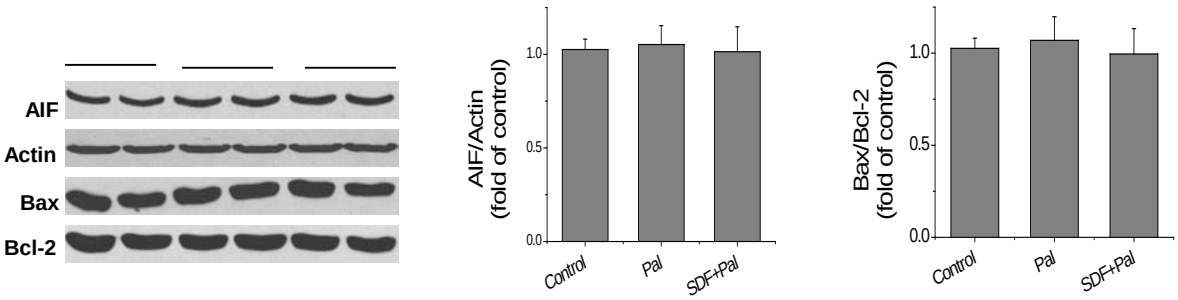
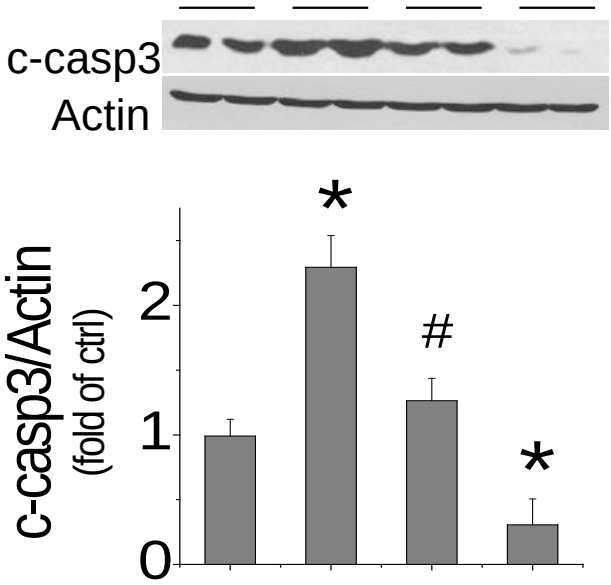
SUPPLEMENTARY DATA

Supplementary Figure 1. Pal-induced cardiac cell death via mitochondria independent pathway. H9C2 cells were exposed to Pal at 62.5 μM for 15 h and then expression of AIF, Bax, and Bcl-2 was examined by Western blotting assay. Ratio of Bax to Bcl-2 expression was calculated and presented. Data are presented as mean ± SD from at least three separate experiments. Pal: palmitate

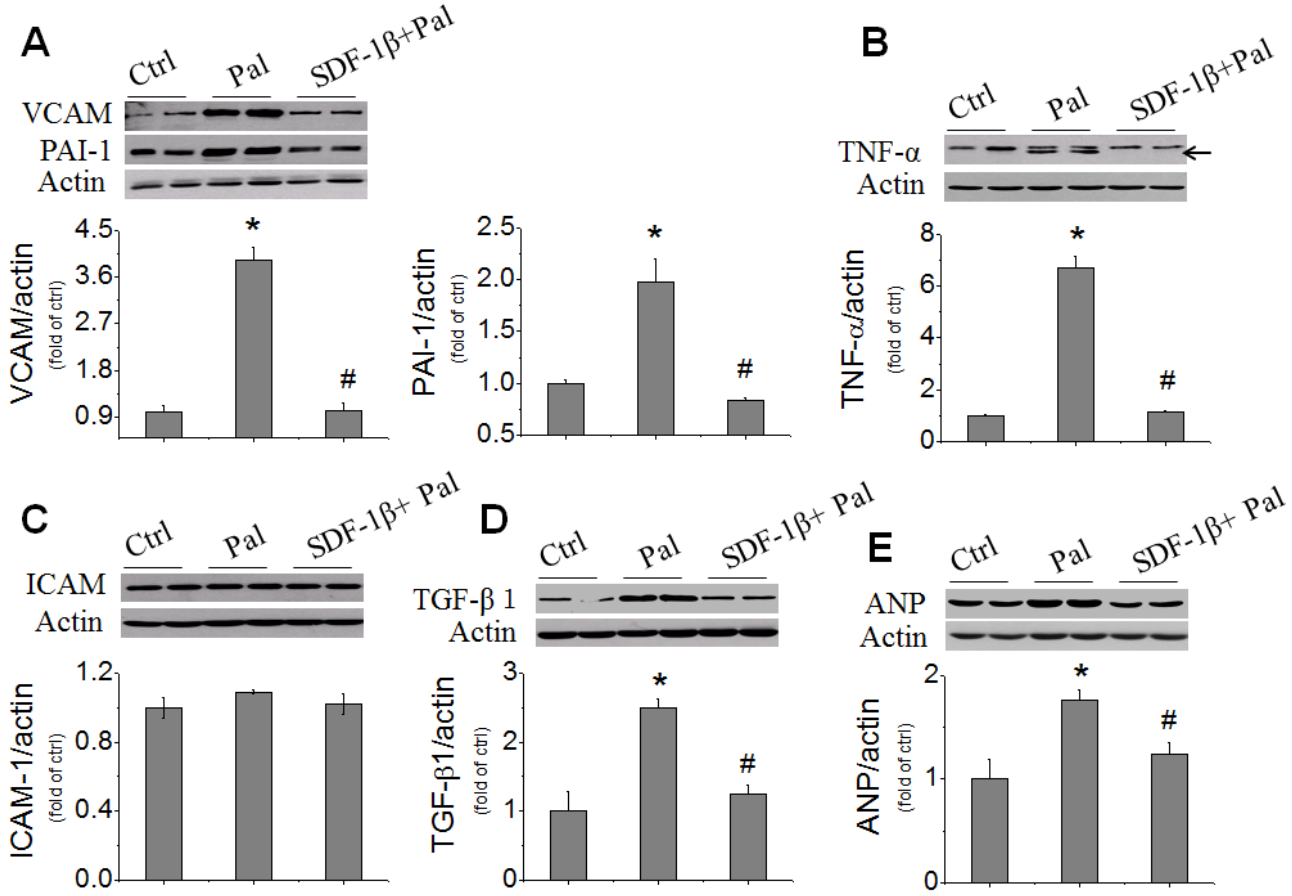


Supplementary Figure 2. Protective effect of SDF-1β on spontaneous and Pal-induced apoptotic cell death. Effects of SDF-1β at 100 nM on the cell death in the cells with and without exposure of palmitate at 62.5 μM for 15 h were examined. *, P<0.05 vs control group; #, P<0.05 vs palmitate group.



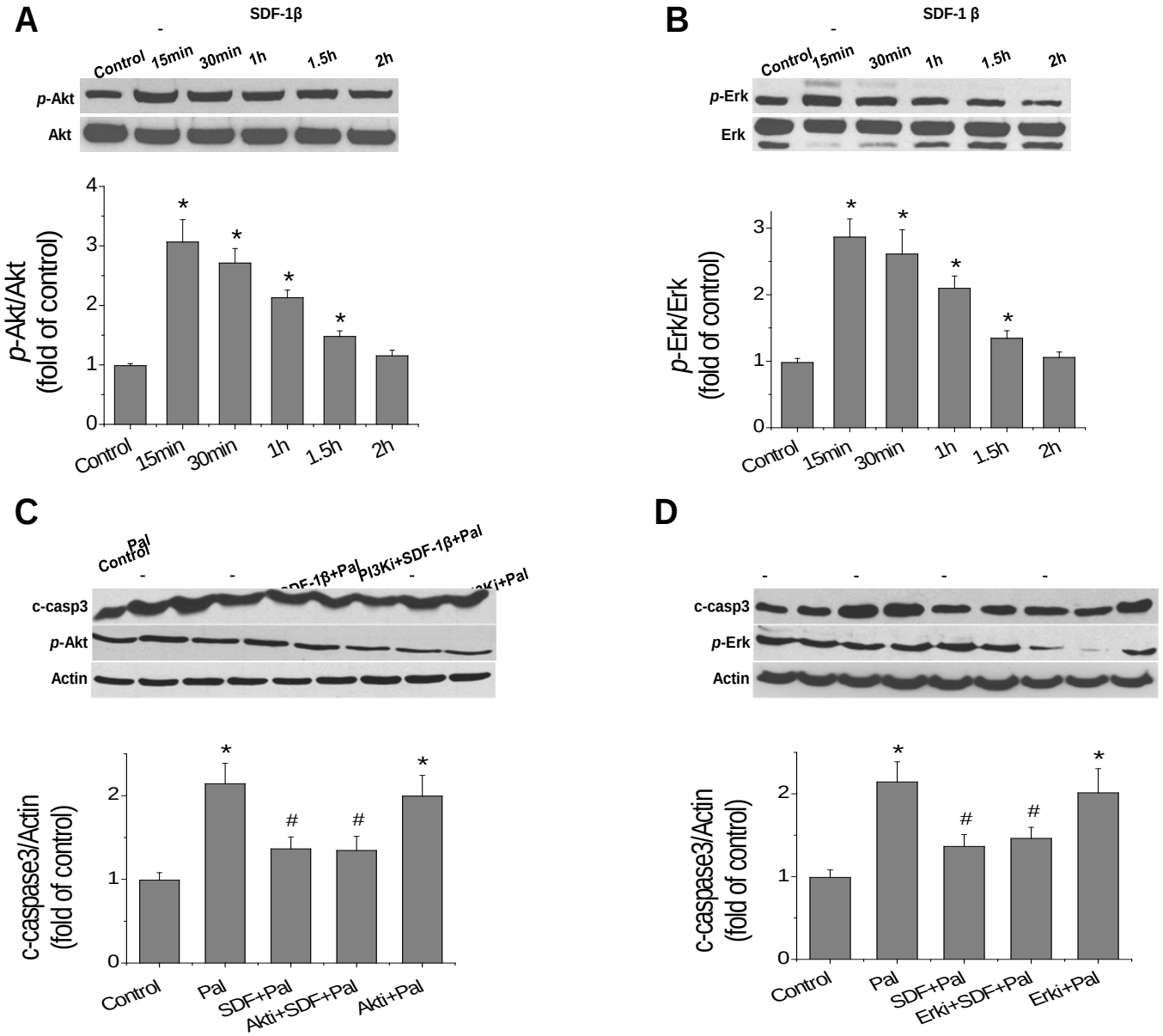
SUPPLEMENTARY DATA

Supplementary Figure 3. Preventive effect of SDF-1β on Pal-induced expression of cardiac inflammatory and pro-fibrotic cytokines. H9C2 cells were exposed to Pal at 62.5 μM for 15 h and then the expression of VCAM & PAI-1 (A), TNF-α (B), ICAM (C), TGF-β1 (D) and ANP (E) was examined by Western blotting assay. The expression of actin was used as loading control. Data are presented as mean ± SD from at least three separate experiments. *, P<0.05 vs control group; #, P<0.05 vs palmitate group. Ctrl: control; Pal: palmitate.



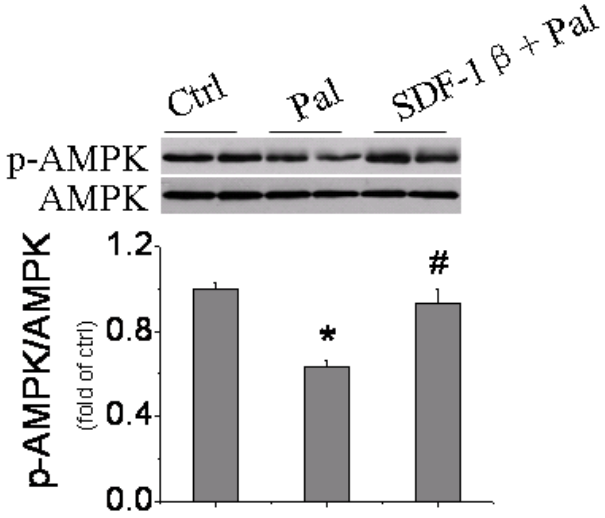
SUPPLEMENTARY DATA

Supplementary Figure 4. Preventive effect of SDF-1β on Pal-induced cardiac cell death, which was independent on the activation of Akt and ERK1/2. H9C2 cells were treated by SDF-1β for different time (A, B) and then expression of *p*-Akt and Akt (A) or *p*-ERK1/2 and ERK1/2 (B) were examined by Western blotting assay. H9C2 cells were pretreated by LY294002 at 50 μM (C) or U0126 at 10 μM (D) for 1 h and then treated by SDF-1β and Pal in the presence of either LY294002 (C) or U0126 (D) for another 15 h, for which cell death was determined by cleaved caspase 3. Data are presented as mean ± SD from at least three separate experiments. Pal: palmitate; *p*-Akt: phosphorylated Akt; *p*-ERK1/2: phosphorylated ERK1/2; c-caspase 3: cleaved caspase 3. *, $P<0.05$ vs control group; #, $P<0.05$ vs palmitate group.

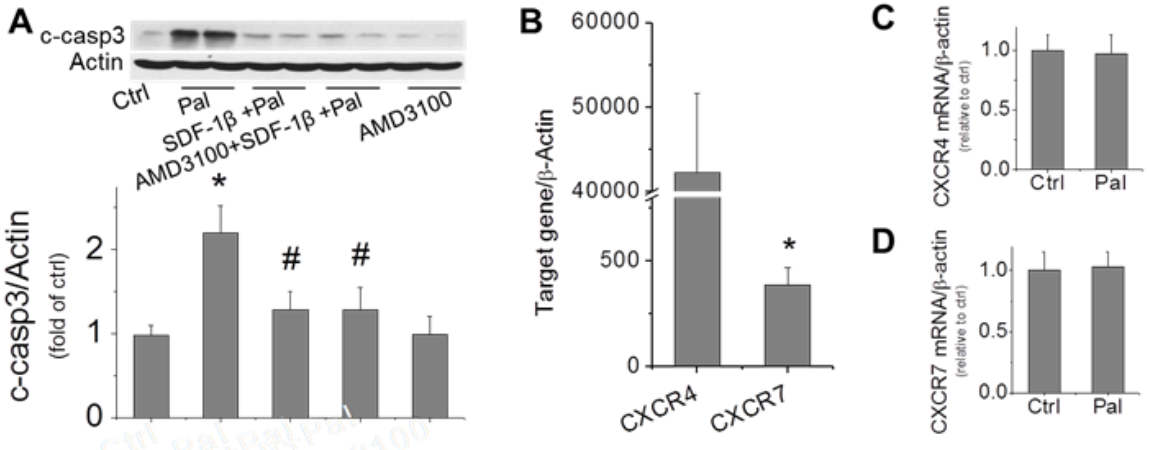


SUPPLEMENTARY DATA

Supplementary Figure 5. Protective effect of SDF-1β on palmitate-induced AMPK inactivation in primary cultures of neonatal cardiomyocytes. Effects of SDF-1β on AMPK phosphorylation (activation) were examined in primary cultures of neonatal cardiomyocytes with and without exposure to palmitate at 62.5 μM for 3 h in presence or absence of SDF-1β at 100 nM, by Western blotting. Data are presented as mean ± SD from at least three separate experiments. *, P<0.05 vs control group; #, P<0.05 vs palmitate group. Ctrl: control; Pal: palmitate.

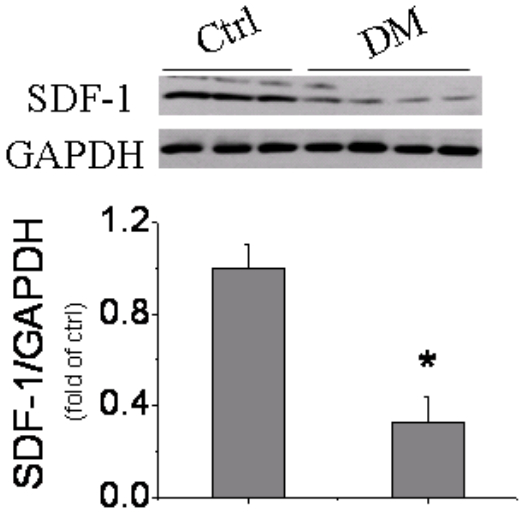


Supplementary Figure 6. Protective effect of SDF-1β on palmitate-induced cell death is not mediated through CXCR4 receptor. H9C2 cells were pretreated with AMD3100 at 0.5 μM for 1 h before treatment with SDF-1β, palmitate and AMD3100 together for another 15 h and then cell death was measured by activation of caspase 3 (A). H9C2 cell mRNA expressions of CXCR4 and CXCR7 in normal condition (B) and palmitate-treated conditions (C, D) was determined by qPCR. Ctrl: Control; Pal: palmitate; *, *P*<0.05 vs control group; #, *P*<0.05 vs palmitate group.



SUPPLEMENTARY DATA

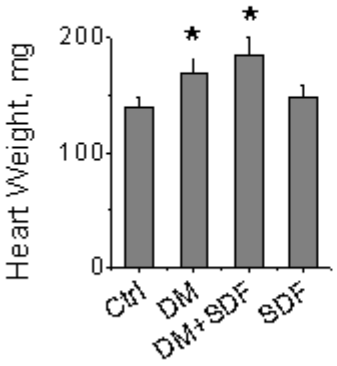
Supplementary Figure 7. Diabetic down-regulation of SDF-1 expression in cardiac tissues. Male C57BL/6 mice (Jackson Lab., Bar Harbor, ME) at 8 weeks old were fed with a high fat diet (HFD, Rodent Diet with 60% kcal% fat, D12492, Research Diets, Inc., New Brunswick, NJ) to induce type 2 diabetes, while the control mice were fed with low fat diet (LFD) (Rodent Diet with 10% kcal% fat, D12450B). After 3 months on the HFD, the fasting blood glucose and glucose tolerance testing were performed. Then the mice were sacrificed for measuring the plasma insulin and triglyceride levels using an ultrasensitive Mouse Insulin ELISA kit (Crystal Chem Inc., IL) and a Triglyceride Colorimetric Assay kit (Cayman Chemical, MI). All these parameters indicate the development of type 2 diabetes (data not shown). The expression of SDF-1 in cardiac tissue was examined by Western blotting assay. Data are presented as mean ± SD (n≥7); * $p < 0.05$ vs. LFD control. Ctrl: control. DM: HFD-induced diabetes mellitus.



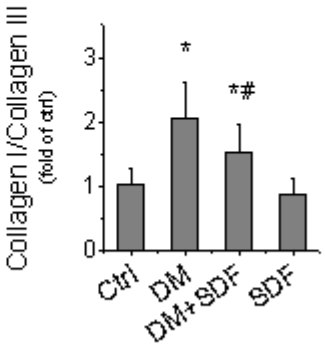
Supplementary Figure 8. Protective effect of SDF-1β on diabetes-induced heart weight changes and cardiac fibrosis and hypertrophy. Heart weight (A), Collagen I and III were detected by Western blotting assay (B). Collagen accumulation was observed by Siruis Red staining (C), and the hypertrophy of myocytes was observed under microscope by hemotoxylin and eosin (H&E) staining (D). Data are presented as mean ± SD (n=6 at least in each group). DM: diabetes; *, $P<0.05$ vs control group; #, $P<0.05$ vs DM.

SUPPLEMENTARY DATA

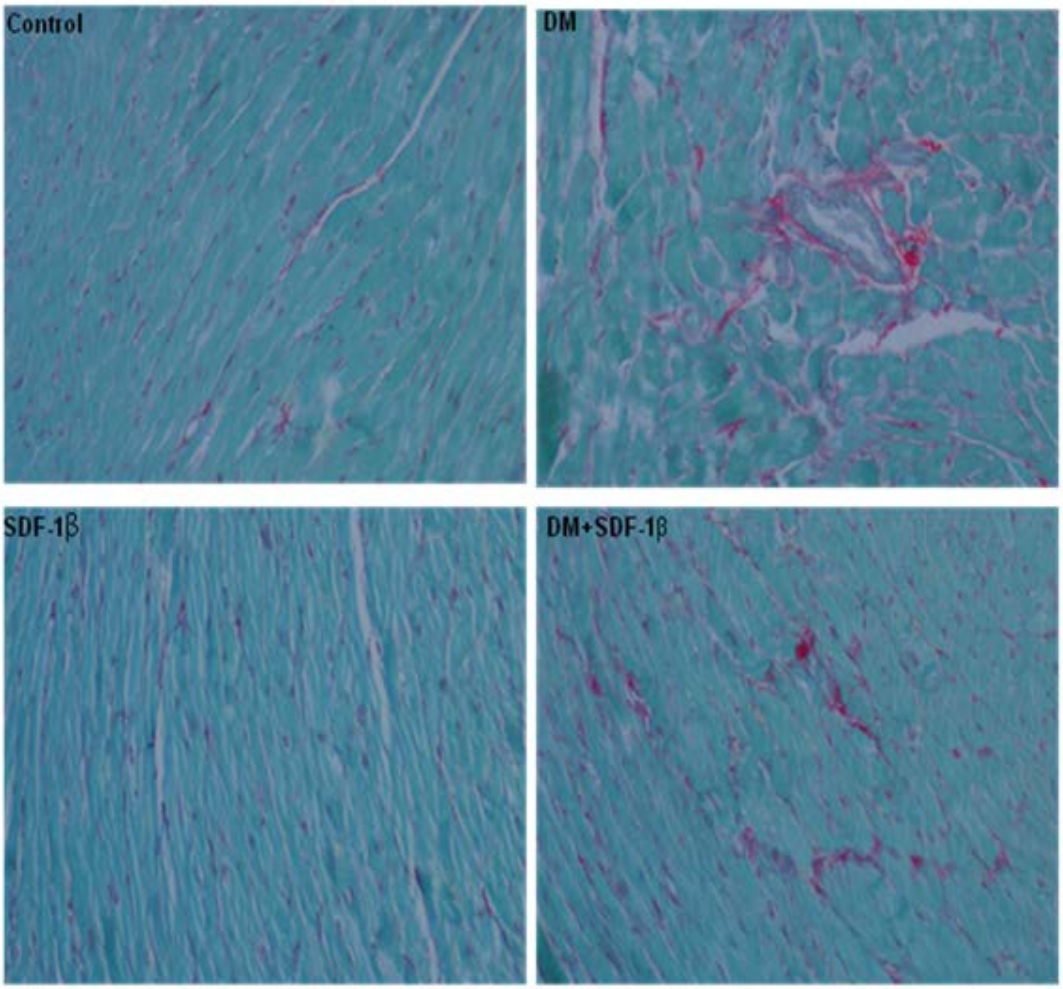
A. Heart weight



B. Collagen I/ Collagen III

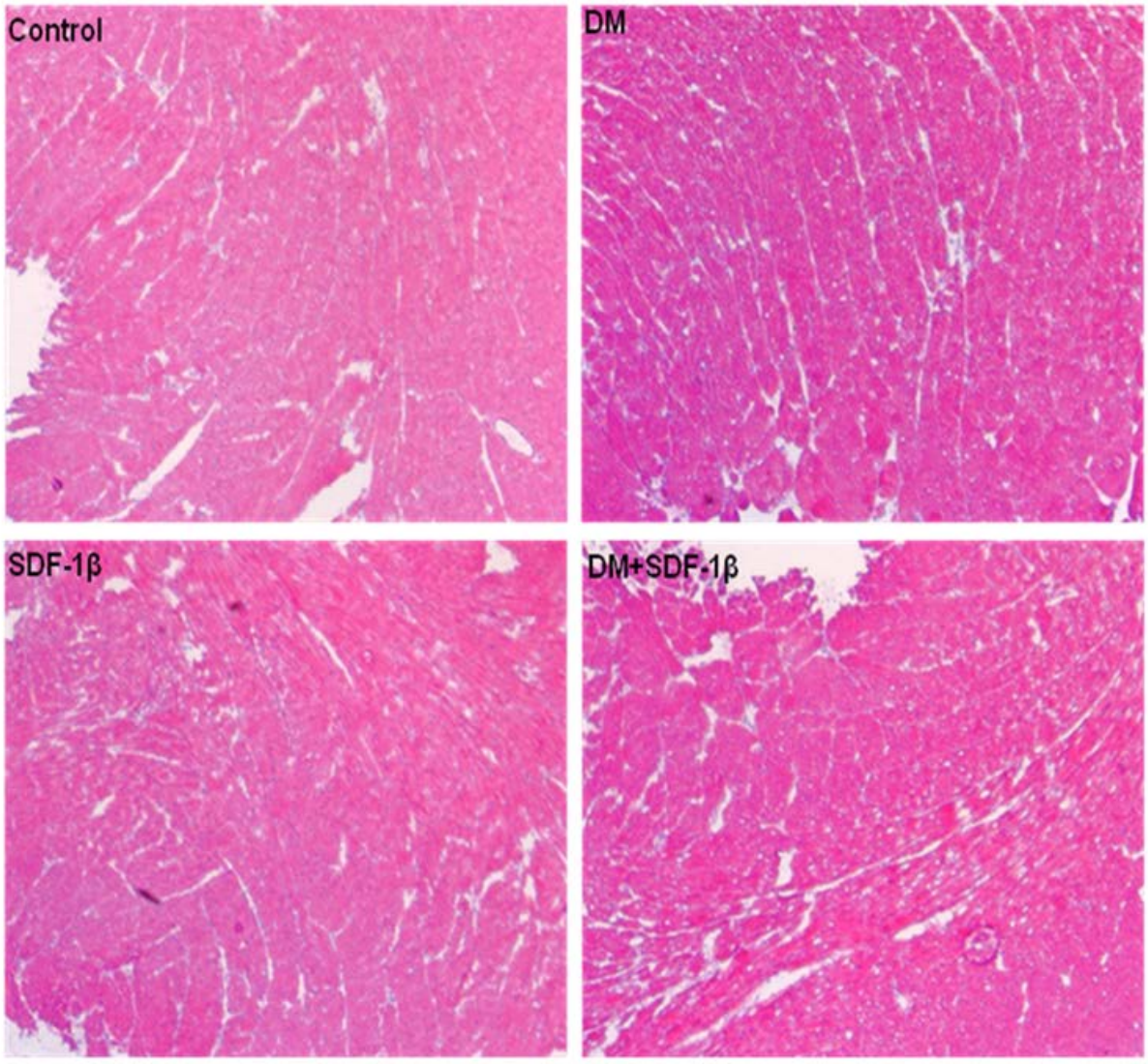


C. Sirius-red staining



SUPPLEMENTARY DATA

D. H&E for hypertrophy



SUPPLEMENTARY DATA

Supplementary Figure 9. Illustration of the hypothesis for the protective effect of SDF-1β on palmitate-induced cardiac cell death. Exposure of H9C2 cells to palmitate causes nitrosative damage that causes ER stress and related apoptotic cell death; SDF-1β can protect cardiac cells from palmitate-induced nitrosative damage, ER stress and cell death; Cardiac protective effect of SDF-1β is mediated through its interaction with CXCR7, and activation of AMPK and p38 β MAPK mediated IL-6 production.