# STING Modulates CD4 T Cell Necroptosis via Activation of PARP-1/PAR Following Acute Systemic Inflammation

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**Research**

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# Abstract

## Background

Regulated cell death profoundly affects on the progress of inflammatory and immune responses in various acute inflammatory diseases, as seen in sepsis and trauma. However, the mechanisms underlying CD4 T cells death have not yet been fully addressed.

## Results

We demonstrated that interferon genes (STING) promoted excessive Poly (ADP-ribose) polymerase 1 (PARP-1) activity stimulated by endotoxin, which in turn induced apoptosis-inducing factor (AIF)-independent but PARP-1 dependent programmed cell death. Elevated PARP-1 activity triggered a cascade of molecular events, including PAR polymer release from the nucleus and the nicotinamide adenine dinucleotide ($NAD^+$) and ATP depletion. Interestingly, translocation of AIF, a biochemical signature for PARP-1-dependent parthanatos, was not observed in the present study, suggesting a non-canonical mechanism of CD4 T cells parthanatos. In this study, we also identify a STING-mediated mechanism of necroptosis in CD4 T cells in septic animals. Furthermore, we revealed wider effects of STING on the immune functions of CD4 T cells when PARP-1 gene inhibited.

## Conclusions

These findings reveal that STING signaling and targeting PARP-1/PAR pathway in CD4 T cells may present a new therapeutic strategy for the treatment of acute systemic inflammatory diseases.

# Background

Sepsis is a common complication of severe trauma, shock, infection, and major surgical operation, and can lead to septic shock and multiple organ dysfunction syndrome (MODS). The immune system responds to injury by means of rapidly producing early and late inflammatory cytokines, and also affecting proliferation and differentiation of T cells[1–3]. Despite the evolution of therapeutic strategies such as aggressive surgical techniques, extensive methods of supportive care, unfortunately, they have failed to reduce mortality significantly in severely septic patients in many countries. Therefore, it is of great significance to further elucidate the pathophysiological mechanisms, and to seek scientific interventional approaches for prevention and rational treatment of septic complications secondary to acute insults.

The cyclic GMP-AMP synthase-stimulator of interferon genes (STING) pathway is a component of the innate immune system that functions to detect the presence of cytosolic DNA and, in response, trigger expression of inflammatory genes in the nucleus of the cell[4]. STING ligands are usually pathogenic components, including cyclic dinucleotides (CDNs), DNA, etc. As one of the sting dependent DNA receptors, cyclic GMP amp synthase (cGAS) is a cytoplasmic DNA pattern recognition receptor, which

mainly relies on the sting signal pathway to play its immune function. As a cytoplasmic DNA sensor, cGAS generates the second messenger cGAMP after recognizing cytoplasmic DNA, and then starts the sting signal pathway. In this process, after cross-linking to form a dimer, cGAS protein binds to dsDNA to form cGAS DNA complex, which catalyzes ATP and GTP to produce cGAMP. cGAMP furtherly binds and activates STING. Subsequently, interferon regulatory factor 3 (IRF3) is phosphorylated and induces the production of nuclear interferons (IFNs) and other proinflammatory cytokines[5–8]. However, the mechanisms by STING regulates T cell fate are not fully understood.

Regulated necrosis is a form of genetically programmed necrotic cell death, and it encompasses a range of pathways. Poly (ADP-ribose) polymerase 1 (PARP-1)-mediated regulated necrosis, referred to as parthanatos, has been shown to be a distinct pathway in regulated necrosis, although there may be shared molecular components[9]. Activated PARP-1 consumes nicotinamide adenine dinucleotide ($NAD^+$) and depletes cellular ATP, causes large-scale DNA fragmentation and chromatin condensation, leading to cell death[10]. Likely, PARP-1 activation results in PAR polymer formation, the translocation of apoptosis-inducing factor (AIF) from mitochondria to the nucleus, fragmenting DNA[11]. Parthanatos can be triggered by various pathological stimuli, such as oxidative stress, stroke, traumatic brain injury, or ischemic reperfusion injury. Recent studies have shown that activation of STING also induces apoptosis and necroptosis in a variety of immune cells, including T cells[12, 13]. However, the roles of STING or PARP-1 in regulating T cell survival during a systemic inflammatory response, as seen in sepsis, are not known. Here, we demonstrate that STING promoted PARP-1-mediated necrosis constitutes a substantial portion of the death of CD4 T cells in response to LPS. Next, PAR polymer release and translocation were observed downstream of PARP-1 activation. However, AIF was irrelevant in this death process, indicating that such type of cell death is the uncanonical parthanatos in which AIF translocation does not follow PARP-1 activation. Further, we addressed the involvement of STING in CD4 T cell necrotic death in a cecal ligation and puncture (CLP) animal model *in vivo*. We also confirmed the marked effect of STING on the immune function of CD4 T cells when PARP-1 gene inhibited after sepsis, suggesting a pathophysiological significance of STING and PARP-1 activation in the pathogenesis of septic complications.

## Results

# STING modulates LPS-induced necroptosis in CD4 T cells in mice

To determine the role of STING in the mechanism of regulatory CD4 T cell death, we isolated CD4 T cells from wildtype (WT) C57B/L mice and treated with LPS at concentrations of 1μg/ml for 12h and 24h followed by measurement of cell death using 7-AAD/Annexin V double staining, which differentiates healthy (double negative), apoptotic (Annexin V single positive), and necrotic (7-AAD /Annexin V double positive) cells. WT CD4 T cells exposed to LPS exhibited necrotic cell death and STING promotes necroptosis in CD4 T cells (Fig. 1A). Simultaneously, STING expression was elevated in response to LPS

(Fig. 1C and Fig. 1D). Our results clearly demonstrate that STING might modulate necrotic death in CD4 T cells induced by LPS.

## STING promotes activation of PARP-1 and PAR polymer in CD4 T cells in mice

We further determined whether PARP-1 is involved in the STING mediated CD4 T cell necrotic death. CD4 T cells were stimulated with LPS at 1μg/ml for 24 h, and PARP-1 expression in the CD4 T cells was detected by confocal microscopy and Western blot. We found that PARP-1 remained in nuclear, and was markedly increased in CD4 T cells after treatment with LPS, and decreased when STING gene knock out (Fig. 2A and 2B). The increased PARP-1 expression was associated with elevated cytosolic PAR polymer formation (Fig. 2A and 2B). Furthermore, silencing of PARP-1 gene using siRNA approach in the $STING^{-/-}$ mice significantly decreased PARP-1 expression in response to LPS (Fig. 2C). These results suggest a role for PARP-1 in STING mediated CD4 T cell necroptosis.

## AIF is dispensable for STING modulated necrotic death through PARP-1 activation in CD4 T cells in mice

We examined the translocation of AIF from mitochondria into the nucleus, a biochemical signature of parthanatos, when CD4 T cells were cultured in the presence of 1μg/ml for 24 h. AIF mainly remained in the mitochondria, and did not transfer to nucleus upon LPS stimulation in $STING^{-/-}$ mice by Western blot (Fig. 3A and 3B). Furthermore, silencing of PARP-1 gene using siRNA approach decreased CD4 T cell necrotic death in $STING^{-/-}$ mice (Fig. 3C). Then, we measured the cellular $NAD^+$ and ATP levels following exposure to LPS. $NAD^+$ and ATP levels were significantly decreased in response to LPS for 24 h, and they were prevented by the STING gene deleted and the PARP-1 gene silence in CD4 T cells (Fig. 3D, E), suggesting that activated PARP-1 by STING triggers cellular energy depletion with decline in cellular $NAD^+$ and ATP in CD4 T cells. Our findings indicate the presence of a novel necrotic pathway that is distinctive from canonical parthanatos, AIF is dispensable for STING modulated necroptosis in LPS-exposed CD4 T cells.

## PARP-1 activity is associated with STING modulated necroptosis in human CD4 T cells

To address whether the *in vitro* observation of STING-PARP-1 signaling also be active in human CD4 T cells, we determined the expressions of PARP-1, PAR polymer, and levels of cellular $NAD^+$ and ATP in the CD4 T cells from healthy human peripheral blood. We firstly observed the human CD4 T cell death in the presence of LPS for 24 h, and found that necrosis rate was 10.6% at 24 h, whereas rise to 23.7% by activation of STING by 2,3-cGAMP in human CD4 T cells (Fig. 4A). AIF was not delivered into nucleus has

revealed using AIF antibody and Confocal observation after LPS treatment in the presence of 2,3-cGAMP (Fig. 4B). Western blot study showed that expression of PARP-1 and PAR was increased in human CD4 T cells after 2,3-cGAMP treatment (Fig. 4C and Fig. 4D). In addition, $NAD^+$ and ATP levels were increased in human CD4 T cells by the PARP-1 siRNA, and the increases were prevented by 2,3-cGAMP (Fig. 4E and Fig. 4F). Taken together, our data show that STING appears to be related with the human CD4 T cell necrotic death via affecting PARP1 activity, PAR polymer formation, and depletion of cellular $NAD^+$ and ATP.

# STING modulates necroptosis of CD4 T cells in the CLP mouse model

To address whether the *in vitro* observation of the STING pathway in CD4 T cells also be active *in vivo*, we determined the expressions of PARP-1 and levels of cellular $NAD^+$ and ATP in CD4 T cells in the spleens of CLP-induced sepsis mice. We firstly observed the CD4 T cells death by flow cytometry using the CLP model, and found that necrosis rate of CD4 T cells was drop in the $STING^{-/-}$ and PARP-1 inhibitor group (Fig. 5A). Western blot study showed that expression of STING was enhanced in CD4 T cells after CLP, while decreased in CD4 T cells at 12 h, 24 h after CLP in PARP-1 inhibition mice (Fig. 5B). Moreover, expression of PARP-1 was elevated in CD4 T cells after CLP, but PARP-1 was decreased in CD4 T cells at 12 h, 24 h after CLP in $STING^{-/-}$ mice (Fig. 5C). In addition, $NAD^+$ and ATP levels were reduced in CD4 T cells at 24 h after CLP, and the decreases were prevented by the pharmacological inhibitor of PARP-1 in the $STING^{-/-}$ mice (Fig. 5D and Fig. 5E). Taken together, our data show that activated PARP-1 by STING appears to be related with the CD4 T cell necrotic death via affecting PARP1 activity, and depletion of cellular $NAD^+$ and ATP during the CLP-induced sepsis. Further, STING deficiency significantly reduced circulating IL-6 levels and mortality during CLP injury. Inhibition of PARP-1 using olaparib significantly increased had marked effects on cell proliferation, IL-6 levels and mortality in $Sting^{-/-}$mice after CLP injury. Together, our results reveal that the STING signaling regulates PARP-1 activation and promotes necroptosis in CD4 T cell.

# Disscussion

It has been proposed that T lymphocyte might play a central role in the initiation and control of the adaptive immune response in the peripheral lymphatic system[14–17]. Uncontrolled inflammation and immunosuppressive response followed by tissue and organ dysfunction are associated with CD4 T loss and cell death after septic challenge. It is our belief that further investigation into the mechanisms underlying the host immune suppression might contribute to formulate better therapeutic modalities for acute insults. These findings have implications for the regulation of CD4 T cell fate in settings associated with pathologic necroptosis of T cells during systemic inflammation as seen during sepsis.

The STING pathway appeared recently as an important hub of the cytosolic surveillance system[18], which activates in APCs by cytosolic DNA, provides a critical source of such a priming signal. DNA normally resides in the nucleus and mitochondria, and its presence in the cytoplasm serves as a danger-associated molecular pattern (DAMP) to trigger immune responses. Decades of evidence proved that STING can accelerate the inflammatory reaction, organ disorder and even death after being activated by bacteria, thus STING gene knockout has a protective effect on severe sepsis mice[19, 20]. STING-IRF3 signaling has also been shown to plays an adverse role in the pathogenesis of sepsis[21]. Furthermore, STING signaling is involved in autophagy, induced autophagy is the original function of cGAS sting pathway, STING can activate autophagy through a mechanism independent of TBK1 activation and interferon induction[21–23]. STING signaling may play a protective role in the early stage of infection, with the aggravation of infection, STING is over activated, resulting in the imbalance of inflammatory/anti-inflammatory response. Nevertheless, the biological significance of the diversity of cell death initiated by STING has not yet been fully elucidated. Herein, we confirmed the higher sensitivity of CD4 T cells to necrotic death during endotoxemia and demonstrate the role of the STING pathway as a major driver of T cell necrotic death in the spleen.

PARP-1 plays a pivotal role in multiple neurologic diseases by mediating caspase-independent cell death[24, 25]. Currently, three major pathways have been made responsible for PARP-mediated necroptotic cell death[26–30]: compromised cellular energetic mainly due to depletion of NAD, the substrate of PARPs; PAR mediated translocation of AIF from mitochondria to nucleus (parthanatos); and a most elusive crosstalk between PARylation and cell death/survival kinases and phosphatases. Thus, the PARP-1-mediated parthanatos would be a future focus of this study. Our data indicated that the STING activated a cascade of intracellular events leading to uncanonical parthanatos, which was different from other forms of cell death such as apoptosis and necrosis. The PAR polymer, generated when PARP-1 is overactivated, is now recognized as a key signaling molecule in the parthanatos cascade[31–39]. Here, STING gene knockout reduced the expression of PARP-1 and PAR polymer in CD4 T cells in response to LPS. In vivo experiment regarding STING gene deficient during sepsis showed a decreased CD4 T cell death, involving down-regulation of PARP-1 and PAR polymer. Our results show that STING modulates necroptosis of CD4 T cells, together with the DNA damage response factors PARP-1, and is also involved in the host response to sepsis in vivo.

Mitochondrial AIF release and translocation to the nucleus secondary to PARP-1 activation is the signature of parthanatos, and AIF is required as an executioner of parthanatos[4–45]. Although AIF is a bigger protein, there is a small pool (30%) of AIF on the outer mitochondrial membrane (cytosolic side), which is essential to induce cell death in parthanatos[46–50]. In the current study, we did not observe that AIF was released from the mitochondria and translocated into the nucleus in CD4 T cells after 2,3-cGAMP stimulation or STING gene deletion, suggesting that AIF did not participate in the execution of cell death (parthanatos) mediated by STING in CD4 T cells. These findings suggest the potent role for STING in induction of regulated necrotic cell in CD4 T cells which is distinct from that of the canonical parthanatos. The effect of STING on human CD4 T cell necrosis was evident by the fact that 2,3-cGAMP

treatment elevated activity of PARP-1, PAR polymer release and increased depletion of cellular $NAD^+$ and ATP. Based on the above observations, STING modulates CD4 T cell necrosis. We further determined the effect of STING on immune regulation of CD4 T cells via PARP-1 signaling in the CLP induced sepsis. Our data showed that STING gene knockout increased the proliferation and by the PARP-1 inhibitor. Understanding the definitely pathophysiological significance of PARP-1 in the STING modulated CD4 T cell necroptosis will be our next focus of study.

## Conclusions

In summary, STING causes regulated necrotic cell death in CD4 T cells, with PARP-1 serving as the main perpetrator of necrosis. Unlike in canonical PARP1-mediated cell death known as parthanatos, cell death in this model proceeds in the absence of AIF translocation. STING mediated CD4 T cells are fully promoted AIF independent parthanatos, as STING triggers over-activation of PARP-1, accumulation of PAR polymer, and depletion of ATP and $NAD^+$. Simultaneously, STING initiated human CD4 T cell necrosis, together with the DNA damage response factors PARP-1. STING further influences the immune function of CD4 T cells via PARP-1 signaling. Dysregulation of CD4 T cell necrosis is involved in a variety of pathological conditions, such as immunosuppression in acute critical illness. We believe that our data may provide insight into the complex cellular events underlying in vitro conditions. The findings shed light on the regulatory effect of STING and PARP-1 pathway on cell fate, which may play an important role in directing the progress of acquired immunity in the setting of infection and acute insults.

## Methods

# Ethics Statement.

All the mice used in the experiments were 9–10 weeks old and on a C57BL/6 background. All experimental manipulations were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. CD4 T cells from healthy human peripheral blood was purchased from Bioscience. Lonza.com (Bend, OR, USA).

# Animal CLP model.

All animals were housed in separate cages in a temperature-controlled room with 12-h light and 12-h darkness, experimental sepsis was induced by the CLP procedure. Some mice were intravenously injected with 15 mg/kg PARP-1 inhibitor (olaparib)(Selleck Chemical LLC, USA) for 4-h before CLP model. After anesthesia, the mice were fixed on the operating table, and the abdominal skin was disinfected with iodine and alcohol. A 1.5 cm incision was made along the midline of the abdomen, and the peritoneum was opened to find the free root of the cecum. The cecum was ligated at the middle and punctured twice with a 21-gauge (0.723 mm) needle to induce a moderate severity of sepsis. Sham-operated mice underwent the same surgical procedure except the ligation and perforation step.

# Generation of STING-deficient mice.

Age and sex matched littermates were used as controls. Mice were housed in BioMedical Research Institute of Nanjing University, Nanjing, China. All procedures used were pre-approved by the Institutional Animal Care and Use Committee, Nanjing University, Nanjing, China. Two sgRNAs-targeting the exon2 of STING gene were constructed and transcribed in vitro. Cas9 mRNA and sgRNAs were coinjected into 0.5-day-old C57BL/6J mouse zygotes.

# Isolation of splenic CD4 T cells.

All procedures used were pre-approved by the Institutional Animal Care and Use Committee, Nanjing University, Nanjing, China. Spleens were obtained from WT C57BL/6 mice, $STING^{-/-}$ mice and septic mice, and they were teased in 5 ml RPMI 1640. Mononuclear cells were isolated and then CD4 T cells were isolated from them. A MiniMACS™ Separator with a negative selection LS column by following the manufacturer's instructions (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The pellets of selected CD4 T cells were obtained by centrifugation (1500 rpm, 10 min), then the supernatant was discarded, and the cells were cultured in medium RPMI 1640, supplemented with 10% heat-inactivated fetal calf serum (FCS).

# PARP-1 depletion.

A total of $1\times10^6$ CD4 T cells were planted in twelve-well plates and cultured overnight. Predesigned siRNAs against mouse PARP-1was purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology, USA), and control scrambled siRNA was purchased from Abcam (Abcam, Cambridge, MA, USA). The transfection procedure followed the protocol of Santa Cruz Biotechnology, USA. 48 h after transfection, the depletion of PARP-1 was confirmed by Western blotting, and cells were used in subsequent experiments.

# Flow cytometric analysis for cell death.

CD4 T cells were treated with the indicated concentrations of LPS (1μg/ml) for the indicated time points. CD4 T cells were harvested and washed using Annexin V buffer provided by the supplier (BD Biosciences, San Jose, CA, USA) and then stained with Annexin V. Next, Bv-421-CD4 antibody (CD4 T cell maker) was added and stained for 1 h. Cell death was also measured, 7-AAD was added at a final concentration of 5 μg/ml. The cells were then analyzed by flow cytometry. Acquisition was performed on 10000 events using a FACScalibur cytometer (BD Biosciences, San Jose, CA, USA) or BD LSR II (BD Biosciences, San Jose, CA, USA) with CellQuestPro (BD Biosciences, San Jose, CA, USA) and FlowJo-V10 software (Tree Star, Ashland, OR, USA).

# Immunofluorescence confocal microscopy.

After treatment with LPS for 24 h, CD4 T cells were washed with PBS for three times, and fixed with 4% paraformaldehyde in PBS for 20 min, then permeabilized with 0.02% Triton X-100 for 20 min at room temperature. Sections were preblocked with 1% bovine serum albumin in PBS for 30 min and stained with anti- PARP-1 and PAR antibody (Abcam, Cambridge, MA, USA) (1:200) overnight at 4°C. Anti-AIF antibody (Invitrogen, USA) were stained overnight at 4°C. After being washed in PBS for three times, CD4 T cells were stained with goat anti-mouse IgG H&L (Alexa Fluor® 647) and goat anti-rabbit IgG H&L (Alexa Fluor® 488) (Abcam, Cambridge, MA, USA) as the second antibody for 1 h at room temperature followed by 3×PBS washes. After being washed, the nuclei were stained with Heochst (Sigma-Aldrich, USA) for 5 min. The cells were observed with a laser scanning confocal microscope.

# Western blotting.

Western blot was performed to determine expressions of PARP-1, PAR polymer, COXIV, STING in CD4 T cells. Protein concentrations were measured using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Grand Island, NY, USA). Proteins of CD4 T cells were separated by SDS PAGE and then transferred electrophoretically onto polyvinylidenedifluoride (PVDF) membranes. The membranes were probed with anti-PARP-1 antibody, anti-PAR polymer antibody (Abcam, Cambridge, MA, USA), anti-COXIV antibody (Invitrogen, USA), anti-STING antibody (Abcam, Cambridge, MA, USA) and polyclonal anti-actin antibodies (Santa Cruz Biotechnology, USA). Protein bands were detected using Odyssey System from LI-COR Biosciences, USA.

# Measurement of $NAD^+$ and ATP levels.

CD4 T cells were seeded into 96-well plates ($1\times10^4$ cells/well) with or without transfecting siRNA-PARP-1 for 48 h. The cells then were treated with LPS (1μg/ml) for 24h. The cellular $NAD^+$ levels were measured using the Fluoro NAD/NADH detection kit (Cell Technology, USA) according to the manufacturer's instruction. Additionally, CD4 T cells in 96-well plates were treated with LPS (1μg/ml) for the indicated intervals. Cellular ATP levels were measured using the Enzylight ATP assay kit (BioAssay Systems, Hayward, CA, USA) according to the manufacturer's instructions. The ATP assay kit provided a rapid method to measure intracellular ATP. Luminescence was measured on BioTek Synergy Mx luminometer (BioTek Instruments, Winooski, VT, USA) and quantitated to ATP standards.

# Enzyme-linked immunosorbent assay (ELISA).

IL-6 level in culture supernatants was determined with ELISA kits (R&D System Inc., Minneapolis, MN, USA) according to the manufacturer's instructions.

# Statistical analysis.

Data were represented as mean ± standard deviation (SD). Data sets were examined by one-way ANOVA, and individual group means were then compared with Student paired t test. All statistical tests were two sides and a *P* value of 0.05 or less was considered to indicate statistical significance.

## Abbreviations

PARP-1: Poly (ADP-ribose) polymerase 1

AIF: apoptosis-inducing factor

$NAD^+$: nicotinamide adenine dinucleotide

MODS: multiple organ dysfunction syndrome

CDNs: cyclic dinucleotides

Cgas: cyclic GMP amp synthase

IRF3: interferon regulatory factor 3

IFNs: nuclear interferons

CLP: cecal ligation and puncture

LPS: lipopolysaccharide

## Declarations

# Acknowledgements

Not applicable.

# Authors' contributions

Ying-yi Luan and Cheng-hong Yin conceptualized the project. Ying-yi Luan and performed all the experimental studies. Xiao-peng Cao assisted the protein extraction and WB. Lei Zhang assisted with preparing samples for immunofluorescence confocal and analysis. Yi-qiu Peng and Ying-ying Li performed data analysis. Ying-yi Luan wrote the manuscript, which was further revised by Cheng-hong Yin. All authors read and approved the final manuscript.

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# Availability of data and materials

Not applicable.

# Ethics approval and consent to participate

All the mice used in the experiments were 9-10 weeks old and on a C57BL/6 background. All experimental manipulations were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

# Consent for publication

Not applicable.

# Competing interests

The authors declare that they have no competing interests.

# Competing Interests

The authors have declared that no competing interest exists.

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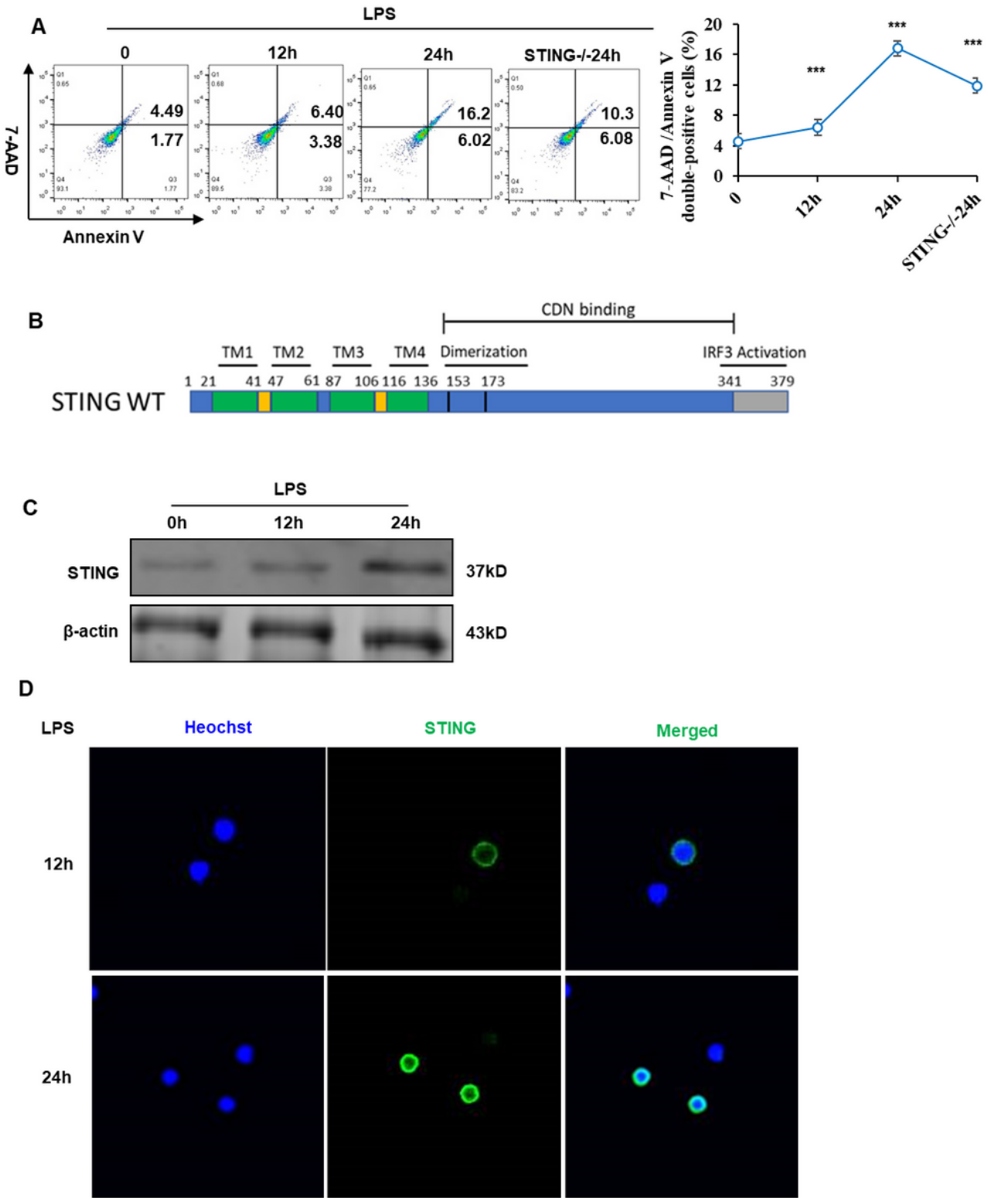
# Figures




## Figure 1

STING modulates LPS-induced necroptosis in CD4 T cells in mice. (1A) CD4 T cells were treated with 1μg/ml LPS for 12h, 24 h in WT mice and STING-/-mice. Cells were analyzed by flow cytometry using double staining with Annexin V and 7-AAD. Annexin V-7-AAD double negative cells were considered “live”, Annexin V single positive cells as “apoptotic”, and Annexin V/7-AAD double-stained cells as “necrotic”. (1B) Structure of STING in WT mice. (1C) Western blot analysis revealed the expression of STING. (1D)

Immunofluorescence of STING (green) were detected. The nuclei were counterstained with Heochst (blue). Original magnification×600, results were representative of three independent experiments. The values were the mean ± S.D., from triplicate independent experiments (n=8 each) with statistical significances: *P<0.05, **P<0.01, ***P<0.001.

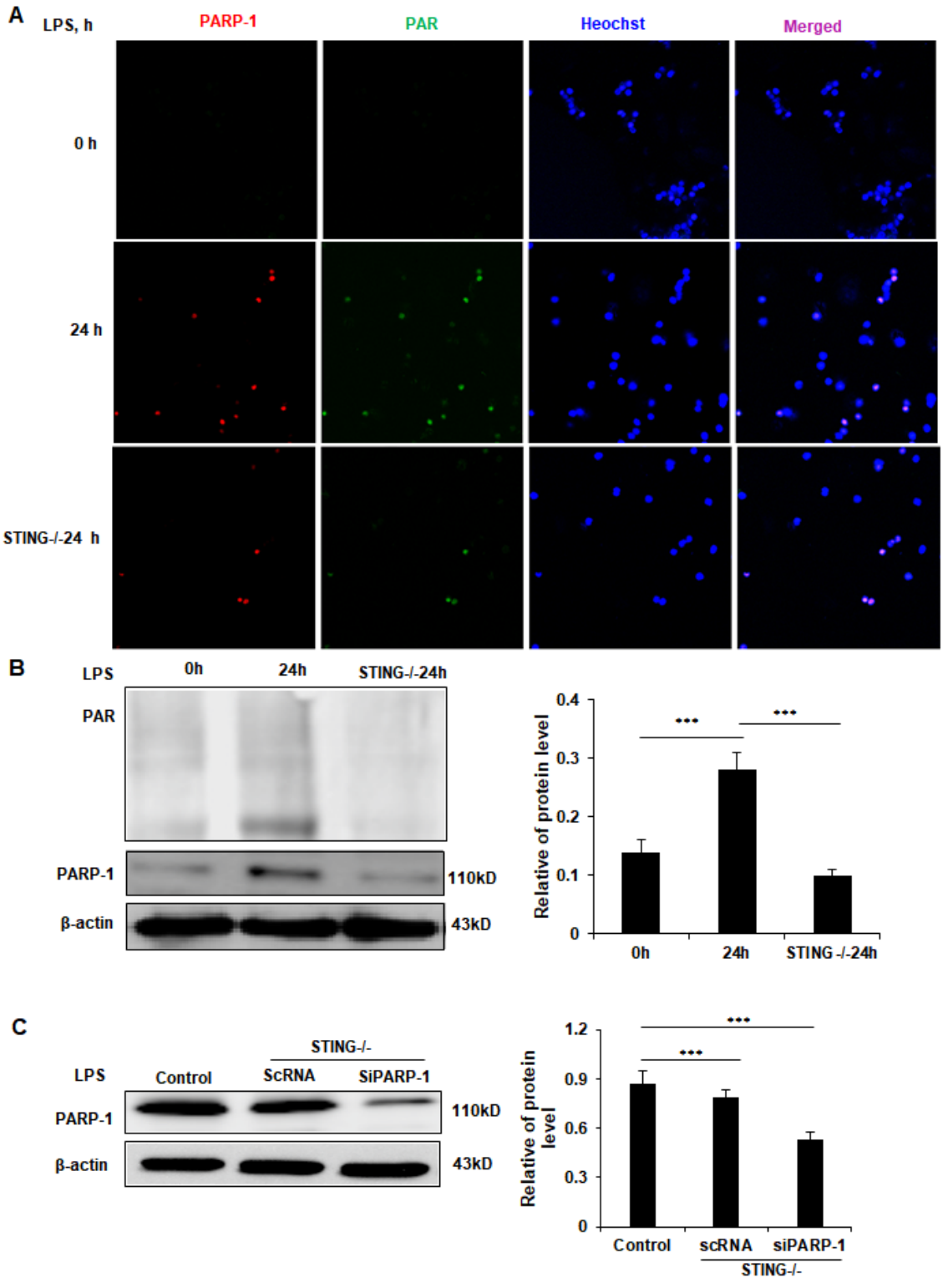
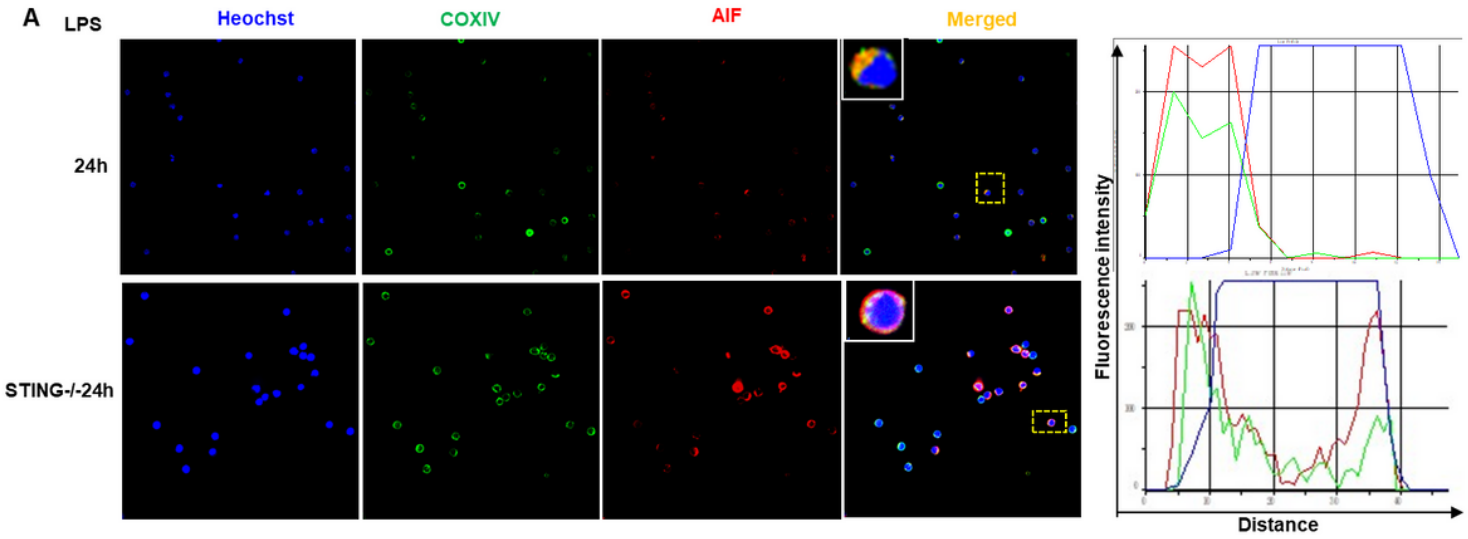
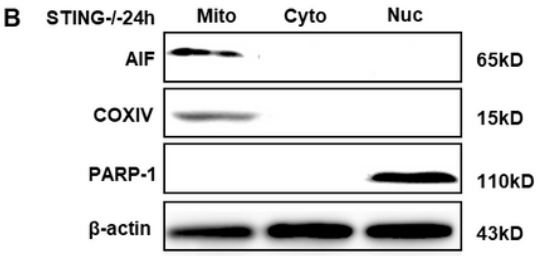
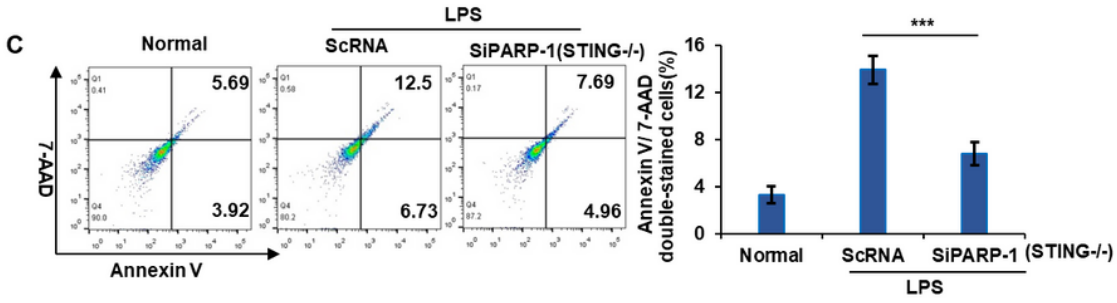


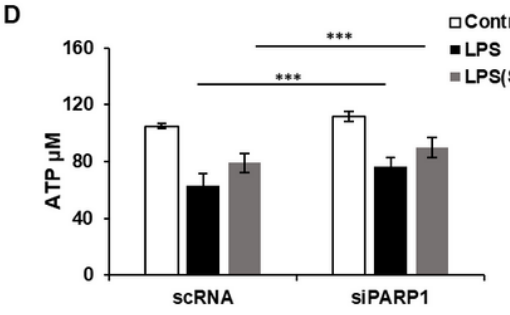

Figure 2

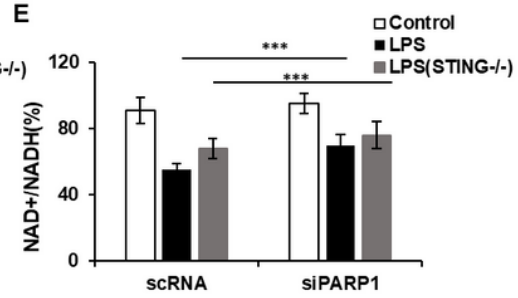
STING promotes activation of PARP-1 and PAR polymer in CD4 T cells in mice. (2A) CD4 T cells were treated with 1μg/ml LPS for 24 h, then immunofluorescence of PARP-1 (red) and PAR (green) were detected. The nuclei were counterstained with Heochst (blue). Original magnification×600, results were representative of three independent experiments. (2B) Western blot analysis revealed the expression of PARP-1 and PAR. (2C) CD4 T cells were transfected with scrambled-siRNA (scRNA) or PARP-1 targeting siRNA (siPARP-1) for 48 h. Then, cells were treated with 1μg/ml LPS for 24 h in WT mice and STING-/- mice. PARP-1 expression was detected by western blot. The values were the mean ± S.D., from triplicate independent experiments (n=8 each) with statistical significance: *P<0.05.



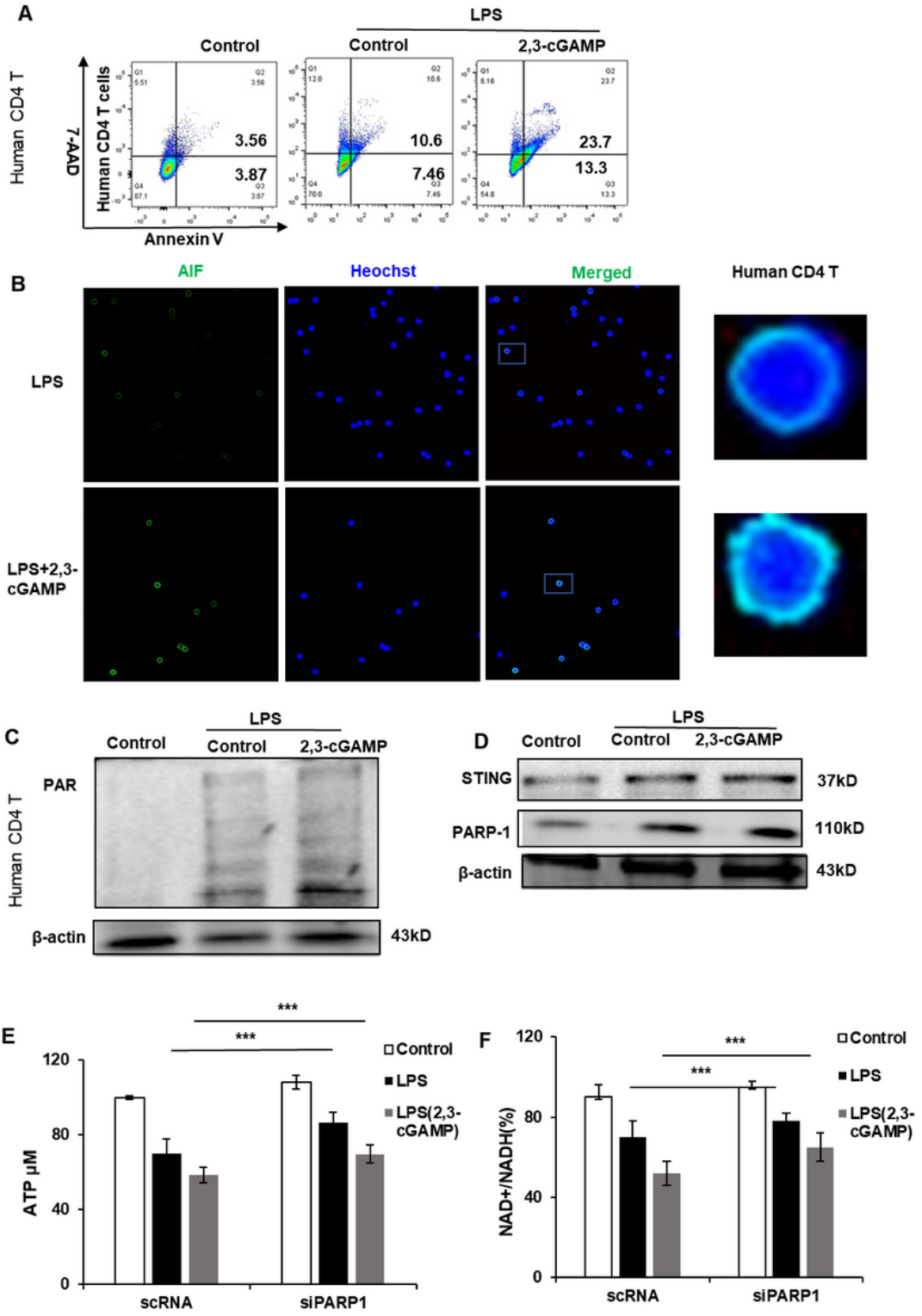



## Figure 3

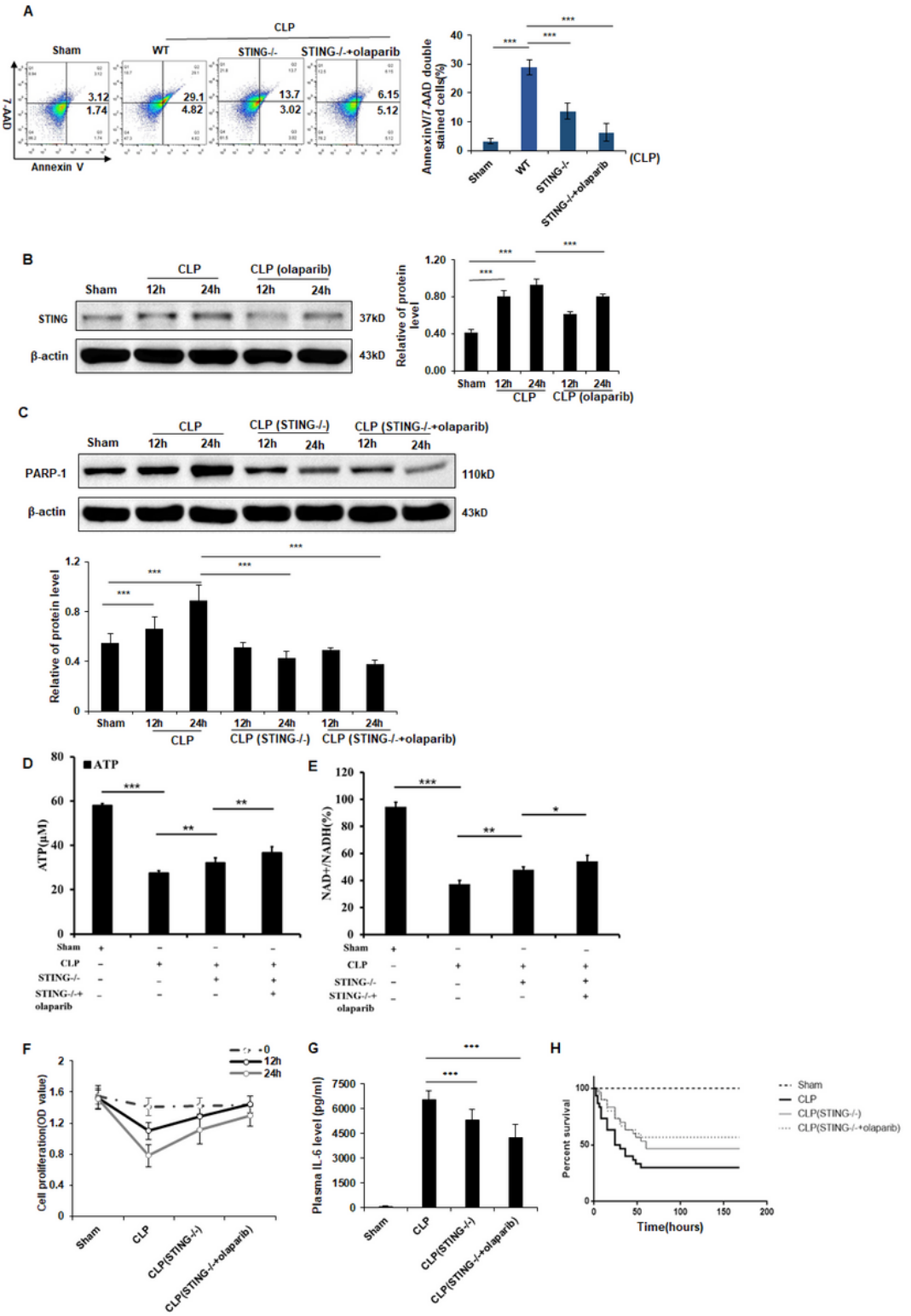
AIF is dispensable for STING modulated necrotic death through PARP-1 activation in CD4 T cells in mice. (3A) CD4 T cells were treated with 1μg/ml LPS for 24 h and immunostained with anti-AIF antibody to determine the localization of AIF. CD4 T cells were immunostained with AIF Ab (red) and COXIV Ab (green). The nuclei were counterstained with Heochst (blue). Original magnification×600, these experiments were replicated in separate experiments at least three times with similar results. (Scalebar, 10 μm). (3B) The cytosolic (cyto), mitochondrial (mito), and nuclear fractions (nuc) were recovered and examined using an immunoblot assay with anti-AIF antibody and anti-PARP-1 antibody. COXIV, β-actin, and PARP-1 were used as mitochondria, cytosolic and nuclear markers, respectively. (4D) CD4 T cells were transfected with scRNA or siPARP-1 for 48 h, and then cells were treated with LPS for 24 h at concentrations of 1μg/ml. The ATP level was measured, luminescence was read on a BioTek Synergy Mx luminometer. The cellular level of NAD+/NADH was determined using fluorescent 96 well plate reader readout (excitation: 530-570 nm and emission at 590-600 nm). The values were the mean ± S.D., from triplicate independent experiments (n=8 each) with statistical significance: **P<0.01.




## Figure 4

PARP-1 activity is associated with STING modulated necroptosis in human CD4 T cells. (4A) Human CD4 T cells were treated with LPS (1μg/ml) ± 2,3-cGAMP (2.5 mg/ml) for 24 h. Cell death was evaluated by flow cytometry. (4B) Human CD4 T cells were immunostained with AIF Ab (green). The nuclei were counterstained with Heochst (blue). Original magnification×600, these experiments were replicated in separate experiments at least three times with similar results. (Scalebar, 10 μm). (4C) Human CD4 T cells

were transfected with scRNA or siPARP-1 for 48 h, and then cells were treated with LPS (1μg/ml) ± 2,3-cGAMP (2.5 mg/ml). The expression levels of PARP-1 and PAR were detected by Western blotting. (4D) The expression levels of STING were detected by Western blotting. (4E, F) Human CD4 T cells were transfected with scRNA or siPARP-1 for 48 h, and then cells were treated with LPS (1μg/ml) ± 2,3-cGAMP (2.5 mg/ml). The ATP level was measured, luminescence was read on a BioTek Synergy Mx luminometer. The cellular level of NAD+/NADH was determined using fluorescent 96 well plate reader readout (excitation: 530-570 nm and emission at 590-600 nm). The values were the mean ± S.D., from triplicate independent experiments with statistical significances: *P<0.05, **P<0.01, ***P<0.001.




## Figure 5

Activation of PARP-1 in CD4 T cell is associated with STING in the CLP mouse model. (5A) Mice underwent a sham procedure or cecal ligation and puncture (CLP). STING-/-mice were administrated with PARP-1 inhibitor olaparib (100 mg/kg, 3 hours before CLP injury). Cell death was evaluated by flow cytometry at 24h after CLP injury. (5B) Using Western blot analysis, STING expression was determined. (5C) Using Western blot analysis, expression of PARP-1 was determined. (5D, 5E) The pharmacological

inhibition of PARP-1 was used. The cellular level of NAD+/NADH was determined using fluorescent 96 well plate reader readout (excitation: 530-570 nm and emission at 590-600 nm). The ATP level was measured, luminescence was read on a BioTek Synergy Mx luminometer. (5F) The proliferation of CD4 T cells was detected by CCK-8. (5G) Plasma IL-6 level was determined by ELISA in WT versus STING−/−mice at 24 hours after indicated treatments. (5H) Seven-day survival. STING−/−mice were administrated with or without olaparib before CLP injury (100 mg/kg, 3 hours before CLP injury). n= 15 in STING−/− CLP group; n = 15 in STING−/− CLP +olaparib group. The values were the mean ± S.D., from triplicate independent experiments (n=8 each, A-G) with statistical significances: *P<0.05, **P<0.01, ***P<0.001.