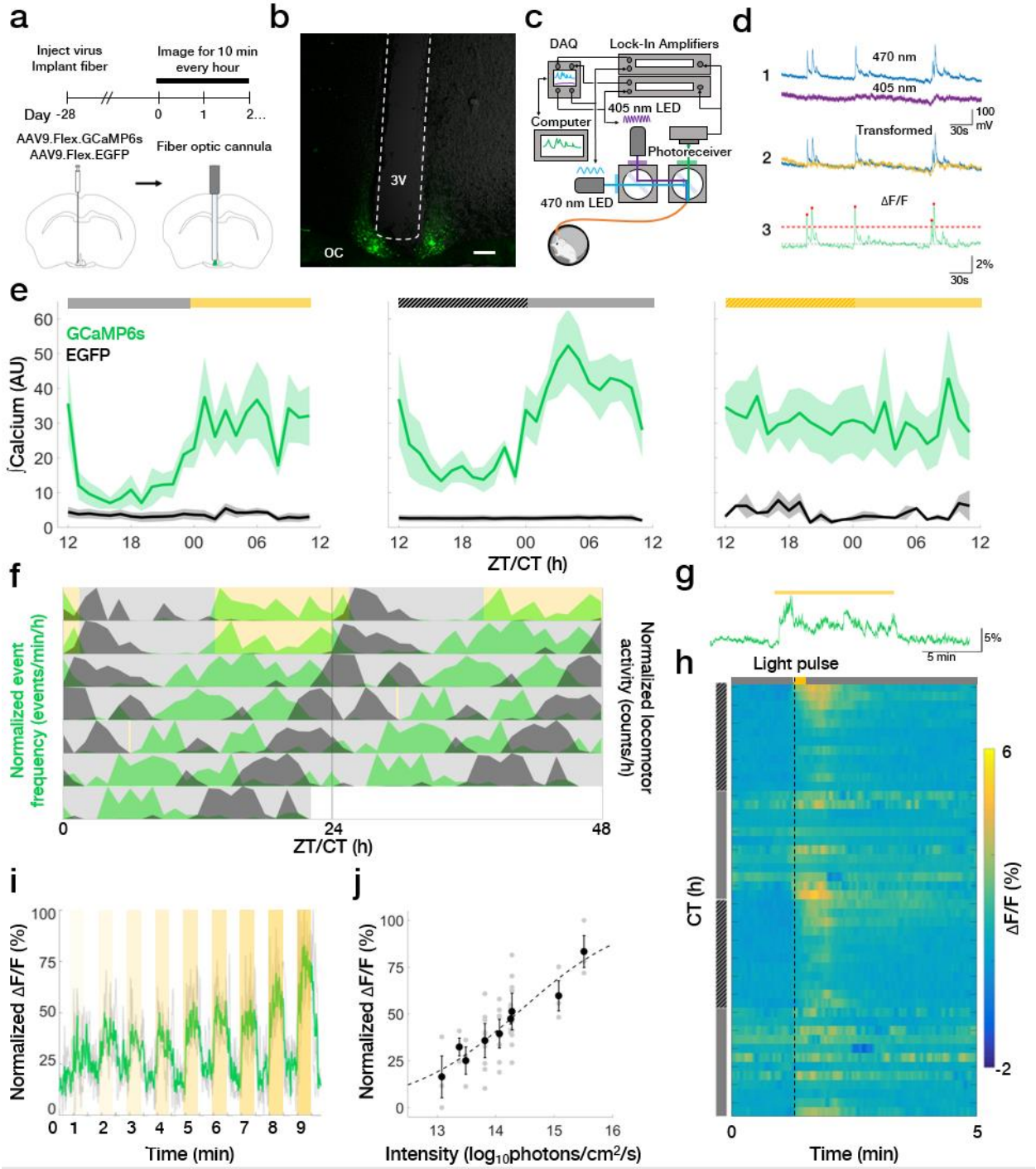
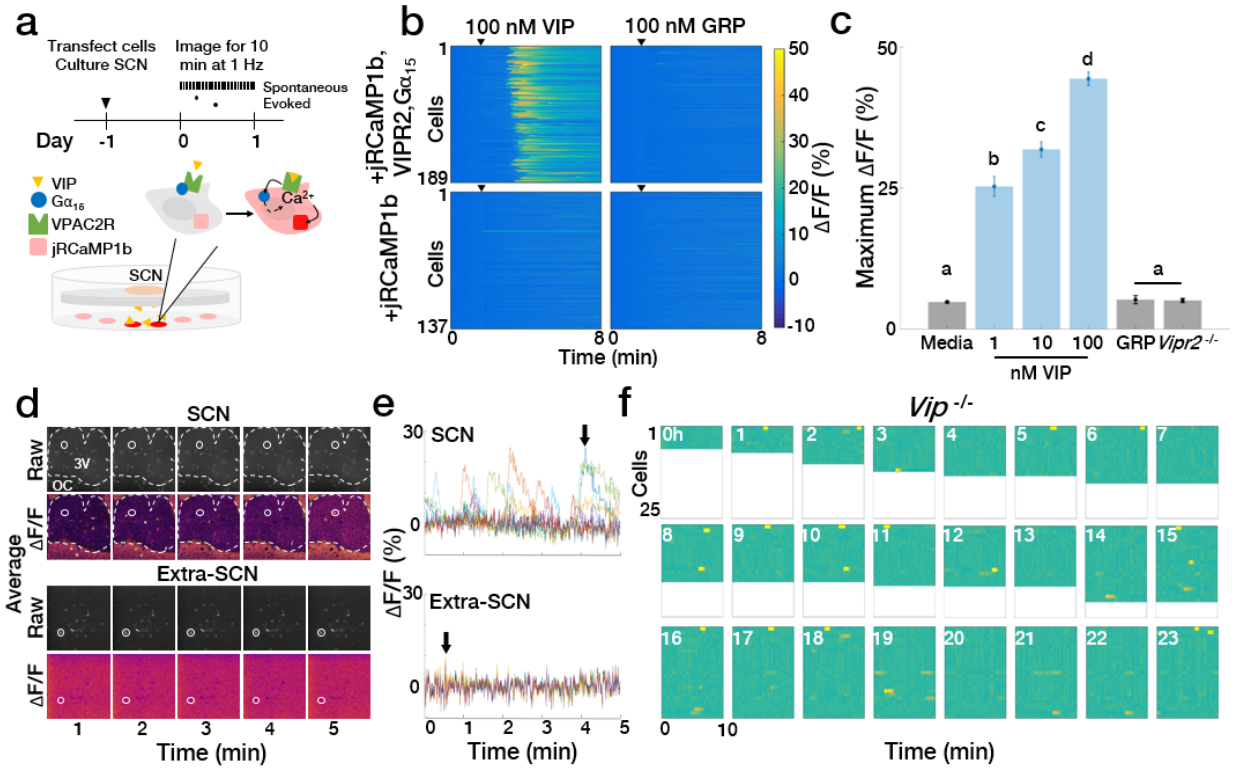


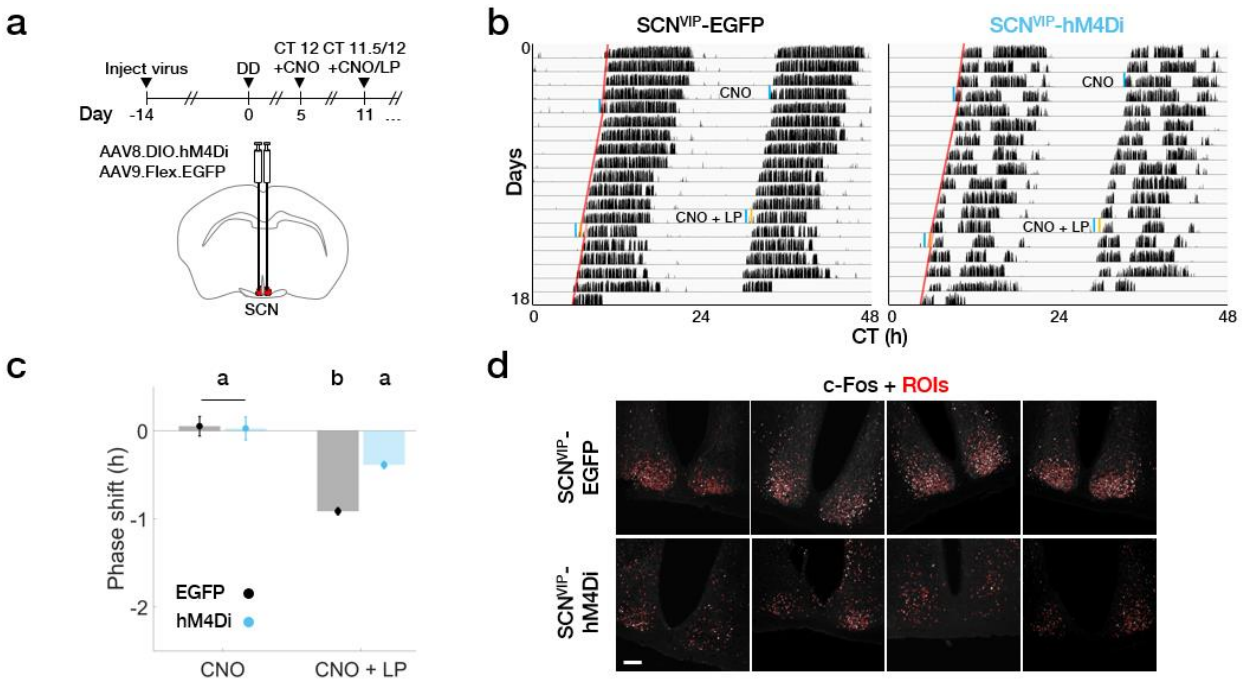
Supplementary Figure 1. ***In vivo*** **fiber photometry recording of rhythms in spontaneous and light-evoked calcium activity in SCN VIP neurons.**

a) Fiber photometry experimental timeline and schematic. **b**) Representative image of a fiber optic cannula (white dashed lines) positioned above a GCaMP6s^{+} SCN. OC, optic chiasm; 3V, third ventricle. Scale bar = 200 µm. **c**) Schematic of the two-color excitation system used to

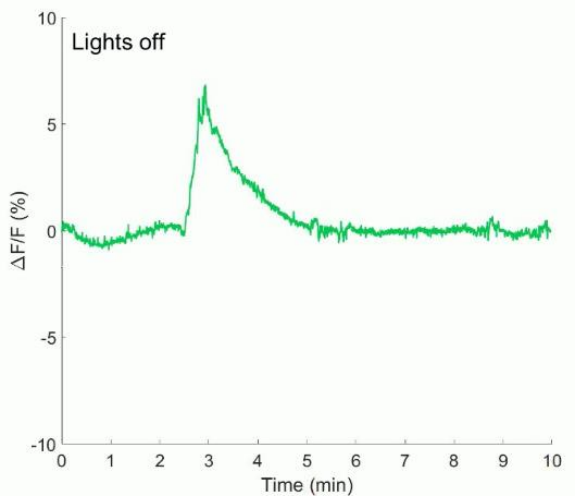
measure Ca+2 (470nm)- relative to background (405nm)-evoked fluorescence. **d**) Illustration of Ca+2 event detection. Blue, raw 470 nm signal; purple, raw 405 nm signal; yellow, detrended 405 nm signal; green, ΔF/F; dashed line, event threshold value; red circles, events. **e**) VIP neuron integrated calcium levels depended on circadian time and light in LD, DD, and constant light (LL; light yellow = subjective day) in GCaMP6s mice (n = 5, 5, 4; JTK Cycle, $p<0.001$). EGFP mice showed no changes in fluorescence with time or light (n = 5, 4, 2; JTK Cycle, $p>0.05$). **f**) Double-plotted actogram of normalized event frequency per hour (green) and normalized locomotor activity counts per hour (black) recorded from an individual GCaMP6s^{+} mouse housed in LD (gray and yellow shading) and DD (gray shading). A 10-min light pulse (yellow) was given at CT 0 on day five. **g**) Representative ΔF/F trace in response to a 10-min light pulse depicted in **e**. **h**) Representative heatmap depicting ΔF/F traces in response to a 15-s light pulse (dashed line) given every hour for 48 h. Hatched gray bar, subjective night, gray bar, subjective day. **i**) Representative ΔF/F trace in response to 30 s pulses of increasing light intensity (yellow bars, shading indicates brighter light) given at CT 12. Green line indicates the mean response of three repeated trials (gray) normalized within trial to the maximum ΔF/F response. **j**) Maximum ΔF/F response to 30-s light pulses given at CT 12 increased with light intensity. n = 3 mice, 3-9 trials per light intensity. Dashed line depicts sigmoid fit, $r^2 = 0.4865$.



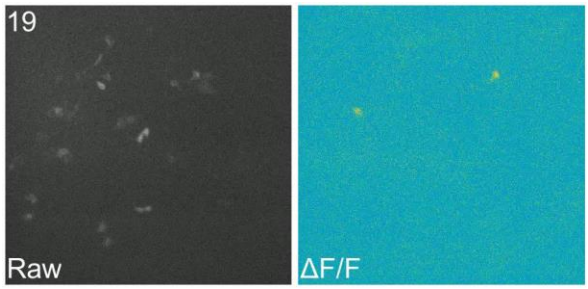
Supplementary Figure 2. VIP reporter cells detect the release of VIP from SCN neurons. **a**) Experimental outline and VIP sensor schematic. **b**) jRCaMP1b calcium fluorescence in VIP reporter cells stably transfected with $G\alpha_{15}$ and VPAC2R increases in the presence of VIP but not gastrin releasing peptide (GRP; n = 189, 137 cells). **c**) VIP sensors exhibit a dose-dependent response to VIP concentration but do not respond to GRP or in the absence of VPAC2R (n = >500 cells, 4 wells per group; One-Way ANOVA, $F(5, 18) = 242.2$, $p < 0.0001$ with post-hoc Tukey's MCT. Error bars depict means ± SEM; letters indicate statistically different groups. **d**) Representative 1-min binned average raw pixel intensity and average ΔF/F images of VIP sesnors cultured underneath an acute SCN slice (top) or in the same dish but away from the SCN (bottom). White dashed outline delineates the boundary of the SCN. 3V, third ventricle; OC, optic chiasm. **e**) ΔF/F traces of the cells depicted in **d**. Colors represent individual reporter cells; black arrows indicate the cell circled in **d**. **f**) Co-culturing VIP sensors with a *Vip*$^{-/-}$ SCN slice did not produce daily rhythms in VIP release.



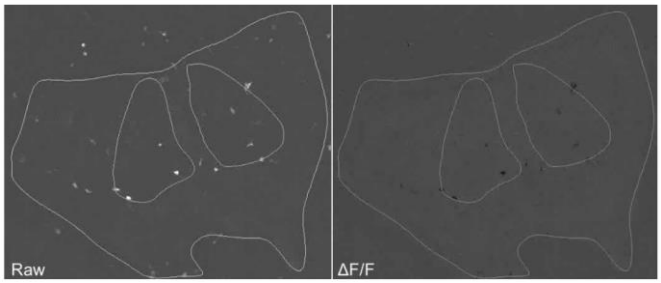
Supplementary Fig. 3. ***In vivo*** **SCN VIP neuron inhibition attenuates VIP neuron activity and circadian behavioral responses to light. a)** Experimental outline and DREADD schematic. **b**) SCN from individual EGFP and hM4Di mice (n = 4 per group) taken one hour after CNO administration plus a 15 minute light pulse. c-Fos is depicted in grayscale; regions of interest (ROIs) for automated cell counting were determined by Shanbhag auto-thresholding and are depicted as red outlines. Scale bar 100 μm. **c)** Representative double-plotted actograms from transgenic mice free-running in DD without (EGFP) or with (hM4Di) SCN VIP neurons expressing an inhibitory DREADD. Mice were given 1 mg/kg CNO alone or CNO plus a 15 min dim light pulse at CT 12. Blue, time of CNO administration; yellow, 15 min dim light pulse; red, activity onset lines of best fit. **d)** CNO attenuates phase delays in response to a dim light pulse in DREADD-expressing mice. n = 4 mice per group; Two-Way Repeated Measures ANOVA, $F(1, 6) = 9.632$, $p = 0.0210$ with post-hoc Sidak's MCT. Error bars depict means ± SEM; letters indicate statistically different groups.



Supplementary Video 1.



Supplementary Video 2.



Supplementary Video 3.