

Supplementary Figures

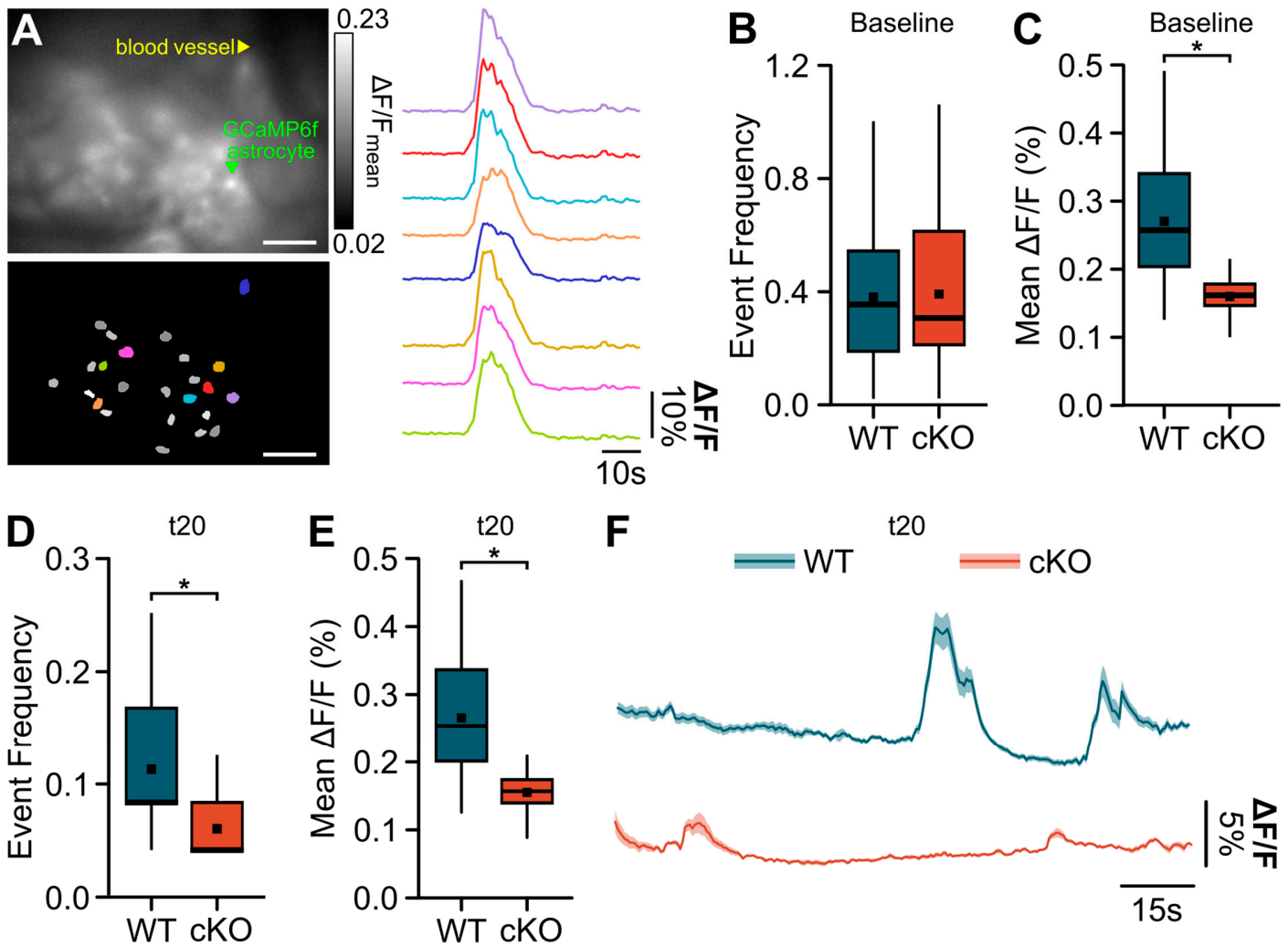


Figure S1. Verification of astroglial β 2-AR cKO. (A) Top left panel is a representative maximum projection image from the miniature microscope. The yellow triangle points to a blood vessel, and the green triangle shows an example of a GCaMP6f⁺ astrocyte. Bottom left panel shows locations of identified GCaMP6f⁺ astrocytes/ROIs that correspond with the top left projection image. Right panel shows fluorescent GCaMP6f Ca²⁺ traces that correspond to the color-coded ROIs in the bottom left spatial map. Scale bars = 100 μm. Distribution of (B) frequency of events (event # per 5 s) and (C) mean $\Delta F/F$ values under baseline conditions (Mann-Whitney U). Black squares denote means. Note that the event frequency does not differ between WT and cKO mice despite overall lower mean $\Delta F/F$ values for cKO. Values are from $n = 115$ individual ROIs from 3 WT mice and $n = 84$ individual ROIs from 3 cKO mice. Distribution of (D) frequency of events (event # per 5 s) and (E) mean $\Delta F/F$ values at 20 min (t20) following intraperitoneal (i.p.) injection of the β -AR agonist isoproterenol (5 mg/kg) (Mann-Whitney U). Black squares denote means. Note that both event frequency and mean $\Delta F/F$ values are blunted for cKO mice in response to the β -AR agonist compared to WT mice. Values are from $n = 115$ individual ROIs from 3 WT mice and $n = 84$ individual ROIs from 3 cKO mice. (F) Representative Ca²⁺ traces ($\Delta F/F$) from a WT mouse (23 ROIs) and a cKO mouse (23 ROIs) at 20 min (t20) following injection of isoproterenol (5 mg/kg, i.p.). Values are means \pm standard deviation. * $p < 0.05$.

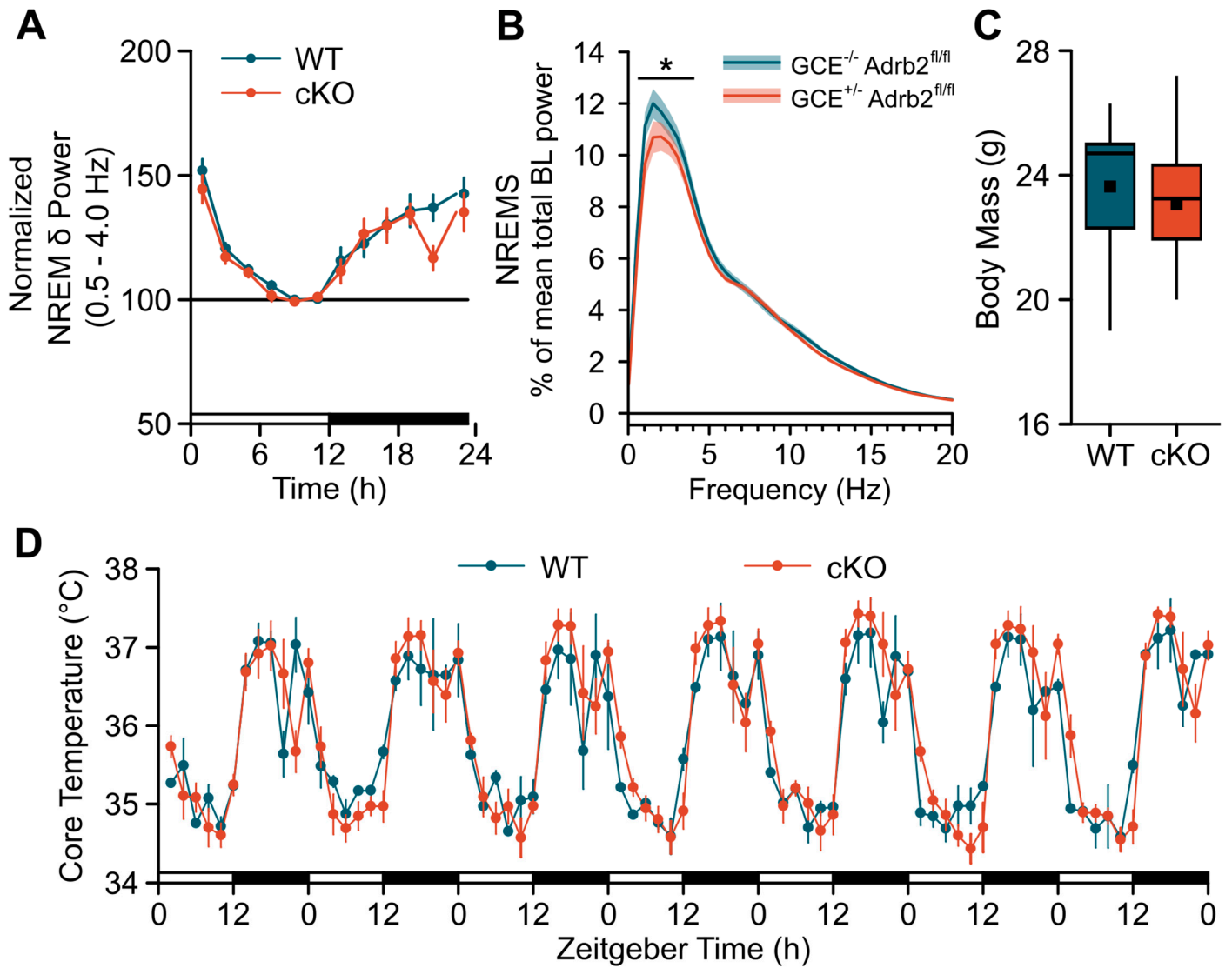


Figure S2. Assessment of astroglial $\beta 2$ -AR cKO on electroencephalographic activity and physiology. (A) Baseline normalized NREM SWA (i.e., delta (δ) power) shown in 2 h bins (Kruskal-Wallis). Open and closed bars on the x-axis denote the light and dark periods, respectively. Values are means \pm SE. (B) Normalized EEG spectral power for NREMS during the first 6 h of recovery sleep post-sleep deprivation (ANOVA over delta (0.5 – 4 Hz; $F(1,205.87) = 9.07$, $p = 0.003$) and upper (4.5 – 20 Hz) bands). Values are means \pm SE. (C) Body mass distribution of WT and cKO mice at time of surgery (Mann-Whitney U). Black squares denote means. (D) Continuous core body temperature measurements shown across 7 days in 2 h bins for WT and cKO mice (repeated measures ANOVA). Open and closed bars on the x-axis denote the light and dark periods, respectively. Values are means \pm SE. $n = 13$ WT and $n = 13$ cKO mice for A – C; $n = 2$ WT and $n = 5$ cKO mice for D.

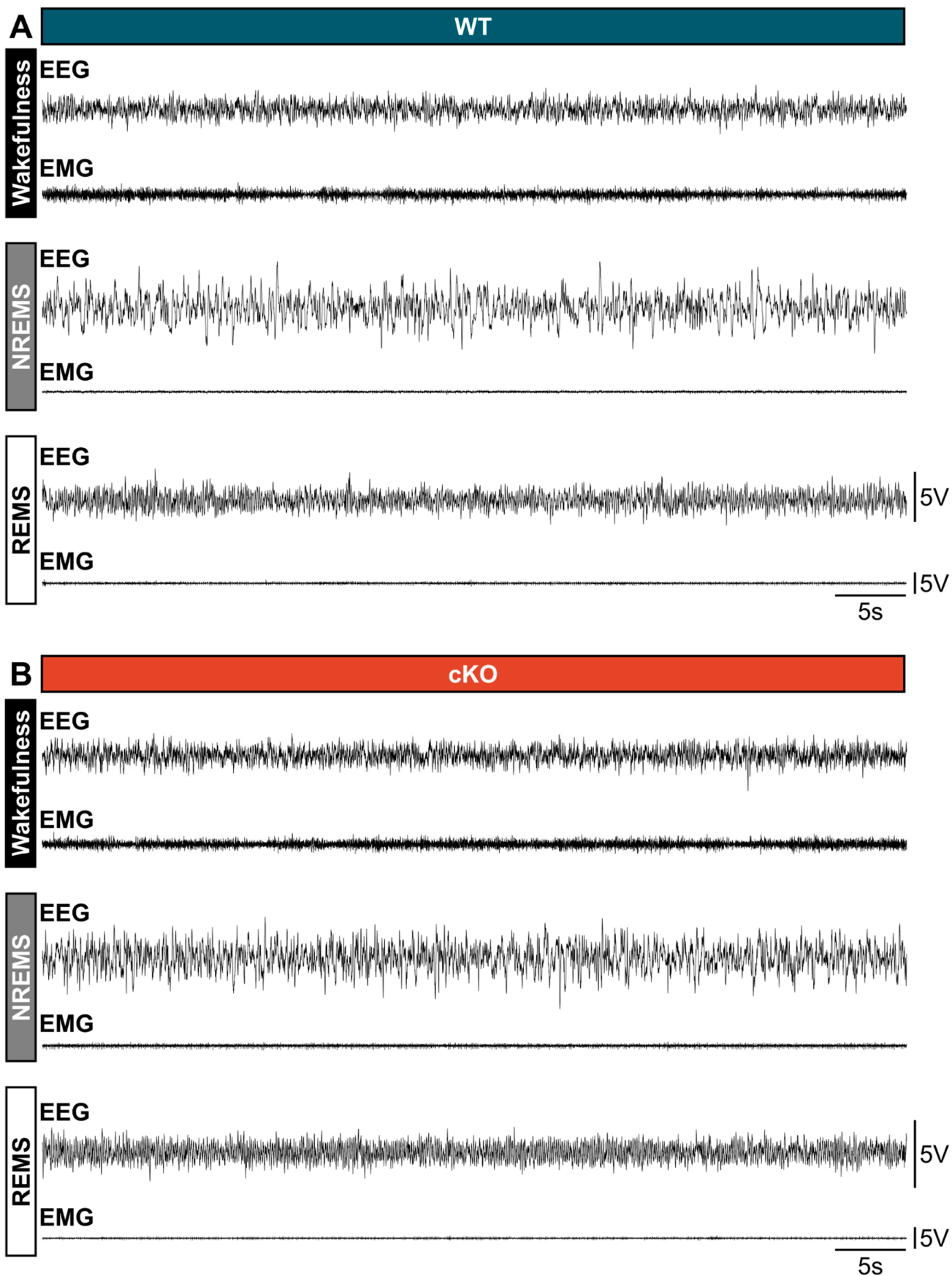


Figure S3. Arousal state-specific electroencephalography (EEG) and electromyography (EMG) from a WT mouse and a cKO mouse. Representative baseline EEG and EMG traces for a (A) WT mouse and a (B) cKO mouse shown for wakefulness (top), NREMS (middle), and REMS (bottom) for each mouse.