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Reporting Summary

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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	Flow Cytometry data was acquired using FACS DIVA (BD-Biosciences). Confocal imaging data was obtained using ZEN Black software (ZEISS).
Data analysis	Flow Cytometry data was analyzed using FlowJo software (BD-Biosciences). Immunoblots were analyzed using Image Lab 5.2.1 Software (Bio-Rad). A voxel-based measurement of the tresholded PCC and Manders coefficients M1 en M2 was carried out in Volocity 6.3.0 (Perkin Elmer). Gene expression was analyzed using qbase+ software version 2.6 (Biogazelle). FlowSOM analysis was performed in R.

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. 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 data that support the findings of this study are available from the corresponding author upon request. The flowcytometry dataset used for FlowSOM analysis has been deposited in the public database FlowRepository as experiment FR-FCM-Z267.

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	In case of studies performed on human material, sample size was dependent on the number of patients available for this study. No statistical method was used to predetermine sample size of mouse experiments. Instead sample size was determined based on the numbers required to generate statistical power and preliminary experiments.
Data exclusions	No data exclusion was performed for this study.
Replication	All experiments were repeated at least twice. Data presented in this study is representative for the different repeats OR combined in one final figure.
Randomization	In the case of human studies, patients samples were compared to healthy controls of similar age. In the case of murine studies, both mutant and wild type mice were randomized over housing cages. Mutant mice were compared to age and sex matched controls.
Blinding	No blinding was performed for these studies as it was deemed as not relevant.

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
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

CD11b ICRF44 BV480 BD Biosciences 746704; CD11c B-Ly6 BV650 BD Biosciences 563403; CD123 32703 BB630-P BD Biosciences CUSTOM; CD127 11A9 BUV737 BD Biosciences 564300; CD14 M5E2 BV750 BD Biosciences 746920; CD16 3G8 BV570 Biolegend 302035; CD183 1C6 AF488 BD Biosciences 561730; CD185 J252D4 BV421 Biolegend 356919; CD194 L291H4 PE Biolegend 359411; CD196 11A9 BUV496 BD Biosciences 564659; CD197 3D12 BUV615-P BD Biosciences CUSTOM; CD20 2H7 BB700 BD Biosciences 745889; CD24 ML5 BUV395 BD Biosciences 566221; CD25 2A3 BUV573 BD Biosciences 565700; CD27 O323 PE-Cy7 Biolegend 302837; CD38 HIT2 APC-R700 BD Biosciences 564980; CD3e UCHT1 BV711 Biolegend 301637; CD4 SK3 BUV805 BD Biosciences 564911; CD45RA HI100 APC Biolegend 304111; CD56 NCAM16-2 BB790-P BD Biosciences CUSTOM; CD8 RPA-T8 BV786 BD Biosciences 563824; CRTH2 BM16 APC-Cy7 Biolegend 350113; FoxP3 PCH101 AF647 eBioscience 77-5776-40; HLA-DR G46-6 BUV667 BD Biosciences 565074; ICOS C398.4A PerCP-Cy5-5 Biolegend 313517; IFNg B27 FITC BD Biosciences 552887; IgD IA6-2 BV605 Biolegend 348231; IL17a eBio64DEC17 APC Thermofisher 17-7179-41; OX40 ACT35 APC Biolegend 350007; PD1 EH12.1 BB660-P BD Biosciences CUSTOM; Streptavidin PE-Cy5 BD Biosciences 554062; TCR gd B1 BUV395 BD Biosciences 564155; Va24-Ja18 6B11 Biotin Thermofisher 13-5806-82; Va7.2 3C10 BV605 Biolegend 351719; XCR1 S15046E APC-Fire750 Biolegend 372607; anti-goat Polyclonal AF647 ThermoFisher A27018; B220 RA3-6B2 BV650 BD Biosciences 563893; CD115 AFS98 PE ThermoFisher 12-1152-83; CD11b M1/70 BUV395 BD Biosciences 565976; CD11b M1/70 APC-eFluor780 ThermoFisher 47-0112-82; CD11c N418 PE-eFluor610 ThermoFisher 61-0114-82; CD11c N418 PE-Cy7 ThermoFisher 25-0114-82; CD134 OX-86 PE Thermofisher 12-1341-82; CD16/32 2.4G2 Unconjugated In-house; CD16/32 2.4G2 PE BD Biosciences 561727; CD161 PK136 BV605 Biolegend 108739; CD19 eBio1D3 PE-Cy5 ThermoFisher 15-0193-82; CD21/35 7G6 PE BD Biosciences 552957; CD23 B3B4 PE-Cy7 ThermoFisher 25-0232-82; CD26 H194-112 FITC BD Biosciences 559652; CD27 LG.7F9 PE-Cy7 ThermoFisher 25-0271-82; CD279 RMP1-30 PE-Cy7 Thermofisher 12-9981-82; CD3 145-2C11 BUV737 BD Biosciences 564618; CD3 145-2C11 BV510 BD Biosciences 563024; CD3 145-2C11 PE-Cy5 Thermofisher 15-0031-82; CD335 29A1.4 eFluor450 Thermofisher 48-3351-82; CD38 90 AF700 Thermofisher 56-0381-82; CD4 GK1,5 APC-Cy7 BD Biosciences 552051; CD44 IM7 RedFluor710 Tonbo Biosciences 80-0441-U025; CD45 30-F11 AF700 Thermofisher 56-0451-82; CD45.1 A20 BV605 Biolegend 110737; CD45.2 104 BUV737 BD

	Biosciences 564880; CD62L MEL-14 FITC Thermofisher 11-0621-82; CD64 X54-5/7.1 BV711 Biolegend 139311; CD64 X54-5/7.1 AF647 Biolegend 139321; CD8 53-6.7 PE-Cy7 Thermofisher 25-0081-82; CD8 53-6.7 eFluor450 Thermofisher 48-0081-82; CD93 AA4.1 Biotin Thermofisher 13-5892-82; Clec4F Polyclonal Goat Bio-Techne AF2784; cRel 1RELAH5 PE ThermoFisher 12-6111-80; CTLA4 UC10-4F10-11 APC BD Biosciences 564331; CXCR5 2G8 Biotin BD Biosciences 551960; F4/80 BM8 BV786 Biolegend 123141; FoxP3 FJK-16s APC Thermofisher 17-5773-82; IgD 11-26c.2a V450 BD Biosciences 560869; IgM II/41 FITC BD Biosciences 553437; IL-17A TC11-18H10.1 PE Biolegend 506903; IL-2 JES6-5H4 APC ThermoFisher 503809; Ly6-C HK1.4 eFluor450 ThermoFisher 48-5932-82; Ly6-G 1A8 BV650 BD Biosciences 740554; MHCII M5/114,15,2 AF700 Thermofisher 56-5321-82; mPDCA1 120g8 FITC In-house
Validation	All antibodies used in this manuscript have been validated for this specific use by the respective vendor.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK 293T cells were obtained from the American Type Culture Collection.
Authentication	HEK 293T cells were not authenticated.
Mycoplasma contamination	HEK293T were tested negative for Mycoplasma contamination prior to use in experiments.
Commonly misidentified lines (See ICLAC register)	No cell lines that have been reported as misidentified have been used in this study.

Animals and other organisms

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

Laboratory animals	Sanroque mice carrying the M199R mutation in the ROQ domain of Rc3h1 were backcrossed to the C57Bl/6 background. C57Bl/6 CD45.1/CD45.2 mice were used to generate bone marrow chimeras. Rc3h1-2fl/fl; CD4-Cre-ERT2 mice were crossed with rtTA transgenic mice to generate C57Bl/6 Rc3h1-2fl/fl; CD4-Cre-ERT2; rtTA mice. Both male and female mice were used in the experiments. In the case of bone marrow transplantation, mice were tested 8 weeks post irradiation. Details are reported in Methods.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All experimental procedures involving mice were performed in accordance with the regulations of and were approved by the Ludwig-Maximilians-Universität München or the ethical committee of the Science Department at Ugent University.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	The patient studied in this manuscript is an 18yr old male of caucasian origin. A nonsense mutation (R688*) in the RC3H1 gene was identified by WES.
Recruitment	Patient recruitment is clinician guided on a case by case basis. No criteria of inclusion or exclusion were formulated for this study.
Ethics oversight	This study was approved by the ethical committee of Ghent University Hospital

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

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	In general, cells were first stained with FcR block (human; Miltenyi; 130-059-901, mouse; in-house developed; 2.4G2) together with biotin conjugated antibodies and Fixable Viability dye eFluor 506 (eBioscience; 65-0866-14) or Fixable Viability Stain 620 (BDBiosciences; 564996). In a second step, remaining surface markers were stained with a mixture of antibodies in FACS buffer (DPBS pH7.4, 1% Bovine Serum Albumin, 0,05% NaN3, 1 mM EDTA). If staining of intracellular antigens was required, cells were fixed 30 minutes in 2% paraformaldehyde at room temperature and subsequently permeabilized with FoxP3 permeabilization buffer (ebioscience; 00-5523-00).
Instrument	LSRFortessa and BDFACSymphony
Software	FlowJo10 software (BD biosciences)
Cell population abundance	Not relevant for this manuscript.
Gating strategy	In this manuscript we identified the following populations: B cells: FSC-A/SSC-A (lymphocytes) --> FSC-A/FSC-H (singlets) --> Viability dye/FSC-A (Viability dye -)-->CD45+ cells -->CD19+ T cells: FSC-A/SSC-A (lymphocytes) --> FSC-A/FSC-H (singlets) --> Viability dye/FSC-A (Viability dye -)-->CD45+ cells -->CD3+ Representative plots of Naive (CD62L+) or central memory (CD44+/CD62L+) and effector memory (CD62L+) are given. Regulatory T cells were identified as FoxP3+, Follicular Th cells were identified as CXCR5+/PD-1+ gamma delta T cells were identified as TCRgd+ Monocytes: FSC-A/SSC-A (non-debris) --> FSC-A/FSC-H (singlets) --> Viability dye/FSC-A (Viability dye -)-->CD45+ cells -->CD3-/CD19- cells-->Ly6G- cells --> CD11b+/Ly6Chi Neutrophils: FSC-A/SSC-A (non-debris) --> FSC-A/FSC-H (singlets) --> Viability dye/FSC-A (Viability dye -)-->CD45+ cells -->CD3-/CD19- cells -->Ly6G+/CD11b+ cells Macrophages: FSC-A/SSC-A (non-debris) --> FSC-A/FSC-H (singlets) --> Viability dye/FSC-A (Viability dye -)-->CD45+ cells -->CD3-/CD19- cells -->CD64+/F4/80+ cells. cDCs: FSC-A/SSC-A (non-debris) --> FSC-A/FSC-H (singlets) --> Viability dye/FSC-A (Viability dye -)-->CD45+ cells -->CD3-/CD19- cells --> CD64- cells --> CD11c+ MHCII+ cells pDCs: FSC-A/SSC-A (non-debris) --> FSC-A/FSC-H (singlets) --> Viability dye/FSC-A (Viability dye -)-->CD45+ cells -->CD3-/CD19- cells --> CD64- cells --> CD11b- cells--> B220+/120g8+ cells NK cells: FSC-A/SSC-A (non-debris) --> FSC-A/FSC-H (singlets) --> Viability dye/FSC-A (Viability dye -)-->CD45+ cells -->CD3-/CD19- cells --> NK1.1+/NKp46+ cells. Representative plots of different NK cell subsets are given (CD27/CD11b). In the setting of gating on liver derived immune cells (e.g. Kupffer cells) an additional step outgating CD26+ hepatocytes was included in the gating strategy.

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