nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒	A statement on 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 statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data were collected with the LightCycler 480 Real-Time PCR System (Roche), the Varioskan Lux, the Chemidoc (Bio-Rad), the NovaSeq 6000 system (Illumina), the LSRII cytometer (BD Biosciences), the Fortessa X20 cytometer (BD Biosciences), the FACS ARIA II cytometer (BD Biosciences), the Opera Phenix confocal HCS device (Perkin Elmer), the spinning disk confocal microscope (Yogokawa head, Hamamatsu CMOS Flash4 camera, 20X objective NA 0.75), the UltiMate 3000 RS nanoLC system (ThermoFisher Scientific) coupled to the TIMS-TOF SCP mass spectrometer (Bruker).
Data analysis	Data were analyzed using the LightCycler 480 software, Image Lab (Bio-Rad), Imaris (Oxford Instruments), MatLab (Mathworks), Harmony (Perkin Elmer), FlowJo (TreeStar), pyGenomeTracks, Metamorph, pheatmap R package, QuPath, Graphpad Prism.

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

- The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository.
Project Name: Comparative analysis of the proteome of Th2 lymphocytes from WT or ASB2 KO mice
Project accession: PXD044062
Reviewer account details:
Username: reviewer_pxd044062@ebi.ac.uk
Password: GwxLcBI2

- RNA-seq data used in this study have been deposited at GEO under accession number GSE251847.
To review GEO accession GSE251847:
Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE251847
Enter token ktgxoygebxylbmx into the box

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Because mice experiments were performed with female, human samples were from women.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	8 healthy individuals (age from 21 to 43 year old) were enrolled in this study.
Recruitment	PBMCs were obtained from Etablissement Français du Sang, and all human participants provided written informed consent.
Ethics oversight	Etablissement Français du Sang

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Sample size was determined from similar experiments in the literature. Sample size and the number of independent experiments are stated in the figure legends and in supplementary table 4. Four to more independent results were used for statistical analysis
Data exclusions	No data was excluded
Replication	The experimental results were consistently reproduced, and the figure legends and supplementary table 4 specify the exact number (n) of biological replicates used in the study.
Randomization	All samples of the same genotype were randomly assigned for the control and experimental groups, and all cells analyzed were randomly selected from in vivo and in vitro samples.
Blinding	Investigators were blinded during data analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Anti-CD45 AF700 (Clone 30-F11), anti-Siglec-F FITC (Clone S17007L), anti-CD11c APC (Clone N418), anti-CD4 PerCP (Clone RM4-5), anti-IL4 A488 (Clone 11B11), anti-IL5 APC (Clone TRFK5), anti-IFN PE (Clone XMG1.2), anti-TCR Vb5.1, 5.2 Pacific Blue (Clone MR9-4), anti-TCR Va2 PE-Cy7 (Clone B20.1), anti-GATA3 PE (Clone 16E10A23), anti-CD45RO Pacific Blue (Clone UCHL1), anti-CD4 PerCP (Clone RPA-T4), anti-CCR6 APC/Cy7 (Clone G034E3), anti-CRTH2 PE (Clone BM16), anti-human CXCR3 PE-Cy7 (Clone G025H7), anti-mouse CXCR3 PE-Cy7 (Clone CXCR3-173), anti-CD61 PE (Clone 2C9.G2) , anti-CD51 APC (Clone RMV-7), anti-T-BET PE-Cy7 (Clone 4B10), anti-CD3ε (Clone 145-2C11) and anti-CD28 (Clone 37.51) were purchased from BioLegend. Anti-Siglec-F PE (Clone E50-2440) and anti-CD51 (Clone 21/CD51) were purchased from BD Pharmingen. Anti-T1/ST2 PE (Clone DJ8) was purchased from MD Bioproducts. Anti-IL13 PE-Cy7 (Clone eBio13A) was purchased from eBioscience. The anti-FLNa (Clone EP2405Y) was purchased from Abcam. The anti-FLNb (Clone GT1372) was purchased from Sigma-Aldrich. The anti-CD61 (Clone SJ19-09) was purchased from Invitrogen. The anti-GAPDH (Clone 14C10) was purchased from Cell Signaling Technologies. The anti-Ubiquitylated proteins (Clone FK2) was purchased from Enzo Life Sciences. Goat anti-rabbit IgG(H+L) Alexa Fluor 488, goat anti-mouse IgG2a Alexa Fluor 488, goat anti-mouse IgG2a Alexa Fluor 647 were purchased from Invitrogen. Goat anti-rabbit IgG(H+L) HRP, goat anti-rabbit IgG(L) HRP and goat anti-mouse IgG(H+L) HRP were purchased from Jackson Laboratories. The anti-FLNa rabbit serum has been previously described (Lamsoul et al., 2012).

Validation

All of the commercially available antibodies used in this study were validated for the use in human or mouse specimens by the manufacturers and for the respective methods used in this article. The anti-FLNa rabbit serum has been previously validated (Lamsoul et al., 2012).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The HeLa cell line was from the American Type Culture Collection (ATCC, USA).

Authentication

The HeLa cell line was authenticated by ATCC.

Mycoplasma contamination

The HeLa cell line was tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

Female Asb2fl/fl and VE-cadherin (VEC) -Cre;Asb2fl/fl transgenic mice were generated as described previously (Lamsoul et al., 2013; Metais et al., 2018). TCR transgenic OT2 mice were crossed with VEC-Cre;Asb2fl/fl to generate female OT2;VEC-Cre;Asb2fl/fl or OT2;Asb2fl/fl mice. Female C57BL/6J mice were purchased from Janvier Labs. All mice were housed under specific pathogen-free conditions.

Wild animals

No wild animals were involved in this study.

Reporting on sex

Aged-matched female mice were used in this study.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

Mice studies were handled according to the Centre National de la Recherche Scientifique (CNRS) and the Institut national de la santé et de la recherche médicale (Inserm) ethical guidelines and approved by the French Ministry ethic committees (CEEA-122 & CEEA-001).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	Splenocytes, lung cells, and lymph node cells were harvested from C57Bl6, control and ASB2 knockout mice.
Instrument	LSRII cytometer (BD Biosciences), Fortessa X20 cytometer (BD Biosciences), FACS ARIA II cytometer (BD Biosciences)
Software	FlowJo (TreeStar)
Cell population abundance	N/A
Gating strategy	Cell debris were excluded by SSC and FSC gating.

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