Supplemental Data

1. Materials and Methods

1.1 Animal models

Ventricular tachypaced (VTP)-dogs were anesthetized under diazepam (0.25 mg/kg IV)/ketamine (5.0 mg/kg IV)/halothane (1% to 2% PI) anesthesia, intubated and ventilated. Bipolar pacing leads were fixed to the right-ventricular apex via the left jugular vein, and connected to pacemakers (St. Jude Medical, Minneapolis, MN) implanted subcutaneously in the neck. Following twenty-four hours for postoperative recovery, ventricular-pacing was initiated at 240 bpm. The duration of AF induced by 10 Hz burst pacing was measured repeatedly to measure mean AF-duration as an index of AF-sustainability. The AF-duration for 10 inductions was averaged for each dog. AF-duration was measured during open-chest terminal study under morphine (2 mg/kg im)/alpha-chloralose (120 mg/kg iv) anesthesia, with the heart exposed through a left thoracotomy. Following these measurements, the heart was removed for tissue/cell isolation and study.

All experimental mice were fed and housed under a 12:12-hour light/dark cycle at 21°C and 30% humidity. Mice were housed in individual cages and had free access to tap water and food. MI mice were anesthetized with 2% isoflurane, intubated, and ventilated after preoperative buprenorphine (0.1 mg/kg S.C.) injection. The thorax was shaved and sterilized with 2% w/v chlorhexidine gluconate in 70% v/v isopropyl alcohol. Body temperature was maintained at 37°C on a heating pad (Harvard Apparatus, Holliston, MA). The beating heart was accessed via a left thoracotomy. The left anterior descending coronary artery was then ligated with 10-0 silk. Ligation was confirmed by the whitening of a region of the left ventricle, immediately post-ligation. MI mice were compared with sham controls that underwent similar procedures but

without left anterior descending artery ligation. The chest was closed with 5-0 silk and the skin closed with autoclips. The animals were placed in a prone position until the occurrence of spontaneous breathing. Additional doses of ketoprofen (5 mg/kg) and buprenorphine (0.1 mg/kg) were administered subcutaneously immediately after surgery, and 18 hours postoperatively. Euthanasia for cardiac tissue procurement was performed under deep isoflurane anesthesia (absent pain reflexes) followed by cervical dislocation.

1.2 Mouse echocardiography

Echocardiography was performed under 2%-isoflurane anesthesia. Left ventricular (LV) regional wall motion was scored in LV short axis view at the level of the papillary muscle for the 6 segments viewed in this view as follows: (1) normal, (2) hypo-kinesia, (3) akinesia, (4) dyskinesia, and (5) aneurysmal. Wall motion score index (WMSI) was the mean value of all scores. LV area at end cardiac diastole (LVA_d) and systole (LVA_s) were measured in this view. LV fractional area change was calculated as FAC = (LVA_d-LVA_s) X 100%. Thickness of LV anterior and posterior wall at end cardiac diastole ($LVAW_d$, $LVPW_d$), LV dimension at end cardiac diastole (LVD_d) and systole (LVD_s) were measured by M-mode echocardiography (M-mode) also in this view. LV fractional shortening (FS) and ejection fraction (EF) were obtained by software algorithms within the Vivid 7 system. LV mass was calculated using the formula developed by Liao Y et al, and indexed to body weight (BW).[1] Left atrial dimension at end cardiac systole (LAD_s) and diastole (LAD_d) were measured by M-mode in parasternal long axis view. The average of 3 consecutive cardiac cycles was used for each measurement, with the operator being blinded to treatment assignment.

1.3 Mouse electrocardiography

At baseline and 2 weeks post-MI, mice were anesthetized with 2% isoflurane for electrocardiogram (ECG) recording. Body temperature was maintained at 37°C with a heating pad. A surface ECG (lead I) was obtained with four 25-gauge subcutaneous electrodes and transmitted to a computer via an analogue-digital converter (IOX v1.585, EMKA Technologies) for monitoring and later analysis with ECG-Auto 2.8.1.18 software (EMKA Technologies). Recordings were filtered between 0.5 and 500 Hz. Measurements were based on averages of a minimum of 10 complexes. Standard criteria were used to measure *P*-wave duration.

1.4 Canine fibroblast and cardiomyocyte isolation

The heart was removed after intra-atrial injection of heparin (10,000 U), immersed in 2 mmol/L Ca^{2+}- containing Tyrode's solution containing (in mM): NaCl 136, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 2, NaH_2PO_4 0.33, HEPES 5 and dextrose 10, pH 7.35 (NaOH). The left circumflex coronary artery was cannulated and all leaking branches were ligated, followed by perfusion with Ca^{2+}-free Tyrode's solution for 10 minutes. The preparation was then perfused at 10 mL/min with Ca^{2+}-free Tyrode solution containing type II collagenase (0.48 mg/mL, Worthington, OH) and albumin (0.1%, Bioshop Canada Inc. Burlington, ON) for one hour. The harvested cells were collected in DMEM medium and dispersed by gentle trituration with a pipette. Filtration (500-μm nanomesh) was used to remove debris and cells were centrifuged at 800 rpm for 5 minutes to pellet cardiomyocytes. The supernatant was collected and filtered through 20 μm nanomesh and centrifuged at 2,000 rpm for 10 minutes to pellet fibroblasts. Pelleted, freshly isolated fibroblasts and cardiomyocytes were immediately frozen in liquid-N_2 and stored for RNA extraction.

1.5 Protein extraction and immunoblots

Protein extracts (30 μg) of snap-frozen LA and LV tissue from dogs or cultured fibroblasts were separated by electrophoresis on 12% SDS-PAGE (Bio-Rad) and transferred to PVDF membranes (EMD Millipore, Billerica, MA). For secreted collagen type I, supernatants of medium (32 μl) from cultured fibroblasts were separated by electrophoresis on 7.5% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad). Membranes were blocked and incubated overnight with the primary antibodies and then with secondary antibodies. All of the primary antibodies are listed in Table 1. Secondary antibodies were horseradish peroxidise–conjugated anti-mouse or anti-rabbit (1:10000; Jackson Immuno Research Laboratories). Antibody signals were visualized with enhanced chemiluminescence (PerkinElmer, Waltham, MA). Data were normalized to GAPDH band intensity from the same samples on the same membranes and/or normalized to cell number (medium supernatant).

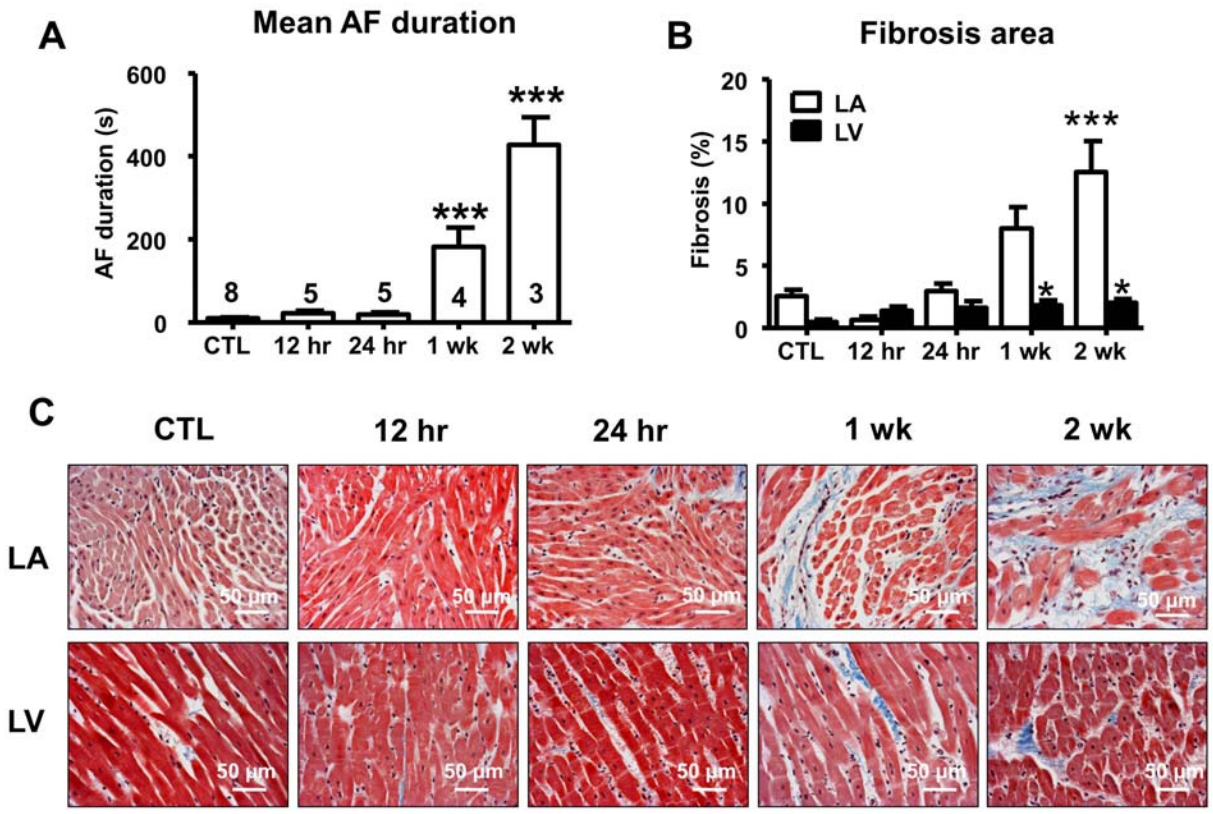
Supplemental Table 1. Primary antibodies.

Name (anti-)	Species	Type	Dilution	Source
PDGFR-α	Rabbit	Polyclonal	1:2,000	Santa Cruz Biotechnology, Santa Cruz, CA
PDGFRβ	Rabbit	Polyclonal	1:2,000	Santa Cruz Biotechnology
STAT3	Rabbit	Polyclonal	1:2,000	Cell signaling Technology, Danvers, Mass
Phosphorylated - STAT3	Mouse	Monoclonal	1:2,000	Cell signaling Technology
Collagen type I	Rabbit	Polyclonal	1:25,000	MD Biosciences, Zurich Switzerland
GAPDH	Mouse	Monoclonal	1:10,000	Research Diagnostics Inc. RDI, Fitzgerald Industries, NJ

References

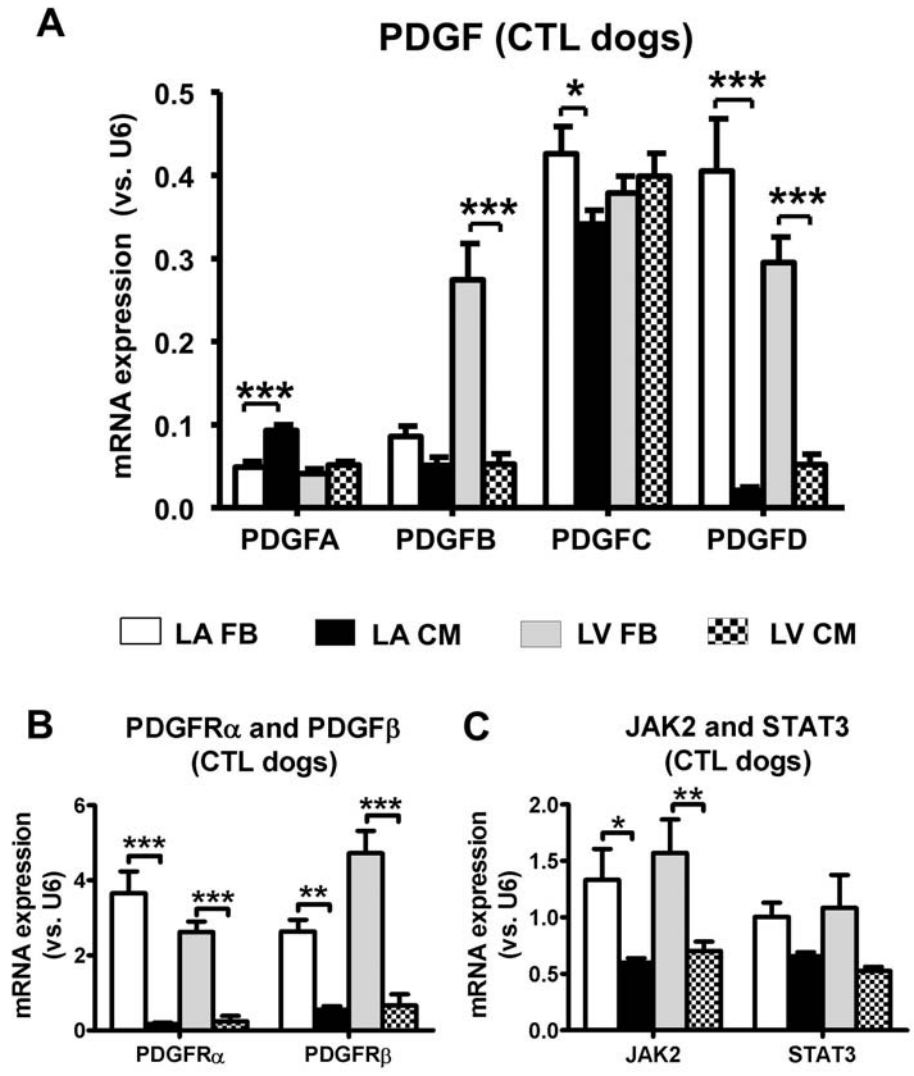
1. Liao Y, Ishikura F, Beppu S, Asakura M, Takashima S, Asanuma H, Sanada S, Kim J, Ogita H, Kuzuya T, Node K, Kitakaze M, Hori M. Echocardiographic assessment of LV hypertrophy and function in aortic-banded mice: necropsy validation. *Am J Physiol Heart Circ Physiol*. 2002 **282**:H1703-H1708.

Supplemental Figure 1



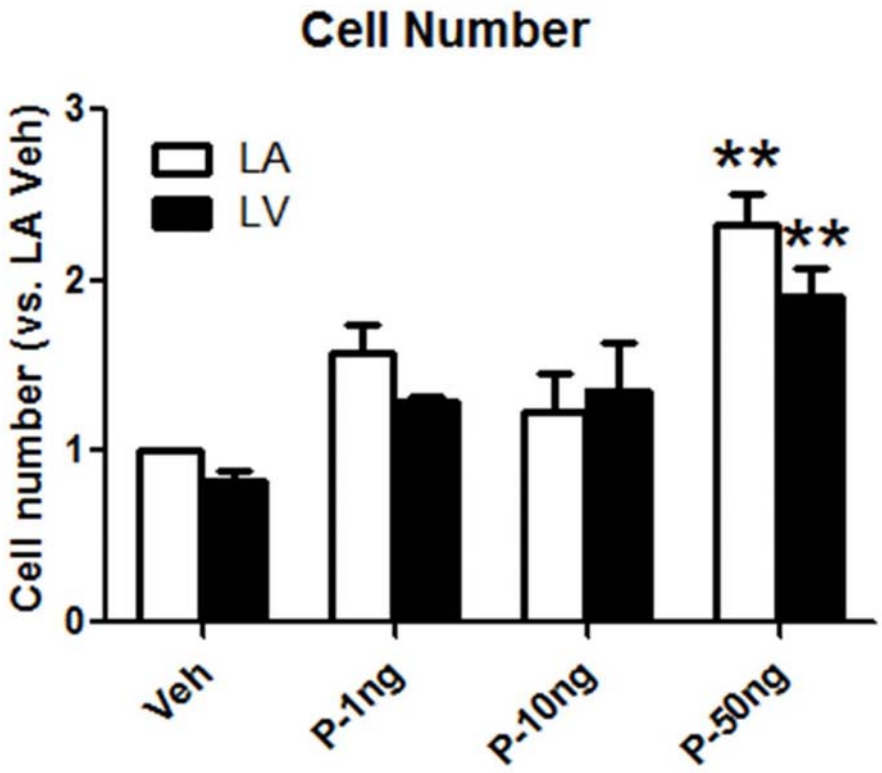
Supplemental Figure 1. Ventricular tachypacing (VTP) dogs developed progressive AF susceptibility and atrial fibrosis. (A) Mean AF duration increased substantially at 1-week and 2-week VTP. *n*'s are shown on corresponding bars. (B) Quantification of fibrosis during the course of CHF induced by VTP. (C) Representative histological tissue sections (Masson Trichrome) of left atria (LA) and left ventricles (LV). Area stained with blue color is collagen. Mean±SEM, *n*=3-8/group, **P*<0.05, ***P*<0.01, ****P*<0.001 vs. corresponding CTL. One-way ANOVA with Dunnett's tests was used for statistical analysis. AF, atrial fibrillation; CTL, non-pacing control.

Supplemental Figure 2

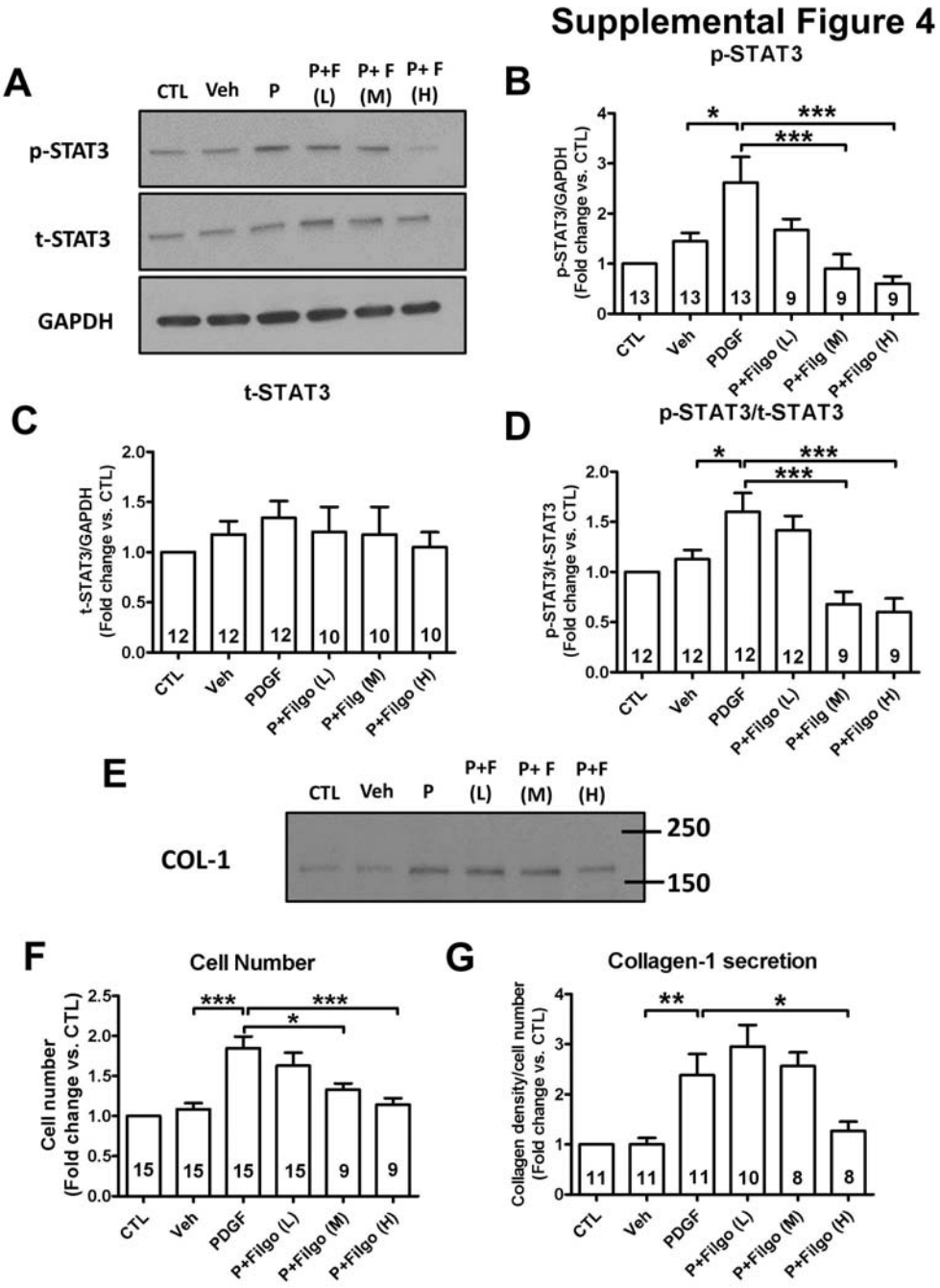


Supplemental Figure 2. mRNA expression of (A) PDGF isoforms, (B) PDGF receptor (PDGFR) isoforms, and (C) JAK2 and STAT3 in fibroblasts and cardiomyocytes from control dogs. Mean±SEM, n=4/group. *P<0.05, **P<0.01, ***P<0.001. One-way One-way ANOVA with Bonferroni-corrected t-tests was used for statistical analysis. LA, left atrium; LV, left ventricle; FB, fibroblast; CM, cardiomyocyte.

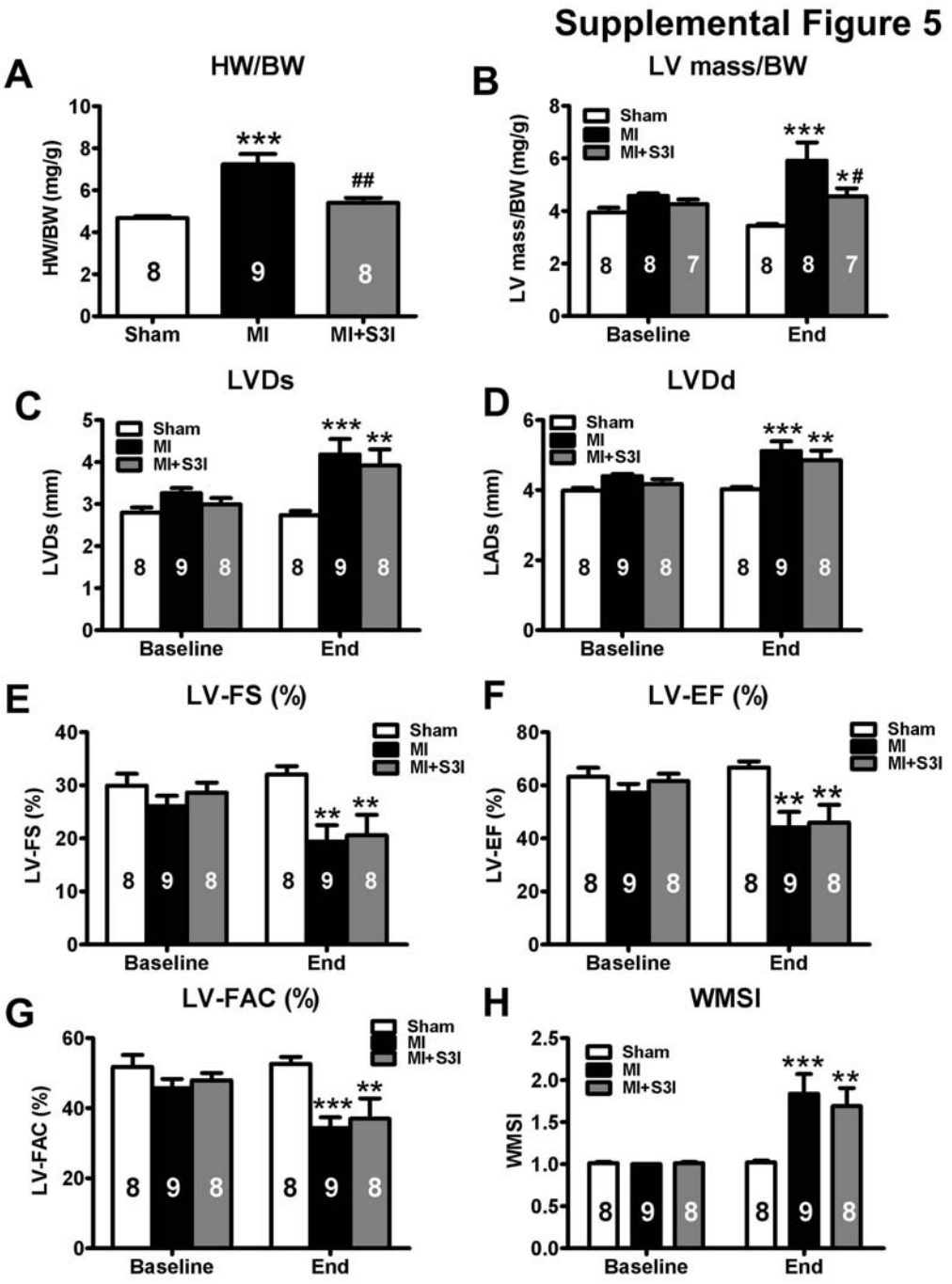
Supplemental Figure 3



Supplemental Figure 3. Fibroblast cell-count (cells/culture-dish) after 24-hour incubation with PDGF-AB at indicated concentrations. Mean±SEM, *n*=3-4/group. ***P*<0.01 vs. corresponding vehicle. One-way ANOVA with Dunnett's tests were used for statistical analysis. LA, left atrium; LV, left ventricle; Veh: vehicle; P, PDGF.

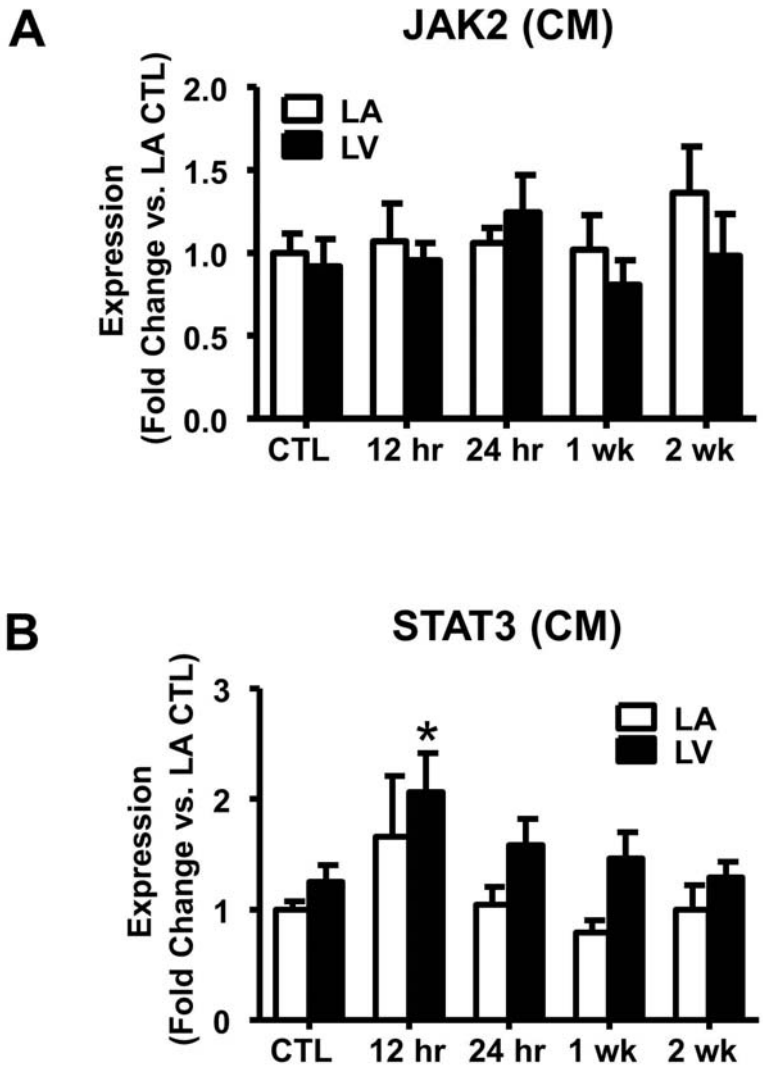


Supplemental Figure 4. The effects of the JAK inhibitor filgotinib (GLPG0634) on PDGF-AB action in atrial fibroblasts. (A) Representative immunoblots of p-STAT3 and t-STAT3 in fibroblasts stimulated with PDGF-AB with or without filgotinib at different concentrations. (B-D) Protein expression of p-STAT3, t-STAT3 and p/t-STAT3 ratio. (E) Representative immunoblots of secreted collagen-1 from culture-media of LA fibroblasts stimulated with PDGF-AB with or without filgotinib. (F) Fibroblast cell-count (cells/culture-dish) after 48-hour incubation with PDGF-AB with or without filgotinib. (G) Band-intensities for secreted collagen-1 (normalized to cell number). *n*'s are shown on corresponding bars. Mean±SEM, **P*<0.05, ***P*<0.01, ****P*<0.001. One-way ANOVA with Bonferroni-corrected *t*-tests were used for statistical analysis. COL-1, collagen type I; Veh, vehicle; P, PDGF; F or Filgo, filgotinib; L-low concentration (10 nM); M, medium (100 nM); H, high concentration (1000 nM).

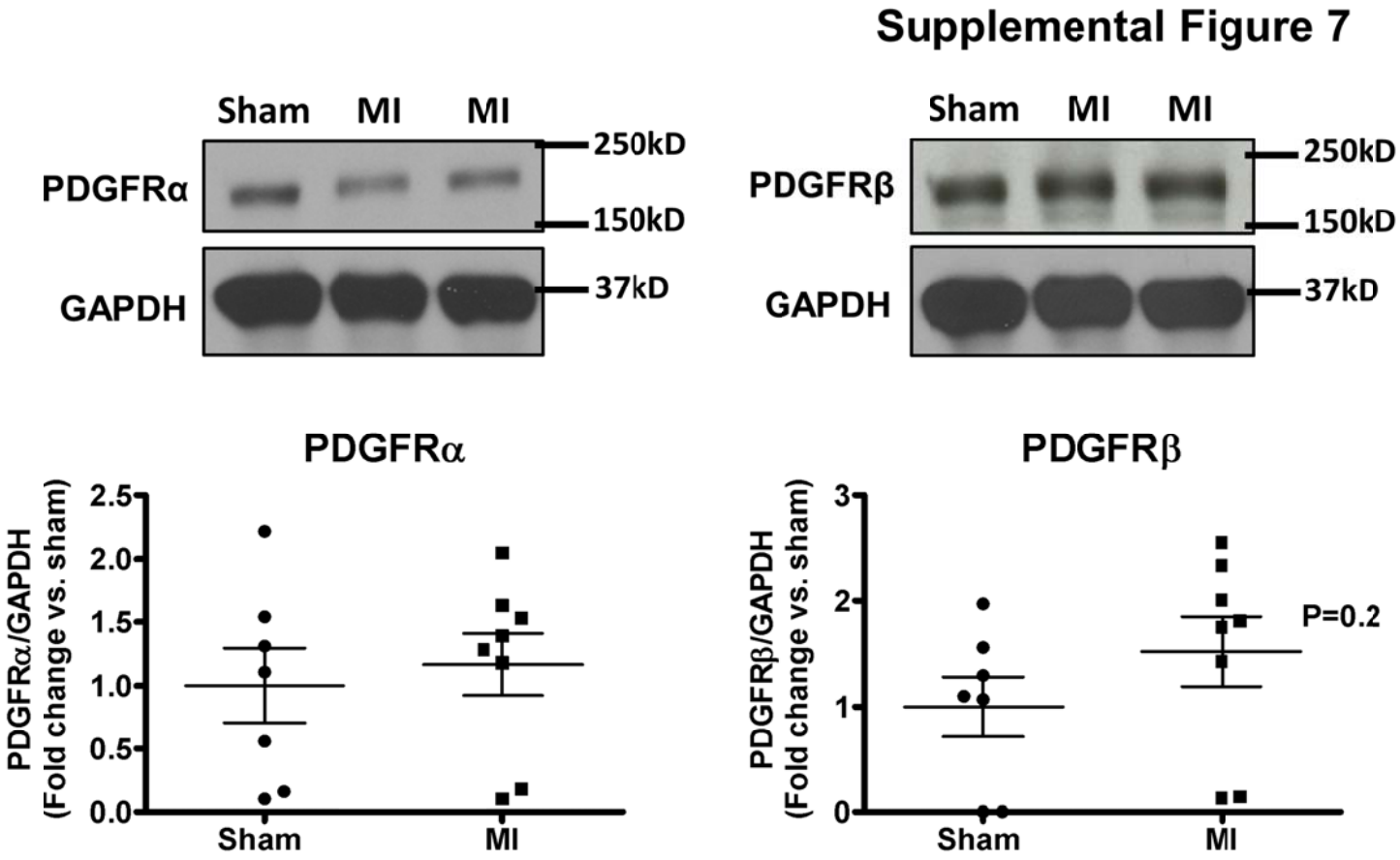


Supplemental Figure 5. Effects of S3I-201 on LV-remodeling. (A-B) Ratio of heart weight to body weight and LV mass to body weight were increased 2 weeks after myocardial infarction (MI), changes attenuated by S3I-201 treatment. (C-H) LV dilation and LV contractile dysfunction were induced by MI but not improved by S3I-201. *n*'s are shown on corresponding bars. Mean±SEM, **P*<0.05, ***P*<0.01, ****P*<0.001 vs. sham; $^{\#}P$<0.05, $^{\#\#}P$<0.01 vs. MI. Two-way ANOVA with Bonferroni post-tests was used for statistical analysis. HW, heart weight; BW, body weight; LV, left ventricular; LVDs, left ventricular dimension at end systole; LVDd, left ventricular dimension at end diastole; FS, fractional shortening; EF, ejection fraction; FAC, fractional area change; WMSI, Wall motion score index; Sham, sham surgery without ligation; MI, myocardial infarction; MI+S3I, myocardial infarction with S3I-201 treatment.

Supplemental Figure 6

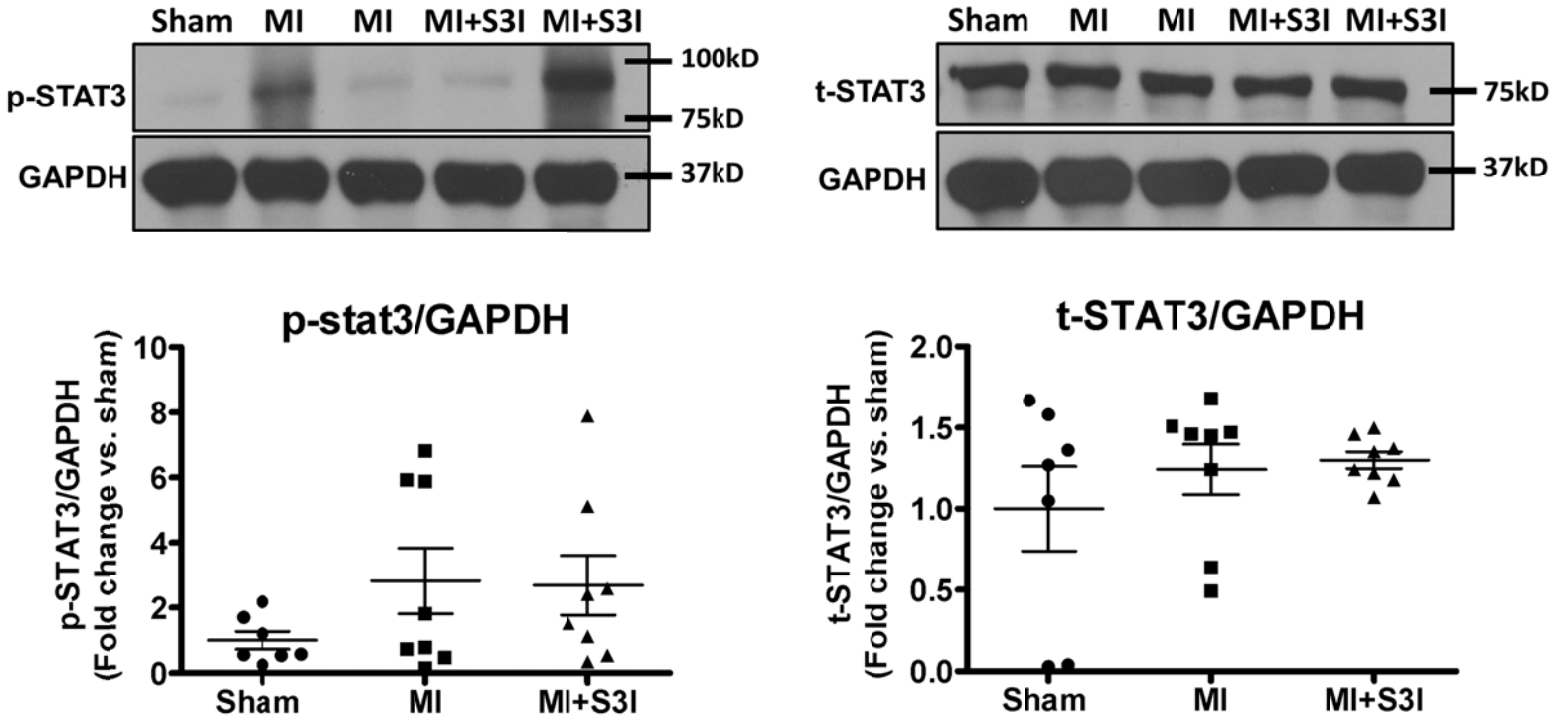


Supplemental Figure 6. mRNA expression of (A) JAK2 and (B) STAT3 in left atrial (LA) and left ventricular (LV) cardiomyocytes during the ventricular tachypacing (VTP) time-course. Mean±SEM, *n*=5-12/group, **P*<0.05 vs. corresponding CTL. One-way ANOVA with Dunnett's tests was used for statistical analysis. CM, cardiomyocyte; LA, left atrium; LV, left ventricle; CTL, non-paced control.



Supplemental Figure 7. Western blot for PDGF receptors α and β in right atrial samples from MI mice (top), along with individual and mean data (±SEM) at the bottom. PDGF receptor β showed a trend to increase in MI, but there was no statistically significant change.

Supplemental Figure 8



Supplemental Figure 8. Western blot for phosphorylated STAT3 (p-STAT3) and total STAT3 (t-STAT3) in right atrial samples from MI mice (top), along with individual and mean (±SEM) data at the bottom. No clear changes were seen.