Propofol Improves Survival In A Murine Model of Sepsis Via Inhibiting Rab5a -Mediated Intracellular Trafficking of TLR4

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Research

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Abstract

Background: Propofol is a widely used anesthetic and sedative, which has been reported to exert an anti-inflammatory effect. TLR4 plays a critical role in coordinating the immuno-inflammatory response during sepsis. Whether propofol can act as an immunomodulator through regulating TLR4 are still unclear. In view of its potential as a sepsis therapy, we investigated the mechanisms underlying the immunomodulatory activity of propofol.

Methods: The effects of propofol on TLR4 and Rab5a (a master regulator involved in intracellular trafficking of immune factors) were investigated in macrophage (from $Rab5a^{-/-}$ and WT mice) following treatment with lipopolysaccharide or cecal ligation and puncture in vitro and in vivo, and in peripheral blood monocyte from sepsis patients and healthy volunteers.

Results: We showed that propofol reduced membrane TLR4 expression on macrophage in vitro and in vivo. Rab5a participated in TLR4 intracellular trafficking and both Rab5a expression and the interaction between Rab5a and TLR4 were inhibited by propofol. We also showed Rab5a upregulation in peripheral blood monocyte of septic patients, accompanied by increased TLR4 expression on the cell surface. Both were correlated with SOFA score of sepsis patients and higher expression of Rab5a were found in septic non-survivors. Propofol downregulated the expression of Rab5a and TLR4 in these cells.

Conclusions: We demonstrated that Rab5a regulates intracellular trafficking of TLR4 and that propofol reduces membrane TLR4 expression on macrophages by targeting Rab5a. Our study not only reveals a novel mechanism for the immunomodulatory effect of propofol but also indicates that Rab5a may be a potential therapeutic target against sepsis.

Background

Sepsis has been defined as life-threatening organ dysfunction caused by a dysregulated host inflammatory immune response to infection [1, 2]. Although the case fatality rate of sepsis has decreased to ~20% in some surveys in recent years [3, 4], it remains the leading cause of death in critically ill patients. Many clinical trials have focused on seeking an effective therapeutic target for sepsis, but these have met with very limited success[5]. Consequently, a better understanding of the molecular mechanisms involved in the pathogenesis of sepsis is imperative.

Propofol is most widely used in anesthesia induction and maintenance in the operating room and also in sedation for ICU patients [6]. Previous studies by our group and others have revealed that propofol possesses anti-inflammatory and antioxidant properties in many conditions including ischemia/reperfusion injuries of organs and cardiopulmonary bypass[6]. It is well established that propofol suppresses production of proinflammatory cytokines, reduces expression of nitric oxide, and inhibits the immune activities of macrophages and neutrophils[7]. Moreover, propofol was proved to be beneficial to the survival of mice challenged with CLP[8]. Nevertheless, the precise mechanism that governs these protective effects of propofol is still not well understood.

The toll-like receptor 4 (TLR4) receptor plays an important role in regulating the innate immune in response to bacterial infection [9, 10]. The activation and upregulation of TLR4 expression on the macrophage cell membrane surface are critical in mediating immuno-inflammatory responses [11]. Most TLR4 receptor molecules are stored in subcellular compartments such as the Golgi apparatus and endosomes, indicating that the expression of cell surface TLR4 is determined by both receptor trafficking from the Golgi apparatus to the cell membrane and internalization of the cell surface receptor into endosomal compartments [11-13]. However, the molecular mechanisms of TLR4 intracellular trafficking are unclear. As propofol is an immunomodulating agent with anti-inflammatory effects, it is reasonable to hypothesize that it may influence cell surface TLR4 expression, but whether this occurs is also unclear.

Rab GTPases are a large family of small GTPases which are known as key coordinators of vesicle traffic, and are involved in recycling of cell surface receptors [14-17]. Rab5a, one of the Rab GTPases, has been recognized as a master regulator of endocytosis [16, 18, 19]. Rab5a is also involved in the intracellular trafficking of cell surface receptors for hormones, cytokines, and chemokines [20]. However, whether Rab5a regulates intracellular trafficking of TLR4, and whether propofol inhibits cell surface TLR4 expression via targeting Rab5a, have not yet been reported.

We hypothesized that Rab5a regulates intracellular trafficking of TLR4 in macrophages and that propofol exerts its anti-inflammatory role by reducing membrane TLR4 expression on macrophages via targeting Rab5a. To investigate these hypotheses, we employed a series of experiments with macrophages following LPS treatment in vitro, and CLP in vivo. We found that Rab5a is critical in TLR4 intracellular trafficking, and regulates membrane TLR4 expression on macrophages. Propofol exerts its anti-inflammatory role by reducing TLR4 expression on macrophages via inhibiting Rab5a expression and the interaction between Rab5a and TLR4.

Methods

Animals

C57/BL mice were obtained from Guangdong Medical Laboratory Animal Center (Guangzhou, China). Rab5a knockout mice were purchased from Cyagen Biosciences (Guangzhou, China). All animal experiments were reviewed and approved by the Ethics Committee of Southern Medical University.

BMDM isolation and culture

BMDMs were isolated and cultured as previously described [21]. Briefly, 6-8-week-old male C57BL mice were executed by neck breakage and the tibia and femur were isolated. Bones were kept on ice and rinsed in a sterile dish with DMEM (Gibco), and bone marrow was then flushed out with DMEM using a 30-gauge needle. Cells were harvested and plated at 10^6 cell/ml with DMEM containing 10% fetal bovine serum (FBS) (Gibco), 20% L929 supernatant, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were

cultured at 37 °C in 5% CO_2 for differentiation into macrophages before further experiments. BMDMs were treated with LPS (1μg/ml), and propofol (100μM) were added 30 min before LPS treated[22].

CLP procedure

The CLP-induced mouse sepsis model was performed according to general guidelines [23, 24]. Male 6-8-week-old C57BL mice were anesthetized with 1% pentobarbital (0.01 mg/g) by intraperitoneal injection. For the CLP procedure, in brief, a 2-cm midline laparotomy was performed to expose the cecum. The distal 5 mm of the tip was tightly ligated with 3.0 silk suture and punctured once using an 18-gauge needle. A small amount of fecal material was then squeezed to extrude into the peritoneal cavity. The cecum was returned to the abdominal cavity, and the abdomen and skin were respectively closed using 4.0 silk. Following the surgery, 1 ml of saline was subcutaneously administered in the neck. In the sham group, mice underwent the same procedure, but without the cecal ligation or puncture. In the propofol-treated group, mice were pretreated with a 50 mg/kg propofol (dissolved in fat emulsion) intraperitoneally 30 min before CLP. This subhypnotic dosage of propofol used was based on previous studies[25, 26]. Imipenem was administered subcutaneously at a dose of 0.5 mg/d after CLP[27].

Tissue histology

After animals were euthanized, segments of the small intestine, lung, liver and kidney were fixed using 4% paraformaldehyde, embedded in paraffin, used to prepare 5 μm sections, and hematoxylin and eosin (H&E) stained. Two pathologists blinded to study groups then independently evaluated and scored the injury severity of stained sections as in prior studies[24].

Plasmid transfection

Mouse Rab5a (GV141) plasmid was constructed by Genechem (Shanghai, China). BMDMs were transfected with control GV141, or GV141-mRab5a (2.5 μg/10^5 cells) using Lipofectamine 3000 reagent (Life technologies) according to the manufacturer's protocol. Twenty-four hours after transfection, cells were stimulated with LPS (1 μg/ml) for 24 h and then harvested for membrane TLR4 detection using flow cytometry.

Immunoprecipitation and immunoblot

Immunoprecipitation and immunoblot were performed as previously described [28]. Cells were lysed in IP lysis buffer (Life technologies) at 4 °C for 30 min. After centrifugation at 12,000 × g for 20 min, 500 μl supernatant (500 μg protein) was collected and TLR4 (Abcam) antibody was added, followed by incubation overnight at 4 °C. Magnetic beads (20 μl) were used to capture the protein and antibody complex. The beads were incubated for 4-6 h and washed three times in PBS. Proteins were subjected to SDS-PAGE (6–20% gel) and then transferred to Immobilon-P membranes for Western blotting.

Immunofluorescence

Cells were fixed and permeabilized with 0.5% TritonX-100, followed by blocking with 1% BSA for 30 min at room temperature. Thereafter, the primary antibodies for TLR4 (BD Biosciences), Rab5a (Santa Cruz Biotechnology), or GM130 (Abcam) were added and incubated at 4 °C overnight. The following steps were as described previously [29].

Flow Cytometric Analysis.

To detect membrane TLR4 expression and other markers, cells were incubated with fluorescently labeled antibodies against mouse TLR4 or F4/80 (eBioscience) for 30 min on ice, washed, and analyzed in a FACScalibur flow cytometer (BD).

Antibodies and Reagents

Antibodies used were as follows: TLR4 antibody (610458; BD Biosciences, San Jose, CA, USA), p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb (695; Cell Signaling Technology, Danvers, MA, USA), GAPDH (D16H11) XP Rabbit mAb (5174; Cell Signaling Technology, Danvers, MA, USA), Rab5a antibody (NB120-13253; Novus, Grand Island, NY, USA), and GM130 (PA1-077; Thermo Scientific, Pittsburgh, PA,USA).

Reagents used included a Pierce co-immunoprecipitation kit (26149; Thermo Fisher Scientific, Pittsburgh, PA, USA), lipopolysaccharides from *Salmonella minnesota*, Alexa Fluor® 488 conjugate (L23356; Thermo Fisher Scientific, Pittsburgh, PA, USA), and chlorpromazine hydrochloride (C-8138, Sigma, Louis, MO, USA).

Clinical samples

Patients were included if they were at least 18 years of age, and met sepsis criteria within the first 24 hours after admitted to the Intensive Care Unit of Nanfang Hospitals between March 2020 and June 2020. Exclusion criteria were age younger than 18 yrs, immunosuppressed, treatment with hemodialysis, chemotherapy within 4 weeks, and unable to sign informed consent. A total of 19 septic patients were enrolled between March 2020 and June 2020. The clinical characteristics, including SOFA score, APACHE II score, causes of sepsis, length of Intensive Care Unit stay, and he 28-day mortality, were recorded in Table 1. Eleven healthy donors with no medical problems in the medical examination center of Nanfang Hospital were included as controls. Peripheral blood was collected within the first 24 hours of Intensive Care Unit admission. Monocytes were prepared as previously described[30]. Expression of TLR4 on monocytes surface was detected using flow cytometry and expression of Rab5a mRNA was detected using qPCR.

Statistical analysis

Results are presented as mean ± SD. Differences between two groups were analyzed by Student's t-test. For multi-group comparisons, One-way ANOVA was used followed by Tukey's post hoc test. $P<0.05$ was considered statistically significant. Graphs and figures were made with Graphpad Prism 5.

Results

Propofol inhibits membrane TLR4 expression on macrophages in response to LPS stimulation and CLP

Consistent with previous results[7, 31], we found that propofol can effectively inhibit the expression of pro-inflammatory cytokines in BMDMs after LPS treatment, and can reduce the level of pro-inflammatory cytokines in the serum of mice challenged with CLP (Figure 1A and 1B). Recent evidence suggests that the amount of TLR4 on the surface of macrophages significantly increases following LPS treatment, reflecting involvement in the inflammatory response [32, 33]. To determine whether propofol reduces plasma membrane TLR4 in macrophages, we measured plasma membrane TLR4 after LPS stimulation using flow cytometry. At 6, 12, and 24 h after LPS stimulation, TLR4 expression on BMDM cell membranes was significantly increased compared to the control group. However, when propofol was added 30 mins before LPS stimulation, membrane TLR4 expression on BMDMs decreased significantly at all time points (Figure 1C). To further confirm these findings, we isolated cytomembrane from BMDMs and then detected the expression of TLR4 by western blotting. As shown in Figure 1D, propofol significantly inhibited the cell surface expression of TLR4 in response to LPS.

To explore the influence of propofol on macrophage plasma membrane TLR4 in vivo, we performed standardized CLP on male mice, a gold standard model in sepsis research. Propofol (50 mg/kg) was injected intraperitoneally 30 min prior to CLP. Six hours after surgery, peritoneal macrophages were collected and marked with the cell surface marker F4/80. Similar to the vitro results, CLP induced an increase in the amount of TLR4 on the peritoneal macrophage surface, and propofol eliminated the TLR4 response otherwise induced by CLP (Figure 1E). Moreover, propofol significantly reduced organ injury of CLP mice indicated by hematoxylin and eosin [H&E] staining and serum markers (Figure S1A and S1B). Propofol also increased the survival of CLP mice(Figure S1C). Taken together these results indicate that propofol reduces the amount of cell surface TLR4 induced by immune challenge.

Knockout of Rab5a attenuates membrane TLR4 expression on macrophages and inhibits the activation of macrophages in response to LPS and CLP

It is well established that Rab5a is a key regulator of endocytosis and transportation of plasma membrane compartments [19, 34]. Therefore, we asked whether Rab5a participates in the transport of TLR4 and/or regulates the expression of TLR4 on the cell membrane. Flow cytometry results revealed that TLR4 expression on the cell surface of BMDMs from Rab5a$^{-/-}$ mice was significantly lower than that in the WT group after LPS stimulation (Figure 2A). Furthermore, knockout of Rab5a inhibited the expression of proinflammatory cytokines and activation of the MAPK pathway in BMDMs upon LPS treatment. In vivo, we also found decreased release of proinflammatory cytokines in serum of Rab5a$^{-/-}$ mice compared to WT mice subjected to CLP (Figure 2B and 2C). We further identified an interaction between TLR4 and Rab5a by co-immunoprecipitation and confocal immune-fluorescence microscopy. LPS promoted interaction and co-localization between TLR4 and Rab5a (Figure 3B-3D). To confirm the

role of Rab5a in TLR4 intracellular trafficking, we labeled the Golgi apparatus in BMDMs with GM130 antibody. We found that TLR4 was partially localized in the Golgi before LPS stimulation. LPS weakened the co-localization of TLR4 and Golgi, indicating a translocation of TLR4 from Golgi to cell membrane, and this response was attenuated by Rab5a knockout (Figure 2D).

To further investigate the role of Rab5a in septic mice, we performed CLP on Rab5a-/- mice. Peritoneal macrophages from $Rab5a^{-/-}$ CLP mice showed reduced cell surface TLR4 expression compared to the WT group (Figure 3A). The expression of serum cytokines in $Rab5a^{-/-}$ CLP mice was also significantly lower than WT CLP mice (Figure 3B). Further, the survival of $Rab5a^{-/-}$ CLP mice was significantly increased compared to the WT group and histopathological sections suggested that the damage to lung, liver, kidney, and intestinal tissue in $Rab5a^{-/-}$ mice with CLP surgery was significantly alleviated compared to WT mice (Figure 3C and 3D). Taken together, these data indicate that Rab5a is essential for the intracellular trafficking of TLR4 from Golgi to cell membrane, and for the upregulation of cell surface TLR4 upon LPS treatment. Knockdown of Rab5a can attenuate the inflammatory response and organ damage in septic mice, suggesting it as a potential target for treating sepsis.

Propofol inhibits Rab5a expression and the interaction between Rab5a and TLR4 in macrophages in response to LPS and CLP

Rab5a has previously been shown to play a significant role in membrane receptor trafficking [35]. We hypothesized that Rab5a may bind to TLR4 and help it to traffic between membrane and cytoplasm during LPS treatment, and that this may be suppressed by Propofol. We first evaluated the expression of Rab5a in BMDMs treated with LPS, with or without propofol pretreatment. Western blotting analysis showed that LPS increased the expression of Rab5a, and this effect was suppressed by propofol pretreatment (Figure 4A). Co-immunoprecipitation and confocal immune-fluorescence microscopy revealed that propofol pretreatment weakened the interaction and co-localization between TLR4 and Rab5a (Figure 4B and 4C). Collectively, these data reveal that propofol inhibits both Rab5a expression, and the interaction between Rab5a and TLR4.

Propofol reduces membrane TLR4 expression on macrophages upon LPS treatment through downregulating Rab5a

To gain further insight into whether propofol inhibits cell surface TLR4 expression by LPS-treated macrophages through regulating Rab5a, we analyzed the amount of cell surface TLR4 in Rab5a-overexpressing BMDMs in response to LPS in the presence or absence of propofol pretreatment. As shown in Figure 5A and B, the amount of cell surface TLR4 was increased by Rab5a upregulation and abolished by propofol pretreatment in BMDM treated with LPS compared to an empty-vector-transfected

group. Furthermore, propofol could not effectively reduce the expression of pro-inflammatory factors in Rab5a-overexpressing BMDMs treated with LPS compared to the control group (Figure 5C).

In summary, these results reveal that propofol attenuates the expression of cell surface TLR4 by downregulating Rab5a.

Propofol inhibits the expression of Rab5a and TLR4 on peripheral blood monocytes from healthy volunteers as well as septic patients

In order to validate the results detailed above with in vitro and in vivo experiments, and to exclude differences between mice and humans, we extracted peripheral blood monocytes from healthy volunteers and verified the effects of propofol on Rab5a and TLR4 following LPS stimulation. We found that the expression of Rab5a and TLR4 on the surface of human peripheral blood monocytes increased after LPS stimulation, and propofol pretreatment inhibited the expression of Rab5a and cell surface TLR4 (Figure 6A and 6B). In addition, lower levels of proinflammatory cytokines were also observed in the propofol pretreatment group (Figure 6C). Further, we extracted peripheral blood mononuclear cells in sepsis patients and found a significant increase in both Rab5a and cell surface TLR4 expression compared with Health volunteers. Propofol was able to inhibit the expression of Rab5a and cell surface TLR4, and the release of inflammatory factors (Figure 6D-6F). Moreover, Rab5a and membrane TLR4 expression were positively associated with the Sequential Organ Failure Assessment score of the sepsis patients (Figure 7A-7B) and more importantly, the septic non-survivors had significantly increased Rab5a mRNA levels when compared to the septic survivors (Figure 7C). The above findings showed that the expression levels of Rab5a and TLR4 are elevated in peripheral blood mononuclear cells of patients with sepsis, and the mechanisms by which propofol exerts its anti-inflammatory effects include inhibiting the expression of Rab5a and TLR4, which is in accordance with the results of our other in vitro and in vivo experiments.

Discussion

Our first principal finding was that Rab5a interacts with TLR4 and facilitates the transport of TLR4 from intracellular space to the cell membrane. Possibly as a result of its involvement in TLR4 intracellular trafficking, Rab5a is critical for macrophage activation and TLR4-mediated inflammatory reaction. These findings were consistent across experiments in vivo with $Rab5a^{-/-}$ mice subjected to CLP, and in vitro with LPS-treated BMDMs from $Rab5a^{-/-}$ mice, and reveal a novel mechanism of TLR4 intracellular trafficking.

Another important finding of this study was that propofol exerts its anti-inflammatory role by reducing membrane TLR4 expression on macrophages via inhibiting Rab5a expression and the interaction between Rab5a and TLR4. Consistently, we also showed that both Rab5a, and cell surface TLR4 expression, were upregulated in LPS-treated peripheral blood monocytes from healthy volunteers, and in peripheral blood monocytes from septic patients, and that expression of both factors was downregulated by propofol. The correlation between the expression of both factors and the SOFA score of sepsis

patients and the high expression of Rab5a in septic non-survivors peripheral blood monocytes further enhanced the clinical relevance of our study. In this study, we demonstrated that propofol, acting as an immunomodulating agent, exerts its anti-inflammatory role in sepsis by targeting Rab5a and thereby downregulating membrane TLR4 expression by macrophages.

The TLR4 receptor is a key signaling molecule in initiating the immune response to exogenous bacteria and endogenous damage-associated molecular patterns during endotoxemia or sepsis. It is specifically activated by LPS, a major component of the cell walls of gram-negative bacteria, heat shock proteins released by necrotic cells, heparin sulfate, polysaccharide degraded from hyaluronate, and other factors [36-38]. Numerous studies have suggested that TLR4 is a "double-edged sword" during sepsis [36, 39]. On the one hand, after activation, it can mediate the inflammatory reaction to remove exogenous microorganisms and damage cells in the body. On the other hand, excessive inflammation can cause damage to normal tissue and may lead to multiple organ failure. Therefore, as a crucial regulation molecule of endotoxemia or sepsis, TLR4 expression on the cell membrane surface is tightly regulated [40, 41]. Here, we showed that propofol prevents excessive activation of inflammatory responses by reducing TLR4 expression on the surface of macrophages exposed to exogenous bacteria. Therefore, propofol may be a useful immunomodulating agent to return an aberrant immune response to homeostasis in sepsis.

It has been demonstrated that the expression of cell-surface TLR4 is determined by both endocytosis of cell-surface TLR4 into endosomes and receptor trafficking from the Golgi apparatus to the cell membrane[40]. However, the molecular events involved in the membrane trafficking of TLR4 are still not well understood. It is also unknown whether there is a crosstalk between endocytosis and replenishment of TLR4. Many studies have shown that LPS not only causes endocytic degradation of TLR4 but also promotes intracellular TLR4 transport to the cell membrane [42, 43]. Rab5a has been recognized as master regulator of endocytosis and is involved in the intracellular trafficking of cell surface receptors [20]. Of interest, we showed that knockout of the Rab5a gene not only reduces endocytosis of TLR4, but also abolishes the replenishment of TLR4 from Golgi to cell membrane. This indicates that Rab5a is a key molecule in membrane trafficking of TLR4 for its role in bridging TLR4 endocytosis and replenishment.

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection [44]. In sepsis, the immune response that is initiated by an invading pathogen is refractory to a return to homeostasis, thus culminating in a pathological syndrome that is characterized by sustained excessive inflammation and immune suppression [45]. Immunomodulation has been considered an important avenue of treatment for sepsis, but anti-inflammatory agents against inflammatory factors have been shown to fail to improve patient outcomes [46]. New therapeutic targets and individualized treatment options are urgently needed. Our study revealed that propofol inhibits the interaction between TLR4 and Rab5a and abolishes Rab5a-mediated TLR4 membrane trafficking, indicating that propofol may enact its anti-inflammatory properties through mediating TLR4 expression via targeting of Rab5a. Moreover, higher expression of Rab5a and surface TLR4 were found in peripheral blood monocytes of

patients with sepsis than in the normal control group, and both were correlated with SOFA score of sepsis patients and higher expression of Rab5a were found in septic non-survivors. Given the importance of these factors to the immune response, therapeutic methods focusing on exploring the possible mechanisms involved in TLR4 trafficking, the regulation of Rab5a expression, and the amount of TLR4 on macrophage surfaces, may be effective strategies for treating sepsis.

However, our clinical data suggest that TLR4 expression is not associated with patient survival and studies of Eritorin (a TLR-4 antagonist) have failed in clinical trials[47], which may raise questions about the clinical application of our findings. As TLR4 not only mediates inflammatory responses leading to organ damage but also plays an important role in maintaining the normal function of the immune system, direct blocking of TLR4 may not benefit all patients. Based on the failure of eritoran clinical trial, some scholars proposed new strategies for enrolling only patients who are likely to respond to the drug on the basis of appropriate biochemical screening[48]. Rab5a is elevated and is associated with survival in sepsis patients. Given that Rab5a is critical in TLR4 trafficking, high expression of Rab5a may indicate abnormal transport and overactivation of TLR4. Therefor, Rab5a maybe a good maker for screening patients sensitive to TLR4 inhibitor.

Limitations of this study include our inability to explore the exact mechanisms of propofol's effect on Rab5a, and the regulation by Rab5a of TLR4 recycling from membrane to subcellular compartments such as Golgi and endocytosomes. Propofol was initially defined as a gamma-aminobutyric acid (GABA) receptor antagonist. Recent studies have demonstrated that immune cells, including macrophages, also express GABA receptors [31]. The function of GABA in the immune system is at an early stage of study, but inhibitory effects have been observed in autoimmune diseases [49]. The questions of whether Rab5a interacts with GABA receptors, and whether propofol regulates Rab5a through GABA receptors, are of interest for future research.

Conclusion

We demonstrated that Rab5a regulates intracellular trafficking of TLR4 and that propofol reduces membrane TLR4 expression on macrophages by targeting Rab5a. Our study sheds light on the mechanism underlying the anti-inflammatory effect of propofol from the aspects of receptor transport and immune regulation. This informs the potential clinical application of propofol to septic patients, and indicates that Rab5a may be a potential therapeutic target against sepsis.

Abbreviations

TLR4: toll-like receptor 4; ICU: intensive care unit; CLP: cecal ligation and puncture; BMDM: bone marrow derive macrophage; LPS: lipopolysaccharide; DMEM: Dulbecco's modified eagle medium; FBS: fetal bovine serum; SOFA: sequential organ failure assessment; APACHE II: Acute Physiology and Chronic Health Evaluation II.

Declarations

Ethics approval and consent to participate

Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Southern Medical University (SMU). Ethical approval for clinical samples was obtained from the institutional review board of SMU. All patients gave informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

BWZ and FLZ performed most experiments and contributed to writing the manuscript. WJZ, BCZ, JZ reviewed and edited the manuscript. WJF and XDC analyzed data. CL and TJ supervised the project. KXL conceived and designed the study.

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Tables

Table 1. Characteristics of sepsis patients and health control

Characteristics	Septic Survivors (n=15)	Septic Non-survivors (n=4)	Health (n=11)	P value
Age (yr)	57.73±15.57	63.25±13.35	52.27±14.77	1
Sex, male (%)	11 (73.33%)	3 (75%)	5 (45.45%)	0.91
APACHE II score	20.40±5.49	31.25±6.09		<0.01
SOFA score	9.27±4.73	14.25±1.71		0.06
Sepsis due to				
Peritonitis	2 (13.33%)	2 (50%)		
Pneumonia	5 (33.33%)	2 (50%)		
Urinary tract infections	1 (6.67%)	0		
Skin and soft tissue infections	4 (26.67%)	0		
Other	3 (20%)	0		
Length of ICU stay	11.4±12.71	15.25±6.13		0.57

Figures

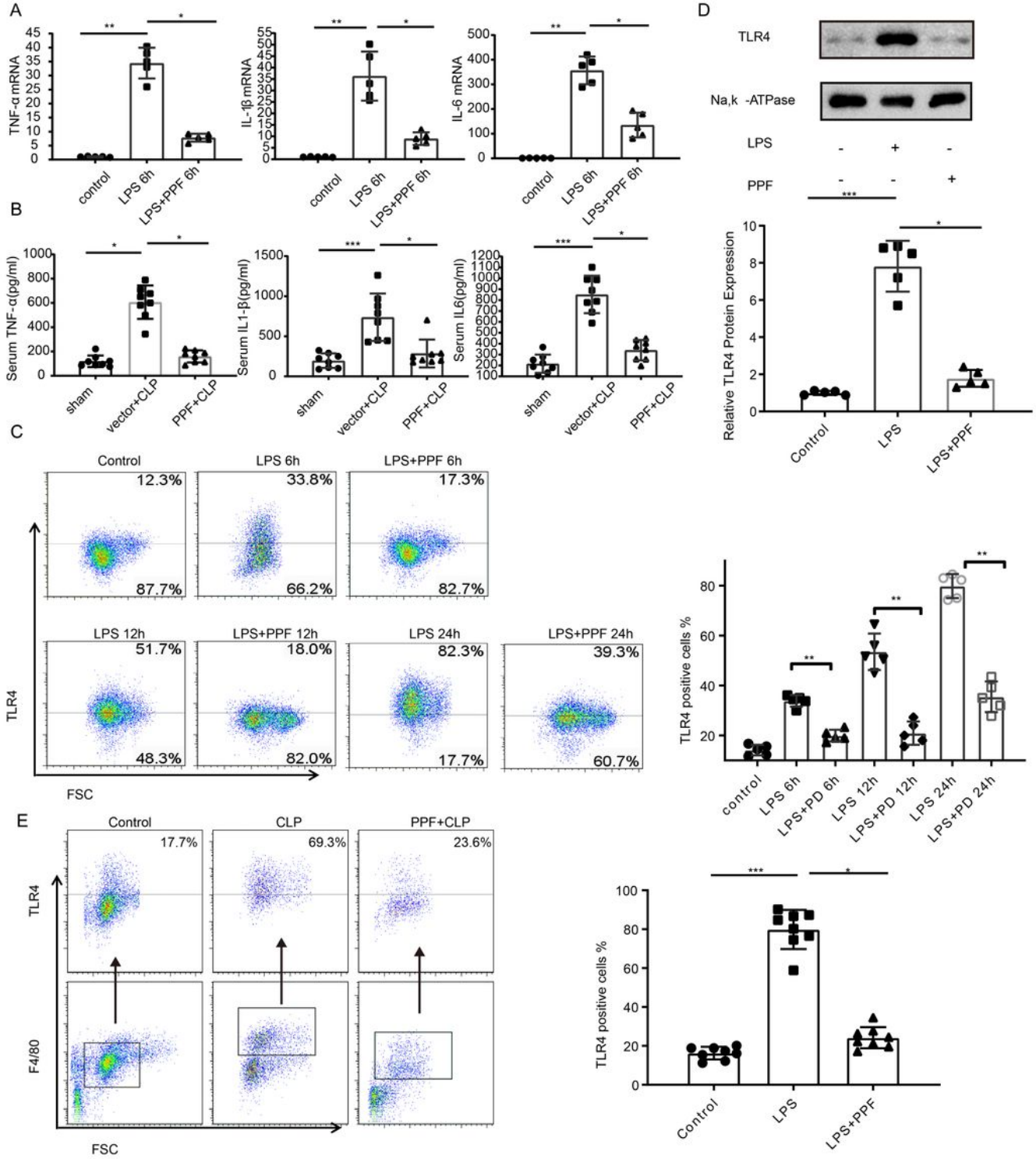

Figure 1

Propofol inhibits membrane TLR4 expression of macrophages after LPS stimulation or CLP. BMDMs were treated with LPS (1μg/ml) for indicated time with or without pretreatment of propofol (100μm) for 30min. C57BL mice that underwent CLP were pretreated with propofol (50mg/kg) or equal volume of fat emulsion for 30min and sacrificed after 12 hours. (A) proinflammatory factor mRNA levels in BMDMs were measured by qPCR. (B) serum proinflammatory factor levers of CLP mice were assessed by ELISA. (C) membrane TLR4 expression of BMDMs was analyzed by flow cytometry. (D) Western blot analysis of membrane TLR4 expression of BMDMs (E) Membrane TLR4 expression of peritoneal macrophages from CLP mice was detected by flow cytometry. n = 3-5, Data are expressed as the mean ± SD, n = 5–7, *p < 0.05, **p < 0.01,***p < 0.001, 1-way ANOVA with Tukey's post hoc test. Abbreviations: PPF: propofol.

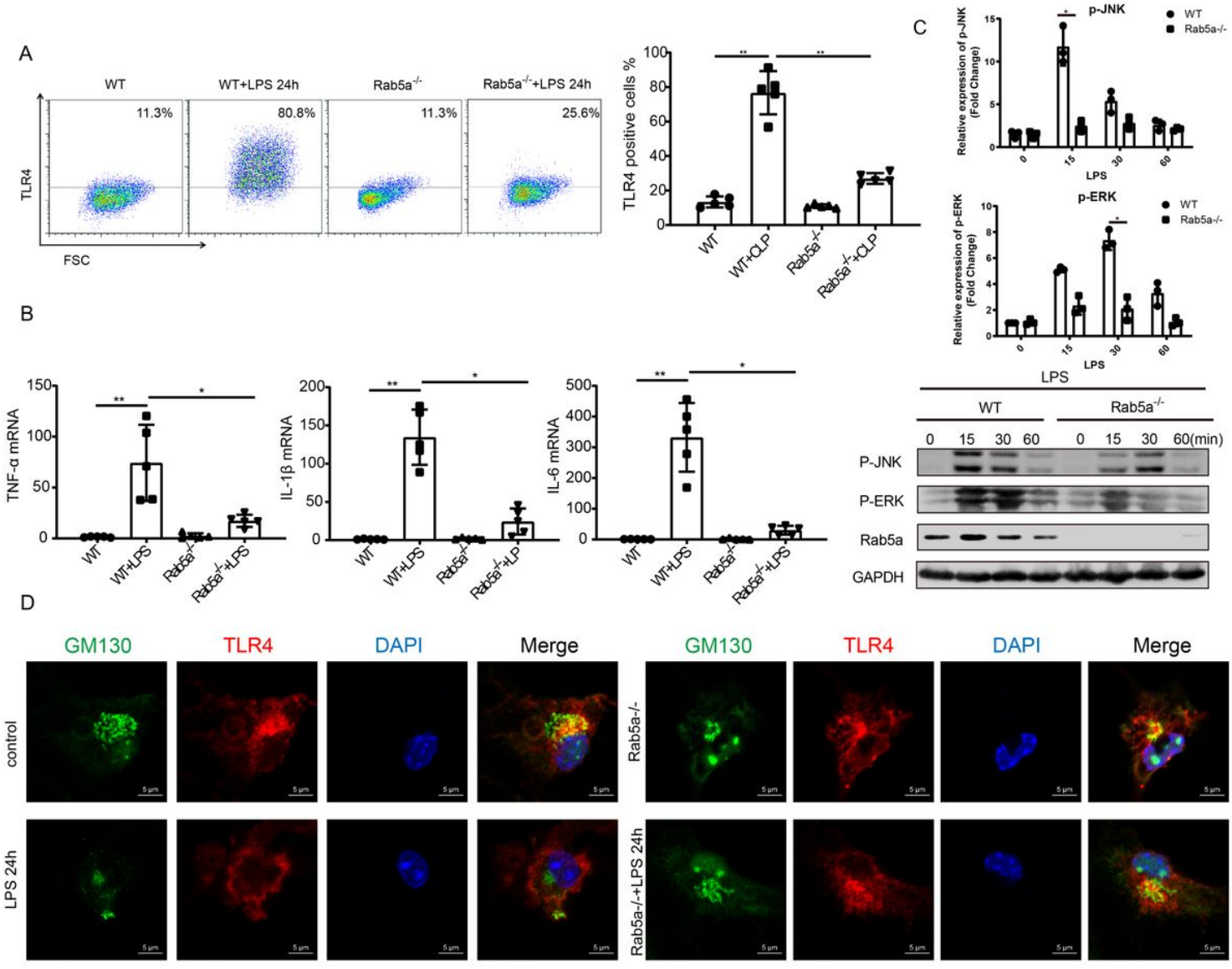

Figure 2

Knockout of Rab5a attenuates TLR4 expression on macrophages and inhibits the activation of macrophages. BMDMs from wild type and Rab5a-/- C57BL mice were treated with LPS (1μg/ml) for indicated time. (A) membrane TLR4 expression of BMDMs was analyzed by flow cytometry. (B) proinflammatory factor mRNA levels in BMDMs were measured by qPCR. (C) Western blotting of MAPK

levels in BMDMs. (D) Representative image of TLR4 (red) and GM130 (green) immunofluorescence staining in BMDMs, scale bars: 5μm. Data are expressed as the mean ± SD, n = 3–5, *p < 0.05, **p < 0.01,***p < 0.001, 1-way ANOVA with Tukey's post hoc test.

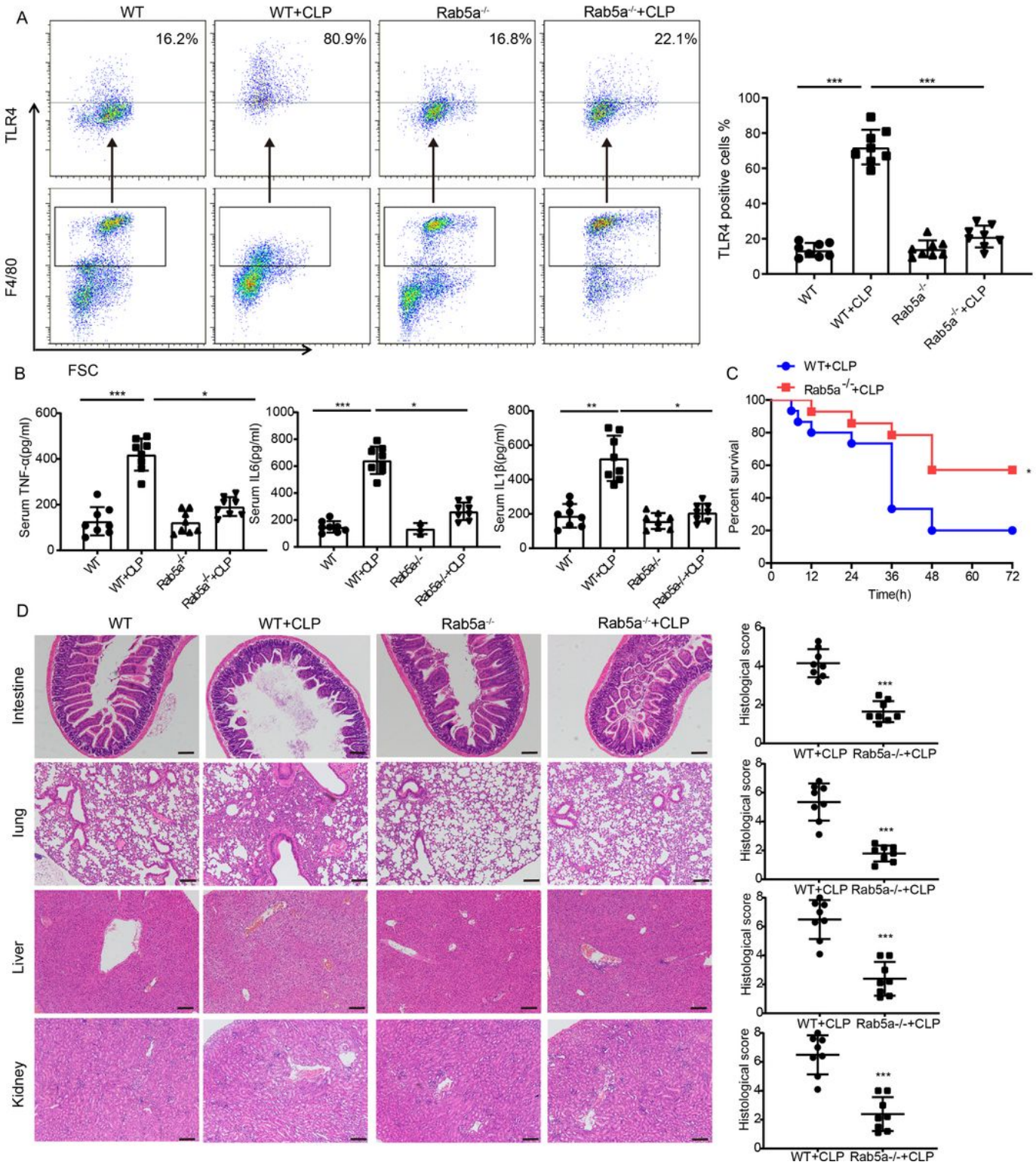

Figure 3

Knockout of Rab5a attenuates membrane TLR4 expression on macrophages in response to CLP. Wild type and Rab5a-/- mice that underwent CLP were sacrificed after 12 hours. (A) Membrane TLR4

expression of peritoneal macrophages from CLP mice was detected by flow cytometry. (B) serum proinflammatory factor levers of CLP mice were assessed by ELISA. (C) H&E staining and histological score of lung, liver, kidney, and intestinal in wild type and RAB5a−/− mice, Scale bars: 50μm. Data are expressed as the mean ± SD, n = 5–7, *p < 0.05, **p < 0.01,***p < 0.001, unpaired t test or 1-way ANOVA with Tukey's post hoc test.

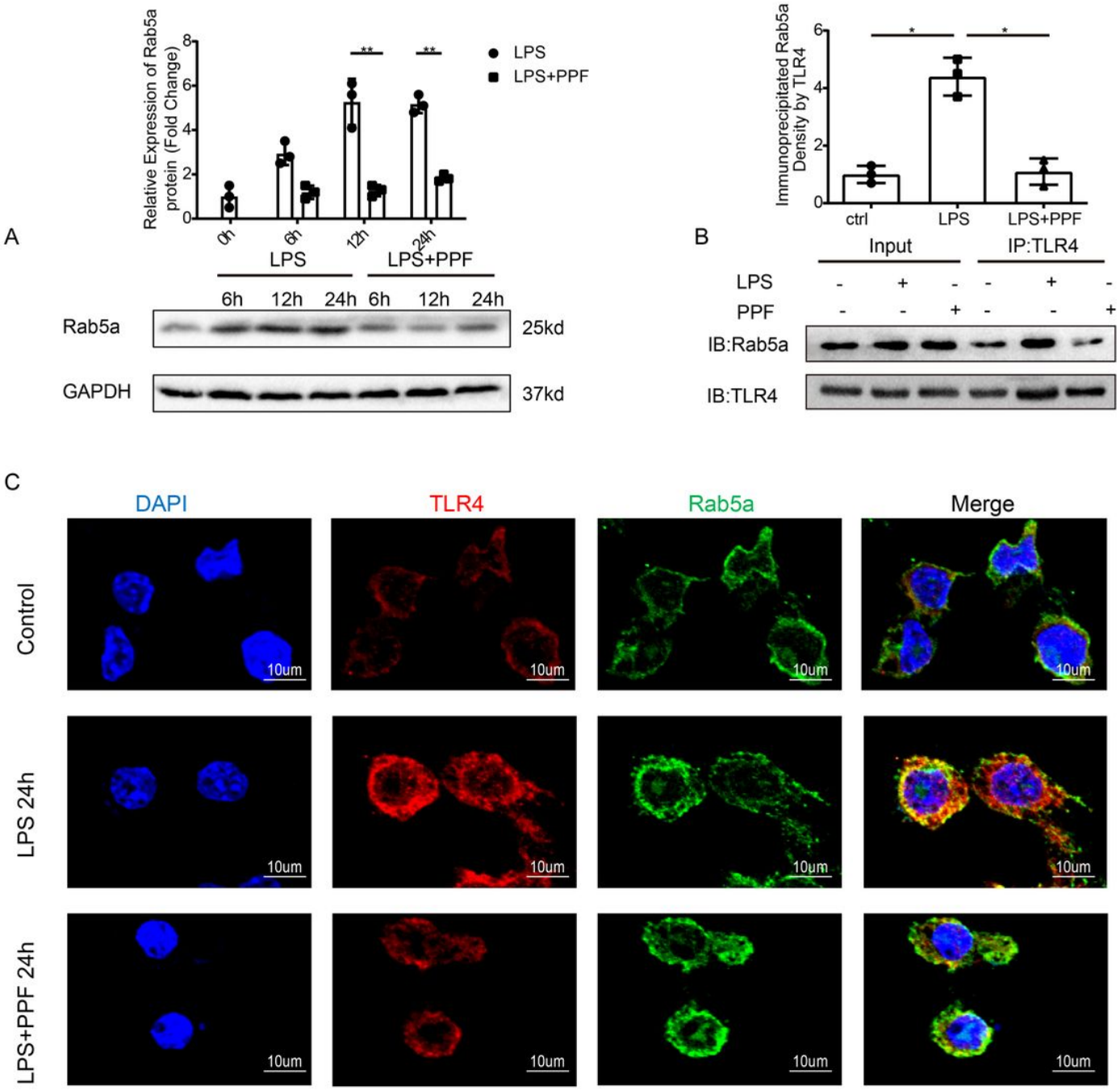

Figure 4

Propofol inhibits Rab5a expression and the interaction between Rab5a and TLR4 in macrophages. BMDMs were treated with LPS (1μg/ml) for indicated time with or without pretreatment of propofol (100μm) for 30min. (A) Western blotting of Rab5a in BMDMs. (B) Interaction of TLR4 withRab5a

determined by Co-IP analyses in BMDMs. immunoprecipitation (IP) and immunoblotting (IB) were performed with anti-TLR4 and anti-Rab5a antibodies. (C) Representative image of TLR4 (red) and Rab5a (green) immunofluorescence staining in BMDMs, scale bars: 10μm. Data are expressed as the mean ± SD, n = 3–5, *p < 0.05, **p < 0.01,***p < 0.001, 1-way ANOVA with Tukey's post hoc test, Abbreviations: PPF: propofol.

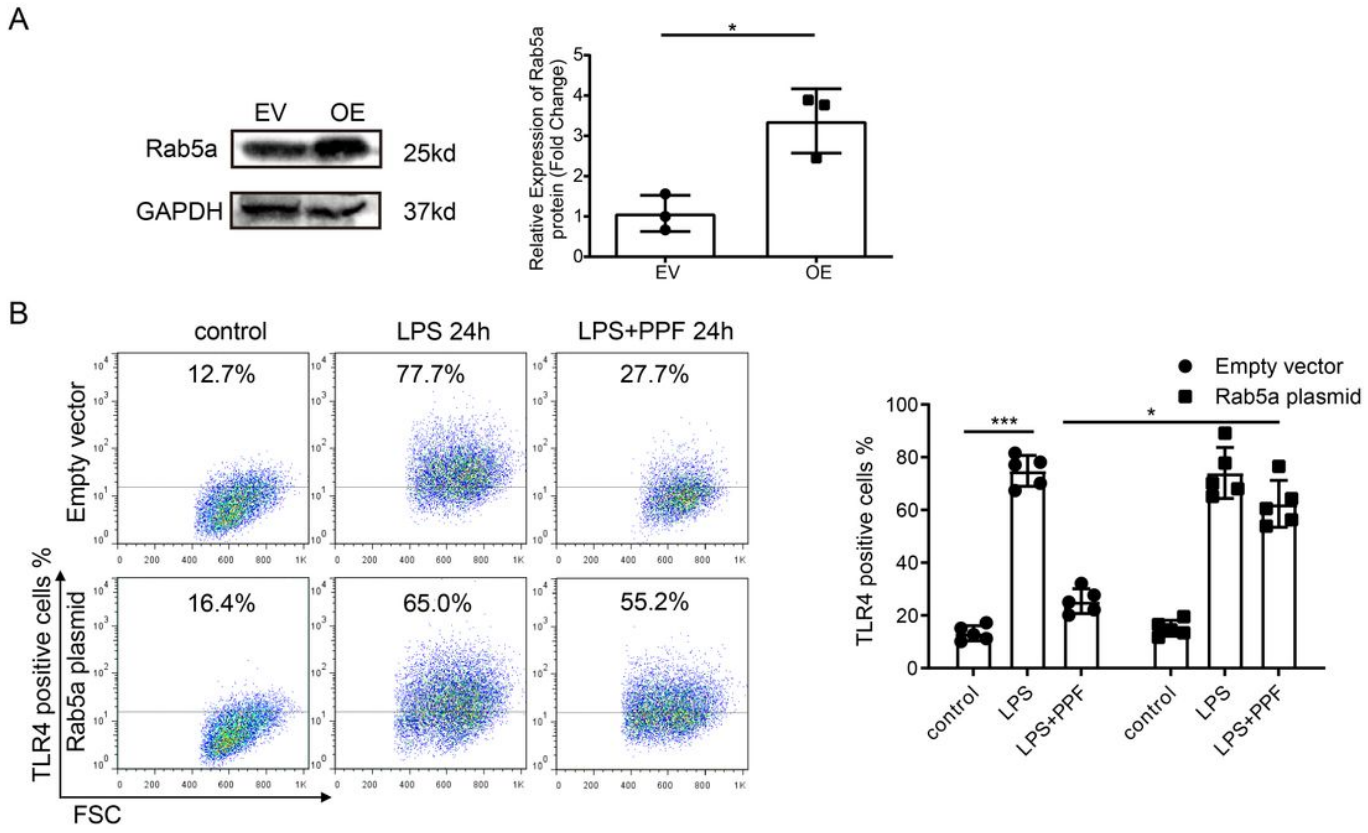

Figure 5

Propofol reduces membrane TLR4 expression on macrophages upon LPS treatment through downregulating Rab5a. BMDMs were transfected with GV141-mRab5a or empty vector and stimulated with LPS (1μg/ml) for 24h with or without pretreatment of propofol (100μm) for 30min. (A) Overexpression efficiency of Rab5a was confirmed by western blotting. (B) membrane TLR4 expression of BMDMs was analyzed by flow cytometry. Data are expressed as the mean ± SD, n = 3–5, *p < 0.05, **p < 0.01,***p < 0.001, 1-way ANOVA with Tukey's post hoc test.

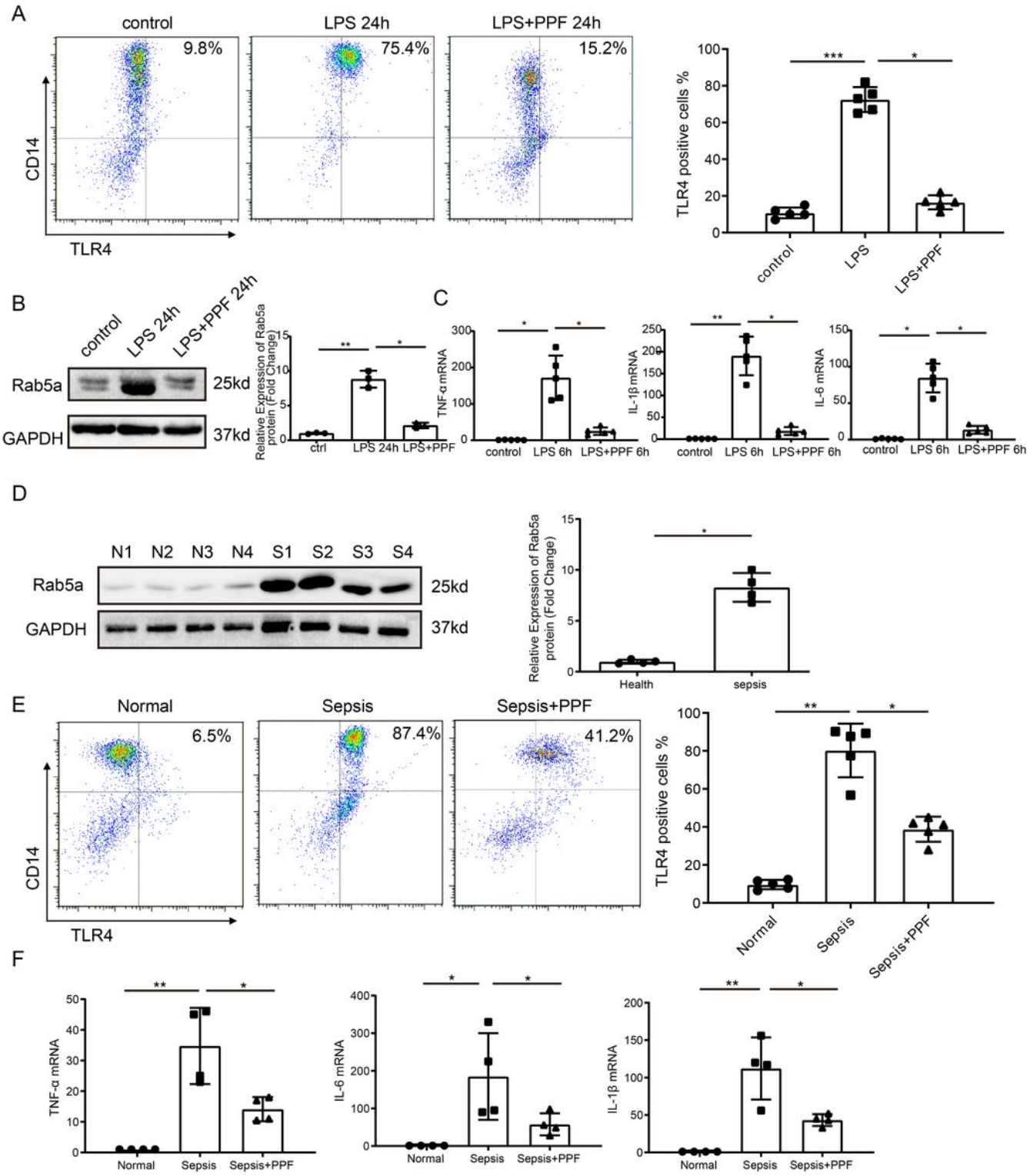

Figure 6

Propofol inhibits the expression of Rab5a and TLR4 on peripheral blood monocytes. Peripheral blood monocytes from healthy volunteers were treated with LPS treated with LPS (1μg/ml) for indicated time with or without pretreatment of propofol (100μm) for 30min. (A) Membrane TLR4 expression of PMBCs from healthy volunteers was analyzed by flow cytometry. CD14 antibody was used to mark monocyte. (B) Western blotting of Rab5a in peripheral blood monocytes from healthy volunteers. (C) proinflammatory

factor mRNA levels in peripheral blood monocytes from healthy volunteers were measured by qPCR. (D) Western blotting of Rab5a in peripheral blood monocytes from healthy volunteers and sepsis patients. (E) Membrane TLR4 expression of peripheral blood monocytes from healthy volunteers and sepsis patients were measured by flow cytometry. Peripheral blood monocytes from sepsis patients were treated with propofol (100μm) for 3h. (F) proinflammatory factor mRNA levels in Peripheral blood monocytes from healthy volunteers and sepsis patients were measured by qPCR. Peripheral blood monocytes from sepsis patients were treated with propofol (100μm) for 3h. Data are expressed as the mean±SD, n = 3–5, *p < 0.05, **p < 0.01, ***p < 0.001, 1-way ANOVA with Tukey's post hoc test.

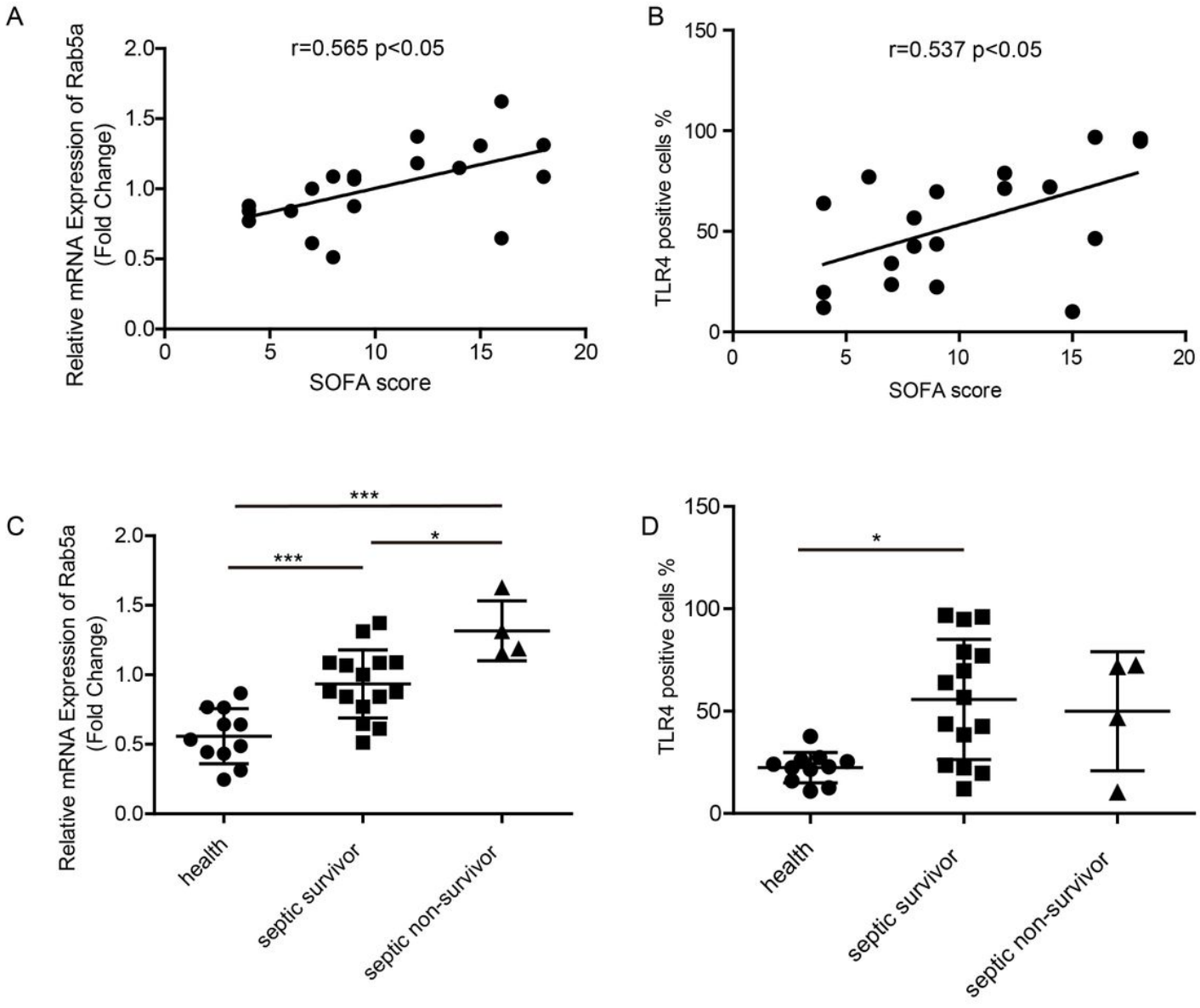

Figure 7

Rab5a expression in peripheral blood monocytes from sepsis patients is associated with SOFA scores. (A) The correlation between Rab5a mRNA lever in peripheral blood monocytes from sepsis patients and

SOFA scores. The data were analyzed using Pearson correlation test (r=0.565, P<0.05, n=19). (B) The correlation between membrane TLR4 in peripheral blood monocytes from sepsis patients and SOFA scores. The data were analyzed using Pearson correlation test (r=0.537, P<0.05, n=19). (C) Rab5a mRNA lever in peripheral blood monocytes from health control, septic survivor and septic non-survivor (n=11, 15, 4). (D) Membrane TLR4 in peripheral blood monocytes from health control, septic survivor and septic non-survivor (n=11, 15, 4). Data are expressed as the mean±SD, *p < 0.05, **p < 0.01, ***p < 0.001, Kruskal-Wallis ANOVA with Dunn-Bonferroni post hoc test.

Supplementary Files

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