

File S1

CAT GFP expression cassette

Cassette, bordered by LoxP sites, to express the fusion protein CAT-GFP driven by the promoter of the *T gondii* α -tubulin gene (α TUB) and the 3' untranslated region of the *T. gondii* SAG1 gene (3'UTR SAG1)

Color code :

HindIII LoxP PromotorTUB CAT AvrII GFP 3'UTR SAG1 loxP XbaI

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Expression cassette

Cassette to express a protein of interest driven by a modified promoter of the *T gondii* α -tubulin gene (α TUB8) and the 3' untranslated region of the *T. gondii* SAG1 gene (3'UTR SAG1)

Length: 1018 bp

5 repeat motifs underlined (Soldati et al, 1995)

Promotor α TUB8: XbaI/PmeI (509 bp)

3'UTR SAG1 : NotI/AvrII (345 bp)

Cloning sites : PmeI (510), NotI (532)

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XbaI PmeI NotI AvrII

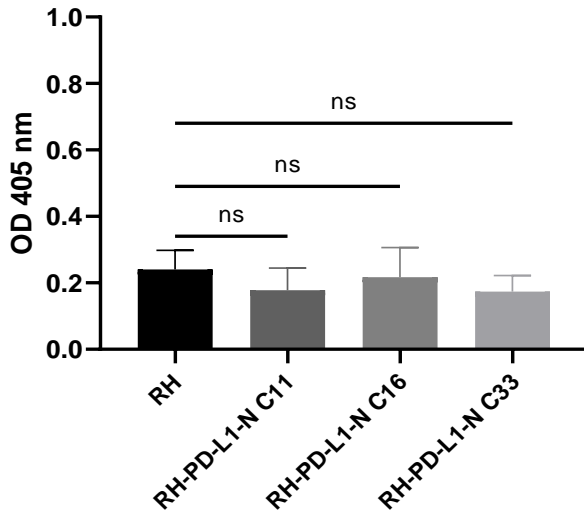
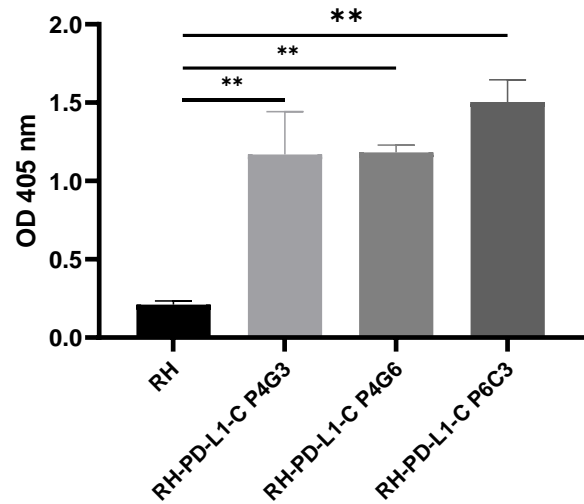
A**Binding of PD-L1 protein to RH-PD-L1-N Clones****B****Binding of PD-L1 protein to RH-PD-L1-C Clones**

Figure S1. Binding of RH-PD-L1-N and RH-PD-L1-C clones to PD-L1 protein. Tachyzoites of the selected clones of RH-PD-L1-N (**A**) and RH-PD-L1-C (**B**) recombinant strains were incubated with histidine-tagged PD-L1 recombinant protein and bound protein was assessed by ELISA using anti-His tag antibody (n= 3 replicates). Optical densities were read at 405 nm. Statistical significance is indicated by **p < 0.01. ns: not significant. All experiments were performed at least three times.

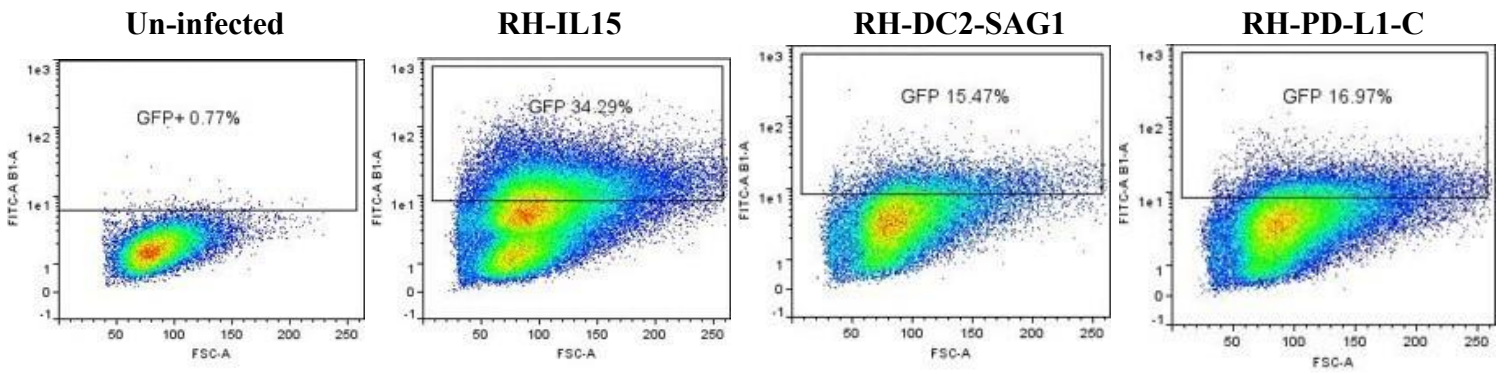
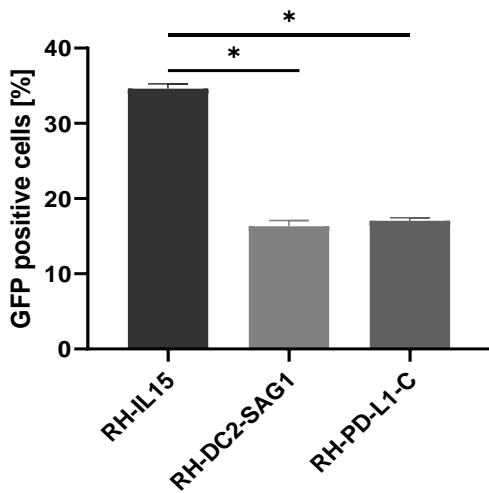
A**B**

Figure S2. Replication of RH-PD-L1-C and RH-DC2-SAG1 recombinant tachyzoites in non-targeted cells. The replication of RH-PD-L1-C and RH-DC2-SAG1 tachyzoites in HFF cells was compared to the recombinant RH strain engineered to secrete IL-15 (as a surface-non-modified, GFP positive strain). HFF cells were infected with each of the indicated strain at MOI 1, then, incubated for 1 h at 37 °C to allow for invasion. Following incubation, cells were washed to remove extracellular tachyzoites and incubated for 24 h. Invasion was analyzed by flow cytometry. **(A)** Representative flow cytometry dot plots showing the infected cells indicated by the GFP positive cells. **(B)** Quantification of invasion in 3 replicates. Statistical significance is indicated by *p < 0.05. Experiments were performed three times.