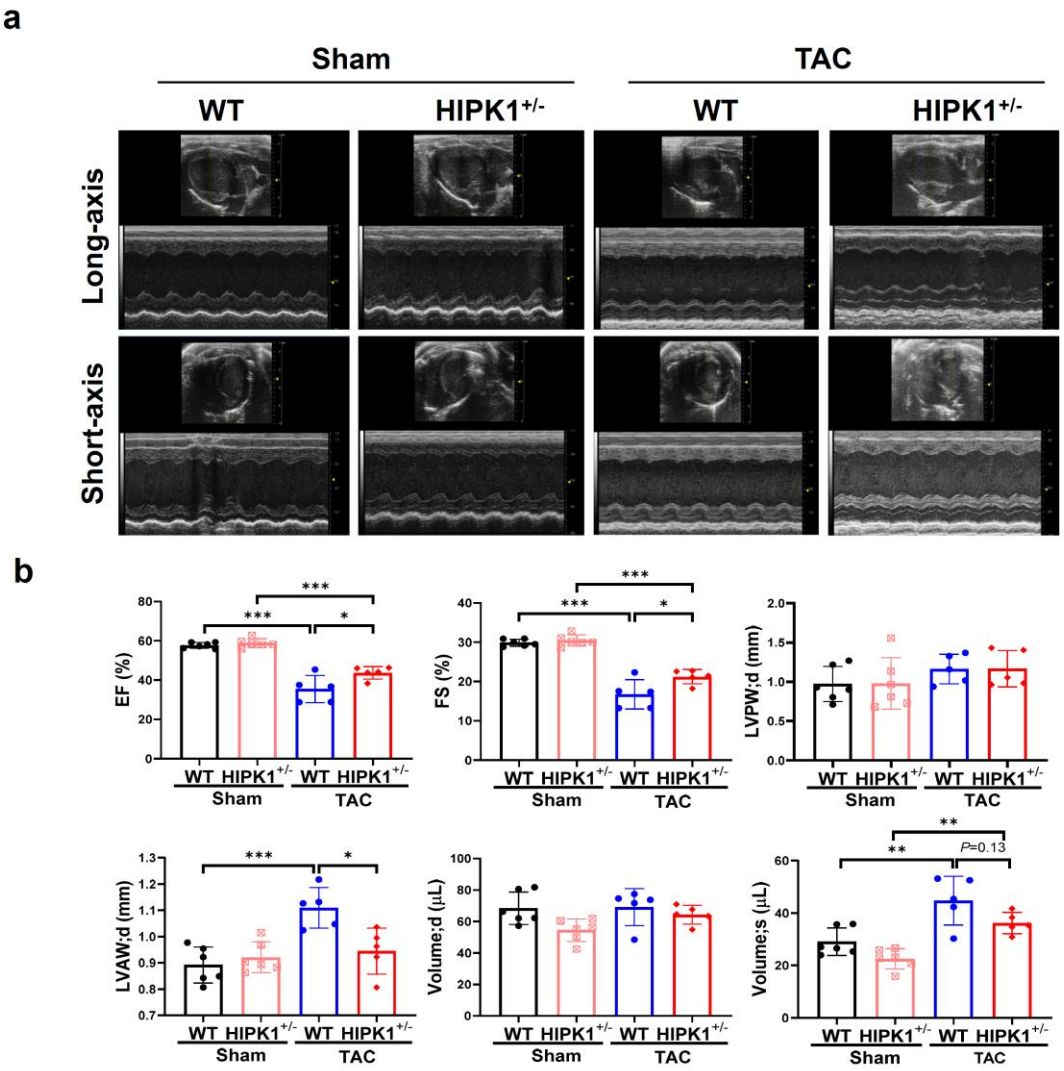

Figure S14. HIPK1 hetero-knockout mice have improved cardiac function at 8 weeks after transaortic constriction (TAC) surgery. a, Echocardiography for left ventricle (LV) systolic function of mice with representative images showing the long-axis view and the corresponding short-axis view of LV at the papillary muscle level. **b,** Statistical analysis of LV ejection fraction (EF) and fractional shortening (FS), end-diastolic LV posterior wall thickness (LVPW;d) and anterior wall thickness (LVAW;d), and end-diastolic LV volume (Volume;d) and end-systolic LV volume (Volume;s) according to measurements of three consecutive cardiac cycles from the long-axis view of LV at the papillary muscle level (n=5-6). 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 S15

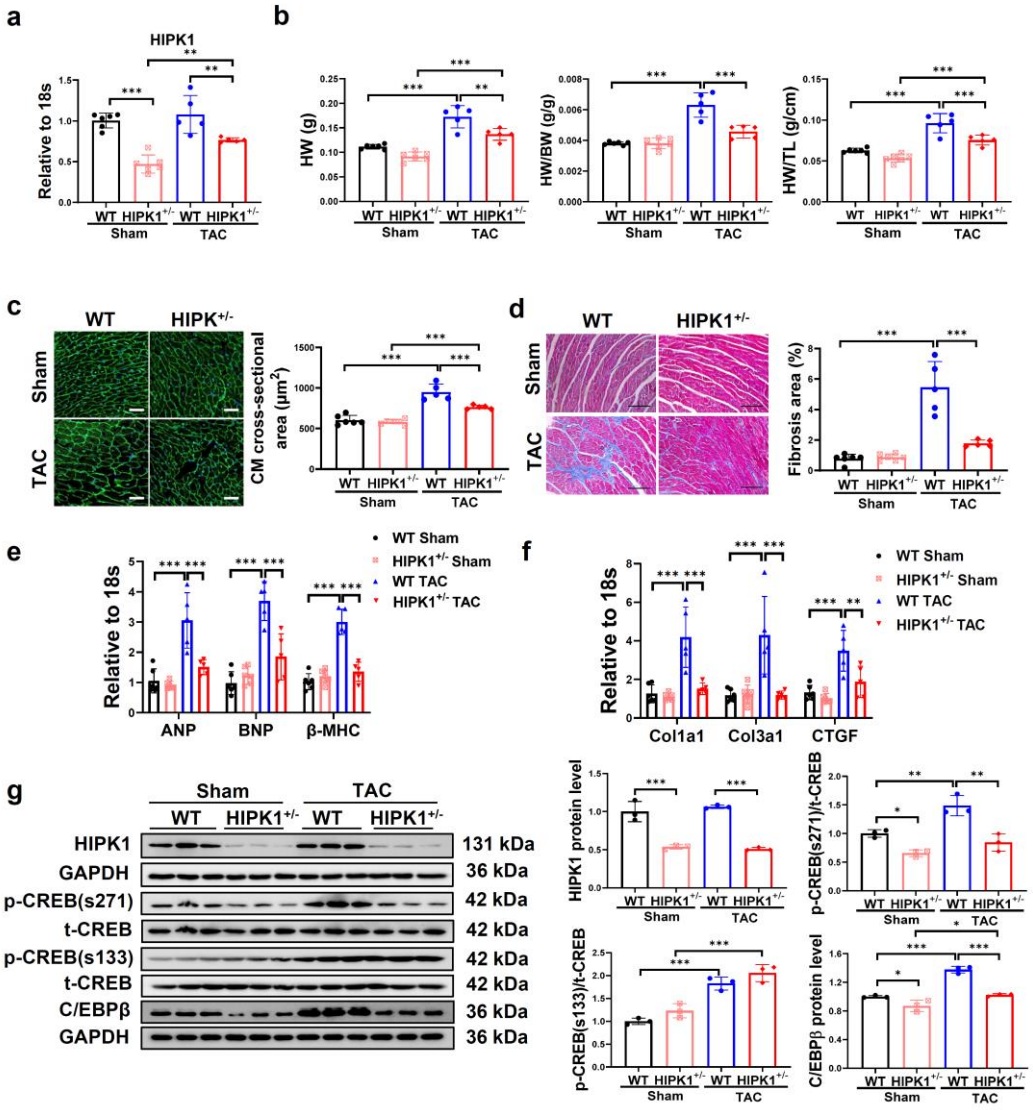

Figure S15. HIPK1 hetero-knockout is sufficient to attenuate transaortic constriction (TAC)-induced pathological cardiac hypertrophy. a, qRT-PCR for HIPK1 in heart tissues from sham-operated mice or mice at 8 weeks post TAC surgery (n=5-6). **b,** Heart weight (HW), heart weight/body weight (HW/BW) ratio, and heart weight/tibia length (HW/TL) ratio of mice (n=5-6). **c,** Wheat germ agglutinin (WGA) staining for cardiomyocyte (CM) cross-sectional area (n=5-6). Scale bar=50 μm. **d,** Masson staining for cardiac fibrosis area (n=5-6). Scale bar=100 μm. **e and f,** qRT-PCR for ANP, BNP, and β-MHC **(e)** and Col1a1, Col3a1, and CTGF **(f)** in mice heart tissues after sham or TAC surgery (n=5-6). **g,** Western blot for HIPK1, CREB phosphorylation, and C/EBPβ expressions in mice heart tissues after sham or TAC surgery (n=3). 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 S16

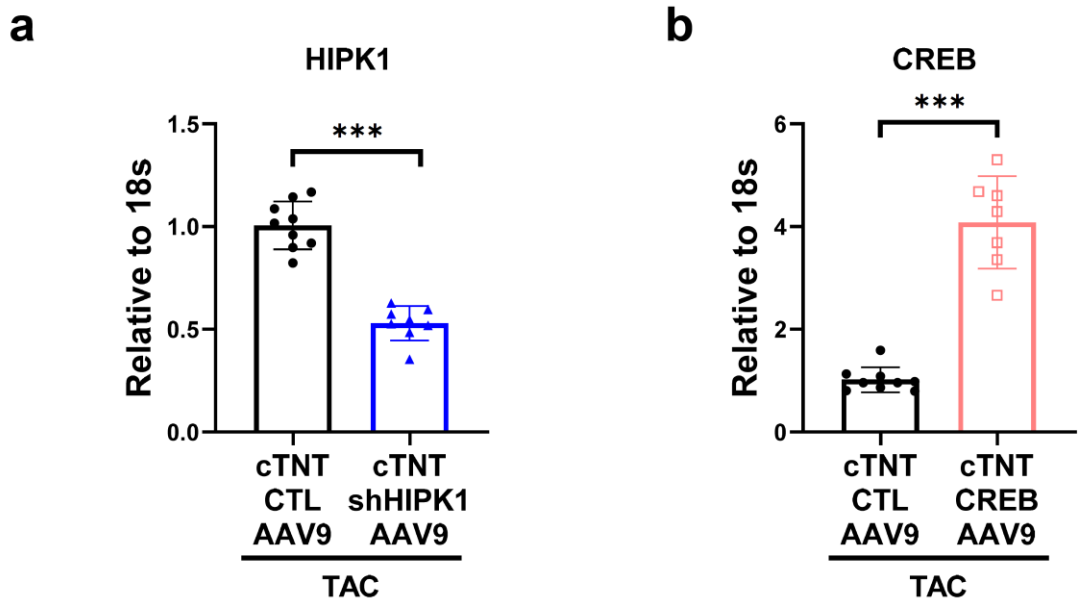

Figure S16. Validation of AAV9-mediated gene expression changes in mice hearts. Mice were treated via tail vein injections of cTnT promoter-mediated knockdown of HIPK1 AAV9 (cTnT-shHIPK1-AAV9), cTnT promoter-mediated overexpression of CREB AAV9 (cTnT-CREB-AAV9), or control AAV9 vectors (cTnT-CTL-AAV9) and subsequently followed 1 week later by transaortic constriction (TAC) surgery to induce pathological cardiac hypertrophy. **a and b**, at 6 weeks post TAC, qRT-PCR was used to determine HIPK1 **(a)** and CREB **(b)** mRNA levels in mice heart tissues (n=7-9). Data between 2 groups were compared by independent-sample two-tailed Student's t-test. ***, $P<0.001$.

Figure S17

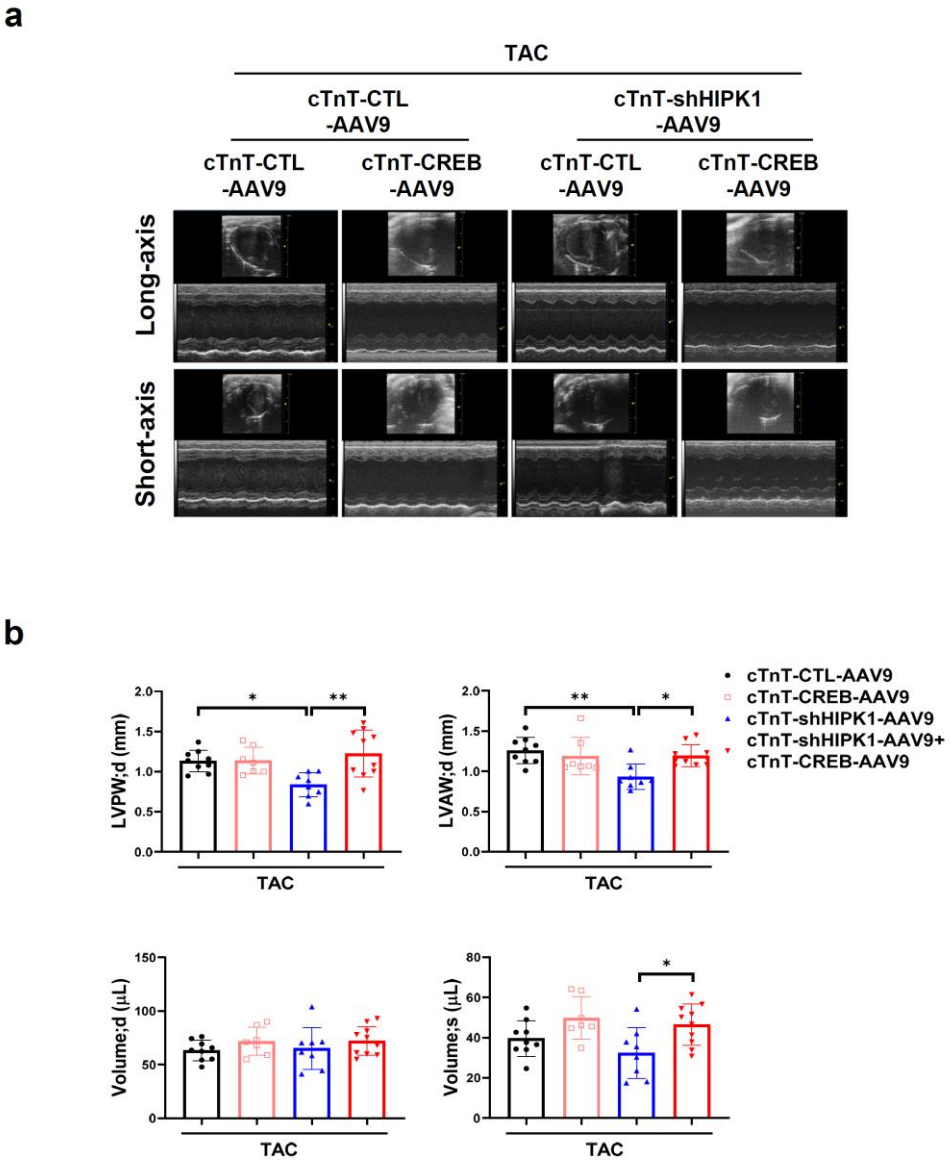

Figure S17. Echocardiography for left ventricle (LV) cardiac function of mice with cTnT-shHIPK1-AAV9 and/or cTnT-CREB-AAV9 injections at 6 weeks after transaortic constriction (TAC) surgery. a, Representative images showing the long-axis view and the corresponding short-axis view of left ventricle (LV) at the papillary muscle level. **b,** Statistical analysis of end-diastolic LV posterior wall thickness (LVPW;d) and anterior wall thickness (LVAW;d), and end-diastolic LV volume (Volume;d) and end-systolic LV volume (Volume;s) according to measurements of three consecutive cardiac cycles from the long-axis view of LV at the papillary muscle level (n=7-10). 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 S18

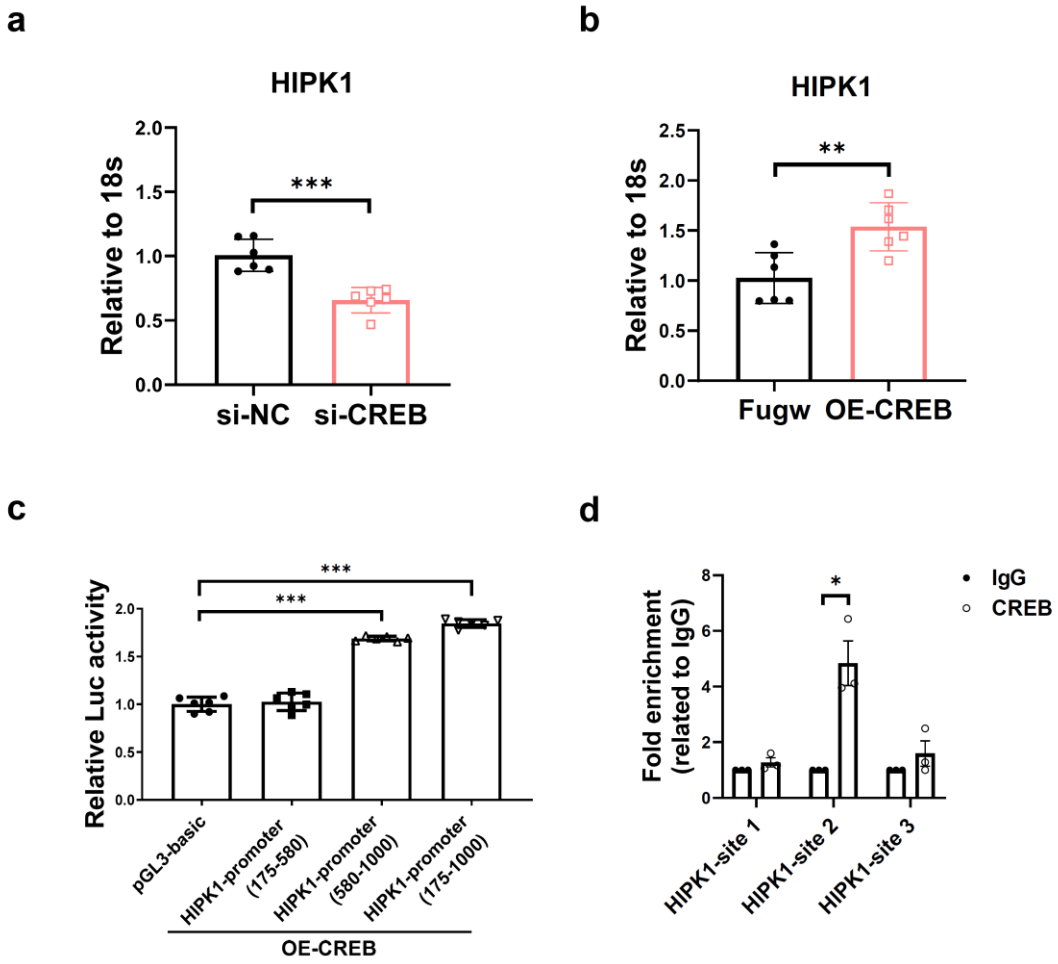

Figure S18. CREB positively regulates HIPK1 in cardiomyocytes. a and b, qRT-PCR for HIPK1 in neonatal rat cardiomyocytes (NRCM) transfected with CREB siRNA (si-CREB) and negative control (si-NC) (**a**, n=6), or with OE-CREB plasmid and Fugw control vector (**b**, n=6). **c,** Luciferase reporter assay for detection of the regulation between CREB and HIPK1 (n=6). **d,** ChIP-PCR assay for detection of the enrichment of CREB to the promoter region of HIPK1 (n=3). Data between 2 groups were compared by independent-sample two-tailed Student's t-test. Data among 4 groups were compared by one-way ANOVA test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Figure S19

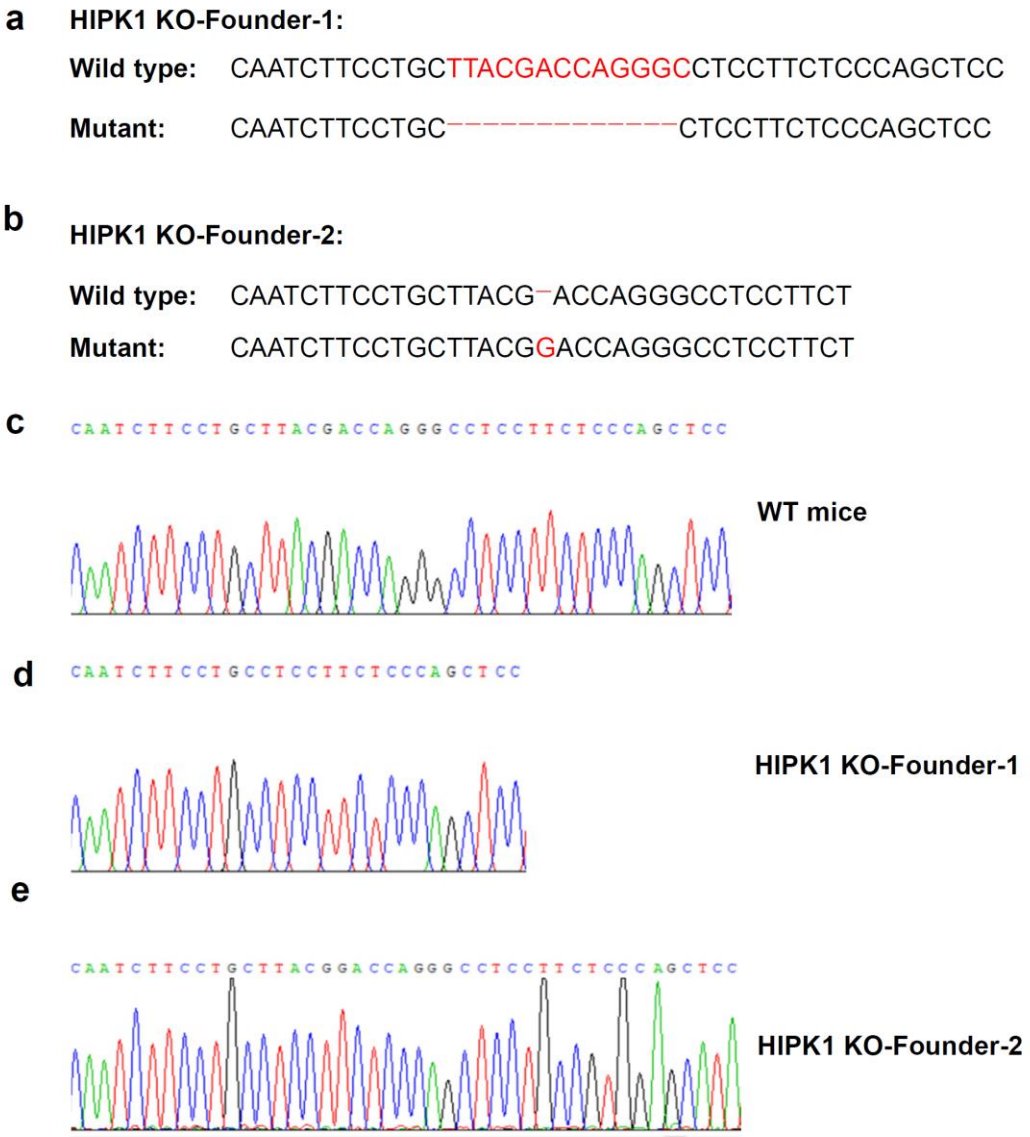

Figure S19. Generation and DNA sequences of HIPK1 knockout (KO) mice. a, HIPK1 KO mice (Founder-1) was generated with 13 bp deletion before CTCCTTCTCCCAGCTCC in HIPK1 genomic DNA. **b,** HIPK1 KO mice (Founder-2) was generated with one G insertion before ACCAGGGCCTCCTTCT in HIPK1 genomic DNA. **c-e,** Genotypes were confirmed by DNA sequencing of tail DNA as demonstrated for wild type (WT) mice **(c)**, Founder-1 **(d)** and Founder-2 **(e)**, respectively.

Supplemental Tables

Table S1. Echocardiography parameters of wild type (WT) mice *versus* HIPK1 knockout (KO) mice (Line-1) at 4 weeks after TAC surgery.

	WT+Sham (n=7)	HIPK1 KO+Sham (n=8)	WT+TAC 4w (n=10)	HIPK1 KO+TAC 4w (n=11)
Heart rate (bpm)	470.20±41.25	487.30±41.93	474.20±50.57	478.90±33.39
EF (%)	60.25±8.05	60.63±6.16	31.19±6.51***	54.63±5.58$^{###}$
FS (%)	31.74±5.23	31.81±4.20	14.54±3.34***	27.86±3.50$^{###}$
LV Mass (mg)	118.5±29.2	101.5±22.6	211.7±44.7***	135.3±15.9$^{###}$
LVPW;s (mm)	1.26±0.26	1.18±0.16	1.35±0.17	1.31±0.14
LVPW;d (mm)	0.88±0.19	0.80±0.14	1.19±0.20**	0.99±0.11$^{#}$
LVAW;s (mm)	1.30±0.17	1.27±0.18	1.42±0.11	1.34±0.09
LVAW;d (mm)	0.88±0.18	0.87±0.16	1.20±0.13***	0.94±0.11$^{##}$
Diameter;s (mm)	2.53±0.31	2.41±0.38	3.45±0.47***	2.69±0.29$^{###}$
Diameter;d (mm)	3.70±0.34	3.51±0.35	4.03±0.44	3.72±0.27
Volume;s (μL)	23.42±6.69	21.14±7.80	50.56±16.81***	27.15±7.39$^{###}$
Volume;d (μL)	58.71±12.53	52.10±12.03	72.55±19.12	59.21±10.51

BPM, beat per minute; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; LVPW;s, end-systolic LV posterior wall thickness; LVPW;d, end-diastolic LV posterior wall thickness; LVAW;s, end-systolic LV anterior wall thickness; LVAW;d, end-diastolic LV anterior wall

thickness; Diameter;s, end-systolic LV diameter; Diameter;d, end-diastolic LV diameter; Volume;s, end-systolic LV volume; Volume;d, end-diastolic LV volume. Data are presented as mean±SD. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. **, $P<0.01$ compared to corresponding Sham group; ***, $P<0.001$ compared to corresponding Sham group; ##, $P<0.01$ compared to WT+TAC group; ###, $P<0.001$ compared to WT+TAC group.

Table S2. Echocardiography parameters of wild type (WT) mice *versus* HIPK1 knockout (KO) mice (Line-1) at 8 weeks after TAC surgery.

	WT+Sham (n=13)	HIPK1 KO+Sham (n=9)	WT+TAC (n=13)	HIPK1 KO+TAC (n=9)
Heart rate (bpm)	498.20±40.52	458.00±43.84	488.80±40.54	447.40±43.11
EF (%)	58.44±6.46	54.62±5.76	36.83±8.98***	48.79±5.86$^{\#\#}$
FS (%)	30.46±4.31	27.91±3.76	17.59±4.74***	24.38±3.49$^{\#\#}$
LV Mass (mg)	110.9±14.3	99.8±17.6	168.2±30.5***	118.0±22.1$^{\#\#\#}$
LVPW;s (mm)	1.12±0.20	1.01±0.15	1.15±0.25	0.99±0.09
LVPW;d (mm)	0.82±0.16	0.73±0.05	0.99±0.20*	0.75±0.07$^{\#\#}$
LVAW;s (mm)	1.29±0.20	1.19±0.17	1.36±0.30	1.16±0.19
LVAW;d (mm)	0.85±0.13	0.78±0.14	1.08±0.24*	0.83±0.15$^{\#}$
Diameter;s (mm)	2.62±0.39	2.74±0.23	3.33±0.45***	3.06±0.29
Diameter;d (mm)	3.75±0.37	3.80±0.22	4.03±0.34	4.04±0.22
Volume;s (μL)	25.89±9.14	28.28±5.69	46.28±15.71***	37.21±8.78
Volume;d (μL)	60.75±13.88	62.15±8.59	71.76±14.59	71.94±9.34

BPM, beat per minute; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; LVPW;s, end-systolic LV posterior wall thickness; LVPW;d, end-diastolic LV posterior wall thickness; LVAW;s, end-systolic LV anterior wall thickness; LVAW;d, end-diastolic LV anterior wall

thickness; Diameter;s, end-systolic LV diameter; Diameter;d, end-diastolic LV diameter; Volume;s, end-systolic LV volume; Volume;d, end-diastolic LV volume. Data are presented as mean±SD. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$ compared to corresponding Sham group; ***, $P<0.001$ compared to corresponding Sham group; #, $P<0.05$ compared to WT+TAC group; ##, $P<0.01$ compared to WT+TAC group; ###, $P<0.001$ compared to WT+TAC group.

Table S3. Echocardiography parameters of wild type (WT) mice *versus* HIPK1 knockout (KO) mice (Line-2) at 6 weeks after TAC surgery.

	WT+Sham (n=5)	HIPK1-KO-2+Sham (n=5)	WT+TAC (n=6)	HIPK1-KO-2+TAC (n=8)
Heart rate (bpm)	461.5±32.8	448.3±24.7	455.6±34.7	461.3±21.6
EF (%)	58.73±3.44	57.91±3.51	28.45±7.13***	49.32±4.32*,###
FS (%)	30.71±2.28	30.14±2.30	13.42±3.58***	24.58±2.49**,###
LV Mass (mg)	141.2±22.1	106.7±10.9	240.2±21.2***	131.2±24.7###
LVPW;s (mm)	1.24±0.04	1.16±0.18	1.24±0.05	1.10±0.12
LVPW;d (mm)	0.94±0.11	0.74±0.16	1.06±0.03	0.88±0.13#
LVAW;s (mm)	1.35±0.16	1.18±0.08	1.18±0.22	1.27±0.06
LVAW;d (mm)	0.85±0.08	0.74±0.06	0.95±0.17	0.90±0.06#
Diameter;s (mm)	2.81±0.34	2.82±0.18	4.45±0.48***	2.94±0.33###
Diameter;d (mm)	4.05±0.47	4.03±0.16	5.13±0.41***	3.89±0.33###
Volume;s (μL)	30.35±8.81	30.10±4.71	91.20±21.98***	33.89±8.96###
Volume;d (μL)	73.23±19.68	71.27±6.92	126.30±22.39***	66.09±13.08###

BPM, beat per minute; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; LVPW;s, end-systolic LV posterior wall thickness; LVPW;d, end-diastolic LV posterior wall thickness; LVAW;s, end-systolic LV anterior wall thickness; LVAW;d, end-diastolic LV anterior wall

thickness; Diameter;s, end-systolic LV diameter; Diameter;d, end-diastolic LV diameter; Volume;s, end-systolic LV volume; Volume;d, end-diastolic LV volume. Data are presented as mean±SD. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$ compared to corresponding Sham group; **, $P<0.01$ compared to corresponding Sham group; ***, $P<0.001$ compared to corresponding Sham group; #, $P<0.05$ compared to WT+TAC group; ###, $P<0.001$ compared to WT+TAC group.

Table S4. Echocardiography parameters of mice injected with cTnT-shHIPK1-AAV9 *versus* control AAV9 at 6 weeks after TAC surgery.

	cTnT-CTL-AAV9+Sham (n=11)	cTnT-shHIPK1-AAV9+Sham (n=11)	cTnT-CTL-AAV9+TAC (n=11)	cTnT-shHIPK1-AAV9+TAC (n=10)
Heart rate (bpm)	504.6±43.2	508.9±37.9	497.3±46.3	492.4±45.6
EF (%)	59.82±3.92	58.69±4.72	38.50±5.46***	48.07±8.35***,##
FS (%)	31.31±2.52	30.5±3.07	18.38±2.90***	23.99±4.72***,##
LV Mass (mg)	111.6±20.9	111.5±20.8	181.4±26.8***	136.9±30.5##
LVPW;s (mm)	1.12±0.13	1.13±0.13	1.30±0.17*	1.07±0.18##
LVPW;d (mm)	0.76±0.09	0.76±0.12	1.04±0.14***	0.82±0.14##
LVAW;s (mm)	1.31±0.16	1.36±0.13	1.39±0.13	1.31±0.30
LVAW;d (mm)	0.89±0.15	0.93±0.14	1.17±0.10***	0.97±0.21#
Diameter;s (mm)	2.59±0.27	2.56±0.33	3.22±0.26***	3.03±0.36**
Diameter;d (mm)	3.77±0.27	3.68±0.34	3.94±0.25	3.98±0.32
Volume;s (μL)	24.92±6.81	24.41±8.48	41.92±7.91***	36.72±10.77*
Volume;d (μL)	61.23±10.65	58.13±13.35	67.93±10.29	69.89±12.41

BPM, beat per minute; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; LVPW;s, end-systolic LV posterior wall thickness; LVPW;d, end-diastolic LV posterior wall thickness; LVAW;s, end-systolic LV anterior wall thickness; LVAW;d, end-diastolic LV anterior wall

thickness; Diameter;s, end-systolic LV diameter; Diameter;d, end-diastolic LV diameter; Volume;s, end-systolic LV volume; Volume;d, end-diastolic LV volume. Data are presented as mean±SD. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$ compared to corresponding Sham group; **, $P<0.01$ compared to corresponding Sham group; ***, $P<0.001$ compared to corresponding Sham group; #, $P<0.05$ compared to WT+TAC group; ##, $P<0.01$ compared to WT+TAC group.

Table S5. Echocardiography parameters of wild type (WT) *versus* HIPK1 hetero-knockout mice at 8 weeks after TAC surgery.

	WT+Sham (n=6)	HIPK1$^{+/-}$+Sham (n=6)	WT+TAC (n=5)	HIPK1$^{+/-}$+TAC (n=5)
Heart rate (bpm)	505.9±20.2	504.9±29.3	477.2±53.3	508.1±14.6
EF (%)	57.65±1.50	58.86±2.24	35.43±6.93***	43.71±3.21***,#
FS (%)	29.88±0.89	30.47±1.42	16.76±3.74***	21.23±1.82***,#
LV Mass (mg)	143±27.3	125.9±21.7	192.8±43.2	163.9±27.0
LVPW;s (mm)	1.32±0.23	1.30±0.31	1.32±0.18	1.42±0.20
LVPW;d (mm)	0.97±0.22	0.98±0.33	1.16±0.19	1.17±0.23
LVAW;s (mm)	1.37±0.07	1.34±0.07	1.40±0.13	1.28±0.06
LVAW;d (mm)	0.89±0.07	0.92±0.06	1.11±0.08***	0.94±0.09#
Diameter;s (mm)	2.77±0.20	2.50±0.18	3.30±0.30**	3.04±0.14**
Diameter;d (mm)	3.95±0.25	3.59±0.20	3.97±0.30	3.86±0.15
Volume;s (μL)	29.06±5.28	22.51±3.90	44.74±9.31**	36.19±4.08**
Volume;d (μL)	68.38±10.34	54.45±7.17	69.22±11.77	64.29±6.04

BPM, beat per minute; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; LVPW;s, end-systolic LV posterior wall thickness; LVPW;d, end-diastolic LV posterior wall thickness; LVAW;s, end-systolic LV anterior wall thickness; LVAW;d, end-diastolic LV anterior wall

thickness; Diameter;s, end-systolic LV diameter; Diameter;d, end-diastolic LV diameter; Volume;s, end-systolic LV volume; Volume;d, end-diastolic LV volume. Data are presented as mean±SD. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. **, $P<0.01$ compared to corresponding Sham group; ***, $P<0.001$ compared to corresponding Sham group; #, $P<0.05$ compared to WT+TAC group.

Table S6. Echocardiography parameters of mice injected with cTnT-shHIPK1-AAV9 and/or cTnT-CREB-AAV9 at 6 weeks after TAC surgery.

	cTnT-CTL-AAV9+TAC (n=9)	cTnT-CREB-AAV9+TAC (n=7)	cTnT-shHIPK1-AAV9+TAC (n=8)	cTnT-shHIPK1-AAV9+ cTnT-CREB-AAV9+TAC (n=10)
Heart rate (bpm)	490.9±32.3	506.1±34.1	492.8±23.0	492.4±30.5
EF (%)	37.85±6.00	30.84±4.33	51.5±6.77***	35.83±4.65$^{\#\#\#}$
FS (%)	17.98±3.19	14.32±2.20	25.97±4.06***	16.95±2.48$^{\#\#\#}$
LV Mass (mg)	195.1±35.7	201.9±34.8	128.9±38.2**	215.7±38.7$^{\#\#\#}$
LVPW;s (mm)	1.33±0.10	1.31±0.18	1.13±0.19	1.39±0.30
LVPW;d (mm)	1.13±0.13	1.14±0.17	0.84±0.15*	1.23±0.29$^{\#\#}$
LVAW;s (mm)	1.53±0.14	1.42±0.29	1.32±0.16	1.46±0.15
LVAW;d (mm)	1.26±0.16	1.19±0.23	0.93±0.16**	1.20±0.14$^{\#}$
Diameter;s (mm)	3.14±0.29	3.46±0.31	2.86±0.47	3.36±0.31$^{\#}$
Diameter;d (mm)	3.82±0.24	4.03±0.31	3.85±0.48	4.04±0.32
Volume;s (μL)	39.62±8.88	49.85±10.59	32.37±12.63	46.52±10.20$^{\#}$
Volume;d (μL)	63.19±9.46	71.84±13.08	65.20±19.47	72.13±13.49

BPM, beat per minute; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; LVPW;s, end-systolic LV posterior wall thickness;

LVPW;d, end-diastolic LV posterior wall thickness; LVAW;s, end-systolic LV anterior wall thickness; LVAW;d, end-diastolic LV anterior wall thickness; Diameter;s, end-systolic LV diameter; Diameter;d, end-diastolic LV diameter; Volume;s, end-systolic LV volume; Volume;d, end-diastolic LV volume. Data are presented as mean±SD. Data among 4 groups were compared by two-way ANOVA test followed by Tukey post hoc test. *, $P<0.05$ compared to corresponding Sham group; **, $P<0.01$ compared to corresponding Sham group; ***, $P<0.001$ compared to corresponding Sham group; #, $P<0.05$ compared to WT+TAC group; ##, $P<0.01$ compared to WT+TAC group; ###, $P<0.001$ compared to WT+TAC group.

Table S7. List of PCR primers

Gene	Primer sequence
rno-HIPK1-Forward	CAGCATCAGCCAATCATC
rno-HIPK1-Reverse	ATTAGACCTCGCCTTCAG
rno-cTnI-Forward	ATGGCGGATGAGAGCAG
rno-cTnI -Reverse	GTTTTCTGGAGGCGGAGA
rno-cTnT-Forward	GCCATCGACCACCTGAATGA
rno-cTnT-Reverse	TTTGGCCTTCCCACGAGTTT
rno-ANP-Forward	GAGCAAATCCCGTATACAGTGC
rno-ANP-Reverse	ATCTTCTACCGGCATCTTCTCC
rno-BNP-Forward	CTGCTTGCGGAGGCGAGAC
rno-BNP-Reverse	TGTTCTGGAGACTGGCTAGGACTTC
rno-β-MHC-Forward	TCCAGCTGAAAGCAGAAAGAG
rno-β-MHC-Reverse	CCTGGGAGATGAACGCATAAT
rno-Col1a1-Forward	GAGCGGAGAGTACTGGATCGA
rno-Col1a1-Reverse	TGCCATTGCTGGAGTTGGA
rno-Col3a1-Forward	TGCCATTGCTGGAGTTGGA
rno-Col3a1-Reverse	GAAGACATGATCTCCTCAGTGTTGA
rno-C/EBPβ-Forward	CAAGCTGAGCGACGAGTACAA
rno-C/EBPβ-Reverse	ACAGCTGCTCCACCTTCTTCT
rno-CREB-Forward	TTCCACTTCTGCCCTCAC
rno-CREB-Reverse	GGTCTCCTCATGGTTCCTG
rno-skeletal α-actinin-Forward	GGTTATGCCCTGCCACA
rno-skeletal α-actinin-Reverse	GCGCACAATCTCACGTTC
rno-cardiac α-actinin-Forward	CCAACCGTGAGAAGATGAC
rno-cardiac α-actinin-Reverse	GTCGCCAGAATCCAGAAC
rno-α-MHC-Forward	CAGCTCAGTCAGGCCAATAGAA
rno-α-MHC-Reverse	CGTTCCTACACTCCTGCACC
rno-SERCA2a-Forward	TGGCAGCAGACTTAGGAAA
rno-SERCA2a-Reverse	AGGGAGTGGCAAACCAA
mmu-HIPK1-Forward	AATCTTCCTGCTTACGGAC
mmu-HIPK1-Reverse	CTCACTCTTTCTCTTCAATCC
mmu-ANP-Forward	AGCCGTTCGAGAACTTGTCTT
mmu-ANP-Reverse	CAGGTTATTGCCACTTAGGTTCA
mmu-BNP-Forward	GAGTCCTTCGGTCTCAAGGC
mmu-BNP-Reverse	TACAGCCCAAACGACTGACG
mmu-β-MHC-Forward	CTTCAACCACCACATGTTCG
mmu-β-MHC-Reverse	TCTCGATGAGGTCAATGCAG
mmu-Col1a1-Forward	CAGAGGCGAAGGCAACA
mmu-Col1a1-Reverse	GTCCAAGGGAGCCACATC
mmu-Col3a1-Forward	AGAACCTGGCCGAGATG
mmu-Col3a1-Reverse	TGGACTTCCGGGCATAC
mmu-CTGF-Forward	TAAGACCTGTGGGATGGG
mmu-CTGF-Reverse	GCAGCCAGAAAGCTCAA
mmu-C/EBPβ-Forward	GCCAACTTCTACTACGAGCCC

mmu-C/EBPβ-Reverse	ACTCGTCGCTCAGCTTGTC
mmu-CREB-Forward	AAAACTCCTAAACCCCAAGC
mmu-CREB-Reverse	GCCTACCATCAAAACACATCT
mmu-skeletal α-actinin-Forward	AAGACGAGACCACCGCTCT
mmu-skeletal α-actinin-Reverse	ACACCGTCCCCAGAATCCAAC
mmu-cardiac α-actinin-Forward	ATGTGTGACGACGAGGAGAC
mmu-cardiac α-actinin-Reverse	GCCATGTTCAATGGGGTACT
mmu-α-MHC-Forward	CAGAGAACGGGGAGTTGG
mmu-α-MHC-Reverse	CTTGGCCTTGCCTTCCT
mmu-SERCA2a-Forward	GGCAGGATGAGGATGTGA
mmu-SERCA2a-Reverse	CTGGGCTGAAGGGCTTA
rno/mmu-18s-Forward	TCAAGAACGAAAGTCGGAGG
rno/mmu-18s-Reverse	GGACATCTAAGGGCATCAC