SUPPLEMENTARY DATA

In vitro FFA2 Assays

CHO-K1 cells stably expressing human or mouse FFA2 were seeded in cell culture medium in black, clear-bottom 384-well plates and grown overnight at 37 °C, 5% CO_2. For the Ca^{2+} flux assay, on the day of the assay, cell culture media was removed and cells were loaded with Calcium 5 Dye for 1 h at 37 °C, 5% CO_2. Cpd 1 was added to wells in the absence or presence of a final concentration of sodium acetate that produces approximately 20% maximal response (EC_{20}), to detect agonist or positive allosteric modulation (PAM) activity respectively. Intracellular Ca^{2+} levels were measured using a Ca^{2+} sensitive fluorescent dye and a Fluorescent Imaging Plate Reader (FLIPR^{TM}).

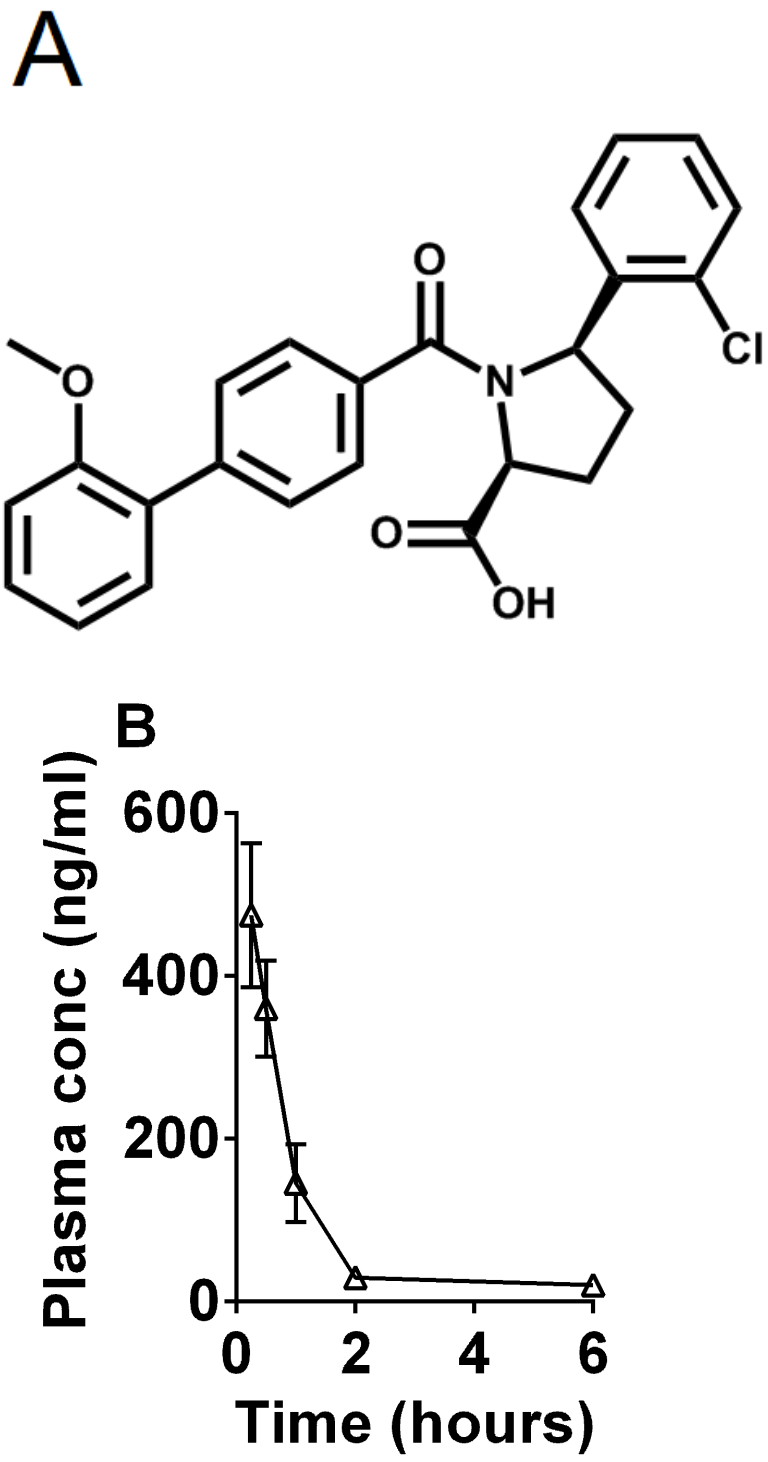
For the cAMP assay on the day of the assay, cell culture media was removed and Cpd 1 and 1 µM forskolin (final concentration) were incubated for 1 h at 37 °C, 5% CO_2. Intracellular cAMP levels were detected using the Cisbio HTRF dynamic 2 cAMP detection reagent. D2-labeled cAMP tracer and Eu-cryptate–conjugated anti-cAMP antibody in lysis buffer were added and incubated for 1 h at room temperature. The ratiometric data (665 nm read/615 nm read) x10,000 were then converted to cAMP (nM) based on a standard curve for cAMP (replacing the cell addition step) run at the same time and under identical conditions to the assay. Forskolin-stimulated intracellular FFA2 agonist activity and/or PAM were determined by measuring inhibition of forskolin-stimulated intracellular cAMP levels.

Pharmacokinetics of Cpd 1

Cpd 1 was formulated in 1:99 Tween 80:Methylcellulose (0.5%, w/v in water) at a dose volume of 5 ml/kg *i.p.* and administered to C57Bl6 male. At pre-determined times thereafter animals were asphyxiated by a rising concentration of CO_2 and a terminal blood sample taken by cardiac puncture. Analysis to determine the concentration of Cpd 1 in plasma was performed by LC-MS.

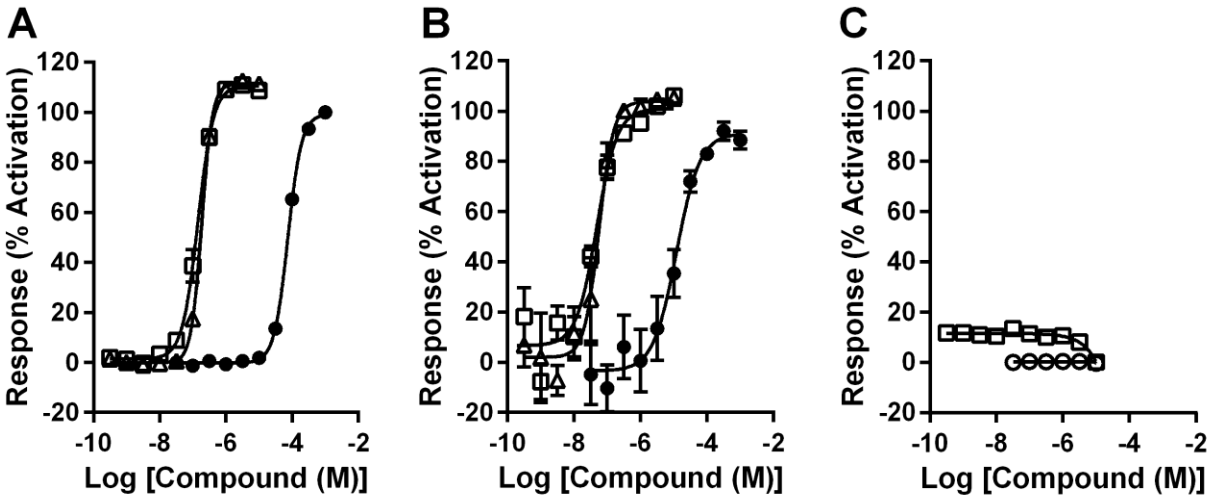
SUPPLEMENTARY DATA

Supplementary Figure 1. The chemical structure of Cpd 1 (A). In (B) the pharmacokinetic profile of Cpd 1 following a 10 mg/kg *i.p*. dose to C57Bl6 mice (aged 6-8 weeks) is shown. The concentrations of Cpd 1 were determined by HPLC-MS from terminal plasma samples and reached 474.7 ng/ml at 15 min after dosing, which was the first time-point tested. Cpd 1 was rapidly cleared from the plasma by first order kinetics, with the majority of the compound removed by 2 h and levels remained low up to 6 h. Values in B are the mean ±1 SEM from 3 observations.



SUPPLEMENTARY DATA

Supplementary Figure 2. Cpd 1 is a potent agonist at human FFA2 *in vitro* in recombinant functional assays with selectivity over human FFA1 and FFA3 receptors. Concentration-response relationships for acetate (black circles), Cpd 1 (open triangles), or Cpd 1 in the presence of an EC_{20} acetate concentration (open squares) in (A) Ca^{2+} flux assay or, (B) cAMP assay in CHO cells stably expressing FFA2. Data is representative from 4 independent experiments, expressed as the percentage of acetate maximum activity at 1 mM. EC_{50} values were determined from concentration-response curves generated using the average of two wells for each data point. In (C) Cpd 1 is tested in the presence of an EC_{20} concentration of proprionate in CHO cells stably expressing FFA3 (open squares) or in the absence of SCFA in CHO cells stably expressing FFA1 (open circles). Data is expressed as the percentage of maximal activity compared to a reference FFA1 agonist or 1 mM proprionate for FFA3 and generated using the average of two wells for each data point.



SUPPLEMENTARY DATA

Supplementary Figure 3. Biphasic changes in I_{sc} (primary, 1˚; and secondary, 2˚) in response to the apical addition of acetate or propionate (5 mM) to WT colon mucosa. The 2˚ response to apical acetate was significantly reduced in FFA2-/- colon mucosa compared to WT responses (** $P < 0.01$, Student's *t*-test). Each bar is the mean ± 1SEM from *n* numbers shown in parenthesis.