The lysine methyltransferase DOT1L controls CD4$^+$ T cell-dependent immunity and inflammation

Authors

S. Scheer[1*], M. Bramhall[1], J. Runting[1], B. Russ[1], Q. Zhang[2], S. F. Flanigan[2], A. Zaini[1], J. Ellemor[1], G. Rodrigues[1], J. Ng[1], C. Davidovich[2,3] and C. Zaph[1*†]

Affiliations

[1]Infection and Immunity Program, Monash Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, Monash University, Clayton VIC 3800, Australia.

[2]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton VIC 3800, Australia.

[3]EMBL-Australia and the ARC Centre of Excellence in Advanced Molecular Imaging, Clayton, VIC 3800, Australia.

*Corresponding authors: colby.zaph@monash.edu, sebastian.scheer@monash.edu,

†Lead contact

Abstract

$CD4^+$ T helper (Th) cell differentiation is controlled by lineage-specific expression of transcription factors and effector proteins, as well as silencing of lineage-promiscuous genes. Lysine methyltransferases (KMTs) comprise a major class of epigenetic enzymes that are emerging as important regulators of Th cell biology. Here, we show that the KMT DOT1L regulates Th cell function and lineage integrity. DOT1L-dependent dimethylation of lysine 79 of histone H3 (H3K79me2) is associated with lineage-specific gene expression. However, DOT1L-deficient Th cells overproduce IFN-γ under lineage-specific and lineage-promiscuous conditions. Consistent with the increased IFN-γ response, mice with a T cell-specific deletion of DOT1L are susceptible to infection with the helminth parasite *Trichuris muris* and resistant to the development of allergic lung inflammation. These results identify a central role for DOT1L in Th cell lineage commitment and stability, and suggest that inhibition of DOT1L may provide a novel therapeutic strategy to limit type 2 immune responses.

Introduction

Upon encounter of foreign antigens in the periphery, $CD4^+$ T helper (Th) cells can differentiate into several cell lineages that have distinct physiological properties and functions. For example, Th1 cells are induced following viral or intracellular bacterial infection, produce the cytokine interferon-γ (IFN-γ) and activate macrophages to kill infectious organisms. In contrast, Th2 cells express interleukin (IL)-4, IL-5 and IL-13 following helminth infection and are required for immunity to this class of pathogens. These cells show a distinct and mutually exclusive cell fate, as the induction of the Th1 cell lineage program by the cytokine IL-12 and the transcription factor (TF) TBET leads to the production of IFN-γ, while repressing genes that characterize differentiated Th2 cells, such as IL-4 and IL-13 and the TF GATA3. Maintenance of lineage integrity is critical for optimal responses to a wide variety of pathogens. In addition, an imbalanced Th1/Th2 immune response may be responsible for the onset of maintenance of T cell-mediated inflammatory disorders.

Epigenetic modifiers, such as histone lysine methyltransferases (KMTs) have emerged as critical regulators of Th cell differentiation and function as their activity results in a defined state of chromatin, which enables the regulation of gene expression and in turn regulates cellular development and differentiation[1]. We and others have previously shown that modulation of KMT activity has a profound impact on Th cell differentiation, lineage stability and function. For example, EZH2-dependent trimethylation of histone H3 at lysine 27 (H3K27me3) and SUV39H1/2-dependent H3K9me3 are important for lineage integrity of Th1 and Th2 cells[2,3], while G9a-mediated H3K9me2 has been shown to control inducible regulatory T (iTreg) and Th17 cell differentiation[4,5]. Importantly, KMTs are viable drug targets in a wide range of diseases[6]. Thus, a better understanding of the epigenetic

mechanisms controlling Th cell differentiation and lineage stability could offer new strategies to modulate dysregulated Th cell function in disease.

Recently, in a screen using chemical probes targeting KMTs, H3K79 methyltransferase Disruptor of telomeric silencing 1-like (DOT1L) was shown to limit Th1 cell differentiation in both murine and human Th cells in vitro[7]. DOT1L is the sole KMT for H3K79, performing mono-, di- and trimethylation in vivo[8,9] since its knockout leads to a complete loss of H3K79 methylation[10]. DOT1L-dependent H3K79 methylation has been suggested to directly promote transcriptional activation[11,12]. However, H3K79me2 has also been correlated to silenced genes[13] and was further associated with both gene activation and repression in the same cell for separate genes[14]. These reports highlight the lack of clarity of H3K79 methylation in mammalian gene transcription, and the role of H3K79 methylation in Th cells has not been described in detail. In addition, the in vivo role of DOT1L in T cells is unknown, yet may lead to the development of new therapeutics against inflammatory disorders.

Here, we identify a key role for DOT1L in limiting the Th1 cell differentiation program in both Th1 and Th2 cells in vivo. DOT1L-dependent H3K79me2 is tightly associated with lineage-specific gene expression in Th1 and Th2 cells. However, loss of DOT1L results in increased production of Th1 cell-associated genes and IFN-γ production under both lineage-specific and -promiscuous conditions. Further, the absence of DOT1L in Th cells leads to an increased Th1 and an impaired Th2 cell response during infection with the helminth parasite *Trichuris muris* or during allergic airway inflammation, identifying DOT1L as a potential therapeutic target to treat diseases associated with dysregulated Th2 cell responses at mucosal sites.

Results

DOT1L limits IFN-γ expression in both Th1 and Th2 cells

To begin to understand the precise role of DOT1L and H3K79 methylation in Th cell differentiation, we generated a T cell-specific DOT1L-deficient mouse strain (DOT1L$^{\Delta T}$ mice) by crossing *Dot1l*$^{fl/fl}$ mice with *Cd4*-Cre transgenic mice and observed a specific reduction in DOT1L-dependent H3K79me2 levels within DOT1L-deficient T cells (Fig. 1a). Analysis of DOT1L-sufficient or -deficient Th cells from the spleen and peripheral lymph nodes that were cultured under Th1 polarising conditions ex vivo showed highly significant increased expression of IFN-γ under Th1 cell-polarising conditions in the absence of DOT1L. We also observed an increase in IL-13 production by Th2 cells (Fig. 1b), suggesting that DOT1L is critical for restraining lineage-specific gene expression. However, most strikingly, a significant proportion of cells stimulated under Th2 cell-inducing conditions produced IFN-γ (Fig. 1, b-d). The increased frequency of IFN-γ-producing cells was associated with reduced frequencies of GATA3-positive cells and a concomitant increase in TBET-positive cells (Fig. 1e). Thus, DOT1L appears to be critically required for silencing of lineage-promiscuous expression of TBET and IFN-γ in Th cells. This increased IFN-γ production occurred rapidly after activation (Fig. 1f), but was not due to increased proliferation (Fig. 1g). Titration of αCD3 antibody demonstrated that loss of DOT1L rendered T cells hyper-sensitive to activating signals, with increased IFN-γ observed at all concentrations of αCD3 (Fig. 1h). In addition, stimulation of freshly purified Th cells with phorbol-12-myristate-13-acetate and calcium ionophore (PMA/Iono) in the absence of direct T cell receptor stimulation further demonstrated that DOT1L is required to limit Th cell activation and IFN-γ production independently of the T cell receptor (Fig. 1i). Importantly, when we cultured sorted naive (CD44^{-} CD62L^{+}) Th cells from control or DOT1L-deficient

mice under Th1 polarising conditions, we found that DOT1L was still required to limit IFN-γ production (Fig. 1j). Taken together, these results identify DOT1L as a cell-intrinsic regulator of Th cell lineage choice that is critical for limiting Th1 cell differentiation.

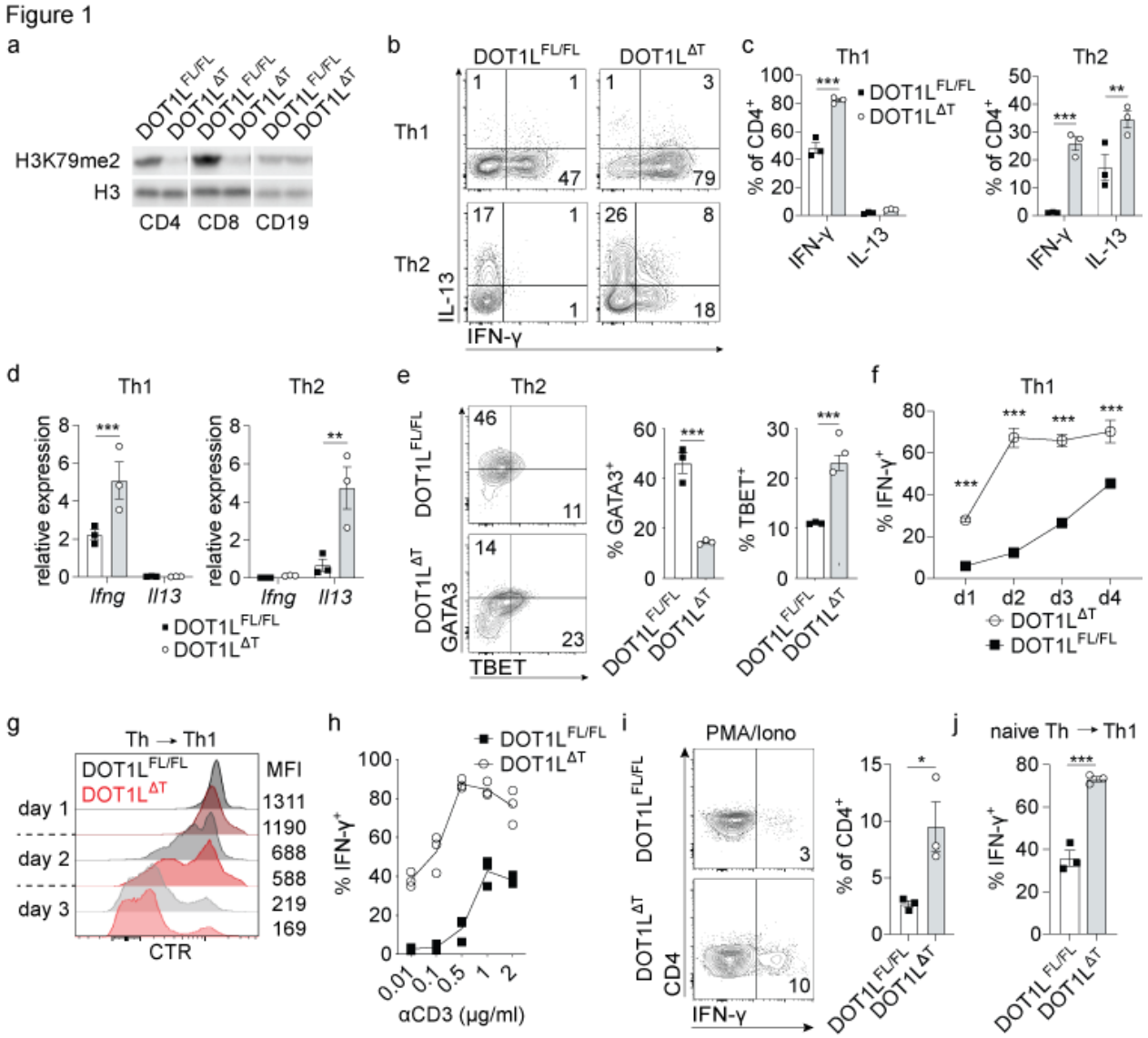

Fig.1. DOT1L-deficient Th cells show increased production of IFN-γ.

(a) Western blot of histone extracts from sorted TCRb^{+} CD4^{+} (CD4), TCRb^{+} CD8^{+} (CD8) T cells and CD19^{+} B cells (CD19) from control (DOT1L$^{FL/FL}$) or T cell conditional knockout mice for DOT1L (DOT1L$^{\Delta T}$) for H3K79me2 and pan-H3 (control). **(b)** Enriched Th cells from control (DOT1L$^{FL/FL}$) or DOT1L$^{\Delta T}$ mice under

Th1 and Th2 polarising conditions. **(c)** Quantification of (b). **(d)** Quantitative PCR analysis of indicated genes of cells described in (b). **(e)** Signature Th1 and Th2 transcription factor analysis of the Th2 cells described in (a). **(f)** Kinetics of IFN-γ expression of enriched Th cells from control ($DOT1L^{FL/FL}$) or $DOT1L^{\Delta T}$ mice under Th1 polarising conditions. **(g)** Proliferative capacity of Th cells from control ($DOT1L^{FL/FL}$) or $DOT1L^{\Delta T}$ mice at indicated days after stimulation with plate bound αCD3 and αCD28 in the presence of IL-2. **(h)** Enriched peripheral Th cells from control ($DOT1L^{FL/FL}$) or $DOT1L^{\Delta T}$ mice cultured for four days under Th1 polarising conditions at indicated concentrations of plate-bound αCD3. **(i)** Freshly isolated and PMA/Iono stimulated peripheral Th cells from control ($DOT1L^{FL/FL}$) or $DOT1L^{\Delta T}$ mice in the absence of direct T cell receptor stimulation. **(j)** IFN-γ expression of sorted naive Th cells ($CD44^{-}$ $CD62L^{+}$) from control ($DOT1L^{FL/FL}$) or $DOT1L^{\Delta T}$ mice cultured for three days under Th1 polarising conditions. Error bars represent mean ± S.E.M. Data shown is representative of two (a, e-g, i, j) and three (b-d, h) independent experiments (n=3 mice per group). MFI: mean fluorescence intensity, CTR: CellTrace far red. Statistical significance was determined by 2-tailed Student's t test (c, d, e, i, j) or 2-way-ANOVA (f). *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

DOT1L maintains Th2 cell lineage integrity

As we observed lineage-promiscuous expression of IFN-γ in the absence of DOT1L, we next tested whether DOT1L was required for lineage stability of Th cells[2,3]. We stimulated control or DOT1L-deficient Th cells under Th1- or Th2 cell-inducing conditions for three days, then washed and restimulated under opposing conditions for a further two days (Fig. 2a). We found that the absence of DOT1L resulted in a loss of lineage integrity of both Th1 and Th2

cells. However, most striking was the loss of Th2 cell stability and the acquisition of IFN-γ production by a significant proportion of ex-Th2 cells (Fig. 2b), suggesting DOT1L is critical for silencing of Th1 cell lineage-specific genes in Th2 cells. To directly test if DOT1L was required to limit the Th1 cell differentiation program in *bona fide* Th2 cells or was more important in uncommitted activated Th cells in these cultures, we made use of IL-4/IL-13/IFN-γ triple reporter mice (IL-4-AmCyan/IL-13-dsRed/IFN-γ-YFP; CRY mice) mice to distinguish between committed Th1 (YFP^+) or Th2 ($AmCyan^+$ and/or $dsRed^+$) cells, or uncommitted but activated cells (Fig. 2c). We activated Th cells from DOT1L-sufficient and -deficient CRY mice for three days under Th1- or Th2 cell-inducing conditions, sorted IFN-γ/YFP^+ (Th1) or IL-4/$AmCyan^+$ and/or IL-13/$dsRed^+$ (Th2) cells and restimulated under the opposing condition for two days. Committed Th1 and Th2 cells generated from DOT1L-sufficient mice were stable, with <4% of cells switching following reactivation. Interestingly, loss of DOT1L had no effect on the switching of committed Th1 cells, suggesting that DOT1L was dispensable for Th1 cell lineage stability. However, strikingly a significant frequency (>30%) of DOT1L-deficient Th2 cells stimulated under Th1 cell polarising conditions produced IFN-γ (Fig. 2d), showing that DOT1L is critically required to maintain Th2 cells through the inhibition of IFN-γ production.

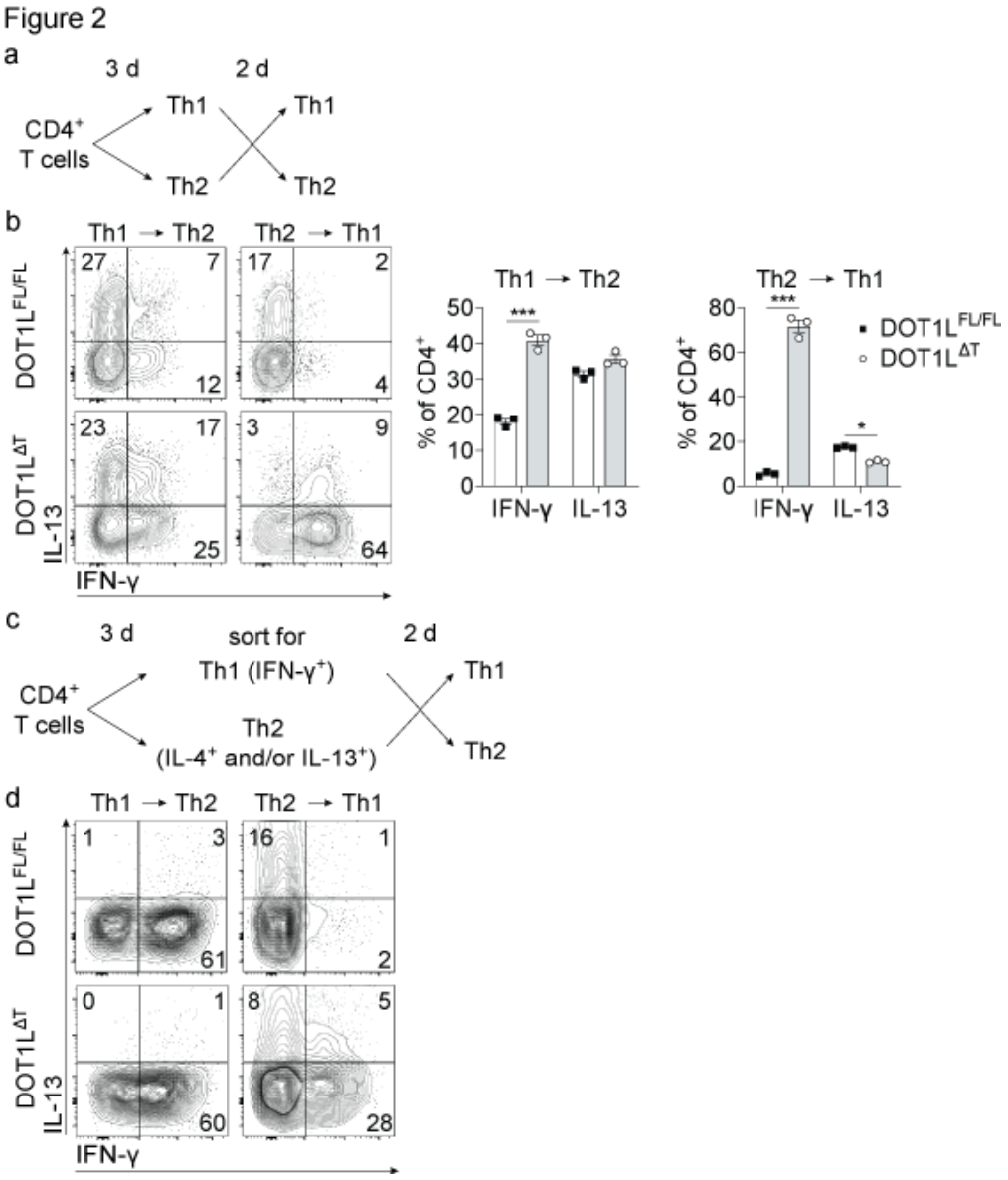

Fig. 2. DOT1L maintains Th2 cell integrity.

(a) Experimental setup of (b). **(b-d)** Enriched Th cells from control ($DOT1L^{FL/FL}$) or $DOT1L^{\Delta T}$ mice were cultured under Th1 or Th2 polarising conditions for three days and re-polarised under opposite conditions for an additional two days. **(b)** Th cells from C57BL/6J mice were cultured for three days, washed and re-polarised under opposite conditions for another two days. **(c)** Experimental setup using enriched Th cells from control ($DOT1L^{FL/FL}$-IL-4-AmCyan/IL-13-dsRed/IFN-γ-YFP (CRY)) or $DOT1L^{\Delta T}$-CRY mice in (d). **(d)** Th cells from indicated mice were cultured under Th1

or Th2 polarising conditions for three days, FACS-sorted for *bona fide* Th1 (YFP^+) or *bona fide* Th2 ($IL\text{-}4^+$ and/or $IL\text{-}13^+$) cells and re-polarised under opposite conditions for two days. Error bars represent mean ± S.E.M. Data shown is representative of three (b) and two (d) independent experiments (b: n=3 mice per group; d: n=3 mice per group, pooled after sort). Statistical significance was determined by 2-tailed Student's t test. *: $p \leq 0.05$, ***: $p \leq 0.001$.

Dysregulated IFN-γ production in DOT1L-deficient Th cells is dependent upon TBET

We next tested whether the increased early expression of IFN-γ in the absence of DOT1L leads to heightened IFN-γ expression through a feed-forward loop in Th cells. Treatment of WT Th1 cells with neutralising antibodies against IFN-γ (αIFN-γ) resulted in a significant reduction in expression of IFN-γ (Fig. 3, a and b). In contrast, IFN-γ expression was not reduced by αIFN-γ treatment in the absence of DOT1L, highlighting that expression of IFN-γ in the absence of DOT1L is independent of an IFN-γ feed-forward mechanism (Fig. 3, a and b). In line with this, DOT1L-deficient Th2 cells express IFN-γ despite being generated in the presence of αIFN-γ and in the absence of IL-12 (Fig. 3, c and d), identifying DOT1L as a critical cell-intrinsic regulator of the Th1 cell differentiation program. As αIFN-γ treatment failed to abolish IFN-γ production under both Th1- and Th2 cell-promoting conditions in the absence of DOT1L, we next analyzed whether the transcription factor TBET was required for the expression of IFN-γ in these cells (Fig. 3, a and c). To do this, we made use of T cell-specific TBET and DOT1L/TBET double knockout mice ($TBET^{\Delta T}$ and $DOT1L/TBET^{\Delta T}$ mice). While TBET-deficient Th cells fail to differentiate into IFN-γ-producing Th1 cells, the absence of DOT1L allows for moderate IFN-γ expression in the absence of TBET (Fig. 3, a and b). Although the addition of αIFN-γ to the Th1 cell-promoting conditions had no effect in

the absence of DOT1L, the combined deletion of DOT1L and TBET resulted in a further reduction in the frequency of IFN-γ-producing cells, showing that the increased frequency of IFN-γ-producing cells in DOT1L-deficient mice is dependent on the expression of TBET. Consistent with this finding, the lineage-promiscuous expression of IFN-γ in Th2 cells was entirely dependent upon TBET, as DOT1L/TBET-deficient Th cells activated under Th2 cell-polarising conditions failed to produce any detectable levels of IFN-γ (Fig. 3, c and d). Thus, the dysregulated production of IFN-γ in Th1 and Th2 cells is TBET-dependent, suggesting that DOT1L deficiency affects the upstream Th1 cell lineage differentiation program rather than directly controlling IFN-γ expression.

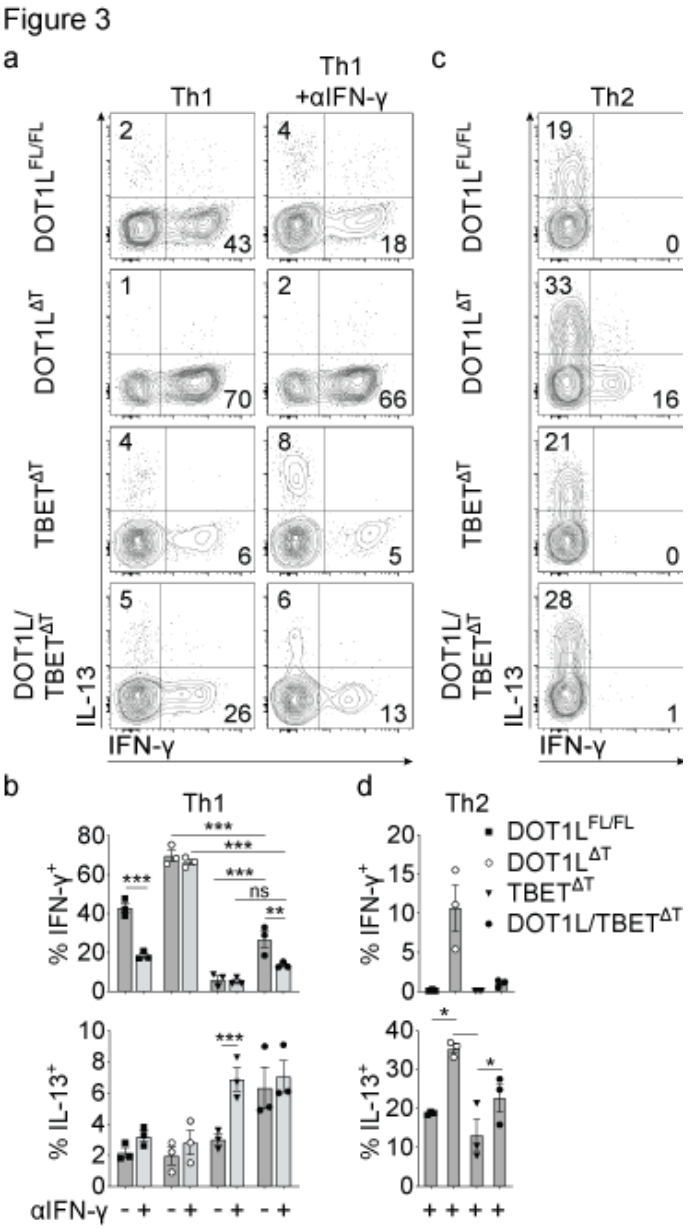

Fig. 3. Heightened IFN-γ expression in the absence of DOT1L is dependent on TBET.

(a, c) Ex vivo cultured Th cells from control ($DOT1L^{FL/FL}$), $DOT1L^{\Delta T}$, $TBET^{\Delta T}$ or $DOT1L/TBET^{\Delta T}$ mice under **(a)** Th1 or **(c)** Th2 polarising conditions in the absence (Th1) or presence (Th1+αIFN-γ, Th2) of neutralising antibodies against IFN-γ. **(b)** Quantification of (a). **(d)** Quantification of (c). Error bars represent mean ± S.E.M. Data shown is representative of two independent experiments (n=3 mice per group).

ns: not significant. Statistical significance was determined by 2-tailed Student's t test. *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

DOT1L-dependent H3K79me2 is associated with lineage-specific gene expression

Next, we made use of CRY mice to analyze the distribution of H3K79me2 in naive, or cultured and FACS-sorted *bona fide* Th1 or *bona fide* Th2 cells across the genome. Principal coordinate analysis (PCA) of three biological replicates showed high consistency within the samples of Th cell subsets and high diversity between groups (Fig. 4a). H3K79me2 plot profiles of all genes ± 3 Kb around the transcriptional start site (TSS) revealed increased coverage of H3K79me2 in all Th cell subsets downstream of the TSS[15]. Overall levels of H3K79me2 were increased in Th1 and Th2 cells compared to naive Th cells (Fig. 4b), suggesting increased H3K79me2 coverage at genes in activated Th cells. In general, H3K79me2 levels were high in all Th cell subsets at lineage-specific and low at non-Th lineage specific genes, such as for *Cd4* and *Myod1* (Fig. 4c), respectively. Increased coverage at the genes for *Ifng* and *Tbx21* in Th1 (Fig. 4d), and *Il13* and *Gata3* in Th2 (Fig. 4e) suggests that H3K79me2 is correlated with active, Th cell lineage-specific gene expression.

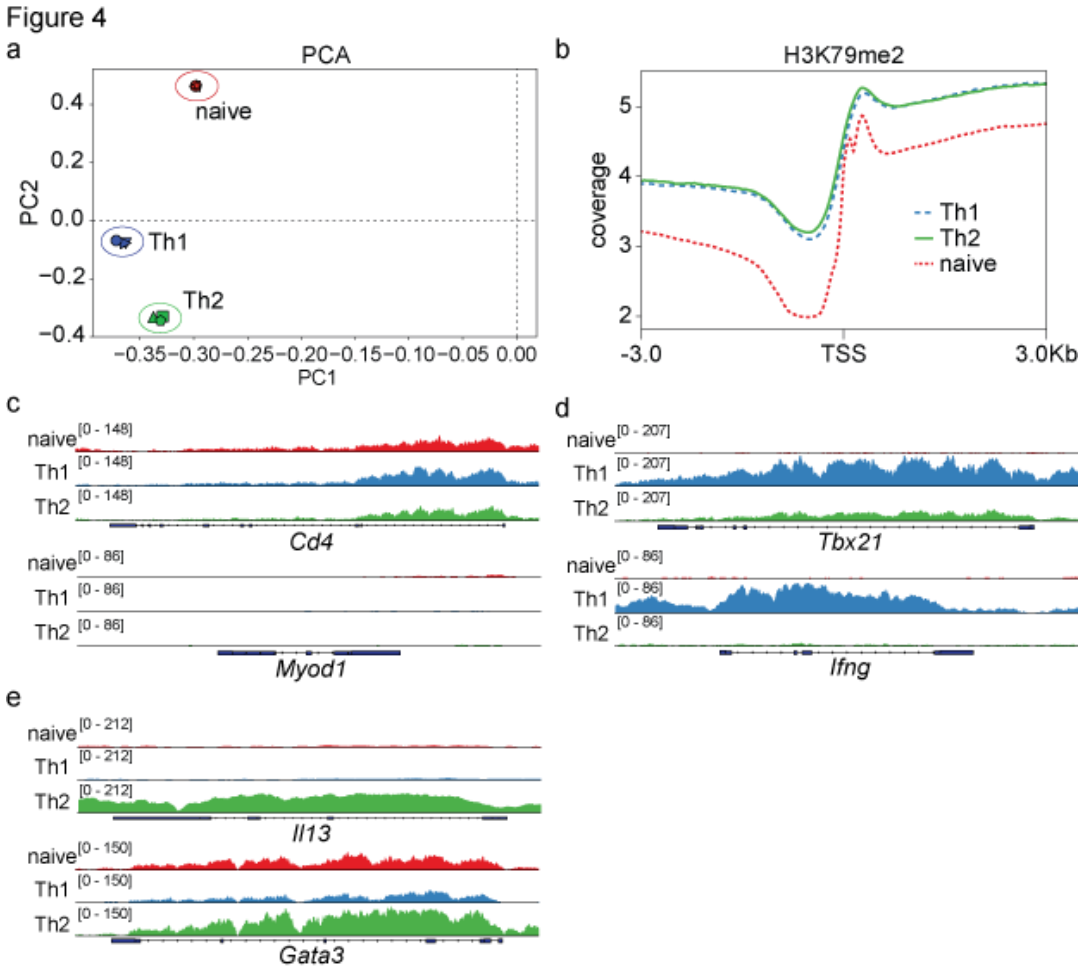

Fig. 4. DOT1L-dependent H3K79me2 is associated with lineage-specific genes.

(a) Principal coordinates analysis (PCA) of H3K79me2 ChIP-sequencing from naive ($CD44^{-}$ $CD62L^{+}$) Th, *bona fide* Th1 ($IFN\text{-}\gamma^{+}$), and *bona fide* Th2 ($IL\text{-}4^{+}$ and/or $IL\text{-}13^{+}$) cells from WT mice. **(b)** Analysis of H3K79me2 coverage ± 3 Kb around the TSS of all peak-called genes in naive Th, *bona fide* Th1 and *bona fide* Th2 cells. **(c)** H3K79me2 coverage at indicated genes. Data shown is from one H3K79me2 ChIP-seq experiment with three biological replicates and visualised with deepTools plotProfile or the Integrative Genomics Viewer (IGV).

DOT1L limits Th1 cell-associated gene expression

Our in vivo and ex vivo results using DOT1L$^{\Delta T}$ mice suggest that DOT1L is a negative regulator of the Th1 cell differentiation program. However, our results show that methylation of H3K79 by DOT1L is primarily associated with transcriptional activity. We therefore performed genome-wide expression analysis by RNA-sequencing to understand the role of DOT1L-dependent H3K79me2 for specific gene expression in Th cells. PCA showed high similarities between the two biological replicates of each, naive Th, Th1 and Th2 samples from DOT1L$^{\Delta T}$ mice or *Cd4*-Cre^{+} controls (Fig. 5a). Analysis of the number of up- and downregulated genes in Th cell subsets showed an increased number of genes with heightened levels in DOT1L-deficient Th cells (Fig. 5b, Table S1), identifying a potential inhibitory role for DOT1L in regulation of gene expression, despite the association of H3K79me2 with lineage-specific gene expression. MA-plots of significantly dysregulated genes (Th1/*Cd4*-Cre^{+} or Th2/*Cd4*-Cre^{+}; FDR cut-off 0.05, absolute log fold >0.585 ≍ >1.5-fold expression) in the absence of DOT1L show similar Th1 cell differentiation program-associated upregulated genes in Th1 and Th2 polarised cells (Fig. 5, c and d), which was partially already manifested in the unpolarised, naive state of the cells (Fig. 5e). We further compared the results from our RNA-seq for Th2 polarised cells with published data on a set of *bona fide* Th1 genes[16]. Strikingly, we found that 73% of all the Th1-specific genes were significantly increased in DOT1L-deficient Th2 cells (Fig. 5f), further highlighting that DOT1L deficiency is associated with a significant upregulation of the Th1 gene program.

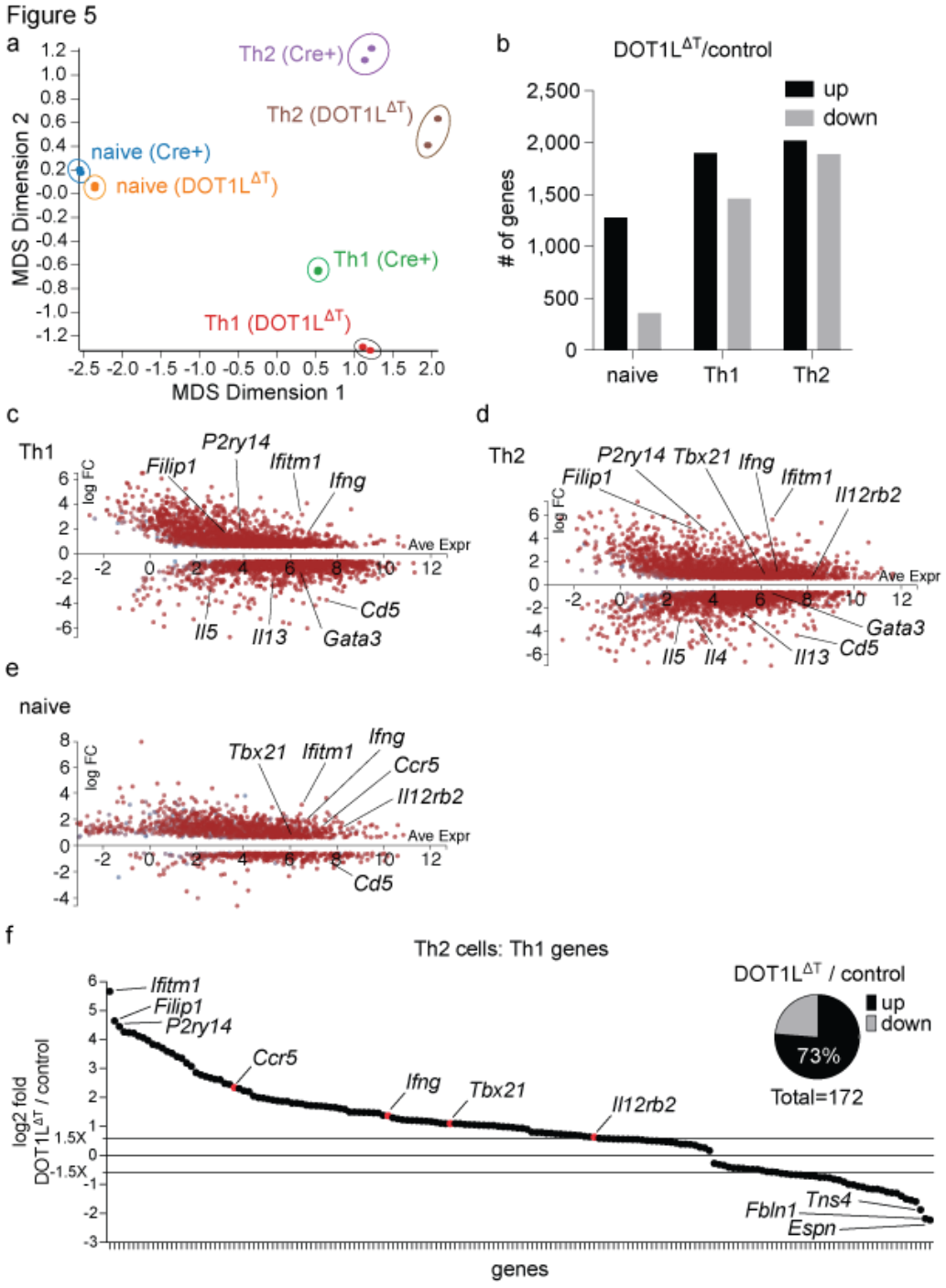

Fig. 5. DOT1L limits Th1 cell-associated gene expression.

(a) Principal coordinates analysis (PCA) of RNA-sequencing (Table S1) from naive ($CD44^{-}$ $CD62L^{+}$) Th, *bona fide* Th1, and Th2 cells from control (Cre^{+}) and $DOT1L^{\Delta T}$ mice. **(b)** Number of up- and down-regulated genes from RNA-seq analysis (FDR cut-off 0.05, absolute log fold >0.585 ≃ >1.5-fold) of indicated cells. **(c-e)** MA-plots

for differentially expressed genes (see (b)) in the absence of DOT1L in *bona fide* Th1 **(c)**, Th2 **(d)** and naive **(e)** Th cells. **(f)** Signature Th1 genes[16] that overlap with differentially expressed genes in the absence of DOT1L (DOT1L$^{\Delta T}$ / control) in Th2 cells. Data shown is from one experiment (n=2).

DOT1L-dependent H3K79me2 is associated with a subset of highly expressed genes

As our data suggests a correlation of H3K79me2 coverage with gene expression, we clustered genes from each Th cell subset by gene expression into low (<20 counts), mid (~500 counts) or high (top 450 expressed genes) and assessed the presence of H3K79me2 ± 3 Kb around the TSS. Analysis of the clusters revealed that strength of gene expression correlated to coverage for H3K79me2, with highly expressed genes showing highest average H3K79me2 coverage and little coverage for low expressed genes (Fig. 6a). However, our analysis also revealed the heterogeneity within the group of highly expressed genes with regard to H3K79me2 coverage at the TSS. While some highly expressed genes are also highly enriched for H3K79me2 (subcluster "a"), other highly expressed genes show little to no enrichment for H3K79me2 (subcluster "b") (Fig. 6b, high gene expression). This observation was less prominent in the clusters of intermediary expressed genes or in the cluster that showed low gene expression (Fig. 6b, mid and low gene expression). We therefore analyzed the genes in subclusters "a" and "b" for their expression in the absence of DOT1L. While genes in subcluster "a" showed a highly significantly reduced fold expression in the absence of DOT1L, genes in subcluster "b" showed a moderately reduced expression in the absence of DOT1L (Fig. 6c), suggesting that DOT1L-dependent H3K79me2 is specifically required for maintaining the expression of a subset of highly expressed genes.

As we observed increased gene expression in the presence of H3K79me2 across the genome and a reduced Th1 cell differentiation program in the absence of DOT1L, we analyzed whether the absence of DOT1L is associated with decreased expression of master regulators, such as TFs that inhibit the Th1 cell differentiation program. We found that 15 TFs were significantly downregulated across all Th cell subsets, seven of which are known to inhibit the Th1 cell differentiation program (red), five of which have not been defined with regard to their influence of the Th1 cell differentiation program (black) and three of which promote the Th1 cell differentiation program (green). Most prominently, the TFs BACH2, FOXP3 and NR4A3 were among the highest downregulated TFs across naive Th, Th1 and Th2 cells in the absence of DOT1L (Fig. 6d), all of which have been correlated to limiting the Th1 cell differentiation program[17–19]. As an example, the Th1 program-inhibiting TF BACH2 was one of the highest downregulated TF across all Th cell subsets and clustered within the highest H3K79me2 covered genes (Fig. 6e), suggesting a role of DOT1L and H3K79me2 in maintaining its gene expression across Th cell subsets and thereby limiting the Th1 cell differentiation program. Thus, DOT1L controls Th cell differentiation through direct and indirect mechanisms.

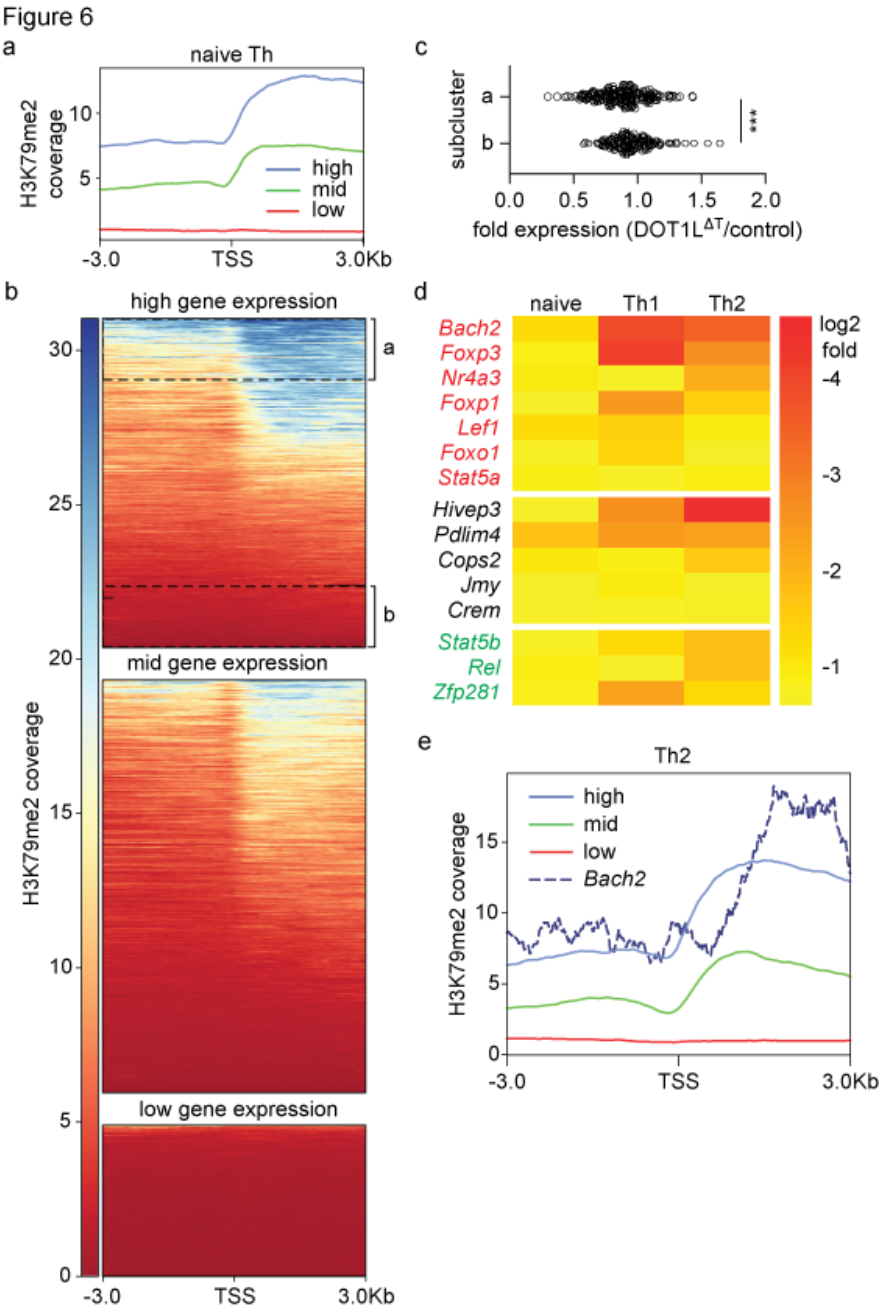

Fig. 6. DOT1L-dependent H3K79me2 is associated with a subset of highly expressed genes.

(a) Coverage of H3K79me2 ± 3 Kb around the TSS in wild-type naive Th cells grouped by expression strength: low (<20 counts), mid (~500 counts), high (top 450 expressed genes). **(b)** Heatmap of H3K79me2 coverage ± 3 Kb around the TSS, clustered in high, mid and low expressed genes. Subclusters "a" and "b" comprise of

the top and bottom 1000 peaks (185 different genes for "a" and 143 different genes for "b"), regarding H3K79me2 coverage scores within the highly expressed genes, respectively. **(c)** Fold expression of genes in subcluster "a" and "b" (Fig. 6b, high gene expression) in the presence or absence of DOT1L in naive Th cells. **(d)** Transcription factors (from RNA-seq experiment, Fig. 5, Table S1) that were downregulated across all Th cell subsets (naive Th, Th1, Th2) in the absence of DOT1L compared to control mice. Red indicates genes that inhibit the Th1 cell differentiation program, black shows unknown genes with regard to regulation of the Th1 cell differentiation program and green represents genes that are promoting the Th1 cell differentiation program. **(e)** Coverage of H3K79me2 ± 3 Kb around the TSS in Th2 cells grouped by expression strength: low (<20 counts), mid (~500 counts), high (top 450 expressed genes) and the coverage of H3K79me2 at the TSS of the TF BACH2. Data was visualised using deepTools[20]. Data shown is representative of one experiment (n=3). Statistical significance was determined by 2-tailed Student's t test. ***: $p \leq 0.001$.

DOT1L is not a direct activator of the PRC2 complex

The loss of Th cell lineage stability is reminiscent of mice with a deletion in EZH2, the catalytic component of the PRC2 complex that methylates H3K27[2]. As H3K27 methylation is a critical component of the gene repression machinery and loss of H3K27-dependent silencing could explain the increased gene expression observed in DOT1L-deficient cells, we sought to examine whether lack of DOT1L affected the H3K27 methylation state. We first examined the global levels of H3K27me3 and consistent with our previous studies using the specific DOT1L inhibitor SGC0946[7], found that loss of DOT1L had minimal effects on

H3K27me3 levels (Fig. 7a). However, when we examined levels of H3K27me3 at the *Ifng* promoter, we found that naive DOT1L-deficient Th cells had slightly reduced levels of H3K27me3, suggesting that DOT1L-dependent H3K79 methylation may be required for optimal PRC2 complex activity at specific loci (Fig. 7b). Previous studies have shown that certain methylated peptides can activate the PRC2 complex by binding to accessory proteins such as JARID1A[21,22]. However, unlike an H3K27-associated methylated peptide that induced PRC2 activity, mono-, di- or trimethylated H3K79 peptides failed to activate PRC2 complex (Fig. 7c), suggesting that DOT1L-dependent H3K79 methylation does not directly induce PRC2-dependent H3K27 methylation.

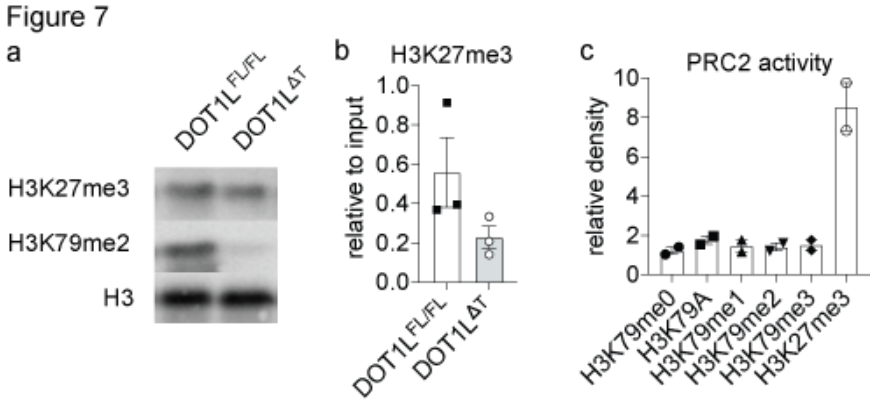

Fig. 7. DOT1L is not a direct activator of the PRC2 complex.

(a) Western blot of histone extracts from sorted Th cells of DOT1L$^{\Delta T}$ or control (DOT1L$^{FL/FL}$) mice for H3K79me2, H3K27me3 and pan-H3 (control). **(b)** H3K27me3 ChIP-qPCR of the *Ifng* promoter region from sorted Th cells of DOT1L$^{\Delta T}$ or control (DOT1L$^{FL/FL}$) mice. **(c)** Histone methyltransferase (HMTase) assays using the human PRC2 core complex (EZH2-SUZ12-EED-RBBP4) in the presence of mono-nucleosome substrates. Data shown is representative of two experiments (a, c: n=2; b: n=3).

T cell-intrinsic expression of DOT1L is required for Th2 cell-dependent immunity

Our results demonstrate that DOT1L is critical for limiting Th1 cell responses and maintaining Th2 cell lineage integrity in vitro. To test whether DOT1L is required for maintaining a protective Th2 cell responses in vivo, we infected control DOT1L$^{FL/FL}$ or DOT1L$^{\Delta T}$ mice with the intestinal helminth parasite *Trichuris muris*. In this model, a high dose infection (~200 eggs) of C57BL/6J mice results in the development of a protective Th2 cell-biased immune response, associated with goblet cell hyperplasia and mucus production, ultimately leading to worm expulsion. Following infection, DOT1L$^{\Delta T}$ mice maintained a significant worm burden at day 21 post-infection (Fig. 8, a and e), developed a non-protective Th1 cell response, with high levels of IFN-γ and low levels of IL-13 (Fig. 8, b and c), and produced low levels of *Trichuris*-specific IgG1 (Fig. 8d). Further, treatment of mice with monoclonal antibodies against IFN-γ (αIFN-γ) failed to reduce the levels of IFN-γ and promote resistance to infection in the absence of DOT1L (Fig. 8, a-e). Thus, consistent with our in vitro results, DOT1L appears to be a T cell-intrinsic factor that is critical for limiting the development of Th1 cell responses.

T cell-intrinsic expression of DOT1L is required for Th2 cell-dependent inflammation

Although protective in the context of helminth infection, Th2 cells are pathogenic in allergic inflammatory diseases such as asthma. To test if DOT1L deficiency prevented the development of pathogenic Th2 cells, we treated control DOT1L$^{FL/FL}$ or DOT1L$^{\Delta T}$ mice intranasally with house dust mite antigen (HDM). HDM induces a cascade of type 2 immune responses, including group 2 innate lymphoid cells, Th2 cells, and culminating with the recruitment and activation of eosinophils into the lungs. Consistent with our findings in vitro and during helminth infection, loss of DOT1L in T cells during allergic lung inflammation

resulted in a significant increase in IFN-γ expression and reduced levels of IL-13 (Fig. 8f). This further led to significantly fewer eosinophils ($CD45^+$ $SiglecF^+$ $CD11c^{neg}$, Fig. 8g) and a significantly reduced expression of *Muc5AC* as a marker of mucous production in the lungs of $DOT1L^{\Delta T}$ mice (Fig. 8h). These results suggest that targeting DOT1L could provide a novel therapeutic strategy to limit pathogenic Th2 cell responses.

In summary, the experiments described here provide the first evidence of a pivotal role for DOT1L and H3K79me2 in Th1/Th2 cell differentiation and lineage integrity, which has a significant impact on immunity to infection and the development of inflammation. These findings place DOT1L as a central regulator of Th cell differentiation and identify this pathway as a potential therapeutic target to treat diseases associated with dysregulated Th cell responses.

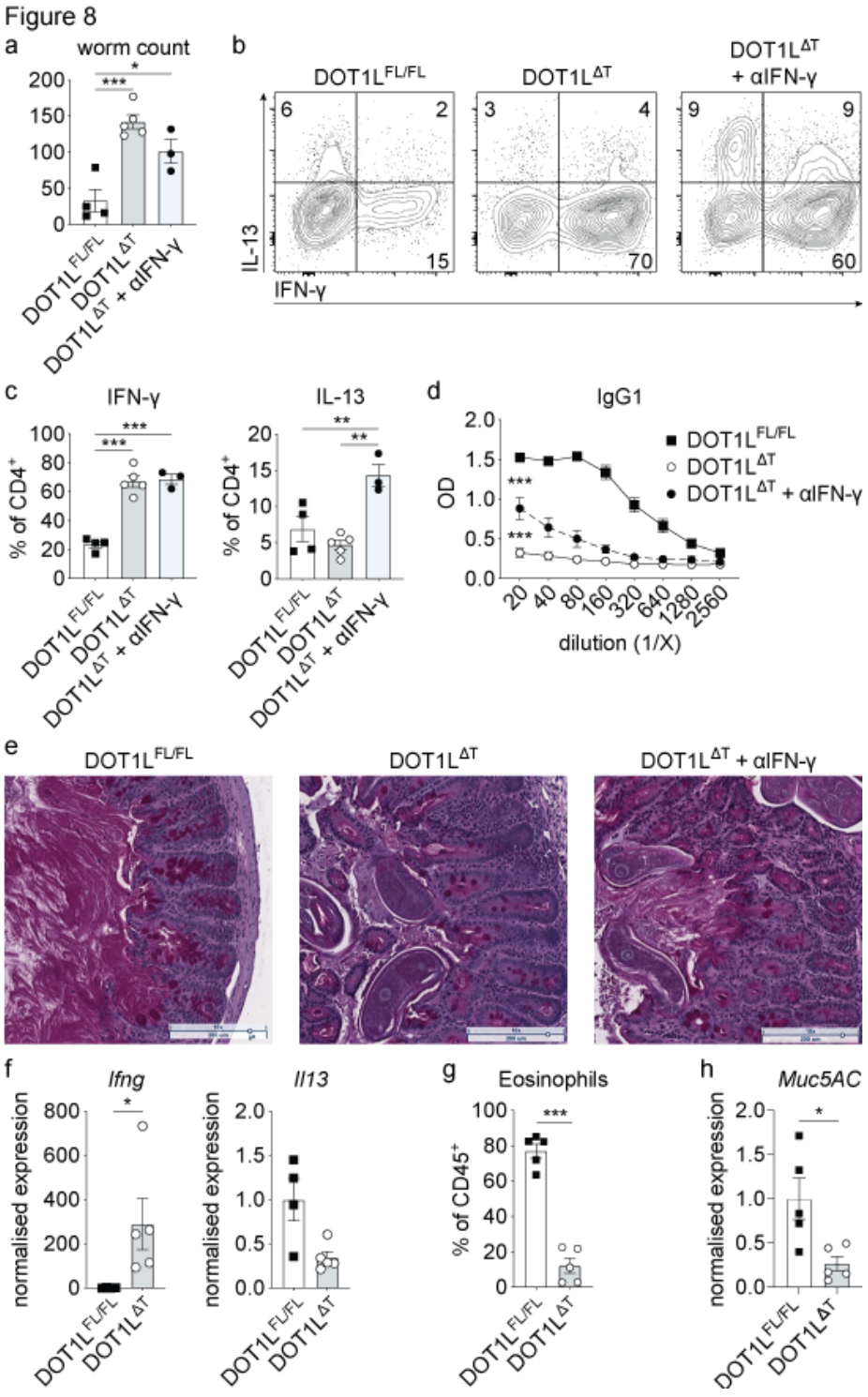

Fig. 8. T cell-intrinsic expression of DOT1L is required for Th2 cell-dependent immunity and infection.

(a) Worm count in the cecum 21 days after the infection of control (DOT1L$^{FL/FL}$) or DOT1L$^{\Delta T}$ mice with 200 eggs of *Trichuris muris*. Where indicated, some DOT1L$^{\Delta T}$

mice were additionally treated with neutralising antibodies against IFN-γ. **(b)** Production of IFN-γ and IL-13 from mesenteric lymph node cells (gated on viable $CD4^+$ T cells) three days post restimulation with αCD3 and αCD28 ex vivo. **(c)** Quantification of (b). **(d)** *Trichuris*-specific serum IgG1 from the same mice as (a). **(e)** Histology (PAS stain) of cecum tips from representative mice in (a). **(f, h)** QPCR analysis of indicated genes from whole lung tissue from mice sensitized and challenged with house dust mite allergen (HDM). **(g)** Eosinophils in the bronchoalveolar lavage from mice in (f). **(h)** QPCR analysis of the lungs for *Muc5AC*. Data shown is representative of two independent experiments (n=4-5 mice per group). Statistical significance was determined by 2-tailed Student's t test (f, g, h) or 2-way-ANOVA (a, c, d). *: $p \leq 0.05$, **:$p \leq 0.01$, ***: $p \leq 0.001$.

Discussion

KMTs are a fundamental part of the epigenetic machinery that can influence the outcome of the Th cell mediated immune response. As such, the KMTs G9a, EZH2 or SUV39H1 have previously been shown to be important factors in Th cell differentiation and Th cell-dependent immune response in infection and inflammation[2–5]. In the present study, we identify a role for DOT1L in Th cell differentiation and lineage integrity, which has a key impact on Th cell function during infection and inflammation in vivo.

Our finding that DOT1L-dependent H3K79me2 is associated with a subset of highly expressed genes is consistent with several previous studies that correlate the presence of H3K79me2 with gene expression and transcriptional elongation[11,12,14,15,23–25]. Despite our own findings and previous reports that correlate DOT1L-dependent H3K79me2 with gene expression, we found an increased overall gene expression and an increased Th1 cell differentiation program in the absence of DOT1L in Th cells. There are several possible mechanisms that could explain this. First, our gene expression data and H3K79me2 coverage analysis shows that a subset of highly expressed genes are dependent on the presence of H3K79me2. In the absence of DOT1L, some of these H3K79me2-dependent genes are significantly downregulated across Th cell subsets, among which are the Th1 cell differentiation program-inhibiting TFs BACH2, NR4A3 and FOXO1[17,19,26]. For example, BACH2 has been described as a potent and central regulator of the Th cell differentiation program[27]. In the absence of BACH2, T cells show increased gene expression for Th1 or Th2 genes under respective conditions[17,28] and its decreased expression in the absence of DOT1L could explain the increased expression of Th1 genes in Th cells. Another potential mechanism is cross-talk between DOT1L and the PRC2 complex. Interestingly, T cell-specific deletion of a member of the canonical PRC2 complex, EZH2, shows a similar

phenotype of increased production of IFN-γ under Th1 polarising conditions[2]. However, as we did not observe a direct impact on the activity of the PRC2 complex in the absence of H3K79 methylation, and did not observe a global reduction of PRC2-mediated H3K27me3 after inhibiting DOT1L in our previous report[7], we conclude that these similar phenotypes may be based on independent mechanisms. Finally, while we show that H3K79me2 is important for the expression of a subset of genes that regulate the Th1 cell differentiation program, DOT1L-dependent H3K79me3 may play an additional role in the gene expression program of Th cells, as this mark has been described to be negatively associated with gene expression in cells of the immune system[29]. Therefore, the absence of H3K79me3 in DOT1L-deficient Th cells may account for an increase in the expression of genes that depend on H3K79me3 for their suppression.

We found that the T cell-intrinsic expression of DOT1L was required to restrain Th1 cell lineage during immunity and inflammation in vivo. Following infection with the helminth parasite *Trichuris muris*, DOT1L$^{\Delta T}$ mice failed to develop a protective Th2 cell response and were susceptible to infection. Treatment of mice with αIFN-γ slightly increased the production of type 2 associated factors such as IL-13, IgG1 and mucous but failed to induce immunity to infection, further highlighting the T cell-intrinsic role for DOT1L to limit Th1 cell differentiation. This phenotype is similar to mice with a T cell-specific deletion of the KMT G9a as both mice fail to mount an efficient Th2 immune response[4]. However, unlike DOT1L-deficient Th cells, G9a-deficient Th cells do not show increased production of IFN-γ under Th2 polarising conditions, highlighting that distinct epigenetic mechanisms operate to control Th cell differentiation and function.

Our data shows that inhibition of DOT1L may be a highly efficient way of treating Th2-dependent inflammation, e.g. in patients with asthma. DOT1L is an especially

interesting target as the small molecule inhibitor pinometostat is currently approved for use in patients with mixed lineage leukemia (MLL) rearranged (MLL*r*) leukemia[30,31], which offers the possibility of repurposing this drug for other diseases. Interestingly, to date there are no reports on the effect of pinometostat on other cells in patients with MLL*r* leukemia, both highlighting the need for further studies in these patients with regard to Th cell differentiation, and accentuating the possibility that changes in T cell biology may account for the effect of pinometostat in these patients.

Taken together, we show that DOT1L is a central regulator of Th cell differentiation and function and identify DOT1L as a potential therapeutic target to treat diseases associated with dysregulated type 2 immunity.

Materials and Methods

Mice

To create DOT1L$^{FL/FL}$ mice, we derived mice from DOT1L targeted ES cells (Dot1ltm1a(KOMP)Wtsi) and crossed them with FLP mice (Monash University). Subsequently, DOT1L$^{FL/FL}$ mice were crossed with *Cd4*-Cre^{+} (C57BL/6 background) to generate DOT1L$^{\Delta T}$ mice. DOT1L$^{\Delta T}$ mice were crossed with IFN-γ-YFP reporter mice[32] to create DOT1L$^{\Delta T}$IFN-γYFP mice. DOT1L$^{\Delta T}$CRY mice were generated by crossing DOT1L$^{\Delta T}$IFN-γYFP with 4C13R[33] mice. DOT1L/TBET$^{\Delta T}$ mice were created by crossing DOT1L$^{\Delta T}$ and TBET$^{\Delta T}$ [34]. Animals were maintained in a specific pathogen-free environment and used at 6-10 weeks of age. Experiments and the animals' care were in accordance with the animal ethics committee of Monash University.

HDM model of allergic asthma

For the first 3 days of airway sensitization, mice were anesthetized under aerosolized isoflurane and intranasally instilled daily with 100 µg of house dust mite (HDM) antigen (Greer, Lenoir, NC) in 40 µl PBS. On days 13 to 17 post sensitization, mice were intranasally challenged daily with 25 µg of HDM antigen in 40 µl PBS before analysis on day 18. Bronchoalveolar lavage (BAL) was performed from the right lobes of the lung with three flushes of 800 µl PBS after clamping off the left lobe of the lung (used for histology). BAL fluid and tissues were processed as previously described[35].

Trichuris muris infection

Propagation of *Trichuris muris* eggs and infections were performed as previously described[36]. Mice were infected with 200 embryonated *T. muris* eggs by oral gavage to induce an

intestinal infection over a period of 21 days. Sacrificed mice were assessed for worm burdens by manually counting worms in the ceca using a dissecting microscope.

T cell assays

$CD4^+$ T cells were isolated from spleen and peripheral lymph nodes from indicated mice by negative selection using the EasySep™ Mouse $CD4^+$ T Cell Isolation Kit (StemCell Technologies Inc). 1.75 x 10^5 cells were cultured for 4 days in RPMI1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 25 mM HEPES, and 5×10^{-5} M 2-mercaptoethanol with 1 µg/ml each of plate-bound αCD3 (clone 145-2C11) and αCD28 (clone 37.51). Cells were cultured under Th0 (IL-2, 10 ng/ml), Th1 (IL-2 and IL-12, 10 ng/ml each; αIL-4 10 µg/ml).

Western blot

T cells ($CD4^+$ or $CD8^+$) and B cells ($CD19^+$) were sorted from the spleens of $DOT1L^{\Delta T}$ or CD4-Cre mice and pellets were frozen at -80˚C. Histones were extracted from frozen cell pellets by incubating in 0.2 N HCl overnight at 4˚C. Supernatants were run on 12% SDS-PAGE gels. H3K79me2 was detected using clone ab3594 (Abcam). A pan H3 antibody (ab1791, Abcam) was used as a loading control.

ELISA

T. muris-specific IgG1 was analyzed as previously described. In short, ELISA plates were coated with o/n supernatant of adult worms, blocked with 10% NCS and serum was added in serial dilutions (1/20-1/2560). Secondary antibody (IgG1-HRP) was added at 1/1000 and

incubated with 50 µl of TMB substrate until adequately developed. TMB substrate reaction was stopped by adding 50 µl of 1 N HCl and the plate was read at 450 nm.

RNA-seq

RNA was isolated from viable *bona fide* Th1 (YFP^+) or viable Th2 polarised cells of control ($CD4Cre^+$) and $DOT1L^{\Delta T}$ mice after 4 days of culturing under Th1 and Th2 polarising conditions, respectively, using the NucleoSpin RNA Kit from Macherey&Nagel according to the manufacturer's instructions and sequenced on a MiSeq paired-end run (75 x 75, v3; Illumina). Samples were aligned to the mm10 transcript reference using TopHat2, and differential expression was assessed using Cufflinks (Illumina). Visualization of the data was performed using DEGUST (https://github.com/drpowell/degust) and represent the average expression from two biological replicates. Raw data is available in Table S1.

ChIP-qPCR and ChIP-seq preparation

1.5 x 10^6 sorted naive T cells ($CD4^+$ $CD44^-$ $CD62L^+$), *bona fide* Th1 (YFP^+) and *bona fide* Th2 ($IL\text{-}4/IL\text{-}13^+$) cells from control (CRY mice) or $DOT1L^{\Delta T}$-CRY mice were fixed for 8 min with 0.6% formaldehyde in 10 ml complete RPMI1640 media on an overhead rotator at room temperature. Fixing was stopped by adding 1 ml of 1.25 M glycine and incubating for 5 min on an overhead rotator at room temperature. Cells were pelleted at 600 x g for 5 min at 4˚C and washed twice with 10 ml ice cold PBS. Pellets were resuspended in 250 µl ChIP lysis buffer and stored at -80˚C. Cells were sonicated in 3 sets of 10 x 30 s ON, 30 s OFF at 4˚C using a Bioruptor (Diagenode) with intermittent quick vortex and centrifugation using polystyrene tubes. 200 µl of the supernatant was collected after centrifugation at 15,000 x g for 10 min at 4˚C and diluted in 800 µl ChIP dilution buffer containing 1:20 protease

inhibitor. 40 µl of washed protein A and protein G magnetic beads (BioRad) and 2 µg of αH3K79me2 (ab3594) were added and incubated o/n at 4˚C on an overhead rotator. 20 µl of the supernatant (input) were diluted in 180 µl ChIP dilution buffer containing 0.3 M NaCl and incubated at 65˚C o/n for decrosslinking before using the PCR purification kit (Macherey&Nagel) for isolation of DNA. Next day, IP samples were washed consecutively twice with 1 ml of each ChIP low salt, ChIP high salt, ChIP LiCl and TE buffer using a magnet. Chromatin was eluted after the last wash by incubating twice with 150 µl ChIP elution buffer on an overhead rotator at room temperature. Both elutions were pooled and incubated at 65˚C o/n in the presence of 0.3 M NaCl. Next day, IP samples were purified using the PCR purification kit (Macherey&Nagel) and DNA was stored at -80˚C until QC and library preparation.

ChIP-sequencing

ChIP samples were processed using the MGITech MGIEasy DNA FS Library preparation kit V1 (according to the manufacturer's instructions: document revision A0) and sequenced using one lane of the MGISEQ-2000RS using an MGISEQ-2000RS High-Throughput Sequencing Set (PE100), yielding paired-end 100 base reads (according to the manufacturer's instructions: document revision A1).

PRC2 activity assay

Histone methyltransferase (HMTase) assays were carried out using the 4-subunit human PRC2 core complex (EZH2-SUZ12-EED-RBBP4) in the presence of mono-nucleosome substrates. SDS-PAGE with autoradiography was carried out to quantify the HMTase activity and the same gels were stained using Coomassie to ensure even loading. Quantification of

14C-methyl-transfer to H3 histones was done though densitometry of the radiograms, with the values represented in bar plots. Error bars represent two independent replicates that were carried out in two different days. As the H3K79 methylated peptides have low solubility in water, DMSO was included also in the positive (H3K27me3 peptide) and negative (no peptide) controls, to account for residual DMSO carried from the peptides stock (the final DMSO concentration was less than 3.5% in all cases.

Statistics

Statistical significance was determined by 2-tailed Student's t test or 2-way-ANOVA using GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA). Results were considered statistically significant with $P \leq 0.05$. *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

Acknowledgments

We would like to thank Dr Kim Jacobson for constructive comments and advice on the manuscript. We thank the Monash animal facility, Micromon, the Monash flow core facility and the Monash bioinformatics core facility for their excellent technical support and assistance. This work was supported by NHMRC Project grants (APP1104433 and APP1104466).

Author contributions

SS, CD and CZ designed the experiments. SS, MB, JR, BR, QZ, SFF, AZ, JE, GR and JN performed experiments. SS performed all mouse studies, ChIP-seq and RNA-seq experiments, ex vivo assays and performed final RNA- and ChIP-seq analysis. SS and CZ wrote the manuscript.

Competing interests

The authors declare no competing interests.

Data and materials availability

We thank Dr Simon Phipps (QIMR Berghofer) for providing 4C13R mice. We further thank Dr Ron Germain (NIAID) for providing the MTA for these mice[33]. RNA- and ChIP-Seq datasets described in this article are available at the National Center for Biotechnology Information (accession numbers GSE123966, GSE138821).

Supplementary Materials

Table S1. Raw data of RNA-seq as Excel sheet.

References

1. He, S., Tong, Q., Bishop, D. K. & Zhang, Y. Histone methyltransferase and histone methylation in inflammatory T-cell responses. *Immunotherapy* **5**, 989–1004 (2013).
2. Tumes, D. J. *et al.* The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. *Immunity* **39**, 819–832 (2013).
3. Allan, R. S. *et al.* An epigenetic silencing pathway controlling T helper 2 cell lineage commitment. *Nature* **487**, 249–253 (2012).
4. Lehnertz, B. *et al.* Activating and inhibitory functions for the histone lysine methyltransferase G9a in T helper cell differentiation and function. *J. Exp. Med.* **207**, 915–922 (2010).
5. Antignano, F. *et al.* Methyltransferase G9A regulates T cell differentiation during murine intestinal inflammation. *J. Clin. Invest.* **124**, 1945–1955 (2014).
6. Schapira, M. & Arrowsmith, C. H. Methyltransferase inhibitors for modulation of the epigenome and beyond. *Curr. Opin. Chem. Biol.* **33**, 81–87 (2016).
7. Scheer, S. *et al.* A chemical biology toolbox to study protein methyltransferases and epigenetic signaling. *Nat. Commun.* **10**, 19 (2019).
8. Min, J., Feng, Q., Li, Z., Zhang, Y. & Xu, R.-M. Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell* **112**, 711–723 (2003).
9. Frederiks, F. *et al.* Nonprocessive methylation by Dot1 leads to functional redundancy

of histone H3K79 methylation states. *Nat. Struct. Mol. Biol.* **15**, 550–557 (2008).

10. Jones, B. *et al.* The histone H3K79 methyltransferase Dot1L is essential for mammalian development and heterochromatin structure. *PLoS Genet.* **4**, e1000190 (2008).
11. Guenther, M. G., Levine, S. S., Boyer, L. A., Jaenisch, R. & Young, R. A. A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* **130**, 77–88 (2007).
12. Steger, D. J. *et al.* DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. *Mol. Cell. Biol.* **28**, 2825–2839 (2008).
13. Zhang, W. *et al.* Aldosterone-sensitive repression of ENaCalpha transcription by a histone H3 lysine-79 methyltransferase. *Am. J. Physiol. Cell Physiol.* **290**, C936–46 (2006).
14. Chen, X. *et al.* Methyltransferase Dot1l preferentially promotes innate IL-6 and IFN-β production by mediating H3K79me2/3 methylation in macrophages. *Cell. Mol. Immunol.* (2018). doi:10.1038/s41423-018-0170-4
15. Wang, Z. *et al.* Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat. Genet.* **40**, 897–903 (2008).
16. Stubbington, M. J. *et al.* An atlas of mouse CD4(+) T cell transcriptomes. *Biol. Direct* **10**, 14 (2015).
17. Edwards, C. L. *et al.* The Role of BACH2 in T Cells in Experimental Malaria Caused by Plasmodium chabaudi chabaudi AS. *Front. Immunol.* **9**, 2578 (2018).
18. Yang, C., Huang, X.-R., Fung, E., Liu, H.-F. & Lan, H.-Y. The Regulatory T-cell Transcription Factor Foxp3 Protects against Crescentic Glomerulonephritis. *Sci. Rep.* **7**, 1481 (2017).

19. Sekiya, T. *et al.* The nuclear orphan receptor Nr4a2 induces Foxp3 and regulates differentiation of CD4+ T cells. *Nat. Commun.* **2**, 269 (2011).

20. Ramírez, F. *et al.* deepTools2: a next generation web server for deep-sequencing data analysis. *Nucleic Acids Res.* **44**, W160–5 (2016).

21. Pasini, D. *et al.* Coordinated regulation of transcriptional repression by the RBP2 H3K4 demethylase and Polycomb-Repressive Complex 2. *Genes Dev.* **22**, 1345–1355 (2008).

22. Peng, J. C. *et al.* Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell* **139**, 1290–1302 (2009).

23. Im, H. *et al.* Dynamic regulation of histone H3 methylated at lysine 79 within a tissue-specific chromatin domain. *J. Biol. Chem.* **278**, 18346–18352 (2003).

24. Kryczek, I. *et al.* IL-22(+)CD4(+) T cells promote colorectal cancer stemness via STAT3 transcription factor activation and induction of the methyltransferase DOT1L. *Immunity* **40**, 772–784 (2014).

25. Pursani, V., Bhartiya, D., Tanavde, V., Bashir, M. & Sampath, P. Transcriptional activator DOT1L putatively regulates human embryonic stem cell differentiation into the cardiac lineage. *Stem Cell Res. Ther.* **9**, 97 (2018).

26. Kerdiles, Y. M. *et al.* Foxo transcription factors control regulatory T cell development and function. *Immunity* **33**, 890–904 (2010).

27. Roychoudhuri, R. *et al.* BACH2 represses effector programs to stabilize T(reg)-mediated immune homeostasis. *Nature* **498**, 506–510 (2013).

28. Kim, E. H. *et al.* Bach2 regulates homeostasis of Foxp3+ regulatory T cells and protects against fatal lung disease in mice. *J. Immunol.* **192**, 985–995 (2014).

29. Chory, E. J. *et al.* Nucleosome Turnover Regulates Histone Methylation Patterns over the Genome. *Mol. Cell* **73**, 61–72.e3 (2019).

30. Stein, E. M. *et al.* A Phase 1 Study of the DOT1L Inhibitor, Pinometostat (EPZ-5676), in Adults with Relapsed or Refractory Leukemia: Safety, Clinical Activity, Exposure and Target Inhibition. *Blood* **126**, 2547–2547 (2015).

31. Stein, E. M. *et al.* The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. *Blood* **131**, 2661–2669 (2018).

32. Reinhardt, R. L., Liang, H.-E. & Locksley, R. M. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat. Immunol.* **10**, 385–393 (2009).

33. Huang, Y. *et al.* IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells. *Nat. Immunol.* **16**, 161–169 (2015).

34. Intlekofer, A. M. *et al.* Anomalous type 17 response to viral infection by CD8+ T cells lacking T-bet and eomesodermin. *Science* **321**, 408–411 (2008).

35. Chenery, A. L. *et al.* Low-Dose Intestinal Trichuris muris Infection Alters the Lung Immune Microenvironment and Can Suppress Allergic Airway Inflammation. *Infect. Immun.* **84**, IAI.01240–15–501 (2015).

36. Antignano, F., Mullaly, S. C., Burrows, K. & Zaph, C. Trichuris muris infection: a model of type 2 immunity and inflammation in the gut. *J. Vis. Exp.* (2011).