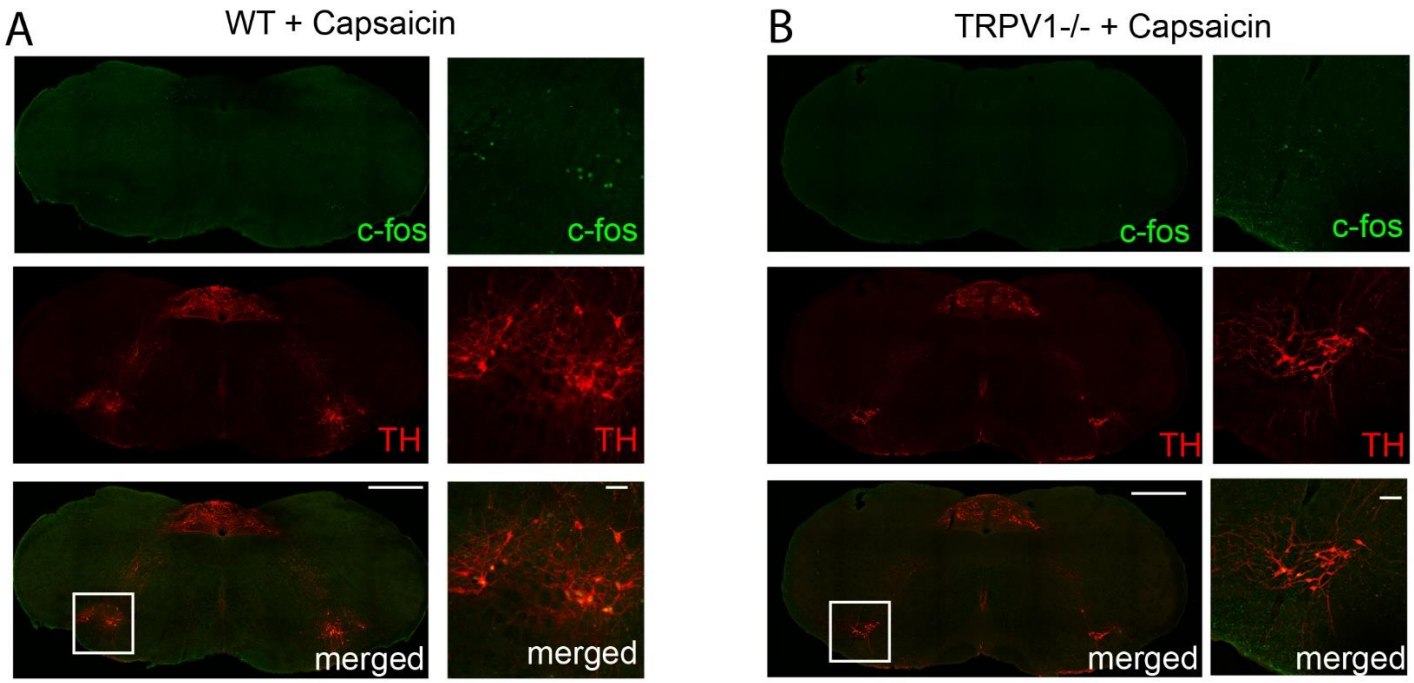

Fig S1 related to Fig 1**.** A-C. Representative images of the caudal medulla and magnified ventral lateral medulla regions where tyrosine hydroxylase immune-positive neurons are found (images to the right of the main panels). A. In control mice injected with capsaicin in the hind-paw cfos expression is found prominently in TH-positive neurons. B. By contrast, in the cVLM of Trpv1KO mice injected with capsaicin there were very few cfos-positive neurons. Scale bars: 500 µm for coronal sections and 50 µm for magnified field images.

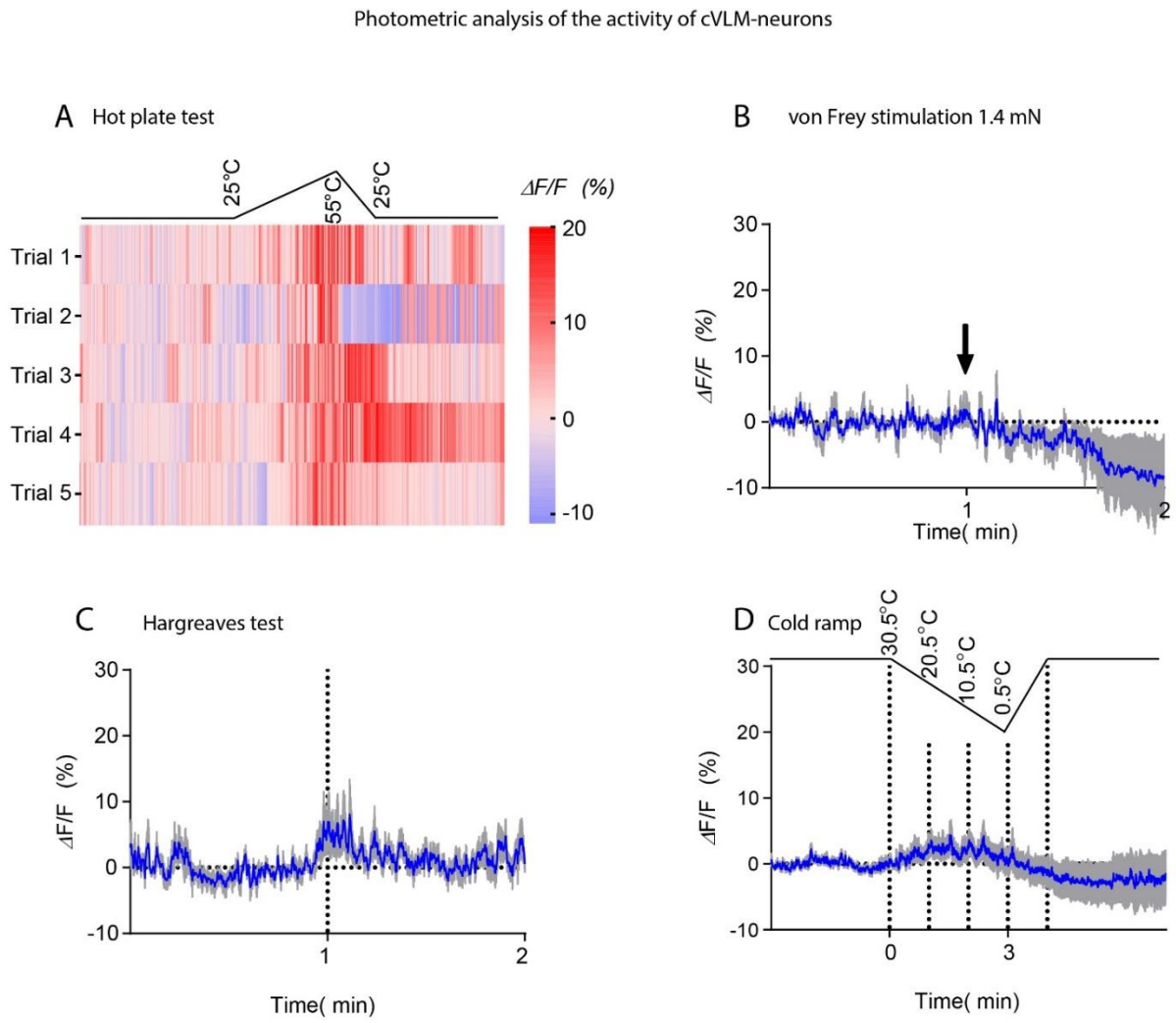

Fig S2 related to Fig 1**.** A. Heatmap of intracellular calcium responses of cVLMTH-neurons to a heat ramp, using *in vivo* fiber photometry. A Results from a single mouse tested repeatedly with the same heat-ramp challenge, show that these responses are reproducible. B-D. *In vivo* fiber photometry results for mild mechanical (von Frey filament on plantar surface of hind-paw), localized heating (Hargreaves test on plantar surface of hind-paw), and cold (plantar surface of hind-paw) stimulations show, only modest increases in intracellular calcium in cVLMTH-neurons; n=6 mice, data are represented as mean results (blue) ± SEM (grey).

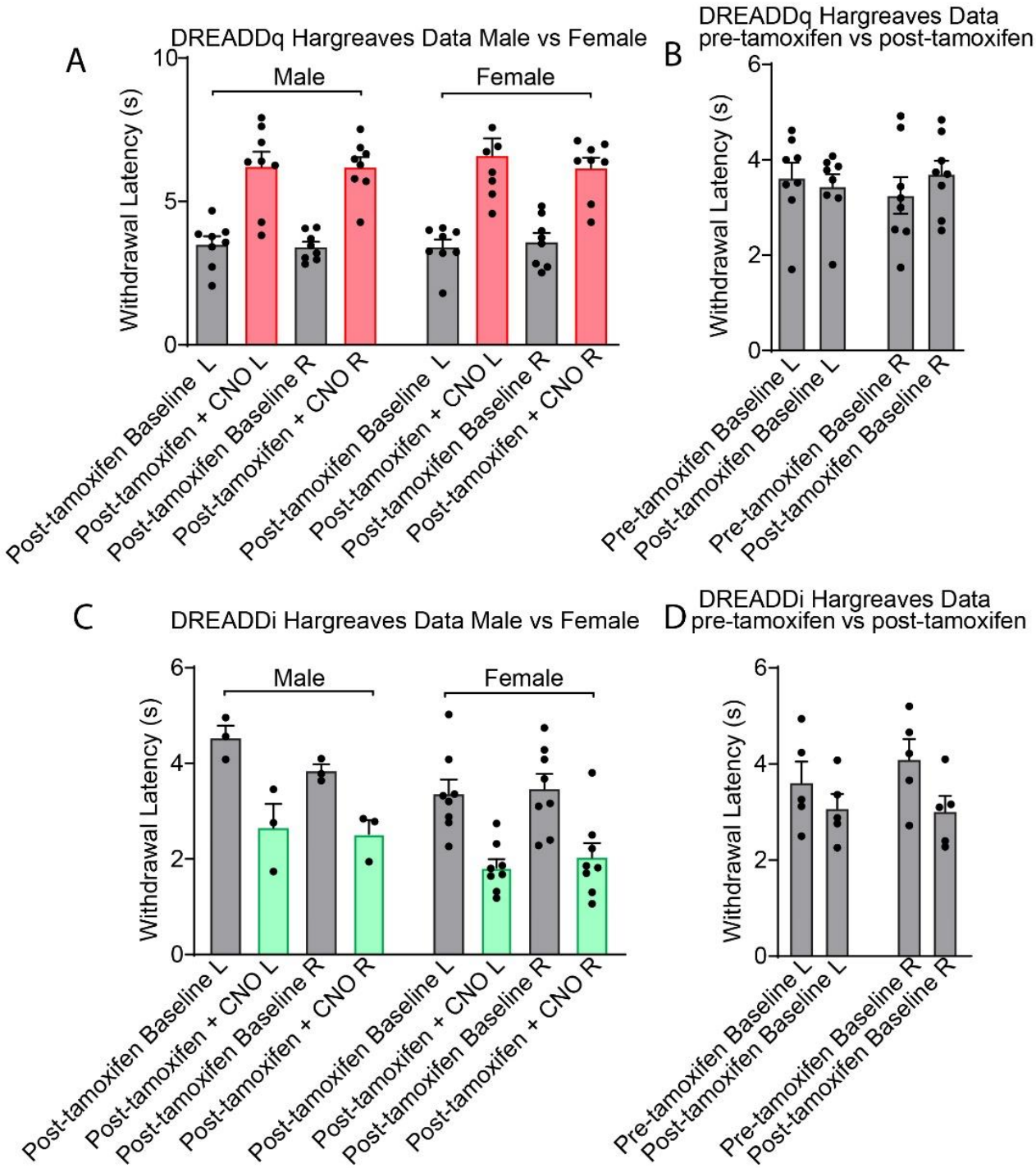
A
DREADDq Hargreaves Data Male vs Female
Withdrawal Latency (s)
Male
Female
Post-tamoxifen Baseline L
Post-tamoxifen + CNO L
Post-tamoxifen Baseline R
Post-tamoxifen + CNO R
B
DREADDq Hargreaves Data
pre-tamoxifen vs post-tamoxifen
Pre-tamoxifen Baseline L
Post-tamoxifen Baseline L
Pre-tamoxifen Baseline R
Post-tamoxifen Baseline R
C
DREADDi Hargreaves Data Male vs Female
D
DREADDi Hargreaves Data
pre-tamoxifen vs post-tamoxifen

Fig S3 related to Fig 2**.** A. For both male and female mice, withdrawal latencies were significantly increased, in Hargreaves tests, after chemogenetic activation of cVLMTH-neurons (CNO administration) compared to saline injected mice (L and R indicate left and right hind-paws respectively), n=8 male n=8 female mice, $p<0.001$ Student T-test, data represent means $\pm$ SEM. There were no significant difference in responses between male and female mice, $p=0.63$ for L and $p = 0.95$ for R, Student T-test, data represent means $\pm$ SEM. B. Hargreaves test responses of mice before and after administration of tamoxifen (to induce translocation of CreERT2 and recombination) were not significantly different, n=8, $p=0.42$ for L and 0.09 for R, Student T-test, data represent means $\pm$ SEM. C. For both male and female mice withdrawal latencies were significantly decreased, in Hargreaves tests, after chemogenetic inhibition of cVLMTH-neurons (CNO administration) compared to saline injected mice, n=3 male n=8 female mice, $p<0.05$ Student paired T-test. There were no significant differences in responses between male and female mice. $p=0.09$ for L and $p=0.38$ for R. D. Hargreaves test responses of mice before and after administration of tamoxifen. The were no significant differences between treatment groups, n=5, $p=0.30$ for L and $p=0.08$ for R, Student T-test, data represent means $\pm$ SEM.

Analysis of effects of stereotaxic injection of AAV-EF1a-flex DREADDq into the cVLM of TH-CreER mice

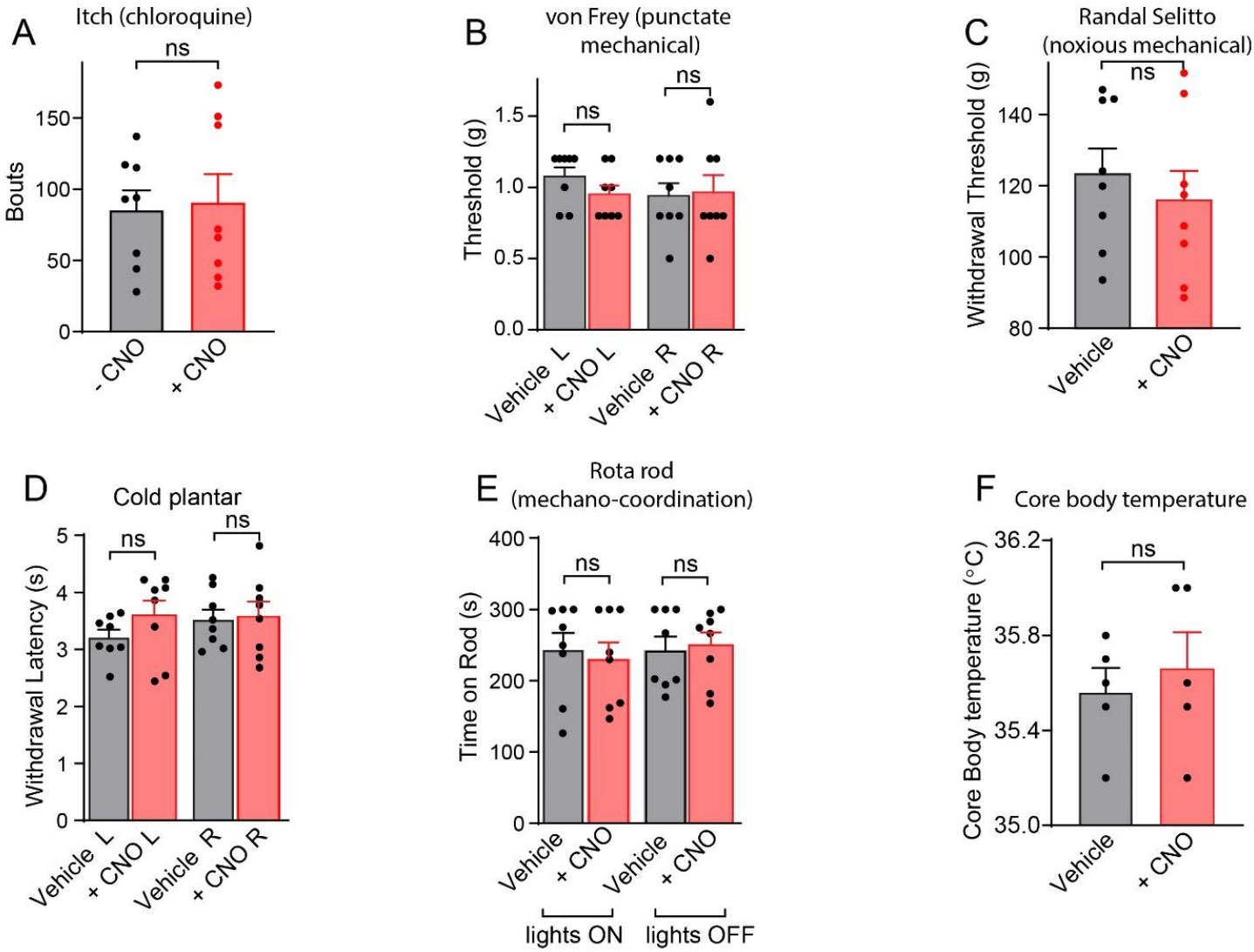

Fig S4 related to Fig 2**.** A-F Analysis of behavioral responses in TH-CreER mice injected unilaterally in the cVLM with AAV2-hSyn-DIO-hM3D(Gq)-mCherry and tested in behavioral assays following chemogenetic activation of cVLMTH-neurons (CNO). A. Number of scratching bouts over 30 minutes to intradermal injection of chloroquine (200 µg) in the nape of the neck was not significantly different between treatment groups ($\pm CNO$) n=8 mice, p=0.76, Student T-test, data represent means $\pm$ SEM. B. Threshold responses to von Frey filament stimulation was not significantly different between treatment groups ($\pm$CNO) n=8 mice, p=0.80, Student T-test, data represent means $\pm$ SEM. C. Mechanical pinch responses (Randal Selitto method) were not significantly different between treatment groups ($\pm CNO$) n=8 mice, p=0.47, Student T-test, data represent means $\pm$ SEM. D. Latencies for withdrawal in plantar reflex responses to cold stimulation were not significantly different between treatment groups ($\pm CNO$) n=8 mice, p=0.11, Student T-test, data represent means $\pm$ SEM. Motor coordination was not significantly different between treatment groups ($\pm CNO$) n=8 mice, p=0.43 Student T-test, data represent means $\pm$ SEM. F. Core body temperature measured with a rectal thermal probe was not significantly different between treatment groups ($\pm CNO$) n=5 mice, p=0.58, Student T-test, data represent means $\pm$ SEM.

Analysis of effects of stereotaxic injection of AAV2-hSyn-DIO-hM4Di into the cVLM of TH-CreER mice

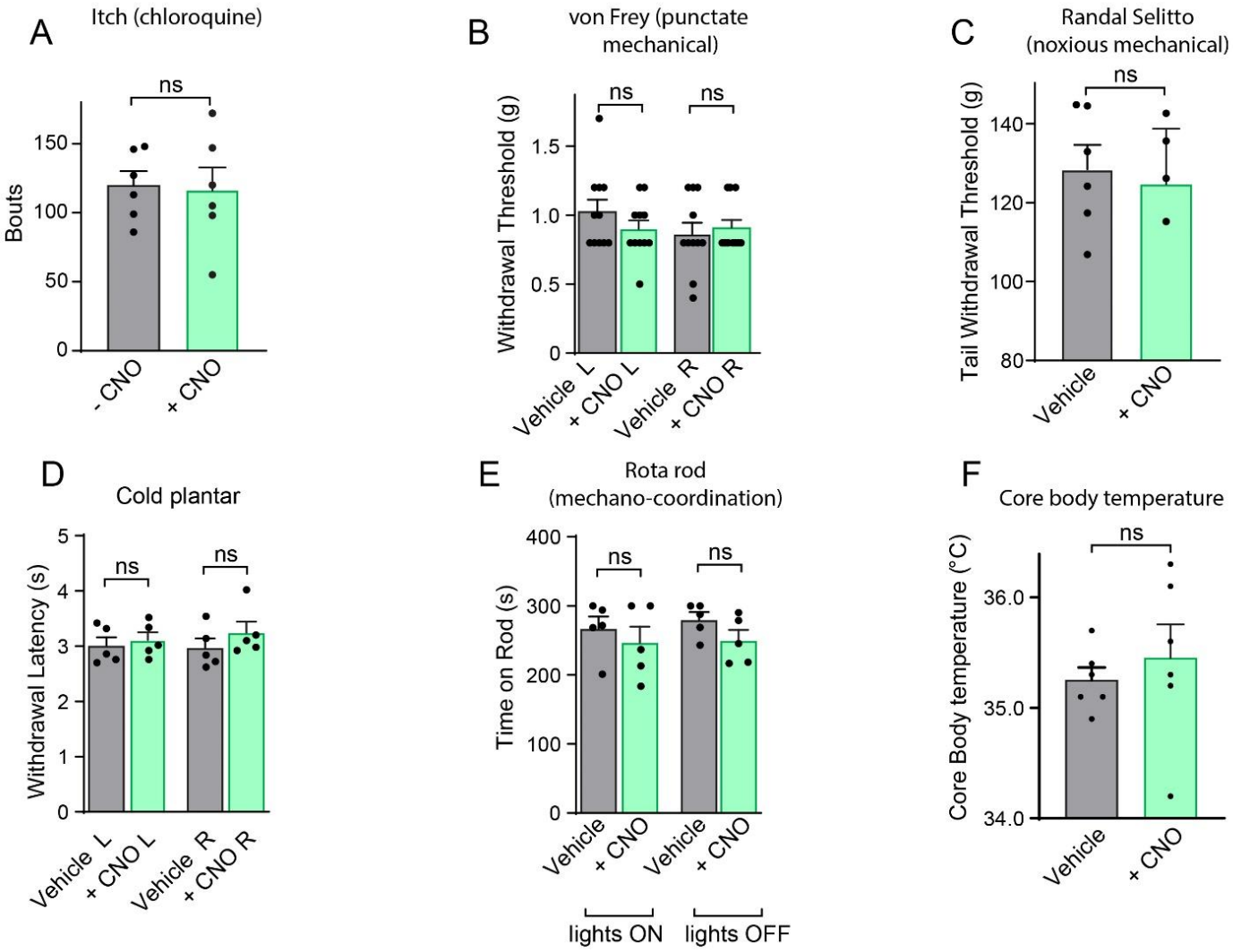

Fig S5 related to Fig 2**.** A-F Analysis of behavioral responses in TH-CreER mice injected unilaterally in the cVLM with AAV2-hSyn-DIO-hM4D(Gi)-mCherry and tested in behavioral assays following chemogenetic inhibition of cVLMTH-neurons (CNO). A. Number of scratching bouts over 30 minutes to intradermal injection of chloroquine (200µg) in the nape of the neck was not significantly different between treatment groups $(\pm CNO)$ n=6 mice, p=0.83 Student T-test, data represent means $\pm$ SEM. B, Threshold responses to von Frey filament stimulation was not significantly different between treatment groups $(\pm CNO)$ n=11 mice, p=0.30 for L and 0.55 for R. Student T-test, data represent means $\pm$ SEM. C. Mechanical pinch responses (Randal Selitto method) were not significantly different between treatment groups $(\pm CNO)$ n=6 mice, p=0.75. Student T-test, data represent means $\pm$ SEM. D. Latencies for withdrawal in plantar reflex responses to cold stimulation were not significantly different between treatment groups $(\pm CNO)$ n=5 mice, p=0.64 for L and 0.08 for R. Student T-test, data represent means $\pm$ SEM. Motor coordination was not significantly different between treatment groups $(\pm CNO)$ n=5 mice, p=0.13. Student T-test, data represent means $\pm$ SEM. F. Core body temperature measured with a rectal thermal probe was not significantly different between treatment groups $(\pm CNO)$ n=6 mice, p=0.41. Student T-test, data represent means $\pm$ SEM.

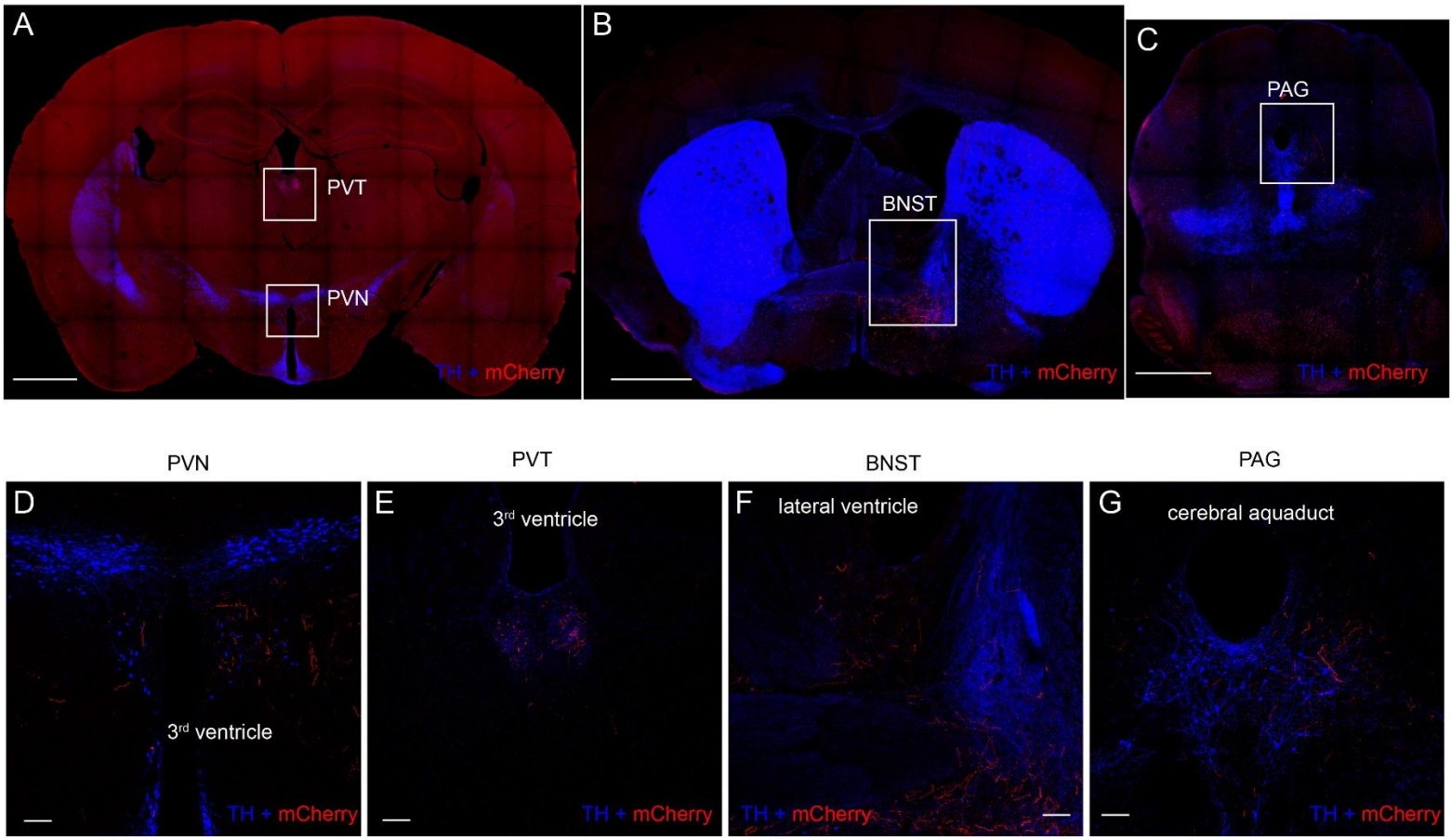

Fig S6 related to Fig 3**.** A-G. Representative images of the pattern of fiber projections of TH-Cre mice injected in the cVLM with AAV2-Syn-DIO-TVA-mCherry, showing labeled fibers (red) and TH-staining (blue), n=3 mice. A. Representative image of a coronal section of the midbrain showing cVLMTH-neuron projections to the PVT and PVN. B. Section through midbrain showing cVLMTH-neuron projections to the BNST. C. Section of the hindbrain showing cVLMTH-neuron projections to the PAG. D and E. Magnified view of boxed areas in A. F. Magnified view of boxed areas in B. G. Magnified view of boxed areas in C. Scale bars: 1 mm in A-C and 100 µm in D-G.

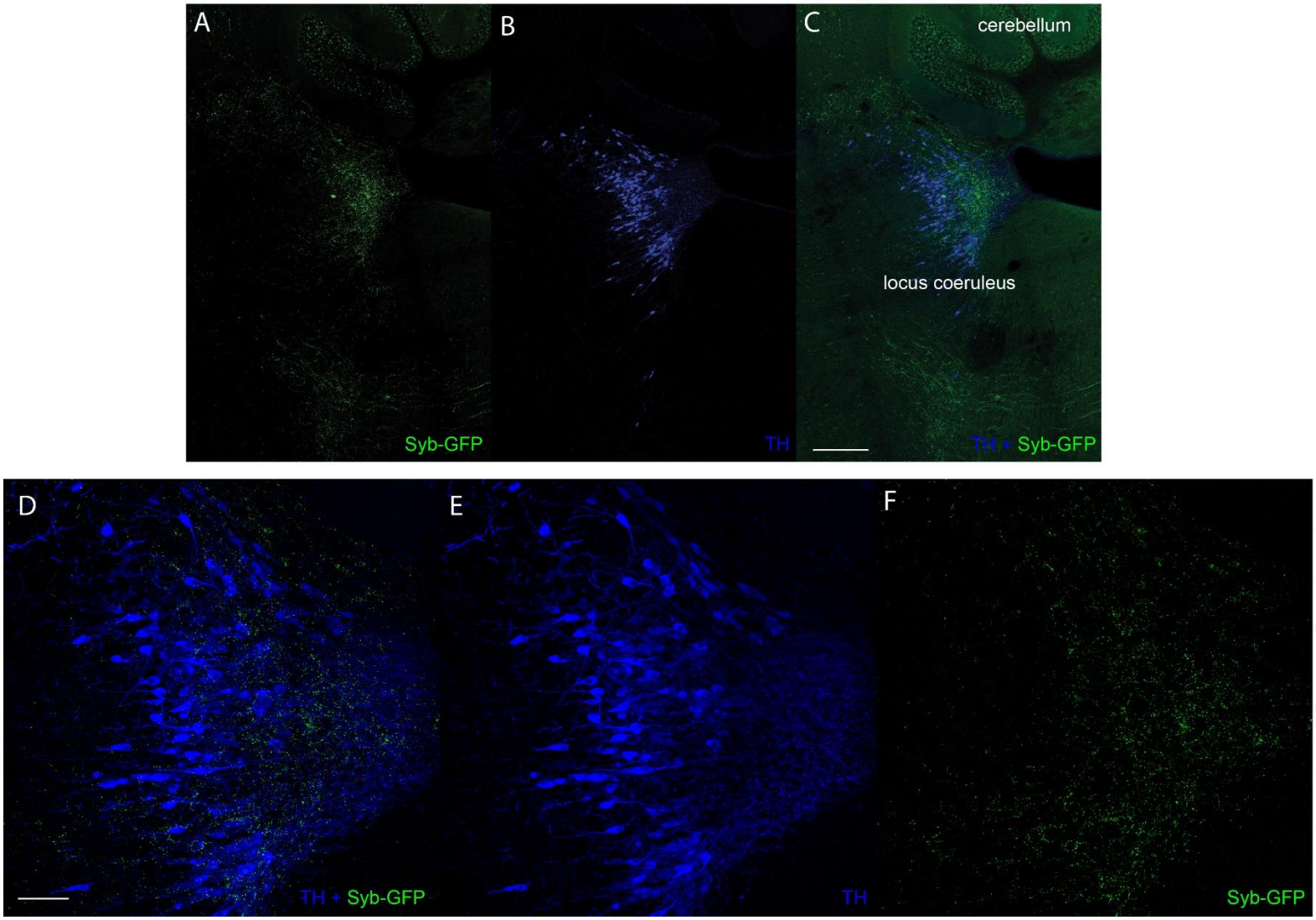

Fig S7 related to Fig 3**.** A-F. Representative images of the pattern of fiber projections from TH-Cre mice injected in the cVLM with AAV9-hSyn-DIO-mCherry-2A-SybGFP, showing synaptic boutons (green) on projecting axons and TH-staining of LC-neurons (blue), n=3 mice. A-C. Representative image of a sagittal section of the hindbrain showing cVLMTH-neuron projections to the LC. D-F. Magnified view of LC. Scale bars, 500 µm in A-C and 50 µm in D-F.

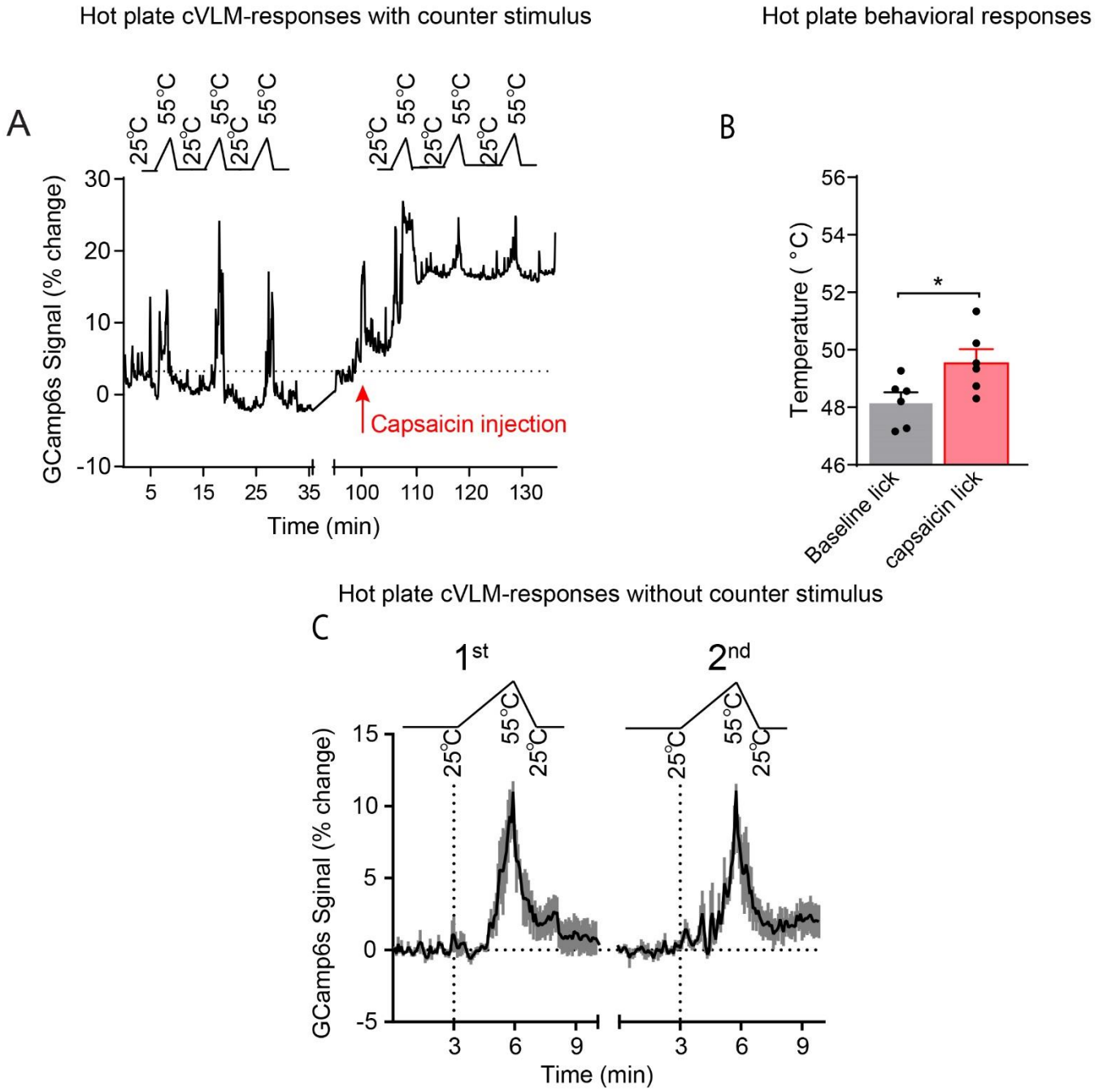
Hot plate cVLM-responses with counter stimulus
Hot plate behavioral responses
A
B
25°C
55°C
GCamp6s Signal (% change)
Capsaicin injection
Time (min)
Temperature (°C)
Baseline lick
capsaicin lick
Hot plate cVLM-responses without counter stimulus
C
1st
2nd
GCamp6s Sginal (% change)
Time (min)

Fig S8 related to Fig 4**.** A. Representative example of calcium responses of cVLMTH-neurons, measured using *in vivo* fiber photometry, from a single mouse to repeated noxious heat stimulation (on a hot plate; temperature ramps indicated above trace) before and after injection of capsaicin counter-stimulus into the fore paw (indicated with red arrow). A 1-hour rest period was included between naïve and counter-stimulus trials. B. Behavioral responses to heat challenge (heat ramp to 55 ºC) on a hot plate before and after injection of capsaicin in the forepaw; latency to first lick (left columns). There was a significant difference between trial groups for first lick ($\pm$ capsaicin), n=6 mice, p=0.043, paired Student T-test, data represent means ± SEM. C. Averaged *in vivo* photometry responses of cVLMTH-neurons for three trials (combined and averaged) before and after a 1-hour rest period to heat challenges (3 x heat ramp to 55 ºC) on a hot plate, showed that averaged responses were not altered to repeated noxious thermal insult.