Supplementary Figure 1

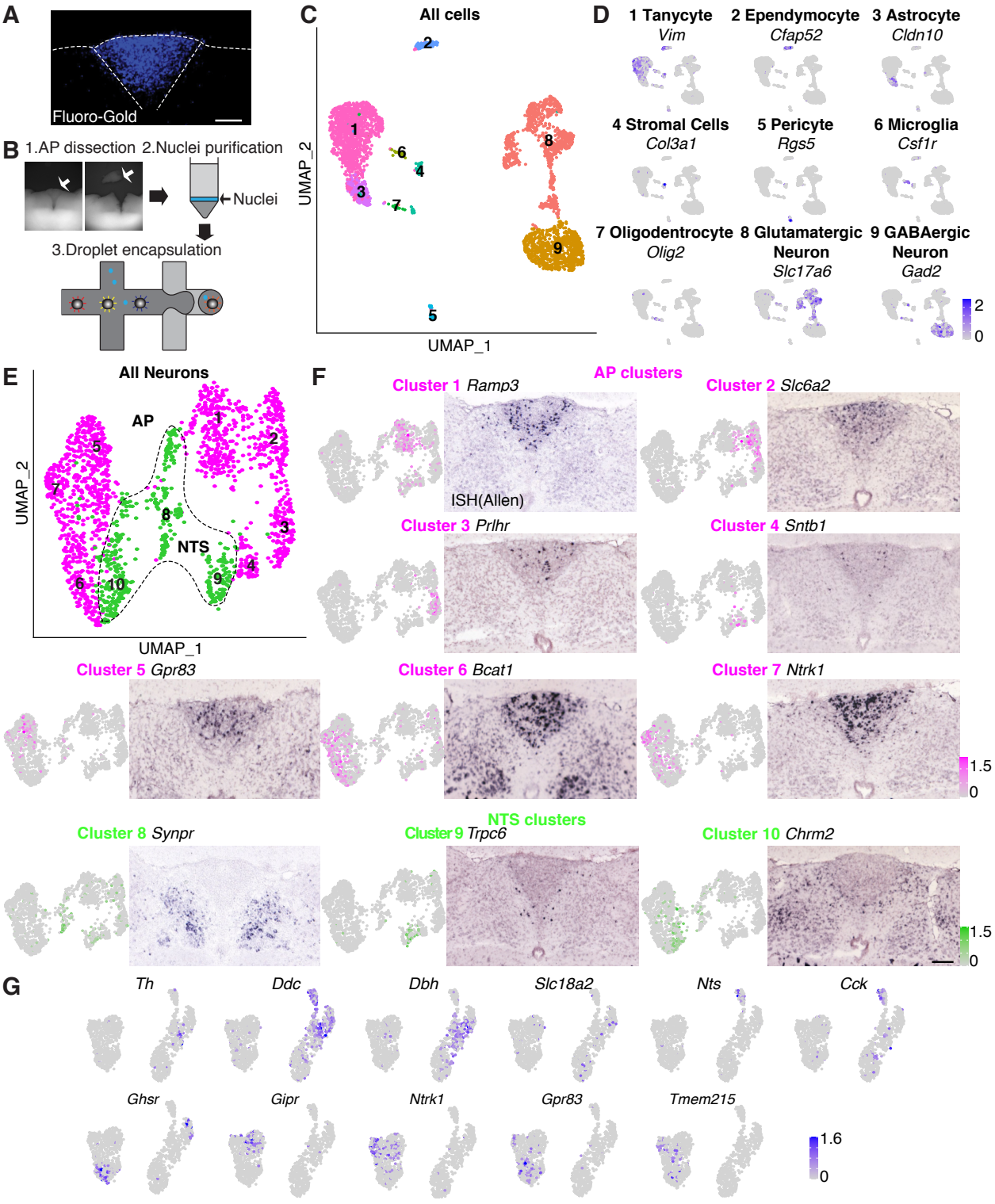

Figure S1. Area postrema cell types revealed by single-nucleus RNA sequencing, related to Figure 1.

(A) Native Fluoro-Gold fluorescence is visualized in coronal area postrema cryosections after intraperitoneal injection. (B) Workflow for preparation of single-nucleus RNA. (C) UMAP plot of all cell types analyzed by single-nucleus RNA sequencing. (D) Cell type assignments for cell clusters in (C) and associated UMAP plots indicating marker gene expression. (E) UMAP plot based on transcriptome data from all neurons, including seven area postrema cell types (pink, clusters 1-7) and three NTS cell types (green, clusters 8-10). (F) UMAP plots (left) and RNA *in situ* hybridization data from the Allen Brain Atlas (right) showing expression of signature genes in area postrema (pink, clusters 1-7) or NTS (green, clusters 8-10) neurons. (G) UMAP plots indicating expression of some neurotransmitter-related genes in area postrema excitatory neurons and enriched receptor genes in area postrema inhibitory neurons.

Supplementary Figure 2

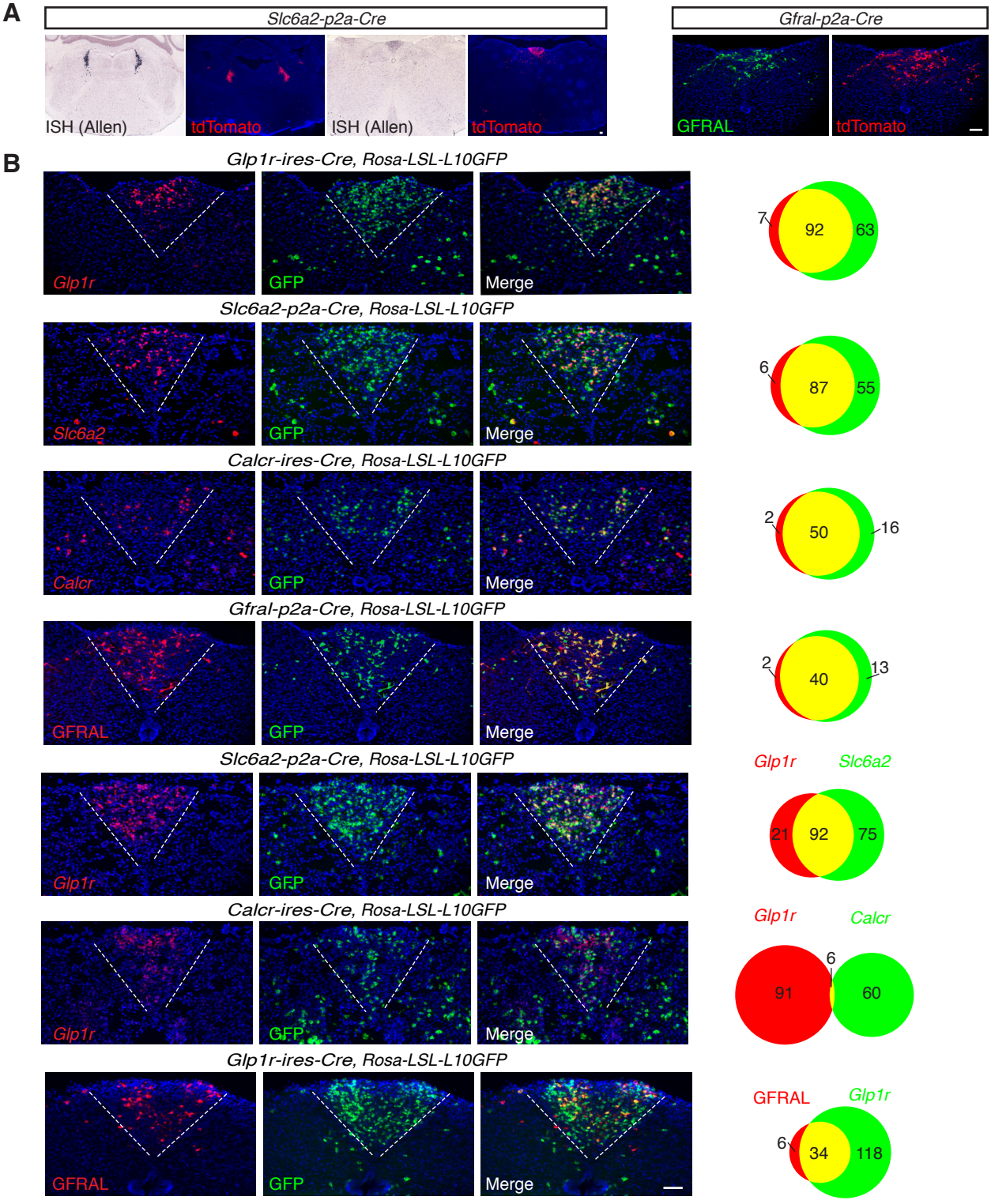

Figure S2. Validation of genetic tools, related to Figure 1.

(A) In coronal brain cryosections, RNA *in situ* hybridization data from the Allen Brain Atlas for *Slc6a2* (left) or GFRAL immunostaining (right) was compared with native tdTomato fluorescence observed in *Slc6a2-p2a-Cre; Rosa-LSL-tdTomato* (left) or *Gfral-p2a-Cre; Rosa-LSL-tdTomato* (right) mice, scale bars: 100 μm. (B) Two color expression analysis in coronal area postrema cryosections of mice indicated, including either RNA *in situ* hybridization for *Glp1r*, *Slc6a2*, or *Calcr* (red), immunochemistry for GFRAL (red), and native GFP fluorescence (green), scale bar: 100 μm. The numbers of co-labeled (yellow) or individually labeled (red, green) cells were counted (right).

Supplementary Figure 3

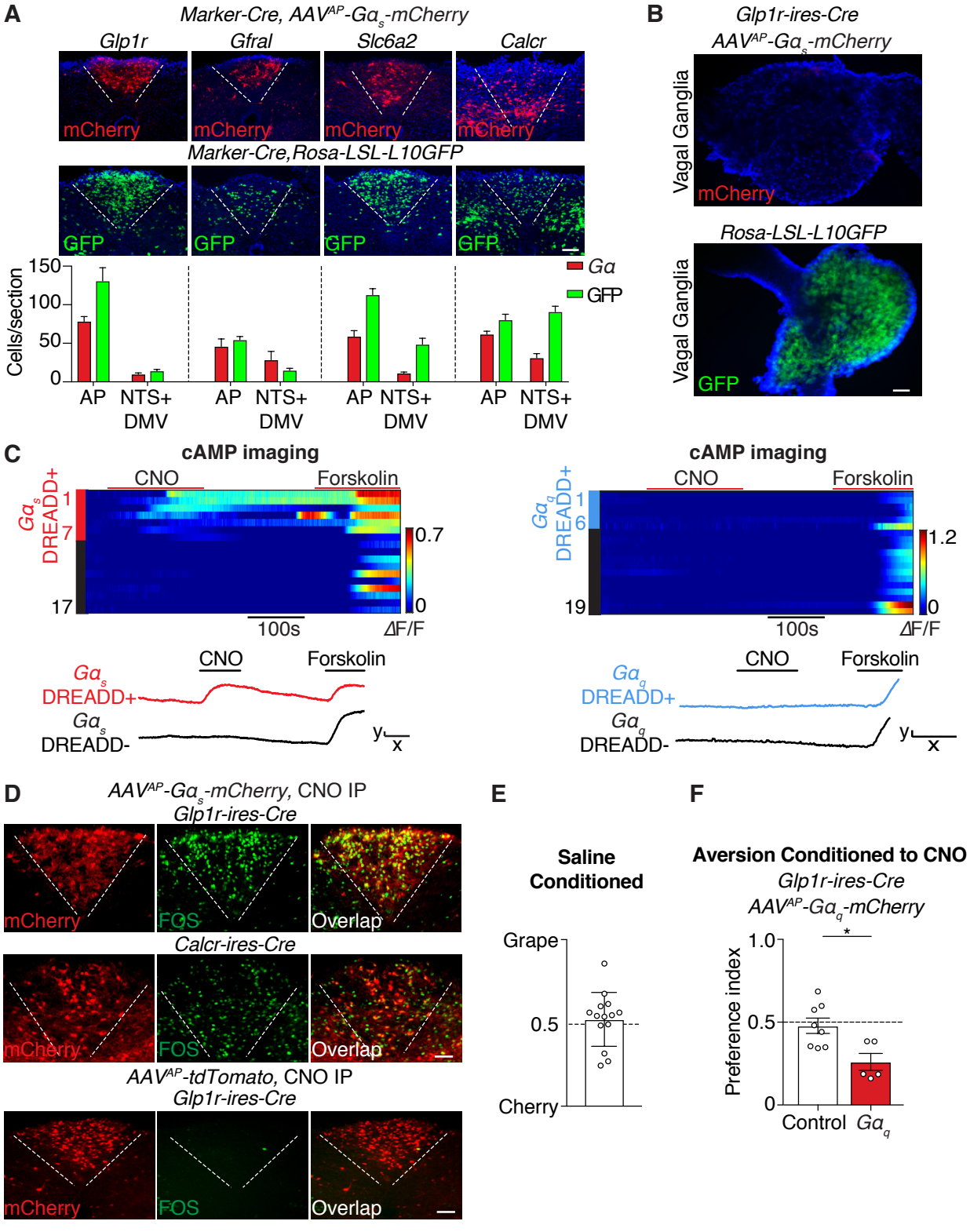

Figure S3. Validating chemogenetic control of area postrema neurons, related to Figure 3.

(A) Cre lines indicated were either injected in the area postrema with an AAV encoding Cre-dependent *Gα_s-DREADD-mCherry* (top) or crossed to mice with a *Rosa-LSL-L10GFP* reporter allele (bottom). Native GFP and mCherry fluorescence was visualized in coronal area postrema cryosections (scale bar: 100 μm), with the number of positive cells per section counted in caudal brainstem regions indicated. (B) *Glp1r-ires-Cre* mice were either injected in the area postrema with *AAV-Flex-Gα_s-DREADD* (left) or crossed to mice with a *Rosa-LSL-L10GFP* reporter allele (right). Native GFP and mCherry fluorescence was visualized in wholemounts of vagal ganglia (scale bar: 100 μm). (C) The area postrema of *Glp1r-ires-Cre* mice was injected with *AAV-Flex-Gα_s-DREADD* (red) or *AAV-Flex-Gα_q-DREADD* (blue). Area postrema neurons were acutely dissociated and transfected with the cAMP sensor cADDis. Top: Rows indicate responses (*F/F*, color scale) of individual neurons over time, with CNO (30 μM) and forskolin (25 μm) applied at times indicated (red bars). Y-axis colored bars (left: red; right: blue) and black bars indicated DREADD-positive and DREADD-negative neurons respectively. Bottom: Representative responses of DREADD-positive (red, blue) and DREADD-negative (black) neurons are shown to CNO (30 μM) and forskolin (25 μM); x: 100 seconds, y: 0.1 *F/F*. (D) The area postrema of *Glp1r-ires-Cre* and *Calcr-ires-Cre* mice were injected with *AAV-Flex-Gα_s-DREADD* or *AAV-Flex-tdTomato*. CNO was injected intraperitoneally (IP), and two-color expression analysis was subsequently performed in coronal area postrema cryosections for native mCherry fluorescence (red) and Fos immunofluorescence (green), scale bar: 100 μm. (E) No preference for grape or cherry flavor was observed in the absence of malaise induction. During conditioning, mice randomly received either cherry or grape stimuli, and all received saline injections (IP), n=14, mean ± sem, circles: individual data points. (F) *Glp1r-ires-Cre* mice were injected with AAVs encoding Cre-dependent *Gα_q-DREADD-mCherry* (Gα_q) or tdTomato (Control), and analyzed for CNO-evoked flavor avoidance, n=5-8, mean ± sem, circles: individual data points, *p<.05.

Supplementary Figure 4

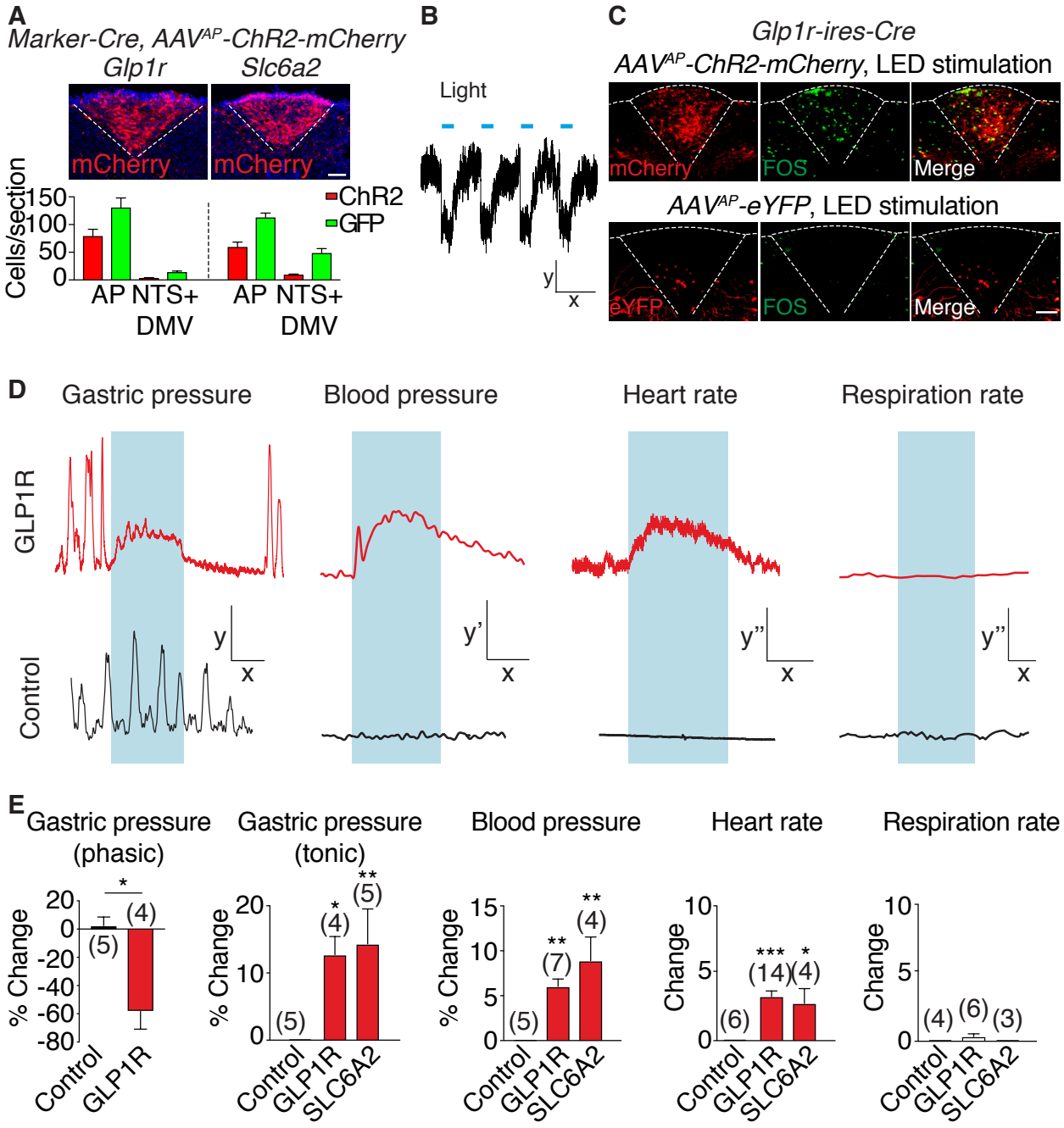

Figure S4. Physiological responses to optogenetic stimulation of area postrema neurons, related to Figure 3.

(A) *Glp1r-ires-Cre* (left) or *Slc6a2-ires-Cre* (right) mice were either injected in the area postrema with *AAV-Flex-ChR2* or crossed to mice with a *Rosa-LSL-L10GFP* reporter allele. mCherry immunofluorescence was visualized in coronal area postrema cryosections (scale bar: 100 μm), with the number of mCherry (ChR2) and GFP positive cells per section counted in caudal brainstem regions indicated. (B) The area postrema of *Glp1r-ires-Cre* mice was injected with *AAV-Flex-ChR2* (*AAV^{AP}-ChR2-mCherry* mice). Area postrema neurons were acutely harvested and responses to light (blue bar) were measured by whole-cell voltage clamp recordings, x: 2 seconds, y: 20 picoamperes. (C) Two-color expression analysis in area postrema cryosections after area postrema illumination in *Glp1r-ires-Cre* mice injected with *AAV-Flex-ChR2* (top) or *AAV-Flex-Yfp* (bottom), red: native mCherry fluorescence (top) or pseudocolored native YFP fluorescence (bottom); green: Fos immunofluorescence, scale bar: 100 μm. (D) Physiological responses to area postrema illumination (blue bar) in *Glp1r-ires-Cre* mice previously injected in the area postrema with either *AAV-Flex-ChR2* (GLP1R) or *AAV-Flex-Yfp* (Control), x: 100 seconds, y: 1 cmH_2O, y': 5 mmHg, y'': 1 beat/breath per minute. (E) Quantifying physiological changes to stimulating area postrema neuron subtypes indicated (n=3-14, mean ± sem, *p<.05, **p<.01, ***p<.001, statistical comparisons to control).

Supplementary Figure 5

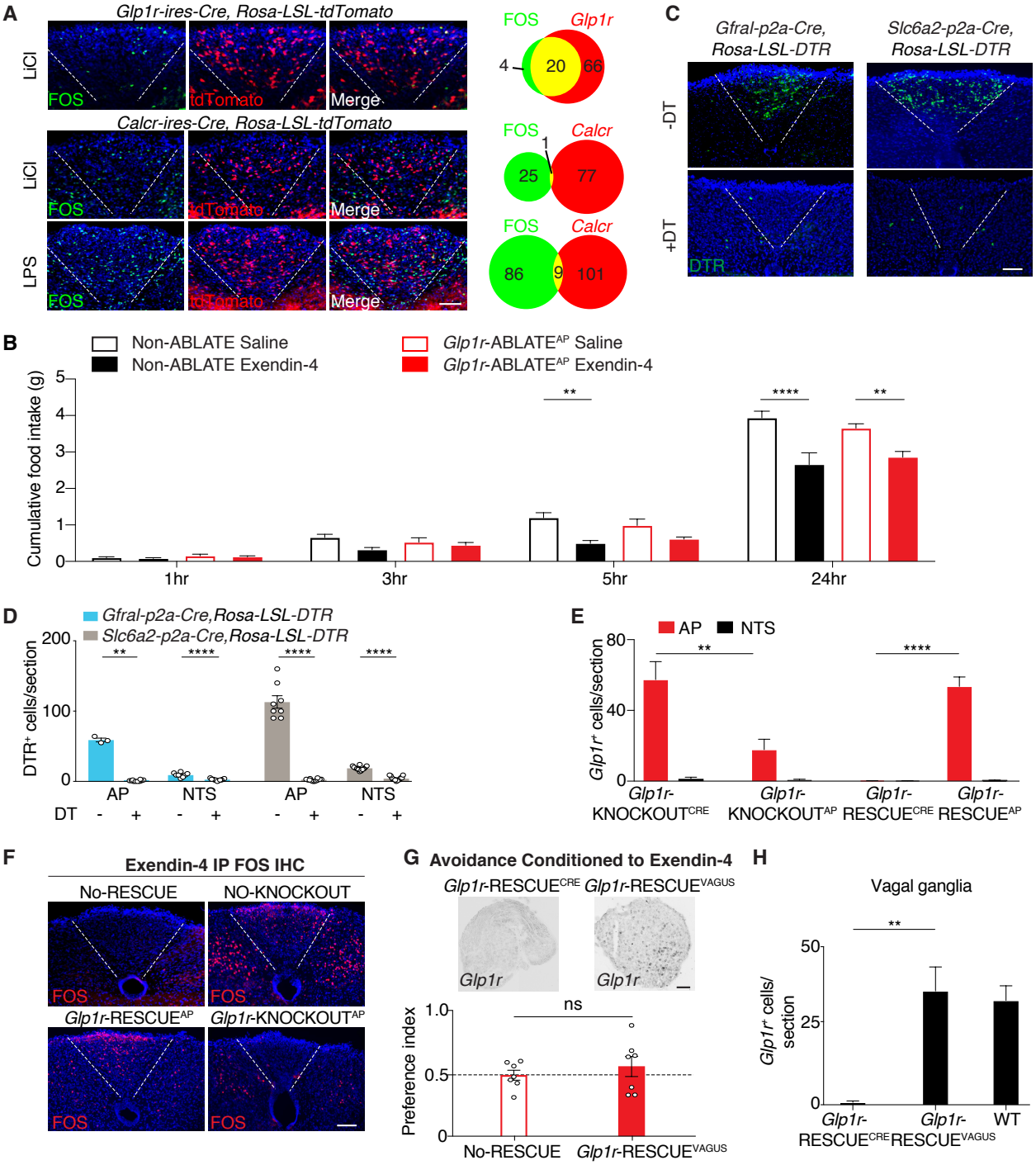

Figure S5. Validating and analyzing loss-of-function approaches in area postrema neurons, related to Figures 4, 5.

(A) Two-color expression analysis involving Fos immunohistochemistry (green) and native tdTomato fluorescence (red) in coronal area postrema cryosections after intraperitoneal injection of lithium chloride (LiCl) or lipopolysaccharide (LPS) in *Glp1r-ires-Cre; Rosa-LSL-tdTomato* or *Calcr-ires-Cre; Rosa-LSL-tdTomato* mice, scale bar: 100 μm. The numbers of co-labeled (yellow) or individually labeled (red, green) cells were counted (right). (B) *Ad libitum* fed Non-ABLATE (black) and *Glp1r*-ABLATE^{AP} (red) mice were injected with either exendin-4 (filled bars) or saline (unfilled bars), and cumulative food consumption was measured at 1, 3, 5, and 24 hours after injection, n=3-8, mean ± sem, **p<.01, ****p<.0001. (C) The areas postrema of *Gfral-p2a-Cre; Rosa-LSL-DTR* and *Slc6a2-p2a-Cre; Rosa-LSL-DTR* mice were injected with DT (+DT) or saline (-DT). Immunohistochemistry for DTR was performed in coronal area postrema sections, scale bar: 100 μm. (D) Counts of DTR-expressing cells in area postrema (AP) and NTS from mice in C, n=3-13 sections from 2-5 mice, circles: individual data points, **p<.01, ****p<.0001. (E) Counts of *Glp1r*-positive cells across sections from *in situ* hybridization experiments described in Figure 5A, n=6-10 sections from 2-4 mice, mean ± sem, **p<.01, ****p<.0001. (F) Fos immunohistochemistry in area postrema sections of mice indicated after intraperitoneal exendin-4 injection, scale bar: 100 μm. (G) RNA *in situ* hybridization for *Glp1r* in vagal ganglia cryosections (top, scale bar: 100 μm) and flavor avoidance responses conditioned to exendin-4 (bottom, n= 7 mice, mean ± sem, circles: individual data points) in mice indicated. (H) Counts of *Glp1r*-positive cells from RNA *in situ* hybridization experiments in vagal ganglia of mice indicated, n=4-9 sections from 3-4 mice, mean ± sem, **p<.01.

Supplementary Figure 6

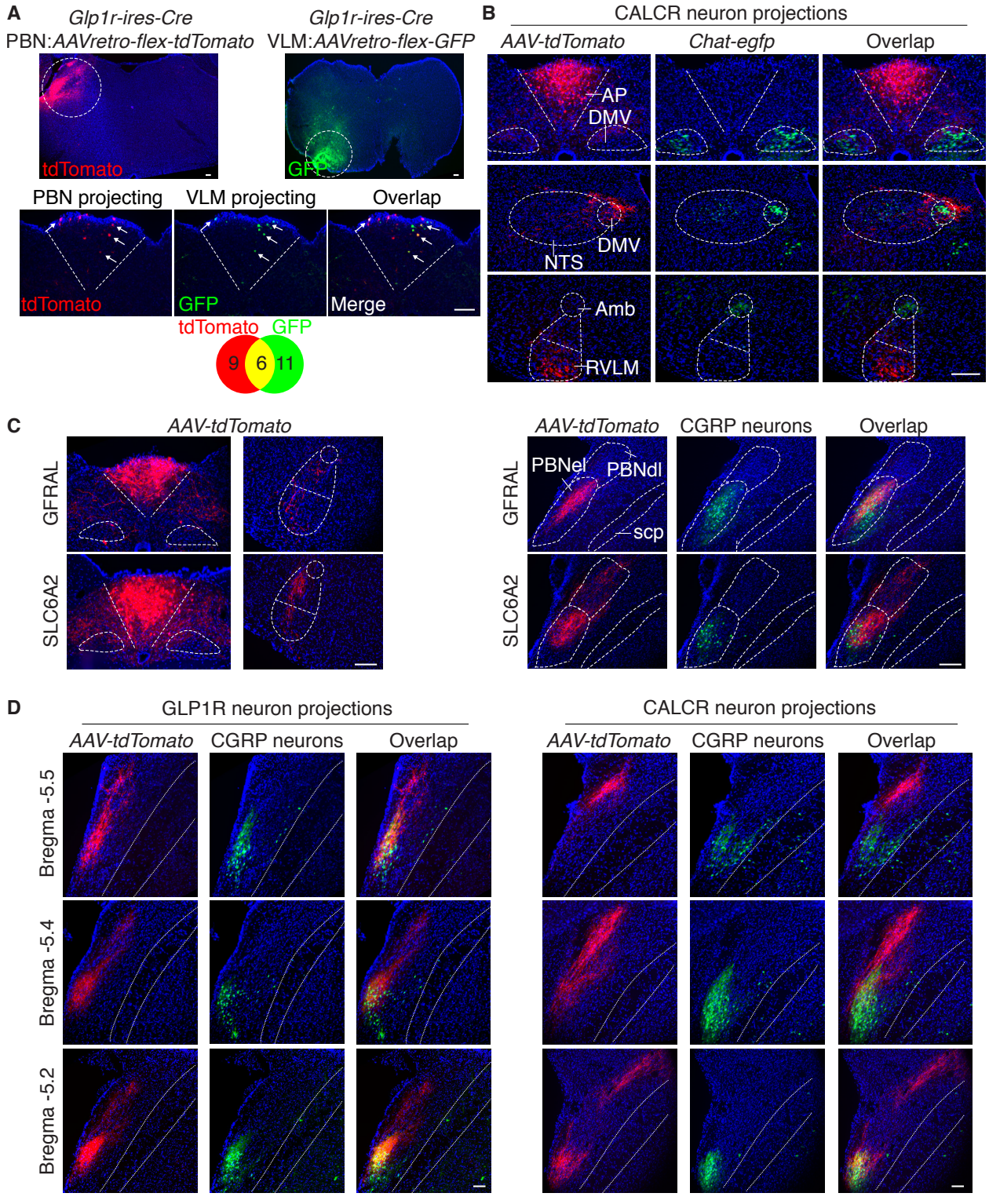

Figure S6. Anatomical mapping of area postrema neuron projections, related to Figure 6. (A) The PBN and VLM (ventrolateral medulla) of *Glp1r-ires-Cre* mice were injected with *AAVretro-flex-tdTomato* and *AAVretro-flex-GFP* respectively, and tdTomato and GFP immunofluorescence was visualized in coronal brain cryosections containing injection sites (top) or area postrema (bottom), arrows: co-labeled cells, scale bar: 100 μm. The numbers of co-labeled (yellow) or individually labeled (red, green) cells were counted in a representative section. (B) *AAV-Flex-tdTomato* was injected into the area postrema of *Calcr-ires-Cre; Chat-gfp* mice. GFP was visualized by native fluorescence and tdTomato-positive fibers were visualized by immunohistochemistry. (C) *AAV-Flex-tdTomato* was injected into the area postrema of *Gfral-p2a-Cre; Calca-gfp* (top) and *Slc6a2-p2a-Cre; Calca-gfp* (bottom) mice. GFP was visualized by native fluorescence (CGRP neurons) and tdTomato-positive fibers were visualized by immunohistochemistry. (D) *AAV-Flex-tdTomato* was injected into the area postrema of *Glp1r-ires-Cre*; *Calca-gfp* and *Calcr-ires-Cre*; *Calca-gfp* mice. GFP was visualized by native fluorescence (CGRP neurons) and tdTomato-positive fibers were visualized by immunohistochemistry in coronal sections of Bregma indicated, AP: area postrema; DMV: dorsal motor nucleus of the vagus; Amb: nucleus ambiguus; RVLM: rostral ventrolateral medulla; PBN: parabrachial nucleus; dl: dorsolateral; el: external lateral; scp: superior cerebellar peduncle; scale bars: 100 μm.

Supplementary Figure 7

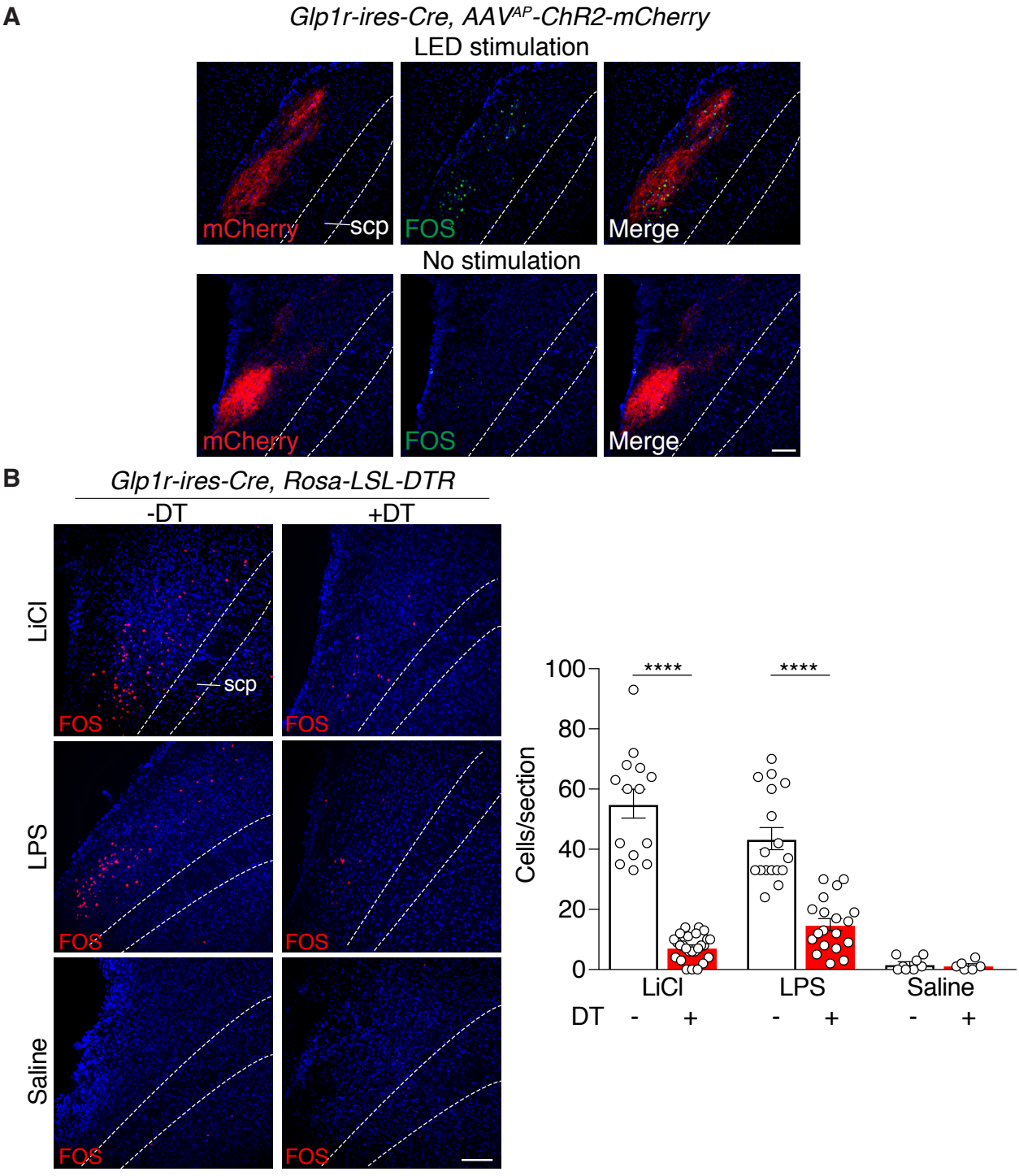

Figure S7. Area postrema GLP1R neurons are functionally connected to PBN neurons, related to Figure 6.

(A) *Glp1r-ires-Cre* mice previously injected in the area postrema with *AAV-flex-ChR2*, which encodes a ChR2-mCherry fusion protein, were treated with (LED stimulation) or without (No stimulation) area postrema optogenetic stimulation (5 Hz, 1 hour, awake mice). Two-color expression analysis was then performed in coronal cryosections of PBN regions involving Fos immunochemistry (green) and native mCherry fluorescence (red), scale bar: 100 μm, scp: superior cerebellar peduncle. (B) Fos immunohistochemistry (red) in coronal PBN cryosections 2.5 hours after visceral injection of lithium chloride (LiCl), lipopolysaccharide (LPS) or saline in *Glp1r-ires-Cre*; *Rosa-LSL-DTR* mice previously injected in the area postrema with DT (+DT) or saline (-DT). Left: representative images, scale bar: 100 μm, Right: quantification of Fos-positive cells per section, n=6-14, mean ± sem, ****p<.0001.

Supplementary Table 1

Cluster	Gene numbers	Signature genes
1	1	*Olfr78*
	2	*D130009I18Rik*
	3	*Gal*
	4	*Calcr*
	5	*Ebf1*
2	6	*Casr*
	7	*Slc6a2*
	8	*Rprm*
	9	*Cyb561*
	10	*Acsl1*
3	11	*Agtr1a*
	12	*Pter*
	13	*Prlhr*
	14	*Tox*
	15	*Tafa1*
4	16	*Gpr88*
	17	*Nts*
	18	*Gfral*
	19	*Man1a*
5	20	*Gpr83*
	21	*Slc5a7*
	22	*Ptprk*
	23	*Spon1*
	24	*Trhde*
6	25	*Gipr*
	26	*Chn2*
	27	*Abcc9*
	28	*Ngef*
	29	*Ndnf*
7	30	*Insrr*
	31	*F5*
	32	*Phf24*
	33	*Etv5*
	34	*Sst*

Table S1. Signature genes in area postrema neuron subtypes, Related to Figure 1.

A list of the 4-5 most enriched genes for each area postrema neuron cluster, with gene numbers corresponding to rows in Figure 1B.