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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Softmax Pro 5.4.5, LASX 1.6 software, Zeiss ZEN blue pro 2011, BD FACSDIVA™ SOFTWARE.
Data analysis	Softmax Pro 5.4.5, Bitplane Imaris, ImageJ 1.50i, Photoshop, Flowjo9.9, FlowJo v10.1r5, Genepix Pro 6.0 softwares, Multi experiment viewer software (MeV, DFCI Boston, MA), Prism 6 (GraphPad Software), exactRankTests' R package and the 'Hmisc' package.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The materials, data, and any associated protocols that support the findings of this study are available from the corresponding authors upon request. Accession codes for microbiome sequences are publically available at: (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP132959).

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size. Sample size was determined based on previous and/pilot studies. In most experiments between 4-6 mice were used per group and guided by the 3R principles to reduce animal numbers.
Data exclusions	No data were excluded from the analysis.
Replication	Biological and technical replicates have been performed. Most of the experiments have been reproduced 2-3 independent times with comparable results. Information on replicates is included in figure legends. Where representative data were shown, the experimental findings were reproduced with similar results.
Randomization	For human samples: PI3Kd patients have been screened genetically prior to this work. Healthy donors (>18y age) were received from NIH blood bank, no information on these individuals is available. For mouse experiments: littermates from multiple breeding were randomly allocated to experiments but based on genotype, age and sex to control for covariates.
Blinding	For most of the experiments, investigators were not blinded. However, in most mouse experiments, isolation of organs and cells from mice were performed in a blinded fashion by a third party. The genotypes of patients and mice were known during the data collection and/or analysis because we chose objective readouts as measure of our experiments. Therefore, the outcome and results were not prone to be influenced by subjective evaluation. Sup. Fig8b was blinded during data collection and analysis. Quantifications of cells in Figure 3g and Sup. Fig. 7a were performed by software independently of human factor.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Antibody, clone, vendor, cat.#, lot#, dilution: MOUSE ANTIBODIES:

CD3-BV421, 145-2C11, BioLegend, 100335, B237072, 1:200
CD4-BV605, RM4-5, BioLegend, 100548, B244808, 1:500
CD8-APC-Cy7, 53- 6.7, eBioscience, 47-0081-82, 4322567, 1:200
CD19-APC-Cy7, 1D3, BioLegend, 115530, B253924, 1:100
CD19-PerCP/Cy5.5, 1D3, BioLegend, 152406, B241294, 1:100
CD25-PE, PC61, BioLegend, 102008, B191542, 1:300
CD25-FITC, PC61, BioLegend, 102006, B152996, 1:100
CD62L-PE/Dazzle 594, MEL-14, BioLegend, 104448, B219195, 1:400
CD62L-AF647, MEL-14, BioLegend, 104421, B239264, 1:400
CD44-AF700, IM7, eBioscience, 56-0441-82, 4329936, 1:100
CD45.1-PE/Dazzle 594, A20, BioLegend, 110747, B211844, 1:300
CD45.1-BV421, A20, BioLegend, 110732, B211844, 1:300
CD45.2-FITC, 104, eBioscience, 11-0454-85, E00316-1632, 1:300
CD86-AF488, GL-1, BioLegend, 105017, B178153, 1:100
PD- 1- PE-Cy7, RMP1-30, BioLegend, 109110, B218703, 1:200
ICOS-APC-Cy7, C398.4A, BioLegend, 313530, B234257, 1:100
B220-PerCP, RA3-6B2, BioLegend, 103234, B232968, 1:100
B220-APC-eFluor780, RA3-6B2, eBioscience, 47-0452-82, 4317799, 1:100
FAS-PE-Cy7, Jo2, BD Biosciences, 557653, 6214949, 1:200
FAS-BV421, Jo2, BD Biosciences, 562633, 7047594, 1:200
GL-7-APC, BioLegend, 144606, B207559, 1:400
GL-7-Pacific Blue, BioLegend, 144613, B209951, 1:200
CXCR5-biotin, 2G8, BD Biosciences, 551960, 7068617, 1:50
CXCR5-purified, 2G8, BD Biosciences, 551961, 6343832, 1:100
CXCR4-biotin, 12G5, BD Biosciences, 551968, 6028693, 1:50
CD21-FITC, 7E9, eBioscience, 11-0212-82, 4293549, 1:200
CD23-PE, B3B4, eBioscience, 12-0232-82, E01138-226, 1:200
CD138-BV605, 281-2, BioLegend, 142516, B232065, 1:500
IgM-FITC, II/41, eBioscience, 11-5790-81, 4282654, 1:100
IgM-PE-Cy7, R6-60.2, BD Biosciences, 552867, 6083739, 1:100
IgD-AF647, 11-26c-2a, BioLegend, 405707, B247514, 1:300
IgD-PE, 11-26c-2a, BioLegend, 405705, B241543, 1:300
IgG1-BB515, X56, BD Biosciences, 565104, 5344921, 1:200
IgG1-APC, X56, BD Biosciences, 550874, 7128657, 1:200
CD43-FITC, S7, BD Biosciences, 553270, 2317604, 1:200
Foxp3-eFluor450, FJK-16s, eBioscience, 48-5773-82, E10036-1634, 1:200
MCL-1-PE, Cell Signaling Technology, D2W9E, 65617S, 1:100
TCR Va2-APC, B20.1, eBioscience, 17-5812-82, E10745-1634, 1:300
polyclonal phospho-FOXO1(Ser256) (antibody #9461; Cell Signaling Technology), 1:100
IgA-PE, 11-44-2, eBioscience, 12-5994-81, 4296926, 1:40
Rat IgG1 K Isotype control-PE, eBRG1, eBioscience, 12-4301-81, 4291957
IgGa/b-PE, X57, BD Biosciences, 340269, 6223621, 1:20
IgG1-PE, A85-1, BD Biosciences, 550083, 7132872, 1:200
IgG3-PE, Southern Biotech, 110009S, B0712-Y887, 1:40
NP(37)-PE, Biosearch technologies, N-5070-1, 1:200
p-AKT S473-PETexas red, M89-61, BD Biosciences, 562465, 1:50
p-S6 S240/244 AF647, D68F8, Cell Signaling Technology, 5044S, 1:500
p-S6 S235/236 PE-Cy7, D57.2.2E, Cell Signaling Technology, 34411S, 1:500
CD93-BV421, AA4.1, eBioscience, 48-5892-80, E17714-102, 1:50
BCL-6-PE, K112-91, BD Bioscience, 561522, 6308845, 1:20
CD5-APC, 53-7.3, eBioscience, 17-0051-82, 4318517, 1:200
CD69-PE-Cy7, H1.2 F3, BioLegend, 104512, B253212, 1:200
HA-AF647, 6E2, Cell Signaling Technology, 3444, 1:50
Recombinant Mouse IL-21 R Fc Chimera, 596-MR, R&D, 1:20
R-Phycoerythrin AffiniPure F(ab')2 Fragment Goat Anti-Human IgG, Fcy Fragment Specific, 109-116-098, Jackson Laboratories, 1:50

MOUSE ANTIBODIES USED FOR IMMUNOFLUORESCENCE:
BCL-6 AF647, K112-91, BD Bioscience, 561525, 7055649, 1:50
Foxp3 AF488, FJK-16s, eBioscience 53-5773-82, 4306753, 1:50
PD-1 BV421, 29F.1A12, BioLegend, 135217, B231473, 1:100
B220 AF700, RA3-6B2, BioLegend, 103232, B175437, 1:100
CD4 AF594, GK1.5, BioLegend, 100446, B221002 ,1:100
CD31 PE, MEC13.3, BD Bioscience, 553373, 553373, 1:200
CD35 BV510, 8C12, BD Bioscience, 740132, 7102546, 1:400

HUMAN ANTIBODIES:
CD4-PerCP, OKT4, BioLegend, 317427, B204491, 1:100
CD3-PE-Cy7, SP34-2, BD Biosciences, 557749, 5044831, 1:100
CD45RA-eFluor450, HI100, eBioscience, 48-0458-42, E08506-42, 1:100
CXCR5-AF647, RF8B2, BD Biosciences, 558113, 5016980, 1:100
PD-1-PE, eBioJ105, eBioscience, 12-2799-41, E11943, 1:100
CD25-PE-CF594, M-A251, 562403, 5267661, 1:100
CCR6-BV605, 11A.9, BD Biosciences, 562724, 7268728, 1:100
CXCR3-AF488, G025H7, BioLegend, 353709, B219004, 1:100

SECONDARY ANTIBODIES
AF488 goat anti-rabbit IgG (H+L), Invitrogen, A11034, 1885241, 1:500
Biotin-SP (long spacer) AffiniPure Fab Fragment Goat Anti-Rat IgG (H+L), Jackson ImmunoResearch, 112-067-003, 1:1000
Brilliant Violet 421™ Streptavidin, BioLegend, 405226, 1:500
Streptavidin-APC, BD Biosciences,554067, 1:500

Validation

All antibodies used in this study are from commercial sources and have been validated by the vendors. Validation data are available on the manufacturer's website. Appropriate antibody dilutions were performed based on preliminary experiments and intensity of fluorescent signals. Dilutions for flow cytometry antibodies are referred to a staining volume of 20-30ul per sample (1-3 × 106 cells).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK 293 T cells were obtained from ATCC.
Authentication	No method for cell authentication was used.
Mycoplasma contamination	No mycoplasma contamination test was performed because: cells are kept in culture for no more than 12 passages and new lots were obtained from ATCC every 6-12 months.
Commonly misidentified lines (See ICLAC register)	Used cell lines are not listed in the ICLAC database.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Male and female Mus musculus mice have been used. C57BL/6 (000664), OT- II (004194), ZP3-Cre (003651) MD4 (002595) and BLIMP-1-YFP (008828), mice were obtained from The Jackson Laboratory. Pik3cdE1020K/+ mice have been generated as described in the method section. In most of the experiments mice have been analyzed at 8 or 16 weeks of age. In certain experiments, 12-14 month old mice were analyzed. Age information is provided in each figure legend. Mice were maintained and treated under specific pathogen-free (SPF) conditions in accordance with the guidelines of the NHGRI Animal Care and Use Committee at the National Institutes of Health under protocol number NHGRI G98-3.
Wild animals	n/a
Field-collected samples	n/a

Human research participants

Policy information about studies involving human research participants

Population characteristics	PBMC samples from seven PI3Kd patients (PASLI/APDS) have been used in this study, with the following characteristic: age, sex, ethnicity, point mutation in p110d, treatment (TRX): 25 years, M, CAUC, E525K, NO TRX 5.5 years, F, CAUC, E1021K, NO TRX 15 years, F, AA, N334K, SIROLIMUS 25 years, F, CAUC, E1021K, NO TRX 16 years, F CAUC, E525K, NO TRX 11 years, M, CAUC, E1021K, EVORILIMUS 29 years, F, ASIAN, E1021K, NO TRX Blood from healthy donors, age 18 or older, was obtained at the NIH Clinical Center under approved protocols.
Recruitment	PASLI/APDS patient samples were selected based on genetic screening for mutation is PI3Kd. No information was obtained regarding these patients based on NIH blood bank policy.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For human samples: Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque gradient centrifugation. For FACS staining: cells were washed and resuspended in MACS buffer (PBS with 2%FBS and 2µM EDTA), block with 10% human serum in PBS, washed twice and stained in MACS buffer with antibody mix. For mouse samples: Spleens and peripheral lymph nodes were minced between the 3ml syringe plunger and fine mesh in MACS buffer (PBS with 2%FBS and 2µM EDTA) to obtain single-cell suspensions that were then filtered through a 70 micron filter. After ACK (Ammonium Chloride) lysis of RBCs, cells were washed once and Fc receptors blocked with anti-mouse CD16/32 (2.4G2, Bio X Cell). Cells (1-3 × 106 cells) were incubated with antibodies for 45/60 min on ice. Mouse and human samples have been stained in round bottom 96 well plate in 20-30ul of antibody mix.
Instrument	LSRII (BD Biosciences). FACSAria IIIu cell sorter (BD Biosciences).
Software	BD FACSDIVA™ was used to collect flow cytometry data. FlowJo 9.9 software (TreeStar) was used to analyze data.
Cell population abundance	Post-sort fractions had more than 95% purify that was verified through flow cytometry analysis on the same FACSAria machine used to sort the cells.
Gating strategy	Initial gating strategy performed: FSC-A/SSC-A, exclusion of doublets (through SSC-H/SSC-W and FSC-H/FSC-W), live cells (negative for Aqua or Near-IR dead stain kit). These were followed by specific gating strategy reported in the figure legends of main and supplementary figures.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.