Supporting Information

Grosse et al. 10.1073/pnas.1411413111

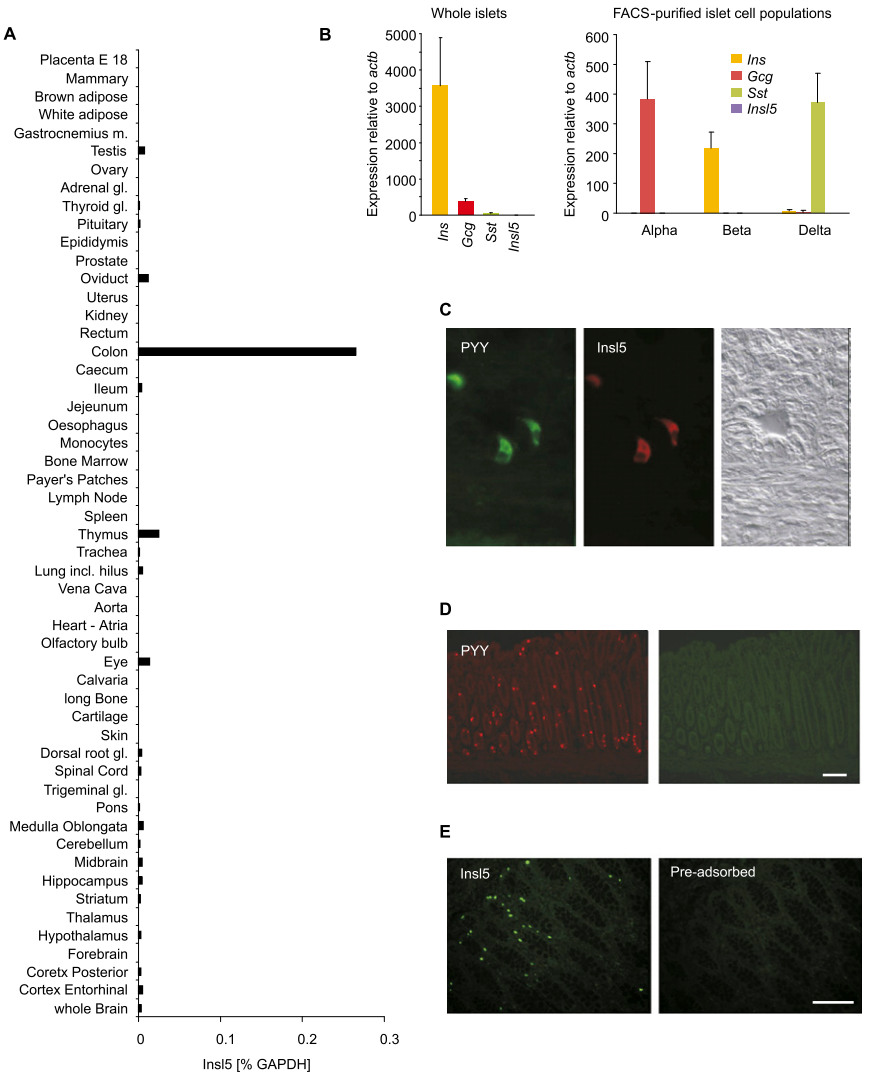

Fig. S1. Expression of insulin-like peptide 5 (*Insl5*) in a range of mouse tissues and cells. (*A*) *Insl5* mRNA was quantified by quantitative RT-PCR (qRT-PCR) in a range of mouse tissues. (*B*) Detailed expression analysis of *insl5*, *gcg*, insulin (*ins*), and somatostatin (*sst*) in pancreatic endocrine cells. Taqman qRT-PCR analysis was performed on whole mouse islets or from FACS-purified murine pancreatic alpha, beta, and delta cells. Alpha and beta cells were purified from GLU-Venus mice, as described (1). Delta cells were purified from Sst-Cre × ROSA26-EYFP mice. b-actin (*Actb*) was used as a housekeeper. Columns show mean + 1 SEM. (*C*) Colocalization of PYY (*Left*) and Insl5 (*Center*) in mouse colon. (*C*, *Right*) Differential interference contrast. (*D*) Immunohistochemistry controls for the specificity of the Insl5 signal. Insl5 antibody was omitted but not the secondary antibody (green channel, *Right*). PYY was detected as described (red, *Left*). There was no cross-channel contamination observed and no nonspecific signal arising from the secondary anti-rat antibody in the green channel. (Scale bar: 100 μm.) (*E*) Insl5 is detected using hybridoma clone NSFL7-18H1 at a dilution of 1:5,000 (*Left*). In an adjacent section no specific signal was detected after preadsorption of a 1:1,000 dilution with 0.5 μg/mL Insl5 (*Right*). (Scale bar: 100 μm.)

1. Reimann F, et al. (2008) Glucose sensing in L cells: A primary cell study. *Cell Metab* 8(6):532–539.

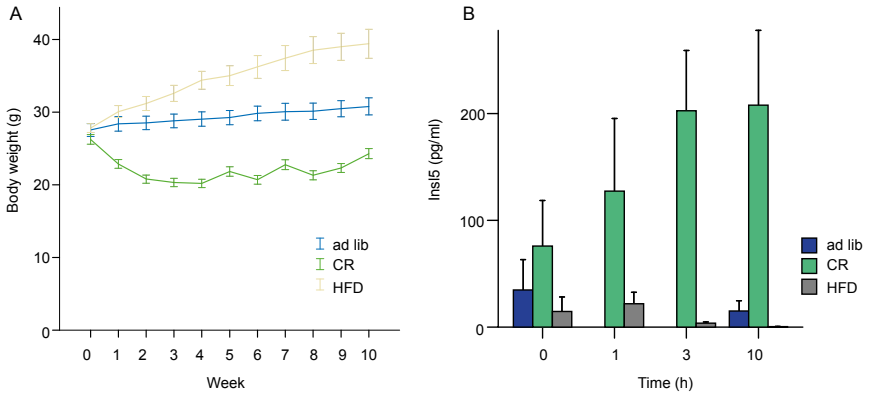

Fig. S2. Plasma Insl5 levels after dietary intervention. (*A* and *B*) Effect of long-term dietary alterations on Insl5 levels. C57BL/6J mice were fed with standard chow ad libitum (ad lib) or restricted to 60% of the former (calorie restriction; CR), or with a high-fat diet (HFD, 45% fat) for 10 wk. (*A*) Body weight changed as expected in the cohorts, represented as mean ± 95% CI. (*B*) After an overnight fast, terminal blood samples were taken before or at the indicated time after refeeding. Insl5 levels were higher in the CR cohort (ANOVA with time and diet as factors; $P < 0.001$; post hoc Bonferroni: $P < 0.001$ for CR vs. HFD. Ad libitum cohort was not included in the analysis as only two time points were measured for this cohort. $n \geq 7$ for each time point, diet and genotype, respectively (mean ± SEM).

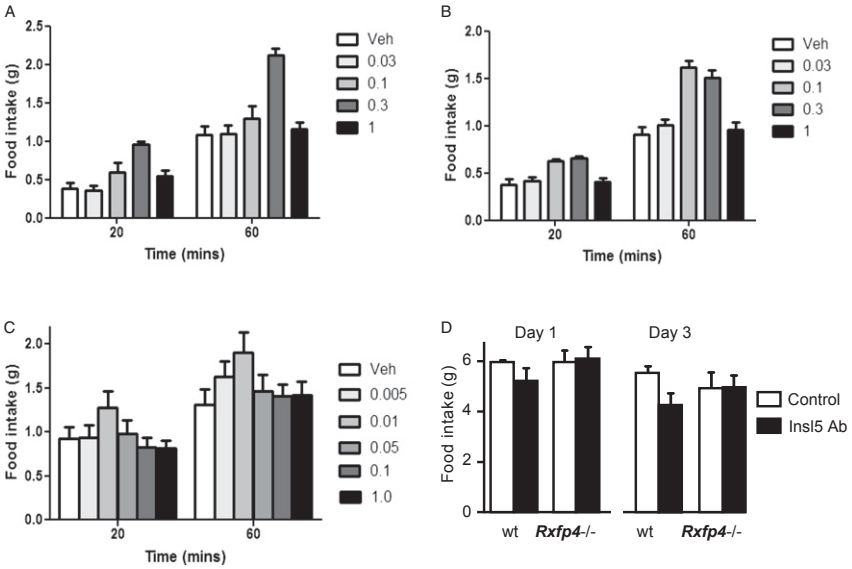

Fig. S3. Effect of Insl5 on food intake in mice. (*A*) Animals were adapted to a palatable wet mash meal (made from chow diet) at a fixed time of day. Commercial Insl5 (Phoenix Pharmaceuticals), at the doses indicated, was injected and food intake was monitored. Information about the nature of the C terminus (acidic or amide) of Insl5 purchased from Phoenix Pharmaceuticals was not available. (*B*) As in *A*, using a synthetic Insl5 preparation with free C-terminal acid, which is substantially more potent than the amide form in vitro (1). (*C*) Consumption of wet peachy porridge following administration of the Insl5 preparation with free C-terminal acid. Consumption in the vehicle-treated groups was higher than in the wet mash groups (*A* and *B*), suggesting that this might be even more palatable. The apparent difference in potency compared with experiments in *A* or *B* is most likely attributable to reduced adsorption by the addition of 0.5% mouse albumin and use of siliconized glassware. In all experiments (*A*–*C*), Insl5 (dose given as μg per 25 g of body weight) caused an increase in food intake (two-way ANOVA with time and dose as factor, $P < 0.001$, $n \geq 6$). Data are represented as mean ± SEM. (*D*) The effect of AB-mediated neutralization of endogenous Insl5 is abrogated in receptor-deficient mice: Wild-type or *Rxfp4*$^{-/-}$ mice were treated as described in Fig. 4*C* with 50 μg of anti-Insl5 antibody (black bars) or rabbit normal serum (white bars). Insl5 Ab resulted in a reduced food intake in wild-type but not *Rxfp4*$^{-/-}$ mice ($P < 0.001$, GLM for repeated measures, $n = 5$; Tukey HSD post hoc: $P < 0.001$ for the wild-type group treated with anti-Insl5 Ab vs. all other groups). Data are presented as mean + 95% CI.

1. Belgi A, et al. (2011) Structure and function relationship of murine insulin-like peptide 5 (INSL5): free C-terminus is essential for RXFP4 receptor binding and activation. *Biochemistry* 50(39):8352–8361.

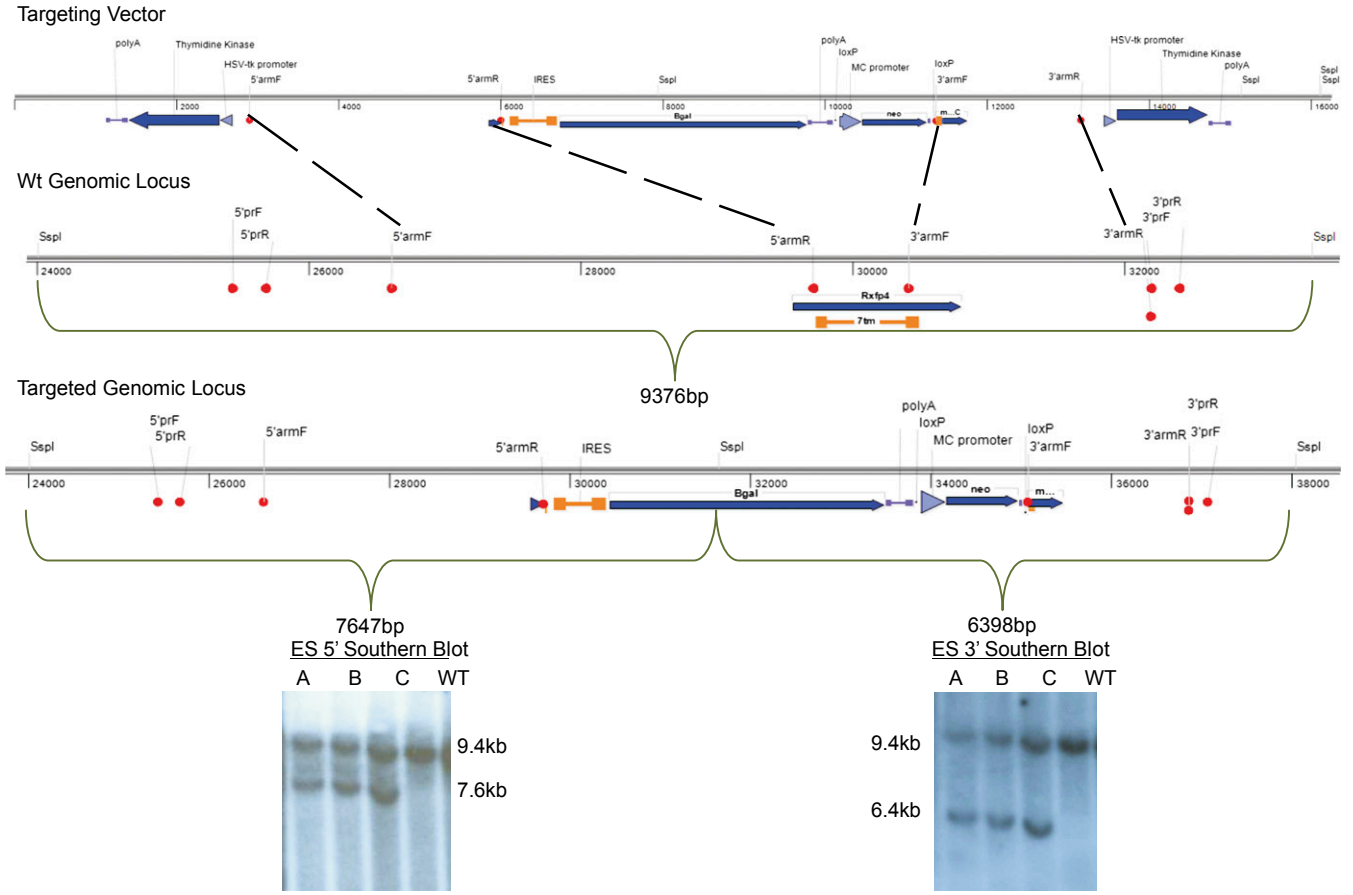

Fig. S4. Generation of Rxfp4$^{-/-}$ mice. To generate the targeting construct 3,125-bp 5′ and 1,810-bp 3′ homology arms were PCR amplified from genomic DNA and cloned into a vector containing an IRES–βgal reporter gene, a Neomycin selection marker under the control of a MC1 promoter, and two Thymidine Kinase negative selection markers. The linearized vector was electroporated into ES cells (129SvEv). Homologous recombination was detected by screening PCR and SspI-digested Southern blotting of genomic DNA of individual clones as well as the parental cell line (wt).

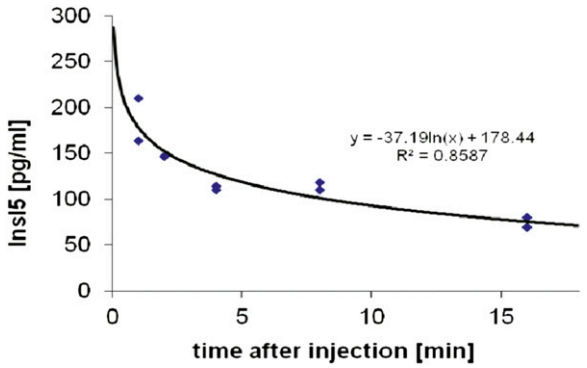

Fig. S5. Pharmacokinetics of Insl5. Mice were injected i.v. with 5 μg of Insl5 (Phoenix), and plasma was taken at either 1, 2, 4, 8, or 16 min after administration. Insl5 levels were measured using an RIA (Phoenix).