SUPPLEMENTAL METHODS

Generation of ILDR1 antibody

The initial attempt to generate an ILDR1 peptide antibody (388-407 aa) in rabbits was unsuccessful. Therefore, mouse ILDR1α (accession # BC057644) amino acids 84-159 and 357-380, were amplified by PCR and cloned in the *Nhe*I and *Xho*I sites of pET28a (Life Technologies). The clones were sequenced and used for expression of a His-tag fusion protein in BL21(DE3) strain of *E. coli*. Bacterial cultures were induced with 1 mM IPTG for 4h at 37°C and bacterial cell pellets were extracted with 20 mM sodium phosphate, pH7.4, 8 M urea, 0.5 M NaCl, 0.2% Tween 20, and 20 mM imidazole. The extract was passed over a Ni^{2+} Sepharose column (GE Healthcare Life Sciences), and the bound His-tag ILDR1 proteins were eluted with 0.2 M imidazole in the extraction buffer. The protein was dialyzed, in 10% glycerol, 10 mM sodium phosphate buffer, pH 7.4, 150 mM NaCl and sent to Open Biosystems for immunization in rabbits. The serum was affinity purified over a column of purified bacterial protein conjugated with CNBr-activated Sepharose (GE Healthcare Life Sciences). Antibody for ILDR1 was produced in rabbits immunized with peptide 357-380, as judged by western blots against the bacterially expressed protein.

Western Blotting

For western blots, CHO and ILDR1-transfected stable cells were washed with PBS, and resuspended in PBS containing protease inhibitors (Complete Protease Inhibitor Cocktail tablets, Roche Applied Science,). After addition of gel loading buffer, the gel samples were sonicated, heated to 95°C for 5 min and loaded on a 4-12% Bis-Tris gel (Novex®, Life Technologies). Following transfer of proteins to PVDF membrane (Perkin Elmer), blots were incubated either with a monoclonal antibody to HA tag, (Clone 16B12, Covance) or with the rabbit ILDR1 polyclonal (amino acids 357-380). After reaction with goat anti-mouse or anti rabbit HRP, bands were visualized by Enhanced Chemiluminescence reagent (Perkin Elmer).

Immunochemistry of transfected cells

Cells on the chamber slides were fixed with 10% formalin for 10 min at 4°C. The slides were washed, blocked in 10% donkey serum and incubated with 1:1000 dilution of HA.11 Clone 16B12 monoclonal

antibody (Covance). ILDR1 rabbit polyclonal was used at a 1:100 dilution for double immunofluorescence imaging. Donkey anti-mouse $F(ab')_2$ conjugated with DyLight 488 and donkey anti-rabbit $F(ab')_2$ conjugated with Cy3 (Jackson ImmunoResearch Laboratories Inc.) were used as secondary antibodies for detection of ILDR1 expression. Images were acquired using a Zeiss 510 inverted confocal microscope as described under Methods.

Detection of HDL in intestinal lumen

Proximal duodenum (1 cm) from fasted wild type mice was harvested, gently flushed with either 300 µl sterile PBS or human HDL (100 µg protein/ml) and the flow through was collected (Supplemental Figure 5, mice 1 and 2). Alternatively, mice were deeply anesthetized and 300 µl of sterile PBS was slowly passaged from the proximal to distal portion of a 1 cm ligated segment of the duodenum (Supplemental Figure 5, mouse 3) and collected with a syringe. The washes were centrifuged (15,000 rpm, 30 sec) and 180 µl of the supernatant was added to 50 µl SDS-PAGE sample buffer (Life Technologies Corporation). Gel samples of human HDL (100 µg protein/ml) and five serial dilutions were added to SDS-PAGE sample buffer in order to quantitate HDL present in the intestinal lumen. Mouse HDL (61) was used as control to evaluate the specificity of the antibody. Protein samples were electrophoresed on 4-12% Bis-Tris polyacrylamide gel as described above, transferred to PVDF membrane and incubated with goat anti-mouse Apo A-I primary antibody (Rockland Inc., catalog # 600-101-196) followed by rabbit anti-goat HRP secondary antibody (Jackson ImmunoResearch). Blots were developed with Enhanced Chemiluminescence reagent as described above.

Measurement of blood triglyceride levels

Wild type or *Ildr1*$^{-/-}$ mice (male, age 8 weeks) were fed a normal (5001 Laboratory Rodent Diet, Lab Diet) or 60% high fat diet (Research Diets Inc.) for three weeks. Blood was collected in EDTA, and triglyceride levels were measured in plasma of non-fasted mice using a colorimetric kit from Cayman Chemical Company.

Weight gain on high fat diet

Wild type or *Ildr1*$^{-/-}$ mice (male, age 4 weeks) were fed a 10% control or 45% high fat diet (Research Diets Inc.,) for 26 weeks with ad libitum food and water. Body weights were recorded weekly.

SUPPLEMENTAL FIGURE 1

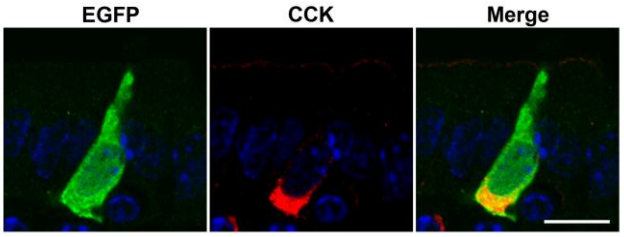

Figure 1 *Colocalization of CCK and endogenous EGFP in intestinal cells of CCK-EGFP mice.* CCK expressing cells of CCK-EGFP mice have endogenous EGFP fluorescence. This fluorescence was utilized for purification of CCK cells by FACS. Scale bar = 10 µm.

SUPPLEMENTAL FIGURE 2

Figure 2A

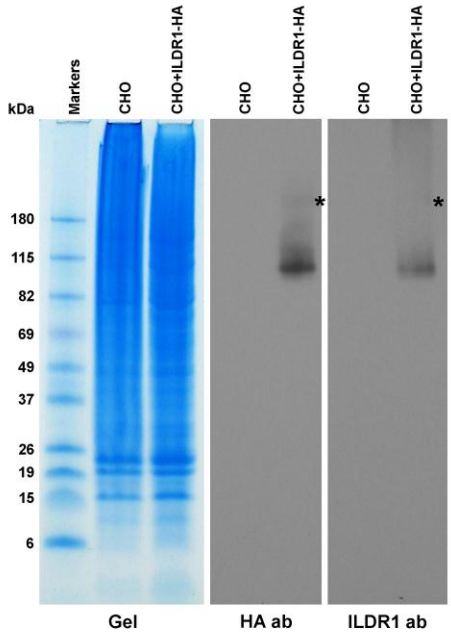

Figure 2B

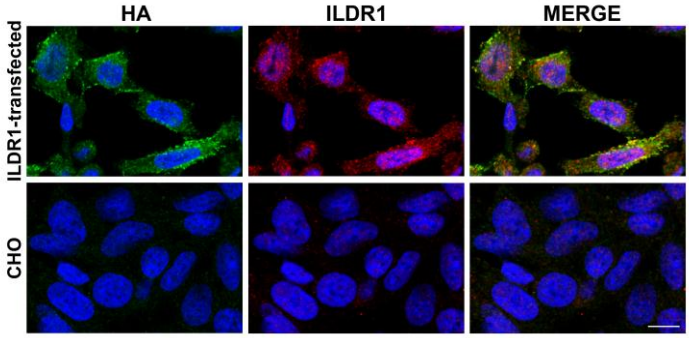

Figure 2 *Expression of HA-tagged ILDR1.* HA-tagged ILDR1 was detected in transfected and untransfected CHO cells by immunoblotting (A) and immunostaining (B) with antibodies directed against the HA tag and ILDR1 C-terminus (amino acids 357-380). (A) A band corresponding to ~100 kDa was detected by HA and ILDR1 antibodies in transfected, but not untransfected cells. A faint band corresponding to a higher molecular of ~198 kDa (marked with asterisk) was also detected, suggesting that the ILDR1 protein can dimerize. (B) Immunostaining of ILDR1-transfected and CHO cells with antibody against the HA tag (green) or ILDR1 (red), showed significant colocalization (merge). Scale bar = 10 µm.

SUPPLEMENTAL FIGURE 3

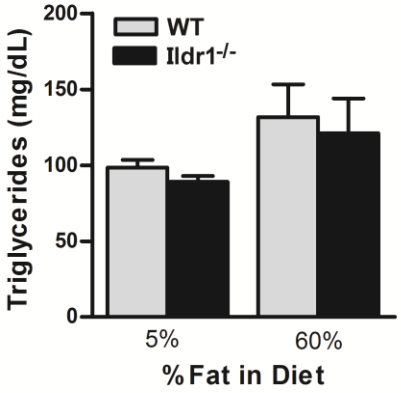

Figure 3 *Triglyceride levels in wild type and Ildr1$^{-/-}$ mice.* Triglyceride levels were measured in duplicate from non-fasted mice that were fed a 5% fat control diet or a 60% high fat diet for three weeks. n=4 for all groups. Triglyceride levels were not significantly different between wild type and *Ildr1$^{-/-}$* mice.

SUPPLEMENTAL FIGURE 4

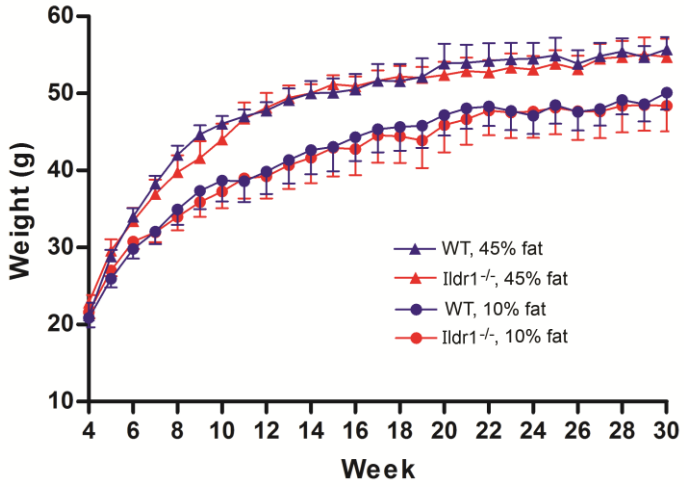

Figure 4 *Weight gain in wild type and Ildr1$^{-/-}$ mice.* Male mice at age 4 weeks were fed a 10% control or 45% high fat diet and body weights were recorded weekly. Body weights increased with high fat feeding in both wild type and *Ildr1*$^{-/-}$ mice compared to control diet ($p<0.05$) but there were no differences between genotypes (n=4 or 5).

SUPPLEMENTAL FIGURE 5

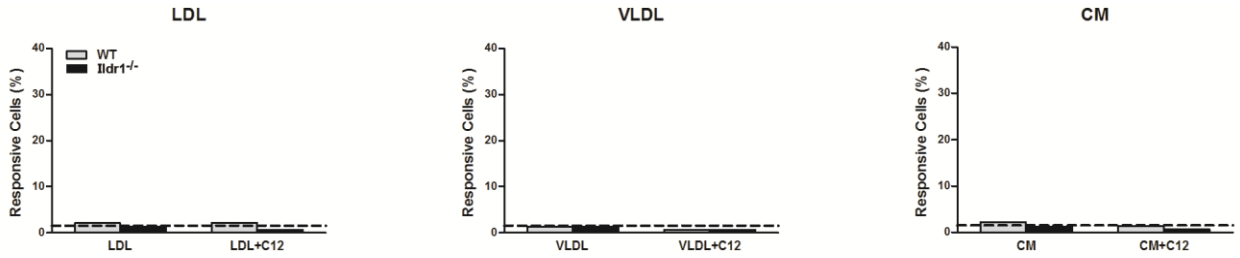

Figure 5 *Effects of fatty acids and lipoproteins on intracellular Ca^{2+} fluorescence in CCK cells.* CCK-EGFP cells were isolated from the intestine of wild type CCK-EGFP and *Ildr1*$^{-/-}$ CCK-EGFP mice and enriched by FACS. Cells were loaded with the calcium sensitive dye (X-rhod-1) and incubated first with either lipoproteins (100 µg protein/ml) or C12 (100 µM), followed by lipoprotein+C12 and finally with KCl (50 mM). The percentage of wild type CCK-EGFP and *Ildr1*$^{-/-}$ CCK-EGFP cells that elicited a >10% increase in Ca^{2+} fluorescence in response to lipoprotein or lipoprotein+C12 is shown. Percent of cells responding to C12 alone are represented by the dashed line. The y-axis scale is identical to Figure 6C.

SUPPLEMENTAL FIGURE 6

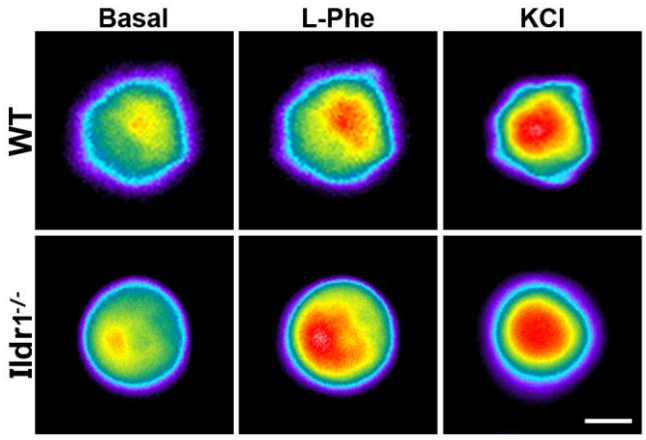

Figure 6 *Effect of phenylalanine on Ca^{2+} fluorescence in wild type and Ildr1$^{-/-}$ CCK-EGFP cells.* CCK-EGFP cells were isolated from the intestine of wild type CCK-EGFP and *Ildr1*$^{-/-}$ CCK-EGFP mice and enriched by FACS. Cells were loaded with the calcium sensitive dye (X-rhod-1) and incubated with first with 10 mM L-phenylalanine and then exposed to KCl (50 mM). (A) Response of representative wild type CCK-EGFP or *Ildr1*$^{-/-}$ CCK-EGFP cells to L-phenylalanine and KCl. Change of fluorescence from green to yellow to red indicates increase in $[Ca^{2+}]_i$. Results are representative of three experiments. Scare bar = 5 μm.

SUPPLEMENTAL FIGURE 7

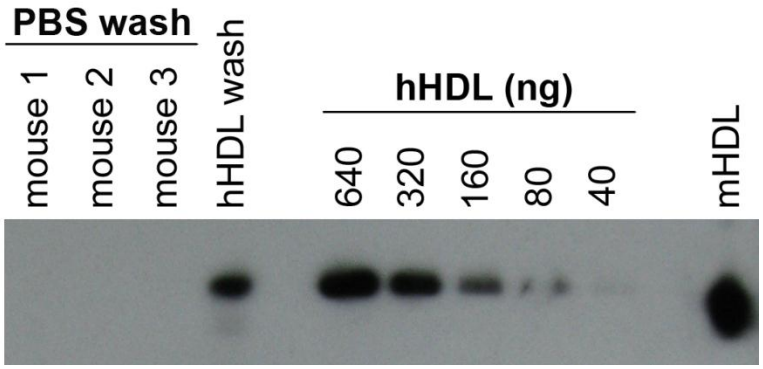

Figure 7 *Detection of HDL in lumen of mouse intestine.* SDS-PAGE analysis of protein samples made from PBS washes of mouse duodenum (n = 3) did not reveal detectable Apo A-I which is a major apolipoprotein present in HDL. Human HDL (hHDL) wash sample shows recovery of HDL after passing it through the intestinal segment. At least 40 ng hHDL (total protein) could be detected. The goat anti-mouse Apo A-I antibody recognized mouse HDL.