SCIENTIFIC REPORTS

OPEN

Maf deficiency in T cells dysregulates T_{reg} - T_H17 balance leading to spontaneous colitis

Received: 28 November 2018
Accepted: 1 April 2019
Published online: 16 April 2019

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The maintenance of homeostasis in the gut is a major challenge for the immune system. Here we demonstrate that the transcription factor MAF plays a central role in T cells for the prevention of gastro-intestinal inflammation. Conditional knock out mice lacking *Maf* in all T cells developed spontaneous late-onset colitis, correlating with a decrease of FOXP3$^+$RORγt$^+$ T cells proportion, dampened IL-10 production in the colon and an increase of inflammatory T_H17 cells. Strikingly, FOXP3$^+$ specific conditional knock out mice for MAF did not develop colitis and demonstrated normal levels of IL-10 in their colon, despite the incapacity of regulatory T cells lacking MAF to suppress colon inflammation in *Rag1*$^{-/-}$ mice transferred with naïve CD4$^+$ T cells. We showed that one of the cellular sources of IL-10 in the colon of these mice are T_H17 cells. Thus, MAF is critically involved in the maintenance of the gut homeostasis by regulating the balance between T_{reg} and T_H17 cells either at the level of their differentiation or through the modulation of their functions.

Maf encodes for a transcription factor belonging to the AP-1 family (MAF or c-MAF). It has been studied for many years in T cells, and more specifically in CD4$^+$ T helper (T_H) cell differentiation. Its role in *Il4* transcriptional regulation and T_H2 regulation was first established using a transgenic mouse system for *Maf*[1,2]. *Maf* was also shown to play a prominent role in T_H17 cells in both mice and human, through the regulation of *Il10* and *Il23r* expression[3,4] and in T_{FH} cells together with the transcription factor *Bcl6*[5]. MAF inactivation in T cells affects susceptibility to disease in a context-specific manner, depending on the T cell polarization induced by this disease with a general tendency to increase inflammatory responses over tolerance[6]. In invariant natural killer T (iNKT) cells, MAF regulates the expression of IL-17A and RORγt[7]. Interestingly, *Maf* was found upregulated in CD8$^+$ T cells infiltrated in human and murine melanoma. This expression led to intratumoral T cell dysfunction through the regulation of genes involved in T cell exhaustion[8].

The potential role of *Maf* in regulatory T cells (T_{reg}) is less clear. MAF cooperates with AhR in FOXP3$^-$ T regulatory 1 (T_{R1}) cells to control *Il10* expression[9]. Recently, a subset of T_{reg} expressing both FOXP3 and RORγt, the master transcription factor of T_H17 cells, has been characterized in more detail[10]. Mostly present in the gut, RORγt$^+$ T_{reg} have enhanced suppressive activity compared to RORγt$^-$ T_{reg} and maintain gut homeostasis to microbiota[11–13]. The development of this population, described in both mice and human[11,12], is tightly linked to the presence of the microbiota but not to dietary antigens[14]. Transcriptomic analysis showed an enriched expression of *Maf* in RORγt$^+$ T_{reg}[13] and it was shown that the inactivation of *Maf* in T_{reg} strongly affects its function and differentiation into RORγt$^+$ T_{reg}[15,16]. Recent studies have linked MAF to the modulation of a large immunoregulatory and tissue-residency program in human T_H17 cells producing Il-10. These results are reminiscent of the role of MAF in the regulation of tolerance in the gut that is dependent on induced Treg cells[17]. MAF can bind in the vicinity of many genes expressed in recently activated T_H17 cell subsets. This study suggests that binding with other transcriptional partners to regulatory regions of the genome would explain the different effect of MAF on the expression of genes encoding either tolerogenic or inflammatory molecules.

In an attempt to clarify the role of *Maf* in T cells *in vivo*, we studied mice inactivated for *Maf* in all T cells (*Maf*$^{\Delta Tcells}$). Interestingly, these mice developed late onset colitis correlating with a decrease of RORγt$^+$ T_{reg} and an increase of T_H17 cells in the colon and the mesenteric LNs. The disease was associated with increased production

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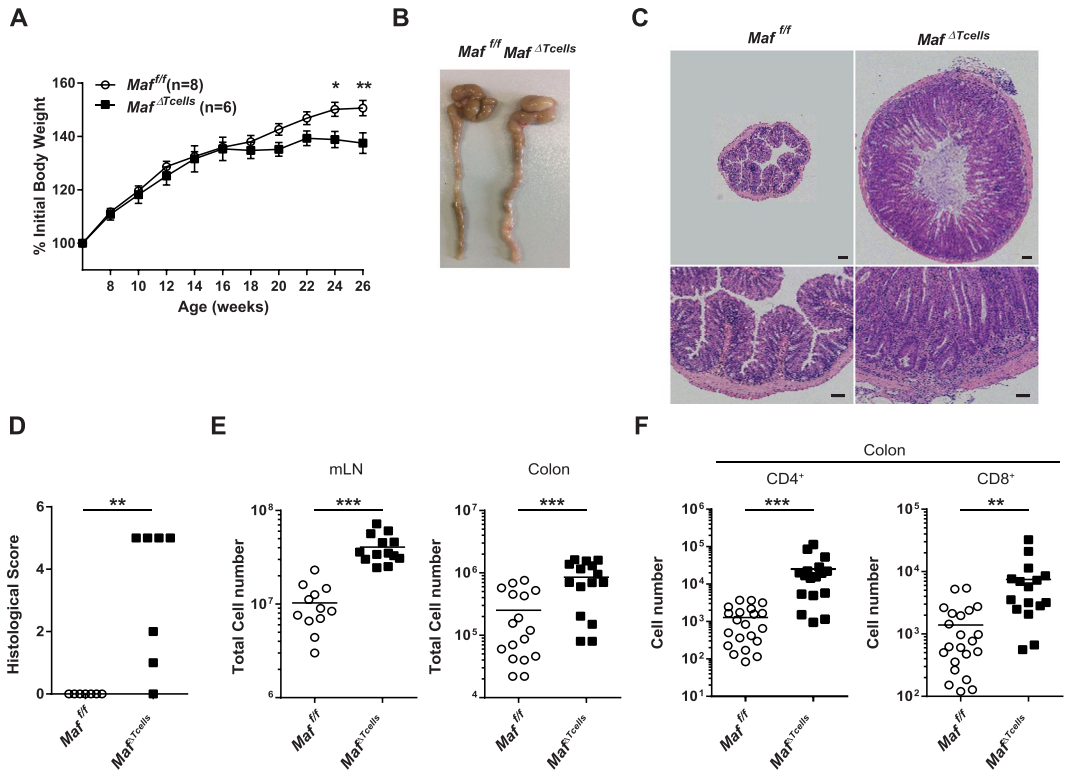

Figure 1. *Maf*$^{\Delta Tcells}$ mice develop late-onset spontaneous colitis. (**A**) Body weight curves of *Maf*$^{f/f}$ (n = 8) and *Maf*$^{\Delta Tcells}$ (n = 6) male littermates. Statistical significances were calculated by a Bonferroni test after a significant two-way ANOVA. (**B**) Representative pictures of colons from *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice. (**C**) Haematoxylin and eosin-stained sections of colons (upper picture: scale bar 200 µm; bottom pictures: scale bar 100 µm) and (**D**) histological scores (from 0 to 6) of microscopic changes in the colon from *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice (n = 7 per group). (**E**) Total immune cell numbers in mLN (left) and colon (right) of *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice (n > 10 per group). (**F**) Absolute cell numbers of CD4$^+$ and CD8$^+$ T cells in colons of *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice (n > 10 per group). **(D–F)** Each symbol represents an individual mouse. **(E,F)** Data are representative of at least 2 independent experiments with at least 4 mice per group. All mice were over 20 weeks old. All graphs indicate means. Error bars display Standard Error Mean (SEM).

of TNFα, IFNγ, IL-1β and a lack of IL-10 in the colon of *Maf*$^{\Delta Tcells}$ mice. Using an adoptive cell transfer model in *Rag1*$^{-/-}$ mice, we showed that *Maf*-deficient T_{reg} are inefficient in preventing colitis, demonstrating the role of *Maf* in regulating T_{reg} function. Moreover, we observed that *Maf*$^{\Delta Tcells}$ mice develop exacerbated T_H17 response against *Helicobacter pylori*, a human pathobiont colonizing the stomach mucosa, leading to the inability of the bacteria to establish a chronic state of infection in *Maf*$^{\Delta Tcells}$ mice. Strikingly, mice deficient for *Maf* in T_{reg} alone (*Maf*$^{\Delta Treg}$ mice) did not develop colitis and demonstrated normal levels of IL-10 in their colon. Compared to *Maf*$^{\Delta Tcells}$, we observed that T_H17 cells of *Maf*$^{\Delta Treg}$ mice produce IL-10. Taken together, our data demonstrated that *Maf* is playing a major role in the maintenance of gastro-intestinal homeostasis through the regulation of functions of both T_{reg} and T_H17 cells. *Maf* regulates the differentiation of RORγt$^+$ T_{reg}, the suppressive activities of T_{reg} as well as the activity of T_H17 cells from the gut.

Results

T cell specific Maf-deficient mice develop spontaneous colitis. To study the role of *Maf* in T cells, we generated *CD4*^{cre} *Maf*$^{fl/fl}$ mice (*Maf*$^{\Delta Tcells}$), in which all T cells (CD4$^+$ and CD8$^+$) are inactivated for the expression of *Maf*. Interestingly, these mice spontaneously developed strong colitis, associated with a defect in weight gain starting at 16 weeks of age (Fig. 1A), concomitant with the appearance of diarrhea. These symptoms increased as the mice got older – with up to a 15% difference of body weight at 26 weeks of age between *Maf*$^{\Delta Tcells}$ mice and Cre-negative littermates (*Maf*$^{f/f}$) (Fig. 1A). Macroscopic and microscopic examination of the gastrointestinal system revealed severe inflammation of all the parts of the colon with a clear enlargement in most of the mice (Fig. 1B,C) but no clear difference in the length of the colon (Supplementary Fig. 1A). We did not observe similar features in other organs such as liver or kidney (Supplementary Fig. 1B-D). Hematoxylin and eosin staining on histological sections of colon samples showed massive infiltration of cells in the lamina propria and submucosal spread (Fig. 1C). The scoring of histological sections revealed that the majority of mice had severe colitis (4/7 mice with the score of 5) (Fig. 1D). We found a significant increase in the number of total immune cells both in the mesenteric LN (mLN) and in the colon of *Maf*$^{\Delta Tcells}$ mice compared to the one from *Maf*$^{f/f}$ littermates (Fig. 1E). Both CD4$^+$ and CD8$^+$ T cells were more abundant in the colon of *Maf*$^{\Delta Tcells}$ mice compared to *Maf*$^{f/f}$ littermates (Fig. 1F). *Maf*$^{\Delta Tcells}$ mice treated with antibiotics developed no or only mild colitis (Supplementary Fig. 1E,F)

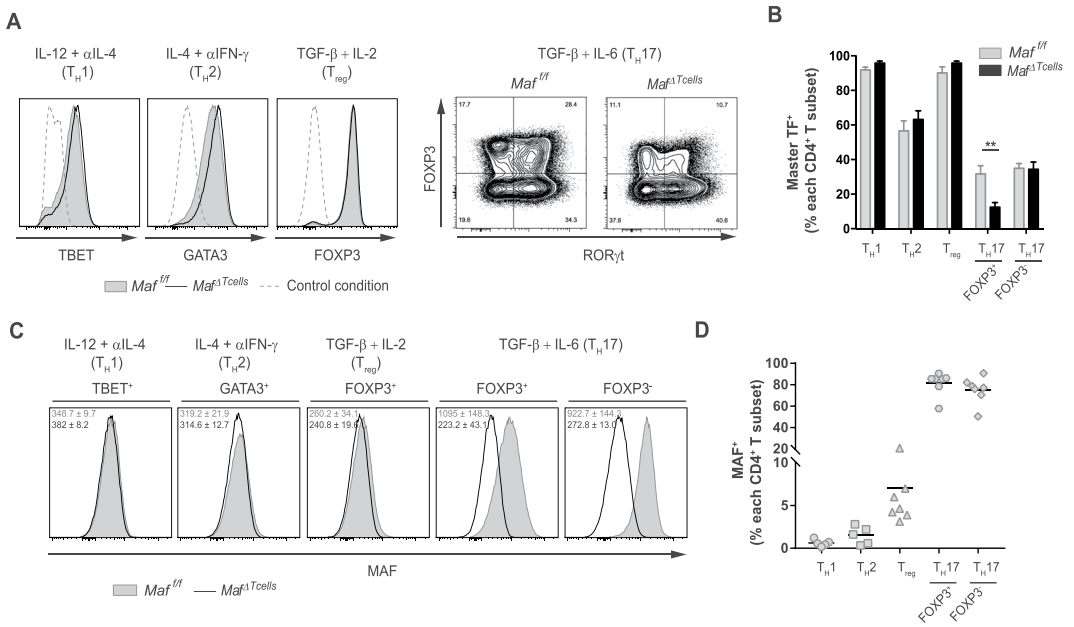

Figure 2. *In vitro* differentiation of *Maf*-deficient CD4$^+$ T cells in RORγt$^+$ T$_{reg}$ is defective. CD4$^+$ T cells isolated from splenocytes of *Maf$^{f/f}$* and *Maf$^{\Delta Tcells}$* mice were cultured for 5 days in T$_H$1 (IL-12 + anti-IL-4), T$_H$2 (IL-4 + anti-IFN-γ), T$_{reg}$ (TGF-β +IL-2) or T$_H$17 (TGF-β +IL-6) polarizing conditions. (**A**) Representative histograms for the expression of master transcription factors associated with each helper subset: TBET (T$_H$1), GATA3 (T$_H$2), FOXP3 (T$_{reg}$) and RORγt (T$_H$17 FOXP3$^+$ and FOXP3$^-$) from *Maf$^{f/f}$* and *Maf$^{\Delta Tcells}$* mice. (**B**) Percentage of each master transcription factor according to the condition of polarization among CD4$^+$ T cells (**C**) Representative histograms for the expression of MAF in *in vitro* differentiated CD4$^+$ T cell subsets according to the expression of their master transcription factor from *Maf$^{f/f}$* and *Maf$^{\Delta Tcells}$* mice. MFI is indicated for T cells from *Maf$^{f/f}$* (grey) or *Maf$^{\Delta Tcells}$* (black) mice. (**D**) Percentage of MAF$^+$ cells among each T$_H$ subset according to the expression of their master transcription factor from WT mice. Each symbol represents an individual mouse. Data are representative of 2 independent experiments with at least 2 mice per group. All graphs indicate means. Error bars display SEM.

demonstrating the requirement of microbiota in the development of this phenotype. Thus, the absence of *Maf* in T cells leads to spontaneous colitis in mice.

***In vitro* differentiation of Maf-deficient CD4$^+$ T cells in RORγt$^+$ T$_{reg}$ is defective.** As previously described, MAF has been shown to regulate cytokine production in various CD4$^+$ T$_H$ subsets. To determine whether MAF deficiency in CD4$^+$ T cells can also alter the differentiation of any of these subsets, we differentiated naïve CD4$^+$ T cells with polarizing medium to obtain T$_H$1, T$_H$2, T$_{reg}$ and T$_H$17 (FOXP3$^-$ or FOXP3$^+$) cells *in vitro* (Supplementary Fig. 2A–C). Comparison of the expression of the master transcription factors TBET (for T$_H$1), GATA3 (for T$_H$2), FOXP3 (for T$_{reg}$) and RORγt (for T$_H$17) between CD4$^+$ T cells from *Maf$^{\Delta Tcells}$* and *Maf$^{f/f}$* mice did not show significant differences (Fig. 2A,B). However, in the T$_H$17 polarizing condition, we observed the development of two distinct populations when analyzing FOXP3 expression. For the FOXP3$^+$ population, also named RORγt$^+$ T$_{reg}$, there was a significant decrease in RORγt level in CD4$^+$ T cells from *Maf$^{\Delta Tcells}$* mice compared to the one from *Maf$^{f/f}$* mice, from 35% to 10% of CD4$^+$ T cells respectively (Fig. 2A,B). In these polarizing conditions, MAF levels were the highest in RORγt$^+$ T$_{reg}$ and T$_H$17, with approximately 80% of MAF- expressing cells in both subsets. MAF is also expressed in RORγt$^-$ T$_{reg}$, though at lower level - around 8% of the cells - whereas it is almost absent in T$_H$1 and T$_H$2 (Fig. 2C,D). These *in vitro* experiments of differentiation suggest that MAF plays a role for the differentiation of RORγt$^+$ T$_{reg}$ and might impact the physiological functions of T$_H$17 cells.

T cell specific Maf-deficient mice are deprived of RORγt$^+$ T$_{reg}$. To confirm the results obtained *in vitro*, we first looked at the abundance of transcripts encoding for cytokines in the total colon of aged *Maf$^{f/f}$* or *Maf$^{\Delta Tcells}$* mice (20 weeks or more). We found higher expression of *Tnfa, Ifng and Il1b* in *Maf$^{\Delta Tcells}$* mice compared to *Maf$^{f/f}$* littermates (Fig. 3A), in accordance with a higher infiltration of immune cells and a stronger inflammation in the colon. The transcripts encoding for *Il4* were slightly less abundant in *Maf$^{\Delta Tcells}$* mice and, strikingly, *Il10* expression level was much lower in colons from *Maf$^{\Delta Tcells}$* mice (Fig. 3A). We then determined the *in vivo* expression of MAF in the various subsets. MAF was expressed in RORγt$^+$ FOXP3$^+$ T$_{reg}$ and RORγt$^+$ FOXP3$^-$ T$_H$17 cells- around 40% and 20%, respectively - from the colon (Fig. 3B). In the colon, around 15% of RORγt$^-$ T$_{reg}$ expressed MAF (Fig. 3B). The proportion of RORγt$^+$ T$_{reg}$ was dramatically decreased in spleens, mLN and colons of *Maf$^{\Delta Tcells}$* mice, while we found a significant increase in percentages as well as total numbers of RORγt$^-$ T$_{reg}$ from these organs compared to *Maf$^{f/f}$* mice (Fig. 3C,D and Supplementary Fig. 3A). We also observed elevated percentages and cell numbers of T$_H$17 cells in spleens, mLN and colons of *Maf$^{\Delta Tcells}$* mice compared to *Maf$^{f/f}$* mice

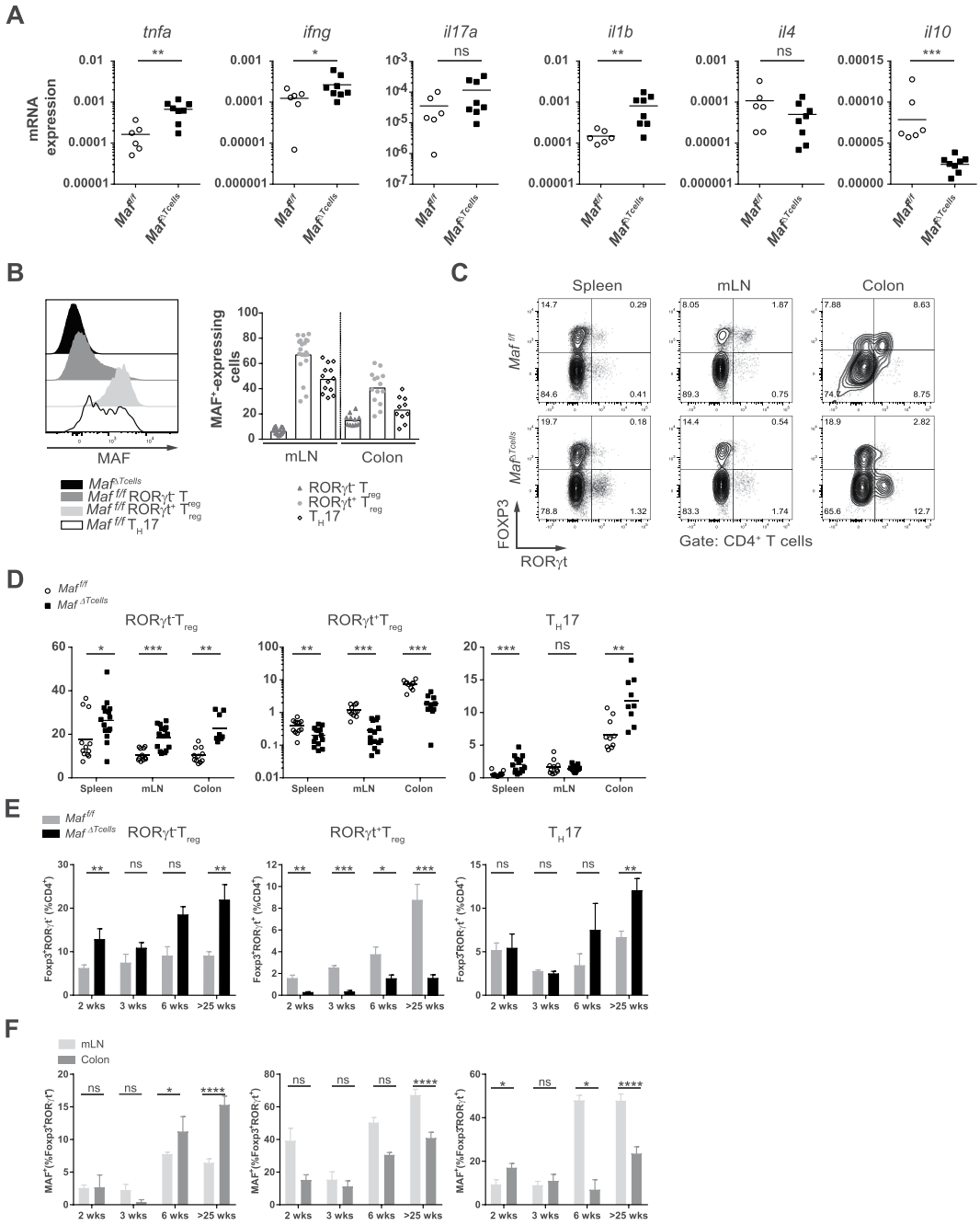

Figure 3. *Maf*$^{\Delta Tcells}$ mice are deprived of RORγt$^+$ T$_{reg}$. **(A)** Quantitative RT–PCR of colonic tissues from *Maf*$^{f/f}$ (n = 6) and *Maf*$^{\Delta Tcells}$ (n = 8) mice for the indicated transcripts. Gene expression levels were normalized to *Beta2microglobulin*. **(B)** Representative histogram of the expression of MAF in mLN (left) and percentage of MAF$^+$ cells (right) in mLN and colon in RORγt$^-$ T$_{reg}$, RORγt$^+$ T$_{reg}$ and T$_H$17 cells from *Maf*$^{f/f}$ mice (n > 10). **(C)** Representative contour plot of the expression of FOXP3 and RORγt in CD4$^+$ T cells from spleens, mLN and colons of *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice. **(D)** Percentage of RORγt$^-$ T$_{reg}$, RORγt$^+$ T$_{reg}$ and T$_H$17 cells from spleens, mLN and colons of *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice (n > 10 per group). **(E)** Percentage of RORγt$^-$ T$_{reg}$, RORγt$^+$ T$_{reg}$ and T$_H$17 cells in CD4$^+$ T cells from colons of *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice at indicated ages (n > 4 per group). **(F)** Percentage of MAF$^+$ cells among RORγt$^-$ T$_{reg}$, RORγt$^+$ T$_{reg}$ and T$_H$17 cells from mLN and colons of *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice at indicated ages (n > 4 per group). **(A–C)** Each symbol represents an individual mouse. Data are representative of at least 2 independent experiments with at least 3 mice per group. **(E,F)** Data are representative of at least 2 independent experiments with at least 2 mice per group. All graphs indicate means. Error bars display SEM.

(Fig. 3C,D and Supplementary Fig. 3A). In *Maf*$^{f/f}$ mice, RORγt$^+$ T$_{reg}$ proportions increased with age to reach 10% of the total CD4$^+$ T cell population in the colon at 25-weeks age mice (Fig. 3E). The defect in RORγt$^+$ T$_{reg}$ in *Maf*$^{\Delta Tcells}$ mice is already detected at 2 weeks of age (i.e. the weaning period) compared to *Maf*$^{f/f}$ mice (Fig. 3E).

Interestingly, the level of MAF in these three populations followed the same upward trend with time (Fig. 3F). RORγt$^-$ T_{reg} increase was already present in $Maf^{\Delta Tcells}$ mice at the age of 2 weeks and tended to expand over time (Fig. 3E). However, the increase of T_H17 cells was only observed at an advanced age in $Maf^{\Delta Tcells}$ mice, correlating with the development of colitis. These data indicate that the deletion of MAF in T cells alters differentiation of highly suppressive RORγt$^+$ T_{reg} whereas it favors RORγt$^-$ T_{reg} accumulation and later T_H17 cells expansion. Loss of the equilibrium between these subsets in the colon is associated with colitis onset.

Maf-deficient T_{reg} fail to prevent colitis development *in vivo*. The dramatic decrease in RORγt$^+$ T_{reg} was associated with an increase in classical RORγt$^-$ T_{reg} in $Maf^{\Delta Tcells}$ mice (Fig. 3D). However, the development of spontaneous colitis in $Maf^{\Delta Tcells}$ mice implies that *Maf*-deficient T_{reg} are unable to offset the decrease in highly suppressive RORγt$^+$ T_{reg}. This would suggest that the remaining *Maf*-deficient T_{reg} are dysfunctional and thus inefficient at suppressing T_H17 cells. To test this hypothesis, we examined the capacity of *wild type (WT)* or *Maf*-deficient T_{reg} to suppress gut inflammation driven by the injection of naïve CD4$^+$ T cells into $Rag1^{-/-}$ mice[18]. We transferred either WT or *Maf*-deficient naïve CD4$^+$ T cells alone or in the presence of either WT or *Maf*-deficient T_{reg} into $Rag1^{-/-}$ recipient mice. Both WT and *Maf*-deficient naïve CD4$^+$ T cells induced weight loss and cell infiltration in the colon when transferred alone (Fig. 4A,B) without any significant difference in the histologic score between the two groups (Fig. 4C). When we co-transferred WT T_{reg}, the weight loss and the infiltration of cells in the colon were prevented with WT and *Maf*-deficient naïve CD4$^+$ T cells (Fig. 4A,B). Strikingly, *Maf*-deficient T_{reg} failed to prevent colitis in all transferred groups compared to WT T_{reg}, with a significant difference for the weight loss and the histological score (Fig. 4A–C). Histological sections of colons from recipient mice transferred with *Maf*-deficient T_{reg} displayed massive infiltration of cells in the associated lamina propria, with a disorganized architecture that was not observed in mice transferred with WT T_{reg} (Fig. 4B,C). We confirmed the presence of T_{reg} in all co-transferred groups (Fig. 4D) and the expression of MAF in WT transferred T_{reg} (Fig. 4E). Taken together, these results demonstrated that *Maf*-deficient T_{reg} are at least partially dysfunctional since they cannot prevent inflammation and colitis development.

T_{reg} specific Maf-deficient mice do not develop spontaneous colitis. We showed that both T_{reg} differentiation and function were largely affected in $Maf^{\Delta Tcells}$ mice (Figs 3 and 4). To test whether the defect of MAF in Treg was sufficient to recapitulate the phenotype of colitis, we generated $Foxp3^{YFPcre}$ $Maf^{fl/fl}$ mice ($Maf^{\Delta Treg}$), in which only T_{reg} are inactivated for the expression of *Maf*. We observed a strong decrease in percentages as well as total numbers of RORγt$^+$ T_{reg} in the colon and in the mLN, mirrored by a slight increase in percentages of RORγt$^-$ T_{reg} and T_H17 cells (Fig. 5A,C and Supplementary Fig. 3). We confirmed the complete deletion of *Maf* only in T_{reg} from $Maf^{\Delta Treg}$ mice (Fig. 5B). However, we did not observe any signs of colitis, confirmed by histological colon section and body weight measurement of these mice compared to $Maf^{f/f}$ mice (Fig. 5D,E) even in aged (6–12 months) $Maf^{\Delta Treg}$ mice (Fig. 5F). Similarly, the number of immune cells infiltrated in the colon of $Maf^{f/f}$ and $Maf^{\Delta Treg}$ was not different (Fig. 5G). We noticed a slight increase in cells in mLN (Fig. 5G) and no difference in the number of CD4$^+$ or CD8$^+$ T cells in the colon of $Maf^{\Delta Treg}$ mice compared to WT (Fig. 5H). This showed that *Maf* inactivation in T_{reg} is not sufficient to induce spontaneous colitis.

Maf deletion impairs IL-10 production by T_{reg} and T_H17 cells. To uncover why $Maf^{\Delta Treg}$ mice do not develop colitis, we measured transcript levels of cytokines encoding genes by quantitative RT-PCR in total colon of $Maf^{f/f}$, $Maf^{\Delta Treg}$ or $Maf^{\Delta Tcells}$ mice aged of more than 20 weeks. As expected, we found higher expression of transcripts encoding for *Maf* in colon from $Maf^{\Delta Treg}$ mice compared to colon from $Maf^{\Delta Tcells}$ mice, confirming that conventional T cells infiltrated in the colon still expressed *Maf* in $Maf^{\Delta Treg}$ mice (Fig. 6A). When looking at transcripts encoding for *Il10*, we found a similar pattern of expression than the one for *Maf*, with a higher level of transcript in $Maf^{f/f}$ and $Maf^{\Delta Treg}$ mice compared to $Maf^{\Delta Tcells}$ mice (Fig. 6A). To confirm that T_{reg} from $Maf^{\Delta Treg}$ are really deficient for *Maf* and *Il10* production, T_{reg} from the spleen and LN of $Maf^{f/f}$, $Maf^{\Delta Treg}$ or $Maf^{\Delta Tcells}$ mice were sorted using flow cytometry. We measured high levels of transcripts for *Maf* and *Il10* in T_{reg} cells from $Maf^{f/f}$ mice, while the levels of transcripts for these genes were low in T_{reg} cells from both $Maf^{\Delta Treg}$ and $Maf^{\Delta Tcells}$ mice (Fig. 6B). Similarly, we confirmed that upon restimulation, T_{reg} from mLN and colon of $Maf^{\Delta Treg}$ or $Maf^{\Delta Tcells}$ mice expressed decreased levels of IL-10 compared to $Maf^{f/f}$ counterparts (Fig. 6C,D). This reduction is largely observed in RORγt$^+$ T_{reg} but also to a lesser extent in RORγt$^-$ T_{reg} from mLN of $Maf^{\Delta Treg}$ mice, suggesting a dependence for MAF for IL-10 production in T_{reg}. These data suggest that *Maf* expression in FOXP3$^-$ T cells is able to compensate for sufficient amounts of *Il10* production to prevent colitis development in $Maf^{\Delta Treg}$ mice. To determine which other T cell type could produce IL-10, we stimulated *ex-vivo* CD4$^+$ T cells from mLN and colon of mice. We observed that IL-10 production by T_H17 cells from mLN of $Maf^{\Delta Tcells}$ mice was decreased compared to production from $Maf^{f/f}$ and $Maf^{\Delta Treg}$ mice (Fig. 6E). This suggests that IL-10 production by T_H17 cells in the gut is also regulated by *Maf*. The reduction of IL-10 expression not only by T_H17 cells, but also T_{reg}, could explain colitis onset observed only in mice where all the T cells are inactivated for *Maf*. Furthermore, we observed an increased percentage in IFNγ$^+$$T_H17$ cells while the percentage of IL-17A$^+$ T_H17 cells remained similar in $Maf^{\Delta Tcells}$ mice compared to $Maf^{f/f}$ or $Maf^{\Delta Treg}$ mice (Fig. 6F). Together, these data demonstrate that *Maf* is an essential driver of T_{reg} and T_H17 immunoregulatory function, especially through IL-10 regulation.

***$Maf^{\Delta Tcells}$* mice eliminate *Helicobacter pylori* through exacerbated T_H17 response.** Recently, Gabrysova L *et al*. demonstrated that T_H17 response was inhibited in $Maf^{\Delta Tcells}$ mice in an experimental autoimmune encephalomyelitis (EAE) model[6]. This conclusion is not in line with our observation that $Maf^{\Delta Tcells}$ mice develop a colitis (Fig. 1C) characterized by the accumulation of T_H17 cells in the colon lamina propria (Fig. 3D). In order to re-evaluate our results in a different experimental setting, we infected $Maf^{\Delta Tcells}$ mice with the human pathobiont *Helicobacter pylori*. *H. pylori* infects the stomach mucosa and relies on T_{reg} and IL-10 to chronically

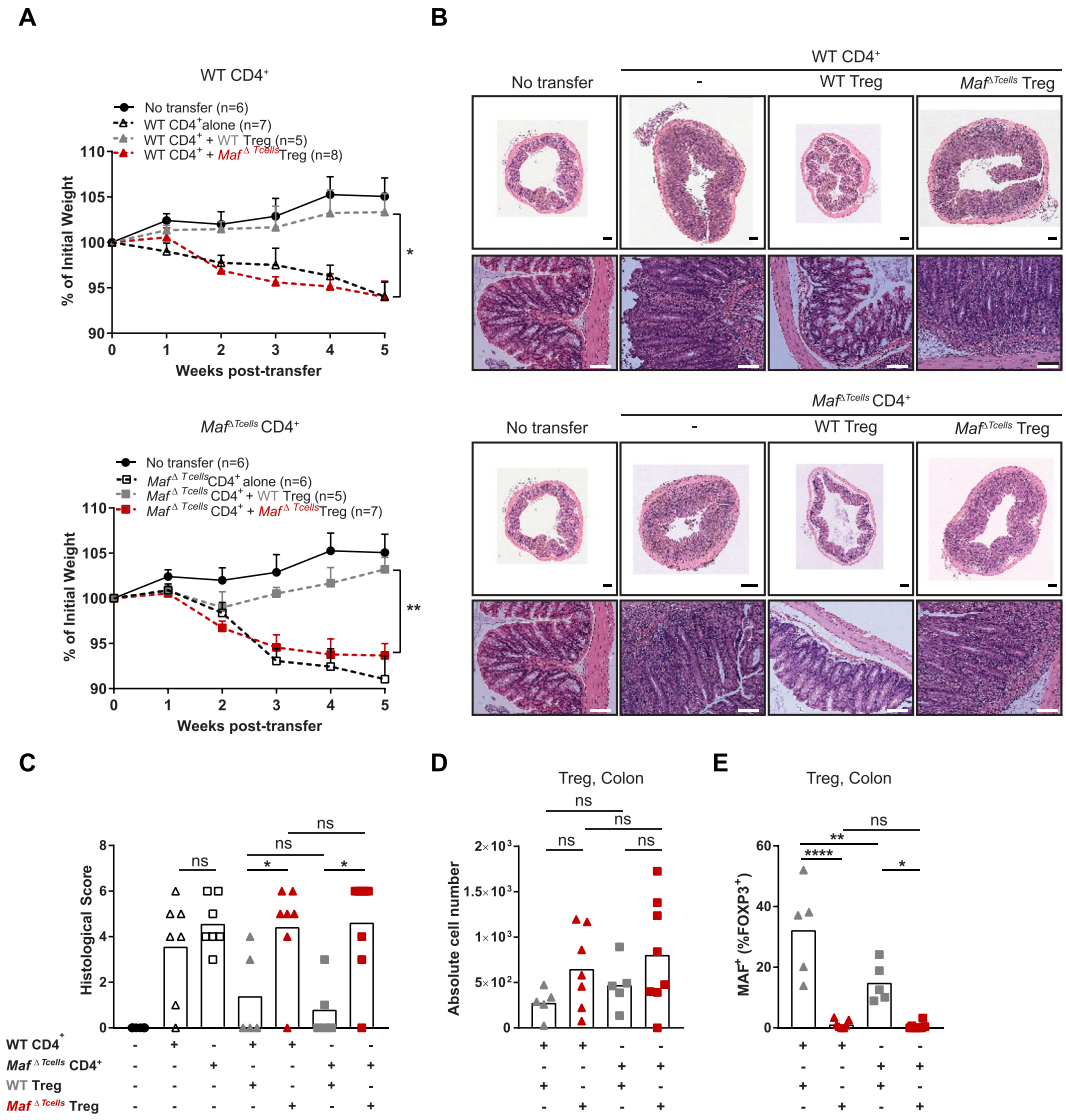

Figure 4. *Maf*-deficient T_{reg} fail to prevent colitis *in vivo*. $CD4^+CD45RB^{hi}CD25^{lo}$ cells (0.5×10^6) were intravenously injected alone or in combination with $CD4^+CD45RB^{lo}CD25^{hi}$ cells (0.4×10^6) into males and females $Rag1^{-/-}$ mice from 10 to 15 weeks age. (**A**) Body weight curve of $Rag1^{-/-}$ mice injected with either WT (upper) or $Maf^{\Delta Tcells}$ naïve $CD4^+$ T cells (bottom) alone or in combination with WT or $Maf^{\Delta Tcells}$ T_{reg}. Statistical significances were calculated by a Bonferroni test after a significant two-way ANOVA. (**B**) Haematoxylin and eosin-stained sections of colons (Upper picture: scale bar, 200 µm. Bottom pictures: scale bar, 100 µm) and **(C)** Histological scores (from 0 to 6) for microscopic changes in the colons of indicated groups of mice. (**D**) Absolute cell numbers of total $FOXP3^+$ T cells in the colons of the indicated groups of mice ($n > 5$ per group). (**E**) Percentage of MAF^+ cells among $FOXP3^+$ T cells in the colons of the indicated groups of mice ($n > 5$ per group). (**A,C,D,E**) All results are representative of 2 independent experiments with at least 2 mice per group. (**C–E**), Each symbol represents an individual mouse. All graphs indicate means. Error bars display SEM.

infect its host[19,20]. Moreover, it has been established that *H. pylori* can be cleared from the stomach mucosa by a T_H17 response triggered by vaccination[21]. Two months after infection, quantification of the bacterial burden by numeration of colony-forming unit (CFU) in the mouse stomach showed that $Maf^{\Delta Tcells}$ mice cannot be infected by *H. pylori* (Fig. 7A). Remarkably, as compared to infected $Maf^{f/f}$ mice, we observed increased mRNA levels encoding for *Cd4*, *Tnfα*, *Inos*, *Il17*a, *Il22* and the antimicrobial peptides *RegIIIβ* and *RegIIIγ* in the stomach mucosa of $Maf^{\Delta Tcells}$ mice (Fig. 7B–E). This pattern of increased expression, which is characteristic of a T_H17 response, is very similar to the vaccine-induced T_H17 response that clear *H. pylori* infection in WT mice[22]. In addition, we found higher *Il2* production as well as higher *Foxp3* level in $Maf^{\Delta Tcells}$ mice (Supplementary Fig. 4). Collectively, these data suggest that $Maf^{\Delta Tcells}$ mice can eliminate *H. pylori* infection by generating an exacerbated T_H17 inflammatory program.

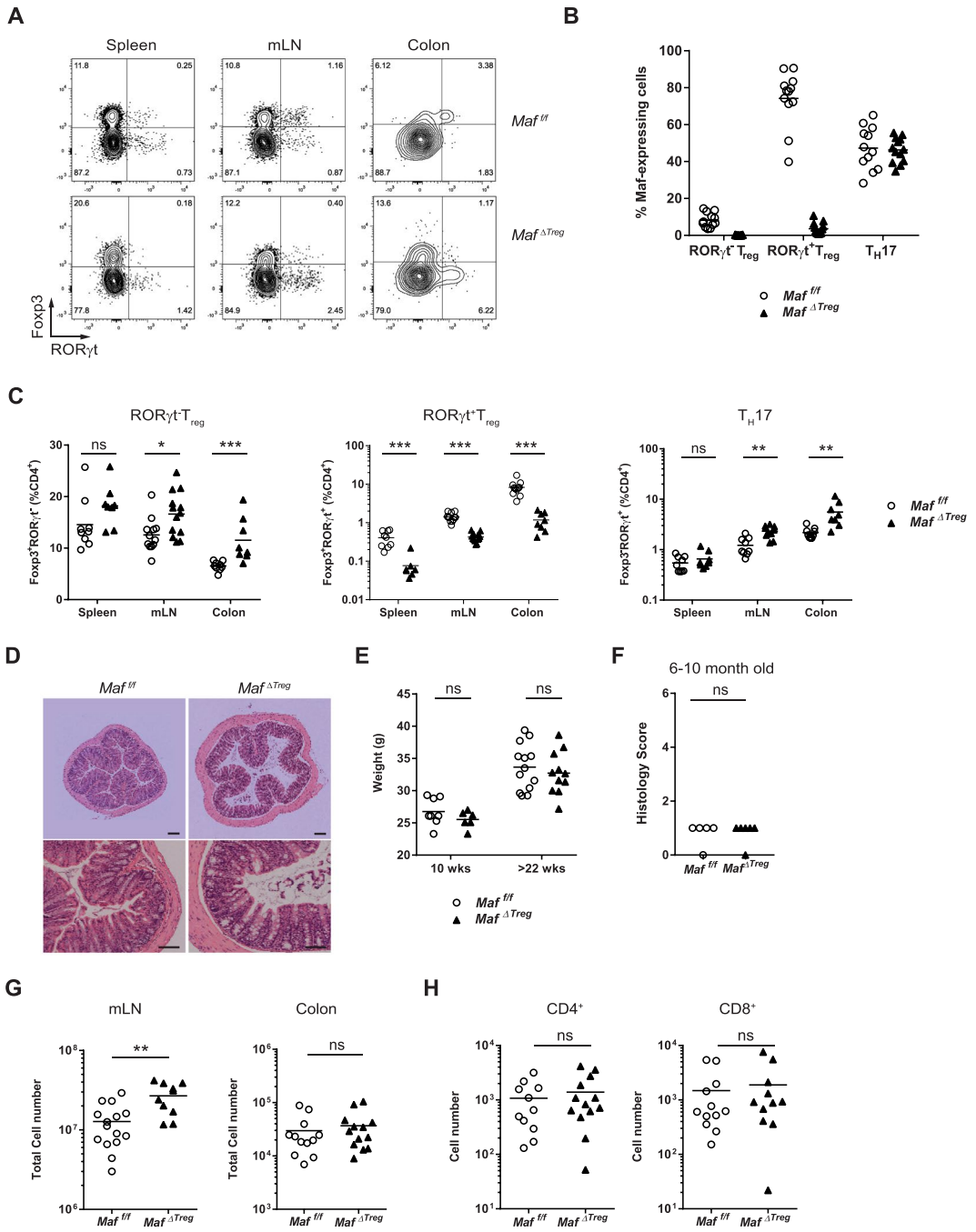

Figure 5. *Maf*$^{\Delta Treg}$ mice do not develop colitis. (**A**) Representative contour plot of the expression of FOXP3 and RORγt in CD4$^+$ T cells from spleens, mLN and colons of *Maf*$^{f/f}$ and *Maf*$^{\Delta Treg}$ mice. (**B**) Percentages of RORγt$^-$ T_{reg}, RORγt$^+$ T_{reg} and T_H17 cells from spleens, mLN and colons of *Maf*$^{f/f}$ and *Maf*$^{\Delta Treg}$ mice (n > 8 per group). **(C)** Percentage of MAF$^+$ cells among RORγt$^-$ T_{reg}, RORγt$^+$ T_{reg} and T_H17 cells from mLN of *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ (n > 10 per group). **(D)** Haematoxylin and eosin-stained sections of colons from *Maf*$^{f/f}$ and *Maf*$^{\Delta Treg}$ mice (Upper picture: scale bar, 200 µm; bottom pictures: scale bar, 100 µm). **(E)** Histological scores (from 0 to 6) of microscopic changes in the colon from *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice at the age of 6 to 10 months (n = 5 per group). **(F)** Body weight of *Maf*$^{f/f}$ and *Maf*$^{\Delta Treg}$ male mice of 10 and 22 weeks old (n > 6 per group). (**G**) Total cell numbers in mLN (left) and colons (right) of *Maf*$^{f/f}$ and *Maf*$^{\Delta Treg}$ mice (n > 10 per group). (**H**) Absolute cell numbers of CD4$^+$ and CD8$^+$ T cells in colons of *Maf*$^{f/f}$ and *Maf*$^{\Delta Treg}$ mice (n > 10 per group). (**B,C,E,H**) Each symbol represents an individual mouse. (**B,C,G,H**) Data are representative of at least 2 independent experiments with at least 4 mice per group. All mice were over 20 weeks old. All graphs indicate means.

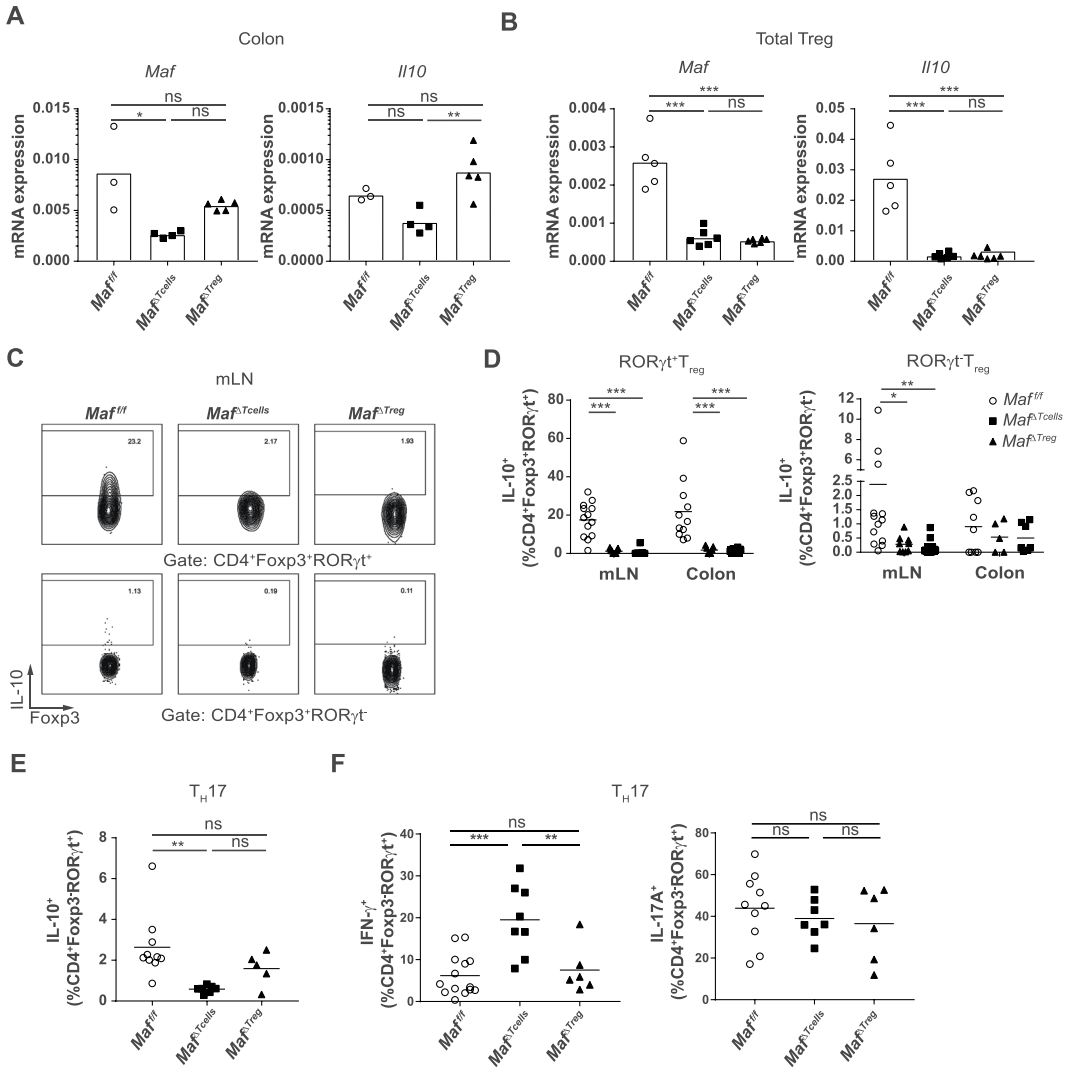

Figure 6. IL-10 expression is regulated by MAF in T_{reg} and T_H17 cells. (**A**) Quantitative RT–PCR of colonic tissues from $Maf^{f/f}$ (n = 3), $Maf^{\Delta Tcells}$ (n = 4) and $Maf^{\Delta Treg}$ (n = 5) mice for the indicated transcripts. Gene expression levels were normalized to *Beta2microglobulin*. (**B**) Quantitative RT–PCR of isolated T_{reg} ($CD4^+CD25^+CD45RB^{lo}$) of spleens and LN of $Maf^{f/f}$ (n = 5), $Maf^{\Delta Tcells}$ (n = 6) and $Maf^{\Delta Treg}$ (n = 6) mice for the indicated transcripts. Gene expression levels were normalized to *Beta2microglobulin*. (**C**) Representative contour plot of the expression of IL-10 and RORγt in T_{reg} from mLN of $Maf^{f/f}$, $Maf^{\Delta Tcells}$ and $Maf^{\Delta Treg}$ mice. (**D**) Percentage of $IL\text{-}10^+$ cells among $ROR\gamma t^-$ (left) and $ROR\gamma t^+$ T_{reg} (right) in mLN and colon of $Maf^{f/f}$, $Maf^{\Delta Tcells}$ and $Maf^{\Delta Treg}$ mice after *ex vivo* restimulation (n > 8 per group). **(E)** Percentage of $IL\text{-}10^+$ cells among T_H17 cells from mLN of $Maf^{f/f}$, $Maf^{\Delta Tcells}$ and $Maf^{\Delta Treg}$ mice after *ex vivo* restimulation (n > 5 per group). **(F)** Percentage of IFN-γ^+ and $IL\text{-}17A^+$ cells among T_H17 cells from colons of Cre-negative littermates, $Maf^{\Delta Tcells}$ and $Maf^{\Delta Treg}$ mice after *ex vivo* restimulation (n > 6 per group). (**A,B,D–F**) Each symbol represents an individual mouse. Data are representative of 2 independent experiments. All mice were over 20 weeks old. All graphs indicate means. Statistical significances were calculated by a Tukey test after a significant one-way ANOVA.

Discussion

Our study shows that the deletion of *Maf* in all T cells ($Maf^{\Delta Tcells}$ mice) drives spontaneous late-onset colitis (Fig. 1). To date, no study has shown that the deletion of *Maf* in T cells was sufficient and necessary to drive colitis. The development of the colitis is associated with the dysregulation of the T_{reg}-T_H17 equilibrium (Fig. 3D,E) and a large decrease in the production of IL-10 (Fig. 3A). Inactivation of *Maf* in T cells impaired the differentiation of $ROR\gamma t^+$ T_{reg} (Fig. 3C) and favoured the accumulation of colitogenic T_H17 cells in the colon of the mice (Fig. 3D). The onset of the colitis is dependent on the presence of microbiota since $Maf^{\Delta Tcells}$ mice treated with antibiotics develop only mild colitis (Supplementary Fig. 1E,F). The first visible signs of colitis developed around the age of 15 weeks (Fig. 1A). It correlates with the natural differentiation and/or accumulation of $ROR\gamma t^+$ T_{reg} and MAF expression in these cells in the colon of WT animals (Fig. 3E,F). This $ROR\gamma t$ T_{reg} population is preferentially found in the colon (Fig. 3C) and represents the main producer of IL-10 (Fig. 6D)[10,16]. The role of MAF in the regulation of the $ROR\gamma t$ T_{reg} population was demonstrated very recently using mice in which *Maf* is deleted in

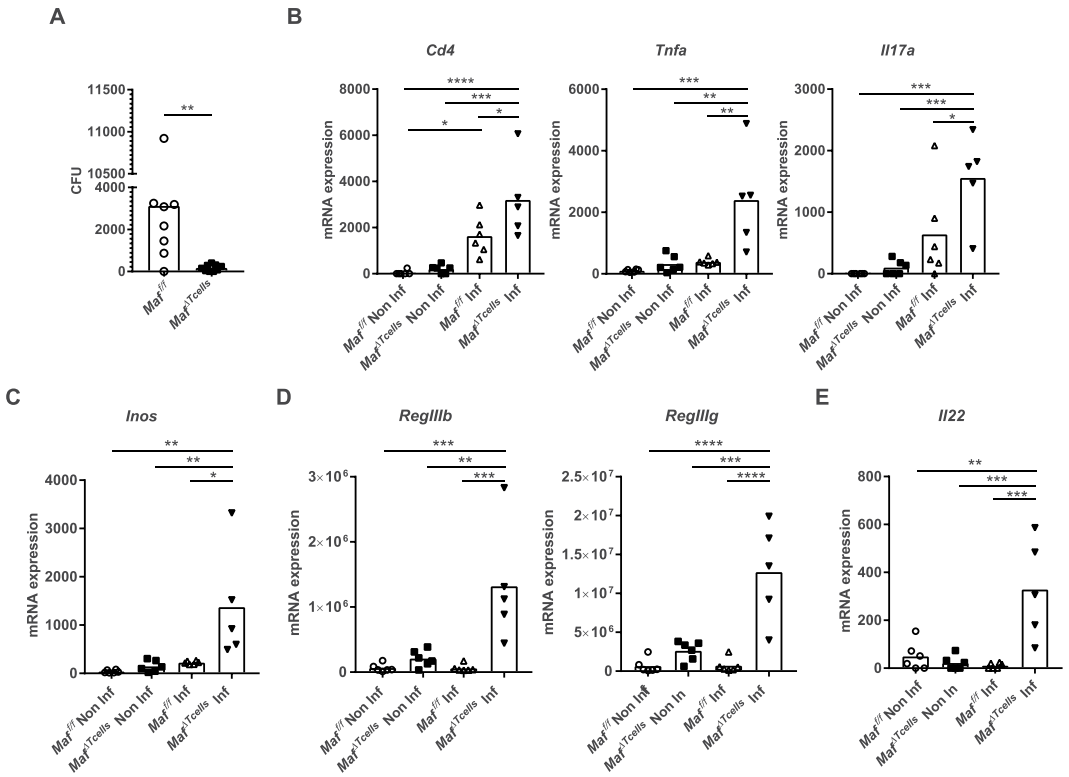

Figure 7. Elimination of Helicobacter pylori by $Maf^{\Delta Tcells}$ mice through exacerbated T_H17 response. (**A**) Numeration of CFU from stomachs of *Helicobacter pylori* infected $Maf^{f/f}$ (n = 6) and $Maf^{\Delta Tcells}$ (n = 5) mice. (**B–E**) Quantitative RT–PCR of stomachs from non-infected $Maf^{f/f}$ (n = 6) and $Maf^{\Delta Tcells}$ (n = 6) mice and *Helicobacter pylori* infected $Maf^{f/f}$ (n = 6) and $Maf^{\Delta Tcells}$ (n = 5) mice for the indicated transcripts. Gene expression levels were normalized to *Gapdh*. Each symbol represents an individual mouse. Data are representative of at least 2 independent experiments with at least 2 mice per group. Statistical significances were calculated by a Tukey test after a significant one-way ANOVA.

T_{reg} ($Maf^{\Delta Treg}$)[15,16]. In these two studies, the authors showed that the differentiation of this particular regulatory population is altered in the absence of *Maf*.

The low proportion of RORγt$^+$ T_{reg} in the colon of $Maf^{\Delta Tcells}$ mice was paradoxically associated with an increase in proportion and numbers of RORγt$^-$ T_{reg}. We provide evidence that T_{reg} in $Maf^{\Delta Tcells}$ mice are not fully functional. Indeed, compared to WT T_{reg}, *Maf*-deficient T_{reg} do not control the development of colitis induced by the transfer of naïve CD4$^+$ T cells into $Rag1^{-/-}$ mice (Fig. 4). Moreover, we observed that *Maf*-deficient RORγt$^-$ T_{reg} produce lower level of IL-10 as compared to WT RORγt$^-$ T_{reg} (Fig. 6C,D). These findings are in appearance not entirely in line with the study of Xu *et al*. showing that *Maf* -deficient nT_{reg} are still able to suppress colon inflammation in $Rag1^{-/-}$ mice transferred with naïve CD4$^+$ T cells[15]. However, given the nature of their experimental setting in which *Maf* -deficient nT_{reg} were isolated from *H. hepaticus* colonized mice and transferred in *H. hepaticus* infected mice, we assume that the antigen specificity of some *Maf*-deficient nT_{reg} could compensate for their partial loss of function. One other possibility would be the effect of potentially contaminating Maf-deficient T cells transferred with sorted Maf-deficient Treg. We assume that this potentially pathogenic T cells are also present in the "naïve" Maf-deficient T cells transferred in the mice. However, control Treg transferred together with "naïve" Maf-deficient T cells are still able to control colitis, which make this possibility unlikely. Altogether, it can be suggested that despite their normal differentiation, *Maf*-deficient RORγt$^-$ T_{reg} are unable of preventing colitis due to their loss of function (decreased IL-10 production), combined to their low specificity to the gut microbiota in our experimental setting.

An impaired differentiation of colon RORγt$^+$ T_{reg} was also observed in $Maf^{\Delta Treg}$ mice (Fig. 5A). Despite this absence, the mice did not develop colitis even at old ages (Fig. 5D–F). The absence of colitis onset is associated with a very limited accumulation of T_H17 cells (Fig. 5C) and normal expression level of IL-10 in the colon (Fig. 6A). This result is similar to what is described previously in these mice, with only a mild inflammation detected in *H. hepaticus* free animals aged of 6 months[15].

One of the differences between $Maf^{\Delta Treg}$ and $Maf^{\Delta Tcells}$ mice is that RORγt$^+$ T_H17 cells do not express *Maf* in $Maf^{\Delta Tcells}$ mice (Fig. 2C). It has been shown that MAF plays a prominent role in T_H17 cells through the regulation of IL-10 production[4,17]. We indeed observed *in vivo* that RORγt$^+$ T_H17 cells from $Maf^{\Delta Tcells}$ mice do not produce IL-10 while $Maf^{f/f}$ and $Maf^{\Delta Treg}$ counterparts do (Fig. 6E). Furthermore, we showed an increase of IFN-γ$^+$ T_H17 cells from $Maf^{\Delta Tcells}$ compared to $Maf^{f/f}$ and $Maf^{\Delta Treg}$ mice (Fig. 6F).

Maf can affect other T cell subtypes that might participate to colitis development. A recent study on the role of MAF in iNKT cells showed that MAF inactivation in these cells leads to a decrease in IL-17a production[7].

In our system this would mean that inactivation of MAF in these cells would limit the development of colitis, which is however not the case. MAF regulates IL-4 but not IL-10 production in T_H2 cells[23]. The absence of MAF leads to a decreased expression of Il-4[23], which is not in accordance with an increased pathology. T_H2 cells are a minority of the CD4 T cells that we find in the colon of the mice and do not increase among CD4 T cells in $Maf^{\Delta Tcells}$ (Supplementary Fig. 3C). Despite the involvement of T_H2 cells in the development of ulcerative colitis in human[24], we think for these reasons that it is unlikely that the development of colitis after MAF inactivation is related to T_H2 cells. T_R1 cells, may also be a source of IL-10. Indeed, it was described that MAF is present in this population and regulates the expression of *Il10* together with AhR[9]. We did not find any IL-10 producing RORgt-Foxp3- CD4 T cells in the colon of our mice, which limits the potential role of Tr1 in our system (Supplementary Fig. 3C). Altogether, the absence of IL-10 production by both RORγt$^{+/-}$ T_{reg} and T_H17 cells associated with the increase production of IFN-γ by T_H17 cells in $Maf^{\Delta Tcells}$ mice most probably lead to colitis onset.IL-10 production by T_{reg} is essential to maintain homeostasis at environmental surfaces by directly suppressing pathogenic T_H17 cells and T_H17/T_H1 cells[25]. Several years ago, work led by Rudensky *et al*. showed that selective disruption of *Il10* in T_{reg} leads to spontaneous colitis[26]. However, mice lacking IL10RA[27] in T_{reg} that show reduced expression of IL-10, develop a more severe colitis, suggesting that compensatory mechanisms orchestrated by non-T_{reg} can participate to maintain homeostasis through regulation of T_{reg} function. Although restricted to the colon, the phenotype observed in our model looks similar to the colitis observed in T cell–specific IL-10 mutant mice[28] or $Il10^{-/-}$ mice[29]. This is in line with recent study showing that *Maf* is a common regulator of IL-10 in CD4$^+$ T cells[6]. We were able to link this phenotype to decreased suppressive capacity of all *Maf*-deficient T_{reg}, but particularly RORγt$^+$ T_{reg}, leading to uncontrolled T_H17-driven inflammation.

Recent reports suggested that RORγt is induced after the differentiation of naïve cells into Foxp3$^+$ T_{reg}[13,30]. How MAF is regulating the development of these cells remains an open question. MAF is induced by TGF-β and IL-6 in CD4$^+$ T_H subsets and CD8$^+$ T cells[3,4,8,15]. Wheaton *et al*. proposed that IL-6 can upregulate MAF in pre-differentiated T_{reg} leading to RORγt acquisition. Indeed, RORγt has been identified as a direct target of MAF[6]. Observation that selective disruption of STAT3 in T_{reg} leads to a decrease of RORγt$^+$ T_{reg} (11) in mice suggest that STAT3 may also be required in this process of differentiation. Thus, MAF appears to be at least in part regulated through STAT3 activating cytokines such as IL-6. However, what is exactly regulating MAF expression in the gut remains to be precisely determined.

A recent study showed that additionally to its direct regulation of *Il10* expression, *Maf* is also an inhibitor of *Il2*[6]. In the *Maf*-deficient T cells, the increased production of IL-2 has been shown to inhibit the T_H17 cell response in the EAE induced model[6]. In our study, we observed that after infection with *H. pylori*, the bacteria is cleared from the stomach mucosa of $Maf^{\Delta Tcells}$ mice but establish a chronic infection in $Maf^{f/f}$ mice. We measured an increased level in transcripts coding for *Il2* and *Foxp3* in the stomach mucosa of *H. pylori*-infected $Maf^{\Delta Tcells}$ mice (Supplementary Fig. 4), leading to the possibility that increased number of classical T_{reg} are recruited in the stomach mucosa of $Maf^{\Delta Tcells}$ mice through an *Il2*-dependent pathway. However, we also found high amount of *Il17a* and *Il22*, indicating the recruitment of T_H17 cells (Fig. 7B,E) leading to the clearance of *H. pylori* from the stomach mucosa of $Maf^{\Delta Tcells}$ mice. These results clearly demonstrate that MAF plays a major role in maintaining the T_{reg} -T_H17 equilibrium not only in the colon but also in the gastric mucosa. In addition, it can be suggested that the development of a vaccine directed against a pathobiont, such as *H. pylori*, might be facilitated by a vaccine formulation that prevent MAF expression in primed T cells.

Altogether, we established that the role of *Maf* was not only restricted to T_{reg} but also to conventional T cells, especially T_H17 cells, establishing *Maf* as a major regulator of the T_{reg} – T_H17 balance in the gastro-intestinal tract. On a broader perspective, MAF expression in colonic lymphocytes appears to be strongly associated with an anti-inflammatory type of response. However, the implication of *Maf* in immune responses appears to be largely location- and context-dependent[6].

Material and Methods

Mice. $Rag1^{-/-}$ and $Foxp3^{YFPcre}$ [26] mice were kindly provided by P.C Ho. $Foxp3^{YFPcre}$ and $CD4^{cre}$ [31] mice were crossed with $Maf^{fl/fl}$ mice[32] to generate $Foxp3^{YFPcre}$ $Maf^{fl/fl}$ ($Maf^{\Delta Treg}$) and $CD4^{cre}$ $Maf^{fl/fl}$ ($Maf^{\Delta Tcells}$), respectively. Mice were bred and maintained in a specific-pathogen-free (SPF) environment, which excludes *Helicobacter hepaticus*, of the animal facility of the University of Lausanne. The animals used were cohoused and littermate controls are referred to as $Maf^{f/f}$ mice. Experiments were performed in compliance with the University of Lausanne Institutional regulations and were approved by the veterinarian authorities of the Canton de Vaud (Switzerland).

Isolation of cells. Single-cell suspensions from spleens and mesenteric lymph nodes were obtained after mashing the organs through a 70 µM cell strainer. Colons were collected in calcium and magnesium free Hank's Balanced Salt Solution (HBSS) (ThermoFisher Scientific) supplemented with 2% of Fetal Calf Serum (FCS) (HBSS 2%) on ice. Samples were further flushed with HBSS 2% and cut longitudinally into 3–4 pieces (2–3 cm). Minced tissues were treated with EDTA 1 mM (ThermoFisher Scientific) and DTT 1 µM (AppliChem) in HBSS 10% solution for 20 min under shaking at 37 °C. After intraepithelial lymphocytes (IEL) removal, cells from the lamina propria were washed twice in HBSS 10% and incubated with Collagenase D (1 mg/ml) (Roche) and complete RPMI (ThermoFisher Scientific) for 30 min under shaking at 37 °C. To isolate leucocytes, supernatants were centrifuged in density gradients 40%/70% Percoll (GE Healthcare Life Sciences) for 30 min at 2000 rpm. All isolated cells were washed in complete RPMI and filtrated before staining.

T cell isolation and transfer into $Rag1^{-/-}$ mice. Donor cells were obtained from spleens and LN of both WT (obtained from Cre-negative littermates and C57Bl6 mice) and $Maf^{\Delta Tcells}$ female mice. CD4$^+$ T cells were enriched using EasySep Mouse CD4$^+$ T Cell Isolation Kit (StemCell Technologies) according to the

Antibody	Clone	Reference	Fluoresence Labelling	Supplier	Dilution
CD25	PC61	20251–83	PE	eBioscience	1/3000
CD4	RM4–5	100549	BV711	Biolegend	1/100
CD45.2	104	109835	BV650	Biolegend	1/50
CD45RB	C363-16A	103307	PE	Biolegend	1/200
CD8	53–6.7	100743	BV605	Biolegend	1/500
C-MAF	sym0F1	50-9855-82	eFluor 660	eBioscience	1/100
FOXP3	FJK-16s	48-5773-82	eFluor 450	eBioscience	1/200
	MF-14	126406	Alexa Fluor 488	Biolegend	1/200
GATA-3	TWAJ	46-9966-42	PerCP-eFluor710	eBioscience	1/200
IFN-γ	XMG1.2	505826	PE/Cy7	Biolegend	1/200
IL-10	JES5-16E3	505007	PE	Biolegend	1/100
IL-17A	eBio17B7	53-7177-81	Alexa Fluor 488	eBioscience	1/200
RORγt	B2D	61-6981-82	PE-eFluor 610	eBioscience	1/200
T-BET	4B10	644814	APC	Biolegend	1/200

Table 1. List of the antibodies used for flow cytometry.

Gene	Reverse (5′-3′)	Forward (5′-3′)
beta2microglobulin	AGACTGATACATACGCCTGCAG	GCAGGTTCAAATGAATCTTCAG
ifnγ	CAACAGCAAGGCGAAAAA	GGACCACTCGGATGAGCTC
il10	ACCTGCTCCACTGCCTTGCT	GGTTGCCAAGCCTTATCGGA
il17a	GCTCCAGAAGGCCCTCAGA	AGCTTTCCCTCCGCATTGA
il1b	TCGAGGCCTAATAGGCTCATCT	GCTGCTTCAGACACTTGCACAA
il4	GAAGCCCTACAGACGAGCTCA	GGGACGCCAT
maf	AACATATTCCATGGCCAGGG	GGATGGCTTCAGAACTGGCA
tnfa	TGGAAGTAGACAAGGTACAACCC	CATCTTCTCAAAATTCGAGTGACAA
inos	CATTGGAAGTGAAGCGTTTCG	CAGCTGGGCTGTACAAACCTT
gapdh	TCACCACCACCATGGAGAAGG	GCTAAGCAGTTGGTGGTCA
regIIIb	QT00239302 (Quiagen)	
regIIIg	QT00147455 (Quiagen)	
foxp3	QT00138369 (Quiagen)	

Table 2. List of the primers used for quantitative RT-PCR.

manufacturer's recommendations. $CD4^+CD45RB^{hi}CD25^{lo}$ and $CD4^+CD45RB^{lo}CD25^{hi}$ were isolated by sorting on a FACS Aria cell sorter (BD Biosciences). $CD4^+CD45RB^{hi}CD25^{lo}$ cells (0.5×10^6) were intravenously injected alone or in combination with $CD4^+CD45RB^{lo}CD25^{hi}$ cells (0.4×10^6) into males and females *Rag1*$^{-/-}$ mice from 10 to 15 weeks age. Mice were weighed and monitored weekly. 6 weeks after transfer, mice were sacrificed.

Histopathology and scoring. Colons were fixed with histological tissue fixative (Sigma), embedded in paraffin and stained with haematoxylin and eosin. Histopathological score (0–6) was calculated based on inflammatory cells infiltration (0–3) and tissues abnormalities (0–3) by a pathologist.

***In vitro* T cell differentiation.** Naïve $CD4^+CD25^{lo}CD62^{hi}CD44^{lo}$ cells were isolated by using EasySep Mouse Naïve $CD4^+$ T Cell Isolation Kit (Stemcell Technologies) from spleens and LN of *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ mice, and activated with plate-bound anti-CD3 (Biolegend, 5 μg/ml) and soluble anti-CD28 (Biolegend, 1 μg/ml) supplemented with mIL12 (10 ng/ml) and neutralizing antibody anti-IL-4 (clone 11B11, 10 μg/ml) (T_H1), mIL-4 (10 ng/ml) and anti-IFN-γ (clone: XMG-121,10 μg/ml) (T_H2), hIL-2 (50U/ml) and TGF-β (10 ng/ml) (T_{reg}), TGF-β (5 ng/ml) and IL-6 (40 ng/ml) ($FOXP3^+$ and $FOXP3^-$ T_H17). Cells were incubated 5 days at 37 °C, 5% CO_2.

Antibodies, intracellular staining and flow cytometry. All antibodies used are listed in Table 1. For intracellular cytokine staining, cells were restimulated with PMA (Sigma, 50 ng/ml) and Ionomycin (Sigma, 100 ng/ml) for 4 h at 37 °C in presence of Golgi Plug (BD Biosciences). After staining for viability using LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (ThermoFisher Scientific) and extracellular markers, cells were fixed and permeabilized with the FOXP3 Transcription Factor Staining Buffer Set (eBiosciences) according to manufacturer's recommendations. Following permeabilization, cells were intracellularly stained for specific cytokine and acquired on a LSRII flow cytometer (BD). Data were analysed with FlowJo software V10.

Real-time qPCR analysis. Colons were collected, dried and ground into a powder using liquid nitrogen. Total RNA was isolated using RNAeasy Mini Kit (Qiagen, #74194) according to the manufacturer's

recommendations. cDNA was retro-transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, # 4368814) and used for quantitative PCR. The KAPA SYBR® FAST qPCR Master Mix (2×) Kit (Sigma, #KK4618) was used for SYBR analysis. The housekeeping gene *beta2microglobulin* was used to normalize gene expression. Sequences of primers used are listed in Table 2.

Bacteria and infection. H. pylori P49, kindly provided by Harry Kleanthous (Acambis, Cambridge, MA), is a human clinical isolate adapted to mice. H. pylori P49 expresses VacA but not CagA. H. pylori P49 was grown on Helicobacter pylori-selective agar plate (Oxoid, Basingstoke, UK) and brain heart infusion broth supplemented with 0.25% yeast extract and 10% fetal calf serum (PAA, Pasching, Austria) under microaerophilic conditions, as previously describe[33]. Mice were treated on day 4 and 5 after birth with 5×10^8 H. pylori P49, administered by orogastric gavage in 200 µL BHI.

Antibiotics treatment. *Maf*$^{f/f}$ and *Maf*$^{\Delta Tcells}$ pregnant females were fed with antibiotics in their drinking water consisting of 0.5 mg/ml Amoxicillin (Mepha Pharma AG) and 5 mg/ml Enrofloxacine (Bayer). Antibiotic-containing drinking water was changed once a week until analysis. Offspring were then treated until their sacrifice at 17 weeks age.

Statistical analysis. Unless otherwise stated, unpaired non-parametric Mann-Whitney t tests were used to calculate statistical significance using GraphPad Prism software. P values: *$P < 0.05$, **$0.01 < P < 0.05$, ***$P < 0.001$.

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Acknowledgements

We thank the group of Dr. Caroline Pot (D. Duc; S. Vigne) for technical help and Dr. Connie Gilfillan for critical reading of the manuscript. This work was supported by grants from the Swiss Cancer League (G.V: 3679-08-2015), the Max Cloëtta Foundation (G.V), the Cancer Research Institute, USA (G.V, D.E.S), by the Swiss National Foundation grant (D.V: 310030_173197) and Ludwig Cancer Research, USA.

Author Contributions

C.I., M.M.L., D.V. and G.V. designed, performed and analysed the data. H.B. performed the analysis for the histology. G.V., C.I., D.V. and D.E.S. wrote, reviewed and revised the paper.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-42486-2.

Competing Interests: The authors declare no competing interests.

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