Supplemental Materials

Materials and methods

Patients and settings

Demographic characteristics and clinical presentations, such as cough, dyspnea, hemoptysis, weight loss (≥ 10% reduction in six months), and fever, were recorded during the enrollment interviews. Radiological presentations, including cavitary lesions and pleural effusion, at the time of TB diagnosis were independently reviewed by a chest physician. Two patients with active pulmonary TB who underwent surgical procedures and had available lung tissue were prospectively enrolled. Both lung specimens exhibited necrotizing granulomatous inflammation in the presence of acid-fast bacilli, and culture confirmed the presence of MTB. The lung tissues were used for IHC analysis.

MTB-related materials

To prepare MTB whole-cell lysates, MTB cells were suspended in phosphate buffered saline (PBS) and disrupted using a French press, resulting in approximately 90% cell breakage. The lysates were then centrifuged to separate and pellet the intact cells. The whole cell lysates contained proteins, lipids, and carbohydrates. Recombinant EsxA was expressed in *Escherichia coli* BL21 (DE3) pLysS and subsequently purified using immobilized metal affinity chromatography.

IHC and IF analysis

For IHC analysis, 4 μm sections of lung tissues were incubated with anti-PD-L1 antibody (R&D Systems) and anti-TET1 antibody (GeneTex; Irvine, CA, USA) at 10 mg/mL (diluted 1:100) for 60 min at room temperature. Immunohistochemistry (IHC) analysis was performed using a Leica Bond-MAX automatic IHC staining system. IF was performed to evaluate PD-L1 and TET1 expression in THP-1 cells before and after stimulation with MTB whole-cell lysate and EsxA protein. Alexa Fluor conjugated (Invitrogen) secondary antibodies were used for detection. Negative control experiments were performed in the absence of primary antibodies to determine the background autofluorescence levels.

Western blot assay

After stimulation with MTB-related materials, THP-1 cells were harvested and lysed in a lysis buffer containing a protease inhibitor cocktail. After centrifugation, the protein concentration in each sample was measured. Equal amounts of protein were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to polyvinylidene fluoride membranes. The membranes were incubated for 1 h at room temperature in a blocking solution (5% nonfat milk in PBS with 0.1% Tween 20), followed by incubation with primary antibodies against PD-L1 (Cell Signaling Technology, Danvers, MA, USA) at room temperature for 3 h. The membranes were then washed thrice with PBST and incubated with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibodies at room temperature for 1 h. The intensities of the PD-L1 bands in the western blot assays were quantified by measuring the peak areas of the densitogram using ImageJ software.

TET1 knockdown with shRNA

For the *TET1* knockdown experiment, we initially tested the optimal concentration of the selection agent (puromycin dihydrochloride, sc-108071, Santa Cruz (Dallas, TX, USA)) required to kill 100% of THP-1 cells. Puromycin (1 μg/mL) was used for the selection protocol. Transduction of THP-1 cells was performed in the presence of 8 μg/mL polybrene (sc-134220, Santa Cruz Biotechnology, Dallas, TX, USA), and viral stocks were added to the cells (1×10^4 cells/well in 96-well plates) at a multiplicity of infection (MOI) of 10. After 24 h of transduction, the cells were collected and allowed to proliferate until a sufficient number of cells was obtained for puromycin selection. The cell culture medium was replaced with fresh medium containing 1 μg/mL puromycin every 2–3 days until resistant clones emerged. After 3–4 weeks, the cells were collected and examined for GFP and TET1 expression by RT-PCR. The selected clones were maintained in fresh puromycin-containing medium for further experiments.

Promoter methylation analysis using bisulfite sequencing

Genomic DNA was extracted from peripheral PBMCs collected from patients with active TB, and bisulfite treatment was performed using an EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA). Subsequently, PCR was performed on the bisulfite-treated DNA to amplify the promoter regions of PD-L1 using hot-start TaKaRa Taq DNA polymerase (TaKaRa Bio, Shiga, Japan). Considering the previously reported high methylation

status of cg19724470 and cg14305799 (16), methylation-specific primers were designed to cover these two CpG loci and then used for further investigation. The primers used for bisulfite sequencing are listed in Appendix 1.

Supplementary Table 1. Sequences of primers used in real-time PCR

Target	Forward	Reverse
PD-L1	TGGCATTTGCTGAACGCATTT	TGCAGCCAGGTCTAATTGTTTT
DNMT1	GAATCAGTTATGTGACTTGGAAACC	CTAGACGTCCATTCACTTCCC
DNMT3A	TGACCTCTCCATCGTCAACC	GGTTGGACTCGAGAAATCGC
DNMT3B	CCCATTCGAGTCCTGTCATTG	TTGATATTCCCCTCGTGCTTC
TET-1	CATCAGTCAAGACTTTAAGCCCT	CGGGTGGTTTAGGTTCTGTTT
TET-2	GATAGAACCAACCATGTTGAGGG	TGGAGCTTTGTAGCCAGAGGT
Bisulfite seq-Cg14305799	GYGGGATTTYGTTTTYGGGTTTGGYGTAAYGTTGAGTAGTTGGYGYGTTTYGYGYGGTT TTAGTTTTGYGTAGTTTTTYGAGGTTTYGTATTAGTYG	
Bisulfite seq-Cg19724470	TTGGATAYGGGTTTAAGTTTATYGTTAGTTGTTTGTTAGTAATATGAT	
shRNA-TET1 knockdown	GCAGCTAATGAAGGTCCAGAA	

Supplementary Table 2. Information of antibodies used in the western-blot, and immunohistochemistry (IHC), and immunofluorescence (IF) analyses

Experiment	Antibody	Manufacturer	Catalog number
Western blot	PD-L1	Cell signaling	13684s
Western blot	GAPDH	Abcam	Ab9484
IHC	PD-L1	R&D Systems	ab217313
IHC	TET-1	GeneTex	MAB1187
IHC	CD68 (Human)	Dako	M0814
IF	PD-L1	GeneTex	GTX104763
IF	TET-1	GeneTex	GTX627420

Supplementary Figure 1. Comparisons of *PD-L1, DNMTs*, and *TETs* expression in THP-1 cells treated with *Mycobacterium tuberculosis*-related materials. THP-1 cells were treated with H37Rv whole cell lysate (10 and 20 μg/ml) and EsxA protein (0.5 and 2 μg/ml) for 24 hours.