Benzene-induced mouse hematotoxicity is regulated by a protein phosphatase 2A complexes that stimulates transcription of *cytochrome P4502E1*

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Supplementary Table 1

Periphery blood cytometry in mice exposed to benzene by inhalation and oral gavage

Exposure route	Exposure level (mean ± SD, ppm or mg/kg)			
	0	1	10	100
Dynamic inhalation (n=10)				
HGB (g/L)	92.90±9.97	91.10±14.93	93.80±8.53	77.40±11.59 [a]
MCH (pg)	12.30±0.52	12.00±0.50	12.42±0.45	14.05±0.78 [a]
MCHC (g/L)	290.17±10.32	285.68±6.53	282.53±5.40 [a]	301.25±7.80 [a]
RDW-CV (%)	19.92±1.47	19.56±1.64	20.83±1.26	24.47±0.69 [a]
HCT (%)	49.68±3.11	49.37±6.86	50.36±4.40	44.80±2.64 [a]
MPV (f l)	3.94±0.21	3.97±0.17	3.83±0.28	3.93±0.40
MID	0.23±0.11	0.17±0.07	0.19±0.08	0.07±0.03 [a]
NEU (10^9/L)	1.84±0.46	1.84±0.35	1.77±0.15	0.79±0.19[a]
LYM %	56.19±3.04	49.85±2.59 [a]	45.77±5.46 [a]	48.12±6.87 [a]
NEU %	38.82±4.08	45.79±3.22 [a]	49.01±6.08 [a]	47.60±7.14 [a]
MID%	5.00±2.41	4.36±1.94	5.22±1.60 [a]	4.28±1.17 [a]
Oral gavage (n=6)				
HGB (g/L)	132.83±4.62	129.58±10.63	126.75±10.63	115.00±7.04 [a]
MCH (pg)	15.96±0.14	16.09±0.31	16.33±0.37	17.47±0.44 [a]
MCHC (g/L)	279.50±7.07	306.70±18.29 [a]	283.30±5.71	301.30±15.06[a]
RDW-CV (%)	18.55±0.44	19.02±0.58	19.41±1.63	22.01±1.71 [a]
HCT (%)	47.50±1.88	42.43±4.10	44.07±1.82	38.24±4.29 [a]
MPV (f l)	4.60±0.13	4.62±0.12	4.60±0.09	4.48±0.11[a]
MID	0.22±0.06	0.17±0.05 [a]	0.17±0.05 [a]	0.07±0.01 [a]
NEU (10^9/L)	2.04±0.48	2.18±0.50	1.61±0.35	0.89±0.09[a]
LYM %	53.17±6.11	41.98±5.33	47.75±11.00	43.55±5.08 [a]
NEU %	42.24±5.64	54.00±5.32	47.56±10.81	52.18±4.73 [a]
MID%	4.59±0.87	4.02±0.55	4.69±0.61	4.28±0.56

[a] $P<0.05$, compared with the corresponding control group, as determined by Independent-sample *t*-test or assessed with One-way ANOVA followed by Bonferroni post-test.

Supplementary Table 2

Primers for RT-qPCR analysis

Gene	Forward Primer	Reverse Primer
cyp1a2	GAGGTATCCTGACCCTGAAGTA	CACACGCAGCTCATTGTAGA
cyp2e1	GTCTTCCTCTTCTTAGCCATCC	GCTTCATGGTCAACCCATAGT
cyp2a22	GGCACTGATGTGTTCCCTATAA	CCTGACCAAATGGCTGTAGAT
cyp2a4	CTTCATCGACTCCTTCCTCATC	GTGCCAGCAAAGAAGAGATTTAG
cyp2a5	GAGGAGATTGATCGGGTGATTG	CATGGATTACAGCCTCCGTATAG
cyp3a11	TGAATATGAAACTTGCTCTCACTAAAA	CCTTGTCTGCTTAATTTCAGAGGT
cyp2c29	CACAGCTAAAGTCCAGGAAGAG	GAATCATGGCGTCTGTATAGGG
cyp2c37	CTGCCAATCCTTCACCAATTTATC	CTTCCTTCACTGCCTCATATCC
cyp2d22	TTGAACTACAGGGCTTCCTTATC	TCTCCCAGACAGTCTCATCTT
cyp2d9	CAGAAGTCCTTCATCGCCATAC	CCAGGAAGGCATCAGTCAAA
cyp2f2	TGCTCACCATCATCCACTTTAT	CACCACACGGTCAATCTCTT
gstp1	GAGACCTCACCCTTTACCAATC	CCCATCATTCACCATATCCATCT
Gsta1	GCTTCTCTAGCTCACGCTATTC	CCCAGTCAAGGCTCTACTTATTG
Gstm1	GACTTTCCCAATCTGCCTTACT	CTCCTCCTCTGTCTCTCCATC
Gstm2	GAGACAGAGGAGGAGAGGATT	GGGAAGAGGAAATGGAGGAATAG
Gstm3	GTTATGGACACCCGCATACA	CCCACTGGGCTATCTTAGTAAAC
Gstm4	GCTTCTCTAGCTCACGCTATTC	CCCAGTCAAGGCTCTACTTATTG
β-actin	GAGGTATCCTGACCCTGAAGTA	CACACGCAGCTCATTGTAGA

Supplementary table 3

The values of *m/z* and mass error of benzene and its metabolites

Chemical	*m/z* of Target ion	mice	Δ*m/z* (mmu)				
			M1	M2	M3	M4	M5
Benzene	79.0542 (+)	WT	0.733	0.533	0.533	0.473	0.49
		HO	0.223	0.463	0.443	0.743	0.433
Phenol	93.0346 (-)	WT	-0.271	-0.261	-0.311	-0.341	-0.371
		HO	-0.321	-0.261	-0.291	-0.241	-0.231
Hydroquinone	109.0295 (-)	WT	-0.076	-0.236	-0.276	-0.282	-0.346
		HO	-0.266	-0.216	-0.346	-0.046	-0.196
tt-MA	141.0193 (-)	WT	-0.095	-0.105	-0.155	-0.175	-0.245
		HO	-0.225	-0.235	-0.085	-0.045	-0.005

"+" and "-" were indicated as positive ions and negative ions, respectively.

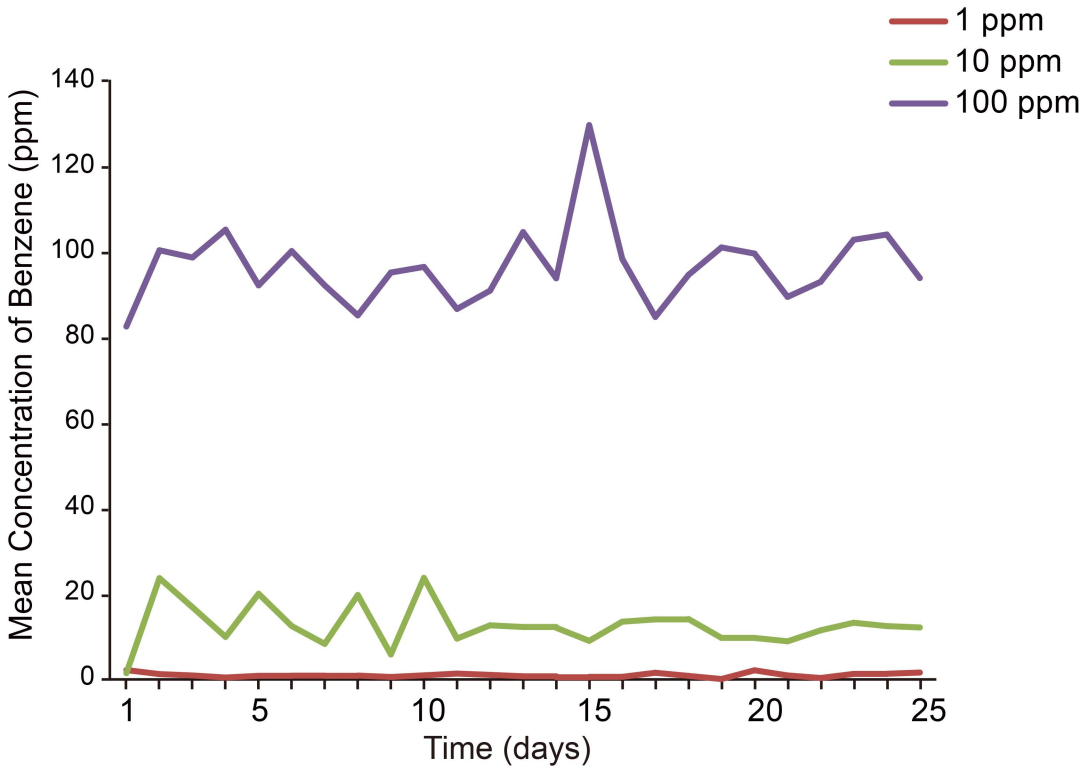

Figure S1 The benzene concentrations in inhalation chambers
The daily mean concentrations of benzene vapor in the inhalation chambers were monitored by gas chromatography-mass spectrometry (GC-MS) every day during the inhalation exposure period.

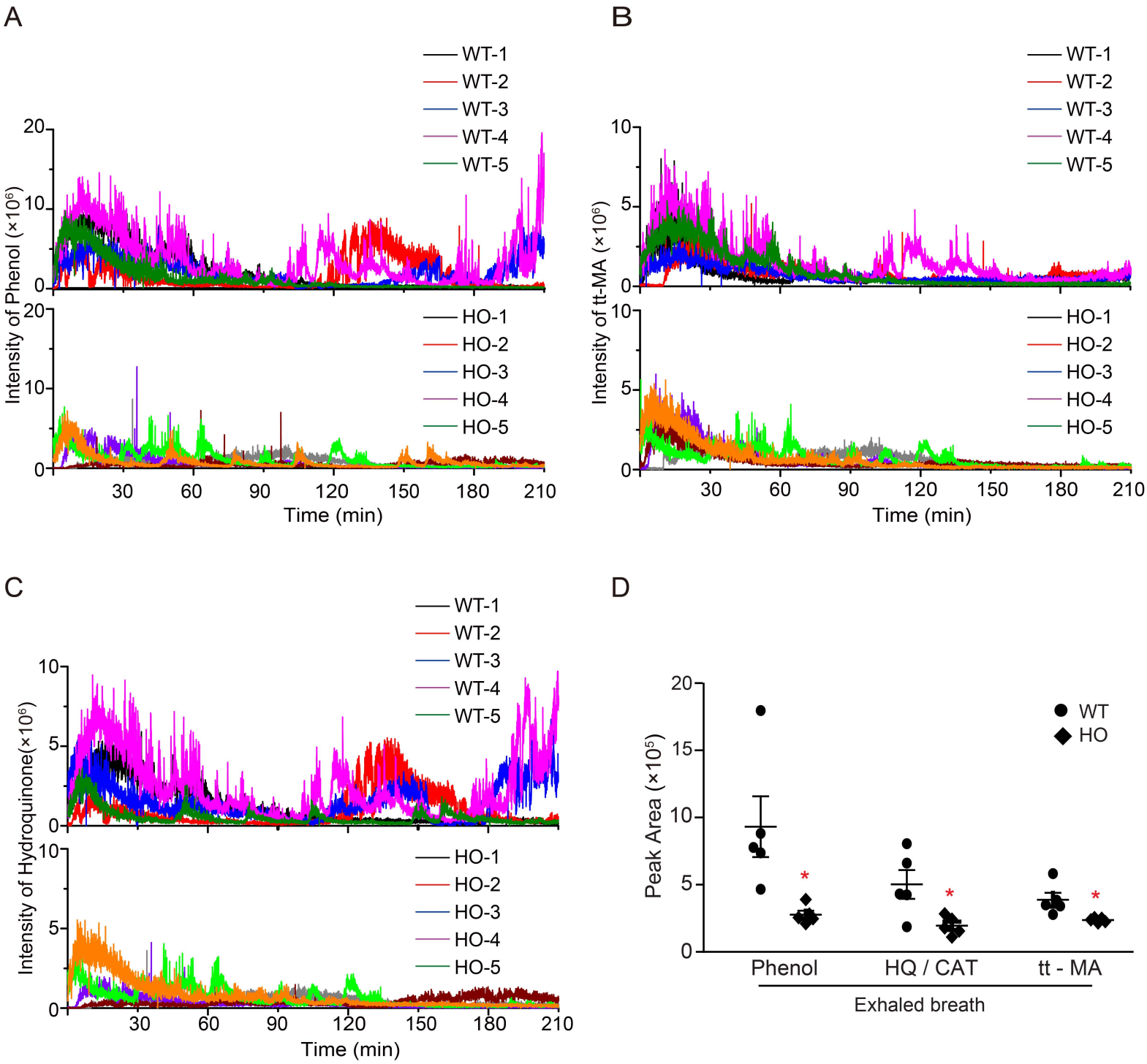

Figure S2 The effect of PP2A Aα deletion on the contents of exhaled benzene metabolites
WT and HO mice (n=5) were administrated with benzene at dose of 100 mg/kg and the components were monitored dynamically over 210 min in total. The contents of exhaled metabolites including (a) phenol, (b) tt-MA, and (c) hydroquinone was determined by a real time MI-SPI-TOFMS. Representative images showed two-dimensional mass spectrum data with standardized m/z intensity and elusion time. (d) The contents of exhaled benzene metabolites were determined based on the peak area, $P<0.05$ (*) compared to WT mice, as analyzed by the Student t-test.

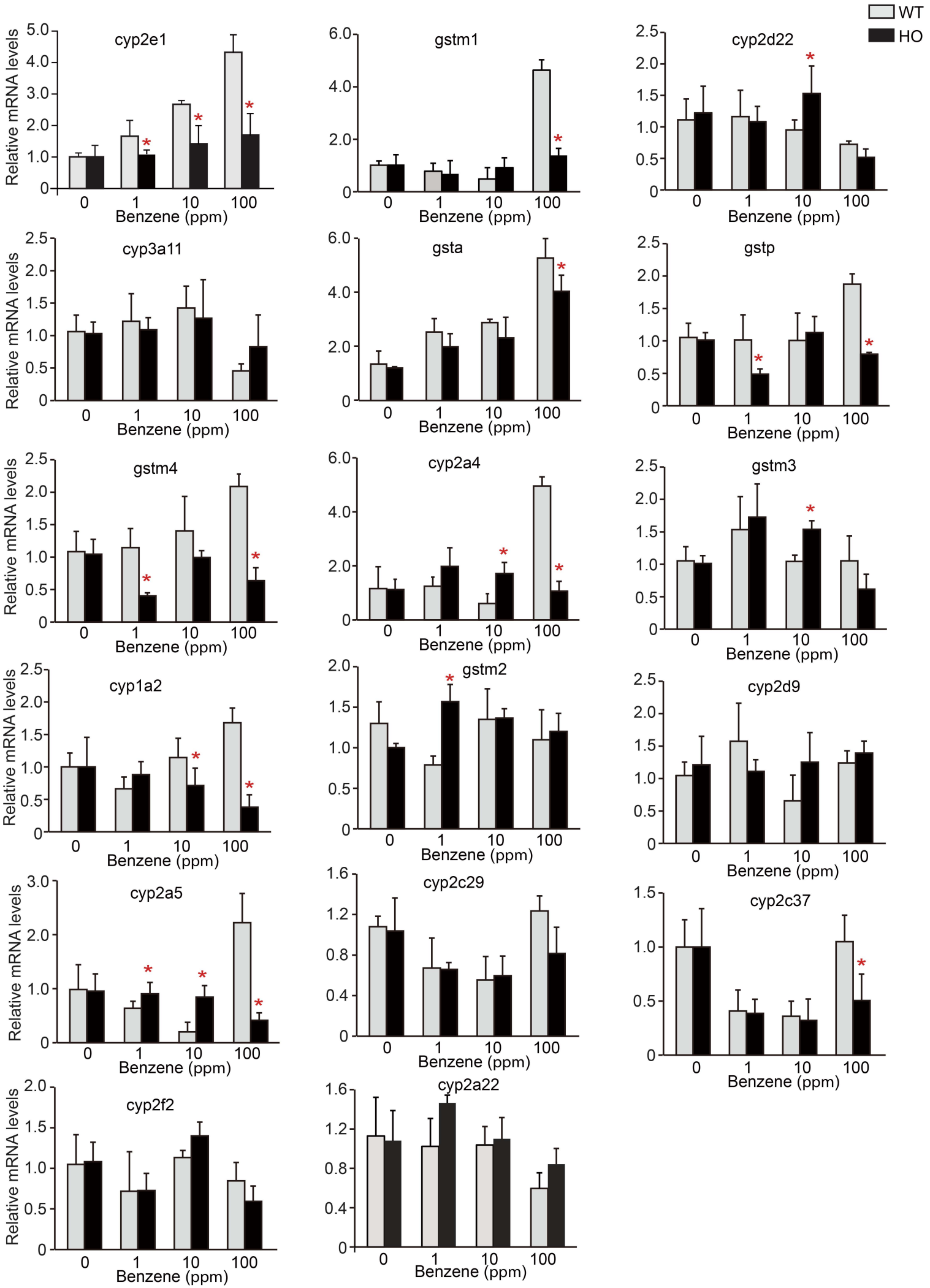

Figure S3 The effect of PP2A Aα deletion on the expressions of metabolic enzymes in benzene-treated mice

The relative mRNA levels of 17 metabolic enzymes were examined in WT and HO mice (n=3) exposed to benzene at indicated doses. The data were shown as mean ± SEM for three independent experiments. *P<0.05, compared with the respective control group, as analyzed by the Student t-test.

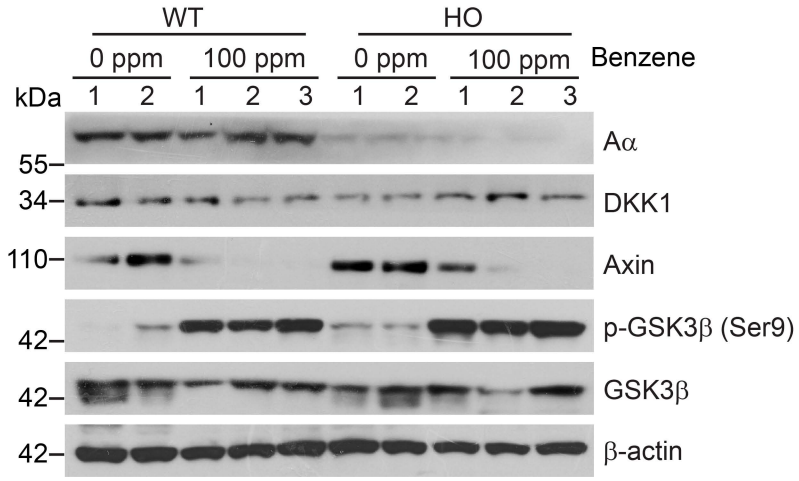

Figure S4 The expressions of Wnt/β-catenin components in benzene-treated mice
Immunoblotting analysis of Aα, DDK1, Axin, GSk3β, and phosphorylated GSk3β in WT and HO mice liver treated with 100 ppm benzene.

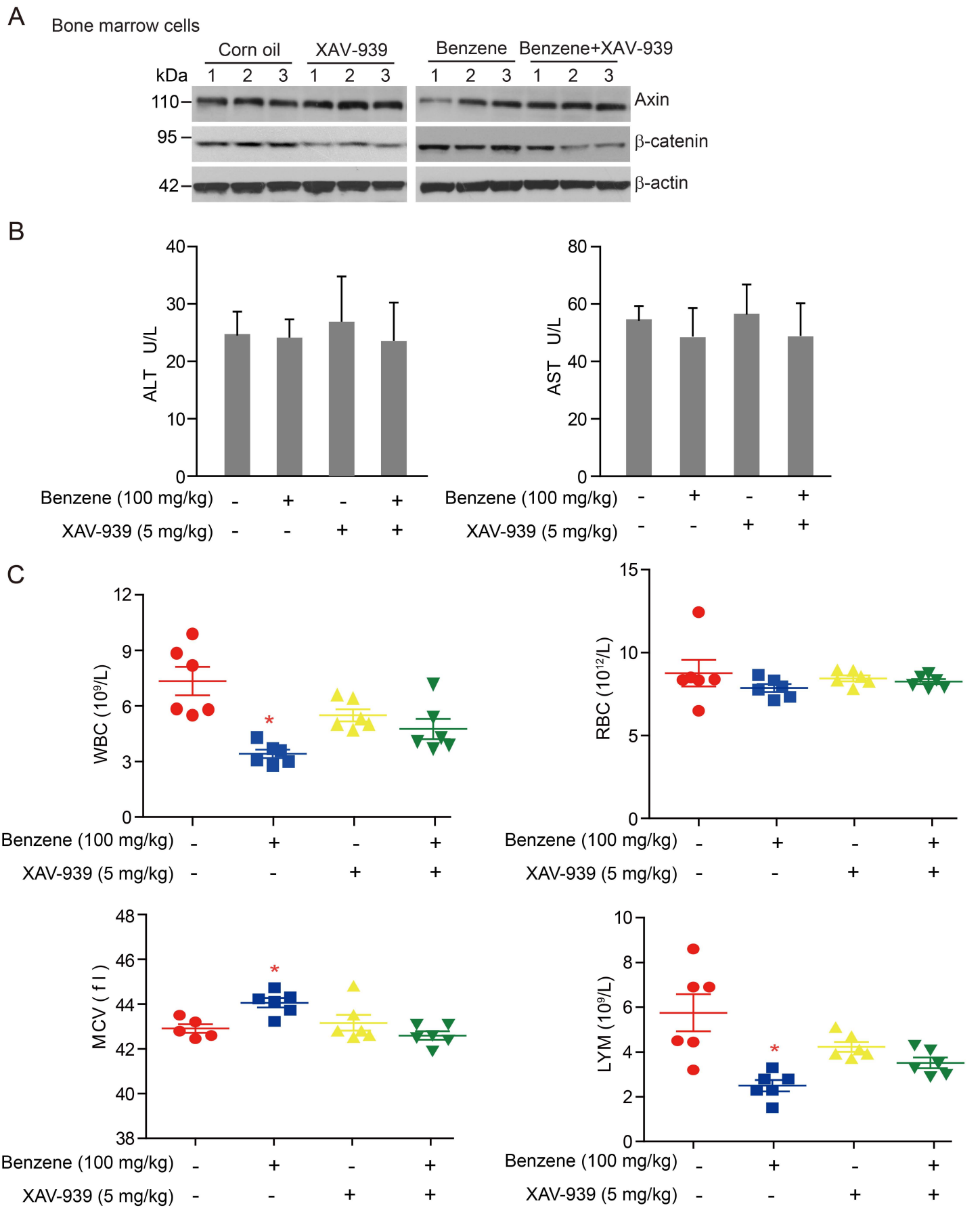

Figure S5 The effect of β-catenin suppression on the hematotoxicity induced by benzene
WT mice (n=6) were administrated with benzene at dose of 100 mg/kg and/or 5 mg/kg XAV-939 for 7 days.
(A) Immunoblotting analysis of Axin and β-cateinin in bone marrow cells at indicated treatment group. (B) The ALT and AST levels of plasma at indicated group mice. (C) Peripheral blood counts including WBC, RBC, MCV, and LYM. Data were shown as mean ± SD.*P<0.05, compared with the control mice.

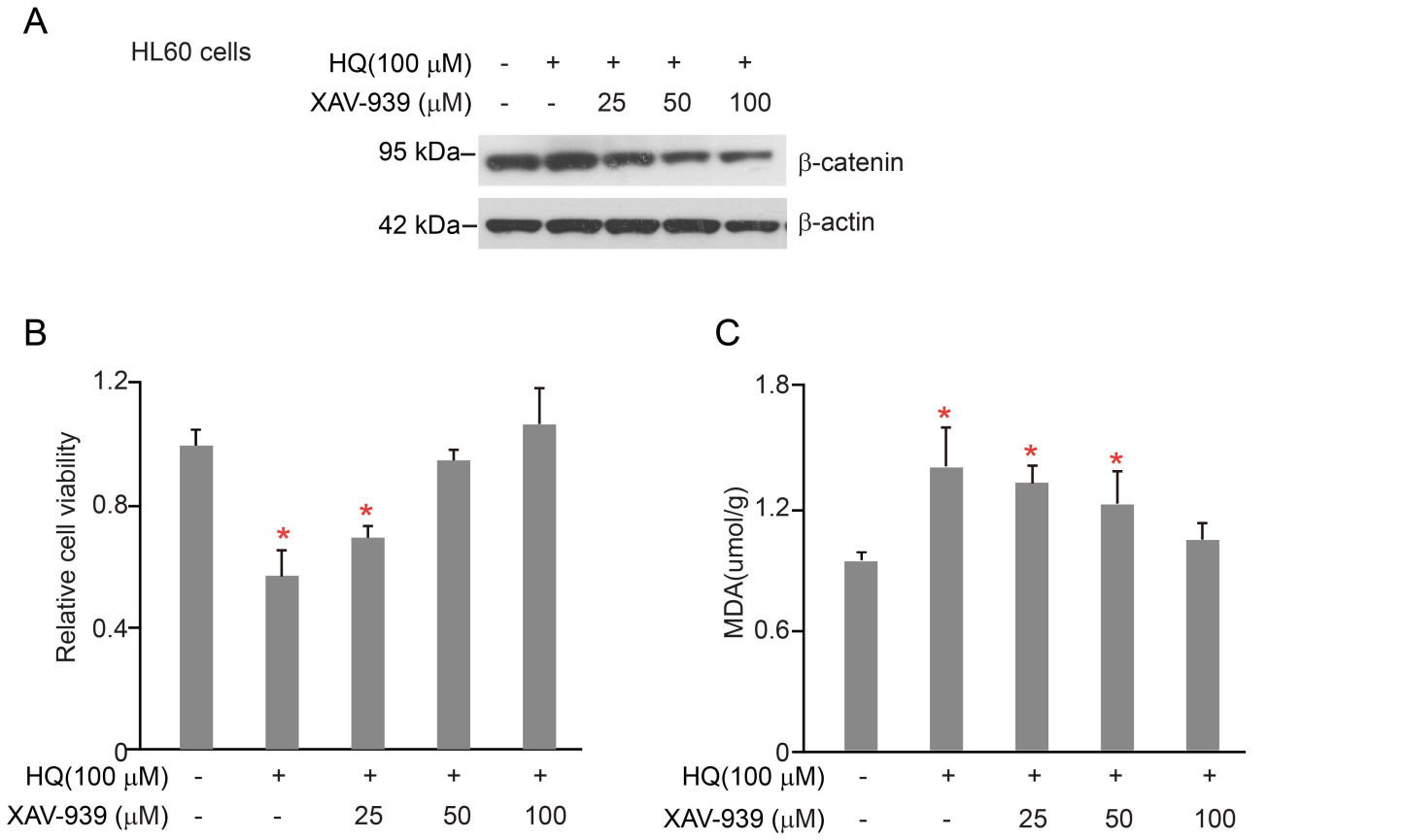

Figure S6 The effect of β-catenin suppression on the toxicity induced by HQ
HL60 cells were treated with 100 μM HQ and/or XAV-939 at the indicated concentrations for 24 h, respectively. (A) Immunoblotting analysis of β-catenin in HL60 cells at the indicated treatment. (B) Relative cell viability was measured by MTT assay. (C) MDA content was determined and calculated as μmol/g protein. Data were shown as mean±SD from three independent experiments. *P<0.05, compared with the control cells.

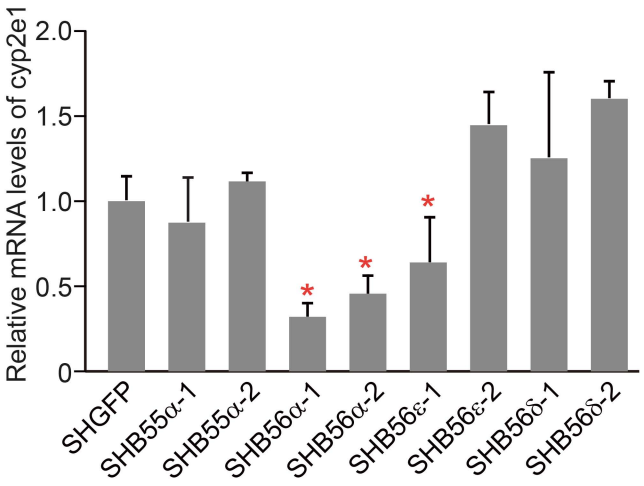

Figure S7 The effects of suppression of PP2A B subunits on the expressions of cyp2e1
The relative mRNA level of cyp2e1 was examined in HepG2 cells expressing shRNA targeting B55α, B56α, B56δ, or B56ε subunit. Data were shown as mean ± SEM for three independent experiments. *P<0.05, compared with the respective control group.

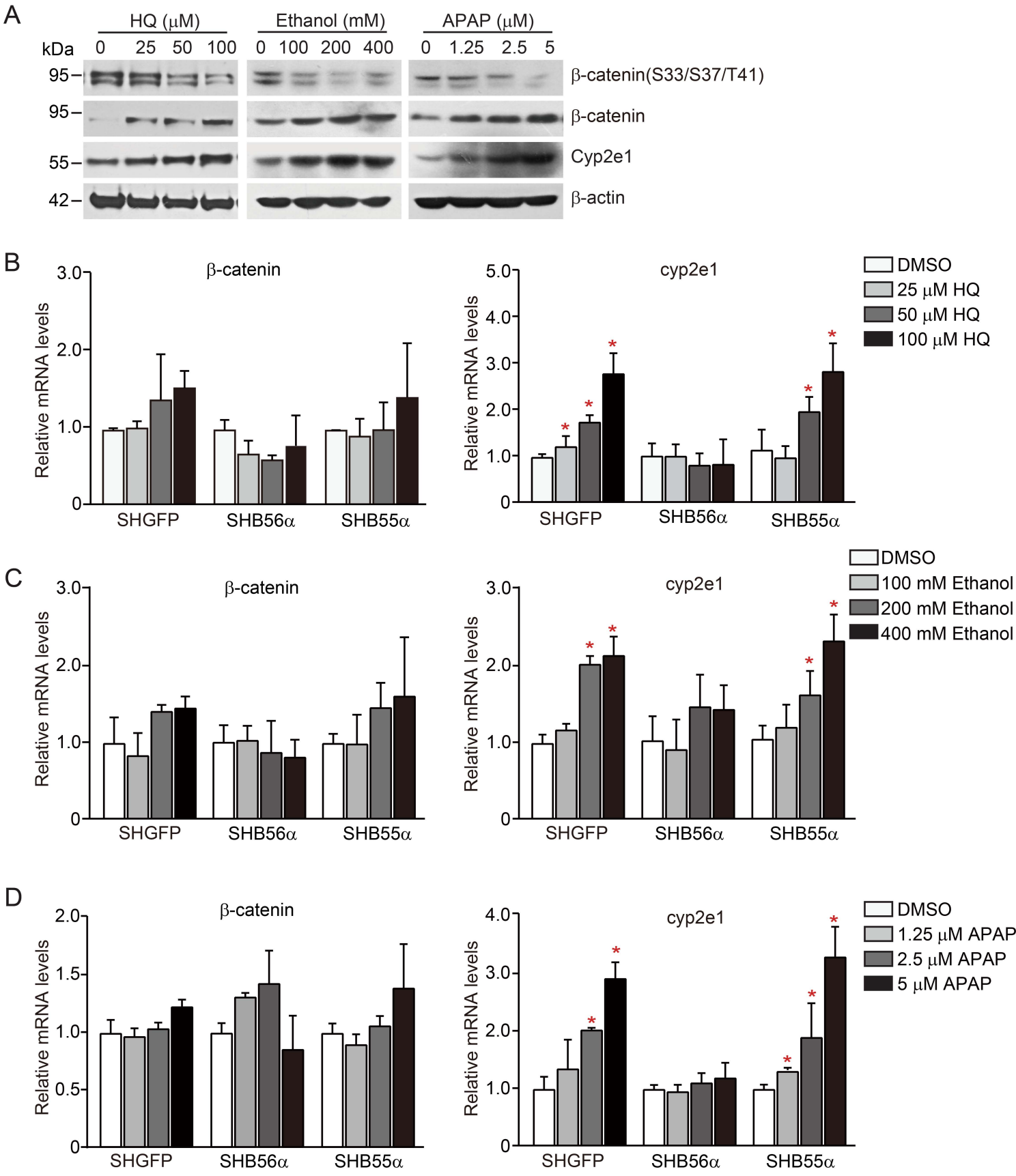

Figure S8 The induction of Cyp2e1 and β-catenin expression upon agonists
(A) HepG2 cells were treated with ethanol, HQ, or APAP at the indicated concentrations for 24 h, respectively, and followed by immunoblotting analysis with an antibody against β-catenin, β-catenin (S33/S37/T41), and Cyp2e1. HepG2-SHGFP, HepG2-SHB56α and HepG2-SHB55α cells were treated with (B) HQ, (C) Ethanol, and (D) APAP at the indicated concentrations for 24 h, respectively. The relative mRNA levels of β-catenin and cyp2e1 were examined by Q-PCR. The data were shown as mean ± SEM for three independent experiments. *P<0.05, compared with the respective control group.