Supplementary Files

Loss of *Snord116*, sleep and food-related behaviors: a report of changes in lateral hypothalamus

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Methods

Sleep deprivation procedures

Total sleep deprivation was performed by gentle handling techniques, consisting of introducing novel objects into the cage, knocking or shaking the cage whenever animals are inactive or display behaviors associated with sleep (asleep account for inactivity, twitching or if mice eyes are closed). Animals were subjected to SD during the last 6h of the light period, were the sleep pressure is high. Animals were visual inspected throughout all SD procedure.

Thermal imaging

Surface body temperature was continuously recorded in $PWScr^{m+/p-}$ and $PWScr^{m+/p+}$ mice for 24 h at 22°C and 30°C using an infrared thermocamera (FLIR A20). The thermocamera was positioned above the cage where the mice were individually housed. Head (T-head) and tail temperatures (T-tail) were obtained by manually analysing the recorded video (1 frame/s) using a dedicated software program (Thermocam Research, FLIR). T-head was used to compute the heat loss index (HLI). HLI was calculated by the equation (T-head - Ta)/(T-tail - Ta) [67]. T-head, T-tail and HLI values were collapsed into 2-h bins and compared between conditions, before and after drug treatment, in the two experimental groups.

Additionally, body weight was measured weekly in small groups of both $PWScr^{m+/p-}$ and $PWScr^{m+/p+}$ mice for 5 weeks at 22°C and at the TNZ (n= 10); the first body weight measurement was recorded at 15 weeks of age. Another group of animals (n=10) born and raised at the TNZ was also investigated. Body weight was assessed from 9 to 20 weeks of age.

A two-way ANOVA with repeated measures on both factors (group x time) was used for the statistical analysis of T-head, T-tail, and HLI over the conditions investigated between the two genotypes ($^{+/+}$ vs. $^{-/-}$). A two-way ANOVA was employed for the statistical analysis of body weight (group x time).

Surgery

Mice were anaesthetised using 1.5%–2.5% isoflurane in oxygen and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). To assess the sleep-wake cycle, mice were surgically implanted with a telemetric transmitter (volume, 1.9 cm 3; total weight, 3.9 g; TL11M2-F20-EET; DSI, St. Paul, MN, USA) connected to electrodes for continuous EEG/EMG recordings. A wireless EEG transmitter/receiver, which also contained a sensor to detect body temperature, was subcutaneously implanted. Specifically, EEG wire electrodes were implanted into the frontal cortex (coordinates: 2 mm posterior to the bregma and 2 mm lateral to the midline in the right parietal skull) and the parietal cortex (coordinates: 3 mm anterior to the lambda and 2 mm lateral to the midline in the right frontal skull). EMG was recorded by two stainless steel wires inserted bilaterally into the neck muscles. Subsequently, to record SUA, a tetrode-based 16-channel micro-wire array (4 x 4, 5.3 mm, 100-200-1250) of silicon probes (NeuroNexus Technologies) was implanted into the LH (coordinates relative to the bregma: –1.45 mm anteroposterior, –1.0 mm mediolateral, and –4.8 mm dorsoventral) of the contralateral hemisphere with respect to the EEG electrodes (Figure 1A). Mice were operated on by performing a small craniotomy, and the dura mater was removed for placement of the tetrodes in the LH. Two screws (1 mm diameter) were used to anchor the implant. One screw placed in the cerebellum was wrapped by the electrode ground. Next, the tetrode and the ground wire were covered with dental acrylic. Following surgery, all animals were administered paracetamol (200 mg/kg; once a day; PO; Tempra) and enrofloxacine (10 mg/kg; once a day; SC; Baytril) for two days.

Histology

At the end of the last recording session, animals were sacrificed, and the locations of the recording electrodes were verified histologically. Mice were anaesthetised using pentobarbital anaesthesia (100 mgr/kg, i.p.), and microlesions were made at the tip of one or two microwires by passing a small current (5 m A, 10 s). The animals were transcardially perfused with 10 ml of phosphate-buffered saline (PBS) before infusion of 4% paraformaldehyde (PFA) in PBS. The brains were removed and

equilibrated in 30% sucrose, sectioned at 40 µm on a freezing microtome, mounted onto gelatin-coated slides, air dried, dehydrated in ethanol, stained with Nissl substance, cleared with xylene, and cover slipped with DPX (Figure 1A). Three mice were excluded from the study because the electrode was not correctly placed in the LH (Figure 1A).

Real-time quantitative PCR

Thirty PWScr$_{m+/p-}$ and PWScr$_{m+/p+}$ mice were sacrificed by cervical dislocation at three different time points: at ZT 6 (T0), immediately after 6-h of total SD (T1), and 1 h after previous SD (ZT 7; T2). Total RNA was extracted from the hypothalamus by the Trizol method (Sigma-Aldrich) according to the manufacturer's instructions [68]. RNA concentrations were then determined by a NanoDrop 2000c spectrophotometer. The complementary DNA was obtained from up to 2 mg of total RNA by using a high-capacity RNA-to-cDNA kit (Invitrogen) and then analysed with SYBR GREEN qPCR mix. Reactions were performed in three technical replicates using an AB 7900HT fast real-time PCR system (Applied Biosystems). The relative expression levels were quantified according to the previously described $\Delta\Delta$CT calculation method [69]. *Gapdh* was used as a reference gene. The specific primer pairs used for the analysis were designed using Primer3 (Supplementary Tables 5 and 6). An unpaired t-test was used to compare the differences between the genes investigated in the two genotypes.

Perfusion and immunohistochemistry (IHC)

To determine the number of OX neurons PWScr$_{m+/p-}$ and PWScr$_{m+/p+}$ mice were sacrificed at beginning of the dark period, since a recent study [70] has reported a diurnal variation in the number of immunolabeled OX neurons, which was significantly increased during the active phase of mice (dark period). Regarding the MCH neurons, it has been observed no significant difference in the number of MCH-immunoreactive neurons between the animals in either phases [70].

PWScr$^{m+/p-}$ and PWScr$^{m+/p+}$ mice were transcardially perfused with 10 ml of PBS before infusion of 4% PFA in PBS. Perfused brains were postfixed for 24 h in 4% PFA at 4°C before immersion in 30% sucrose. To quantify lateral hypothalamic neurons expressing OX and MCH neurons, serial cryosections were cut coronally at 40 μm intervals to include brain regions within–1.10/–1.90 mm of the bregma. Before IHC staining, the sections were washed three times with PBS for 10 min each and then blocked in 5% of the appropriate serum (normal goat serum or normal donkey serum) in 0.1% Triton X-100 in PBS for 1 h. The sections were incubated with rabbit PPOX polyclonal (1:20; Millipore) and pro-melanin-concentrating hormone (PMCH; 1:50; Invitrogen) primary antibodies in 1% serum in 1% Triton X-100/PBS at room temperature overnight. The sections were then washed three times in PBS for 5 min each. They were incubated with appropriate secondary antibodies (1:1000) (goat anti-rabbit IgG, Alexa Fluor 488, Invitrogen) in 1% serum in 1% Triton X-100/PBS for 2 h at room temperature in the dark, washed three times in PBS for 5 min each, and counterstained with Hoechst (1:400 in PBS; Sigma-Aldrich). Finally, the sections were washed in PBS and mounted on glass slides using ProLong gold antifade reagent (Invitrogen). The sections were imaged with an upright Widefield Epifluorescence Olympus BX51 microscope equipped with a 4x UPLFLN N.A objective. The microscope was controlled by Neurolucida software.

The percentage of OX- and MCH-positive cells was manually scored using NIH ImageJ software. A total of 3-5 sections were evaluated for each mouse (n= 4 for genotype), and the neuron counts were normalized to the total number of DAPI-stained nuclei (approximately 300 nuclei per microscopic field). All digital images were processed in the same way between experimental conditions to avoid artificial manipulation between different data sets. An unpaired t-test was used to compare differences between the two genotypes.

Chromatin immunoprecipitation (ChIP)

For the analysis of PEG3 binding, H3K4me2/3 and H3K27me3, ChIP was performed on formaldehyde cross-linked chromatin isolated from the hypothalamus of 28 PWScr$^{m+/p-}$ and

PWScr$^{m+/p+}$ mice. Briefly, the tissue of seven different hypothalamic was minced. Formaldehyde was added to the tissue, which was resuspended in PBS at a final concentration of 1% and incubated at room temperature for 15 min while shaking. The reaction was stopped by the addition of glycine to a final concentration of 0.125 M. The tissue was washed twice with ice-cold PBS, centrifuged and resuspended in lysis buffer 1 (50 mM HEPES pH 8, 10 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40 and 0.25% Triton X-100) for 90 min at 4°C. Isolated nuclei were lysed in lysis buffer 2 (10 mM Tris–HCl pH 8.0, 200 mM NaCl, 1 mM EDTA and 0.5 mM EGTA) for 60 min at 4°C. The chromatin was sheared in sonication buffer (10 mM Tris–HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate and 0.5% N-lauroylsarcosine) to an average size of 100–400 bp using the Sonifier 150 (Branson).

For each IP, 100 µg of sonicated chromatin was diluted in a final volume of 600 µl with sonication buffer and pre-cleared with 30 µl protein A/G agarose beads (Santa Cruz) for 4 h at 4°C on a rotating wheel. Anti-PEG3 antibody (7 µg, Abcam Ab99252), anti-histone H3K4me3 (7 µg, Abcam Ab8895), and anti_Histone H3K27me3 (7 µg, Abcam Ab195477) or rabbit IgG were added to the pre-cleared chromatin and incubated overnight at 4°C on a rotating wheel. Chromatin was precipitated with 30 µl protein A/G agarose beads for 4 h at 4°C with rotation. The beads were then washed five times with 500 µl RIPA buffer (10 mM Tris–HCl pH 8.0, 140 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate, 1% Triton X-100 and 0.1% SDS) and once with each of the following buffers: WASH buffer (50 mM HEPES, 0.5% sodium deoxycholate, 1% Triton X-100, 1 mM EDTA, 500 mM NaCl and 0.2% NaN3), LiCl buffer (0.25 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA and 10 mM Tris pH 8) and TE buffer (10 mM Tris pH 8, 1 mM EDTA). The bound chromatin was eluted in 100 µl TE buffer. Crosslinks were reversed by incubation with O/N at 65°C after the addition of 1 µl RNAse cocktail (Ambion) and 2 h at 50°C after the addition of 2.5 µl SDS 20% + 2.5 µl 20 mg/ml proteinase K (Sigma). DNA was extracted by using a QIAquick Gel Extraction Kit (Qiagen). Immunoprecipitated or 1% input DNAs were analysed by real-time PCR using SYBRGreen PCR Master Mix (Bio-Rad) on a 7900HT Fast Real-Time PCR System (Applied

Biosystems). Each reaction was performed in triplicate, and experiments were performed twice. The specific primer pairs used for the analysis were designed using Primer3 (Supplementary Table 7). An unpaired t-test was used to compare differences between the two genotypes.

Hypothalamic cell culture transfected with *Snord116*-siRNA

Embryonic rat hypothalamus cell line R7 (rHypoE-7 – Tebu-bio) cells were cultured in Dulbecco's modified Eagle's medium (Sigma Co. Ltd, St Louis, MO, USA) supplemented with 10% foetal bovine serum, 50 units of penicillin and 50 μg/ml of streptomycin at 37°C under an atmosphere of 5% CO2. Cells were seeded in six-well plates (Nunc Co., Roskilde, Denmark) at a density of $1 \times 10_5$ cells per well. The day after cell seeding, 20 μM of Silencer® Snord116-siRNA (Ambion®) was mixed with RNAiMAX Lipofectamine (Life Technologies) reagent, and the mixture was added to each dish. We used MISSION® siRNA universal negative control #1 (Sigma) as a negative control at 20 μM. Cells were incubated for 48-h after transfection, and RNA was extracted by using the Trizol method for gene expression analysis (Supplementary Table 6). Experiments were conducted in triplicate. An unpaired t-test was used to compare differences between the two genotypes.

Narcoleptic mice were sacrificed between ZT 6 and ZT 7, and the hypothalamus and cortex of the brain were dissected and immediately frozen.

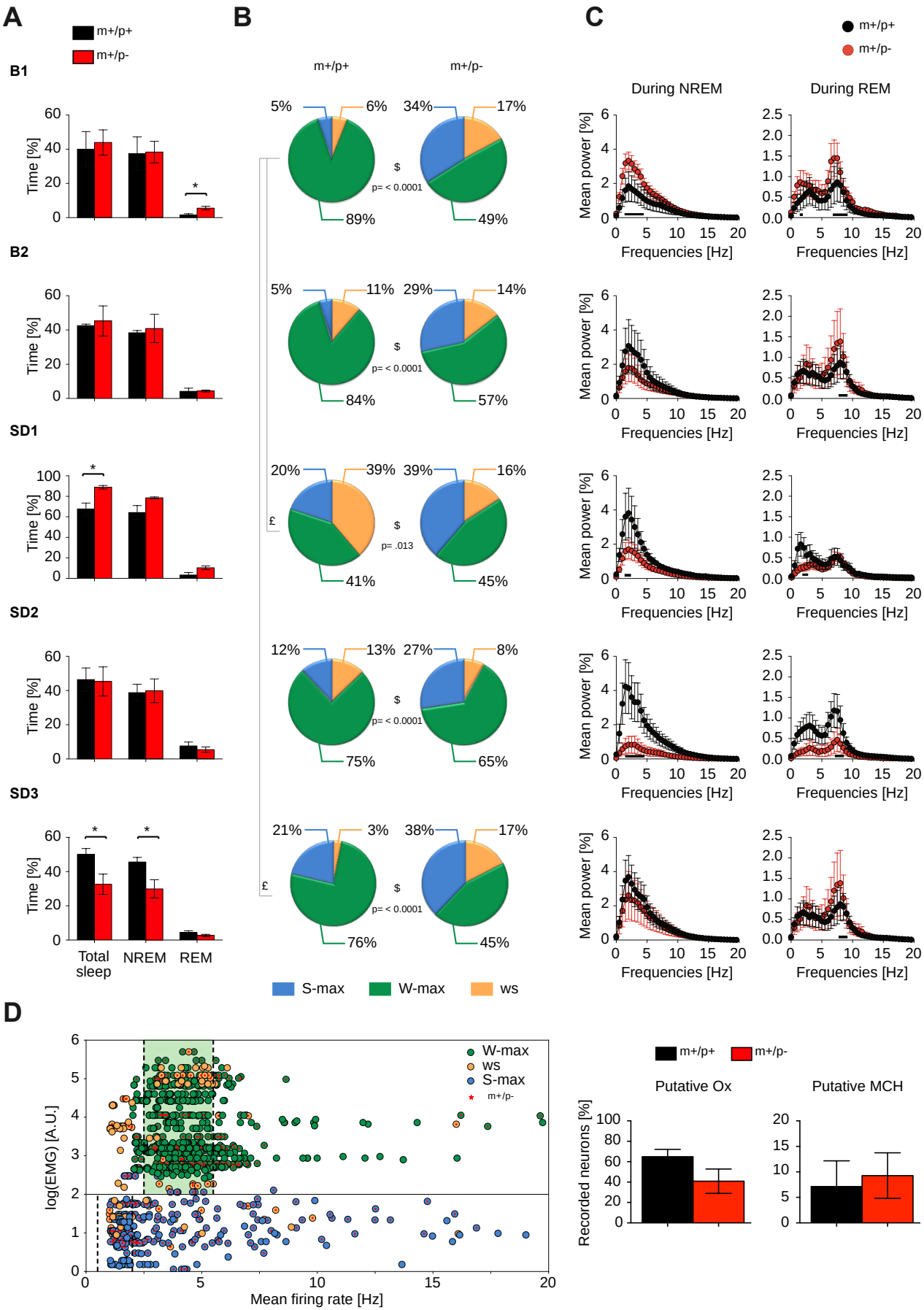

A
m+/p+
m+/p-
B1
B2
SD1
SD2
SD3
Time [%]
Total sleep
NREM
REM
B
m+/p+
m+/p-
5%
6%
89%
34%
17%
49%
p= < 0.0001
5%
11%
84%
29%
14%
57%
p= < 0.0001
20%
39%
41%
39%
16%
45%
p= .013
12%
13%
75%
27%
8%
65%
p= < 0.0001
21%
3%
76%
38%
17%
45%
p= < 0.0001
S-max
W-max
ws
C
m+/p+
m+/p-
During NREM
During REM
Mean power [%]
Frequencies [Hz]
D
log(EMG) [A.U.]
Mean firing rate [Hz]
W-max
ws
S-max
m+/p-
m+/p+
m+/p-
Putative Ox
Putative MCH
Recorded neurons [%]

Figure S1

PWScr$_{m+/p-}$ mice have altered neuronal dynamics of the LH in relation to sleep and food. The results of single unit activity (SUA) combined with EEM/EMG recordings. **A)** The percentage of total time spent in sleep stages (NREM plus REM sleep), NREM sleep and REM sleep, in PWScr$_{m+/p-}$ mice (red bar) compared with control mice (black bar). EEG/EMG/SUA were recorded at different time points and are shown in each row: B1 and B2, baseline; SD1, 1-h immediately after 6 h of SD; and SD2 and SD3, two time points during the 18-h recovery period. At B1, REM sleep was increased in PWScr$_{m+/p-}$ mice (unpaired t-test: $t(6) = 3.06$, $p = .02$) relative to PWScr$_{m+/p+}$ mice. At B2, no differences between the two genotypes were observed in any of the sleep stages investigated. At SD1, total sleep was increased in PWScr$_{m+/p-}$ mice (unpaired t-test: $t(6) = 3.53$, $p = .01$) relative to controls. At SD2, no differences between the two genotypes were observed in any of the sleep stages investigated. At SD3, total sleep (unpaired t-test: $t(6) = 2.52$, $p = .04$) and NREM sleep (unpaired t-test: $t(6) = 2.64$, $p = .03$) were decreased in PWScr$_{m+/p-}$ mice relative to PWScr$_{m+/p+}$ mice. Data are presented as the mean ± SEM. Asterisks (*) indicate a significant difference between genotypes: * $P \leq .05$. **B)** The panel shows the neuronal distribution of recorded neurons (according to the classification described in the Methods session) in a pie chart for each time point. W-max neurons are shown in green, S-max neurons are shown in blue, and ws neurons are shown in yellow. Differences between the two genotypes are indicated by $, while differences within groups across time points are indicated by §. Significance was computed with the chi-square test. **C)** The normalized power density of the whole spectrum during NREM and REM sleep (left and right, respectively) for both genotypes; red circles depict PWScr$_{m+/p-}$ mice, and black circles depict PWScr$_{m+/p+}$ mice. **D)** Neuronal classification in a 2D scatter plot. The x-axis plots the mean firing rate of each cell in the state in which they maximally fire, and the y-axis plots the mean logarithm of EMG signals according to the selected sleep-wake state. W-max neurons are shown in green, S-max neurons in blue, and ws neurons in yellow. Neurons recorded from mutant mice are marked by red dots. Putative OX neurons (green squares) and MCH putative neurons (blue squares) are also shown (see Methods). The two genotypes investigated were PWScr$_{m+/p-}$ mice (n=4) and PWScr$_{m+/p+}$ mice (n= 4).

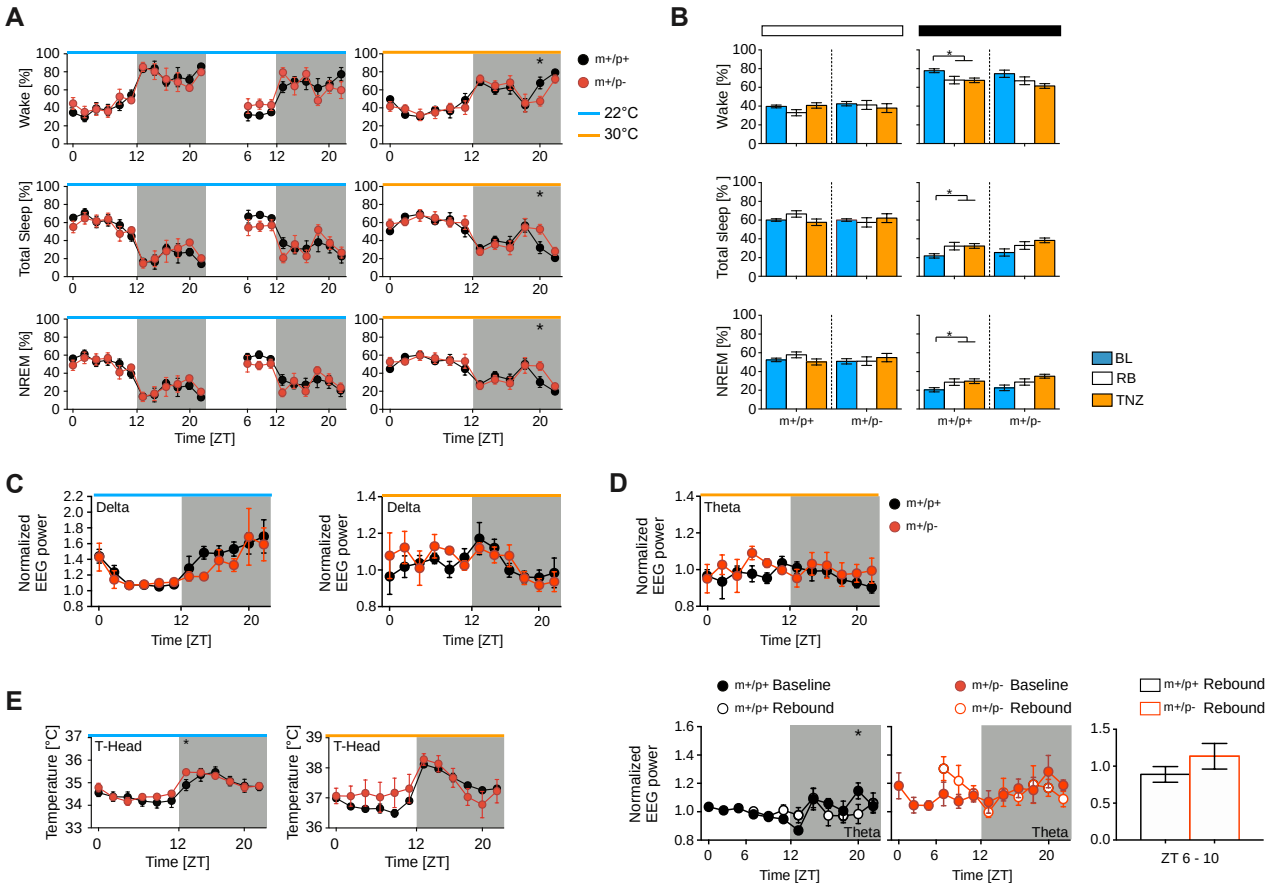


Figure S2

Sleep-wake cycle and temperature profile recorded. **A)** The panel shows the hourly percentage of time spent in wakefulness, total sleep (including both NREM sleep and REM sleep), NREM sleep, and REM sleep in PWScr$_{m+/p-}$ mice (red) versus control mice (black), recorded over an uninterrupted 24-h period of the 12-h light/dark cycle for baseline (BL) and during the 18-h after SD. Recordings were conducted in animals maintained at 22°C (cyan bar in each graph) and at 30°C (orange bar in each graph). No significant differences were found at 22°C between genotypes among all sleep stages investigated. At 30° C, a two-way repeated-measures ANOVA revealed significant main effects of time for wakefulness ($F(11,88) = 13.70$, $p < .0001$ "time"), for total sleep ($F(11,88) = 13.51$, $p < .0001$ "time") and for NREM sleep ($F(11,88)= 12.50$, $p = <.0001$ "time"). Data are presented as the 2-h mean values ± SEM. **B)** Cumulative amount of time spent in wakefulness, total sleep and NREM sleep for PWScr$_{m+/p-}$ and PWScr$_{m+/p+}$ mice over the 12-h light period and the 12-h dark period. Cyan bar indicates the baseline (BL) value, the white bar indicates the 18-h recovery (RB) period following 6 h of SD, and the orange bar indicates recordings at 30°C. Statistical analysis was performed by one-way ANOVA followed by post hoc analysis with the Bonferroni multiple comparison test. Dark period: PWScr$_{m+/p+}$ mice showed a difference in the percentage of wakefulness ($F(1.79, 7.19)= 6.85$, $p = .02$), total sleep ($F(1.80, 7.20)= 7.35$, $p = .01$) and NREM sleep ($F(1.86, 7.46)= 5.40$, $p = .03$). Data are presented as the mean ± SEM. Asterisks (*) indicate a significant difference between genotypes: * $P \leq .05$. **C-D)** Spectral analysis. Normalized delta and theta power during NREM sleep (C-upper) and REM sleep (D-upper) at 22°C (cyan bar above the graph) and at 30°C (orange bar above the graph) and after SD and relative to baseline for PWScr$_{m+/p-}$ mice (D-button) were recorded between genotypes. No substantial differences were observed between genotypes and conditions investigated. Data are presented as the means of 2-h bins ± SEM. Asterisks (*) indicate a significant difference between genotypes: * $P \leq .05$; **$p \leq .01$; *** $p \leq .001$; **** $p \leq .0001$. The bottom right shows the normalised theta power recorded from ZT 6 to ZT 10, and no differences were observed between genotypes. Two genotypes were investigated: PWScr$_{m+/p-}$ mice (n= 10, 5 mice at 22°C and

5 mice at 30°C) and PWScr$_{m+/p+}$ mice (n= 10, 5 mice at 22°C and 5 mice at 30°C). **E)** Head temperature (Head-T) profiles for PWScr$_{m+/p+}$ and PWScr$_{m+/p-}$ mice were recorded with an infrared thermocamera over 24 h. Recordings were performed under two different environmental conditions: at 22°C (cyan bar over the graph, shown on the left) and at 30°C, representing the thermoneutrality zone (TNZ) (orange bar over the graph, shown on the right). T-head was slightly increased in PWScr$_{m+/p-}$ mice relative to control mice during the dark period at ZT 14 at 22°C. Asterisks (*) indicate a significant difference between genotypes: * $P \leq .05$.

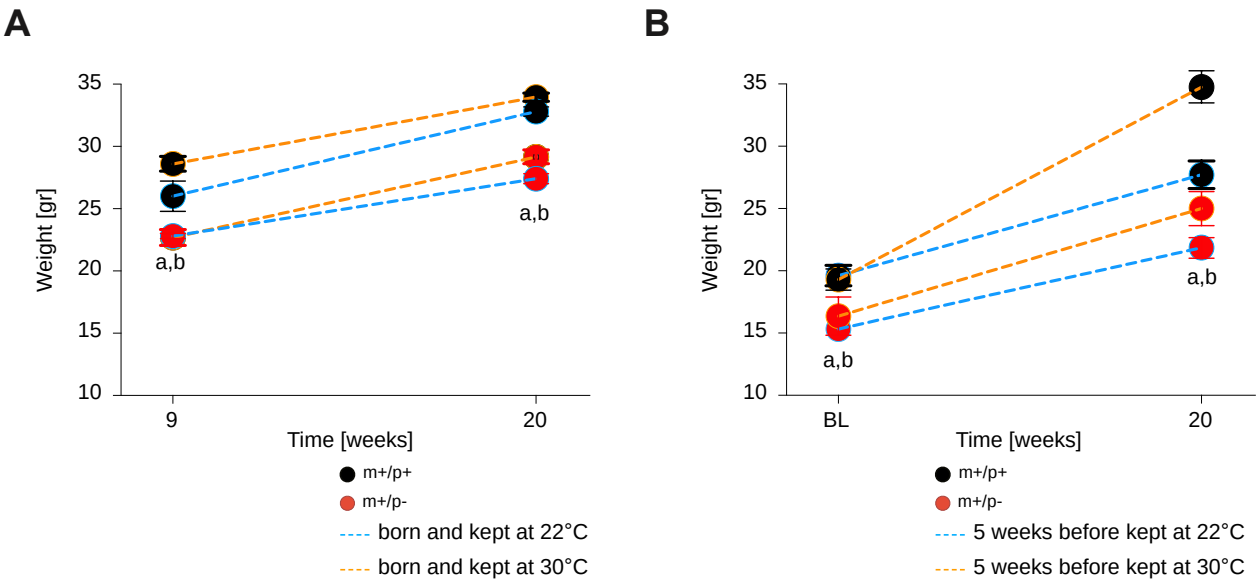
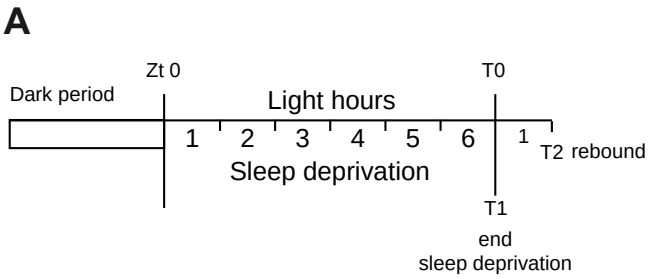
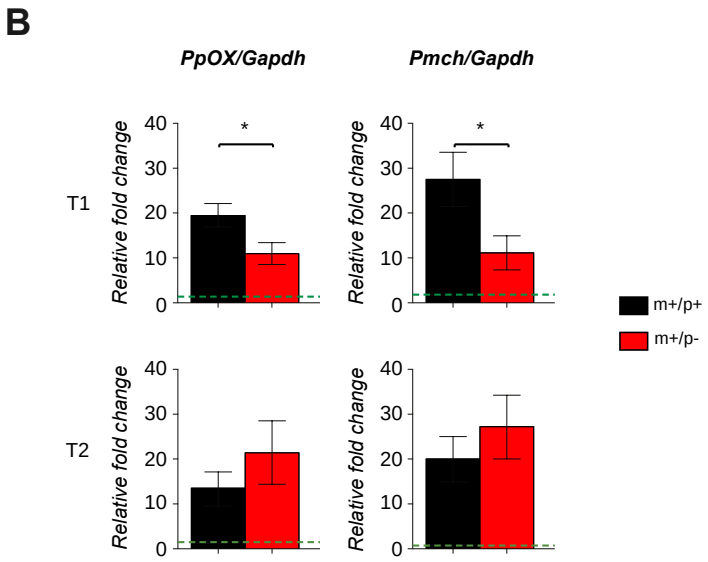


Figure S3

***Snord116* loss induces growth retardation.** Body weight was assessed at 22°C and 30°C (TNZ). **A)** The absolute weekly body weights of mice born at 22°C (cyan connection) and mice born at 30°C (orange connection) were assessed. PWScr$^{m+/p-}$ mice are indicated by red circles, while PWScr$^{m+/p+}$ mice are represented by black circles. Two-way ANOVA revealed that PWScr$^{m+/p-}$ mice had significant growth retardation compared with control mice when raised at either 22°C or 30°C ($F(3, 32)= 179.5$, $p = < .0001$ "time"; $F(3, 32)= 45.07$, $p = < .0001$ "genotypes"). a indicates differences between genotypes at 22°C, while b indicates differences between genotypes at 30°C. Two genotypes were investigated: PWScr$^{m+/p-}$ mice (n= 10, 5 mice at 22°C and 5 mice at 30°C) and PWScr$^{m+/p+}$ mice (n= 10, 5 mice at 22°C and 5 mice at 30°C). **B)** At 15 to 20 weeks of age (at the time point when the sleep-wake cycle was recorded), the absolute weekly body weight was assessed in mice after housing for 5 weeks either at 22°C or 30°C. Two-way ANOVA revealed that PWScr$^{m+/p-}$ mice had significant growth retardation compared with control mice housed at either 22°C or 30°C ($F(3, 32)= 10.58$, $p = < .0001$ "interaction"). a indicates differences between genotypes at 22°C, while b indicates differences between genotypes at 30°C. Two genotypes were investigated: PWScr$^{m+/p-}$ mice (n= 10, 5 mice at 22°C and 5 mice at 30°C) and PWScr$^{m+/p+}$ mice (n= 10, 5 mice at 22°C and 5 mice at 30°C).


A
Zt 0
T0
Dark period
Light hours
1
2
3
4
5
6
1
T2 rebound
Sleep deprivation
T1
end
sleep deprivation


B
PpOX/Gapdh
Pmch/Gapdh
T1
T2
Relative fold change
m+/p+
m+/p-

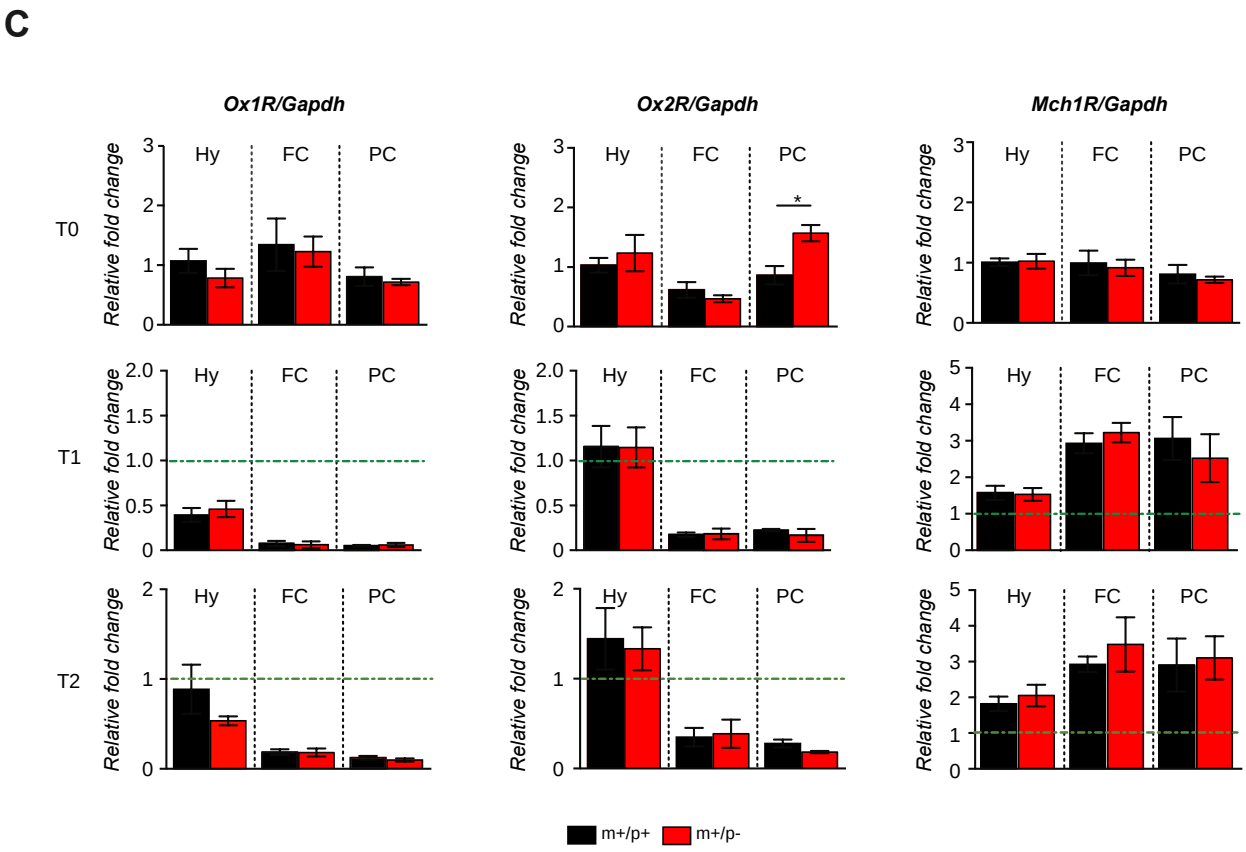

C
Ox1R/Gapdh
Ox2R/Gapdh
Mch1R/Gapdh
T0
T1
T2
Hy
FC
PC
Relative fold change
m+/p+
m+/p-

Figure S4

Gene expression analysis of the OX amd MCH systems. **A)** Experimental timeline. PWScr$^{m+/p-}$ and PWScr$^{m+/p+}$ mice were sacrificed at three different time points to assess the gene expression of prepro-OX (*Ppox*) and the precursor of MCH (*Pmch*). Mice were sacrificed at Zeitgaber 6 (ZT 6; T0), after 6-h of SD at ZT 6 (T1), and after 6-h of SD (ZT 7; T2). **B)** At T1, PWScr$^{m+/p-}$ mice showed reduced *Ppox* (unpaired t-test: $t(8) = 3.14$, $p = .01$) and *Pmch* (unpaired t-test: $t(8) = 2.66$, $p = .02$) compared with PWScr$^{m+/p+}$ mice. At T2, no differences were observed between the two genotypes for both genes. **C)** Gene expression analysis of the OX receptor 1 (Ox1R), the OX receptor 2 (Ox2R) and the MCH receptor 1 (*Mch1R*) in the hypothalamus (Hy), the frontal cortex (FC) and the parietal cortex (PC). The green lines in T1 and T2 represent the baseline value (T0). Only OxR2 in the PC was found to be increased in PWScr$^{m+/p-}$ mice (unpaired t-test: $t(8) = 3.44$, $p = .008$). Gene expression (mean ± SEM) was assessed by qRT-PCR in mice of the 2 genotypes: PWScr$^{m+/p-}$ mice (n= 15, 5 mice for each time point) and PWScr$^{m+/p+}$ mice (n= 15, 5 mice for each time point). Gapdh was used as a reference gene.

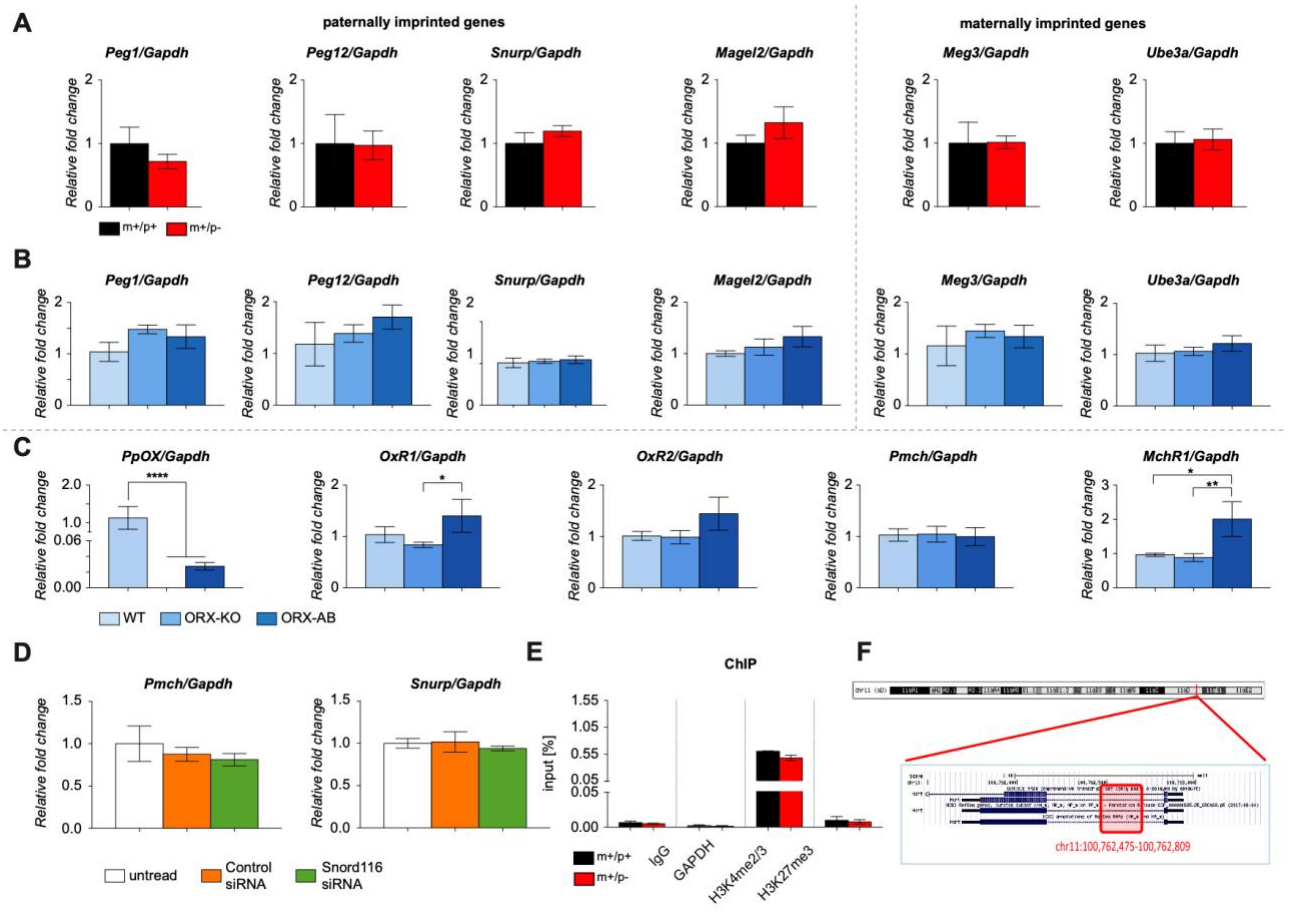


Figure S5

Gene expression analysis of the maternally and paternally imprinted genes in PWScr$_{m+/p-}$ mice and in mice of two narcoleptic models. **A)** Gene expression analysis of *Peg1, Peg12, Snurp Magel2 Meg3* and *Ube3a* in PWScr$_{m+/p-}$ mice relative to control mice. Unpaired t tests did not reveal any significant changes between the two genotypes. **B)** Gene expression of *Peg1, Peg12, Snurp Magel2 Meg3* and *Ube3a* in WT, KO and Atx mice. One-way ANOVA did not reveal any significant changes between genotypes. **C)** Gene expression analysis of *Ppox, OxR1, OxR2, Pmch* and *MchR1* in WT, KO and Atx mice. We confirmed a significant reduction in *Ppox* in both strains of narcoleptic mice relative to WT mice (one-way ANOVA: F(3,16)= 11.63, Bonferroni post hoc test p = <.0001). *OxR1* (one-way ANOVA: F(3,16)= 7.034, Bonferroni post hoc test p = <.03) and *MchR1* (one-way ANOVA: F(2,15)= 5.17, Bonferroni post hoc test p = <.01) were reduced only in Atx mice relative to WT and KO mice. Gene expression (mean ± SEM) was assessed by qRT-PCR in mice of the 2 genotypes: PWScr$_{m+/p-}$ mice (n= 5) and PWScr$_{m+/p+}$ mice (n=5). KO (n= 12) and Atx (n=4) narcoleptic mice were investigated and compared with WT mice (n=4). *Gapdh* was used as a reference gene. **D)** Gene expression analysis of *Pmch* and *Snurp* in the *Snord116*-siRNA-treated immortalised hypothalamic rat cell line (green bars). Both genes were unchanged compared with untreated cells or scrambled siRNA-treated cells (white and orange bars). **E)** ChIP analysis of PEG3 binding on the *Gapdh* promoter region in PWScr$_{m+/p-}$ mice (red) versus controls (black). Values are expressed as the mean of the input ± standard deviation. **F)** A cartoon showing the details and coordinates of the genomic region assessed in the ChIP analysis of PEG3 binding to the *Ppox* promoter region.

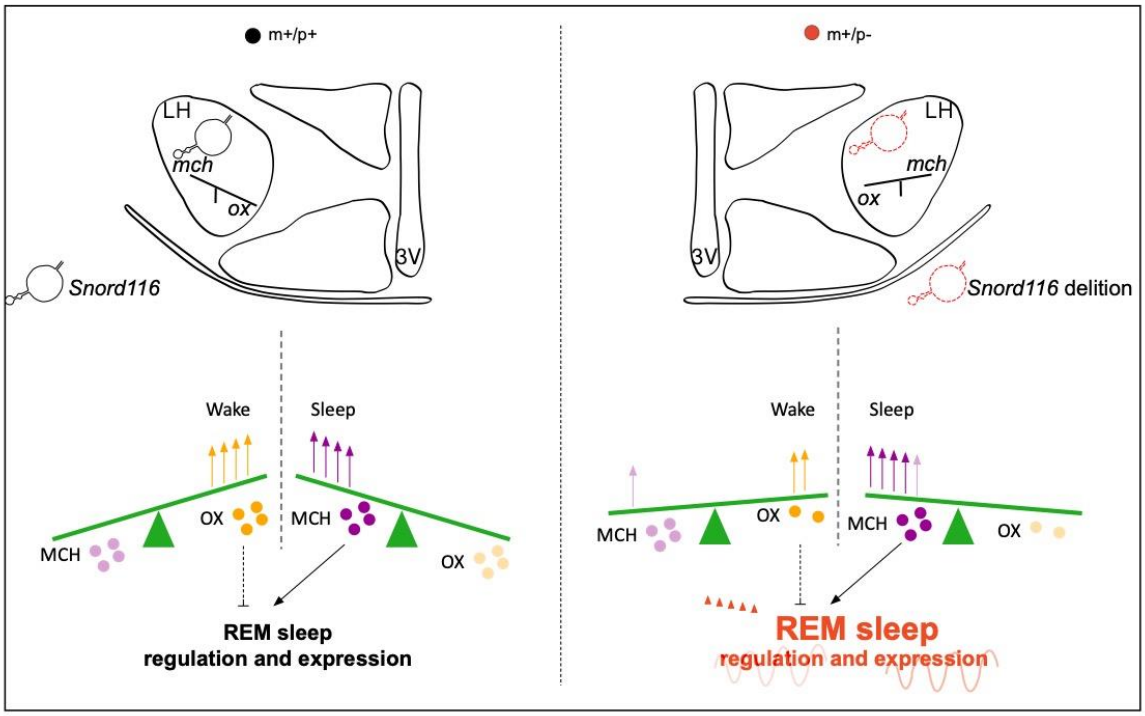


Figure S6
Snord116 is highly expressed in the hypothalamus, which is one of the brain regions that plays a pivotal role in controlling the sleep-wake cycle. Specifically, in the lateral hypothalamus (LH), there are two groups of neurons, melanin-concentrating hormone (MCH) neurons and orexin (OX) neurons, which exert antagonistic actions on the sleep-wake cycle. MCH neurons promote sleep and maximally fire during REM sleep, while OX neurons promote wakefulness and suppress REM sleep; both contribute to the sleep-wake switch. MCH and OX neurons are important in thermoregulation, which is tightly integrated with the sleep-wake cycle. REM sleep increases when the need for thermoregulatory defence is minimized in TNZ conditions. Our model suggests that Snord116 loss induces a 60% reduction in OX neurons in the LH, while MCH neurons located near OX neurons are unaffected, resulting in an imbalance between MCH and OX neurons. This imbalance between these two types of neurons may explain the sleep alterations and REM sleep disturbances observed in our model, which are also characteristic of PWS syndrome. Thus, the model suggests that *Snord116* regulates REM sleep expression induced by the TNZ via orexin neurons, resulting in an altered thermoregulatory response.

Supplementary Tables

Table S1: The number of neurons recorded over different time points (B1; B2; SD; SD1; SD2) for both genotypes. Neurons were classified as sleep (S-max) or wakefulness (W-max). S-max neurons were further distinguished between NREM (NR-max), REM (R-max) neurons, and neurons firing in both stages (NRR-max). Neurons that did not show significant changes in their firing rate across the sleep-wake states were called as neurons "ws" (wake and sleep).

Phase:	B1		B2		SD		SD1		SD2	
Genotype:	m+/p+	m+/p-	m+/p+	m+/p-	m+/p+	m+/p-	m+/p+	m+/p-	m+/p+	m+/p-
W-max	90	60	104	43	48	55	77	73	96	54
Wake										
NR - max	2	21	1	19	6	27	6	17	24	23
R - max	2	4	2	4	2	17	5	5	2	11
NRR - max	1	5	3	7	15	3	1	9	1	12
Sleep										
wsp	9	11	1	9	14	11	13	5	4	16
WR - max	3	4	6	6	31	8	0	4	0	5
None										
Total	107	105	117	88	116	121	102	113	127	121

Genotype:	m+/p+	m+/p-
W-max	415	285
Wake		
NR - max	39	107
R - max	13	41
NRR - max	21	36
Sleep		
wsp	41	52
WR - max	40	27
None		
Total	569	548

Table S2: Statistical analysis was performed by using X_2 test in which we compared one class of neurons to all other classes over different time points (B1; B2; SD; SD1; SD2). The three classes of sleep neurons were W-max S-max and ws.

B1	m+/p+	m+/p-	Totals
W-Max	90	60	150
Others	17	45	62
Totals	107	105	212
	p-value		<.0001

	m+/p+	m+/p-	Totals
S-max	5	30	35
Others	102	75	177
Totals	107	105	212
	p-value		<.0001

	m+/p+	m+/p-	Totals
ws	12	15	27
Others	95	90	185
Totals	107	105	212
	p-value		ns

B2	m+/p+	m+/p-	Totals
W-Max	104	43	147
Others	13	45	58
Totals	117	88	205
	p-value		<.0001

	m+/p+	m+/p-	Totals
S-max	6	30	36
Others	111	58	169
Totals	117	88	205
	p-value		<.0001

	m+/p+	m+/p-	Totals
ws	7	15	22
Others	110	73	183
Totals	117	88	205
	p-value		.02

SD	m+/p+	m+/p-	Totals
W-Max	48	55	103
Others	68	66	134
Totals	116	121	237
	p-value		ns

	m+/p+	m+/p-	Totals
S-max	23	47	70
Others	93	74	167
Totals	116	121	237
	p-value		.0017

	m+/p+	m+/p-	Totals
ws	45	19	64
Others	71	102	173
Totals	116	121	237
	p-value		<.0001

SD1	m+/p+	m+/p-	Totals
W-Max	77	73	150
Others	25	40	65
Totals	102	113	215
	p-value		ns

	m+/p+	m+/p-	Totals
S-max	12	31	43
Others	90	82	172
Totals	102	113	215
	p-value		.059

	m+/p+	m+/p-	Totals
ws	13	9	22
Others	89	104	193
Totals	102	113	215
	p-value		ns

SD2	m+/p+	m+/p-	Totals
W-Max	96	54	150
Others	31	67	98
Totals	127	121	248
	p-value		<.0001

	m+/p+	m+/p-	Totals
S-max	27	46	73
Others	100	75	175
Totals	127	121	248
	p-value		.052

	m+/p+	m+/p-	Totals
ws	4	21	25
Others	123	100	223
Totals	127	121	248
	p-value		.0002

Table S3: Statistical analysis performed by using X_2 test between the baseline and all others time points (B2; SD; SD1; SD2) between the two genotypes

B2; SD; SD1; SD2

m+/p+	B1	B2	Totals
ws	12	7	19
W-max	90	104	194
S-max	5	6	11
Totals	107	117	224
		p-value	ns

m+/p-	B1	B2	Totals
ws	15	15	30
W-max	60	43	103
S-max	30	30	60
Totals	105	88	193
		p-value	ns

m+/p+	B1	SD	Totals
ws	12	45	57
W-max	90	48	138
S-max	5	23	28
Totals	107	116	223
		p-value	< .0001

m+/p-	B1	SD	Totals
ws	15	19	34
W-max	60	55	115
S-max	30	47	77
Totals	105	121	226
		p-value	ns

m+/p+	B1	SD1	Totals
ws	12	13	25
W-max	90	77	167
S-max	5	12	17
Totals	107	102	209
		p-value	ns

m+/p-	B1	SD1	Totals
ws	15	9	24
W-max	60	74	134
S-max	30	31	61
Totals	105	114	219
		p-value	ns

m+/p+	SD1	SD2	Totals
ws	12	4	16
W-max	90	96	186
S-max	5	27	32
Totals	107	127	234
		p-value	.0001

m+/p-	B1	SD2	Totals
ws	15	21	36
W-max	60	54	114
S-max	30	46	76
Totals	105	121	226
		p-value	ns

Table S4: Statistical analysis was performed by using X_2 test in which we compared one class of neurons to all other classes over different time points (B1; B2; SD; SD1; SD2). The three classes of food-related neurons were Type I, Type II, Type III.

	m+/p+	m+/p-	Totals
Type I	28	16	44
Others	119	58	177
Totals	147	74	221
		p-value	ns

	m+/p+	m+/p-	Totals
Type II	105	22	127
Others	42	52	94
Totals	147	74	221
		p-value	< .0001

	m+/p+	m+/p-	Totals
Type III	14	36	50
Others	133	38	171
Totals	147	74	221
		p-value	< .0001

Table S5: List of primers selected for Real-time quantitative PCR

	m+/p+	m+/p-	Totals
Type I	28	16	44
Others	119	58	177
Totals	147	74	221
		p-value	ns

	m+/p+	m+/p-	Totals
Type II	105	22	127
Others	42	52	94
Totals	147	74	221
		p-value	< .0001

	m+/p+	m+/p-	Totals
Type III	14	36	50
Others	133	38	171
Totals	147	74	221
		p-value	< .0001

Table S6: List of primers selected for Chromatin immunoprecipitation (ChIP)

CHIP Primers		
GENE	**FORWARD**	**REVERSE**
PPHCRT	5′ – TCCTTTGCTGGGGAAACTGT – 3	5′ – AGAGAGGAGTATGGGTGGGT – 3′
SNRPN	5′ – TAACACACCCAAGGAGTCCG – 3	5′ – GACTAGCGCAGAGAGAGGAGAG –
GAPDH	5′ – CCCAGCCAAGTTTGAAAGGG – 3	5′ – GCATCTCCCTCACACACCTCTT – 3′

Table S7: List of primers selected for *Snord116* and *Peg3* gene expression analysis in the *Snord116* siRNA–treated immortalized hypothalamic rat cell line.

RATS primers		
GENE	**FORWARD**	**REVERSE**
PPHCRT	5′ – AACCTTCCTTCTACAAAGGTTCC – 3′	5′ – CAGCTCCGTGCAACAGTTC– 3′
PMCH	5′ – CACAAAGAACACAGGCTCCA – 3′	5′ – TTCCCTCTTTTCCTGTGTGG – 3′
Peg3	5′ – GGGGAGTGCTACCTTCTTGA – 3′	5′ – CTGTTTTGCTCACACCCAAG – 3′
Snord116	5′ – TGCTTGGATCGATGATGATTT – 3′	5′ – CTGGACCTCAGTCACGATGAT – 3′
Snrpn/Snurf	5′ – CAGCAATCATGACTGTGGGTA – 3′	5′ – TCTTTGGCTTGATCTTCCTGA – 3′
Gapdh	5′ – GAACATCATCCCTGCATCCA – 3′	5′ – CCAGTGAGCTTCCCGTTCA – 3′

Table S8: List of differentially expressed genes (DEGs) in the both PWScr$^{m+/p+}$ mice and PWScr$^{m+/p-}$ mutant mice at three different time points at the beginning of the light period ZT 0 (group 1; G1), 6 hours later at ZT 6 (G2), and at ZT 6 but following 6 hours of SD (G3). Additionally, DEGs derived from postmortem hypothalamic data from PWS patients versus healthy controls were also included. All genes listed passed the criteria of $P < 0.05$ P and with > 2-fold change.

Table S9: Complete list of differentially expressed genes (DEGs) presented in Venn diagrams.