in vitro electroporation

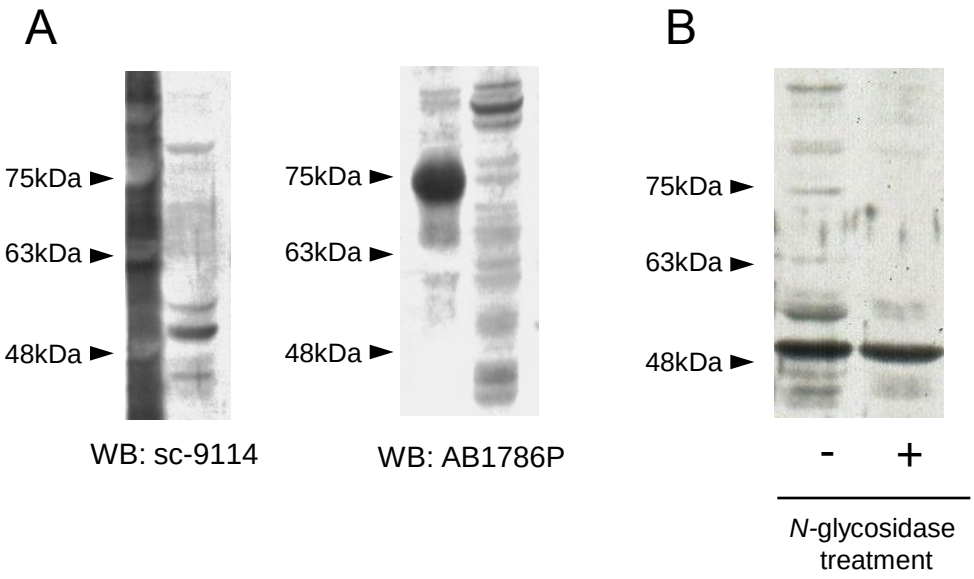
The cell suspension ($1X10^6$ cells) was mixed with plasmid 1 μg pcDNA3.1 intact vector or pcDNA3.1 human drd3 in Opti-MEM™ Media (GIBCO). The expression plasmid of pcDNA3.1 was obtained by Missouri S&T cDNA Resource Center. The cell and plasmid suspension was then transferred to a cuvette, and the plasmids were transferred to the cells by electroporation using Super Electroporator NEPA21 (NEPA GENE, Co. Ltd, Ichikawa, Japan). Square electric pulses were applied at 150 V (pulse length, 0.5 ms; two pulses; interval, 50 ms), followed by additional pulses at 20 V (pulse length, 50 ms; five pulses; interval, 50 ms).

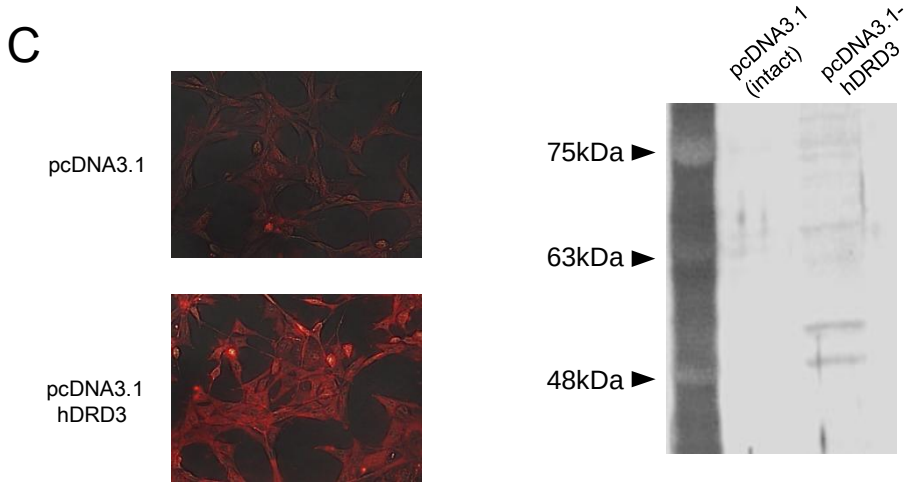
Cell culture and immunocytochemistry

The transfected cells were cultured in the same medium for 2 days in vitro. The cells were then fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature (RT). Subsequently, cells were treated with PBS containing 3% Bovine Albumin (BSA) for 1 h at RT. This was followed by incubation in the primary antibody diluted in PBS with 0.1% BSA for 2 h at overnight at 4 °C. The primary antibodies used were as follows: rabbit anti-DRD3 (1 : 250; Santa Cruz Biotechnology). The sections were incubated for 1 h at RT in the secondary antibody diluted in PBS with 0.1% BSA. The secondary antibodies used were as follows: Cy3 anti-rabbit (1 : 1000; Sigma–Aldrich, St. Louis, MO). Photographs were taken with a fluorescence microscope (Biozero BZ-9000 Keyence).

Deglycosylation Assay

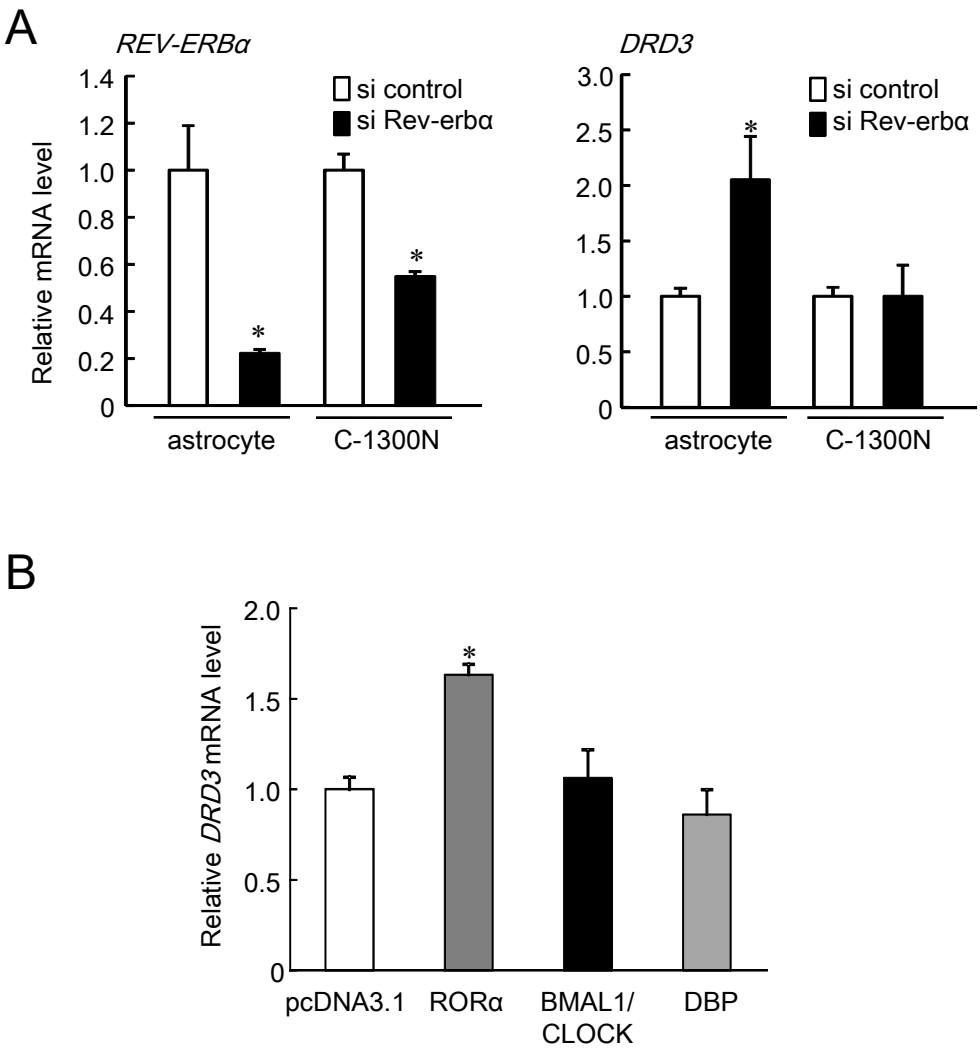
Protein samples were incubated with *N*-glycosidase F for 17 h at 37°C according to the supplier's instructions (TaKaRa Bio Inc.). We used 1mU *N*-glycosidase F per 25 μg of glycoprotein. Controls were incubated without enzyme. Analysis was carried out by 8 % SDS_PAGE under reducing conditions, and immunoblots were revealed by DRD3 antibody (Santa Cruz).



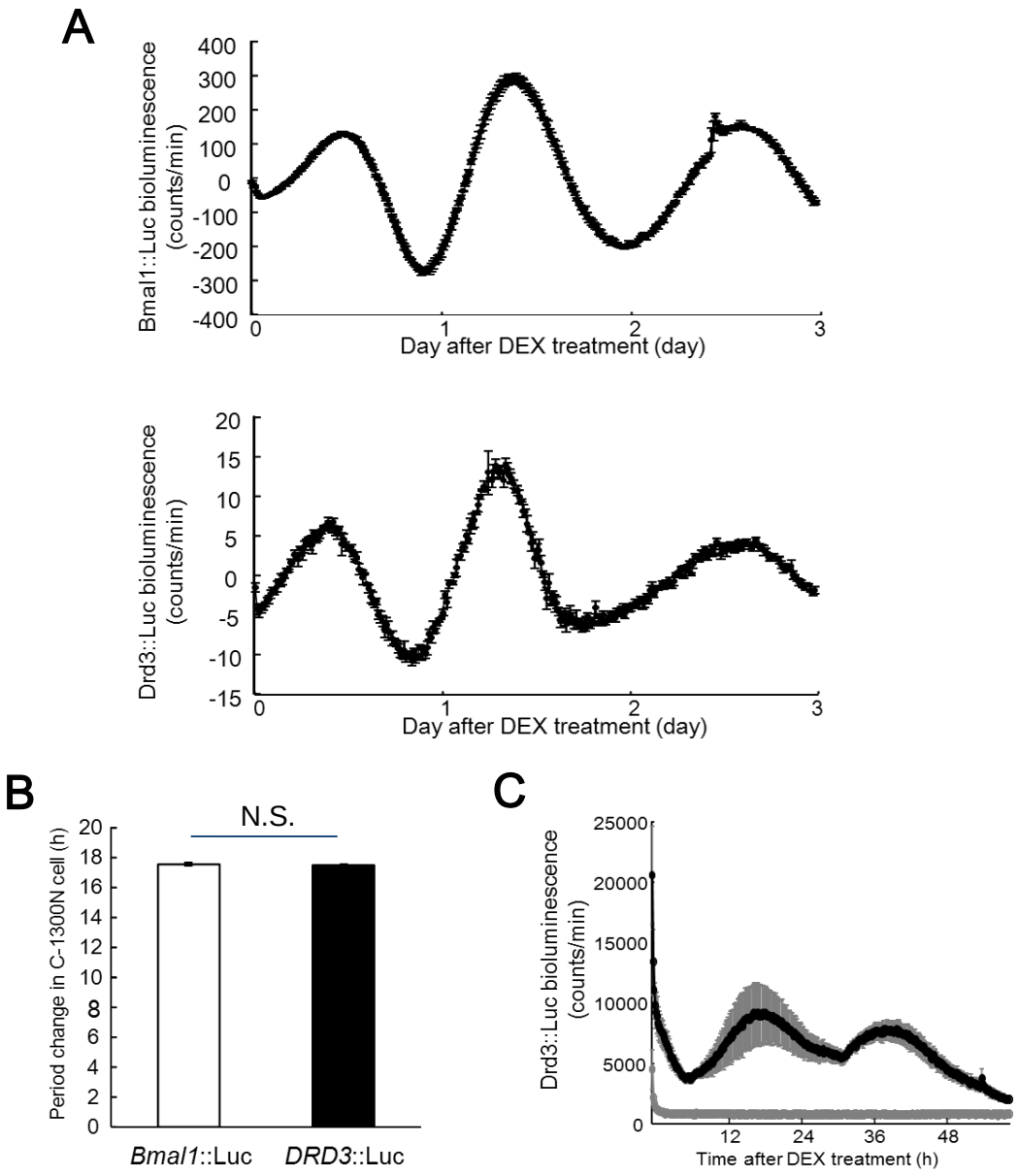


Supplemental Figure 1 Recognition of both glycosylation and unglycosylation forms of DRD3 protein by anti-DRD3 antibody (sc-9114). (A) Representative photographs of western blot analysis for DRD3 protein in ventral striatum of mice using two different antibodies, sc-9114 (left) and AB1786P (right). The band around 50kDa reveals immature DRD3 protein. Several bands above 50kDa indicate glycosylation form of DRD3 protein. Data shown were confirmed in three independent experiments. (B) Representative photographs of western blot analysis for DRD3 protein in ventral striatum of mice after treatment with *N*-glycosidase. Protein samples prepared form ventral striatum of mice were treated with *N*-glycosidase (Takara Bio Inc.) at 37 ^{o}C for 17 h. The digested protein samples were subjected to western blot analysis using anti-DRD3 antibody (sc-9114). Data shown were confirmed in three independent experiments. (C) Representative photographs of immunohistochemical (left) and western blot (right) analyzes for DRD3 protein in NIH3T3 cells transfected with human DRD3 expression constructs or pcDNA 3.1 empty vectors. Data shown were confirmed in three independent experiments.

Supplemental Figure 1

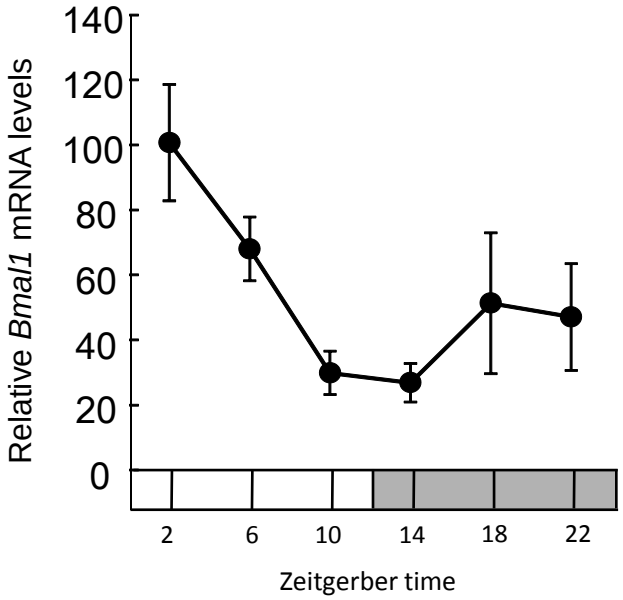


Supplemental Figure 2 Influence of siDRD3 on expression of DRD3 in astrocyte cell. (A)The expression level of *REV-ERBα or DRD3* mRNA in siRNA transfected astrocyte or C-1300N cell at 44 hr after serum shocked. Each value is the mean ± S.E. (n=3). *; P<0.05 compared with si control. (B) Transcriptional regulation of endogenous *DRD3* mRNA by clock genes. Astrocytes were transfected with expression plasmids (2 μg; each of RORα, BMAL1, CLOCK and DBP). Each value is the mean ± S.E. (n=3). *; P<0.05 compared with pcDNA.



Supplemental Figure 3 Influence of REV-ERBα on circadian oscillation. (A) Upper panel: Representative traces of bioluminescent oscillations driven by *Bmal1::luc* in C-1300N cells with the lumicycle (neuroscience). Lower panel: Representative traces of bioluminescent oscillations driven by *Drd3::luc* in C-1300N cells.(mean ±S.E. (n=3)) (B) The period change in C-1300N cells. Each column represents the mean ±S.E. (n=3) (*Bmal1*::Luc vs *DRD3*::Luc；Student‘t Test；N.S) (C) Representative traces of bioluminescent oscillations driven by *Drd3::luc* in C1300N cells with the kuronos (ATTO). The C-1300N cells were transfected with 2 μg of pcDNA (black line) or 2 μg of REV-ERBα (gray line) using Lipofectamin 2000. (pcDNA; $P < 0.05$, cosinor analysis,mean ±S.E. (n=3))

Supplemental Figure 3



Supplemental Figure 4 Temporal expression profile of *Bmal1* mRNA in the ventral striatum. The data was normalized using β-actin as a control. For intensity plots, the mean value of ZT2 was a set at 100. Each value represents the mean ± S.E. (n=6. $p<0.05$; cosinor analysis)