

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <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	scRNA-seq libraries were prepared using the Chromium Single Cell 3' Library & Gel Bead Kit v3 (10× Genomics) following standard protocols. Cells were encapsulated in nanoliter-scale droplets for cDNA generation with cell barcodes and UMIs, followed by sequencing on the Novaseq platform (Illumina) at ~50,000 reads per cell. Data processing was conducted with Cell Ranger v3.0.2 (10× Genomics) for demultiplexing and alignment to the GRCh38 human genome reference (v3.0.0). Flow cytometry data were collected using FACSDiva v8.01 Software (Becton Dickinson).
Data analysis	Data analysis was conducted using R 4.1.3 and Python 3. For scRNA-seq analysis, R was utilized with the dplyr package for data merging and subsetting. Seurat v4 was used for analysis, and batch correction was performed with Harmony, producing UMAP visualizations. SCTransform with Poisson distribution was applied for normalization. Visualizations in R were performed using ggplot2 v3.4.0 and ggpubr v0.5.0. In Python, analysis was conducted using pySCENIC. The codes of this study can be found in https://github.com/swoouj/TNFi-refractorness-in-AS . Flow cytometry data were further analyzed using FlowJo v10.7.2 (BD Biosciences), and finalized data visualizations were created with Prism. Figures created with BioRender.com were exported under a paid subscription with an associated publication license.

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](#)

The published article includes all datasets generated or analyzed as part of this study. Individual source data are provided with the associated figures where applicable, in accordance with the data sharing agreement authorized by Professor Jeong Seok Lee (Korea Advanced Institute of Science and Technology, KAIST). Raw flow cytometry source files are available upon reasonable request. Source data are included with this paper. All scRNA-seq data have been uploaded to GSE277117 and GSE277791, with access codes for reviewers to be provided separately.

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

All sex data refers to sex and not gender and is presented in Supplementary Data 1. This study involved 255 participants in total (198 males and 57 females), with an age range of 18 to 78 years. Sex was self-reported by the participants.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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

Sample size was determined based on availability, with the aim of including as many samples as possible to ensure comprehensive analysis. The study included scRNA-seq performed on PBMC samples from a total of 22 ankylosing spondylitis (AS) patients, comprising 12 TNF inhibitor responders and 10 non-responders. For hypothesis validation, two cohorts were utilized: one for mRNA measurement through qPCR and bulk RNA-seq with 18 samples, and another for protein-level validation using flow cytometry and ELISA, encompassing 117 samples. Overall, the study analyzed a large cohort of 167 AS patient samples across different experimental setups, as detailed in the text and figures.

Data exclusions

No data exclusions were made; all available samples were included in the analyses.

Replication

Due to the constraint of conducting all experiments on human specimens with restricted availability, it was not feasible to replicate the identical experiment within the same subject. We endeavored to incorporate the maximum number of specimens feasible in each experimental iteration, ensuring that the cases and controls represented biologically analogous samples. Furthermore, we executed comprehensive orthogonal validation experiments to corroborate significant findings through supplementary assays.

Randomization

Samples were allocated based on clinical disease category.

Blinding

No blinding. Prospective observational study; Patients fulfilling the 2009 Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axial spondyloarthritis and treated with TNFi.

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		Methods	
n/a	Involved in the study	n/a	Involved in the study
☐	☒ Antibodies	☒	☐ ChIP-seq
☒	☐ Eukaryotic cell lines	☐	☒ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☒	☐ Animals and other organisms		
☒	☐ Clinical data		
☒	☐ Dual use research of concern		
☒	☐ Plants		

Antibodies

Antibodies used

For flow cytometry (FACS) experiments, we used the following antibodies: anti-CD3 (BV605; BD Biosciences), anti-CD4 (BV510; BD Biosciences), anti-CD14 (PE-eFluor610; eBioscience), anti-CD19 (PE-eFluor610; eBioscience), anti-CD45RA (APC-Cy7; BioLegend), anti-CCR6 (BV421; BD Biosciences), and anti-IL-1R1 (PE; R&D). Stained cells were permeabilized using the Foxp3 staining buffer kit, and intracellular markers were subsequently stained with anti-RORyt (Alexa Fluor 488; eBioscience), anti-T-bet (PE-Cy7; BD Biosciences), anti-TNF (APC; BD Biosciences), anti-IFN- γ (Alexa Fluor 488; eBioscience or APC-R700; BD Biosciences), and anti-IL-17A (BV711; BioLegend). For in vitro TNF inhibition experiments, Adalimumab and an IgG1 isotype control were obtained from Invitrogen.

Validation

All antibodies were purchased from commercial suppliers, including BD Biosciences, BioLegend, ThermoFisher, Sigma, and eBioscience, with validation data and applicable citations available on the product listings for each antibody (see individual catalog numbers). Antibodies that have previously been validated in the literature were prioritized, and each was used at the specified dilution or according to the manufacturer's specifications.

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, mosaicism, 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

For the AIF-1 experiment, cryopreserved PBMCs were thawed, resuspended in RPMI 1640 containing 5% fetal bovine serum and 2 mmol/L L-glutamine, and rested for 12 hours at 37°C. PBMCs were then cultured in the presence or absence of 100 ng/ml AIF-1 (Cloud-Clone Corp., Katy, TX) for 3 days and stimulated with anti-CD3 antibody (0.1 mg/ml OKT-3; eBioscience) and

anti-CD28 antibody (1 mg/ml; BD Biosciences). For T cell experiments, CD4⁺ T cells were isolated from the flow-through of the PBMCs using the CD4⁺ T Cell Isolation Kit (Miltenyi Biotec). Isolated CD4⁺ T cells were stimulated with Dynabeads Human T-Activator CD3/CD28 (ThermoFisher Scientific, Waltham, MA) at a 4:1 cell-to-bead ratio. Brefeldin A (GolgiPlug, BD Biosciences) and monensin (GolgiStop, BD Biosciences) were added after 1 hour, and cells were further incubated for 5 hours. Cells were stained using Live/Dead fixable cell stain to exclude dead cells and subsequently stained with fluorochrome-conjugated antibodies for surface markers.

Instrument

Data were collected using an LSR II instrument with FACSDiva software (BD Biosciences).

Software

FlowJo software v10.7.2 (Treestar, San Carlos, CA) was used for data analysis.

Cell population abundance

The gating strategy for CD4 T cells from PBMCs is illustrated in Extended Data Fig. S8a, with specific abundance details provided in Fig. 7 and Extended Data Figs. S8 and S9.

Gating strategy

A general gating strategy is shown in Extended Data Fig. S8a. Initially, a broad gate was applied to capture lymphocytes using FSC-A/SSC-A. Singlets were identified with an FSC-H/FSC-A gate, followed by SSC-H/SSC-A gating. Live cells were gated by excluding CD14, CD19, and dead cells (Live/Dead stain). T cells were identified by CD3 surface staining, and CD4⁺ T cells were further gated. CD45RA⁻ memory CD4⁺ T cells were identified, followed by IL-17A and IFN- γ staining to calculate the fractions of IL-17A⁺, IFN- γ ⁺ single-positive, and double-positive cells.

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