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GPR120 inhibits colitis through regulation of CD4+T cell IL-10 production

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Abstract

BACKGROUND & AIMS: G protein-coupled receptor (GPR) 120 has been implicated in regulating metabolic syndromes with anti-inflammatory function. However, the role of GPR120 in intestinal inflammation is unknown. Here, we investigated whether and how GPR120 regulates $CD4^+$T cell function to inhibit colitis development.

METHODS: Dextran sodium sulfate (DSS)-induced colitis model, *Citrobacter rodentium* infection model, and $CD4^+$T cell adoptive transfer model were utilized to analyze the role of

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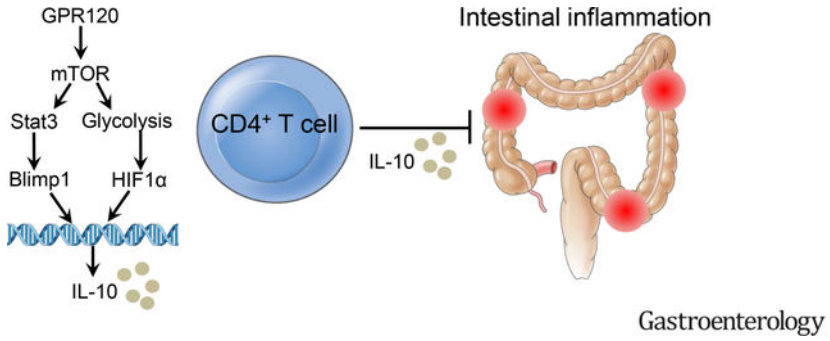
GPR120 in regulating colitis development. The effect of GPR120 on CD4^{+}T cell functions was analyzed by RNA sequencing, flow cytometry, and Seahorse metabolic assays. Mice were administered GPR120 agonist for investigating the potential of GPR120 agonist in preventing and treating colitis.

RESULTS: Deficiency of GPR120 in CD4^{+}T cells resulted in more severe colitis in mice upon DSS insult and enteric infection. Transfer of GPR120-deficient CD4^{+}CD45Rb^{hi}T cells induced more severe colitis in *Rag*$^{-/-}$ mice with lower intestinal IL-10^{+}CD4^{+}T cells. Treatment with GPR120 agonist, CpdA, promoted CD4^{+}T cell production of IL-10 by upregulating Blimp1 and enhancing glycolysis, which was regulated by mTOR. GPR120 agonist-treated wild-type but not IL-10-deficient and Blimp1-deficient Th1 cells induced less severe colitis. Furthermore, oral administration of GPR120 agonist protected mice from intestinal inflammation in both prevention and treatment schemes. *Gpr120* expression was positively correlated with *Il10* expression in the human colonic mucosa, including patients with inflammatory bowel diseases (IBD).

CONCLUSIONS: Our findings demonstrate the role of GPR120 in regulating intestinal CD4^{+}T cell production of IL-10 to inhibit colitis development, which identifies GPR120 as a potential therapeutic target for treating IBD.

Graphical Abstract



Lay Summary

Long-chain fatty acid receptor G protein-coupled receptor 120 induces interleukin-10 production in CD4^{+}T cells to inhibit colitis development through upregulation of B lymphocyte-induced maturation protein 1 and enhancing glycolysis.

Keywords

Effector CD4^{+}T cells; Glycolysis; Blimp1; Intestinal homeostasis; Inflammatory bowel diseases

Introduction

It has been well-established that CD4^{+}T cell responses to gut microbiota play crucial roles in the pathogenesis of inflammatory bowel disease (IBD). Among CD4^{+}T cells, Th1 and Th17 cells are central to the pathogenesis of certain types of IBD[1], which can be inhibited by multiple mechanisms, including CD4^{+}T cell production of IL-10, a critical immunosuppressive cytokine for regulating intestinal homeostasis. Polymorphisms in the

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Il10 locus confer risk for IBD[2, 3]. Deficiency in IL-10 or IL-10 receptor (IL-10R) in mice and humans causes severe intestinal inflammation[4–6]. IL-10-IL-10R signaling is essential in regulatory T cells (Tregs) for their suppressive functions in IBD, in $CD4^+$T effector cells for inhibiting exaggerated T cell responses in mucosal compartments, and also in innate cells for regulating mucosal homeostasis[7–9]. However, how $CD4^+$T cell production of IL-10 is regulated is still not completely understood. Accumulating evidence indicates that dietary compositions regulate health and disease, regardless of energy intake[10]. It has been shown that dietary fatty acids, the primary dietary components, affect host immune responses[11]. Long-chain fatty acids (LCFA) are the major dietary fatty acids absorbed and sensed by host cells. Omega 3 fatty acids (ω-3 FA), which belong to LCFA and are commonly consumed as fish products, dietary supplements, and pharmaceuticals, have been demonstrated to have potent anti-inflammatory effects and several health benefits, including amelioration of atherosclerosis and increased insulin sensitivity[12, 13]. The intestinal tract directly interacts with dietary components[14]. High dietary ω-3 FA has been associated with a low risk of IBD[15]. However, the underlying mechanisms are still unclear. G protein-coupled receptor (GPR) 120, which is recently identified as a receptor for ω-3 FA[16], has a critical role in various physiological homeostasis such as adipogenesis[17, 18]. Deficiency of GPR120 leads to obesity in both humans and mice, and $Gpr120^{-/-}$ mice are more susceptible to insulin resistance and have higher expression of the genes related to inflammation[19], whereas GPR120 agonists inhibit pro-inflammatory cytokine production by dendritic cells (DCs) and macrophages[16, 20]. However, it is still unknown how GPR120 regulates the pathogenesis of IBD.

In this study, we demonstrate that $Gpr120^{-/-}$ mice and $CD4^+$T cell-specific GPR120 knockout $Cd4^{cre}Gpr120^{fl/fl}$ mice develop more severe colitis than wild-type (WT) mice. $Gpr120^{-/-}CD4^+$T cells induce more severe colitis than WT $CD4^+$T cells when transferred into $Rag^{-/-}$ mice. The GPR120 agonist promotes $CD4^+$T cell production of IL-10 through upregulation of Blimp1 and enhancing glycolysis. Importantly, *Gpr120* expression is positively correlated with *Il10* expression in the human mucosa, including patients with IBD.

Materials and Methods

Mice

C57BL/6J WT mice, B6.129-Prdm1tm1Clme/J ($Prdm^{fl/fl}$) mice, B6.Cg-Tg(Cd4-cre)1Cwi/BfluJ ($Cd4^{cre}$) mice, and B6.SJL-$Ptprc^a$ $Pepc^b$/BoyJ (CD45.1) mice were purchased from Jackson Laboratory. $Gpr120^{-/-}$ mice were provided by Bristol-Myers Squibb. $Gpr120^{fl/fl}$ mice were generated by inserting two loxP sites in the *Gpr120* allele using CRISPER/Cas9 gene-editing technology in C57BL/6J background in Viewsolid Biotech and crossed to $Cd4^{cre}$ to generate $Cd4^{cre}Gpr120^{fl/fl}$ mice. IL-$10^{-/-}$ mice were provided by Dr. Cohn at the University of the Texas Medical Branch (UTMB). $Prdm1^{fl/fl}$ mice were crossed to $Cd4^{cre}$ to generate $Cd4^{cre}Prdm^{fl/fl}$ mice. All the mice were maintained on a 12 hour-light/dark cycle with the temperature of 20–26°C and 30–70% humidity in the specific pathogen-free animal facility of UTMB. All the experimental mice were sex-matched and age-matched littermates

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and cohoused after weaning. All the animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of UTMB.

Human

All the patients with Crohn's disease (CD) and ulcerative colitis (UC) were recruited at the Gastroenterology Department of the First Affiliated Hospital of Nanjing Medical University. The diagnosis of CD and UC was based on the combination of clinical signs and symptoms, endoscopic features, and histological results. Colonic tissues were collected from 14 healthy volunteers, and inflamed mucosa biopsies were obtained from 21 active CD patients and 10 active UC patients. Ethical approval was obtained from the Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Nanjing Medical University, and informed consent was obtained from all the participants. The characteristics of all the human subjects were included in Supplementary Table 1.

Statistical analysis

Data were analyzed by the two-way Student's *t*-test or Mann–Whitney U test or one-way ANOVA test or Spearman's correlation analysis using GraphPad Prism 9. All data are presented as mean ± SEM, and the P-value < .05 was considered statistically significant.

All the other methods are included in the online supplemental materials.

Results

1. Gpr120$^{-/-}$ mice develop more severe colitis upon inflammatory insult and enteric infection.

To determine whether GPR120 regulates intestinal homeostasis, we induced colitis in WT and *Gpr120*$^{-/-}$ mice by administering DSS. First, we confirmed that *Gpr120* expression was knockout in *Gpr120*$^{-/-}$ mice (Supplementary Figure 1A). *Gpr120*$^{-/-}$ mice demonstrated more weight loss, suffered from more severe colitis, and produced more TNF-α and IL-6 than WT mice (Supplementary Figure 2A–D). There were no differences in IFN-γ and IL-17A production (Supplementary Figure 2D). However, IL-10 was lower in colonic tissues of *Gpr120*$^{-/-}$ mice than WT mice (Supplementary Figure 2D). There were no differences of IFN-γ^{+}CD4^{+}T cells, IL-17A^{+}CD4^{+}T cells, and Foxp3^{+}Tregs in the colonic lamina propria (LP) between WT and *Gpr120*$^{-/-}$ mice, but fewer intestinal IL-10^{+}CD4^{+}T cells were present in *Gpr120*$^{-/-}$ mice (Supplementary Figure 2E–I).

Similar results were obtained in WT and *Gpr120*$^{-/-}$ mice infected with *Citrobacter rodentium (C. rodentium)*, an enteric bacterial strain similar to IBD-associated human enteropathogenic *Escherichia coli*[21]. *Gpr120*$^{-/-}$ mice demonstrated more weight loss than WT mice (Supplementary Figure 2J). While there were no differences in the bacterial burdens at day 4, colony counts of fecal *C. rodentium* were higher in the *Gpr120*$^{-/-}$ mice on day 7, and the difference was even more significant from day 10 (Supplementary Figure 2K). On day 14, *C. rodentium* was no longer detectable in WT mice, but counts were still high in *Gpr120*$^{-/-}$ mice (Supplementary Figure 2K), suggesting the importance of GPR120 in adaptive immune responses against intestinal pathogens. When sacrificed 14 days post-

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infection, *Gpr120*$^{-/-}$ mice developed more severe colitis and produced more TNF-α, IL-6, and IFN-γ but less IL-10 in the colon than WT mice (Supplementary Figure 2L–N). There were no differences in colonic IL-17A production (Supplementary Figure 2N). These data indicate that GPR120 inhibits intestinal inflammation.

2. *Gpr120*$^{-/-}$ CD4^{+}T cells induce more severe colitis.

While intestinal epithelial cells (IECs), DCs, and macrophages have been shown to express GPR120[16, 20, 22], its expression by CD4^{+}T cells has not been defined. We found that activated CD4^{+}T cells expressed GPR120 at a higher level than BMDCs and small bowel IECs, and GPR120 levels in activated CD4^{+}T cells were higher than naïve T cells (Figure 1A–B). Besides, Th1 cells, Th17 cells, and Treg cells expressed GPR120 at similar levels but higher than DCs and IECs (Figure 1A–B).

We next investigated the role of CD4^{+}T cell expression of GPR120 in intestinal homeostasis. First, we determined that *Gpr120* was specifically knockout in CD4^{+}T cells from *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice (Supplementary Figure 1B–C). We next induced colitis in *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice and WT *Cd4*^{cre}*Gpr120*$^{fl/+}$ mice using DSS. *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice developed more severe colitis with more weight loss, higher pathological scores in the colon, and produced more TNF-α, IL-6, and IL-17A, but less IL-10 in the colon of *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice than WT mice (Figure 1C–F). There was no difference in IFN-γ (Figure 1F). LP IL-10^{+}CD4^{+}T cells were decreased in *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice, whereas there were no differences in IFN-γ^{+}Th1 cells, IL-17^{+}Th17 cells, and Foxp3^{+}Tregs (Supplementary Figure 3A–E), indicating that CD4^{+}T cell expression of GPR120 suppresses colitis, possibly through induction of IL-10.

Next, we infected *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice and WT *Cd4*^{cre}*Gpr120*$^{fl/+}$ mice with *C. rodentium*. *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice demonstrated more weight loss compared with WT *Cd4*^{cre}*Gpr120*$^{fl/+}$ mice (Figure 1G). While there were no differences in the bacterial burdens at day 4, fecal *C. rodentium* levels were higher in *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice than WT mice on days 7, 10, and 14 (Figure 1H). When mice were sacrificed 14 days post-infection, *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice developed more severe colitis and produced more TNF-α, IL-6, IFN-γ, and IL-17A, but less IL-10 in the colons (Figure 1I–M). More IFN-γ^{+}Th1 cells and IL-17A^{+}Th17 cells but fewer IL-10^{+}T cells were present in LP of *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice, while there was no difference in Foxp3^{+}Tregs (Supplementary Figure 3F–J).

To further verify the role of GPR120 in CD4^{+}T cells in regulating colitis, we utilized the CD4^{+}CD45RB^{hi}T cell adoptive transfer model[23]. WT and GPR120-deficient CD4^{+}CD45RB^{hi}T cells, isolated from WT *Cd4*^{cre}*Gpr120*$^{fl/+}$ mice and *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice, were transferred into *Rag*$^{-/-}$ mice. The recipient mice were sacrificed 6 weeks later. GPR120-deficient CD4^{+}CD45RB^{hi}T cells induced more severe colitis in *Rag*$^{-/-}$ mice (Figure 2A–E), and produced more TNF-α, IL-6, IFN-γ, and IL-17A, but less IL-10 in colons than did WT CD4^{+}CD45RB^{hi}T cells (Figure 2F). Fewer intestinal IL-10^{+}CD4^{+}T cells and more IFN-γ^{+}Th1 cells and IL-17A^{+}Th17 cells were present in *Rag*$^{-/-}$ mice receiving GPR120-deficient T cells compared with recipient mice reconstituted with WT T cells, while there was no difference in Foxp3^{+}Tregs between the two groups of mice

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(Figure 2G–K). These data indicate that GPR120 in CD4$^+$T cells is critical in regulating intestinal homeostasis.

3. GPR120 agonist promotes CD4$^+$T cell production of IL-10 to suppress colitis.

To investigate whether GPR120 indeed regulates CD4$^+$T cell functions, we cultured CD4$^+$T cells with or without a GPR120 agonist, CpdA[20], for 2 days. Cells were collected, and the differences in the transcriptome were analyzed by RNA sequencing (RNA-Seq). GPR120 agonist altered the gene expression profile (Figure 3A and Supplementary Figure 4A), in which 1749 genes were upregulated and 1709 genes were downregulated (Supplementary Figure 4B). Gene Ontology functional enrichment analysis demonstrated that the differentially expressed genes were enriched in various functions regarding metabolism, function, and biological processes, including primary metabolic process, glycolytic process, regulation of immune system process, regulation of T cell-mediated immunity, regulation of T cell cytokine production, and cellular response to hypoxia (Supplementary Figure 4C). Furthermore, KEGG pathway enrichment analysis demonstrated that the differentially expressed genes were enriched in 46 pathways, including inflammatory bowel disease (Figure 3B). CpdA promoted *Il10* but inhibited *Ifng* and *Il17* at the mRNA levels, whereas it did not affect *Foxp3* expression (Figure 3C).

To verify the effect of CpdA on CD4$^+$T cell cytokine production at the protein level, we cultured CD4$^+$T cells with or without CpdA for 5 days to measure cytokine production using flow cytometry. CpdA promoted CD4$^+$T cell IL-10 production, but it did not affect IFN-γ and IL-17A under neutral conditions (Supplementary Figure 5A–F), which might be attributed to low protein expressions of IFN-γ and IL-17A in CD4$^+$T cells under neutral conditions. GPR120 agonist promoted IL-10 in CD4$^+$T cells under Th1 conditions (Figure 3D–F), but not under Th17 and Treg conditions (Supplementary Figure 5A–B). Furthermore, CD4$^+$T cells expressed higher levels of IL-10 under Th1 conditions than under other polarization conditions (Figure 3D–E and Supplementary Figure 5A–B). Interestingly, CpdA promoted IFN-γ$^+$IL-10$^+$T cells but not IFN-γ$^+$IL-10$^-$T cells under Th1 conditions (Supplementary Figure 5G–I), indicating that CpdA renders self-regulatory function to Th1 effector cells. CpdA suppressed IL-17A production under Th17 conditions (Supplementary Figure 5J–K), but it did not affect Treg differentiation (Supplementary Figure 5L–M). Besides, CpdA at the indicated dose did not affect CD4$^+$T cell viability and proliferation (Supplementary Figure 6A–C). To determine whether the natural ligand for GPR120 also promotes IL-10 production in CD4$^+$T cells, we treated CD4$^+$T cells with DHA, a ω-3 FA, under Th1 conditions. As shown in Supplementary Figure 7A–B, DHA increased T cell IL-10 production in a dose-dependent manner.

As GPR120 promoted CD4$^+$T cell production of IL-10, especially under Th1 conditions, and Th1 cells expressed higher levels of IL-10, we next investigated whether GPR120 agonist-treated Th1 cells induced less severe colitis through induction of IL-10. We treated WT and IL-10-deficient CD4$^+$T cells with or without the GPR120 agonist CpdA for 5 days, then transferred them into *Rag*$^{-/-}$ mice. The mice were sacrificed 6 weeks later. The *Rag*$^{-/-}$ recipient mice of CpdA-treated WT Th1 cells developed less severe colitis and produced less TNF-α, IL-6, and IFN-γ, but more IL-10 in colons compared with

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Rag$^{-/-}$ mice reconstituted with untreated WT Th1 cells (Figure 3G–J). However, the *Rag*$^{-/-}$ mice receiving control IL-10-deficient Th1 cells or CpdA-treated IL-10-deficient Th1 cells developed severe colitis at similar levels with no differences in colonic cytokines (Figure 3G–J). We also used the anti-IL-10R antibody to inhibit the IL-10-IL-10R pathway in *Rag*$^{-/-}$ mice receiving CpdA-treated Th1 cells or control Th1 cells. Anti-IgG antibody was used as a control. CpdA-treated Th1 cells induced less severe colitis than control Th1 cells in *Rag*$^{-/-}$ mice treated with control anti-IgG antibody (Supplementary Figure 8A–F). However, there were no differences in disease severity and cytokine production when the mice were treated with anti-IL-10R antibody (Supplementary Figure 8A–F).

As GPR120 did not affect Treg differentiation (Supplementary Figure 5L–M), we then investigated whether GPR120 affects Treg suppressive functions. CD45.1 WT CD4^{+}CD45RB^{hi}T cells were transferred into *Rag*$^{-/-}$ mice together with or without CD45.2 WT or GPR120-deficient Tregs, differentiated from naïve CD4^{+}T cells of WT *Cd4*^{cre}*Gpr120*$^{fl/+}$ mice or *Cd4*^{cre}*Gpr120*$^{fl/fl}$ mice. Both WT and GPR120-deficient Tregs suppressed colitis induced by CD4^{+}CD45RB^{hi} T cells at similar levels (Supplementary Figure 9A–F). These data indicate that GPR120 does not affect Treg function in suppressing colitis.

4. Blimp1 mediates GPR120 induction of IL-10 through the mTOR-Stat3 pathway.

Next, we investigated the potential mechanisms underlying the GPR120 induction of IL-10 in CD4^{+}T cells. RNA-Seq showed an increased tendency of transcription factors *Irf4*, *Prdm1* (encodes Blimp1), and *Maf*, but a decreased expression of *Ahr* (Supplementary Figure 10A), all of which have been implicated in regulating IL-10 production in CD4^{+}T cells[24, 25]. To evaluate the transcriptome data, we treated CD4^{+}T cells with or without CpdA with increased sample size. We found that CpdA significantly increased *Prdm1* expression (Figure 4A), but not *Irf4* and *Maf* (Supplementary Figure 10B–C). To determine if Blimp1 mediates GPR120 induction of IL-10, we utilized *Cd4*^{cre}*Prdm1*$^{fl/fl}$ mice, in which GPR120 was specifically knockout in CD4^{+}T cells (Supplementary Figure 1D–E). We found that CpdA induction of IL-10 was compromised in Blimp1-deficient CD4^{+}T cells (Figure 4B–D), indicating that Blimp1, at least partially, mediates GPR120 induction of IL-10.

We then investigated how GPR120 regulates Blimp1 expression in CD4^{+}T cells. It has been reported that GPR120 activates mTOR in bone marrow-derived mesenchymal stem cells[26], which has been shown to regulate IL-10 production in T cells[27]. KEGG pathway enrichment analysis demonstrated that GPR120 agonist altered mTOR upstream AMPK signaling pathway in T cells (Figure 3B). Treatment with GPR120 agonist CpdA promoted mTOR activation in CD4^{+}T cells (Figure 4E–F). Treatment with rapamycin, the mTOR inhibitor, decreased T cell expression of *Prdm1* (Figure 4G) and IL-10 production induced by CpdA (Figure 4H and M–O). Given that Stat3 promotes Blimp1 expression and mTOR positively regulates Stat3[28–30], and RNA-seq data suggested an altered JAK-STAT signaling pathway in CpdA-treated T cells (Figure 3B), we hypothesized that GPR120 activation of mTOR induces IL-10 production through the Stat3-Blimp1 pathway. Indeed, CpdA activated Stat3, which was inhibited by the mTOR inhibitor rapamycin (Figure 4I–J). Stattic, a selective Stat3 inhibitor, suppressed CpdA-induced *Prdm1* expression and IL-10

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production (Figure 4K–O). Furthermore, both mTOR and Stat3 inhibitors suppressed IL-10 production induced by DHA (Supplementary Figure 7C–D). These data suggest that Blimp1 mediates GPR120 induction of IL-10 through the mTOR-Stat3 pathway.

5. Blimp1 is essential for GPR120 inhibition of T effector cell induction of colitis.

We then investigated whether Blimp1 is essential for GPR120 inhibition of T effector cell induction of colitis. We treated Blimp1-deficient and WT $CD4^+$T cells from *Cd4*^{cre}*Prdm1*$^{fl/fl}$ mice and *Cd4*^{cre}*Prdm1*$^{fl/+}$ mice with or without the CpdA for 5 days, then transferred them into *Rag*$^{-/-}$ mice. *Rag*$^{-/-}$ recipient mice of CpdA-treated WT Th1 cells developed less severe colitis and produced less TNF-α, IL-6, and IFN-γ, but more IL-10 in the colon than those with WT Th1 cells (Figure 4P–S). However, both CpdA-treated Blimp1-deficient Th1 cells and control Blimp1-deficient Th1 cells induced severe colitis and led to similar levels of pro-inflammatory cytokines in the colon of *Rag*$^{-/-}$ recipient mice (Figure 4P–S). While intestinal IFN-γ^+CD4^+T cells were decreased, and IL-10^+CD4^+T cells were increased in *Rag*$^{-/-}$ mice reconstituted with CpdA-treated WT Th1 cells compared with the mice receiving control WT Th1 cells, similar levels of intestinal IL-10^+CD4^+T cells and IFN-γ^+CD4^+T cells were present in recipient mice receiving control Blimp1-deficient Th1 cells or CpdA-treated Blimp1-deficient Th1 cells (Supplementary Figure 11A–B, and E). All the recipient mice showed similar levels of intestinal IL-17^+CD4^+T cells and $Foxp3^+$Tregs (Supplementary Figure 11A, and C–D).

6. Glycolysis is involved in GPR120 induction of IL-10 in $CD4^+$T cells.

Because transcriptome data indicated that GPR120 affects the metabolism of $CD4^+$T cells (Figure 3B and Supplementary Figure 4C), we next investigated whether GPR120 modulates mitochondrial oxidation and glycolysis, the major events of metabolism, which differentially regulate T cell functions[31]. First, we measured the oxygen consumption rate (OCR), primarily attributed to mitochondrial oxidation, and the extracellular acidification rate (ECAR), which represents glycolysis, in $CD4^+$T cells using an extracellular flux Seahorse analyzer. GPR120 agonist, CpdA, enhanced OCR and ECAR levels in both naïve T cells (Supplementary Figure 12A–D) and anti-CD3/anti-CD28-activated T cells (Supplementary Figure 12E–H). We also checked the key parameters of mitochondrial oxidation using the Mito stress test kit. CpdA-treated $CD4^+$T cells exhibited enhanced OCR with higher basal respiration, ATP-related respiration, maximal respiration, and spare respiratory capacity than control T cells (Figure 5A–B). To further assess the key parameters of glycolytic flux and exclude the non-glycolytic acidification, we measured ECAR levels in activated $CD4^+$T cells using the Glycolysis stress test kit. CpdA promoted glycolysis and glycolytic capacity but not glycolytic reserve, and slightly increased the ECAR levels related to the non-glycolytic activity (Figure 5C–D). In line with elevated glycolysis, CpdA promoted $CD4^+$T cell glucose uptake (Supplementary Figure 12I–J).

Glycolysis has been shown to regulate IL-10 production in several types of immune cells[32, 33]. We then investigated whether GPR120-enhanced glycolysis contributes to upregulated IL-10 production in $CD4^+$T cells. We found that blocking glycolysis suppressed IL-10 production induced by CpdA and DHA (Figure 5E–G, and Supplementary Figure 7C–D). Additionally, inhibition of the mTOR using rapamycin inhibited CpdA-induced

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glycolysis and glycolytic capacity (Figure 5H–I) and glucose uptake (Supplementary Figure 12K–L), suggesting that GPR120 activation of mTOR regulates glycolysis. Glycolysis has been reported to promote HIF1α expression[34], which increases IL-10 production in B cells [33]. KEGG pathway enrichment analysis suggested that GPR120 regulates the HIF1 signaling pathway in T cells (Figure 3B), and GPR120 agonist CpdA treatment promoted HIF1α expression in $CD4^+$T cells (Supplementary Figure 10), which was suppressed by the glycolysis inhibitor, 2-DG (Figure 5J). Furthermore, treatment with YC-1, a HIF1α inhibitor, decreased CpdA-induced IL-10 production (Figure 5K–M). Collectively, these data indicate that GPR120-elevated HIF1α expression also contributes to GPR120 induction of IL-10.

7. Oral feeding of GPR120 agonist inhibits colitis.

As the GPR120 agonist promoted $CD4^+$T cell production of IL-10 and GPR120 regulates intestinal inflammation, we then investigated whether administering the GPR120 agonist could prevent and treat colitis. We first transferred WT $CD4^+CD45RB^{hi}$T cell to *Rag*$^{-/-}$ mice and gave the mice orally with or without CpdA daily from the day of cell transfer until the mice were sacrificed. Administering CpdA suppressed colitis, characterized by less weight loss, increased colon length, decreased pathology scores, and inhibited TNF-α, IL-6, IFN-γ, and IL-17A, but promoted IL-10 production in the colon (Figure 6A–F). However, CpdA did not alleviate colitis severity in *Rag*$^{-/-}$ recipients of GPR120-deficient $CD4^+CD45RB^{hi}$ T cells (Supplementary Figure 13A–F), suggesting that $CD4^+$T cells are indispensable in GPR120 regulation of colitis. Similar results were obtained in mice infected with *C. rodentium*, which were treated orally with or without CpdA daily for 10 days. The CpdA-treated mice developed less severe colitis with less weight loss, lower intestinal bacteria load, decreased pathological scores, and lower pro-inflammatory cytokines in the intestine than those treated with carrier alone (Supplementary Figure 14A–E). Administering CpdA promoted IL-10 and IL-22 production in the colon and increased intestinal expression of antimicrobial peptide regenerating islet-derived 3 gamma (Reg3γ) (Supplementary Figure 14E–F). Furthermore, CpdA treatment decreased intestinal IFN-γ^+CD4^+T cells and IL-$17A^+CD4^+$T cells but increased intestinal IL-10^+CD4^+T cells and IL-22^+CD4^+T cells (Supplementary Figure 14 G–K). However, CpdA treatment did not affect the levels of intestinal $Foxp3^+$Treg (Supplementary Figure 14 G and L).

Next, we investigated whether GPR120 agonist treats colitis. We transferred WT $CD4^+CD45RB^{hi}$T cells into *Rag*$^{-/-}$ mice. Two weeks after cell transfer, when mice have developed mild colitis (Supplementary Figure 15), the mice were treated orally with or without CpdA daily for additional 4 weeks. Administering CpdA inhibited colitis progression with decreased weight loss, increased colon length, lower pathological scores, and lower pro-inflammatory cytokine production but increased IL-10 production in the intestine than carrier alone-treated mice (Figure 6 G–L). Collectively, these data suggest that GPR120 agonist prevents and treats colitis.

8. GPR120 is positively correlated with IL-10 in IBD patients.

To investigate whether GPR120 is differentially expressed in the intestinal mucosa of IBD patients, we retrieved the data from GSE11223 in the GEO database. *Gpr120* expression

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values were increased in the colonic mucosa of UC patients compared with healthy controls (Figure 7A). The higher expression of intestinal GPR120 in UC patients suggests the potential for oral GPR120 agonists as therapeutics in IBD patients. Additionally, colonic *Gpr120* expression values were positively correlated with *Il10* in healthy controls, and there was a tendency correlation in UC patients (Figure 7B–C). To further verify the correlation between colonic GPR120 and IL-10 expression, we collected colonic biopsies from 14 healthy controls, 10 active UC patients, and 21 active CD patients, and measured GPR120 and IL-10 expression. Consistent with the result retrieved from the database, *Gpr120* expression was positively correlated with *Il10* expression in colonic biopsies of healthy controls and UC patients (Figure 7D–E). CD patients also showed the correlation between *Gpr120* and *Il10* in the mucosa (Figure 7F).

Discussion

Dietary regulation has been shown to play essential roles in health and diseases. GPR120, the receptor for ω-3 FA, has been implicated in regulating metabolic diseases and several types of immune cells[16, 19]. However, the role of GPR120 in the regulation of intestinal inflammation is unknown. In this study, we demonstrated that GPR120 promotes $CD4^+$T cell production of IL-10 by upregulating Blimp1 and enhancing glycolysis to inhibit colitis, thus, providing novel insights into a critical role for GPR120 in maintaining intestinal homeostasis through the modulation of $CD4^+$T cell functions.

Dietary ω-3 FA are beneficial for preventing metabolic syndrome and inflammation[12]. As a receptor of ω-3 FA, GPR120 regulates insulin signaling and inflammation in adipose tissue and controls the energy balance[17, 19]. GPR120 agonist increases insulin sensitivity, indicating that GPR120 might be a promising target for diabetes[16, 20, 35]. The intestinal tract directly interacts with dietary components, and several intestinal disorders have been linked to diet[14]. Nevertheless, the exact role of diet in IBD pathogenesis remains poorly understood. ω-3 FA consumption is negatively associated with IBD incidence[15]. We found that DHA, a ω-3 FA, promoted T cell production of IL-10. It has been reported that dietary intake of ω-3 FA increased the blood levels of ω-3 FA[36], suggesting that dietary ω-3 FA could be absorbed in the intestine and transferred into circulation to regulate circulating $CD4^+$T cell production of IL-10. IL-10^+CD4^+T cells could be recruited to the inflamed intestines to suppress colitis. It is also possible that dietary ω-3 FA might directly affect $CD4^+$T cell functions in the gut.

GPRs are actively involved in most physiological responses in humans and have become one of the successful targets of pharmaceuticals[37]. Previous studies have demonstrated the potential treatment of the GPR120 agonist for treating diabetes and metabolic syndrome[16, 20, 35, 37]. We showed that *Gpr120*$^{-/-}$ mice developed more severe colitis upon inflammatory insult and enteric infection, and oral administration of the GPR120 agonist prevented and treated colitis, indicating that GPR120 agonists might be potential therapeutic targets for IBD.

Excessive $CD4^+$T cell responses have been considered critical in driving intestinal inflammation, whereas IL-10 restricts pro-inflammatory responses to maintain intestinal

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homeostasis. IL-10 produced by $CD4^+$ effector T cells exerts a self-limiting mechanism to prevent an exaggerated T cell response in the intestines, which otherwise would be detrimental[38]. Although several types of cells, including IECs, macrophages, and adipocytes, have been shown to express GPR120, it was unknown whether $CD4^+$T cells express GPR120. We demonstrated that activated $CD4^+$T cells express GPR120 at high levels, and $CD4^+$T cell-specific GPR120 knockout mice developed more severe colitis. Furthermore, transfer of GPR120-deficient $CD4^+CD45Rb^{hi}$T cells induced more severe colitis than WT $CD4^+CD45Rb^{hi}$T cells with lower levels of IL-10 in the intestine. GPR120 agonist treatment inhibited WT but not IL-10-deficient T cell induction of colitis, indicating that GPR120 promotes T cell IL-10 production to inhibit colitis development. It has been shown that the lack of IL-10 in $CD4^+$T cells worsens colitis but does not affect or slightly increases the clearance of *C. rodentium*[39, 40]. In our study, GPR120 agonist also increases IL-22 production and Reg3γ expression in the intestine, which could promote the intestinal clearance of *C. rodentium*[41]. Meanwhile, higher levels of IL-10 suppress excessive inflammation. The combination of IL-10 and IL-22 might contribute to the decreased severity of colitis and enhanced *C. rodentium* clearance. Variants in *Prdm1*, which encodes Blimp1, have been associated with the susceptibility of developing IBD[42]. Blimp1 plays an essential role in promoting IL-10 production in $CD4^+$T cells[43]. We showed that GPR120 agonist inhibited WT but not $Blimp^{-/-}$ Th1 cell induction of colitis with higher levels of IL-10 production, indicating that Blimp1 mediates GPR120 regulation of colitis through IL-10 production.

Different $CD4^+$T cells require distinct metabolic programs compatible with their functional demands. While quiescent $CD4^+$T cells are characterized by mixed fuel oxidative phosphorylation, activated T cells become more glycolytic with increased oxidative phosphorylation for fulfilling the requirement of rapid cell growth and proliferation[31]. Metabolic programs have been shown to involve and regulate $CD4^+$T cell functions. However, how the metabolic pathways affect T cell production of IL-10 has not been fully defined. Our study found that the GPR120 agonist increased $CD4^+$T cell oxygen consumption and glycolysis, leading to higher ATP production and energy levels. Blocking glycolysis compromised GPR120-induced IL-10 production, indicating that enhanced glycolysis mediates GPR120-promotion of IL-10 production in $CD4^+$T cells. Additionally, the GPR120 agonist increased T cell expression of HIF1α expression. Although HIF1α has been reported to suppress mitochondrial respiration[44], the increased oxidation in $CD4^+$T cells might be attributed to other pathways affected by GPR120. A recent report showed that distinct mitochondrial metabolism determines $CD4^+$T cell differentiation and function[45]; thus, it will be interesting to investigate whether the mode of mitochondrial metabolism is also involved in GPR120 induction of IL-10 in T cells.

In summary, our study demonstrated that GPR120 suppresses $CD4^+$T cell induction of colitis through promoting IL-10 production. Oral administration of the GPR120 agonist inhibits colitis, which presents GPR120 as a potential therapeutic target for IBD treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BMDCs	Bone-marrow dendritic cells
C. rodentium	*Citrobacter rodentium*
DCs	Dendritic cells
DSS	Dextran sulfate sodium
DHA	Docosahexaenoic acid
ECAR	Extracellular acidification rate
GPR	G protein-coupled receptor
IL-10R	IL-10 receptor
IBD	Inflammatory bowel disease
IECs	Intestinal epithelial cells
LP	Lamina propria
LCFA	Long-chain fatty acids
ω-3 FA	Omega 3 fatty acids
OCR	Oxygen consumption rate
Tregs	Regulatory T cells
RNA-Seq	RNA sequencing

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT:

G protein-coupled receptor (GPR) 120 has been implicated in regulating metabolic syndromes with anti-inflammatory function. However, the role of GPR120 in the regulation of intestinal inflammation is unknown.

NEW FINDINGS:

We discover that GPR120 induces IL-10 production in $CD4^+$T cells through upregulation of Blimp1 and enhancing glycolysis to inhibit colitis. Administration of GPR120 agonist protected mice against intestinal inflammation.

LIMITATIONS:

A limitation of the current study is that it was mostly performed in animal models. The effect of GPR120 in human $CD4^+$T cells and patients with inflammatory bowel disease (IBD) needs to be further validated in the clinical setting.

IMPACT:

This study identifies GPR120 as a potential therapeutic target for treating IBD.

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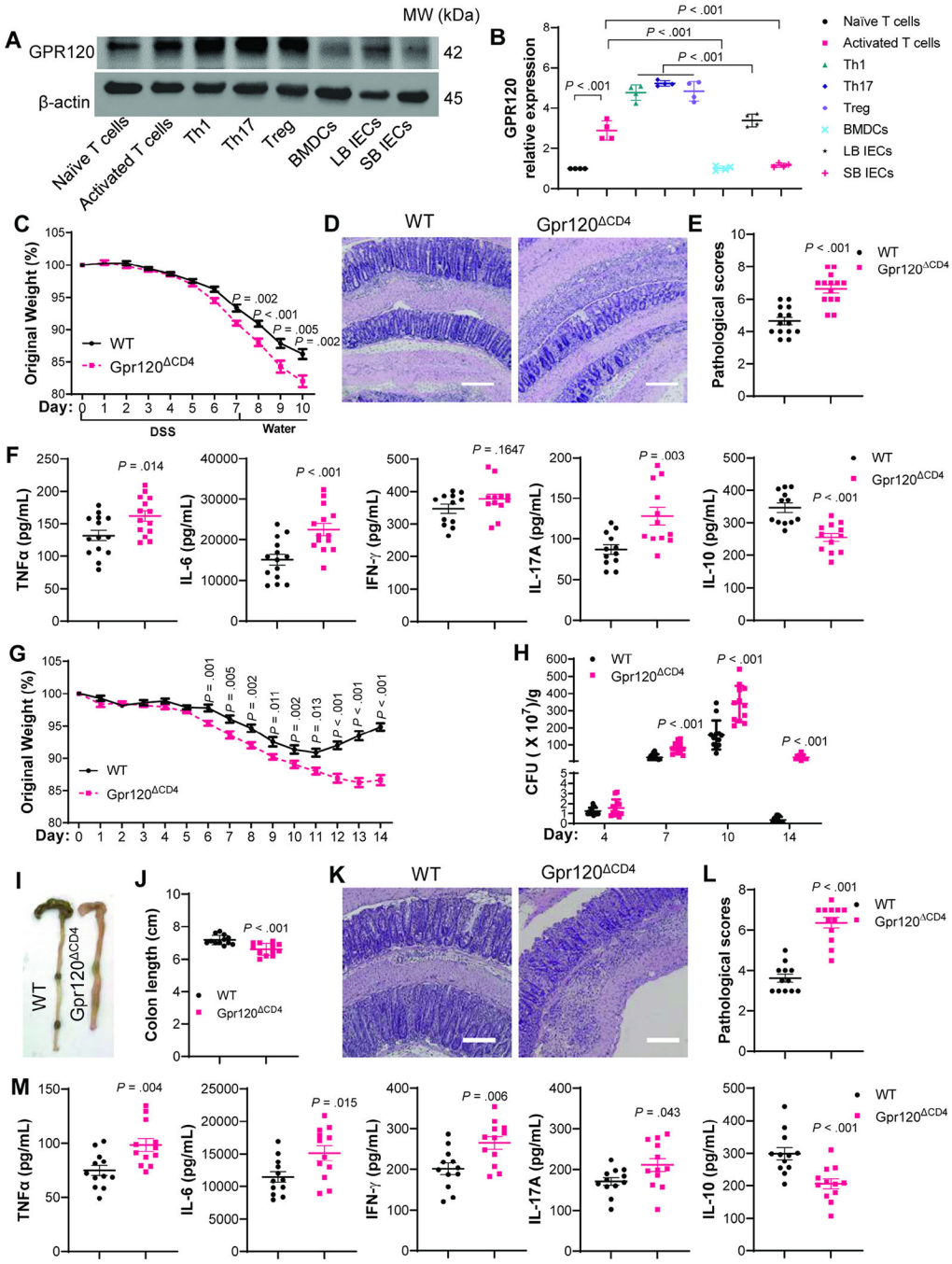

Fig. 1. CD4^{+}T cell-specific GPR120 KO mice develop more severe colitis upon DSS insult and *C. rodentium* infection.

(A–B) GPR120 expression was measured in naïve CD4^{+}T cells, activated CD4^{+}T cells, Th1, Th17, Treg, bone-marrow dendritic cells (BMDCs), large bowl epithelial cells (LB IECs), and small bowel epithelial cells (SB IECs) (N=4/group). Western bolts (A) and GPR120 protein relative expression (B). (C–F) *Cd4*^{cre}*Gpr120*$^{fl/+}$ (WT) mice and *Cd4*^{cre}*Gpr120*$^{fl/fl}$ (*Gpr120*$^{\Delta CD4}$) mice (N=14/group) were administrated with 1.65% DSS (w/v) in drinking water for 7 days followed by drinking water alone for additional 3 days. (C) Mouse weight change. (D) Representative intestinal H&E staining. (E) Pathological score. (F)

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Colonic secretion of cytokines. (G–M) *Cd4*^{cre}*Gpr120*$^{fl/+}$ (WT) mice and *Cd4*^{cre}*Gpr120*$^{fl/fl}$ (*Gpr120*$^{\Delta CD4}$) mice (N=12/group) were orally infected with *C. rodentium* on day 0, and sacrificed on day 14. (G) Weight change. (H) Fecal *C. rodentium* counts. (I) Representative gross morphology of the colon. (J) Colon length. (K) Representative intestinal H&E staining. (L) Pathological score. (M) Colonic secretion of cytokines. All data are pooled from three independent experiments. (D and K) Scale bar, 300 μm. (A) one-way ANOVA; (C, F, G, H, J, and M) unpaired Student t-test; (E and L) Mann–Whitney U test.

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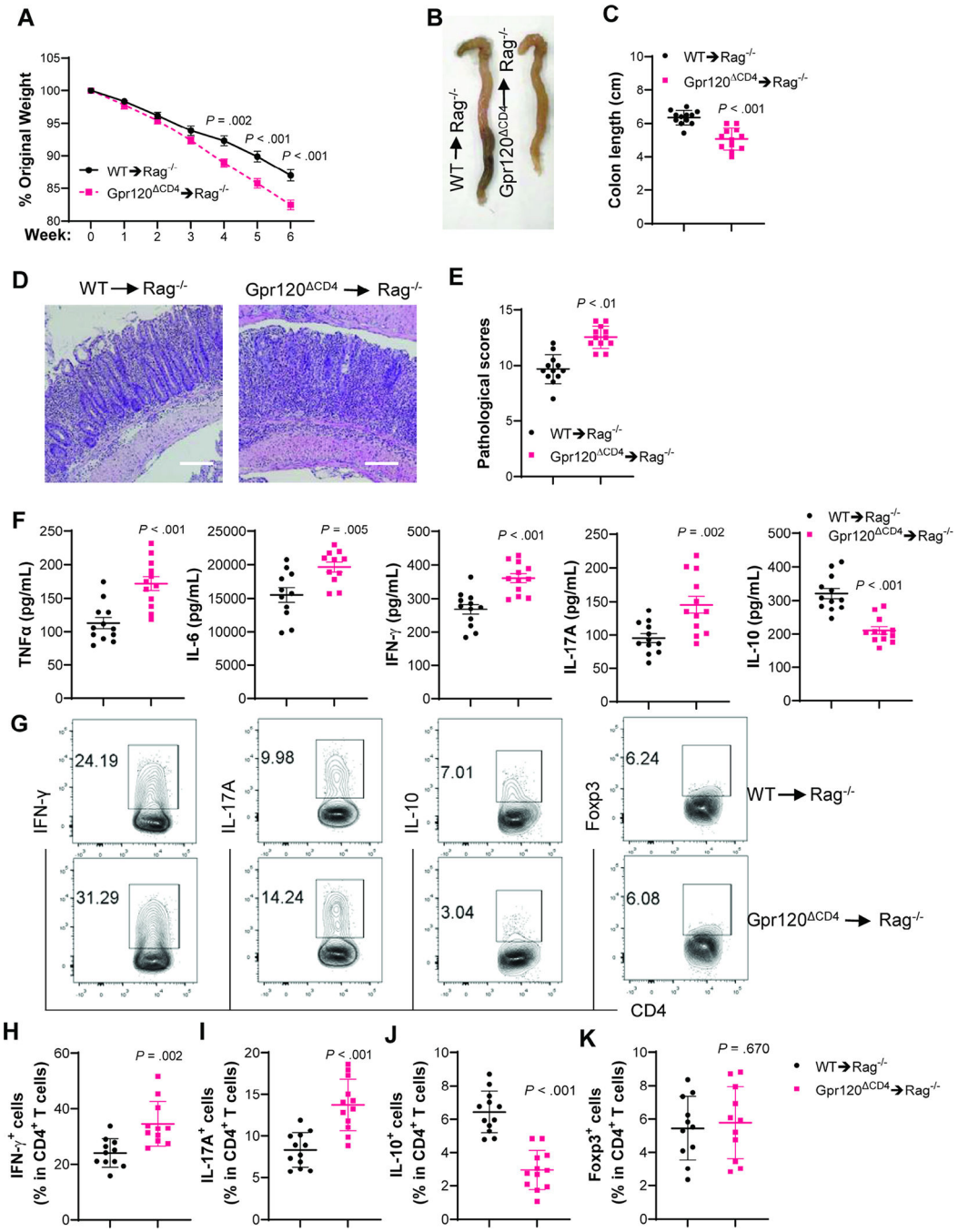

Fig. 2. GPR120-deficient CD4$^+$CD45RB^{hi}T cells induce more severe colitis in *Rag*$^{-/-}$ mice.

WT and GPR120-deficient CD4$^+$CD45Rb^{hi}T cells (1×10^5 cells/mouse) were intravenously transferred to *Rag*$^{-/-}$ mice (N=12/group). (A) Mouse weight changes. (B) Representative gross morphology of the colon. (C) Colon length. (D) Representative intestinal H&E staining. (E) Pathological score. (F) Colonic secretion of cytokines. (G) Representative flow cytometry profile of LP CD4$^+$T cells. (H–K) Bar charts of IFNγ^+, IL-17A$^+$, IL-10$^+$, and Foxp3$^+$CD4$^+$T cells. All data are pooled from three independent experiments. (D) Scale bar, 300 μm. (A, C, F, and H–K) unpaired Student t-test; (E) Mann–Whitney U test.

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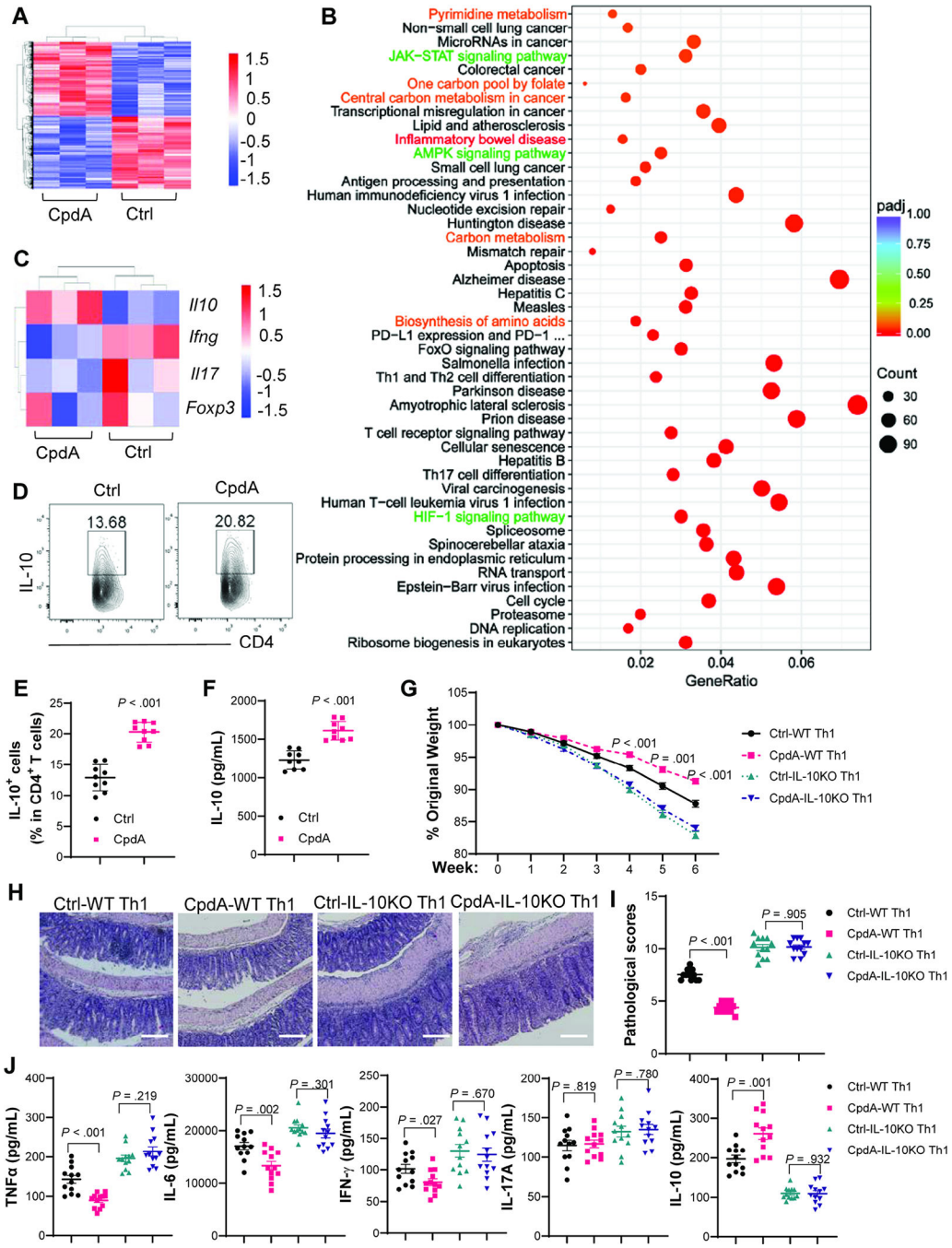

Fig. 3. GPR120 promotes CD4^{+}T cell production of IL-10 to suppress colitis.

(A–C) Splenic CD4^{+}T cells were activated with anti-CD3/anti-CD28±CpdA for 48 hours to analyze gene expression by RNA-Seq (N=3/group). (A) Differentially expressed genes between CD4^{+}T cells treated with or without CpdA in Heatmap. Arbitrary units. (B) KEGG pathway enrichment analysis. (C) Specific gene expressions in Heatmap. Arbitrary units. (D–F) CD4^{+}T cells were activated with anti-CD3/anti-CD28±CpdA under Th1 conditions (N=9/group). Flow cytometry profile of IL-10^{+}CD4^{+}T cells after 5 days (D–E). IL-10 in culture supernatants after 2 days (F). (G–J) WT and IL-10$^{-/-}$ CD4^{+}T cells were activated

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with anti-CD3/anti-CD28±CpdA under Th1 conditions for 5 days and transferred to *Rag*$^{-/-}$ mice (N=12/group). (G) Mouse weight change. (H) Representative intestinal H&E staining. (I) Pathological score. (J) Colonic secretion of cytokines. (D–J) Data were pooled from three independent experiments. (H) Scale bar, 300 μm. (E, F, G, and J) unpaired Student's *t*-test; (I) Mann–Whitney U test.

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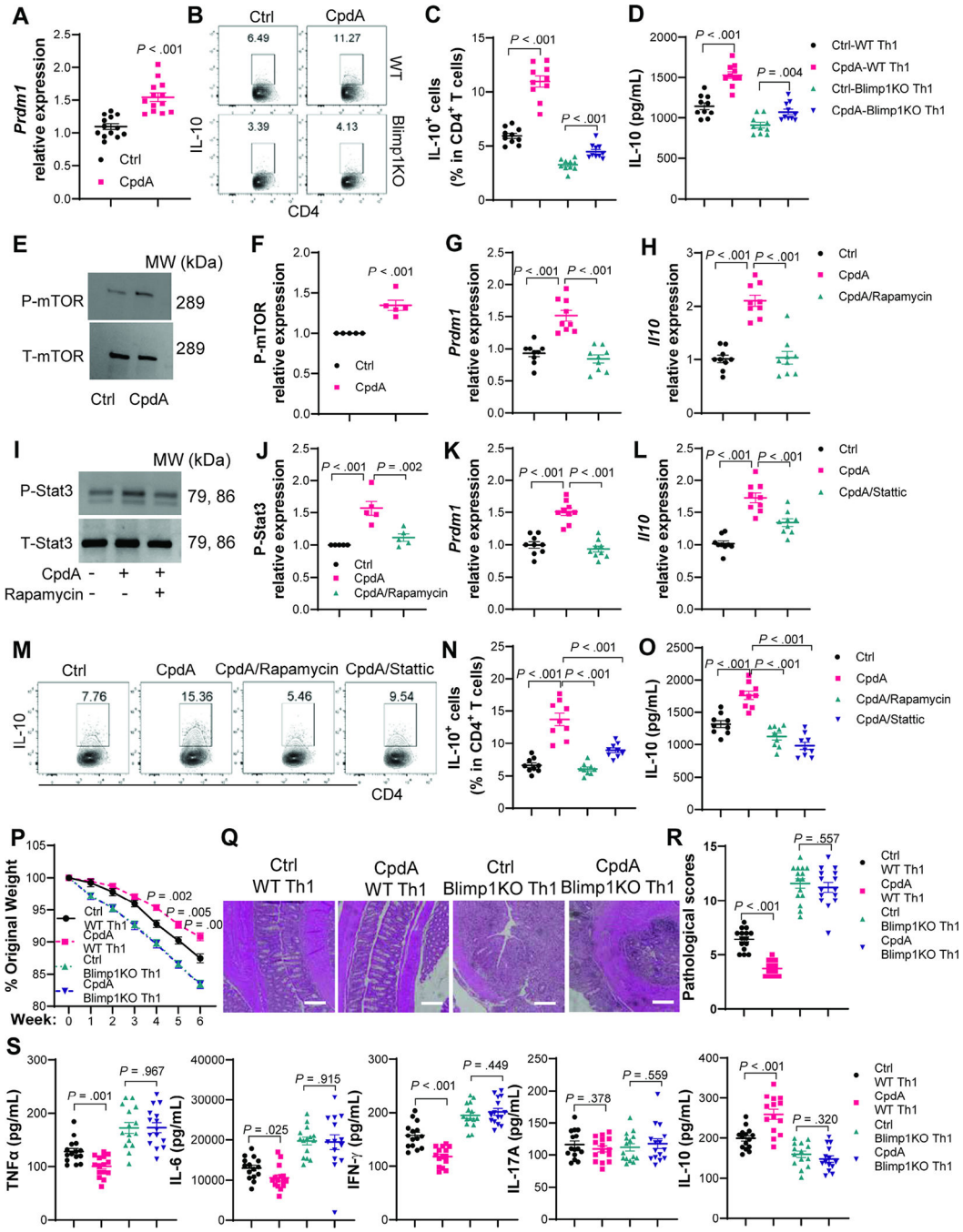

Fig. 4. Blimp1 mediates GPR120 induction of IL-10 through the mTOR-Stat3 pathway.

Splenic CD4$^+$T cells were activated with anti-CD3/anti-CD28±CpdA and various inhibitors under Th1 conditions. (A) *Prdm1* mRNA expression on day 2 (N=13/group). (B–C) Flow cytometry profile of IL-10$^+$CD4$^+$T cells in WT and Blimp$^{-/-}$ CD4$^+$T cells on day 5. (D) IL-10 in culture supernatants on day 2 (N=10/group). (E–F) Phosphorylated and total mTOR expressions at 30 minutes (N=5/group). (G) *Prdm1* and (H) *Il10* mRNA expression in CD4$^+$T cells treated with or without CpdA±rapamycin on day 2 (N=9/group). (I–J) Phosphorylated and total Stat3 expressions at 2 hours in CD4$^+$T cells treated with or without

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CpdA±rapamycin (N=5/group). (K) *Prdm1* and (L) *Il10* mRNA expression in $CD4^+$T cells treated with or without CpdA±Static (N=9/group). (M–N) Flow cytometry profile of IL-10^+CD4^+T cells on day 5, and (O) IL-10 in culture supernatants on day 2 (N=9/group). (P–S) WT and Blimp$1^{-/-}$ $CD4^+$T cells from *Cd4*^{cre}*Prdm1*$^{fl/+}$ mice and *Cd4*^{cre}*Prdm1*$^{fl/fl}$ mice were activated with anti-CD3/anti-CD28±CpdA under Th1 conditions for 5 days and transferred to *Rag*$^{-/-}$ mice (N=15/group). (P) Mouse weight change. (Q) Representative intestinal H&E staining. (R) Pathological score. (S) Colonic secretion of cytokines. All data are pooled from three independent experiments. (A, C, D, F, J, P, and S) unpaired Student's *t*-test; (G, H, N, O) one-way ANOVA; (R) Mann–Whitney U test.

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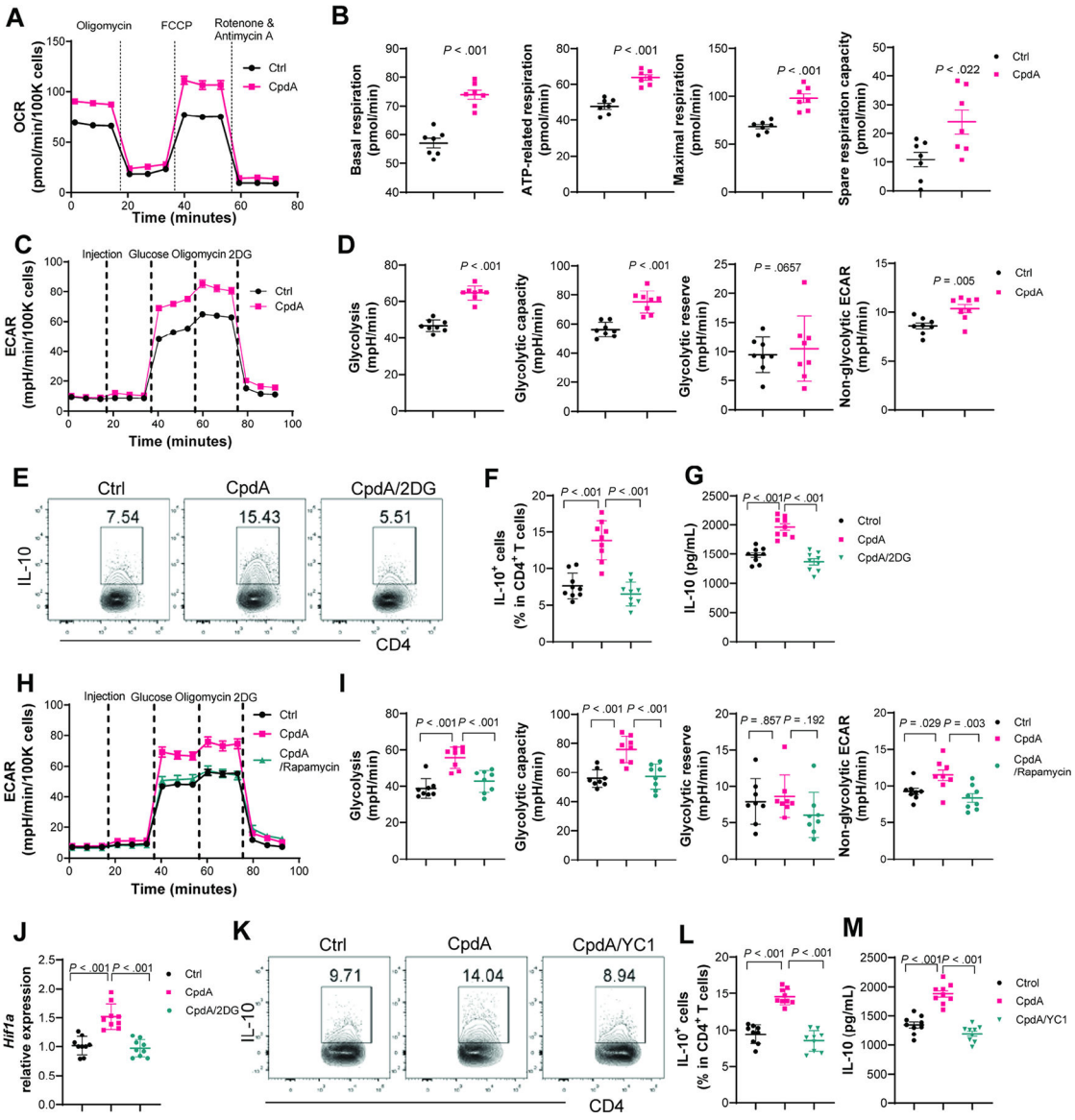

Fig. 5. GPR120 agonist promotes IL-10 production through the glycolysis-HIF1α pathway.

(A–B) Splenic CD4$^+$T cells were activated with anti-CD3/anti-CD28±CpdA under Th1 conditions for 48 hours, and the Mito stress test kit was used to measure the parameters of mitochondrial respiration (N=7/group). The OCR profile, basal respiration, ATP-related respiration, maximum respiration, and spare respiration capacity. (C–D, and H–I) Splenic CD4$^+$T cells were activated with anti-CD3/anti-CD28 under Th1 conditions for 48 hours, and a Glycolytic stress test kit was used to measure the key parameters of glycolysis (N=8/group). The ECAR profile, glycolysis, glycolytic capacity, glycolytic reserve, and non-glycolytic ECAR. (E–G) CD4$^+$T cells were treated with or without CpdA±2-DG (N=9/group). IL-10$^+$CD4$^+$T cells on day 5 (E–F), and IL-10 in culture supernatants on day 2 (G). (J) *Hif1a* mRNA expression in CD4$^+$T cells treated with or without CpdA±2-DG at 48 hours (N=9/group). (K–M) CD4$^+$T cells were treated with or without CpdA±YC-1 (N=9/group). Flow cytometry profile of IL-10$^+$CD4$^+$T cells at day 5 (K–L) and IL-10 production in

culture supernatants on day 2 (M). (E–G, and J-M) Data are pooled from three independent experiments. (B and D) Unpaired Student's *t*-test (F, G, I, L, and M) one-way ANOVA.

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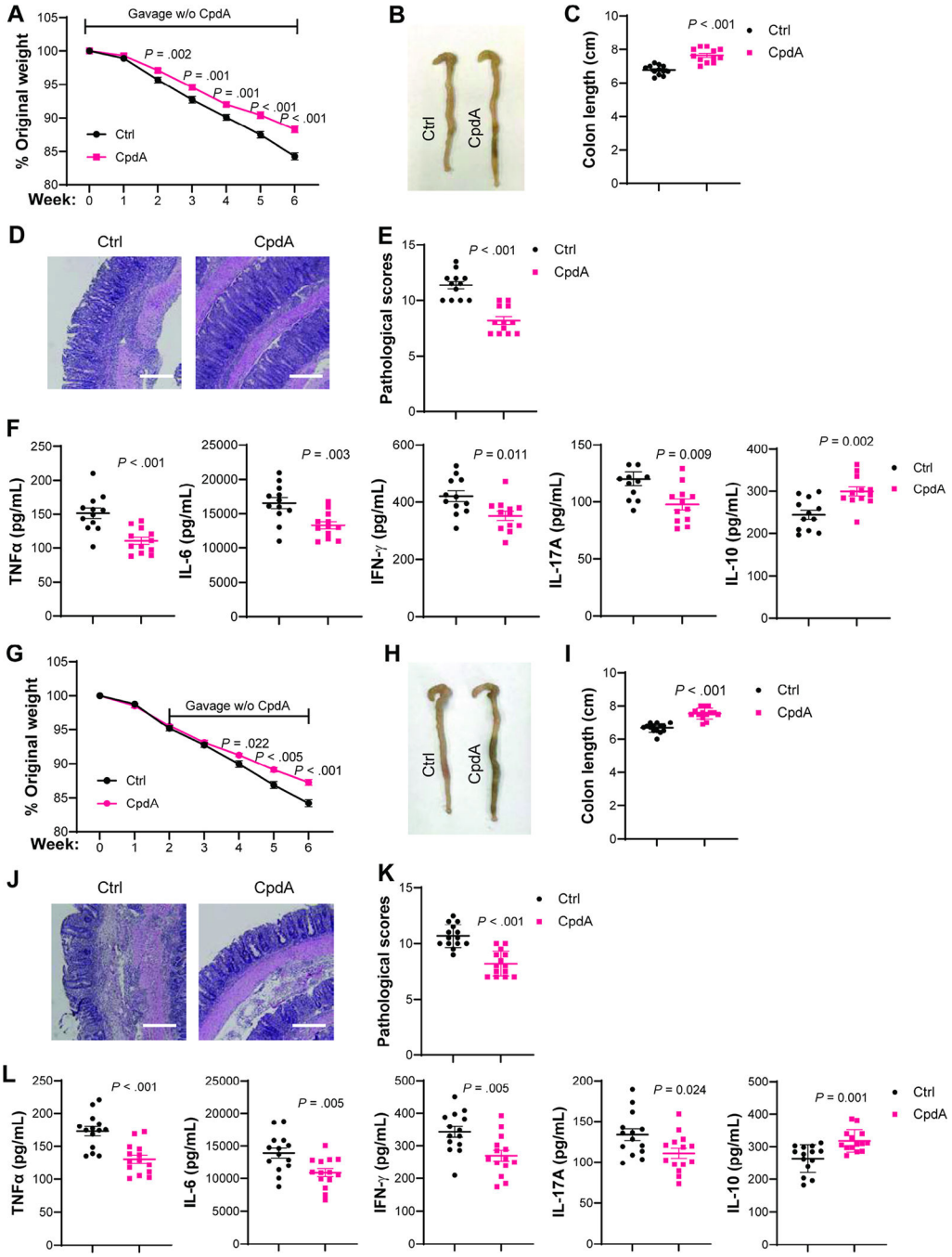

Fig. 6. Oral feeding of GPR120 agonist prevents and treats colitis.

(A–F) WT $CD4^+CD45Rb^{hi}T$ cells (1×10^5 cells/mouse) were intravenously transferred to *Rag*$^{-/-}$ mice and orally administered with or without CpdA (N=12/group) daily from the day of cell transfer until the mice were sacrificed 6 weeks after cell transfer. (A) Mouse weight change. (B) Gross morphology of colon. (C) Colon length. (D) Representative intestinal H&E staining. (E) Pathological score. (F) Colonic secretion of cytokines. (G–L) WT $CD4^+CD45Rb^{hi}T$ cells (1×10^5 cells/mouse) were intravenously transferred to *Rag*$^{-/-}$ mice and orally administered with or without CpdA (N=12/group) daily from 2 weeks post

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cell transfer for additional 4 weeks. (G) Mouse weight change. (H) Gross morphology of colon. (I) Colon length. (J) Representative intestinal H&E staining. (K) Pathological score. (L) Colonic secretion of cytokines. All data are pooled from two independent experiments. (D and J) Scale bar, 300 μm. (A, C, F, G, I, and L) unpaired Student t-test; (E and K) Mann–Whitney U test.

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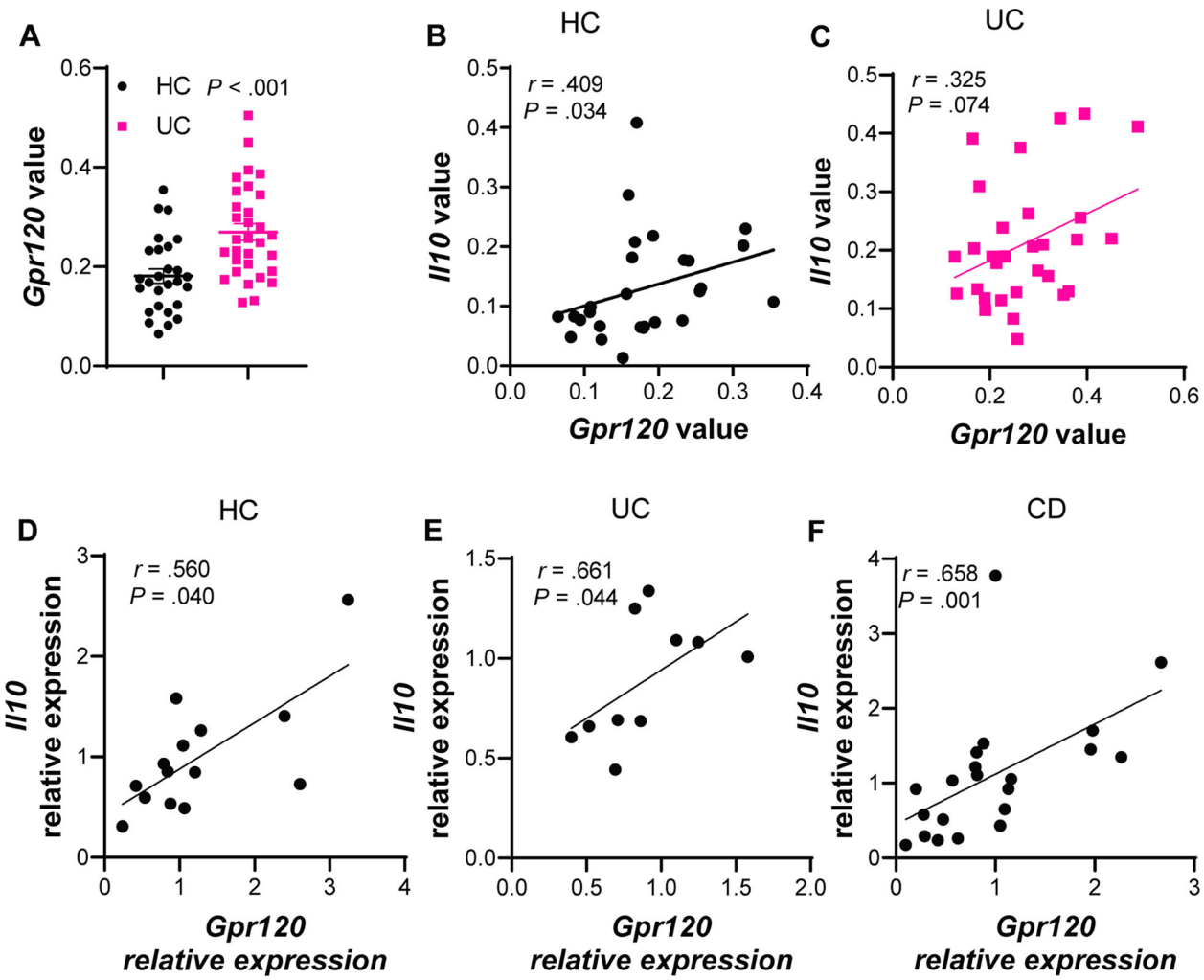

Figure 7. *Gpr120* is positively correlated with *Il10* in human colonic mucosa.

(A–C) The data from GSE11223 in the GEO database were retrieved. (A) Gpr120 expression in the colonic mucosa of healthy controls (HC, N=27) and patients with ulcerative colitis (UC, N=31). (B–C) The correlation between colonic *Gpr120* and *Il10* in HC (B) and UC patients (C). (D–F) Intestinal biopsies were collected from HC (N=14), UC patients (N=10), and patients with Crohn's disease (CD, N=21). The correlation between intestinal *Gpr120* and *Il10* in HC (D), UC patients (E), and CD patients (F). (A) unpaired Student t-test; (B–F) Spearman correlation.