Supporting Information

Disruption of Thioredoxin Reductase 1 Protects Mice from Acute Acetaminophen-Induced Hepatotoxicity through Enhanced Nrf2 Activity

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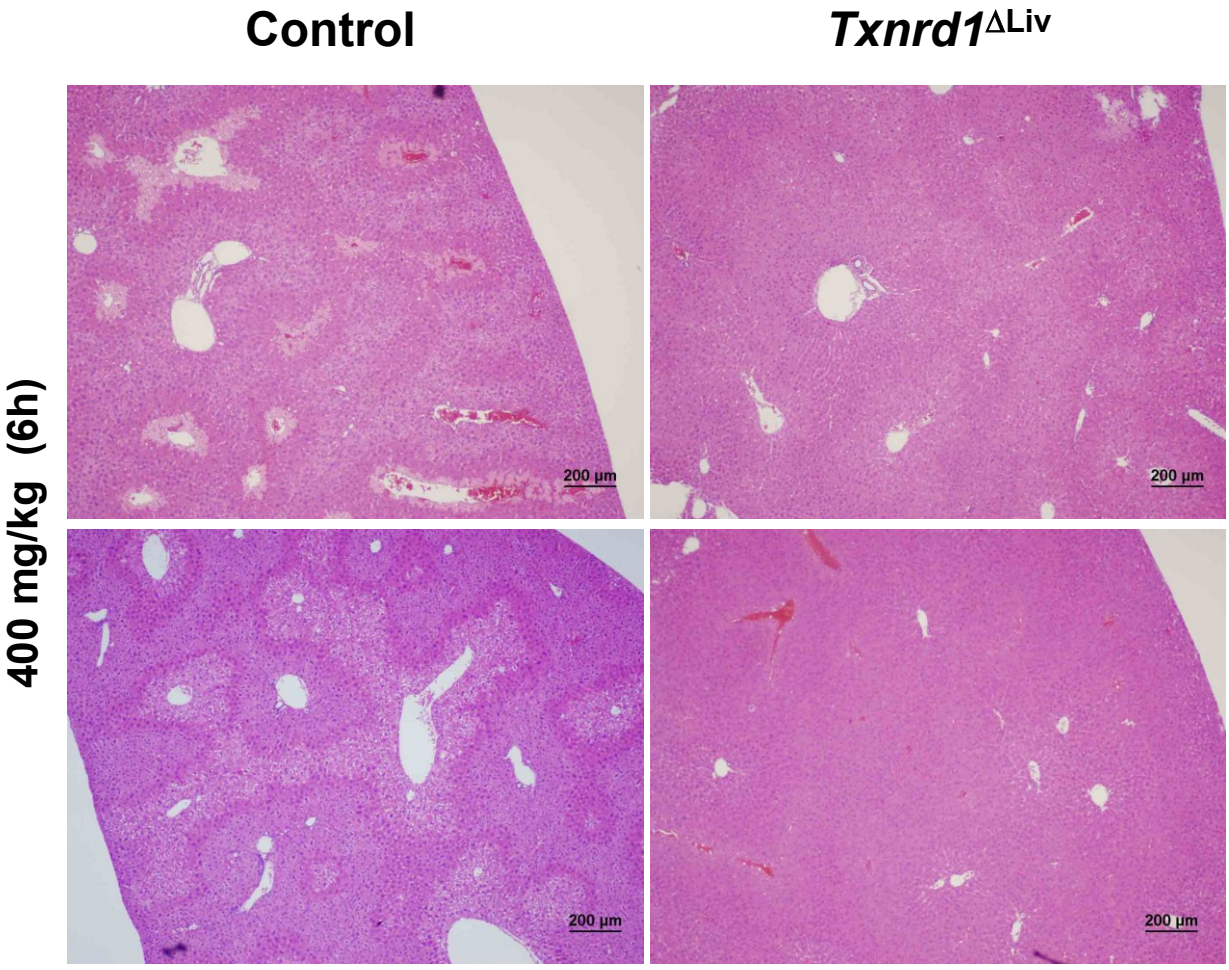

Figure S1. Histopathological analysis of additional mouse livers from control (left panel) or *Txnrd1*$^{\Delta Liv}$ mice (right panel) following H&E staining. Mice received 400 mg/kg of acetaminophen.

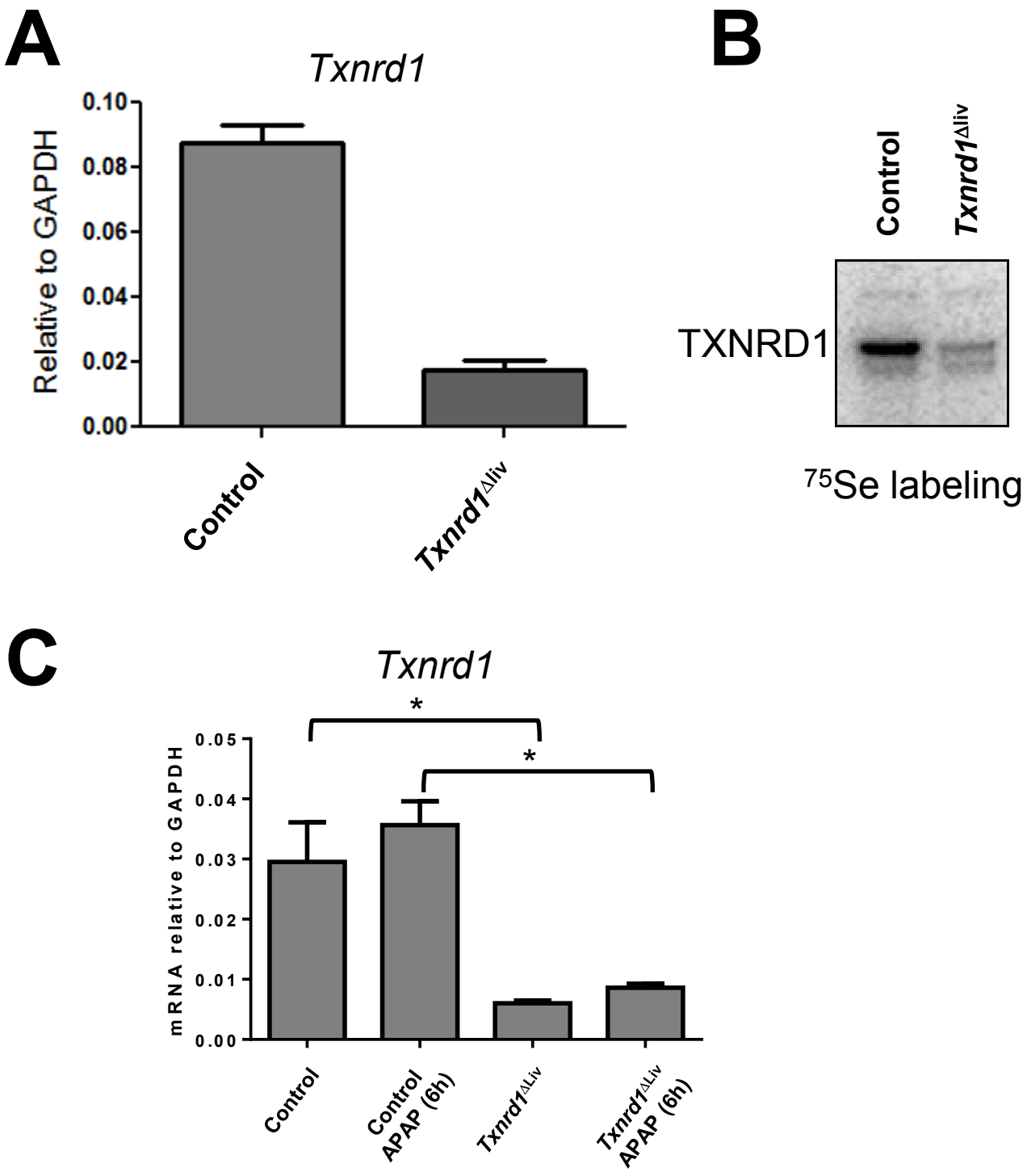

Figure S2. Characterization of livers from control and *Txnrd1*$^{\Delta Liv}$ mice. (A) The levels of *Txnrd1* mRNA in livers from control and *Txnrd1*$^{\Delta Liv}$ mice were determined by QPCR analysis using *gapdh* as an internal control as described in Experimental Procedures. (B) Mice were given I.P. injections of ^{75}Se, livers harvested after 24 h and the level of TXNRD1 was visualized by PhosporImager analysis following SDS electrophoresis. (C) Levels of *Txnrd1* in livers from control and *Txnrd1*$^{\Delta Liv}$ mice with and without acetaminophen (400 mg/kg) treatment. *P<0.05

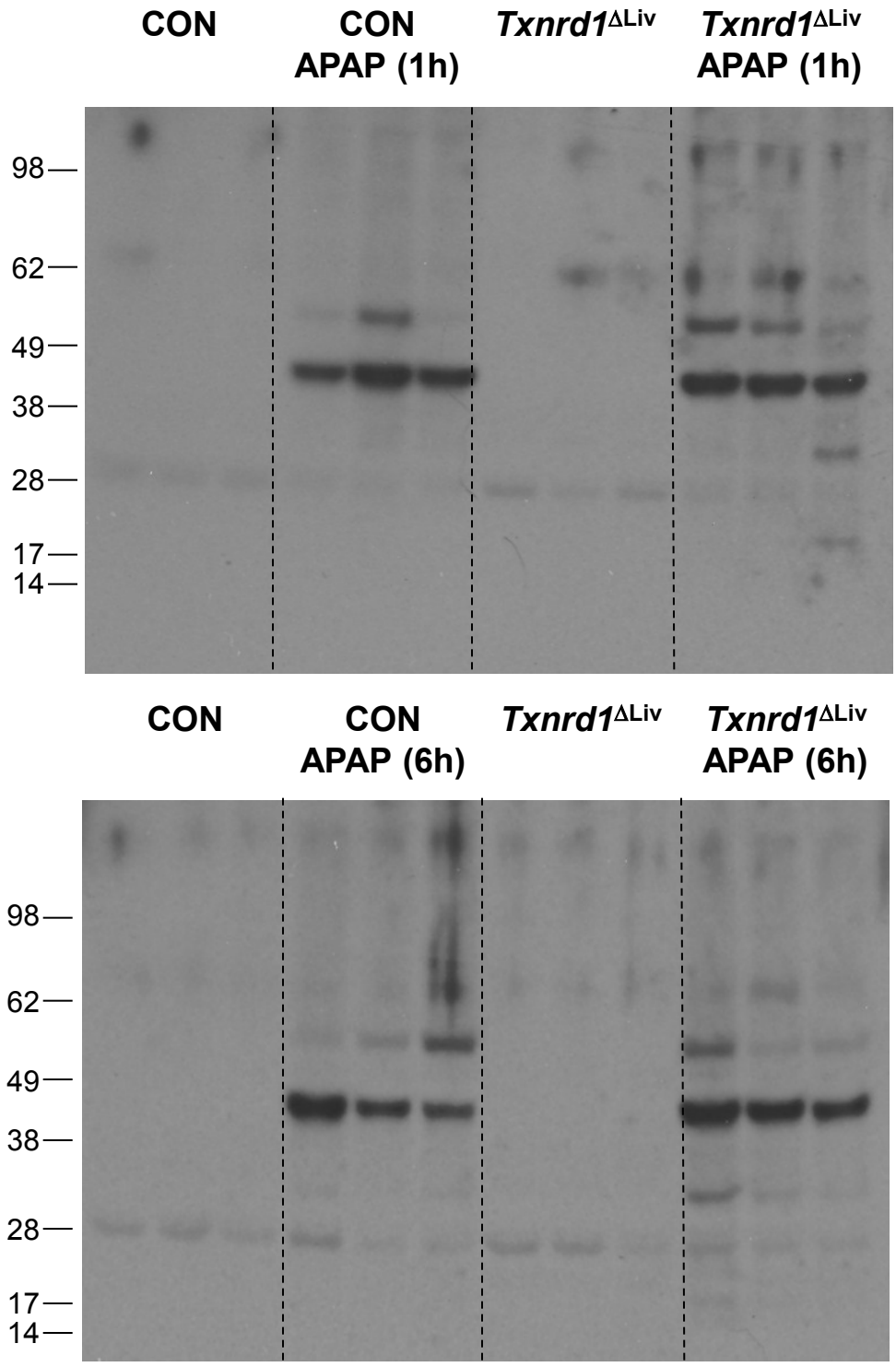

Figure S3. Western blot analysis of APAP protein adducts. Control and *Txnrd1*$^{\Delta Liv}$ mice (n=3) were treated or untreated with a hepatotoxic dose of APAP and liver extracts from 1 h and 6 h after treatment were analyzed by Western blotting of APAP protein adducts.

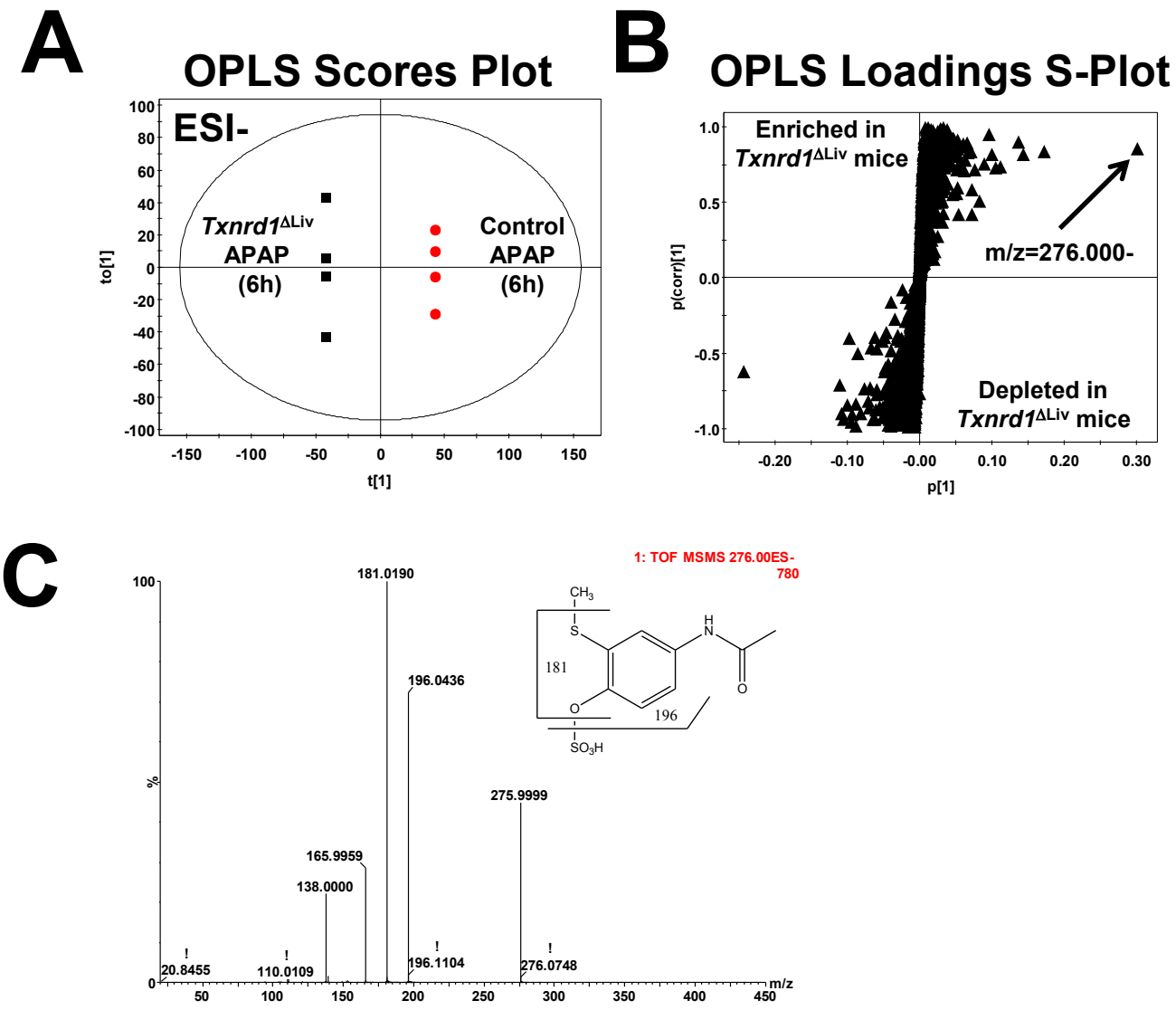

Figure S4. Identification of APAP metabolites in urine from control and *Txnrd1*$^{\Delta Liv}$ mice. Control and *Txnrd1*$^{\Delta Liv}$ mice were treated with 400 mg/kg APAP for 6 h using UPLC-ESI-QTOFMS-based metabolomics; here operating in ESI- mode. (A) OPLS analysis of ESI- data shows clear separation of control and *Txnrd1*$^{\Delta Liv}$ mice. The OPLS loadings plot indicates APAP metabolite and fragments ions enriched or depleted in each genotype. (B) OPLS analysis of ESI- data. (C) Tandem MS and the chemical structure of 3-thiomethyl-APAP-sulfate. Tandem MS was conducted by ramping the collision voltage from 5-35 V. Additional information about m/z=276.000- is provided in Table 1.

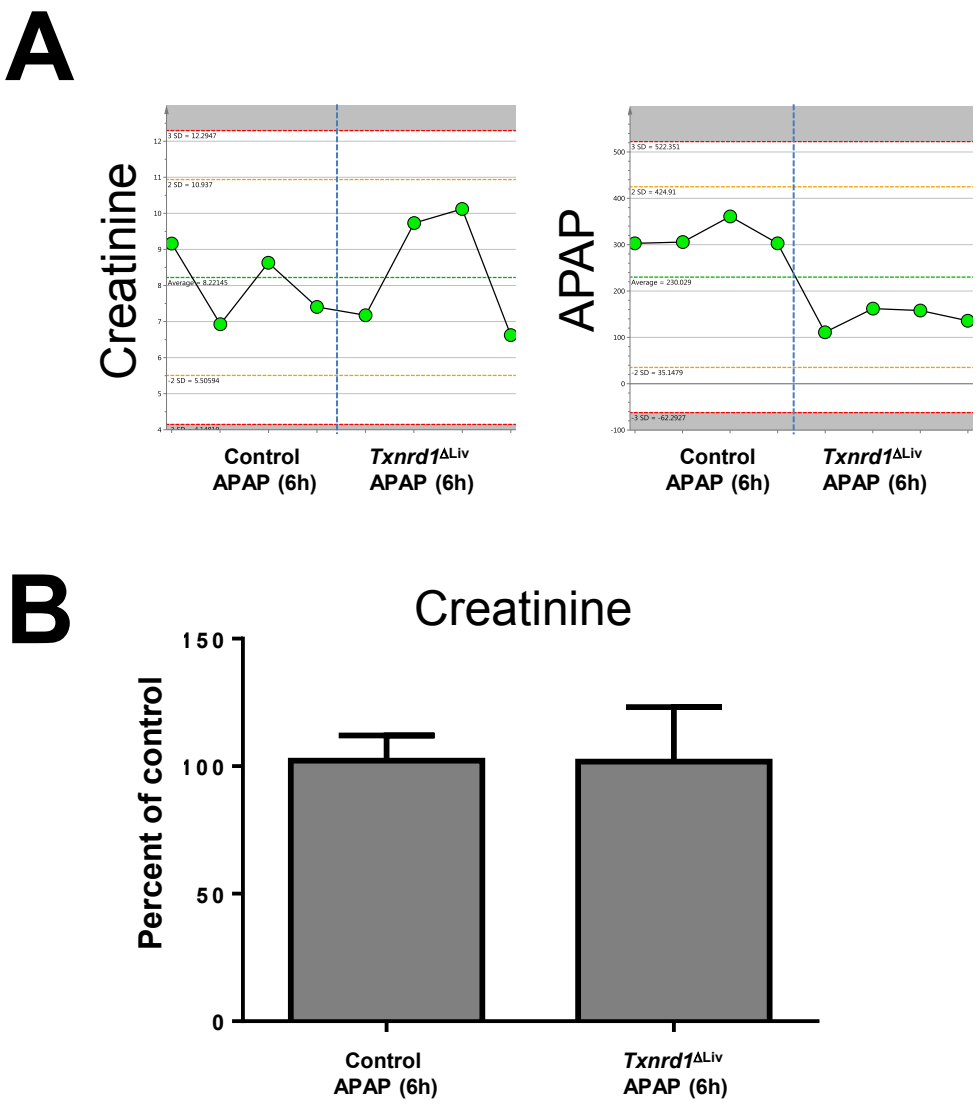

Figure S5. Characterization of creatinine in control and *Txnrd1*$^{\Delta Liv}$ mice. Treatment with 400 mg/kg APAP for 6 h does not impact kidney function in terms of creatinine output. (A) The m/z corresponding to creatinine (m/z = 114.0667+) was extracted from the multivariate data analysis and the levels plotted for each control and *Txnrd1*$^{\Delta Liv}$ mouse. Levels of free APAP in the urine are shown for comparison. (B) Peak areas corresponding to creatinine were determined from the raw UPLC-ESI-QTOFMS data. No significant difference was observed.

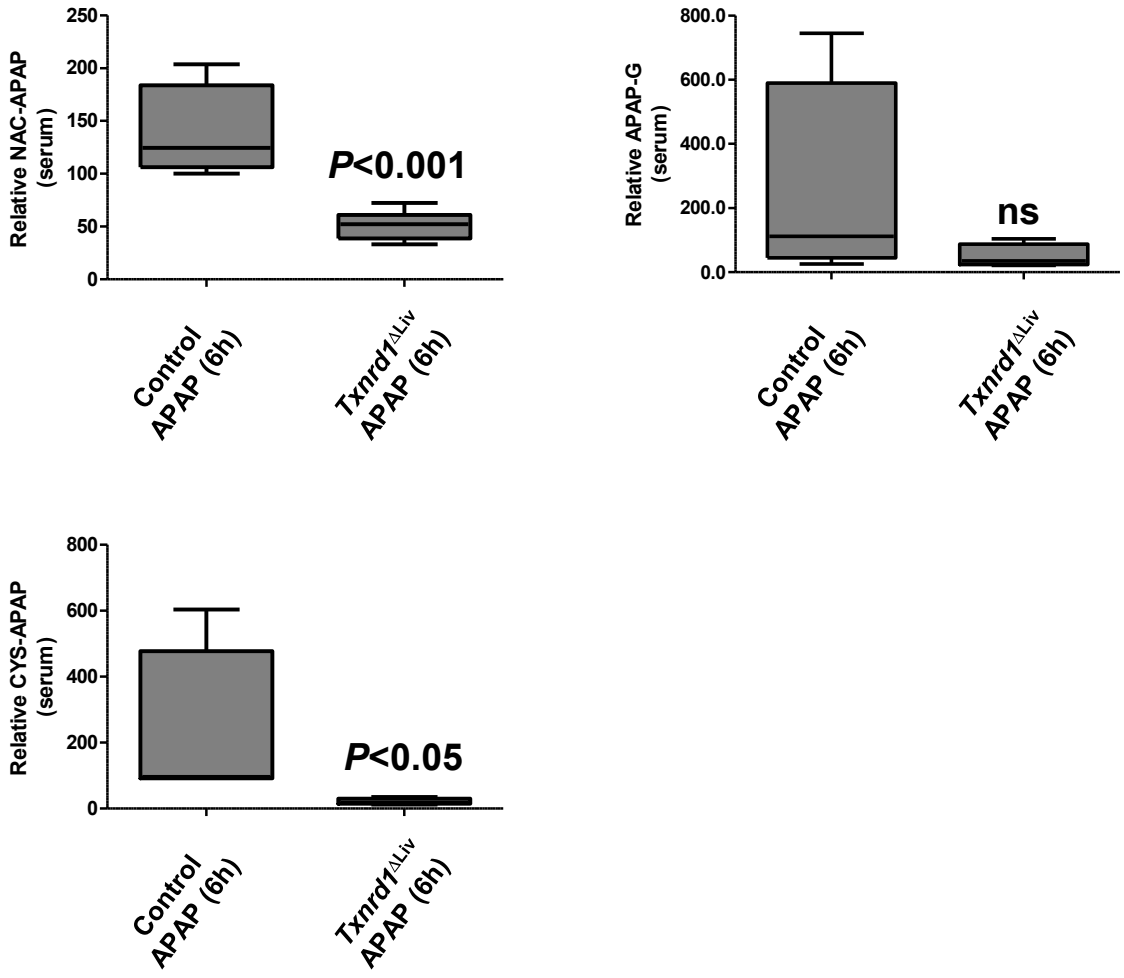

Figure S6. Quantitation of APAP metabolites in serum. For serum APAP metabolism analysis, one volume of serum was deproteinated by 20 volumes of 66% aqueous acetonitrile and spun at 14000xg for 10 min at 4C. Conjugates of APAP (Cys-APAP, NAC-APAP, APAP-Glucuronide) in serum of control and *Txnrd1*$^{\Delta Liv}$ mice were separated by reverse phase chromatography on a 50 x 2.1-mm ACQUITY 1.7 µm BEH C_{18} column (Waters Corp., Milford, MA) using an ACQUITY ultraperformance liquid chromatography system (Waters Corp) and monitored using a Xevo TQ triplequadrupole mass spectrometry (Waters Corp). The following multiple reaction monitoring transitions were used: Cys-APAP (271→139.9), NAC-APAP (393.1→139.9), APAP-Glucuronide (328.1→152), wherein Chlorpropamide (MRM transition 277→110.9) was used as an internal standard. Serial dilution calibration curves were generated for each metabolite. Results are expressed as micromolar (µM).