Supporting Information

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SI Materials and Methods

Cloning of Mouse *Hiomt* cDNA. The progress of the cloning and the locations of the primers used are shown in Fig. S1. Based on the Celera assembly sequences (NW_001038117.1 and NW_001030776.1) and rat *Hiomt* cDNA (L78306), we designed several PCR primers to obtain a partial sequence of mouse *Hiomt* from the C3H pineal RNA, which was reverse transcribed using SuperScript III reverse transcriptase (Invitrogen) and oligo(dT) primer. A primer pair (F1, 5′-CAG GGA AGG GCG GAR CCA GTA-3′ and R1, 5′-AGY RAC AGC AGS AGC GWC CGC-3′) successfully amplified a fragment of mouse *Hiomt* cDNA. We used several thermostable DNA polymerases in the PCR, and *LA Taq* (Takara Bio) gave the best result in the PCR and was used in all of the subsequent experiments. Next, 5′ RACE was performed according to cRACE method (1) with mouse *Hiomt*-specific primers (F2, 5′-TTC AGG GTC ATC TGC GAC CTC GG-3′ and R2, 5′-CAC AGG CTC CAG GTC TCC TGC-3′) using R3 (5′-AAG AAG TCG CCT GAC AGG AAG-3′)-primed C3H pineal RNA as a template. Because the cRACE product did not contain the start codon of *Hiomt*, we again performed cRACE with new primers (F2, 5′-TTC AGG GTC ATC TGC GAC CTC GG-3′ and R4, 5′-CTG GGA GGC CAT GAA GCC GTG G-3′), which were designed from the sequence determined by the first cRACE. We obtained a fragment containing a sequence upstream of the start codon that was deduced from the rat *Hiomt*. Finally, the full-length coding sequence of mouse *Hiomt* was isolated by RT-PCR using the following primer pair: F3, 5′-TCA GGC TGA GCA GCT CGC GTC-3′ and R5, 5′-CTC ACA GTC TGT GAC CCT ACT GC-3′.

Cloning of Mouse *Hiomt* Gene. Mouse *Hiomt* gene was amplified by PCR from genomic DNA in three overlapping fragments and subjected to direct sequencing. Fragment A, from exon 1 to exon 4, was amplified using the following primer pair: AF, 5′-AGG CTS AGT AKC TCG CGT CCC ACG ATG-3′ and AR, 5′-ACC TGT AGA TGG CGG TGA AGG-3′; and sequenced using S1, AGG CTG AGC AGC TCG CGT CCC ACG ATG CAC; S2 (= R4); S3, 5′-CGC GCT CCG CGT GTT CGA C-3′; S4, 5′-CCC TGC GTC TCC SCA GCA G-3′; S5, 5′-CCA ACT CCC CCC TGG CGT CCA C-3′; and S6, 5′-CCG TCC GCC AGG TGC CCC CAG C-3′. Fragment B, from exon 3 to exon 6, was amplified using the following primer pair: BF (= S5) and BR, 5′-GTC TCG AAC ACG GTG ACC TCG-3′; and sequenced using S7, 5′-CCC GTG ACC TTT GAC CTT C-3′; S8, 5′-GGT CGG AGG CCG AGC GCC TGC-3′; and S9, 5′-CCA CCG AGG TCG CAG ATG ACC-3′. Fragment C, from intron 5 to exon 8, was amplified using the following primer pair: CF, 5′-CAA GCC CTC AGG GTT CAG GA-3′ and CR, 5′-AGC CCC ACG CCC CRC TTC CTG CCT CA-3′; and sequenced using S10, 5′-GCA AAG TGA ACG CCG ATG TG-3′; S11, 5′-CCC CAG GCG ACT TCT TC-3′; S12, 5′-CGC CGC AGC AGC TCC ACG CAG-3′; S13, 5′-GTG CTG CTG GTG GAG AGC-3′; and S14, 5′-GGC CAT CAT GGC GTG GTA GGG-3′.

Direct Sequencing of *Hiomt* cDNA and Gene. A PCR product, which was amplified by *LA Taq* with GC buffer I (Takara Bio), was treated with ExoSAP-IT (Affymetrix). After heat inactivation, the sample was used in the fluorescence dideoxy terminator cycle sequencing reaction (BigDye Terminator Cycle Sequencing FS Ready Reaction Kit v3.1; Applied Biosystems) and analyzed by ABI 3730*xl* DNA sequencer. Because of the high GC content of mouse *Hiomt*, we routinely added DMSO (final concentration, 7.5%) and a higher concentration of a primer (5 μM) in the sequencing reaction, and we used a high denaturing temperature (98 °C for 10 s) of each cycle. If a clear sequencing result was still not obtained, we used dGTP BigDye Terminator v3.0 Kit (Applied Biosystems) or a 1:1 mixture of the two reagents.

Transient Expression of Mouse HIOMT. Silent mutations were introduced by site-directed mutagenesis into the C3H-type mouse *Hiomt* cDNA for easier handling (ggg ctg ctg cgg aga cgc agg ggg gcg ggg cct cgc ggc ccc gcc → ggT ctg ctg cgT aga cgc agA ggT gcT ggA cct cgT ggT ccA gcT; corresponding to the region of 75–89 of the encoded protein) before insertion of the cDNA into a mammalian expression vector pcDNA3.1 (Invitrogen). R78G and R242C mutations were subsequently introduced. These constructs were transfected into HEK293 cells using Lipofectamine LTX and PLUS reagent (Invitrogen). HEK293 cells were cultured in DMEM/F12 medium (Sigma-Aldrich) containing 10% FBS and penicillin/streptomycin. Cells were harvested 48 h after transfection.

HIOMT Enzyme Assay. HEK293 cells plated on a 10-cm dish were transfected with the C3H-type mouse *Hiomt* cDNA as described above. Cells were harvested 48 h after transfection and homogenized with a glass–Teflon homogenizer in 1,300 μL of ice-cold 50 mM sodium phosphate (pH 7.9). After centrifugation at 18,000 × *g* for 20 min at 4 °C, the supernatant (50 μL) was mixed with NAS (final concentration, 0.5 mM), SAM (0.1 mM), and [^{3}H]SAM (0.5 μCi) in 50 mM sodium phosphate (pH 7.9). SAH (final concentration, 0.1 mM) was added as a potential inhibitor of mouse HIOMT. After incubation at 37 °C for 30 min, the reaction (200 μL) was stopped by addition with 200 μL of 50 mM sodium borate (pH 10.0). Melatonin was extracted with water-saturated chloroform (1 mL), and radioactivity was counted after washing with the borate buffer. NAS-independent, background radioactivity was determined by the reactions without NAS. Protein content was determined by the Bradford method using BSA as a standard.

Generation of Mouse HIOMT Antiserum. A heptadecapeptide (CTSQTGSGTGSEVGAQD) corresponding to the C-terminal part of mouse HIOMT was synthesized, purified using HPLC, and conjugated to keyhole limpet hemocyanin (KLH). Rabbits were immunized with the KLH-conjugated peptide emulsified in complete Freund's adjuvant. Antiserum was obtained 1 week after the fourth boosting with the same antigen. For immunoblot analysis, the antiserum was diluted with 4% skim milk in PBS at 1:400 and incubated with a blot overnight at 4 °C.

Melatonin Assay. Mice were deeply anesthetized with diethyl ether in a very dim red light (at night), and blood was collected from the inferior vena cava followed by sampling of the pineal gland in dim red light (at night). Plasma was separated from the blood treated with EDTA-2Na by centrifugation. Melatonin was extracted from the plasma by using a C18 column (Sep-Pak Vac 1cc C18 cartridge; Waters). The plasma sample (250 μL) was applied to the column equilibrated with distilled water and passed through the column by centrifugation at 200 × *g* for 1 min. After washing the column with 10% methanol (1 mL) twice, melatonin was extracted with methanol (1 mL). The extracted melatonin was reconstituted with PBS (250 μL), after methanol was evaporated under N_2 gas. Pineal gland was homogenized in PBS (1 mL) with a glass–Teflon homogenizer, and the homogenate was directly assayed. Both plasma and pineal melatonin contents were as-

sayed by ELISA (Direct Saliva Melatonin ELISA kit; Bühlmann Laboratories) according to manufacturer's instructions.

Quantitative RT-PCR. Pineal glands were collected in TRIzol reagent (Invitrogen). Total RNA was extracted according to the manufacturer's instructions, when two or more glands were used. When RNA was extracted from one pineal gland, we used the PureLink RNA Micro kit (Invitrogen). In every case, RNA was treated with DNase I following to RT using SuperScript III reverse transcriptase (Invitrogen) and oligo(dT) primer. Mouse *Aanat*, *Hiomt*, and *Actb* mRNA levels were assayed by real-time PCR technology with Power SYBR Green PCR Master Mix (Applied Biosystems). The relative level of each mRNA was calculated by 2^{-Ct} (Ct indicating the cycle number at which the signal reached the threshold). Each Ct value used for these calculations was the mean of quadruplicate of the same reaction. The following primers were used: *Aanat*, 5′-GTC ACT GGG CTG GTT TGA GG-3′ and 5′-CTC CGG GCC TGT GTA GTG TC-3′; *Hiomt*, 5′-GCA GCC TCC TGC TCT ACC TG-3′ and 5′-ACC TGT AGA TGG CGG TGA AGG-3′; and *Actb*, 5′-CGY GGC TAC AGC TTC ACC AC-3′ and 5′-AGC TCR TAG CTC TTC TCC AG-3′. An external control standard curve was determined by PCR with serial dilutions of a template plasmid containing mouse (MSM) *Aanat*, *Hiomt*, or *Actb* cDNA (ranging from 3 to 10^6 molecules in a single well of a 384-well plate).

Recording of Circadian Behaviors. Wheel-running activity was measured as previously described (2). Mice were individually housed in cages (24 cm × 11 cm × 14 cm high) equipped with a steel wheel (5 cm wide × 14 cm in diameter). Wheel-running activity (three counts per rotation) was monitored by an online PC computer system (O'Hara & Co.). Light/dark cycles were controlled by an automatic timer, and light was provided by a white fluorescent tube and the light intensity was 30–50 lx at the level of the mouse's eyes in the cage. Food and water were available ad libitum at all time.

1. Maruyama IN, Rakow TL (1995) Maruyama HI. cRACE: A simple method for identification of the 5′ end of mRNAs. *Nucleic Acids Res* 23:3796–3797.

2. Kasahara T, et al. (2006) Mice with neuron-specific accumulation of mitochondrial DNA mutations show mood disorder-like phenotypes. *Mol Psychiatry* 11:577–593.

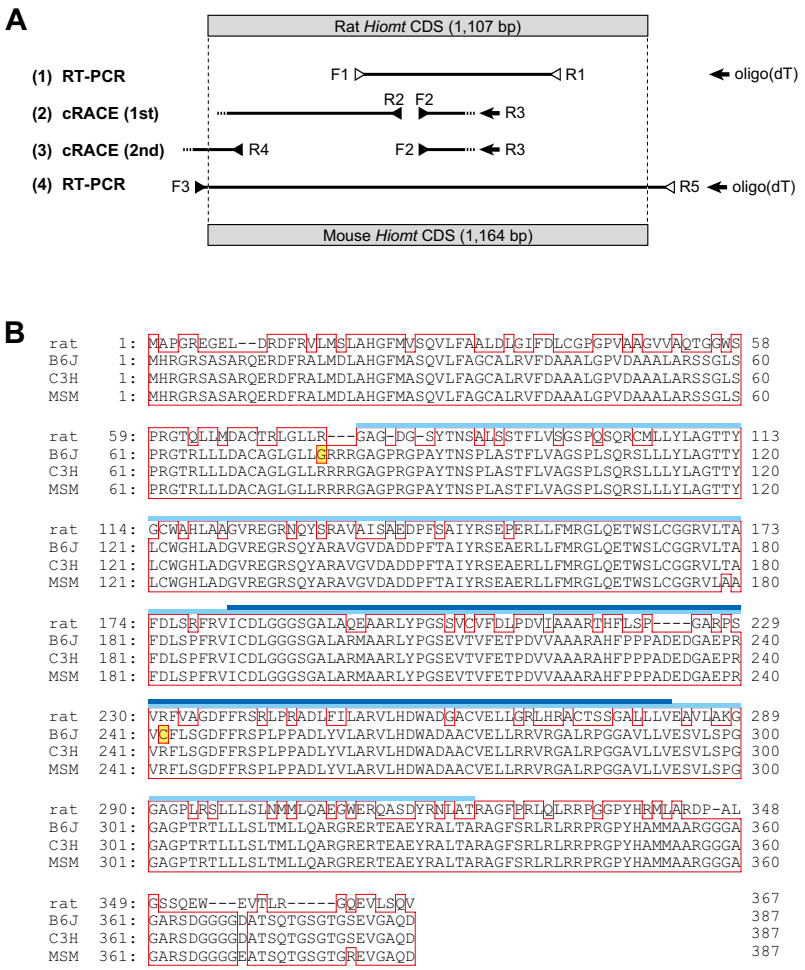

Fig. S1. Cloning of mouse cDNA. (*A*) Strategy. We cloned the mouse cDNA from C3H pineal RNA by using a PCR-based strategy. (Row 1) Degenerate primers (open triangles) designed based on the rat Hiomt sequence (L78306) and Celera's data (NW_001038117 and NW_001030776) were used to amplify a part of mouse *Hiomt* cDNA from oligo(dT)–primed RNA. (Rows 2 and 3) cRACE was performed using mouse *Hiomt*-specific primers (closed triangles) to amplify unknown 5′ regions of *Hiomt* cDNA. (Row 4) The entire CDS of mouse *Hiomt* was obtained using a mouse *Hiomt*-specific primer and a degenerate one. (*B*) Alignment of HIOMT sequences of rat and three mouse strains, B6J, C3H, and MSM. Gaps (–) are introduced in the sequences to optimize alignment. Amino acid residues conserved among more than three are boxed in red, and unconserved residues of B6J are indicated in yellow. The domain conserved among *S*-adenosylmethionine (SAM)–dependent methyltransferases and the amino acid sequence highly conserved among vertebrate HIOMT proteins are indicated by blue and cyan lines, respectively. R242C is located in the functional domain of SAM-dependent methyltransferases, and R78G is at the region with a relatively weak structural organization.

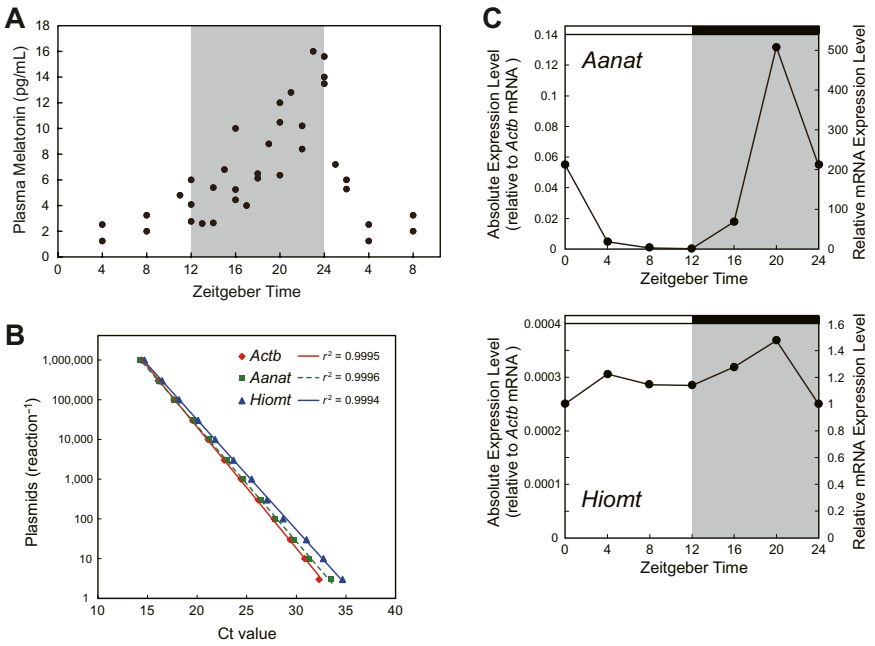

Fig. S2. Diurnal variation in plasma melatonin and pineal *Aanat* and *Hiomt* mRNA levels of MSM mice. (*A*) Plasma samples were collected from MSM mice maintained under LD12:12, and melatonin levels were assayed by ELISA. Light and dark periods are indicated by white and gray backgrounds, respectively. (*B*) A standard curve showing the correlation between PCR cycle threshold (Ct) values and the absolute amounts of plasmid containing mouse *Actb*, *Aanat*, or *Hiomt* cDNA. (*C*) Diurnal *Aanat* and *Hiomt* mRNA profiles. Pineal glands were collected from MSM mice maintained under LD12:12. RNA was extracted from a pool of three glands, and *Aanat*, *Hiomt*, and *Actb* mRNAs were assayed by RT-PCR. *Aanat* and *Hiomt* mRNA levels determined by the standard curves (*B*) and shown as values relative to *Actb* mRNA level (left axes) and relative to the lowest values (right axes; *Aanat*, ZT12 and *Hiomt*, ZT0).

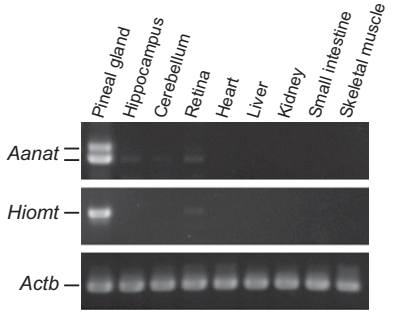

Fig. S3. Tissue distribution of mouse *Hiomt* mRNA. Various tissues were collected from MSM mice at ZT18–19. Total RNA was extracted and reverse transcribed, and *Hiomt* as well as *Aanat* and *Actb* were assayed by PCR. In this assay, almost half length of coding region (CDS) of *Hiomt*, full length CDSs of *Aanat*, and *Actb* were amplified using the following primer pairs: *Hiomt*, 5′-TGA GGG AAG GGC GGA GCC AGT A-3′ and 5′-GCG ACA GCA GCA GCG TCC GC-3′; *Aanat*, 5′-GGG AGG GKT CAG TGG CCA GA-3′ and 5′-CAC CCT CGC CAT GGC CAA G-3′; and *Actb*, 5′-CAT GTT TGA GAC CTT CAA CAC C-3′ and 5′-GGC ATA GAG GTC TTT ACG GAT G-3′. Two splicing variants of mouse *Aanat* were detected: a shorter one encoding authentic AANAT enzyme, and a longer one containing a pseudoexon insertion causing a frameshift and premature termination. The pseudoexon insertion was reported in B6J strain, and the mutant variant was exclusively expressed in the pineal gland of B6J mice (1).

1. Roseboom PH, et al. (1998) Natural melatonin 'knockdown' in C57BL/6J mice: Rare mechanism truncates serotonin *N*-acetyltransferase. *Brain Res Mol Brain Res* 63:189–197.

***Mid1* (exon 10)**

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                                  ** **** **********************  * ************************ **** * ***************
B6J     atcggcctggcgtacagatcGCGCCGAAACACGAGTGGATCGGGAAGAACGCGGCGTCCTGGGCCCTCTGCCGCTGCCACAACCACTGGGCGGTGCGACA
MSM     atcggcctggcgtacagatcGCTCCGAGACACGAGTGGATCGGGAAGAATCCCGCGTCCTGGGCCCTCTGCCGCTGCAACAATCGCTGGGCGGTGCGACA

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B6J     CGACGGCAAGGAGACCCCCATCGCGCCGGCCCCTCACCTCAGGCGCGTCGGCGTCCTGCTGGACTACGACAACGGCTCCATCGCCTTCTACGACGCTCTG
MSM     CGACGGCAAGGAGACCCCCATCGAGCCGGCCCCTCACCTCAGGCGCGTGGGCGTCCTGCTGGACTACGACAACGGCTCCATCGCCTTCTACGACGCTCTC

         ****** ****************************************************
B6J     AGCTCCGTCCACCTCCACACCTTCCACGCGGCGCTCGCGCAGCCCGTGTGCCCCACCTTCACcgtgtggaacaagtgtctgacc
MSM     GGCTCCGGCCACCTCCACACCTTCCACGCGGCGCTCGCGCAGCCCGTGTGCCCCACCTTCACcgtgtggaacaagtgtctgacc
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***Sts* (exon 1)**

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                          *** ****                                                  ****************************
B6J     gctcgctgacatcatcctgGCCGCGGAT-------------------------------------------CCCGCCCCCGCAGGCCCCGCCCCCCGGC
MSM     gctcgctgacatcatcctgGCCACGGACCCCGCCCCGGCAGGCCCCGCCCCGCAGGCCCCGCCCCCGCAGGCCCCGCCCCCGCAGGCCCCGCCCCCCGGC

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B6J     CGCCCAACTTCCTGCTCATCATGGCCGACGACCTGGGCATCGGTGACCTCGgctgctacggcaacaaaacg
MSM     CGCCCAACTTCCTGCTCATCATGGCCGACGACCTGGGCATCGGGGACCTCGgctgctacggcaacaaaacg
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***Hiomt* (intron 5 – exon 6 – intron 6)**

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B6J     CAAGCCCTCAGGGTTCAGGAAGTGACATCACTCCAAGCTTCATAACAGGATGTGACCTCATGGTGGGCGGGCCTCACACGCCAGGCCTGCGGAACAGGAA
C3H     CAAGCCCTCAGGGTTCAGGAAGTGACATCACTCCAAGCTTCATAACAGGATGTGACCTCATGGTGGGCGGGCCTCACACGCCAGGCCTGCGGAACAGGAA
MSM     CAAGCCCTCAGTGTTCAGGAAGTGACATCACTCCAAGCTTCATAACAGGATGTGACCTCATGGTGGGCGGGCCTCACACGCCAGGCCTGCGGAACAGGAA

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B6J     GTGACCTCACCGGAGACAGGAAGTGACGTCACC----CCCCCCCCCCGCAGGTGGGTCCGGGGGCGCTGGCCCGCATGGCCGCCCGGCTCTACCCGGGCAG
C3H     GTGACCTCACCGGAGACAGGAAGTGACGTCACCACCGCCCCCCCCCCGCAGGTGGGTCCGGGGGCGCTGGCCCGCATGGCCGCCCGGCTCTACCCGGGCAG
MSM     GTGACCTCACCGGAGACAGGAAGTGACGTCCCC------CCCCCCCCGCAG-TGGGTCCGGGGGCGCTGGCCCGCATGGCCGCCCGGCTCTACCCGGGCAG

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B6J     CGAGGTCACCGTGTTCGAGACGCCCGACGTCGTCGCTGCCGCCCGCGCCCACTTCCCGCCCCCAGCGGACGAGGACGGGGCGGAGCCTCGTGTGTGCTTC
C3H     CGAGGTCACCGTGTTCGAGACGCCCGACGTCGTCGCCGCCGCCCGCGCCCACTTCCCGCCCCCAGCGGACGAGGACGGGGCGGAGCCTCGTGTGCGCTTC
MSM     CGAGGTCACCGTGTTCGAGACGCCCGACGTCGTCGCCGCCGCCCGCGCCCACTTCCCGCCCCCCGCGGACGAGGACGGGGCGGAGCCTCGCGTGCGCTTC

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B6J     CTGTCAGGTGGGCGCGGGGCAGGCGTCTGGGCGGGGCTCAGTGAGGGGCGGGGCTTGGCGAGTGACATCATCCCGGGCCTTCAATCACACAGCCCTGGCC
C3H     CTGTCAGGTGGGCGCGGGGCAGGCGTCTGGGCGGGGCTCAGTGAGGGGCGGGGCTTGGCGAGTGACATCATCCCGGGCCTTCAATCACACAGCCCTGGCC
MSM     CTGTCAGGTGGGCGCGGGGCGGGCGTCTGGGCGGGGCTTGGCGAGGGGCGGGGCTTGCTGGGTGACATCATCCCGGGCCTTCAATCACACAGCCCTGGCC

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B6J     ACAACTTCAAGTCCATACAAATTTATGCAAATTTATGCAAATTTATACAAACGCACATCGGCGTTCACTTTGC
C3H     ACAGCTTCAAGTCCATACAAATTTATGCAAATTTATGCAAATTTATACAAACGCACATCGGCGTTCACTTTGC
MSM     ACAACTTCAAGTCCATACAAATTTATGCAAATTTATGCAAATTTATACAAACGCACATCGGCGTTCACTTTGC
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***Aanat* (intron 3)**

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B6J     TGGTTCTCAACCTTCCTAAAGGTGAGACACTTTAACACAGTTCCTCATGTTGTGGTGACCCCCCAACCATAAAATCATTTTCATTGCTACTTCACAACTA
C3H     TGGTTCTCAACCTTCCTAAAGGTGAGACACTTTAACACAGTTCCTCATGTTGTGGTGACCCCCCAACCATAAAATCATTTTCATTGCTACTTCGCAACTA
MSM     tggttctcaaccttcctaaagcTGAGACACTTTAACACAGTTCCTCATGTTGTGGTGACCCCCCAACCATAAAATCATTTTCATTGCTACTTCACAACTA

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B6J     TAATCTTGTTACTGCTATAAATTGTAATCTAAATCTCTGTGTTTTCTTTTTTTTACAGTCTTAGTCGACTCCTATGAAACAGTCGTTTGACCTCCAAAGGG
C3H     TAATCTTGTTACTGCTATAAATTGTAATCTAAATCTCTGTGTTTTCTTTTTTTTACGGTCTTAGTCGACTCCTATGAAACAGTCGTTTGACCTCCAAAGGG
MSM     TAATCTTGTTACTGCTATAAATTGTAATCTAAATCTCTGTGTTTTCTTTTTTTTACGGTCTTAGTCGACTCCTATGAAACAGTCGTTTGACCTCCAAAGGG

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B6J     GTCACAACCCACAGGTTGAGAATTGCTGCTTTAGAGTCCCGAACTGTAGGACTTTAGATGTAGGTAGCAGTGGGGTCCTGTCTGGTAAGGGAACCCTGGA
C3H     GTCACAACCCACAGGTTGAGAATTGCTGCTTTAGAGTCCCGAACTGTAGGACTTTAGATGTAGGTAGCAGTGGGGTCCTGTCTGGTAAGGGAACCCTGGA
MSM     GTCACAACCCACAGGTTGAGAATTGCTGCTTTAGAGTCCCGAACTGTAGGACTTTAGATGTAGGTAGCAGTGGGGTCCTGTCTGGTAAGGGAACCCTGGA

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B6J     TGTTCCTGGTACCCAGGATCCTTTTGGTCTGGTTGGGTTTGGAGAAACAGGTGTAGC
C3H     TGTTCCTGGTACCCAGGATCCTTTTGGTCTGGTTGGGTTTGGAGAAACAGGTGTAGC
MSM     TGTTCCTGGTACCCAGGATCCTTTTGGTCTGGTTGggtttggagaaacaggtgtagc
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Fig. S4. Nucleotide sequences used for genotyping. Primer sequences for PCR amplification and for sequencing are indicated by cyan and green backgrounds, respectively. Direct sequencing of the PCR products of *Mid1*, *Sts*, and *Aanat* were performed with the forward primers. Nucleotide sequences in lowercase are not determined, but the corresponding primers were hybridized to the sequences.

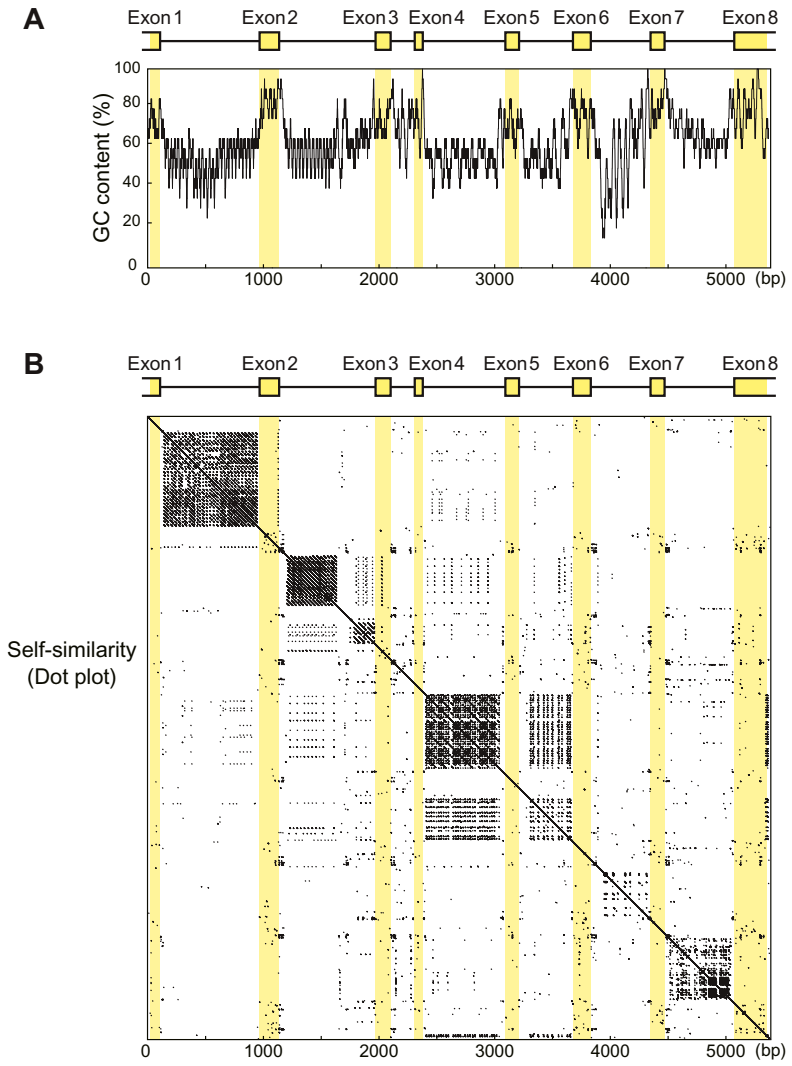

Fig. S5. Characterization of mouse *Hiomt* gene. (*A*) GC content. All exons markedly show higher GC content. (*B*) Self-similarity dot plot. Introns 1, 2, 4, 5, and 7 have remarkable self-similarity with repetitive sequences.

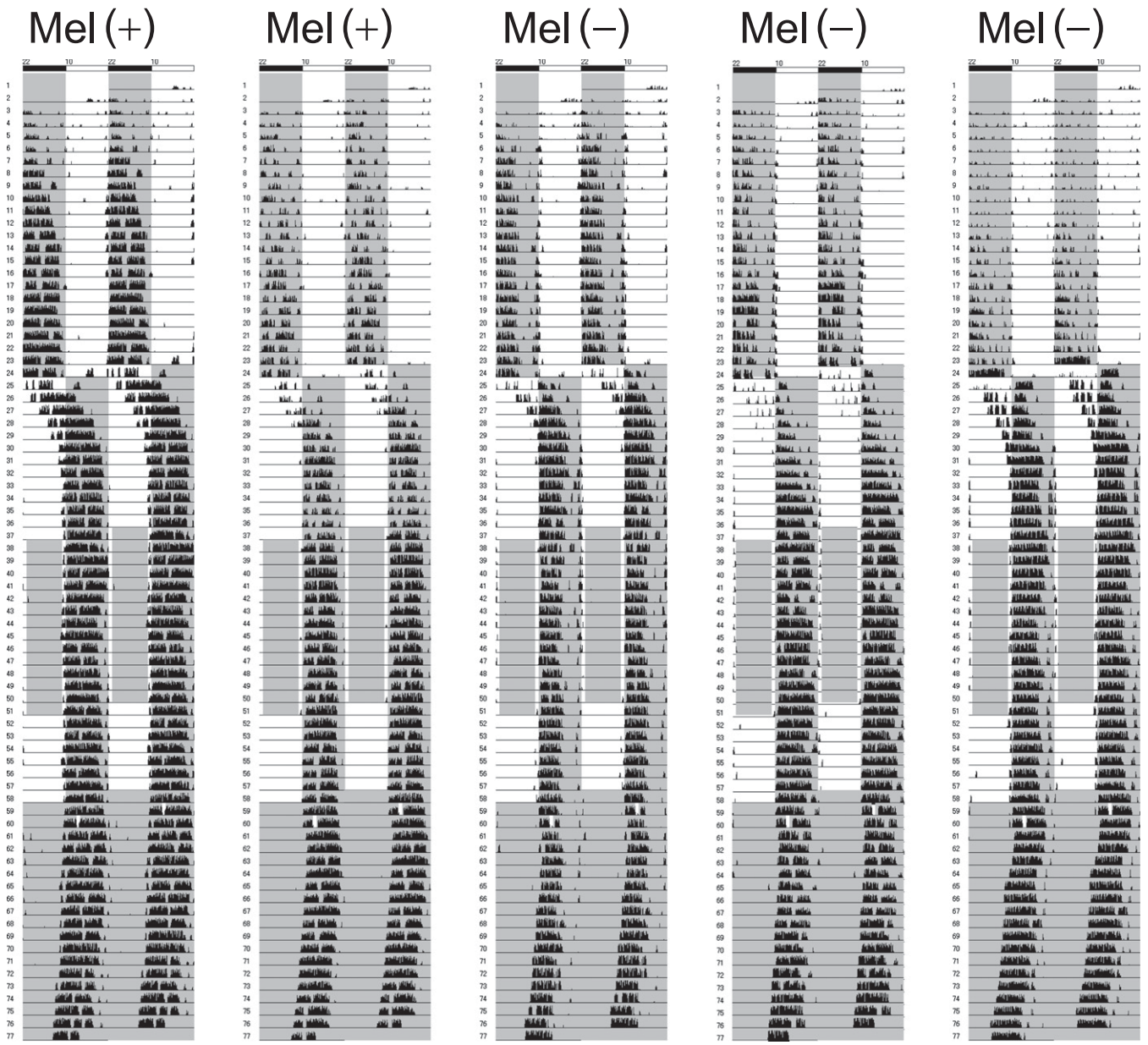

Fig. S6. Representative records of wheel-running activity of F2 intercrosses (C3H × B6J). Actogram is shown as double plot, and each horizontal sequence of colored bars represents 48 consecutive hours. Melatonin productivity is indicated above the actogram. Mice were maintained under LD12:12 for 3 weeks, then an inverted LD cycle for 2 weeks, a skeleton light condition (L1:D10:L1:D12) for 2 weeks, an LD cycle for 1 week, and finally in a constant darkness condition. Light and dark periods are indicated by white and gray backgrounds, respectively.

Table S1. Interstrain variations in AANAT and HIOMT activities and melatonin production

	Inbred strain	AANAT activity	HIOMT activity	Melatonin	Ref.
Classical laboratory mouse	129/Sv			–	(3)
	AKR/J	–	–	–	(2)
	BALB/c	–	–	–	(2)
	C3H/He	+	+	+	(3)
	C57BL/6J	–	–	–	(1)
	CBA/Ms	+	+	+	(3)
	CF-1			–	(3)
	DBA/2	–	+	–	(3)
	NZB/BLNJ	+	–	–	(1)
M. m. domesticus–derived	IS/CamEi			–	(1)
	SK/CamEi	(+)	(+)	+	(1)
	SK/Nga	+	–	–	(3)
	SF/CamEi	(+)	(+)	+	(1)
	PERU-A./CamEi	(+)	(+)	+	(1)
M. m. casteneus–derived	CAST/Ei			–	(1)
M. m. molossinus–derived	MOM	(+)	(+)	+	(3)
	Mol-Nis	(+)	(+)	+	(3)
	Mol-A	(+)	(+)	+	(3)
	MSM/Ms	(+)	(+)	+	(Current study)

+ Active/proficient, experimentally validated; (+) active, inferred from melatonin productivity; – inactive/deficient.

1. Ebihara S, Marks T, Hudson DJ, Menaker M (1986) Genetic control of melatonin synthesis in the pineal gland of the mouse. *Science* 231:491–493.
2. Ebihara S, Hudson DJ, Marks T, Menaker M (1987) Pineal indole metabolism in the mouse. *Brain Res* 416:136–140.
3. Goto M, Oshima I, Tomita T, Ebihara S (1989) Melatonin content of the pineal gland in different mouse strains. *J Pineal Res* 7:195–204.

Table S2. List of SNPs/deletion in *Hiomt* CDS of different mouse strains*

Nucleotide number	18	27	30	69	90	105	114	189	232	255	271	306	379	496	535	555	579	666	677	693	720	724	820	846	871	939	1002	1030	1035	1073	1080	1091	1101	1110	1143
C3H/He, CBA/J, etc.†	G	C	C	C	G	C	C	G	C	T	A	G	G	C	A	C	T	C	C	A	T	C	G	C	C	G	C	C	G	G	C	G	C	C	T
C57BL/6J, 129/Sv, etc.‡		T							G	G								T				T		T			T								
MBT/Pas													T	T												A					del10				
A/J		T	A	T	A		T									T				C									A				A		
BFM/2Ms								A				T				T				C	C	T													
NOD/Shi, NZW/N		T							G	G										C	C	T					T				T				
RIII/Imr		T							G							T				C	C	T									T				
DDD/Sgn, KK/Sgn		T							G		T					T				C	C	T									T				
MYS/Mz	A														G		C			C	C		C		T				A				A		
JF1/Ms	A														G		C		T	C	C												A		G
MSM/Ms	A					T									G		C			C	C												A		G
Amino acid substitution									R78G		T91S		A127S		T179A				A226V			R242C	V274L					R344C		G358D		Del+FS§		D370E	S381R

Hydroxyindole *O*-methyltransfeases

SAM-dependent methyltransferases

*Nucleotides different from the C3H-type seuqence are shown in boldface type. Nonsynonymous variations are shown in red, and the substituted amino acid residues are indicated on the lowest row.

†C3H/He, CBA/J, DBA/1J, and SM/J have the identical sequence.

‡C57BL/6J, 129/Sv, AKR/N, BALB/cN, RFM/Ms, NC/Nga, CF1/Sgn, FVB/N, and NZB/N have the identical sequence.

§There is a 10-bp deletion (del10) causing a frameshift (FS).

Table S3. Comparison of two mouse PAR genes

	Gene	*HIOMT/ASMT*		*STS*	
	Enzyme encoded	Hydroxyindole *O*-methyltransferase		Steroid sulfatase	
	Tissue distribution	Pineal gland and retina		Ubiquitous	
Human	Chromosomal location	PAR1 (Xp 22, Yp 11)		Xp 22 (proximal to PAR1)	
	Disorders	(Autistic spectrum disorders)		X-linked ichthyosis (1, 2)	
	Gene size	28 kb		97 kb	
	GC content in CDS	56%		52%	
	GC3	68%		61%	
Rat	Chromosomal location	12q 11		Xq 12–14	
	Gene size	~5–6 kb		9 kb	
	GC content in CDS	65%		63%	
	GC3	74%		75%	
Mouse	Chromosomal location	PAR		PAR	
	Gene size	5.4–5.7 kb		9 kb	
	GC content in CDS	76%		75%	
	GC3	95%		97%	
	Activity diversity*	C3H/He	+	C57BL/6J	+++ (3, 4)
		CBA/Ms	+	C3H/HeJ	++
		BALB/c	–	A/J	+
		C57BL/6J	–	C3H/An	–

*Symbols represent relative levels of the enzymatic activity: +++ > ++ > + > – (almost no activity).

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