

Supplementary information

Sub-chronic treatment in C57BL/6J mice with native peptides

C57BL/6J mice (Janvier Labs, Saint Berthevin, Cedex, France), 8 weeks of age, were offered regular chow diet (Altromin 1324, Brogaarden A/S, Denmark). Mice were randomized according to body weight into four individual study groups (n=10 per group). Group 1: Vehicle (SC, BID). Group 2: native GLP-1 (3 mg/kg, SC, BID). Group 3: native GLP-2 (3 mg/kg, SC, BID). Group 4: native GLP-1 (3 mg/kg, SC, BID) + native GLP-2 (3 mg/kg, SC, BID). Compounds were dissolved in PBS buffer containing 3% mannitol and 0.6% L-His (pH 9.0) and dosing volume was 5 ml/kg. On day 8, animals were fasted for 4 hours before termination in the light phase, the intestines were collected and the length of the small and large intestine was measured. Intestines were cleaned by flushing with saline and finally the weight was measured.

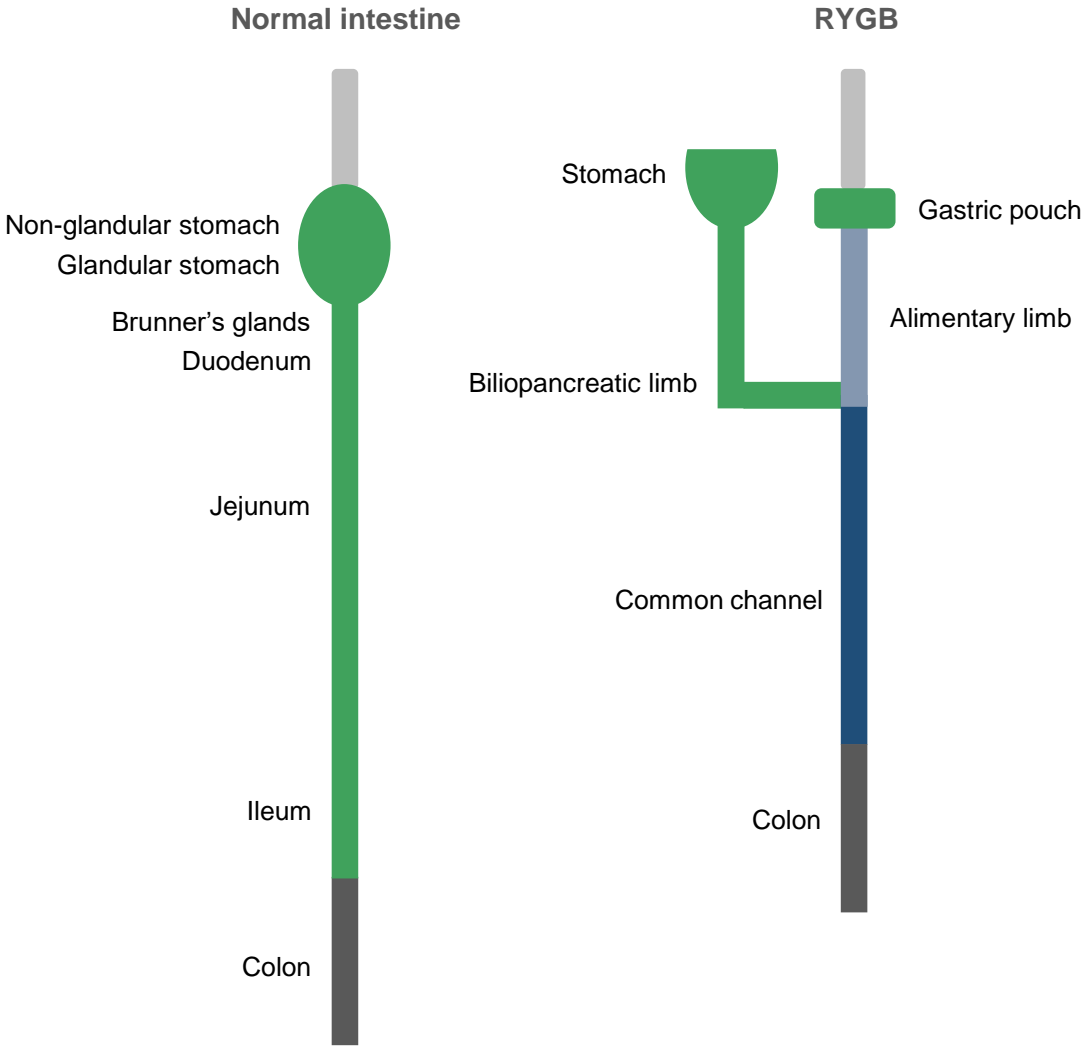
RYGB surgery

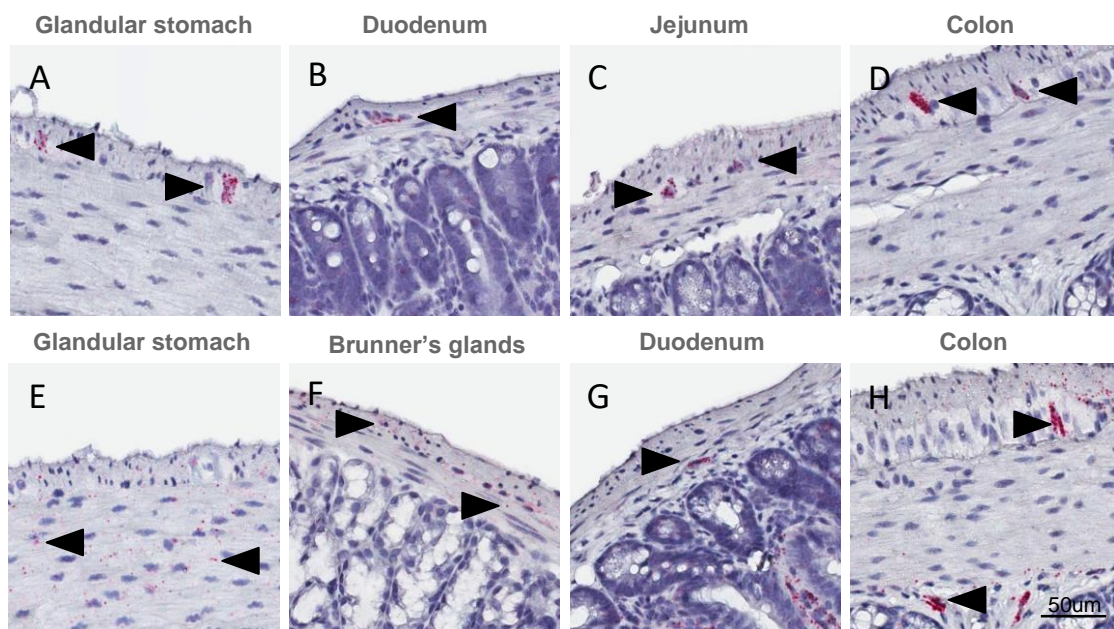
Sprague-Dawley rats were made obese by access to a two-choice diet for approximately 12 weeks before experimentation. The diets consisted of regular Altromin 1324 rodent chow (Brogaarden, Denmark) and a high-fat paste made from Chocolate spread (Nutella, Ferrero Italy), Peanut butter (pcd, Holland) and powdered chow. In the peri-surgery period (day -3 to 9) the rats in all groups were offered a liquid diet (Fresubin® Original, 1 kcal pr. ml). Rats were randomized based on body weight on day -7 into two experimental groups: RYGB and sham. The RYGB procedure was performed by creating a 17cm long biliopancreatic limb. This was attached to the common channel approx. 70 cm proximal from the caecum. The remaining alimentary limb was approx. 28 cm long and attached to the newly created gastric pouch while the stomach remnant was stapled together. The sham operation followed the RYGB operation to the point of the transverse incision 17 cm caudal to the ventricle. This incision was re-sutured to reconnect the jejunum. The stomach was taken out of the abdominal cavity, freed from ligaments and repositioned in the abdominal cavity. Pain relief was provided by subcutaneous injections of 5-10 mg/kg Enrofloxacin and 5 mg/kg Carprofen were administered from day -1 until day 5 post surgery. 10 ml/kg of warm saline solution were administered on day 0 until 5 days post-operatively. The animals were terminated 9 days post-surgery, euthanized via CO₂ inhalation and the entire GI tract from the stomach to the rectum was removed. The small and large intestines were flushed with PBS to remove contents, blotted dry, weighed and placed in 10% natural buffered formalin until further processing. The intestine was sampled using systematic random uniform sampling (SURS) principles, providing a minimum of 4 systematically placed biopsies from each segment (biliopancreatic limb, alimentary limb, common channel and colon). All biopsies were embedded in blocks of paraffin, cut into 5 µm thick sections and stained with hematoxylin-eosin for subsequent stereology-based area estimations. Stereological area estimations were performed by point-counting on digitally scanned slides using the newCAST system (Visiopharm, Denmark).

Double KO Mice:

Glp1^{r/-}:Glp2^{r/-} double knockout (DKO) were generated by crossing double heterozygote *Glp1^{r+/-}: Glp2^{r+/-}* mice. All experiments were carried out in 24-week old male mice from the same litter or family. Mice were housed under specific pathogen-free conditions in microisolator cages and maintained on a 12-h light/dark cycle with free access to standard rodent diet and water. All experiments were carried out in accordance with protocols and guidelines approved by the Animal Care Committee at the Toronto Centre for Phenogenomics. Mice were euthanized via CO₂ inhalation, weighed and the entire GI tract from the stomach to the rectum was removed. The small intestine and colon was dissected away from the caecum, flushed with PBS to remove contents, blotted dry and weighed.

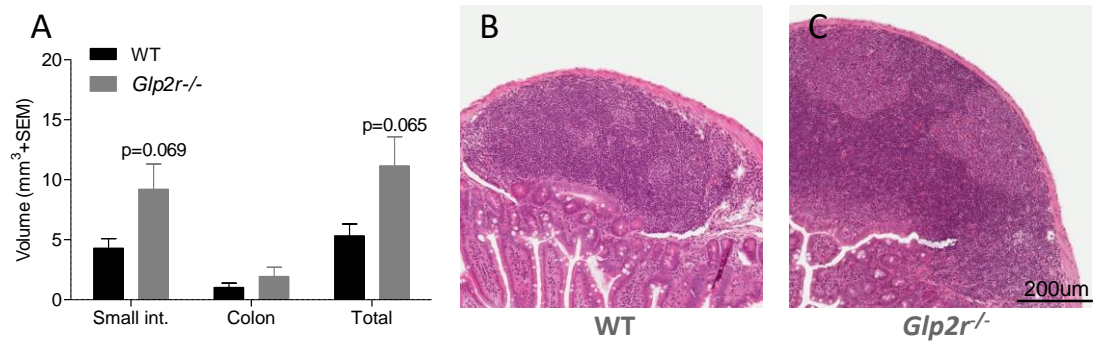
Schematic overview of normal rodent intestine and RYGB surgery





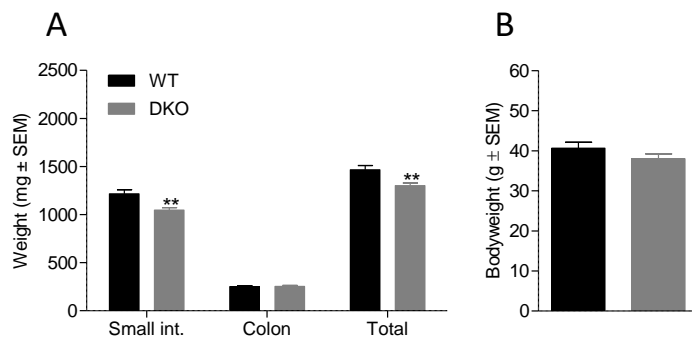
Supplementary Figure 1: *Glp1r* and *Glp2r* mRNA is localized in enteric neurons in the mouse gastrointestinal tract.

Localization of *Glp1r* (A-D) and *Glp2r* (E-H) mRNA in mouse gastrointestinal tract. Glandular stomach muscularis nerve plexi of (A, E), small intestine muscularis nerve plexi (B, C, F, G) and colon muscularis and submucosa nerve plexi (D, H) using RNA scope 2.5 in situ hybridization.



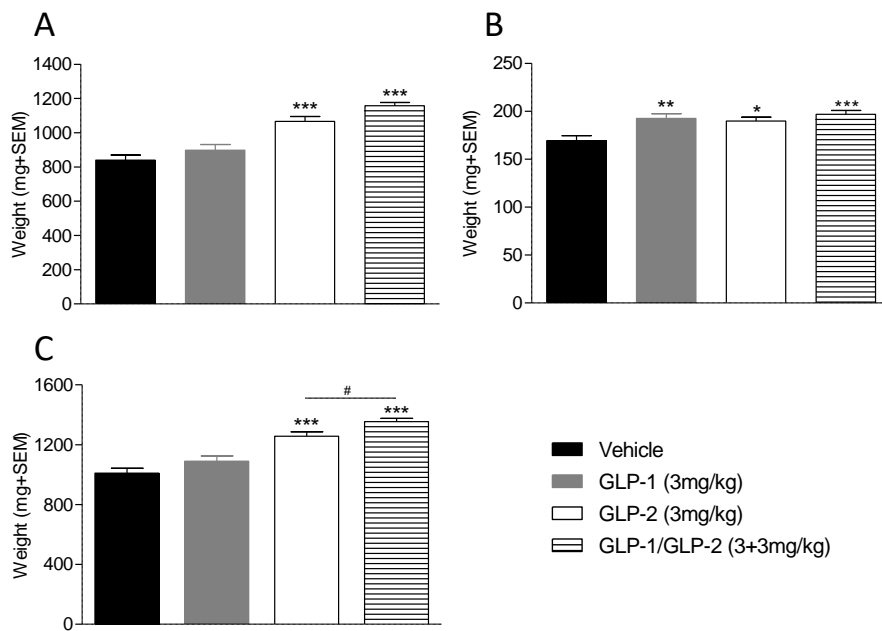
Supplementary Figure 2: *Glp2r*^{-/-} mice display a tendency towards increased volume of immune cells in the small intestine.

Intestine immune cell volume in small intestine, colon and total intestine in WT C57BL/6J or *Glp2r*^{-/-} mice (A). Picture depicts examples of small intestine lymphoid tissue in WT mice (B) and *Glp2r*^{-/-} mice (C).



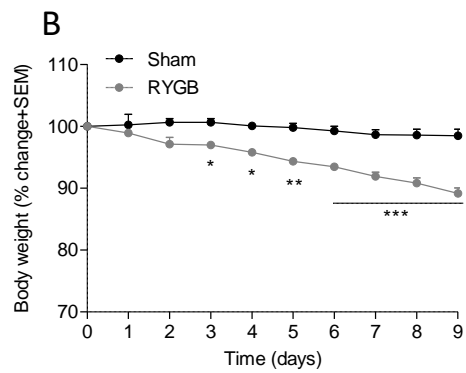
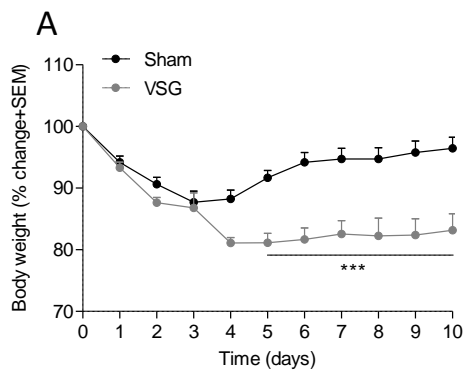
Supplementary Figure 3: Double knockout (DKO) *Glp1r^{-/-}:Glp2r^{-/-}* mice display a slight reduction of small intestine weight.

Weight of small intestine, colon, total intestine (A) and body weight (B) in WT and double KO (DKO) mice.



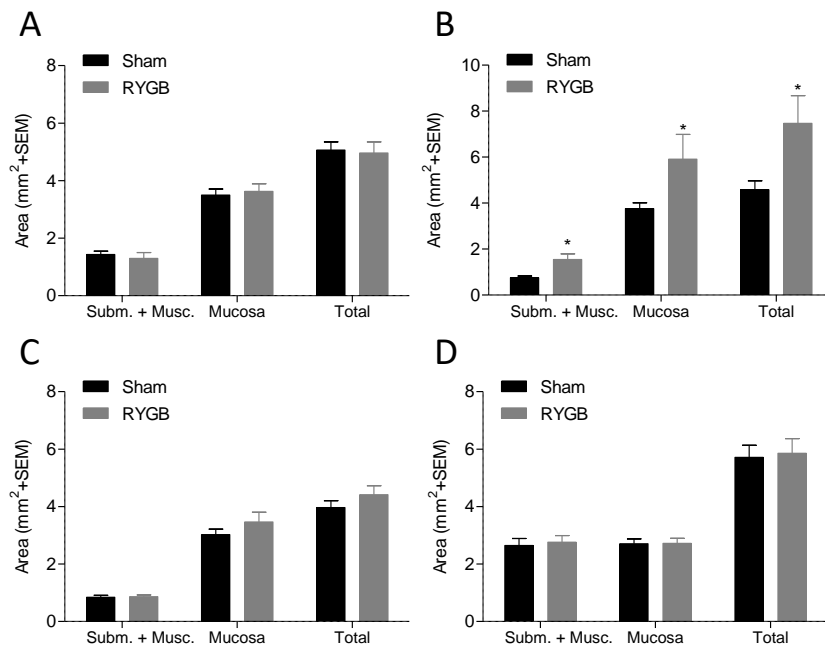
Supplementary Figure 4: Native GLP-2, and to some extent GLP-1, increase intestinal weight in mice.

The effect of native GLP-1 and GLP-2 mono or co-agonism on small intestine (A), colon (B) and total intestinal weight (C) measured at termination.



Supplementary Figure 5: VSG and RYGB surgery significantly decrease body weight in rodents.

Body weight change of mice subjected to either sham or VSG surgery (A), and rats subjected to either sham or RYGB surgery (B).



Supplementary Figure 6: RYGB significantly increases alimentary limb area 9 days after surgery.

The effect of RYGB or sham operation on area of biliopancreatic limb (A), alimentary limb (B), common channel (B) and colon (C) as measured by stereology.