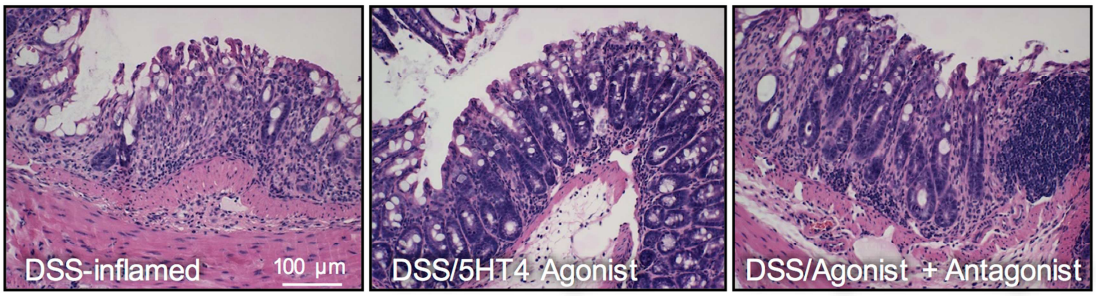
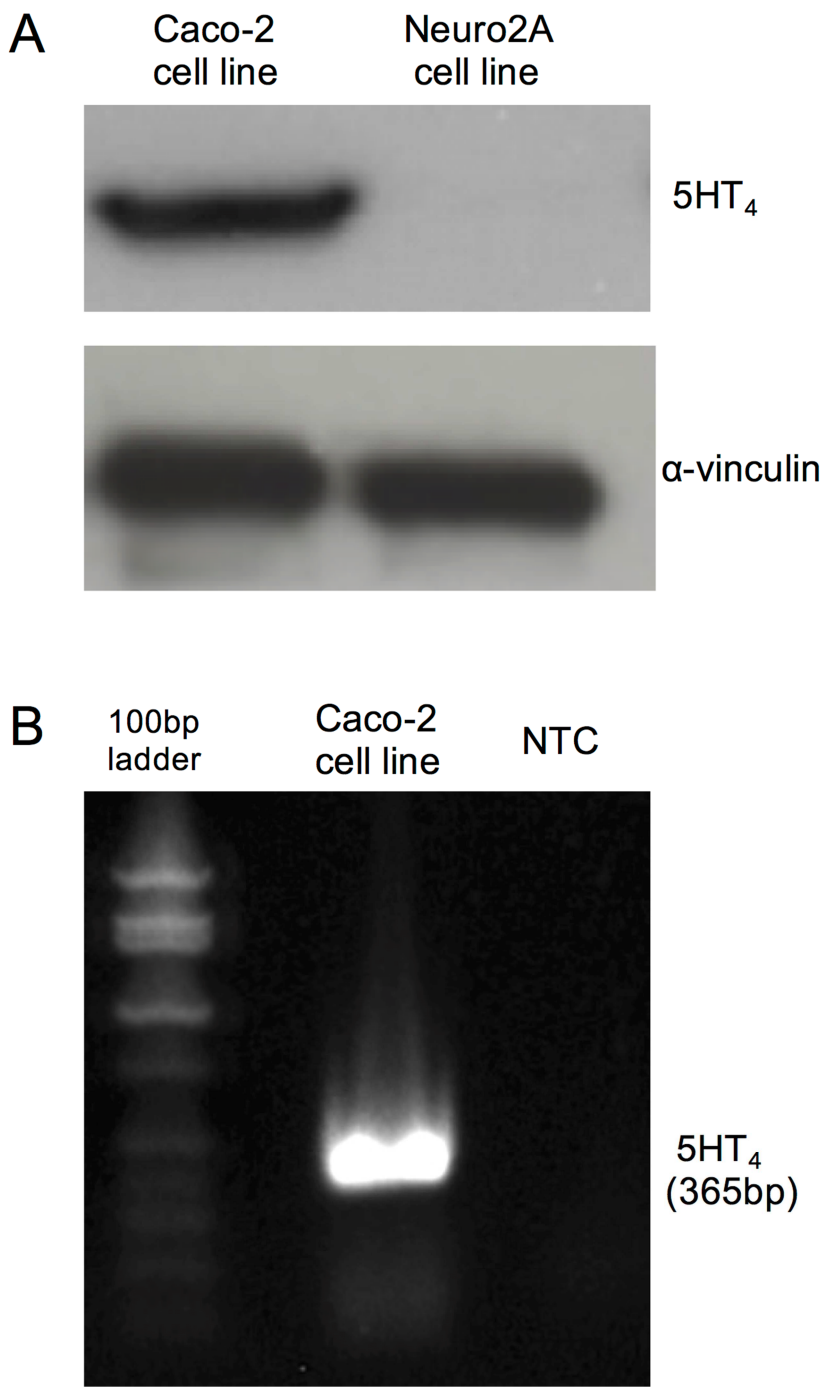
Supplemental figure 1. Daily intraluminal treatment with the 5-HT_4 agonist, tegaserod (1mg/Kg) reduced the extent of DSS colitis, and this protective effect was inhibited by antagonist treatment. Micrographs illustrating typical histological features in colons from the three treatment groups.

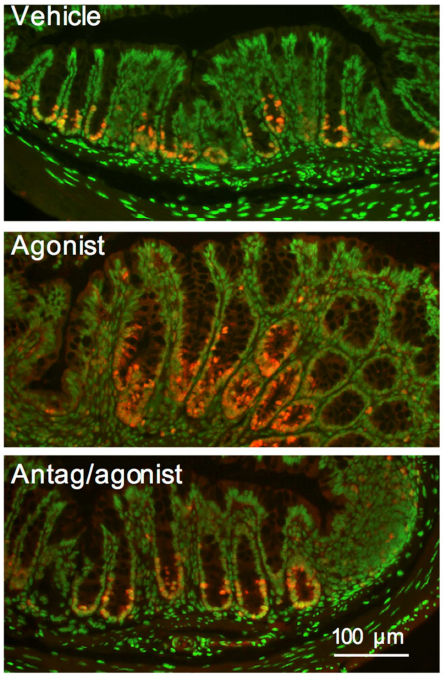
Supplemental Figure 2. Evidence for expression of the 5-HT_4 receptor by Caco-2 cells. A. Western blot analysis of $5HT_4$ expression in human CaCo-2 cell line (lane 1) using a human specific $5HT_4$ antibody (1:200, Abcam, Cambridge, UK); the murine Neuro2A neuroblastoma cell line (lane 2), which does not express 5-HT_4, was used as negative control. An internal control for loading was performed using an anti-vinculin antibody (1:50000, Sigma-Aldrich, Saint Louis, MO, USA). B. A *$5HT_4$* transcript is detectable by RT-PCR in RNA extracted from Caco-2 cells supporting the presence of this receptor in this cell line. A low diffuse primer-dimer band was present in both Caco-2 cells and no template control (NTC) lanes (l00bp DNA ladder). Total RNA was isolated from Caco-2 using the RNAeasy Kit (Qiagen) according to the manufacturer's specifications and cDNA was generated from 2 ug of RNA using M-MLV Reverse Transcriptase (Promega) . 5HT4R expression was determined using SYBR GreenER qPCR SuperMix (Invitrogen) with the following primers: forward 5'-GGCCTTCTACATCCCATTTCTCCT-3'; reverse: 5'- CTTCGGTAGCGCTCATCATCACA-3'. The predicted product size is 365 bp.

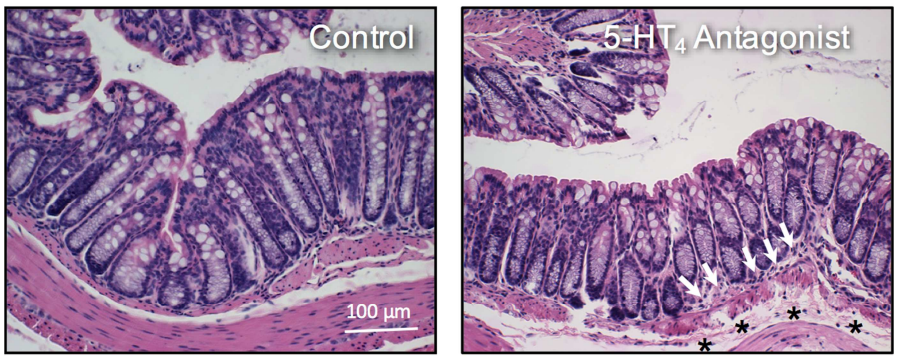
Supplemental Figure 3. Intraluminal treatment with tegaserod increased epithelial proliferation. Representative photomicrographs demonstrating Ki67 immunoreactivity (red) and DAPI counterstaining (green).

Supplemental figure 4. Intraluminal administration of the 5-HT4 antagonist GR113808 (1 mg/Kg) induced colitis in mice. Micrographs illustrating typical histological features in colons from mice treated with a vehicle or with the antagonist. Indications of inflammation in the antagonist-treated micrograph include signs of edema (*), cellular infiltration (arrows), and increased spacing between the base of the crypts and the muscularis mucosa.


DSS-inflamed
100 µm
DSS/5HT4 Agonist
DSS/Agonist + Antagonist


A
Caco-2
cell line
Neuro2A
cell line
5HT4
α-vinculin
B
100bp
ladder
Caco-2
cell line
NTC
5HT4
(365bp)


Vehicle
Agonist
Antag/agonist
100 µm


Control
100 µm
5-HT4 Antagonist