

Fig S1. Knockout and add-back of *PP2Acα* gene in tachyzoites of *Toxoplasma gondii*.

(A) Schematic illustration of knocking out *PP2Ac* subfamily by CRISPR/Cas9-mediated homologous gene replacement in the $RH\Delta ku80\Delta hxpRT$ cells ($RH\Delta ku80$). (B) Electrophoresis of PCR amplicons as indicated WT: Parental cells. (C) Schematic illustration of adding back *PP2Acα* by CRISPR/Cas9-mediated homologous gene replacement in the $\Delta PP2Ac\alpha$ knockouts. (D) Electrophoresis of PCR amplicons as indicated. WT: Parental cells. (E) Positive/negative (P/N) OD ratios of antibodies in the serum of mice survived from $\Delta PP2Ac\alpha$ tachyzoites infection. P/N ratio greater than 2.1 is considered positive for *Toxoplasma* infection.

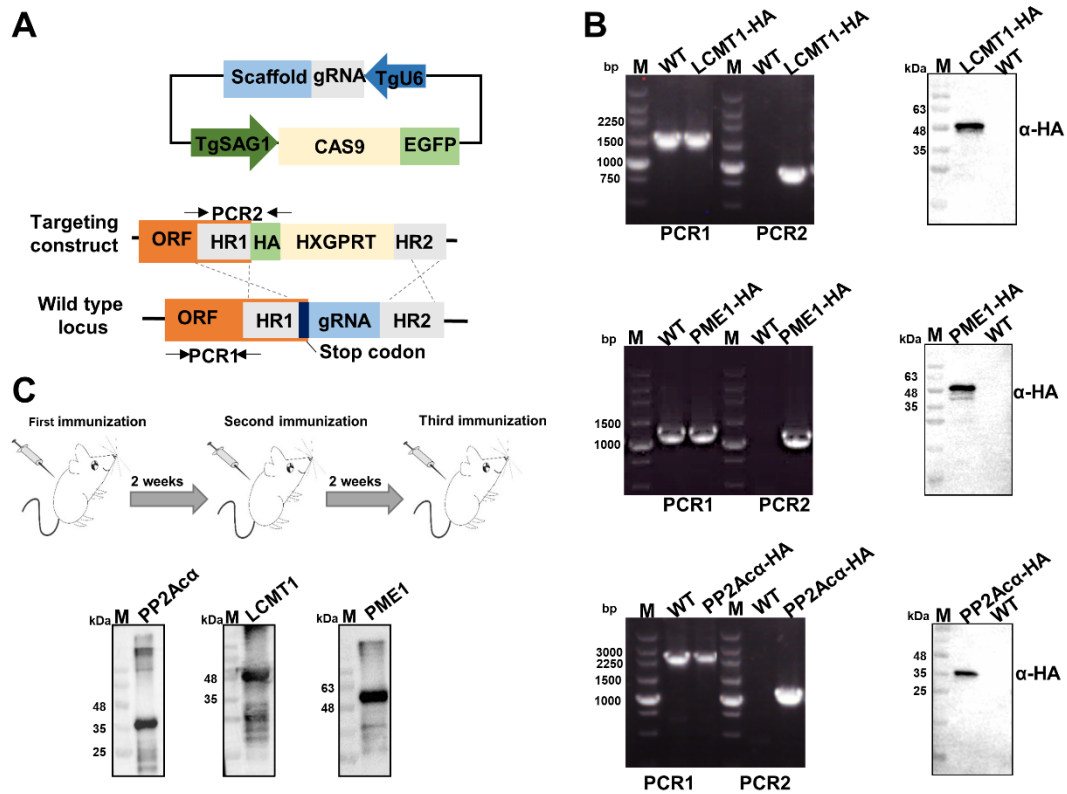


Fig S2. Endogenous 3 \times hemagglutinin tagged cells and polyclonal antibodies against PP2Ac α and its methylation regulators. (A) Schematic illustration of PP2Ac α -3HA, leucine carboxyl methyl transferase 1 (LCMT1) -3HA and protein phosphatase methylesterase 1 (PME1)-3HA by CRISPR/Cas9 tagging strategy in *RH Δ ku80*. Three consecutive hemagglutinin (HA) tags are included. (B) PCR (left) and Western blot (WB, right) of representatives expressing PP2Ac α -HA, LCMT1-3HA and PME1-3HA. Anti-HA antibodies are used in Western blot. (C) Western blotting analysis of polyclonal antibodies against PP2Ac α , LCMT1 or PME1.

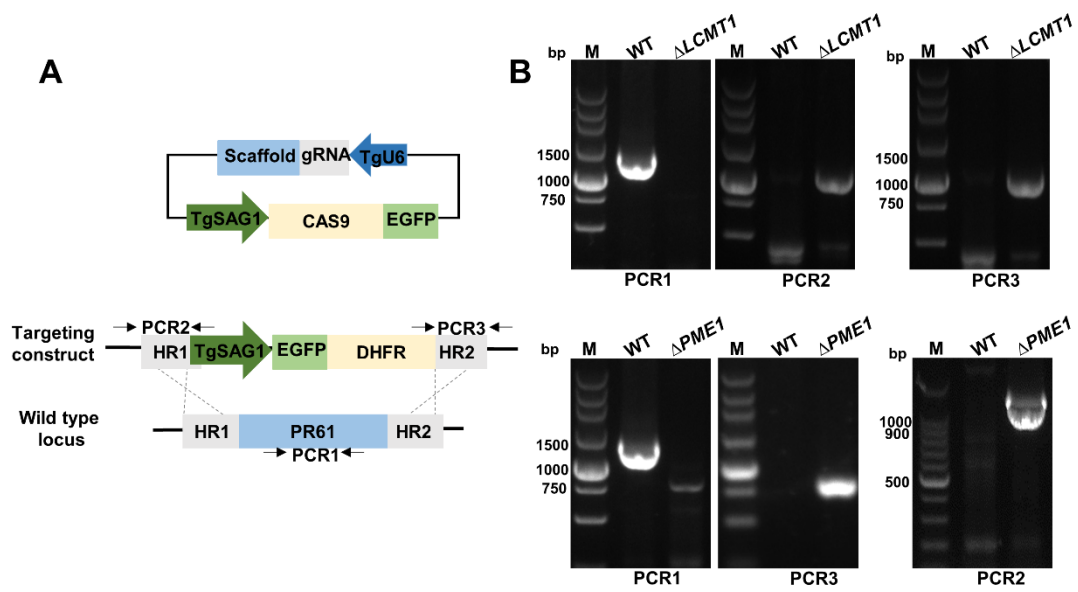


Fig S3. Knockout of *LCMT1* and *PME1* genes in tachyzoites of *Toxoplasma gondii*. (A) Schematic illustration of the genetic deletion of *LCMT1* and *PME1* by CRISPR/Cas9 tagging strategy in RH Δ ku80. WT: parental cells; Δ *LCMT1*: tachyzoite with *LCMT1* knockout; Δ *PME1*: tachyzoite with *PME1* knockout. (B) PCR identification of the Δ *LCMT1* or Δ *PME1* representative clones.

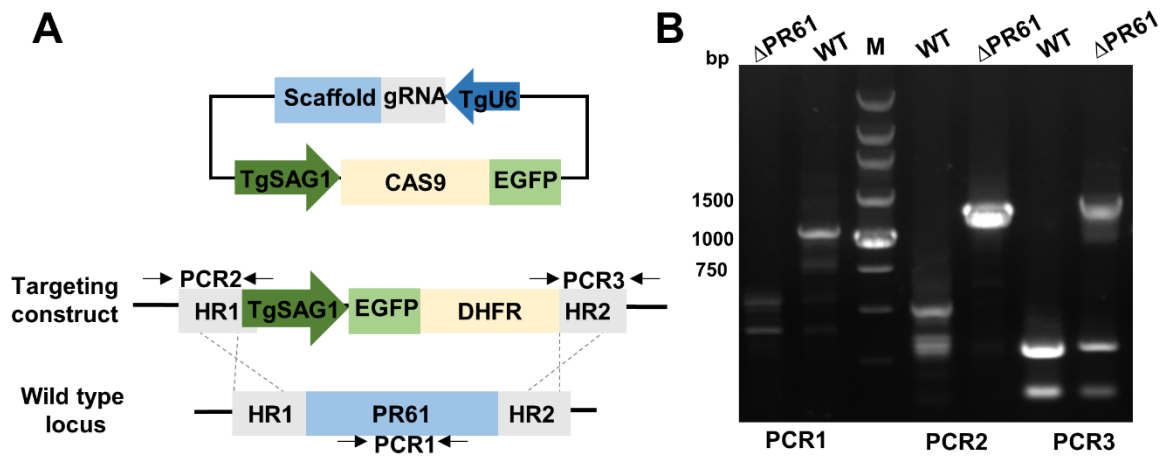


Fig S4. Knockout of B'/PR61 in tachyzoites of *Toxoplasma gondii*. (A) Schematic illustration of knocking out B'/PR61 by CRISPR/Cas9-mediated homologous gene replacement in the RH $\Delta ku80$. WT: parental cells; $\Delta PR61$: tachyzoite with PR61 knockout; (B) PCR identification of the $\Delta PR61$ tachyzoites representative clones.

Table S1 Primers used in the current study.

Name	Sequence (5' - 3')
PP2A α -KO sgRNA F	AGTTGTTGGGGCAAGAAGAGTTGCG
PP2A α -KO sgRNA R	AAACCGCAACTCTTCTTGCCCAAC
PP2A α -KO donor F	TTTTTCTTCCACACACTCTGCCGTTTCTCCTTTCTTGTACCTCCGCAAAGCTTTTACATCCGTTGCCTTTTC
PP2A α -KO donor R	CGTTTGTCTGGCCTCCTCAAGCCTTCGTTTGTGTTTGTTCGGTCTCCCCGGGGCAAGAATTGTGTTAACCGGT
PP2A α -KO screen F1	GAGTGCCTGCGCAAATATGG
PP2A α -KO screen R1	GCAGAAGCGACGGATTGAAC
PP2A α -KO screen F2	TCAGATCCCTGGAGACATCCGAA
PP2A α -KO screen R2	GTTGCGGCACTGTGAGCACTGAT
PP2A α -KO screen F3	GCTTCCTATTCGCTTTTGACGCT
PP2A α -KO screen R3	TTCTGTTACCGACTTTTTTTGC
PP2A α -Comp sgRNA F	AGTTACACGCTTTGTGGTGAGAAG
PP2A α -Comp sgRNA R	AAACCTTCTCACCACAAAGCGTGT
PP2A α -Comp donor F	TTTTTCTTCCACACACTCTGCCGTTTCTCCTTTCTTGTACCTCCGCAATGTCGGGTGCAACTCCA
PP2A α -Comp donor R	TCTTTTCTTCGCATTCTTCTCATCGCCTCGCATCCTTCTCGCCTGTGATAGGGCGAATTGGAGCTCC
PP2A α -Comp screen F1	GAGTGCCTGCGCAAATATGG
PP2A α -Comp screen R1	GCAGAAGCGACGGATTGAAC
PP2A β -KO sgRNA F	AGTTAGAGGCGCCGGGATGCTCTG
PP2A β -KO sgRNA R	AAACCAGAGCATCCCGGCGCCTCT
PP2A β -KO donor F	GCTGCACAAGTTGAGCACTCCCGTTCTTGACGCGAGTAGCTGTACCAAGCTTTTACATCCGTTGCCTTTTCCA
PP2A β -KO donor R	GTTGAACAGCAAGCGGTCGAGCGAAGAAGACTGCAGCCTAGTCATCTCGGGGCAAGAATTGTGTTAACCGGTTC
PP4c-KO sgRNA F	AGTTATGGGAGCAGCACCGTTTGG
PP4c-KO sgRNA R	AAACCCAAACGGTGTCTGCCAT
PP4c-KO donor F	CCTCCTCCTACTCTTCCGTTCTACGCTTCTGCTGCTCTCCTCTCAAGCTTTTACATCCGTTGCCTTTTCCA
PP4c-KO donor R	AGAGACGCGGAATTCGAAGAGAACGTTGAACTCCAAACGTCGCCTGCTGGGGCAAGAATTGTGTTAACCGGTTC

PP6c-KO sgRNA F	AGTTGCGACTGGCAAGACTTGGTCA
PP6c-KO sgRNA R	AAACTGACCAAGTCTTGCCAGTCGC
PP6c-KO donor F	GCATCGCTTCGTTCTCCCACAGACCAGCACTGGCGGGTCCGACACCAGAAGCTTTTACATCCGTTGCCTTTTCCA
PP6c-KO donor R	TCCCCCTGCCTACCTCCTCCTCCCCCTCCCCCTACGCATACGCTGTATCGGGGGCAAGAATTGTGTTAACCGGTC
LCMT1-KO sgRNA F	AGTTGTCAGGCTCACTACTCGATTG
LCMT1-KO sgRNA R	AAACCAATCGAGTAGTGAGCCTGAC
LCMT1-KO donor F	GAGTCGCGTGTCTCAGTGATCAGTCACGCCCCGAGCACACGAGATAATTAAGCTTTTACATCCGTTGCCTTTTC
LCMT1-KO donor R	GAGGTGCCACGAGGACAAAGAATTTGAGTGTGGAACCTAGTGGACTTCGGGGGCAAGAATTGTGTTAACCGGT
LCMT1-KO screen F1	TGAAATCCGCTCCCAGTGTC
LCMT1-KO screen R1	GGGTCATCTTCTCGTTGCCA
LCMT1-KO screen F2	GGACGGTTTGAAGGCGTTTC
LCMT1-KO screen R2	CACGCGTCTCAAGGTTTGTG
LCMT1-KO screen F3	TGCAGGTGTCACATCCACAA
LCMT1-KO screen R3	CTCCTAGGTGTCTCGTTGGC
PME1-KO sgRNA F	AGTTAAAGTGGGCGGAGGCTCCCCG
PME1-KO sgRNA R	AAACCGGGAGCCTCCGCCACTTT
PME1-KO donor F	GTCGCTTGCTCCTCGAGTGTCAAGCCGGCCTCGCCTCTCGTCGAAAAGAAGCTTTTACATCCGTTGCCTTTTC
PME1-KO donor R	CGTGGGGTTCGTCGACTTCAGGCGGCTCCTAGTGTATGCACACTGCGTGGGGGCAAGAATTGTGTTAACCGGT
PME1-KO screen F1	AAGAGGCTTCCAACGGTGTT
PME1-KO screen R1	CCCTGCACAGATAAGCACCT
PME1-KO screen F2	AGGACTTTATGCGGCCTTCC
PME1-KO screen R2	CTACGAAACGTTACACGCC
PME1-KO screen F3	TTCACCGCCGAGGATTTTGA
PME1-KO screen R3	GCAACGAAACAGACAACGCA
PR61-KO sgRNA F	AGTTGCCTCCGCCAGGAGGAGGCG
PR61-KO sgRNA R	AAACCGCCTCCTCCTGGCGGAGGC

PR61-KO donor F	GGTCTGCCTTGCGTCTCAGTTTGCTCCCATAATGCTTTCGACTCTCTCCCAGGTTAGCCTCCAAGCTTTTACATCCGTTGCCTTTTC
PR61-KO donor R	GTCAGAAACTTTGAGGAAGACGTGCGAACGCGCACACCTTCTCCCAGGAGAGCGGAGACGGGGCAAGAATTGTGTTAACCGGT
PR61-KO screen F1	ATTCTGCTGAACACGCAACCTC
PR61-KO screen R1	AAAAGTCGCGTCGGCGGA
PR61-KO screen F2	CGGCATTTTCCATTCGGCA
PR61-KO screen R2	TCAAATGCCTCGTGTGGGAA
PR61-KO screen F3	TCAAATGCCTCGTGTGGGAA
PR61-KO screen R3	GCTGCGCCTTCTGTCAAAA
PP2A α -HA sgRNA F	AGTTGAACCACGGCGCGTTTGTC
PP2A α -HA sgRNA R	AAACGGACAAACGCGCCGTGGTTC
PP2A α -HA donor F	CAAGCCTCGGGAGCAGACATGCAGAAACGAATTCCGGACTACTTCTGGGCGGAGAAATCTCCCTGAGAA
PP2A α -HA donor R	TCTTTTCTTCGCATTCTTCTCATCGCCTCGCATCCTTCTCGCCTGTGATAGGGCGAATTGGAGCTCC
PP2A α -HA screen F	GCCGAACTATTGCTACCGCTGT
PP2A α -HA screen R	GCCATCGGACATAGGACTCATT
LCMT1-HA sgRNA F	AGTTGGACTCCAATCAGTTAGCCG
LCMT1-HA sgRNA R	AAACCGGCTAACTGATTGGAGTCC
LCMT1-HA donor F	GAGAAGATGACCCTGCCGCGCCTGAAGCAGATCTACGAAGGCATGAATGGCGGAGAAATCTCCCTGAGAA
LCMT1-HA donor R	AAAAGCGCGGCTTCCGAGGAGTCGGAGGAAAGAATCTCAATTCACAGAATAGGGCGAATTGGAGCTCC
LCMT1-HA screen F	GTGGGGCAGAGTTCTCAAAG
LCMT1-HA screen R	CCATCGGACATAGGACTCAT
PME1-HA sgRNA F	AGTTGTGGGGTTCGTCGACTTCAGG
PME1-HA sgRNA R	AAACCCTGAAGTCGACGACCCAC
PME1-HA donor F	TCTGTCCAGCCTTGCGCGTTTACCCTCTGCGCCGAAAGCCTGCGAGTGGCGGAGAAATCTCCCTGAGAA
PME1-HA donor R	CATGTACAGACATTACCAAACCTGAGGATTTACATGTGGGGACAGGTGAATAGGGCGAATTGGAGCTCC
PME1-HA screen F	CTGGACCTGGCGAACAGATT
PME1-HA screen R	CTCCTATCGGAGAGGGGGAG

PR61-HA sgRNA F	AGTTGTACACATATACGTGAGGAG
PR61-HA sgRNA R	AAACCTCCTCACGTATATGTGTAC
PR61-HA donor F	GGCGGGGAGGACGCGTTCTTGGTTCGCTTTTCCGCCGACGCGACTTTTGGCGGAGAAATCTCCCTGAGAA
PR61-HA donor R	AACTTTGAGGAAGACGTCGAACGCGCACACCTTCTCCCGCAAGAGCATAGGGCGAATTGGAGCTCC
PR61-HA screen F	ACCAGCAACTCACCTACTGC
PR61-HA screen R	CCCCCGGACAGAACAATACC
PP2Ac α qPCR F	GTCACCATTTTCTCTGCGCC
PP2Ac α qPCR R	CTTGCAGAAGCGACGGATTG
PP2Ac β qPCR F	ATTGGGTCCGAGTGCTTCTG
PP2Ac β qPCR R	TGTCGTAGGGGTCTGACCAT
PP4c qPCR F	GAGCTCTTTCGTGTGGGGAA
PP4c qPCR R	GGGTACCGAACTTTCAGGG
PP6c qPCR F	GGGGCCTCACATGGAATCAA
PP6c qPCR R	ACGCCTTCTTGTCGAGAGTG
PP1c qPCR F	CACGGATTGCTTCAACTGCC
PP1c qPCR R	CAGTGGGCCTGACAATCCTT
PR53 qPCR F	TTCTTCCCCGCTTTTCGAC
PR53 qPCR R	GGAAACGCATGAAAGCCTGG
PR61 qPCR F	GTGGAACAACGAGCACATCG
PR61 qPCR R	CCTCTTGCTGCTTGCCTTTT
PR70 qPCR F	CCACTACCTCTGGTCTCGGA
PR70 qPCR R	CCAAGCACCGTGGTTTTTCAG
LCMT1-HA F	TAGAAGACACCGACTCTAGAATGTCGTCTTATTTATCGTC
LCMT1-HA R	TTGTAATCCAGAGGTTGATTTCAAGCGTAGTCTGGGACGTCGTATGGGTAATTCATGCCTTCGTAGATCT
PME1-HA F	TAGAAGACACCGACTCTAGAATGGATAACGACTTCTTTCTTCC
PME1-HA R	TTGTAATCCAGAGGTTGATTTCAAGCGTAGTCTGGGACGTCGTATGGGTAACCTCGCAGGCTTCGGCGCA

PP2A α -Flag F	TAGAAGACACCGACTCTAGAATGTCGGGTGCAACTCCA
PP2A α -Flag R	TTGTAATCCAGAGGTTGATTTTACTTATCGTCATCGTCTTTGTAATCCAGGAAGTAGTCCGGAATTC
LCMT1-pET30a F	CGGGATCCATGTCGTCTTATTTATCGTCTCCCC
LCMT1-pET30a R	GCTCTAGAATTCATGCCTTCGTAGATCTGCTT
PME1-pET30a F	CGGAATTCATGGATAACGACTTCTTTCTTCCAC
PME1-pET30a R	CCGCTCGAGACTCGCAGGCTTCGGCGCAGAGTG
PP2A α -pET30a F	CGGGATCCATGTCGGGTGCAACTCCAG
PP2A α -pET30a R	GCTCTAGATCACAGGAAGTAGTCCGGAATTCGT
