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Sulforaphane reduces adipose tissue fibrosis via promoting M2 macrophages polarization in HFD fed-mice

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Article

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Abstract

Background/Objectives: Adipose tissue fibrosis has been identified as a novel contributor to the pathomechanism of obesity associated metabolic disorders. Sulforaphane (SFN) has been shown to have an anti-obesity effect. However, the impact of SFN on adipose tissue fibrosis is still not well understood.

Methods: In this study, obese mice induced by high-fat diets (HFD) were used, and SFN was administered through subcutaneous injection to examined the effects on adipose tissue fibrosis. Analysis included quantification of: (i) body weight, food intake, fat mass, glucose tolerance and insulin tolerance; (ii) the relative mRNA and protein levels of fibrosis, inflammation and macrophages polarization related genes; (iii) tissue histology using Hematoxylin-Eosin (H&E), immunohistochemistry and immunofluorescent staining; and (iv) the levels of inflammatory cytokinesin serum.

Results: According to the current findings, SFN dramatically enhanced glucose tolerance and decreased body weight in diet-induced-obesity (DIO) mice. Additionally, SFN therapy significantly reduced extracellular matrix (ECM) deposition and altered the expression of genes related to fibrosis. Furthermore, SFN also reduced inflammation and promoted macrophages polarization towards to M2 phenotype in adipose tissue, which protected adipose tissue from fibrosis. Notably, SFN-mediated nuclear factor E2-related factor 2 (Nrf2) activation was crucial in decreasing adipose tissue fibrosis.

Conclusions: These results implied that SFN had favorable benefits in the management of adipose tissue fibrosis, which consequently ameliorates obesity-related metabolic problems. Our research provides new treatment strategies for obesity.

Introduction

Obesity, a global prevalent disease, is intimately linked to diets, which exhibit the "three highs" phenomena of high fat, protein, and sugar in recent decades [1]. Numerous metabolic problems, including type 2 diabetes (T2D), insulin resistance (IR), cardiovascular disease (CVD) and inflammation have close relationships with obesity [2]. Therefore, there is an urgent need to treat obesity and associated disorders.

White adipose tissue (WAT) is essential for systemic energy homeostasis. In addition to lipid storage and mobilization, WAT also severs as a secretory organ that secretes a large number of signaling molecules [3]. During the development of obesity, WAT undergoes massive expansion as a result of adipocytes proliferation and hypertrophy, accompanied by continuous remodeling and functional alterations. WAT becomes dysfunctional and immune cells like mast cells and macrophages are stimulated to be recruited. An abundance of proinflammatory chemokines is released and produces a chronic low-grade inflammatory microenvironment where extracellular matrix (ECM) is over-deposited through interaction with adipocytes and leads to fibrosis [4, 5]. The stiff ECM is unable to remodel dynamically to prevent expanding in a healthy manner, leading to metabolic dysfunction [6, 7]. Therefore, prevention of WAT fibrosis is crucial for improving systemic health.

Clinical researches have shown that WAT fibrosis can be utilized to predict results in terms of health, underscoring its significance in the treatment. The regulatory impact of factors on WAT fibrosis is frequently investigated using the diet-induced obesity models in mice. Several factors, including medications, cytokines, and important genes, have been proven in studies to attenuate WAT fibrosis [8–11], and the underlying molecular mechanisms are a very intricate regulatory network. Fibrosis in WAT still has a very limited clinical therapy option, nonetheless.

Sulforaphane (SFN) is a kind of isothiocyanate compound, and produced by the hydrolysis of glucosinolates under the action of endogenous myrosinase [12]. SFN is widely exists in cruciferous vegetables and has a variety of effects, which has attracted extensive attention. Studies has showed that SFN exhibited antioxidant, antibacterial, anti-inflammatory, and anti-aging properties, and it is also widely known as one of the most potent anti-cancer components [13, 14]. Nuclear factorE2-related factor 2 (Nrf2) is one of the most important targets of SFN, with the capacity to regulate oxidative stress, mitochondrial dysfunction, and protein misfolding, all of which contribute to a variety of metabolic diseases [14, 15]. Multiple studies have indicated that SFN has crucial functions in regulating glucose and lipid metabolism [16, 17]. However, few research have looked into whether SFN effectively prevents the development of WAT fibrosis in obesity.

Therefore, we attempted to elucidate the impacts of SFN on fibrosis in WAT of obese mice and *in vitro* cultured adipocytes, as well as to uncover the potential mechanisms in this study.

Methods

Animals and Experimental Design

6-week-old male C57BL/6J mice (Chengdu Dossy Experimental Animals Co., Ltd, Chengdu, China) were housed in cages at room temperature and subjected to a 12-hour cycle of light and darkness with free access to food and water. Before the experiment, mice were acclimated to the laboratory conditions for 1 week.

Then, mice were divided into 2 groups randomly, in which one group was fed with a regular normal chow diet (NC, calories consist of 10% fat, 20% protein and 70% carbohydrate), and the other one was fed with a high fat diet (HFD, calories consist of 60% fat, 20% protein and 20% carbohydrate) for 12 weeks to build an obesity model.

SFN (MCE, New Jersey, USA) was dissolved in dimethyl sulfoxide (DMSO, Solarbio, Beijing, China) and administered through subcutaneous injection five times per week at the concentration of 10 mg/kg/day as previously reported [18]. Mice fed with HFD were subdivided into two parts, half of which were administrated with SFN (HS group, n = 8), and the rest were treated with DMSO (HF group, n = 8). At the same time, mice in NC group were also treated with DMSO for 4 weeks (NC group, n = 8). Body weight and food intake were measured every four days. Mice handling protocols were conducted following the

guidelines and regulations approved by the Animal Ethics Committee of Southwest University of Science and Technology.

Oral glucose tolerant test (OGTT) and insulin tolerance test (ITT)

Mice were fasted overnight and then were given 1 g/kg dextrose by gavage for OGTT. As for ITT, mice were fasted for 4 hours and then were intraperitoneal injected with 0.75U/Kg insulin (Sigma-Aldrich, Shanghai, China). Blood glucose was assessed from tail vein at 0, 15, 30, 45 and 60 minutes.

Biochemical analysis

Blood samples from mice were centrifuged for 10 minutes at 3000 rpm after resting for an hour at room temperature. The supernatant was separated and stored at -80℃. The concentrations of insulin, leptin, free fatty acids (FFAs), triglycerides (TG), tumor necrosis factor α (TNF-α), Interleukin (IL)-1β, IL-6 and lipopolysaccharide (LPS) in serum were detected with ELISA kits (Solarbio, Beijing, China) according to the manufacturer's instructions.

Cell culture and treatment

The RAW264.7 cells were maintained in DMEM supplemented with 10% FBS. For the experiments, cells were pretreated for 24 hours with 10 ng/ml LPS (Sigma-Aldrich, Shanghai, China) to stimulate macrophage M1 polarization, or 20 ng/ml IL-4 (PeproTech, New Jersey, USA) to stimulate macrophage towards M2 polarization. Then the culture medium was changed to DMEM containing 10 μM SFN. After 24 hours of treatment, the cells were washed twice with PBS and cultivated in fresh medium for another 24 h before being harvested as well as the medium.

The proposal of primary adipocyte culture was conducted as described [19]. 2.5 μg/mL TGFβ1 (Sigma-Aldrich, Shanghai, China) was added in the differential medium to induce fibrosis. 6 days after differentiation, the mature adipocytes were incubated with 1 μM SFN dissolved in DMSO for 48 h. On the other hand, the adipocytes were treated with the medium of RAW264.7 cells collected above for 24 h to detect the roles of macrophages polarization on fibrosis.

Quantitative Real-time PCR (qPCR)

Total RNAs from tissue and cells were isolated with RNAiso Plus reagent (Takara Biotechnology Co., Ltd., Dalian, China) following manufacturer's instructions. Then cDNA was synthesized using PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time). Quantitative PCR was used to detect the relative mRNA expression levels with TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) (Takara Biotechnology Co., Ltd., Dalian, China). The $2^{-\Delta\Delta Ct}$ technique was used to calculate the relative gene expressions [20–22].

Immunoblotting

Protein levels were detected using immunoblotting [19, 21]. The primary antibodies used were anti-Collagen I, anti-Collagen III, anti-Collagen VI, anti-LOX, anti-αSMA, anti-Nrf2, anti-iNOS, anti-Arg1, anti-

Fibronectin, anti-GAPDH, anti-Tublin and anti-β-actin (Abcam, Cambridge, UK). The rabbit secondary antibody was purchased from Baoshen (Beijing, China).

Histological staining analysis

For morphological and fibrotic analysis, adipose tissue sections were stained with Hematoxylin-Eosin (H&E) and Masson's Trichrome Stain Kit (Solarbio, Beijing, China). The proposals were conducted following by manufacturer's instruction.

Immunohistochemistry and immunofluorescence Staining

Immunofluorescence and immunofluorescence staining were performed using the standard method. For each section, three high-power fields from four mice in each group were examined and analyzed.

Active Nrf2 binding assay

Following the manufacturer's directions, the Nuclear Extract Kit was used to extract nuclei from adipose tissues. And then an Nrf2-DNA-binding ELISA kit (Active Motif, Carlsbad, CA) was used to detect Nrf2 activity in nuclear extracts as previously reported [23].

RNA sequencing

Total RNA was extracted and the quality was detected with Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Then, RNA sequencing was conducted by Novogene Co., Ltd. (Beijing, China). The main functions of differential expression genes (DEGs) were analyzed and the Gene Ontology (GO) enrichment of DEGs were carried out using R version 4.2.1 as previous reports [24, 25].

Statistical analyses

All data are presented as mean ± standard deviation (SD) and were analyzed using GraphPad Prism 8. The unpaired Student's t test was used when two groups were compared and a one-way analysis of variance (ANOVA) was used when three or more groups were compared. It was considered difference when a threshold p value < 0.05. In this study, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Results

SFN improved obesity induced IR in mice

After 12 weeks of receiving either a standard chow (NC) or high fat diet (HFD), mice in HS group were injected with SFN (10 mg/kg/day), and the others were treated with DMSO for 4 weeks (Fig. 1A). We found mice fed with HFD gained significantly more body weight compared to those mice with NC diet. Compared with HF mice, SFN administration significantly reduced body weight in HS group with no difference in food intake (Fig. 1B and 1C), which may be related to reduced fat accumulation (Fig. 1D). Meanwhile, SFN therapy significantly reduced fasting insulin levels and FFA levels in serum of HFD mice (Fig. 1E and 1F). Furthermore, we found that the systemic glucose tolerance and insulin sensitivity were

improved following SFN treatment (Fig. 1G and 1H). These results suggested that SFN attenuated the HFD-induced obesity phenotype and improved IR in diet-induced-obesity (DIO) mice.

RNA-seq analysis of gene expression in iWAT of mice

To identify the function of SFN, inguinal white adipose tissue (iWAT) was collected after mice sacrificed and performed RNA sequencing. Here, we identified differential expression genes (DEGs) with a statistical cutoff of $\log_2|\text{foldchange}| \geq 1$ and p value ≤ 0.05. Data showed that there was 354 DEGs in HS group compare to genes in HF group, which contained 129 up-regulated and 225 down-regulated genes (Fig. 2A and Supplemental Table S1). GO analysis of DEGs showed a significant enrichment in ECM remodeling (Fig. 2B and Supplemental Table S2). In addition, we also noticed that there was an inflammatory genes enrichment (Fig. 2B). RNA-seq analysis also confirmed that SFN treatment suppressed the expressions of fibrosis and inflammation related genes, which were upregulated in HF group relative to NC group (Fig. 2C). These data indicated SFN had a positive regulatory function in regulating WAT fibrosis.

SFN restrained the inguinal white adipose tissue (iWAT) fibrosis in HFD mice

To further verify the effect of SFN on iWAT fibrosis, H&E and Masson's trichrome staining were performed. Results revealed that collagen fibers in iWAT were increased to 1.77-fold in HF mice compared to the NC mice. However, the aberrant collagen deposition in HF mice was greatly decreased by 0.54-fold after SFN delivery (Fig. 3A). The transcription levels of genes associated with fibrosis, including *collagen type* (*Col1a1*), *type III* (*Col3a1*), *type* (*Col6a1*), *lysyl oxidase* (*Lox*), and *fibronectin*, were elevated in HF group compared to NC group, but this trend was reversed in HS group (Fig. 3B). The same results were observed in ECM-regulating genes, such as *matrix metalloproteinase 2* (*Mmp-2*), *Mmp-14*, and tissue inhibitors of MMPs (*Timp-1, Timp-2, Timp-3*) (Fig. 3C). The effect of SFN on the protein levels of fibrosis genes was also investigated using western blotting. Our findings demonstrated that the levels of collagen I and III, Lox, and α-smooth muscle actin (α-SMA) protein expression were decreased in HS mice compare to those in HF mice (Fig. 3D). Taken together, these results suggested that SFN restrained excessive collagen deposition in iWAT of HFD mice.

SFN inhibited inflammation and M1 macrophages polarization in iWAT of DIO mice

Studies have shown that inflammation may act as a trigger for fibrosis [26]. In our study, we had noticed that SFN have a mitigating effect on inflammation (Fig. 2B and 2C). We examined the levels of inflammatory factors further, and found HFD fed mice had higher serum levels of proinflammatory cytokines, including lipopolysaccharide (LPS), TNFα, IL-1β, and IL-6, compared with those in mice fed NC diet (Fig. 4A). However, this phenomenon was disappeared following SFN treatment in HFD fed mice (Fig. 4A). The changes were also observed in serum leptin and TG levels (Fig. 4A). We also measured the

mRNA levels of inflammatory cytokines in iWAT. The transcription levels of the pro-inflammatory genes *Tnfa*, *Il-1β* and *Il-6* were also shown to be reduced in the HS group compared with HF group (Fig. 4B). Furthermore, F4/80 immunohistochemistry revealed that HFD significantly enhanced macrophages population and the number of crown-like structures (CLS) by 3.26-fold (Fig. 4C). In the HS group, CLS formation was markedly diminished to 0.70-fold as compared to the HF group (Fig. 4C). Of which, we found M1 macrophage markers *Monocyte chemoattractant protein 1* (*Mcp1*), *inducible nitric oxide synthase* (*iNos*), and *Cyclooxygenase 2* (*Cox2*) were downregulated, while M2 macrophage markers including *Arginase 1* (*Arg1*) and *Il-10* were concurrently elevated (Fig. 4B and 4D). These results demonstrated SFN inhibited M1 macrophage polarization and promoted M2 macrophage polarization, and thus inhibited inflammation.

SFN protected adipocytes from excessive matrix deposition *in vitro*

To evaluated the effect of SFN on matrix deposition *in vitro*, we cultured primary adipocytes treating with TGF-β1 to simulate fibrosis. An increase of fibronectin mRNA and protein levels were observed, which was prevented following SFN treatment (Fig. 5A-5C). Also, the inhibiting effects of SFN on α-SMA, Col1a1 and Col6a1 were also detected (Fig. 5B and 5C). These data demonstrated that SFN inhibited fibrosis *in vitro* cultured adipocytes.

The polarization of M1 and M2 macrophages were induced by LPS or IL-4 respectively, and then treated with SFN. Results showed that SFN suppressed the expression of M1 macrophage markers *CD86*, *iNos*, *Mcp-1* and *Cox2* (Fig. 5D), and enhanced the mRNA levels of M2 macrophage markers *Il-10*, *Arg1*, *CD206* and *CD163* (Fig. 5E), indicating SFN had the ability to promote M2 macrophage polarization and inhibit M1 macrophage polarization. Next, mature adipocytes were cultured with the collected supernate from macrophages to mimic the co-culture system. We found the supernate from M1 macrophages increased the mRNA levels of collagen genes, including *Col1a1*, *Col6a1*, *fibronectin* and *Lox*, which were reversed by the supernate from SFN treated M1 macrophages (Fig. 5F). On the other hand, the expressions of collagen genes were decreased following the supernate from M2 macrophages treatment, and the supernate from M2 macrophages treated with SFN aggravated the downregulation of these genes (Fig. 5G). These findings suggested that SFN may prevent fibrosis by promoting M2 macrophage polarization and inhibiting M1 macrophage polarization.

SFN activated Nrf2 signaling pathway in iWAT

As SFN is a pharmacological activator of Nrf2, we investigated the levels of Nrf2 expression to further clarify the mechanism of SFN-mediated reduction in WAT fibrosis of HFD mice. As reported, the levels of Nrf2 in nucleus was reduced under oxidative stress in obesity [27, 28]. SFN therapy boosted the translocation of Nrf2 to the nucleus and activated Nrf2 signaling (Fig. 6A). We examined the mRNA levels of *Glutathione S-transferase alpha 4* (*Gsta4*), *Glutamate-cysteine ligase modifier subunit* (*Gclm*), *NAD(P)H quinone dehydrogenase 1* (*Nqo-1*), *Heme oxygenase-1* (*Ho-1*) and *Superoxide dismutase 2* (*Sod2*), whose

promoter regions revealed a shared ARE-binding site for Nrf2 [29]. We observed a considerable rise in transcriptional levels of these target genes of Nrf2 in HS group compared to the HF group, which had been limited compared to NC group (Fig. 6B). Additionally, we contrasted the Nrf2 ARE-binding activity in those groups as well. Comparatively to HF group, Nrf2 activity was increased in the nuclear extracts of iWAT from HS mice (Fig. 6C). In conclusion, these findings revealed the Nrf2 pathway was activated and increased protective mechanisms in iWAT of obese mice following SFN treatment.

Discussion

Adipose tissue in obesity is exposed to a chronic inflammatory milieu due to the production of huge number of proinflammatory substances. Adipocytes hypertrophy and proliferation result in the expansion of fat pads, which can store extra energy. The morphological changes ultimately result in fibrosis and malfunction in adipose tissue. WAT fibrosis is caused by an increase in ECM protein buildup and has been linked to local hypoxia and inflammation [6, 7]. Therefore, preventing WAT fibrosis is a useful tactic for enhancing systemic glucose and energy balance. In this investigation, we found that SFN significantly decreased WAT fibrosis and improved IR in DIO mice, which may present novel therapeutic targets for the treatment of obesity and related metabolic illnesses.

Adipose tissue homeostasis depends on the ECM, a noncellular component [7, 30]. Unbalanced production/degradation leads to abnormal ECM protein accumulation in WAT of obesity. Increased collagens, including type I, III, V, and VI, as well as fibronectin, have been seen in obesity-related WAT [31, 32], which is consistent with our findings. Aside from that, MMPs have the ability to dissolve ECM components, and certain TIMPs can restrict the actions of MMPs. The activities of MMPs and MMPs are also altered in obesity to exacerbate the expression of ECM-encoding genes [33, 34]. As a final result, an excess of ECM proteins is accumulated, forming WAT fibrosis and impaired systematic metabolism [30, 31, 35].

SFN has been proven to reduce fibrosis in the liver, kidney, lungs, and muscle [36–39]. However, little is known about SFN's function in regulating WAT fibrosis. In this study, we found that SFN therapy decreased ECM accumulation and inhibited WAT fibrosis, led to rigid recovery of adipocytes, removed physical constraints on healthy WAT expansion, and enhanced lipid metabolism. SFN may modulate macrophages polarization as part of its regulatory mechanism for suppressing WAT fibrosis. Fibrosis is frequently associated with inflammation [40]. Evidence suggests that macrophages have a role in obesity-related WAT fibrosis [41–44]. The macrophage-inducible C-type lectin is recruited by activated TLR4 in WAT, which triggers involved in ECM remodeling and results in WAT fibrosis [45]. SFN has the capacity to inhibit TLR4 activation, promote macrophages polarization towards M2 phenotype [46–48], and suppress inflammation in WAT. M2 macrophages contribute to the removal of the ECM by consuming and degrading collagen [49]. As a result, there is an improvement in the development of obesity-related WAT fibrosis.

In response to cellular stimuli, Nrf2 activates defense mechanisms as a master regulator of cellular redox state. Nrf2 is generally degraded constitutively by binding to Kelch-like ECH-associated protein 1 (Keap1). Under oxidative stress, the rate of Nrf2 breakdown slows and transfers to the nucleus, where Nrf2 modulates target gene transcription [27]. There is growing evidence that Nrf2 pathway activation inhibits the onset of obesity and IR [50, 51]. In addition to reducing the oxidative stress in obesity [52], Nrf2 can also decrease the production of inflammatory markers in macrophages [53]. The complicated mechanism of Nrf2 system has made it a desirable therapeutic target [15, 54].

SFN is a typical Nrf2 activator. According to researches, SFN activates the Nrf2 pathway, alters redox equilibrium, and assists the system recover from stress [55, 56]. Axelsson et al found that SFN inhibited glucose synthesis in hepatic cells by nuclear translocation of Nrf2 to reverse the T2D hallmark [17]. Research in NAFLD model of rats demonstrated that the consumption of lipids increased with SFN in a Nrf2 dependent way [57]. The ability of Nrf2 binding with DNA is compromised in the adipose tissue of obesity due to mitochondrial dysfunction. The Nrf2 pathway was boosted by SFN therapy, which also had anti-inflammatory effects, restored normal ECM remodeling, and maintained healthy adipocytes growth.

In conclusion, the WAT fibrosis and metabolic abnormalities in obesity were dramatically improved following SFN treatment. The molecular mechanism pointed to macrophages polarization. SFN was able to stimulate M2 macrophages polarization and inhibit M1 macrophages polarization to protect WAT from inflammation and aberrant ECM deposition. Moreover, SFN-mediated pharmacological activation of Nrf2 was expected to reduce obesity-induced oxidative stress and inflammation, maintain oxidant and antioxidant homeostasis, and decrease WAT fibrosis to improve insulin resistance.

Declarations

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Author contributions

Zhenzhen Zhang and Huali Chen conceived the study. All authors performed the study, carried out data statistics and discussed the results. Zhenzhen Zhang and Huali Chen wrote the manuscript. Wangsheng Zhao and Tianzeng Song provided the Funding and revised the manuscript.

Competing Interests

None

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Figures

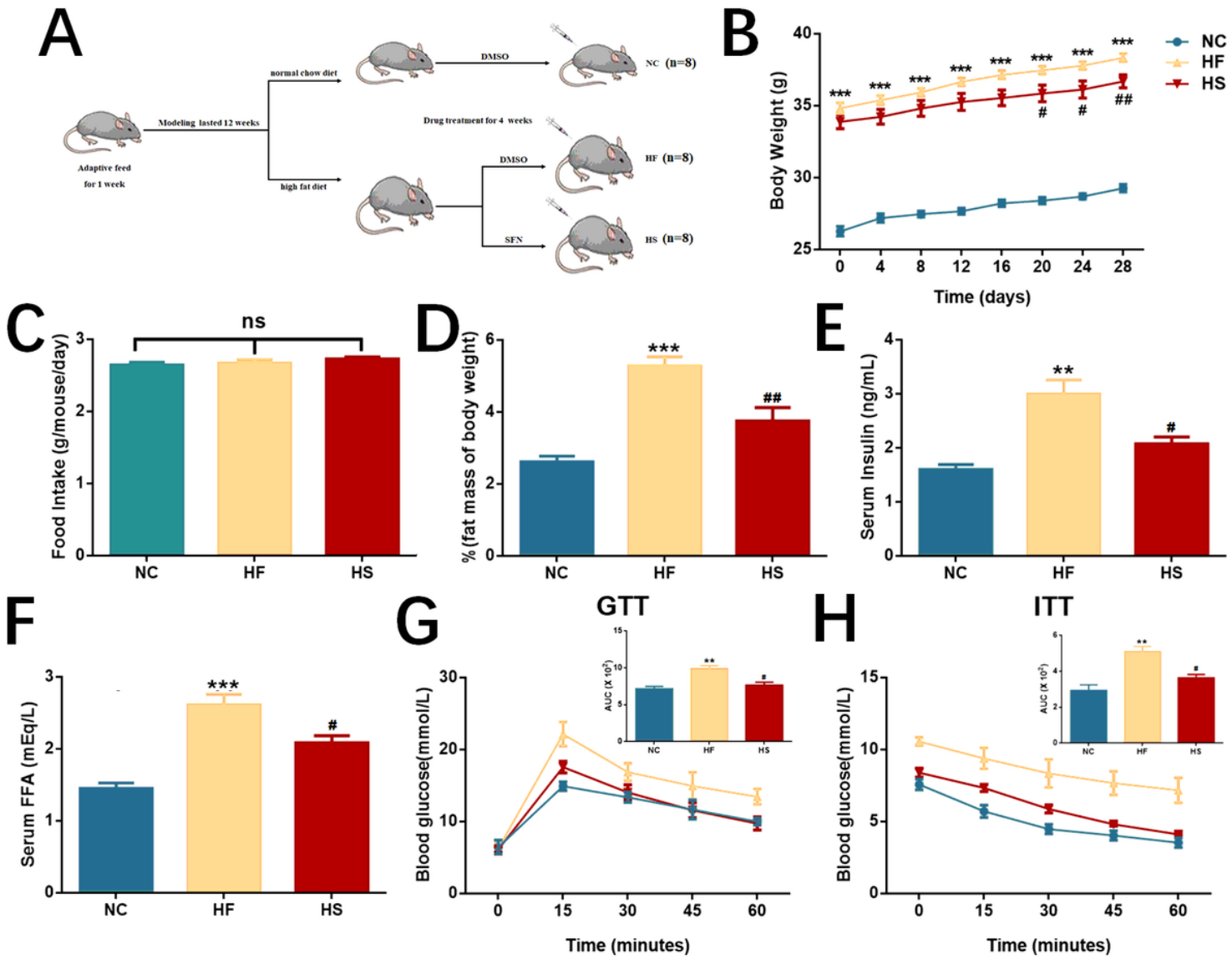

Figure 1

SFN suppressed HFD-induced obesity and improved IR in mice. (A) Schematic diagram of processing flow in mice. Experiment design was divided into three groups: normal chow diet group (NC), high-fat diet group (HF), and high-fat diet combined with SFN treatment group (HS) (n=8). (B) Body weight and (C) food intake were monitored every four days during experiment. (D) The proportion of iWAT mass. (E and F) Fasting insulin and FFA levels in serum were evaluated by ELISA. (G and H) GTT and ITT were conducted. The HF group compared with NC group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data was presented as mean ±SD. The HS group compared with the HF group: #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$. ns means no significance.

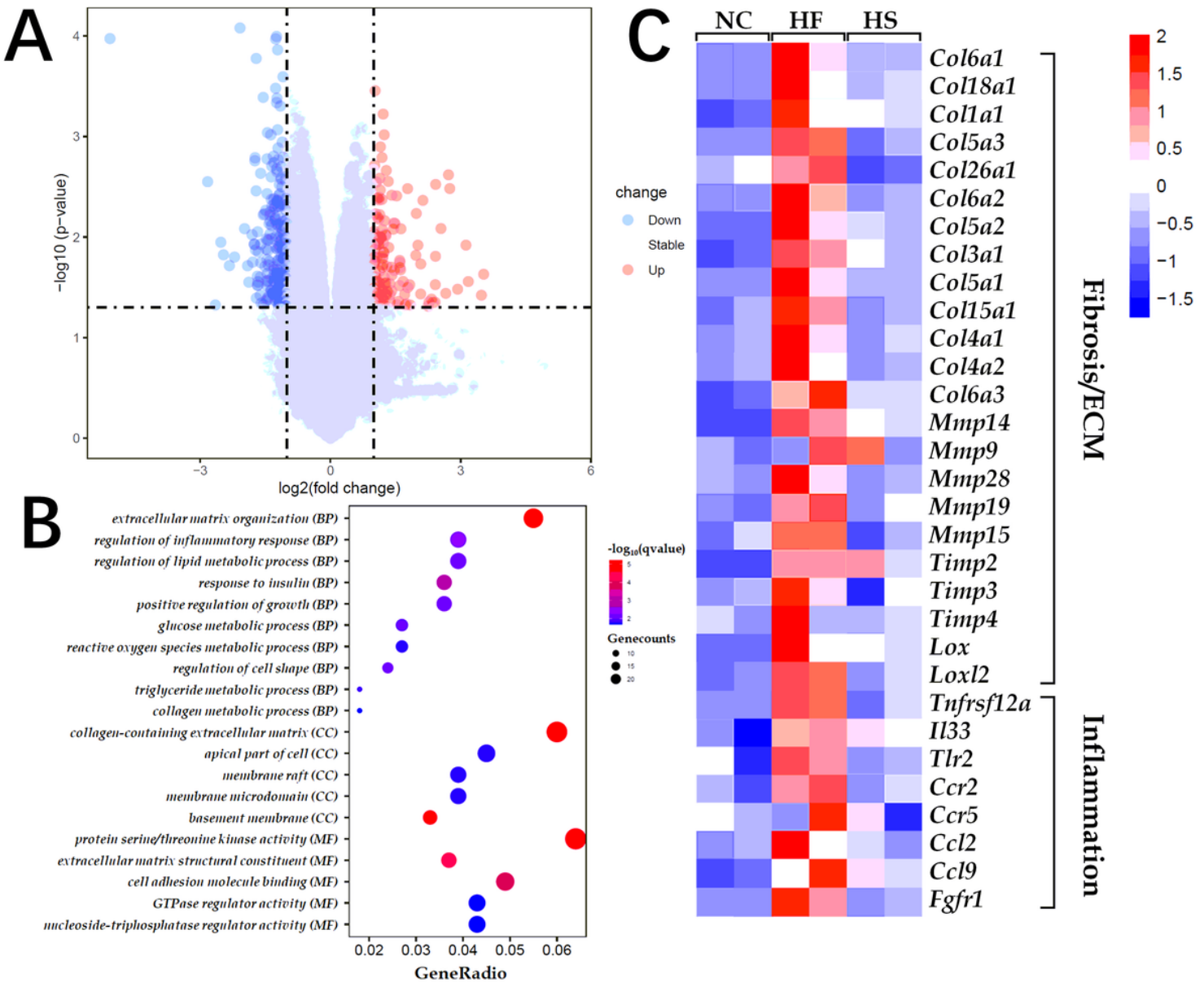

Figure 2

RNA-seq analysis of gene expression in iWAT of mice. (A) DEGs among HF and HS groups were showed by volcanic maps with a statistical cutoff of log2foldchange ≥1 and p value ≤0.05. (B) GO functional enrichment analysis was shown in a bubble diagram. (C) Heatmap showing relative expression of chosen genes related to fibrosis and inflammation. N=2 in NC, HF and HS groups.

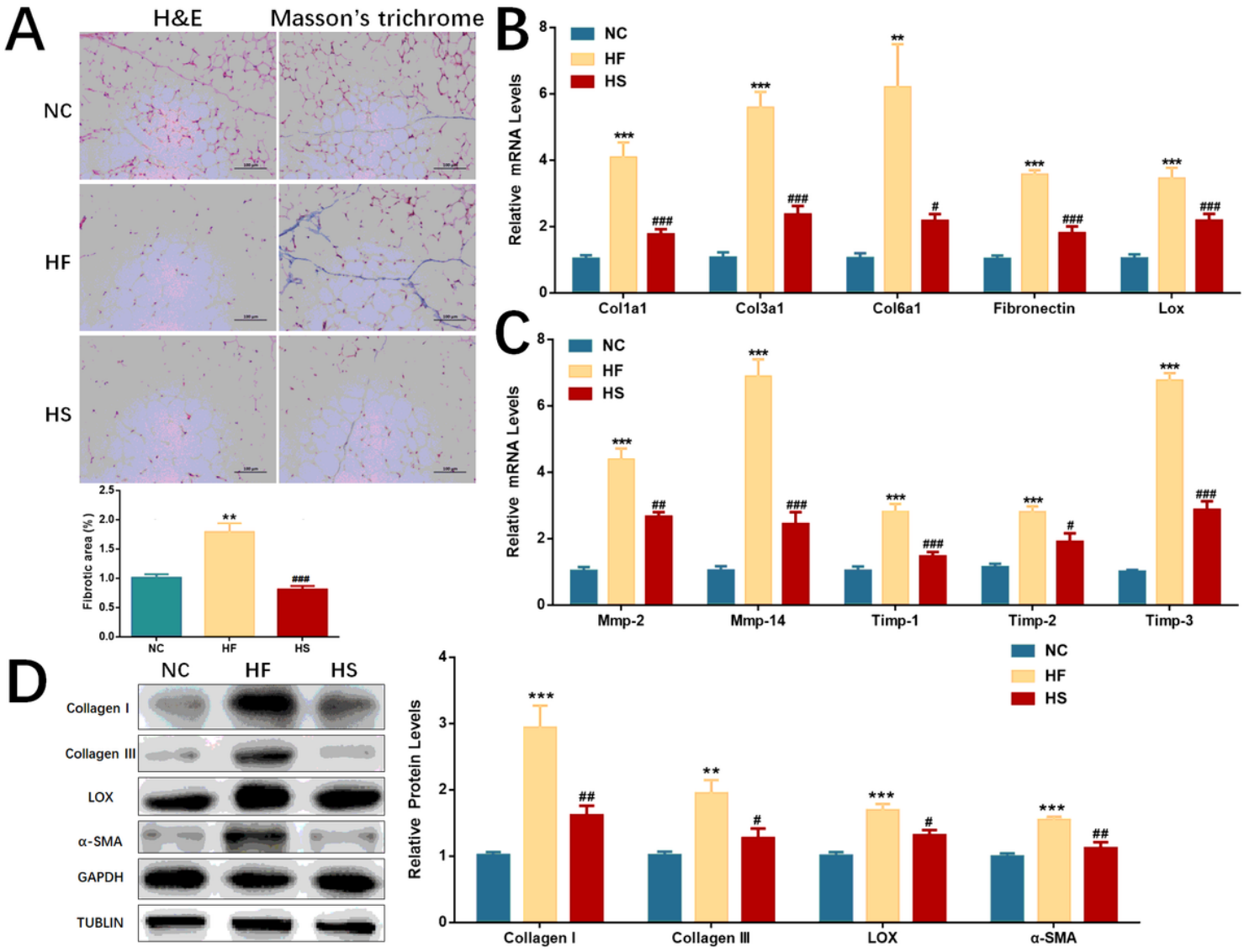

Figure 3

SFN restrained the iWAT fibrosis in HFD mice. (A) H&E (left) and Masson's trichrome (right) staining of iWAT (scare bar: 100 μm). (B and C) The transcriptional levels of fibrogenic genes *Col1a1*, *Col3a1*, *Col6a1*, *fibronectin* and *Lox* (B) and ECM regulator genes *Mmp-2*, *Mmp-14*,*Timp-1*, *Timp-2* and *Timp-3* (C) were detected by QPCR. (D) Western blot and quantification analysis of fibrogenesis-related genes extracted from iWAT. Data was presented as mean ±SD. The HF group compared with NC group: *p < 0.05, **p < 0.01, ***p< 0.001. The HS group compared with the HF group: #p < 0.05, ##p<0.01, and ###p < 0.001. ns means no significance.

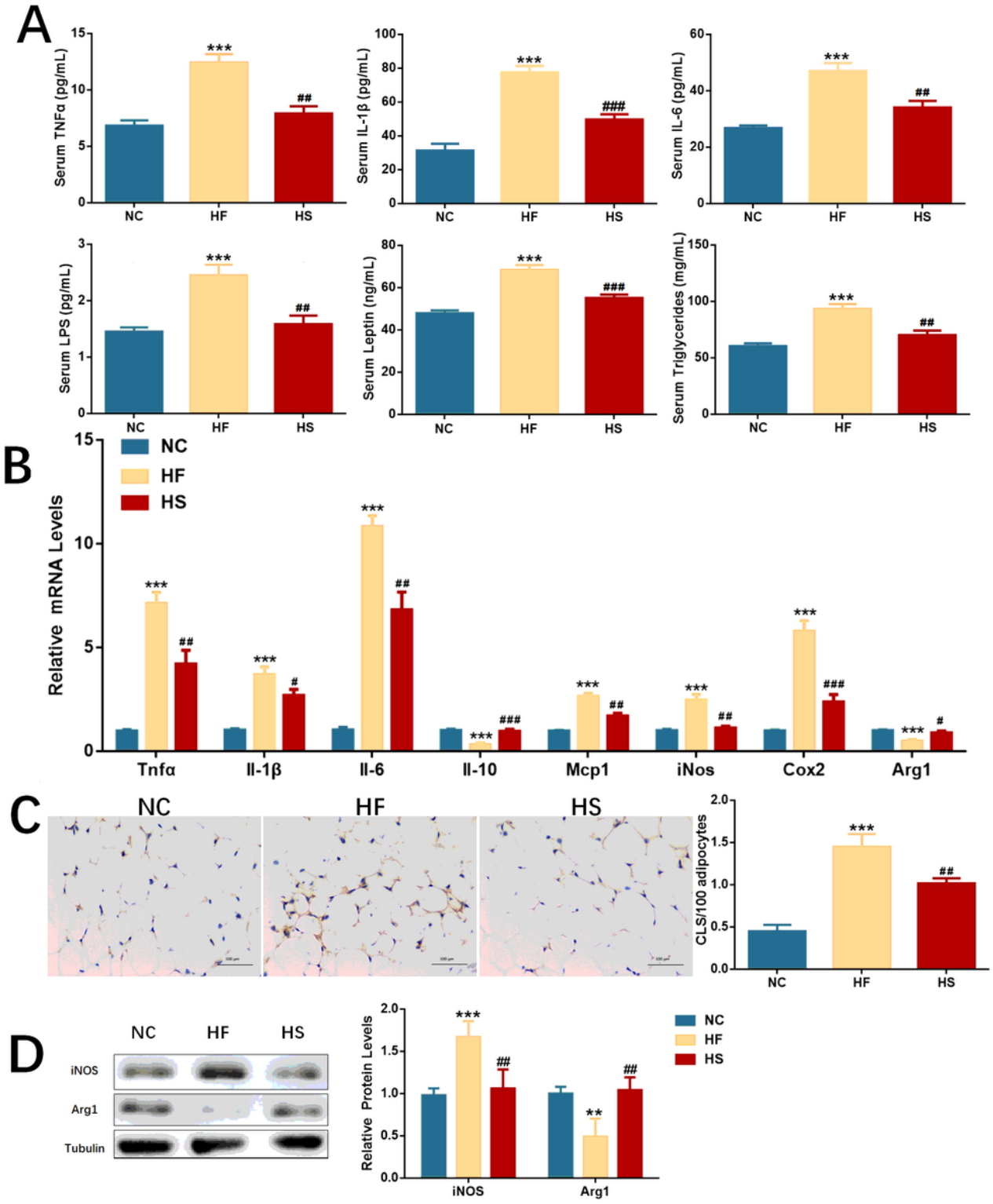

Figure 4

SFN inhibited inflammation and M1 macrophages polarization in iWAT of DIO mice. (A) Inflammatory factors in serum were determined by ELISA. (B) Quantitative PCR analysis of inflammatory genes *Tnfα*,*Il-1β*, *Il-6*, *Il-10*, *Mcp-1*, *iNos*, *Cox2*and *Arg1*. (C) F4/80 immunohistochemical analysis in iWAT of mice (scare bar: 100 μm). (D) Expression analysis of macrophages markers protein iNos and Arg1. Data was presented as mean ±SD. The HF group compared with NC group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. The

HS group compared with the HF group: #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$. ns means no significance.

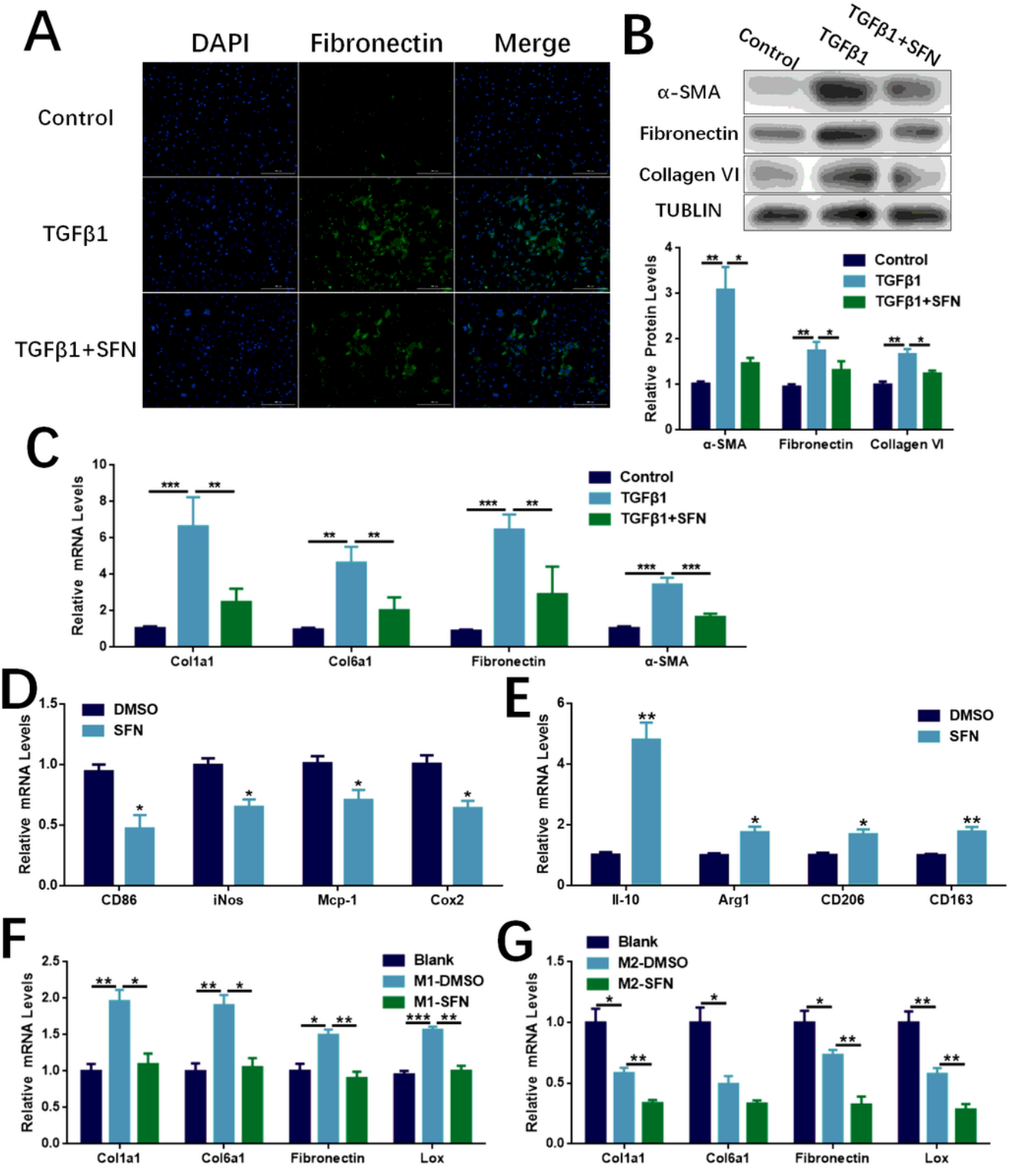

Figure 5

SFN protected adipocytes from excessive matrix deposition *in vitro*. (A) Immunofluorescence staining of TGFβ1 (scare bar: 100 μm). (B) Western blot detected the expression of protein levels of α-SMA,

fibronectin and Collagen VI in adipocytes (n=3). (C) QPCR analysis of fibrosis genes *Col1a1*, *Col6a1*, *fibronectin* and *α-SMA* in adipocytes (n=3). (D) Relative gene expression of M1 macrophages markers *CD86*, *iNos*, *Mcp-1* and *Cox2* and (E) M2 macrophages markers *Il-10*, *Arg1*, *CD206*and *CD163* in RAW264.7 cells (n=3). (F and G) The mRNA levels of fibrosis genes *Col1a1*, *Col6a1*, *fibronectin* and *Lox* in adipocytes after treated with supernate from M1 macrophages (F) and M2 macrophages (G). Data was presented as mean ±SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

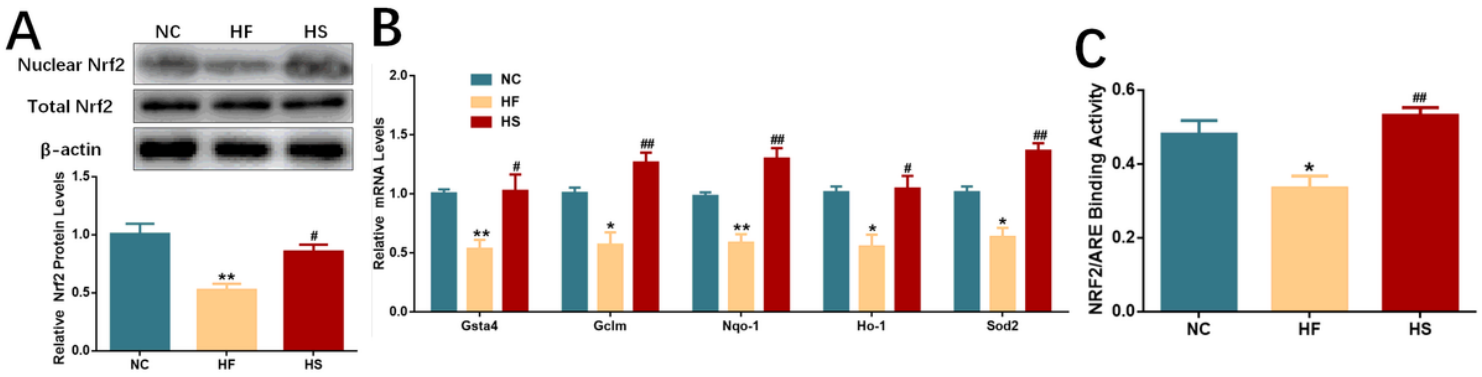

Figure 6

SFN activated Nrf2 signaling pathway in iWAT. (A) Western blot was used to detect the expression of Nrf2. (C) Quantitative PCR analysis of Nrf2 target genes. (D) Nrf2-ARE-binding activity was measured by ELISA. Data was presented as mean ±SD. The HF group compared with NC group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. The HS group compared with the HF group: #$p < 0.05$, ##$p<0.01$, and ###$p < 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTableS1.xlsx
- SupplementalTableS2.xlsx