Supplemental Data

Manuscript title:

Small heterodimer partner regulates circadian cytochromes p450 and drug-induced hepatotoxicity

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Materials and Methods

Materials

Acetaminophen (APAP), phenacetin, collagenase IV, mitoxantrone (MTX) and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). NAC-APAP and Cys-APAP were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Aminophylline, 1,3-dimethyluric acid (1,3-DMU), resorufin, coumarin, testosterone, tolbutamide, retinoic acid, alamethicin, 7-hydroxycoumain, reduced glutathione (GSH), arachidonic acid, and 4-nitrophenol were purchase from Aladdin (Shanghai, China). 15α-hydroxytestosterone (15α-OH testosterone) and 6β-hydroxyltestosterone (6β-OH testosterone) were obtained from Steraloids (Wilton, NH, USA). 20-hydroxy arachidonic acid (20-HETE) was purchased from MedChem Express (Monmouth Junction, NJ, USA). Aflatoxin B1 (AFB1) was purchased from Fermentek Ltd. (Jerusalem, Israel). Pentoxyresorufin was purchased from ApexBio Technology (Houston, TX, USA).

Plasmids

pRL-TK vector was purchased from Promega (Madison, WI). mCyp1a2 (2 kb)-Luc, mCyp2a5 (2 kb)-Luc, mCyp2a5 (1500)-Luc, mCyp2a5 (600)-Luc, mCyp2a5 (100)-Luc, mCyp2b10 (2.5 kb)-Luc, mCyp2b10 (2.3 kb)-Luc, mCyp2b10 (2 kb)-Luc, mCyp2b10 (600)-Luc, mCyp2c38 (2 kb)-Luc, mCyp2c38 (1200)-Luc, mCyp2c38 (700)-Luc, mCyp2c38 (80)-Luc, mCyp2e1(2 kb)-Luc, mCyp3a11 (2 kb)-Luc, mCyp3a11 (1650)-Luc, mCyp3a11 (1400)-Luc, mCyp3a11 (1000)-Luc, mCyp3a11 (400)-Luc, mCyp4a10(2 kb)-Luc, mCyp4a10(1500)-Luc, mCyp4a10(1350)-Luc, mCyp4a10(180)-Luc, mCyp4a14(2 kb)-Luc, mCyp4a14(1700)-Luc, mCyp4a14(700)-Luc, mCyp4a14(400)-Luc, mCyp4a14(200)-Luc, pcDNA-His-mNr1d1, pcDNA-mDec2, pcDNA-Flag-mE4bp4, pcDNA-mLrh-1, pcDNA-HA-mShp, pcDNA-mHnf1α, pcDNA-mHnf4α and siRNA were synthesized by Transheep (Shanghai, China).

Table S1. siRNA sequences used in this study.

Gene	Forward (5'-3' sequence)	Reverse (3'-5' sequence)
siE4BP4	GCACAAGCUUCGGAUUAAATT	UUUAAUCCGAAGCUUGUGCTT
siRev-erbα	CUUCGUUGUUCAACGUGAATT	UUCACGUUGAACAACGAAGTT
siLrh-1	GCAGAAGAAAGCCCUCAUUTT	AAUGAGGGCUUUCUUCUGCTT
siHnf4α	CCUGCAGGUUUAGCCGACAAUGUGU	ACACAUUGUCGGCUAAACCUGCAGG
siHnf1α	CACCCAUUCUGAAAGAGCUGGAGAA	UUCUCCAGCUCUUUCAGAAUGGGUG
siDec2	CAGCCUUAACUGAGCAGCAGCAUCA	UGAUGCUGCUGCUCAGUUAAGGCUG
Negative	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Table S2. Mouse primer sequences for quantitative real-time PCR (qPCR).

Gene	Gene bank no.	Forward (5'-3' sequence)	Reverse (3'-5' sequence)
Cyp1a1	NM_009992.4	GGTTAACCATGACCGGGAACT	TGCCCAAACCAAAGAGAGTGA
Cyp1a2	NM_009993.3	AGTACATCTCCTTAGCCCCAG	GGTCCGGGTGGATTCTTCAG
Cyp1b1	NM_009994.1	TTCATCTCCATCCTCGCTCA	CAGCCTTCCTCAGTTTGCAG
Cyp2a4	NM_009997.2	AACGGTGCTTTCATTGACCC	CCTCATAGTCAAAGCGGTCCC
Cyp2a5	NM_007812.4	GGACAAAGAGTTCCTGTCACTGCTTC	GTGTTCCACTTTCTTGGTTATGAAGTCC
Cyp2a12	NM_133657.1	TTAGCTCCATTGTCTTCGGC	GGTACTTCATAACTGAATGG
Cyp2a22	NM_001101467.1	ATCAAGAGGGAGGGAGAGCC	GACCCTTTCTGGATGAGTGACA
Cyp2b9	NM_010000.2	GACTTTGGGATGGGAAAGAG	CCAAACACAATGGAGCAGAT
Cyp2b10	NM_009999.4	AGACATCCTTATTTCTATGCCC	CTGCATACTGGAGCACTTCATT
Cyp2b13	NM_007813.2	TTTTCTTCCAGTGTGTTACAGCC	AACGCAGGAACTGTTCATCTG
Cyp2c29	NM_007815.3	TGGTCCACCCAAAAGAAATTGA	GCAGAGAGGCAAATCCATTCA
Cyp2c37	NM_010001.2	CACGAGGCGTTTCTCACTCA	AGGGCTGCTCAGAATCTTTGT
Cyp2c38	NM_010002.3	CACTATGGAGACAGAGGTCTA	CCAAATACAGAGTGAAAACG
Cyp2c39	NM_010003.2	GGAGACAGAGCTGTGGC	TAAAAACAATGCCAAGGCCG
Cyp2c40	NM_010004.2	TCCGGTTTTTGACAAGGTTTCTAC	TGCCCAAGTTCCTCAAGGTATTC
Cyp2c44	NM_001001446.3	GGCTATGATGTGGTGAAGGAAGC	TCGCAGGAGTTTCCACCTCTCT
Cyp2c50	NM_001167877.1	ATGGAAAAACACAAGGCGCTTC	GCAACCAAGGGGTGCTCATT
Cyp2c54	NM_206537.2	TTGACCATGGGGATGTCTTT	ATCCAATGCCCATTCCATTA
Cyp2c55	NM_028089.3	AATGATCTGGGGGTGATTTTCAG	GCGATCCTCGATGCTCCTC
Cyp2c65	NM_028191.2	ACAACCCAAAGACGGAGTTTAC	CTGTCCCAGCAGCAAACAGAT
Cyp2c66	NM_001011707.1	GGAGTTTGCTGGAAGAGGAACC	GCGTCTCATCTCTTTCCAGGTC
Cyp2c69	NM_001104525.1	TGTAGTCTTGGTGCTTTGTCTG	ACAATAGGCTGTGAGCCAAAATA
Cyp2c70	NM_145499.2	AGTATGGCCCTGTGTTTACTGT	GCCTTGGCTGGTTCTACTGAG
Cyp2d9	NM_010006.2	AGTCTCTGGCTTAATTCCTGAGGTT	CGCAAGAGTATCGGGAATGC
Cyp2d10	NM_010005.3	TTCCGGTTGCCCCCTACCCC	TCAGCCTGCCCTGCCTACCA
Cyp2d11	NM_001104531.1	TCTCAGTGCCTGATGGACAG	CACAGAGCTGGTAGGGGAAG
Cyp2d22	NM_001163472.1	CAGTGGTTGTACTAAATGGGCT	GCTAGGACTATACCTTGAGAGCG
Cyp2d26	NM_029562.2	TTCAAAAGCCTGGAAGCAGT	TCCACCAGAAGCAGGAAGAT
Cyp2d40	NM_023623.2	GTCGAGCATGTCTGGGTGAG	CCATCCGGTACTGAGAAGCTA
Cyp2e1	NM_021282.2	CGTTGCCTTGCTTGTCTGGA	AAGAAAGGAATTGGGAAAGGTCC
Cyp2f2	NM_007817.2	TGCGTCACGCCTTCCTTATT	ACATCTGTGCCCTTGGGTATC
Cyp2g1	NM_013809.1	AGGAGGAGGCTGGCTACC	TCAGGTATAAGGTGGGATCTATGG
Cyp2j5	NM_010007.4	CAGACATGGAAGGAGCAAAGG	GAATGCGCTCCTCCAAGCT
Cyp2j6	NM_010008.4	ATGCTCGCTGCTACCGGCTC	GTGCTTCTTTGATTAAGGGC
Cyp2r1	NM_177382.4	TGGTGAGGTAAATGAGGCTTTC	TGCCAGTGCTCCAGTCTTC
Cyp2s1	NM_028775.3	GGTCACGGAGTTTTCTTTGCC	ATAGGGCAAACGGATGCCAA
Cyp2u1	NM_027816.3	GGCTGAAGTGTTCAGTGACC	CGTATGCAAACTCCTCGATG

Gene	Gene bank no.	Forward (5'-3' sequence)	Reverse (3'-5' sequence)
Cyp3a11	NM_007818.3	CGCCTCTCCTTGCTGTCACA	CTTTGCCTTCTGCCTCAAGT
Cyp3a13	NM_007819.4	CCTCTGCCTTTCTTGGGGACGAT	CCGCCGGTTTGTGAAGGTAGAGT
Cyp3a16	NM_007820.2	CTGAAATTAAGCAGAGAACT	ATCACGCTCCAGTTATGA
Cyp3a25	NM_019792.2	CTTCACTGTCCAGCCTTGTGAA	AATTGGTTCCCTGCTGATCTTC
Cyp3a41	NM_017396.3	GTGGAGAAAGCCAAAGGGATT	GAAGACCAAAGGATCAAAAAAGTCA
Cyp3a59	NM_001105160.1	ACTACTTTGATGGCATGTGGA	GCCCAAGAGTCGGACGG
Cyp4a10	NM_010011.3	TTCCCTGATGGACGCTCTTTA	GCAAACCTGGAAGGGTCAAAC
Cyp4a12a/b	NM_172306.2	GGGGAGATCAGACCCAAAAGC	ATTCGTCGGTGCTGAAACCAT
Cyp4a14	NM_007822.2	CCCAAAGGTATCACAGCCACAA	CAGCAATTCAAAGCGGAGCAG
Cyp4a31	NM_201640.3	AGCAGTTCCCATCACCGCCC	TGCTGGAACCATGACTGTCCGTTT
Cyp4a32	NM_001100181.1	AAGGCGAACAAGAACTAA	GCAAACCATACCCAATCC
Cyp4b1	NM_007823.2	CTGCATGGCCCTTTATCCTA	GAAGCACTCCTTCATGCACA
Cyp4f13	NM_130882.1	CGACAGCAACTGCCAAGAGT	CAGTGAGATAGTACAGGAGGTCC
Cyp4f14	NM_001357788.1	ACTGGCTTATGGGTCACGTG	ACCCACCAAACGAGTCAATTC
Cyp4f15	NM_134127.1	CCCCCAAATGGAATTGGTTCT	ACCAGGTTAGTCACCTCCTTC
Cyp4f16	NM_024442.1	CTGCGGCTAAGTGTGTCTGGGC	TGGAAAGTCTGGCCCATTTCGGTA
Cyp4f17	NM_001101445.1	TGACACCTGCATTCCACTTTG	GCAGTTCCTTTGGTGGTCAG
Cyp4f18	NM_024444.2	TGTTTTTGAGAACTCTCTTCGCC	TGGAATATACGGATGACTGGG
Cyp4v3	NM_133969.2	TCCGAGTTTTCCCATCTGTC	CGGTGTAGTGCGTAGGGAAT
Cyp4x1	NM_001003947.1	GCCGTGAAGCTCTACCTGAG	GGTCTTTGGGTCTGTTCTGC
Cyp7a1	NM_007824.2	AGCAACTAAACAACCTGCCAGTACTA	GTCCGGATATTCAAGGATGCA
Cyp7b1	NM_007825.4	AATTGGACAGCTTGGTCTGC	TTCTCGGATGATGCTGGAGT
Cyp8a1	NM_008968.3	ATGCCATCAACAGCATCAAA	AAACTCAGGAACCTCTGTGTCC
Cyp8b1	NM_010012.3	GCCTTCAAGTATGATCGGTTCCT	GATCTTCTTGCCCGACTTGTAGA
Cyp17a1	NM_007809.3	CATCCCACACAAGGCTAACA	CAGTGCCCAGAGATTGATGA
Cyp20a1	NM_030013.3	CTGGACTTCGCCATCTTCG	CCGGGTAGAGGTACAGCAC
Cyp21a1	NM_009995.2	AGGAATTCTCCTTCCTCACTTGT	TCTGTACCAACGTGCTGTCC
Cyp26a1	NM_007811.2	CGAGAAGAGATAAAGAGCAAG	TCAGAGCAACCCGAAACC
Cyp26b1	NM_175475.3	GCAAGATCCTACTGGGCGAAC	TTGGGCAGGTAGCTCTCAAGT
Cyp27a1	NM_024264.5	GTGGACAACCTCCTTTGGGA	TTGCTCTCCTTGTGCGATGAA
Cyp39a1	NM_018887.4	ATCCAGCCACACTCAATACCC	GGAGCCATACTCAAAGCCCTC
Cyp46a1	NM_010010.1	ATGCCAGGGAAGAGAAAGC	CTTTGAGGATCTGCGTGAG
Cyp51a1	NM_020010.2	GTCTACCTGTTCCGTCTCGC	TCATATGCAACTCCCTTCCC
Ugt1a1	NM_201645.2	GCTTCTTCCGTACCTTCTGTTG	GCTGCTGAATAACTCCAAGCAT
Ugt1a2	NM_013701.3	ATGGACACGGGACTATGTGTG	CATGGGTAACACCAGCACTTTT
Ugt1a5	NM_201643.2	TGAGAAGGTGCTAGTGTTTCCT	GGGAACGGCATAGACTTTGAA
Ugt1a6a	NM_145079.3	GTTTCTCTTCCTAGTGCTTTGGG	CCTCGTTCACTGAGATGTTCTAC
Ugt1a9	NM_201644.2	TTTCGATGTGTGCGGCTTAAC	GGTTCCGAGTTCTTTCCTTGAA
Ugt2a1	NM_053184.2	TTGAGGAGTTTCATTTGGTGTCC	GGGAAAACCGCAAGGAATAGATA
Ugt2a2	NM_001024148.1	TGTGGTAGTTTGGCCTACAGA	TTGTGTACGAAACCGGAATCTC

Gene	Gene bank no.	Forward (5'-3' sequence)	Reverse (3'-5' sequence)
Ugt2a3	NM_028094.3	GATGTTGAGTTTCCTCGTCCAT	GCCTGAGTATCTCCACAAAACCT
Ugt2b1	NM_152811.1	TTGATGTCGTTCTAGCAGATGC	TCAAAAAGCAACACCTGCAAC
Ugt2b34	NM_153598.2	TGAAGTGATGGTTCTGAGACCT	ACTGCTTTGGCAGCTCATAAAT
Ugt2b35	NM_172881.3	CTTGTACAGAGGGGCCATGAA	TGGGACCTCAAAAGTCCATTC
Ugt2b36	NM_001029867.1	CTACAAGTGGCTTCCCCAAAA	ATGGATCGCCTCGTAGAGTCC
Ugt2b37	NM_053215.3	ACAGCTTTTGAATGTGTGGACT	GCGATTAGCTCCCCACCAAA
Ugt2b38	NM_133894.2	TGCGCCACAAAAGGGCTAA	ACACAAGAGAGTAGGAAGCCG
Ugt2b5	NM_009467.3	TGTCTATGAGGCCATCTATC	ATATTCAGTGTAACAGCTGC
Ugt3a1	NM_207216.2	ATGGCTGCACATCGGAGTTG	AGAATTTGCGACACACGGTTC
Ugt3a2	NM_144845.3	TGATAAGCCGTGTGTCGCA	AGTCGATGCCTAAGATCAGCA
Ugt8a	NM_011674.5	ACTCCATATTTCATGCTCCTGTG	AGGCCGATGCTAGTGTCTTGA
Sult1a1	NM_133670.1	CACAAGGGTCCTCTCCTTAGC	TGACAGCGGAACGTGAAGTC
Sult1b1	NM_001356943.1	GAGGTGCTCCATCTCAGGTCA	GCAGGCTTCAATCAGAACGA
Sult1c2	NM_026935.4	GGCGAACCATCATTCAACACC	AGGAAACCATGCAGTCTTTAGC
Sult1d1	NM_016771.3	GGGATGCAATCTACAAACGAGT	CCACATCTTTGGCATTCCGTG
Sult1e1	NM_023135.2	ATGGAGACTTCTATGCCTGAGT	ACACAACTTCACTAATCCAGGTG
Sult2a1	NM_001111296.2	CCAGGGTCACTCGGAACTTA	GCTCAAACCATGATCCGAAT
Sult2a7	NM_001184981.1	TTGTTATGAGAGAAGACACAGTCGT	GCAGACAATCTCAACAAGCCAG
Sult2a8	NM_175250.5	GACCTTACTCCATGTAAAAGCCTAT	AGTTGCTGAAGCAGGGTAATTGAG
Sult5a1	NM_020564.3	ATGACTGAGCGCATGAACACC	CCACAAGTGACCCTCACAGA
Sult6b1	NM_001163625.1	AACTTTCCGAGCTTTGGATGC	TTGAACCACACTTCGGGTAGG
Lxra	NM_001177730.1	AGGAGTGTCGACTTCGCAAA	CTCTTCTTGCCGCTTCAGTTT
Pparα	NM_013839.4	AGGCCGTTGCCACTGTTCAG	AGCCCTCTTCATCCCCAAGC
Vdr	NM_009504.4	GCATCCAAAAGGTCATCGGC	AGCGCAACATGATCACCTCA
Ahr	NM_013464.4	AGCCGGTGCAGAAAACAGTAA	AGGCGGTCTAACTCTGTGTTC
Car	NM_009803.5	CCCTGACAGACCCGGAGTTA	GCCGAGACTGTTGTTCCATAAT
Pxr	NM_010936.3	GAGATCTCCATGTGCAGCCA	CTCCAGCTCTCCTCAGGTCT
Nrf2	NM_010902.4	TAGATGACCATGAGTCGCTTGC	GCCAAACTTGCTCCATGTCC
Dec2	NM_024469.2	CCCTCATTTGCAAGAGAGACAG	AGGTATCCTTGGTATCGTCTCG
E4bp4	NM_017373.3	CTTTCAGGACTACCAGACATCCAA	GATGCAACTTCCGGCTACCA
Shp	NM_011850.3	CGATCCTCTTCAACCCAGATG	AGGGCTCCAAGACTTCACACA
Rev-erbα	NM_145434.4	TTTTTCGCCGGAGCATCCAA	ATCTCGGCAAGCATCCGTTG
Lrh-1	NM_030676.3	GAACTGTCCAAAACCAAAAAAGG	CTTCCAGCTTCATCCCAAC
Hnf4α	NM_008261.3	GACCATGGGCAATGACACGTCC	TTGCAGCCGTCACAGCTCGA
Cyclophilin b	NM_011149.2	TCCACACCCTTTTCCGGTCC	CAAAAGGAAGACGACGGAGC

Table S3. The probe substrates, monitored metabolites, and incubation conditions for the measurements of enzymatic activities.

P450 isoform	Substrate	Concentration (μM)	Incubation time (h)	Metabolite
Cyp1a2	Phenacetin	50	2	Acetaminophen
Cyp2a4	Testosterone	100	2	15α-OH Testosterone
Cyp2a5	Coumarin	5	2	7-OH Coumarin
Cyp2b10	Pentoxyresorufin	10	1	resorufin
Cyp2c39	Retinoic Acid	100	2	4-OH Retinoic acid
Cyp2e1	4-Nitrophenol	50	2	4-OH Nitrophenol
Cyp3a11	Testosterone	100	2	6β-OH Testosterone
Cyp4a	Arachidonic acid	100	2	20-HETE

Table S4. Chromatographic and mass spectrometric parameters for quantification of chemicals in this study.

Analyte	**Retention time (min)**	**[M+H]$^+$**	**[M-H]$^-$**	**Analytical column**	**Analytical gradient**
Phenacetin	2.45	180.101		Luna Omega polar C18 1.6 µm, 100 × 2.1 mm	10% B at 0-1.0min, 10-90% B at 1.0-3.5 min, 90% B 3.5min, and 90-10 % B at 4.5-5.0 min.
APAP	2.61	152.070			
APAP-Cys	2.70	271.075			
APAP-NAC	2.72	313.081			
APAP-G	2.10	328.100			
APAP-S	2.30	232.145			
Aflatoxin B1	3.54	313.216			
Coumarin	3.19	147.056			
7-OH Coumarin	2.75	163.053			
Theophyline	2.41	181.245			
1,3-DMU	1.93	197.234			
Mitoxantrone	2.96	445.424			
Testosterone	4.75	289.212			5% B at 0-0.5min, 35-45% B at 0.5-3.5 min, 45-95% B at 3.5-4.0 min, 95% B at 4.0-5.0 min, and 95-5% B at 5.0-
6β-	2.37	305.212			
15α-	2.73	305.212			
Retinoic acid	3.74	301.139		ACE Ultracore 2.5 Supper C18, 50 × 2.1 mm	10% B at 0-1.0min, 10-90% B at 1.0-3.5 min, 90% B 3.5min, and 90-10 % B at 4.5-5.0 min.
4-OH retinoic acid	2.89	317.140			
Arachidonic acid	4.22	303.234			
19/20-HETE	2.89	319.140			
4-Nitrophenol	2.74		138.020	ACE Excel 2 C18-PFP, 50 × 2.1 mm	
4-nitrocatechol	2.65		154.100		
Resorufin					λex = 530 nm, λem = 580 nm

Table S5. Oligonucleotide sequences for EMSA assays.

Oligonucleotide	Forward (5'-3' sequence)
Cyp2a5-E4bp4RE (-927/-901)	CAGTAGGGCTTTTGTAAATTAGAACTT
Cyp2b10-RevRE (-1963/-1937)	ATTATGAGTTCAAAGTCAGCCTGGGAT
Cyp2c38-Lrh-1RE (-1066/-1040)	GTGCATGGTTCAAGGTCAAGAAATTGT
Cyp2c38-Hnf4αRE (-143/-117)	CTATCAAGGAACAAAGTTTATTTGAAT
Cyp4a10-RevRE (-1105/-1078)	AGAGTTCCCTTGGTCATGGTGTTTGCT
Cyp4a14-RevRE (-1715/-1688)	GCTTACCATAATATGGGTCAAACAAAA

Table S6. Primer sequences for ChIP assays.

Gene	Forward (5'-3' sequence)	Reverse (3'-5' sequence)
Cyp2a5_ E4BP4	AATAACAGGAGCAATGAG	GACAACATCTGAGGGTCT
Cyp2a5_Distal	AAAGGCAGATTGAAGTTTAG	TTCCTCCTGATAGTAATGGT
Cyp3a11_Hnf1α	ATCTTGGTGGGTATGTGG	TGTGGCCTTGAAGTCTAA
Cyp3a11_Distal	CCAGGTGGAACAGAAGGG	GCAATGGTGTCAGCGTTT
Cyp2b10_ Rev-erbα	CACATCTGTGGTCCCAGTA	TCCCAGGTGTCAGGATTTCAG
Cyp2b10_ Distal	GAAGTTCTGCTGTGGGTC	GTAACATTACTATCTAGCCTCT
Cyp2c38_Lrh-1	ATTGACATTCTAAGCATTAC	TTCTTGACCTTGAACCAT
Cyp2c38_Hnf4α	GTAGCAATACTTGGACAT	TGTTGAGTAACCCATTAG
Cyp2c38_ Distal	GAATACCAAATGGCAGAG	CACCAACAATGGAGGAGT
Cyp7a1_ Lrh-1/Hnf4α	GCTTATCGACTATTGCAGCTCTCT	CTGGCCTTGAACTAAGTCCATCT
Cyp4a10_Rev-erbα	TGTAAGCCAGGCCCAATT	GGTGCCCATGTATTTCTC
Cyp4a10_ Distal	TGTAAGGGTGGCATAAGG	TAATGGGACATGGTCTGG

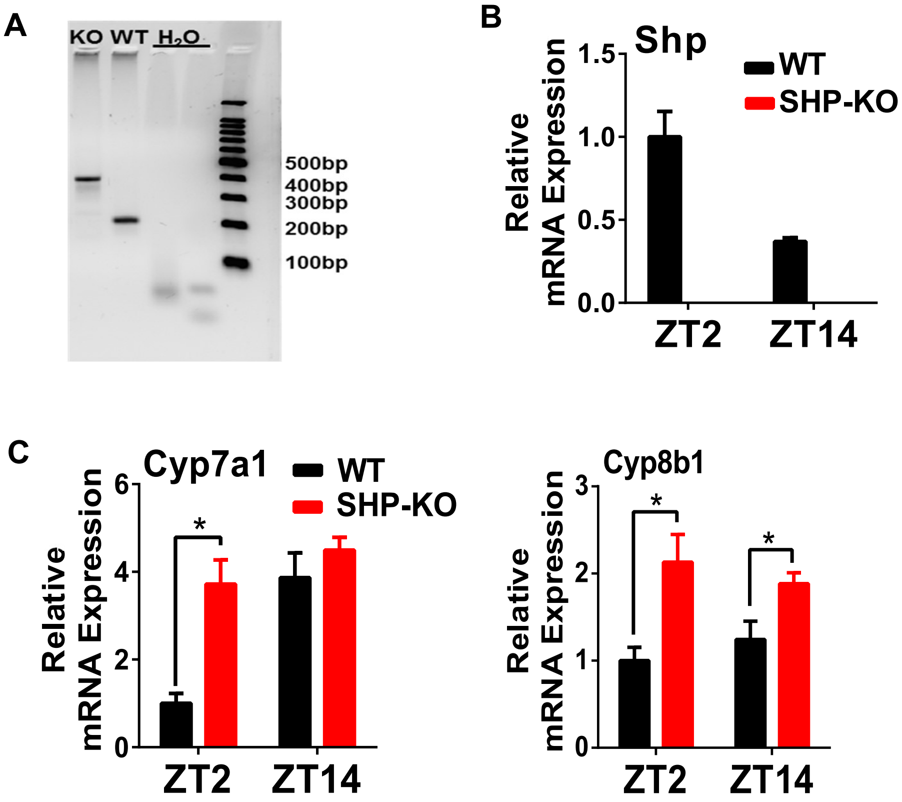

Figure S1. (A) Genotypes of SHP-knockout (SHP-KO) and wild-type (WT) mice were identified using genomic DNAs prepared from mouse tail. Expression of hepatic SHP **(B)**, Cyp7a1 and Cyp8b1**(C)** at ZT2 and ZT14 in WT and SHP-KO mice. mRNA levels were measured by qPCR and normalizede to cyclophilin b. Data are mean ± SD from *n* = 5 mice for each time point, *p < 0.05 versus WT group.

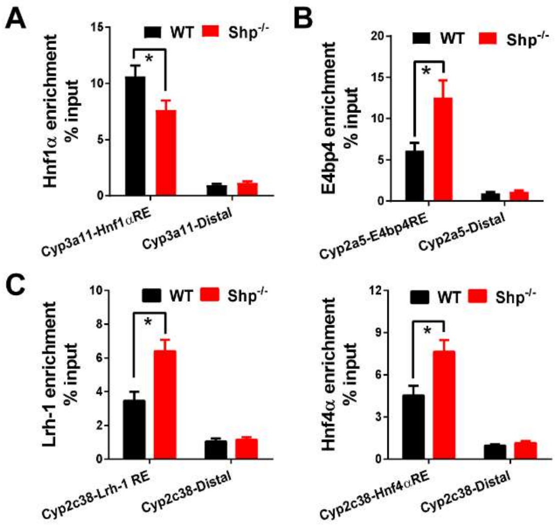

Figure S2. **(A)** ChIP assays showing the relative enrichment of Hnf1α to the promoter region of Cyp3a11 in the livers of WT and SHP$^{-/-}$ mice. **(B)** ChIP assays showing the relative enrichment of E4bp4 to the promoter region of Cyp2a5 in the livers of WT and SHP$^{-/-}$ mice. **(C)** ChIP assays showing the relative enrichment of Lrh-1 and Hnf4α to the promoter region of Cyp2c38 in the livers of WT and SHP$^{-/-}$ mice. Data are presented as mean ± SD (n = 5). *p < 0.05 versus IgG.

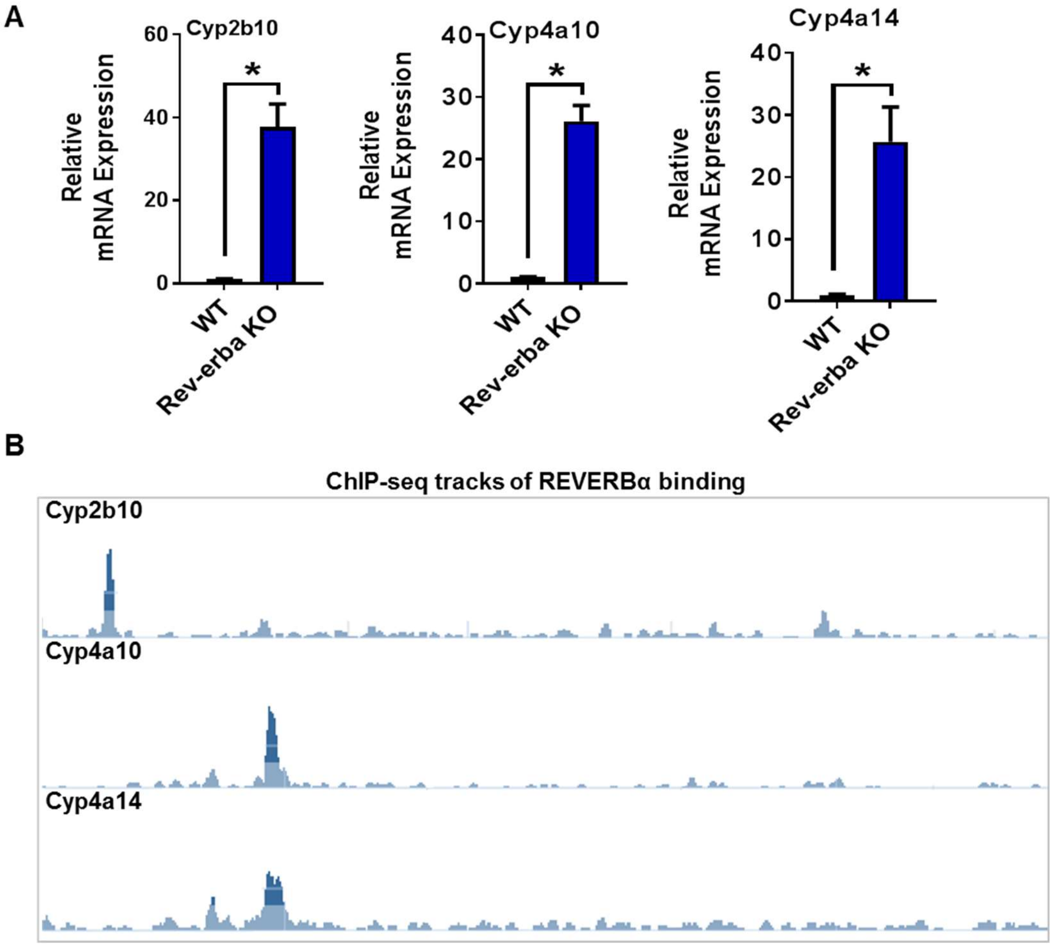

Figure S3. Rev-erbα regulates Cyp2b10, Cyp4a10 and Cyp4a14. (A) Cyp2b10, Cyp4a10 and Cyp4a14 mRNA expression in the livers from WT and Rev-erbα KO mice. **(B)** Representative ChIP-seq tracks of REVERBα binding on Cyp2b10, Cyp4a10 and Cyp4a14 genes. The data were retrieved from the gene expression omnibus database (GSE59486 and GSE67973)

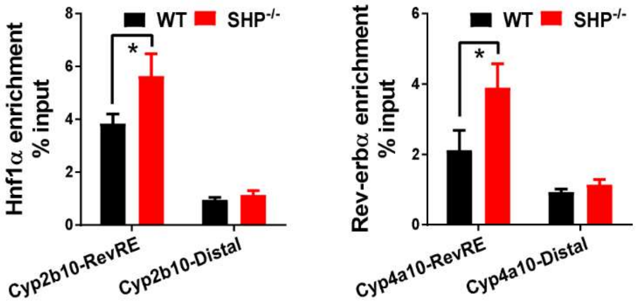

Figure S4. ChIP assays showing the relative enrichment of Rev-erbα to the promoter region of Cyp2b10 and Cyp4a10 in liver of WT and SHP$^{-/-}$ mice. Data are presented as mean ± SD (*n* = 5). $^*p < 0.05$ versus IgG.

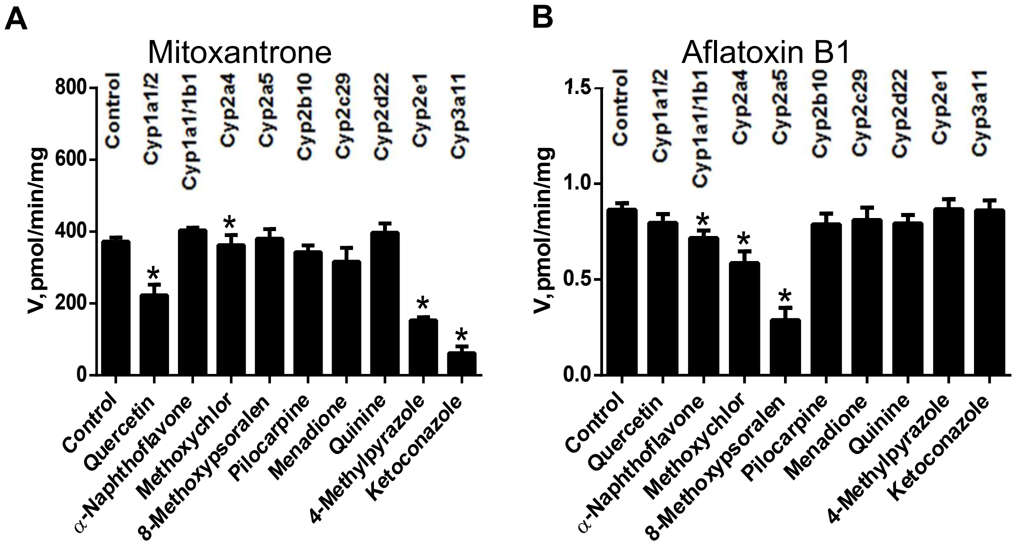

Figure S5. The effects of CYP enzyme inhibitors on mitoxantrone **(A)** and aflatoxin B1 **(B)** metabolism. The metabolic rates of mitoxantrone and aflatoxin B1 were measured after incubation for 2 h with mouse liver microsome (1 mg/ml) at 37℃ with DMSO (control), quercetin (2 µM), a-naphthoflavone (10 µM), methoxychlor (2 µM), 8-methoxypsoralen (5 µM), pilocarpine (10 µM), menadione (10 µM), quinine (10 µM), 4-methylpyrazole (20 µM) , or ketoconazole (2 µM). Data are presented as mean ± SD (*n* = 5). $^*p < 0.05$ versus control.

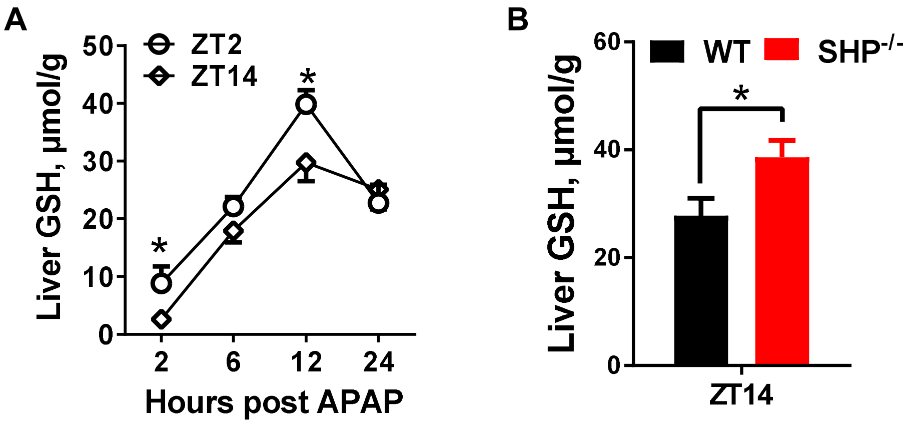

Figure S6. (A) Liver levels of GSH determined at various time points after APAP administration (500 mg/kg, i.p.) at ZT2 and ZT14. Data are presented as mean ± SD (*n* = 5). *p < 0.05 versus ZT14. **(B)** Liver GSH levels in WT and SHP$^{-/-}$ mice 12 h after APAP administration (500 mg/kg, i.p.) at ZT14. Data are presented as mean ± SD (*n* = 5). *p < 0.05.

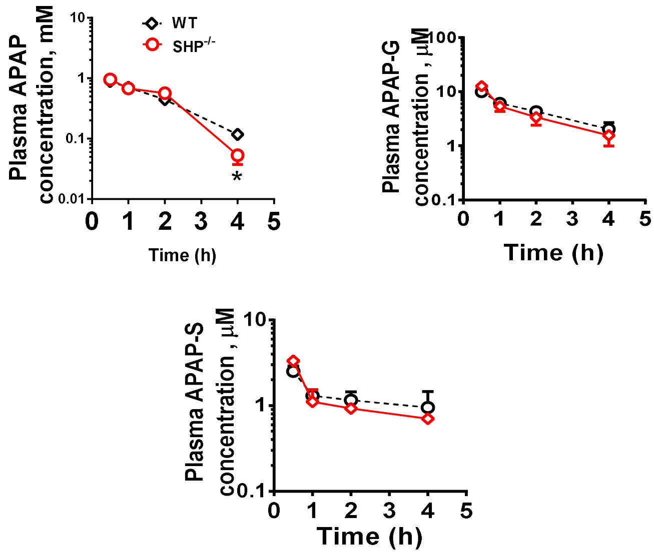

Figure S7. Plasma concentrations of acetaminophen (APAP), acetaminophen-O-glucuronide (APAP-G) and acetaminophen-O-sufate (APAP-S) in WT and SHP$^{-/-}$ mice at 0.5, 1, 2, and 4 h after APAP treatment (500 mg/kg, i.p.). Data are presented as mean ± SD (*n* = 3).

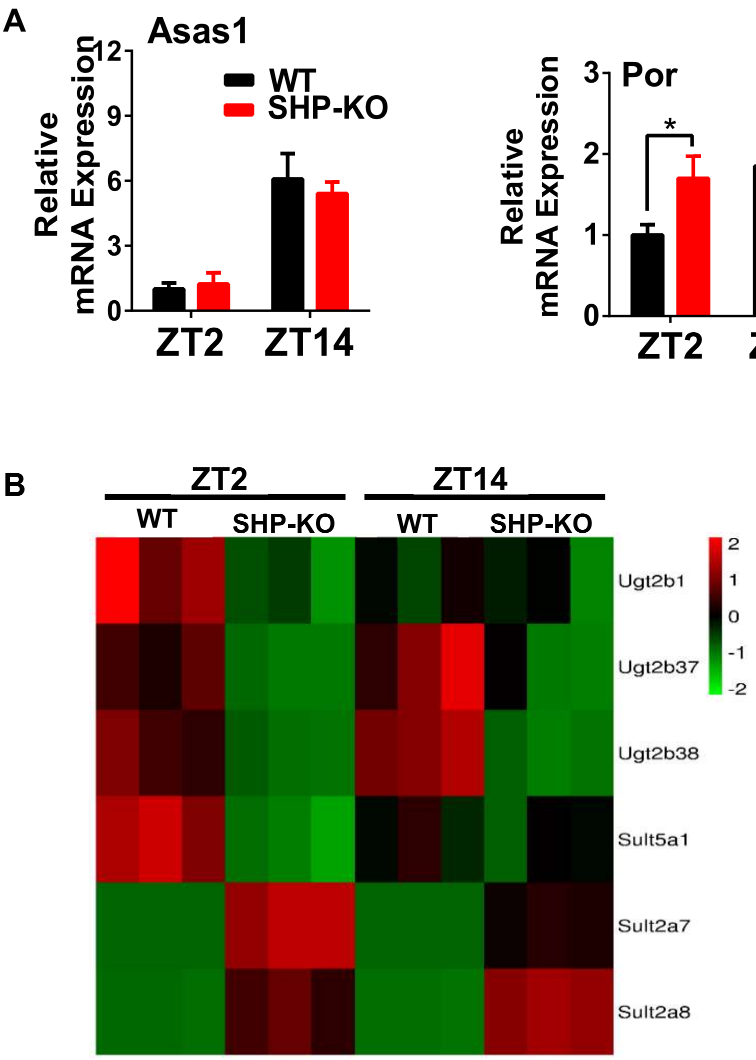

Figure S8. (A) Expression of hepatic Alas1 and Por at ZT2 and ZT14 in WT and SHP-KO mice. **(B)** Heatmap of relative mRNA levels of UGT and SULT at ZT2 and ZT14 in WT and SHP-KO mice (Fold change >2). mRNA levels were measured by qPCR and normalizede to cyclophilin b. Data are mean ± SD from *n* = 5 mice for each time point, *p < 0.05 versus WT group.

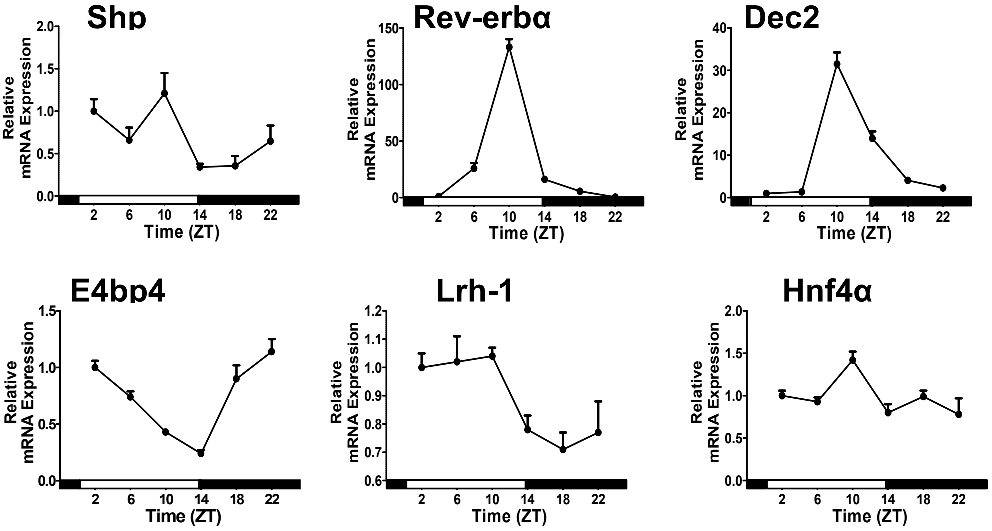

FIGURE S9. Circadian experssion of Shp, Rev-Erbα, Dec2, E4bp4, Lrh-1 and Hnf4α in WT mice. mRNA levels were measured by qPCR and normalizede to *cyclophilin b*. Data are mean ± SD (*n* = 5).

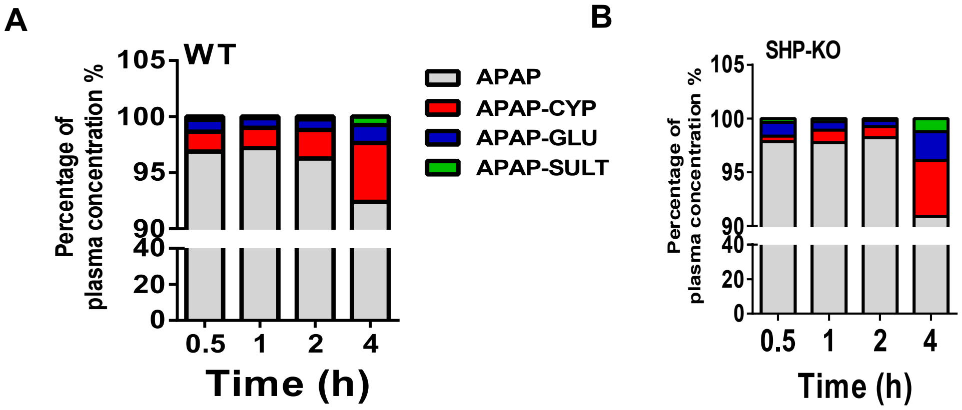

Figure S10. Percentages of APAP, APAP-CYP (APAP-Cys and APAP-NAC), APAP-G and APAP-S in WT **(A)** and SHP-KO mice **(B)** plasma at 0.5, 1, 2, and 4 h after APAP treatment (500 mg/kg, i.p.). APAP, acetaminophen; APAP-S, acetaminophen-O-sulfate; APAP-Cys, 3-cysteinylacetaminophen; APAP-NAC-, 3-N-acetylcysteinylacetaminophen; APAP-G, acetaminophen-O-glucuronide. Data are presented as mean ± SD (*n* = 3).

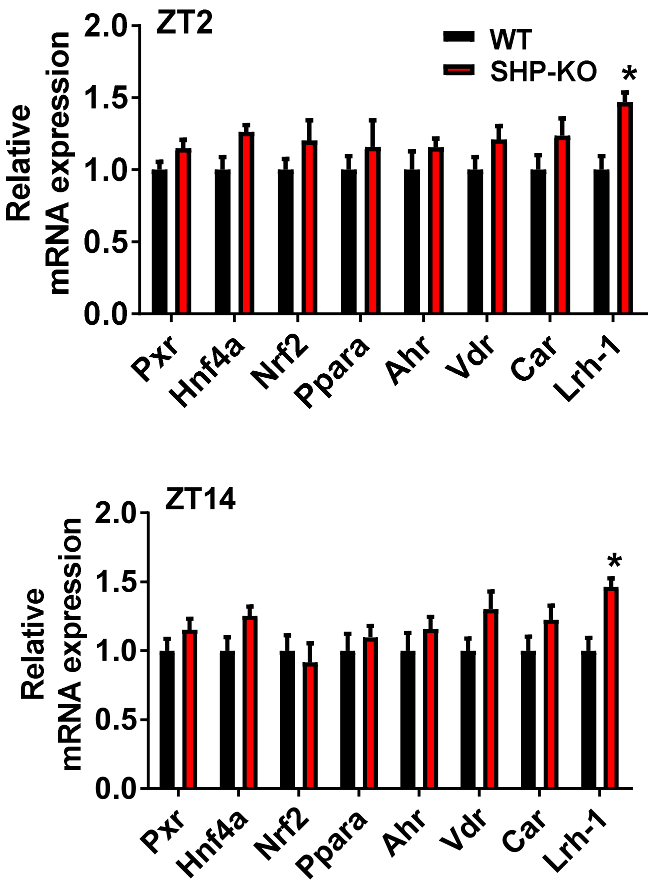

Figure S11. Expression of hepatic nuclear receptors/transcriptional factors involved in xenobiotic metabolism at ZT2 and ZT14 in WT and SHP-KO mice. mRNA levels were measured by qPCR and normalizede to *cyclophilin b*. Data are mean ± SD (*n* = 5). *p < 0.05 versus WT group.

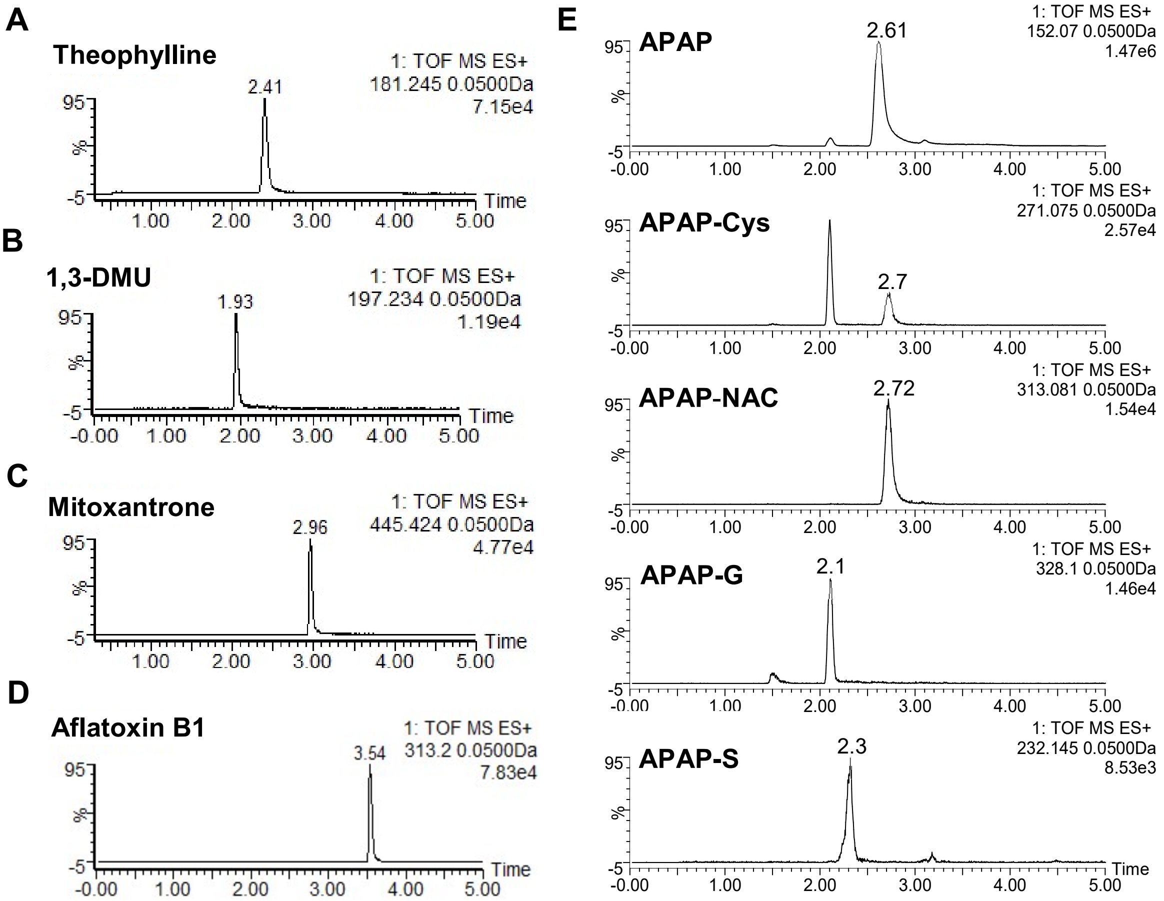

Figure S12. Representative chromatograms for quantification of drugs/xenobiotics and their metabolites. 1,3-DMU, 1,3-dimethyluric acid; APAP, acetaminophen; APAP-S,acetaminophen-O-sulfate; APAP-Cys, 3-cysteinylacetaminophen; APAP-NAC, 3-N-acetylcysteinylacetaminophen; APAP-G, acetaminophen-O-glucuronide.