Anti-Infection Roles of mir155-5p Packaged in Exosomes Secreted by Dendritic Cells Infected with *Toxoplasma Gondii*

Dan Jiang
Southern Medical University
Shuizhen Wu
Southern Medical University
Liqing Xu
Southern Medical University
Guantai Xie
Southern Medical University
Hong-Juan Peng (floriapeng@hotmail.com)
Southern Medical University

Research

Keywords: Toxoplasma gondii, exosomes, RAW264.7 cells, mir155-5p, SOCS1

Posted Date: March 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-287510/v1

Abstract

Background: *Toxoplasma gondii* is a zoonotic intracellular protozoon that is estimated to infect about 30% of the world's population, resulting in toxoplasmosis in immuno-compromised patients and adverse outcomes in the primary infection during pregnancy. Exosomes are tubular vesicles secreted by cells, and they function in intercellular communication. It has been reported that the exosomes secreted by *T. gondii*-infected immune cells transmit infection signals to the uninfected cells. However, the mechanism and effect of the exosome transmission are still vague. We therefore investigated the function of the exosomes transmitted from DC2.4 cells infected with *T. gondii* RH strain (*Tg*-DC-Exo) to the uninfected cells, as well as their roles in anti-infection.

Methods: We conducted exosome isolation and identification with ultracentrifugation, transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and Western blotting. Recipient cells taken up exosomes by PKH67 assay. The signal transmission and the abundance of mir155-5p were determined using transwell assay and qRT-PCR. For the immune responses, cytokines was evaluated. *T. gondii* B1 gene was determined to evaluate tachyzoites proliferation.

Results: We observed that *Toxoplasma* infection up-regulated mir155-5p expression in DC2.4 cells secreted exosomes, and those exosomes can be ingested by murine macrophage RAW264.7 cells. *Tg*-DC-Exo and mir155-5p stimulating host proinflammatory immune responses such as proinflammatory cytokine (IL6,iNOS and TNF-α) production increased and NF-κB signal pathway activation by down regulating SOCS1, leading to inhibit *T. gondii* tachyzoite proliferation in RAW264.7 cells.

Conclusion: Our findings provide a novel mechanism for how infected cells transmit infection signals to the uninfected cells through exosome secretion after *T. gondii* infection, followed by inflammatory responses and anti-infection reactions, which may help us develop a new intervention strategy for toxoplasmosis prevention, especially in immuno-compromised patients.

Introduction

Toxoplasma gondii is an obligate intracellular protozoon that infects almost one-third of the world's population [1]. The infection is usually asymptomatic, but causes a lifelong threat to immune-competent populations; however, it may result in severe toxoplasmosis and even death in immuno-compromised patients, and primary infection during pregnancy may result in adverse pregnancy outcomes [1]. Hosts' immune responses mediate the results of *T. gondii* infection [2]. For example, interferon-γ (IFN-γ), Interleukin-12 (IL-12), and IL-1α have been reported to promote protective immunity against *T. gondii* infection [3–5]. Macrophages are important components of the innate immune system; they scavenge pathogens by activating innate immunity or promoting adaptive immunity through antigen presentation [6]. Many *T. gondii* secretions, such as ROP16, GRA12, GRA15, and GRA2, have been reported to regulate the host immune response [7–9].

Exosomes—nano-sized vesicles that encapsulate proteins, nucleic acids (with the highest abundance of microRNAs (miRNAs)), and other biological substances—are powerful tools for intercellular communication that mediate biological, physiological, and pathological states related to immune responses. It has been reported that *T. gondii* exosomes can trigger humoral and cellular immune responses and can promote the secretion of proinflammatory cytokines by macrophages for immune protection of the host [10, 11]. Nematode exosomes can inhibit the innate type 2 response [12]. Proteomic and microRNA (miRNA) analyses of the exosome-like vesicles from *Schistosoma mansoni* implicated that exosomes have potential for use in vaccine development and therapeutic innovation [13].

Small non-coding RNAs (miRNAs) inhibit the transcription and translation of the target genes, mainly by binding to the 3'UTR regions. Exosomes released by immune cells can carry miRNAs from donor cells to recipient cells to regulate biological processes [14]. Except Rather than regulating their own cells, exosomic miRNAs can regulate proximal and distal target cells through exosome transportation or other means. However, how exosomic miRNA works has not been fully characterized. Several highly expressed miRNAs have been identified from the extracellular vesicles of *Schistosoma japonicum* and have been found to be able to suppress target genes' expression in RAW264.7 cells [15].

The activation of $CD8^+$T cells is essential for inhibition of *T. gondii* infection [16]. Dendritic cells and macrophages are important antigen-presenting cells for T cells, which play important roles in anti-infection [17, 18]. The communication between these immune cells is particularly important. However, it is not known if the exosomes secreted by dendritic cells can transmit the anti-infection signals to macrophages. This study was conducted to answer this question.

Materials And Methods

Parasites and Cell Lines

Murine dendritic cell line DC2.4, human foreskin fibroblast cell line HFF, and murine macrophage cell line RAW264.7 were purchased from the ATCC (American Type Culture Collection, Manassas, USA) and preserved in our laboratory. The DC2.4 cells were cultured in RPMI-1640 (Roswell Park Memorial Institute-1640, bought from Gibco/Invitrogen, Waltham, MA, USA); the HFF and RAW264.7 cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium, bought from Gibco/Invitrogen). Both of the culture mediums were supplemented with 10% fetal bovine serum (FBS) (Gibco/Invitrogen) and 1% gentamicin (10 mg/mL, Invitrogen, USA). The cells were cultured with 5% CO_2 at 37°C. The *T. gondii* RH strain was propagated in HFF cells in our lab.

Exosome Isolation and Identification

The DC2.4 cells were cultured with RPMI-1640 supplemented with 10% exosome-free FBS (Gibco) in three 75 cm² cell culture flasks to 100% confluence and were infected with RH tachyzoites with a multiplicity of

infection of 3 (MOI = 3) for 28 h. Supernatants were collected and exosomes were extracted as described previously [19]. Isolated exosomes were examined using transmission electron microscopy (TEM) (Hitachi, Northeastern Honshu, Japan) at a voltage of 80 kV, as well as the exosomic proteins were detected with Western blotting [19] and the size of exosomes was determined by nanoparticle tracking analysis(NTA) using ZetaView (Particle Metrix, Germany) as previously described [15]. Exosome concentration was determined using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Massachusetts, USA) as manufacturer's described.

Western Blot

The isolated exosomes and the transfected cells were collected and lysed using lysis buffer (Beyotime Biotechnology, Shanghai, China), total proteins were loaded for SDS-PAGE, and immunoblotting was performed as described previously [20]. The following primary antibodies were used: rabbit mAb anti-CD9 (1:1000), rabbit mAb anti-TSG101 antibody (1:1000), rabbit mAb anti-HSP70 antibody (1:1000), rabbit mAb anti-iNOS (1:1000), rabbit mAb anti-p65 (1:1000) and rabbit mAb anti-SOCS1 (1:1000) ,which were purchased from Abcam (Massachusetts, USA); mouse mAb anti-GAPDH (1:1000), rabbit mAb anti-phosphorylated IκB alpha (Ser32/Ser36) (1:1000), and rabbit mAb anti-phosphorylated NF-κB p65 (S536) (1:1000), which were purchased from Affinity Biosciences (OH,USA); rabbit mAb anti-IKBα(1:1000) was purchased from GeneTex (Santa Cruz, USA). The secondary antibodies used for the Western blot were HRP goat anti-mouse IgG (1:5000) and HRP goat anti-rabbit IgG (1:5000), which were purchased from abclonal (Wuhan, China).

Uptake of *Tg*-DC-Exo and DC-Exo by RAW264.7 Cells

RAW264.7 cells were seeded on coverslips in a 12-well plate, approximate 1.1×10^6 cells per well, and were cultured with DMEM supplemented with 10% exosome-free FBS for 12 h. Exosomes extracted from the supernatants of *T. gondii*-infected DC2.4 cells (Tg-DC-Exo) and DC2.4 cells (DC-Exo) were labeled with green fluorescence using a PKH67 Green Fluorescent Cell Linker Mini Kit (Sigma-Aldrich, USA) according to the experimental procedures described by Lin WC et al. [21]. Briefly, Tg-DC-Exo and DC-Exo (5μg each) were respectively mixed in 40μl of PBS with 50μl Diluent C and 0.25μl PKH67 dye, mixed gently for 5 min at room temperature and 1% bovine serum albumin (BSA) was added to terminate the dying process. PKH67-stained Tg-DC-Exo and DC-Exo were recollected at 100,000×g for 2h at 4°C and resuspended in PBS. PKH67-stained exosomes or the same volume of the PKH67-PBS control were separately added to the RAW264.7 cells and incubated for 6 h. The cells were washed with PBS three times and fixed with 4% paraformaldehyde (Dingguo, China). Subsequently stained with DAPI (Dingguo, China). The coverslips were taken out and then observed under a fluorescence microscope (Nikon, Tokyo, Japan) with a Green Fluorescence Protein (GFP) filter, and images were captured.

Detection of Macrophage Polarization after Treatment of RAW264.7 Cells with *Tg*-DC-Exo and DC-Exo

The RAW264.7 cells were seeded in 24-well plates, grown overnight to 80% confluence, and then treated with 120 μg/mL of *Tg*-DC-Exo or DC-Exo for 24 h. The cells were washed with PBS three times and harvested after scraping. Total RNA was extracted to detect the transcription of TNF-α, iNOS, and IL6. The RAW264.7 cells were treated with 120 μg/mL of *Tg*-DC-Exo or DC-Exo for 48 h. The macrophage M1-type cytokine iNOS and M2-type cytokine Arg1 were detected with a Western blot.

RNA and DNA Isolation and qRT-PCR

The total RNA of exosomes was extracted with an exoRNeasy Serum/Plasma MidiKit (QIAGEN, Duesseldorf, Germany) according to the manufacturer's protocol. The total RNA of cells was extracted with Trizol reagent (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's protocol. Genomic DNA was removed with a One-Step gDNA Removal kit (Trans Gen Biotech, Beijing, China). For miRNA analysis, exosomic RNA was reverse-transcribed using Super Script™ II Reverse Transcriptase (Thermo Fisher Scientific, Wilmington, USA), and cell RNA was reverse-transcribed using Easy Script® All-in-One First-Strand cDNA Synthesis Super Mix. To evaluate the relative amount of *T. gondii* tachyzoites, the total DNA of infected cells was extracted with a DNeasy Blood and Tissue Kit and Proteinase K (QIAGEN, Duesseldorf, Germany) according to the manufacturer's protocol. The specific detection method of the *T. gondii* B1 gene followed previously described experimental procedures [22]. Real-time polymerase chain reaction (PCR) was performed by using Hieff® qPCR SYBR® Green Master Mix (Yeasen, Shanghai, China) and the Quant Studio™ real-time PCR system (Thermo Fisher Scientific, Wilmington, USA). The primers for quantitative PCR (qPCR) are shown in Table S1. The relative mRNA level was measured with $2^{-\Delta\Delta Ct}$.

Detection of mir155-5p Transportation to RAW264.7 Cells through Exosome Uptake with a Transwell Experiment

DC2.4 cells were seeded in the upper chambers of 24-well transwell inserts (Corning, New York, USA) and cultured with DMEM supplemented with 10% exosome-free FBS and 1% gentamicin to 100% confluence. The cells were divided into four groups: two groups were left uninfected, and two groups were infected with the *T. gondii* RH strain (MOI = 3). After infection for 30 min, the culture medium was aspirated and the DC2.4 cells in the upper chambers were washed with PBS three times to remove the unrecruited *T. gondii* tachyzoites. Meanwhile, RAW264.7 cells were seeded in the lower chambers. The four groups of DC2.4 cells (two infected with *T. gondii* and two uninfected) in the upper chambers and the RAW264.7 cells in the lower chambers were co-cultured in DMEM supplemented with 10% exosome-free FBS and 1% gentamicin, with or without 100 μL of 10 μm GW4869 (inhibitor of exosome secretion, MedChemExpress), for 52h. Each of these groups was labeled as Normal, DC2.4 + GW4869, DC2.4 + RH, or DC2.4 + RH +

GW4869. After that, the RAW264.7 cells were harvested and lysed with lysis buffer (Beyotime Biotechnology, China). The total RNAs were extracted for detection of mir155-5p abundance via quantitative real-time PCR (qRT-PCR). Each group was prepared in triplicate, and the experiment was repeated three times for statistical analysis.

Cell Transfection with Synthesized miRNA or siRNA and Treatment with Exosomes

RAW264.7 cells were seeded in 6-well plates to 80% confluence and were divided into 4 groups. Two groups were transfected with 10 μL of 20 nM mir155-5p mimics and mimic-NC(miRNA mimic-Normal Control), mir155-5p inhibitors and miRNA inhibitors-NC (miRNA inhibitor-Normal Control) (Gene Pharma, Suzhou, China), respectively. The other two groups were transfected with 10 μL of 20 nM si-SOCS1 (siRNA targeting SOCS1) and si-NC (siRNA-Normal Control) (Ribobio, Guangzhou, China), respectively. The transfection was performed using Lipofectamine 3000 (Thermo Fisher Scientific) according to the protocol provided by the manufacturer. The exosomes extracted from the cell culture supernatant of the DC2.4 (DC-Exo) and the DC2.4 infected with *T. gondii* (*Tg*-DC-Exo) were added to the 80% confluent RAW264.7 cells at 120 ug/well, respectively.

Cell activity, Proliferation, and Polarization Detection

The RAW264.7 cells were incubated with exosomes or transfected with miRNA/siRNA. At 24 h post-treatment, the transcription of the related cytokines was detected via qRT-PCR and Western blotting, and a CCK8 kit (Cell Counting Kit, Trans Gen Biotech, Beijing, China) was used to detect the cell viability and proliferation according to the instructions. In the same way, cell proliferation activity was evaluated by detecting cell absorbance after treatment for 0, 24, 48, 72, and 96 h. Each group was prepared in triplicate, and the experiment was repeated three times for statistical analysis.

Statistical Analysis

The differences between two or three groups were analyzed with Prism (GraphPad Software) using Student's t-test and one-way analysis of variance (ANOVA). SPSS (version 20) was used to analyze multiple comparisons (Tukey's), and $p < 0.05$ indicated that the difference was statistically significant.

Results

Characterization of the Exosomes Derived from DC2.4 Cells Infected or Uninfected with *T. gondii* RH Strain

The exosomes extracted from the DC2.4 cells infected or uninfected with *T. gondii* presented a specific saucer structure when observed with a transmission electron microscope (TEM) (Fig. 1A). The

nanoparticle tracking analysis showed that the particles had a diameter of 30–200 nm diameter, which was the characteristic size of the exosomes (Fig. 1B1 and Fig. 1B2). Several specific exosomic protein makers, such as TSG101, CD9, CD81, and HSP70, were detected in the exosome extracts by the Western blot (Fig. 1C).

Macrophages' (RAW264.7) Uptake of Exosomes Secreted by DC2.4 Cells

To determine whether immune cells can communicate with each other through exosome transmission, we labeled exosomes with PKH67 and incubated with RAW264.7 for 6 h in vitro. As the confocal microscope results shown in (Fig. 2), the RAW264.7 cells could engulf the exosomes successfully (including *Tg*-DC-Exo and DC-Exo) extracted from DC2.4 cells; however this uptake phenomenon was not observed in PKH67-PBS and PBS groups.

Tg-DC-Exo Results in Polarization of Macrophages toward M1 and Promotion of Inflammation Progress

Exosomes play a key role in intercellular communication by transmitting signals and molecules to other cells [23]. Based on the observation that RAW264.7 cells ingest exosomes secreted by DC2.4 cells, we hypothesized that exosomes function in the intercellular communication between different types of immune cells. To test this hypothesis, we treated RAW264.7 cells with *Tg*-DC-Exo and DC-Exo for 24 h, and evaluated the proinflammatory cytokine production in these RAW264.7 cells. The transcription levels of the proinflammatory cytokines (TNF-α, iNOS, and IL6) relative to the housekeeping gene—glyceraldehyde-3-phosphate dehydrogenase (GAPDH)—in these RAW264.7 cells were detected with real-time PCR. The results showed that the transcription levels of IL6, iNOS, and TNF-α were remarkably increased in the RAW264.7 cells treated with *Tg*-DC-Exo compared with those of the cells treated with DC-Exo (Fig. 3A). Consistently with the RT-PCR results, in our Western-blot detection, *Tg*-DC-Exo significantly promoted iNOS translation compared with DC-Exo treatment and non-treatment (Fig. 3B). Taken together, these results indicated that *Tg*-DC-Exo could induce RAW264.7 M1 macrophage polarization in vitro.

Mir155-5p Is Abundantly Packed in *Tg*-DC-Exo and Delivered to Recipient Cells via Exosomes

Our exosomic transcriptome sequencing identified a bunch of differentially enriched exosomic miRNAs from the DC2.4 cells infected with *T. gondii* RH tachyzoites; unlike those identified from the uninfected DC2.4 cells, the mir146a, let7k, and mir155-5p were upregulated [19]. Upon *T. gondii* infection, high expression of mir-155 was detected in immune cells [24]. Given the unique functions of mir-155 in immune system, such as controlling inflammatory response, regulating immune memory[25] and others highly related to immunity[26, 27]. We first confirmed the transcriptome sequencing result of the upregulated mir155-5p expression in *Tg*-DC-Exo compared with that in DC-Exo with qRT-PCR (Fig. 4A). To

determine whether mir155-5p can be transported to the recipient cells through the exosomes secreted by the DC 2.4 cells infected with *T. gondii*, RAW264.7 cells were co-cultured with *Tg*-DC-Exo or DC-Exo for 24 h, and the abundance of mir155-5p was detected with qRT-PCR. The level of mir155-5p relative to U6 increased by several times in *Tg*-DC-Exo-treated RAW264.7 cells compared with DC-Exo-treated RAW264.7 cells (Fig. 4B).

In our transwell experiment, DC2.4 cells infected with *T. gondii* and uninfected DC2.4 cells were pre-treated with GW4869 (an inhibitor of exosome secretion) for 24h to prevent the production of exosomes, or were left untreated with GW4869 as a control. After that, RAW264.7 cells were cultured in the lower chambers for 28 h, and were then harvested for detection of mir155-5p transcription with qRT-PCR. We found that the relative transcription level of mir155-5p in the RAW264.7 cells was reduced significantly in the DC2.4 + RH + GW4869 treatment group compared with that in the DC + RH treatment group (Fig. 4C). All these data indicated that the mir155-5p enriched in the exosomes derived from DC2.4 cells infected with *T. gondii w*as transported to and absorbed in the RAW264.7 cells.

The mir155-5p Packed in the Exosomes Secreted by DC2.4 Cells Infected with *T. gondii* (*Tg*-DC-Exo) Modulates Macrophage Polarization

It has been reported that mir155-5p induces a robust pro-inflammatory response in macrophages [28]. In this study, we found that mir155-5p was highly enriched in *Tg*-DC-Exo and *Tg*-DC-Exo-induced RAW264.7 M1 polarization. We therefore hypothesized that mir155-5p could be a key factor that plays an important role in *Tg*-DC-Exo-induced M1 polarization of RAW264.7. We first transfected mir155-5p mimics and mir155-5p inhibitors into RAW264.7 cells and mir155-5p inhibitors into DC2.4 cells, consistent levels of mir155-5p were detected with qRT-PCR in the transfected RAW264.7 cells (Fig. 5A□Figure S1A). Proliferation of these RAW264.7 cells was detected with a CCK8 kit at different times post-transfection. However, no significant differences were found in cell proliferation and viability at different time points among these transfected or un-transfected RAW264.7 cells (Fig. 5B). We further speculated that mir155-5p modulated the polarization direction of macrophages. We found in our experiments that mir155-5p mimics led to a significant increase in proinflammatory cytokine (IL-6, iNOS, and TNF-α) transcription in RAW264.7 cells compared to miRNA mimic-NC and mock treatments (Fig. 5C). To further verify the role of miR155-5p in macrophages, we transfected a miR155-5p inhibitor to DC2.4 and then extracted the exosomes (miR155-5p inhibitor-DC-Exo). The miR155-5p inhibitor-DC-Exo and the exosomes extracted from the normal DC2.4 cells (negative control-Exo) were added to RAW264.7 cells and incubated for 24 h, after that, the cytokines in the culture medium were detected. However, no significant differences in cytokine' levels were found between these groups (Figure S1B). Considering the low background value of miR155-5p [24] and loss of exosomes in extraction, the inhibition effect of the miR155-5p in exosomes may not be obvious. Therefore, we transfected a miR155-5p inhibitor to RAW264.7 cells. The mir155-5p inhibitor significantly inhibited the transcription of IL-6, iNOS, and TNF-α in RAW264.7 cells compared to

miRNA-NC inhibitor (miRNA Normal Control inhibitor) and mock treatments (Fig. 5D). The Western blot showed a result consistent with that of the qRT-PCR: mir155-5p mimics significantly up-regulated iNOS expression in RAW264.7 cells compared to miRNA mimic-NC and mock treatments (Fig. 5E). All of this evidence illustrated that *Tg*-DC-Exo-derived mir155-5p contributes to M1 macrophage polarization.

The mir155-5p Packed in *Tg*-DC-Exo Inhibits the Multiplication of *T. gondii* RH Tachyzoites

The production of proinflammatory factors is conducive for restraining *T. gondii* multiplication [29], and we had identified that *Tg*-DC-Exo-derived mir155-5p up-regulated the transcription of some proinflammatory cytokines (Fig. 3 and Fig. 5). However, whether the *Tg*-DC-Exo-derived mir155-5p inhibits *T. gondii* multiplication or not is still unknown. Therefore, we also found in our study that *Tg*-DC-Exo treatment of RAW264.7 cells resulted in the inhibition of *T. gondii* replication in the RAW264.7 cells compared to DC-Exo treatment and non-treatment (Fig. 6A). We assumed that the *Tg*-DC-Exo significantly enriched with mir155-5p also had the same effect on *T. gondii* replication. Firstly, a CCK8 was used to examine the effects of mir155-5p mimics and mir155-5p inhibitors on cell activity, and no significant differences were found between the RAW264.7 cell groups that were transfected or un-transfected with the miRNAs (Fig. 6D). Secondly, the *T. gondii* B1 gene copies (indicating the amount of *T. gondii* tachyzoites) in the cells transfected with mir155-5p mimics and mir155-5p inhibitors were evaluated with qRT-PCR. The results showed that the amount of B1 gene copies was significantly lower in the RAW264.7 cells transfected with the mir155-5p mimic than in the cells transfected with the miRNA mimic-NC and in the mock cells (Fig. 6B). On the contrary, the amount of B1 gene copies was significantly greater in the RAW264.7 cells transfected with mir155-5p inhibitors than in the cells transfected with the miRNA-NC inhibitor and in the mock cells (Fig. 6C). Overall, these results indicated that *Tg*-DC-Exo treatment and highly expressed mir155-5p in RAW264.7 cells significantly inhibit the multiplication of *T. gondii* in these cells.

Tg-DC-Exo-Derived mir155-5p Targets SOCS1 to Activate the NF-κB Signaling Pathway in Macrophages

We previously reported that the target genes of the significantly enriched miRNAs (such as mir155-5p) in *Tg*-DC-Exo are mainly related to immunity and are aggregated in NF-κB, MAPK, P13K-AKT, and the calcium signaling pathway [19]. To identify the target genes of mir155-5p in RAW264.7, online bioinformatics tools (Target Scan and mirWalk) were used to predict the potential target genes. Among the potential targets, the suppressor of cytokine signaling (SOCS1), which acts in both immune modulation and cell cycle regulation, has been reported to be a negative feedback regulator of cytokines and to be involved in multiple signaling pathways [30]. Hence, we assumed that SOCS1 is targeted by *Tg*-DC-Exo and mir155-5p and that it acts in the promotion of M1 macrophage polarization. We found that the transcription and translation of SOCS1 were significantly inhibited by mir155-5p mimics (compared to

miRNA mimic-NC transfection and non-transfection); These statements were verified with qRT-PCR and Western blotting, respectively (Fig. 7A–Fig. 7B). Considering the fact that the activation of NF-κB plays a crucial role in M1 macrophage polarization and inhibition of *T. gondii* proliferation [31], we speculated that the mir155-5p packed in *Tg*-DC-Exo was transported to the target RAW264.7 cells and that it targeted SOCS1 to the activate NF-κB signaling pathway. As a result, the multiplication of *T. gondii* in RAW264.7 cells was inhibited. To test our hypothesis, SOCS1 was knocked down in RAW264.7 cells by using small interfering RNAs targeting SOCS1 (si-SOCS1) (Fig. 7C–Fig. 7D). We found that mir155-5p mimics and si-SOCS1 transfection promoted the phosphorylation of IKBα and p65 more in the RAW264.7 cells compared with the si-NC transfection and un-transfected groups (Fig. 7E–Fig. 7F). Thus, NF-κB was activated, and macrophage polarization towards M1 was subsequently induced. We also found that the si-SOCS1 transfection into RAW264.7 did not affect the cells' proliferation or viability (Fig. 7G –Fig. 7H). Next, we investigated the role of SOCS1 in *T. gondii* RH proliferation in RAW264.7 cells by detecting the copy number of the *T. gondii* B1 gene in the si-SOCS1 and si-NC treatment groups with qRT-PCR. No significant differences in *T. gondii* RH proliferation were found between the groups (Fig. 7H). Interestingly, when pretreated with IFN-γ, si-SOCS1 can play a synergistic role in inhibition of *T. gondii* proliferation (Fig. 7H). Collectively, these results demonstrated that the mir155-5p-targeting SOCS1 gene activated the NF-κB signal pathway and inhibited the proliferation of *T. gondii*.

Discussion

Hosts' immune sensors can recognize *T. gondii* infection and activate immune cells in order to produce pro-inflammatory cytokines to resist infection [32–34]. Here, we found that exosomes derived from dendritic cells infected with *T. gondii* (*Tg*-DC-Exo) could be ingested by RAW264.7 cells and that mir155-5p was highly enriched in *Tg*-DC-Exo, but not in DC-Exo. Mir155-5p was found to target the SOCS1 gene to activate the NF-κB pathway, and it subsequently inhibited *T. gondii* proliferation in the RAW264.7 cells.

Macrophages are the first line of immune cells to respond to an infection, and together with DC2.4 cells, they present antigens to T cells, which are as important as macrophages in anti-infection responses. Therefore, the RAW264.7 cells we used here could be a model for observing the intercellular transmission of infection signals between different types of immune cells [35–37]. In our study, we found that the intercellular communication between dendritic cells and macrophages could be realized through exosome transmission (Fig. 2). This could be a mechanism for how infection signals from infected cells are transmitted to uninfected cells.

T. gondii infection altered the miRNA profiles of exosomes secreted from DC2.4 cells [19]. Mir155-5p is abnormally expressed in activated dendritic cells, macrophages, T cells, and B cells, it is an important regulatory factor that plays a key role in hematopoietic differentiation, immunity, inflammation, and pathogen infection [24, 38]. We deduced that the highly enriched mir155-5p in the exosomes released

from the dendritic cells infected by *T. gondii* was transmitted to RAW264.7 cells to activate the immune signaling pathways.

We found that *Tg*-DC-Exo and mir155-5p mimics boosted the transcription levels of IL6, iNOS, and TNF-α, which are macrophage M1-associated marker cytokines (Fig. 3 and Fig. 5). In *T. gondii*(RH)infection, dendritic cells transmitted immune signals to macrophages by secreting exosomes enriched with mir155-5p. As a result, the macrophages were driven to M1 polarization and participated in the positive immune responses after receiving mir155-5p. This phenomenon we found happened in late phase of infection resulted by exosomes transmission. However, it has been reported that *T. gondii* (types I and III) direct infection drives the polarization of macrophages towards M2 [39]. It is possible that *T. gondii* (types I and III) ROP16 activates M2 macrophages by arousing STAT6, which reduces the secretion of pro-inflammatory factors, such as IL12, in the early infection stage, thus helping to reduce the response of the host.

It is generally believed that M1 macrophages exert positive immune-modulatory effects and inhibit *T. gondii* proliferation by secreting proinflammatory cytokines, including TNF-α, IL6, and iNOS [40]. Therefore, the inhibited proliferation of *T. gondii* in the treatment with mir155-5p mimics was verified (Fig. 6). The downstream genes of mir155-5p in RAW264.7 cells were predicted with an informatic analysis, and SOCS1 was proposed. SOCS1 was verified as the target gene of mir155-5p through an experiment with mir155-5p mimic transfection (Fig. 7A, B). This result is consistent with that of the previous report by Ye J. et al. [41]. This previous study showed that, as a negative feedback cytokine regulator of the host cells, SOCS1 is involved in multiple immune-related pathways [42–45]; as the NF-κB signaling pathway is a crucial one for inflammatory response regulation, it can be activated by a variety of proinflammatory factors [46]. In our studies, after mir155-5p mimics were transfected into RAW264.7 cells, the NF-κB signaling pathway was significantly activated (Fig. 7D). However, multiple pathways are involved in proinflammatory cytokine secretion, and SOCS1 plays a key role in these pathways. The modulation of SOCS1 by miR-155-5p may also affect the other cellular functions; therefore, the function of the enriched mir155-5p in *Tg*-DC-Exo in anti-*T. gondii* infection remains to be further investigated.

Host interferon-γ (IFN-γ) plays a crucial role in the control of *T. gondii* infection [47, 48]. We found in our research that SOCS1, an effective inhibitor of the interferon-γ (IFN-γ) pathway, showed no effect on the proliferation of *T. gondii* in RAW264.7 cells without IFN-γ stimulation; however, when the RAW264.7 cells were treated with IFN-γ, the proliferation of *T. gondii* in the SOCS1 knockdown group was significantly more inhibited than in the normal control siRNAs (si-NC) transfected cells and the normal control cells (stimulated or not stimulated by IFN-γ) (Fig. 7F). This result implied an inhibitory role of SOCS1 in IFN-γ's anti-infection function, which is consistent with the report that *T. gondii* induces host cell SOCS1 to block IFN-γ signaling for immune evasion [49].

Conclusions

The exosomes secreted by the DC2.4 cells infected with *T. gondii* (*Tg*-DC-Exo) transmitted *T. gondii* infection signals to RAW264.7 cells through the latter's exosome uptake. The enriched mir155-5p in *Tg*-DC-Exo targeted the SOCS1 gene to activate the NF-κB pathway and then to promote the transcription of inflammatory factors, including TNFα, IL6, and iNOS. As a result, *T. gondii* proliferation was inhibited in these RAW264.7 cells. This finding may provide a new insight into intercellular communication for transmission of infection signals between host cells, and may have implications for the role of exosomic mir155-5p in anti-*T. gondii* infection.

Abbreviations

T. gondii: *Toxoplasma gondii*; Exo: Exosomes; miRNA: MicroRNA HFF: human foreskin fibroblast; RAW264.7: leukemia cells in mouse macrophage; FBS: Fetal bovine serum; TSG101: Tumor susceptibility gene 101; CD9: CD9 molecule; HSP70: Heat shock protein 70; CD81:CD81 molecule; LPS: Lipopolysaccharide; NF-κB: Nuclear factor kappa B; CCK8: Cell Counting Kit; iNOS: inducible nitric oxide synthase; TNF-α: tumor necrosis factor-α; IL-6: Interleukin-6; SOCS1: Suppressor of cytokine signaling1.

Declarations

Acknowledgments

The authors are grateful to the participants in this study and the anonymous reviewers and editors for their comments and valuable inputs.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Funding

This research was supported by National Key R&D Program of China (2017YFD0500400) , National Natural Science Foundation of China (81772217, 201828006, 81971954, 81572012), Science and Technology Planning Project of Guangdong Province (2018A050506038), and Key project of Guangzhou science research（201904020011）to HJP

Author Contributions

Conceptualization, data analysis and validation: Jiang D, Peng HJ; Funding acquisition: Peng HJ; Investigation: Jiang D, Wu S; Methodology: Jiang D, Liqing Xu, Guantai Xie; Project administration, resources and supervision: Peng HJ; Writing: Jiang D (original draft, and revision), Peng HJ (review, editing, and revision). All authors have read and agreed to the manuscript's publishing.

References

1. Montoya JG, Liesenfeld O. Toxoplasmosis Lancet. 2004;363(9425):1965–76.
2. Milne G, Webster JP, Walker M: *Toxoplasma gondii*: An Underestimated Threat? Trends Parasitol 2020.
3. Batista SJ, Still KM, Johanson D, Thompson JA, O'Brien CA, Lukens JR. Gasdermin-D-dependent IL-1α release from microglia promotes protective immunity during chronic *Toxoplasma gondii* infection. Nat Commun. 2020;11(1):3687.
4. Nishiyama S, Pradipta A, Ma JS, Sasai M, Yamamoto M. T cell-derived interferon-γ is required for host defense to *Toxoplasma gondii*. Parasitology international 2020, 75:102049.
5. Ivanova DL, Mundhenke TM, Gigley JP. The IL-12- and IL-23-Dependent NK Cell Response Is Essential for Protective Immunity against Secondary *Toxoplasma gondii* Infection. J Immunol. 2019;203(11):2944–58.
6. Essandoh K, Li Y, Huo J, Fan GC. MiRNA-Mediated Macrophage Polarization and its Potential Role in the Regulation of Inflammatory Response. Shock (Augusta Ga). 2016;46(2):122–31.
7. Chen L, Christian DA, Kochanowsky JA, Phan AT, Clark JT, Wang S, Berry C, Oh J, Chen X, Roos DS, et al: The *Toxoplasma gondii* virulence factor ROP16 acts in cis and trans, and suppresses T cell responses. The Journal of experimental medicine 2020, 217(3).
8. Wang JL, Bai MJ, Elsheikha HM, Liang QL, Li TT, Cao XZ, Zhu XQ. Novel roles of dense granule protein 12 (GRA12) in *Toxoplasma gondii* infection. FASEB journal: official publication of the Federation of American Societies for Experimental Biology 2020, 34(2):3165–3178.
9. Mukhopadhyay D, Arranz-Solís D. *Toxoplasma* GRA15 and GRA24 are important activators of the host innate immune response in the absence of TLR11. PLoS Pathog. 2020;16(5):e1008586.
10. Li Y, Liu Y, Xiu F, Wang J, Cong H, He S, Shi Y, Wang X, Li X, Zhou H. Characterization of exosomes derived from *Toxoplasma gondii* and their functions in modulating immune responses. Int J Nanomed. 2018;13:467–77.

11. Silva VO, Maia MM, Torrecilhas AC, Taniwaki NN, Namiyama GM, Oliveira KC, Ribeiro KS, Toledo MDS, Xander P. Extracellular vesicles isolated from *Toxoplasma gondii* induce host immune response. Parasite Immunol. 2018;40(9):e12571.
12. Buck AH, Coakley G, Simbari F, McSorley HJ, Quintana JF, Le Bihan T, Kumar S, Abreu-Goodger C, Lear M, Harcus Y, et al. Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. Nature communications. 2014;5:5488.
13. Samoil V, Dagenais M, Ganapathy V, Aldridge J, Glebov A, Jardim A, Ribeiro P. Vesicle-based secretion in schistosomes: Analysis of protein and microRNA (miRNA) content of exosome-like vesicles derived from Schistosoma mansoni. Scientific reports. 2018;8(1):3286.
14. Manca S, Upadhyaya B. Milk exosomes are bioavailable and distinct microRNA cargos have unique tissue distribution patterns. Scientific reports. 2018;8(1):11321.
15. Liu J, Zhu L, Wang J. Schistosoma japonicum extracellular vesicle miRNA cargo regulates host macrophage functions facilitating parasitism. PLoS Pathog. 2019;15(6):e1007817.
16. Dupont CD, Christian DA, Selleck EM, Pepper M, Leney-Greene M, Harms Pritchard G, Koshy AA, Wagage S, Reuter MA, Sibley LD, et al. Parasite fate and involvement of infected cells in the induction of CD4 + and CD8 + T cell responses to *Toxoplasma gondii*. PLoS pathogens. 2014;10(4):e1004047.
17. Poncet AF, Blanchard N, Marion S. Toxoplasma and Dendritic Cells: An Intimate Relationship That Deserves Further Scrutiny. Trends Parasitol. 2019;35(11):870–86.
18. Delgado Betancourt E, Hamid B, Fabian BT, Klotz C, Hartmann S, Seeber F. From Entry to Early Dissemination-*Toxoplasma gondii*'s Initial Encounter With Its Host. Frontiers in cellular and infection microbiology 2019, 9:46.
19. Li DL, Zou WH, Deng SQ, Peng HJ. Analysis of the Differential Exosomal miRNAs of DC2.4 Dendritic Cells Induced by *Toxoplasma gondii* Infection. International journal of molecular sciences 2019, 20(21).
20. Kong L, Jiang D, He C, Xia J, Wei H, Zhou L, Peng H. TgROP18 targets IL20RB for host-defense-related-STAT3 activation during *Toxoplasma gondii* infection. Parasit Vectors. 2020;13(1):400.
21. Lin WC, Tsai CY, Huang JM, Wu SR, Chu LJ, Huang KY. Quantitative proteomic analysis and functional characterization of Acanthamoeba castellanii exosome-like vesicles. Parasit Vectors. 2019;12(1):467.
22. Wu SZ, Wei HX, Jiang D, Li SM, Zou WH, Peng HJ. Genome-Wide CRISPR Screen Identifies Host Factors Required by *Toxoplasma gondii* Infection. Frontiers in cellular and infection microbiology 2019, 9:460.
23. Pegtel DM, Gould SJ. Exosomes. Annual review of biochemistry 2019, 88:487–514.
24. Elton TS, Selemon H, Elton SM, Parinandi NL. Regulation of the MIR155 host gene in physiological and pathological processes. Gene. 2013;532(1):1–12.
25. Vigorito E, Kohlhaas S, Lu D, Leyland R. miR-155: an ancient regulator of the immune system. Immunological reviews. 2013;253(1):146–57.

26. Lim KH, Staudt LM. Toll-like receptor signaling. Cold Spring Harb Perspect Biol. 2013;5(1):a011247.
27. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci USA. 2007;104(5):1604–9.
28. Mantuano E, Brifault C, Lam MS, Azmoon P, Gilder AS, Gonias SL. LDL receptor-related protein-1 regulates NFκB and microRNA-155 in macrophages to control the inflammatory response. Proceedings of the National Academy of Sciences of the United States of America 2016, 113(5):1369–1374.
29. Yap GS, Sher A. Cell-mediated immunity to *Toxoplasma gondii*: initiation, regulation and effector function. Immunobiology. 1999;201(2):240–7.
30. Ilangumaran S, Bobbala D, Ramanathan S. SOCS1: Regulator of T Cells in Autoimmunity and Cancer. Current topics in microbiology and immunology 2017, 410:159–189.
31. Mukhopadhyay D, Sangaré LO, Braun L, Hakimi MA. Toxoplasma GRA15 limits parasite growth in IFNγ-activated fibroblasts through TRAF ubiquitin ligases. EMBO J. 2020;39(10):e103758.
32. Sasai M, Pradipta A, Yamamoto M. Host immune responses to *Toxoplasma gondii*. Int Immunol. 2018;30(3):113–9.
33. Lima TS, Lodoen MB. Mechanisms of Human Innate Immune Evasion by *Toxoplasma gondii*. Frontiers in cellular and infection microbiology 2019, 9:103.
34. Suzuki Y. The immune system utilizes two distinct effector mechanisms of T cells depending on two different life cycle stages of a single pathogen, *Toxoplasma gondii*, to control its cerebral infection. Parasitol Int. 2020;76:102030.
35. Tsitsiklis A, Bangs DJ, Robey EA. CD8(+) T Cell Responses to *Toxoplasma gondii*: Lessons from a Successful Parasite. Trends Parasitol. 2019;35(11):887–98.
36. Kumar R, Loughland JR, Ng SS, Boyle MJ, Engwerda CR. The regulation of CD4(+) T cells during malaria. Immunol Rev. 2020;293(1):70–87.
37. Tiwari A, Hannah R, Lutshumba J, Ochiai E, Weiss LM, Suzuki Y. Penetration of CD8(+) Cytotoxic T Cells into Large Target, Tissue Cysts of *Toxoplasma gondii*, Leads to Its Elimination. The American journal of pathology 2019, 189(8):1594–1607.
38. Croston TL, Lemons AR, Beezhold DH, Green BJ. MicroRNA Regulation of Host Immune Responses following Fungal Exposure. Frontiers in immunology 2018, 9:170.
39. Wang C, Cheng W, Yu Q, Xing T, Chen S, Liu L, Yu L, Du J, Luo Q, Shen J, et al. Toxoplasma Chinese 1 Strain of WH3Δrop16(I/III) /gra15(II) Genetic Background Contributes to Abnormal Pregnant Outcomes in Murine Model. Frontiers in immunology. 2018;9:1222.
40. Park J, Hunter CA. The role of macrophages in protective and pathological responses to *Toxoplasma gondii*. Parasite Immunol. 2020;42(7):e12712.
41. Ye J, Guo R, Shi Y, Qi F, Guo C, Yang L. miR-155 Regulated Inflammation Response by the SOCS1-STAT3-PDCD4 Axis in Atherogenesis. Mediators Inflamm 2016, 2016:8060182.

42. Shi D, Li D, Wang Q, Kong X, Mei H, Shen Y, Liu W. Silencing SOCS1 in dendritic cells promote survival of mice with systemic Candida albicans infection via inducing Th1-cell differentiation. Immunology letters. 2018;197:53–62.
43. He Q, Sun C, Lei W, Ma J. SOCS1 Regulates Apoptosis and Inflammation by Inhibiting IL-4 Signaling in IL-1β-Stimulated Human Osteoarthritic Chondrocytes. Biomed Res Int 2017, 2017:4601959.
44. Yoshimura A, Ito M, Chikuma S, Akanuma T, Nakatsukasa H. Negative Regulation of Cytokine Signaling in Immunity. Cold Spring Harbor perspectives in biology 2018, 10(7).
45. Liang YB, Tang H, Chen ZB, Zeng LJ, Wu JG, Yang W, Li ZY, Ma ZF. Downregulated SOCS1 expression activates the JAK1/STAT1 pathway and promotes polarization of macrophages into M1 type. Molecular medicine reports 2017, 16(5):6405–6411.
46. Thoma A, Lightfoot AP. NF-kB and Inflammatory Cytokine Signalling: Role in Skeletal Muscle Atrophy. Advances in experimental medicine and biology 2018, 1088:267–279.
47. Bando H, Lee Y, Sakaguchi N, Pradipta A, Sakamoto R, Tanaka S, Ma JS, Sasai M, Yamamoto M. Toxoplasma Effector GRA15-Dependent Suppression of IFN-γ-Induced Antiparasitic Response in Human Neurons. Frontiers in cellular and infection microbiology 2019, 9:140.
48. Liau NPD, Laktyushin A, Lucet IS, Murphy JM, Yao S, Whitlock E, Callaghan K, Nicola NA, Kershaw NJ, Babon JJ. The molecular basis of JAK/STAT inhibition by SOCS1. Nature communications 2018, 9(1):1558.
49. Stutz A, Kessler H, Kaschel ME, Meissner M, Dalpke AH. Cell invasion and strain dependent induction of suppressor of cytokine signaling-1 by *Toxoplasma gondii*. Immunobiology. 2012;217(1):28–36.
50. **Data Availability Statement**.
51. The raw data. supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Figures

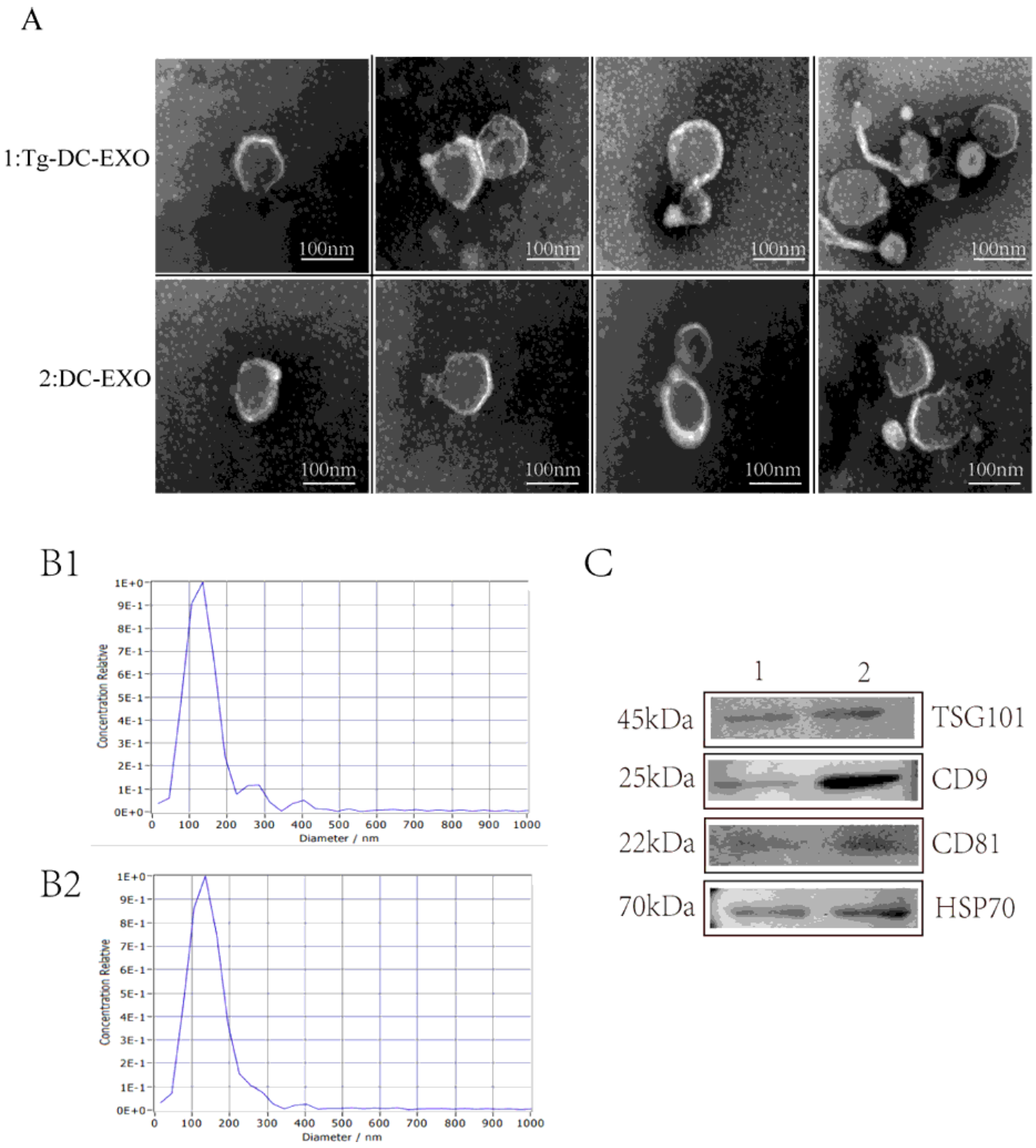

Figure 1

Identification of the extracted exosomes. Exosomes were extracted from dendritic cells infected or uninfected with T. gondii (RH strain) for 28 h. (A) Transmission electron microscopy was used to analyze the morphological structure of the exosomes obtained from the infected (Tg-DC-Exo) or uninfected dendritic cells (DC-Exo). Particle sizes and concentrations in Tg-DC-Exo (B1) and DC-Exo (B2) were

detected with nanoparticle tracking analysis (NTA). (C) Exosome-specific proteins TSG101, CD9, and CD81 and heat-shock protein HSP70 were detected in Tg-DC-Exo (1) or DC-Exo (2) using Western blotting.

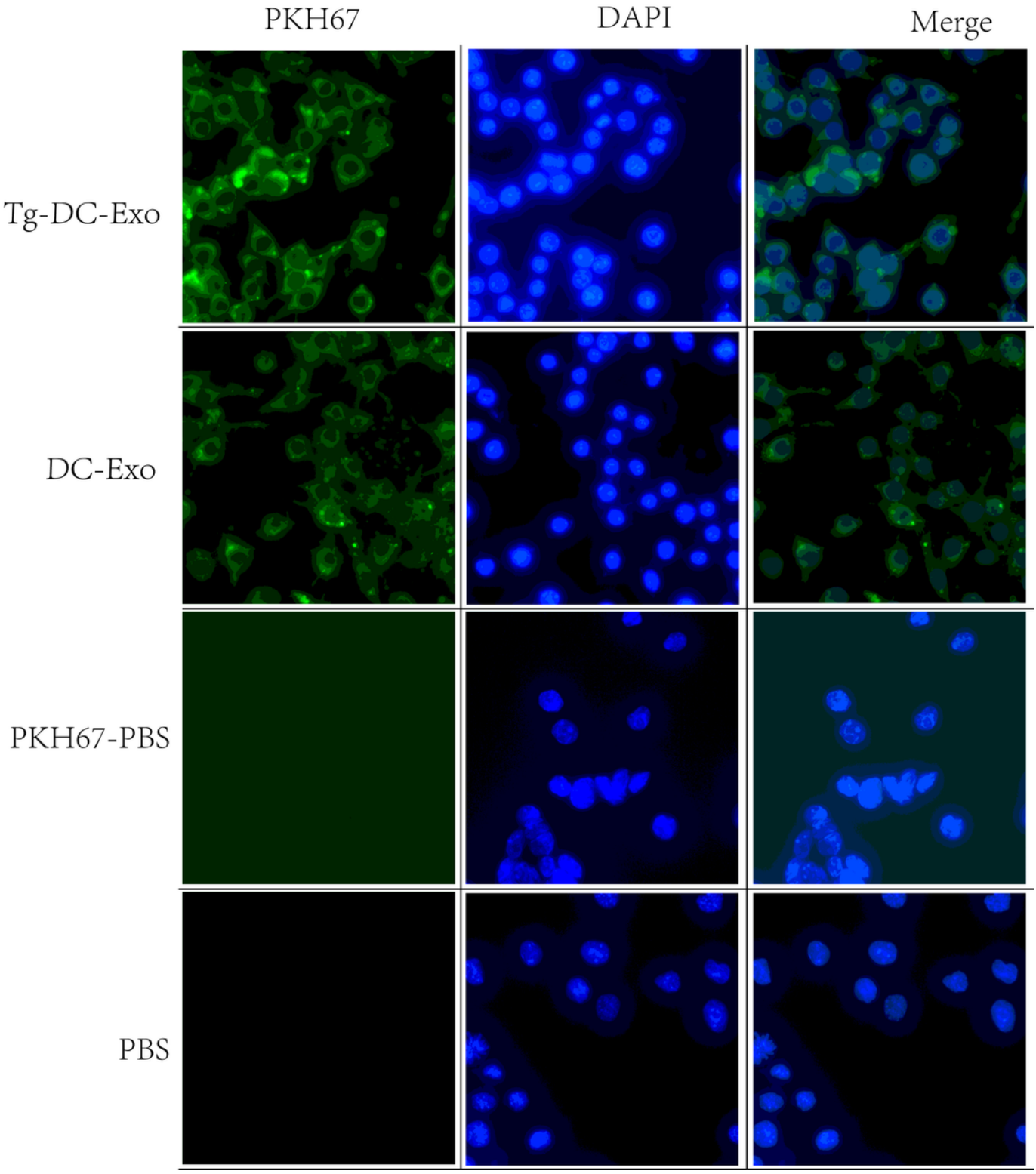

Figure 2

Tg-DC-Exo and DC-Exo are taken up by macrophages. Tg-DC-Exo, DC-Exo and PBS were labeled using PKH67 exosome labeling kit and were incubated with RAW264.7 cells for 6 h. PKH67-PBS and PBS were

set as negative control. Green indicates the labeled Tg-DC-Exo and DC-Exo. Cell nucleus were stained blue with DAPI.

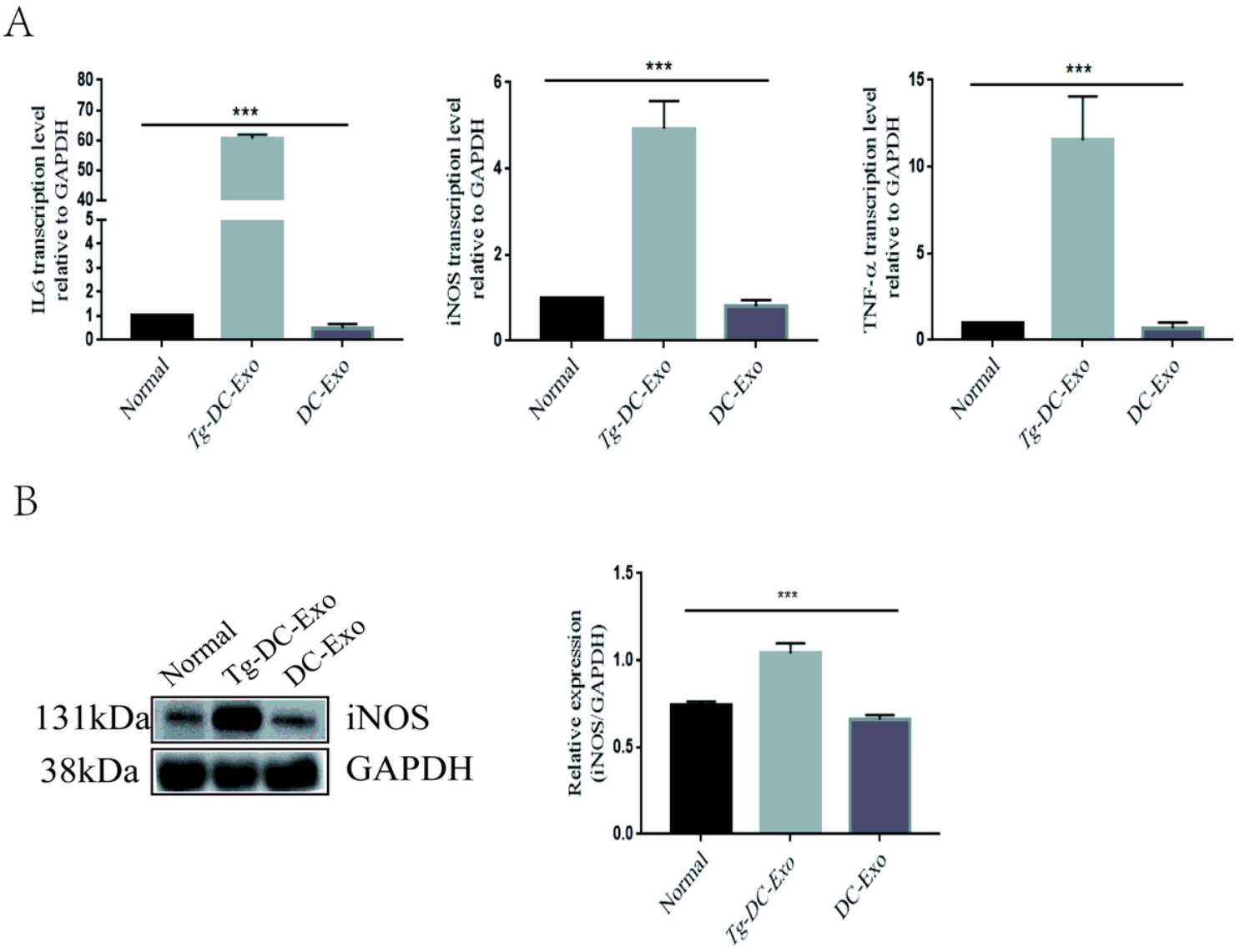

Figure 3

Findings of the different macrophage polarizations stimulated by Tg-DC-Exo and DC-Exo. (A) The relative transcription levels of IL-6, iNOS, and TNF-α in the RAW264.7 cells were quantified with quantitative real-time polymerase chain reaction (qRT-PCR) 24 h after the treatment with 120μg/ml Tg-DC-Exo and DC-Exo; they are represented as the fold change relative to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) level (set to 1). (B) The macrophage M1-type cytokine iNOS was detected by Western blotting. Each band densitometric quantitation in B was applied using Image J. Statistical analysis. The differences between two or three groups were analyzed with Prism (GraphPad Software) or SPSS (version 20) using Student's t-test or one-way analysis of variance (ANOVA). Tukey's multi-group test was used for multi-group comparisons. Each experiment was repeated three times (***p-value < 0.001).

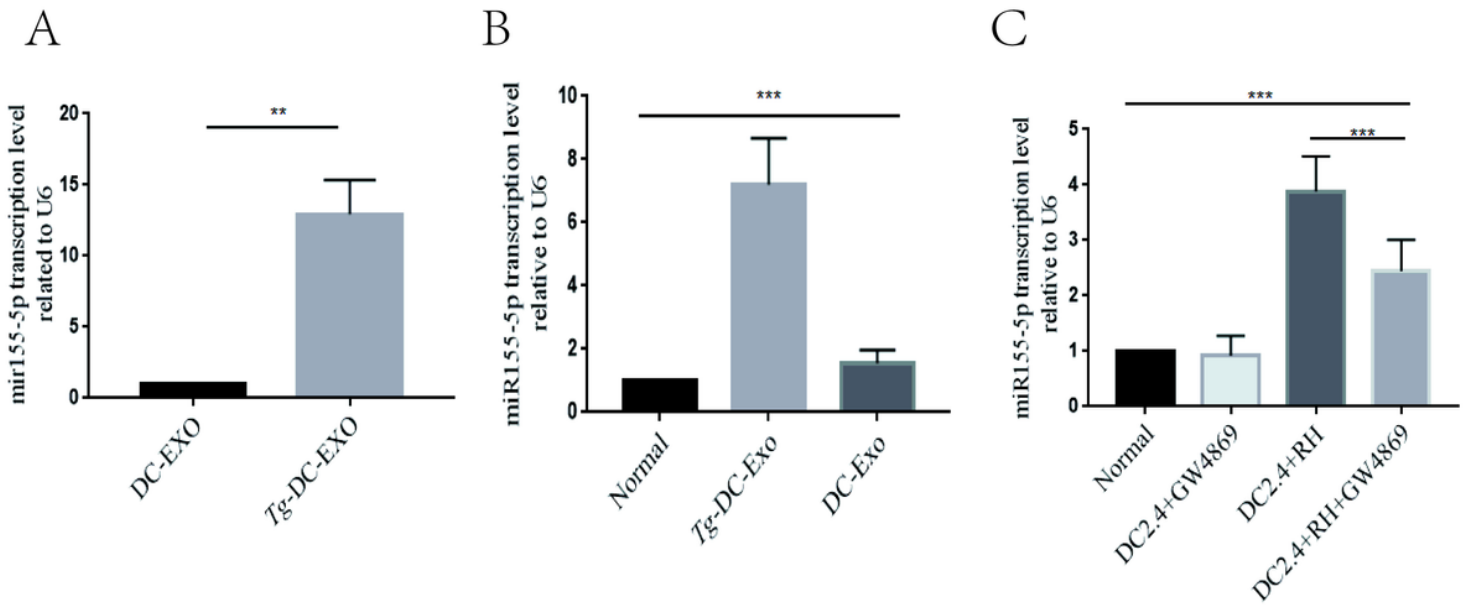

Figure 4

Evidence of mir155-5p being enriched in Tg-DC-Exo and delivered to recipient cells via exosomes. (A)qRT-PCR confirmation of the abundance of mir155-5p in Tg-DC-Exo and DC-Exo. (B) After RAW264.7 cells were treated with 120μg/ml Tg-DC-Exo or DC-Exo for 24 h, the mir155-5p transcription levels were quantified with qRT-PCR and are represented as the fold change relative to the U6 level (set to 1); normal cells were set as the negative control. (C) DC2.4 cells were pre-cultured in upper transwell chambers overnight; as indicated, the DC2.4 cells were pre-treatment or no treatment with GW4869. Followed with RH strain infected (for 30 min, and then the un-recruited tachyzoites were washed off) or left uninfected, synchronously RAW264.7 cells were cultured in the lower chambers for 28 h. The mir155-5p transcription levels in each lower chamber were detected with qRT-PCR, and are represented as the fold change relative to the U6 level (set to 1); normal cells were set as the negative control. Data are presented as mean ± standard deviation (SD). Student's t-test and one-way ANOVA were used for the significance analysis, and Tukey's multi-group test was used for multi-group comparisons. Each experiment was repeated three times (** p-value < 0.01 and***p-value < 0.001).

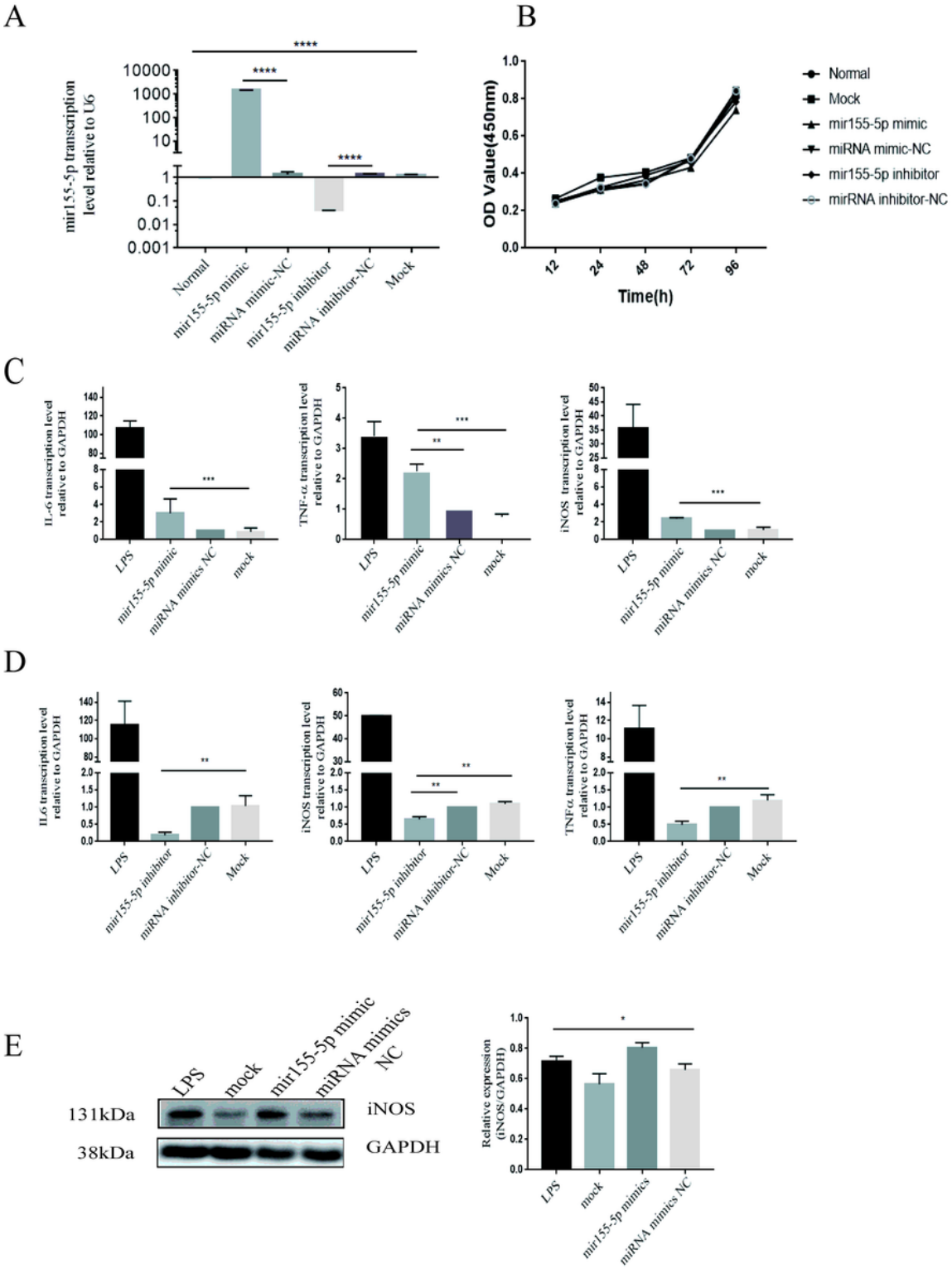

Figure 5

Tg-DC-Exo-enriched mir155-5p promoted macrophage M1 polarization. After the indicated treatment, the levels of mRNAs in RAW264.7 cells were quantified with qRT-PCR and are represented as the fold change relative to U6 (set to 1) for mir155-5p or relative to GAPDH (set to 1) for IL-6, iNOS, and TNF-α. (A) Detection of the transfection efficiency of mir155-5p mimics and inhibitors in RAW264.7cells. (B) RAW264.7 cells' proliferation was detected after transfection with mir155-5p mimics or mir155-5p

inhibitors for 12, 24, 48, 72, and 96 h, as indicated; the mimic control, inhibitor control, and normal cells were used as negative controls. The relative transcription levels of IL6, iNOS, and TNF-α in the mir155-5p mimic transfection group at 18 h post-transfection (C) and in the mir155-5p inhibitor transfection group at 24 h post-transfection (D). Western blot assay for iNOS expression in the mir155-5p mimic transfection group at 48 h post-transfection, (E). Densitometric quantitation of each band in E was applied using Image J . The mimics control transfection and mock cells were set as negative controls, and the lipopolysaccharide (LPS) treatment was set as the positive control. The SPSS software was used for statistical analysis. One-way ANOVA was used for between-group comparisons, and Tukey's multi-group test was used for multi-group comparisons. Each experiment was repeated three times (*p-value < 0.05, ** p-value < 0.01 and *** p-value < 0.001).

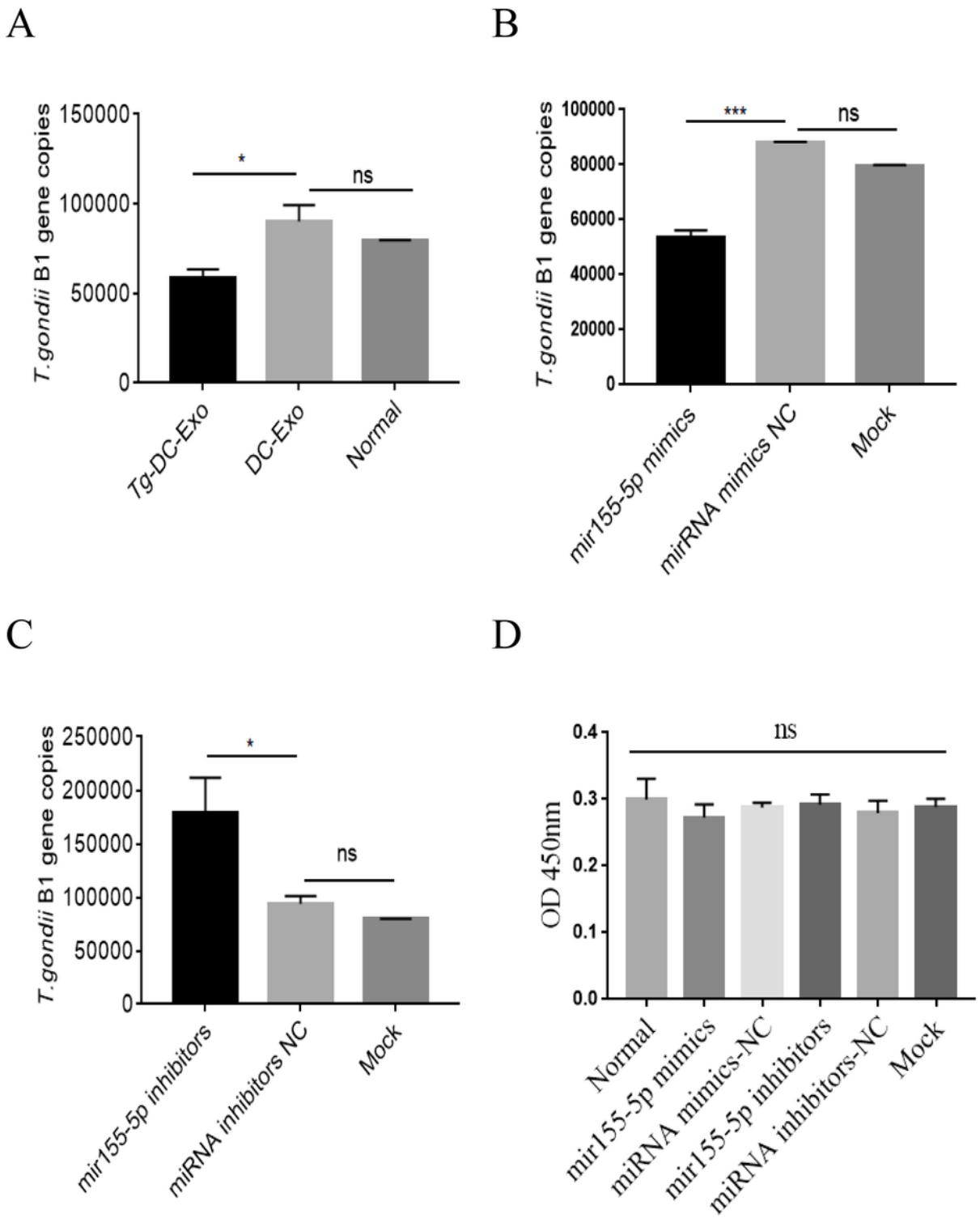

Figure 6

The inhibited proliferation of T. gondii in the Tg-DC-Exo treatment and mir155-5p transfection groups. T. gondii B1 gene copies were quantified with qPCR. (A) The RAW264.7 cells were treated with 120μg/ml Tg-DC-Exo or DC-Exo for 24 h and then infected with T. gondii for 24 h. (B) The RAW264.7 cells were transfected with mir155-5p mimics or (C) mir155-5p inhibitors for 24 h and then infected with T. gondii for 24 h; the mimic control transfection and mock cells were set as negative controls. (D) The cell viability

detection of the RAW264.7 cells transfected with mir155-5p mimics or inhibitors for 24 h. The SPSS software was used for statistical analysis. One-way ANOVA was used for between-group comparisons, and Tukey's multi-group test was used for multi-group comparisons. Each experiment was repeated three times (*p-value < 0.05, ***p-value < 0.001).

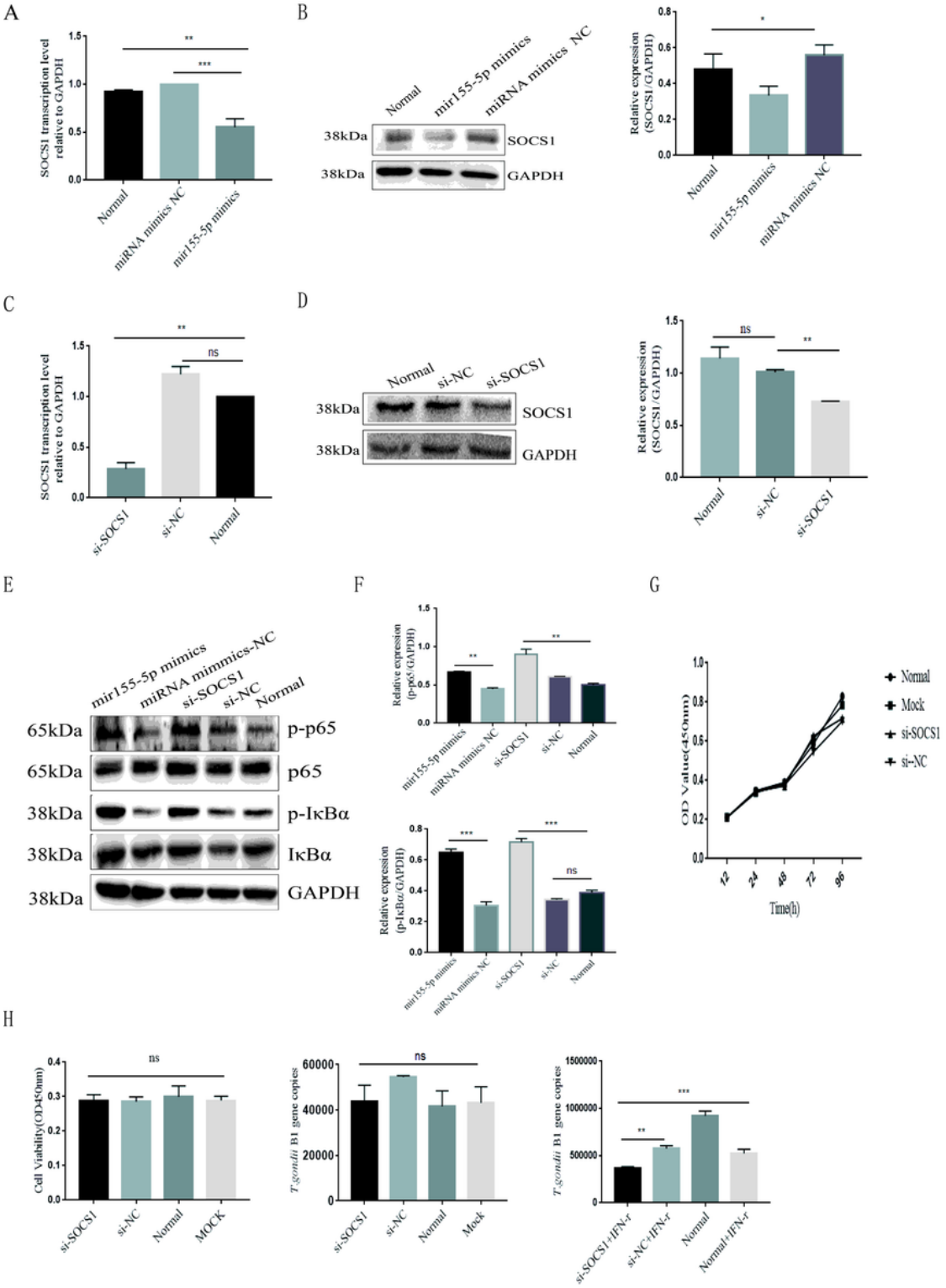

Figure 7

Tg-DC-Exo mir155-5p target SOCS1 to promote the inhibitory effect of interferon-γ (IFN-γ) on T. gondii proliferation through the NF-κB pathway. (A-B) Detection of SOCS1 transcription with qRT-PCR and translation with Western blotting in RAW264.7 cells transfected with mir155-5p mimics or Normal Control miRNA (mimic-NC) at 24 and 48 h post-transfection. (C-D) Detection of SOCS1 transcription with qRT-PCR and translation with Western blotting in siRNA targeting SOCS1 (si-SOCS1) and Normal Control siRNA (si-NC) transfected RAW264.7 cells at 24 and 48 h post-transfection. (E- F) Detection of the phosphorylation levels of p65 and IKBα in the NF-κB pathway in the RAW264.7 cells transfected with mir155-5p mimics, Normal Control siRNA (mir-NC), si-SOCS1, or si-NC for 36 h. (G) RAW264.7 cells' proliferation was detected after transfection with si-SOCS1, and si-NC for 12, 24, 48, 72, and 96 h. (H) Detection of the T. gondii B1 gene copies in the RAW267.4 cells transfected with si-SOCS1 or si-NC and stimulated with IFN-γ for 24 h, then infected with T. gondii. Densitometric quantitation of each band in B, D and E was applied using Image J .One-way ANOVA was used for between-group comparisons, and Tukey's multi-group test was used for multi-group comparisons. Each experiment was repeated three times (**p-value < 0.01, ***p-value < 0.001).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- FigureS1.tif
- GraphicalAbstract.tif