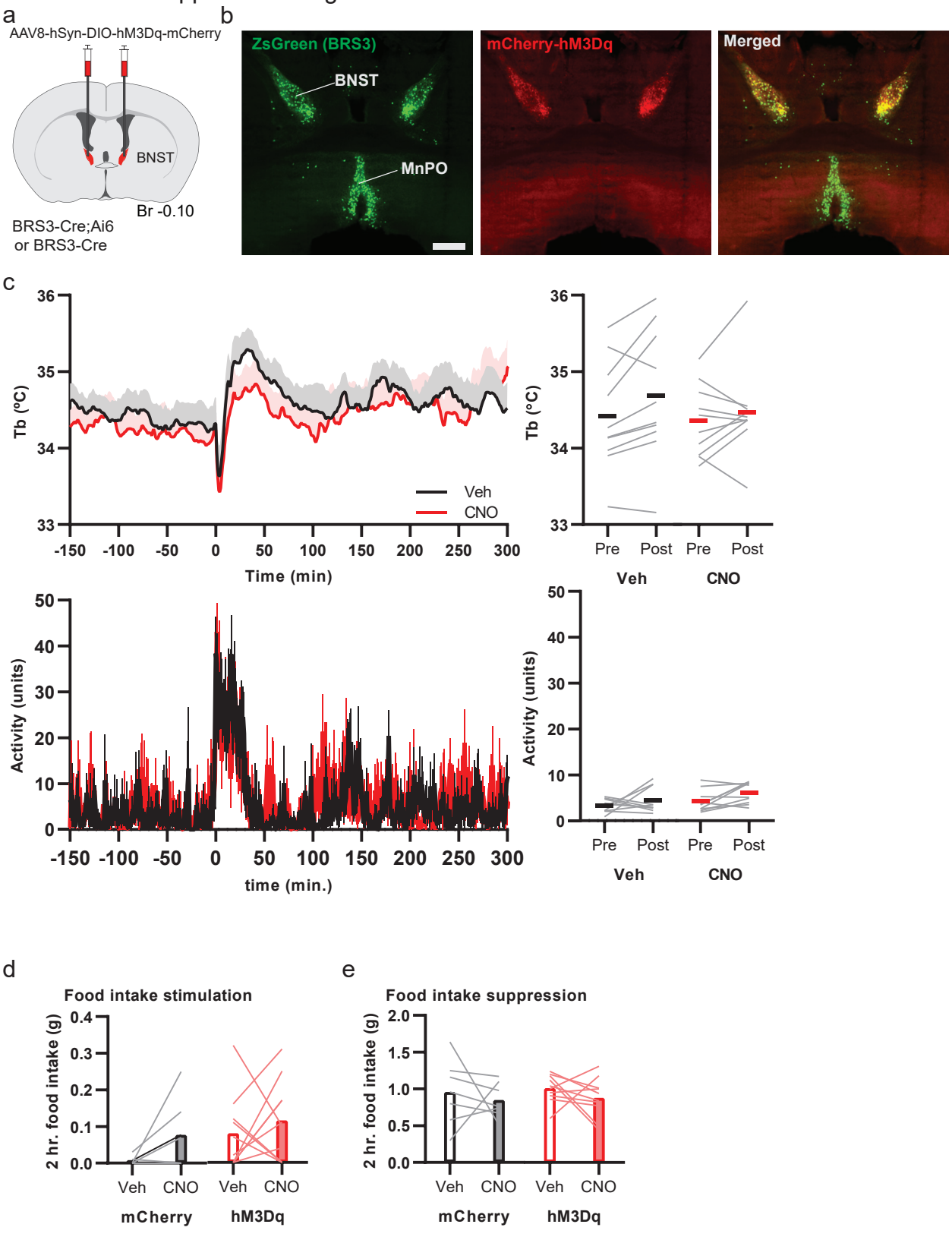
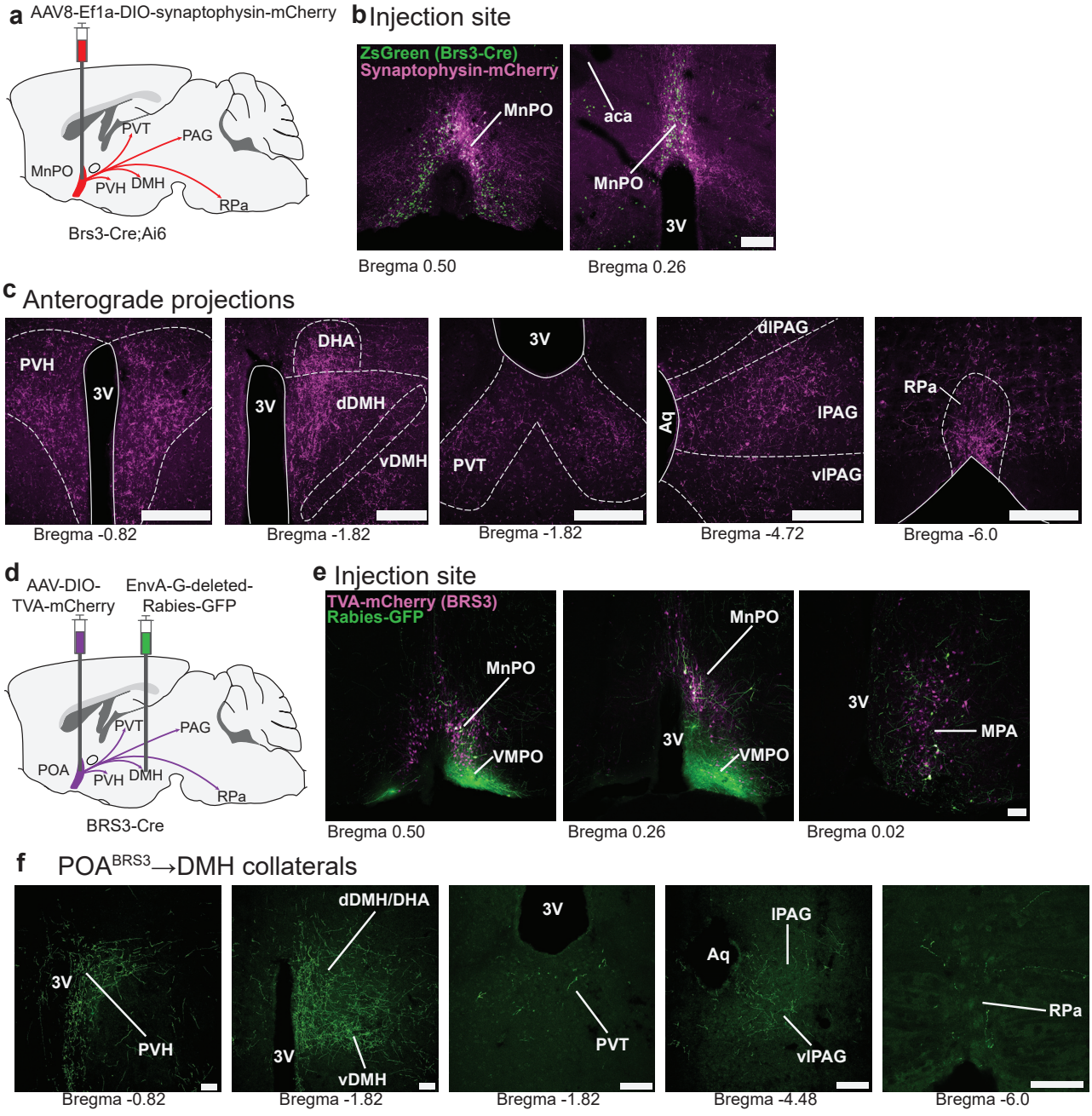
Supplemental Fig 1

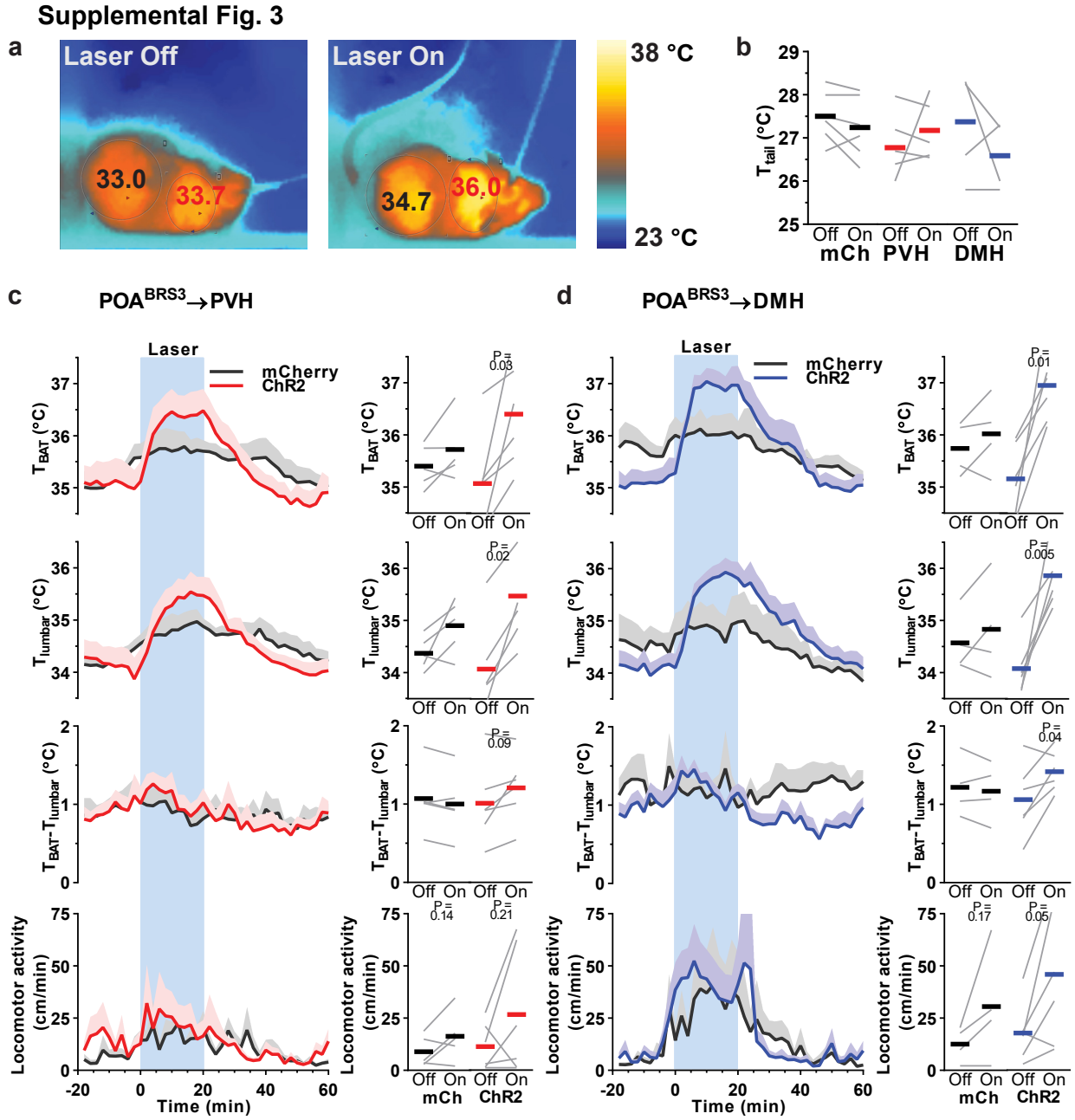


Supplemental Figure 1. Chemogenetic activation of $BNST^{BRS3}$ neurons does not change Tb, physical activity or food intake. Related to Figure 3. a) Schematic of virus injection. b) hM3Dq expression (red) in BRS3 (green) neurons in the BNST of a BRS3-Cre;Ai14 mouse. Angle of coronal slice was adjusted to include the MnPO and BNST in the same slice. Scale bar is 500 µm c,d) Tb and physical activity response to CNO (1 mg/kg) or vehicle in $BNST^{BRS3}$::hM3Dq mice (n = 10). For quantification, Pre is mean from -150 to -30 and Post from 60 to 180 min. No significant difference in paired two-sided t test on change from baseline, CNO vs vehicle. Data are mean + s.e.m. in top left panel. S.e.m. not graphed for visual clarity in bottom left. Black (vehicle) and red (CNO) bars represent means. d) Effect of CNO (1 mg/kg) on food intake at onset of light cycle in satiated $BNST^{BRS3}$::hM3Dq (n = 10/group) and control $BNST^{BRS3}$::mCherry mice (n = 7/group). e) Effect of CNO (1 mg/kg) on food intake at onset of dark cycle after 5 h fast in $BNST^{BRS3}$::hM3Dq mice (n = 10/group) and control $BNST^{BRS3}$::mCherry mice (n = 7/group). (c-e) Crossover design.

Supplemental Fig. 2

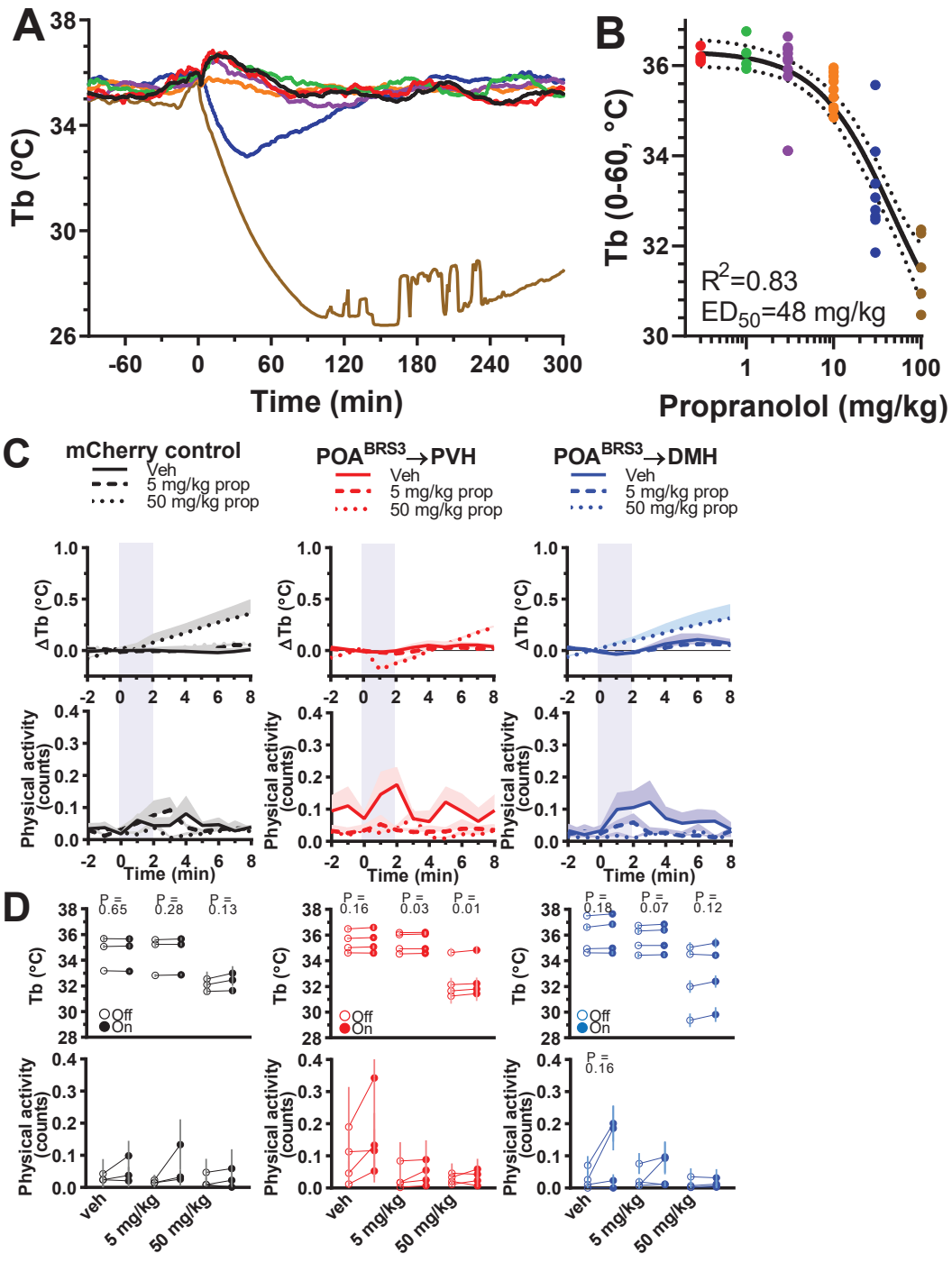


Supplemental Figure 2. POA^{BRS3} neurons project widely in the brain with collaterals. Related to Figure 4. a) Schematic of virus injection into BRS3-Cre;Ai14 mice for Cre-dependent synaptophysin mCherry expression and the major projections. b) Injection site verification showing that virus was limited to MnPO region of POA. BRS3 neurons are green and viral synaptophysin is magenta. c) Projection targets of $MnPO^{BRS3}$ neurons. b,c) Scale bar is 200 µm. d) schematic of virus injection strategy in BRS3-Cre mice; Rabies-GFP was injected in the DMH >4 weeks after first injections and tamoxifen treatment. e) POA^{BRS3} TVA-mCherry expressing neurons (magenta) and Rabies-GFP-expressing neurons (green), retrogradely infected through axon projections to DMH. f) Projection targets of POA^{BRS3}→DMH neurons. e,f) Scale bar is 100 µm. 3V – third ventricle; aca – anterior commissure; Aq – aqueduct; dDMH/DHA – dorsal part of the dorsomedial hypothalamus/dorsal hypothalamic area; dlPAG - dorsolateral periaqueductal grey; MnPO – median preoptic area; MPA – medial preoptic area; PVH – paraventricular nucleus of the hypothalamus; PVT – paraventricular nucleus of the thalamus; lPAG - lateral periaqueductal grey; RPa – raphe pallidus; vDMH – ventral part of the dorsomedial hypothalamus; vlPAG –ventrolateral periaqueductal grey; VMPO – ventromedial preoptic area.



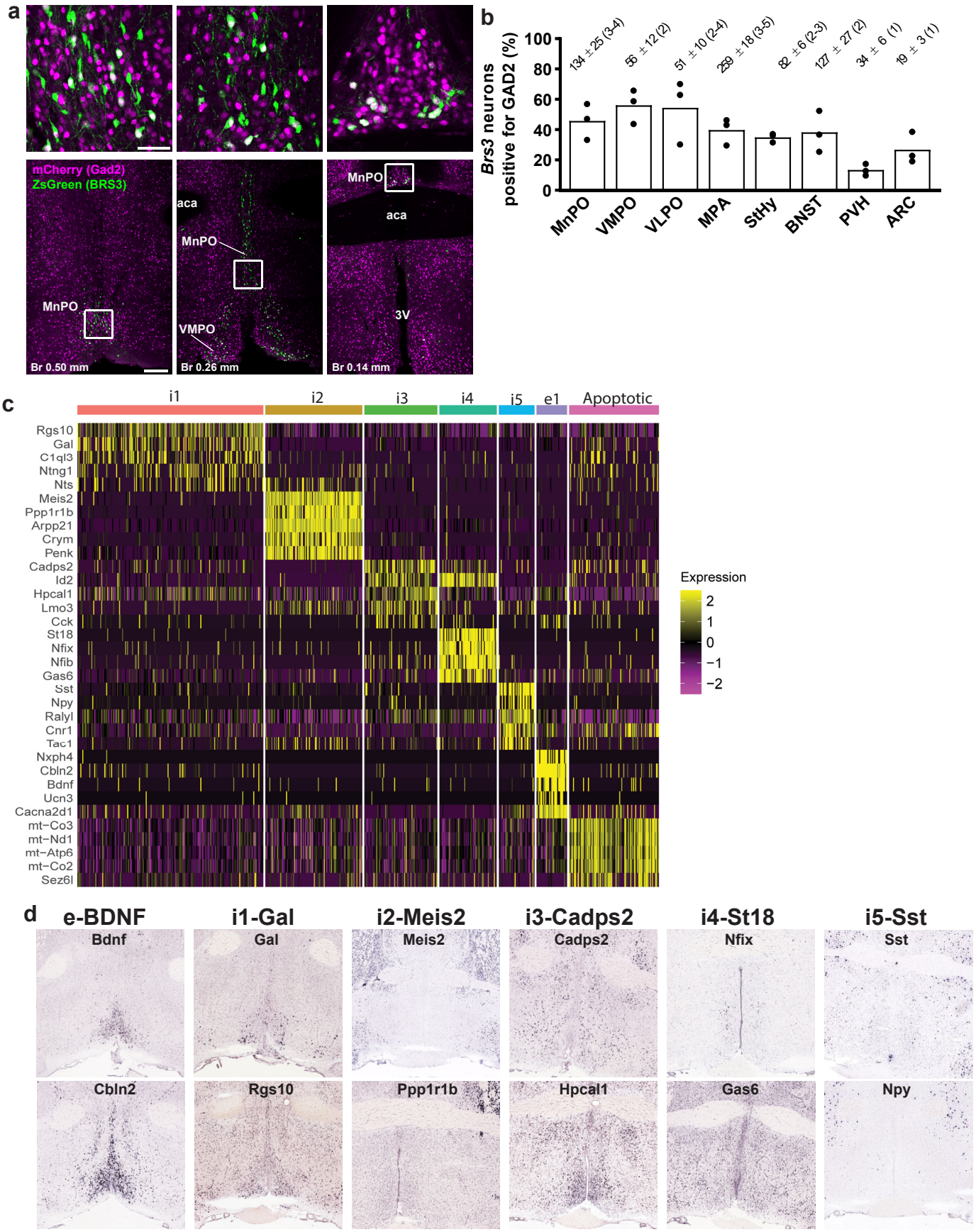
Supplemental Figure 3. POA^{BRS3}→PVH and POA^{BRS3}→DMH neurons increase Tb through BAT activation. Related to Figure 4. a) POA^{BRS3}→PVH mouse with laser off (left) and on for 6 minutes (right). Infrared camera interscapular (T_{BAT}; red) and lumbar (T_{lumbar}; black) skin temperature. b) Mean (bars) and individual (gray lines) tail temperature (T_{tail}) before and during stimulation. POA^{BRS3}→PVH::mCherry and POA^{BRS3}→DMH::mCherry mice are combined in the control group. c,d) T_{BAT}, T_{lumbar}, their difference (T_{BAT}-T_{lumbar}), and physical activity during optogenetic stimulation (blue interval; 1s on 3s off; 20 Hz; 10 ms pulses) of POA^{BRS3}→PVH (c, red, n= 6) and POA^{BRS3}→DMH (d, blue, n= 6) projections and respective mCherry controls (black, n = 4-5). Data are average of 3 epochs/mouse, relative to epoch baseline (-20 to -1 min); mean ± s.e.m Quantitation in right panels uses intervals: Off, -10 to -2 min; On, 10 to 18 min for T_{BAT} and T_{lumbar}, 0 to 8 min for locomotor activity, and Off, -4 to 0 min; On, 2 to 6 min for T_{BAT}-T_{lumbar}. Experiments were performed at 25 °C. Bars are means; gray lines, individual animals; P values from paired t test, Off vs On.

Supplemental Fig. 4



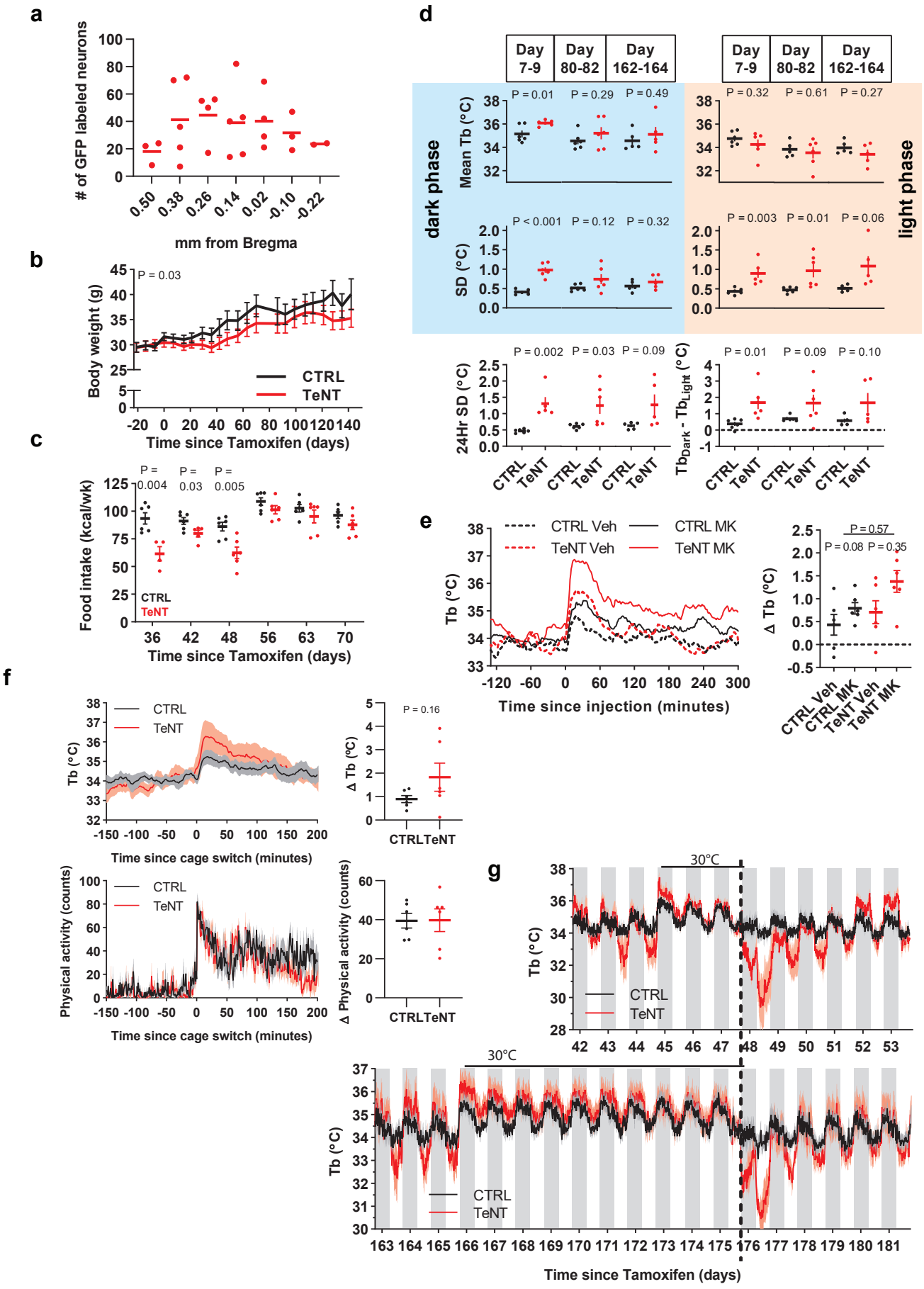
Supplemental Figure 4. Effect of propranolol on body temperature. Related to Figure 4. a) Wild-type mice were treated with the indicated dose of propranolol (color key in b) or vehicle (10% DMSO for 30 and 100 mg/kg; water for other doses) at time 0. b) Tb (mean of 0-60 min after dosing) data were non-linearly fit using Prism to Tb = bottom+(top-bottom)/(1+(dose/ED_{50})), giving parameters ED_{50} = 48 mg/kg, bottom = 29.0 °C, and top = 36.1 °C, with R^2 = 0.83 and DF = 47. The fitted curve and its 95% confidence interval are in black. c) Effect of stimulating POA^{BRS3}→PVH or POA^{BRS3}→DMH neurons on Tb and physical activity during propranolol treatment. mCherry controls (black, n = 3), POA^{BRS3}→PVH (red, n = 4), POA^{BRS3}→DMH (blue, n = 4). Data in each epoch was normalized to its baseline (-1 to 0 min) and the effect of laser stimulation (blue shading) is depicted as change from baseline. Data are mean + s.e.m. In d, the individual mouse data are presented. Open symbols are baseline (Off, from -1 to 0 minutes) and closed symbols are stimulated (On, from 1 to 2 minutes for physical activity and from 4 to 5 minutes for Tb). P values from paired t test, Off vs On. Data are mean ± s.e.m. of 8 epochs (during 75-155 min).

Supplemental Fig. 5



Supplemental Figure 5. POA^{BRS3} neurons are a mix of excitatory and inhibitory clusters. Related to Figure 5. a) BRS3 (green) and GAD2 (magenta) are expressed in overlapping populations in the MnPO and VMPO in BRS3-Cre;Ai6;Gad2-mCherry mice. Scale bar overview image is 200 µm, inset 50 µm. b) Quantification of BRS3 neurons expressing GAD2. The number of double positive neurons ± s.e.m. in the indicated region and (number of slices/mouse counted) are indicated; n = 3 mice. c) Expression profile of several marker mRNAs for the POA region BRS3 clusters. Data from Moffit et al., 2018. d) Allen Brain Atlas ISH images (http://mouse.brain-map.org/) for two of the marker mRNAs (aside of BRS3 and Vglut2 or Vgat) for each cluster for the POA region.

Supplemental Figure 6



Supplemental Figure 6. Silencing POA^{BRS3} neurons increases Tb variability and exaggerates Tb changes. Related to Figure 7. a) Number of EYFP labeled (TeNT) neurons in the POA at the indicated distance from Bregma. Every third section was counted from each mouse. b) POA^{BRS3}::TeNT mice gain less body weight than do controls. P value, repeated measures ANOVA. c) Reduced food intake in POA^{BRS3}::TeNT mice at 4-6 weeks after starting tamoxifen treatment. P values, unpaired t test. d) At indicated number of days after starting tamoxifen treatment, Tb was measured each minute during 72 h intervals and the Tb and standard deviation (SD) during dark (left) and light (right) phases was measured. The circadian amplitude (Tb_{dark}-Tb_{light}) and the Tb span (95^{th} – 5^{th} Tb percentiles) of the full intervals were also calculated. Data are mean ± s.e.m. P values, unpaired t test. e) Tb response to MK-5046 (10 mg/kg, i.p.) or vehicle (saline) and ΔTb ($Tb_{60to180}$ minus $Tb_{-150to-30}$). Data are mean ± s.e.m. (s.e.m. omitted from left for visual clarity); P value, paired t test between vehicle and MK-5046 and unpaired t test with unequal variance between delta Tb (MK-5046 minus vehicle) of CTRL and TeNT groups. f) Tb and physical activity response to switching mice to a clean cage. ΔTb is Tb_{0to60} minus $Tb_{-90to-30}$; Δ Physical activity was calculated the same way. Data are mean ± s.e.m. g) Acclimation and Tb response in mice exposed for 3 days (top) vs 10 days (bottom) to 30 °C, otherwise at 22 °C. Data are mean ± s.e.m. In all panels, n=6 mice/group, except for a) with n=5 mice.