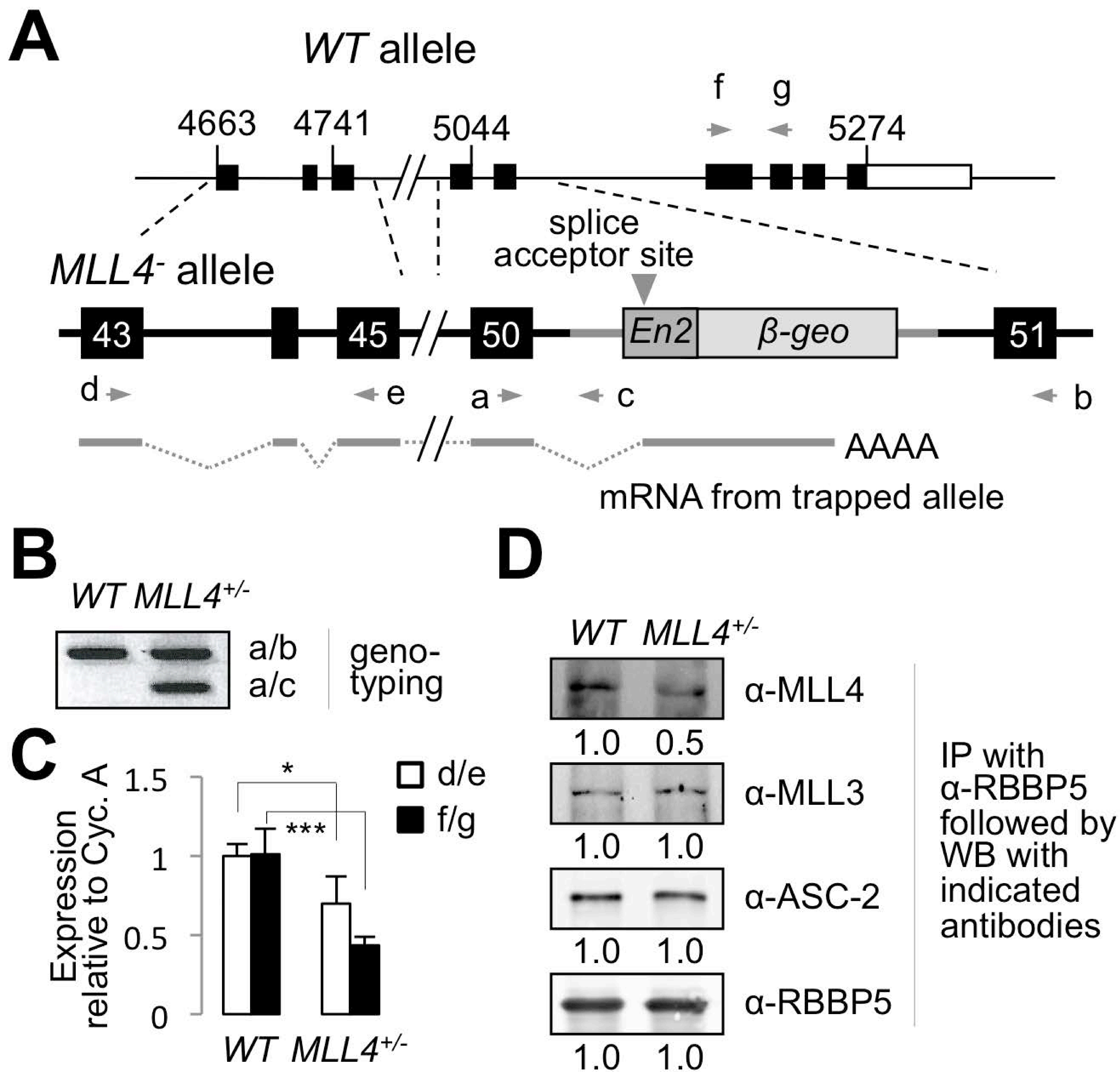
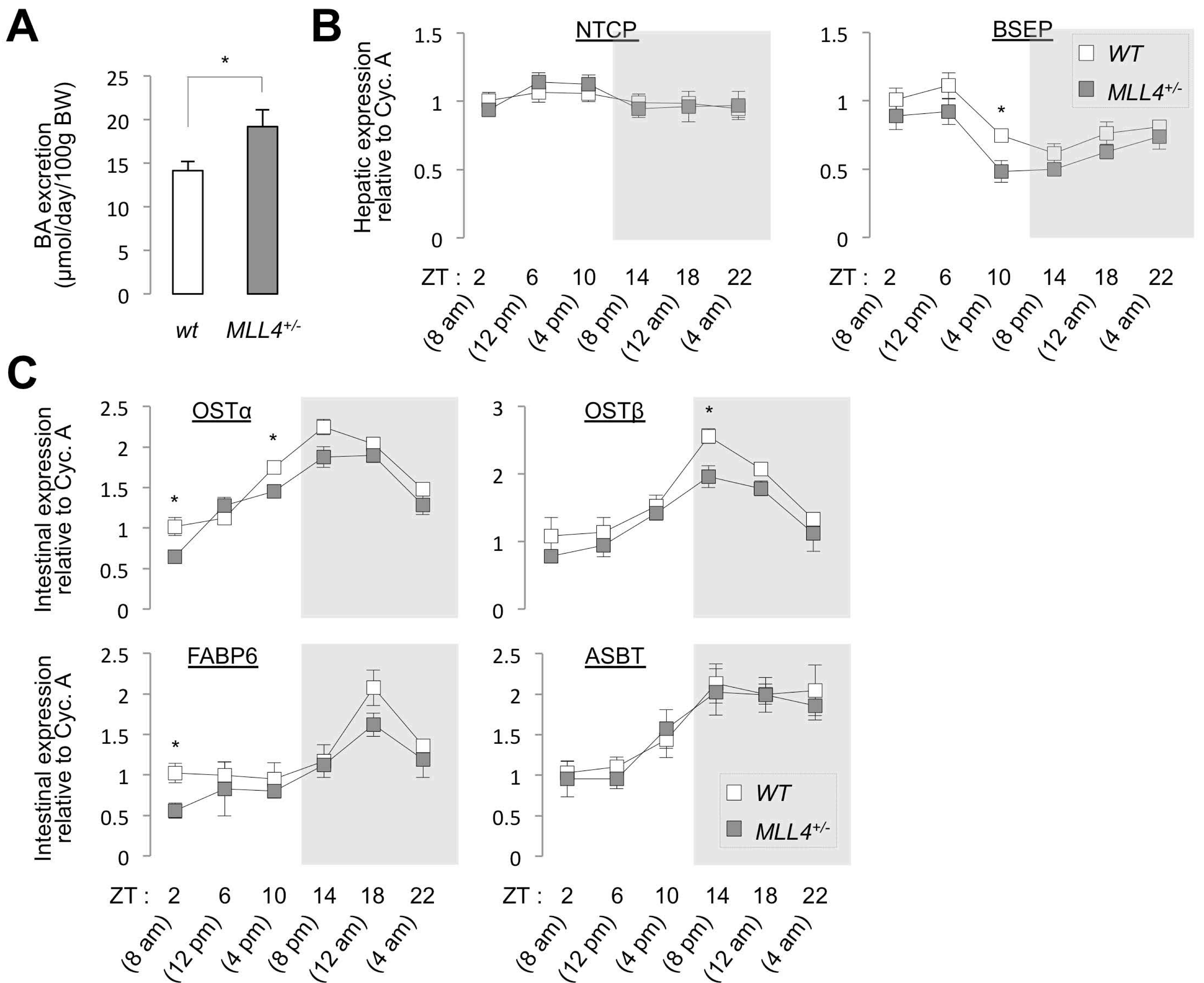


S-Fig. 1. Construction of *MLL4*$^{+/-}$ mice. (A) Schematic representation of *WT* and mutants *MLL4* alleles. (B) Genotyping PCR confirmed the expected targeting of *MLL4* allele. (C, D) Results with qRT-PCR (C) and immunoprecipitation followed by western blotting (D) revealed the instability of the targeted MLL4 mRNA. The *MLL4*$^{-}$ allele appears to produce no MLL4 protein (D).



S-Fig. 2. Roles of MLL4 in BA homeostasis. (A) Measurement of BA excretion in feces in 5-month-old female *WT* and *MLL4*$^{+/-}$ mice (n=8). (B, C) Determination of hepatic mRNA levels of NTCP and BSEP (B) and intestinal mRNA levels of OSTs, FABP6 and ASBT in 5-month-old female *WT* and *MLL4*$^{+/-}$ mice (n=4) throughout the circadian cycle using qRT-PCR. *P* values are for differences between *WT* and *MLL4*$^{+/-}$ mice.

Supplementary Tables

S-Table 1. 726 target genes of MLL3 and/or MLL4 identified in our RNA-seq

S-Table 2. GO analysis of common targets of MLL3 and MLL4

Supplementary Methods

Generation of MLL4$^{+/-}$ mice. *MLL4$^{+/-}$* mice were generated using BayGenomics gene trap embryonic stem cell line RRT024 in collaboration with the MMRRC at University of California, Davis. In the mutant *MLL4^{-}* allele, an *En2-β-geo* gene with a splice acceptor site was inserted between exon 50 and exon 51 of the *MLL4* gene (S-Fig. 1A, 1B). The *MLL4^{-}* allele was designed to produce a truncated form of MLL4 protein at amino acid 5044, lacking the C-terminal region (amino acids 5045-5274) that contains the catalytic SET domain. To examine whether MLL4 mRNA is expressed from the *MLL4^{-}* allele, we performed quantitative RT-PCR (qRT-PCR) in the livers of *MLL4$^{+/-}$* and littermate *WT* mice. When the primers for the region of MLL4 that is no longer transcribed the *MLL4^{-}* allele were used, the levels of MLL4 mRNA in *MLL4$^{+/-}$* livers were decreased to nearly half of those in *WT* livers as expected (see qRT-PCR results with primers f and g, S-Fig. 1B). Interestingly, qRT-PCR analyses with the primers amplifying the MLL4 mRNA region transcribed from the *MLL4^{-}* allele revealed that MLL4 mRNA levels were reduced to 40-70% in *MLL4$^{+/-}$* livers compared to *WT* livers (see qRT-PCR results with primers d and e, Fig. S-1B and data not shown), suggesting that MLL4 mRNA from the *MLL4^{-}* allele is unstable. Next, to determine the level of MLL4 protein in *MLL4$^{+/-}$* livers, we performed co-immunoprecipitation assays with antibodies against RBBP5, a component of MLL3/4-complexes, followed by the immunoblotting assays with antibodies against MLL3, MLL4, ASC-2 and RBBP5. These experiments uncovered that MLL4 protein levels were reduced to approximately half in *MLL4$^{+/-}$* livers relative to *WT* livers, while levels of MLL3, ASC-2 and RBBP5 were comparable between *MLL4$^{+/-}$* and *WT* livers (Fig. 1C). Together, our data establish that the *MLL4* gene trapped allele (i.e. *MLL4^{-}* allele) is an MLL4-null allele that does not express MLL4 protein.

Glucose and Insulin Tolerance Tests. For glucose tolerance test, 18 h fasted mice were injected intraperitoneally with 2 g/kg glucose. Blood samples were taken at multiple time points between 0 to 120 min after injection, and glucose levels in blood collected from the tail vein were measured using a glucometer (One Touch Ultra, Lifescan). For insulin tolerance test, mice were fasted 6 h and injected intraperitoneally with 0.5 units of insulin per kg body weight. Blood glucose levels were then measured at multiple time points between 0 to 90 minutes.

RT/qRT-PCR Analysis. HepG2 cells were transfected with control si-RNA, si-MLL3 or si-MLL4 using calcium phosphate method. Cells were harvested at 48 h after transfection and a second round of transfections was carried out under identical conditions along with additional Bmal1- or RORγ-expression vector. Hepa1c1c7 cells were transfected with flag-tagged RORα or RORγ using Lipofectamine 2000 (Invitrogen). Total RNA from these cell lysates (24 h post-transfection) or mouse liver samples was isolated upon lysis in TRIzol (Invitrogen), and RT- or qRT-PCRs were then performed.

RNA-seq Experiments. Total RNA of liver tissues was isolated using TRIzol (Invitrogen). Then, RNA samples were subjected to DNase (Invitrogen) treatment and the total RNA was purified using RNeasy mini kit (Qiagen). The integrity of the total RNA was verified by running samples on the bioanalyzer. RNA-seq libraries were prepared according to the Illumina TruSeq protocol. The RNA-seq libraries were validated using the bioanalyzer and qRT-PCR, which provides the adaptor concentration for application to the flow cell. Illumina version 3 flow cells were assembled on the Illumina cBot. Illumina HiSeq 2000 was used to sequence the RNA-seq libraries. The base call files were converted to fastq format using Illumina's CASAVA package.

RNA-seq Data Analysis. All the raw reads of 101 bases were filtered and only the passed filtering (PF) reads were kept. Those PF reads were aligned to NCBI37/mm9 genome using Tophat (version 1.3.3) (1) with the guidance of mm9 RefSeq gene annotations. RefSeq annotations in gene transfer format were downloaded from University of California Santa Cruz Genome Browser. To eliminate the artifacts of multihit reads, only the reads mapped to a single unique locus on the genome were kept for further analysis. Some entries in gene transfer format were manually corrected.

The number of reads assigned to each individual transcript was counted by the tools in DEXSeq, an R package (2), with the guidance of RefSeq annotations. The count data were normalized by median. The differentially expressed genes were determined by a negative binomial distribution model in DESeq package (3). Other analysis tools were implemented in Perl and R. Analysis of gene ontology (GO) terms was performed using DAVID (3).

ChIP Analysis. ChIP assays for Hepa1c1c7 cells and mouse livers were performed as described (4). The antibodies used for ChIP assays were α-flag M2 antibody (Sigma) and α-H3K4me3 (ab8580, Abcam). The antibodies against ASC-2, MLL3 and RBBP5 were as described (5). The antibody against MLL4 was a kind gift from Dr. Robert Roeder.

Co-immunoprecipitation (Co-IP). Whole cell lysates of either mouse liver tissues homogenized in lysis buffer consisting of 50mM Tris·HCl (pH 8.0), 150mM NaCl, 1mM DTT, 0.5mM EDTA, 1% Nonidet P-40, 10% glycerol, and protease inhibitor cocktail tablet (Complete EDTA-free, Roche), HEK293 cells transfected with vectors encoding Flag-tagged RORα or RORγ and subsequently incubated with 20 μM of T0901317 (Sigma) for 24 h, HEK293 cells transfected with vectors encoding Flag-tagged RORα and either HA-tagged ASC-2-DN1 or ASC-2-DN1-m2, or WT and ASC-2-null mouse embryo fibroblast cells transfected with Flag-tagged RORα-expression vector were subjected to immunoprecipitation (IP) experiments with indicated antibodies, followed by Western blotting (WB) analyses for co-immunopurified proteins, which were visualized using the Odyssey System (LI-COR).

Primers. The following primers were utilized in this study.
The sequences of the primers a-g, which were used for genomic PCR and RT-PCR for *WT* and mutant *MLL4* loci (Fig. 1):
a) CGAAGAACTCTTTGGGCTGACAGTG
b) ATCTGCATCTCAAACCCTCAGAAGG
c) CGCTCTTACCAAAGGGCAAACC
d) GTGCAGCAGAAGATGGTGAA
e) GCACAATGCTGTCAGGAGAA
f) GCACCGAGTGGAAGAACAAT
g) TAAATACCGCGGTTCTGCTC

The RT/qRT-PCR primers for mouse and human GADPH:
CACCATCTTCCAGGAGCGAGA
GCTAAGCAGTTGGTGGTGCA

The RT/qRT-PCR primers for human Rev-erbα:
CAAGGCTGTCCCACCTACTT
ACGACGAGGAAGATGAGGAA

The RT/qRT-PCR primers for mouse Cyclophilin A:
GTCTCCTTCGAGCTGTTTGC
GATGCCAGGACCTGTATGCT

The RT/qRT-PCR primers for mouse Clock:
CACTCTCACAGCCCCACTGTAC
CCCCACAAGCTACAGGAGCAGT

The RT/qRT-PCR primers for mouse Bmal1:
TGACCCTCATGGAAGGTTAGAA
GGACATTGCATTGCATGTTGG

The RT/qRT-PCR primers for mouse Cry1:
CACTGGTTCCGAAAGGGACTC

CTGAAGCAAAAATCGCCACCT

The RT/qRT-PCR primers for mouse Per2:
GAAAGCTGTCACCACCATAGAA
AACTCGCACTTCCTTTTCAGG

The RT/qRT-PCR primers for mouse Rev-erbα:
CATGGTGCTACTGTGTAAGGTGTGT
CACAGGCGTGCACTCCATAG

The RT/qRT-PCR primers for mouse Rev-erbβ:
GGAGTTCATGCTTGTGAAGGCTGT
CAGACACTTCTTAAAGCGGCACTG

The RT/qRT-PCR primers for mouse ASC-2:
GAAGTCCAAAACCCTGACGA
TTCCAGCTCGCTTACTTGGA

The RT/qRT-PCR primers for mouse MLL3:
GCAACCTCTTACCGGTTGAA
GTTCTCTCGGGAACCCTTGT

The RT/qRT-PCR primers for mouse UTX:
CTGAAGGGAAAGTGGAGTCTG
TCGACATAAAGCACCTCCTG

The RT/qRT-PCR primers for mouse CYP7A1:
GGTCTCTGAACTGATCCGTCTACG
AGTCCTCCTTAGCTGTCCGGATATT

The RT/qRT-PCR primers for mouse SHP:
ACGATCCTCTTCAACCCAGA
TGATAGGGCGGAAGAAGAGA

The RT/qRT-PCR primers for mouse FGF15:
GCTGGTCCCTATGTCTCCAA
CAGTCCATTTCCTCCCTGAA

The RT/qRT-PCR primers for mouse NTCP:
CAAACCTCAGAAGGACCAAACA
GTAGGAGGATTATTCCCGTTGTG

The RT/qRT-PCR primers for mouse BSEP:
ATGGAAATCGGATGGTTTGACTG
CCCTAAAAGGAGCCCAGACAA

The RT/qRT-PCR primers for mouse OSTα:
AGGCAGGACTCATATCAAACTTG
TGAGGGCTATGTCCACTGGG

The RT/qRT-PCR primers for mouse OSTβ:
AGATGCGGCTCCTTGGAATTA

TGGCTGCTTCTTTCGATTTCTG

The RT/qRT-PCR primers for mouse FABP6:
CTTCCAGGAGACGTGATTGAAA
CCTCCGAAGTCTGGTGATAGTTG

The RT/qRT-PCR primers for mouse ASBT:
GTCTGTCCCCCAAATGCAACT
CACCCCATAGAAAACATCACCA

The ChIP primers for mouse *Bmal1*-RORE:
GGATTGGTCGGAAAGTAGGTTAG
GGTAAACAGGCACCTCCGTC

The ChIP primers for mouse *Rev-erbα*-RORE:
GTAGACTACAAATCCCAACAATCCTG
TGGAGCAGGTACCATGTGATTC

siRNA sequences.
The sequences for si-MLL3: GCUUAUCUUCCUGUCCAGU
The sequences for si-MLL4: UCGCAUGCGUUGCCCCAAU

References

1. Trapnell C, Pachter L, Salzberg SL. TopHat: Discovering splice junctions with RNA-Seq. Bioinformatics 2009;25:1105-1111.
2. Anders S, Rejes A. DEXSeq: Inference of differential exon usage in RNA-Seq. 2010;R package version 1.0.2.
3. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol 2010;11:R106.
4. Lee S, Lee B, Joshi K, Pfaff SL, Lee JW, Lee SK. A regulatory network to segregate the identity of neuronal subtypes. Dev Cell 2008;14:877-889.
5. Lee S, Lee DK, Dou Y, Lee J, Lee B, Kwak E, et al. Coactivator as a target gene specificity determinant for histone H3 lysine 4 methyltransferases. Proc Natl Acad Sci USA 2006;103:15392-15397.