Supplemental Materials

Title: glycyrrhizin protects against acetaminophen-induced acute liver injury via alleviating TNFα-mediated apoptosis;

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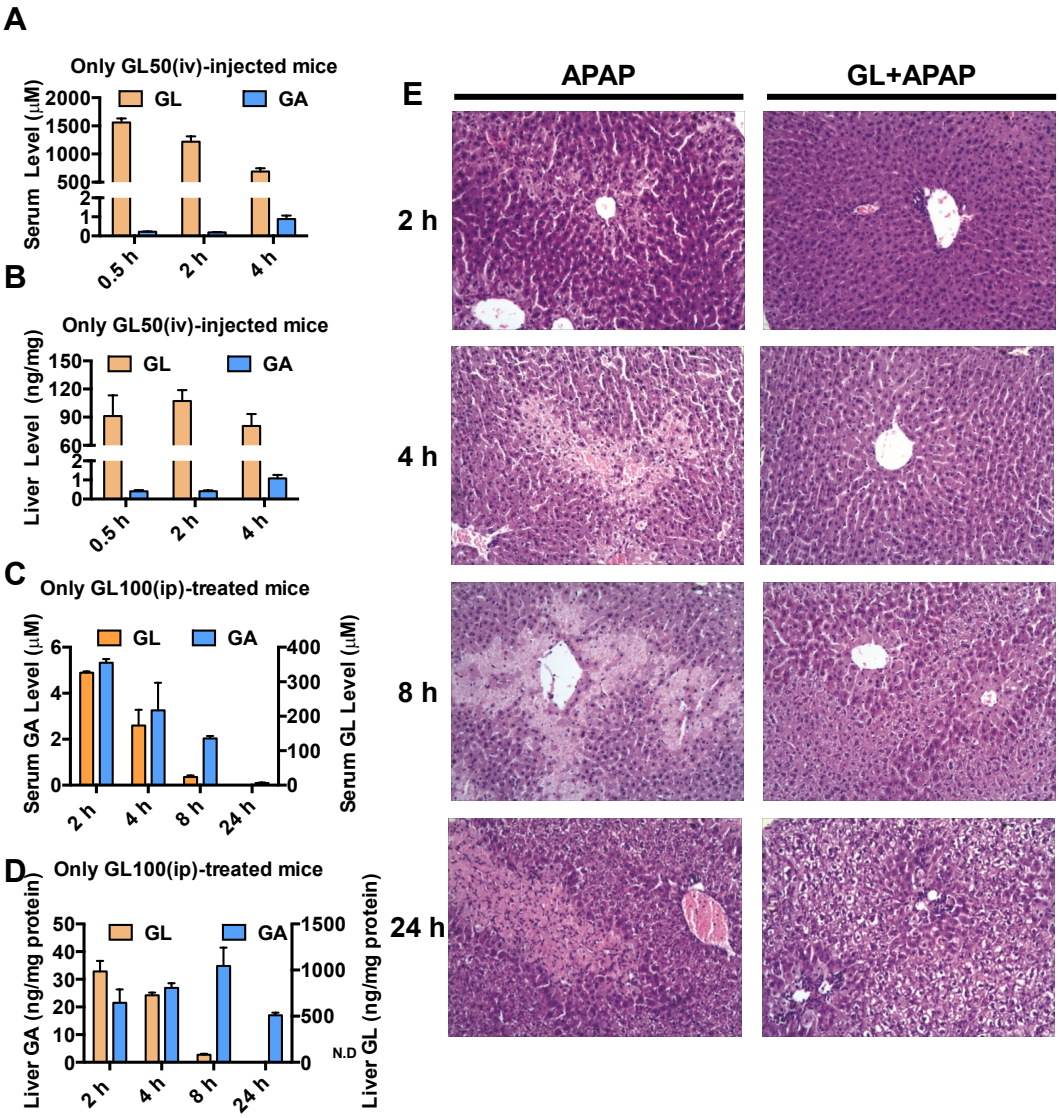
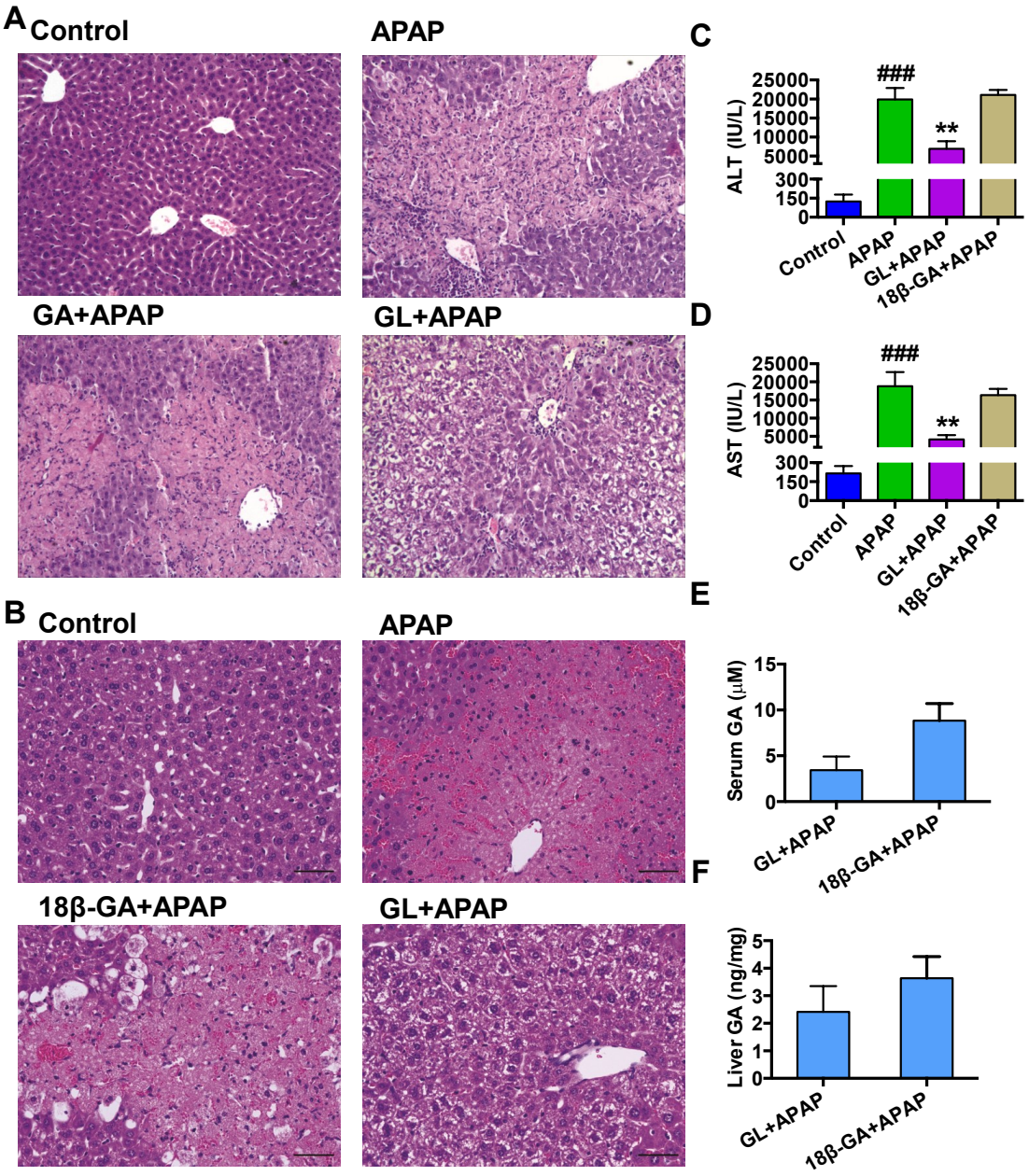
Journal title: Drug Metabolism and Disposition

Supplemental Table 1. List of mouse primers:

Primers	Forward 3'-5'	Reverse 5'-3'
Gapdh	TTGAGGTCAATGAAGGGGTC	TCGTCCCGTAGACAAAATGG
Ripk3	GGTGGTGCTACCAAGGAGTT	GAGATGGAAGACACGGCACT
Mlkl	ATAGAGGAGAGTATCACA	AATATACGCAAGATGTTG
Il6	CACAAGTCCGGAGAGGAGAC	CAGAATTGCCATTGCACAAC
Il1β	CCTCTGCCAAGTCA-GGTCTC	GAATGTGCCACGGTTTTCTT
Tnfα	GAGAGATTGGCTGCTGGAAC	TGGAGACCATGATGACCGTA
Cyp2e1	GACCACCAGCACAACTCTGA	CCCAATCACCCTGTCAATTT
Cyp3a11	AGCAGGGATGGACCTGG	CGGTAGAGGAGCACCAA
Cyp1a2	TCCTGGACTGACTCCCACAAC	GAACGCCATCTGTACCACTGAA
Bcl2a1c	CCTGGCTGAGCACTACCTTCA	TGAACGGAGAAAGCAACTCTTTG

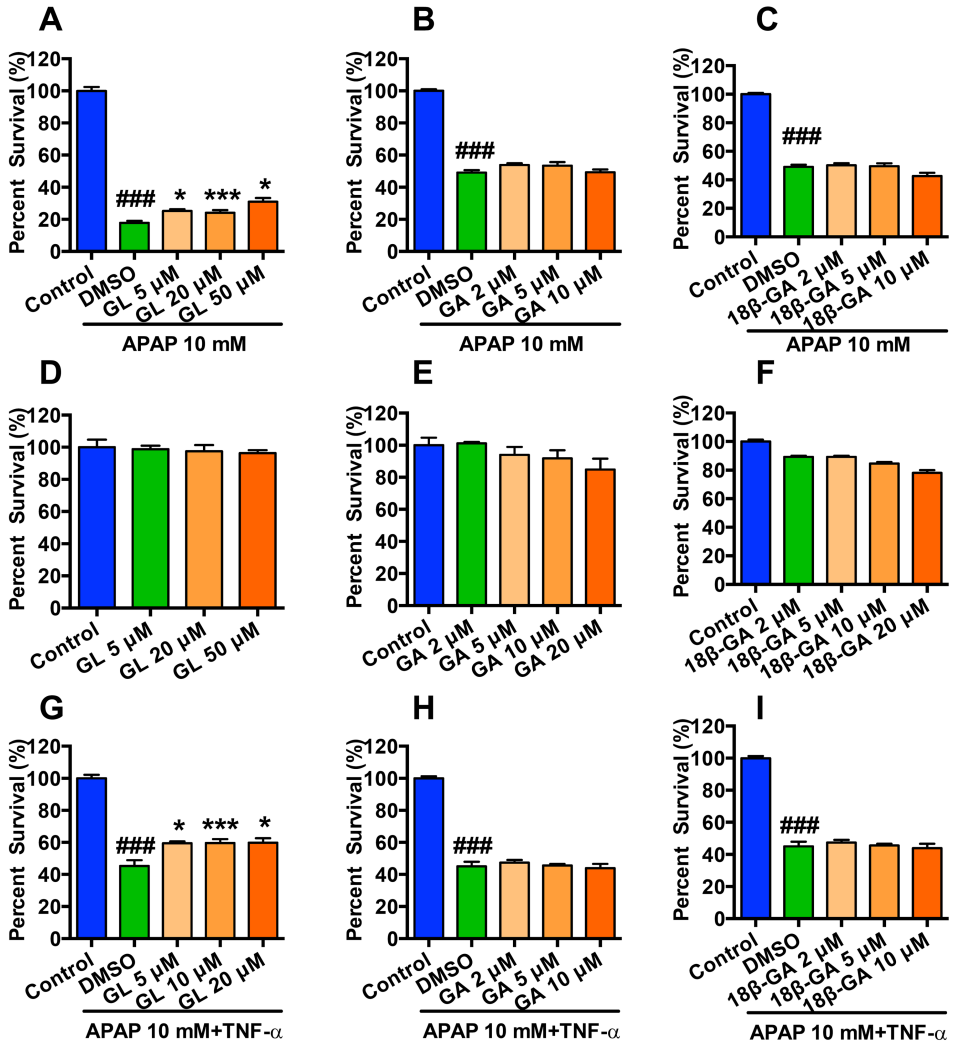


Supplemental Figure 1. Time course of GL and GA in only GL-treated mice and histological analyses of APAP or GL/APAP-treated livers. (A), serum GL and GA in only GL 50 mg/kg (iv)-injected mice. (B), liver GL and GA in only GL 50 mg/kg (iv)-injected mice. n=3-6 mice for each group; (C), serum GL and GA in only GL 100 mg/kg (ip)-treated mice. (D), liver GL and GA in only GL 100 mg/kg (ip)-treated mice. (E), H & E-stained liver sections of saline/APAP and GL/APAP-treated mice original magnification, 20×. APAP, saline/APAP-treated mice; GL+APAP, GL 100 mg/kg /APAP-treated mice. n=3 mice in each group.

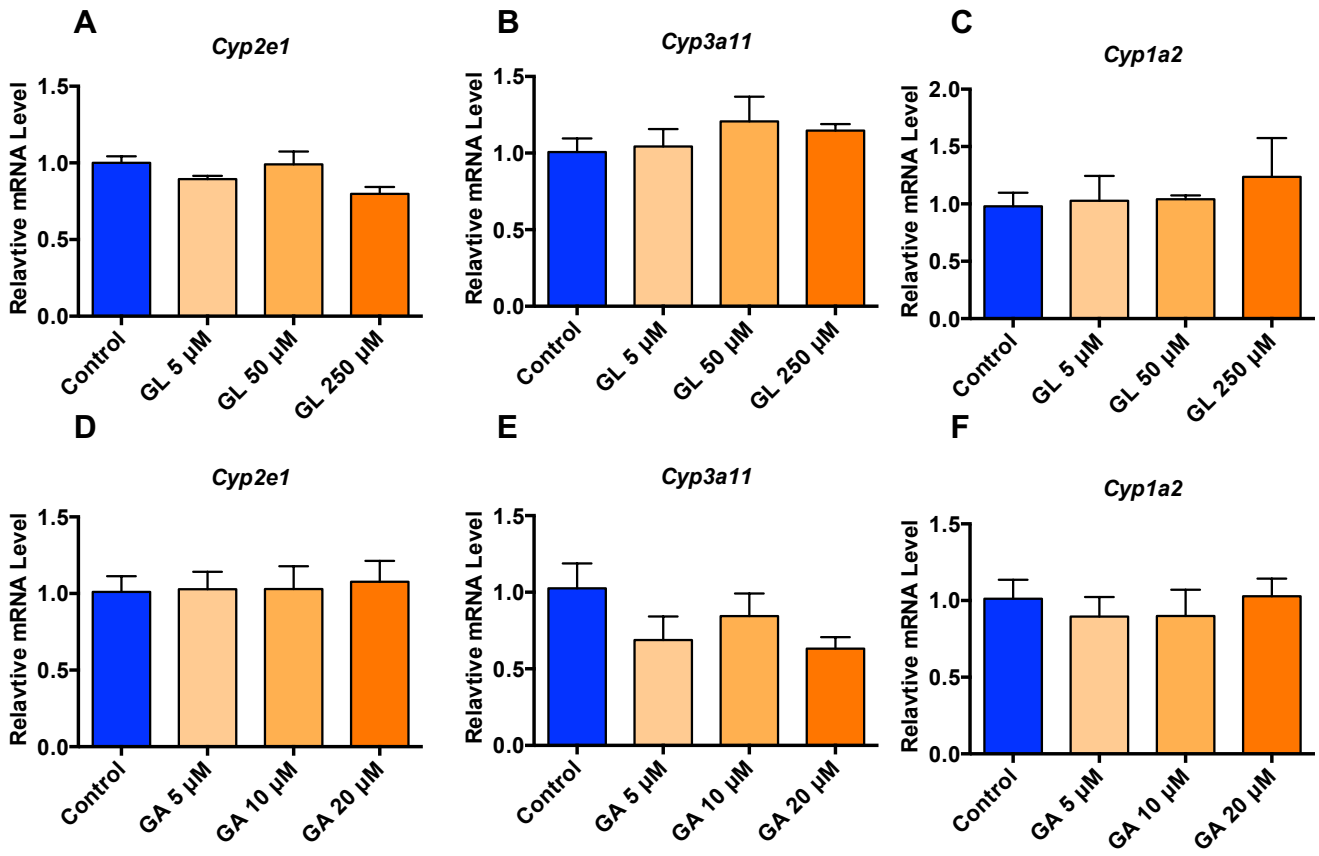


Supplemental Figure 2. GA/18β-GA fails to prevent APAP-induced liver injury. (A and B), H & E-staining of liver sections in GA/APAP-treated mice (A) and 18β-GA/APAP-treated mice (B) at 24 h after APAP challenge, original magnification, 20×. (C and D), Serum ALT and AST levels. (E and F), GA exposure in serum (E) and in liver (F) in18β-GA/APAP-treated mice. Data

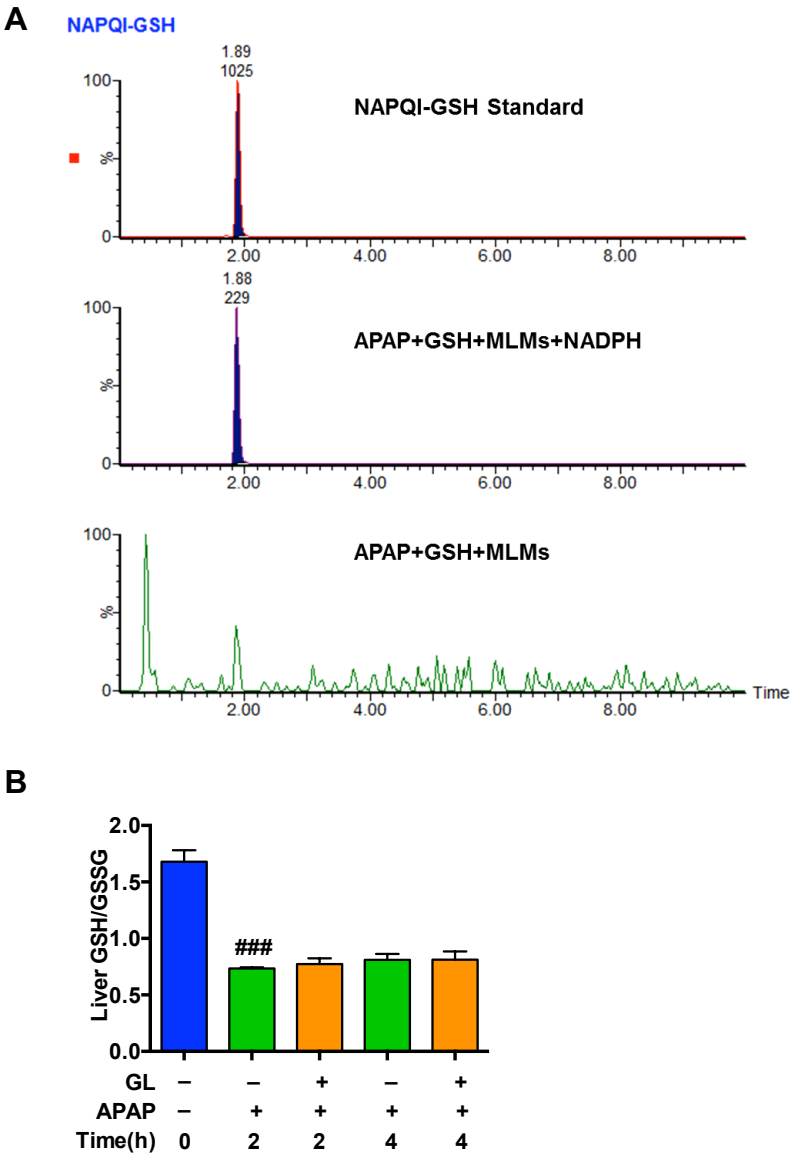
are expressed as mean ± SEM, and n=4-6 for both animal experiments. Control, saline-treated mice; APAP, saline-treated APAP-overdosed mice; GA+APAP, GA 50 mg/kg (ip)-treated APAP-overdosed mice; GL+APAP, GL 50 mg/kg (ip)-treated APAP-overdosed mice; 18β-GA+APAP, 18β-GA 30 mg/kg (ip)-treated APAP-overdosed mice. ###$p<0.001$ versus control mice. **$p<0.01$ versus APAP-overdosed mice.



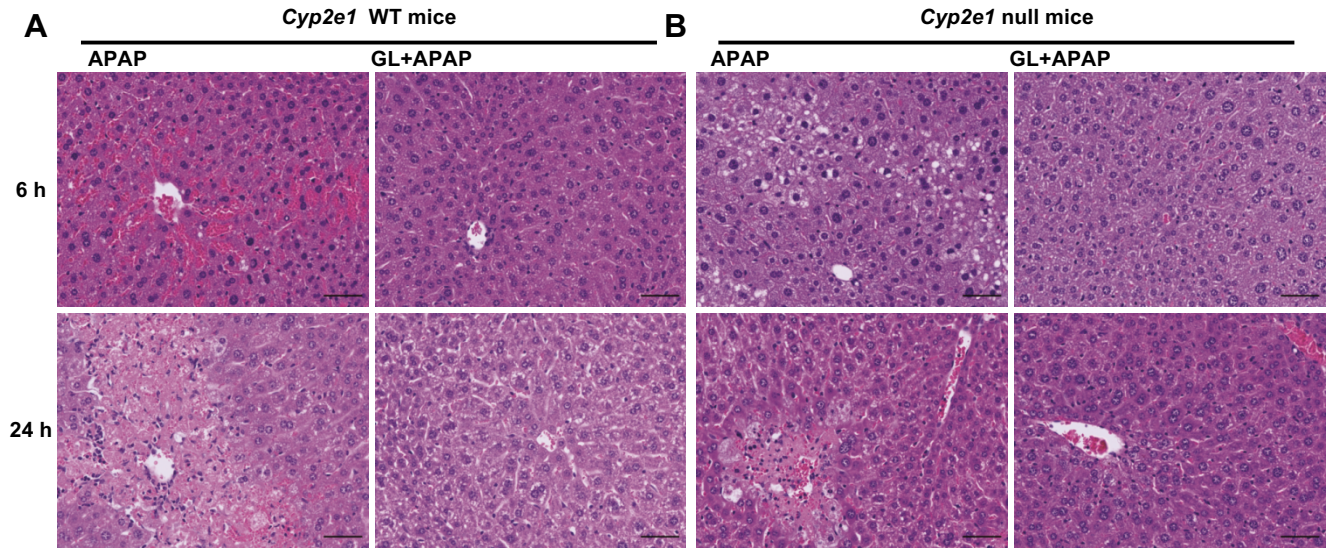
Supplemental Figure 3. GL, and not GA and 18β-GA, ameliorates APAP or TNF-α/APAP-induced hepatocyte damage. (A, B and C), the effect of GL (A), GA (B) and 18β-GA (C) in APAP 10 mM-caused LO2 cell death. (D, E and F), cell toxicity of GL (E), GA (F), and 18β-GA. (G, H and I), the effect of GL (G), GA (H), and 18β-GA (I) in TNF-α/APAP-induced LO2 cell death in TNF-α/APAP-induced LO2 cell death. Data are expressed as mean ± SEM, and n=5-6 for each group. #p<0.05, ##p<0.01 and ###p<0.001 versus 0.1% DMSO-treated control LO2 cells. *p<0.05, **p<0.01 and ***p<0.001 versus 0.1% DMSO/APAP-treated LO2 cells.



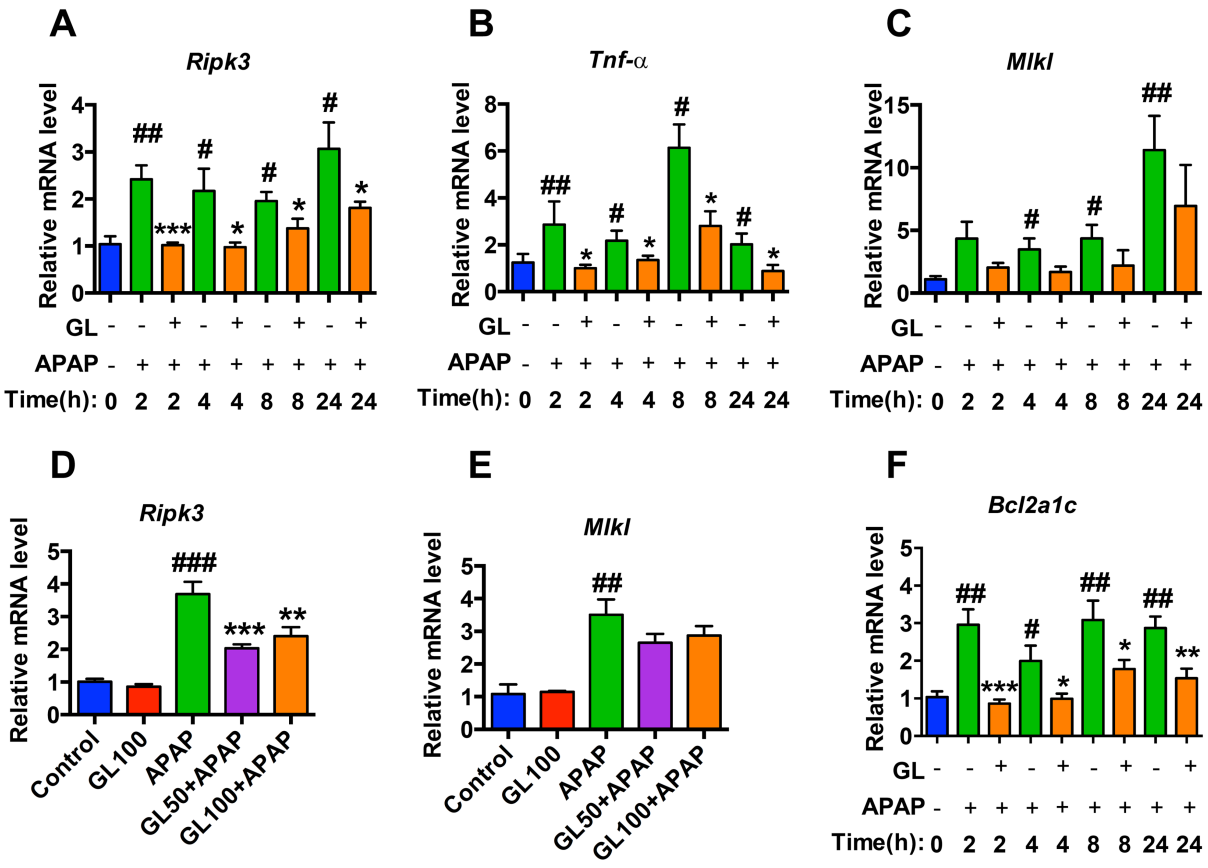
Supplemental Figure 4. GL and GA have no significant effect in *Cyp2e1*, *Cyp3a11*, and *Cyp1a2* expression in mouse primary hepatocytes. (A, B and C), the effect of GL in mouse *Cyp2e1*, *Cyp3a11*, and *Cyp1a2* expression. (D, E and F), the effect of GA in mouse *Cyp2e1*, *Cyp3a11*, and *Cyp1a2* expression. Data are expressed as mean ± SEM, and n=5-6 for each group. C57BL/6 mice as isolated by 3-step treated with 0.1% DMSO (Control) or various concentrations of GL, GA/18βGA for 24 h.



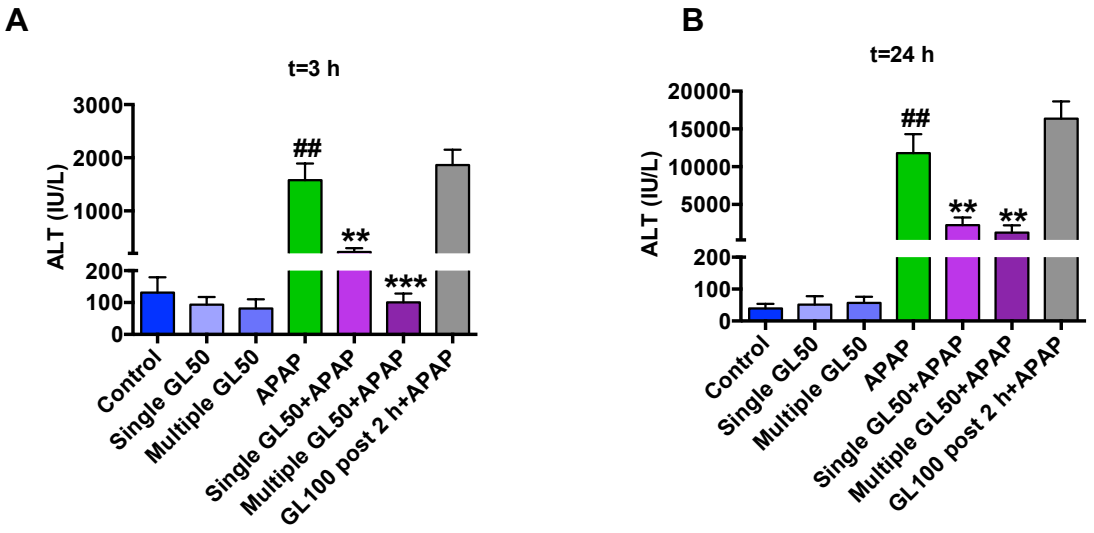
Supplemental Figure 5. GL fails to rescue APAP-induced GSH depletion in liver. (A), Representative chromatograms (peak at m/z 457.1393) of NAPQI-GSH authentic standard, incubation product from APAP/MLMs incubation system, and incubation product from APAP/MLMs incubation system without adding NADPH. (B), Hepatic GSH/GSSG ratio of saline or GL treated mice after APAP challenge at 2 h and 4 h.



Supplemental Figure 6. GL rescues APAP-induced histological injury in both wild-type and Cyp2e1-null mice. (A), H & E staining of saline/APAP and GL/APAP-treated livers in wild-type mice at 6 h and 24 h. (B), H & E staining of saline/APAP and GL/APAP-treated livers in cyp2e1-null mice at 6 h and 24 h. APAP, saline/APAP-treated mice; GL+APAP, GL 50 mg/kg/APAP-treated mice. Original magnification, 20×, and black scale bar, 50 μm.



Supplemental Figure 7. The effect of APAP and GL in Ripk3 signaling. (A), time course of *Ripk3* mRNA levels. (B), time course of *Tnfa* mRNA levels. (C), time course of *Mlkl* mRNA levels. (D and E), effect of GL 50 mg/kg and GL 100 mg/kg in *Ripk3* (D) and *Mlkl* (E) mRNA level at 24 h after APAP challenge. (F), Time course of Bcl2a1c mRNA levels. Data are expressed as mean ± SEM, and n=5-8 mice for each group. Control, saline-treated control mice; GL100, only GL 100 mg/kg-treated mice; APAP, saline/APAP-treated mice; GL50+APAP, GL 50 mg/kg-treated APAP-overdosed mice; GL100+APAP, GL 100 mg/kg-treated APAP-overdosed mice. #p<0.05, ##p<0.01 and ###p<0.001 versus control mice. *p<0.05, **p<0.01 and ***p<0.001 versus APAP-overdosed mice.



Supplemental Figure 8. Intravenous injection of GL at 2 h post APAP challenge fails to combat APAP-induced liver injury, while both multiple injections of GL and single injection of GL via intraperitoneal injection significantly prevent APAP-induced toxicity. (A), Serum ALT levels at 2 h after APAP challenge. (B), Serum ALT levels at 24 h after APAP challenge. Control, saline-treated mice; Single GL50, only single GL 50 mg/kg-treated mice; APAP, saline/APAP-treated mice; Single GL50+APAP, single injection of GL 50 mg/kg (ip)/APAP-treated mice; Multiple GL50+APAP, multiple injections of GL 50 mg/kg (ip)/APAP-treated mice; GL100 post 2 h+APAP, mice were intravenously injected with 100 mg/kg of GL at 2 h post APAP treatment. Data are expressed as the mean ± SEM, n=5 mice in each group. #P<0.05, ##P<0.01 and ###P<0.001 versus control mice. *P<0.05, **P<0.01 and ***P<0.001 versus APAP-overdosed mice.