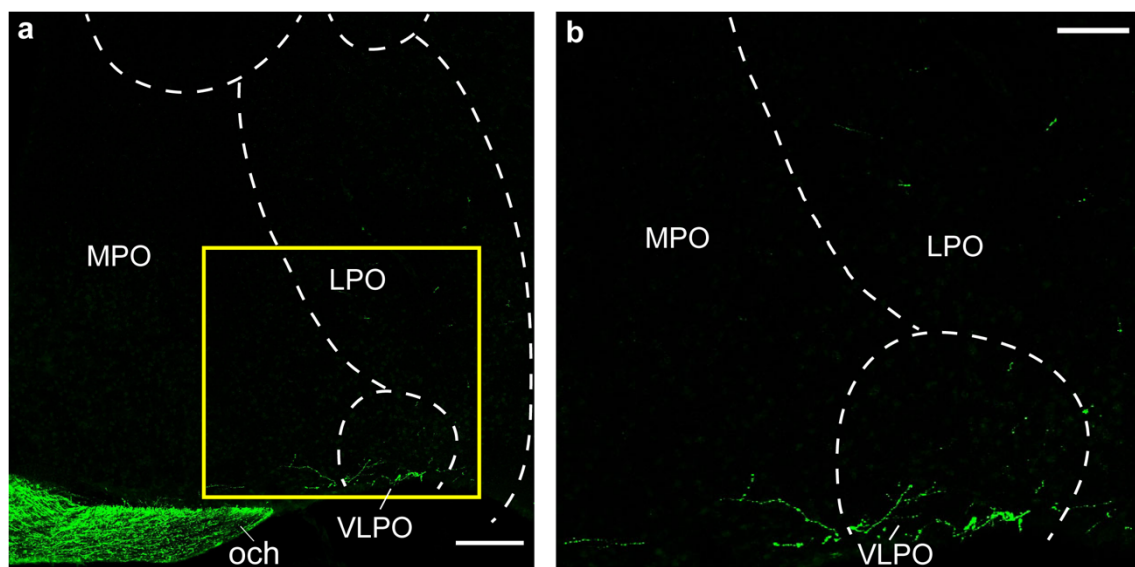
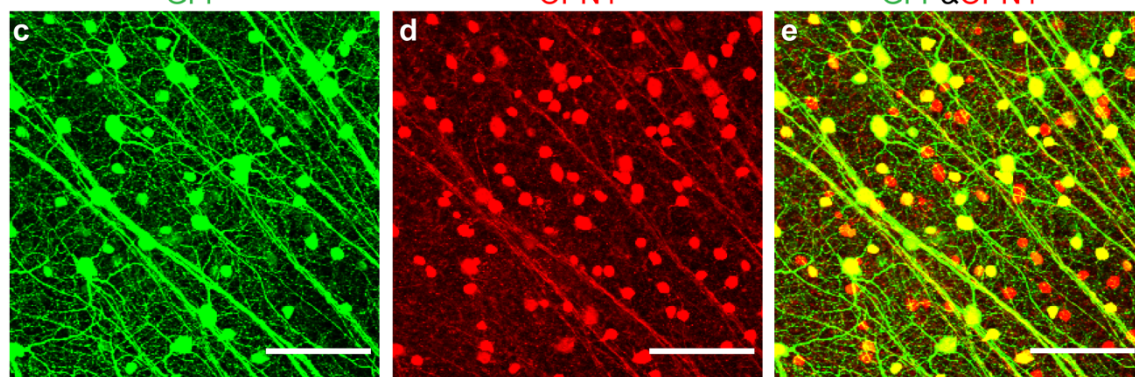




GFP

OPN4

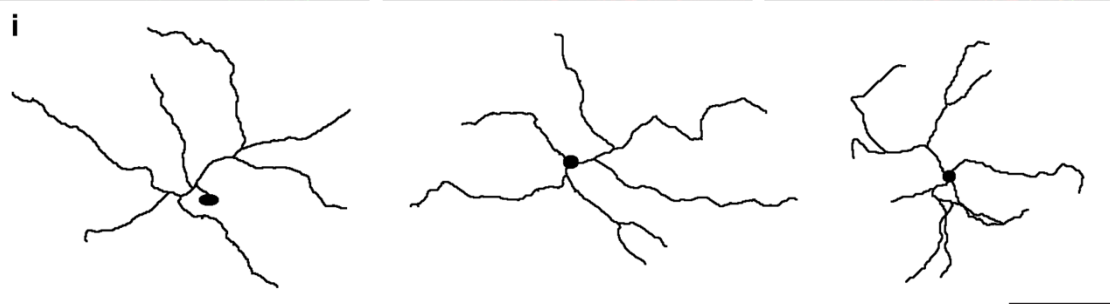
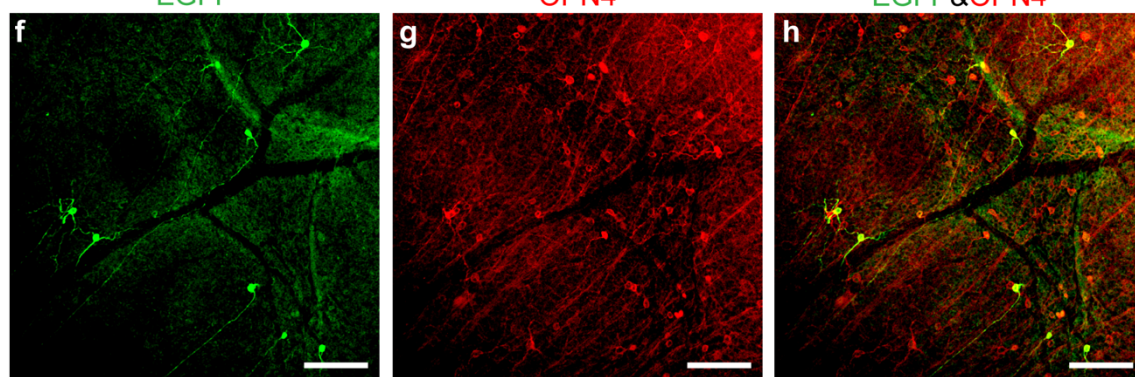
GFP&OPN4



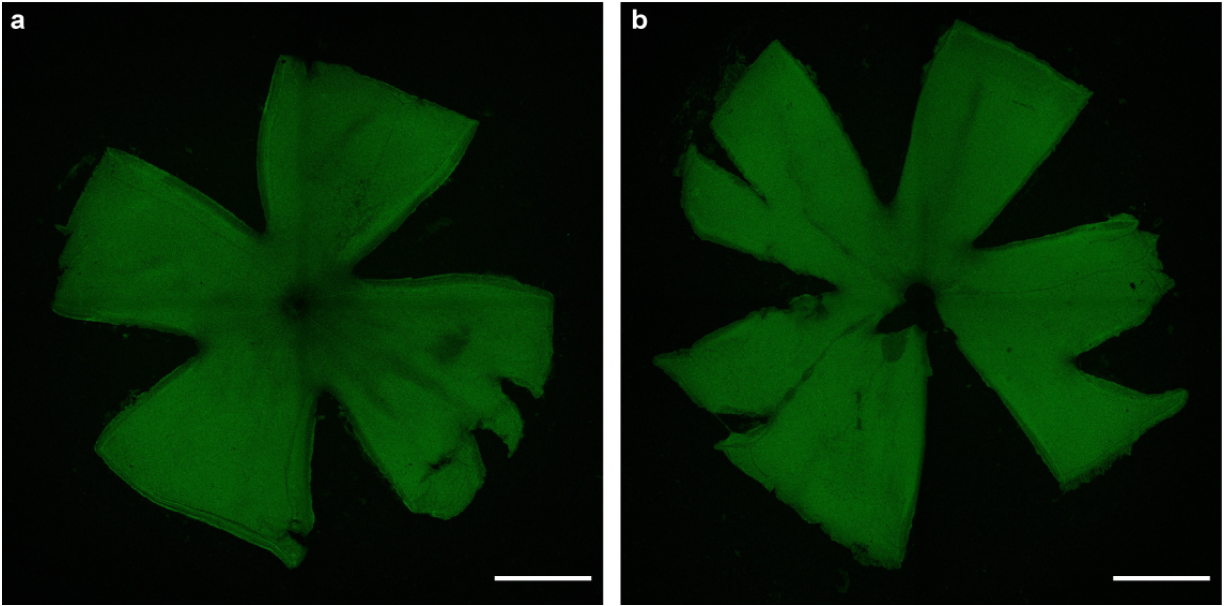
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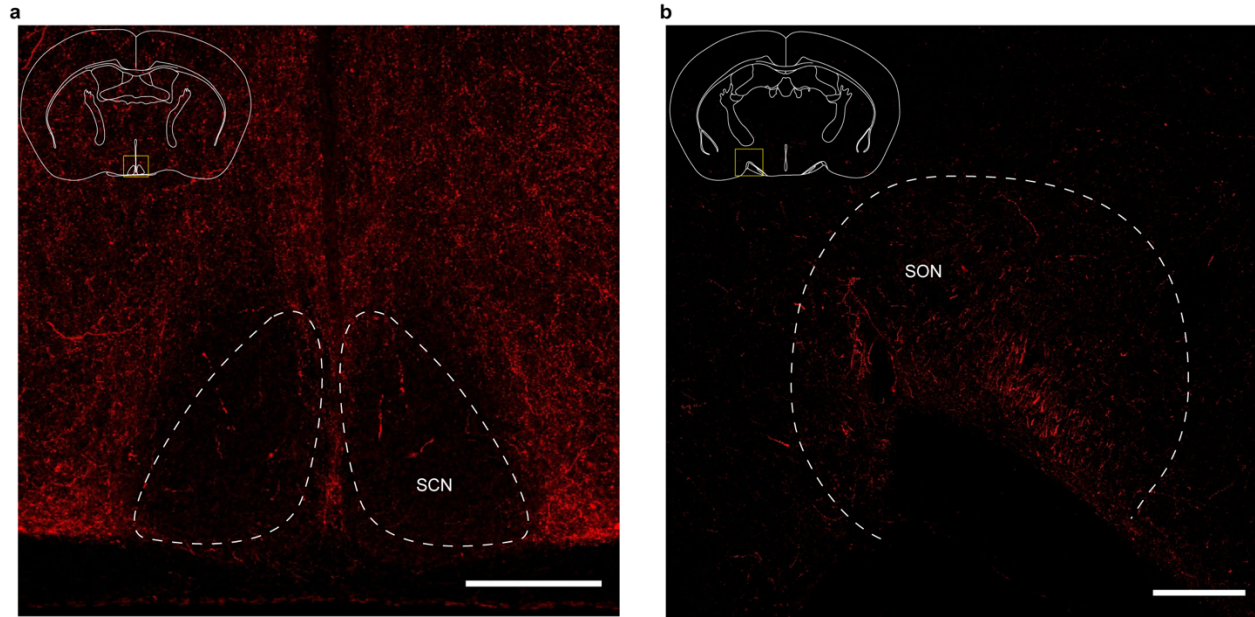
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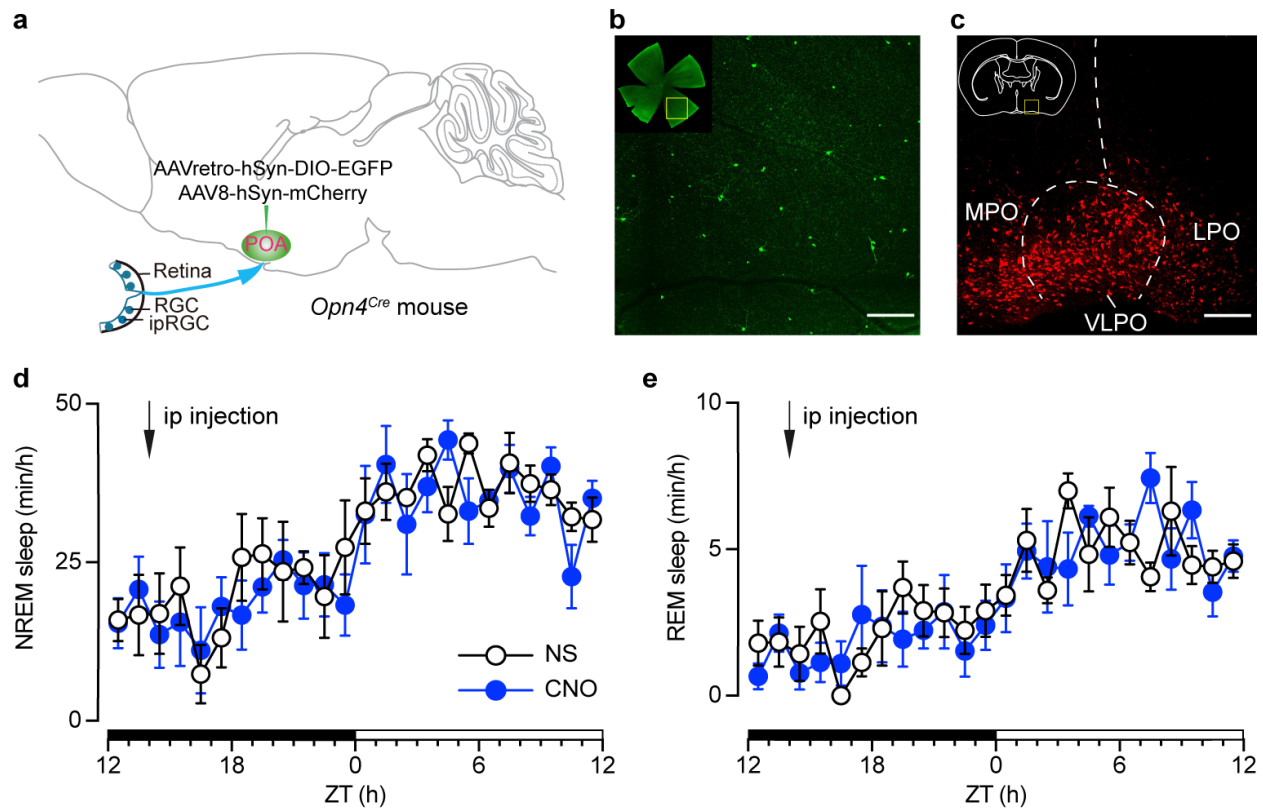
Supplementary Figure 1 M1 ipRGCs are the predominant subtype that projects to the POA and ipRGCs project beyond the VLPO to the MPO and the LPO. **a**, Immunostaining of GFP in the MPO, the LPO, and the VLPO in *Opn4^{Cre}* mice that are injected with AAV2-CAG-DIO-GFP virus in the eye (n = 10 retinas from 5 animals; scale bar, 200 μ m). The boxed region in **a** is enlarged in **b** showing fibers outside the VLPO (n = 10 retinas from 5 animals; scale bar, 100 μ m). **c-e**, Distribution of GFP antibody-positive cells (**c**), OPN4 mCherry-expressing cells (**d**), and colocalization (**e**) in the retina of *Opn4^{Cre};Ai9* mice that are injected with AAV2-CAG-DIO-GFP virus in the eye (n = 4 retinas from 2 animals; scale bar, 100 μ m). **f-h**, Distributions of EGFP positive cells (**f**), OPN4 antibody-positive cells (**g**), and colocalization (**h**) in the retina of *Opn4^{Cre}* mice that are injected with AAV2-Efl α -DIO-Flp virus in the eye and AAVretro-Efl α -fDIO-hM3D(Gq)-EGFP virus in the POA (n = 4 retinas from 2 animals; scale bar, 100 μ m). **i**, Retinal reconstruction of ipRGCs that project to the POA. All three reconstructed cells belong to the M1 ipRGC subtype (Scale bar, 100 μ m). MPO, medial preoptic area; LPO, lateral preoptic area; VLPO, ventrolateral preoptic nucleus; och, optic chiasm.



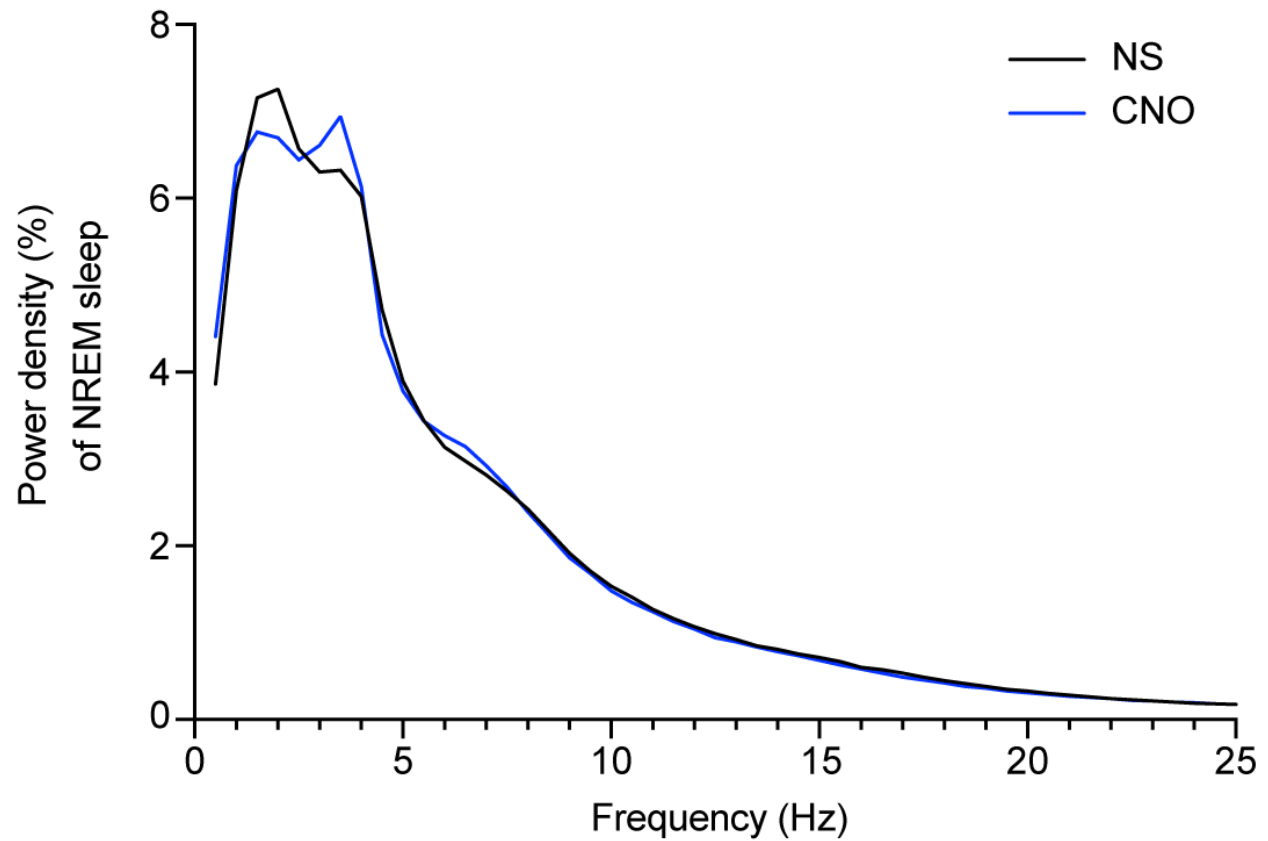
Supplementary Figure 2 Viral expression depends on Cre. **a, b**, No GFP expression in the retina of wild type mouse that was injected AAV2-CAG-DIO-GFP in the eye (**a**) ($n = 4$ retinas from 2 animals; scale bar, 1000 μm); or that was injected AAV2-Efl α -DIO-Flp in the eye and AAVretro-Efl α -fDIO-hM3D(Gq)-EGFP in the POA (**b**) ($n = 6$ retinas from 3 animals; scale bar, 1000 μm).



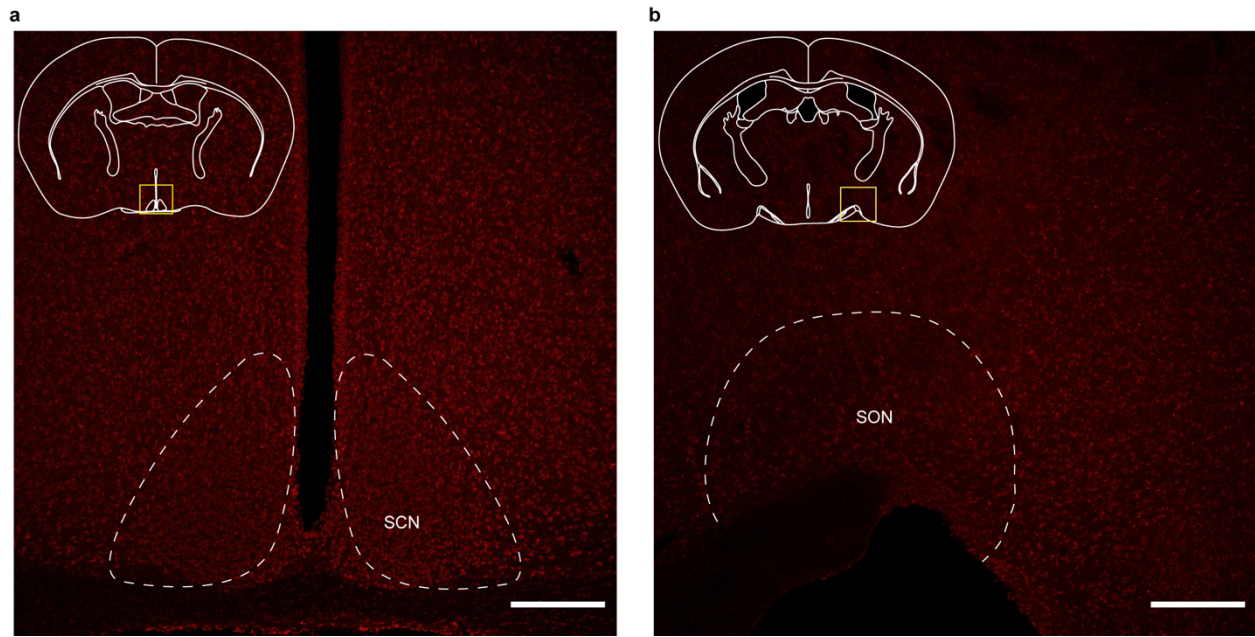
Supplementary Figure 3 The SCN and the SON did not show mCherry expression after viral infection. **a, b**, AAV8-hSyn-mCherry injection in the POA of *Opn4^{Cre}* mice did not show expression in the somas of the SCN (**a**), or the SON (**b**). The fibers in the SCN and SON arise from the injection of non-Cre dependent virus expressing mCherry in the POA. n = 8 animals. Scale bars denote 200 μm. SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; POA, preoptic area.



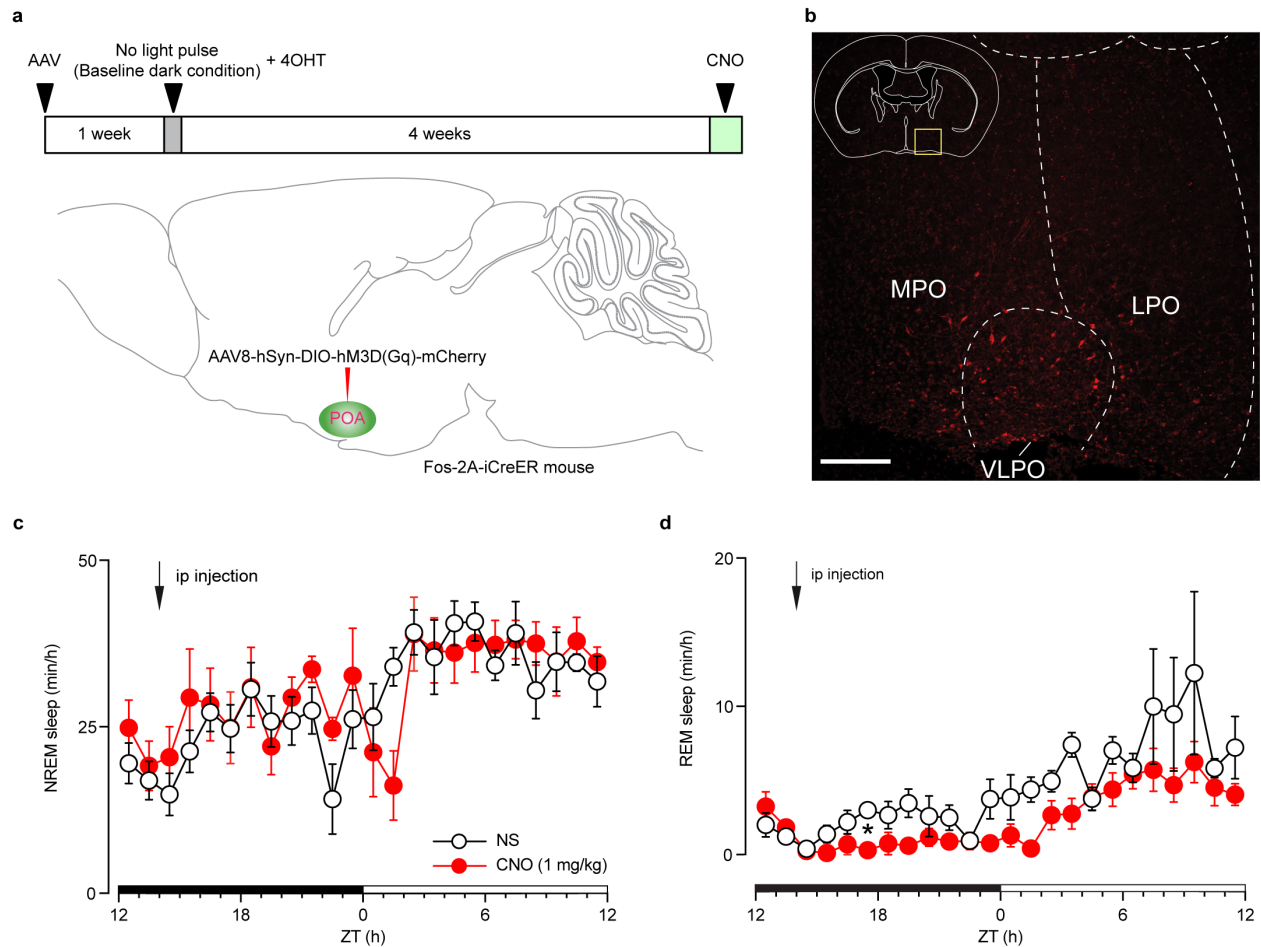
Supplementary Figure 4 CNO injection did not affect NREM or REM sleep in *Opn4^{Cre}* mice injected with AAVretro-hSyn-DIO-EGFP, that expresses only EGFP in the retina. **a**, Schematic of the viral strategy employed. **b**, Immunostaining of EGFP in the retina shows labeling of ipRGCs with EGF (n = 10 retinas from 5 animals; scale bar, 200 μ m). **c**, Immunostaining of mCherry in the POA to delineate the injection site (n = 5 animals; scale bar, 200 μ m). **d**, Time course changes in NREM sleep after NS or CNO injection (n = 5 animals; two-way ANOVA, $F_{1,8} = 1.508$, $P = 0.2544$, Bonferroni post hoc test). The black and white bars on the x axes indicate light-off and light-on periods, respectively. **e**, Time course changes in REM sleep after NS or CNO injection (n = 5 animals; two-way ANOVA, $F_{1,8} = 0.5040$, $P = 0.4979$, Bonferroni post hoc test). All error bars denote SEM. ipRGC, intrinsically photosensitive retinal ganglion cell; MPO, medial preoptic area; LPO, lateral preoptic area; VLPO, ventrolateral preoptic nucleus; NREM, non-rapid eye movement.



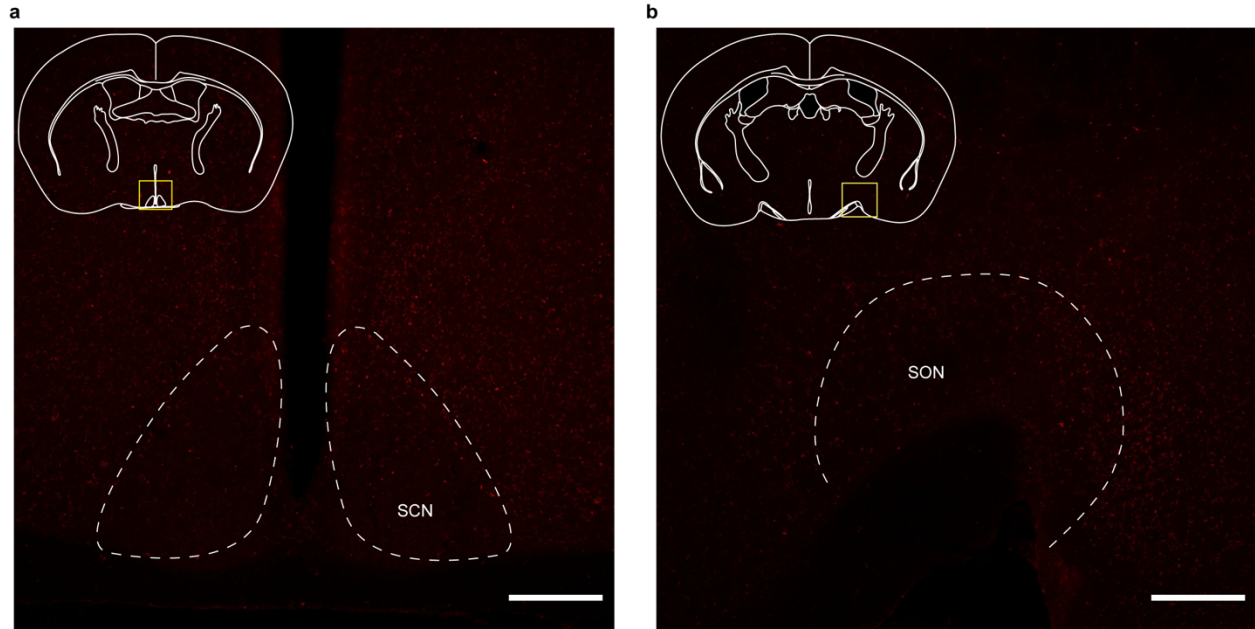
Supplementary Figure 5 Activation of POA light-responsive neurons did not change the power density of NREM sleep. Although NREM sleep was increased, the power density was not affected between the normal saline (NS) and clozapine-N-oxide (CNO) treated animals. $n = 5$ animals. NREM, non-rapid eye movement.



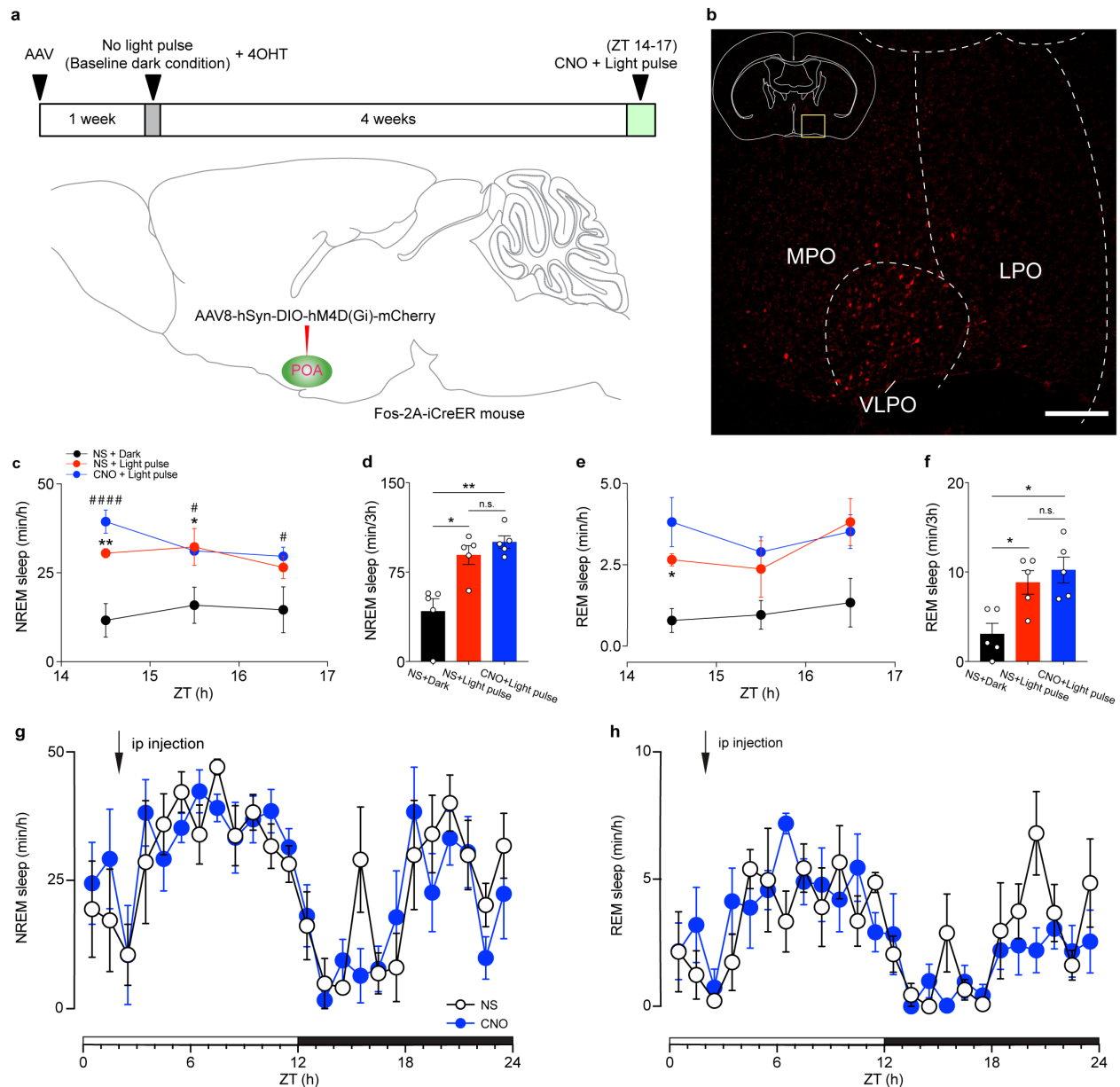
Supplementary Figure 6 The SCN and SON did not show mCherry expression after viral infection in Fos-2A-iCreER mice. **a, b**, AAV8-hSyn-DIO-hM3D(Gq)-mCherry injected in the POA of Fos-2A-iCreER mice treated with light and 4-Hydroxytamoxifen did not show mCherry expression in somas of the SCN (**a**), or the SON (**b**). Note the absence of fiber innervation from the activated light-responsive POA neurons in comparison to Supplementary Figure 3. $n = 5$ animals. Scale bars denote 200 μm . SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; POA, preoptic area.



Supplementary Figure 7 The effect of activation of night-active POA neurons on sleep. **a**, Schematic showing viral, 4-Hydroxytamoxifen (4-OHT) and CNO injections in Fos-2A-iCreER mice. **b**, 4-OHT induced the expression of mCherry in the POA (n = 5 animals; scale bar, 200 μ m). **c**, Time course changes in NREM sleep after normal saline (NS) or clozapine-N-oxide (CNO) injection (n = 5 animals; two-way ANOVA, $F_{1,10} = 1.426$, $P = 0.2599$, Bonferroni post hoc test). Each cycle represents the hourly mean $\pm$ SEM of sleep. The black and white bars on the x axes indicate light-off and light-on periods, respectively. **d**, Time course changes in REM sleep after NS or CNO injection (n = 5 animals; two-way ANOVA, $F_{1,10} = 5.697$, $P = 0.0382$, Bonferroni post hoc test). All error bars denote SEM. MPO, medial preoptic area; LPO, lateral preoptic area; VLPO, ventrolateral preoptic nucleus; ZT, zeitgeber time.

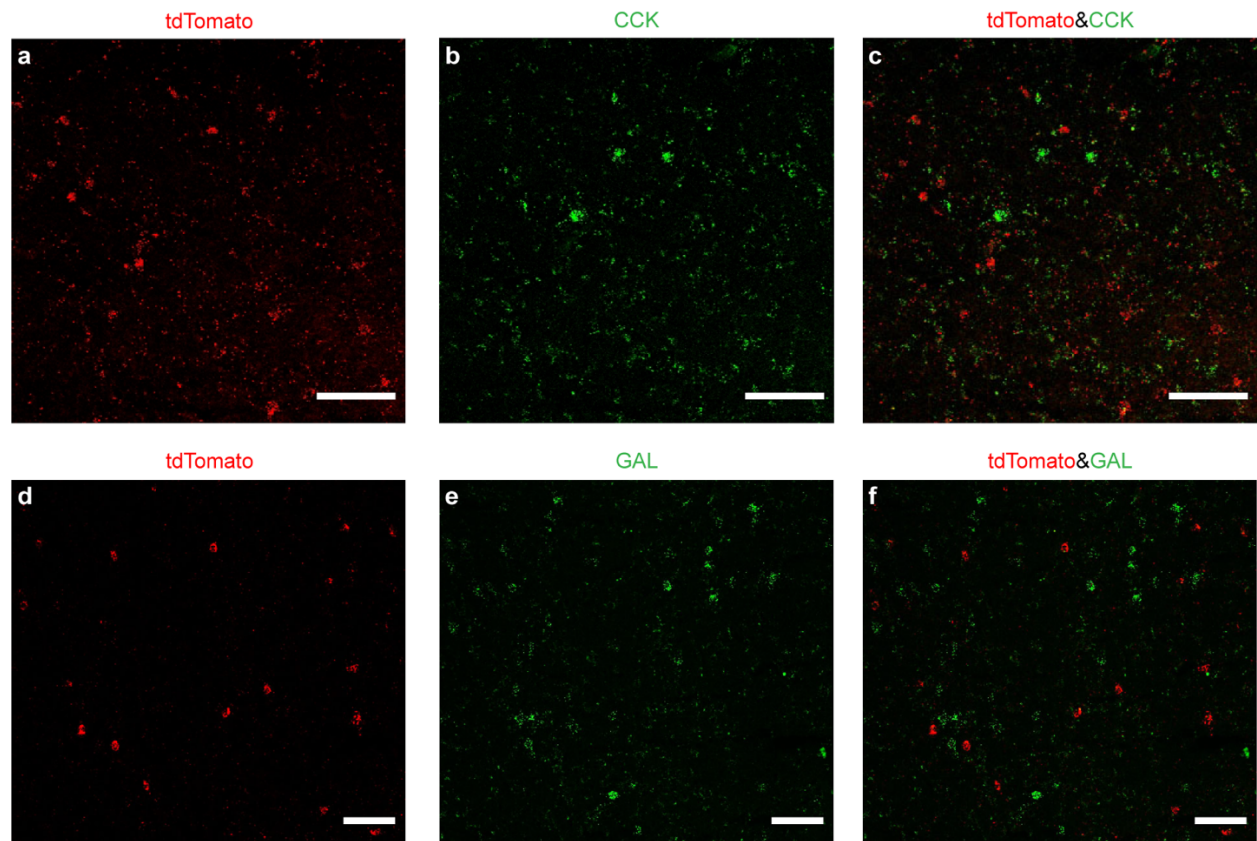


Supplementary Figure 8 The SCN and SON did not show mCherry expression after viral infection in Fos-2A-iCreER mice. **a, b**, AAV8-hSyn-DIO-hM4D(Gi)-mCherry injected in the POA of Fos-2A-iCreER mice treated with light and 4-Hydroxytamoxifen did not show mCherry expression in somas of the SCN (**a**), or the SON (**b**). $n = 6$ animals. Scale bars denote 200 μm . SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; POA, preoptic area.

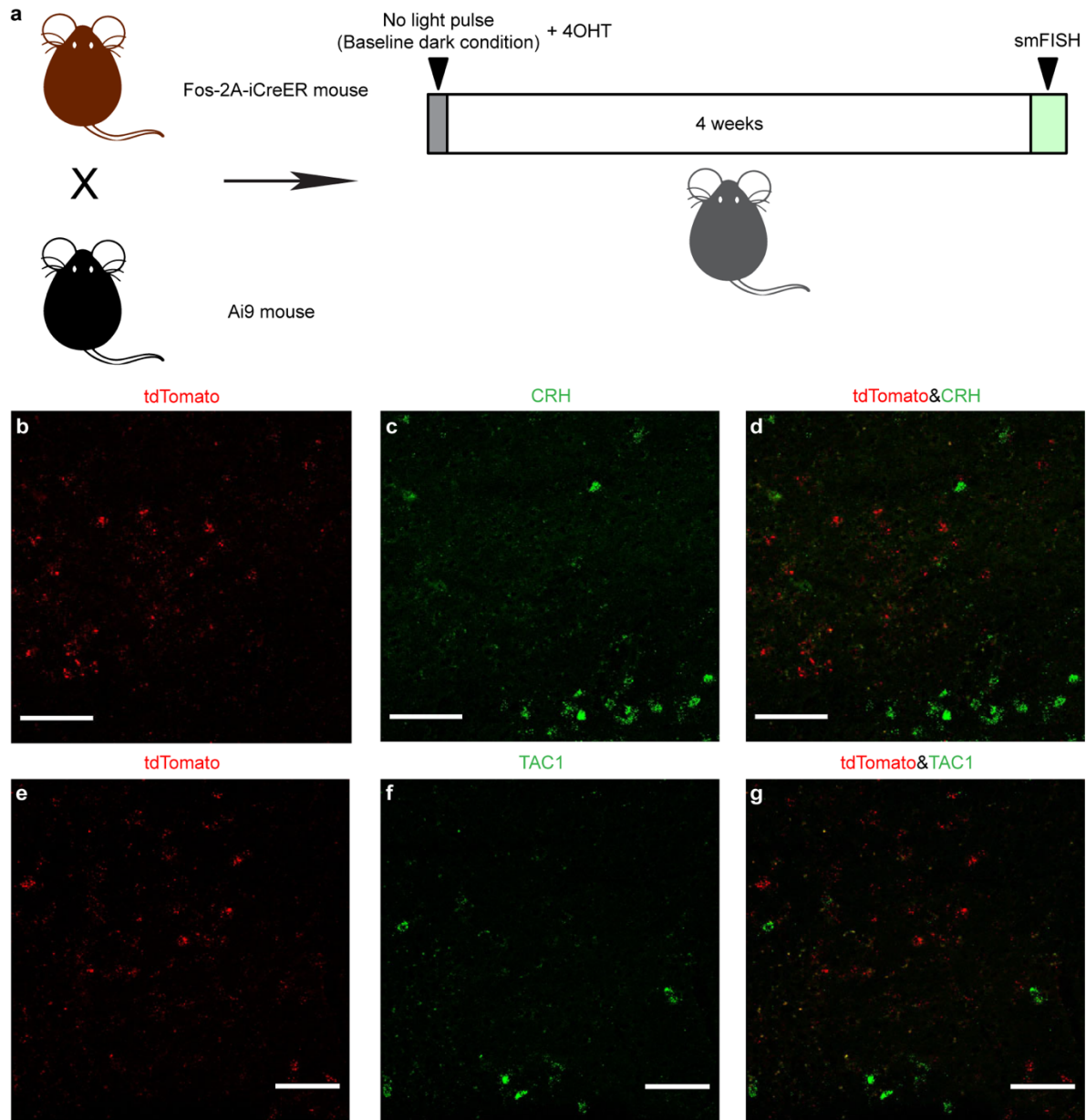


Supplementary Figure 9 Inhibiting night-active POA neurons does not influence sleep. **a**, Schematic showing viral, 4-Hydroxytamoxifen (4-OHT) and clozapine-N-oxide (CNO) injections in Fos-2A-iCreER mice. **b**, 4-OHT induced expression of mCherry in night-active POA neurons (n = 5 animals; scale bar, 200 μ m). **c**, Time course changes in NREM sleep after normal saline (NS) or CNO injection during light pulse from zeitgeber time (ZT) 14 to ZT 17 (n = 5 animals; two-way ANOVA, $F_{2,12} = 14.00$, $P = 0.0007$, Bonferroni post hoc test. * $P < 0.05$, * $P < 0.01$ indicates significant difference between NS+Light pulse and NS+Dark. # $P < 0.05$, #### $P < 0.0001$ indicates significant difference between CNO+Light pulse and NS+Dark.). Each

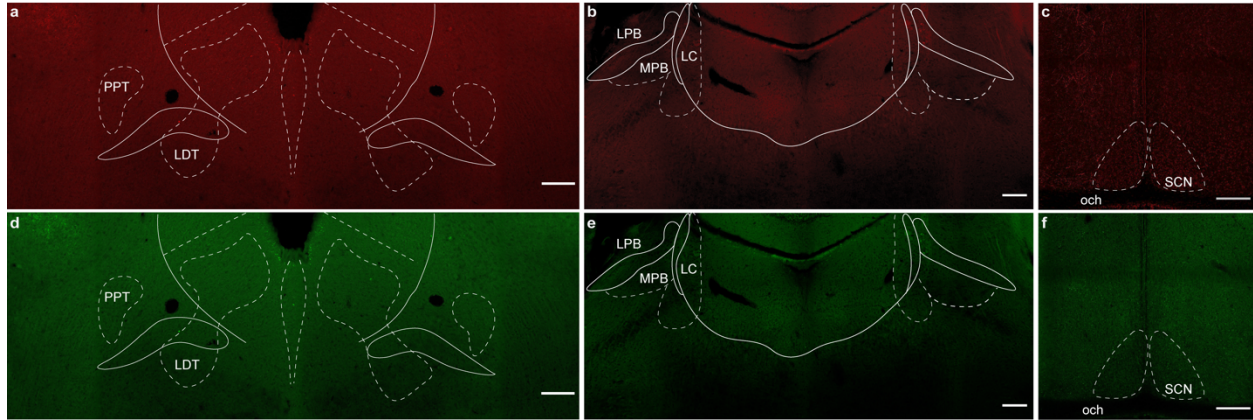
cycle represents the hourly mean $\pm$ SEM of sleep. **d**, Total time in NREM sleep for 3 h after NS or CNO injection (n = 5 animals; one-way ANOVA, $F_{1,608, 6.432} = 20.81$, $P = 0.0019$, Bonferroni post hoc test, $*P < 0.05$, $**P < 0.01$). **e**, Time course changes in REM sleep after NS or CNO injection during light pulse from ZT 14 to ZT 17 (n = 5 animals; two-way ANOVA, $F_{2, 12} = 8.257$, $P = 0.0056$). Each cycle represents the hourly mean $\pm$ SEM of sleep. **f**, Total time in REM sleep for 3 h after NS or CNO injection (n = 5 animals; one-way ANOVA, $F_{2,000, 11.71} = 8.257$, $P = 0.0058$, Bonferroni post hoc test, $*P < 0.05$). **g**, Time course changes in NREM sleep after NS or CNO injection (n = 4 animals; two-way ANOVA, $F_{1, 6} = 0.2188$, $P = 0.6565$, Bonferroni post hoc test). Each cycle represents the hourly mean $\pm$ SEM of sleep. The white and black bars on the x axes indicate light-on and light-off periods, respectively. **h**, Time course changes in REM sleep after NS or CNO injection (n = 4 animals; two-way ANOVA, $F_{1, 6} = 0.2236$, $P = 0.6530$). All error bars denote SEM. MPO, medial preoptic area; LPO, lateral preoptic area; VLPO, ventrolateral preoptic nucleus.



Supplementary Figure 10 Characterization of light-responsive neurons in the POA. **a-c**, Distributions of tdTomato positive neurons (**a**), CCK positive neurons (**b**), and colocalization (**c**) in the POA. **d-f**, Distributions of tdTomato positive neurons (**d**), GAL positive neurons (**e**), and colocalization (**f**) in the POA. Note that both CCK and GAL are not co-labeled with tdTomato. $n = 4$ animals. Scale bars denote 100 μm . CCK, cholecystokinin; GAL, galanin.



Supplementary Figure 11 Characterization of night-active neurons in the POA. **a**, Schematic showing Fos-2A-iCreER and CAG-FLEX-tdTomato (Ai9) mice maintained in baseline dark condition, 4-Hydroxytamoxifen (4-OHT) injection and single-molecule fluorescence in situ hybridization (smFISH). **b-d**, Distributions of tdTomato positive neurons (**b**), CRH positive neurons (**c**), and colocalization (**d**) in the POA. **e-g**, Distributions of tdTomato positive neurons (**e**), TAC1 positive neurons (**f**), and colocalization (**g**) in the POA. Note that in contrast to Figure 4, there are no co-labeling of CRH and TAC1 neurons. $n = 3$ animals. Scale bars denote 100 μ m. CRH, corticotropin-releasing hormone, TAC1, tachykinin-1.



Supplementary Figure 12 POA light-responsive neurons do not project to the SCN, the PPT, the LDT, the LPB, the MPB or the LC. **a**, Distributions of tdTomato in the PPT and the LDT. **b**, Distributions of tdTomato in the LPB, the MPB and the LC. **c**, Distributions of tdTomato in the SCN. **d**, Distribution of EGFP in the PPT and the LDT. **e**, Distributions of EGFP in the LPB, the MPB and the LC. **f**, Distribution of EGFP in the SCN. PPT, pedunculopontine nucleus; LDT, laterodorsal tegmental nucleus; LPB, lateral parabrachial nucleus; MPB, medial parabrachial nucleus; LC, locus coeruleus; SCN, suprachiasmatic nucleus; och, optic chiasm. $n = 3$ animals. Scale bars denote 200 μm .