# natureresearch

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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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

### 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 <u>exact sample size</u> (*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 repeate	dly

The statistical test(s) used AND whether they are one- or two-sided

_	$\square$	Only a	common	tests sho	ould be	described	solely	by name;	describe r	more cor	nplex	techniques	in the	Methods	s section
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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 <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (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

 BD FACS Diva 8.0.1 software was used for cytofluorimetric data collection.

 Data analysis

 GraphPad Prism7 was used for the statistical analysis. FlowJo10 was used for the analysis of the FACS data. CaseViewer from 3dhistech was used for the morphometric assessment of histological slides. BioMark HD (Fluidigm) was used for gene expression analysis. HiSat2, bowtie2, MarkDuplicates, featureCounts v1.5.2 and DESeq v1.16.1 were used for bioinformatics analysis.

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.

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

Behavioural & social sciences

- 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 RNA sequencing data have been deposited in the GEO repository under accession code GSE114827.

Ecological, evolutionary & environmental sciences

# 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

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

# Life sciences study design

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

Sample size	No sample size calculation was performed, but a reasonable sample size was chosen to ensure adequate reproducibility of results and was based on our previous studies.
Data avelusions	No data was avoluded
Data exclusions	No data was excluded.
Replication	Experiments were replicated several times with reproducible results, as indicated in each figure legend.
Randomization	Mice were assigned to experimental groups according to sex and age.
Blinding	In the in vivo experiments the investigators were not blinded to the genotype of the experimental groups. Anatomopathological analysis of

# Reporting for specific materials, systems and methods

#### Materials & experimental systems

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n/a | Involved in the study  $\boxtimes$ Unique biological materials Antibodies Eukaryotic cell lines Palaeontology Animals and other organisms Human research participants

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- Involved in the study n/a
- $\left|\times\right|$ ChIP-sea
- Flow cytometry
- MRI-based neuroimaging

## Antibodies

Antibodies used

The following antibodies were used for flow cytometry: CD45.2 (1:200, clone 104, #560693), CD3 (1:100, clone 145-2C11, #562286 and #557596), CD19 (1:200, clone 1D3, #562291), NK1.1 (1:50, clone PK136, #563096), CD49a (1:400, clone Ha31/8, #562113), CD11b (1:400, clone M1/70, #562950), NKp46 (1:50, clone 29A1.4, #562850), MHCII (1:200, clone M5/114.15.2, #563413), TCRb (1:400, clone H57-597, #553171), PD1 (1:50, clone J43, #744546), Hamster IgG2 isotype control (1:50, clone B81-3, #563860), PDL1 (1:100, clone MIH5, #558091), granzyme B (1:50, clone GB11, #561142), Rorgt (1:100, clone Q31-378, #562684), IL17 (1:100, clone TC11-18H10, #559502), CD107a (1:60, clone 1D4B, #553793), Fc block CD16/CD32 (1:200, clone 24G2, #553142) and Ki67 (1:50, clone B56, #5560027) from BD Biosciences; NKp46 (1:50, clone 29A1.4, #46-3351-82 and #25-3351-82), CD49b (1:200, clone DX5, #103518), F4/80 (1:100, clone BM8, #17-4801-82), Ly49H (1:200, clone 3D10, #17-5886-82) and IL22 (1:200, clone IL22JOP, #16-7222-82 coupled to AlexaFluor647) from eBioscience; CD19 (1:200, clone 6D5, #115530), CD11c (1:200, clone N418, #117318), PD1 (1:50, clone RMP1-30, #109103), Rat IgG2b isotype control (1:50, clone RTK4530, #400607), PDL2 (1:100, clone TY25, #107206), Ly6G (1:200, clone 1A8, #127624), and IFN-g (1:100, clone XMG1.2,

	#505829) from Biolegend; GR (1:50, clone D8H2, #12007) and rabbit mAb IgG XP (1:100, clone DA1E, #2795) from Cell Signaling
	Technology.
	For PD1 blockade experiments in vivo anti-PD1 antibody (clone J43 #BE0033-2 or clone RMP1-14 #BE0146) and Armenian
	Hamster IgG (#BE0091) or IgG2a mAb (clone 2A3, #BE0089) from BioXCell were used. For IFN-g neutralization in vivo anti-IFN-g
	(clone XMG1.2 #BE0055) or Rat IgG1 (clone HRPN #BE0088) both from BioXCell were used.
Validation	All the antibodies used are from commercial sources and have been validated by the vendors. Validation data for species
Vandation	(mouse) and application (flow cytometry and in vivo injection) are available on the manufacturer's website.

### Animals and other organisms

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

All mice used for experiments were females on a C57BL/6J genetic background and 7-10 weeks-old. Wild-type mice littermates (Ncr1iCre/+Nr3c1+/+) of GRNcr1-iCre mice (Ncr1iCre/+Nr3c1LoxP/LoxP) were used as a control of the mutant strain, or were purchased from Janvier Labs otherwise. All the mice used were bred and maintained under specific pathogen-free conditions at the Centre d'Immunophenomique (Ciphe) de Marseille and the Centre d'Immunologie de Marseille Luminy. Mice were housed under a standard 12 h:12 h light-dark cycle with ad libitum access to food and water.					

### 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	Single-cell suspensions were obtained from the spleen by scratching it through 70um cell strainer, or from the liver by scratching it through 100um cell strainer and subsequent lymphocyte isolation on a 37.5-67.5% Percoll gradient. For isolation of small intestine lamina propria cells, intestines were cut longitudinally, then transversally in 2-3 cm pieces, thoroughly rinsed with PBS, and shaken for 30 min in PBS containing 10% FBS, 15mM Hepes and 5mM EDTA to remove intraepithelial and epithelial cells. Intestines were then digested with collagenase VIII (300UI/ml) in complete RPMI for 45 min at 37'C under agitation, and lamina propria lymphocytes were isolated on a 40-100% Percoll gradient.
Instrument	BD LSRII and BD FACSAria II
Software	BD FACS Diva 8.0.1 software was used for data collection and FlowJo v.10 was used for data analysis.
Cell population abundance	Post-sort cells were not reanalyzed because cells were sorted in RLT lysis buffer for RNA extraction
Gating strategy	Among live, single, CD45+ cells, NK cells were gated as: CD3-CD19-NK1.1+NKp46+CD49b+ (and in the liver CD49a-); liver lLC1s were gated as: CD3-CD19-NK1.1+NKp46+CD49b-CD49a+ T cells were gated as: CD3+NK1.1- or TCRb+ B cells were gated as: CD19+ Neutrophils were gated as: CD11c-CD11b+Ly6G+ Macrophages were gated as: CD11c-CD11b+F480+ DCs were gated as: CD11c+MHCIIhi small intestine ILC3s were gated as: CD3-CD19-NKp46+Rorgt+

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