

**IDENTIFICATION OF NOVEL TOXICITY-ASSOCIATED METABOLITES BY  
METABOLOMICS AND MASS ISOTOPOMER ANALYSIS OF ACETAMINOPHEN  
METABOLISM IN WILD-TYPE AND CYP2E1-NULL MICE**

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**Supplemental Tables and Figures**

**Supplemental Table 1.** Comparison of major APAP metabolites in the serum of wild-type (*Cyp2e1*<sup>+/+</sup>) and *Cyp2e1*-null (*Cyp2e1*<sup>-/-</sup>) mice. Data were processed by MarkerLynx software. Relative abundances of APAP and its metabolites (mean  $\pm$  SD in parts per ten thousand) were determined by normalizing the single ion counts (SIC) of each metabolite *versus* the total ion counts (TIC) of each serum sample (n=4, \* for  $P < 0.05$ , \*\* for  $P < 0.01$ , ND for not detected).

Metabolites	1 h		2 h		4 h	
	<i>Cyp2e1</i> <sup>+/+</sup>	<i>Cyp2e1</i> <sup>-/-</sup>	<i>Cyp2e1</i> <sup>+/+</sup>	<i>Cyp2e1</i> <sup>-/-</sup>	<i>Cyp2e1</i> <sup>+/+</sup>	<i>Cyp2e1</i> <sup>-/-</sup>
APAP (I)	224.12 $\pm$ 44.11	228.54 $\pm$ 8.12	114.00 $\pm$ 5.19	111.37 $\pm$ 7.43	22.51 $\pm$ 8.62**	1.75 $\pm$ 3.14**
Cys-APAP (II)	6.83 $\pm$ 2.19	7.19 $\pm$ 1.32	8.09 $\pm$ 0.58*	5.26 $\pm$ 1.65*	2.82 $\pm$ 1.21*	0.55 $\pm$ 0.89*
NAC-APAP (III)	15.42 $\pm$ 3.97	14.93 $\pm$ 3.26	8.17 $\pm$ 0.77	10.24 $\pm$ 2.47	2.66 $\pm$ 1.89	1.62 $\pm$ 1.50
APAP-G (IV)	10.81 $\pm$ 1.56	11.55 $\pm$ 2.15	7.47 $\pm$ 0.36	8.23 $\pm$ 0.93	0.97 $\pm$ 0.66	0.18 $\pm$ 0.35
APAP-S (V)	5.91 $\pm$ 1.29	6.48 $\pm$ 0.59	4.59 $\pm$ 0.93*	6.81 $\pm$ 1.11*	2.72 $\pm$ 0.96**	ND
GS-APAP	12.57 $\pm$ 2.11	13.46 $\pm$ 9.56	44.37 $\pm$ 5.58*	21.11 $\pm$ 15.50*	12.50 $\pm$ 5.76**	ND

## SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure 1.** Influence of APAP treatment on the GSSG level in liver and GSH level in mitochondria of wild-type and *Cyp2e1*-null mice. Liver samples collected at 0, 1, 2, 4, 24 h after i.p. administration of 400 mg/kg APAP were processed as described in the *Experimental procedures* (n=4, \* for  $P < 0.05$  and \*\* for  $P < 0.01$ ). Both oxidized and reduced glutathione levels were determined by LC-MS analysis, and normalized by liver weight. **A.** GSSG level in the liver of wild-type and *Cyp2e1*-null mice. **B.** Relative GSH level in the liver mitochondria of wild-type and *Cyp2e1*-null mice. Determined mitochondrial GSH level in control wild-type mice was arbitrarily set as 100%.

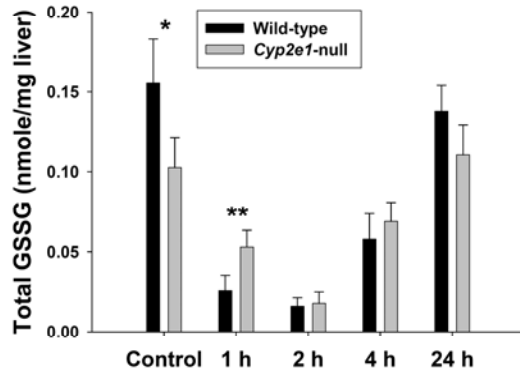
**Supplemental Figure 2.** 3-D scores plot of a PCA model on 24-h urine samples from control and APAP-treated wild-type and *Cyp2e1*-null mice. A three-component PCA model was constructed to characterize the relationship among 6 mouse groups (8 mice/group), including wild-type mice (control, 200 mg/kg APAP, 400 mg/kg APAP) and *Cyp2e1*-null mice (control, 200 mg/kg APAP, 400 mg/kg APAP). The t[1], t[2] and t[3] values represent the scores of each sample in principal component 1, 2 and 3, respectively.

**Supplemental Figure 3.** **A.** MS<sup>2</sup> fragmentation of APAP (**I**). **B.** MS<sup>2</sup> fragmentation of Cys-APAP (**II**). **C.** MS<sup>2</sup> fragmentation of NAC-APAP (**III**). **D.** MS<sup>2</sup> fragmentation of APAP-G (**IV**). Major fragment ions were interpreted in the inlaid structural diagrams.

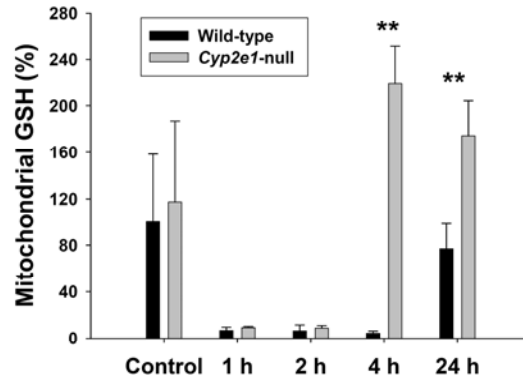
**Supplemental Figure 4.** MS<sup>2</sup> fragmentation of *N*-acetylamino-1,4-benzothiazine formed by HRP-catalyzed reaction between Cys-APAP and H<sub>2</sub>O<sub>2</sub>.

Supplemental Figure 1

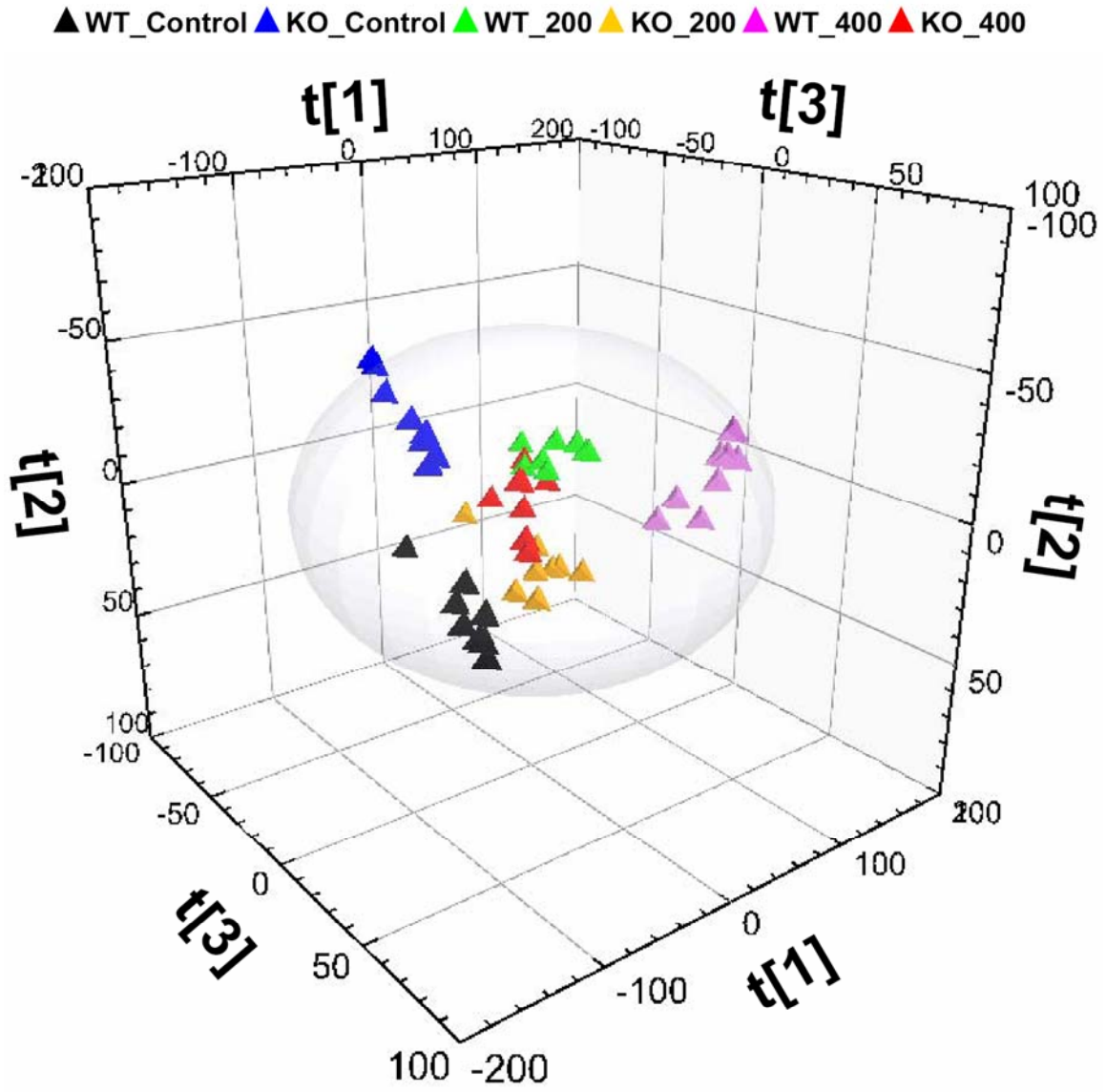
**A**



**B**

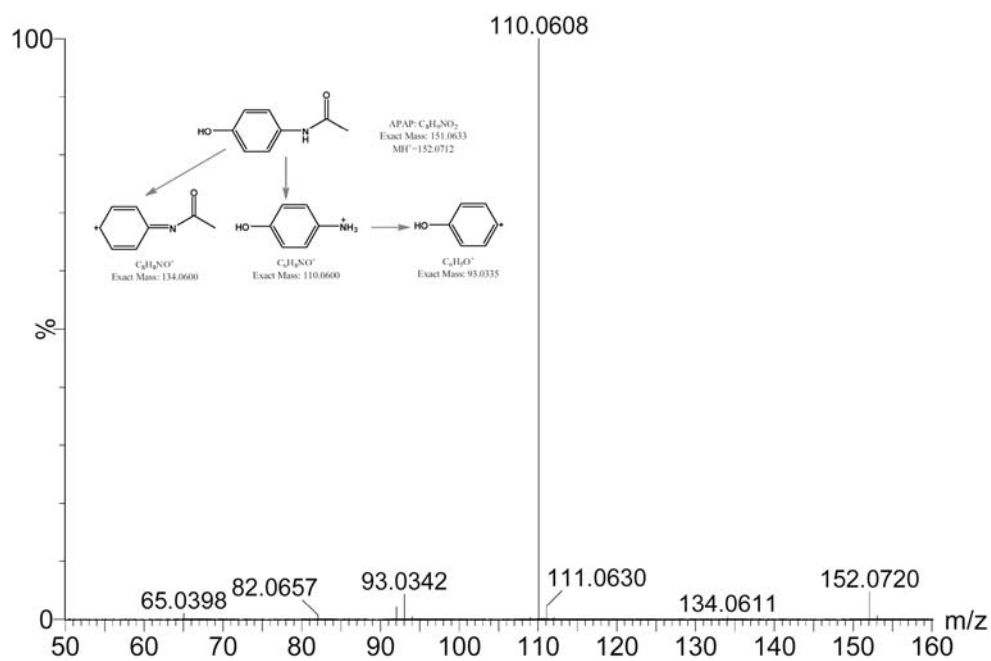


Supplemental Figure 2

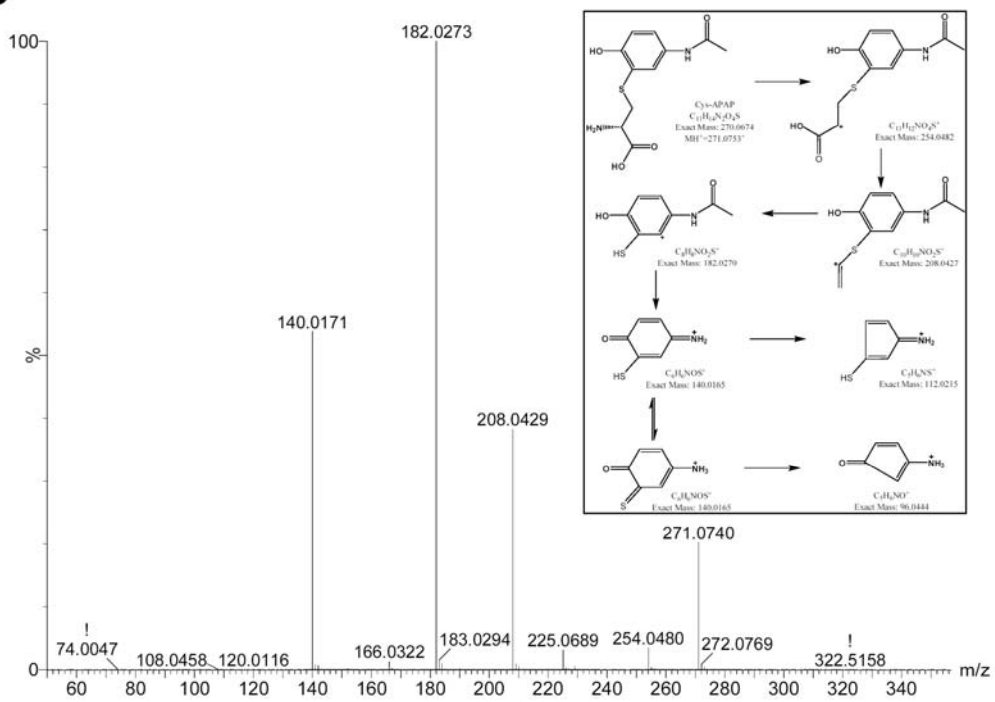


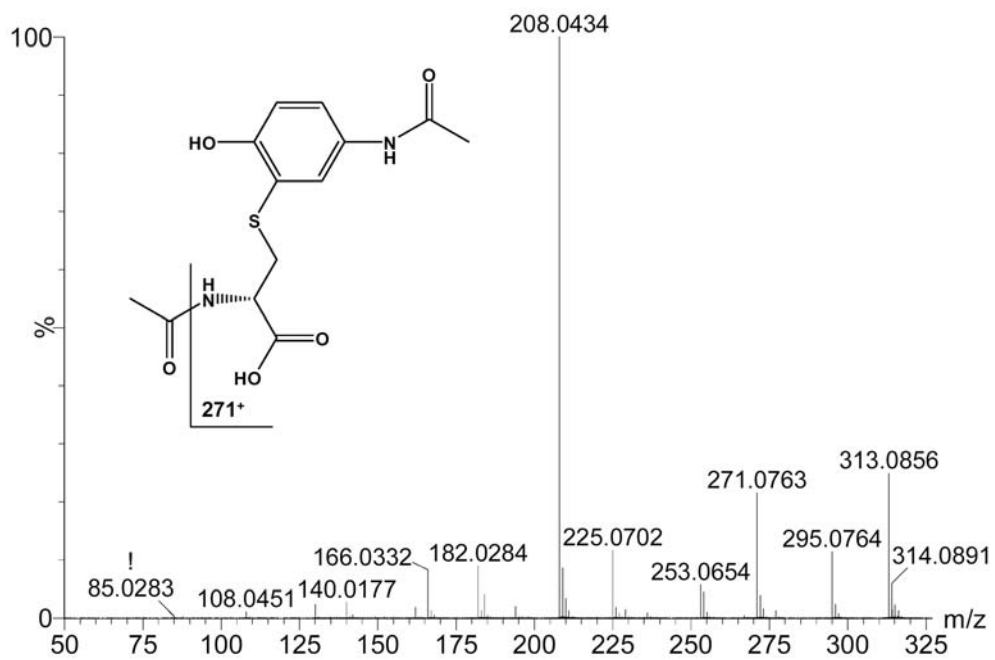
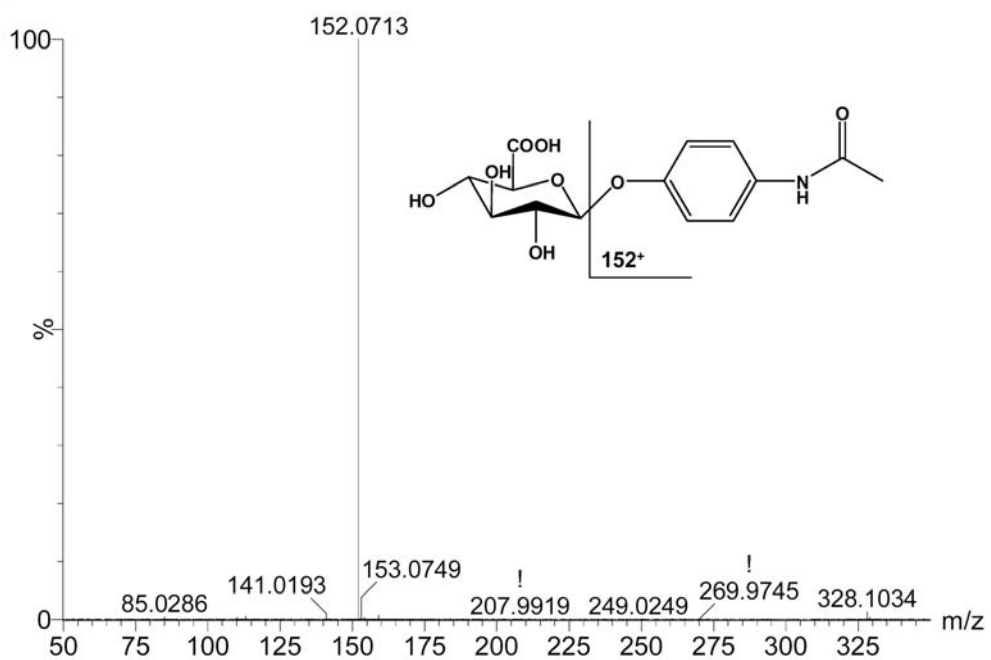
Supplemental Figure 3

**A**



**B**



**C****D**

Supplemental Figure 4

