Supplementary Materials and Methods

Genotyping and Southern Blot Analysis of GPR40$^{-/-}$ Mice

PCR primers coding for eGFP and the endogenous GPR40 (wild-type) gene were used to differentiate the knockout and the wild-type mouse, respectively (Supplementary Table 1). For Southern blot, genomic tail DNA was digested with Kpn1 and analyzed using DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Applied Science, Indianapolis, IN). DIG-labeled probe was amplified by PCR using PCR DIG Probe Synthesis Kit (Roche Applied Science, Indianapolis, IN) with primers CD22 S4:TACAGATAGGAGTAACCATGCCCAG and CD22-AS4: ATTCTCATAATCCCCCTGCAAAGCAC.

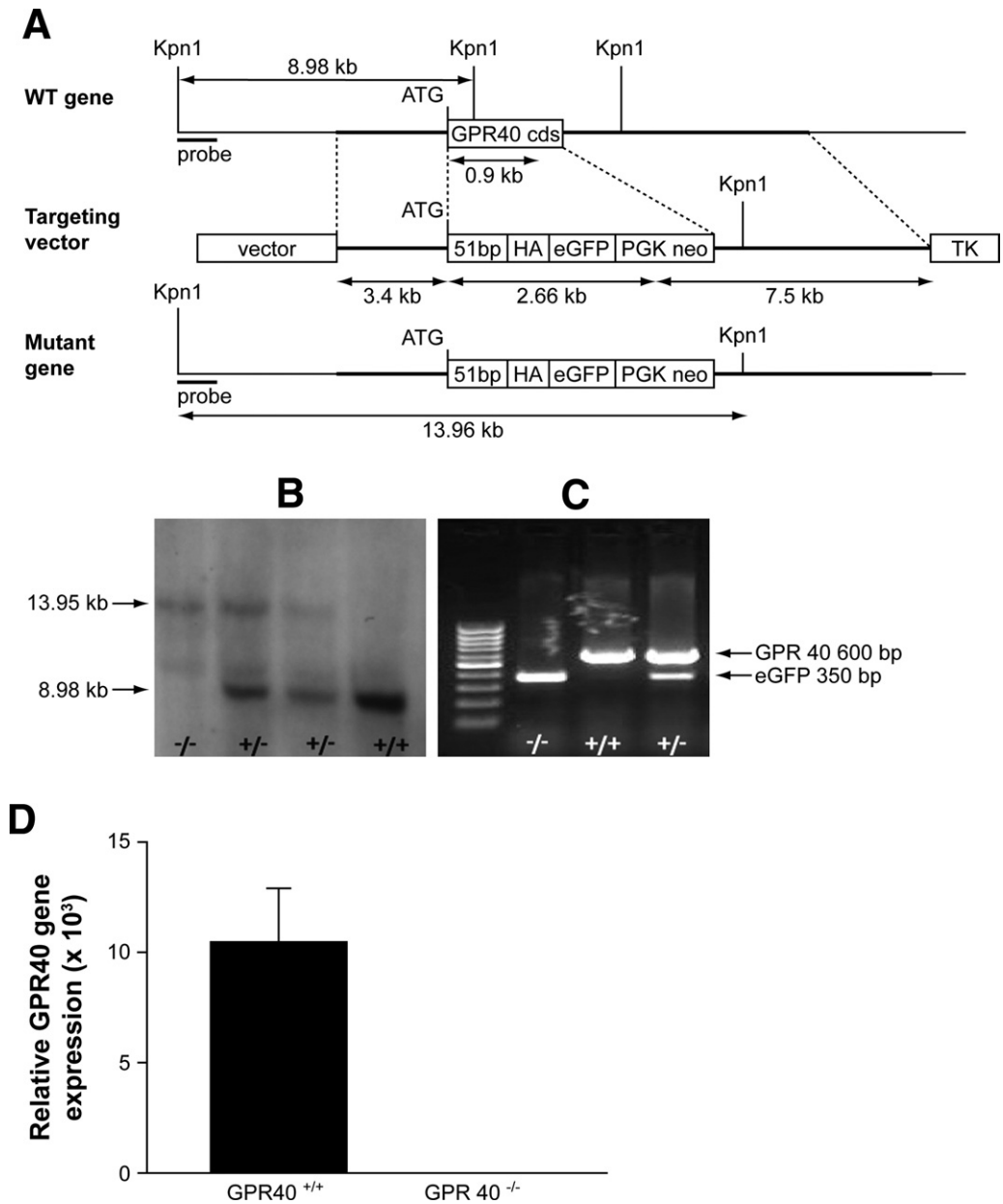
Genotyping of CCK-eGFP GPR40 Mice

Because both CCK-eGFP and GPR40$^{-/-}$ mice express eGFP, new primers were designed to incorporate both eGFP and their flanking gene-specific sequence (either CCK or GPR40) to genotype the presence or absence of their respective genes (see Supplementary Table 1). All PCR reactions were performed using the Invitrogen Platinum Taq DNA Polymerase High Fidelity system (Invitrogen) with 35 cycles of 94°C for 2 minutes, 94°C for 30 seconds, 55°C for 30 seconds, and 68°C for 1 minute, with a final 7-minute extension at 68°C. Amplified PCR products were resolved on a 1.5% ethidium-bromide−stained agarose gel and examined for their expected band sizes.

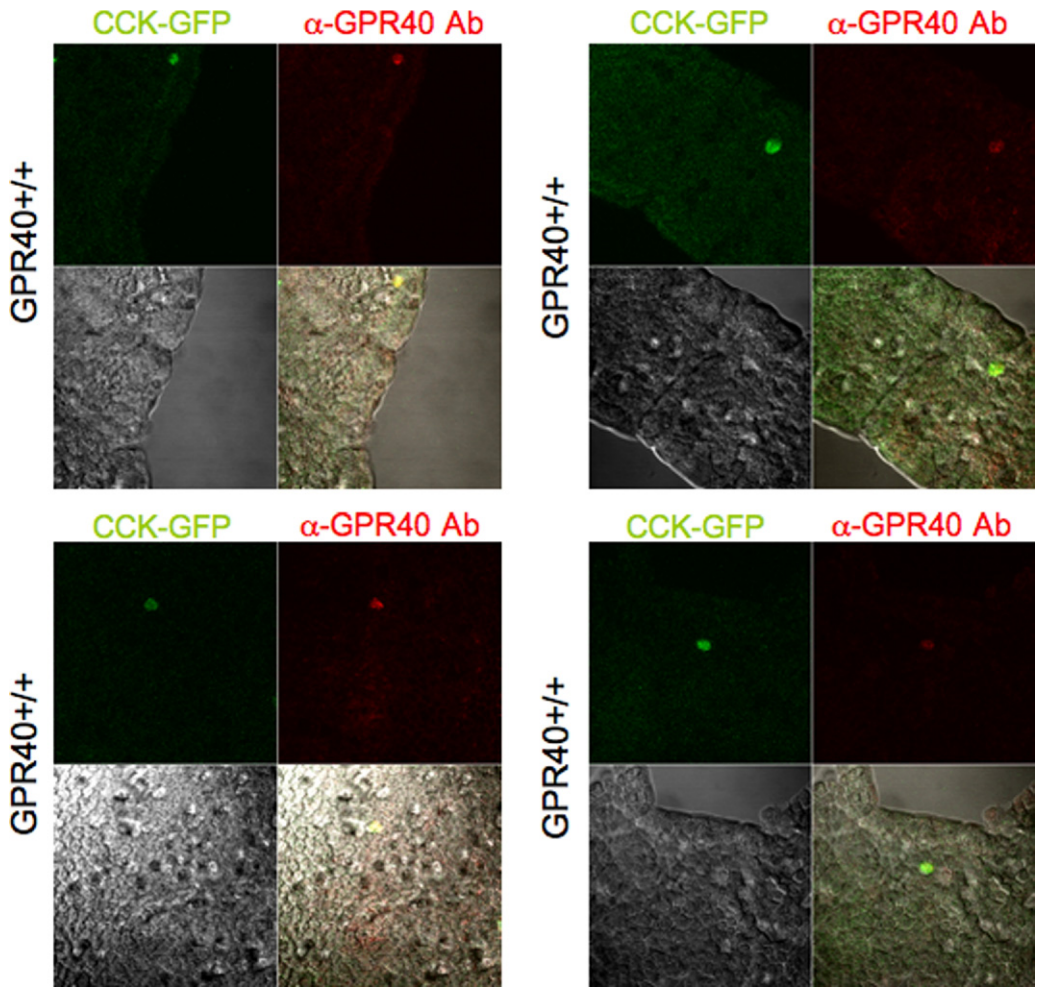
RNA Extraction and RT-PCR

Cells were washed with cold PBS, resuspended in ~100−200 uL Trizol Reagent (Invitrogen), and extracted with 20% phenol:chloroform isoamyl-alcohol (Invitrogen). The aqueous phase was ethanol precipitated and resuspended in Diethyl pyrocarbonate water, and RNA quantity was measured with a Nanodrop ND1000 Spectrophotometer (Nanodrop Technologies, Inc., Wilmington, DE). Total RNA was reversed transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, San Jose, CA) under the following conditions: 10 minutes at 25°C, 60 minutes at 50°C, and 5 minutes at 94°C. The quantitative PCR reaction was performed using the Applied Biosystems Step One System preset cycling conditions with a 20 uL reaction volume made of 2× Master Mix (with 1 uL target probe; see Supplementary Table 2), 20× Assay Mix, 10× amplified complementary DNA sample, and water. Detection of amplification relied on monitoring a reporter dye (6-FAM). Mouse β-actin was used as the housekeeping gene to normalize for RNA sample input variability. Samples without template were used as a negative control for contaminating genomic DNA.

Gene expression was analyzed using the comparative CT method (ABI User Bulletin #2). Briefly, gene expression was normalized to the constitutively expressed gene β-actin to obtain a ΔC_T value. The ΔC_T of the CCK-eGFP cell type was normalized to the ΔC_T value of the non-eGFP cell, which we defined as the calibrator cell. This value (RQ) was calculated as $2^{-\Delta\Delta CT}$. Statistical significance was determined by a Student's *t* test of the ΔC_T values between the eGFP and non-eGFP cells. For graphical purposes, the relative gene expression compared to β-actin (expressed as $2^{-\Delta\ CT} \times 1000$) was also used to demonstrate gene expression for both eGFP and non-eGFP cells.



Supplementary Figure 1. Characterization of G-protein–coupled receptor 40 (GPR40) knockout mouse. Diagram of GPR40 knockout construct used for targeted disruption of the GPR40 gene (*A*). Except for the first 51 nucleotides, the coding sequence of GPR40, which contains a Kpn1 site, was replaced by a DNA fragment that included the 21 nucleotides encoding influenza hemagglutinin antigen (HA), enhanced green fluorescence protein (eGFP), and the neomycin (neo) cassette. Targeted disruption was confirmed with Southern blot analysis (*B*) and polymerase chain reaction (PCR) of genomic DNA (*C*) in GPR40 knockout ($GPR40^{-/-}$), heterozygous ($GPR40^{+/-}$), and wild-type (WT) ($GPR40^{+/+}$) mice. PCR primers were designed to amplify the endogenous GPR40 gene (WT) and the inserted eGFP gene (knockout). GPR40 messenger RNA expression is undetectable in duodenal mucosal scrapings of $GPR40^{-/-}$ mice compared to WT ($GPR40^{+/+}$; D).



Supplementary Figure 2. Colocalization of G-protein–coupled receptor 40 (GPR40) (*red*) onto 4 cholecystokinin (CCK)–enhanced green fluorescent protein (eGFP) cells (*green*) from freshly isolated duodenal villi of GPR40$^{+/+}$ CCK-eGFP mice. Images were acquired at 20× objective. A set of images in each panel includes CCK-eGFP only (*top left; green*), anti-GPR40 (*top right; red*), transmitted light (bottom left), and all merged (*bottom right*).

Supplementary Table 1. Polymerase Chain Reaction Primers and Expected Band Sizes for Genotyping CCK-eGFP BAC Transgenic Mice, GPR40$^{+/+}$, and GPR40$^{-/-}$ Mice, and Cross-Bred Mice

Target sequence	Primers	Primer sequence	Band size (*bp*)
eGFP	eGFP-F1	5′ CCA CCA GTT CAG CGT GTC C 3′	350
	eGFP-R1	5′ GTT GTA CTC CAG CTT GTG C 3′	
GPR40 WT	GPR40 WT REV	5′ CTG TTC CCA AGT AGC CAG TGA CCA G 3′	565
	GPR40 WT FOR	5′ GGA GGC TTC CTA GCT GCT CTC AGC 3′	
GPR40 KO	GPR40xFOR2	5′ CAC AGC TCT CCT TCG CTC TCT A 3′	289
	Gensat GFP REV	5′ TAG CGG CTG AAG CAC TGC A 3′	
CCK-eGFP	M249 (CCK) FOR	5′ TAG GAA CTT CGC TTG GCT ACG G 3′	374
	Gensat GFP REV	5′ TAG CGG CTG AAG CAC TGC A 3′	

CCK, cholecystokinin; eGFP, enhanced green fluorescent protein; GPR40, G-protein–coupled receptor 40.

Supplementary Table 2. Taqman Gene Expression Assay Probes, All Inventoried and Optimized by Applied Biosystems (San Jose, CA)

Gene symbol	Gene name	Catalog no.
CCK	Cholecystokinin	Mm00446170_m1
GPR40	Free fatty acid receptor 1 (FFAR1)	Mm00809442_m1
GPR120	Long chain fatty acid receptor	Mm00725193_m1
Akp3	Alkaline phosphatase (enterocyte marker)	Mm00475847_g1
Clca3	Chloride channel 3, gob5 (goblet cell marker)	Mm00489959_m1

All probes span an intron to ensure RNA-specific amplification.