 DOI: https://doi.org/10.5114/ceji/208684

Glutaminase 1-mediated glutaminolysis regulates tuberculosis progression by modulating Th1 and Th17 immune responses

XIAOZHEN CAI, YIDAN HUANG, LILI ZENG, RONG RONG

Department of Respiratory Medicine, Houjie Hospital of Dongguan, Dongguan, China

Abstract

Introduction: *Mycobacterium tuberculosis-mediated tuberculosis (TB) is an infectious disease that results in approximately 1.2 million deaths annually. Glutaminase (GLS1) is a metabolic enzyme involved in glutaminolysis. The current study examined the roles and mechanisms of GLS1 in TB progression.*

Material and methods: *Peripheral blood mononuclear cells (PBMCs) were extracted from TB patients and healthy individuals, and $CD4^+$ T and $CD14^+$ monocytes were sorted. PBMCs were incubated with M. tuberculosis strain H37Rv lysate to stimulate immune responses and treated with BPTES, a GLS1 inhibitor. RT-qPCR and western blotting were employed to detect mRNA and protein levels, respectively. Immunophenotyping of cells was performed using flow cytometry. ELISA was used to determine cytokine levels. Colony-forming unit assays were used to evaluate M. tuberculosis survival in macrophages. ChIP assays were used to detect the enrichment of H3K9ac/H3K27ac at the gene promoters.*

Results: *GLS1 was elevated in $CD4^+$ T cells from TB patients and H37Rv lysate-stimulated PBMCs. GLS1-mediated glutaminolysis promoted Th1 and Th17 cell differentiation. Inhibition of GLS1 by BPTES facilitated M. tuberculosis survival in macrophages. GLS1 inhibition reduced H3K9ac and H3K27ac epigenetic modification in the promoter region of interferon γ and interleukin 17.*

Conclusions: *GLS1-mediated glutaminolysis may regulate TB progression by modulating Th1 and Th17 immune responses via epigenetic regulation.*

Key words: *tuberculosis, GLS1, glutaminolysis, Th1, Th17 immune response.*

(Cent Eur J Immunol 2026; 51:)

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a chronic infectious disease that primarily affects the lungs [1]. It is a leading cause of death worldwide, accounting for more than 1.4 million deaths annually [2]. Approximately 95% of primary *M. tuberculosis* infections are asymptomatic, progressing to latent infections, while 5-10% develop into active disease [3]. Established risk factors for active TB include human immunodeficiency virus (HIV) infection, malnutrition, and young age [4]. Patients with active TB may be afflicted with symptoms such as coughing, chest pain, weight loss, fever, and fatigue [5]. Conventional bacteriological tests, despite being the gold standard, have a low positivity rate of approximately 30% [6]. Isoniazid and rifampicin are employed as the first-line anti-TB drugs [7]. However, the nonadherence of patients to anti-tubercular drugs leads to the development of multidrug resistance, resulting in treatment challenges [8]. Thus, novel diagnostic biomarkers and therapeutic strategies for TB are urgently needed.

The immune response to *M. tuberculosis* is critical in regulating TB initiation, progression, and outcomes [9]. Immune activation and inflammatory response are essential for host protection against mycobacteria, while dysregulated responses can exacerbate the disease [10]. Antigen-specific $CD4^+$ T cells are central to adaptive immunity against *M. tuberculosis*, with T helper cell 1 (Th1) and Th17 as key effector subsets [11]. Th1 cells produce interferon γ (IFN-γ) and mediate protection mainly by activating macrophages which destroy intracellular pathogens [12]. The main effector cytokine of Th17 cells is interleukin (IL)-17, which can eliminate the primary infection and establish an effective memory response [13].

Glutaminolysis is a metabolic process that produces various substrates for anabolism in effector T cells. In this process, glutamine is lysed to glutamate, which later con-

Correspondence: Yidan Huang, Houjie Hospital of Dongguan, Houjie Town, Dongguan City, Guangdong Province, China, e-mail: ydhdoctor@hotmail.com
Submitted: 12.02.2025, Accepted: 27.07.2025

verts to α-ketoglutarate that then enters the tricarboxylic acid (TCA) cycle and generates multiple metabolites such as acetyl-CoA [14]. This metabolic pathway provides energy for T cell activation by regulating multiple enzymatic activities [15, 16]. Glutaminase 1 (GLS1), the rate-limiting enzyme in glutaminolysis, converts glutamine to glutamate [17]. Evidence suggests that GLS1-mediated glutaminolysis can enhance Th17 cell generation and promote psoriasis pathogenesis in mice [18]. Additionally, GLS1 inhibition by BPTES can normalize the effector functions of Th17 and Th1 cells and alleviate Sjögren's syndrome [19]. Moreover, Zhang *et al*. reported that GLS1 inhibition affects the Th1/Th2 and Th17/Treg imbalance in lupus models [16]. However, the role of GLS1 in TB pathogenesis remains underexplored.

This study aimed to investigate the role and potential mechanism of GLS1 in Th1/Th17 cell differentiation. We hypothesized that GLS1-mediated glutaminolysis may regulate TB progression by modulating Th1 and Th17 immune responses.

Material and methods

Sample collection

Peripheral blood was collected from 30 TB patients and 30 healthy donors at Houjie Hospital of Dongguan. TB patients were diagnosed according to clinical manifestations, bacteriological identification, and genotyping. TB patients aged 18-64 years with a smear-positive result for acid-fast bacilli or a GeneXpert MTB/RIF positive result were enrolled in our study. The exclusion criteria included: 1) requirement for hospital admission, 2) pregnancy, 3) diagnosis of multidrug-resistant or extensively drug-resistant TB, 4) HIV infection, 5) other concurrent infectious diseases. Healthy volunteers undergoing annual health checkups during the same period were recruited. The inclusion criteria included: 1) normal chest X-ray, 2) no recent TB patient contact, 3) negative purified protein derivative test, and 4) no history of immunosuppressive drug use, autoimmune diseases, acquired immunodeficiency, hypertension, diabetes, hepatitis, or tumors. TB patients and healthy controls were matched for age and gender, with no significant differences. Written informed consent was obtained from each participant. This study was approved by the Ethics Committee of Houjie Hospital of Dongguan (approval No. 2022-007; March 22, 2022).

Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated through Ficoll-Hypaque density gradient centrifugation with Lymphoprep solution (Stemcell Technologies, Shanghai, China). The buffy coat layer (containing PBMCs) at the interface between the plasma and Ficoll-Hypaque was gently aspirated using a pipette and transferred to a new 15 ml sterile conical tube, followed by washing twice with phosphate-buffered saline (PBS). The isolated PBMCs (2.5×10^6 cells/ml) were resuspended in RPMI-1640 culture medium containing 10% fetal bovine serum and 1% penicillin/streptomycin (Yeasen, Shanghai, China) in a humidified 5% CO_2 atmosphere at 37°C.

Cell sorting

For $CD4^+$ T cell isolation, freshly isolated PBMCs were incubated with anti-human CD4 microbeads (NovoBiotechnology Co., Ltd, Beijing, China) for 15 min at 4°C, followed by passing through the magnetic bead separation column to retain labeled $CD4^+$ cells. After the removal of the column, $CD4^+$ cells were eluted and collected. Similarly, $CD14^+$ monocytes were sorted by incubating PBMCs with anti-human CD14 microbeads before magnetic separation. The purities of $CD4^+$ T cells and CD14 monocytes were determined by flow cytometry using FITC-CD3 (1 : 100, clone UCHT1, ab34275), PE-CD4 (1 : 200, clone MEM-241, ab18282), and FITC-CD14 (1 : 100, clone MEM-15, ab28061) antibodies (all from Abcam, Shanghai, China). Mouse IgG1-FITC (clone B11/6, ab91356, Abcam) or IgG1-PE (clone B11/6, ab91357, Abcam) was used as an isotype control to exclude the interference of fluorescein.

Cell culture

Mycobacterium tuberculosis strain H37Rv (ATCC, Manassas, VA, USA) was cultured in Middlebrook 7H9 broth medium (Thermo Fisher, Shanghai, China) supplemented with 10% oleic acid albumin dextrose catalase enrichment (OADC; Thermo Fisher) at 37°C for three weeks. Bacterial cells were harvested by centrifugation, resuspended in PBS, and lysed by ultrasonication. To stimulate immune responses, PBMCs were incubated with H37Rv whole cell lysate (10 μg/ml) for 48 hours. To investigate the role of GLS1-mediated glutaminolysis, PBMCs were treated with 10 μM BPTES (a GLS1 inhibitor; MedChemExpress, Shanghai, China) and 1 mM glutamate (Sigma-Aldrich, St. Louis, MO, USA).

Flow cytometry analysis

After the indicated treatments, PBMCs were harvested and washed twice with PBS. Cells were resuspended in the staining buffer and stained with FITC-conjugated anti-CD4 antibody (1 : 1000, clone B-A1, ab59474, Abcam). The isotype control was Mouse IgG1 (clone B11/6, ab91356, Abcam) at the same dilution. For intracellular cytokine detection, cells were stained with PE-conjugated anti-IFN-γ (1 : 200, clone BYGAMMA3-11.1, AC0799, Beyotime, Shanghai, China) or anti-IL-17 (clone BYBL23, 1 : 200, AC0804, Beyotime) antibodies. Corresponding isotype controls for intracellular staining were Mouse IgG1 (clone B11/6, ab91357, Abcam) for both IFN-γ and IL-17A antibodies, used at matching dilutions to assess non-specific

binding. The stained cells were washed twice and analyzed on a FACSCalibur flow cytometer (BD Biosciences, Beijing, China), and the data were analyzed using FlowJo software.

Enzyme-linked immunosorbent assay

Culture supernatants from PBMCs were collected after the indicated treatments. Concentrations of IFN-γ and IL-17 were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) as per the manufacturer's instructions.

Measurement of ATP production

The concentration of intracellular ATP was determined using an ATP Assay Kit (S0026, Beyotime) according to the manufacturer's instructions. Briefly, PBMCs were lysed to isolate total protein and then centrifuged at 12,000 g and 4°C for 5 minutes. Next, 20 µl of the sample or standard solution was added to 100 µl of ATP detection solution, mixed, and luminescence was measured with a luminometer (Promega, Madison, WI, USA).

Assessment of OCR and ECAR

Cells were incubated with an oxygen consumption rate (OCR; ab197243, Abcam) or extracellular acidification rate (ECAR; ab197244, Abcam) reagent as recommended by the manufacturer. After incubation according to the instructions, a microplate reader (Thermo Scientific) was used to record the final assay signals at 10 minutes intervals for approximately 120 minutes, employing excitation and emission wavelengths of 380 and 650/615 nm, respectively.

RT-qPCR

Total RNA was extracted from PBMCs or $CD4^+$ T cells using TRIzol reagent (Sigma-Aldrich). RNA concentration and purity were assessed at 260 nm/280 nm using a NanoDrop Spectrophotometer (Thermo Fisher). Total RNA (1 µg) was reverse transcribed into cDNA using the PrimeScript RT reagent kit (Takara, Beijing, China). RT-qPCR was performed via the TaqMan Gene Expression Assays Protocol (Thermo Fisher). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method [20], normalized to GAPDH. Primer sequences are listed in Table 1.

Western blotting

Total protein was extracted from PBMCs or $CD4^+$ T cells using RIPA lysis buffer (Beyotime) supplemented with 1% phenylmethylsulfonyl fluoride (PMSF) and a protease inhibitor cocktail (Beyotime). Equal amounts of protein samples (40 µg) were separated by electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Beyotime). Subsequently, the membranes were blocked with 5% skimmed milk for 1 hour at room temperature and incubated overnight at 4°C with primary antibodies against GLS1 (1 : 1000, clone EP7212, ab156876), GAPDH (1 : 2500, ab9485), H3K9ac (1 : 500, clone Y28, ab32129), H3K14ac (1 : 2000, clone EP964Y, ab52946), H3K23ac (1 : 1000, clone EPR17712, ab177275), H3K27ac (1 : 2500, ab4729), and total H3 (1 : 1000, ab1791) (all from Abcam). After washing three times with Tris Buffered Saline Tween 20 (TBST) three times, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1 : 3000, ab6721, Abcam) at room temperature for 1 hour. Finally, the membranes were washed three times in TBST, and protein bands were visualized with an enhanced chemiluminescence reagent (Thermo Fisher). Band intensities were quantified using Image J software.

Colony-forming unit (CFU) assays

To induce macrophage differentiation, the isolated $CD14^+$ monocytes were incubated in RPMI-1640 medium supplemented with 10% FBS and 50 ng/ml recombinant human macrophage colony-stimulating factor (P5313, Beyotime) for 7 days. The medium was refreshed every 2 days. Differentiated macrophages were infected with H37Rv at a multiplicity of infection (MOI) of 5 : 1 (bacteria : macrophage) for 4 hours at 37°C, followed by washing with PBS to remove extracellular bacteria. Then, infected macrophages were treated with 10 µM BPTES or 1 mM glutamate for 48 hours. To evaluate intracellular bacterial survival, macrophages were lysed with 0.1% Triton X-100 for 10 minutes. Cell lysates were serially diluted (10-fold) in PBS, and the dilutions were plated on Middlebrook 7H10 agar plates (Solarbio, Beijing, China). The plates were incubated at 37°C for 3 weeks, and then the colonies were quantified manually.

Induction of Th1 and Th17 cell differentiation

$CD4^+$ T cells isolated from healthy controls were stimulated with plate-bound anti-human CD3 (5 µg/ml) and

Table 1. Sequences of primers used for RT-qPCR

Gene (human)	Sequence (5′–3′)
GLS1 forward	AGTTGCTGGGGGCATTCTTTTAGTT
GLS1 reverse	CCTTTGATCACCACCTTCTCTTCGA
IFN-γ forward	GAGTGTGGAGACCATCAAGGA
IFN-γ reverse	GTATTGCTTTGCGTTGGACA
IL-17 forward	AACGATGACTCCTGGGAAGA
IL-17 reverse	CTCAGAATTTGGGCATCCTG
GAPDH forward	GGAGCGAGATCCCTCCAAAAT
GAPDH reverse	GGCTGTTGTCATACTTCTCATGG

anti-human CD28 (1 μg/ml) antibodies for 5 days under different induction conditions. To induce Th1 differentiation, IL-12 (10 ng/ml) and IL-4 (10 μg/ml) were added. To induce Th17 differentiation, transforming growth factor β (TGF-β; 5 ng/ml), IL-6 (20 ng/ml), IL-1β (10 ng/ml), IL-23 (20 ng/ml), anti-IFN-γ (10 μg/ml), and anti-IL-4 (10 μg/ml) were added. All the antibodies were obtained from Abcam. Cell culturing was performed in 48-well plates with a total volume of 0.5 ml/well culture medium with 2.5×10^5 $CD4^+$ T cells. The medium was refreshed on day 3.

Measurement of acetyl-CoA

Th1/Th17 cells were lysed and centrifuged at 20,000 g for 10 minutes at 4°C. Then, the pellets were discarded, and the supernatant was collected. The concentration of acetyl-CoA in the supernatant was determined using an acetyl-CoA Assay kit (MAK039, Sigma-Aldrich) according to the manufacturer's protocols.

Chromatin immunoprecipitation assay

The cells were cross-linked with 1% formaldehyde, and the reaction was quenched with 125 mM glycine. Cells were lysed, and nuclei were isolated using a chromatin immunoprecipitation (ChIP) assay kit (P2078, Beyotime) following the manufacturer's instructions. Chromatin was sonicated to fragments of 200-500 bp. Then, 50 μl of sonicated chromatin was immunoprecipitated overnight with 2 μg of anti-H3K27ac (ab4729, Abcam) or anti-H3K9ac (ab32129, Abcam) antibodies. Beads with bound immunocomplexes were washed and eluted, followed by DNA isolation to perform RT-qPCR. Input DNA (10% of sonicated chromatin) was used as a reference, and results were expressed as percentage of input.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Data are presented as the mean ± standard deviation. Differences between two groups were evaluated using Student's *t*-test, while those among multiple groups were assessed using one-way analysis of variance followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$.

Results

GLS1 is upregulated in PBMC-derived CD4+ T cells of TB patients

First, we evaluated the expression of GLS1 in $CD4^+$ T cells sorted from PBMCs of TB patients and healthy volunteers. Flow cytometry showed that the purity of isolated $CD4^+$ T cells was > 95% (Supplementary Fig. 1A). As RT-qPCR and western blotting demonstrated, TB patients exhibited significantly higher GLS1 mRNA and protein levels in $CD4^+$ T cells compared to healthy controls (Fig. 1A-C). Additionally, no significant difference was found in GLS2 expression between the two groups (Supplementary Fig. 1B). Then, PBMCs were stimulated with *M. tuberculosis* strain H37Rv lysate. Notably, GLS1 expression was upregulated after H37Rv lysate stimulation in PBMCs from healthy donors and TB patients (Supplementary Fig. 1C). Compared with those from healthy donors, PBMCs derived from TB patients had higher levels of GLS1 in both the control and stimulated groups (Supplementary Fig. 1C). We then selected PBMCs from healthy donors for further experiments. Moreover, treatment with BPTES, a GLS1 inhibitor, effectively inhibited GLS1 activity in H37Rv lysate-stimulated PBMCs (Fig. 1D-F).

GLS1-mediated glutaminolysis promotes Th1/Th17 cell differentiation

Then, the effect of GLS1 on Th1/Th17 cell differentiation was examined. As flow cytometry revealed, *M. tuberculosis* H37Rv lysate stimulation significantly promoted Th1 and Th17 cell differentiation, as evidenced by higher frequencies of $CD4^+IFN\text{-}\gamma^+$ and $CD4^+IL\text{-}17^+$ cells compared to the control group. However, the inhibition of glutaminolysis by BPTES significantly reduced the proportions of $CD4^+IFN\text{-}\gamma^+$ and $CD4^+IL\text{-}17^+$ cells, which were recovered by supplementation of glutamate (Fig. 2A-D). Moreover, we found that ATP production was increased in PBMCs stimulated with H37Rv lysate, indicating increased metabolic activity. Treatment with BPTES reduced ATP levels, while glutamate supplementation partially restored this effect caused by BPTES (Fig. 2E).

In addition, basal OCR and ECAR were measured to further evaluate the metabolic fitness of PBMCs. As shown in Figure 2F, G, lysate stimulation increased OCR and ECAR, which was partially counteracted by BPTES treatment. Addition of glutamate rescued BPTES-mediated reduction in OCR and ECAR under H37Rv lysate stimulation. These results indicate the critical role of GLS1-mediated glutaminolysis in Th1/Th17 differentiation and metabolic fitness during *M. tuberculosis* antigen stimulation.

Inhibition of GLS1 by BPTES protects *M. tuberculosis* survival in macrophages

Moreover, we determined the levels of cytokines secreted by Th1/Th17 cells. As demonstrated by ELISA, the concentrations of IFN-γ and IL-17 were higher in H37Rv lysate-stimulated PBMCs compared with the unstimulated cells. BPTES reduced IFN-γ and IL-17 levels in H37Rv lysate-stimulated PBMCs, while this effect was reversed by glutamate supplementation (Fig. 3A, B). Consistent results were observed in RT-qPCR analysis showing changes in IFN-γ and IL-17 mRNA levels in

each group (Fig. 3C, D). Then, PBMC-derived CD14$^+$ monocytes (purity > 95%, as shown in Supplementary Fig. 1D) were differentiated into macrophages, followed by H37Rv infection.

CFU assays demonstrated that BPTES treatment markedly increased the survival of *M. tuberculosis* from infected macrophages, whereas supplementation of glutamate significantly abolished the effect of BPTES (Fig. 3E, F).

GLS1-mediated glutaminolysis promotes H3K9ac and H3K27ac epigenetic modification in the IFN-γ or IL-17 gene promoters

We then explored the regulatory mechanisms by which GLS1-mediated glutaminolysis modulates Th1/Th17 cell differentiation. We assessed the acetyl-CoA concentration in Th1 and Th17 cells. Treatment with BPTES significantly reduced acetyl-CoA production, which was res-

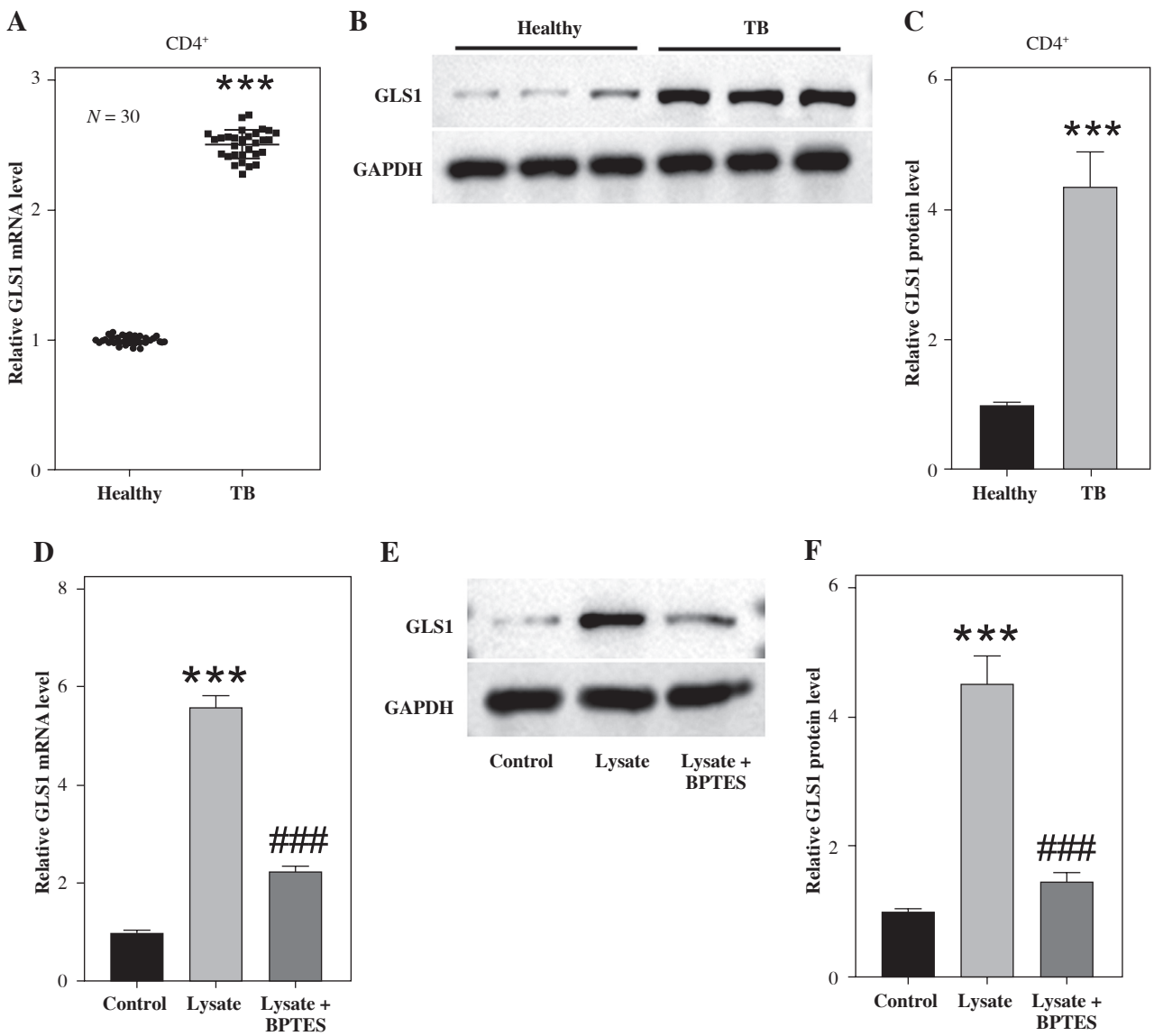

Fig. 1. Glutaminase 1 (GLS1) is upregulated in PBMC-derived CD4$^+$ T cells of TB patients. **A**) The mRNA level of GLS1 in CD4$^+$ T cells derived from PBMCs of TB patients and healthy donors was measured by RT-qPCR (n = 30). **B**, **C**) The protein level of GLS1 in CD4$^+$ T cells derived from PBMCs of TB patients and healthy donors was evaluated by western blotting (n = 30). **D**) The mRNA level of GLS1 in healthy PBMCs treated with or without H37Rv lysate and BPTES (a GLS1 inhibitor) was measured by RT-qPCR. **E**, **F**) The protein level of GLS1 in healthy PBMCs under different treatments was measured by western blotting. n = 3/group. ***$p < 0.001$ vs. healthy group or control group, $^{###}p < 0.001$ vs. lysate group

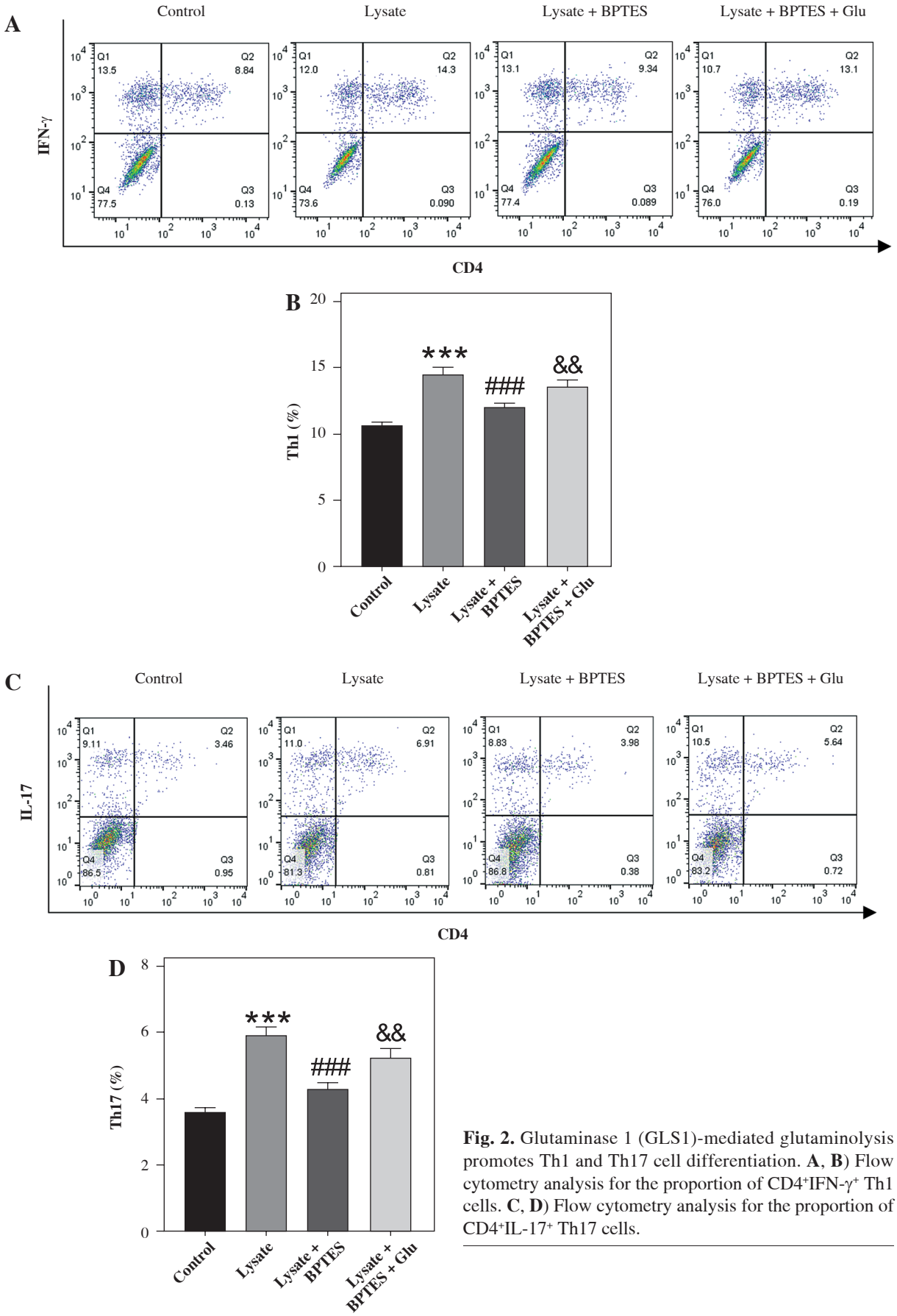

Fig. 2. Glutaminase 1 (GLS1)-mediated glutaminolysis promotes Th1 and Th17 cell differentiation. **A**, **B**) Flow cytometry analysis for the proportion of $CD4^+IFN\text{-}\gamma^+$ Th1 cells. **C**, **D**) Flow cytometry analysis for the proportion of $CD4^+IL\text{-}17^+$ Th17 cells.

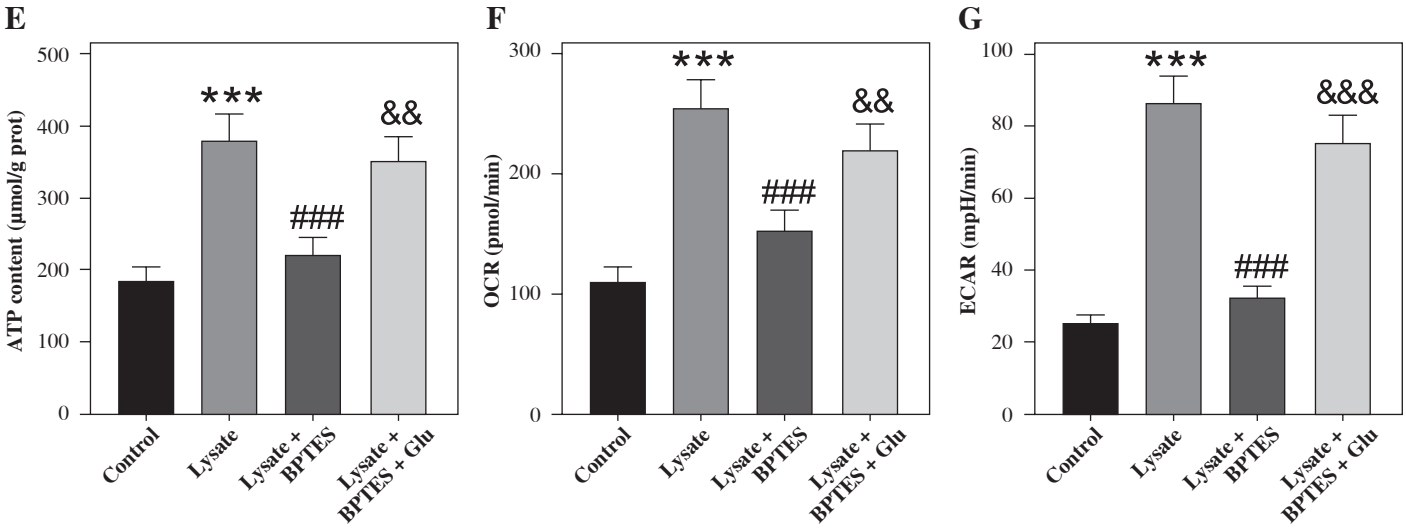

Fig. 2. Cont. **E**) Measurement of ATP production in PBMCs under different treatments. **F**) Evaluation of basal OCR. **G**) Evaluation of ECAR. n = 3/group. $^{***}p < 0.001$ vs. control group, $^{\#\#\#}p < 0.001$ vs. lysate group, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs. lysate + BPTES group

cued by supplementation of glutamate (Fig. 4A). To investigate the link between GLS1-mediated glutaminolysis and histone acetylation, we assessed histone acetylation levels (H3K9ac, H3K14ac, H3K23ac, and H3K27ac) in Th1/Th17 cells using western blotting. BPTES treatment markedly reduced H3K9ac and H3K27ac levels compared to the control group, whereas this effect was rescued by glutamate supplementation in Th1 cells (Fig. 4B). Similar results were observed in Th17 cells (Fig. 4B). Additionally, no significant difference was observed in H3K14ac or H3K23ac among these groups (Fig. 4B). ChIP assays revealed that BPTES treatment significantly reduced H3K9ac and H3K27ac enrichment at the IFN-γ and IL-17 gene promoters, while supplementation of glutamate restored these epigenetic marks (Fig. 4C). Furthermore, we evaluated the role of acetate, a key nutrient contributing to acetyl-CoA synthesis. We found that acetate supplementation counteracted BPTES-mediated suppression of acetyl-CoA production and restored IFN-γ and IL-17 expression levels in Th1 and Th17 cells, respectively (Fig. 4D, E). Additionally, acetate addition abolished BPTES-mediated reduction in H3K9ac and H3K27ac enrichment at the promoter region of IFN-γ and IL-17 (Fig. 4F). The above results indicate that GLS1-mediated glutaminolysis regulates Th1 and Th17 cell differentiation by modulating histone acetylation at key gene promoters.

Discussion

The present study demonstrated that GLS1 was upregulated in T cells from TB patients. BPTES, a GLS1 inhibitor, suppressed *M. tuberculosis* H37Rv lysate-stimulated Th1 and Th17 differentiation in PBMCs. Moreover, BPTES increased *M. tuberculosis* survival in infected macrophages. Additionally, glutamate supplementation could reverse these effects mediated by BPTES. Mechanistically, GLS1 inhibition suppressed acetyl-CoA-dependent histone acetylation at the IFN-γ and IL-17 promoters.

Macrophages, the primary host cells for *M. tuberculosis*, serve as the first line of defense against respiratory pathogens [21]. Macrophage phagocytosis of *M. tuberculosis* initiates granuloma formation, which can sequester the bacteria but also provides a niche for their persistence and reactivation [22]. *M. tuberculosis* has evolved mechanisms to survive and replicate within macrophages, thus escaping the body's immune system [23]. Glutamine is the main nitrogen source for *M. tuberculosis* [24]. Previous evidence has indicated that mycobacterium can modulate macrophage autophagy to enhance intracellular survival by manipulating GLS1-mediated glutaminolysis [25]. Consistent with this, we found that BPTES significantly increased *M. tuberculosis* survival in infected macrophages, whereas glutamate supplementation reversed this effect, indicating the critical role of GLS1-mediated glutaminolysis macrophage function.

Emerging evidence indicates that the immune response against *M. tuberculosis* infection depends on the activation of $CD4^+$ T cells, with Th1 and Th17 cells as critical effector subsets [26]. IFN-γ-producing Th1 cells defend against TB by activating macrophages and stimulating phagocytosis, phagosome maturation, and antigen presentation [27]. A strong IFN-γ-mediated Th1 immune response is considered the primary protective mechanism of anti-TB immunity [28]. Additionally, elevated plasma levels of IFN-γ have been observed in TB patients, and depressed IFN-γ production in diabetic patients is related to a decreasing immune

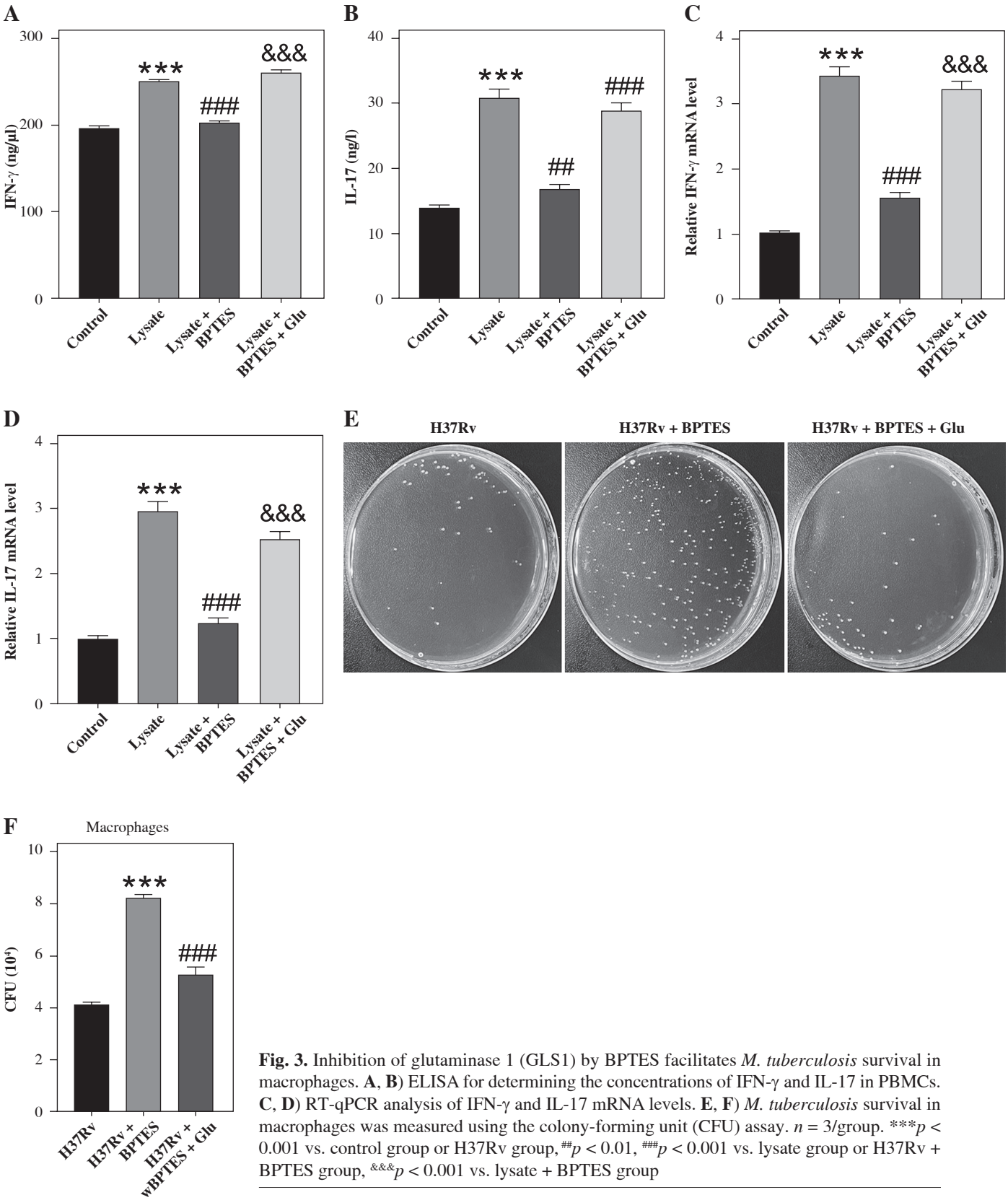

Fig. 3. Inhibition of glutaminase 1 (GLS1) by BPTES facilitates *M. tuberculosis* survival in macrophages. **A**, **B**) ELISA for determining the concentrations of IFN-γ and IL-17 in PBMCs. **C**, **D**) RT-qPCR analysis of IFN-γ and IL-17 mRNA levels. **E**, **F**) *M. tuberculosis* survival in macrophages was measured using the colony-forming unit (CFU) assay. $n = 3$/group. $^{***}p < 0.001$ vs. control group or H37Rv group, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. lysate group or H37Rv + BPTES group, $^{\&\&\&}p < 0.001$ vs. lysate + BPTES group

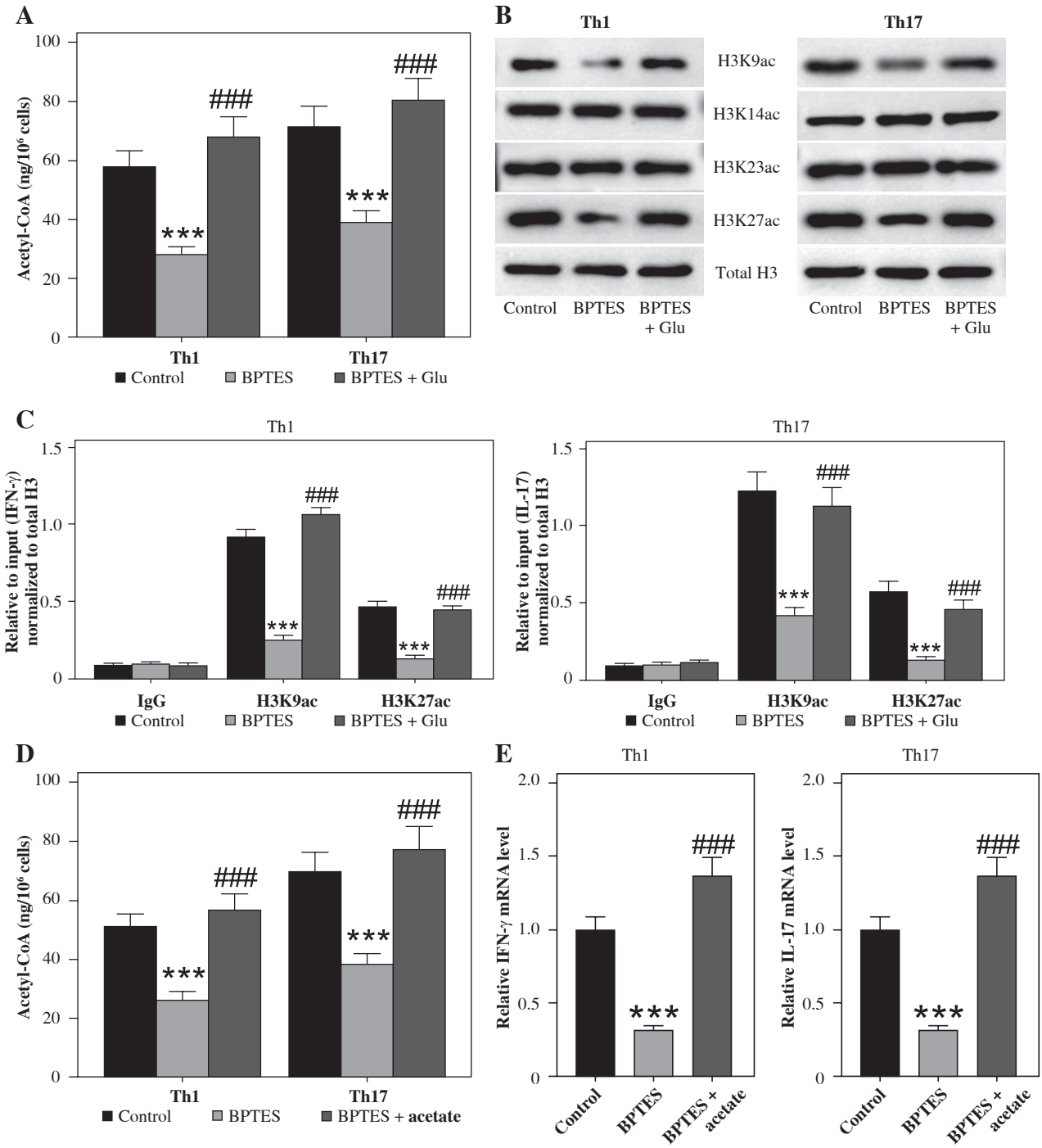

Fig. 4. Glutaminase 1 (GLS1)-mediated glutaminolysis affects Th1/Th17 cell differentiation *via* histone acetylation. **A**) Measurement of acetyl-CoA concentrations in Th1 and Th17 cells in the control, BPTES, and BPTES + glutamate groups. **B**) The levels of H3K9ac, H3K14ac, H3K23ac, and H3K27ac in Th1 and Th17 cells were evaluated by western blotting. **C**) ChIP assays for detecting H3K9ac and H3K27ac enrichment at the promoter region of IFN-γ or IL-17. **D**) Measurement of acetyl-CoA concentrations in Th1 and Th17 cells in the control, BPTES, and BPTES + acetate groups. **E**) RT-qPCR of IFN-γ and IL-17 mRNA levels in Th1 or Th17 cells, respectively. ***$p < 0.001$ vs. control group, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. BPTES group

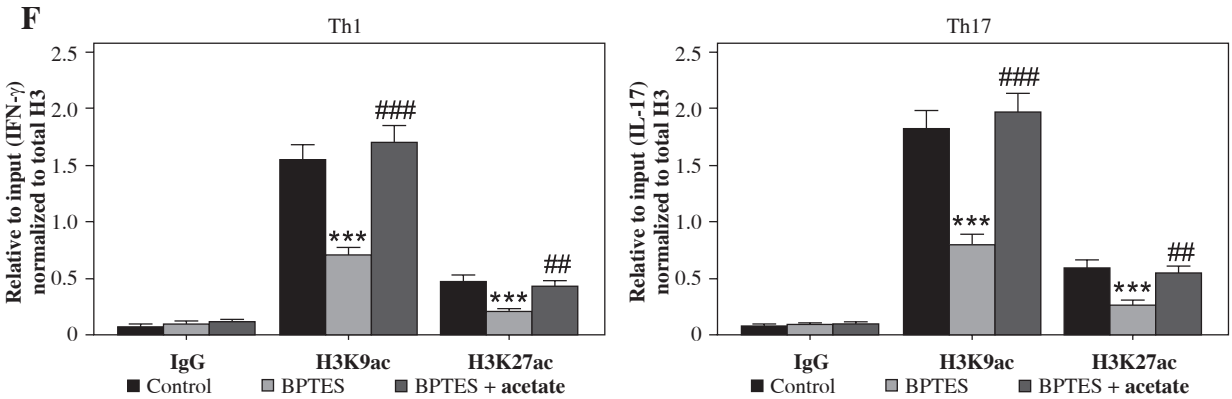

Fig. 4. Cont. **F**) ChIP assays for detecting H3K9ac and H3K27ac enrichment at the promoter region of IFN-γ or IL-17. n = 3/group. ***$p < 0.001$ vs. control group, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. BPTES group

response to *M. tuberculosis* infection [29]. However, an IFN-γ response is not an optimal correlation of protection, because IFN-γ alone is insufficient to control *M. tuberculosis* infection [30]. Th17 cells are a unique subset of effector T cells that are distinct from the Th1 and Th2 subsets [31]. They also play a key role in immunity against TB, with IL-17 as the main effector cytokine. $CD4^+IL\text{-}17^+$ T-cells play a particularly crucial role in vaccine-mediated immunity *via* prompt recruitment to the lungs, leading to early control of *M. tuberculosis* replication in infected mice [32]. A reduced Th17 response has been associated with severe outcomes of *M. tuberculosis* infection [33]. Ritter *et al*. reported that IL-17A-deficient mice exhibited high susceptibility to *M. tuberculosis* infection due to the strong accumulation of neutrophils in the infected lung tissue [34]. A previous study revealed that IFN-γ production was elevated in PBMCs stimulated with *M. tuberculosis* lysate [35]. Consistently, our study showed that the proportion of $CD4^+IFN\text{-}\gamma^+$ T cells and the expression levels of IFN-γ were significantly elevated in *M. tuberculosis* strain H37Rv lysate-stimulated PBMCs. Additionally, the lysate also induced increases in $CD4^+IL\text{-}17^+$ T cell proportion and IL-17 expression, indicating that the antigen stimulation induces immune responses of Th1 and Th17 cells.

The conversion of glutamine to glutamate catalyzed by GLS1 is critical during the differentiation of $CD4^+$ T cells [36]. It has been reported that both GLS1 shRNA and its selective inhibitor BPTES restrain Th17 differentiation [37]. Johnson *et al*. demonstrated that GLS inhibition reduced cytokine production in Th17 cells and increased Th1 cell numbers, which exhibited functional exhaustion over time [38]. A previous study also demonstrated that BPTES divergently inhibited IFN-γ-producing cells in the infiltrated $CD4^+$ T cell population [19]. Moreover, evidence suggests that cellular glutamine metabolism is involved in effective host responses against *M. tuberculosis* [39]. However, it is unclear whether GLS1-mediated glutamine metabolism affects Th1/Th17 differentiation in TB. Herein, GLS1 inhibition by BPTES led to reduction in Th1/Th17 proportions and cytokine expression, suggesting that glutaminolysis is a key metabolic checkpoint for Th1/Th17 responses. Targeting GLS1 with inhibitors such as BPTES could modulate excessive Th1/Th17-driven inflammation, potentially reducing tissue damage in severe TB. Additionally, BPTES reduced ATP production, OCR, and ECAR in *M. tuberculosis* lysate-stimulated PBMCs, reflecting impaired mitochondrial and glycolytic metabolism, which are critical for T cell proliferation and cytokine production. These effects caused by BPTES were partially reversed by glutamate supplementation, indicating the role of downstream glutamine metabolites.

Glutaminolysis provides α-ketoglutarate to fuel the TCA cycle, generating acetyl-CoA for energy and epigenetic modifications [40]. Consistently, our study revealed that the acetyl-CoA concentration was reduced following BPTES treatment. Studies have suggested that aberrant epigenetic modifications are involved in the pathogenesis of TB [41-43]. This study showed that BPTES led to a decrease in H3K9ac and H3K27ac at IFN-γ and IL-17 promoters in Th1 and Th17 cells, respectively, suggesting that GLS1-mediated acetyl-CoA production supports histone acetylation, thereby enhancing gene transcription. Additionally, our analysis was limited to promoter regions, and the genome-wide distribution of these histone modifications and their roles in enhancer regions remain unexplored. Future studies are needed to employ ChIP-seq to systematically map epigenetic marks (H3K9ac, H3K27ac, H3K4me1, and H3K4me3) in Th1 and Th17 cells under GLS1 inhibition.

In conclusion, this study demonstrated that GLS1-mediated glutaminolysis regulates TB progression by modulating Th1 and Th17 immune responses *via* acetyl-CoA-dependent epigenetic regulation. These findings highlight the role of GLS1 in TB immune imbalance and identify it as a potential therapeutic target for modulating host immunity in TB.

Acknowledgements

The authors appreciated the help of Houjie Hospital of Dongguan.

Funding

The work was supported by Dongguan Social Development Science and Technology Project in 2022 (20221800905862).

Disclosures

The study was approved by the Ethics Committee of Houjie Hospital of Dongguan (Houjie Medical Ethics Review No. 2022-007; March 22, 2022).

The authors declare no conflict of interest.

Supplementary material is available on the journal's website.

References

1. Trajman A, Campbell JR, Kunor T, et al. (2025): Tuberculosis. Lancet 405: 850-866.
2. Bouzeyen R, Javid B (2022): Therapeutic vaccines for tuberculosis: an overview. Front Immunol 13: 878471.
3. Nachiappan AC, Rahbar K, Shi X, et al. (2017): Pulmonary tuberculosis: role of radiology in diagnosis and management. Radiographics 37: 52-72.
4. Narasimhan P, Wood J, Macintyre CR, et al. (2013): Risk factors for tuberculosis. Pulm Med 2013: 828939.
5. Suárez I, Fünger SM, Kröger S, et al. (2019): The diagnosis and treatment of tuberculosis. Dtsch Arztebl Int 116: 729-735.
6. Heydari AA, Movahhede Danesh MR, Ghazvini K (2014): Urine PCR evaluation to diagnose pulmonary tuberculosis. Jundishapur J Microbiol 7: e9311.
7. Grace AG, Mittal A, Jain S, et al. (2019): Shortened treatment regimens versus the standard regimen for drug-sensitive pulmonary tuberculosis. Cochrane Database Syst Rev 12: CD012918.
8. Farhat M, Cox H, Ghanem M, et al. (2024): Drug-resistant tuberculosis: a persistent global health concern. Nat Rev Microbiol 22: 617-635.
9. Ravesloot-Chávez MM, Van Dis E, Stanley SA (2021): The innate immune response to Mycobacterium tuberculosis infection. Annu Rev Immunol 39: 611-637.
10. Dorhoi A, Reece ST, Kaufmann SH (2011): For better or for worse: the immune response against Mycobacterium tuberculosis balances pathology and protection. Immunol Rev 240: 235-251.
11. Jasenosky LD, Scriba TJ, Hanekom WA, et al. (2015): T cells and adaptive immunity to Mycobacterium tuberculosis in humans. Immunol Rev 264: 74-87.
12. Lyadova IV, Panteleev AV (2015): Th1 and Th17 cells in tuberculosis: protection, pathology, and biomarkers. Mediators Inflamm 2015: 854507.
13. Shen H, Chen ZW (2018): The crucial roles of Th17-related cytokines/signal pathways in M. tuberculosis infection. Cell Mol Immunol 15: 216-225.
14. Ou LP, Liu YJ, Qiu ST, et al. (2024): Glutaminolysis is a potential therapeutic target for kidney diseases. Diabetes Metab Syndr Obes 17: 2789-2807.
15. Chakraborty S, Khamaru P, Bhattacharyya A (2022): Regulation of immune cell metabolism in health and disease: Special focus on T and B cell subsets. Cell Biol Int 46: 1729-1746.
16. Zhang X, Wang G, Bi Y, et al. (2022): Inhibition of glutaminolysis ameliorates lupus by regulating T and B cell subsets and downregulating the mTOR/P70S6K/4EBP1 and NLRP3/caspase-1/IL-1β pathways in MRL/lpr mice. Int Immunopharmacol 112: 109133.
17. Yoshikawa S, Nagao M, Toh R, et al. (2022): Inhibition of glutaminase 1-mediated glutaminolysis improves pathological cardiac remodeling. Am J Physiol Heart Circ Physiol 322: H749-H761.
18. Xia X, Cao G, Sun G, et al. (2020): GLS1-mediated glutaminolysis unbridled by MALT1 protease promotes psoriasis pathogenesis. J Clin Invest 130: 5180-5196.
19. Fu J, Pu Y, Wang B, et al. (2022): Pharmacological inhibition of glutaminase 1 normalized the metabolic state and CD4+ T cell response in Sjogren's syndrome. J Immunol Res 2022: 3210200.
20. Livak KJ, Schmittgen TD (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408.
21. Cadena AM, Flynn JL, Fortune SM (2016): The importance of first impressions: Early events in Mycobacterium tuberculosis infection influence outcome. mBio 7: e00342-16.
22. Wang GY, Lu B, Cui X, et al. (2022): An intelligent peptide recognizes and traps Mycobacterium tuberculosis to inhibit macrophage phagocytosis. J Mater Chem B 11: 180-187.
23. Wager CML, Arnett E, Schlesinger LS (2019): Mycobacterium tuberculosis and macrophage nuclear receptors: What we do and don't know. Tuberculosis (Edinb) 116S: S98-S106.
24. Shaku MT, Ocius KL, Apostolos AJ, et al. (2023): Amidation of glutamate residues in mycobacterial peptidoglycan is essential for cell wall cross-linking. Front Cell Infect Microbiol 13: 1205829.
25. Yu J, Yan N, Gong Z, et al. (2024): Mycobacterium manipulate glutaminase 1 mediated glutaminolysis to regulate macrophage autophagy for bacteria intracellular survival. Cell Signal 124: 111422.
26. Roy A, Agnivesh PK, Sau S, et al. (2023): Tweaking host immune responses for novel therapeutic approaches against Mycobacterium tuberculosis. Drug Discov Today 28: 103693.
27. Gong W, Liang Y, Wang J, et al. (2022): Prediction of Th1 and cytotoxic T lymphocyte epitopes of Mycobacterium tuberculosis and evaluation of their potential in the diagnosis of tuberculosis in a mouse model and in humans. Microbiol Spectr 10: e0143822.
28. Luo Y, Xue Y, Lin Q, et al. (2022): CD39 pathway inhibits Th1 cell function in tuberculosis. Immunology 166: 522-538.
29. Rumende CM (2018): Risk factors for multidrug-resistant tuberculosis. Acta Med Indones 50: 1-2.
30. Park HS, Choi S, Back YW, et al. (2021): Mycobacterium tuberculosis RpfE-induced prostaglandin E2 in dendritic cells induces Th1/Th17 cell differentiation. Int J Mol Sci 22: 7535.
31. Luo W, Hu J, Xu W, et al. (2022): Distinct spatial and temporal roles for Th1, Th2, and Th17 cells in asthma. Front Immunol 13: 974066.
32. Choi HG, Kwon KW, Choi S, et al. (2020): Antigen-specific IFN-γ/IL-17-co-producing CD4(+) T-cells are the determi-

nants for protective efficacy of tuberculosis subunit vaccine. Vaccines (Basel) 8: 300.
33. Li HM, Wang LJ, Huang Q, et al. (2022): Exploring the association between Th17 pathway gene polymorphisms and pulmonary tuberculosis. Front Immunol 13: 994247.
34. Ritter K, Behrends J, Rückerl D, et al. (2022): High-dose Mycobacterium tuberculosis H37rv infection in IL-17A- and IL-17A/F-deficient mice. Cells 11: 2875.
35. Pellegrini JM, Martin C, Morelli MP, et al. (2021): PGE2 displays immunosuppressive effects during human active tuberculosis. Sci Rep 11: 13559.
36. Liu T, Ren S, Sun C, et al. (2023): Glutaminolysis and peripheral CD4(+) T cell differentiation: from mechanism to intervention strategy. Front Immunol 14: 1221530.
37. Yang L, Zheng Y, Miao YM, et al. (2022): Bergenin, a PPARγ agonist, inhibits Th17 differentiation and subsequent neutrophilic asthma by preventing GLS1-dependent glutaminolysis. Acta Pharmacol Sin 43: 963-976.
38. Johnson MO, Wolf MM, Madden MZ, et al. (2018): Distinct regulation of Th17 and Th1 cell differentiation by glutaminase-dependent metabolism. Cell 175: 1780-1795.e19.
39. Koeken V, Lachmandas E, Riza A, et al. (2019): Role of glutamine metabolism in host defense against Mycobacterium tuberculosis infection. J Infect Dis 219: 1662-1670.
40. Chisolm DA, Weinmann AS (2018): Connections between metabolism and epigenetics in programming cellular differentiation. Annu Rev Immunol 36: 221-246.
41. Khadela A, Chavda VP, Postwala H, et al. (2022): Epigenetics in tuberculosis: Immunomodulation of host immune response. Vaccines (Basel) 10: 1740.
42. Thomas SS, Abhinand K, Menon AM, et al. (2024): Epigenetic mechanisms induced by Mycobacterium tuberculosis to promote its survival in the host. Int J Mol Sci 25: 11801.
43. Wazahat R, Zaidi R, Kumar P (2024): Epigenetic regulations in Mycobacterium tuberculosis infection. Indian J Tuberc 71: 204-212.