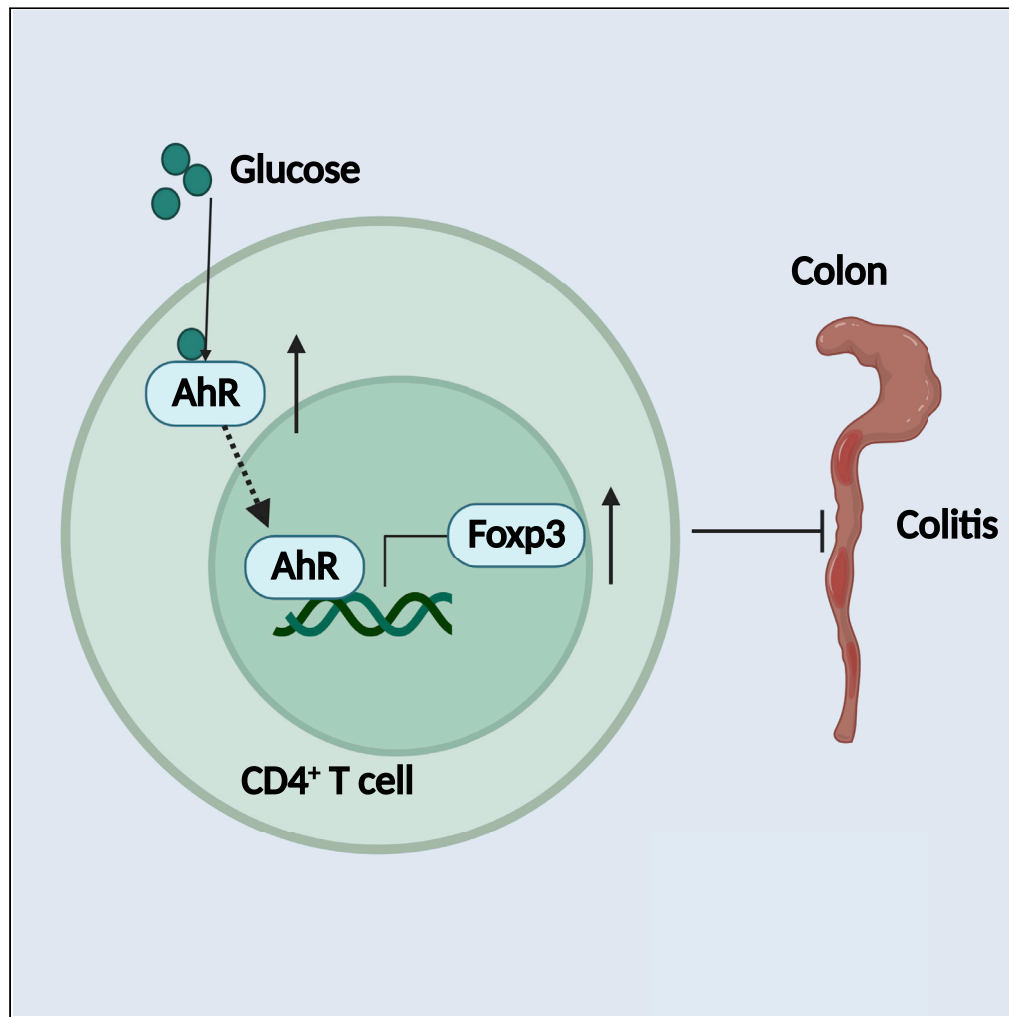


## Article

## Glucose promotes regulatory T cell differentiation to maintain intestinal homeostasis



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**Highlights**

A moderate dose of  
glucose suppresses  
intestinal inflammation

Glucose promotes Treg  
differentiation but not  
Treg stability

Microbiota are not  
involved in the glucose  
induction of Treg cells

AhR mediates the glucose  
induction of Treg cells

## Article

## Glucose promotes regulatory T cell differentiation to maintain intestinal homeostasis

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## SUMMARY

**Glucose, the critical energy source in the human body, is considered a potential risk factor in various autoimmune diseases when consumed in high amounts. However, the roles of glucose at moderate doses in the regulation of autoimmune inflammatory diseases and CD4<sup>+</sup> T cell responses are controversial. Here, we show that while glucose at a high concentration (20% w/v) promotes intestinal inflammation, it suppresses colitis at a moderate dose (6% w/v), which increases the proportion of intestinal regulatory T (Treg) cells but does not affect effector CD4<sup>+</sup> T cells. Glucose treatment promotes Treg cell differentiation but it does not affect Treg stability. Feeding glucose alters gut microbiota compositions, which are not involved in the glucose induction of Treg cells. Glucose promotes aryl hydrocarbon receptor (AhR) activation to induce Treg polarization. These findings reveal the different effects of glucose at different doses on the intestinal immune response.**

## INTRODUCTION

It has been well-established that chronic intestinal inflammation is caused by a dysregulated host immune response to microbiota that involves a state of local immune hyper-reactivity (Zhang et al., 2017b), in which the mucosal CD4<sup>+</sup> T cell responses to gut microbiota antigens are crucial (Bamias et al., 2017; Elson et al., 2005; Mizoguchi et al., 2016). Among T cells, Th1 cells and Th17 cells have been implicated in the pathogenesis of IBD, whereas regulatory T (Treg) cells play a critical role in maintaining intestinal homeostasis and inhibiting intestinal inflammation (Barnes and Powrie, 2009; Raza et al., 2012; Wallace et al., 2014). It has been shown that Treg cells were involved in immune regulation by inhibiting the proliferation and cytokine production of effector T cells (Schmidt et al., 2012). Foxp3 has been demonstrated as a crucial transcription factor in driving Treg cell differentiation (Hori et al., 2003). Various environmental factors, including allergens, diet, and microbiota, regulate Treg cells through the regulation of Foxp3 expression (Apostolou et al., 2002; Kretschmer et al., 2005; Lathrop et al., 2011). However, how Treg cells are regulated in the intestines is still not completely understood.

It has been shown that diet strongly influences intestinal immunity by directly regulating mucosal immune cells (Childs et al., 2019; Siracusa et al., 2018) or indirectly by shaping the composition of the gut microbiome and altering the microbial-derived molecules, which are present in the intestinal lumen and circulation (Hubbard et al., 2017; Singh et al., 2017; Zallot et al., 2013). A high sugar diet (HSD) has been associated with several metabolic and autoimmune inflammatory diseases, including IBD, in Western countries (Siracusa et al., 2018). This is likely owing to the effects of an HSD on immune cell responses to autoantigens or gut microbiota antigens (Kang et al., 2009; Wegorzewska et al., 2019), as evidenced by recent studies showing that intake of simple sugars is inclined to induce colitis and promote pathogenesis through the regulation of gut microbiota (Khan et al., 2020). A recent study further showed that high glucose intake could promote Th17 differentiation and exacerbate autoimmunity and intestinal inflammation (Zhang et al., 2019). However, while high dietary sugar poses several health risks, simple sugar plays a critical role in the human body as the most important energy source in our daily life (Slavin and Carlson, 2014). Different types of monosaccharides have been shown to regulate immune response differentially. Although mannose in fruit exhibits strong anti-inflammatory properties, fructose has been shown to induce chronic inflammation (DiNicolantonio et al., 2018; Liu et al., 2020; Zhang et al., 2017a). Glucose serves as a

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primary fuel to generate energy in the cells to carry out their metabolic and biological functions, which is critical in the differentiation of naive T cells into an effector state upon antigen recognition (van der Windt and Pearce, 2012). However, how glucose at moderate levels regulates T cell function and intestinal inflammation is still poorly understood.

In this report, we show that while glucose at a high dose accelerates intestinal inflammation, a moderate amount of glucose alleviates intestinal inflammation in the CD4<sup>+</sup> T cell transfer model of colitis by promoting Treg cell differentiation. Glucose promotes Treg cell differentiation by activating aryl hydrocarbon receptor (AhR). These findings reveal a previously unrecognized function of glucose in maintaining intestinal homeostasis by enhancing Treg cell differentiation.

## RESULTS

### Glucose at different doses differentially affects intestinal inflammation

To investigate whether glucose at a moderate amount affects the pathogenesis of colitis, we induced chronic colitis in *Rag*<sup>-/-</sup> mice by transferring CBir1 TCR transgenic (CBir1 Tg) CD4<sup>+</sup> T cells, which are specific for an immunodominant microbiota antigen CBir1 flagellin (Cong et al., 2009; Yang et al., 2021). Recipient *Rag*<sup>-/-</sup> mice were administered with or without 6% or 20% (w/v) glucose in drinking water on the same day of T cell transfer for the whole duration of the experiments. Six weeks later, the mice were sacrificed, and the intestinal histopathology was examined. CD4<sup>+</sup> T cell phenotypes in the intestinal immune compartment, the mesenteric lymph node (MLN), were analyzed by flow cytometry. Consistent with the previous reports (Khan et al., 2020; Zhang et al., 2019), feeding glucose at 20% (w/v) aggravated colitis development as evidenced by more infiltrates and higher histopathological scores than the control mice (Figures 1A and 1B). However, the recipient mice that received 6% (w/v) glucose developed less severe colitis than the control group, as evidenced by fewer infiltrates and lower histopathological scores (Figures 1A and 1B). Feeding 6% (w/v) glucose increased the percentages of Foxp3<sup>+</sup> Treg cells but not IFN- $\gamma$ <sup>+</sup> and IL-17<sup>+</sup> CD4<sup>+</sup> T cells in the MLN (Figures 1C and 1D), while 20% glucose (w/v) did not affect CD4<sup>+</sup> T cell subsets (Figures 1C and 1D). These data suggest that glucose at different doses differentially regulates intestinal inflammation, which might be attributed to its effect on tipping the balance between Treg cells and effector T cells.

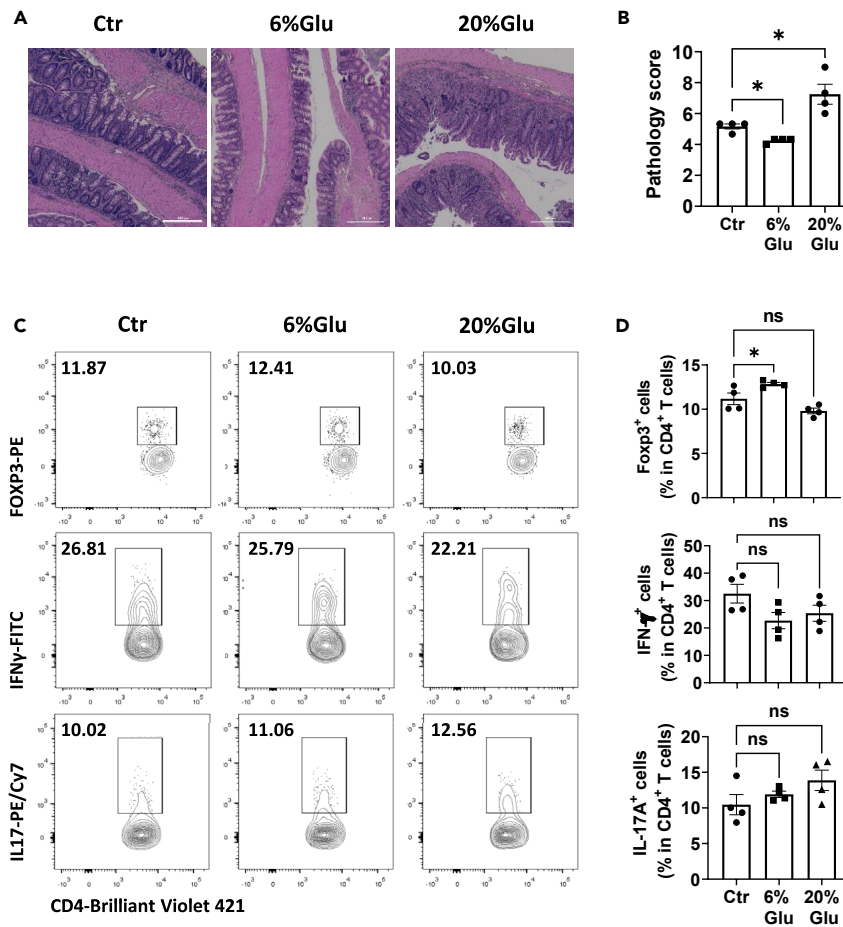
### Glucose induces Treg cells *in vitro*

Next, we asked whether glucose could affect CD4<sup>+</sup> T cell differentiation. To this end, we cultured CBir1 Tg naive CD4<sup>+</sup> T cells with glucose at a series of doses under neutral, Th1, Th17, and Treg cell polarization conditions. Glucose did not affect Th1 or Th17 cell differentiation under Th1 (Figures 2A and 2B) and Th17 conditions (Figures 2C and 2D), respectively. However, glucose promoted Treg cell development in a dose-dependent manner, which peaked at 7000  $\mu$ g/mL (Figures 2E and 2F). Furthermore, IL-10 and TGF $\beta$ , the two characteristic cytokines of Treg cells, were increased after glucose treatment under Treg conditions (Figures 2G and 2H), suggesting that glucose specifically promotes Treg cell differentiation. Then, we determined whether glucose affects T cell activation, apoptosis, and proliferation. Glucose at a dose of 7000  $\mu$ g/mL did not affect CD4<sup>+</sup> T cell activation, apoptosis, or proliferation under Th1 and Treg conditions. However, while glucose did not affect T cell proliferation, it promoted T cell activation and apoptosis under Th17 conditions (Figures S1A–S1E).

To investigate whether the glucose-induced Treg cells inhibits naive T cell proliferation, we treated CBir1 Tg CD4<sup>+</sup> T cells (CD45.2) with or without glucose under Treg conditions for 5 days and then co-cultured with CD45.1<sup>+</sup> naive CD4<sup>+</sup> T cells. We found that glucose promoted Treg cell differentiation to suppress naive CD4<sup>+</sup> T cell proliferation *in vitro* (Figures 2I and 2J).

### Glucose promotes Treg cell differentiation *in vivo*

To investigate whether glucose induces Treg cells under steady conditions *in vivo*, we treated C57BL/6 (B<sub>6</sub>) mice with or without 6% (w/v) glucose in drinking water. Two weeks later, the mice were sacrificed to analyze different subsets of CD4<sup>+</sup> T cells. Consistent with *in vitro* data, glucose increased Treg cells in the MLN (Figures 3A and 3B). However, glucose did not affect the percentages of IFN $\gamma$ -producing and IL-17-producing CD4<sup>+</sup> T cells (Figures 3A and 3B). To extend this finding to intestinal microbiota antigen-specific T cells, we transferred CBir1 Tg naive CD4<sup>+</sup> T cells to *Rag*<sup>-/-</sup> mice. The recipient mice were treated with or without 6% (w/v) glucose in drinking water for 2 weeks. Consistently, glucose promoted CBir1 Tg naive CD4<sup>+</sup> T cell

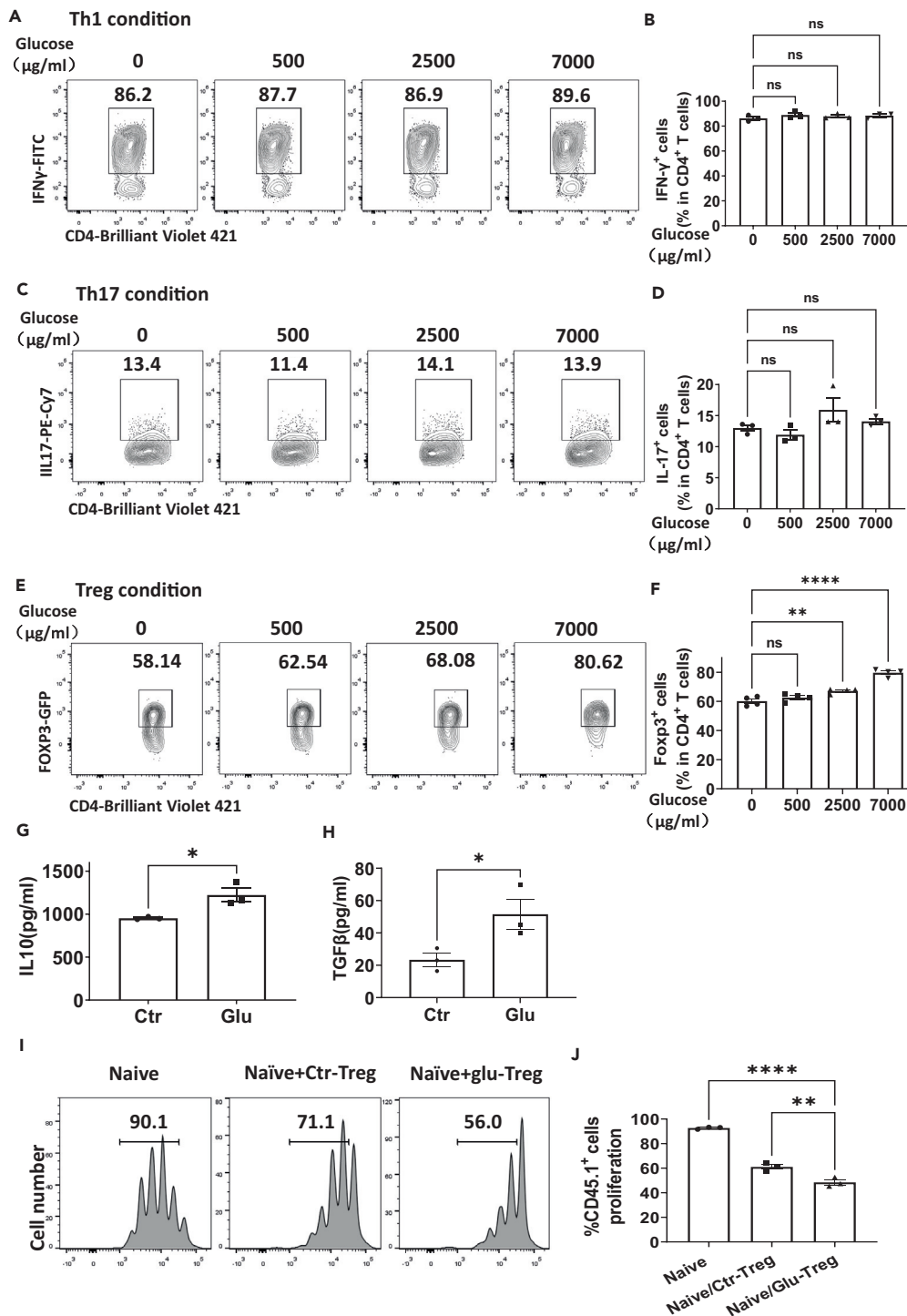


**Figure 1. Glucose at a moderate amount inhibits colitis development**

*Rag*<sup>-/-</sup> mice were transferred with naive CB1r Tg CD4<sup>+</sup> T cells and treated with drinking water (Ctr) or 6%/20% glucose in drinking water (Glu) for six weeks (n = 4/group). Colonic histopathology (A) and pathological scores (B), and representative FACS plots and frequency of CD4<sup>+</sup> Foxp3<sup>+</sup> cells, CD4<sup>+</sup> IFNγ<sup>+</sup> cells, and CD4<sup>+</sup>IL-17<sup>+</sup> cells in MLN were determined (C and D). One representative of three independent experiments with similar results was shown. Scar bar, 300 μm (A). Data were expressed as mean ± SEM. Statistical significance was tested by the non-parametric two-tailed Mann-Whitney U test (B) or two-tailed unpaired Student's t test (D). Scale bar, 100 μm; ns, not significance; \*p < 0.05.

differentiation into Treg cells in MLN (Figures 3C and 3D). In a complementary approach, we transferred CD45.1 naive CD4<sup>+</sup> T cells to immunocompetent CD45.2 WT mice and treated them with or without 6% (w/v) glucose in drinking water for 2 weeks post cell transfer. We found that oral feeding glucose (6% (w/v)) increased the Treg population in CD45.1 CD4<sup>+</sup> T cells (Figures 3E and 3F). These data suggest that moderate amounts of glucose promote Treg cell differentiation.

To investigate whether the glucose-induced Treg cells could suppress intestinal inflammation, we utilized the CD45RB<sup>hi</sup> CD4<sup>+</sup> T cell adoptive transfer model, a well-established colitis model in which transfer of CD45RB<sup>hi</sup> CD4<sup>+</sup> T cells into *Rag*<sup>-/-</sup> mice induces chronic colitis in the recipient mice (Powrie et al., 1994). We transferred CD45.1<sup>+</sup> CD45RB<sup>hi</sup> CD4<sup>+</sup> T cells into *Rag*<sup>-/-</sup> mice with or without CD45.2 CD4<sup>+</sup> T cells pretreated for 5 days with or without glucose under Treg conditions. Mice were sacrificed 4 weeks later, and histopathology of the intestine was examined. CD4<sup>+</sup> T cells pretreated either with or without glucose under Treg conditions suppressed colitis induced by CD45RB<sup>hi</sup> CD4<sup>+</sup> T cells (Figures S2A and S2B). Interestingly, recipient mice that received CD4<sup>+</sup> T cells pretreated with glucose under Treg conditions showed a tendency of less severe intestinal inflammation than the mice that received CD4<sup>+</sup> T cells without glucose treatment under Treg conditions (Figures S2A and S2B). The ratio of CD45.2<sup>+</sup> CD4<sup>+</sup> cells to CD45.1<sup>+</sup> effector CD4<sup>+</sup> T cells, which inversely reflects the proliferation of CD45.1<sup>+</sup> effector CD4<sup>+</sup> T cells,



**Figure 2. Glucose promotes Treg cell differentiation *in vitro***

(A–D) CBir1 Tg CD4<sup>+</sup> T cells were cultured with CBir1 peptide and glucose at different concentrations ( $n = 3/\text{group}$ ). (A and B) Representative FACS plots and the frequency of IFN $\gamma$ <sup>+</sup> cells after 5 days of culture under Th1 polarization condition. (C and D) Representative FACS plots and frequency of IL-17<sup>+</sup> cells after 5 days of culture under Th17 polarization condition.

**Figure 2. Continued**

(E and F) Splenic CD4<sup>+</sup> T cells were isolated from Foxp3-GFP mice and cultured under Treg conditions (n = 4/group). Representative FACS plots and frequency of Foxp3<sup>+</sup> cells after 5 days of culture. (G and H) CD4<sup>+</sup> T cells were activated with anti-CD3/CD28 mAbs under Treg conditions and treated with or without glucose (7000 μg/mL) for two days (n = 3/group). IL-10 (G) and TGFβ (H) production were measured by ELISA. (I and J) CD45.2 CBir1 Tg CD4<sup>+</sup> T cells were cultured with APCs and CBir1 peptide in the presence (Glu) or absence (Ctr) of 7000 μg/mL glucose (n = 3/group) under Treg polarization conditions for 5 days. The cells were harvested and then co-cultured with CFSE-labeled CD45.1 naive CD4<sup>+</sup> T cells at the ratio of 1:1 for 60h. (I) The CFSE dilution and (J) the CD45.1 cell proliferation were determined by flow cytometry. One representative of 3-5 independent experiments with similar results was shown. Data were expressed as mean ± SEM. Statistical significance was tested by two-tailed one-way ANOVA (B, D, F, J) or two-tailed unpaired Student's t test (G, H). ns, not significance; \*p < 0.05; \*\*p < 0.01; \*\*\*\*p < 0.0001. See also [Figure S1](#).

was increased in mice that received CD4<sup>+</sup> T cells with glucose treatment under Treg conditions compared with the mice that received CD4<sup>+</sup> T cells without glucose treatment under Treg conditions ([Figures S2C and S2D](#)), indicating that glucose promotes Treg cell differentiation to suppress T cell proliferation and induction of colitis *in vivo*.

**Glucose does not affect Treg stability**

Maintaining Foxp3 expression is essential for the stability of Treg cells to regulate intestinal homeostasis -. To investigate whether glucose enhances Treg's stability, we cultured CD4<sup>+</sup> T cells isolated from GFP-Foxp3 reporter mice with or without glucose under Treg conditions. Five days later, we sorted GFP<sup>+</sup> Foxp3<sup>+</sup> Treg cells to determine the methylation of promoters and Treg-specific demethylated region (TSDR), which are crucial for Treg's stability ([Polansky et al., 2008](#)). We found no difference in the methylation of Foxp3 TSDR between control and glucose-treated Tregs ([Figure 4A](#)). Furthermore, the methylation levels were similar between the two groups in sites of several Treg function-related genes, including PDL1, CTLA4, CD25, GITR, HELIOS, and EOS ([Figures 4B–4F](#)). These data indicate that glucose did not affect Treg's stability. However, glucose-treated Treg cells showed decreased methylation levels in several sites of distal promoters ([Figure 4G](#)), which further indicates that glucose promotes Treg differentiation.

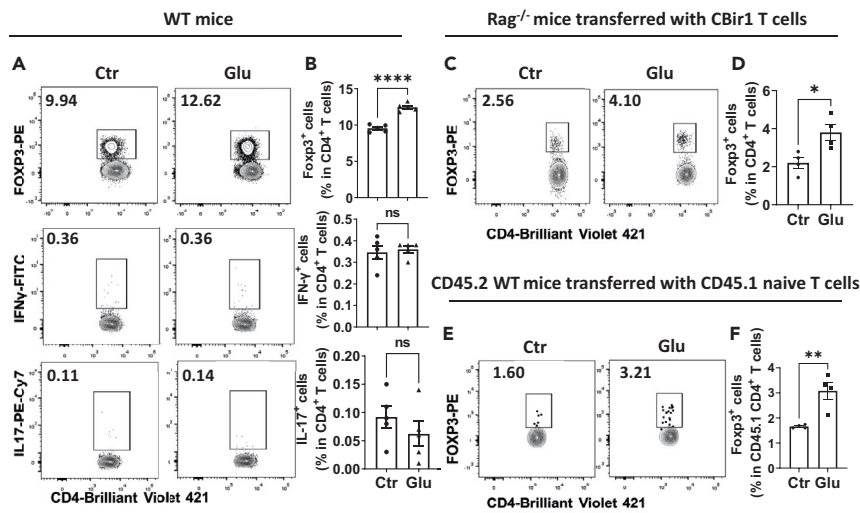
**Gut microbiota is not involved in the glucose induction of Treg *in vivo***

The microbiota plays a crucial role in regulating intestinal immune responses ([Wu and Wu, 2012](#)) and dietary factors have been considered critical for shaping the composition of the gut microbiome ([Conlon and Bird, 2014](#); [Singh et al., 2017](#)). To investigate whether glucose affects gut microbiota, we treated B<sub>6</sub> mice with 6% (w/v) glucose in drinking water for two weeks. Fecal pellets were collected on day 0 and day 14 for analysis of gut microbiota. Analysis of α-diversity using Shannon indices showed no differences in microbiota abundance between these two groups ([Figure 5A](#)). However, the β-diversity of the microbial communities was significantly altered in glucose-treated mice ([Figure 5B](#)). Glucose treatment significantly affected the relative abundance of multiple bacterial families ([Figure 5C](#)). Ruminococcaceae and Tannerellaceae were increased, whereas Mariniflaccaceae, Eggerthellaceae, and Bifidobacteriaceae were decreased after glucose treatment ([Figures 5C and 5D](#)).

Next, we investigated whether altered gut microbiota mediates the glucose induction of Treg cells *in vivo*. We treated B<sub>6</sub> mice with broad-spectrum antibiotics for two weeks, and then these antibiotic-pretreated mice were administered by oral gavage twice a week for one month with feces collected from the mice before or after two-week treatment with 6% (w/v) glucose in drinking water. Mice were then sacrificed to analyze CD4<sup>+</sup> T cell phenotypes by flow cytometry. There were no differences in percentages of Foxp3<sup>+</sup> Treg cells, IFNγ-producing and IL-17-producing CD4<sup>+</sup> T cells in MLN of these 2 groups of mice ([Figures 5E and 5F](#)). Altogether, these data indicated that gut microbiota is not involved in the glucose induction of Treg cells, although feeding glucose alters gut microbiota compositions.

**Glucose promotes Treg cell differentiation through the activation of AhR**

We then investigated the mechanism underlying glucose induction of Treg cells. First, we checked glucose uptake capacity among Th1, Th17, and Treg cells. We found that Treg cells showed a lower glucose uptake capacity than Th1 cells ([Figures S3A and S3B](#)), which is consistent with previous reports that the glycolytic levels are higher in effector T cells than Treg cells ([Palmer et al., 2015](#)), but there was no difference in glucose uptake between Th17 and Treg cells ([Figures S3A and S3B](#)). Furthermore, a previous study has shown that glucose has no effect on T cell oxidation and glycolysis measured by Seahorse ([Zhang et al.,](#)



**Figure 3. Glucose promotes Treg cell differentiation *in vivo***

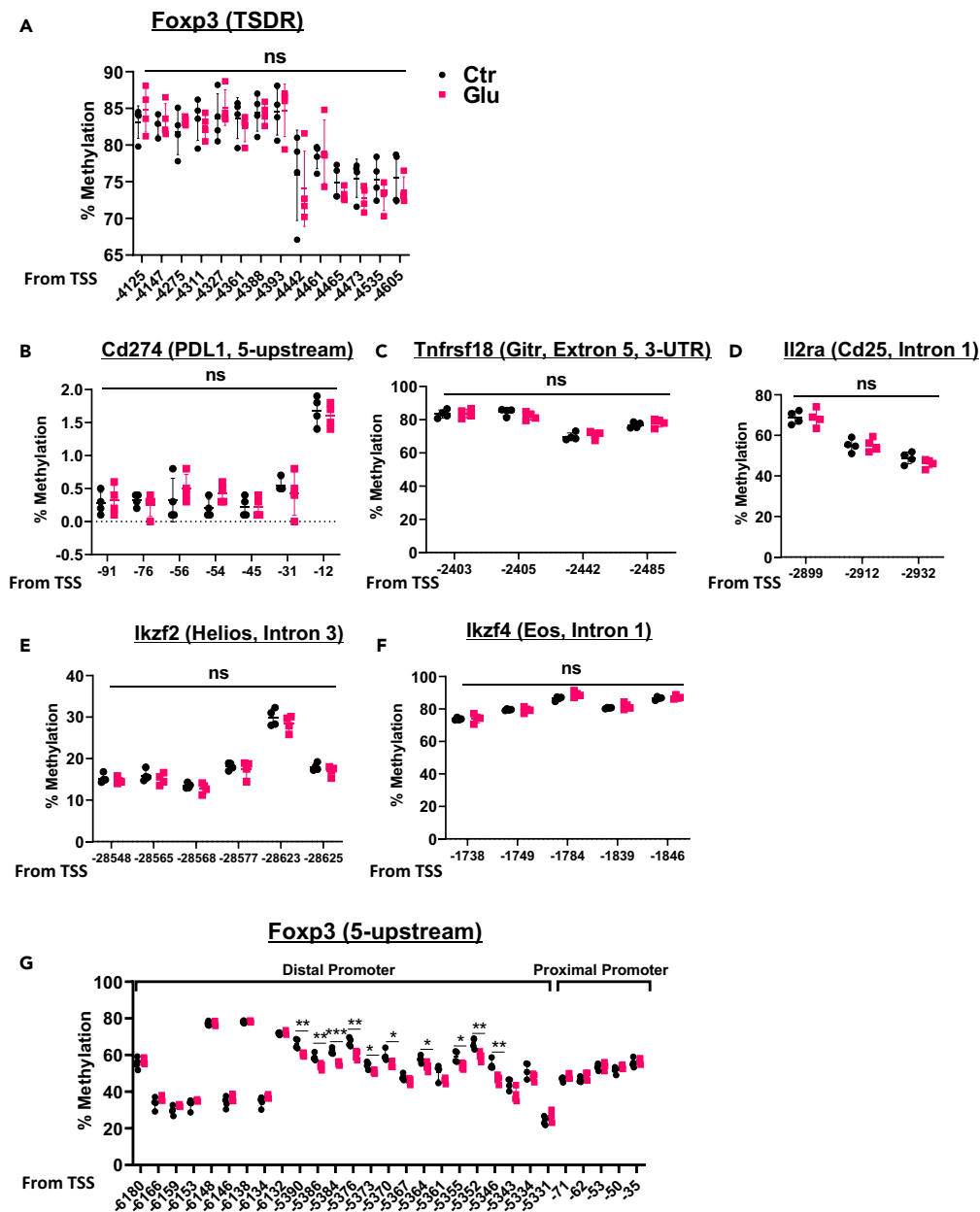
(A and B) The  $B_6$  mice were treated with drinking water (Ctr) or 6% glucose in drinking water (Glu) for 2 weeks ( $n = 5$ /group). Representative FACS plots (A) and frequency of CD4<sup>+</sup>Foxp3<sup>+</sup> cells, CD4<sup>+</sup>IFN $\gamma$ <sup>+</sup> cells and CD4<sup>+</sup>IL-17<sup>+</sup> cells in MLN were determined (B).

(C and D)  $Rag^{-/-}$  mice were transferred with naive CBir1 CD4<sup>+</sup> T cells and treated with drinking water (Ctr) or 6% glucose in drinking water (Glu) for 2 weeks ( $n = 4$ /group). Representative FACS plots (C) and frequency of CD4<sup>+</sup>Foxp3<sup>+</sup> cells MLN were determined (D).

(E and F) CD45.2 WT mice were transferred with naive CD45.1<sup>+</sup> CD4<sup>+</sup> T cells and treated with drinking water (Ctr) or 6% glucose in drinking water (Glu) for 2 weeks ( $n = 4$ /group). Representative FACS plots (E) and frequency of Foxp3<sup>+</sup> cells in MLN CD45.1<sup>+</sup> CD4<sup>+</sup> T cells were determined (F). One representative of three independent experiments was shown. Data were expressed as mean  $\pm$  SEM. Statistical significance was tested by a two-tailed unpaired Student's *t* test (B, D, F). ns, not significance; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ . See also Figure S2.

2019). Next, we checked whether glucose affects the mRNA expression of *Tbx21*, *Gata3*, *Rorgt*, and *Foxp3*, the transcription factors driving CD4<sup>+</sup> cell differentiation into different subsets. Glucose promoted the expression of *Foxp3* (Figure 6A) and *Rorgt*, but not *Tbx21* and *Gata3* (Figure S4A), under Treg conditions. To check whether ROR $\gamma$ t mediates the glucose induction of Treg cells, we culture T cells with or without ROR $\gamma$ t inhibitor, GSK803, in the presence of glucose. We found that the inhibition of ROR $\gamma$ t even slightly increased the Treg differentiation (Figure S4B), indicating that increased ROR $\gamma$ t does not contribute to Treg differentiation induced by glucose. It has been reported that AhR promotes Treg cell differentiation through binding to CABS and NCABS-2 (Crunkhorn, 2018; Quintana et al., 2008). Furthermore, glucose has been shown to activate AhR in endothelial cells (Dabir et al., 2008). We then investigated whether glucose activates AhR, which could mediate the glucose induction of Treg cells. We cultured naive T cells with the indicated dose of glucose under Treg conditions and found that glucose promoted the expression of *Cyp1a1* and *Cyp1a2* (Figure 6B), two canonical gene targets of AhR (Hankinson, 2016; Mescher and Haarmann-Stemann, 2018; Vogel et al., 2020), suggesting that glucose activates AhR. To determine whether glucose activates AhR *in vivo*, we isolated the MLN CD4<sup>+</sup> T cells from WT mice treated with or without glucose (6% (w/v)) and found that MLN CD4<sup>+</sup> T cells expressed higher levels of *Cyp1a1*, and a tendency of increased *Cyp1a2*, in mice fed with glucose compared with control mice (Figure 6C), indicating that oral feeding glucose increases AhR activity *in vivo*. In addition, glucose promoted AhR protein expression, as illustrated by both Western blot and flow cytometry analysis (Figures 6D-6G). To determine whether glucose induces Treg cell differentiation through the activation of AhR, we added CH223191, an AhR-specific inhibitor, to T cell cultures with glucose under Treg conditions. Blockade of AhR inhibited Treg cell induction by glucose (Figures 6H and 6I), suggesting that the glucose induction of Treg cells is mediated through the activation of AhR.

Next, we investigated the role of AhR in glucose induction of Treg cells *in vivo*. We transferred AhR-deficient naive CD4<sup>+</sup> T cells from CD4 T cell-specific *Ahr* knock-out (*Cd4<sup>cre</sup>Ahr<sup>fl/fl</sup>*, *Ahr<sup>ΔCD4</sup>*) mice into  $Rag^{-/-}$  mice and fed them with or without 6% (w/v) glucose in drinking water for 2 weeks. Glucose did not promote AhR-deficient CD4<sup>+</sup> T cell differentiation into Treg cells as well as IFN- $\gamma$ <sup>+</sup> Th1 in the MLN and spleens

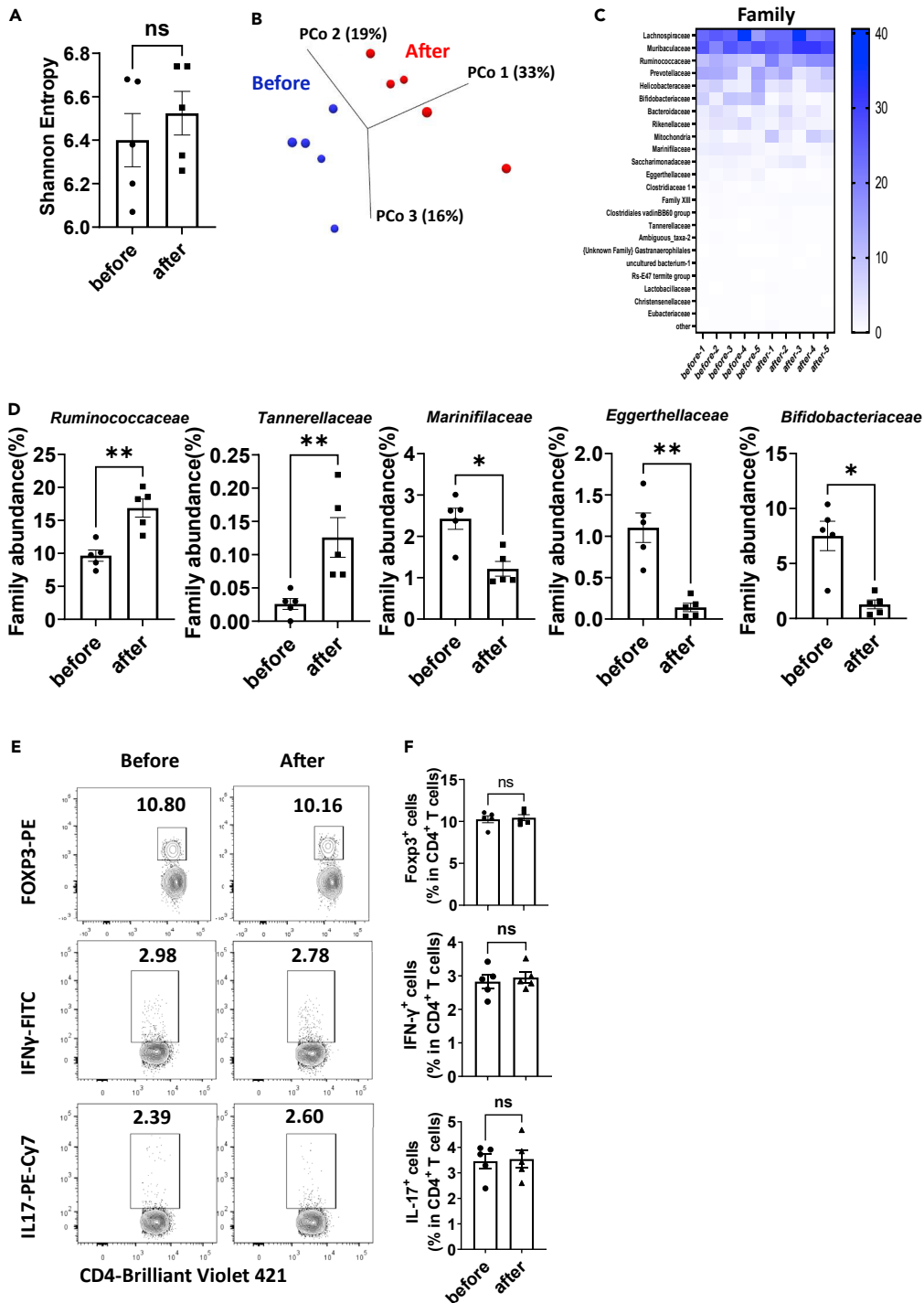


**Figure 4. Glucose does not affect Treg stability**

(A–G) Splenic CD4<sup>+</sup> T cells were isolated from Foxp3-GFP mice and cultured in the presence or absence of glucose (7000 μg/mL) under Treg conditions (n = 4/group) for 5 days. GFP<sup>+</sup> Foxp3<sup>+</sup> Treg cells were isolated by FACS sorter, and the methylation levels in sites of different genes were determined. The methylation levels were shown in Treg specific demethylated region (TSDR) (A), upstream of PDL1 (B), Extron in GITR (C), Intron 1 in CD25 (D), Intron 3 in HELIOS (E), Intron 1 in EOS (F), and upstream of Foxp3 (G). Data were expressed as mean ± SEM. Statistical significance was tested by a two-tailed unpaired Student's t test. ns, not significance; \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001.

(Figures 7A, 7B, S5A, and S5B). However, while glucose did not affect Th17 differentiation in spleens (Figures S5A and S5B), it promoted Th17 differentiation in MLN (Figures 7A and 7B), which might be attributed to irresponsive Treg differentiation to glucose in AhR-deficient CD4<sup>+</sup> T cells. To further confirm the role of AhR in glucose induction of Treg cells, we transferred naive CD4<sup>+</sup> T cells isolated from Treg cell-specific AhR knock-out (*Foxp3<sup>cre</sup>Ahr<sup>fl/-</sup>*, *Ahr<sup>Δfoxp3</sup>*) mice into *Rag<sup>-/-</sup>* mice and treated them with or without 6% (w/v) glucose in drinking water for 2 weeks. We found that glucose treatment did not affect Treg cell





**Figure 5. Treatment with glucose alters gut microbiota composition**

B<sub>6</sub> mice were treated with 6% (w/v) glucose in drinking water for two weeks. Gut microbiota before and after treatment with glucose was determined by 16s rRNA sequencing.

(A and B)  $\alpha$ -diversity was calculated by Shannon index, and (B)  $\beta$ -Diversity analysis of gut microbiota before and after glucose treatment. Ordination plots based on the PCoA using Bray-Curtis metrics.

(C and D) Relative abundances of a common set of microbial families were compared before and after glucose treatments.

**Figure 5. Continued**

(E and F) B<sub>6</sub> mice were pretreated with antibiotics (1 g/L metronidazole, 0.5 g/L vancomycin, 1 g/L ampicillin, and 1 g/L kanamycin) in drinking water for two weeks. The antibiotic-pretreated mice were then administered twice a week for one month with feces collected from the mice before or after two-week treatment with 6% (w/v) glucose in drinking water. Representative FACS plots (E) and frequency of CD4<sup>+</sup>Foxp3<sup>+</sup> cells, CD4<sup>+</sup>IFN $\gamma$ <sup>+</sup> cells and CD4<sup>+</sup>IL-17<sup>+</sup> cells in MLN were determined (F). Data were expressed as mean  $\pm$  SEM (n = 5 mice/group). Statistical significance was tested by Bray-Curtis PERMANOVA analysis (B) or non-parametric two-tailed Student's t test. (A, D). ns, not significance; \*p < 0,05; \*\*p < 0.01. One representative of two independent experiments was shown.

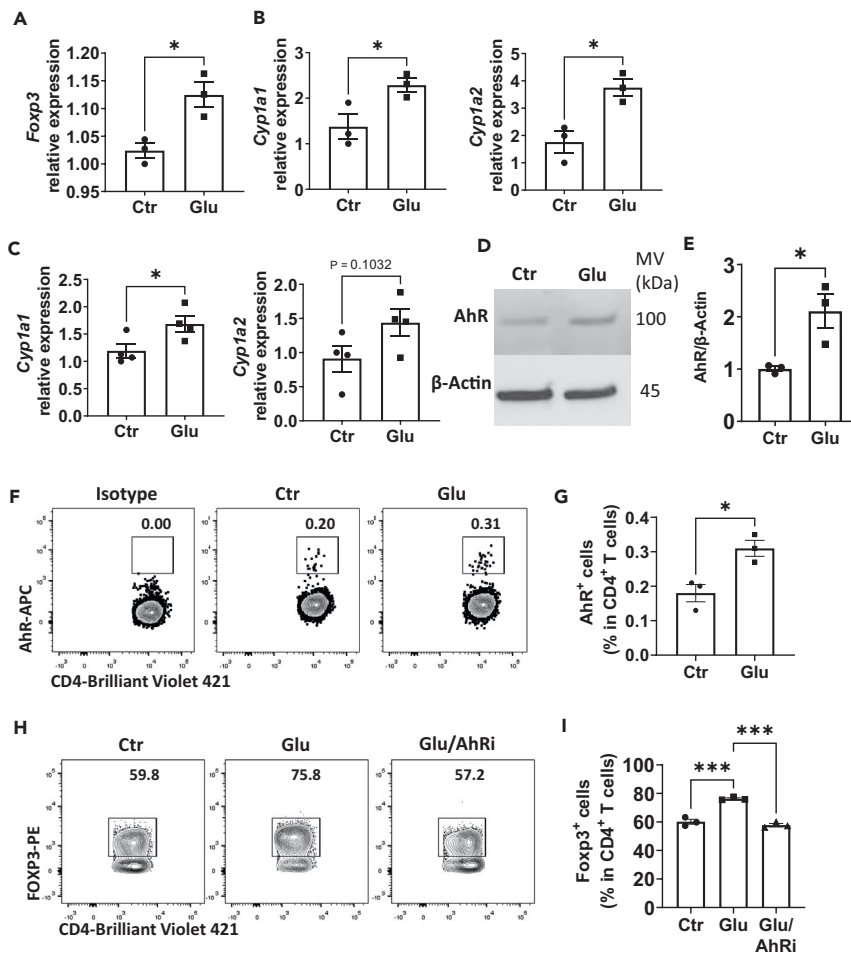
generation and Th1 and Th17 cell differentiation in mice transferred with Treg cell-specific *Ahr*-deficient T cells (Figures 7C, 7D, S5C, and S5D). Besides, *Rag*<sup>-/-</sup> recipients of Treg cell-specific *Ahr* knock-out T cells showed intestinal inflammation 2 weeks after cell transfer (Figure S6). Put all together, these data indicated that AhR mediates the glucose induction of Treg cells.

**DISCUSSION**

An unhealthy diet has long been considered a potential environmental risk factor for the development of autoimmune diseases (Childs et al., 2019; Manzel et al., 2014). The common dietary carbohydrate, glucose, is a critical nutrient for humans and is considered the major fuel source used by all cells for their biological function (Mardis, 2001; Qi and Tester, 2019). On the other hand, increased sugar consumption has been associated with an increased incidence of various autoimmune diseases and metabolic syndromes, such as obesity, IBD, and diabetes (Hu et al., 2018; Manzel et al., 2014; Qi et al., 2012). However, whether the increased glucose intake is a cause or consequence of these diseases is still controversial (Cashman and Shanahan, 2003; Ruxton et al., 2010). Although recent studies have demonstrated that high glucose intake promotes the susceptibility and development of colitis (Khan et al., 2020; Zhang et al., 2019), our current study revealed that different doses of glucose differentially regulate the development of intestinal inflammation. Although a high amount of glucose promoted intestinal inflammation, a moderate dose of glucose-induced Treg cells and alleviated colitis development. These findings thereby reveal an immunoregulatory function of glucose at an appropriate amount in the maintenance of intestinal homeostasis.

CD4<sup>+</sup> T cells are one of the major components of the adaptive immune system, which play a critical role in mediating an effective immune response to gut microbiota and pathogens (Luckheeram et al., 2012). Accumulating evidence suggests that the dysregulation of CD4<sup>+</sup> T cells is one of the major initiators in the pathogenesis of IBD (Imam et al., 2018). As the primary source of cellular energy, glucose is critical in T cell activation, differentiation, and function (Palmer et al., 2015; van der Windt and Pearce, 2012). Our study demonstrated that a moderate glucose treatment promotes Treg cell but not Th1 and Th17 cell differentiation *in vivo* and *in vitro*. CBir1 TCR transgenic mice were generated in which CD4<sup>+</sup> T cells are specific for CBir1 flagellin. Thus, CBir1 TCR transgenic T cells represent T cells specific for a single gut bacterial antigen (Yang et al., 2021). Wild-type polyclonal CD4<sup>+</sup> T cells can be activated by various antigens in the intestine of *Rag1*<sup>-/-</sup> recipient mice. The strength of using CBir1 TCR transgenic T cells is to investigate the effect of glucose on gut bacterial-specific T cell responses while using polyclonal CD4<sup>+</sup> T cells is to better reflect the role of glucose in regulating T cell responses in a more actual condition. Glucose promoted Treg cell differentiation in a dose-dependent manner. High glucose intake promoted colitis development with no effect on Treg cell generation, consistent with previous studies (Zhang et al., 2019). One possible explanation is that high glucose intake could promote inflammatory cytokine production in the inflammatory environment, which may, in turn, inhibit Treg cell differentiation (Hu et al., 2018; Shanmugam et al., 2003). Interestingly, D-mannose, a C-2 epimer of glucose, has also been shown to promote Treg cell differentiation by promoting TGF- $\beta$  activation (Zhang et al., 2017a), suggesting that moderate levels of simple sugars which usually present in the diet may share a common feature in the induction of Treg cell differentiation to maintain immune homeostasis.

Treg cells have been critical in the maintenance of immune homeostasis and the protection of autoimmune diseases (Okeke and Uzonna, 2019; Sakaguchi et al., 1995). Immunosuppressive cytokine production by Treg cells and Treg cell-mediated effector cell apoptosis are considered two efficient mechanisms of Treg cell-mediated suppression (Schmidt et al., 2012; Shevryev and Tereshchenko, 2019). Our study demonstrated that glucose-induced Treg cells produced higher levels of anti-inflammatory cytokines TGF $\beta$  and IL-10 to suppress naive CD4<sup>+</sup> T cell proliferation.



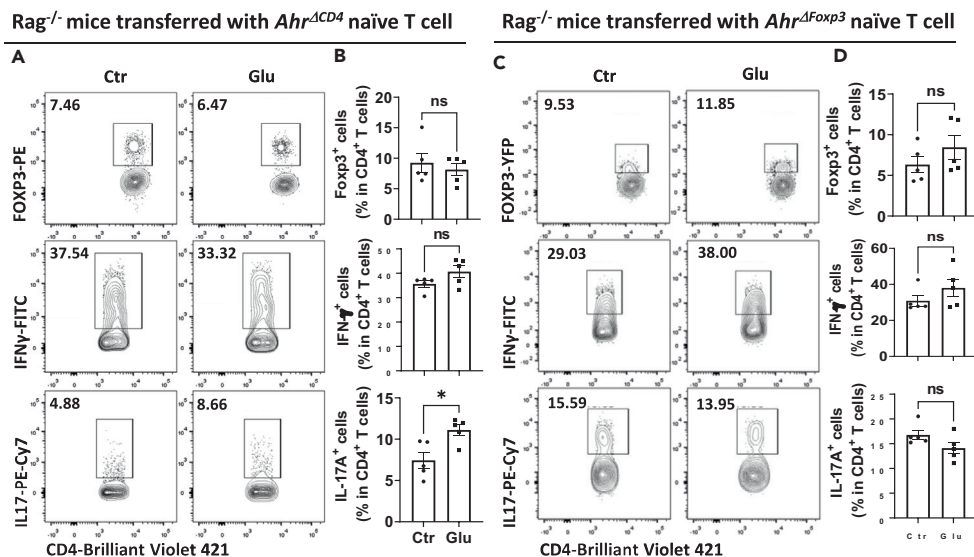
**Figure 6. Glucose promotes CD4<sup>+</sup> T cell AhR expression and activation**

(A and B) CD4<sup>+</sup> T cells were activated with anti-CD3/CD28 mAbs in the presence (Glu) or absence (Ctr) of 7000 μg/mL glucose for 2 days under Treg conditions (n = 3/group). Foxp3 (A) and CYP1a1 and CYP1a2 (B) mRNA were measured by qRT-PCR.

(C) WT mice were treated with drinking water (Ctr) or 6% glucose in drinking water (Glu) for 2 weeks (n = 4/group). CYP1a1 and CYP1a2 expression in MLN CD4<sup>+</sup> T cells was by qRT-PCR.

(D-I) CD4<sup>+</sup> T cells were activated with anti-CD3/CD28 mAbs in the presence (Glu) or absence (Ctr) of 7000 μg/mL glucose ± CH223191 (3 μM) (n = 3/group) under Treg polarization conditions. AhR proteins were analyzed by Western blot (D-E) and FACS on day 3 (F-G). (H) Representative FACS plots showing Foxp3<sup>+</sup> cells in CD4<sup>+</sup> T cells and (I) frequency of Foxp3<sup>+</sup> Treg cells in CD4<sup>+</sup> cells after 5 days of culture. One representative of three independent experiments was shown. Data were expressed as mean ± SEM. Statistical significance was tested by two-tailed unpaired Student's t test (A-C, E, G) or two-tailed one-way ANOVA(I). \*p < 0,05; \*\*\*p < 0.001. See also Figures S3 and S4.

Although Treg cells rely on glycolysis which is indispensable for Treg induction (de Kivit and Mensink, 2020; De Rosa et al., 2015; Li et al., 2019; Procaccini et al., 2016), we found that Treg cells showed less capacity of glucose uptake but there was no difference in glucose uptake between Treg cells and Th17 cells. Additionally, increased glucose concentration does not affect T cell oxidation and glycolysis *in vitro* (Zhang et al., 2019). Accumulating evidence indicates that AhR ligands produced by dietary nutrients and specific gut microbiota play essential roles in the development of T cells (Kiss et al., 2011; Qiu et al., 2012). Glucose has been shown to activate AhR in vascular endothelial cells (Dabir et al., 2008). As a ligand-dependent transcription factor, AhR regulates the expression and function of target genes by translocating to the nucleus and binding with the XRE consensus sequence, essential for the development and function of Treg cells (Oh-Oka et al., 2017; Ye et al., 2017). Our study demonstrated that glucose activated AhR and enhanced AhR expression during the differentiation of Treg cells. Blockade of AhR both chemically and genetically abolished the Treg cell generation induced by glucose both *in vitro* and *in vivo*, suggesting



**Figure 7. Deficiency of AhR in CD4 T cells impairs the glucose induction of Treg cells**

(A and B) *Rag*<sup>-/-</sup> mice transferred with *Cd4*<sup>cre</sup>*Ahr*<sup>fl/fl</sup> (*Ahr*<sup>ΔCd4</sup>) naive T cells were treated with (Glu) or without (Ctr) 6% glucose in drinking water for 2 weeks (A) Representative FACS plots and (B) frequency of Foxp3<sup>+</sup>, IL-17<sup>+</sup>, and IFN $\gamma$ <sup>+</sup> CD4<sup>+</sup> T cell in MLN were determined.

(C and D) *Rag*<sup>-/-</sup> mice transferred with *Foxp3*<sup>cre</sup>*Ahr*<sup>fl/fl</sup> (*Ahr*<sup>ΔFoxp3</sup>) naive T cells were treated with (Glu) or without (Ctr) 6% glucose in drinking water for 2 weeks (c) Representative FACS plots and (D) frequency of Foxp3<sup>+</sup>, IL-17<sup>+</sup>, and IFN $\gamma$ <sup>+</sup> CD4<sup>+</sup> T cell in MLN were determined. One representative of three independent experiments was shown. Data were expressed as mean  $\pm$  SEM. Statistical significance was tested by a two-tailed unpaired Student's *t* test. ns, not significance; \**p* < 0.05. See also Figure S5.

that glucose could serve as an AhR ligand, and activation of AhR mediates the glucose induction of Treg cells.

Previous studies have revealed the critical role of diet in shaping gut microbiota composition (Conlon and Bird, 2014; David et al., 2014; Singh et al., 2017). Consistent with a previous report (Khan et al., 2020), oral feeding of glucose altered the composition of gut microbiota. The abundance of Ruminococcaceae was increased after glucose treatment. It has been reported that patients with ulcerative colitis had decreased Ruminococcaceae, whose abundance is positively correlated with secondary bile acid and plays an important role in alleviating intestinal inflammation (Duboc et al., 2013; Sinha et al., 2020; Theriot et al., 2016). In addition to the high abundance of Ruminococcaceae, the abundance of Tannerellaceae, which decreased in abundance in patients with IBD (Alam et al., 2020), was also increased. In contrast, Marinifilaceae, which is increased in experimental colitis models (Shao et al., 2020), was reduced upon glucose consumption. However, we found that the altered gut microbiota is not involved in glucose induction of Treg cells *in vivo*. We cannot exclude the possibility that altered microbiota in glucose-treated mice may contribute to decreased intestinal inflammation in a Treg-independent manner, which needs to be investigated in future studies.

In summary, our study demonstrated that moderate glucose intake alleviates intestinal inflammation and promotes Treg cell differentiation by promoting AhR-mediated Foxp3 expression. This finding identifies the previously unrecognized function of glucose at an appropriate amount in inhibiting colitis. Thus, it provides a potential target for preventing intestinal inflammatory diseases and promoting intestinal health.

### Limitations of the study

Here, we investigated the role of glucose at a moderate dose in modulating Treg cell differentiation in the intestine, thereby controlling colitis. However, the effect size of glucose on Treg, which might be attributed to the short term- of glucose treatment. Using a chronic colitis model would be a good way to explore whether longer treatment of glucose can induce Treg more dramatically in the future.

## STAR★METHODS

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## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.105004>.

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## AUTHOR CONTRIBUTIONS

Conceptualization: Y.Y. (Yu Yu), W.Y., and Y.C.; Methodology: Y.Y. (Yu Yu), W.Y., G.G., and Y.C.; Investigation: Y.Y. (Yu Yu), W.Y., T.Y., X.Z., Z.Z., Y.Y. (Yanbo Yu), H.Y., A.J.B., S.Y., and Y.C.; Resources: A.P., L.X., F.J.Q., L.Z., and Y.L.; Writing – original draft preparation: Y.Y. (Yu Yu), W.Y., and Y.C.; Writing – review and editing: Y.Y. (Yu Yu), W.Y., F.J.Q., L.Z., Y.L., and Y.C. with input from all other authors; Supervision: Y.C.; Funding acquisition: Y.C.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Purified anti-mouse CD3(145-211)	Bio X Cell	Cat#BE0001-1; RRID:AB_1107634
Purified anti-mouse CD28(37.51)	Bio X Cell	Cat#BE00015-1
anti-IFN- $\gamma$ -FITC	Biolegend	Cat#505806; RRID:AB_315400
anti-CD4-Brilliant Violet 421	Biolegend	Cat#100544; RRID:AB_11219790
anti-CD4-APC	Biolegend	Cat#100516; RRID:AB_312719
anti-CD25-Percp 5.5	Biolegend	Cat#102030; RRID:AB_893288
anti-CD62L-PE	Biolegend	Cat#104408; RRID:AB_313095
anti-CD45RB-PE	Biolegend	Cat#103308; RRID:AB_313015
anti-CD45.1- PE/cyanine 7	Biolegend	Cat#110729; RRID:AB_1134170
anti-IFN- $\gamma$ -FITC	Biolegend	Cat#505831; RRID:AB_11142685
anti-IL-17-PE/cyanine 7	Biolegend	Cat#506922; RRID:AB_2125010
anti-Foxp3 PE	Invitrogen	Cat#12-5773-82; RRID:AB_465936
AHR Monoclonal Antibody eFluor 660	eBioscience	Cat#50-5925-80; RRID:AB_2574254
anti-mouse CD16/CD32 antibody	Biolegend	Cat#101302; RRID:AB_312801
anti-mouse-IL4	Bio X Cell	Cat#BE0045; RRID:AB_1107707
anti-mouse-IFN $\gamma$	Bio X Cell	Cat#BE0055; RRID:AB_1107694
Aryl hydrocarbon receptor polyclonal antibody	Enzo Life Sciences	Product# BML-SA210; RRID:AB_10540536
$\beta$ -Actin (D6A8) Rabbit mAb	Cell Signaling Technology	Cat#8457; RRID:AB_10950489
<b>Chemicals, peptides, and recombinant proteins</b>		
IL12	Biolegend	Cat# 577004
IL6	Biolegend	Cat# 575708
TGF $\beta$	Biolegend	Cat# 580706
CH223191	Sigma	Cat#C8124
D-glucose	Fisher chemical	Cat#D16-3
PMA	Sigma Aldrich	Cat# P8139
ionomycin	Invitrogen	Cat# I24222
Golgi-stop	BD Biosciences	
RPMI 1640	GE healthcare	Cat#SH30027.01
Penicillin/streptomycin	GE healthcare	Cat# SV300100
Fetal Bovine Serum (FBS)	ATLANTA biologicals	Cat#S11550
Hematoxylin Solution (Mayer's, Modified)	Abcam	Cat# ab220365
Eosin Y Solution	Abcam	Cat# ab246824
<b>Critical commercial assays</b>		
CD4 <sup>+</sup> CD62L <sup>+</sup> T cell Isolation Kit, mouse	BD Biosciences	Cat#551539
Foxp3/Transcription Factor Staining Buffer Set	ThermoFisher	Cat#00-5523-00
Pierce BCA Protein Assay Kit	ThermoFisher Scientific	Cat#23225
Annexin V/ 7AAD apoptosis kit	Biolegend	Cat# 640934
CellTrace CFSE Cell Proliferation Kit	Invitrogen	Cat#C34554
ELISA MAXTM Deluxe Sets ELISA kit, Mouse IL-10	Biolegend	Cat#431411
TGF beta-1 Mouse Uncoated ELISA kit	Biolegend	Cat#88-8350-88

(Continued on next page)



**Continued**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Experimental models: Organisms/strains</b>		
Mouse: Rag1 <sup>-/-</sup>	Jackson laboratory	Stock# 002216
Mouse: CD45.1	Jackson laboratory	Stock# 002014
Mouse: Cd4 <sup>cre</sup> Ahr <sup>fl/fl</sup>	Dr. Francisco Quintana	N/A
Mouse: Foxp3 <sup>cre</sup> Ahr <sup>fl/-</sup>	Dr. Liang Zhou	N/A
Mouse: Foxp3-GFP	Jackson laboratory	Stock# 006772
<b>Oligonucleotides</b>		
Primers for quantitative PCR, see <a href="#">Table S1</a>	This paper	N/A
universal 16S rRNA V3-V4 region primers	<a href="#">Klindworth et al., 2013</a>	N/A
<b>Software and algorithms</b>		
GraphPad Prism 9.0	GraphPad Software	N/A
FlowJo_V10		N/A
BD FACSAria™ Fusion Cell Sorter	BD Biosciences	N/A
LSRII Fortessa flow cytometer	BD Biosciences	N/A
ChemiDoc™ Imaging System	BIO-RAD	N/A
Gen5 software	Biotek	N/A
<b>Data and code availability in resource availability</b>		
16s rRNA seq	BioProject	#PRJNA721957
<b>Other</b>		
anti-mouse CD4-magnetic beads	BD Biosciences	Cat#551539
LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit	Invitrogen	Cat#L10119
DAPI	Invitrogen	REF: D1306
QIAAMP PowerFecal DNA kit	Qiagen	Cat# 12830-50

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead contact, Yingzi Cong ([yicong@utmb.edu](mailto:yicong@utmb.edu)).

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

All data supporting the findings of this study are available from the corresponding author upon reasonable request. 16s rRNA data has been deposited in SRA database under the accession number PRJNA721957. This paper does not report the original code. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Mice

C57BL/6 Wild type (WT), Rag<sup>-/-</sup>, CD45.1 mice, and Foxp3-GFP mice were purchased from Jackson laboratory. CBir1 TCR Tg mice were bred in the animal facilities of UTMB. Cd4<sup>cre</sup>Ahr<sup>fl/fl</sup> mice were kindly provided by Dr. Francisco Quintana of Harvard medical school. Foxp3<sup>cre</sup>Ahr<sup>fl/-</sup> mice were kindly provided by Dr. Liang Zhou of the University of Florida. Both male and female mice were used in this study. 6-12 weeks age-matched mice were used at the beginning of the experiments. All experiments were reviewed and

approved by the Institutional Animal Care and Use Committees (IACUC) of UTMB and were carried out following the approved guidelines.

### CD4<sup>+</sup> T cell isolation and cell culture

CD4<sup>+</sup> T cells were isolated from spleens using anti-mouse CD4-magnetic beads (BD Biosciences). CD4<sup>+</sup> T cells were cultured with CBir1 peptides (ThermoFisher Scientific) and irradiated splenic antigen-presenting cells (APCs) or were activated with 5 µg/ml plate-bound anti-mouse-CD3 (Bio X Cell) and 2 µg/ml soluble anti-mouse CD28 (Bio X Cell). Cells were treated with different concentrations of D-glucose (Fisher chemical) in RPMI 1640 medium in the presence or absence of 3 µM Ahr inhibitor CH223191 (Sigma) and cultured under neutral (without exogenous cytokines), Th1 (10 ng/mL IL-12), Th17 (2 ng/mL TGFβ, 50 ng/mL IL6, 10 µg/ml anti-IFNγ mAb, 5 µg/ml anti-IL-4 mAb), or Treg (2 ng/mL TGFβ) polarization conditions. All recombinant antibodies were obtained from Biolegend.

## METHOD DETAILS

### Flow cytometry

Cells were stimulated with PMA (50 ng/mL, Sigma Aldrich) and ionomycin (750 ng/mL, Invitrogen), followed by Golgi-stop (0.75 µL/mL, BD Biosciences) at 37°C for 5h, then stained with Live/dye using a LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit (Invitrogen), followed by surface staining with mouse CD4 (1:200, Biolegend) and then fixing with the Foxp3/Transcription Factor Fixation/Permeabilization set (ThermoFisher) according to the manufacturer's instructions. Intracellular cytokines and transcriptional factors were then stained with different antibodies (anti-IFN-γ-FITC, 1:200, Biolegend; anti-IL-17-PE/cyanine 7, 1:200, Biolegend; anti-Foxp3, 1:200, Invitrogen; AHR Monoclonal Antibody, eFluor 660, Invitrogen). For cell apoptosis staining, cells were stained with Annexin V and 7-AAD for 15 min at room temperature. Stained cells were analyzed on an LSRII Fortessa flow cytometer (BD Biosciences), and data were analyzed with FlowJo 10 software. The gating strategy was shown in [Figure S7](#).

### Cell sorting

Splenic cells were prepared and stained with relevant surface antibodies and incubated for 30 min at 4°C. For naive T cell sorting, cells were stained with anti-mouse CD4-APC (Biolegend), anti-CD25-Percp 5.5 (Biolegend), anti-CD62L-PE (Biolegend), and DAPI (Invitrogen). For CD45RB<sup>hi</sup> T cell sorting, cells were stained with anti-mouse CD4-APC (Biolegend), anti-CD45RB-PE (Biolegend), and DAPI. After incubation, cells were washed and suspended in 2% FBS in PBS. Cells were then sorted by BD FACSAria™ Fusion Cell Sorter.

### Foxp3 methylation Panel

Methylation in sites of different genes was analyzed by NextGen Sequencing (EpigenDx), and methylation levels were calculated in Bismark by dividing the number of methylated reads by the total number of reads.

### T cell suppression assay

Naïve CD4<sup>+</sup> T cells were labeled with CFSE using a CellTracker™ CFSE proliferation kit (Invitrogen). 5 × 10<sup>4</sup> CFSE-labeled CD4<sup>+</sup>CD45.1<sup>+</sup> naïve T cells were cultured with 2 × 10<sup>5</sup> irradiated splenic APCs and soluble anti-CD3 (5 µg/ml) and co-cultured with or without 5 × 10<sup>4</sup> control Treg cells or glucose-treated Treg cells. The cells were harvested 60 h later. CFSE intensity was analyzed by flow cytometry by gating on CD45.1<sup>+</sup> CD4<sup>+</sup> cells.

### Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted with TRIzol (Invitrogen) and followed by cDNA synthesis with reverse transcriptase. qPCR was performed using TaqMan Gene Expression Assays. Primers are indicated in [Table S1](#). Data were normalized to Gapdh mRNA expression.

### Western Blot

CD4<sup>+</sup> T cells were activated with anti-CD3 (Bio X Cell) and anti-CD28 mAb (Bio X Cell) in the presence or absence of glucose (7000 µg/ml) under Treg polarization conditions. Cells were collected on day 3. Proteins were extracted using a radio-immunoprecipitation buffer containing protease inhibitor cocktail, phosphatase inhibitor cocktail, and PMSF. Protein concentrations were determined using a Pierce BCA Protein

Assay Kit (ThermoFisher Scientific). 10  $\mu$ g protein of each sample was loaded into the Mini-PROTEAN TGX Stain-free Gels (BIO-RAD) and separated electrophoretically. Proteins were then transferred to a PVDF membrane. After blocking with 5% non-fat milk, the membrane was incubated with primary antibodies overnight at 4°C and then with secondary antibodies (1:5000) for 1 h at room temperature. After incubating with the substrate, blots were detected using the ChemiDoc™ Imaging System. Primary antibodies: anti-AhR, 1:2000, Enzo Life Sciences; anti- $\beta$ -actin, 1:2000, Cell Signaling Technology.

### Glucose uptake assay

CD4<sup>+</sup> T cells were activated and cultured under Th1, Th17, and Treg conditions for 5 days. Cells were then collected and cultured (50,000 cells/well) in the glucose-free medium for 2 h. Cells were then incubated with 2-NBDG (150  $\mu$ g/mL) for 2 h. After washing, cells were stained with PI in assay buffer. Stained cells were analyzed on an LSRII Fortessa flow cytometer (BD Biosciences), and data were analyzed with FlowJo 10 software.

### Naïve T cell-transfer model

Naïve CD4<sup>+</sup> T cells were isolated from spleens of CBir1 Tg mice, WT mice, *Cd4<sup>cre</sup>Ahr<sup>fl/fl</sup>* mice, or *Foxp3<sup>cre</sup>Ahr<sup>fl/fl</sup>* mice, and adoptively transferred into *Rag1<sup>-/-</sup>* mice or WT mice by intravenous injection. Two weeks later, mice were sacrificed for immunological analysis of MLN and spleen cells.

### CBir1 T cell-transfer colitis model

CD4<sup>+</sup>CD25<sup>-</sup>CD62L<sup>+</sup>T cells were sorted from the spleens of CBir1 Tg mice and adoptively transferred into *Rag1<sup>-/-</sup>* mice by intravenous injection. Six weeks later, mice were sacrificed, and the colon and cecum were used for organ culture and histopathological analysis. MLN and spleens were harvested for immunological analyses.

### CD45RB<sup>hi</sup> T cell-transfer colitis model

CD45.2<sup>+</sup> CD4<sup>+</sup> T cells were cultured under Treg polarization conditions in the presence or absence of the indicated glucose concentration. Five days later, cells were harvested ( $0.5 \times 10^6$  cells per mouse) and co-transferred with CD4<sup>+</sup>CD45.1<sup>+</sup>CD45RB<sup>hi</sup> T cells ( $0.1 \times 10^6$  cells per mouse) to *Rag1<sup>-/-</sup>* mice by intravenous injection. The bodyweights of mice were measured every two or three days. The colon and cecum were used to isolate lamina propria lymphocytes, organ culture, and histopathological analysis. MLN and spleens were harvested for immunological analyses.

### Glucose administration

To investigate the effect of glucose on Treg cells *in vivo*, WT mice were administered drinking water with or without glucose at 6% (w/v) for 2 weeks. For the naïve T cell transfer model, mice that were transferred with naïve T cells were then treated for 2 weeks after injection with or without 6% (w/v) glucose in drinking water. For the naïve T cell transfer colitis model, mice transferred with sorted naïve T cells were treated with or without 6% (w/v) or 20% (w/v) glucose in drinking water on the same day T cell transfer and for 6-7 weeks after.

### Fecal microbiota transplantation model

WT mice were pretreated with 1 g/l metronidazole, 0.5 g/l vancomycin, 1 g/l ampicillin, and 1 g/l kanamycin in drinking water for two weeks. Antibiotic-pretreated recipient mice were then administered twice a week for one month with feces collected from the mice before or after two-week treatment with 6% (w/v) glucose in drinking water.

### Histopathology assessment

Colon and cecum samples were collected and rolled into a Swiss Roll, then fixed in 10% buffered formalin in the tissue cassettes for 24h. Samples were then paraffin-embedded, sliced, and stained with Haematoxylin and Eosin (H&E). Pathology scores were assessed by hyperplasia; goblet cell number; crypt abscesses; ulceration; mucosa and submucosa inflammatory cell infiltration. A score of 0-3, denoting increasingly severe abnormality, was assigned for each parameter, and then the sum of their parameters was the total histological score. Three colleagues in the lab participated in the assessment of each sample, and each sample was read blindly.

### 16S rRNA high throughput sequencing and analysis

The microbiomes samples were analyzed using barcoded high-throughput amplicon sequencing of the bacterial 16S rRNA gene. Fecal bacterial DNA was isolated using a QIAAMP PowerFecal DNA kit (Qiagen) following the manufacturer's guidelines. Sequencing libraries for each isolate were generated using universal 16S rRNA V3-V4 region primers (Klindworth et al., 2013) in accordance with Illumina 16S rRNA metagenomic sequencing library protocols. The samples were barcoded for multiplexing, and sequencing was performed on an Illumina MiSeq instrument. Reference-based OTU picking was performed using the SILVA SSU v132 97% database (Yilmaz et al., 2014). Sequences present in more than one copy but not clustered to the database were then placed into *de novo* OTUs (97% similarity) and aligned against the reference database with 80% similarity threshold to assign the "closest" taxonomical name where possible. Chimeras were removed from the dataset if the absolute crossover cost was 3 using a k-mer size of 6. Alpha diversity was measured using Shannon entropy (OTU level).

### ELISA

IL-10 and TGF $\beta$  production was measured using ELISA MAXTM Deluxe Sets ELISA kit (Mouse IL-10, Biolegend) and TGF beta-1 Mouse Uncoated ELISA kit (Biolegend). The detection for each cytokine was performed following the manufacturer's instructions.

### QUANTIFICATION AND STATISTICAL ANALYSIS

Mann-Whitney U test, two-tailed one-way ANOVA, and Student's t test were used to determine significance levels for comparisons between groups using Prism 9.0 (Graphpad). All the specific analysis can be found in the figure legends. As to animal experiments, age and sex-matched mice were randomly divided into different groups. No data were excluded from analysis in this study. Mean  $\pm$  SEM is represented on graphs. A p value < 0.05 was considered statistically significant and shown as an asterisk (\*).