Supplemental information

Cardiac mast cells cause atrial fibrillation through PDGF-A-mediated fibrosis in pressure-overloaded hearts

Chien-hui Liao, Hiroshi Akazawa, Masaji Tamagawa, Kaoru Ito, Noritaka Yasuda, Yoko Kudo, Rie Yamamoto, Yukako Ozasa, Masanori Fujimoto, Ping Wang, Hiromitsu Nakauchi, Haruaki Nakaya, Issei Komuro

SUPPLEMENTAL METHODS

ECG telemetry

A 24-h ambulatory ECG trace was obtained using the transmitters (TA10ETA-F20, Data Sciences International) implanted in the abdominal cavity of TAC- and sham-operated mice with subcutaneous electrodes in a lead-II configuration. The ECG signal from the transmitter was monitored by the telemetry receiver (PRC-1, Data Sciences International) placed underneath the animal's cage. The data were then digitalized and analyzed using the A/D convertor (PowerLab, ADInstruments) and software (Chart v5.0.1, ADInstruments). Data acquisition was initiated at 24 h after implantation of the transmitter.

Measurement of cardiomyocyte cross-sectional area

Sections of ventricle at 5 μm were stained with tetramethylrhodamine isothiocyanate (TRITC) -conjugated wheat germ agglutinin (Sigma) and Hoechst 33258. Suitable cross sections were defined as having round-to-oval cardiomyocytes sections and nearly round-shape capillaries that perfused in the region. A cross-sectional area of myocytes in the left ventricular wall was determined by tracing TRITC-labeled lectin with use of image processing software (Adobe Photoshop CS4, Adobe Systems).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. TAC-operated mice do not develop spontaneous AF.

(A) ECG sample in lead-II deflection recorded for conscious sham-operated mice by telemetry.

(B) ECG sample in lead-II deflection recorded for conscious TAC-operated mice by telemetry.

Supplemental Figure 2. Cromolyn treatment does not affect hypertrophic growth of cardiomyocytes after TAC operation.

(A) Sections of left ventricle stained with TRITC-conjugated wheat germ agglutinin and Hoechst 33258. Cell boundaries and nuclei are stained in red and blue, respectively. Scale bars, 5 μm.

(B) Cross-sectional areas of ventricular myocytes (100 individual cardiomyocytes in each group). The data are presented as mean ± s.e.m. $^{**}P<0.01$.

Supplemental Figure 3. The mRNA expressions of fibrogenic cytokines in BMMCs after co-culture with cardiac myocytes or fibroblasts.

(A) The mRNA expressions of fibrogenic cytokines in BMMCs at baseline, 6 h and 24 h after co-culture with cardiac myocytes. Experiments were repeated four times in triplicate, and the data are presented as mean ± s.e.m.

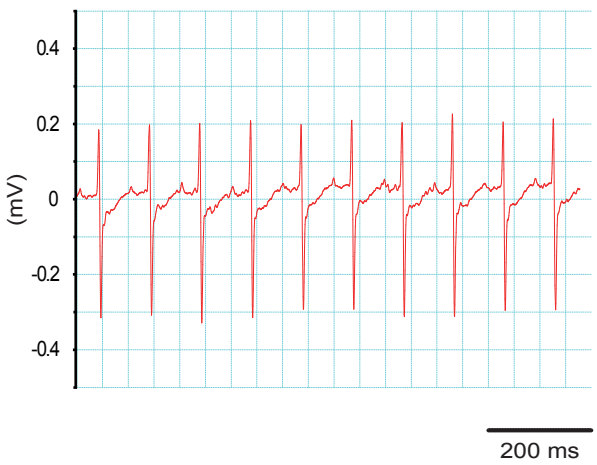
(B) The mRNA expressions of fibrogenic cytokines in BMMCs at baseline, 6 h and 24 h after co-culture with cardiac fibroblasts. Experiments were repeated five times in

triplicate, and the data are presented as mean ± s.e.m. $^{**}P<0.01$ versus baseline.

The primer sequences and Universal Probe numbers were designed with the ProbeFinder software as following: *Tgfb1*, 5'-tggagcaacatgtggaactc-3' and 5'-cagcagccggttaccaag-3', No. 72; *Tnfa*, 5'-tgcctatgtctcagcctcttc-3' and 5'-gaggccatttgggaacttct-3', No. 49; *Il6*, 5'-gctaccaaactggatataatcagga-3' and 5'-ccaggtagctatggtactccagaa-3', No. 6; *Il1a*, 5'-ttggttaaatgacctgcaaca-3' and 5'-gagcgctcacgaacagttg-3', No. 52; *Il1b*, 5'-tgtaatgaaagacggcacacc-3' and 5'-tcttctttgggtattgcttgg-3', No. 78; *Ifng*, 5'-atctggaggaactggcaaaa-3' and 5'-ttcaagacttcaaagagtctgaggta-3', No. 21; *fgf2*, 5'-cggctctactgcaagaacg-3' and 5'-tgcttggagttgtagtttgacg-3', No. 4; mouse *Gapdh*, 5'- tgtccgtcgtggatctgac-3' and 5'-cctgcttcaccaccttcttg-3', No. 80.

Supplemental Figure 1

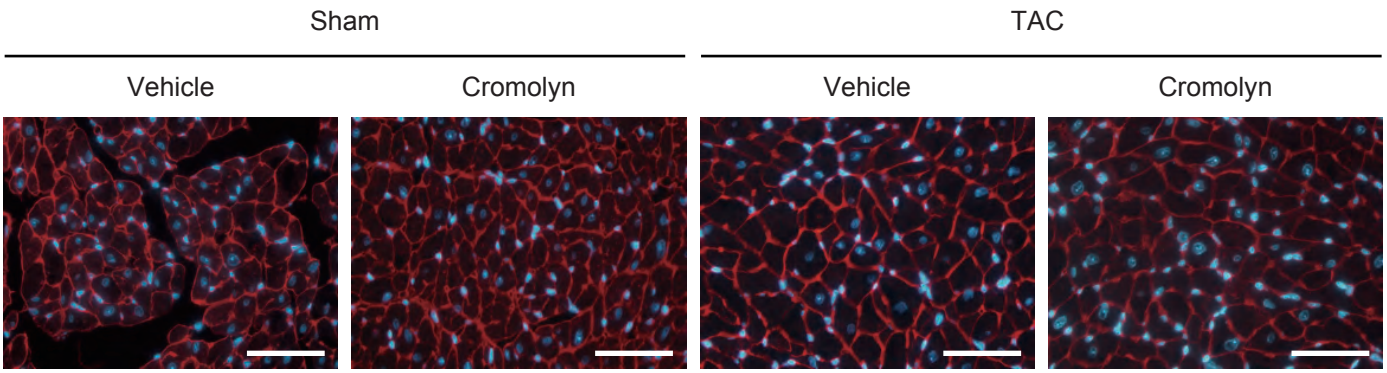
A.



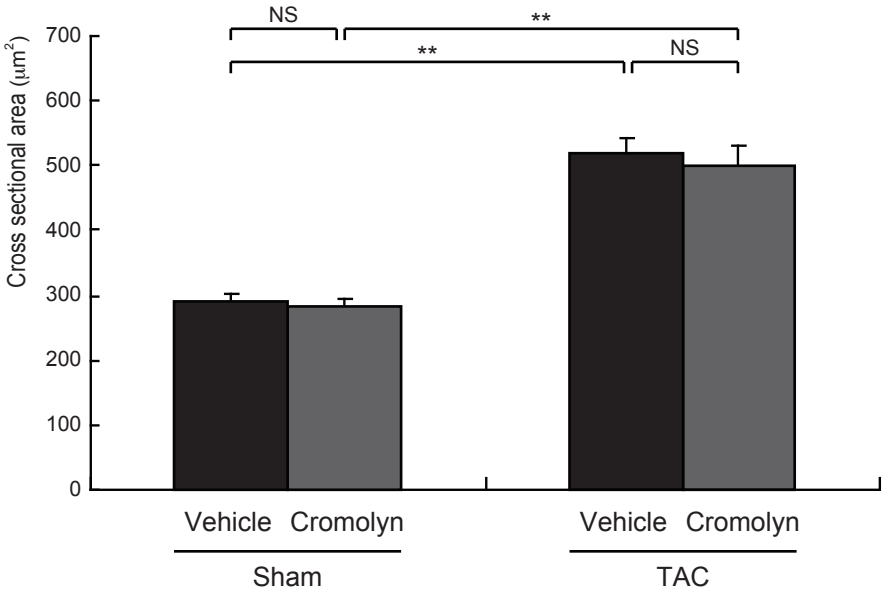
B.

Supplemental Figure 2

A.



B.



Supplemental Figure 3