

**Supplementary Table 7.** Examples of heterologous prime-boost immunization against *T. gondii* in mouse models

Antigen/Adjuvant	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
rROP2-SAG1/FCA pcROP2-SAG1 pcROP2-SAG1 boost rROP2-SAG1/FIA	s.c i.m	BALB/c	NR	↑ IgG antibody (especially in mice immunized with rROP2-SAG1 compared with pcROP2-SAG1 and pcROP2-SAG1+rROP2-SAG1 groups after 70 days of the first immunization, p<0.05) Predominance of IgG1 over IgG2a (significant for mice immunized with rROP2-SAG1 and pcROP2-SAG1+rROP2-SAG1 groups, p<0.05) More vigorous specific lymphoproliferative responses in mice of group rROP2-SAG1 ↑ IFN-γ in groups rROP2-SAG1, or pcROP2-SAG1, or pcROP2-SAG1 boosted with rROP2-SAG1 (non-significant between these groups)	NR	NR	The results indicate that fusion proteins ROP2-SAG1 exhibit immunogenicity by three immunization procedures, using a recombinant protein vaccine, or DNA vaccine, or DNA boosted with protein. Immune effects based on the recombinant protein are stronger than that of the DNA vaccine.	[56]
ROP18 Encoding MAS and UMAS ROP18 <sub>297-386</sub> , SAG3 <sub>101-144</sub> , MIC6 <sub>288-347</sub> , GRA7 <sub>182-224</sub> , MAG1 <sub>158-125</sub> , BAG1 <sub>158-211</sub> , and SPA <sub>42-200</sub> DNA vaccine or/and adenovirus vaccine Prime/bopst. DNA/DNA (p-UMAS/p-UMAS) Ad/Ad (Ad-UMAS/Ad-UMAS) DNA/Ad (p-UMAS/Ad-UMAS) Ad/DNA (Ad-UMAS/p-UMAS)	The combination of DNA vaccine (p-UMAS, 100 µg each) and recombinant adenovirus vaccine (Ad-UMAS virus, 3 × 10 <sup>8</sup> PFU each), i.m	BALB/c	Acute: 1 × 10 <sup>3</sup> tachyzoites, RH strain (genotype I), i.p Chronic: 20 cysts PRU strain (genotype II), i.g via oral gavage	Highest levels of humoral antibodies and cellular immune responses were achieved in mice immunization priming with the DNA vaccine and boosting with the Ad-UMAS vaccine Compared with p-UMAS or Ad-UMAS immunization alone, higher levels of a specific IgG (predominance of IgG2a) and higher levels of cytokines (IFN-γ and IL-2) were obtained by priming with p-UMAS and boosting with Ad-UMAS (p<0.05) Priming with p-UMAS and boosting with Ad-UMAS demonstrated higher proliferation activity, compared with the other immunization strategy (p<0.05)	Reduced (p<0.01) The most significant reduction of brain cyst burden was observed by the DNA prime-Ad boost approach.	Increased survival rate 67% Survival in mice vaccinated with p-UMAS prime and Ad-UMAS boost 28 days after challenge Control mice were died within 8-10 days	Priming vaccination with DNA vaccine and boosting with the recombinant Ad vaccine encoding ubiquitin conjugated multi-stage antigens of <i>T. gondii</i> was proved to be a potential strategy against the infection of type I and type II parasite.	[54]

↑, increase; Ad-UMAS, adenovirus expressing ubiquitin-conjugated multistage antigen segments; Ad, adenovirus; Ag, antigen; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; GRA, dense granule antigens; i.g, intragastrically; i.m, intramuscular; i.p, intraperitoneally; IFN-γ, interferon-γ; IL, interleukin; MAS, multi-stage antigen segments; MIC, microneme proteins; NR, not reported; ROP, rhoptry protein or rhoptry antigens; s.c, subcutaneous; SAG, surface antigens; *T. gondii*, *Toxoplasma gondii*; UMAS, ubiquitin-conjugated multistage antigen segments.