To Per-Cyst or Not: Unravelling the Secrets Behind an Attenuated *Toxoplasma* Strain

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Toxoplasmosis is a zoonotic parasitic disease caused by the obligate intracellular protozoa *Toxoplasma gondii*, which threatens a range of warm-blooded mammals including humans, livestock and zoo animals. To date, the only commercial vaccine to treat toxoplasmosis is Toxovax, comprising an 'incomplete' tachyzoite S48 strain. The molecular and cellular basis of attenuation of the strain remains a mystery.

The aim of this project is to characterise the S48 strain, which will give a deeper understanding of apicomplexan biology and *Toxoplasma* persistence. It could uncover previously unidentified genes essential for parasite persistence and has the potential to identify new therapeutic targets. Furthermore, from a biotechnology and synthetic biology standpoint, understanding the genetic basis of *T. gondii* attenuation can inform the development of *Toxoplasma* as a gene delivery vehicle, in both humans and animals.

We show S48 has an incomplete differentiation phenotype *in vitro*, where it is not able to fully differentiate from the fast-growing tachyzoites to the slow growing, persistent bradyzoite form. In order to identify the genomic cause of this differentiation defect we assembled a high-quality genome of the S48 strain using Oxford Nanopore long reads and DNBseq short reads. GC content was used to separate genomic DNA from the mitochondrial and apicoplast genomes, giving rise to 13 complete chromosomes.

SNP calling identified nine possible loss of gene function mutations unique to S48 relative to five other normally differentiating Toxoplasma strains. Based on a range of parameters, we selected two genes with unknown function to focus on. Currently, we are using CRISPR-Cas9 to characterise these genes by both tagging and deleting the gene in the cyst forming Type II ME49 strain. Initial characterisation will focus on determining the effect of gene knockouts on parasite differentiation, including cyst formation and bradyzoite gene expression.

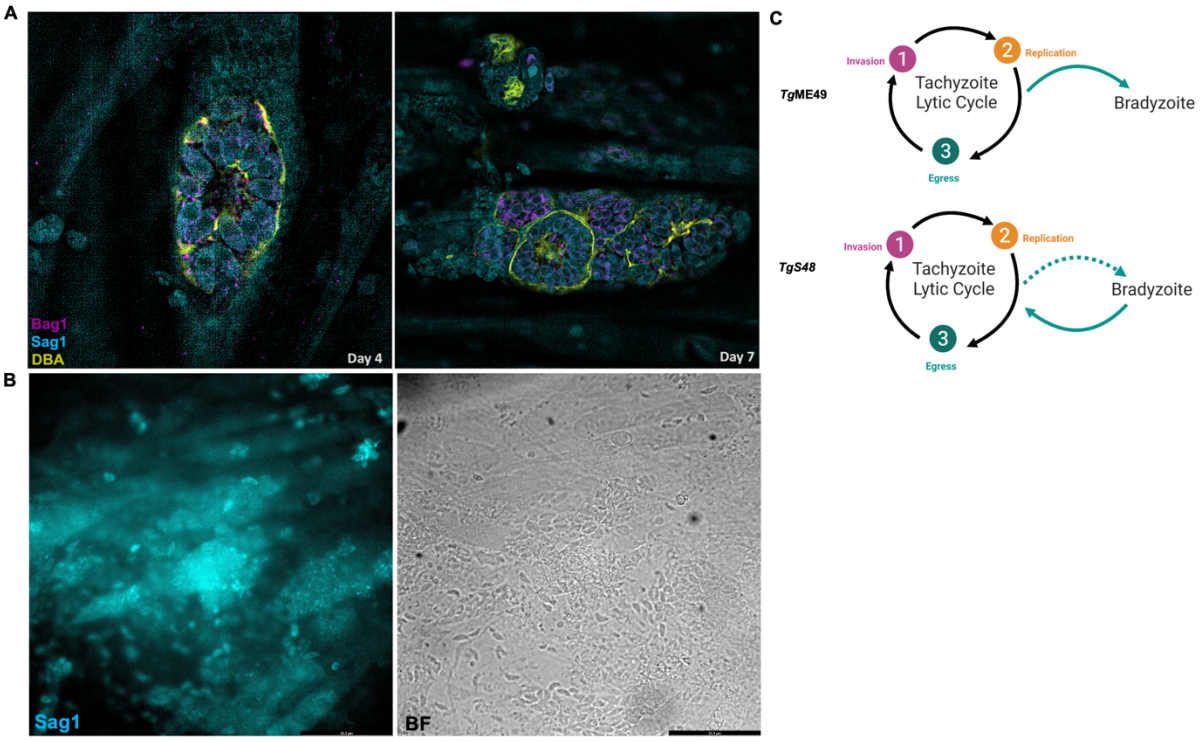

Figure 1. S48 is not able to fully differentiate into bradyzoite cysts. A. Super resolution microscopy shows S48 parasites expresses both bag1 and sag 1 markers at day 4 and day 7 **B.** A high number of extracellular parasites were observed at day 7 in S48 **C.** Hypothesis figure graphically displaying *Tg*S48 is locked in the lytic cycle whereas ME49 displays normal differentiation behaviour where tachyzoites convert to bradyzoites and do not reactivate under alkaline stress conditions.