SUPPLEMENTAL MATERIALS

Numbers of figures: eight; Numbers of tables: one.

Supplemental Methods

Quantitative real-time PCR

Total RNA was isolated from fresh mouse hearts or cultured cardiac fibroblasts using the TRIzol method (Ambion, Life Technologies, USA). RNA (1 μg) was reverse-transcribed into first-strand cDNA using a reverse transcription kit (Promega, USA). Quantitation of mRNA levels was performed via real-time PCR using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Mouse GAPDH was used as a reference standard. The experiment is undertaken in duplicate, this technical replicate were used to ensure the reliability of single values. Reactions were heated to 95°C for 2 min followed by 40 cycles at 95°C for 15 sec, 55°C for 15 sec and 72°C for 30 sec. The primer sequences are listed in Table S1.

In vivo HNF4α siRNA sequence

Chemically modified anti-HNF4α siRNA and negative control (NC) siRNA were synthesized by GenePharma Co., Ltd. (Shanghai, China).

HNF4α-mus-1316: sense 5'-3': CCAAUGUCAUUGUUGCUAAdTdT, antisense 5'-3': UUAGCAACAAUGACAUUGGdTdT.

HNF4α-mus-1512: sense 5'-3': CAACGAUCACCAAGCAAGAdTdT, antisense 5'-3': UCUUGCUUGGUGAUCGUUGdGdC.

For use of HNF4α siRNA, equal amounts of HNF4α-mus-1316 and HNF4α-mus-1512 were mixed before injection.

Reverse transcription PCR (RT-PCR)

Total RNA was extracted from tissues with TRIzol and 1 μg RNA was reverse-transcribed using oligo-dT in a total volume of 20 μL, and 0.5μL was used for RT-PCR. The amplification conditions were 95 °C, 30 s; 58 °C, 30 s; 68 °C, 30 s for 35 cycles. The primer sequences are listed in Table S1. P1 derived HNF4α transcript is detected by HNF4α α2 variant, and P2 derived HNF4α transcript is detected by

HNF4α α10-12 variant.

Table S1. Primer sequences used in PCR analysis

Gene name	Primer sequences
Primers used for real-time PCR	
TGFβ1	Forward: 5'-CTCCCGTGGCTTCTAGTGC-3'
	Reverse: 5'-GCCTTAGTTTGGACAGGATCTG-3'
Collagen I	Forward: 5'-GTAACTTCGTGCCTAGCAACA-3'
	Reverse: 5'-CCTTTGTCAGAATACTGAGCAGC-3'
Collagen III	Forward: 5'-ACGTAGATGAATTGGGATGCAG-3'
	Reverse: 5'-GGGTTGGGGCAGTCTAGTG-3'
GAPDH	Forward: 5'-TCCTGGTATGACAATGAATACGGC-3'
	Reverse: 5'-TCTTGCTCAGTGTCCTTGCTGG-3'
Primers used for ChIP	
TGFβ1	Forward: 5'-CGAGGCCAGACTTGACTTGA-3'
	Reverse: 5'-TGCTCAAAACCCTCCCATGA-3'

Supplemental figures and figure legends

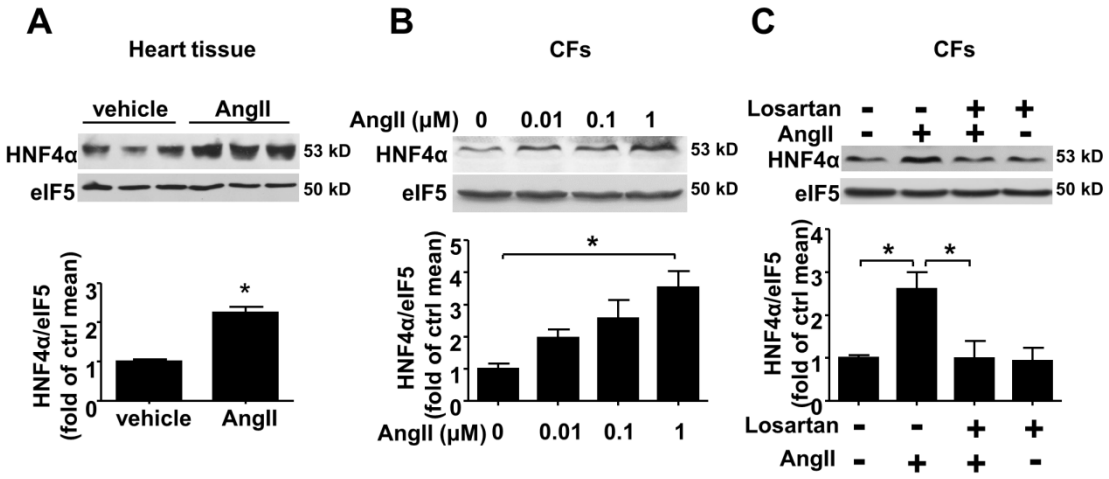

Fig. S1. AngII increased the HNF4α protein levels via AT1 receptor in cardiac fibroblasts. (A) Western blot analysis of HNF4α expression in hearts treated with saline or AngII for 7 days. n=8. (B) CFs were treated with AngII at a dose of 0.01 μM, 0.1 μM or 1 μM for 24 h, and HNF4α protein expression was then determined via western blot analysis, n=5. (C) Western blot analysis of HNF4α expression in CFs treated with AngII and/or AT1 receptor antagonist losartan (1 μM), n=5. *P <0.05, Student's unpaired two-tailed t-test was used (A), One-way ANOVA with the Bonferroni post hoc test was used (B), and Two-way ANOVA with the Bonferroni post hoc test was used (C).

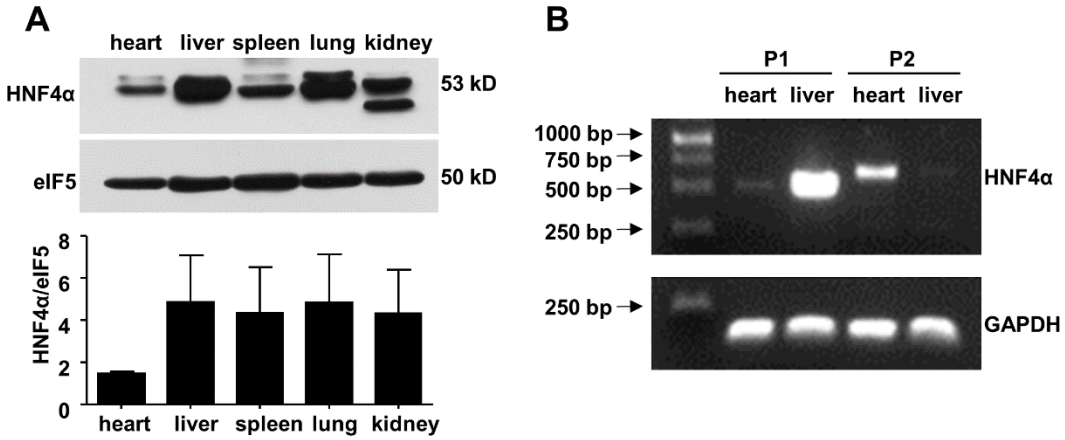

Fig. S2. HNF4α is differentially expressed in the heart and other organs. (A) Western blot analysis of HNF4α in different organs. n = 6. Kruskal–Wallis ANOVA

with post-hoc Dunn's multiple comparison test was used. (B) Different expression of HNF4α variants derived from P1 or P2 promoters in heart and liver via RT-PCR.

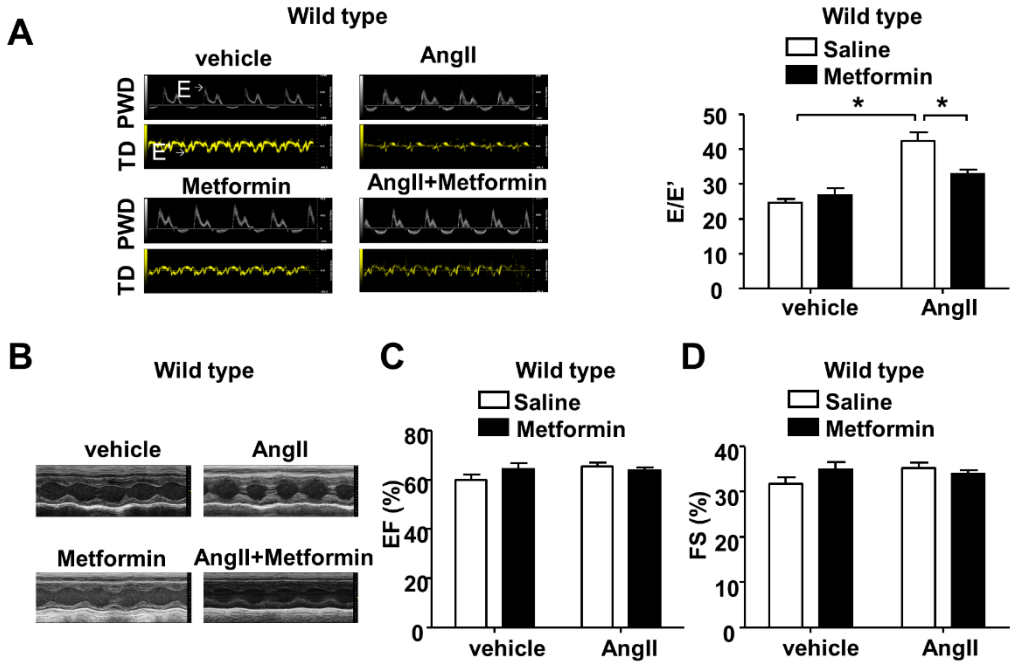

Fig. S3. Metformin improved cardiac diastolic function upon AngII exposure in wild type mice. (A) Left panel: Representative PWD images showing the mitral flow and TD images of the mitral valve ring on the 7th day of AngII infusion in wild type mice. Right panel: E/E'. (B) Representative echocardiograms on the 7th day of AngII or saline infusion in wild type mice. (C) Left ventricular ejection fraction (EF%) on the 7th day of AngII infusion in wild type mice. (D) Left ventricular fractional shortening (FS%) on the 7th day of AngII infusion in wild type mice. Data are expressed as means±SEM of 8 mice per group. *P <0.05, NS=not significant. Two-way ANOVA with the Bonferroni post-hoc test (A) or two-way ANOVA (C and D) was used.

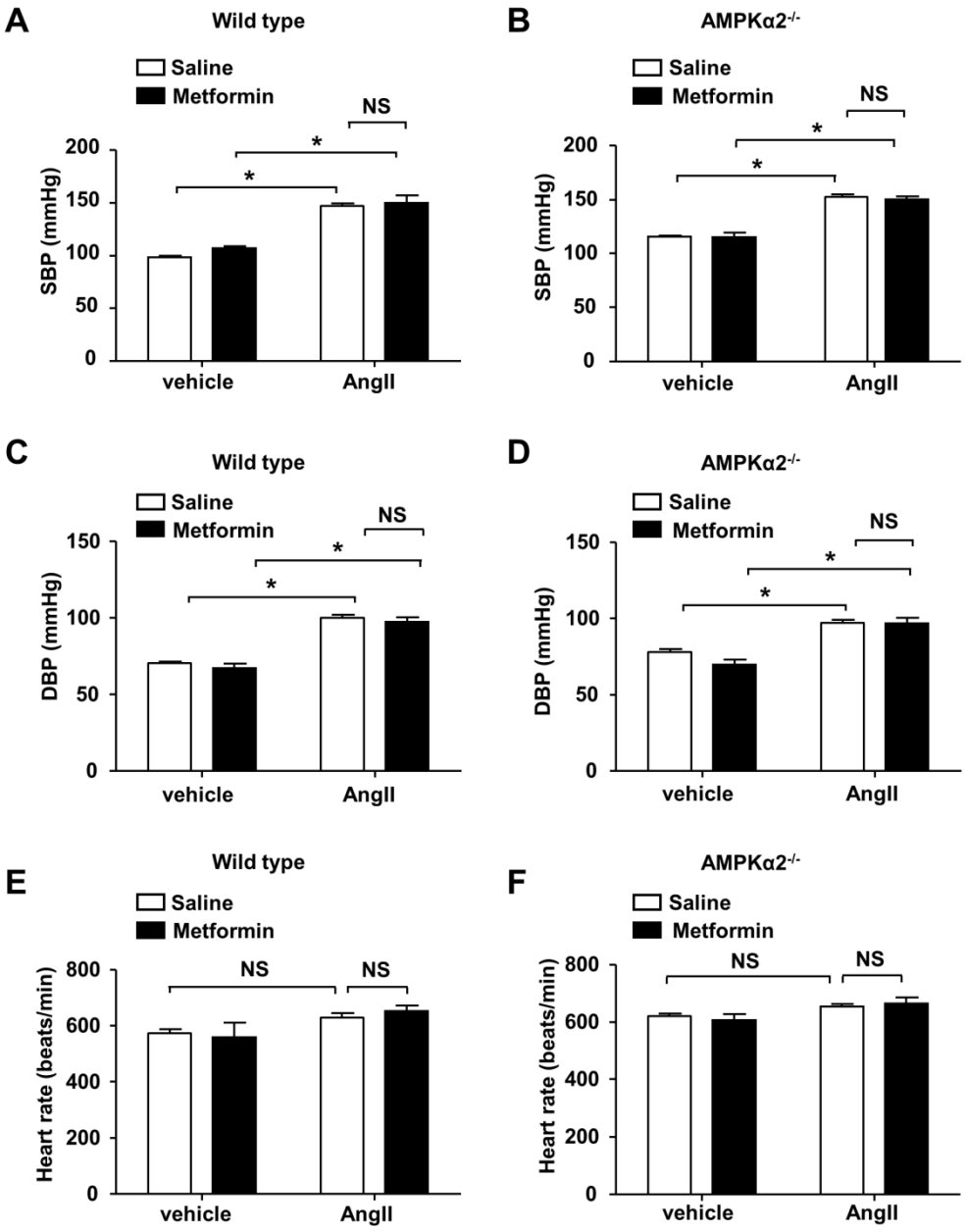

Fig. S4. Metformin does not change blood pressure or heart rate in either wild type or AMPKα2$^{-/-}$ mice. Systolic blood pressure (SBP) of (A) wild type mice and (B) AMPKα2$^{-/-}$ mice after 7 days of AngII infusion. (C) Diastolic blood pressure (DBP) of wild type mice and (D) AMPKα2$^{-/-}$ mice after 7 days of AngII infusion. (E) Heart rate of wild type mice after 7 days of AngII infusion. (F) Heart rate of AMPKα2$^{-/-}$ mice after 7 days of AngII infusion. n = 8. Data are expressed as means±SEM. *P <0.05, NS=not significant. Two-way ANOVA with the Bonferroni post hoc test was used (C and D). Welch's ANOVA with post hoc Games-Howell test was used for the other panels.

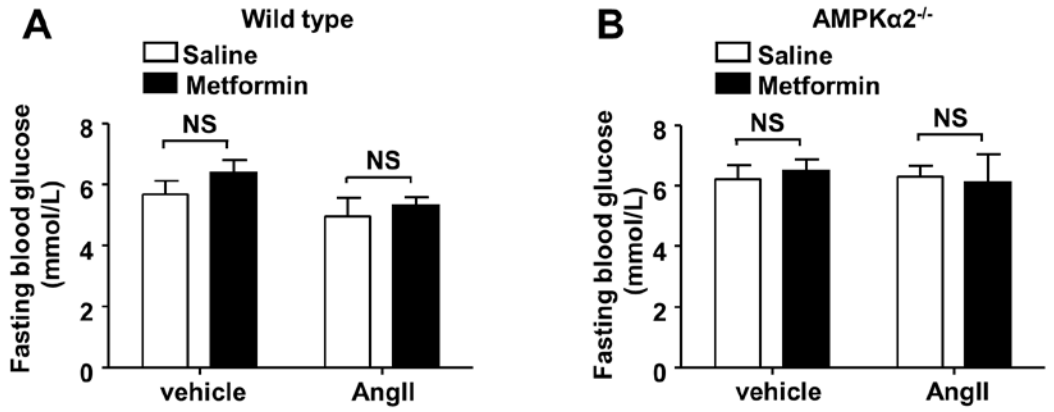

Fig. S5. Metformin has no effect on fasting blood glucose levels in wild type or AMPKα2$^{-/-}$ mice. (A) Fasting blood glucose levels of wild type mice after 7 days of AngII infusion. (B) Fasting blood glucose levels of AMPKα2$^{-/-}$ mice after 7 days of AngII infusion. n = 7. Data are expressed as means±SEM. NS = not significant. Two-way ANOVA was used.

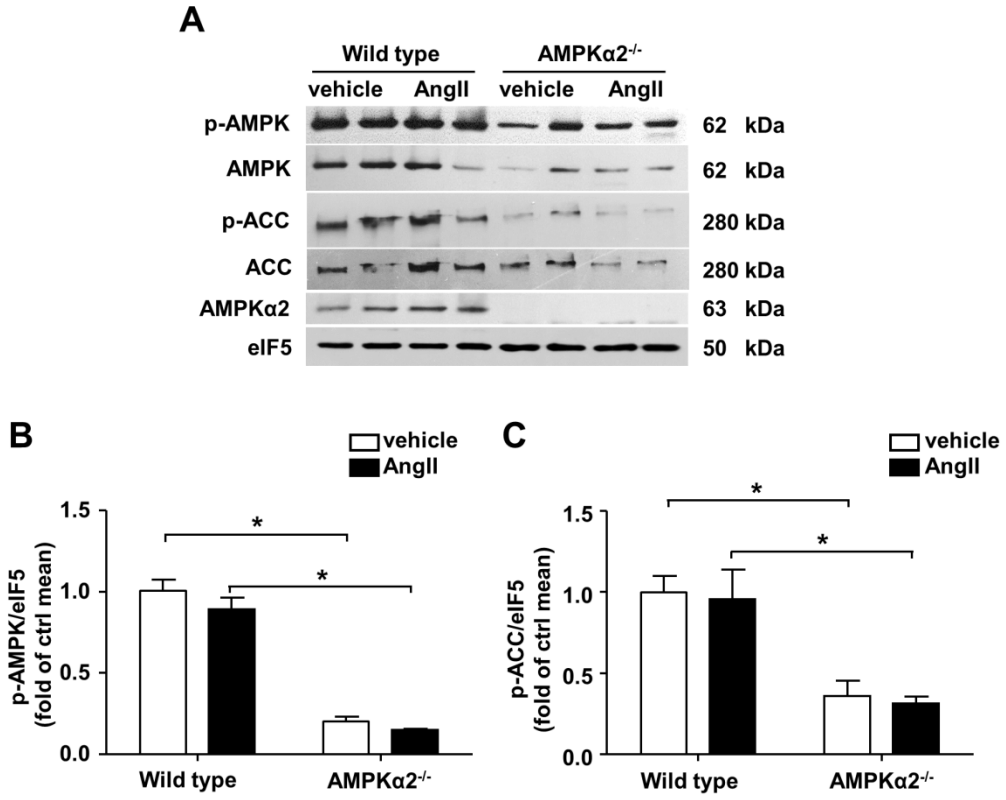

Fig. S6. Myocardial AMPK activity was decreased in AMPKα2$^{-/-}$ mice. (A) Western blot analysis of p-AMPK, AMPK, p-ACC, ACC and AMPKα2 in wild type and AMPKα2$^{-/-}$ mice after 7 days of AngII infusion. (B) Quantification of the p-AMPK levels shown in (A). (C) Quantification of the p-ACC levels shown in (A), n=8. Data are expressed as means±SEM. *P <0.05. Welch's ANOVA with post hoc Games-Howell test was used.

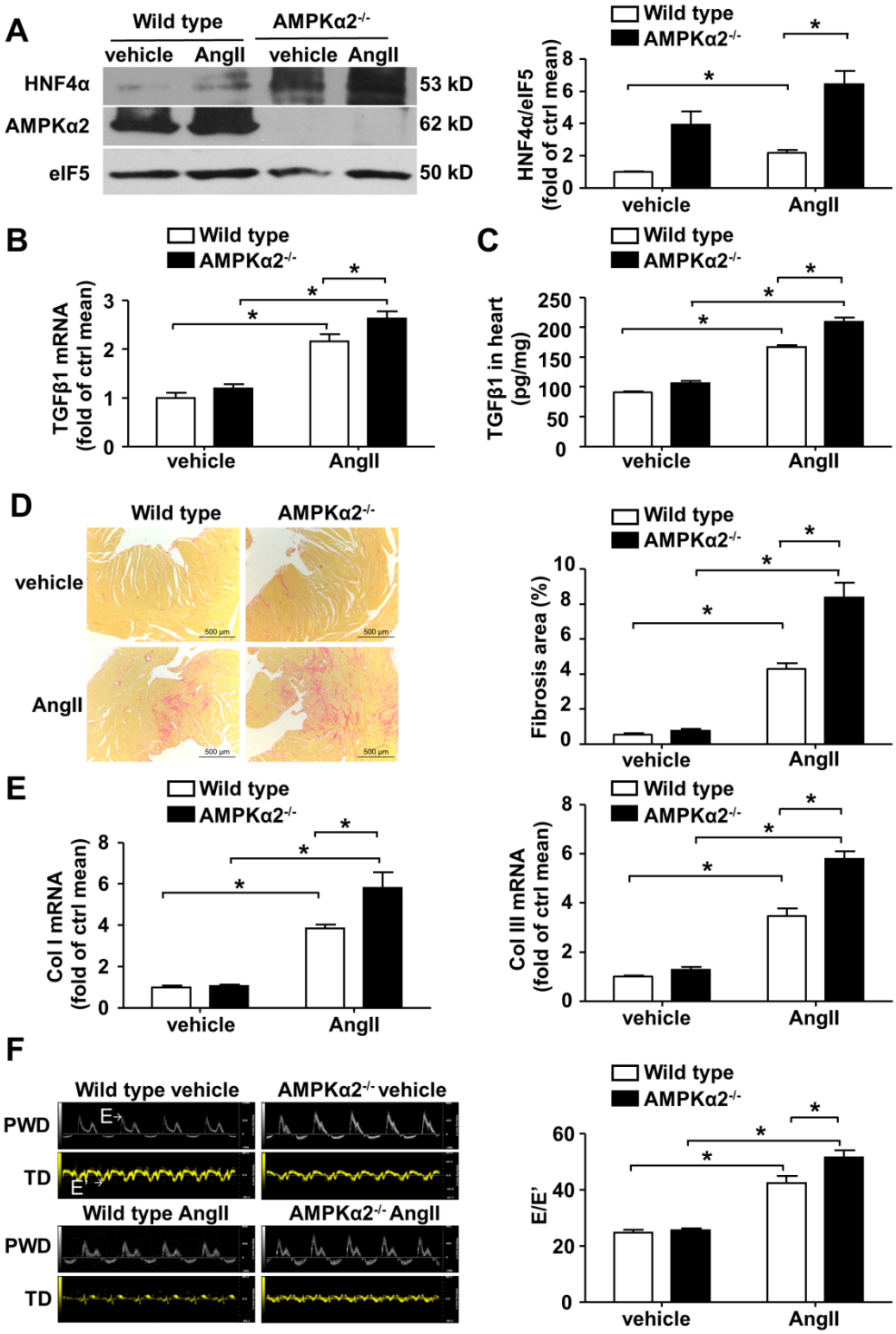

Fig. S7. AMPKα2 knockout exacerbates AngII-induced HNF4α expression, TGFβ1 expression and cardiac fibrosis. (A) Left panel: western blot analysis of HNF4α expression in the heart. The right panel shows quantification of the HNF4α protein levels. (B) Quantitative real-time PCR analysis of TGFβ1 mRNA expression in heart lysates. (C) The TGFβ1 protein level was determined via ELISA. (D) Left

panel: representative micrographs of Sirius red-stained heart sections; the red area represents collagen. Bars = 500 μm. Right panel：Quantification of the fibrotic area is expressed as the percentage of the total cardiac area. (E) Collagen I (left panel) and collagen III (right panel) mRNA expression was measured via real-time PCR analysis. (F) Left panel: representative pulsed wave Doppler (PWD) images across the mitral flow and tissue Doppler (TD) images of the mitral valve ring on the 7th day of AngII infusion in wild type mice. Right panel: E/E'. The E wave and E' wave are indicated by arrows. Data are expressed as means±SEM of 8 mice per group. *P <0.05. Two-way ANOVA with the Bonferroni post hoc test was used (B and C). Welch's ANOVA with post hoc Games-Howell test was used for the other panels.

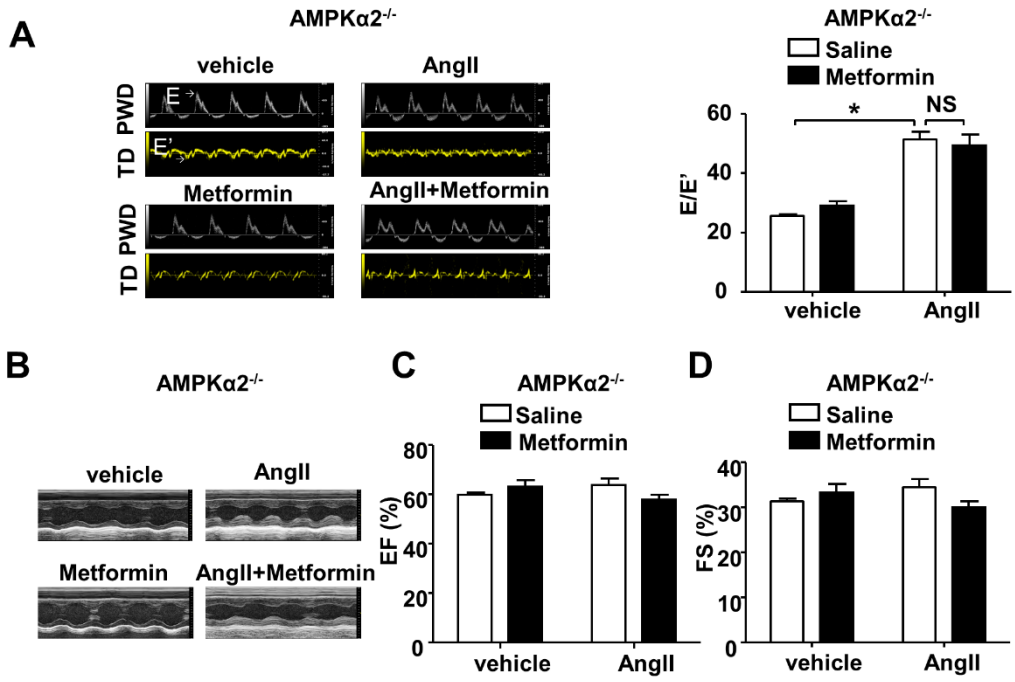

Fig. S8. Metformin did not improve cardiac diastolic function upon AngII exposure in AMPKα2$^{-/-}$ mice. (A) Left panel: Representative PWD images showing the mitral flow and TD images of the mitral valve ring on the 7th day of AngII infusion in AMPKα2$^{-/-}$ mice. Right panel: E/E'. (B) Representative echocardiograms on the 7th day of AngII or saline infusion in AMPKα2$^{-/-}$ mice. (C) EF% on the 7th day of AngII infusion in AMPKα2$^{-/-}$ mice. (D) Left ventricular shortening fraction (FS%) on the 7th day of AngII infusion in AMPKα2$^{-/-}$mice. Data are expressed as means±SEM of 8 mice per group. *P <0.05, NS=not significant. Welch's ANOVA

with post hoc Games-Howell test (A) or two-way ANOVA (C and D) was used.