Role and species-specific expression of colon T cell homing receptor GPR15 in colitis

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Lymphocyte recruitment maintains intestinal immune homeostasis but also contributes to inflammation. The orphan chemoattractant receptor GPR15 mediates regulatory T cell homing and immunosuppression in the mouse colon. We show that GPR15 is also expressed by mouse T H17 and T H1 effector cells and is required for colitis in a model that depends on the trafficking of these cells to the colon. In humans GPR15 is expressed by effector cells, including pathogenic T H2 cells in ulcerative colitis, but is expressed poorly or not at all by colon regulatory T (T reg) cells. The T H2 transcriptional activator GATA-3 and the T reg-associated transcriptional repressor FOXP3 robustly bind human, but not mouse, GPR15 enhancer sequences, correlating with receptor expression. Our results highlight species differences in GPR15 regulation and suggest it as a potential therapeutic target for colitis.

Recruitment of lymphocytes from the circulation is a tissue- and cell-specific process that is mediated by adhesion and chemoattractant receptors1,2. The interaction of lymphocyte adhesion receptors with their ligands on vascular endothelium allows endothelial capture of blood-borne lymphocytes and mediates lymphocyte rolling, but arrest and firm adhesion of rolling cells, as well as subsequent diapedesis, require the engagement of lymphocyte receptors of the chemotactic GPCR family3. Signaling via these chemoattractant receptors triggers rapid integrin-dependent lymphocyte adhesion on the endothelium and activates and drives programs of lymphocyte motility. Chemoattractant receptors thus play critical roles in the recruitment of lymphocyte subsets from the blood, and they direct the trafficking of lymphocyte subsets in both homeostatic and inflammatory states1,4. As examples, CCR7 helps target naïve lymphocytes and subsets of memory and effector cells to lymph nodes and tertiary lymphoid tissues in chronic inflammation; CCR10, CCR8 and CCR4 participate selectively, though not exclusively, in skin homing by memory and effector T cells; and CCR9 serves as a specific T cell and plasmablast homing receptor for the small intestines5. Interestingly, CCR10 also targets IgA plasmablast homing to mucosal tissues, including the bronchial tree and the colon, but it is not expressed by gut-homing T cells: CCR10 expression on T cells is largely mutually exclusive with that of the integrin intestinal homing receptor α4β7 (ref.5), which is required for efficient lymphocyte homing to the intestines through its recognition of the mucosal vascular addressin MadCAM1 (refs. 3,6). Although inflammatory chemokinases and their receptors may participate in homing7,8, whether colon effector and memory T cells have specific chemoattractant trafficking receptors analogous to skin and small intestinal T cell–selective chemokine receptors has remained unclear.

GPR15 is an ‘orphan’ GPCR and an HIV co-receptor that is structurally related to known lymphocyte trafficking receptors9,10. A recent study has implicated this receptor in colon homing of T reg cells in the mouse11, but its role in effector T cell trafficking and function is not known. Here we examine the expression and function of GPR15 on effector T cells in mouse and man. Our results show that GPR15 is important for effector as well as regulatory T cell localization to the mucosal colon and implicate GPR15-dependent effector cell recruitment in mouse colitis. We also describe substantial differences in GPR15 expression in the human, with expression by T H2 cells but not T reg cells in the normal and inflamed human colon. We relate the expression of GPR15 by human T H2 cells and by mouse, but not human, T reg cells to interspecies differences in binding of transcriptional regulators to Gpr15 enhancer sequences.

RESULTS

Activated colon CD4+ T cells express GPR15

Using Gpr15-GFP knock-in mice11, we confirmed that GFP+ CD4+ cells are most frequent among T cells in colon lamina propria (LP) as compared to small intestine (SI) and peripheral lymphoid...
tissues. They were infrequent among the intraepithelial lymphocytes of the colon or SI, and absent in naive CD4+ T cells of all tissues (Supplementary Fig. 1a; ref. 11). GFP+ CD4+ T cells in the colon display an activated phenotype, characterized by high expression of activation antigens CD44, CD69 and CD25 and low expression of homeostatic conditions most, although not all, colon LP GFP+ CD4+ T cells express markers of Treg differentiation, including Foxp3, CD25, GITR and Helios, and lack effector cytokine expression (Supplementary Fig. 1c).

**Gpr15 mediates CD4+ TEM accumulation in the colon**

To assess the importance of GPR15 to effector-memory T cells (TEM) in different tissues, we reconstituted irradiated mice with a mixture of allotype-marked Gpr15<sup>+/+<sup> (Gpr15-knockout; KO) and Gpr15<sup>+/+<sup> (Gpr15-heterozygous; het) bone marrow cells, allowing Gpr15-KO and Gpr15-het lymphocytes to compete for niches within a common host environment. Gpr15-KO CD4+ T cells repopulated the colon LP with significantly lower efficiency than their Gpr15-het counterparts (Supplementary Fig. 2a,b and Fig. 1a). In contrast, Gpr15 deficiency had no effect on the reconstitution of CD4+ T cells in lymphoid tissues or SI LP; nor did it alter colon naive CD4+ T cell frequency (Fig. 1a). Notably, accumulation of TEM (CD45RB<sup>hi</sup>, CD44<sup>hi</sup>, Foxp3<sup>-</sup>) cells was reduced to the same extent as that of Treg cells (CD25<sup>high</sup>, Foxp3<sup>-</sup>; Fig. 1a). This accumulation is likely to be mediated by GPR15-dependent recruitment because GPR15 effects colon homing of in vitro–generated TEM (Supplementary Fig. 3), Treg cells and transduced T cells11; however, effects of GPR15 on retention, survival or proliferation cannot be excluded. These results demonstrate an important role for GPR15 in long-term TEM as well as in Treg reconstitution and homeostasis in the mouse colon.

**Gpr15 required for CD45RB<sup>hi</sup> T cell transfer colitis**

Having shown that GPR15 contributes to colon effector T cell recruitment and accumulation, we next assessed its importance in colon inflammation. We used a well-established model of colitis, the CD45RB<sup>hi</sup> CD4+ T cell transfer model15, in which colitis is dependent on effector T cell expression of intestinal trafficking receptors16,17. In the absence of Treg cells, transferred T cells develop into pathogenic effector T cells that home to the colon and induce disease15. Rag2<sup>−/−</sup> mice that received naive Gpr15-KO T cells were protected from developing colitis (Fig. 2), consistent with a critical function...
for GPR15 on pathogenic effector T cell colon trafficking. Inhibition of colitis by Treg cells in this model does not require colon homing18, and accordingly, GPR15-deficient Treg cells and WT Treg cells prevented or delayed colitis in a comparable manner (Fig. 2). In the acute DSS-induced model of colitis, in which innate immunity plays a predominant role and colitis is not dependent on T cell colon homing19,20, as well as in the acute TNBS model, Gpr15-KO and WT mice were similarly susceptible to intestinal inflammation (Supplementary Fig. 4). The inefficiency of Gpr15-deficient cells in inducing colitis in the CD45RBhi transfer model thus reveals a role of GPR15 in the colon trafficking of pathogenic proinflammatory T cells.

Colitis in the naive T cell transfer model depends on the generation of pathogenic IFN-γ− and IL17-producing T H1 and T H17 effector CD4+ T cells21−23. To determine whether a deficiency in generation or localization of such effector cells might underlie or contribute to the reduced ability of KO T cells to induce colitis, we transferred allotype-marked naive Gpr15-KO and Gpr15-het CD4+ T cells together into Rag2−/− mice and assessed the presence of effector T cells in recipient tissues 2 weeks later, before the clinical onset of colitis (Supplementary Fig. 5a). Compared to GFP+ Gpr15-KO T cells, GFP+ Gpr15-het (GPR15-expressing) T cells preferentially accumulated in the colons of Rag2−/− recipients, consistent with a prominent role for GPR15 in effector T cell localization (Fig. 3a). On the other hand, GPR15 deficiency had no significant effect on the frequency of T H1 and T H17 effector T cells among the donor populations (Fig. 3b and Supplementary Fig. 5b−d); on the fraction of proliferating T cells, as indicated by the extent of BrdU incorporation (Fig. 3c); or on that of apoptotic CD4+ T cells, as indicated by annexin V staining (data not shown). In related Rag2−/− transfer studies (Supplementary Fig. 5e), we found that GFP-expressing subsets of colon-localized Gpr15-KO- and Gpr15-het-derived cells also displayed similar expression of cytokines, Foxp3 and Ki67 (Supplementary Fig. 5f). These data suggest that the failure of GPR15-deficient T cells to induce colitis is not due to alterations in T cell activation or production of proinflammatory cytokines.

**GPR15 expression on human CD4+ T cells**

To determine the relevance of these mouse studies to humans, we assessed GPR15 expression by colon CD4+ T cells from individuals with ulcerative colitis (UC), a ‘T H12-like’ disease in which inflammation is limited to the colon24 or Crohn’s disease (typically a T H1 and T H17 disease25) and in colons from individuals without UC that included healthy or normal margins of cecal or colorectal adenocarcinomas. Unexpectedly, GPR15 was highly enriched among UC colon T H2 cells (IL-5+ or IL-13+; Fig. 4a). Few IFN-γ− or IL-17+ effector T cells expressed the receptor (Fig. 4a) and, in contrast to the

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**Figure 2** GPR15-deficient naive T cells fail to induce colitis in the CD45RBhi CD4+ T cell transfer model. (a) Weight loss of Rag2−/− mice that received either WT naive CD4+ T cells, Gpr15-KO naive CD4+ T cells, WT naive plus WT Treg cells, WT naive plus KO Treg cells, or KO naive plus WT Treg cells. n = 5 for each condition; mean ± s.e.m., *P < 0.05, **P < 0.01 between WT naive (disease control) and Gpr15 KO naive (two-way ANOVA, Bonferroni post-test). Representative of three experiments. (b) Clinical score for histological inflammation in colon. *P < 0.05, **P < 0.01, ***P < 0.001 (one-way ANOVA, Tukey’s post-test). (c) Hematoxylin and eosin staining of distal colon section: WT naive (top left), Gpr15-KO naive (top right), WT naive + WT Treg (middle left), WT naive + Gpr15-KO Treg (middle right) and Gpr15-KO naive and WT Treg (bottom). Scale bar, 10 μm.

**Figure 3** GPR15 deficiency inhibits effector T cell accumulation, but not T H1 or T H17 differentiation, in the colon. Naive CD45RBhi CD4+ T cells sorted from Gpr15-KO (CD45.2) or Gpr15-het (CD45.1, CD45.2) mice were co-injected into Rag2−/− recipients in equal proportion, as in Supplementary Figure 5a. (a) Ratios Gpr15-het to Gpr15-KO GFP+ or GFP− CD4+ T cells in recipient tissues after 2 weeks, normalized to those in the spleen. n = 5 for each condition; mean ± s.e.m., *P < 0.001 (two-way ANOVA, Bonferroni post-test). (b) Left, similar percentage of Gpr15-het and GFP-KO-derived IFN-γ+ and IL-17+ CD4+ T cells in recipient colon after 2 weeks. Right, ratio of Gpr15-het-derived to GFP-KO-derived IFN-γ+ or IL-17+ CD4+ T cells, normalized to that of the spleen. Representative of two experiments. (c) BrdU incorporation 2 weeks after cell transfer as in a; baseline (gray shade). n = 3 for each condition; mean ± s.e.m.
T cells express GPR15 in the colon. (a) Top, expression of GPR15 on cytokine-producing CD45RO+ CD4+ T cells from colons of individuals with ulcerative colitis (UC colons) or non-UC colons. Bottom, in UC colons, GPR15 is expressed on IL-17+, IL-5+, and IL-13+ T cells. n = 5 UC specimens, n = 11 non-UC or normal samples, and n = 2 colons from individuals with Crohn’s disease (CD), which showed cytokine expression among GPR15+ cells similar to those of normal samples. Mean ± s.e.m.; *P < 0.05, **P < 0.01, ***P < 0.001 (two-way ANOVA, Bonferroni post-test). Statistics not determined for CD samples. (b) Lower expression of GPR15 on Treg cells (CD127lo, Foxp3+; red histogram) as compared to non-Treg memory CD4+ T cells (CD127hi, Foxp3− blue).

Figure 4  Human proinflammatory CD4+ T cells express GPR15 in the colon. (a) Top, expression of GPR15 on cytokine-producing CD45RO+ CD4+ T cells from colons of individuals with ulcerative colitis (UC colons) or non-UC colons. Bottom, in UC colons, GPR15 is expressed on IL-17+, IL-5+, and IL-13+ CD45RO+ CD4+ T cells. n = 5 UC specimens, n = 11 non-UC or normal samples, and n = 2 colons from individuals with Crohn’s disease (CD), which showed cytokine expression among GPR15+ cells similar to those of normal samples. Mean ± s.e.m.; *P < 0.05, **P < 0.01, ***P < 0.001 (two-way ANOVA, Bonferroni post-test). Statistics not determined for CD samples. (b) Lower expression of GPR15 on Treg cells (CD127lo, Foxp3+; red histogram) as compared to non-Treg memory CD4+ T cells (CD127hi, Foxp3− blue).

Figure 5  Distinct patterns of GPR15 expression by in vitro-polarized human and mouse T cells. (a, b) Naive CD4+ T cells enriched from human peripheral blood mononuclear cells (a) or mouse Gpr15−/− spleen (b) were polarized as described in the Online Methods. GPR15 is expressed on human but not mouse in vitro–polarized Treg cells. Human T cells were gated on CD4+CD45RO+ CD4+ T cells, and mouse T cells were gated on CD45th CD4+ T cells. Combined results of three mouse and four human experiments; mean ± s.e.m.
was confirmed by ChIP-qPCR, with binding to the upstream site and to the IL4 CNS2 element remaining constant between human and mouse (Fig. 6c). Consistent with this species-specific binding, consensus GATA-3 sites could be identified at this site in human but not in mouse (Supplementary Fig. 7). Notably, a tandem GATA-3–STAT-6 motif is conserved in multiple species, but it contains an A-to-G nucleotide difference in the mouse (Supplementary Fig. 7), a mutation known to attenuate GATA-3 binding33 (T.T.D. and J.P., unpublished data).

Histone marks indicative of active chromatin characteristic this region in the GPR15-expressing ENCODE cell line GM12878 (Supplementary Fig. 7), whereas repressive chromatin modifications are observed in cell lines that do not express GPR15. We therefore hypothesized that the downstream site is an enhancer that is active in human T_{H2} cells. To test this, we performed ChIP-Seq for the active enhancer mark H3K4me3 and the transcriptional initiation mark H3K4me3 in in vitro–polarized human T_{H1} and T_{H2} cells (Fig. 6a). We found that the site was indeed associated with H3K27ac, and more so in T_{H2} than in T_{H1} cells, mirroring the expression of GPR15. The upstream site and the GPR15 gene were also associated with greater abundance of the H3K4me3 mark in T_{H2} cells. In contrast, no H3K4me3 is present at the downstream site or at the GPR15 gene in mouse (Fig. 6b), consistent with the lack of GATA-3 binding. We conclude that GATA-3 exhibits differential binding to an enhancer at the GPR15 locus between human and mouse and that this contributes to the differing patterns of enhancer activation and GPR15 expression in T_{H2} cells between the two species.

Foxp3 is responsible for the repression of T effector genes in T_{reg} cells34. We therefore considered that differential binding of this factor could be responsible for the differential expression of GPR15 between mouse and human T_{reg} cells. ChIP-Seq for FOXP3 in human and mouse T_{reg} cells identified significant binding to the GPR15 enhancer in human but not mouse (Supplementary Fig. 8). In human T_{reg} cells, the GPR15 enhancer was one of the most significant binding sites, having P values between 1.7 × 10^{-11} and 1.7 × 10^{-80} and being in the top 1% of sites in four different experiments. By contrast, in replicate experiments in mouse, the P values for Foxp3 binding to the Gpr15 enhancer were 1.4 × 10^{-4} and 2.6 × 10^{-4}, below the default MACS significance threshold of 10^{-5} and only within the top 13% or 23%, respectively, of all sites with P < 10^{-3}. Therefore, the low expression of GPR15 in human T_{reg} cells may reflect efficient binding and repression by FOXP3.

DISCUSSION

GPR15 is an HIV co-receptor and an orphan GPCR with structural homology to known chemokine receptors. Recently, GPR15 was shown to be expressed by colon T_{reg} cells and to contribute to their homing to, and function in, the colon. Our studies reveal that GPR15 is also an effector-memory CD4+ T cell receptor with a role in CD4+ T_{EM} cell homing and colitis pathogenesis, whose expression is differentially regulated in CD4+ T cell subsets between humans and mice. We show that GPR15 expression is enriched on human T_{H2} colon cells, but in striking contrast to the data from mouse, most human colon T_{reg} cells (whether in colons from individuals with or without inflammatory bowel disease) fail to express GPR15. We identified mutations that disrupt GATA-3 binding and GPR15 enhancer activation in the mouse enhancer region, and described differences in GATA-3 and FOXP3 binding to the enhancer in mice versus humans that may underlie the species-specific regulation of GPR15 on effector versus regulatory T cell subsets. Moreover, ChIP-Seq data indicate that FOXP3, a repressor of T_{H2}-associated genes in T_{reg} cells, binds with high efficiency to the human but not mouse enhancer in T_{reg} cells, correlating with inhibition of GPR15 expression in human regulatory T cells.

Our results are consistent with, but extend, those of another study11. The study’s authors used transfected naïve lymphocytes to show convincingly that GPR15 functions as a colon homing receptor, and they demonstrated its role in T_{reg} cell localization and function in the colon as well. Our results confirm the receptor’s role in T_{reg} cell localization in the mouse, but they also demonstrate a similarly
important role in \(T_{EM}\) cell homing and accumulation in the large intestine. Although GPR15's action as a T cell trafficking receptor for the colon is now established, additional roles for the receptor in cell retention or survival are not ruled out, nor is its involvement in homing to other epithelial surfaces. Expression of GPR15 on \(T_{reg}\) cells was previously shown to be required for transferred \(T_{reg}\) cells to prevent mortality and severe colitis associated with Citrobacter rodentium, a widely accepted model of infectious colitis with properties similar to the colitis caused by human pathogen enteropathogenic Escherichia coli. We, however, employed the widely used CD45RB\(^{hi}\) CD4\(^{+}\) T cell transfer colitis model that does not require \(T_{reg}\) cell homing to the colon for disease control. \(T_{reg}\) cell suppression of colitis in the CD45RB\(^{hi}\) CD4\(^{+}\) T model does not appear to require gut homing; \(T_{reg}\) cells deficient in integrin \(\beta_7\) are fully capable of suppressing disease, whereas \(T_{reg}\) cell suppression of colitis in this model requires \(T_{reg}\) cell migration to lymph nodes; CCR7-deficient \(T_{reg}\) cells were less efficient at preventing colitis than were CCR7-sufficient \(T_{reg}\) cells. In contrast, the pathogenic T cells in the CD45RB\(^{hi}\) CD4\(^{+}\) T cell transfer model do require intestinal trafficking receptors for colitigenesis: colitis in this model is inhibited by antibody blockade of the gut homing receptor \(\alpha_4\beta_7\) or by genetic deficiency of integrin \(\beta_7\). We showed that it is also inhibited by GPR15 deficiency on transferred T cells. \(\beta_7\) integrin deficiency does not alter the course of DSS-induced colitis, correlating with the lack of effect of GPR15 deficiency, and likely owing to the noncritical role of T cells in the acute chemically induced models of colitis. Together, the results highlight the importance of GPR15 expression for T cell localization and function inthe colon. GPR15-dependent T cell homing can mediate immune pathogenesis in T effector–cell driven colitis when \(T_{EM}\) cell activity is required locally in the colon, but it can contribute to immune regulation in models in which \(T_{reg}\) cell responses are important locally in the gut wall. In this context, we note that the CD45RB\(^{hi}\) CD4\(^{+}\) T cell transfer model has been used to identify and validate antibody inhibitors of intestinal lymphocyte trafficking that have led to effective therapeutics for human colitis.

These considerations may have significant implications for the translation of mouse studies to the human. Our data show that patterns of GPR15 expression are quite different in man. In sharp contrast to the mouse, in humans few if any colon \(T_{reg}\) cells isolated from individuals with colon cancer (normal adjacent tissue), ulcerative colitis or Crohn’s disease expressed the receptor. The highest frequency of GPR15 expression was found on \(T_{H2}\) effector T cells, an important population of pathogenic \(T_{EM}\) cells in the colon of ulcerative colitis patients. Notably, in vitro–generated human, but not mouse, \(T_{H2}\) cells expressed GPR15. Although it is not feasible to carry out functional studies of the orphan receptor in humans, GPR15 is highly conserved in mammalian species including man. Thus our results suggest that GPR15 may help target pathogenic \(T_{H2}\) cells to the colon in humans but is probably less important in humans than in mice for the homing and function of regulatory T cells in the gut wall.

We identified an active GPR15 enhancer region with marked differences in sequence and cell-type-specific transcription factor binding and chromatin marks in human and mouse. The human enhancer comprises canonical GATA-3 binding motifs that are conserved in multiple species but missing in the mouse. GATA-3 plays key roles in \(T_{H2}\) cell differentiation and cytokine expression. GPR15 ChIP-Seq data and targeted ChIP-qPCR revealed that GATA-3 binds to the 3' (downstream) GPR15 enhancer in human but not mouse \(T_{H2}\) cells, whereas binding to a site upstream at human CLDN1 and mouse Clhn25 and to the established IL4 CNS locus is conserved between the two species. The active enhancer mark H3K27ac and the transcriptional initiation mark H3K4me3 in human \(T_{H1}\) and \(T_{H2}\) cells confirmed the increased activity of the enhancer and increased expression of GPR15 in human \(T_{H2}\) cells. Unlike in the human, H3K4me3 is not observed at Gpr15 or its enhancer in mouse \(T_{H2}\) cells. We conclude from these experiments that GATA-3 only binds, and the enhancer is only activated, in human \(T_{H2}\) cells and not in mouse \(T_{H2}\) cells.

We also found that Gpr15 expression in \(T_{reg}\) cells is species specific, being suppressed in human cells. Potentially explaining this, ChIP-Seq for Foxp3, a transcriptional regulator necessary for repression of T effector cell genes in \(T_{reg}\) cells, demonstrates significant binding to the GPR15 enhancer only in human and not mouse \(T_{reg}\) cells. Foxp3 is thought to bind DNA with cofactors, including GATA-3, and thus the reduced binding of Foxp3 to the GPR15 enhancer in mouse \(T_{reg}\) cells may be due to differences in GATA motifs and loss of GATA-3 binding. Additional factors are also likely to be involved in the differential expression of GPR15 between human and mouse T cell subsets, including those responsible for the increased expression in mouse \(T_{H1}\) cells.

Although differences in transcription factor binding between species have been reported, to our knowledge, the differential binding of GATA-3 at GPR15 between human and mouse is the first example in which defined differences in transcription factor binding have been associated with species differences in targeting mechanisms of functionally distinct T cell populations, differences expected to result in altered patterns of local (\(T_{H1}\) cell–derived) cytokine production and thus mechanisms of pathogenesis. Manipulation of the enhancer sequences in T cells in vitro and in vivo will be required to elucidate the specific consequences of the species differences in GATA-3 binding shown here.

In conclusion, our studies show that GPR15 can control the colon localization of T effector cells, and we demonstrate its key role in a model of colitis that requires effector cell homing to and function in the gut wall. Our studies also identify significant differences in \(T_{reg}\) as compared to T effector cell expression of the receptor between mice and humans, differences that suggest a predominant proinflammatory role for GPR15 in man. These species differences may fundamentally restrict the relevance of mouse models for the study of Treg trafficking in colitis. Indeed, given its preferential expression by pathogenic effector cells in man, inhibition of GPR15 may present a therapeutic approach to controlling inflammatory T cell recruitment in ulcerative colitis.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** GEO accession codes for published ChIP-Seq data are provided in Supplementary Table 1.

**Note:** Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
J.P., E.O.H., A. Habetzion, E.C.B. and L.P.N. performed exploratory gene profiling that identified GPR15 as a candidate colon-homing receptor. L.P.N. performed experiments and wrote the manuscript. H.H. and A.E. performed mouse T cell polarization and short-term homing experiments. J.P. identified the Gpr15 enhancer and the Gata3 mutation and J.P. and T.T.D. characterized it. A. Hertweck and M.R.G. performed ChIP-Seq and ChIP-qPCR. G.M.L. and R.G.I. initiated and supervised the ChIP experiment. R.G.J. performed ChIP, analyzed ChIP-Seq data and wrote parts of the manuscript. E.C.B. initiated the project, analyzed data, provided overall supervision and wrote the manuscript. A. Habetzion supervised the project and wrote the manuscript.

COMPETING FINANCIAL INTERESTS
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ONLINE METHODS

Animals. BALB/c, C57BL/6 and B6.SJL mouse strains were purchased from Jackson Laboratory and bred in house. Gpr15-GFP mice, generated as described31, were backcrossed 10 times onto the C57BL/6 and BALB/c backgrounds before use in experiments, or for mating with B6.SJL and DO11.10 breeders to obtain CD45.1+ and CD45.2+ KO (Gpr15−/−) mice. Rag2 constitutive knockout (Rag2−/−) mice were obtained from Taconic and housed for at least 1 week before being used as recipients in colitis studies. Animals were maintained in accordance to US National Institutes of Health guidelines, and experiments were approved by Stanford University Institutional Animal Care and Use Committee.

Cell isolation. Spleen, Peyer’s patches, and mesenteric and peripheral (including inguinal, brachial and axillary) lymph nodes were mechanically dispersed through a stainless steel 200 µm wire mesh to yield single-cell suspensions in Hank’s buffered salt solution (HBSS; Mediatech, Inc.) containing 2% BCS. Splenocytes were incubated in Red Blood Cell Lysing buffer (Sigma-Aldrich) and the cells washed by centrifugation for 5–10 min at 300g, 4 °C. Intraperitoneal and lamina propria lymphocytes were isolated from the small intestine and colon as described previously32. After excision of the Peyer’s patches, the intestines were cut into ~0.5 cm pieces and cleansed in HEPES-buffered HBSS containing 2% BCS. To harvest IEL, the tissues were incubated twice with 1 mM dithiothreitol at 37 °C for 20 min and the supernatant collected. To isolate LP lymphocytes, the tissue pieces were incubated in 2 mM EDTA for 15 min (~2 rounds) followed by 3 rounds of collagenase digestion at 37 °C (30–45 min each), and the supernatant was collected, pooled and washed. Centrifugation in a 30%/70% Percoll step-gradient produced enriched IEL or LP lymphocytes at the interface, and the cells were then washed, counted and stained for flow cytometry analysis.

Antibodies and fluorescence-activated cytometry. The following antibodies and dilutions were used for flow cytometry in the mouse studies: CD62L-APC (MEL-14, 1:500), CD103-PE (M290, 1:100) (BD Pharmingen); CD25–PE-Cy7 (PC61, 1:300), Foxp3–PE, Foxp3–FITC, Foxp3–APC (FK-16s, 1:150), CD4–APC, CD45RB–APC-Cy7 (C363-16A, 1:300), CD44–Pacific Blue (IM7) (BioLegend, 1:300). For staining human blood and tissues, these antibodies were used: rabbit anti-human GPR15 (1 µg, Abcam), rabbit IgG isotype control (Sigma-Aldrich), goat anti–rabbit–AF488 secondary antibody (1:500, Invitrogen) and goat anti–mouse–AF488 (1:500, Invitrogen); IL-13–Pe (JES10-5A2), IFN-γ–PE (B72, IL-17A–PE-Cy7 (BL168), CD45RO-APC-Cy7 (UCHL1) and CD25–AF700 (BC96) were purchased from Biolegend; Foxp3–APC (PCH101) from eBioscience; CD4–Pacific Blue (RPA-T4) and IL-5–PE (TRFK5) from BD Biosciences. The mouse anti-human GPR15 mAb (Clone 367902; R&D Systems) and corresponding mouse IgG2b isotype control (R&D Systems) were used only in side-by-side assessment of the GPR15 Pab, as described in below. Where not otherwise specified, 5 µL of antibodies to human proteins was used per test. Where indicated, cells were activated with phorbol myristate acetate (PMA; 50 ng/mL) and ionomycin (1 µg/mL) in the presence of brefeldin A (to prevent cytokine release into medium) for 4 h at 37 °C before surface staining46. Staining of mouse and human Foxp3 and intracellular cytokine antibodies was performed using Foxp3 fixation and permeabilization solutions as recommended (eBioscience). Data was acquired on an LSRII or Fortessa cytometer (BD Biosciences) and analyzed with FlowJo software (FlowJo, LLC).

CD45RB+CD4+ T cell transfer model of colitis. CD4+ T cells were enriched from spleen of WT or Gpr15 KO mice by negative selection using the CD4+ T cell Isolation Kit II (Miltenyi Biotec). The bead-enriched CD4+ T cells were stained and sorted by flow cytometry using the ARIA II or ARIA III cell sorter (BD Biosciences) to separate naive cells (CD4+, CD45RB+, CD25−) and Treg cells (CD4+, CD45RB+, CD25+). Cells were washed with sterile HBSS, and 0.5 × 106 naive CD4+ T cells alone or with 0.1 × 106 Treg cells were transferred iv. into each Rag2−/− mouse. The mice were weighed weekly and assessed for weight loss, stool consistency, bloody stools and general well-being. The mice were killed at week 6 (or sooner if moribund or if weight loss exceeded 20%), the spleen, MLN and colon were harvested, and colon length and stool consistency were assessed. For histologic assessment, the distal-most 1 cm section of colons was fixed in 10% buffered formalin for tissue sectioning and hematoxylin and eosin staining21. The histology was assessed and scored in a blinded in fashion as previously described48.

GPR15 expression in human colon samples. Colon resection samples were obtained from patients undergoing ulcerative-colitis– or adenocarcinoma–indicated colectomies with approval by the Stanford University Institutional Review Board and after obtaining informed patient consent. After removal of the serosa, mesentery fat and muscularis externa, lymphocytes were isolated from the lamina propria as described49, and the cells activated and stained for flow cytometry analysis.

Chromatin IP and ChiP-Seq data analysis. Human and mouse GATA-3 ChiP-Seq has previously been published30,31. Human and mouse H4K4me3 ChiP-Seq data have also been published50,51. Mouse GATA-3 and Foxp3 and human Foxp3 ChiP-Seq data were downloaded as fastq files from GEO (accession codes listed in Supplementary Table 1) and aligned to mm9 or hg18 with bowtie2 (default settings); bigwig files were generated for visualization in the UCSC Genome Browser as described52, and binding sites were identified with MACS (using input DNA as background, if available). ChiP for H3K27ac in in vitro–polarized human T1/2 and T1/2 cells was performed as described53 using the antibody ab4729 (10 µg, Abcam). ChiP for GATA-3 was performed with the D16 antibody (10 µg, Santa Cruz Biotechnologies, Inc.) in mouse T1/2 cells polarized as described54. ChiP for Foxp3 in mouse induced Treg (Treg) cells is described in Supplementary Figure 8. DNA enriched by GATA-3 ChiP was measured by quantitative PCR relative to input DNA and normalized to a control region at the GPR15 locus that lacked GATA-3 binding. The primers used are listed in Supplementary Table 2.

Mixed bone-marrow chimeras. Bone marrow was extracted from femur and tibia of Gpr15-KO (Gpr15fl/fl; CD45.2) and Gpr15-het (Gpr15fl/wt; CD45.1) mice and red blood cells lysed. Cells were combined in equal ratios and injected into lethally irradiated Fl0 offspring of C57BL/6 × B6.SJL breeding (CD45.1, CD45.2) to generate experimental KO/Het mixed chimeras. In parallel, cells from Gpr15-het (CD45.2) and congenic Gpr15-het (CD45.1) mice were similarly transferred to generate Het/Het control chimeras. After 8–12 weeks, tissues from Fl0 recipients were harvested and prepared for analysis by flow cytometry. For each mouse, the ratio of CD45.2+ CD4+ to CD45.1+ CD4+ T cells recovered from each organ was calculated. Since bone marrow ‘take’ from different donors can vary between recipients, the CD45.2/CD45.1 ratio of T cells in different organs was normalized to that of CD19+ B cells, and the relative ratio of cells in each tissue was additionally normalized to that of the spleen as described33.

Generation of stable GPR15 transfectants. Mouse pre-B cell lymphoma L1.2 cells were transfected (Nucleofector, Lonza Inc.) with a mammalian expression vector pCMV6-AC-GFP containing the human GPR15 cDNA with a C-terminal tGFP tag (Origene). G418-selected transfectants with highest levels of GFP expression were sorted for testing of antibody to human GPR15. The mouse Gpr15 ORF was subcloned into the pCMV6-AC-GFP vector 3′ of the Kozak consensus sequence to similarly generate stable L1.2 transfectants.

Assessment of rabbit polyclonal antibody to human GPR15. To determine the specificity of the polyclonal GPR15 antibody (Abcam) used throughout the study, L1.2 transfectants (1 × 106 cells) of human (or mouse) GPR15 were incubated with 0.5 or 1 µg of the GPR15 polyclonal antibody for 30 min, followed by 30 min with PE–donkey anti–rabbit IgG (Jackson Immunoresearch Laboratories, Inc.; Supplementary Fig. 9a). The specific staining of the polyclonal antibody was also tested side by side with a monoclonal antibody to human GPR15 (Clone 367902; R&D Systems) on freshly isolated human colon cells (Supplementary Fig. 9b,c), and the two antibodies produced similar staining.

DSS-induced colitis. Two-month-old gender-matched Gpr15-KO and wild-type BALB/c mice were given drinking water containing 4.5% DSS (w/v; dextran sulfate sodium salt, 36,000–50,000 Da; MP Biomedical) beginning day 0 (ref. 34). The mice were weighed daily and assessed for the presence of bloody stool and general well-being. They were killed on day 9.
TNBS-induced colitis. Two-month-old gender-matched Gpr15-KO and wild-type BALB/c mice were rectally instilled with 2 mg of TNBS (2,4,6-trinitrobenzensulfonic acid solution; Sigma-Aldrich) in 100 μL of 40% ethanol on day 0 (ref. 54). The mice were assessed daily for weight loss, bloody stool and general well-being, and they were killed on day 4.

Co-transfer of Gpr15 KO and WT naive CD4+ T cells into Rag2−/− mice. Similar to the cell transfer model of colitis, splenocytes from Gpr15−/− (Gpr15-KO; CD45.2) or Gpr15+/+ (Gpr15-het; CD45.1, CD45.2) mice were sorted by flow cytometry to enrich naive CD4+ T cells of >90% purity. The cells were combined at a 1:1 ratio, and a total of 1 × 106 cells were i.v. injected into each Rag2−/− recipient. After 2 weeks, lymphocytes from MLN, spleen and colon were isolated, and cells activated for 4 h before staining with surface markers and intracellular cytokines. Where indicated, GFP+ CD4+ T cells were sorted from pooled MLN or colon, activated and stained to assess GPR15 expression among effector/memory CD4+ T cells. For data analysis, normalization to spleen is used to control for variability between mice in injected cell numbers. The spleen lacks high endothelial venules, and unlike the other tissues examined, it is empirically less affected by homing or trafficking effects, making it a preferable (and commonly employed) internal reference tissue.

In vitro–polarized T helper cells. Naive CD62L-CD4+ T cells were enriched using the Naive CD4+ T Cell Isolation Kit II (Miltenyi Biotec) from human blood or chicken eggs and sorted into 96-well plates (0.2 × 10^6 cells/well, precoated with 1 μg/mL anti-CD3 antibody; clone HIT3a). To generate T11 cells, IFN-γ (10 ng/mL) and recombinant IL-12 (5 ng/mL) were added. To polarize T12 cells, IL-4 (10 ng/mL) and anti–IL-12 antibody (10 μg/mL) were used; for T17 cells, IL-1β, IL-23, IL-6, TGF-β (10 ng/mL each) and 10 μg/mL of anti–IFN-γ and anti–IL-1 antibodies; for Treg cells, 5 ng/mL TGF-β. Cells were cultured for 5–6 d in the presence of IL-2 (10 ng/mL) and soluble anti-CD28 (1 μg/mL; clone CD28.2). Cells were washed, activated for 4 h and stained with surface and intracellular markers as described above. Mouse spleen cells from Gpr15−/− mice were subjected to similar polarizing conditions as described previously35–37. Cytokines and antibodies were purchased from Peprotech Inc. or eBioscience.

Competitive short-term homing of effector CD4+ T cells. Bead-enriched CD4+ T cells (MACS kit; Miltenyi Biotec) from spleen of allotype-marked Gpr15-het and Gpr15-KO mice were cultured under T1117-polarizing conditions using plate-bound anti-CD3 mAb (1 μg/mL; clone 145-2C11), soluble anti-CD28 mAb (1 μg/mL; clone 37.51), rmIL-2 (20 ng/mL), rmIL-6 (20 ng/mL), rhTGF-β (5 ng/mL) and retinoic acid (100 pM) as previously described32. After 3 d, cells were washed and i.v. injected into normal hosts. After 6–8 h, host lymphocytes were assessed for presence of donor cells.

Antigen-specific T cell accumulation. Splenocytes (25 × 10^6) from DO11.10, Gpr15-het or DO11.10, Gpr15-KO were i.v. injected into Balb/c (Thy-1.1) mice. After 24 h, anesthetized recipients were treated iarectally with ovalbumin from chicken egg white (500 μg; Sigma-Aldrich) and cholera toxin (10 μg; Sigma-Aldrich) in a 150 μL volume. At day 5 post treatment, recipient tissues were i.v. injected in equal proportion (15 × 10^6 total cells) into Thy-1.1 Balb/c mice. After 24 h, mice were treated iarectally with an Ovalbumin (OVA; 500 μg) and cholera toxin (CT; 10 μg) mixture. At indicated time, mice were i.p. injected with FTY720 or vehicle (PBS) and their tissues collected. Stock solutions of FTY720 (Sigma-Aldrich; 10 mg/mL ethanol) were diluted with PBS to 100 μg/mL and i.p. injected at 1 mg/kg.

Comparative sequence analyses. We analyzed a highly conserved noncoding region downstream of mouse, chimpanzee, monkey, rat and human GPR15. The conserved region is located −23 kb and 15 kb 3′ of GPR15 in human and mouse, respectively. The human sequence is within a region (chr3:98,272,711–98,276,110 in Hg19) highlighted in the ENCODE/BROAD histone modification track (UCSC Genome Browser) as an active promoter region in the GPR15-expressing B lymphoblastoid cell line GM12878. D1α5 hypersensitivity marks from human T12 cells, as well as histone modification comparisons with GM12878 and non–GPR15-expressing H1-hESC embryonic stem cells, HepG2 hepatocyte line, HUVEC umbilical vein endothelial cells, HMEC microvascular endothelial cells, HSMM smooth muscle myoblasts, NHEK epidermal keratinocytes and NHLF lung fibroblasts, were from the UCSC Genome Browser and the ENCODE project consortium. To verify the A-to-G mutation in the mouse GATA-3 consensus sequence adjacent to the conserved STAT6 binding motif, we surveyed mouse SNP databases (http://www.sanger.ac.uk/sanger/Mouse_SnpViewer/rel-13-09) and found no variation in this nucleotide, indicating that it is a species- (not strain-) specific variant.

Foxp3 ChIP-Seq in mouse iTreg cells. CD4+ T cells from spleens and lymph nodes of 4- to 10-week-old mice were purified by CD4+ selection (Miltenyi Biotec) followed by sorting of naive CD4+, CD25+, CD62L+CD4+ cells using a FACS Aria II (BD Biosciences). Cells were cultured by plate-bound anti-CD3 and anti-CD28 (both 10 μg/mL; clones 145-2C11 and 37.51, respectively; Bio X Cell). iTreg cells were generated by culturing in recombinant human TGF-β (33 ng/mL) and IL-2 (20 ng/mL; R&D Systems) for 7 d. Following activation, cross-linking and sonication30, Foxp3-bound genomic DNA was isolated from whole-cell lysate using a mix of two antibodies (5 μg of each): Santa Cruz S-31738 and ebioscience JFK-16. Libraries were constructed and sequenced as described30.

Statistical analysis. Prism software (GraphPad Software) was used for statistical analyses and statistical tests employed are indicated in the figure legends, with P values of <0.05 considered as significant.

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