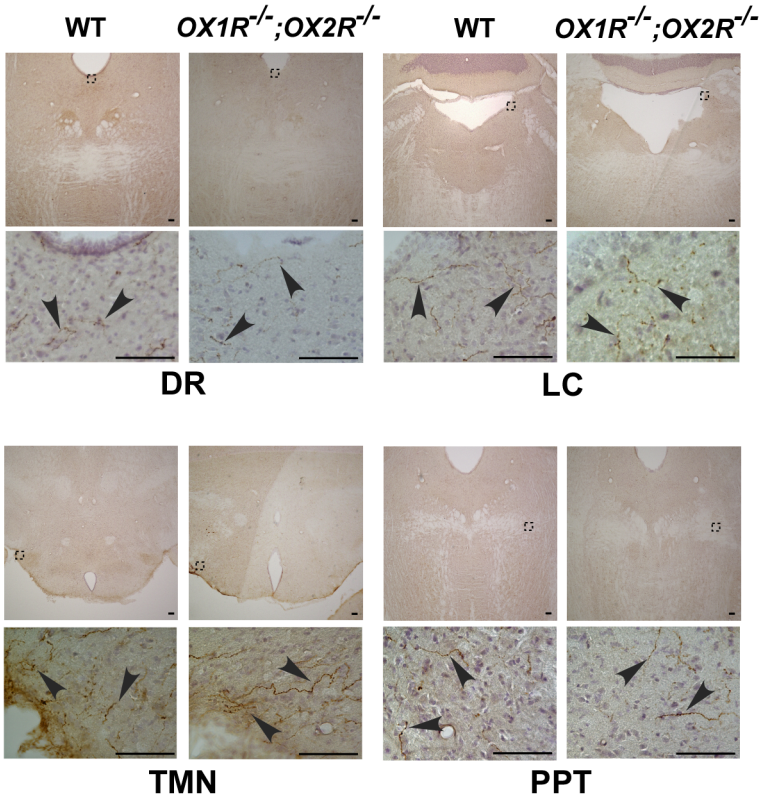
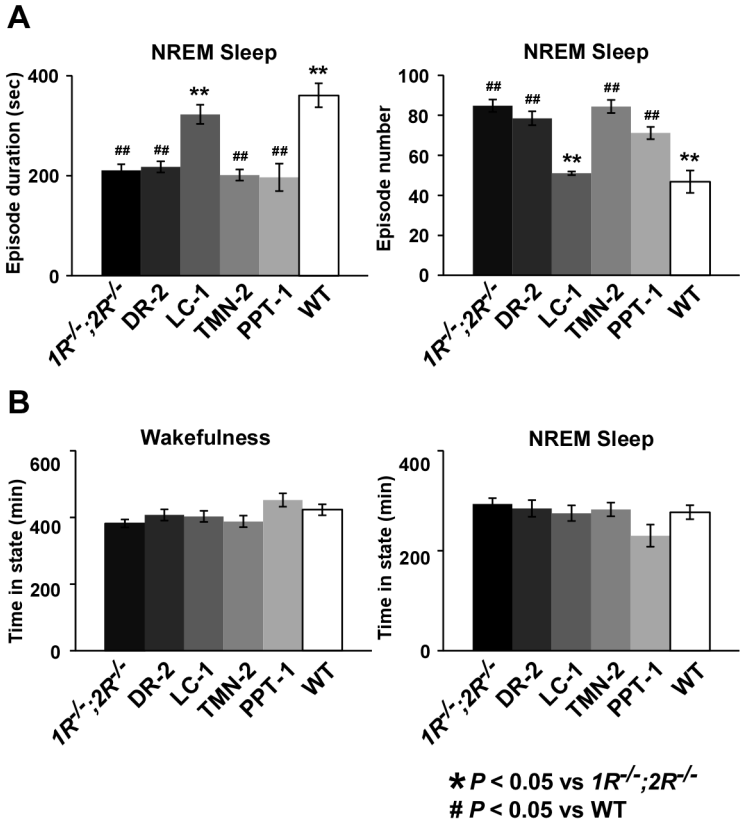
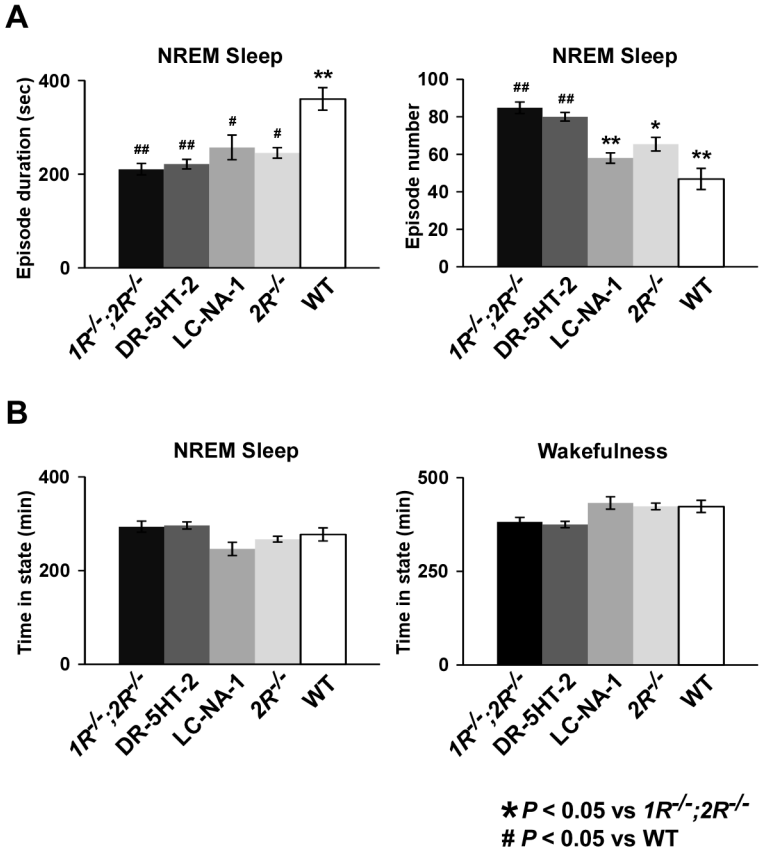
## Supplemental Figures



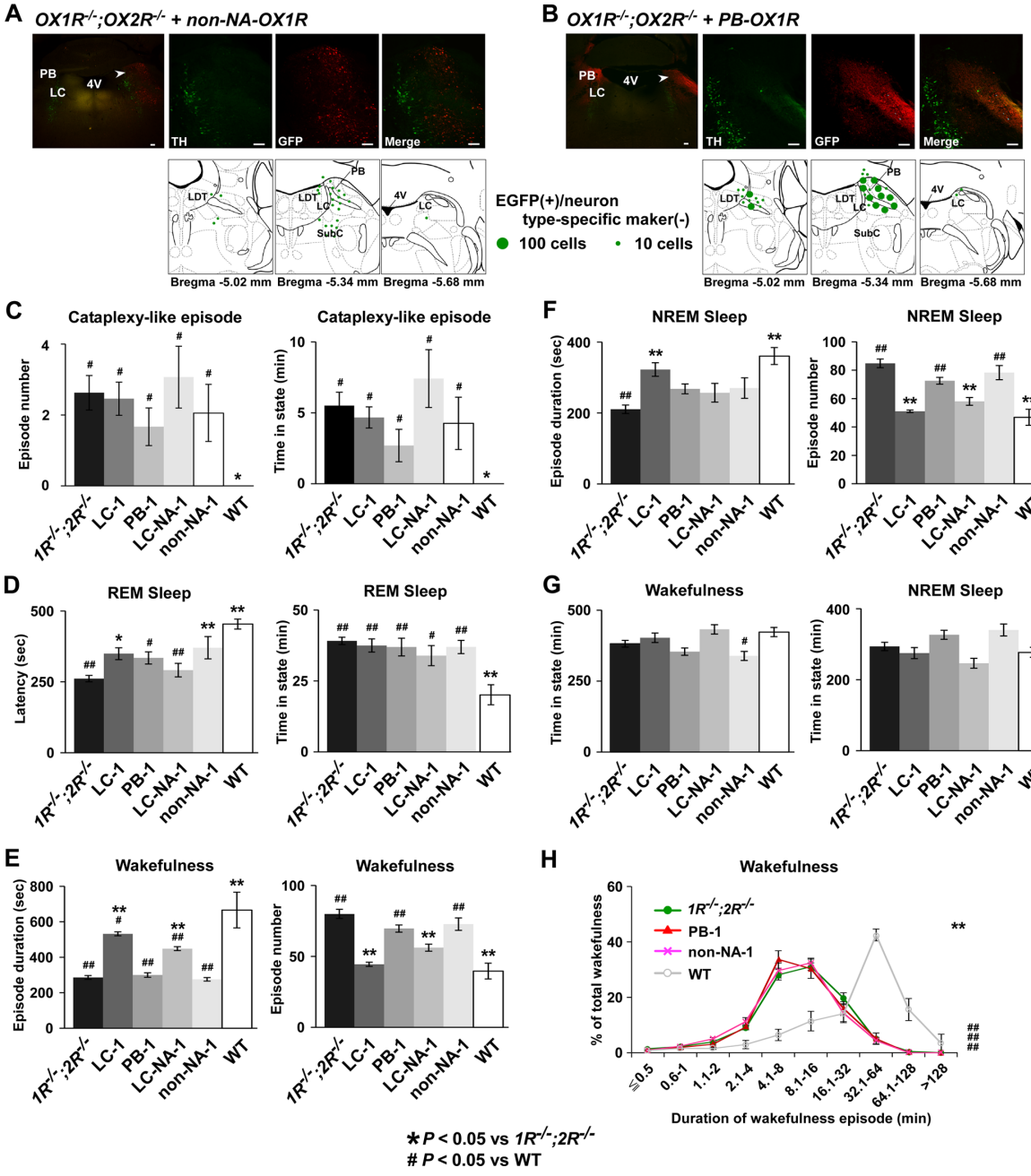

**Supplemental Figure 1.** Projections of orexin neurons are preserved in *OX1R*$^{-/-}$;*OX2R*$^{-/-}$ mice. Coronal brain sections prepared from *OX1R*$^{-/-}$;*OX2R*$^{-/-}$ and wild-type mice were stained brown with anti-orexin A antibody and counterstained with hematoxylin. Orexin-immunoreactive nerve fibers are indicated by arrowheads. The locations of the magnified images are indicated by boxes in the low-power images. Scale bars, 100 $\mu$m.




**Supplemental Figure 2.** Region-specific restoration of orexin receptor expression in *OX1R*$^{-/-}$*;OX2R*$^{-/-}$ mice. (A) Mean duration and number of NREM sleep episodes. (B) Total time spent in wakefulness and NREM sleep during the dark phase in *OX1R*$^{-/-}$*;OX2R*$^{-/-}$ mice with or without the targeted restoration of orexin receptor expression in the indicated nuclei (and the subtype of orexin receptor) and wild-type (WT) mice in the dark phase. Mean values of three nights' recordings are shown. Control (*1R*$^{-/-}$*;2R*$^{-/-}$) was *OX1R*$^{-/-}$*;OX2R*$^{-/-}$ mice with AAV-CAG/EGFP injection into the DR or LC, AAV-Pet1/ChR2::EYFP in DR, or AAV-PRSx8/ChR2::EYFP in LC. *$P < 0.05$, **$P < 0.01$ vs. control; #$P < 0.05$, ##$P < 0.01$ vs. wild-type mice. Values are mean ± S.E. ($n = 16$ for control, $n = 7$ for DR-restored, $n = 8$ for LC-restored, $n = 4$ for TMN-restored, $n = 4$ for PPT-restored, $n = 5$ for PPT-restored, $n = 4$ for wild-type mice).

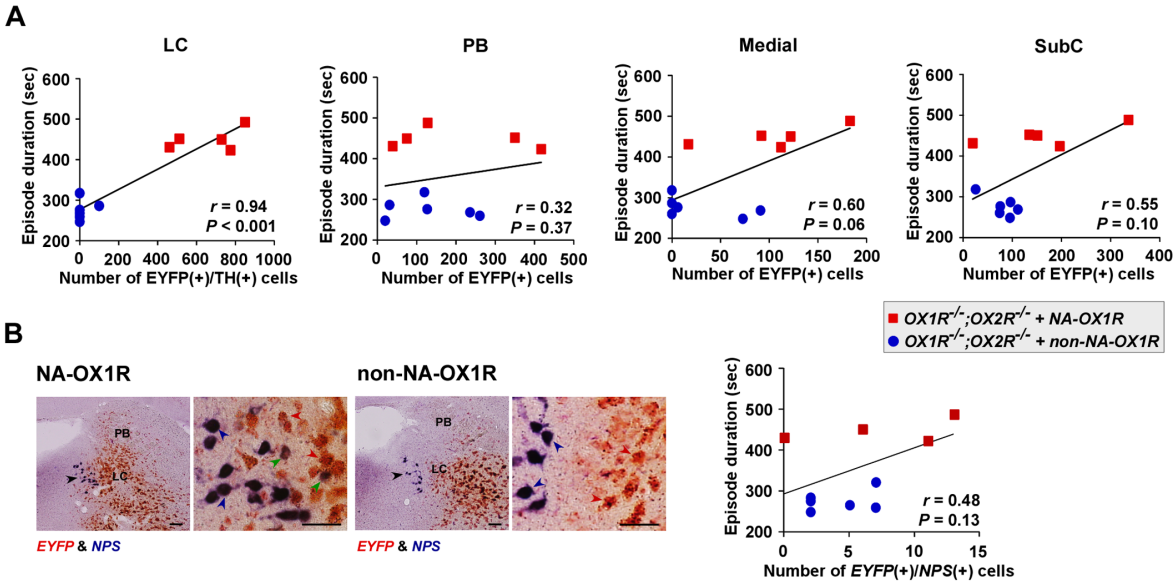



**Supplemental Figure 3.** Neuronal type-selective restoration of orexin receptor expression in the DR and LC of *OX1R*$^{-/-}$*;OX2R*$^{-/-}$ mice. (A) Mean duration and number of NREM sleep episodes. (B) Total time spent in wakefulness and in NREM sleep during the dark phase in *OX1R*$^{-/-}$*;OX2R*$^{-/-}$ mice with control virus injection (*1R*$^{-/-}$*;2R*$^{-/-}$: same as in Supplemental Figure 2), those with neuron type-selective restoration of orexin receptor expression (DR-5HT-2: DR serotonergic-selective OX2R restoration = *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+5HT-OX2R* mice; LC-NA-1: LC noradrenergic-selective OX1R restoration = *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+NA-OX1R* mice), *OX2R*$^{-/-}$, and wild-type mice (data from the Supplemental Figure 2 are included for comparison) in the dark phase. Mean values of three nights' recordings are shown. **P* < 0.05, ***P* < 0.01 vs. control; #*P* < 0.05, ##*P* < 0.01 vs. wild-type mice.

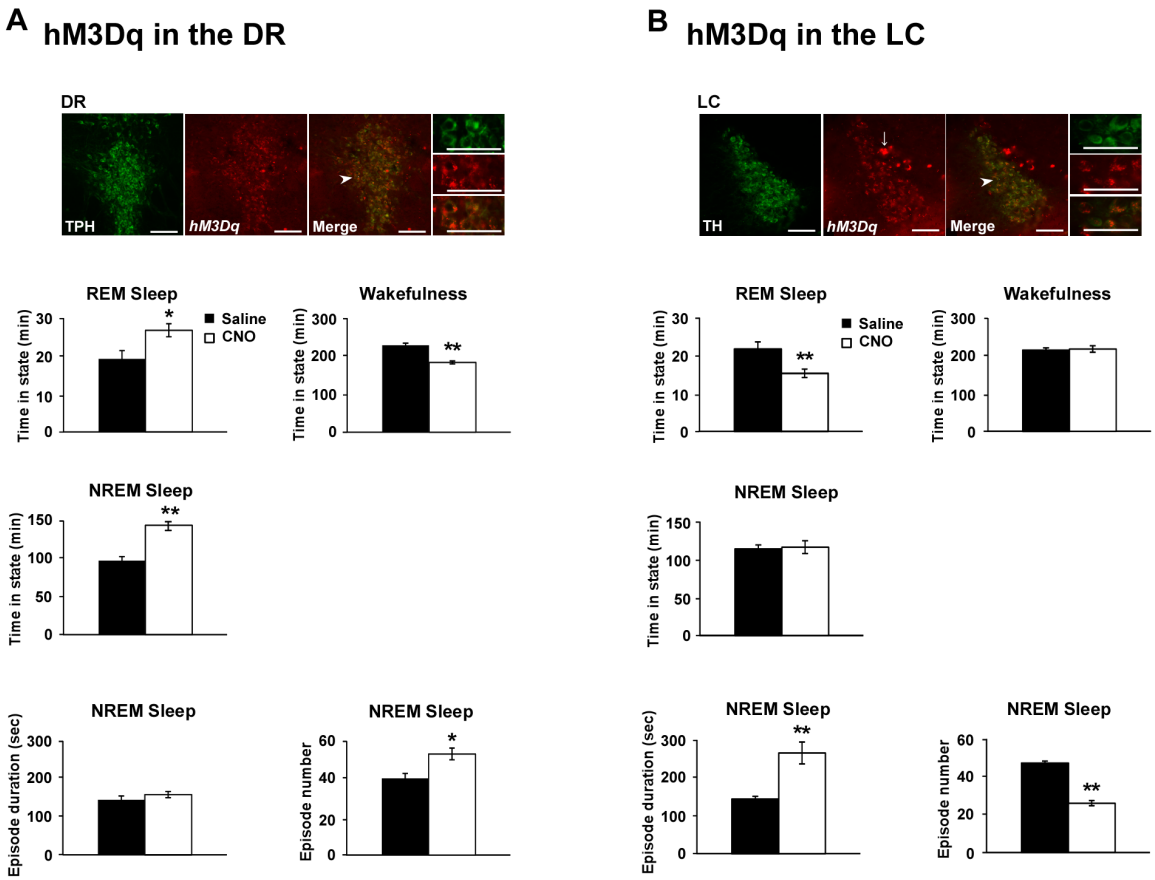



**Supplemental Figure 4.** The restoration of orexin receptor expression in the regions surrounding the LC does not ameliorate narcoleptic symptoms. (A, B) Expression of OX1R::EYFP (A) or OX1R::EGFP (B) in *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+non-NA-OX1R* mice (A), which have similar numbers of EYFP(+)/TH(-) cells but have few EYFP(+)/TH(+) cells as

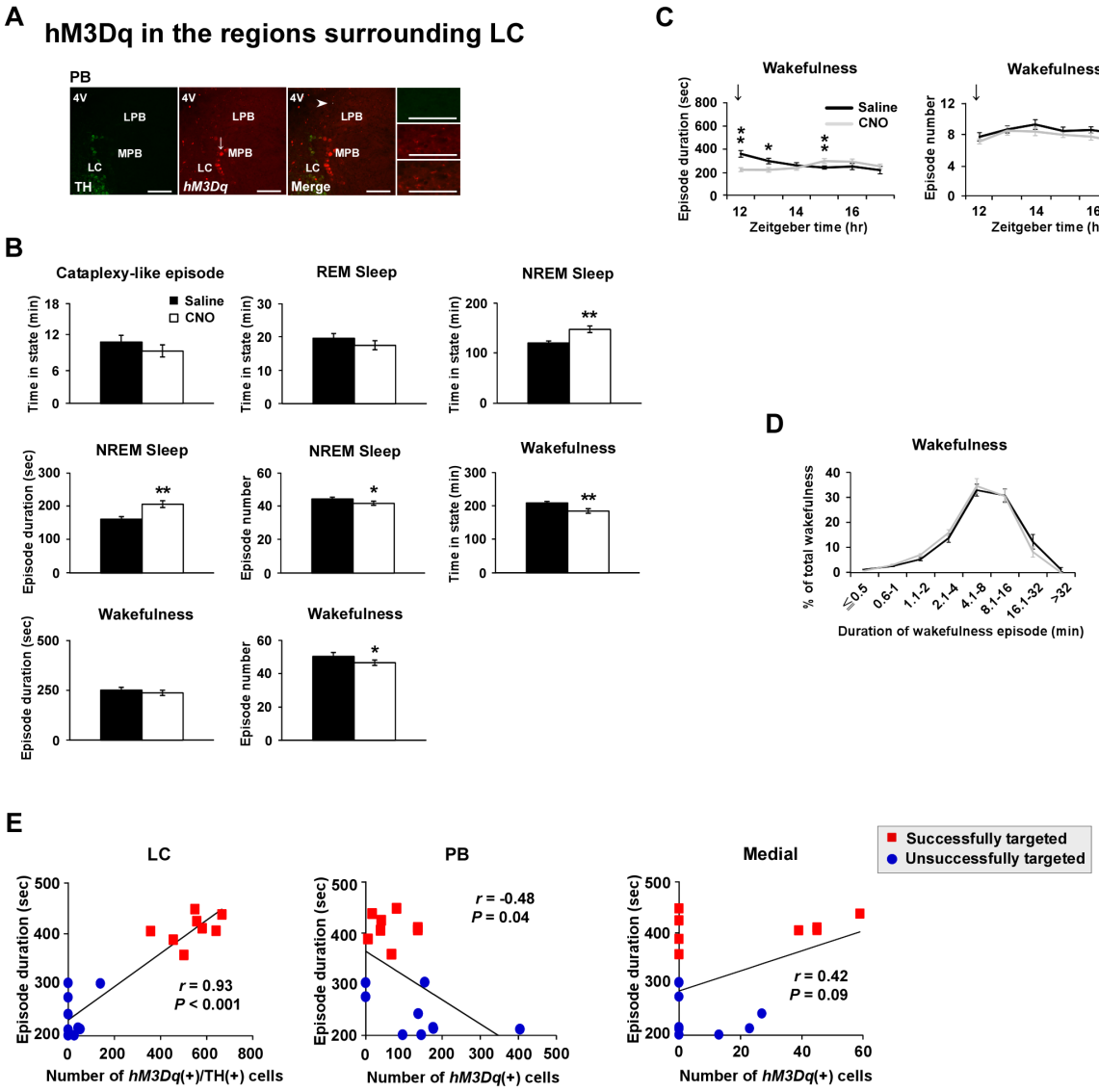
compared to *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+NA-OX1R* mice, and in *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+PB-OX1R* mice (B), which received the focal injection of AAV-EF1α/OX1R::EGFP in their PB. (C-H) Sleep/wakefulness parameters of *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+non-NA-OX1R* and *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+PB-OX1R* mice during the dark phase. (C) Number of and time spent in cataplexy-like episodes. (D) Mean REM sleep latency and time spent in REM sleep. (E) Mean duration and number of wakefulness episodes. (F) Mean duration and number of NREM sleep episodes. (G) Time spent in wakefulness and NREM sleep. **P* < 0.05, ***P* < 0.01 vs. control; #*P* < 0.05, ##*P* < 0.01 vs. wild-type mice. (H) Time-weighted frequency histograms of wakefulness duration showing the proportion of wakefulness that occurred in episodes of each length to the total amount of wakefulness in the dark phase. *1R*$^{-/-}$*;2R*$^{-/-}$, control mice as in Supplemental Figure 2 (*n* = 16); LC-1, *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+LC-OX1R* mice (*n* = 8); PB-1, *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+PB-OX1R* mice (*n* = 6); LC-NA-1, *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+NA-OX1R* mice (*n* = 5); non-NA-1, *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+non-NA-OX1R* mice (*n* = 6); WT, wild-type mice (*n* = 4).




**Supplemental Figure 5.** Consolidation of fragmented wakefulness is correlated with the number of noradrenergic neurons restored with OX1R in the LC. (A) Correlation between the mean duration of wakefulness episodes and the number of EYFP(+) cells in the LC noradrenergic neurons, or in the region lateral (including parabrachial nucleus: PB), medial, or ventral (including subcoeruleus region: SubC) to the LC of *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+NA-OX1R* mice and *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+non-NA-OX1R* mice. Pearson's correlation coefficients (*r*), *P* values, and regression lines are also shown. (B) Expression of *OX1R::EYFP* (orange stain) and *NPS* mRNA (blue stain) around the LC of *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+NA-OX1R* mice and *OX1R*$^{-/-}$*;OX2R*$^{-/-}$*+non-NA-OX1R* mice, and correlation between the mean duration of wakefulness episodes and the number of *EYFP*(+);*NPS*(+) cells in these mice. Representative *EYFP*(+);*NPS*(-), *EYFP*(-);*NPS*(+), and *EYFP*(+);*NPS*(+) cells are indicated by red, blue, and green arrowheads, respectively.

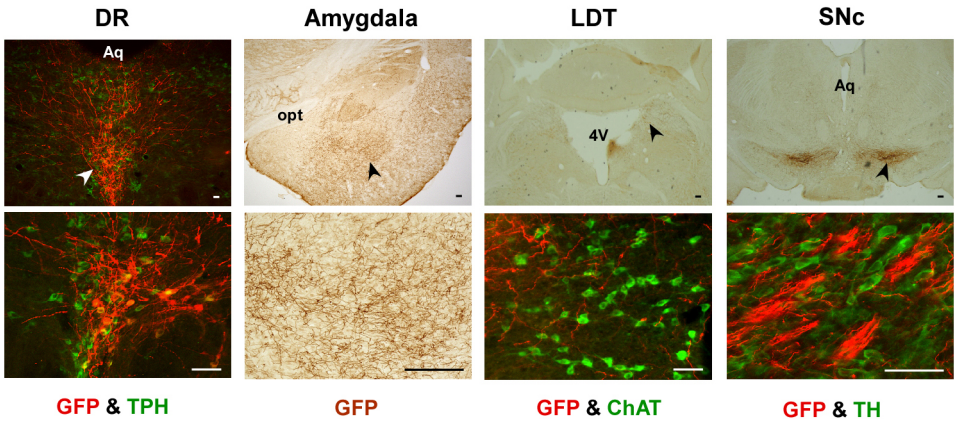



**Supplemental Figure 6.** Pharmacogenetic activations of LC noradrenergic and DR serotonergic neurons in *orexin/ataxin-3* mice. Expression of hM3Dq and sleep parameters after the injection of CNO or saline in *orexin/ataxin-3* mice with DR serotonergic (A) or LC noradrenergic (B) neuron-selective expression of hM3Dq. For expression analyses, coronal brain sections containing the DR or LC were initially hybridized *in situ* to an *hM3Dq* antisense probe (red), then were immunostained with anti-TPH or anti-TH antibodies. The locations of the magnified images are indicated by white arrowheads in the low-power images (green). The intense red staining indicated by an arrow (B) was likely to be non-specific, because similar staining was observed also in sections of mice that had not received AAV injections. Total time spent in REM sleep, wakefulness, and in NREM sleep, mean duration and number of NREM sleep episodes within 6 h after saline or CNO administration at ZT12 are shown. $*P < 0.05$, $**P < 0.01$; two-tailed Student's paired *t* test. Values are mean ± S.E. ($n = 7$ for DR, $n = 8$ for LC).




**Supplemental Figure 7.** Consolidation of fragmented wakefulness by CNO administration is correlated with the number of *hM3Dq*-expressing cells in the LC noradrenergic neurons. (A) The wake-stabilizing effect of CNO was not observed in *orexin/ataxin-3* mice with off-target hM3Dq expression, which have similar numbers of hM3Dq-expressing cells in the regions surrounding the LC but have almost no such cells in the LC. Expression of *hM3Dq* mRNA (A), time spent in, mean duration and number of each stage within 6 h after

saline or CNO administration at ZT12 are shown (B), as well as hourly plots of mean wakefulness duration and number after administrations (C) and time-weighted frequency histogram of wakefulness duration (D) within 6 h after saline or CNO administration at ZT12. *$P < 0.05$, **$P < 0.01$; two-tailed Student's paired *t* test. Values are mean ± S.E. ($n = 10$). The intense red staining indicated by an arrow was likely to be non-specific, because similar staining was observed also in sections of mice that had not received AAV injections. (E) Correlation between the mean duration of wakefulness episodes within 6 h after CNO administration and the number of *hM3Dq*(+) cells in the LC TH(+) neurons, LC TH(-) neurons, or in the region lateral (including PB) or medial to the LC of *orexin/ataxin-3* mice with LC noradrenergic neuron-selective (Successfully targeted) or off-target (Unsuccessfully targeted) hM3Dq expression. *hM3Dq*(+) cells were not detected in the SubC area. Pearson's correlation coefficients (*r*), *P* values, and regression lines are also shown.




**Supplemental Figure 8.** DR serotonergic neurons innervate the nuclei involved in the regulation of sleep/wakefulness and emotion. Coronal brain sections prepared from *OX1R*$^{-/-}$;*OX2R*$^{-/-}$ mice with the targeted injection of AAV-Pet1/ChR2::EYFP in the DR, which were analyzed as a part of control mice for orexin receptor restoration experiments, were stained brown with anti-GFP antibody or double-stained with anti-GFP antibody (red fluorescence) and one of the antibodies for neuronal type-specific markers (green fluorescence): TH for dopaminergic neurons of substantia nigra compacta (SNc), TPH for DR serotonergic neurons, and ChAT for cholinergic neurons of LDT. EYFP-positive fibers were observed in many areas, with particularly dense innervations to the central and basolateral nuclei of the amygdala, SNc, and PPT/LDT. The PPT/LDT contains REM-active cholinergic neurons implicated in the initiation of REM sleep and REM atonia. Cataplexy is often triggered by strong emotions, which are controlled by the amygdala. Thus, DR serotonergic neurons may coordinately control multiple brain regions involved in the regulation of REM sleep and emotion under the control of orexin neurons, to prevent cataplexy-like episodes. The locations of magnified images are indicated by arrowheads in the low-power images. 4v, fourth ventricle; Aq, aqueduct; opt, optic tract. Scale bars, 100 $\mu$m.