SUPPLEMENTAL INFORMATION

IDENTIFICATION OF *N*-ACETYLTAURINE AS A NOVEL METABOLITE OF ETHANOL THROUGH METABOLOMICS-GUIDED BIOCHEMICAL ANALYSIS

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Preparation of semi-solid ethanol diet. 0.5% (w/w) agar powder is dissolved in warm water (1/2 of the total water volume). The control dextrose diet powder and the Lieber-DeCarli ethanol diet powder (Bio-Serv) are mixed in a ratio based on desired ethanol concentration and then suspended in water (the other 1/2 of the total water volume). Two solutions are blended together and ethanol is added to reach to desired concentration. Prepared semi-solid ethanol diet is stored in air-tight container at 4°C and is used within a week.

Comparison of the feeding tubes for semi-solid ethanol diet and liquid ethanol diet. The customized feeding tube for semi-solid ethanol diet (**A**) and the standard feeding tube for liquid ethanol diet (**B**) are shown below.

Determining the factors affecting the NAT biosynthesis in vitro. Liver homogenates of the wild-type mice were incubated with 20 mM taurine, 2.5 mM acetate, and various compounds including metal ions ($CaCl_2$, $CsCl_2$, $FeCl_2$, $HgCl_2$, $MgCl_2$), chelator (EDTA), esterase inhibitors (PMSF, Diazinon) and substrate analogs (β-alanine, α-alanine, cysteine, propionic acid, butyric acid). The production of NAT after 30-min incubation was quantitated by the LC-MS analysis.

SUPPLEMENTAL FIGURES

Figure S1. The scores plot of a PLS-DA model on urine samples from the wild-type and *Cyp2e1*-null mice fed with control and ethanol diets. All samples from the wild-type and *Cyp2e1*-null mice with no ethanol exposure were labeled as WT_Ctl (■) and KO_Ctl (◊), respectively, while the samples from both mouse lines with ethanol treatment were labeled as WT_EtOH (Δ) and KO_EtOH (♦), respectively, which include the samples from 7-day, 14-day and 21-day ethanol treatment. The *t*[1] and *t*[2] values represent the scores of each sample in the principal component 1 and 2, respectively.

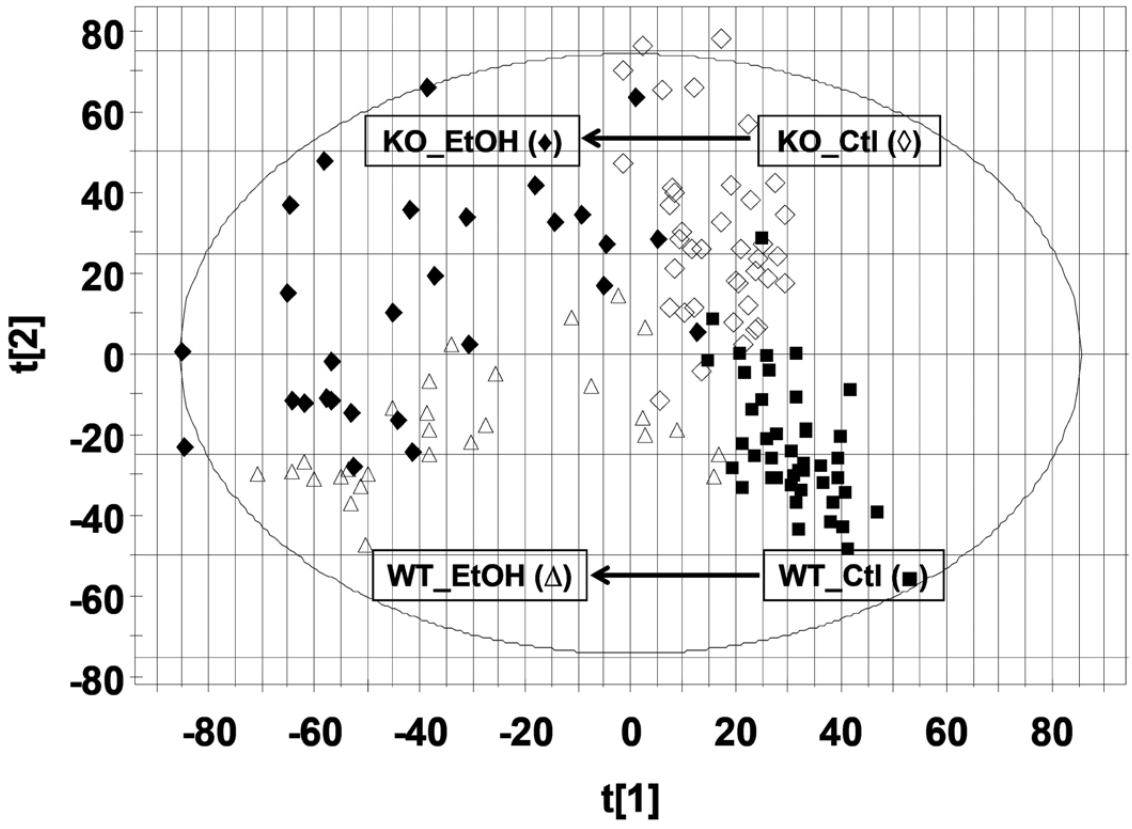
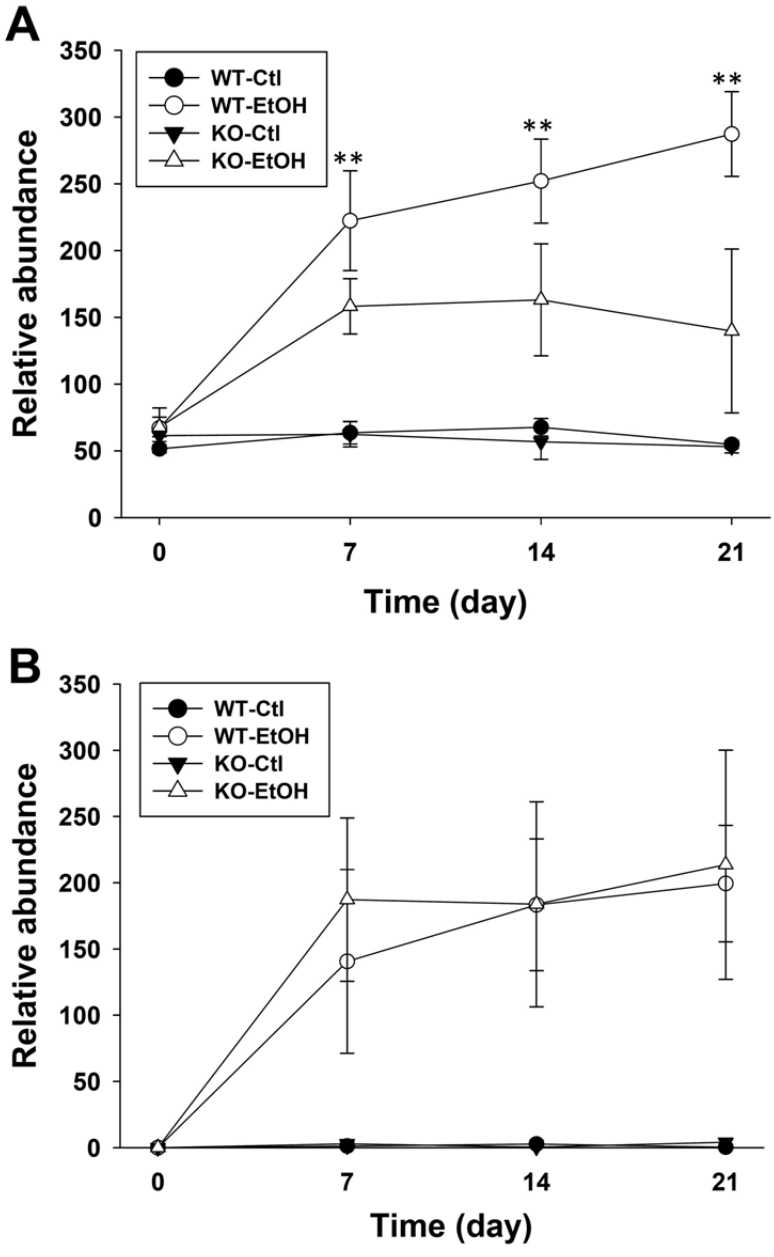


Figure S2. Relative abundance of urinary biomarkers of ethanol exposure in the wild-type (WT) and *Cyp2e1*-null (KO) mice during the 21-day ethanol treatment. The abundance of a urinary metabolite was expressed as a value that is 10,000 fold of the ratio between the single ion count (SIC) of a metabolite and the total ion count (TIC) of a urine sample detected by mass spectrometer (Relative abundance = 10000 × SIC/TIC). ***A.*** Relative abundance of urinary NAT. ***B.*** Relative abundance of urinary EtG. Values were presented as mean ± S.D (n=8). * (p<0.05) and ** (p<0.01) indicate statistical significance between WT and KO samples at the same time point.



SUPPLEMENTAL TABLE

Table S1: The effects of metal ions, chelator, esterase inhibitors, and substrate analogs on the production of NAT *in vitro*. The conditions of the *in vitro* assay are described in the *Supplemental Experimental Procedures*. The enzymatic activity of the control mouse liver homogenate (no compound added) is defined as 100%. The effects of treatment compounds on the activity of NAT synthase are represented by the changes (%) in the NAT production in comparison with the control (+, stimulation; -, inhibition).

Metal ions (mM)			**Chelator (mM)**			**Substrate analogs (mM)**		
	0.1	**1**		**1**	**10**		**1**	**2.5**
$CaCl_2$	+21.8	+10	**EDTA**	-33.6	-72.7	**β-alanine**	-25.5	-36.4
$CsCl_2$	+16.4	+31.8	**Esterase inhibitors (mM)**			**L-alanine**	-25.5	-24.6
$FeCl_2$	+11.8	+3.6		**0.1**	**1**	**Cysteine**	-23.6	-32.7
$HgCl_2$	-45.5	-57.3	**PMSF**	-19.1	-40.9	**Propionic acid**	-36.4	-61.8
$MgCl_2$	-10.9	-21.8	**Diazinon**	-8.2	-40.9	**Butyric acid**	-35.5	-50.9