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TIPE2 modulates macrophage polarization *via* targeting Rac1/NF-κB and PI3K/AKT pathways

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Abstract

Introduction: *Chronic rhinosinusitis with nasal polyps (CRSwNP) is a chronic inflammatory disorder that affects up to 12% of the general population. It is associated with significant morbidity and a considerable burden on the healthcare system. Tumor necrosis factor α-induced protein 8-like 2 (TIPE2) is an immune-related protein potentially involving the pathogenesis of autoimmune diseases, and depletion of TIPE2 can cause inflammatory diseases. In this study, we aimed to investigate the roles and mechanisms of TIPE2 in inflammation and macrophage polarization.*

Material and methods: *A model of macrophage polarization was set up in phorbol-12-myristate-13-acetate-differentiated THP-1 monocytes. Once differentiated, THP-1 cells were treated with interleukin (IL)-4 to obtain M2 polarized macrophages or with interferon (IFN)-γ and lipopolysaccharide (LPS) for classical macrophage activation (M1). Cell surface markers (CD11b and CD206) were analyzed by flow cytometry. Protein levels of TIPE2, M1/M2 macrophages-associated inflammatory factors, and pathway-related genes were measured by western blotting. ELISA was conducted to measure the level of iNOS.*

Results: *TIPE2 upregulation inhibited the M1 polarization of macrophages. TIPE2 downregulation inactivated the M2 polarization of macrophages. TIPE2 overexpression inactivated the Rac1/NF-κB pathway in LPS/IFN-γ-induced M1 macrophages. TIPE2 downregulation inactivated the PI3K/AKT pathway in IL-4-induced M2 macrophages.*

Conclusions: *TIPE2 mediates macrophage polarization caused by LPS/IFN-γ and IL-4 via the Rac1/NF-κB and PI3K/AKT pathways.*

Key words: *chronic rhinosinusitis with nasal polyps, TIPE2, macrophage, polarization, Rac1/NF-κB, PI3K/AKT.*

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Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a chronic inflammatory disorder [1]. It is estimated that approximately 13 million people are diagnosed with CRSwNP in China, and the global number is in the range 70-180 million [2, 3]. Nasal blockage, anosmia, rhinorrhoea, and facial pain are the most common symptoms associated with CRSwNP [4, 5]. Genetic, immunological, environmental, and mucosal factors are involved in the pathogenesis of CRSwNP [6]. CRSwNP presents remarkable heterogeneity in inflammation patterns. A majority of patients with CRSwNP in East Asia show non-eosinophilic inflammation [7]. Nevertheless, CRSwNP in whites is primarily characterized by eosinophilic inflammation [8]. Eosinophilic CRSwNP mostly displays a type 2 immune response with more severe symptoms and a higher recurrence rate than non-eosinophilic CRSwNP [9]. Currently, endoscopic sinus surgery is widely used for CRSwNP treatment, but factors such as allergic fungal rhinosinusitis, asthma, and respiratory disorder may lead to the recurrence of nasal polyps [10]. Therefore, more insights into CRSwNP pathogenesis are required to initiate novel therapeutic options.

Macrophages, innate immune cells differentiated from monocytes, are critical players in tissue homeostasis, innate immunity, and inflammation [11, 12]. Antigen presentation and cytokine production are two major patterns of macrophage reaction [13, 14]. Macrophages can be polarized into the classically activated macrophage (M1) and alternatively activated macrophage (M2) under microenvironmental stimuli [15, 16]. M1 polarization develops under

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stimulation with interferon γ (IFN-γ) and bacterial products including lipopolysaccharide (LPS) [17]. Secretion of inflammatory cytokines, including interleukin (IL)-1β, IL-12, IL-18, and IL-23, can be induced by M1 phenotype [18]. M1 macrophages have also been shown to promote the activation of inducible nitric oxide synthase (iNOS) [19]. M1 macrophages play an important role in initiating and maintaining inflammation [20]. M2 macrophages can be activated by IL-4 [21]. They can release immunomodulatory cytokines such as IL-10 and transforming growth factor β (TGF-β) [22]. M2 macrophages have the potential to facilitate tissue remodeling and inhibit inflammation [23]. Previous studies have indicated that macrophage polarization plays a crucial role in tissue repair and remodeling. This process involves the secretion of diverse cytokines and inflammatory mediators [24, 25]. Recently, growing evidence has suggested that macrophage polarization patterns are involved in the pathogenesis of CRSwNP and associated tissue heterogeneity [26, 27]. However, the regulatory mechanisms driving macrophage polarization remain incompletely understood.

Tumor necrosis factor-α-induced protein 8 (TIPE) is implicated in immune homeostasis. TIPE2, belonging to the TIPE family, is preferentially expressed by hematopoietic cells including macrophages [28-30]. The expression of TIPE2 is found to be upregulated in eosinophilic polyps of patients with CRSwNP, and TIPE2-positive M2 macrophages potentially contribute to eosinophilic inflammation and disease progression in CRSwNP [31]. Additionally, TIPE2 inhibits the inflammatory response by switching arginine metabolism from nitric oxide synthase to arginase in macrophages [32]. Moreover, TIPE2 negatively regulates M1 macrophage differentiation, induces M2 macrophage differentiation, and alleviates experimental systemic lupus erythematosus in mice [33]. However, the mechanisms of TIPE2 in CRSwNP pathogenesis remain uncertain.

The PI3K/Akt pathway can be activated by IL-4 treatment, and the PI3K signaling pathway can promote the transformation of macrophages from M1 phenotype to M2 phenotype [34-36]. TIPE2 has been reported to activate PI3K/AKT signaling, thereby facilitating M2 macrophage differentiation [37]. The NF-κB pathway is a classical inflammatory mediating pathway involved in regulating the cellular inflammatory responses [38]. Activated NF-κB has been observed following CRSwNP [39]. Accumulating evidence has demonstrated that TIPE2 can alleviate various inflammatory diseases via inactivation of the NF-κB pathway, such as rheumatoid arthritis [40], acute lung injury [41], and *Pseudomonas aeruginosa* keratitis [42]. Rac family small GTPase 1 (Rac1) can lead to the activation of NF-κB [43]. This study aimed to investigate the roles of TIPE2 in macrophage polarization and determine whether its functions were associated with the PI3K/AKT and Rac1/NF-κB pathways.

Material and methods

Cell culture

The human leukemia monocytic/macrophage THP-1 cell line (ATCC, Rockville, MA, USA) was incubated in RPMI 1640 (PM150110B; Procell, Wuhan, China) containing 10% FBS and 1% penicillin/streptomycin (CL12331; ChemeGen, Shanghai, China). The THP-1 cells were differentiated into macrophages (M0) using 10 ng/ml of phorbol-12-myristate-13-acetate (PMA; HY-18739; MedChemExpress, Shanghai, China). This stimulation was performed by culturing the THP-1 cells in six-well plates (5×10^6 cells/well) with PMA for 48 h. The M0 macrophages were polarized into M1 macrophages by further stimulation with lipopolysaccharide (LPS; CX40002; ChemeGen) and IFN-γ (CY33258; ChemeGen) at 100 ng/ml, or into M2 macrophages by stimulation with IL-4 (HY-P7042; MedChemExpress) at 40 ng/ml, for 7 days. Subsequently, the cells were washed with FACS buffer (PBS with 2% FBS and 1 mM of EDTA) twice and blocked with anti-CD32 (ab281898, 1 μg/ml; Abcam) in FACS buffer at 4°C for 15 min. The cells were then stained with antibodies against CD11b (ab28101, 20 μl for 10^6 cells; Abcam) and CD206 (ab270647, 4 μl for 10^6 cells; Abcam) at 4°C for 40 min. The cells were also incubated with the control isotype corresponding to each primary antibody. Finally, the cells were analyzed using a flow cytometer. Data analysis was performed using the FlowJo software.

Cell transfection

The cells were seeded into a six-well plate (5×10^4 cells/well) and incubated for cell transfection until confluence reached 70-80%. To construct the TIPE2-overexpression plasmid (pcDNA3.1/TIPE2), TIPE2 was cloned into the pcDNA3.1 vector. The constructed pcDNA3.1/TIPE2 plasmid was transfected with THP-1 cells to overexpress TIPE2. The THP-1 cells transfected with the pcDNA3.1 plasmid were used as negative controls. The TIPE2-knockdown THP-1 cells were constructed by transfection with specific siRNA-targeting TIPE2 (si-TIPE2), and THP-1 cells transfected with si-NC were used as the negative control. Transient transfection was achieved using Lipofectamine 2000 (Yeasen, Shanghai, China).

Western blotting

The differentiated THP-1 cells were further incubated with LPS and IFN-γ at 100 ng/ml or IL-4 at 40 ng/ml, for 24 h, and the cells were harvested at different time points (0 h, 4 h, 8 h, 16 h, and 24 h) for western blotting analysis. Total protein was isolated from the THP-1 cells using RIPA lysis buffer (abs9228; Absin Biotech, Shanghai, China) with a cocktail protease and phosphatase inhibitors (HY-L081; MedChemExpress). Protein concentration was examined using an Enhanced

BCA Protein assay kit (P0012S; Beyotime, Shanghai, China). Proteins (30 μg) were separated on 10% acrylamide gels using SDS-PAGE and subsequently transferred onto PVDF membranes (Millipore, Shanghai, China). After 2-h blocking in 5% skimmed milk, the membranes were incubated overnight with primary antibodies against MGL1 (ab168613, 1 μg/ml), TIPE2 (ab169616, 1 μg/ml), tubulin (ab7291, 1 : 5000), p-IκB (ab133462, 1 : 10000), TNF-α (ab6671, 1 : 1000), MIF (ab176565, 1 : 1000), MCP-1 (ab214819, 1 : 1000), IL-6 (ab6672, 1 : 1000), ARG-1 (ab133543, 1 : 2000), p-p65 (ab76311, 1 : 2000), MGL2 (ab24701, 1 : 200), IL-10 (ab34843, 1 : 2000), Rac1 (ab155938, 1 : 1000), GAPDH (ab37168, 1 μg/ml), phospho-Y458-PI3K (ab278545, 0.5 μg/ml), PI3K (ab32089, 1 : 1000), phospho-T308-AKT (ab38449, 1 : 1000), phospho-S473-AKT (ab81283, 1 : 5000), and AKT (ab32505, 1 : 2000) at 4°C followed by TBST washing three times. Then the membranes were incubated with secondary antibodies for 2 hours at room temperature. Antibodies were purchased from Abcam. The blots were then developed using enhanced chemiluminescence (Yeasen) and imaged using the chemiluminescence detection system (Bio-Rad, Hercules, CA, USA). The bands were quantified by measuring the intensity of signals using ImageJ software. Tubulin and GAPDH served as control proteins to quantify the expression of target proteins. The ladder for the western blotting images is included in the supplementary file labeled "Western blotting".

Measurement of iNOS

The level of iNOS in LPS/IFN-γ-treated THP-1 cells was measured using an ELISA kit (ab253217; Abcam) according to the manufacturer's instructions.

Statistical analysis

All experiments were performed in at least three independent replicates. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Data were presented as the mean ± standard deviation. One-way analysis of variance followed by Tukey's post hoc analysis and Student's *t* test were used for comparative analyses. $P < 0.05$ was considered statistically significant.

Results

Induction of macrophage polarization

M1 polarization was induced by LPS and IFN-γ. Flow cytometry was used to analyze the CD11b positive cells. An increase in the number of CD11b positive cells with time was observed (Fig. 1A, B). Meanwhile, the results of western blotting demonstrated that LPS/IFN-γ led to time-dependent increases in the protein levels of M1 macrophage-associated factors (MIF, MCP-1, TNF-α, and IL-6) in THP-1 cells (Fig. 1C). Next, IL-4 was used to induce M2 phenotype, and induction of M2 polarization was determined by flow cytometry. We found that the number of CD206 positive cells increased time-dependently (Fig. 1D, E). Concomitantly, the protein levels of M2 macrophage-associated factors (ARG-1, MGL1, MGL2, and IL-10) were time-dependently upregulated in THP-1 cells by IL-4 treatment (Fig. 1F). Morphological evidence showed that THP-1 cells exposed to PMA for 48 h became adherent, a characteristic of M0 cells. These cells acquired a rounded and flat M1 phenotype after exposure to LPS/IFN-γ. The M0 cells became highly elongated following treatment with IL-4, a characteristic of the M2 phenotype (Fig. 1G). Moreover, as western blotting revealed, in LPS/IFN-γ-treated M1 macrophages, the protein level of TIPE2 decreased time-dependently, while IL-4-challenged M2 macrophages showed a time-dependent increase in TIPE2 protein level (Fig. 1H-K). In conclusion, M1 and M2 polarizations of macrophages regulate the expression of TIPE2.

TIPE2 upregulation inhibits the M1 polarization of macrophages

To detect the role of TIPE2 in the M1 polarization of macrophages, we overexpressed TIPE2 in THP-1 monocytes stimulated with PMA (used as the model of undifferentiated M0 macrophages) or M0 macrophages treated with LPS/IFN-γ (used as the model of differentiated M1 macrophages) using pcDNA3.1/TIPE2 (Fig. 2A, B). Then as flow cytometry revealed, there was a significant increase in the number of CD11b positive cells following LPS/IFN-γ stimulation of M0 macrophages, whereas TIPE2 upregulation significantly reduced the number of CD11b positive cells. However, the number of CD11b positive cells exhibited a non-significant change in undifferentiated M0 macrophages with TIPE2 overexpression (Fig. 2C, D). The level of iNOS was dramatically reduced in LPS/IFN-γ-stimulated M1 macrophages following TIPE2 upregulation (Fig. 2E). Finally, the protein levels of M1 macrophage-associated factors (MIF, MCP-1, TNF-α, and IL-6) were measured by western blotting, and we found that TIPE2 upregulation significantly downregulated their protein levels in M1 macrophages (Fig. 2F). These results show that TIPE2 upregulation prevents M1 macrophage differentiation.

TIPE2 downregulation inhibits the M2 polarization of macrophages

Considering the elevated level of TIPE2 in IL-4-treated THP-1 cells, we effectively knocked down its level *via* transfection of si-TIPE2 in undifferentiated M0 macrophages and differentiated M2 macrophages, and the knockdown efficiency was examined by western blotting (Fig. 3A, B). As flow cytometry demonstrated, TIPE2

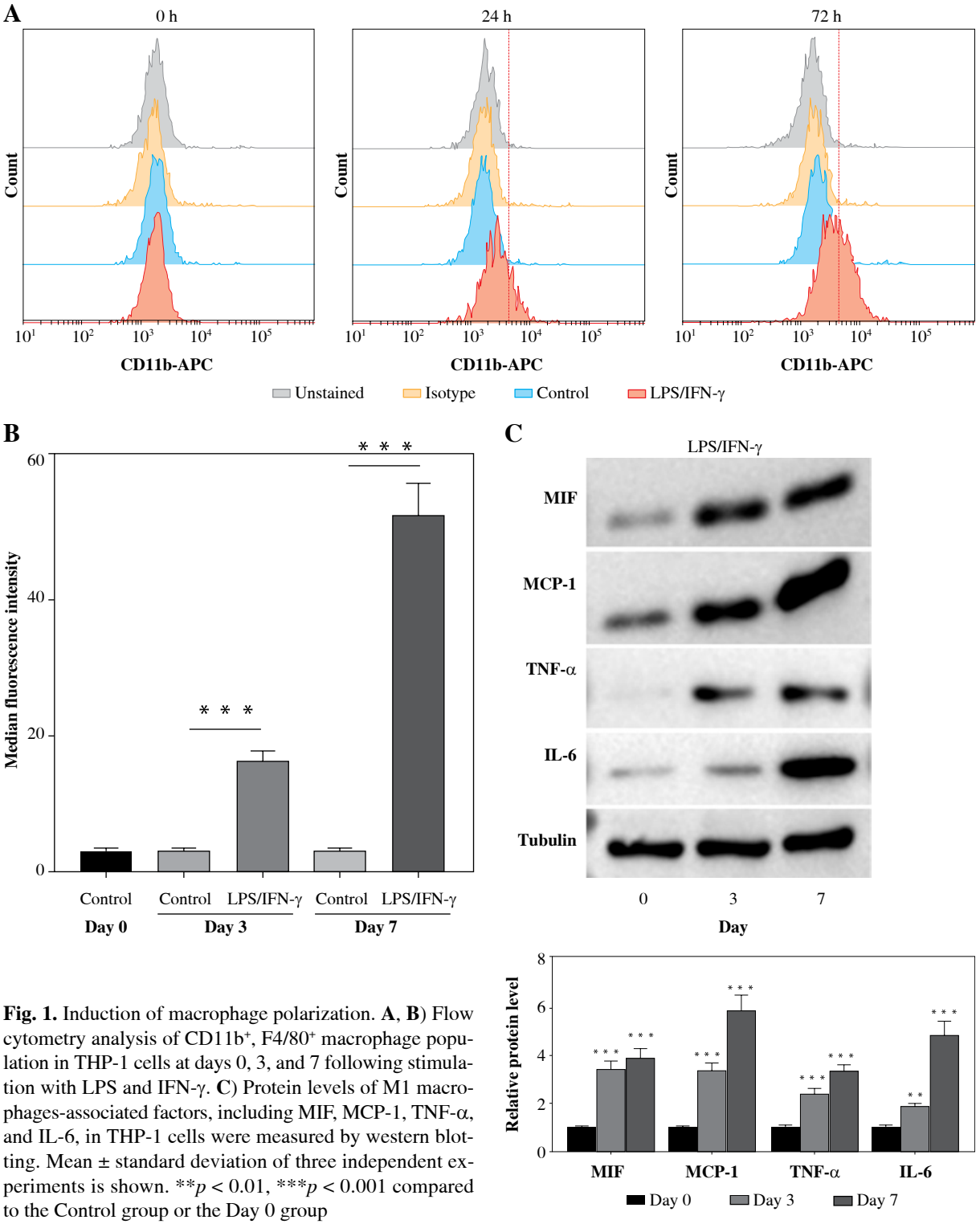

Fig. 1. Induction of macrophage polarization. **A**, **B**) Flow cytometry analysis of CD11b$^+$, F4/80$^+$ macrophage population in THP-1 cells at days 0, 3, and 7 following stimulation with LPS and IFN-γ. **C**) Protein levels of M1 macrophages-associated factors, including MIF, MCP-1, TNF-α, and IL-6, in THP-1 cells were measured by western blotting. Mean ± standard deviation of three independent experiments is shown. $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the Control group or the Day 0 group

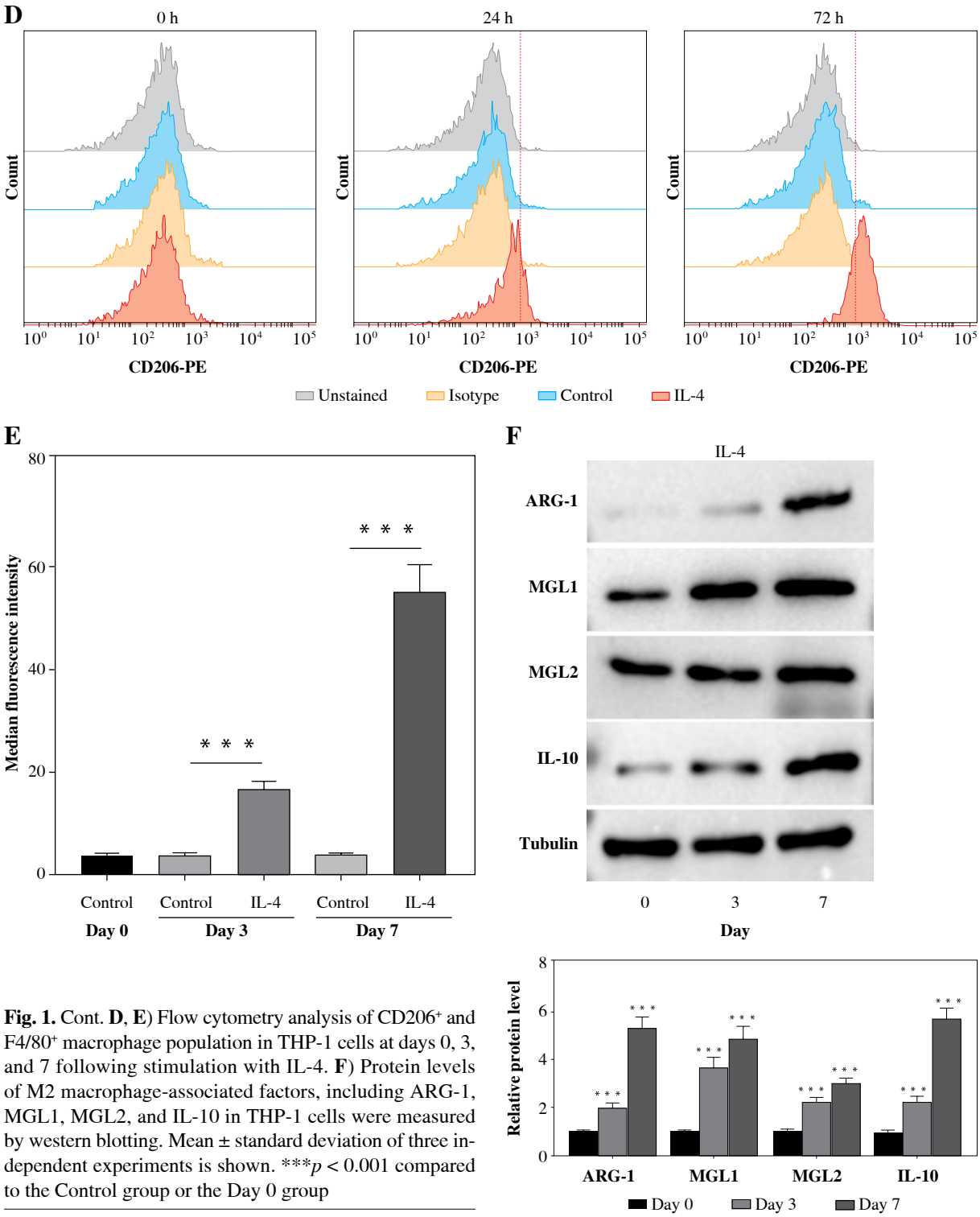

Fig. 1. Cont. **D**, **E**) Flow cytometry analysis of CD206⁺ and F4/80⁺ macrophage population in THP-1 cells at days 0, 3, and 7 following stimulation with IL-4. **F**) Protein levels of M2 macrophage-associated factors, including ARG-1, MGL1, MGL2, and IL-10 in THP-1 cells were measured by western blotting. Mean ± standard deviation of three independent experiments is shown. ***$p < 0.001$ compared to the Control group or the Day 0 group

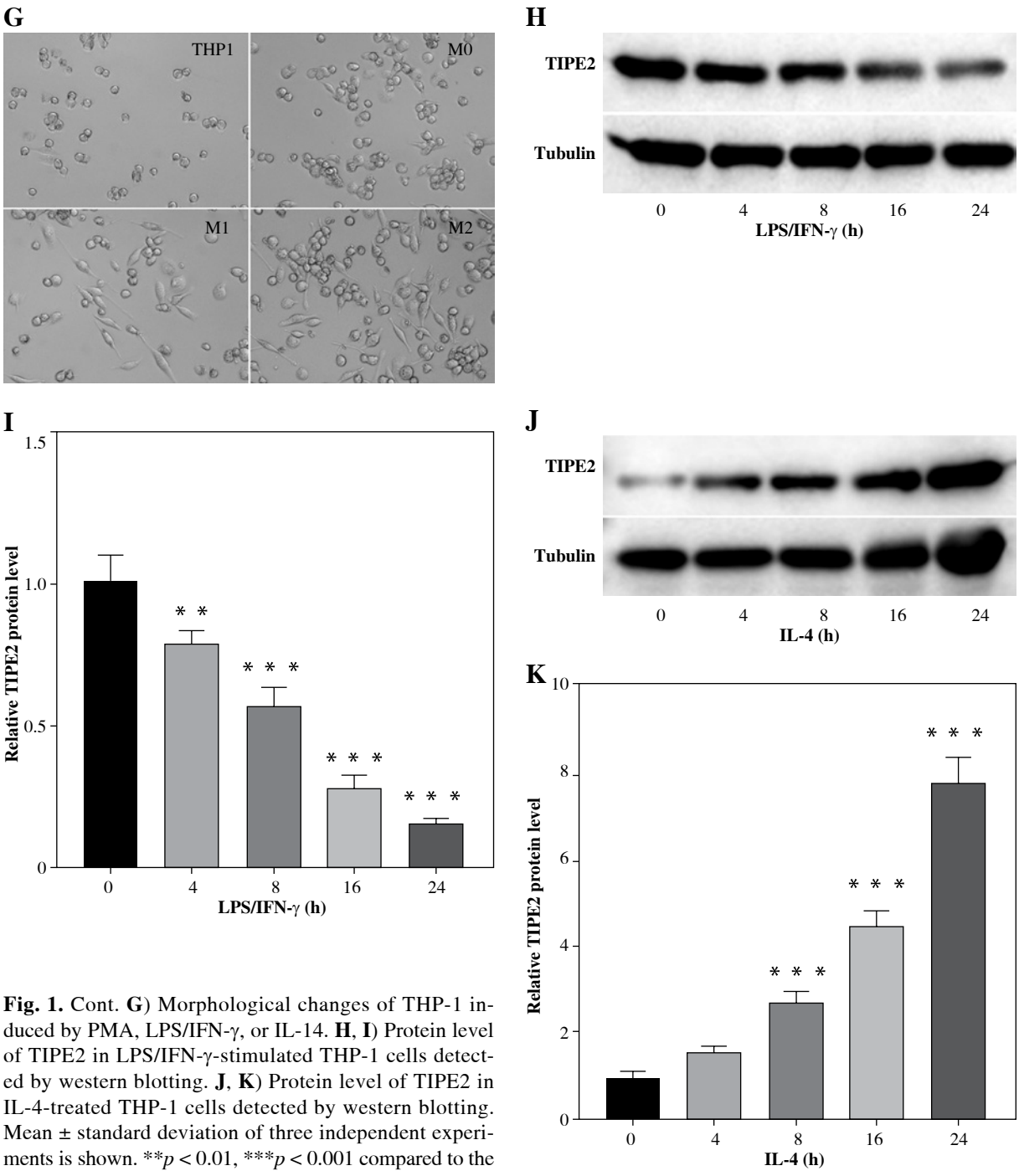

Fig. 1. Cont. **G**) Morphological changes of THP-1 induced by PMA, LPS/IFN-γ, or IL-14. **H**, **I**) Protein level of TIPE2 in LPS/IFN-γ-stimulated THP-1 cells detected by western blotting. **J**, **K**) Protein level of TIPE2 in IL-4-treated THP-1 cells detected by western blotting. Mean ± standard deviation of three independent experiments is shown. $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the LPS/IFN-γ (0 h) group or the IL-4 (0 h) group

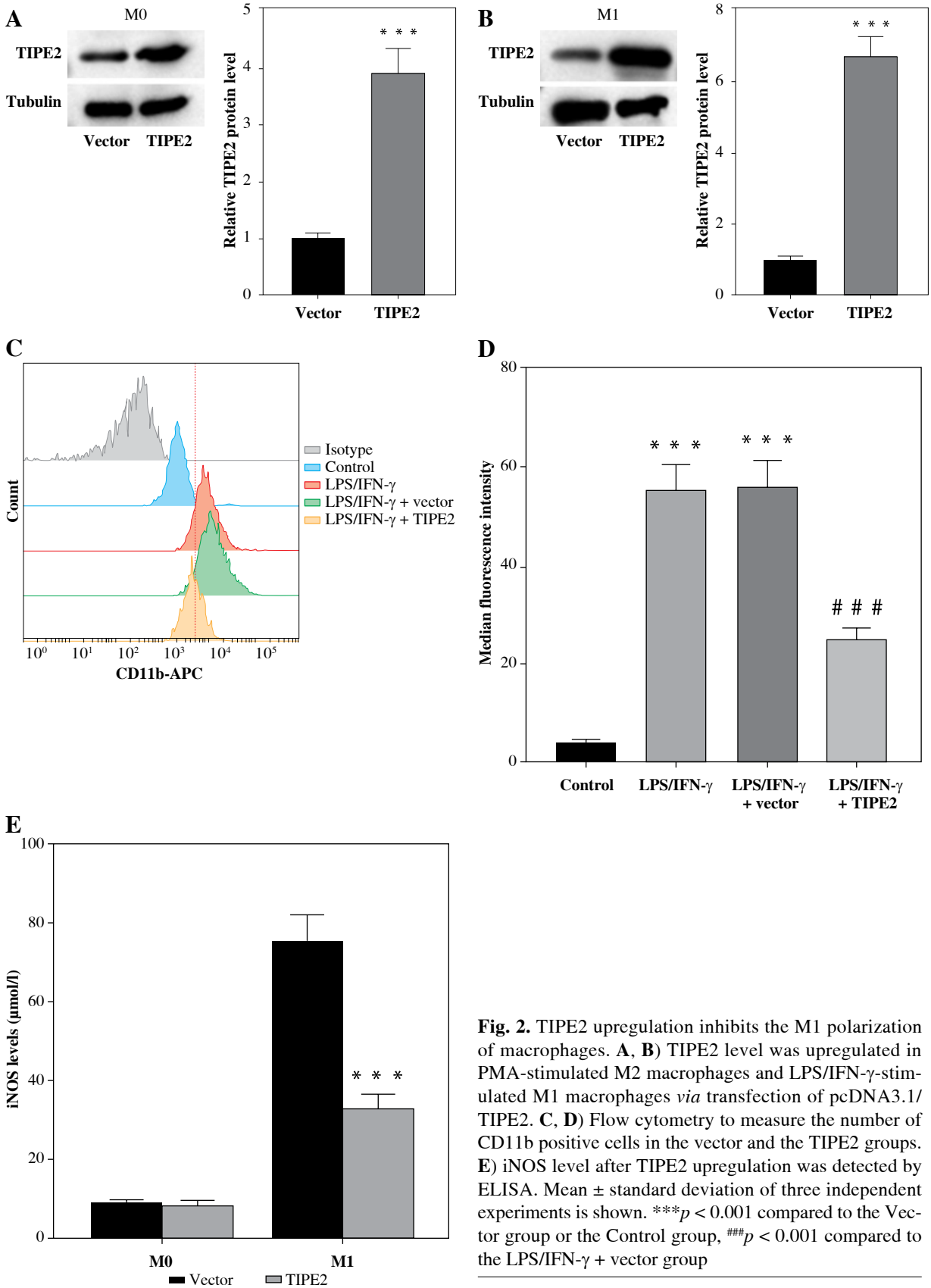

Fig. 2. TIPE2 upregulation inhibits the M1 polarization of macrophages. **A**, **B**) TIPE2 level was upregulated in PMA-stimulated M2 macrophages and LPS/IFN-γ-stimulated M1 macrophages *via* transfection of pcDNA3.1/TIPE2. **C**, **D**) Flow cytometry to measure the number of CD11b positive cells in the vector and the TIPE2 groups. **E**) iNOS level after TIPE2 upregulation was detected by ELISA. Mean ± standard deviation of three independent experiments is shown. ***$p < 0.001$ compared to the Vector group or the Control group, ###$p < 0.001$ compared to the LPS/IFN-γ + vector group

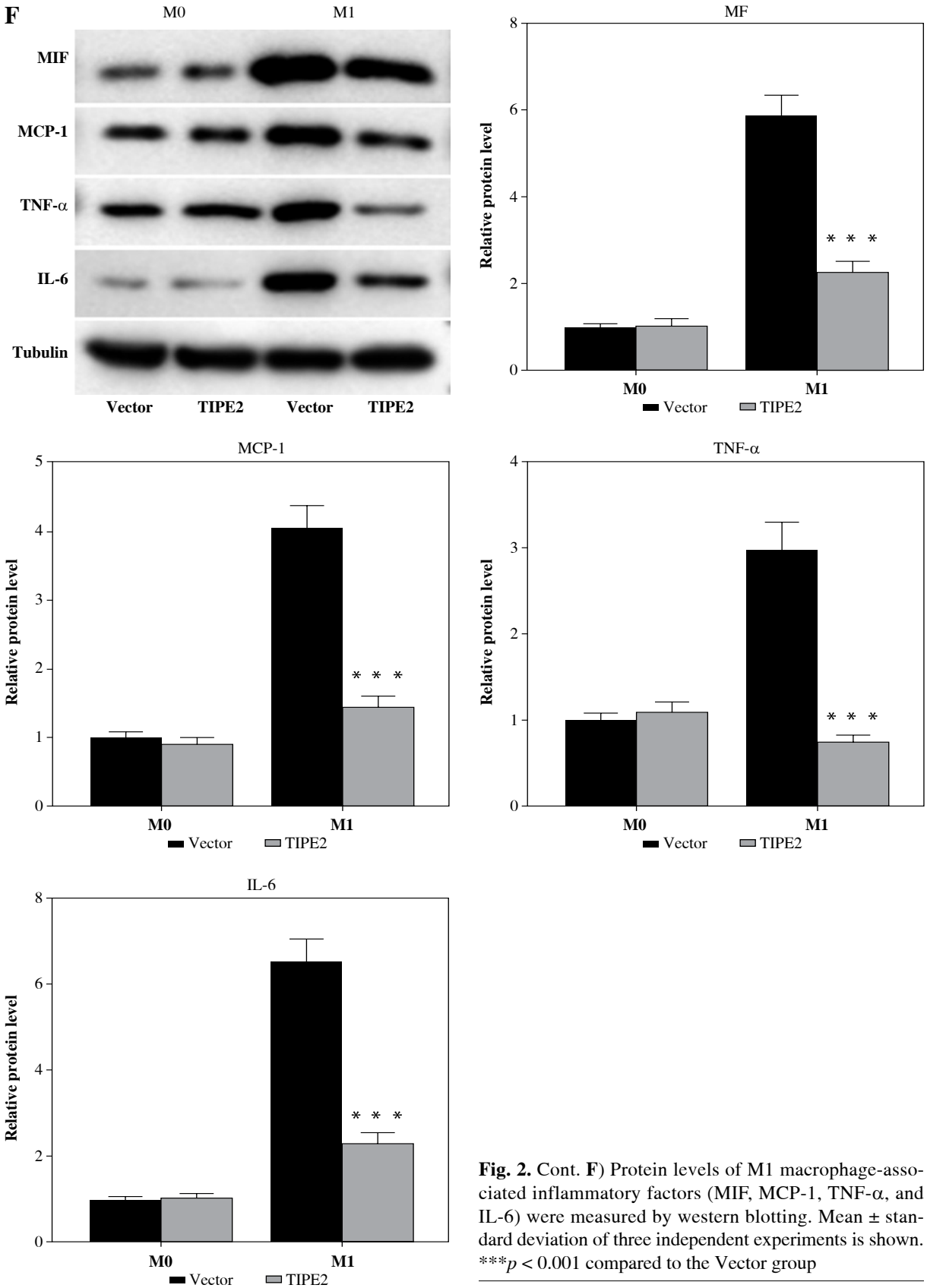

Fig. 2. Cont. **F**) Protein levels of M1 macrophage-associated inflammatory factors (MIF, MCP-1, TNF-α, and IL-6) were measured by western blotting. Mean ± standard deviation of three independent experiments is shown. ***$p < 0.001$ compared to the Vector group

downregulation significantly reduced the number of CD206 positive cells in M2 macrophages (Fig. 3C, D). The results of western blotting revealed that the protein levels of M2 macrophages-associated effectors (ARG-1, MGL1, MGL2, and IL-10) were decreased in M2 macrophages following TIPE2 downregulation (Fig. 3E). These results indicate that TIPE2 downregulation inhibits M2 macrophage differentiation.

TIPE2 inactivates the Rac1/NF-κB pathway in LPS/IFN-γ-induced M1 macrophages

The protein levels of Rac1 and p-p65 increased following LPS/IFN-γ stimulation, while TIPE2 upregulation reversed the promoting effects of LPS/IFN-γ stimulation, as western blotting revealed. In parallel, the protein level of p-IκB decreased in LPS/IFN-γ-treated THP-1 cells, while TIPE2 upregulation rescued the LPS/IFN-γ-stimulated inhibition (Fig. 4A). These results reveal that TIPE2 inactivates the Rac1/NF-κB pathway in LPS/IFN-γ-induced M1 macrophages.

TIPE2 knockdown inactivates the PI3K/AKT pathway in IL-4-induced M2 macrophages

As shown by western blotting, the protein levels of phospho-Y458-PI3K, phospho-T308-AKT, and phospho-S473-AKT were elevated following IL-4 stimulation,

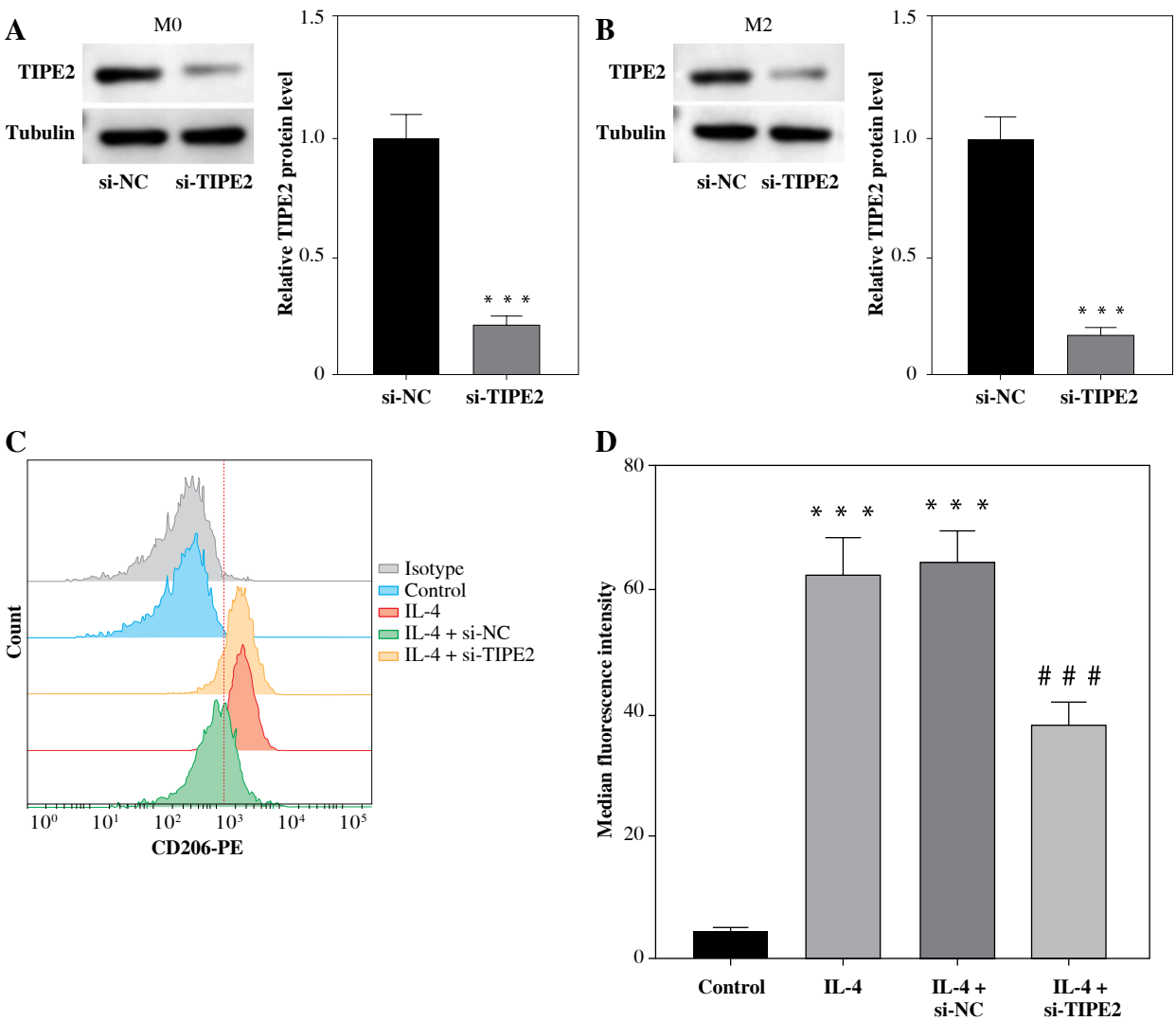

Fig. 3. TIPE2 downregulation inhibits the M2 polarization of macrophages. **A**, **B**) TIPE2 level was downregulated in PMA-induced M0 macrophages or IL-4-induced M2 macrophages *via* transfection of si-TIPE2, and the knockdown efficiency was estimated by western blotting. **C**, **D**) Flow cytometry to evaluate the number of CD206 positive cells after TIPE2 downregulation. Mean ± standard deviation of three independent experiments is shown. ***$p < 0.001$ compared to the si-NC or Control group, $^{\#\#\#}p < 0.001$ compared to the IL-4 + si-NC group

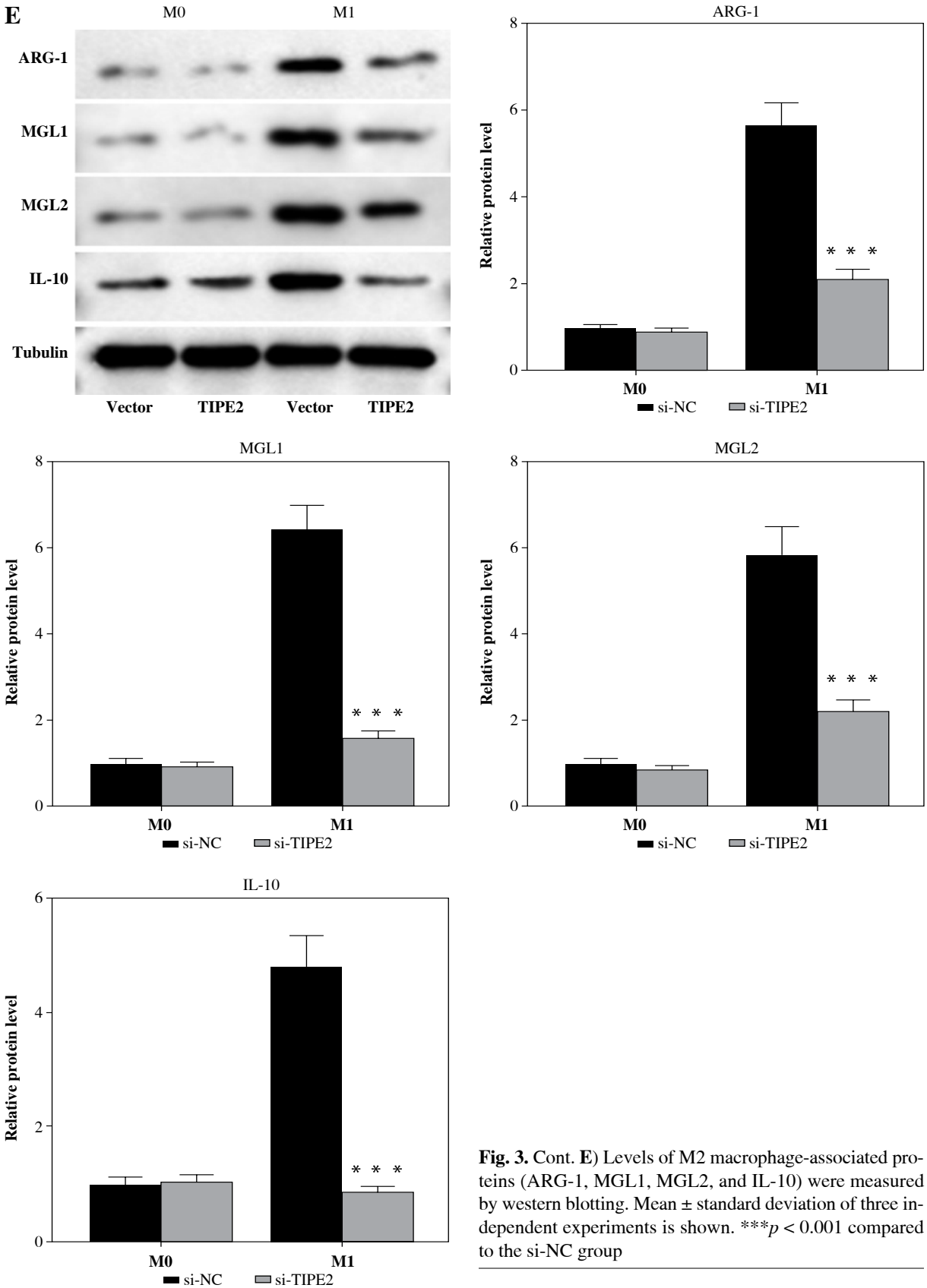

Fig. 3. Cont. **E**) Levels of M2 macrophage-associated proteins (ARG-1, MGL1, MGL2, and IL-10) were measured by western blotting. Mean ± standard deviation of three independent experiments is shown. ***$p < 0.001$ compared to the si-NC group

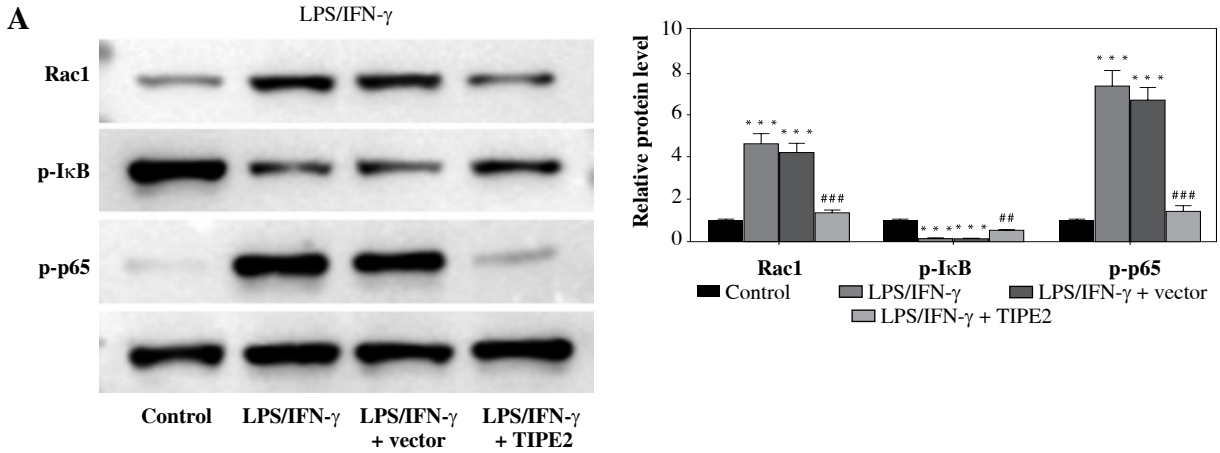

Fig. 4. TIPE2 inactivates the Rac1/NF-κB pathway in LPS/IFN-γ-induced M1 macrophages. **A**) Protein levels of Rac1, p-IκB, and p-p65 were measured by western blotting. Mean ± standard deviation of three independent experiments is shown. ***$p < 0.001$ compared to the Control group, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to the LPS/IFN-γ + vector group

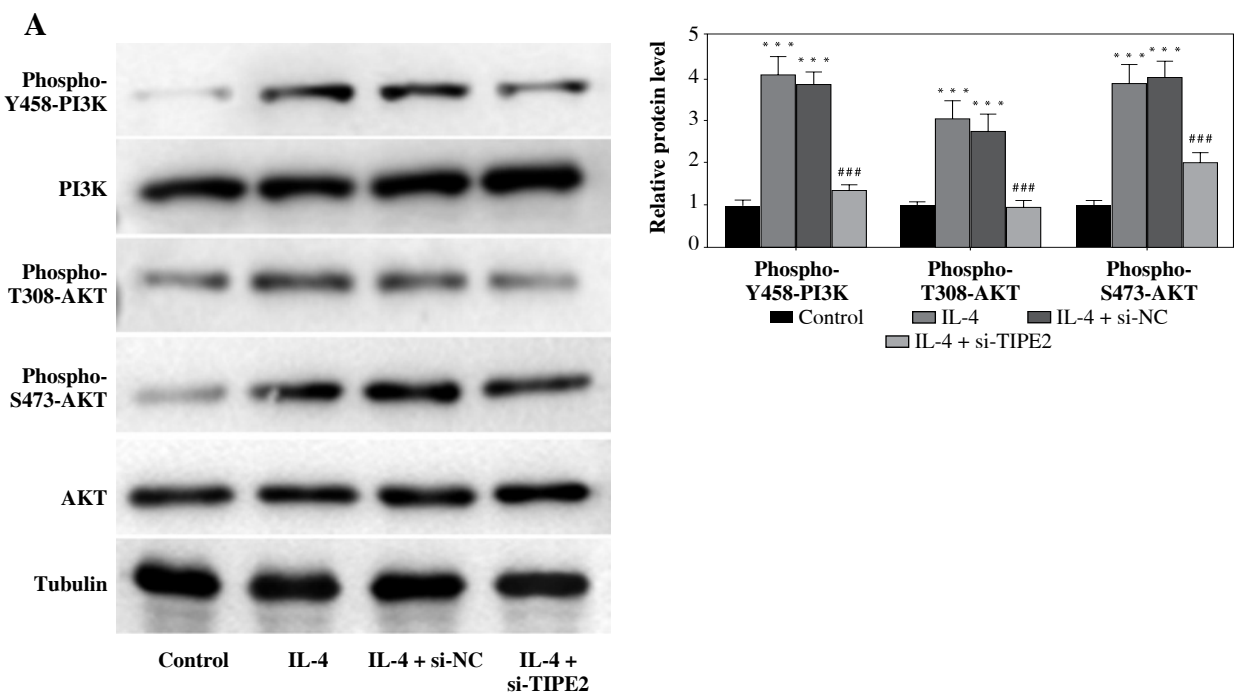

Fig. 5. TIPE2 activates the PI3K/AKT pathway in IL-4-induced M2 macrophages. **A**) Protein levels of phospho-Y458-PI3K, phospho-T308-AKT, and phospho-S473-AKT were measured by western blotting. Mean ± standard deviation of three independent experiments is shown. ***$p < 0.001$ compared to the Control group, $^{\#\#\#}p < 0.001$ compared to the IL-4 + si-NC group

while TIPE2 downregulation restored the IL-4-induced promotion in the pathway-associated proteins (Fig. 5A). These results show that TIPE2 knockdown inactivates the PI3K/AKT pathway in IL-4-induced M2 macrophages.

Discussion

Chronic rhinosinusitis with nasal polyps is an important clinical entity diagnosed by the presence of both subjective symptoms and objective evidence of chronic sinonasal inflammation [44]. Increasing evidence has indicated that the infiltration of macrophages, especially M2 macrophages, plays pivotal roles in the pathogenesis of CRSwNP [45, 46]. However, the underlying mechanisms remain undetermined. TIPE2 is found to be upregulated in eosinophilic polyps of patients with CRSwNP compared with non-eosinophilic polyps and control mucosa, and the number of TIPE2 cells, especially TIPE2 positive M2 macrophages, is correlated positively with the number of eosinophils and total inflammatory cells in sinonasal mucosa, as well as disease duration, CT scores, hyposmia scores, and polyp size in CRSwNP [46]. Our study aimed to investigate the roles and mechanisms of TIPE2 in inflammation and macrophage polarization.

The THP-1 cell line has been widely used as a macrophage model [47, 48]. Mucosal macrophages can differentiate into M1 and M2 phenotypes [49]. M1 phenotype is strongly associated with upregulation of integrin CD11b [50]. Upregulated CD206 is implicated in M2 macrophages [51]. M0 macrophages can be polarized into M1 macrophages through LPS/IFN-γ stimulation, or into M2 macrophages by stimulation with IL-4 [52]. In the current study, we treated THP-1 cells with LPS/IFN-γ and IL-4 and observed elevated CD11b positive LPS/IFN-γ-stimulated THP-1 cells and CD206 positive IL-4-stimulated THP-1 cells, indicating that LPS/IFN-γ successfully induced M1 polarization and IL-4 successfully promoted M1 polarization.

M1 macrophages secrete pro-inflammatory cytokines including iNOS, IL-6, and TNF-α, MIF as well as chemokines such as MCP-1 [53-56]. M2 macrophages express Arg-1 and IL-10, which play an anti-inflammatory role [57]. MGL1 and MGL2 are known as M2 macrophage markers [58]. A previous study suggested that the TIPE2 level is decreased following LPS/IFN-γ stimulation of M0 macrophages to induce polarization into M1 macrophages, while the TIPE2 level is elevated in IL-4-challengened M0 macrophages polarized into M2 macrophages [59]. Additionally, TIPE2 has been reported to promote M2 but inhibit M1 macrophage differentiation in vitro [37]. Moreover, TIPE2 induces macrophage polarization to M2 phenotype to alleviate experimental systemic lupus erythematosus [33]. In this study, we found that TIPE2 upregulation reduced the number of CD11b positive cells and the protein levels of MIF, MCP-1, TNF-α, and IL-6. Meanwhile, TIPE2 knockdown reduced the number of CD206 positive cells and downregulated the protein levels of ARG-1, MGL1, MGL2, and IL-10, indicating that TIPE2 promoted the transformation of macrophages from M1 phenotype to M2 phenotype. Accumulating evidence has suggested the inhibitory effects of TIPE2 on inflammatory responses in various diseases. For example, in hyperstretched bronchial epithelial cells, TIPE2 attenuates the release of asthma-related inflammatory factors [60]. Additionally, muscular dystrophy phenotype is alleviated by TIPE2 *via* inhibition in inflammation [61], Moreover, in hepatocytes exposed to metabolic stimulation, TIPE2 upregulation ameliorates lipid accumulation and inflammation [62]. We herein found that TIPE2 upregulation reduced the secretion of pro-inflammatory cytokines and chemokines, while TIPE2 downregulation reduced the levels of anti-inflammatory factors, indicating that TIPE2 alleviated the inflammation in THP-1 cells.

According to the findings of previous studies, TIPE2 regulates the Rac1/NF-κB pathway. For example, TIPE2 upregulation alleviates inflammatory pain by inhibiting the Rac1/NF-κB pathway [63]. Additionally, TIPE2 inhibits inflammation by inactivating NF-κB [64]. Moreover, TIPE2 negatively regulates inflammation following myocardial ischemia/reperfusion injury via inhibition of the NF-κB pathway [65]. Here we found that TIPE2 upregulation reduced the protein levels of Rac1 and p-p65, while increasing the protein level of IκB, suggesting that TIPE2 inhibited activation of the Rac1/NF-κB pathway.

As reported, TIPE2 can exert functions in the PI3K/AKT pathway. For example, TIPE2 upregulation inactivates PI3K/AKT signaling in airway smooth muscle cells [66]. TIPE2 suppresses atherosclerosis *via* inactivation of the PI3KAKT pathway [67]. Oppositely, TIPE2 enhances M2 macrophage polarization by activating the PI3K/AKT pathway [37]. During the differentiation of dendritic cells (DC), TIPE2 activates PI3K to increase the expression of CD80 and CD86 (DC maturation markers) [68]. Here we found that TIPE2 downregulation reduced the protein levels of phospho-Y458-PI3K, phospho-T308-AKT, and phospho-S473-AKT, indicating that TIPE2 knockdown inhibited the PI3K/AKT pathway.

However, this study has some limitations. First, our study only illuminated the regulatory effect of TIPE2 on macrophage M1/M2 polarization and its related signaling pathways. The biological functions of TIPE2 in CRSwNP progression should be investigated. Second, sinonasal mucosal tissues obtained from patients with CRSwNP and *in vivo* experiments are needed for further study.

In conclusion, this study demonstrated for the first time that TIPE2 could promote M2 but inhibit M1 macrophage differentiation and attenuate the inflammatory response in THP-1 cells through inhibition of the Rac1/NF-κB pathway and activation of the PI3K/AKT pathway. These findings provide insights into CRSwNP and help to identify novel treatment strategies.

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Disclosures

Approval of the Bioethics Committee was not required. The authors declare no conflict of interest.

Supplementary material is available on the journal's website.

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