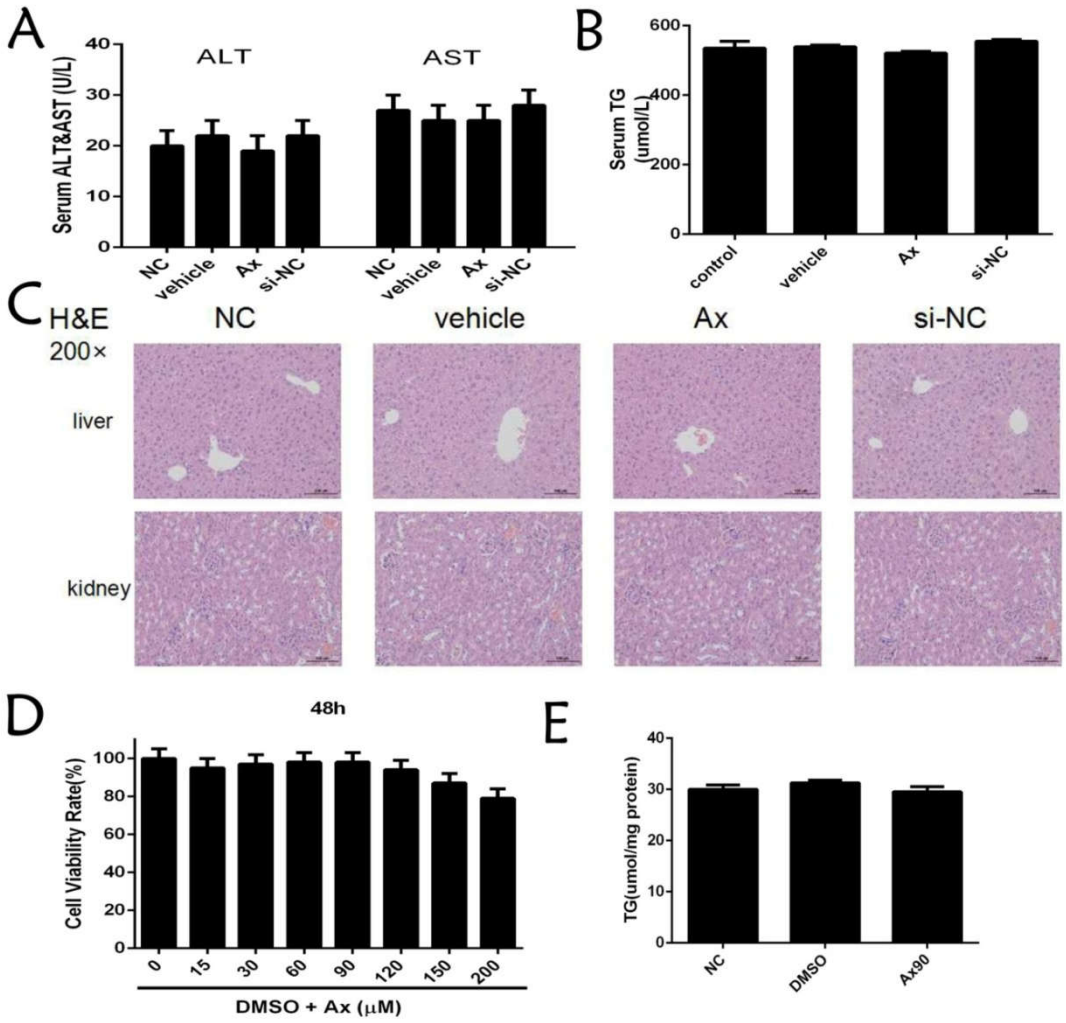
Supplymentary Figures for Ax-NAFLD

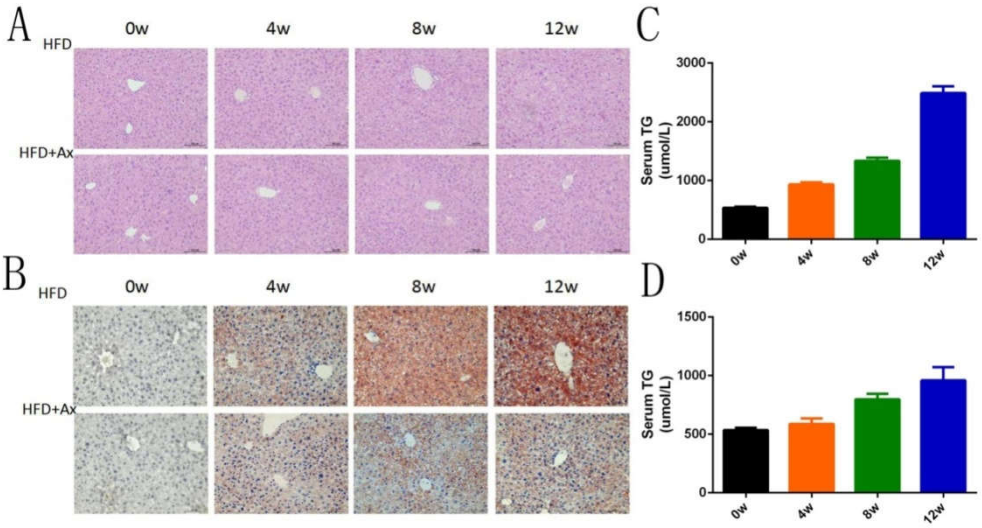
1.Ax ,vehicle(saline or DMSO), and si-NC had no toxicity on liver and hepatocytes.

To study the toxicity of Ax , vehicle(saline or DMSO), and si-NC on liver in mice or hepatocytes and make our experiments more reliable, we did some preliminary studies to examine the safety of Ax. ALT and AST are usually regarded as hallmarks of liver injury, SF1A showed that compared with NC group, the treatment of Ax, vehicle(saline) and si-NC didn't elevate them. And TG, a biomarker for lipotoxicity, also wasn't influenced obviously(SF 1B). H&E staining in SF 1C showed that Ax, vehicle(saline) and si-NC had no toxic effects on liver and kidney pathology. Besides, we examined the cytotoxicity of Ax and vehicle(DMSO) by CCK8, and results were showed in SF 1D. Also, TG wasn't changed among these groups(SF 1E). Therefore, we confirmed that Ax , vehicle(saline or DMSO), and si-NC had no obvious toxicity on liver and hepatocytes.



2. Pathological changes in liver of HFD-fed mice after 0,4,8 and 12 weeks

SF2A showed the liver H&E staining results of HFD-fed mice with or without Ax treatment. We found that obvious hepatic fatty infiltration appeared at 8 and 12 weeks, and Ax treatment alleviated HFD-induced liver damages. To examine the lipid accumulation in the liver, Oil Red O staining was used in our study, and SF2B exhibited lipid deposition in the liver.In results of 8 and 12 weeks, Ax administration significantly suppressed lipid accumulation in liver.Serum TG levels of mice fed with HFD were shown in SF2C,and SF2D was for mice with both HFD and Ax treatment.The data showed that Ax reduced the TG deposion caused by HFD.Thus, the liver damage was more severe after 12 weeks of feeding,and Ax did its job,too. So, we choose mice of 12 weeks treatment for our following experiments.



3. Lentivirus screening

Lentiviruses, which contained different sh-FGF21 sequence, or the empty vector were transfected into L02 cells.SF3A showed the sequence and transfection results.Then we detected the mRNA and protein expression of FGF21, and found the FGF21-homo-434 was the most effective(SF3B).