

# Supervillin contributes to LPS-induced inflammatory response in THP-1 cell-derived macrophages

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## Research Article

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# Abstract

Supervillin (SVIL), the largest member of villin/gelsolin family, is an actin-binding and membrane-associated protein, that can also be localized to the nucleus. It has been reported that the mRNA expression of SVIL in neutrophils could be increased by lipopolysaccharide (LPS), but the underlying mechanisms remain unknown. Moreover, SVIL was also observed to be involved in the regulation of macrophages' movement. However, it is not clear whether SVIL is involved in the LPS-induced inflammatory response in macrophages. This work was to investigate the underlying molecular mechanisms of LPS regulating SVIL expression in macrophages and hence the possible role of SVIL in LPS-induced inflammation. Our data showed that in THP-1-derived macrophages, LPS stimulation significantly increased SVIL mRNA and protein expression. Inhibition of TLR4 by Resatorvid (Res) completely reversed the expression of SVIL and inflammatory cytokines (IL-6, IL-1 $\beta$  and TNF- $\alpha$ ) induced by LPS. Additionally, ERK1/2 and NF- $\kappa$ B inhibitors (U0126 and BAY) significantly reduced SVIL and IL-6, IL-1 $\beta$  & TNF- $\alpha$  expression. Furthermore, down-regulation of SVIL by SVIL-specific shRNA significantly attenuated the expression of IL-6, IL-1 $\beta$  & TNF- $\alpha$  induced by LPS. Taken together, as a downstream molecule of TLR4/NF- $\kappa$ B and ERK1/2, SVIL was involved in the inflammatory response of LPS-induced elevated IL-6, IL-1 $\beta$  and TNF- $\alpha$  in macrophages.

## 1. Introduction

Supervillin (SVIL), a member of villin/gelsolin family, is an actin-binding and membrane-associated protein participating in cell proliferation, motility, and signal transduction etc [1, 2]. Current research on SVIL mostly focuses on cancers and muscular tissue. It was reported that SVIL were involved in many cellular processes of tumor development and muscular function [3, 4]. SVIL was found to localize with the nucleus to participate in the intracellular signal transduction and early myogenesis of myoblasts [5, 6]. Recent evidence indicated that SVIL was a component of podosome and could bind to actin isoforms and myosin regulators contributing to regulation of podosome turnover, migration and invasion in primary human macrophages [7, 8]. However, the knowledge about the functions of SVIL in macrophage is still limited. It is far from clear whether and how SVIL participates in macrophage inflammatory response.

Lipopolysaccharide (LPS) is a key component in the outer membrane of Gram-negative bacteria. LPS was observed to stimulate macrophages to produce pro-inflammatory cytokines, IL-1 $\beta$ , IL-6 and TNF- $\alpha$  [9]. It was once found that the mRNA expression of SVIL was elevated by LPS stimulation in human neutrophils [10]. However, there is no more evidence of how LPS regulates SVIL, and what role SVIL plays after LPS stimulation. It might be speculated that SVIL could also response to this LPS stimulation and participate in the subsequent pro-inflammatory process in macrophage.

Toll-like receptor 4 (TLR4) is a definite receptor of LPS, which belongs to pattern recognition receptor (PRR) and triggers all the responses to LPS stimulation [11, 12]. The mitogen-activated protein kinase (MAPK) signal molecules (ERK1/2, JNK, P38) are activated as the downstream of TLR4 after LPS stimulation [13-15], leading to activation of NF- $\kappa$ B and hence the expression of inflammatory factors [16, 17]. In smooth muscle cells, SVIL has been identified as a ERK scaffolding protein and contributed to the regulation of ERK binding, activation, and release from the signaling complex [18, 19]. SVIL also activated EGFR-ERK1/2 and raised the motility in Hela cells [20]. In natural killer (NK) cells, SVIL was necessary for KIR2DL1-mediated ERK activation [21]. SVIL promoted liver cancer cell migration and invasion via the activation of the RhoA/ROCK-ERK/p38 pathway under hypoxia [22]. These reports highly suggest that SVIL may be closely related to TLR4-MAPK-NF- $\kappa$ B pathway and hence participate in macrophage inflammation response.

In this study, we identified that LPS stimulation markedly upregulated the expression of SVIL in macrophage via activating TLR4/NF- $\kappa$ B and ERK1/2 MAPK signaling pathway. The deficiency of SVIL significantly attenuated the expression of IL-6, IL-1 $\beta$  and TNF- $\alpha$  in macrophages induced by LPS. This novel finding helps elucidate the involvement of SVIL in LPS-induced inflammation.

## 2. Materials And Methods

### 2.1 Cell culture and reagents

THP-1 (human monocytic cells) cell line was obtained from Zhong Qiao Xin Zhou Biotechnology Company (shanghai, china). The THP-1 cells were grown in suspension in RPMI 1640 medium (Gibco, USA) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 0.05 mM 2-mercaptoethanol (Sigma, Saint Louis, USA), and cultured in a 5% CO<sub>2</sub> incubator with humidified atmosphere at 37°C. During the experiment, 100 ng/ml PMA (phorbol 12-myristate 13-acetate) induced THP-1 cells to differentiate from monocytes into macrophages. After attachment of the bottom surface, PMA-contained medium were removed and cells were washed with D-Hanks. Then, cells were starved with serum-free RPMI 1640 medium for 24 h. The macrophages were treated with drugs according to the needs of experiments.

Phorbol 12-myristate 13-acetate (PMA,) and lipopolysaccharide (LPS) were purchased from Sigma (Saint Louis, USA). Lipofectamine TM 2000 transfection reagent and RNA isolation kit (TRIzol) were purchased from Invitrogen (California, USA). Annexin V-Alexa Fluor 647/PI Apoptosis Assay Kit (FMSAV647-100, FcMACS, NanJing, China) was used to detect cell apoptosis. HiScript<sup>®</sup> Q-RT SuperMix for qPCR and ChamQ SYBR qPCR Master Mix (without ROX) were purchased from Vazyme (Nanjing, China). Cell Counting Kit-8 (CCK-8) was obtained from Yeasen Biotech company (Shanghai, China). TLR4 inhibitor Resatorvid (Res), NF-κB inhibitor BAY11-7082 (BAY), ERK inhibitor U0126, P38 inhibitor SB203580 (SB), JNK inhibitor SP600125 (SP) were acquired from Selleck Chemicals Company (Houston, USA). Antibodies against NF-κB p65, p-NF-κB, p38 MAPK, p-p38 MAPK, ERK, p-ERK, JNK, p-JNK were purchased from Cell Signaling Technologies (MA, USA). Antibodies to Tubulin and GAPDH were received from Proteintech (Chicago, USA). anti-supervillin (SVIL) was purchased from Sigma(Saint Louis, USA).

### 2.2 Real-time PCR (RT-PCR)

Total RNA was extracted from treated THP-1-derived macrophages by using TRIzol reagent. For cDNA synthesis, HiScript II Q-RT superMix was applied to reverse transcription reaction. The cDNA was used for qRT-PCR with SYBR green under standard reaction condition by Bio-Rad CFX Connect System (Bio-Rad, Hercules, CA, USA). The primers for SVIL, IL-1β, IL-6, TNF-α, GAPDH were provided by GENERAY (Shanghai, China) and the sequence were shown in Table 1.

### 2.3 Protein isolation and western blots analysis

The proteins were obtained from treated cells, which were lysed by RIPA buffer, which contained PMSF (1%), protease (10%) inhibitor and phosphatase (10%) inhibitor (Roche). and the concentration of protein was assessed by BCA (Bicinchoninic Acid) method. 30 μg of protein lysate was added to each lane and separated by SDS-PAGE, then transferred electrically to polyvinylidene fluoride (PVDF) membranes (Millipore). Then the PVDF membranes were blocked with 5% non-fat dry milk or BSA in TBST solution for 90 minutes and incubated with primary antibodies overnight at 4°C. Membranes were washed with TBST and incubated with HRP-conjugated secondary antibodies for 1h. After washed with TBST, membranes were added with SuperBright Subpico ECL substrate and visually observed by Chemiluminescence gel imaging system (SYNGENE). The Band densities were quantified by GELPRO4 software. The dilution ratio of primary antibody as follow: SVIL (1:500), GAPDH (1:5000), Tubulin (1:5000), p-ERK1/2 (1:1000), p-P38 (1:1000), p-JNK (1:1000), p-NF-κB (1:1000), ERK1/2 (1:1000), P38 (1:1000), JNK (1:1000), NF-κB (1:1000).

### 2.4 Lentiviral encapsulation and infection

The sequences of shRNAs targeting SVIL (shSVIL) and a scrambled shRNA (shNC) were designed and constructed by Gene Pharma (shanghai, China). ShRNAs (shNC or shSVIL) and Lentiviral plasmids were packaged in HEK293FT cells with Lipofectamine 2000 and then lentiviral particles containing shRNA (shNC or shSVIL) were produced.

THP-1 cells were infected with Lentiviruses containing shNC or shSVIL by polybrene (8 µg/ml), then the infected cells were selected by puromycin (1 µg/ml) for about 2 weeks. Cells expressing green fluorescence were also differentiated by PMA and were used in subsequent experiments.

## 2.5 Flow cytometry analysis

The treated THP-1 Cells were harvested and stained with the Annexin V-FITC and PI to detect cell apoptosis. Briefly, the harvested cells were resuspended in 500 µl binding buffer (1x) and incubation with 5 µl Annexin V-FITC and 10 µl PI for 15 min in the dark, then cell apoptosis was detected by flow cytometry (BD FACS Calibur, NY) according to the manufacturer's instructions.

Cell cycle distribution changes were also measured by flow cytometry (BD FACS Calibur, NY). The cells were collected and fixed with 75% ethanol and stored at 4°C. Then, cell cycle was detected by flow cytometry according to the manufacturer's instructions.

## 2.6 Cell proliferation assay

Cell proliferation was measured by CCK-8. The cells were seeded into 96-well plates at a density of  $1 \times 10^4$  cells/well and incubated at 37°C. Then, cells were treated with/without indicated drugs and indicated times. Next, 10 µl CCK-8 solution was added into each well and incubated for 2.5 h. The cells viability was measured by microplate reader at the absorbance of 450nm.

## 2.7 Statistical analysis

The data was analyzed with GraphPad Prism 5.0 by two-tailed t-test (comparison of two groups) or one-way analysis followed by Turkey tests (comparison of multiple groups). \* $p < 0.05$  was considered to be statistically significant. In our study, each experiment was repeated more than three times respectively.

# 3. Results

## 3.1 LPS induced SVIL expression in THP-1 macrophages

Here, we detected the mRNA and protein expression levels of SVIL in THP-1-derived macrophages after LPS stimulation. LPS treatment significantly induced the mRNA expression (Fig. 1A) and protein expression (Fig. 1B) of SVIL in a dose-dependent manner and the concentration (100 ng/ml) of LPS was selected for following experiments. As shown in Fig. 1C and D, LPS treatment induced the highest mRNA expression level of SVIL at 3 h and the protein expression reached the peak at 9 h. These results indicated that LPS stimulation could significantly enhance the expression of SVIL in macrophages.

## 3.2 TLR4/NF-κB mediated LPS-induced SVIL expression in macrophages

TLR4 has been assumed to trigger all responses to LPS since it was identified as the LPS receptor [23-25]. However, whether TLR4 participates in LPS-induced expression of SVIL is not known yet. Here, Resatorvid (Res), a specific inhibitor of TLR4, to detect whether it could inhibit the expression of SVIL induced by LPS in macrophages. As shown in Fig. 2A and B, LPS led to a significant increase of SVIL mRNA expression level, and the protein expression level was also increased remarkably. LPS-induced SVIL expression was significantly suppressed by pretreatment of Res at both mRNA (Fig. 2A) and protein (Fig. 2B) levels.

As the above data show, LPS caused a significant up-regulation in the mRNA level of SVIL via TLR4 (Fig. 1A and C, Fig. 2A). As known, the nuclear transcription factor NF-κB is a classical downstream signal molecule of TLR4. Hence, we

speculated that NF- $\kappa$ B was also involved in the regulation of SVIL by LPS. As shown in Fig. 2C, LPS stimulation increased the phosphorylation level of NF- $\kappa$ B, which could be significantly reversed by Res. To investigate whether NF- $\kappa$ B participated in LPS-induced SVIL expression, NF- $\kappa$ B inhibitor BAY were used and significant inhibition of LPS-induced phosphorylation of NF- $\kappa$ B was observed (Fig. 2D). As expected, pretreatment of the cells with BAY reversed LPS-induced SVIL mRNA (Fig. 2E) and protein expression (Fig. 2F). Therefore, these results suggested that TLR4/NF- $\kappa$ B mediated LPS-induced SVIL expression in macrophages.

### 3.3 ERK1/2 MAPK mediated LPS-induced SVIL expression in macrophages

Although inhibition of NF- $\kappa$ B could reverse the up-regulation of SVIL induced by LPS, the reversion was not complete (Fig. 2E and F). Thus, we wondered if there were other signaling pathways involved in this process. Many studies demonstrated that TLR4 activation can activate MAPK signal pathway and initiate intracellular response [26-28]. In order to verify whether MAPK family was involved in the regulation of SVIL by LPS, we pretreated cells with ERK1/2 inhibitor U0126, JNK inhibitor SP or P38 inhibitor SB, respectively, before LPS stimulation. Interestingly, only U0126 inhibited LPS-induced mRNA expression of SVIL, while SP and SB did not reverse this efficiency of LPS (Fig. 3A). Therefore, the following research mainly focused on the ERK1/2 MAPK signaling pathway.

As shown in Fig. 3B, LPS stimulation increased the phosphorylation of ERK1/2 and this effect could be reversed by Res. U0126 markedly inhibited LPS-induced ERK1/2 phosphorylation (Fig. 3C). Western blot analysis showed that SVIL protein expression induced by LPS was also significantly down-regulated by U0126 (Fig. 3D). Additionally, U0126 could partially inhibit LPS-induced NF- $\kappa$ B phosphorylation (Fig. 3E). These results indicated that ERK1/2 MAPK was at least partially involved in the regulation of SVIL by LPS.

### 3.4 SVIL Knockdown efficiency in THP-1 cells

To further investigate the role of SVIL in LPS-induced macrophage inflammatory response, we used SVIL-targeted shRNA to interfere with its genetic expression. Expression of LV(H1/GFP&Puro)-shNC and LV(H1/GFP&Puro)-shSVIL was confirmed by detection of green fluorescent protein (GFP)-fluorescence in cells (Fig. 4A). Then, we detected the mRNA expression (Fig. 4B), protein expression (Fig. 4C) of SVIL in the transfected cells. As shown, the data indicated that SVIL was significantly knocked down by shRNA. The shSVIL THP-1 cells with the highest downregulation efficiency of SVIL expression was selected for the present study.

### 3.5 The influence of SVIL knockdown on cell cycle, cell proliferation & apoptosis

The effects of SVIL depletion on the THP-1 viability was detected by cell flow cytometry and cell proliferation assay. Cell cycle was evaluated by measuring DNA content, as shown in Fig. 5A and B, PMA significantly reduced the proportion of cells in G<sub>0-1</sub> and S phases, while the proportion of cells in G<sub>2</sub>/M phases was increased remarkably in shNC THP-1 cells. After PMA stimulation, the number of cells were significantly inhibited compared with the control group (Fig. 5C). These results demonstrated that PMA significantly inhibited the proliferation of THP-1 cells in the process of inducing THP-1 to differentiate into macrophages (Fig. 4A).

As shown in Fig. 5A and B, without PMA stimulation, SVIL ablation increased the proportion of cells in G<sub>0-1</sub> phase and decreased the proportion of cells in G<sub>2</sub>/M phase, while the proportion of cells in S phase with no significant difference. After THP-1 cells were induced to differentiate into macrophages by PMA, the knockdown of SVIL caused similar changes in G<sub>0-1</sub> and G<sub>2</sub>/M phases, and significantly increased the proportion of cells in S phase. However, CCK8 data showed that SVIL knockdown did not affect the final change in cell numbers compared with control group with or without PMA incubation (Fig. 5C). As shown in Fig. 5D, SVIL depletion also could not induce apoptosis of THP-1 cells.

### 3.6 SVIL depletion inhibited LPS-induced expression of inflammatory cytokines

LPS stimulation induces pro-inflammatory responses of macrophages and promotes the production of inflammatory cytokines, such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$  [9, 15]. Moreover, SVIL has been identified as an interacting protein of Sphingosine kinase 1 (sphk1) [29], and sphk1 is reported to involve in macrophage inflammation induced by LPS [30, 31]. Therefore, it was of interest to examine if SVIL depletion could change the regulation of LPS-induced pro-inflammatory responses in THP-1 macrophages. Indeed, LPS stimulation significantly induced the expression of inflammatory cytokines, TNF- $\alpha$ , IL-6 and IL-1 $\beta$  (Fig. 6A-I). The mRNA expression of all the three inflammatory cytokines induced by LPS could be inhibited by TLR4 inhibitor Res (Fig. 6A-C), NF- $\kappa$ B inhibitor BAY (Fig. 6D-F) and ERK1/2 inhibitor U0126 (Fig. 6G-I).

Importantly, knockdown of SVIL produced opposite effects of LPS. As shown in Fig. 7A, LPS significantly increased the expression of SVIL in shNC macrophages, while the same stimulation condition could not induce the SVIL expression in shSVIL macrophages. Interestingly, we found that the depletion of SVIL in macrophages significantly reversed the mRNA expression of inflammatory factors IL-1 $\beta$ , IL-6 and TNF- $\alpha$  induced by LPS stimulation (Fig. 7B-D).

## 4. Discussion

As the first vital barrier for the prevention of cardiovascular diseases in the human cardiovascular system, macrophages respond to a variety of pathophysiological changes through the transformation of different functional phenotypes [32]. In the development of atherosclerosis, a chronic inflammatory disorder, macrophages are critically involved in all stages [33-35]. The persistent inflammatory state of macrophages can lead to the accumulation of fragments and apoptotic cells, promoting the deterioration of atherosclerotic plaques [36]. The occurrence of macrophage inflammation in atherosclerotic plaque is affected by various factors, and the infection of various bacteria is also one of the important factors [36, 37]. Lipopolysaccharide (LPS), a key component in the outer membrane of Gram-negative bacteria, induced inflammatory response of macrophages in vitro. Just as LPS induces the expression of SVIL in neutrophils [10], here we demonstrate that LPS could enhance the mRNA and protein expression of SVIL in THP-1-derived macrophages. SVIL has been reported to be involved in the regulation of podosome function in macrophages [7, 8]. As far as we know, the specific mechanism of LPS's SVIL-increasing effect has not been reported yet. In this paper, we found the signaling pathway of LPS regulating SVIL in macrophages. Our results may provide some useful basis for discussing the relationship between LPS and SVIL, and hence gaining a new insight into some inflammatory conditions like atherosclerosis.

Since TLR4 was firstly identified as the receptor of LPS in 1998, various studies have shown that it is the most important cell surface receptor of LPS, in almost all responses caused by LPS [25]. The activation of TLR4 by LPS cause various intracellular changes, i.e. the production of inflammatory factors in macrophages [38]. And our data showed that LPS increased SVIL expression in macrophages. Therefore, in the process of regulating SVIL expression, TLR4 was the first receptor that we considered in response to LPS stimulation. As expected, LPS activated the TLR4 receptor and markedly enhanced the expression of SVIL in macrophages. The inhibition of TLR4 with Res could significantly reverse this SVIL-inducing effect of LPS. Thus, TLR4 played a critical role in the process of LPS regulating the expression of SVIL in macrophages.

Our results showed that LPS could significantly increase the mRNA expression of SVIL in macrophages, which was consistent with Morozumin T's report in neutrophils [10]. In this study, we further investigated the transcription factors mediating the transcription of SVIL induced by LPS. The nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B), is known to be a key downstream nuclear transcription signaling effector of the TLR4 pathway [39, 40]. Therefore, it is believable that NF- $\kappa$ B would mediate the up-regulation of SVIL mRNA expression by LPS. As expected, LPS stimulation induced the phosphorylation of NF- $\kappa$ B and this effect could be inhibited by TLR4 inhibitor Res pre-treatment. BAY, the NF- $\kappa$ B inhibitor also significantly attenuated LPS-induced mRNA and protein expression of SVIL in macrophages. These results indicated that TLR4-NF- $\kappa$ B contributed to LPS-induced SVIL expression in macrophages. To our knowledge, it is the first time to demonstrate that LPS-induced SVIL expression is mediated by TLR4-NF- $\kappa$ B pathway. Moreover, we found that inhibition of TLR4 almost completely reversed the expression of SVIL protein induced by LPS, but inhibition of NF- $\kappa$ B could not achieve

the same efficacy. Therefore, these results suggest that in addition to NF- $\kappa$ B, there may exist other signaling molecules in the downstream of TLR4 that participated in the regulation of SVIL by LPS.

MAPKs are another TLR4-mediated activated protein kinases, which are also responsible for cellular responses induced by LPS, such as the production of proinflammatory cytokines [39, 41]. Therefore, we used ERK, JNK and P38 inhibitors to investigate the effect of MAPK on LPS-induced SVIL expression. Although TLR4 mediated LPS-induced phosphorylation of ERK, P38 and JNK, it was found that only ERK inhibitor U0126 could partially reverse LPS-induced SVIL mRNA and protein expression, while P38 inhibitor SB and JNK inhibitor SP had no significant effect. This result is consistent with previous studies that SVIL participated in ERK signal transduction and promoted epithelial-mesenchymal transformation [20-22]. Furthermore, U0126 was observed to inhibit LPS-induced NF- $\kappa$ B phosphorylation, indicating that U0126 inhibited LPS-induced SVIL expression perhaps partly through NF- $\kappa$ B pathway. Taken together, our research provided a certain basis for exploring the relationship between SVIL and ERK.

SVIL contributed to cytokinesis and mainly played a regulatory role in earlier cytokinesis by interacting with central spindle proteins and myosin II [42, 43]. Similarly, SVIL could also enhance cell survival and maintain the cell proliferative activity [3, 44]. Our results showed that SVIL depletion increased the proportion of cells in S phase and decreased the proportion of cells in G<sub>2</sub>/M phase. However, CCK8 data showed that cell proliferation was not affected. This result is partially consistent with the report that SVIL had some effect on cell division in M phase [42, 43]. The S-phase-related effect remains to be elucidated.

SVIL was reported to be an interacting protein of sphk1 [29], and involved in cellular inflammation together with sphk1. However, there were no more reports on the relationship between inflammation and SVIL, which was a macromolecular protein closely related to cytoskeleton. Due to the lack of literature about the role of SVIL in inflammation, it was challenging but interesting to demonstrate whether SVIL was involved in the regulation of LPS-induced macrophage inflammation. In our study, LPS induced the expressions of inflammatory cytokines and SVIL in macrophages. Interestingly, we found that after interfering with the expression of SVIL, the expressions of inflammatory cytokines (IL-6, IL-1 $\beta$  and TNF- $\alpha$ ) in macrophages induced by LPS were significantly inhibited. These data suggested that SVIL played an important role in LPS-induced inflammation.

In conclusion, this is the first study investigating the involvement of SVIL in LPS-induced macrophages inflammatory response. As depicted in Fig. 8, our current study clearly showed that LPS induced the mRNA and protein expressions of SVIL in macrophages via activating TLR4/NF- $\kappa$ B and ERK1/2 MAPK signaling pathway. Additionally, depletion of SVIL could significantly reverse the expression of inflammatory factors in macrophages induced by LPS. The present study provides a basis for exploring the relationship between SVIL and inflammation, and SVIL may be a potential target for the therapy of vascular inflammatory diseases such as atherosclerosis.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

The materials and analyzed data sets generated during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

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## Authors' contributions

Shengnan Li and Jun Zhou designed the study, and drafted the manuscript. Jun Zhou, Yuhui Que, Lihua Pan and Xu Li performed the experiments and analyzed the data. Lai Jin contributed essential reagents and revised the manuscript. Chao Zhu revised the manuscript. All authors read and approved the final manuscript.

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## References

1. Pestonjamas, K.N., Pope, R.K., Wulfschlegel, J.D., Luna, E.J. 1997. Supervillin (p205): a novel membrane-associated, F-actin-binding protein in the villin/gelsolin superfamily. *J Cell Biol* 139: 1255-1269.
2. Crowley, J.L., Smith, T.C., Fang, Z., Takizawa, N., Luna, E.J. 2009. Supervillin reorganizes the actin cytoskeleton and increases invadopodial efficiency. *Mol Biol Cell* 20: 948-962.
3. Fang, Z., Luna, E.J. 2013. Supervillin-mediated suppression of p53 protein enhances cell survival. *J Biol Chem* 288: 7918-7929.
4. Spinazzola, J.M., Smith, T.C., Liu, M., Luna, E.J., Barton, E.R. 2015. Gamma-sarcoglycan is required for the response of archvillin to mechanical stimulation in skeletal muscle. *Hum Mol Genet* 24(9): 2470-2481.
5. Ting, H.J., Yeh, S., Nishimura, K., Chang, C. 2002. Supervillin associates with androgen receptor and modulates its transcriptional activity. *Proc Natl Acad Sci USA* 99(2): 661-666.
6. Oh, S.W., Pope, R.K., Smith, K.P., Crowley, J.L., Nebl, T., Lawrence, J.B., et al. 2003. Archvillin, a muscle-specific isoform of supervillin, is an early expressed component of the costameric membrane skeleton. *J Cell Sci* 116: 2261-2275.
7. Bhuvania, R., Cornfine, S., Fang, Z., Kruger, M., Luna, E.J., Linder, S. 2012. Supervillin couples myosin-dependent contractility to podosomes and enables their turnover. *J Cell Sci* 125: 2300-2314.
8. Cervero, P., Wiesner, C., Bouissou, A., Poincloux, R., Linder, S. 2018. Lymphocyte-specific protein 1 regulates mechanosensory oscillation of podosomes and actin isoform-based actomyosin symmetry breaking. *Nat Commun* 9(1): 515.
9. Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaili, S.A., Mardani, F., Seifi, B., Mohammadi, A., Afshari, J.T., Sahebkar, A. 2018. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol* 233(9): 6425-6440.
10. Morozumi, T., Kubota, T., Sugita, N., Ohsawa, Y., Yamazaki, K., Yoshie, H. 2001. Elevated mRNA expression for supervillin and vascular endothelial growth factor in human neutrophils stimulated with lipopolysaccharide from *Porphyromonas gingivalis*. *J Periodont Res* 36(3): 160-168.
11. Peri, F., Piazza, M. 2012. Therapeutic targeting of innate immunity with Toll-like receptor 4 (TLR4) antagonists. *Biotechnol Adv* 30(1): 251-260.



12. Di-Lorenzo, F., De-Castro, C., Silipo, A., Molinaro, A. 2019. Lipopolysaccharide structures of Gram-negative populations in the gut microbiota and effects on host interactions. *FEMS Microbiol Rev* 43(3): 257-272.
13. Dhande, I., Ma, W., Hussain, T. 2015. Angiotensin AT2 receptor stimulation is anti-inflammatory in lipopolysaccharide-activated THP-1 macrophages via increased interleukin-10 production. *Hypertens Res* 38(1): 21-29.
14. Liu, D., Cao, G., Han, L., Ye, Y., SiMa, Y., Ge, W. 2016. Flavonoids from Radix Tetrastigmae inhibit TLR4/MD-2 mediated JNK and NF- $\kappa$ B pathway with anti-inflammatory properties. *Cytokine* 84: 29-36.
15. Bode, J.G., Ehltling, C., Häussinger, D. 2012. The macrophage response towards LPS and its control through the p38(MAPK)-STAT3 axis. *Cell Signal* 24(6): 1185-1194.
16. Guha, M., Mackman, N. 2001. LPS induction of gene expression in human monocytes. *Cell Signal* 13(2): 85-94.
17. Mandrekar, P., Szabo, G. 2009. Signalling pathways in alcohol-induced liver inflammation. *J Hepatol* 50(6): 1258-1266.
18. Gangopadhyay, S.S., Kengni, E., Appel, S., Gallant, C., Kim, H.R., Leavis, P., et al. 2009. Smooth muscle archvillin is an ERK scaffolding protein. *J Biol Chem* 284(26): 17607-17615.
19. Li, Y., Reznichenko, M., Tribe, R.M., Hess, P.E., Taggart, M., Kim, H., DeGnore, J.P., Gangopadhyay, S., Morgan, K.G. 2009. Stretch activates human myometrium via ERK, caldesmon and focal adhesion signaling. *PLoS ONE* 4(10): e7489.
20. Fang, Z., Takizawa, N., Wilson, K.A., Smith, T.C., Delprato, A., Davidson, M.W., et al. 2010. The membrane-associated protein, supervillin, accelerates F-actin-dependent rapid integrin recycling and cell motility. *Traffic* 11(6): 782-799.
21. Liu, H.P., Yu, M.C., Jiang, M.H., Chen, J.X., Yan, D.P., Liu, F., Ge, B.X. 2011. Association of supervillin with KIR2DL1 regulates the inhibitory signaling of natural killer cells. *Cell Signal* 23(2): 487-496.
22. Chen, X., Zhang, S., Wang, Z., Wang, F., Cao, X., Wu, Q., Zhao, C., Ma, H., Ye, F., Wang, H., Fang, Z. 2018. Supervillin promotes epithelial-mesenchymal transition and metastasis of hepatocellular carcinoma in hypoxia via activation of the RhoA/ROCK-ERK/p38 pathway. *J Exp Clin Cancer Res* 37(1): 128.
23. Poltorak, A., He, X., Smirnova, I., Liu, M.Y., Van-Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenburg, M., Ricciardi-Castagnoli, P., Layton, B., Beutler, B. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282(5396): 2085-2088.
24. Beutler, B., Du, X., Poltorak, A. 2001. Identification of Toll-like receptor 4 (Tlr4) as the sole conduit for LPS signal transduction: genetic and evolutionary studies. *J Endotoxin Res* 7(4): 277-280.
25. Płóciennikowska, A., Hromada-Judycka, A., Borzęcka, K., Kwiatkowska, K. 2015. Co-operation of TLR4 and raft proteins in LPS-induced pro-inflammatory signaling. *Cell Mol Life Sci* 72: 557-581.
26. Palsson-McDermott, E.M., O'Neill, L.A. 2004. Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4. *Immunology* 113: 153-162.
27. Moresco, E.M., LaVine, D., Beutler, B. 2011. Toll-like receptors. *Curr Biol* 21: 488-493.
28. Mausumee, Guha., Nigel, Mackman. 2001. LPS induction of gene expression in human monocytes. *Cell Signal* 13: 85-94.
29. Yagoub, D., Wilkins, M.R., Lay, A.J., Kaczorowski, D.C., Hatoum, D., Bajan, S., Hutvagner, G., Lai, J.H., Wu, W., Martiniello-Wilks, R., Xia, P., McGowan, E.M. 2014. Sphingosine kinase 1 isoform-specific interactions in breast cancer. *Mol Endocrinol* 28(11): 1899-1915.
30. Jin, J., Lu, Z., Li, Y., Ru, J.H., Lopes-Virella, M.F., Huang, Y. 2018. LPS and palmitate synergistically stimulate sphingosine kinase 1 and increase sphingosine 1 phosphate in RAW264.7 macrophages. *J Leukoc Biol* 104(4): 843-853.
31. Pchejetski, D., Nunes, J., Coughlan, K., Lall, H., Pitson, S.M., Waxman, J., Sumbayev, V.V. 2011. The involvement of sphingosine kinase 1 in LPS-induced Toll-like receptor 4-mediated accumulation of HIF-1 $\alpha$  protein, activation of ASK1 and production of the pro-inflammatory cytokine IL-6. *Immunol Cell Biol* 89: 268-274.

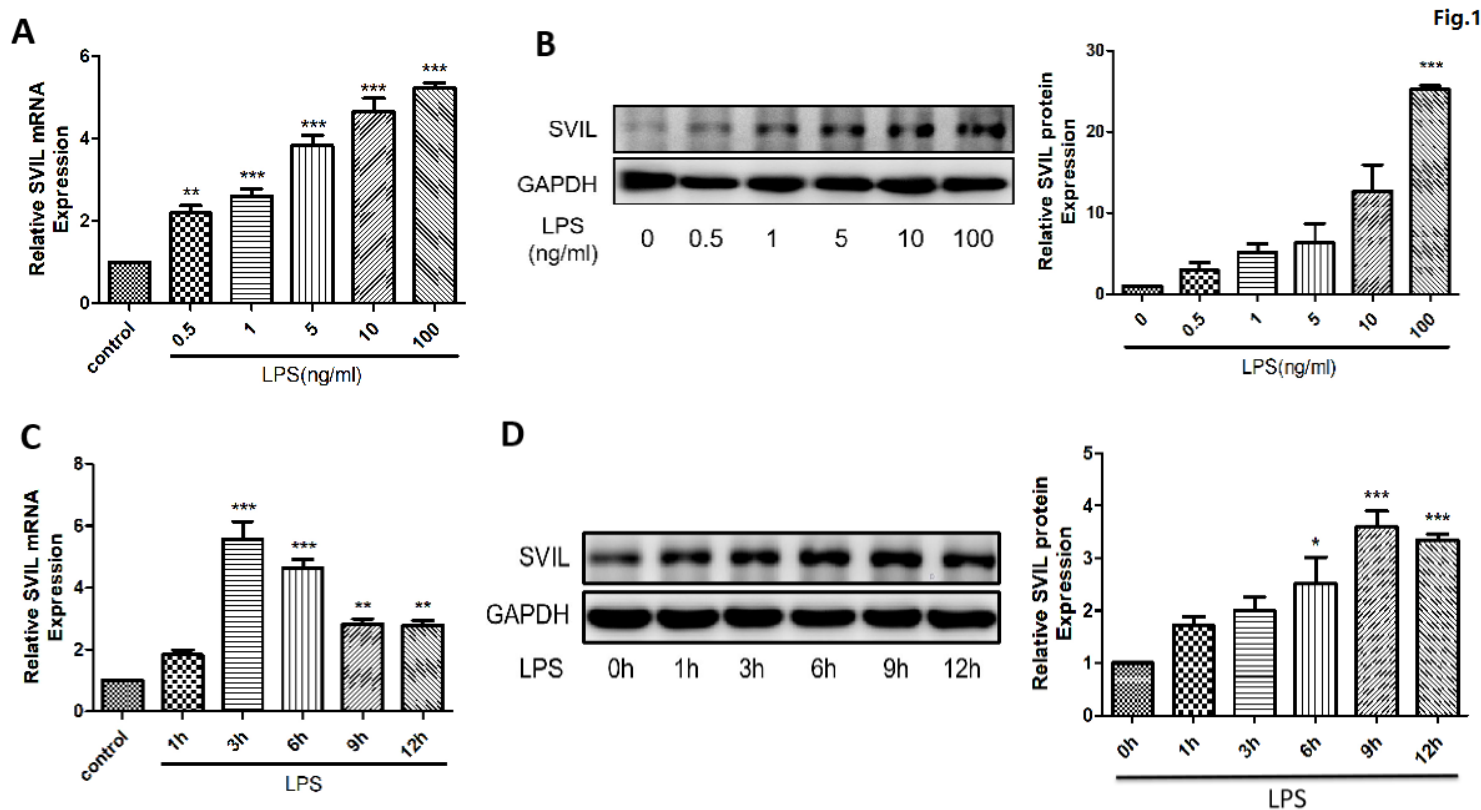
32. Nasser, M.I., Zhu, S., Huang, H., Zhao, M., Wang, B., Ping, H., Geng, Q., Zhu, P. 2020. Macrophages: First guards in the prevention of cardiovascular diseases. *Life Sci* 250: 117559.
33. Fiordelisi, A., Iaccarino, G., Morisco, C., Coscioni, E., Sorriento, D. 2019. NFkappaB is a Key Player in the Crosstalk between Inflammation and Cardiovascular Diseases. *Int J Mol Sci* 20(7): 1599.
34. Honold, L., Nahrendorf, M. 2018. Resident and Monocyte-Derived Macrophages in Cardiovascular Disease. *Circ Res* 122(1): 113-127.
35. Bäck, M., Yurdagul, A., Tabas, I., Öörni, K., Kovanen, P.T. 2019. Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. *Nat Rev Cardiol* 16(7): 389-406.
36. Barrett, T.J. 2020. Macrophages in Atherosclerosis Regression. *Arterioscler Thromb Vasc Biol* 40: 20-33.
37. Kavurma, M.M., Rayner, K.J., Karunakaran, D. 2017. The walking dead: macrophage inflammation and death in atherosclerosis. *Curr Opin Lipidol* 28(2): 91-98.
38. Fu, Y.J., Xu, B., Huang, S.W., Luo, X., Deng, X.L., Luo, S., Liu, C., Wang, Q., Chen, J.Y., Zhou, L. 2021. Baicalin prevents LPS-induced activation of TLR4/NF-κB p65 pathway and inflammation in mice via inhibiting the expression of CD14. *Acta Pharmacol Sin* 42(1): 88-96.
39. Shang, L., Wang, T., Tong, D., Kang, W., Liang, Q., Ge, S. 2018. Prolyl hydroxylases positively regulated LPS-induced inflammation in human gingival fibroblasts via TLR4/MyD88-mediated AKT/NF-κB and MAPK pathways. *Cell Prolif* 51: e12516.
40. Luo, H., Wang, J., Qiao, C., Ma, N., Liu, D., Zhang, W. 2015. Pycnogenol attenuates atherosclerosis by regulating lipid metabolism through the TLR4-NF-κB pathway. *Exp Mol Med* 47: e191.
41. Kim, H.G., Shrestha, B., Lim, S.Y., et al. 2006. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-κB through Akt and p38 inhibition in RAW 264.7 macrophage cells. *Eur J Pharmacol* 545: 192-199.
42. Hasegawa, H., Hyodo, T., Asano, E., Ito, S., Maeda, M., Kuribayashi, H., et al. 2013. The role of PLK1-phosphorylated SVIL in myosin II activation and cytokinetic furrowing. *J Cell Sci* 126(16): 3627-3637.
43. Smith, T.C., Fridy, P.C., Li, Y., Basil, S., Arjun, S., Friesen, R.M., et al. 2013. Supervillin binding to myosin II and synergism with anillin are required for cytokinesis. *Mol Biol Cell* 24(23): 3603-3619.
44. Chen, X., Yang, H., Zhang, S., Wang, Z., Ye, F., Liang, C., et al. 2017. A novel splice variant of supervillin, SV5, promotes carcinoma cell proliferation and cell migration. *Biochem Biophys Res Commun* 482: 43-49.

## Tables

**Table 1: primer sequences for RT-PCR**

Name	Sequences	
	Forward primer (5'→3')	Reverse primer (5'→3')
SVIL	TTTCCAGCCTGTCCAACCTCA	CGTCACCTACTGCCATAACCC
IL-1β	GAAATGATGGCTTATTACAGTGGCA	GTAGTGGTGGTGGGAGATTCGTAG
IL-6	GCTCTGGCTTGTTCCCTCACTA	AATCATCACTGGTCTTTTGGAG
TNF-α	AGGACACCATGAGCACTGAAAGC	AAGGAGAAGAGGCTGAGGAACAAG
GAPDH	CGCTGAGTACGTCGTGGAGTC	GCTGATGATCTTAGGCTGTTGTC

## Figures



**Figure 1**

LPS induced SVIL expression in THP-1 macrophages. THP-1 cells were seeded in 6-well plates and differentiated into macrophages by PMA (100 ng/ml). The cells were treated with LPS at indicated concentrations and time after differentiation and starvation. (A and B) The macrophages were treated with LPS at concentrations ranging from 0 to 100 ng/ml. Results and statistical graphs of mRNA (A) and protein (B) expressions were represented. (C and D) THP-1 macrophages were treated with LPS (100 ng/ml) for the indicated times, mRNA (C) and protein (D) expressions were detected by RT-PCR and western blot respectively. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . \* versus control group. Data were expressed as the means  $\pm$  S.E.M. of more than three independent experiments and a representative result was shown.

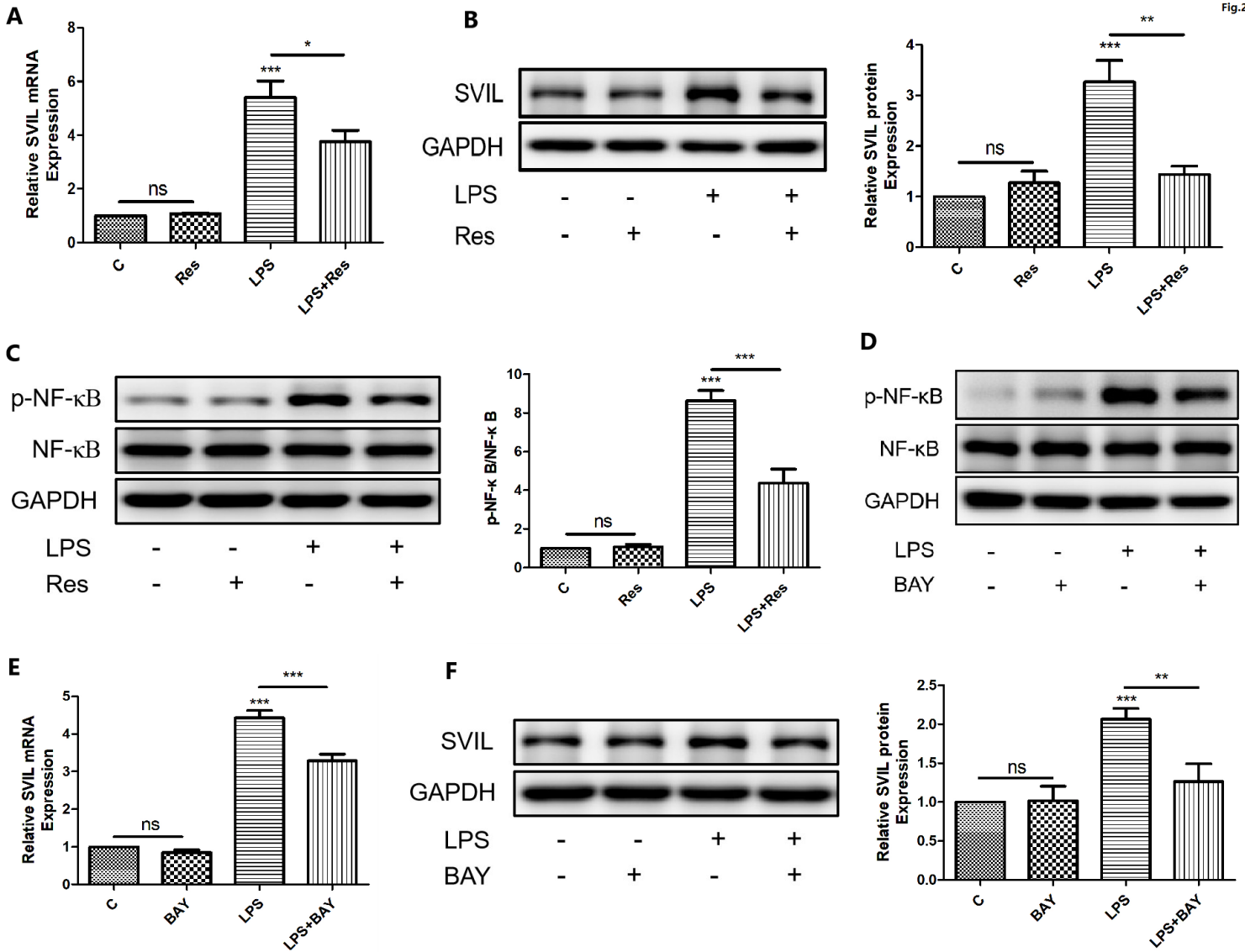
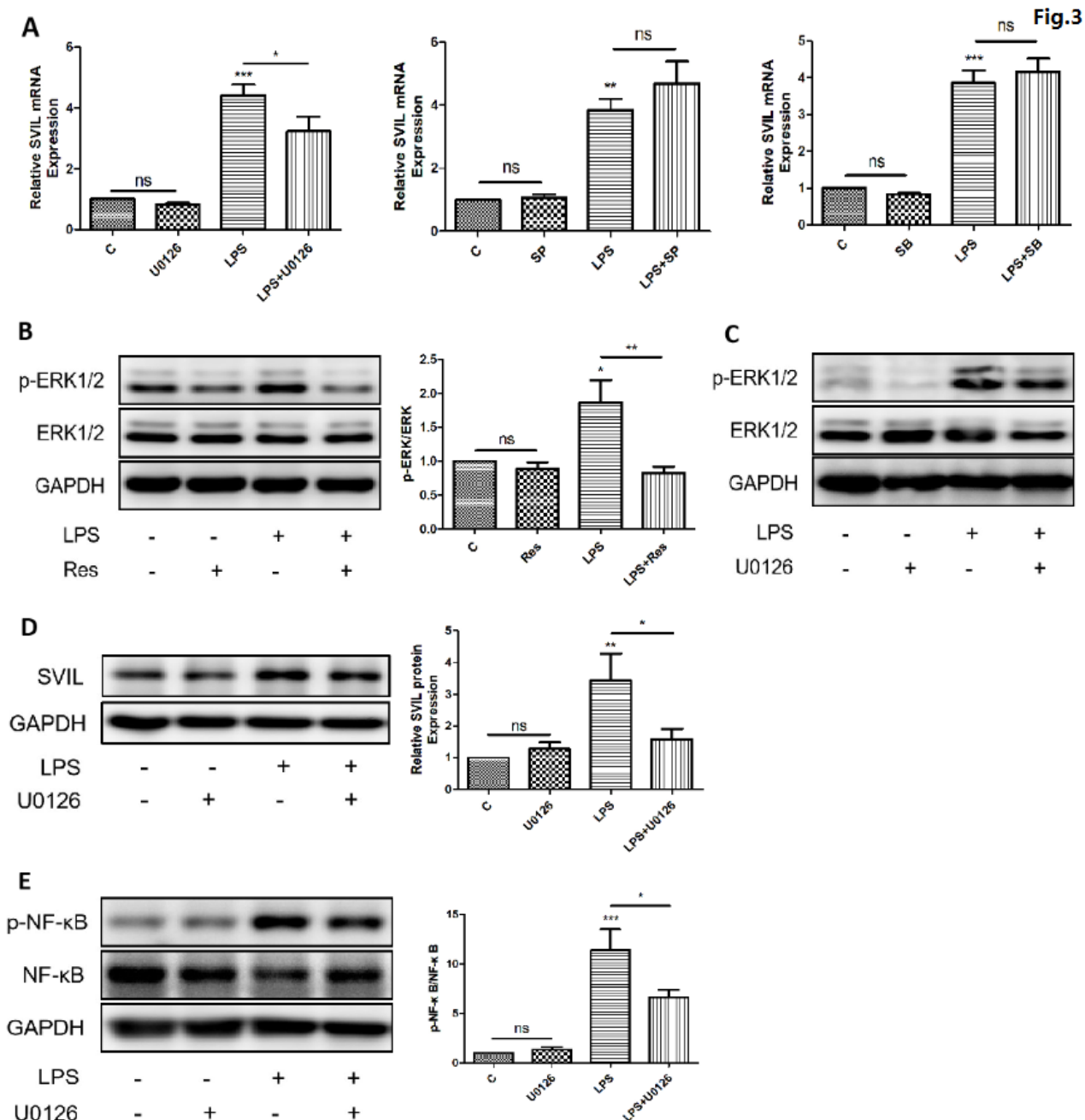


Figure 2

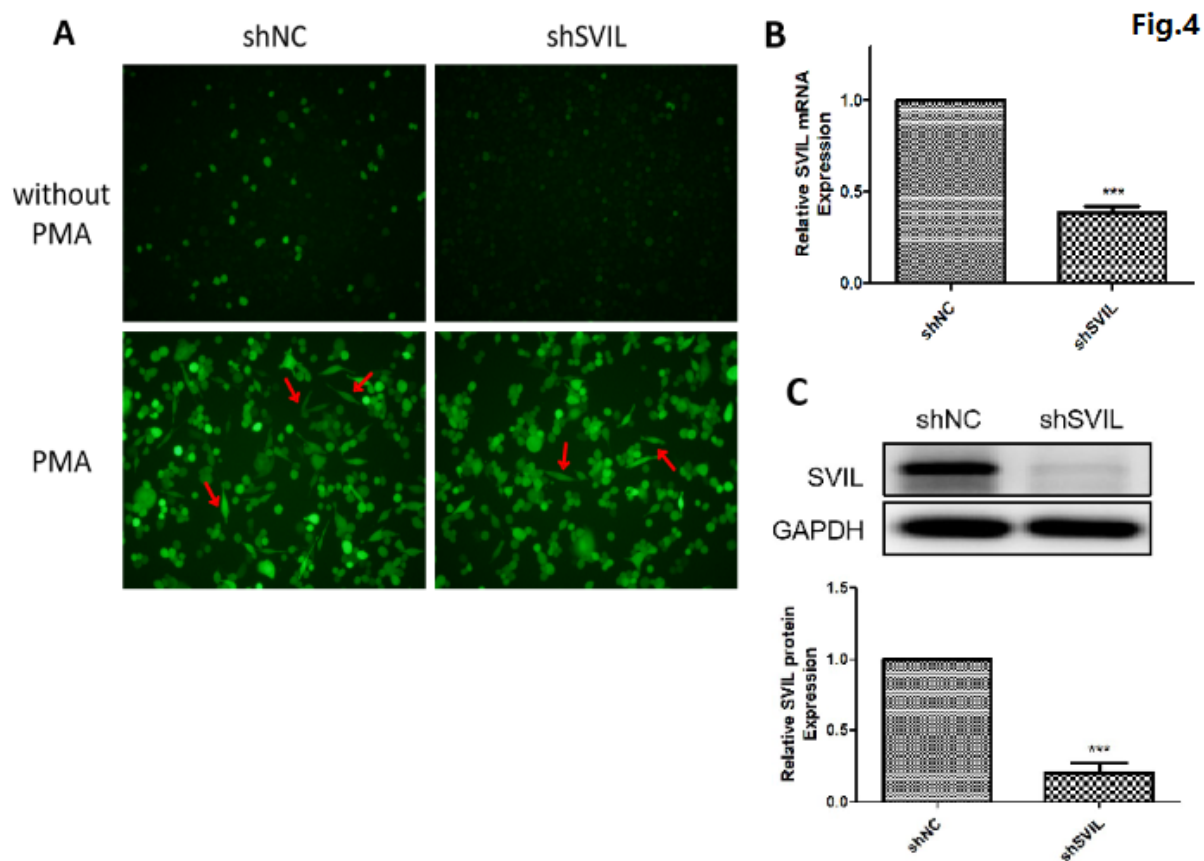
TLR4/NF- $\kappa$ B pathway mediated LPS-induced SVIL expression in macrophages. THP-1 macrophages were pretreated with or without TLR4 inhibitor Res (10  $\mu$ M) for 1 h prior to LPS stimulation for 3 h or 9 h. (A) The cell samples collected at 3 h were used to detect the SVIL mRNA expression by RT-PCR. And cells collected at 9 h were used to detect the SVIL protein level by western blot (B). The phosphorylation level of NF- $\kappa$ B was detected by western blot (C). Cells were pretreated with or without NF- $\kappa$ B inhibitor BAY (10  $\mu$ M) for 1 h prior to LPS stimulation. (D) BAY decreased LPS-induced phosphorylation of NF- $\kappa$ B. The SVIL mRNA (E) and protein expressions (F) were measured by RT-PCR and western blot respectively.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . \* versus control group. Data were expressed as the means  $\pm$  S.E.M. of more than three independent experiments and a representative result was shown. ns: no statistical significance.



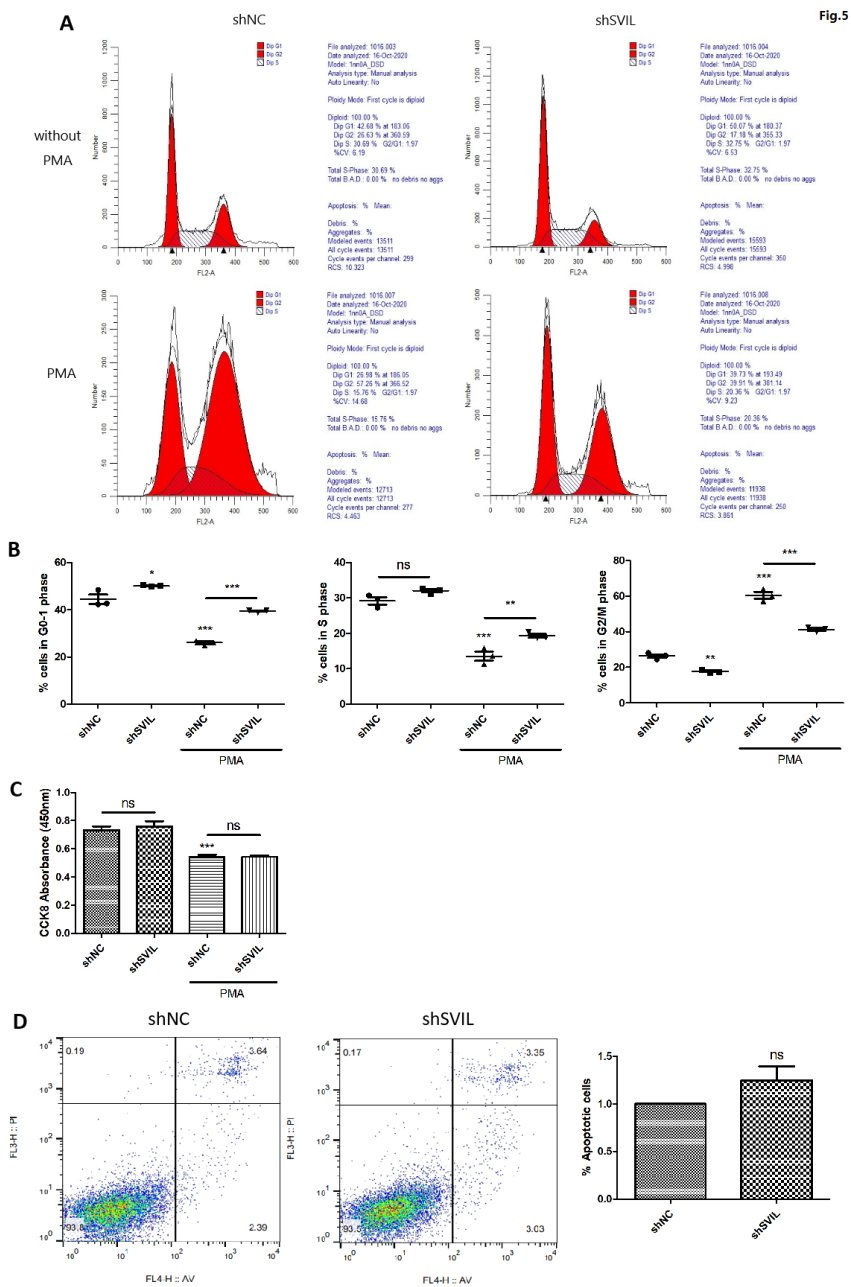
**Figure 3**

LPS induced SVIL expression via ERK1/2 MAPK in THP-1 macrophages. (A) Cells were pretreated with or without ERK inhibitor U0126, P38 inhibitor SB203580 and JNK inhibitor SP600125 for 1 h prior to LPS stimulation, the SVIL mRNA expressions were detected by RT-PCR. (B) Cells were pretreated with or without TLR4 inhibitor Res (10  $\mu$ M) for 1 h prior to LPS stimulation, the phosphorylation of ERK1/2 was detected by western blot. The phosphorylation level of ERK1/2 (C), SVIL protein expression (D) and the phosphorylation level of NF- $\kappa$ B (E) were measured by western blot after cells pretreated with or without ERK inhibitor U0126 for 1 h prior to LPS stimulation. \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ < 0.001. \* versus control group. Data were expressed as the means  $\pm$  S.E.M. of more than three independent experiments and a representative result was shown. ns: no statistical significance.



**Figure 4**

SVIL was knocked down in THP-1 macrophages. THP-1 cells were transfected with viruses containing shNC or shSVIL and screened by puro. (A) The Fluorescence of GFP (green) was detected after transfected cells incubated with or without PMA (100 ng/ml). The SVIL mRNA (B) and protein (C) expressions in both shNC and shSVIL THP-1 macrophages were detected by RT-PCR and western blot, respectively. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . \* versus shNC group. Data were expressed as the means  $\pm$  S.E.M. of more than three independent experiments and a representative result was shown. ns: no statistical significance. Red arrow: representative macrophage.



**Figure 5**

The influence of SVIL knockdown on cell cycle, cell proliferation & apoptosis. (A) The representative flow cytometry images showed the proportion of cells in different phases of cell cycle, G1, G2 and S. (B) The cell percentage in each phase of different treated cells was shown as scatter plot graph. (C) Cell proliferation was measured by CCK8 assay and the statistical data was shown as histogram. (D) The representative apoptotic images detected by flow cytometry were selected and the percentage of apoptotic cell was shown as histogram. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . \* versus shNC group. Data were expressed as the means  $\pm$  S.E.M. of more than three independent experiments and a representative result was shown. ns: no statistical significance.

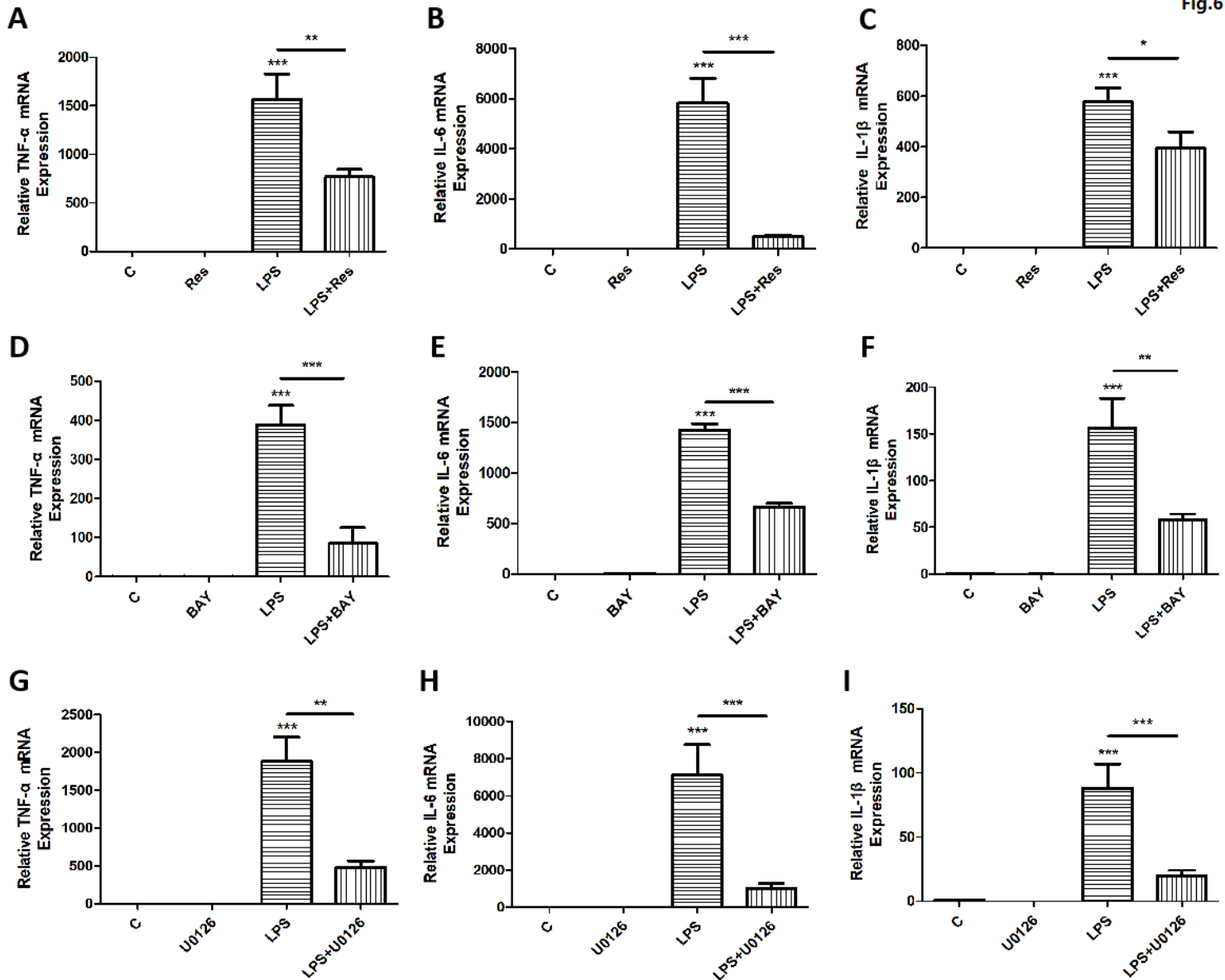


Figure 6

TLR4 inhibitor, NF-κB inhibitor and ERK inhibitor reversed LPS-induced inflammatory cytokines expression in THP-1 macrophages. The THP-1 macrophages were respectively pretreated with or without TLR4 inhibitor Res (10 μM), NF-κB inhibitor BAY (10 μM) and ERK inhibitor U0126 (10 μM) for 1 h prior to LPS stimulation, the inflammatory cytokines (TNF-α, IL-6 and IL-1β) were detected. (A-I) The mRNA expression level of inflammatory cytokines (TNF-α, IL-6 and IL-1β) were measured by RT-PCR after macrophages pretreated with Res (A-C), BAY (D-F) and U0126 (G-I) respectively. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . \* versus control group. Data were expressed as the means  $\pm$  S.E.M. of more than three independent experiments and a representative result was shown. ns: no statistical significance.



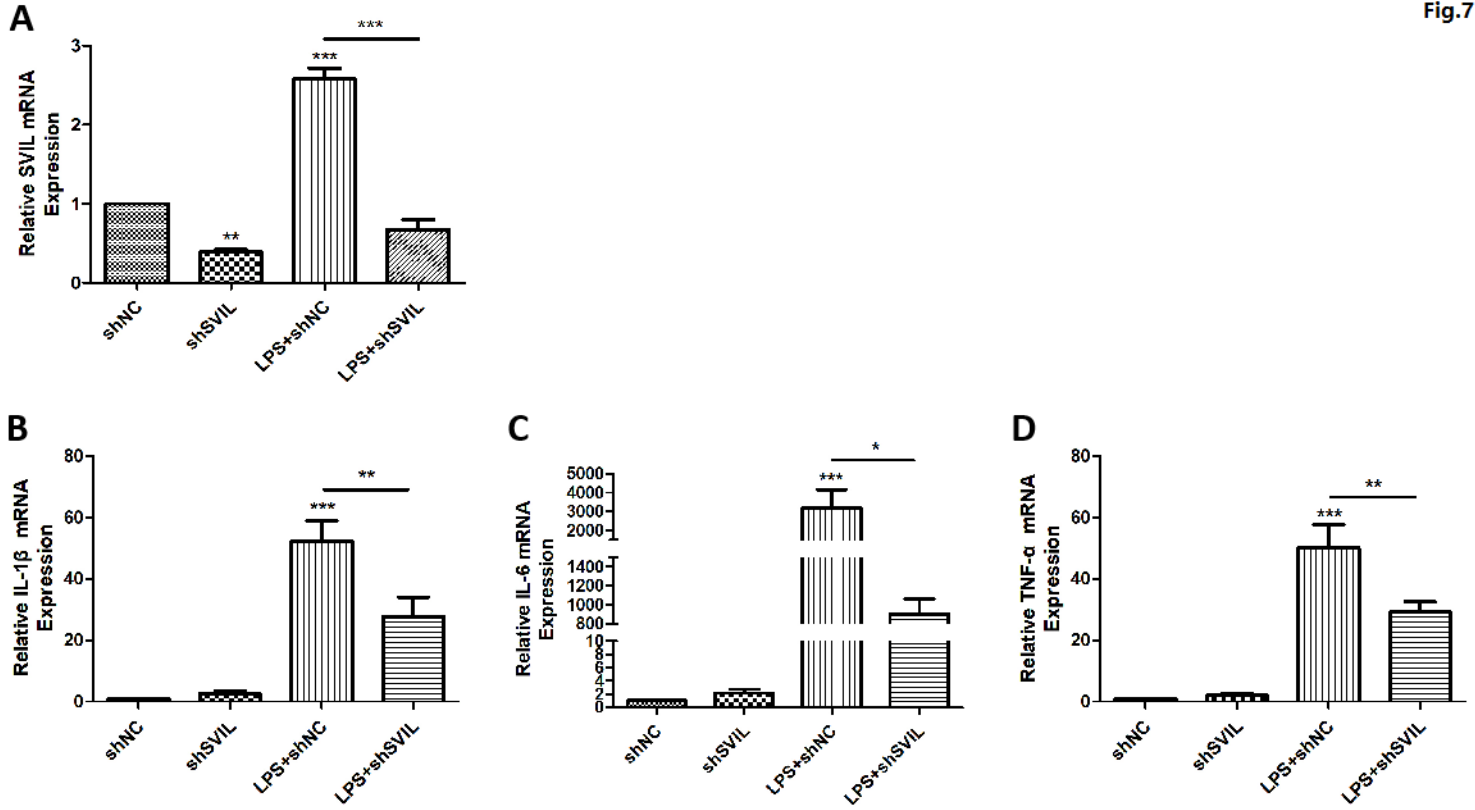


Figure 7

SVIL depletion inhibited LPS-induced expression of inflammatory cytokines. The shNC and shSVIL THP-1 cells were differentiated to macrophages and stimulated with or without LPS. The SVIL expressions (A) in shNC and shSVIL THP-1 macrophages with or without LPS stimulation were measured. The mRNA expressions of inflammatory cytokines IL-1 $\beta$  (B), IL-6 (C) and TNF- $\alpha$  (D) were detected by RT-PCR. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . \* versus control group. Data were expressed as the means  $\pm$  S.E.M. of more than three independent experiments and a representative result was shown. ns: no statistical significance.

Fig.8

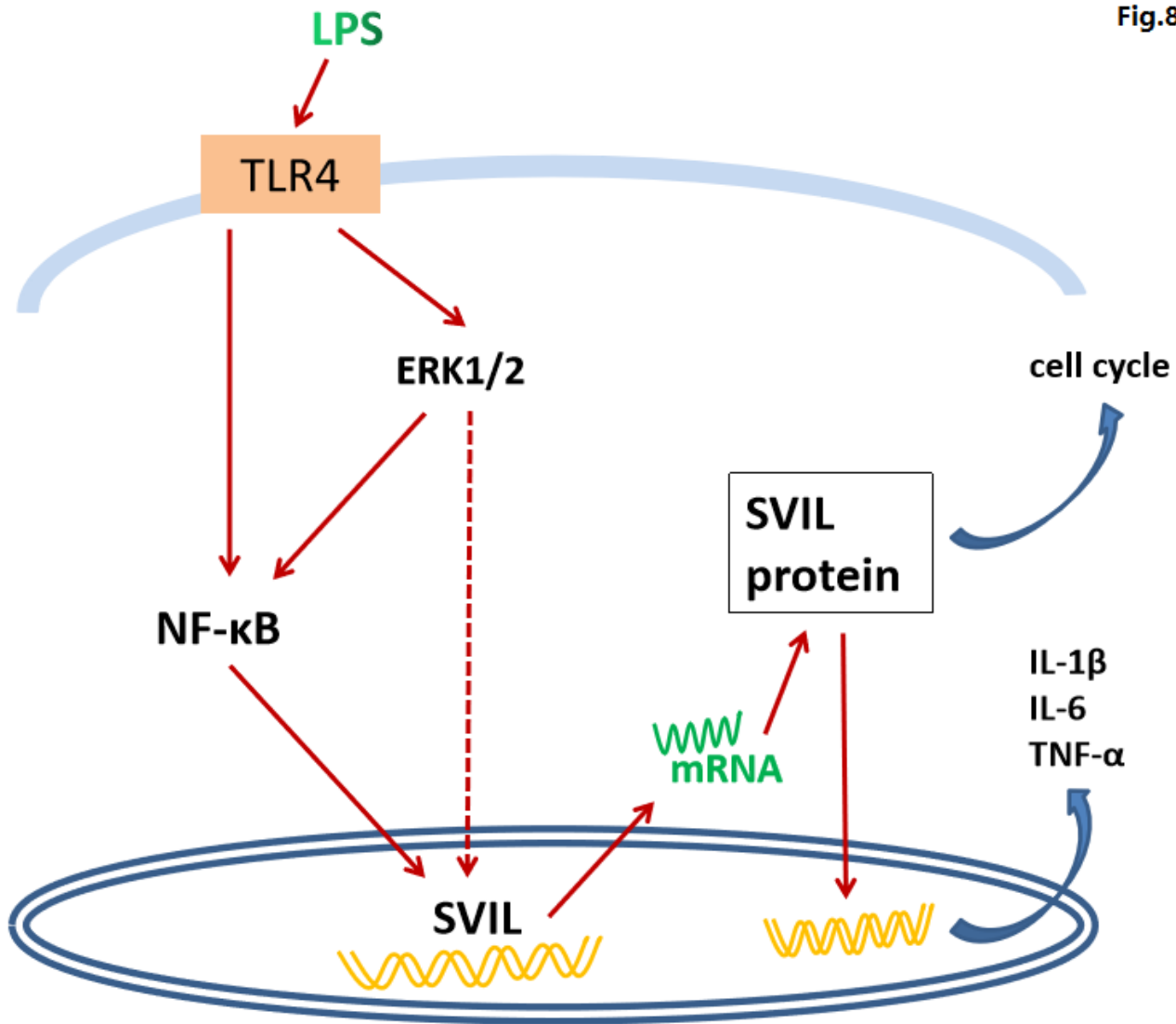


Figure 8

Schematic diagram of SVIL involved in LPS-induced inflammatory response in macrophages. LPS induced SVIL expression by activating TLR4/NF-κB signaling pathway. ERK1/2 MAPK pathway also participated in this process as a downstream of TLR4. Elevated SVIL was involved in the production and release of inflammatory cytokines.