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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	FACSDiva 8.0.1 (BD). XCalibur 4.0.27.42 (Thermo Fisher Scientific). Microplate Manager 6.2 (Bio-Rad). Wallac 1450 MicroBeta Workstation (Perkin Elmer)
Data analysis	Graphpad Prism 6.0, PC. GSEA using Broad Institute software. Imaris software (Bitplane). PEAKS 8.0 (Bioinformatics Solutions). DESeq2 R package. Flowjo 7.5 and Flowjo 10.0.

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.

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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 RNAseq data have been deposited in the Gene Expression Omnibus under accession number GSE114824. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD009919 and 10.6019/PXD009919.

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	For two-photon analysis, previous studies have shown that the mean track velocity ranges between 7 and 12 micrometers/min with a standard deviation of approximately 4. To have a power of 0.95 and an alpha 0.05, we require a minimal of ~50-60 samples. All of our calculations were derived from a minimal of 60 counted tracks. For competitive ELISA analysis, we have determined that the optical density measurement ranged from a min of 0.3 to 0.6 with a standard deviation of 0.05. To have a power of 0.95 and an alpha of 0.05, we require a minimal of 3 biological replicates, and in all the experiments we included 6 samples. For RNAseq, based on our previous studies we expected an average gene expression level standard deviation of 1-1.5. To have a power of 0.95 and an alpha of 0.05, we required 4 biological replicates per group. For diabetes incidence, with an equal number of animals monitored per group and a relative hazard ratio of 0.5, a minimal of 4 mice per group were required. We included 6 mice per group.
Data exclusions	We did not exclude any data from our studies.
Replication	For two-photon analysis, we analyzed 60-200 individual T cells from 2-4 mice per experiment, and the statistical analysis was based 2-4 independent experiments. For ELISA, the statistical analysis was based on 6 independent experiments containing 4-8 mice per experiment. For mass spectrometry, we analyzed two independent experiments with 16-24 mice per experiment. For RANseq, we included 4 biological sample pairs and each pair was from a total of 8-10 mice. For diabetes incidence, we monitored 6 mice per group from 3 independent experiments.
Randomization	Mice were age and gender matched. Among the mice with matched ages and genders, they were randomized and distributed equally into experimental groups.
Blinding	All the measurements were performed by instruments or computing devices and therefore blinding is not necessary.

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	The following fluorescently conjugated antibodies were purchased from BioLegend: anti-B220 (RA3-6B2), anti-CD11c (N418), anti-CD4 (RM4-5), anti-CD45 (30-F11), anti-CD45.1 (A20), anti-CD45.2 (104), anti-CD8a (53-6.7), anti-F4/80 (BM8), anti-Vβ8.1/8.2

(KJ16-133.18), anti-CD44 (IM7), anti-CD62L (MEL-14), anti-CD25 (PC61.5) and anti-TNFα (MP6-XT22). Unconjugated or Alexa Fluor 647-labelled Rabbit anti-insulin MoAb (C27C9) was purchased from Cell Signaling Technology. Unconjugated mouse anti-insulin MoAb (E11D7) was purchased from Millipore. Alexa Fluor 594 F(ab)2 donkey anti-mouse IgG and HRP-conjugated goat anti-mouse IgG (Fcγ portion specific) were purchased from Jackson ImmunoResearch.
All the commercial antibodies were validated by the vendor.
The 6F3.B8 antibody recognizing mouse insulin B-chain was generated in the laboratory. The reactivity was confirmed using ELISA.

Validation

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Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

The T cell hybridomas used in this study were generated in the laboratory.

Authentication

The reactivity of the T cell hybridomas to their specific antigens was tested using by their ability to produce interleukin-2 when stimulated by corresponding antigens presented by antigen presentation cells.

Mycoplasma contamination

All the cell lines were confirmed mycoplasma free.

Commonly misidentified lines (See ICLAC register)

Not applicable.

Animals and other organisms

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

Laboratory animals

NOD/ShiLtJ (NOD), NOD.129S7(B6)-Rag1tm1Mom/J (NOD.Rag1−/−), NOD.Cg-Tg(Ins2*Y16A)1EllIns1tm1JjaIns2tm1Jja/GseJ (NOD.B16A), NOD.Cg-Tg(TcraTcrbNY8.3)1Pesa/DvsJ (8.3), NOD.129S2(B6)-Ighmtm1Cgn/DoiJ (µMT), NOD.C-(Ptprc-D1Mit262)/WehiJ (NOD.CD45.2), NOD.B10Sn-H2b/J (NOD.H2b) and B6.NOD-(D17Mit21-D17Mit10)/LtJ (B6g7) mice were originally obtained from the Jackson Laboratory.
For most of the studies, 4-8 week old female or male mice were used.

Wild animals

The study did not involve wild animals

Field-collected samples

The study did not involve field-collected samples

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 prepared and incubated with FcR blocking media containing 2.4G2 antibody for 30 min on ice. The cells were then stained for surface markers with corresponding fluorescent antibodies on ice for 25 min.

Instrument

The flow cytometry samples were collected using a FACSCanto II (BD Biosciences). T cell sorting was performed using FACSAria II (BD Biosciences).

Software

Data were collected using FACS DIVA (BD Biosciences) and were analyzed using FlowJo software (Tree Star Software).

Cell population abundance

The post sort was performed to confirm a purity above 99%.

Gating strategy

The starting cell population is gated on appropriate FSC/SSC with the default setting of the FACSDiva software. The singlet gate, based on FSH/SSW is applied in all the analyses to exclude doublets. The positive and negative boundaries were determined using isotype control staining or biological control samples. An example of gating strategy is shown in Extended data Figure 8a.

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