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Supplemental information

Toxoplasma gondii SAG1 targeting host cell S100A6 for parasite invasion and host immunity

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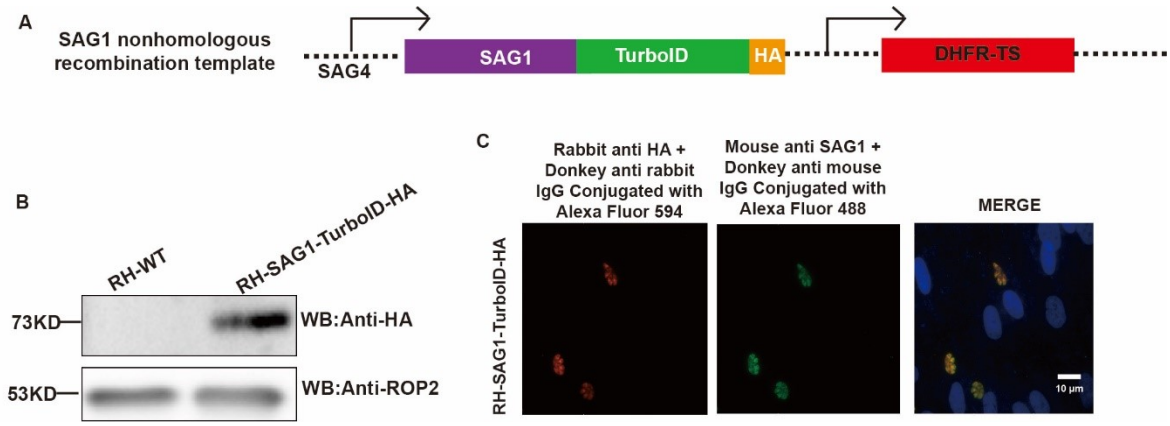

Figure S1. Construction and Identification of RH-SAG1-TurboID-HA strain, Related to Figure 1 (A) Diagram of the nonhomologous recombination template: a reading frame composed of the SAG4 promoter followed by SAG1-TurboID-HA coding sequence, and the coding sequence of dihydrofolate reductase-thymidylate synthase conferring pyrimethamine resistance. (B) WB detection of the expression of SAG1-TurboID-HA with the whole-cell lysates of RH-WT and RH-SAG1-TurboID-HA parasites. HA antibody was used to detect SAG1-TurboID-HA (73 KD), ROP2 (53 KD) was used as a loading control. (C) Detection of the localization and colocalization of SAG1 and SAG1-TurboID-HA with IFA. The nuclei were visualized with Alexa Fluor-405, SAG1 was visualized with Alexa Fluor-488, and HA-tag was visualized with Alexa Fluor-594 (scale bars, 10μm).

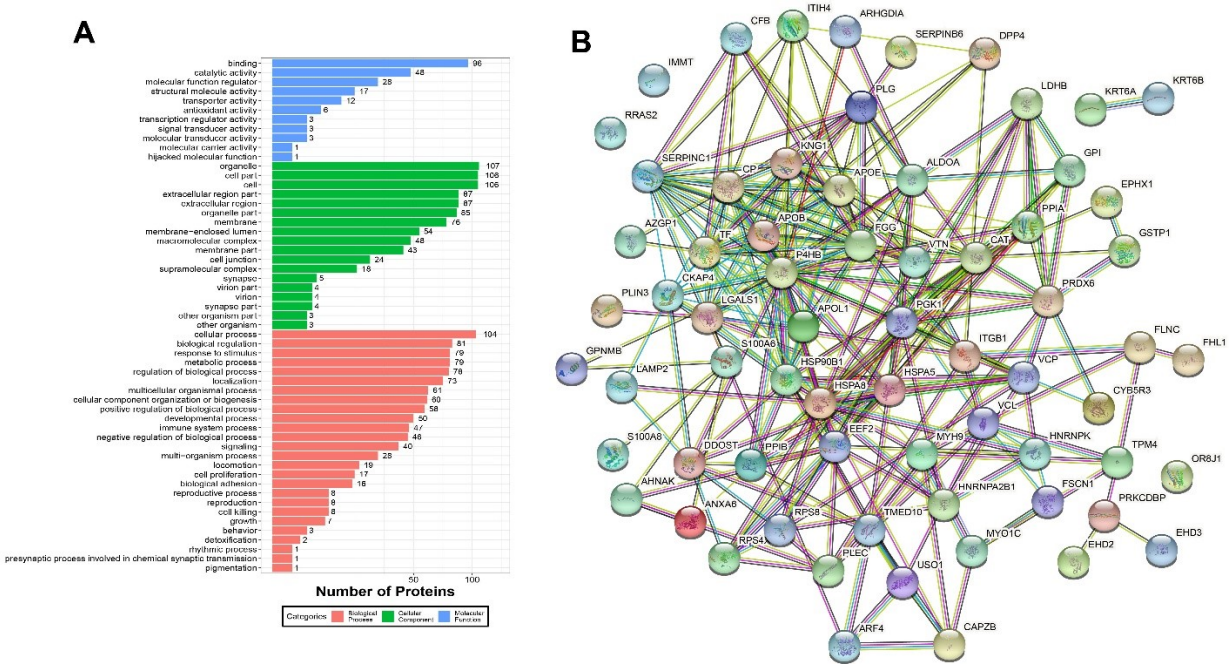

Figure S2. Gene Ontology (GO) and Protein-Protein Interaction (PPI) analysis for the *Tg*SAG1 interactome, Related to Figure 2 (A) GO analysis showed the 119 *Tg*SAG1 interaction proteins were enriched into 3 GO categories: biological processes, cellular components, and molecular functions. The cellular process, organelles, and binding are the mostly enriched groups for these three categories, respectively. (B) PPI analysis for the 76 *Tg*SAG1 candidate interactive membrane proteins using STRING 11.0 (http://string-db.org/). Criteria for interactions are shown by colored lines as follows: red line, gene fusion events; green line, gene neighborhood; blue line, gene cooccurrence; purple line, experimental evidence; yellow line, text mining; light blue line, database; black line, co-expression.

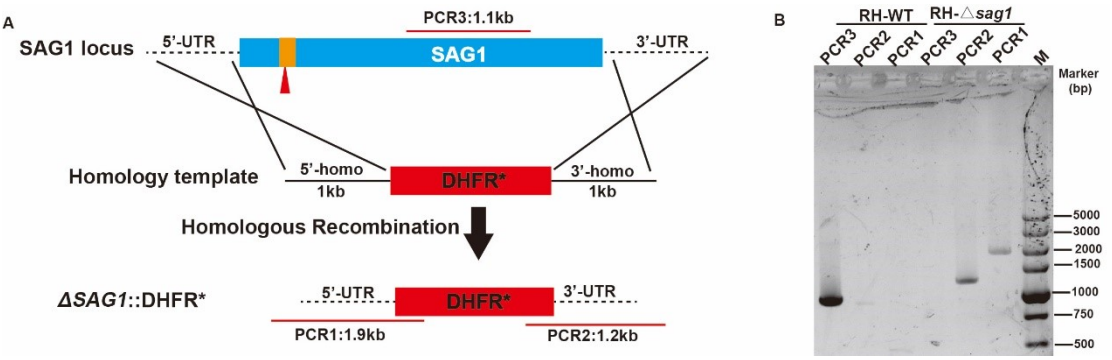

Figure S3. CRISPR/Cas9-mediated gene disruption of the SAG1 locus, Related to Figure 6, Figure 7 and Figure 8 (A) Schematic of the CRISPR/Cas9 strategy used to inactivate *sag1* by insertion of pyrimethamine-resistant DHFR (DHFR*). (B) Verification PCR demonstrating the homologous integration of DHFR and gene disruption of *sag1* in a representative clone, compared with its parental RH tachyzoites.

Table S1. Gene name and primers used in qRT-PCR analysis or RNAi transfection. Related Figure 7

Gene	Primer name*	Primer sequence (5' to 3')
Vimentin	Vimentin-h-F	5'-CACTGAGTACCGGAGACAGG-3'
	Vimentin-h-R	5'-GAAGGTGACGAGCCATTTCC-3'
S100A6	S100A6-h-F	5'-AAGGCTGATGGAAGACTTGG-3'
	S100A6-h-R	5'-CCTTGAGGGCTTCATTGTAGAT-3'
IL-12	IL12-h-F	5'-GATGTACCAGGTGGAGTTCAAG
	IL12-h-R	5'-GCCTGCATCAGCTCATCAATA-3'
TNF-α	TNFα-h-F	5'-CCAGGGACCTCTCTCTAATCA-3'
	TNFα-h-R	5'-TCAGCTTGAGGGTTTGCTAC-3'
IFN-γ	IFNγ-h-F	5'-GTGAAGACCTCTGTACCAAGA-3'
	IFNγ-h-R	5'-CATTGAGAGCTGGCTCCTTTA-3'
GAPDH	GAPDH-h-F	5'-GTCAACGGATTTGGTCGTATTG-3'
	GAPDH-h-R	5'-TGTAGTTGAGGTCAATGAAGGG-3'
TNF-α	TNFα-m-F	5'-TTGTCTACTCCCAGGTTCTCT-3'
	TNFα-m-R	5'-GAGGTTGACTTTCTCCTGGTATG-3'
Actin	Actin-m-F	5'-GCCTTCCTTCTTGGGTATGGAA-3'
	Actin-m-R	5'-CAGCTCAGTAACAGTCCGCC-3'
S100A6	S100A6 siRNA 1	5'-GCAGGATGCTGAAATTGCA-3'
	S100A6 siRNA 2	5'-TGGCCATCTTCCACAAGTA-3'
	S100A6 siRNA 3	5'-GGCTGATGGAAGACTTGGA-3'
Vimentin	Vimentin siRNA 1	5'-CAGACAGGATGTTGACAATGCGTCT-3'
	Vimentin siRNA 2	5'-TTTGCGTTCAAGGTCAAGACGTGCC-3'
	Vimentin siRNA 3	5'-TTGATAACCTGTCCATCTCTAGTTT-3'

*Forward (F) and reverse (R) primers. h: Human, m: Mouse