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ORIGINAL ARTICLE

Shenfu Injection Alleviates Pancreatitis-Induced Damage and Oxidative Stress by Upregulating CLDN4 to Protect Endothelial Cells

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SUMMARY

Background: **Endothelial dysfunction represents a critical pathological feature of acute pancreatitis (AP). Shenfu injection (SFI) has been demonstrated to protect both endothelial cells and pancreatic tissues affected by AP; however, the precise mechanisms underlying its protective effects remain incompletely understood. The study investigated the protective role of SFI in oxidative-stressed endothelial cells and in acute pancreatitis through the regulation of CLDN4.**

Methods: **Human umbilical vein endothelial cells (HUVECs) were treated with oxidized low-density lipoprotein (ox-LDL) to create an oxidative stress environment, followed by SFI treatment to assess cell viability, apoptosis, and reactive oxygen species (ROS) levels. Additionally, shRNA technology was employed to knock down CLDN4 expression in order to evaluate its role in SFI-mediated protection. Finally, an AP rat model was established to investigate the effects of SFI on AP-induced pancreatic damage and the role of CLDN4.**

Results: **SFI significantly enhances cell viability, reduces apoptosis, and lowers ROS levels in HUVECs under oxidative stress. At the molecular level, SFI upregulates CLDN4 expression, and depletion of CLDN4 attenuates the protective effects of SFI against oxidative stress. In the AP rat model, SFI administration alleviated pancreatic tissue damage and reduced inflammation, while CLDN4 knockdown diminished these protective effects.**

Conclusions: **These findings identify CLDN4 as a key mediator of SFI's protective effects on both oxidative-stressed endothelial cells and inflamed pancreatic tissues, underscoring its potential as a therapeutic target in AP.**
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Supplementary Data

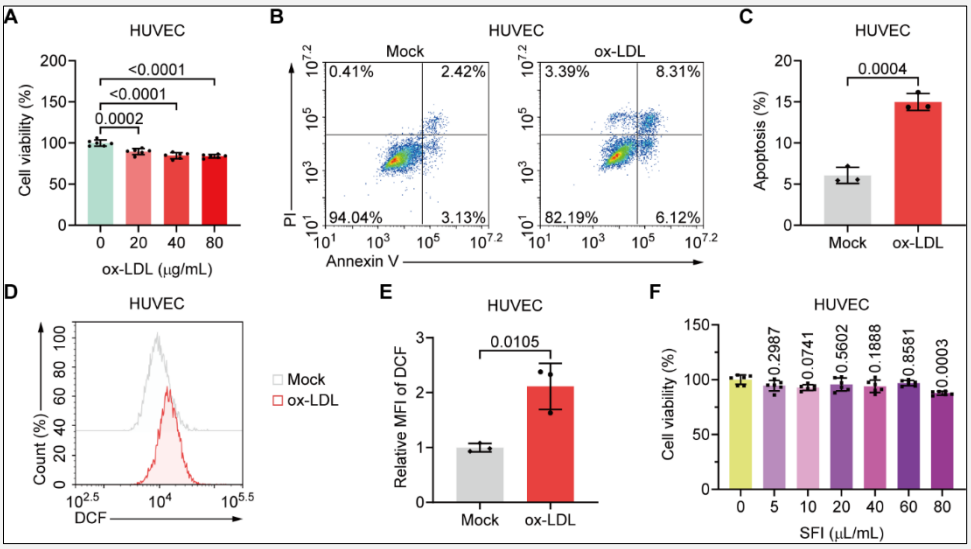

Figure S1. ox-LDL and SFI affect HUVECs.

A - CCK-8 data indicating the viability of HUVECs treated with the specified concentrations of ox-LDL. B, C - Annexin V/PI staining results showing the apoptosis of HUVECs exposed to PBS (mock) or 40 μg/mL ox-LDL. D, E - DCF-DA staining outcomes revealing the ROS levels of HUVECs exposed to PBS or 40 μg/mL ox-LDL. F - CCK-8 results illustrating the viability of HUVECs treated with the specified concentrations of SFI.

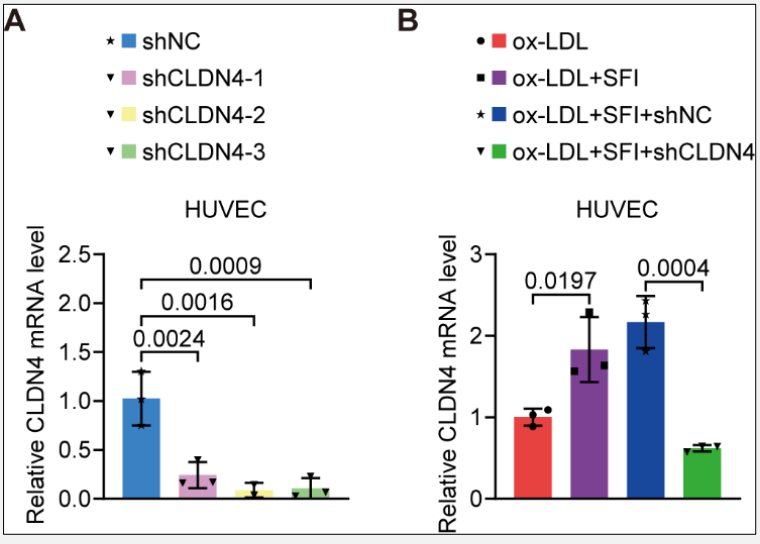

Figure S2. Verification of the efficiency of siRNAs targeting CLDN4.

A - qPCR data showing the mRNA expression level of CLDN4 in HUVECs transfected with the specified shRNAs. B - qPCR results depicting the mRNA levels of CLDN4 in HUVECs with the specified treatments.