

## RESEARCH ARTICLE

# Label-free quantitative proteomic analyses of mouse astrocytes provides insight into the host response mechanism at different developmental stages of *Toxoplasma gondii*

Huanhuan Xie<sup>1</sup>, Hang Sun<sup>1</sup>, Hongjie Dong<sup>1</sup>, Lisha Dai<sup>1</sup>, Haozhi Xu<sup>1</sup>, Lixin Zhang<sup>1,2</sup>, Qi Wang<sup>1</sup>, Junmei Zhang<sup>1</sup>, Guihua Zhao<sup>1</sup>, Chao Xu<sup>1</sup>, Kun Yin<sup>1\*</sup>

**1** Shandong Institute of Parasitic Diseases, Shandong First Medical University & Shandong Academy of Medical Sciences, Jining, Shandong, China, **2** Xingan League Center for Disease Control and Prevention, Ulanhot, Inner Mongolia, China

\* [kyin@sdfmu.edu.cn](mailto:kyin@sdfmu.edu.cn)



## OPEN ACCESS

**Citation:** Xie H, Sun H, Dong H, Dai L, Xu H, Zhang L, et al. (2023) Label-free quantitative proteomic analyses of mouse astrocytes provides insight into the host response mechanism at different developmental stages of *Toxoplasma gondii*. *PLoS Negl Trop Dis* 17(9): e0011102. <https://doi.org/10.1371/journal.pntd.0011102>

**Editor:** Luisa Magalhães, Universidade Federal de Minas Gerais, BRAZIL

**Received:** January 12, 2023

**Accepted:** August 7, 2023

**Published:** September 18, 2023

**Copyright:** © 2023 Xie et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [1] partner repository with the dataset identifier PXD038919. <https://www.ebi.ac.uk/pride/archive/projects/PXD038919>.

**Funding:** YK was supported by Natural Science Foundation of Shandong Province (ZR2022MH197). YK and XHH were funded by the the Medicine and Health Science Technology

## Abstract

*Toxoplasma gondii* (*T. gondii*) is an opportunistic parasite that can infect the central nervous system (CNS), causing severe toxoplasmosis and behavioral cognitive impairment. Mortality is high in immunocompromised individuals with toxoplasmosis, most commonly due to reactivation of infection in the CNS. There are still no effective vaccines and drugs for the prevention and treatment of toxoplasmosis. There are five developmental stages for *T. gondii* to complete life cycle, of which the tachyzoite and bradyzoite stages are the key to the acute and chronic infection. In this study, to better understanding of how *T. gondii* interacts with the host CNS at different stages of infection, we constructed acute and chronic infection models of *T. gondii* in astrocytes, and used label-free proteomics to detect the proteome changes before and after infection, respectively. A total of 4676 proteins were identified, among which 163 differentially expressed proteins (fold change  $\geq 1.5$  or  $\leq 0.67$  and  $p$ -value  $\leq 0.05$ ) including 109 up-regulated proteins and 54 down-regulated proteins in C8-TA vs C8 group, and 719 differentially expressed proteins including 495 up-regulated proteins and 224 down-regulated proteins in C8-BR vs C8-TA group. After *T. gondii* tachyzoites infected astrocytes, differentially expressed proteins were enriched in immune-related biological processes to promote the formation of bradyzoites and maintain the balance of *T. gondii*, CNS and brain. After *T. gondii* bradyzoites infected astrocytes, the differentially expressed proteins up-regulated the host's glucose metabolism, and some up-regulated proteins were strongly associated with neurodegenerative diseases. These findings not only provide new insights into the psychiatric pathogenesis of *T. gondii*, but also provide potential targets for the treatment of acute and chronic Toxoplasmosis.

## Author summary

*Toxoplasma gondii* is an extremely widespread and a worldwide health problem protozoon, which can infect the central nervous system, inducing acute and chronic infection

Development Plan of Shandong Province (202101050270, 202201050457). XHH was supported by the Youth Science Foundation of Shandong First Medical University (Shandong Academy of Medical Sciences) (202201-042). YK was funded by the Taishan Scholars Project of Shandong Province (tsqn202103186), Open research foundation of Key Laboratory for parasitic disease (NHCKFKT2022-15), key research and development plan of Jining (2022YXNS152), the Academic Promotion Program of Shandong First Medical University (NO.2019QL005). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

and causing severe toxoplasmosis and behavioral cognitive impairment. However, there is still no effective vaccines to prevent infection, drugs used to treat the acute infected individuals have a lot of limitations, and no drugs to treat chronic infected individuals. In this study, we established an acute and chronic infection model of *T. gondii* in astrocytes, and then used label-free quantitative proteomics to detect the proteome changes before and after infection, respectively. We aimed to understand the molecular mechanism and find special targets of *T. gondii* infection at different stages by analyzing the biological processes and signaling pathways, which the differential expressed proteins were involved in. Our study may provide new insights for further exploring the parasite-host interaction, and new targets for developing anti-*Toxoplasma* vaccines and drugs for the treatment of acute and chronic toxoplasmosis.

## 1. Introduction

*Toxoplasma gondii* (*T. gondii*) is an obligate intracellular parasite that can infect almost all warm-blooded animals, including humans and livestock, and causes zoonotic parasitic diseases [1–4]. It is estimated that 30% of the global population was infected by *T. gondii* [5]. In China, approximately 8.2% of Chinese population individuals have been affected by this parasite [6]. *T. gondii* has a complex life cycle, and almost all warm-blooded animals can become intermediate hosts. During the intracellular life, *T. gondii* undergoes five distinct developmental stages including tachyzoite, bradyzoite, sporozoite, schizont, and gametocyte. Tachyzoites reside within pseudocyst, while bradyzoites are present in the tissue cyst. The schizont and gametophyte are the sexual reproductive stages, ultimately formed into oocyst. In the intermediate hosts, there are two infectious stages: tachyzoite and bradyzoite. Due to the minimal morphological disparity between tachyzoites and bradyzoites, the bradyzoite-specific expression protein BAG1 is usually used for the identification of bradyzoites [7].

The mutual transformation of tachyzoites and bradyzoites is the central link in the pathogenesis of *T. gondii*. Tachyzoites can cause acute infection, while bradyzoites are the main cause of chronic infection. Acute toxoplasmosis is more prevalent in immunodeficiency patients [8–10]. However, when the tachyzoites are transformed into bradyzoites and form cysts in normal immune responses, they will latently parasitize in the host's brain and ocular chorioretinal regions. When the host's immune system is impaired, the latent bradyzoites (in the CNS) could burst out of the cysts, revert to replicative tachyzoites and triggering a new round of infection [11]. In the chronic stages of infection, the host may have alterations in behavior and cognition [12–15]. For example, the chronic infection in rodent hosts, can lead to excessive exercise, decreased anxiety, reduced new phobias, and predator vigilance. In particular, *T. gondii* could induce changes in rodent olfactory preferences, converting an innate aversion for cat odor into attraction, in order to enhance their own transmission [16]. Nevertheless, Boillat *et al* found that *T. gondii* infection could commonly shift the host's aversion to predators, which is not unique to cats. And this alternation may be related to cysts in the host brain [17]. As far as humans are concerned, a large amount of seroepidemiological data have indicated that *T. gondii* infection has increased the incidence of mental illness, especially focusing on the relationship among schizophrenia, suicide and traffic accidents [18–21].

*T. gondii* can infect a variety of cells in the CNS, including neuronal cells, astrocytes, microglia and Purkinje cells. Previous studies have reported that astrocytes in CNS were more susceptible to tachyzoites [22]. Astrocytes are the most abundant glial cell type in CNS, constitute a heterogeneous cell population and could maintain neural homeostasis [23]. Astrocytes play

an important role in regulation of energy metabolism, brain barrier, synaptic structure and plasticity [24–27]. They can also interact with CD4+ and CD8+ T cells to prevent neuronal damage [28]. However, the regulation details of astrocytes infected with tachyzoites or / and bradyzoites are still unknown. Therefore, exploring the response of astrocytes infected with different stages of *T. gondii* will help to understand the mechanism of interaction between *T. gondii* and host CNS, and lay a solid foundation for the study of acute and chronic Toxoplasmosis.

The aim of the present study was to figure out the changes of host protein expression before and after infection with tachyzoite and bradyzoite in C8-D1A cells, respectively by using the label-free proteome detection. Exploring the function of differentially expressed proteins to reveal the molecular mechanism of astrocytes to parasites at different stages of *T. gondii* infection, which can provide new insights into the pathogenic mechanism of acute and chronic toxoplasmosis, and show new targets for the development of anti-*T. gondii* therapeutic drugs.

## 2. Materials and methods

### 2.1 *T. gondii* and cell culture

*T. gondii* tachyzoites (RH strain) were maintained using human foreskin fibroblast (HFF) cells. Cells were cultured in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 100 IU/mL penicillin, and 100 µg/mL streptomycin (Beyotime, China) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. After the infected HFF monolayer was lysed, cell mixture was collected and filtered through a 5 µm filter to obtain *T. gondii* tachyzoites, counted and stored at -80°C. C8-D1A mouse astrocytes were cultured under the identical conditions as HFF cells.

### 2.2 Sample preparation and collection

C8-D1A ( $5 \times 10^5$  cells) were seeded on 8 mm coverslips in 6-well plates (Costar, USA), and cultured in 37°C with 5% CO<sub>2</sub>. When cells formed confluent monolayers, replace medium with 3% FBS. RH strain of *T. gondii* were added to C8-D1A (The ratio of cells to tachyzoites was 10:1) for 24 h. PBS washed two times and collected samples, centrifuged at 1000 g for 5 minutes, stored at -80°C, and the group was named C8-TA. Alkaline-induced transformation of tachyzoites *in vitro* were performed. The *T. gondii* tachyzoites were added to cells at a ratio of 1:10 and cultured for 8 h at 37°C with 5% CO<sub>2</sub>, then continued to culture for 96 h in alkaline media (pH 8.2) and changed the medium every 1–2 days. PBS washed two times and collected samples, centrifuged at 1000 g for 5 minutes, stored at -80°C, the group was named C8-BR. The uninfected group was named C8. Because of three replicates for each group, nine samples were used for the following experiment.

### 2.3 RT-PCR

The expression of bradyzoite antigen 1 (BAG1) was detected using RT-PCR. Total RNA was extracted from the C8-BR group and the C8-TA group with TRIzol (Sigma-Aldrich, USA). The isolated RNA was converted to cDNA using Takara PrimerScript RT reagent Kit (Takara, Japan) according to the instructions. BAG1 primers were as follows:

Forward primer: 5'-TCGCCTCTCAACAGCTAGAC-3';

Reverse primer: 5'-CCCTGAATCCTCGACCTTGAT-3';

The PCR reaction conditions were 94°C for 5 min, 94°C for 40 s, 56°C for 40 s, and 72°C for 1 min. PCR products were analyzed by 1% agarose gel electrophoresis.

## 2.4 Protein extraction and trypsin digestion

All of the samples were sonicated three times on ice using a high intensity ultrasonic processor (Scientz, China) in lysis buffer (8 M urea, 1% Protease Inhibitor Cocktail). Centrifuged at 12,000 g at 4°C for 10 min to remove remaining debris. BCA kit was used to determine the protein concentration according to the manufacturer's instructions. For digestion, the protein solution of each sample was reduced with 5 mM dithiothreitol for 30 min at 56°C and then alkylated with 11 mM iodoacetamide in the dark for 15 min at room temperature. 100 mM TEAB was added to dilute the sample. Finally, trypsin was added for the first digestion overnight (trypsin-to-protein mass ratio was 1:50) and performed a subsequent 4 h-digestion (trypsin-to-protein mass ratio was 1:100).

## 2.5 LC-MS/MS analysis

The tryptic peptides were dissolved in 0.1% formic acid (solvent A), and directly loaded onto a home-made reversed-phase analytical column (15-cm length, 75 µm i.d.). The gradient was comprised of an increase from 6% to 23% solvent B (0.1% formic acid in 98% acetonitrile) over 26 min, 23% to 35% in 8 min and climbing to 80% in 3 min then holding at 80% for the last 3 min, all at a constant flow rate of 400 nL/min on an EASY-nLC 1000 UPLC system. The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q Exactive Plus (Thermo, USA) coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The m/z scan range was 350 to 1800 for full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 28 and the fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans with 15.0 s dynamic exclusion. Automatic gain control (AGC) was set at 5E4.

## 2.6 Database search

The resulting MS/MS data were processed using Maxquant search engine (v.1.5.2.8). Tandem mass spectra were searched against *Mus musculus* data in the Uniprot database concatenated with reverse decoy database. Trypsin/P was specified as cleavage enzyme allowing up to 2 missing cleavages. Mass tolerance for precursor ions was set as 20 ppm in First search and 5 ppm in Main search, and the mass tolerance for fragment ions was set as 0.02 Da. Carbamidomethyl on Cys was specified as fixed modification, oxidation on Met was specified as variable modifications. Label-free quantification method was LFQ, FDR was adjusted to < 1% and the minimum score for peptides was set > 40.

## 2.7 Bioinformatic analysis

Wolfpsort ([http://www.genscript.com/psort/wolf\\_psort.html](http://www.genscript.com/psort/wolf_psort.html)) was used to predicate subcellular localization of differentially expressed proteins. Eukaryotic orthologous group (<http://genome.jgi.doe.gov/help/kogbrowser.jsf>) was performed on all differentially expressed proteins for further functional classification by aligning their sequences with the KOG database. The UniProt-GOA database (<http://www.ebi.ac.uk/GOA/>) together with InterProScan soft (<http://www.ebi.ac.uk/InterProScan/>) were used to analyze biological process, cellular component and molecular function of differentially expressed proteins. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.genome.jp/kegg>) was used to analyze signaling pathways involved in differentially expressed proteins. A two-tailed Fisher's exact test was employed to test the enrichment of the differentially expressed protein against all identified proteins. A corrected *p*-value < 0.05 was considered significant in database analysis.

Cluster membership was visualized by a heat map using the “heatmap.2” function from the “gplots” R-package.

## 2.8 Parallel Reaction Monitoring (PRM) Validation

To verify the accuracy of label-free proteome quantification analysis, we selected 18 differentially expressed proteins for PRM assay. The methods of protein extraction and trypsin digestion were as described above. In LC-MS/MS Analysis, the gradient was comprised of an increase from 6% to 23% solvent B (0.1% formic acid in 98% acetonitrile) over 38 min, 23% to 35% in 14 min and climbing to 80% in 4 min then holding at 80% for the last 4 min, all at a constant flow rate of 700 nL/min on an EASY-nLC 1000 UPLC system. Automatic gain control (AGC) was set at 3E6 for full MS and 1E5 for MS/MS. The maximum IT was set at 20 ms for full MS and auto for MS/MS. The isolation window for MS/MS was set at 2.0 m/z. Peptide parameters were as follows: enzyme was set as Trypsin [KR/P], Max missed cleavage set as 2. The peptide length was set as 8–25. precursor charges were set as 2, 3, ion charges were set as 1, 2, ion types were set as b, y, p. The product ions were set as from ion 3 to last ion, the ion match tolerance was set as 0.02 Da.

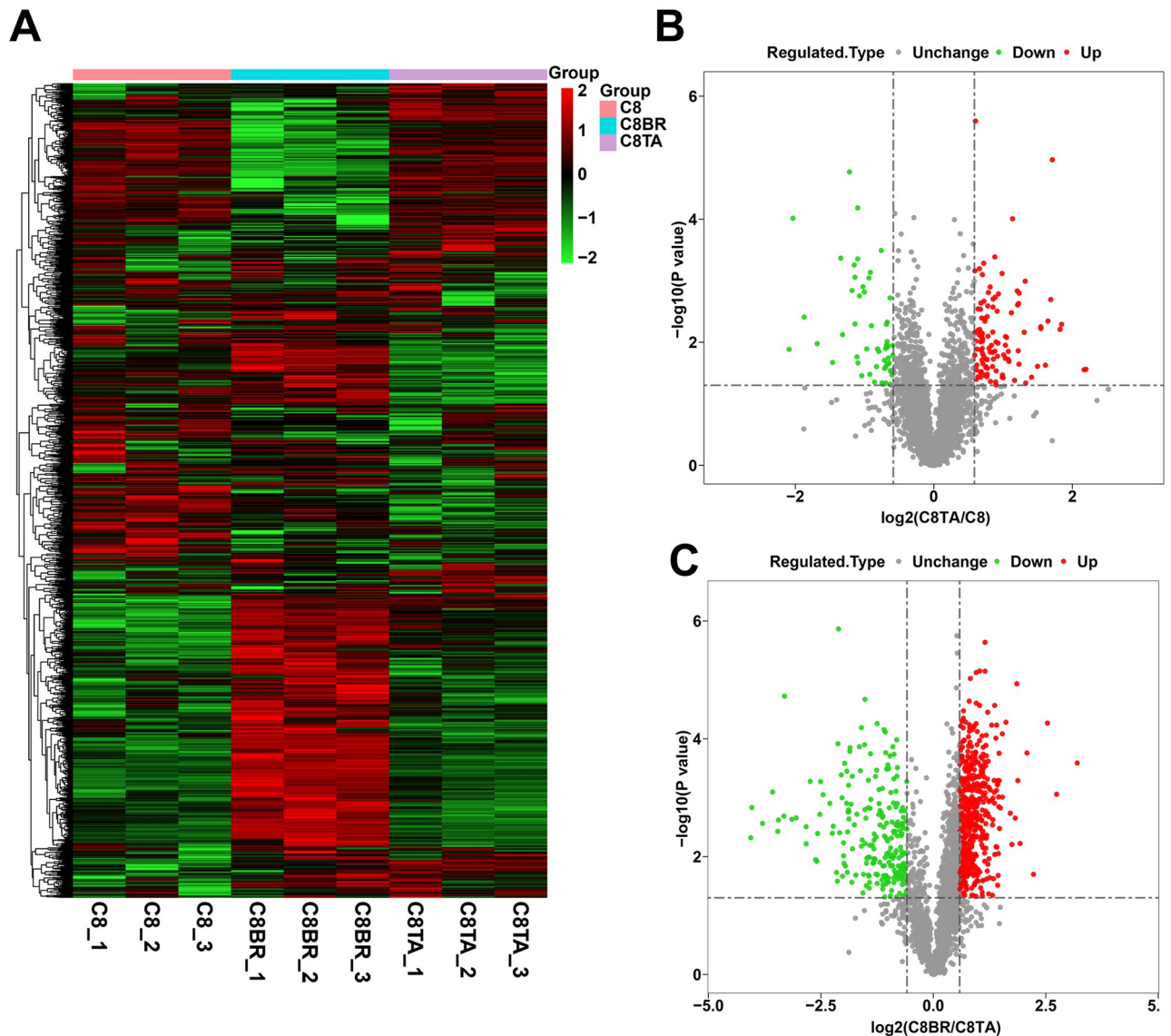
## 2.9 Western blotting

The cell pellets of C8, C8-TA and C8-BR group were lysed on ice in RIPA lysis buffer (Beyotime, China) for 30 min. Proteins were separated using 10% SDS PAGE and transferred to a PVDF membrane (Beyotime, China). The membrane was blocked in 5% milk powder for 2 h at 37°C. The membrane was incubated with primary antibodies at 4°C overnight. The membrane was then washed with TBST (0.1% Tween 20) three times and incubated with goat anti-rabbit IgG (H+L) HRP conjugate (1:5000, Proteintech, China) for 2 h at 4°C and detected by chemiluminescence (Proteintech, China) method. The densities of the bands were analyzed using iBright 1500 v1.4.3 software (Thermo, USA). The information for antibodies are as follows: DDX58 (1:3000, Proteintech, China), GOT1 (1:1000, Proteintech, China), SIRT2 (1:8000, Proteintech, China), DLD (1:1000, Bioworld, China), ECHS1 (1:5000, Proteintech, China) and GAPDH (1:10000, Bioworld, China).

## 3. Results

### 3.1 Identification of differentially expressed proteins between C8-TA, C8-BR and C8 groups

We used *T. gondii* tachyzoites (*RH* strain) infected C8-D1A cells. After 24 h, the pseudocysts were observed in the cells (S1 Fig). Next, we performed Alkaline-induced transformation of tachyzoites *in vitro*. After 96 h, the bradyzoite-specific gene BAG1 was detectable (S2 Fig). These results indicated that we have successfully constructed *in vitro* models of tachyzoite and bradyzoite infection in C8-D1A astrocytes. We used label-free proteome to quantitatively analyze the host proteins and in tachyzoite and tachyzoite to bradyzoite transformation stages of *T. gondii* infection. Among the 9 samples, a total of 32906 peptides and 30808 unique peptides were identified. We identified 4676 proteins as host protein, of which 3415 proteins were quantified (S1 Table). We defined fold change  $\geq 1.5$  or  $\leq 0.67$  and  $p$ -value  $\leq 0.05$  as the criteria to analyze the differentially expressed proteins of C8-TA, C8-BR and C8 group (Fig 1A). There were a total of 163 differentially expressed proteins, of which 109 were up-regulated and 54 were down-regulated in C8-TA group compared with C8 group (S2A Table and Fig 1B). In addition, there were 719 differentially expressed proteins, of which 495 host proteins were up-



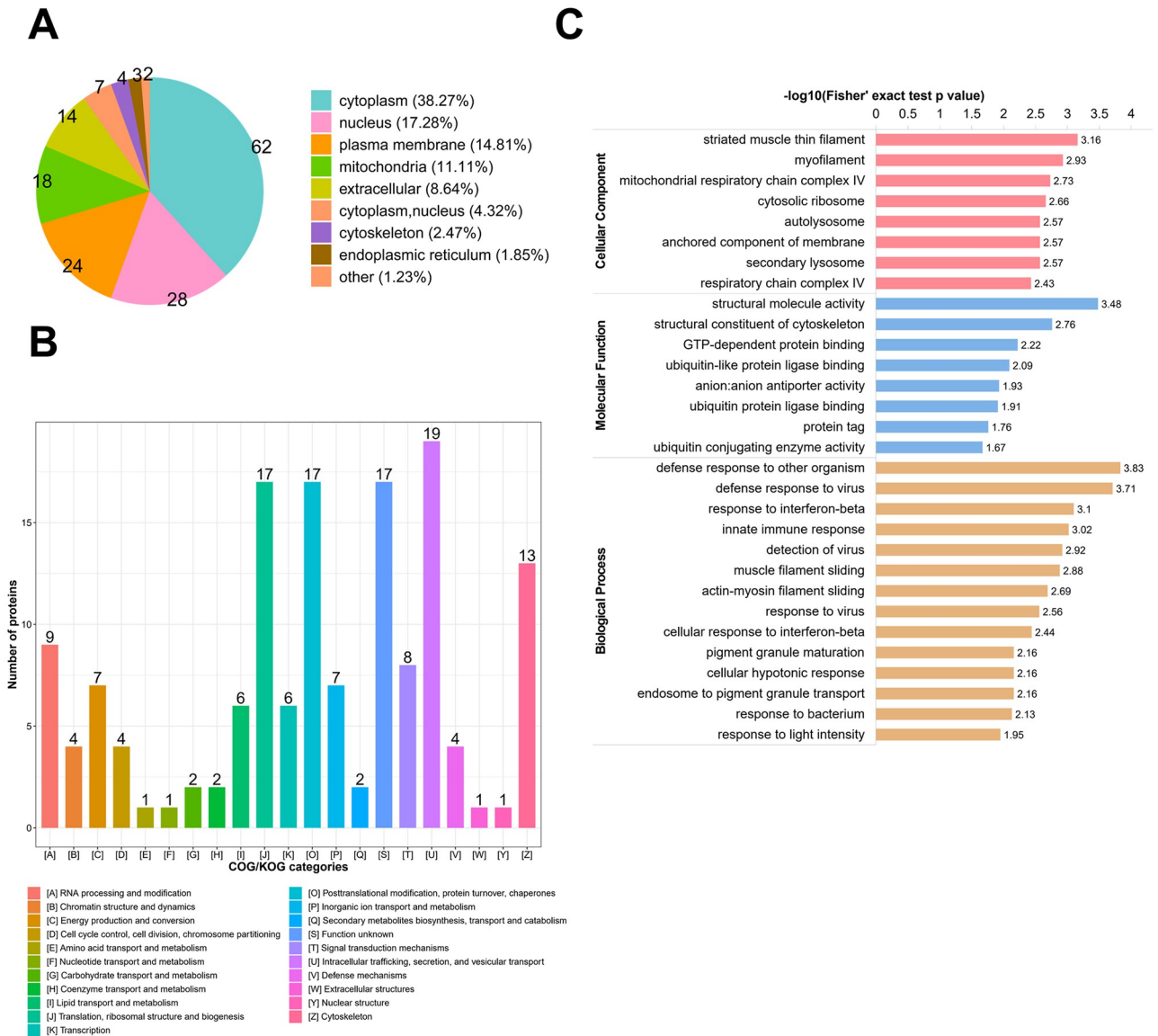
**Fig 1. Proteome analysis of differentially expressed proteins in tachyzoite and tachyzoite to bradyzoite transformation stages of *T. gondii* infection.** (A) Heat map shows the differentially expressed proteins in the three groups. (B) Volcano plot shows differentially expressed proteins between the C8-TA group and the C8 group. (C) Volcano plot shows differentially expressed proteins between the C8-BR group and the C8-TA group.

<https://doi.org/10.1371/journal.pntd.0011102.g001>

regulated and 224 host proteins were down-regulated in C8-BR group compared with C8-TA group (S2B Table and Fig 1C).

### 3.2 Tachyzoite infection altered multiple immunoregulatory processes in astrocytes

To investigate the biological functions of the differentially expressed proteins between the C8-TA group and the C8 group, we firstly analyzed the subcellular localization of the differentially expressed proteins. As shown in Fig 2A, 38.27% differentially expressed proteins were located in cytoplasm, 14.81% differentially expressed proteins were located in plasma membrane, and 17.28% differentially expressed proteins were located in nucleus in C8-TA vs C8



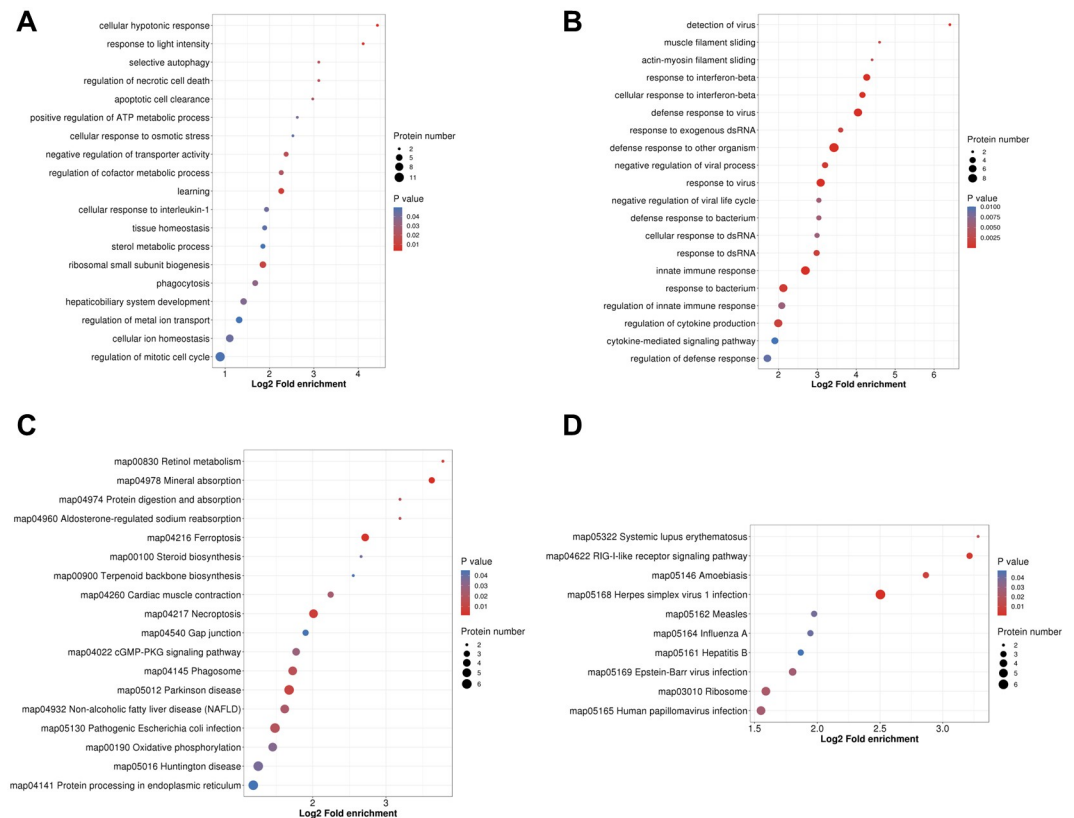
**Fig 2. Location and functional classification of differentially expressed proteins between the C8-TA group and the C8 group.** (A) Location of subcellular structures of differentially expressed proteins between the C8-TA group and the C8 group. (B) KOG classification of differentially expressed proteins between the C8-TA group and the C8 group. (C) GO enrichment analysis of differentially expressed proteins between C8-TA vs C8 group included biological process, cellular composition, and biological function.

<https://doi.org/10.1371/journal.pntd.0011102.g002>

group. KOG (EuKaryotic orthologous groups) was used to predict the potential functions of differentially expressed proteins in the C8-TA vs C8 group (S3 Table and Fig 2B). The results showed that the top five KOG classifications were [U] Intracellular trafficking, secretion, and vesicular transport, [J] Translation, ribosomal structure and biogenesis, [O] Posttranslational modification, protein turnover, chaperones, [Z] Cytoskeleton, [A] RNA processing and modification. GO enrichment analysis was performed for the functional annotation of differentially expressed proteins including three categories: biological process (GO-BP), cellular compartment (GO-CC) and molecular function (GO-MF) (S4A Table and Fig 2C). We focused on the biological processes and found that the differentially expressed proteins mainly enriched in

immune-related biological processes, such as defense response to other organism, defense response to virus, response to interferon-beta, innate immune response, detection of virus, which indicated that the differentially expressed proteins were primarily involved in immune regulations.

Next, we comprehensively analyzed the biological functions of up-regulated and down-regulated proteins in C8-TA vs C8 group. The GO-BP results showed that, the up-regulated proteins in C8-TA vs C8 group were mainly involved in the following biological processes: cellular hypotonic response, response to light intensity, learning, ribosomal small subunit biogenesis, negative regulation of transporter activity (S4B Table and Fig 3A). The down-regulated proteins were mainly related to immune responses, such as defense response to other organisms, defense response to virus, response to interferon-beta, response to virus, and innate immune response (S4C Table and Fig 3B). KEGG enrichment analysis of up-regulated and down-regulated proteins revealed the signaling pathways during the infection of *T. gondii* tachyzoites (S5A and S5B Table). As shown in Fig 3C and 3D, the up-regulated proteins were mapped to 18 signaling pathways, and the down-regulated proteins were mapped to 10 signaling pathways in C8-TA vs C8 group. As expected, the down-regulated proteins were enriched in inflammatory signaling pathways, such as Herpes simplex virus 1 infection, RIG-I-like receptor signaling pathway, Amoebiasis, Systemic lupus erythematosus. The above findings indicated that the host immune response was activated during *T. gondii* invasion. Interestingly,



**Fig 3. GO and KEGG pathway enrichment analysis of up-regulated and down-regulated proteins between C8-TA group and C8 group.** (A) GO enrichment analysis of up-regulated proteins. (B) GO enrichment analysis of down-regulated proteins. (C) KEGG pathway enrichment analysis of up-regulated proteins. (D) KEGG pathway enrichment analysis of down-regulated proteins.

<https://doi.org/10.1371/journal.pntd.0011102.g003>



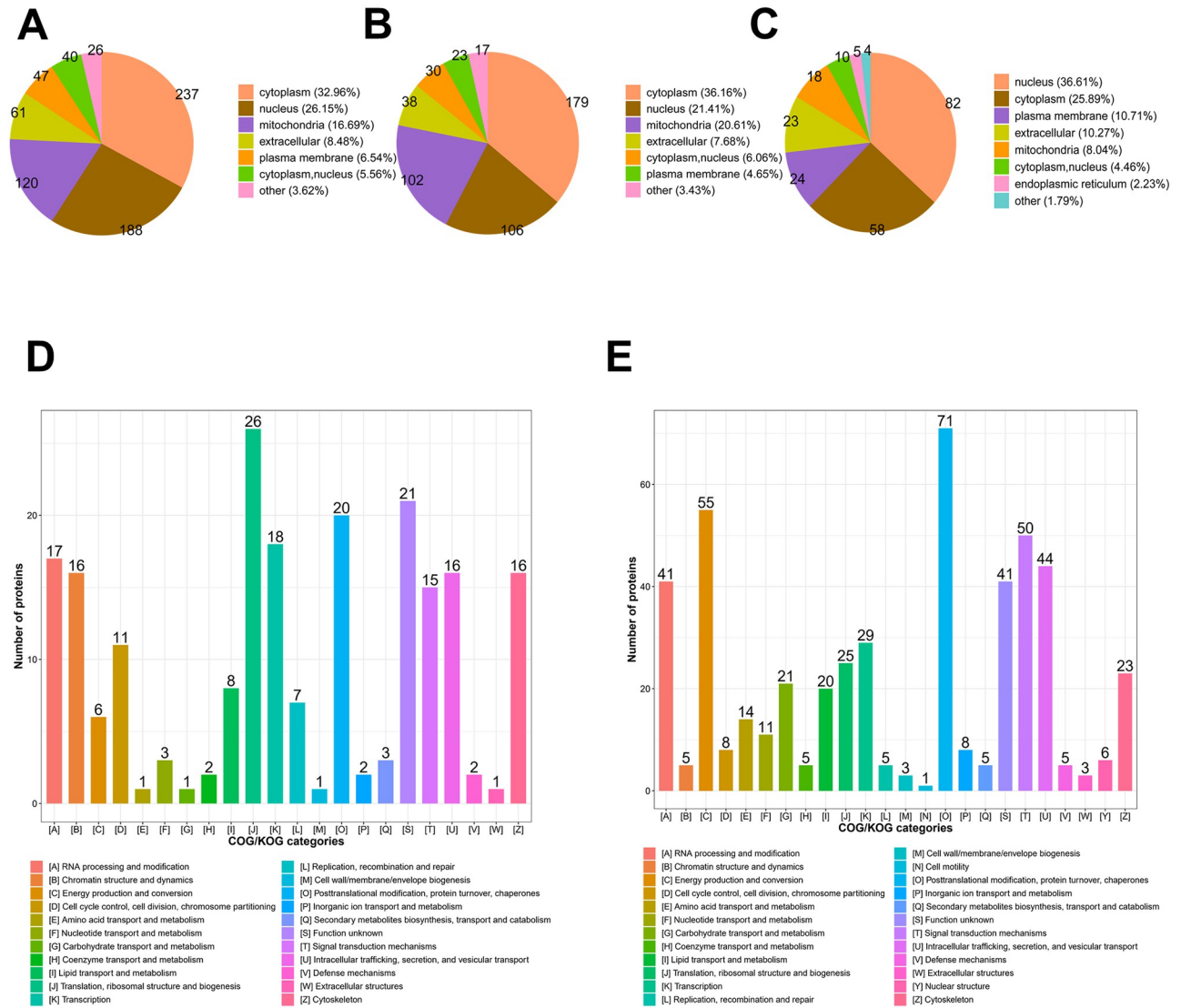
our results showed that the down-regulated proteins may play a major role in immune regulation. This was different from the biological traits of *T. gondii* invaded epithelial cells, which indicated that the immune response of the host was gradually weakened when tachyzoites invaded astrocytes for 24 h. This might be a protective measurement for *T. gondii* to avoid host astrocytes generating strong neuro-immune response.

### 3.3 The conversion of the tachyzoites into bradyzoites upregulated glucose metabolism of astrocytes

Tachyzoite to bradyzoite differentiation is a key aspect of *T. gondii* biology and pathogenesis. To better understand the mechanism of interaction between *T. gondii* and the host during differentiation of *T. gondii*, we performed a comprehensive bioinformatics analysis in C8-BR vs C8-TA group. First, we analyzed the sublocalization of all differentially expressed proteins by WoLF PSORT software (Fig 4A). The results showed that 33% of the differentially expressed proteins were localized in cytoplasm, 26% in nucleus, and 17% in mitochondria. In up-regulated proteins, most of them were located in the cytoplasm (36%), followed by nucleus (21.41%) and mitochondria (20.61%) (Fig 4B). In down-regulated proteins, most of them were located in the nucleus (37%), followed by cytoplasm (26%) and plasma membrane (11%) (Fig 4C).

We used KOG database to predict the function and classification of up and down-regulated proteins (S6A and 6B Table and Fig 4D and 4E). The results showed that the functions of up-regulated proteins were consistent with down-regulated proteins, and the differentially expressed proteins were mainly enriched in the following categories: [O] Posttranslational modification, protein turnover, chaperones, [T] Signal transduction mechanisms, [C] Energy production and conversion, [U] Intracellular trafficking, secretion, and vesicular transport, [A] RNA processing and modification pathway, suggested that during the chronic infection stage, epigenetic mechanisms may mediate the effects on host nutrient metabolism in the coexistence of host and bradyzoites. GO enrichment analysis showed that the differentially expressed proteins were mainly involved in metabolism-related biological processes including dicarboxylic acid metabolic process, oxidoreduction coenzyme metabolic process, hexose biosynthetic process, monocarboxylic acid metabolic process, tricarboxylic acid metabolic process (S7A Table and S3A Fig). Among them, glucose metabolism related biological processes were up-regulated, such as dicarboxylic acid metabolic process, oxidoreduction coenzyme metabolic process, carboxylic acid metabolic process, tricarboxylic acid metabolic process, nucleoside phosphate metabolic process (S7B Table and Fig 5A). However, there were no significant biological processes enrichment for down-regulated proteins. The top five categories of down-regulated proteins were protein localization to endoplasmic reticulum, ribosomal large subunit biogenesis, peptide biosynthetic process, DNA conformation change, peptide metabolic process (S7C Table and Fig 5B).

KEGG pathway enrichment was used to identify pathways of differentially expressed proteins (S8A Table and S3B Fig), the results showed that differentially expressed proteins significantly enriched in metabolism-related pathways, the top five were Glycolysis/Gluconeogenesis, Citrate cycle (TCA cycle), Phenylalanine metabolism, Phagosome, Pyruvate metabolism. We further investigated the function of up and down-regulated proteins and found that up-regulated proteins were mainly involved in metabolism-related signaling pathways including Citrate cycle (TCA cycle), Glycolysis/Gluconeogenesis, Proteasome, Phagosome, Pyruvate metabolism, which was consistent with the GO-BP (S8B Table and Fig 5C). The part of down-regulated proteins involved in amino acid metabolism, such as Tyrosine metabolism, Phenylalanine metabolism (S8C Table and Fig 5D). During the transformation



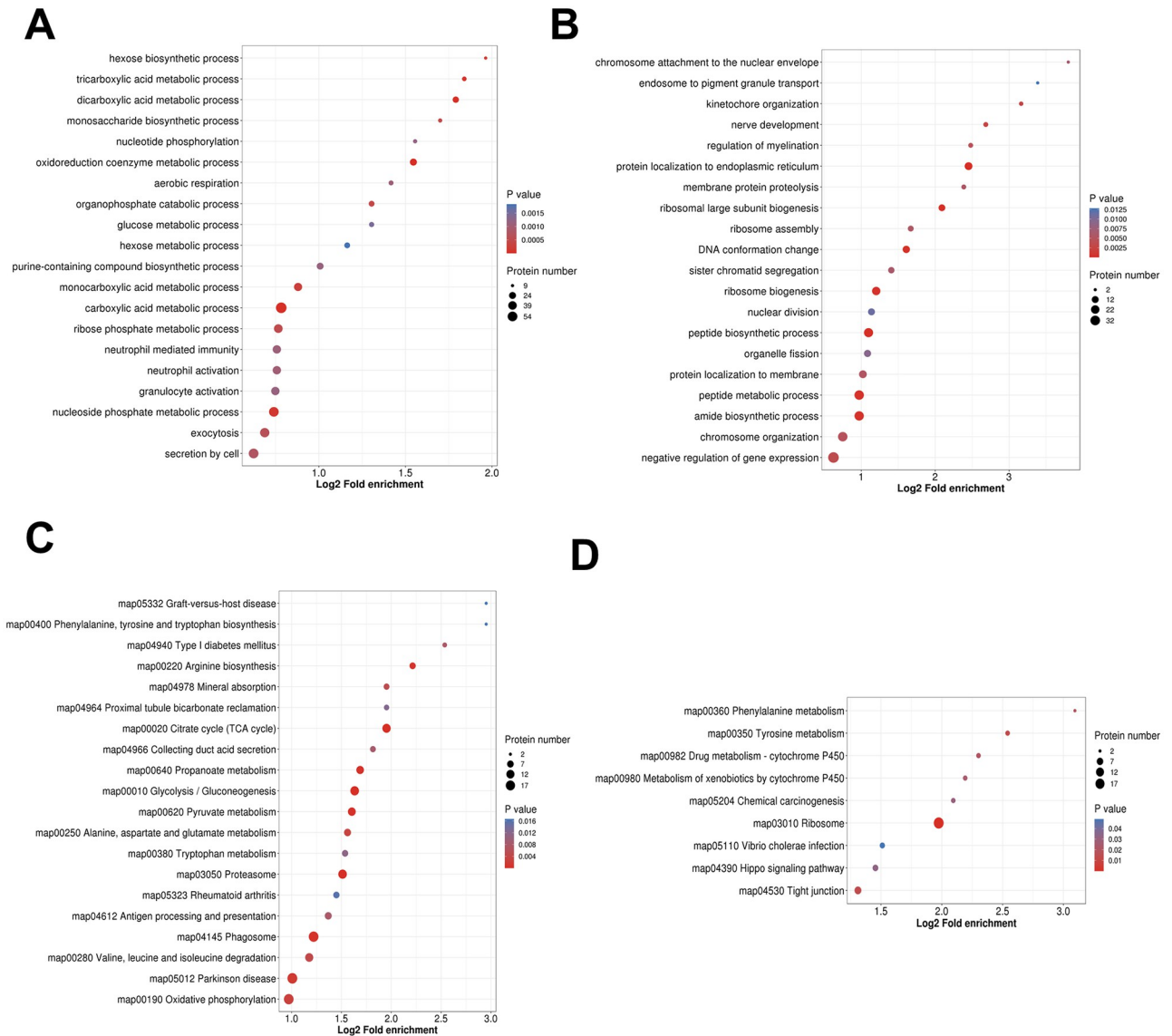
**Fig 4. Subcellular localization and functional classification of differentially expressed proteins between C8-BR group and C8-TA group.** (A) The location of subcellular structures of all differentially expressed proteins. (B) The location of subcellular structures of up-regulated proteins. (C) The location of subcellular structures of down-regulated proteins. (D) The KOG classification of up-regulated proteins. (E) The KOG classification of down-regulated proteins.

<https://doi.org/10.1371/journal.pntd.0011102.g004>

from tachyzoite infection stage to bradyzoite infection stage, the function of host proteins may have undergone a gradual transition from immune emergency mode to up-regulation metabolic pathways. The effects on host metabolism during transformation suggested that the increased host's metabolism could accelerate the decomposition of glucose, so the metabolism of *T. gondii* was decreased, which was more conducive to its long-lived in their hosts.

### 3.4 Verification of differentially expressed proteins

To evaluate the accuracy of label-free proteome quantification techniques, a total of 18 differentially expressed proteins were selected for PRM analysis. Based on GO and KEGG annotations, we chose Bystin (BYSL), Fibronectin (FN1), Signal transducer and activator of



**Fig 5. GO and KEGG pathway enrichment analysis of up-regulated and down-regulated differentially expressed proteins between C8-BR group and C8-TA group.** (A) GO enrichment analysis of up-regulated proteins. (B) GO enrichment analysis of down-regulated proteins. (C) KEGG pathway enrichment analysis of up-regulated proteins. (D) KEGG pathway enrichment analysis of down-regulated proteins.

<https://doi.org/10.1371/journal.pntd.0011102.g005>

transcription 1 (STAT1), Serpin B6 (SERPINB6), Guanylate-binding protein 4 (GBP4), Interferon-induced protein with tetratricopeptide repeats 3 (IFIT3), Antiviral innate immune response receptor RIG-I (DDX58), 60S ribosomal protein L23 (RPL23), 40S ribosomal protein S24 (RPS24) in C8-TA vs C8-group and Dihydrolipoyl dehydrogenase (DLD), Aspartate aminotransferase, cytoplasmic (GOT1), DNA (cytosine-5)-methyltransferase 1 (DNMT1), Nischarin (NISCH), Enoyl-CoA hydratase, mitochondrial (ECHS1), Dihydrolipoylsine-residue acetyltransferase component of pyruvate dehydrogenase complex (DLAT), Tricarboxylate transport protein (SLC25A1), NAD-dependent protein deacetylase sirtuin-2 (SIRT2), Omegaamidase NIT2 (NIT2) in C8-BR vs C8-TA group (S9 Table and Fig 6). The results showed that the changing trends of 18 differentially expressed proteins in PRM were consistent with label-

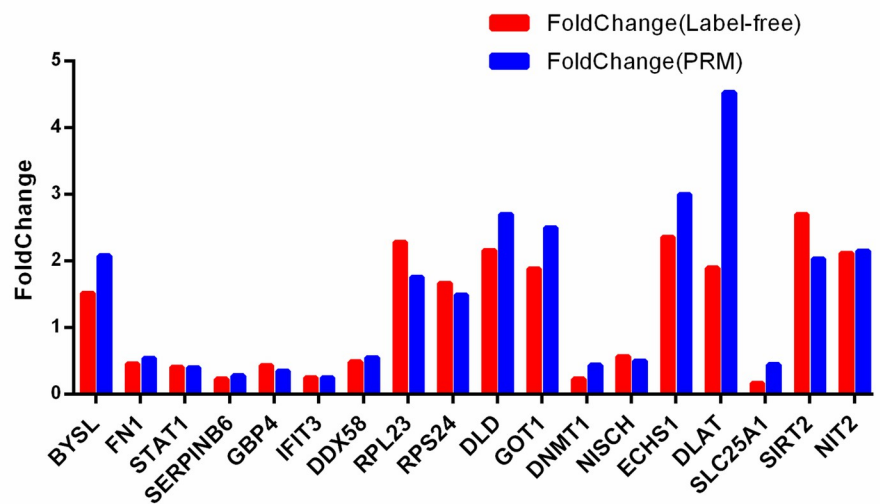


Fig 6. Comparative analyses of label-free proteomics and PRM results for 18 screened differentially expressed proteins.

<https://doi.org/10.1371/journal.pntd.0011102.g006>

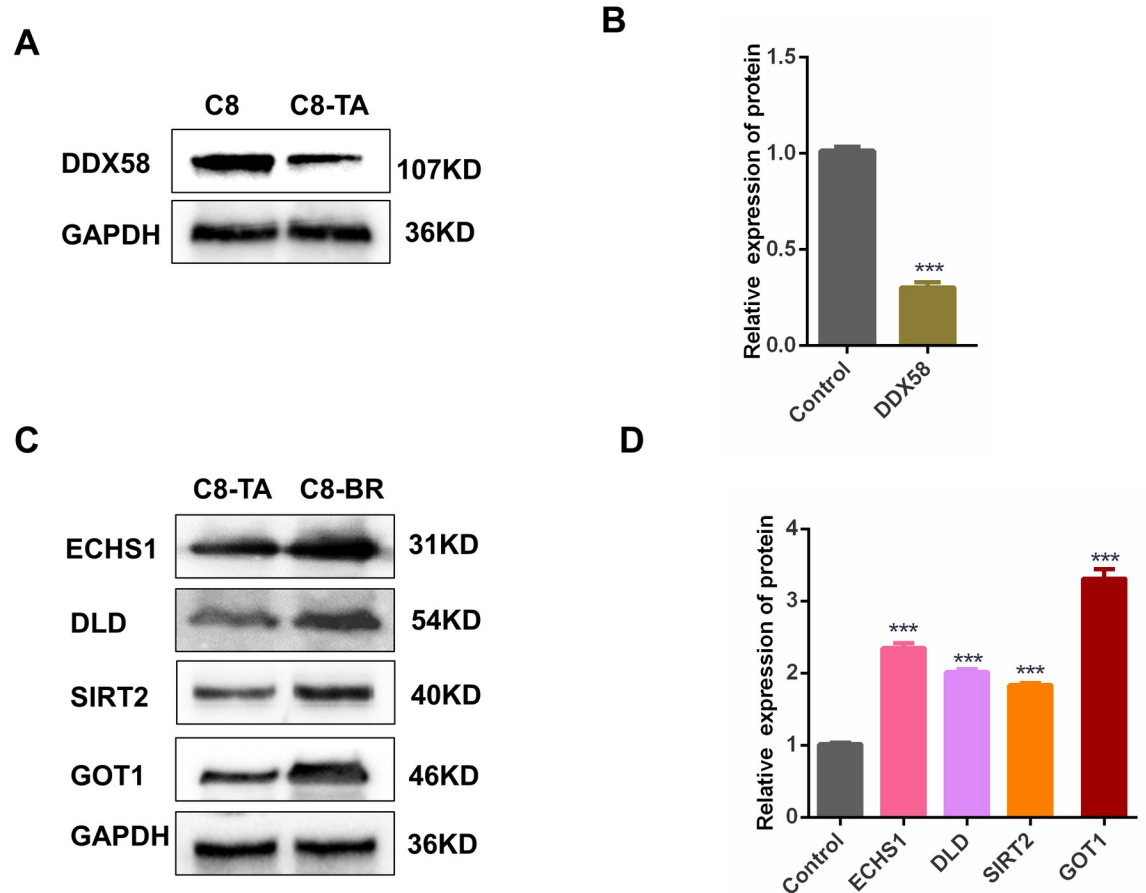
free proteome quantification. Next, we used western blotting to verify the expression of DDX58, ECHS1, DLD, SIRT2, GOT1 (Fig 7). The results were also consistent with label-free proteome quantification. The above results suggested that label-free proteome quantification outcomes were relatively reproducible and reliable.

#### 4. Discussion

The invasion of *T. gondii* tachyzoites and bradyzoites in the host causes acute infection and chronic infection respectively, resulting in clinical manifestations of toxoplasma encephalopathy, chorioretinitis, miscarriage, stillbirth and schizophrenia [18,29–31]. Since there is no effective method to eliminate tissue cysts up to now, the damage of host CNS caused by *T. gondii* accompanied with mental and behavioral disorders has been brought into focus. Therefore, understanding the proteomics changes in host neurogliaocytes after tachyzoites/bradyzoites infection would identify the negative effects of different stages on the host, and provide targets for developing new vaccines and drugs to treat brain damages caused by *T. gondii*.

In the present study, our results showed that the differentially expressed proteins in the acute infection stage were mainly enriched in immune-related biological processes, while the differentially expressed proteins in the chronic infection stage were mainly enriched in metabolic-related biological processes. Therefore our results suggested that *T. gondii* would regulate host neurogliaocytes by distinct modes in each of the two stages. Similarly, previous research on tachyzoite infection stage indicated that the up-regulated proteins were also involved in immune inflammation-related pathways to prevent acute infection [32], while those similar studies were mainly based on epithelial cells such as HFF cells.

As we know, invasion of *T. gondii* can cause inflammation in the host, and astrocytes have been proven to be a pivotal regulator of CNS inflammatory responses [33]. The borders and scars of astrocytes could serve as functional barriers that restrict the entry of inflammatory cells into CNS parenchyma in health and disease, simultaneously, it also has powerful pro-inflammatory potential. Therefore, we have established acute/chronic infection models with



**Fig 7. Verification of the differentially expressed proteins in different stages (tachyzoite and bradyzoite) of *T. gondii* infection.** (A) Western blotting analysis was used to detect the protein expression level of differentially expressed proteins in C8 group and C8-TA group. (B) Histogram analysis of the western blotting results in C8 group and C8-TA group. Statistical significance was analyzed using an unpaired Student's t-test (\*\* $p < 0.001$ ). (C) Western blotting analysis was used to detect the protein expression level of differentially expressed proteins in C8-TA group and C8-BR group. (D) Histogram analysis of the western blotting results in C8-TA group and C8-BR group. Statistical significance was analyzed using an unpaired Student's t-test (\*\* $p < 0.001$ ).

<https://doi.org/10.1371/journal.pntd.0011102.g007>

mouse astrocytes. Interestingly, although our differentially expressed proteins results in tachyzoites infection models were also involved in immune inflammation-related pathways, changes in protein expression were very different to the results of previous studies in epithelial cells.

In our study, the down-regulation proteins rather than up-regulation proteins were involved in immune regulation-related processes, especially in defense response pathways and innate immune response pathways. Consistently, Cekanaviciute reported that after *T. gondii* infection in the host CNS, the immune function must be restricted to prevent excessive neuronal damage, in order to keep a balance among *T. gondii*, brain and the immune system [34]. As a result, we have focused on four down-regulated and immune-related proteins including STAT1, GBP4, IFIT3 and DDX58 after tachyzoite infection. STAT1 is an important immune inflammatory factor in CNS and is involved in the immune regulation of various cells. Studies have shown that inhibition of STAT1 can promote bradyzoite formation [35]. In this study, the expression of STAT1 was significantly decreased, suggesting that after tachyzoite infection

in astrocytes, the down-regulated STAT1 might participate in promoting the transformation of tachyzoites to bradyzoites, as well as inhibiting the persistent infection of tachyzoites. GBP4 and IFIT3 are IFN- $\gamma$ -inducing proteins. Hu *et al* found that GBP4 could negatively regulate virus-induced type I IFN and antiviral responses by interacting with IFN regulatory factor (IRF) 7 during viral infection, thus the following researches on their roles in astrocytes during *T. gondii* infection may reveal a new mechanism by which astrocytes against parasites [36]. DDX58, also known as RIG-I, can function as an innate antiviral immune response receptor and play an important role in antiviral innate immunity [37–39]. In vesicular stomatitis virus (VSV)-infected astrocytes, RIG-I knockdown significantly reduced inflammatory cytokine production [40], so the down regulation of DDX58 may be also involved in the transformation of tachyzoites to bradyzoites in host CNS.

The conversation from tachyzoites to bradyzoites of *T. gondii* is a key to establish chronic infection and an important link in the pathogenesis of *T. gondii*. We comprehensively analyzed the differentially expressed proteins between the bradyzoites infection group and the tachyzoite infection group. The results showed that the differentially expressed proteins were mainly involved in metabolism-related biological processes and signaling pathways, and the detailed GO and KEGG analyses indicated that the host's glucose metabolism and a part of the amino acid metabolism process have been changed significantly during chronic infection. Further, we found that the up-regulated proteins were mainly involved in glucose metabolism and entered the TCA process, especially for ECHS1.

ECHS1 is a key enzyme involved in the metabolism of fatty acyl-CoA esters [41]. In fatty acid  $\beta$ -oxidation, it could increase the synthesis of acetyl-CoA and promote the TCA cycle, thereby increasing the process of glucose metabolism. It has been reported that ECHS1 deficiency (ECHS1D) led to the impaired ATP production and metabolic acidosis in patients [42]. As an obligate intracellular parasite, *T. gondii* obtains all nutrients from host cells to support its intracellular growth and proliferation. Glucose and glutamine are raw materials for tachyzoites to complete the classic TCA and then synthesize ATP [43,44]. Therefore the increase of host glucose metabolism would accelerate the consumption of glucose, resulting in the decreasing uptake of glucose by *T. gondii*. In the present study, the up-regulated ECHS1 may be an important host receptor of invading parasites, and associated with the inhibition of parasites self-glucose metabolism, thereby promoting the formation of intracellular bradyzoites and establish long-term latent infection in the host.

Neuronal degeneration caused by chronic infection of *T. gondii* is an important pathogenesis of neurodegenerative diseases, but the mechanism has not been fully elucidated. Our results also found important clues and potential targets for this process, such as DLD, SIRT2 and GOT1.

DLD, also known as dihydrolipoyl dehydrogenase, is a mitochondrial enzyme that is essential for eukaryotic cell metabolism [45]. Ahmad reported that DLD was related to Alzheimer's disease (AD), and inhibition of DLD expression would lead to a significant recovery of A $\beta$  pathological degradation. This could be explained that the inhibition of DLD would down-regulate metabolism-related signaling pathways and reduce the host's energy metabolism, which would be beneficial to alleviate the symptoms of AD [46]. In this study, we found that DLD was significantly up-regulated in the bradyzoites infection group, which may provide a new insight for explaining the mechanism of *T. gondii* infection-induced Alzheimer's disease. In addition, DLD is also associated with severe diseases in infants, causing developmental delay, hypotonia and metabolic acidosis [47]. Recent studies have shown that *Leishmania*'s self-encoded DLD induced a protective cellular immune response in *L. major*-infected mice, which could serve as a design site for Duchenne vaccines for kala-azar prevention [25,48].

SIRT2 is an NAD<sup>+</sup>-dependent deacetylase that is widely involved in cell division, angiogenesis [49–51], energy metabolism [52] and neurodegenerative [53,54], cardiovascular disease [55,56], oxidative stress [57] and many cancers [58,59], etc. Studies have found that SIRT2 was the most abundant sirtuin expressed in mammalian CNS, especially in cortex, striatum, hippocampus, and spinal cord, suggesting that it might have a role in CNS [60]. Dopaminergic (DA) neurons play a vital role in CNS, and DA neuron hyperfunction was involved in several neurological disorders including schizophrenia and Parkinson's Disease. SIRT2 expression was dramatically increased during the differentiation of human embryonic stem cells (hESCs) into midbrain DA neurons [61]. In addition, SIRT2 knockout (SIRT2<sup>-/-</sup>) mice displayed fewer DA neurons and less dense striatal fibers in the substantia nigra [62]. SIRT2 inhibition also improves cognitive impairment in different AD animal models and promotes neuronal survival [63,64]. This suggested that SIRT2 can be a potential therapeutic target for neurodegenerative disease. Moreover, SIRT2 is also involved in ATP synthesis. Recent studies have shown that SIRT2 localized to the inner mitochondrial membrane of the mouse brain, and mice lacking SIRT2 showed decreased ATP levels in the striatum [65]. In our study, the expression of SIRT2 was up-regulated after chronic infection, which is consistent with McConkey's report, they found that dopaminergic cells and brain tissues encysted with cerebral cysts have increased levels of dopamine synthesis and release [66]. Moreover, treatment of rats and mice with dopamine receptor antagonists could inhibit the behavioral changes induced by *T. gondii* infection [67,68]. In addition, some parasites such as *Leishmania*, *T. brucei*, and *Schistosoma* could encode SIRT2 homologous protein, and more importantly, it was essential for parasite growth [69–71]. Thus SIRT2 might serve as a potential treatment target for psychiatric disorders induced by manipulative parasites.

Neuroendocrine programs and neurotransmitter imbalance may act as the physiological basis for *T. gondii* induced psychiatric and behavioral disorders [72]. Glutamate (GLU) is the most abundant neurotransmitter in the brain, and its excitability plays a crucial role in brain structure and function. GOT1 aspartate aminotransferase is a type of aminotransferase that catalyzes the transamination of aspartate and  $\alpha$ -ketoglutarate to form glutamate and oxaloacetate. Glutamate dehydrogenase (GLUD1) is a mitochondrial enzyme that catalyzes the reductive fixation of ammonia to  $\alpha$ -ketoglutarate to form glutamate. Both GOT1 and GLUD1 expression were found to be up-regulated in our study, suggesting that the glutamate synthesis might be increased in the chronic infection group. Our finding is also consistent with a recent study, which showed that chronic infection with *T. gondii* could cause an increase in extracellular glutamate and a two-fold decrease in glutamate transporter expression in glial cells [73]. As hyperexcitability of GLU is neurotoxic and leading to brain damage, neurological disorders (eg, ALS, multiple sclerosis, AD, Huntington's disease, Parkinson's disease) and psychiatric disorders (eg, schizophrenia, depression, bipolar disorder). Our finding provides new evidence to explain the host mental behavioral disorders induced by chronic *T. gondii* infection.

In conclusion, our results systematically analyzed the proteomic changes in astrocytes infected with not only tachyzoites, but also bradyzoites, respectively. We surprisingly discovered that *T. gondii* tachyzoites can cause down-regulation of immune-related pathways in astrocytes, and the inhibited expression of DDX58 might be associated with the transformation of tachyzoites to bradyzoites. However, during the bradyzoites infection stage, metabolism rather than immune pathways of the host was changed significantly. Both the glucose metabolism pathways and the expression of some metabolism-related enzymes in astrocytes were significantly up-regulated, such as ECHS1, DLD, SIRT2, and GOT1. Since their expression was closely related to chronic degenerative diseases and psychiatric diseases, which could provide a new explanation for host mental and behavioral disorders induced by chronic infection of *T. gondii*.

## Supporting information

**S1 Fig. The *vitro* model of *T. gondii* tachyzoites (RH strain) infected mouse C8-D1A astrocytes.**

(TIF)

**S2 Fig. Agarose gel electrophoresis of PCR product for BAG1 gene.**

(TIF)

**S3 Fig. GO and KEGG enrichment analysis of all differentially expressed proteins between C8-BR group and C8-TA group.** (A) GO enrichment analysis of all differentially expressed proteins. (B) KEGG enrichment analysis of all differentially expressed proteins.

(TIF)

**S1 Table. Qualitative and quantitative annotation of astrocytes proteome.**

(XLSX)

**S2 Table.** A. The differentially expressed proteins in the C8-TA vs C8 group. B. The differentially expressed proteins in the C8-BR vs C8-TA group.

(XLSX)

**S3 Table. KOG classification of differentially expressed proteins in the C8-TA vs C8 group.**

(XLSX)

**S4 Table.** A. GO enrichment analysis of differentially expressed proteins in C8-TA vs C8 group. B. The GO enrichment analysis of up-regulated proteins in C8-TA vs C8 group. C. Table The GO enrichment analysis of down-regulated proteins in C8-TA vs C8 group.

(XLSX)

**S5 Table.** A. The KEGG pathway enrichment analysis of up-regulated proteins in the C8-BR vs C8-TA group. B. The KEGG pathway enrichment analysis of down-regulated proteins in the C8-BR vs C8-TA group.

(XLSX)

**S6 Table.** A. The KOG classification of up-regulated proteins in the C8-BR vs C8-TA group. B. The KOG classification of down-regulated proteins in the C8-BR vs C8-TA group.

(XLSX)

**S7 Table.** A. The GO enrichment analysis of all differentially expressed proteins in the C8-BR vs C8-TA group. B. The GO enrichment analysis of up-regulated proteins in the C8-BR vs C8-TA group. C. The GO enrichment analysis of down-regulated proteins in the C8-BR vs C8-TA group.

(XLSX)

**S8 Table.** A. The KEGG pathway enrichment analysis of all differentially expressed proteins in the C8-BR vs C8-TA group. B. The KEGG pathway enrichment analysis of up-regulated proteins in the C8-BR vs C8-TA group. C. The KEGG pathway enrichment analysis of down-regulated proteins in the C8-BR vs C8-TA group.

(XLSX)

**S9 Table. The quantification results of PRM analysis of differentially expressed proteins in the C8-TA vs C8 and C8-BR vs C8-TA groups.**

(XLSX)



## Author Contributions

**Conceptualization:** Huanhuan Xie, Kun Yin.

**Data curation:** Hongjie Dong, Qi Wang, Chao Xu.

**Formal analysis:** Huanhuan Xie, Haozhi Xu.

**Funding acquisition:** Huanhuan Xie, Kun Yin.

**Investigation:** Huanhuan Xie, Lisha Dai, Junmei Zhang.

**Methodology:** Huanhuan Xie, Hang Sun, Lixin Zhang, Guihua Zhao.

**Project administration:** Huanhuan Xie.

**Resources:** Hang Sun, Lisha Dai, Lixin Zhang, Guihua Zhao.

**Software:** Hongjie Dong, Haozhi Xu.

**Supervision:** Huanhuan Xie, Junmei Zhang, Kun Yin.

**Validation:** Hang Sun, Hongjie Dong.

**Visualization:** Hongjie Dong.

**Writing – original draft:** Huanhuan Xie.

**Writing – review & editing:** Huanhuan Xie, Chao Xu, Kun Yin.

## References

1. Elmore S A, Jenkins E J, Huyvaert K P, Polley L, Root J J, Moore C G. *Toxoplasma gondii* in circum-polar people and wildlife. Vector borne and zoonotic diseases (Larchmont, NY). 2012; 12(1):1–9. <https://doi.org/10.1089/vbz.2011.0705> PMID: 21995261
2. Chen J, Xu M J, Zhou D H, Song H Q, Wang C R, Zhu X Q. Canine and feline parasitic zoonoses in China. Parasit Vectors. 2012; 5:152. <https://doi.org/10.1186/1756-3305-5-152> PMID: 22839365
3. Boughattas S, Bergaoui R, Essid R, Aoun K, Bouratbine A. Seroprevalence of *Toxoplasma gondii* infection among horses in Tunisia. Parasit Vectors. 2011; 4:218. <https://doi.org/10.1186/1756-3305-4-218> PMID: 22107730
4. Montoya J G, Liesenfeld O. Toxoplasmosis. Lancet (London, England). 2004; 363(9425):1965–1976. [https://doi.org/10.1016/S0140-6736\(04\)16412-X](https://doi.org/10.1016/S0140-6736(04)16412-X) PMID: 15194258
5. Tenter AM, Heckeroth AR, Weiss L M. *Toxoplasma gondii*: from animals to humans. International journal for parasitology. 2000; 30(12–13):1217–1258. [https://doi.org/10.1016/s0020-7519\(00\)00124-7](https://doi.org/10.1016/s0020-7519(00)00124-7) PMID: 11113252
6. Dong H, Su R, Lu Y, Wang M, Liu J, Jian F, et al. Prevalence, Risk Factors, and Genotypes of *Toxoplasma gondii* in Food Animals and Humans (2000–2017) From China. Frontiers in microbiology. 2018; 9:2108. <https://doi.org/10.3389/fmicb.2018.02108> PMID: 30254613
7. Bohne W, Gross U, Ferguson D J, Heesemann J. Cloning and characterization of a bradyzoite-specifically expressed gene (*hsp30/bag1*) of *Toxoplasma gondii*, related to genes encoding small heat-shock proteins of plants. Molecular microbiology. 1995; 16(6):1221–1230. <https://doi.org/10.1111/j.1365-2958.1995.tb02344.x> PMID: 8577255
8. Luft B J, Remington J S. Toxoplasmic encephalitis. Journal of Infectious Diseases. 1988; 157(1):1–6.
9. Antinori A, Larussa D, Cingolani A, Lorenzini P, Bossolasco S, Finazzi M G, et al. Prevalence, associated factors, and prognostic determinants of AIDS-related toxoplasmic encephalitis in the era of advanced highly active antiretroviral therapy. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2004; 39(11):1681–1691. <https://doi.org/10.1086/424877> PMID: 15578371
10. Mohraz M, Mehrkhani F, Jam S, Seyedalinalaghi S, Sabzvari D, Fattahi F, et al. Seroprevalence of toxoplasmosis in HIV(+)/AIDS patients in Iran. Acta medica Iranica. 2011; 49(4):213–218. PMID: 21713730
11. Watts E, Zhao Y, Dhara A, Eller B, Patwardhan A, Sinai A P. Novel Approaches Reveal that *Toxoplasma gondii* Bradyzoites within Tissue Cysts Are Dynamic and Replicating Entities In Vivo. mBio. 2015; 6(5):e01155–01115. <https://doi.org/10.1128/mBio.01155-15> PMID: 26350965

12. Quatrini L, Vivier E, Ugolini S. Neuroendocrine regulation of innate lymphoid cells. *Immunological reviews*. 2018; 286(1):120–136. <https://doi.org/10.1111/imr.12707> PMID: 30294960
13. Xiao J, Savonenko A, Yolken R H. Strain-specific pre-existing immunity: A key to understanding the role of chronic *Toxoplasma* infection in cognition and Alzheimer's diseases? *Neuroscience and biobehavioral reviews*. 2022; 137:104660. <https://doi.org/10.1016/j.neubiorev.2022.104660> PMID: 35405182
14. Stock A K, Heintschel Von Heinegg E, Köhling H L, Beste C. Latent *Toxoplasma gondii* infection leads to improved action control. *Brain, behavior, and immunity*. 2014; 37:103–108. <https://doi.org/10.1016/j.bbi.2013.11.004> PMID: 24231154
15. Flegr J. How and why *Toxoplasma* makes us crazy. *Trends in parasitology*. 2013; 29(4):156–163. <https://doi.org/10.1016/j.pt.2013.01.007> PMID: 23433494
16. Vyas A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky R M. Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(15):6442–6447. <https://doi.org/10.1073/pnas.0608310104> PMID: 17404235
17. Boillat M, Hammoudi P M, Dogga S K, Pagès S, Goubran M, Rodriguez I, et al. Neuroinflammation-Associated Aspecific Manipulation of Mouse Predator Fear by *Toxoplasma gondii*. *Cell reports*. 2020; 30(2):320–334. e326. <https://doi.org/10.1016/j.celrep.2019.12.019> PMID: 31940479
18. Hamidinejat H, Ghorbanpoor M, Hosseini H, Alavi S M, Nabavi L, Jalali M H, et al. *Toxoplasma gondii* infection in first-episode and inpatient individuals with schizophrenia. *International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases*. 2010; 14(11): e978–981. <https://doi.org/10.1016/j.ijid.2010.05.018> PMID: 20843718
19. Ansari-Lari M, Farashbandi H, Mohammadi F. Association of *Toxoplasma gondii* infection with schizophrenia and its relationship with suicide attempts in these patients. *Tropical medicine & international health: TM & IH*. 2017; 22(10):1322–1327. <https://doi.org/10.1111/tmi.12933> PMID: 28734096
20. Oncu-Oner T, Can S. Meta-analysis of the relationship between *Toxoplasma gondii* and schizophrenia. *Annals of parasitology*. 2022; 68(1):103–110. <https://doi.org/10.17420/ap6801.414> PMID: 35491856
21. Sutherland A L, Kuin A, Kuiper B, Van Gool T, Leboyer M, Fond G, et al. Driving us mad: the association of *Toxoplasma gondii* with suicide attempts and traffic accidents—a systematic review and meta-analysis. *Psychological medicine*. 2019; 49(10):1608–1623. <https://doi.org/10.1017/S0033291719000813> PMID: 31010440
22. Halonen S K, Lyman W D, Chiu F C. Growth and development of *Toxoplasma gondii* in human neurons and astrocytes. *Journal of neuropathology and experimental neurology*. 1996; 55(11):1150–1156. <https://doi.org/10.1097/00005072-199611000-00006> PMID: 8939198
23. Eroglu C, Barres B A. Regulation of synaptic connectivity by glia. *Nature*. 2010; 468(7321):223–231. <https://doi.org/10.1038/nature09612> PMID: 21068831
24. Morita M, Ikeshima-Kataoka H, Kreft M, Vardjan N, Zorec R, Noda M. Metabolic Plasticity of Astrocytes and Aging of the Brain. *International journal of molecular sciences*. 2019; 20(4):941. <https://doi.org/10.3390/ijms20040941> PMID: 30795555
25. Daneman R, Prat A. The blood-brain barrier. *Cold Spring Harbor perspectives in biology*. 2015; 7(1): a020412. <https://doi.org/10.1101/cshperspect.a020412> PMID: 25561720
26. Allen N J, Eroglu C. Cell Biology of Astrocyte-Synapse Interactions. *Neuron*. 2017; 96(3):697–708. <https://doi.org/10.1016/j.neuron.2017.09.056> PMID: 29096081
27. Perez-Catalan N A, Doe C Q, Ackerman S D. The role of astrocyte-mediated plasticity in neural circuit development and function. *Neural development*. 2021; 16(1):1. <https://doi.org/10.1186/s13064-020-00151-9> PMID: 33413602
28. Blanchard N, Dunay I R, Schlüter D. Persistence of *Toxoplasma gondii* in the central nervous system: a fine-tuned balance between the parasite, the brain and the immune system. *Parasite immunology*. 2015; 37(3):150–158. <https://doi.org/10.1111/pim.12173> PMID: 25573476
29. Caselli D, Andreoli E, Paolicchi O, Savelli S, Guidi S, Pecile P, et al. Acute encephalopathy in the immune-compromised child: never forget toxoplasmosis. *Journal of pediatric hematology/oncology*. 2012; 34(5):383–386. <https://doi.org/10.1097/MPH.0b013e318257a15c> PMID: 22627571
30. Montoya J G, Remington J S. Toxoplasmic chorioretinitis in the setting of acute acquired toxoplasmosis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*. 1996; 23(2):277–282. <https://doi.org/10.1093/clinids/23.2.277> PMID: 8842263
31. Alvarado-Esquivel C, Pacheco-Vega S J, Hernández-Tinoco J, Centeno-Tinoco M M, Beristain-García I, Sánchez-Anguiano L F, et al. Miscarriage history and *Toxoplasma gondii* infection: A cross-sectional study in women in Durango City, Mexico. *European journal of microbiology & immunology*. 2014; 4(2):117–122. <https://doi.org/10.1556/EuJMI.4.2014.2.4> PMID: 24883197

32. Sun H, Li J, Wang L, Yin K, Xu C, Liu G, et al. Comparative Proteomics Analysis for Elucidating the Interaction Between Host Cells and *Toxoplasma gondii*. *Frontiers in cellular and infection microbiology*. 2021; 11:643001. <https://doi.org/10.3389/fcimb.2021.643001> PMID: 34055664
33. Sofroniew M V. Astrocyte barriers to neurotoxic inflammation. *Nature reviews Neuroscience*. 2015; 16(5):249–263. <https://doi.org/10.1038/nrn3898> PMID: 25891508
34. Cekanaviciute E, Dietrich H K, Axtell R C, Williams A M, Egusquiza R, Wai K M, et al. Astrocytic TGF- $\beta$  signaling limits inflammation and reduces neuronal damage during central nervous system *Toxoplasma* infection. *Journal of immunology* (Baltimore, Md: 1950). 2014; 193(1):139–149. <https://doi.org/10.4049/jimmunol.1303284> PMID: 24860191
35. Hidano S, Randall L M, Dawson L, Dietrich H K, Konradt C, Klover P J, et al. STAT1 Signaling in Astrocytes Is Essential for Control of Infection in the Central Nervous System. *mBio*. 2016; 7(6): e01881–16. <https://doi.org/10.1128/mBio.01881-16> PMID: 27834206
36. Hu Y, Wang J, Yang B, Zheng N, Qin M, Ji Y, et al. Guanylate binding protein 4 negatively regulates virus-induced type I IFN and antiviral response by targeting IFN regulatory factor 7. *Journal of immunology* (Baltimore, Md: 1950). 2011; 187(12):6456–6462. <https://doi.org/10.4049/jimmunol.1003691> PMID: 22095711
37. Yoneyama M, Fujita T. Function of RIG-I-like receptors in antiviral innate immunity. *The Journal of biological chemistry*. 2007; 282(21):15315–15318. <https://doi.org/10.1074/jbc.R700007200> PMID: 17395582
38. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nature immunology*. 2004; 5(7):730–737. <https://doi.org/10.1038/ni1087> PMID: 15208624
39. Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, et al. Cell type-specific involvement of RIG-I in antiviral response. *Immunity*. 2005; 23(1):19–28. <https://doi.org/10.1016/j.immuni.2005.04.010> PMID: 16039576
40. Furr S R, Moerdyk-Schauwecker M, Grdzlishvili V Z, Marriott I. RIG-I mediates nonsegmented negative-sense RNA virus-induced inflammatory immune responses of primary human astrocytes. *Glia*. 2010; 58(13):1620–1629. <https://doi.org/10.1002/glia.21034> PMID: 20578054
41. Finsterer J. Metabolische Myopathien—Teil I: Störungen des Kohlehydratstoffwechsels. *Fortschritte der Neurologie- Psychiatrie*. 2011; 79(10):598–606.
42. Brown G K, Hunt S M, Scholem R, Fowler K, Grimes A, Mercer J F, et al. beta-hydroxyisobutyryl coenzyme A deacylase deficiency: a defect in valine metabolism associated with physical malformations. *Pediatrics*. 1982; 70(4):532–538. PMID: 7122152
43. Blume M, Rodriguez-Contreras D, Landfear S, Fleige T, Soldati-Favre D, Lucius R, et al. Host-derived glucose and its transporter in the obligate intracellular pathogen *Toxoplasma gondii* are dispensable by glutaminolysis. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106(31):12998–13003. <https://doi.org/10.1073/pnas.0903831106> PMID: 19617561
44. Macrae JI, Sheiner L, Nahid A, Tonkin C, Striepen B, Mcconville M J. Mitochondrial metabolism of glucose and glutamine is required for intracellular growth of *Toxoplasma gondii*. *Cell host & microbe*. 2012; 12(5):682–692. <https://doi.org/10.1016/j.chom.2012.09.013> PMID: 23159057
45. Babady N E, Pang Y P, Elpeleg O, Isaya G. Cryptic proteolytic activity of dihydroliipoamide dehydrogenase. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(15):6158–6163. <https://doi.org/10.1073/pnas.0610618104> PMID: 17404228
46. Ahmad W, Ebert P R. Suppression of a core metabolic enzyme dihydroliipoamide dehydrogenase (DLD) protects against amyloid beta toxicity in *C. elegans* model of Alzheimer's disease. *Genes & diseases*. 2021; 8(6):849–866. <https://doi.org/10.1016/j.gendis.2020.08.004> PMID: 34522713
47. Ambrus A, Adam-Vizi V. Human dihydroliipoamide dehydrogenase (E3) deficiency: Novel insights into the structural basis and molecular pathomechanism. *Neurochemistry international*. 2018; 117:5–14. <https://doi.org/10.1016/j.neuint.2017.05.018> PMID: 28579060
48. Mou Z, Barazandeh AF, Hamana H, Kishi H, Zhang X, Jia P, et al. Identification of a Protective Leishmania Antigen Dihydroliipoamide Dehydrogenase and Its Responding CD4(+) T Cells at Clonal Level. *Journal of immunology* (Baltimore, Md: 1950). 2020; 205(5):1355–1364. <https://doi.org/10.4049/jimmunol.2000338> PMID: 32727889
49. Voelter-Mahlknecht S, Ho A D, Mahlkecht U. FISH-mapping and genomic organization of the NAD-dependent histone deacetylase gene, Sirtuin 2 (SIRT2). *International journal of oncology*. 2005; 27(5):1187–1196. <https://doi.org/10.3892/ijo.27.5.1187> PMID: 16211212
50. Li C, Zhou Y, Rychahou P, Weiss H L, Lee E Y, Perry C L, et al. SIRT2 Contributes to the Regulation of Intestinal Cell Proliferation and Differentiation. *Cellular and molecular gastroenterology and hepatology*. 2020; 10(1):43–57. <https://doi.org/10.1016/j.jcmgh.2020.01.004> PMID: 31954883

51. Hu F, Sun X, Li G, Wu Q, Chen Y, Yang X, et al. Inhibition of SIRT2 limits tumour angiogenesis via inactivation of the STAT3/VEGFA signalling pathway. *Cell death & disease*. 2018; 10(1):9. <https://doi.org/10.1038/s41419-018-1260-z> PMID: 30584257
52. Park S, Chung M J, Son J Y, Yun H H, Park J M, Yim J H, et al. The role of Sirtuin 2 in sustaining functional integrity of the liver. *Life sciences*. 2021; 285:119997. <https://doi.org/10.1016/j.lfs.2021.119997> PMID: 34597608
53. Zhang Y, Anoopkumar-Dukie S, Arora D, Davey A K. Review of the anti-inflammatory effect of SIRT1 and SIRT2 modulators on neurodegenerative diseases. *European journal of pharmacology*. 2020; 867:172847. <https://doi.org/10.1016/j.ejphar.2019.172847> PMID: 31812544
54. Manjula R, Anuja K, Alcain F J. SIRT1 and SIRT2 Activity Control in Neurodegenerative Diseases. *Frontiers in pharmacology*. 2020; 11:585821. <https://doi.org/10.3389/fphar.2020.585821> PMID: 33597872
55. Zhang N, Zhang Y, Wu B, Wu S, You S, Lu S, et al. Deacetylation-dependent regulation of PARP1 by SIRT2 dictates ubiquitination of PARP1 in oxidative stress-induced vascular injury. *Redox biology*. 2021; 47:102141. <https://doi.org/10.1016/j.redox.2021.102141> PMID: 34555594
56. Wu B, You S, Qian H, Wu S, Lu S, Zhang Y, et al. The role of SIRT2 in vascular-related and heart-related diseases: A review. *Journal of cellular and molecular medicine*. 2021; 25(14):6470–6478. <https://doi.org/10.1111/jcmm.16618> PMID: 34028177
57. Yang M, Peng Y, Liu W, Zhou M, Meng Q, Yuan C. Sirtuin 2 expression suppresses oxidative stress and senescence of nucleus pulposus cells through inhibition of the p53/p21 pathway. *Biochemical and biophysical research communications*. 2019; 513(3):616–622. <https://doi.org/10.1016/j.bbrc.2019.03.200> PMID: 30981502
58. Farooqi A S, Hong J Y, Cao J, Lu X, Price I R, Zhao Q, et al. Novel Lysine-Based Thioureas as Mechanism-Based Inhibitors of Sirtuin 2 (SIRT2) with Anticancer Activity in a Colorectal Cancer Murine Model. *Journal of medicinal chemistry*. 2019; 62(8):4131–4141. <https://doi.org/10.1021/acs.jmedchem.9b00191> PMID: 30986062
59. Zhao N, Guo Y, Liu P, Chen Y, Wang Y. Sirtuin 2 promotes cell stemness and MEK/ERK signaling pathway while reduces chemosensitivity in endometrial cancer. *Archives of gynecology and obstetrics*. 2022; 305(3):693–701. <https://doi.org/10.1007/s00404-021-06216-2> PMID: 34476599
60. Maxwell M M, Tomkinson E M, Nobles J, Wizeman J W, Amore A M, Quinti L, et al. The Sirtuin 2 microtubule deacetylase is an abundant neuronal protein that accumulates in the aging CNS. *Human molecular genetics*. 2011; 20(20):3986–3996. <https://doi.org/10.1093/hmg/ddr326> PMID: 21791548
61. Cha Y, Han M J, Cha H J, Zoldan J, Burkart A, Jung J H, et al. Metabolic control of primed human pluripotent stem cell fate and function by the miR-200c-SIRT2 axis. *Nature cell biology*. 2017; 19(5):445–456. <https://doi.org/10.1038/ncb3517> PMID: 28436968
62. Szegő É M, Gerhardt E, Outeiro T F. Sirtuin 2 enhances dopaminergic differentiation via the AKT/GSK-3 $\beta$ / $\beta$ -catenin pathway. *Neurobiology of aging*. 2017; 56:7–16. <https://doi.org/10.1016/j.neurobiolaging.2017.04.001> PMID: 28478325
63. Biella G, Fusco F, Nardo E, Bernocchi O, Colombo A, Lichtenthaler S F, et al. Sirtuin 2 Inhibition Improves Cognitive Performance and Acts on Amyloid- $\beta$  Protein Precursor Processing in Two Alzheimer's Disease Mouse Models. *Journal of Alzheimer's disease: JAD*. 2016; 53(3):1193–1207. <https://doi.org/10.3233/jad-151135> PMID: 27372638
64. Wang Y, Yang J Q, Hong T T, Sun Y H, Huang H L, Chen F, et al. RTN4B-mediated suppression of Sirtuin 2 activity ameliorates  $\beta$ -amyloid pathology and cognitive impairment in Alzheimer's disease mouse model. *Aging cell*. 2020; 19(8):e13194. <https://doi.org/10.1111/ace1.13194> PMID: 32700357
65. Liu G, Park S H, Imbesi M, Nathan W J, Zou X, Zhu Y, et al. Loss of NAD-Dependent Protein Deacetylase Sirtuin-2 Alters Mitochondrial Protein Acetylation and Dysregulates Mitophagy. *Antioxidants & redox signaling*. 2017; 26(15):849–863. <https://doi.org/10.1089/ars.2016.6662> PMID: 27460777
66. Mcconkey G A, Martin H L, Bristow G C, Webster J P. *Toxoplasma gondii* infection and behaviour—location, location, location? *The Journal of experimental biology*. 2013; 216(Pt 1):113–119. <https://doi.org/10.1242/jeb.074153> PMID: 23225873
67. Webster J P, Lambertson P H, Donnelly C A, Torrey E F. Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on *Toxoplasma gondii*'s ability to alter host behaviour. *Proceedings Biological sciences*. 2006; 273(1589):1023–1030. <https://doi.org/10.1098/rspb.2005.3413> PMID: 16627289
68. Skallová A, Kodym P, Frynta D, Flegr J. The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study. *Parasitology*. 2006; 133(Pt 5):525–535. <https://doi.org/10.1017/S0031182006000886> PMID: 16882355

69. Roiko MS, Lafavers K, Leland D, Arrizabalaga G. Toxoplasma gondii-positive human sera recognise intracellular tachyzoites and bradyzoites with diverse patterns of immunoreactivity. *International journal for parasitology*. 2018; 48(3–4):225–232. <https://doi.org/10.1016/j.ijpara.2017.08.016> PMID: 29170086
70. Ronin C, Costa D M, Tavares J, Faria J, Ciesielski F, Ciapetti P, et al. The crystal structure of the Leishmania infantum Silent Information Regulator 2 related protein 1: Implications to protein function and drug design. *PLoS one*. 2018; 13(3):e0193602. <https://doi.org/10.1371/journal.pone.0193602> PMID: 29543820
71. García-Salcedo J A, Gijón P, Nolan D P, Tebabi P, Pays E. A chromosomal SIR2 homologue with both histone NAD-dependent ADP-ribosyltransferase and deacetylase activities is involved in DNA repair in Trypanosoma brucei. *The EMBO journal*. 2003; 22(21):5851–5862. <https://doi.org/10.1093/emboj/cdg553> PMID: 14592982
72. Yin K, Xu C, Zhao G, Xie H. Epigenetic Manipulation of Psychiatric Behavioral Disorders Induced by Toxoplasma gondii. *Frontiers in cellular and infection microbiology*. 2022; 12:803502. <https://doi.org/10.3389/fcimb.2022.803502> PMID: 35237531
73. David C N, Frias E S, Szu J I, Vieira P A, Hubbard J A, Lovelace J, et al. GLT-1-Dependent Disruption of CNS Glutamate Homeostasis and Neuronal Function by the Protozoan Parasite Toxoplasma gondii. *PLoS pathogens*. 2016; 12(6):e1005643. <https://doi.org/10.1371/journal.ppat.1005643> PMID: 27281462