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 *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 *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	Annotated repertoire sequences were obtained from Cogent™ NGS Immune Profiler Software (Takara) by using raw data.
Data analysis	WES: SEQ Platform v8.6.0 Over-representation analysis (ORA): WebGestalt (WEB-based Gene SeT AnaLysis Toolkit) Immune repertoire analys.is: MGT/V-QUEST version: 3.6.3 and Immunarch 1.0.0 Flow cytometry: FlowJo 10.8.1 and BD FACSDiva™ Software Western blot band analysis: Image Lab 6.1 (BioRad) Cytokine measurements: LEGENDplex™ Oognit

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

Bulk RNA-seq datasets have been deposited in NCBI Gene Expression Omnibus (GEO) under accession code GSE256535. Immune repertoire sequencing data is available in figshare.

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 patients in our study were female. We used sex-matched healthy controls whenever possible. Gender was not a factor in our study design.
Reporting on race, ethnicity, or other socially relevant groupings	All patients and healthy controls are of Turkish origin.
Population characteristics	All clinical details of patients were extensively described in Supplementary Text of the manuscript.
Recruitment	Patients were identified through collaborating clinicians in Turkish hospitals. Healthy controls were also from the same hospitals.
Ethics oversight	Hacettepe University Ethics Committee.

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	Three patients with the indicated variant (IKKα G167R) were identified. Given the small sample size, a formal sample size calculation was not performed. At least 3 healthy controls were included for comparison, which provided sufficient statistical power for meaningful analysis.
Data exclusions	No data were excluded from the study.
Replication	Whenever possible, cells from all patients were used as distinct biological replicates. However, due to limited sample availability, some experiments were performed with only one or two patient samples. At least 3 healthy controls were included for comparison. Due to the limited number of patient cells, the Treg function assay was performed only once. All attempts at replication were successful.
Randomization	IKK alpha deficiency is a very rare condition and thus, no randomization was applicable.
Blinding	Blinding was not relevant to this study.

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 name Cat number Company Assay Conjugate
IKKα A19694 ABclonal Western Blotting
IKKα 621401 BioLegend Western Blotting
NIK 4994 CST Western Blotting
NF-κB2 p100/p52 695902 BioLegend Western Blotting
Phospho-NF-κB2 p100 (Ser866/870) 4810 CST Western Blotting
FLAG F9291 Sigma Western Blotting
β-Actin 4970 CST Western Blotting
GAPDH 2118 CST Western Blotting
α-Tubulin ab7291 Abcam Western Blotting
CD19 363010 BioLegend Flow cytometry APC-Cy7
CD19 302206 Biolegend Flow cytometry FITC
CD27 356410 BioLegend Flow cytometry APC
IgD 348225 BioLegend Flow cytometry BV421
IgM 314506 BioLegend Flow cytometry FITC
CD38 356606 BioLegend Flow cytometry APC
CD21 354908 BioLegend Flow cytometry PERCP
CD3 317318 317308 BioLegend Flow cytometry APC, PE
CD8 345774 BD Flow cytometry PerCP
CD4 300546 BioLegend Flow cytometry BV510
CD8 344748 BioLegend Flow cytometry BV421
CD56 362508 BioLegend Flow cytometry PE
CD161 339926 BioLegend Flow cytometry PB
TCRV α7.2 351728 BioLegend Flow cytometry AF700
CD25 302604 BioLegend Flow cytometry FITC
FOXP3 320108 BioLegend Flow cytometry PE
CXCR5 356914 BioLegend Flow cytometry FITC
CD31 303106 BioLegend Flow cytometry PE
CD16 360706 Biolegend Flow cytometry APC
CD16 302017 Biolegend Flow cytometry APC-Cy7
ICOSL 309403 Biolegend Flow cytometry PE
CD69 310906 Biolegend Flow cytometry PE
CD45RA 304112 Biolegend Flow cytometry APC
IFNGamma 69007 Proteintech Flow cytometry CL488
ICOS 313508 Biolegend Flow cytometry PE

Validation

Validation of all antibodies was provided on manufacturer's website with their references used in published studies.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T, ATCC
Authentication	HEK293T cells were not authenticated.
Mycoplasma contamination	The cell lines were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	NA

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	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	Cells were gated based on FSC and SSC first. After live/dead cell determination, live cells were gathered in lymphocyte gate for subpopulation phenotyping, activation, proliferation and intracellular cytokine production analyses.

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