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<br>*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<br>*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 | Not applicable |
| Data analysis | Imaging mass Cytometry: Visualization of the global single-cell landscape was performed in OMIQ after arcsinh transformation (factor 5). Expression of steady state and activation markers were gated in OMIQ<br>Microarray: Microarray analysis was performed using Affymetrix Clariom S Mouse arrays. Arrays were normalized using Robust Multi-Array Average expression measure, and probe expression values summarized to the gene level using the R/Bioconductor package pd.clariom.s.mouse. Differential analysis: A linear model based approach (limma R package) was used to identify the differentially regulated RNA between untreated and treated cells. Regulated RNA with Benjamini Hochberg adjusted P-value <0.05 was set as significant.<br>For statistical analysis, an unpaired or paired t-test (two-sided) or when required a Two-way ANOVA test was applied. If the data did not meet the criteria for normality, the Mann-Whitney U test was applied unless stated otherwise in the Figure legend. Data are presented as mean and standard deviation (error bars). Differences were considered significant when the P-value was <0.05. Survival data were plotted using the Kaplan-Meier method and compared by using a Log-rank (Mantel-Cox) test. Statistical analysis was performed using GraphPad Prism (GraphPad Software V9,; San Diego, CA). Data are presented as mean and s.e.m. (error bars). Differences were considered significant when the P-value was <0.05. |

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

Data and materials availability:
The Microarray data in this study was deposited in the GEO repository under accession code GSE193696. The Single nucleus transcriptomic data was deposited in the GEO repository accession number: GSE223647. Kinase assay data was deposited in the GEO repository under accession code GSE225811. IIMC data was deposited in Zenodo, doi: 10.5281/zenodo.10695442.

## 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 | Gender of participants was determined based on self-report. Our study group (ICANS) included 4 female and 3 male patients (Suppl. Table S4, Patient characteristics- IMC). Since CNS tissue from patients that died of ICANS is rare we analyzed all patients that were available. The relatively equal distribution (4 vs 3) was by chance and not predefined.<br>Our research findings do not apply to only one sex or gender.<br>Informed consent for sharing individual level data was obtained prior to the study. |
| Reporting on race, ethnicity, or other socially relevant groupings | N/A Race and ethinicity were not considered during the study design |
| Population characteristics | Patients with B cell malignancies that received CAR T cells were considered as eligible study population. The population included r/r DLBCL, Mantel cell lymphoma, multiple myeloma and controls |
| Recruitment | Patients were recruited at Department of Nuclear Medicine, LMU Munich and at the Department of Nuclear Medicine, Medical Center – University of Freiburg. |
| Ethics oversight | Human sample collection and analysis were approved by the Institutional Ethics Review Board of the Medical center, University of Freiburg, Germany ). Written informed consent was obtained from each patient. All analysis of human data was carried out in compliance with relevant ethical regulations.<br>The study was approved by the local ethics committee (project-number: 20-1223). TSPO- PET images of 22 age-matched healthy controls were obtained from previous cohorts (64, 65), approved by the local ethics committee (project-numbers: 17-569, 19-022). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. |

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 | For sample size in the murine survival experiments a power analysis was performed. A sample size of at least n=8 per group was determined by 80% power to reach a statistical significance of 0.05 in order to detect an effect size of at least 1.06.<br>All other experiments were performed with 4-15 biological individuals, usually in 2-3 independent experiments. The sample size was chosen based on typical sample sizes in GVHD experiments as previously reported e.g. Schwab et al., Nat. Med. 2014. |
| Data exclusions | No data were excluded from the analyses. |
| Replication | Survival studies were performed two times with 5 biological replicates per group. For flow cytometry based analyses the experiment was performed at least two times (some experiments were done 3-4 times). The imaging experiments including 3D reconstruction, IHC, FITC-dextran were performed at least two times. Microarray, Single cell analysis, kinase assay and Imaging mass cytometry analysis were |

performed once.

Randomization

Mice were randomly allocated to control and treatment groups prior to any interventions. There was no randomization of mice or samples before analysis. Therefore, all samples or mice were included in our analysis.

Blinding

The experiments were performed in a non-blinded manner, because the survival, FACS, Histology are not a subjective parameter. Behavior studies with KO strains were mostly performed in blinded fashion.

# 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 and archaeology |
| ☐ | ☒ Animals and other organisms |
| ☐ | ☒ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

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

## Antibodies

Antibodies used

Antibody Clone Catalog number Fluorochrome Dilution Vendor
Anti-mouse CD3 17A2 100220 PE-Cy7 1:100 Biolegend
Anti-mouse CD45 30-F11 101132 PerCP-Cy5.5 1:100 Biolegend
Anti-mouse CD45 30-F11 103126 PB 1:100 Biolegend
Anti-mouse CD80 16-10A1 104708 PE 1:100 Biolegend
Anti-mouse CD86 GL-1 105021 PB 1:50 Biolegend
Anti-mouse CX3CR1 Polyclonal FAB5825G Alexa flour 488 1:50 R&D systems
Anti-mouse p70s6k IC8692P PE 1:100 R&D systems IC8692P

Anti- mouse p2ry12 S16007D 848006 APC 1:100 Biolegend
Anti-mouse/human B220 RA3-6B2 103247 BV510 1:100 Biolegend
Anti-mouse CD11b M1/70 25-0112 PE-Cy7 1:100 eBioscience
Anti-mouse IL-6 MP5-20F3 12-7061-41 PE 1:20 eBioscience
Anti-mouse MHC- II AMS-32.1 17-5323-82 APC 1:100 eBioscience
Anti-mouse NK1.1 PK136 BLD-108737 BV510 1:50 Biolegend
Anti-mouse p38 MAPK (pT180/pY182) 36/p38 612565 PE 1:50 BD Bioscience
Anti-mouse TNF-α MP6-XT22 506327 BV421 1:100 Biolegend
Anti-mouse Trem2 FAB17291P PE 1:100 R&D Systems
Anti-mouse CD11c N418 117312 APC 1:100 Biolegend
Protein L –biotin 29997 1:100 Thermofisher
Streptavidin - 349023 PE 1:50 Biolegend
Anti-mouse GM-CSF MP1-22E9 505405 PE 1:50 Biolegend
Anti-mouse MCP-1 2H5 505904 PE 1:50 Biolegend
Anti-Mouse CD3 17A2 100220 PE-Cy7 1:100 Biolegend
Anti-Mouse CD3 17A2 100213 PB 1:100 Biolegend
Anti-Mouse IFNγ XMG1.2 12-7311-82 / 81 PE 1:50 eBioscience
Anti-mouse CSF1R AFS98 12-1152 PE 1:100 Biolegend
Anti-Mouse Ly6C AL-21 560525 PerCP-Cy5.5 1:100 BD Bioscience
Anti-Mouse Ly6G 1A8 560600 APC-Cy7 1:100 Biolegend
Anti-Mouse F4/80 BM8 123112 PE-Cy5 1:100 Biolegend
Anti-Mouse CD45 30-F11 103114 PE-Cy7 1:100 Biolegend
Anti-Mouse CD45 30-F11 103116 APC-Cy7 1:100 Biolegend
Anti-Mouse CD45 30-F11 103106 PE 1:100 Biolegend
Anti- Mouse CD8a 53-6.7 560182 APC-H7 1:100 BD Pharmingen
Anti-Mouse CD4 GK1.5 100434 PerCP-Cy5.5 1:100 Biolegend
Anti-Mouse CD11c N418 117307 PE 1:100 Biolegend
Anti- Mouse VCAM 429 Percpcy5.5 1:50 Biolegend
Anti- Mouse ICAM1 HA58 17-0549-42 PB 1:50 Biolegend
Anti- Mouse ICAM2 3C4(MIC2/4)105606FITC 1:50 Biolegend
Anti- Mouse VCAM 429 (MVCAM.A)105722PB 1:50 Biolegend
Anti-mouse Perforin eBioOMAK-D 17-9392 APC 1:50 Biolegend
Anti- mouse CD69 H1.2F3 11-0691PE 1:100 Biolegend
Anti- mouse CD25 PC61 102011APC 1:100 Biolegend

Anti-Mouse CD3 17A2100220PECy7 1:100 Biolegend
Anti-Mouse CD31 380 12-0311-81 PE 1:100 Ebioscience
Anti-mouse TMEM119 V3RT1GOsz 12-6119-82 PE 1:50 Ebioscience
Anti-mouse CD107 1D4B 121612 PE 1:50 BioLegend
Anti-mouse granzyme B NGZB 25-8898-82 PE-Cy7 1:50 Ebioscience
Anti-mouse CTLA-4 UC10-4B9106310APC 1:100 BioLegend
MHC-II (I-Ab) AF6120.1 116408PE 1:100 Biolegend
MHC-II (I-Ab) AF6120.1 116418APC 1:100 Biolegend

Validation

Antibodies were validated by titration experiments where the base line was the recommended working concentration provided by the manufacturer. Validation was performed per experimental setup and used cell type.
The dosage for the tak1 inhibitor was based on previous publications (Nimitha and Vinnakota et al., JCI., 2020)

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

We used the following mouse cell lines:
- A20 Luc+GFP+ (A20 Cell line procured from ATCC)
- E2a-PBX1 (Cell line generated at Stanford University and provided by Federico Simontetta, Stanford, USA, description of the cell line: Duque-Afonso J, Feng J, Scherer F, Lin CH, Wong SH, Wang Z, et al. Comparative genomics reveals multistep pathogenesis of E2A-PBX1 acute lymphoblastic leukemia. J Clin Invest. 2015;125(9):3667–80.)
- CD19.28z CAR producer cell line (Cell lines generated at Stanford University and provided by Federico Simontetta, Stanford, USA)

Authentication

The cell lines used in this study were obtained from a batch that had been prevoiusly authenticated at DSMZ, Germany.

Mycoplasma contamination

The cell lines used in this study were obtained from a batch that had been prevoiusly tested for Mycoplasma contamination (PCR analysis) and were found to be negative. Cell lines were subsequently routinely tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No such 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

C57BL/6 (H-2Kb) and BALB/c (H-2Kd) mice were purchased from Janvier Labs (France) or from the local stock of the animal facility at University of Freiburg. Mice were used between 6 and 14 weeks of age, and only female or male donor/recipient pairs were used. Housing conditions: dark/light 12/12 hours, temperature: 20-24°C, humidity 45-65%

Wild animals

The study did not involve wild animals.

Reporting on sex

To rule out sex specific differences we used female donors and female recipients or male donors and male recipients.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All mouse experiments were approved by the Federal Ministry for Nature, Environment and Consumers' Protection of the state of Baden-Württemberg (Regierungspräsidium Freiburg, Freiburg, Germany) (No: G17/093, G-22/052, G-20/075,

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

## Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

NCT05811117

Study protocol

Immunotherapy with chimeric antigen receptor (CAR) T cell therapy can cause immune effector cell-associated neurotoxicity syndrome (ICANS). However, the molecular mechanisms leading to ICANS are not well understood. In this study, the investigators plan to examine the role of microglia as the primary parenchymal immune cells of the central nervous system (CNS) during ICANS in human samples. The samples will be analyzed using imaging-mass-cytometry-based analysis (IMC). Single-cell data will be obtained through machine learning supervised segmentation of IMC data. Single-cell marker expression in all cells and in microglia (Iba1+ cells) will be analyzed in patients with ICANS.

| | |
|---|---|
| Data collection | formaldehyde-fixed paraffin-embeeded (FFPE) sections post mortem brain samples were collected from University of Pennyslvenia, USA and Germany - Uniklinik Erlangen, Uniklinik Tübingen, Uniklnik Mainz, Uniklinik Münster, LMU Munich, Uniklinik Würzburg |
| Outcomes | Primary Outcome Measure:<br>- Imaging mass cytometry of patient samples-<br>secondary outcome:<br>Analysis of myeloid immune cell activation patterns<br>Immunoflourescence of Microglia and other cell types |

## Plants

| | |
|---|---|
| Seed stocks | *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.* |
| Novel plant genotypes | *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.* |
| Authentication | *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.* |

## 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 | Mice were anesthesized and duly perfused with PBS. Brain was isolated and homogenized in Dissection medium (Home made). The cells were resuspended in 37% Percol and subjected to density gradient centrifugation.<br>Splenocytes from mice were obtained by smashing whole spleen through an EASYstrainer (70µm – grainer - 542070). Bone marrow cells from mice were obtained by isolating hip, tibia and femur and subsequent flushing of these bones with fresh PBS. For FACS analysis, erythrocytes in whole splenocytes and bone marrow cell samples were killed using home-made erythrocyte lysis buffer. |
| Instrument | The majority of data were acquired on a BD LSR Fortessa and BD Fusion for cell sorting. |
| Software | The majority of data were analyzed using FlowJo (Flowjo 10.4 or 10.6, LLC) software. |
| Cell population abundance | The gating strategy was determined using unstained controls, single stains and fluorescence minus one (FMO) controls. |
| Gating strategy | All experiments were performed using appropiated controls and compensation controls were also considered. Cells were first gated using SSC-A vs FSC-A, doublet cells were excluded by gating on FSC-H vs FSC-A and subsequent SSC-H vs SSC-A, dead cells were excluded using LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (L34966) or Zombie Red™ Fixable Viability Kit (423109), Subsequently, the desired paramiters were gated. Unstained controls, single stains and fluorescence minos one (FMO) controls were used for appropiate gating on desired parameters and differentiation between positive and negative cells. Gating strategy will be provided upon request |

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

## Magnetic resonance imaging

### Experimental design

| | |
|---|---|
| Design type | *Indicate task or resting state; event-related or block design.* |
| Design specifications | *Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.* |

Behavioral performance measures

*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

## Acquisition

Imaging type(s)

*Specify: functional, structural, diffusion, perfusion.*

Field strength

*Specify in Tesla*

Sequence & imaging parameters

TSPO PET Munich : Patients with r/r DLBCL and controls were scanned at the Department of Nuclear Medicine, LMU Munich, using a Biograph 64 PET/CT scanner (Siemens, Erlangen, Germany). In brief, [18F]GE-180 TSPO-PET recordings (average dose: 182 ± 10 MBq) with an emission window of 60-80 min after injection were obtained to measure microglial activation

TSPO PET. Freiburg: Emission scans were acquired 60-90 min after intravenous injection of 524 ± 30 MBq and 562 ± 62 MBq [11C]ER176, respectively (before and after CAR T cell transfer)

Area of acquisition

TSPO PET Munich : Per patient, the standardized regional deviation of global mean scaled images (z-score) was calculated versus the age-matched normal cohort (5). The obtained VOI set was used to extract TSPO PET z-score values of predefined brain regions matching the immunohistochemistry analysis (grey matter of the right frontal cortex, bilateral grey and white matter of the occipital lobe, left putamen, cerebellum) or to manually define matching subregions by 3 mm spheres within the brainstem VOI (locus coeruleus, midbrain, olive).
TSPO PET Freiburg: Brain

Diffusion MRI ☐ Used ☐ Not used

## Preprocessing

Preprocessing software

TSPO PET Munich: To outline the brain into the 83 atlas volumes of interest (VOIs) of the Hammers atlas (4) and to transform the TSPO PET image into the atlas space using the PNEURO tool implemented by PMOD (V3.9, PMOD Technologies LLC, Zurich, Switzerland). Obtained spatial transformations were used to perform all quantitative analyses in the individual T1 MRI space. An axial MRI FLAIR sequence was co-registered for visual comparison of white matter hyperintensities and TSPO PET images
TSPO PET. Freiburg: We used an in-house pipeline in MATLAB (The MathWorks, Inc.) and Statistical Parametric Mapping (SPM) 12 software (www.fil.ion.ac.uk/spm) for PET data processing

Normalization

TSPO PET Munich : Intensity normalization of all TSPO PET images was performed by global mean scaling to ensure robust semi-quantification

TSPO PET. Freiburg:TSPO-PET scans were stereotactically normalized to an in-house [18F]fluorodeoxyglucose (FDG)-PET template in MNI space using individual [18F]FDG-PET brain scans obtained on the same PET/CT system 1 – 2 days before/ after first TSPO PET

Normalization template

TSPO PET Munich : Per patient, the standardized regional deviation of global mean scaled images (z-score) was calculated versus the age-matched normal cohort
TSPO PET. Freiburg: in-house [18F]fluorodeoxyglucose (FDG)-PET template in MNI space using individual [18F]FDG-PET brain scans obtained on the same PET/CT system 1 – 2 days before/after first TSPO PET

Noise and artifact removal

*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring

*Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

## Statistical modeling & inference

Model type and settings

*Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*

Effect(s) tested

*Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

*Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

(See Eklund et al. 2016)

Correction

*Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

## Models & analysis

| n/a | Involved in the study |
|---|---|
| ☐ | ☐ Functional and/or effective connectivity |
| ☐ | ☐ Graph analysis |
| ☐ | ☐ Multivariate modeling or predictive analysis |

Functional and/or effective connectivity

*Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis

*Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis

*Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*