Figure S1, related to Figure 2

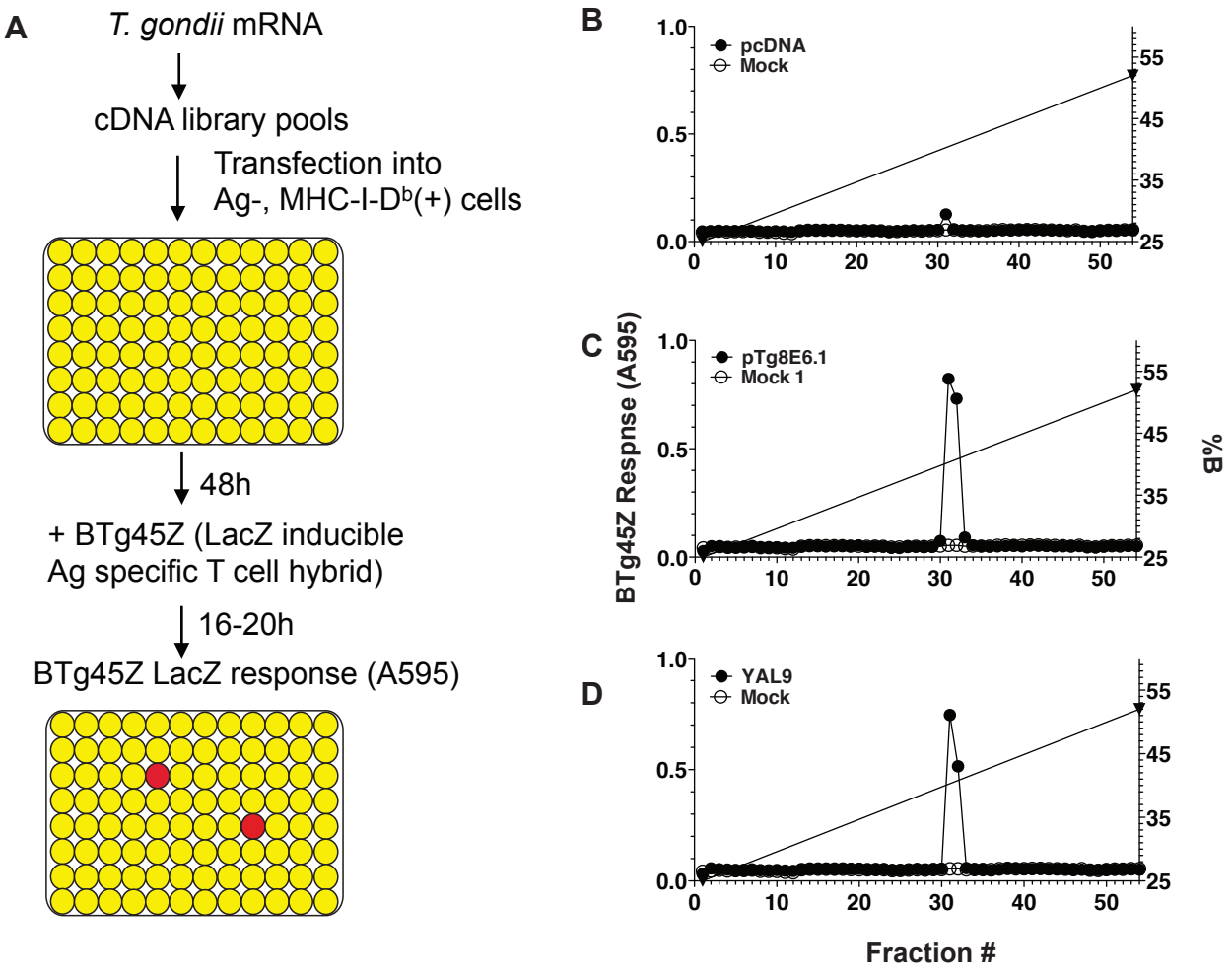


Figure S1: The strategy used for identifying the antigen recognized by BTg45Z

A) Schematic of experimental strategy. The approach was similar to that described previously (Karttunen et al., 1992). In brief, a *T. gondii* cDNA expression library was prepared in a eukaryotic expression vector. DNA was extracted from recombinant E.coli expressing the different *T. gondii* cDNAs, and then transfected into APCs expressing H-2D^b MHC I molecule. To probe the library, *T. gondii* specific CD8 hybrid, BTg45Z were incubated with the APCs 48h post-transfection, and positive pools were identified using a colormetric assay.

B - D) HPLC analysis of T cell-stimulatory peptide. Cellular extracts from COS cells transfected with B) pcDNA vector alone or C) cDNA clone pTg8E6.1 were generated and fractionated through HPLC. D) As a control, cellular extracts were spiked with synthetic 9-mer peptide, YAL9, alone was also fractionated. Lyophilized peptides fractions were pulsed onto H-2D^b expressing APCs and incubated with BTg45Z hybrid. Mock represents response to HPLC buffer alone.

Figure S2, related to Figure 3

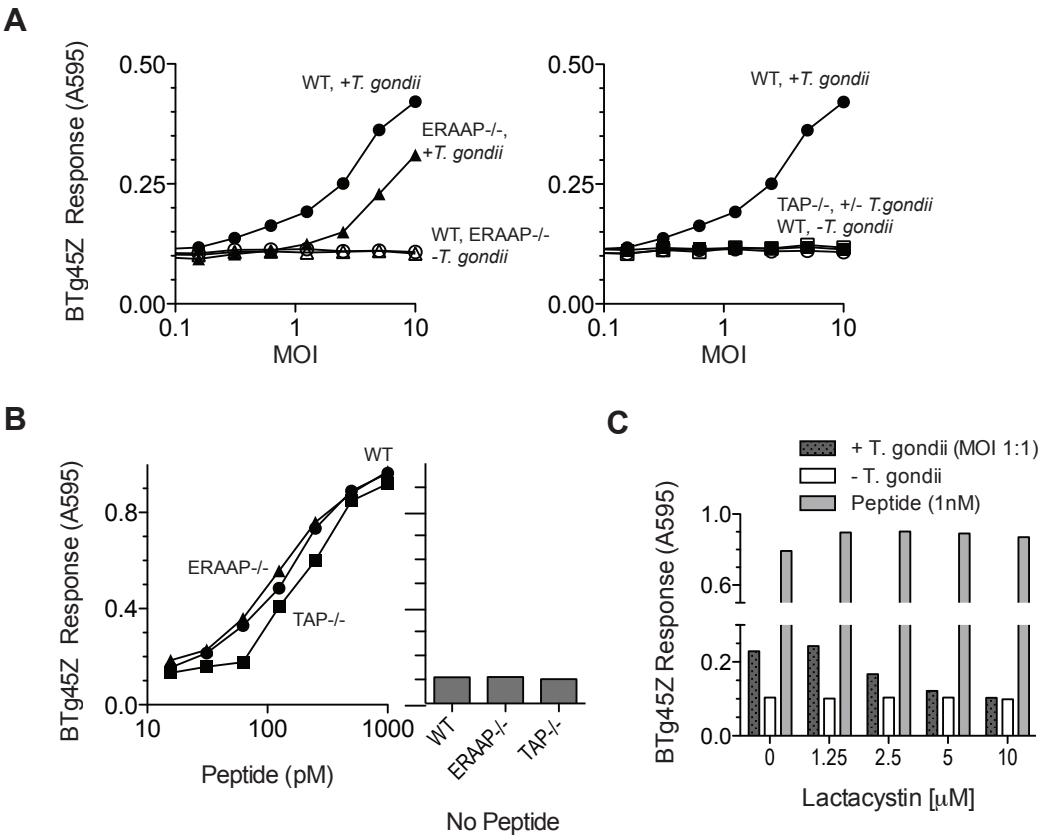


Figure S2: Presentation of YAL9 requires proteasome and TAP, and is partially dependent on ERAAP.

BMDCs from C57BL/6, ERAAP-/- or TAP-/- were A, C) infected in-vitro with irradiated *T. gondii*, ME49 strain or B, C) pulsed with YAL9 antigenic peptide. BTg45Z lacZ response after an overnight stimulation against wild-type, ERAAP-/- or TAP-/- A) +/- *T. gondii* or B) BMDCs that were pulsed with varying concentrations of YAL9 peptide. C) BTg45Z lacZ response against wild-type BMDCs that were treated with lactacystin and then +/- T. gondii or pulsed with the antigenic peptide, YAL9.

Figure S3, related to Figure 4

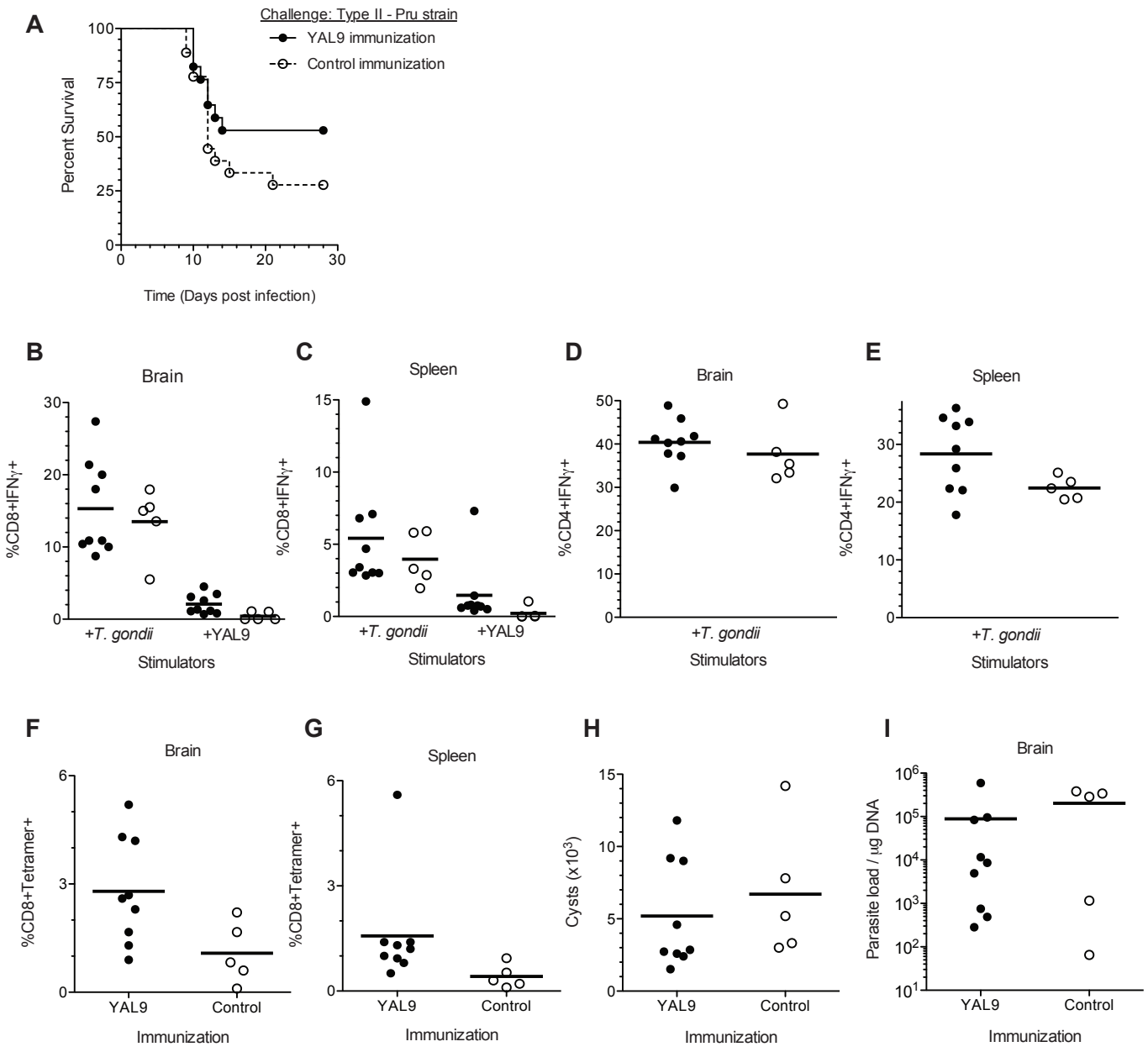
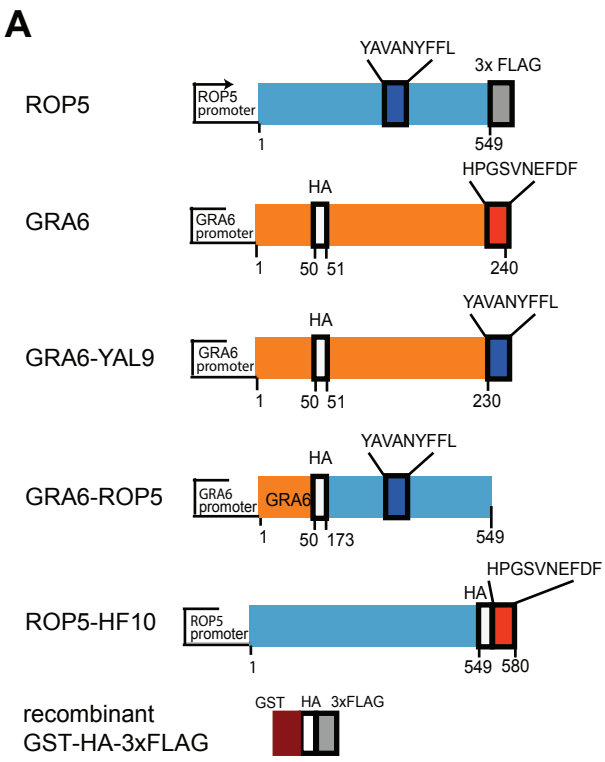
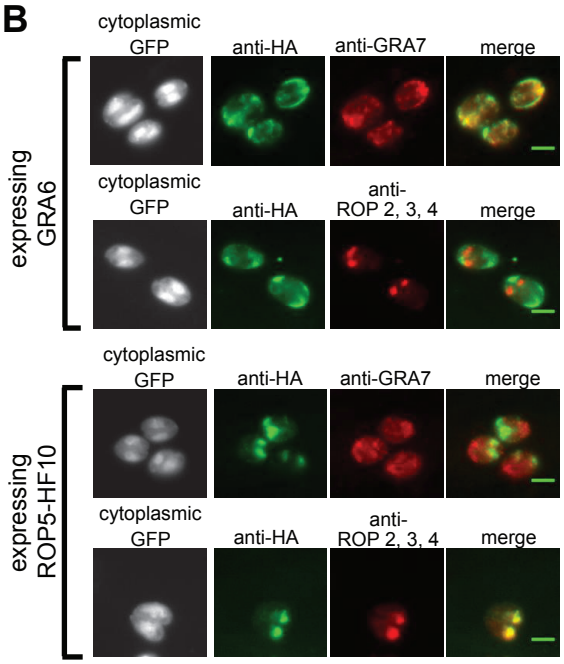


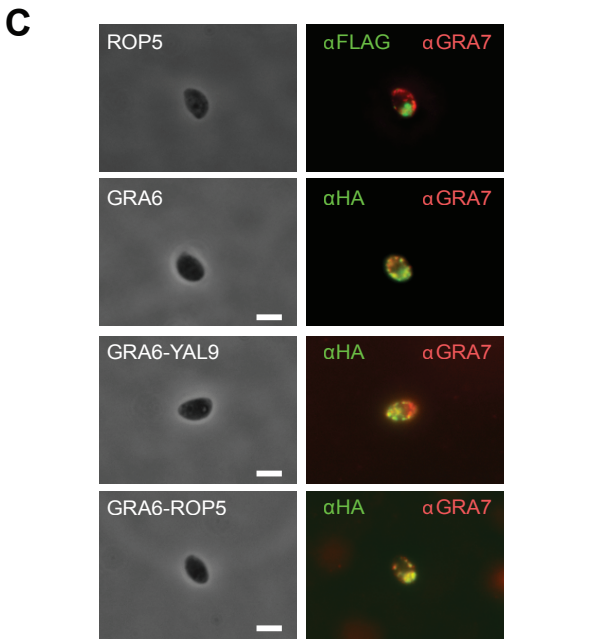
Figure S3: Immunization with YAL9 does not protect C57BL/6 mice from lethal challenge with T. gondii.

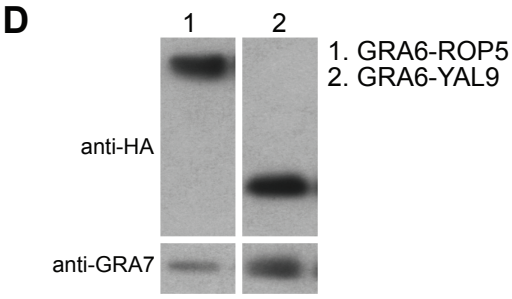
C57BL/6 mice were immunized with LPS activated BMDCs pulsed with YAL9 or controlpeptide, WI9. 7-21 days post immunization, these mice were infected with $1x10^4$ live type II, Pru strain, *T. gondii* tachyzoites, intraperitoneally. A) Kaplan-Meier survival curves between the two groups. B-I) Analysis of the T cell responses and parasite loads from surviving mice. IFN-γ response by B, C) CD8 T cells, D, E) CD4 T cells, as measured by ICCS for IFN-γ using flow cytometry after ex-vivo restimulation with *T. gondii*-infected APCs or peptide-pulsed APCs in B, D) brain C, E) spleen. Data are background corrected based on the values from uninfected APCs or APCs pulsed with irrelevant peptide. F, G) MHC class I H-2D^b –YAL9 tetramer staining on F) brain leukocytes G) splenocytes. Cells were also co-stained with CD8 antibody. H) Number of cysts in the brain as measured by staining a portion of the brain with fluorescent lectin to detect the cysts. I) The parasite load in the brain measured using semi-quantitative PCR on genomic DNA extracted from the tissue. Data is representative of at least three experiments with at least 4 mice per condition in each experiment.

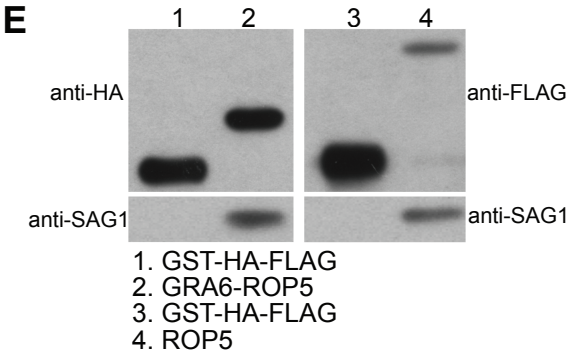
Figure S4, related to Figure 5











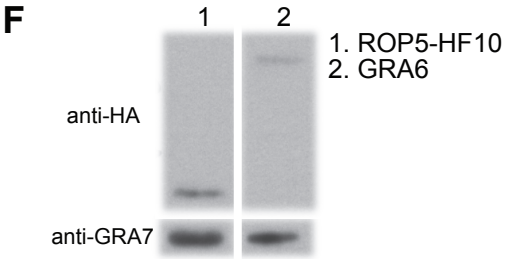


Figure S4: Characterization of transgenic parasites.

A) Schematic of constructs designed to express T cell stimulatory epitopes in different secretory contexts, as described in text and in Figure 5 legend. B) Immunofluorescence staining confirming the localization of protein in transgenic parasites expressing the GRA6 epitope containing constructs. Parasites were stained with antibodies to the HA epitope tags to reveal the transgenic proteins, and co-stained with antibody to GRA7 to reveal dense granules, or antibody to ROP2,3,4 to reveal rhoptries. Cytosol of parasites is labeled with GFP. Scale bars represent 5μm. C) Immunofluorescence staining confirming the localization of protein in transgenic parasites expressing the ROP5 epitope containing constructs. Parasites were stained with antibodies to either the HA or FLAG epitope tags to reveal the transgenic proteins, and co-stained with antibody to GRA7 to reveal dense granules. Scale bars represent 5μm. D) Western blot comparing the levels of precursor proteins in CTG parasites expressing the ROP5 epitope. E) Western blot comparing the levels of the HA-tagged GRA6-ROP5 and the FLAG-tagged ROP5. The left-upper panel is probed with an anti-HA antibody and the right-upper panel is probed with an anti-FLAG antibody. In both panels an equal amount of recombinant GST-HA-FLAG protein was run to allow comparison of the HA- and FLAG- tagged proteins. Both panels are probed with anti-SAG1 antibody as a loading control. F) Western blot comparing the transgenic protein levels of CTG parasites expressing the GRA6 epitope containing constructs.

Figure S5, related to Figure 5

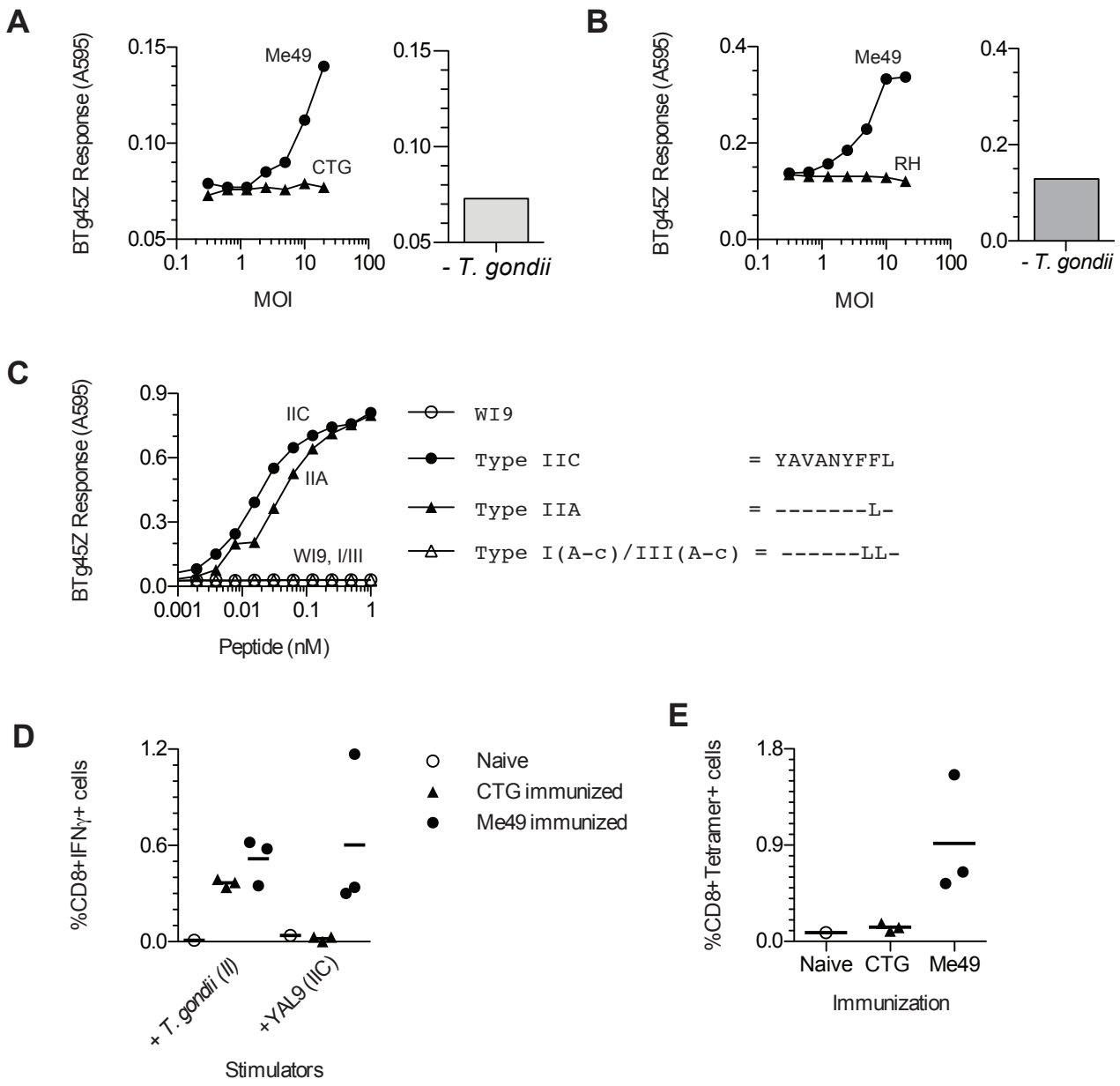


Figure S5: BTg45Z hybridoma does not recognize the polymorphic versions of the 9-mer epitope, YAL9, from different strains of T. gondii.

A, B) BTg45Z lacZ response after an overnight stimulation with BMDCs infected with irradiated type I (RH), type II (ME49) or type III (CTG) strain of *T. gondii*. Background signal with uninfected BMDC is indicated in the right panel. C) BTg45Z lacZ response after an overnight stimulation with BMDCs pulsed with varying concentrations of polymorphic YAL9 peptides and irrelevant WI9 peptide. D, E) Mice were immunized with irradiated parasites from type II (ME49) and type III (CTG) strain. Splenocytes were harvested from mice 2 wks post immunization and *T. gondii* or YAL9-specific CD8 T cell responses were measured by intracellular cytokine staining for IFN-γ or by staining with tetramers. D) Compiled data showing CD8 T cell responses from immunized or naïve mice towards *T. gondii* infected or YAL9 (type IIC) peptide pulsed APCs. Each dot represents an individual mouse. Data are corrected for background based on CD8 T cell responses towards uninfected or irrelevant peptide pulsed APCs. E) Compiled data showing MHC class I H-2D^{b}-YAL9 tetramer staining on splenocytes from immunized or naïve animals. Each dot represents an individual mouse. Cells were also co-stained with CD8 antibody. Data are representative of three independent experiments.

Figure S6, related to Figure 6

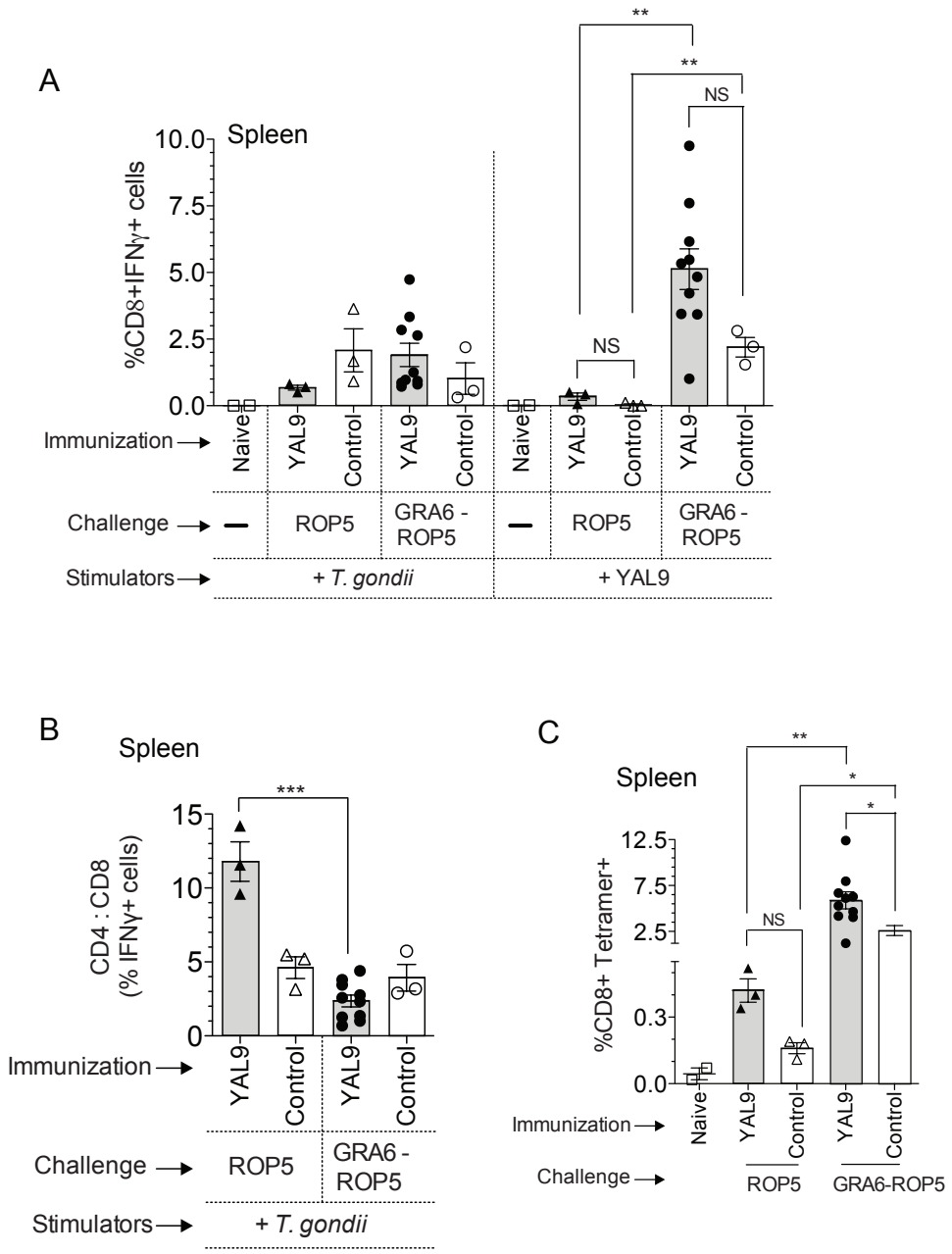


Figure S6: Immunization with the YAL9 peptide protects C57BL/6 mice from lethal challenge with transgenic T. gondii that express ROP5IIC as a dense granule protein .

A-C) C57BL/6 mice were immunized with LPS activated BMDCs pulsed with YAL9 or control peptide, WI9 followed by a booster dose 2 wks post primary immunization. 3 wks post boost immunization, these mice were infected with $5x10^5$ live transgenic *T. gondii* tachyzoites (either CTG+ROP5 or CTG+GRA6-ROP5), intraperitoneally. Analysis of the T cell responses from surviving mice.
A) IFN-γ response by CD8 T cells B) ratio of *T. gondii* specific CD4 to CD8 T cells in the spleen as measured by ICCS for IFN-γ using flow cytometry after ex-vivo restimulation with *T. gondii*-infected APCs or peptide-pulsed APCs. Data are background corrected based on the values from uninfected APCs or APC pulsed with irrelevant peptide. C) MHC class I H-$2D^b$ –YAL9 tetramer staining on splenocytes. Cells were also co-stained with CD8 antibody. Data is pooled from two representative experiments with at least 5 mice per condition in each experiment. (*=p<0.05, **=p<0.01, ***=p<0.01, ****=p<0.0001, NS = not significant)

Supplementary References

Karttunen, J., Sanderson, S., and Shastri, N. (1992). Detection of rare antigen-presenting cells by the lacZ T-cell activation assay suggests an expression cloning strategy for T-cell antigens. Proc Natl Acad Sci U S A 89, 6020-6024.