Data in Brief 59 (2025) 111359

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

A dataset on NFKB1/NFKB2 knockout Jurkat cells and primary CD4+ T cells generated by CRISPR/Cas9 mediated gene disruption

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ARTICLE INFO

Article history:
Received 13 December 2024
Revised 14 January 2025
Accepted 27 January 2025
Available online 31 January 2025

Dataset link: A dataset on NFKB1/NFKB2 knockout Junket cells and primary CD4+ T cells generated by CRISPR/Cas9 mediated gene disruption (Original data)

Keywords:
NFKB1
NFKB2
Jurkat cells
CD4+ T cells
CRISPR/Cas9

ABSTRACT

Specific biological roles of non-canonical nuclear factor-kappa B (NF-kB) signaling in human T cells remain unclear. Therefore, we genetically modified Jurkat leukemic cells using the clustered regularly interspaced palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) technology to better understand the NF-kB signaling mechanisms. IKAROS family zinc finger-2 levels were downregulated in *NFKB2*-knocked-out cells but upregulated in *NFKB1*-knocked-out cells without stimulation. To further understand the biological roles of NF-kB signaling in CD4+ T cells, NFKB1/NFKB2 loci of human primary CD4+ T cells were genetically edited using the CRISPR/Cas9 technology. Quantitative polymerase chain reaction and fluorescence-activated cell sorting revealed the similar phenotypes and gene expression profiles of *NFKB1/NFKB2*-knocked-out human CD4+ T cells at a resting state.

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https://doi.org/10.1016/j.dib.2025.111359

Specifications Table

Subject	Health Sciences, Medical Sciences & Pharmacology
Specific subject area	Immune engineering for the understating CD4+ T cell biology.
Type of data	Analyzed images, Analyzed graphs
Data collection	Immunophenotype of engineered T cells and T cell-lines were analyzed by flow cytometry. Genotype of edited cells were analyzed by sanger sequencing. Gene expression profiles were analyzed by real-time PCR. Protein expressions were quantified by western blot.
Data source location	Immunophenotype of engineered T cells and T cell-lines were analyzed by flow cytometry. Genotype of edited cells were analyzed by sanger sequencing. Gene expression profiles were analyzed by real-time PCR. Protein expressions were quantified by western blot.
Data accessibility	Repository name: Mendeley Data Data identification number: 10.17632/w24t4trkv5.2 Direct URL to data: https://data.mendeley.com/datasets/w24t4trkv5/2
Related research article	Y. Sato, E. Osada, Y. Manome, Non-canonical NFKB signaling endows suppressive function through FOXP3-dependent regulatory T cell program, Heliyon 9(12) (2023) e22911. https://doi.org/10.1016/j.heliyon.2023.e22911.

1. Value of the Data

- We have developed the methods for NFKB1/NFKB2 knockout by CRISPR/Cas9.
- Our CRISPR/Cas9 gene editing strategy provides successful knockout both in cell lines and primary cells.
- It would be possible to generate NFKB1/NFKB2 knockout cells other than cell lines and primary cells we have generated.

2. Background

Nuclear factor-kappa B (NF-kB) signaling is crucial for cell proliferation, survival, differentiation, and apoptosis. Recent studies have demonstrated the importance of canonical NF-kB signaling in T cells and revealed the roles of non-canonical signaling in regulatory T cells of humans [1] and mice [2]. Owing to recent advances in the clustered regularly interspaced palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) gene editing technology, gene disruption and correction can be evaluated in T cells via target gene integration using both viral (adeno-associated virus-6) and non-viral approaches [3,4]. We hypothesized that NF-kB signaling plays crucial roles in effector T cells. To verify this, we performed *NFKB1/NFKB2* knockout in effector T cells using the CRISPR/Cas9 technology.

3. Data Description

NFKB1 and *NFKB2* were knocked-out in Jurkat cells using the CRISPR/Cas9 technology (Fig. 1A). To confirm the knockout, we established a system in which the NFKB1 and NFKB2 loci were replaced with the enhanced green fluorescent protein (EGFP) and performed fluorescence-activated cell sorting (FACS; Fig. 1B). Examination of the knockout strains via western blotting and FACS confirmed the decrease in NFKB1 and NFKB2 levels (Fig. 1C–D). Notably, forkhead box P3 (FOXP3) levels in Jurkat cells were unaffected by *NFKB1/NFKB2* knockout. Raw FCS files and MFI values of Jurkat cells were archived at Mendeley Data (FCS files for FOXP3 staining) [5]. Integration of the EGFP cassette was verified via in–out polymerase chain reaction (PCR) and Sanger sequencing (Fig. 2A–B). Agarose gel (In-out PCR.tif) and immunoblot images (Western blot.tif) of Jurkat cells were archived at Mendeley Data [5].

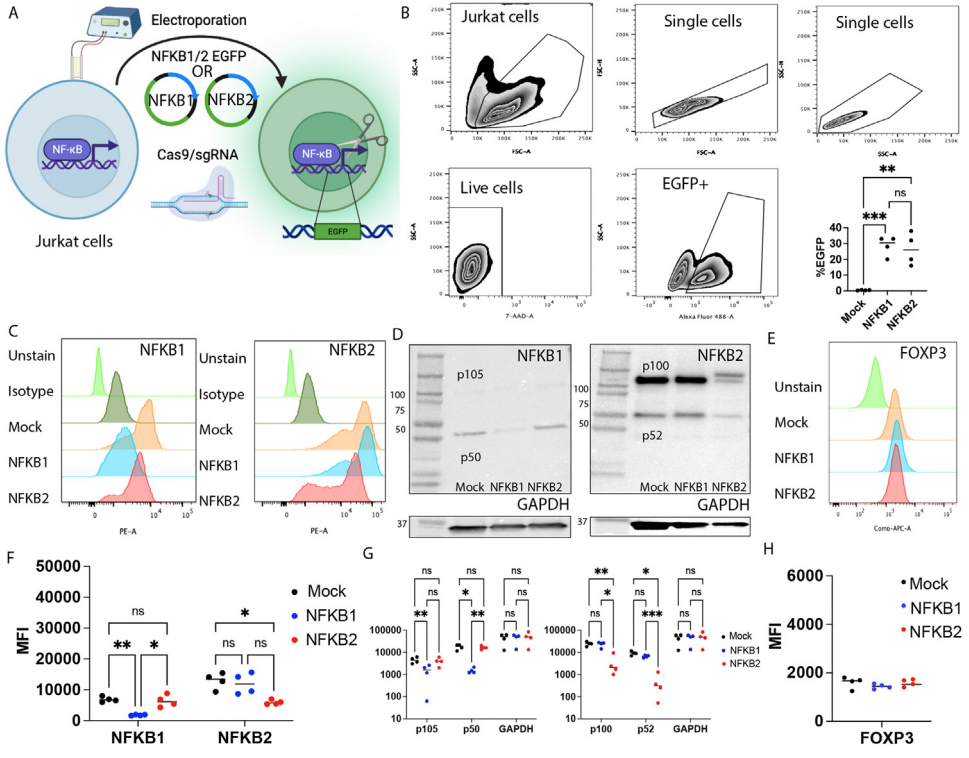

Fig. 1. Nuclear factor-kappa B (NFKB)-1/NFKB2 knockout in Jurkat cells using the clustered regularly interspaced palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) technology. (**A**) NFKB1/NFKB2 editing process in Jurkat cells. (**B**) Enhanced green fluorescent protein (EGFP) knock-in cassette was successfully inserted at the NFKB1/NFKB2 loci. NFKB1/NFKB2 knockout was confirmed via fluorescence-activated cell sorting (FACS; **C and F**) and western blotting (**D and G**). (**E and H**) Forkhead box P3 (FOXP3) levels in *NFKB1/NFKB2*-knocked-out Jurkat cells were determined via FACS.

Next, we determined the levels of FOXP3 and regulatory T cell (Treg)-related molecules (cytotoxic T-lymphocyte-associated protein-4, inducible T cell co-stimulator, IKAROS family zinc finger 2 [IKZF2], tumor necrosis factor receptor superfamily member 18, and T cell immunoreceptor with Ig and ITIM domains). mRNA expression was evaluated via quantitative PCR (qPCR; Fig. 2C), and protein expression was analyzed via FACS (Fig. 2D). Raw FCS files and MFI values of Jurkat cells were archived at Mendeley Data (FCS files for FOXP3 related molecules) [5]. Only *IKZF2* mRNA levels differed between the wild-type and knockout strains, with no significant differences observed in the protein levels in both strains.

To closely mimic the in vivo conditions, we knocked-out both *NFKB1* and *NFKB2* in CD4+ T cells isolated from healthy donors (Fig. 3A). Upon incorporating an EGFP/tdTomato cassette, we identified a double-positive population, confirming the knockout of both *NFKB1* and *NFKB2* (Fig. 3B). Subsequently, *NFKB1* and *NFKB2* and integration of the EGFP/tdTomato cassette were verified using techniques similar to those used for Jurkat cells (Fig. 3C–D). Agarose gel images of CD4+ T cells were archived at Mendeley Data (In-out PCR.tif) [5]. No significant changes were observed in the expression levels of FOXP3 in the knockout cells (Fig. 3E–F). Raw FCS files and MFI values of CD4+ T cells were archived at Mendeley Data (FCS files for FOXP3 staining) [5]. Immunoblot images of CD4+ T cells were archived at Mendeley Data (Western blot.tif) [5].

Similar to that in the Jurkat cells, integration of the EGFP cassette was verified in the CD4+ T cells via in–out PCR and Sanger sequencing (Fig. 4A–B). We also analyzed the expression levels of FOXP3 and Treg-related molecules (cytotoxic T-lymphocyte-associated protein-4, inducible T

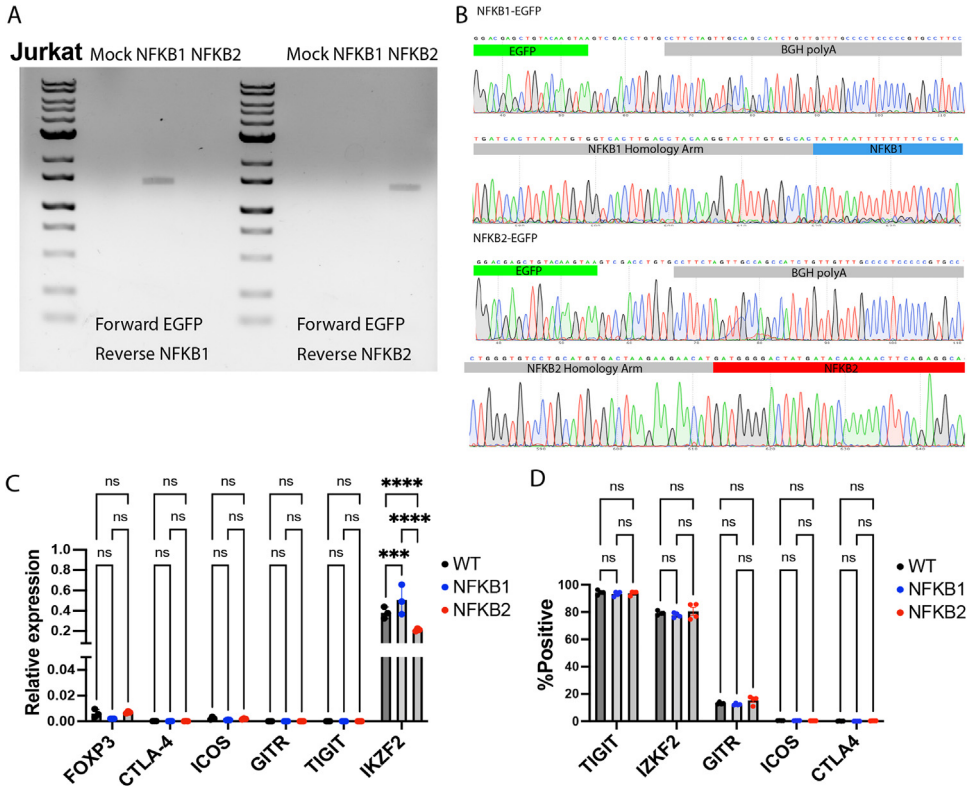

Fig. 2. Editing of the NFKB1/NFKB2 loci in Jurkat cells using the CRISPR/Cas9 technology. **(A)** Homologous recombination of EGFP was confirmed via in–out polymerase chain reaction (PCR). **(B)** Sanger sequencing of in–out PCR products confirmed the integration of EGFP at the NFKB1/NFKB2 loci. **(C)** Gene expression profile of the edited Jurkat cells (n = 3). **(D)** FACS analysis of the edited Jurkat cells (n = 4).

cell co-stimulator, IKZF2, tumor necrosis factor receptor superfamily member 18, and T cell immunoreceptor with Ig and ITIM domains). mRNA levels were determined via qPCR (Fig. 4C), and protein levels were determined via FACS (Fig. 4D). Raw FCS files and MFI values of CD4+ T cells were archived at Mendeley Data (FCS files for FOXP3 related molecules) [5]. No differences in mRNA and protein levels were observed between the wild-type and knockout strains.

4. Experimental Design, Materials and Methods

4.1. Cell lines and isolation

Jurkat cells were provided by the Japan National Institute of Biomedical Innovation, Health and Nutrition, Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). Peripheral blood mononuclear cells (PBMCs) isolated from healthy subjects were purchased from Precision for Medicine (n = 4) and Lonza (n = 2). Biological profiles of the PBMC donors are presented in Supplementary Table 1. Teffs were enriched from PBMCs using the EasySep Human CD4+ Isolation Kit (STEMCELL Technologies). Purity of enriched Teffs (%CD4) was >95% immediately after isolation.

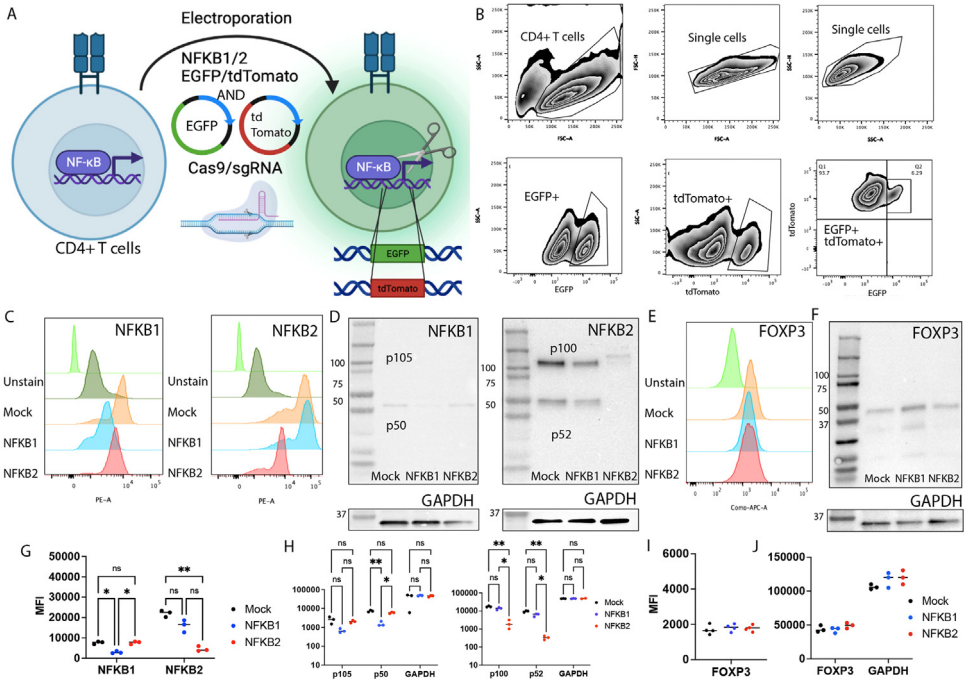

Fig. 3. Bi-allelic *NFKB1/NFKB2* knockout in primary CD4+ T cells. (**A**) NFKB1/NFKB2 editing process in CD4+ T cells. (**B**) EGFP/tdTomato knockout cassette was inserted at the NFKB1/NFKB2 loci. *NFKB1/NFKB2* knockout was confirmed via FACS (**C and G**) and western blotting (**D and H**). FOXP3 levels in *NFKB1/NFKB2*-knocked-out effector T cells (Teffs) were analyzed via FACS (**E and I**) and western blotting (**F and J**).

4.2. Cell culture

Cell lines were cultured in the Roswell Park Memorial Institute medium (Life Technologies) supplemented with 10% fetal bovine serum (Gibco) and routinely tested for *Mycoplasma* using the MycoAlert Quick Mycoplasma Detection Kit (Lonza). No *Mycoplasma* contamination was detected throughout the study. Teffs were cultured in the ImmunoCult XF T-cell expansion medium (STEMCELL Technologies) in the presence of 100 U/mL of recombinant human-IL-2 (Peprotech). ImmunoCult Human CD3/CD28/CD2 T-cell activator (STEMCELL Technologies) was added on days 0 (activation) and 10–13 (re-stimulation), according to the manufacturer's protocol.

4.3. CRISPR/Cas9

Freshly isolated Teffs were activated for 3–5 d using the ImmunoCult Human CD3/CD28/CD2 T-cell activator (STEMCELL Technologies). Then, a ribonucleoprotein (RNP) complex was constructed by incubating 15 μg of Cas9 protein (Alt-R S.p. Cas9 Nuclease V3; Integrated DNA Technologies) with 8 μg of synthetic single guide RNA (Synthego) targeting NFKB1 with the sequence, 5′-2′O-methyl [OMe](U(phosphorothioate [ps])A(ps)U(ps))AUAGAUCUGCAACUAUG, or NFKB2 with the sequence, 5′-2′OMe(U(ps)C(ps)C(ps))CUCGUAGUUACAGAUCU, followed by incubation with an 80-mer SpCas9 scaffold at room temperature for 15 min. This ribonucleoprotein complex, together with 5 μg of donor plasmid containing homologous arms and EGFP or tdTomato expression cassette under the control of the PGK promoter (Genscript), was transduced into Teffs using the NEPA21 Electroporator (Nepagene). The electroporation conditions are described in Supplementary Table 2. Subsequently, the EGFP/tdTomato-positive population was

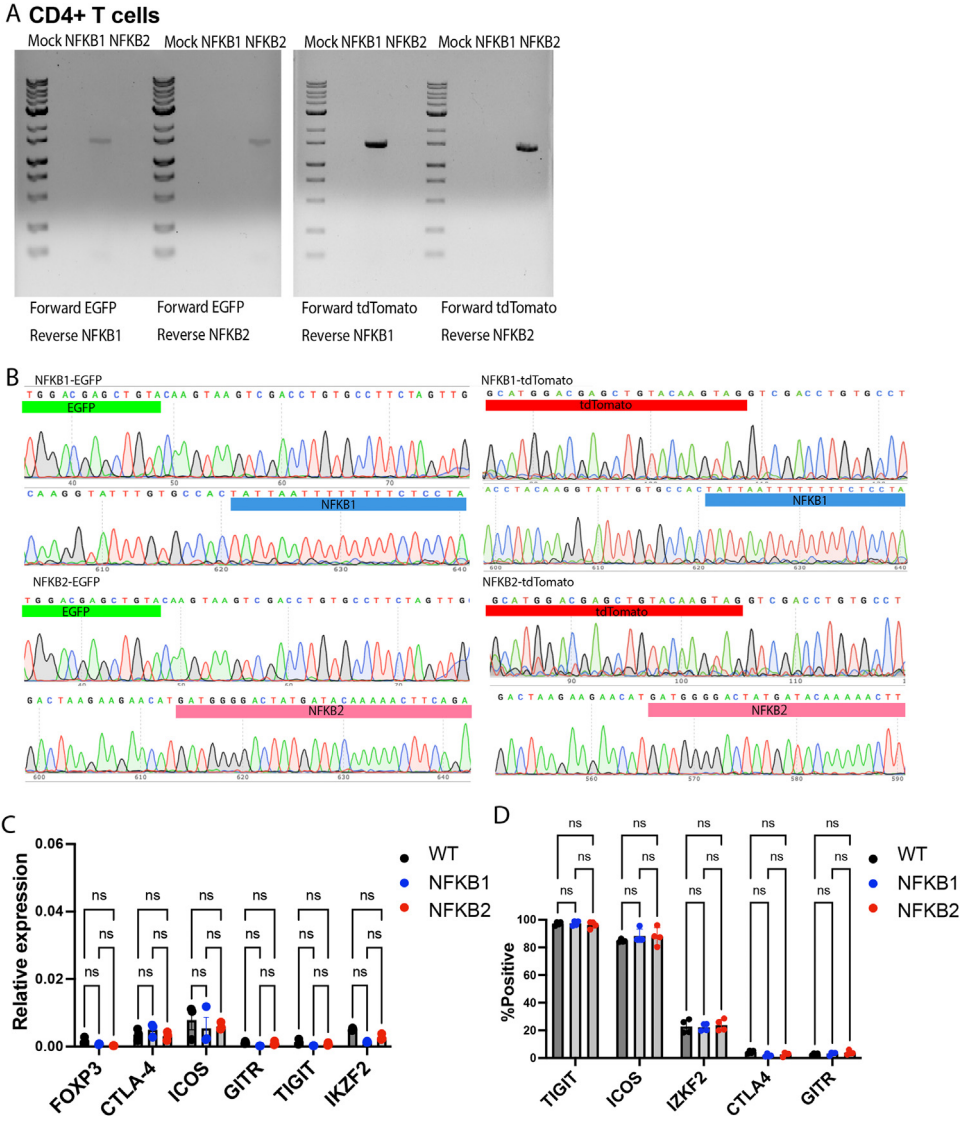

Fig. 4. Biallelically editing of the NFKB1/NFKB2 loci in CD4+ T cells using the CRISPR/Cas9 technology. **(A)** Homologous recombination of EGFP/tdTomato was confirmed via in–out PCR. **(B)** Sanger sequence of in–out PCR products confirmed the integration of EGFP/tdTomato at the NFKB1/NFKB2 loci. **(C)** Gene expression levels of regulatory T cell (Treg)-related molecules (n = 3). **(D)** FACS analysis of Treg-related molecules (n = 4).

sorted via FACS 4–5 d after transduction. After sorting, the edited cells were expanded using the CD3/CD28/CD2 T-cell activator and harvested 10–13 d after restimulation.

4.4. PCR

Genomic DNA was isolated from the edited cells using the DNeasy kit (QIAGEN). The following primer sequences were used for in–out PCR: *EGFP*, forward primer 5′-CACCGGTCGCCACCA

TGGTGAGCAAGGGCGAGGA-3′; tdTomato, forward primer 5′-ATGGTGAGCAAGGGCGAGGA-3′; *NFKB1*, reverse primer 5'-GCAGCAGAGAGCTGAAATGA-3′; *NFKB2*, reverse primer 5′-GGGCAGTAGGTACATGTGTGA-3′. PCR was performed using the KOD One PCR Master Mix (TOYOBO) with the T100 Thermal Cycler (Bio-Rad). Finally, PCR products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific).

4.5. qPCR

Total RNA was extracted from 1×10^6 Teffs using the RNeasy Plus Mini Kit (QIAGEN). Then, RNA (100 ng) was reverse-transcribed into cDNA using Superscript IV VILO (Thermo Fisher Scientific). qPCR was performed using the TaqMan Gene Expression Assay and TaqMan Universal Master Mix II (Thermo Fisher Scientific) with QuantStudio 5 Real-Time PCR (Thermo Fisher Scientific). Gene expression was calculated using ΔCT, where $\Delta CT = CT_{target} - CT_{housekeeping}$. Hypoxanthine-guanine phosphoribosyltransferase was used as an internal control. TaqMan probes are listed in Supplementary Table 1.

4.6. Western blotting

Next, immunoblotting was performed to analyze NFKB1/NFKB2 expression. Briefly, total protein was extracted from the cell lines and primary cells using the EzRIPA lysis kit (ATTO). Then, protein concentration was measured using the Pierce BC protein assay kit (Thermo Fisher Scientific). Proteins (5 µg) were loaded into each lane of a 4–20% gradient sodium dodecyl sulfate-polyacrylamide gel (Bio-Rad), subjected to electrophoresis, and transferred to a polyvinylidene fluoride membrane (Bio-Rad) using the Trans-Blot Turbo Transfer System (Bio-Rad). After incubation with primary antibodies (1:1000) overnight, the membrane was incubated with the anti-rabbit horseradish peroxidase-conjugated IgG secondary antibodies (1:1000; Cell Signaling Technologies) for 1 h. Then, a chemiluminescent substrate (Clarity Western ECL Substrate; Bio-Rad) was added to the membrane, and the signals were visualized using the ChemiDoc Touch Imaging System (Bio-Rad). Protein expression was quantified using the ImageJ software v.1.54. The antibodies are listed in Supplementary Table 2.

4.7. Flow cytometry

Teffs were resuspended in the FACS buffer (phosphate-buffered saline supplemented with 0.5% bovine serum albumin and 2 mM EDTA) and stained with a cocktail of surface-staining antibodies for 30 min. After surface staining, intracellular staining was performed using the Foxp3/Transcription Factor Staining buffer set (eBioscience) with an intracellular staining antibody cocktail for 30 min. For the unconjugated antibodies (NFKB1 and NFKB2), phycoerythrin-conjugated anti-rabbit IgG antibodies were added as the secondary antibodies and incubated for 30 min. Data were acquired using FACSAria III (BD Biosciences) and analyzed using the FlowJo 10.7 software (FlowJo LLC). The antibodies are listed in Supplementary Table 3.

4.8. Statistical analyses

GraphPad Prism Software v.9.3.1 (GraphPad Software) was used for statistical analysis. One-way analysis of variance, followed by Tukey's multiple-comparison test or Kruskal–Wallis test, was used to compare multiple conditions. Statistical significance was set at $P < 0.05$. Data are represented as the mean $\pm$ standard deviation.

Limitations

Not applicable.

Ethics Statement

This study was performed in accordance with the Declaration of Helsinki. PBMC from normal healthy donors were purchased from a commercial source and it was declared that the cells were isolated after obtaining permission for their use in research applications by informed consent or legal authorization. Institutional Review Board (IRB) of the Jikei University School of Medicine granted the exemption of research use of deidentified PBMCs provided from commercial source isolated after obtaining permission for their use in research applications by informed consent or legal authorization. The use of deidentified cell lines was also considered exempt and do not require IRB review of the Jikei University School of Medicine.

Data Availability

A dataset on NFKB1/NFKB2 knockout Junket cells and primary CD4+ T cells generated by CRISPR/Cas9 mediated gene disruption (Original data) (Mendeley Data).

CRediT Author Statement

Erika Osada: Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing; **Yoshinobu Manome:** Project administration, Supervision, Validation, Writing – review & editing; **Yohei Sato:** Data curation, Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Acknowledgements

We express our gratitude to Dr. Toshiko Yamazawa (Core Research Facilities, Jikei University School of Medicine) and Mr. Kiyoshi Hayakawa (NEPA gene) for their technical assistance with electroporation. Additionally, we extend our thanks to Dr. Saishu Yoshida and Dr. Kiyotsugu Yoshida (Department of Biochemistry, Jikei University School of Medicine) for granting us access and providing technical support for the BD Aria III. This work was supported by JSPS KAKENHI Grant Number 22K20868.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2025.111359.

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