Supplemental Methods

Cell isolation and culture

Murine bone marrow-derived dendritic cells (BMDCs), murine T cells from the spleen, and Lewis lung cancer (LLC), mouse melanoma B16-F10 and the OVA-transfected clone derived from E.G7 (E.G7-OVA) cell lines were used for *in vitro* studies. BMDCs and T cells were isolated and cultured in RPMI-1640 medium. Briefly, the bone marrow cells from mice were pooled, filtered through a 70 μm nylon mesh filter (Corning, USA), centrifuged and resuspended in ACK lysis buffer for 5 min of incubation on ice. After rinsing by medium, the bone marrow cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS (Gibco, USA), 100 U/mL penicillin/streptomycin (Gibco, USA) and additions of 1 mM sodium pyruvate, 50 μM β-mercaptoethanol (β-ME), 20 ng/mL GM-CSF and 15 ng/mL IL-4 for BMDCs differentiation. The non-adherent and loosely adherent cells were collected as BMDCs. The spleen was isolated from mice, grinded and filtered throng a 70 μm nylon mesh filter for lymphocytes processing using a lymphocyte separation solution kit (Dakewe, China). The isolated lymphocytes were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin/streptomycin and additions of 1 mM pyruvate, 50 Mm β-ME and 100 U/mL IL-2 for T cells differentiation. Murine LLC, B16-F10, and E.G7-OVA cells were purchased from American Type Culture Collection (ATCC) and cultured in DMEM except for E.G7-OVA cells in RPMI-1640 medium. G418 (0.4 mg/ml) was added to the E.G7-OVA culture. All cells were maintained in a humidified atmosphere containing 5% CO_2 at 37℃.

Tumor model

To mimic tumor lung metastasis models, female or male *Tfam*$^{-/-}$ mice were intravenously injected with a total of 5×10^5 LLC cells or 2×10^5 B16-F10 cells. Specially, for survival statistics of the lung metastasis B16-F10 model, mice were intravenously injected with a total of 5×10^5 B16-F10 cells. *Tfam*$^{fl/fl}$ littermates and

wild type (WT) mice were used as control. The survival rate was figured. After inoculation for 24 days (LLC models) or 14 days (B16-F10 models), the mice were sacrificed and lung metastasis was evaluated. To investigate the immunological responses, mice were subcutaneously injected with 10 μg OVA in PBS in a total volume of 100 μL (*Tfam*$^{-/-}$ + OVA/Control + OVA group) or 100 μL PBS alone (*Tfam*$^{-/-}$ + PBS/Control + PBS group) antigen to the left flank on days of 0, 14, and 21. Then subcutaneously injected with a total of 5×10^5 E.G7-OVA cells to the right flank on day 28. Tumor size was monitored and measured using a vernier caliper every third day. Mice were sacrificed between days of 16 and 19. For Survival statistics, mice were humanely sacrificed when the volume of tumors reached about 2000 mm^3.

Further, we blocked the STING signaling in mice via C-176 (MCE, #HY-112906), a strong and covalent STING inhibitor [1]. Mice were intravenously injected with a total of 5×10^5 LLC cells, and then intraperitoneally administrated with 13.4 mg/kg of C-176 dissolved in 10% DMSO and 90% saline containing 20% SBE-*β*-CD or equal volume of vehicle (10% DMSO and 90% saline containing 20% SBE-*β*-CD) daily during the course [2].

Stimulation of BMDCs and T cells

To study the induced responses by tumor cells to BMDCs from *Tfam*$^{-/-}$ mice, the tumor supernatant (TS) of LLC cells was harvested, filtrated by 0.22 μm filters, and added to the BMDCs at a final dilution of 1:3 for 24 h of incubation. To block the STING signal, 2 μM H-151 (MCE, #HY-112693) was added to the diluted TS. The supernatant and cells were collected for further study. To remove nucleic acid in TS, the filtrated TS was incubated with 10 U/mL SuperNuclease (Sino Biological, China) for 1-2 h at 37℃.

To investigate cell-mediated immune responses, T cells from spleens of *Tfam*$^{-/-}$ and WT mice immunized with 10 μg OVA in PBS were obtained, and $CD8^+$ T cells were sorted using a negative magnetic bead screening kit (Stemcell Technologies, #19853) following the manufacturer's instruction. Cells were cultured with $OVA_{257-264}$ peptides

(10 μg/mL) for 24 h or 72 h. The supernatant and cells were collected for further study.

Cell staining for flow cytometry (FCM)

Mice were euthanized for lungs with tumor nodules and tumor tissues processing. Briefly, tissues were dissected from mice, minced on ice into small pieces of less than 1 mm^3 and then suspended in a 10 mL of digestion buffer consisting of 1 mg/mL collagenase I (Gibco), 0.5 mg/mL collagenase IV (Gibco) and 40 U/mL DNase I (Sigma) in RPMI-1640 medium (Gibco, USA). The digestion buffer was incubated with frequent agitation at 37℃ for 1 h. Subsequently, the suspensions were passed through a 70 μm nylon mesh filter and centrifuged at 400×g for 5 min. All pelleted cells were resuspended into ice-cold PBS and counted. Besides, mice were also euthanized for inguinal lymph nodes and spleens processing. Cells were collected from inguinal lymph nodes and spleens by grinding and passing through a 70 μm nylon mesh filter. For *in vitro* studies, cultured cells were collected at the indicated end point.

Cells were pre-treated with FcR blocking (BD Biosciences, #553142, USA) to avoid non-specific staining. Then the cells were incubated with live/dead staining dye (Invitrogen, #L34974) for 30 min at 4 °C. For surface staining, cells were stained with the indicated antibodies at 4 °C. For intracellular staining, cells pre-treated with Brefeldin A for 4-6 h were then fixed using 4% paraformaldehyde (PFA) and permeabilized by the 1% Triton X-100, and were incubated with fluorochrome-labeled antibodies specific for the mouse. Fluorescence minus one (FMO) control was used for gates strategy. Samples were performed using BD LSRFortessa, and data were analyzed by FlowJo 10.7.1 or Novoexpress. Antibodies used for FCM analysis included BV650 anti-CD45, PerCP-Cy5.5 anti-CD11b, BV711 anti-CD11c, PE anti-F4/80, APC anti-MHCII, BV421 anti-Ly6C, BV510 anti-Ly6G, FITC anti-CD206, PerCP-Cy5.5 anti-CD3, BV421 anti-CD4, BV510 anti-CD8, FITC anti-PD-1, PE anti-IFN-γ, APC anti-GzmB, PE anti-CD69, FITC anti-Foxp3, FITC

anti-CD62L, APC anti-CD44, APC anti-11c, PE anti-CD25, APC anti-CD40, PE-anti-CD86, FITC anti-CD80, PerCP-Cy5.5-anti-CD115, and BV421-anti-CD135 (all from BD Biosciences or BioLegend, USA), and APC H-2K^b MuLV p15E Tetramer (MBL, Japan). For TFAM staining, permeabilized cells were stained by rabbit anti-TFAM antibody (Abcam, UK) followed by PE anti-rabbit IgG (BioLegend, USA). For apoptosis analysis, cells were stained by FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, USA).

Histology, immunohistochemistry (IHC) and immunofluorescence

Lungs were fixed in 4% PFA at RT, embedded in paraffin and sectioned at 3 μm, or frozen in OCT compound (Sakura Finetek, Japan) and sectioned at 10 μm. Haematoxylin and eosin (H&E) staining was used to assess pathology and lung tumor metastasis. The lung metastatic area was calculated using ImageJ software.

Paraffin-embedded sections were incubated with 3% H_2O_2 to block endogenous peroxidases, and then subjected to an EDTA buffer for antigen retrieval. OCT-embedded sections were post-fixed with ice-cold acetone and incubated with 3% H_2O_2. Cell slides were fixed with 4% PFA. The samples were then incubated with 5% goat serum for blocking. Primary antibodies used for immunohistochemistry or immunofluorescence analysis included rabbit anti-Hsp60 (Abcam, #ab137706), hamster anti-CD11c (BioLegend, #117301), rabbit anti-CD8α (CST, #98941), rabbit anti-CD45 (Servicebio, #GB11066), rabbit anti-CD3 (Servicebio, #GB13014), rabbit anti-CD31 (Abcam, #ab28364) and rabbit anti-cleaved caspase-3 (CST, #9664). The immunofluorescent images were captured using a Zeiss LSM880 laser confocal microscope, and immunohistochemistry and H&E images were captured using the Pannoramic MIDI scanner (3DHISTECH, Hungary).

ELISA assay

To investigate the serum antibodies against the OVA, mice immunized with 10 μg OVA on days of 0,14 and 21 and sera were collected by retro-orbital puncture on day 28. OVA was performed to coat flat-bottom 96-well plates (NUNC-MaxiSorp,

Thermo Fisher Scientific) at a final concentration of 10 μg/mL in 50 mM carbonate coating buffer (pH 9.6) at 4 °C overnight. Then blocking solution containing 5% BSA in PBST was added for 1 h of incubation at RT. Diluted sera were added and incubated at 37 °C for 1 h. Antibodies, including anti-mouse IgG, IgG1, IgG2b, IgG2c and IgG3 horseradish peroxidase (HRP)-conjugated antibody, were diluted 1:5,000 in blocking solution and added to wells (100 μl per well) for 1 h of incubation at RT. After development using 3,3′,5,5′-tetramethylbiphenyldiamine (TMB) and subsequent stop of reactions by 50 μL/well of 1 M H_2SO_4 solution, the absorbance was measured at 450 nm using a microplate reader (Biotek, USA).

Cytokines *in vitro* were measured following the manufacturer's instructions, including IL-6 Mouse Uncoated ELISA Kit (Thermo Fisher Scientific, #88-7064-88), TNF alpha Mouse Uncoated ELISA Kit (Thermo Fisher Scientific, #88-7324-88), IL-1 beta Mouse Uncoated ELISA Kit (Thermo Fisher Scientific, #88-7013-88), IFN gamma 'Femto-HS' High Sensitivity Mouse Uncoated ELISA Kit (Thermo Fisher Scientific, #88-8314-88), and Mouse IL-12 p40 ELISA Kit (Abcam, #ab236717).

Antigen uptake function detection of BMDCs

BMDCs were treated with 1mg/mL FITC-Dextran (Sigma, #60842-46-8) at 37°C for 1 h, rinsed with ice-cold PBS and then fixed with 4% PFA. The fluorescent images were captured using a fluorescence microscopy. For FCM analysis, BMDCs treated with FITC-Dextran at 4°C were performed as control of the spontaneous FITC-dextran phagocytic.

T cell proliferation and cross-presentation assay

To investigate the antigen cross-presentation, the $CD8^+$ T cell proliferation assay was performed [3]. Briefly, BMDCs were isolated from *Tfam*$^{-/-}$ and control mice on day 28 pre-immunized with OVA (10 μg) on days of 0, 14 and 21. After incubation for 7 days for BMDCs maturation, 10 μg/mL of $OVA_{257-264}$ peptide was added to cells following by 24 h of incubation at 37 °C, and then BMDCs were collected and counted.

Simultaneously, $CD8^+$ T cells were isolated from spleens of OT-I mice, sorted using a negative magnetic bead screening kit (Stemcell Technologies, #19853), and then labelled with CFSE (Invitrogen, #C34554) by incubation with CFSE (2.5 μM, prepared with 0.1% FBS/PBS solution) for 15 min in the dark at 37℃. For co-culture system preparation, 2×10^5 BMDCs and 1×10^6 $CD8^+$ T cells were mixed in 1 mL of medium and seeded into a 24-well plate following by 72 h of incubation at 37 °C. Terminally, the cells were collected for flow cytometric analysis of CFSE fluorescence value of T cells.

T cell activation assay

To detect the activation of OVA-specific cytotoxic T cells (CTLs), splenic lymphocytes were isolated from mice on day 28 pre-immunized with OVA (10 μg) on days of 0, 14 and 21. Subsequently, 10 μg/mL of $OVA_{257-264}$ peptide was added to activate cells following by 72 h of incubation at 37 °C. Terminally, the cells were collected and labeled with T-select MHC Tetramer /H-$2K^b$-OVA (SIINFEKL) (MBL, #TS-5001-1C) and anti-CD8 antibody for flow cytometric analysis.

Adoptive immunity assay

To investigate the adoptive immunity, control and *Tfam*$^{-/-}$ mice were immunized with 10 μg of OVA on days of 0, 14 and 21, and then $CD8^+$ T cells were isolated from the immunized spleen on days of 28, 30 and 32 and sorted using a negative magnetic bead screening kit (Stemcell Technologies, #19853). A total of 5×10^5 E.G7-OVA cells was subcutaneously injected to the right flank of WT mice. Meanwhile, a total of 5×10^6 $CD8^+$ T cells from control or *Tfam*$^{-/-}$ mice was intravenously injected to the mice on 1-day ahead-inoculation of tumor cells, and on 1-day and 3-days post-inoculation of tumor cells, respectively. All the tumour grew in the WT.

For adoption of DCs and macrophages, BMDCs and macrophages were differentiated from bone marrow cells with 100 ng/ml FLT-3L (R&D Systems, USA) or 20 ng/ml M-CSF (novoprotein, China) respectively. Totally 1×10^6 *Tfam*$^{-/-}$/control BMDCs or

macrophages were intravenously injected into WT LLC lung metastasis models on day 0, 1, 4, 7, and 14. Mice were sacrificed on day 24 to collect the lungs. The gross appearance of lungs were photographed, and the lung metastatic nodules were counted. Three representative lungs were selected from each group for formaldehyde fixation, paraffin embedding, and HE staining. The lung metastatic area was quantified by ImageJ software.

Quantitative PCR (qPCR)

Total RNA was extracted using the RNA Extraction Kit (TianGen, #DP419), and first-strand cDNA was synthesized using a PrimeSriptTM RT reagent kit (Takara, #RR036) following manufacturer's protocols. qPCR was performed in triplicate at a 20 μL total volume using SsoFast EvaGreen (Bio-Rad, #1725202) on a Bio-Rad iCycler RT–PCR detection system. Mitochondrial DNA copy number was measured using primers specific to nuclear Tert and the D-loop region of mtDNA. The expression levels of target genes were normalized to β-actin using $2^{-\Delta\Delta CT}$ method. All primers used in this study are listed in Supplementary Table 2.

Measurement of cytosolic mitochondrial DNA (mtDNA)

Cytosolic mtDNA was extracted from BMDCs using the mitochondrial DNA isolation kit (Abcam, #ab65321), purified using the DNeasy Blood & Tissue Kit (Qiagen, #USA), and quantified by PCR using a TaqMan probe as previously described [4].

ROS measurement

The ROS level was detected using a DCF-DA (Sigma, #35845) probe. Briefly, cells seeded in 96-well plates were incubated with or without the tumor supernatant (TS) for 24 h, and then treated with 10 μM DCF-DA (prepared with phenol red-free medium) for 30 min. The fluorescence intensity was determined by a microplate reader (Biotek, USA) at the excitation/emission wavelengths of 485/530 nm. All procedures were carried out in the dark.

Extracellular oxygen consumption measurement

The mitochondrial metabolism was measured using the Cell Metabolism kit (Abcam), and the extracellular oxygen consumption rates (OCR) were measured using the Extracellular Oxygen Consumption Assay kit (Abcam). All procedures were performed according to the manufacturer's protocols, and the fluorescence intensity was determined by a microplate reader (Biotek, USA).

Characterization of mitochondrial structure

Transmission electron microscopy was used to characterize the mitochondrial structure. Briefly, murine BMDCs were fixed with 2.5% glutaraldehyde, dehydrated and then embedded in resin. The ultrathin section (~50 nm) was obtained and performed on a copper grid by staining with uranyl acetate and lead citrate. The images were captured using a JEM-2100PLUS electron microscope (JEOL, Japan).

Western blot

Total protein was extracted using the ice-cold enhanced RIPA lysis buffer supplemented with a phosphatase inhibitor cocktail (Millipore, #524629) and measured using the Bradford dye (BIO-RAD, #5000205) by Eppendorf Bio-photometer Plus (Eppendorf). Western blotting was performed using standard protocols according to the manufacturer's recommendations. Primary antibodies used for western blot analysis included rabbit, rabbit anti-Stat1 (CST, #9172), rabbit anti-STING (CST, #13647), rabbit anti- Phospho-TBK1 (CST, #5483), rabbit anti-TBK1 (CST, #3504), rabbit anti-IRF-3 (CST, #4302), rabbit anti-Phospho-IRF-3 (CST, #79945) and mouse anti-β-actin (Santa Cruz, #sc-47778). The β-actin protein was used as the loading control.

RNA sequencing

Total RNA was isolated from BMDCs (treated with or without TS) of *Tfam*$^{-/-}$ and control mice using the TRIzol reagent kit. RNA integrity was assessed using the RNA Nano 6000 Assay Kit by the Bioanalyzer 2100 system (Agilent Technologies, CA, USA), and samples with RIN scores above 6 and a minimum total RNA of 100 ng

were used for further library construction. PCR products were purified using the AMPure XP system, and library quality was assessed using the Agilent Bioanalyzer 2100 system. Libraries were sequenced on Illumina Novaseq6000 (Illumina) following PE150 sequencing strategy. Differential expression analysis was performed using the DESeq2 R package (1.20.0), and mRNAs with a FDR <0.001 and fold-change >2 were considered as significant differential expression between two groups. GSEA (gene set enrichment analysis) were used for functional annotation of the differentially expressed genes. The experiment was conducted in triplicate.

References

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244 .**Table.1 Primer used in PCR for genotyping**

Gene name	Forward sequences Reverse sequences	Reaction
m*Tfam*	CTT GTA GGT CCT CCC CAC TG	A
	ACA GCA CCA ACC CAA AGT GT	A
m*Lyz2-cre*	CCC AGA AAT GCC AGA TTA CG	Mutant (A)
	CTT GGG CTG CCA GAA TTT CTC	Common (A, B)
	TTA CAG TCG GCC AGG CTG AC	Wild type (B)

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246 **Table.2 Primer used in qPCR**

Gene name	Forward sequences Reverse sequences
β-actin	CTGAGAGGGAAATCGTGCGT
	CCACAGGATTCCATACCCAAGA
m*Tfam*	AAGGATGATTCGGCTCAGG
	GGCTTTGAGACCTAACTGG
mIfna4	CTTTCCTCATGATCCTGGTAATGAT
	AATCCAAAATCCTTCCTGTCCTTC
m*Ifnb1*	CCCTATGGAGATGACGGAGA
	CCCAGTGCTGGAGAAATTGT
m*Irf7*	CAATTCAGGGGATCCAGTTG
	AGCATTGCTGAGGCTCACTT
m*Cxcl10*	CCAAGTGCTGCCGTCATTTTC
	GGCTCGCAGGGATGATTTCAA
m*Irf3*	CGGAAAGAAGTGTTGCGGTTAGC
	CAGGCTGCTTTTGCCATTGGTG
m*Ddx58*	GAGTACCACTTAAAGCCAGAG
	AATCCATTTCTTCAGAGCATCC

m*Tnfa*	CATCTTCTCAAAATTCGAGTGACAA
	CCAGCTGCTCCTCCACTTG
m*Il1b*	TGGACCTTCCAGGATGAGGACA
	GTTCATCTCGGAGCCTGTAGTG
m*Il12*	CCATTGAACTGGCGTTGGAAG
	ACTTGAGGGAGAAGTAGGAATGG
m.mtDNA Dloop 3	TCCTCCGTGAAACCAACAA
	AGCGAGAAGAGGGGCATT
m.nucDNA Tert	CTAGCTCATGTGTCAAGACCCTCTT
	GCCAGCACGTTTCTCTCGTT

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