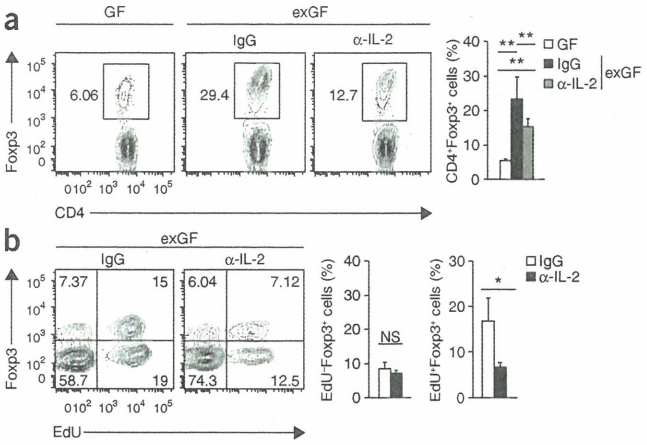

Figure 2 IL-2 is responsible for the vigorous proliferation of colonic T_{reg} cells after inoculation of commensal bacteria. (**a**) Foxp3 expression by CD4+ T cells in the cLP of GF mice left untreated and exGF mice inoculated with bacteria (as in **Fig. 1a**) and, 3 d later, given intravenous injection of control IgG or IL-2-neutralizing antibody (α-IL-2) and assessed 2 d later. Numbers adjacent to outlined areas (left) indicate percent Foxp3+CD4+ T cells. (**b**) Proliferation of CD4+ T cells in the cLP of exGF mice treated as in **a**. Numbers in quadrants (left) indicate percent cells in each. $*P < 0.05$ and $**P < 0.01$ (one-way analysis of variance (ANOVA) followed by Tukey's test (**a**) or Student's *t*-test (**b**)). Data are representative of at least three independent experiments (error bars, s.d. of three mice).

T_{reg} cells with neutralizing antibodies to integrin $\alpha_4\beta_7$ subunits[11] before administering the thymidine analog EdU to exGF mice. This treatment affected the abundance of proliferative T_{reg} cells in the colon only marginally (**Fig. 1d**). Collectively, these results suggested that colonization by commensal bacteria induced extensive proliferation of T_{reg} cells mainly in the colonic mucosa.

IL-2 is responsible for colonic T_{reg} cell population expansion

IL-2, which is well documented as promoting the proliferation of T_{reg} cells[22], was induced in the colonic mucosa, particularly in CD4+ T cells after inoculation of mice with bacteria (**Fig. 1a**). Similarly, colonic CD4+ T cells of infant mice (around 2 weeks old) housed in SPF conditions had high expression of IL-2; during this time, T_{reg} cells displayed active proliferation in the cLP but not in the spleen (**Supplementary Fig. 1b,d**). We therefore postulated that early induction of IL-2 may have been responsible for the local proliferation of T_{reg} cells. To test this idea, we treated exGF mice with neutralizing antibody to IL-2. As expected, abrogation of IL-2 strongly suppressed the induction of colonic T_{reg} cells (**Fig. 2a**). There was also a significantly lower abundance of EdU+ proliferating T_{reg} cells in the antibody-treated exGF mice than in their counterparts treated with the control antibody immunoglobulin G (IgG) (**Fig. 2b**). On the basis of these observations, we reasoned that an early IL-2 response was indispensable for the proliferation of T_{reg} cells in the colon.

IL-2 upregulates *Uhrf1* in colonic T_{reg} cells

We explored the molecular machinery that mediates the proliferation of colonic T_{reg} cells. First we profiled genes selectively upregulated in T_{reg} cells from exGF mice (**Fig. 3a**, cluster I). We also categorized IL-2-responsive genes (**Fig. 3b**, cluster II). After comparison of the two clusters, followed by gene ontology–based functional analysis, we selected several candidate genes encoding molecules potentially associated with the proliferation of colonic T_{reg} cells in an IL-2-dependent manner (**Fig. 3c**). Among those specifically upregulated in colonic T_{reg} cells was *Uhrf1* (**Fig. 3d**). *Uhrf1* expression was highest in T_{reg} cells among colonic CD4+ T cell subsets in SPF mice (**Fig. 3e**). We confirmed that IL-2 was essential for *Uhrf1* expression by colonic T_{reg} cells after inoculation of commensals, since neutralization of IL-2 in exGF mice significantly inhibited *Uhrf1* expression (**Fig. 3f**). In contrast, *Uhrf1* was not induced in splenic T_{reg} cells from exGF mice (**Supplementary Fig. 1e**). Consistent with our observations of exGF mice, there was substantial upregulation of *Uhrf1* in colonic T_{reg} cells during the population-expansion phase in infant SPF mice (**Supplementary Fig. 1c**).

To rigorously confirm the role of bacterial colonization in *Uhrf1* expression, we analyzed gnotobiotic mice colonized with a 17-strain mixture of Clostridia bacteria ('17-mix'), which efficiently induces the population expansion of T_{reg} cells in the colon[23]. Inoculation of GF mice with 17-mix significantly augmented IL-2 expression by T_{conv} cells (**Fig. 3g**), which led to upregulation of *Uhrf1* in T_{reg} cells, with a concomitant increase in their proliferation (**Fig. 3h,i**). We also confirmed the upregulation of *Uhrf1* in cultured T_{reg} cells stimulated with IL-2 (**Fig. 3j**), in which accumulation of the transcription factor STAT5 on the promoter region of *Uhrf1* was also evident (**Fig. 3k**). Together these results indicated that commensal bacteria upregulated *Uhrf1* in T_{reg} cells by eliciting IL-2 production from effector T cells (T_{eff} cells) in the colonic mucosa.

Uhrf1 is critical for colonic T_{reg} cell proliferation

To investigate the role of Uhrf1 in the homeostasis of colonic T_{reg} cells, we generated mice with T cell–specific deficiency in Uhrf1 (mice with *lox*P-flanked alleles (*Uhrf1*$^{fl/fl}$) deleted by Cre recombinase expressed from the Cd4 promoter (*Uhrf1*$^{fl/fl}$*Cd4*-Cre mice); **Supplementary Fig. 2a**) and crossed them with *Foxp3*^{hCD2} reporter mice (which have sequence encoding a reporter fusion of human CD52 and CD2 inserted into *Foxp3*), to easily detect T_{reg} cells[14], and thus generated *Uhrf1*$^{fl/fl}$*Cd4*-Cre*Foxp3*^{hCD2} progeny (called '*Uhrf1*$^{fl/fl}$*Cd4*-Cre' here). In young *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice reared under SPF conditions, the overall composition of B lymphocytes and T lymphocytes was intact (**Supplementary Fig. 2b,c**). However, these mice had a considerable defect in the development of colonic T_{reg} cells (**Fig. 4a**) indicative of the importance of Uhrf1 in the homeostasis of T_{reg} cells in the colonic mucosa. We observed a slightly lower abundance of T_{reg} cells in the spleen and thymus of *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice than in those of their *Uhrf1*$^{+/+}$*Cd4*-Cre (control) littermates (**Supplementary Fig. 2d**). We also confirmed the lower abundace of colonic T_{reg} cells in chimeras reconstituted with a mixture of bone marrow progenitor cells from Uhrf1-deficient and congenic wild-type mice. T_{reg} cells derived from the bone marrow of Uhrf1-deficient mice were nearly completely absent from the chimeras (**Supplementary Fig. 3a–c**). Thus, a T_{reg} cell–intrinsic defect was the cause of the lower abundance of these cells. Collectively, these data demonstrated that Uhrf1 was essential for the maintenance of colonic T_{reg} cells but not for the maintenance of extracolonic T_{reg} cells.

We further investigated whether Uhrf1 deficiency affected the differentiation or proliferation of T_{reg} cells by both *in vitro* and *in vivo* experiments. Uhrf1 deficiency did not influence the differentiation or stability of Foxp3 expression by T_{reg} cells in an *in vitro* culture system (**Fig. 4b** and data not shown). To rigorously confirm those results, we transferred naive CD4+ T cells from Uhrf1-deficient or Uhrf1-sufficient CD45.2+ mice into CD45.1+ mice. The efficiency of T_{reg} cell differentiation *in vivo* was similar for Uhrf1-deficient and Uhrf1-sufficient naive T cells (**Supplementary Fig. 4a,b**). In contrast,

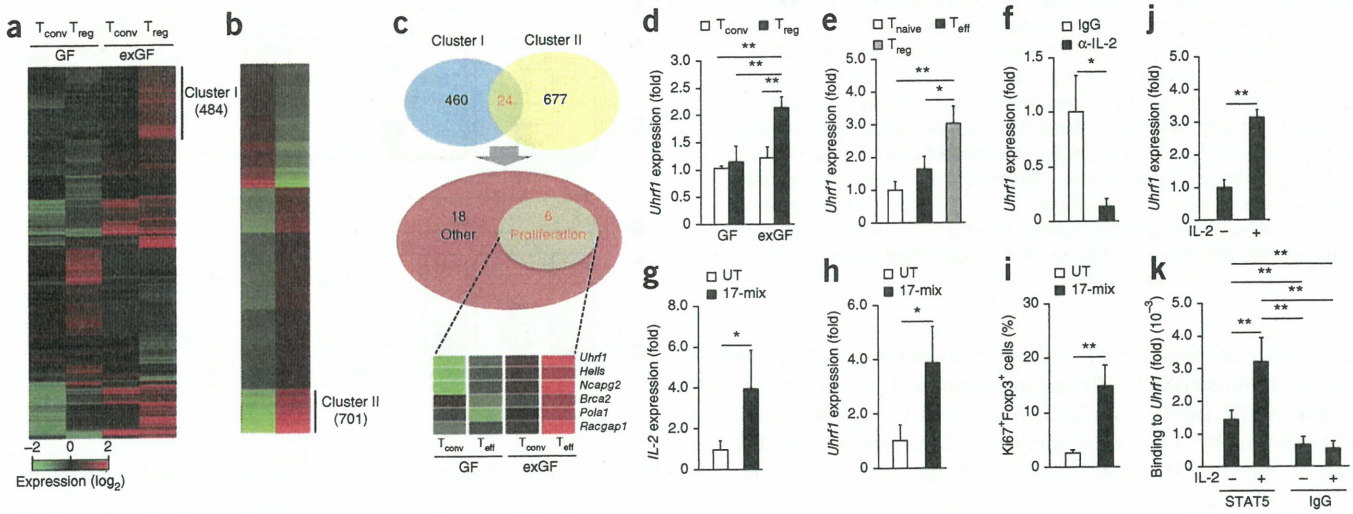

Figure 3 Colonization with commensal bacteria induces *Uhrf1* expression in colonic T_{reg} cells in IL-2-dependent manner. (**a**) Gene-expression profiles of T_{conv} cells ($CD3\varepsilon^+CD4^+CD25^-FR4^-$) and T_{reg} cells ($CD3\varepsilon^+CD4^+CD25^+FR4^+$) isolated from the cLP of GF and exGF mice. (**b**) Gene-expression profile of T_{reg} cells obtained from SPF mice and cultured *in vitro* and stimulated for 2 d with IL-2 in the presence of TGF-β. (**c**) Gene ontology–enrichment analysis of genes common to clusters I and II in **a**,**b**. (**d**) Quantitative PCR analysis of *Uhrf1* expression in T_{conv} cells and T_{reg} cells from the cLP of GF and exGF mice at 7 d after oral inoculation with feces from SPF C57BL/6 mice; results were normalized to those of the gene encoding β-actin (*Actb*) and are presented relative to those of T_{conv} cells from GF mice, set as 1. (**e**) *Uhrf1* expression in naive T cells (T_{naive}; $CD3\varepsilon^+CD4^+hCD2^-CD44^{lo}CD62L^{hi}$), T_{eff} cells ($CD3\varepsilon^+CD4^+hCD2^-CD44^{hi}CD62L^{lo}$) and T_{reg} cells $CD3\varepsilon^+CD4^+hCD2^+$) from SPF *Foxp3*^{hCD2} mice; results were normalized as in **d** and are presented relative to those of naive T cells, set as 1. (**f**) Quantitative PCR analysis of *Uhrf1* expression in cells from the cLP of mice inoculated orally with feces from SPF C57BL/6 mice and then, 3 d later, given intravenous injection of control IgG or neutralizing antibody to IL-2, followed by analysis 2 d later (at day 5); results were normalized as in **d** and are presented relative to those of cells from mice treated with IgG, set as 1. (**g**,**h**) Quantitative PCR analysis of the expression of *Il2* in T_{conv} cells (**g**) and *Uhrf1* in T_{reg} cells (**h**) from GF mice at day 3 (**g**) or day 6 (**h**) after inoculation with 17-mix; results were normalized to those of the gene encoding ribosomal protein L13A (*Rpl13a*) are presented relative to those of cells from untreated GF mice (UT), set as 1. (**i**) Frequency of $Foxp3^+Ki67^+$ cells in the cLP of GF mice at day 6 after inoculation with 17-mix, analyzed by flow cytometry (presented as in **g**,**h**). (**j**) Quantitative PCR analysis of *Uhrf1* expression in splenic $CD4^+CD25^+$ T cells cultured for 3 d with beads coated with mAb to CD3 and mAb to CD28 in the presence of IL-2 and TGF-β, allowed to 'rest' for 6 h and then stimulated 24 h with (+) or without (–) IL-2; results were normalized as in **g**,**h** and are presented relative to those of cells not stimulated with IL-2, set as 1. (**k**) ChIP–quantitative PCR analysis of the binding of STAT5 or IgG to the *Uhrf1* promoter region in splenic T cells cultured with beads as in **j**, allowed to 'rest' for 6 h and then stimulated for 1.5 h with or without IL-2; results are presented relative to total input. $^*P < 0.05$ and $^{**}P < 0.01$ (one-way ANOVA followed by Tukey's test (**d**,**e**,**k**), Mann-Whitney *U*-test (**f**,**g**,**i**) or Student's *t*-test (**h**,**j**)). Data are representative of one experiment (**a**–**c**,**g**–**k**;) or two experiments (**d**–**f**; error bars, s.e.m (**d**) or s.d. (**e**–**k**) of three mice per group).

Uhrf1 deficiency substantially adversely affected proliferation due to cell-cycle arrest at the G1-S transition (**Fig. 4c**,**d**). The same was true for Uhrf1-deficient T_{reg} cells in the cLP, as the frequency of Ki67$^+$ proliferating Foxp3$^+$ T_{reg} cells in the colon of *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice was diminished (**Fig. 4e**,**f**). In contrast, the proliferation of T_{conv} cells was unaffected by Uhrf1 deficiency (**Fig. 4e**).

We further confirmed the role of Uhrf1 in T_{reg} cell homeostasis by another *in vivo* experiment. Although there was no difference between *Uhrf1*$^{fl/fl}$*Cd4*-Cre and *Uhrf1*$^{+/+}$*Cd4*-Cre mice in their proportion of T_{reg} cells under GF conditions (**Fig. 4g**), *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice had defective population expansion of T_{reg} cells in response to colonization by chloroform-resistant bacteria, which consist of spore-forming bacteria mainly of the class Clostridia[1,24] (**Fig. 4h**). Thus, Uhrf1 was indispensable for the local population expansion of colonic T_{reg} cells.

Given that *Uhrf1* was an IL-2-responsive gene, we postulated the IL-2–Uhrf1 axis may serve a key role in the extensive proliferation of T_{reg} cells. To further investigate this possibility, we treated *Uhrf1*$^{fl/fl}$ *Cd4*-Cre and *Uhrf1*$^{+/+}$*Cd4*-Cre mice with exogenous IL-2 mixed with monoclonal antibody (mAb) to IL-2 (i.e., as a complex of IL-2 and mAb to IL-2). Consistent with a published report[25], this treatment potently induced a proliferative response in the systemic T_{reg} cell population specifically in *Uhrf1*$^{+/+}$*Cd4*-Cre mice; however, this response was substantially attenuated in the same population from *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice (**Fig. 4i**). The complex of IL-2 and mAb to IL-2 also induced the proliferation of T_{conv} cells, albeit to a lesser extent than that of T_{reg} cells regardless of the presence of Uhrf1. These data provided evidence of the notable role of the IL-2–Uhrf1 axis in T_{reg} cell proliferation but the lesser role of this axis for T_{conv} cells.

Proliferation may confer functional maturity to T_{reg} cells[26], as shown by upregulation of the expression of molecules with a suppressive function in the proliferative compartment (**Supplementary Fig. 5a**). We hypothesized that diminished proliferative activity in the absence of Uhrf1 may affect the suppressive activity of T_{reg} cells. Indeed, ablation of Uhrf1 impaired the expression of functional molecules, including IL-10 and the immunomodulatory receptor CTLA-4 (CD152) (**Fig. 4j** and **Supplementary Fig. 5b**). Accordingly, Uhrf1-deficient T_{reg} cells exhibited attenuated immunosuppressive function and failed to prevent the development of experimental colitis (**Supplementary Fig. 6**). Given these observations, we concluded that Uhrf1 serves an essential role in the functional maturation of T_{reg} cells in the colonic mucosa, probably by regulating proliferation.

Uhrf1 epigenetically represses *Cdkn1a* expression

The Uhrf1-Dnmt1 complex has a critical role in the accurate maintenance of DNA methylation, which contributes to gene repression[16,17]. To define the targets of Uhrf1 that encode molecules involved in T_{reg} cell proliferation, we first profiled the subset of genes specifically derepressed only in T_{reg} cells (**Supplementary Fig. 7a**). Gene-function–enrichment analysis of the genes profiled identified at the top of the

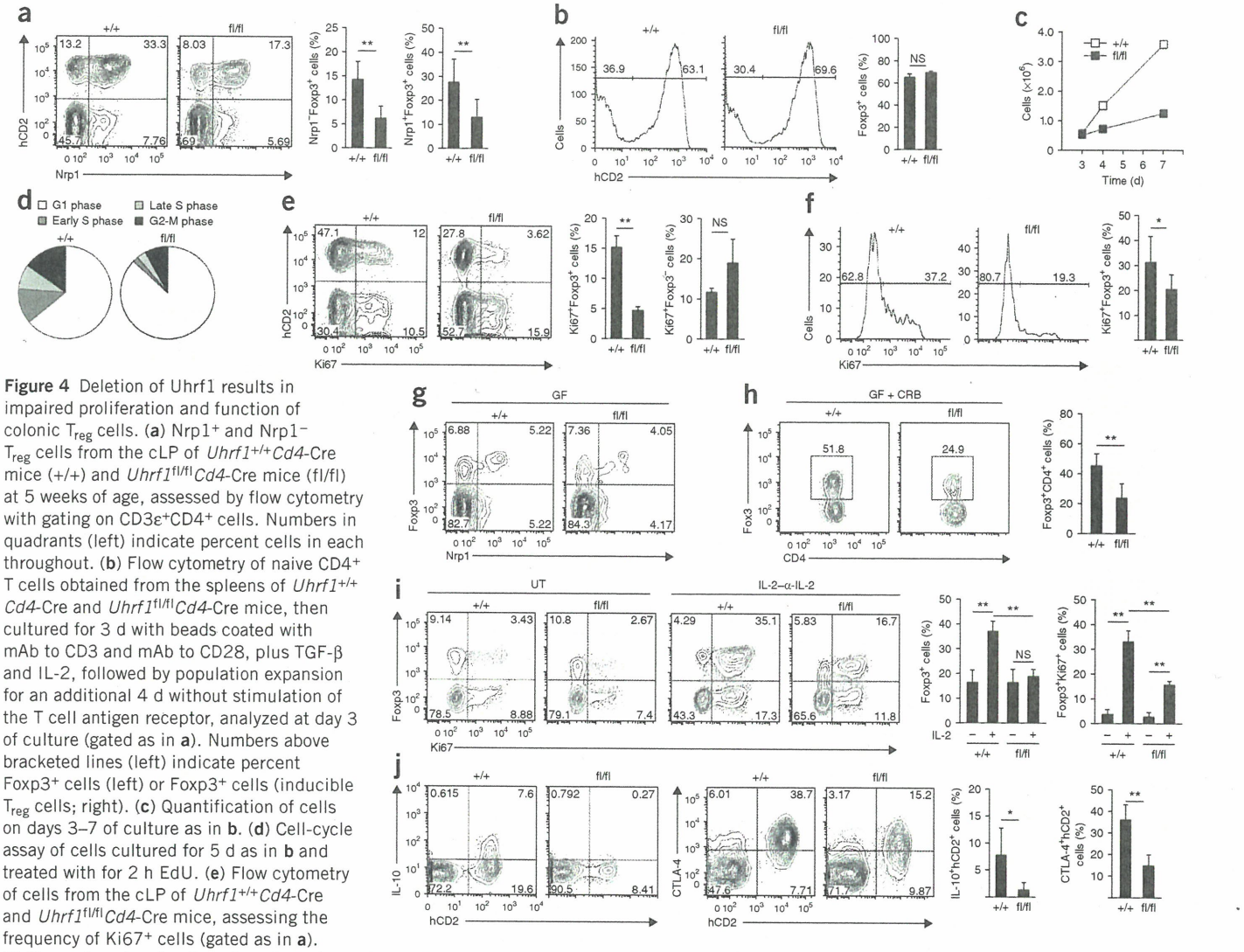

Figure 4 Deletion of Uhrf1 results in impaired proliferation and function of colonic T_{reg} cells. (**a**) Nrp1$^+$ and Nrp1$^-$ T_{reg} cells from the cLP of *Uhrf1*$^{+/+}$*Cd4*-Cre mice (+/+) and *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice (fl/fl) at 5 weeks of age, assessed by flow cytometry with gating on CD3ε$^+$CD4$^+$ cells. Numbers in quadrants (left) indicate percent cells in each throughout. (**b**) Flow cytometry of naive CD4$^+$ T cells obtained from the spleens of *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice, then cultured for 3 d with beads coated with mAb to CD3 and mAb to CD28, plus TGF-β and IL-2, followed by population expansion for an additional 4 d without stimulation of the T cell antigen receptor, analyzed at day 3 of culture (gated as in **a**). Numbers above bracketed lines (left) indicate percent Foxp3$^+$ cells (left) or Foxp3$^+$ cells (inducible T_{reg} cells; right). (**c**) Quantification of cells on days 3–7 of culture as in **b**. (**d**) Cell-cycle assay of cells cultured for 5 d as in **b** and treated with for 2 h EdU. (**e**) Flow cytometry of cells from the cLP of *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice, assessing the frequency of Ki67$^+$ cells (gated as in **a**). (**f**) Flow cytometry of cells as in **e**, but with gating on CD3ε$^+$CD4$^+$Foxp3$^+$ cells. (**g**) Expression of Foxp3 and Nrp1 by CD4$^+$ T cells from the cLP of 8-week-old *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice reared under GF conditions, analyzed by flow cytometry (gated as in **a**). (**h**) Flow cytometry of CD4$^+$ T cells from the cLP of mice as in **g**, inoculated with chloroform-resistant bacteria (+ CRB) and analyzed 4 weeks later (gated as in **a**). Numbers adjacent to outline areas indicate percent Foxp3$^+$CD4$^+$ T cells. (**i**) Expression of Foxp3 and Ki67 by splenic CD4$^+$ T cells from *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice left untreated (UT) or treated with complexes of IL-2 and mAb to IL-2 (IL-2–α-IL-2), analyzed by flow cytometry (gated as in **a**). (**j**) Expression of IL-10 and CTLA-4 by Foxp3$^+$ cells from the cLP of *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice raised under SPF conditions, analyzed by flow cytometry (gated as in **a**). $*P < 0.05$ and $**P < 0.01$ (Student's *t* test (**a**,**e**,**f**,**h**,**j**), Mann-Whitney *U*-test (**b**) or one-way ANOVA followed by Tukey's test (**i**)). Data are representative of three independent experiments (error bars, s.d. of four to nine mice per group).

list (that is, among genes with the highest statistical significance) a group of genes encoding molecules in the category of 'cellular growth and proliferation' (**Supplementary Fig. 7b,c**). Furthermore, we used an integrated '-omics' approach with data sets obtained from the transcriptome and analysis of the 'methylome' (the pattern of methylated DNA in the genome) based on precipitation of methylated DNA followed by sequencing (MeDP-seq) (**Fig. 5a**) and identified *Cdkn1a* as a target of Uhrf1 (**Fig. 5b**). The product of *Cdkn1a*, p21, is a cell-cycle regulator that induces cell-cycle arrest at the G1-S transition[27]. We confirmed that there was substantially more *Cdkn1a* mRNA and p21 protein in *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{reg} cells than in *Uhrf1*$^{+/+}$*Cd4*-Cre T_{reg} cells (**Fig. 5c,d**). The derepression of *Cdkn1a* most probably resulted from hypomethylation of CpG islands in the distal promoter region of *Cdkn1a* in the absence of Uhrf1 (**Fig. 5e–g**), an outcome that was more prominent in T_{reg} cells than in T_{conv} cells (**Fig. 5f**). To further explore whether the derepression of *Cdkn1a* caused the cell-cycle arrest of *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{reg} cells, we induced *Uhrf1*$^{fl/fl}$*Cd4*-Cre cells *in vitro* to differentiate into T_{reg} cells, then treated those cells with small interfering RNA (siRNA) targeting *Cdkn1a* and analyzed their cell-cycle status. Knockdown of *Cdkn1a*, which diminished *Cdkn1a* expression by 45% (data not shown), at least partially rescued cells from the arrest at G1, as indicated by the greater proportion of cells in S phase than in G2-M phases (**Fig. 5h**). From these data, we concluded that Uhrf1-dependent repression of *Cdkn1a* was critical for the maintenance of T_{reg} cell proliferation.

Uhrf1-deficient mice spontaneously develop colitis

Intestinal T_{reg} cells orchestrate the immunoregulatory system that suppresses inappropriate immune responses to commensal bacteria[28].

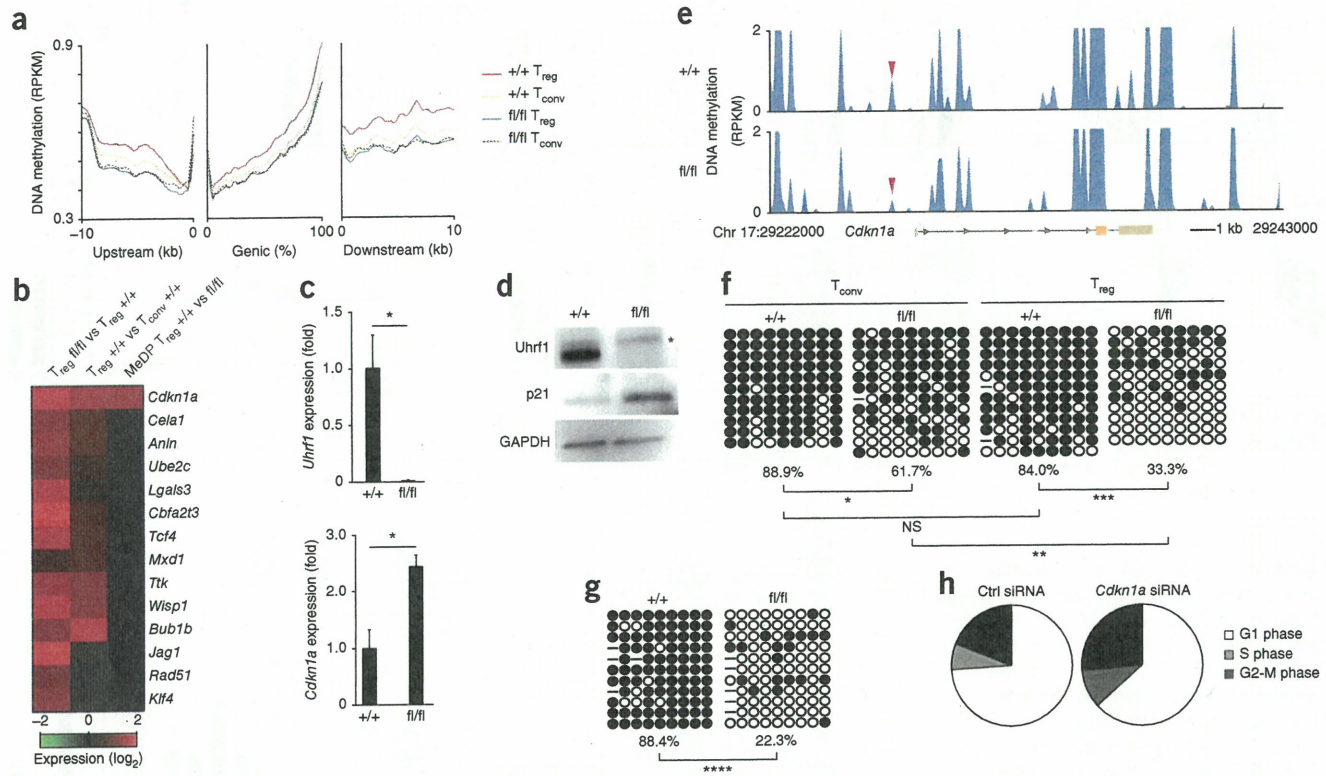

Figure 5 *Cdkn1a* is upregulated in Uhrf1-deficient T_{reg} cells because of hypomethylation of the *Cdkn1a* promoter region. (**a**) Genome-wide MeDP-seq analysis of DNA from *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{conv} and T_{reg} cells, showing the average DNA methylation status (presented as 'reads' per kilobase of exon model per million mapped 'reads' (RPKM)) of upstream, genic and downstream regions. (**b**) Comparison of transcriptome analysis of *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{reg} cells with the MeDP-seq analysis data to identify potential targets of Uhrf1 (right margin). (**c**) Quantitative PCR analysis of *Uhrf1* and *Cdkn1a* in *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{reg} cells cultured *in vitro*; results are presented relative to those of *Uhrf1*$^{+/+}$*Cd4*-Cre cells, set as 1. $*P < 0.01$ (Student's *t*-test). (**d**) Immunoblot analysis of Uhrf1 and p21 in *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{reg} cells cultured *in vitro*; GAPDH (glyceraldehyde phosphate dehydrogenase) serves as a loading control. *, nonspecific signal. (**e**) MeDP-seq analysis of *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre CD3ε^{+}CD4^{+}hCD2^{+} cells *ex vivo*: red downward arrowheads, distal promoter region of *Cdkn1a* (exon structure below: orange, coding sequences; tan, UTR; arrows, direction of transcription). (**f**,**g**) Bisulfite-sequencing analysis of the methylation of CpG islands on the distal promoter region of *Cdkn1a* in *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{conv} and T_{reg} cells *ex vivo* (**f**) and T_{reg} cells cultured *in vitro* (**g**): filled circles, methylated; open circles, demethylated; –, undetermined (due to noise signals); below, methylated CpG/total CpG. $*P = 5.94 \times 10^{-5}$, $**P = 4.34 \times 10^{-13}$, $***P = 7.18 \times 10^{-14}$ and $****P = 3.12 \times 10^{-21}$ (hypergeometric distribution). (**h**) Cell-cycle assay of *Uhrf1*$^{+/+}$*Cd4*-Cre naive CD4^{+} T cells differentiated into Foxp3^{+} cells, then treated for 24 h with fluorescence-labeled *Cdkn1a*-specific siRNA (*Cdkn1a* siRNA) or nontargeting (control) siRNA (Ctrl siRNA). Data are representative of two (**a**,**b**,**e**–**g**) or three (**c**,**d**,**h**) independent experiments.

Given that the absence of Uhrf1 led to a considerable defect in the accumulation of colonic T_{reg} cells, we reasoned that Uhrf1 might be fundamental to the maintenance of intestinal immunological homeostasis. In support of that proposal, *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice spontaneously developed colitis characterized by thickening of the colonic wall, epithelial hyperplasia, loss of goblet cells and massive cellular infiltrates into the colonic mucosa and submucosa before 10 weeks of age (**Fig. 6a–d** and **Supplementary Fig. 8a**). Nearly all of the *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice eventually succumbed to death within 6 months due to the exacerbated colitis (data not shown). In contrast, there were no inflammatory symptoms in the other peripheral tissues examined, including liver, kidney, lung, skin, pancreas, stomach, salivary gland and small intestine (**Supplementary Fig. 9**), consistent with the observation that the Uhrf1-regulated population expansion of T_{reg} cells occurred principally in the local colonic mucosa (**Figs. 1–4** and **Supplementary Figs. 1** and **2**). Commensal bacteria were the causative agent of this chronic inflammatory response, because *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice raised under GF conditions did not display any inflammation (**Supplementary Fig. 8b–d**).

We subsequently examined the immunological phenotype of the spontaneous colitis. The expression of genes encoding proinflammatory cytokines was upregulated considerably in *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice relative to their expression in *Uhrf1*$^{+/+}$*Cd4*-Cre mice (**Fig. 6e**). In keeping with that, the frequency of T_{eff} cells expressing the proinflammatory cytokines interferon-γ, IL-17A and tumor-necrosis factor in the cLP was much greater in *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice than in *Uhrf1*$^{+/+}$*Cd4*-Cre mice (**Fig. 6f,g**). Conversely, IL-10-expressing T_{reg} cells were nearly absent from colitic *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice (**Fig. 6f,g**). This was also the case even in younger mice before the development of frank colitis (**Fig. 4j**). These results suggested that activation of responses of the T_H1 and T_H17 subsets of helper T cells due to compromised T_{reg} cell function mediated the development of colitis in *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice.

To investigate the possibility that excessive activation of T_{eff} cells due to the loss of Uhrf1 might cause the development of colitis, we assessed the *in vivo* function of T_{eff} cells independently of the effect of T_{reg} cell dysfunction through the use of a mixed–bone marrow chimera system. We transferred CD45.1^{+} wild-type and Uhrf1-deficient

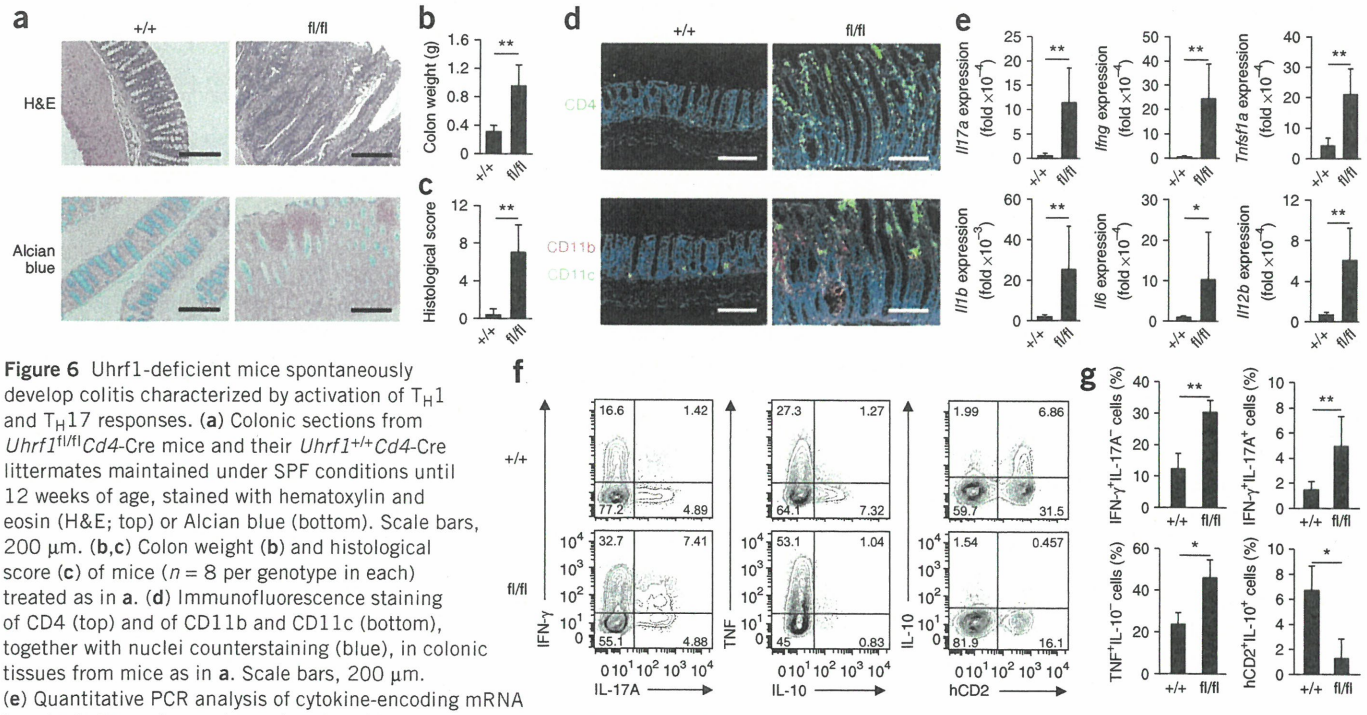

Figure 6 Uhrf1-deficient mice spontaneously develop colitis characterized by activation of T_H1 and T_H17 responses. (**a**) Colonic sections from *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice and their *Uhrf1*$^{+/+}$*Cd4*-Cre littermates maintained under SPF conditions until 12 weeks of age, stained with hematoxylin and eosin (H&E; top) or Alcian blue (bottom). Scale bars, 200 μm. (**b**,**c**) Colon weight (**b**) and histological score (**c**) of mice ($n = 8$ per genotype in each) treated as in **a**. (**d**) Immunofluorescence staining of CD4 (top) and of CD11b and CD11c (bottom), together with nuclei counterstaining (blue), in colonic tissues from mice as in **a**. Scale bars, 200 μm. (**e**) Quantitative PCR analysis of cytokine-encoding mRNA in colonic tissue from mice as in **a** ($n = 11$); results are presented relative to *Actb* expression. (**f**,**g**) Cytokine expression in cLP-infiltrating CD4$^+$ T cells from mice as in **a**, analyzed by flow cytometry (**f**) and summarized (**g**). $^*P < 0.05$ and $^{**}P < 0.01$ (Mann-Whitney *U* test (**b**,**c**,**e**) or Student's *t*-test (**g**)). Data are representative of three independent experiments (error bars (**b**,**c**,**e**,**g**), s.d.).

bone marrow cells together into irradiated recipient mice with congenital deficiency in mature B cells and T cells (deficient in recombination-activating gene 1). We confirmed that these chimeras did not show any signs of inflammation in the colon (data not shown). Under these non-inflammatory conditions, the frequency of T_{eff} cells expressing interferon-γ and IL-17A in the cLP was similar for Uhrf1-deficient and Uhrf1-sufficient populations (**Supplementary Fig. 3d**). In addition, Uhrf1 deficiency did not influence the *in vitro* differentiation or function of the T_{eff} cells (**Supplementary Fig. 10a**,**b**). Examination of methylated DNA by MeDP-seq analysis also confirmed that the methylation status of genes encoding proinflammatory cytokines, as well as those encoding key transcription factors for T_H1 or T_H17 differentiation, was normal in the absence of Uhrf1 (**Supplementary Fig. 10c–f**). These results excluded the possibility that deficiency of Uhrf1 influenced the function of colonic T_{eff} cells.

We finally investigated whether the defect in T_{reg} cell proliferation was responsible for the development of colitis. To address this issue, we gave young (4- to 5-week-old) *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice wild-type T_{reg} cells (CD3$^+$CD4$^+$CD45.1$^+$hCD2$^+$ cells) from congenic *Foxp3*^{hCD2} reporter mice. Adoptive transfer of the wild-type T_{reg} cells prevented the development of colitis (**Fig. 7a**), concomitant with suppression of the T_{eff} cell response (**Fig. 7b**). Collectively, these data illustrated that the aberrant activation of T_{eff} cells caused by Uhrf1 deficiency resulted from the breakdown of the colonic immunoregulatory system. From this model, we concluded that the proliferative response of T_{reg} cells mediated by Uhrf1 was a prerequisite for their functional maturation in colonic mucosa.

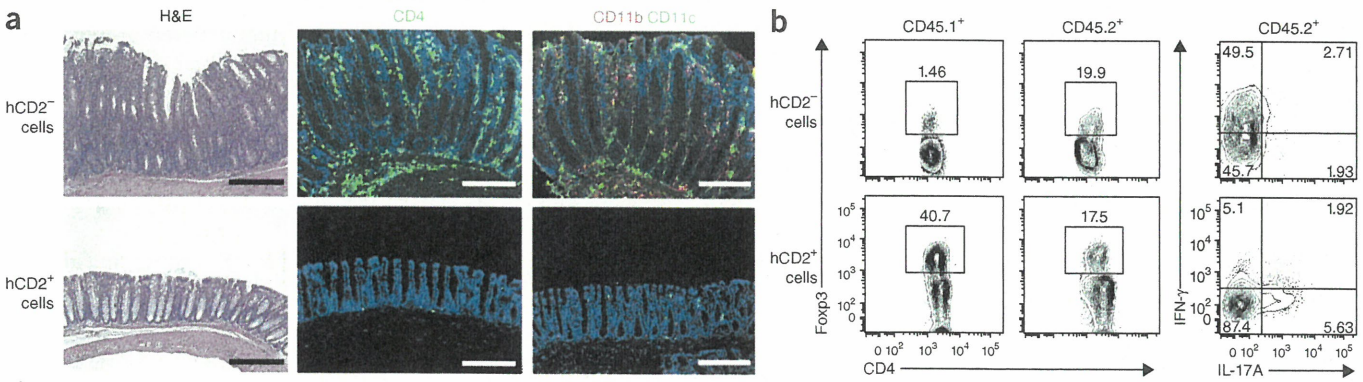

Figure 7 A defect in the proliferation of colonic T_{reg} cells is responsible for the development of spontaneous colitis in *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice. (**a**) Histology and immunofluorescence staining of colonic tissues from *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice (6 weeks of age) at 6 weeks after transfer of hCD2$^+$ or hCD2$^-$ cells from CD45.1$^+$ congenic *Foxp3*^{hCD2} mice. Scale bars, 200 μm. (**b**) Flow cytometry of CD4$^+$ T cells from cLP of the mice in **a**. Numbers adjacent to outlined areas (left and middle) indicate percent Foxp3$^+$CD4$^+$ cells. Data are representative of two independent experiments (with a total of four mice per group) with similar results.