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ORIGINAL ARTICLE

Defects in aminoacyl-tRNA synthetase cause partial B and T cell immunodeficiency

Ju A. Shim[1,2] · Yuna Jo[1,2] · Hyunju Hwang[3] · So Eun Lee[1,2,4] · Dahyeon Ha[1,2,4] · Jun Hwa Lee[1,2,4] · Jayoung Kim[2,4] · Parkyong Song[2,4] · Dongjun Lee[2,4] · Changwan Hong[1,2,4]

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Abstract

Aminoacyl-tRNA synthetases (ARSs) are emerging as important regulators in various immune diseases; however, their roles in immune cells remain unclear. In this study, using alanyl-tRNA synthetase (AARS) mutant (*sti*) mice with neurodegenerative disorder, we investigated the effect of translational fidelity in immune cells. Dysfunctional AARS caused disorders in immune cell responses and cellularity. The impairment was caused by dampened TCR signaling than cytokine signaling. Therefore, *sti* mutant inhibits TCR signaling, impeding T cell survival and responses. B cell numbers were decreased in *sti* mice. Despite low B cell cellularity, serum IgM, IgA, and IgE levels were higher in *sti* mice than in wild-type mice. Misacylation of ARS and the consequent translational infidelity induce disturbances in signaling pathways critical for immune cell survival and responses. Our findings provide a novel mechanism by which translational fidelity might play a critical role in cellular and humoral immune responses.

Keywords TCR signaling · T cell homeostasis · Protein misfolding disorder · Protein aggregation · Neurological disorder

Introduction

During protein synthesis, aminoacyl-tRNA synthetases (ARSs) catalyze the aminoacylation of transfer (t)RNAs [1]. When a tRNA is covalently linked to the cognate amino acid, it is released from the enzyme and transported into the ribosome–mRNA complex by elongation factor-Tu (EF-Tu) [2] or elongation factor 1 alpha (eEF1α) [3]. The polypeptide chain is continuously elongated until a stop codon occurs [4]. When then polypeptide chains are fully synthesized, they are folded and assembled into a functional protein with the aid of chaperones [5]. When a tRNA is mischarged with a non-cognate amino acid, ARSs induce the de-acylation of the tRNA to prevent mistranslation [6]. Alanyl-tRNA synthetase (AARS) catalyzes the de-acylation of Ser-tRNA^{Ala} and Gly-tRNA^{Ala} [7]. Impaired editing activities result in the generation of mis- and/or unfolded proteins, which can accumulate in the neuronal cytoplasm, resulting in neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease [8]. Dominant mutations in tRNA modification enzymes and proteins involved in tRNA charging can induce diseases, such as type 2 diabetes mellitus, breast cancer, and Dubowitz syndrome. tRNA mutations can induce neuronal disorders, such as mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes syndrome, myoclonus epilepsy with ragged-red fibers syndrome, and ataxia [9].

One would expect that mutations perturbing tRNA-mediated translation would affect all cells in the body, as all cells carry the same genetic information. Nevertheless, it has been demonstrated that such mutations predominantly affect the central nervous system, pancreas, and muscle cells, and

Ju A. Shim and Yuna Jo have contributed equally to this work.

✉ Changwan Hong
chong@pusan.ac.kr

1 Department of Anatomy, Pusan National University School of Medicine, Yangsan 50612, Republic of Korea

2 Department of Convergence Medical Science, Pusan National University School of Medicine, Yangsan 50612, Republic of Korea

3 Asan Institute for Life Sciences and Department of Convergence Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, Republic of Korea

4 PNU GRAND Convergence Medical Science Education Research Center, Pusan National University School of Medicine, Yangsan 50612, Republic of Korea

deficiency in ARS-mediated proofreading function induces cardioproteinopathy through perturbed cardiac homeostasis [10]. Further, ARSs play important non-canonical roles in immune regulation and inflammatory responses [11–14]. Therefore, we hypothesized that tRNA-related mutation would affect other types of cells, especially, immune cells. To address this hypothesis, in this study, we utilized *sti* mice, which harbor a point mutation in the gene region encoding the AARS editing domain [15]. Moreover, AARS mutation has been associated with the induction of skin inflammation, which is an immune-mediated disease. Although *sti* mice typically have neuronal disorders, such as loss of cerebellar Purkinje cells, ataxia, and follicular dystrophy [15], the effects of AARS mutation in other tissue and cell types have not been fully clarified.

In this study, we first assessed immune cell cellularity in primary and peripheral lymphoid organs. We found that cellularity was dramatically reduced in *sti* mice compared to their wild-type (WT) counterparts. Further, we demonstrate that tRNA misacylation induces not only loss of cerebellar Purkinje cells and cardiomyocytes, but also loss of immune cells. Surprisingly, T cell responses were notably suppressed and B cell responses were disturbed in *sti* mice, suggesting that *sti* mice may be more susceptible to infectious diseases or allergic diseases.

Materials and methods

Animals

C57BL/6 (B6) mice were obtained from Orient Bio, Korea. RAG1$^{-/-}$ and congenic C57BL/6.SJL (CD45.1) mice of both sexes were obtained from the Jackson Laboratory. *Aars*$^{sti/sti}$ mice (*sti* mice) were described and provided from Dr. Jeong-Woong Lee at the Korea Research Institute of Bioscience and Biotechnology (KRIBB) [15]. Animal experiments were performed using young (6–16 weeks of age) and old (18–25 weeks of age) mice of both sexes. Animal experiments were approved by the Pusan National University (PNU) Institutional Animal Care and Use Committee (PNU-2020–2714) and the KRIBB Animal Care and Use Committee (KRIBB-AEC-18156). All mice were cared for in accordance with the guidelines set forth by the PNU School of Medicine and the KRIBB.

Flow cytometry

Cells were fixated and permeabilized using a Foxp3 Transcription Factor Staining Buffer kit (eBiosciences/Thermo Fisher Scientific) according to the manufacturer's instructions. Antibodies targeting the following proteins were used for staining: Foxp3 (FJK-16), TCRβ (H57- 597), IL-7Rα (A7R34), CD45.1 (Ly5.1; A20), CD45.2 (Ly5.2; 104), CD44 (IM7), and IL-15Rα (DNT15Rα) from eBiosciences/Thermo Fisher Scientific; γc (4G3), CD4 (GK1.5), CD25 (PC61.5), pSTAT5 (pY694), CD122 (TMb1), and CD8α (53–6-7) from BD Biosciences; B220(RA3-6B2), TCRγ/δ (GL3), PD-1 (RMP1-30), and CXCR5 (L138D7) from BioLegend. Fluorochrome-conjugated CD1d tetramers loaded with PBS-567 and unloaded controls were obtained from the NIH Tetramer Facility (Emory University, Atlanta, GA). For Foxp3 staining, a Foxp3 intracellular staining buffer set (eBiosciences/Thermo Fisher Scientific) was used according to the manufacturer's instructions. Flow-cytometric data were acquired using a FACS Canto II (BD Biosciences) or Attune NxT (Invitrogen) instrument. Live cells were identified based on forward scatter exclusion of dead cells stained with propidium iodide. Data were analyzed using FlowJo version 10 (TreeStar).

Calculation of cell numbers

Single-cell suspensions were prepared from lymphoid organs of the indicated mice. Viable cell numbers were enumerated using Trypan Blue exclusion assay with a hemocytometer. The viable cell concentration, the total cell count and specific cell count in the original sample were calculated using the formula:

Cells/ml (C) = (total cell counted * dilution factor * 10^4/ml)/number of squares counted,

Total cells in sample (T) = C * total sample volume,

X cell numbers = (T * % of *X* cells in FACS plot or histogram)/100.

Assessment of intracellular pSTAT5 levels

To determine the extent of cytokine-induced STAT5 phosphorylation, LN T cells from WT and *sti* mice were treated with IL-2, IL-7, or IL-15 (PeproTech) for 30 min, and phospho-STAT5 (pSTAT5) levels were determined after methanol/acetone fixation by intracellular staining, as previously described [16].

In vivo homeostatic proliferation assay

Naïve CD44^{lo} LN T cells were harvested from congenic CD45.1$^+$ B6 mice and CD45.2$^+$ *sti* mice by cell sorting using BioMag goat anti-mouse IgG beads (Qiagen). CD45.1$^+$ WT and CD45.2$^+$ *sti* T cells labeled with CFSE fluorescent dye (Invitrogen) were mixed at a 1:1 ratio, and a total of 1×10^6 naïve T cells were adoptively transferred into lymphopenic RAG1$^{-/-}$ host mice via intravenous injection. After 5 days, LNs and spleens were harvested from the host mice, and

the homeostatic proliferation of donor T cells was assessed based on CFSE dilution by flow cytometry.

In vitro T cell proliferation assay

Splenic T cells were labeled with CFSE (Invitrogen) and stimulated with plate-bound αCD3 (1 μg/mL; BioLegend) and αCD28 (1 μg/mL; Thermo Fisher) for 2 days. Cell proliferation was assessed by flow cytometry based on CFSE dye dilution, per the manufacturer's protocol.

Immunoblotting

Proteins were purified from WT and *sti* LNT cells stimulated with αCD3/αCD28, using RIPA buffer (Abcam). The purified proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electrophoretically transferred to polyvinylidene fluoride membranes (Amersham Biosciences). The membranes were incubated with antibodies against the following proteins: Zap70, LAT, TCRζ, AKT, ERK, STAT1,5 and phospho-Zap70 (Y319), LAT (Y191), TCRζ (Y142), AKT (S473), ERK (Tyr204), STAT1 (Tyr701), 5 (Tyr694), and HRP-conjugated anti-mouse β-actin (Cell Signaling and Santa Cruz). Then, the membranes were incubated with enhanced chemiluminescence reagents (Amersham Biosciences) and exposed in an Amersham Imager 680 (GE Healthcare).

ELISA

T cells were stimulated with plate-bound αCD3/αCD28 for 16 h and culture supernatants were collected at the indicated time points. sγc levels in the supernatants were measured by sandwich ELISA using a mouse γc-specific polyclonal antibody (R&D Systems) as a capture antibody and a biotin-conjugated mouse γc-specific monoclonal antibody (4G3; BD) as a detection antibody [16, 17]. Recombinant sγc protein was used as a positive control. IL-2 and IFNγ levels in the culture supernatants were measured by ELISA (BioLegend and R&D Systems, respectively) according to the manufacturers' instructions. Sera obtained from WT and *sti* mice were used for Ig isotyping (Invitrogen) and IgE (BD Biosciences) measurement by ELISA, according to the manufacturers' instructions.

Statistical analysis

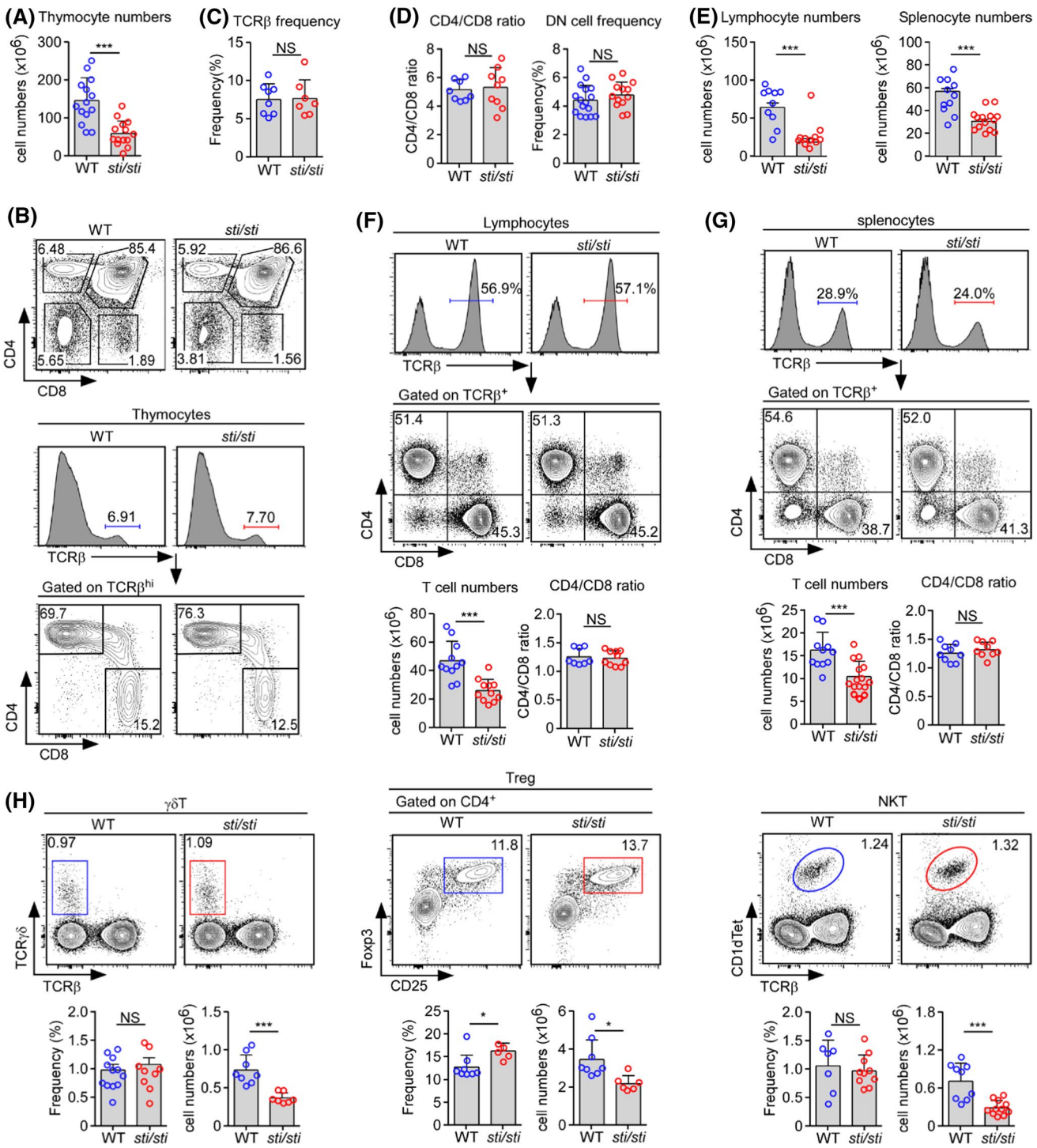
Data are reported as the mean ± SD. Means were compared using an unpaired two-tailed Student's *t*-test. $P < 0.05$ was considered significant. All statistical analyses were performed using GraphPad Prism.

Results

sti mice have reduced numbers of thymocytes and peripheral T cells

As the accumulation of misfolded protein due to misacylated tRNA increases with age, we divided *sti* and WT mice into two age groups: young (6–16 weeks of age) and aged (18–25 weeks of age), and we investigated the numbers of thymocytes and peripheral T cells. AARS mutation had a significant effect on thymic cellularity in aged, but not young *sti* mice (Supplementary Fig. 1A). Thymus size and total thymocyte numbers were reduced by approximately 55% when compared to those in WT mice (Fig. 1A). All further experiments were conducted using aged mice. To assess the effects of AARS mutation on T cell development, thymocytes from *sti* and WT mice were analyzed by flow cytometry for CD4 and CD8 coreceptor expression (Fig. 1B). Four subpopulations of thymocytes (DN, DP, CD8SP, and CD4SP) were not significantly altered by AARS mutation (Fig. 1B and Supplementary Fig. 1B). Upon assessing thymocyte profiles of *sti* mice, we found no significant changes in the $TCR\beta^{hi}$ mature T cell generation (Fig. 1B,C) and CD4/CD8 lineage commitment (Fig. 1D).

As the reduced thymocyte numbers were likely a result of developmental blockade at the DN stage, we investigated the frequency and subpopulations (DN1–DN4) of DN thymocytes expressing the markers CD44 and CD25. There were no substantial differences between *sti* and WT mice (Fig. 1D and Supplementary Fig. 1C), implying that there was no developmental blockade in early T cell development in *sti* mice. Next, we investigated peripheral lymphoid organs of *sti* mice and found that total numbers of old *sti* lymph-node (LN) and spleen (SP) cells were significantly diminished (Fig. 1E), but not in young *sti* mice (Supplementary Fig. 2A) like thymus. To assess whether AARS mutation affects peripheral T cells, LN and SP cells were analyzed by flow cytometry for CD4 versus CD8 gated on T cells. Mature T cells including CD8 and CD4 T cells in the peripheral LN and SP were significantly reduced in numbers in *sti* mice compared to WT mice; however, the ratio of CD4 to CD8 subpopulations and frequencies of LN and SP T cells were comparable (Fig. 1F,G and Supplementary Fig. 2B-D). There was no significant difference in the ratio of naïve to memory T cells between *sti* and WT mice (Supplementary Fig. 2E). To further assess the impact of AARS mutation, we analyzed the distributions of individual peripheral T cell subsets, γδT cells, Foxp3 + T regulatory (Treg) cells, and natural killer T (NKT) cells. We found a significant decrease in their numbers, but not in the percentages of γδT, Treg,



and NKT cells in *sti* mice (Fig. 1H), which indicated that the reduced T cell number is not cell-type specific and is due to an overall impairment in homeostasis. Collectively, these data demonstrated that AARS mutation is detrimental for T cell development, and that AARS affects the overall cellularity of T cells during development and in homeostasis, without targeting a specific developmental stage or subset of T cells.

AARS mutation interferes with TCR signaling, but not cytokine signaling, in T cells

A disruption of homeostatic cytokine signaling can cause T cell loss in *sti* mice. As γ-chain (γc) cytokines, especially, IL-2, IL-7, and IL-15, are essential for T cell development and homeostasis [18–21], we next examined IL-2, IL-7, and IL-15 responsiveness. We first evaluated the expression

◂**Fig. 1** T cell development, homeostasis and cellularity in *sti* mice. **A** Total thymocyte numbers in *sti* and WT mice. Results are the mean and SD of nine independent experiments. **B** CD4 versus CD8 profile in total (top) and TCRβ^{hi}-gated (bottom) thymocytes of WT and *sti* mice. **C** Frequencies of TCRβ cells in WT and *sti* thymocytes. **D** CD4/CD8 ratio in mature SP thymocytes (left) and frequency of DN thymocytes (right) in WT and *sti* mice. Data are the mean and SD from five independent experiments with three mice per group. **E** Total lymphocytes (left) and splenocytes (right) numbers in WT and *sti* mice. **F-G** CD4/CD8 profiles of LN and SP T cells in the indicated mouse genotypes. Histograms showing TCRβ^{+} mature T cells in total LN cells (upper panel) and contour plot showing CD4 versus CD8 expression in TCRβ^{+}-gated cells (middle panel). Frequencies and numbers of T cells in WT and *sti* mice (lower panel). The data are representative of at least five independent experiments. **H** Frequency and total numbers of γδT, Treg, and NKT cells in LN and SP from the indicated mouse genotypes. Contour plots showing TCRβ/γδTCR (left), CD25/Foxp3 (middle) and TCRβ/CD1dtet (right) profiles and percentages of γδT, Treg, and NKT cells, respectively. Summary of γδT, Treg, and NKT cell frequencies, and numbers are shown in the lower panel. Each symbol represents an individual mouse. Horizontal lines indicate the mean and SD. $*P<0.05$, $***P<0.001$, *NS* not significant

of surface cytokine receptors, including IL-2Rα, IL-2Rβ, IL-2Rγ (γc), IL-7Rα, and IL-15Rα, which are the signaling units of functional IL-2, IL-7 and IL-15, in *sti* and WT T cells. We found that *sti* T cells had similar receptor expression as WT T cells (Fig. 2A). In addition, the expression pattern of cytokine receptors in *sti* T cells upon TCR stimulation was comparable to that in WT T cells (Supplementary Fig. 3). To demonstrate whether AARS mutation interferes with IL-2, IL-7, and IL-15 signaling, we stimulated *sti* and WT T cells with IL-2, IL-7, and IL-15 in vitro and assessed their intracellular phospho-STAT5 contents. IL-2, IL-7, and IL-15 induced comparable levels of STAT5 phosphorylation in WT and *sti* T cells (Fig. 2B). Thus, cytokine responsiveness was not dampened by AARS mutation.

However, in vivo, we had observed a significant reduction in the T cell population in *sti* mice compared to WT mice (Fig. 1). Therefore, we reasoned that the decreased T cell numbers were not due to hampered cytokine signaling, but presumably due to distorted proliferation activity, which is critical for T cell homeostasis [20]. Thus, we tested whether AARS mutation diminished cytokine-dependent T cell proliferation under severe T cell lymphopenia, in which homeostatic cytokines are abundant. WT and *sti* naïve T cells were isolated and coinjected into Rag1-deficient lymphopenic host mice, and the proliferation of donor T cells was analyzed. To monitor T cell proliferation, donor naïve T cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE), and to distinguish the donor origin of injected T cells, CD45.1 (WT) and CD45.2 (*sti*) congenic markers were used. We did not observe a substantial difference in lymphopenia-induced proliferation between WT and *sti* T cells (Fig. 2C), indicating that AARS mutation does not affect homeostatic cytokine-induced proliferation.

As cytokine responsiveness and biological effects were intact in *sti* T cells, we next investigated whether the low cellularity was caused by an impairment in TCR signaling, which is another critical factor in T cell development and homeostasis [22, 23]. CFSE-labeled naïve T cells were stimulated with anti-CD3 and anti-CD28 antibodies, and cell proliferation was analyzed based on CFSE dilution. *sti* T cells showed markedly reduced proliferation (Fig. 3A) and IL-2, IFNγ and soluble γc (sγc) levels when compared to WT T cells (Fig. 3B), indicating that *sti* T cells are hyporesponsive to TCR stimulation. As *sti* T cells were hyporesponsive to TCR signaling but had intact cytokine signaling, we examined TCR signaling intermediates. Phosphorylation of CD3ζ, which is the most proximal event upon TCR stimulation, was suppressed in *sti* T cells (Fig. 3C). Furthermore, the phosphorylation of other proximal TCR signaling molecules, including zeta chain-associated protein kinase 70 (Zap70) and linker for activation of T cells (LAT), upon TCR stimulation was also dramatically reduced when compared to that in WT T cells (Fig. 3C). The phosphorylation of protein kinase B (AKT) and extracellular signal-regulated kinase (ERK), which are TCR signaling molecules downstream of Zap70 and LAT, after short-term TCR stimulation was diminished in *sti* T cells (Fig. 3C). The total protein abundances of these factors were not substantially altered. These data showed that the phosphorylation of CD3ζ and its downstream molecules upon TCR stimulation were suppressed, which indicates that AARS mutation inhibits proximal TCR signaling. As the production of IL-2 and IFNγ in activated *sti* T cells after long-term TCR stimulation was inhibited (Fig. 3B), we tested cytokine signaling after long-term TCR stimulation. The phosphorylation of STAT5 and STAT1, which are responsive to IL-2 and IFNγ, respectively, was significantly impaired in *sti* T cells (Fig. 3D). Consistent with the low levels of cytokines observed in activated *sti* T cells, the phosphorylation of molecules downstream of IL-2 and IFNγ was not effectively induced (Fig. 3D), and ERK and AKT phosphorylation was also inhibited in long-term TCR-stimulated *sti* T cells (Fig. 3D). Collectively, these data indicated that the low cellularity of T cells in *sti* mice is due to dampened TCR signaling.

The cellularity of B cells is impaired by AARS mutation

AARS mutation showed no difference in the frequency of T cells in LN and SP, but showed a decrease in the T cell numbers (Fig. 1F,G). Here, we investigated whether AARS mutation also affects the responses and cellularity of B cells. B cell frequencies in the LN and SP of *sti* mice were similar to those in WT mice; however, the numbers of B cells in *sti* mice were significantly reduced compared to those in WT mice (Fig. 4A). As follicular helper T (Tfh) cells have been

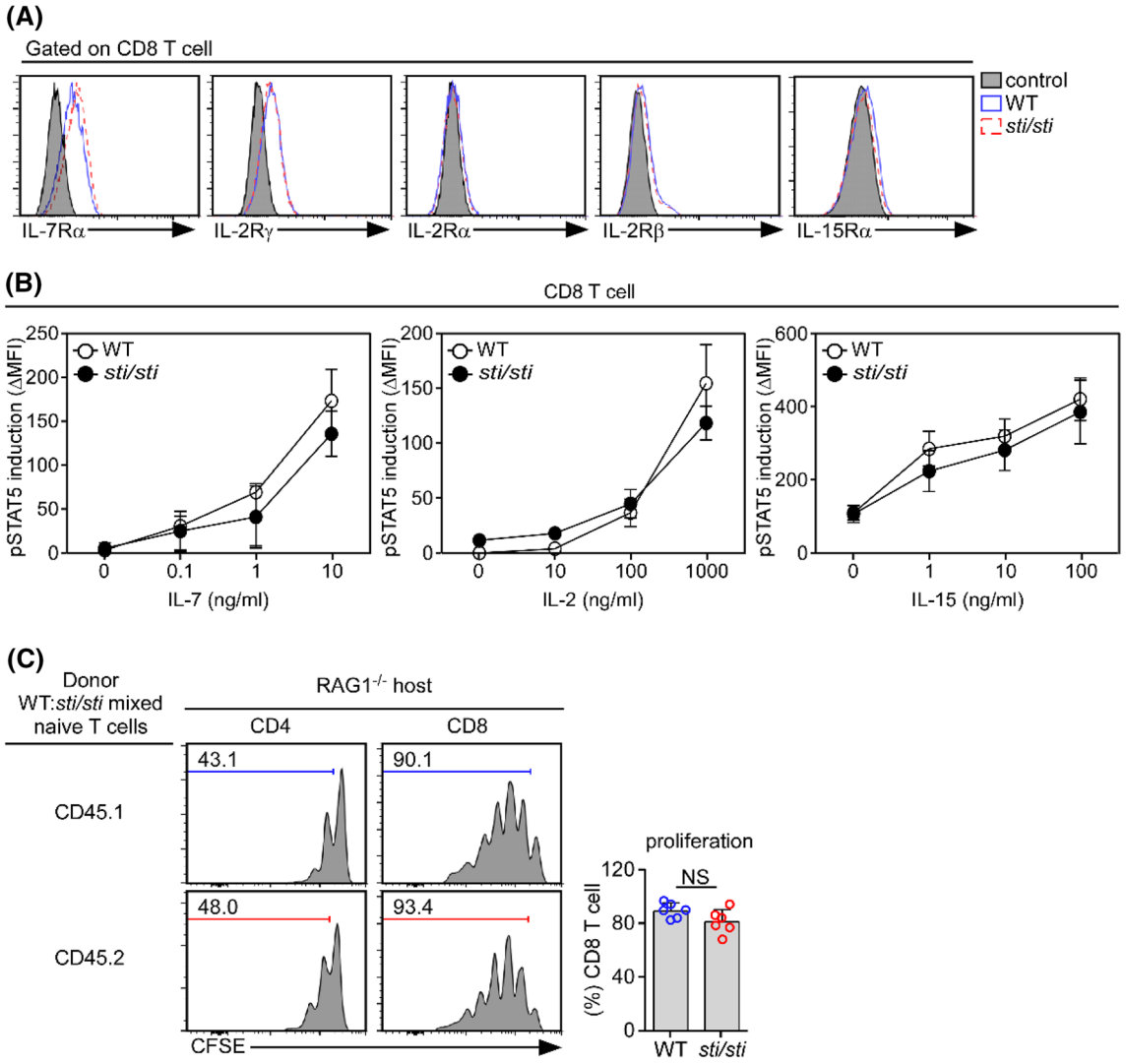


Fig. 2 Cytokine signaling and cytokine-dependent *sti* T cell proliferation. **A** Surface staining for γc cytokine receptor family molecules on WT and *sti* CD8 T cells. Data are representative of three independent experiments. **B** STAT5 phosphorylation induced by IL-2, IL-7, and IL-15 in CD8 T cells of WT and *sti* mice. Intracellular pSTAT5 contents were assessed after 30 min of IL-2, IL-7, or IL-15 stimulation. Data are representative of three independent experiments. **C** Homeostatic proliferation of WT and *sti* donor LN T cells in RAG1$^{-/-}$ mice. Histograms showing CFSE dilution of adoptively transferred donor naive T cells from CD45.1^{+} WT and CD45.2^{+} *sti* mice. Donor origin was identified using CD45.1/2 congenic markers, and cell proliferation was determined for donor T cells recovered from the LNs. Data are representative of three independent experiments. *NS* not significant

identified as a distinct lineage of helper T (Th) cells, which are involved in B cell homeostasis and responses [24, 25], we assessed the frequencies and numbers of Tfh cells. The frequency of Tfh cells in *sti* mice was similar to that in WT mice (Fig. 4B), and the Tfh cell number was slightly, albeit not significantly, decreased (Fig. 4B). Next, to determine whether AARS mutation affects the functional antibody production responses of B cells, serum immunoglobulin (Ig) isotype levels were measured using enzyme-linked immunosorbent assay (ELISA). Surprisingly, despite the reduced B cell numbers, serum levels of IgE, IgA, and IgM were significantly increased in *sti* mice compared to WT mice (Fig. 4C), indicating that *sti* mice easily suffer from infectious or allergic diseases.

Discussion

The present study aimed to clarify the effects of translational fidelity in the development and responses of immune cells. Despite the long-term research on AARSs [10, 15], their function remains ambiguous as they seem to have various roles in different cell types. Studies have shown that AARS mutation induces the generation and accumulation of un- and

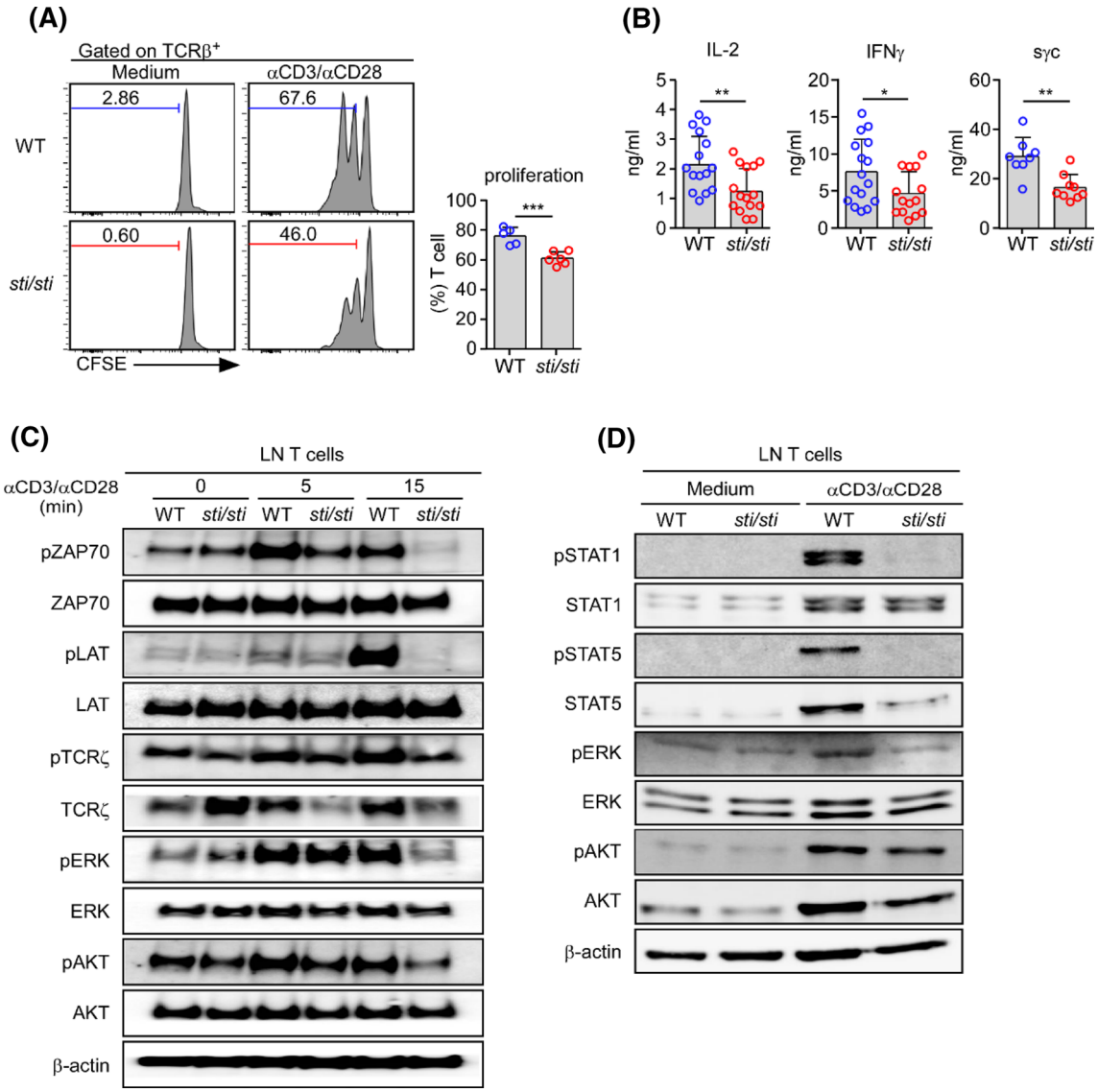


Fig. 3 Impaired *sti* T cell proliferation by dampened TCR signaling. **A** FACS-purified LN T cells were labeled with CFSE and stimulated with αCD3/αCD28 in vitro for 2 days. CFSE dilutions were analyzed at the indicated time points. Data are representative of five independent experiments. **B** LN T cells were stimulated with αCD3/αCD28 for 16 h. Culture supernatants were harvested and assessed for IL-2, IFNγ, and sγc by ELISA. **C, D** Proximal events and multiple distal pathways of TCR signaling. **C** Immunoblot analysis of total and phosphorylated Zap70, LAT, TCRζ, ERK, and AKT in WT and *sti* T cells stimulated for different periods (indicated above the lanes) with αCD3/αCD28. **D** Immunoblot analysis of total and phosphorylated STAT1, STAT5, ERK, and AKT in WT and *sti* T cells stimulated with αCD3/αCD28 for 16 h. Error bars indicate SDs. $*P<0.05$, $**P<0.01$, $***P<0.001$

misfolded proteins in non-immune cells, such as neuronal and cardiac cells, and is associated with neurodegenerative disorders and cardioproteinopathy [10, 15, 26–28]. Immune cell analysis using AARS-mutant (*sti*) mice in the current study revealed that AARS regulates TCR signaling and T cell responses. AARS mutation induced hyporesponsiveness of T cells in terms of cell proliferation and cytokine production, resulting in decreased numbers of thymocytes and peripheral T cells. Interestingly, B cell cellularity was also decreased, and serum levels of isotypes of antibodies were altered in *sti* mice. Collectively, these findings indicate that the impairment of adaptive immune cells due to a defect in translational fidelity of AARS may be closely related to the phenotype of *sti* mice, which has rough and thin hair falling out in patches.

We postulated that the decreased numbers of T cells in *sti* mice might be attributed to cytokine hyporesponsiveness. Cytokine signaling is mainly regulated by the expression level of cytokine receptors and is measured as STAT phosphorylation [16, 21, 29]. Although the common γc

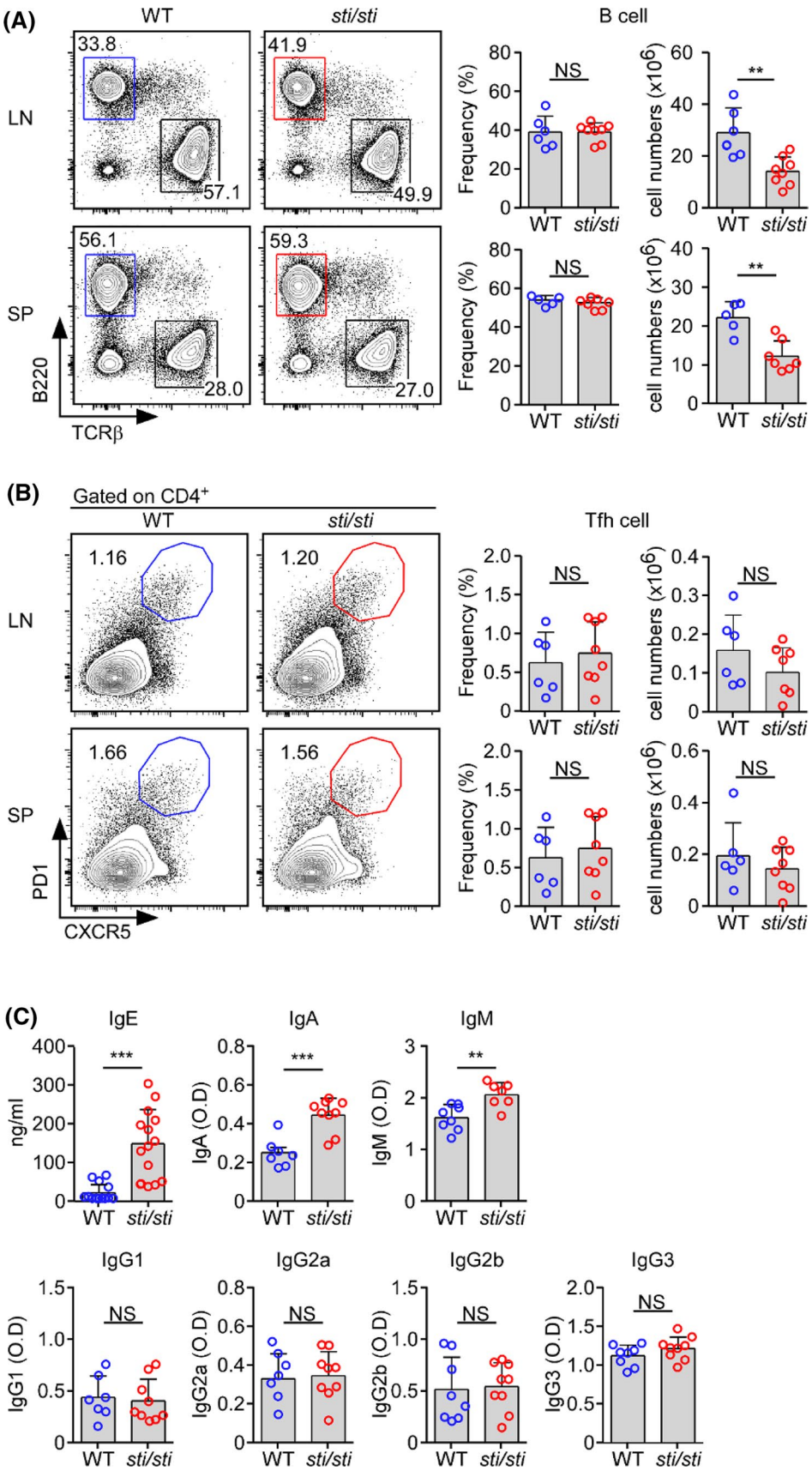


Fig.4 B cell responses and cellularity in *sti* mice. **A** Contour plots showing CD4/CD8 expression in total LN and SP cells from WT and *sti* mice (left). Frequencies and numbers of B cells in the indicated mouse genotypes (right). **B** Tfh cell frequencies in WT and *sti* CD4 T cells. Tfh cells were identified based on $CXCR5^{hi}PD\text{-}1^{hi}$ expression. Contour plots (left) and the bar graph (right) are representative of three independent experiments. **C** Serum Ig concentrations in *sti* mice. Sera were collected from more than eight *sti* and WT control mice of the same age (18–25 weeks). The concentrations of each Ig isotype were determined in triplicate by ELISAs, along with appropriate Ig standards. Each circle represents an individual mouse. Data are the mean ± SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, *NS* not significant

family cytokines, IL-2, IL-7 and IL-15, are major factors in T cell development and homeostasis [16, 20, 25], we found no defect in cytokine receptor expression or STAT5 phosphorylation, nor did we find a difference in proliferation between WT and *sti* T cells from a chronic lymphopenic Rag-deficient animal model. These data indicated that cytokine receptors and their signaling pathways were unaffected by AARS mutation. Thus, we next postulated that TCR signaling might be impaired in *sti* T cells, as tonic TCR signaling is required to maintain T cell homeostasis as well as homeostatic cytokine signaling [20, 23]. *sti* T cell proliferation upon TCR stimulation was significantly reduced, which was associated with hyporesponsiveness in early TCR signaling. When TCR is engaged with ligand, the immunoreceptor tyrosine-based activation motifs in the cytosolic domain of CD3 are phosphorylated, and Zap70 is recruited to the phosphorylated CD3 and phosphorylated by Lck or Fyn [30, 31]. The main substrate of activated Zap70 is the scaffolding transmembrane adaptor, LAT [31, 32]. ERK and AKT play critical roles as downstream molecules in early TCR signaling [30]. Therefore, we investigated TCR signaling intermediates, and we found that *sti* T cells were hyporesponsive in proximal TCR signaling, with reduced phosphorylation of CD3ζ, Zap70, LAT, and the downstream molecules ERK and AKT. This showed that the decrease in active ERK and AKT induced by AARS mutation was closely linked to the impaired cell proliferation. In addition, the impaired cytokine production in *sti* T cells due to dampened TCR signaling was associated with reduced phosphorylation of STAT1 and STAT5. Although AARS is pivotal in protein synthesis, there was no abnormalities in the total levels of TCR signaling proteins in *sti* T cells. Thus, we speculate that irrelevant amino acid substitutions in key functional domains of enzymes and proteins involved in TCR signaling or conformational changes in their structures due to the translational infidelity of AARS—as evidenced by the synthesis and accumulation of dysfunctional un- or misfolded proteins in *sti* mice[15]—may result in suboptimal TCR signal transduction, which would eventually lead to an overall reduction in T cell cellularity and a low number of T cells in *sti* mice. Clinically, patients with progressive leukodystrophy caused by pathogenic variants in lysyl-tRNA synthetase have immune-deficient diseases, such as anemia and pancytopenia [33]. The cause and molecular mechanism of immunodeficiency in these patients have not been defined. Our study provides important clues for the potential treatment of immunodeficiency in such patients; however, further studies are needed to provide specific and direct evidence of our speculation.

Concomitant with the decrease in T cell numbers in *sti* mice, the numbers of B cells were also decreased. Tonic B cell receptor signaling is important for the development and homeostasis of B cells [34] and is regulated by several cascades, including PI3K-AKT, PLCγ2-PKC, and RAS-Raf-ERK [35–38]. As we observed dampened ERK and AKT activation in *sti* T cells, we speculated that their phosphorylation might be suppressed, adversely affecting *sti* B cell cellularity. Although the B lymphocyte stimulator family of cytokines is important for the survival and maintenance of B cells [24, 39], it is doubtful that they were involved in the reduction in B cell numbers as cytokine signals were intact in *sti* T cells. The exact cause of this reduction requires further molecular studies.

ARSs play critical roles in immune regulation and inflammatory responses in non-canonical manners [11, 40, 41]. AARSmutant mice have rough, thin hair that fall out in patches [15]. This abnormal skin phenotype may be associated with inflammatory skin disease caused by the collapse of cellular and humoral immune responses [42, 43]. Our analysis of *sti* T cells suggested that a defect in T cell responsiveness and effector cytokine production might result in increased susceptibility to pathogens and might be linked to an infectious skin inflammatory phenotype. This was supported by the observed reductions in nonconventional T cells, such as γδT and NKT cells, which are regulatory cells in infectious skin disease [44, 45], in *sti* mice. Chronic inflammation due to susceptibility to infection may have driven the IgM and IgA isotype antibody predominance [46, 47] in *sti* mice, despite the overall reduction in B cell numbers, suggesting that antibody production functioned normally in these mice. Nevertheless, further study is required to clarify how and why these specific isotypes of antibodies were dominantly produced in *sti* mice. Alternatively, the *sti* phenotype may have been due to atopic dermatitis, given the predominance of IgE in *sti* serum. A major hallmark of atopic dermatitis is an elevated serum IgE level [48], which is generated as B cells undergo IgE isotype switching driven by Th2 cytokines, including IL-4 and IL-13 [49]. Thus, study on effects of AARS in the Th differentiation of CD4 T cells is worthy of further study. Taken together, the above data indicate that AARS defect causes disturbances in the adaptive immune system that may lead to several infectious or atopic inflammatory diseases.

Our finding that comprehensive translational infidelity negatively affects TCR signaling warrants further proteomics studies. A complex network of TCR signaling cascades drives dynamic changes in transcription and facilitates differentiation, proliferation, and survival. Although key factors of this pathway have been extensively characterized, signaling protein interactions and collaboration with T cells are not fully understood. Comprehensive proteomics studies using ARS mutant T cells are expected to provide new information on the proteins that affect TCR signaling and identify new proteins or domains that regulate TCR signals. Such data will pave the way to the development of small-molecule modulators to inhibit or enhance TCR signaling

and will be useful in the development of disease-specific immunotherapies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00018-021-04122-z.

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Author contributions CH conceived and designed the study. JAS, YJ, HH, DL, SEL, DH, JK and CH performed experiments and analyzed data. JAS, YJ, DL, JHL, PS and CH analyzed and interpreted the results. JAS and CH wrote the manuscript. All authors read and approved the manuscript.

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Data availability All relevant data are within the manuscript and Supplementary Information Appendix.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing financial interests.

Ethical approval All the procedures involving animals in this study complied with Pusan National University (PNU) Institutional Animal Care and Use Committee (PNU-2020-2714) and the KRIBB Animal Care and Use Committee (KRIBB-AEC-18156).

Consent for publication All the authors have approved and agreed to publish this manuscript.

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