Supplementary Table 1. Baseline characteristics of included studies based on immunization experiments with *T. gondii* DNA-encoding ROPs in mouse models (single antigens)

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP1	-	Plasmid, i.m	BALB/c	NR	Elicited cellular and humoral immune responses ↑ Proliferative activity of spleen T lymphocytes ($p<0.01$) ↑ Percentages of $CD8^+$ T cells ($p<0.05$) The numbers of $CD4^+$ T cells showed no obvious increase compared with the control group ↑ NK cell activity ($p<0.05$)	NR	NR	pcROP1 could elicit both cellular and humoral immune responses in vaccinated mice.	[1]
	pcIFN-γ	Plasmid, i.m	BALB/c	NR	↑ Levels of IgG antibody in sera of pcROP1 and pcROP1+pcIFN-γ groups, compared with the control group ($p<0.01$) ↑ NK cell activity, especially in pcROP1+pcIFN-γ group ($p<0.05$) ↑ proliferative activity of spleen T lymphocytes in mice immunized with pcROP1 and pcROP1+pcIFN-γ (especially in the latter ($p<0.01$) The levels of IFN-γ and NO in the pcROP1+pcIFN-γ group was significantly higher than that with pcROP1 alone ($p<0.05$) ↑ IL-2 in vaccinated mice with pcROP1+pcIFN-γ, compared with the controls ($p<0.05$) The levels of IL-2 in the pcROP1+pcIFN-γ group was significantly higher than that with pcROP1 alone ($p<0.05$)	NR	NR	The genetic adjuvant pcIFN-γ could enhance the cellular immune response induced by DNA vaccine of pcROP1 in mice against *T. gondii* infection.	[2]
	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 Predominance of the levels of IgG2a over IgG1 (especially in mice immunized with pROP1+pIL-12) ↑ IL-10, IFN-γ, and TNF-α ($p<0.05$) ↑ splenocyte proliferation ($p<0.05$)	Reduced ($p<0.01$)	Increased survival rate ($p<0.01$)	The study showed that a DNA vaccine expressing the *T. gondii* ROP1 Ag induced specific humoral and cellular immune responses and immunization with vaccine combined with pIL12 induces greater Th1-type immune responses and protective efficacy against *T. gondii* infection.	[3]
	Alum	Plasmid, i.m	BALB/c	5×10^5 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ IgG2a ($p<0.05$) ↑ IFN-γ (1,161.00±76.10 pg/mL and 433.00±51 pg/mL for pcROP1 and pcROP1+alum groups, respectively, $p<0.05$)	NR	Increased survival time (7 days compared with 6 days in control)	The study showed that ROP1 DNA vaccine can induce partial protective response against toxoplasmosis.	[4]
	-	Plasmid, i.m	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	Induced humoral immune response ↑ SI (2.04±0.12, $p<0.001$) ↑ IFN-γ (712±28.1 pg/mL, $p<0.001$) and IL-4 (94±14.5 pg/mL, $p<0.01$)	NR	Increased survival rate (50%, 23-day post challenge, $p<0.05$) All control mice died within 9 days.	These findings proposed that the ROP1 Ag is a potential candidate for the development of vaccine against toxoplasmosis. Complete protection may be achieved by combining ROP1 with other immunogenic rhoptry antigens.	[5]

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Supplementary Table 1. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP2	-	Plasmid, i.m	C57BL/6 (H-2^b), BALB/c (H-2^d) and CBA/J (H-2^k)	6×10^3 Tachyzoites, RH strain, s.c	↑ IgG in mice of all three strains (especially in BALB/c mice) ↑ IgG1 in mice of all three strains (especially in BALB/c and C57BL/6 mice) ↑ IgG2a (BALB/c: homogeneous response; CBA/J: heterogeneous response) ↑ IgG2c in C57BL/6 mice Induced mixed Th1/Th2 response	NR	All DNA-immunized mice of the three strains died after the challenge. Nevertheless, in BALB/c mice a slight delay (2 days) was observed, compared with control (p=0.04).	These results suggest that plasmid immunization using the ROP2 gene generates a mixed Th1/Th2 response against ROP2.	[6]
	-	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	↑ IgG antibodies ($p<0.05$) ↑ Splenocyte proliferation ↑ IFN-γ (335.00 ± 9.7982 pg/mL, $p<0.05$), IL-2 (200.82 ± 8.7593 pg/mL, $p<0.05$), and TNF-α (198.91 ± 9.2450 pg/mL, $p<0.05$)	NR	Increased survival time (7 days compared with 5 days in control)	-	[7]
	-	Plasmid, i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	↑ Ratio IgG2a to IgG1 ↑ IFN-γ (651 ± 120 pg/mL) and IL-12 (430 ± 36 pg/mL)	NR	Increased survival time (10 days compared with 7-8 days in control)	-	[8]
	-	Plasmid, i.m	C57BL/6 (H-2^b), BALB/c (H-2^d), and C3H (H-2^k)	Acute: C57BL/6: 10 cysts of strain IPB-G (a zymodeme II type strain) BALB/c: either 50 or 200 cysts of strain IPB-G, orally C3H: 50 cysts of strain IPB-G or 76K, orally Chronic: BALB/c and C3H: 25 cysts of strain IPB-G, orally C57BL/6: 10 cysts of strain 76K, i.p	Induced a strong antibody response ↑ Splenocyte proliferation in BALB/c ($p<0.05$) and C3H ($p<0.01$) mice ↑ IFN-γ in BALB/c ($p<0.01$) and C3H ($p<0.01$) mice	C3H: Reduced ($p<0.05$)	C3H (challenged with 50 cysts of strain IPB-G): 90% survival during 20 days ($p<0.001$) C3H (challenged with 50 cysts of strain 76K): 100% survival during 20 days ($p<0.02$) C57BL/6 (challenged with 10 cysts of strain IPB-G): 20% survival during 20 days BALB/c (challenged with 200 cysts of strain IPB-G): 20% survival during 20 days BALB/c (challenged with 50 cysts of strain IPB-G): 90% survival during 20 days	In this study, we show that DNA immunization with potentially protective *T. gondii* Ag induces both humoral and cellular immune responses in mice of three different genetic backgrounds. In addition, we show that in one mouse strain, DNA vaccination not only reduces the mortality associated with the acute phase of infection, but also limits the parasite load during the chronic phase of the disease.	[9]

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Supplementary Table 1. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP5	-	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 ($p<0.05$) Predominance of IgG2a over IgG1 (IgG2a/IgG1 ratio: 1.51 ± 0.03) ↑ Splenocyte proliferation (SI: 3.32 ± 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 (57.4%, $p<0.05$)	Increased survival time (19.4 ± 4.9 days, $p<0.05$) All control mice died within 9 days.	Results demonstrated that a potential DNA vaccine expressing the *T. gondii* ROP5 elicit Th1-biased responses, as well as $CD8^+$ cell-mediated cytotoxic T cell response, against acute or chronic *T. gondii* infection in mice.	[10]
	-	Plasmid, i.m	BALB/c	Acute: 1×10^4 tachyzoites, RH strain, i.p Chronic: 20 cysts PRU strain type, i.g	↑ IgG antibodies ($p<0.05$) The predominance of IgG2a over IgG1 ↑ IFN-γ (672.6 ± 43.17 pg/mL) and IL-2 (256.89 ± 11.81 pg/mL) ($p<0.05$)	Reduced ($p<0.05$)	Increased survival time ($p<0.05$)	Our results showed that a DNA vaccine encoding ROP5 significantly enhanced protection against *T. gondii* challenge.	[11]
ROP7	-	Plasmid, i.m	BALB/c	Acute: 1×10^4 tachyzoites, RH strain, i.p Chronic: 20 cysts PRU strain, i.g	↑ IgG antibodies ($p<0.05$) The predominance of IgG2a over IgG1 ↑ IFN-γ (662.76 ± 42.42 pg/mL) and IL-2 (264.42 ± 18.31 pg/mL) ($p<0.05$)	Reduced ($p<0.05$)	Increased survival time ($p<0.05$)	Our results showed that a DNA vaccine encoding ROP7 significantly enhanced protection against *T. gondii* challenge.	[11]
ROP8	-	Plasmid, i.m	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ production (816 ± 26.3 pg/mL, $p<0.05$) and IL-4 (148 ± 18.3 pg/mL, $p<0.05$)	NR	Increased survival rate (50%, 29- day post challenge, $p<0.05$) Control mice died within 9 days.	Results presented in this study suggest that ROP8 DNA is a promising and otential vaccine candidate against toxoplasmosis.	[12]
ROP9	-	Plasmid, i.m	Kunming	1×10^3 Tachyzoites, RH strain, i.p	↑ IgG antibodies ($p<0.05$) High ratio (1.69) of IgG2a to IgG1 demonstrating that immunization of pVAX-ROP9 primarily induced a Th1 type response. ↑ Splenocyte proliferation ($p<0.05$) ↑ Percentages of $CD4^+$ and $CD8^+$ T cells ($p<0.05$) ↑ IFN-γ (466.62 ± 12.72 pg/mL, $p<0.05$), IL-2 (305.88 ± 15.02 pg/mL, $p<0.05$), IL-4 (231.72 ± 9.89 pg/mL, $p<0.05$), and IL-10 (212.00 ± 16.23 pg/mL, $p<0.05$)	NR	Prolonged survival time in mice (12.9 ± 2.9 days; $p<0.05$) Control mice were died within 6 days.	The results of the present study showed that immunization with a TgROP9 plasmid induced strong humoral and cellular Th1-type immune responses, and prolonged survival time against lethal challenge. Although TgROP9 elicited only partial protection against acute toxoplasmosis, it could be used as a potential vaccine candidate in further studies of multi-component. *T. gondii* vaccines against toxoplasmosis in the mice model.	[13]

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Supplementary Table 1. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP13	pIL-18	Plasmid, i.m	Kunming	Acute: 1×10^3 tachyzoites, RH strain (type I), i.p Chronic: 10 cysts PRU strain (type II), orally	↑ Level of IgG antibodies in the sera of mice immunized with pVAX-ROP13 and pVAX-ROP13 plus pVAX-IL-18 ($p<0.05$) ↑ Lymphocyte response compared with the control ($p<0.05$) ↑ IFN-γ in mice immunized with pVAX-ROP13 plus pVAX-IL-18 (1,107.28±26.74 pg/mL, $p<0.05$) and pVAX-ROP13 (826.75±18.91 pg/mL, $p<0.05$) ↑ IL-2 in mice immunized with pVAX-ROP13 plus pVAX-IL-18 (934.52±13.03 pg/ml, $p<0.05$) and pVAX-ROP13 (793.07±22.09 pg/mL, $p<0.05$) ↑ IL-4 in mice immunized with pVAX-ROP13 plus pVAX-IL-18 (257.54±4.17 pg/mL, $p<0.05$) and pVAX-ROP13 (163.23±6.05 pg/mL, $p<0.05$) ↑ IL-10 in mice immunized with pVAX-ROP13 plus pVAX-IL-18 (246.02±10.61 pg/mL, $p<0.05$) and pVAX-ROP13 (160.49±3.14 pg/mL, $p<0.05$)	pVAX-ROP13 plus pVAX-IL-18: Reduction in brain tissue cyst load (66.03%, $p<0.05$) pVAX-ROP13: Reduction in brain tissue cysts load (39.82%, $p<0.05$)	Increased survival time pVAX-ROP13: 24.9±2.3 days, $p<0.05$ pVAX-ROP13 plus pVAX-IL-18: 32.3±2.7 days, $p<0.05$ Control mice were died within 10 days.	The results suggest that ROP13 DNA vaccine induced strong protective humoral and cellular responses against *T. gondii*, indicating that it has the potential to be a vaccine candidate worthy of further development. The use of an IL-18-encoding plasmid as an adjuvant successfully enhanced the immune protection and survival time of immunized mice. Further studies are warranted to evaluate the immune efficacy of this DNA vaccine construct in other animal host species against toxoplasmosis.	[14]
ROP16	-	Plasmid, i.m	Kunming	1×10^3 Tachyzoites, RH strain, i.p	↑ Specific anti-ROP16 IgG ($p<0.05$) ↑ Th1 immune responses ↑ Splenocyte proliferation (~8-fold higher than control, $p<0.05$) ↑ IFN-γ (918±12.77 pg/mL, $p<0.05$), IL-2 (887.33±24.94 pg/mL, $p<0.05$), IL-4 (172.67±7.51 pg/mL, $p<0.05$), and IL-10 (168±19.52 pg/mL, $p<0.05$)	NR	Increased survival time (21.6±9.9 days), compared with that of control mice, which died within 7 days after challenge ($p<0.05$).	*T. gondii* ROP16 should provide a promising vaccine candidate against toxoplasmosis, worth further evaluation and development using other animal species.	[15]
	pB7-2	Plasmid, i.m	Kunming	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) The predominance of IgG2a over IgG1 ($p<0.05$) ↑ Percentage of $CD8^+$ T cells ($p<0.05$) ↑ IFN-γ ($p<0.05$)	NR	Increased survival time ($p<0.01$)	The formulation of pB7-2 with pROP16, resulted in dramatically enhanced antibody titers, both Thl nd $CD8^+$ T cell mediated immune responses.	[16]
ROP17	-	Plasmid, i.m	BALB/c	1×10^3 Tchyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.01$) Elicited both Th1- and Th2-specific responses (IgG2a/IgG1 ratio >1) ↑ IFN-γ (186.17±11.47 pg/mL) and IL-2 (158.41±11.38 pg/mL), $p<0.05$ Increased number of $CD8^+$ T cells ($p<0.05$)	NR	Prolonged survival time (15.6±5.4 days, $p<0.05$) Control mice were died within 4 to 8 days.	Despite the partial protective efficacy of the DNA vaccine, ROP17 appears to be a potential candidate for the development of vaccines against toxoplasmosis.	[17]

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Supplementary Table 1. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP18	pIL-12	Plasmid, i.m	CBA/J ($H\text{-}2^k$)	60 Cts of the 76 K strain, orally	↑ Secific IgG antibody Mixed Th1/Th2 response The predominance of IgG2a over IgG1 in mice immunized with pROP18 and pROP18-pIL-12 ↑ IFN-γ and IL-2 in mice immunized with pROP18 and pROP18-pIL-12, compared to controls ($p<0.05$) Similar percentage of $CD8^+$ T cells between vaccinated and control group ($p>0.05$)	None significant	NR	These results suggest that ROP18 could be component of a subunit vaccine against toxoplasmosis and could lead to more encouraging results.	[18]
	-	Plasmid, i.m	Kunming	1×10^3 Tchyzoites, RH strain, i.p	↑ Secific IgG antibody ($p<0.05$) ↑ Ratio IgG2a to IgG1 ↑ $CD4^+$ and $CD8^+$ T cells in the spleen ($p<0.05$) ↑ IFN-γ (1,008.67 ± 32.47 pg/mL, $p<0.05$), IL-2 (980 ± 54.84 pg/mL, $p<0.05$), IL-4 (149 ± 12.49 pg/mL, $p<0.05$), and IL-10 (143 ± 9.64 pg/mL, $p<0.05$)	NR	Increased survival time (27.9 ± 15.1 days) Control mice were died within 7 days.	For the first time was shown that a ROP18 vaccine construct can enhance the *T. gondii*-specific CTL. Th1 responses and increased survival suggested that ROP18 is a promising vaccine candidate against infection with *T. gondii*.	[19]
	-	Plasmid, i.m	ICR	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response with predominance of IgG2a over IgG1 ($p<0.05$) ↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ (427 ± 40 pg/mL, $p<0.05$) and IL-4 (56 ± 9 pg/mL, $p<0.05$)	NR	Increased survival time (16 days compared with 7 days in control, $p<0.05$)	The study indicates that the introduction of multiantigenic DNA vaccine is more powerful and efficient than single-gene vaccine.	[20]
ROP19	-	Plasmid, i.m	BALB/c	20 Cysts PRU strain, i.g	↑ Levels of IgG antibodies ($p<0.05$) ↑ IFN-γ (485.04 ± 64.559 pg/mL, <0.05)	Reduced ($p<0.05$)	NR	The results suggest that the DNA vaccine encoding ROP19 induced a significant immune response and provided protection against a challenge with *T. gondii* strain PRU cysts.	[21]
ROP38	-	Plasmid, i.m	Kunming	Acute: 1×10^3 tachyzoites, RH strain (type I), i.p Chronic: 10 cysts PRU strain (type II), orally	↑ Level of IgG antibody ($p<0.01$) The predominance of IgG2a over IgG1 Proliferation SI measured at $OD_{570\,nm}$ in mice vaccinated with pVAX-ROP38 (0.90 ± 0.02) was similar to that immunized with PBS (0.91 ± 0.01), pVAX I (0.89 ± 0.07), and blank control (0.97 ± 0.01) ($p>0.05$) ↑ Percentages of $CD4^+$ and $CD8^+$ T cells than control ($p<0.01$) No significant differences in the ratio of $CD8^+/CD4^+$ between mice immunized with pVAX-ROP38 and in controls ($p>0.05$) ↑ IFN-γ (575.2 ± 123.0, $p<0.01$) and IL-2 (195.3 ± 28.4, $p<0.05$) ↓ IL-10 ($p<0.01$)	Reduction in brain tissue cyst burden (76.6%, $p<0.01$)	Increased survival time (10 days compared with 8 days in control, $p>0.05$)	The present study revealed that the pVAX-ROP38 vaccine could elicit strong humoral and cellular immunity response against chronic *T. gondii* infection in mice, resulting in the reduction of the brain cyst formation effectively, which suggests that TgROP38 is a desirable vaccine candidate against chronic *T. gondii* infection.	[22]

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Supplementary Table 1. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP54	-	Plasmid, i.m	Kunming	Acute: 1×10^3 tachyzoites, RH strain, i.p Chronic: 10 cysts of the PRU strain, orally	Induced a strong IgG antibody response ($p<0.05$) Mixed IgG1/IgG2a response with the predominance of IgG2a over IgG1 ↑ Proliferation SI (1.90 ± 0.02, $p<0.05$) ↑ IFN-γ (986.9 ± 14.74 pg/mL, $p<0.05$), IL-2 (360.98 ± 20.45 pg/mL, $p<0.05$), IL-12 p-70 (310.4 ± 21.57 pg/mL, $p<0.05$), IL-4 (90.59 ± 8.45 pg/mL, $p<0.05$), and IL-10 (131.71 ± 15.22 pg/mL, $p<0.05$)	Reduced (35.9%, $p<0.05$)	Increased survival time (13.0 ± 1.15 days, $p<0.05$) All control mice died within 8 days	These results indicate that the recombinant ROP54 plasmid can provide partial protection and might be a potential vaccine candidate against acute and chronic toxoplasmosis.	[23]

↑, increase; Ag, antigen; CTLs, cytotoxic T lymphocytes; i.g, intragastrically; i.m, intramuscular; i.p, intraperitoneally; IFN-γ, interferon-γ; IL, interleukin; NK cells, natural killer cells; NO, nitric oxide; NR, not reported; PBS, phosphate buffered saline; ROP, rhoptry protein or rhoptry antigens; s.c, subcutaneous; SI, stimulation index; *T. gondii*, *Toxoplasma gondii*; Th, T helper; TNF-α, tumor necrosis factor α.

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