

Supplement Material

Genistein alleviates pressure overload-induced cardiac dysfunction and interstitial fibrosis in mice

Running Title: Genistein prevents cardiac fibrosis

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Authorship contribution

Yong Zhang and Zhimin Du supervised the project and wrote the manuscript; Wei Qin, Ning Du and Zhiwei Fang designed the experiments and conducted the animal model; Longyin Zhang and Xianxian Wu performed the cell culture and western blot; Yingying Hu and Xiaoguang Li performed Real Time RT-PCR experiments; Nannan Shen performed immunofluorescent staining; Yang Li and Chaoqian Xu participated in statistical analysis; Baofeng Yang and Yanjie Lu reviewed and edited the manuscript.

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Supplemental Methods

Echocardiography

Mice were anesthetized with pentobarbital sodium (6 mg kg⁻¹, i.p.) for cardiac echocardiography with an ultrasound machine (Vivid 7, GE Medical System, USA). M-mode tracings were recorded from the long-axis view at the high papillary muscle level. Left ventricular inner diameter in diastole (LVIDd), left ventricular inner diameter in systole (LVIDs), left ventricular posterior wall in diastole (LVPWd), left ventricular posterior wall in systole (LVPWs) and left ventricular mass (LV mass) were assessed in end-systolic or end-diastolic situation.

Picrosirius red staining

Hearts from mice were harvested 8 weeks post-TAC surgery. Then the samples were embedded in paraffin and cut into 5 µm sections. To determine changes of collagen deposition, tissue sections were stained with picrosirius red as previously described (Chen *et al.*, 2014). Stained area was analyzed as percentage per field of vision at 200 magnification.

Supplemental Table

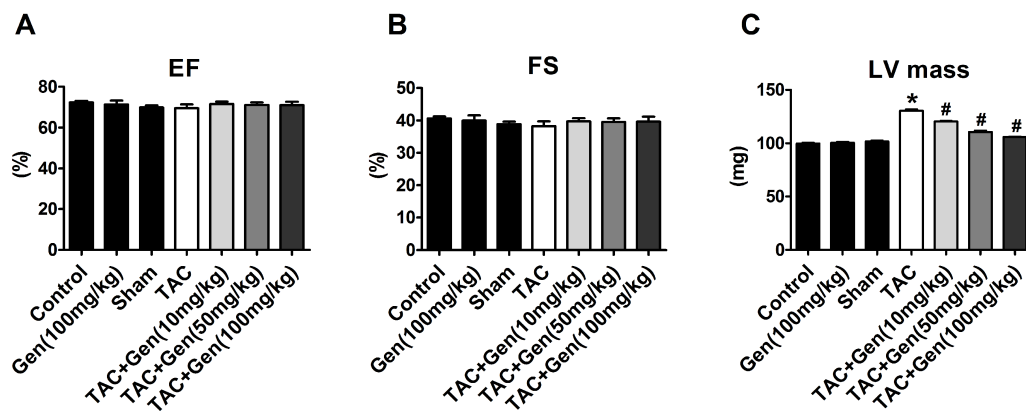
Supplemental Table 1. Echocardiography of mice before and after TAC and treatment with genistein (Gen)

	Control	Gen (100mg kg ⁻¹ day ⁻¹)	Sham	TAC	TAC+Gen (10mg kg ⁻¹ day ⁻¹)	TAC+Gen (50mg kg ⁻¹ day ⁻¹)	TAC+Gen (100mg kg ⁻¹ day ⁻¹)
LVIDd (mm)	3.51±0.03	3.53±0.03	3.53±0.01	4.04±0.03*	3.68±0.05 [#]	3.56±0.01 [#]	3.54±0.02 [#]
LVIDs (mm)	2.10±0.05	2.13±0.04	2.13±0.02	3.07±0.05*	2.64±0.05 [#]	2.41±0.03 [#]	2.23±0.02 [#]
LVPWd (mm)	0.99±0.06	1.00±0.04	1.00±0.05	1.26±0.03*	1.16±0.02	1.13±0.02	1.07±0.01 [#]
LVPWs (mm)	1.43±0.04	1.42±0.04	1.43±0.02	1.67±0.04*	1.59±0.04	1.55±0.01	1.46±0.01 [#]
LV mass (mg)	106±1.64	107±1.14	108±0.84	154±3.36*	135±3.05 [#]	120±1.16 [#]	109±0.5 [#]

Data are expressed as mean±SEM. LVIDd, left ventricular inner diameter in diastole; LVIDs, left ventricular inner diameter in systole; LVPWd, left ventricular posterior wall in diastole; LVPWs, left ventricular posterior wall in systole. LV mass, left ventricular mass. n=7. *P < 0.05 vs sham; [#]P < 0.05 vs TAC.

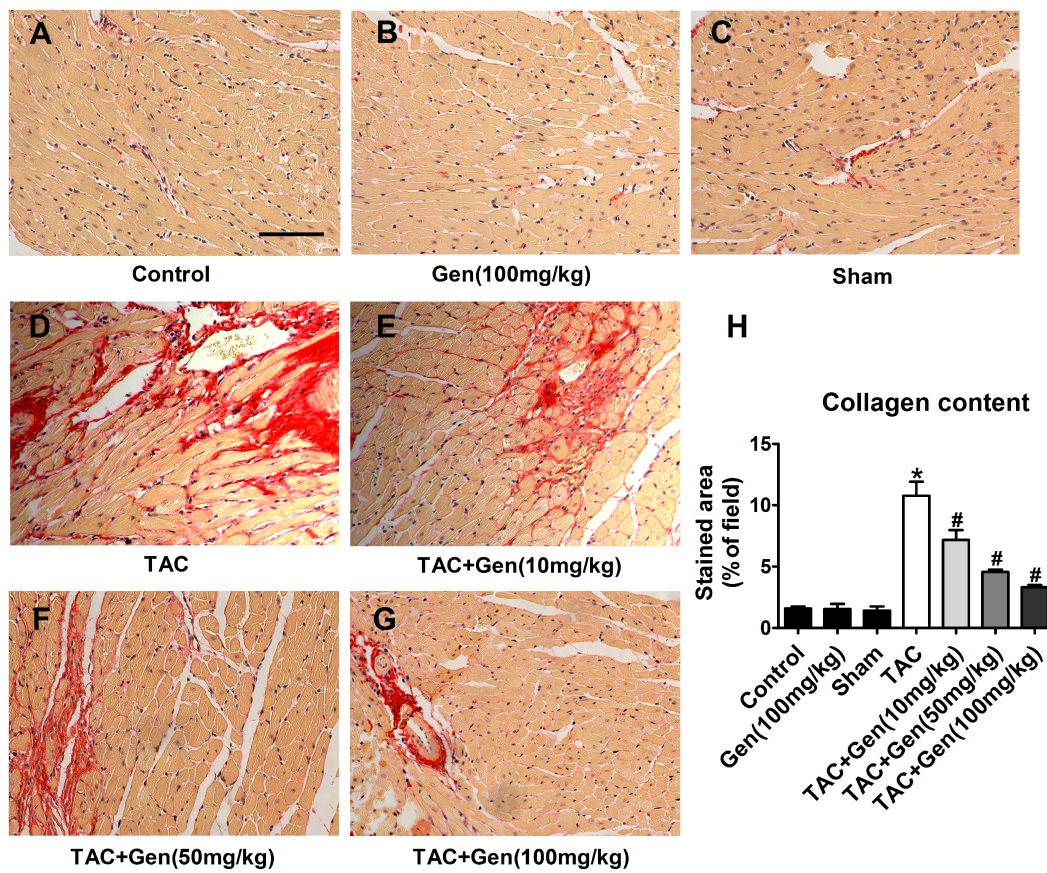
Supplemental Figures

Supplemental Figure 1



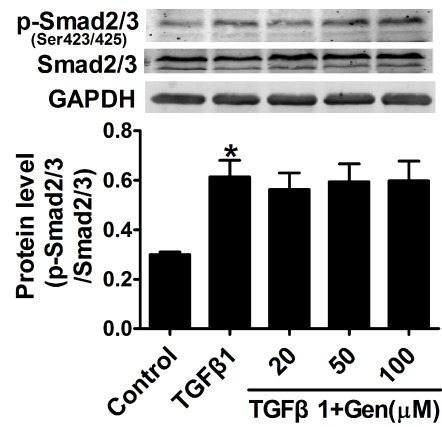
Legend S1. Effects of Genistein (Gen) on EF, FS values and LV mass of 4-week TAC-treated hearts. (A) Eject fraction (EF) levels evaluated by echocardiography. n=7. (B) Fraction shortening (FS) levels evaluated by echocardiography. n=7. (C) Left ventricular (LV) mass evaluated by echocardiography. n=7. *P<0.05 vs sham; #P<0.05 vs TAC.

Supplemental Figure 2



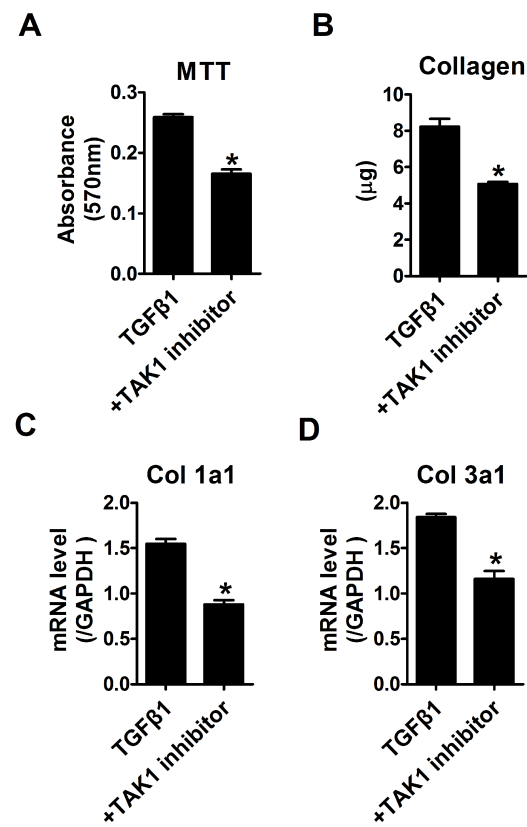
Legend S2. Effects of genistein (Gen) on pressure overload-induced cardiac interstitial fibrosis. (A-G) Representative fields of Sirius red–stained heart sections in control, Gen, sham, TAC and TAC+Gen animals. Scale bar indicates 100μm. (H) The summarized data showed that Gen treatment dose-dependently abolished TAC-induced cardiac interstitial fibrosis. n=5. *P<0.05 vs sham; #P<0.05 vs TAC.

Supplemental Figure 3



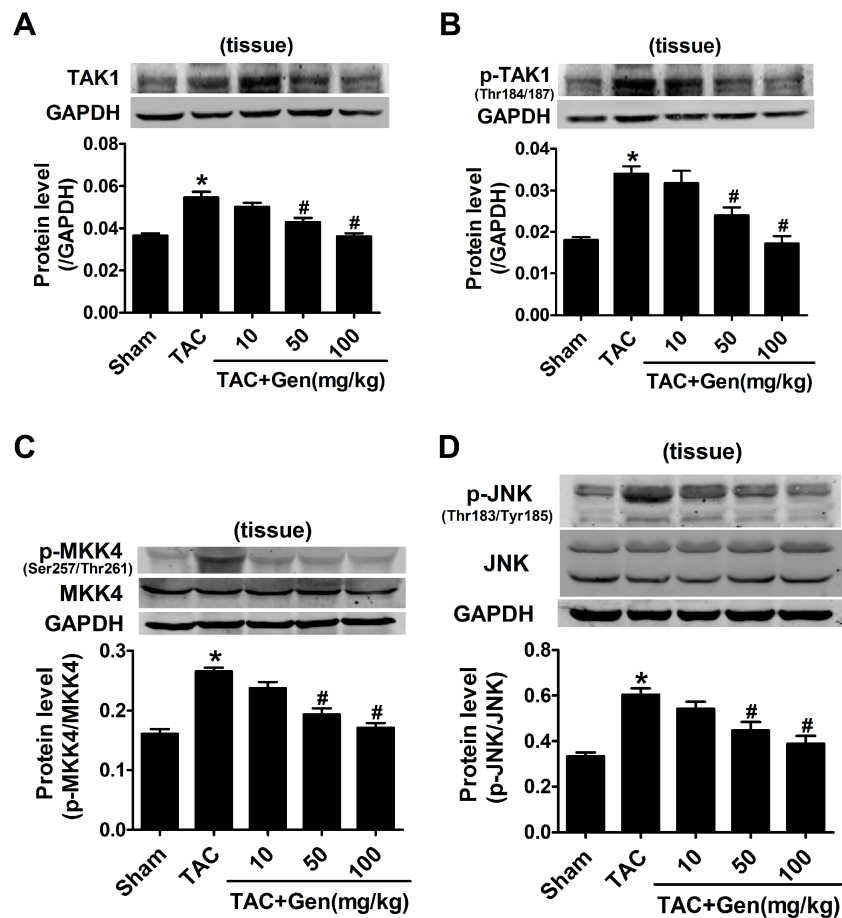
Legend S3. Genistein (Gen) treatment has no effects on Smad signaling. The expression of p-Smad2/3 was increased after TGFβ1 treatment. Co-application with Gen did not inhibit p-Smad2/3/Smad2/3 ratio. n=5. *P<0.05 vs control.

Supplemental Figure 4



Legend S4. TAK1 inhibition suppresses TGFβ1-induced proliferation and collagen production of cultured CFs. Cells were treated with TGFβ1 in the presence of DMSO or 500 nM TAK1 inhibitor (5Z)-7-oxozeaenol for 24h. (A) MTT test. (B) Collagen production assay. (C, D) Col 1a1 and Col 3a1 mRNA expressions. n=5. *P<0.05 vs TGFβ1.

Supplemental Figure 5



Legend S5. Genistein (Gen) represses TAK1 expression and blocks TAK1/MKK4/JNK signaling in TAC treated mice. (A) Representative western blots and analyzed data for total TAK1 in heart tissue. (B) Representative western blots and analyzed data for phosphorylated TAK1 (p-TAK1) in heart tissue. (C) Representative western blots and analyzed data for p-MKK4 and MKK4 in heart tissue. (D) Representative western blots and analyzed data for p-JNK and JNK in heart tissue. n=5. *P<0.05 vs sham; #P<0.05 vs TAC.

References

Chen L, Schrementi ME, Ranzer MJ, Wilgus TA, DiPietro LA (2014). Blockade of mast cell activation reduces cutaneous scar formation. *PLoS One* 9(1): e85226.