

Supplementary Information

SIRP γ Promotes CD8⁺ T Cell Exhaustion via Metabolic-Epigenetic Reprogramming in Tuberculous Pleural Effusion

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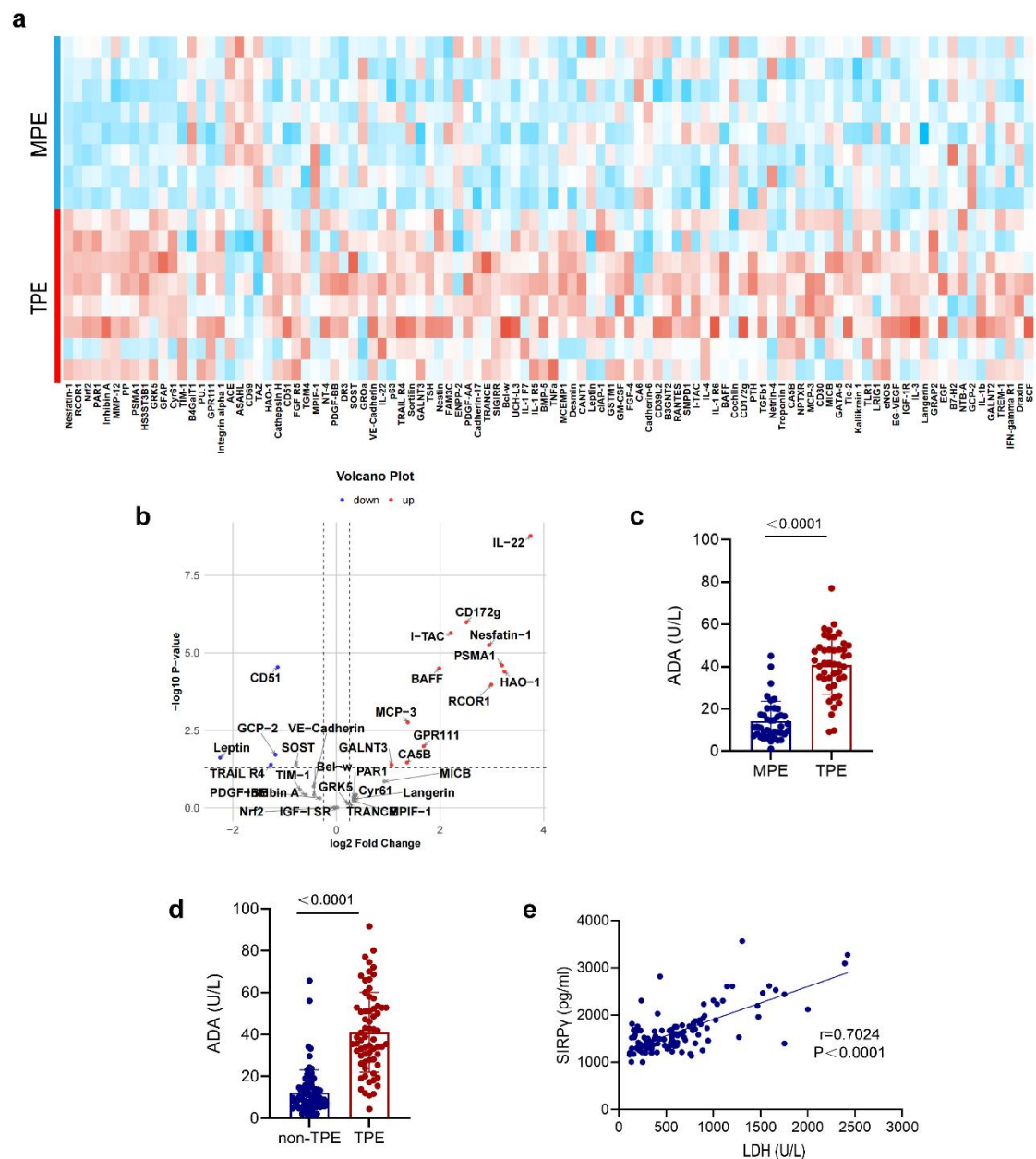
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Supplementary Figures 1 to 16.

Supplementary Tables 1 to 7

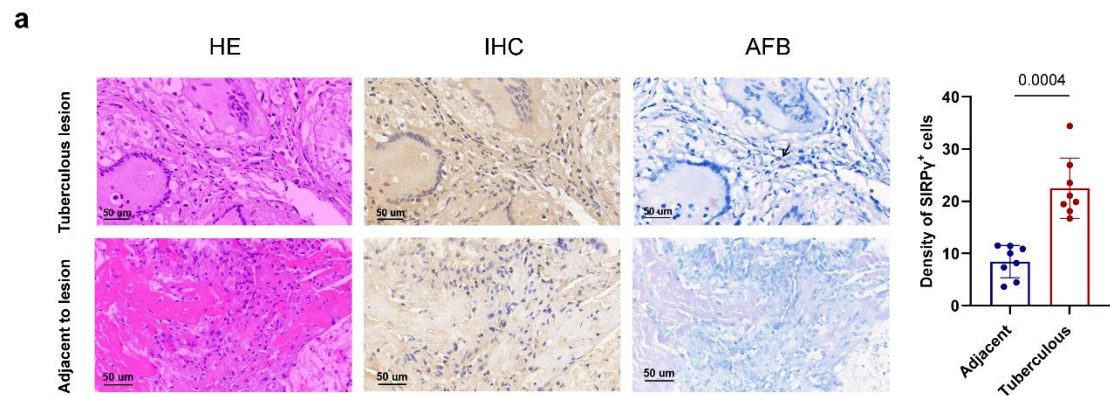
Supplementary Figure 1



Supplementary Figure 1 SIRPy serves as a diagnostic biomarker for TPE, related to Figure 1. (a) Heatmap depicting protein expression profiles in 16 pleural effusion samples (8 MPE vs. 8 TPE) from the preliminary high-throughput cytokine array analysis. (b) Volcano plot identifying 17 differentially expressed proteins in the secondary antibody microarray validation cohort (50 MPE vs. 50 TPE). (c) Comparative analysis of ADA levels in TPE (n= 44 patients) versus MPE (n= 40 patients) from the training cohort. (d) Comparative analysis of ADA levels in TPE (n = 66 patients) versus non-TPE including MPE (n = 35 patients), parapneumonic pleural

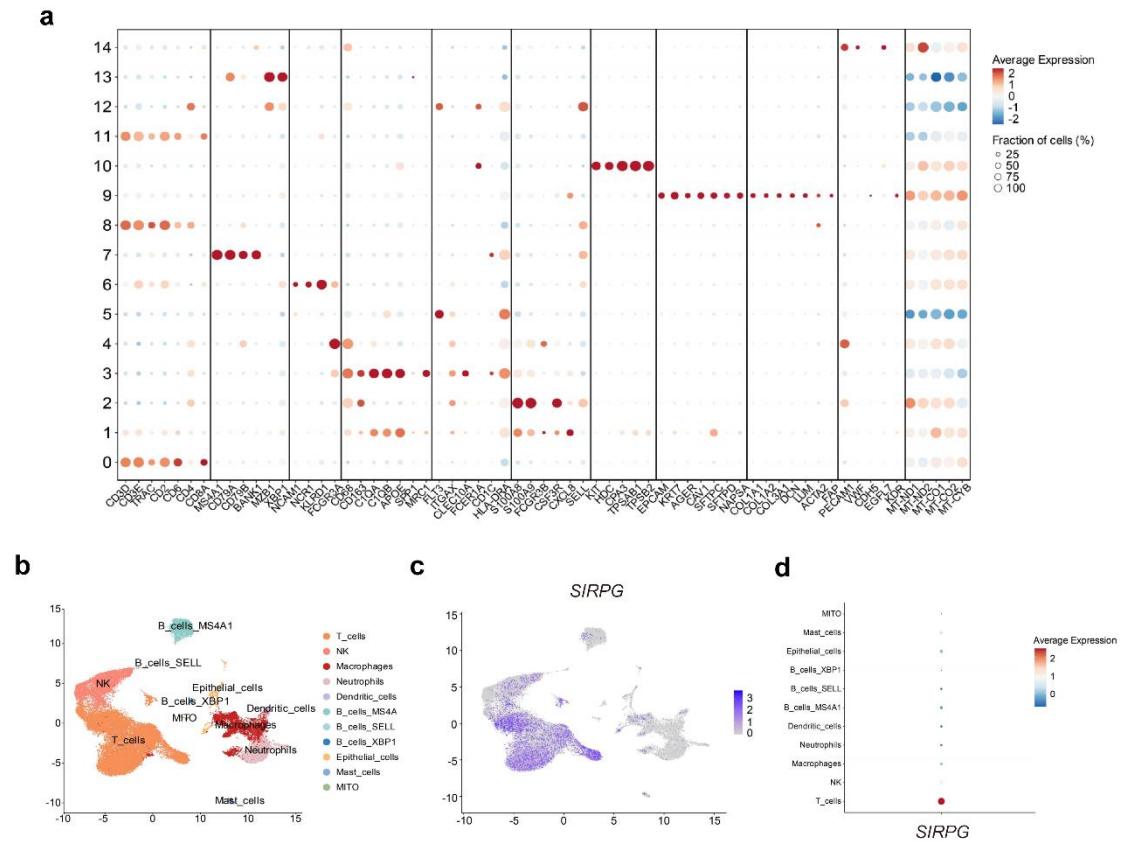
effusion (PPE) (n = 27 patients), and miscellaneous (n = 16 patients) from validation cohort. **(e)** Correlation between pleural fluid SIRP γ levels and LDH concentrations ($Y = 0.6938 \times X + 1216$) (n = 111 patients). Statistical analyses were performed using unpaired two-tailed t-tests **(c, d)** and Pearson correlation coefficient **(e)**. Data are presented as mean values $\pm$ SD **(c, d)**.

Supplementary Figure 2



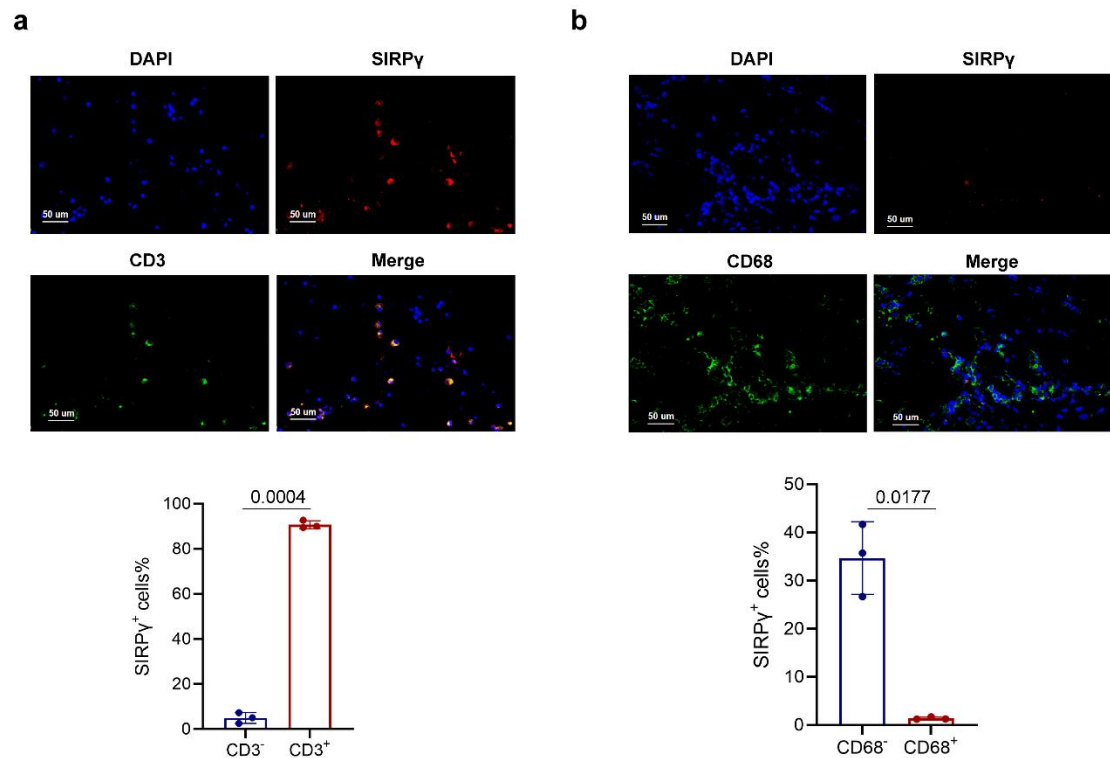
Supplementary Figure 2 SIRP γ serves as a diagnostic biomarker for TPE, related to **Figure 1**. **(a)** Representative hematoxylin and eosin (H&E), immunohistochemistry (IHC) detection of SIRP γ , and acid-fast bacteria stain (AFB) stains in pleural tissues are displayed with corresponding quantitative results of SIRP γ ⁺ cell density (n = 8 patients). Statistical analyses were performed using paired two-tailed t-tests **(a)**. Data are presented as mean values $\pm$ SD **(a)**.

Supplementary Figure 3



Supplementary Figure 3 *SIRPγ* is highly expressed in exhausted $CD8^+$ T cells in TPE, related to Figure 2. (a) Gene expression dot plot showing unique expression patterns to define 11 distinct cell populations in (b). (b) UMAP representation of cell clusters from combined 5 pulmonary tuberculosis tissue samples. (c) UMAP feature plot showing the expression of *SIRPG* of all cells. (d) Dot plot showing *SIRPG* expression patterns of the annotated cell populations. NK, natural killer cells; MITO, mitochondrial transcripts.

Supplementary Figure 4



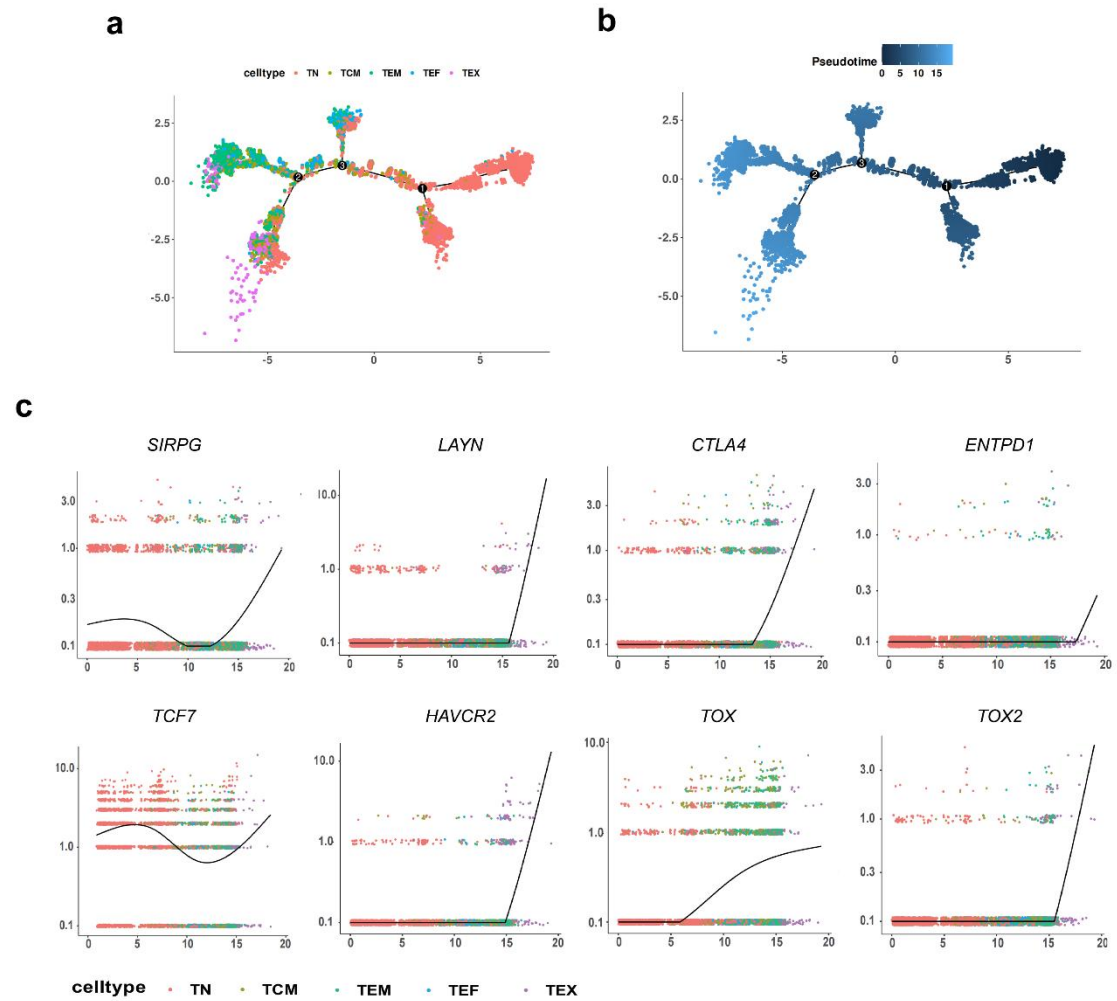
Supplementary Figure 4 SIRP γ is highly expressed in exhausted CD8⁺ T cells in TPE, related to Figure 2. (a) Representative images and quantitative analysis demonstrating the colocalization of SIRP γ and CD3⁺T cells from tuberculous lesions. Scale bar 50 μ m. (b) Representative images and quantitative analysis demonstrating the colocalization of SIRP γ and macrophages from tuberculous lesions. Scale bar 50 μ m. Statistical significance determined by paired two-tailed t-test (a, b). Data are presented as mean values $\pm$ SD (a, b).

[illegible]

Supplementary Figure 5 SIRP γ is highly expressed in exhausted CD8⁺ T cells in TPE, related to Figure 2. (a) t-distributed stochastic neighbor embedding (t-SNE) projection of single-cell RNA sequencing data demonstrates five immune cell clusters in peripheral blood, including B, NK, Macrophage, CD4⁺ T, and CD8⁺ T. (b) Expression

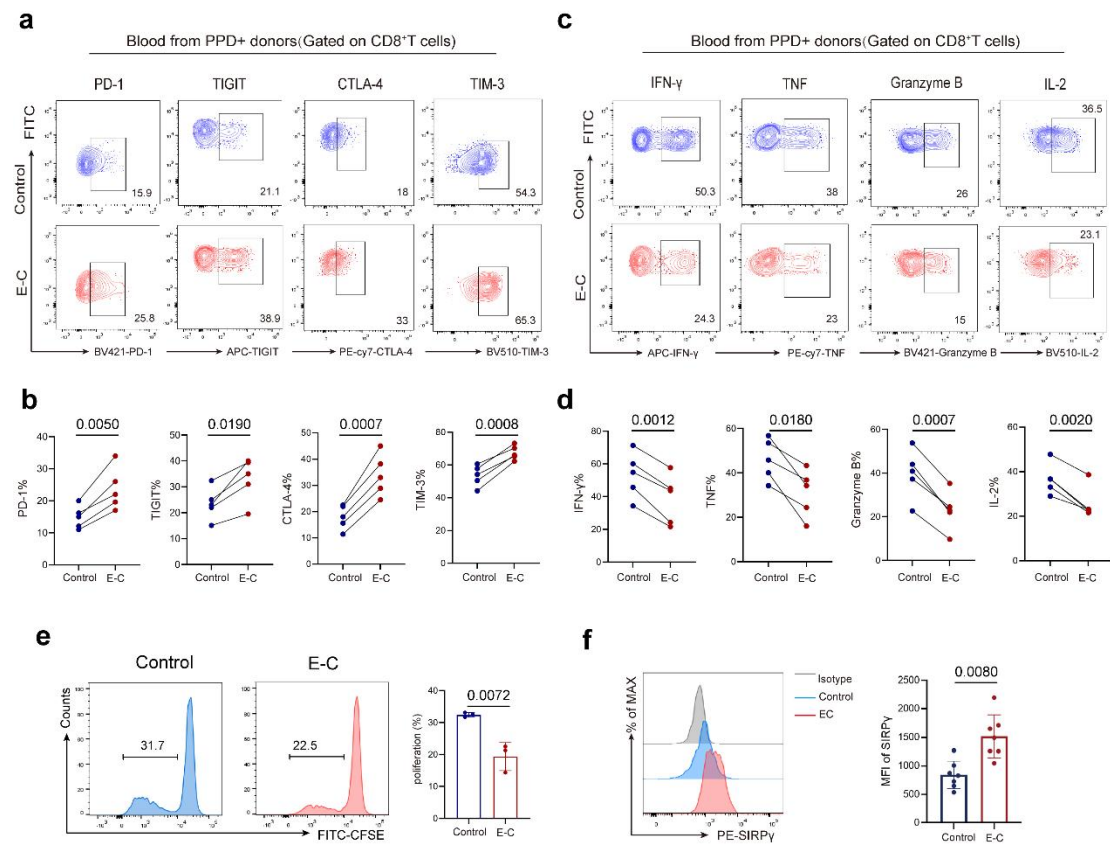
levels of the *SIRPG* gene visualized within the t-SNE space. **(c)** Comparative *SIRPG* expression levels across blood immune subsets. **(d, e)** Immunofluorescence co-localization of SIRP γ and CD4 in TPE-derived T cells: Representative images **(d)** and quantitative analysis **(e)** ($n = 3$ independent experiments). Scale bar 5 μm . **(f)** Representative flow cytometric plots and corresponding statistical analyses showing the proportions of SIRP γ^+ cells from MPE, including SIRP γ^+ B cells (CD45 $^+$ CD19 $^+$ SIRP γ^+), SIRP γ^+ natural killer cells (CD45 $^+$ CD3 $^-$ CD56 $^+$ SIRP γ^+), SIRP γ^+ macrophages (CD45 $^+$ CD68 $^+$ SIRP γ^+), SIRP γ^+ CD4 $^+$ T cells (CD3 $^+$ CD4 $^+$ SIRP γ^+), and SIRP γ^+ CD8 $^+$ T cells (CD3 $^+$ CD8 $^+$ SIRP γ^+) ($n = 10$ patients). **(g)** Comparative analysis of SIRP γ expression levels in different immune cell populations between MPE and TPE (TPE, $n = 11$; MPE, $n = 10$ biologically independent samples per group). **(h, i)** Flow cytometric quantification of SIRP γ^+ CD4 $^+$ T cells (CD3 $^+$ CD4 $^+$ SIRP γ^+) frequencies in TPE versus matched peripheral blood: Representative plots **(h)** and statistical analysis **(i)** ($n=10$ patients). **(j)** Single-cell RNA sequencing analysis comparing *SIRPG* expression in CD4 $^+$ T cells from pleural effusion versus peripheral blood. **(k)** t-SNE representation of CD8 $^+$ T cells revealing 12 clusters. **(l)** Bubble plot visualizing subset-defining gene signatures across CD8 $^+$ T cell subpopulations. Statistical significance determined by paired two-tailed t-test **(e, i)** and two-way ANOVA **(g)**. Data are presented as mean values $\pm$ SD **(e-g, i)**.

Supplementary Figure 6



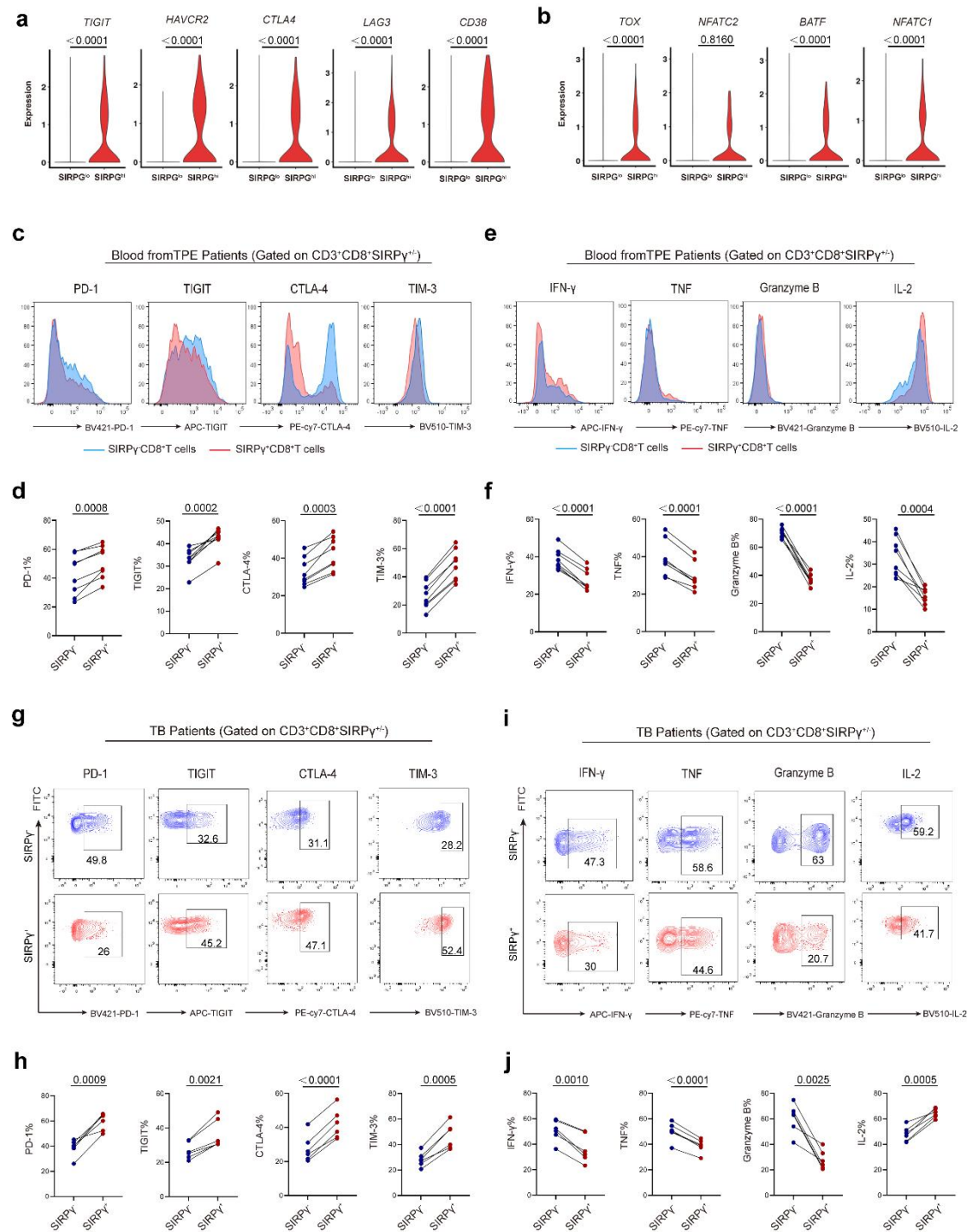
Supplementary Figure 6 SIRP γ is highly expressed in exhausted CD8⁺ T cells in TPE, related to Figure 2. (a) The differentiation trajectory of CD8⁺ T cells clusters. (b) Monocle2 pseudotime analysis showing the transition tendency among different sub-clusters of CD8⁺ T cells. (c) Expression dynamics of marker gene signatures of exhausted CD8⁺ T cells along the pseudotime axis. TEX, exhausted T cells; TEM, effector memory-like T cells; T_{EFF}, effector T cells; T_{CM}, central memory-like T cells; T_N, naïve-like T cells.

Supplementary Figure 7



Supplementary Figure 7 SIRPy serves as a potential mediator of T cell exhaustion in TPE, related to Figure 3. (a–f) Peripheral blood mononuclear cells were isolated from PPD+ donors using MACS and activated with CD3/CD28 antibodies for 24 hours. Subsequently, cells were treated with recombinant fusion antigen ESAT-6-CFP-10 (E-C). Flow cytometric analyses were conducted to measure the expression levels of inhibitory receptors (PD-1, TIGIT, CTLA-4, TIM-3) (a, b) and cytokines (IFN-γ, TNF, Granzyme B, IL-2) (c, d) (n = 5 biological replicates). Proliferation was evaluated using CFSE fluorescence labeling (e) (n = 3 independent experiments), while flow cytometric analysis was used to detect changes in SIRPy expression in CD8⁺ T cells following E-C treatment (n = 7 biological replicates per group). Statistical significance was determined by unpaired two-tailed Student's t-test (e) and paired two-tailed t-tests (b, d, f). Data are presented as mean values ± SD (b–f).

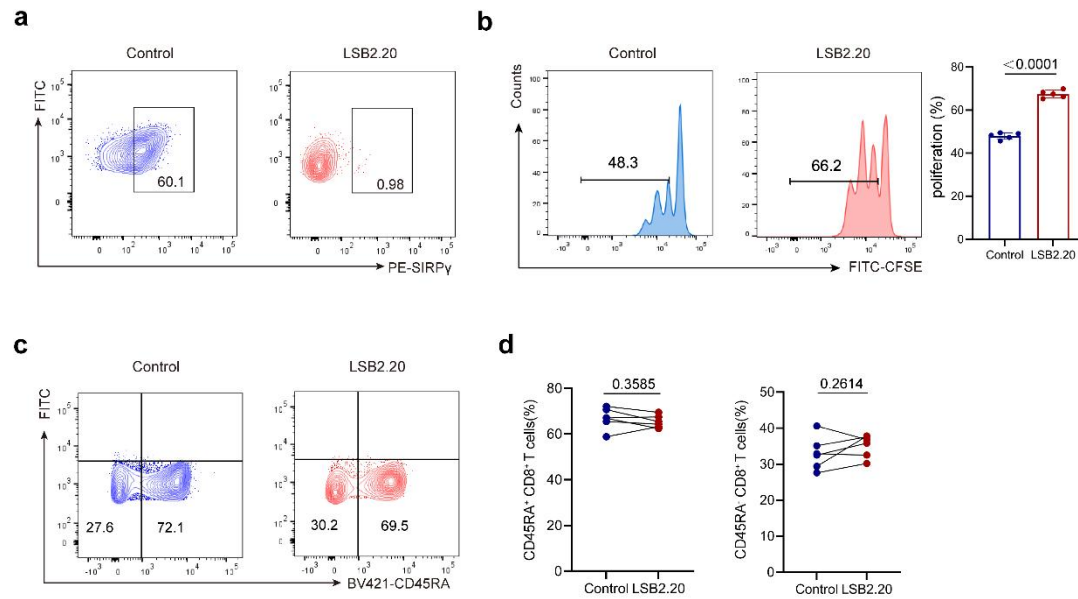
Supplementary Figure 8



Supplementary Figure 8 SIRPγ serves as a potential mediator of T cell exhaustion in TPE, related to Figure 3. (a, b) Violin plots depict differential gene expression between SIRPγ⁺ and SIRPγ⁻ CD8⁺ T cells in TPE. (c-f) CD8⁺ T cells isolated from peripheral blood of TPE patients were analyzed via flow cytometry for expression of inhibitory receptors (PD-1, TIGIT, CTLA-4, TIM-3) (c, d) and cytokines (IFN-γ, TNF,

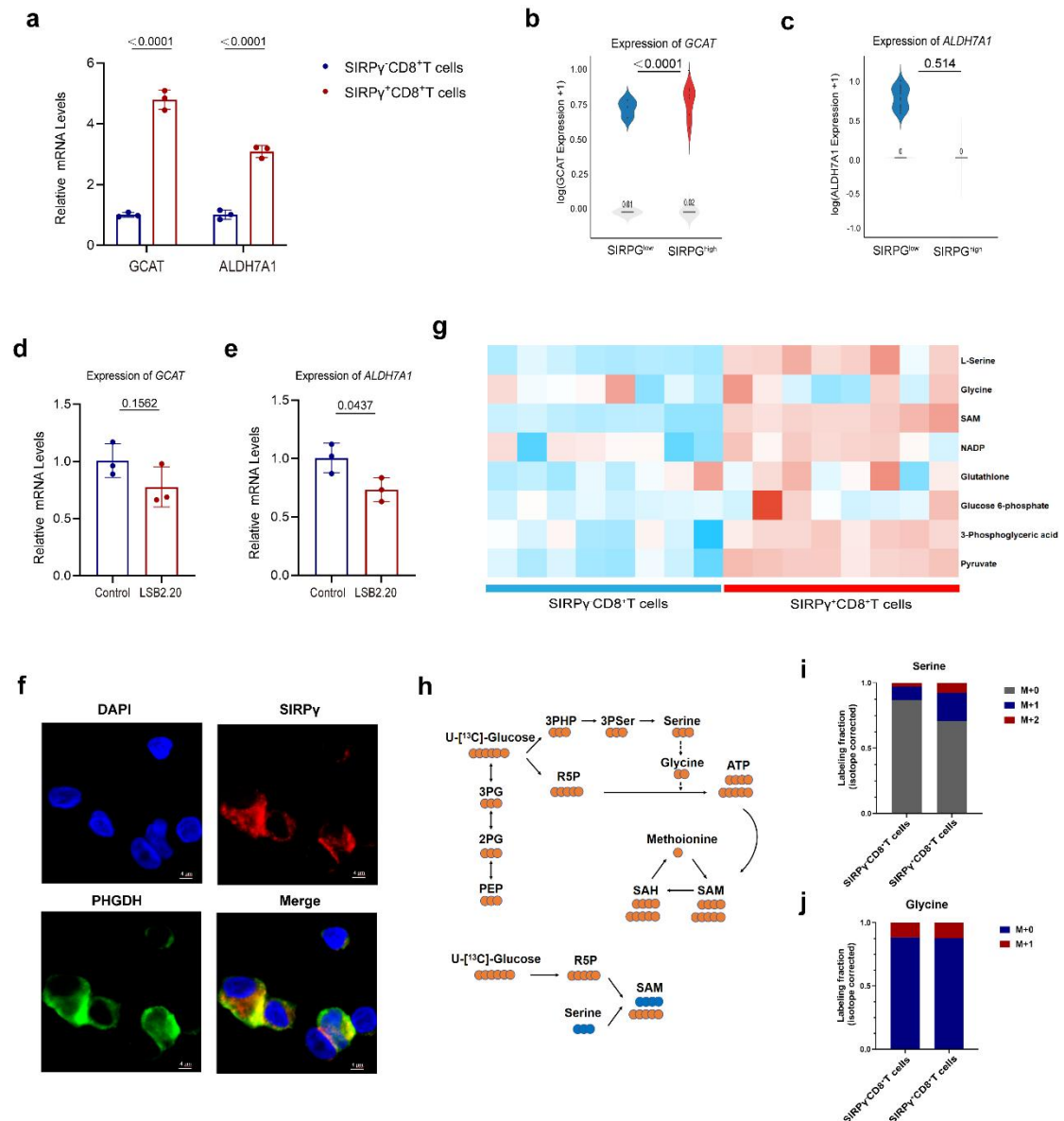
Granzyme B, IL-2) (**e, f**) in SIRP γ^+ and SIRP γ^- subsets. n = 8 patients for (**d**) and (**f**). (**g-j**) CD8 $^+$ T cells from tuberculosis (TB) patients were assessed by flow cytometry for inhibitory receptors (PD-1, TIGIT, CTLA-4, TIM-3) (**g, h**) and cytokines (IFN- γ , TNF, Granzyme B, IL-2) (**i, j**). n = 6 patients for (**h**) and (**j**). Statistical significance was determined by paired two-tailed t-tests (**d, f, h, j**).

Supplementary Figure 9



Supplementary Figure 9 SIRP γ serves as a potential mediator of T cell exhaustion in TPE, related to Figure 3. (a) Schematic illustration of SIRP γ blockade using the specific anti-SIRP γ monoclonal antibody LSB2.20. (b-d) CD8⁺ T cells isolated from TPE were activated with anti-CD3/CD28 antibodies for 24 hours, followed by 48-hour treatment with LSB2.20 (10 μ g/mL). Proliferation of SIRP γ -blocked CD8⁺ T cells was assessed via CFSE dilution (n = 5 independent experiments) (b). Flow cytometry quantified proportions of naïve (CD45RA⁺CD8⁺) and memory-like (CD45RA⁻CD8⁺) T cell subsets (n=5 patients per group) (c, d). Statistical significance was determined by unpaired two-tailed Student's t-test (b) and paired two-tailed t-tests (d). Data are presented as mean values $\pm$ SD (b).

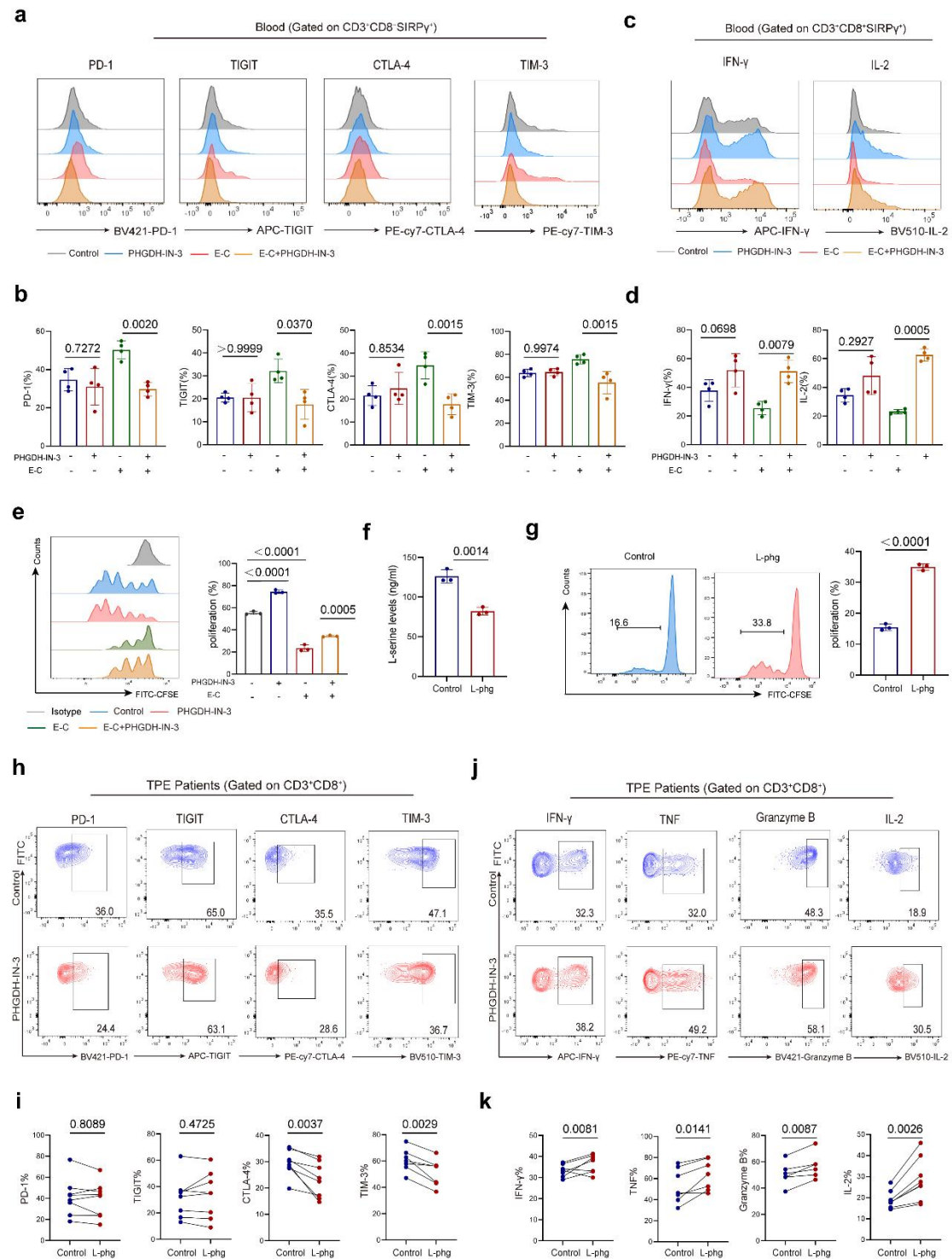
Supplementary Figure 10



Supplementary Figure 10 SIRPy promotes CD8⁺ T cell exhaustion by reprogramming serine metabolism in TPE, related to Figure 4. (a) Relative *GACT* and *ALDH7A1* expression in sorted SIRPy⁻ and SIRPy⁺ CD8⁺ T cells from TPE assessed by qPCR (n = 3 independent experiments). (b, c) *GCAT* (b) and *ALDH7A1* (c) expression SIRPy^{low} and SIRPy^{high} CD8⁺ T cells derived from single-cell RNA sequencing data. (d, e) CD8⁺ T cells isolated from TPE were activated with anti-CD3/CD28 antibodies for 24 hours, followed by 48-hour treatment with LSB2.20 (10 μg/mL). *GCAT* (d) and *ALDH7A1* (e) expression were analyzed by qPCR (n = 3 independent experiments). (f) Representative images of SIRPy and PHGDH staining in

CD8⁺ T cells of TPE. Scale bar 4μm. **(g–j)** SIRPγ[−] and SIRPγ⁺ CD8⁺ T cells isolated from TPE were incubated with U-[¹³C]-glucose for six hours. Heatmaps **(g)** and schematic representations **(h)** illustrate the incorporation of U-[¹³C]-glucose into various metabolites. The percent distribution of each isotopic isomer in the serine **(i)** and glycine **(j)** pool is shown (n = 8 patients per group). Statistical significance was determined by unpaired two-tailed Student's t-test **(a, d, e)**. Data are presented as mean values ± SD **(a, d, e)**.

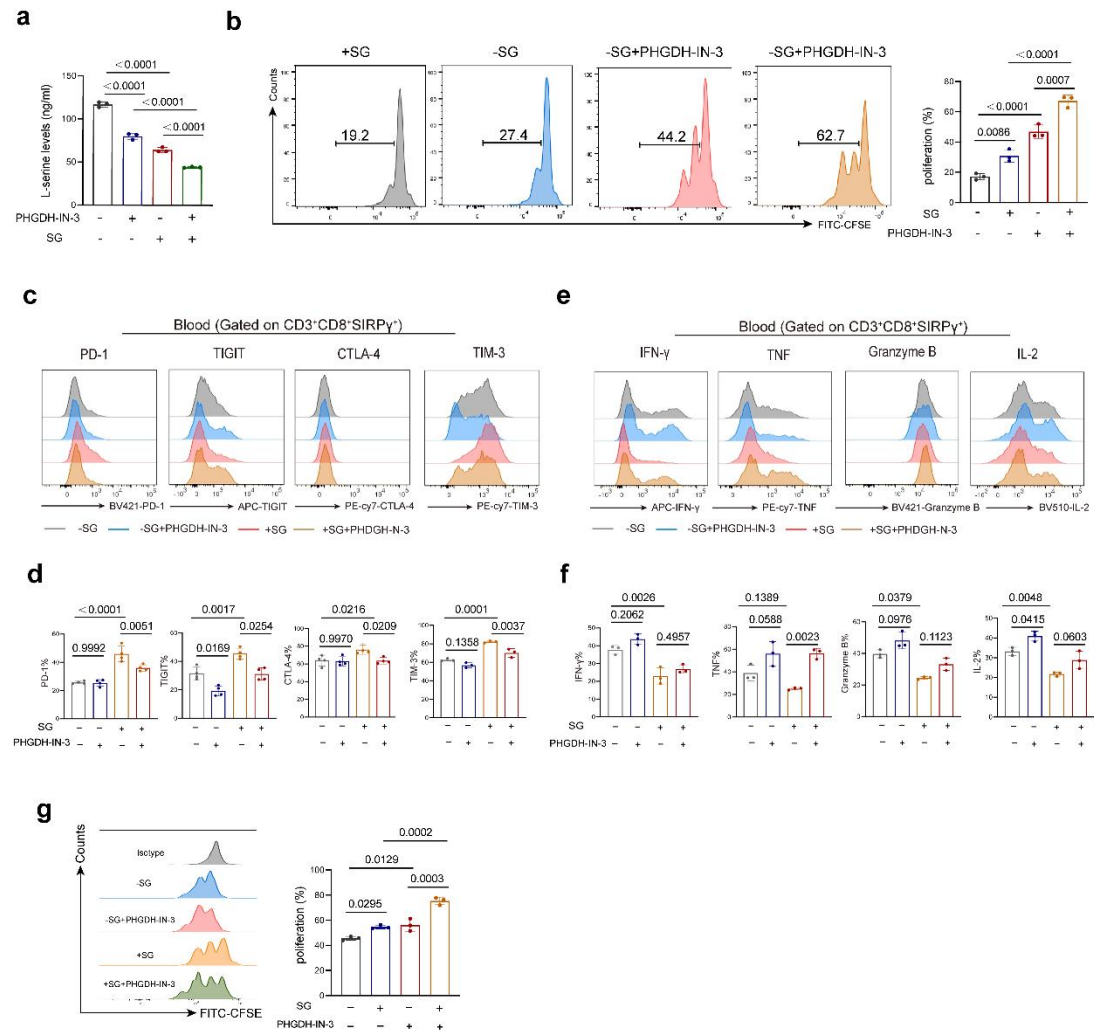
Supplementary Figure 11



Supplementary Figure 11 SIRPγ drives T cell exhaustion through PHGDH-mediated serine metabolism in TPE, related to Figure 5. (a-e) Mononuclear cells were isolated from the peripheral blood of TPE patients and treated with recombinant fusion antigen ESAT-6-CFP-10 (E-C) (10 μg/ml) for 20h. Subsequently, they were

exposed to the PHGDH inhibitor PHGDH-IN-3 (10 μ M) for 48h. Flow cytometry was employed to analyze the levels of inhibitory receptors (PD-1, TIGIT, CTLA-4, TIM-3) (**a, b**) and cytokines (IFN- γ , IL-2) (**c, d**) in CD8⁺ T cells. n = 4 biological replicates per group for (**b**) and (**d**). Proliferation was assessed using CFSE fluorescence labeling (**e**) (n = 3 independent experiments). (**f-k**) CD8⁺ T cells were isolated from TPE and activated with CD3/CD28 antibodies for 24h, followed by treatment with the alanine-serine-cysteine transporters (ASCT)-specific inhibitor L-phenylalanine (L-phg) (50 μ M) for 48h. Intracellular L-serine levels were measured via ELISA (**f**) (n = 3 independent experiments). Proliferation was evaluated via CFSE fluorescence labeling (**g**) (n = 3 independent experiments). Flow cytometry was performed to assess inhibitory receptor levels (PD-1, TIGIT, CTLA-4, TIM-3) (**h, i**) and cytokine expression (IFN- γ , TNF, Granzyme B, IL-2) (**j, k**). The number of independent replicates is indicated as follows: n = 9 (TIM-3), n = 8 (PD-1, TIGIT, CTLA-4, IL-2), n = 7 (IFN- γ , TNF), n = 6 (Granzyme B) per group. Statistical analysis was determined by unpaired two-tailed t-test (**f, g**), paired two-tailed t-tests (**i, k**), and one-way ANOVA (**b, d, e**). Data are presented as mean values $\pm$ SD (**b, d-g**).

Supplementary Figure 12

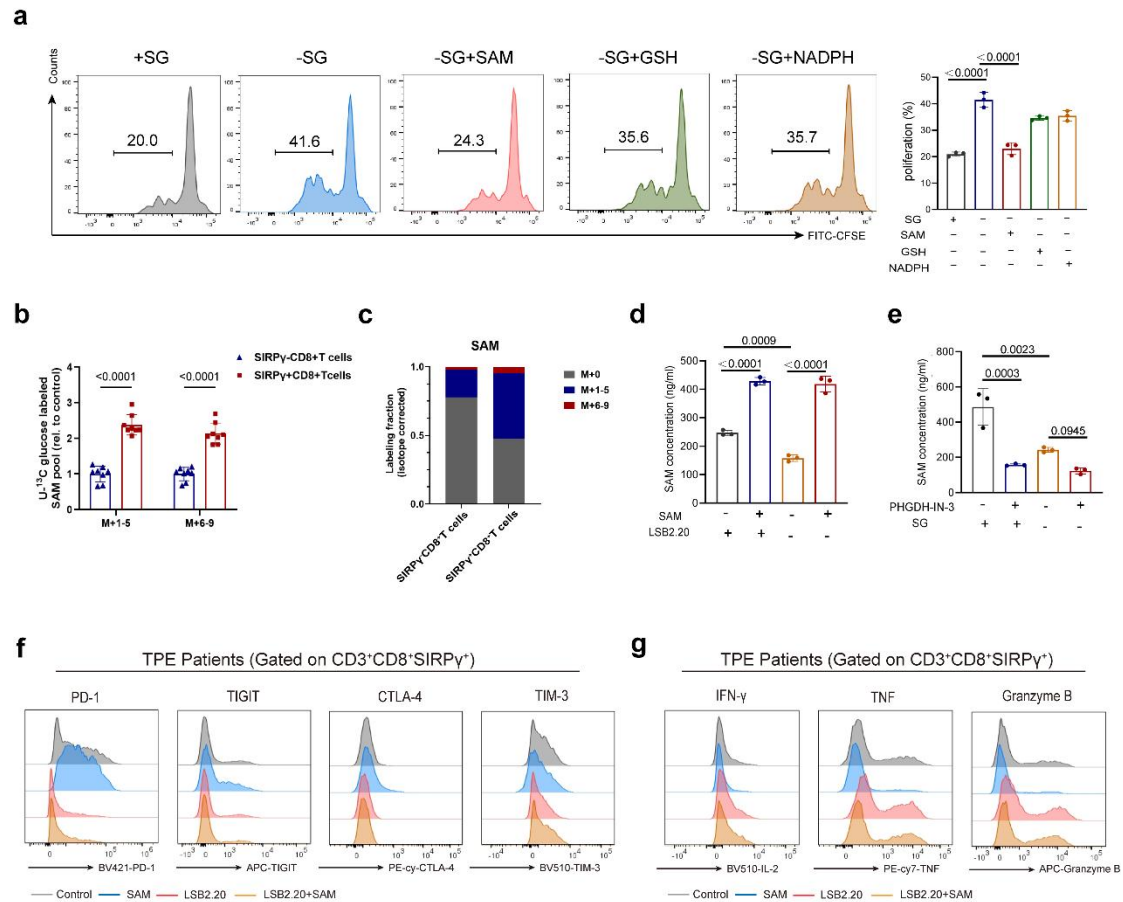


Supplementary Figure 12 SIRPy drives T cell exhaustion through PHGDH-mediated serine metabolism in TPE, related to Figure 5. (a, b) TPE-isolated CD8⁺ T cells were treated with or without PHGDH-IN-3 (24 h), with or without serine/glycine (SG) deprivation (3d). Intracellular L-serine levels were quantified by ELISA (a) (n = 3 independent experiments). Proliferation was assessed via CFSE dilution (b) (n = 3 independent experiments). (c-g) Mononuclear cells were isolated from the peripheral blood of TPE patients and treated with recombinant fusion antigen ESAT-6-CFP-10 (E-C) (10 µg/ml) for 2 days. They were treated with or without PHGDH-IN-3 (24 h), with or without serine/glycine (SG) deprivation (3d). Flow cytometry quantified expression of inhibitory receptors (PD-1, TIGIT, CTLA-4, TIM-3) (c, d) and cytokines (IFN-γ, TNF, Granzyme B, IL-2) (e, f). n = 3 biological replicates experiments per group for (d) and (f). Proliferation was assessed via CFSE dilution (g) (n = 3 independent

experiments). Statistical analysis was determined by one-way ANOVA (**a, b, d, f, g**).

Data are presented as mean values $\pm$ SD (**a, b, d, f, g**).

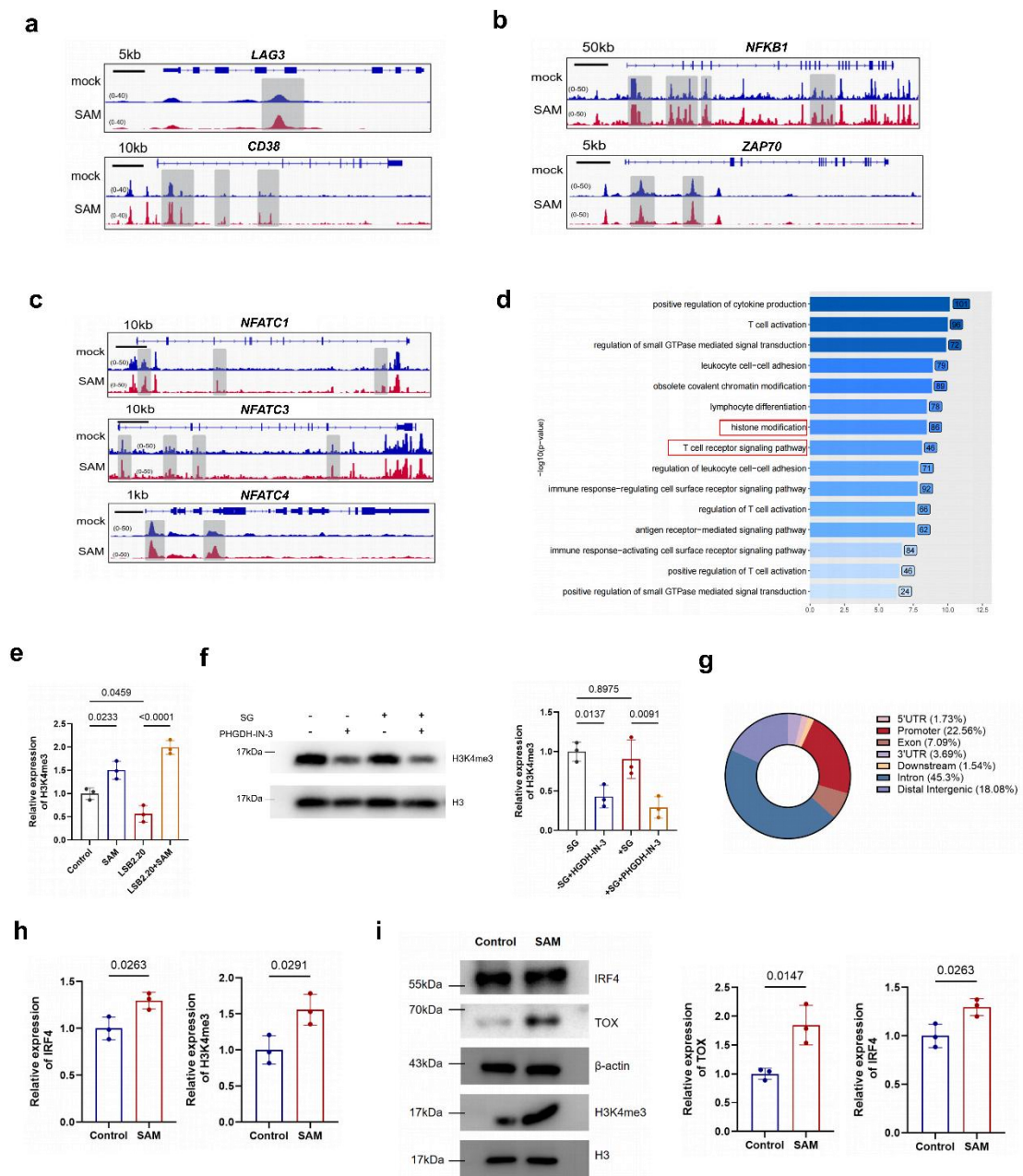
Supplementary Figure 13



Supplementary Figure 13 SIRPy drives T cell exhaustion through PHGDH-mediated serine metabolism in TPE, related to Figure 6. (a) CD8⁺ T cells isolated from TPE were activated with CD3/CD28 antibodies for 24h and subjected to serine/glycine (SG) medium starvation for 3 days. Cells were subsequently treated with 400 µM NADPH, GSH, or SAM for 48 h. Proliferation was assessed via CFSE dilution (n = 3 independent experiments). (b, c) Incorporation of U-[¹³C]-glucose carbon into SAM (b) and the percentage distribution of isotopologues in the SAM pool (c) in sorted SIRPy⁻ and SIRPy⁺ CD8⁺ T cells derived from TPE (n = 8 patients per group). (d) CD8⁺ T cells isolated from TPE were treated with the specific anti-SIRPy monoclonal antibody LSB2.20 (10 µg/mL) for 24h, followed by the addition of SAM (400 µM) for 48h. Intracellular SAM levels were measured using ELISA (n = 3 independent experiments). (e) CD8⁺ T cells isolated from TPE samples were treated with or without the PHGDH inhibitor PHGDH-IN-3 for 24h, followed by SG medium starvation for 3

days. Intracellular SAM levels were measured using ELISA (n = 3 independent experiments). **(f, g)** CD8⁺ T cells from TPE were treated with the specific anti-SIRP γ monoclonal antibody LSB2.20 (10 μ g/mL) for 24h, followed by 400 μ M SAM treatment for 48h. Flow cytometry was performed to assess the expression levels of inhibitory receptors (TIGIT, TIM-3, CTLA-4, PD-1) **(f)** and cytokines (IL-2, IFN- γ , Granzyme B, TNF) **(g)**. Statistical analysis was determined by one-way ANOVA **(a, d, e)** and two-tailed Student's t-test **(b)**. Data are presented as mean values $\pm$ SD **(a, b, d, e)**.

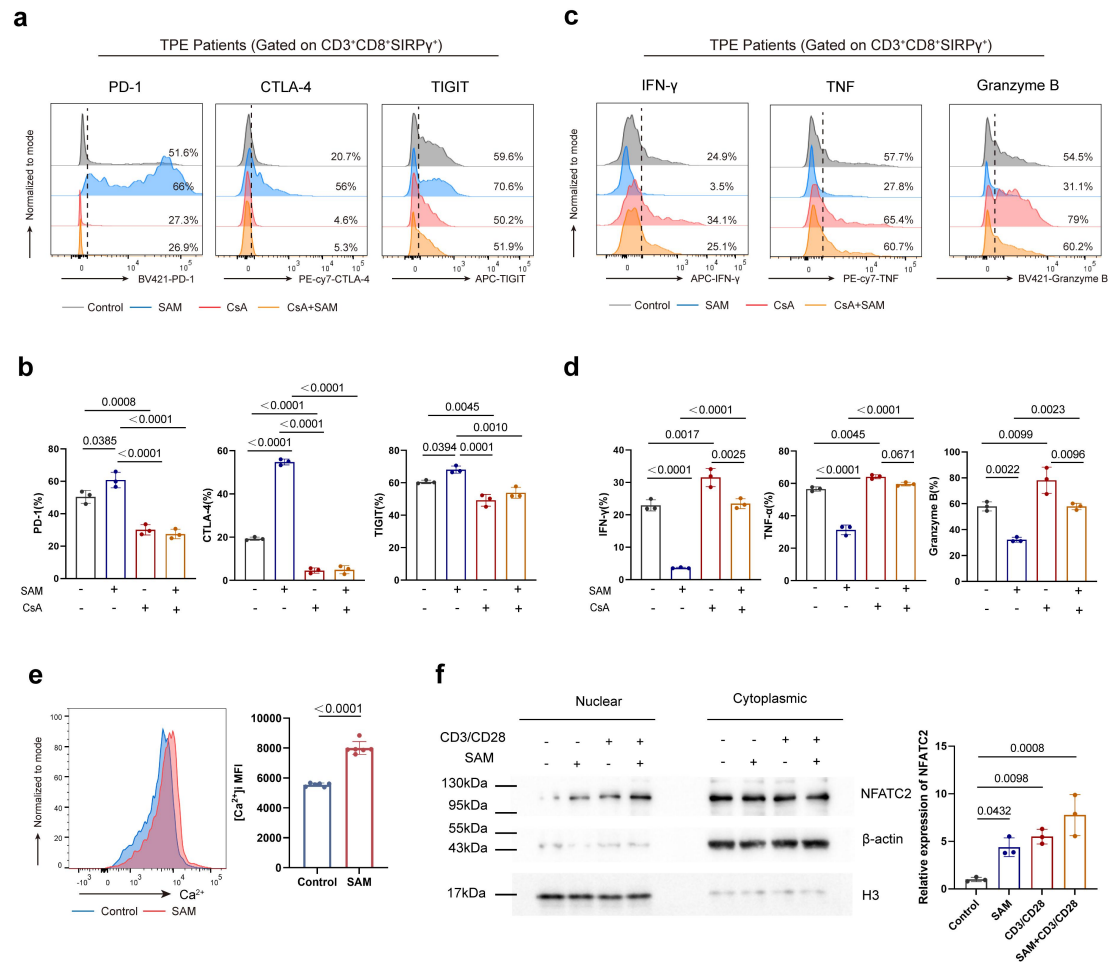
Supplementary Figure 14



Supplementary Figure 14 Serine-derived SAM promotes an active epigenetic state at the NFATC2 locus to drive T cell exhaustion, related to Figure 7. (a-c) ATAC-seq coverage at *LAG3* and *CD38* (**a**); *NFKB1* and *ZAP70* (**b**); *NFTAC1*, *NFATC3*, and *NFATC4* (**c**). All peaks were highlighted in gray bars. (**d**) GO pathway enrichment analysis of upregulated differentially expressed genes (DEGs) in mock- or SAM-treated CD8⁺ T cells. (**e**) Western blot analysis was performed to assess H3K4me3 levels markers in CD8⁺ T cells derived from TPE (n = 3 independent experiments). Following

stimulation with anti-CD3/CD28 antibodies for 24 h, the cells were treated with the anti-SIRP γ monoclonal antibody LSB2.20 (10 μ g/mL), and then supplemented with SAM (400 μ M) for 48 h. **(f)** CD8⁺T cells isolated from TPE were activated with anti-CD3/CD28 antibodies for 24 h, treated with or without the PHGDH inhibitor PHGDH-IN-3 for 24 h, followed by serine/glycine (SG) starvation for 3 days. H3K4me3 levels were analyzed by immunoblotting (n = 3 independent experiments). **(g)** Pie chart illustrating the genomic distribution of H3K4me3 CUT&Tag-seq peaks in mock- or SAM-treated CD8⁺T cells. **(h)** Protein expression of NFATC2 and H3K4me3 was analyzed by Western blot in mock- or SAM-treated CD8⁺ T cells (n = 3). **(i)** Western blot analysis of TOX, IRF4, and H3K4me3 in mock- or SAM-treated CD8⁺T cells (n = 3 independent experiments). Statistical significance was determined by one-way ANOVA (**e**, **f**) and unpaired two-tailed t-test (**h**, **i**). Data are presented as mean values $\pm$ SD (**e**, **f**, **h**, **i**).

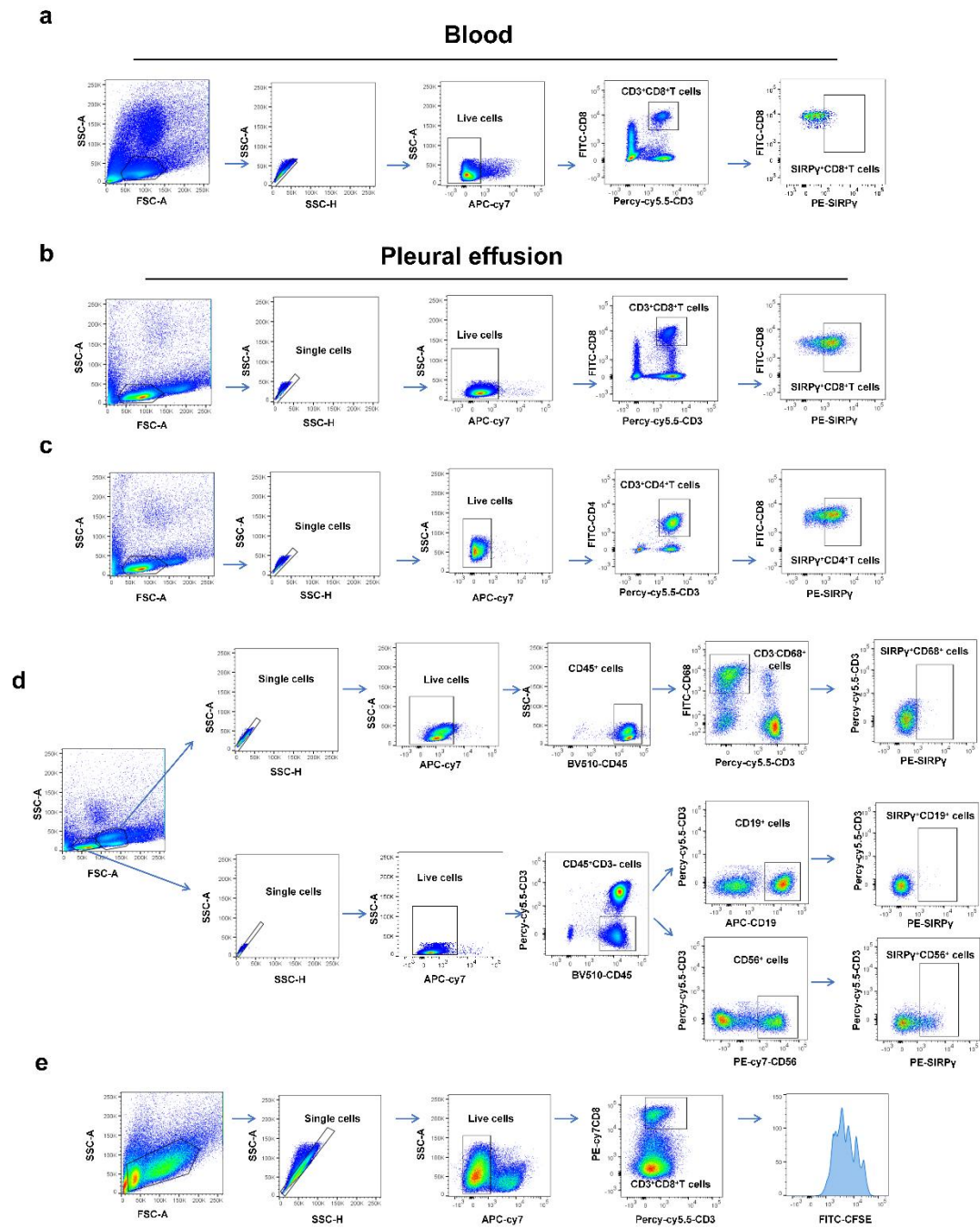
Supplementary Figure 15



Supplementary Figure 15 Serine-derived SAM promotes an active epigenetic state at the NFATC2 locus to drive T cell exhaustion, related to Figure 7. (a-d) CD8⁺ T cells isolated from TPE were activated ex vivo and treated with or without 400 μ M SAM $\pm$ cyclosporin A (CsA) (1 μ M). The expression levels of inhibitory receptors (TIGIT, CTLA-4, PD-1) (a, b) and cytokines (Granzyme B, IFN- γ , TNF) (c, d) were measured by flow cytometry. n = 3 biological replicates experiments per group for (b) and (d). (e) CD8⁺ T cells from TPE were incubated with SAM (400 μ M) for 20 h, after which intracellular Ca²⁺ levels ([Ca²⁺]_i) were assessed via flow cytometry (n = 6 biological replicates experiments per group). (f) Representative Western blots of nuclear and cytoplasmic extracts from CD8⁺ T cells isolated from TPE, either untreated or treated with CD3/CD28 antibodies (1 μ g/mL) $\pm$ SAM (400 μ M), showing NFATC2 protein expression (n = 3 independent experiments). Statistical analysis was determined

by unpaired two-tailed t-test (**e**) and one-way ANOVA (**b, d, f**). Data are presented as mean values $\pm$ SD (**b-f**).

Supplementary Figure 16



Supplementary Figure 16 Gating strategy for immune cells from pleural effusion and blood. (a, b) Gating strategy for SIRPy⁺CD3⁺CD8⁺T cells isolated from blood **(a)** and pleural effusion **(b)**; **(c)** Gating strategy for SIRPy⁺CD3⁺CD4⁺T cells isolated from pleural effusion; **(d)** Gating strategy for isolating SIRPy⁺ immune cells from pleural effusion. Shown are the strategies for identifying SIRPy⁺ macrophages (CD45⁺CD3⁻

CD68⁺), SIRP γ ⁺ B cells (CD45⁺CD3⁻CD19⁺), and SIRP γ ⁺ natural killer cells (CD45⁺CD3⁻CD56⁺). (e) Gating strategy for SIRP γ ⁺CD3⁺CD8⁺T cells used in the proliferation assay.

**Supplementary Table 1 Diagnostic performance of pleural SIRP γ , ADA, and
SIRP γ ×ADA between TPE and MPE patients**

Variables	Cut-off value	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)
SIRP γ	1324 pg/ml	0.9037 (0.8375 - 0.9698)	84.09	90.00	8.409	0.1768	89.37	84.98
ADA	24.2 U/L	0.9361 (0.884 - 0.9882)	86.36	87.50	6.9088	0.1559	87.36	86.52
SIRP γ ×ADA	39414 ng U/L ²	0.9585 (0.9190 - 0.9980)	93.18	92.50	12.424	0.0737	94.52	83.94

TPE, tuberculous pleural effusion; MPE, malignant pleural effusion; SIRP γ , signal regulatory protein γ ; ADA, adenosine deaminase; AUC, area under the curve; PLR, positive likelihood ratio; NPV, negative likelihood ratio; PPV, Positive Predictive Value; NLR, negative predictive value.

**Supplementary Table 2 Diagnostic performance of pleural SIRP γ , ADA, and
SIRP γ ×ADA between TPE and non-TPE patients**

Variables	Cut-off value	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)
SIRP γ	1324 pg/ml	0.9481 (0.9162 - 0.9801)	82.09	91.03	9.152	0.1967	87.10	85.71
ADA	24.2 U/L	0.9238 (0.8778 - 0.9699)	80.60	92.31	10.481	0.2102	88.33	85.71
SIRP γ ×ADA	39414 ng U/L ²	0.9732 (0.9510 - 0.9954)	81.82	97.44	31.961	0.1866	94.55	84.44

TPE, tuberculous pleural effusion; SIRP γ , signal regulatory protein γ ; ADA, adenosine deaminase; AUC, area under the curve; PLR, positive likelihood ratio; NPV, negative likelihood ratio; PPV, Positive Predictive Value; NLR, negative predictive value.

Supplementary Table 3 Demographic, cytological and biochemical characteristics in pleural effusions

	Training Cohort			Validation Cohort				
Parameters	TPE (n=44)	MPE (n=40)	P value	TPE (n=66)	MPE (n=35)	PPE (n=27)	Miscellaneous (n=16)	P value
Sex, male, %	61.4	52.5	0.5090	71.6	67.7	74.1	35.3	0.1602
Age, years	48.43 ± 21.81	63.73 ± 14.27	0.0003	58.37± 19.63	61.50 ± 14.08	64.58 ± 14.95	60.44 ± 16.31	0.4623
ADA, U/L	40.65 ± 13.69	14.25 ± 9.371	<0.0001	38.56 ± 21.70	10.24 ± 6.619	16.35 ± 17.01	11.79 ± 6.091	<0.0001
LDH, U/L	644.5 ± 595.0	711.1 ± 671.4	0.6311	486.1 ± 298.6	272.3 ± 234.7	466.0 ± 701.0	267.7 ± 171.7	0.0228
Protein, g/L	46.88 ± 5.706	41.82 ± 5.706	0.0038	42.42 ± 11.08	40.74 ± 8.347	34.15 ± 11.28	30.14 ± 10.66	<0.0001
Cell counts, cells/mm3	4198 ± 2663	3685 ± 2114	0.3699	2597 ± 1902	4077 ± 3166	5622 ± 7889	685.8 ± 748.6	0.0009
Mononuclear cells, %	77.7 ± 17.15	79.55 ± 20.01	0.6625	79.09 ± 20.25	80.06 ± 11.96	83.23 ± 20.01	84.25 ± 8.729	0.6155

P<0.05 represents statistical significance; Data are presented as mean ± SEM. In training cohort, comparisons were performed using unpaired two-tailed t-test (age, ADA, LDH, glucose, protein, cell counts, lymphocyte) and two-sided Fisher's exact test (sex). In validation cohort, comparisons were performed using unpaired t one-way ANOVA (age, ADA, LDH, glucose, protein, cell counts, lymphocyte) and Fisher's exact test (sex). LDH, lactate dehydrogenase; ADA, adenosine deaminase; MPE, malignant pleural effusion; PPE, parapneumonic pleural effusion; TPE, tuberculous pleural effusion.

Supplementary Table 4 Material and Methods

REAGENT	SOURCE	IDENTIFIER
Antibodies		Cat# and dilutions
Fixable Viability Stain 780	BD Pharmingen	Cat# 565388 (1: 50)
Human TruStain FcX™	Biolegend	Cat# 422302 (1: 50)
Brilliant Violet 510™ anti-human CD45 Antibody	Biolegend	Cat# 304036 (1: 50)
PerCP/Cyanine5.5 anti-human CD3	Biolegend	Cat# 317336 (1: 50)
FITC anti-human CD8a	Biolegend	Cat# 301050 (1: 20)
PE/Cyanine7 anti-human CD8a	Biolegend	Cat# 301012 (1: 20)
FITC anti-human CD4	Biolegend	Cat# 300538 (1: 50)
FITC anti-human CD68	Biolegend	Cat# 333806 (1: 50)
APC anti-human CD19	Biolegend	Cat# 982406 (1: 50)
PE/Cyanine7 anti-human CD56	Biolegend	Cat# 985912 (1: 50)
PE anti-human CD172g (SIRPγ)	Biolegend	Cat# 336606 (1: 100)
Brilliant Violet 421™ anti-human CD279 (PD-1)	Biolegend	Cat# 329920 (1: 50)
APC anti-human TIGIT (VSTM3)	Biolegend	Cat# 372706 (1: 50)
Brilliant Violet 510™ anti-human CD366 (TIM-3)	Biolegend	Cat# 345030 (1: 50)
PE/Cyanine7 anti-human CD366 (Tim-3) Antibody	Biolegend	Cat# 364820 (1: 50)
PE/Cyanine7 anti-human CD152 (CTLA-4)	Biolegend	Cat# 349914 (1: 50)
APC anti-human IFN-γ	Biolegend	Cat# 502512 (1: 50)
BD Horizon™ BV421 Mouse Anti- Human IFN-γ	BD Horizon™	Cat# 562988 (1: 50)

PE/Cyanine7 anti-human TNF	Biolegend	Cat# 502930 (1: 50)
Brilliant Violet 510™ anti-human IL-2	Biolegend	Cat# 500338 (1: 20)
Brilliant Violet 421™ anti-human/mouse Granzyme B	Biolegend	Cat# 515410 (1: 100)
APC anti-human/mouse Granzyme B Recombinant Antibody	Biolegend	Cat# 372204 (1: 50)
Ultra-LEAF™ Purified anti-human CD28 Antibody	Biolegend	Cat# 302934 (1: 1000)
Ultra-LEAF™ Purified anti-human CD3 Antibody	Biolegend	Cat# 317326 (1: 1000)
CFSE	BD Pharmingen	Cat# 565082
Antibodies		Cat# and dilutions
Anti-Histone H3	Abcam	Cat# ab12079 (1:1500)
Tri-Methyl-Histone H3 (Lys4)/H3K4me3 Antibody	Affinity	Cat# DF6935 (1:1500)
Tri-Methyl-Histone H3 (Lys9)/H3K9me3 Antibody	Affinity	Cat# DF6938 (1:1500)
Anti-Trimethyl-Histone H3 (Lys27)/H3K27me3 Rabbit mAb	PTM Bio	Cat# PTM-647RM (1:1500)
SIRPG Antibody	Affinity	Cat# DF4508 (WB, 1:1500; IF, 1:200)
PHGDH Polyclonal antibody	proteintech	Cat# 14719-1-AP (WB, 1:1500; IF, 1:200)
Anti-IRF4 Rabbit pAb	ABclonal	Cat# A0524 (1:1000)
Anti-TOX Antibody	Abcam	Cat# ab237009 (1:1500)
Anti-NFATC2 Antibody	Abcam	Cat# ab283691 (1:1000)
mTOR Antibody	Abclonal	Cat# A11355 (1:1500)
Phospho-mTOR	Abclonal	Cat# AP0115 (1:1000)

(Ser2448) Antibody		
PI3-kinase p85-alpha/gamma pAb	Abmart	Cat# T40064 (1:1000)
Phospho-PI3-kinase p85- alpha/ gamma (Tyr467/199) pAb	Abmart	Cat# T40116 (1:1000)
AKT1/2/3 Antibody	Abmart	Cat# T55561 (1:1000)
Phospho-pan-AKT1/2/3 (Ser473) Antibody	Abmart	Cat# TA0016 (1:1000)
β -Actin Antibody	Cell Signaling Technology (CST)	Cat# 4967 (1:3000)
anti-CD4 Antibody	Abcam	Cat# ab133616 (1:100)
anti-CD8Antibody	Abcam	Cat# ab237709 (1:100)
anti-CD68 Antibody	Abcam	Cat# ab283654 (1:100)
Chemicals, peptides, and recombinant proteins		Cat#
Fetal Bovine Serum	Gibco	Cat# 16140071
RPMI 1640 Medium	Gibco	Cat# C11875500BT
1640 Medium (excluding serine and glycine)	Boster	Cat# DZPYG0062
Phorbol 12-myristate 13-acetate (PMA)	ENZO	Cat#BML-PE160-0001
Brefeldin A(BFA)	Biolegend	Cat# 420601
Ionomycin	ENZO	Cat#ALX-450-007-M001
EasySep Human CD8 Positive Selection Kit II	STEMCELL	Cat# 17853
Ficoll-Paque™	STEMCELL	Cat# 7851
Protease inhibitor	Servicebio	Cat# G2006
PMSF	Servicebio	Cat# G2008
RIPA Lysis Buffer	Servicebio	Cat# G2002
180 kDa Prestained Protein Marker	Vazyme	Cat# MP102-01

ColorMixed Protein Marker 250 (10-250 kDa)	ABclonal	Cat# RM02949
TRIzol Reagent	TAKARA	Cat# 9109
Invitrogen™ eBioscience™ IC Fixation Buffer	Invitrogen	Cat# 00-8222-49
Foxp3/Transcription Factor Staining Buffer	eBioscience	Cat# 00-5523-00
ECL Prime Western Blotting Detection Reagent	Servicebio	Cat# 32209
Hiscript qRT-PCR SuperMix	Vazyme	Cat# R122-01
AceQ qPCR SYBR Green Master Mix	Vazyme	Cat# R323-01
BCA Protein Assay Kit	Beyotime	Cat# P0012
Human Signal Regulatory Protein Gamma (SIRPg) ELISA Kit	GenMed	Cat# JYM2810Hu
Human L-Serine ELISA Kit	Camilo	Cat# 2H-KMLJh315544
General SAM/ S-Adenosyl methionine ELISA Kit	ABclonal	Cat# RK04519
Purified anti-human CD172g (SIRPγ)	BioLegend	Cat# 336602
PHGDH-IN-3	proteintech	Cat# CM11658
Purified anti-human CD172g (SIRPγ) Antibody	Biolegend	Cat# 336602
serine	Sigma-Aldrich	Cat#S4311
glycine	Sigma-Aldrich	Cat# G5417
L-Phenylglycine	Sigma-Aldrich	Cat# 237647
AL34662 (formate)	GLPBIO	Cat# GC 1703
NADPH tetrasodium sat	GLPBIO	Cat# GC34058

L-Glutathione reduced	GLPBIO	Cat# GC12203
S-Adenosy---methionine tosylate	GLPBIO	Cat# GC61259
Deposited data		
Human scRNA-seq of TPE		https://ngdc.cncb.ac.cn/
RNA-seq	This paper	
ATAC-seq	This paper	
CUT&Tag	This paper	

Supplementary Table 5 Flow cytometry panels

Cell Subsets								
	APC-cy7	Percy-cy5.5	FITC	PE	APC	PE-cy7	BV421	BV510
Human-macrophage cells	FVS	CD3	CD68	SIRP γ				CD45
Human-B cells	FVS	CD3		SIRP γ	CD19			CD45
Human-natural killer cells	FVS	CD3		SIRP γ		CD56		CD45
Human-CD4 ⁺ T cells	FVS	CD3	CD4	SIRP γ				CD45
Human-CD8 ⁺ T cells-1	FVS	CD3	CD8	SIRP γ				CD45
Human-CD8 ⁺ T cells-2	FVS	CD3	CD8	SIRP γ	IFN- γ	TNF	Granzyme B	IL-2
Human-CD8 ⁺ T cells-3	FVS	CD3	CD8	SIRP γ	TIGIT	CTLA-4	PD-1	TIM-3
Human-CD8 ⁺ T cells-4	FVS	CD3		SIRP γ		CD8		
Human-CD8 ⁺ T cells-5	FVS	CD3	CD8	SIRP γ		CD45RO	CD45RA	
Human-CD8 ⁺ T cells-6	FVS	CD3	CD8	SIRP γ	Granzyme B	TIM-3	IFN- γ	IL-2

The table details the fluorochrome-conjugated antibodies used for flow cytometric analysis. The specific panels correspond to the following figures: Human-macrophage cells, Human-B cells, Human-CD4⁺T cells, Human-CD8⁺T cells-1 were used for immune cell phenotyping in Fig. 2d-i, Fig S3f-i; Human-CD8⁺T cells-2 and Human-CD8⁺T cells-3 was used for T cell phenotype assays in Fig. 3c-j, Fig. 5c-j, Fig. 6a-j; Fig S5a-d, f, Fig S6c-j, Fig S7a, Fig S9a, b, h-k, Fig. S10c-f, Fig. S11f, Fig. S12a-d; Human-CD8⁺T cells-4 was used for CSFE and [Ca²⁺]_i assays in Fig. 5b, Fig S5e, Fig S7b, Fig. S9e, g, Fig. S10b, g, Fig. S11a ; Human-CD8⁺T cells-5 was used for T cell phenotype assays in Fig S7c, d; Human-CD8⁺T cells-6 was used for T cell phenotype assays in Fig. 6a-d, Fig. S9a-d, Fig. S10c, d, Fig. S11g.

Supplementary Table 6 Primer sequences for qPCR

Genes	Sequence (5' to 3')
<i>SIRPG-F</i>	CTCCTGGTCCTTTCCTGCTTCT
<i>SIRPG-R</i>	AGTGCAGAGTGGCTGTCTTTCC
<i>PHGDH-F</i>	AGGTCCAAGCCAGCGAAAGAT
<i>PHGDH-R</i>	AAGATGCCCTTCCTTGTTGCG
<i>ALDH7A1-F</i>	ACCAAGCAGGCAGTGAGCAT
<i>ALDH7A1-R</i>	AGGTCCAAGCCAGCGAAAGAT
<i>GCAI-F</i>	AGCTGGCACTTGGAAGAGTGA
<i>GCAI-R</i>	ATAGCGGTACTTGTGGGCCTT
<i>GAPDH-F</i>	ACTCCTCCACCTTTGACGCT-3
<i>GAPDH-R</i>	CACCCTGTTGCTGTAGCCAAAT

Supplementary Table 7 Primer sequences for ChIP-qPCR

Genes	Sequence (5' to 3')
<i>HAVCR2-F</i>	TGGCTCTTATCTTCGGCTCT
<i>HAVCR2-R</i>	TCACGACCCAGTGTTAAAGG
<i>PDCD1-F</i>	TCATAAACGAGAGCTTCCTCGC
<i>PDCD1-R</i>	ACTGCTGTGGCCTCTTTCCA
<i>CTLA4-F</i>	ATCTCCAGGCAAAGCCACTGA
<i>CTLA4-R</i>	CCATCAGCACCACACTCACCAT
<i>TIGIT-F</i>	TGGAAACCTACCTACAGCGCA
<i>TIGIT-R</i>	CAGCCGGAGCCATTACCTTTCT
<i>TOX-F</i>	ACCCAGGCAATGGAGTATGTGC
<i>TOX-R</i>	CAAGAGAACGATCAACTAGGGACG
<i>IRF4-F</i>	ACACTCCTACCATAGTGGCTTCC
<i>IRF4-R</i>	GCTGTCCTCCCTGGTTCTTTGA