

# Differential Expression of TgMIC1 in Isolates of Chinese<sup>1</sup> Toxoplasma With Different Virulence

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## Research

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# Abstract

The predominant genotype of *T. gondii* in China is Chinese 1 (ToxoDB#9) lineage. TgCtwh3 and TgCtwh6 are two representative strains of Chinese 1, exhibiting high virulence and low virulence to mice, respectively. Little is known about the virulence mechanism of this non classical genotype. Our previous RNA sequencing data revealed that differential mRNA level of TgMIC1 in TgCtwh3 and TgCtwh6. To further confirm the differential expression of TgMIC1 and its significance in this atypical genotype, quantitative real-time PCR was used to verify the RNA sequencing data firstly, and then polyclonal antibodies against TgMIC1 were prepared and identified. Moreover, the invasion and proliferation of the parasite in HFF cells were observed after treatment with TgMIC1 polyclonal antibody or not. The data showed the protein level of TgMIC1 was significantly higher in high virulence strain TgCtwh3 than that in low virulence strain TgCtwh6, and the invasion and proliferation of TgCtwh3 were inhibited by TgMIC1 polyclonal antibody. Differential expression of TgMIC1 in TgCtwh3 and TgCtwh6 may explain, at least partly, the virulence mechanism of this atypical genotype

## Introduction

*Toxoplasma gondii* is a intracellular parasite belonging to the phylum Apicomplexa that infects a wide range of warm-blooded vertebrates, including approximately one-third of humans [1]. In most hosts, this intracellular protozoan parasite escaping immunologic surveillance and cross the blood-brain and blood-retina barrier reaching alleged immune privileged regions to establish a life-long, latent infection [2]. Opportunistic characteristic of *T. gondii* has decided the majority of infected healthy individuals are asymptomatic. However, in immunosuppressed or congenitally infected individuals, infection can cause encephalitis, cerebral, ocular disease or even death, is of great clinical importance [3]. Therefore, a better understanding the molecular mechanism of virulent difference in *T. gondii* is needed to develop effective vaccines or drugs to control the spread of toxoplasmosis.

It is perhaps not surprising that *T. gondii* subverts host protein networks depend on the cell type infected and the parasite genotype. Differences of *T. gondii* strains (e.g. types I, II and III are the classical North American and European strains) in their genomes were typically revealed by techniques such as restriction fragment length polymorphisms or isoenzyme markers [4]. Types I strains are high level of acute virulence in laboratory mice, with an LD<sub>100</sub> as low as a single tachyzoite. Although types II strains are relatively reduced virulence, they are most commonly associated with human infection. Type III is avirulent and always exist in domestic and wild animals, it hardly found in human infection [5]. In comparison to strains in North America and Europe, the genetic polymorphisms are much more complex and diverse in South America. More importantly, the predominant genotype of *T. gondii* in China is Chinese I (ToxoDB#9) lineage (more than 79% based on 60 isolates) [6]. Furthermore, our previous studies have demonstrated that the isolates of Chinese I vary in their virulence to mice, of these TgCtwh3 exhibits high virulence, while TgCtwh6 displays low virulence [6, 7, 8].

Upon infection, *T. gondii* tachyzoites are surrounded by a non-fusogenic compartment, termed parasitophorous vacuole (PV), to survive in host cytoplasm by avoiding lysosomal degradation [9, 10]. This active process depends on the discharge of parasite proteins from its specialized set of secretory organelles called micronemes, rhoptries and dense granules. Among these, proteins secreted from the micronemes (MICs) are involved in the initial recognition, attachment and invasion, whereas dense granules (GRAs) and rhoptries (ROPs) participate in modulating a variety of host signals to establish a suitable environment for parasite growth [11, 12]. Revealed by genetic manipulation, handful TgMIC proteins are proved to be critical virulence factors including TgMIC1. Rather than relying on transmembrane (TM) domains or glycosylphosphatidylinositol (GPI) anchors, TgMIC1 have contained two microneme adhesive repeat (MAR) domains, which is able to form complex with TgMIC4/TgMIC6 to achieve surface localization [13, 14]. The critical role of TgMIC1 in parasite invasion and contributes to virulence in mice has been reported. In addition, a recent research shows that TgMIC1 directly binding to N-glycans of TLR2, thereby to impact systemic levels of IL-12 and IFN- $\gamma$  in vivo [15]. In this study, a different expression of TgMIC1 gene was described for TgCtwh3 and TgCtwh6. Given its influential role in *T. gondii* infection competency and murine pathogenesis, TgMIC1 could be a critical virulence factor between these two strains. To investigate this possibility, the difference of TgMIC1 protein expression in TgCtwh3 and TgCtwh6 was indicated by using polyclonal antibody.

## Materials And Methods

### 2.1 Cells and parasites

Human foreskin fibroblast (HFF) cells were purchased from ATCC (SCRC-1041) and routinely maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, BI, Israel), 100  $\mu$ g/mL penicillin and 100  $\mu$ g/mL streptomycin (Sigma, USA), and maintained in an incubator at 37°C and 5% CO<sub>2</sub>. *Toxoplasma gondii* RH strain, green fluorescent protein-RH strain (GFP-RH), TgCtwh3 and TgCtwh6 were propagated in HFF cells.

### 2.2 RNA extraction and quantitative real-time PCR assays

Total RNA was extracted from the parasites, using Trizol Reagent (Invitrogen, USA). Total RNA was used for reverse transcription with the cDNA synthesis kit (TaKaRa, Japan), according to the manufacturer's protocol. cDNA synthesis was performed using SYBR-Green Master Mix (TaKaRa, Japan). The primer sequences used were as follows:

TgMIC1 forward, TCGGTTTATGCTGAGTGTGC;

TgMIC1 reverse, GGCGAATTCCTTCCTCTTCT;

TgMIC4 forward, GACATGACGGGATCCAGAAC;

TgMIC4 reverse, CATGCAACTTGGCAGTCTGT;

TgMIC6 forward, CATATCACCTGCAAGCGTGT;

TgMIC6 reverse, GGCTCACGACTTTCACCTTC;

$\beta$ -tublin forward, GTCTCCACTTCTTCCTCATTG;

$\beta$ -tublin reverse, GTTCTTTGCGTCGAACATC.

$\beta$ -tublin was used as an internal control. Relative expression levels were calculated according to the standard  $2^{-\Delta\Delta C_t}$  method. All experiments were performed in triplicate and repeated at least three times.

### 2.3 Protein isolation and western blot assays

Parasites was lysed with RIPA lysis buffer (Beyotime, China) with 1% phenyl methyl sulfonyl fluoride and total proteins were subjected to electrophoresis in 8-10% polyacrylamide gel. The proteins were transferred onto a polyvinylidene fluoride membrane (Millipore, USA) by a standard western blot procedure. The membrane was blocked with 5% non-fat milk and incubated with specific primary antibodies diluted in the blocking buffer overnight. Following incubation with the corresponding secondary antibodies conjugated to horseradish peroxidase for 1 h. The membranes were visualized using ECL Western blotting substrate (Bio-RAD, USA).

### 2.4 Expression and purification of recombinant TgMIC1 proteins

The Coding sequence (CDS) of TgMIC1 was optimized by using the Optigene™ codon optimized analysis platform (Shanghai Jierui Bioengineering Co., Ltd.). The optimized TgMIC1 sequence was synthesized and cloned into pET30a. The recombinant pET30a-MIC1 plasmid was transformed into *Escherichia coli* BL21 (DE3), cultivated in Luria-Bertani (LB) at 37 °C. The recombinant protein expression was induced by adding 0.5mM isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG, Sigma, USA) at 16°C for overnight with constant shaking at 200 rpm. Then, the bacterial cells were harvested by centrifugation at 8000 rpm for 6 min, and resuspended in 20ml 10mM Tris-HCL buffer. The bacteria suspension was sonicated on ice (500 W, 180 times, 5 s each time, 5 s interval). The lysate was centrifuged at 12,000 rpm for 10 min to separate the supernatant and bacteria debris. The level of rTgMIC1 expression was analyzed by 12% SDS-PAGE. Furthermore, the separated supernatants were collected and purified by Ni column (Ni Sepharose 6 Fast Flow, GE Healthcare). The purification efficiency was analyzed via 12% SDS-PAGE.

### 2.5 TgMIC1 polyclonal antibody preparation and specific identification

For polyclonal antibody production, about 2 kg New Zealand white rabbits were immunized with 400  $\mu$ g of purified rTgMIC1 diluted in 200  $\mu$ L PBS was mixed with equal volume of complete Freund's adjuvant (Sigma, USA) and multiple injected intradermally to the back of each rabbits. Two weeks later, the immunization was boosted with 200  $\mu$ g rTgMIC1 protein in Freund's incomplete adjuvant, followed by a booster immunization once every 2 weeks. The ear vein blood was collected 7 days after the fourth immunization to measure the titer of antibodies by enzyme-linked immunosorbent assay (ELISA). The

rabbit heart was then bled, and the antibodies were purified by Protein A. The serum mixed with equal volume of the binding buffer to equilibrate the column. After serum sample was loaded, rinse the column with binding buffer until the binding solution contains no protein. The presence of TgMIC1 polyclonal antibody was further identified by western blot and immunofluorescence assays.

## 2.6 ELISA analysis

The titer in the serum from immunized rabbits was performed with ELISA. The purified rMIC1 protein was used to coat plate at 4°C overnight, followed by blocked with 5% BSA at 37°C for 60 min. The serum sample was serially diluted from 1:200 to 1:204800, and then 100 µL of diluted sample was added to each well incubating 60 min at 37°C. Following incubation with the secondary anti-rabbit antibodies conjugated to horseradish peroxidase to each well for 1 h. Thereafter, each well was incubated with 100 µL of tetramethylbenzidine (TMB, Beyotime, China) at 37°C for 10 min. Finally, 2 M H<sub>2</sub>SO<sub>4</sub> (50 µL) was used to stop the reaction in the well, and the absorbance was read at 450 nm.

## 2.7 Immunofluorescence assays (IFA)

HFF monolayers were grown on coverslips placed in wells of a 6-well plate at a density of 10<sup>5</sup> cells per well and were incubated for 24 h. Then, cells were challenged with *T. gondii* GFP-RH strains for 24 h. After washing with phosphate buffered saline (PBS) three times, the coverslips were fixed with 4% paraformaldehyde (PFA) for 20 min. The coverslips were permeabilized with 0.1% Triton X-100 (Sigma, USA) for 30 min, and blocked for 30 min at 37°C with 5% BSA in PBS. After washing again, the coverslips were then incubated with primary antibodies overnight at 4°C, followed by goat anti-rabbit secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 594 (Invitrogen, USA). Images were recorded using a Zeiss LSM880 confocal microscope.

## 2.8 Invasion assay

HFF monolayers were grown on coverslips placed in wells of a 6-well plate at a density of 10<sup>5</sup> cells per well and were incubated for 24 h. Then, cells were challenged with *T. gondii* TgCtwh3 and TgCtwh6 strains for 1 h. Non adherent parasites were washed away with PBS before fixation with 4% PFA for 20min. Adherent external parasites were detected using rabbit anti-*T. gondii* glides associated protein 45 (TgGAP45) antibodies, followed by secondary anti-rabbit antibodies coupled to Alexa488. After cell permeabilization with 0.1% Triton X-100 for 30 min, invaded intracellular and adherent external parasites were labeled with anti-GAP45 antibodies, followed by secondary anti-rabbit antibodies coupled to Alexa594. Fields were randomly selected in the same pattern for all samples and the number of external and internal parasites was counted.

## 2.9 Intracellular growth assay

The TgCtwh3 or TgCtwh6 parasites were allowed to invade HFF cells for 24 h. After washing with PBS three times, the coverslips were fixed with methyl alcohol for 5 min, followed by Wright Giemsa method

and observed under microscope. About 50 PVs were randomly selected and the number of *T. gondii* tachyzoites in each PV was counted.

## 2.10 Statistical analysis

Typical results are shown with values expressed as means  $\pm$  standard deviation of at least three independent experiments. The statistical significance of differences was performed using one-way ANOVA and t-test. The value of  $p < 0.05$  was considered statistically significant.

# Results

## 3.1 Differential mRNA expression of TgMIC1/4/6 in TgCtwh3 and TgCtwh6 strains

To seek the virulence genes through comparing and studying the different genes expression between TgCtwh3 and TgCtwh6 strains. Quantitative real-time PCR was used to identify the mRNA expression of TgMIC1, TgMIC4 and TgMIC6 in TgCtwh3 and TgCtwh6 strains. Significant high expression of TgMIC1, TgMIC4 and TgMIC6 were observed in virulent TgCtwh3 when compared with less virulent TgCtwh6 (Fig. 1).

## 3.2 Codon optimization and efficient of TgMIC1 gene

Optigene™ Codon Optimization Analysis Platform was used to optimize multiple important parameters of the TgMIC1 gene to stabilize DNA fragments, and in turn increase gene expression efficiency. After optimized, TgMIC1 Codon Adaption Index (CAI) increased from 0.74 to 0.91, reaching a high gene expression level (Fig. 2A). Frequency of Optimal codons (FOP) for TgMIC1 was maintained above 80, which was significantly improved compared to the original sequence (Fig 2B). In the comparison of the number of secondary structures of mRNA, the secondary structure of the TgMIC1 optimized group was slightly less (Fig. 2C). Moreover, peaks of GC% has been removed in a 60 bp window, the ideal percentage range of GC content is between 30% and 70%. The GC content of the optimized group was reduced compared with the previous one (Fig. 2D). The results revealed that optimized sequence of TgMIC1 more suitable than original sequence for next experiment.

## 3.3 Expression, purification and identification of recombinant TgMIC1 protein

Recombinant plasmid pET-30a-MIC1 was successfully constructed. The rTgMIC1 was expressed in *Escherichia coli* (*E. coli*) strain BL21 (Fig. 3A-B) and identification by HIS monoclonal antibodies (Fig. 3C). Western blot and SDS-PAGE analysis indicated that the rTgMIC1 had a molecular weight of approximately 60-kDa, which was consistent with the predicated combined sizes of the protein (49-kDa) encoded by the TgMIC1 gene and HIS-tag from the vector. In addition, the rTgMIC1 protein was purified by Ni<sup>2+</sup>-affinity chromatograph (Fig. 3D). Thus, these results suggested that rTgMIC1 had been successfully expressed and purified.

## 3.4 Generation and specific identification of TgMIC1 polyclonal antibody

To further research, the purified rTgMIC1 protein was injected into the New Zealand white rabbit by multiple back injections, and antiserum was collected after the fourth booster immunization. The polyclonal antibody (pAb) against TgMIC1 purified by Protein A, the titer approximately was 1:12800 estimated by ELISA assay (Fig. 4A), and Purity > 90% indicated by SDS-PAGE analysis (Fig. 4B). The specificity of rTgMIC1 and pET30a-MIC1 were identified by the polyclonal antibodies against TgMIC1 as a clearly band of approximately 60kDa, in addition, other parasite proteins recombinant bacteria, pET30a-MIC4, pET30a-MIC6 or pET28a-ROP18 was not identified by the TgMIC1 antibodies (Fig. 4C). Moreover, different type of *T. gondii* strains, RH, TgCtwh3 or TgCtwh6 total protein was identified by TgMIC1 antibodies, other parasites, *Plasmodium* or *Schistosoma* total protein was not detected by antibodies against TgMIC1 (Fig. 4D). Immunofluorescence with TgMIC1 (red) was performed to determine the localization of TgMIC1 in GFP-RH infected HFF cells (Fig. 4E). These results indicated that the obtained TgMIC1 antibodies which located in the apex of parasite with high specificity and can be used for further experiments.

### 3.5 Differential protein expression of TgMIC1 in TgCtwh3 and TgCtwh6 strains

Western bolt analysis revealed that the relative protein expression of TgMIC1 in TgCtwh3 tachyzoites was 2-fold higher compared to TgCtwh6 tachyzoites (Fig. 5), which in agreement with the differential mRNA expression of TgMIC1. Inspired by these results, we hypothesis that differentially expressed TgMIC1 is likely one of virulence regulators between TgCtwh3 and TgCtwh6 strains.

### 3.6 Evaluation the role of TgMIC1 for parasite invasion and replication

From the superimposed picture, red parasites are the parasites that invaded into the cell, yellow ones are the parasites outside the cell, and the nucleus is blue. The results showed that the invasion efficiency of TgCtwh3 was 67%, that of TgCtwh6 was 40%, and that of TgCtwh3 plus anti-mic1 was 43% (Fig. 6A-B). The invasion efficiency of TgCtwh3 is higher than that of TgCtwh6, and the invasion efficiency of TgCtwh3 can be reduced by TgMIC1 polyclonal antibody to some extent ( $p < 0.001$ ). Furthermore, intracellular replication capability of TgCtwh3 also attenuated by TgMIC1 polyclonal antibody comparing with untreated group (Fig. 6C-D). These results indicated that the level of TgMIC1 impacts TgCtwh3 attachment and replication capability in HFF cells.

## Discussion

Much progress has been made toward the understanding of *T. gondii* strain-specific virulence factors and their effects on the host immune response. However, most attention focused on the dominant strains in North America and Europe, the picture is less clear when considering the dominant strains in others regions, especially in China [16, 17, 18]. A great deal of evidence demonstrates that ROP18/ROP5/ROP17 specifically counteract murine defense mechanisms, in phosphorylating and inactivating immunity-related GTPases (IRGs). Moreover, ROP18 can phosphorylate the host activating transcription factor 6 $\beta$  (ATF6 $\beta$ ), leading to the proteasomal degradation of ATF6 $\beta$ , in turn interrupt its role in antigen presentation by DCs [19, 20]. Generally, the aforementioned parasite-derived polymorphic effectors

represent most of the differences in acute virulence between these classically three lineages. But that seem to inapplicable to explain the different toxicity between TgCtwh3 and TgCtwh6 strains, neither genetic variation in exon regions nor differential expression of ROP5 and ROP18 was noted between these two strains. Given its role in altering host gene transcription, ROP16 has been extensively studied as an important virulence related molecule. More interestingly, studies reported that both TgCtwh3 and TgCtwh6 of type Chinese I carry ROP16<sub>I/III</sub> and GRA15<sub>II</sub> effectors [21], and there was no significantly difference of the virulence to mice between TgCtwh3 $\Delta$ rop16 and TgCtwh3 WT strains [22]. The genotype varies amongst different strains of *T. gondii*, resulting in divergent resistance to host defense mechanisms, this implies that type Chinese I strains may has unique virulence characteristics and pathogenesis.

Recent advances within the field of *T. gondii* research have unraveled the secretion of microneme was regulated by phosphatidic acid, cyclic guanosine monophosphate and calcium [23, 24]. These TgMIC proteins cooperate with actomyosin system control tachyzoites invasion and egress from infected cells [13, 25]. Our results demonstrated that the mRNA level of TgMIC1 in TgCtwh3 apparently higher than in TgCtwh6. Given its remarkable role, in next research, we developed rTgMIC1 protein and pAbs against TgMIC1 to identify the protein expression of TgMIC1 in these two strains. In view of the native gene of TgMIC1 has several features which may lead to poor expression, we strategically optimizing the underlying DNA sequence of TgMIC1 to improve its mRNA stability and recombinant protein expression in *E. coli*. Compared with actual codon frequency, the squared difference of the preferred codon frequency has been changed from 1.85 to 3.17. The GC content throughout the sequence has been homogenized, in order to increase the half-life of the mRNA. Moreover, the mRNA secondary structure has been reduced, which improved the translation efficiency. These modifies were benefit for us to obtain the high yield and high concentration of TgMIC1 protein. In our study, the produced pAbs could recognize not only the recombinant TgMIC1 but also the natural TgMIC1 protein from the *T. gondii* not *Plasmodium* and *Schistosoma*.

Invasiveness, intracellular replication capability and the strength and characteristics of the induced immune response in host cells, both of these factors combine to determine *T. gondii* virulence. Previous study reported that the invasion by TgMIC1ko parasites was reduced by half compared to wild type [26]. One of the important observations in the present study is that the protein level of TgMIC1 in TgCtwh3 was significantly higher than that in TgCtwh6, which may cause the difference invasiveness or replication capability of *T. gondii* to host cells. Consistent with our hypothesis, the two-color and experimental analysis of *T. gondii* infected HFF cell revealed that elimination of TgMIC1 protein by add exogenous polyclonal antibodies hinder invasiveness and intracellular replication capability of TgCtwh3. Taken together, we have successfully developed rTgMIC1 and its pAb, these outcomes can be applied to many experiments, from western blotting, immunofluorescence to in vivo function analysis. Furthermore, our work shows a clearly impact of differences in TgMIC1 level on the Chinese I *Toxoplasma* intracellular invasion and proliferation, and this represents an important step toward our understanding how TgCtwh3 and TgCtwh6 vary in their virulence to mice.

# Declarations

## Author Contribution

LY conceived and designed the study, and critically revised the manuscript. YW, CH and RZ carried out the experiments and drafted the manuscript. JZ, FZ, JL, QL, JD, DC, YC and JS contributed to the revision of the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

All animal experiments were conducted in strict accordance with the Chinese National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Review Board of Anhui Medical University Institute of Biomedicine (Permit Number: AMU26-081108).

## Consent for publication

Not applicable.

## Competing interests

There are no conflicts of interest to declare.

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## Figures

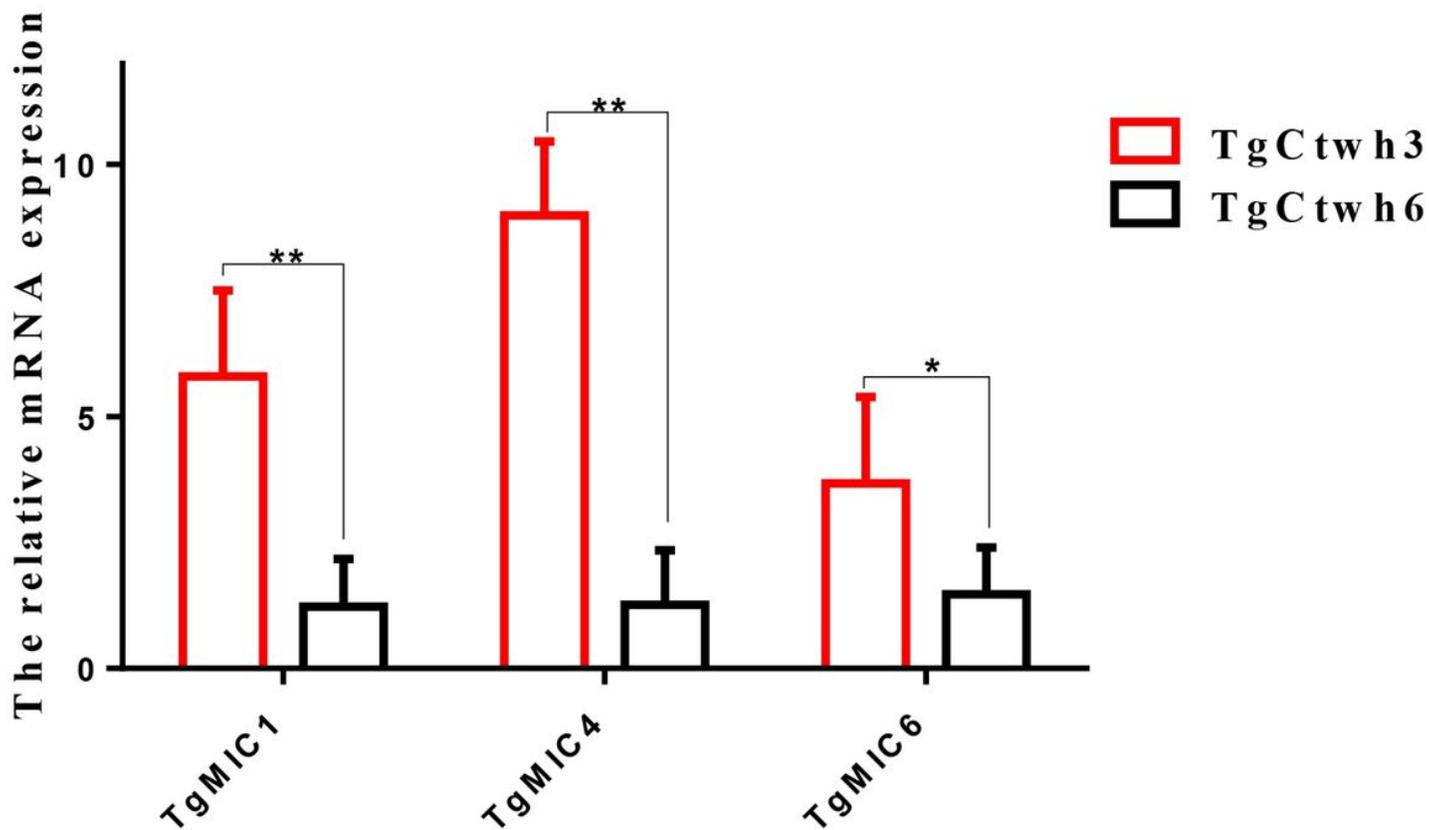
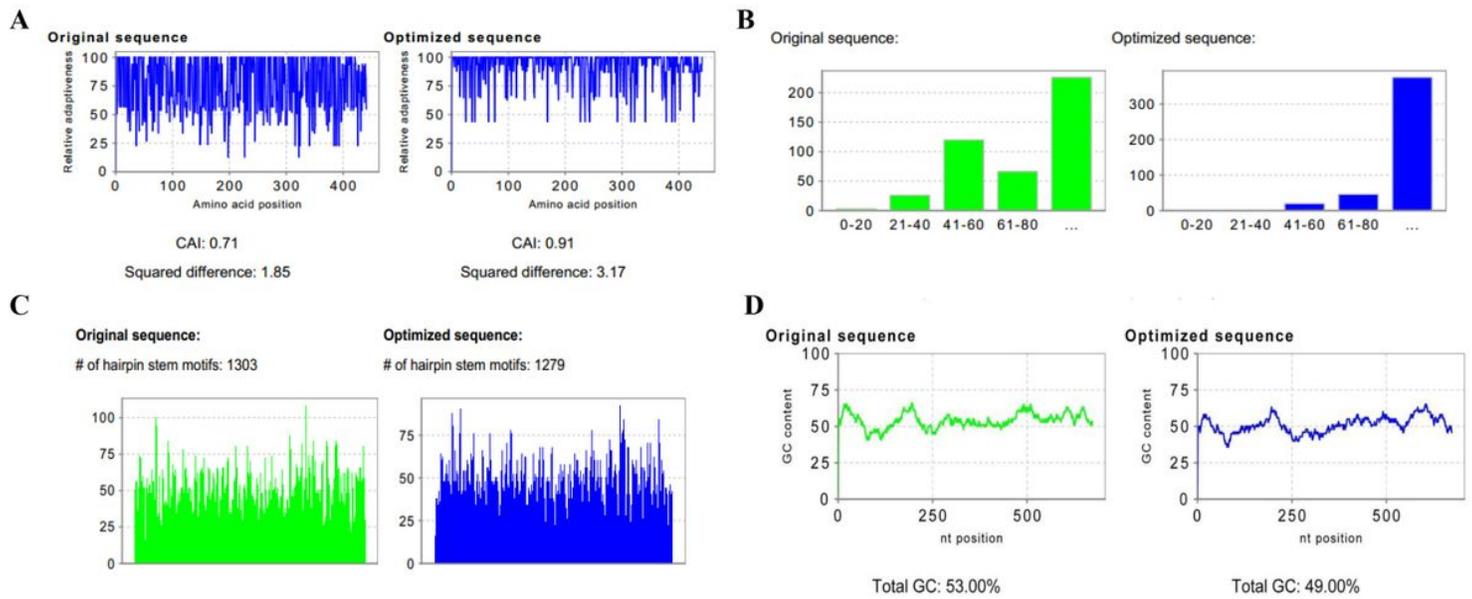


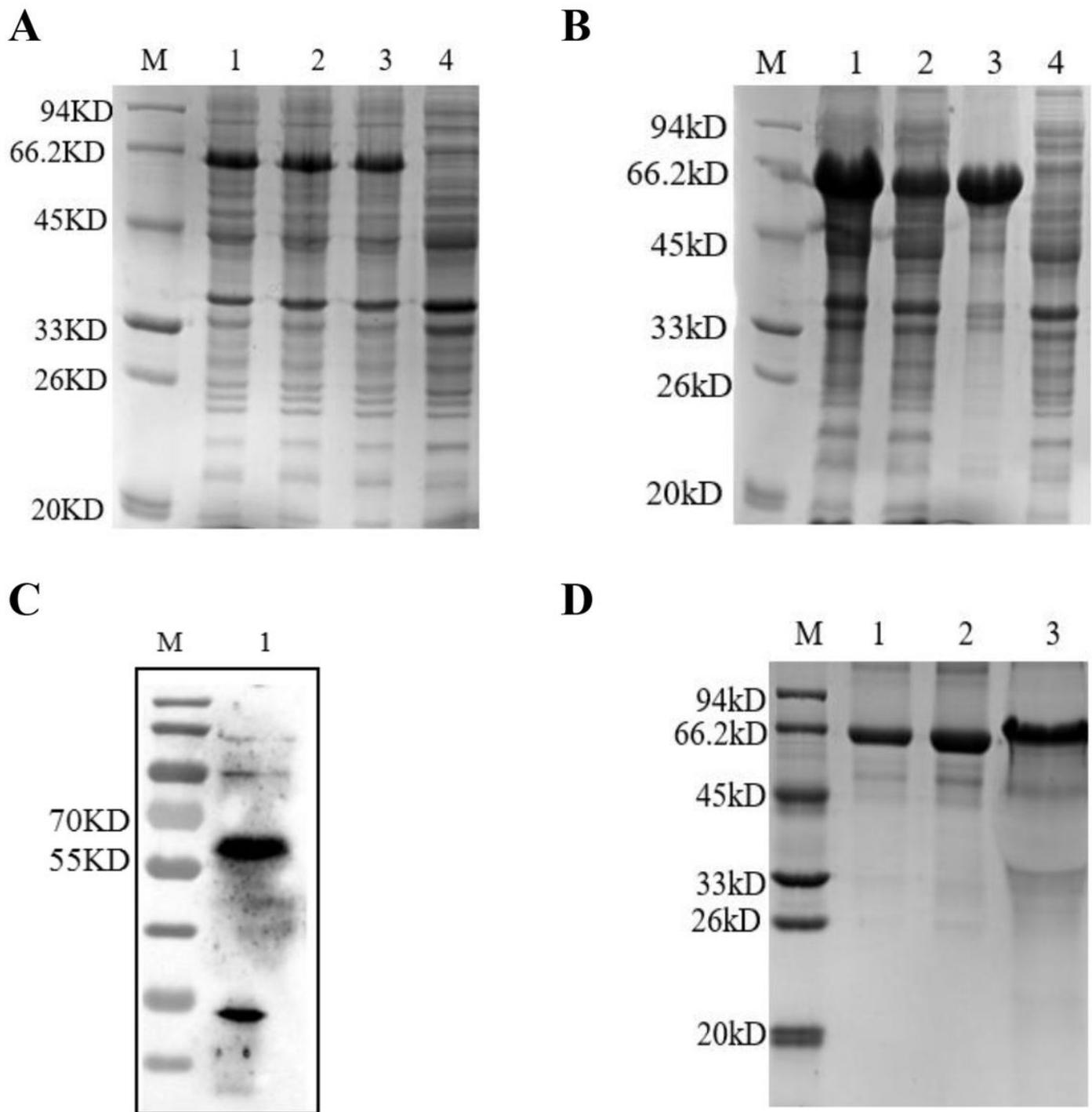
Figure 1

Quantitative real-time PCR analysis of the TgMIC1, TgMIC4 and TgMIC6 mRNA expression in TgCtwh3 and TgCtwh6 strains. \* $p < 0.05$ , \*\* $p < 0.01$  versus TgCtwh6 group. Data are represented as mean  $\pm$  SD for three independent experiments.



**Figure 2**

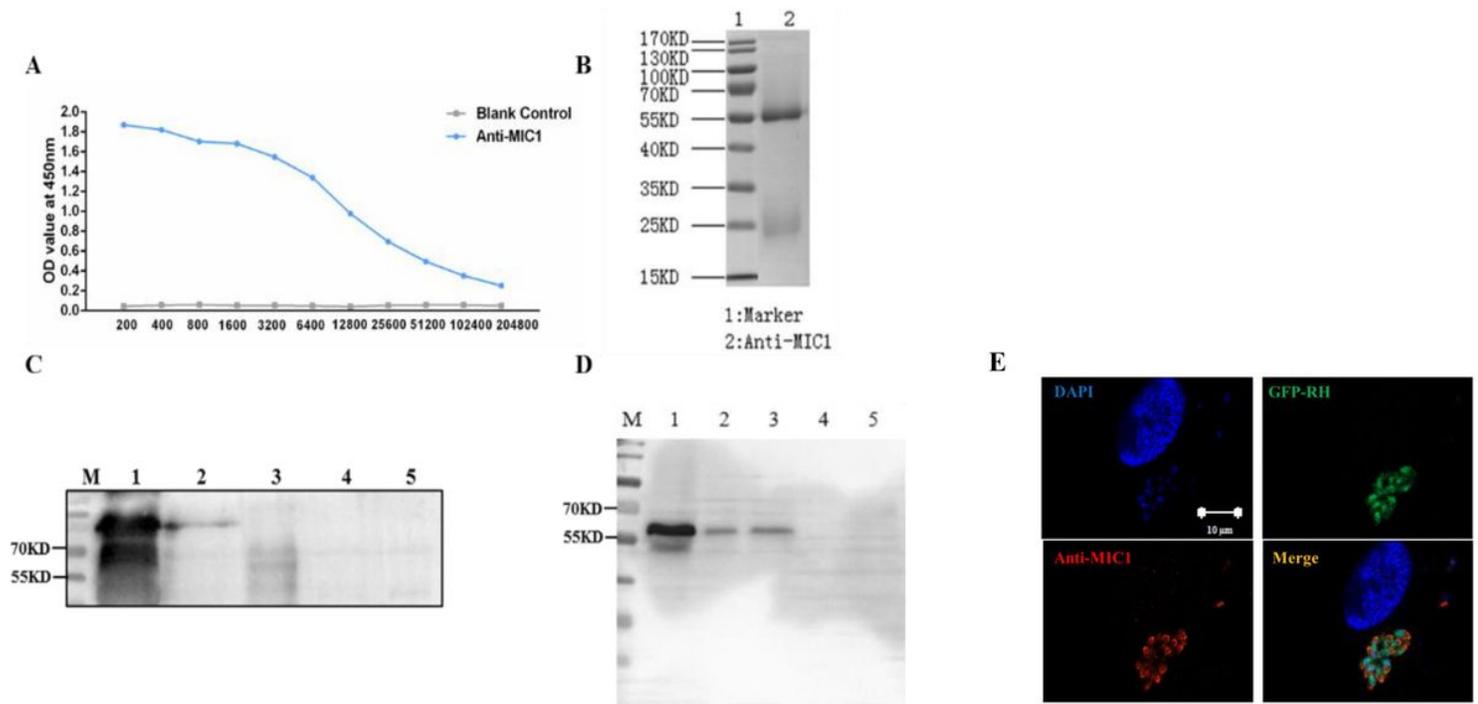
Optimization of TgMIC1 codon sequences. (A): The original and optimized gene sequence relative adaptiveness distribution. A CAI of  $> 0.9$  is regarded as very good, in terms of high gene expression level. (B): The percentage distribution of codons in original and optimized groups. The value of 100 is set for the codon with the highest usage frequency for a given amino acid in the desired expression organism. (C): Total GC content of original and optimized genes. (D): Comparison of the mRNA secondary structure between the original and optimized groups.



**Figure 3**

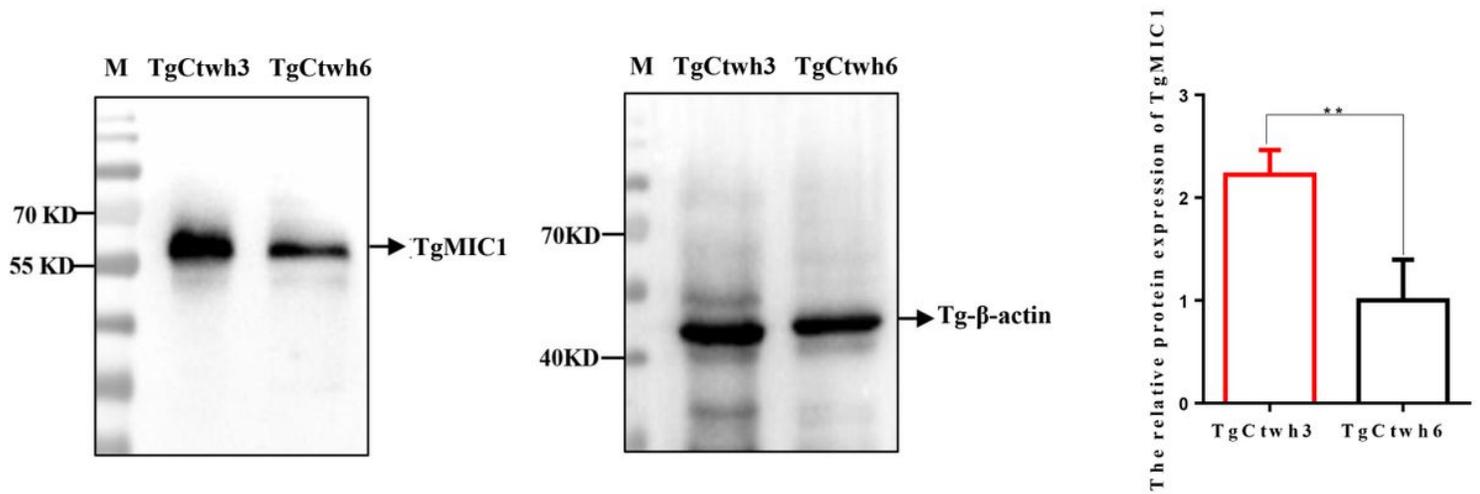
SDS-PAGE and Western blot analysis of the rTgMIC1 protein. (A): Expression of pET30a-MIC1/BL21 in small amounts. Lane M: Protein marker; Lane 1-3: Mono-clone picked from conversion plates after induction with IPTG; Lane 4: Uninduced control. (B): Large amounts of expression detection. Lane M: Protein marker; Lane 1: Proteins in pET30a-MIC1/BL21 after induction with IPTG; Lane 2: Supernatant of pET30a-MIC1/BL21 bacteria after ultrasonication; Lane 3: Precipitation of pET30a-MIC1/BL21 bacteria after ultrasonication; Lane 4: Uninduced control. (C): Western blot analysis of the rTgMIC1. Lane M:

Protein marker; Lane 1: rTgMIC1 detected by HIS monoclonal antibodies. (D): The rTgMIC1 purified by Ni<sup>2+</sup>-affinity chromatograph. Lane M: Protein marker; Lane 1: 0.5mg/ml BSA; Lane 2: 1.0mg/ml BSA; Lane 3: Purified rTgMIC1 proteins.



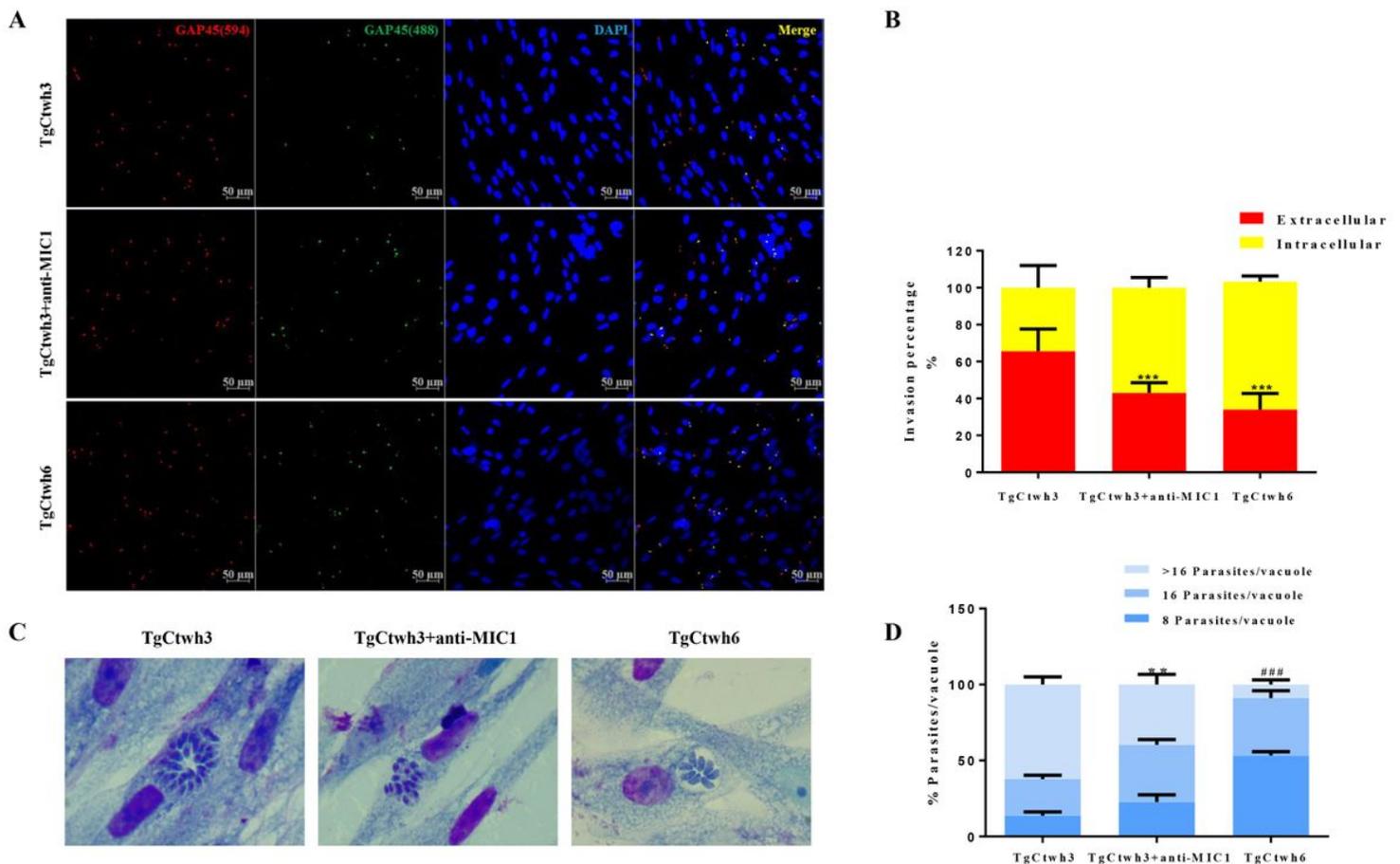
**Figure 4**

Preparation and specific detection of polyclonal antibody against TgMIC1. (A): The titer of the purified TgMIC1 polyclonal antibody was detected by indirect ELISA. The titer is the dilution corresponding to the minimum OD reading greater than the maximum OD/2. (B): The purity of TgMIC1 polyclonal antibody indicated by SDS-PAGE. Lane M: Protein marker; Lane 1: Heavy and light chains of the purified TgMIC1 polyclonal antibody. (C): Different recombinant bacteria from Toxoplasma proteins reacted with the TgMIC1 polyclonal antibody. Lane M: Protein marker; Lane 1: rTgMIC1; Lane 2: pET30a-MIC1 bacteria; Lane 3-5: pET30a-MIC4, pET30a-MIC6 and pET28a-ROP18 respectively. (D): The TgMIC1 polyclonal antibody reacted with proteins from different type of *T. gondii* strains and parasite. Lane M: Protein marker; Lane 1-6: RH, TgCtwh3, TgCtwh6, Plasmodium and Schistosoma respectively. (E): Confocal microscopy images showing the localization of TgMIC1 (red) in GFP-RH tachyzoites (green) challenges HFF cells for 24 h. DAPI (blue) was used to stain the nuclei. The scale bars are 10µm.



**Figure 5**

Differential expression of TgMIC1 protein in TgCtwh3 and TgCtwh6 strains. The total proteins extracted from TgCtwh3 and TgCtwh6 tachyzoites were examined to the expression of TgMIC by Western blot analysis. Tgβ-actin used as a reference protein to analyze the *T. gondii* protein quantitatively. \*\*p < 0.01 versus TgCtwh6 group. Data are represented as mean ± SD for three independent experiments.



**Figure 6**

TgMIC1 polyclonal antibody regulates parasite invasion and growth. (A): Representative images of invaded and attached tachyzoites with HFF cells. (B): Histogram of *T. gondii* invasion. Infected HFF cells were incubated with TgGAP45 polyclonal antibody, and then analyzed by IFAT to evaluate the invade rate of *T. gondii*. Tachyzoites with yellow being extracellular and red intracellular; DAPI (blue) was used to stain the nuclei. (C): Microscopy showing the growth of parasites in HFF monocytes. (D): The percentage of parasites/vacuole histogram. \*\*p < 0.01, \*\*\*p < 0.001 versus TgCtwh3 group. Data are represented as mean ± SD for three independent experiments.

## Supplementary Files

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