

## **Serum Amyloid A Proteins Induce Pathogenic T<sub>H</sub>17 Cells and Promote Inflammatory Disease**

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**Summary:** Lymphoid cells that produce IL-17 cytokines protect barrier tissues from pathogenic microbes, but are also prominent effectors of inflammation and autoimmune disease. T-helper (T<sub>H</sub>17) cells, defined by ROR $\gamma$ t-dependent production of IL-17A and IL-17F, exert homeostatic functions in the gut upon microbiota-directed differentiation from naïve CD4<sup>+</sup> T cells. In the non-pathogenic setting, their cytokine production is regulated by serum amyloid A proteins (SAA1 and SAA2) secreted by adjacent intestinal epithelial cells. However, T<sub>H</sub>17 cell behaviors vary markedly according to their environment. Here we show that SAAs additionally direct a pathogenic pro-inflammatory T<sub>H</sub>17 cell differentiation program, acting directly on T cells in collaboration with STAT3-activating cytokines. Using loss- and gain-of-function mouse models, we show that SAA1, SAA2, and SAA3 have distinct systemic and local functions in promoting T<sub>H</sub>17-mediated inflammatory diseases. These studies suggest that T cell signaling pathways modulated by the SAAs may be attractive targets for anti-inflammatory therapies.

**Key-words:** experimental autoimmune encephalomyelitis (EAE), autoimmunity, inflammatory bowel disease, TGF- $\beta$ , IL-23, T<sub>H</sub>1\*, *Helicobacter hepaticus*, segmented filamentous bacteria (SFB), T cell transfer colitis

## Introduction

T-helper 17 (T<sub>H</sub>17) cells and related IL-17-producing T cells perform critical roles at mucosal surfaces, mediating protection from pathogenic bacteria and fungi and contributing to regulation of the mutualistic organisms that comprise the microbiota (Honda and Littman, 2016). T<sub>H</sub>17 cells also drive the pathogenesis of multiple inflammatory diseases (McGeachy et al., 2019; Patel and Kuchroo, 2015; Stockinger and Omenetti, 2017). Yet, despite extensive studies conducted both *in vitro* and *in vivo*, the differentiation cues that distinguish T<sub>H</sub>17 cells that execute homeostatic functions, such as maintenance of barrier epithelial integrity, from those with potentially harmful inflammatory functions as observed in autoimmune diseases, remain an enigma. Differentiation of the latter, which have a distinct transcriptional program with features resembling those of T<sub>H</sub>1 cells, requires IL-23 and IL-1 $\beta$  (Chung et al., 2009; Hirota et al., 2011; Komuczki et al., 2019; McGeachy et al., 2009). Mice deficient for either of those cytokines retain the capacity to induce commensal microbe-specific T<sub>H</sub>17 cells (Ivanov et al., 2008), but are largely resistant to autoimmune disease in multiple animal models (Kullberg et al., 2006; Langrish et al., 2005). *In vitro* differentiation of the “homeostatic” T<sub>H</sub>17 cells can be achieved by stimulation of antigen-activated T cells with TGF- $\beta$  and either IL-6 or IL-21 that induce STAT3 phosphorylation, which is prerequisite for expression of the T<sub>H</sub>17-defining transcription factor, ROR $\gamma$ t (Ivanov et al., 2006; Zhou et al., 2007). When myelin-reactive T cells were generated *in vitro* with TGF- $\beta$  and IL-6, they failed to provoke autoimmune disease following transfer into mice (Lee et al., 2012). However, additional *in vitro* exposure to IL-23 rendered these cells pathogenic, as did their differentiation in the absence of TGF- $\beta$ , with a combination of IL-6, IL-1 $\beta$ , and IL-23 (Ghoreschi et al., 2010; Lee et al., 2012).

The SAAs are a family of acute phase response proteins that were recently associated with gut microbial ecology and inflammation and are encoded by at least four closely-linked genes that likely arose from gene duplication events (Lloyd-Price et al., 2019; Tang et al., 2017; Uhlar et al., 1994). SAA1,2, and 3 are elicited by inflammatory cues, while SAA4 is constitutively produced and regulated independently of the inflammatory state (De Buck et al., 2016; Yarur et al., 2017; Ye and Sun, 2015). Previously, we demonstrated that colonization of mice with an epithelial cell associated commensal microbe, segmented filamentous bacteria (SFB), triggered local secretion of SAA1 and SAA2 by the IEC. The SAAs directly potentiated local  $T_{H17}$  cell effector cell function (Ivanov et al., 2009; Sano et al., 2015). In this context, SAA1/2 remained confined to the ileum and did not enter into the circulation (Sano et al., 2015). However, SAA1 and SAA2 are most prominently induced upon liver injury and/or inflammation, with concentrations in serum increased by as much as a thousand-fold over basal levels (De Buck et al., 2016; Yarur et al., 2017; Ye and Sun, 2015). Serum concentrations of SAA1 and SAA2 are also consistently elevated in various  $T_{H17}$ -mediated autoimmune diseases, including rheumatoid arthritis, Crohn's disease (CD), ulcerative colitis (UC), and multiple sclerosis, in addition to numerous cancers (Ye and Sun, 2015). Systemic SAA levels have long served as biomarkers, but whether they contribute to these diseases is not known. The secreted proteins form hexamers that associate in serum with high density lipoprotein, and are thought to be involved in the maintenance of lipid homeostasis (Wang and Colon, 2004; Wang et al., 2002). There is also evidence that SAAs, particularly SAA3, may form tetramers and associate with derivatives of vitamin A (Derebe et al., 2014). The anatomical distribution and cellular targets of SAAs in chronic disease are largely undefined, but the SAAs were recently implicated in conditioning the liver microenvironment to provide a niche for tumor metastases (Lee et al., 2019).

Here, we investigate the role of SAAs in the differentiation and function of potentially pathogenic T<sub>H</sub>17 cells. We find that the SAAs can substitute for TGF- $\beta$  in the induction of Th17 cells, but engage a distinct signaling pathway that results in a pro-inflammatory program of differentiation. As a consequence, SAAs contribute in vivo to T<sub>H</sub>17-mediated pathogenesis, revealed in inflammatory bowel disease and experimental autoimmune encephalomyelitis with both loss- and gain-of-function models. Collectively, our findings show that the SAAs contribute selectively to T<sub>H</sub>17 cell functions in vivo, and suggest strategies for therapeutic modulation in T<sub>H</sub>17-mediated inflammatory disease.

## Results

### **The SAAs direct a distinct T<sub>H</sub>17 differentiation program independently of TGF- $\beta$ .**

*In vitro* activation of naïve murine CD4<sup>+</sup> T cells in the presence of IL-6 alone is insufficient to promote T<sub>H</sub>17 cell differentiation (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006). Addition of either TGF- $\beta$  or IL-1 $\beta$  plus IL-23 is required for the T cells to up-regulate ROR $\gamma$ t and produce T<sub>H</sub>17 cytokines. Unexpectedly, we observed that a combination of SAA1 and IL-6 (referred to T<sub>H</sub>17-SAA1 condition), in the presence of TGF- $\beta$  neutralizing antibody, elicited potent T<sub>H</sub>17 cell differentiation in a dose-dependent manner, with expression of the signature cytokines, IL-17A and IL-17F (Figures 1A-C and Figure S1A). Similar results were obtained using T cells expressing the dominant negative form of TGF $\beta$ RII (TGF $\beta$ RII DNtg), while addition of SAA1 neutralizing antibodies prevented T<sub>H</sub>17 cell induction under T<sub>H</sub>17-SAA1 conditions (Figures S1B and S1C). Expression of ROR $\gamma$ t was similar between T<sub>H</sub>17-SAA1 cells and T<sub>H</sub>17 cells induced by the standard polarization cocktail consisting of IL-6 + TGF- $\beta$  (T<sub>H</sub>17-TGF $\beta$ ) (Figure 1D). Notably, naïve murine CD8<sup>+</sup> T cells responded similarly to CD4<sup>+</sup> cells under the different polarization

conditions (Figures S1D and S1E). To determine whether SAA-directed signaling converged with the TGF- $\beta$  signaling pathway, we assessed levels of phosphorylated SMAD2/3 (pSMAD2/3). As expected, TGF- $\beta$  rapidly induced pSMAD2/3. However, this was not observed with SAA1, which instead was found to rapidly engage the MAPK pathway, inducing p38 phosphorylation (Figure 1E and S1F). These findings demonstrate that SAA1 promotes Th17 cell differentiation through a TGF- $\beta$ -independent mechanism.

To compare *in vitro* Th17 cell programming by SAA1 and TGF- $\beta$ , we performed RNA sequencing (RNAseq) of cells differentiated under Th17-SAA1 or Th17-TGF $\beta$  conditions for 3h, 12h, and 48h (Figure S2A). After 48h, there were 3537 differentially expressed (DE) genes between Th17-SAA1 and Th17-TGF $\beta$  cells (Figure 2A). Compared to Th cells cultured in IL-6 alone or the Th17-TGF $\beta$  condition, Th17-SAA1 cells exhibited a profound induction of hallmark chronic inflammatory disease-associated genes, such as *Il23r* (Abdollahi et al., 2016; Duerr et al., 2006; Gaffen et al., 2014; Hue et al., 2006), *Il1r1* (Shouval et al., 2016), and *S100a4* (Oslejskova et al., 2009) (Figure S2B and S2C). Gene set enrichment analysis using previously defined Th17 datasets revealed a striking correlation with the “pathogenic” Th17 signature, but an anti-correlation with the “non-pathogenic” Th17 signature, as early as 3h after SAA treatment (Lee et al., 2012) (Figure 2B). Examples of increased pathogenic-Th17 signature genes in differentiating Th17-SAA1 cells include *Csf2*, *Tbx21* and *Gzmb*, while decreased non-pathogenic-Th17 signature genes include *Maf*, *Ahr* and *Il10* (Figures S2D and S2E) (Lee et al., 2012). We validated elevated protein expression of GM-CSF (*Csf2*) and T-bet (*Tbx21*) in Th17-SAA1- compared to Th17-TGF $\beta$ -differentiated cells (Figures S2F-H). SAA1 had no measurable effect in Th1 differentiation culture conditions (Figure S2I and S2J). Using CD4<sup>+</sup> T cells from *IL23r<sup>eGFP</sup>* reporter mice, we confirmed that SAA1, compared to TGF- $\beta$ , potently augmented IL-6-mediated *IL23r* induction

(Figure 2C). This translated into stronger STAT3 activation (pSTAT3) in SAA-differentiated T<sub>H</sub>17 cells upon exposure to IL-23 (Figure 2D). These *in vitro* studies thus suggest that SAA1 sensitizes T<sub>H</sub>17 cells to inflammatory cytokines and poises them for pathological responses.

### **SAA1s direct human Th17 cell differentiation and expression is associated with inflamed colon of IBD patients**

Multiple genes involved in the T<sub>H</sub>17 pathway have been described as contributing to inflammatory bowel disease (IBD) in humans. In particular, mutations in *IL23R* result in either protection or predisposition to disease (Abdollahi et al., 2016; Duerr et al., 2006; Gaffen et al., 2014). To determine if the SAA1s also contribute to human T<sub>H</sub>17 cell differentiation, we subjected naïve CD4<sup>+</sup> T cells from cord blood to T<sub>H</sub>17 differentiation conditions with and without inclusion of recombinant human SAA1 or SAA2. Both SAA1s substantially enhanced ROR $\gamma$ T expression and IL-17A production in cells differentiated from multiple donors (Figures 3A-C and S3A). As in mouse, SAA1s also promoted human T<sub>H</sub>17 cell differentiation in absence of TGF- $\beta$ , based on upregulated expression of the ROR $\gamma$ T-dependent chemokine receptor CCR6 (Figure S3B and S3C). Increased *SAA1/2* gene expression in inflamed tissues and elevated SAA serum concentrations have been reported in IBD patients with UC and CD (Lloyd-Price et al., 2019; Tang et al., 2017; Yarur et al., 2017). Using an antibody recognizing SAA1 and SAA2, we observed that SAA was prominently expressed by epithelia and lamina propria cells of inflamed but not non-inflamed adjacent regions of biopsies from UC patients (Figure 3D, 3E and S3D). Further, in a single cell RNAseq dataset of the terminal ileum lamina propria (Martin et al., 2018), there was selective heterogeneous expression of *SAA1* and *SAA2* in activated, *podoplanin*<sup>+</sup> (*PDPN*) fibroblast-like cells isolated from inflamed lesions of CD patients (Figure 3F). Of note, this population was part



of a highly pathogenic module characterized by an inflammatory mononuclear phagocyte (MNP)-associated cellular response organized around IgG plasma cells, inflammatory MNP, activated T and stromal cells (GIMATS) that could predict resistance to anti-TNF $\alpha$  therapy (Martin et al., 2018) and is reminiscent of the oncostatin M receptor-expressing PDPN<sup>+</sup> population identified by Powrie and colleagues that also correlated with IBD severity and resistance to anti-TNF $\alpha$  therapy (West et al., 2017).

### **SAAs are required for differentiation and pathogenicity of colitogenic T<sub>H</sub>17 cells**

The shared ability of mouse and human SAAs to propel *in vitro* T<sub>H</sub>17 cell differentiation, and the observation that SAA production was elevated in inflamed lesions in IBD patients, compelled us to examine whether SAAs exert important functions in mouse colitis models. We generated *Saa* isotype 1, 2, 3 triple-deficient (SAA<sup>TKO</sup>) mice and colonized these and WT littermates with *Helicobacter hepaticus* (*H. hepaticus*) (Xu et al., 2018). We then transferred into these mice naïve *H. hepaticus*-specific TCR transgenic (HH7-2Tg) CD4<sup>+</sup> T cells and tracked the anti-bacterial T cell response (Xu et al., 2018) (Figure 4A and Figure S4A). Previously, we showed that T cells primed by *H. hepaticus* in WT animals differentiate into predominantly ROR $\gamma$ <sup>+</sup>Foxp3<sup>+</sup> induced regulatory T cells (iTreg) or Bcl6<sup>+</sup> follicular helper cells (T<sub>FH</sub>) (Xu et al., 2018). In this regard, examination of HH7-2Tg cells in the colonic lamina propria 2 weeks post-adoptive transfer revealed that *H. hepaticus* elicited similar numbers and frequencies of HH7-2Tg iTreg and T<sub>FH</sub> cells in healthy SAA<sup>TKO</sup> and WT littermate animals, indicating that SAAs did not affect T cell differentiation at steady state (Figures S4B-C). Using the same scheme, we subjected animals to continuous IL-10RA blockade, which subverts the function and maintenance of iTreg cells, resulting in expansion of cognate pathogenic T<sub>H</sub>17 cells and colitis (Kullberg et al., 2006; Xu et

al., 2018) (Figure 4A). Sustained IL-10RA blockade resulted in abundant SAA1/2 in the serum of WT mice and upregulation of *Saa1-3* transcripts in the proximal colon, specifically, *Saa1* and *Saa2* in epithelium and *Saa3* in monocytes/macrophages and dendritic cells (Figures 4B-D and Figures S4D and S4E). By contrast, in the terminal ileum of the same mice, which were also colonized with SFB, only transcripts for *Saa1* and *Saa2* were detected and there was little change upon IL-10RA blockade (Figure 4D and Figure S4F).

We then assessed the HH7-2Tg cell phenotype in the mesenteric lymph nodes (mLN) of WT and SAA<sup>TKO</sup> recipients at 5d after transfer. We observed reduced numbers of ROR $\gamma$ <sup>+</sup> T<sub>H</sub>17 cells in SAA<sup>TKO</sup> mice compared to WT littermates, in which T-bet upregulation was also markedly decreased (Figure 4E and 4F). Phenotypic analysis of HH7-2Tg cells in the colon lamina propria at two weeks after transfer revealed striking reductions in both proportion and absolute number of HH7-2Tg T<sub>H</sub>1 and T<sub>H</sub>17 cells, corresponding to profoundly decreased IFN- $\gamma$ <sup>+</sup>, IL-17A<sup>+</sup>, and IFN- $\gamma$ <sup>+</sup>IL-17A<sup>+</sup> cells in SAA<sup>TKO</sup> recipients (Figures 4G-I). Notably, previous IL-17A fate mapping experiments performed in mice treated with anti-IL-10RA during *H. hepaticus* infection (Morrison et al., 2013), and other pathogenic T<sub>H</sub>17 responses, such as experimental autoimmune encephalomyelitis (EAE), demonstrated that a significant proportion of IFN $\gamma$ <sup>+</sup> cells previously expressed or descended from cells that expressed the T<sub>H</sub>17 program (Harbour et al., 2015; Hirota et al., 2011). Therefore, reduced numbers of HH7-2Tg IFN- $\gamma$ <sup>+</sup> cells in SAA<sup>TKO</sup> mice could be explained, at least in part, by the effect of SAAs on T<sub>H</sub>17 cells. Despite lower T<sub>H</sub>1 and T<sub>H</sub>17 responses in SAA<sup>TKO</sup> mice, we observed a similar reduction in both the number and frequency of HH7-2Tg T<sub>reg</sub> cells in both mutant and WT recipients after  $\alpha$ IL10RA blockade (Figure 4J and Figure S4G). In accord with their reduced pathogenic T<sub>H</sub> response, SAA<sup>TKO</sup> mice exhibited significantly attenuated histological features of colitis compared to WT littermates following

chronic IL-10RA blockade (Figure 4K). Altogether, these findings argue that the SAAs have a major role in the induction of pathogenic T<sub>H</sub>17 responses both *in vitro* and *in vivo*, and thus contribute to exacerbated colonic inflammation.

### **Distinct sources of SAA promote experimental autoimmune encephalomyelitis at discrete stages of disease**

To address the impact of SAAs on another T<sub>H</sub>17-dependent autoimmune disease model, we immunized mice with myelin oligodendrocyte glycoprotein (MOG) to induce EAE. SAA<sup>TKO</sup> mice displayed delayed EAE onset and significantly milder disease compared to WT littermates, and had reduced T<sub>H</sub>17 cell accumulation in the central nervous system (CNS) (Figures 5A-C). Following MOG immunization, transcription of *Saa1* and *Saa2*, but not *Saa3*, increased in the liver, which corresponded to high concentrations of SAA1/2 in the serum beginning at the preclinical stage of disease (Figures 5D and S5A). At the peak of disease, we also detected SAA1/2 in efferent lymph, though not in the CNS (Figure S5B-C). In contrast, *Saa3* was the only *Saa* induced in the CNS during EAE, and was expressed by both microglia and monocyte-derived macrophages, particularly at the peak of disease (Figures 5E and S5D-F). Immunofluorescence staining also revealed colocalization of SAA3 with IBA1, a specific marker of microglia and monocyte-derived macrophages (Ajami et al., 2011) (Figures 5F and S5G). Thus, SAAs are broadly induced during EAE; however, their temporal and spatial sequestration suggests that they differentially influence the fate of pathogenic T<sub>H</sub>17 responses.

To distinguish the role of liver- from CNS-derived SAAs, we compared the course of EAE in SAA1 and SAA2 double-deficient (SAA<sup>DKO</sup>) and SAA3-deficient (SAA<sup>3KO</sup>) mice, respectively. Like SAA<sup>TKO</sup> mice, SAA<sup>DKO</sup> mice exhibited delayed disease onset and milder symptoms than WT

littermates (Figure 5G). SAA<sup>3KO</sup> mice, on the other hand, did not exhibit delayed disease onset; however, the magnitude of disease, particularly during the chronic stage of EAE, tapered faster in these animals than in WT littermates (Figure 5J). T<sub>H</sub>17 responses were also in striking concordance with these phenotypes, such that T<sub>H</sub>17 cell differentiation was impaired in the draining lymph nodes of SAA<sup>DKO</sup> mice (Figures 5H and 5I), while the T<sub>H</sub>17 response in the CNS of SAA<sup>3KO</sup> mice was not sustained during EAE (Figure 5K). Taken together, these findings argue that systemic SAA1 and SAA2 exert functions early in EAE pathogenesis, while SAA3 functions subsequently to sustain inflammation locally in the CNS.

### **Local SAA expression fuels pathogenicity of activated T<sub>H</sub>17 cells**

To further explore the distinct functions of SAAs in EAE pathogenesis, we employed an adoptive T cell transfer model of EAE in concert with either loss- or gain-of-function of the SAAs. We differentiated T<sub>H</sub>17-TGFβ cells *in vitro* from naive MOG peptide-specific 2D2 TCR transgenic T cells. When supplemented with IL-23 (T<sub>H</sub>17-IL-23 condition), these cells readily induce EAE in recipient mice (Lee et al., 2012). Following adoptive transfer of T<sub>H</sub>17-IL-23 cells into WT or SAA<sup>DKO</sup> mice, all recipients developed EAE with similar kinetics and severity (Figures 6A-C). Moreover, the numbers of IL-17A<sup>+</sup> 2D2 cells recovered from the CNS were indistinguishable between the two sets of recipients (Figure 6D). In contrast, when T<sub>H</sub>17-IL-23 2D2 cells were transferred into SAA<sup>3KO</sup> recipients, the majority did not develop EAE, and those that did exhibited significantly milder symptoms than WT littermates (Mean max score: WT = 5.9, SAA<sup>3KO</sup> = 2.3) (Figures 6E-H). Underscoring this impaired response was a significant reduction in the number of 2D2 cells infiltrating the CNS of SAA<sup>3KO</sup> mice at the peak of disease, with lower frequencies of T<sub>H</sub>17 cells and fewer IL-17A<sup>+</sup> cells among those (Figures S6A-C). These results suggest that

SAA3, produced by microglia and monocytes during EAE, engages a feed-forward loop that fuels the  $T_{H17}$  niche in inflamed tissue. Of note, although the combination of SAA3 with IL-6 had a much less striking effect than SAA1 and IL-6 on *in vitro*  $T_{H17}$  differentiation, in other  $T_{H17}$  culture settings, such as  $T_{H17}$ -TGF $\beta$  or in combination with IL-6, IL-1 $\beta$ , and IL-23, SAA3 potentiated  $T_{H17}$  differentiation to a similar degree as SAA1. These findings indicate that SAA3 can also signal directly in T cells (Figures S6D-G).

We next sought to probe the effect of SAAs on  $T_{H17}$  responses in the absence of other inflammatory cues. Therefore, we generated mice capable of over-expressing SAA1, by placing the coding sequence of *Saa1* downstream of a *LoxP*-flanked STOP cassette in the *Rosa26* locus (*R26<sup>Saa1</sup>*) (Figure S6H). When these mice expressed a *Cre* transgene under control of albumin (*Alb-Cre<sup>Tg</sup>*), they exhibited high concentrations of SAA1 in serum, due to constitutive production in liver. Other  $T_{H17}$  mediators, such as IL-6, were not detected (Figure S6I). Consistent with the ability of SAAs to promote  $T_{H17}$  cell differentiation *in vitro*, we found that numbers of  $T_{H17}$ , but not  $T_{H1}$  cells, were increased in secondary lymphoid tissue of *Alb-Cre<sup>Tg</sup>;Rosa26<sup>Saa1</sup>* (liver<sup>SAA1</sup>) mice (Figures S6J-M). To investigate the consequence of systemically raised SAA1 levels, we transferred 2D2  $T_{H17}$ -TGF $\beta$  cells that, under normal settings, fail to efficiently evoke a pathogenic response (Figure 6I). Accordingly, few of the control *Alb-Cre<sup>Tg</sup>* recipient mice developed disease (2/11), but the majority of littermate liver<sup>SAA1</sup> mice (10/13) developed EAE (Figure 6J). In addition, we recovered significantly higher numbers of ROR $\gamma$ <sup>+</sup>  $T_{H17}$ -2D2 cells, of which a greater proportion expressed IL-17A, from the CNS of liver<sup>SAA1</sup> recipients (Figures 6K and 6L). Therefore, serum SAA1/2 elevation supplants IL-23 preconditioning of  $T_{H17}$ -TGF $\beta$  cells, consistent with a direct *in vivo* effect of SAAs on the myelin-specific T cells and suggesting that SAAs and IL-23 have convergent functions in  $T_{H17}$  autoimmune pathogenesis.

## Discussion

$T_H17$  cells exert beneficial or detrimental functions under context-specific conditions at multiple body sites. In response to colonization of the small intestine with SFB, a microbe that enforces protection from enteropathogenic bacteria,  $T_H17$  cells promote strengthening of the epithelial barrier. Production of cytokines by SFB-specific  $T_H17$  cells is enhanced by local epithelial cell-derived SAA1 and SAA2, as a consequence of a signaling circuit that involves myeloid cell-derived IL-23 inducing ILC3 to produce IL-22 that, in turn, stimulates the epithelial cells. These observations prompted us to propose that  $T_H17$  cell effector functions are acquired in two steps, with SAA-independent priming of naïve SFB-specific T cells in the draining mesenteric lymph nodes, accompanied by their differentiation into  $ROR\gamma^t^+$  poised  $T_H17$  cells, followed by migration to the lamina propria, where exposure to the SAAs results in up-regulation of IL-17 cytokines. The protective function of this type of  $T_H17$  cells is highlighted by the findings that blockade of IL-17A promoted epithelial barrier permeability, exacerbating DSS-induced colitis (Lee et al., 2015), and by clinical trials targeting IL-17A or IL-17RA, that failed to ameliorate CD and resulted in higher rates of adverse events or disease worsening (Hueber et al., 2012; Targan et al., 2016). In light of the previous results, which suggested that SAAs act on poised  $ROR\gamma^t^+$  cells, we were surprised to find that SAAs act directly on naïve T cells, instructing their differentiation into  $T_H17$  cells in combination with the STAT3-activating cytokine IL-6. This SAA activity was critical for pathogenicity of  $T_H17$  cells in models of colitis and EAE, and likely reflects a program of differentiation distinct from that mediated by other combinations of cytokines both *in vitro* and *in vivo*.

### **SAA-dependent differentiation of pathogenic T<sub>H</sub>17 cells.**

*In vitro*, SAA1 induced a pathogenic T<sub>H</sub>17 program independently of TGF- $\beta$  signaling. TGF- $\beta$  has been shown to be dispensable for T<sub>H</sub>17 differentiation under some conditions both *in vitro* and *in vivo*. For example, genetic repression of T<sub>H</sub>1 and T<sub>H</sub>2 cell differentiation was shown to relieve the requirement for TGF- $\beta$  in IL-6-mediated T<sub>H</sub>17 induction and, indeed, T<sub>H</sub>17 cells can be differentiated *in vitro* by a combination of IL-6, IL-23 and IL-1 $\beta$  (Das et al., 2009; Ghoreschi et al., 2010). Recently, Wan and colleagues revealed that SKI, via SMAD4, transcriptionally repressed *Rorc* and that TGF- $\beta$  signaling degraded or modified SKI to relieve the inhibition and promote T<sub>H</sub>17 cell differentiation in the presence of IL-6 (Zhang et al., 2017). Our studies indicate that SAAs function independently of STAT3 or the SMAD transcription factors, and that they do not regulate SKI degradation (unpublished). Elucidation of the receptor(s) and signaling pathway(s) engaged by the SAAs will be needed to provide insight into how they promote distinct differentiation programs for homeostatic and pathogenic T<sub>H</sub>17 cells.

### **Context-dependent regulation of Th17 cell differentiation by SAAs**

The requirement for inducible SAAs in mouse inflammatory disease models and the selective expression of SAAs in inflamed tissue from human IBD patients support a role for these secreted proteins in T<sub>H</sub>17-mediated inflammatory disease. Our results reveal that the effects of SAAs are subject to the context of T<sub>H</sub>17 cell differentiation. In SFB-mediated T<sub>H</sub>17 induction, the SAAs do not contribute to priming, but rather amplify the effector functions of T<sub>H</sub>17 cells in SFB-colonized regions. These “homeostatic” T<sub>H</sub>17 cells do not become pathogenic even under pro-colitogenic conditions, as occurs upon blockade of the IL-10 signaling pathway (Xu et al., 2018). By contrast, in the absence of IL-10, *H. hepaticus*-specific CD4<sup>+</sup> T cells no longer adopt a T<sub>reg</sub> cell fate, but

instead differentiate into colitis-inducing pathogenic T<sub>H</sub>17 cells with a transcriptome that is strikingly different from that of homeostatic T<sub>H</sub>17 cells (Chai et al., 2017; Xu et al., 2018). The SAAs support this *H. hepaticus*-driven colitis, inducing pathogenic features exemplified by T-bet expression during T<sub>H</sub>17 priming and sensitization of the T cells to cytokines essential for T<sub>H</sub>17-mediated autoimmune pathogenesis, such as IL-23 and IL-1 $\beta$ .

The SAA proteins thus have critical roles during T<sub>H</sub>17 cell induction and potentiation of effector functions. It remains unclear what distinguishes these properties that are associated with pathogenic and homeostatic T<sub>H</sub>17 cells. It may be a feature of the microenvironment of T cell priming, e.g. in lymph nodes draining a healthy small intestine versus an inflamed large intestine, and of the other cytokines that are present; of the concentration of SAAs that are encountered; or of the cell types that produce the SAAs. A better understanding of how SAAs participate in T<sub>H</sub>17 cell differentiation may permit selective targeting of potentially harmful T<sub>H</sub>17 cells while sparing the beneficial cells.

### **SAAs in mouse and human**

In mouse inflammatory models, we found that SAA1 and SAA2 were prominently produced by hepatocytes after systemic immune activation and by intestinal epithelial cells adjacent to colonizing bacteria, whereas SAA3 was produced by myeloid cells in both the gut and CNS. We discovered that SAA3 advanced a feed-forward loop that sustained murine T<sub>H</sub>17 responses and drove EAE pathology. Although *SAA3* does not encode a full protein in humans, there are reports of inducible SAA1/2 expression by macrophages in inflamed tissues of multiple chronic inflammatory diseases (De Buck et al., 2016; Meek et al., 1994; Ye and Sun, 2015). Moreover, we and others found that tissue-derived SAA1 and SAA2 are not limited to the epithelium during



inflammation. Fibroblast-like synoviocytes were shown to express and secrete SAA1/2 in rheumatoid arthritis patients (O'Hara et al., 2000), and we observed that activated fibroblasts in the inflamed parenchyma of CD patients expressed *SAA1* and *SAA2*. Given our findings and links between activated fibroblast abundance and primary anti-TNF $\alpha$  resistance in Crohn's disease (West et al., 2017), it will be important to investigate whether SAAs play a causal role in this disease setting. Taken together, these findings suggest that the evolution of *SAA3* into a pseudogene in humans may be ascribed to functional redundancies with *SAA1* and *SAA2*. However, we cannot rule out that SAA3 has a unique role in the mouse, particularly since it is considerably less effective than SAA1 and SAA2 in directing T<sub>H</sub>17 cell differentiation in the absence of TGF- $\beta$ .

### **SAA signaling to T<sub>H</sub>17 cells in inflammatory disease**

Although our results clearly demonstrate that SAAs interact directly with T cells and promote T<sub>H</sub>17 cell programming and response amplification, these secreted proteins also have effects on multiple other cell types. Several receptors have been described for the SAAs, including TLR2 and FPRL-1 (Ye and Sun, 2015), but inactivation of the genes for these candidate molecules had no effect on SAA1-induced Th17 cell differentiation (J-Y.L., unpublished). While we cannot rule out SAA functions through other cell types during in vivo pathogenic T<sub>H</sub>17 cell differentiation, our results strongly support a substantial contribution through direct interaction with T cells. In particular, the finding that enforced expression of SAA1 by hepatocytes overcame non-pathogenic T<sub>H</sub>17-TGF $\beta$  priming to promote EAE is consistent with sensitization of the T cells to render them more responsive to IL-23 and potentially other pro-inflammatory cytokines.

The general importance of the SAAs in maintaining the pathogenic programming of T<sub>H</sub>17 cells was underscored by the reductions in colitis and EAE observed when tissue SAAs were absent in mice. Elucidation of the receptor(s) for SAAs on T cells will resolve the relative contributions of direct and indirect signaling cues delivered by SAAs to T cells. However, the combined results showing that SAAs provide a previously unappreciated means for inducing inflammatory T<sub>H</sub>17 cells and that adoptively transferred antigen-specific T cells confer pathogenicity based on their response to SAAs argue strongly that the disease phenotypes were due, at least in part, to the breach of direct SAA interactions with T cells. Together, these findings suggest that a better understanding of T<sub>H</sub>17 cell responses modulated by SAAs will facilitate therapeutic efforts to ameliorate inflammatory diseases and enforce epithelial barrier integrity.

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**Author Contributions:** J.-Y.L. and D.R.L. conceived the project; J.-Y.L. and J.A.H. performed the experiments; L.W. and H.M.S performed RNA sequencing of microglia and monocytes from EAE spinal cords; T.N. generated and purified recombinant SAA proteins; L.K. performed bioinformatic analyses; H.H.N. performed blinded histology scoring on murine colitis sections; W.-Y.L. and D.L. assisted with experiments.; S.T.Y. and K.M.K. performed tissue immunofluorescence staining; A.H., P.L., D.H., J.C.M, E.K., and M.M. provided human IBD biopsies and bioinformatics resources; J.-Y.L., J.A.H and D.R.L. wrote the manuscript.

**Declaration of Interests:** D.R.L. consults for and has equity interest in Chemocentryx, Vedanta Biosciences, and Pfizer Pharmaceuticals.

## Figure Legends

**Figure 1. SAAs act directly on mouse T cells to induce  $T_H17$  cell differentiation *in vitro* in absence of TGF- $\beta$ .**

**(A)** Experimental scheme for *in vitro* differentiation of naïve CD4<sup>+</sup> or CD8<sup>+</sup> T cells.

**(B and C)** Flow cytometric analysis of IL-17A and IL-17F expression **(B)** and summary of IL-17A frequency **(C)** among re-stimulated cells. Summary of 3 experiments, with n = 11. Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*\*\*\*p < 0.0001.

**(D)** ROR $\gamma$ t expression in T<sub>H</sub>17 cells. Geometric mean fluorescence intensities (gMFI) are included in parentheses. Representative data of n > 10 experiments.

**(E)** Immunoblotting for SMAD2/3 phosphorylation (pSMAD2/3) of primed CD4<sup>+</sup> T cells upon rmSAA1 (10 $\mu$ g/ml) or TGF- $\beta$  (1ng/ml) treatment for indicated times. Total SMAD2/3 is shown as a loading control.

See also Figure S1.

**Figure 2. SAA1 elicits a pathogenic T<sub>H</sub>17 program *in vitro*.**

**(A and B)** RNA sequencing analysis of temporal gene expression in IL6 +  $\alpha$ TGF- $\beta$  + SAA1 (T<sub>H</sub>17-SAA1, n = 3) and IL6 + TGF- $\beta$  (T<sub>H</sub>17-TGF $\beta$ , n = 3) differentiated CD4<sup>+</sup> T cells. **(A)** MA plot depicts differentially expressed (DE) genes of T<sub>H</sub>17-SAA1 versus T<sub>H</sub>17-TGF $\beta$  cells at 48h *in vitro* polarization. Colored dots are significant DE genes. Red dots or green dots highlight pathogenic or non-pathogenic T<sub>H</sub>17 signature genes, respectively. DE genes were calculated in DESeq2 using the Wald test with Benjamini-Hochberg correction to determine the false discovery rate (FDR < 0.01). **(B)** GSEA plots of pathogenic (top) or non-pathogenic (bottom) T<sub>H</sub>17 signatures amongst T<sub>H</sub>17-SAA1 and T<sub>H</sub>17-TGF $\beta$  gene sets. NES, normalized enrichment score.

**(C)** Summary of eGFP expression in T cells from *I123 $\gamma$ <sup>eGFP</sup>* mice following indicated condition and time of polarization. The gMFI of non-reporter, wild-type cells was subtracted from gMFI of eGFP to obtain the normalized gMFI. Representative data of two independent experiments. Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*p < 0.05, \*\*p < 0.01, and \*\*\*\*p < 0.0001.

**(D)** Amount of Y705 phosphorylated STAT3 following IL-23 treatment (10ng/ml) of T<sub>H</sub>17 cells differentiated under different conditions for indicated times. Total STAT3 is shown as a loading control.

See also Figure S2.

**Figure 3. Human SAA expression in inflamed tissue and induction of T<sub>H</sub>17 cell differentiation.**

**(A-C)** Naïve human CD4<sup>+</sup> T cells were isolated from cord blood and differentiated for 6 days in T<sub>H</sub>17 polarizing conditions ± recombinant human (rh) SAA1 or rhSAA2. **(A)** Summary of IL-17A production from in vitro polarized human T<sub>H</sub>17 cells. Connecting lines signify cells from the same donor. **(B)** Stacked histogram illustrates representative RORγT expression using the indicated polarizing conditions. **(C)** Summary of RORγT gMFI. Summary of 2 experiments with n = 6 donors for rhSAA1 and with n = 3 donors for rhSAA2.

**(D and E)** Representative confocal images (D) and quantification of SAA1/2 by fluorescence integrated optical density (FIOD) levels (E) show prominent SAA expression in biopsies of inflamed tissue from ulcerative colitis (UC, n = 7) patients. Panels from left to right: Healthy (n = 2), UC-uninflamed, and UC-inflamed. Scale bar corresponds to 50µm. SAA1/2 (green), EPCAM (red) and nucleus (DraQ7; blue).

**(F)** Violin plots showing the log<sub>2</sub> normalized UMI of SAA1 and SAA2 genes in the fibroblast cluster associated with the GIMATS module (IgG plasma cells, inflammatory MNP, activated T and stromal cells).

Statistics were calculated using the paired two-tailed Student's t-test. \*\*p < 0.01, \*\*\*\*p < 0.0001.

See also Figure S3.

**Figure 4. SAAs drive pathogenic T<sub>H</sub> cell responses in IL-10 deficiency-dependent colitis.**

**(A)** Experimental scheme to examine HH7-2tg cells in SAA1/2/3 triple knock-out (SAA<sup>TKO</sup>) and WT littermate recipient mice colonized with *H. hepaticus* ± IL-10RA blockade (αIL10RA).

**(B)** Serum concentrations of SAA1/2 of recipient mice at day 21 post *H. hepaticus* colonization.

**(C and D)** Normalized expression of *Saa1/2* in proximal colon **(C)** and *Saa3* in ileum and colon **(D)** of recipient mice at day 21 post *H. hepaticus* colonization.

**(E and F)** Characterization of HH7-2tg donor-derived cells 5 days post-adoptive transfer in mesenteric lymph nodes of recipient SAA<sup>TKO</sup> (blue boxes, n = 8) and WT (red boxes, n = 10) littermates injected with αIL10RA. Number of RORγt expressing HH7-2 cells **(E)** and T-bet gMFI level in the RORγt<sup>+</sup> HH7-2 cells **(F)**.

**(G-J)** Characterization of HH7-2tg donor-derived cells two weeks post-adoptive transfer in colon lamina propria of recipient mice injected with αIL10RA. Summary of 2 experiments with SAA<sup>TKO</sup> (blue boxes, n = 8) and WT (red boxes, n = 10) littermates. Frequency **(G)** and number of the indicated T<sub>H</sub> cells based on transcription factor expression **(H)** or cytokine expression after restimulation **(I)**. Numbers of Foxp3<sup>+</sup> iTreg cells in isotype-treated recipients (hollow boxes) versus αIL10RA-treated recipients **(J)**.

**(K)** Representative H&E staining (left) and summary of histology scores (right) of colon sections harvested from mice with or without *H. hepaticus* colonization, following twelve weeks of αIL10RA injection. Summary of two separate experiments is shown. Uncolonized mice (open boxes): WT + αIL10RA (red, n = 5), SAA<sup>TKO</sup> + αIL10RA (blue, n = 5); or colonized with *H. hepaticus* (closed boxes): WT + αIL10RA (red, n = 10), SAA<sup>TKO</sup> + αIL10RA (blue, n = 13).

**(B-F and K)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

**(G-J)** Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*p < 0.05, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

See also Figure S4.

**Figure 5. Distinct sources of SAAs promote and sustain autoimmune encephalomyelitis.**

**(A)** Mean EAE scores of myelin oligodendrocyte glycoprotein (MOG)-immunized SAA<sup>TKO</sup> (blue boxes, n = 18) and WT littermate mice (red boxes, n = 22). Summary of 3 experiments.

**(B and C)** Number of CD4<sup>+</sup> T cells **(B)** and frequency of ROR $\gamma$ t<sup>+</sup> T<sub>H</sub>17 cells among Foxp3<sup>Neg</sup> CD44<sup>hi</sup> CD4<sup>+</sup> T cells **(C)** in the CNS of mice at the indicated stage of EAE. day 10 = pre-clinical, day 15 = acute, day 32 = chronic. Pre-clinical, (SAA<sup>TKO</sup> = 6, WT = 9), acute, (SAA<sup>TKO</sup> = 4, WT = 6) and Chronic, (SAA<sup>TKO</sup> = 10, WT = 11). Data combined two, two, and three experiments for the pre-clinical, acute, and chronic stages of disease, respectively.

**(D)** Longitudinal mean serum concentrations of SAA1/2 in MOG-immunized mice. Error bars denote the s.d.

**(E)** Normalized relative expression of *Saa3* in CNS of MOG-immunized mice, measured by qPCR. Spinal cords were isolated at days 0, 10, 18, and 38 post-immunization. For WT, SAA<sup>DKO</sup>, and SAA<sup>TKO</sup>, the number of samples at each time point were 13, 7, and 13 (day 0); 5, 6, and 8 (day 10); 17, 11, and 10 (day 18); and 10, 3, 6 (day 38).

**(F)** Representative confocal image of spinal cord cross section isolated from a WT mouse (n = 2) at the peak of EAE (score 4). IBA1 (red), SAA3 (green), and CD4 (aqua). White arrows pointed at yellow regions indicate IBA1/SAA3 colocalization.

**(G)** Mean EAE scores of MOG-immunized SAA1/2 DKO (SAA<sup>DKO</sup>, orange boxes, n = 17) and WT littermate mice (red boxes, n = 19). Summary of 4 experiments.

**(H and I)** Number of ROR $\gamma$ <sup>+</sup> T<sub>H</sub>17 cells among CD44<sup>hi</sup> effector/memory CD4<sup>+</sup> T cells isolated from draining lymph nodes (dLNs) **(H)** and spinal cord **(I)** of SAA<sup>DKO</sup> (n = 6) or WT (n = 9) littermates at day 10 post immunization.

**(J)** Mean EAE scores of MOG-immunized SAA3 KO (SAA<sup>3KO</sup>, purple boxes, n = 14) and WT littermate mice (red boxes, n = 16). Summary of 3 experiments.

**(K)** Number of ROR $\gamma$ <sup>+</sup> T<sub>H</sub>17 and Foxp3<sup>+</sup> Treg cells at pre-clinical (day 10) and chronic (day 32) stages of EAE. Summary of 3 experiments, with SAA<sup>3KO</sup> (n = 14) and WT (n = 16) littermates.

**(A-C, E, G, J, and K)** Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean  $\pm$  s.e.m **(A, G, and J)** or mean  $\pm$  s.d. **(B, C, E, H, I, and K)**. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

**(H and I)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

See also Figure S5.

## **Figure 6. SAA regulation of pathogenicity of *in vitro*-differentiated encephalitogenic T<sub>H</sub>17 cells**

**(A-D)** Examination of EAE development and the 2D2tg cell response in SAA<sup>DKO</sup> and WT littermate recipients of 2D2tg T<sub>H</sub>17-IL-23 cells. Summary of 2 experiments, with SAA<sup>DKO</sup> (orange,



n = 9) and WT (red, n = 10) mice. Experimental scheme (**A**), EAE incidence (**B**) and mean score (**C**), and number of 2D2tg IL17A<sup>+</sup> T<sub>H</sub>17 cells in spinal cords (**D**).

(**E-H**) Examination of EAE development and the 2D2tg cell response in SAA<sup>3KO</sup> and WT littermate recipients of 2D2tg T<sub>H</sub>17-IL-23 cells. Summary of 4 experiments, with SAA<sup>3KO</sup> (purple, n = 24) and WT (red, n = 24) mice. Experimental scheme (**E**), EAE incidence (**F**) and mean score (**G**), and maximal disease score (**H**).

(**I-L**) Examination of EAE development and the 2D2tg cell response in liver-specific SAA1tg (liver<sup>SAA1</sup>) and control Alb-Cre<sup>tg</sup> littermates following transfer of 2D2tg T<sub>H</sub>17-TGFβ cells. Summary of 2 experiments, with liver<sup>SAA1</sup> (red circles, n = 13) and Alb-Cre<sup>tg</sup> (white circles, n = 11) mice. Experimental scheme (**I**), incidence of EAE onset (**J**), number of 2D2tg RORγt<sup>+</sup> T<sub>H</sub>17 cells (**K**) and frequency of IL17A<sup>+</sup> cells amongst RORγt<sup>+</sup> T<sub>H</sub>17 cells in spinal cords at day 40 post-adoptive transfer (**L**).

(**B, F, and J**) Statistics were calculated by log-rank test using the Mantel-Cox method.

(**C and G**) Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean ± s.e.m. \*p < 0.05, \*\*p < 0.01, and \*\*\*\*p < 0.0001,

(**D, H, K, and L**) Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean ± s.d. ns = not significant, \*p < 0.05, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

See also Figure S6.

## Tables

**Table 1 -sgRNA and HDR donor sequence**

Oligonucleotide	Sequence	Note
Mouse <i>Saa3</i> guide	taaatacgaactactatagGATCTTTGGCTGTCAACTCCC	T7 promoter Linker

		<u>sgRNA</u>
HDR donor DNA template	CCACCCCACGAAATGGCATGCTCATCTTTGTTTTCTGGCC CTATAGCAGGATGAAGCCTTCCATTGCCATCATTCTTTGCA TCTTGATCTAGTAAAGCTTGTGACAGCCAAAGATGGG TCCAGTTCATGAAAGAAGCTGGTCAAGGTAGGCTTCAAGG ATGGGGGCTGGAGTTGTATTCTGAGTTTT	Premature stop codons

**Table 2 -Antibodies and staining conditions for mouse tissues**

Antibody	Species	Conc.	Clone	Catalog #	Lot #	Vendor
SAA1/2	Goat	1:300		AF2948	VWJ0417091	R&D Systems
SAA3	Rat	1:50		Ab231680	GR32218061	Abcam
Draq-7		1:1000		564904		R&D Systems
FC Block		1:200	93	101302	3255480	Biolegend
Donkey anti-goat 488		1:300		A11055	1827671	Life Technologies
Donkey anti-rat 594		1:300		A21209	1979379	Life Technologies
Iba-1	Rabbit	1:500		019-19741	LKH4161	Wako
Donkey anti-rabbit 647		1:300		A21447	1977345	Life Technologies
CD4-BV510	Rat	1:100	RM4-5	100559	B227810	Biolegend

**Table 3 -Antibodies and staining conditions for human tissues**

Antibody	Species	Conc.	Clone	Catalog #	Lot #	Vendor
SAA	Rabbit	1:500		Ab207445	GR270796-2	Abcam
SAA	Mouse	1:50	mc1	M0759	20059191	Dako
CD326-BV605	Mouse	1:100	9C4	324224	B251369	Biolegend
Draq-7		1:1000		564904		R&D Systems
Goat anti-Rabbit 488		1:300		A11070	1775509	Life Technologies
FC Block		1:200		14-9161-73	4350496	Invitrogen

## STAR Methods

## KEY RESOURCE TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Flow Cytometry: anti-mouse CD3 (17A2) AlexaFluor700	ThermoFisher	Cat. 56-0032
Flow Cytometry: anti-mouse CD4 (RM4-5) eFluor450	ThermoFisher	Cat. 48-0042
Flow Cytometry: anti-mouse CD11b (M1/70) PerCP-cy5.5	ThermoFisher	Cat. 45-0112
Flow Cytometry: anti-mouse CD11c (N418) PerCP-cy5.5	ThermoFisher	Cat. 45-0114
Flow Cytometry: anti-mouse CD14 (Sa2-8) FITC	ThermoFisher	Cat. 11-0141
Flow Cytometry: anti-mouse CD14 (Sa2-8) PerCP-cy5.5	ThermoFisher	Cat. 45-0141
Flow Cytometry: anti-mouse CD19 (1D3) PerCP-cy5.5	TONBO	Cat. 65-0193
Flow Cytometry: anti-mouse CD25 (PC61) PE-Cy7	TONBO	Cat. 60-0251
Flow Cytometry: anti-mouse CD44 (IM7) BV500	BD Bioscience	Cat. 563114
Flow Cytometry: anti-mouse CD45.1 (A20) BV650	BD Bioscience	Cat. 563754
Flow Cytometry: anti-mouse CD45.2 (104) APC-e780	ThermoFisher	Cat. 47-0454
Flow Cytometry: anti-mouse CD62L (MEL-14) APC	ThermoFisher	Cat. A14720
Flow Cytometry: anti-mouse TCR $\beta$ (H57-597) PerCP-cy5.5	ThermoFisher	Cat. 45-5961
Flow Cytometry: anti-mouse TCR $\beta$ (H57-597) BV711	BD Bioscience	Cat. 563135
Flow Cytometry: anti-mouse TCR V $\alpha$ 3.2 (RR3-16) FITC	ThermoFisher	Cat. 11-5799
Flow Cytometry: anti-mouse TCR V $\beta$ 6 (RR4-7) FITC	BD Bioscience	Cat. 553193
Flow Cytometry: anti-mouse MHCII (M5/114.15.2) PE	ThermoFisher	Cat. 12-5321
Flow Cytometry: anti-mouse MHCII (M5/114.15.2) PerCP-cy5.5	BD Bioscience	Cat. 562363
Flow Cytometry: anti-mouse NK1.1 (PK136) PerCP-cy5.5	ThermoFisher	Cat. 45-5941
Flow Cytometry: anti-mouse KLRG1 (2F1) PerCP-eFluor710	ThermoFisher	Cat. 46-5893
Flow Cytometry: anti-mouse Bcl6 (K112-91) BV421	BD Bioscience	Cat. 563363
Flow Cytometry: anti-mouse FoxP3 (FJK-16s) FITC	ThermoFisher	Cat. 53-5773
Flow Cytometry: anti-mouse ROR $\gamma$ t (B2D) PE	ThermoFisher	Cat. 12-6981
Flow Cytometry: anti-mouse ROR $\gamma$ t (Q31-378) BV421	BD Bioscience	Cat. 562894
Flow Cytometry: anti-mouse T-bet (eBio4B10) PE-cy7	ThermoFisher	Cat. 25-5825
Flow Cytometry: anti-mouse IL-17A (eBio17B7) eFluor660	ThermoFisher	Cat. 50-7177
Flow Cytometry: anti-mouse IL-17F (9D3.1C8) AlexaFluor488	Biologend	Cat. 517006
Flow Cytometry: anti-mouse GM-CSF (MP1-22E9) PE	ThermoFisher	Cat. 12-7331
Flow Cytometry: anti-mouse IFN $\gamma$ (XM61.2) eFluor450	ThermoFisher	Cat. 48-7311
Flow Cytometry: anti-human CD4 (RPA-T4) PacBlue	BD Pharmingen	Cat. 558116
Flow Cytometry: anti-human CD25 (2A3) PE	BD Pharmingen	Cat. 341009
Flow Cytometry: anti-human CD45RA (HI100) FITC	ThermoFisher	Cat. 11-0458
Flow Cytometry: anti-human CD45RO (UCHL1) APC	ThermoFisher	Cat. 17-0457
Flow Cytometry: anti-human CCR6 (11A9) BV421	BD Pharmingen	Cat. 565925
Flow Cytometry: anti-human HLA-DR (LN3) APC-eFluor780	ThermoFisher	Cat. 47-9956
Flow Cytometry: anti-human IL-17A (Bio64DEC17) PE	ThermoFisher	Cat. 12-7179
Flow Cytometry: anti-human IFN $\gamma$ (4S.B3) eFluor450	ThermoFisher	Cat. 48-7319
Flow Cytometry: anti-human ROR $\gamma$ t (Q21-559)	BD Pharmingen	Cat. 563081

IEC sorting: anti-mouse CD326 (G8-8) PeCY7	ThermoFisher	Cat. 11-5791
IEC sorting: anti-mouse CD326 (G8-8) FITC	ThermoFisher	Cat. 25-5791
IEC sorting: anti-mouse CD11b (M1/70) APC	ThermoFisher	Cat. 17-0112
IEC sorting: anti-mouse CD31 (390) APC	ThermoFisher	Cat. 17-0311
IEC sorting: anti-mouse TCRgd (eBioGL3) APC	ThermoFisher	Cat. 17-5711
IEC/APC sorting: anti-mouse CD3 (145-2C11) APC	TONBO	Cat. 20-0031
APC sorting: anti-mouse CD19 (eBio1D3) APC	ThermoFisher	Cat. 17-0193
APC sorting: anti-mouse NK1.1 (PK136) APC	ThermoFisher	Cat. 17-5941
APC sorting: anti-mouse CD11c (N418) PeCY7	ThermoFisher	Cat. 17-0114
APC sorting: anti-mouse CD14 (Sa2-8) FITC	ThermoFisher	Cat. 11-0141
APC sorting: anti-mouse MHCII (M5/114.15.2) PE	ThermoFisher	Cat. 12-5321
Immunoblotting: anti-Smad2/3 (D7G7) XP® Rabbit	Cell Signaling technology	Cat. 8685
Immunoblotting: anti-Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit	Cell Signaling technology	Cat. 8828
Immunoblotting: anti-p38 MAPK	Cell Signaling technology	Cat. 9212
Immunoblotting: anti-Phospho-p38 MAPK (Thr180/Tyr182)	Cell Signaling technology	Cat. 9211
Immunoblotting: anti-β-Tubulin	Cell Signaling technology	Cat. 2146
Immunoblotting: anti- mouse Serum Amyloid A1/A2	R&D systems	Cat. AF2948
<i>In vitro</i> T cell differentiation: anti-hamster IgGs	MP Biomedicals Catalog	Cat. 55398
<i>In vitro</i> T cell differentiation: anti-mouse CD3ε (145-2C11)	BioXCell	Cat. BP0001-1
<i>In vitro</i> T cell differentiation: anti-mouse CD28 (37.51)	BioXCell	Cat. BE0015-1
<i>In vitro</i> T cell differentiation: anti-mouse IL-4 (11B11)	BioXCell	Cat. BP0045
<i>In vitro</i> T cell differentiation: anti-mouse IFNγ (XMG1.2)	BioXCell	Cat. BP0055
<i>In vitro</i> T cell differentiation: anti-TGFβ (1D11.16.8)	BioXCell	Cat. BP0057
<i>In vitro</i> T cell differentiation: anti-human CD3ε (OKT-3)	BioXCell	Cat. BE0001-2
<i>In vitro</i> T cell differentiation: anti-human IL-4 (8D4-8)	ThermoFisher	Cat. 14-7049-85
<i>In vitro</i> T cell differentiation: anti-human IFNγ (MD-1)	ThermoFisher	Cat. 14-7317-85
Dynabeads™ Human T-Activator CD3/CD28 for T Cell Expansion and Activation	Thermo Fisher Scientific	Cat. 11131D
<i>In vivo</i> IL-10R blockade: anti-mouse IL10R (1B1.3A)	BioXCell	Cat. BE0050
Immunohistochemistry: anti-mouse SAA1/2	R&D Systems	Cat. AF2948
Immunohistochemistry: anti-mouse SAA3	Abcam	Cat. Ab231680
Immunohistochemistry: anti-mouse Iba-1	Wako	Cat. 019-19741
Immunohistochemistry: anti-mouse CD4-BV510	Biologend	Cat. 100559
Immunohistochemistry: anti-human SAA1/2	Abcam	Cat. Ab207445
Immunohistochemistry: anti-human SAA	Dako	Cat. M0759
Immunohistochemistry: anti-human CD326-BV605	Biologend	Cat. 324224
Immunohistochemistry: Donkey anti-goat 488	Life Technologies	Cat. A11055
Immunohistochemistry: Donkey anti-rat 594	Life Technologies	Cat. A21209
Immunohistochemistry: Donkey anti-rabbit 647	Life Technologies	Cat. A21447
Immunohistochemistry: Goat anti-Rabbit 488	Life Technologies	Cat. A11070
Bacterial and Virus Strains		

<i>Helicobacter Hepaticus</i>	Gift from James Fox (MIT)	N/A
Biological Samples		
Fetal Bovine Serum	Atlanta Biologicals	Cat. S11195 Lot. A16003
Chemicals, Peptides, and Recombinant Proteins		
EDTA, 0.5M, pH8.0	Ambion	Cat. AM9260G
Collagenase D	Roche	Cat. 11088882001
Dispase	Worthington	Cat. LS02104
DNase I	Sigma	Cat. DN25
DTT	Sigma	Cat. D9779
Percoll	GE Healthcare Life Sciences	Cat. 45001747
Ficoll-Paque Premium	GE Healthcare Life Sciences	Cat. 17-5442-02
2-Mercaptoethanol (BME)	ThermoFisher	Cat. 21985023
Phorbol Myristate Acetate	Sigma	Cat. P1585
Ionomycin	Sigma	Cat. I0634
Recombinant Human IL-2	NIH AIDS Reagent Program	Cat. 136
Recombinant Human IL-23 Protein	R&D systems	Cat. 1290-IL
Recombinant Human IL-1 beta/IL-1F2 Protein	R&D systems	Cat. 201-LB
Recombinant Human TGF $\beta$ Protein	Peprtech	Cat. 100-21-10ug
Recombinant Human Serum Amyloid A1 Protein	CEPTER Biopartners	N/A
Recombinant Human Serum Amyloid A2 Protein	CEPTER Biopartners	N/A
Recombinant Mouse Serum Amyloid A1 Protein	R&D systems	Cat. 2948-SA
Recombinant Mouse Serum Amyloid A3 Protein	CEPTER Biopartners	N/A
Recombinant Mouse IL-6 Protein	R&D systems	Cat. 406-ML-200/CF
Recombinant Mouse IL-1 beta/IL-1F2 Protein	R&D systems	Cat. 401-ML
Recombinant Mouse IL-23 Protein	R&D systems	Cat. 1887-ML
Recombinant Mouse IL-12 Protein	R&D systems	Cat. 419-ML
Critical Commercial Assays		
SAA Mouse ELISA Kit	ThermoFisher	Cat. KMA0021
IL-6 Mouse ELISA Kit	ThermoFisher	Cat. KMC0061
LIVE/DEAD® Fixable Blue Dead Cell Stain Kit	ThermoFisher	Cat. L34961
CountBright™ absolute counting beads	ThermoFisher	Cat. C36950
BD Cytotfix/Cytoperm Plus Fixation/Permeabilization Solution Kit	BD Biosciences	Cat. 554714
eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set	ThermoFisher	Cat. 00-5523-00
LightCycler® 480 SYBR Green I Master	Roche Life Science	Cat. 04707516001
SuperScript™ III First-Strand Synthesis System	ThermoFisher	Cat. 18080051
RNeasy Mini Kit	QIAGEN	Cat. 74104
RNeasy MinElute Cleanup Kit	QIAGEN	Cat. 74204

RNase-Free DNase Set	QIAGEN	Cat. 79254
TRIzol™ Reagent	ThermoFisher	Cat. 15596026
BD GolgiPlug Protein Transport Inhibitor	BD Biosciences	Cat. 555029
BD GolgiStop Protein Transport Inhibitor	BD Biosciences	Cat. 554724
Deposited Data		
RNA-seq from Th17-SAA1 and Th17-TGFβ		
Experimental Models: Cell Lines		
Plat-E Retroviral Packaging Cell Line	Cell Biolabs, INC.	RV-101
Experimental Models: Organisms/Strains		
C57BL/6J	The Jackson Laboratory	JAX:000664
C57BL/6-II17a <sup>tm1Bcgen</sup> /J	The Jackson Laboratory	JAX: 018472
B6. SJL Ptprca <sup>a</sup> Pepcb <sup>b</sup> /BoyJ	The Jackson Laboratory	JAX:002014
C57BL/6-Tg(Tcra2D2,Tcrb2D2)1Kuch/J	The Jackson Laboratory	JAX:006912
B6.Cg-Tg(Cd4-TGFBR2)16Flv/J	The Jackson Laboratory	JAX:005551
B6.129P2(Cg)-Cx3cr1tm2.1(cre/ERT2)Litt /WganJ	The Jackson Laboratory	JAX: 021160
B6.Cg-Speer6-ps1Tg(Alb-cre)21Mgn/J	The Jackson Laboratory	JAX:003574
Rosa26 <sup>LoxP-STOP-LoxP-DsRed</sup>	Gift of the Wenbiao Gan laboratory	<a href="https://www.sciencedirect.com/science/article/pii/S0092867413014815?via%3Dihub">https://www.sciencedirect.com/science/article/pii/S0092867413014815?via%3Dihub</a>
IL23R-GFP knock-in reporter mice	Gift of the Mohamed Oukka laboratory	<a href="http://www.jimmunol.org/content/182/10/5904.long">http://www.jimmunol.org/content/182/10/5904.long</a>
LoxP-STOP-LoxP-SAA1; LSL-SAA1	This paper	N/A
SAA1/2 double knock-out (DKO)	Gift from Frederick De Beer	<a href="https://www.cell.com/cell/fulltext/S0092-8674(15)01113-7">https://www.cell.com/cell/fulltext/S0092-8674(15)01113-7</a>
SAA1/2/3 triple knock-out (TKO)	This paper	N/A
SAA3 knock-out (3KO)	This paper	N/A
Oligonucleotides		
Saa1 qRT-PCR forward primer: CATTTGTTACGAGGCTTTCC	Derebe MG. et al., 2014	<a href="https://elifesciences.org/articles/03206">https://elifesciences.org/articles/03206</a>
Saa1 qRT-PCR reverse primer: GTTTTCCAGTTAGCTTCCTTCATGT	Derebe MG. et al., 2014	<a href="https://elifesciences.org/articles/03206">https://elifesciences.org/articles/03206</a>
Saa2 qRT-PCR forward primer: TGATGCTGCCCAAAGG	Liu J. et al., 2016	<a href="https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-016-0493-y">https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-016-0493-y</a>

Saa2 qRT-PCR reverse primer: GCCAGGAGGTCTGTAGTAA	Liu J. et al., 2016	<a href="https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-016-0493-y">https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-016-0493-y</a>
Saa3 qRT-PCR forward primer: CGCAGCACGAGCAGGAT	This paper	
Saa3 qRT-PCR reverse primer: TGGCTGTCAACTCCCAGG	This paper	
Gapdh qRT-PCR forward primer: AATGTGTCCGTCGTGGATCT	Sano, T. et al., 2015	<a href="https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7">https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7</a>
Gapdh qRT-PCR forward primer: CATCGAAGGTGGAAGAGTGG	Sano, T. et al., 2015	<a href="https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7">https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7</a>
Universal 16S qPCR forward primer: ACTCCTACGGGAGGCAGCAGT	Sano, T. et al., 2015	<a href="https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7">https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7</a>
Universal 16S qPCR reverse primer: ATTACCGCGGCTGCTGGC	Sano, T. et al., 2015	<a href="https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7">https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7</a>
SFB 16S qPCR forward primer: GACGCTGAGGCATGAGAGCAT	Sano, T. et al., 2015	<a href="https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7">https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7</a>
SFB 16S qPCR reverse primer: GACGGCACGAATTGTTATTCA	Sano, T. et al., 2015	<a href="https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7">https://www.cell.com/cell/pdfExtended/S0092-8674(15)01113-7</a>
<i>H. hepaticus</i> 16S qPCR forward primer: CAACTAAGGACGAGGGTTG	This paper	
<i>H. hepaticus</i> 16S qPCR reverse primer: TTCGGGGAGCTTGAAAAC	This paper	
<b>Software and Algorithms</b>		
FlowJo	9.9.6	<a href="https://www.flowjo.com/">https://www.flowjo.com/</a>
Prism	8.1.0	<a href="https://www.graphpad.com/scientific-software/prism/">https://www.graphpad.com/scientific-software/prism/</a>
Imaris software	9.0.1	Oxford Instruments
DEseq2	1.22.2	<a href="https://bioconductor.org/packages/release/bioc/html/DESeq2.html">https://bioconductor.org/packages/release/bioc/html/DESeq2.html</a>
Gene Set Enrichment Analysis tool	3.0	<a href="http://software.broadinstitute.org/gsea/index.jsp">http://software.broadinstitute.org/gsea/index.jsp</a>

## CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dan R. Littman (Dan.Littman@med.nyu.edu).

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Mouse Strains

C57BL/6J mice were purchased from The Jackson Laboratory. All transgenic animals were bred and maintained in specific-pathogen free (SPF) conditions within the animal facility of the Skirball Institute (NYU School of Medicine). *Saa1/2* double-knockout (SAA<sup>DKO</sup>) mice were previously described (Eckhardt et al., 2010) and maintained bred to the II17a-GFP reporter strain (JAX; C57BL/6-II17a<sup>tm1Bcgen/J</sup>). *H.hepaticus* (Hh)-specific Th17-TCR tg (HH7) mice were previously described (Xu et al., 2018) and maintained on a Ly5.1 background (JAX; B6.SJLPtpr<sup>a</sup> Pepc<sup>b</sup>/BoyJ). MOG-specific TCR transgenic (2D2, JAX; C57BL/6-Tg (Tcr $\alpha$ 2D2,Tcr $\beta$ 2D2)1 Kuch/J) mice were purchased from Jackson Laboratories, and maintained on an Ly5.1 background. CD4-dnTGFBRII (JAX; B6.Cg-Tg(Cd4-TGFB2)16Flv/J) mice were purchased from Jackson Laboratories. Il-23r-GFP mice were provided by M. Oukka (Awasthi et al., 2009). *Cx3cr1*<sup>CreER</sup> (JAX; B6.129P2(Cg)-Cx3cr1<sup>tm2.1(cre/ERT2)Litt</sup>/WganJ) mice were bred with Rosa26<sup>LoxP-STOP-LoxP-DsRed</sup> (R26<sup>DsRed</sup>) mice to fate-label microglia (Parkhurst et al., 2013). *Saa3* knockout (SAA<sup>3KO</sup>) and *Saa1/2/3* triple-knockout (SAA<sup>TKO</sup>) mice were generated using CRISPR-Cas9 technology. A premature stop codon was inserted into exon 2 of the *Saa3* locus in WT (for SAA<sup>3KO</sup>) or *Saa1/2* knock-out (for SAA<sup>TKO</sup>) zygotes. Guide RNA and HDR donor template sequences are listed in Table 1. Inducible SAA1 knock-in mice (LoxP-STOP-LoxP-SAA1; LSL-SAA1) mice were generated by targeted insertion of the STOP-eGFP-ROSA26TV cassette (Addgene Plasmid 15912: CTV vector), in which the mouse *Saa1* gene was sub-cloned, into the ROSA26 locus (Figure S6H).



Without Cre-recombinase LSL-SAA1 mice were healthy, fertile, and born in Mendelian ratios. All in-house developed strains were generated by the Rodent Genetic Engineering Core (RGEC) at NYULMC. LSL-SAA1 mice were bred with Albumin-Cre (JAX; B6.Cg-Speer6-ps1Tg(Alb-cre)21Mgn/J) mice to generate liver-specific SAA1 overexpressing mice (LSL-SAA1/Alb-cre) (Figure S6H). Age-(6-12 weeks) and sex-(both males and females) matched littermates stably colonized with Segmented Filamentous Bacteria (SFB) were used for all experiments. To assay SFB colonization, SFB-specific 16S primers were used and universal 16S and/or host genomic DNA were quantified simultaneously to normalize SFB colonization in each sample. All animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Usage Committee of New York University School of Medicine

### **Human IBD samples**

Pinch biopsies were obtained from the colon tissues (distal and rectum) of adult UC patients undergoing surveillance colonoscopy using 2.8 mm standard biopsy forceps, after protocol review and approval by the New York University School of Medicine Institutional Review Board (Mucosal Immune Profiling in Patients with Inflammatory Bowel Disease; S12-01137). All biopsies were collected in ice cold complete RPMI (RPMI 1640 medium, 10% fetal bovine serum (FBS), penicillin/streptomycin /glutamine, 50  $\mu$ M 2-mercaptoethanol). Inflammation status of rectal tissue included in the study was confirmed by pathological examination as chronic active colitis.

## **METHOD DETAILS**

### **Flow cytometry**

Single cell suspensions were pelleted and resuspended with surface-staining antibodies in HEPES Buffered HBSS. Staining was performed for 20-30min on ice. Surface-stained cells were washed and resuspended in live/dead fixable blue (ThermoFisher) for 5 minutes prior to fixation. For transcription factor staining, cells were treated using the FoxP3 staining buffer set from eBioscience according to the manufacturer's protocol. Intracellular stains were prepared in 1X eBioscience permwash buffer containing anti-CD16/anti-CD32, normal mouse IgG (conc), and normal rat IgG (conc). Staining was performed for 30-60min on ice. For cytokine analysis, cells were initially incubated for 3h in RPMI or IMDM with 10% FBS, phorbol 12-myristate 13-acetate (PMA) (50 ng/ml; Sigma), ionomycin (500 ng/ml;Sigma) and GolgiStop (BD). After surface and live/dead staining, cells were treated using the Cytotfix/Cytoperm buffer set from BD Biosciences according to the manufacturer's protocol. Intracellular stains were prepared in BD permwash in the same manner used for transcription factor staining. Absolute numbers of isolated cells from peripheral mouse tissues in all studies were determined by comparing the ratio of cell events to bead events of CountBright™ absolute counting beads. Flow cytometric analysis was performed on an LSR II (BD Biosciences) or an Aria II (BD Biosciences) and analyzed using FlowJo software (Tree Star).

### ***In vitro* T cell culture and phenotypic analysis**

Mouse T cells were purified from lymph nodes and spleens of six to eight week old mice, by sorting live (DAPI-), CD4<sup>+</sup>CD25<sup>-</sup>CD62L<sup>+</sup>CD44<sup>low</sup> naïve T cells using a FACSAria (BD). Detailed antibody information is provided above (Flow cytometry). Cells were cultured in IMDM (Sigma) supplemented with 10% heat-inactivated FBS (Hyclone), 10U/ml penicillin-streptomycin (Invitrogen), 10µg/ml gentamicin (Gibco), 4 mM L-glutamine, and 50 µM β-mercaptoethanol. For

T cell polarization,  $1 \times 10^5$  cells were seeded in 200  $\mu$ l/well in 96-well plates that were pre-coated with a 1:20 dilution of goat anti-hamster IgG in PBS (STOCK = 1mg/ml, MP Biomedicals Catalog # 55398). Naïve T cells were primed with anti-CD3 $\epsilon$  (0.25 $\mu$ g/mL) and anti-CD28 (1 $\mu$ g/mL) for 24 hours prior to polarization. Cells were further cultured for 48h under T<sub>H</sub>-lineage polarizing conditions; Control (Con. : 20 ng/mL IL-6, 2.5  $\mu$ g/mL anti-IL-4, 2.5  $\mu$ g/mL anti-IFN $\gamma$ , 10  $\mu$ g/mL anti-TGF $\beta$ ), T<sub>H</sub>17-TGF $\beta$  (0.3 ng/mL TGF- $\beta$ , 20 ng/mL IL-6, 2.5  $\mu$ g/mL anti-IL-4, 2.5  $\mu$ g/mL anti-IFN $\gamma$ ), T<sub>H</sub>17-SAA1 (0.15-10  $\mu$ g/mL rmSAA1 (R&D systems or homemade), 20 ng/mL IL-6, 2.5  $\mu$ g/mL anti-IL-4, 2.5  $\mu$ g/mL anti-IFN $\gamma$ , 10  $\mu$ g/mL anti-TGF $\beta$ ) and T<sub>H</sub>1 (100U/ml IL-2, 20ng/ml IL-12, 2.5  $\mu$ g/mL anti-IL-4). For the SAA1 blocking assay, 2.5 $\mu$ g/ml of rmSAA1 was preincubated with various amount (6.25 $\mu$ g/ml, 12.5 $\mu$ g/ml, 25 $\mu$ g/ml, 50 $\mu$ g/ml) of anti-mSAA1 (R&D systems, polyclonal goat IgG, antigen affinity-purified) or control polyclonal goat IgG (R&D systems) antibodies for 30 minutes prior to the treatment. Human naïve CD4<sup>+</sup> T cells were isolated from cord blood of healthy donors using anti-human CD4 MACS beads (Miltenyi), followed by CD4<sup>+</sup>CD25<sup>-</sup>HLA-DR<sup>-</sup>CD45RO<sup>-</sup>CD45RA<sup>hi</sup> staining and sorting using a FACS Aria (BD). Human naïve CD4<sup>+</sup> T cells were cultured for 6 days in 96-well U bottom plates with 10 U/ml of IL-2, 20 ng/mL of IL-1 $\beta$ , 20 ng/ml of IL-23, 1 ng/ml of TGF- $\beta$ , 2.5  $\mu$ g/ml of anti-IL-4, 2.5  $\mu$ g/mL of anti-IFN $\gamma$  and anti-CD3/CD28 activation beads (LifeTechnologies) at a ratio of 1 bead per cell, as previously described (Manel et al., 2008). For monitoring cell surface expression of CCR6 on human T<sub>H</sub>17 cells by recombinant SAA treatment (Figure S3C and S3D), isolated human naïve CD4<sup>+</sup> T cells were cultured in anti-human CD3 (OKT3)-coated 96-well flat bottom plates with 50 ng/mL of IL-1 $\beta$  and 50 ng/ml of IL-23, as previously described (Revu et al., 2018). 20  $\mu$ g/ml of recombinant human SAA (rhSAA) proteins and 10  $\mu$ g/ml anti-TGF $\beta$  neutralizing antibodies (BioXcell) were added into the culture medium during the T<sub>H</sub>17 differentiation.

## **Isolation of Colonic Epithelial Cells**

The large intestine was removed immediately after euthanasia, carefully stripped of mesenteric fat and the cecal patch, sliced longitudinally and vigorously washed in cold HEPES buffered (25mM), divalent cation-free HBSS to remove all fecal traces. The tissue was cut into 1-inch fragments and placed in a 50ml conical containing 10ml of HEPES buffered (25mM), divalent cation-free HBSS and 2 mM of fresh DTT. The conical was placed in a bacterial shaker set to 37 °C and 200rpm for 7 minutes. After 20 seconds of vigorously shaking the conical by hand, the process was repeated once more. After 20 seconds of vigorously shaking the conical by hand, the tissue was moved to a fresh conical containing 10ml of HEPES buffered (25mM), divalent cation-free HBSS and 5 mM of EDTA. The conical was placed in a bacterial shaker set to 37 °C and 200rpm for 7 minutes. After 20 seconds of vigorously shaking the conical by hand, the EDTA wash was repeated once more. Both EDTA washes were combined, passed through a 100 $\mu$ M filter, then spun down at 1000rpm for 5 minutes with no brake. The supernatant was carefully aspirated and the pellet resuspended in 8ml of HEPES buffered (25mM), 5% FBS-supplemented, divalent cation-free HBSS containing 4mM CaCl<sub>2</sub>, DNase I (100  $\mu$ g/ml; Sigma), dispase (0.05 U/ml; Worthington) and then transferred into a 15ml conical. The conical was placed horizontally in a bacterial shaker set to 37 °C and 180rpm for 7 minutes. The suspension was pipetted up-down multiple times to ensure homogenization and before adding HEPES buffered (25mM), divalent cation-free HBSS to volume. The sample was spun down at 1000rpm for 5 minutes with no brake and resuspended in an Ab cocktail to enrich for IECs (See Ab table under IEC sorting) and stained for 15 minutes on ice. The cells were washed, spun down and resuspended in 2% FBS-supplemented DMEM

containing DAPI and 1mM EDTA. CD326<sup>+</sup> (Epcam1) cells were isolated through a 100  $\mu$ M nozzle using a Sony SH800S.

### **Isolation of Lamina Propria Lymphocytes**

The intestine (small and/or large) was removed immediately after euthanasia, carefully stripped of mesenteric fat and Peyer's patches/cecal patch, sliced longitudinally and vigorously washed in cold HEPES buffered (25mM), divalent cation-free HBSS to remove all fecal traces. The tissue was cut into 1-inch fragments and placed in a 50ml conical containing 10ml of HEPES buffered (25mM), divalent cation-free HBSS and 1 mM of fresh DTT. The conical was placed in a bacterial shaker set to 37 °C and 200rpm for 10 minutes. After 45 seconds of vigorously shaking the conical by hand, the tissue was moved to a fresh conical containing 10ml of HEPES buffered (25mM), divalent cation-free HBSS and 5 mM of EDTA. The conical was placed in a bacterial shaker set to 37 °C and 200rpm for 10 minutes. After 45 seconds of vigorously shaking the conical by hand, the EDTA wash was repeated once more in order to completely remove epithelial cells. The tissue was minced and digested in 5-7ml of 10% FBS-supplemented RPMI containing collagenase (1 mg/ml collagenaseD; Roche), DNase I (100  $\mu$ g/ml; Sigma), dispase (0.05 U/ml; Worthington) and subjected to constant shaking at 155rpm, 37 °C for 35 min (small intestine) or 55 min (large intestine). Digested tissue was vigorously shaken by hand for 2 min before adding 2 volumes of media and subsequently passed through a 70  $\mu$ m cell strainer. The tissue was spun down and resuspended in 40% buffered percoll solution, which was then aliquoted into a 15ml conical. An equal volume of 80% buffered percoll solution was underlaid to create a sharp interface. The tube was spun at 2200rpm for 22 minutes at 22 °C to enrich for live mononuclear cells. Lamina propria (LP) lymphocytes were collected from the interface and washed once prior to staining.

For antigen presenting cell (APC) isolation, the cells were resuspended in an Ab cocktail to enrich for APCs (See Ab table under APC sorting) and stained for 15 minutes on ice. The cells were washed, spun down and resuspended in 2% FBS-supplemented DMEM containing DAPI. Dendritic cells and macrophage/inflammatory monocytes were sorted based on MHCII<sup>+</sup>CD11c<sup>+</sup>CD14<sup>neg</sup> and MHCII<sup>+</sup>CD11c<sup>+</sup>CD14<sup>+</sup> expression, respectively, through a 100  $\mu$ M nozzle using a Sony SH800S.

For ILC3 isolation, the cells were resuspended in an Ab cocktail to remove cells positive for the following lineage markers (CD3, CD11b, CD11c, CD14, CD19, TCR $\beta$ , TCR $\gamma$ , NK1.1, KLRG1). CD127<sup>+</sup>CD90<sup>+</sup> were sorted through a 70 $\mu$ M nozzle on an Aria II (BD Biosciences).

### ***H. hepaticus*-induced colitis**

*H. hepaticus* was provided by J. Fox (MIT) and grown on blood agar plates (TSA with 5% sheep blood, Thermo Fisher) as previously described (Xu et al., 2018). *H. hepaticus*-induced colitis was induced as described (Kullberg et al., 2006). Briefly, mice were colonized with *H. hepaticus* by oral gavage on days 0 and 4 of the experiment. 1 mg of an IL-10R-blocking antibody (clone 1B1.2) was administered by intraperitoneal injection once weekly starting at day 0. After 7 days, naïve HH7-2 TCRtg CD4<sup>+</sup> T cells were adoptively transferred into the *H. hepaticus* colonized recipients. Briefly, spleens from donor HH7-2 TCRtg mice were collected and mechanically disassociated. Red blood cells were lysed using ACK lysis buffer (Lonza). Naive HH7-2 Tg CD4<sup>+</sup> T cells were sorted as CD4<sup>+</sup>CD3<sup>+</sup>CD44<sup>lo</sup>CD62L<sup>hi</sup>CD25<sup>-</sup>V $\beta$ 6<sup>+</sup> on an Aria II (BD Biosciences). Cells were resuspended in ice-cold PBS and transferred into congenic recipient mice via intravenous injection. In order to confirm *H. hepaticus* colonization, *H. hepaticus*-specific 16S primers were used on

DNA extracted from fecal pellets. Universal 16S were quantified simultaneously to normalize *H. hepaticus* colonization of each sample.

To score colitis severity, colon and cecum samples were surgically removed from all groups of mice at 12 weeks post IL-10R-blockade injection. The samples were gently swiss rolled from the distal end and fixed in 4% paraformaldehyde (Electron Microscopy Science, Hatfield USA). Formalin-fixed tissues were then processed for paraffin embedding, cut into 5-micron thick sections and stained with hematoxylin and eosin (H&E) as per protocol by the Experimental Pathology Core Laboratory at New York University (NY, USA). H&E scoring was performed in a blinded fashion using coded slides. Total scores for colonic and cecum inflammation were comprised of individual scores from 4 categories: 1) Goblet cells per High Power Field (HPF) (Score of 1: 11 to 28 goblet cells per HPF; Score of 2: 1 to 10 goblet cells per HPF; Score of 3:  $\leq$  1 goblet cell per HPF). 2) Submucosa edema (Score of 0: no pathological changes; Score of 1: Mild edema accounting for <50% of the diameter of the entire intestinal wall; Score of 2: moderate edema involving 50-80% of the diameter of the entire intestinal wall; Score of 3: profound edema involving >80% of the diameter of the entire intestinal wall). 3) Inflammatory Infiltration Depth (Score of 0: No infiltrate; Score of 1: Infiltration above muscularis mucosae; Score of 2: Infiltration extending to include submucosa; Score of 3: Transmural Infiltration). 4) Epithelial Changes (Score of 0: No changes; Score of 1: Upper third or only surface epithelial missing; Score of 2: Moderate epithelial damage with intact base of crypts; Score of 3: Severe with missing crypts).

### **Induction of EAE by MOG-immunization or 2D2 transfer**

For induction of active experimental autoimmune encephalomyelitis (EAE), mice were immunized subcutaneously on day 0 with 100 $\mu$ g of MOG<sub>35-55</sub> peptide, emulsified in CFA

(Complete Freund's Adjuvant supplemented with 2mg/mL Mycobacterium tuberculosis H37Ra), and injected i.p. on days 0 and 2 with 200 ng pertussis toxin (Calbiochem). For 2D2 transfer EAE experiments, *in vitro* polarized 2D2 Th17 cells were injected intravenously into recipient mice at  $3 \times 10^6$  IL-17A producing 2D2 cells per recipient. Naive 2D2 TCR-transgenic CD4<sup>+</sup> T cells from the spleen and lymph nodes of 2D2 mice were electronically sorted (CD4<sup>+</sup>Vβ11<sup>+</sup>CD62L<sup>hi</sup>) and activated under TH17-polarizing conditions, in the presence of irradiated wild-type splenocytes at a 5:1 ratio, with the following: anti-CD3 (2.5μg/ml) (145-2C11, BioXCell), anti-IL-4 (20μg/ml) (11B11, BioXCell), anti-IFN-γ (20μg/ml) (XMG1.2, BioXCell), mIL-6 (30ng/ml) (Miltenyi Biotec) and hTGF-β1 (3ng/ml) (Miltenyi Biotec). After 48 h of activation, mIL-23 (10 ng/ml; R&D Systems) was added, as necessary. On day 5 of culture, cells were reactivated on plates precoated with 2 μg/ml of anti-CD3 and anti-CD28 (PV1, BioXCell) for an additional 48 h, before adoptive transfer. The EAE scoring system was as follows: 0-no disease, 1- Partially limp tail; 2- Paralyzed tail; 3- Hind limb paresis, uncoordinated movement; 4- One hind limb paralyzed; 5- Both hind limbs paralyzed; 6- Hind limbs paralyzed, weakness in forelimbs; 7- Hind limbs paralyzed, one forelimb paralyzed; 8- Hind limbs paralyzed, both forelimbs paralyzed; 9- Moribund; 10- Death. For isolating mononuclear cells from spinal cords during EAE, spinal cords were mechanically disrupted and dissociated in RPMI containing collagenase (1 mg/ml collagenaseD; Roche), DNase I (100 μg/ml; Sigma) and 10% FBS at 37 °C for 30 min. Leukocytes were collected at the interface of a 40%/80% Percoll gradient (GE Healthcare). Efferent lymph fluids of pre-clinical stage mice were collected from cisterna chyli as previously described (Matloubian et al., 2004).

### **Microglia and monocyte-derived macrophage isolation from EAE spinal cords**



*Cx3cr1<sup>CreERT2/+</sup>/R26<sup>DsRed/+</sup>* mice were gavaged with 10mg of tamoxifen diluted in corn oil at the age of 28 and 30 days. On day 60 EAE was induced by MOG-immunization and pertussis toxin injection as described. Mice were evaluated daily for weight loss and clinical development of hind limb paralysis. Mice were euthanized at the pre-clinical (day 9-10 post immunization), peak (acute; day 15-17), and chronic (day 25) stages of disease. Spinal cords were harvested, minced and digested as described. After obtaining a single cell suspension, samples were stained with the appropriate antibodies. Microglia cells and infiltrating monocyte-derived macrophage cells were sorted as DsRed<sup>+</sup>CX3CR1<sup>+</sup>CD11b<sup>+</sup>CD45<sup>int</sup>MHCII<sup>lo</sup>B220<sup>-</sup>CD3<sup>-</sup> (microglia) and DsRed<sup>-</sup>CX3CR1<sup>+</sup>CD11b<sup>+</sup>CD45<sup>hi</sup>F480<sup>+</sup>MHC<sup>hi</sup>B220<sup>-</sup>CD3<sup>-</sup> (monocyte-derived cells) on the Aria II (BD Biosciences) respectively.

### **Tissue Preparation for Immunofluorescence, Confocal Microscopy, and Image Analysis**

Tissue preparation for immunofluorescence, confocal microscopy, and image analysis was conducted as described (Perez et al., 2017). Briefly, tissues were fixed in paraformaldehyde, lysine, and sodium periodate buffer (PLP, 0.05 M phosphate buffer, 0.1M L-lysine, p.H. 7.4, 2 mg/mL NaIO<sub>4</sub>, and 10 mg/mL paraformaldehyde) overnight at 4°C followed by 30% sucrose overnight at 4°C and subsequent OCT media embedding. 20-μm frozen tissue sections were sectioned using a Leica CM3050S cryostat. FcR block was with anti-CD16/32 Fc block antibody (Biolegend) diluted in PBS containing 2% goat or donkey serum and 2% fetal bovine serum (FBS) for 1 hour at room temperature. Sections were stained with the indicated antibodies (Tables 2 and 3) diluted in PBS containing 2% goat or donkey serum, 2% FBS, and 0.05% Fc block for 1 hour at room temperature. For the intracellular staining, all antibodies including the Fc block were diluted in PBS containing 2% goat or donkey serum, 2% FBS, and 0.1% Triton-X. Images were acquired using a Zeiss LSM

880 confocal microscope (Carl Zeiss) with the Zen Black software. The imaging data were processed and analyzed using Imaris software version 9.0.1 (Bitplane; Oxford Instruments). Human SAA1/2 confocal images were analyzed by Image J software (NIH, Bethesda, MD) to measure signal intensity. Average signal intensity for each region of interest was normalized to healthy control.

### **Expression and purification of mouse recombinant SAAs**

*Escherichia coli* codon optimized DNA sequences, encoding SAA isotypes (without the signal sequence), were chemically synthesized (GenScript) and cloned into the pET26(b)+ (Novagen) expression vector between NdeI and HindIII restriction endonuclease sites, with a C-terminal hexa-histidine. Proteins were purified following the previously published protocol (Derebe et al., *eLife* 2014;3:e03206) with slight modifications. Briefly, proteins were overexpressed in *E. coli* BL21(DE3) cells by induction with 0.4 mM isopropyl- $\beta$ -D-galactoside (IPTG) for ~4 hr at 25°C for mSAA1, and at 37°C for mSAA3. Cells were harvested by centrifugation at 5000 $\times$ g for 20 min at 4°C and re-suspended in ice-cold lysis buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 500 mM NaCl, 10 mM imidazole for mSAA1 and 500 mM NaCl, 50 mM Tris pH 8.0, 10 mM imidazole, 15 mM  $\beta$ -mercaptoethanol for mSAA3). After sonication of cell suspension, decyl maltopyranoside (DM) (Avanti Polar Lipids) was added to a final concentration of 40 mM and incubated for ~3 hr at 4°C followed by centrifugation at 14,000 $\times$ g for 45 min. The supernatant was collected and incubated with Ni-NTA resin (GE HealthCare) pre-equilibrated with ice-cold corresponding lysis buffer containing 4 mM DM (DM buffer) for 3 h at 4 °C under mild mixing conditions to facilitate binding. The column was washed thrice with 25 mM imidazole in DM buffer to remove the non-specific contaminants. Finally, proteins were eluted using an imidazole gradient (in DM

buffer) under gravity flow. Fractions containing the protein of interest were pooled and dialyzed against 20 mM Tris-HCl (pH 8.2) containing 500 mM NaCl, 0.1% (w/v) PEG 3350 and 20 mM Imidazole. The dialyzed protein was concentrated in an Amicon stirrer cell apparatus (Millipore) to a final concentration of ~1 mg/mL. Protein purity was assessed by SDS/PAGE.

### **RNA isolation and cDNA preparation**

Total RNAs from *in vitro* polarized T cells or sorted cell populations were extracted using TRIzol (Invitrogen) followed by DNase I (Qiagen) treatment and cleanup with RNeasy MinElute kit (Qiagen) following manufacturer protocols. For total tissue RNA isolation, dissected tissues were homogenized in TRIzol. cDNA was generated using a SuperScript™ III First-Strand Synthesis System (ThermoFisher).

### **qRT-PCR and qPCR**

Quantitative RT-PCR and PCR were performed using the Hot Start-IT SYBR Green (Affymetrix) on the Roche real-time PCR system (Roche 480). For analysis of mRNA transcripts, RNA samples were treated with DNase (Roche) prior to cDNA synthesis to avoid effect of DNA contamination. Gene specific primers spanning exons were used. Values were normalized to GAPDH for each sample.

### **Library preparation for RNA sequencing**

RNA-seq libraries for *in vitro* polarized TH17 lineages were prepared with the TruSeq Stranded Total RNA Library Prep Gold Kit (Illumina, 20020598). The sequencing was performed using the

Illumina NovaSeq or NextSeq. RNA-seq libraries were prepared and sequenced by the Genome Technology Core at New York University School of Medicine.

RNAseq libraries for isolated microglia and CNS-infiltrating monocytes were prepared and sequenced by the Illumina RapidRun at the Genome Services Laboratory, HudsonAlpha.

## **QUANTIFICATION AND STATISTICAL ANALYSIS**

### **Transcriptome analysis**

Fastq files were aligned to the mouse Ensemble genome GRCm38 with STAR v 2.6.1d. Read pairs were counted using featurecounts from the Subread package v 1.6.2, prior to normalization and differential expression analysis which were performed using DESeq2. Gene set enrichment analyses were performed with the Broad Institute's GSEA tool. Potential enrichment of pathogenic and non-pathogenic gene sets defined from previous studies (Lee et al., 2012) were analyzed with the RNAseq data at each time point.

The single cell RNA sequencing data of SAA1 and SAA2 genes in the fibroblast clusters was analyzed as described in (Martin et al., 2018).

### **Statistical analysis**

Differences between groups were calculated using the unpaired two-sided Welch's t-test or the two-stage step-up method of Benjamini, Krieger and Yekutieliun. For EAE disease induction, log-rank test using the Mantel-Cox method was performed. For RNA-seq analysis, differentially expressed genes were calculated in DESeq2 using the Wald test with Benjamini-Hochberg correction to determine the FDR. GSEA p value was calculated with gene set permutations. Genes were considered differentially expressed with  $FDR < 0.01$  and  $\log_2$  fold change  $> 1.2$ . Data was

processed with GraphPad Prism, Version 8 (GraphPad Software). We treated less than 0.05 of p value as significant differences. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ . Details regarding number of replicates and the definition of center/error bars can be found in figure legends.

## Supplemental Information

### Supplemental figure legends

#### Figure S1. Mouse $T_H17/Tc17$ cell differentiation by SAAs, Related to Figure 1

(A) IL-17A dose response curve to rmSAA1.

(B) Representative IL-17A versus IFN $\gamma$  expression (left) and summary (right) in WT and TGF $\beta$ RII DNtg CD4 $^+$  T cells following SAA1-induced differentiation.

(C) Dosage-dependent inhibition of rSAA1-induced Th17 cell differentiation by SAA1-neutralizing antibody.

(D, E) Representative IL-17A versus IFN $\gamma$  expression (D) and ROR $\gamma$ t expression (E) in CD8 $^+$  T cells cultured under SAA1 or TGF- $\beta$  differentiation conditions. gMFI of ROR $\gamma$ t is in parentheses.

(F) Immunoblotting for p38 MAPK phosphorylation (p-p38) at T180/Y182 of primed CD4 $^+$  T cells upon rmSAA1 (10 $\mu$ g/ml) or vehicle (Veh.) treatment for indicated times. Total p38 is shown as a loading control. A summary of 2 experiments with at least 2 biological replicates per experiment.

(A-E) Experiments were conducted based using the same scheme as in Figure 1A.

(A-C) All statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure S2. Transcriptional responses and validation of differentially expressed genes in  $T_H17$  cells differentiated with IL6 plus SAA1 or TGF- $\beta$ , Related to Figure 2**

**(A)** Experimental scheme. Cells were harvested and RNA extracted at 3, 12 and 48 h after polarization.

**(B)** Clustered heatmap of differentially expressed genes at 48 h timepoint. Color scale is based on normalized read counts. Genes listed on the righthand margin are color coded. Green = non-pathogenic  $T_H17$  signature. Maroon = pathogenic  $T_H17$  signature. Black = Autoimmune disease associated.

**(C-E)** Normalized counts of autoimmune disease-associated (*Il23r*, *Il1r1*, *S100a4*) **(C)**, pathogenic (*Csf2*, *Tbx21*, *Gzmb*) **(D)**, or non-pathogenic (*Maf*, *Ahr*, *Il10*) **(E)** genes at the indicated time points.

**(F-H)** GM-CSF **(F and G)** and T-bet **(H)** expression at 48 h. gMFI of T-bet is in parentheses.

**(I and J)** Representative expression of IFN $\gamma$  versus IL-17A **(I)** and T-bet **(J)** in  $T_H1$  cells induced *in vitro* with IL-2 and IL-12, with and without SAA1, for 48 hrs.

**(C-E)** Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean  $\pm$  s.d.

**(F)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*p < 0.05, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

**Figure S3. Role of SAA in human  $T_H17$  cell differentiation and protein expression in inflamed colon tissue from UC patients, Related to Figure 3**

**(A)** Representative FACS plots of IL-17A versus IFN $\gamma$  expression by *in vitro* polarized human  $T_H17$  cells upon restimulation.

**(B and C)** Representative FACS plots (B) and summaries (C) indicating cell surface expression of CCR6 on *in vitro* polarized human T<sub>H</sub>17 cells upon human recombinant SAA treatment. Connecting lines signify the same donor. Summary of 3 experiments with n = 8 donors. Statistics were calculated using the paired two-tailed Student's t-test. \*p < 0.05,

**(D)** Confocal images show prominent SAA expression in biopsies of inflamed tissues from UC patients. Scale bar corresponds to 100µm. SAA1/2 (green), EPCAM (red) and nucleus (Draq7; blue).

**Figure S4. Role of SAAs in IL-10R blockade-induced colitis in response to *Helicobacter hepaticus*, Related to Figure 4**

**(A)** Gating strategy to identify all T<sub>H</sub> populations amongst HH7-2tg donor-derived cells in the colon lamina propria of representative homeostatic (isotype control IgG-injected) or colitis-developing (anti-IL10R- injected) recipients at 2 weeks post transfer.

**(B and C)** Characterization of HH7-2tg donor cells in colon lamina propria of recipient mice injected with isotype control Ab. SAA<sup>TKO</sup> (blue boxes, n = 4) and WT (red boxes, n = 4) littermates. Frequencies **(B)** and numbers **(C)** of indicated T<sub>H</sub> cells based on transcription factor staining.

**(D and E)** Normalized expression of *Saa1* **(D)** and *Saa3* **(E)** in the indicated sort-purified populations from colons of mice treated as in Figure 4A. Tissues were harvested 3-5 days after transfer of HH7-2tg cells.

**(F)** Normalized expression of *Saa1* and *Saa2* in ileum of recipient mice.

**(G)** Percentage of Foxp3<sup>+</sup> iTreg cells in isotype-treated recipients (open boxes) versus αIL10RA-treated recipients (solid boxes).

**(A-G)** Experiments were conducted based on the same scheme as in Figure 4A.

Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean  $\pm$  s.d. ns = not significant. \* $p < 0.05$ , \*\*\* $p < 0.001$ .

### **Figure S5. Distinct sources of SAA production in EAE, Related to Figure 5**

**(A)** Normalized expression of *Saa* isotypes in the liver at day 15 post MOG-immunization.

**(B)** SAA1/2 concentrations in lymph fluid collected from thoracic duct, at day 10 post immunization (pre-clinical stage) of EAE.

**(C)** Expression of SAA1/2 in the spinal cord and liver of WT (n=2) and SAA<sup>TKO</sup> (n=2) at day 15 of EAE.  $\beta$ -tubulin is shown as a loading control. Center lanes marked (M) are molecular weight markers, 14kD for the top and 64kD for the bottom.

**(D)** Normalized expression of *Saa* isotypes in the spinal cord at day 15 post MOG-immunization.

**(E and F)** Expression of SAAs in myeloid cells of the CNS. In order to distinguish microglia (DsRed<sup>+</sup>) from infiltrating monocyte-derived macrophages, *Cx3cr1<sup>creER</sup>:R26-DsRed* mice were injected with tamoxifen at day 28 and day 30, then rested for 30 days before MOG-immunization. RNAseq was performed on sort-purified microglia and infiltrating monocytes. Normalized RNAseq counts of *Saa* isotypes in microglia (**E**) and monocyte (**F**) isolated from CNS of WT mice at the indicated stages of EAE. Data for each condition are the mean of 2 biological replicates.

**(G)** Representative confocal image of spinal cord cross sections illustrating the specificity of the  $\alpha$ -SAA3 Ab used. IBA1 (red), SAA3 (green), and CD4 (aqua).

**(A, B, and D)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ .



**Figure S6. Role of SAAs in the TH17-dependent 2D2 transfer EAE model.**

**(A-C)** Total number **(A)**, percentage that are ROR $\gamma$ <sup>+</sup> TH17 **(B)** and quantification of IL-17A<sup>+</sup> **(C)** transferred 2D2tg donor cells in the CNS of WT (red boxes, n = 8) or SAA<sup>3KO</sup> (purple boxes, n = 9) recipients on day 17 post-adoptive transfer. Summary of 3 experiments.

**(D)** In vitro Th17 cell differentiation in response to SAA1 versus SAA3. The experiment was conducted based on the scheme in Figure 1A. Expression of IL-17A versus IFN- $\gamma$  in *in vitro* polarized CD4<sup>+</sup> T cell with IL-6 and TGF $\beta$  neutralizing antibodies  $\pm$  rmSAA1 (10 $\mu$ g/ml) or  $\pm$  rmSAA3 (10 $\mu$ g/ml) following restimulation.

**(E-G)** Comparison of cytokine production following different Th17 cell differentiation conditions. Experiments were conducted based on the scheme in Figure 1A. **(E)** Expression of IL-17A versus GM-CSF in *in vitro* polarized CD4<sup>+</sup> T cell with TH17-TGF $\beta$  (Top: IL6 + TGF $\beta$ ) or TH17-IL23 (Bottom: IL-6 + IL-1 $\beta$  + IL-23) conditions  $\pm$  rmSAA1 (10 $\mu$ g/ml) or  $\pm$  rmSAA3 (10 $\mu$ g/ml) following restimulation. **(F and G)** Summary of IL-17A frequency. Summary of 4 experiments, with 8 biological replicates.

**(H)** Schematic of conditional *Saa1* targeting construct and generation of liver<sup>Saa1</sup> mouse.

**(I)** Serum concentrations of SAAs and IL-6 in liver-overexpressing mice. Left: SAA1/2 in serum of liver<sup>Saa1</sup> (red circles, n = 27) or Alb-Cre<sup>Tg</sup> (white circles, n = 25) and right: IL-6 liver<sup>Saa1</sup> (red circles, n = 6) or Alb-Cre<sup>Tg</sup> (white circles, n = 6) in 6-week-old mice.

**(J and K)** Percent **(J)** and total number **(K)** of ROR $\gamma$ <sup>+</sup> TH17 cells amongst Foxp3<sup>Neg</sup> CD44<sup>hi</sup> CD4<sup>+</sup> T cells in secondary lymph nodes of 6-week-old mice with liver<sup>Saa1</sup> (red circles, n = 10) or Alb-Cre<sup>Tg</sup> (white circles, n = 10) summarized over two experiments.

**(L and M)** As in **(J and K)**, but of Tbet<sup>+</sup> TH1 cells.

**(A-G and I-M)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

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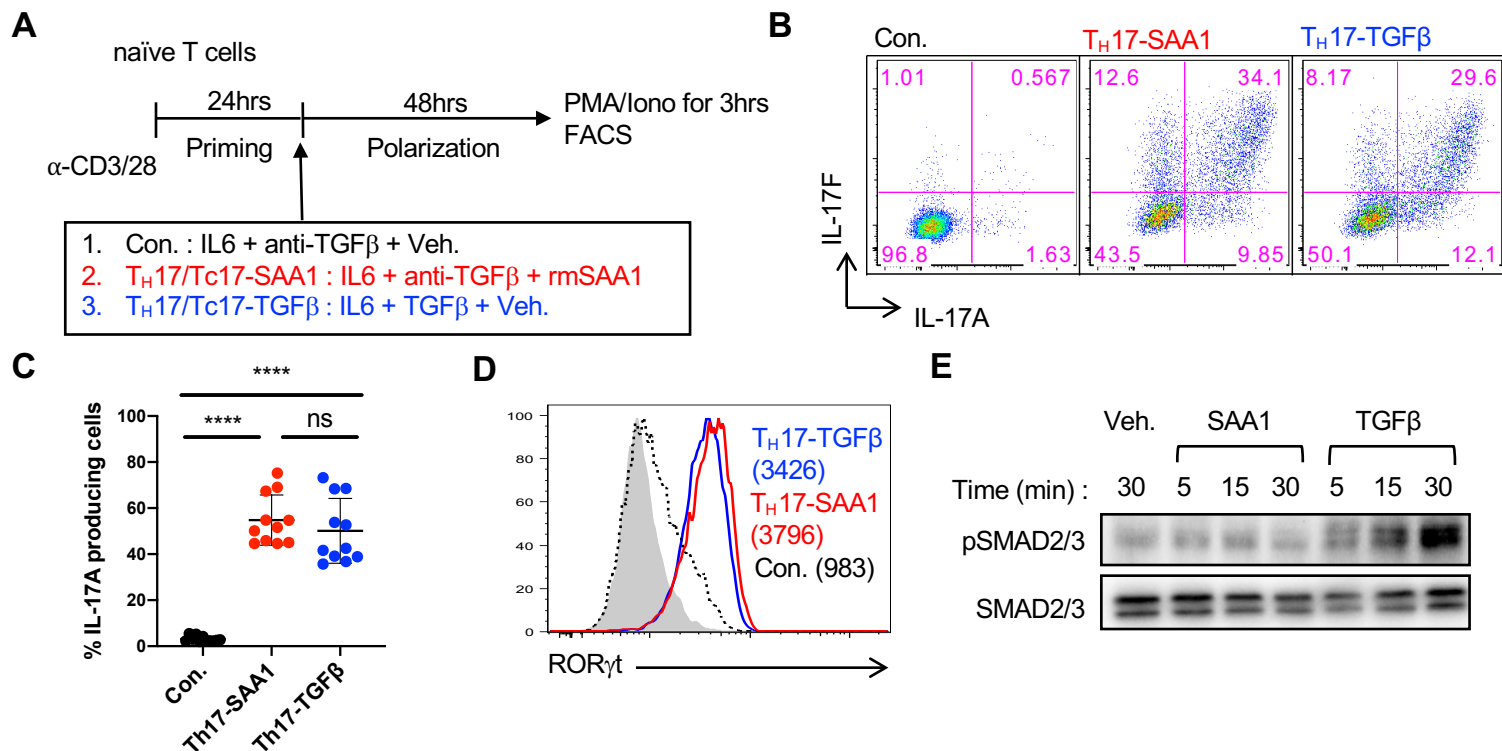
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## Figure 1



**Figure 1. SAAs act directly on mouse T cells to induce T<sub>H</sub>17 cell differentiation *in vitro* in absence of TGF- $\beta$ .**

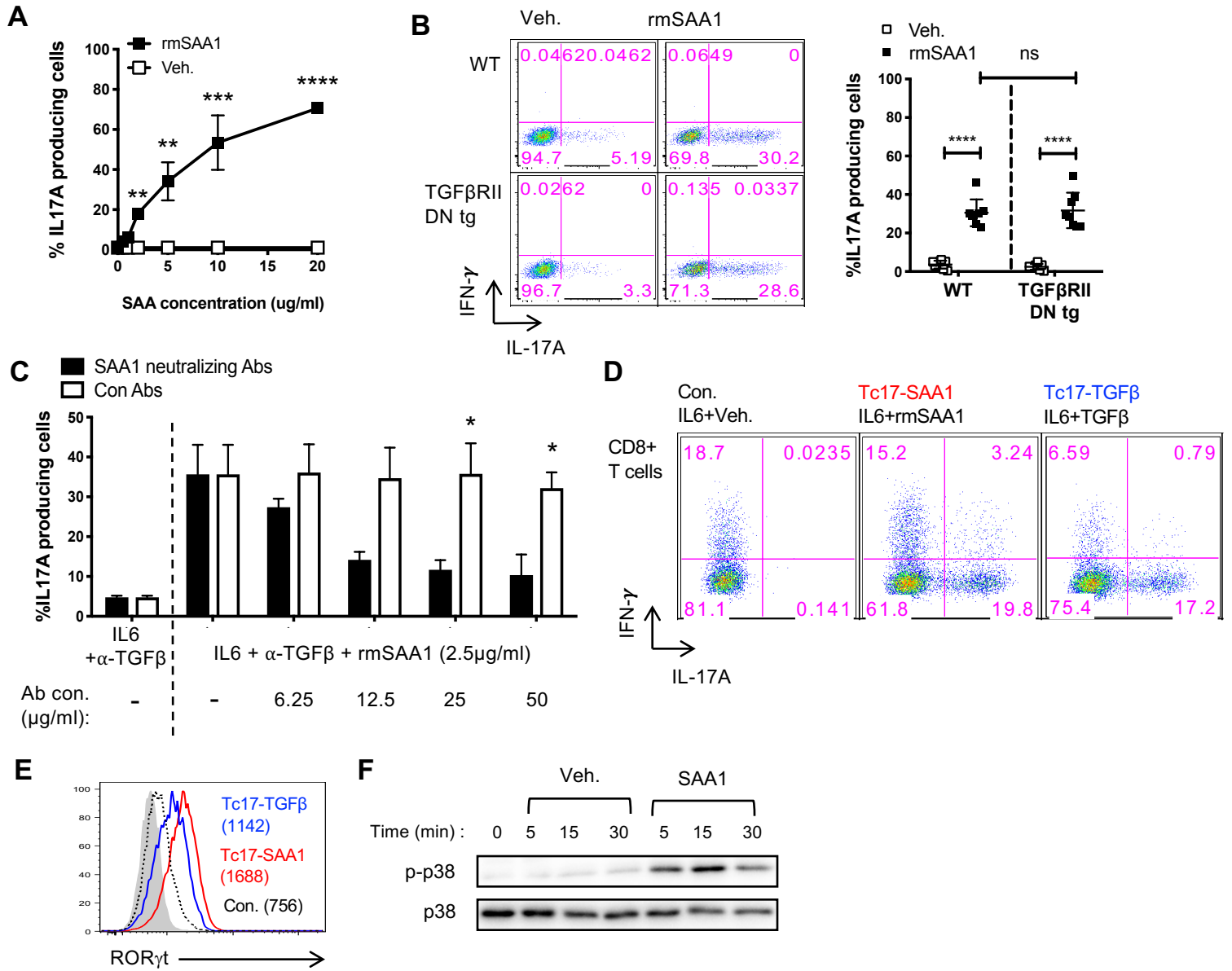
**(A)** Experimental scheme for *in vitro* differentiation of naïve CD4<sup>+</sup> or CD8<sup>+</sup> T cells.

**(B and C)** Flow cytometric analysis of IL-17A and IL-17F expression **(B)** and summary of IL-17A frequency **(C)** among re-stimulated cells. Summary of 3 experiments, with n = 11. Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*\*\*\*p < 0.0001.

**(D)** ROR $\gamma$ t expression in T<sub>H</sub>17 cells. Geometric mean fluorescence intensities (gMFI) are included in parentheses. Representative data of n > 10 experiments.

**(E)** Immunoblotting for SMAD2/3 phosphorylation (pSMAD2/3) of primed CD4<sup>+</sup> T cells upon rmSAA1 (10mg/ml) or TGF- $\beta$  (1ng/ml) treatment for indicated times. Total SMAD2/3 is shown as a loading control. See also Figure **S1**.

**Figure S1**



**Figure S1. Mouse T<sub>H</sub>17/Tc17 cell differentiation by SAAs, Related to Figure 1**

**(A)** IL-17A dose response curve to rmSAA1.

**(B)** Representative IL-17A versus IFN $\gamma$  expression (left) and summary (right) in WT and TGF $\beta$ RII DNtg CD4<sup>+</sup> T cells following SAA1-induced differentiation.

**(C)** Dosage-dependent inhibition of rSAA1-induced Th17 cell differentiation by SAA1-neutralizing antibody.

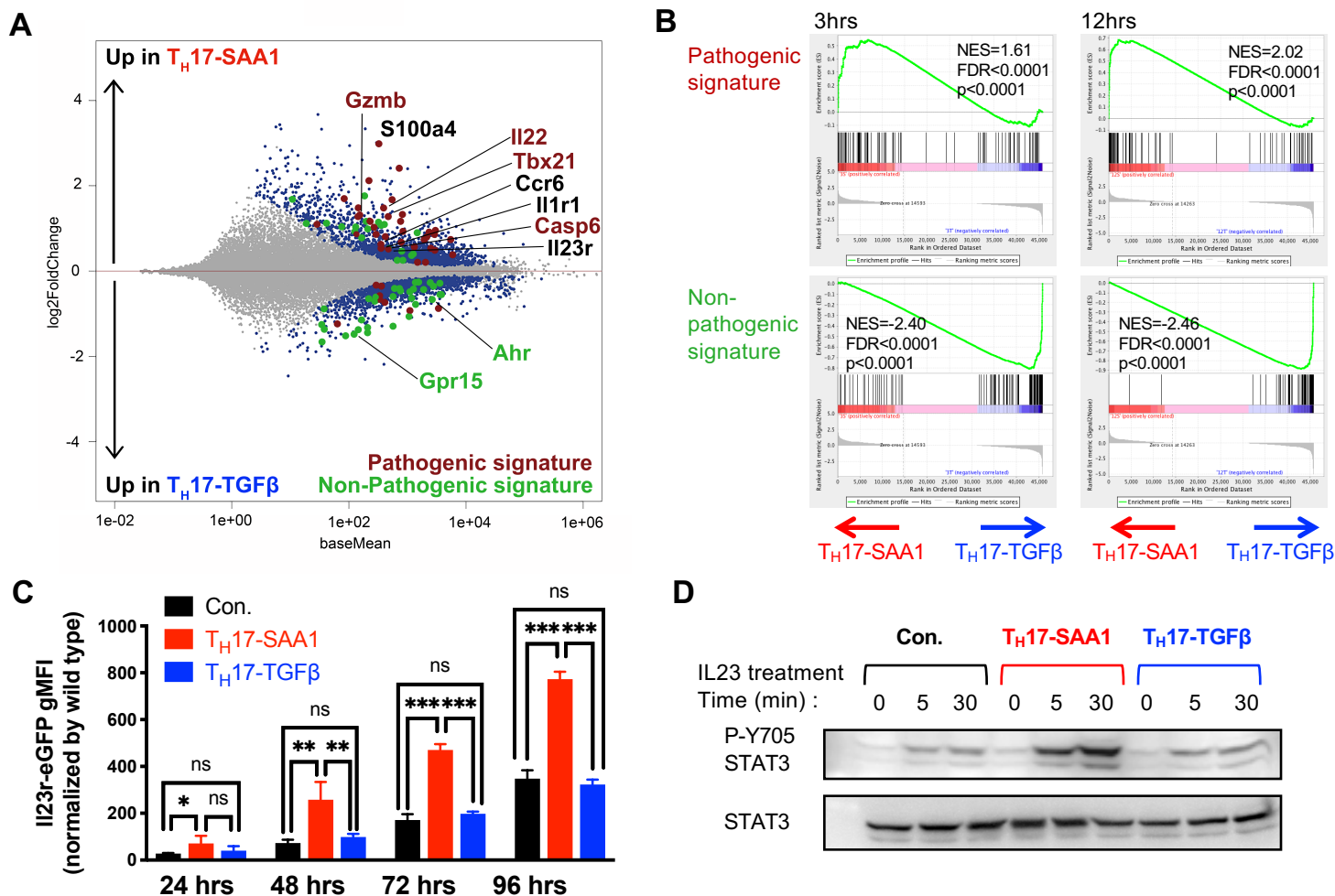
**(D, E)** Representative IL-17A versus IFN $\gamma$  expression (**D**) and ROR $\gamma$ t expression (**E**) in CD8<sup>+</sup> T cells cultured under SAA1 or TGF- $\beta$  differentiation conditions. gMFI of ROR $\gamma$ t is in parentheses.

**(F)** Immunoblotting for p38 MAPK phosphorylation (p-p38) at T180/Y182 of primed CD4<sup>+</sup> T cells upon rmSAA1 (10mg/ml) or vehicle (Veh.) treatment for indicated times. Total p38 is shown as a loading control. A summary of 2 experiments with at least 2 biological replicates per experiment.

**(A-E)** Experiments were conducted based using the same scheme as in Figure 1A.

**(A-C)** All statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

**Figure 2**



**Figure 2. SAA1 elicits a pathogenic  $T_H17$  program *in vitro*.**

**(A and B)** RNA sequencing analysis of temporal gene expression in IL6 + aTGF- $\beta$  + SAA1 ( $T_H17$ -SAA1, n = 3) and IL6 + TGF- $\beta$  ( $T_H17$ -TGF $\beta$ , n = 3) differentiated CD4<sup>+</sup> T cells. **(A)** MA plot depicts differentially expressed (DE) genes of  $T_H17$ -SAA1 versus  $T_H17$ -TGF $\beta$  cells at 48h *in vitro* polarization. Colored dots are significant DE genes. Red dots or green dots highlight pathogenic or non-pathogenic  $T_H17$  signature genes, respectively. DE genes were calculated in DESeq2 using the Wald test with Benjamini-Hochberg correction to determine the false discovery rate (FDR < 0.01). **(B)** GSEA plots of pathogenic (top) or non-pathogenic (bottom)  $T_H17$  signatures amongst  $T_H17$ -SAA1 and  $T_H17$ -TGF $\beta$  gene sets. NES, normalized enrichment score.

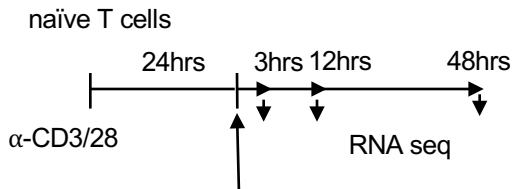
**(C)** Summary of eGFP expression in T cells from *I123r<sup>eGFP</sup>* mice following indicated condition and time of polarization. The gMFI of non-reporter, wild-type cells was subtracted from gMFI of eGFP to obtain the normalized gMFI. Representative data of two independent experiments. Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieli. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*p < 0.05, \*\*p < 0.01, and \*\*\*\*p < 0.0001.

**(D)** Amount of Y705 phosphorylated STAT3 following IL-23 treatment (10ng/ml) of  $T_H17$  cells differentiated under different conditions for indicated times. Total STAT3 is shown as a loading control.

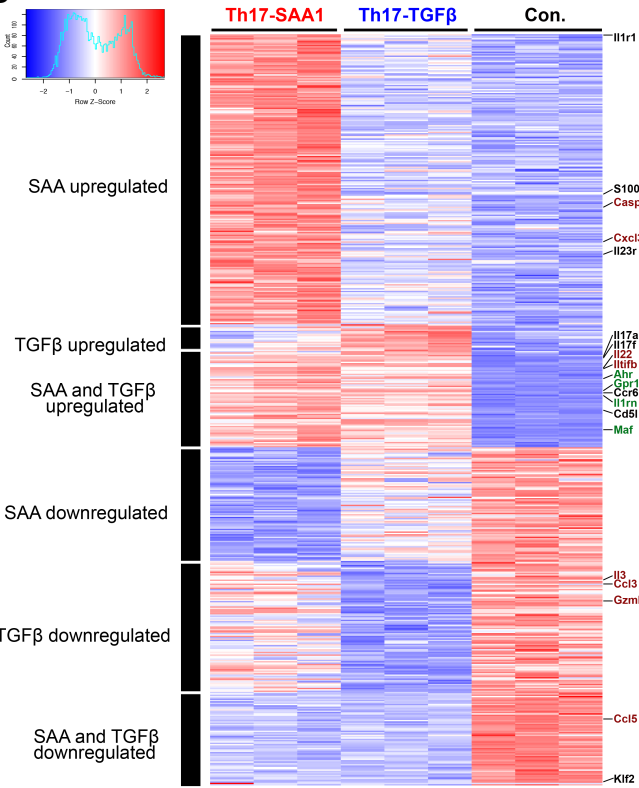
See also Figure S2.

**Figure S2**

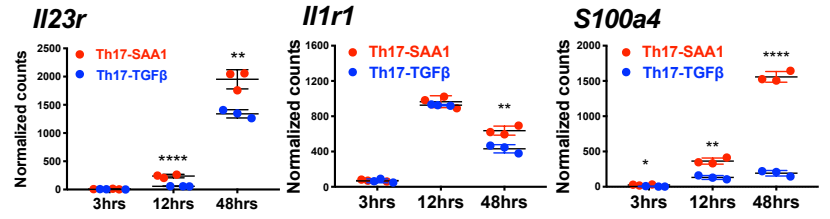
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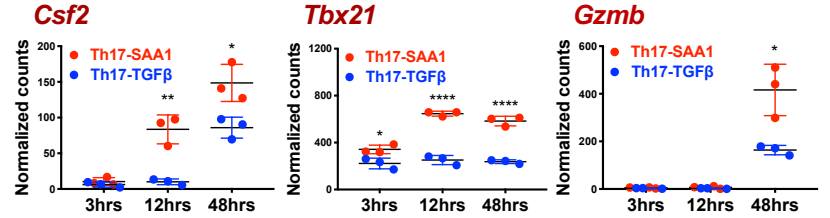
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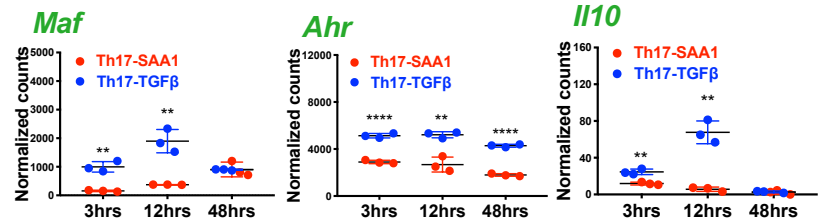
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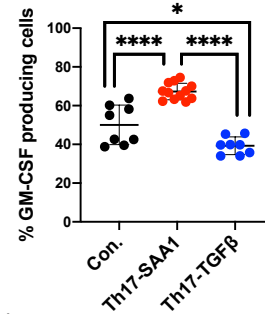
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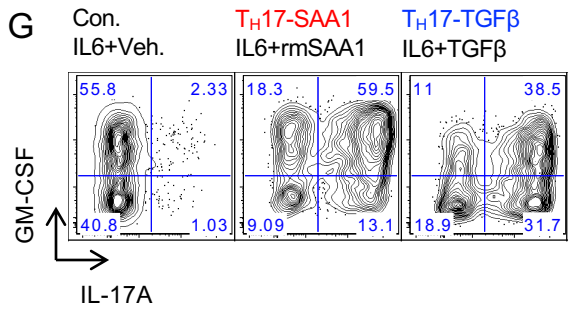
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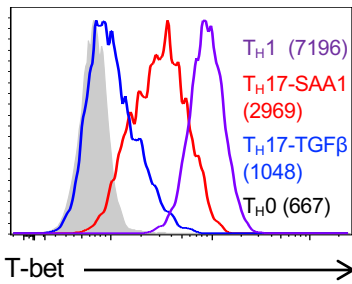
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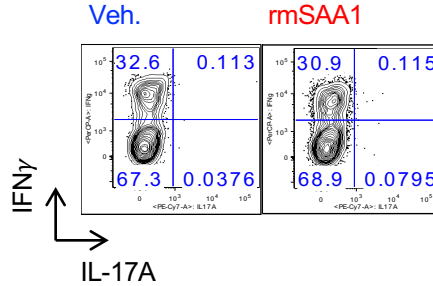
**G**



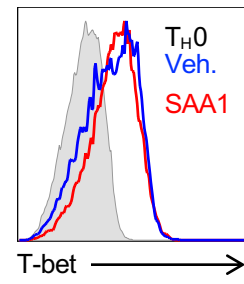
**H**



**I**  $T_H1$  (IL2+IL12)



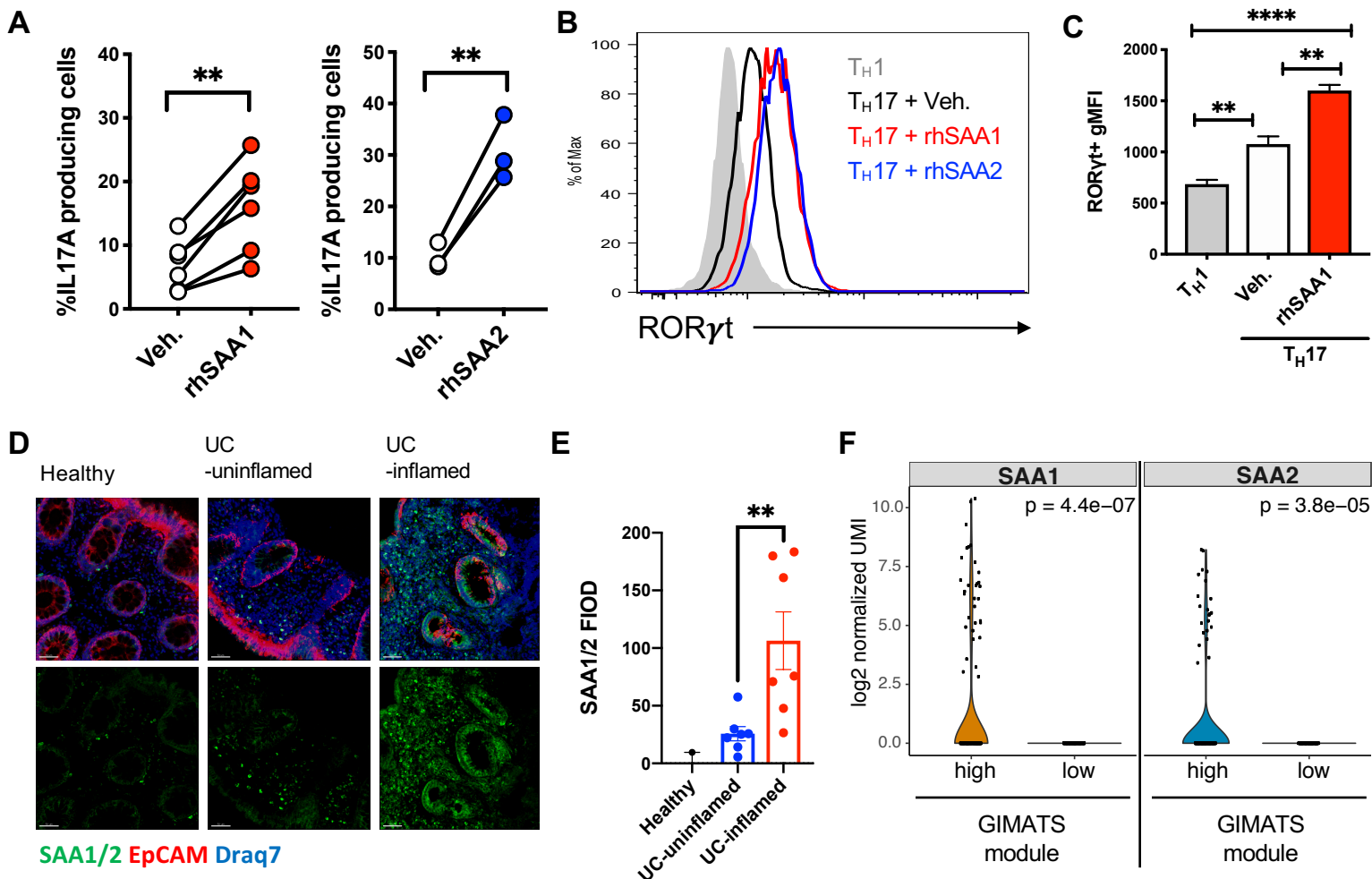
**J**



**Figure S2. Transcriptional responses and validation of differentially expressed genes in T<sub>H</sub>17 cells differentiated with IL6 plus SAA1 or TGF- $\beta$ , Related to Figure 2**

- (A)** Experimental scheme. Cells were harvested and RNA extracted at 3, 12 and 48 h after polarization.
- (B)** Clustered heatmap of differentially expressed genes at 48 h timepoint. Color scale is based on normalized read counts. Genes listed on the righthand margin are color coded. Green = non-pathogenic T<sub>H</sub>17 signature. Maroon = pathogenic T<sub>H</sub>17 signature. Black = Autoimmune disease associated.
- (C-E)** Normalized counts of autoimmune disease-associated (*Il23r*, *Il1r1*, *S100a4*) **(C)**, pathogenic (*Csf2*, *Tbx21*, *Gzmb*) **(D)**, or non-pathogenic (*Maf*, *Ahr*, *Il10*) **(E)** genes at the indicated time points. **(F-H)** GM-CSF **(F and G)** and T-bet **(H)** expression at 48 h. gMFI of T-bet is in parentheses.
- (I and J)** Representative expression of IFN $\gamma$  versus IL-17A **(I)** and T-bet **(J)** in T<sub>H</sub>1 cells induced *in vitro* with IL-2 and IL-12, with and without SAA1, for 48 hrs.
- (C-E)** Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean  $\pm$  s.d.
- (F)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \* $p < 0.05$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ .

**Figure 3**



**Figure 3. Human SAA expression in inflamed tissue and induction of T<sub>H</sub>17 cell differentiation.**

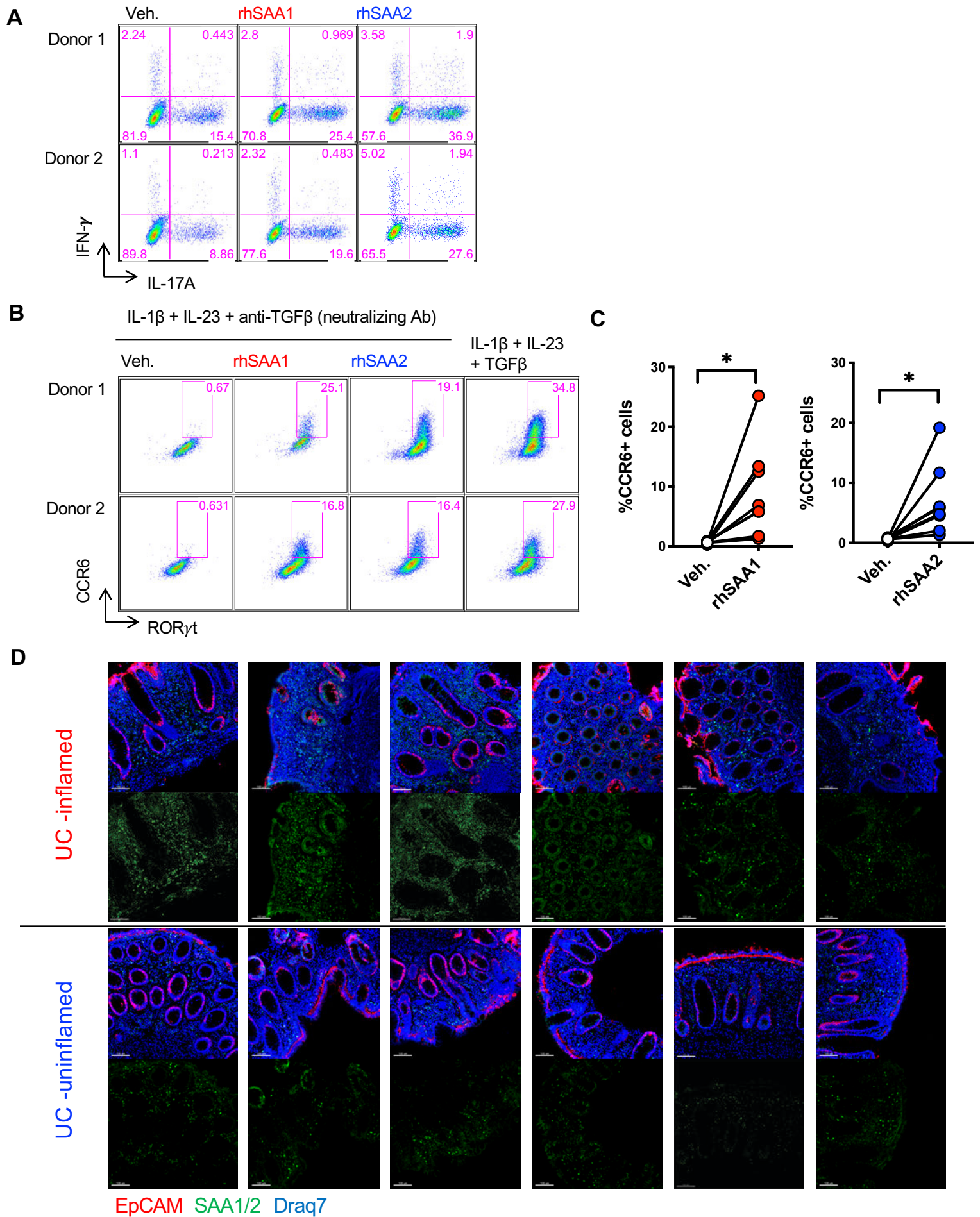
**(A-C)** Naïve human CD4<sup>+</sup> T cells were isolated from cord blood and differentiated for 6 days in T<sub>H</sub>17 polarizing conditions ± recombinant human (rh) SAA1 or rhSAA2. **(A)** Summary of IL-17A production from in vitro polarized human T<sub>H</sub>17 cells. Connecting lines signify cells from the same donor. **(B)** Stacked histogram illustrates representative RORγT expression using the indicated polarizing conditions. **(C)** Summary of RORγT gMFI. Summary of 2 experiments with n = 6 donors for rhSAA1 and with n = 3 donors for rhSAA2.

**(D and E)** Representative confocal images (D) and quantification of SAA1/2 by fluorescence integrated optical density (FIOD) levels (E) show prominent SAA expression in biopsies of inflamed tissue from ulcerative colitis (UC, n = 7) patients. Panels from left to right: Healthy (n = 2), UC-uninflamed, and UC-inflamed. Scale bar corresponds to 50µm. SAA1/2 (green), EPCAM (red) and nucleus (Draq7; blue).

**(F)** Violin plots showing the log<sub>2</sub> normalized UMI of SAA1 and SAA2 genes in the fibroblast cluster associated with the GIMATS module (IgG plasma cells, inflammatory MNP, activated T and stromal cells). Statistics were calculated using the paired two-tailed Student's t-test. \*\*p < 0.01, \*\*\*\*p < 0.0001.

See also Figure S3.



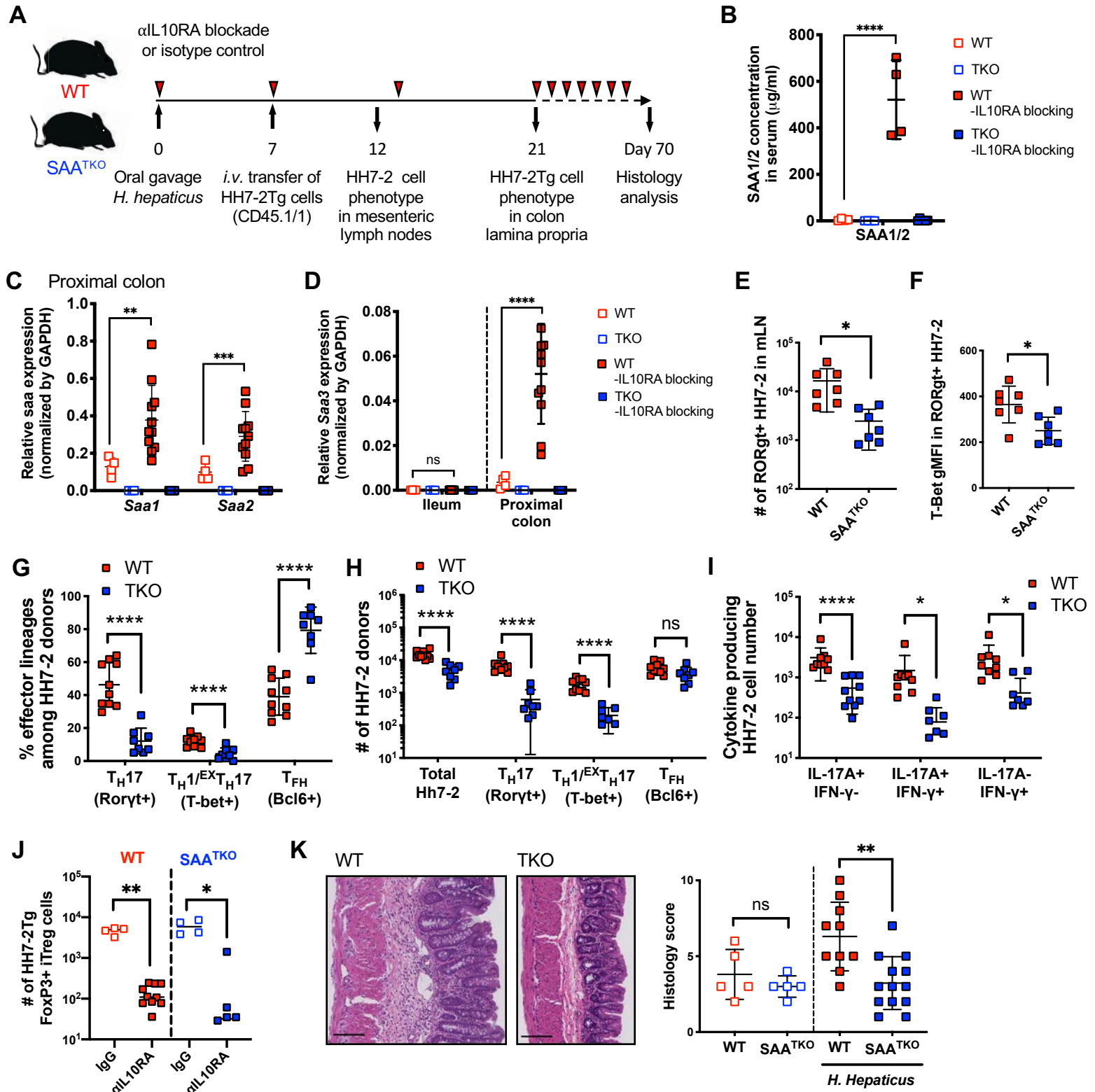
**Figure S3**

**Figure S3. Role of SAA in human T<sub>H</sub>17 cell differentiation and protein expression in inflamed colon tissue from UC patients, Related to Figure 3**

**(A)** Representative FACS plots of IL-17A versus IFN $\gamma$  expression by *in vitro* polarized human T<sub>H</sub>17 cells upon restimulation.

**(B and C)** Representative FACS plots (B) and summaries (C) indicating cell surface expression of CCR6 on *in vitro* polarized human T<sub>H</sub>17 cells upon human recombinant SAA treatment. Connecting lines signify the same donor. Summary of 3 experiments with n = 8 donors. Statistics were calculated using the paired two-tailed Student's t-test. \*p < 0.05,

**(D)** Confocal images show prominent SAA expression in biopsies of inflamed tissues from UC patients. Scale bar corresponds to 100 $\mu$ m. SAA1/2 (green), EPCAM (red) and nucleus (Draq7; blue).

**Figure 4**

**Figure 4. SAAs drive pathogenic T<sub>H</sub> cell responses in IL-10 deficiency-dependent colitis.**

**(A)** Experimental scheme to examine HH7-2tg cells in SAA1/2/3 triple knock-out (SAA<sup>TKO</sup>) and WT littermate recipient mice colonized with *H. hepaticus* ± IL-10RA blockade (aIL10RA).

**(B)** Serum concentrations of SAA1/2 of recipient mice at day 21 post *H. hepaticus* colonization.

**(C and D)** Normalized expression of *Saa1/2* in proximal colon **(C)** and *Saa3* in ileum and colon **(D)** of recipient mice at day 21 post *H. hepaticus* colonization.

**(E and F)** Characterization of HH7-2tg donor-derived cells 5 days post-adoptive transfer in mesenteric lymph nodes of recipient SAA<sup>TKO</sup> (blue boxes, n = 8) and WT (red boxes, n = 10) littermates injected with aIL10RA. Number of ROR $\gamma$ t expressing HH7-2 cells **(E)** and T-bet gMFI level in the ROR $\gamma$ t<sup>+</sup> HH7-2 cells **(F)**.

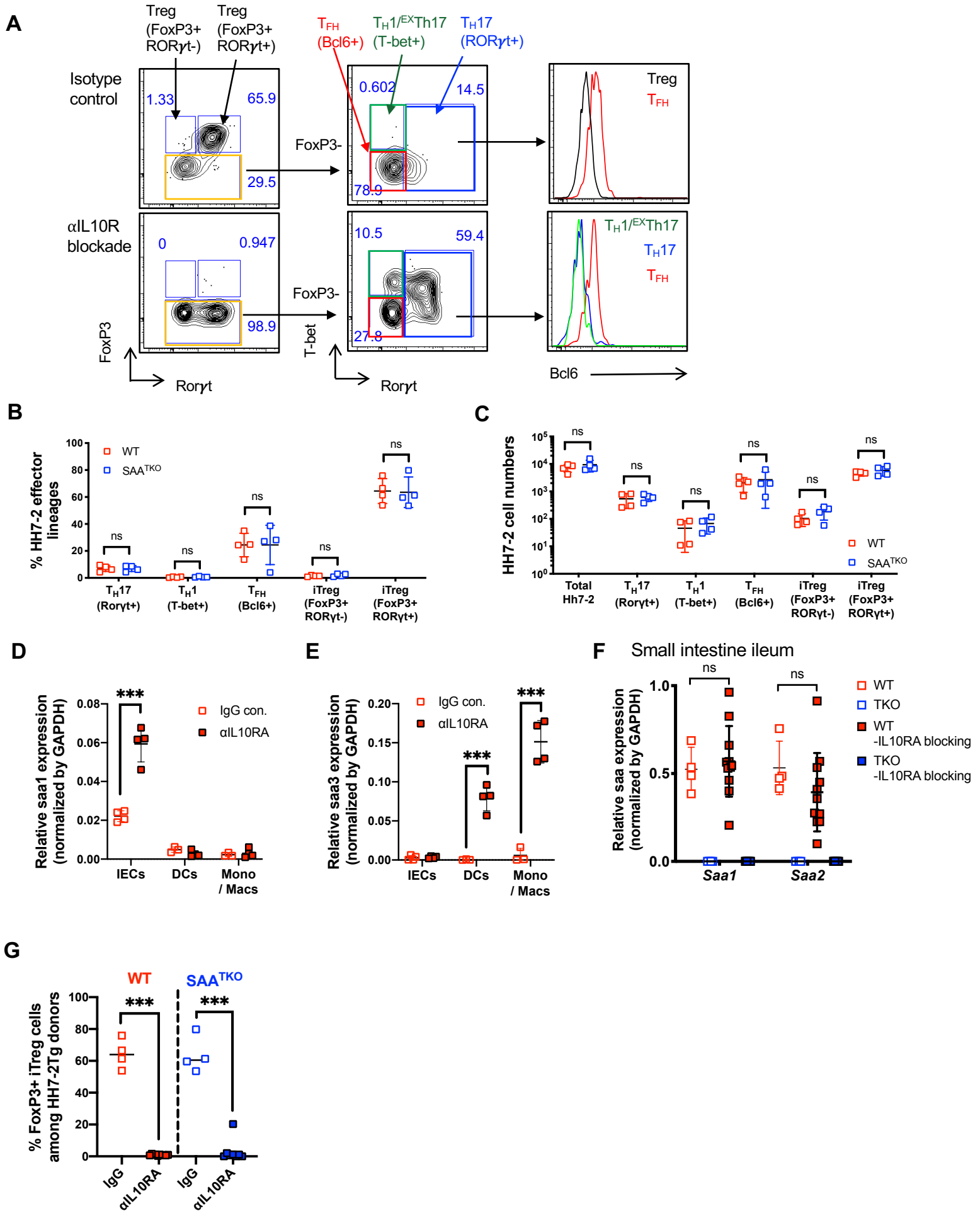
**(G-J)** Characterization of HH7-2tg donor-derived cells two weeks post-adoptive transfer in colon lamina propria of recipient mice injected with aIL10RA. Summary of 2 experiments with SAA<sup>TKO</sup> (blue boxes, n = 8) and WT (red boxes, n = 10) littermates. Frequency **(G)** and number of the indicated T<sub>H</sub> cells based on transcription factor expression **(H)** or cytokine expression after restimulation **(I)**. Numbers of Foxp3<sup>+</sup> iTreg cells in isotype-treated recipients (hollow boxes) versus aIL10RA-treated recipients **(J)**.

**(K)** Representative H&E staining (left) and summary of histology scores (right) of colon sections harvested from mice with or without *H. hepaticus* colonization, following twelve weeks of aIL10RA injection. Summary of two separate experiments is shown. Uncolonized mice (open boxes): WT + aIL10RA (red, n = 5), SAA<sup>TKO</sup> + aIL10RA (blue, n = 5); or colonized with *H. hepaticus* (closed boxes): WT + aIL10RA (red, n = 10), SAA<sup>TKO</sup> + aIL10RA (blue, n = 13).

**(B-F and K)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean ± s.d. ns = not significant, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

**(G-J)** Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean ± s.d. ns = not significant, \*p < 0.05, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001. See also Figure **S4**.

Figure S4



**Figure S4. Role of SAAs in IL-10R blockade-induced colitis in response to *Helicobacter hepaticus*, Related to Figure 4**

**(A)** Gating strategy to identify all T<sub>H</sub> populations amongst HH7-2tg donor-derived cells in the colon lamina propria of representative homeostatic (isotype control IgG-injected) or colitis-developing (anti-IL10R-injected) recipients at 2 weeks post transfer.

**(B and C)** Characterization of HH7-2tg donor cells in colon lamina propria of recipient mice injected with isotype control Ab. SAA<sup>TKO</sup> (blue boxes, n = 4) and WT (red boxes, n = 4) littermates. Frequencies **(B)** and numbers **(C)** of indicated T<sub>H</sub> cells based on transcription factor staining.

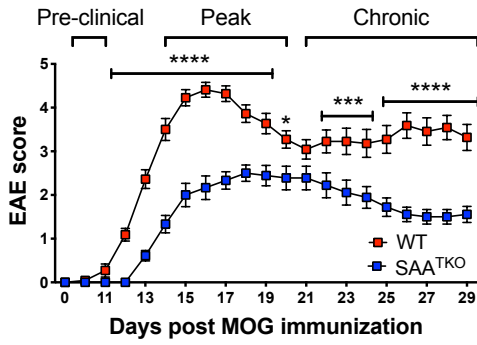
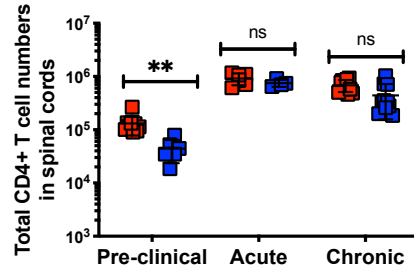
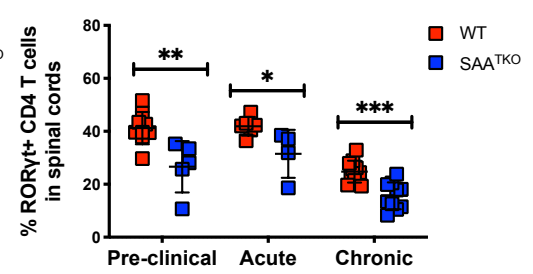
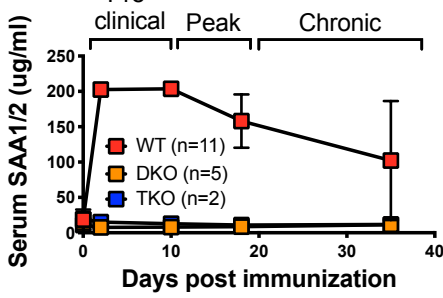
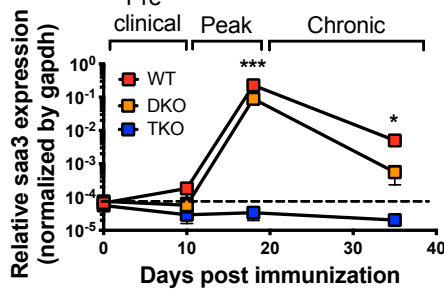
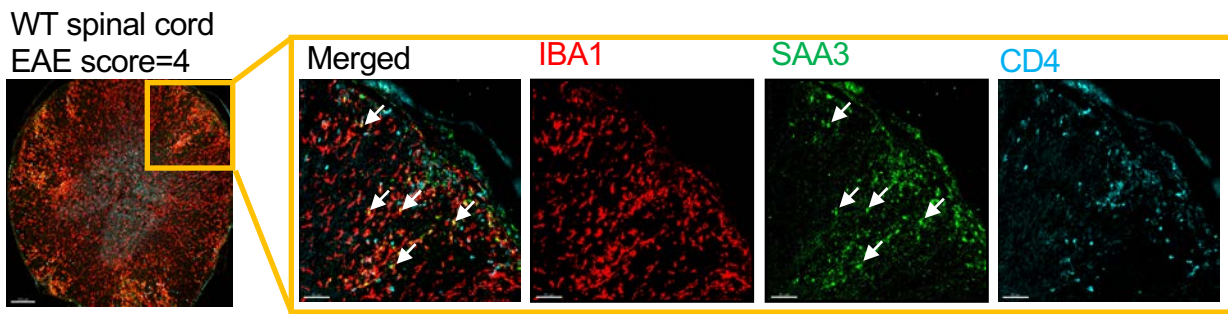
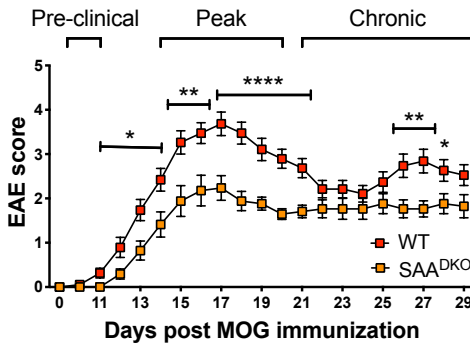
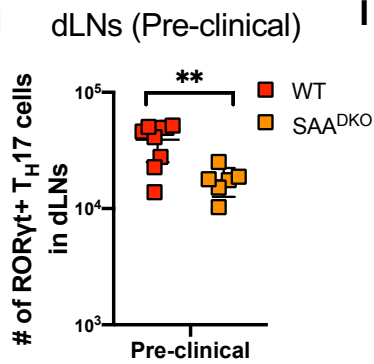
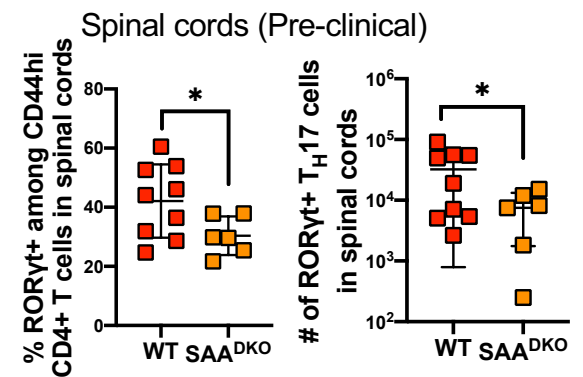
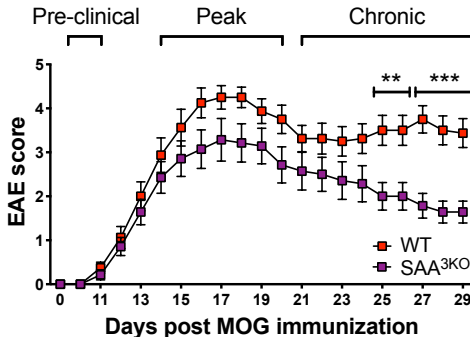
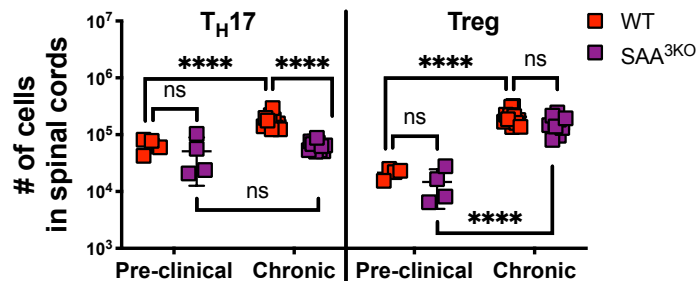
**(D and E)** Normalized expression of *Saa1* **(D)** and *Saa3* **(E)** in the indicated sort-purified populations from colons of mice treated as in Figure 4A. Tissues were harvested 3-5 days after transfer of HH7-2tg cells.

**(F)** Normalized expression of *Saa1* and *Saa2* in ileum of recipient mice.

**(G)** Percentage of Foxp3<sup>+</sup> iTreg cells in isotype-treated recipients (open boxes) versus all10RA-treated recipients (solid boxes).

**(A-G)** Experiments were conducted based on the same scheme as in Figure 4A.

Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean ± s.d. ns = not significant. \*p<0.05, \*\*\*p < 0.001.

**Figure 5****A****B****C****D****E****F****G****H****I****J****K**

## Figure 5. Distinct sources of SAAs promote and sustain autoimmune encephalomyelitis.

**(A)** Mean EAE scores of myelin oligodendrocyte glycoprotein (MOG)-immunized SAA<sup>TKO</sup> (blue boxes, n = 18) and WT littermate mice (red boxes, n = 22). Summary of 3 experiments.

**(B and C)** Number of CD4<sup>+</sup> T cells **(B)** and frequency of ROR $\gamma$ <sup>t</sup> T<sub>H</sub>17 cells among Foxp3<sup>Neg</sup> CD44<sup>hi</sup> CD4<sup>+</sup> T cells **(C)** in the CNS of mice at the indicated stage of EAE. day 10 = pre-clinical, day 15 = acute, day 32 = chronic. Pre-clinical, (SAA<sup>TKO</sup> = 6, WT = 9), acute, (SAA<sup>TKO</sup> = 4, WT = 6) and Chronic, (SAA<sup>TKO</sup> = 10, WT = 11). Data combined two, two, and three experiments for the pre-clinical, acute, and chronic stages of disease, respectively.

**(D)** Longitudinal mean serum concentrations of SAA1/2 in MOG-immunized mice. Error bars denote the s.d.

**(E)** Normalized relative expression of *Saa3* in CNS of MOG-immunized mice, measured by qPCR. Spinal cords were isolated at days 0, 10, 18, and 38 post-immunization. For WT, SAA<sup>DKO</sup>, and SAA<sup>TKO</sup>, the number of samples at each time point were 13, 7, and 13 (day 0); 5, 6, and 8 (day 10); 17, 11, and 10 (day 18); and 10, 3, 6 (day 38).

**(F)** Representative confocal image of spinal cord cross section isolated from a WT mouse (n = 2) at the peak of EAE (score 4). IBA1 (red), SAA3 (green), and CD4 (aqua). White arrows pointed at yellow regions indicate IBA1/SAA3 colocalization.

**(G)** Mean EAE scores of MOG-immunized SAA1/2 DKO (SAA<sup>DKO</sup>, orange boxes, n = 17) and WT littermate mice (red boxes, n = 19). Summary of 4 experiments.

**(H and I)** Number of ROR $\gamma$ <sup>t</sup> T<sub>H</sub>17 cells among CD44<sup>hi</sup> effector/memory CD4<sup>+</sup> T cells isolated from draining lymph nodes (dLNs) **(H)** and spinal cord **(I)** of SAA<sup>DKO</sup> (n = 6) or WT (n = 9) littermates at day 10 post immunization.

**(J)** Mean EAE scores of MOG-immunized SAA3 KO (SAA<sup>3KO</sup>, purple boxes, n = 14) and WT littermate mice (red boxes, n = 16). Summary of 3 experiments.

**(K)** Number of ROR $\gamma$ <sup>t</sup> T<sub>H</sub>17 and Foxp3<sup>+</sup> Treg cells at pre-clinical (day 10) and chronic (day 32) stages of EAE. Summary of 3 experiments, with SAA<sup>3KO</sup> (n = 14) and WT (n = 16) littermates.

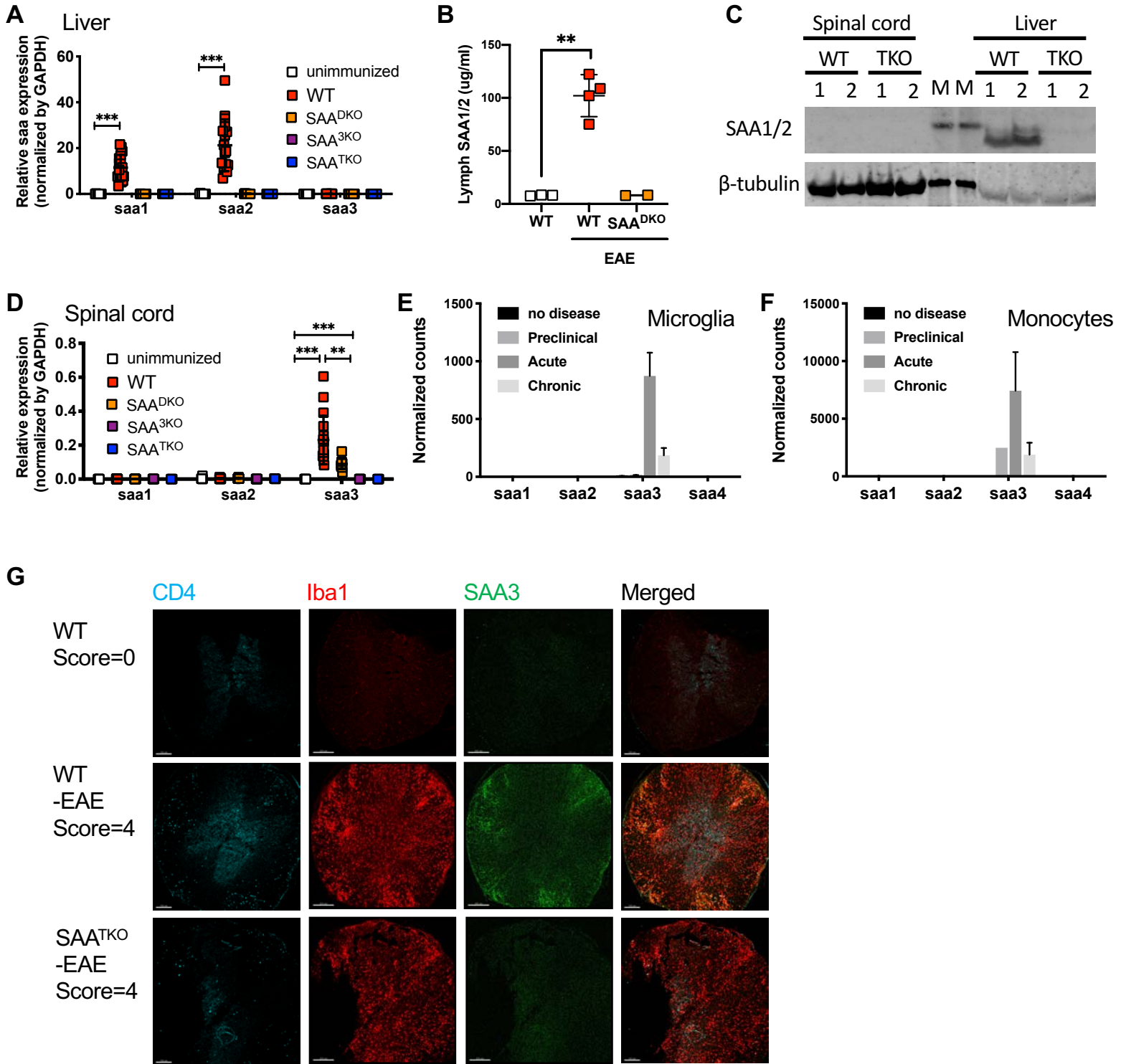
**(A-C, E, G, J, and K)** Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean  $\pm$  s.e.m **(A, G, and J)** or mean  $\pm$  s.d. **(B, C, E, H, I, and K)**. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

**(H and I)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

See also Figure S5.



**Figure S5**



## Figure S5. Distinct sources of SAA production in EAE, Related to Figure 5

**(A)** Normalized expression of *Saa* isotypes in the liver at day 15 post MOG-immunization.

**(B)** SAA1/2 concentrations in lymph fluid collected from thoracic duct, at day 10 post immunization (pre-clinical stage) of EAE.

**(C)** Expression of SAA1/2 in the spinal cord and liver of WT (n=2) and SAA<sup>TKO</sup> (n=2) at day 15 of EAE.  $\beta$ -tubulin is shown as a loading control. Center lanes marked (M) are molecular weight markers, 14kD for the top and 64kD for the bottom.

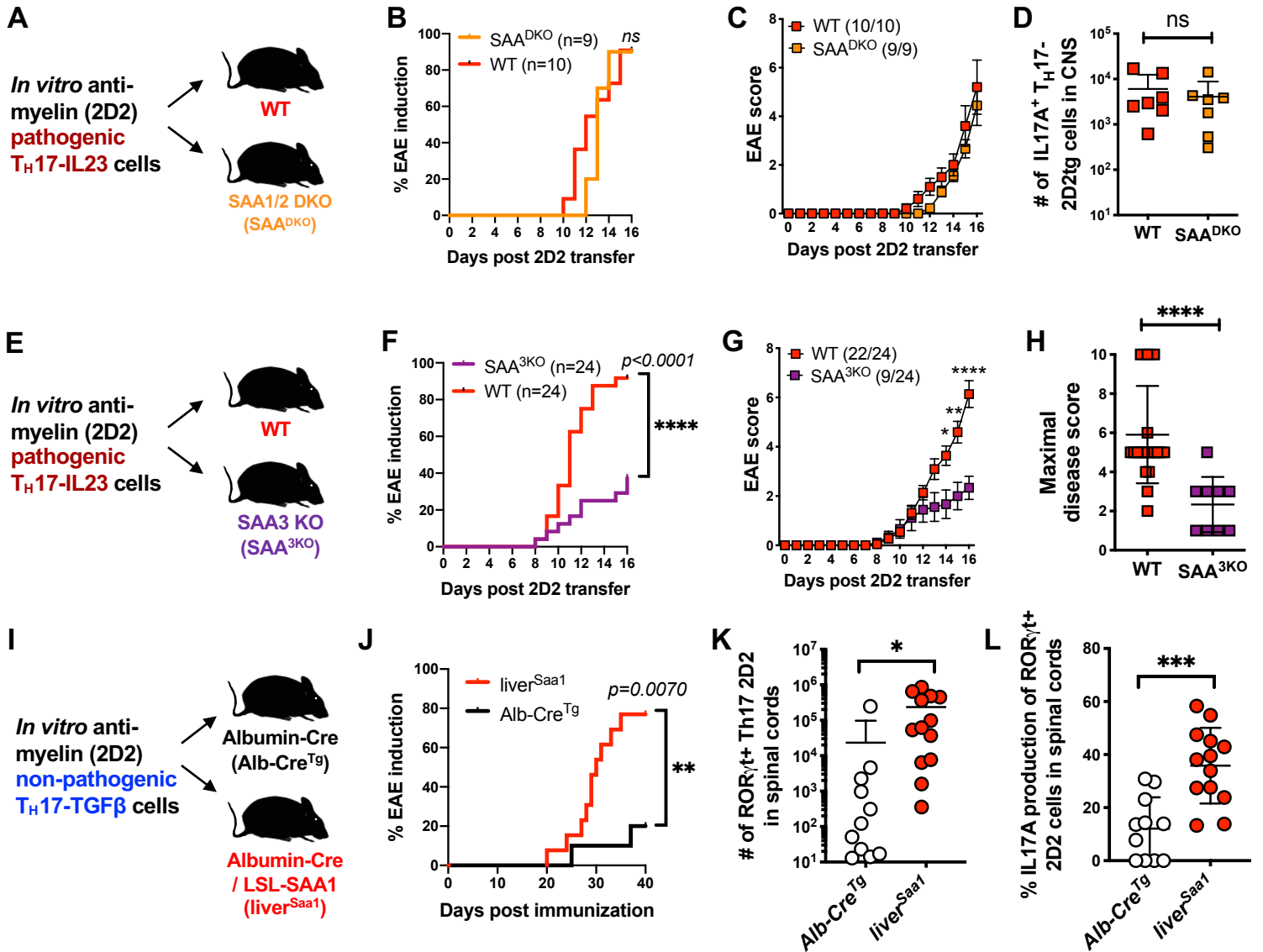
**(D)** Normalized expression of *Saa* isotypes in the spinal cord at day 15 post MOG-immunization.

**(E and F)** Expression of SAAs in myeloid cells of the CNS. In order to distinguish microglia (DsRed<sup>+</sup>) from infiltrating monocyte-derived macrophages, *Cx3cr1<sup>creER</sup>:R26-DsRed* mice were injected with tamoxifen at day 28 and day 30, then rested for 30 days before MOG-immunization. RNAseq was performed on sort-purified microglia and infiltrating monocytes. Normalized RNAseq counts of *Saa* isotypes in microglia **(E)** and monocyte **(F)** isolated from CNS of WT mice at the indicated stages of EAE. Data for each condition are the mean of 2 biological replicates.

**(G)** Representative confocal image of spinal cord cross sections illustrating the specificity of the  $\alpha$ -SAA3 Ab used. IBA1 (red), SAA3 (green), and CD4 (aqua).

**(A, B, and D)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. \*\*p < 0.01, and \*\*\*p < 0.001.

**Figure 6**



## Figure 6. SAA regulation of pathogenicity of *in vitro*-differentiated encephalitogenic T<sub>H</sub>17 cells

**(A-D)** Examination of EAE development and the 2D2tg cell response in SAA<sup>DKO</sup> and WT littermate recipients of 2D2tg T<sub>H</sub>17-IL-23 cells. Summary of 2 experiments, with SAA<sup>DKO</sup> (orange, n = 9) and WT (red, n = 10) mice. Experimental scheme (**A**), EAE incidence (**B**) and mean score (**C**), and number of 2D2tg IL17A<sup>+</sup> T<sub>H</sub>17 cells in spinal cords (**D**).

**(E-H)** Examination of EAE development and the 2D2tg cell response in SAA<sup>3KO</sup> and WT littermate recipients of 2D2tg T<sub>H</sub>17-IL-23 cells. Summary of 4 experiments, with SAA<sup>3KO</sup> (purple, n = 24) and WT (red, n = 24) mice. Experimental scheme (**E**), EAE incidence (**F**) and mean score (**G**), and maximal disease score (**H**).

**(I-L)** Examination of EAE development and the 2D2tg cell response in liver-specific SAA1tg (liver<sup>SAA1</sup>) and control Alb-Cre<sup>tg</sup> littermates following transfer of 2D2tg T<sub>H</sub>17-TGFb cells. Summary of 2 experiments, with liver<sup>SAA1</sup> (red circles, n = 13) and Alb-Cre<sup>tg</sup> (white circles, n = 11) mice. Experimental scheme (**I**), incidence of EAE onset (**J**), number of 2D2tg RORγt<sup>+</sup> T<sub>H</sub>17 cells (**K**) and frequency of IL17A<sup>+</sup> cells amongst RORγt<sup>+</sup> T<sub>H</sub>17 cells in spinal cords at day 40 post-adoptive transfer (**L**).

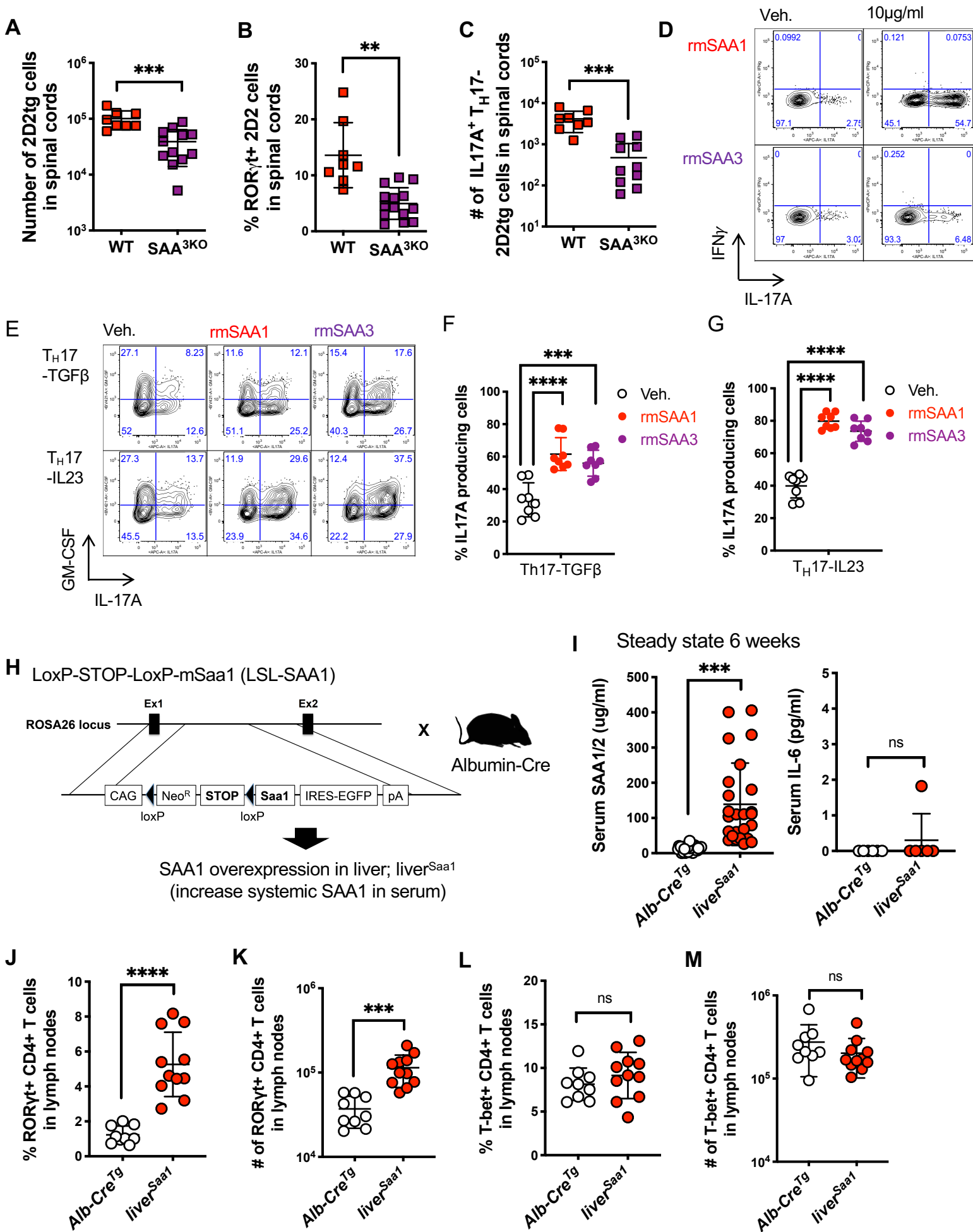
**(B, F, and J)** Statistics were calculated by log-rank test using the Mantel-Cox method.

**(C and G)** Statistics were calculated using the two-stage step-up method of Benjamini, Krieger and Yekutieliun. Error bars denote the mean ± s.e.m. \*p < 0.05, \*\*p < 0.01, and \*\*\*\*p < 0.0001,

**(D, H, K, and L)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean ± s.d. ns = not significant, \*p < 0.05, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

See also Figure **S6**.

**Figure S6**



**Figure S6. Role of SAAs in the T<sub>H</sub>17-dependent 2D2 transfer EAE model.**

**(A-C)** Total number **(A)**, percentage that are ROR $\gamma$ <sup>t</sup> T<sub>H</sub>17 **(B)** and quantification of IL-17A<sup>+</sup> **(C)** transferred 2D2tg donor cells in the CNS of WT (red boxes, n = 8) or SAA<sup>3KO</sup> (purple boxes, n = 9) recipients on day 17 post-adoptive transfer. Summary of 3 experiments.

**(D)** In vitro Th17 cell differentiation in response to SAA1 versus SAA3. The experiment was conducted based on the scheme in Figure 1A. Expression of IL-17A versus IFN- $\gamma$  in *in vitro* polarized CD4<sup>+</sup> T cell with IL-6 and TGF $\beta$  neutralizing antibodies  $\pm$  rmSAA1 (10 $\mu$ g/ml) or  $\pm$  rmSAA3 (10 $\mu$ g/ml) following restimulation.

**(E-G)** Comparison of cytokine production following different Th17 cell differentiation conditions. Experiments were conducted based on the scheme in Figure 1A. **(E)** Expression of IL-17A versus GM-CSF in *in vitro* polarized CD4<sup>+</sup> T cell with T<sub>H</sub>17-TGF $\beta$  (Top: IL6 + TGF $\beta$ ) or T<sub>H</sub>17-IL23 (Bottom: IL-6 + IL-1 $\beta$  + IL-23) conditions  $\pm$  rmSAA1 (10 $\mu$ g/ml) or  $\pm$  rmSAA3 (10 $\mu$ g/ml) following restimulation. **(F and G)** Summary of IL-17A frequency. Summary of 4 experiments, with 8 biological replicates.

**(H)** Schematic of conditional *Saa1* targeting construct and generation of liver<sup>Saa1</sup> mouse.

**(I)** Serum concentrations of SAAs and IL-6 in liver-overexpressing mice. Left: SAA1/2 in serum of liver<sup>Saa1</sup> (red circles, n = 27) or Alb-Cre<sup>Tg</sup> (white circles, n = 25) and right: IL-6 liver<sup>Saa1</sup> (red circles, n = 6) or Alb-Cre<sup>Tg</sup> (white circles, n = 6) in 6-week-old mice.

**(J and K)** Percent **(J)** and total number **(K)** of ROR $\gamma$ <sup>t</sup> T<sub>H</sub>17 cells amongst Foxp3<sup>Neg</sup> CD44<sup>hi</sup> CD4<sup>+</sup> T cells in secondary lymph nodes of 6-week-old mice with liver<sup>Saa1</sup> (red circles, n = 10) or Alb-Cre<sup>Tg</sup> (white circles, n = 10) summarized over two experiments.

**(L and M)** As in **(J and K)**, but of Tbet<sup>+</sup> T<sub>H</sub>1 cells.

**(A-G and I-M)** Statistics were calculated using the unpaired two-sided Welch's t-test. Error bars denote the mean  $\pm$  s.d. ns = not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.