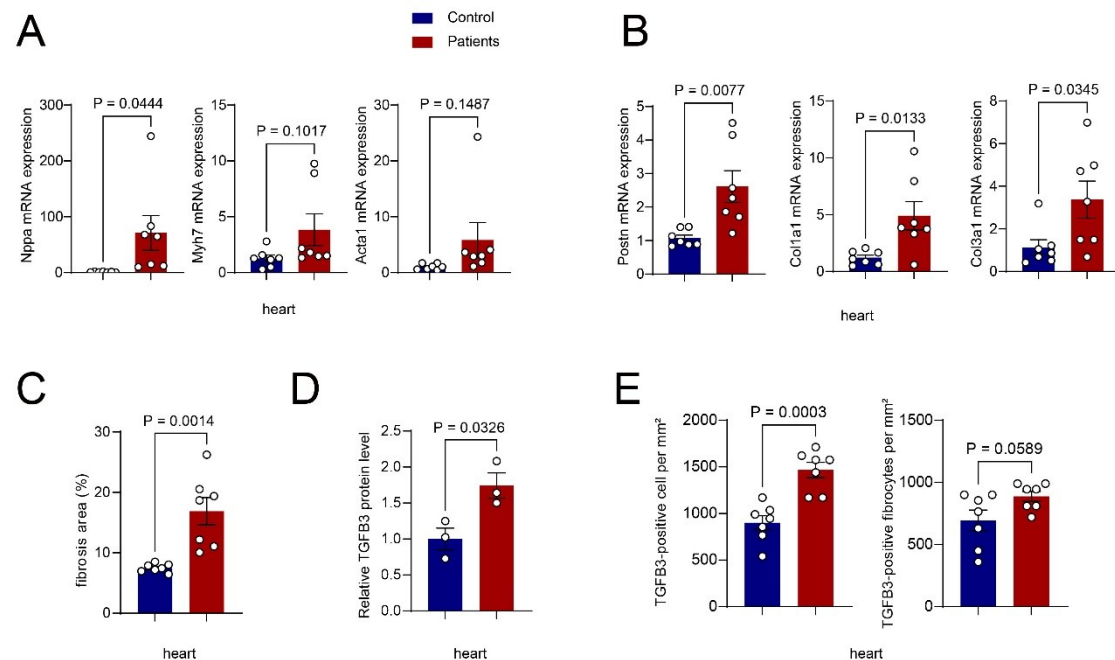


## Supplement Fig. 1

### Figure S1



**A,B** qPCR analysis of hypertrophic genes (A) and fibrogenic genes (B) in myocardial tissues from control and heart failure patients ( $n = 7$  per group).

**C.** Quantification of fibrotic area by Sirius Red staining. Fibrosis is expressed as the percentage of Sirius Red-positive area ( $n = 7$  per group).

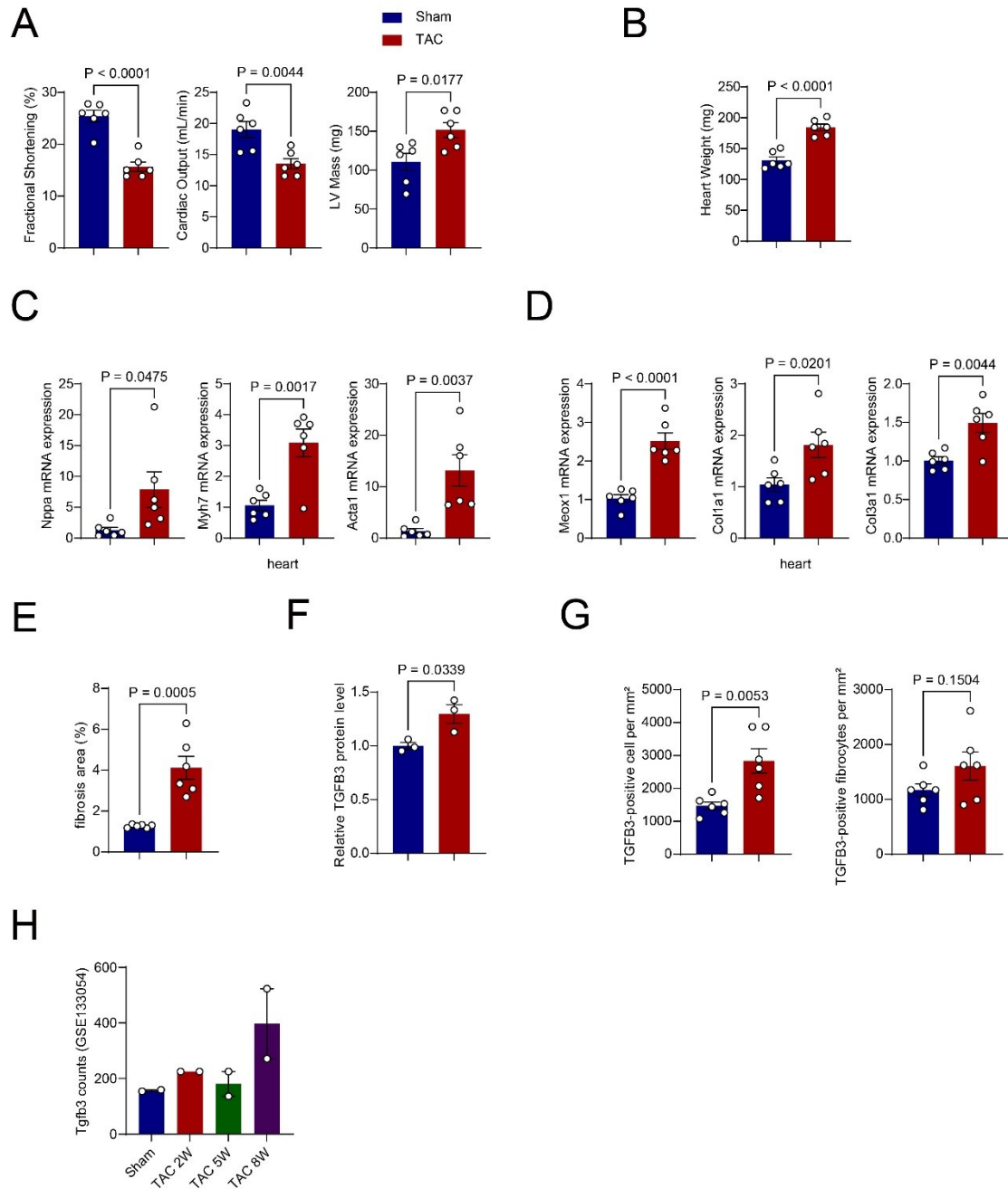
**D.** Western blot quantification of TGFB3. Protein levels were normalized to HSP90 and expressed relative to controls ( $n = 3$ ).

**E.** Quantification of TGFB3-positive cells and fibroblasts by immunofluorescence ( $n = 7$  per group).

Data are presented as mean  $\pm$  SEM from three independent experiments. Statistical significance was tested by two-tailed unpaired Student's t test (A-E). p-values are indicated above each comparison.

## Supplement Fig. 2

Figure S2



**A.** Echocardiographic analysis of Left Ventricular Fractional Shortening, Cardiac Output and Left Ventricular (LV) Mass in sham and TAC groups (n = 6 per group).

**B.** Comparison of heart weight between sham and TAC groups (n = 6 per group).

**C.D** qPCR analysis of cardiac hypertrophic genes (C) and fibrogenic genes (D) in sham and TAC hearts (n = 6 per group).

**E.** Quantification of fibrotic area by Sirius Red staining. Fibrosis is expressed as the percentage of Sirius Red-positive area (n = 6 per group).

**F.** Western blot quantification of TGFB3. Protein levels were normalized to HSP90 and expressed relative to controls (n = 3).

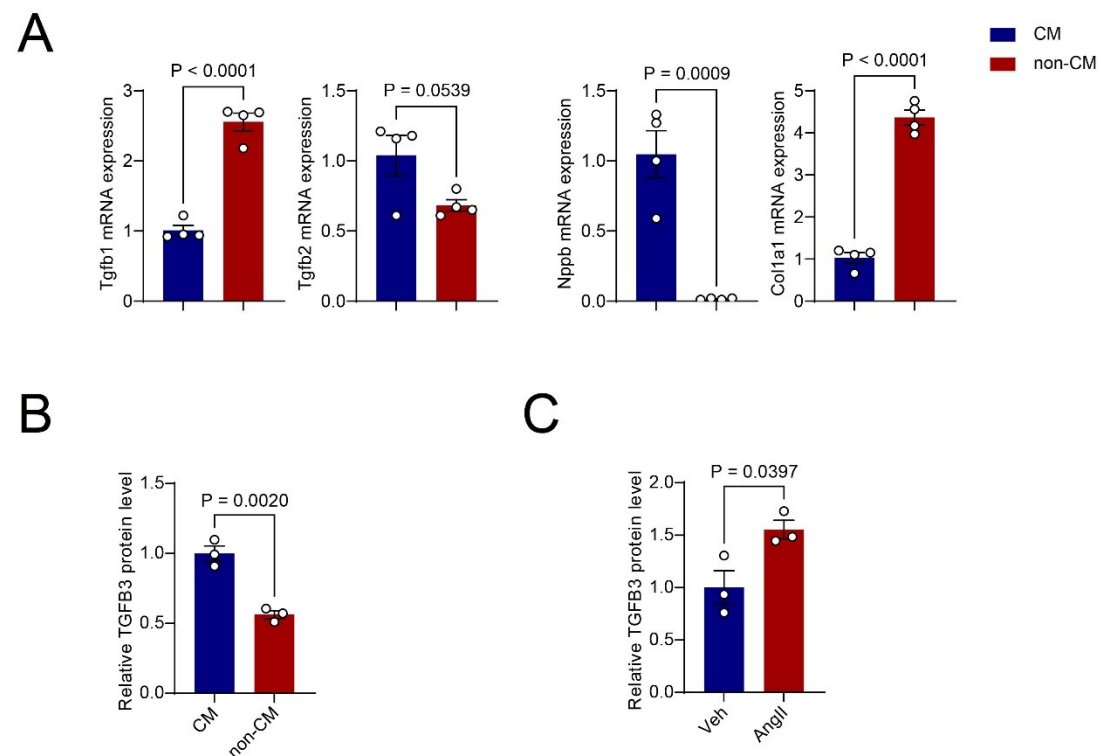
**G.** Quantification of TGFB3-positive cells and fibroblasts by immunofluorescence (n = 6 per group).

**H.** TGFB3 expression levels in sham and TAC 2W, TAC 5W, TAC 8W mouse hearts, obtained from a publicly available RNA-seq dataset (GEO accession: GSE133054).

Data are presented as mean  $\pm$  SEM from three independent experiments. Statistical significance was tested by two-tailed unpaired Student's t test (A-G). p-values are indicated above each comparison.

### Supplement Fig. 3

## Figure S3



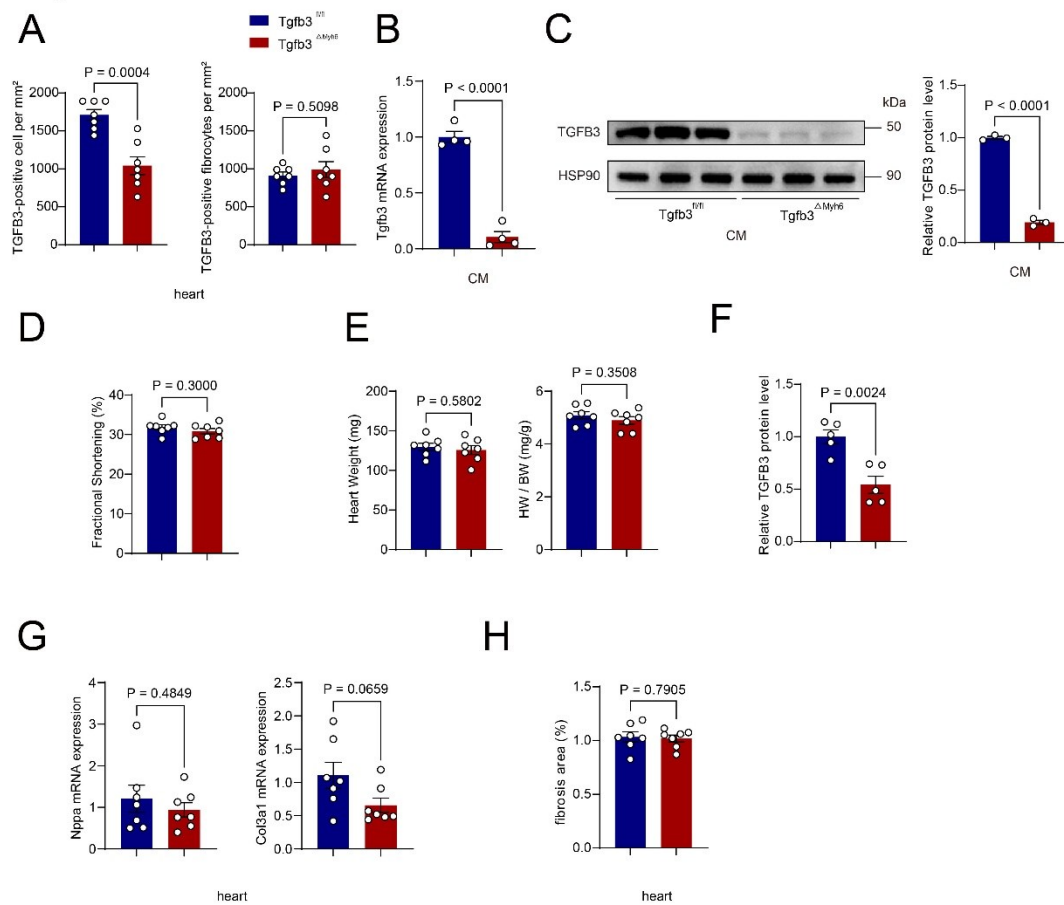
**A.** qPCR analysis of Tgfb1, Tgfb2, Nppb and Col1a1 expression in isolated cardiomyocyte (CM) and non-cardiomyocyte (non-CM) fractions (n = 4 per group).

**B.C.** Western blot quantification of TGFB3. Protein levels were normalized to HSP90 and expressed relative to controls (n = 3).

Data are presented as mean  $\pm$  SEM from three independent experiments. Statistical significance was tested by two-tailed unpaired Student's t test (A-C). p-values are indicated above each comparison.

### Supplement Fig. 4

Figure S4



**A.** Quantification of TGFB3-positive cells and fibroblasts by immunofluorescence (n = 7 per group).

**B.** qPCR analysis of Tgfb3 in isolated cardiomyocytes from Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>ΔMyh6</sup> mice (n = 4 per group).

**C.** Immunoblot analysis and quantification of TGFB3 protein levels in isolated cardiomyocytes from Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>ΔMyh6</sup> mice. HSP90 served as a loading control (n = 3 per group).

**D.** Echocardiographic analysis of Left Ventricular Fractional Shortening in Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>ΔMyh6</sup> mice (n = 7 per group).

**E.** Heart Weight and Heart weight-to-body weight ratio (HW/BW) in Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>ΔMyh6</sup> mice (n = 7 per group).

**F.** Western blot quantification of TGFB3. Protein levels were normalized to HSP90 and expressed relative to controls (n = 5 per group).

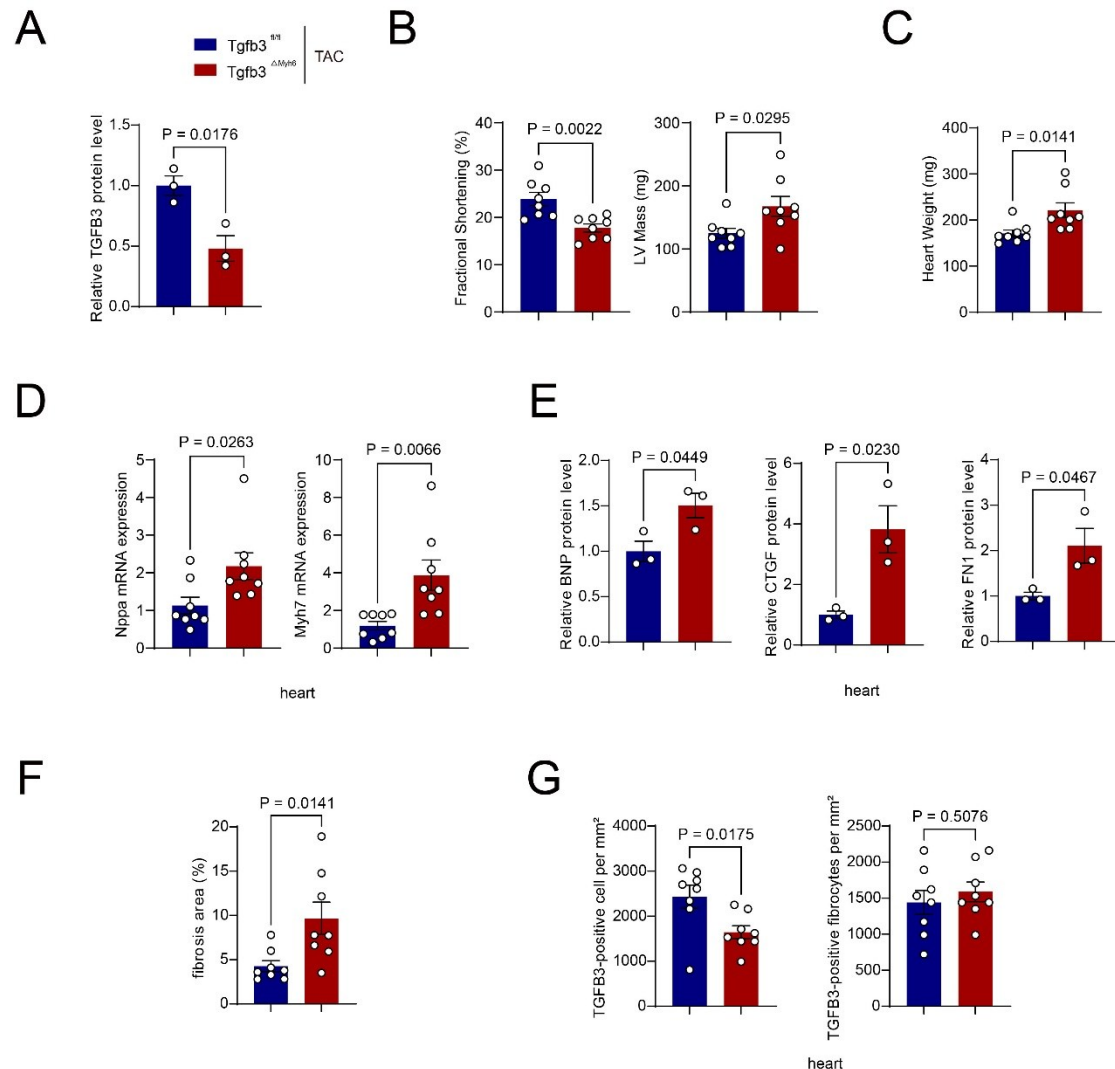
**G.** qPCR analysis of cardiac hypertrophic genes (A) and fibrogenic genes (B) in Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>ΔMyh6</sup> mice (n = 7 per group).

**H.** Quantification of fibrotic area by Sirius Red staining. Fibrosis is expressed as the percentage of Sirius Red-positive area (n = 7 per group).

Data are presented as mean ± SEM from three independent experiments. Statistical significance was tested by two-tailed unpaired Student's t test (A-H). p-values are indicated above each comparison.

**Supplement Fig. 5**

**Figure S5**



**A.** Western blot quantification of TGFB3. Protein levels were normalized to HSP90 and expressed relative to controls (n = 8 per group).

**B.** Echocardiographic analysis of Left Ventricular Fractional Shortening and LV Mass in Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>ΔMyh6</sup> mice 8 weeks after TAC (n = 8 per group).

**C.** Heart Weight in between Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>ΔMyh6</sup> mice 8 weeks after TAC (n = 8 per group).

**D.E.** qPCR analysis of hypertrophic and HF marker genes in Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>ΔMyh6</sup> mice 8 weeks after TAC (n = 8 per group).

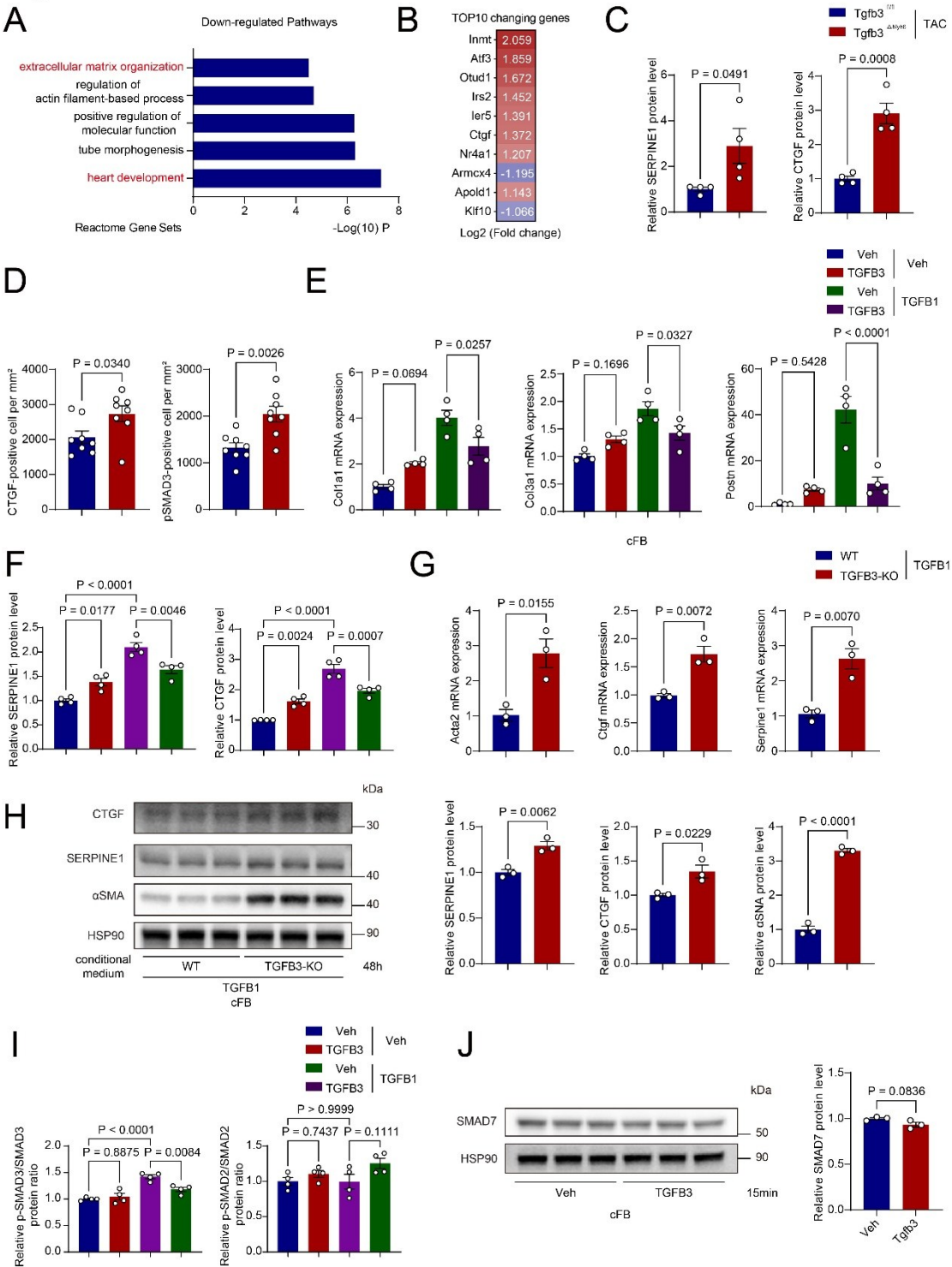
**F.** Quantification of fibrotic area by Sirius Red staining. Fibrosis is expressed as the percentage of Sirius Red-positive area (n = 8 per group).

**G.** Quantification of TGFB3-positive cells and fibroblasts by immunofluorescence (n = 8 per group).

Data are presented as mean  $\pm$  SEM from three independent experiments. Statistical significance was tested by two-tailed unpaired Student's t test (A-G). p-values are indicated above each comparison.

Supplement Fig. 6

Figure S6



A. Reactome pathway enrichment analysis of downregulated genes from RNA-seq of

Tgfb3<sup>Δ</sup>Myh6 hearts.

**B.** Top 10 changing genes ranked by Log2 (Fold change) from enrichment analysis of Tgfb3<sup>Δ</sup>Myh6 hearts.

**C.** Western blot quantification of SERPINE1 and CTGF in Tgfb3<sup>fl/fl</sup> and Tgfb3<sup>Δ</sup>Myh6 mice 8 weeks after TAC. Protein levels were normalized to HSP90 and expressed relative to controls (n = 4 per group).

**D.** Quantification of CTGF-positive and p-SMAD3-positive cells by immunofluorescence (n = 8 per group).

**E.** qPCR analysis of Col1a1, Col3a1 and Postn expression in primary cardiac fibroblasts (cFB) treated with vehicle, TGF-β1 (5 ng/mL), and/or TGF-β3 (5 ng/mL) for 24 h (n = 4 per group).

**F.** Western blot quantification of SERPINE1 and CTGF in cFB. Protein levels were normalized to HSP90 and expressed relative to controls (n = 4 per group).

**G.** qPCR analysis of Acta2, Ctgf and Serpine1 expression in cFB treated with conditional medium from primary cardiomyocytes of WT and TGFB3-KO group for 24 h (n = 3 per group).

**H.** Immunoblot analysis and quantification of αSMA, SERPINE1 and CTGF in cFB treated with conditional medium from primary cardiomyocytes of WT and TGFB3-KO group for 48 h. Protein levels were normalized to HSP90 and expressed relative to controls (n = 3 per group).

**I.** Western blot quantification of ratio of p-SMAD3/SMAD3 and p-SMAD2/SMAD2 in cFB treated with TGFB1 and TGFB3. Protein levels were expressed relative to controls (n = 4 per group).

**J.** Immunoblot analysis and quantification of SMAD7 protein levels in cFB treated with TGFB3. HSP90 served as a loading control (n = 3 per group).

Data are presented as mean ± SEM from three independent experiments. Statistical significance was determined using a two-tailed unpaired Student's t-test (C, D, E, G, H, J) and one-way ANOVA by Tukey's multiple comparisons test (F, I). p-values are indicated above each comparison.

Figure 1E

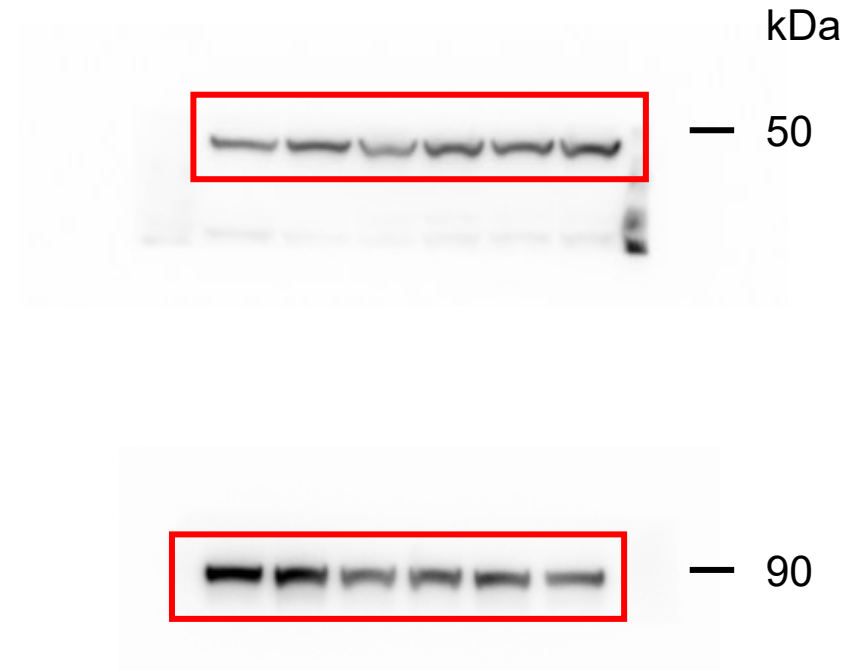
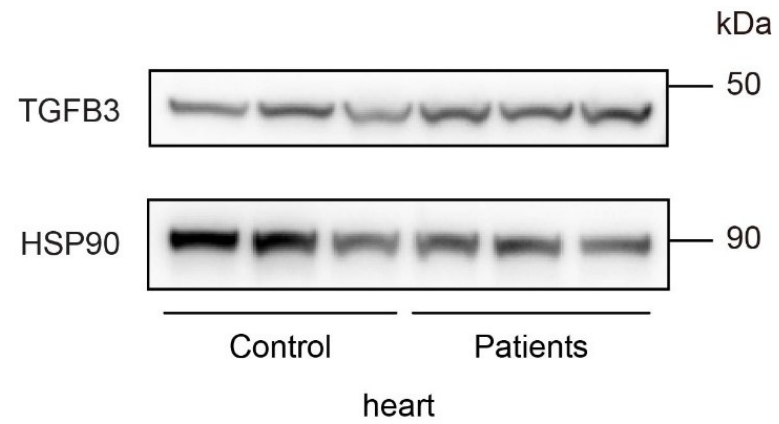


Figure 2I

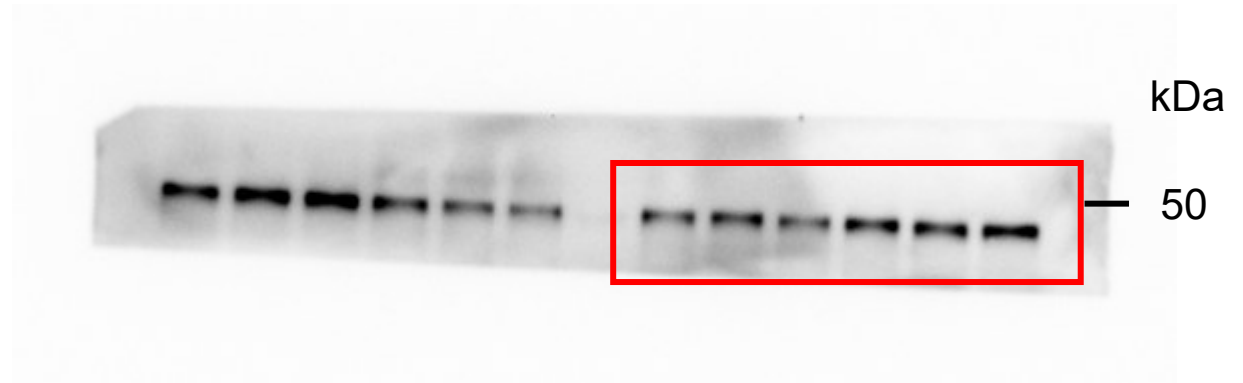
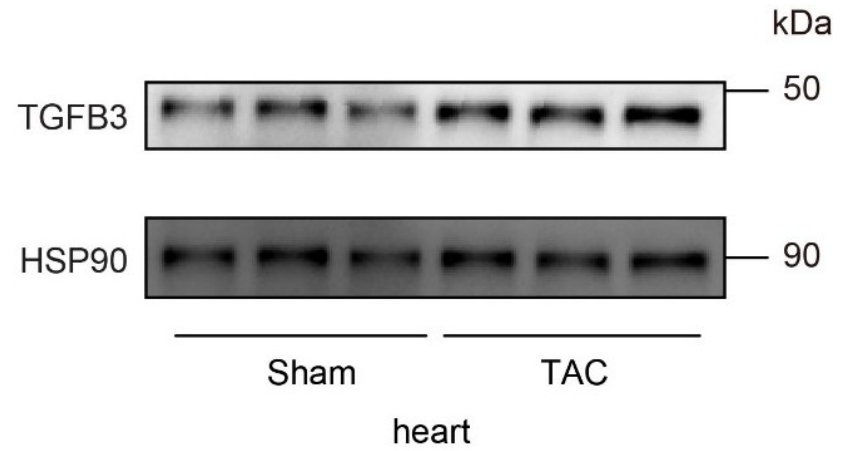
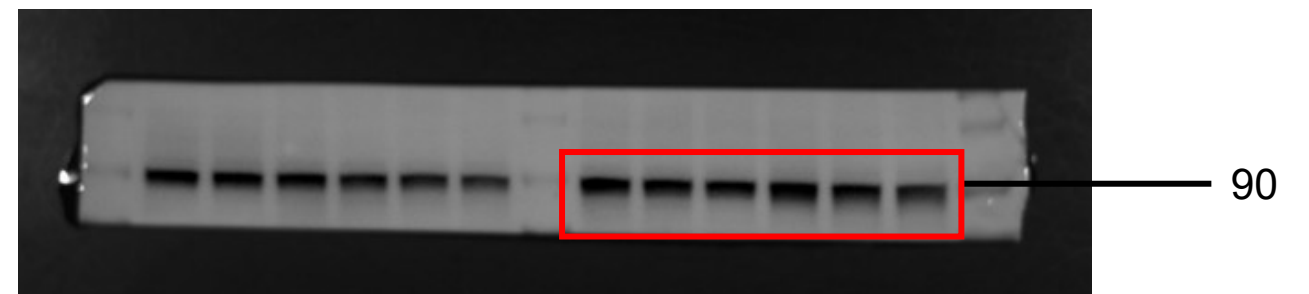
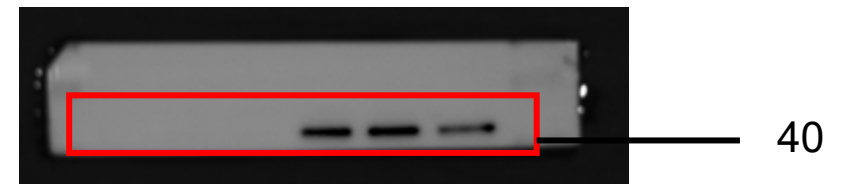
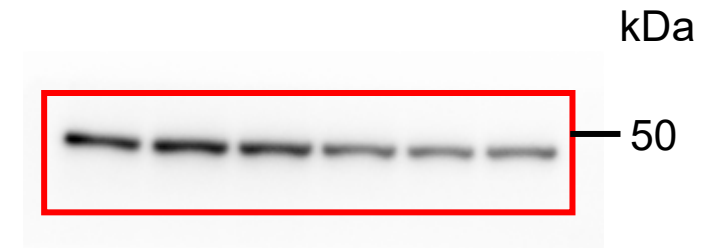
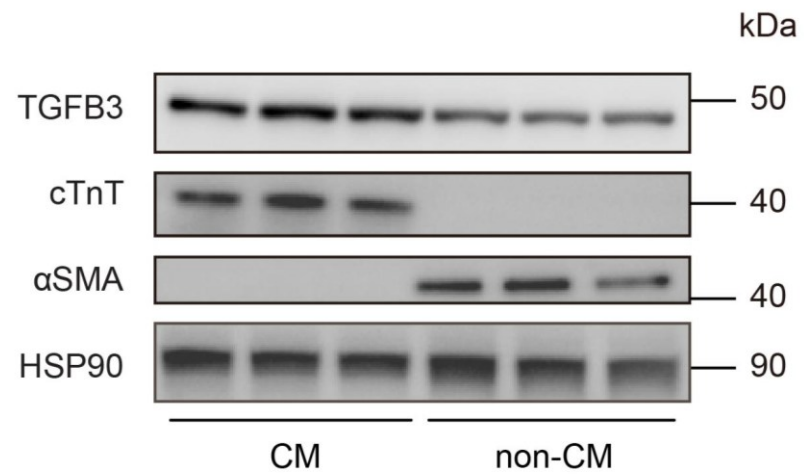


Figure 3D



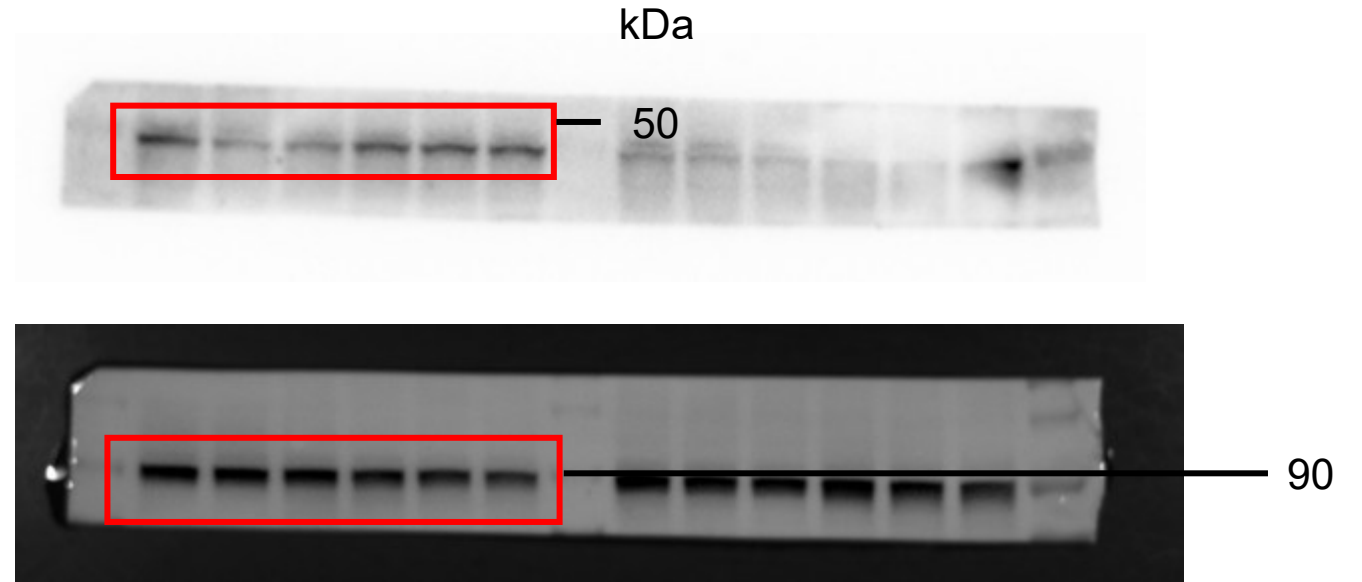


Figure 4E

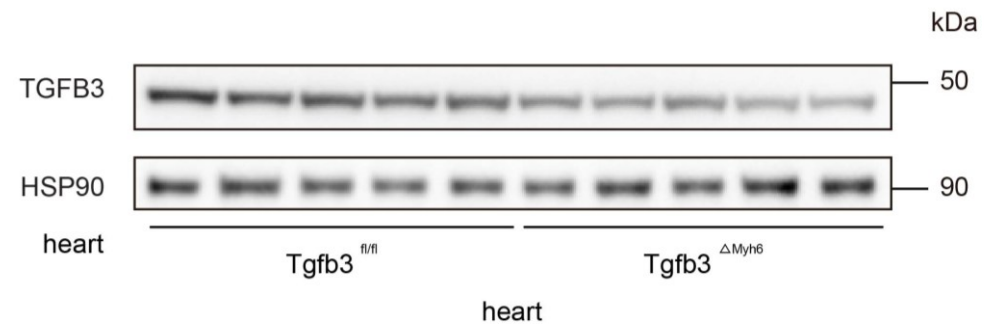


Figure 5C

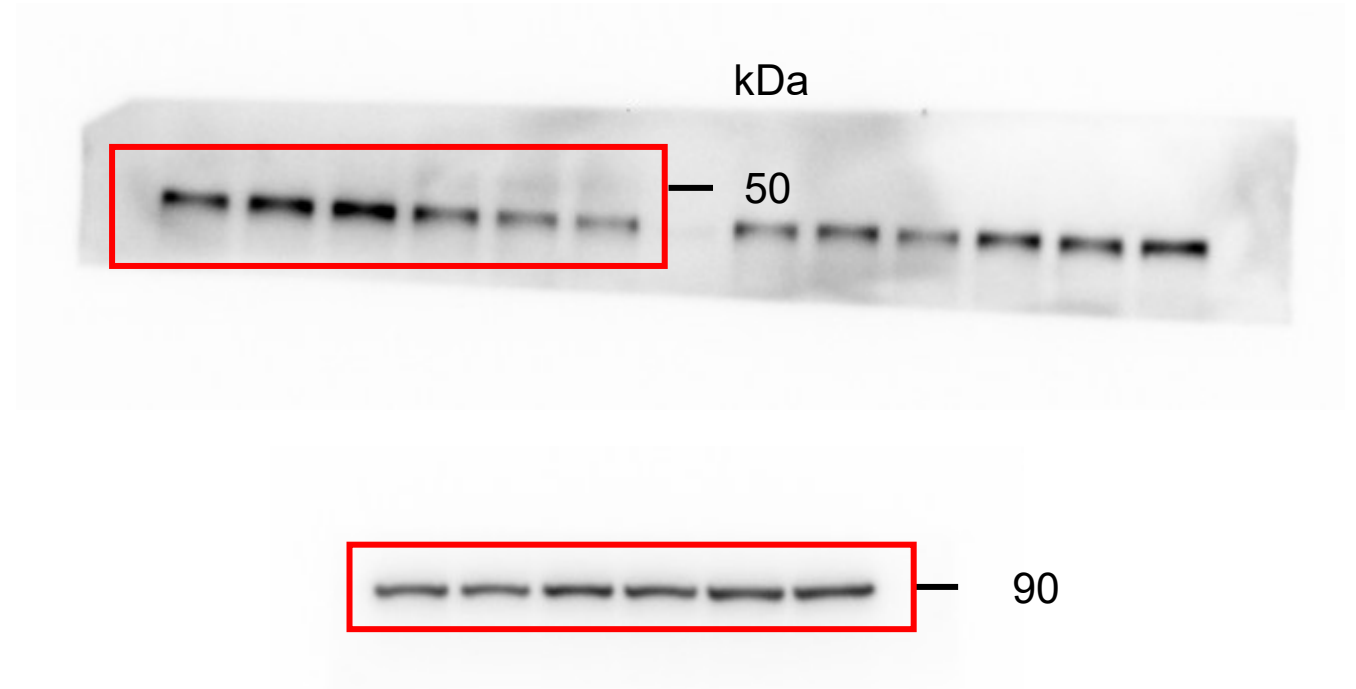
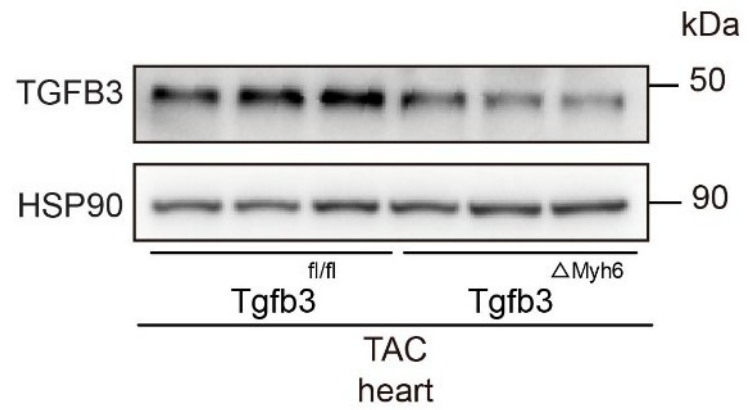


Figure 5J

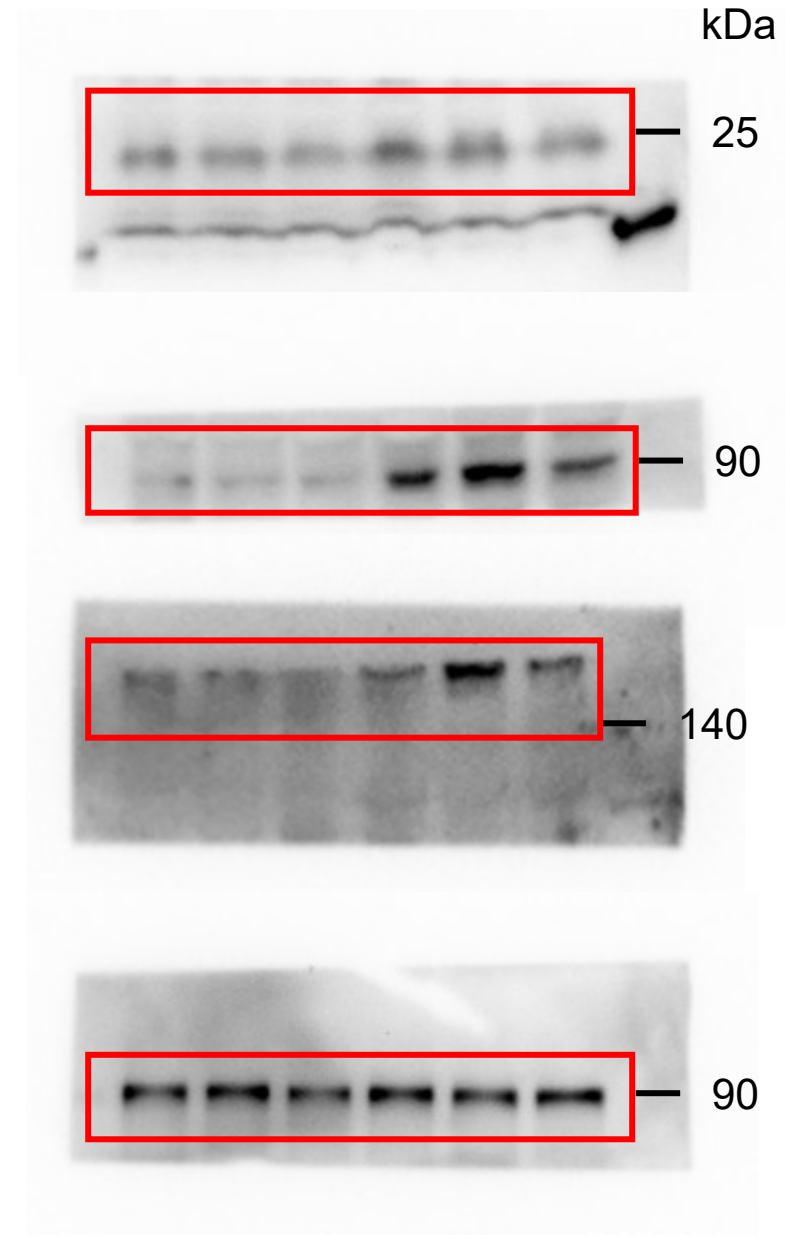
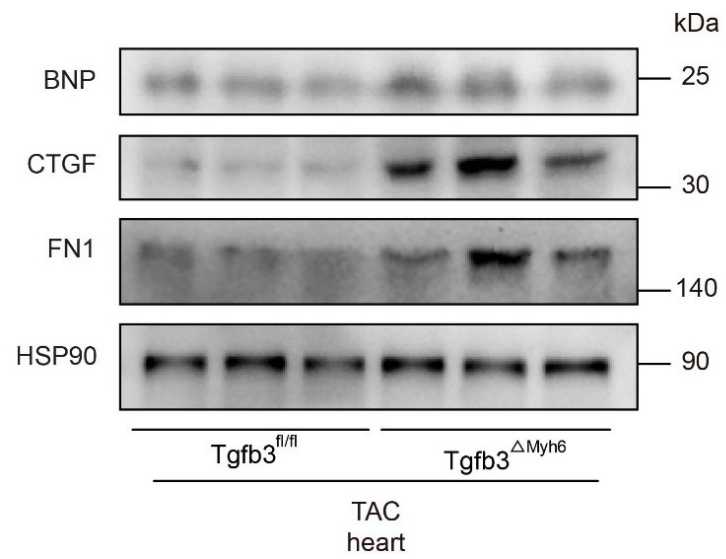


Figure 6E

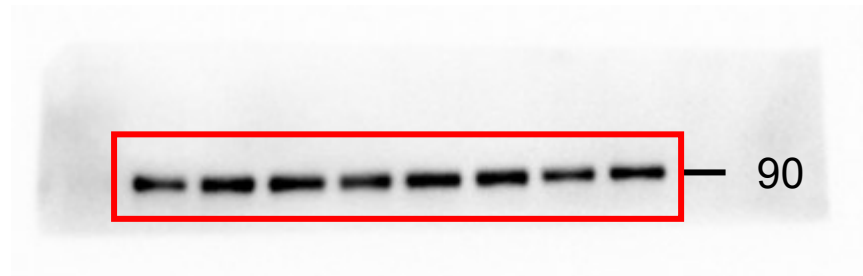
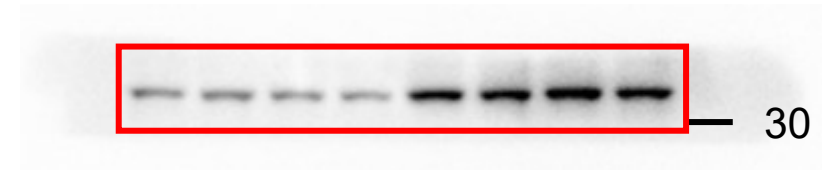
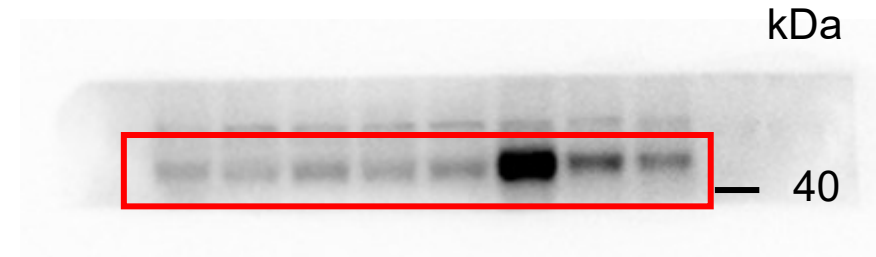
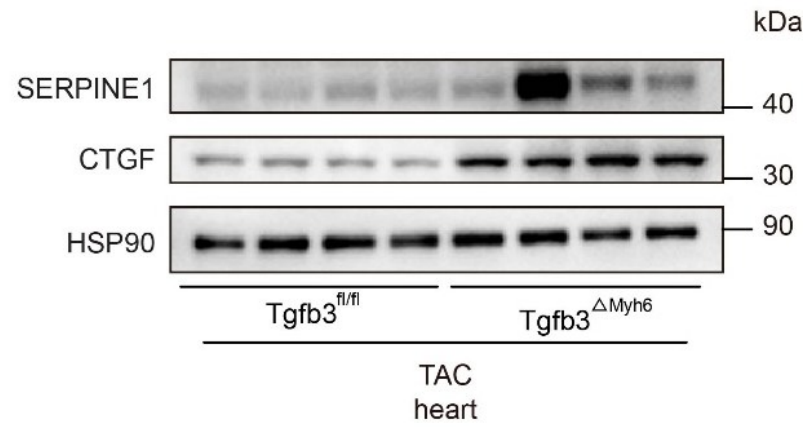


Figure 6H

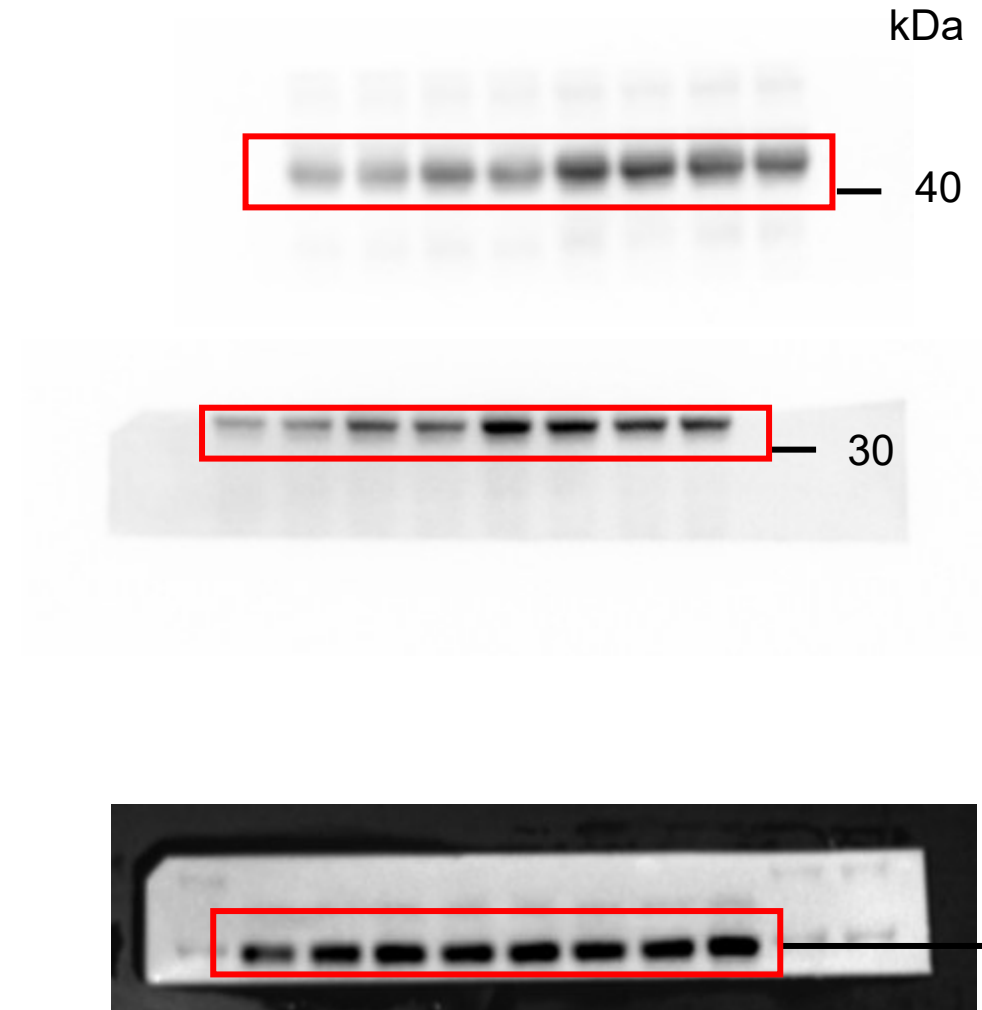
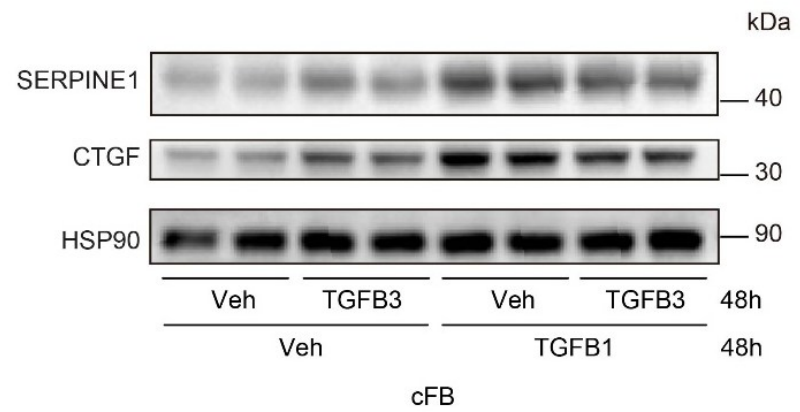
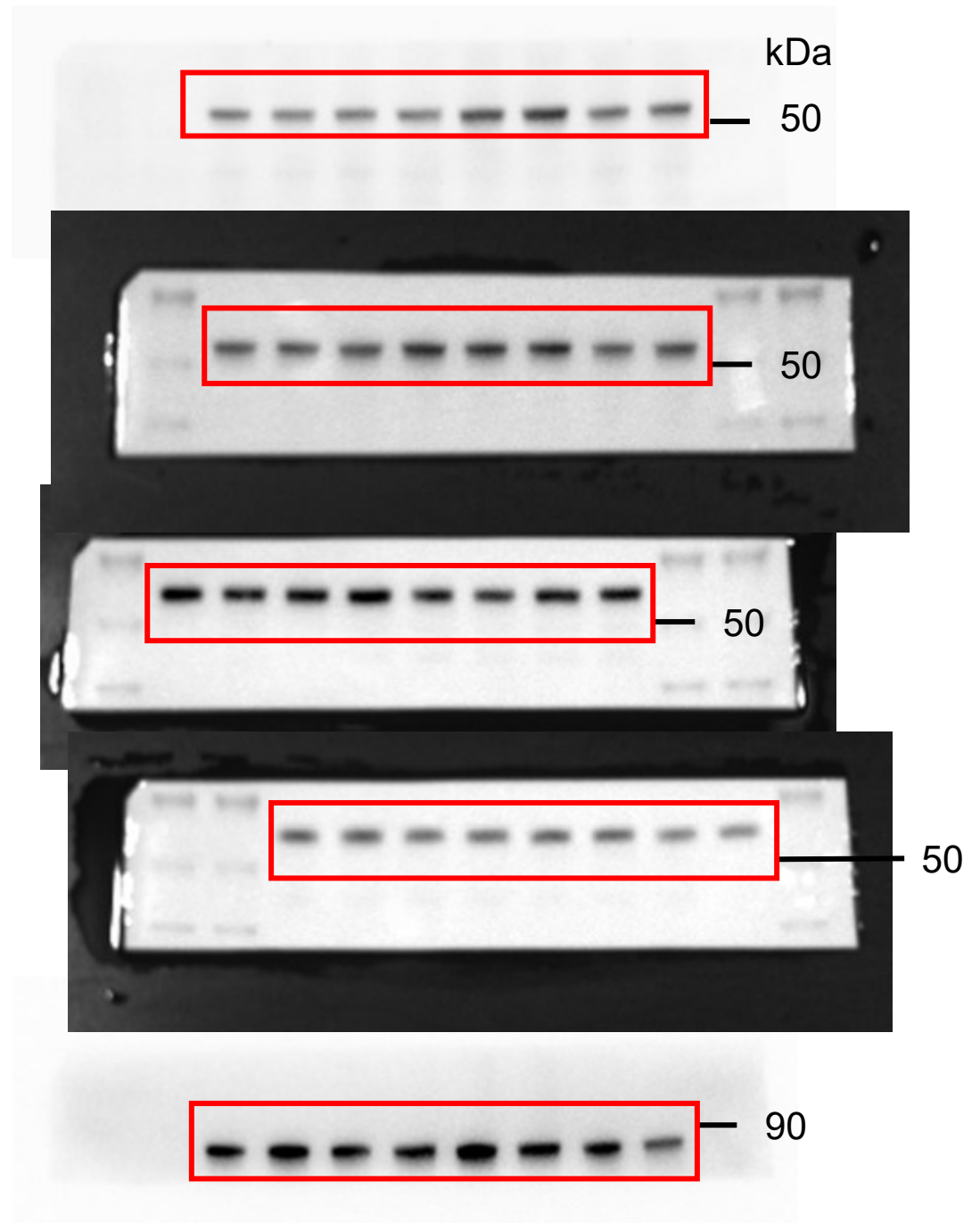
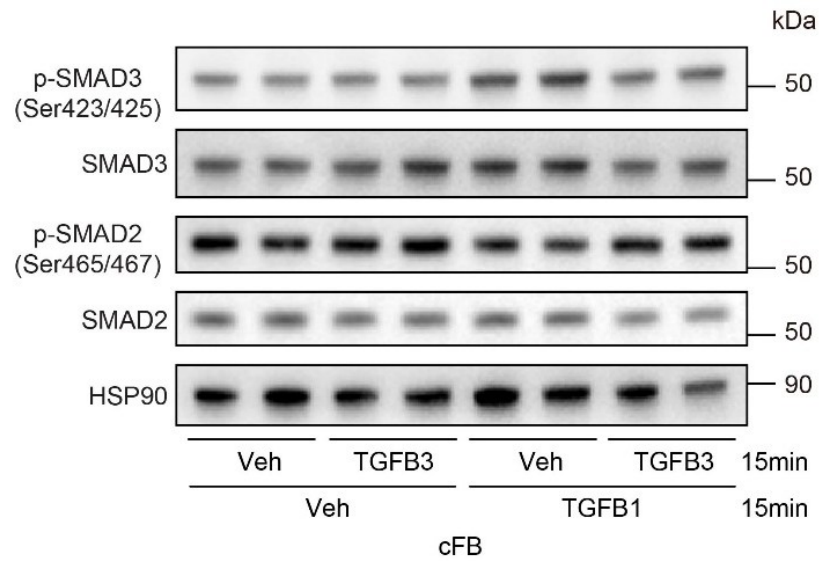


Figure 6l



# Figure 6K

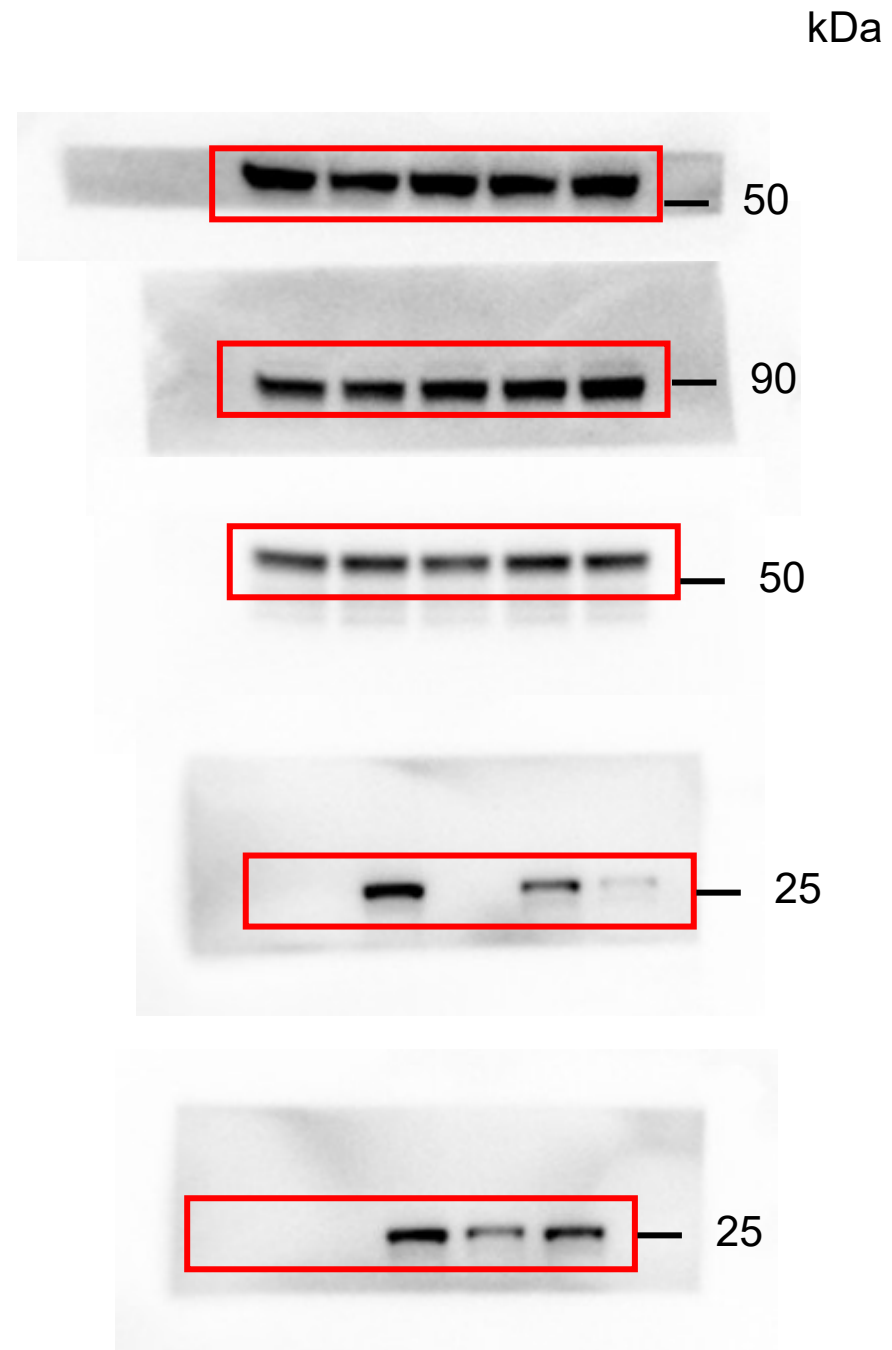
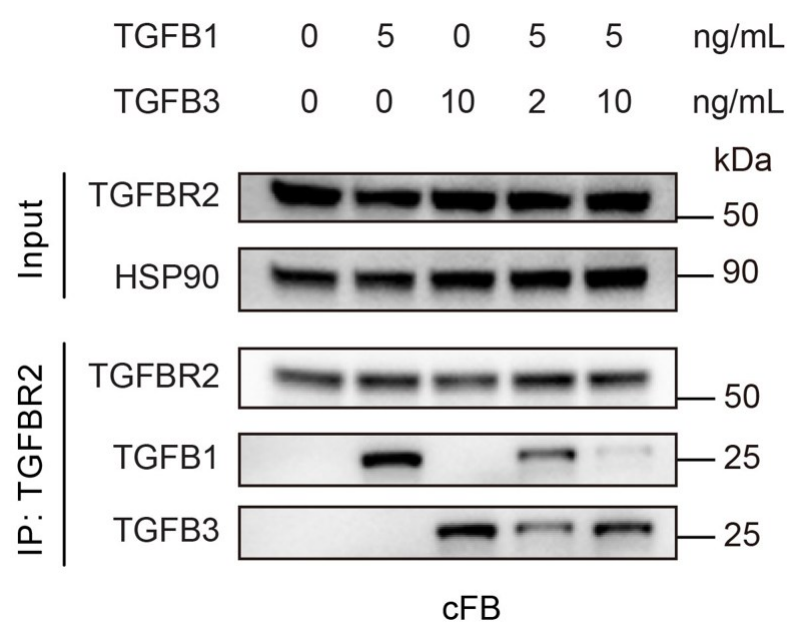


Figure S4B

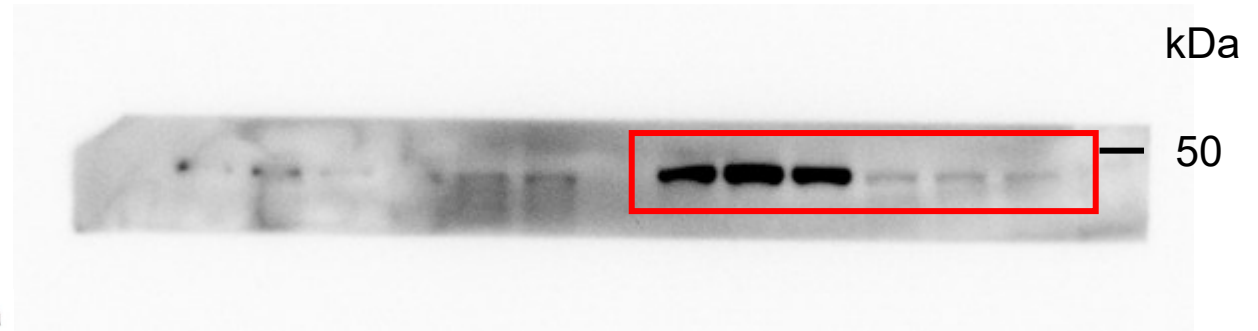
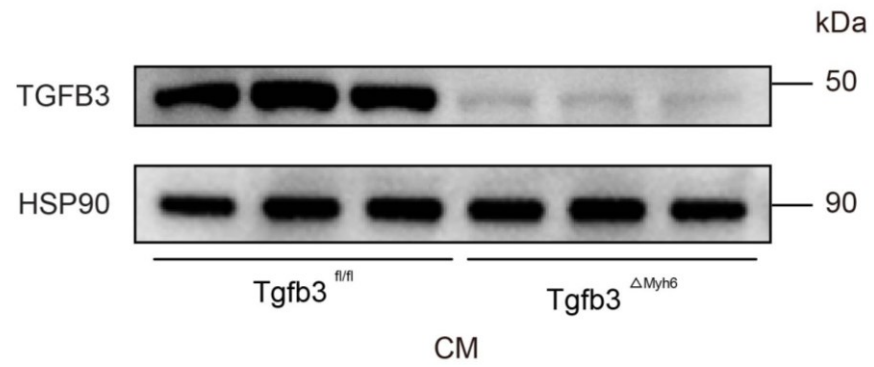


Figure S4H

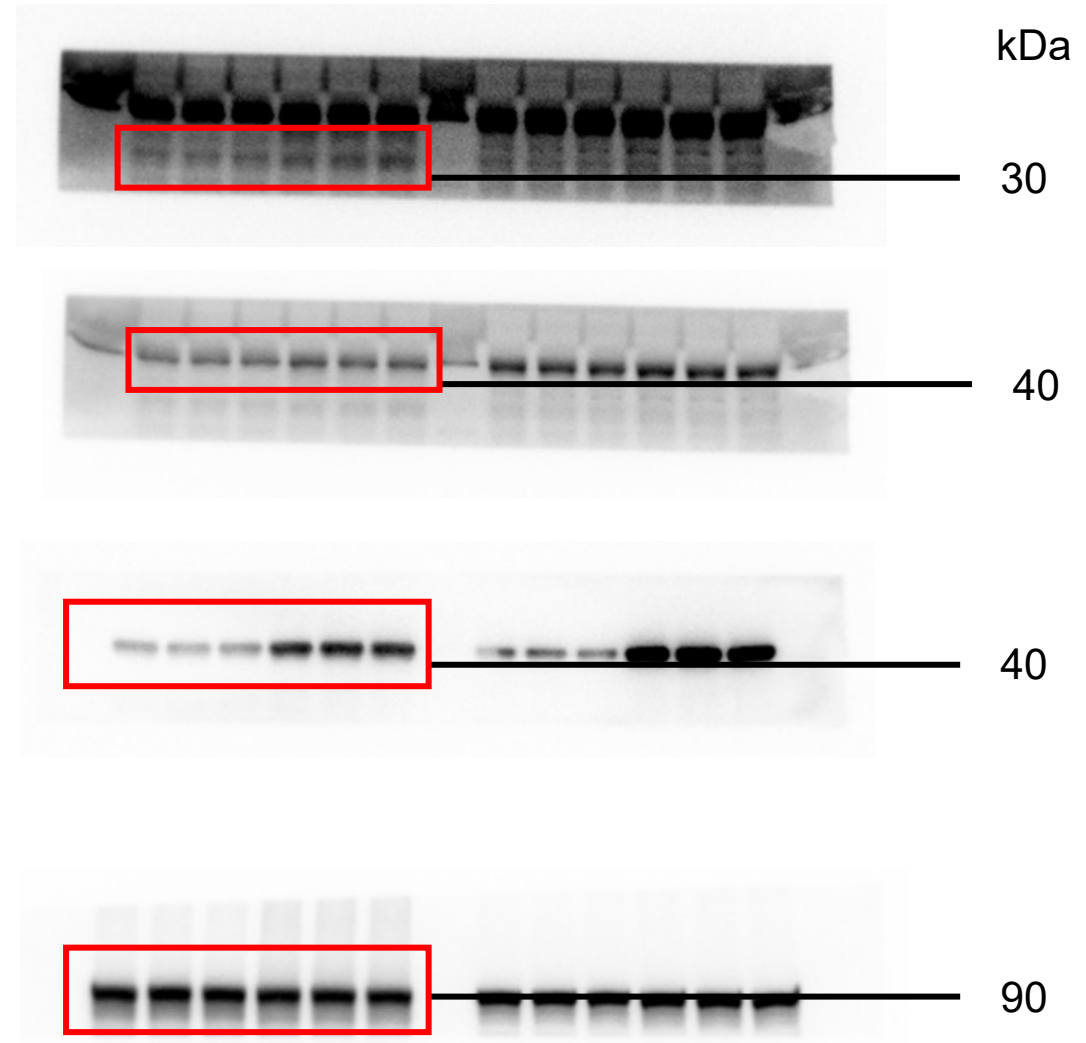
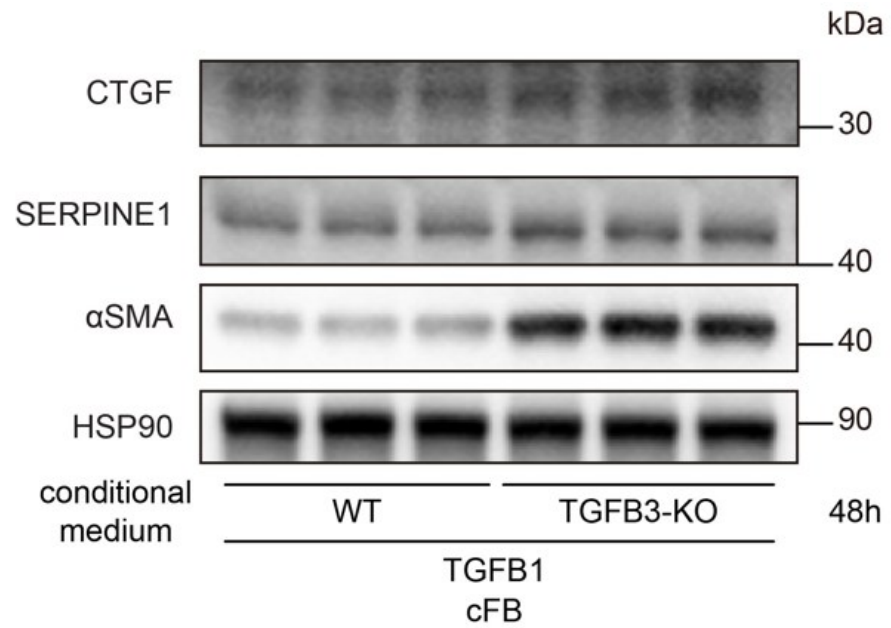


Figure S4J

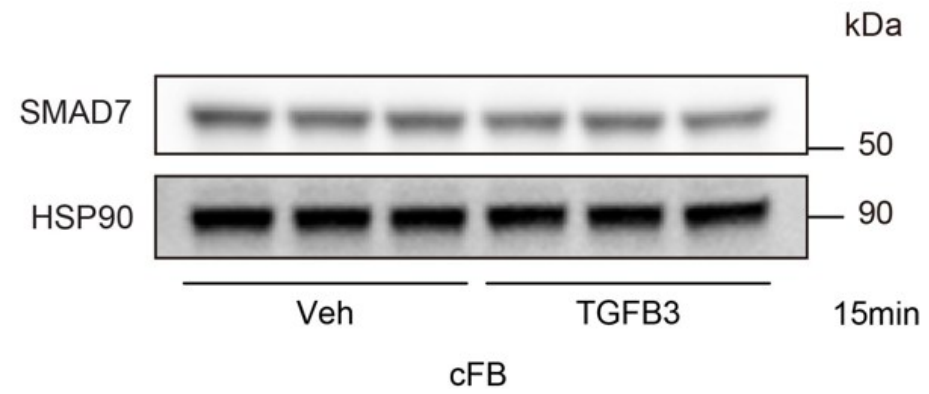


Table S1

| Antibodies         | Source      | Catalog Number | Dilution Ratio for WB | Dilution Ratio for IF |
|--------------------|-------------|----------------|-----------------------|-----------------------|
| TGFB3              | Proteintech | 18942-1-AP     | 1:1000                | 1:200                 |
| HSP90              | Proteintech | 13171-1-AP     | 1:5000                |                       |
| Cardiac Troponin I | Proteintech | 21652-1-AP     |                       | 1:1000                |
| Cardiac Troponin T | Proteintech | 68300-1-Ig     | 1:20000               |                       |
| PDGFRA             | R&D         | AF1062         |                       | 1:50                  |
| $\alpha$ SMA       | Proteintech | 67735-1-Ig     | 1:20000               | 1:500                 |
| BNP                | Invitrogen  | PA5-96084      | 1:5000                |                       |
| CTGF               | Proteintech | 25474-1-AP     | 1:2000                | 1:500                 |
| FN1                | Proteintech | 15613-1-AP     | 1:5000                |                       |
| SERPINE1           | Epizyme     | R011006        | 1:1000                |                       |
| SMAD2              | CST         | #5339          | 1:1000                |                       |
| p-SMAD2            | CST         | #18338         | 1:1000                |                       |
| SMAD3              | CST         | #9523          | 1:1000                |                       |
| p-Smad3            | CST         | #9520          | 1:1000                | 1:200                 |
| TGFBR2             | Proteintech | 66636-1-Ig     | 1:5000                |                       |

Table S2

| Gene               | Former                 | Reverse                   |
|--------------------|------------------------|---------------------------|
| mouse qPCR primers |                        |                           |
| Nppa               | GCTTCCAGGCCATATTGGAG   | GGGGGCATGACCTCATCTT       |
| Nppb               | GAGGTCACTCCTATCCTCTGG  | GCCATTTCTCCGACTTTTCTC     |
| Myh7               | ACTGTCAACACTAAGAGGGTCA | TTGGATGATTTGATCTTCCAGGG   |
| Acta1              | CCCAAAGCTAACCGGGAGAAG  | CCAGAATCCAACACGATGCC      |
| Acta2              | GTCCCAGACATCAGGGAGTAA  | TCGGATACTTCAGCGTCAGGA     |
| Meox1              | GGGGAGGATTGCATGGTACTT  | GGGTCAGGTAGTTGTGGTGG      |
| Ccn2               | CAGGTCCCATCAGCCTCG     | CTGCAGTCCTGGCCATA         |
| Col1a1             | AAGAGGCGAGAGAGGTTTCC   | AGAACCATCAGCACCTTTGG      |
| Col3a1             | CTGTAACATGGAACTGGGGAAA | CCATAGCTGAACTGAAAACCACC   |
| Tgfb1              | GAGCCCGAAGCGGACTACTA   | TGGTTTTCTCATAGATGGCGTTG   |
| Tgfb2              | CTTCGACGTGACAGACGCT    | GCAGGGGCAGTGTAACCTTATT    |
| Tgfb3              | CAGGCCAGGGTAGTCAGAG    | ATTTCCAGCCTAGATCCTGCC     |
| Serpine1           | TGAGCGACAGCCAACAAGAGCC | ACCCTTTCCCAGAGACCAGAACCAG |
| BNP                | GAGGTCACTCCTATCCTCTGG  | GCCATTTCTCCGACTTTTCTC     |
| human qPCR primers |                        |                           |
| Nppa               | CAACGCAGACCTGATGGATTT  | AGCCCCCGCTTCTTCATTC       |
| Nppb               | TGGAAACGTCCGGGTACAG    | CTGATCCGGTCCATCTTCCT      |
| Myh7               | ACTGCCGAGACCGAGTATG    | GCGATCCTTGAGGTTGTAGAGC    |
| Acta1              | GGCATTACAGAGACCACCTAC  | CGACATGACGTTGTTGGCATA     |
| Acta2              | GTGTTGCCCCTGAAGAGCAT   | GCTGGGACATTGAAAGTCTCA     |
| Postn              | CTCATAGTCGTATCAGGGGTCG | ACACAGTCGTTTTCTGTCCAC     |
| Ccn2               | CGCACAAGGGCCTATTCTGT   | AGCACCATCTTTGGCGGT        |
| Col1a1             | GTGCGATGACGTGATCTGTGA  | CGGTGGTTTCTTGGTCGGT       |
| Col3a1             | GGAGCTGGCTACTTCTCGC    | GGGAACATCCTCCTTCAACAG     |
| Tgfb1              | CTAATGGTGGAAACCCACAACG | TATCGCCAGGAATTGTTGCTG     |
| Tgfb2              | CCCCGGAGGTGATTTCCATC   | TGGCATCAAGGTACCCACAG      |
| Tgfb3              | GGAAAACACCGAGTCGGAATAC | GCGGAAAACCTTGGAGGTAAT     |

Table S3

| NAME | Age | Sex    | Height (cm) | Weight (kg) | Diagnosis                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | NT-proBNP (pg/ml) | Preoperative LVEF (%) | Group         |
|------|-----|--------|-------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------------|---------------|
| N/A  | 36  | Male   | 171         | 65          | 1. Dilated Cardiomyopathy [Congestive Cardiomyopathy] 2. Heart Failure 3. Cardiac Function Class IV (NYHA Classification) 4. Mitral Regurgitation 5. Ventricular Thrombus                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 4963.55           | 16                    | heart failure |
| N/A  | 55  | Male   | 175         | 70          | 1. Valvular Heart Disease 2. Hypertrophic Cardiomyopathy 3. Left Ventricular Outflow Tract Obstruction 4. Mitral Regurgitation 5. Tricuspid Regurgitation 6. Ascending Aortic Dilation 7. Heart Failure 8. Cardiac Function Class III (NYHA Classification) 9. Hypertension                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 322.79            | 49                    | heart failure |
| N/A  | 28  | Male   | 175         | 65          | 1. Valvular Heart Disease 2. Aortic Valve Stenosis 3. Aneurysm 4. Ascending Aortic Dilation 5. Heart Failure 6. Cardiac Function Class III (NYHA Classification)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 4613.15           | 48                    | heart failure |
| N/A  | 44  | Female | 166         | 57          | 1. Valvular Heart Disease 2. Heart Failure 3. Rheumatic Mitral Stenosis with Regurgitation 4. Aortic Valve Stenosis with Regurgitation 5. Tricuspid Regurgitation 6. Pulmonary Hypertension 7. Patent Foramen Ovale (PFO) 8. Cardiac Function Class III (NYHA Classification)                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 1234.14           | 46                    | heart failure |
| N/A  | 71  | Male   | 175         | 73          | 1. Valvular Heart Disease 2. Heart Failure 3. Mitral Regurgitation 4. Tricuspid Regurgitation 5. Coronary Atherosclerosis 6. Cardiac Function Class III-IV (NYHA Classification) 7. Hypertension 8. Lacunar Infarction                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 5439.4            | 39                    | heart failure |
| N/A  | 73  | Male   | 173         | 70          | 1. Rheumatic Aortic Valve Stenosis with Regurgitation 2. Severe Aortic Valve Stenosis 3. Moderate Aortic Valve Regurgitation 4. Rheumatic Mitral Stenosis 5. Rheumatic Mitral Regurgitation 6. Mild to Moderate Mitral Regurgitation 7. Rheumatic Tricuspid Regurgitation 8. Moderate Tricuspid Regurgitation 9. Persistent Atrial Fibrillation 10. Rheumatic Heart Disease 11. Valvular Heart Disease 12. Possible Left Atrial Thrombus 13. Heart Failure 14. Cardiac Function Class III (NYHA Classification) 15. Cerebral Infarction (Recovery Phase) 16. Hypertension Grade 2 (High Risk) 17. Multiple Large Artery Occlusions and Stenoses 18. History of Gastric Hemorrhage 19. Hemoptysis 20. Chronic Renal Insufficiency | 1038.07           | 47                    | heart failure |

|     |    |        |     |    |                                                                                                                                                                                                                                                                                                                                           |         |    |               |
|-----|----|--------|-----|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|----|---------------|
| N/A | 60 | Male   | 172 | 75 | 1. Valvular Heart Disease 2. Hypertrophic Cardiomyopathy 3. Left Ventricular Outflow Tract Obstruction 4. Mitral Regurgitation with Aortic Valve Stenosis and Regurgitation 5. Tricuspid Regurgitation 6. Post Cardiac Interventional Closure Status 7. Heart Failure 8. Cardiac Function Class III (NYHA Classification) 9. Hypertension | 1274.39 | 45 | heart failure |
| N/A | 44 | Female | 160 | 52 | 1. Valvular Heart Disease 2. Mitral Regurgitation 3. Tricuspid Regurgitation 4. Cardiac Function Class II-III (NYHA Classification) 5. Left Ventricular Outflow Tract Muscle Bundle Hypertrophy 6. Hypertension                                                                                                                           | 99.15   | 64 | control       |
| N/A | 42 | Male   | 170 | 70 | 1. Cardiomyopathy 2. Mitral Regurgitation 3. Cardiac Function Class II-III (NYHA Classification)                                                                                                                                                                                                                                          | 67.75   | 62 | control       |
| N/A | 54 | Male   | 175 | 75 | 1. Hypertrophic Obstructive Cardiomyopathy 2. Mitral Regurgitation 3. Aortic Valve Regurgitation 4. Malignant Neoplasm of Esophagus 5. Anemia 6. Hypercholesterolemia 7. Hypertension                                                                                                                                                     | 75.39   | 64 | control       |
| N/A | 72 | Female | 164 | 55 | 1. Valvular Heart Disease 2. Hypertrophic Obstructive Cardiomyopathy 3. Mitral Regurgitation 4. Tricuspid Regurgitation 5. Cardiac Function Class III (NYHA Classification)                                                                                                                                                               | 88.19   | 61 | control       |
| N/A | 69 | Female | 168 | 62 | 1. Valvular Heart Disease 2. Hypertrophic Obstructive Cardiomyopathy 3. Mitral Regurgitation 4. Cardiac Function Class III (NYHA Classification)                                                                                                                                                                                          | 72.58   | 63 | control       |
| N/A | 48 | Male   | 175 | 70 | 1. Aortic Valve Stenosis with Insufficiency 2. Valvular Heart Disease 3. Bicuspid Aortic Valve 4. Cardiac Function Class II-III (NYHA Classification) 5. Hypertension 6. Post Cardiac Radiofrequency Ablation Status                                                                                                                      | 68.45   | 65 | control       |
| N/A | 61 | Female | 160 | 60 | 1. Valvular Heart Disease 2. Hypertrophic Obstructive Cardiomyopathy 3. Cardiac Function Class II-III (NYHA Classification) 4. Mitral Regurgitation 5. Hypertension Stage 2                                                                                                                                                               | 91.26   | 60 | control       |