

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                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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 | Blood was isolated from patients via venipuncture. PBMCs were isolated from blood by layering diluted blood (1:1 in PBS) on top of an equal volume of Ficoll, followed by centrifugation and isolation of the buffy coat. PBMCs were frozen in a mixture of fetal bovine serum (FBS; 90%) and dimethyl sulfoxide (DMSO; 10%) overnight at -80°C in a Mr. Frosty freezing container (Thermo Fisher) and transferred the following day to liquid nitrogen for storage. PBMCs were sorted on sytox blue live/dead dye on a Sony SH800 in FACS buffer containing PBS with 0.4% BSA. 100,000 cells were sorted into 750 ul FACS buffer, centrifuged at 350G for 7 min and resuspended in 85 ul FACS buffer to achieve ~1,000 cells/ul. 16.5 ul of cells were mixed with 23 ul water then prepared according to 10X Genomics NextGEM v1.1 5' protocol with single indexes. cDNA was processed for whole RNA and TCR libraries. To avoid batch effects, all 40 samples were sorted and loaded into GEMs on the same day and all libraries were generated simultaneously using a 96-well PCR plate and a plate magnet. Single-cell RNA libraries were processed using the CRISPRclean Single Cell RNA Boost Kit (Jumpcode Genomics, KIT1018) to remove highly expressed ribosomal and mitochondrial transcripts, as well as non-variable genes, prior to sequencing. Following cDNA amplification and adapter ligation with the 10X Genomics Chromium Next GEM Single Cell 3' Reagent Kit v3.1 (10X Genomics, 1000268), CRISPR ribonucleoprotein (RNP) complexes were formed by incubating Cas9 nuclease (Jumpcode Genomics, REA1000) with guide RNAs (Jumpcode Genomics, REA1025-003, REA1120-001) targeting undesired sequences. CRISPR digestion was performed at 42°C for 60 minutes, followed by AMPure XP bead-based size selection (Beckman Coulter, A63881) to remove cleaved fragments. The remaining library was eluted in nuclease-free water and carried forward to sample index PCR and double-sided size selection using SPRIselect beads (Beckman Coulter, B23318), as per the 10X Genomics Next GEM v3.1 single-cell 3' gene expression protocol. Libraries were quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Q32854) on a Qubit Fluorometer (Thermo Fisher Scientific, Q33238), and fragment distributions were assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies) before sequencing on an Illumina NovaSeq 6000 (Illumina, Inc.). Single-cell RNA sequencing (scRNA-seq) and T-cell receptor sequencing (TCR-seq) of PBMCs were performed using the 10x Genomics Chromium Next GEM Single Cell 5' v2 platform with the immune profiling kit. Library preparation followed the standard 10x Genomics protocol. Sequencing was conducted by Novogene on an Illumina NovaSeq 6000 system, with base calling performed using the Illumina RTA3 method. RNA reads were aligned to the hg38 reference genome, |
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and gene expression matrices were generated using Cell Ranger 8.0.0. TCR reads were also aligned to hg38, and clonotype/contig matrices were produced using Cell Ranger.

FFPE spinal cord sections were cut at 5µm thickness onto glass slides. Tissues were deparaffinized, decrosslinked and stained with a combination of DAPI (1:100; #62248; ThermoFisher), goat anti-IBA1 (1:250; #107159; Abcam), chicken anti-MAP2 (1:2500; ab5392; Abcam) and rabbit anti-TDP-43 (1:500; 80001-1-RR; ProteinTech). Notably, we used TrueBlack Plus Lipofuscin Autofluorescence Quencher (#23014; Biotum) according to the manufacturer's instructions. Tissues were imaged on a Nikon A1 confocal imaging system using a 20x-objective. Post imaging, spatial gene and protein expression libraries were immediately prepared according to the user guide for Visium CytAssist Spatial Gene and Protein Expression Reagent Kits (CG000494; Rev B; 10x Genomics). We employed the Visium Human Transcriptome Probe Set version 2.0 for RNA transcript detection, along with the Human FFPE Immune Profiling Panel, which includes a 35-plex CytAssist Panel of antibodies, both intracellular and extracellular, sourced from BioLegend and Abcam for protein detection. This panel also comprises four isotype controls. Final libraries were sequenced as detailed above for spatial transcriptomics. The targeted sequencing depth was 25,000 reads per tissue-covered spot for gene expression libraries, and 5,000 reads per tissue-covered spot for protein expression libraries, as recommended.

ST data was processed with 10X Space Ranger version 2.1.1. Immune profiling data was processed with Cell Ranger version 8.0.0.

## Data analysis

Code used to generate the figures of this study can be found at [https://github.com/gatelabNW/als\\_immune\\_public](https://github.com/gatelabNW/als_immune_public) and <https://github.com/KiskinisLab/TargetALS-BulkAnalysis>.

Images are analyzed and quantified with Fiji without knowledge about sample identification. A Wilcoxon rank-sum test is used by default to compute statistical significance to account for non-normal distributions by default. Exceptions to this, such as computing significance of differentially expressed gene, are described in the methods section.

All single cell and spatial transcriptomics analysis are performed with R v4.2.3 using primarily the following packages: Seurat\_5.0.1, SingleCellExperiment\_1.26.0, MAST\_1.30.0, SoupX\_1.6.2, tidyr\_1.3.1, dplyr\_1.1.4, DoubletFinder\_2.0.4, data.table\_1.16.0, tidyverse\_2.0.0, fgsea\_1.28.0, msigdb\_7.5.1, ShinyCell v2.1.0, cell2location v0.1.3. Bulk transcriptomics analysis is performed with R v4.4.2 primarily using cutadapt (v3.4), STAR (v2.7.5a), FeatureCounts (v2.0.1), DESeq2 (v1.46.0), sva (v3.54.0), edgeR (v4.4.2), and CIBERSORTx (through website).

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](#)

Spatial RNA and single-cell RNA-seq data have been deposited at GEO under accession numbers GSE288365 and GSE268995, respectively.

## 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

For the PBMC subject, age and sex are well matched except for the C9-ALS, which only has females. 10 out of the 18 controls are female, and 12 out of the 22 ALS patients are female. To account for variance due to age and sex, both are used as covariates when possible. For C9-ALS comparison which includes only females, age is used as the covariate.

For the cervical spinal cord samples, age and sex are well matched. 5 out of the 9 controls are male. 2 controls are female. 2 controls do not have sex information. 4 out of the 9 ALS samples are male. 3 ALS samples are female. 2 ALS samples do not have sex information.

### Reporting on race, ethnicity, or other socially relevant groupings

5 of the 40 PBMC samples come from non white race (35 White, 1 Asian, 1 Black, and 3 Hispanics).

Spatial cohort do not have racial information.

### Population characteristics

The PBMC samples are obtained from 22 ALS patients and 18 healthy controls. For the PBMC cohort, age spans from 44 to 76 for controls (mean=60.2) while the age of ALS patients spans from 40 to 75 (mean=59.86).

For the spatial cohort, age spans from 40 to 80 for ALS samples (mean = 59.2) while the age of controls spans from 51 to 75 (mean = 61.6).

### Recruitment

PBMCs were acquired through the Houston Methodist Neurological Institute. All subjects were free from acute infectious diseases and in good physical condition. Bulk RNAseq data were acquired from the Target ALS Data Engine. Spinal cord sections were acquired from the Target ALS Multicenter Human Postmortem Tissue Core.

### Ethics oversight

The study of PBMCs and spinal cord tissues approved by the Institutional Review Board of Northwestern University

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](https://www.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     | Both PBMC and cervical spinal cord samples analyzed in this study are unique. Every sample that passes quality check are used in the analysis. Controls and patients are matched regarding sex, age and race as closely as possible.                                                                                                                                   |
| Data exclusions | Two of the spinal cord samples are discarded due to low RNA quality, resulting in 18 total spinal cord section (9 control, 5 sALS, and 4 C9-ALS). All PBMC samples are included in the analysis. Individual cells and spatial spots are further excluded based on metrics such as mitochondrial expression and extreme UMI counts as discussed in the methods section. |
| Replication     | There are no replicates.                                                                                                                                                                                                                                                                                                                                               |
| Randomization   | <i>Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.</i>                                                                                                                                               |
| Blinding        | During the ST workflow, it was not possible to identify the samples being used because the sample IDs were obscured by the cassette used in the ST process. For scRNAseq, the samples were coded to ensure unbiased analysis. Additionally, immunohistochemistry analyses were conducted by a researcher who was blinded to the group IDs.                             |

## 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                                  |
|-------------------------------------|--------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                        |

### Methods

| n/a                                 | Involved in the study                           |
|-------------------------------------|-------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Antibodies

|                 |                                                                                                     |
|-----------------|-----------------------------------------------------------------------------------------------------|
| Antibodies used | All the relevant information on antibodies used as described in the corresponding methods sections. |
| Validation      | All primary antibodies used were validated by manufacturers or internally within our labs.          |

## Plants

|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Seed stocks           | <i>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.</i>                                                                                                                                                                                                                                                                                          |
| Novel plant genotypes | <i>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.</i> |
| Authentication        | <i>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, mosaicism, off-target gene editing) were examined.</i>                                                                                                                                                                                                                                       |