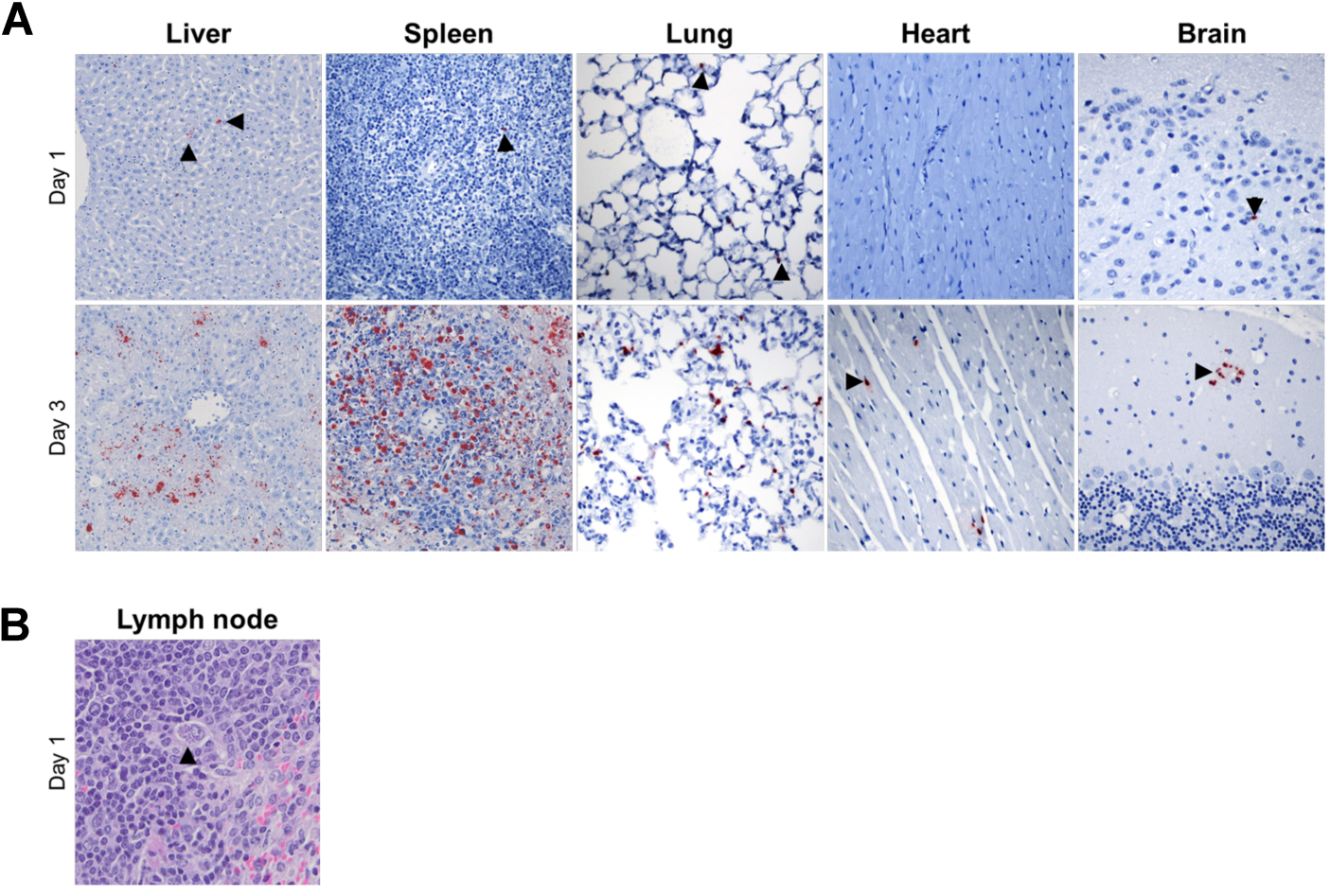
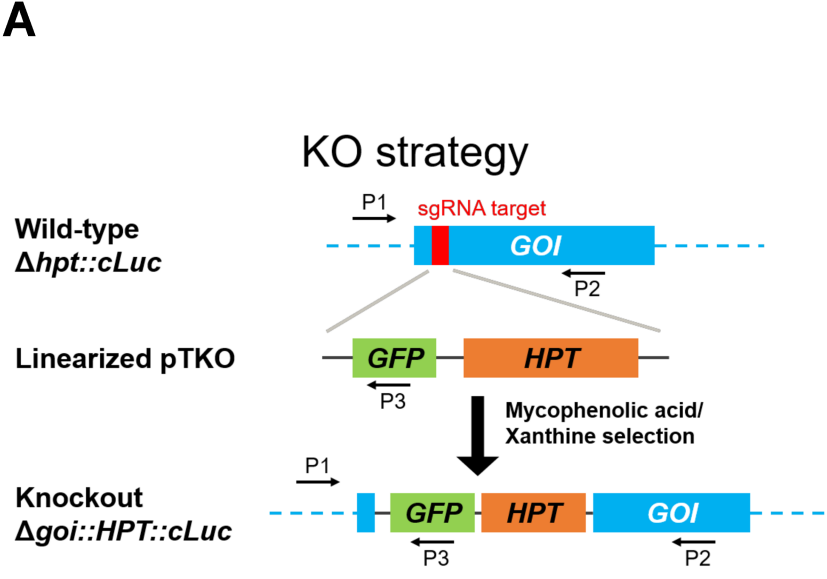


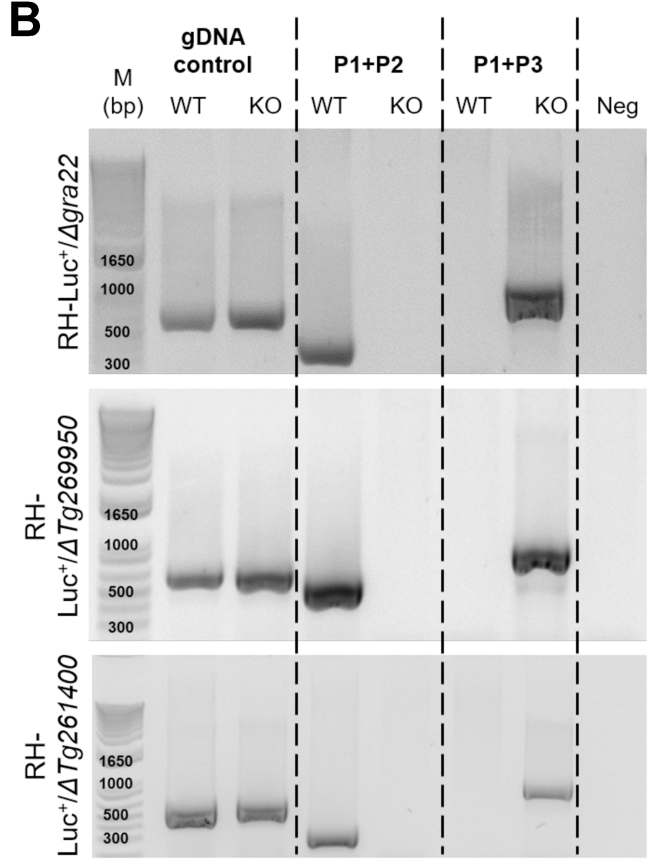
Figure S1

Figure S1. Related to Figure 1

(A) Day 1: Within the liver, scattered single parasites are present within the sinusoids (arrowheads). A similar and random number are found within the cortical sinus of the spleen (arrowhead) and the alveolar capillaries (lung). On day 1 no parasites were visible in the atrial or ventricular sections of the heart, and only a single parasite was present within a brain capillary (arrowhead). **Day 3:** Multiple parasites, often clustered, are present within sinusoids disrupting the hepatic parenchyma and within alveolar macrophages. Hepatocytes adjacent to the parasites and in regions remote from infection are shrunken and mineralized. The follicles of the spleen contain abundant intra and extracellular parasites. Immunoreactivity in the lung is within the alveolar walls and lumen. Within the left ventricle scattered myocytes, usually, near parasites, are disrupted and contain karyorrhectic debris and minerals. The parasites within the molecular layer of the cerebellum are within the neuropil, and there is vacuolation of the white matter in the region of clustered parasites. (**B)** The cytoplasm of a macrophage trafficking within the cortical sinus of a mesenteric lymph node is distended with numerous tachyzoites that peripheralize the nucleus.



	P1+P2	P1+P3	P2+P3
Wild-type	+	-	-
Knockout with forward insertion	-	+	-
Knockout with reverse insertion	-	-	+



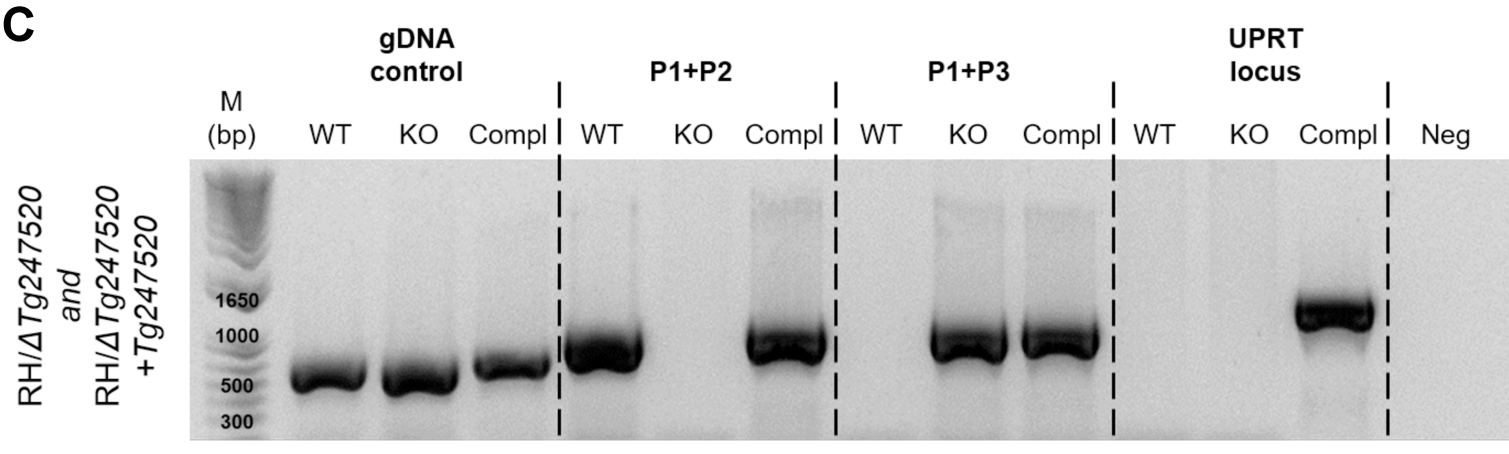
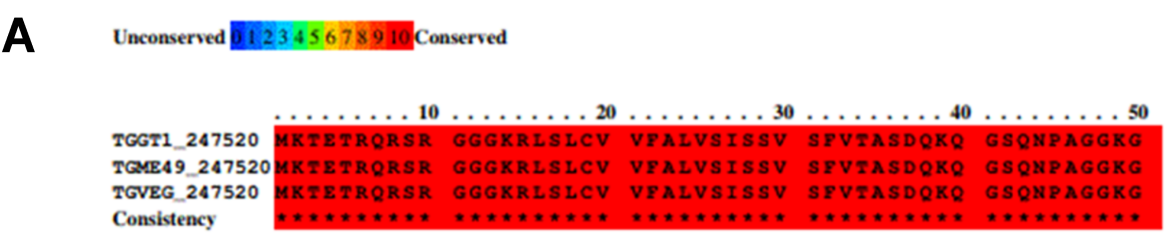


Figure S2

Figure S2. Confirmation of parasite knockouts, Related to Figure 2

PCR on genomic DNA from knockout parasites and respective wild-type parasites. **(A)** Schema representing the strategy used to generate individual knockout strains. **(B) Top panel** *gra22* knockout in RH-Luc+/*Δhpt* parasites; **middle panel** *Tg269950* knockout in RH-Luc+/*Δhpt* parasites and **bottom panel** *Tg261400* knockout in RH-Luc+/*Δhpt* parasites. **(C)** *Tg247520* knockout in RH*Δhpt* parasites and complementation of the gene in the *UPRT* locus. From the left: genomic DNA quality control with a primer flanking 500 bp of a non-related gene *Tg309160*. P1+P2 amplification with primers that flanked the cutting site of the sgRNA in the gene locus. P1+P3 amplification of GFP present in the repair template and the 5' UTR of the gene of interest. *UPRT* locus amplification in the *UPRT* locus and the 5' UTR of *Tg247520* (See **Table S6** for the primer sequences).


A
Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved
WIRS motif
SH3 motif
SH3 motif
TGGT1_247520
TGME49_247520
TGVEG_247520
Consistency

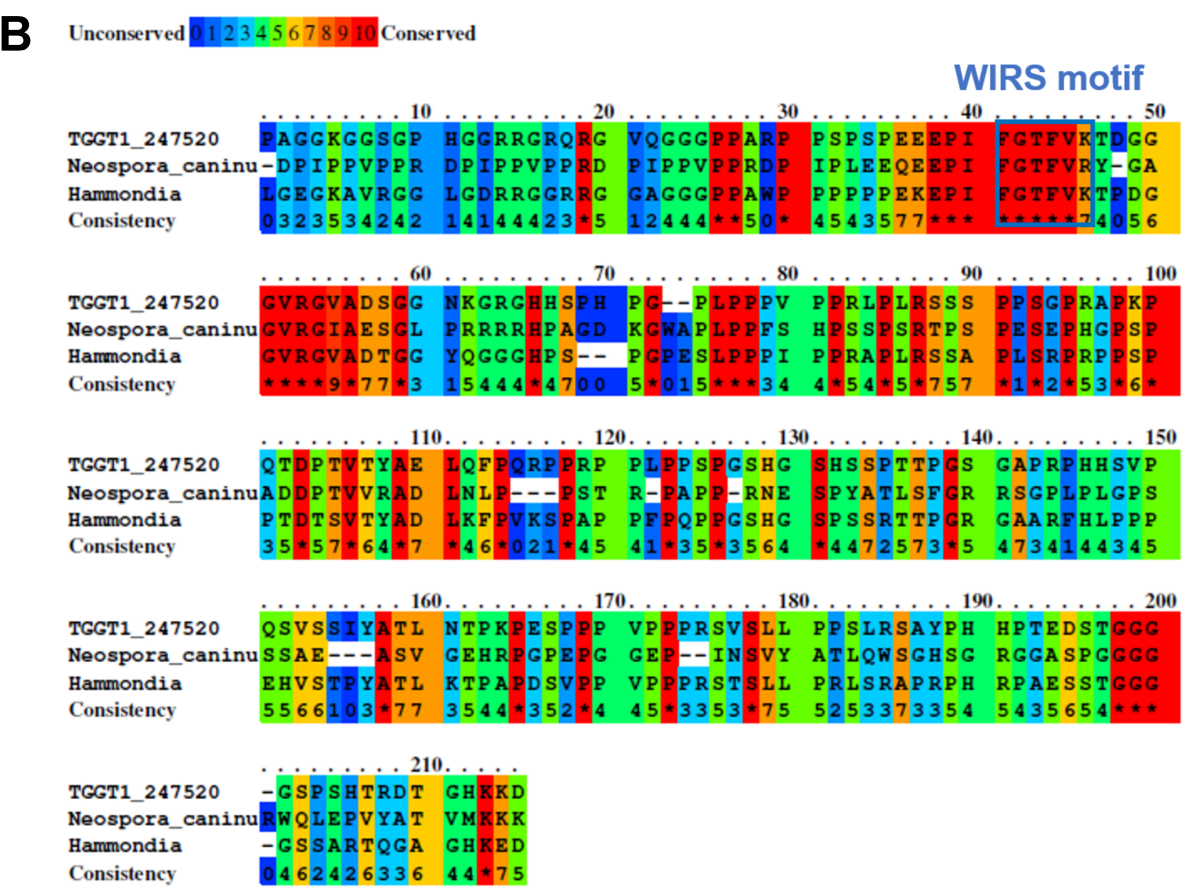

B
Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved
WIRS motif
TGGT1_247520
Neospora_caninu
Hammondia
Consistency

Figure S3

Figure S3. *Tg*WIP protein sequence alignment shows conservation between *Toxoplasma* strains and in the apicomplexan phylum, Related to Figure 3

(A) *Tg*WIP protein sequence alignment between type I (TGGT1_247520), type II (TGME49_247520) and type III (TGVEG_247520) strains using the online software PRALINE multiple sequence alignment. Regions with high conservation are presented in red color (*) and regions with low conservation are presented in blue (0). The WIRS motif in the blue box was identified manually using data in (Chen *et al.*, 2014). SH3 motifs in the green box were identified by using the online software *MoDPepInt* (Modular Domain Peptide Interaction). **(B)** *Tg*WIP protein sequence alignment between *Toxoplasma gondii* type I (TGGT1_247520), *Neospora caninum* (BN1204_065230) and homolog in *Hammondia hammondi*, using the online software PRALINE multiple sequence alignment. The WIRS motif is represented in the blue box.

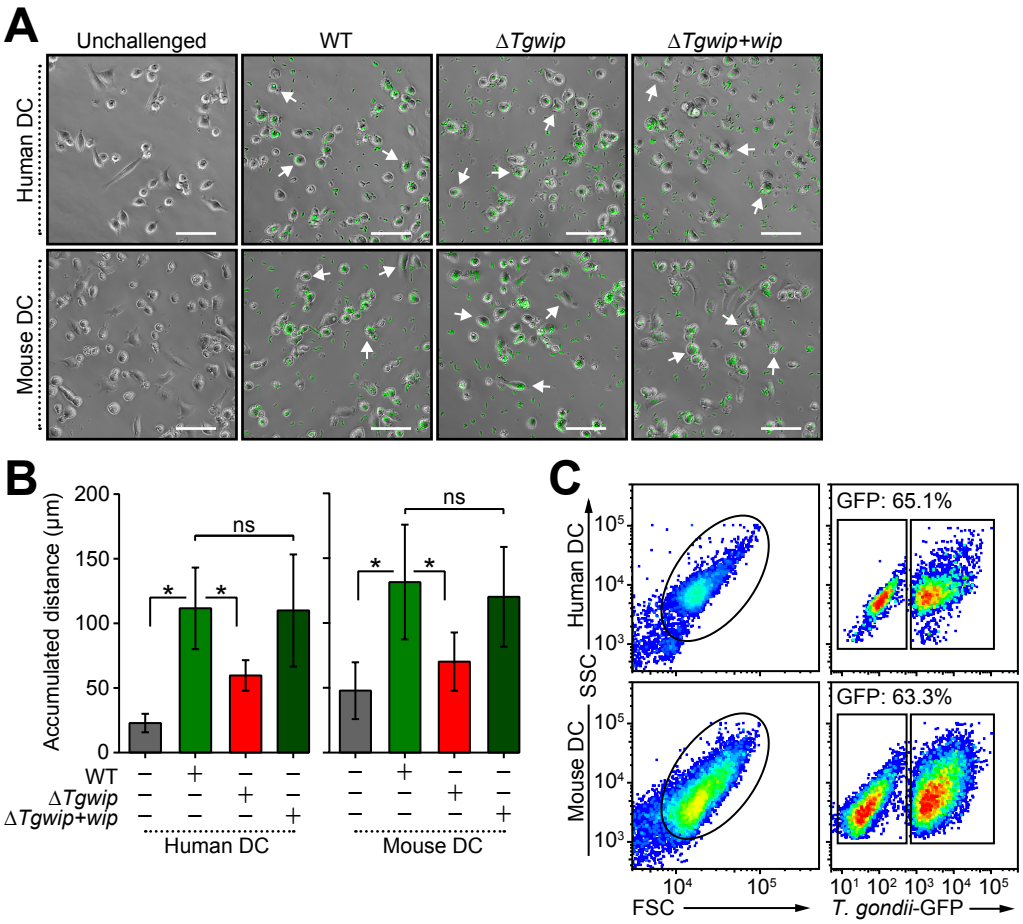

A
Unchallenged
WT
ΔTgwip
ΔTgwip+wip
Human DC
Mouse DC
B
Accumulated distance (μm)
200
150
100
50
0
ns
ns
*
*
*
*
WT
ΔTgwip
ΔTgwip+wip
Human DC
Mouse DC
C
Human DC
Mouse DC
SSC
GFP: 65.1%
GFP: 63.3%
FSC
T. gondii-GFP

Figure S4

Figure S4. Infections of DCs with *Toxoplasma* lines and determination of infection frequencies, Related to Figure 4 and 5

(A) Representative micrographs of unchallenged or *Toxoplasma*-challenged primary human and murine DCs. DCs were infected with freshly egressed GFP^+ wild type (WT), *ΔTgwip* or *ΔTgwip+Tgwip* complemented parasites. Arrowheads indicate DCs infected by GFP^+ parasites assessed in the assay. Scale bars: 100 µm. Micrographs are representative of 3 independent experiments. **(B)** Accumulated migrated distance of unchallenged or *Toxoplasma*-infected primary human and murine DCs, performed as in **Figure 4A**. Bar graphs show the average accumulated distance (± SD) from 3 independent experiments (*n= 3*). **(C)** The infection rate was determined by flow cytometry as detailed in materials and methods. Representative dot plots of gates used in flow cytometry analysis to define cells (FSC/ SSC), noninfected cells (GFP^-) and *Toxoplasma*-infected cells (GFP^+). The percentage (%) of GFP^+ cells is presented. Data are representative of 3 independent experiments. Asterisks (*) indicate significant difference, ns: non-significant difference: repeated measures one-way ANOVA, Tukey's post-hoc test (B).

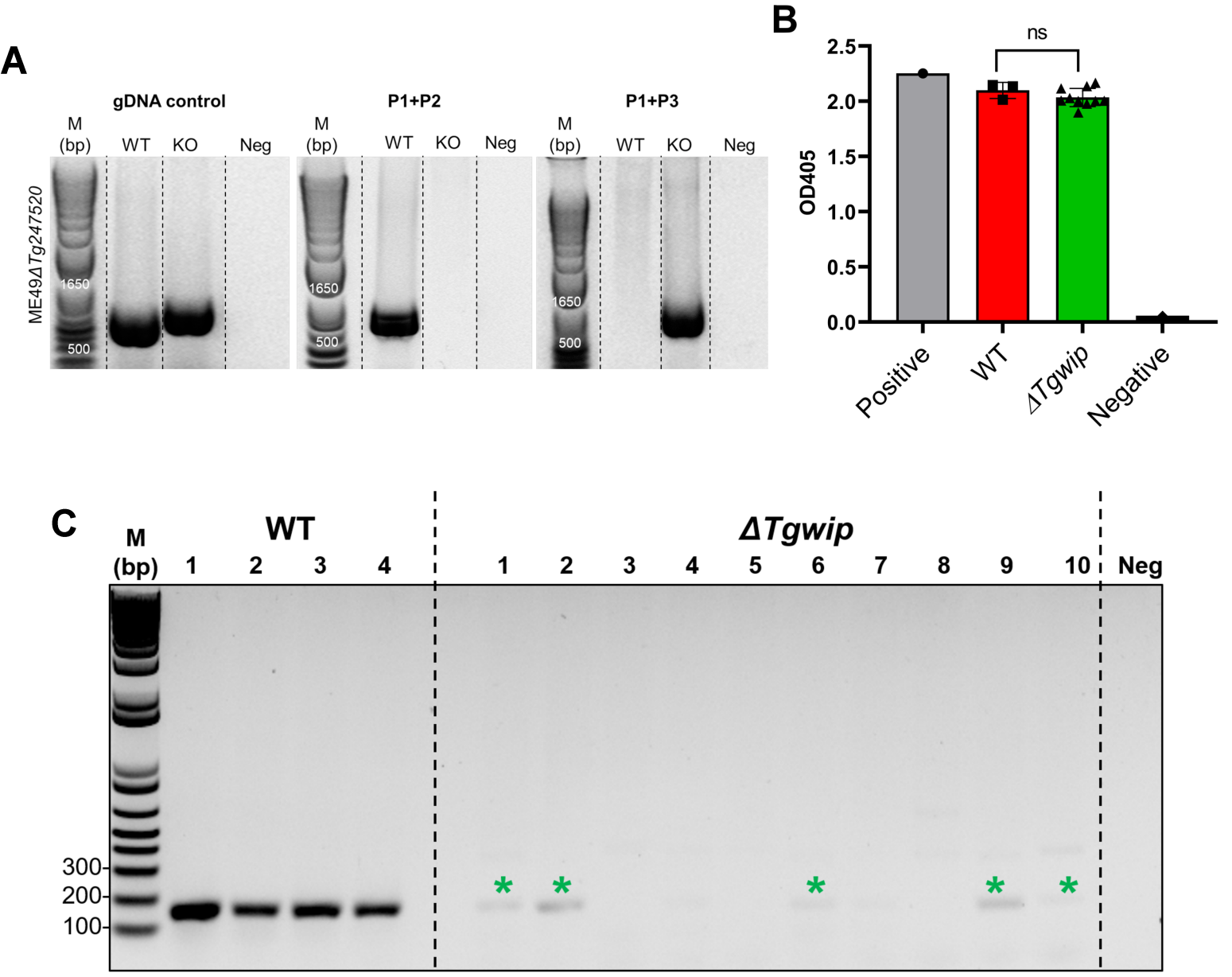

A
gDNA control
P1+P2
P1+P3
M (bp)
WT
KO
Neg
ME49ΔTg247520
1650
500
B
ns
OD405
2.5
2.0
1.5
1.0
0.5
0.0
Positive
WT
ΔTgwip
Negative
C
WT
ΔTgwip
M (bp)
1
2
3
4
5
6
7
8
9
10
Neg
300
200
100

Figure S5

Figure S5. Related to Figure 6

(A) PCR on genomic DNA from *Tgwip* knockout parasites and respective ME49 wild-type parasites. From the left: genomic DNA quality control with a primer flanking 500 bp of a non-related gene *Tg309160*. P1+P2 amplification with primers that flanked the cutting site of the sgRNA in the gene locus. P1+P3 amplification of GFP present in the repair template and the 5' UTR of the gene of interest (See **Table S6** for the primer sequences). Dotted line indicates where lanes from different part of the gel were spliced together. (**B**) The serum of the mice in **Figure 6A** (3. from the mice infected with wild type ME49 and 10 from mice infected with ME49*ΔTgwip* parasites) was obtained 27 days p.i. The anti-*Toxoplasma* IgG titers were quantified by ELISA. The graph represents the OD_{405} reading at least 2-fold higher than the mean background in the uninfected mouse control. Results are presented as average OD_{405} (± SD) obtained from infected mice. **ns** (no significant difference), non-parametric unpaired t-test. (**C**) The presence of *Toxoplasma* genomic DNA in the brain of infected mice in **Figure 6A** (4 from the mice infected with wild-type ME49 and 10 from mice infected with ME49*ΔTgwip*) was detected by diagnostic PCR targeting the multicopy *B1* gene. The green Asterisks (*) indicates the presence of positive bands in the mice infected with ME49*ΔTgwip*).