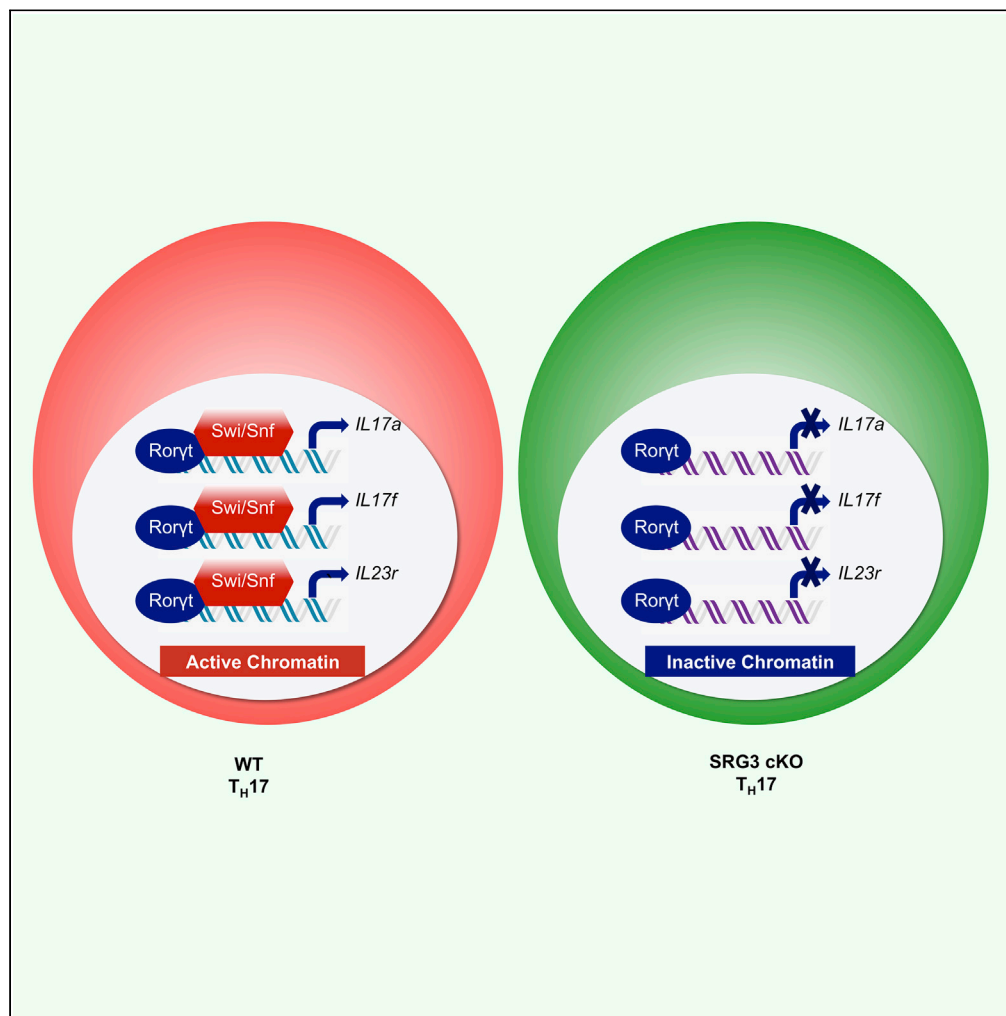


## Article

ROR $\gamma$ t-driven T<sub>H</sub>17 Cell Differentiation Requires Epigenetic Control by the Swi/Snf Chromatin Remodeling Complex

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## HIGHLIGHTS

The Swi/Snf complex plays essential roles for T<sub>H</sub>17 differentiation

SRG3/mBAF155 deficiency abrogates the expression of major target genes of ROR $\gamma$ t

ROR $\gamma$ t-dependent T<sub>H</sub>17 transcriptional program requires the Swi/Snf complex

The Swi/Snf complex is required for ROR $\gamma$ t-driven histone modifications

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## Article

ROR $\gamma$ t-driven T<sub>H</sub>17 Cell Differentiation Requires Epigenetic Control by the Swi/Snf Chromatin Remodeling ComplexSungkyu Lee,<sup>1</sup> Jieun Kim,<sup>1</sup> Hyungyu Min,<sup>1</sup> and Rho H. Seong<sup>1,2,\*</sup>

## SUMMARY

Epigenetic regulation, including chromatin accessibility and posttranslational modifications of histones, is of importance for T cell lineage decision. T<sub>H</sub>17 cells play a critical role in protective mucosal immunity and pathogenic multiple autoimmune diseases. The differentiation of T<sub>H</sub>17 cells is dictated by a master transcription factor, ROR $\gamma$ t. However, the epigenetic mechanism that controls T<sub>H</sub>17 cell differentiation remains poorly understood. Here we show that the Swi/Snf complex is required for T<sub>H</sub>17-mediated cytokine production both *in vitro* and *in vivo*. We demonstrate that ROR $\gamma$ t recruits and forms a complex with the Swi/Snf complex to cooperate for the ROR $\gamma$ t-mediated epigenetic modifications of target genes, including both permissive and repressive ones for T<sub>H</sub>17 cell differentiation. Our findings thus highlight the Swi/Snf complex as an essential epigenetic regulator of T<sub>H</sub>17 cell differentiation and provide a basis for the understanding of how a master transcription factor ROR $\gamma$ t collaborates with the Swi/Snf complex to govern epigenetic regulation.

## INTRODUCTION

The differentiation of naive CD4<sup>+</sup> T cells into functionally distinct effector T<sub>H</sub> cell subsets, including T<sub>H</sub>1, T<sub>H</sub>2, T<sub>H</sub>9, and T<sub>H</sub>17, is an essential process for adaptive immunity (Zhu et al., 2010). The functional specialization is directed by induction of distinct master transcription factors, depending on the external cues from a diverse array of pathogenic agents. In addition, dynamic changes in chromatin structure and histone modifications are also considered a key underlying mechanism that directs lineage-specific gene expression (Lim et al., 2013). Gaining insight into the mechanisms by which master transcription factors and epigenetic changes establish and maintain lineage-specific programs of gene expression is an area of intense interest.

Interleukin-17 (IL-17)-producing T<sub>H</sub> cells (T<sub>H</sub>17 cells) play important roles in protective mucosal immunity against extracellular pathogens and also promote autoimmune and chronic inflammation (Korn et al., 2009; Muranski and Restifo, 2013; Stockinger and Omenetti, 2017; Weaver et al., 2013). T<sub>H</sub>17 cell can be differentiated *in vitro* either by transforming growth factor  $\beta$  (TGF- $\beta$ ) and IL-6 (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006) (hereafter referred to as T<sub>H</sub>17( $\beta$ )) or by IL-6, IL-1 $\beta$ , and IL-23 (Ghoreschi et al., 2010) (hereafter referred to as T<sub>H</sub>17(23)) to mimic distinct functional subsets. Identification of key transcription factors and a coherent regulatory network has contributed to the understanding of T<sub>H</sub>17 cell differentiation (Ciofani et al., 2012). Although additional transcription factors are required to promote full T<sub>H</sub>17 differentiation program (Ciofani et al., 2012; Oestreich and Weinmann, 2012), retinoic acid-related orphan receptor  $\gamma$ t (ROR $\gamma$ t) is considered as the master transcription factor for T<sub>H</sub>17 cell differentiation that is necessary and sufficient to induce IL-17A expression (Ivanov et al., 2006). Moreover, ROR $\gamma$ t-driven T<sub>H</sub>17 transcriptional program is essential for the expression of a core subset of T<sub>H</sub>17 signature genes, including IL-17F and IL-23R as well as IL-17A (Ciofani et al., 2012; Wang et al., 2015).

Numerous studies have reported that epigenetic programming commanded by master transcription factors is key to cellular differentiation (Boller et al., 2016; Ghisletti et al., 2010; Heinz et al., 2010; Johnson et al., 2018; Natoli, 2010; Sartorelli and Puri, 2018). It is not clear yet whether the mechanism by which ROR $\gamma$ t controls target genes is a simple transcriptional regulation or whether ROR $\gamma$ t plays a more fundamental role for establishing permissive chromatin environments by actively remodeling chromatin structure. If

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the latter is the case, it also remains to be determined which enzymes capable of altering chromatin structure are required to mediate ROR $\gamma$ t-driven epigenetic regulation.

The Swi/Snf chromatin remodeling complex is a group of epigenetic regulators that physically remodel DNA-nucleosomal architecture to regulate gene expression with the energy derived from ATP hydrolysis (de la Serna et al., 2006; Mathur and Roberts, 2018). The Swi/Snf complex is composed of multiple subunits including Brg1 with ATPase activity, Srg3/mBaf155, Baf170, and Baf47/Snf5 as the core subunits of the complex. SRG3, a murine homolog of human BAF155, serves as a scaffold protein that controls the stability of the Swi/Snf complex through direct interaction with the other subunits of the complex (Panamarova et al., 2016; Sohn et al., 2007). As no components of the Swi/Snf complex have intrinsic DNA sequence specificity, the Swi/Snf complex is recruited to its genomic targets by sequence-specific transcription factors and serves as a coactivator for transcriptional activation.

In this study, we uncover the Swi/Snf complex as a critical epigenetic regulator in T<sub>H</sub>17 cell differentiation. Specifically, unbiased transcriptomic analyses comparing wild-type (WT) and SRG3-deficient T<sub>H</sub>17-polarized cells reveal that loss of SRG3 expression results in the specific downregulation of ROR $\gamma$ t target genes such as IL-17A, IL-17F, and IL-23R. We also reveal that ROR $\gamma$ t augments the accumulation of the Swi/Snf complex in *IL17a-IL17f* and *IL-23R* gene loci and functions as a key epigenetic regulator of those T<sub>H</sub>17 signature genes. Indeed, the Swi/Snf complex serves an indispensable role for T<sub>H</sub>17 cell differentiation by coordinating multiple layers of ROR $\gamma$ t-mediated epigenetic program to govern histone modifications.

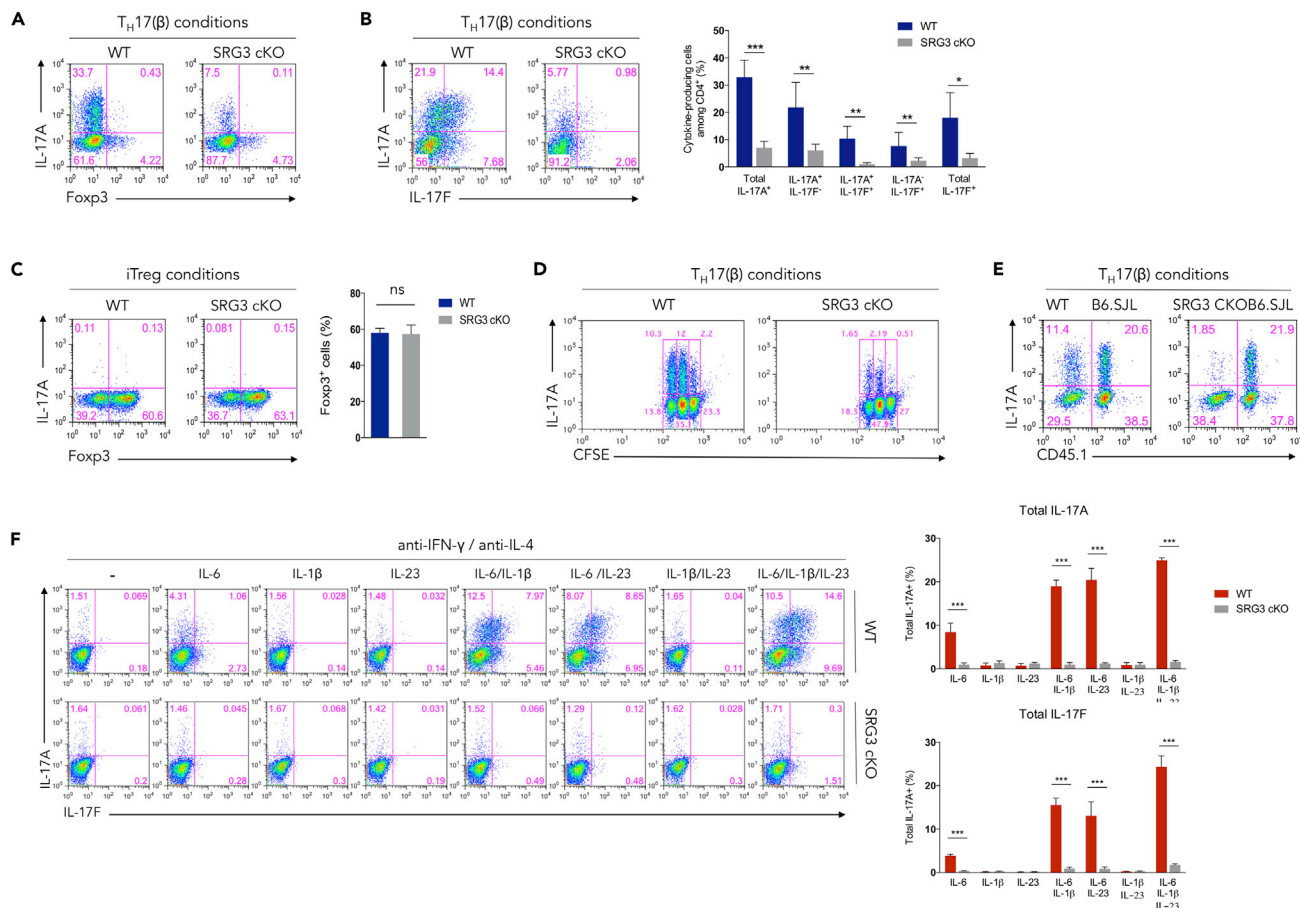
## RESULTS

### The Swi/Snf Complex Is Essential for *In Vitro* T<sub>H</sub>17 Differentiation

To investigate the role of the Swi/Snf complex in T<sub>H</sub>17 differentiation, we generated mice with conditional deficiency of SRG3 in CD4<sup>+</sup> T cells (*CD4<sup>Cre</sup>SRG3<sup>fl/fl</sup>* or *SRG3 cKO*) by crossing *SRG3<sup>fl/fl</sup>* mice (Choi et al., 2012) to mice expressing Cre recombinase from the CD4 promoter (*CD4<sup>Cre</sup>* mice). As reported in our previous study (Choi et al., 2015), in comparison with their WT littermates (*CD4<sup>Cre</sup>SRG3<sup>wt/wt</sup>*), SRG3-deficient mice exhibited comparable characteristics in terms of the frequencies and numbers of peripheral CD4/CD8 T cells, the ratio of naive to memory CD4<sup>+</sup> T cells, and their rate of proliferation. These allowed for further analysis of effector differentiation. Naive CD4<sup>+</sup> T cells were isolated from *SRG3 cKO* mice and their WT littermates and differentiated under T<sub>H</sub>17( $\beta$ ) conditions. We found that SRG3-deficient CD4<sup>+</sup> T cells showed a marked reduction in IL-17A and IL-17F production compared with WT CD4<sup>+</sup> T cells (Figures 1A and 1B). In addition, knockdown of BRG1 by retroviral transduction greatly reduced IL-17A production, indicating that BRG1 is also required for T<sub>H</sub>17 differentiation (Figure S1). Contrary to the remarkable decrease in the expression of IL-17A and IL-17F, SRG3-deficient CD4<sup>+</sup> T cells displayed a normal level of Foxp3 expression both in iT<sub>reg</sub> and T<sub>H</sub>17( $\beta$ ) conditions (Figures 1A and 1C). These results, given the possible reciprocal regulation of differentiation between iT<sub>reg</sub> and T<sub>H</sub>17 cells (Bettelli et al., 2006; Zhou et al., 2008), indicate that the defect of T<sub>H</sub>17 cell differentiation in SRG3-deficient CD4<sup>+</sup> T cells was not attributed to dysregulation of iT<sub>reg</sub> cell differentiation. In addition, we found that SRG3-deficient CD4<sup>+</sup> T cells were indistinguishable from WT CD4<sup>+</sup> T cells in proliferative response, as monitored by CFSE staining, which indicates that the impaired production of IL-17A in SRG3-deficient CD4<sup>+</sup> T cells was not due to a defect in culture proliferation (Figure 1D). To assess whether the Swi/Snf complex plays a cell-intrinsic role in T<sub>H</sub>17 differentiation, we mixed WT or SRG3-deficient CD4<sup>+</sup> T cells (CD45.1<sup>-</sup>) with congenic naive CD4<sup>+</sup> T cells (CD45.1<sup>+</sup>) and cultured them in T<sub>H</sub>17( $\beta$ ) conditions (Figure 1E). We found a cell-intrinsic defect in the production of IL-17A in SRG3-deficient CD4<sup>+</sup> T cells, indicative again of the Swi/Snf complex as an essential cell-intrinsic factor for T<sub>H</sub>17 differentiation. To further confirm the importance of the Swi/Snf complex in T<sub>H</sub>17 cell differentiation, we cultured WT and SRG3-deficient CD4<sup>+</sup> T cells under pathogenic T<sub>H</sub>17(23) conditions with individual cytokines or several cytokine combinations (Figure 1F). Robust IL-17A and IL-17F production were detected in WT CD4<sup>+</sup> T cells under three combinations of cytokines (IL-6/IL-1 $\beta$ , IL-6/IL-23, and IL-6/IL-1 $\beta$ /IL-23), whereas SRG3 deficiency severely diminished IL-17A and IL-17F production in all conditions. These results indicate that the Swi/Snf complex is essential for T<sub>H</sub>17 cell differentiation *in vitro*.

### The Swi/Snf Complex Is Required for the *In Vivo* Generation of T<sub>H</sub>17 Cells

We next examined gut-resident homeostatic T<sub>H</sub>17 cells in WT and SRG3-deficient mice at steady state (Figure 2A). A substantial proportion of WT CD4<sup>+</sup> T cells in the small intestinal lamina propria (SI LP) expressed IL-17A alone or both IL-17A and IL-17F. However, SRG3-deficient SI LP CD4<sup>+</sup> T cells showed a marked



**Figure 1. The Swi/Snf Complex Is Essential for the In Vitro TH17 Differentiation**

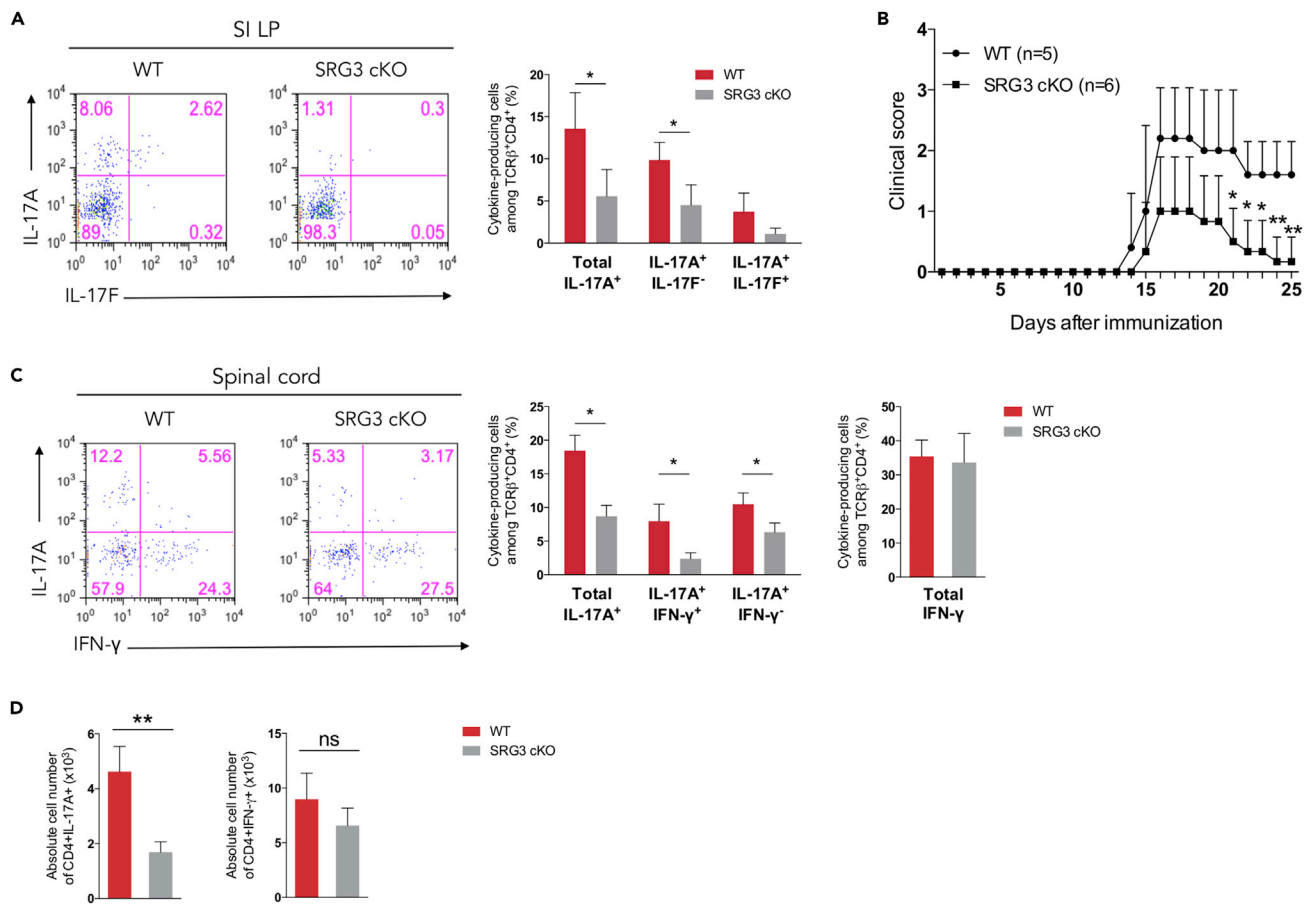
(A–C and F) Flow cytometry of intracellular staining for IL-17A/Foxp3 (A and C) or IL-17A/IL-17F (B and F) in WT or SRG3 cKO naive CD4<sup>+</sup> T cells cultured under  $T_H17(\beta)$  conditions (A and B), iTreg conditions (C), or in the presence of cytokines as indicated (F) for 60 h and restimulated with PMA/ionomycin. (D and E) (D) Flow cytometry of intracellular staining for IL-17A in CFSE-labeled WT or SRG3 cKO naive CD4<sup>+</sup> T cells cultured under  $T_H17(\beta)$  conditions for 60 h followed by restimulation. (E) Flow cytometry of intracellular staining for IL-17A in CD45.1<sup>+</sup> WT naive CD4<sup>+</sup> T cells plus CD45.1<sup>+</sup> WT or SRG3 cKO naive CD4<sup>+</sup> T cells cultured under  $T_H17(\beta)$  conditions for 60 h followed by restimulation. Data are representative of four (A and C), six (B), or two (D–F) independent experiments with consistent results. (B, C, and F) Representative FACS data are shown in the left or top panel, and pooled data with mean  $\pm$  SD from six (B), three (C), or four (F) independent experiments are shown on the right or bottom. Statistical analysis was performed using unpaired two-tailed t test (\* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001).

reduction of IL-17A and IL-17F production. Thus, the Swi/Snf complex is indispensable for the generation of a subset of  $T_H17$  cells in SI LP.

Next, we immunized SRG3-deficient and WT littermate control mice with myelin oligodendrocyte glycoprotein (MOG) peptide to induce experimental autoimmune encephalomyelitis (EAE) in which  $T_H17$  cells are the major pathogenic population. SRG3-deficient mice exhibited remarkably reduced EAE incidence and severity compared with WT mice (Figure 2B). Analysis of spinal cord infiltrates revealed that IL-17A production was also greatly reduced in immunized SRG3-deficient mice, whereas IFN- $\gamma$  levels were largely unaltered (Figures 2C and 2D). Given that commensal microbiota has been implicated in the generation of IL-17-producing T cells in the intestine and spinal cord (Lee et al., 2011), we cannot rule out that altered microbiota may contribute to an unfavorable environment for IL-17 production in SRG3-deficient mice. However, these data collectively show a central role for the Swi/Snf complex in both *in vivo* and *in vitro* differentiation of  $T_H17$  cells.

### The Loss of SRG3 Impairs the Expression of ROR $\gamma$ t Target Genes

To identify the basis for the defects of  $T_H17$  differentiation in SRG3-deficient CD4<sup>+</sup> T cells, we performed three biological replicate microarray analysis comparing WT and SRG3-deficient CD4<sup>+</sup> T cells cultured



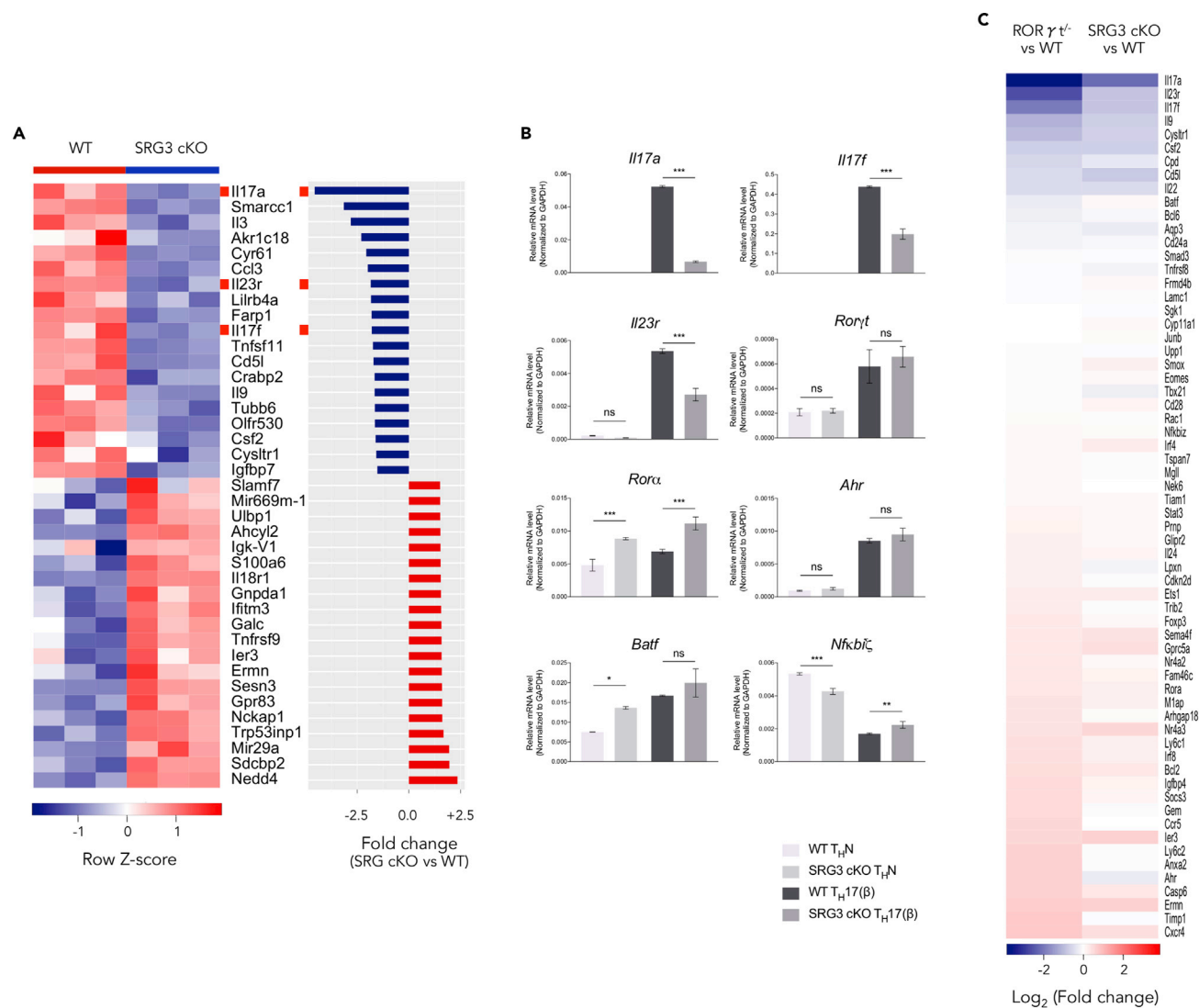
**Figure 2. The Swi/Snf Complex Is Required for the In Vivo Generation of TH17 Cells**

(A) Flow cytometry of intracellular staining for IL-17A/IL-17F in CD4<sup>+</sup> T cells isolated from lamina propria in WT or SRG3 cKO mice and stimulated with PMA/ionomycin for 4 h. (B) EAE disease scores in WT and SRG3 cKO mice. (C and D) Flow cytometry of intracellular staining for IL-17A/IFN-γ or the absolute number of IL-17A<sup>+</sup> or IFN-γ cells in WT or SRG3 cKO CD4<sup>+</sup> T cells isolated from spinal cords 21 days after EAE induction and stimulated with PMA/ionomycin for 4 h. (A and C) Representative FACS data are shown in the left panel and pooled data with mean ± SD from four (A) or three (C and D) independent experiments are shown on the right. Statistical analysis was performed using unpaired two-tailed t test (\*p < 0.05, \*\*p < 0.01).

under TH17(β) conditions (Figure 3A). We identified a total of only 39 genes with more than 1.5-fold differential expression in SRG3-deficient TH17(β)-polarized cells compared with WT TH17(β)-polarized cells. Of note, *IL17a* was topping the list of downregulated genes in SRG3-deficient TH17(β)-polarized cells. Moreover, other TH17 signature genes, *IL17f* and *IL23r*, were prominently featured in the downregulated gene category. These results also indicate that the failure to produce IL-17A and IL-17F in SRG3-deficient TH17-polarized cells lies at the level of cellular transcription.

Verification of the expression of a subset of TH17 signature genes by quantitative PCR with reverse transcription (qRT-PCR) confirmed that mRNA expression of *IL17a*, *IL17f*, and *IL23r* is consistently much lower in SRG3-deficient TH17(β)-polarized cells than in WT counterparts (Figure 3B). However, the expression of *RORγt* mRNA was comparable between WT and SRG3-deficient cells. In addition, the mRNA expression of other transcription factors that facilitate TH17 differentiation, including *RORα*, *Nfkbiz*, *Batf*, and *Ahr*, was also largely unchanged by the loss of SRG3. We also validated that RORγt protein expression and nuclear localization are similar between WT and SRG3-deficient TH17-polarized cells (Figure S2).

Considering reduced expression of RORγt target genes in SRG3-deficient TH17-polarized cells despite the normal induction of RORγt expression, we then decided to further clarify the relationship between RORγt and the Swi/Snf complex by performing the comparative analysis of the transcription profiles on a restricted panel of genes involved in the TH17 cell biology (Hasan et al., 2017; Wu et al., 2013) from WT, SRG3-



**Figure 3. The Loss of SRG3 Impairs the Expression of ROR $\gamma$ t Target Genes**

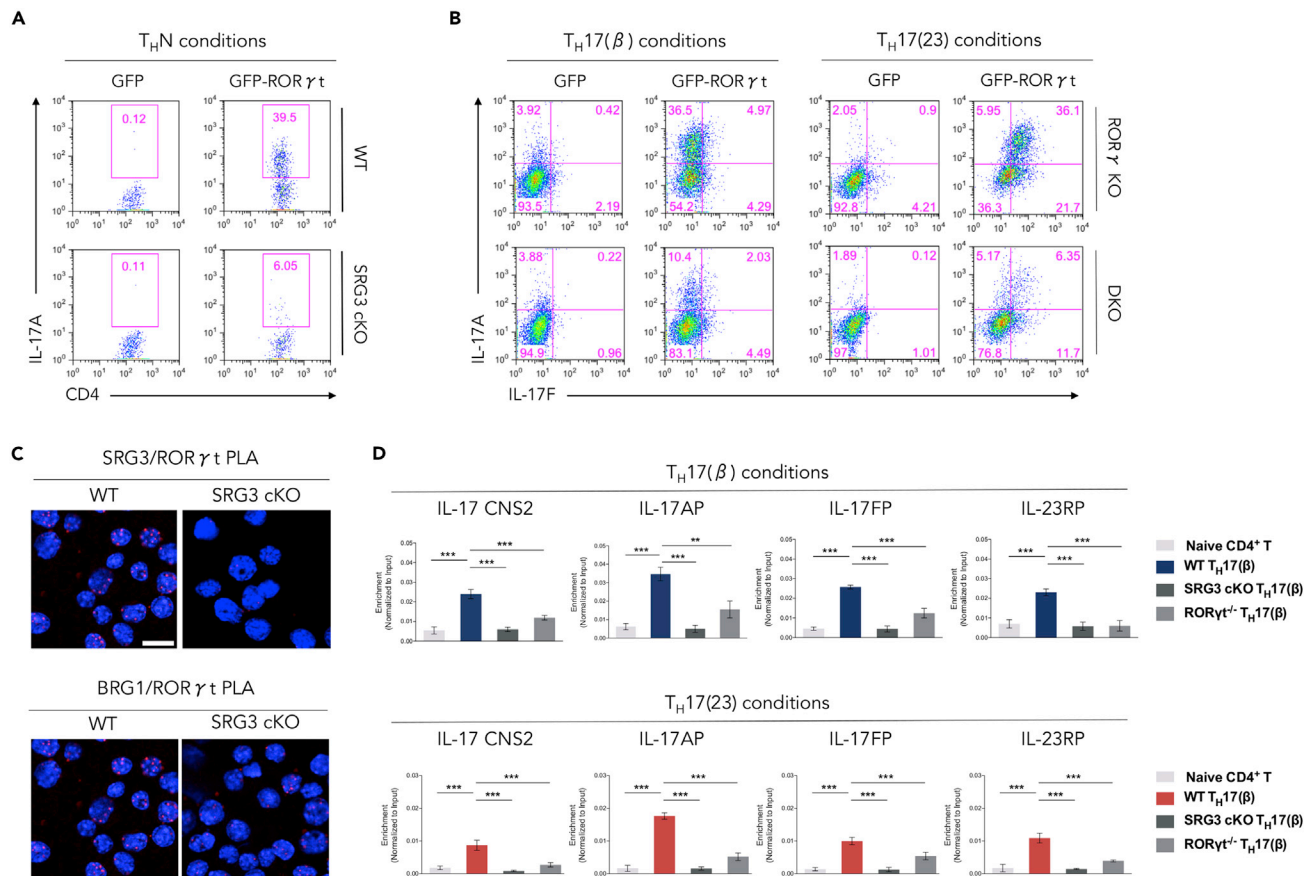
(A) Heatmap of relative expression (Z score) (left) and gene expression fold change (right) displaying microarray analysis of WT and SRG3 cKO naive CD4<sup>+</sup> T cells cultured under T<sub>H</sub>17(β) conditions for 60 h. Genes with at least 1.5-fold change are shown.

(B and C) (B) qRT-PCR analysis of WT or SRG3 cKO naive CD4<sup>+</sup> T cells cultured under T<sub>H</sub>1N or T<sub>H</sub>17(β) conditions. Error bars, SD. Statistical analysis was performed using one-way ANOVA. (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001; ns, not significant) (C) Heatmap displaying microarray data, log<sub>2</sub>(fold change) of T<sub>H</sub>17-related gene subsets in the two experimental settings: ROR $\gamma$ t<sup>-/-</sup> versus WT and SRG3 cKO versus WT T<sub>H</sub>17(β)-polarized cells. (A and C) Data represent the combined analysis of three biologically independent samples.

deficient, and ROR $\gamma$ t-deficient T<sub>H</sub>17(β)-polarized cells. Surprisingly, microarray analysis of SRG3-deficient versus WT T<sub>H</sub>17(β)-polarized cells exhibited a marked overlap with ROR $\gamma$ t-deficient versus WT T<sub>H</sub>17(β)-polarized cells in differentially regulated genes (Figure 3C), implying the possibility that the activation of ROR $\gamma$ t-dependent core T<sub>H</sub>17 transcriptional program might rely on the Swi/Snf complex.

### The Activation of ROR $\gamma$ t-Dependent T<sub>H</sub>17 Transcriptional Program Requires the Swi/Snf Complex

To address this issue, we directly investigated whether SRG3 deficiency compromises the functional activity of ROR $\gamma$ t in T<sub>H</sub>17 differentiation. We first employed retroviral transduction to deliver ROR $\gamma$ t to naive CD4<sup>+</sup> T cells to compare the ability of ROR $\gamma$ t to induce IL-17A expression in WT and SRG3-deficient CD4<sup>+</sup> T cells. In the absence of exogenous T<sub>H</sub>17-polarizing cytokines (T<sub>H</sub>1N conditions), ectopic expression of ROR $\gamma$ t induced 39.5% IL-17A production, compared with only 0.12% IL-17A production by a control retrovirus



**Figure 4. The Activation of ROR $\gamma$ t-dependent  $T_H$ 17 Transcriptional Program Requires the Swi/Snf Complex**

(A) Flow cytometry of intracellular staining for IL-17A in WT or SRG3 cKO naive CD4<sup>+</sup> T cells transduced with control retrovirus expressing GFP only (GFP) or retrovirus encoding GFP and ROR $\gamma$ t (GFP-ROR $\gamma$ t) and cultured under  $T_H$ N conditions followed by restimulation. (B) Flow cytometry of intracellular staining for IL-17A/IL-17F in ROR $\gamma$ t<sup>-/-</sup> or ROR $\gamma$ t<sup>-/-</sup> SRG3 cKO (DKO) naive CD4<sup>+</sup> T cells transduced with control retrovirus (GFP) or retrovirus encoding GFP and ROR $\gamma$ t (GFP-ROR $\gamma$ t) and cultured under  $T_H$ 17( $\beta$ ) (left) or  $T_H$ 17(23) (right) conditions followed by restimulation. (A and B) Data are representative of three independent experiments with consistent results. Dot plots are gated on CD4<sup>+</sup>GFP<sup>+</sup>. (C) Proximity ligation assay (PLA) in WT or SRG3 cKO naive CD4<sup>+</sup> T cells cultured under  $T_H$ 17( $\beta$ ) conditions using anti-ROR $\gamma$ t, anti-SRG3, or anti-BRG1 antibodies (blue, DAPI; red, PLA signal), scale bar (20  $\mu$ m). (D) ChIP-qPCR analysis of WT naive and WT, SRG3 cKO or ROR $\gamma$ t<sup>-/-</sup>  $T_H$ 17( $\beta$ )-polarized CD4<sup>+</sup> T cells (upper) or  $T_H$ 17(23)-polarized CD4<sup>+</sup> T cells (bottom) using anti-SRG3 antibody. (C and D) Data are representative of at least two independent experiments. Error bars, SD. Statistical analysis was performed using one-way ANOVA. (\*\*p < 0.01, \*\*\*p < 0.001).

(Figure 4A). Strikingly, SRG3-deficient CD4<sup>+</sup> T cells showed a marked reduction in IL-17A production (6.05%) upon ectopic expression of ROR $\gamma$ t. To further validate impaired ROR $\gamma$ t-directed IL-17 production in SRG3-deficient CD4<sup>+</sup> T cells in the presence of  $T_H$ 17-polarizing cytokines, we used ROR $\gamma$ -deficient or ROR $\gamma$ /SRG3 double-deficient (DKO) CD4<sup>+</sup> T cells. Again, re-expression of ROR $\gamma$ t rescued IL-17A/IL-17F production in ROR $\gamma$ t-deficient cultures, whereas it failed to potentiate IL-17A and IL-17F production in DKO cultures under both  $T_H$ 17( $\beta$ ) and  $T_H$ 17(23) conditions (Figure 4B). Thus, the Swi/Snf complex is required for ROR $\gamma$ t to activate *IL17a* and *IL17f* gene expression.

### The Swi/Snf Complex Physically Interacts with ROR $\gamma$ t

We next examined the possibility that the Swi/Snf complex might be in close proximity to ROR $\gamma$ t and form a functional complex during  $T_H$ 17 differentiation. To validate this, we performed the proximity ligation assay (PLA), a sensitive technique to visualize protein-protein interactions via a fluorescent signal when proteins neighbor each other. We found that SRG3 and BRG1 are in close proximity with ROR $\gamma$ t *in situ* in WT  $T_H$ 17( $\beta$ )-polarized cells, indicating that the Swi/Snf complex interacts with ROR $\gamma$ t (Figure 4C). We detected no PLA signal between ROR $\gamma$ t and SRG3 in SRG3-deficient  $T_H$ 17( $\beta$ )-polarized cells as a negative control. In addition, the PLA signals between BRG1 and ROR $\gamma$ t were substantially reduced in SRG3-deficient  $T_H$ 17( $\beta$ )-

polarized cells compared with WT T<sub>H</sub>17(β)-polarized cells, which could result from the decreased expression of BRG1 in the absence of SRG3 (Figure S2A). In addition, we confirmed the interaction between RORγt and the Swi/Snf complex through conventional immunoprecipitation assay followed by immunoblot analysis (Figure S3). Taken together, our results demonstrate the physical association of the Swi/Snf complex and RORγt in T<sub>H</sub>17 cells.

### The Swi/Snf Complex Directly Targets *IL17a-IL17f* and *IL-23r* Loci in an RORγt-Dependent Manner

To investigate whether the Swi/Snf complex directly targets *IL17a-IL17f* and *IL-23r* loci, we performed the chromatin immunoprecipitation assay followed by quantitative PCR (ChIP-qPCR). These loci were chosen because they were among the most differentially expressed T<sub>H</sub>17-specific genes in SRG3-deficient T<sub>H</sub>17-polarized cells compared with WT T<sub>H</sub>17-polarized cells. We observed that SRG3 bound to *IL17* conserved noncoding region 2 (*IL17* CNS2, essential for *IL17a-IL17f* transcription [Wang et al., 2012]), the *IL17a* promoter (IL-17AP), *IL17f* promoter (IL-17FP), and *IL23r* promoter (IL-23RP) in both WT T<sub>H</sub>17(β)-polarized and WT T<sub>H</sub>17(23)-polarized cells (Figure 4D). Of note, we observed a substantial reduction of the occupancy of SRG3 in the absence of RORγt, indicating that RORγt increases the recruitment of the Swi/Snf complex to these sites.

### The Pattern of Alterations of Histone Modifications by the Loss of SRG3 Closely Parallels that by the Loss of RORγt

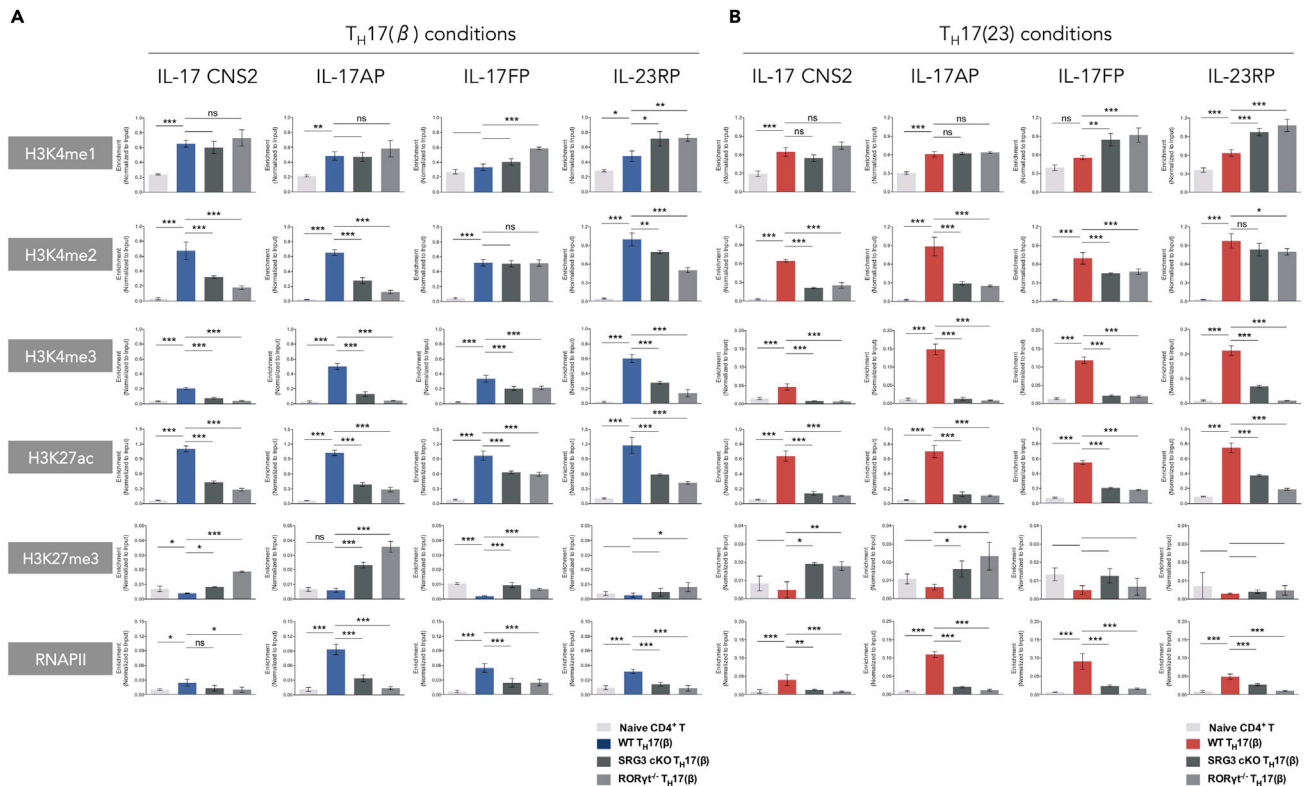
If RORγt and the Swi/Snf complex act through collaborative interactions, we would expect that epigenetic regulations mediated by either of them would be similar. Based on this hypothesis, we first examined the role of the Swi/Snf complex and RORγt in modulating chromatin accessibility. We used restriction enzyme accessibility assay coupled with ligation-mediated quantitative PCR (LM-qPCR) to assess the accessible regions in IL-17AP. The restriction enzymes XmnI, PstI, PvuII, and HaeIII were used to measure the accessibility (Figure S4). WT T<sub>H</sub>17(β)-polarized cells showed a great increase in chromatin accessibility in PstI-282 and PstI+758 sites compared with naive CD4<sup>+</sup> T cells. However, SRG3-deficient T<sub>H</sub>17(β)-polarized cells exhibited reduced accessibility compared with WT T<sub>H</sub>17(β)-polarized cells, indicating that the Swi/Snf complex is required to facilitate the accessible chromatin structure in IL-17AP. Interestingly, the two PstI sites in RORγt-deficient T<sub>H</sub>17(β)-polarized cells were as less accessible as were those in SRG3-deficient T<sub>H</sub>17(β)-polarized cells, which reveals the role of RORγt as an epigenetic regulator.

Chromatin accessibility is typically associated with permissive histone modifications. To characterize chromatin features accompanying the loss of SRG3 or RORγt, we focused on the deposition of six marks and factors: H3K4me1, H3K4me2, H3K4me3, H3K27ac, H3K27me3, and RNA polymerase II. H3K4me3 is a key feature of active promoters, whereas H3K4me1, H3K4me2, and H3K27ac are present in both promoters and enhancers (Cao and Wysocka, 2013; Heintzman et al., 2009). H3K27me3 is associated with gene repression (Cao et al., 2002). Naive CD4<sup>+</sup> T cells exhibited little constitutive enrichment for all the histone modifications tested, with the exception of considerable amounts of H3K4me1 (Figures 5A and 5B). Remarkably, WT T<sub>H</sub>17-polarized cells showed high levels of H3K4me2, H3K4me3, H3K27ac, and RNAPII at IL-17 CNS2, IL-17AP, IL-17FP, and IL-23RP in both T<sub>H</sub>17(β) and T<sub>H</sub>17(23) conditions, reflective of robust expression of those genes. Interestingly, the levels of these enrichments were severely diminished in RORγt-deficient T<sub>H</sub>17-polarized cells with the only exception of H3K4me2 at IL-17FP in T<sub>H</sub>17(β) conditions and IL-23RP in T<sub>H</sub>17(23) conditions. Furthermore, as was the case in the absence of RORγt, SRG3-deficient T<sub>H</sub>17-polarized cells exhibited similar, albeit to a lesser extent, reduction of enrichment. On the other hand, the absence of SRG3 or RORγt significantly increased the deposition of repressive H3K27me3 compared with WT T<sub>H</sub>17-polarized cells. The levels of H3K4me1 were largely independent of SRG3 or RORγt. Taken together, SRG3 and RORγt seem to make a substantial contribution to shaping the active epigenetic landscape. In addition, these data, along with results of restriction enzyme accessibility assay, reveal that the SRG3-deficient and RORγt-deficient T<sub>H</sub>17-polarized cells closely resemble each other in the altered patterns of epigenetic structure and modifications, strongly suggesting a high degree of correlation between RORγt and the Swi/Snf complex for epigenetic regulation.

### The Swi/Snf Complex Is Required for RORγt-Driven Histone Modifications

To directly investigate the ability of RORγt and a functional link between RORγt and the Swi/Snf complex in the histone modifications, we re-expressed RORγt in RORγt-deficient and RORγt/SRG3 double-deficient (DKO) CD4<sup>+</sup> T cells and cultured them under T<sub>H</sub>17(β) conditions followed by ChIP-qPCR (Figure 6).





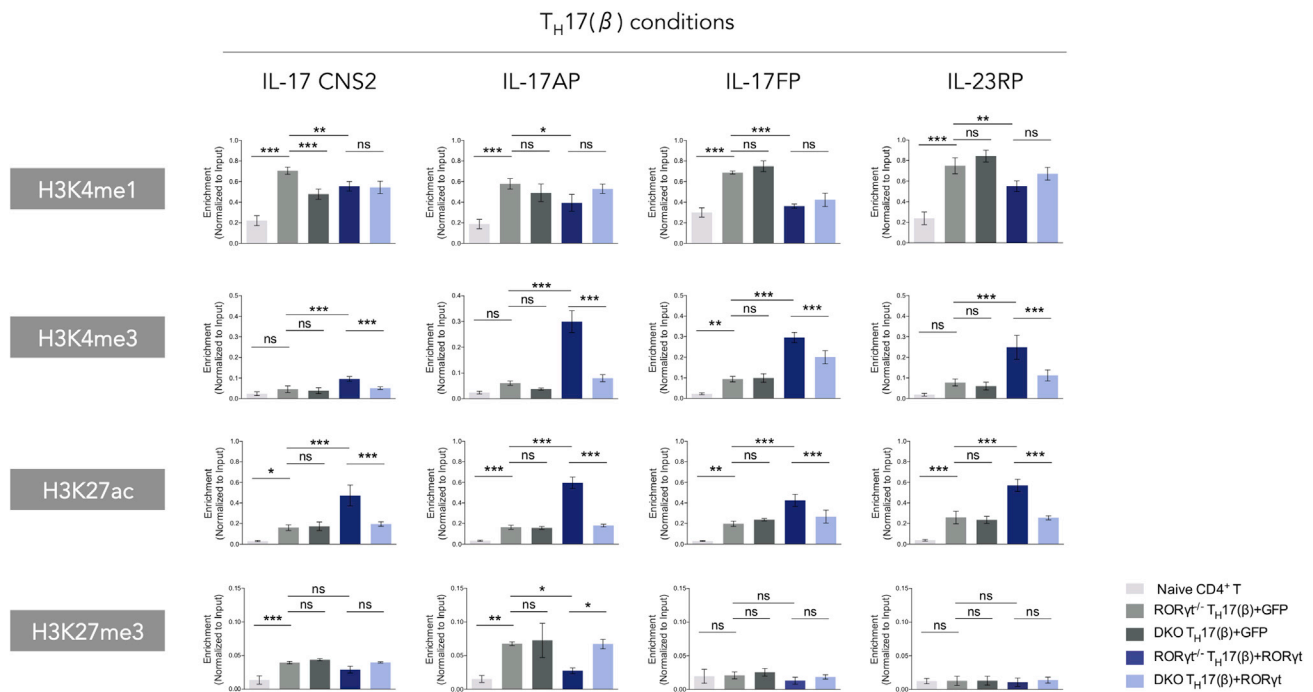
**Figure 5. The Pattern of Alterations of Histone Modifications by the Loss of SRG3 Closely Parallels That by the Loss of ROR $\gamma$ t** (A and B) ChIP-qPCR analysis of WT naive and WT, SRG3 cKO or ROR $\gamma$ t<sup>-/-</sup> T<sub>H</sub>17(β)-polarized CD4<sup>+</sup> T cells or T<sub>H</sub>17(23)-polarized CD4<sup>+</sup> T cells using anti-H3K4me1, anti-H3K4me2, anti-H3K4me3, anti-H3K27ac, anti-H3K27me3, and anti-RNA PolII-S2P antibodies. Data are representative of at least two independent experiments. Error bars, SD. Statistical analysis was performed using one-way ANOVA. (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001; ns, not significant).

Although ROR $\gamma$ t re-expression had little if any effect on the levels of H3K4me1, it led to a dramatic increase in the accumulation of H3K4me3 and H3K27ac and a significant decrease in the deposition of H3K27me3, which further confirm the active role of ROR $\gamma$ t for establishing epigenetic landscape. However, in the absence of SRG3, ROR $\gamma$ t re-expression was not able to rescue the induction of H3K4me3 and H3K27ac and the reduction of H3K27me3 in DKO T<sub>H</sub>17(β)-polarized cells, supporting a model wherein the Swi/Snf complex is required for ROR $\gamma$ t-mediated histone modifications and complete activation of T<sub>H</sub>17 signature genes.

## DISCUSSION

This study reveals a critical requirement for the Swi/Snf complex in T<sub>H</sub>17 cell differentiation. Although the Swi/Snf complex does not regulate the expression of T<sub>H</sub>17-related transcription factors including ROR $\gamma$ t, a master transcription factor of T<sub>H</sub>17 cells, we showed that defective T<sub>H</sub>17 differentiation of SRG3-deficient CD4<sup>+</sup> T cells is primarily due to impaired ROR $\gamma$ t activity to drive T<sub>H</sub>17 differentiation. Mechanistically, SRG3 loss results in the impaired expression of ROR $\gamma$ t target genes by preventing ROR $\gamma$ t-mediated histone modifications, which is a possible mechanism by which ROR $\gamma$ t contributes to transcriptional control of a core subset of T<sub>H</sub>17 signature genes such as IL-17A, IL-17F, and IL-23R. Our results thus uncover that the Swi/Snf complex partners with ROR $\gamma$ t and coordinates the epigenetic regulation of target genes of ROR $\gamma$ t and ultimately T<sub>H</sub>17 cell differentiation.

It has been recognized that ROR $\gamma$ t is a master transcription factor in T<sub>H</sub>17 cell differentiation (Ivanov et al., 2006). However, less is appreciated about how ROR $\gamma$ t integrates with and regulates epigenetic framework to control lineage-specific gene expression in T<sub>H</sub>17 cells in spite of its pivotal role in defining the T<sub>H</sub>17 transcriptional program. In general, a set of pioneer factors are needed to actively open the otherwise inherent repressive chromatin architecture and shape the epigenetic landscape during CD4<sup>+</sup> T cell differentiation, in turn allowing the binding of other transcriptional regulators in



**Figure 6. The Swi/Snf Complex Is Required for RORγt-Driven Histone Modifications**

ChIP-qPCR analysis of WT naive or, RORγt<sup>-/-</sup> or RORγt<sup>-/-</sup> SRG3 cKO (DKO) naive CD4<sup>+</sup> T cells transduced with control retrovirus (GFP) or retrovirus encoding GFP and RORγt (GFP-RORγt) and cultured under T<sub>H</sub>17(β) conditions using anti-H3K4me1, anti-H3K4me3, anti-H3K27ac, and anti-H3K27me3 antibodies. Data are representative of at least two independent experiments. Error bars, SD. Statistical analysis was performed using one-way ANOVA. (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001; ns, not significant).

differentiating T cells. In T<sub>H</sub>17 cells, BATF and IRF4 were recently shown to cooperatively serve as pioneer factors to regulate initial chromatin accessibility without help from the specialized chromatin remodeling enzymes, thereby setting up the genomic landscape for T<sub>H</sub>17 cell differentiation (Ciofani et al., 2012). Although chromatin remodeling appears to be initiated by the cooperation between BATF and IRF4, our present study reveals a number of intriguing aspects regarding the epigenetic role of RORγt in the regulation of IL-17 gene expression. First, we found unexpected critical contributions of RORγt to not only establishing the permissive epigenetic landscape but also preventing the repressive one, as evidenced by comparing WT and RORγt-deficient T<sub>H</sub>17-polarized cells, indicating that RORγt has key roles for setting up the epigenetic landscape to govern a diverse array of histone modifications. Second, RORγt is involved in the regulation of chromatin accessibility to a comparable degree as SRG3, as shown by restriction enzyme accessibility assay. Of particular note, the epigenetic alterations such as chromatin accessibility and histone modifications in the absence of RORγt are dramatically paralleled by those in the absence of SRG3, which calls attention to the notion that the ability of transcription factors to remodel chromatin is dependent on the recruitment of dedicated chromatin remodeling complex. Re-expression of RORγt in RORγt-deficient and DKO cells led us to find that the Swi/Snf complex orchestrates the RORγt-mediated epigenetic regulations, including both histone methylation and acetylation. Thus, our results identify the Swi/Snf complex as a central link between the master transcription factor and its epigenetic control of target regions. Third, H3K4me1 deposition is largely unaffected by loss of SRG3 or RORγt, which indicates that H3K4me1 deposition is not regulated by SRG3 and RORγt. In addition, it also implies that the levels of H3K4me1 do not reflect reduced gene expression in the absence of SRG3 or RORγt. Given that H3K4me1 marks genomic regulatory elements irrespective of their activation status (Calo and Wysocka, 2013), it is likely that other transcription factors including pioneer factors are responsible for the deposition of H3K4me1 prior to binding or even expression of RORγt to mark specific genomic regulatory sites to recruit additional transcriptional activators. In this context, it is reasonable to assume that pre-conditioning of genomic target sites of RORγt accompanied by H3K4me1 deposition is a prerequisite for RORγt binding and that their activation states are subsequently potentiated by RORγt in conjunction with the Swi/Snf complex to culminate in robust gene expression.

Obviously, ROR $\gamma$ t increased the accumulation of the Swi/Snf complex at IL-17 CNS2, IL-17AP, IL-17FP, and IL-23RP. However, it is worth noting that substantial levels of residual SRG3 and BRG1 were observed in the absence of ROR $\gamma$ t. Thus, we cannot rule out the possibility that a significant amount of the Swi/Snf complex is recruited in an ROR $\gamma$ t-independent manner and plays ROR $\gamma$ t-independent roles for T<sub>H</sub>17 differentiation. However, the environmental cues or intracellular transcriptional factors to prompt the targeting of the Swi/Snf complex to IL17 locus in the absence of ROR $\gamma$ t remain to be determined. Furthermore, it is also unclear whether the residual Swi/Snf complex is involved in subsequent ROR $\gamma$ t binding and transcriptional activity. The answers to these questions are required to better understand the role of the Swi/Snf complex for T<sub>H</sub>17 differentiation.

Our data also suggest a potential role for chromatin remodeling by the Swi/Snf complex in the histone modifications. In line with this, recent studies have revealed the link between the Swi/Snf complex and a number of histone modifications (Alver et al., 2017; Hodges et al., 2018; Kia et al., 2008; Stanton et al., 2017; Wang et al., 2017; Wilson et al., 2010). Although histone modifying enzymes and ATP-dependent chromatin remodeling complex serve totally distinct biochemical functions, they act in a cooperative and closely integrated manner, thereby forming a reciprocal regulatory network to determine the chromatin states and the levels of gene expression. It is likely that most of these chromatin regulators exert their function as a large multiprotein complex that contains both histone modifying and nucleosome remodeling activities to regulate gene expression in a cooperative manner. We speculate that the Swi/Snf complex may regulate histone modifications by recruitment or expulsion of relevant enzymes in the target regions.

In this study, we demonstrate that ROR $\gamma$ t requires the Swi/Snf complex to establish chromatin landscape to fully activate T<sub>H</sub>17 cell phenotype. However, in addition to its role in T<sub>H</sub>17 cell differentiation, ROR $\gamma$ t plays essential roles in the development of thymocytes, lymphoid tissue inducer (LTi) cells, type 3 innate lymphoid cells, and IL-17 production in Tc17, TCR $\gamma$  $\delta$ , and natural killer T cells. Therefore, it will be interesting to examine the correlation between ROR $\gamma$ t and the Swi/Snf complex in other cell types to determine whether it is a cell-type-specific mechanism.

Delineating the precise molecular mechanisms that dictate specification to the T<sub>H</sub>17 cell lineage is critical to the development of potential therapeutic applications that target T<sub>H</sub>17 cells. Collectively, our data may provide a new perspective on the epigenetic approach for therapeutic intervention in T<sub>H</sub>17-related diseases.

### Limitations of the Study

The aim of this study is to investigate the role of the Swi/Snf complex in the T<sub>H</sub>17 generation. Even though we performed extensive *in vitro* molecular analyses to determine the mechanism by which the Swi/Snf complex regulates ROR $\gamma$ t-mediated transcriptional program, further work is needed to validate the ROR $\gamma$ t-independent role of the Swi/Snf complex during T<sub>H</sub>17 differentiation. Also, detailed study is necessary to reveal which histone-modifying enzymes require and cooperate with the Swi/Snf complex to understand their comprehensive epigenetic networks for the regulation of T<sub>H</sub>17 differentiation.

### Resource Availability

#### Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Rho. H. Seong (rhseong@snu.ac.kr).

#### Materials Availability

This study did not generate new unique reagents.

#### Data and Code Availability

GEO, GSE129132 (Microarray analysis).

## METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

## SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2020.101106>.

## ACKNOWLEDGMENTS

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## AUTHOR CONTRIBUTIONS

S.L. performed most of the experiments with help from J.K. and H.M. J.K. performed restriction enzyme accessibility experiments. S.L. and R.H.S. designed the research, analyzed results, and wrote the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## **Supplemental Information**

### **ROR $\gamma$ t-driven T<sub>H</sub>17 Cell Differentiation Requires Epigenetic Control by the Swi/Snf Chromatin Remodeling Complex**

**Sungkyu Lee, Jieun Kim, Hyungyu Min, and Rho H. Seong**

# Supplemental information

ROR  $\gamma$  t-driven T<sub>H</sub>17 cell differentiation  
requires epigenetic control  
by the Swi/Snf chromatin remodeling complex

Sungkyu Lee, Jieun Kim, Hyungyu Min & Rho H. Seong

# Supplemental Figure 1

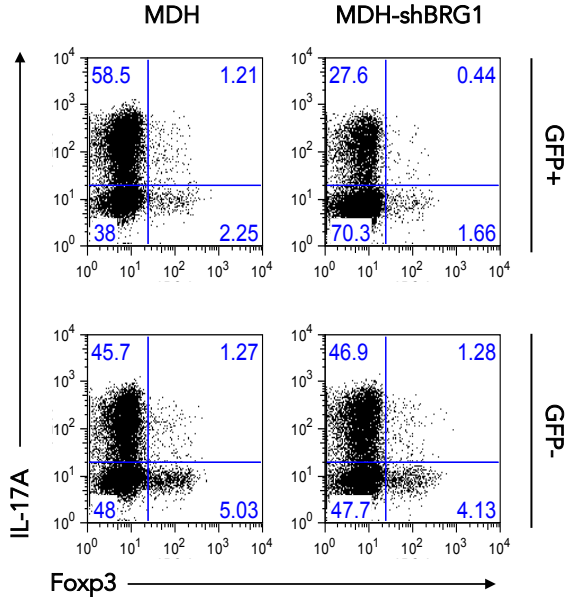


Figure S1. BRG1 is required for the T<sub>H</sub>17 differentiation, Related to Figure 1.

Flow cytometry of intracellular staining for IL-17A/Foxp3 in naïve CD4<sup>+</sup> T cells transduced with control retrovirus (MDH) expressing GFP only or retrovirus expressing GFP and shBRG1 (MDH-shBRG1), cultured under T<sub>H</sub>17( $\beta$ ) conditions and followed by restimulation. Dot plots are gated on GFP<sup>+</sup> (top) or GFP<sup>-</sup> (bottom)



# Supplemental Figure 2

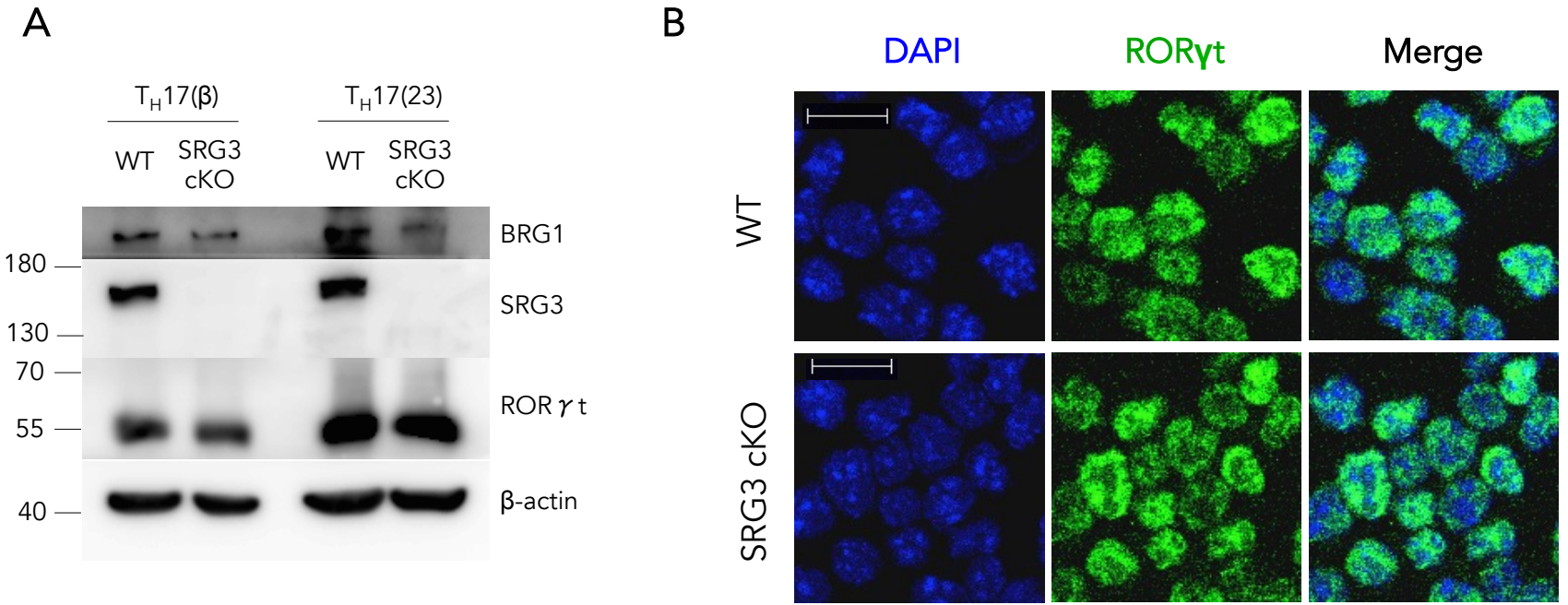


Figure S2. The expression level and nuclear localization of Ror  $\gamma$  t protein are similar between WT and SRG3-deficient T<sub>H</sub>17 polarized cells, Related to Figure 3.

(A) Immunoblot analysis for Ror  $\gamma$  t, Brg1 and SRG3 in whole-cell lysate of cultured T<sub>H</sub>17 cells from WT or SRG3 cKO mice (B) Immunofluorescence staining of ROR  $\gamma$  t in cultured T<sub>H</sub>17(β) cells from wild-type or SRG3 cKO mic. Scale bars (20μm)

Supplemental Figure 3

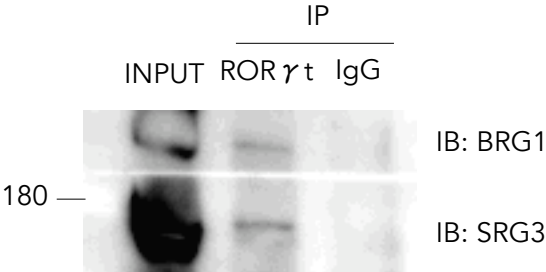


Figure S3. Co-immunoprecipitation of SRG3 and BRG1 with anti-ROR  $\gamma$  t antibodies in lysates of  $T_H17(\beta)$ -polarized cells, Related to Figure 4.

# Supplemental Figure 4

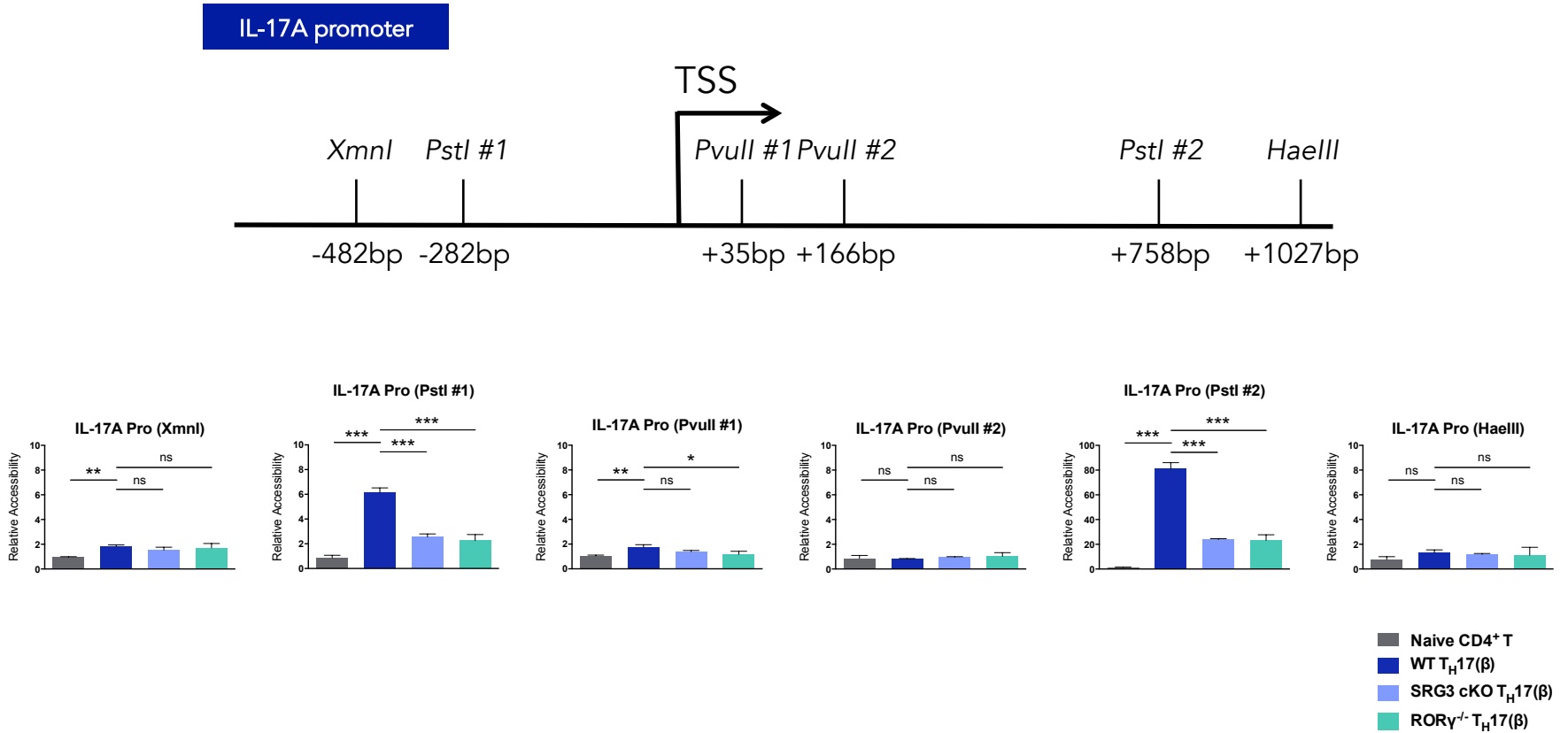


Figure S4. SRG3 and ROR  $\gamma$  t are required to modulate chromatin accessibility at *Il17a* promoter, Related to Figure 5.

Restriction enzyme accessibility assay followed by LM-PCR in WT naïve and WT, SRG3 cKO or ROR  $\gamma$  t<sup>-/-</sup> T<sub>H</sub>17(β)-polarized CD4<sup>+</sup> T cells restimulated with PMA/ionomycin for 4h. Data are representative of at least two independent experiments. Error bars, s.d. Statistical analysis was performed using one-way ANOVA. (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001; ns, not significant)

# Supplemental Table 1

Primer name	Sequence (5'-3')	Use
<i>IL17</i> CNS2 Forward	TCAGTGACATCCGTTTACTTGGAA	ChIP qPCR
<i>IL17</i> CNS2 Reverse	TCCAACCAAGTACATTTAGAATCAAGT	ChIP qPCR
<i>IL17a</i> Promoter Forward	AGTTTCTATCTGGAGGATAGCTGGT	ChIP qPCR
<i>IL17a</i> Promoter Reverse	TGCACTAGTGTTCTTAGTCACTCCTT	ChIP qPCR
<i>IL17f</i> Promoter Forward	ATCTGTAGTAAAGGTAATGGGAGTGG	ChIP qPCR
<i>IL17f</i> Promoter Reverse	AGTTGTGGTTAACCGAGAGTTACTG	ChIP qPCR
<i>IL23r</i> Promoter Forward	ACTAGTCATTATGAAATGGACACGAG	ChIP qPCR
<i>IL23r</i> Promoter Reverse	TCTTTTCATTTGTTTGCATTACTTTC	ChIP qPCR
<i>Il17a</i> Promoter XmnI Forward	GTAACCTCATGTCTCTCGCTACTC	REAA LM-qPCR
<i>Il17a</i> Promoter XmnI Reverse	CCGGGAGATCTGAATTCCGTTCTATTT	REAA LM-qPCR
<i>Il17a</i> Promoter PstI#1 Forward	GTTTCTTTGGGCAAGGGATG	REAA LM-qPCR
<i>Il17a</i> Promoter PstI#1 Reverse	CCGGGAGATCTGAATTCTGCAGAGCTG	REAA LM-qPCR
<i>Il17a</i> Promoter PstI#2 Forward	AGGGAGGTTTCTATCTGG	REAA LM-qPCR
<i>Il17a</i> Promoter PstI#2 Reverse	CCGGGAGATCTGAATTCTGCAGAAAAC	REAA LM-qPCR
<i>Il17a</i> Promoter PvuII#1 Forward	ACTCTTGACGTAAGTGACCACAGA	REAA LM-qPCR
<i>Il17a</i> Promoter PvuII#1 Reverse	CCGGGAGATCTGAATTCCTGGTGCT	REAA LM-qPCR
<i>Il17a</i> Promoter PvuII#2 Forward	AGCTTCATCTGTGGTGAGTCCT	REAA LM-qPCR
<i>Il17a</i> Promoter PvuII#2 Reverse	CCGGGAGATCTGAATTCCTGGAATA	REAA LM-qPCR
<i>Il17a</i> Promoter HaeIII Forward	AAGACATCACATGATTGTCCTCAG	REAA LM-qPCR
<i>Il17a</i> Promoter HaeIII Reverse	CCGGGAGATCTGAATTCCTGGAATA	REAA LM-qPCR
Control GAPDH Forward	GTGTCCTACCGAAGAACAACGAG	REAA LM-qPCR
Control GAPDH Reverse	GTAATAATGGGAGAACAAGGGGAAAT	REAA LM-qPCR

Table S1. Primers used in this study, Related to Figures 4,5,6.

## Transparent Methods

### Mice

*SRG3<sup>fl/fl</sup>* mouse was generated by (Choi et al., 2012). The information on targeting constructs and strategy for the generation of *SRG3<sup>fl/fl</sup>* mice were provided (Choi et al., 2012, Fig. 1D). *RORγt<sup>-/-</sup>* mouse was purchased from The Jackson Laboratories (Stock No. 007572). All mice were bred and maintained in specific pathogen-free barrier facilities at Seoul National University and were used in accordance with protocols approved by the Institutional Animal Care and Use Committees (IACUC) of Seoul National University.

### Antibodies

The following antibodies were used for flow cytometry analysis and cell sorting: anti-CD4 (GK1.5, eBioscience), anti-CD25 (PC61, eBioscience), anti-CD44 (IM7, eBioscience), anti-CD62L (MEL-14, BD Pharmingen), anti-CD45.1 (A20, eBioscience), anti-IL-17A (TC11-18H10, BD Pharmingen), anti-IL-17F (eBio18F10, eBioscience), anti-Foxp3 (FJK-16s, eBioscience), anti-IFN- $\gamma$  (XMG1.2, BD Pharmingen). For proximity ligation assay, anti-ROR $\gamma$ t (Q31-378, BD Pharmingen; RRID:AB\_2651150), anti-serum against SRG3 and anti-serum against BRG1 were used. For ChIP assay, anti-H3K4me1 (Abcam, ab8895; RRID:AB\_306847), anti-H3K4me2 (Millipore, 07-030; RRID:AB\_310342), anti-H3K4me3 (Abcam, ab8580; RRID:AB\_306649), anti-H3K27ac (Abcam, ab4729; RRID:AB\_2118291), anti-H3K27me3 (Millipore, 07-449; RRID:AB\_310624), anti-RNAPIIS2 (Abcam, ab5095; RRID:AB\_304749) were used.

### *In vitro* T cell culture

Naïve CD4<sup>+</sup> T cells were purified from pooled spleen and lymph nodes or spleen alone by using Sony cell sorter SH800. In short, red blood cells were removed with ACK lysis buffer and B cells were depleted of single-cell suspensions using anti-biotin magnetic beads (Miltenyi biotech). Thereafter, cells were stained with antibodies against CD4, CD25, CD44 and CD62L, and FACS-sorted to isolate CD4<sup>+</sup>CD25<sup>-</sup>CD44<sup>low</sup>CD62L<sup>high</sup>

naïve T cells. Cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM, Gibco) supplemented with 10% heat-inactivated FBS (Gibco), 50U penicillin-streptomycin and 50 $\mu$ M  $\beta$ -mercaptoethanol. For T cell polarization, naïve CD4<sup>+</sup> T cells were cultured for 48~60 h in 96-well flat-bottom plates pre-coated with anti-CD3 (145-2C11;10 $\mu$ g/ml) and anti-CD28 (37.51;10 $\mu$ g/ml) antibody under T<sub>H</sub>17( $\beta$ ) conditions (TGF- $\beta$ 1(1ng/ml), IL-6 (30 $\mu$ g/ml), anti-IL-4 (11B11;2 $\mu$ g/ml), anti-IFN- $\gamma$  (XMG1.2;2 $\mu$ g/ml)), T<sub>H</sub>17(23) conditions (IL-6(20 $\mu$ g/ml), IL-1 $\beta$ (20 $\mu$ g/ml), IL-23 (40 $\mu$ g/ml), anti-IL-4 (1B11;2 $\mu$ g/ml), anti-IFN- $\gamma$  (XMG1.2;2 $\mu$ g/ml)) or iTreg conditions (TGF- $\beta$ 1(1ng/ml), anti-IL-4 (1B11;2 $\mu$ g/ml), anti-IFN- $\gamma$  antibody (XMG1.2;2 $\mu$ g/ml))

### **Flow cytometry**

For cytokine analysis, cells were stimulated for 4h with phorbtor 12-myristate 13-acetate (PMA) (50ng/ml;Sigma), ionomycin (500ng/ml;Sigma) in the presence of Brefeldin A (eBioscience) in a 37°C tissue culture incubator. Intracellular staining was performed according to the manufacturer's instruction (Cytofix/Cytoperm buffer; BD Biosciences). FACS Canto (BD Biosciences) and FlowJo software were used for flow cytometry and analysis, respectively. For analysis of cell proliferation, cells were labeled with CFSE (Cell Trace CFSE Cell Poliferation Kit; Invitrogen) in suspension at 10<sup>6</sup> cells/ml with 5 $\mu$ M CFSE for 5min.

### **Retroviral transduction**

Naïve CD4<sup>+</sup> T cells were activated for 24h with plate-bound anti-CD3 (145-2C11;10 $\mu$ g/ml) and anti-CD28 (37.51;10 $\mu$ g/ml) antibody under T<sub>H</sub>17( $\beta$ ) conditions or T<sub>H</sub>N conditions, and spin-infected for 1h with retroviral supernatant containing 8 $\mu$ g/ml polybrene from Phoenix packaging cells transfected with retroviral expression plasmids.

### **Microarray analysis**

Microarray services were provided by MacroGen (MacroGen Inc., Seoul, Korea). Total RNA was extracted and then cDNA was synthesized using the GeneChip WT (Whole Transcript) Amplification kit as described by the manufacturer. The sense cDNA was then fragmented and biotin-labeled with TdT (terminal deoxynucleotidyl transferase)

using the GeneChip WT Terminal labeling kit. Approximately 5.5 µg of labeled DNA target was hybridized to the Affymetrix GeneChip Array (GeneChip® Mouse Gene 2.0 ST Array) at 45°C for 16h. Hybridized arrays were washed and stained on a GeneChip Fluidics Station 450 and scanned on a GCS3000 Scanner (Affymetrix).

### **EAE induction**

8 to 10-week old, female mice were immunized subcutaneously with MOG<sub>35-55</sub> peptide (200µg/mouse) emulsified with complete Freund's adjuvant (CFA) (100µl/mouse, Difco) containing *Mycobacterium tuberculosis* (4 mg/ml). Pertussis toxin (200 ng /mouse, Sigma) was also intraperitoneally injected into the mice twice on day 0 and on day 2 post immunization. Disease severity was evaluated on a scale of 0–5 as follows: 0, no signs of disease; 1, limp tail; 2, partially paralyzed hind legs; 3, completely paralyzed hind legs; 4, complete hind and partial front leg paralysis; 5, completely paralyzed hind and front legs. Mice with disease score 5 were considered moribund and euthanized. Minced cells from spinal cords were re-suspended in 30% Percoll (GE Healthcare), mixed with 70% Percoll and then centrifuged for 20min at 20°C. Mononuclear cells were isolated from the interface, stimulated with PMA/ionomycin for 4h and analyzed for cytokine expression by flow cytometry.

### **Isolation of lamina propria lymphocytes**

Small intestines were placed in ice-cold PBS. Residual fat tissue and Peyer's patches were carefully removed. The intestine was opened longitudinally and then thoroughly washed in ice-cold PBS and cut into 1.0cm pieces. The pieces were incubated in EDTA buffer (0.5mM EDTA, 2% FBS RPMI) for 20min at 37°C with slow rotation. Intestinal pieces were collected and washed twice with ice-cold PBS. Pieces were minced with scissors and incubated in collagenase buffer (100µg/ml DNaseI (Sigma), 0.5mg/ml collagenase (Sigma), 10% FBS RPMI) for 20min. The resulting suspension was passed through a 70µm cell strainer and subject to a discontinuous 30:70 Percoll gradient (GE Healthcare). Mononuclear cells were collected at the Percoll interphase.

### **Quantitative real-time PCR**

Quantitative real-time PCR was performed on ABI StepOnePlus real-time PCR system using SYBR green PCR master mix (Applied Biosystems).

#### ***In situ* proximity ligation assay (PLA)**

PLA was performed according to the manufacturers protocol (Duolink In Situ Red Starter Kit Mouse/Rabbit, DUO92101, Sigma).

#### **Chromatin immunoprecipitation coupled with qPCR (ChIP-qPCR)**

ChIP assay was carried out according to the manufacturer's guide (Upstate/Millipore) with minor modifications. Briefly, activated T cells ( $0.5\sim 1.5 \times 10^6$  cells) were crosslinked in culture medium containing 1% formaldehyde at 37°C for 15min and sonicated with Bioruptor to generate fractionated genomic DNA. Primers used for qPCR are listed in supplementary table 1.

#### **Restriction enzyme accessibility assay (REAA)**

Cells were pelleted, washed with ice-cold PBS, and then lysed with NP-40 lysis buffer (10mM Tris-Cl (pH7.4), 10mM NaCl, 3mM MgCl<sub>2</sub>, 0.5% NP-40, 0.15mM spermine, 0.5mM spermidine) on ice for 5min. The nuclei were carefully pelleted, washed with restriction enzyme digestion buffer (10mM Tris-Cl (pH7.4), 50mM NaCl, 10mM MgCl<sub>2</sub>, 0.2mM EDTA, 0.2mM EGTA, 0.15mM spermine, 0.5mM spermidine, 1mM β-mercaptoethanol), and then incubated for 10min at 37°C with restriction enzyme plus restriction enzyme digestion buffer supplied with the enzyme (NEB). DNA was purified using the DNeasy Blood & Tissue kit (Qiagen) in accordance with the manufacturer's instructions. Next, ligation-mediated PCR (LM-PCR) was performed according to (Ohkawa et al., 2012). In Brief, DNA was ligated with Linker A and Linker B and then restriction enzyme accessibility was assessed with primers listed in supplementary table 1 by quantitative real-time PCR.

#### **Western blotting and immunoprecipitation assay**



For western blotting, proteins were extracted with RIPA buffer, separated by SDS-PAGE, and transferred to a nitrocellulose membrane. The membrane was incubated in TBS-T solution containing primary antibodies (Roryt 1:1000, 562607 BD Pharmingen; Srg3 1:4000, in our lab; Brg1 1:4000, in our lab). It was washed three times in TBS-T solution then incubated in a diluted specific secondary antibody (1:4000) for 1 hour at room temperature and washed in TBS-T solution. The blot was developed using enhanced chemiluminescence (Roryt, Thermo Fisher Supersignal™ West Femto Maximum Sensitivity Substrate (Cat# 34095); SRG3, BRG1, b-actin, AbClon Absignal (Cat# ABC-3001))

For immunoprecipitation, T<sub>H</sub>17 polarized cells were lysed with IP buffer (Thermo Fischer, Pierce™ IP lysis buffer (Cat# 87787). 500µg of whole cell extracts were incubated with either normal mouse IgG or anti-Roryt antibody (562607 BD Pharmingen) for 6 hours. Protein G Magnetic Beads were added to the cell lysates and incubated for 1 hour. Subsequently, the magnetic beads were separated from the lysates with a magnet and washed three times with IP buffer. The washed beads were eluted with loading buffer and the eluate was analyzed by western blotting.

### **Data and Software Availability**

GEO, GSE129132 (Microarray analysis)

### **Supplemental References**

Choi, J., Ko, M., Jeon, S., Jeon, Y., Park, K., Lee, C., Lee, H., and Seong, R.H. (2012). The SWI/SNF-like BAF complex is essential for early B cell development. *J Immunol* 188, 3791-3803.

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