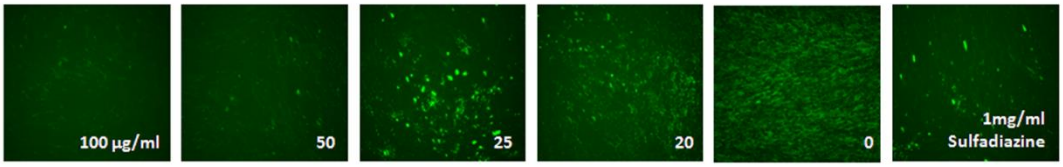

FIG S1 The fluorescence intensity of RH-GFP on HFF cells after treatment with increasing concentration of CAR or sulfadiazine.

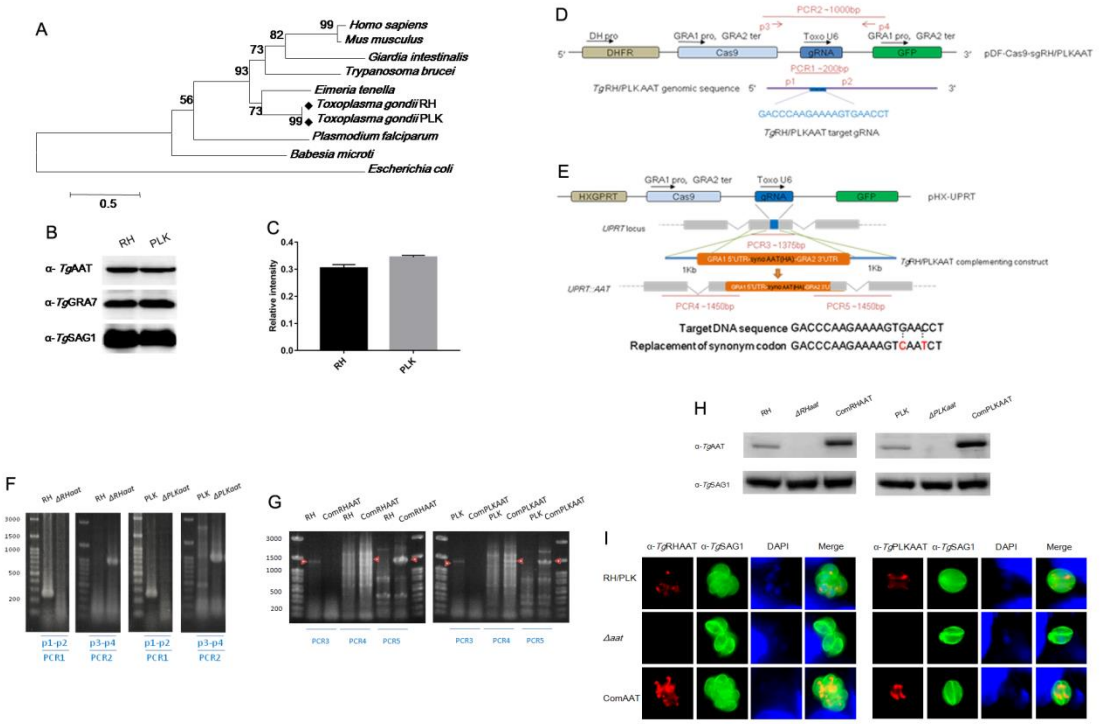

FIG S2 Generation and confirmation of *Δaat* deletion mutants and ComAAT complementing strains. (A) Phylogenetic tree of AATs from different species. (B and C) Expression levels of native AAT protein in RH and PLK strains. Means ± SEM of three independent experiments are shown, unpaired Student's *t* test. (D) Schematic illustration of knocking out AAT by CRISPR/Cas9 system both in RH and PLK strains, including Cas9, GFP, guide RNA and dihydrofolate reductase DHFR expression cassettes expressed in different colors. (E) Schematic of CRISPR/Cas9 strategy for insertion of synoAAT in UPRT locus. The blue bar in UPRT gene represents the region targeted by the gRNA. (F and G) Confirmation of knockout by PCR. PCR1/PCR2 denote the amplification products of diagnostic polymerase chain reactions (PCRs) used in D. PCR3/PCR4/PCR5 denote the amplification products of PCRs used in E. (H) Western blot analysis of *Δaat* and ComAAT parasites. Wild-type strains RH and PLK were used as control. (I) IFAT analysis of *Δaat* and ComAAT parasites. Wild-type strains RH or PLK were used as control. Mouse α-*Tg*RH/PLKAAT and rabbit α-*Tg*SAG1 antibody was used for staining.

Table S1. Primers used for generation of *ΔRH/PLKaat* and ComRH/PLKAAT strains

Primer name	Sequence(5'→ 3')	Use
RH/PLKAATgRNA-KOF1	CGAATTGGAGCTCCACCGCGGGAGCTCCAAGTAAGCAGAAG	Construction of pDF-Cas9-sgRH/PLKAAT
RH/PLKAATgRNA-KOR1	AGGTTCACTTTTCTTGGGTCAACTTGACATCCCCATTTAC	Construction of pDF-Cas9-sgRH/PLKAAT
RH/PLKAATgRNA-KOF2	GACCCAAGAAAAGTGAACCTGTTTTAGAGCTAGAAATAGC	Construction of pDF-Cas9-sgRH/PLKAAT
RH/PLKAATgRNA-KOR2	TCTAGAGCGGCCGCCACCGCGGGAGCTGATACCGCTCGCC	Construction of pDF-Cas9-sgRH/PLKAAT
RH/PLKsynoAAT-COF1	CTTTGAAGAAATCAAGCAAGGAATTCATGTTTCCAACTCTTAGTGA	Construction of pB-synoRH/PLKAAT
RH/PLKsynoAAT-COR1	AGATTGACTTTTCTTGGGTCTTGGTCTGCCCTGAACGCGA	Construction of pB-synoRH/PLKAAT
RH/PLKsynoAAT-COF2	GACCCAAGAAAAGTCAATCTCGGCATCGGAGCCTACCGAA	Construction of pB-synoRH/PLKAAT
RH/PLKsynoAAT-COR2	TTAAGCGTAATCTGGAACATCGTATGGGTACATGCTTGCAGGAACTGCCCGCA	Construction of pB-synoRH/PLKAAT
ΔRH/PLKaat-CheckP1	GCACTCAACAGAGGGTATTT	Validation of *Δaat*, PCR1
ΔRH/PLKaat-CheckP2	CACCCGAGCTGGCTTGCTTA	Validation of *Δaat*, PCR1
ΔRH/PLKaat-CheckP3	CGAATTGGAGCTCCACCGCGGGAGCTCCAAGTAAGCAGAAG	Validation of *Δaat*, PCR2
ΔRH/PLKaat-CheckP4	TCTAGAGCGGCCGCCACCGCGGGAGCTGATACCGCTCGCC	Validation of *Δaat*, PCR2
ComRH/PLKAAT-CheckP5	TCTTCTACGCCGACCGCCTGATT	Validation of ComRH/PLKAAT, PCR3
ComRH/PLKAAT-CheckP6	CAGGCAGCTTCTCGTAGATCAG	Validation of ComRH/PLKAAT, PCR3
ComRH/PLKAAT-CheckP7	TTCAGACTCTCTGTGGTCGGCGAG	Validation of ComRH/PLKAAT, PCR4
ComRH/PLKAAT-CheckP8	ATGGTCAACAAAACAGCATATTCCTCCC	Validation of ComRH/PLKAAT, PCR4
ComRH/PLKAAT-CheckP9	GTAGAGAGGACCAAAAGACGATTGC	Validation of ComRH/PLKAAT, PCR5
ComRH/PLKAAT-CheckP10	CGAACCGATATAAATGCATGGCAT	Validation of ComRH/PLKAAT, PCR5

SUPPLEMENTARY METHODS

DNA isolation and quantitative PCR (qPCR) detection of parasite burdens in infected mice tissues

DNA was extracted from the tissues (brain, liver and spleen) of parasite challenged mice by DNeasy Blood & Tissue Kit (QIAGEN, Germany), according to the manufacturer's instructions. The 200 ng tissues DNA was then amplified with primers specific to the *T. gondii* B1 gene (Table S2) by qPCR. A standard curve was constructed using 10-fold serial dilutions of *T. gondii* DNA extracted from 10^5 parasites; thus, the curve ranged from 0.01 to 10,000 parasites. The parasite number was calculated from the standard curve as described above.

Total RNA extraction and qPCR analysis of gene expression

Total RNA was extracted from homogenized tissues (brain, liver and spleen) using TRI Reagent (Sigma-Aldrich). The cDNA was amplified by Reverse transcription of 800ng RNA with PrimeScript™ RT Master Mix (Perfect Real Time) (TaKaRa Bio, Inc., Shiga, Japan) according to the manufacturer's instructions. qPCR was carried out as described above. All primers were used in this study are listed in Table S2. The expression levels of target genes were normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels using the $2^{-\Delta\Delta Ct}$ method.

Cytokine ELISA

Previous studies reported that anti-*Toxoplasma* defense mechanisms depend largely on IFN-γ production by immune cells (1). As well as, TNF-α as a critical pro-inflammatory cytokine, is involved in the host's immune responses against parasites (2, 3), and mediates apoptosis for T cells and NK cells predominantly (4). Therefore, to explain currently enhanced acute virulence whether the AAT-deleted parasites in mice are associated with overstimulation of Th1 cytokines which led to lethal infections (4), mouse serum was collected for measurement of IFN-γ and TNF-α levels using Mouse IFN gamma ELISA and TNF alpha Mouse ELISA Kit

((Thermo Fisher Scientific, Inc., Waltham, MA)) according to the manufacturer's recommendations.

Statistical analysis

To graph the data, GraphPad Prism 7 software (GraphPad Software Inc., USA) was used. Statistical analyses were performed using a one-way ANOVA plus Tukey-Kramer *post hoc* analysis in this study. Data represent the mean ± Standard Error of Mean. A P value < 0.05 was considered statistically significant.

SUPPLEMENTARY RESULTS

Absence of *Tg*AAT in PLK strain does not decrease parasite burdens in mice

To better clarify the virulence on *ΔPLKaat* parasites in mice, we increased the doses to 5×10^4 tachyzoites in the survival assay, and meanwhile determined the interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) level in mouse sera at 2, 4, 6 and 8 days post-infection (dpi). All *ΔPLKaat* infected mice died at 7 and 8 dpi, while 83.3% mortality was obtained in PLK-infected mice however even at the highest infection dose of 5×10^4 tachyzoites per mouse (Fig. 6C and D). In addition, IFN-γ and TNF-α levels in sera from mice with *ΔPLKaat* infection were present in higher level at 2 and 4 dpi, and in significantly elevated levels at 6 and 8 dpi, compared to that of with PLK infection (Fig. S3A and B). Collectively, these current results indicated that loss of PLKAAT caused a stronger as well as more serious immune response in mice during infection correlating with lethality.

The above results showed that *aat* inactivation leads to enhanced virulence in mice. To elucidate the mechanisms underlying these changes, mice were infected with 1×10^5 purified tachyzoites challenge of either PLK, mutant or ComPLKAAT strains by intraperitoneal injection (i.p.) to promote acute infection. At 3 and 6 dpi, tissues (brain, liver and spleen) were collected, and the number of parasites they contained was determined by quantitative PCR (qPCR) targeting the *Tg*B1 gene using extracted DNA. As shown in Fig. S3C, the 200 ng tissues DNA were used to determine the

number of parasites in brain, liver and spleen tissues during different tachyzoites infection, revealing that the number of PLK, *ΔPLKaat* and ComPLKAAT parasites found in all three tissues of infected mice with different loads on 3 and 6 dpi. Although the parasite number in liver increased to 580.2177 parasites at 6 dpi from 14.467 parasites at 3 dpi, parasite loads of *ΔPLKaat*-infected mouse liver (8960.715 parasites per 200 ng DNA) was 15.44 times than that of the PLK-infected mouse at 6 dpi. Nonetheless, the parasite load in *ΔPLKaat* mouse spleen (408.7724 parasites per 200 ng DNA) was similar to wild-type (414.663 parasites per 200 ng DNA) at 6 dpi. These data suggest that loss of AAT in PLK strains resulted in increasing parasite burdens in tissues during acute infection. Subsequently, high burdens led to up-regulated mRNA expression levels of interleukin-12p40 (IL12p40), IFN-γ, TNF-α, IL10 and inducible nitric oxide synthase (iNOS) activities (Fig. S3D). In particular, the expression of IFN-γ in *ΔPLKaat* mice liver was 4.44-fold than that PLK mice at 6 dpi and 21.4-fold than that of *ΔPLKaat*-infected mice at 3 dpi. Moreover the iNOS of *ΔPLKaat* mouse liver was up-regulated by 9.44-fold compared to that of PLK mouse liver at 6 dpi. Taken together, mice were infected with AAT-deficient parasites resulted to increased parasite numbers leading to up-regulate expression of inflammatory cytokines or iNOS in tissues as infection progressed, which induced overexpression of IFN-γ and TNF-α until a lethal outcome.

SUPPLEMENTARY REFERENCES

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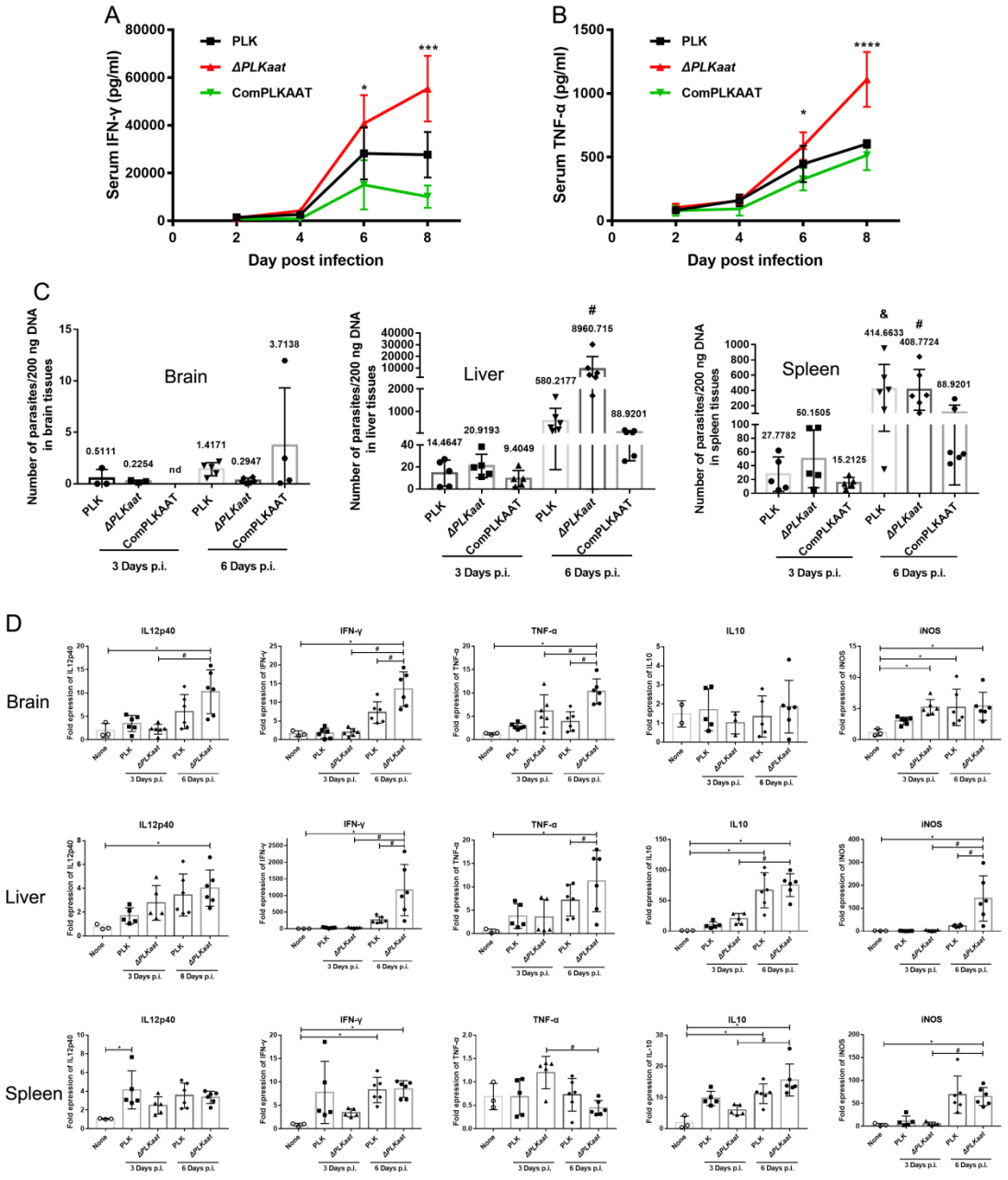

FIG S3 *ΔPLKaat* infection causes higher parasite burdens in mice. (A and B) Serum IFN-γ (A) and TNF-α (B) level of 50,000 *ΔPLKaat* tachyzoites challenge by intraperitoneal injection. Mouse sera were collected at 2, 4, 6 and 8 dpi by Tail tip. IFN-γ and TNF-α was detected by ELISA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA plus Tukey-Kramer *post hoc* analysis. (C) Parasite burdens during acute infection. Six mice were infected by intraperitoneal injection with 100,000 tachyzoites, and then tissues including brain, liver and spleen were collected at 3 or 6 dpi. Parasite burdens were determined from tissues DNA by qPCR. # $p < 0.05$ vs. day

6 *ΔPLKaat* group; & $p < 0.05$ vs. day 6 PLK group, one-way ANOVA plus Tukey-Kramer *post hoc* analysis. (D) The expression of inflammatory cytokines and iNOS during acute *ΔPLKaat* infection in mice. * $p < 0.05$ vs. no-infection group; # $p < 0.05$ vs. day 6 *ΔPLKaat* group, one-way ANOVA plus Tukey-Kramer *post hoc* analysis.

Table S2. Primers used quantitative PCR (qPCR)

Target gene		Forward Primer (5'→ 3')	Reverse Primer (5'→ 3')
T. gondii	B1	AACGGGCGAGTAGCACCTGAGGAG	TGGGTCTACGTCGATGGCATGACAAC
Mouse	IL-12p40	GAGCACTCCCCATTCCTACT	ACGCACCTTTCTGGTTACAC
	IFN-γ	GCCATCAGCAACAACATAAGCGTC	CCACTCGGATGAGCTCATTGAATG
	TNF-α	GGCAGGTCTACTTTGGAGTCATTGC	ACATTCGAGGCTCCAGTGAA
	IL10	TGGACAACATACTGCTAACCGAC	CCTGGGGCATCACTTCTACC
	iNOS	ACCCCTGTGTTCCACCAGGAGATGTTGAA	TGAAGCCATGACCTTTCGCATTAGCATGG
	GAPDH	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGAT