CD4+ T cell lineage integrity is controlled by the histone deacetylases HDAC1 and HDAC2

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Molecular mechanisms that maintain lineage integrity of helper T cells are largely unknown. Here we show histone deacetylases 1 and 2 (HDAC1 and HDAC2) as crucial regulators of this process. Loss of HDAC1 and HDAC2 during late T cell development led to the appearance of major histocompatibility complex (MHC) class II–selected CD4+ helper T cells that expressed CD8-lineage genes such as Cd8a and Cd8b1. HDAC1 and HDAC2–deficient T helper type 0 (Th0) and Th1 cells further upregulated CD8-lineage genes and acquired a CD8+ effector T cell program in a manner dependent on Runx-CBFβ complexes, whereas Th2 cells repressed features of the CD8+ lineage independently of HDAC1 and HDAC2. These results demonstrate that HDAC1 and HDAC2 maintain integrity of the CD4 lineage by repressing Runx-CBFβ complexes that otherwise induce a CD8+ effector T cell–like program in CD4+ T cells.

Dynamic changes in the acetylation of core histones have a key role in the regulation of gene expression during development and differentiation. Modification of core histones by lysine acetylation is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs), which are major epigenetic regulators. Eighteen HDACs grouped in four classes have been identified in mammalian organisms. These HDAC subgroups differ in their structure, tissue expression, intracellular localization and target specificity. Class I comprises HDAC1, HDAC2, HDAC3 and HDAC8, and the class II group, which is subdivided into class Ia and class Ib, includes HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10. HDAC11 is the only member representing class IV HDACs. The class III group of HDACs comprises sirtuins (Sirt1–7), which differ in their cofactor requirement for enzymatic activity.

The use of HDAC inhibitors revealed important immunological processes dependent on HDAC activity, and several mammalian deacetylases, including HDAC1, HDAC2, HDAC3, HDAC7 and HDAC9, have been implicated in regulation of T cell development and function. Nevertheless, unique functions of individual HDAC members in specific T cell functions are still only poorly understood. T cell–specific loss of HDAC1 (using a Cd4-Cre deletion) leads to enhanced allergic airway inflammation and increased Th2 cytokine production, but T cell development is not impaired in these mice. However, HDAC2 is upregulated in the absence of HDAC1; thus, compensatory pathways or functions between these two closely related class I HDAC family members could occur in T cells, as described for several other cell systems. Indeed, mice with an Lck-Cre–mediated T cell–specific combined deletion of HDAC1 and HDAC2 display severely reduced thymic cellularity, accompanied by a block at the CD4+CD8+ double-negative (DN) to CD4+CD8+ double-positive (DP) transition. A block at the pre-pro-B to pre-B transition during B cell development also has been observed after the B cell–specific loss of HDAC1 and HDAC2 (ref. 10). Mice with a reduced combined activity of HDAC1 and HDAC2 (i.e., both Hdac1 alleles and one Hdac2 allele deleted) undergo neo-plastic transformation of immature thymocytes. These tumor cells become aneuploid and display enhanced expression of c-Myc. Together, this indicates that HDAC1 and HDAC2 are essential for early T cell development and the control of genomic stability in immature T cells.

Here we investigated the role of HDAC1 and HDAC2 at later stages during T cell development. We show that Cd4-Cre–mediated T cell–specific loss of HDAC1 and HDAC2 led to the appearance of MHC class II–selected CD4+ helper T cells that spontaneously...
expressed CD8-lineage genes such as Cd8a, Cd8b1 and Eomes (encoding eomesodernin). HDAC1 and HDAC2–deficient (Hdac1loxP/loxP/Hdac2loxP/loxP/Cd4-Cre here abbreviated Hdac1−−/−−Hdac2−−/−−Cd4-Cre and designated ‘HDAC1-2’ conditional knockout (cko)), T cells of the CD4 lineage had normal expression of Th-POK protein under homeostatic conditions and upregulated CD154 upon activation, which are characteristic features of helper T cells. However, activated HDAC1-2 cKO CD4+ T cells acquired CD8+ effector T cell features, indicated by a strong further upregulation of eomesodernin, by high production of interferon gamma (IFN-γ), and by the increased expression of Runx3, T-bet, granzyme B and perforin. Expression of genes characteristic of CD8+ effector T cells in activated HDAC1-2 cKO CD4+ T cells required Runx-CBFβ, and correlated with the presence of Runx-CBFβ complexes and local histone hyperacetylation at the promoter regions of these genes. These observations revealed that HDAC1 and HDAC2 are essential to maintain integrity of the CD4 lineage by repressing genes of the CD8 lineage in CD4+ T cells.

RESULTS
Appearance of CD4+CD8+ T cells in HDAC1-2 cKO mice
To investigate redundant and nonredundant functions of HDAC1 and HDAC2, we generated mice with a conditional T cell–specific (using Cd4-Cre) combined loss of HDAC1 and HDAC2. Similar to H dac1−−/−−Cd4−−/−−Cd4-Cre mice, Hdac2−−/−−Cd4-Cre (here referred to as HDAC2 cKO) mice did not display developmental alterations in the thymus and had normal CD4+ and CD8+ T cell numbers in the spleen in comparison to wild-type mice (data not shown). HDAC2 protein was progressively lost during T cell development and was not detectable in peripheral HDAC2 cKO T cells, which showed efficient deletion (Supplementary Fig. 1a–c). Naive and effector T cells as well as Foxp3+ regulatory T cells were present at numbers similar to those in wild-type mice (Supplementary Fig. 1d,e), which indicated normal T cell homeostasis, although HDAC1 was upregulated in the absence of HDAC2 (Supplementary Fig. 1f).

HDAC1-2 cKO mice displayed a 2–3-fold reduction of peripheral T cell numbers (Fig. 1a,b). However, a large population of CD4+CD8+ peripheral T cells (up to 16%) was present in HDAC1-2 cKO mice (Fig. 1a). These CD4+CD8+ T cells expressed CD8α–CD8β heterodimers (Fig. 1c) with similar TCRβ and reduced CD4 expression compared to wild-type CD4+ T cells (Supplementary Fig. 2a,b). In addition, a fraction of Foxp3+ HDAC1-2 cKO CD4+ T cells (~3–4%) also expressed CD8 (Supplementary Fig. 2c). The percentage of CD44hiCD4+ and CD44hiCD8+ T cells was not changed in HDAC1-2 cKO mice. However, CD44hi subsets in the CD4+CD8+ T cell population were reduced (~2.6–3-fold) compared to CD4+ and CD8+ T cells in HDAC1-2 cKO mice (Supplementary Fig. 2d). Upon ex vivo short-term activation, HDAC1-2 cKO CD4+CD8+ T cells displayed a cytokine profile characteristic of naive cells (Supplementary Fig. 2f) and did not show upregulation of memory markers under homeostatic conditions (Supplementary Fig. 2g). This suggests that HDAC1-2 cKO CD4+CD8+ T cells are naive T cells. HDAC1-2 cKO mice displayed an ~1.6-fold enhanced Annexin V fraction of peripheral CD8+ T cells, whereas there was no change in the CD4+ T cell population (Fig. 1d,e). Moreover, HDAC1-2 cKO CD4+CD8+ T cells displayed a similar percentage of Annexin V cells as the CD8+ T cell subset (Fig. 1d,e). Together, this indicates that loss of HDAC1 and HDAC2 leads to reduced peripheral T cell numbers and to the appearance of CD4+CD8+ T cells.

A detailed analysis of thymocyte subsets in HDAC1-2 cKO mice showed normal percentages of CD4+ single-positive (SP) (CD4SP) and CD8SP thymocytes as well as normal percentages and numbers of TCRβhi cells (Fig. 2a–d). Expression of CD5, CD69 and CD24 in HDAC1-2 cKO DP thymocytes was similar to wild-type DP thymocytes, and TCRβhiCD4SP and TCRβhiCD8SP cells upregulated CD5 normally,
indicating no major alteration in TCR signaling during positive selection (Fig. 2a). However, the percentage and numbers of mature TCRβCD24hi cells were ~70% reduced in HDAC1-2 cKO mice, whereas the number of TCRβCD24bi cells was similar to that in wild-type mice (Fig. 2a,c,d). There was also a small reduction of the percentage of CD69+SP cells (data not shown). Mature HDAC1-2 cKO TCRβCD24bi thymocytes contained a substantial CD4+CD8+ T cell population (up to 7%) in addition to regular CD4SP and CD8SP thymocytes (Fig. 2c), indicating that CD4+CD8+ T cells are emerging already during T cell development. In vivo 5-bromodeoxyuridine (BrDU) labeling experiments showed that the frequency of BrDU+ TCRβCD24hi thymocytes was similar in wild-type and HDAC1-2 cKO thymi (Fig. 2f), whereas the percentage of Annexin V+ TCRβCD24bi was ~1.4-fold increased (although not significant) in HDAC1-2 cKO mice compared to wild type (Fig. 2g). This suggests a partial loss of mature TCRβCD24bi cells resulting from apoptosis rather than a developmental block at the CD24bi to CD24hi transition. Moreover, the generation of either wild-type (CD45.1+) and wild-type (CD45.2+) or wild-type (CD45.1+) and HDAC1-2 cKO (CD45.2+) mixed bone marrow (BM) chimeric mice indicated that the reduction in T cell numbers and the appearance of CD4+CD8+ T cells are cell-intrinsic effects (Supplementary Fig. 3a). Taken together, the data indicate T cell–intrinsic defects in HDAC1-2 cKO mice that lead to reduced T cell numbers and the appearance of peripheral CD4+CD8+ T cells.

**HDAC1-2 cKO CD4+CD8+ T cells are helper T cells**

To test whether HDAC1-2 cKO CD4+CD8+ T cells, like CD4+ T cells, were selected on MHC class II, we generated MHC class I–deficient (B2m−/−) wild-type or HDAC1-2 cKO BM chimeric mice. Wild-type BM chimeric B2m−/− mice developed peripheral CD4+ T cells but not CD8+ T cells in the absence of MHC class I (Fig. 3a). In HDAC1-2 cKO chimeric B2m−/− mice both CD4+ T cells and CD4+CD8+ peripheral T cell subsets developed (Fig. 3a), whereas both subsets were absent or markedly reduced in HDAC1-2 cKO mice that have been crossed onto a MHC class II–deficient (H2-Ab1−/−) background (Fig. 3b). These results indicated that the peripheral CD4+CD8+ T cells in HDAC1-2 cKO were MHC class II–restricted (Fig. 3a). Moreover, the CD4+CD8+ T cells isolated from HDAC1-2 cKO chimeric B2m−/− mice or from HDAC1-2 cKO mice upregulated CD154 upon activation (Fig. 3c and Supplementary Fig. 3b), thus displaying features of the helper lineage.

Only a fraction of the HDAC1-2 cKO CD4+ T cells upregulated CD8 under homeostatic conditions in vivo. We excluded inefficient deletion and thus escape of cells that retained Hdc1 or Hdc2 alleles, because a PCR analysis revealed that the majority of cells (>95%) had deleted Hdc1 and Hdc2 alleles (Supplementary Fig. 3c). Intracellular staining showed that >99% of wild-type CD4+ T cells expressed high amounts of HDAC1 and HDAC2 (Fig. 3d). In HDAC1-2 cKO CD4+ T cells, residual HDAC1 and HDAC2 protein expression was still detectable (Fig. 3d,e). However, the percentage of cells expressing HDAC1 and HDAC2 was reduced by ~50%, and the amounts of HDAC1 and HDAC2 protein were reduced by ~60% in HDAC1-2 cKO CD4+ T cells compared to wild-type CD4+ T cells (Fig. 3e,f) and protein amounts were even further reduced (by ~25–30%) in HDAC1-2 cKO CD4+CD8+ T cells compared to HDAC1-2 cKO CD4+ T cells (Fig. 3f). This indicates a slow disappearance of HDAC1...
and HDAC2 proteins upon Cd4-Cre–mediated deletion of the Hdac1 and Hdad2 alleles. Together, these data indicate that peripheral HDAC1-2 cKO CD4+CD8+ T cells represent helper-lineage T cells and show that the upregulation of CD8 in HDAC1-2 cKO CD4-lineage T cells under homeostatic conditions inversely correlated with the decreasing amounts of HDAC1 and HDAC2 protein upon deletion of Hdac1 and Hdad2 alleles.

Activated HDAC1-2 cKO CD4+ T cells upregulate CD8

Next, we tested whether CD8 is stably silenced in HDAC1-2 cKO CD4+ T cells that did not upregulate CD8 in vivo. Sorted wild-type and HDAC1-2 cKO CD4+ T cells showed a similar proliferative response (Fig. 4a) and upregulation of CD25 and CD69 (Fig. 4b) to anti-CD3 and anti-CD28 stimulation, which suggested that early T cell activation pathways are not altered in the absence of HDAC1 and HDAC2. A large proportion (up to 45%) of HDAC1-2 cKO CD4+ T cells upregulated CD8 (Fig. 4c), whereas control wild-type CD4+ T cells (Fig. 4c) or single Hdac1/2Cd4-Cre or Hdad2/2Cd4-Cre CD4+ T cells (data not shown) remained CD8−. HDAC1-2 cKO CD8+ T cells did not upregulate CD4 (Fig. 4c). Upregulation of CD8 expression was not potentiated by TGF-β (data not shown), which is known to induce CD8 expression on a fraction of CD4+ T cells13. Because the Cd8 enhancer E8β directs CD8 expression in mature CD8+ T cells14–16, we tested whether E8β is required for CD8 expression in HDAC1-2 cKO CD4+CD8+ T cells. In comparison to E8β+/−/−HDAC1-2 cKO mice, in E8β−/−/−HDAC1-2 cKO mice CD4+CD8+ T cell numbers were reduced by ~50%, and cells from the latter mice had lower CD8 expression (~30% less mean fluorescence intensity) (Fig. 4d–f). Moreover, ex vivo–activated E8β−/−/−HDAC1-2 cKO CD4+ T cells showed impaired upregulation of CD8 in comparison to activated E8β+/+ HDAC1-2 cKO CD4+ T cells (Supplementary Fig. 3d). These data indicate derepression of Cd8β in all HDAC1-2 cKO CD4-lineage T cells and show that CD8 expression in HDAC1 and HDAC2–deficient CD4-lineage T cells is largely dependent on E8β.

HDAC1-2 cKO CD4+ T cells acquire CD8+ effector T cell features

To characterize HDAC1-2 cKO CD4+ and CD4+CD8+ T cell subsets, we analyzed gene expression using Agilent arrays. 450 genes were upregulated and 162 genes were downregulated in HDAC1-2 cKO CD4+ T cells compared to wild-type CD4+ T cells (Supplementary Fig. 4a,b and Supplementary Table 1). Moreover, upregulation of Cd8α and Cd8β1 in HDAC1-2 cKO CD4+CD8+ T cells was accompanied by the enhanced expression of Eomes (5.4-fold), Runx3 (1.9-fold) and Tbx21 (encoding T-bet; 3.6-fold) (Supplementary Fig. 4c,d), which are essential transcription factors required for induction of the cytotoxic effector T cell program in CD8+ T cells17,18. In contrast, the expression of genes of the CD4 lineage, such as Cd4 and Zbtb7b...
Upon activation, HDAC1 association with CD8-CD7 γ10 + CD8α-10e+44. This suggests I10+ H10140.1 Supplementary Fig. 5a 16.20.8.1 11.6+α-d+21x306 © 2014 with enhanced H3K9Ac histone marks at the promoter regions of Runx3 making it unlikely that the appearance of HDAC1-2 cKO CD4+ T cells also upregulated CD8 upon activation (Fig. 4f). Next, we tested whether the increased CD8-lineage gene expression in HDAC1-2 cKO CD4-lineage T cells correlated with the acquisition of CD8α effector T cell functions upon activation. Because we observed a strong induction of apoptosis after 3 d of activation (Fig. 4g) during the culture of HDAC1-2 cKO CD4+ T cells, similar to observations reported for B cells, fibroblasts and keratinocytes7,9,12, naive HDAC1-2 cKO CD4+ T cells and CD8α T cells were activated with anti-CD3 and anti-CD28 only for 60 h. Under these non-polarizing ’T0’ conditions, HDAC1-2 cKO CD4+ T cells produced increased amounts of IFN-γ (Fig. 5a,c) and displayed increased expression of Granzyme B (approximately sevenfold on mRNA level) in comparison to activated wild-type CD4+ T cells (Fig. 5b,d). Moreover, Prf1 (encoding Perforin) expression was fourfold higher (although not significant; Fig. 5b). In addition, HDAC1-2 cKO CD4+ T cells upregulated eomesodermin (~23-fold on mRNA level) to a level equivalent to that in CD8α T cells and expressed significantly higher amounts of T-bet (encoded by Tbx21) (~2.6-fold on mRNA level) and Runx3 (~2.5-fold), while Zbtb7b expression was decreased (approximately twofold) (Fig. 5b,c). As observed for ex vivo-activated HDAC1-2 cKO CD4+ T cells (Fig. 4c), sorted naive HDAC1-2 cKO CD4+ T cells also upregulated CD8 upon activation (Fig. 5d). Those HDAC1-2 cKO CD4+ T cells that upregulated CD8α exhibited a similar cell division rate compared to the cells that were still CD8α− (Fig. 5e), making it unlikely that the appearance of HDAC1-2 cKO CD4+CD8α T cells upon activation is due to preferential proliferation of cells that had already upregulated CD8α. Moreover, expression of Cds8a, Runx3 and Eomes in activated HDAC1-2 cKO CD4+ T cells correlated with enhanced H3K9Ac histone marks at the promoter regions of these gene loci and at the Cds8 enhancer E8α, in comparison to activated wild-type CD4+ T cells, whereas repressive H3K27me3 marks were reduced (Fig. 5f). We also examined the recruitment of HDAC1 and HDAC2 to the Cds8a, Cds8b1, Runx3 and Eomes promoter regions, and to E8α, using chromatin immunoprecipitation (ChIP) assays. We observed HDAC1 and HDAC2 binding to the regulatory regions of CD8-lineage genes both in CD4+ and CD8α T cells (Supplementary Fig. 5a). Upon activation, HDAC1 association with CD8α lineage genes was slightly increased in CD4+ T cells but was reduced in activated CD8α T cells. In contrast, HDAC2 association with regulatory regions of CD8-lineage genes was increased upon activation both in CD4+ and CD8α T cells (Supplementary Fig. 5a). This suggests that binding of HDAC1 and HDAC2 is not indicative of whether the analyzed genes are repressed by HDAC1 and HDAC2 or not.

To investigate the role of HDAC1 and HDAC2 in the repression of a CD8α effector T cell lineage program, we polarized wild-type and HDAC1-2 cKO CD4+ T cells under T11- and T12-polarizing conditions. T11-polarized HDAC1-2 cKO CD4+ T cells displayed a similar upregulation of features of the CD8 lineage as observed for HDAC1-2 cKO CD4+ T cells (Fig. 5a). In contrast, T12-polarizing conditions repressed the induction of a CD8α T cell program in the absence of HDAC1 and HDAC2 (Fig. 6a,b). Of note, the addition of IL-4 alone (in the absence of anti-IFN-γ or anti–IL-12 blocking antibodies) was sufficient to repress the induction of CD8 in activated HDAC1-2 cKO CD4+ T cells (Fig. 6c). In contrast, the addition of IL-12 or IFN-γ did not alter the extent of CD8 upregulation upon activation (Fig. 6c). Together, these data indicate that HDAC1 and HDAC2 are required for the repression of a CD8α-lineage program in T10 and T11 cells, whereas HDAC1-2 cKO T12-polarized cells maintain the repression of a CD8α program.

Normal Th-POK expression in HDAC1-2 cKO CD4+ T cells

The transcriptional regulator Th-Pok has been implicated in the repression of the Cds8 loci and other CD8-lineage genes in CD4-lineage T cells15–22. Zbtb7b mRNA levels were reduced by 40–50% in HDAC1-2 cKO CD4+CD8α T cells compared to HDAC1-2 cKO CD4+ T cells and wild-type CD4+ T cells (Supplementary Fig. 4c,d). Immunoblot analysis indicated that Th-Pok expression in CD4+ and CD4+CD8α T cells from HDAC1-2 cKO mice was similar...
Figure 5 A CD8 effector program is induced in activated HDAC1-2 cKO CD4+ T cells. (a) IFN-γ in supernatants of wild-type (WT) and HDAC1-2 cKO CD4+ and CD8+ T cells activated with anti-CD3 and anti-CD28 for 60 h. *P < 0.05, unpaired two-tailed Mann-Whitney test. (b) Quantitative real-time PCR (qRT-PCR) analysis showing the expression relative to Hprt of several genes in WT and HDAC1-2 cKO CD4+ and CD8+ T cells activated as described in a. *P < 0.05, **P < 0.01, ***P < 0.001, unpaired two-tailed Student’s t-test. (c,d) Flow cytometric analysis showing intracellular expression of IFN-γ and eomesodermin and of GATA-3 and T-bet and of granzyme B (Gz B) and surface expression of CD8α and CD8β in WT and HDAC1-2 cKO CD4+ and CD8+ T cells activated as described in a. (e) Flow cytometry analysis showing cell divisions of cell division dye eFluor 450–labeled naive HDAC1-2 cKO CD4+ T cells activated as described in a. Cells were gated on CD4+CD8α− and CD4+CD8α+ subsets shown in the CD4 and CD8α contour plots (left). (f) qRT-PCR analysis with primers specific for Runx3, Eomes and Cd8a promoter regions and for Cd8 enhancer E8 from chromatin of nonactivated (N) and activated (A; anti-CD3 plus anti-CD28 for 60 h) WT and HDAC1-2 cKO CD4+ T cells immunoprecipitated with anti-H3K9Ac or anti-H3K27me3 antibodies (or IgG as control). ND, not detected. Values are corrected to H3 levels and shown as % input. Numbers in contour plots indicate the percentage of cells in the respective quadrant (c,e). Means ± s.d. are shown. Data are representative (c-f) or show the summary (a,b) of four (a,e), of three (CD4+ T cells) and two (CD8+ T cells) (b) and of two (c,d,f) independent samples that were analyzed in three (a), in two (CD4+ T cells) and one (CD8+ T cells) (b), and in two (c-f) independent experiments.

To study that in wild-type CD4+ T cells (Fig. 7a,b). Normal Th-POK protein amounts in CD4+CD8+ T cells, despite reduced mRNA expression under homodynamic conditions, might indicate increased Th-POK protein stability in the absence of HDAC1 and HDAC2. To study Zbtb7b expression in single cells, we crossed HDAC1-2 cKO mice with green fluorescent protein (GFP) reporter knock-in mice, in which GFP expression is driven by the endogenous Zbtb7b regulatory elements23. We found similar GFP expression in CD4+ T cells from Zbtb7b+/−GFP mice and Zbtb7b+/GFP HDAC1-2 cKO mice, although the percentage of GFP+ Th-POK–expressing CD4+ T cells was slightly reduced in HDAC1-2 cKO mice (Fig. 7c). Of note, a fraction (up to 25%) of HDAC1-2 cKO CD8+ T cells expressed GFP (Fig. 7c). There was also high expression of GFP in CD4+CD8+ HDAC1-2 cKO T cells (Fig. 7c). Thus, CD8-lineage genes are derepressed in HDAC1-2 cKO helper T cells under homodynamic conditions despite normal expression of Th-POK protein.

The CD8+ effector T cell program depends on Runx-CBFβ

Runx-CBFβ complexes are essential for the differentiation of CD8+ effector T cells17,18,24. Runx3 protein was highly upregulated (~6–7-fold) in activated HDAC1-2 cKO CD4+ T cells in comparison to wild-type CD4+ T cells (Fig. 8a). Runx3 expression was also induced in activated wild-type CD4+ T cells in the presence of MS-275, a specific inhibitor of most class I HDACs (i.e., HDAC1, HDAC2 and HDAC3)25. To test whether the induction of CD8-lineage genes in HDAC1-2 cKO CD4+ T cells is dependent on Runx factors, we crossed HDAC1-2 cKO mice with Cbfβ−/− mice26 to induce conditional deletion of CBFβ. Activated Cbfβ−/−HDAC1-2 cKO CD4+ T cells displayed a marked reduction of CD8+ effector T cell program induction compared to HDAC1-2 cKO CD4+ T cells, indicated by the diminished expression of CD8α, eomesodermin, granzyme B, T-bet and IFN-γ (Fig. 8b,c). Runx-CBFβ complexes bind to CD8-lineage effector genes such as Prf1, Gzbm, Ifng18 and to Cd8 enhancer E8,16,27 in activated CD8+ T cells. Runx-CBFβ complexes bind to these gene regions in activated HDAC1-2 cKO CD4+ T cells but not in wild-type CD4+ T cells (Fig. 8d), which suggests that Runx-CBFβ complexes are required for induction of CD8-lineage genes in HDAC1-2 cKO helper T cells.

Next, we expressed Runx3 from a retrovirus in wild-type CD4+ T cells in the presence or absence of MS-275. The retroviral construct contained an internal ribosomal entry site followed by GFP to
distinguish between transduced and nontransduced cells. Treatment with MS-275 of CD4+ T cells transduced with the retroviral control vector (MIGR) induced the expression of CD8α, eomesodermin, IFN-γ and T-bet (Fig. 8e), which indicates that class I HDACs are required to maintain the repression of these genes characteristic of CD8+ effector T cells, in addition to Runx3 (Fig. 8a), in CD4-lineage T cells. Treatment of activated CD8+ T cells with MS-275 did not upregulate CD4 expression (data not shown). Enforced retroviral expression of Runx3 induced expression of CD8α, granzyme B and IFN-γ, which were additionally upregulated in synergy with MS-275 (Fig. 8e). In contrast, treatment of Runx3–transduced cells with the HAT inhibitor anacardic acid reduced the effect of Runx3 on the induction of CD8 but not on expression of granzyme B or IFN-γ (Fig. 8e). Similarly, treatment with anacardic acid antagonized the upregulation of CD8 in HDAC1-2 cKO CD4+ T cells (Fig. 8f). Finally, MS-275–treated E81-α or Cbfbδ–/–Cd4-T-Cre CD4+ T cells displayed impaired upregulation of CD8 (Fig. 8g) and the upregulation of CD8 upon retroviral expression of Runx3 and MS275 treatment was reduced in E81-α–/–CD4+ T cells in comparison to wild-type CD4+ T cells (data not shown), which suggests an important role for E81 and Runx-CBFβ in the induction of CD8 expression.

The CD8+ effector T cell program was not induced in HDAC1-2 cKO CD4+ T cells under T12 polarizing conditions (Fig. 6a,b). GATA-3, which is a key factor driving T12 differentiation, inhibited T12 differentiation and was shown to repress Runx3-mediated Ifng induction.29,30 To test whether MS-275 might counteract the MS-275–mediated induction of CD8, we retrovirally expressed GATA-3 in wild-type CD4+ T cells in the presence or absence of MS-275. Whereas MIGR-control vector transduced cells upregulated CD8 expression upon MS-275 treatment, GATA-3 expressing cells showed a reduced upregulation of CD8 (Supplementary Fig. 5b). This indicates that GATA-3 impairs the upregulation of CD8 upon inhibition of class I HDACs. Together, these data suggest that the induction of a CD8+ effector T cell program in the absence of HDAC1 and HDAC2 is dependent on the activity of Runx-CBFβ complexes and that class I HDACs are required to maintain the repression of a CD8+ effector T cell program in CD4-lineage T cells.

Loss of HDAC1 and HDAC2 affects potential nonhistone targets HATs and HDACs mediate dynamic changes in the acetylation of core histones at lysine residues and are thus considered key epigenetic regulators of gene expression. Many nonhistone targets of HATs and/or HDACs, however, are emerging in which lysine acetylation has been shown to affect protein-protein and protein-DNA interactions, protein stability and intracellular localization.31 To identify potential nonhistone HDAC targets that might have an altered stability due to the lack of HDAC1 and HDAC2, we performed a mass spectrometry analysis of wild-type and HDAC1-2 cKO CD4+ T cells under homeostatic conditions. This revealed 255 proteins as differentially abundant in the absence of HDAC1 and HDAC2.
In this study we provide genetic evidence that HDAC1 and HDAC2 control CD4+ T cell lineage integrity and T cell homeostasis. CD4+ and CD4+CD8+ T cells in HDAC1-2 cKO mice were MHC class II-restricted, expressed Th-POK under homeostatic conditions and upregulated CD154 upon activation, thus displaying defined features of CD4-lineage cells. However, the observation that a Runx-CBFβ complex–dependent CD8+ effector T cell program is upregulated in HDAC1-2 cKO CD4-lineage cells upon activation revealed that HDAC1 and HDAC2 are essential to repress features of the CD8 lineage in CD4+ T cells. Moreover, our study showed that late (Cd4-Cre) deletion of HDAC1 and HDAC2 led to reduced numbers of peripheral T cells and to a strong induction of apoptosis in CD4+ T cells after 3 d of activation. This indicates that HDAC1 and HDAC2 are also essential for the generation of the peripheral T cell pool and for the survival of proliferating CD4+ T cells.

Our data show that HDAC1 and HDAC2 repress features of the CD8 lineage in CD4+ T cells. The presence of a small fraction of HDAC1-2 cKO CD4+CD8+ T cells in MHC class I chimeric mice might suggest
that some MHC class II–restricted HDAC1–2 cKO CD4+ T cells were redirected into the CD8+ lineage. Runx-CBFβ complexes are essential for the differentiation of CD8+ effector T cells17,18,24. Based on our data, we propose that HDAC1 and HDAC2 maintain integrity of the CD4 lineage by repressing Runx-CBFβ complexes. Our mRNA expression analysis and ChIP experiments suggested that HDAC1 and HDAC2 directly suppress the transcription of the Runx3 gene in CD4+ T cells. It is conceivable that the strong induction of Runx3 expression in HDAC1–2 cKO CD4-lineage T cells is the initiating event in the induction of a CD8+ effector T cell program, in part via recruitment of Runx-CBFβ complexes to target genes such as Cd8, GzmB, Prf1 and Ifng. Within the Cd8 enhancer gene complex, Runx-CBFβ complexes were bound to the Cd8 enhancer E87, and the induction of Cd8 in HDAC1–2 cKO CD4+ T cells was largely dependent on E87. Preliminary data using Cd8 enhancer E87, E87-deficient mice22 also indicate a role for E87 in this process, because HDAC1–2 cKO CD4+ T cells did not upregulate Cd8 under homeostatic conditions in the combined absence of E87 and E87 (data not shown). Moreover, eomesodermin and T-bet, which are important for the induction of the cytotoxic program17, were upregulated in HDAC1–2 cKO CD4-lineage T cells in a Runx-CBFβ–dependent manner, and it is conceivable that Runx3, eomesodermin and T-bet together regulate the induction of the CD8+ effector T cell program in HDAC1–2 cKO CD4+ T cells. Th-POK, which is a master commitment factor for the development of CD4-lineage T cells33,34, represses the expression of genes specific to the CD8 lineage20–22. Loss of HDAC1 and HDAC2 recapitulates in part the phenotype of a ‘hypomorphic’ Zbtb7b allele in CD4+ T cells, which acquire some CD8-lineage characteristics and start to express certain cytotoxic effector genes such as perforin and granzyme B21,122. Ex vivo, HDAC1–2 cKO CD4+CD8+ T cells had similar Th-POK protein expression as wild-type and HDAC1–2 cKO CD4+ T cells, although Zbtb7b mRNA expression was slightly reduced in HDAC1–2 cKO CD4+CD8+ T cells in comparison to wild-type and HDAC1–2 cKO CD4+ T cells. This might indicate that HDAC1 and HDAC2 are part of a regulatory complex that controls stability of Th-POK, because acetylation of Th-POK, mediated by the HAT p300, has been shown to stabilize Th-POK35. Thus, the derepression of certain CD8-lineage genes in CD4+ T cells in the absence of HDAC1 and HDAC2 under homeostatic conditions is not due to reduced expression of Th-POK protein, although we cannot rule out that Th-POK requires (in part) HDAC1 and HDAC2 to exert its activity. However, HDAC1–2 cKO CD4+ T cells upon activation downregulated Zbtb7b expression compared to activated wild-type CD4+ T cells; thus it is likely that reduced expression of Zbtb7b might enhance and/or contribute to the induction of a CD8+ effector T cell program upon activation in the absence of HDAC1 and HDAC2.

The induction of a CD8+ effector T cell program was repressed in HDAC1–2 cKO CD4+ T cells under Th12-polarizing conditions. This demonstrates that the upregulation of CD8+ effector T cell genes is not a ‘general’ feature of HDAC1–2 cKO CD4+ T cells. GATA-3, the key transcription factor for Th12 cell differentiation inhibits Th1 cell differentiation and was shown to repress Runx3-mediated Ifng induction29,30. Our observation that enforced GATA-3 expression in MS-275–treated wild-type CD4+ T cells impaired the upregulation of CD8 suggests that GATA-3 contributes to the repression of a CD8 program in Th12 cells in the absence of HDAC1 and HDAC2, perhaps via blocking the activity of Runx3. In contrast, CD8 upregulation was not influenced by enforced expression of eomesodermin (data not shown). Preliminary results indicate that HDAC1–2 cKO Th17 cells also repress the induction of a CD8+ effector T cell program (data not shown), which suggests that other factors in addition to GATA-3 might interfere with the activity of Runx3 in the absence of HDAC1 and HDAC2 as well. Additional studies are required to investigate the regulatory hierarchy among HDAC1, HDAC2, Runx3, Th-POK, eomesodermin, T-bet and GATA-3.

Our study also provides insight into HDAC1 and HDAC2–mediated transcriptional regulation of the T cell transcriptome. The expression of a small number of genes was altered in HDAC1–2 cKO CD4+ T cells (612 genes, >twofold absolute change; P < 0.05, two-tailed unpaired Student’s t-test), which indicates a high specificity of HDAC1 and HDAC2 in the regulation of gene expression. More genes were upregulated than downregulated in HDAC1–2 cKO CD4+ T cells, which demonstrated the repressive function of complexes containing HDAC1 and HDAC2. Future experiments using ChIP following by sequencing (ChIP-seq) approaches will be important to determine which of the deregulated genes are direct HDAC1 and HDAC2 target genes, although our ChIP assays on selected CD8-lineage genes suggest that HDAC1 and HDAC2 binding is not indicative whether a gene is repressed by HDAC1 and HDAC2 or not.

HDACs are considered general chromatin-modifying regulators of gene expression, although many nonhistone targets of HATs and HDACs are currently emerging. Lysine acetylation of proteins has been shown to affect protein–protein and protein–DNA interactions, protein stability and intracellular localization31. The correlative analysis of our proteomic data with gene expression arrays identified 189 genes as differentially expressed between wild-type and HDAC1–2 cKO CD4+ T cells at the protein but not at the mRNA level. This indicates potential nonhistone targets of HDAC1 and HDAC2 that might have altered protein stability because of changes in post-translational lysine-acetylation patterns. Thus, these data suggest that loss of HDAC1 and HDAC2 affects CD4+ T cells at the transcriptional (chromatin) as well as post-translational modification level. Runx3 protein amounts can be controlled by competitive lysine acetylation and deacetylation36, which suggests an additional post-translational regulation of Runx3 expression, although preliminary data using cycloheximide approaches suggest that Runx3 stability is not increased in activated HDAC1 and HDAC2–deficient CD4+ T cells (data not shown). Additional studies are required to determine whether post-translational modifications of Runx3 are altered in the absence of HDAC1 and HDAC2 that affect Runx3 activity and/or stability.

Finally, our study also shows that HDAC2 is not required for (late) T cell development and the generation of the peripheral T cell pool. As HDAC1-deficient or HDAC2-deficient T cells displayed a compensatory upregulation of HDAC2 or HDAC1, respectively, it is likely that a combined activity of HDAC1 and HDAC2 above a certain threshold is sufficient to allow proper development of T cells. Loss of HDAC1 and HDAC2 in mouse embryonic fibroblasts8, keratinocytes9 or B cells10 leads to a cell-cycle block at the G1 to S transition and an induction of apoptosis. Similarly, loss of HDAC1 and HDAC2 at early stages of T cell development (Lck-Cre) leads to a severe reduction of thymocyte numbers, most likely owing to a block in cell-cycle progression at the pre-TCR stage11,12. A potential explanation why peripheral T cells are still present, although at reduced numbers, upon simultaneous ablation of HDAC1 and HDAC2 using Cd4-Cre might be the low proliferation rate of mature CD4+ SP and CD8+ SP thymocytes and naive peripheral T cells, which allows those cells to keep residual HDAC1 and HDAC2 protein upon deletion of the Hdac1 and Hdac2 alleles in amounts that are sufficient for survival. However, the increase in Annexin V+ T cells under homeostatic conditions and the strong induction of apoptosis in CD4+ T cells after 3 d of activation indicates that HDAC1 and HDAC2 are also essential for the generation of the
Role of caspase-8 in thymus function.

Control of the development of CD8αα+ intestinal β

Repression of interleukin-4 in T helper type 1 cells by Runx/Cbf +

Hdac1 and Hdac2 act redundantly to control p63 and p53 +

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ONLINE METHODS

Animal models. All animal experiments were evaluated by the ethics committees of the Medical University of Vienna and approved by the Federal Ministry of Science, Research and Economy, BMWFW (GZ:BMWFW-66.009/0057-II/10b/2010 and GZ:BMWFW-66.009/58-II/10b/2010). Animal husbandry and experimentation was performed under the national laws (Federal Ministry for Science and Research, Vienna, Austria) and ethics committees of the Medical University of Vienna and according to the guidelines of the Federation of Laboratory Animal Science Associations (FELASA), which match that of Animal Research: Reporting In Vivo Experiments (ARRIVE).

Mice carrying loxp-flanked (floxed) Hdacl and Hdacl alleles have been described. Cd4-Cre mice were provided by C. Wilson. MHC class II-deficient (H2-Ab1−/) and MHC class I-deficient (B2m−/) mice were obtained from Taconic. OT- II TCR transgenic mice were provided by D. Stoiber-Sakaguchi (Medical University of Vienna). E8γ−/−, E8γ−/−Eβ−/−, Zbtb7b-GFP knock-in, Runx3f/f and Cbybfl mice have been described. All mice analyzed were 8–12 weeks of age and of mixed sex unless otherwise stated. Littermate controls were used for flow cytometry antibody stainings within one experiment.

Generation of bone marrow chimeric mice. BM transplantation was done as described. Six to eight weeks after transplantation, the reconstituted mice were killed and analyzed by flow cytometry (LSRII; BD Biosciences).

Flow cytometry analysis and antibodies. Antibodies used in this study are listed in Supplementary Table 4.

Isolation and activation of T cells. CD4+ and CD8+ T cells were isolated from 6-week-old mice as previously described. Cells (1 × 10^6 cells in 1 ml) were seeded onto 48-well cell culture plates coated with anti-CD3e (1 µg/ml) and anti-CD28 (3 µg/ml) (BD Pharmingen). Cells were then harvested at various time points dependent on the experimental setting for flow cytometric analysis as indicated in figure legends. For cell-proliferation experiments, purified CD4+ T cells were labeled with CFSE (Molecular Probes) or eFluor 450 proliferation dye (eBioscience) before activation as previously described.

Differentiation of CD4+ T cells and cytokine measurements. Sorted naive CD44−/lowCD62L−/− CD4+ T cells were stimulated (day 0) with plate-bound anti-CD3ε (1 µg/ml) and anti-CD28 (3 µg/ml) on 96-well plates (2 × 10^5 cells/well) in 200 µl T cell medium (RPMI GlutaMAX-I supplemented with 10% FCS, antibiotics and 2-mercaptoethanol; all from Invitrogen) under various cytokine conditions. For TGFβ conditions (non-polarizing) recombinant human IL-2 (HI-2; 20 U/ml) and anti-IL-4 was added; for Th1 conditions, IL-2 (20 U/ml), IL-12 (5 ng/ml) and anti–IL-4 was used; for Th2 conditions, IL-2 (10 U/ml), IL-4 (250 U/ml), anti–IFN-γ (10 µg/ml) and anti–IL-12 (10 µg/ml). Cells were analyzed after 60 h.

Intracellular cytokine, transcription factor and granzyme B stainings. T cells were cultured under various conditions as described above. For intracellular cytokine detection, cells were stimulated in 96-well plates at 10^6 cells/ml for 4 h with PMA (50 ng/ml) and ionomycin (500 ng/ml) (Sigma-Aldrich) in the presence of GolgiStop (BD Biosciences). After harvesting, T cells were surface-stained with appropriate antibodies. Subsequently, cells were fixed with cytofix Fixation Buffer (BD Biosciences), permeabilized with Perm/Wash Buffer (BD Biosciences), and stained for intracellular proteins according to the manufacturer’s protocol.

BrdU incorporation experiments. Mice were injected intraperitoneally with 1 mg (in 200 µl) BrdU. 4 d later, thymocytes were isolated, stained for surface markers. Subsequently, BrdU was detected with the Apoptosis, DNA Damage and Cell Proliferation Kit (BD Biosciences) according to the manufacturer’s protocol.

Apoptosis detection assay. Apoptosis of ex vivo–analyzed and of cultured T cells was detected by Annexin V and 7-AAD stainings following the Annexin V Apoptosis detection kit (eBioscience), according to the manufacturer’s instructions.

Intracellular HDAC1 and HDAC2 staining. Splenocytes were preincubated with Fc-block (BD Pharmingen) and stained with CD8ε, CD4 and TCRβ antibodies. For immunodetection of HDAC1 and HDAC2, cells were fixed and permeabilized using Fixp3 Fixation/Permeabilization Concentrate and Diluent (eBioscience) according to the manufacturer’s instructions. Subsequently, cells were blocked in 5% normal goat serum and then incubated with rabbit anti-mouse HDAC1 and mouse anti-mouse HDAC2 antibodies in permeabilization buffer (eBioscience) for 1 h. Cells were washed with permeabilization buffer and incubated with Alexa Fluor 488–conjugated goat anti-rabbit IgG1 and biotinylated anti-mouse IgG1 antibodies, followed by a streptavidin secondary staining.

cDNA synthesis and quantitative real-time PCR. Cells were harvested with Trizol reagent (Invitrogen), and total RNA was isolated according the manufacturer’s instructions. RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad). Quantitative real-time PCR (qRT-PCR) analysis was performed with the SuperScript III qPCR MasterMix (Invitrogen) on the CFX 96 Real-Time PCR detection system (Bio-Rad).

Chromatin immunoprecipitation assays. Preparation of soluble chromatin was carried out as previously described. For HDAC1 and HDAC2 ChIP assays, T cells were pretreated with the cross-linker disuccinimidyl glutarate (2 mM; AppliChem) for 25 min at room temperature. Chromatin was sonicated with the Bioruptor Sonication System (Diagenode). For ChIP assays, equal amounts of sonicated chromatin were diluted tenfold and precipitated overnight with the following antibodies: rabbit anti-mouse HDAC1 (affinity-purified polyclonal serum), mouse anti-mouse HDAC2 (Abcam ab12169), anti–H3K9ac (Millipore 07-449), C-terminal H3 (Abcam ab1791), rabbit IgG (Invitrogen), mouse IgM (Invitrogen) and CFBP26 as a control. Chromatin/antibody complexes were isolated using Protein A or Protein G magnetic beads (Dynabeads, Invitrogen). The extracted DNA was used for qRT-PCR analysis with an iCycler IQ system (Bio-Rad) and KAPA SYBR FAST qPCR MasterMix (Peqlab). In parallel, PCR reactions with 1:20 dilutions of genomic DNA (input) were carried out. Values for histone H3 modification marks were corrected to the H3 values obtained with C-terminal H3 antibody.

Primers. Primers used in this study are listed in Supplementary Table 5.

HDAC and HAT inhibitor experiments. CD4+ or CD8+ T cells were isolated from lymph nodes and spleens of C57BL/6, Eβ−/− or Cbyb−/−Cd4-Cre mice and activated as described above. After activation for 24 h, either MS-275 (Selleck Chemicals, at a final concentration of 2 µM with DMSO as a carrier control) or anacardic acid (Sigma-Aldrich, used at a final concentration of 20 µM with EtOH as a carrier control) was added and cells were cultured for additional 24 h. Afterward, cells were stained as described and analyzed on a FACScalibur or LSRII flow cytometer (BD Biosciences).

Retroviral expression of Runx3 and GATA-3. The cDNA encoding for the distal promoter–derived Runx3 protein was cloned into the MIGR retroviral vector. GATA-3 vector was provided by J. Zhu. High-titer viral preparations were generated as described. Wild-type C57BL/6 CD4+ T cells (0.5 × 10^6 cells in 1 ml) were activated in 48-well tissue-culture plates coated with anti-CD3ε (0.5 µg/ml) and anti–CD28 (1 µg/ml) in the presence of 10 U/ml rhIL-2. After 24 h, the culture medium was aspirated and 1 ml virus-containing supernatant was added per well. Spin infection was performed by centrifugation of the plates at 600g for 1 h at 32 °C. The supernatant was removed, and fresh medium containing 10 U/ml rhIL-2 was added. Twenty-four hours later, MS-275 (2 µM, with DMSO as carrier control) or anacardic acid (Sigma-Aldrich, used at a final concentration of 20 µM with EtOH used as carrier control) was added and cells were cultured for additional 24 h. Afterward, cells were stained as described and analyzed on a LSRII flow cytometer.

Immunoblots analysis. T cells (0.6 × 10^6) were lysed in (35 µl) Carlin lysis buffer (20 mM Tris–HCl (pH 8.0), 138 mM NaCl, 10 mM EDTA, 100 mM NaF, 1% (vol/vol) Nonidet P-40, 10% (vol/vol) glycerol, 2 mM Na vanadate supplemented with complete protease inhibitors (Roche). Proteins were separated on
The acquired raw MS data files were processed with msconvert (ProteoWizard Library v2.1.2708) and converted into Mascot generic format (mgf) files. Peptide identification was performed by searching the resultant peak lists against the SwissProt mouse database version v2013.01_20130110 (24,615 sequences; 14,280,050 residues) with the search engines Mascot (v2.3.02, MatrixScience) and Phenyx (v2.5.14, GeneBio) \(^\text{34}\). Submission to the search engines was via a Perl script that performs an initial search with relatively broad mass tolerances (Mascot only) on both the precursor and fragment ions (±0.1 p.p.m. and ±0.6 Da, respectively). High-confidence peptide identifications were used to recalculate all precursors and fragment-ion masses before a second search with narrower mass tolerances (±0.05 p.p.m. and ±0.025 Da). Trypsin was chosen as cleavage specificity with the maximum of 1 miscleavage site allowed. Carbamidomethyl cysteine, N-terminal and lysine-modified iTRAQ 4-plex were set as fixed modifications, whereas oxidized methionine was set as a variable modification.

To validate the proteins, Mascot and Phenyx output files were processed by internally developed parsers. Proteins with ≥2 unique peptides above a score T1, or with a single peptide above a score T2, were selected as unambiguous identifications. Additional peptides for these validated proteins with score > T3 were also accepted. The threshold set (T1, T2, T3) was equal to ion score (16, 40, 10) for Mascot, and z-score (5.5, 9.5, 3.5) for Phenyx. In addition, peptide P-values output by Phenyx were required to be less than 10\(^{-2}\). The validated proteins retrieved by the two algorithms were merged, any spectral conflicts were discarded, and protein groupings according to common peptides. A false positive detection rate (FDR) of <1% and <0.1% (including the peptides exported with lower scores) was determined for proteins and peptides, respectively, by applying the same procedure against a decoy database with reversed protein sequences.

**Bioinformatic analysis: array data.** Gene expression study was performed with Agilent Whole Genome Microarrays (Agilent’s SurePrint G3 Mouse GE 8x60K Microarray, Agilent Microarray Design ID 028005). The data analysis was performed using GeneSpring software 11.5 (Agilent Technologies). According to the manufacturer, the array contains 39,430 RNAs from Entrez Gene and 16,251 large intergenic noncoding RNAs (lincRNAs). The lincRNAs were not considered in the analysis. The cutoffs for differential expression were set as absolute fold change > 2 and a corrected P < 0.05 (two-tailed unpaired Student’s t-test).

**Bioinformatic analysis: proteomic data.** Quantitative proteomics data were analyzed with isobar version 1.5.3 (ref. 44). iTRAQ intensities were corrected for isotope impurities according to the instructions provided by the manufacturer, and channels were normalized to equal summed intensities. Protein identifications were grouped by peptides, and only group-specific peptides were used for quantitation. In total, 5,167 protein groups were identified, and 5,138 were quantitated. The intraclass ratios were calculated (114:115 and 116:117) to estimate the biological variability and fit a T-distribution. The HDAC1-2 cKO wild-type (WT) ratios were summarized from interclass ratios, and proteins were selected as significantly different that passed all of the following criteria: ratio P < 0.05, FDR-adjusted sample P < 0.05, fold change > 1.3. With these criteria, 206 upregulated and 49 downregulated proteins in HDAC1-2 cKO compared to WT data were selected from 5,138 quantitated proteins.

**Bioinformatic comparison of array data and proteomic data.** The mapping from probe identifiers to gene product was performed using the Gene Expression Omnibus (GEO) Platform file for the microarray (GPL10877) and Ensembl Biomart (version 72, accessed using the R package biomart 2.16.0). 31,185 CDNs could be mapped to Uniprot accession codes, 4,760 of the 5,167 protein groups identified from the proteomic experiment could be matched to microarray probe identifiers. A subset of protein accession codes matched to multiple probe identifiers (because of splice variants and incorrect annotations) and these were processed to generate a unique mapping: (i) matches...
in which the gene name differed between transcript and protein (as stated by the Gene Expression Omnibus microarray platform annotation and Uniprot) were removed; (ii) remaining rows were summarized (average ratio), unless the mRNA ratios were considered significant. Correlation of the log-ratios is in the expected range for microarray transcriptomic versus proteomics data\(^5\). The Pearson's correlation coefficient was 0.4357, and Spearman's rank coefficient was 0.4219. Out of the subset of significantly different species, 134 transcripts could be matched to proteomic data, and 238 proteins to transcriptomic data.

**Statistical analysis.** No statistical methods were used to predetermine the sample size. The data shown are expressed as mean ± s.d. or as mean ± s.e.m. (as indicated in the figure legends). All experiments that required a statistical analysis were performed at least three times. The statistical analyses were performed using Prism Software (GraphPad Inc). As indicated in each figure legend, \(P\) values were calculated with either an unpaired two-tailed Student's \(t\)-test (if data showed a Gaussian distribution; variances were assessed and if necessary an unpaired \(t\)-test with Welch's correction was applied), with an unpaired two-tailed Mann-Whitney test or with a Wilcoxon signed-rank test. For qRT-PCR expression data, a normal distribution was assumed, and data were analyzed using an unpaired two-tailed Student's \(t\)-test (Fig. 5a and Supplementary Fig. 4b, indicated in the figure legends). The relative reduction in the percentage of CD8\(^+\) cells in Supplementary Fig. 5b was analyzed using a one sample two-tailed \(t\)-test. No data were excluded and no specific randomization of animals or blinding of investigators was applied.

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Corrigendum: CD4⁺ T cell lineage integrity is controlled by the histone deacetylases HDAC1 and HDAC2

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In the version of this article initially published, Lisa Göschl’s surname was spelled incorrectly. The error has been corrected in the HTML and PDF versions of the article.