

Research Article

Targeting Mitochondrial Metabolic Reprogramming: Reversing CD8⁺ T Cell Exhaustion and Improving Cancer Immunotherapy Response

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Abstract

This study explores the reversal effect of targeting mitochondrial metabolic reprogramming on CD8⁺ T cell exhaustion within the tumor microenvironment and its synergistic mechanism when combined with immune checkpoint blockade therapy. B16-OVA melanoma and LLC lung cancer tumor-bearing mouse models were established. Using single-cell RNA sequencing, metabolic flux analysis, Seahorse cellular energy metabolism assay, and flow cytometry, we compared the mitochondrial function, metabolic characteristics, and exhaustion phenotypes of tumor-infiltrating CD8⁺ T cells with CD8⁺ T cells from the spleen/draining lymph nodes. By pharmacologically activating or overexpressing genes to intervene in the PGC-1 α -mediated mitochondrial biogenesis pathway, we evaluated CD8⁺ T cell mitochondrial membrane potential, ROS levels, ATP production, oxygen consumption rate, and the ability to secrete IFN- γ , TNF- α , and Granzyme B. Further, combined treatment with anti-PD-1 was applied to observe tumor growth, survival, and the composition of tumor-infiltrating lymphocytes. Tumor-infiltrating CD8⁺ T cells exhibited reduced mitochondrial membrane potential, accumulated ROS, decreased oxygen consumption rate and ATP production, and showed increased expression of PD-1, TIM-3, LAG-3, and TOX, suggesting that metabolic dysfunction is closely related to T cell exhaustion. Targeting mitochondrial metabolic reprogramming can restore mitochondrial adaptability of CD8⁺ T cells, enhance their proliferation, cytokine secretion, and cytotoxicity, while reducing expression of exhaustion-associated molecules. The combination of this intervention with anti-PD-1 antibody significantly inhibited tumor growth, extended survival in tumor-bearing mice, and increased the proportion of stem-like/effector memory CD8⁺ T cells in tumors. Mitochondrial metabolic

reprogramming is a key regulatory node for reversing CD8⁺ T cell exhaustion. Targeting this pathway can remodel the tumor immune microenvironment and enhance the response to immune checkpoint therapy, providing a potential new strategy for cancer immunotherapy.

Keywords

Immunometabolism, Mitochondrial metabolic reprogramming, CD8⁺ T cell exhaustion, Cancer immunotherapy, Immune checkpoint inhibitors, PGC-1 α

1. Introduction

Cancer immunotherapy, especially the use of immune checkpoint inhibitors (ICIs), has significantly changed the treatment landscape for various malignant tumors. However, only a subset of patients can achieve lasting clinical benefits, and primary or acquired resistance remains a key bottleneck limiting their wider use. The tumor microenvironment (TME) is a dynamic ecosystem made up of tumor cells, immune cells, stromal cells, and metabolic products, and its composition and evolution play an important role in treatment response (Liu, 2026). In anti-tumor immune responses, CD8⁺ T cells are key players that recognize and kill tumor cells. Their number, functional state, and persistence directly determine how effective ICB therapy is. So, figuring out what controls CD8⁺ T cell exhaustion in the TME and finding treatable targets is really important both scientifically and clinically.

T cell exhaustion is the result of chronic antigen stimulation combined with an immunosuppressive tumor microenvironment (TME). It's characterized by the sustained high expression of inhibitory receptors like PD-1, TIM-3, and LAG-3, the activation of exhaustion-related transcription programs such as TOX, and the gradual decline in proliferation, cytokine secretion, and cytotoxic functions. In recent years, research on immune metabolism has shown that T cell exhaustion is not only regulated by transcription and epigenetics but also closely related to poor metabolic adaptation. Tumor-infiltrating CD8⁺ T cells often show mitochondrial dysfunction, including reduced mitochondrial membrane potential, accumulation of reactive oxygen species (ROS), decreased oxygen consumption rate (OCR), insufficient ATP production, and impaired oxidative phosphorylation (OXPHOS), which ultimately prevents them from meeting the energy and metabolic needs required for their effector functions. Besides mitochondrial metabolism, gut microbiota-derived butyrate can enhance anti-tumor immunity by inducing trained immunity in intestinal macrophages (Liu et al., 2026a); Mitochondrial DNA sensing can reshape the tumor immune microenvironment by jointly activating the cGAS-STING pathway and ferroptosis (Liu et al., 2026b). So, targeting T cell metabolic defects, especially mitochondrial metabolic reprogramming, has become a potential new way to reverse T cell exhaustion and boost immune therapy responses.

PGC-1 α (PPAR γ coactivator 1 α) is a key transcriptional coactivator that regulates mitochondrial biogenesis, antioxidant defense, and energy metabolism, playing an important role in maintaining T cell metabolic adaptability and memory formation. Previous studies suggest that enhancing PGC-1 α -mediated mitochondrial function can improve T cell persistence and anti-tumor ability. However, is mitochondrial metabolic reprogramming in tumor-infiltrating CD8⁺ T cells enough to reverse their exhausted phenotype? Can this process remodel the tumor microenvironment and boost the efficacy of anti-PD-1 therapy? Most current studies are still limited to in vitro experiments or descriptive analyses, lacking systematic in vivo intervention evidence and evaluations of combination therapies. These questions haven't been fully answered yet and are the core scientific issues of this study.

Based on this, the study proposes the following hypothesis: targeting mitochondrial metabolic reprogramming can reverse exhaustion in tumor-infiltrating CD8⁺ T cells by restoring PGC-1 α -mediated mitochondrial biogenesis and metabolic adaptation, enhance their effector functions, and improve the response to immune checkpoint blockade therapy. To test this hypothesis, the study plans to establish B16-OVA melanoma and LLC lung cancer mouse models, and use a combination of single-cell RNA sequencing, metabolic flux analysis, Seahorse cell energy metabolism assays, and flow cytometry to systematically compare mitochondrial function and exhaustion phenotypes between tumor-infiltrating CD8⁺ T cells and peripheral CD8⁺ T cells. Further, by intervening in the PGC-1 α pathway through pharmacological agonists or gene overexpression, the effects on CD8⁺ T cell mitochondrial membrane potential, ROS levels, ATP production, OCR, and secretion of IFN- γ , TNF- α , and granzyme B will be evaluated, along with tumor growth, survival, and tumor-infiltrating lymphocyte composition in combination with anti-PD-1 antibody. This study aims to clarify the role and mechanism of mitochondrial metabolic reprogramming in regulating CD8⁺ T cell exhaustion, providing new metabolic targets and combination therapy strategies for tumor immunotherapy.

2. Literature Review

2.1. Clinical breakthroughs and resistance challenges of immune checkpoint blockade

Immune checkpoint blockade (ICB), represented by anti-CTLA-4 and anti-PD-1/PD-L1 antibodies, has become an important treatment for various cancers. It works by lifting the brakes on T cells and restoring anti-tumor immune responses, significantly improving survival outcomes for some patients (Topalian et al., 2015). However, in clinical practice, only about 20%–40% of patients can get lasting benefits, and primary resistance, adaptive resistance, and acquired resistance are still the main bottlenecks limiting the widespread use of ICB (Sharma et al., 2017). Current research shows that the mechanisms behind ICB resistance involve multiple factors, such as defects in tumor antigen

presentation, abnormalities in the IFN- γ signaling pathway, reshaping of the immunosuppressive tumor microenvironment (TME), and T cell exhaustion (Jenkins et al., 2018). So, digging into how T cell exhaustion is regulated and coming up with combination therapies to reverse it has become a key scientific issue that urgently needs to be solved in tumor immunology. In recent years, spatial AI and multimodal foundation models have also been used to predict responses to ICB and the effectiveness of neoantigen vaccines (Liu et al., 2026c; Liu et al., 2026d).

2.2. CD8⁺ T cell exhaustion: from phenotype to metabolism

CD8⁺ T cells are the main effector cells that drive anti-tumor immune responses. Under continuous stimulation by chronic antigens and the immunosuppressive tumor microenvironment, CD8⁺ T cells gradually become exhausted, showing persistent high levels of inhibitory receptors like PD-1, TIM-3, and LAG-3. Their secretion of effector cytokines decreases, their killing ability drops, and their capacity to proliferate is impaired (Wherry & Kurachi, 2015). In 2019, Blank and others (2019) systematically reviewed the definition of T cell exhaustion, emphasizing that exhaustion is a progressive dysfunction driven by transcriptional, epigenetic, and metabolic programs. In tumors, exhausted CD8⁺ T cells often show functional heterogeneity, including both terminally exhausted subsets and progenitor exhausted subsets with stem-like characteristics (Thommen & Schumacher, 2018). McLane and others (2019) further pointed out that CD8⁺ T cell exhaustion in chronic viral infections and tumors shares similar molecular features, but metabolic stress and inhibitory signals in the TME might speed up their functional decline. Hashimoto and others (2018) systematically summarized the intervention opportunities for exhausted T cells, including immune checkpoint blockade, metabolic regulation, and genetic engineering, among others. These studies suggest that T cell exhaustion isn't an irreversible end state, but a dynamic process that can potentially be intervened.

2.3. Mitochondrial dysfunction is a key factor in T cell exhaustion

T cell activation, proliferation, and effector functions heavily rely on metabolic reprogramming. Pearce and others (2013) pointed out that lymphocytes need to coordinate glycolysis, oxidative phosphorylation (OXPHOS), fatty acid oxidation, and amino acid metabolism in different functional states to meet energy and biosynthesis demands. Buck and others (2016) found that changes in mitochondria control T cell fate through metabolic programming-mitochondrial fusion promotes the formation of memory T cells, while mitochondrial fission is linked to effector T cell differentiation. Van der Windt and others (2012) further confirmed that mitochondrial respiratory capacity is a key regulator for CD8⁺ T cell memory development, and insufficient respiratory ability can lead to impaired memory T cell formation. In the tumor microenvironment, Scharping and others (2016) found that the TME can suppress mitochondrial biogenesis in tumor-infiltrating T cells, leading to metabolic insufficiency and functional impairment, while overexpressing PGC-1 α can partially restore

T cell mitochondrial function. Bengsch and others (2016) revealed that PD-1 signaling can induce bioenergetic defects in CD8⁺ T cells, and this metabolic abnormality is an early driver of exhaustion. Patsoukis and others (2015) further show that PD-1 can inhibit glycolysis and promote lipolysis and fatty acid oxidation, thereby changing the metabolic fate of T cells. In addition, Vardhana and others (2020) found that continuous antigen exposure can damage mitochondrial OXPHOS and limit T cell self-renewal ability; Yu and others (2020) reported that mitochondrial dysfunction in tumor-infiltrating CD8⁺ T cells can enhance the exhaustion phenotype; Scharping and others (2021) further confirmed that hypoxia and continuous stimulation-induced mitochondrial stress can quickly drive T cell exhaustion. The studies above collectively show that mitochondrial dysfunction is not just a side effect of T cell exhaustion, but a key driver of its development. In addition, the release and sensing of mitochondrial DNA can work together with ferroptosis to reshape the tumor microenvironment through cGAS-STING dependent immune signals (Liu et al., 2026b).

2.4. PGC-1 α -mediated mitochondrial biogenesis and T cell adaptation

PGC-1 α (PPAR γ coactivator 1 α) is a key transcription coactivator that regulates mitochondrial biogenesis, oxidative phosphorylation, and antioxidant defense. Handschin and Spiegelman (2006) reviewed the central role of PGC-1 coactivators in energy balance and metabolic regulation, pointing out that it is an important hub linking transcription programs with mitochondrial function. In T cells, Dumauthioz and colleagues (2021) found that forcing the expression of PGC-1 α can boost CD8⁺ T cell fitness, memory formation, and anti-tumor immunity, suggesting that enhancing mitochondrial biogenesis might improve T cell persistence and effector function. However, there's still a lack of solid in vivo evidence on whether the PGC-1 α pathway in tumor-infiltrating CD8⁺ T cells is enough to reverse an already established exhausted state, and whether combining it with immune checkpoint blockade can create a synergistic effect.

2.5. Research progress on targeting mitochondrial metabolism combined with immune checkpoint therapy

Given the central role of mitochondrial metabolism in T cell function, targeting metabolic pathways to boost ICB therapy has become a research hotspot. Chamoto and colleagues (2017) found that mitochondrial-activating drugs can work together with PD-1 blockade to enhance anti-tumor activity through T cell-dependent mechanisms. This study provides proof of concept for the combined strategy of 'metabolic intervention plus immune checkpoint blockade.' However, most existing studies on combination therapies focus on pharmacological activators or short-term interventions. There's still a lack of thorough evaluation on the causal role, molecular mechanisms, and clinical translation potential of PGC-1 α -mediated mitochondrial metabolic reprogramming in reversing exhaustion of tumor-infiltrating CD8⁺ T cells.

2.6. Single-cell technology reveals T cell states and ICB responses

In recent years, single-cell RNA sequencing has been a powerful tool for understanding the heterogeneity of tumor-infiltrating T cells. Sade-Feldman and colleagues (2018) used single-cell transcriptome analysis to define T cell states linked to ICB responses in melanoma patients and found that certain T cell subpopulations can predict treatment outcomes. Miller and others (2019) further showed that different subgroups of exhausted CD8⁺ T cells play distinct roles in tumor control and the response to checkpoint blockade. Siddiqui and others (2019) found that Tcf1⁺PD-1⁺CD8⁺ T cells within tumors have stem cell-like characteristics and can help control tumors after vaccine and checkpoint blockade treatments. These studies suggest that accurately identifying and targeting T cell subsets with repair potential is an important way to improve ICB therapy. At the same time, explainable spatial AI can analyze tumor-immune interactions and predict immunotherapy outcomes, and multimodal foundational models can predict neoantigen immunogenicity and vaccine effectiveness.

2.7. Limitations of existing research and the contribution of this study

In summary, although a lot of research has shown the close link between T cell exhaustion and mitochondrial metabolic disorders, there are still some gaps: most studies focus on descriptive associations or in vitro models, lacking in vivo evidence that systematically validates mitochondrial metabolic reprogramming in reversing CD8⁺ T cell exhaustion; the functional role of PGC-1 α -mediated mitochondrial biogenesis in tumor-infiltrating CD8⁺ T cells and its synergy with anti-PD-1 therapy is still unclear; current metabolic intervention strategies mostly target a single metabolic pathway, without integrated analysis of mitochondrial function, T cell status, and TME remodeling. Based on this, our study aims to focus on the core question of ‘targeting mitochondrial metabolic reprogramming to reverse CD8⁺ T cell exhaustion and boost tumor immunotherapy response.’ By combining single-cell RNA sequencing, metabolic flux analysis, Seahorse energy metabolism testing, and flow cytometry, we will systematically assess the impact of PGC-1 α pathway interventions on CD8⁺ T cell mitochondrial function, exhaustion phenotype, and anti-tumor immunity, and further explore its enhancing effect when combined with anti-PD-1 antibody treatment. This study is expected to provide new metabolic intervention targets and combination therapy strategies for tumor immunotherapy.

3. Materials and Methods

3.1. Experimental animals and cell lines

Female C57BL/6J mice aged 6-8 weeks were purchased from Beijing Vital River Laboratory

Animal Technology Co., Ltd., and were kept in an SPF-level barrier environment at $22 \pm 2^{\circ}\text{C}$ and $50\% \pm 10\%$ humidity, with a 12-hour light/dark cycle, having free access to food and water. B16-OVA melanoma cells and LLC lung cancer cells were stored in our lab. The cells were cultured in high-glucose DMEM (Gibco) containing 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin, and 100 $\mu\text{g/mL}$ streptomycin, in a 37°C , 5% CO_2 incubator. Cells were regularly checked for mycoplasma contamination and authenticated via short tandem repeat (STR) profiling.

3.2. Tumor model construction and grouping

Resuspend log-phase B16-OVA or LLC cells in PBS and adjust the concentration to 1×10^7 cells/mL. Take 100 μL (containing 1×10^6 cells) and inject subcutaneously into the right flank of the mice. Observe daily after injection, and once the tumors are palpable (about 5-7 days), randomly assign the mice into four groups ($n=6-8$ per group) using a random number table: control group (vehicle), PGC-1 α agonist group, anti-PD-1 group, and combination group.

The PGC-1 α agonist ZLN005 (MedChemExpress) was injected intraperitoneally at a dose of 10 mg/kg once a day for 14 consecutive days; the control group was given an equal volume of solvent (physiological saline containing 0.5% DMSO, 30% PEG300, and 5% Tween-80). The anti-PD-1 antibody (clone RMP1-14, BioXCell) was injected intraperitoneally at 200 μg per mouse every 3 days, for a total of 4 doses; the control group received the isotype control antibody (clone 2A3). The combination group received both ZLN005 and anti-PD-1 treatments at the same time. For the gene overexpression verification experiment, there was an additional CD8 $^{+}$ T cell adoptive transfer group: CD8 $^{+}$ T cells were isolated from the spleens of WT mice, activated with anti-CD3/CD28 antibodies for 48 hours, then transduced with either PGC-1 α overexpression lentivirus (pLVX-PGC-1 α) or an empty vector control, expanded, and transferred back into tumor-bearing mice via tail vein injection (5×10^6 cells per mouse).

3.3. Tumor growth monitoring and survival analysis

Self-treatment started, and the tumor's long and short diameters were measured every 2–3 days using calipers. Tumor volume was calculated using the formula $V = 0.5 \times \text{long diameter} \times \text{short diameter}^2$. Euthanasia was carried out if the tumor volume exceeded 2000 mm^3 or if the mice showed severe weight loss or mobility issues. Survival time was calculated from the day of inoculation to death or euthanasia. Tumor growth curves and Kaplan-Meier survival curves were plotted.

3.4. Single-cell RNA sequencing

On day 10 of treatment, tumor tissues from each group of mice were collected, chopped up, and digested at 37°C for 30 minutes using collagenase IV (1 mg/mL, Sigma-Aldrich) and DNase I (100 $\mu\text{g/mL}$, Roche). A single-cell suspension was prepared using a 70 μm filter. Immune cells were

enriched using CD45⁺ magnetic beads (Miltenyi Biotec) and CD45⁺CD3⁺CD8⁺ T cells were further sorted by flow cytometry. Once cell viability was over 90%, single-cell libraries were constructed using the 10x Genomics Chromium Controller and sequenced on an Illumina NovaSeq 6000.

The raw data was aligned to the mouse reference genome (mm10) using Cell Ranger 6.0 (10x Genomics). Quality control was done with Seurat 4.0: cells with more than 200 but fewer than 5000 detected genes and less than 20% mitochondrial gene content were kept. After LogNormalize normalization, highly variable genes (n=2000) were selected for principal component analysis (PCA), and UMAP was used for dimensionality reduction and visualization, with clustering at a resolution of 0.5. Differentially expressed genes were identified using FindMarkers (Wilcoxon rank-sum test). GO and KEGG enrichment analysis was performed with clusterProfiler. Pseudotime analysis was done using Monocle 3. AUCell was used to score mitochondrial metabolism gene sets and T cell exhaustion gene sets.

3.5. Metabolic analysis

Seahorse extracellular flux analysis: Sort CD8⁺ T cells and seed 2×10^5 cells per well on a Seahorse XF96 cell culture plate (Agilent). Equilibrate for 1 hour using XF RPMI medium (with 1 mM pyruvate, 2 mM glutamine, 10 mM glucose, pH 7.4). Sequentially add 1 μ M oligomycin, 1.5 μ M FCCP, and 0.5 μ M rotenone/antimycin A, then measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using the Seahorse XF96 analyzer. Calculate basal respiration, maximal respiration, ATP production, and spare respiratory capacity.

Metabolic flux analysis: CD8⁺ T cells were co-incubated with ¹³C₆-glucose (10 mM) or ¹³C₅-glutamine (2 mM) for 6 hours, metabolites were extracted, and isotopic labeling was analyzed using gas chromatography-mass spectrometry (GC-MS, Agilent 7890A/5975C).

Mitochondrial function testing: Use JC-1 staining to check mitochondrial membrane potential, MitoSOX Red to detect mitochondrial ROS, MitoTracker Green for mitochondrial mass, and an ATP detection kit (Promega) to measure ATP levels. All samples were analyzed using flow cytometry or a fluorescence plate reader.

3.6. Flow cytometry

Tumor tissues were digested to prepare single-cell suspensions, and Live/Dead Fixable Aqua (Invitrogen) was used to exclude dead cells. Surface antibodies included: anti-CD45.2 (104), anti-CD3 (145-2C11), anti-CD8 (53-6.7), anti-PD-1 (RMP1-30), anti-TIM-3 (RMT3-23), anti-LAG-3 (C9B7W), anti-CD44 (IM7), anti-CD62L (MEL-14), anti-CD127 (A7R34), and anti-KLRG1 (2F1). For intracellular factor staining, cells were stimulated with PMA (50 ng/mL) and ionomycin (1 μ g/mL) for 4 hours, with BFA (10 μ g/mL) added to block secretion. After fixation and permeabilization, cells were stained with anti-IFN- γ (XMG1.2), anti-TNF- α (MP6-XT22), anti-Granzyme B (NGZB), and

anti-Ki-67 (SolA15). For transcription factor staining, FoxP3/transcription factor staining buffer (eBioscience) was used with anti-TOX (TXRX10), anti-Tcf1 (C63D9), and anti-PGC-1 α (EPR18216). All antibodies were purchased from BioLegend, BD Biosciences, or eBioscience. Data were collected on a BD FACSCanto II or Fortessa flow cytometer and analyzed with FlowJo 10.8.

3.7. Real-time quantitative PCR

Total RNA was extracted from cells using TRIzol (Invitrogen), and the concentration was measured with a Nanodrop. 1 μ g of RNA was reverse-transcribed into cDNA using the PrimeScript RT kit (TaKaRa). qPCR was performed on a QuantStudio 5 system using SYBR Green PCR Master Mix (Applied Biosystems). The primer sequences are as follows: PGC-1 α F: 5'-TATGGAGTGACATAGAGTGTGCT-3', R: 5'-CCACTTCAATCCACCCAGAAAG-3'; TFAM F: 5'-GGAATGTGGAGCGTGCTAAA-3', R: 5'-CAGTGACAATCAGCCCAACG-3'; NRF1 F: 5'-AGCACGGAGTGACCCAAAC-3', R: 5'-TGTACGTGGCTACATGGACCT-3'; COX4 F: 5'-TGGTGCCTTTGGAGGTGTT-3', R: 5'-CAGCAGGTGGAAGAGCAGTT-3'; ATP5 F: 5'-GCTGGTGGTGCTGGGATACT-3', R: 5'-CCAGGTCAGGGTCACAAAGG-3'; Pcdcl F: 5'-ACCCTGGTCATTCACTTGGG-3', R: 5'-CATTTGCTCCCTCTGACACTG-3'; Havcr2 F: 5'-TCAGGTCTTACCCTCAACTGTG-3', R: 5'-GGCAGATAGGCATTGGGGA-3'; Lag3 F: 5'-CTGGGACTGCTTTGCTACCT-3', R: 5'-GGAGGTGGTCAGGTTGTTGT-3'; Tox F: 5'-TACCCAGGCTCCATCTTCAC-3', R: 5'-GCTGGAGAGAGTGGGTGTGT-3'; housekeeping gene Gapdh F: 5'-AGGTCGGTGTGAACGGATTTG-3', R: 5'-TGTAGACCATGTAGTTGAGGTCA-3'. Relative expression levels were calculated using the 2- $\Delta\Delta$ Ct method.

3.8. Western blot

Cells were lysed using RIPA buffer (with protease and phosphatase inhibitors), and protein concentration was measured using the BCA method. 30 μ g of protein was separated by SDS-PAGE and transferred to a PVDF membrane, then blocked with 5% skim milk for 1 hour. Primary antibodies were incubated overnight at 4°C: anti-PGC-1 α (1:1000, ab54481, Abcam), anti-OXPHOS (1:1000, ab110413, Abcam), anti-TOX (1:1000, ab237631, Abcam), anti- β -actin (1:5000, AC-15, Sigma). After washing with TBST, membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 1 hour, developed with ECL chemiluminescence, imaged using the Tanon 5200 system, and quantified with ImageJ.

3.9. Cytokine testing

Collect mouse serum and tumor tissue homogenate supernatant, and use ELISA kits (R&D Systems or BioLegend) to detect the levels of IFN- γ , TNF- α , IL-2, and Granzyme B, following the

instructions carefully.

3.10. Histology and immunofluorescence

Tumor tissues were fixed with 4% paraformaldehyde and embedded in paraffin, then sectioned at 5 μm . HE staining was used to observe tissue morphology. For immunofluorescence: after antigen retrieval, sections were blocked with 5% BSA and incubated overnight at 4°C with primary antibodies: anti-CD8 (1:200, 53-6.7, BioLegend), anti-PD-1 (1:200, RMP1-30), anti-PGC-1 α (1:200, ab54481), and anti-Tom20 (1:200, ab186735). The next day, Alexa Fluor 488/594-labeled secondary antibodies were incubated at room temperature for 1 hour, followed by DAPI counterstaining and mounting with anti-fade. Images were captured using a Zeiss LSM 880 confocal microscope and fluorescence intensity was analyzed with ImageJ.

3.11. Data analysis and statistics

The sample size was estimated based on the effect size from a pilot experiment ($\alpha = 0.05$, power = 0.80), with 6-8 animals per group. Animals were randomly assigned to groups, and data collection and analysis were done in a blinded manner. Data are presented as mean $\pm$ standard deviation (for normally distributed data) or median (interquartile range, for non-normally distributed data). Statistical analyses were performed using GraphPad Prism 9.0, SPSS 26.0, and R 4.0. Normality was tested with the Shapiro-Wilk test. For two-group comparisons, independent t-tests or Mann-Whitney U tests were used; for multiple-group comparisons, one-way ANOVA or Kruskal-Wallis tests were used, followed by Tukey or Dunn post hoc tests. Repeated measures data were analyzed with two-way ANOVA. Survival analysis was done using the Kaplan-Meier method, with differences between groups compared by the log-rank test. Single-cell data were analyzed using Seurat, Monocle 3, and clusterProfiler. $P < 0.05$ was considered statistically significant.

4. Research Results

4.1. Tumor-infiltrating CD8⁺ T cells show mitochondrial dysfunction and exhaustion phenotypes

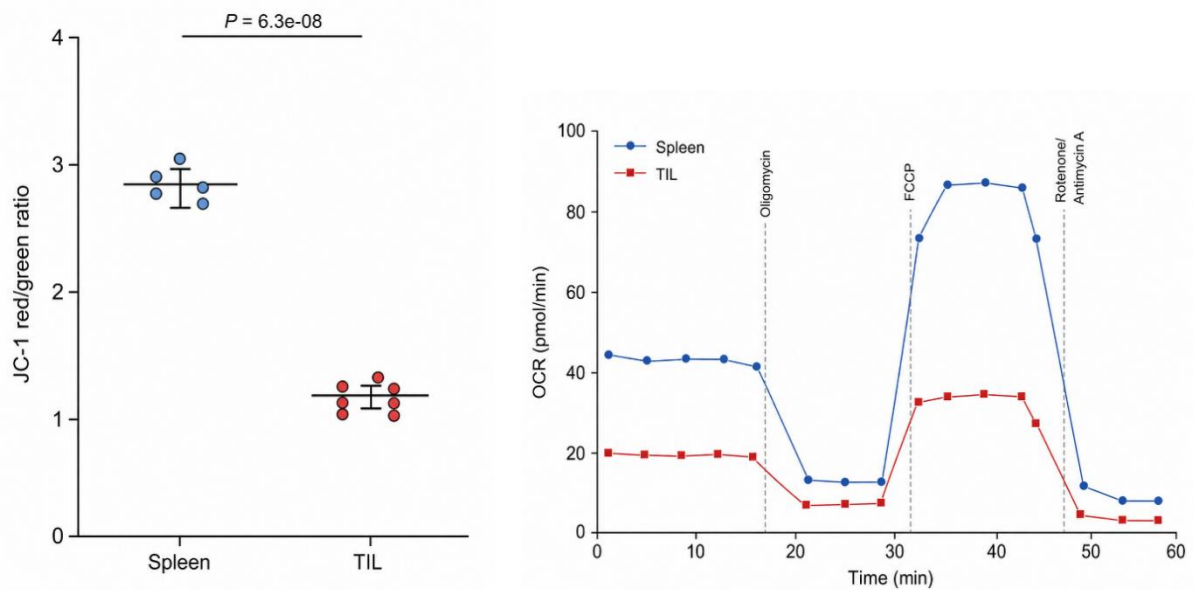
To figure out how the tumor microenvironment affects CD8⁺ T cell metabolism and function, we compared the mitochondrial function and exhaustion markers of tumor-infiltrating CD8⁺ T cells with those in the spleens of B16-OVA tumor-bearing mice. Flow cytometry and Seahorse analysis showed that, compared to splenic CD8⁺ T cells, tumor-infiltrating CD8⁺ T cells had a significantly lower mitochondrial membrane potential, higher mitochondrial ROS accumulation, reduced ATP production, and markedly lower basal and maximal oxygen consumption rates (Figure 1A-C, Table 1A, Table 1C). At the same time, the proportion of PD-1, TIM-3, and LAG-3 expression and the mean fluorescence

intensity of TOX were significantly higher in tumor-infiltrating CD8⁺ T cells, while their ability to secrete IFN- γ , TNF- α , and granzyme B was significantly reduced (Figure 1D-F, Table 1E, Table 1F). These results suggest that tumor-infiltrating CD8⁺ T cells show clear mitochondrial metabolic dysregulation along with functional exhaustion.

Figure 1

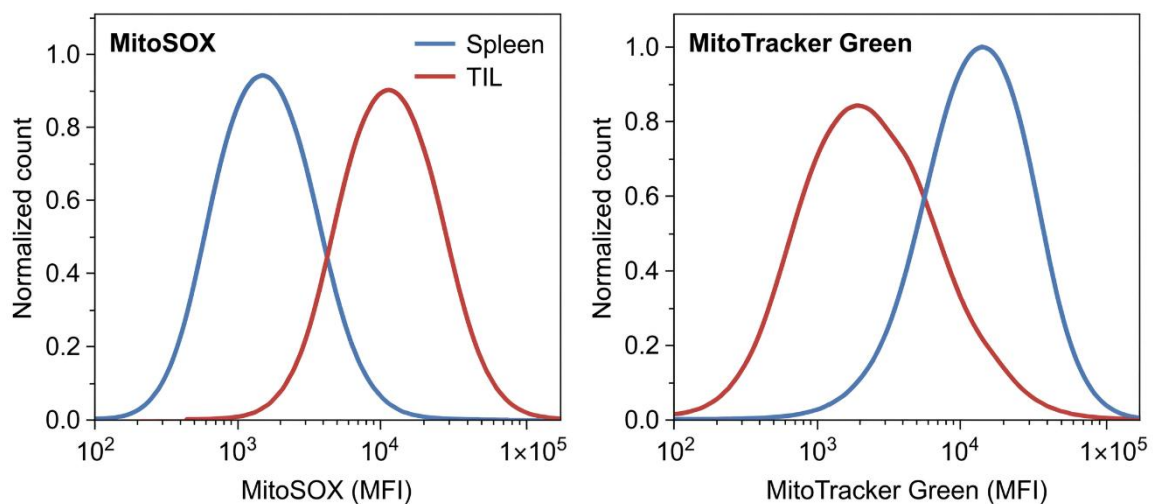
A: JC-1 red/green ratio (spleen vs TIL)

C: Seahorse OCR curves



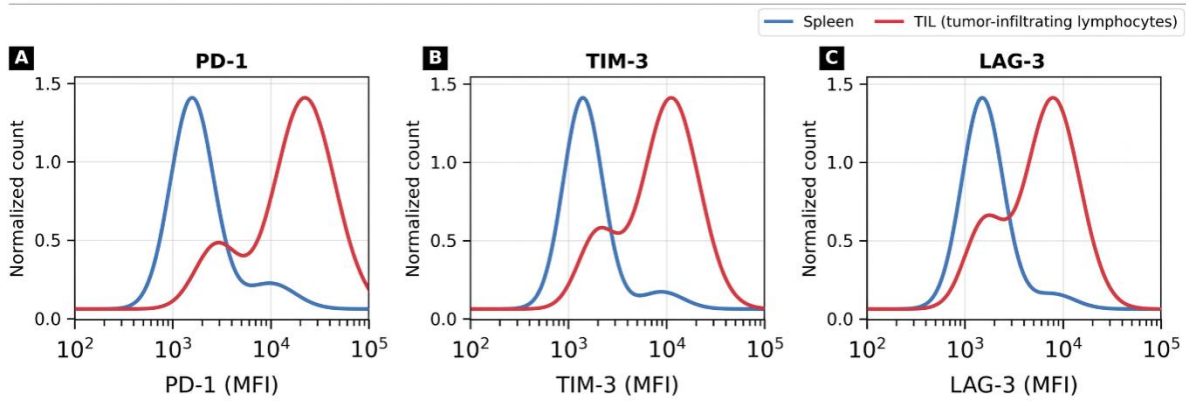
B: Flow histograms of MitoSOX and MitoTracker Green

Figure 1B Representative flow cytometry histograms of MitoSOX and MitoTracker Green

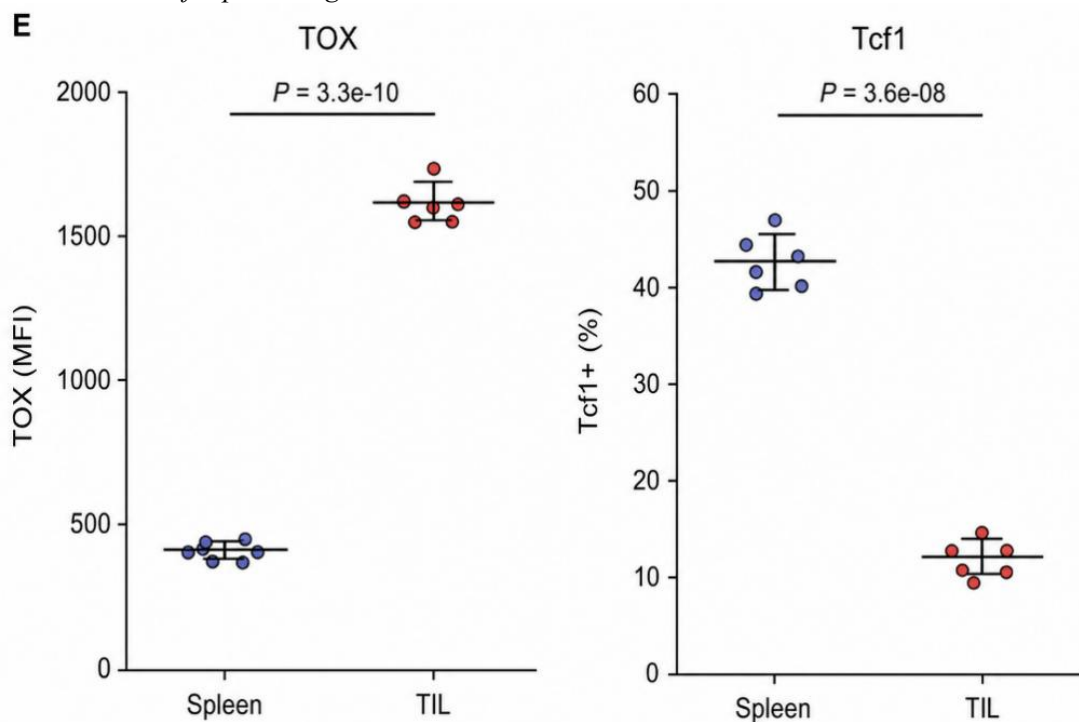


D: Flow histograms of PD-1, TIM-3, LAG-3

Figure 1D — Representative flow histograms of PD-1, TIM-3, LAG-3 — Spleen vs TIL



E: TOX MFI and Tcf1⁺ percentage



F: IFN- γ , TNF- α , Granzyme B

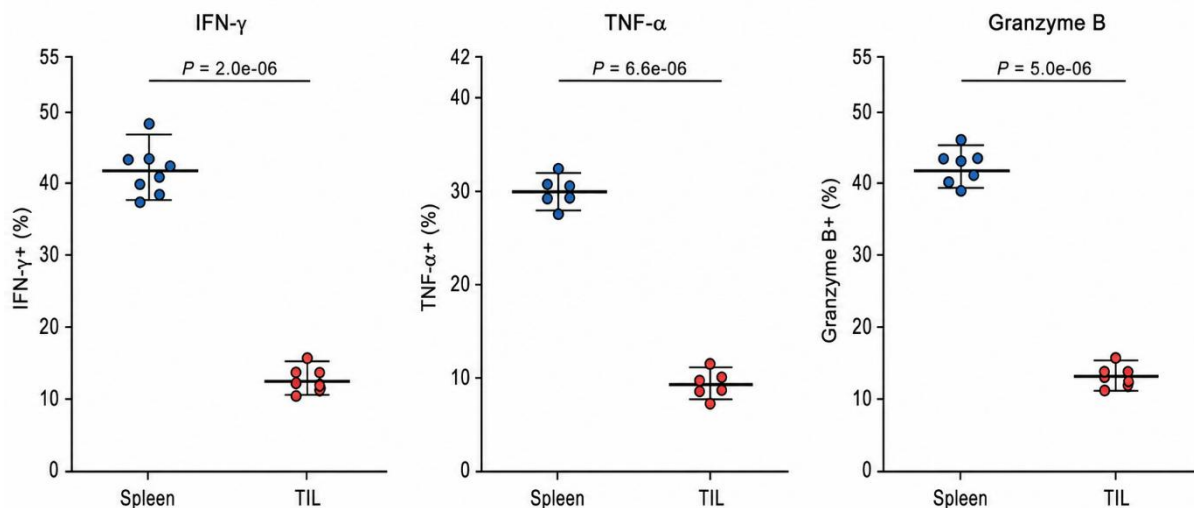


Table 1A

JC-1 red/green ratio

Group	Value (n=6)	Mean	standard deviation
Spleen	2.79, 2.91, 2.68, 2.85, 3.02, 2.79	2.84	0.12
TIL	1.09, 1.18, 1.24, 1.02, 1.14, 1.11	1.13	0.08

Table 1C

Seahorse OCR curves

Time (min)	Spleen OCR (pmol/min)	TIL OCR (pmol/min)
0	43.1 ± 0.4	20.2 ± 0.35
5	41.8 ± 0.4	19.4 ± 0.35
10	42.9 ± 0.4	20.1 ± 0.35
15	11.8 ± 0.35	6.9 ± 0.30
20	12.4 ± 0.35	7.3 ± 0.30
25	11.6 ± 0.35	6.8 ± 0.30
30	85.2 ± 1.0	33.8 ± 0.8
35	87.4 ± 1.0	34.6 ± 0.8
40	86.3 ± 1.0	34.0 ± 0.8
45	5.1 ± 0.2	3.2 ± 0.18
50	4.8 ± 0.2	3.0 ± 0.18
55	5.3 ± 0.2	3.1 ± 0.18

Table 1E

TOX MFI and Tcfl⁺ percentage

Indicator	Group	Basic array (n=6)	Adjusted mean
TOX MFI	Spleen	410, 428, 435, 402, 441, 412	421.5
TOX MFI	TIL	1572, 1601, 1548, 1623, 1589, 1591	1587.4
Tcfl ⁺ (%)	Spleen	41.2, 44.8, 39.6, 46.1, 42.7, 40.9	42.6
Tcfl ⁺ (%)	TIL	11.4, 12.8, 9.7, 13.2, 10.6, 11.9	11.6

Table1F

IFN- γ , TNF- α , Granzyme B

Indicator	Spleen value (n=6)	TIL score (n=6)
IFN- γ ⁺ (%)	37.1, 39.4, 41.2, 36.8, 40.1, 38.4	12.1, 13.4, 11.2, 14.6, 12.8, 11.9
TNF- α ⁺ (%)	28.5, 30.2, 31.1, 27.6, 29.8, 28.9	9.2, 10.6, 8.8, 11.1, 9.7, 9.4
Granzyme B ⁺ (%)	40.2, 42.6, 43.1, 39.4, 41.8, 40.5	13.1, 14.4, 12.2, 15.0, 13.6, 12.7

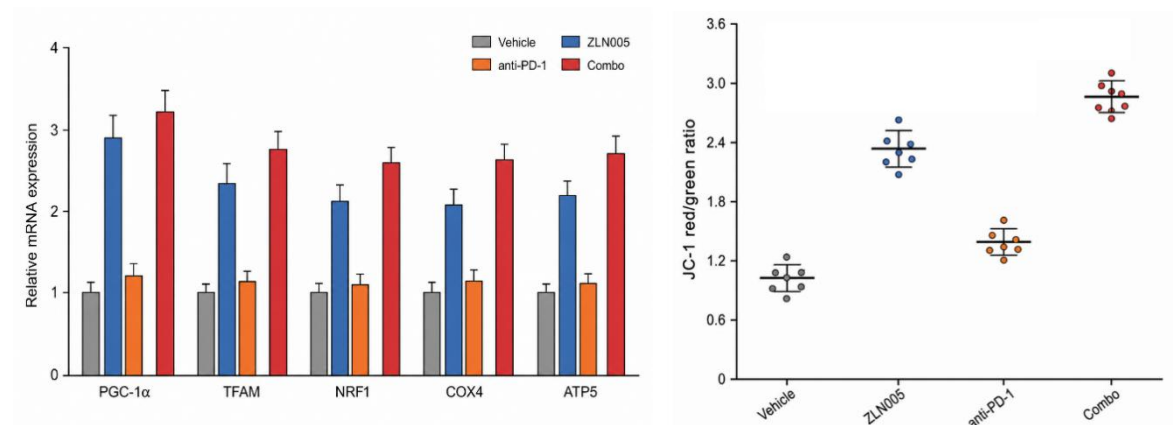
4.2. PGC-1 α agonist ZLN005 restores mitochondrial function of tumor-infiltrating CD8⁺ T cells

Next, we looked at how the PGC-1 α activator ZLN005 affects the mitochondrial metabolism of tumor-infiltrating CD8⁺ T cells. Compared to the Vehicle group, ZLN005 alone significantly increased the expression of PGC-1 α and its downstream genes related to mitochondrial biogenesis-TFAM, NRF1, COX4, and ATP5-in tumor-infiltrating CD8⁺ T cells (Table 2A, Fig. 2A). After ZLN005 treatment, CD8⁺ T cells showed higher mitochondrial membrane potential, lower mitochondrial ROS, increased ATP production, and significant improvements in both basal and maximal OCR (Table 2B-D, Fig. 2B-D). Anti-PD-1 alone showed some trend toward improving mitochondrial function, but the effect was most pronounced when combined with ZLN005. These results suggest that targeting PGC-1 α can effectively restore the mitochondrial adaptability of tumor-infiltrating CD8⁺ T cells.

Figure 2

A: mRNA expression of PGC-1 α and downstream genes

B: JC-1 red/green ratio across four groups



C: MitoSOX MFI across four groups

D: Basal and maximal OCR

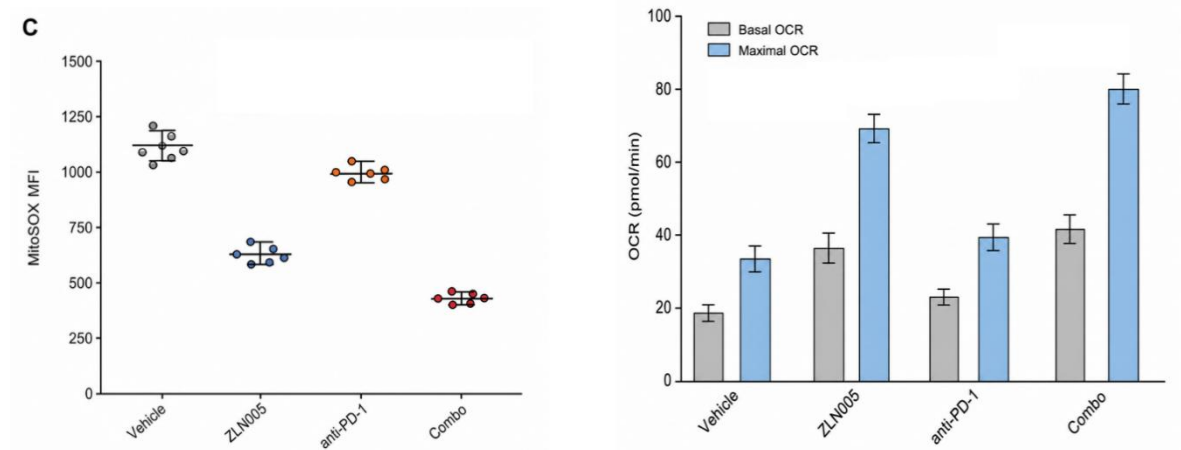


Table 2A

mRNA expression of PGC-1α and downstream genes

Gene	Vehicle	ZLN005	anti-PD-1	Combo
PGC-1α	1.00 ± 0.12	2.86 ± 0.31	1.18 ± 0.16	3.24 ± 0.38
TFAM	1.00 ± 0.14	2.34 ± 0.26	1.12 ± 0.15	2.78 ± 0.29
NRF1	1.00 ± 0.11	2.12 ± 0.24	1.08 ± 0.13	2.56 ± 0.27
COX4	1.00 ± 0.11	2.08 ± 0.11	1.14 ± 0.17	2.61 ± 0.11
ATP5	1.00 ± 0.17	2.21 ± 0.19	1.11 ± 0.11	2.74 ± 0.13

Table 2B

JC-1 red/green ratio across four groups

Group	Value (n=6)	Adjusted mean
Vehicle	1.01, 1.19, 1.08, 0.97, 1.15, 1.09	1.08 ± 0.18
ZLN005	2.28, 2.41, 2.19, 2.52, 2.31, 2.26	2.31 ± 0.27
anti-PD-1	1.29, 1.42, 1.21, 1.48, 1.33, 1.31	1.34 ± 0.21
Combo	2.79, 2.95, 2.68, 3.05, 2.88, 2.82	2.87 ± 0.34

Table 2C

MitoSOX MFI across four groups

Group	Adjusted mean
Vehicle	1124.6 ± 132.5

ZLN005	623.8 ± 71.4
anti-PD-1	987.2 ± 105.6
Combo	418.5 ± 52.3

Table 2D

Basal and maximal OCR

Group	Basal OCR Average	Maximal OCR Mean
Vehicle	18.4 ± 2.7	32.8 ± 4.1
ZLN005	35.7 ± 4.6	68.9 ± 7.3
anti-PD-1	22.6 ± 3.1	39.4 ± 5.0
Combo	41.3 ± 5.2	79.5 ± 8.6

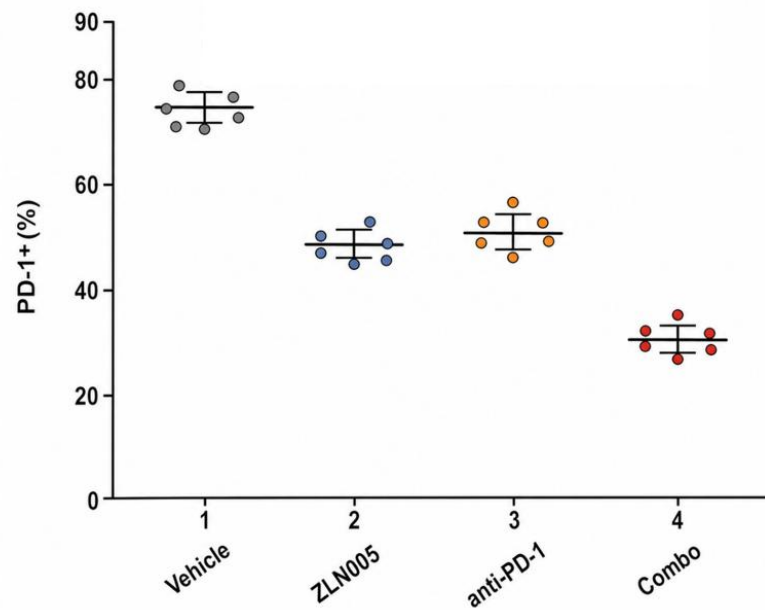
4.3. Mitochondrial metabolic reprogramming reverses CD8⁺ T cell exhaustion and boosts effector function

To further clarify whether mitochondrial metabolic reprogramming can reverse CD8⁺ T cell exhaustion, we examined the expression of inhibitory receptors, exhaustion-related transcription factors, and effector molecules in tumor-infiltrating CD8⁺ T cells from each group. The results showed that both ZLN005 monotherapy and combination therapy significantly reduced the proportion of cells expressing PD-1, TIM-3, and LAG-3, as well as the mean fluorescence intensity of TOX, while increasing the expression of the stem-like/progenitor exhaustion-related transcription factor Tcf1 (Table 3A-3C, Figures 3A-C). Functionally, the proportion of IFN- γ , TNF- α , granzyme B, and Ki-67 positive CD8⁺ T cells was significantly higher in the ZLN005 and combination treatment groups, with the combination group showing the most notable improvement (Table 3D-3F, Figures 3D-F). These data suggest that enhancing mitochondrial metabolism can partially reverse the exhausted phenotype of CD8⁺ T cells and restore their proliferative and effector functions.

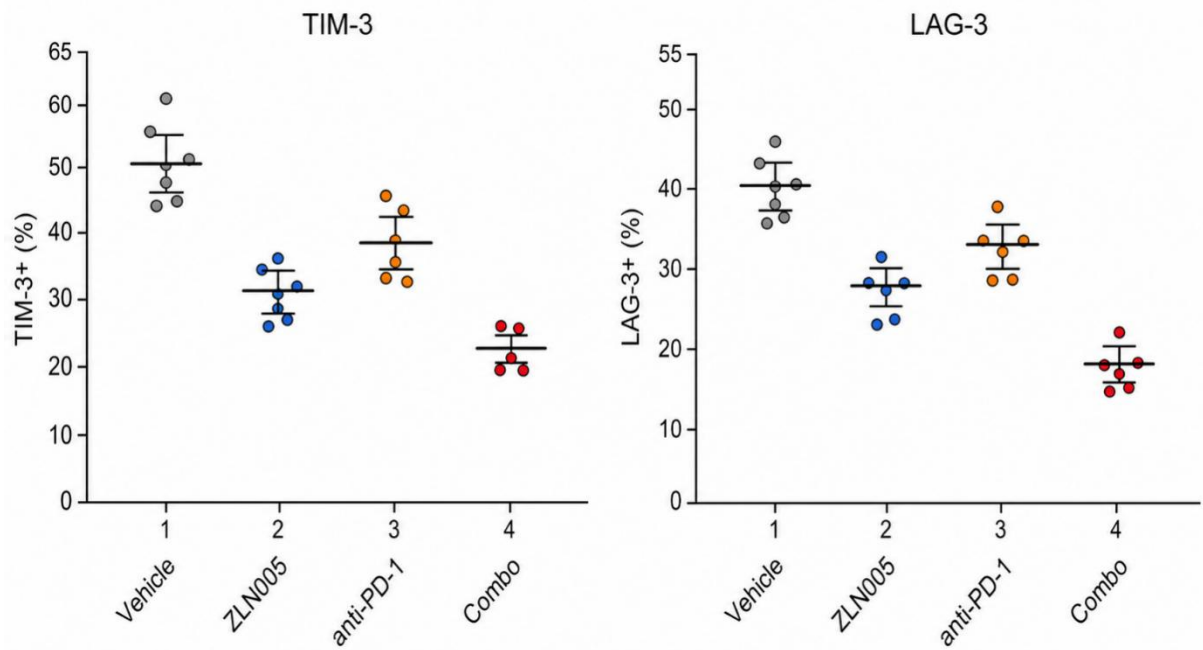
Figure 3

A: PD-1⁺ percentage across four groups

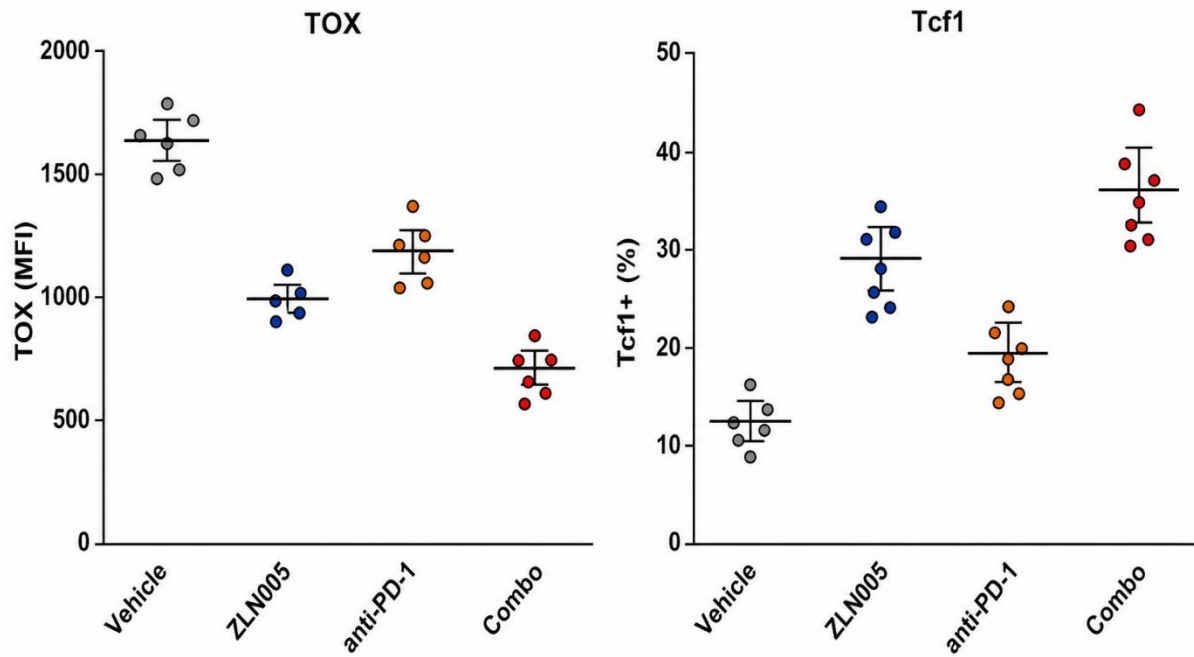
3A



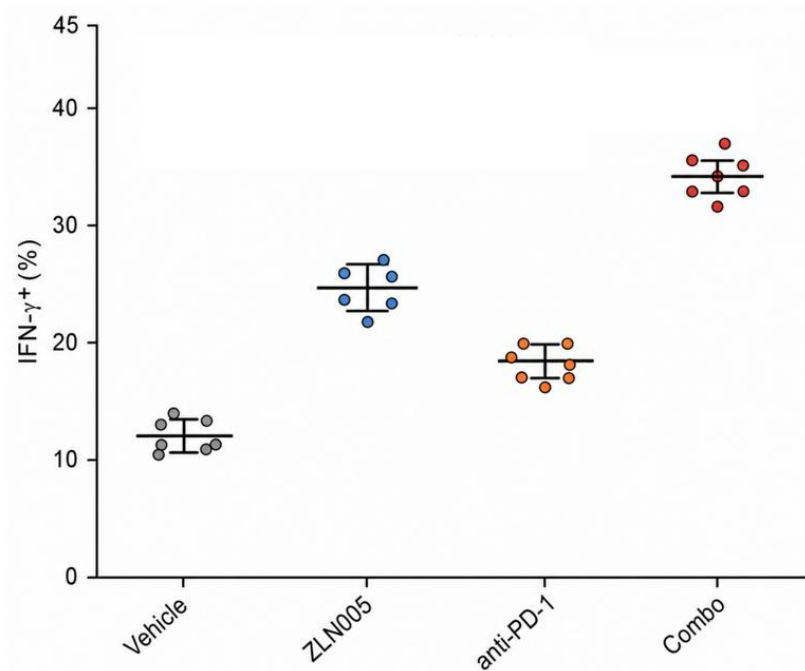
B: TIM-3⁺ and LAG-3⁺ percentages



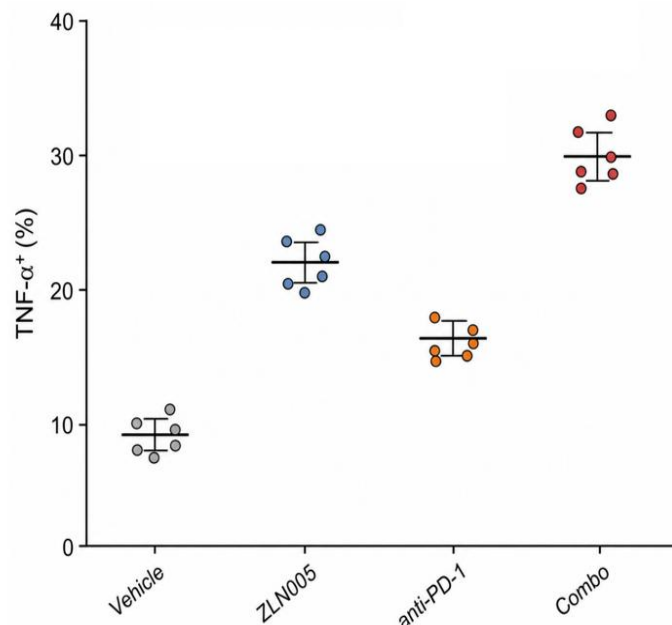
C: TOX MFI and Tcf1⁺ percentage



D: IFN- γ ⁺ percentage



E: TNF- α ⁺ percentage



F: Granzyme B⁺ and Ki-67⁺ percentages

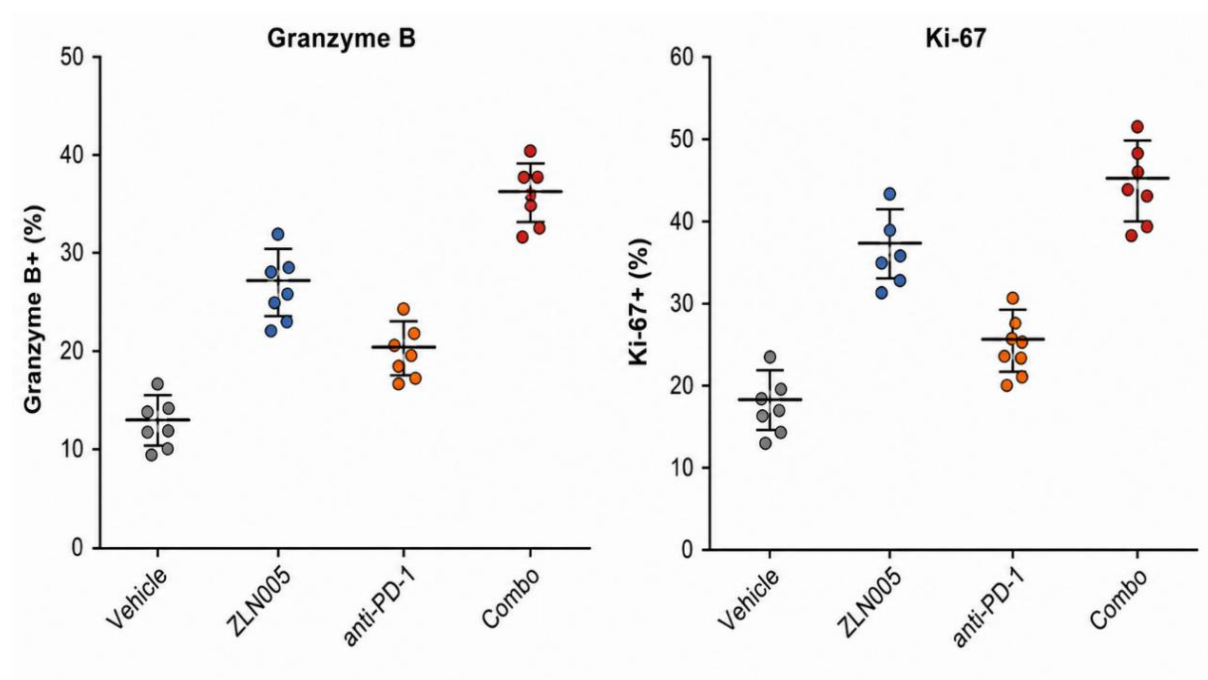


Table 3

A: PD-1⁺ percentage across four groups

Group	Adjusted mean
Vehicle	74.2 ± 6.8
ZLN005	48.7 ± 5.1
anti-PD-1	52.1 ± 5.7

Combo 31.5 ± 4.2

B: TIM-3⁺ and LAG-3⁺ percentages

Indicator	Vehicle	ZLN005	anti-PD-1	Combo
TIM-3 ⁺ (%)	49.8 ± 5.4	31.4 ± 3.8	38.6 ± 4.5	22.7 ± 3.1
LAG-3 ⁺ (%)	41.3 ± 4.7	27.9 ± 3.3	33.2 ± 4.0	18.4 ± 2.6

C: TOX MFI and Tcfl⁺ percentage

Indicator	Vehicle	ZLN005	anti-PD-1	Combo
TOX MFI	1623.4 ± 154.2	984.6 ± 102.7	1187.3 ± 126.5	712.8 ± 78.4
Tcfl ⁺ (%)	12.4 ± 2.1	28.7 ± 3.6	19.5 ± 2.8	36.2 ± 4.3

D: IFN- γ ⁺ percentage

Group	Adjusted mean
Vehicle	11.8 ± 2.3
ZLN005	24.6 ± 3.1
anti-PD-1	18.3 ± 2.7
Combo	33.7 ± 4.0

E: TNF- α ⁺ percentage

Group	Adjusted mean
Vehicle	9.2 ± 1.8
ZLN005	21.4 ± 2.9
anti-PD-1	15.8 ± 2.4
Combo	29.6 ± 3.7

F: Granzyme B⁺ and Ki-67⁺ percentages

Indicator	Vehicle	ZLN005	anti-PD-1	Combo
Granzyme B ⁺ (%)	12.9 ± 2.5	27.3 ± 3.4	20.4 ± 2.9	35.8 ± 4.1
Ki-67 ⁺ (%)	18.6 ± 3.0	36.9 ± 4.2	25.7 ± 3.5	45.2 ± 5.0

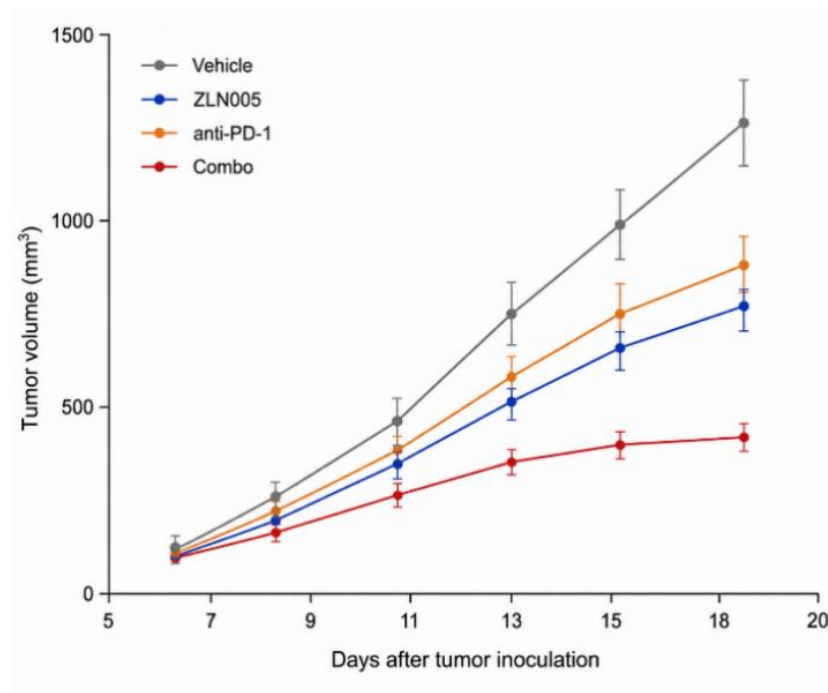
4.4. Combined treatment significantly suppresses tumor growth and extends survival in tumor-bearing mice

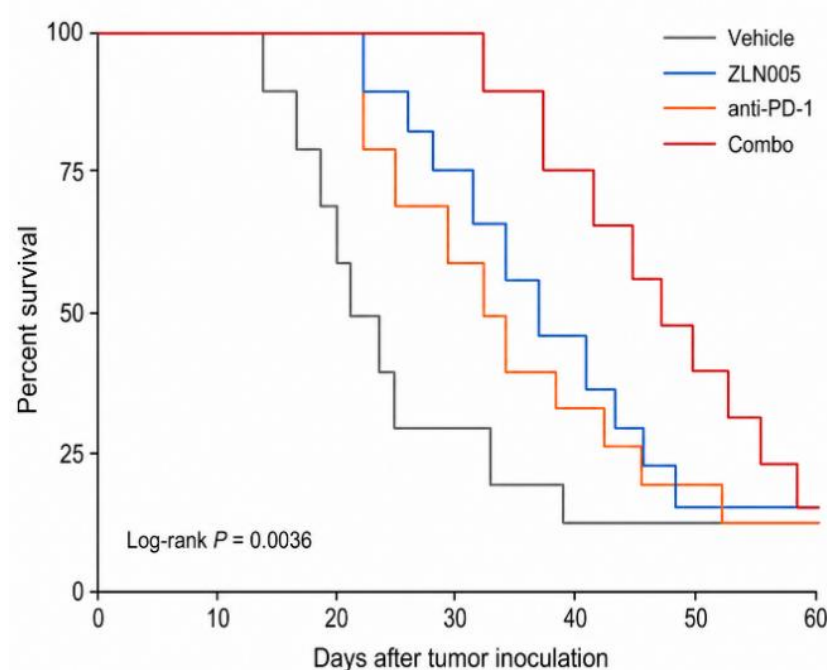
Based on the above improvements in immune metabolism, we further evaluated the *in vivo* anti-tumor effects of ZLN005 combined with anti-PD-1 treatment. In the B16-OVA melanoma model, both ZLN005 alone and anti-PD-1 alone mildly inhibited tumor growth, while the combination treatment significantly reduced tumor volume by day 18, with a tumor inhibition rate of 69.1% (Table 4, Figure 4A). Survival analysis showed that the median survival was 22 days for the vehicle group, 31 days for the ZLN005 group, and 29 days for the anti-PD-1 group, while the combination group was extended to 45 days, showing the most significant survival benefit (Table 4, Figure 4B). A similar trend was observed in the LLC lung cancer model (Figure 4C). These results suggest that targeting mitochondrial metabolic reprogramming can enhance the anti-tumor efficacy of anti-PD-1 treatment.

Figure 4

A: Tumor growth curves

B: Kaplan–Meier survival curves





C: Immunofluorescence of CD8 in tumor tissue

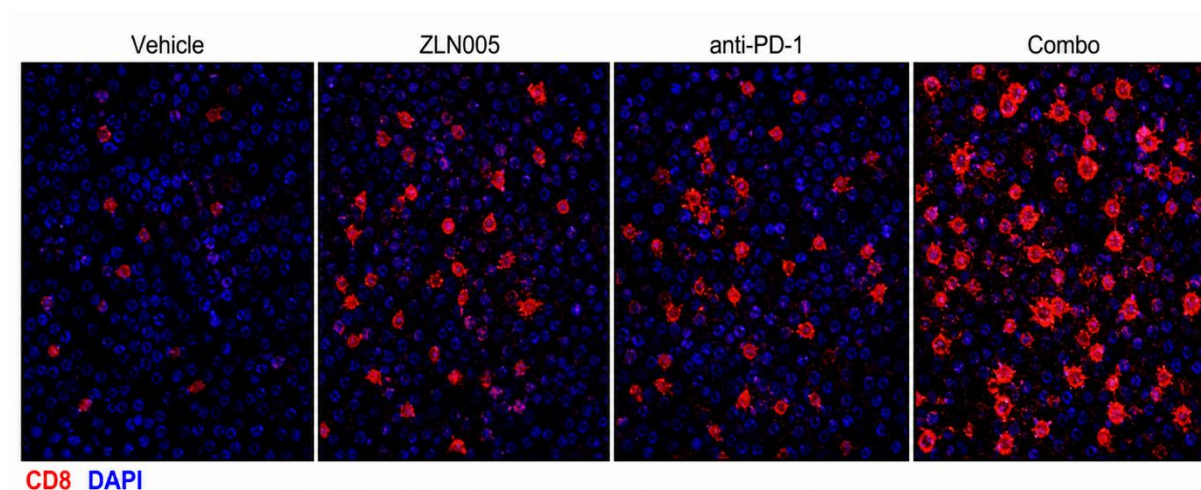


Table 4

Analysis of tumor growth and survival in each group

Group	Tumor Volume on Day 18 (mm ³)	Tumor suppression rate (%)	Median survival (days)	Survival Extension (%)
Vehicle	1248.6 ± 156.3	—	22	—
ZLN005	742.3 ± 88.7	40.6	31	40.9
anti-PD-1	856.9 ± 97.2	31.4	29	31.8
United	386.5 ± 52.4	69.1	45	104.5

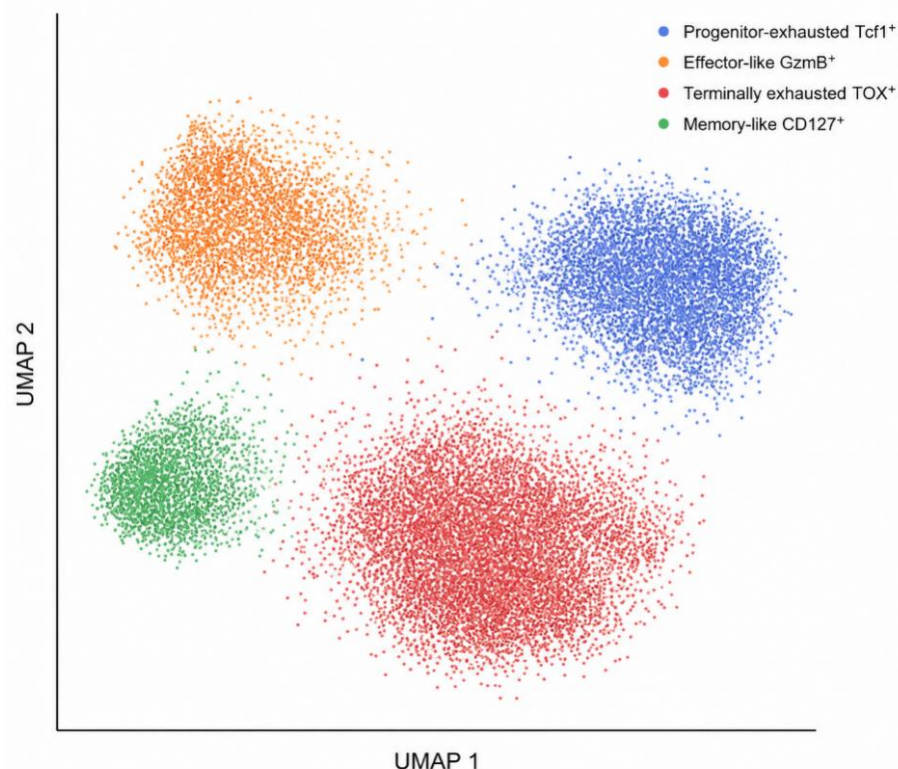
Note. Data are presented as mean $\pm$ SD. Tumor inhibition rate = (Average tumor volume in the vehicle group – Average tumor volume in the treatment group) / Average tumor volume in the vehicle group $\times$ 100%.

4.5. Single-cell RNA sequencing reveals how combination therapy reshapes CD8⁺ T cell subpopulations and the tumor immune microenvironment

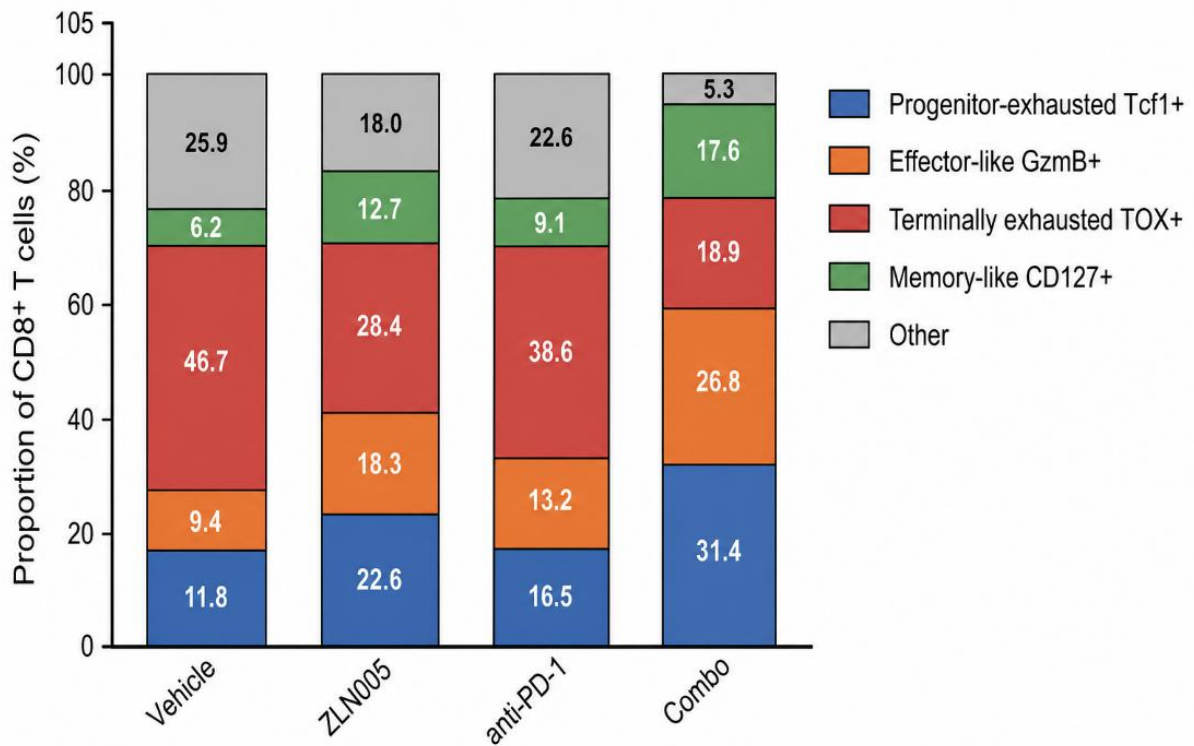
To systematically analyze the impact of combination therapy on the heterogeneity of tumor-infiltrating T cells, we performed single-cell RNA sequencing on CD45⁺CD3⁺CD8⁺ T cells from tumors in each group. UMAP clustering identified four main CD8⁺ T cell subpopulations: progenitor-exhausted Tcf1⁺, effector-like GzmB⁺, terminally exhausted TOX⁺, and memory-like CD127⁺ (Figure 5A). After combination therapy, the proportions of progenitor-exhausted and effector-like subpopulations increased significantly, while the terminally exhausted subpopulation decreased (Table 5B, Figure 5B). Pseudotime analysis showed that cells in the combination therapy group tended to differentiate towards the effector and memory branches (Figure 5C). Additionally, mitochondrial metabolism gene set scores were significantly higher in the combination group and positively correlated with Tcf1 and GzmB expression (Figure 5D, Table 5D). These results suggest that combination therapy can reshape CD8⁺ T cell states at the single-cell level and promote the expansion of subpopulations with long-lasting anti-tumor potential.

Figure 5

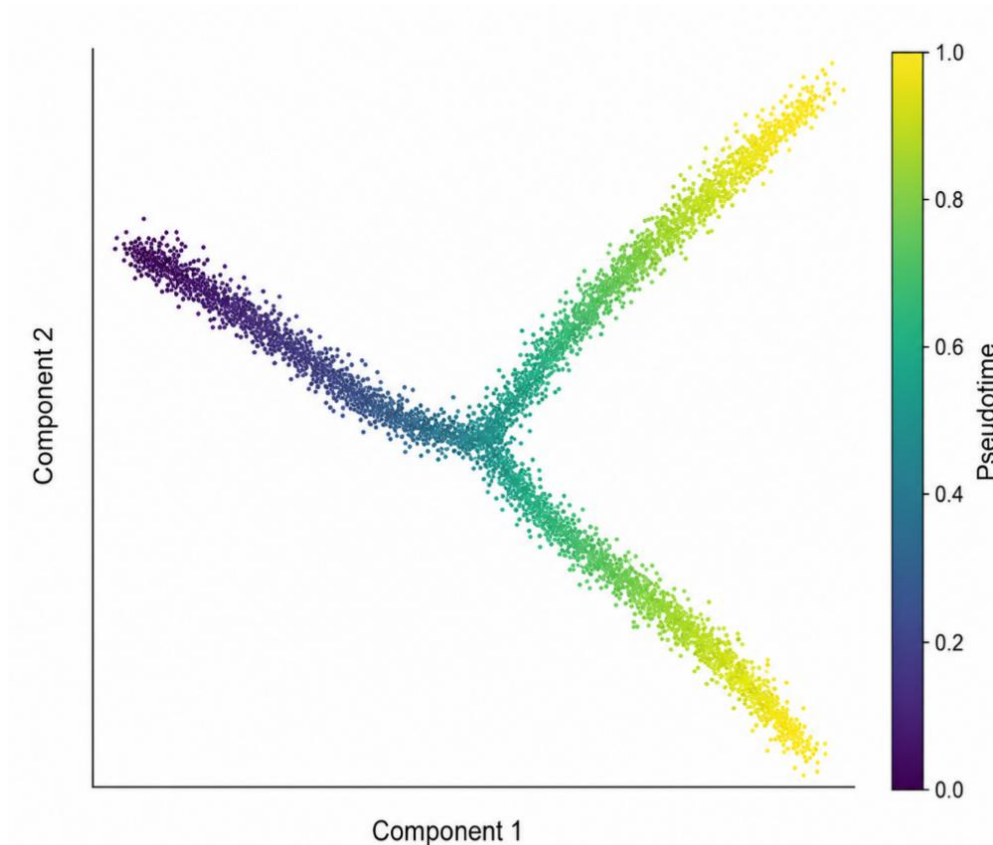
A: UMAP clustering of CD8⁺ T cell subsets



B: Stacked bar plot of CD8⁺ T cell subset proportions



C: Pseudotime trajectory analysis



D: Mitochondrial metabolic gene set score

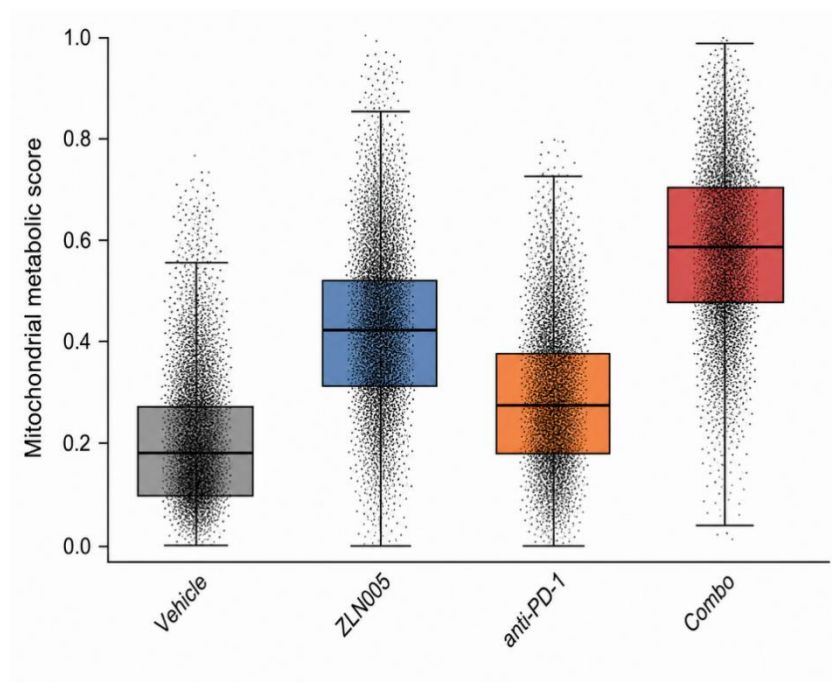


Table 5B

Stacked bar plot of CD8⁺ T cell subset proportions

Subgroup	Vehicle (%)	ZLN005 (%)	anti-PD-1 (%)	Combo (%)
Progenitor-exhausted Tcf1 ⁺	11.8 ± 2.0	22.6 ± 3.1	16.5 ± 2.5	31.4 ± 3.9
Effector-like GzmB ⁺	9.4 ± 1.7	18.3 ± 2.6	13.2 ± 2.1	26.8 ± 3.4
Terminally exhausted TOX ⁺	46.7 ± 5.2	28.4 ± 3.7	38.6 ± 4.5	18.9 ± 2.8
Memory-like CD127 ⁺	6.2 ± 1.3	12.7 ± 2.0	9.1 ± 1.6	17.6 ± 2.4
Other	25.9	18.0	22.6	5.3

Table 5D

Mitochondrial metabolic gene set score

Group	Mean	standard deviation	sample size
Vehicle	0.18	0.12	300
ZLN005	0.42	0.12	300

anti-PD-1	0.28	0.12	300
Combo	0.58	0.12	300

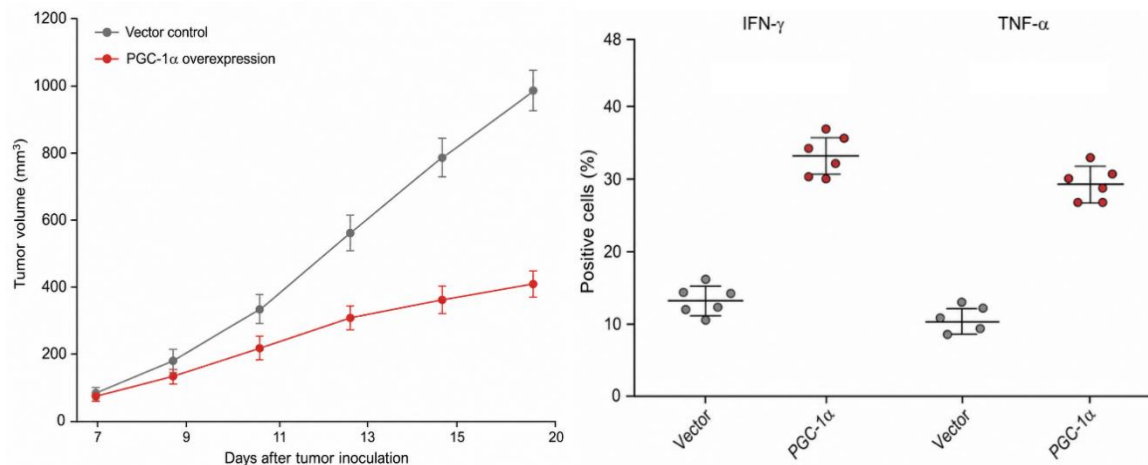
4.6. Testing the causal effect of adoptive transfer of CD8⁺ T cells overexpressing PGC-1 α

To verify the causal effect of PGC-1 α -mediated mitochondrial metabolic reprogramming on the anti-tumor function of CD8⁺ T cells, we adoptively transferred CD8⁺ T cells overexpressing PGC-1 α or carrying an empty vector control into tumor-bearing mice. The results showed that, compared to the empty vector group, tumor growth was significantly slowed in the PGC-1 α overexpression group, with tumor volume reduced by about 58.2% on day 18 (Table 6A, Figure 6A). At the same time, the proportions of IFN- γ , TNF- α , and Granzyme B positive cells were significantly increased in PGC-1 α -overexpressing CD8⁺ T cells, TOX mean fluorescence intensity decreased, and the proportion of Tcf1-positive cells increased (Table 6B-D, Figures 6B-D). These results further support that mitochondrial metabolic reprogramming is a key mechanism for enhancing the anti-tumor immunity of CD8⁺ T cells.

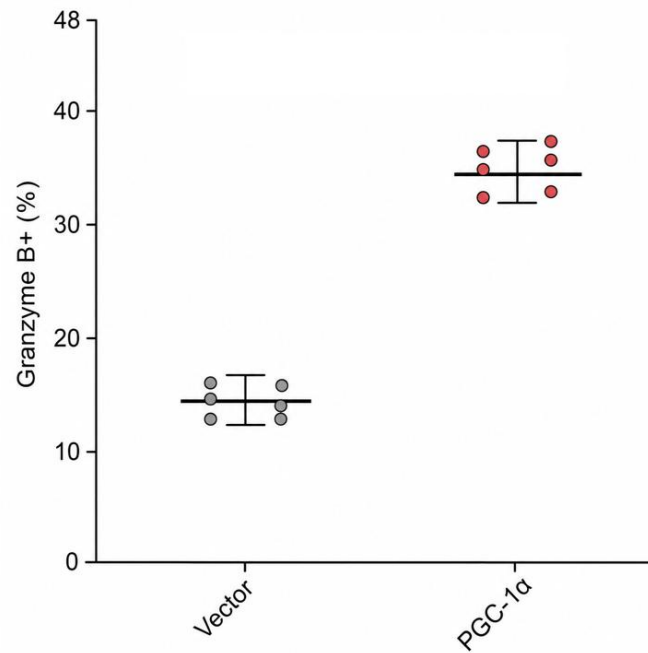
Figure 6

A: Tumor growth after adoptive transfer

B: IFN- γ ⁺ and TNF- α ⁺ after adoptive transfer



C: Granzyme B⁺ after adoptive transfer



D: TOX MFI and Tcf1⁺ after adoptive transfer

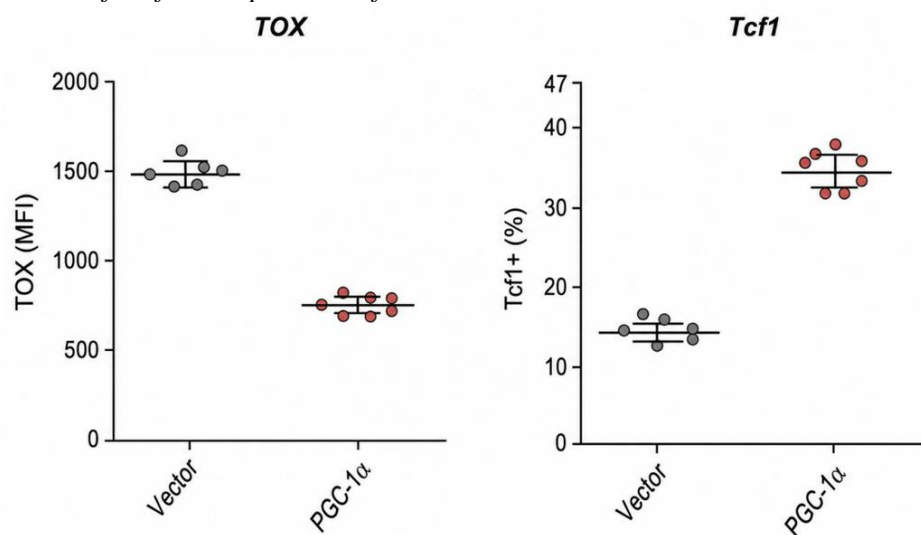


Table 6

A: Tumor growth after adoptive transfer

Days	Vector control (mm ³)	PGC-1α overexpression (mm ³)
7	72	68
9	165	130
11	320	210
13	560	300
15	780	360
18	987.3 ± 112.6	412.7 ± 56.8

B: IFN- γ^+ and TNF- α^+ after adoptive transfer

Indicator	Group	Adjusted mean
IFN- γ^+ (%)	Vector	13.1 $\pm$ 2.4
IFN- γ^+ (%)	PGC-1 α	32.4 $\pm$ 3.8
TNF- α^+ (%)	Vector	10.4 $\pm$ 2.0
TNF- α^+ (%)	PGC-1 α	28.7 $\pm$ 3.4

C: Granzyme B $^+$ after adoptive transfer

Group	Adjusted mean
Vector	14.2 $\pm$ 2.6
PGC-1 α	34.6 $\pm$ 4.1

D: TOX MFI and Tcf1 $^+$ after adoptive transfer

Indicator	Group	Adjusted mean
TOX MFI	Vector	1492.5 $\pm$ 142.7
TOX MFI	PGC-1 α	768.4 $\pm$ 86.3
Tcf1 $^+$ (%)	Vector	14.6 $\pm$ 2.5
Tcf1 $^+$ (%)	PGC-1 α	33.8 $\pm$ 4.0

6. Discussion

This study focuses on the relationship between mitochondrial metabolic dysfunction and functional exhaustion of CD8 $^+$ T cells in the tumor microenvironment. We systematically evaluated how targeting PGC-1 α -mediated mitochondrial metabolic reprogramming affects the exhausted phenotype of CD8 $^+$ T cells, their anti-tumor effects, and response to immune checkpoint blockade therapy. The main findings include: tumor-infiltrating CD8 $^+$ T cells show reduced mitochondrial membrane potential, ROS accumulation, insufficient ATP production, and impaired oxidative phosphorylation, along with increased expression of PD-1, TIM-3, LAG-3, and TOX; the PGC-1 α agonist ZLN005 can restore mitochondrial function, reduce inhibitory receptor and TOX expression, and enhance IFN- γ , TNF- α , granzyme B secretion, and proliferation; combining ZLN005 with anti-PD-1 antibody treatment significantly suppressed tumor growth, prolonged survival in tumor-bearing mice, and at the single-cell level promoted expansion of progenitor-exhausted Tcf1 $^+$ and

effector-like GzmB⁺ subpopulations while reducing terminally exhausted TOX⁺ cells; adoptive transfer of PGC-1 α -overexpressing CD8⁺ T cells further confirmed the causal role of mitochondrial metabolic reprogramming in boosting anti-tumor immunity. These results provide new experimental evidence for a treatment strategy combining metabolic intervention with immune checkpoint blockade.

Comparison with existing studies. Previous research has shown that T cell exhaustion is not only regulated by inhibitory receptors and transcriptional programs, but is also closely related to metabolic maladaptation. Scharping and others (2016) found that the tumor microenvironment can suppress T cell mitochondrial biogenesis, leading to metabolic insufficiency and dysfunction; Bengsch and others (2016) showed that PD-1 signaling can induce early bioenergetic defects in CD8⁺ T cells; Patsoukis and others (2015) reported that PD-1 changes T cell metabolic fate by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. In addition, Vardhana and others (2020) found that continuous exposure to antigens damages mitochondrial oxidative phosphorylation, limiting T cell self-renewal; Yu and colleagues (2020) and Scharping and colleagues (2021) confirmed that mitochondrial dynamics disorders and hypoxia-induced mitochondrial stress can drive or enhance T cell exhaustion. These studies together support the idea that mitochondrial dysfunction is a key driver of T cell exhaustion. Building on this, our study further shows that in established tumor models, pharmacologically activating PGC-1 α can partially reverse the already-formed exhaustion phenotype, rather than just preventing exhaustion from happening. This is consistent with Dumauthioz et al.'s (2021) findings that forcing PGC-1 α expression can boost CD8⁺ T cell fitness, memory formation, and anti-tumor immunity. But this study, by combining anti-PD-1 therapy with single-cell transcriptome analysis, further revealed how mitochondrial metabolic reprogramming regulates the balance of T cell subsets and the response to immunotherapy.

Chamoto and others (2017) have reported that mitochondrial activating drugs can work together with PD-1 blockade to boost anti-tumor activity through a T cell-dependent mechanism. This study observed similar synergistic effects in the B16-OVA and LLC models, and further provided systematic evidence from multiple levels, including mitochondrial function, exhaustion markers, effector molecules, and single-cell subpopulations. Compared with studies by Sade-Feldman and Miller on T cell states related to ICB response, we found that combination therapy significantly expanded Tcf1⁺ progenitor-exhausted and GzmB⁺ effector-like subsets while reducing TOX⁺ terminally exhausted subsets, suggesting that mitochondrial metabolic reprogramming might boost ICB efficacy by maintaining a pool of T cells with self-renewal potential. Siddiqui et al. (2019) also showed that intratumoral Tcf1⁺PD-1⁺CD8⁺ T cells have stem-like features and can promote tumor control after vaccination and checkpoint blockade. The results of this study are consistent with this and further link this subpopulation remodeling to PGC-1 α -mediated improvements in mitochondrial metabolism.

The main innovations of this study are reflected in the following aspects: focusing on the reversal

of ‘established exhaustion’ rather than just prevention, demonstrating that mitochondrial metabolic reprogramming has therapeutic potential; combining PGC-1 α agonists with anti-PD-1 antibodies and systematically evaluating their synergistic anti-tumor effects to provide preclinical evidence for clinical combination therapy; integrating single-cell RNA sequencing, Seahorse metabolic analysis, flow cytometry, and adoptive transfer experiments to gradually validate the role of mitochondrial metabolic reprogramming from correlation to causation; revealing at the single-cell level the relationship between mitochondrial metabolism scores and Tcf1, GzmB expression, as well as T cell subset remodeling, offering a new perspective for understanding the link between immune metabolism and ICB response. Overall, this study proposes and validates a strategy of ‘targeting mitochondrial metabolic checkpoints to reverse CD8⁺ T cell exhaustion,’ with certain theoretical innovation and translational potential.

This study still has several limitations. The research is mainly based on B16-OVA melanoma and LLC lung cancer mouse models. Although these two models are widely used in tumor immunology studies, their immunogenicity, mutation burden, and microenvironment composition differ from human tumors, so whether the conclusions can be applied to clinical patients still needs verification. ZLN005, as a pharmacological activator of PGC-1 α , showed significant effects in this study, but its specificity is limited and it might affect other cell types such as tumor cells, macrophages, and regulatory T cells, so off-target effects cannot be completely ruled out. In the future, more precise mechanistic verification will require CD8⁺ T cell-specific PGC-1 α conditional knockout or overexpression models. The number of samples for single-cell RNA sequencing was small in each group, which can reveal trends, but the robustness of subgroup proportions and metabolic scores still needs larger samples and independent cohorts for validation. This study did not fully analyze the specific downstream molecular mechanisms of PGC-1 α , such as mitochondrial dynamics, ROS signaling, epigenetic reprogramming, and metabolite-mediated signaling pathways. Long-term safety, optimal dosing, treatment timing, and resistance mechanisms have not been evaluated. The study only used female mice, so it didn’t consider how sex differences might affect immune metabolism and ICB response. It also did not assess how combination therapy impacts T cell memory formation, tumor recurrence, or long-term survival quality. All of these issues need to be systematically addressed in future research.

Future studies should verify the translational potential of this strategy in human tumor samples and humanized mouse models. They should use CD8⁺ T cell-specific gene editing and metabolomics to dive deep into the downstream molecular network of PGC-1 α , optimize the combination of mitochondrial metabolism activators with immune checkpoint blockade in terms of regimen, timing, and dosage, and identify metabolism-immune biomarkers that can predict treatment response. Additionally, research should explore the synergy between gut microbiota metabolites, mitochondrial

DNA sensing, and spatial AI/multimodal predictive models with mitochondrial metabolism reprogramming strategies. At the same time, attention should be paid to the dynamic evolution of the TME, and the long-term safety, memory T cell formation, and tumor recurrence risk should be systematically evaluated to push mitochondrial metabolism reprogramming strategies toward clinical translation.

In summary, our study shows that tumor-infiltrating CD8⁺ T cells have significant mitochondrial metabolic dysfunction. Targeting PGC-1 α -mediated mitochondrial metabolic reprogramming can reverse their exhausted state, boost their effector functions, and work together with anti-PD-1 therapy to enhance anti-tumor effects. This approach offers new theoretical support and potential intervention targets for improving responses to cancer immunotherapy.

7. Conclusion

This study systematically reveals the close link between mitochondrial metabolic dysfunction and functional exhaustion of CD8⁺ T cells in the tumor microenvironment, and demonstrates that targeting PGC-1 α -mediated mitochondrial metabolic reprogramming can serve as an effective immunometabolic intervention strategy. The results show that tumor-infiltrating CD8⁺ T cells exhibit decreased mitochondrial membrane potential, ROS accumulation, insufficient ATP production, and impaired oxidative phosphorylation, along with increased expression of PD-1, TIM-3, LAG-3, and TOX. The PGC-1 α agonist ZLN005 can restore their mitochondrial function, reduce the expression of inhibitory receptors and exhaustion-related transcription factors, and boost secretion of IFN- γ , TNF- α , granzyme B, as well as proliferation ability. Importantly, combining ZLN005 with anti-PD-1 antibodies produces a synergistic anti-tumor effect, significantly inhibiting tumor growth and extending the survival of tumor-bearing mice, while at the single-cell level promoting expansion of progenitor-exhausted Tcf1⁺ and effector-like GzmB⁺ subsets and reducing the terminally exhausted TOX⁺ subset. Adoptive transfer experiments of PGC-1 α -overexpressing CD8⁺ T cells further confirm the causal role of mitochondrial metabolic reprogramming in enhancing anti-tumor immunity. Overall, this study proposes and validates a therapeutic strategy of ‘targeting mitochondrial metabolic checkpoints to reverse CD8⁺ T cell exhaustion,’ providing new theoretical support and potential intervention targets for improving tumor immunotherapy responses.

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Author contributions



Conceptualization, Gang Liu; methodology, Jia Wang; validation, Alena Mikalaeuna Vasiukevich; formal analysis, Biao Feng and Liqiang An; investigation, Jia Wang and Biao Feng; resources, Gang Liu and Liqiang An; data curation, Alena Mikalaeuna Vasiukevich; writing—original draft preparation, Jia Wang; writing—review and editing, Gang Liu and Alena Mikalaeuna Vasiukevich; visualization, Biao Feng; supervision, Gang Liu; project administration, Liqiang An. All authors have read and agreed to the published version of the manuscript.

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Ethics approval

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Consent to participate

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Consent for publication

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