SUPPLEMENTARY DATA

Animals. Mice were housed in ventilated cages with controlled light cycle (6AM-6PM) and temperature (22±1°C) with *ad libitum* access to water and standard rodent chow (LabDiet 5L0D) unless otherwise noted. GFP reporter mice were generated by crossing vGAT$^{Cre/+}$, vGlut2$^{Cre/+}$, and Glp1r$^{Cre/+}$ males to female *L10*-GFP homozygotes and double heterozygotes of both sexes were pooled together for study. For the initial longitudinal study, male *vGAT*$^{Cre/+}$*Glp1r*$^{flox/+}$ and *vGlut2*$^{Cre/+}$*Glp1r*$^{flox/+}$ were bred to female *Glp1r*$^{flox/+}$ mice. This breeding strategy generated six study groups. The two experimental groups, *vGAT*$^{Cre/+}$*Glp1r*$^{flox/flox}$ and *vGlut2*$^{Cre/+}$*Glp1r*$^{flox/flox}$, are designated vGAT$^{\Delta Glp1r}$ and vGlut2$^{\Delta Glp1r}$, respectively. Four control groups, *vGAT*$^{Cre/+}$*Glp1r*$^{+/+}$, *vGlut2*$^{Cre/+}$*Glp1r*$^{+/+}$, *Glp1r*$^{flox/flox}$, and mice wild-type for all alleles were studied separately, but no phenotypic difference in any measure studied was found and results have been pooled. For the conditioned taste avoidance and chronic liraglutide experiment, male *vGAT*$^{Cre/+}$*Glp1r*$^{flox/flox}$ or *vGlut2*$^{Cre/+}$*Glp1r*$^{flox/flox}$ were crossed with female *Glp1r*$^{flox/flox}$ to generate vGAT$^{\Delta Glp1r}$, vGlut2$^{\Delta Glp1r}$, and *Glp1r*$^{flox/flox}$ controls.

Immunohistochemistry. Primary antibodies used were monoclonal rabbit anti-Fos (1:2000, Cell Signaling Technology #2250) and chicken anti-GFP (1:2000, Aves #GFP-1020). Secondary antibodies used were goat anti-chicken-488 (1:500, Invitrogen #A11039), donkey anti-rabbit-568 (1:500, Invitrogen #A10042), and biotinylated donkey anti-rabbit (1:500, Jackson Immunoresearch #711-065-152). For colorimetric assay, sections were incubated for one hour in VectaStain ABC-HRP kit (Vector #PK-4000), developed with DAB substrate (Pierce #34065), mounted onto slides and counterstained with 50% Gill's Hematoxylin (Electron Microscopy Sciences #26801-01), dehydrated through ethanol gradients, cleared with xylenes and cover-slipped with DPX mounting media. Fluorescent sections were coverslipped with ProLong Antifade Gold (Invitrogen #P36931).

Microscopy and Image Analysis. Images of fluorescent ISH and IHC were taken with an Olympus BX53 and QImaging Retiga 6000 monochrome camera, whereas images of DAB-developed IHC were taken with a Nikon 90i upright microscope and DS-Fi1 color camera. For ISH, three images per section were captured under 40X objective, then processed and stitched together using Photoshop (Adobe). IHC images were taken under 20X objective, except where noted. For fluorescent IHC images, CellProfiler (1) was used to threshold background and identify and count Fos-positive cells which were positive or negative for GFP. For DAB images, the number of Fos+ cells per image was analyzed with ImageJ particle analyzer following thresholding of brown DAB signal (2). For *Glp1r*-GFP expression atlas, 2-11 images per section were taken under 4X objective and stitched together, then brightness and contrast were adjusted identically for each image using Photoshop (Adobe).

Semi-Quantitative Real-Time PCR. Primers were designed using Primer3 (3) with sequences as follows: Glp1r-F 5'-AAGATGCTGCCCTCAAGTGGATG-3'; Glp1r-R 5'-AGTACTGCATGAGCAGGAACACC-3'; Hprt-F 5'-GATTAGCGATGATGAACCAGGTT-3'; Hprt-R 5'-CCTCCCATCTCCTTCATGACA-3'. The relative quantity of *Glp1r* mRNA in each sample was calculated from a standard curve, normalized to *Hprt* and then normalized to *Glp1r*-flox controls.

Conditioned Taste Avoidance. Assay was modified from a previously-described protocol (4). Mice were single-housed and their lixits replaced by two 50-mL conical tubes fitted with a sipper and containing sterile tap water. Standard chow was provided *ad libitum* for the duration of the test. After 1 week of habituation, access to water was restricted to two one-hour periods (9-10am and 4-5pm) each day. On day 5 of scheduled water, the morning water was replaced by 0.15% saccharin sodium (Sigma #S6047). 15 minutes after saccharin removal, mice were injected subcutaneously with either saline, liraglutide (400 µg/kg) or lithium chloride (0.3M, Sigma #L4408) in a constant volume (10 mL/kg). That afternoon and the next day, scheduled water was resumed to allow for recovery. On day 7, one bottle in each cage was randomly selected and the water replaced by 0.15% saccharin. The amount consumed from each bottle during the one hour was measured by weighing. Preference ratio was calculated as amount of saccharin consumed divided by total amount of liquid consumed.

SUPPLEMENTARY DATA

References

1. Lamprecht MR, Sabatini DM, Carpenter AE: CellProfiler(TM): free, versatile software for automated biological image analysis. Biotechniques 2007;42:71-75
2. Schneider CA, Rasband WS, Eliceiri KW: NIH Image to ImageJ: 25 years of image analysis. Nature Methods 2012;9:671-675
3. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG: Primer3-new capabilities and interfaces. Nucleic Acids Research 2012;40
4. Ding HK, Teixeira CM, Frankland PW: Inactivation of the anterior cingulate cortex blocks expression of remote, but not recent, conditioned taste aversion memory. Learning & Memory 2008;15:290-293
5. Paxinos G, Franklin KBJ: *The Mouse Brain in Stereotaxic Coordinates*. Amsterdam, Elsevier Academic Press, 2008

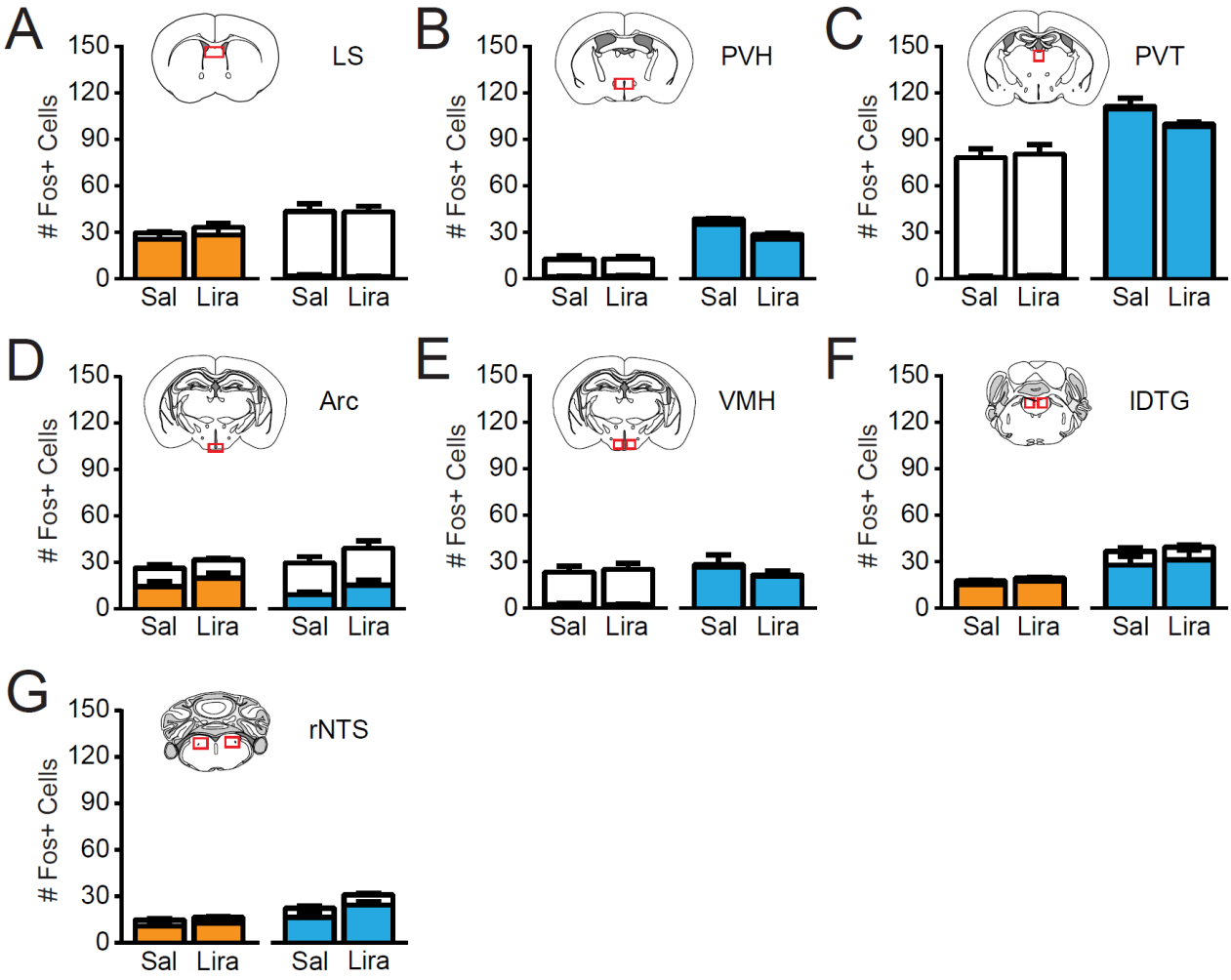
SUPPLEMENTARY DATA

Supplementary Table 1. Coordinates of nuclei imaged for Fos counts (5).

Region	Position from Bregma (mm)
Lateral Septal Nucleus	+0.62 +0.50 +0.38 +0.26
Bed Nucleus of the Stria Terminalis	+0.26 +0.14
Paraventricular Hypothalamus	-0.58 -0.70 -0.82 -0.94
Paraventricular Thalamus	-0.94 -1.06 -1.22 -1.34
Central Nucleus of the Amygdala	-1.34 -1.46 -1.58 -1.70
Arcuate Nucleus of the Hypothalamus	-1.46 -1.58 -1.70 -1.82 -1.94
Ventromedial Nucleus Hypothalamus	-1.46 -1.58 -1.70 -1.82
Lateral Dorsal Tegmental Nucleus	-5.02 -5.20 -5.34 -5.40
Lateral Parabrachial Nucleus	-5.20 -5.34
Nucleus of the Solitary Tract (rostral)	-6.72 -6.84 -6.96 -7.08
Nucleus of the Solitary Tract (caudal)	-7.20 -7.32 -7.48 -7.56
Area Postrema	-7.48 -7.56

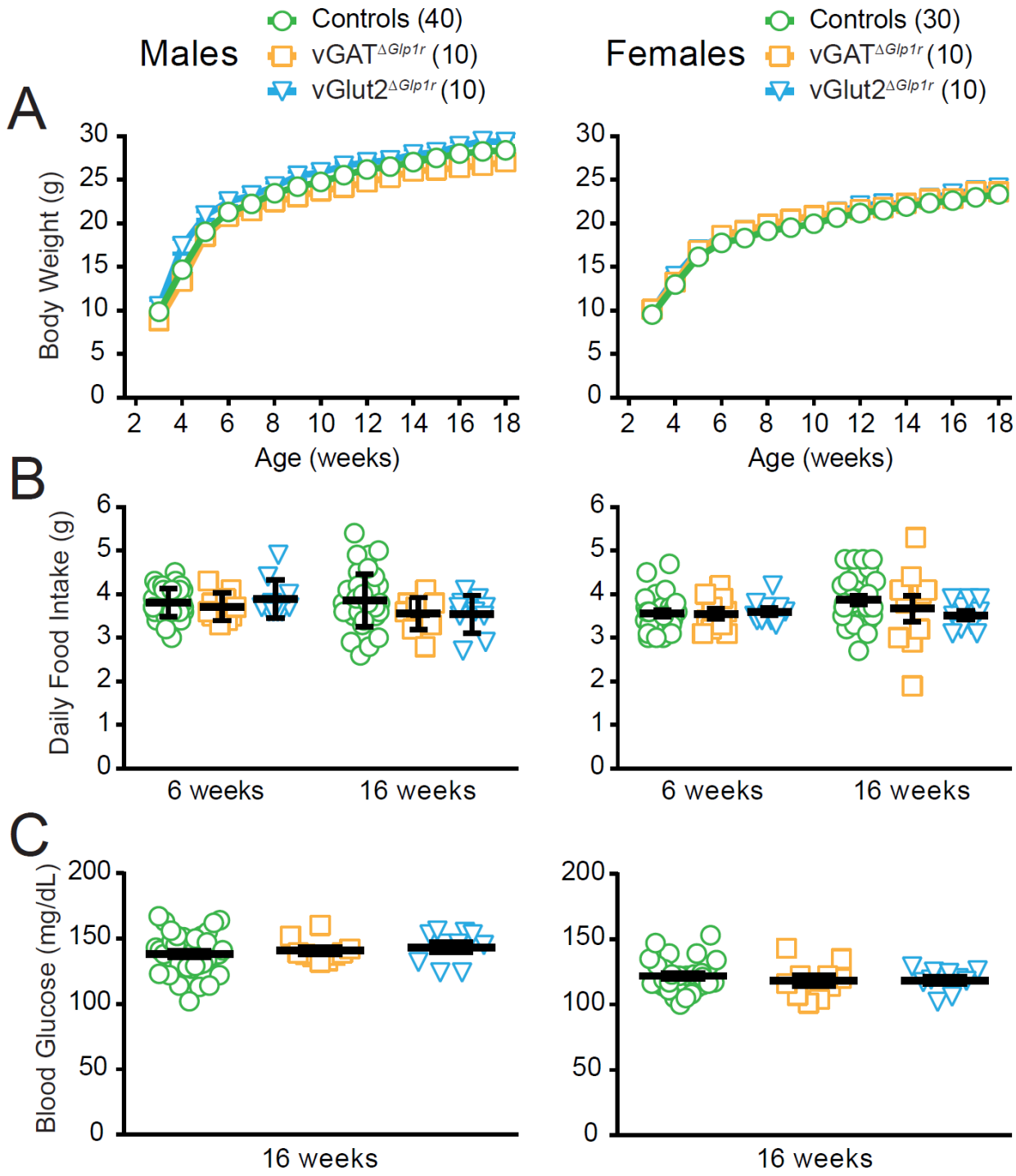
SUPPLEMENTARY DATA

Supplementary Figure 1. Additional nuclei quantified for neuronal activation following liraglutide injetion. In each column, the proportion of Fos-positive neurons positive for *vGAT*-GFP (orange) or *vGlut2*-GFP (blue) is indicated. **(A)** Lateral septum **(B)** Paraventricular hypothalamus **(C)** Paraventricular thalamus **(D)** Arcuate hypothalamus **(E)** Ventromedial hypothalamus **(F)** Lateral dorsal tegmental nucleus **(G)** Rostral nucleus of the solitary tract. N≥6. Data were analyzed by unpaired Student's t-test between drug treatments, but no significant differences were found.



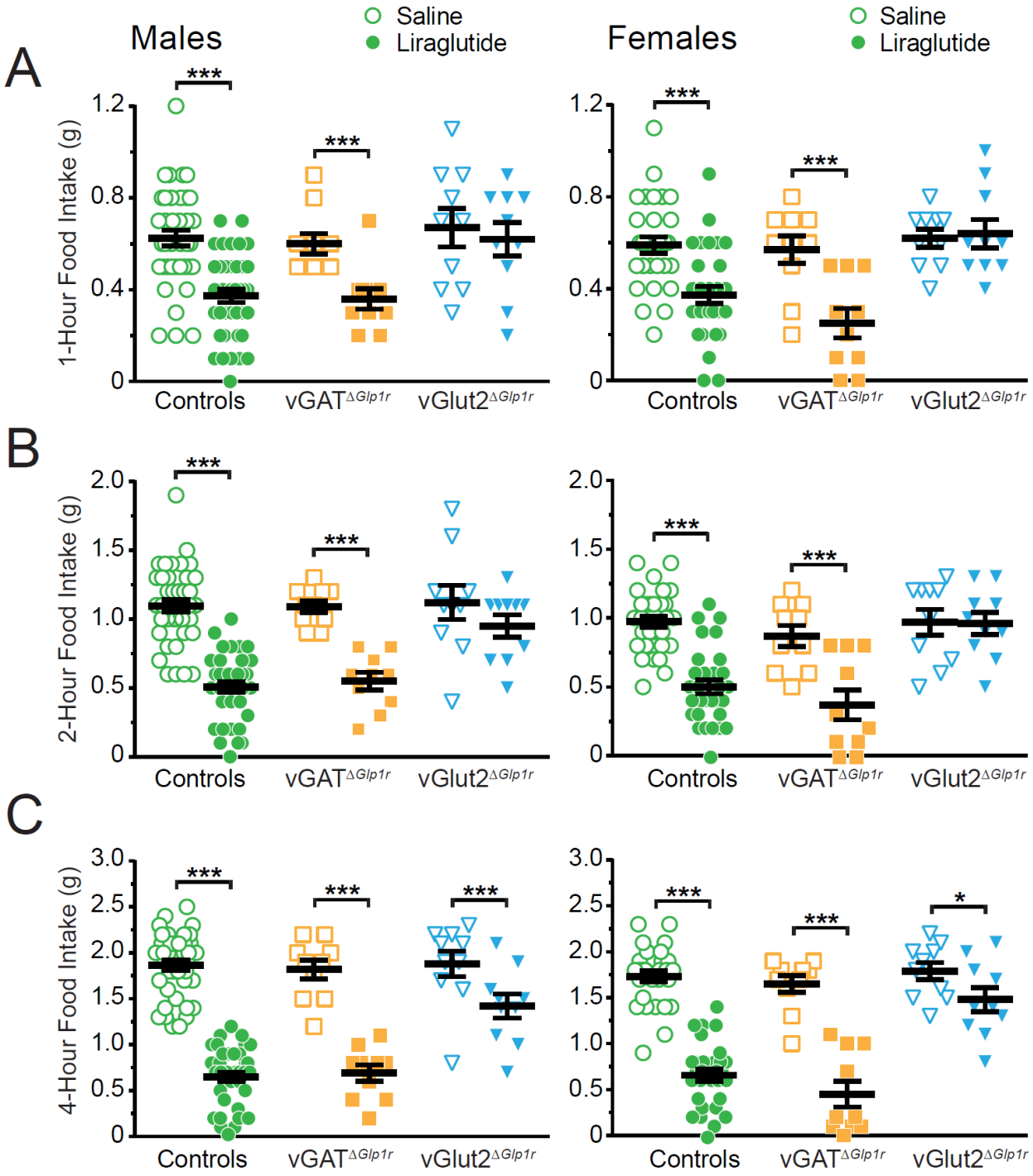
SUPPLEMENTARY DATA

Supplementary Figure 2. Baseline characterization of vGAT$^{\Delta Glp1r}$ and vGlut2$^{\Delta Glp1r}$ chow-fed mice. Males (left) and females (right) are shown for all panels. **(A)** Body weight of singly-housed mice from weaning to age 18 weeks. **(B)** Average daily food intake at age 6 weeks and age 16 weeks. **(C)** 4-hour fasting blood glucose at age 16 weeks. N≥10, data analyzed by repeated measures two-way ANOVA **(A)** and one-way ANOVA **(B,C)**, but no differences between groups were found.



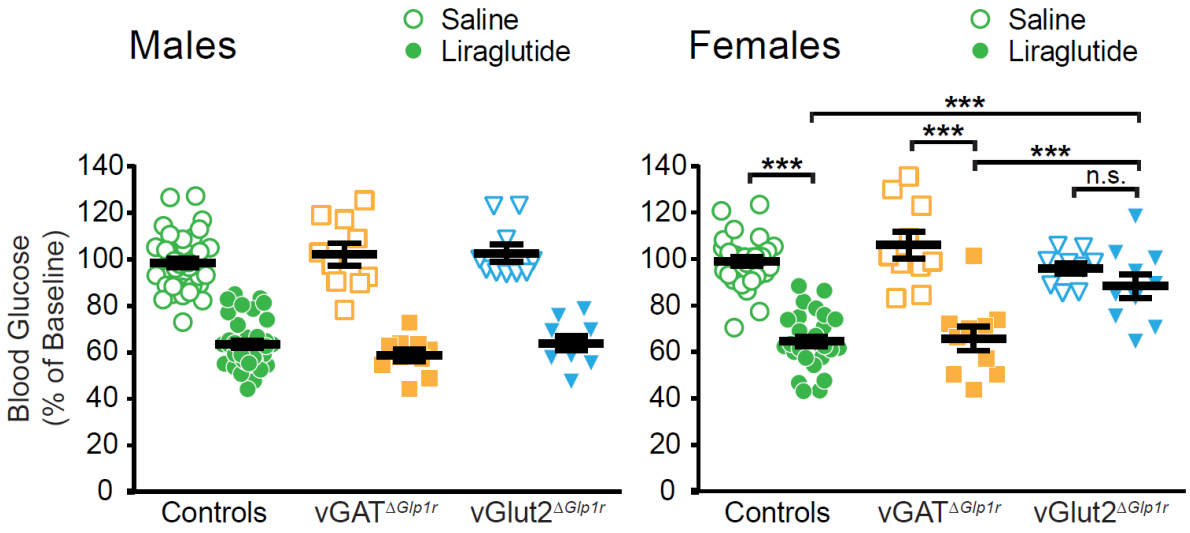
SUPPLEMENTARY DATA

Supplementary Figure 3. Short-term effects of acute liraglutide in vGAT$^{\Delta Glp1r}$ and vGlut2$^{\Delta Glp1r}$ chow-fed mice. Males (left) and females (right) are displayed separately. Change in total food intake 1 hour **(A)**, 2 hours **(B)** and 4 hours **(C)** following liraglutide or saline control injection at the onset of the dark cycle. N≥10. Repeated measures two-way ANOVA was performed followed by Sidak's multiple comparisons test. * $P < 0.05$; *** $P < 0.001$.

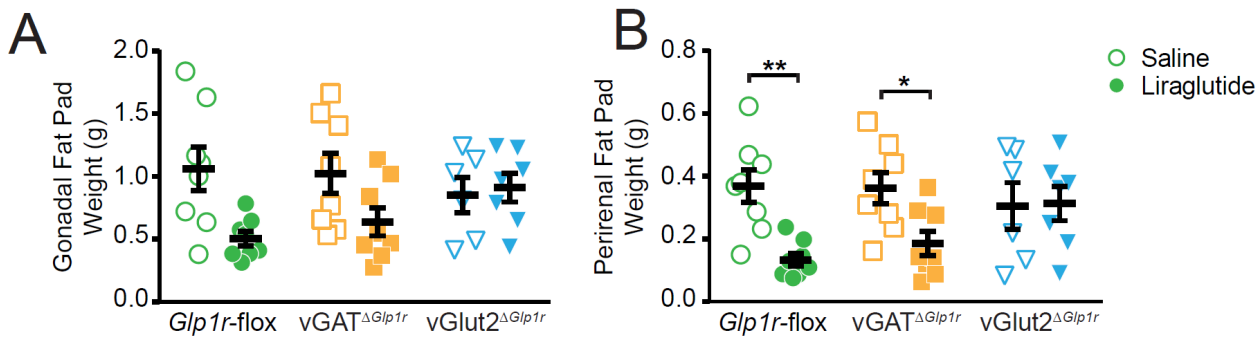


SUPPLEMENTARY DATA

Supplementary Figure 4. Acute effect of liraglutide on blood glucose levels in vGAT$^{\Delta Glp1r}$ and vGlut2$^{\Delta Glp1r}$ mice. Males (left) and females (right) are shown. Blood glucose is expressed as a percentage of baseline (see Supplemental Figure 2) 2 hours following liraglutide or saline control injection. N≥10, data analyzed by repeated measures two-way ANOVA. In males, only a significant effect of drug treatment was found ($P < 0.001$), whereas in females, a significant interaction between drug and genotype was found and Sidak's multiple comparisons test performed. *** P < 0.001.



Supplementary Figure 5. Chronic effect of liraglutide on fat pad mass in vGAT$^{\Delta Glp1r}$ and vGAT$^{\Delta Glp1r}$ HFD-fed mice. Gonadal **(A)** and perirenal **(B)** fat pad weights following 8 weeks of high fat diet, the last two weeks of which mice were administered once-daily saline or liraglutide. N≥6, data analyzed by two-way ANOVA. In gonadal fat, only a significant effect of drug treatment was found ($P < 0.05$), whereas in perirenal fat, a significant interaction between drug and genotype was found and Sidak's multiple comparisons test performed. * $P < 0.05$; ** $P < 0.01$.



SUPPLEMENTARY DATA

Supplementary Figure 6. Expression pattern of *Glp1r*-GFP across the brain. Coronal sections approximately 500 µm apart were immunostained for GFP (green) and counterstained with DAPI (blue). Coordinates in mm from Bregma were estimated for each section from the Paxinos and Franklin atlas (4) and are listed above each section. Scale bar = 2 mm.