1. Supplementary material and methods

1.1. Roux-en-Y gastric bypass

RYGB surgery was performed as previously described (Seyfried et al. 2012). In brief, surgical anaesthesia was induced and maintained throughout the procedure with isoflurane (2–3 % with O₂). Mice were placed on a heating pad to avoid hypothermia and the abdomen was shaved and disinfected before a 4-cm midline laparotomy was performed. The esophagogastric junction was exposed and the esophagus mobilized. The left gastric vessels and the vagal nerve branches were dissected away from the esophagus to avoid respectively consecutive ischemia of the remaining stomach and damage to the vagal nerve. The small vessels on the cardia were cauterized. The stomach was transected at the gastro-esophageal junction. The native stomach was closed with non-resorbable sutures. At 4 cm distal from the pylorus the jejunum was incised for 2 mm on the antimesenterial side and anastomosed in an end-to-side fashion to the esophagus with two continous, non resorbable sutures. The first 5 cm of the jejunum was used to create a Roux limb. Here, an end-to-side jejunoojejunostomy between the biliopancreatic (4 cm) and Roux limb was performed to create the common limb. Hereafter the jejunum was double ligated and transected just proximal of the esophagojejunostomy to create the biliopancreatic limb. The abdominal wall and skin were closed.

In the sham groups, the small bowel and the gastro-esophageal junction were mobilized and a gastrostomy (5 mm) on the anterior wall of the stomach was performed. The gastrostomy was closed subsequently.

To prevent postoperative pain, all mice received carprofen (3mg/kg) and buprenorphine (0.1mg/kg) twice daily the first 2 days after surgery. A western style diet was continued in all groups for 7 weeks till sacrifice. The first three postoperative days, manually crushed chow together with 20ml of Nutridrink® was provided at the bottom of the cage in order to facilitate food intake.

1.2. Plasma ghrelin Measurement

Plasma samples were extracted on a SEP-Pak C18 cartridge (Waters Corporation, Milford, MA), vacuum-dried and subjected to ghrelin radioimmunoassay (RIA) as previously described (Janssen et al.)
For determination of octanoyl ghrelin a rabbit anti-human ghrelin [1-8] antibody was used which does not recognize desoctanoyl ghrelin. Total ghrelin levels were determined using a rabbit anti-human ghrelin [14-28] antibody, which recognizes both octanoyl and desoctanoyl ghrelin.

1.3. Plasma Glucagon-Like Peptide-1, Peptide YY, insulin and leptin measurement

Samples were analysed for plasma GLP-1 (GLP-1(7-36)amide and GLP-1 (7-37)) using the Active GLP-1 (ver. 2) Kit (Mesoscale Discovery) and plasma leptin and serum insulin levels were determined using the Mouse metabolic Kit (Mesoscale Discovery), according to the manufacturer’s instructions. Plasma PYY levels (PYY(1-36) and PYY(3-36)) were determined using a PYY ELISA (Phoenix pharmaceuticals), according to the manufacturer’s instructions.

1.4. Oral Glucose Tolerance Test

An Oral glucose tolerance test (OGTT) was performed 2 weeks before and 5 weeks after surgery. Mice were fasted for 6 hours and gavaged with 2g/kg D-glucose. Tail vein glucose levels were measured at 0, 15, 30, 60 and 120 minutes after glucose administration with a glucometer (Glucomen LX®, A. Menarini Diagnostics) and serum was collected after centrifugation for 7 min at 2000 rpm (4°C) and subsequently snap frozen in liquid nitrogen for insulin measurements. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the equation 

\[ \frac{G_0 \times I_0}{405} \]

where \( G_0 \) and \( I_0 \) refer to 6-h fasting plasma glucose and insulin values.

1.5. Reverse transcription and quantitative real-time polymerase chain reaction

Total RNA was isolated from mouse tissue segments using the RNeasy kit (Qiagen). Isolated RNA was treated with Turbo DNAfree kit (Ambion) and was reversed transcribed to cDNA using Superscript II Reverse Transcriptase (Invitrogen). The real-time PCR reaction was performed as described previously, using the Lightcycler 480 (Roche Diagnostics) with the Lightcycler 480 Sybr Green I Master mix (Roche Diagnostics). Results were expressed relative to the geometric mean of the normalized expression of the three most stable housekeeping genes tested; hypoxanthine phosphoribosyltransferase 1 (Hprt1), ribosomal protein L13a (RPL13a) and \( \beta \)-actin.
1.6. Histology and Immunohistochemistry

Gastric or colonic tissues and segments from the RL and BPL, or corresponding segments were sampled as illustrated (Fig. 3a). Samples (5 mice/group) were immediately fixed with 4% paraformaldehyde for 2h (4°C) followed by cryoprotection in 30% sucrose at 4°C overnight. Cryostat sections (8 µm) were incubated for 2 h in 0.1 M phosphate-buffered saline (PBS) containing 10% donkey serum and 0.3% Triton X-100 and incubated with one of the following primary antibodies: rabbit anti-octanoyl ghrelin (1/500 for limbs and 1/1000 for gastric tissue, Ab5004, in-house developed), goat anti-ghrelin (1/1000, sc-10368, Santa Cruz), goat anti-GLP-1 (1/150, SC-7782, Santa Cruz Biotechnology) or rabbit anti-PYY (1/1000, Ab22663, Abcam). Substitution of the primary antibody with PBS was used as a negative control. Subsequently, tissues were incubated with secondary antibody for 2 hours, after washing: donkey anti-rabbit Alexa488 (1/1500, A21206, Thermofisher), anti-goat Alexa594 (1/1000, A11058, Thermofisher) or donkey anti-rabbit AMCA (1/250, 711-225-152, Jackson Immuno Labs). For the double-immunofluorescence staining, after being incubated with the secondary antibody and washed three times, sections were incubated for 24 hours at room temperature with the second primary antibody. Subsequently, tissues were incubated with the second secondary antibody for 2 hours. Sections were mounted in Citifluor and visualized under a fluorescence microscope (Olympus BX41). Of each tissue segment three sections were analyzed using Cell^F Imaging Software (Olympus Soft Imaging Solutions GmbH). Octanoyl ghrelin-, ghrelin-, GLP-1- or PYY-positive cells, were counted in 5 randomly chosen fields (20x) for the stomach and expressed per mm², or in the whole section (20x) for the segments from colon, RL, BPL or corresponding segments.

Morphological measurements were performed on hematoxylin and eosin (H&E) sections. Mucosal thickness was measured at 15 randomly chosen representative fields (4x).

1.7. SCFA Analysis

SCFAs were extracted from the fecal samples using an ether extraction. 50 mg of fecal sample was suspended in 1 ml saturated NaCl (36%). Ether (3 ml), Na₂SO₄ (50 mg), H₂SO₄ (150 μl) and internal
standard (50 µl) (2-ethylbutyric acid (Merck)) was added to each sample. The ether layer was collected and the SCFA were analyzed on a gas chromatography–mass spectrometry quadrupole (Finnigan Trace GC, Thermoquest) as previously described. (De Preter et al. 2009) Samples were absolutely quantified using an appropriate calibration curve. The primers used are represented in Supplementary table 1.

1.8. Gut Permeability Assays

The distal colon or tissue from the CL or corresponding segment, was dissected in carbogenated Krebs-Ringer buffer (11mM D-glucose). Intestinal segments of approximately 0.5 cm² (n=3/mouse) were cut and mounted in modified Ussing chamber (Mussler Scientific Instruments) with an area of 0.017cm² for colonic segments, and 0.096cm² for segments from CL or the corresponding tissue. Potential difference (PD) was continuously recorded and transepithelial electrical resistance (TEER) (Ωxcm²) was calculated from the voltage deflections induced by bipolar constant-current pulses of 16 mA every 60 s with duration of 200 ms and was recorded over 2h. Meanwhile, permeability was measured, by adding fluorescein (376 Da, Sigma Aldrich) to the mucosal side after a calibration period of 30 min. Regular (every 30 min) sampling from the serosal side allowed to detect the degree of mucosal permeability. The fluorescence level (ng/ml*cm²) was measured using a fluorescence reader (FLUOstar Omega; BMG Labtech).

Short circuit current was calculated using the measured TEER and recorded potential difference (voltage) using following formula; Isc = V/R where V and R refer to the measured Voltage (V) in micro Ampère and Resistance (R) in Ohm.

1.9. Blinding

Genotype paradigms were blinded during the sample collecting and genotype and treatment paradigms were blinded during the analysis.
2. Supplementary figure legends

Fig. S1. Experimental design
Scheme of the experimental design of the study. WT and α-gust⁻/⁻ mice (6 weeks old) were fed a western diet for 12 weeks. Mice were randomized in three groups: a sham-group fed ad libitum (ALF), a sham-group, pair-fed to the RYGB group (PF), and a RYGB group fed ad libitum fed (RYGB). All mice received the western diet for 7 weeks after surgery till sacrifice. An oral glucose tolerance test was performed 2 weeks before surgery and 2 weeks before sacrifice. Seven weeks post-surgery, (6h) fasted mice (between 7.30-9 a.m. – 1.30-3 p.m. to minimize circadian fluctuations) were gavaged with 200µL Nutridrink®, 15 minutes before sacrifice.

Fig. S2. Pre-surgical weight gain and glucose tolerance
(A) Pre-surgical time-dependent changes in body weight of WT and α-gust⁻/⁻ mice fed a western style diet (n= 21-23). (B) Plasma glucose levels during an OGTT (2g/kg) in WT and α-gust⁻/⁻ mice fed a western style diet, 2 weeks before surgery (n=21-23). * P<0.05 compared to WT mice.

Fig. S3. RYGB increased plasma total ghrelin levels in α-gust⁻/⁻ mice
Number of (A-C) octanoyl ghrelin and (D-F) total ghrelin IR cells in the stomach (A, D), BPL (B, E), RL (C, F) and corresponding small intestine (sham groups) in ALF, PF and RYGB WT and α-gust⁻/⁻ mice (n= 5-8).
(G, H) Relative mRNA levels of GOAT in the stomach, BPL, RL (RYGB) or corresponding small intestine (sham groups) in PF and RYGB groups in WT and α-gust⁻/⁻ mice (n= 6-8). The dotted line indicates the mean relative GOAT mRNA levels in ALF mice.
**Fig. S4.** RYGB increased L-cell density in the foregut, but not the distal gut of WT mice in an α-gustducin independent manner

(A) Morphometric analysis showing the mucosal height from sections of the distal colon from ALF, PF and RYGB groups in WT and α-gustducin mice. Density of GLP-1 IR cells in sections of the RL (B) and distal colon (E) in RYGB groups or corresponding segments (ALF, PF groups) in WT and α-gustducin mice (n= 5).

Relative mRNA levels of neurogenin 3 in the RL (C, D) and distal colon (F, G) or corresponding segment (ALF-PF) in WT and α-gustducin mice (n= 6-8). The dotted line indicates the mean relative neurogenin 3 mRNA levels in ALF mice.

* P<0.05 compared to ALF groups. # P<0.05 compared to PF groups. £ P<0.05, ££ P<0.01 genotype*operation effect between the indicated groups.

**Fig. S5.** α-gustducin plays a role in the RYGB-induced changes in ion transport across the epithelium (secretory and/or absorption properties) of the diverged small intestine

Equivalent short circuit current (Isc) in the (A, B) CL (RYGB groups) and (D, E) distal colon or corresponding segment (sham-operated groups) in ALF, PF and RYGB WT and α-gustducin mice (n= 8-9).

(C, F) Average equivalent short circuit current (Isc).

*** P<0.001 compared to ALF groups. #### P<0.001 compared to PF groups. ££ P<0.01 genotype*operation effect between the indicated groups.

**Fig. S6.** RYGB improved colonic permeability in an α-gustducin independent manner

(A, B) Colonic transepithelial electrical resistance (TEER) 7 weeks after surgery in ALF, PF and RYGB WT and α-gustducin mice (n= 6-8). (C, D) Colonic fluorescein passage 7 weeks after surgery in ALF, PF and RYGB WT and α-gustducin mice (n= 6-8). (E, F) Relative occludin mRNA levels in the distal colon in PF and RYGB WT and α-gustducin mice (n= 6-9). The dotted line indicates the mean relative occludin mRNA levels in ALF mice.
* P<0.05, ** P<0.01, *** P<0.001 compared to ALF groups. ## P<0.01, ### P<0.001 compared to PF group
3. Supplementary references

