Epithelial MHC class II directs microbiota-specific intestinal immune homeostasis

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Abstract

Dysregulated immune responses to resident microbes promote pathologic inflammation, however, the mechanisms instructing commensal-specific T cells remain poorly understood. Here, we find that non-hematopoietic intestinal epithelial cells (IECs) represent the primary cells expressing major histocompatibility complex (MHC) II at the intestinal host-microbiota interface. Interestingly, epithelial MHCII and commensal-specific $CD4^+$ T cells were concurrently induced by post-natal microbiota colonization, provoking the hypothesis that epithelial MHCII regulates local commensal-specific $CD4^+$ T cells. While MHCII on classical antigen presenting cells directs expansion of antigen-specific $CD4^+$ T cells, loss of IEC-intrinsic MHCII surprisingly led to elevated commensal-specific $CD4^+$ T cells in the intestine. Further, epithelial MHCII expression actively limited accumulation of antigen-specific $CD4^+$ T cells in adult mice. Expansion of commensal-specific Th17 cells was restricted by epithelial MHCII, and remarkably mice lacking epithelial MHCII were highly susceptible to microbiota-triggered inflammation. Collectively, these data indicate that impaired epithelial MHCII-T cell regulation within mucosal tissues alters microbiota-specific immunity and predisposes to chronic inflammation.

Introduction

The mammalian gastrointestinal tract is home to trillions of microorganisms, collectively termed the microbiota, that form beneficial symbiotic relationships with host cells and play a significant role in health and disease. Extensive experiments utilizing broad-spectrum antibiotic or germ-free (GF) mouse models, which lack the microbiota, have demonstrated the necessity of a diverse and intact microbiota on mediating intestinal homeostasis[1,2]. These studies have highlighted that the microbiota directly interact with host cells to influence normal host physiology and tissue development. Digestion, intestinal barrier function, and immune cell development all rely on signals from the microbiota. Further, changes in the containment or diversity of the microbiota have been associated with the development of several chronic diseases including inflammatory bowel disease (IBD), asthma, cancer, and diabetes[1,3].

Intestinal immune responses are tightly regulated to allow protective immunity against invading pathogens, while limiting responses to innocuous commensals. It is well appreciated that the microbiota plays an important role in shaping $CD4^+$ T cell differentiation[4,5]. Commensal bacteria have been shown to drive regulatory T cell differentiation[6], and microbiota-reactive effector and memory T cells are present in both mice and humans[7–11]. Studies have demonstrated that commensal-specific T cells can contribute to intestinal homeostasis by promoting barrier protective cytokines and providing cross-reactivity to pathogens[10,12,13]. However, aberrant immune responses to the microbiota also promote inflammatory conditions, such as IBD[1,14–16]. In mouse models of colitis, intestinal dysbiosis drives inflammation in part by stimulating microbiota-reactive $CD4^+$ T cells[17–19]. Further, recent work found that microbiota-specific $CD4^+$ T cells in IBD patients were functionally altered and produced more pro-inflammatory cytokines compared

to healthy controls[10]. However, the mechanisms directing this critical balance between commensal-specific effector and regulatory T cells in the intestine remain poorly understood.

Auto-reactive $CD4^+$ T cell responses are limited by presentation of self-antigens in the thymus by cells expressing major histocompatibility complex-II (MHCII)[20–22]. In contrast, microbiota-responsive $CD4^+$ T cells are unlikely to encounter their specific antigen within the thymus, and are thus not subject to negative selection before entering peripheral tissues[23,24]. Previous work described that negative selection of activated commensal-specific T cells can be mediated by MHCII-expressing group 3 innate lymphoid cells (ILC3)[25,26]. However, ILC3s are relatively rare cells at the luminal surface of the intestine. In contrast, intestinal epithelial cells (IECs) are uniquely positioned at the direct interface between the microbiota and host immune cells and express necessary machinery for antigen uptake, processing, and presentation[27–31]. While MHCII expression by IECs was described over two decades ago, studies have only recently started to investigate its function in inflammation and tissue homeostasis[32–35]. Results from models of intestinal inflammation have demonstrated that epithelial MHCII regulation can be either protective or detrimental depending on the inflammatory context[33–36]. However, it remains unknown whether IEC expression of MHCII functionally regulates microbiota-triggered immune responses.

Surprisingly, we found that IECs were the dominant cells expressing MHCII at the intestinal luminal interface. Antibiotic-based approaches and germ-free mouse models demonstrated that IEC-intrinsic MHCII expression was dramatically upregulated by the microbiota following birth. Interestingly, this induction of MHCII was concurrent with the expansion of microbiota-specific

CD4$^+$ T cells in post-natal mice. This relationship provoked the hypothesis that IEC-intrinsic MHCII may regulate immune responses to the microbiota. In contrast to a predicted activating role of MHCII, we unexpectedly found that IEC-specific loss of MHCII *in vivo* resulted in increased accumulation of microbiota-specific intestinal CD4$^+$ T cells. Further, IEC-intrinsic MHCII actively limited commensal-specific CD4$^+$ T cells. Mice lacking IEC-intrinsic MHCII expression also exhibited increased accumulation of RORγt$^+$ and decreased FoxP3$^+$ commensal-specific CD4$^+$ T cells and were more susceptible to spontaneous, microbiota-triggered intestinal inflammation. Taken together, these data reveal that microbiota colonization promotes intestinal tolerance by inducing epithelial MHCII expression and thereby limiting local expansion of microbiota-specific T cells.

Results

Commensal bacteria induce intestinal epithelial MHCII expression.

Antigen presentation via MHCII is critical for instructing CD4$^+$ T cell responses, however we have limited understanding for how commensal-specific immune responses are established and maintained in mucosal tissues colonized by microbes. Hematopoietic-derived antigen presenting cells can regulate commensal-specific responses[37,38]. Surprisingly, though, we found that intestinal epithelial cells (IECs), and not CD45$^+$ hematopoietic cells, expressed the majority of MHCII at the intestinal-microbiota interface (**Fig. 1a, b**). IECs integrate microbial cues and instruct downstream immune responses, thus, we next assessed whether the microbiota plays a role in regulating IEC-intrinsic MHCII expression. Interestingly, IECs isolated from conventionally-housed (CNV) mice displayed higher expression of *H2-Ab1* and *H2-Aa*, which encode MHCII, compared to IECs isolated from GF mice (**Fig. 1c**). Consistent with this, surface MHCII protein expression was also

highly enriched in IECs from CNV mice compared to GF mice (**Fig. 1d, e**), whereas MHCII expression on hematopoietic cells at the luminal interface was not altered by microbiota (**Supplementary Fig. 1a**).

Microbiota colonization begins at birth with the complexity and density of microbiota increasing most significantly during early life and into adulthood[39–41]. To next determine whether IEC-intrinsic MHCII expression is modulated with microbiota colonization, IECs were assessed for *H2-Ab1* expression during development. Young wildtype pups with normal microbiota colonization exhibited epithelial *H2-Ab1* expression on IECs that increased into adulthood, whereas pups reared under GF conditions demonstrated low epithelial *H2-Ab1* expression that was minimally altered with age (**Fig. 1f**), indicating that the microbiota strongly controls the induction of epithelial MHCII expression with age. These results were recapitulated following antibiotic-induced microbiota depletion (**Fig. 1g**), as pups born to mothers treated with antibiotics failed to induce epithelial *H2-Ab1* expression (**Fig. 1h, i**). Taken together, these data demonstrate that post-natal microbiota colonization triggers elevated epithelial MHCII expression in the intestine.

Epithelial MHCII expression can be regulated by local immune cells[33,42–44]. Therefore, to evaluate whether MHCII is modulated in IECs in the absence of these cues, intestinal epithelial organoids were derived from the colon of wildtype mice. These three-dimensional organoid cultures possess physiologically relevant intestinal features including a polarized epithelial monolayer but are devoid of immune cells (**Supplementary Fig. 1b**). Interestingly, incubation of intestinal organoids with luminal contents collected from the colon of CNV mice robustly upregulated MHCII expression (**Supplementary Fig. 1c**). However, contents collected from GF mice failed to induce

similar expression of MHCII (**Supplementary Fig. 1c**), indicating that microbiota are required for triggering MHCII on IECs and that this can occur without external signals from other cells. Heat-killing bacteria in intestinal contents of CNV mice completely ablated this induction (**Supplementary Fig. 1d**), suggesting that MHCII expression is actively regulated by live bacteria or heat-liable bacterial products. Past studies have indicated that IFNγ signaling can induce IEC expression of MHCII[33,42–44]. However, microbiota exposure of IFNγ-deficient organoids still induced MHCII expression (**Supplementary Fig. 1e**), further supporting that the microbiota regulates epithelial MHCII in part via IEC-intrinsic mechanisms.

Commensal-specific CD4$^+$ T cells expand following post-natal microbial colonization.

Recent studies have highlighted that microbiota exposure early in life is required for effective immune education and that disruption to the microbiota during this early window of development may increase susceptibility to inflammatory disorders[40,41,45–47]. Therefore, to investigate whether microbiota instruct development of commensal-specific T cell responses, intestinal CD4$^+$ T cells were analyzed for reactivity to the class II-commensal flagella antigen-specific cBir1 tetramer. CNV mice exhibited significantly higher numbers of cBir1$^+$ CD4$^+$ T cells in the large intestine compared to GF mice that lack commensal microbes (**Fig. 2a, b**). Consistent with analyses by others[48,49], intestinal CD4$^+$ T cells were reduced in GF mice relative to CNV mice (**Fig. 2c**) and displayed a more naïve phenotype with decreased CD44 and increased CD62L expression (**Supplementary Fig. 2a, b**). However, day 15 GF and CNV pups did not display differences in CD4$^+$ T cell numbers in the large intestine (**Fig. 2c**). Thus, microbiota-derived signals are necessary for accumulation and maturation of intestinal CD4$^+$ T cell responses. Interestingly, while commensal-specific cBir1$^+$ CD4$^+$ T cells were expanded in adult CNV mice relative to adult GF

mice, commensal-specific $CD4^+$ T cells were similar between GF and CNV pups (**Fig. 2d, e**). Collectively, these data suggest that the microbiota direct development of commensal-specific T cell responses in the intestine with age.

Mice lacking IEC-intrinsic MHCII exhibit elevated microbiota-specific CD4+ T cells.

The temporal association between microbiota exposure, IEC-intrinsic MHCII expression, and expansion of commensal bacterial-specific $CD4^+$ T cells provoked the hypothesis that IEC-intrinsic MHCII expression may mediate the relationship between microbiota colonization and regulation of microbiota-specific T cells. To begin to test this hypothesis, an IEC-specific MHCII knockout mouse model was generated by crossing C57Bl/6 floxed H2-Ab1 mice ($MHCII^{FF}$) to mice expressing cre recombinase downstream of the villin promoter[50,51]. Significant reduction in MHCII expression in IECs was confirmed by mRNA (**Fig. 3a**) and surface protein (**Fig. 3b**) analyses. Importantly, loss of MHCII expression was specific to IECs, as $CD45^+$ cells in the intestine retained MHCII surface expression (**Fig. 3c**). Loss of IEC-intrinsic MHCII had minimal effect on total $CD4^+$ T cell numbers in the intestine (**Fig. 3d**). However, $MHCII^{\Delta IEC}$ mice surprisingly displayed elevated numbers of commensal-specific $cBir1^+$ $CD4^+$ T cells, relative to floxed littermate control mice (**Fig. 3e**), suggesting that expression of MHCII by IECs regulates microbiota-specific T cell homeostasis in the intestine.

Contrary to the canonical role of MHCII in promoting $CD4^+$ T cell expansion, mice with loss of IEC-intrinsic MHCII surprisingly exhibited elevated commensal-specific $CD4^+$ T cells. Therefore, to investigate the mechanism by which epithelial MHCII regulates tissue-intrinsic T cells, proliferation of microbiota-specific T cells was compared in $MHCII^{\Delta IEC}$ mice (**Supplementary**

Fig. 3a). Loss of IEC-intrinsic MHCII expression had little effect on Ki67$^+$ levels in cBir1$^+$ CD4$^+$ T cells (**Supplementary Fig. 3a**), suggesting that epithelial MHCII does not restrict proliferation of commensal-specific T cells. Therefore, to next test whether antigen-experienced CD4$^+$ T cells exhibited altered apoptosis, AnnexinV$^+$ levels were compared. Interestingly, loss of epithelial MHCII expression resulted in decreased AnnexinV$^+$ CD4$^+$ T cells in the intestine compared to littermate controls (**Supplementary Fig. 3b**). These data suggest that IEC-intrinsic MHCII expression promotes deletion of local commensal-specific CD4$^+$ T cells and, therefore, these cells accumulate in the intestine of MHCII$^{\Delta IEC}$ mice relative to control mice.

Epithelial MHCII actively regulates commensal-specific T cells in the intestine.

To evaluate whether IEC-intrinsic MHCII actively regulates CD4$^+$ T cell responses, we employed a fungal commensal model that uses a recombinant *C. albicans* expressing 2W1S$_{55\text{-}68}$ variant of I-Ea epitope (**Fig. 4a**)[52–54]. MHCII^{FF} and MHCII$^{\Delta IEC}$ mice colonized for 2 weeks with *C. albicans*-2W1S$^+$ displayed similar levels of *C. albicans* intestinal colonization (**Fig. 4b**) with no evidence of dissemination or infection. 2W1S$^+$ CD4$^+$ T cells were detected in the large intestine of mice colonized with *C. albicans*-2W1S$^+$ (**Fig. 4c**). Interestingly, MHCII$^{\Delta IEC}$ mice displayed increased numbers of *C. albicans*-2W1S-specific CD4$^+$ T cells relative to MHCII^{FF} mice (**Fig. 4c, d**), indicating that IEC-intrinsic MHCII actively controls commensal-specific CD4$^+$ T cell levels in the intestine of adult mice. Both MHCII^{FF} and MHCII$^{\Delta IEC}$ mice exhibited *Candida*-specific CD4$^+$ T cells that were characterized predominantly as FoxP3$^+$ Tregs, and fewer RORγt$^+$ Th17 cells (**Fig. 4e**). However, differentiation of *Candida*-specific CD4$^+$ T cells harvested from MHCII$^{\Delta IEC}$ mice exhibited an elevated Th17 and diminished Treg profile relative to antigen-specific CD4$^+$ T cells from MHCII^{FF} mice (**Fig. 4e, f**). Together, these results demonstrate that IEC-intrinsic MHCII

expression actively regulates the number and phenotype of commensal-specific $CD4^+$ T cells in the intestine.

MHCII expression on IECs limits microbiota-induced intestinal inflammation.

Dysregulated commensal-specific $CD4^+$ T cells have been found in patients with intestinal inflammation[10]. Remarkably, when $MHCII^{\Delta IEC}$ mice and littermate $MHCII^{FF}$ controls were permitted to age longer, $MHCII^{\Delta IEC}$ mice exhibited an increased incidence of rectal prolapse (**Fig. 5a**), a clinical symptom of intestinal inflammation. Consistent with this, levels of the inflammatory biomarker, lipocalin-2, was elevated in the intestine of $MHCII^{\Delta IEC}$ mice relative to control floxed mice (**Fig. 5b**), while barrier permeability was similar between these genotypes (**Supplementary Fig. 4a, b**). Interestingly, $MHCII^{\Delta IEC}$ mice displaying an increased propensity to prolapse also demonstrated the highest amount of commensal-specific $cBir1^+CD4^+$ T cells within these cohorts (**Fig. 5c**).

In addition, similar to findings with the shorter *C. albicans*-$2W1S^+$ commensal model, the ratio of $FoxP3^+$ Tregs to $ROR\gamma t^+$ Th17 cells was significantly altered in $MHCII^{\Delta IEC}$ mice (**Fig. 5d, e**). The majority of $cBir1^+CD4^+$ T cells were $FoxP3^+$ Tregs (**Fig. 5d**), however, loss of IEC-intrinsic MHCII resulted in a reduction of Tregs and an increase in Th17 commensal-reactive T cells relative to the ratio observed in control $MHCII^{FF}$ mice (**Fig. 5d, e**). IL-10 and IL-17 expression in the large intestine were also correspondingly shifted in $MHCII^{\Delta IEC}$ mice compared to $MHCII^{FF}$ mice (**Fig. 5f, g**). This Treg to Th17 skewing of commensal-specific $CD4^+$ T cells and cytokine expression was more evident in $MHCII^{\Delta IEC}$ mice that developed intestinal inflammation and prolapse (**Fig. 5c-g**).

To test the requirement for the microbiota in this spontaneous intestinal inflammation, MHCII^{FF} and MHCII$^{\Delta IEC}$ mice were compared to littermates of both genotypes in the context of broad-spectrum antibiotics that significantly deplete commensal bacteria (**Supplementary Fig. 4c**). Consistent with earlier results, MHCII$^{\Delta IEC}$ mice exhibited an increased prevalence of rectal prolapse (**Fig. 5h**). However, depletion of the microbiota prevented spontaneous intestinal inflammation in MHCII$^{\Delta IEC}$ mice (**Fig. 5h**). Furthermore, accumulation of commensal-specific Th17 cells did not occur following microbiota depletion (**Fig. 5i**) and loss of microbiota inhibited the IL-17 elevation observed in microbiota-replete MHCII$^{\Delta IEC}$ mice (**Fig. 5j**). Collectively, these data support that regulation by epithelial MHCII promotes T cell tolerance to resident microbes and limits development of microbiota-sensitive Th17-mediated intestinal inflammation.

Discussion

The microbiota is essential for the development and education of the host immune system. However, inappropriate immune reactions to the microbiota have been associated with several chronic inflammatory conditions. In this study, we found that upregulation of epithelial MHCII expression in the large intestine coincided with initial microbiota colonization and expansion of commensal-specific T cells, indicating that IECs may play an important role in early-life immune education during development. Further, deletion of IEC-intrinsic MHCII expression led to accumulation of commensal-specific Th17 cells in the large intestine that reflected impaired apoptosis of commensal-specific T cells. Loss of IEC-intrinsic MHCII also elevated IL-17 in the tissue and increased susceptibility to microbiota-sensitive intestinal inflammation. (**Supplementary Fig. 5**).

Interestingly, patients with active IBD have functionally distinct microbiota-reactive $CD4^+$ T cells, compared to those found in healthy controls, and secrete higher levels of IL-17[10,55]. IL-17 has been linked to development and exacerbation of several autoimmune and inflammatory conditions, including IBD[56]. Furthermore, mouse models with disrupted IL-17 signaling are protected from intestinal inflammation[57,58], supporting a pathogenic role for IL-17 in intestinal inflammation. However, clinical trials depleting IL-17 in IBD patients have been ineffective, and in some cases worsened disease[59]. This pleotropic role for IL-17 in regulating intestinal homeostasis suggests that targeting specific IL-17 producers, instead of broadly neutralizing IL-17 itself, may be a more effective treatment. In fact, work has shown that therapeutics which target commensal-specific Th17 cells, while retaining IL-17 production from other cell types, reduced intestinal inflammation[60]. Our data suggests that promoting epithelial MHCII expression may further restrict pro-inflammatory commensal-specific T cells and promote healthy intestinal homeostasis.

$MHCII^{\Delta IEC}$ mice also exhibited a concurrent decrease in commensal-specific $FoxP3^+$ Treg cells and intestinal IL-10 expression. Interestingly, colonization with a new microbe resulted in active skewing of commensal-specific T cells toward Th17 cells in mice that lack epithelial MHCII, whereas wildtype littermate controls developed more commensal-specific Treg cells. Therefore, epithelial MHCII is necessary to control the delicate balance between suppressive and pro-inflammatory antigen-specific T cells in the intestine. The requirement for IEC-intrinsic MHCII in promoting commensal-reactive Tregs and limiting Th17 cells in the colon is consistent with recent findings that epithelial MHCII expression in the small intestine affects IL-10 production by local intraepithelial lymphocytes[34]. Taken together, these findings support that MHCII expression

specifically by epithelial cells is a dominant mechanism at the luminal surface that limits commensal-specific $CD4^+$ T cells and dampens pathogenic responses to the microbiota. Given the role for MHCII in ILC3s[25,26], complimentary and essential roles for antigen presentation pathways in non-classical cells seem to be essential for establishing and maintaining healthy intestine-intrinsic tissue homeostasis.

Crosstalk between the microbiota and mammalian immune cells involves communication via pattern recognition receptor engagement and microbiota-derived metabolite signaling. While MHCII expression in the intestine has been linked to the microbiota[61], we found that live microbes, or heat-labile microbial components, are necessary for MHCII induction on these cells. Immune cells can induce epithelial MHCII, however we determined that IECs can also intrinsically sense the microbiota to upregulate MHCII expression by employing intestinal organoid cultures that lack immune cells or IFNγ. Therefore, microbiota-dependent epithelial MHCII regulation also occurs, in part, through direct communication that is independent of immune cells. Disruption to canonical pattern recognition pathways leads to loss of intestinal barrier integrity and increased inflammation[62–64] and the toll-like receptor signaling adaptors MyD88/TRAF have been shown to induce epithelial MHCII expression, particularly in the small intestine[33]. However, further investigation will be needed to decipher the host and microbial-derived pathways that are responsible for epithelial MHCII regulation.

Recent studies have begun to investigate epithelial MHCII from multiple approaches. Loss of epithelial MHCII protected mice that were subjected to models of T cell driven intestinal inflammation and graft versus host disease[33,35]. Our findings highlight a broader role in which

epithelial cells are the primary MHC class II-expressing cells in the intestine controlling microbiota-specific T cells and intestinal pathology induced by commensal bacteria (**Supplementary Fig. 5**). Therefore, this work uncovers a fundamental new tenet for immune regulation whereby IEC-intrinsic MHCII is largely induced by the microbiota to dampen self-directed immune responses in early life. Antigen-presenting immune cells are traditionally viewed to be most important in regulation of antigen specificity of CD4 T cells. However, these findings reveal a central and essential epithelial-T cell mechanism that controls mucosal immunity specifically to commensal microbes, and suggest that this regulatory pathway can be targeted to treat microbiota-triggered inflammatory conditions.

Methods

Mice. Conventionally-housed C57Bl/6J mice were purchased from Jackson Laboratories and maintained in our specific-pathogen free colony at Cincinnati Children's Hospital Medical Center (CCHMC). Germ-free (GF) mice were maintained in flexible isolators in the CCHMC Gnotobiotic Mouse Facility, fed autoclaved feed and water, and monitored for absence of microbes. H2-Ab1(MHCII)^{FF} were purchased from Jackson and maintained at CCHMC. MHCII^{FF} mice were crossed to C57Bl/6J-Villin-Cre to generate MHCII$^{\Delta IEC}$ mice. Sex- and age-matched littermate controls were used for all studies. Mice were housed up to 4-per cage in a ventilated cage system in a 12-h light/dark cycle, with free access to water and food. For antibiotic treatment, pregnant mice were provided water supplemented with 1-mg/mL colistin (Sigma), 1-mg/mL ampicillin (Sigma) and 5-mg/mL streptomycin (Sigma) at mid-gestation[65]. Mothers and pups were maintained on antibiotic-water until harvest as noted. For MHCII^{FF} and MHCII$^{\Delta IEC}$ mice, pups were placed on antibiotic-water at weaning and maintained on antibiotics for 16-weeks. Antibiotic-water was refreshed every 7-10 days. *Candida albicans* colonization was conducted as previously described[52,53]. Briefly, mice were pre-treated with ampicillin-water (1-mg/mL) two days prior to colonization and maintained on ampicillin-water for the duration of the experiment. Mice were gavaged with recombinant *C. albicans* expressing 2W1S$_{55-68}$ peptide[54]. Mice were monitored for colonization by plating fecal CFUs and were harvested 14-days post-colonization. All mouse studies were conducted with approval by Animal Care and Use Committees at CCHMC. These protocols follow standards enacted by the United States Public Health Services and Department of Agriculture. All experiments followed standards set forth by Animal Research: Reporting of In Vivo Experiments (ARRIVE).

***Cell Isolation*.** The large intestine was harvested, opened, and washed in PBS. For IECs, tissue was placed in pre-warmed strip buffer (PBS, 5% FBS, 1mM EDTA, 1mM DTT) and incubated at 37°C at 45-degree angle with shaking at 180-rpm for 15-min. For lamina propria isolation, tissue was washed with PBS to remove EDTA and DTT then incubated in pre-warmed digestion buffer (RPMI with 1-mg/mL Collagenase/Dispase (Sigma)) at 37°C at 45-degree angle with shaking at 180-rpm for 30-min. After incubation, the tissue was vortexed and passed through a 70µM cell strainer.

***Flow cytometry*.** Cells were stained for flow cytometry using the following antibodies diluted in FACS Buffer (2% FBS, 0.01% Sodium Azide, PBS): BV711-anti-CD326 (EpCAM) (Clone:G8.8, BioLegend), BUV395-anti-CD45.2 (Clone:104, BD Biosciences), APC-anti-MHCII (Clone:M5/114.15.2, eBioscience), APC-eFluor-780-anti-CD4 (Clone:RM4-5, eBioscience), Pe-Cy7-anti-CD44 (Clone:IM7, eBioscience), AlexaFluor-647-anti-RORγt (Clone:Q31-378, BD Biosciences), PacificBlue-anti-FoxP3 (Clone:FJK-16s, eBiosciences), PerCP-eFluor710-anti-CD8a (Clone:53-6.7, eBiosciences), PerCP-eFluor710-anti-B220 (Clone:RA3-6B2, eBiosciences), PerCP-eFluor710-anti-Ly6G (Clone:1A8-Ly6g, eBiosciences), PerCP-Cy5.5-anti-CD11b (Clone:M1/70, eBiosciences), PerCP-Cy5.5-anti-CD11c (Clone:N418, eBiosciences), PacificBlue-anti-Ki67 (Clone:SolA15, eBiosciences). Dead cells were excluded with the Fixable Aqua Dead Cell Stain Kit (Invitrogen). The BD Fix/Perm kit was used for intracellular staining. Cells were stained with 488-congugated-AnnexinV diluted in AnnexinV staining buffer (BD Pharamgen). Class-II restricted tetramers (cBir1 and 2W1S) were PE-conjugated and were generated and provided by the NIH tetramer core. Samples were acquired on the BD LSRFortessa and analyzed with FlowJo Software (Treestar).

Organoid cultures. Murine organoids were generated from colonic crypts isolated from GF and CNV, or B6.IFNγ$^{-/-}$[66] mice[67]. Briefly, the colon was opened, cleaned, and cut into 1-cm pieces. Tissue was incubated in chelation buffer (2mM EDTA in PBS) for 30-min at 4°C with rotation then transferred into shaking buffer (PBS, 43.3mM sucrose, 54.9mM sorbitol) and shaken by hand. Crypts were plated in Matrigel (Corning) with organoid culture media (60% Advanced DMEM/F12 supplemented with 10mM HEPES, 2mM L-glutamate, 40% L-WRN conditioned media, 1xN2, 1xB27, 50-ng/mL murine EGF, and 10µM Y-27632) overlaid. Media was changed every 2-3 days. For microbial stimulation, stool was collected from either GF or CNV mice and homogenized in PBS using a TissueLyser (Qiagen) at a concentration of 100-mg of stool weight/mL of PBS. Organoid cultures were treated with 100-µL of intestinal contents for 24-hrs. Heat-killing was performed at 95°C for 1-hr. After incubation, cultures were washed with PBS, Matrigel was disrupted, and treated with TrypLE (Gibco) for 3-5 mins to dissociate into single-cell suspension. Cells were stained for flow cytometry. For immunostaining, organoids were plated in 8-well u-slide (ibidi) and fixed in 4% paraformaldehyde for 20-min. Organoids were permeabilized for 20-min in PBS containing 0.5% TritonX-100 followed by a 30-min incubation in Blocking Buffer (PBS, 0.2% TritonX-100, 0.05% Tween, 1% BSA, and 1% normal goat serum). Organoids were stained overnight at 4°C in blocking buffer containing AlexaFluor488-anti-EpCAM (Clone:G8.8, BioLegend). Organoids were washed and imaged using a confocal microscope (Nikon).

RNA and qPCR analysis. RNA was isolated using the RNeasy Kit (Qiagen). For RNA from whole tissue, samples were homogenized in TRIzol. Choroform was added for phase separation and RNA

was precipitated by mixing with isopropanol. cDNA was synthesized using the Verso reverse transcriptase kit (Thermo Fisher) following the manufacturer's protocol. Real-time PCR was performed using SYBR green (Applied Biosystems) and analyzed using the following primers sequences. HPRT forward 5'- GATTAGCGATGAACCAGGT-3', HPRT reverse 5'- CCTCCCATCTCCTTCATGACA-3', H2-Ab1 forward 5'- TGTGAGTCCTGGTGACTGCCATTA-3', H2-Ab1 reverse 5'- TCGCCCATGAACTGGTACACGAAA-3', H2-Aa forward 5'- CAACCGTGACTATTCCTTCC-3', H2-Aa reverse 5'- CCACAGTCTCTGTCAGCTC-3', IL-10 forward 5'- GAGAGCTGCAGGGCCCTTTGC-3', IL-10 reverse 5'- CTCCCTGGTTTCTCTTCCCAAGACC-3', IL-17 forward 5'- ACCGCAATGAAGACCCTGAT-3', IL-17 reverse 5'- TCCCTCCGCATTGACACA-3'.

Lipocalin-2 ELISA and FITC analyses. Fecal pellets were homogenized in PBS at a concentration of 100-mg/mL then centrifuged at high-speed for 10-mins. Supernatants were collected and lipocalin-2 levels were determined using mouse Lipocalin-2/NGAL (R&D Systems) following manufacturer's instructions. For FITC permeability studies, mice were fasted for at least 4-hrs before being gavaged with FITC-dextran (0.6 mg/g of mouse body weight) diluted in PBS. Serum was collected 4-hrs post gavage and fluorescent intensity was measured (excitation 485nm/emission 530nm).

Statistical analysis. Results are expressed as mean ± SEM. Statistical significance was determined with the Student's t-test or one-way ANOVA with all data meeting the assumptions of the statistical test used. Results were considered significant at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$,

****$p < 0.0001$. Statistical significance was calculated using Prism version 7.0 (GraphPad Software).

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FIGURE LEGENDS

Fig. 1. Commensal bacteria induce intestinal epithelial MHCII expression. (**a-b**) MHCII^{+} expressing cells at colonic luminal surface. (**c**) mRNA expression of MHCII genes H2-Ab1 and H2-Aa in IECs isolated from large intestine of GF and CNV mice. (**d**) Surface expression and (**e**) MFI of MHCII on EpCAM^{+} cells. (**f**) mRNA expression of H2-Ab1 in IECs isolated from large intestine of GF and CNV mice at multiple timepoints. (**g**) Relative 16S rRNA gene levels in intestinal contents. (**h**) IEC H2-Ab1 mRNA expression and (**i**) MHCII surface expression (gated on CD45^{-}, EpCAM^{+}) in control and antibiotic (ABX)-treated mice. Data are representative of at least three independent experiments, 3-4 mice per group, per timepoint. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Fig. 2. Commensal-specific CD4^{+} T cells expand following post-natal microbial colonization. (**a-b**) Number of cBir1^{+} tetramer-specific CD4^{+} T cells in large intestine of adult GF and CNV mice. (**c**) Frequency of CD4^{+} T cells and (**d-e**) number of cBir1^{+} tetramer-specific CD4^{+} T cells isolated from large intestine of neonatal and adult GF and CNV mice. cBir1^{+} tetramer cells are gated on live, CD45^{+}, lineage (CD11b, B220, Ly6G, CD11c, CD8a)$^{-}$, CD4^{+}. Data represent at least two independent experiments, 3-4 mice per group. *p<0.05, **p<0.01, ***p<0.001, ns=not significant.

Fig. 3. Mice lacking IEC-intrinsic MHCII exhibit elevated microbiota-specific CD4^{+} T cells. (**a**) H2-Ab1 mRNA expression in IECs isolated from the large intestine of MHCII^{FF} and

MHCII$^{\Delta IEC}$ mice. (**b**) Surface MHCII expression in EpCAM^{+}, CD45^{-} cells from MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. (**c**) Surface MHCII expression in EpCAM^{-}, CD45^{+} cells from MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. (**d**) Frequency of total CD4^{+} and (**e**) number of cBir1^{+} tetramer-specific CD4^{+} T cells isolated from large intestine of MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. Gated on live, CD45^{+}, lineage (CD11b, B220, Ly6G, CD11c, CD8a)$^{-}$, CD4^{+}. Data represent at least three independent experiments, 3-4 mice per group. *p<0.05, **p<0.01, ***p<0.001.

Fig. 4. Epithelial MHCII actively regulates commensal-specific T cells in the intestine. (**a**) Schematic of *C. albicans* commensal colonization. (**b**) Colony forming units (CFUs) of *C. albicans* in stool from MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. (**c, d**) Number of *C. albicans*-2W1S^{+}-specific CD4^{+} cells in large intestine of MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. (**e**) Frequency of FoxP3^{+} and (**f**) RORγt^{+} *C. albicans*-2W1S^{+}-specific CD4^{+} cells in large intestine of MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. Gated on live, CD45^{+}, lineage (CD11b, B220, Ly6G, CD11c, CD8a)$^{-}$, CD4^{+}. Data represent at least two independent experiment, 3-4 mice per group. *p<0.05.

Fig. 5. MHCII expression on IECs limits microbiota-induced intestinal inflammation. (**a**) Frequency of rectal prolapse in MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. (**b**) Lipocalin-2 levels in stool of MHCII^{FF}, MHCII$^{\Delta IEC}$, and prolapsed MHCII$^{\Delta IEC}$ mice. (**c**) cBir1^{+}-specific CD4^{+} T cells in large intestine of MHCII^{FF}, MHCII$^{\Delta IEC}$, and prolapsed MHCII$^{\Delta IEC}$ mice. (**d**) Frequency of FoxP3^{+} and (**e**) RORγt^{+} cBir1^{+}-specific CD4^{+} T cells. (**f**) IL-10 and (**g**) IL-17 mRNA in large intestine of MHCII^{FF}, MHCII$^{\Delta IEC}$, and prolapsed MHCII$^{\Delta IEC}$ mice. (**h**) Frequency of rectal prolapse in control and antibiotic (ABX)-treated MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. (**i**) Frequency of RORγt^{+} cBir1^{+}-specific CD4^{+} T cells in large intestine. (**j**) IL-17 mRNA expression in large intestine of control

and ABX-treated MHCII^{FF} and MHCII$^{\Delta IEC}$ mice. Data are representative of at least two independent experiments, 3-6 mice per group. *p<0.05, **p<0.01, ***p<0.001, ns=not significant.

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Fig. 1. Microbiota colonization induces epithelial MHCII expression.

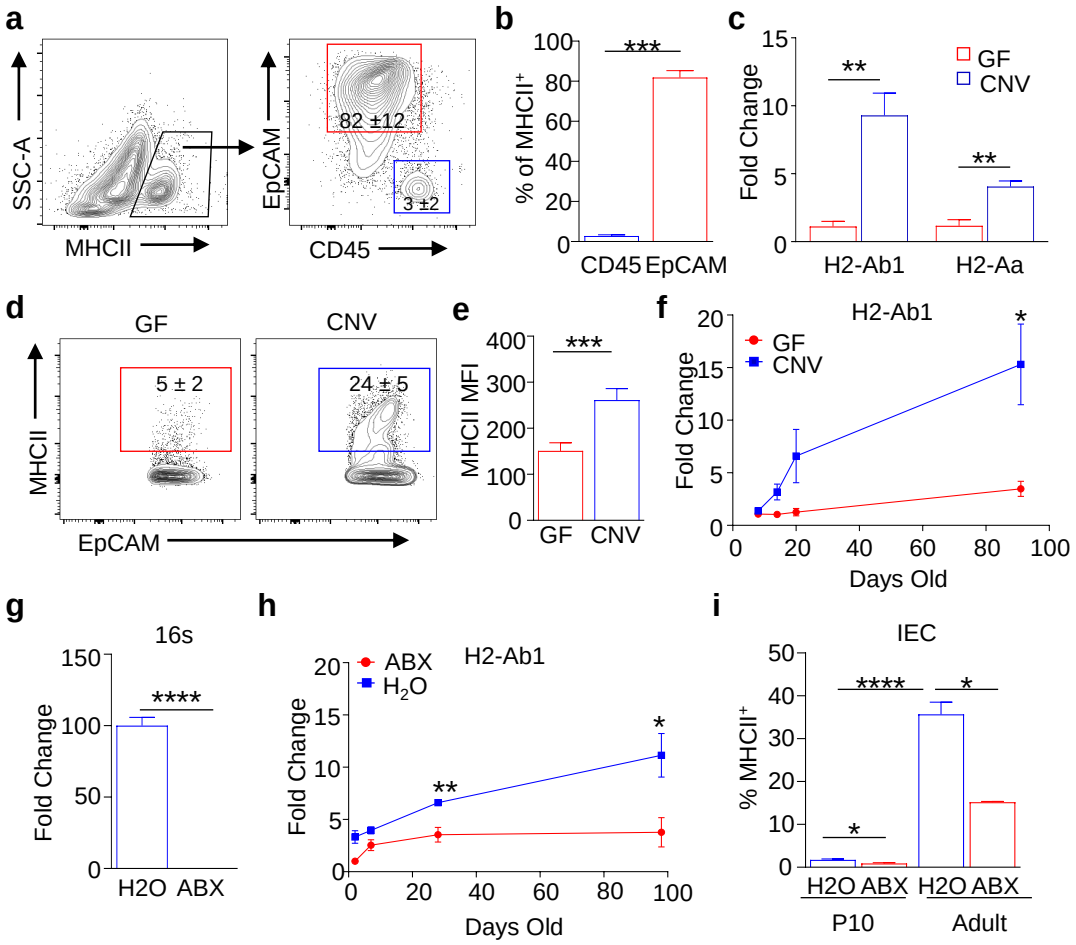

Fig. 2. Commensal-specific CD4+ T cell responses expand with microbiota colonization.

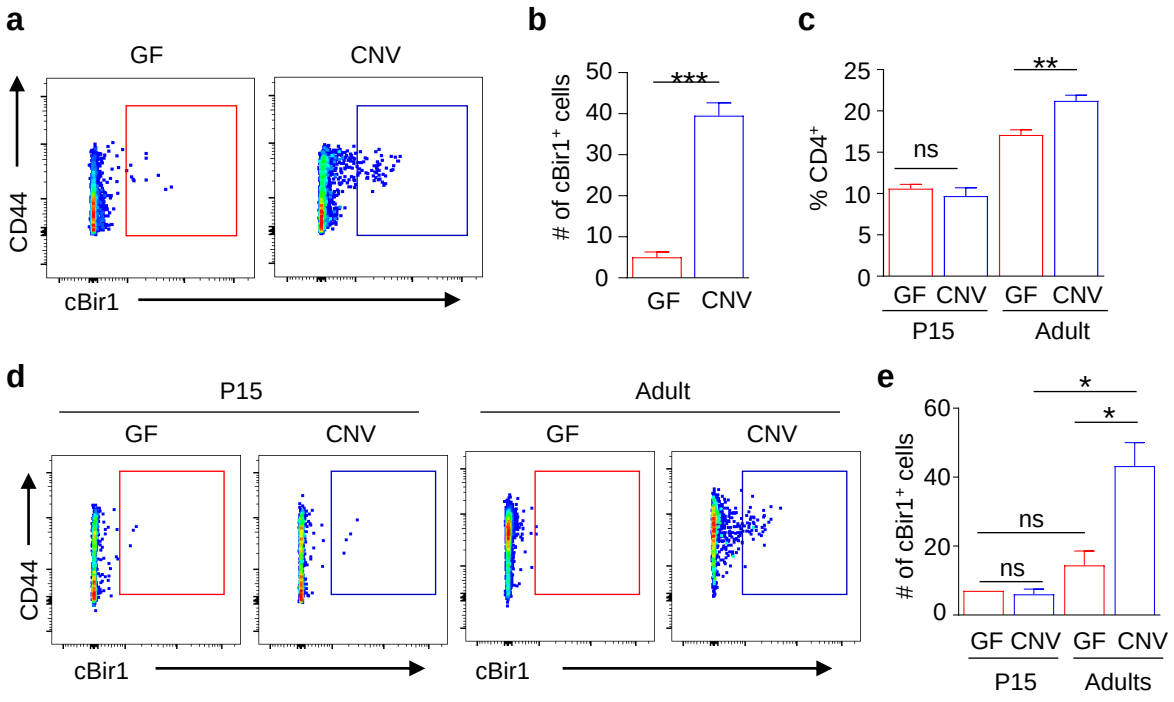

Fig. 3. Microbiota-specific CD4+ T cells are elevated in the colon of MHCII$^{\Delta IEC}$ mice.

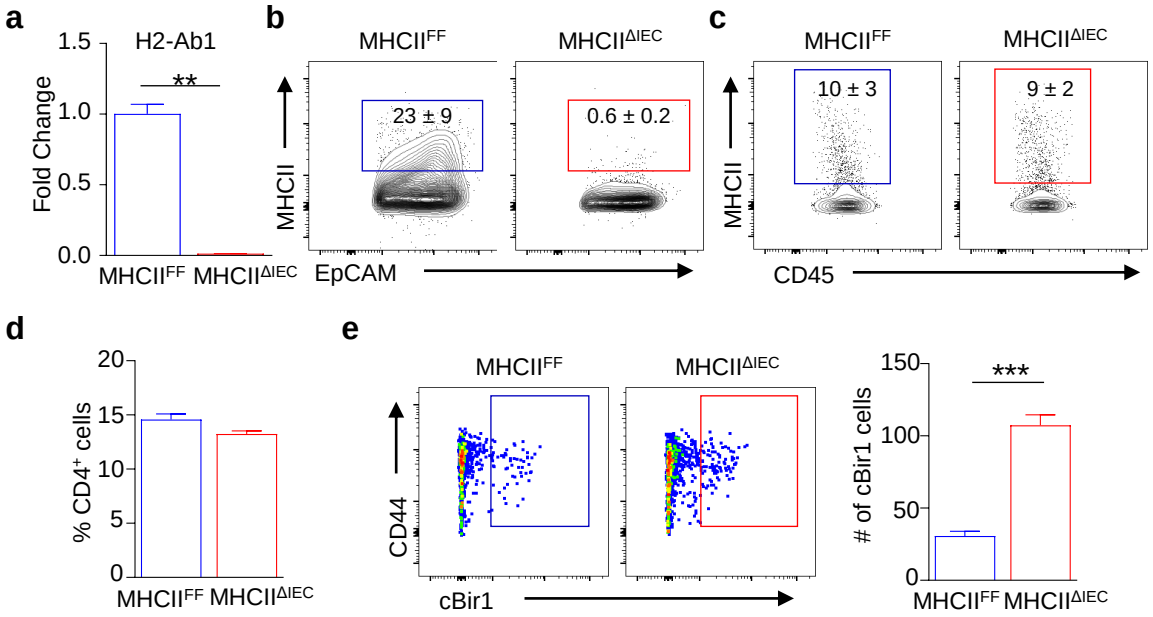

Fig. 4. Epithelial MHCII actively regulates microbiota-specific T cells in the colon.

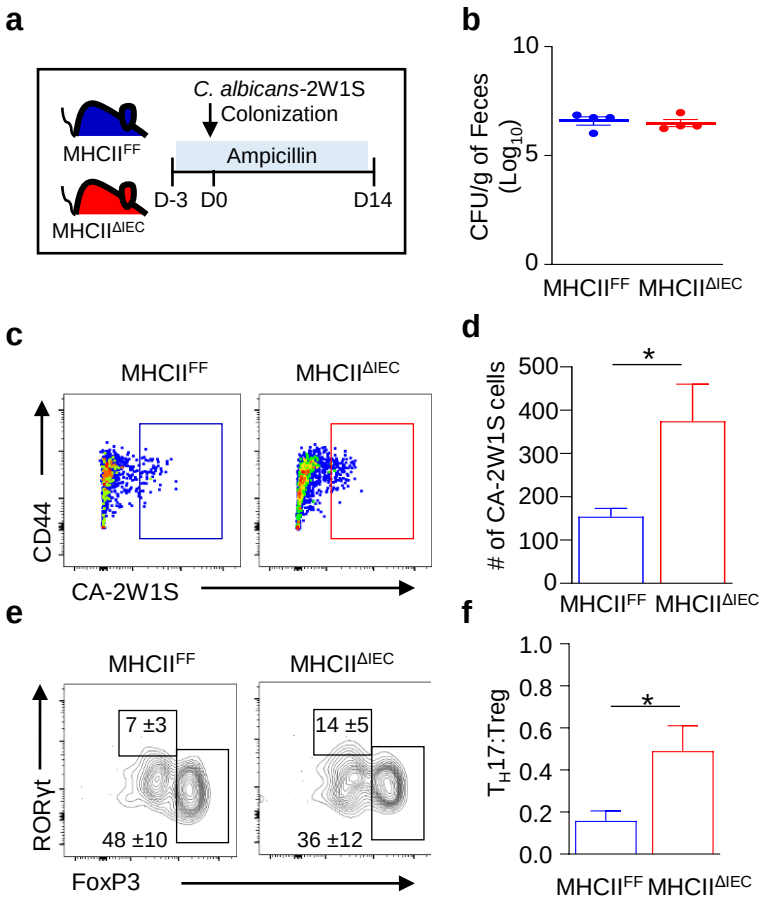
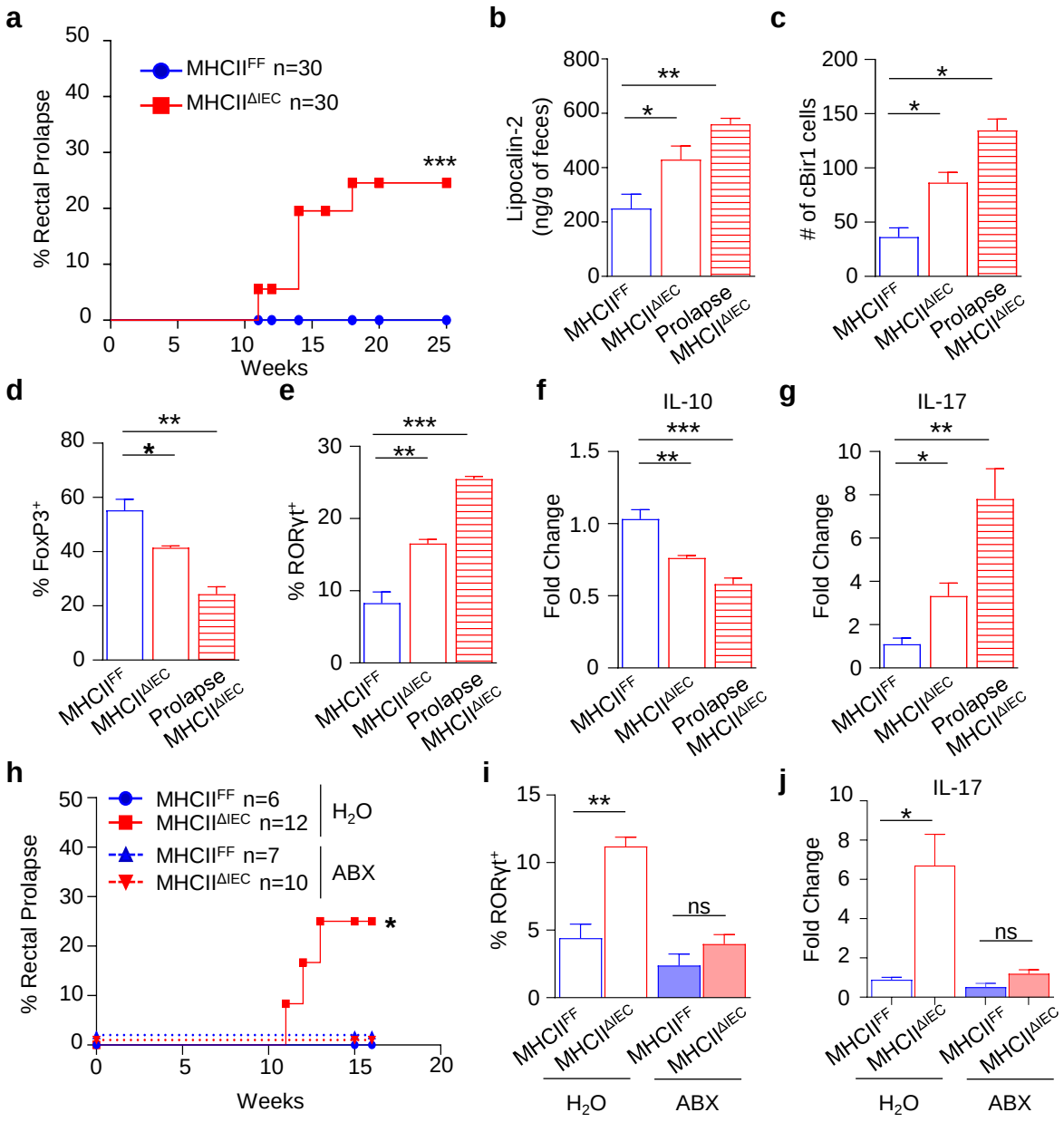
Fig. 5. Epithelial MHCII expression limits microbiota-dependent intestinal inflammation.