Neurobiology of Disease

Supplemental materials

Deficiency of autism-related *Scn2a* gene in mice disrupts sleep patterns and circadian rhythms

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Supplementary figure 1

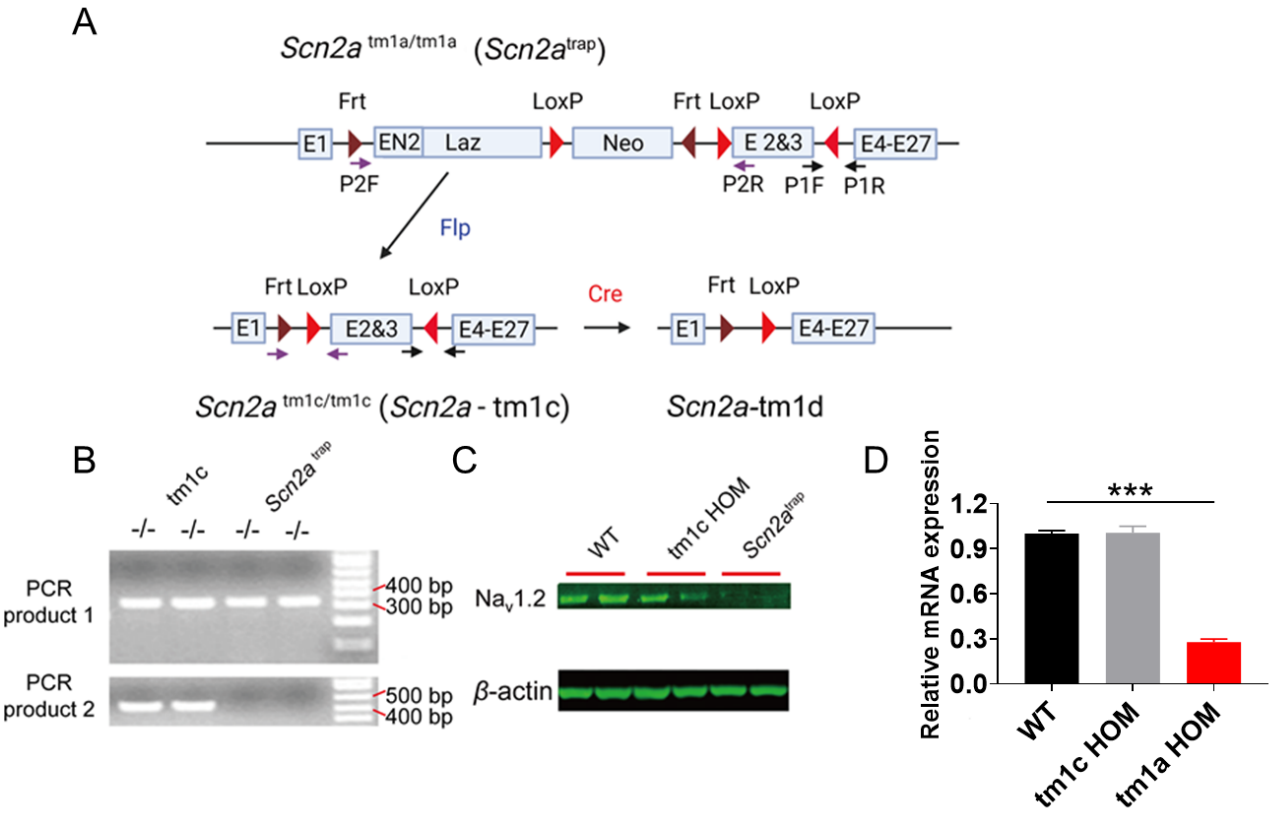

Figure S1. Genetic manipulation of *Scn2a* in mice. A, **upper panel**: Diagram of the tm1a gene-trap cassette between Exon 1 and Exon 2 of *Scn2a* gene in the genome. Abbreviations: *Frt*, Flp recognition target; *En2*, engrailed-2 splice acceptor; *LacZ*, lacZ β-galactosidase; *LoxP*, locus of X-over P1; and *Neo*, neomycin. P1F and P1R: genotyping primer set for *Scn2a*-tm1a, amplified the PCR product 1. P1F, P1R, P2F, and P2R: primer sets for genotyping *Scn2a*-tm1c, amplified the PCR product 1 and 2, separately. **Lower panel, left**: diagram of the *Scn2a*-tm1c (*Scn2a*$^{fl/fl}$) "conditional ready" allele. **Lower panel, right**: Diagram of the conditional knockout which removed the Exon 2 and Exon 3 of *Scn2a* gene by Cre recombinase (*Scn2a*-tm1d). **B**, Representative genotyping results for the *Scn2a*-tm1a (PCR product 1) and *Scn2a*-tm1c (PCR product 1 and 2). **C**, Representative Western blot image for *Scn2a*$^{WT/WT}$, *Scn2a*$^{tm1c/tm1c}$, and *Scn2a*$^{tm1a/tm1a}$ (*Scn2a*^{trap}) mice. **D**, Bar graph for the relative mRNA expression of *Scn2a* (n=4 mice, one-way *ANOVA* analysis, ***p< 0.001).

Supplementary figure 2

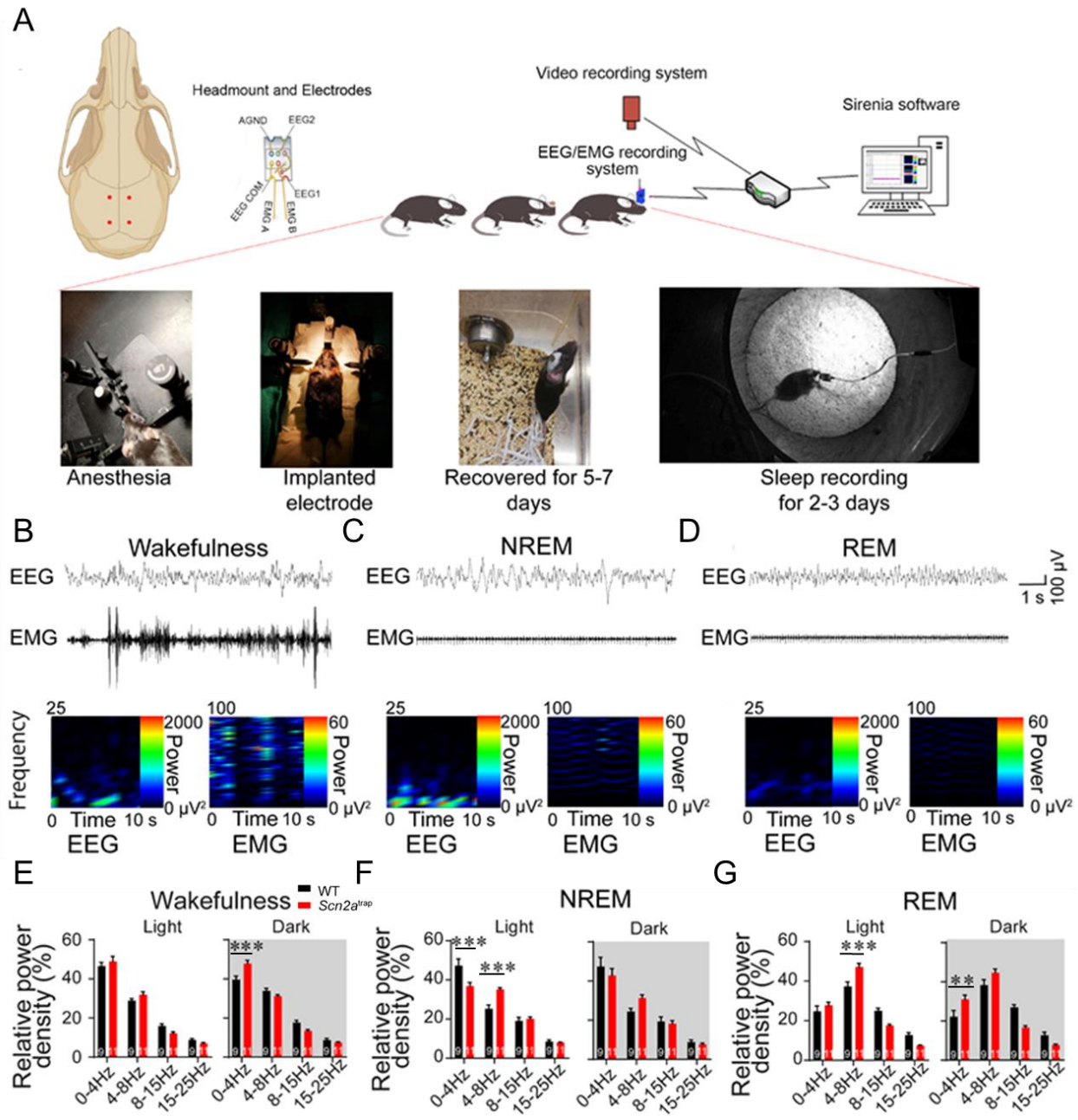

Figure S2. EEG analysis of *Scn2a*-deficient mice. A, Scheme of electrode, screw positions in the skull of mice and video-EEG recording system. **B**-**D**, Representative EEG and EMG coupled with power spectrum heatmap for wakefulness (**B**), NREM (**C**), and REM (**D**) sleep, respectively. **E-G,** power bands under the light period (left) and dark period (right) for wakefulness (**E**), NREM (**F**), and REM (**G**). Each band's spectral power was normalized as a percentage of the total state-specific EEG power to its corresponding time phase. Values are presented as means ± SEM. Data from wild-type (WT, black) and *Scn2a*^{trap} (red) mice were compared by two-way *ANOVA* with post-hoc comparisons using Bonferroni's correction. (*p<0.05; **p<0.01; ***p<0.001; n= at least 9 WT and 11 *Scn2a*^{trap} mice).

Supplementary figure 3

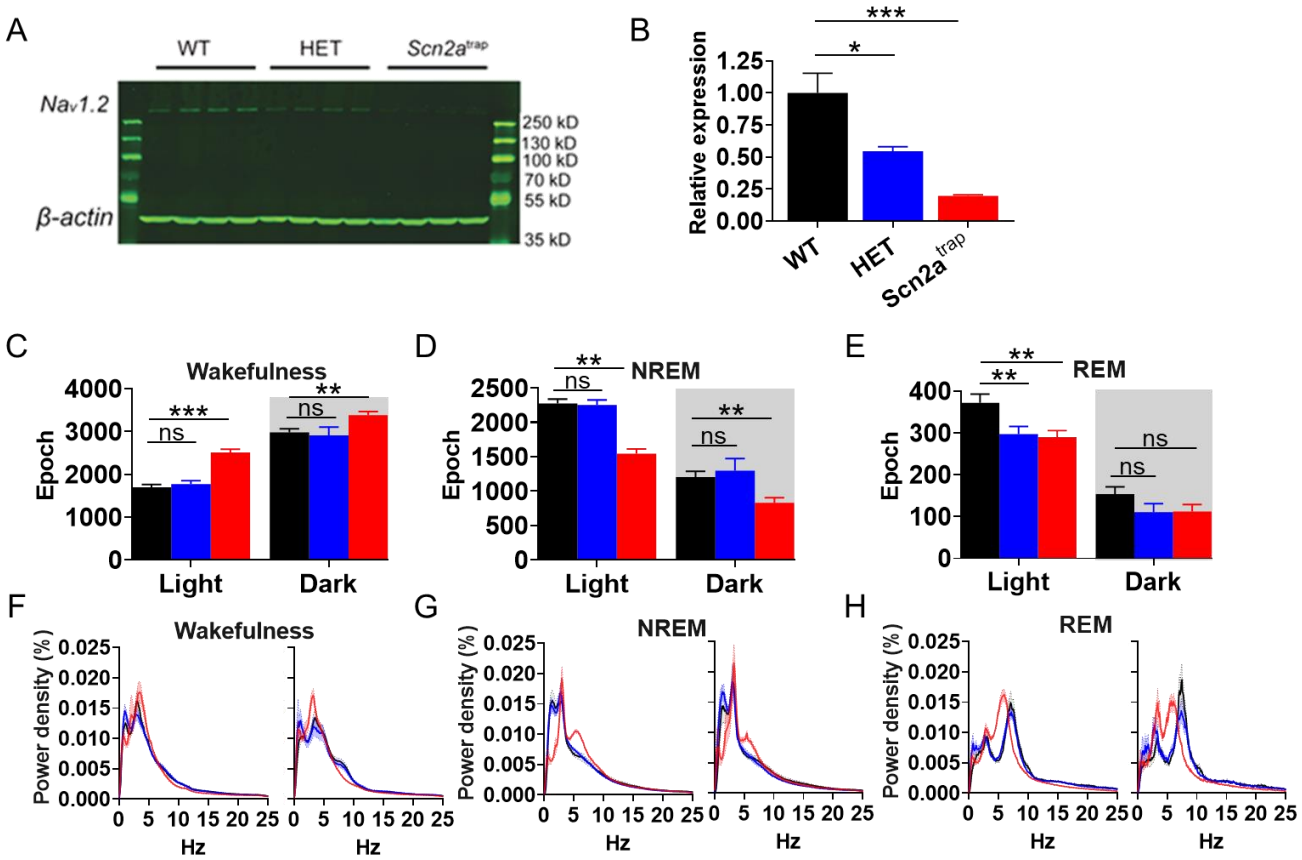

Figure S3. Sleep is disrupted in homozygous (but not heterogenous) gene-trap *Scn2a*-deficient mice. A, Western blot shows the Nav1.2 protein level in WT (n=4), HET (n=4), and *Scn2a*^{trap} (n=4) mice. **B**, Bar graph shows a 100%, ~50%, and ~20% protein level in WT, HET, and *Scn2a*^{trap} mice, respectively. **C-E**, Epoch numbers were counted for wakefulness (**C**), NREM (**D**), and REM (**E**) in light (left) and dark (right) phase among WT, HET, and *Scn2a*^{trap} mice, respectively. Data were analyzed by two-way *ANOVA* with post-hoc Bonferroni test. **F-H,** continuous power density spectrum for wakefulness (**F**), NREM (**G**), and REM (**H**) in light (left) and dark (right) phase among WT, HET, and *Scn2a*^{trap} mice, respectively. Data are presented as means ± SEM (*p<0.05; **p<0.01; ***p<0.001; ns p>0.05).

Supplementary figure 4

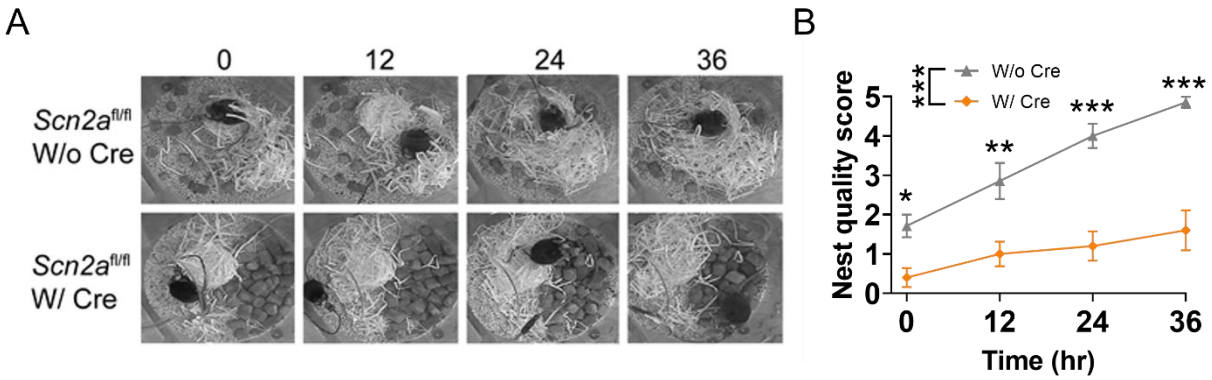

Figure S4. Cre-recombinase mediated *Scn2a* deficiency in SCN containing region impairs nesting behavior. A, Representative nest building of tm1c (*Scn2a*$^{fl/fl}$) mice with Cre-virus (W/ Cre) or control virus (W/o Cre) injection at 0, 12, 24, and 36 hours. **B**, Line graph for nesting score over 36 hours. Data are presented as means ± SEM and analyzed by a repeated measures two-way *ANOVA* with post-hoc Bonferroni's multiple comparisons tests (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Supplementary figure 5

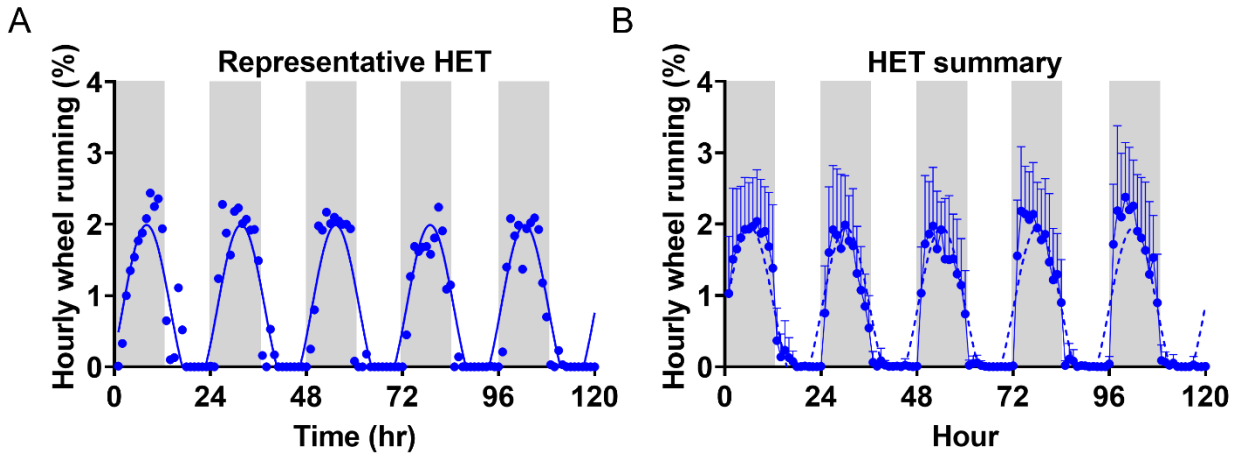

Figure S5. Light-dark wheel-running activity is largely normal in HET *Scn2a*-deficinet mice. **A**: Representative relative wheel-running activity and sine wave fitting for heterozygous (HET) mice. Individual dots show the normalized activity per hour for HET mice, solid lines show the sine wave fitting of the data for HET mice. **B**, averaged relative wheel-running activity and sine wave fitting for HET mice (n = 17 mice). Gray shading represents the dark period. Values represent means ± SEM. Dash line shows the sine wave fitting.

Supplementary figure 6

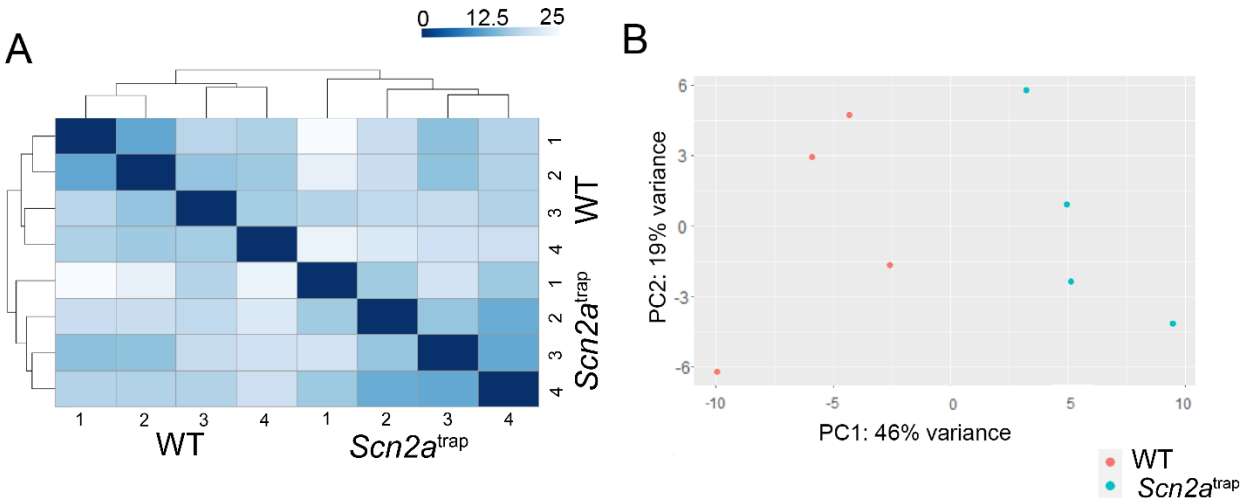

Figure S6. Overall analysis of the RNA-sequencing between *Scn2a*^{trap} and WT mice. A, Relationship of transcriptome profiles among WT and *Scn2a*^{trap} samples (n = 4 mice per group). A heatmap of sample-to-sample distance matrix with hierarchical clustering using counts of detected transcripts. The shade of blue represents the distance between the samples, showing an overview of similarities in the same genotype and dissimilarities between different genotypes. **B**, Principal component analysis (PCA) of RNA-seq data from WT and *Scn2a*^{trap} mice (n = 4 mice per group), showing the primary separation of samples by genotype. The first two principal components (PC1 and PC2) of the gene expression dataset are plotted here for each of the samples. PCA plot showing the variance of the four biological replicates of the two genotypes.

Supplementary figure 7

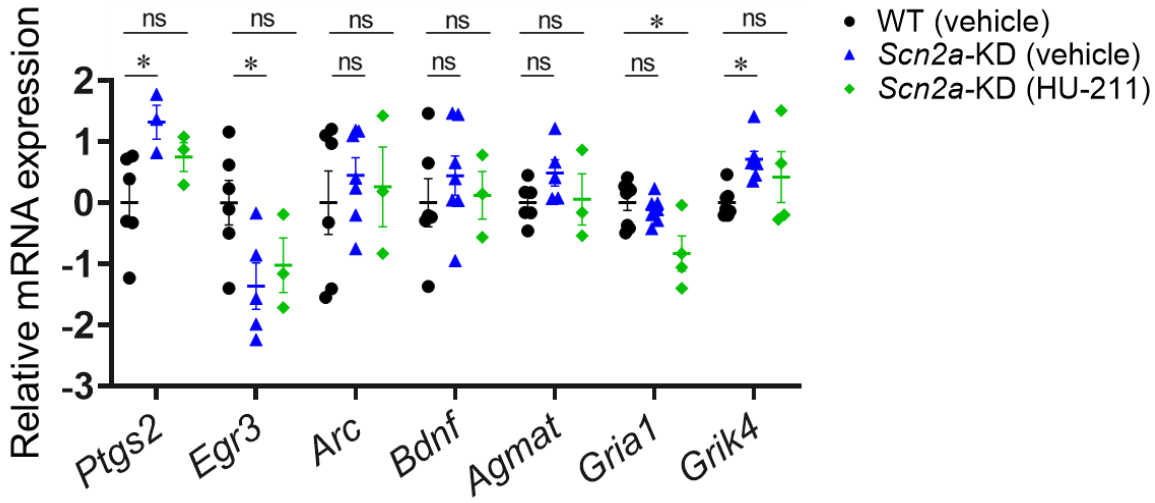

Figure S7. qPCR assay of the gene expression patterns with HU-211 treatment. *Ptgs2*, *Erg3*, *Arc*, *Bdnf*, *Agmat*, *Gria1* and *Grik4* genes predicted by connectivity map was tested in the SCN containing hypothalamus samples of WT mice treated with the vehicle, *Scn2a*-KD mice treated with vehicle, and *Scn2a*-KD mice treated with HU-211 (student's *t-test*; * $p<0.05$; ns $p>0.05$).