Supplemental Data

**Title:** Glycyrrhizin alleviates non-alcoholic steatohepatitis via modulating bile acids and meta-inflammation

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Supplemental Figure 1. GL decreases MCD-induced apoptotic signaling activation, while shows no significant effect in MCD-induced body weight loss, food intake, and liver index. (A), Body weight ratio, the ratio of body weight at the end of experiment to body weight before MCD diet feeding. (B), Average food intake record for the first week after GL injection, all mice were single-caged and food intake was recorded every day for 7 days. (C), Liver index, ratio of liver weight to body weight. (D), Levels of liver apoptotic signaling, Bcl2a1c mRNAs. (E and F), GL treatment alleviates ACTD/TNFα-induced HepG2 cell death at 13 h (E) and 24 h (F) after TNFα challenge by CKK-8 kit. (G), GL treatment alleviates ACTD/TNFα-induced HepG2 apoptosis by FACS assay at 13 h after TNFα challenge. Data are presented as means ± SD. Statistic
differences determined by one-way ANOVA followed by Dunnett’s multiple comparisons test among multiple-group comparisons. For mouse experiments, n=6-8 in each group. Bcl2a1c, B-cell leukemia/lymphoma 2 related protein a1c. MCS, MCS diet-fed mice treated with saline; MCD, MCD diet-fed mice treated with saline; MCD+GL50, MCD diet-fed mice treated with 50 mg/kg of GL. ###p<0.001 versus MCS group. *p<0.05 and **p<0.01 versus MCD group. For cell culture experiments, HepG2 cells were pretreated with 0.1% DMSO or various concentration of GL for 30 minutes in the presence or absence of 0.3 μM of ACTD, and then 30 ng/mL of recombinant human TNFα were added to induce cell death. CCK-8 kit assay or FACS assay was performed at 13 h or 24 h after TNFα treatment to test the cell viability. n=6-12 for F and G, and n=3 for H. ###p<0.001 versus control group. ***p<0.001 versus 0.1% DMSO group.
Supplemental Figure 2. Effect of GL in FXR activation in vivo and in vitro. (A-C), Effect of GL in the mRNA expression of FXR target genes in primary mouse hepatocytes (A), HepG2 cells (B), and in mice (C). Primary hepatocytes isolated from 8-week old male mice or HepG2 cells were treated with 50 μM of GL, 10 μM of GA or control vehicle 0.1% DMSO (n=3-6 per group) for 24 h in 10% DMEM culture medium; mice were treated with control vehicle (Vehicle group), 50 mg/kg of GL for a single injection (Single GL50 group) or 7 consecutive days (Multiple GL50 group), and then livers were collected for mRNA analysis at the end of experiments, n=5 mice in each group. (D), Effect of 200 μM of GL and 15 μM of GA in LPS-induced FXR inactivation in HepG2 cells. (E), Effect of 50 μM or 200 μM of GL, 5 μM or 15 μM of GA, 4 μM of GW4064 in the FXR luciferase reporter activity; HepG2 cells in 10% DMEM culture medium (n=3 per group). Data are presented as means ± SD. Statistic differences determined by one-way ANOVA followed by Dunnett’s multiple comparisons test among multiple-group comparisons. #p<0.05; ##p<0.01 and ###p<0.001 versus control vehicle-treated group. *p<0.05 versus LPS-treated group.
Supplemental Figure 3. GA as well as GL gavage shows no significant effect in body weight, liver weight, and liver index. (A), Body weight ratio, the ratio of body weight at the end of experiment to body weight at Day 0 of the experiment. (B), Liver weight. (C), Liver index, ratio of liver weight to body weight. (D), Body weight ratio, the ratio of body weight at the end of experiment to body weight at Day 0 of the experiment. (E), liver weight. (F), Liver index, ratio of liver weight to body weight. Data are presented as means ± SD. Statistic differences determined by one-way ANOVA followed by Dunnett’s multiple comparisons test among multiple-group comparisons. #p<0.05; ##p<0.01 and ###p<0.001 versus MCS group. n=5 in each group. MCS, MCS diet-fed mice treated with saline; MCD, MCD diet-fed mice treated with saline;
MCD+GL50(P.O.), MCD diet-fed mice treated with 50 mg/kg of GL by gavage. MCD+GA30, MCD diet-fed mice treated with 30 mg/kg of GA.