Supplementary Material and Methods

Plasmids and reagents.

Primary antibodies against cTnI3 (abcam, *Cat#: ab47003*), EGFR (abcam, *Cat#: ab52894*), SCD1 (abcam, Cat#: ab236868), BNP (abcam, Cat#: ab236101), MYH7 (abcam, Cat#: ab50967), PPAR gamma (abcam, Cat#: ab178860), ATP citrate lyase kits (abcam, Cat#: ab40793), Akt (phospho T308) (abcam, Cat#: ab38449) were obtained from Abcam (Cambridge, USA). The specific antibodies to HADHB (Proteintech, Cat#: 29091-1-AP), ACAA2 (Proteintech, Cat#: 11111-1-AP), ACOX1 (Proteintech, Cat#:10957-1-AP), PPARA (Proteintech, Cat#: 15540-1-AP), ANP (Proteintech, Cat#:27426-1-AP), FASN (Proteintech, *Cat#: 66591-1-IG*), Akt (Proteintech, Cat#60203-2-Ig) were purchased from Proteintech Technology (Chicago, IL, USA). Protein A/G beads were obtained from Abmart (Shanghai, China). The FASN inhibitor, C75 were purchased from Selleck Chemicals (Houston, Texas, USA). The other reagents were derived from commercial sources.

Co-immunoprecipitation (co-IP), and western blot.

Co-IP, and western blot assays were performed as described[1]. All the western blot quantification were done according to the Image Lab Software user guide version 6.0, which was provided by Bio-Rad company. Accurate quantitation using the linear dynamic range, which was published[2]. Different bands were normalized to the same internal control, we chose GAPDH as an internal control.

Doppler echocardiography

The echocardiographic examination was briefly described as follows. After anaesthetizing the mouse with 1% isoflurane at a flow of 1.0 L/min, the mouse was

placed supine on the platform for continuous 1% isoflurane. A Vevo2100 system equipped with a 17 MHz transducer (VisualSonics, Toronto, ON, Canada) was used to acquire the long-axis, short-axis, and M-mode views of a two-dimensional echocardiogram.

Histopathological analysis

The mice were euthanized, their hearts were quickly removed, and the heart tissue was washed to remove residual blood, and then fixed with 4% paraformaldehyde for 24-48 hours. The left ventricular myocardium was prepared into 2- or 4-millimetre-thick sections by conventional procedures, and the sections were then embedded in paraffin and stained with wheat germ agglutinin-fluorescein isothiocyanate (WGA) staining, or hematoxylin and eosin (HE) or Masson's trichrome staining.

qRT-PCR, and GST pull-down assays

qRT-PCR and GST pull-down assays were performed using standard methods[3]. **(Supplementary Table 4)**.

Trizol reagent was used to extract total RNA from mouse heart tissue and a quantitative real-time PCR assay was performed to analyze the mRNA levels of various proteins in cardiomyocytes.

The GST pull-down assay was performed as follows. Glutathione Sepharose 4B beads (Sigma) were incubated with GST and GST-cTnI (with or without the R186Q mutation) and His-EGFR in an incubation buffer at 4 °C overnight. The protein was eluted with buffer (50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0), and SDS

buffer was added to the eluted protein and heated at 95 °C for 5 minutes. Finally, SDS-PAGE analyze was used to perform Western blot analysis.

References

[1] R. Wan, Y. Liu, L. Li, C. Zhu, L. Jin, S. Li, Urocortin Increased Endothelial ICAM1 by cPLA2-dependent NF-kappaB and PKA Pathways in HUVECs, J. Mol. Endocrinol., 52 (2014) 43-53. https://doi.org/10.1530/JME-13-0182.

[2] S. C. Taylor, T. Berkelman, G. Yadav, M. Hammond, A Defined Methodology for Reliable Quantification of Western Blot Data, Mol. Biotechnol., 55 (2013) 217-226. https://doi.org/10.1007/s12033-013-9672-6.

[3] Q. Zhou, X. Peng, X. Liu, L. Chen, Q. Xiong, Y. Shen, J. Xie, Z. Xu, L. Huang, J. Hu, R. Wan, K. Hong, FAT10 Attenuates Hypoxia-Induced Cardiomyocyte Apoptosis by Stabilizing Caveolin-3, J. Mol. Cell. Cardiol., 116 (2018) 115-124. https://doi.org/10.1016/j.yjmcc.2018.02.008.

Table S1. Databases used for *TNNI3 p.R186Q* mutation annotation

Databases	URLs	Classification used in analysis
SIFT	http://sift.jcvi.org	T
Polyphen2	http://genetics.bwh.harvard.edu/pph2	B
LRT	http://www.genetics.wustl.edu/jflab/lrt_query.html	Damaging
MutationTaser	http:// www.mutationtaster.org	Damaging
MutationAssessor	http://mutationassessor.org	L
FATHMM	http://fathmm.biocompute.org.uk	Damaging
PROVEAN	http://provean.jcvi.org/index.php	Neutral
MetaLR	https://wglab.org/members/15-member-detail/36-coco-dong	Damaging
M-CAP	http://bejerano.stanford.edu/mcap/	Damaging
fathmm-MKL	http://fathmm.biocompute.org.uk/fathmmMKL.htm	Damaging

Abbreviations: FATHMM= Functional Analysis through Hidden Markov Models Ver.2.3, M-CAP = Mendelian Clinically Applicable Pathogenicity, LRT = likelihood ratio test, SIFT = Sorting Intolerant from Tolerant, PROVEAN =Protein Variation Effect Analyzer;

Table S2. Parameters of heart function and structure detected by Echocardiography of *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice

ECHO	*Tnni3*$^{+/+}$ (*n* = 8)	*Tnni3*$^{R186Q/+}$ (*n* = 8)
LVIDd (mm)	3.92 ± 0.26	3.62 ± 0.23
LVIDs (mm)	2.46 ± 0.29	2.36 ± 0.40
IVSd (mm)	0.80 ± 0.06	0.94 ± 0.13
IVSs (mm)	1.28 ± 0.08	1.43 ± 0.23
LVPWd (mm)	0.86 ± 0.02	0.96 ± 0.15
LVPWs (mm)	1.18 ± 0.11	1.35 ± 0.20
EF (%)	67.91 ± 4.11	68.80 ± 8.30
FS (%)	37.39 ± 3.17	38.15 ± 6.27

Abbreviations: ECHO = echocardiography, LVIDd = left ventricular internal diameter at diastolic phase, LVIDs = left ventricular internal diameter at systolic phase, IVSd = interventricular septal thickness at diastolic phase, IVSs = interventricular septal thickness at systolic phase, LVPWd = left ventricular posterior wall thickness at diastolic phase, LVPWs = left ventricular posterior wall thickness at systolic phase, EF = ejection fraction, FS = left ventricular fractional shortening. **P* < 0.05 Tnni3$^{R186Q/+}$ *vs.* Tnni3$^{+/+}$.

Table S3. Parameters of heart function and structure detected by Echocardiography *Tnni3*$^{+/+}$, *Tnni3*$^{R186Q/R186Q}$ and *Tnni3*$^{R186Q/R186Q}$+ C75 mice

Parameters	*Tnni3*$^{+/+}$ (*n* = 4)	*Tnni3*$^{R186Q/R186Q}$ (*n* = 4)	*Tnni3*$^{R186Q/R186Q}$ + C75 (*n* = 4)
LVIDd(mm)	3.85 ± 0.13	3.23 ± 0.16***	3.28 ± 0.12
LVIDs(mm)	2.51 ± 0.32	1.95 ± 0.11*	2.01 ± 0.33
IVSd(mm)	0.83 ± 0.03	1.00 ± 0.07*	0.98 ± 0.02
IVSs(mm)	1.31 ± 0.15	1.53 ± 0.16*	1.50 ± 0.05
LVPWd(mm)	0.96 ± 0.20	1.10 ± 0.24	0.99 ± 0.13
LVPWs(mm)	1.21 ± 0.14	1.41 ± 0.08*	1.48 ± 0.03
EF(%)	65.25 ± 3.30	71.25 ± 2.87	70.19 ± 2.16
FS(%)	35.75 ± 7.54	38.76 ± 7.36	37.34 ± 4.11

Abbreviations: ECHO: echocardiography; LVIDd: left ventricular internal diameter at diastolic phase; LVIDs: left ventricular internal diameter at systolic phase; IVSd: interventricular septal thickness at diastolic phase; IVSs: interventricular septal thickness at systolic phase; LVPWd: left ventricular posterior wall thickness at diastolic phase; LVPWs: left ventricular posterior wall thickness at systolic phase; EF: ejection fraction; FS: left ventricular fractional shortening. *$P < 0.05$ ***Tnni3***$^{R186Q/R186Q}$ *vs.* ***Tnni3***$^{+/+}$; ***$P<0.001$ ***Tnni3***$^{R186Q/R186Q}$ *vs.* ***Tnni3***$^{+/+}$.

Table S4. Lists of mRNA primer sequence

Gene	Primer sequence
TNNI3	Forward: GACACCCTTCTAAGACCCTCC
	Reverse: CAGCCCATCCAACACCAA
MYH7	Forward: ATGCTGTCCGTGCCAATG
	Reverse: CCTTCTCCTCTGCGTTCCTA
ANP	Forward: GGGCTTCTTCCTCGTCTT
	Reverse: CTTCTCCTCCAGGTGGTCTA
BNP	Forward: GAGGCGAGACAAGGGAGA
	Reverse: AGCGGTGACAGATAAAGGAA
Acly	Forward: CATCATTGGAGGCAGCAT
	Reverse: GCCACATTGGTGAAGTTTG
Fasn	Forward: GCAGCAAGTGTCCACCAAC
	Reverse: ATCCCTGAGCAGATGAACCA
SCD	Forward: ATCATACTGGTTCCCTCCTG
	Reverse: CGTGCCTTGTAAGTTCTGTG
PPAR-γ	Forward: ACCACTACGGAGTTCACG
	Reverse: CCCTTACAGCCTTCACAT
ACOX1	Forward: TTTTCAGACGCAGCAGTAT
	Reverse: GGAGCGGGAAGAGTTTAT
ACAA2	Forward: GCTAACGAGGCTGGCTAC
	Reverse: GACTGTCCCGTCTTTCTTG

	Reverse: GCCAGTTCTGCCACCTCT
	Reverse: GTTGGCCCAGATTTCGTTCA
HADHB	Forward: TCGGGTTTGTTGCATCGGA
	Reverse: GGCCAGAAGCTATCAGACCAA
PPARa	Forward: AACATCGAGTGTCGAATATGTGG
	Reverse: CCGAATAGTTCGCCGAAAGAA
GAPDH	Forward: TGTTTCCTCGTCCCGTAG
	Reverse: CAATCTCCACTTTGCCACT

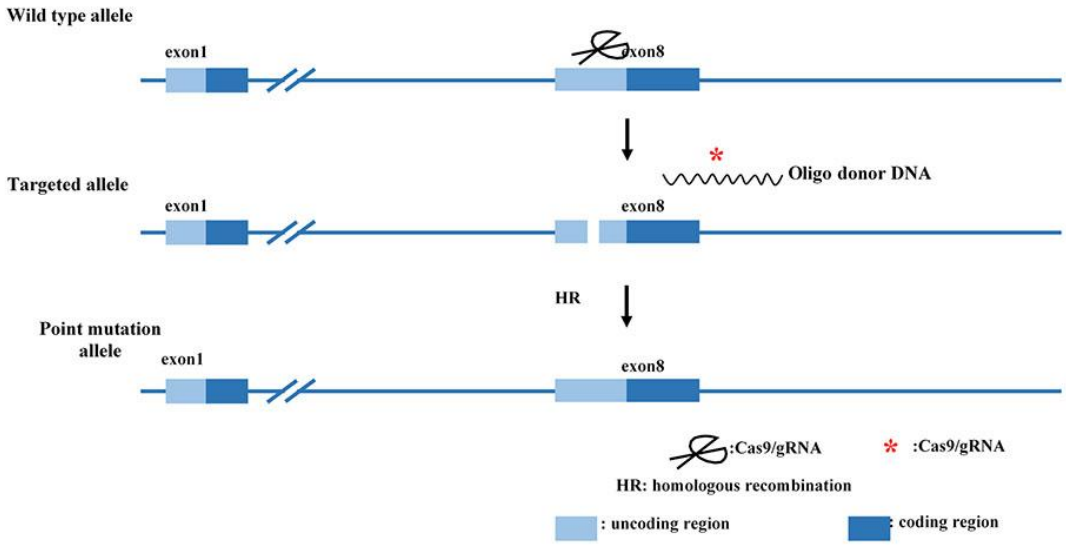

Figure S1. Schematic diagram illustrating the targeting vector and stepwise generation of the *Tnni3*$^{R186Q/R186Q}$ *and Tnni3*$^{+/+}$ mouse model.

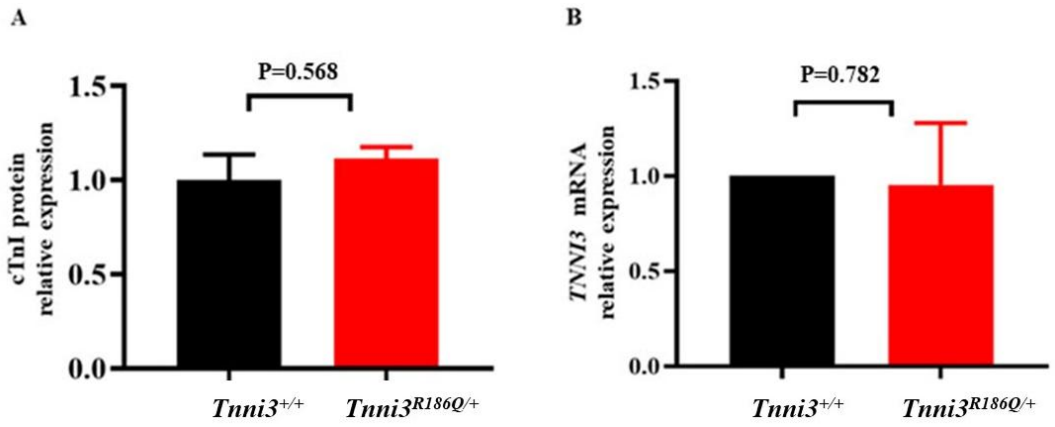

Figure S2. The mRNA and protein expression of *TNNI3* are *not changed* in *Tnni3*$^{R186Q/+}$ mice.

A. Quantitative assessment of cTnI protein expression levels in littermates of *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice ventricular myocardium (n = 3 per group). **B.** Relative cTnI mRNA expression in littermates of *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice ventricular myocardium (n = 3 per group). Ns = not significant. The statistical test was done with unpaired T-test. GAPDH was used as the loading control.

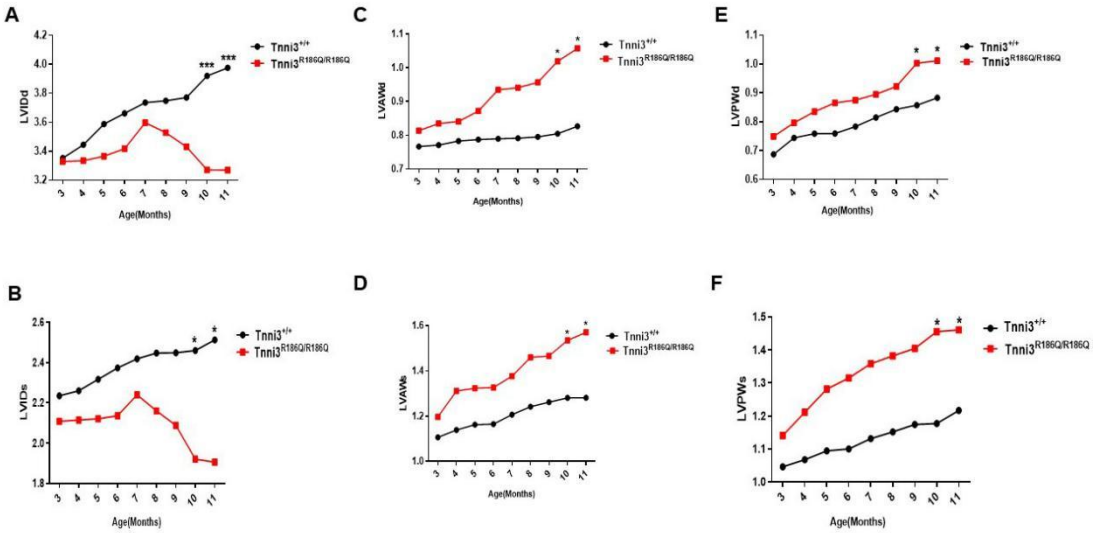

Figure S3. The monthly parameters of heart function and structure detected by Echocardiography of *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/R186Q}$ mice

A. The monthly parameters of LVIDd for *Tnni3*$^{+/+}$ (black) and *Tnni3*$^{R186Q/R186Q}$ (red) mice (n = 8 per group). **B.** The monthly parameters of LVIDs for *Tnni3*$^{+/+}$ (black) and *Tnni3*$^{R186Q/R186Q}$ (red) mice (n = 8 per group). **C.** The monthly parameters of LVAWd for *Tnni3*$^{+/+}$ (black) and *Tnni3*$^{R186Q/R186Q}$ (red) mice (n = 8 per group). **D.** The monthly parameters of LVAWs for *Tnni3*$^{+/+}$ (black) and *Tnni3*$^{R186Q/R186Q}$ (red) mice (n = 8 per group). **E.** The monthly parameters of LVPWd for *Tnni3*$^{+/+}$ (black) and *Tnni3*$^{R186Q/R186Q}$ (red) mice (n = 8 per group). **F.** The monthly parameters of LVPWs for *Tnni3*$^{+/+}$ (black) and *Tnni3*$^{R186Q/R186Q}$ (red) mice (n = 8 per group). The statistical test was done with unpaired T-test.

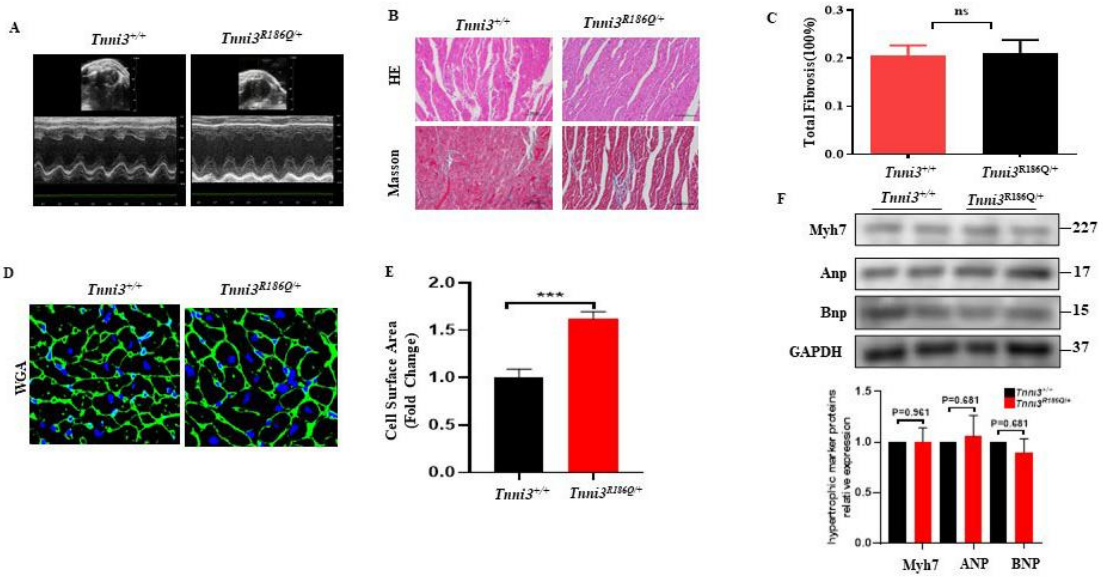

Figure S4. No HCM phenotype is observed in *Tnni3*$^{R186Q/+}$ mice

A. Representative M-mode echocardiographic images of *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice. **B.** HE (pink) and Masson's trichrome staining (red) of *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice ventricular myocardium (cross section). With HE staining (400x), blue represents the nucleus, and pink represents the cytoplasm. With Masson's trichrome staining (400x), the collagen fiber is shown in blue and cardiomyocytes are in red. Scale bar 100 μm. **C.** Quantification of total fibrosis by Masson's trichrome staining in *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice ventricular myocardium (n = 3 per group). **D.** Representative images of WGA staining in *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice hearts, showing the CM cross-sectional area. Scale bar 20 μm. **E.** Quantitative data of CM hypertrophy assessed by cross-sectional area for WGA staining in *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice ventricular myocardium (n = 3 per group with 100 CMs analyzed). **F.** Western blot analysis of the hypertrophic markers MYH7, ANP and BNP in whole heart tissue of *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/+}$ mice (n = 3 per group). The statistical test was done with unpaired T-test. GAPDH was used as the loading control.

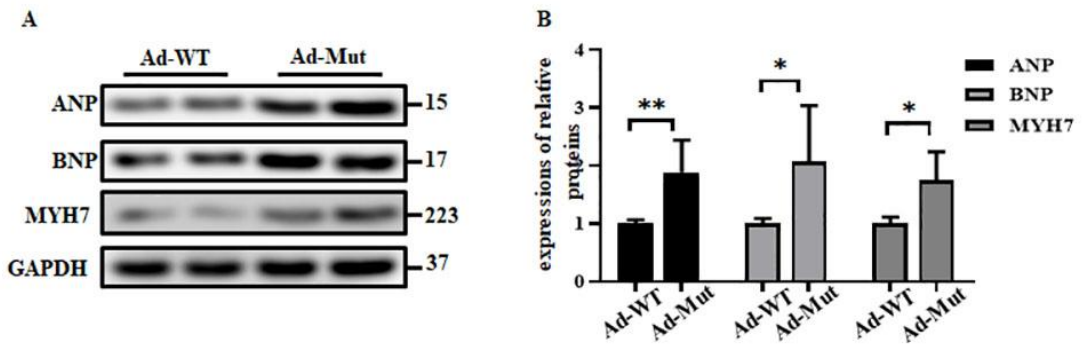

Figure S5. The *TNNI3*-R186Q mutation promotes cardiac hypertrophy *in vitro*.

A. Western blot analysis of the hypertrophic markers MYH7, ANP and BNP in NRCMs of the Ad-WT, Ad-Mut groups (n = 3 per group). The statistical test was done with unpaired T-test. GAPDH was used as the loading control. **B.** Quantitative data of A. *P < 0.05, **P < 0.01, ***P < 0.001 and ns = not significant.

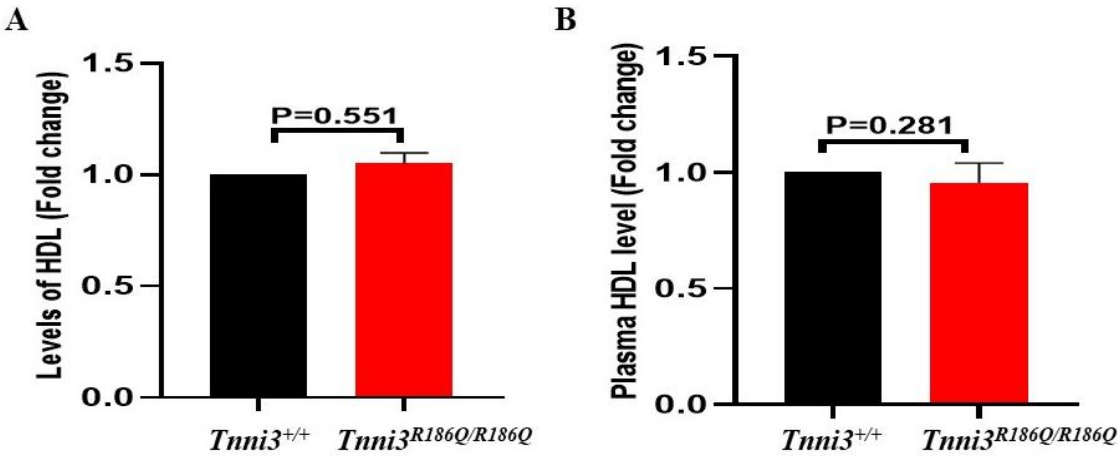

Figure S6. No differences are observed in the content of HDL between *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/R186Q}$ mice from heart tissue and serum.

A. The level of HDL was individually measured using enzyme-linked immunosorbent assay in heart tissues from *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/R186Q}$ mice (n = 3 per group). **B.** The level of HDL was individually measured using enzyme-linked immunosorbent assay in the serum of fasting mice from *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/R186Q}$ mice (n = 3 per group). ns = not significant. The statistical test was done with unpaired T-test.

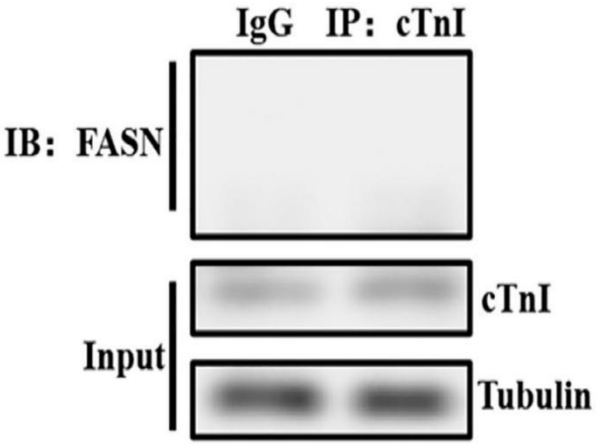

Figure S7. FASN does not bind with cTnI. Co-IP for endogenous FASN and cTnI in cultured cardiomyocytes of Ad-WT and Ad-Mut groups.

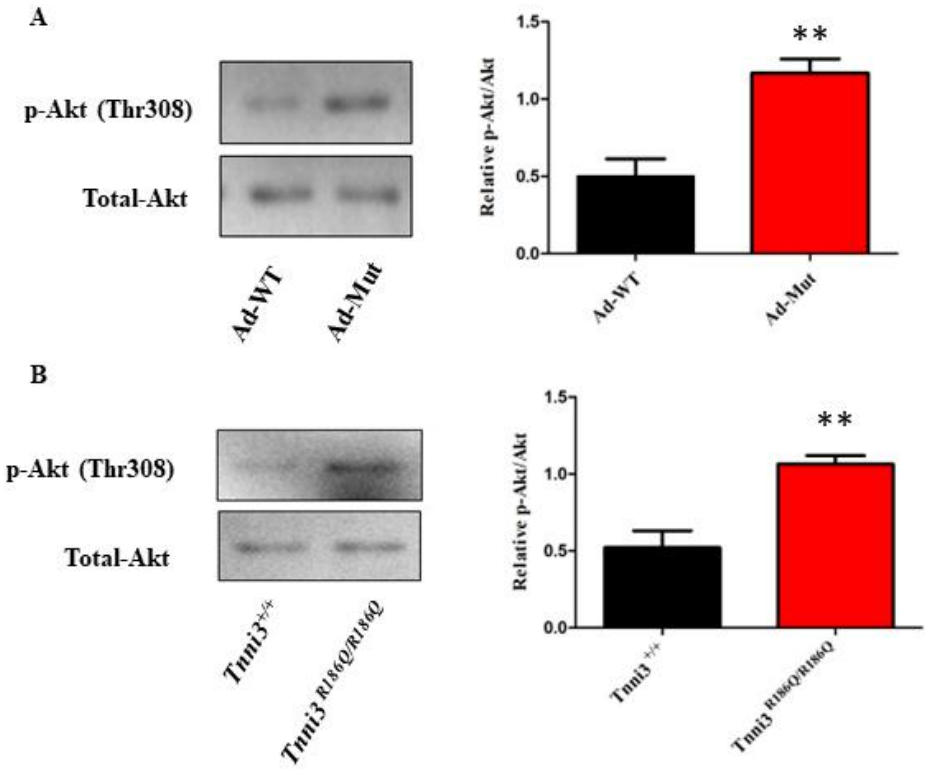

Figure S8. TNNI3 p.R168Q mutation activated EGFR downstream Akt phosphorylation at Thr308.

A. Western blot analysis of Akt phosphorylation at Thr308 in NRCMs of the Ad-WT, Ad-Mut groups (n = 4 per group). **B.** The phosphorylation of Akt at Thr308 levels were measured by Western blot in *Tnni3*$^{+/+}$ and *Tnni3*$^{R186Q/R186Q}$ mice (n = 5 per group).

Abbreviations:

cTnI	cardiac troponin I
HCM	hypertrophic cardiomyopathy
KI	knock-in
NRCMs	neonatal rat cardiomyocytes
EGFR	epidermal growth factor receptor
FASN	fatty acid synthase
MYH7	β-myosin heavy chain
MYBPC3	cardiac myosin binding protein C3
TNNT2	Troponin T2
TNNI3	Troponin I3
TPM1	Tropomyosin 1
FAs	fatty acids
WGA	Wheat germ agglutinin-fluorescein isothiocyanate-staining
HE	hematoxylin and eosin
SEM	standard error of the mean
LVPW	left ventricular posterior wall
LVID	left ventricular internal dimension
CM	cardiac myocyte
TC	total cholesterol
TG	total triglycerides
HDL	high-density lipoprotein
LDL	low-density lipoprotein