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Myeloid ACE2 protects against septic hypotension and vascular dysfunction through Ang-(1–7)-Mas-mediated macrophage polarization

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ABSTRACT

Angiotensin converting enzyme 2 (ACE2) is a new identified member of the renin-angiotensin-aldosterone system (RAAS) that cleaves angiotensin II (Ang II) to Ang (1–7), which exerts anti-inflammatory and antioxidative activities via binding with Mas receptor (MasR). However, the functional role of ACE2 in sepsis-related hypotension remains unknown. Our results indicated that sepsis significantly reduced blood pressure and led to disruption between ACE-Ang II and ACE2-Ang (1–7) balance. ACE2 knock-in mice exhibited improved sepsis-induced mortality, hypotension and vascular dysfunction, while ACE2 knockout mice exhibited the opposite effects. Bone marrow transplantation and *in vitro* experiments confirmed that myeloid ACE2 exerted a protective role by suppressing oxidative stress, NO production and macrophage polarization via the Ang (1–7)-MasR–NF–κB and STAT1 pathways. Thus, ACE2 on myeloid cells could protect against sepsis-mediated hypotension and vascular dysfunction, and upregulating ACE2 may represent a promising therapeutic option for septic patients with hypotension.

1. Introduction

Sepsis has been considered a systemic inflammatory response (SIR) induced by viral or bacterial infection that results in multiple organ failure. Despite improvements in therapies and care, sepsis-associated morbidity and mortality in intensive care units remain unacceptably high. Currently, antibacterial and immunosuppressive drugs are very important options for patients with sepsis, but there are no specific drugs for this disease [1]. Therefore, it is critical to identify new strategies for sepsis treatment. Numerous studies have indicated that endotoxemia is strongly related to SIR. In septic animals induced by lipopolysaccharide (LPS) or cecal ligation and puncture (CLP), pathogenic stimuli such as endotoxin cause activation of the endothelium to release various chemokines and adhesion molecules, such as CXCLs, VCAMs, and ICAMs, which are major factors for recruiting leukocytes at sites of infection [2]. Subsequently, activation of leukocytes produces large amounts of proinflammatory mediators, such as IL-1β and IL-6, and reactive oxygen species (ROS) as well as nitric oxide (NO), which cause vascular dysfunction and systemic hypotension, resulting in reduced peripheral blood flow, oxygen deficit, disseminated intravascular coagulation (DIC), and death [1,3,4]. Therefore, vascular dysfunction leading to hypotension is the central cause of septic shock, multiorgan damage, and poor prognosis in septic patients [5].

The renin-angiotensin-aldosterone system (RAAS) is a critical hormonal cascade consisting of many components that play central roles in regulating blood pressure, inflammation and cardiac and vascular structural remodeling, therefore representing potential therapeutic targets [6,7]. In the classical RAAS, angiotensin converting enzyme (ACE) is an important enzyme that catalyzes the conversion of Ang I (inactive decapeptide) to Ang II (active octapeptide), which interacts with angiotensin II type I receptor (AT1R) to exhibit proinflammatory, prooxidant, and prehypertensive roles. In contrast, ACE2, a human homolog of ACE, is a zinc metallopeptidase that cleaves Ang II to produce Ang (1–7), which has anti-inflammatory and antioxidant activities through bind to the Mas receptor (MasR) [7], indicating a counterbalancing role in regulating cardiovascular physiology and diseases. There are a number of preclinical studies that indicate that the ACE2-Ang (1–7)-MasR pathway displays a protective role in several cardiovascular disorders, including arterial hypertension, cardiac infarction, pulmonary arterial hypertension, and cardiac remodeling [8]. Interestingly, accumulating data have shown that ACE2 as a receptor to mediate severe acute respiratory syndrome coronavirus 2

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(SARS-CoV-2) entry into host cells, thereby resulting in COVID-19 [9]. Moreover, ACE2 is involved in sepsis and sepsis-induced organ damage, such as cardiac dysfunction, lung injury, nephropathy, neuroinflammation, and uveitis after bacterial and viral infection [7,10–15]. Notably, there are some types of immune cells, such as monocytes, macrophages and T cells, that express ACE2 and produce Ang (1–7) [8]. However, the impact of ACE2 on myeloid cells in sepsis-induced vascular dysfunction and hypotension has not been investigated.

In this study, using ACE2 knock-in (ACE2-KI), knockout (ACE2-KO), and bone marrow (BM) chimeric mice, we identified that myeloid ACE2 exerts a beneficial role in sepsis-induced hypotension and vascular dysfunction in a CLP model. Our results revealed that sepsis for 24 h caused an imbalance between the ACE-Ang II and ACE2-Ang (1–7) axes, as evidenced by increased ACE expression and Ang II levels and reduced ACE2 expression levels and Ang (1–7) contents in the mouse aorta following CLP. Overexpression of ACE2 in ACE2-KI mice significantly attenuated CLP-induced mortality, hypotension, vasodilation, increased ROS, NO and Ang II production and M1 polarization and decreased Ang (1–7) levels. However, ACE2-KO mice had the opposite effects. BM transplantation and *in vitro* cell experiments further verified that ACE2 overexpression on myeloid cells protected against the septic response through the Ang (1–7)-MasR–NF–κB and STAT1 pathways. Therefore, these results are the first to elucidate the mechanism by which ACE2 on myeloid cells ameliorates septic hypotension and vascular dysfunction and highlight that upregulating ACE2 activity may represent an effective therapeutic option for sepsis-related diseases.

2. Materials and methods

2.1. Animals

Wild-type (WT) mice on a C57BL/6J background and ACE2 knockout mice (ACE2-KO, KOCMP-70008-Ace2-B6J-VA, strain: C57BL/6J-Ace2em1cyagen) were produced by using CRISPR/Cas9 gene targeting strategy, and obtained from Cyagen Biosciences (Guangzhou, China). F0 founder mice were identified by DNA sequencing and PCR analysis (Forward: 5′-GACGTTGTGCATTGACTGTTCTA-3’; Reverse: 5′-CTACATTACCAGGCAAATGGAAGT-3′) as described [16]. ACE2 knock-in mice (ACE2-KI, strain: C57BL/6J-Ace2 (KI/KI) em1cyagen) were established by Cyagen Biosciences. Briefly, a guide RNA (gRNA) that targets the mouse Ace2 mRNA, the donor mRNA containing human Ace2 gene, and Cas9 mRNA were coinjected into fertilized mouse eggs to generate F0 founder mice, which were identified by PCR analysis (Forward: 5′-CTGGGATCAGAGATCGGAAGAAGAA-3’; Reverse: 5′-CTGATGCTCTTTGGACAACGTTTACT-3′) followed by sequence analysis. All mice were kept in the pathogen-free animal center of Beijing Chao-Yang Hospital and received regular mouse food and water available throughout the study. All study procedures were permitted by the Beijing Chao-Yang Hospital Animal Care and Use Committee (2021-Animal-35, 26 February 2021), and conducted following the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Establishment of a mouse model of sepsis

The cecum ligation/puncture (CLP) surgery was used to establish a sepsis model in mice as we reported recently [17,18]. Briefly, male mice at 8 weeks of age were anesthetized with 2.5 % tribromoethanol injection (Sigma–Aldrich). After ligation of the middle cecum with a 4-0 suture, single puncture was made through the distal cecum with a 21-gauge needle to induce sepsis. Sham mice underwent a similar procedure without CLP surgery. Mice were given subcutaneous injections of analgesic tramadol (12.5 mg/kg) beginning 30 min before CLP and then every 12 h up to the end of modeling as reported previously [19]. All mice were sacrificed by injecting an overdose of tribromoethanol (500 mg/kg, ip) after surgery. Blood samples and aortic tissues were harvested following the experiments.

2.3. Survival analysis

All mice in each group received a sham or CLP operation for survival analysis. The number of deaths was observed, and the percentage of survival was calculated using Kaplan–Meier analysis every 24 h for 48–72 h after CLP.

2.4. Blood pressure monitoring

Arterial systolic and diastolic blood pressure (SBP and DBP) was detected in conscious mice using the tail-cuff method (SoftronBP-98A, Softron, Japan) as described [20]. In brief, mice were inserted gently into the restrainer and warmed at 36 °C for 3–5 min to acclimate, and then noninvasive blood pressure was measured at per min intervals. The average of 5–6 steady-state measurements was recorded for each mouse as described [21].

2.5. Histological analysis

The aortas were quickly removed and preserved in 4 % paraformaldehyde or 20 % sucrose. After 24 h of fixation, all aortas were embedded in paraffin or OCT compound. Immunostaining and immunohistochemistry were conducted as described previously [20,22]. Aortic sections (4 μm) were blocked with goat serum (ZLI-9056, ZSGB-BIO. Beijing, CN) and then incubated with anti-ACE2 (1:200, ARG54913, Arigo, Taiwan, CN), *anti*-F4/80 (1:100, ab111101, Abcam, Cambridge, MA, USA), anti-Mac-2 (1:100, CL488-60207, Proteintech, Chicago, IL, USA), anti-3-nitrotyrosine (1:500, sc-32757, Santa Cruz Biotechnology, Dallas, USA), *anti*-MasR (1:200, 20080-1-AP, Proteintech Group), anti-AT1R1 (1:50, 25343-1-AP, Proteintech Group) or *anti*-IgG (1:200, 30000-0-AP, Proteintech Group) at 4 °C overnight. Aortic cryosections (4 μm) were stained with 10 μM dihydroethidine (DHE) at 37 °C for 30 min. Nuclei were counterstained with DAPI (C1006, Beyotime Biotechnology, Shanghai, CN). Four to five visual fields of each section were photographed randomly with a fluorescence microscope (DM2500, Leica, DE) to obtain fluorescence images, and the quantification of Mac-2^+ macrophages and DHE fluorescence intensity were performed by Image Lab software.

2.6. Evaluation of mesenteric artery function

Vascular relaxation was assessed in isolated rings from mouse mesenteric arteries. Four-millimeter ring segments were gently hung on a tension transducer in organ chambers (Power lab, AD Instrument). After stimulation by phenylephrine (PE, 10^{-6} mol/L, 59-42-7, Sigma, CA) for 5 min, the ring segments were exposed to increasing levels (10^{-9} to 10^{-4} mol/L) of acetylcholine (ACh, 60-31-1, MCE) or levels (10^{-9} to 10^{-5} mol/L) of sodium nitroprusside (SNP, 14402-89-2, MCE). The dynamic vascular responses were recorded and calculated as described previously [20,22].

2.7. Western blot analysis

Fresh aorta issues or *in vitro* cultured macrophages were lysed and homogenized in precooled RIPA lysis buffer. The isolated extracts were used for protein quantification, and immunoblotting analysis was conducted as reported [21,23]. All the primary antibodies used, including anti-ACE antibody (ARG41098, 1:1000, Arigo Biolaboratories Corp), *anti*-MasR (20080-1-AP, Proteintech Group), and anti-AT1R1 (25343-1-AP, Proteintech Group); anti-ACE2 (38241S, 1:1000), *anti*-P-p65 (3033, 1:1000), *anti*-p65 (8242, 1:1000), *anti*-P-Stat1 (9167S, 1:1000), anti-Stat1 (14995S, 1:1000), *anti*-β-actin (4970, 1:1000) or *anti*-GAPDH (5174S), were purchased from Cell Signaling Technology; and anti-3-nitrotyrosine (3-NT, sc-32757, 1:1000) was obtained from Santa Cruz Biotechnology.

2.8. Quantitative PCR analysis

Fresh aortas or cultured macrophages were lysed with TRIzol Reagent (9109, Takara, JP) to purify total mRNAs, and quantitative real-time PCR (qPCR) was conducted as reported [21]. The mRNA levels of all target genes, including NADPH oxidase 2 (NOX2), tumor necrosis factor-α (TNF-α), IL-1β, IL-6, interferon-gamma (IFN-γ), chitinase 3-like protein 3 (YM1), arginase-1 (ARG-1), inducible nitric oxide synthase (iNOS), vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), selectin E (E-selectin), NADPH oxidase 1 (NOX1) and NADPH oxidase 4(NOX4) were evaluated by an iCycler IQ system (QuantStudio 3, Thermo Fisher Scientific, Carlsbad, CA) as described [20,22]. All primer sequences for all genes are listed in Tables S1 and S2. All qPCR values were normalized to GAPDH or β-actin.

2.9. Constitution of bone marrow (BM) chimeric mice

To identify the impact of myeloid ACE2 on CLP-induced hypotension and vascular dysfunction, we generated various BM chimeric mice based on our previous protocols [21]. In brief, WT, ACE2-KI or ACE2-KO mice were anesthetized with 2.5 % tribromoethanol, and BM cells were obtained through flushing the tibia and femur with RPMI-1640 medium containing 10 % fetal bovine serum (10099141, Gibco, USA) and heparin. WT recipients were exposed to irradiation at a dose of 8.5 Gy with cobalt for 8.5 min and then injected with 1–2 x 10^7 BM cells isolated from WT, ACE2-KI or ACE2-KO mice through the tail vein. After eight weeks, all reconstituted mice were subjected to CLP surgery. After 24 h, all mice were sacrificed by injecting an overdose of tribromoethanol (500 mg/kg, ip). The tibias and femurs were flushed with PBS using a 1 ml syringe to obtain BMDCs, which were used to extract DNA with a reagent kit (DC102-01, Vazyme, CN). The genotyping of BMDCs in chimeric mice were validated using PCR analysis as reported previously [24] and the primers are shown in the section of Animals.

2.10. Isolation of peripheral blood leukocytes and bone marrow-derived macrophages

WT, ACE2-KI or ACE2-KO mice were anesthetized with injection of 2.5 % tribromoethanol. Peripheral whole blood was collected into a vacuum tube containing EDTA for anticoagulation. Peripheral blood leukocytes (PBLs) were isolated from blood by the Percoll density gradient centrifugation method (LDS1090, TBD Sciences, CN) and washed 5 times with PBS. BM-derived macrophages (BMDMs) were obtained from WT or ACE2-KI mice by flushing the tibias and femurs with PBS using a 1 ml syringe and were then stimulated with M-CSF (20 ng/ml, #315-02, PEPROTECH, USA) for 72 h to obtain M0 macrophages as reported previously [20]. BMDMs were pretreated with or without A779 (10 μM) and stimulated with LPS (1000 ng/ml) or saline. Total mRNA was purified from PBLs or BMDMs with TRIzol reagent, and the expression levels of iNOS, IL-1β, IL-6, TNF-α, YM1, and ARG-1 mRNAs in PBLs or BMDMs were detected by qPCR analysis. DHE staining of BMDMs was conducted, and five random fields were photographed with a fluorescence microscope.

2.11. Co-culture of macrophages and endothelial cells

WT or ACE2-KI mice were anesthetized with 2.5 % tribromoethanol injection. The tibias and femurs were flushed with PBS using a 1 ml syringe. BM-derived monocytes were cultured in RPMI 1640 medium (C11875500BT, Gibco, USA) with 10 % FBS and 1 % penicillin/streptomycin, and stimulated with colony-stimulating factor (M-CSF, 20 ng/ml, 315-02, PEPROTECH, USA) for 72 h, to obtain M0 macrophages as described previously [25]. Human umbilical vein endothelial cells (HUVECs) were cultured in 10 % DMEM-F12 (C11330500BT, Gibco, USA) medium and maintained in a humidified 5 % CO_2 atmosphere at 37 °C.The co-culture of BM-derived macrophages and HUVECs was performed as previously [22]. HUVECs were seeded in the lower chamber of a 6-well Transwell (0.4 μm) device, and WT or ACE2-KI macrophages were added to the upper chamber and pretreated with or without A779 (10 μM) and stimulated with saline or LPS (1000 ng/mL) for 24 h.

2.12. Measurement of ACE2 activity and total nitrite and MDA levels

The activity of ACE2 in aortic tissues was detected using a fluorometric assay based on the manufacturer's protocols (P0319S, Beyotime, Biotechnology, CN). All samples were analyzed by using a microplate reader (SPARK, AUS) at excitation (325 nm) and emission (393 nm) wavelengths. The total nitrite level in serum supernatant which indirectly indicated NO levels was tested by the microwell plate method based on the manufacturer's protocols at a wavelength of 550 nm (A013-2-1, Nanjing Jiancheng Bioengineering Institute, CN). Malondialdehyde (MDA) levels in aorta tissues were measured using commercial kits (S0131S, Beyotime, CN) at a wavelength of 532 nm.

2.13. Measurement of Ang II, Ang (1–7) and ACE levels by ELISA kits

Fresh aortic tissues of mice were rinsed in ice-cold PBS, weighed and homogenized in fresh cold PBS (PH = 7.4). The isolated tissues homogenates were centrifuged for 10 min at 10,000×*g*, and the supernatants were collected for analysis of Ang II (E-EL-M2612c) and Ang (1–7) (E-EL-M2681c) levels with Elabscience Biotechnology (Wuhan, CN) ELISA kits and ACE (SEA004Mu) levels with CLOUD-CLONE ELISA kit based on the manufacturer's protocols. All samples were monitored on a microplate reader (SPARK, Austria) with a 450 nm filter.

2.14. Statistical analysis

All values are shown as the mean ± standard error of the mean (SEM). Statistical calculations were conducted with GraphPad Prism 8 software. A Shapiro–Wilk test was used to assess the value distribution. If the values were normally distributed and the variances were homogeneous, Student's *t*-test was conducted for the difference comparison between the two groups; otherwise, the Mann–Whitney test was used. Data were evaluated with a 1-way or 2-way ANOVA when the variance was homogenous and the data were normal. Tukey multiple comparison tests were used if ANOVA revealed a significant effect; otherwise, Mann–Whitney tests were conducted when the variance was not homogeneous. Repeated-measures ANOVA was applied to examine Ach- or SNP-stimulated vasodilation in isolated mouse aortic rings. Post hoc comparisons were made using the Fisher least significant difference test if the ANOVA revealed a significant difference.

3. Results

3.1. CLP-induced sepsis alters the balance of RAAS components in the aorta

Given the critical role of the RAAS in sepsis, we first determined the dynamic change in the components of the RAAS in the aorta of CLP-induced septic mice (Fig. 1A). The survival rate and average SBP and DBP were time-dependently reduced compared with the sham control 72 h after sham or CLP surgery (Fig. 1B and C). Consistent with a previous report [26,27], CLP surgery resulted in a marked activation of the classical ACE-Ang II-AT1R axis, as indicated by increased levels of ACE and AT1R proteins and production of ACE and Ang II in the aortic tissues at different time points (Fig. 1D and E). In contrast, activation of ACE2-Ang (1–7)-MasR axis was significantly inhibited in CLP-treated aortas at 24 h, as reflected by the reduction in ACE2 and MasR protein levels, ACE2 activity and Ang (1–7) levels (Fig. 1 D and E). Accordingly, the dynamic changes in MasR and AT1R protein levels were validated in the aortic tissues of mice after sham or CLP treatment using

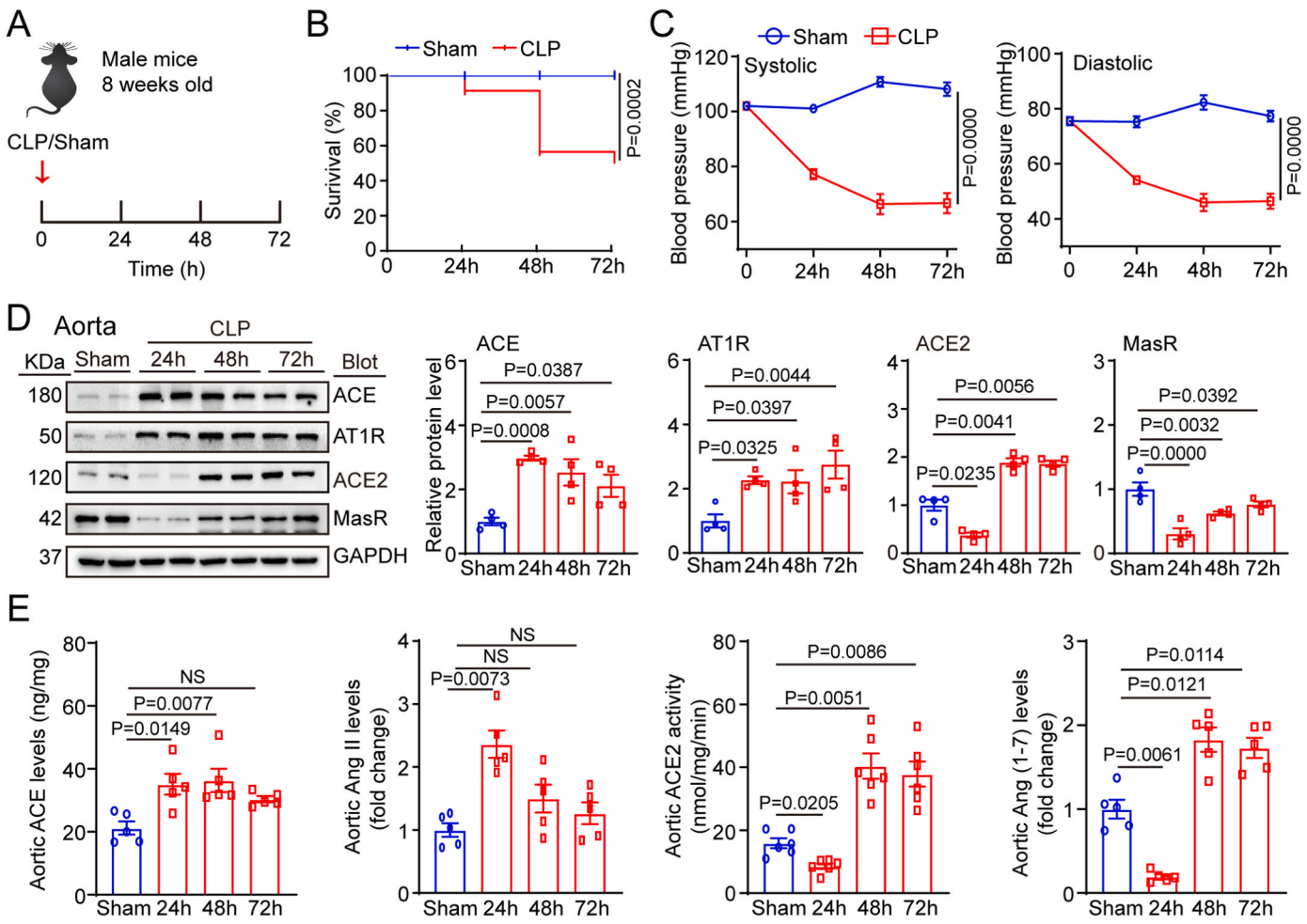

Fig. 1. Imbalance of aortic renin-angiotensin system after CLP-induce sepsis

(A), Schematic of the methods for treatment of wild-type (WT) mice with cecal ligation and puncture (CLP) surgery. **(B),** Survival rate after CLP treatment (n = 24). **(C),** Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured via the noninvasive tail-cuff method in each group (n = 10). **(D),** Immunoblotting analysis of ACE, AT1R, ACE2, MasR protein in the aorta of each group, GAPDH was used as an internal control (n = 4). **(E),** Analysis of ACE, Ang II, and Ang (1–7) levels as well as ACE2 activity in each group (n = 5–6). The data are presented as the mean ± SEM, n represents the samples of each group. Data in (B) were analyzed with Kaplan-Meier; data in C and D were analyzed with 1-way ANOVA by Bonferroni multiple comparison test; data in (E) were analyzed with Mann-Whitney test. NS: No significance.

immunohistochemical staining (Suppl. Fig. S1). Interestingly, ACE2 protein levels and activity markedly increased at 48 and 72 h compared with the sham control after CLP operation (Fig. 1D and E). The Ang (1–7) level was restored in the CLP group at 48 and 72 h (Fig. 1E), probably due to the enhanced expression of ACE2 and Ang II level, a substrate for ACE2 for generation of Ang (1–7). Collectively, these findings indicate that the imbalance of the RAAS components, particularly inactivation of the ACE2-Ang (1–7)-MasR axis at 24 h of sepsis, may be involved in modulating sepsis-related vascular inflammation and dysfunction.

3.2. *Overexpression of ACE2 ameliorates CLP-induced hypotension and vascular dysfunction*

To identify the significance of ACE2 in sepsis, we used ACE2-KI mice overexpressing ACE2 to increase Ang (1–7) production (Suppl. Fig. S2A). Our ELISA data showed that increased ACE2 expression in ACE2-KI mice greatly reversed the CLP-mediated reduction in ACE2 activity and Ang (1–7) contents and the increase in Ang II levels in the aorta compared to those in WT mice (Fig. 2A), implying that ACE2 overexpression improves the Ang (1–7)/Ang II balance. Accordingly, the 48-h survival rate in ACE2-KI mice was dramatically higher than that in WT mice following CLP surgery (Fig. 2B). Then, we tested the effect of ACE2 overexpression on sepsis-induced blood pressure and vascular dysfunction. The noninvasive tail-cuff method showed that the CLP-induced decreases in SBP and DBP in WT mice were markedly ameliorated in ACE2-KI mice (Fig. 2C). Furthermore, mesenteric arteries were isolated from WT or ACE2-KI mice, and an *ex vivo* assay of mesenteric arterial rings confirmed that the sepsis-induced reduction in endothelium-dependent vasodilatation to acetylcholine (ACh) in WT mice was remarkably improved in ACE2-KI mice 24 h after CLP surgery (Fig. 2D). However, endothelium-independent vasodilatation caused by sodium nitroprusside (SNP) was only mildly influenced in ACE2-KI mice following CLP surgery (Fig. 2D), suggesting that ACE2 overexpression attenuates CLP-induced vascular endothelial impairment and hypotension.

3.3. *Deficiency of ACE2 accelerates CLP-induced hypotension and vascular dysfunction*

To validate the impact of ACE2 on the vasculature after sepsis, we used ACE2-KO mice to inhibit aortic ACE2-Ang (1–7) activation (Suppl. Fig. S2B). The results revealed that ACE2-KO further aggravated CLP-mediated inhibition of aortic ACE2 activity and Ang (1–7) contents and upregulation of Ang II contents compared to those in the aortas of

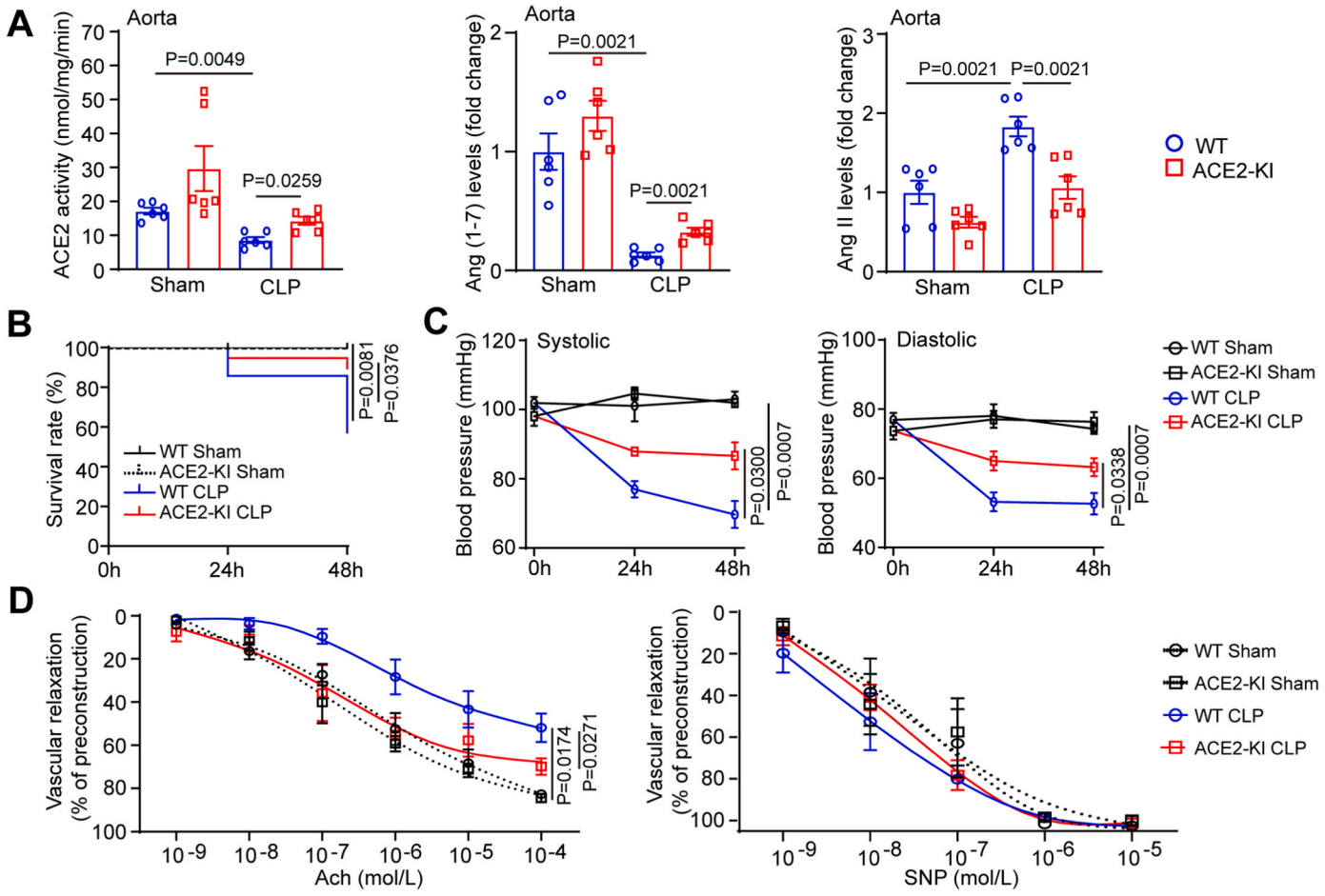

Fig. 2. Overexpression of ACE2 ameliorates sepsis-induced hypotension and vascular dysfunction
(A), WT and ACE2 knock-in (ACE2-KI) mice were subjected to CLP surgery. Measurement of aortic ACE2 activity and Ang (1–7) and Ang II levels in each group at 24 h (n = 6). **(B),** Survival rate of each group after CLP surgery (n = 15). **(C),** Detection of SBP and DBP in each group by the noninvasive tail-cuff method (n = 5). **(D),** Concentration-dependent vasodilation of mesenteric artery rings, including endothelium-dependent (Ach 10^{-9}-10^{-4} mol/L) and non-endothelium-dependent (SNP 10^{-9}-10^{-5} mol/L) (n = 6). The data are presented as the mean ± SEM, n represents the samples of each group. Data in (A) were analyzed by Mann-Whitney test; data in (B) were analyzed by Kaplan-Meier; data in (C) were analyzed with 2-way ANOVA followed by Tukey multiple comparison test; data in (D) were analyzed by repeated-measures ANOVA.

WT controls (Fig. 3A). Moreover, ACE2-KO mice displayed a lower survival rate than WT controls 48 h after CLP surgery (Fig. 3B). CLP-induced decreases in SBP and DBP in WT controls were markedly accelerated in ACE2-KO mice at 24 and 48 h (Fig. 3C). Consistently, the CLP-induced reduction in ACh-mediated vasodilatation in the mesenteric artery of WT mice was further augmented in ACE2-KO mice, while the SNP-mediated response was not influenced in ACE2-KO mice after CLP surgery (Fig. 3D). Thus, these results indicate that ACE2-KO aggravates sepsis-induced vascular dysfunction and hypotension.

3.4. Bone marrow-derived cells expressing ACE2 improve CLP-induced hypotension and vascular dysfunction

ACE2 expression is broadly detected in different tissues and cell types, such as lung, heart, vessels, cardiomyocytes and macrophages [28]. We then examined the expression of ACE2 on BM-derived macrophages. Immunostaining revealed that ACE2 colocalized with F4/80^{+} macrophages and was induced by CLP (Fig. 4A). Moreover, ACE2 was highly expressed on BM-derived macrophages from ACE2-KI animal compared with those from WT controls (Fig. 4B), suggesting that myeloid ACE2 may be important for sepsis-mediated vascular dysfunction and hypotension. First, we generated chimeric mice by translating WT, ACE2-KI or ACE2-KO bone marrow (BM) cells into WT mice. After eight weeks, all chimeric animals were identified by PCR analysis in bone marrow-derived cells (BMDCs) (Suppl. Fig. S3), and then subjected to CLP surgery (Fig. 4C). Consistent with the data from Figs. 2 and 3, CLP surgery after 24 h resulted in a significant decrease in aortic Ang (1–7) concentration and an increase in aortic Ang II level, leading to a reduction in both SBP and DBP in WT controls that received BM cells from WT controls. These actions were further accelerated in WT animals reconstituted with BM cells from ACE2-KO animals but were significantly ameliorated in WT controls that received BM cells from ACE2-KI animals (Fig. 4D and E). Consistently, CLP-induced impairment of ACh-dependent vasodilatation was also enhanced in WT controls that received BM cells from ACE2-KO animals. Conversely, this effect was markedly improved in WT controls that received BM cells from ACE2-KI animals (Fig. 4F). Collectively, our findings confirm that ACE2 on myeloid cells mainly protects against sepsis-induced vascular dysfunction and hypotension.

3.5. ACE2 inhibits CLP-induced oxidative stress, NO production and macrophage polarization in vivo

Due to inflammation, ROS and NO are the main regulators of impaired blood pressure and vascular function [29,30], we examined the effects of ACE2 overexpression on these regulators by multiple assays. Our data indicated that aortic ROS levels (DHE staining), NOX2 mRNA levels (qPCR), MDA contents (ELISA), and serum nitrite levels (a

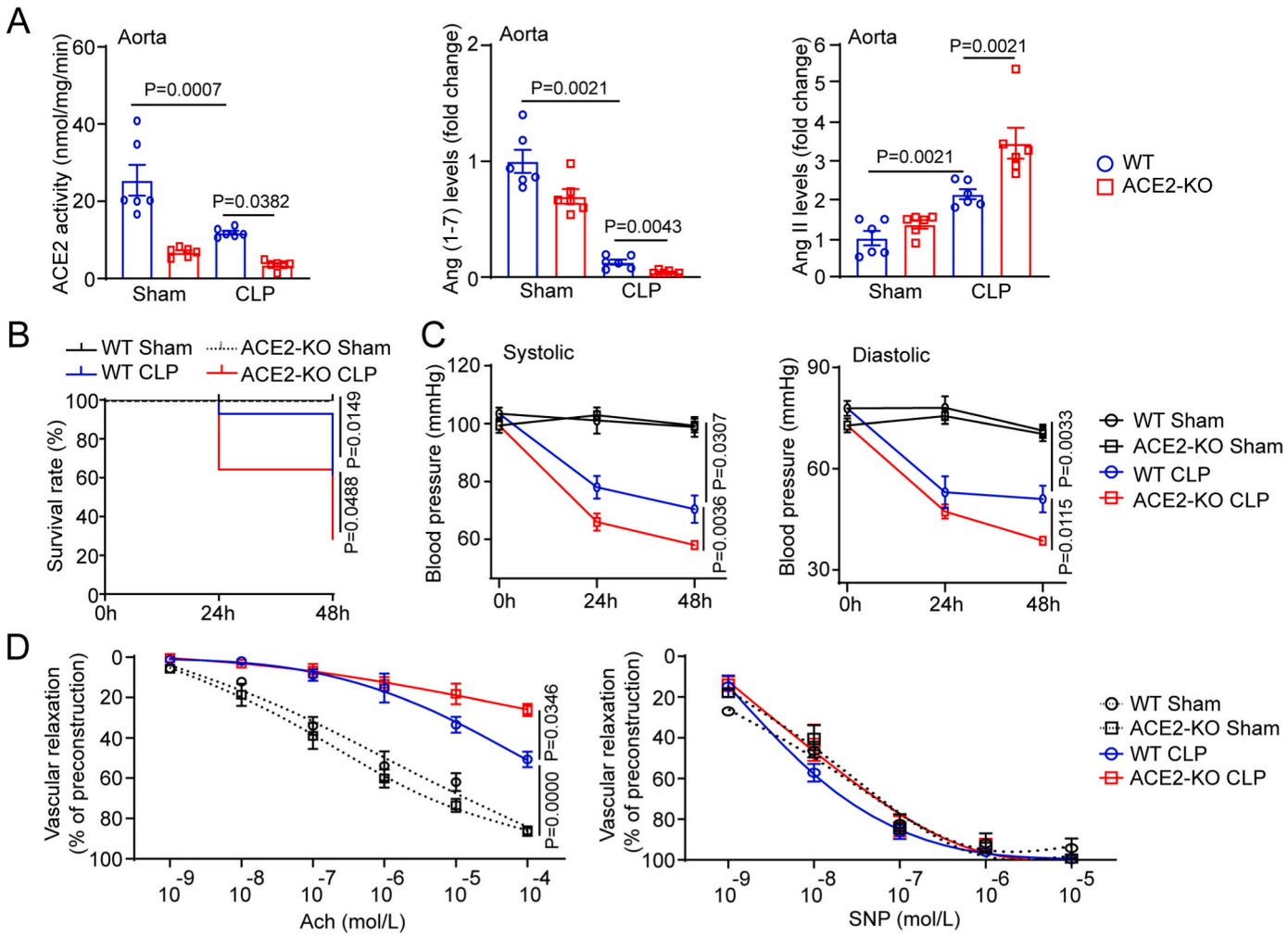

Fig. 3. Deficiency of ACE2 aggravates sepsis-induced hypotension and vascular dysfunction
(A), WT and ACE2 knockout (ACE2-KO) mice were subjected to CLP surgery for 24 h. Analysis of aortic ACE2 activity and Ang (1–7) and Ang II levels in each group (n = 6). **(B),** Survival rate of each group (n = 15). **(C),** Measurement of SBP and DBP in each group by the noninvasive tail-cuff method (n = 5). **(D),** Concentration-dependent vasodilation of mesenteric artery rings, including endothelium-dependent (Ach 10^{-9}-10^{-4} mol/L) and non-endothelium-dependent (SNP 10^{-9}-10^{-5} mol/L) (n = 6). The data are presented as the mean ± SEM, n represents the samples of each group. Data in (A) were analyzed by Mann-Whitney test; data in (B) were analyzed by Kaplan-Meier; data in (C) were analyzed with 2-way ANOVA followed by Tukey multiple comparison test; data in (D) were analyzed by repeated-measures ANOVA.

marker of NO production) as well as 3-NT protein levels (a marker for peroxynitrite) [31] and 3-NT immunoreactive intensities (immunohistochemical staining) were all markedly increased in the aorta of WT controls following CLP surgery, whereas these upregulations were dramatically abrogated in the aorta of ACE2-KI mice (Fig. 5A–F). Meanwhile, the aortic abundance of Mac-2^{+} macrophages (immunostaining) and the mRNA levels of iNOS in peripheral blood leukocytes (PBLs) were also lower in ACE2-KI mice than in WT mice (Fig. 5G and H). Macrophage polarization is a hallmark of sepsis and multiorgan failure [32–34]. We then investigated whether ACE2 is involved in this process in the aorta. qPCR analysis indicated that ACE2-KI mice showed significantly lower mRNA levels of M1-related proinflammatory gene markers (IL-1β, IL-6, TNF-α and IFN-γ) and higher mRNA expression levels of M2-related anti-inflammatory cytokines (Arg-1 and YM-1) than WT mice after CLP surgery (Fig. 5I), suggesting that ACE2 overexpression suppresses macrophage polarization toward the M1 phenotype in the aorta following sepsis.

We next verified the effect of ACE2 on aortic oxidative stress, inflammation and iNOS-NO signaling in ACE2-KO mice. Twenty-four hours after CLP surgery, ACE2-KO mice displayed a marked increase in aortic ROS levels (DHE staining), NOX2 mRNA levels, MDA contents, serum nitrite levels, 3-NT protein levels, and 3-NT immunoreactive intensities compared with WT controls after CLP surgery (Suppl. Figs. S4A–F). Accordingly, the increased percentages of Mac-2^{+} macrophages and iNOS mRNA levels as well as the expression levels of IL-1β, IL-6, TNF-α, and IFN-γ mRNAs and decreased expression levels of Arg1 and YM-1 mRNAs in WT mice were dramatically enhanced in ACE2-KO mice after CLP surgery (Suppl. Figs. S4G–I), indicating that ACE2 deficiency aggravates sepsis-induced effects on the vasculature.

To further demonstrate the protective effect of myeloid ACE2 on vascular function and blood pressure, we performed bone marrow (BM) transplantation. Consistently, CLP-induced upregulation in the ROS levels (Fig. 6A), the number of Mac-2-positive macrophages (Fig. 6B), nitrite contents (Fig. 6C) and the expression levels of NOX2, IL-1β, IL-6, TNF-α, and IFN-γ mRNAs and downregulation in the expression levels of Arg1 and YM1 mRNAs (Fig. 6D and E) were remarkably accelerated in WT controls that received BM cells from ACE2-KO animals but were dramatically reversed in WT controls that received BM cells from ACE2-KI animals compared with WT mice that received BM cells from WT mice (Fig. 6A–E). Together, increased ACE2 expression on myeloid cells suppresses vascular oxidative stress, nitrite production and macrophage polarization induced by sepsis.

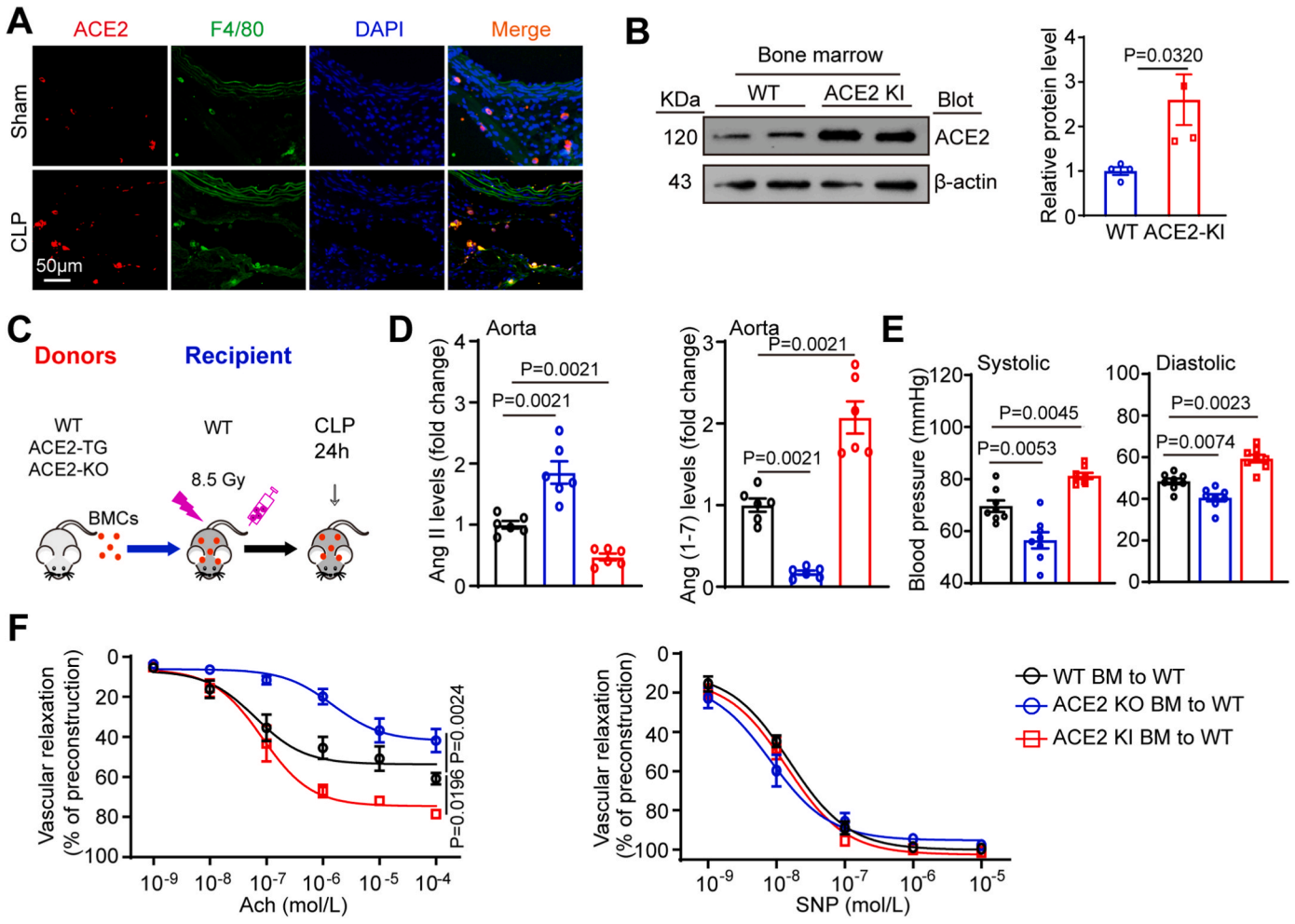

Fig. 4. Bone marrow-derived cells expressing ACE2 improves CLP-caused hypotension and vascular dysfunction
(A), Immunostaining of aorta sections with *anti*-F4/80 and anti-ACE2 antibodies to examine the localization of ACE2 in macrophages (n = 4). Scale bar, 50 μm. **(B)**, Immunoblotting analysis of ACE2 protein in the bone marrow cells of ACE2-KI mice. β-Actin was used as an internal control (n = 4). **(C)**, An experimental protocol showing WT, ACE2-KI and ACE2-KO bone marrow transplantation to irradiated WT mice. **(D)**, The aortic Ang II level in BMT mice after CLP (left, n = 6) and the aortic Ang (1–7) level in BMT mice after CLP (n = 6) **(E)**, Systolic blood pressure and diastolic blood pressure was measured via the noninvasive tail-cuff method in BMT mice after CLP (n = 8). **(F)**, Concentration-dependent vasodilation of aorta ring, including endothelium-dependent (Ach 10^{-9}-10^{-4} mol/L) and non-endothelium-dependent (SNP 10^{-9}-10^{-5} mol/L) (n = 6). The data are presented as the mean ± SEM, n represents the samples of each group. Data in (B) were analyzed by *t*-test; Data in (D and E) were analyzed by Mann-Whitney test; data in (F) were analyzed by repeated-measures ANOVA.

3.6. ACE2 attenuates CLP-induced macrophage polarization through an Ang (1–7)-MasR-dependent mechanism

To elucidate the potential mechanism by which ACE2 regulates macrophage polarization through Ang (1–7)-MasR signaling, BMDMs were obtained from WT or ACE2-KI mice. WT or ACE2-KI BMDMs were treated with PBS or LPS in the presence or absence of the MasR inhibitor A779 *in vitro*. After 24 h, LPS treatment markedly upregulated ROS production and the expression levels of IL-1β, IL-6 and TNF-α mRNAs in WT BMDMs but reduced the expression levels of Arg1 and YM-1 mRNAs compared to PBS treatment, whereas these changes were dramatically attenuated in ACE2-KI BMDMs (Fig. 7A and B). In contrast, A779 treatment significantly reversed ACE2-KI-mediated preventive effects against LPS stimulation (Fig. 7A and B). To determine which signals mediate the effect of ACE2 on macrophage polarization, we examined NF-κB and STAT1, two key modulators of M1 macrophage polarization. Immunoblotting analysis indicated that LPS-upregulated levels of both phosphorylated (p)-P65 and p-STAT1 proteins in WT BMDMs were markedly inhibited in ACE2-KI BMDMs; however, A779 treatment restored the expression levels of both p-p65 and p-STAT1 proteins in ACE2-KI BMDMs following LPS stimulation (Fig. 7C).

Next, we evaluated whether ACE2 overexpression protects endothelial cells from LPS stress by using co-culture of BMDMs and HUVECs. WT or ACE2-KI BMDMs were seeded in the upper chamber and HUVECs were cultured in the lower chamber (Fig. 7D). LPS exposure induced higher levels of E-selectin, ICAM-1, and VCAM-1 mRNA as well as total nitrites in HUVECs co-cultured with WT BMDMs, which were significantly attenuated in HUVECs co-cultured with ACE2-KI BMDMs (Fig. 7E and F). Likewise, LPS-induced increase in ROS generation and the mRNA levels of NOX1 and NOX4 in HUVECs co-cultured with WT BMDMs were also inhibited in HUVECs co-cultured with ACE2-KI BMDMs (Fig. 7G and H). Conversely, A779 treatment significantly reversed ACE2-KI-dependent beneficial actions against LPS stimulation (Fig. 7E–H). Collectively, these data indicate that ACE2 overexpression reduces LPS-stimulated macrophage M1 polarization and subsequent endothelial injury, possibly via MasR–NF–κB and STAT1 signal pathways.

4. Discussion

The current study identified an unknown role of myeloid ACE2 in improving sepsis-induced hypotension and impairment of vascular function. Mechanistically, ACE2 promotes Ang (1–7) production, which

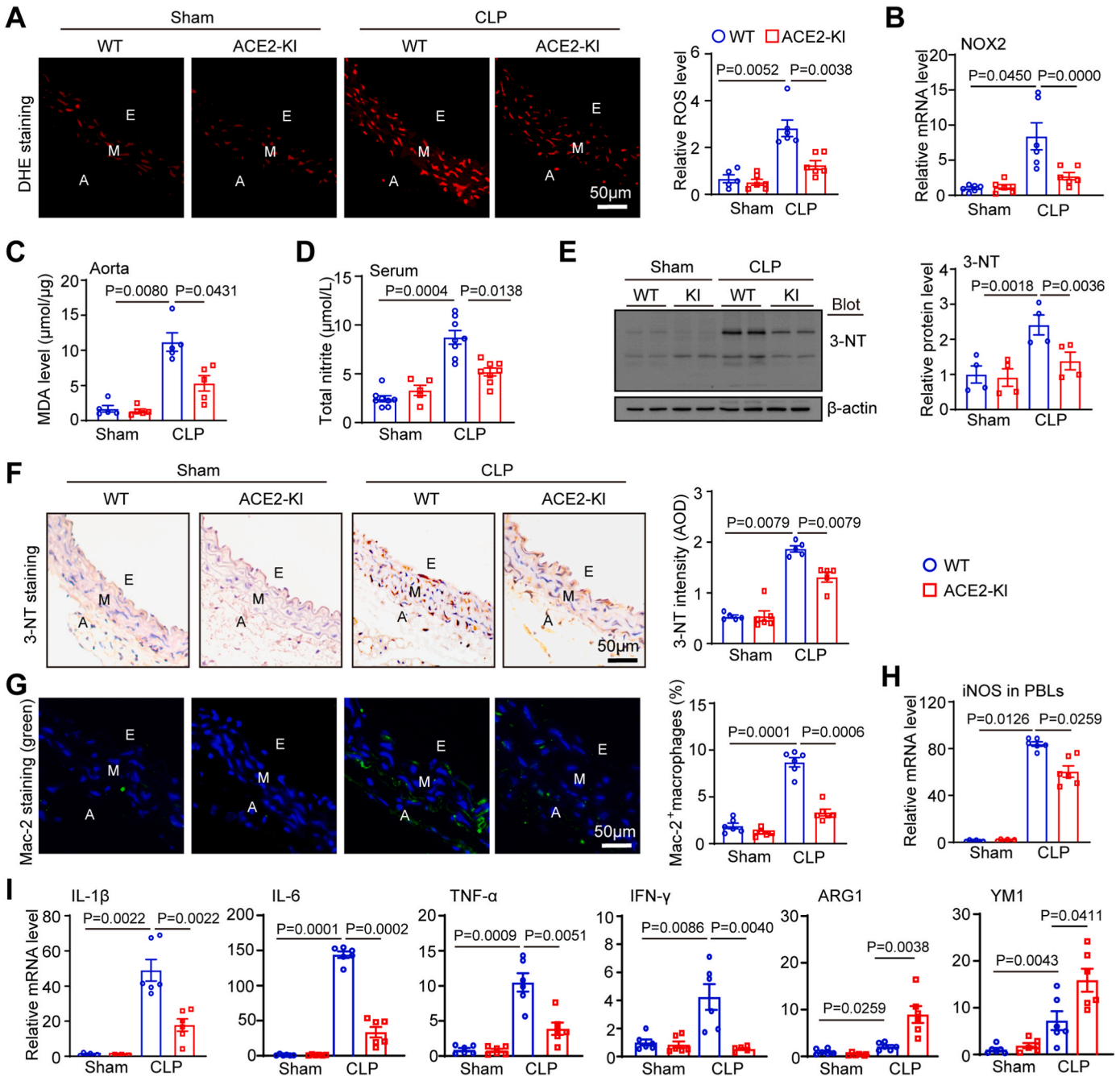

Fig. 5. ACE2 inhibits CLP-induced oxidative stress, NO production and macrophage polarization *in vivo*
(A), WT and ACE2 knockin (ACE2-KI) mice were subjected to CLP surgery for 24 h. Dihydroethidium (DHE) staining of the aortic segments (left, scale bar, 50 μm) and quantification (right, n = 6). **(B),** qPCR analysis of NOX2 mRNA expression level in the aorta (n = 6). **(C),** The MDA level in aorta (n = 6). **(D),** Analysis of serum total nitrite levels (n = 5–8). **(E),** Immunoblotting analysis of 3-NT protein in the aorta. β-Actin was used as an internal control (n = 4). **(F),** Immunohistochemical staining of 3-nitrotyrosine (NT) in the aorta (left, scale bar, 50 μm) and quantification of 3-NT intensity (right, n = 5). **(G),** Immunostaining of aortic segments with anti-Mac-2 antibody (left, scale bar, 50 μm) and quantification of Mac-2-positive macrophages (right, n = 6). **(H),** qPCR analysis of iNOS mRNA expression level in the PBLs (n = 6). **(I),** qPCR analysis of the mRNA levels of IL-1β, IL-6, TNF-α and IFN-γ and ARG1 and YM1 in the aorta (n = 6). The data are presented as the mean ± SEM, n represents the samples of each group. Data in (A) through (C) and (F) and (H) and (I) were analyzed by Mann-Whitney test; data in (D) and (E) and (G) were analyzed with 2-way ANOVA followed by Tukey multiple comparison test.

activates MasR and inhibits NF-κB and STAT1 signals, reducing M1 macrophage polarization and the production of proinflammatory cytokines, ROS, and nitrite, thereby improving endothelial dysfunction and hypotension after sepsis. Consequently, our data highlight that selective stimulation of myeloid ACE2 activity potentially represents a promising therapy for the amelioration of sepsis-related vascular dysfunction. A working model is shown in Fig. 8.

ACE2 can efficiently cleave the C-terminal phenylalanine residue of Ang II to generate Ang (1–7). ACE2, through Ang-(1–7)-MasR signaling, opposes the ACE-Ang II-AT1R axis via its anti-inflammatory and antioxidant activities [35,36]. ACE2 is widely detected in the heart, lung, kidney, brain, vasculature, adipocytes, cardiomyocytes and macrophages [28], although there is some difference between mRNA and protein levels or ACE2 activity [37]. However, ACE2 was barely detected in the endothelial cells and pericytes of intramyocardial microvessels and was expressed at low levels in normal hearts. Interestingly, ACE2

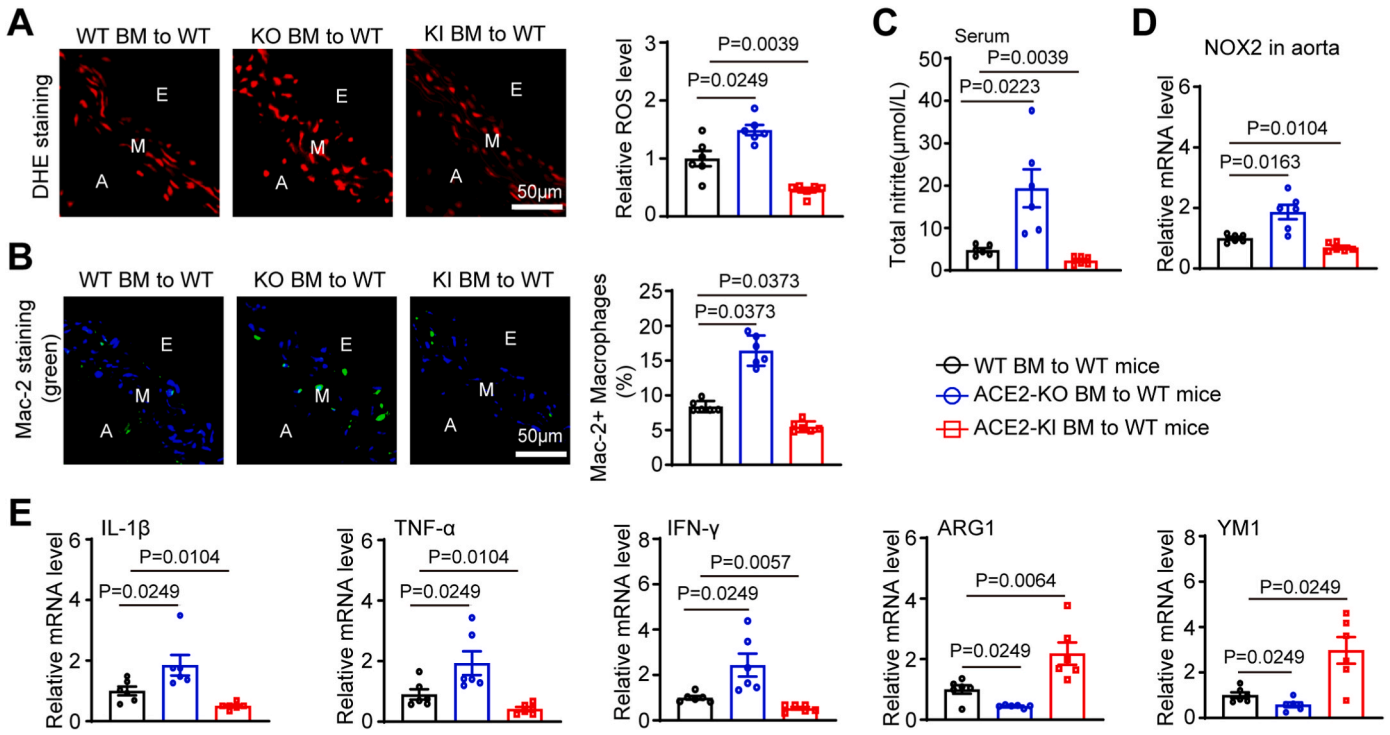

Fig. 6. ACE2 expression on myeloid cells suppresses vascular oxidative stress, NO production and macrophage polarization
(A), WT, ACE2-KI and ACE2-KO BM cell were transplantation to irradiated WT mice and subjected to CLP for 24 h. DHE staining of the aortic segments (left, scale bar, 50 μm) and quantification (right, n = 6). **(B),** Immunostaining of aortic segments with anti-Mac-2 antibody (left, scale bar, 50 μm) and quantification of Mac-2-positive macrophages (right, n = 6). **(C),** Analysis of serum total nitrite levels (n = 8). **(D),** qPCR analysis of NOX2 mRNA expression level in the aorta (n = 6). **(E),** qPCR analysis of the mRNA levels of IL-1β, IL-6, TNF-α and IFN-γ and ARG1 and YM1 in the aorta (n = 6). The data are presented as the mean ± SEM, n represents the samples of each group. Data in (A) and (B) were analyzed with 2-way ANOVA followed by Tukey multiple comparison test; data in (C) through (E) were analyzed by Mann-Whitney test.

was upregulated in dilated cardiomyopathy but was decreased in non-COVID-19 myocarditis and COVID-19 hearts [38]. Recently, a study showed that LPS treatment causes a marked reduction in ACE2, Ang-(1–7), and MasR expression in mouse hearts and primary cardiomyocytes [10]. Consistent with these findings, our current results also showed a disturbance of the RAAS, as evidenced by an increase in the levels of ACE, Ang II and AT1R and a decrease in the levels of ACE2, Ang (1–7) and MasR in the aorta of mice following 24 h of CLP challenge (Fig. 1). Thus, these results indicate a critical role of ACE2-Ang (1–7)-MasR axis in sepsis-induced hypotension.

It is well known that ACE2 plays a protective role in a variety of cardiovascular diseases, which are partly due to the ACE2-mediated hydrolysis of Ang II and production of Ang (1–7) [35,39]. Conversely, these effects are markedly alleviated in ACE2-KO mice administered Ang (1–7) [40]. Importantly, ACE2 is mostly bound to cell membranes as a cellular form of ACE2 (cACE2). However, upon various stresses, such as virus infection, the proteases such as metalloproteinase 17 (ADAM17) can cleave cACE2 to release sACE2 into the plasma, leading to upregulation of sACE2 levels in the circulation [41]. Beyond its cardiovascular protective role, studies have identified that sACE2 is cleaved from the host cell surface as a receptor to crucially mediate virus entry and the pathophysiology of SARS-CoV-2 [42,43]. A case-cohort analysis indicated that upregulated plasma ACE2 levels are strongly related to an enhanced risk of total deaths and major cardiovascular events [44]. Moreover, the sACE2 level was increased in patients with SARS-CoV-2 infection and was independently related to mortality and disease severity [45]. In addition, COVID-19 patients with acute respiratory distress syndrome (ARDS) had higher serum sACE2 and lower eNOS than COVID-19 patients without ARDS [46]. These data suggest that circulating sACE2 might present a predictive marker of complications of COVID-19, and targeting the RAAS to increase cACE2-Ang (1–7) may be a new therapeutic option for treating COVID-19 patients. Indeed, some experimental studies indicate that blocking RAAS with ACE inhibitors, AT1R antagonists, and statins can upregulate cACE2 expression and/or activity, contributing to the benefit of cardiovascular disease [47]. Recent studies have demonstrated that statin administration is a promising therapy for COVID-19. The use of statins is associated with decreased mortality in COVID-19 patients, which may be due to direct or indirect mechanisms, including blocking SARS-CoV-2 entry and replication, inhibiting Toll-like receptor 4 and macrophage IL-6 production, and attenuating COVID-19-mediated endothelial dysfunction and tissue fibrosis [48,49]. Moreover, a study suggested that exogenous supplementation with Ang (1–7) greatly ameliorates LPS-stimulated cardiac inflammation, apoptosis and dysfunction in mice [10]. More recently, administration of recombinant human ACE2 (rhACE2) to mice greatly attenuates LPS-stimulated activation of the NLRP3 inflammasome, NF-κB and p38 MAPK signals, inflammation, and increased Ang II and decreased Ang (1–7), which then protected against LPS-stimulated cardiac pyroptosis and dysfunction [50]. However, the significance of ACE2 in sepsis-induced hypotension and vascular dysfunction remains unclear. Here, our results indicated that ACE2 overexpression improved CLP-induced hypotension, oxidative stress, inflammation and arterial dysfunction by activating Ang (1–7)-MasR signaling (Figs. 2 and 5), and these preventive effects were confirmed in ACE2-KI BM transplanted mice and *in vitro* cultured macrophage experiments (Figs. 4–6). However, ablation of ACE2 prevented these pathophysiologic improvements (Fig. 3 and Suppl. Fig. S2). Therefore, our results illustrate that ACE2 on myeloid cells exerts a protective role in hypotension and vascular dysfunction.

It is well reported that immune response dysfunction is a core mechanism in the pathogenesis of sepsis. Pharmacological inhibition of hyperinflammation and balancing immune function are beneficial

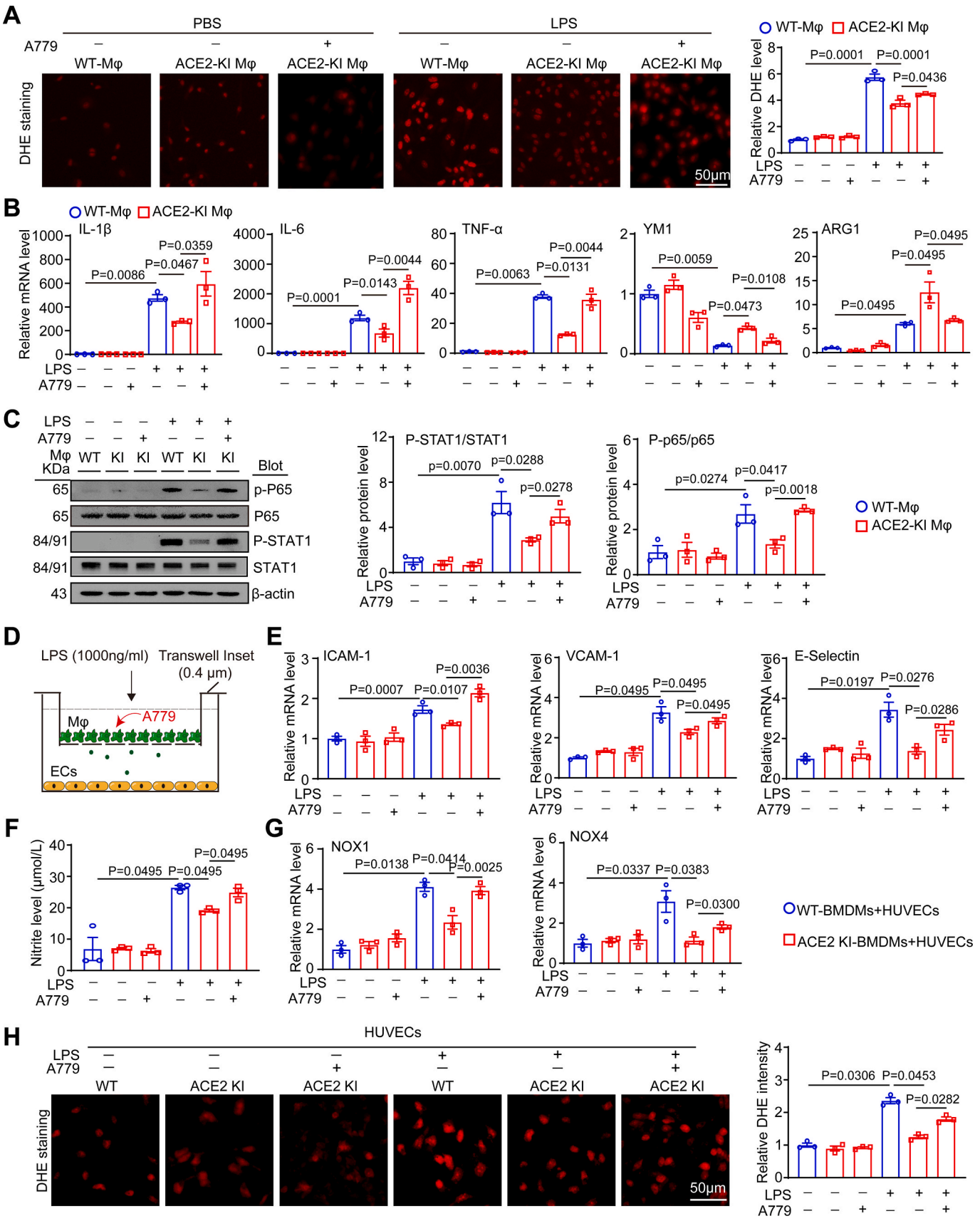

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Fig. 7. ACE2 attenuates CLP-induced macrophage polarization and endothelial cell injury through Ang (1–7)-MasR-dependent mechanism
(A), BMDMs isolated from WT and ACE2-KI mice were stimulated with LPS (1000 ng/ml) for 24 h. DHE staining of ROS production in BMDMs (left), and the quantification of DHE fluorescence intensity in each group (right, n = 3). Scale bar, 50 μm. **(B),** qPCR analysis of IL-1β, IL-6, TNF-α, ARG1 and YM1 mRNA levels in BMDMs (n = 3). **(C),** Immunoblotting analysis of phosphorylated (P)-p65, p65, P-STAT1 and STAT1 proteins in BMDMs (left), and the quantification of protein intensity in each group (n = 3). β-Actin was used as an internal control. **(D),** BMDMs isolated from WT and ACE2-KI mice were pretreated with or without MasR inhibitor A779 (10 μM) for 30 min and then stimulated with LPS (1000 ng/ml) or saline for additional 6 h and placed in transwell inserts above HUVECs for 24 h. **(E),** qPCR analysis of E-selectin, ICAM-1 and VCAM-1 mRNA expression levels of HUVECs co-cultured with WT or ACE2-KI BMDMs (n = 3). **(F),** The detection of total nitrite level in media supernatant released by HUVECs (n = 3). **(G),** qPCR analysis of NOX1 and NOX4 mRNA expression levels of HUVECs co-cultured with WT or ACE2-KI BMDMs (n = 3). **(H),** DHE staining of the HUVECs co-cultured with WT or ACE2-KI BMDMs (left) and quantification of DHE fluorescence intensity (right, n = 3). Scale bar, 50 μm. The data are presented as the mean ± SEM, n represents the number of each group. Data in (A) were analyzed with 2-way ANOVA followed by Tukey multiple comparison test; data in (B) and (C) were analyzed by Mann-Whitney test; data in (E) and (G) and (H) were analyzed with 2-way ANOVA followed by Tukey multiple comparison test; data in (F) were analyzed by Mann-Whitney test.

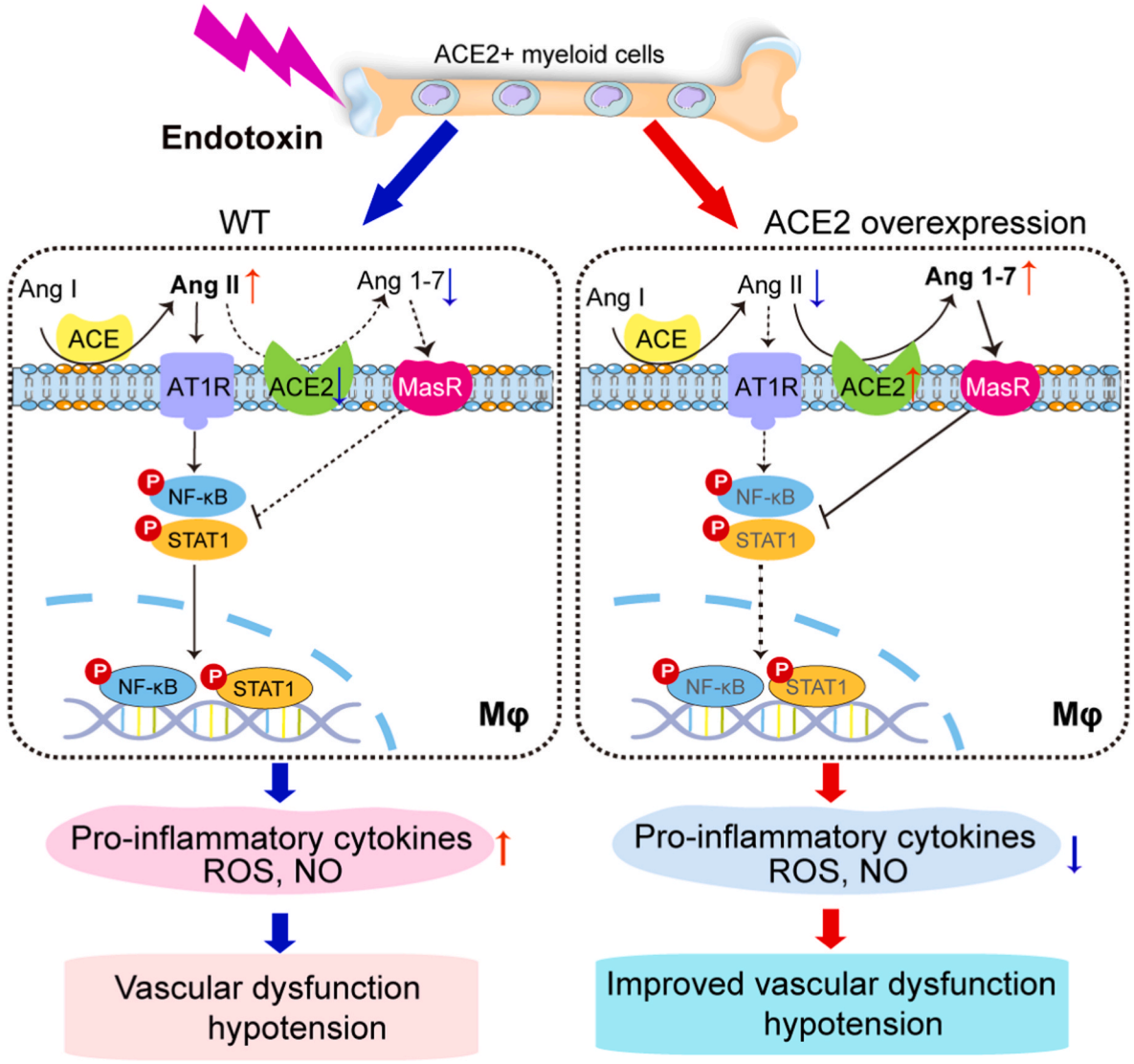

Fig. 8. Analysis of the RAAS components in plasma of patients with sepsis and hypotension
Schematic model for ACE2 in sepsis-induced hypotension and vascular dysfunction. Sepsis reduces myeloid ACE2 expression, which blocks Ang II hydrolysis and Ang (1–7) production, resulting in inhibition of MasR and activation of NF-kB and STAT1 signals, this promotes macrophage M1 polarization and the generation of pro-inflammatory cytokines, ROS, and NO, thereby leading to endothelial dysfunction and hypotension in wild-type mice. In contrast, ACE2 overexpression in mice ameliorates sepsis-induced effects. These data highlight that selective activation of ACE2 may represent promising therapeutic option for sepsis and its related complications.

strategies for therapeutic interventions in patients with sepsis at the early stage [51,52]. Innate immune cells, particularly macrophages, are crucial for host immune balance during sepsis. Macrophages are commonly grouped into two major phenotypes: M1 and M2 macrophages [53]. In the initial sepsis, proinflammatory factors such as IFN-γ and LPS can dramatically induce M1-like macrophage polarization that continues to release extensive proinflammatory cytokines and ROS. However, M2-like macrophages are activated and release abundant anti-inflammatory cytokines in the late stage of sepsis [54,55]. Multiple signaling pathways are involved in this process. LPS and IFN-γ can

activate transcription factors such as NF-κB and STAT1 through binding to TLR4 or IFN-γ receptors, respectively, thereby inducing macrophage M1 polarization. IL-4 and fatty acids promote the activation of STAT6, IRF4, and CREB by binding to their receptors, leading macrophages toward the M2 phenotype [32,33]. Interestingly, both ACE2 and MasR are primarily expressed on macrophages and are markedly reduced upon LPS stimulation *in vitro*. Activation of the Ang-(1–7)-MasR cascade can shift macrophages toward the M2 phenotype by inhibiting MAPK and TLR4-IKK–NF–κB signals in sepsis [34,56]. Moreover, administration of an ACE2 activator reduces the inflammatory effect in retinal pigment epithelium cells by blocking the MAPK–NF–κB pathway following LPS stimulation [57]. In contrast, depletion of ACE2 can cause a cytokines storm such as inflammation in various diseases [58,59]. However, whether ACE2 regulates macrophage polarization through Ang (1–7)-MasR signaling in the aorta during sepsis remains unknown. Here, our data indicated that overexpression of ACE2 dramatically enhanced Ang (1–7) levels, reduced the release of M1-like proinflammatory factors and production of ROS and NO, and upregulated the levels of M2-like anti-inflammatory cytokines (Fig. 5). Conversely, knockout of ACE2 had the opposite effects (Suppl. Fig. S2). These effects were confirmed in ACE2-KI or KO BM-transplanted mice (Fig. 6). Furthermore, *in vitro* culture experiments elucidated the mechanism by which ACE2 reduces macrophages toward the M1 phenotype through Ang (1–7)-MasR-mediated inactivation of the NF-κB and STAT1 pathways (Fig. 7A–C). Moreover, ACE2 expression in macrophages is also important in regulating endothelial cell function during the inflammatory response. The S spike protein is able to induce polarization of THP-1 like macrophages towards the M1 phenotype, which can release large amount of proinflammatory cytokines and ROS, leading to impairment of endothelial function, whereas administration of ACE inhibitors effectively reversed these detrimental effects [60]. Conversely, ACE2 knockout in BMDMs increases the levels of Ang II and proinflammatory cytokines, and promotes monocyte adhesion to endothelial cells, thereby causing endothelial damage. However, these effects were blocked by Ang-(1–7) treatment [61]. Consistently, our data further confirmed that ACE2 overexpression on macrophages also reduced LPS-stimulated macrophage M1 polarization and endothelial dysfunction through Ang(1–7)-MasR–NF–κB and STAT1 signal pathways (Fig. 7). Taken together, these findings suggest that ACE2 is critical for macrophage polarization and subsequent hypotension and vascular injury following sepsis.

Severe sepsis is characterized by peripheral vasodilation, hypotension accompanied by multiorgan failure and high mortality [62]; however, the mechanisms are not fully clarified. It is reported that sepsis/LPS can upregulate iNOS expression and stimulate tissues to produce NO, which critically mediates the sepsis-induced vascular hyporeactivity response to Ang II, and this effect is significantly reversed by iNOS inhibitors in septic mice, implying that NO is a key mechanism for septic hypotension [63–65]. The RAAS plays central roles in vascular and blood pressure homeostasis. Ang II and NO have antagonistic effects on vascular function [62]. Aberrant activation of the RAAS has been observed in severe sepsis, and the roles of Ang II in the regulation of septic hypotension are contradictory. Several clinical studies suggest that Ang II could be a rescue vasopressor that effectively enhances blood pressure in patients with vasodilatory shock and distributive shock [66–68]. However, other studies indicate that the use of Ang II is not able to restore sepsis-related hypotension, but is associated with organ damage and high mortality in septic patients [69]. Interestingly, sepsis reduces AT1R expression in rats and mice [63,65], and sepsis-related production of proinflammatory cytokines and NO can influence AT1R expression or Ang II binding activity [70,71]. The combination of proinflammatory cytokines (IL-1β, TNF-a and IFN-γ) markedly reduces Ang II binding to AT1R in rat renal mesangial cells [65]. Thus, increased proinflammatory cytokines and NO levels as well as reduced AT1R expression/function may account for the diminished effect of Ang II on sepsis-induced hypotension.

4.1. Limitations

We did not elucidate the potential mechanism by which ACE2 expression in the aorta was reduced after 24 h of sepsis. Moreover, the translational significance of septic mice to human patients with sepsis is limited, the sample size is small, and female mice were not examined in this study. We detected ROS levels through DHE staining, which is relatively rough and did not use a more accurate method for testing like HPLC method. The quantifications of Ang (1–7) and Ang II levels in animals by the ELISA kits, Liquid Chromatography (HPLC) or Liquid Chromatograph Mass Spectrometer (LC-MS) have huge variations as reported previously [72–74]. Therefore, we present relative values to indicate the changes of Ang (1–7) and Ang II levels. In addition, several studies indicate that commercial anti-AT1R and MasR antibodies exhibit some nonspecific binding [75–77]. Our results are consistent with reports, and we provide the full blot images of these proteins with weight markers in Suppl. Fig. S5. Finally, the myeloid-specific effect of ACE2 is unclear in other organs, such as the heart.

5. Conclusion

Our study determined that ACE2 expression and Ang (1–7) contents were significantly decreased in CLP-treated mice. ACE2 overexpression highly ameliorated sepsis-induced vascular dysfunction and hypotension potentially by inhibiting oxidative stress, NO production and macrophage M1 polarization via Ang (1–7)-MasR signaling. Thus, our findings demonstrate a protective effect of myeloid ACE2 on sepsis-related hypotension and vascular dysfunction, and suggest that selective upregulation of ACE2 expression may represent a new therapeutic option for treating patients with sepsis and its related complications.

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Availability of data and materials

Data will be made available on request.

CRediT authorship contribution statement

Jia-Xin Li: Writing – review & editing, Investigation, Formal analysis, Data curation. **Xue Xiao:** Methodology, Investigation, Formal analysis, Data curation. **Fei Teng:** Methodology, Formal analysis. **Hui-Hua Li:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have declared that they have no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgments

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2023.103004.

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