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## Supplemental information

# Tanycytes control hypothalamic liraglutide uptake and its anti-obesity actions

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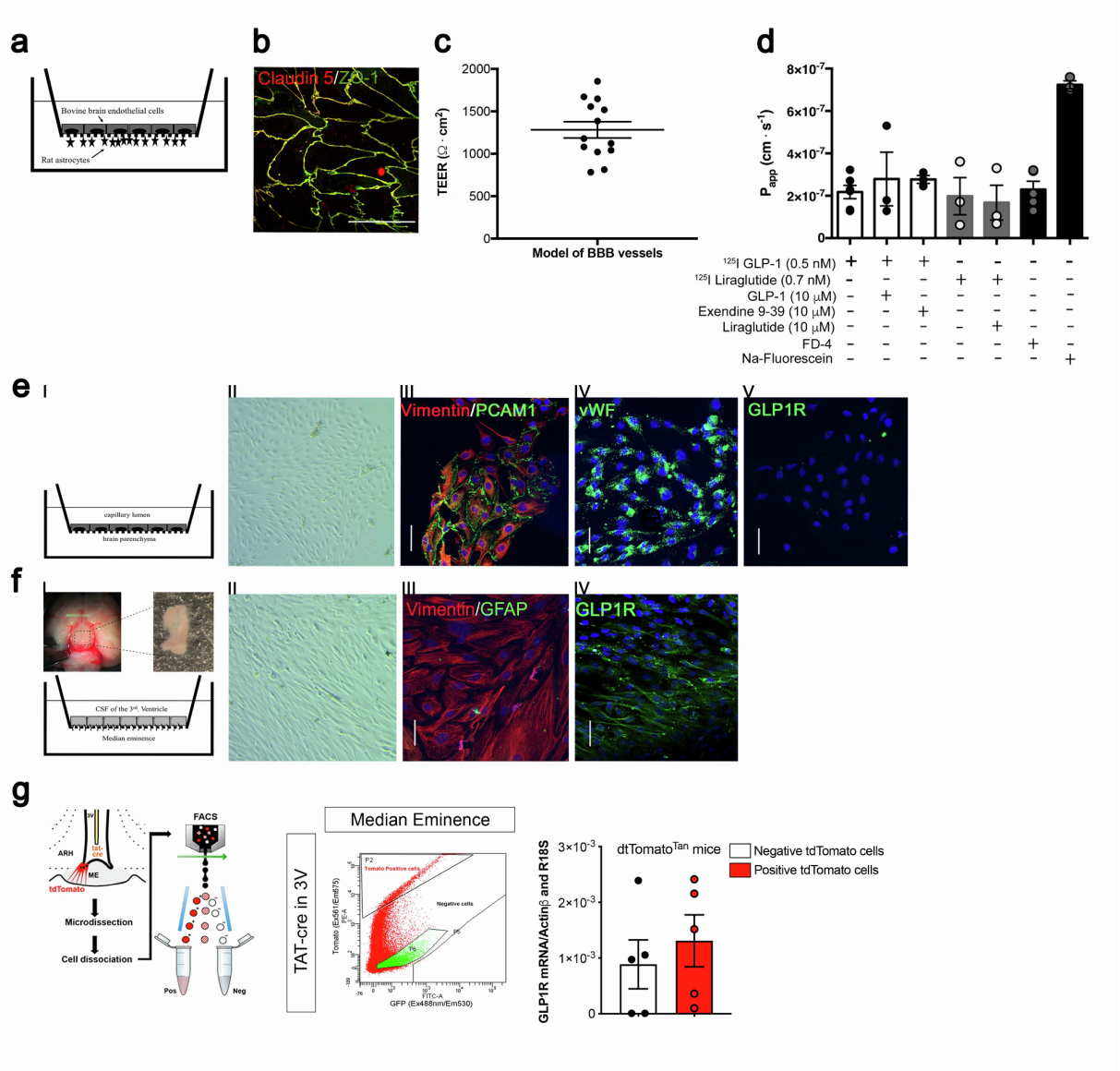
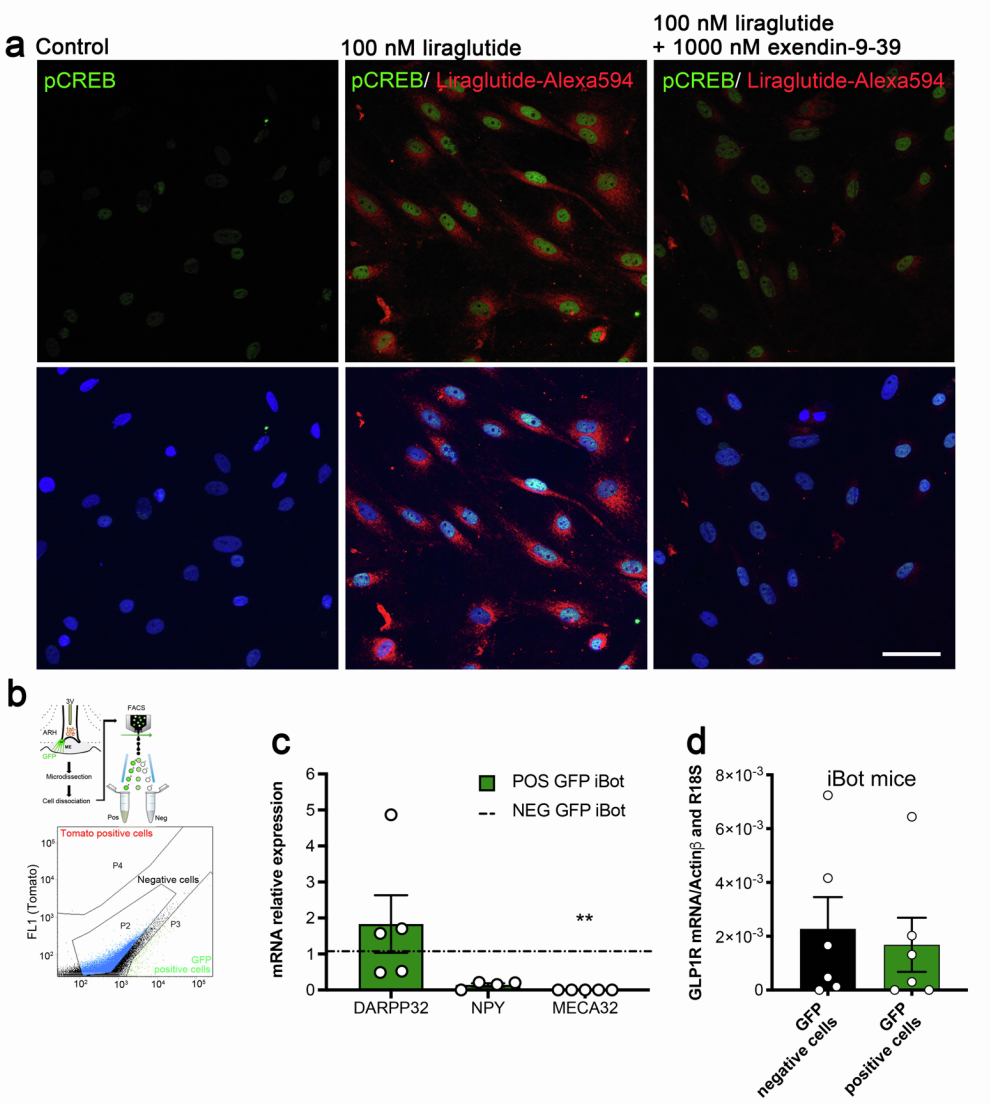
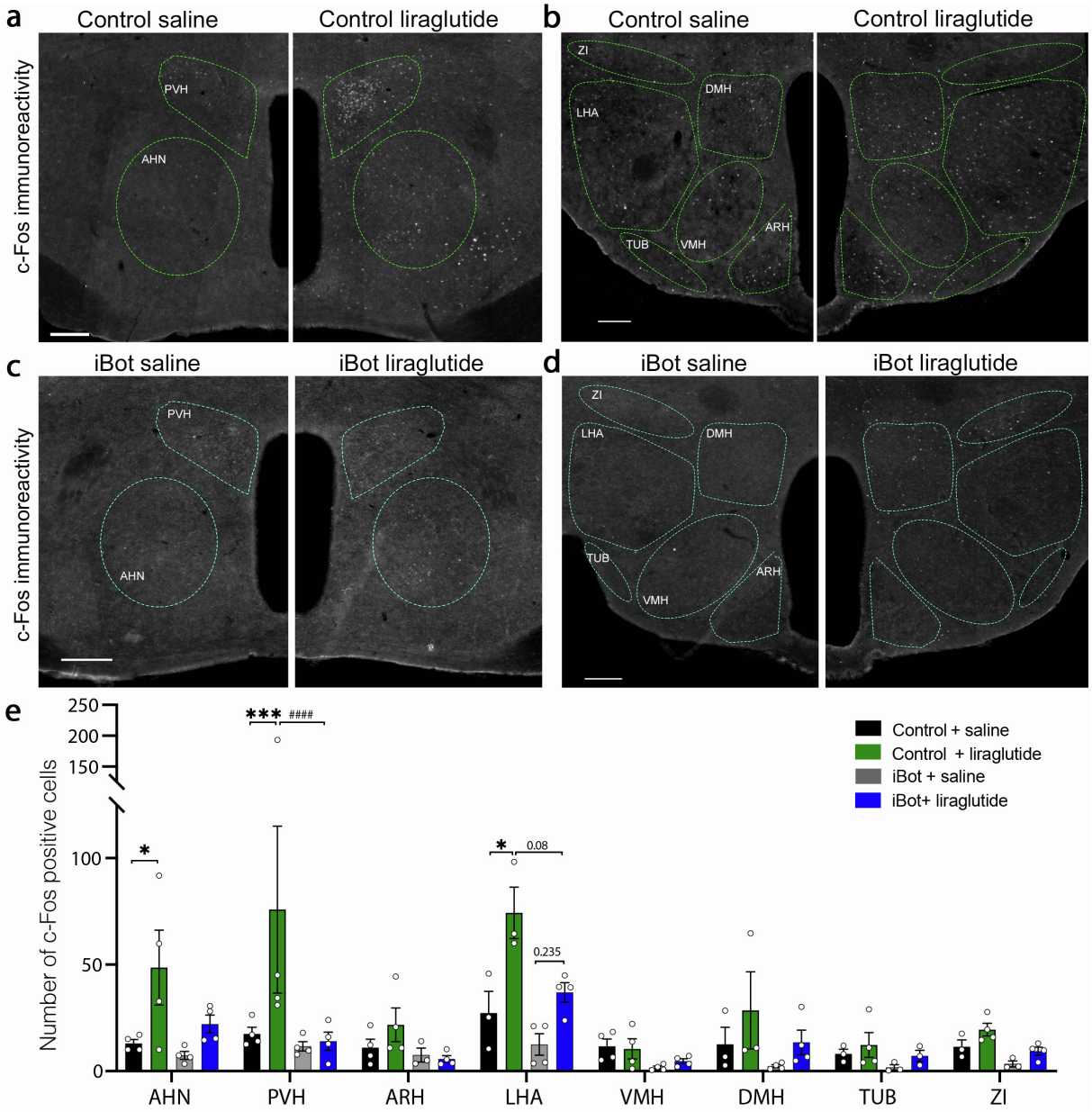



**Supplementary Figure 1. Characterization and validation of *in vitro* blood-brain barrier models** (Related to Fig. 1). (**a**) Illustration of the bovine endothelial cell/rat astrocyte BBB contact co-culture model with endothelial cells cultured on transwell permeable supports and astrocytes covering the lower surface of the support. (**b**) Photomicrograph showing bovine endothelial cells in co-culture with rat astrocytes after immunocytochemical labeling with antibodies against the gap junction markers claudin-5 (green) and ZO-1 (red). Yellow color indicates co-localization of the markers. Scale bar 30 µm. (**c**) Trans-endothelial electrical resistance (TEER) was measured to validate the junctional integrity of the *in vitro* BBB model. Each data point represents the mean of several permeable supports (technical replicates) from an individual preparation. Line and whiskers indicate mean and SEM across biological replicates ($n$ = 13). (**d**) Transcellular permeation of $^{125}$I-labelled GLP-1 (0.5 nM) and liraglutide (0.7 nM) measured in the BBB model and compared to the permeation of 4 kDa FITC-dextran (FD-4) and sodium

fluorescein. Bars show means ± SEM (*n* = 6, 3, 3, 3, 3, 5, 3 wells from 2 independent cultures). (**e**) Rat brain cortical endothelial cells on a transwell insert (I), bright-field photomicrograph of endothelial cell cultures from rat cortical capillaries (II), immunocytochemical labeling for the endothelial marker PECAM-1 (green), vimentin (red), a marker for tanycytes in the hypothalamus, von Willebrand's factor (green), which also labels endothelial cells, and GLP1R (green) (III-V); scale bar: 30 µm. (**f**) Tanycytes cultured on transwell permeable supports (I), bright-field image of tanycytic cultures from median eminence explants (II), immunofluorescence labeling for vimentin (red), GFAP (green) and GLP1R (green) (III-IV) scale bar: 30 µm. (**g**) Diagram and gating strategy for sorting Tomato-positive putative tanycytes following TAT-Cre infusion into the third ventricle (3V) of td*Tomato*$^{\text{loxP-STOP-loxP}}$ mice and GLP1R mRNA expression in tanycytes isolated by FACS from tdTomato mice (*n* = 5).

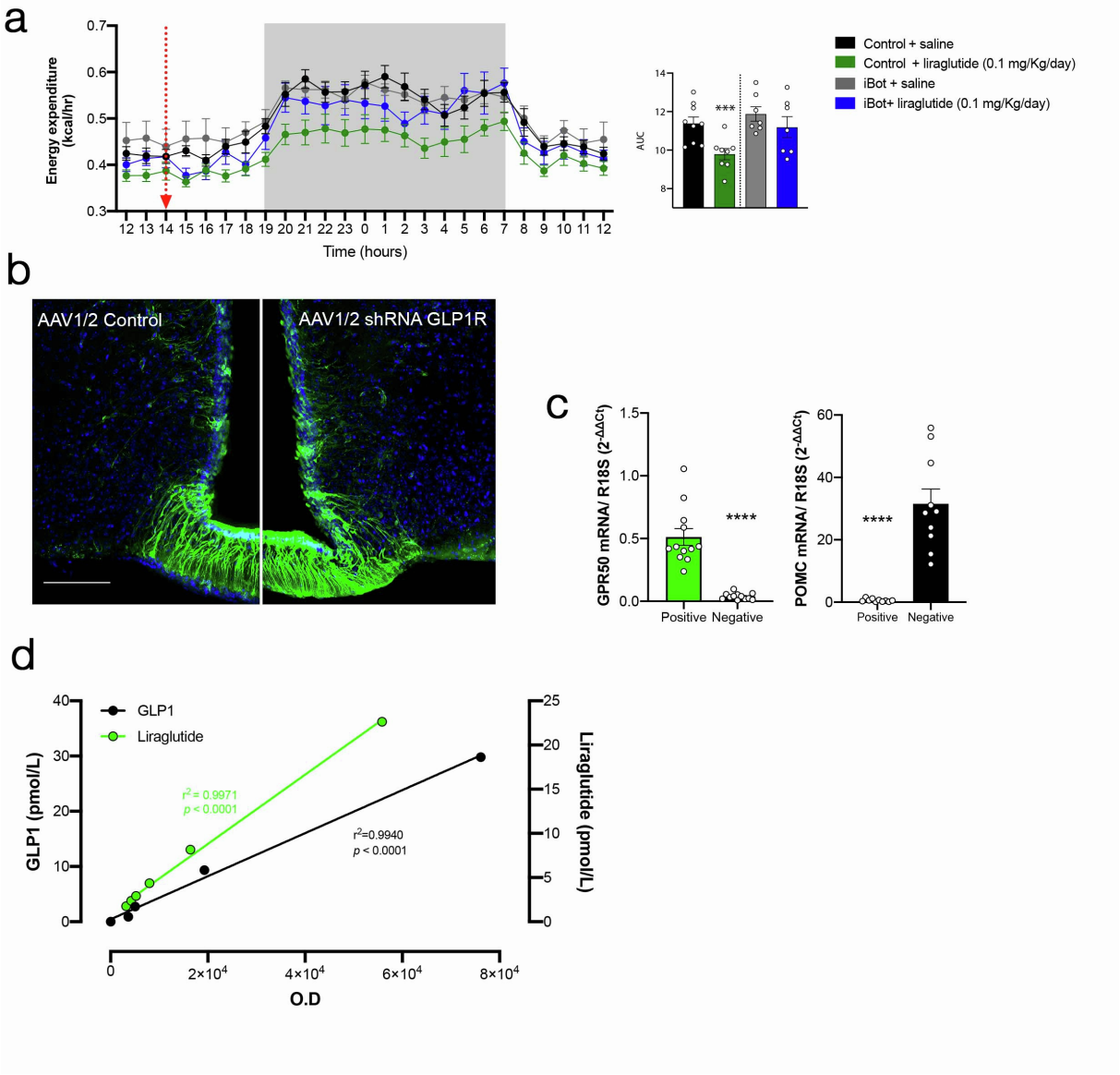



**Supplementary Figure 2. Liraglutide$^{564}$ is taken up by tanycytes, which express GLP1R *in vivo*, in a GLP1R-dependent manner and promotes CREB phosphorylation *in vitro*** (Related to Fig. 1 and Fig. 2). (**a**) Photomicrographs of cultured tanycytes following immunocytochemical staining for pCREB (red) after treatment with control uptake buffer (left panel), 100 nM liraglutide$^{564}$ (middle panel) and 1000 nM exendin 9-39 and subsequently with 100 nM liraglutide$^{564}$; Scale bar: 30 µm. (**b**) Diagram and gating strategy for sorting EGFP-positive putative tanycytes following TAT-Cre infusion into the third ventricle (3V) of BoNTB-EGFP$^{loxP\text{-}STOP\text{-}loxP}$ mice (iBot mice). (**c**) EGFP-positive cells (green) express DARPP-32 mRNA (tanycytic marker), but not NPY mRNA (neuronal marker) or MECA32 mRNA (fenestrated endothelial cell marker). Expression in EGFP-negative cells has been normalized to 1 (dotted line). Mann-Whitney U test, ** $p$=0.0079, $n$ = 5 mice. (**d**) GLP1R mRNA expression in EGFP-negative (black bar) and EGFP-positive (green bar) cells isolated from iBot mice; data are expressed as means ± SEM. ** Mann-Whitney test, $p$=0.0079, $n$ = 6 mice.




**Supplementary Figure 3. Liraglutide-induced c-Fos activation is abolished in the hypothalamus of iBot mice selectively expressing botulinum toxin in tanycytes** (Related to Fig. 2)**.** (**a-d**) Representative photomicrographs of c-Fos immunohistochemistry in the hypothalamus of control (**a, b**) and iBot (**c, d**) mice 10 min after intravenous injection of saline (left panels) or liraglutide (90 nmol/Kg, right panels). AHN: anterior hypothalamic nucleus; ARH: arcuate nucleus of the hypothalamus; DMH: dorsomedial hypothalamic nucleus; LHA: lateral hypothalamic nucleus; PVH: paraventricular nucleus of the hypothalamus; TUB: tuberal nucleus; ZI: zona incerta. Scale bar 200 µm. (**e**) Quantification of the number of c-Fos positive cells in mouse hypothalamic sections 10 minutes after either intravenous saline (black, grey) or liraglutide (90nmol/kg) injection (green, blue) in control (black and green) and iBot mice (grey and blue); *n*= 4 animals per group; 6 to 7 sections per animal. AHN: two-way ANOVA, genotype:

$F_{(1, 12)}$= 3.09, $p$ = 0.104; treatment: $F_{(1, 12)}$= 7.55, $p$ = 0.017; interaction: $F_{(1, 12)}$= 0.276, $p$ = 0.276. Fisher's LSD *post hoc* test, control saline vs. control liraglutide, $p$ = 0.0176. PVH: two-way ANOVA, genotype: $F_{(1, 12)}$= 2.92, $p$ = 0.113; treatment: $F_{(1, 12)}$= 2.39, $p$ = 0.147; interaction: $F_{(1, 12)}$= 2.03, $p$ = 0.1794. Fisher's LSD *post hoc* test, control saline vs. control liraglutide, $p$ = 0.05; control liraglutide vs. iBot liraglutide, $p$ = 0.046. LHA: two-way ANOVA, genotype: $F_{(1, 10)}$= 11.3, $p$ = 0.007; treatment: $F_{(1, 12)}$= 21.31, $p$ = 0.001; interaction: $F_{(1, 10)}$= 2.143, $p$ = 0.174. Fisher's LSD *post hoc* test, control saline vs. control liraglutide, $p$ = 0.002; iBot saline vs. iBot liraglutide, p = 0.0002; control liraglutide vs. iBot liraglutide, $p$ = 0.006. Data are expressed as means ± SEM. *$p$ < 0.05; *** $p$ < 0.001, control saline vs. control liraglutide; #### $p$ < 0.0001, control liraglutide vs. iBot liraglutide.




**Supplementary Figure 4** (Related to Fig. 3 and Fig. 4)**. Effect of liraglutide on energy expenditure in iBot mice.** (**a**) Energy expenditure in iBot and control mice after 3 days of intraperitoneal treatment with saline (baseline, grey and black respectively) or liraglutide (blue and green respectively, 0.1 mg/Kg/day). Line plot shows mean energy expenditure at different times 3 days after liraglutide treatment compared to baseline, and AUC. AUC paired two-tailed t-test, $t_{(7)} = 5.73$, $p = 0.0007$. Dotted arrow indicates saline or liraglutide administration ($n$ = 9, 8, 8, 8 mice). Two-way ANOVA with Tukey's *post hoc* test was performed on AUC (control saline vs. control liraglutide, $p < 0.0001$; control liraglutide vs. iBot liraglutide, $p < 0.0001$) ($n$ = 9, 8, 7, 7 mice). Data are expressed as means ± SEM *** $p$ <0.001. (**b**) Selective expression of GFP in tanycytic cell bodies and processes in animals transduced with control and shRNA viruses. (**c**) Expression of GPR50 mRNA (tanycytic marker) but not POMC mRNA (neuronal marker) by

GFP-positive cells (green), and vice versa in GFP-negative cells (black) FACS-isolated from mice transduced with control-GFP or shRNA-GLP1R-GFP viral vectors. Unpaired one-tailed t-test (GPR50, positive vs. negative, $t_{(22)} = 7.03$, $p < 0.0001$, $n = 12$ mice; POMC, positive vs. negative, $t_{(18)} = 6.49$, $p < 0.0001$, $n = 10$ mice). (**d**) Correlation between optical density (O.D.) with total GLP1 (black line) or liraglutide (green line) amounts detected with the total GLP1 ELISA kit.