Supplemental Data

Manuscript title:

The Circadian Clock Gene Bmal1 Controls Intestinal Exporter MRP2 and Drug Disposition

Authors:

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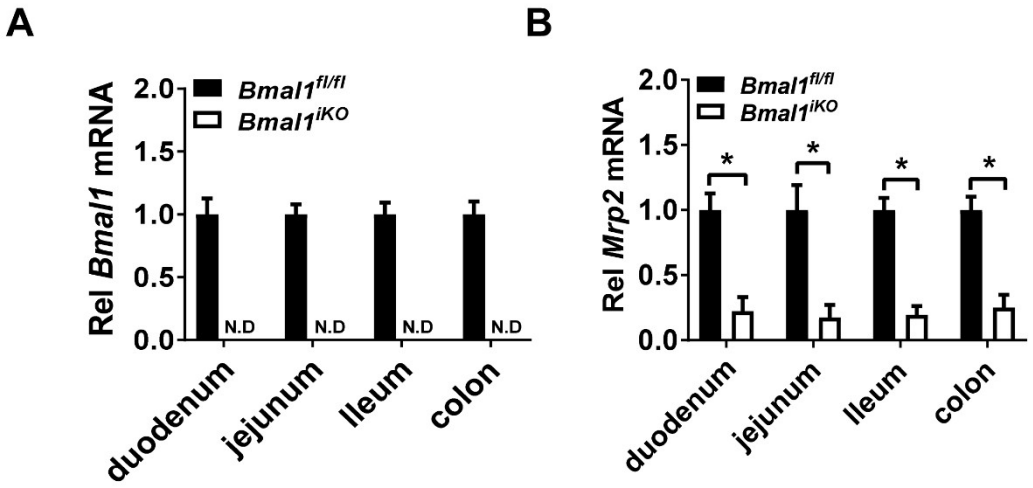
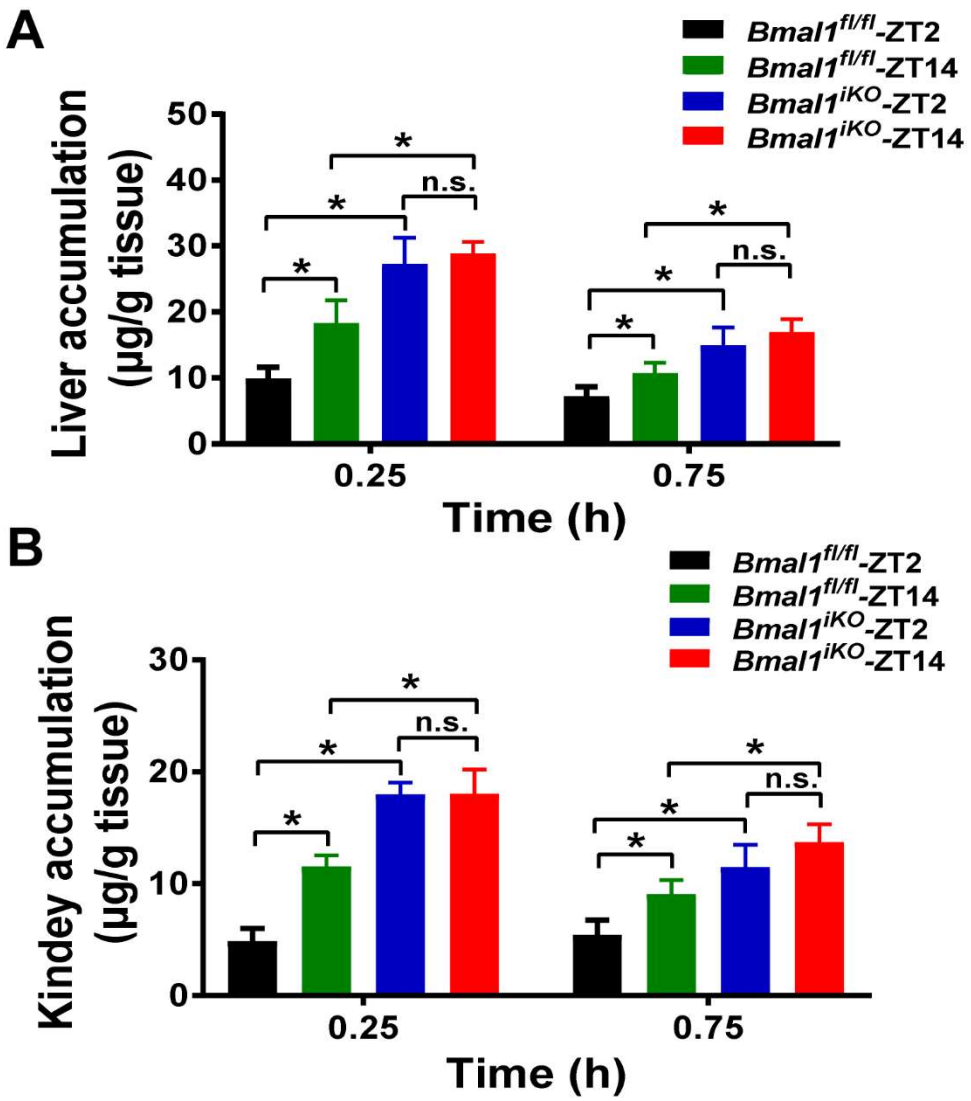
Supplementary Table 1. Oligonucleotides used in this study.

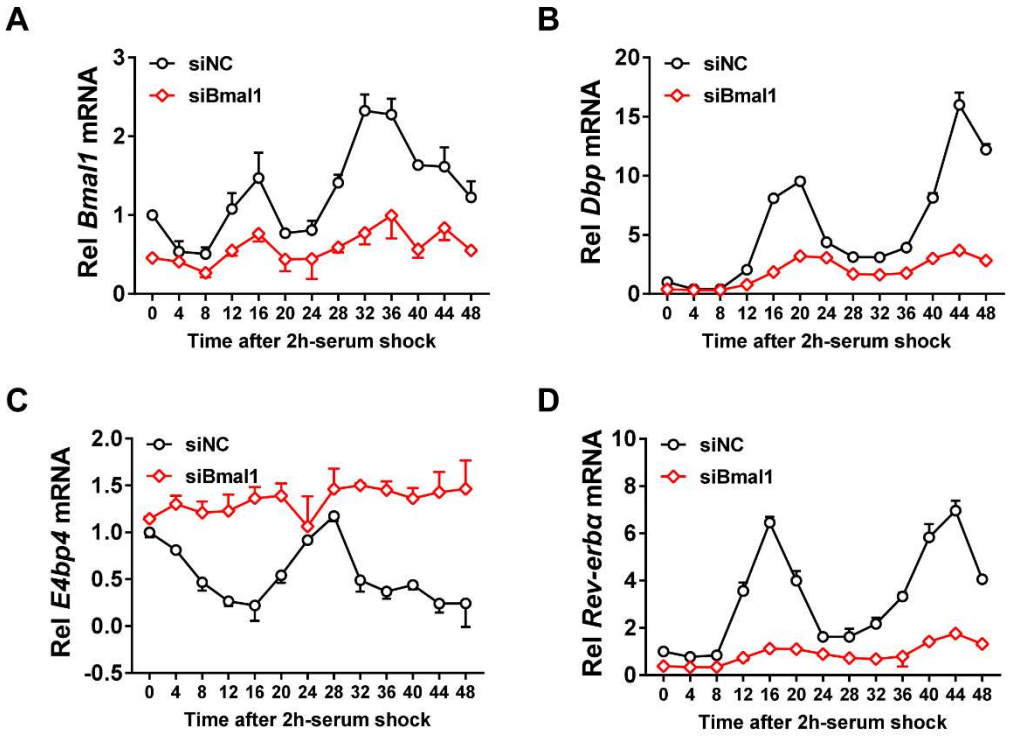
	Forward (5'-3' sequence)	Reverse (3'-5' sequence)
qRT-PCR		
mBmal1	CTCCAGGAGGCAAGAAGATTC	ATAGTCCAGTGGAAGGAATG
mE4bp4	CTTTCAGGACTACCAGACATCCAA	GATGCAACTTCCGGCTACCA
mRev-erbα	TTTTTCGCCGGAGCATCCAA	ATCTCGGCAAGCATCCGTTG
mDbp	ACATCTAGGGACACACCCAGTC	AAGTCTCATGGCCTGGAATG
mHlf	GTGACGACTCCTGCTCCA	CAGCCATACTCCATTCCAAC
mTef	GCATCGCCACCATCTTCCT	GCTGCCACATTGTTCTTCTT
mMrp2	GTGTGGATTCCCTTGGGCTTT	CACAACGAACACCTGCTTGG
mAbcc1	CATGTGGACGTGTTTCGA	CACCATCATCCCTGTAATC
mAbcc3	CTGGGTCCCCTGCATCTAC	GCCGTCTTGAGCCTGGATAAC
mSlc19a1	GCCACAGAACCCTCCTTA	GCATAGTTGAGCCCGACA
mSlc46a1	TCGGGTTCTTCGTGCTTG	GGCTCCTTCACCGTCTCA
mFolr1	CCTGGAATGAAGAAAGCA	AAATACAACCCACCTACTCG
Humbs	CCGAGCCAAGGACCAGGATA	CCGAGCCAAGGACCAGGATA
BMAL1	AAATCGCTTTGAGGTGAC	CTTTCCGTTTGCGGTTGC
MRP2	CCATAGCTTCATTCCTGAGTAGC	TCAGAGGACGCTTGTAGCCTT
GAPDH	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT
EMSA		
Mrp2	CTCACTGGGATGACATAGCATTCATCT	AGATGAATGCTATGTCATCCCAGTGAG
Mrp2(mutant)	CTGACTGGGCTAGCGTCGTATTCATCT	AGATGAATACGACGCTAGCCCAGTCAG
CHIP		
Mrp2	ACTTCCGACCTGCCAACT	CCATCCTCACTGCCCAAT
Mrp2(distal)	ACTTTGCTCTGGGTGTCT	AGGGTTAAGGAAGGGTTT
siRNA		
siE4bp4	GCACAAGCUUCGGAUUAAATT	UUUAAUCCGAAGCUUGUGCTT
siRev-erbα	CUUCGUUGUUCAACGUGAATT	UUCACGUUGAACAACGAAGTT
siBmal1	GCUCUUUCUUCUGUAGAAUTT	AUUCUACAGAAGAAAGAGCTT
siBMAL1	CCGAGGGAAGAUACUCUUUTT	AAAGAGUAUCUUCCCUCGGTT
siNC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT



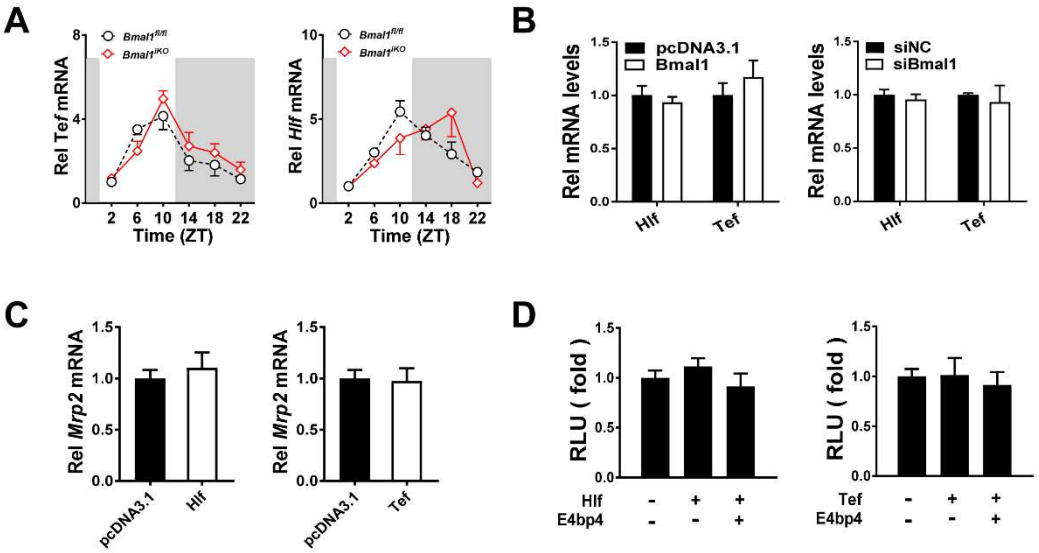
Supplementary Figure 1. (A) qRT-PCR analysis of Bmal1 in the duodenum, jejunum, ileum and colon of *Bmal1*$^{fl/fl}$ and *Bmal1*^{iKO} mice. **(B)** qRT-PCR analysis of Mrp2 in the duodenum, jejunum, ileum and colon of *Bmal1*$^{fl/fl}$ and *Bmal1*^{iKO} mice. Data are mean ± SD (n = 6). Statistics were performed with Student's t test (*$p < 0.05$).



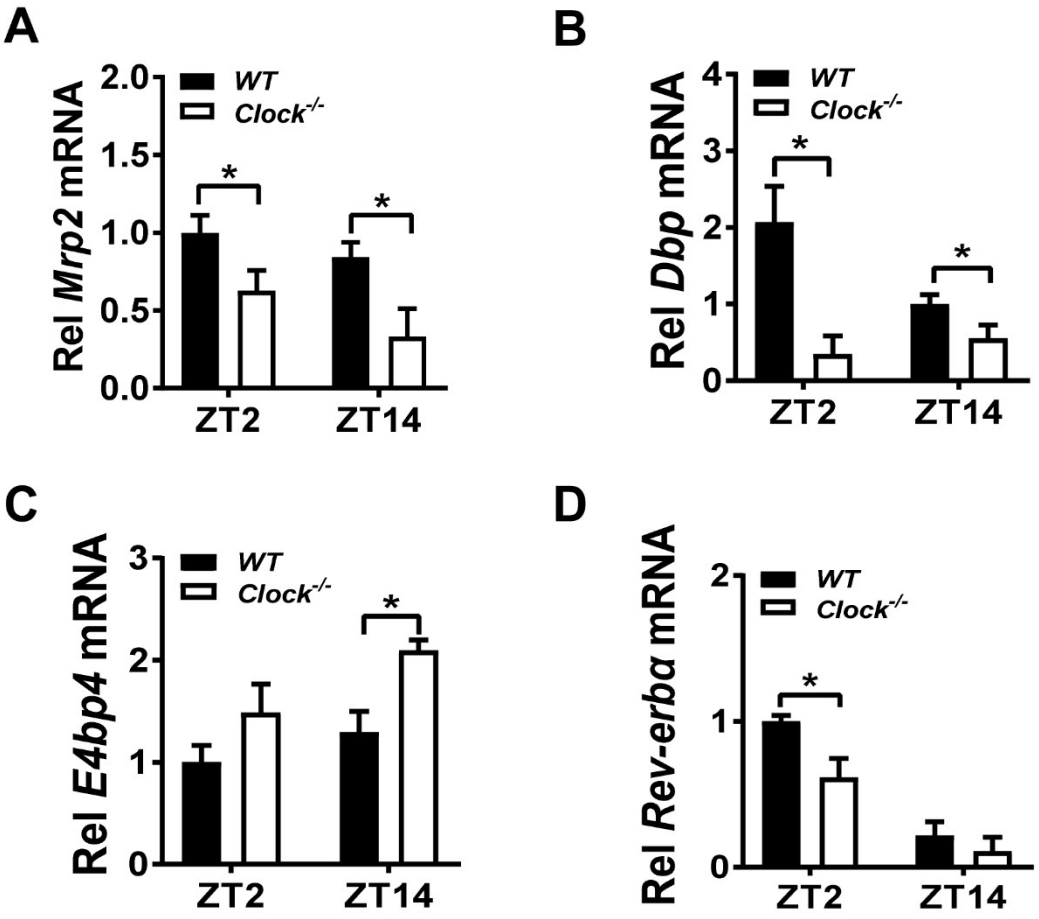
Supplementary Figure 2. (A) The liver accumulation of MTX at 0.25 and 0.75 h post oral administration (50 mg/kg) at dosing time of ZT2 and ZT14 in *Bmal1*$^{fl/fl}$ and *Bmal1*^{iKO} mice. **(B)** The kindey accumulation of MTX at 0.25 and 0.75 h post oral administration (50 mg/kg) at dosing time of ZT2 and ZT14 in *Bmal1*$^{fl/fl}$ and *Bmal1*^{iKO} mice. Data are mean ± SD (n = 5). Statistics were performed with Two-way ANOVA and Bonferroni post hoc test (*$p < 0.05$).



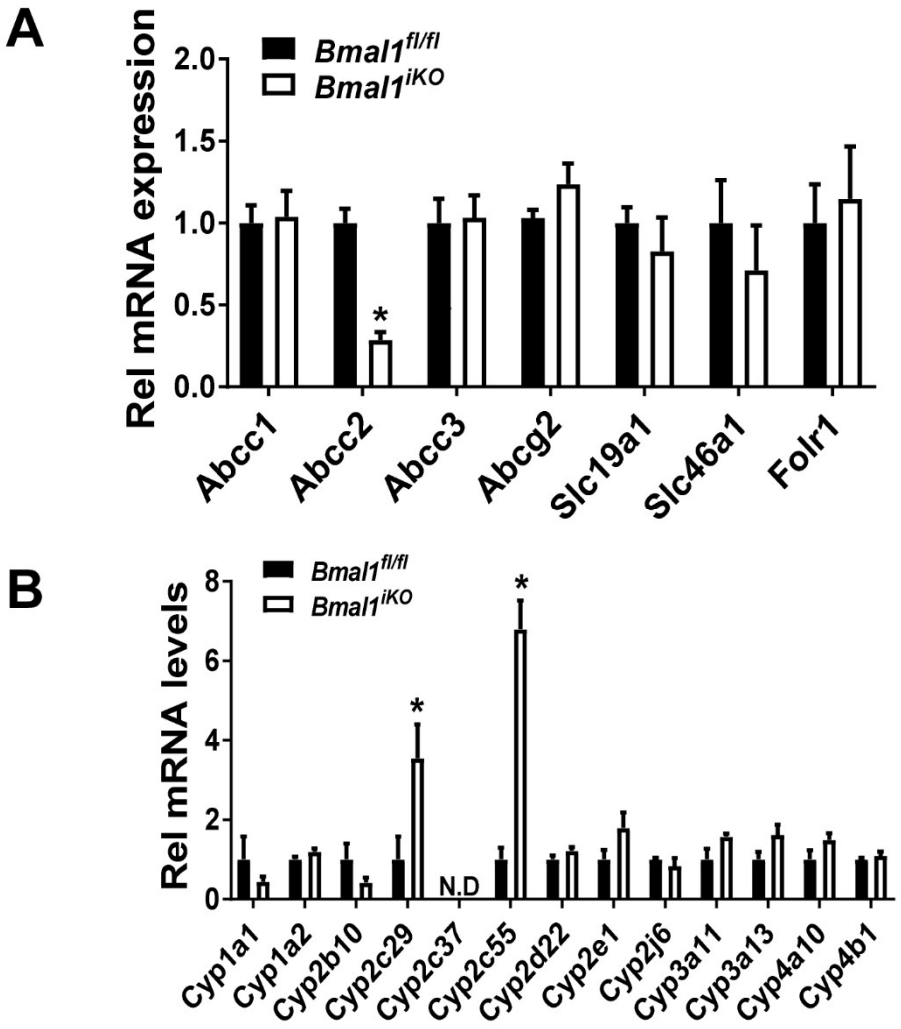
Supplementary Figure 3. (A) qRT-PCR analysis of Bmal1 in serum-shocked CT26 cells treated with siNC or siBmal1. Data are mean ± SD (n = 6). **(B)** qRT-PCR analysis of Dbp in serum-shocked CT26 cells treated with siNC or siBmal1. Data are mean ± SD (n = 6). **(C)** qRT-PCR analysis of E4bp4 in serum-shocked CT26 cells treated with siNC or siBmal1. Data are mean ± SD (n = 6). **(D)** qRT-PCR analysis of Rev-erbα in serum-shocked CT26 cells treated with siNC or siBmal1. Data are mean ± SD (n = 6). Statistics were performed with Two-way ANOVA (*$p < 0.05$).



Supplementary Figure 4. (A) qRT-PCR analysis of *Tef* and *Hlf* mRNA in small intestine of *Bmal1*$^{fl/fl}$ and *Bmal1*^{iKO} mice. **(B)** Overexpression or knockdown of Bmal1 in CT26 cells had no effect on *Tef* and *Hlf* mRNA expression. **(C)** Overexpression of Tef or Hlf in CT26 cells had no effect on *Mrp2* mRNA expression. **(D)** Overexpression of Tef or Hlf had no effect on *Mrp2* promoter activity. NIH3T3 cells were transfected with *Mrp2*-Luc reporter and Tef or Hlf expression plasmids. After 24 h treatment, luciferase reporter activities were measured. Data are mean ± SD (n = 6).



Supplementary Figure 5. (A) Intestinal *Mrp2* mRNA levels in WT and *Clock*$^{-/-}$ mice at ZT2 and ZT14. **(B)** Intestinal *Dbp* mRNA levels in WT and *Clock*$^{-/-}$ mice at ZT2 and ZT14. **(C)** Intestinal *E4bp4* mRNA levels in WT and *Clock*$^{-/-}$ mice at ZT2 and ZT14. **(D)** Intestinal *Rev-erbα* mRNA levels in WT and *Clock*$^{-/-}$ mice at ZT2 and ZT14. Data are mean ± SD (n = 6). Statistics were performed with Two-way ANOVA and Bonferroni post hoc test (*$p < 0.05$).



Supplementary Figure 6. (A) The mRNA levels of transporters involved in intestinal disposition of MTX in small intestine of *Bmal1*$^{fl/fl}$ and *Bmal1*^{iKO} mice. **(B)** Intestinal mRNA levels of CYPs in *Bmal1*$^{fl/fl}$ and *Bmal1*^{iKO} mice. Data are mean ± SD (n = 6). Statistics were performed with Student's t test (*$p < 0.05$).