Supplementary Table 2. Baseline characteristics of included studies based on immunization experiments with *T. gondii* DNA-encoding ROPs in mouse models (mixed antigens)

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP1+SAG1	Liposome	Plasmid, i.m	BALB/c	NR	Induced a strong IgG antibody response ($p<0.05$) ↑ IL-2 (163 ± 25 pg/mL) and IFN-γ (1,950 ± 110 pg/mL) significantly	NR	NR	Immunization with a liposome-encapsulated DNA construct encoding the *T. gondii* SAG1-ROP1 can induce humoral and cell-mediated immune responses.	[24]
ROP1+GRA7	pIL-12	Plasmid, i.m	BALB/c	6 Mice were challenged with a lethal dose of Me49 strains (1,500 cysts per mouse) 5 Mice per group were challenged with a nonlethal dose of strain Me49 (20 cysts per mouse), orally	↑ IgG titers ($p<0.01$) The predominance of the levels of IgG2a over IgG1 ↑ IL-10, IFN-γ, and TNF-α ($p<0.05$) ↑ splenocyte proliferation ($p< 0.05$)	Reduced ($p<0.01$)	Increased survival rate ($p<0.01$) pROP1-GRA7: 33.3% survival rate 4 weeks after infection pROP1-GRA7+pIL-12: 50% survival rate 4 weeks after infection All control mice were died within 18 days.	The study suggest that the multiantigenic DNA antigen pGRA7-ROP1 was very effective in stimulating host protective immune responses than separately injected single antigens, and that IL-12 serves as a good DNA adjuvant.	[3]
ROP2+SAG1	-	Plasmid, i.m	BALB/c	1×10^5 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response Th1-type response with the predominance of the levels of IgG2a over IgG1 ↑ Splenocyte proliferation significantly ↑ IFN-γ, compared with the controls ($p<0.05$) IL-4 was undetectable in splenocyte supernatants from all experimental and control animals	NR	Increased survival time ($p<0.01$)	The results demonstrated that DNA cocktail immunization might be an important approach to achieve a multi-component vaccine against *T. gondii*, particularly with respect to generating an efficient, long-lasting protective immune response.	[25]
	-	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) Predominance of the levels of IgG2a over IgG1 ↑ Splenocyte proliferation ↑ IFN-γ (687 ± 136 pg/mL, $p<0.05$) and IL-12 (456 ± 48 pg/mL, $p<0.05$)	NR	Increased survival time (12 days compared with 8 days in control)	The current study showed that multi-antigenic DNA produced potent, effective and long-term protection against *T. gondii* challenge.	[26]
	pIL-12 and Alum	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	↑ IgG antibodies ($p<0.05$) than control groups (especially in the group immunized with pcSAG1+pcROP2+alum) ↑ IFN-γ ($p<0.05$) ↓ IL-4 in the group immunized with pcROP2+pcSAG1 ($p<0.05$)	NR	Increased survival time (10 days in the group immunized with pcROP2+pcSAG1 compared with 5 days in control, $p<0.05$)	The results of the study showed that use of adjuvants (IL-12 and alum) coincident with DNA cocktail leads to significant change in the survival time of the experiment groups in comparison with control groups. Also, there is no significant difference between adjuvants to induce immune responses.	[27]

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Supplementary Table 2. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
	-	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ IFN-γ (902 ± 155.8 pg/mL, $p<0.05$) and IL-4 (52.0 ± 17 pg/mL, $p<0.05$) The predominance of IgG2a over IgG1	NR	Increased survival time (11 days compared with 6 days in control)	The cocktail DNA containing the recombinant plasmids can be an appropriate candidate for immunization against toxoplasmosis.	[28]
	pIL-12	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) The predominance of IgG2a over IgG1 IgG2a values in the pSAG1-ROP2 plus pIL-12 immunized group were significantly higher than the pSAG1-ROP2 immunized group ($p<0.001$). ↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ and IL-12 significantly ↓ IL-4 significantly	NR	Increased survival time compared to controls, but not complete protection (death within 11 to 16 days) The protection induced by pSAG1-ROP2 was markedly enhanced by pIL-12 co-administration (death within 16 to 22 days)	The study indicates that the introduction of multiantigenic DNA vaccine is more powerful and efficient than the single gene vaccine, and the co-delivery of pIL-12 further enhanced the potency of multiantigenic DNA vaccine.	[8]
	$pCTXA_2$/B and pIL-12	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	Higher levels of IgG antibodies in the sera of mice immunized with pSAG1-ROP2 combined with pIL-12 ($p<0.01$). The predominance of the levels of IgG2a over IgG1 in all three groups (especially in mice immunized with pcDNA3.1-SAG1-ROP2+pIL-12) ↑ Splenocyte proliferation (especially in mice immunized with pcDNA3.1-SAG1-ROP2+pIL-12, $p<0.001$) ↑ IFN-γ and IL-12 (especially in mice immunized with pcDNA3.1-SAG1-ROP2+pIL-12, $p<0.05$) ↓ IL-4 in the group (especially in mice immunized with pcDNA3.1-SAG1-ROP2+pIL-12, $p<0.05$)	NR	Increased survival time (pcDNA3.1-SAG1-ROP2+pIL-12, $p<0.05$)	The results show that the IL-12 is superior to $CTXA_2$/B as vaccine adjuvant of anti-*T. gondii* by i.m challenge. It may be potentially a better choice as a vaccine adjuvant, which provides a basis for further research on cytokine adjuvants and the relationship between the properties of adjuvants and the route of immunization.	[29]

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Supplementary Table 2. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP2+SAG1+SAG2	pIL-12	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	↑ Levels of IgG (especially in mice immunized with pROP2-SAG1-SAG2+pIL-12, $p<0.05$) The predominance of the levels of IgG2a over IgG1 in all groups (especially in mice immunized with pROP2-SAG1-SAG2+pIL-12) ↑ Splenocyte proliferation (pIL-12 augmented splenocytes proliferation over 4 fold more than the group immunized without the co-administration of pIL-12, $p<0.01$). ↑ IFN-γ and IL-12 (especially in mice immunized with pROP2-SAG1-SAG2+pIL-12) ↓ IL-4 in mice immunized with pROP2-SAG1-SAG2+pIL-12 ($p<0.05$)	NR	Increased survival time (pROP2-SAG1-SAG2 and pROP2-SAG1-SAG2+pIL-12: 25 days and 31-32 days respectively, compared with 8 days in control)	Multiantigenic DNA vaccine expressing SAG1, ROP2 and SAG2 is more potent than single gene vaccine and double gene vaccine. Simultaneous murine IL-12 expression plasmid vaccination could enhance the potency of a multiantigenic DNA vaccine. These results will contribute to the development of an efficient and long-term protective immunity against *T. gondii*.	[30]
ROP2+SAG1+GRA2	pIL-12	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) The predominance of the levels of IgG2a over IgG1 ↑ Splenocyte proliferation ($p<0.05$) IL-12 augmented splenocyte proliferation about 2.5 fold more than the group immunized with pSAG1–ROP2–GRA2 ($p<0.01$) ↑ IFN-γ and IL-12 significantly ↓ IL-4 significantly	NR	Increased survival time pROP2-SAG1-GRA2: death within 18 days pROP2-SAG1-GRA2+pIL-12: death within 23 days Control mice were died within 8 days.	The use of IL-12 encoding plasmid as an adjuvant, successfully enhanced the level of protection induced by the multiple antigens encoding plasmid alone, and would be a promising immunization protocol. Thus, this immunization regimen may represent an effective vaccine strategy for generating an efficient long-term protective immunity against *T. gondii* infection.	[26]
ROP2+GRA5	-	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ IFN-γ (892 ± 196 pg/mL, $p<0.05$) and IL-4 (68 ± 28.9 pg/mL, $p<0.05$) The predominance of IgG2a over IgG1	NR	Increased survival time (12 days compared with 6 days in control)	The cocktail DNA containing the recombinant plasmids can be an appropriate candidate for immunization against toxoplasmosis.	[28]
ROP2+GRA5+SAG1	-	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ IFN-γ ($1,278\pm136$ pg/mL, $p<0.05$) and IL-4 (120 ± 48 pg/mL, $p<0.05$) The predominance of IgG2a over IgG1	NR	Increased survival time (12 days compared with 6 days in control)	The cocktail DNA containing the recombinant plasmids can be an appropriate candidate for immunization against toxoplasmosis.	[28]

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Supplementary Table 2. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP2+GRA1+ GRA7	-	Plasmid, i.m	C3H/HeN ($H\text{-}2^k$)	Acute: 100 *T. gondii* 76K cysts, orally Chronic: a sublethal dose of 20 brain cysts of *T. gondii* 76K, orally	↑ Antibody titers against the antigens from two gene DNA vaccine cocktails, but lower titers when immunized with the three-gene cocktail. ↑ IFN-γ	Reduced in the three-gene cocktail DNA vaccinated group (81%, $p<0.01$) Reduced in the pGRA7+pROP2 vaccinated group (79%, $p<0.05$) Reduced in the pGRA1+pROP2 vaccinated group (57%, none-significant) Reduced in the pROP2 vaccinated group (43%, none-significant)	Increased survival time after lethal challenge experiment in mice vaccinated with the three-gene DNA vaccine cocktail (complete protection, $p<0.01$), whereas 56% of control vaccinated mice succumbed to acute toxoplasmosis with a median survival time of 12 days.	The presence of GRA7 in the DNA vaccine formulation was important for optimal protection and this was correlated with GRA7-specific IFN-γ production. We propose GRA7 as a main component in cocktail DNA vaccines for vaccination against *T. gondii*.	[31]
ROP5+ROP7	-	Plasmid, i.m	BALB/c	Acute: 1×10^4 tachyzoites, RH strain, i.p Chronic: 20 cysts PRU strain, i.g	Induced a strong IgG antibody response High level of Th1 type immune response (predominance of IgG2a over IgG1) ↑ IFN-γ (1,109.52±129.66 pg/mL, $p<0.05$) and IL-2 (511.59±70.14 pg/mL, $p<0.05$)	Reduced ($p<0.05$)	Mice vaccinated with pROP5/ROP7 showed a longer survival time than single-gene-immunized mice ($p<0.05$) or control mice ($p<0.05$)	The results suggest that the multiple-gene vaccine had the ability to partly protect mice against virulent and low-virulent *T. gondii* strains.	[11]
ROP5+GRA15	-	Plasmid, i.m	Kunming	Acute: 1×10^3 tachyzoites, RH strain type I), i.p Chronic: 10 tissue cysts PRU strain type II), orally	Induced a strong IgG antibody response ($p<0.05$) The predominance of IgG2a over IgG1 (IgG2a/IgG1 ratio: 1.72±0.03) ↑ Splenocyte proliferation (SI: 4.45±0.05, $p<0.05$) ↑ Levels of the Th1 cytokines IFN-γ, IL-2, IL-12p70, and IL-12p40 ($p<0.05$) ↑ Levels of IL-4 and IL-10 ($p<0.05$) ↑ Percentages of $CD4^+$ T and $CD8^+$ cells ($p<0.05$) Cell-mediated cytotoxic activity with increased frequencies of IFN- γ secreting $CD8^+$ T cells ($p<0.05$)	Reduction in brain tissue cysts load (79%, $p<0.05$)	Increased survival time (22.7±7.2 days, $p<0.05$) All control mice died within 9 days	Co-immunization of pVAX-ROP5 and pVAX-GRA15 boosted the immune responses and increased protective efficacy against *T. gondii* infection compared to single antigen vaccines.	[10]

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Supplementary Table 2. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP16+GRA7	pB7-2	Plasmid, i.m	Kunming	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.01$) The predominance of IgG2a over IgG1 (especially for the pROP16-GRA7 co-delivery with pB7-2 group) ↑ Percentage of $CD4^+$ and $CD8^+$ T cells ($p<0.05$) ↑ IFN-γ especially for the pROP16-GRA7 co-delivery with pB7-2 group ($p<0.05$)	NR	Increased survival time ($p<0.01$)	The formulation of pB7-2 with either a multiantigenic DNA vaccine (pROP16-GRA7) or a single-gene vaccine (pROP16 or pGRA7), all resulted in dramatically enhanced antibody titers, both Thl and $CD8^+$ T cell mediated immune responses, therefore, it might be a feasible method of boosting protective immunity induced by a recombinant DNA vaccine.	[16]
ROP18+MIC3	-	Plasmid, i.m	ICR	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) The predominance of IgG2a over IgG1 (IgG2a values in the pROP18–MIC3 immunized group were significantly higher than the single-gene immunized group ($p<0.05$) ↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ (849±86 pg/mL, $p<0.05$) and IL-4 (66±14 pg/mL, $p<0.05$)	NR	Increased survival time (19 days compared with 7 days in control, $p<0.05$)	Our study indicates that the introduction of multi-antigenic DNA vaccine is more powerful and efficient than single-gene vaccine. These results suggested that multiantigenic DNA immunization might be an important approach to achieve an effective vaccine against *T. gondii*.	[20]

↑, increase; ↓, decrease; Ag, antigen; $CTXA_2/B$, A_2/B subunits of cholera toxin; GRA, dense granule antigens; i.g, intragastrically; i.m, intramuscular; i.p, intraperitoneally; IFN-γ, interferon-γ; IL, interleukin; MIC, microneme antigens or microneme proteins; NR, not reported; ROP, rhoptry protein or rhoptry antigens; SAG, surface antigens; SI, stimulation index; *T. gondii*, *Toxoplasma gondii*; Th, T helper; TNF-α, tumor necrosis factor α.