

**Direct targeting of sEH with alisol B alleviated the apoptosis, inflammation, and oxidative stress in cisplatin-induced acute kidney injury**

Juan Zhang<sup>1,2,+</sup>, Zhi-Lin Luan<sup>1,+</sup>, Xiao-Kui Huo<sup>1,+</sup>, Min Zhang<sup>1</sup>, Christophe Morisseau<sup>3</sup>, Cheng-Peng Sun<sup>1,\*</sup>, Bruce D. Hammock<sup>3,\*</sup>, Xiao-Chi Ma<sup>1,\*</sup>

<sup>1</sup>College of Pharmacy, Second Affiliated Hospital, Dalian Medical University, Dalian 116044, People's Republic of China.

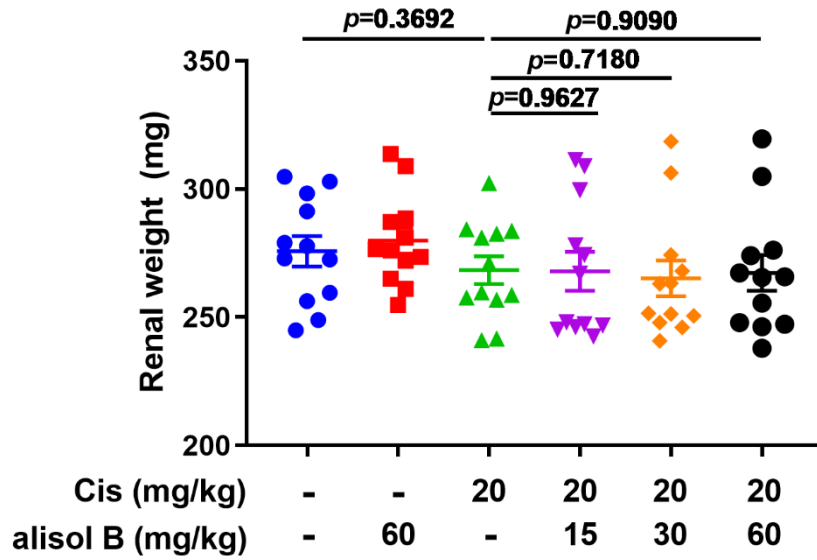
<sup>2</sup>School of Pharmaceutical Sciences, Health Science Center, Shenzhen University, Shenzhen 518061, China

<sup>3</sup>Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis, CA 95616, United States

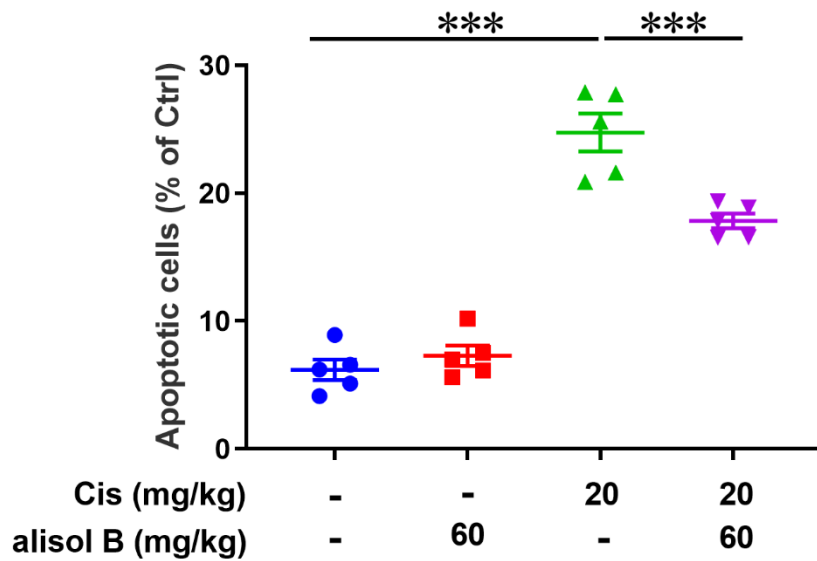
<sup>+</sup>These authors contributed equally to this work.

**Table S1.** Primers used for real-time PCR analysis

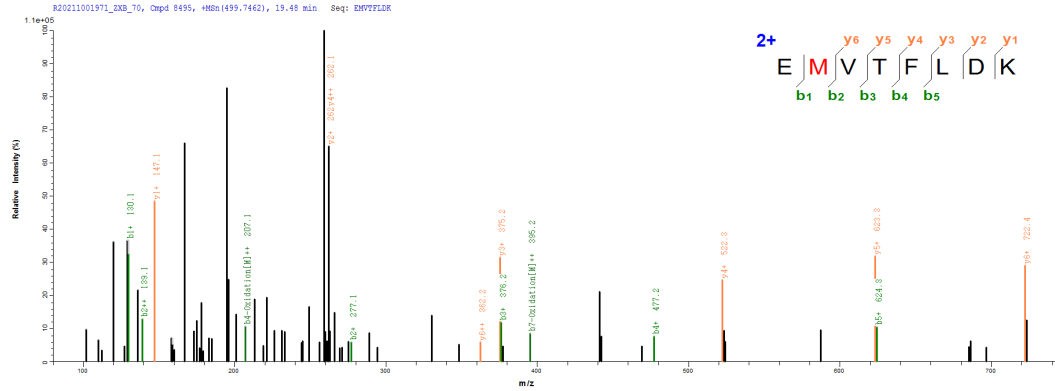
<b>Gene</b>		<b>Sequence (5' to 3')</b>
ICAM-1	Forward	GTGATGCTCAGGTATCCATCCA
	Reverse	CACAGTTCTCAAAGCACAGCG
MCP-1	Forward	TTAAAAACCTGGATCGGAACCAA
	Reverse	GCATTAGCTTCAGATTTACGGGT
IL-6	Forward	TAGTCCTTCCTACCCCAATTTCC
	Reverse	TTGGTCCTTAGCCACTCCTTC
TNF- $\alpha$	Forward	CCCTCACACTCAGATCATCTTCT
	Reverse	GCTACGACGTGGGCTACAG
Nrf2	Forward	CTTTAGTCAGCGACAGAAGGAC
	Reverse	AGGCATCTTGTTTGGGAATGTG
Keap1	Forward	TGCCCTGTGGTCAAAGTG
	Reverse	GGTTCGGTTACCGTCCTGC
$\beta$ -actin	Forward	AGCCATGTACGTAGCCATCC
	Reverse	GCTGTGGTGGTGAAGCTGTA



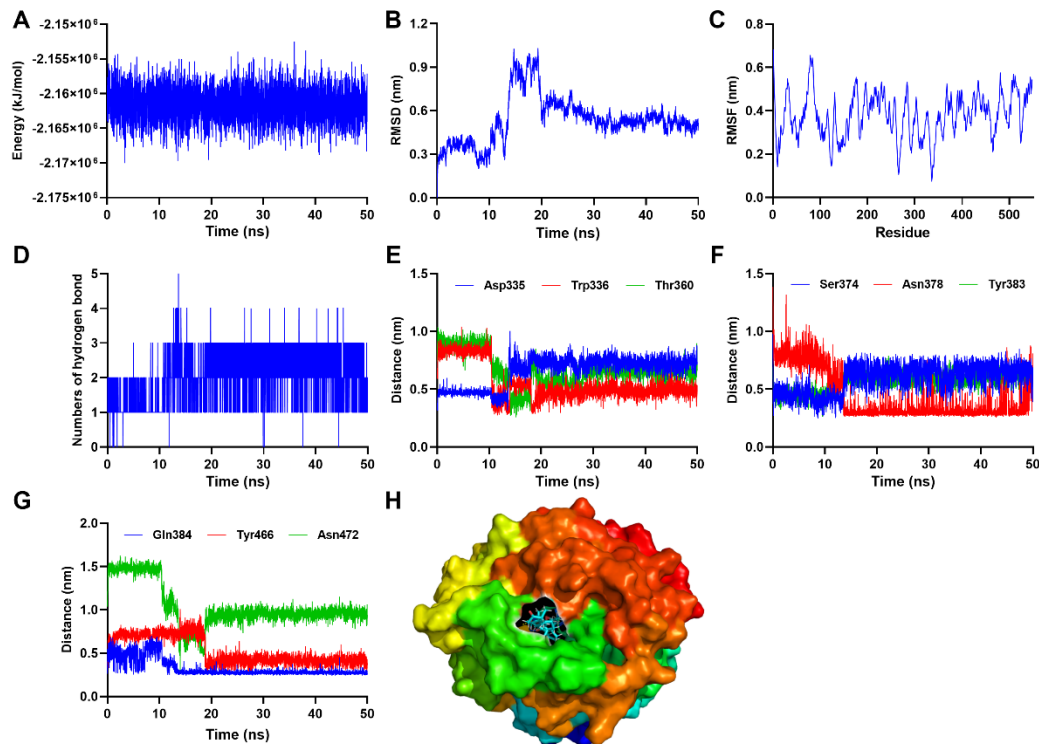
**Figure S1.** Effects of alisol B against renal weight in WT mice, data were presented as mean  $\pm$  SEM, n=12.



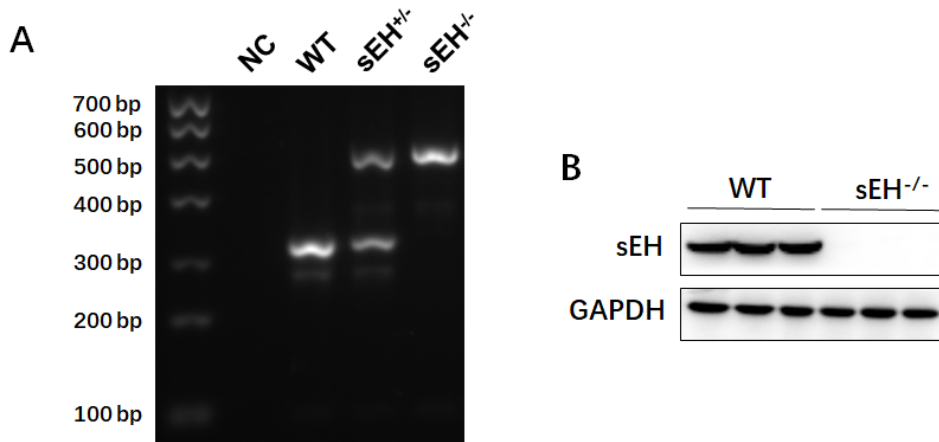
**Figure S2.** Effects of alisol B against renal weight in WT mice, data were presented as mean  $\pm$  SEM, n=5, \*\*\*p<0.001, n.s.= no significance.



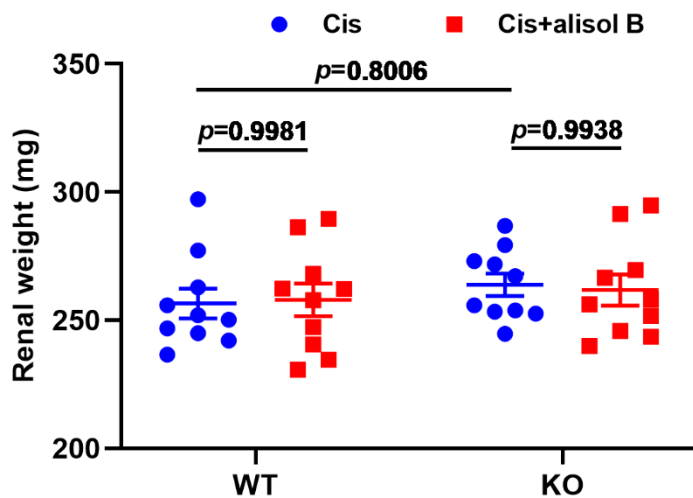
**Figure S3.** Representative MS/MS spectrum of sEH peptide.



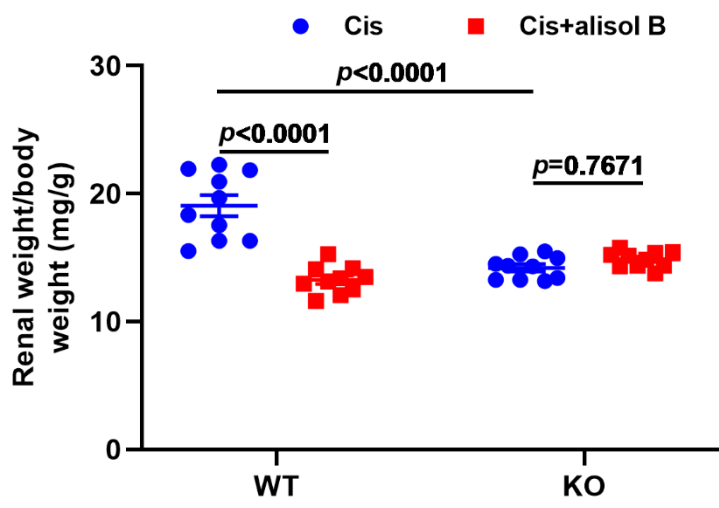
**Figure S4.** The potential energy (A), RMSD (B), RMSF (C), and hydrogen bond number (D) of alisol B with sEH (PDB: 4OCZ) in the 50 ns of MD. (E-G) The distance of alisol B with amino acid residues Asp335, Trp336, Thr360, Ser374, Asn378, Tyr383, Gln384, Tyr466, and Asn472. (H) The cavity of sEH in the presence of alisol B at 50<sup>th</sup> ns of MD.



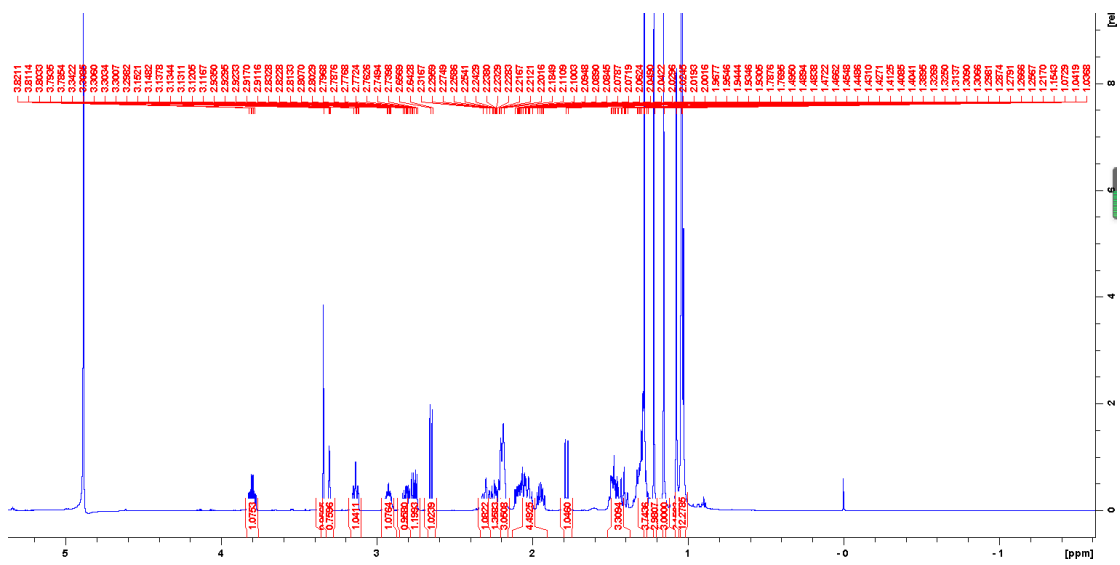
**Figure S5.** Genotyping and confirmation of sEH knockout mice. (A) Genotyping of sEH<sup>-/-</sup> mice. The DNA extracted from mouse tails were used for genotyping and amplified by PCR with three primers: 1) F1-5'- TAA TCC AGC AGC TCT CAT GTC ATC -3'; 2) F2-5'- GAA TCA AAC CAT CCA CCC TCT CT -3'; and 3) R-5'-ATT TCT GCC AGA TGT TTT GAG TAC C-3'. The PCR products from wild-type (WT), heterozygote (sEH<sup>+/-</sup>) and homozygote (sEH<sup>-/-</sup>) include only the 322 bp fragment, both the 322 bp and 529 bp fragments, and only the 529 bp fragment, respectively; (B) Western blotting demonstrating that no signal was observed in renal tissues from sEH<sup>-/-</sup> mice.



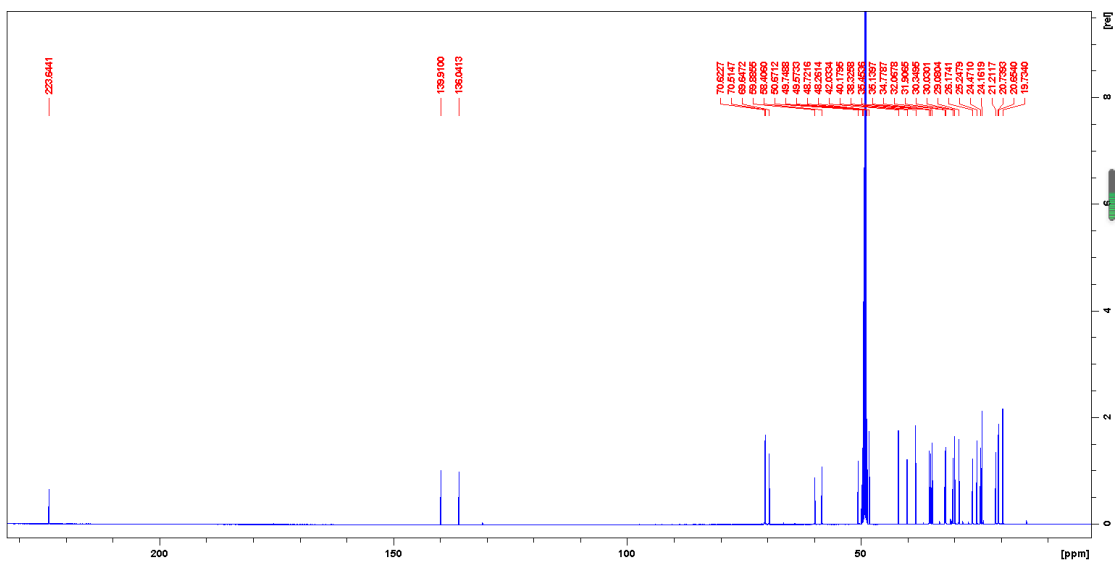
**Figure S6.** Effects of alisol B against renal weight in WT and *Ephx2* KO mice, data were presented as mean  $\pm$  SEM, n=10.



**Figure S7.** The ratio of renal weight versus body weight of wild-type (WT) and sEH knockout mice with cisplatin-induced AKI after alisol B treatment. Data were presented as mean  $\pm$  SEM, n=10 per group.



**Figure S8.** The  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 600 MHz) spectrum of alisol B



**Figure S9.** The  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 150 MHz) spectrum of alisol B