SUPPLEMENTARY MATERIAL

This file includes:

Supplementary Figures and Figure Legends

Figure S17. Expression of TGF-β after miR-486a-5p overexpression or knockdown in CFs

Supplementary Tables

Table S1. Primer sequences (provided as an Excel file)

Table S2. Echocardiographic analysis of Ang II- or saline-infused FcεR1-Flox and FcεR1-cKO mice

Table S3. Candidate miR-486a-5p targets predicted by Targetscan 7.1 and miRanda combined analyses (provided as an Excel file)

Table S4. GO analysis of the candidate miR-486a-5p targets (provided as an Excel file)

Table S5. KEGG analysis of the candidate miR-486a-5p targets (provided as an Excel file)

Table S6. Echocardiographic analysis of Ang II- or saline-infused WT mice treated with lenti-miR-486a-5p or scramble

Supplementary Figures and Figure Legends

Figure S1

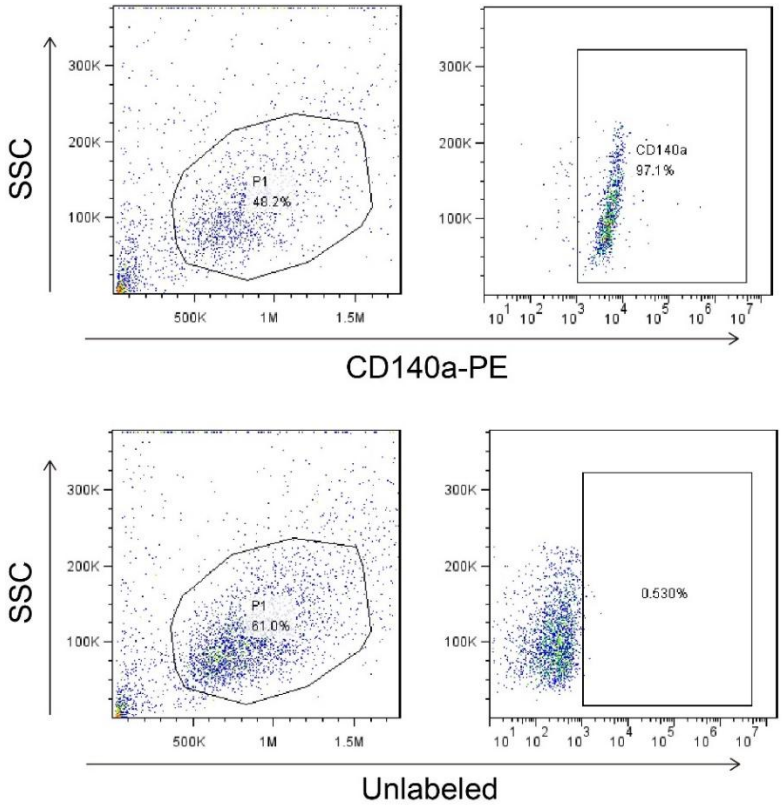

Figure S1. The purity of the extracted CFs

Flow cytometry analysis of PDGFR-α (CD140a) $^{+}$ cells in the isolated primary mouse CFs.

Figure S2

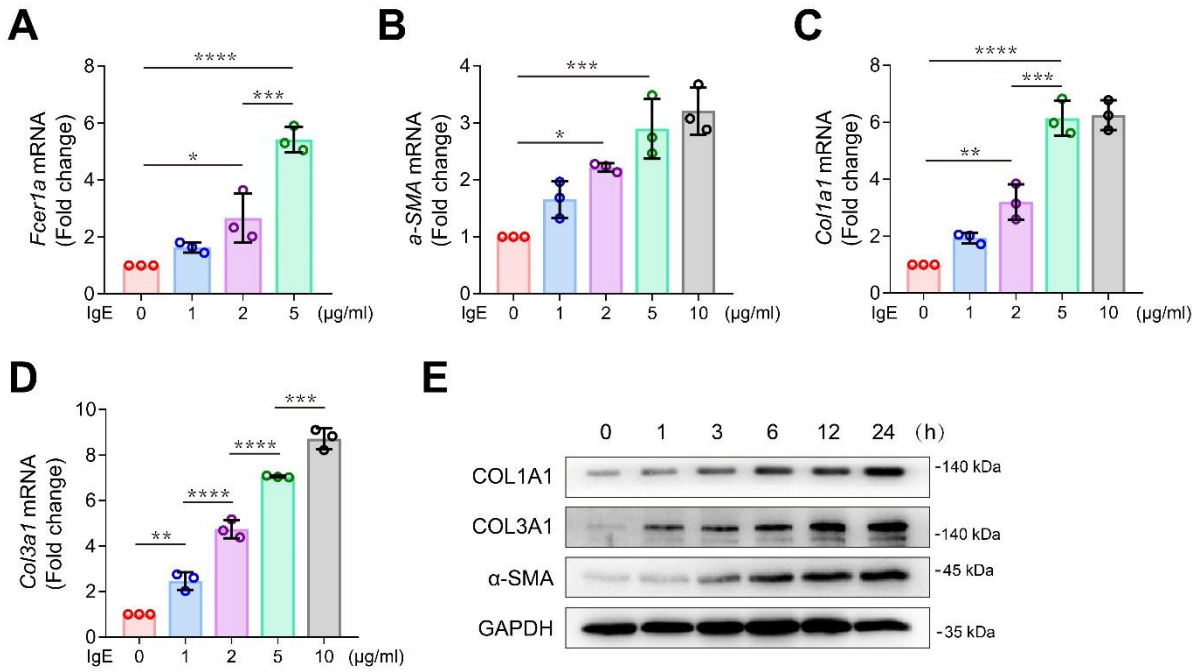

Figure S2. Expression of FcεR1 and fibrotic genes after IgE treatment at different times and doses in CFs

A. *Fcer1a* mRNA expression levels in mouse primary CFs after IgE stimulation at different concentrations (0, 1, 2, and 5 μg/ml). **B–D.** qPCR analysis of key fibrotic genes (*a-SMA*, *Col1a1*, and *Col3a1*) mRNA expression in 0, 1, 2, 5, and 10 μg/ml IgE-stimulated FcεR1-WT CFs. **E.** Immunoblot analysis of α-SMA, COL1A1, and COL3A1 protein expression after IgE treatment of CFs at 0, 1, 3, 6, 12, and 24 hours. Data are mean ± SD from 3 independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ by *one-way ANOVA* with Bonferroni's post hoc test.

Figure S3

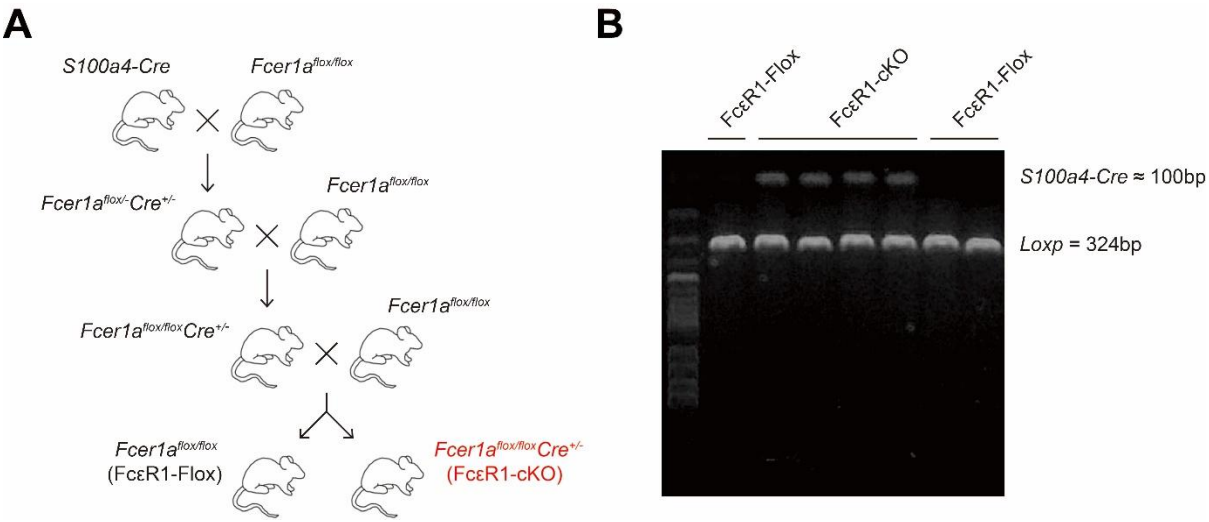

Figure S3. Construction of FcεR1-cKO mice

A–B. The construction pipeline **(A)** and identification **(B)** of FcεR1-cKO mice.

Figure S4

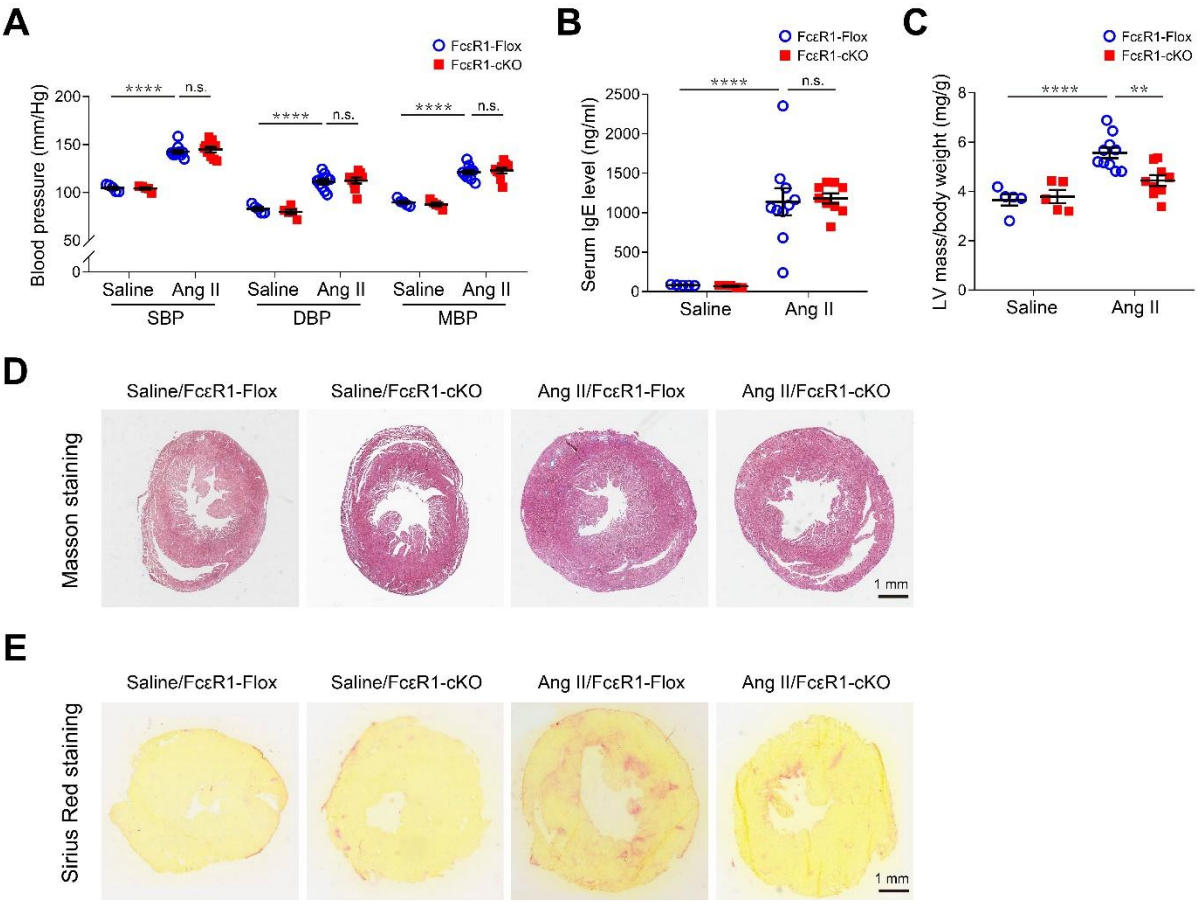

Figure S4. Effect of CFs FcεR1 deletion on Ang II-infused mice

A. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) were measured by non-invasive tail-cuff monitor in Ang II- or saline-infused FcεR1-Flox and FcεR1-cKO mice. **B.** ELISA analysis of serum IgE in Ang II- or saline-infused FcεR1-Flox and FcεR1-cKO mice. **C.** Left ventricular weight versus body weight after 2-week Ang II treatment in FcεR1-Flox and FcεR1-cKO mice. **D-E.** Representative heart sections examined by Masson **(D)** and Sirius Red staining **(E)**. Scale bars, 1 mm. Total n = 5 (Saline/FcεR1-Flox), n = 5 (Saline/FcεR1-cKO), n = 10 (Ang II/FcεR1-Flox) or n = 9 (Ang II/FcεR1-cKO) per group. The results are shown as mean ± SEM. $^{**}p < 0.01$, $^{****}p < 0.0001$, n.s. indicates no significance in *Two-way ANOVA* with Bonferroni's post hoc test.

Figure S5

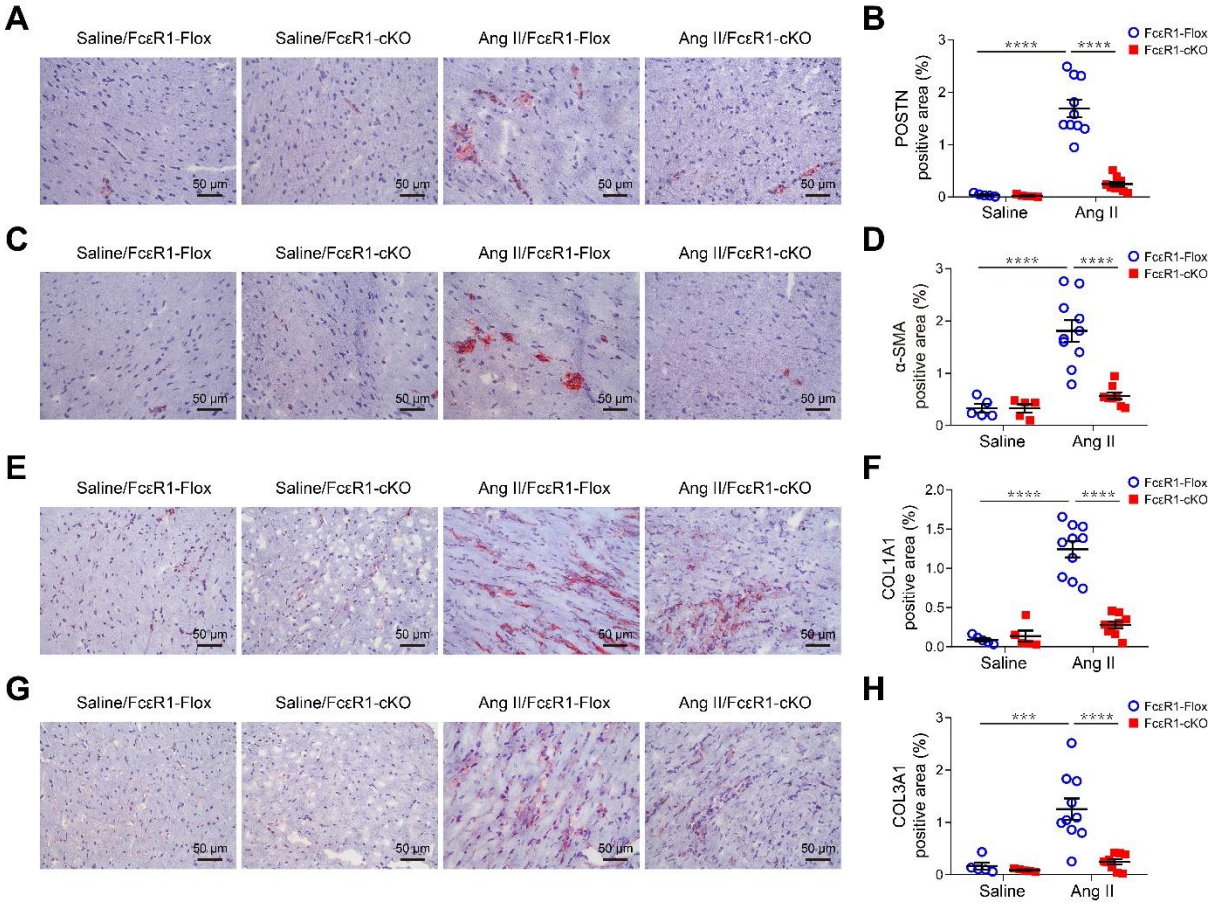

Figure S5. Immunohistochemical staining of fibrotic markers in heart tissues from CF-specific FcεR1 KO mice

A-H. Representative images of POSTN **(A)**, α-SMA **(C)**, COL1A1 **(E)** and COL3A1 **(G)** staining in heart tissues from Ang II- or Saline-infused FcεR1-cKO or FcεR1-Flox mice. Images were taken at 400X magnification. Scale bars, 50 μm. Quantification of POSTN **(B)**, α-SMA **(D),** COL1A1 **(F)** and COL3A1 **(H)** staining. A total of nine fields from three sections (three fields from each section) per mouse were randomly selected for analysis. Total n = 5 (Saline/FcεR1-Flox), n = 5 (Saline/FcεR1-cKO), n = 10 (Ang II/FcεR1-Flox) or n = 9 (Ang II/FcεR1-cKO) per group. The results are shown as mean ± SEM. ***$p < 0.001$, ****$p < 0.0001$, n.s. indicates no significance in *Two-way ANOVA* with Bonferroni's post hoc test.

Figure S6

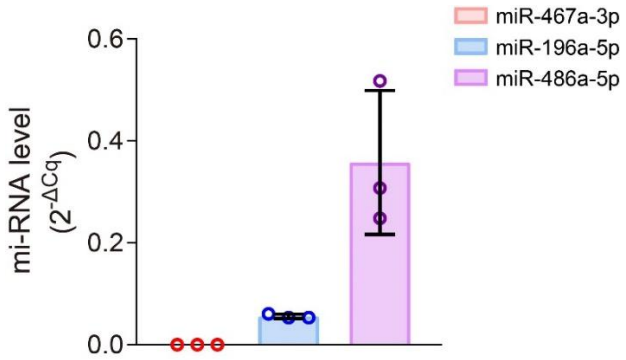

Figure S6. Basal levels of miRNAs in CFs

Basal expressions of three candidate miRNAs (miR-467a-3p, miR-196a-5p, and miR-486a-5p) in CFs detected by qPCR. Results are shown as mean ± SD. Data are mean ± SD from 3 independent experiments.

Figure S7

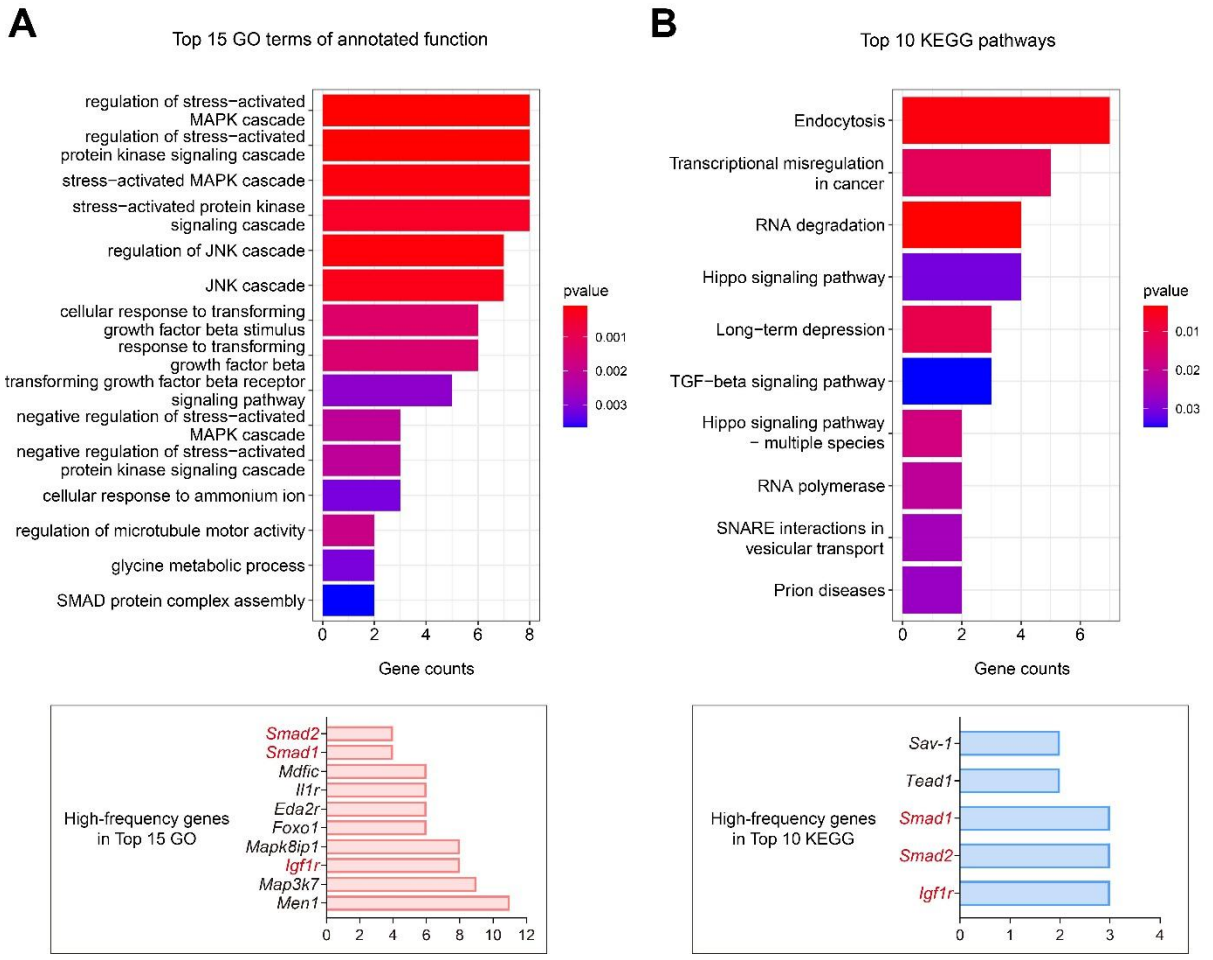

Figure S7. Gene ontology and KEGG Pathway enrichment analyses for screening potential miR-486a-5p targets

A–B. The predicted candidates from the Targetscan7.1 and miRanda intersection were analyzed by GO and KEGG pathway bioinformatic analyses. **(A)** Upper panel: bar chart of the top fifteen GO terms, listed by -log 10 *p* value. The x-axis shows the gene counts in each GO term and y-axis shows GO terms. Lower panel: The high-frequency genes enriched in top 15 GO. **(B)** upper panel: The enriched KEGG pathways of predicted targets of miR-486a-5p. The x-axis shows the gene counts in each KEGG pathway and y-axis shows KEGG pathways. Lower panel: The high-frequency genes enriched in top 10 KEGG pathways. By overlapping the results from GO and KEGG analyses, three genes (*Smad1*, *Smad2* and *Igf1r*) were identified and marked in red.

Figure S8

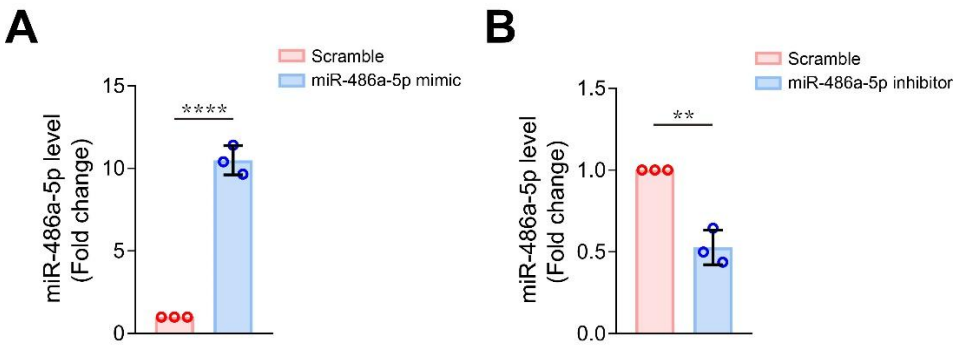

Figure S8. Efficiency data for miR-486a-5p mimic and miR-486a-5p inhibitor

A–B. Expression of miR-486a-5p in CFs after transfected with miR-486a-5p mimic **(A)** or miR-486a-5p inhibitor **(B)** detected by qPCR (fold change versus Scramble controls). Data are mean ± SD from 3 independent experiments. All statistics were performed using Student's *t*-test. ****$p < 0.0001$, **$p < 0.01$.

Figure S9

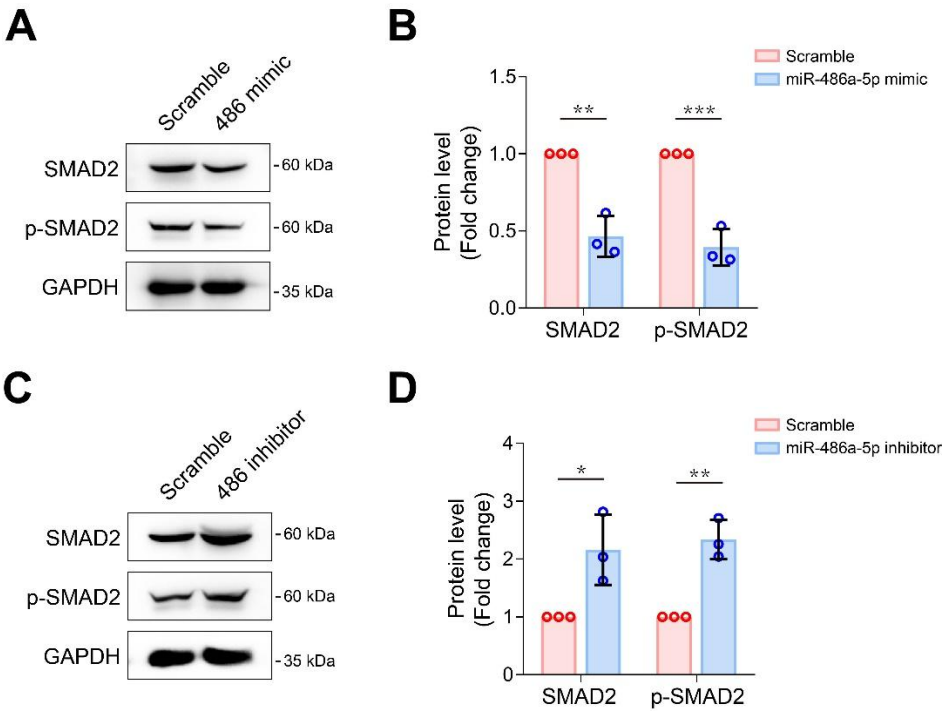

Figure S9. Expression of SMAD2 and phospho-SMAD2 after miR-486a-5p overexpression or knockdown in CFs

A–B. Representative immunoblot **(A)** and quantification analysis **(B)** of SMAD2 and phospho-SMAD2 expression in CFs after transfected with miR-486a-5p mimic or scrambled control. **C–D.** Representative immunoblot **(C)** and quantification analysis **(D)** of SMAD2 and phospho-SMAD2 in CFs after transfected with miR-486a-5p inhibitor or scrambled control. Data are mean ± SD from 3 independent experiments. All statistics were performed using Student's *t*-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure S10

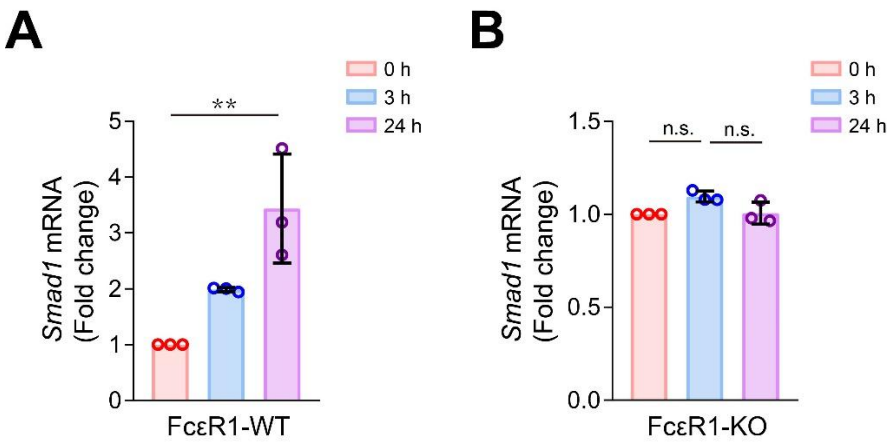

Figure S10. *Smad1* mRNA expression after IgE stimulation in CFs

A–B. qPCR analysis of *Smad1* mRNA expression in IgE-stimulated FcεR1-WT (A) and FcεR1-KO (B) CFs at indicated times (0, 3, 24 h). Data are mean ± SD from 3 independent experiments. $^{**}p < 0.01$, n.s. indicates no significance in *One-way ANOVA* with Bonferroni's post hoc test.

Figure S11

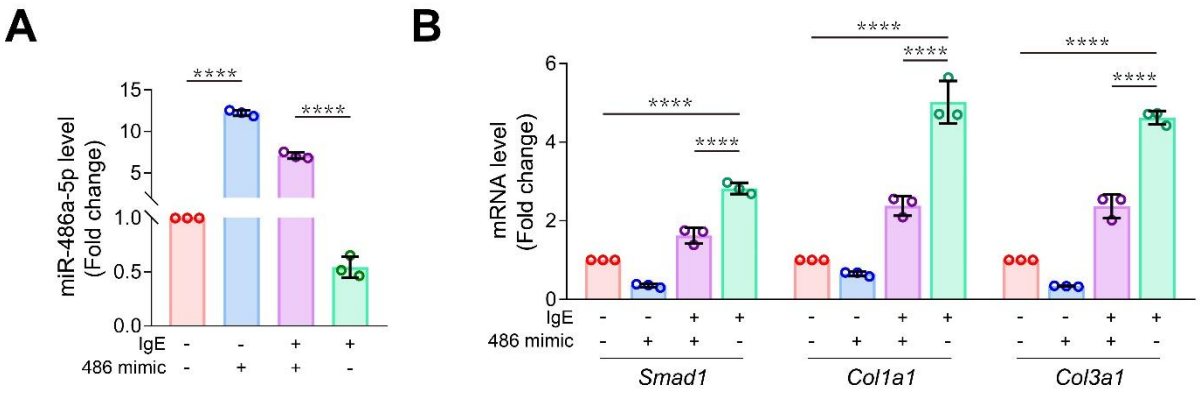

Figure S11. Rescue assays performed in CFs

A–B. CFs were transfected with miR-486a-5p mimic or scrambled control for 24 hours and then treated with IgE for another 24 hours. qPCR analysis of miR-486a-5p **(A)**, *Smad1*, *Col1a1*, and *Col3a1* **(B)** expression. Data are mean ± SD from 3 independent experiments. All statistics were performed using *Two-way ANOVA* with Bonferroni's post hoc test. ****$p < 0.0001$.

Figure S12

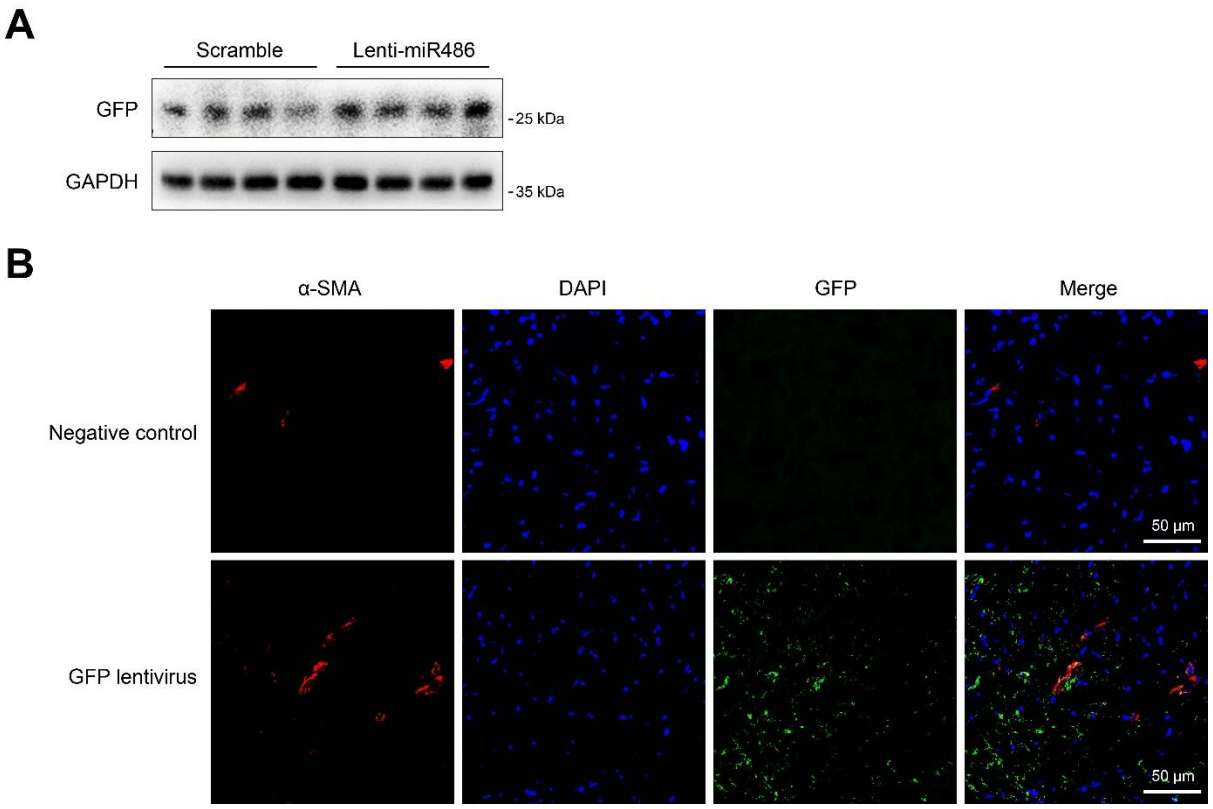

Figure S12. Overexpression of lenti-miR486 indicated by GFP detection

A. Immunoblot analysis of GFP expression to verify that the lentiviruses were delivered successfully to the heart tissue. **B.** Representative images of immunofluorescence analysis of α-SMA (red), GFP (green) and DAPI (blue) on the heart sections from GFP lentivirus-injected mice and negative controls. Scale bars, 50 μm. DAPI, 4'6-diamidino-2-phenylindole.

Figure S13

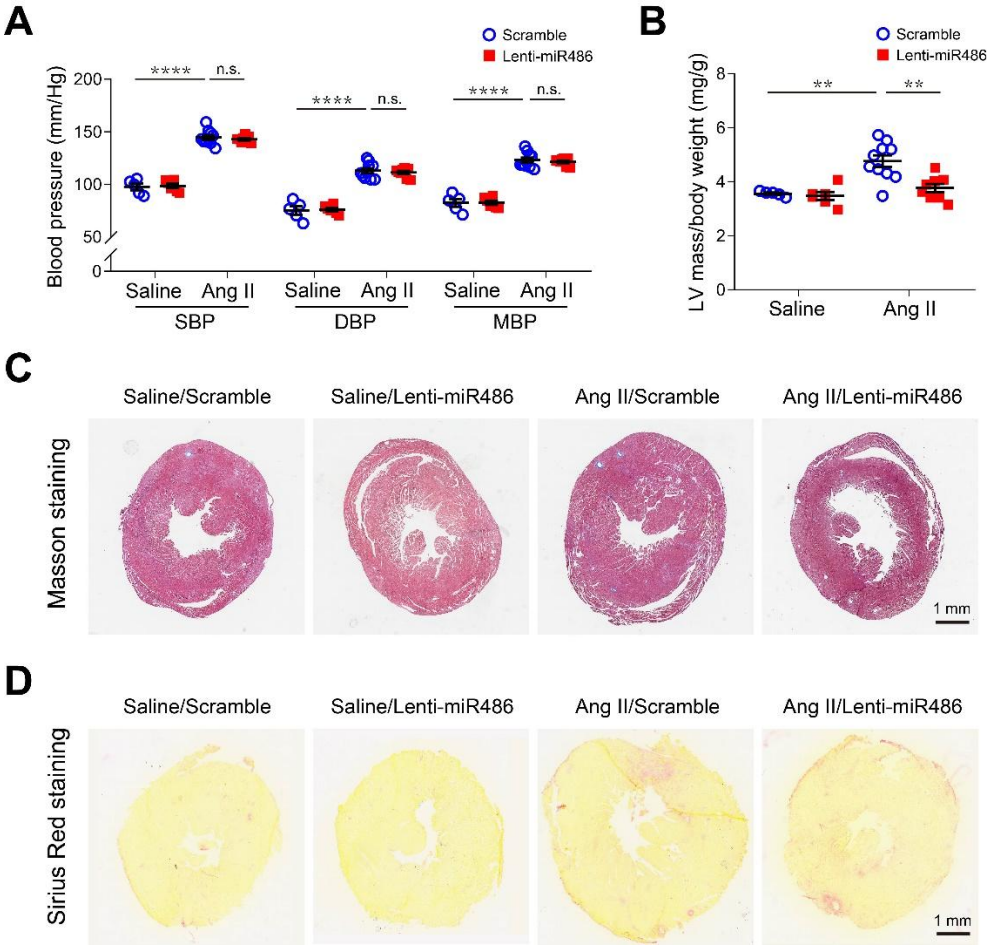

Figure S13. Effect of miR-486a-5p overexpression on Ang II-infused mice

A. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) were measured by non-invasive tail-cuff monitor in lenti-miR486 or scramble-treated Ang II- or saline-infused mice. **B.** Left ventricular weight versus body weight after lenti-miR486 or scramble-treated Ang II-or saline-infused mice. **C–D.** Representative heart sections examined by Masson **(C)** and Sirius Red staining **(D)**. Scale bars, 1 mm. Total n = 5 (Saline/Scramble), n = 6 (Saline/Lenti-miR486), n = 10 (Ang II/Scramble), or n = 8 (Ang II/Lenti-miR486) per group. The results are shown as mean ± SEM. $^{**}p < 0.01$, $^{****}p < 0.0001$, n.s. indicates no significance in *Two-way* ANOVA with Bonferroni's post hoc test.

Figure S14

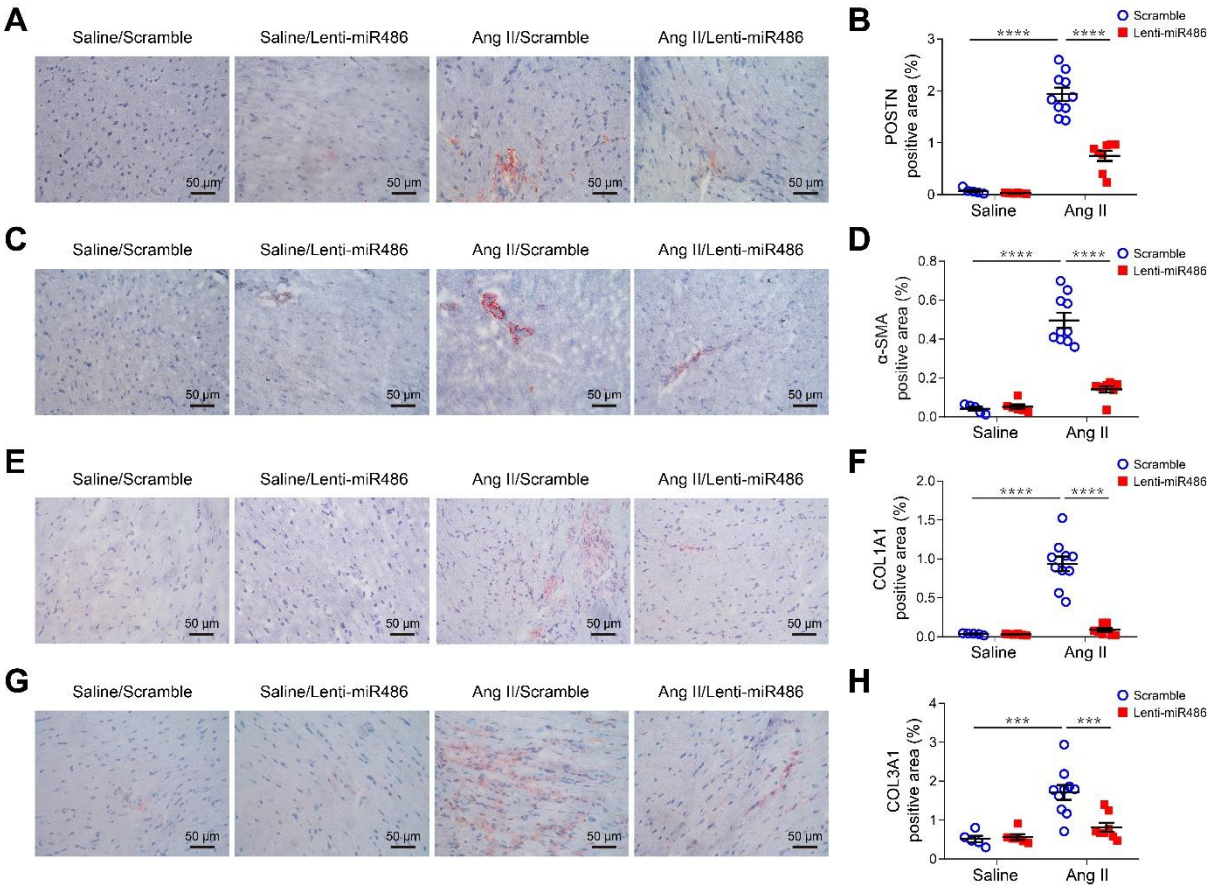

Figure S14. Immunohistochemical staining of fibrotic markers in heart tissues from miR-486a-5p-overexpressed mice

A-H. Representative images of POSTN **(A)**, α-SMA **(C)**, COL1A1 **(E)**, and COL3A1 **(G)** staining of heart tissues from lenti-miR-486a-5p (lenti-miR486) and scramble treated Ang II- or saline-infused WT mice. Images were taken at 400X magnification. Scale bars, 50 μm. Quantification of POSTN **(B)**, α-SMA **(D)**, COL1A1 **(F)**, and COL3A1 **(H)** staining. A total of nine fields from three sections (three fields from each section) per mouse were randomly selected for analysis. Total n = 5 (Saline/Scramble), n = 6 (Saline/Lenti-miR486), n = 10 (Ang II/Scramble), or n = 8 (Ang II/Lenti-miR486) per group. The results are shown as mean ± SEM. ***$p < 0.001$, ****$p < 0.0001$, n.s. indicates no significance in *Two-way* ANOVA with Bonferroni's post hoc test.

Figure S15

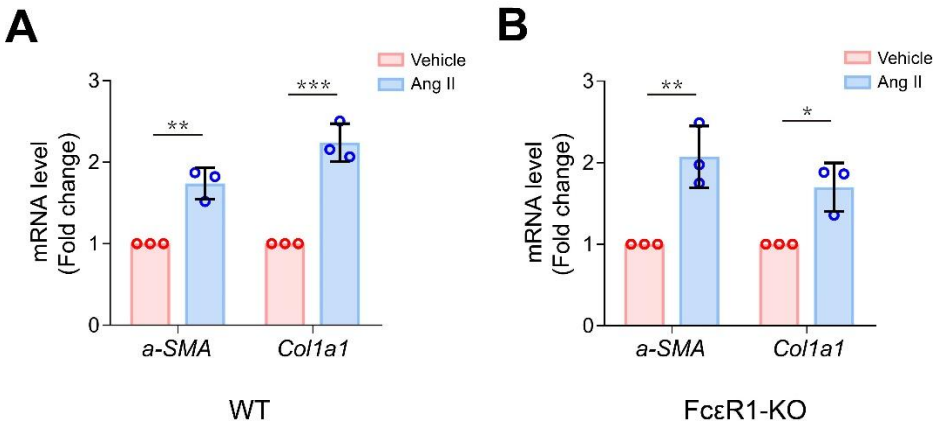

Figure S15. Effect of Ang II on WT and FcεR1-KO CFs *in vitro*

A-B. qPCR analysis of *a-SMA* and *Col1a1* mRNA expression in WT **(A)** and FcεR1-KO **(B)** CFs after IgE after IgE treatment for 24h. Results are shown as mean±SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Student's *t*-test.

Figure S16

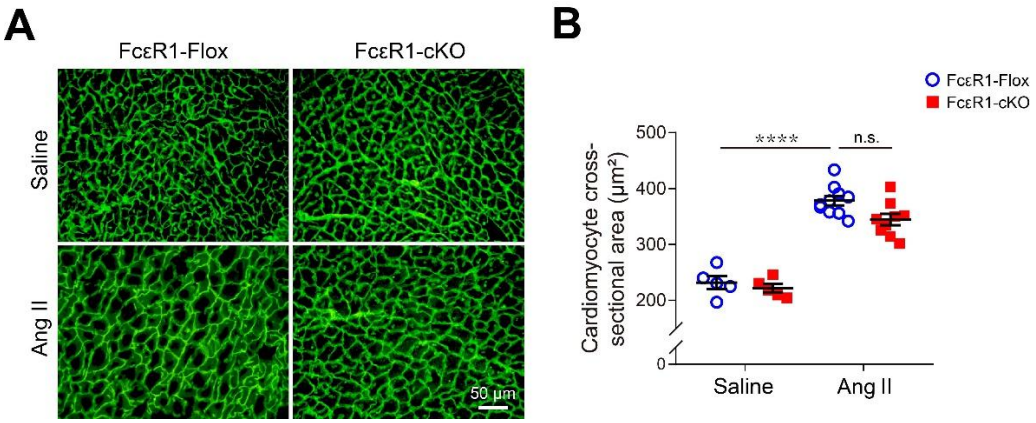

Figure S16. Effect of CF FcεR1 deletion on Ang II-induced cardiomyocyte hypertrophy

A-B. WGA (green) staining of cardiac sections (6 μm) from Ang II- or saline-infused FcεR1-Flox and FcεR1-cKO mice. 450 cells per mouse were randomly selected from 9 fields in three sections (three random fields from each section) were measured. Scale bars, 50 μm. Total n = 5 (Saline/FcεR1-Flox), n = 5 (Saline/cKO), n = 10 (Ang II/FcεR1-Flox), or n = 9 (Ang II/cKO) per group. Results are shown as mean ± SEM. ****$p < 0.0001$, n.s indicates no significance in *Two-way ANOVA* with Bonferroni's post hoc test.

Figure S17

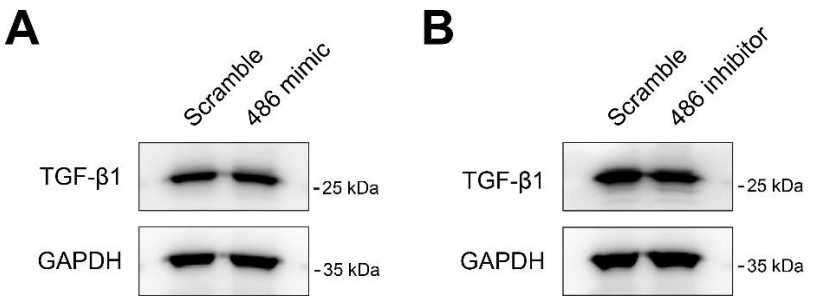

Figure S17. Expression of TGF-β after miR-486a-5p overexpression or knockdown in CFs

A. Western blot analysis of TGF-β1 protein expression in CFs after transfected with miR-486a-5p mimic or scrambled control. **B.** Western blot analysis of TGF-β protein expression in CFs after transfected with miR-486a-5p inhibitor or scrambled control.

Supplementary Tables

Table S2. Echocardiographic analysis of Ang II- or saline-infused FcεR1-Flox and FcεR1-cKO mice

	Saline-treated		Ang II-treated	
	FcεR1-Flox mice (n = 5)	**FcεR1-cKO mice (n = 5)**	**FcεR1-Flox mice (n = 10)**	**FcεR1-cKO mice (n = 9)**
LVAW;d (mm)	0.76 ± 0.01	0.75 ± 0.02	1.11 ± 0.02††††	0.90 ± 0.04****
LVAW;s (mm)	1.17 ± 0.04	1.08 ± 0.06	1.59 ± 0.03††††	1.37 ± 0.06**
LVID;d (mm)	3.99 ± 0.14	4.05 ± 0.09	3.65 ± 0.11	3.94 ± 0.07
LVID;s (mm)	2.80 ± 0.09	2.88 ± 0.07	2.64 ± 0.07	2.93 ± 0.10
LVPW;d (mm)	0.75 ± 0.01	0.82 ± 0.04	1.18 ± 0.08††	0.97 ± 0.04
LVPW;s (mm)	1.04 ± 0.02	1.14 ± 0.10	1.42 ± 0.09†	1.22 ± 0.04
EF (%)	57.62 ± 0.55	56.34 ± 1.03	54.04 ± 2.70	50.97 ± 2.73
FS (%)	29.89 ± 0.42	29.08 ± 0.68	27.57 ± 1.69	25.75 ± 1.65
LV mass AW	108.85 ± 4.73	117.71 ± 5.11	169.02 ± 6.31††††	142.48 ± 6.82*
LV Mass (correct)	87.08 ± 3.78	94.17 ± 4.09	135.22 ± 5.05††††	113.98 ± 5.46*
Heart rate (HR, beats/min)	498.80 ± 15.14	516.80 ± 16.33	482.40 ± 10.76	504.22 ± 10.83

The results are shown as mean ± SEM.

Ang II-treated FcεR1-cKO versus Ang II-treated FcεR1-Flox mice, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

Ang II-treated FcεR1-Flox mice versus Saline-treated FcεR1-Flox mice, $^{†}p < 0.05$, $^{††}p < 0.01$, $^{††††}p < 0.0001$.

Abbreviations: LVAW;d: left ventricular anterior wall thickness in diastole; LVAW;s: left ventricular anterior wall thickness in systole; LVID;d: left ventricular internal diameter in diastole; LVID;s: left ventricular internal diameter in systole; LVPW;s: left ventricular posterior wall thickness in systole; LVPW;d: left ventricular posterior wall thickness in diastole. EF: ejection fraction; FS: fraction shortening; LV mass AW: left ventricle mass anterior wall. LV Vol;d: left ventricular volume in diastole ; LV vol;s: left ventricular volume in systole.

Table S6. Echocardiographic analysis of Ang II- or saline-infused WT mice treated with lenti-miR-486a-5p or scramble

	Saline-treated		Ang II-treated	
	Scramble (n = 5)	**Lenti-miR-486a-5p (n = 6)**	**Scramble (n = 10)**	**Lenti-miR-486a-5p (n = 8)**
LVAW;d (mm)	0.82 ± 0.06	0.87 ± 0.05	1.07 ± 0.05†	0.84 ± 0.04**
LVAW;s (mm)	1.17 ± 0.07	1.25 ± 0.09	1.59 ± 0.05††	1.19 ± 0.09**
LVID;d (mm)	3.72 ± 0.08	3.74 ± 0.13	3.54 ± 0.10	3.79 ± 0.13
LVID;s (mm)	2.50 ± 0.08	2.50 ± 0.14	2.34 ± 0.11	2.71 ± 0.19
LVPW;d (mm)	0.82 ± 0.03	0.80 ± 0.04	1.01 ± 0.05	0.84 ± 0.04
LVPW;s (mm)	1.13 ± 0.03	1.24 ± 0.05	1.41 ± 0.08	1.26 ± 0.06
EF (%)	62.13 ± 1.81	62.59 ± 2.62	63.39 ± 3.45	55.44 ± 4.77
FS (%)	32.89 ± 1.24	33.34 ± 1.88	34.17 ± 2.44	28.89 ± 2.97
LV mass AW	107.95 ± 1.97	110.42 ± 4.80	140.96 ± 6.73††	114.83 ± 5.86*
LV Mass (correct)	86.36 ± 1.58	88.34 ± 3.84	112.77 ± 5.38††	91.86 ± 4.69*
Heart rate (HR, beats/min)	515.20 ± 7.61	509.33 ± 12.71	502.80 ± 10.30	527.25 ± 6.07

The results are shown as mean ± SEM.

Ang II-treated Scramble group versus Ang II-treated Lenti-miR-486a-5p group, *$p < 0.05$, **$p < 0.01$.

Ang II-treated Scramble group versus Saline-treated Scramble group, $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$.

Abbreviations: LVAW;d: left ventricular anterior wall thickness in diastole; LVAW;s: left ventricular anterior wall thickness in systole; LVID;d: left ventricular internal diameter in diastole; LVID;s: left ventricular internal diameter in systole; LVPW;s: left ventricular posterior wall thickness in systole; LVPW;d: left ventricular posterior wall thickness in diastole. EF: ejection fraction; FS: fraction shortening; LV mass AW: left ventricle mass anterior wall. LV Vol;d: left ventricular volume in diastole ; LV vol;s: left ventricular volume in systole.