Supplemental Material for

Fibroblast-specific IKKβ deficiency ameliorates angiotensin II-induced adverse cardiac remodeling in mice

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Supplemental Figure 1. Pharmacological inhibition of IKKβ signaling reduces angiotensin II-induced fibroblast proinflammatory and profibrogenic effects in vitro.

Cardiac fibroblasts (CFs) isolated from male IKKβF/F mice were pretreated with 5 µM of IKKβ inhibitor BMS-345541 or vehicle control for 1 hr, and then incubated with 10^{-6} M of angiotensin (Ang) II or vehicle control for 24 hr. (A) Western blot analysis of the total and phosphorylated (p) IKKβ and NF-κB subunit p65. (B) QPCR analysis of the mRNA levels of proinflammatory cytokines and profibrotic genes (n=3-4, One-way ANOVA, *P < 0.05, **P < 0.01, and ***P < 0.001). (C and D) Representative immunofluorescent images (left) and quantitation (right) of α-SMA+ cells (C) and collagen I (D) (n=3, One-way ANOVA, **P < 0.01 and ***P < 0.001; Scale bar, 100 μm).
Supplemental Figure 2. Deficiency of IKKβ in cardiac fibroblasts attenuates lipopolysaccharide-induced NF-κB activation and inflammation.

(A) Representative immunofluorescent staining of NF-κB p65 subunit in cardiac fibroblasts isolated from IKKβ^{F/F} and IKKβ^{ΔFib} mice that treated with 100 ng/ml of lipopolysaccharide (LPS) or vehicle control for 1 hr (Scale bar, 100μm). (B) QPCR analysis of the mRNA levels of proinflammatory cytokines and adhesion molecules (n=3. Two-way ANOVA **P < 0.01 and ***P < 0.001).
Supplemental Figure 3. Angiotensin II-induced mouse model of cardiac remodeling.
(A) Experimental scheme of mice subjected to 1000 ng/kg/min of angiotensin II (Ang II) or control (saline) infusion for 1 and 4 weeks. Eight-week-old male mice were intraperitoneally injected with tamoxifen (2 mg/per day) for 5 consecutive days to induce CreER<sup>T</sup>-mediated recombination. At the age of 10 weeks, the mice were implanted with mini-osmotic pumps. (B and C) Body weight and mortality rate of male IKKβ<sup>F/F</sup> and IKKβ<sup>ΔFib</sup> mice after 4-week Ang II or control infusion.
Supplemental Figure 4. Fibroblast IKKβ deficiency reduces angiotensin II-induced cardiac fibroblast proliferation.

Eight-week-old male IKKβ<sup>F/F</sup> and IKKβ<sup>ΔFib</sup> mice were intraperitoneally injected with 2 mg tamoxifen per day for 5 days. At the age of ten weeks, those mice were infused with 1000 ng/kg/min of angiotensin II (Ang II) or vehicle control for 1 week. Representative images of immunofluorescence staining of Ki-67 and fibroblast cell marker (A) or cardiomyocyte marker (B) in the hearts of IKKβ<sup>F/F</sup> and IKKβ<sup>ΔFib</sup> mice (Scale bar, A, 50 μm; B, 100 μm).
Supplemental Figure 5. Deficiency of fibroblast IKKβ does not increase cell apoptosis in the heart of angiotensin II-infused mice. (A-B) Eight-week-old male IKKβ^F/F and IKKβ^ΔFib mice were intraperitoneally injected with 2 mg tamoxifen per day for 5 days. At the age of ten weeks, the mice were infused with 1000 ng/kg/min of angiotensin II (Ang II) or vehicle control for 1 week (A) or 4 weeks (B). Representative TUNEL staining of hearts from IKKβ^ΔFib and IKKβ^F/F mice. Apoptotic nuclei fluoresce red. The nuclei were visualized with DAPI (blue), and the TUNEL-positive cells were indicated by arrows (Scale bar, 100 μm).
Supplemental Figure 6. Deficiency of IKKβ abolishes the impact of angiotensin II on proliferation or apoptosis-related gene expression in cardiac fibroblasts.
Cardiac fibroblasts were isolated from male IKKβ^{F/F} and IKKβ^{ΔFib} mice, and then stimulated with 10^{-6} M of angiotensin II (Ang II) or vehicle control for 24 hr. The expression levels of genes related to proliferation and apoptosis were analyzed by QPCR (n=3, Two-way ANOVA, *P < 0.05, **P < 0.01, and ***P < 0.001).
Supplemental Figure 7. Fibroblast IKKβ deficiency modestly affects cardiac inflammation after 4 weeks of angiotensin II infusion.

Eight-week-old male IKKβ^{F/F} and IKKβ^{ΔFib} mice were intraperitoneally injected with 2 mg tamoxifen per day for 5 days. At the age of ten weeks, those mice were infused with angiotensin II (Ang II) at the dose of 1000 ng/kg/min for 4 weeks. (A) QPCR analysis of the mRNA levels of inflammatory cytokines and adhesion molecules in the heart of male IKKβ^{F/F} and IKKβ^{ΔFib} mice (n=5-6, Two-way ANOVA, *P < 0.05, **P < 0.01 and ***P < 0.001). (B) Representative images of immunofluorescence staining of CD68 in the heart of IKKβ^{F/F} and IKKβ^{ΔFib} mice (n=3, Scale bar, 100 μm).
Supplemental Table 1. Echocardiographic parameters of male IKKβ F/F or IKKβ ΔFib mice after 4-week infusion of angiotensin II

| Parameters       | Control (Saline)  | Ang II (1000 ng/kg/min) |
|------------------|-------------------|-------------------------|
|                  | IKKβ F/F          | IKKβ ΔFib               | IKKβ F/F          | IKKβ ΔFib               |
| HR (bpm)         | 359.9±17.55       | 418.66±13.89            | 407±16.66         | 406.1±26.7              |
| IVSd (mm)        | 0.81±0.03         | 0.79±0.03               | 1.05±0.04 ***     | 0.89±0.04 #             |
| IVSs (mm)        | 1.38±0.03         | 1.29±0.05               | 1.50±0.05         | 1.51±0.05               |
| LVPWd (mm)       | 0.62±0.02         | 0.7±0.02                | 1.03±0.05 ***     | 0.80±0.03 ###           |
| LVPWs (mm)       | 1.09±0.03         | 1.03±0.03               | 1.36±0.04 ***     | 1.18±0.04 ##            |
| LVIDd (mm)       | 3.82±0.07         | 3.86±0.09               | 4.16±0.06 **      | 3.76±0.12 #             |
| LVIDs (mm)       | 2.56±0.10         | 2.75±0.08               | 3.14±0.09 ***     | 2.52±0.10 ###           |
| EF (%)           | 66.95±1.22        | 64.52±2.27              | 42.73±1.63 ***    | 67.31±2.56 ###          |
| FS (%)           | 36.55±0.94        | 35±1.69                 | 20.84±0.95 ***    | 37.08±1.91 ###          |

**P < 0.01, ***P <0.001, compared to IKKβ F/F mice infused with control; #P < 0.05, ##P < 0.01, ###P <0.001, compared to IKKβ F/F mice infused with Ang II (n=8-12).

Ang II, angiotensin II; HR, heart rate; IVSd, Inter-ventricular septum diameter at end-Diastole; IVSs, Inter-ventricular septum diameter at end-Systole; LVPWD, Left Ventricular Posterior Wall at end-Diastole; LVPWS, Left Ventricular Posterior Wall at end-Systole; LVIDD, Left Ventricular Internal Dimension at end-Diastole; LVIDS, Left Ventricular Internal Dimension at end-Systole; EF, ejection fraction; FS, Fractional Shortening.
## Supplemental Table 2 Primer sequences for quantitative PCR.

| Name    | Sequence                      | Name    | Sequence                      |
|---------|-------------------------------|---------|-------------------------------|
| IKKβ    | Forward 5'-GAGCTCAGCCCAAAGACAG-3' | CTGF    | Forward 5'-GGGCTCTTCTGCGATTTG-3' |
|         | Reverse 5'-AGGTTCGATCCCCTGCTTG-3' |         | Reverse 5'-ATCCAGGCAATGCTCAGT-3' |
| CD68    | Forward 5'-CTTCCCCACAGGAGCACAG-3' | CCR2    | Forward 5'-GGGTCAAGACCACTCAGT-3' |
|         | Reverse 5'-ATGTTGCAGAGGCCAGAAGG-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| F4/80   | Forward 5'-GACAGAGGAGCAGAGTGTTTCTG-3' | CXCR2   | Forward 5'-GTGCTCCGGTTTATAAGATG-3' |
|         | Reverse 5'-TTGTGCCTTACGCGCGTGTT-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| IL-6    | Forward 5'-TAGTCATCACCTGAGAAGTGTTCTC-3' | CCL4    | Forward 5'-ATCCACTTCTGCTGCTTCTG-3' |
|         | Reverse 5'-AGGTTCTGCATCCCCTGCTTG-3' |         | Reverse 5'-GTGCTCCGGTTTATAAGATG-3' |
| MCP-1   | Forward 5'-TTAAGACCTGGAAGATGAGGG-3' | CDK2    | Forward 5'-CTCGACAGCAGATGCTGCT-3' |
|         | Reverse 5'-GGGTCATGATCCCTATGTGG-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| TNFα    | Forward 5'-CCAGCTCTTCAGAGAGCTTCTC-3' | Cyclin D1 | Forward 5'-TGACAGGCTGAACTGCTG-3' |
|         | Reverse 5'-AACCTGCTCTGGCAGAAGGCAG-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| IL-1β   | Forward 5'-GCAACTGTTCTCAGCTCAGT-3' | c-myc   | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-ATCTTTTGGGTTCGCTAAGCT-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| ICAM-1  | Forward 5'-GTGATCCCTGGCGCTGGTG-3' | Cyclin A | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-GGGAAGAAGTTACAGGATGTTG-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| VCAM-1  | Forward 5'-TACAGCTCCAAAGATTCTG-3' | Cyclin B1 | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-CTATGCTAATCACCAGCTCTG-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| GAPDH   | Forward 5'-AATCCTGGGATGTTGAGG-3' | Cyclin B2 | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-GGATGCTCAAGGATGTTG-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| α-SMA   | Forward 5'-TCCAGCCGCGTGAATTCCGATA-3' | P21     | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-GGCCACACGAGCAGCTGCCT-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| TGF β   | Forward 5'-CAAGGGGTACCCAGCAGACT-3' | P27     | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-ATGGTTGCTGATCCGGCTC-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| Col1a1  | Forward 5'-ATCCCTGGCGATGCTGAT-3' | Bid     | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-CCACAGCAGTCGCTGATG-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| Col3a1  | Forward 5'-CATGACTGGTCCGCAATGGA-3' | BNIP3   | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-ATTCCGCTTATTTGATCCCA-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| Periostin| Forward 5'-CTTGGCCCTTATATGTGCT-3' | Gadd45β  | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-AACAGCTGTCGAAATGAC-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |
| p53     | Forward 5'-CTTCTCGAAGGAGCACAGGATTTTC-3' | NLRP3   | Forward 5'-GCCAGGATGCTGTTGTTG-3' |
|         | Reverse 5'-AAGCAGGCTGAAAGGGATTTTC-3' |         | Reverse 5'-GCCAGGATGCTGTTGTTG-3' |