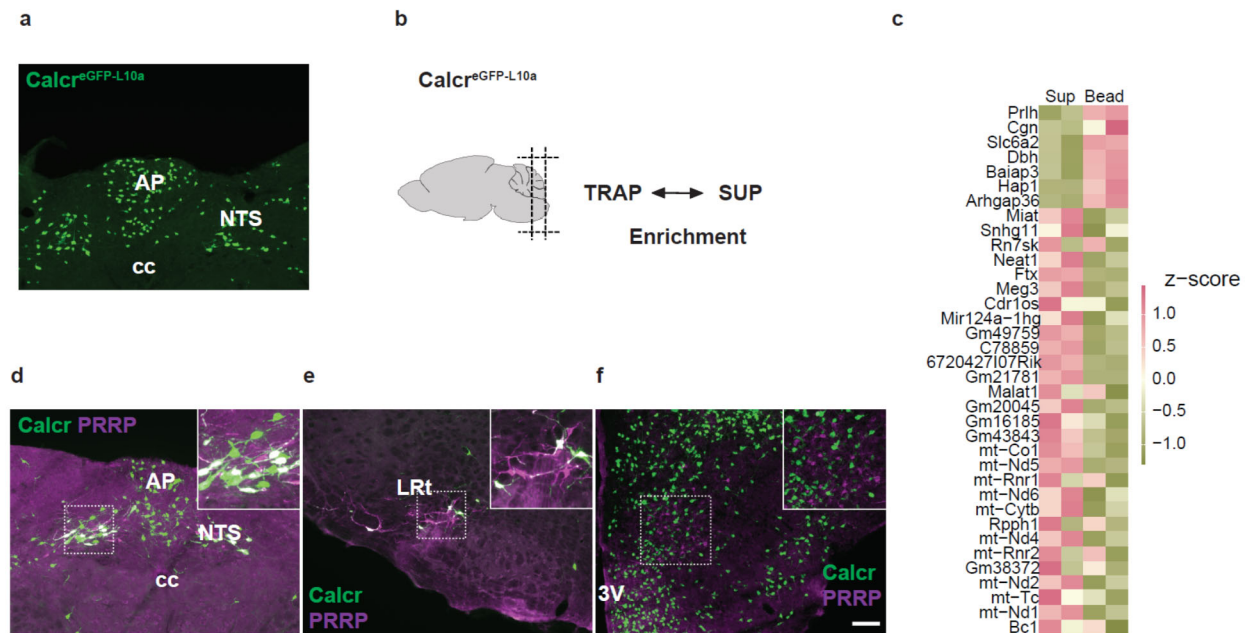
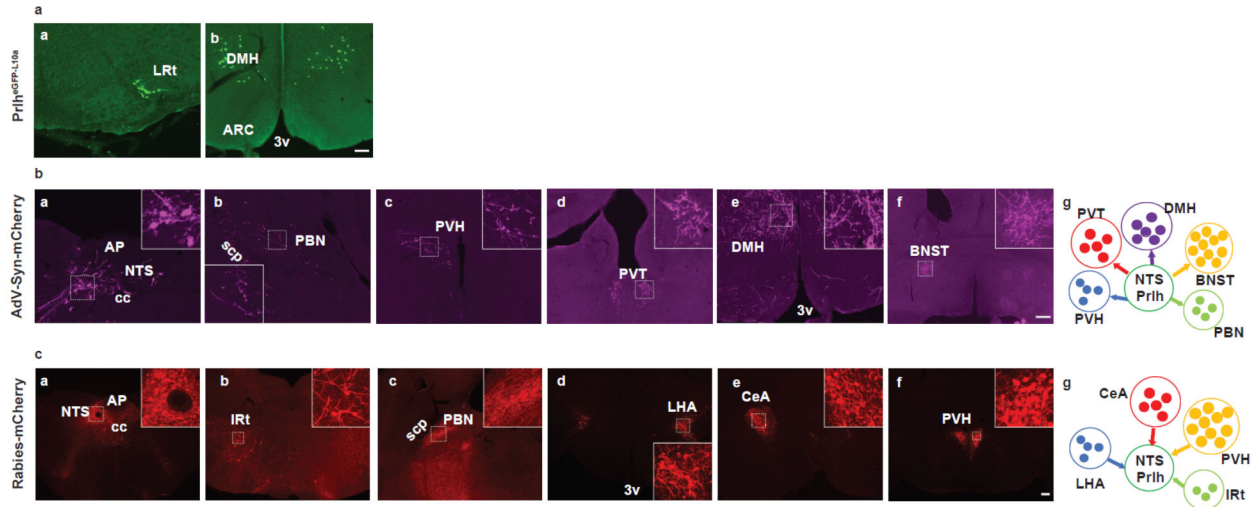


Supplemental Figures

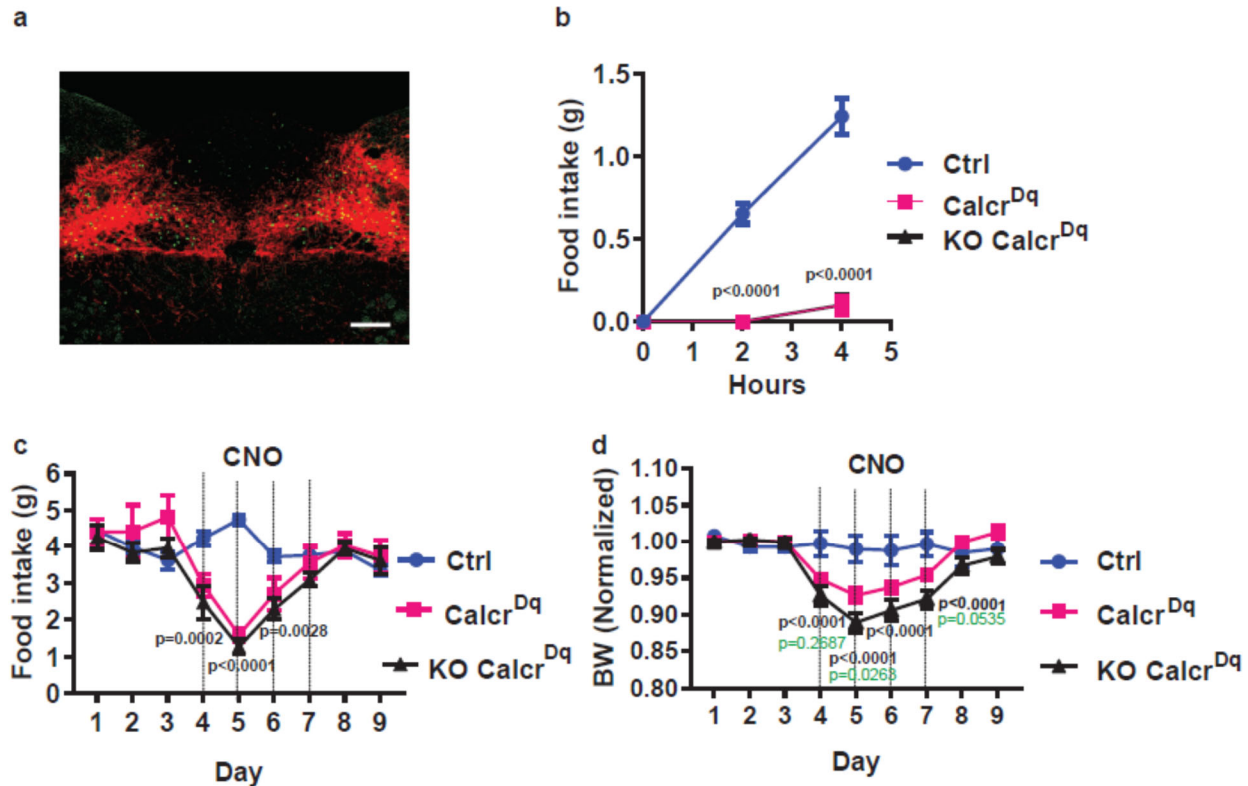


Supplemental figure 1: Expression of *Prlh* in *Calcr* neurons. (A) Image showing GFP-IR (green) in the NTS and AP of a *Calcr*^{eGFP-L10a} mouse; representative of at least three independent experiments with similar results. **(B)** Schematic diagram showing hindbrain dissection for TRAP and the comparison of TRAP-derived and supernatant mRNAs to determine enrichment. **(C)** Heat map showing genes, including *Prlh*, that are enriched in hindbrain *Calcr* neurons. Experiment was performed twice with one sample per condition; results, which were similar between experiments, were combined. **(D-F)** Representative images showing PRRP-IR (magenta) and GFP-IR (green) in PRRP-containing brain regions from a *Calcr*^{eGFP-L10a} mouse: NTS **(D)**, LRT **(E)** and DMH **(F)**. Representative of at least three independent experiments with similar results. All images acquired at the same magnification; scale bar equals 150 μ m. cc: central canal; 3v: third ventricle.



Supplemental figure 2: Distribution of *Prlh*^{cre}-mediated recombination and of afferent and efferent connections of *Prlh*^{NTS} cells. (A) Representative images of GFP-IR (green) in *Prlh*^{eGFP-L10a} mice in the LRt (Aa) and DMH (Ab). (B) Representative images showing the distribution of dsRed-IR (purple) in the brain of *Prlh*^{cre} mice following the intra-NTS injection of AdV-Syn-mCherry (which mediates the cre-dependent expression of a synaptophysin-mCherry fusion protein), showing the NTS injection site (Ba) and projections fields in the PBN (Bb), PVH (Bc), PVT (Bd), DMH (Be) and BNST (Bf). Model of all projections is shown, with relative strength of innervation, in Bg. (C) AAV^{Flex-TVA+G} was injected into the NTS of *Prlh*^{cre} mice; 1 month later the mice were injected in the NTS with pseudotyped mCherry-expressing rabies virus. Representative images show the injection site in the NTS (Ca), and traced cells in the LRt (Cb), PBN (fibers only) (Cc), LHA (Cd), CeA (Ce), and PVH (Cf). Model of all regions with neurons presynaptic to *Prlh*^{NTS} cells, with relative strength of innervation, is shown in Cg. LRt: Lateral reticular nucleus. cc= central canal, scp=superior cerebellar peduncle, LHA-lateral hypothalamus, CeA=The central nucleus of the amygdala, PVH-para ventricular hypothalamus. BNST= Bed Nucleus of the Stria Terminalis. 3v- third

cerebral ventricle. All other abbreviations defined in the main text. Images are representative of at least 3 animals. All images acquired at the same magnification; scale bar equals 150 μm . All data representative of at least two independent experiments consisting of at least two similar replicates.

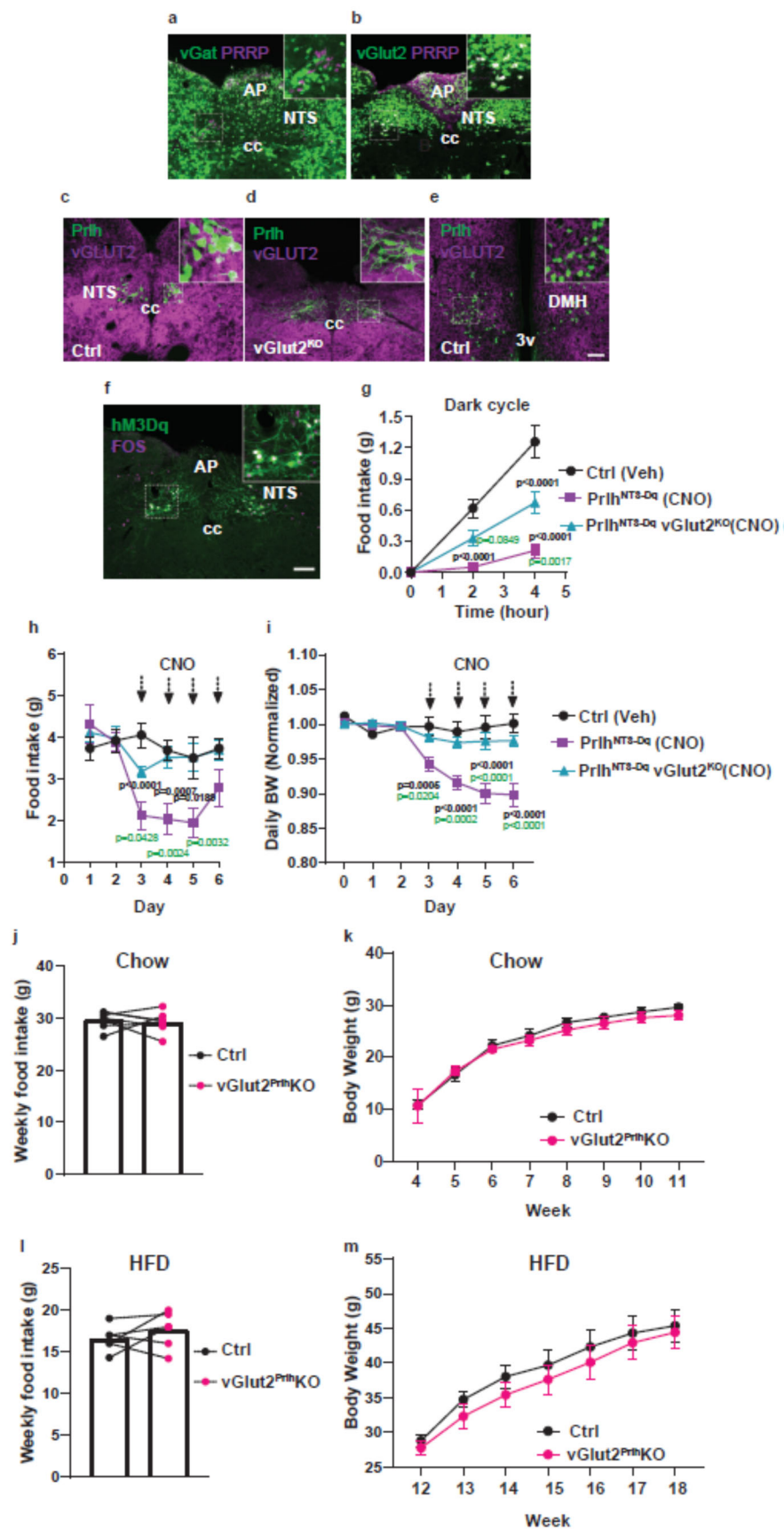


Supplemental figure 3: DREADD-mediated activation of Calcr^{NTS} neurons in

***Prlh*^{Calcr}KO^{Dq} mice suppresses food intake despite lack of *Prlh* expression. (A)**

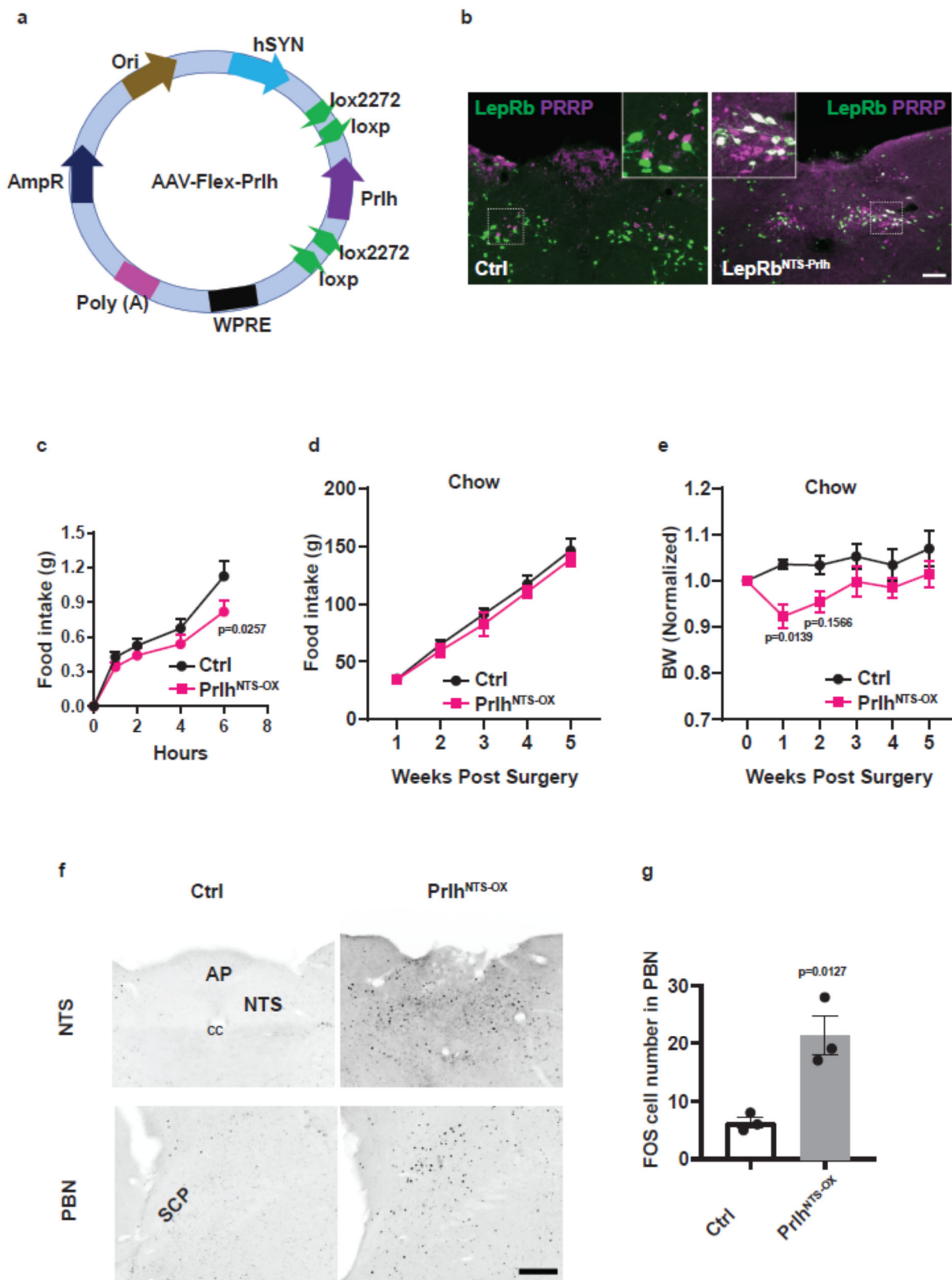
Representative image showing dsRed-IR (red) and FOS-IR (green) in CNO-treated (IP, 1 mg/kg, 2 hr) *Prlh*^{Calcr}KO^{Dq} mice. Scale bar equals 150 μ m. **(B-D)** Food intake at the onset of the dark cycle during CNO (1 mg/kg, IP) treatment (**B**, $n=15$ Ctrl, $n=7$ Calcr^{Dq}, and $n=8$ KO Calcr^{Dq} animals) and food intake (**C**, $n=7$ Ctrl, $n=6$ Calcr^{Dq}, and $n=8$ KO Calcr^{Dq} animals) and body weight (**D**, normalized to initial weight, $n=5$ Ctrl, $n=7$ Calcr^{Dq}, and $n=8$ KO Calcr^{Dq} animals) for control (Ctrl, blue), Calcr^{NTS-Dq} (Calcr^{Dq}, red) and *Prlh*^{Calcr}KO^{Dq} (KO Calcr^{Dq}, black) mice during treatment with vehicle (3 days), CNO (IP, 1 mg/kg BID; 4 days) and washout (2 days). Days of CNO treatment are marked on the graphs in **C**, **D**. Shown is mean $\pm$ SEM. Two-way ANOVA, sidak's multiple comparisons test was used. Significant or near significant p values for comparisons

between Ctrl and KO Calcr^{Dq} or Calcr^{Dq} groups shown in black; those for comparisons between between KO Calcr^{Dq} and Calcr^{Dq} groups shown in green. All experiments were repeated in two independent cohorts of animals with similar results; cohorts were combined for publication.

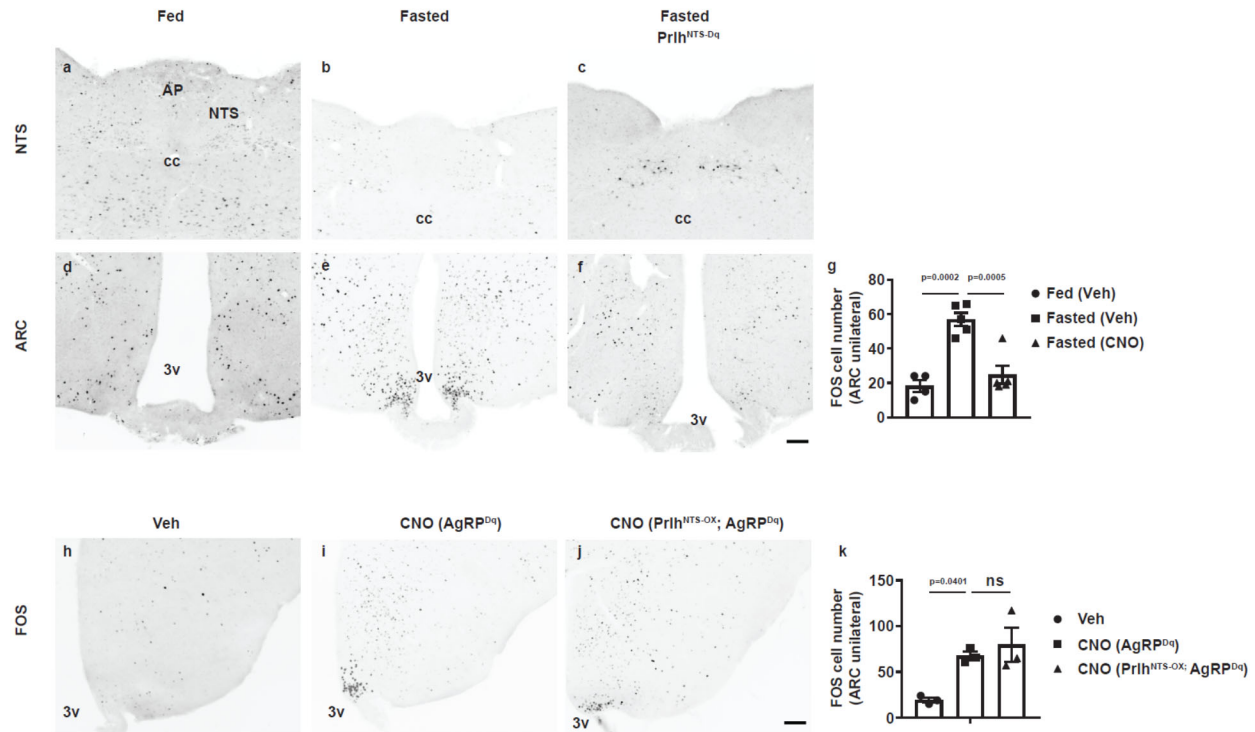


Supplemental figure 4: Glutamatergic transmission contributes to the suppression of feeding during DREADD-mediated activation of Prlh^{NTS} neurons in Prlh^{Calcr}KO^{Dq} mice. (A, B) Representative images showing PRRP-IR (purple) and GFP-IR (green) in the NTS of vGat^{GFP} (A) and vGlut2^{GFP} (B) reporter mice. (C-E) Representative images showing GFP-IR (green, C, E) and PRRP-IR (green, D) and vGLUT2-IR (purple) in the NTS (C, D) and DMH (E) of Prlh^{eGFP-L10a} (Ctrl; C, E) and *Prlh^{cre};vGlut2^{flox/flox}* (vGlut2^{KO}, D) mice. (F) Representative image showing dsRed-IR (green, hM3Dq) and FOS-IR (purple) in a CNO (IP, 1 mg/kg)-treated vGlut2^{KO} mouse that was injected with AAV^{Flex-hm3dq} in the NTS (Prlh^{NTS-Dq};vGlut2^{KO}). (G-I) Food intake at the onset of the dark cycle during CNO (1 mg/kg, IP) (G, n=12 Ctrl animals, n=10 Prlh^{NTS-Dq}, and n=7 Prlh^{NTS-Dq};vGlut2^{KO} animals) and food intake (H, n=6 animals per group for Ctrl and Prlh^{NTS-Dq};vGlut2^{KO}, n=7 for Prlh^{NTS-Dq} animals) and body weight (I, normalized to initial weight, n=6 animals per group for Ctrl and Prlh^{NTS-Dq};vGlut2^{KO}, n=7 for Prlh^{NTS-Dq} animals) for control (Ctrl, black), Prlh^{NTS-Dq} (purple) or Prlh^{NTS-Dq};vGlut2^{KO} (blue) mice during treatment with vehicle (3 days), and then CNO (IP, 1 mg/kg BID; 4 days). Days of CNO treatment are marked on the graphs in H, I. (J-M) The 11th week food intake (J, n=7 animals/group) and weekly body weight (K, n=8 animals/group) of the control (black) and vGlut2^{KO} (red) mice fed with chow, and the 13rd week food (L, n=6 animals/group) and weekly body weight (M, n=7 animals/group) of the control and vGlut2^{KO} mice fed with HFD, all were male. Shown is mean +/- SEM; n=7-12 per group. Two-way ANOVA, sidak's multiple comparisons test was used. Significant or near significant p values for comparisons between Ctrl and Prlh^{NTS-Dq} or Prlh^{NTS-Dq};vGlut2^{KO} shown in black; those for comparisons between Prlh^{NTS-Dq} and Prlh^{NTS-Dq};vGlut2^{KO}

groups shown in green. All images acquired at the same magnification; scale bar equals 150 μm . All experiments were repeated in two independent cohorts of animals with similar results; cohorts were combined for publication.



Supplemental figure 5: Effects of *Prlh* overexpression in *Prlh*^{NTS} neurons in lean mice. (A) Schematic showing AAV^{Flex-*Prlh*} to mediate the cre-dependent overexpression of *Prlh*. (B) Representative images showing PRRP-IR (magenta) and GFP-IR (LepRb, green) in the NTS of LepRb^{eGFP-L10a} control (Ctrl, left panel) or mice injected with AAV^{Flex-*Prlh*} into the NTS (LepRb^{NTS-*Prlh*}, right panel). (C) Food intake during daytime refeeding following an overnight fast for chow-fed control (Ctrl, black) and *Prlh*^{NTS-OX} (red) mice. (D, E) Cumulative weekly food intake (D, n=4 control and n=5 *Prlh*^{NTS-OX} animals/group) and weekly body weight (E, normalized to initial weight, n=4 control and n=6 *Prlh*^{NTS-OX} animals/group) over the five weeks following AAV injection in chow-fed control (Ctrl) and *Prlh*^{NTS-OX} mice. Shown is mean +/- SEM; n=4-7 per group. Two-way ANOVA, sidak's multiple comparisons test was used. Significant or near significant p values are shown for comparisons between Ctrl and *Prlh*^{NTS-OX} groups. (F) Representative images of FOS-IR (black) in the NTS (top panels) and PBN (bottom panels) of control (Ctrl) and *Prlh*^{NTS-OX} mice, n=7 animals/group. All images were acquired at the same magnification; scale bar equals 150 μ m. cc= central canal, scp=superior cerebellar peduncle. (G) PBN FOS-IR cell counts for control (Ctrl) and *Prlh*^{NTS-OX} mice. Shown is mean +/- SEM; n=3 per group. Significant or near significant p values by unpaired t-test are shown for comparisons between Ctrl and *Prlh*^{NTS-OX} groups. All experiments were repeated in two independent cohorts of animals with similar results; cohorts were combined for publication.



Supplemental figure 6: Suppression of mbARC FOS by Prlh^{NTS} neurons and activation of AgRP neurons in Prlh^{NTS-ox};AgRP^{Dq} mice. (A-G) Representative images showing FOS-IR (black) in the NTS (**A-C**) and ARC (**D-F**) of *ad libitum*-fed control animals (Fed; **A, D**), overnight fasted control animals (Fasted; **B, E**), overnight fasted CNO-treated (IP, 1 mg/kg, 2 hours) Prlh^{NTS-Dq} mice (Fasted Prlh^{NTS-Dq}; **C, F**) and FOS positive cell counts in ARC of mice in the condition of being fed (n=4 animals), fasted (n=5 animals) and fasted with CNO injection (IP, 1 mg/kg) (n=5 animals) (**G**). Shown is mean +/- SEM; One-way ANOVA, Dunnett's multiple comparisons test was used. Significant or near significant p values are shown for comparisons to Fasted (Veh) group. (**H-J**) Representative images showing FOS-IR (black) in the ARC of vehicle (Veh) or CNO-treated (IP, 1 mg/kg) AgRP^{Dq} or Prlh^{NTS-ox};AgRP^{Dq} mice. (**K**) Quantification of mbARC fos in mice treated as in H-J; n=3. cc= central canal. 3v: third ventricle. Shown is mean +/- SEM; One-way ANOVA, Dunnett's multiple comparisons

test was used. Significant or near significant p values are shown for comparisons to CNO (AgRP^{Dq}) group. ns: not significant ($p > 0.05$) vs. AgRP^{Dq}. All images taken at same magnification, scale bars= 150 μm . Results shown in the images are representative of at least two independent experiments; quantification was done on one experiment.