AIM2 in regulatory T cells restrains autoimmune diseases

The inflammasome initiates innate defence and inflammatory responses by activating caspase-1 and pyroptotic cell death in myeloid cells. It consists of an innate immune receptor/sensor, pro-caspase-1, and a common adaptor molecule, ASC. Consistent with their pro-inflammatory function, caspase-1, ASC and the inflammasome component NLPR3 exacerbate autoimmunity during experimental autoimmune encephalomyelitis by enhancing the secretion of IL-1β and IL-18 in myeloid cells. Here we show that the DNA-binding inflammasome receptor AIM2 has a T cell-intrinsic and inflammasome-independent role in the function of T regulatory (Treg) cells. AIM2 is highly expressed by both human and mouse Treg cells, is induced by TGFβ, and its promoter is occupied by transcription factors that are associated with Treg cells such as RUNX1, ETS1, BCL11B and CREB. RNA sequencing, biochemical and metabolic analyses demonstrated that AIM2 attenuates AKT phosphorylation, mTOR and MYC signalling, and glycolysis, but promotes oxidative phosphorylation of lipids in Treg cells. Mechanistically, AIM2 interacts with the RACK1–PP2A phosphatase complex to restrain AKT phosphorylation. Lineage-tracing analysis demonstrates that AIM2 promotes the stability of Treg cells during inflammation. Although AIM2 is generally accepted as an inflammasome effector in myeloid cells, our results demonstrate a T cell-intrinsic role of AIM2 in restraining autoimmunity by reducing AKT–mTOR signalling and altering immune metabolism to enhance the stability of Treg cells.

Experimental autoimmune encephalomyelitis (EAE) was induced in wild-type, Aim2, Asc and double-knockout Casp1−/− Casp11−/− (Casp11 is also known as Casp4) mice after immunization with the myelin oligodendrocyte glycoprotein (MOG)35–55 peptide (Methods). Consistent with the literature, Asc and Casp11−/− mice had negligible EAE compared to wild-type controls (Fig. 1a). Notably, Aim2−/− mice developed more severe EAE, with higher clinical score and disease incidence (Fig. 1a) and increased pathology, demyelination and inflammatory immune cell infiltration in the spinal cord when compared to wild-type control mice (Fig. 1b–d), whereas Asc−/− mice showed a reduction in all of these parameters (Fig. 1b, c). These observations questioned the predicted, pro-inflammatory role of AIM2 through inflammasome activation in EAE. Indeed, levels of the cytokines IL-1β, IL-18, IL-6 and TNF were indistinguishable in the spinal cords of Aim2−/− and control mice (Extended Data Fig. 1e). However, levels of the cytokines IFNγ and IL-2 were significantly different in the spleens and draining lymph nodes of wild-type and Aim2−/− mice (Extended Data Fig. 1a, b). Under steady-state conditions, Aim2−/− mice showed normal T cell homeostasis in the thymus, spleen and lymph nodes (Extended Data Fig. 1c–h). These findings indicate that AIM2-dependent changes in T cells occurred at sites of neuroinflammation. Although FOXP3+ Treg cells were reduced in the spinal cords of Aim2−/− mice during EAE (Fig. 1f), wild-type and Aim2−/− Treg cells proliferated, survived and expressed effector markers similarly, under steady state and during EAE (Extended Data Fig. 2a–e). During the late phase of EAE, Aim2 deletion led to reduced expression of Foxp3, Tgfβ1 and Il10 mRNA (Fig. 1h) and IL-10 protein (Fig. 1i) in spinal cords. Consistently, the numbers of FOXP3+ Treg cells were significantly decreased in the spinal cords of Aim2−/− mice, whereas Treg cell numbers were increased (Fig. 1j). Aim2−/− CD4+ T cells proliferated normally in vitro (Extended Data Fig. 2f, g) and Aim2−/− CD8+ T cells were phenotypically normal during EAE (Extended Data Fig. 2h–k). We therefore posit that AIM2 probably controls CD4+ T cell function to mitigate EAE.
Fig. 1 | Aim2−/− and Asc−/− mice have opposing responses to EAE. a, EAE scoring of wild-type (WT) (n = 46), Aim2−/− (n = 45), Asc−/− (n = 40) and Casp1−/−Casp11−/− (n = 4) mice; five experiments for WT and Aim2−/− mice, two experiments for Asc−/− mice and one experiment for Casp1−/−Casp11−/− mice. The last two produced results that are consistent with previous reports. b, Luxol fast blue and periodic acid-Schiff (LFB-PAS) staining of spinal cords. WT (n = 3), Aim2−/− (n = 8) and Asc−/− (n = 4) mice, day 22 of EAE. Representative of 3–8 mice per group, three experiments. c, Quantification of demyelination and inflammatory foci. WT (n = 3), Aim2−/− (n = 8) and Asc−/− (n = 4) mice from b. d, Infiltrating cells in spinal cords of wild-type and Aim2−/− mice, day 22 of EAE, n = 6 per group, two experiments. e, Spinal cord IL-1β, IL-18, IL-6 and TNF, analysed by ELISA; WT (n = 13) and Aim2−/− (n = 16) for IL-1β (3 experiments), n = 5 for other cytokines (2 experiments). f, g, Flow cytometry of CD4+FOXP3+ Treg cells (f), IFNγ+ or IL-17A+ CD4+ cells (g) in spinal cords of wild-type and Aim2−/− mice, days 14–15 during EAE, n = 6 per group, three experiments. h, qRT–PCR of indicated genes, n = 3, 5 and 6 for WT, n = 4, 6 and 8 for Aim2−/− samples, two experiments. i, IL-10 protein analysed by ELISA; n = 10 for WT and 11 for Aim2−/− samples, three experiments. j, Flow cytometry of CD25 FOXP3+FOXP3+IL10+ and IL17A+ CD4+ cells in wild-type and Aim2−/− spinal cords at days 22–23 of EAE, n = 4 for WT, n = 5 for Aim2−/− samples of CD25 FOXP3+ and IL17A− (2 experiments), n = 13 per group for FOXP3+IL10 (3 experiments). k, EAE scores of Rag1−/− mice that received wild-type (n = 21) or Aim2−/− CD4+ T cells (n = 19), three experiments. l, Flow cytometry of spinal cord CD4+FOXP3+ Treg cells from k. n = 6 per group, three experiments. Data are mean ± s.e.m., NS, not significant. **P < 0.01, ***P < 0.001, ****P < 0.0001, two-way ANOVA and Holm–Sidak post hoc test (a, k), two-sided t test (c–j, I).

To assess the CD4+ T cell-intrinsic function of AIM2, we adoptively transferred CD4+ T cells from wild-type or Aim2−/− mice into Rag1−/− mice13 followed by EAE elicitation. Mice that received Aim2−/− CD4+ T cells developed more severe EAE at an earlier time than those that received wild-type CD4+ T cells (Fig. 1k), with concurrent reduced Treg cells in the spinal cord (Fig. II). These findings suggest a previously unappreciated cell-intrinsic function of AIM2 in Treg cells. High levels of AIM2 expression are detected in both mouse (https://www.immgen.org)13 and human (https://www.ebi.ac.uk/kb) Treg cells (Extended Data Fig. 3a–c). We empirically confirmed that isolated Treg cells expressed higher levels of Aim2 than CD4+ conventional T (Tcon) cells, with or without T cell receptor (TCR) activation (Extended Data Fig. 3d–f). Notably, TGFβ, a factor that is vital for Treg cell generation and homeostasis15, increased Aim2 expression in CD4+, but not in CD8+ T cells. Furthermore, genetic abrogation of TGFβ signalling in mice with deletion of the Tgfr2 gene reduced Aim2 expression in Treg cells in vivo (Extended Data Fig. 3g–i). Analysis of chromatin immunoprecipitation followed by high-throughput sequencing (ChIP–seq) datasets (DRP003376)16 of the Aim2 promoter in Treg versus Tcon cells showed that the transcription factors RUNX1, EST1, BCL11B and CREB, which are known to bind the Foxp3 loci to regulate Treg cell stability16, also bound to the Aim2 promoter in Treg cells more than in Tcon cells (Extended Data Fig. 3j). These findings suggest that a Treg cell-specific molecular network favours Aim2 expression.

To assess the function of AIM2 in Treg cells, we first studied a T cell-induced colitis model in which transferred naïve T cells provoke colitis in Rag1−/− recipients, whereas the inclusion of Treg cells offers protection14. CD4+CD45RB+B naïve T cells from wild-type mice were transferred alone or with sorted wild-type or Aim2−/− Treg cells into Rag1−/− recipients (Fig. 2a). Transferred wild-type naïve T cells led to a decline in the body weight of recipient mice (Fig. 2b) and intestinal pathology (Extended Data Fig. 4a, b). Transferred Aim2−/− Treg cells in the colon were present at a reduced frequency compared to wild-type Treg cells (Fig. 2c). The inclusion of wild-type but not Aim2−/− Treg cells mitigated weight loss (Fig. 2b), reduced pathology and suppressed the expression of pro-inflammatory cytokines IL-1α, IL-1β, TNF, IL-12 and IFNγ, but promoted anti-inflammatory IL-10 in colon explants (Extended Data Fig. 4a–c). To determine whether the role of AIM2 in Treg cells can be separated from its conventional role in inflammasome activation, we transferred Asc−/− Treg cells in the colitis model. In contrast to Aim2−/− Treg cells, Asc−/− Treg cells had similar effects to wild-type Treg cells (Extended Data Fig. 4d, e).

We next examined the function of AIM2 in Treg cells in the EAE model. Naïve CD4+ T cells from 2D2 mice that expressed a transgenic T cell receptor for the encephalitogenic autoantigen, MOG35–55, were transferred with sorted Treg cells from either wild-type or Aim2−/− mice into Rag1−/− mice to induce EAE. EAE was then elicited in these recipient mice (Extended Data Fig. 4f, g). Mice that received 2D2 CD4+ T cells alone developed severe EAE (Extended Data Fig. 4h). The addition of wild-type Treg cells modestly attenuated EAE development (Extended Data Fig. 4i, h), with a decrease in Tpl7 cells and an increase in Treg cells in the spinal cord (Extended Data Fig. 4i, j). By contrast, Aim2−/− Treg cells were unable to reduce EAE (Extended Data Fig. 4g–j). Notably,
transferred Aim2<sup>−/−</sup> T<sub>reg</sub> cells gained expression of IFN-17A but had reduced populations of IL-17A IFNγ<sup>+</sup> cells (Extended Data Fig. 4k). These findings suggest a crucial role for AIM2 in promoting T<sub>reg</sub> cell function in vivo. However, the in vitro suppressive function of Aim2<sup>−/−</sup> T<sub>reg</sub> Cells appeared normal (Extended Data Fig. 4l).

Wild-type T<sub>reg</sub> cells did not strongly suppress EAE in 2D2 mice, probably owing to robust T cell activation mediated by the transgenic TCR expressed in these mice. To better address the T<sub>reg</sub> cell-intrinsic role of AIM2, we generated mice with T<sub>reg</sub> cell-specific deletion by creating a mouse strain with a floxed Aim2 allele by gene targeting (Fig. 2d). Mice with a floxed Aim2 allele were bred with mice bearing a T<sub>reg</sub> cell-specific Cre transgene Foxp3-GFP-cre (FGC) and with mice bearing the Rosa26<sup>Tomato</sup> (R26T) lineage-tracing allele (Fig. 2e). T<sub>reg</sub> cell-specific deletion of Aim2 was confirmed by PCR and western blot (Extended Data Fig. 5a, b).

T<sub>reg</sub> cell-specific Aim2<sup>−/−</sup> mice were normal without any obvious defects under steady-state conditions (Extended Data Fig. 5c–i). EAE elicitation resulted in significantly higher clinical scores in T<sub>reg</sub> cell-specific Aim2<sup>−/−</sup> mice compared to wild-type mice, accompanied by lower FOXP3<sup>+</sup> and higher IFNγ<sup>+</sup>CD4<sup>+</sup> T cells in the spinal cord (Fig. 2g, h). Aim2<sup>−/−</sup> FGC R26T and Aim2<sup>−/−</sup> FGC R26T mice were used for lineage tracing based on the schematic depicted in Fig. 2e. Aim2<sup>−/−</sup> FGC R26T mice led to increased levels of T<sub>reg</sub> cells (FOXP3<sup>+</sup>CD4<sup>+</sup> cells) that previously expressed FOXP3<sup>+</sup> and increased IFNγ expression by T<sub>reg</sub> cells in the spinal cord (Fig. 2i, j), but not the spleen or draining lymph nodes during EAE (Extended Data Fig. 5j, k). Aim2-deficient T<sub>reg</sub> cells proliferated and survived normally during EAE and under steady-state conditions (Extended Data Fig. 5l–o). These findings support a crucial biological role for AIM2 in promoting T<sub>reg</sub> cell stability.
We next explored the mechanisms by which AIM2 regulates Treg cells. Previous studies found that AIM2 could attenuate AKT activation in gastrointestinal epithelial cells. AKT–mTOR signalling positively associates with glycolytic metabolism, which negatively affects Treg cell function. To investigate whether AIM2 deletion altered Treg cell metabolism, wild-type or Aim2−/− Treg cells were activated by TCR stimulation. Aim2−/− Treg cells showed higher glycolytic activity assessed by the extracellular acidification rate (ECAR) (Fig. 3a, quantified in Extended Data Fig. 6a) compared to wild-type Treg cells. Aim2−/− Treg cells also had reduced oxygen consumption rate (OCR) and fatty acid oxidation (Fig. 3b, c), indicating impaired lipid oxidative phosphorylation and increased aerobic glycolysis. Global transcriptional profiling by RNA sequencing (RNA-seq) and gene set enrichment analysis (GSEA) revealed that Aim2 deletion led to enhanced interferon (IFN)-responsive signatures (Fig. 3d, e, Extended Data Fig. 6b, c), including IFI15 and IFNγ, both of which are known to attenuate Foxp3 expression and Treg cell function30,31, as well as enhanced MYC-dependent programming (Fig. 3f, Extended Data Fig. 6d). Consistently, Aim2−/− Treg cells had increased expression of MYC protein32, hyperphosphorylation of S6 and 4E-BP1 (an indicator of mTORC1 signalling), and increased levels of phosphorylated AKT (p-AKT) and its downstream targets p-FOXO1 and p-FOXO3A (Fig. 3g), which can block Foxp3 expression33. In addition, p-STAT1, a factor indicative of IFN signalling, was increased in Aim2−/− Treg cells (Fig. 3h). Enhanced mTOR, but not IFNγ, appeared to be central to—and causal for—the above-mentioned alterations in Aim2−/− T cells because treatment with pharmacological inhibitors of mTORC1 (rapamycin) or mTORC1/2 (pp242) blocked excessive MYC, p-S6, p-4E-BP1, p-FOXO1, p-FOXO3A and p-STAT1, whereas anti-IFNγ neutralization only blocked p-STAT1 (Fig. 3i).

We then examined whether AIM2 also controls de novo Treg cell generation by treating wild-type or Aim2−/− CD4+ T cells with TGFβ in vitro. Compared to wild-type cells, fewer Aim2−/− CD4+ T cells differentiated into FOXP3+ cells in the presence of TGFβ (Fig. 4a, b) whereas Treg and TGFβ-induced Treg cell differentiation were similar between wild-type and Aim2−/− cells (Fig. 4c, Extended Data Fig. 7a). To understand how AIM2 controls TGFβ-induced Treg cell generation, we performed RNA-seq analysis to compare gene expression profiles of wild-type and Aim2−/− T cells activated by TGFβ. The analysis revealed that pathways involving P38–AKT–mTOR, TGFβ1, glycolysis, MYC and IFNs are among the top pathways preferentially upregulated in TGFβ-induced Aim2−/− Treg cells (Fig. 4d, Extended Data Figs. 7b–d, 8a–d).

Because TGFβ-induced Treg cell differentiation is under metabolic control34, we investigated whether AIM2 regulates metabolism during such a process. Indeed, TGFβ-induced Aim2−/− Treg cells showed higher ECAR and glycolytic activity, but reduced OCR and fatty acid oxidation, when compared to wild-type cells (Fig. 4e–g), agreeing with what was observed in Treg cells isolated from mice. In addition, AKT–mTOR signalling-related molecular markers, including MYC, p-S6, p-4E-BP1, p-AKT, p-FOXO1 and p-FOXO3A, were upregulated in the absence of AIM2 (Fig. 4h). Biochemical analyses of CD4+ but not CD8+ T cells revealed modestly enhanced AKT–mTOR signalling after AIM2 deletion 24 h after stimulation in vitro (Extended Data Fig. 9a, b).
In addition, Aim2 deletion consistently led to increased Akt–mTOR signalling in Treg cells (Extended Data Fig. 9c) but not in Tconv cells or CD8+ cells (Extended Data Fig. 9d, e) at early time points, further highlighting a preferential role for AIM2 in controlling Treg cell function. The pharmacological mTOR inhibitors, rapamycin and PP242, neutralized these changes in CD4+ cells and reduced p-STAT1, whereas addition of the anti-IFNγ antibody (XMG1.2) only decreased p-STAT1 (Fig. 4h).

These results indicate that IFNγ lies downstream of mTOR. Importantly, rapamycin or PP242 treatment restored TGFβ-induced Treg cell differentiation to a normal level, suggesting that heightened mTOR activity due to Aim2 deletion accounts for defective TGFβ-induced Treg cell differentiation (Extended Data Fig. 10a). These findings suggest that AIM2 controls Treg cell generation and function via the unified mechanism of AKT–mTOR restriction.

To investigate the molecular mechanism by which AIM2 regulates AKT–mTOR, we performed an unbiased mass spectrometric analysis to identify AIM2–interacting proteins during Treg cell differentiation, using a mouse-specific anti-AIM2 antibody for endogenous immunoprecipitation–mass spectrometry (IP–MS) analysis. Red indicates significantly enriched proteins (log2-transformed fold change > 1; t-test adjusted P < 0.05). j, k. The interactions of enriched proteins determined by immunoprecipitation using anti-AIM2 (j) or anti-RACK1 (k), in wild-type and Aim2−− CD4+ T cells activated with TGFβ for 24 h. PP2Aca, catalytic subunit of PP2A. Representative of three experiments. j. Flow cytometry of p-AKT levels (geometric mean) in wild-type and Aim2−− Treg cells transduced with PP2A and RACK1-expressing vector (OE) or vector; n = 6 experiments. m. Flow cytometry of p-AKT in spinal cord Treg cells from mice of indicated genotypes, 28 days after EAE induction; n = 3 experiments. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-sided t-test (a–c, e right, m right), two-way ANOVA (left, f, g), multiple unpaired t-test with Holm–Sidak method (l).
signalling to promote T<sub>reg</sub> cells. Reduced RACK1–PP2A interaction is therefore expected to increase AKT phosphorylation. Although the interaction of RACK1 with PP2A and AKT occurred normally in wild-type T<sub>reg</sub> cells, this interaction was reduced in Aim2<sup>−/−</sup> T<sub>reg</sub> cells (Fig. 4k). The specific interaction between Aim2 and the RACK1–PP2A–AKT complex was observed in TGFβ-induced T<sub>reg</sub> conditions, but not in conventional T cells (Extended Data Fig. 10c). To test the function of RACK1 and PP2A, we ectopically expressed both proteins in TGFβ-induced T<sub>reg</sub> cells and analysed the phosphorylation of AKT (p-AKT). Ectopic expression of RACK1 and PP2A downregulated p-AKT only in wild-type, and not in Aim2<sup>−/−</sup> T<sub>reg</sub> cells, indicating that the effect of RACK1–PP2A on p-AKT was Aim2-dependent (Fig. 4l). In agreement with the observed enhanced p-AKT in Aim2<sup>−/−</sup> T<sub>reg</sub> cells in vitro, p-AKT was increased in Aim2<sup>−/−</sup> T<sub>reg</sub> cells compared to wild-type controls in spinal cords during EAE (Fig. 4m). Therefore, hyperactivation of AKT in Aim2<sup>−/−</sup> T<sub>reg</sub> cells in vitro and in vivo (Figs. 3g, 4h, m) can be attributed to an Aim2-dependent AKT dephosphorylation by the RACK1–PP2A complex<sup>39</sup>.

In summary, AIM2 is an inflammasome effector in myeloid cells but its role in T cells has not been explored. This study reveals a previously unappreciated role of AIM2 in T<sub>reg</sub> cells. It unveils a T<sub>reg</sub> cell-intrinsic, inflammasome-independent function of AIM2 that promotes T<sub>reg</sub> cells to control autoimmunity, specifically in models of multiple sclerosis and inflammatory bowel disease. We demonstrated the mechanism of AIM2 function in T<sub>reg</sub> cells at three levels. At the cellular level, lineage-tracing analysis indicates that AIM2 is needed for the stability of T<sub>reg</sub> cells. At the molecular and metabolic level, AIM2 attenuates the AKT–mTOR pathway to favour oxidative phosphorylation and fatty acid oxidation while mitigating glycolysis, thereby affecting immunometabolism profiles that favour T<sub>reg</sub> cells. At the biochemical level, AIM2 promotes the association of AKT with the RACK1–PP2A axis to restrain AKT activation, thus reprogramming immunometabolism to favour T<sub>reg</sub> cell function (model in Extended Data Fig. 10f).

### Online content

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Methods

Mice

All mice were housed and bred under specific pathogen-free conditions (temperature: 21–23 °C, humidity: 30–70%, 12-h light/dark cycle) in the animal facility at the University of North Carolina at Chapel Hill. All sex- and age-matched (9–12 weeks) mouse experiments were approved by Institution Animal Care and Use Committee of the University of North Carolina. We complied with all relevant ethical regulations. Wild-type and age-matched (9–12 weeks) mouse experiments were approved by animal facility at the University of North Carolina at Chapel Hill. All sex-matched (temperature: 21–23 °C, humidity: 30–70%, 12-h light/dark cycle) in the animal facility. All mice were housed and bred under specific pathogen–free conditions.

Methods

enriched by MACS and then stained with fluorescence-conjugated antibodies. Stained cells were either acquired on LSRII or LSRFortessa (BD biosciences) or sorted on the MoFlow cell sorter (Dako cytometry, Beckman Coulter) by the Flow Core Facility of University of North Carolina at Chapel Hill. FACs data were analysed with FlowJo software (TreeStar). For the gating strategy for FACs analysis, see Supplementary Fig. 2.

CD4+ T and Treg cell adoptive transfer in EAE

Total lymphocytes were isolated from spleens and peripheral lymph nodes of wild-type and Aim2−/− mice. Total CD4+ T cells or CD4+CD25+ Treg cells were enriched by MACS and MoFlow cell sorter. CD4+ T cells (5 × 10⁶ per mouse) were introduced via retro-orbital injection into Rag1−/− female mice. One day later, the recipient mice were immunized with MOG35-55 to induce EAE as described above. To evaluate the function of Treg cells in suppressing EAE, CD4+CD25+ T cells alone (5 × 10⁶ per mouse), CD24+ CD4+ T cells alone (5 × 10⁶ per mouse) with wild-type or Aim2−/− Treg cells (2 × 10⁶ per mouse) were transferred into Rag1−/− mice via retro-orbital injection. One day later, the recipient mice were immunized with MOG35-55 to induce EAE as described above.

CD4+CD45RB+B T cell transfer colitis model

CD4+ T cells from wild-type mice were enriched by anti-CD4 (L3T4) magnetic beads (Miltenyi Biotec) and stained with anti-CD4 Pacific Blue, anti-CD25 PE and anti-CD45RB FITC reagents. Naive CD4+ CD4+CD25+CD45RB+B T cells were sorted by FACS. Treg cells (CD4+CD25+B) of wild-type, Aim2−/− and Aim2−/−, 2D2 T cells were sorted by FACS. Approximately 5 × 10⁶ naive CD4+ T cells alone, or with 2 × 10⁶ wild-type or Aim2−/− Treg cells were transferred into Rag1−/− recipient mice by i.p. injection. The recipient mice were weighed twice every week to measure percentage of body weight change and major organs were collected for analysis at the end of experiment.

In vitro T cell activation, differentiation and proliferation

Lymphocytes were isolated from peripheral lymph nodes and spleens of age- and sex-matched mice and purified with CD4 microbeads (L3T4, Miltenyi Biotec). Purified CD4+ T cells were cultured in RPMI 1640 medium containing 10% FBS, 1% penicillin-streptomycin and 2.6 μl of β-mercaptoethanol and activated with plate-coated 2.5 μg ml−1 anti-CD3 (145-2c11, BioXcell) and 1 μg ml−1 anti-CD28 (37.51, BioXcell) antibodies. For Treg cell differentiation, designated doses of TGFβ (2 ng ml−1) and IL-2 (40 U ml−1) were added into the culture medium. Rapamycin and mTOR inhibitors (pp242) were used as indicated. For T1+ cell differentiation, 20 ng ml−1 IL-12 (Biologend) and 20 μg ml−1 anti-IL-4 (11B11, BioXcell) were added to the culture. For pathogenic T17 cell differentiation, 20 ng ml−1 IL-1β (Biologend), 20 ng ml−1 IL-6 (Biologend), 50 ng ml−1 IL-23 (Biologend) and 10 μg ml−1 anti-IFNγ (XMG1.2, BioXcell) were added to the culture. For classical T17 cell differentiation, 1 ng ml−1 TGFβ (Biologend), 40 ng ml−1 IL-6 (Biologend) and 10 μg ml−1 anti-IFNγ (XMG1.2, BioXcell) were added to the culture. For CFSE proliferation assay, a final concentration of 2 μM of carboxyfluorescein succinimidyl ester (CFSE) (C1517, Life Technologies) was used to label CD4+ T cells.

Ectopic expression of PP2A and RACK1 in Treg cells

To generate the retrovirus expressing PP2A and RACK1, we first cloned PP2A (OriGene Technologies, MR204384L4) and RACK1 (OriGene Technologies, MR204575L3) into retroviral vectors MSCV-ires-Thy1.1 (MIT, Addgene 17442) and MCV-ires-GFP (MIG, Addgene 20672) respectively, and generated MIT-PP2A and MIG-RACK1 retrovirus in 293T cells by transient transfection. For retroviral transduction, isolated CD4+ T cells were stimulated with anti-CD3/CD28 in the presence of IL-2 (40 U ml−1) and TGFβ (2 ng ml−1) on day 0 and then transduced with indicated retroviruses containing 8 μg ml−1 polybrene (Sigma, H9268) by centrifuge at 1,500g at 30 °C for 1.5 h on day 1. Cells were collected and analysed by flow cytometry three days after retroviral transduction.
In vitro T<sub>reg</sub> cell suppression

T<sub>reg</sub> cells (suppressor) from CD45.1.1 wild-type mice and CD45.2.2 Aim2<sup>−/−</sup> mice and naive CD4<sup>+</sup> T cells (responder) from CD45.1.2 wild-type young mice were sorted by FACS. To assess the efficacy of T<sub>reg</sub> cell-mediated immune suppression in vitro, 1 × 10<sup>6</sup> sorted responder T cells were labelled with CFSE and mixed with varying amounts (as indicated) of T<sub>reg</sub> suppressor cells. Cell mixtures were treated with soluble CD3 antibody (0.125 μg ml<sup>−1</sup>) in the presence of 4 × 10<sup>4</sup> irradiated (3,000 cGy) T cell-depleted splenocytes as antigen-presenting cells. The proliferation of responder cells was assessed by CFSE dilution detected by flow cytometric analysis 72 h after stimulation.

Histology

For the assessment of tissue pathology, following an initial perfusion with PBS, mice were subsequently perfused trans-cardially with 4% paraformaldehyde and spinal cords were removed. Tissues were processed and blocked in paraffin wax. Transverse sections of the lumbar spinal cord were stained with haematoxylin and eosin (H&E) or Luxol Fast Blue and periodic acid Schiff (LFB-PAS). The number of inflammatory foci and total demyelination were measured using methods described previously<sup>14</sup>. In brief, the numbers of inflammatory foci that contained at least 20 cells were counted within each H&E-stained section in a blinded fashion. Estimates were made of the number of foci, when foci coalesced. Areas of demyelination were assessed in LFB-PAS-stained sections. ImageJ software was used to manually trace the total cross-sectional area and the demyelinated area of each section. Total demyelination was expressed as a percentage of the total spinal cord area<sup>14</sup>. Colonies were Swiss rolled, fixed in 10% neutral-buffered formalin and routinely paraffin embedded and processed. Five micrometre-thick colon sections were stained with H&E and evaluated by a board-certified veterinary pathologist (A.B.R.) in a blinded manner to perform semiquantitatively scoring of histopathology. Histology scores represented the sum of each histological alterations as outlined here: inflammation, epithelial defects, area of inflammation, area of epithelial defect, crypt atrophy and dysplasia-neoplasia, by giving each parameter a separate score (0–4) for severity and extent as previously described<sup>13</sup>.

Enzyme-linked immunosorbent assay

Both spinal cord homogenates and colon-secreted cytokines were analysed by enzyme-linked immunosorbent assay (ELISA, MCS500, R&D Systems, or by multiplex analyte assay using Luminex technology (EMD Millipore) according to manufacturers’ protocols. IL-1β (Invitrogen BMS618-3), IL-6 (R&D DY406) and TNFα (Biolegend 430904) were measured using ELISA assays according to manufacturer’s instructions. For colon-secreted cytokines, excised colonies were washed and flushed with PBS containing 2 × penicillin/streptomycin. The distal-most 1 cm<sup>2</sup> colon sections were cultured for 15 h in RPMI medium containing 2 × penicillin/streptomycin. The distal-most 1 cm<sup>2</sup> colon sections were cultured for 15 h in RPMI medium containing 2 × penicillin/streptomycin. The distal-most 1 cm<sup>2</sup> colon sections were cultured for 15 h in RPMI medium containing 2 × penicillin/streptomycin.

Immunoblotting, immunoprecipitation and mass spectrometry

CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub>, TGFβ-induced T<sub>reg</sub>, CD4<sup>+</sup> T and CD8<sup>+</sup> T cells were lysed in RIPA buffer supplemented with complete proteinase inhibitor cocktail and PhoSTOP phosphatase inhibitors. Protein lysates were cleaved of insoluble material through centrifugation and the resulting protein lysates were treated with sample buffer and subjected to SDS-PAGE. In brief, total proteins were wet transferred to 0.2 μm nitrocellulose membranes (Bio-Rad Laboratories), which were blocked using 5% BSA in 1 × TBS-T buffer for 1 h at room temperature. The membranes were incubated overnight using the following primary antibodies from Cell Signaling Technology (CST): anti-p-AKT (Ser473) (cat. no. 4060) (WB, 1:1,000), anti-AKT (cat. no. 9272) (WB, 1:1,000), anti-p-FOXO1/3A (cat. no. 5432s) (WB, 1:200) antibodies treated by dimethyl pimelimidate. After overnight incubation, beads were washed four times with lysis buffer. Associated protein was eluted by Laemmli sample buffer (Bio-Rad) and incubated at 95 °C for 5 min. Eluted samples were separated by SDS–PAGE gel and analysed by immuno-blotting.

For mass spectrometry analysis, anti-AIM2 antibody immunoprecipitated proteins were eluted with buffer containing 8 M urea, 50 mM Tris (pH 8.0), reduced with 5 mM DTT and alkylated with 15 mM iodoacetamide. Trypsin digestion was performed at room temperature overnight in 2 M urea buffer. The peptides were desalted on C18 stage-tips and dissolved in 0.1% formic acid. Peptides were loaded on an Acclain PepMap RSLC C18 Column (150 mm × 75 μm ID, C18, 2 μm, Thermo Fisher Scientific) and analysed on a Q-Exactive HF-X coupled with Easy nanoLC 12000 (Thermo Fisher Scientific). Analytical separation of all tryptic peptides was achieved with a linear gradient of 5–30% buffer B over 29 min, 30–45% B over 6 min followed a ramp to 100% B in 1 min and 9 min wash periods with 100% buffer B, where buffer A was aqueous 0.1% formic acid and buffer B contained 80% acetonitrile and 0.1% formic acid. Liquid chromatography–mass spectrometry (LC–MS) experiments were performed in a data-dependent mode with full MS (externally calibrated to a mass accuracy of <5 ppm and a resolution of 60,000 at m/z 200) followed by high energy collision-activated dissociation-MS/MS of the top 15 most intense ions with a resolution of 15,000 at m/z 200. High energy collision-activated dissociation-MS/MS was used to dissociate peptides at a normalized collision energy (NCE) of 27 eV. Dynamic exclusion with 20.0 s was enabled. Then the mass spectra were processed and peptide identification was performed using the Andromeda search engine found in MaxQuant software version 1.6.0.16 (Max Planck Institute, Germany) against the Uniprot mouse protein sequence database (UP00000589). Peptides were identified with a target-decoy approach using a combined database consisting of reverse protein sequences of the database. Up to two missed cleavages was allowed. Peptide identifications were reported by filtering reverse and contaminant entries and assigning to leading razor protein. Peptide inference and protein identification were filtered to maximum 1% and 1% false discovery rate, respectively. Data processing and statistical analysis were performed on Perseus (Version 1.6.0.7). A two sample t-test statistics was used with a P < 0.05 to report statistically significant expression.

Glycolytic and mitochondrial respiration rate measurement

The ECAR and OCR were measured using the Seahorse Extracellular flux XF24e (Agilent) according to industry manuals. CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells were isolated from wild-type and Aim2<sup>−/−</sup> mice and then stimulated with anti-CD3/CD28 and IL-2 (500 U ml<sup>−1</sup>) for 24 h. For in vitro TGFβ-promoted T<sub>reg</sub> cells, wild-type and Aim2<sup>−/−</sup> CD4<sup>+</sup> T cells were...
stimulated with anti-CD3/CD28 in the presence of TGFβ (2 ng ml⁻¹) and IL-2 (40 U ml⁻¹) for 48 h. Before metabolic flux analysis, Treg cells were seeded at a density of 5 × 10⁵ cells per well. ECAR profiles were assessed by real-time measurements at basal condition and after the addition of 10 mM glucose, 1 μM oligomycin and 20 mM 2-deoxyglucose (2-DG).

OCR profiles were assessed by real-time measurements at basal condition and after the addition of 1 μM oligomycin (75351), 4 μM FCCP (C2920), and 1 μM rotenone (R8875), and all the reagents are from Sigma-Aldrich. Fatty acid oxidation assay was performed where cells were starved in substrate-limited medium and given only BSA or palmitate-BSA (cat no. 102720-100) in fatty acid oxidation assay medium. Then OCR was measured to indicate oxidation of fatty acids according to the Agilent fatty acid oxidation assay manual.

RNA preparation and quantitative PCR
Total RNA was prepared from T cells using TRIzol reagent (Invitrogen) according to manufacturer’s instructions and was reverse transcribed into cDNA with iScript cDNA Synthesis Kit (Bio-Rad, cat. no. 1708891). The Taqman probes were purchased from Applied Biosystems and quantitative PCR was performed on the ABI7900 real-time PCR system with QuantStudio software (Applied Biosystems).

RNA-seq
For RNA-seq analysis, totalRNA was extracted from Treg cells using the Direct-zol miniprep kit (Zymo Research, R2060). The RNA samples were first enriched by Oligo(dT) magnetic beads and used to construct BGISEQ-500 libraries. RNA-seq libraries were sequenced using the 50 bp single-end protocol (in vivo isolated Treg cells) or 100 bp paired-end protocol (TGFβ-induced Treg cells) via the BGISEQ-500 sequencer per manufacturer’s protocol. After filtering of adaptors and low quality reads, clean reads (>26 million reads per sample for in vivo isolated Treg cells and >40 million reads per sample for TGFβ-induced Treg cells) were mapped to the mouse reference genome using HISAT/Bowtie2 tool. Mapping results were stored in BAM files using SAMtools.

Heat maps of gene expression were generated based on zscore values of normalized expression matrix from DESeq2 analysis in Gene-E from the Broad Institute (www.broadinstitute.org/GENE-E/).

GSEA
GSEA was performed using the Java application available from The Broad Institute (www.broadinstitute.org/gsea/). Gene set databases including Hallmarks (h.all.v6.1 symbols.gmt), KEGG (c2.cp.kegg.v6.1 symbols.gmt) and Reactome (c2.cp.reactome.v6.1 symbols.gmt) from the Molecular Signatures Database (MsigDB) were used in the analysis. One thousand gene set permutations were performed. An FDR cutoff of <0.05 was used for enriched terms, as is recommended when performing permutations by gene set. R version 3.5.0 was used.

Statistical analysis
Data analysis was processed and represented by Prism (GraphPad, San Diego). Statistical significance was determined by two-sided Student’s t-test, two-way ANOVA followed by Tukey test or Holm–Sidak’s multiple-comparisons tests indicated in figures. A P value of less than 0.05 (confidence interval of 95%) was considered significant. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. The exact P values are shown in the source data. The sample sizes are stated in the figure legends to indicate biologically independent replicates used for statistical analyses.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
The RNA-seq data are available in the Gene Expression Omnibus (GEO) repository at the National Center for Biotechnology Information under accession number GSE133019. The MS proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD015638. All other data supporting the findings of this study are available from the corresponding authors upon reasonable request. Source data are provided with this paper.

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Author contributions W.C. and Z.G. contributed equally to this manuscript. W.C., Z.G., YYW, and J.P.-Y.T. designed experiments and wrote the manuscript. W.C. and Z.G. performed and analysed most of the experiments. H.G., L.C. and G.Z. contributed to the colitis experiments. K.L. and M.D. contributed to the western blots. L.X. and X.C. contributed to IP–MS analysis. S.A.M. contributed to quantitative PCR analysis. X.T. contributed to bioinformatics analysis. E.R. contributed to the metabolism assays. YYW and M.A.S. contributed to the scoring and quantification of spinal cord pathology. S.A.M. contributed to the scoring of colon pathology. H.G., L.C. and G.Z. contributed to the colitis experiments. W.J.B. and J.P.-Y.T. designed experiments and wrote the manuscript. W.C. and Z.G. performed and assembled by the ImmGen consortium and The European Bioinformatics Institute (EMBL-EBI) without prior permission. We thank E. Holley-Guthrie for genotyping mice.

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Additional information
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Extended Data Fig. 1 | Aim2−/− mice show normal T cell homeostasis.

a, b, Flow cytometry of CD4+Foxp3+ Treg cells (a) and IFNγ-, IL-17A-producing CD4+ T cells (b) in the spleen and PLN of wild-type and Aim2−/− mice at day 14 of an EAE course. Representative results (left) and statistical analysis (right) of six experiments are shown. c, Flow cytometry of CD4+Foxp3+ Treg cells in the thymus, spleen and PLN of wild-type and Aim2−/− mice. Representative results (left) and statistical analysis (right) of three experiments are shown. d, Total number of cells isolated from the thymus, spleen and PLN of wild-type and Aim2−/− mice. Experimental design and statistical analysis performed as described in e, f. Flow cytometry of naive, effector/memory CD4+ (e) and CD8+ (f) T cells in the spleen and PLN of wild-type and Aim2−/− mice (n = 3 per group) analysed by CD44 and CD62L expression. Experimental design and statistical analysis performed as described in c, g, h, Flow cytometry of IFNγ-, IL-4- or IL-17A-producing CD4+ cells (g) and IFNγ-producing CD8+ T cells (h) in wild-type and Aim2−/− mice (n = 3 per group). Experimental design and statistical analysis performed as described in c. Representative (top) and composite (bottom) data are shown. Data are mean ± s.e.m. P values determined by two-sided t test. ns, not significant.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | *Aim2*−/− mice show normal T<sub>reg</sub> cell proliferation, survival and ratio of central or effector T<sub>reg</sub> cells at steady state or during EAE in vivo, normal CD4+ T cell proliferation in vitro, and normal CD8+ T cell distribution and cytokine production during EAE. **a**, Flow cytometry of Ki67 to analyse proliferation of wild-type and *Aim2*<sup>−/−</sup> T<sub>reg</sub> cells in the PLN and spleen at steady state. Representative results (top) and statistical analysis (bottom) of five experiments are shown. **b**, Apoptosis of wild-type and *Aim2*<sup>−/−</sup> T<sub>reg</sub> cells in the PLN and spleen at steady state was analysed by flow cytometry using annexin V and 7-aminoactinomycin D (7AAD) staining. Representative results (top) and statistical analysis (bottom) of five experiments are shown. **c**, Flow cytometry of Ki67 to analyse proliferation of wild-type and *Aim2*<sup>−/−</sup> T<sub>reg</sub> cells in the PLN, spleen and spinal cord during EAE. Representative results (top) and statistical analysis (bottom) of six experiments are shown. **d**, Apoptosis of wild-type and *Aim2*<sup>−/−</sup> T<sub>reg</sub> cells in spinal cord during EAE was analysed by flow cytometry using annexin V and 7AAD staining. Representative results (top) and statistical analysis (bottom) of six experiments are shown. **e**, Flow cytometry of CD44 and CD62L in wild-type and *Aim2*<sup>−/−</sup> T<sub>reg</sub> cells isolated from spinal during EAE. Representative results (top) and statistical analysis (bottom) of six experiments are shown. **f**, Flow cytometry of wild-type and *Aim2*<sup>−/−</sup> CD4+ T cell proliferation stimulated with different doses of anti-CD3/CD28, determined by CFSE dilution assay. Representative results of two independent experiments. **g**, Flow cytometry of 2D2 and *Aim2* deficient 2D2 (2D2 × *Aim2*<sup>−/−</sup>) CD4+ T cell proliferation stimulated with bone marrow-derived dendritic cells (BMDCs) pulsed with MOG<sub>35–55</sub> peptide, as determined by CFSE dilution assay. Representative of two independent experiments. **h**, Flow cytometry of wild-type and *Aim2*<sup>−/−</sup> CD4+ or CD8+ T cells in the PLN, spleen and spinal cord during EAE. Representative results (left) and statistical analysis (right) of six experiments are shown. **i–k**, Flow cytometry of IFNγ-, IL-17A-producing (i) or FOXP3+, IFNγ-producing (j) CD8+ T cells in the PLN, spleen and spinal cord during EAE. Representative results (i, j) and statistical analysis (k) of six experiments are shown. Data are mean ± s.e.m. *P* values determined by two-sided *t* test.
Extended Data Fig. 3 | *Aim2* is highly expressed in Treg cells and its promoter is bound by Treg cell-related transcription factors. a, b, Mouse *Aim2* gene expression in different T cell subsets from publicly available gene microarray (a) and RNA-seq (b) databases [Immgen (https://www.immgen.org/)]. c, Human *Aim2* gene expression in regulatory T cell subsets from wild-type and *Aim2*−/− mice. Flow cytometry of CD4+CD25+FOXP3+ Treg cells shows that more than 97% of isolated Treg cells are FOXP3+ cells. Aim2 expression was assessed from isolated CD4+CD25+ Treg cells and CD4+ T cells. *Aim2* mRNA expression was examined by quantitative PCR; *n* = 4 experiments. f, The mRNA expression of *Aim2* in Treg cells and CD4+ T cells stimulated with anti-CD3/CD28 plus IL-2 (500 U ml−1) for 24 h; *n* = 4 experiments. g, The mRNA expression of *Aim2* in Treg cells stimulated with anti-CD3/CD28 plus IL-2 (500 U ml−1) in the absence (−) or presence (+) of TGFβ (1 ng ml−1) for 24 h; *n* = 4 experiments. h, *Aim2* expression was assessed in freshly isolated CD4+CD25+ Treg cells from wild-type and *Tgfbr2fl/fl* Treg mice. *Aim2* expression was examined by quantitative PCR; *n* = 5 experiments. i, The mRNA expression of *Aim2* in naive CD4+ or CD8+ T cells stimulated with anti-CD3/CD28 plus IL-2 (40 U ml−1) in the absence or presence of TGFβ (1 ng ml−1) for 24 h; *n* = 4 experiments. j, ChIP–seq analysis of RUNX1, ETS1, BCL11B and CREB binding to the *Aim2* promoter region in Treg cells and CD4+ Tconv cells (NCBI SRA database number: DRP003376). Data are mean ± s.e.m. **P < 0.01, ***P < 0.001, ****P < 0.0001, two-sided t-test.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | AIM2 is essential for T\textsubscript{reg} cells to suppress T cell-mediated colitis and EAE.

**a**, Haematoxylin and eosin (H&E) staining of colons from T cell-induced colitis mice transferred with wild-type CD4\textsuperscript{+}CD45RB\textsuperscript{hi} T cells (Tn) alone (n = 5) or in combination with wild-type (n = 6) or Aim2\textsuperscript{−/−} (n = 6) CD4\textsuperscript{+}CD25\textsuperscript{+} T\textsubscript{reg} cells, collected 7 weeks after T cell transfer. Scale bars, 1 mm (40×) and 100 μm (100×).

**b**, Statistical analysis of pathology score of colitis mice with biological replicates of each group depicted in **a**. Tn only: n = 5; Tn + WT T\textsubscript{reg}; n = 6; Tn + Aim2\textsuperscript{−/−} T\textsubscript{reg}; n = 6.

**c**, Cytokine levels in the supernatants of colon tissue cultures from mice depicted in **a** measured by Millipore Luminex assay, collected 7 weeks after T cell transfer. Tn only: n = 4; Tn + WT T\textsubscript{reg}; n = 5; Tn + Aim2\textsuperscript{−/−} T\textsubscript{reg}; n = 5.

**d**, Change of body weight of Rag1\textsuperscript{−/−} recipients receiving wild-type naive CD4\textsuperscript{+}CD45RB\textsuperscript{hi} T cells (Tn) alone or in combination with wild-type, Aim2\textsuperscript{−/−} or Asc\textsuperscript{−/−} CD4\textsuperscript{+}CD25\textsuperscript{+} T\textsubscript{reg} cells. Rag1\textsuperscript{−/−} recipients of Tn (n = 8), Tn + WT T\textsubscript{reg} (n = 6), Tn + Aim2\textsuperscript{−/−} T\textsubscript{reg} (n = 9), Tn + Asc\textsuperscript{−/−} T\textsubscript{reg} (n = 7); composite of two independent experiments.

**e**, Flow cytometry of CD4\textsuperscript{+}FOXP3\textsuperscript{+} T\textsubscript{reg} cells in the colons of Rag1\textsuperscript{−/−} recipients of Tn (n = 8), Tn + WT T\textsubscript{reg} (n = 6), Tn + Aim2\textsuperscript{−/−} T\textsubscript{reg} (n = 9), Tn + Asc\textsuperscript{−/−} T\textsubscript{reg} (n = 7), collected 7 weeks after T cell transfer. P value determined by one-way ANOVA with Tukey’s multiple comparisons test.

**f**, Schema of EAE induction in Rag1\textsuperscript{−/−} mice transferred with 2D2 CD4\textsuperscript{+} T cells either alone or in combination with wild-type or Aim2\textsuperscript{−/−} CD4\textsuperscript{+}CD25\textsuperscript{+} T\textsubscript{reg} cells. Lymphocytes and tissues were obtained 14–15 days after initial T cell transfer for further analysis.

**g**, Flow cytometry shows the populations of 2D2 CD4\textsuperscript{+} T cells (V\textbeta11\textsuperscript{+}) or T\textsubscript{reg} cells (FOXP3\textsuperscript{+}) before transfer to Rag1\textsuperscript{−/−} recipient mice.

**h**, Mean EAE clinical score of mice depicted in **f**; n = 5 mice per group. P value by two-way ANOVA and Holm–Sidak post hoc test. Data are representative of three independent experiments. The difference between 2D2 alone and 2D2 with Aim2\textsuperscript{−/−} T\textsubscript{reg} cells is not significant.

**i**, Flow cytometry of IFN\gamma\textsuperscript{+} or IL-17A\textsuperscript{+} CD4\textsuperscript{+}V\textbeta11\textsuperscript{+} T cells in spinal cords from groups depicted in **f**. Left, representative sample; right, composite data pooled of five mice per group from three independent experiments.

**j**, Flow cytometry of CD4\textsuperscript{+}FOXP3\textsuperscript{+} T\textsubscript{reg} cells from spinal cord derived from mice depicted in **f**. Left, representative sample; right, composite data pooled of five mice per group from three independent experiments. P value determined by one-way ANOVA with Tukey’s multiple comparisons test.

**k**, Flow cytometry of IFN\gamma\textsuperscript{+} or IL-17A\textsuperscript{+} CD4\textsuperscript{+}V\textbeta11\textsuperscript{−} T cells in spinal cord from groups depicted in **f**. Left, representative sample; right, composite data summarized from five biological replicates.

**l**, CD25\textsuperscript{−}CD44 lowCD62L\textsuperscript{hi} naive CD4\textsuperscript{+} T cells (Tresp cells) were isolated from wild-type mice and labelled with CFSE. CD4\textsuperscript{+}CD25\textsuperscript{+} T\textsubscript{reg} cells were isolated from wild-type or Aim2\textsuperscript{−/−} mice by FACS. T\textsubscript{reg} cells of different genotypes were mixed at indicated ratios and stimulated with anti-CD3 in the presence of irradiated antigen-presenting cells from mixed spleens and lymph nodes. The suppressive activity of T\textsubscript{reg} cells was assessed by CFSE dilution of Tresp cells. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-sided t test unless specified.
Extended Data Fig. 5} See next page for caption.
Extended Data Fig. 5 | Aim2<sup>fl/fl</sup> FGC R26T mice show normal T cell homeostasis, normal T<sub>reg</sub> cell proliferation and survival at steady state and during EAE. a, Genotyping of FACS-sorted T<sub>reg</sub> cells (CD4<sup>+</sup>CD25<sup>+</sup>GF<sup>+</sup>) and CD4<sup>+</sup> T<sub>conv</sub> (CD4<sup>+</sup>CD25<sup>-</sup>) cells showing efficient deletion of Aim2 genomic DNA in T<sub>reg</sub> cells. The image is representative of three independent experiments. b, Immunoblot analysis of AIM2 protein in T<sub>reg</sub> cells from Aim2<sup>+/+</sup> FGC R26T and Aim2<sup>fl/fl</sup> FGC R26T mice. The image is representative of three independent experiments. c, Images of Aim2<sup>+/+</sup> FGC R26T and Aim2<sup>fl/fl</sup> FGC R26T mice and corresponding lymphoid organs of spleen and lymph nodes. d, Flow cytometry of CD4<sup>+</sup>FOXP3<sup>+</sup>CD25<sup>+</sup>T<sub>reg</sub> cells in the thymus, spleen and PLN of Aim2<sup>+/+</sup> FGC R26T and Aim2<sup>fl/fl</sup> FGC R26T mice. Representative results (top) and statistical analysis (bottom) of four experiments are shown. 

e, Flow cytometry of CD4<sup>+</sup> or CD8<sup>+</sup> T cells in the spleen and PLN of Aim2<sup>+/+</sup> FGC R26T and Aim2<sup>fl/fl</sup> FGC R26T mice. Representative results (left) and statistical analysis (right) of four experiments are shown. f, g, Flow cytometry of naïve, effector/memory CD4<sup>+</sup> (f) and CD8<sup>+</sup> (g) T cells in the spleen and PLN of Aim2<sup>+/+</sup> FGC R26T and Aim2<sup>fl/fl</sup> FGC R26T mice by assessing CD44 and CD62L expression. Representative results (left for f, top for g) and statistical analysis (right for f, bottom for g) of four experiments are shown. h, i, Flow cytometry of IFNγ-, IL-4- or IL-17A-producing CD4<sup>+</sup> cells (h) and IFNγ-producing CD8<sup>+</sup> T cells (i) in Aim2<sup>+/+</sup> FGC R26T and Aim2<sup>fl/fl</sup> FGC R26T mice. Representative results (top) and statistical analysis (bottom) of four experiments are shown. j, Statistical summary of IFNγ-, IL-17A-producing CD4<sup>+</sup>Tomato<sup>+</sup> T<sub>conv</sub> cells in the PLN (left) and spleen (right) of Aim2<sup>+/+</sup> FGC R26T and Aim2<sup>fl/fl</sup> FGC R26T mice at day 28 of an EAE course. Composite data summarized from three biological replicates. k, Statistical summary of IFNγ-, IL-17A-producing CD4<sup>+</sup>Tomato<sup>+</sup> T<sub>reg</sub> cells in the PLN (left) and spleen (right) of Aim2<sup>+/+</sup> FGC R26T and Aim2<sup>fl/fl</sup> FGC R26T mice at day 28 of EAE course. Composite data summarized from three biological replicates. l, Flow cytometry of Ki67 to analyse proliferation of Aim2<sup>+/+</sup> FGC and Aim2<sup>fl/fl</sup> FGC T<sub>reg</sub> cells in the PLN, spleen and spinal cord during EAE. Left, representative sample; right, composite data summarized from three biological replicates. m, Apoptosis of Aim2<sup>+/+</sup> FGC and Aim2<sup>fl/fl</sup> FGC T<sub>reg</sub> cells in the spinal cord during EAE was analysed by flow cytometry using annexin V and 7AAD staining. Left, representative sample; right, composite data summarized from three biological replicates. 

Flow cytometry of Ki67 to analyse proliferation of Aim2<sup>+/+</sup> FGC and Aim2<sup>fl/fl</sup> FGC T<sub>reg</sub> cells in the PLN and spleen at steady state was analysed by flow cytometry using annexin V and 7AAD staining. Left, representative sample; right, composite data of five mice of two independent experiments. Data are mean ± s.e.m. P values determined by two-sided t-test unless specified.
**Extended Data Fig. 6** Enhanced glycolytic, IFN and MYC target signatures are found in Aim2−/− Treg cells isolated in vivo. a, Glycolytic activity of wild-type (n = 7 biological replicates per group) and Aim2−/− (n = 6 biological replicates per group) Treg cells untreated (UNT) or treated (TRE) with anti-CD3/CD28 antibodies plus IL-2 (500 U ml−1) for 24 h. Statistics of glycolysis (ECAR rate after glucose addition) and glycolytic capacity (maximal ECAR after subtracting the ECAR rate after exposure to 2-deoxy-d-glucose (2-DG) calculated from Fig. 3a. Data are mean ± s.e.m. **P < 0.01, ***P < 0.001, two-sided t-test. b, Heat map of IFNα response signature of RNA-seq data from Aim2−/− compared to wild-type Treg cells stimulated with anti-CD3/CD28 plus IL-2 (500 U ml−1) at indicated time points (0 or 24 h). c, Heat map of IFNγ response signature as described in b. d, Heat map of MYC target profiles as described in b.
Extended Data Fig. 7 | Enhanced gene signature found in TGFβ-induced Aim2−/− Treg cells using RNA-seq analysis. 

a, Flow cytometry of IFNγ+ or IL-17A+ CD4+ cells from wild-type and Aim2−/− mice after four days of differentiation under Th1, pTh17 and cTh17 conditions, respectively, as described in Fig. 4c. Data are representative of four independent experiments.

b, Summary of top pathways positively enriched in anti-CD3/CD28 activated Aim2−/− CD4+ T cells in the presence of TGFβ (2 ng ml−1) and IL-2 (40 U ml−1) for 24 h, by GSEA analysis of the RNA-seq dataset.

c, d, Enrichment of IFNγ (c) and IFNα response pathways (d) by GSEA (left) and heat map (right) of pathway-related genes in Aim2−/− versus wild-type CD4+ T cells stimulated with anti-CD3/CD28 in the presence of TGFβ (2 ng ml−1) and IL-2 (40 U ml−1) for 24 h.
Extended Data Fig. 8 | RNA-seq analysis reveals enhanced mTOR, MYC and glycolytic signatures in TGFβ-induced Aim2−/− Treg cells. a, Heat map of PI3K–AKT–mTORC-related gene expression in wild-type and Aim2−/− CD4+ T cells stimulated with anti-CD3/CD28 in the presence of TGFβ (2 ng ml−1) and IL-2 (40 U ml−1) for 24 h. b, Heat map of mTORC1 signalling-related gene expression, with samples described in a. c, Heat map of MYC target-related gene expression, with samples described in a. d, Heat map of glycolysis-related gene expression, with samples described in a.
Extended Data Fig. 9 | AKT–mTOR signalling in wild-type and Aim2−/− CD4+ and CD8+ T cells. a–e, Immunoblot analysis of p-AKT(S473), p-FOXO1, p-FOXO3A, p-S6, p-4E-BP1, MYC and β-actin in wild-type and Aim2−/− CD4+ T cells (a) or CD8+ T cells (b) stimulated with anti-CD3/CD28 plus IL-2 (40 U ml−1) for 24 h. c–e, Immunoblot analysis of p-AKT(S473), p-FOXO1, p-FOXO3A, p-S6, p-4E-BP1, MYC and β-actin in wild-type and Aim2−/− CD4+ T cells (c) or CD4+CD28− Treg cells (d, e) stimulated with anti-CD3/CD28 plus IL-2 (500 U ml−1), or CD4+ (d) and CD8+ (e) T cells stimulated with anti-CD3/CD28 plus IL-2 (40 U ml−1) for indicated time points. Left, representative results; right, quantification for statistics by densitometric analysis using Image Lab software; n = 4 experiments (a–c); n = 3 experiments (d, e). *P < 0.05, **P < 0.01, analysed by two-sided paired t-test.
Extended Data Fig. 10 | AIM2 interacts with the RACK1–PP2A–AKT complex and is critical to regulate AKT–mTOR signalling for T reg cell generation. 

a, Flow cytometry analysis of FOXP3 in wild-type and Aim2−/− CD4+ T cells stimulated by anti-CD3/CD28 plus IL-2 (40 U ml−1) and TGFβ (2 ng ml−1) and then cultured with DMSO (n = 4 biological replicates/group), rapamycin (1 nM) (n = 7 biological replicates/group), or pp242 (0.5 μM) (n = 7 biological replicates/group) for 24 h. Results are representative of six independent experiments. 

b, Schema for AIM2 function shows that AIM2 facilitates the interaction of RACK1 and PP2A to promote AKT dephosphorylation to restrain the activity of the mTOR pathway.

c, Flow cytometry of p-AKT of wild-type and Aim2−/− Treg cells that overexpressed PP2A (Thy1.1+) or RACK1 (GFP+) compared to corresponding vector controls. Representative FACS plots (top) and statistical analysis (bottom) of six experiments are shown. 

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b, Schema of IP–MS approach to identify AIM2 interacting proteins in TGFβ-induced T reg cells. Wild-type and Aim2−/− naive CD4+ T cells were activated with anti-CD3 and CD28 in the presence of TGFβ (2 ng ml−1) and IL-2 (40 U ml−1) for 24 h and protein lysates from each group were collected for further IP–MS analysis. 

c, Interaction of AIM2 and RACK1 detected by immunoprecipitation using anti-RACK1 antibody or anti-IgG as control in TGFβ-induced iTreg cells and CD4+ T cells, and immunoblotted with different antibodies, including anti-PP2Aca, anti-AKT, anti-RACK1 and anti-AIM2. Arrow points to the AIM2 protein. Results are representative of three independent experiments. 

d, Wild-type and Aim2−/− CD4+ T cells were stimulated with anti-CD3 and CD28 plus IL-2 (40 U ml−1) and TGFβ (2 ng ml−1) for 24 h and transduced either with MIT-PP2A and MIG-RACK1, or with MIT and MIG vector controls. The cells were collected 3 days after virus transduction. The populations expressing PP2A (Thy1.1+) or RACK1 (GFP+) were identified by flow cytometry. 

e, Flow cytometry of p-AKT of wild-type and Aim2−/− Treg cells that overexpressed PP2A (Thy1.1+) or RACK1 (GFP+) compared to corresponding vector controls. Representative FACS plots (top) and statistical analysis (bottom) of six experiments are shown. 

f, Model for AIM2 function shows that AIM2 facilitates the interaction of RACK1 and PP2A phosphatase, causing dephosphorylation of AKT to restrain the activity of the mTOR pathway, therein promoting Foxp3 expression and Treg cell stability. Data are mean ± s.e.m. *P < 0.05, ****P < 0.0001.