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Loss of Nr4a1 ameliorates endothelial cell injury and vascular leakage in lung transplantation from circulatory-death donor

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KEYWORDS:
lung transplantation;
ischemia-reperfusion injury;
donation after circulatory death;
nuclear receptor subfamily 4 group A member 1;
endothelial cell

BACKGROUND: Ischemia-reperfusion injury (IRI) stands as a major trigger for primary graft dysfunction (PGD) in lung transplantation (LTx). Especially in LTx from donation after cardiac death (DCD), effective control of IRI following warm ischemia (WIRI) is crucial to prevent PGD. This study aimed to identify the key factors affecting WIRI in LTx from DCD.

METHODS: Previously reported RNA-sequencing dataset of lung WIRI was reanalyzed to identify nuclear receptor subfamily 4 group A member 1 (NR4A1) as the immediate early gene for WIRI. Dynamics of NR4A1 expression were verified using a mouse hilar clamp model. To investigate the role of NR4A1 in WIRI, a mouse model of LTx from DCD was established using *Nr4a1* knockout ($Nr4a1^{-/-}$) mice.

RESULTS: NR4A1 was located around vascular cells, and its protein levels in the lungs increased rapidly and transiently during WIRI. LTx from $Nr4a1^{-/-}$ donors significantly improved pulmonary graft function compared to wild-type donors. Histological analysis showed decreased microvascular endothelial cell death, neutrophil infiltration, and albumin leakage. Evans blue permeability assay demonstrated maintained pulmonary microvascular barrier integrity in grafts from $Nr4a1^{-/-}$ donors, correlating with diminished pulmonary edema. However, NR4A1 did not significantly affect the inflammatory response during WIRI, and IRI was not suppressed when a wild-type donor lung was transplanted into the $Nr4a1^{-/-}$ recipient.

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CONCLUSIONS: Donor NR4A1 plays a specialized role in the positive regulation of endothelial cell injury and microvascular hyperpermeability. These findings demonstrate the potential of targeting NR4A1 interventions to alleviate PGD and improve outcomes in LTx from DCD.
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Background

Lung transplantation (LTx) represents the last hope for individuals grappling with end-stage lung disease. Regrettably, LTx recipients encounter the most unfavorable outcomes among solid organ transplantations.[1] The primary reason for this challenge is primary graft dysfunction (PGD), a major cause of mortality and morbidity after LTx.[2-4] Furthermore, PGD is recognized as a risk factor for chronic lung allograft dysfunction, contributing to increased long-term mortality.[5-7] To achieve a better short- and long-term outcome of LTx, a deeper understanding of the mechanisms underlying PGD is necessary.

Ischemia-reperfusion injury (IRI) is widely acknowledged as a major trigger for PGD. Recently, LTx from donation after circulatory death (DCD) has been increasing to address the shortage of donors.[8] DCD lungs are inevitably exposed to warm ischemia, spanning from the agonal stage to organ preservation. Prolonged warm ischemia significantly influences the development of PGD and subsequent allograft rejection.[9-12] Therefore, it is crucial to elucidate the mechanism of warm ischemia-reperfusion injury (WIRI) for successful LTx from DCD. Although WIRI has been suggested to occur through a mechanism distinct from IRI following cold ischemia (CIRI),[13] most studies on IRI in LTx to date have primarily focused on CIRI, resulting in limited knowledge of WIRI. The aim of this study was to identify key factors contributing to WIRI. To this end, we initially performed RNA sequencing of WIRI lung tissues in mice, identifying candidate genes with altered expression in the early phases of WIRI. Among these candidates, we specifically focused on nuclear receptor subfamily 4 group A member 1 (NR4A1), also known as NUR77, TR3, NGFI-B, and NAC-1 in this study.

NR4A1, an orphan nuclear receptor lacking endogenous ligand, serves various roles as a transcription factor, coregulator, and kinase. Widely expressed in diverse tissues and cells, NR4A1 is involved in numerous physiological activities, including inflammation and apoptosis.[14-18] NR4A1 is required for the differentiation of classical monocytes into nonclassical monocytes.[19] Although NR4A1 is a nuclear receptor, cellular stress can induce its translocation to mitochondria, leading to cytochrome c release and apoptosis.[15] As an immediate early gene (IEG), NR4A1 promptly responds to diverse stimuli.[20-23] Notably, NR4A1 undergoes rapid induction in response to ischemia-reperfusion (IR) stress and contributes to IRI across the brain, heart, liver, and kidney.[24-30] While NR4A1 plays a pivotal role in lipopolysaccharide-induced lung injury,[31-33] scant information is available on its involvement in lung IRI.

In this study, we analyzed the expression pattern of NR4A1 in WIRI using a mouse model of LTx from DCD to investigate its role. We further examined the effects of NR4A1 in various donor-recipient pairs using knockout mice and assessed its potential as a therapeutic target in LTx from DCD. This study not only contributes to our understanding of the role of NR4A1 in WIRI but also lays the foundation for potential interventions to reduce PGD after LTx from DCD.

Material and methods

Animals

Wild-type (WT) C57BL/6 mice (Strain #: 000664) and *Nr4a1*-knockout (*Nr4a1*$^{-/-}$, Strain #: 006187) mice on the C57BL/6 background, aged 8 to 12 weeks, were sourced from Jackson Laboratory (Bar Harbor, ME). Adherence to the principles of laboratory animal care, as established by the National Society for Medical Research, and compliance with the guide for the care and use of laboratory animals (National Academy Press, 1996) was maintained throughout the study. The study protocol received approval from the relevant ethics committee and institutional review board (approval number OKU-2021891).

Lung hilar clamping

Left hilar clamping was performed as previously described[34] (see Supplementary Materials for details). WT mice underwent left lung ischemia for 60 minutes through the left hilar clamp, followed by 0, 0.5, 1, 2, 6, and 24 hours of reperfusion.

Murine lung transplantation

A cuff technique was utilized for orthotopic single left LTx in mice, as described previously[35,36] (see Supplementary Materials for details). The total warm ischemic time was adjusted to 2 hours, with an additional 1 hour of cold ischemic time required for the transplantation procedure. Recipient mice were sacrificed after 4 hours of reperfusion.

Quantitative real-time reverse transcription polymerase chain reaction

Total RNA extraction from lung tissue, cDNA reverse transcription, and real-time reverse transcription polymerase chain reaction (RT-PCR) were performed as previously described[34] (see Supplementary Materials for details), using primers for genes encoding NR4A1 and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Mm01300401_m1 and Mm99999915_g1, respectively).

Western blotting

Cryopreserved lung tissues were employed for Western blotting, as previously described[34] (see Supplementary Materials for details), using primary antibodies for NR4A1 (NB100-57645, Novus Biologicals) and beta-actin (MAB1501, Sigma-Aldrich).

Pulmonary graft function assessment

Arterial blood gases were measured to assess graft function, with oxygen delivered at a FiO_2 of 100% after clamping the right pulmonary hilum for 2 minutes, following established protocols[35] (see Supplementary Materials for details).

Immunohistochemistry, immunofluorescence, and terminal deoxynucleotidyl transferase dUTP nick end labeling assay

Immunohistochemistry was conducted using anti-NR4A1 antibody (#25851-1-AP, Proteintech), antilymphocyte antigen 6G (Ly-6G) antibody (#87048, Cell Signaling Technology), or antialbumin antibody (#A90-134A, Bethyl Laboratories). Immunofluorescence costaining was performed with anti-CD31 antibody (#ab7388, Abcam; DIA-310, dianova), along with a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (DeadEnd Fluorometric TUNEL System, Promega) or anti-NR4A1 antibody (NBP2-66980, Novus Biologicals), as previously described[34,37] (see Supplementary Materials for details).

Evans blue permeability assay

Pulmonary microvascular permeability was assessed through Evans Blue extravasation. Recipient mice were intravenously administered 0.5% Evans blue (20 mg/kg) 15 minutes after reperfusion. Lungs were excised after 4 hours, and Evans blue extravasation was quantified following established protocols[38] (see Supplementary Materials for details).

Quantification of cytokines and chemokines

Concentrations of cytokines and chemokines in lung and serum samples were measured using the Bio-Plex Pro Mouse cytokine 23-plex complete kit (Bio-Rad Laboratories), following the manufacturer's instructions.

Statistical analysis

The sample size for each experiment is stated in the figures, and data are presented as mean ± standard error of the mean (SEM). One-way Analysis of Variance (ANOVA) with the post hoc Šídák correction was used for group comparison. Statistical analysis was performed using GraphPad Prism software (GraphPad Software), with significance considered at a p-value of < 0.05.

Results

Transcriptome analysis identified NR4A1 as an early-response gene of lung WIRI

To identify differentially expressed genes during the early phases of WIRI in the lung, which act as master regulators of lung IRI, we reanalyzed a previously reported RNA-sequencing dataset (GSE203238) of lung tissues at time points 0, 30, 60, and 120 minutes after reperfusion, employing RNA sequencing (Figure 1A).[34] As depicted in the volcano plot, the number of upregulated genes substantially increased with time (Figure 1B). Notably, NR4A1 mRNA levels exhibited a significant upregulation at 30 minutes after reperfusion, with a further increase observed within 120 minutes when compared to the levels at time 0. To better understand the dynamics of NR4A1 in lung IRI, we assessed the expression level of NR4A1 in the lung up to 24 hours after reperfusion in mice hilar clamp model (Figure 1C). The mRNA levels of NR4A1 in the lung were semiquantified using real-time PCR and showed a transient upregulation as early as 0 to 1 hour after reperfusion (Figure 1D). The protein levels of NR4A1 in the lung were also semiquantified through Western blotting, revealing a peak 2 to 6 hours after reperfusion, slightly later than the peak of mRNA expression (Figure 1E).

Deletion of NR4A1 in the donor improved graft function after LTx from DCD

To investigate the functional role of NR4A1 in WIRI after LTx from DCD, we subjected WT and *Nr4a1*$^{-/-}$ mice to orthotopic LTx using the following donor → recipient combinations: WT → WT, *Nr4a1*$^{-/-}$ → WT, WT → *Nr4a1*$^{-/-}$, and *Nr4a1*$^{-/-}$ → *Nr4a1*$^{-/-}$ (Figure 2A). The donor lungs underwent 2 hours of warm ischemia followed by 1-hour cold ischemia and were subsequently subjected to 4 hours of reperfusion after LTx. Graft lung function during IRI after LTx was assessed through arterial blood gas analysis after a 2-minute right hilar clamping, conducted under a FiO_2 of 1.0 (Figure 2B). As indicated by PaO_2 levels, the *Nr4a1*$^{-/-}$ donor group (comprising both *Nr4a1*$^{-/-}$ → WT and *Nr4a1*$^{-/-}$ → *Nr4a1*$^{-/-}$) exhibited significantly improved graft function in comparison to the WT donor group (WT → WT and WT → *Nr4a1*$^{-/-}$).

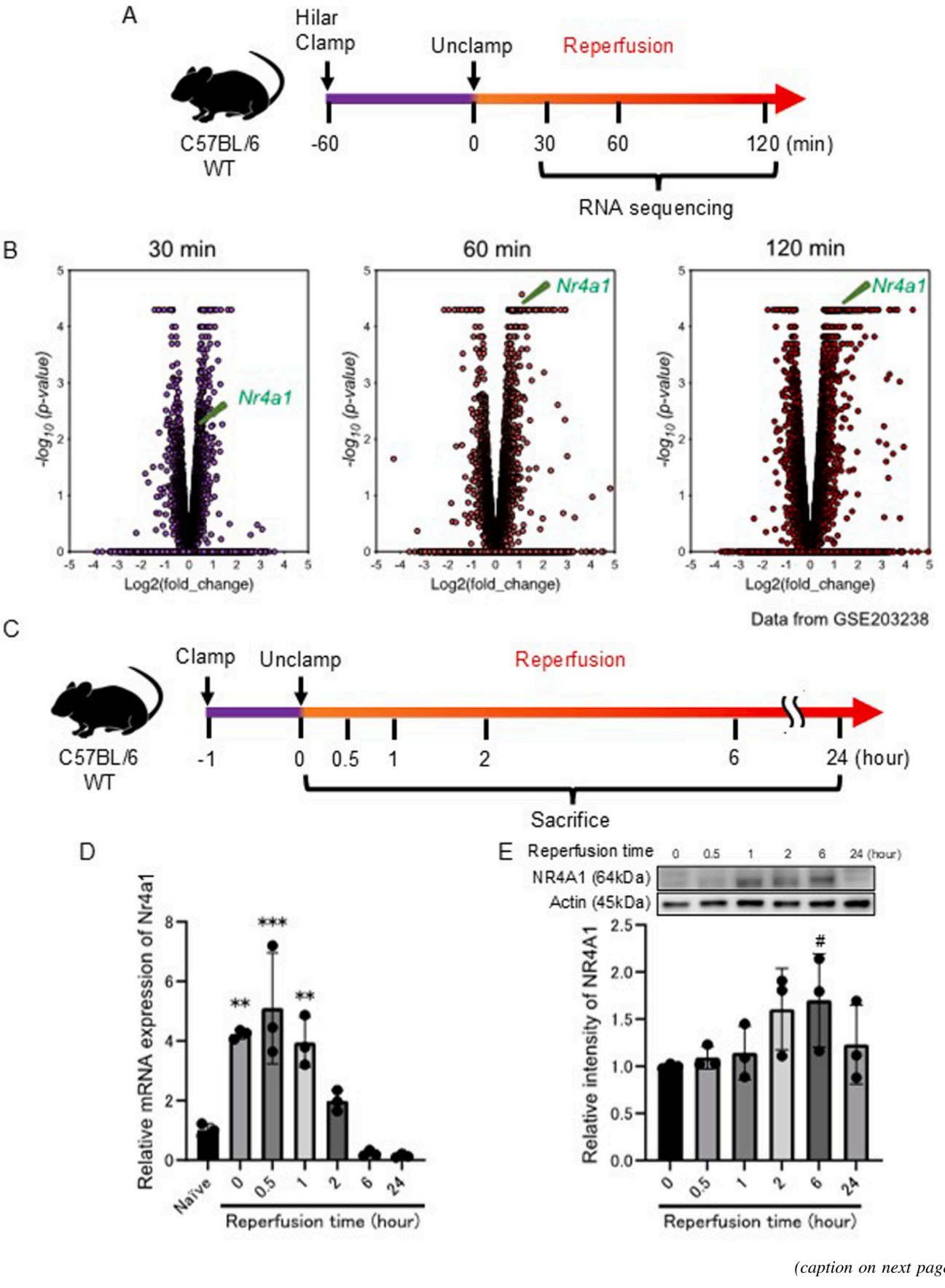

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Figure 1 Exploratory RNA sequencing of the lung ischemia-reperfusion injury (IRI) model and dynamics of NR4A1. (A) Schematic diagram of time course of hilar clamp experiment for RNA sequencing ($n = 3$). (B) Volcano plots of transcriptome data of whole lung tissues at the indicated time after reperfusion. (C) Schematic diagram of time course of hilar clamp experiment for quantification of NR4A1 ($n = 3$). (D) Relative mRNA expression levels of NR4A1 (NR4A1/GAPDH) in lung tissues at the indicated time after reperfusion. (E) Relative intensity of NR4A1 protein (NR4A1/beta-actin) in lung tissues at the indicated time. Data are expressed as mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$ vs naïve; $^{\#}p < 0.05$ vs 0-hour reperfusion. NR4A1, nuclear receptor subfamily 4 group A member 1; WT, wild type.

Conversely, knockout of NR4A1 in the recipient alone did not significantly improve oxygenation capacity compared to the WT recipient. To validate the alterations in NR4A1 expression in *Nr4a1*$^{-/-}$ mice, lung tissues obtained from each experimental group were analyzed by real-time PCR (Figure 2C). Naturally, compared to the WT donor group, *Nr4a1*$^{-/-}$ donor group showed a significant decrease. The expression of NR4A1 protein was further verified by immunostaining, revealing similar findings to mRNA expression (Figure 2D and E). In lung tissue from WT donors, the NR4A1 signal was uniformly distributed throughout the alveolar septum, primarily within the nucleus. In contrast, the NR4A1 signal was not prominent in the alveoli of *Nr4a1*$^{-/-}$ donors. The NR4A1 signal in naïve lung tissue was also observed to be faint, and a marked increase in NR4A1 was detected in the graft lungs from WT donors.

Histological analysis revealed reduced neutrophil infiltration and albumin leakage in the lungs of *Nr4a1*$^{-/-}$ donors

Hematoxylin-eosin staining showed a predominance of polymorphonuclear cell infiltration and thickening of alveolar septal walls in lung grafts from WT donors compared to grafts from *Nr4a1*$^{-/-}$ donors (Figure 3A). Polymorphonuclear cell infiltration in the lung was quantitatively visualized by immunohistochemistry using Ly-6G (Figure 3B). The number of Ly-6G-positive cells was significantly higher in the WT donor group when compared to the *Nr4a1*$^{-/-}$ donor group and the naïve group (Figure 3C). Additionally, immunohistochemistry with albumin was conducted to assess albumin leakage into lung tissue (Figure 3D). The deposition of albumin in lung tissue was significantly greater in the WT donor group than in the *Nr4a1*$^{-/-}$ donor group and the naïve group (Figure 3E).

Donor NR4A1 deletion maintained pulmonary vascular barrier and ameliorated pulmonary edema during IRI after LTx

Based on histological analysis, we hypothesized that NR4A1 is closely related to pulmonary vascular hyperpermeability and subsequent pulmonary edema during IRI after LTx. To investigate this hypothesis, we performed an Evans blue permeability assay. LTx using another series of mice was performed according to the aforementioned protocol, with the intravenous injection of Evans blue dye (20 mg/kg) immediately following reperfusion, and after a 4-hour interval, the graft lungs were procured for subsequent analysis (Figure 4A). The gross appearance of the grafts clearly demonstrated reduced dye deposition in the grafts from *Nr4a1*$^{-/-}$ donors compared to those from WT donors (Figure 4B). Microscopic images of frozen sections further revealed diminished dye deposition within the lung parenchyma in *Nr4a1*$^{-/-}$ donors, as opposed to WT donors (Figure 4C). The leakage of Evans blue dye into the graft was quantified by absorbance of tissue lysate and was significantly decreased in the grafts from *Nr4a1*$^{-/-}$ donors than those from WT donors (Figure 4D).

NR4A1 positively regulated pulmonary endothelial cell death during IRI after LTx

To elucidate the mechanism by which NR4A1 substantially affects pulmonary microvascular permeability, we focused on pulmonary microvascular endothelial cell injury during IRI after LTx. To assess the viability of vascular endothelial cells, we performed immunofluorescent costaining using anti-CD31 antibody as a marker for vascular endothelium and the TUNEL assay as an indicator of cell death (Figure 5A and Figure S1A). The occurrence of double-positive signals for CD31 and TUNEL, representing vascular endothelial cell death, exhibited a significant reduction in the *Nr4a1*$^{-/-}$ donor group in comparison to the WT donor group (Figure 5B). Furthermore, to evidence NR4A1 expression in endothelial cells, the colocalization of NR4A1 and CD31 was also examined (Figure 5C and Figure S1B).

NR4A1 had limited effect on the levels of inflammatory molecules during IRI after LTx

To explore the potential impact of NR4A1 on the inflammatory response and its subsequent influence on pulmonary microvascular permeability and endothelial cell injury, we performed a quantitative analysis of inflammatory molecules using the beads-based multiplex immunoassay. In the lung transplant group, certain inflammatory molecules within the lung tissue exhibited a noteworthy elevation when compared to the naïve group. However, there were only a limited number of significant alterations, regardless of the presence or absence of NR4A1 (Figure 6A-D and Figure S1). These identical observations were consistently made in plasma (Figure 6E-H and Figure S2). These results suggest that the maintained microvascular barrier and subsequent improvement of graft function during IRI after LTx in the *Nr4a1*$^{-/-}$ donor are not based on an alteration of inflammatory response.

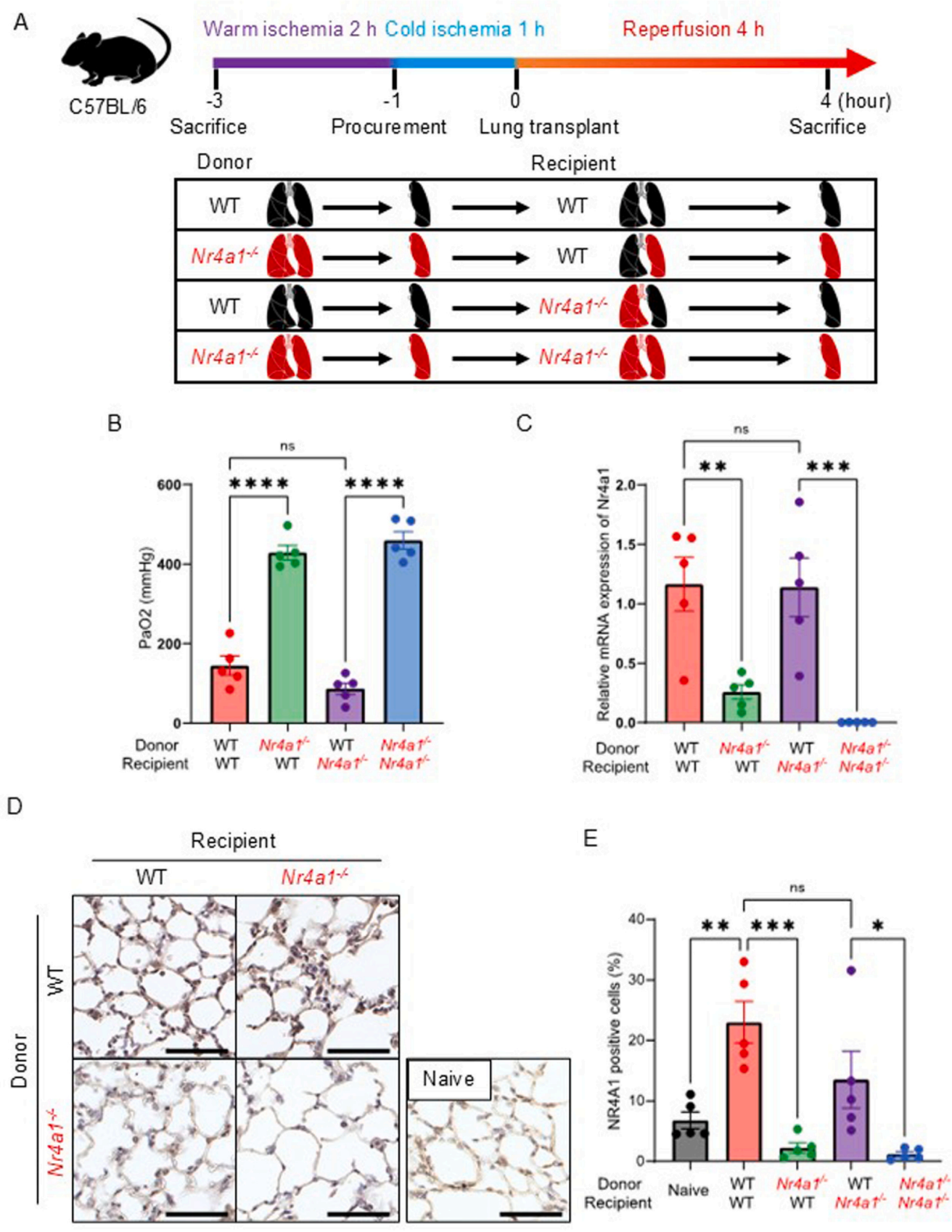

Figure 2 Functional assessment and NR4A1 expression of graft lung in ischemia-reperfusion injury after lung transplantation. (A) Experimental design of mice model of orthotopic lung transplantation ($n = 5$). (B) Graft function measured by PaO_2 on 100% FiO_2 at 4 hours after reperfusion. (C) Alterations in NR4A1 mRNA expression assessed by real-time PCR. (D) Immunohistochemistry of NR4A1. Inset scale bars, 50 μm. Data are expressed as mean ± SEM, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$. NR4A1, nuclear receptor subfamily 4 group A member 1; ns, not significant; WT, wild type.

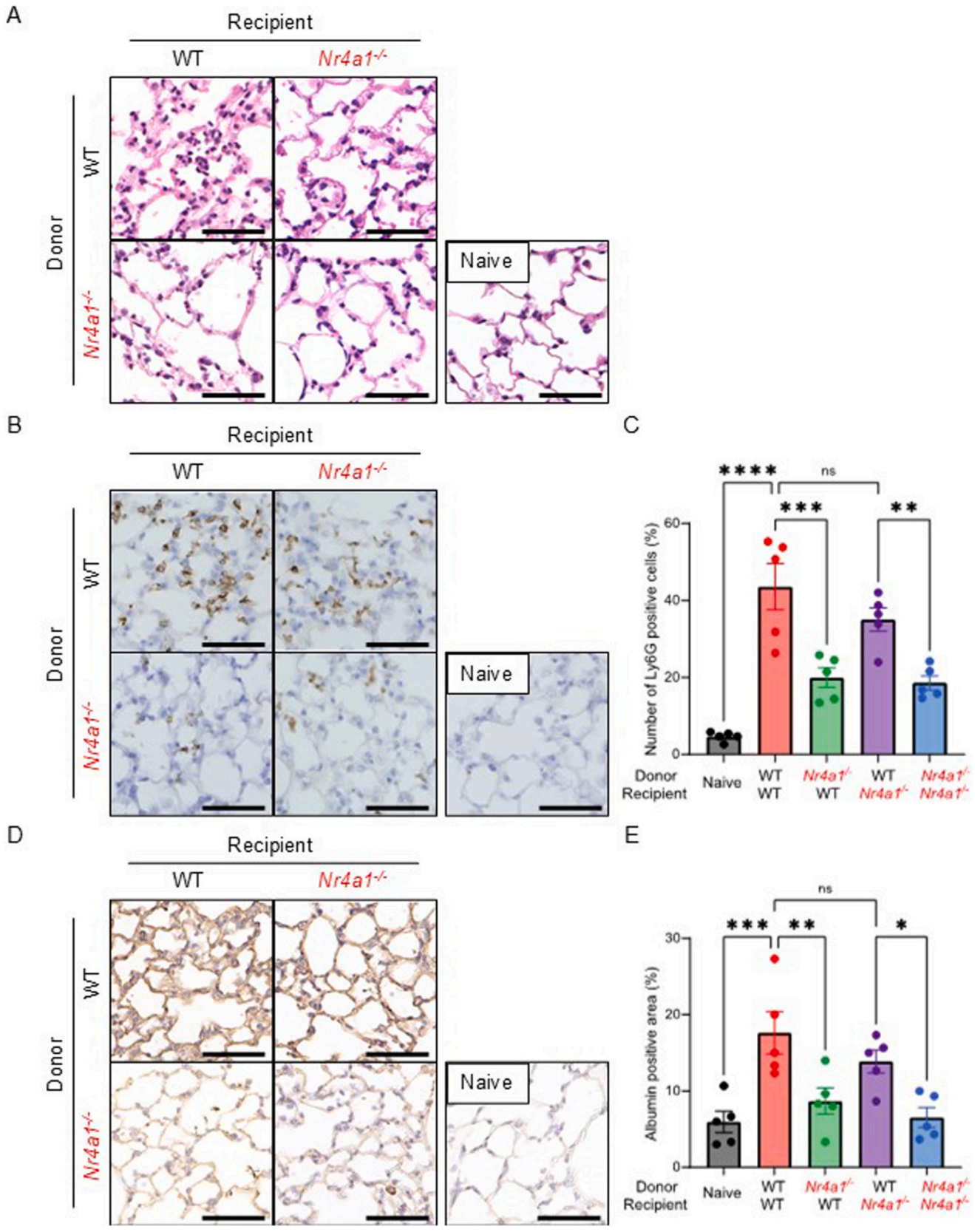

Figure 3 Histological analysis of graft lung in ischemia-reperfusion injury after lung transplantation. (A) Representative hematoxylin and eosin staining images of pulmonary parenchyma sections. (B) Immunohistochemistry of polymorphonuclear cells using the Ly-6G antibody. (C) Number of Ly-6G-positive cells. (D) Immunohistochemistry of albumin. (E) Quantification of area of albumin stain-positive tissue. Inset scale bars, 50 µm. Data are expressed as mean ± SEM, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$. ns, not significant; WT, wild type.

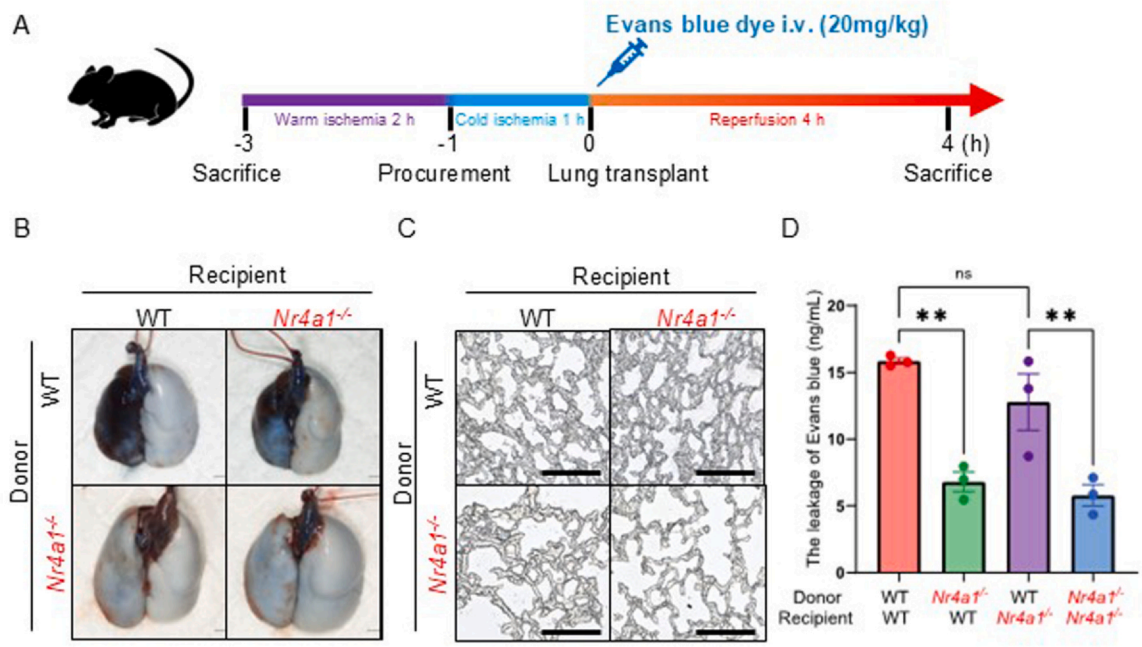

Figure 4 Effects of NR4A1 on microvascular permeability of the graft during ischemia-reperfusion injury after lung transplantation. (A) Schematic diagram of time course of lung transplant experiment for Evans blue permeability assay ($n = 3$). (B) Representative gross images of heart-lung block at 4-hour reperfusion after intravenous Evans blue injection (20 mg/kg). (C) Representative microscopic images of frozen section of pulmonary parenchyma. (D) Quantitative analysis of the leakage of Evans blue into the graft. Data are expressed as mean ± SEM, $^{**}p < 0.01$. NR4A1, nuclear receptor subfamily 4 group A member 1; ns, not significant; WT, wild type.

Discussion

In this study, we first observed the upregulation of NR4A1 during the early phase of WIRI in the lung, which was associated with endothelial cell death, leading to microvascular hyperpermeability and subsequent pulmonary edema following LTx. Notably, the deletion of NR4A1 in the donor alleviated IRI after LTx from DCD. The findings of our study align with previous experiments investigating IRI in other organs.[24-29] However, those experiments did not involve transplant models, and our study is the first to demonstrate the role of NR4A1 in WIRI using a syngenic organ transplantation model from DCD. Using *Nr4a1*$^{-/-}$ mice as donors and/or recipients could distinguish the specific contribution of NR4A1 in the donor and/or recipient during IRI after LTx. Furthermore, our study not only highlighted the impact of NR4A1 on molecular and histological changes, as observed in previous studies, but also revealed its influence on physiological functions in IRI of the lung.

Previous RNA sequencing identified NR4A1 as one of the genes showing early upregulation during lung WIRI. NR4A1 was rapidly upregulated as an IEG in response to warm IR stress in the lung, potentially serving a crucial role in the development of WIRI. RT-PCR and Western blotting further validated the transient upregulation of NR4A1 expression in the initial phases of WIRI. This observation implies a crucial involvement of NR4A1 in the early phases of WIRI. NR4A1 is recognized as an IEG in various biological processes, and its induction mechanism has been extensively investigated.[20-23] Its expression and activation are triggered by diverse stimuli, acting through the Mitogen-activated Protein Kinase (MAPK), Phosphatidylinositol-3 kinase/Protein Kinase B (PI3K/Akt), or cyclic Adenosine Monophosphate (cAMP)/cAMP-responsive element binding protein (PKA) /cAMP-responsive element (CREB) signaling pathways.[15-17,20,21,39] Additionally, NR4A1 serves as a transcriptional processing checkpoint, exerting suppressive control over IEGs by binding to them, including the *Nr4a1* gene itself, during resting conditions. Under stress, NR4A1 undergoes phosphorylation, leading to its dissociation from the binding site of IEGs and the rapid initiation of transcription for these genes, including *Nr4a1*.[22,23] In conjunction with the findings of the present study, it can be concluded that NR4A1 is promptly upregulated as an IEG in response to warm IR stress in the lung and may play a pivotal role in the development of WIRI.

Although IRI after LTx from DCD was suppressed in *Nr4a1*$^{-/-}$ donor lungs compared to the WT, IRI was not suppressed in the WT donor lung when transplanted into the *Nr4a1*$^{-/-}$ recipient. This is an important point to consider when investigating the mechanism by which NR4A1 knockout suppresses IRI and suggests that donor NR4A1 has important role in the early phase in LTx from DCD. Pulmonary vascular hyperpermeability and endothelial death were also suppressed in *Nr4a1*$^{-/-}$ donor lungs compared to the WT, but they were not suppressed in the WT donor lung when transplanted into the *Nr4a1*$^{-/-}$ recipient. Prior studies have demonstrated that NR4A1 is upregulated in vascular endothelial cells in response to various stimuli, leading to endothelial damage.[25,30,39-42] Additionally, numerous studies have shown that upregulated NR4A1 induces apoptosis in various cells, including vascular endothelial cells.[24-26] In response to apoptotic stimuli,

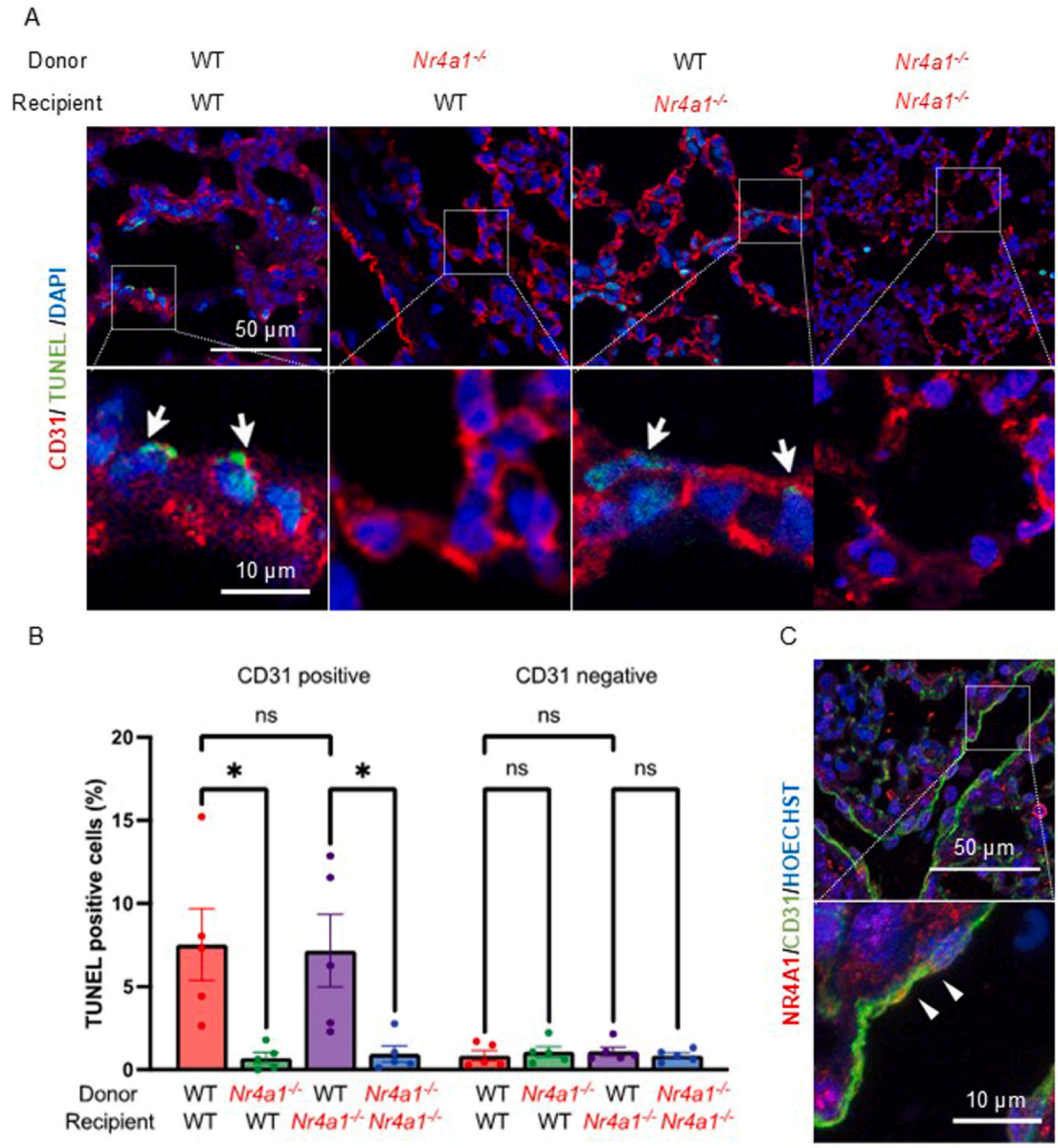

Figure 5 Effects of NR4A1 on the viability of pulmonary microvascular endothelial cells during ischemia-reperfusion injury after lung transplantation. (A) Immunofluorescent costaining using the anti-CD31 antibody and the TUNEL assay. White arrows indicate TUNEL-positive endothelial cells. (B) Number of TUNEL positive and CD31 positive/negative cells. (C) Immunofluorescent costaining of NR4A1 and CD31 in a WT lung graft within a WT recipient. Data are expressed as mean ± SEM, $^{**}p < 0.01$. NR4A1, nuclear receptor subfamily 4 group A member 1; ns, not significant; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; WT, wild type.

NR4A1 translocates from the nucleus to mitochondria to induce cytochrome c release via binding and inhibiting antiapoptotic B cell lymphoma gene 2 family protein, and activates subsequent apoptotic signaling.[15,43-45] As a transcription factor, NR4A1 also induces apoptosis through the transcriptional activation of death ligands and receptors, including FasL, Fas, TRAIL, and DR5.[46] Together with the findings of this study, warm IR stress might upregulate NR4A1 in pulmonary vascular endothelial cells, induce apoptosis, and pulmonary vascular hyperpermeability.

Contrary to expectations, knocking out *Nr4a1* had minimal impact on the inflammatory response, such as proinflammatory cytokines and chemokines. It was suggested that NR4A1 upregulation during WIRI caused pulmonary microvascular hyperpermeability not by promoting inflammation but by inducing endothelial cell death. These findings appear to contradict previous studies where knocking out of donor *Nr4a1* reduced the release of chemokines and the recruitment of monocytes and neutrophils to the graft after allogeneic LTx.[47-49] However, it is important to note that the experimental protocols of those studies did not include warm ischemic time, and the conditions were differed from those of the present study. Thus, the differences between the previous studies and our study

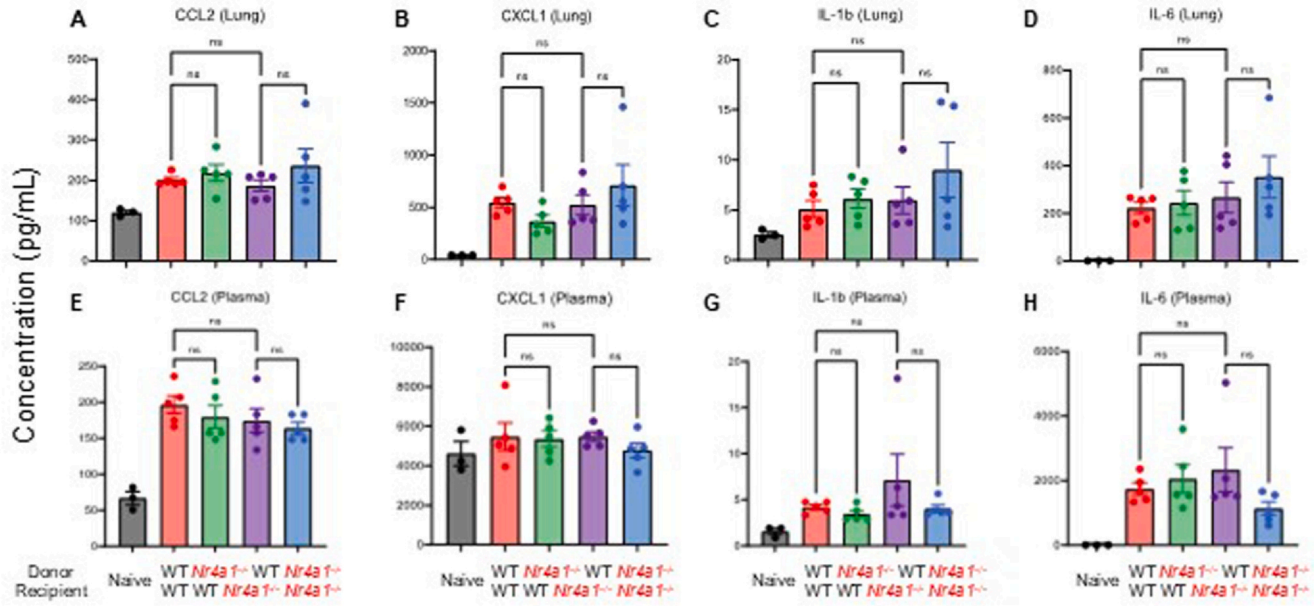

Figure 6 Concentrations of cytokines and chemokines measured by beads-based multiplex immunoassay. Data are expressed as mean ± SEM. ns, not significant; WT, wild type. CCL2, C-C motif chemokine ligand 2. CXCL1, C-X-C motif chemokine ligand 1. IL-1b, interleukin 1 beta. IL-6, interleukin 6

may have been caused by different mechanisms underlying the development of IRI between cold and warm ischemia. These different mechanisms of CIRI and WIRI have also been suggested by transcriptome analysis in clinical samples, comparing the differentially expressed genes between the lungs from DBD and the DCD lungs.[50] In the donation after brain death group, inflammatory cytokines and related pathways were upregulated. In contrast, the DCD group displayed upregulation of the pathways associated with cell death, apoptosis, and necrosis. Although it has been believed that inflammation is deeply involved in the microvascular hyperpermeability in lung IRI, our study suggests that endothelial cell death, rather than inflammation, may be the primary cause of microvascular hyperpermeability in WIRI.

The limitation of this study is that we did not investigate both up- and downstream signaling of NR4A1 in vitro. As these pathways have been extensively examined in previous studies, our research concentrated on exploring the physiological and functional aspects of NR4A1 in vivo. Additionally, while antagonists of NR4A1, such as DIM-C, which blocks NR4A1 translocation from nuclear to mitochondria and inhibits apoptosis, have been identified,[30,51] these drugs were not employed in the present study. To assess their impact on IRI, it is imperative to scrutinize parameters, such as optimal dosage, administration route, timing, etc. Therefore, further research incorporating these drugs is a subject for future study.

In conclusion, the donor NR4A1 emerges as a key player in the early phases of WIRI by regulating microvascular permeability rather than the inflammatory response. Deletion of NR4A1 led to the improvement of graft function in LTx from DCD. Consequently, the donor NR4A1 is proposed as a crucial therapeutic target for addressing PGD in LTx from DCD.

Author contributions

S.K., K.H., and M.O. designed and performed the experiments, analyzed and interpreted the data; S.K. and M.O. wrote the manuscript; T.S. analyzed the RNA-sequencing data; T.O. and A.M. performed histological analyses; all coauthors approved the paper.

Disclosure statement

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.healun.2024.09.028.

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