Comparative miRNA Transcriptomics of Mouse and Macaque Reveals Cytoskeleton is An Inhibitor for *C. neoformans* Invasion into Brain

1 **Short Title:** Reprogramming of Macrophage by MYOC Moderates *C.*
2 *neoformans* Brain Invasion.

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Abstract

Cryptococcal meningitis is a major risk for immune compromised patients mainly caused by the human opportunistic pathogen *Cryptococcus neoformans*, yet mechanisms of the brain or CNS dissemination remain to elucidate, which is the deadliest process for the disease. Meanwhile, illustrations of clinically relevant responses in cryptococcosis were limited, as the low availabilities of clinical samples. In this study, macaque and mouse infection models were employed and miRNA-mRNA transcriptomes were performed and combined, which revealed cytoskeleton was a centric pathway regulated in both two organisms. Notably, myocilin, a novel modulator encoded by MYOC, was identified during cryptococcal pneumonia. An enhanced fungal burden was achieved in the brains of MYOC transgenic mice. Importantly, *C. neoformans* dissemination to the brain was inhibited by cytoskeleton inhibitor, which ruined “Trojan Horse” by dampening migration and phagocytosis of macrophages, but toxic to hosts. Taking together, this study provides new pathways and modulators during *C. neoformans* infections and reveals fundamental roles of cytoskeleton and MYOC in blocking fungal CNS dissemination that facilitate the development of novel drug targets for therapies of meningoencephalitis caused by *C. neoformans* and other pathogenic microorganisms.

Keywords: Cryptococcal meningoencephalitis; Brain dissemination; Host-pathogen interactions; Macaque; miRNA transcriptome; Cytoskeleton; MYOC

47 **Author summary**

48 Fungal CNS dissemination is vital and lethal, which is the most important step
 49 for both fungal pathogenesis and host defense. Many milestones have been
 50 made to illustrate processes of invasion, such as the “Trojan Horse” derived by
 51 macrophages. However, blocking this “road” is still on the way. In our work, to
 52 mimic real responses in humans, macaque and mouse were included, miRNA-
 53 Seq and mRNA-Seq were combined, and we identified the core-pathway
 54 (cytoskeleton) during *C. neoformans* pathogenesis, which plays important roles
 55 in migration and phagocytosis for macrophages, key factors for macrophage
 56 “Trojan horse”, and cytoskeleton was also ruined in HIV/AIDS patients. Fungal
 57 brain dissemination was weakened by cytoskeleton inhibitor in mice.
 58 Meanwhile, we identified a novel modulator for macrophage “Trojan Horse”,
 59 MYOC, involved with *C. neoformans* brain dissemination. Consistently, we
 60 assume macrophages are the key executor cells for fungal CNS dissemination,
 61 and we propose the processes can be shut down by cytoskeleton or MYOC
 62 inhibitors. Our research would not only help to understand the high prevalence
 63 of cryptococcal meningitis in HIV/AIDS but also provides putative ways to block
 64 fungal cells invading the brain.

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Introduction

Occurrences of fungal invasive diseases are always caused by invading fungi and also involved with aspects of hosts closely[1-3]. Studies have shown the raised body temperature, limited nutrients of glucose and metal ions, phagocytosis by phagocytes and cellular immunities are powerful weapons for fungal elimination in hosts[4-10], while fungal evolved strategies in return, such as temperature resistance, polysaccharide capsule and melanin production, morphology switching, and virulence gene expressions[11-16]. Nonetheless, the mechanisms of fungal central nervous system (CNS) invasion have not been determined, which is also a mystery in cryptococcosis, a life-threatening fungal invasive disease, initialized by human pathogen *Cryptococcus neoformans*[17-20]. Cryptococcal diseases are predominant prevalent among immune compromised patients, such as HIV/AIDS, organ transplanted, systemic lupus erythematosus, and other immune associated diseases, and contribute about 15% of deaths in HIV/AIDS patients[21-23]. Seriously, morbidity of cryptococcosis in immune competent is increasing rapidly in China, Australia, Canada, and other countries and regions[24-27].

CNS invasion and colonization is the deadliest factor of fungal diseases. To date, massive researches have focused on CNS invasion during fungal meningitis and numerous milestones achieved, including the fact that cryptococcal cells can interact with brain epithelial cells directly and indirectly by microscopy or electron microscope[18-20, 28]. However, blocking this progression is still on the way. Many studies illustrated the effects of “Trojan horse”, utilized by pathogens, such as *C. albicans*, *C. neoformans*, and *M. tuberculosis* and suggested macrophages play important roles in both fungal

elimination and CNS dissemination, which serve as a double-edged sword[17-20, 28, 29].

Phagocytosis and killing are key factors for effective phagocytes, which involved cellular morphology switching. Meanwhile, cell morphology could be regulated by dynamics of cytoskeleton, the main structure in cells involves cellular transport, cell cycle and cell shape modulation, including three main components, microfilaments, microtubules and intermediate filaments[30-36]. Furthermore, In HIV/AIDS, a main susceptible population for cryptococcosis, studies have shown migration of immune cells was ruined by dampened cytoskeleton functions. The processes can be interrupted by inhibitors of cytoskeleton pathways, such as R10015 and okadaic acid[37, 38]. These indicated the high prevalence of cryptococcal meningitis in HIV/AIDS results from dysfunctional cytoskeleton system, and a relationship between phagocytes cytoskeleton and fungal meningitis derived by macrophage “Trojan Horse”. Collectively, the cytoskeleton may serve as an important part of a successful fungal infection, urgently needing to be deciphered. Identification relationships between the cytoskeleton and fungal infections may provide novel mechanisms of fungal pathogenesis and targets for drug development in mycosis.

Furthermore, responses of hosts during infectious diseases are often divided into DNA, mRNA and protein levels, and also, the processes of post transcriptional and translational modifications[5, 6, 39]. Previous studies identified many responses at mRNA, protein and post translational levels, including key pathways, such as mineral metabolism, IL-17 signaling pathway, platelet activation and sugar metabolisms, also key modulators, such as

MMP12 and OCSTAMP[5, 6]. Post transcriptional modifications, assumed by miRNAs, also play important roles in mRNAs and proteins biosynthesis, and miRNAs participate in processes of many diseases and metabolisms. Recently, several studies have demonstrated key functions of non-coding RNAs during *C. neoformans* infections in vitro, However, little was known in vivo[40-43]. To mimic responses of humans and be more clinically relevant, macaque (*Macaca fascicularis* or crab-eating macaque) was employed, which is 92.83% in sequence identity to human genome[44].

In this study, to demonstrate global responses of hosts at the miRNA-mRNA regulatory level, and identify the mysteries of fungal invasion, comparative miRNA-Seqs in macaque and mouse were employed, continued with networks construction of miRNA-mRNAs. We found that cytoskeleton pathway is the core pathway regulated by *C. neoformans* in both macaque and mice. Furthermore, cytoskeleton and an associated gene, MYOC, are important for “Trojan Horse” invitro and in vivo. Moreover, intervention of cytoskeleton pathway was able to prevent *C. neoformans* from invading the brain in mice. Collectively, these findings demonstrate global responses at the miRNA-mRNA regulatory level and reveal a novel mechanism and modulator of fungal invasion, benefit novel therapies for fungal infections.

Results

MicroRNA transcriptomics in macaque and mouse during cryptococcal pneumonia.

We have previously unveiled transcriptional level responses in macaque and mouse and identified several regulators and pathways during *C. neoformans* infections[6]. To investigate global responses at post transcriptional level in the disease, miRNA-Seqs were performed, followed by mRNA-Seq. In this study, microRNA transcriptomes were performed using lung tissues isolated from macaque and mouse infection models (Figure 1). Mouse is the most used experimental animal model, while macaque is mostly clinically relevant and is a distant relative to humans, so that macaque (*M. fascicularis* or crab-eating monkey) and mouse(C57BL/6) were employed to give a comprehensive response during the fungi-host interplays.

To mimic acute cryptococcal infections, macaques and mice were sacrificed 7 days postinfections. Macaques display infectious signs, such as an increased back temperature, cough and anorexia (data has shown previously [6]). Pathology of both macaque and mouse lung tissue was confirmed by histopathology observations using mucicarmine and hematoxylin/eosin staining, showing the capsular structure of *C. neoformans* (Figure2 A, B). Total RNAs were extracted and miRNA transcriptomes were performed. Total and unique reads from omics were calculated, with a total read number of more than 10^7 per group and 10^6 unique reads for every group (Figure S1 A). Read number around 22 nucleotides were the most abundant in both macaque and mouse, either infected or uninfected groups (Figure S1 B, C). The clean read files were

used to map the corresponding genome, whose outputs were used for miRNA annotation and expression calculation, based on species specificity in miRBase21. However, macaque (crab-eating monkey) has few miRNA annotations and our purpose is to mimic humans, as which miRNA annotations of humans were employed for macaque, and mouse to mouse. As a result, there are 1038 and 1166 expressed miRNAs in lung tissues from macaques and mice during *C. neoformans* infections (Table S1). Principal component analyses (PCAs) were analyzed by using read number of each detected miRNA, as shown in Figure 2 C, D, both infected and uninfected samples were reproducible. Differentially expressed miRNAs were performed by DESeq, with p -value ≤ 0.05 , fold change ≥ 2 or ≤ 0.5 were considered significantly changed. Heatmaps of differentially expressed items were generated, with 32 miRNAs (4 down regulation and 28 up regulation) in macaque and 29 (5 down regulation and 24 up regulation) in mouse, shown as two clusters by column (Figure 2 E, F).

Core responses of host derived from miRNA-mRNA Integrative analyses in response to *C. neoformans* infections.

To mine and mimic responses in human cryptococcosis extremely, omics of miRNA and mRNA were integrated, and homology analyses were performed (Figure 3 A). Eight miRNAs were co-regulated in macaque and mouse during *C. neoformans* infections. Information about the 8 miRNAs were displayed, including IDs, foldchanges and homologous e-values, and RT-qPCRs were performed in mouse for the 8 miRNAs, which were consistent with the miRNA-Seq data (Figure 3 B, C, D).

Targets of the 8 co-regulated miRNAs were predicted and mapped to RNA-Seq, and miRNA-mRNA regulatory network was constructed (Figure S2). The core regulatory target mRNAs, which were co-regulated by the 8 co-regulated miRNAs, were selected for a mini miRNA-mRNA network, with 233 target mRNAs identified, including OCSTAMP, DC-STAMP, IL17A TNF and LIF, which demonstrated anti-microbial activities (Figure 3 E). KEGG and GO analyses were performed of the 8 co-regulated and 223 co-regulated targets (Figure 4, Figure S3, Table S2 and Table S3). KEGG pathways associated immune system, lung diseases, and infectious diseases were significantly enriched in both miRNA and mRNA level, such as TNF signaling pathway, Cytokine-cytokine receptor interaction, Viral protein interaction with cytokine and cytokine receptor, Chemokine signaling pathway, Osteoclast differentiation, NF-kappa B signaling pathway, IL-17 signaling pathway, Complement and coagulation cascades, Small cell lung cancer, Non-small cell lung cancer, Legionellosis, Herpes simplex infection, Chagas disease American trypanosomiasis, Influenza A (Figure S3). GO analyses identified terms involved cytokine activity, histone, immune cells, monocyte, myeloid leukocyte and cell cycle were significantly varied by *C. neoformans* (Figure 4). Interestingly, actin binding, microtubule and their associated complex, which constitute cytoskeleton system, were highly enriched in GO analyses, including cellular component, molecular function and biological process. These results indicated a potent function of cytoskeleton in cryptococcosis.

MYOC is an inhibitor of *C. neoformans* infection by modulating macrophage.

To identify functions of cytoskeleton during *C. neoformans* infections, genes associated with cytoskeleton were screened, and myocilin was selected, a tubulin binding protein, encoded by MYOC gene, which was one of the centric modulators in miRNA-mRNA regulatory network and down regulated significantly during *C. neoformans* infections in both macaque and mouse (Figure 5 A, B). Previous studies revealed the protein was involved with cell migration and adhesion[45].

To our best knowledge, macrophages are powerful weapons engaged in anti-fungal and fungal clearance. Phagocytosis, migration and killing capacity are key factors for phagocytes. To explore functions of MYOC, MYOC overexpressed THP-1 cell lines were constructed and mRNA levels of MYOC were detected by RT-qPCR, which showed a 10-fold induction compared to mock cells (Figure 5 D). Phagocytosis, migration and killing of macrophage cells in MYOC gene-edited cell lines were examined (Figure 5 C, E, F, G). Phagocytosis effectivity was detected by Flow cytometry (Figure 5 C), which was increased significantly in macrophages derived by THP-1 when MYOC was overexpressed (Figure 5 E). Killing capacity was also enhanced by MYOC overexpression, as shown in Figure 5 G, less lived *C. neoformans* were detected by CFU assays (Figure 5 G). However, no changes were observed in migration assays (Figure 5 F).

To test the function of MYOC in vivo, MYOC- transgenic mouse was generated and employed (Figure 5 H), which was confirmed by PCR (Figure 5 I). Six-eight weeks old mice were used for CFU assessments and survival rates. To our surprise, CFU of brains was enhanced in MYOC transgenic mice, either in male or female groups (Figure 5 J), however, lung CFU was decreased in

females, while no changes in the male group (Figure 5 K). Consistent with CFU assays, MYOC transgenic mice showed reduced survival times compared to wild type mice observed in survival rate tests (Figure 5 L, S4 A).

***C. neoformans* brain invasion was inhibited by cytoskeleton pathway inhibitor on effects of “Trojan Horse”.**

To decipher the functions of host cytoskeleton during the battle between host and *C. neoformans*, R10015, a cytoskeleton pathway inhibitor, was employed, which targets LIM Kinase (LIMK) powerfully in cytoskeleton pathway[38]. Firstly, a gradient concentration of R10015 was incubated with *C. neoformans* cells in DMEM medium, including wild type encapsulated H99 strain in a CO₂ incubator with 5% CO₂ at 37 °C. OD 600 was detected 24h and 48h post incubation. R10015 has a low solubility in H₂O and DMEM, as which the ODs were larger when concentration over 40 μM. As a result, R10015 doesn't affect *C. neoformans* growth, and final concentration was set at 14.815 μM (Figure S4 B).

To investigate phagocytosis, migration and killing capacity induced by R10015, THP-1 derived macrophages and human monocyte derived macrophages were employed and pre-treated by R10015 for 2h, washed and then incubated with opsonized *C. neoformans* overnight. Phagocytosis effectivity was detected by Flow cytometry (Figure 5 C). Less fungal cells were phagocytosed in the group of R10015 pre-treated groups either in TDMs or MDMs in vitro (Figure 6 A, D). Trans-well assays were performed for THP-1 suspension cells in 5 μm trans-well inserts, cells from inserts and lower apartments were counted and migration rates were calculated 16h post

incubation. R10015 decreased the motility of THP-1 cells (Figure 6 B). Consistent with THP-1 cells, migration of MDMs was performed in 8 μ m trans-well inserts, compared to control groups, the migration was inhibited by R10015 at 48h (Figure 6 E). For *C. neoformans* killing assays, the incubation time with TDMs or MDMs was extended to 24h post incubation, fungal cells from supernatants and washings were collected, diluted and plated on YPD, and colonies were counted and calculated 2 days later. R10015 did not affect the killing in TDMs in the presence of R10015 and a few more fungal cells lived in MDMs treated by R10015 (Figure 5 C, F).

To confirm the effect of cytoskeleton on *C. neoformans* pathogenicity and host defense, in vivo experiments in C57BL/6 mice were employed. For CFU assessment experiments, twenty mice were divided into 2 groups randomly, pre-treated either R10015 or an equal amount of DMSO 6h before infections. Mice were infected with wild type H99 intranasally and sacrificed at day 7 or day 14 post infections. Brain CFU was decreased significantly in R10015 treatment group at 7-day postinfections without changes in other checked tissues (Figure 6 G). However, adverse results were obtained at day-14, fungal colonies of injected R10015 group were increased over 100 folds in brain tissues compared to DMSO controls, while no significant changes in lung and other tissues (Figure 6 H). The same effects were observed in survival curve experiments. Body weight of mice was examined daily, and death data was about 3 days earlier in the group injected with R10015 in survival rates experiment (Figure 6 I, Figure S4 C), while controls of R10015 injected were healthy with a steady weight gain and none was dead. These results indicate

R10015 plays vital roles, however toxic to hosts, in fungal invading CNS during *C. neoformans* infections.

Discussion

In this study, we demonstrated the landscape at transcriptional and post transcriptional levels in mouse and macaque infection models, which were employed for mimicking and unveiling responses at miRNA-mRNA regulatory levels in humans. Previously studies indicated mouse was the most useful animal model, however, varied from humans a lot, such as the process of immune cells maturation. Our data from *M. fascicularis*, 92.83% sequence identity to human, a little more clinically relevant, may serve as a database for cryptococcosis or mycosis. To investigate core responses during *C. neoformans* infections, miRNA-mRNA combined, GO and KEGG analyses were performed. Eight key miRNAs were identified in our omics, such as miR-146a, miR-223 and miR-155, which were induced in monocytes by co-culture with *C. neoformans* in vitro[40]. We did not focus on the functions of unique miRNAs, and we characterized cytoskeleton pathway as a core regulatory modulator based on enrichment analyses of miRNAs and their targets. Previous studies identified *C. neoformans* disturbed cytoskeleton when invading brain endothelial cells[30].

Our data identified the fundamental roles of cytoskeleton during “Trojan Horse” formation. Studies have indicated the relationship between cytoskeleton and fungal infections[30, 31, 36, 46, 47]. Previous studies highlighted important roles of “Trojan horse” by macrophages during fungal CNS invasion, the processes of which require viability and cellular deformation, and these are

cytoskeleton dependent. Studies have demonstrated migration, adhesion and phagocytosis are key features for phagocytes[48]. Our studies illustrated the basic functions of cytoskeleton dynamics on phagocytosis, migration and killing of macrophages, which are vital for eliminating, blocking fungal cells or fungal cell intracellular proliferation and vomocytosis[49]. Inhibitors of cytoskeleton pathways were efficient for blocking fungal cells from the brain in the early stage of meningitis, however, reciprocal in late stage, these were different phenotypes from “Trojan horse”, which may result from cytoskeletal alteration participates early cellular responses in fungal infections[47]. Furthermore, this phenomenon indicated the toxic of the small molecule drugs, such as vanadate, cytochalasin D, Y27632 and R10015[30, 38, 50], which involves fundamental roles of innate immunity and acquired immunity of macrophages, NK cell and T cells, who also play important roles during fungal, bacterial and virus infections. Overcoming toxicity of cytoskeletal inhibitors is the main barrier to clinical application.

Dysfunctional cytoskeleton may account for the high prevalence of cryptococcal meningitis in HIV/AIDS patients. Studies indicated the same variation of cytoskeleton pathway during HIV infection and *C. neoformans* or *A. fumigates* infections, but different in cells[30-32, 47, 51]. Indeed, *C. neoformans* is an environmental yeast globally ubiquitous, and easily accessible for all individuals[52]. Rates of this invasive disease were severely under-estimated, as the low availability for detection[25, 53]. We speculate two possible reasons, no infectious symptoms and difficulty in detection. However, why do these happen much more in HIV/AIDS? The reasons may hind in the infectious symptoms resulting from *C. neoformans* invasion into brains, such as headache,

dyspnea or altered mental status associated CNS infections[52]. In HIV/AIDS patients, numerous studies have proved cytoskeleton was dampened in immune cells of PBMC[37, 38, 51], which contributes to fungal cells CNS invasion by “Trojan Horse”. Furthermore, a recent study proved vomocytosis was enhanced by HIV infection in macrophage[49], which process was also regulated by the cytoskeleton.

We identified MYOC as a novel modulator for fungal invasion by regulation on macrophages. Mutations of MYOC gene are highly associated with eye diseases, glaucoma. Myocilin was proved co-localized with microtubules, endoplasmic reticulum (ER), and Golgi apparatus[54, 55] and overexpression of MYOC induces a loss of actin stress fibers[56]. Studies showed myocilin promotes cell migration and phagocytic activities of human trabecular meshwork cells[45, 57, 58]. Our data showed a repression of MYOC gene expression in both macaque and mouse lung tissues, and consistent with previous studies overexpressed MYOC in THP-1 cells derived macrophages elevated phagocytic activities and migration, which induced more intracellular cryptococci, enhanced effects of “Trojan Horse”.

In conclusion, our findings therefore described global miRNA-mRNA regulatory responses during *C. neoformans* in primate and rodent animal models, which serves as a clinically relevant database for fundamental and clinical research. This work highlights the importance of MYOC and cytoskeleton pathways during *Cryptococcus* meningoencephalitis and underscores their critical functions in the formation of “Trojan Horse”. This study

357 provides critical roles of cytoskeleton on fungal CNS invasion that create the
358 possibilities of developing an anti-fungal therapy.
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Materials and Methods

Ethics statement

All work with human cells was reviewed and approved by the Medical Research Ethics Committee of the First Affiliated Hospital of China Medical University (Approval no. 2021-63-2). All donors were adults and provided written informed consent prior to research participation. Animal infection experiments in macaques and mice were reviewed and ethically approved by the Research Ethics Committees of the College of Life and Health Sciences of Northeastern University (Approval no. 16099M) and Wincon TheraCells Biotechnologies Co., Ltd. (Approval no. WD-20150701-a). Experiment detailed as described in our last research article. Briefly, Macaques (*M. fascicularis*) are care and used by Wincon TheraCells Biotechnologies Co., Ltd. with unlimited water supply, twice a day food availability and fresh fruits. Macaques were anesthetized by ketamine and pentobarbital, and sacrificed after deep anesthesia. Mice were cared with uninterrupted water and food supply in the College of Life and Health Sciences of Northeastern University, which were anesthetized by ketamine and sacrificed by exposure to CO₂ for 5 min. All animal experiments were carried out according to the Guide for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of the People's Republic of China.

Animal Infection

Macaques and mice were purchased from Grandforest Co., Guangxi, China and Changsheng Biotech, China, respectively, and of which artificial cryptococcal infection processes and infective doses were the same to our

previous article[6]. Briefly, macaque infection care was performed by Wincon TheraCells Biotechnologies Co., Ltd., which was endorsed by the Association for Assessment and Accreditation of Laboratory Animal Care (No. W00099). Six female macaques were divided into two groups: controls and the infection group. Monkeys were anesthetized by ketamine (10 mg/kg) intraperitoneally injection (IP), and then infected via intratracheal injection with 10^8 cells/kg *C. neoformans* H99 cells. Controls were infected using the same volume of PBS. Mice were housed in College of Life and Health Sciences of Northeastern University, anesthetized, and infected intranasally with 10^5 fungal cells in 50 μ L PBS. Macaque and mice were monitored for signs of infection and humanely killed at day 7 or 14 post infections, or used for survival rates detections. Macaque lung tissues were collected, and either fragmented into 0.5 cm $\times$ 0.5 cm $\times$ 0.5 cm cubes prior to suspension in either an RNAlater solution (Ambion) or paraformaldehyde solutions. Mice tissues were isolated and resuspended in RNAlater (Thermofisher) or paraformaldehyde solutions. Tissues were also collected from control animals infected with PBS. Tissue samples were isolated for RNA isolation, CFU analysis and histopathology.

miRNA sequencing library preparation and miRNA transcriptome analysis

Total RNA was isolated using TRIzol (Invitrogen Life Technologies). RNA concentration, quantitation, and integrity assessment were done using a NanoDrop 8000 spectrophotometer (Thermo Scientific). Small RNA-seq libraries were prepared by using TruSeq® Small RNA Library Prep Kit (Illumina, USA) according to the manufacturer's protocol. miRNA libraries construction

and sequencing were entrusted to Shanghai Personal Biotechnology Co., Ltd. (China), and then single-end sequencing was conducted by using Illumina NextSeq 500 sequencing system.

Clean and unique reads were acquired by raw reads filtering, including getting rid of adaptor, reads less than 18 nucleotides, low-quality bases (score ≤ 20) and duplicated reads in a sample. The clean read files in FASTA format were mapped to the corresponding genomes (mouse and macaque) by bowtie in Mirdeep2. Annotation and expression of miRNAs were performed by using quantifier.pl in Mirdeep2 based on miRBase21(<http://www.mirbase.org/>), mouse reference miRNA file used mouse, while macaque used human, as miRNA annotation in crab-eating macaque was not completed. Differentially expressed miRNAs were identified by DESeq, with p -value ≤ 0.05 , fold change ≥ 2 or ≤ 0.5 were considered as differentially expressed miRNAs.

Quantitative PCR of differentially expressed miRNA

Total miRNAs were isolated by miRNeasy Mini Kit (QIAGEN) according to the manufacturer's manuals, and electrophoresis and quantification were performed. First strand cDNA was synthesized using random oligonucleotides and miRcuRY LNA Universal RT microRNA PCR Universal cDNA Synthesis Kit II (EXIQON, USA), U6 was employed as an internal reference. Primers (Table S4) were designed by using miRprimer and the best primer pairs were selected[59]. RT-qPCR was performed by miRcuRY LNA Universal RT

microRNA PCR Exilent SYBR master mix (EXIQON, USA)) and StepOne Plus, biological triplicates were performed for each miRNA. Statistical comparisons were performed with unpaired two-tailed Student *t* test. *p* value ≤ 0.05 was considered significant.

Network construction and analysis

Regulatory network miRNA-mRNA was constructed by the miRNA-seq data (this article) and mRNA-seq data (our previous research, deposited in NCBI's Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>) and GEO Series accession ID was GSE122785 [6]). Targeted genes of selected miRNAs were predicted using miRWalk 3.0 (<http://mirwalk.umm.uni-heidelberg.de/>)[60], and overlapped with differentially expressed mRNA in mRNA-seq. Regulatory network miRNA-mRNA was visualized by Cytoscape (version 3.9.0) based on the relationship of miRNA-mRNA output from miRWalk[61]. Co-regulated genes were acquired by comparison of the gene symbols between macaque and mouse based on human homologous genes, respectively. Annotation of the networks was modified by Cytoscape, which includes regulation type, foldchange, and species-specific.

Bioinformatics Analysis

Gene ontology and KEGG analyses were performed using R version 4.1.2, clusterProfiler v4.2.0, org.Hs.eg.db version 3.14.0, org.Mm.eg.db version 3.14.0 packages [62], and plotted by ggplot2 version 3.3.5. KEGG of miRNAs was performed by using a web-based application named miEAA (<https://ccb-compute2.cs.uni-saarland.de/mieaa2/>)[63] and plotted by ggplot2. Heatmap of

expression was generated by Pheatmap version 1.0.12. Homology analysis was performed based on miRbase search engine database (<https://www.mirbase.org/search.shtml>).

Strains and media

Cryptococcus neoformans H99 (wild type) and H99 GFP-expressed strains (H99, a gift from Professor Tongbao Liu, Southwest University, China) were used in the research. Prior to phagocytosis and intranasal infection, fungal cells were inoculated in 5 mL YPD liquid medium and incubated at 30°C overnight. Harvested cells were then washed three times with sterile PBS buffer, counting by microscope, then diluted in PBS.

Histopathology, colony forming units (CFU) and survival rates assessments

Lung tissues were collected from macaque and mice, as described previously [6, 9, 64]. To perform histopathology analyses, tissue samples were fixed with paraformaldehyde, frozen, and processed using a cryostat microtome (CM1850; Leica). Tissue sections of 10 µm in thickness were stained with mucicarmine or hematoxylin/eosin. For CFUs determination, as described elsewhere[6, 65], YPD agar was used. Homogenized lung, brain, spleen, kidney, and liver tissues were diluted and plated onto YPD agar plates and were then incubated at 30°C for two days. Cell colonies were counted and calculated. For survival rates detection, thirty C57BL/6 mice were divided into 3 groups randomly, named R10015 injected control group, wild type infection group, and R10015 injected infection group. Body weight was examined daily from the day

of infection. Fifteen percentage decrease of initial body weight was identified physiological endpoint.

Construction of MYOC overexpressed THP-1 cell line and THP-1 derived macrophages (TDMs) differentiation

MYOC gene was cloned into pCDH-EF1 α -MCS-T2A-Puro plasmid by restriction enzyme *XbaI* and *EcoRI*. HEK293T cells were seeded on 6-well plates at a density of 10⁶ one day pre-transfection. Lentivirus was packaged by using jetPRIME[®] DNA & siRNA Transfection Reagent (polyplus) according to the instructions and plasmids pCDH-EF1 α -MYOC-T2A-Puro plasmid, pMD2.G, psPAX2 (4:3:1), yet mock lentivirus was packaged. Infections of THP-1 were performed 3 days post transfection by centrifugation at 1200 × g at 37 °C in 24-well plates for 2h with lentivirus, filtrated by 0.45 μ m membranes. Screening of puromycin resistance was conducted 72h post transduction for 2 weeks at 5 μ g·mL⁻¹.

THP-1 derived macrophages (TDM) were differentiated by PMA incubation for 48h in RPMI-1640 medium (10% FBS) containing 1% penicillin/streptomycin and 250 ng/mL PMA (Sangon Biotech, China), and cultured in renewed RPMI-1640 medium (10% FBS) containing 1% penicillin/streptomycin for 24h and ready for use.

Human monocytes isolation and macrophage induction

PBMC were isolated by Ficoll-Paque PLUS density gradient media (Cytiva). Blood samples were centrifuged in a swing bucket rotor at 400 × g for 30 minutes at 25 °C with acceleration set at 5 and break at zero, and followed by

purification in cold PBS for 2 times and centrifuged at 300 × g for 10 minutes at 4 °C with acceleration and break set at 5. 1×10⁶ PBMC were seeded onto 48-well plates in RPMI-1640 media without FBS for 1h to stick monocytes, and fresh RPMI-1640 medium (10% FBS) containing 1% penicillin/streptomycin and 50 ng/mL M-CSF (Sangon Biotech, China) was exchanged, Cells were washed with PBS and fresh media renewed at days 3 and 6 during monocytes differentiation. Monocyte derived macrophages (MDM) were ready to use on day 7.

Phagocytosis effectivity and killing assessments

MDM or TDM cells were seeded on 48-well plates 100,000 cells per well 24h before fungi interaction, and then treated with R10015 (10 mM) or DMSO for 1h. GFP-expressed strains (H99) were incubated overnight at 30 °C, washed three times by sterile PBS, counting and resuspended in PBS, opsonized by 18B7(1mg·L⁻¹) at room temperature for 30 min. Incubate fungal cells with macrophages in CO₂ incubator overnight at MOI=1:10. Macrophages and fungal cells were washed 5 times with PBS buffer thoroughly and then digested by trypsin. Phagocytosis effectivity was detected by flow cytometry. For killing tests, after fungi and macrophage incubation (24h), supernatant was collected and cells were washed with PBS 5 times and collected, subsequently lysed with 0.02% SDS. Total lysates and supernatant were diluted and plated on YPD agar plates and then incubated at 30°C for two days. Colonies were counted and calculated.

Trans-well assay of THP-1 cells and MDMs

Trans-well assays were performed to detect the capacity of migration of THP-1 cells, MYOC gene-edited THP-1 cells or MDMs. THP-1 were seeded onto 5 μ m trans-well inserts at a density of 200,000 cells in 200 μ L in serum free RPMI 1640, and 400 μ L RPMI 1640 contains 10% serum in the lower compartment. Cells were counted by cell counter up and down 16h later respectively, and migration rates were calculated. For MDMs, 50,000 MDMs were seeded onto 8 μ m trans-well insert in 200 μ L serum free RPMI 1640, and 400 μ L RPMI 1640 containing 10% serum in the lower compartment. Migrated cells were counted by microscopy 48h post incubation.

Construction of MYOC transgenic mice

MYOC transgenic mice were produced by Beijing View Solid Biotechnology, China. The linear plasmid pCAG-MYOC cut by the *BstEII* restriction enzyme (NEB) was purified and diluted in RNase-free H₂O (10 ng· μ L⁻¹), which were injected into zygotes of C57BL/6 mice in M2 media (Millipore) using a FemtoJet micromanipulator (Eppendorf, Germany). Microinjected zygotes were transferred into pseudo pregnant female mice. All mice were maintained in a specific pathogen-free facility. Genotype identification was performed by PCR and sequencing from 2-week-old newborn mice with primers (Table S4). Transgenic mice were mated with wild-type C57BL/6 mice to obtain heterozygous mice and colony expansion.

Statistics and reproducibility

Reproducibility of samples used for miRNA-Seq was analyzed using principal component analysis (PCA), performed and plotted by ggplot2 version 3.3.5[66]. All experiments were performed at least biologically triplicated to

ensure reproducibility. Statistics of phagocytosis effectivity, RT-qPCR, CFU were calculated using GraphPad Prism 9.0 with mean and SEM. An unpaired or paired student *t* test was performed. Survival rates were calculated by GraphPad Prism 9.0, Log-rank (Mantel-Cox) test was employed for statistical analysis. When the *p*-value was less than 0.05, statistical significance was recognized.

Data and Software Availability

The RNA transcriptome raw data files have been deposited in NCBI's Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>) with GEO Series accession ID GSE122785 previously[6]. Raw data of microRNA-seq was ready and open to researchers and can be provided upon request.

Author Contributions

H.S. and C.D. conceived the project. H-L.L., X-X.H., W-Q.G. and C.D. designed the study. T.L., S.Y., and C.D. performed the monkey infection experiments. H-L.L., W.D., Y.M. Q-J.L. Y-J.L. and T-S.S. performed mouse infection experiments. H-L.L., X-L.L., M-H.A., Y.Q., H.Z., X-D.G. and Z-N.Z. participated in data analysis. C-H.S. and C.L. performed histopathology staining. H-L.L., W.T. and Y-J.F. performed cell experiments. H-L.L. X-X.H., W-Q.G., C.D., and H.S. composed the manuscript.

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Figure legends

Figure 1. Experimental flow chart

Six macaques were divided into two groups randomly: control group (n=3) and infection group (n=3), which was the same to mouse. Clinically isolated *C. neoformans* H99 were grown in YPD liquid medium at 30 °C overnight. Animals were infected via intratracheal injection in three C57BL/6 female mice and three *M. fascicularis* female macaques by *C. neoformans* cells in PBS. Three macaques and three mice were also infected with PBS buffer as controls. Lung tissues were collected and preserved 7 days post infection. Infectious signs were confirmed by histopathology detections. Total RNAs were isolated for mRNA-seq and miRNA-seq, continued by comparison omics at transcriptional and post-transcriptional levels. Differentially expressed miRNA-mRNA regulatory network was constructed during *C. neoformans* infections based on the responses in macaque and mouse. RNA transcriptome profiles, blocked in were published previously. Figure 1 was created with BioRender.com

Figure 2. miRNA-Seq of mice and macaques in cryptococcal pneumonia.

A B. Histopathology observation of infected macaque and mouse lung tissues. Lung tissues from macaque and mouse were fixed and sectioned at 10 µm thickness and stained by using mucicarmine. Red arrows indicate *C. neoformans* cells, scale bar = 10 µm.

C D. Principal Component Analyses (PCAs) of miRNA-Seq data from macaque and mouse lung tissues. Read number of macaques and mice

obtained from miRNA-Seq was used for PCA analyses (R package ggplot2 [66] version 3.2.1), respectively.

E F. Heatmaps of differentially expressed miRNAs in macaque and mouse in response to *C. neoformans* infections. Normalized read number of differentially expressed miRNAs from infected macaques and mice are plotted. Differentially expressed miRNAs were considered when foldchange was at least 2-fold and *p*-value less than 0.05. Two clusters were generated by Pheatmap based on miRNA expression pattern.

Figure 3. Core regulatory machinery of miRNA-mRNA network in macaque and mouse.

A. Schedule of identification of homologous miRNA between macaque and mouse. Sequences of differentially expressed miRNAs in macaque were used for search homologous miRNAs in mice, based on miRBase21 search engine (<https://www.mirbase.org/search.shtml>). Mouse homologous miRNAs were compared to differentially expressed miRNAs in mice to Identify co-regulated miRNAs.

B. Venn diagram of differentially expressed miRNAs in macaque and mouse. Venn diagram was plotted based on homology analysis. Green represents macaque, while blue represents mouse.

C. Information of 8 co-regulated mature miRNAs in miRNA-seq. Foldchange of each miRNA and homologous e-value of paired miRNAs were shown.

D. RT-qPCR of the 8 co-regulated miRNAs in mouse.

miRNAs were isolated from infected(n=3) and uninfected(n=3) mice and RT-qPCR assays were performed for 147-3, 146-3, 146-5, 155-3, 21a-3, 21a-5, 223-4 and 155-5. Three biological replicates were performed for each miRNA. Mean and SEM were shown. Unpaired student *t* test was performed. **p* < 0.05, ** *p* < 0.01, or ****p* < 0.005.

E. Construction of miRNA-mRNA core regulatory network during cryptococcal pneumonia. Target genes of 8 co-regulated miRNAs were predicted by miRWalk3.0, then mapped to differentially expressed mRNAs in RNA-Seq. Co-regulated miRNAs and mRNAs were shown here. The whole network is shown in Figure S3. Differentially expressed miRNA from mouse tissues are indicated by triangles, those from macaque tissues are indicated by rectangles, and those from both are indicated by circles. Upregulated miRNA or mRNA are shown in red, downregulated are shown in green, and mRNAs that are oppositely regulated between two are shown in purple and blue. The foldchange is indicated by size.

Figure 4. Gene ontology (GO) analysis of co-regulated miRNAs and target mRNAs in macaque and mouse in response to *C. neoformans* infections.

A. GO of miRNAs calculated by miEAA.

B. GO terms of target mRNAs enriched by Clusterprofiler.

Eight co-regulated miRNAs and co-regulated mRNAs from Figure 4E were employed for GO analyses, respectively. Top 10 or all significantly enriched GO terms were plotted. Green, blue and red columns represent cellular component, molecular function and biological process, respectively.

Figure 5. MYOC is an inhibitor of *C. neoformans* invasion.

A. Negative regulation network of miRNAs and targets differentially expressed during *C. neoformans* infections. Networks were constructed by using differentially expressed miRNAs and target mRNAs, who were regulated opposite during *C. neoformans* infections, which is a zoom-in figure for Figure 5E. Red lines indicated miRNAs regulated MYOC predicted by miRWalk.

B. Normalized MYOC reads in mouse and macaque in response to *C. neoformans*. Raw read number were used and normalized by uninfected groups. Mean and SEM calculated by GraphPad Prism 9 were plotted. Student *t* test was performed. ** $p < 0.01$, *** $p < 0.001$.

C. Strategies for phagocytosis assessment by flow cytometry. Cryptococcal internalization was determined by flow cytometry using GFP-expressing *Cryptococcus* strains. During flow cytometry, single cells were selected, cells with negative Uvitex 2B were considered as phagocytes, and in which FITC positive cells were fungi internalized (Uvitex 2B -/ FITC +).

D. Relative expression level of MYOC in MYOC gene edited THP-1 cell lines. Lentivirus were employed for MYOC gene overexpressed THP-1 cell line construction. Total RNA was isolated, and RT-qPCR were performed using MYOC specific primers (Supplemental tables S4). Triplicate was performed. Mean and SEM calculated by GraphPad Prism 9 were plotted. Student *t* test was performed. **** $p < 0.0001$.

E. Effectivity of phagocytosis in MYOC overexpressed THP-1 derived macrophages by flowcytometry. TDM were induced and seeded onto 48-well

plates with the same number, and then incubated with *C. neoformans* for 24h in CO₂ incubator without serum, and phagocytosis was examined by flow cytometry. Four biological replicates were performed. Mean and SEM calculated by GraphPad Prism 9 were plotted. Unpaired Student *t* test was performed. * $p < 0.05$.

F. Capacity of migration in MYOC overexpressed THP-1 cell lines by trans-well assays. Trans-well assays were performed to detect the effect of MYOC on the capacity of migration in THP-1 cell lines. 200,000 cells (200 μ L) THP-1 cells were seeded onto 5 μ m trans-well inserts in serum free RPMI 1640, and 400 μ L RPMI 1640 contain 10% serum in the lower compartment. Cells were counted by cell counter up and down respectively 24h post incubation, and migration rates were calculated. Four biological replicates were performed. Mean and SEM calculated by GraphPad Prism 9 were plotted. Unpaired Student *t* test was performed.

G. Killing tests of MYOC overexpressed THP-1 derived macrophages. TDM were differentiated using mock THP-1 cell line and MYOC overexpressed THP-1 cell line, and then incubated with *C. neoformans* in CO₂ incubator for 24h in serum free medium. Supernatants and washings were collected, diluted and plated onto YPD agar plates and then incubated at 30°C for two days. Colonies were counted and calculated. Triplicates were performed. Mean and SEM calculated by GraphPad Prism 9 were plotted. Unpaired Student *t* test was performed. * $p < 0.05$.

H. Photo of MYOC transgenic mice. The cute picture is one of our MYOC transgenic mice, 5-week-old, which showed healthy and eye-functioned.

I. MYOC gene fragment was checked by agarose electrophoresis in MYOC

transgenic mice. Genome DNA was isolated and PCR and electrophoresis

were performed. Target PCR products of MYOC specific DNA fragment was

about 500 bp.

J. Brain CFU assessments of MYOC transgenic mice, compared to wild

type mice.

K. Lung CFU assessments of MYOC transgenic mice, compared to wild

type mice.

Seven MYOC transgenic mice (3 male and 4 female) and 8 wild type mice (4

male and 4 female) from the same cages were used for CFU assays. Mice were

anesthesia and infected with 10^5 *C. neoformans* intranasally. All mice were

sacrificed at day-14 post infections, brain and lung tissues were isolated,

homogenized, diluted, plated onto YPD agar plates, and were then incubated

at 30°C for two days. Colonies were counted and calculated. Mean and SEM

calculated by GraphPad Prism 9 were plotted. Unpaired Student *t* test was

performed. * $p < 0.05$.

L. Survival tests of MYOC transgenic mice, compared to wild type mice

from the same cages. Eleven MYOC transgenic mice (5 male and 6 female)

and 9 wild type mice (3 male and 6 female) from the same cages were used for

survival assays. Mice were anesthesia and infected with 10^5 *C. neoformans*

intranasally. Body weight was examined. Fifteen percentage decrease of initial

body weight was identified physiological endpoint. *Log-rank* (Mantel-Cox) test

was employed for statistical analysis. * $p < 0.05$.

Figure 6. Invasion into brain tissue of *C. neoformans* is blocked by cytoskeleton inhibitor.

A. Phagocytosis effectivity was inhibited by R10015 in THP-1 derived macrophages. GFP-expressed fungal cells (H99, a gift from Professor Tongbao Liu, Southwest University, China) were harvested and washed 5 times by PBS buffer, counted by microscopy and opsonized by 18B7 at room temperature for 30min. THP-1 derived macrophages (TDM) were induced by PMA for 48h, washed by PBS, and cultured for 24h, and then digested and seeded onto 48-well plated at 50,000 cells per well the day before phagocyte, and R10015 were incubated for 2h before fungal cell added to pre-treat TDM cells, then washed and renewed with serum free RPMI 1640 medium. Fungal cells were resuspended in RPMI 1640, and added to TDM plates at MOI 10, and incubated in CO₂ incubator overnight. Phagocytosed fungal cells were labeled by GFP, and detected by flow cytometry as shown in Figure 7A. Four biological replicates were performed. Mean and SEM calculated by GraphPad Prism 9 were plotted. Student *t* test was performed. **p* < 0.05.

B. THP-1 cells migration was dampened by R10015. Trans-well assays were performed to detect the capacity of migration of THP-1 cells in the presence of R10015. THP-1 were pre-treated by R10015 for 2h, and then seeded onto 5 μ m trans-well inserts at a density of 200,000 cells (200 μ L) in serum free RPMI 1640, and 400 μ L RPMI 1640 contain 10% serum in the lower compartment. Cells were counted by cell counter up and down respectively, and migration rates were calculated. Four biological replicates were performed. Mean and

SEM calculated by GraphPad Prism 9 were plotted. Student *t* test was performed. ** $p < 0.01$.

C. R10015 does not affect killing *C. neoformans*. Fungal cells and TDM were cultured, differentiated, and incubated in CO₂ incubator for 24h in serum free medium. Supernatants were collected and washed 5 times, and washings were collected. Phagocytes were treated with 0.01(W/V) SDS for 15min, and then cells were collected. Collections were diluted and plated onto YPD agar plates and then incubated at 30°C for two days. Cell colonies were counted and calculated. Four biological replicates were performed. Mean and SEM calculated by GraphPad Prism 9 were plotted. Student *t* test was performed.

D. Phagocytosis effectivity was inhibited by R10015 in human primary monocyte derived macrophages. PBMC were isolated by using Ficoll-Paque from bloods of 12 healthy volunteers (1 mL per individual), and monocytes were collected onto 48-well plates, and then differentiated in RPMI 1640 containing 10% serum, 1% penicillin/streptomycin, and 20ng·mL⁻¹ M-CSF for 7 days and fresh media were renewed on days 3 and 6 of differentiation. A yield of 1×10⁵ monocyte derived macrophages (MDMs) per well was estimated. MDMs from one person were divided into two wells, one is incubated with R10015 and another with an equal volume of DMSO for 2h. R10015/DMSO was washed, renewed with serum free RPMI 1640 and fungal cells were added at MOI 10, and incubated for 24h. The effectivity of phagocytosis was determined by flow cytometry. Twelve volunteers were included, and paired student *t* test was performed by GraphPad Prism 9 plotted, * $p < 0.05$.

E. MDMs migration was dampened by R10015. MDMs were differentiated and pre-treated by R10015/DMSO (MDMs from the same individual), and 50,000 MDMs were seeded onto 8 μ M trans-well insert in 200 μ L serum free RPMI 1640, and 400 μ L RPMI 1640 containing 10% serum in the lower compartment. Cells were counted by microscopy 48h post incubation. Six volunteers were included, and paired student *t* test was performed by GraphPad Prism 9 plotted, * $p < 0.05$.

F. R10015 has no effect on killing *C. neoformans* in MDMs. MDMs were differentiated and pre-treated by R10015/DMSO (MDMs from the same individual), R10015/DMSO were washed, renewed with serum free RPMI 1640 and fungal cells were added at MOI 10, and incubated for 24h. Killing assays collections were performed as Figure 7D, washings were collected, diluted and plated onto YPD agar plates and incubated at 30°C. Cell colonies were counted and calculated two days later. Five volunteers were included. Paired student *t* test was performed by GraphPad Prism 9 plotted.

G. Early CFU assessment in vivo in the presence of R10015. R10015 was injected intraperitoneally (IP) at a concentration of 6.868 mg/kg 6h before infection[38]. PBS buffer injected mice were used for controls. Wild-type *C. neoformans* H99 (10^5 cells) were infected intranasally. Mice were sacrificed 7 days post infections. Brain, lung, liver, kidney and spleen tissues were isolated, homogenized, diluted and plated onto YPD agar plates and then incubated at 30°C for two days. Cell colonies were counted and calculated. Five biological replicates were performed. Mean and SEM calculated by GraphPad Prism 9 were plotted. Student *t* test was performed. * $p < 0.05$.

H. Late CFU assessment in vivo in the presence of R10015. R10015 was injected intraperitoneally (IP) at a concentration of 6.868 mg/kg 6h before infection[38]. PBS buffer injected mice were used for controls. Wild-type *C. neoformans* H99 (10^5 cells) were infected intranasally. Mice were sacrificed 14 days post infections. Brain, lung, liver, kidney and spleen tissues were isolated, homogenized, diluted and plated onto YPD agar plates and then incubated at 30°C for two days. Cell colonies were counted and calculated. Five biological replicates were performed. Mean and SEM calculated by GraphPad Prism 9 were plotted. Student *t* test was performed. ** $p < 0.01$.

I. Effects of R10015 on survival rates of *C. neoformans* infected mice

Thirty C57BL/6 mice were divided into 3 groups randomly, named R10015 injected control group(n=10), wild type infection group(n=10), and R10015 injected infection group(n=10). Same to CFU experiments, R10015 were injected intraperitoneally (IP) at a concentration of 6.868 mg/kg 6h before infection[38]. Mice were infected intranasally with 10^5 fungal cells in 50 μ L PBS. Body weight was examined almost once a day post H99 infections. Fifteen percentage decrease of initial body weight was identified physiological endpoint. *Log-rank* (Mantel-Cox) test was employed for statistical analysis. * $p < 0.05$.

Striking image

Based on “Trojan Horse”, cryptococci trigger cytoskeleton dynamics, enhance capacities of motility and phagocytosis of macrophages, and then phagocytosed by macrophages. Phagosomes, containing fungal cells

intracellular proliferated, migrate, pass through the blood-brain barrier (BBB) and arrive at the brain eventually (shown in blue lines). Inhibitors of cytoskeleton pathways or MYOC could control migration and phagocytosis, dampen “Trojan Horse” and decrease the number of cryptococci in the brain transported by “Trojan Horse” (shown in red lines).

Supporting information

Figure S1. Read number of miRNA-Seq.

A. Total and unique read number of miRNA-Seq. Blue represents all reads, red unique reads.

B. Distribution of different lengths of nucleotides from macaques. Total reads and unique reads were plotted.

C. Distribution of different lengths of nucleotides from mice. Total reads and unique reads were plotted.

Figure S2. Network of differentially expressed miRNA-mRNA during cryptococcal pneumonia. The whole network of 8 co-regulated miRNAs and their target genes in RNA-Seq, which is the intact network of Figure 4E.

Figure S3. KEGG analysis of co-regulated miRNA and targeted mRNA in macaque and mouse in response to *C. neoformans* infections. Eight co-regulated miRNAs and co-regulated mRNAs from Figure 4E were employed for KEGG analyses, respectively. KEGG of miRNAs was calculated by miEAA, while mRNA by Clusterprofiler. Comparative analysis was performed by ggplot2, and KEGG pathways with p -value ≤ 0.05 were plotted. Gradient colors

represent p -value, while size of ring represents the number of miRNAs or mRNAs in the pathway.

Figure S4. Growth curve of *C. neoformans* and body weight loss of mice in survival rates during *C. neoformans* infections.

A. Body weight was examined daily in survival rates experiment. Blue line indicated wild type control group(n=11) and red line MYOC transgenic mice group(n=12) Dash dot lines represent 85% of the initial average body weight (physiological endpoint of mouse).

B. Growth curve of H99 in different concentrations of R10015. Fungal cells were incubated in YPD Liquid culture medium overnight at 30 °C, washed three times using sterile PBS buffer, and quantified by microplate reader. Initial concentration of *C. neoformans* is OD=0.02. Then incubated fungal cells with different concentrations R10015, an inhibitor of cytoskeleton pathway, and check OD 24h and 48h post incubation. Mean and SEM calculated by GraphPad Prism 9 were shown.

C. Body weight was examined daily in survival rates experiment. Blue line indicated R10015 injected control group(n=10), red line wild type infection group(n=10) and green indicated R10015 injected infection group. Dash dot lines represent 85% of the initial average body weight (physiological endpoint of the mouse).

Table S1. Differentially expressed miRNAs in macaque and mouse lung tissues during *C. neoformans* infections.

- 1154 **Table S2.** GO and KEGG analyses of 8 co-regulated miRNAs in response to
 1155 *C. neoformans* infections.
- 1156 **Table S3.** GO and KEGG analyses of target genes for co-regulated 8 miRNAs
 1157 in mouse and macaque.
- 1158 **Table S4.** Primers used in the study.

Figure 3

A

B

■ Cellular Component

■ Molecular Function

■ Biological Process

Figure 4

Figure 5

Figure 6

Figure S1

Figure S2

Figure 1

Figure S3

Figure S4