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Xylose (D-xylose) inhibits the macrophage high-expression gene LYZ to ameliorate non-alcoholic fatty liver disease

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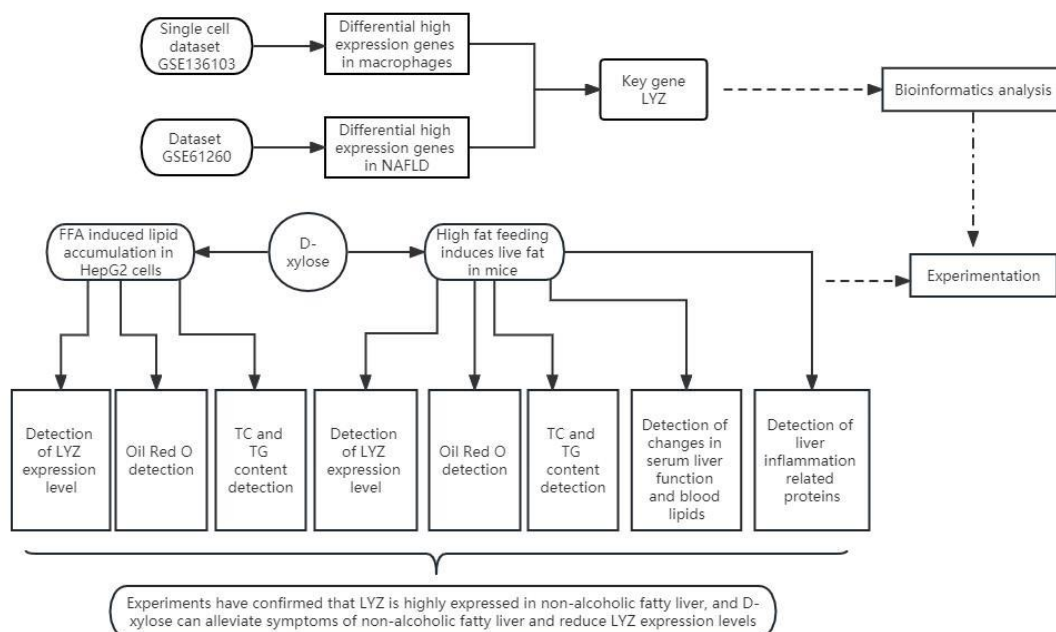
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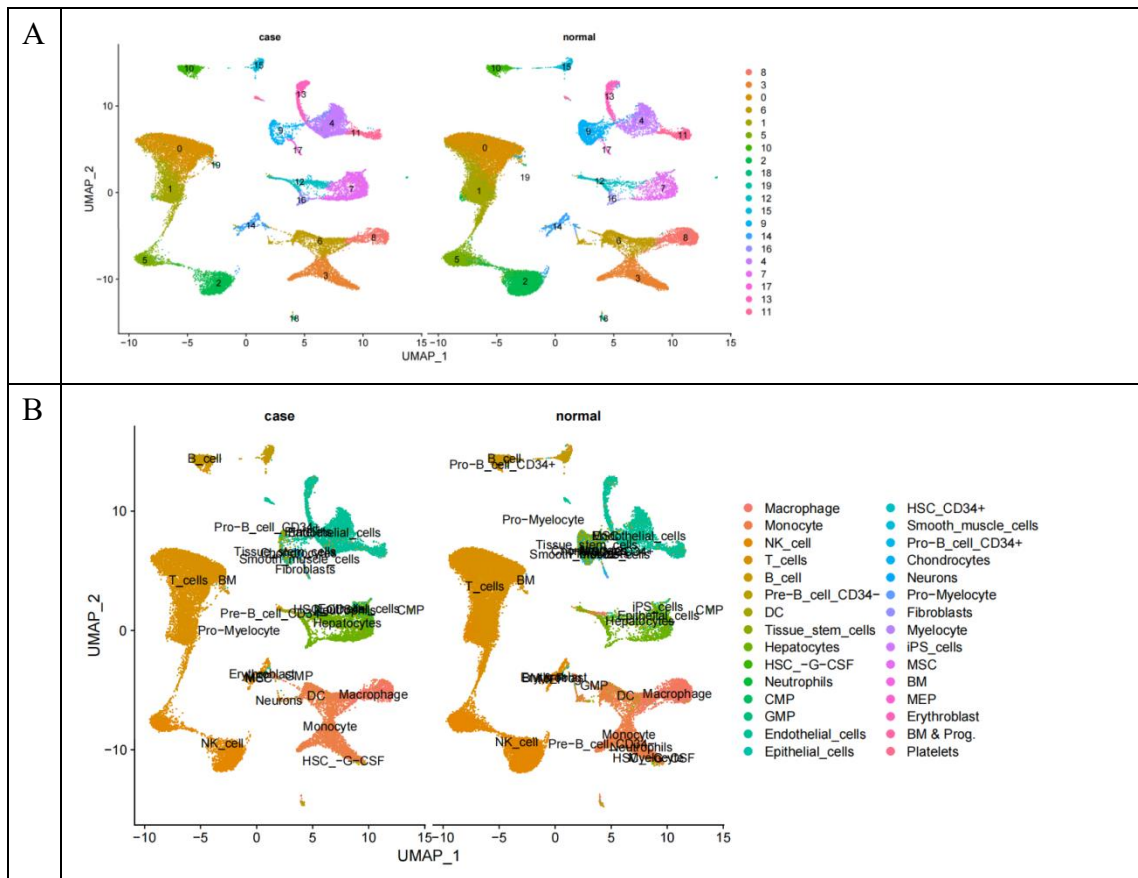
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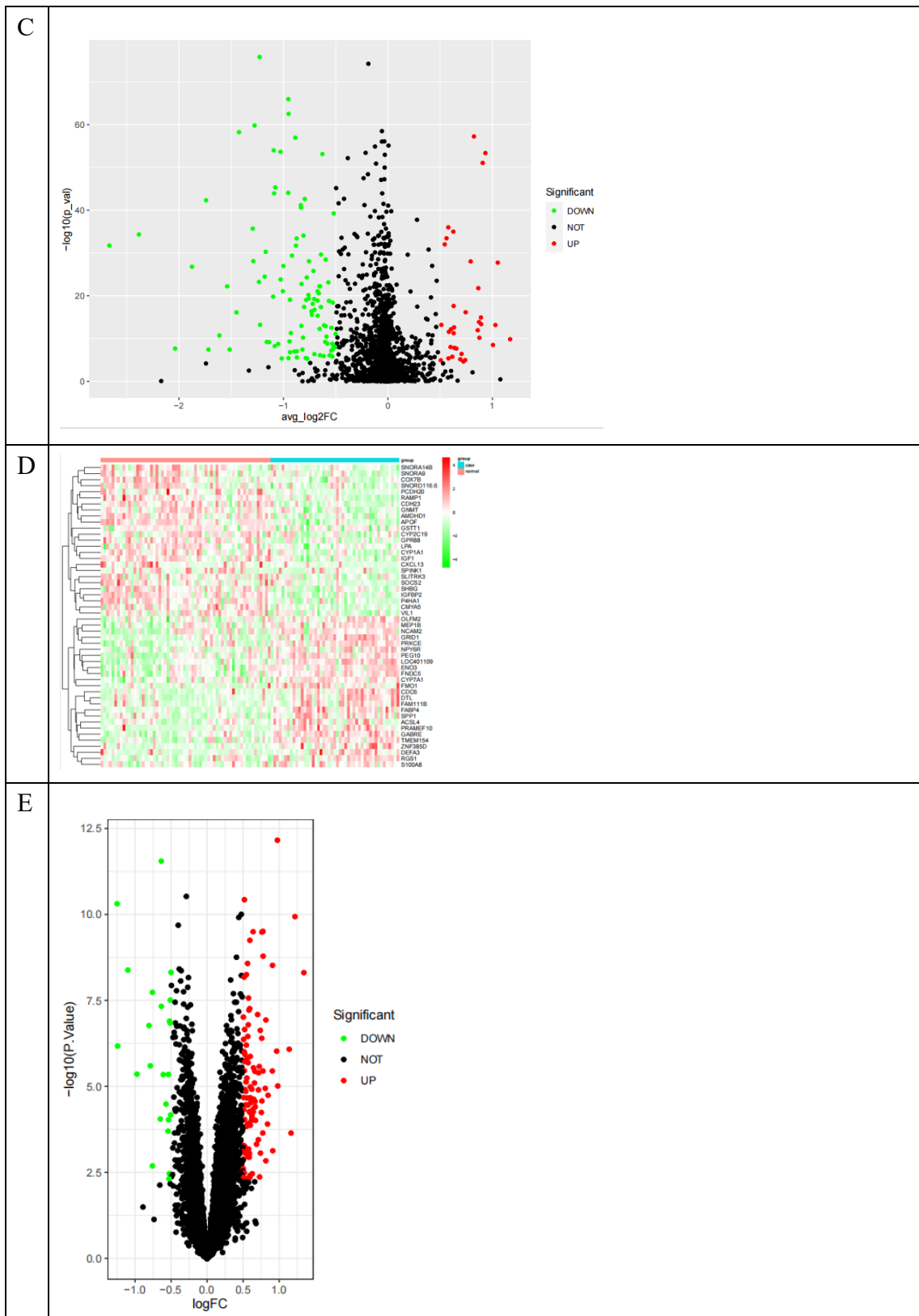


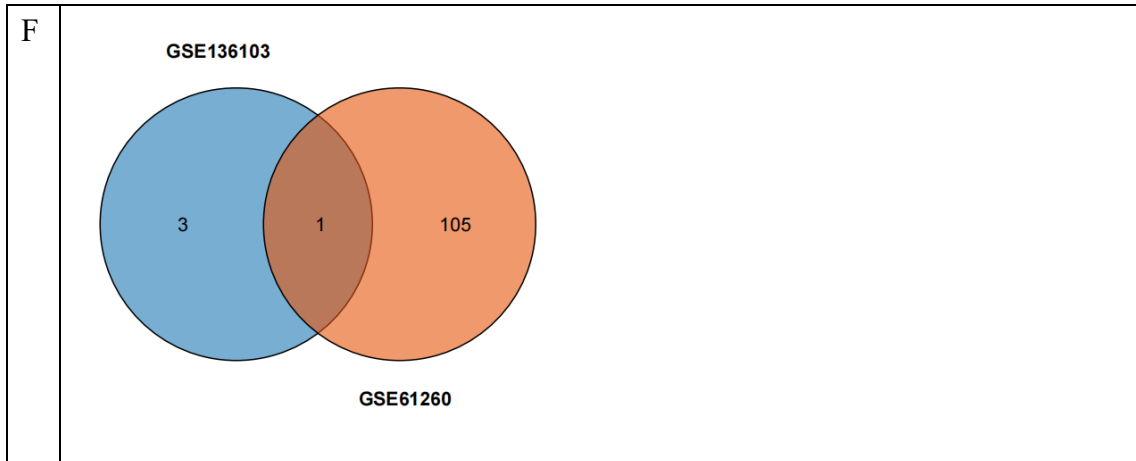
1. Identify crucial genes associated with NAFLD

The study involved the extraction of 9 NAFLD samples from the NAFLD single-cell dataset GSE136103, which was obtained from the GEO database

(www.ncbi.nlm.nih.gov/geo). The data were analyzed using the "Seurat" package in R software version 4.0.5 (FIG 1A-B). The annotated macrophage population was compared to other populations (FIG 1C) simultaneously, and a set of differentially highly expressed genes in macrophages was obtained (Table 1). The NAFLD dataset GSE61260 was downloaded from the GEO database (www.ncbi.nlm.nih.gov/geo), in which the expression matrices of the whole genome of 47 NAFLD and 62 normal control samples were downloaded, and utilized the "limma" package of the R software(version 4.0.5) to " package for differential analysis of the above data (FIG 1D-E). The single-cell dataset GSE136103 was used to obtain the macrophage differentially highly expressed gene set and the GSE61260 differentially highly expressed gene set was intersected to obtain the key gene LYZ (FIG 1F).

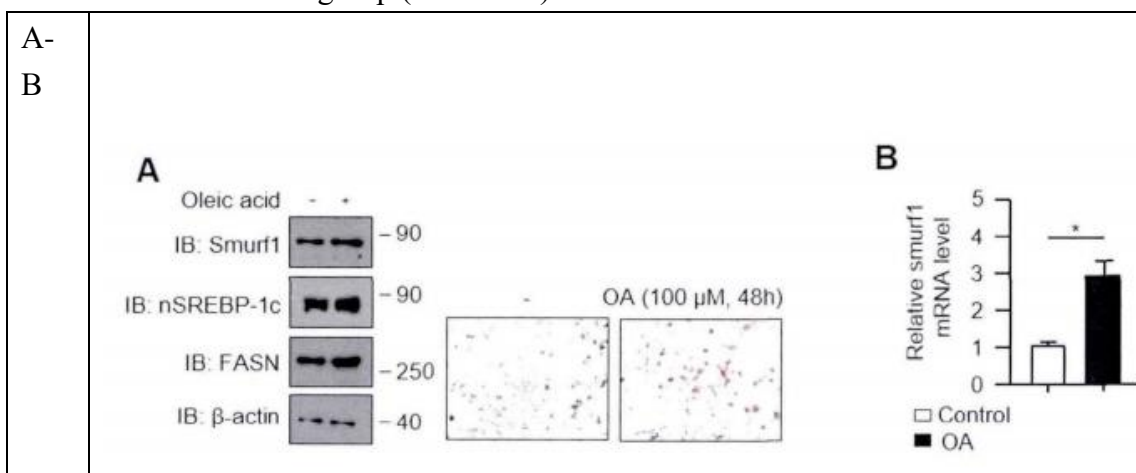


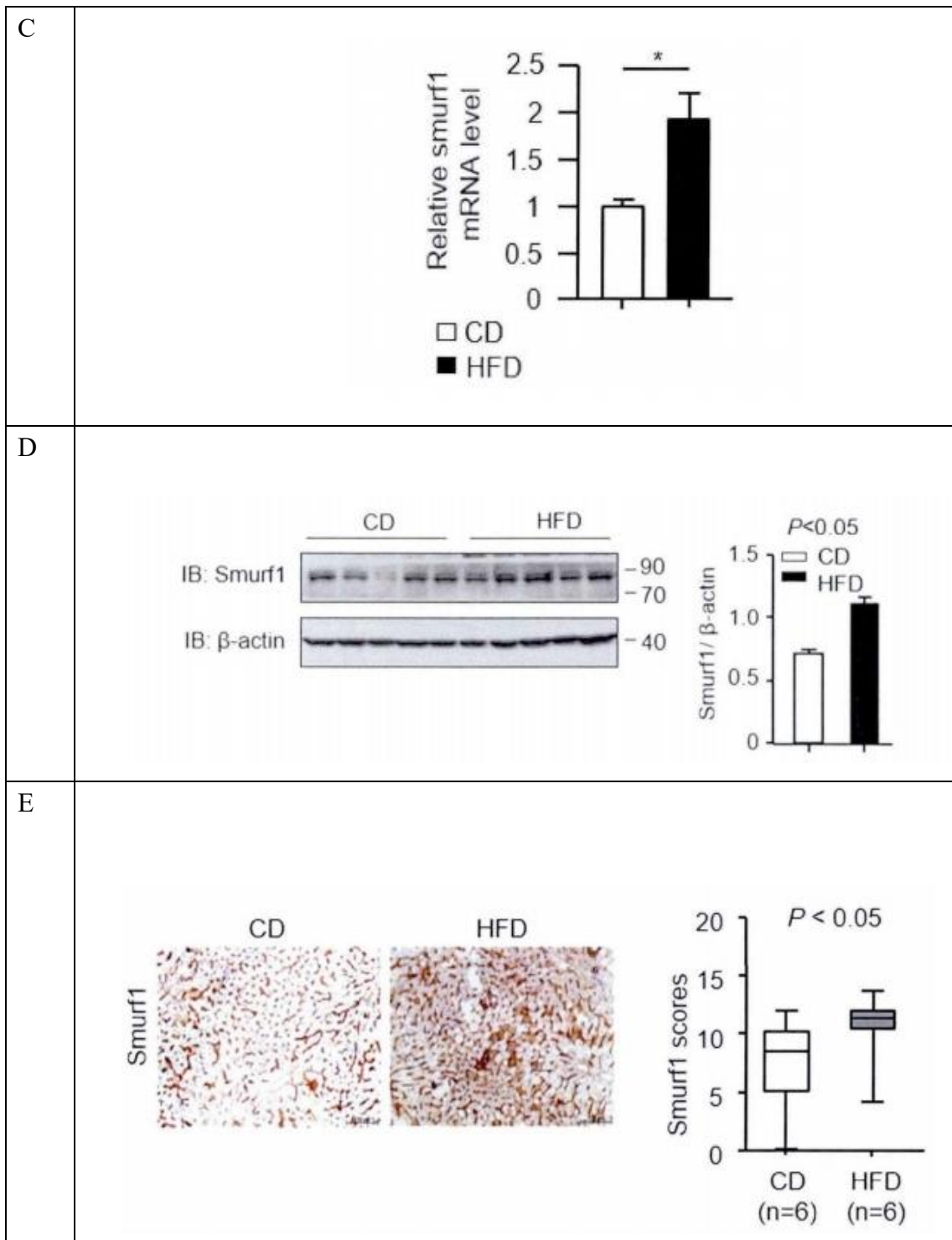




2.LYZ is highly expressed in NAFLD cells and in mouse liver tissues

A NAFLD cell model was constructed in vitro. FFA mixtures (OA:PA=2:1) were prepared by combining sodium oleate (OA) and sodium palmitate (PA) with medium containing 1% bovine serum albumin (BSA). HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin in 5% CO₂ at 37°C(Reference: doi: 10.7150/ijbs.68873). HepG2 cells were incubated with 1 mM FFA for 24 h to promote lipid accumulation, which was detected by Oil Red “O”. qPCR and WB were performed to detect the expression of LYZ in the cells. The results demonstrated that cells in the FFA group exhibited enhanced oil red O staining and a significant upregulation of LYZ expression(FIG 2A-B). A mouse model was constructed and divided into control group (normal feeding) and model group (high-fat feeding). Liver tissues of mice were taken and the protein and mRNA expression of LYZ were detected. qPCR assay showed LYZ was significant increase in the liver tissues of mice in the model group (FIG 2C). wb and immunohistochemistry results showed that the protein level of LYZ was significantly increased in the model group (FIG 2D-E).

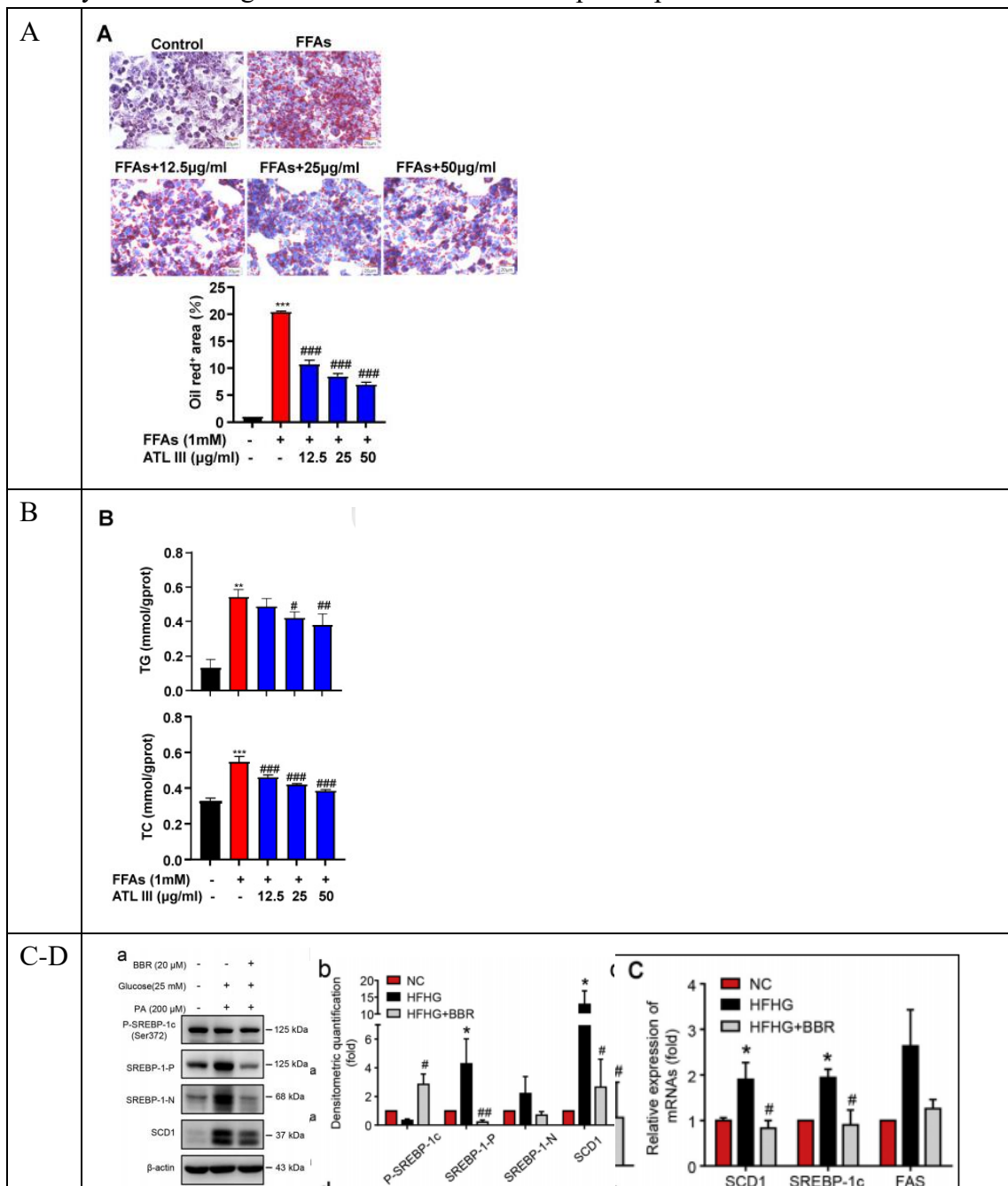




In vitro experiments to verify the effect of xylose on lipid accumulation and LYZ expression

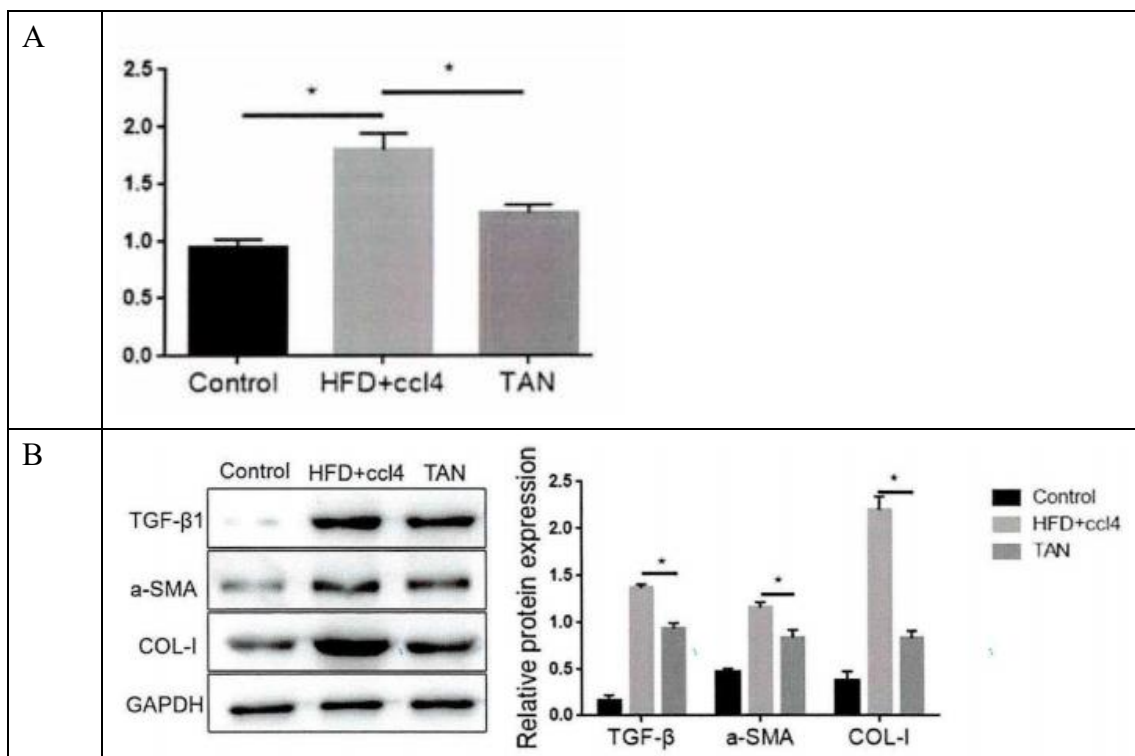
HepG2 cells were incubated with 1 mM FFA for 24h to promote lipid accumulation, then treated with xylose for 24h to assess its effect on lipid metabolism. Control group and FFA-treated group were treated with the same volume of XXX. Oil red “O” staining showed a significant increase in lipid accumulation in the FFA group compared to the control group. The accumulation of lipids in the cells of the

xylose-treated group was significantly reduced compared to the FFA group (FIG3 A). At the same time, we detected triglyceride (TG) and total cholesterol (TC) content in the cells of each group. The results showed that the content of TG and TC in the cells of the FFA group was significantly increased compared to the control group(FIG3 B). Next, we explored whether xylose could reduce LYZ in vitro. We found that both protein and mRNA of LYZ were increased in the FFA group compared to the control group, and these expression were reduced after xylose treatment (FIG 3C-D). These findings suggest that LYZ is highly expressed in hepatocytes with fat accumulation, and xylose can mitigate overnutrition-induced hepatic lipid accumulation and LYZ.



Xylose inhibits overnutrition-induced increase in LYZ expression in mouse liver

A mouse model was constructed, which was divided into a control group (normal feeding), a model group (high-fat feeding), and a dosing group (high-fat feeding along with xylose intervention). The liver tissues were obtained from mice fed on three different diets, and subsequent RNA extraction was performed after tissue grinding. The mRNA of LYZ in control group was significantly higher than that of control group, and the expression of LYZ was significantly lower than that of the model group after administration of LYZ (FIG 4A). WB analysis showed that, comparing with control group, the protein of LYZ in the livers of model group was significantly higher, and the expression of LYZ was significantly lower than that of the model group after administration of LYZ (FIG 4B).

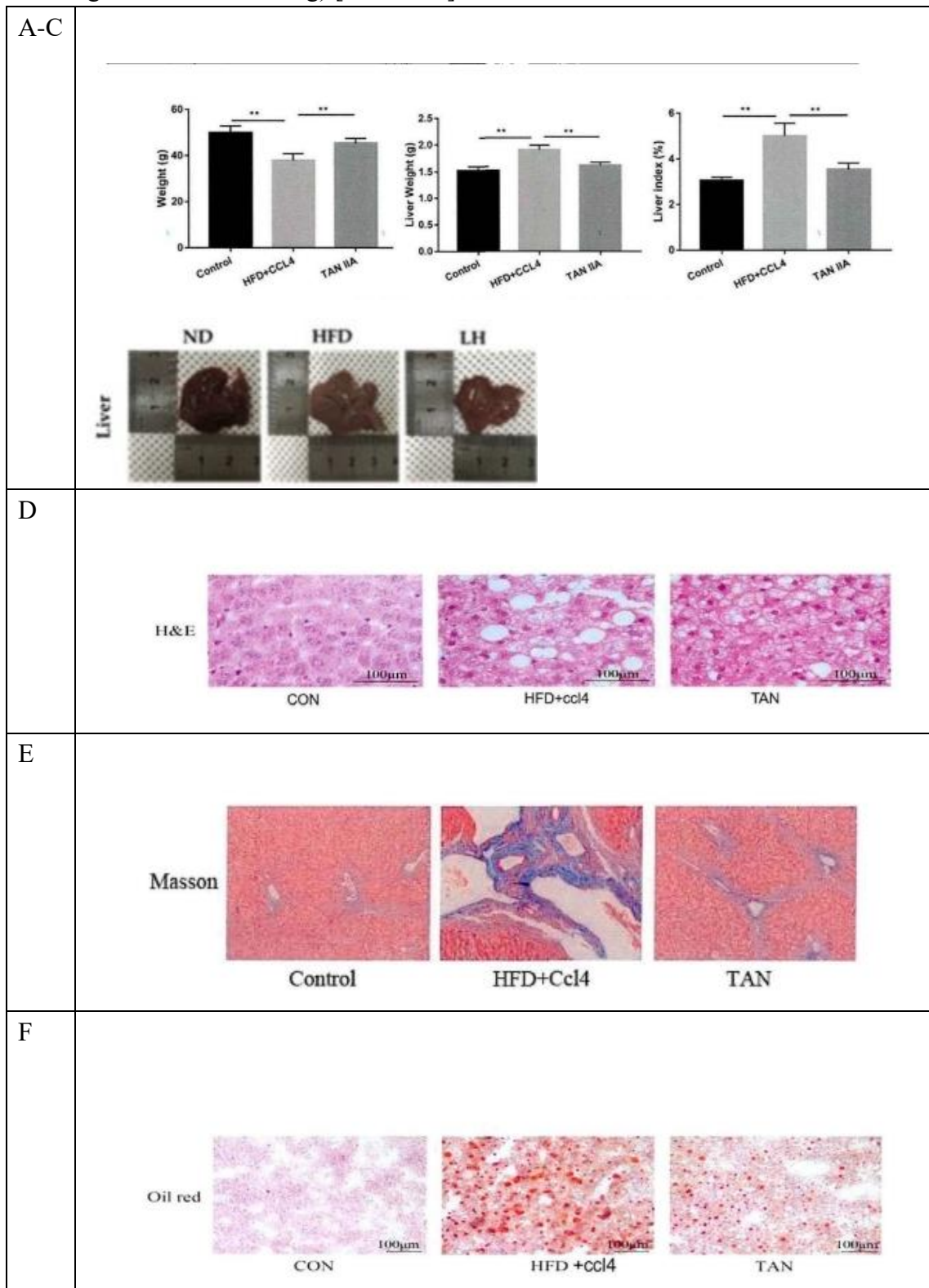


Xylose attenuates fat accumulation in mouse liver induced by overnutrition

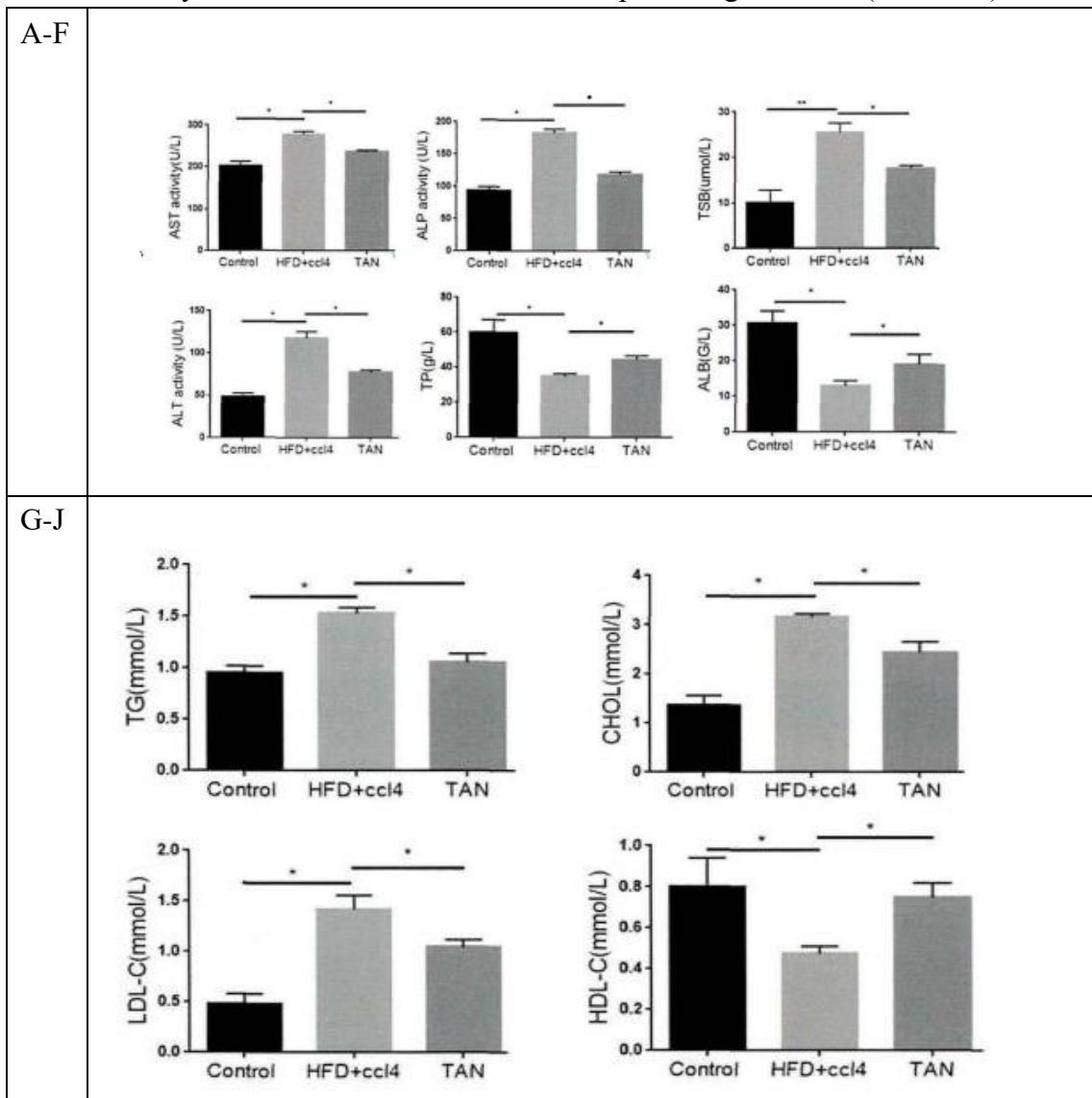
1. The effects of xylose on the changes of body weight, liver weight and liver index in mice were detected.

The results showed that the body weight of the model group was significantly lower than that of the control group, and the body weight of the dosing group was larger than that of the model group and smaller than that of the control group (FIG 5A). The liver of the model group exhibited a yellowish-brown coloration with visible lipid droplets, accompanied by an increase in both liver weight and liver index when compared to the control group. In contrast, the dosing group showed a significant reduction in both liver weight and index compared to the model group, along with a lighter red coloration of the liver (FIG 5B-C).

2. Effects of xylose on histopathological changes in mouse liver (HE staining, Masson staining, oil red “O” staining) [FIG 5D-F].



3. Effect of xylose on serum liver function and lipid changes in mice(FIG6 A-J).



4. Effect of xylose on inflammatory response in mouse liver (FIG 7).

