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

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Adjuvant or carrier</th>
<th>Ag delivery</th>
<th>Mouse strain</th>
<th>Challenge</th>
<th>Immune responses</th>
<th>Brain cyst load</th>
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<th>Conclusions or suggestions</th>
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</thead>
<tbody>
<tr>
<td>MIC2</td>
<td>Gold particles</td>
<td>Gene gun into abdomen</td>
<td>BALB/c (H-2d) and C57BL/6 (H-2b)</td>
<td>20 Cysts of the <em>T. gondii</em> Beverley strain, orally</td>
<td>Induce the production of specific antibodies ↑ IFN-γ</td>
<td>NR</td>
<td>BALB/c: Increased survival rate (40%, 30-day post challenge, p=0.015) C57BL/6: Increased survival rate (37.5%, 30-day post challenge, p=0.0151)</td>
<td>- [1]</td>
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<tr>
<td>MIC3</td>
<td>pGM-CSF</td>
<td>i.m</td>
<td>CBA/J (H-2k)</td>
<td>70 Cysts of the 76K strain, orally</td>
<td>Induced a strong IgG antibody response (p&lt;0.05) ↑ Splenocyte proliferation p&lt;0.05 ↑ IFN-γ in mice immunized with pMIC3+pGM-CSF (111.9±11 pg/mL, p&lt;0.05) ↑ IL-2 (210±45 pg/mL, p&lt;0.05 and 242±57 pg/mL, p&lt;0.05 for pMIC3 and pMIC3+pGM-CSF groups, respectively) Similar levels of IL-4 and IL-10 between all groups (p&lt;0.05)</td>
<td>Reduced (p&lt;0.05) pMIC3: 58% pMIC3+pGM-CSF: 74%</td>
<td>NR</td>
<td>Increased survival rate (p&lt;0.05)</td>
<td>[2]</td>
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<tr>
<td></td>
<td>Footpad</td>
<td>i.m</td>
<td>Kunming</td>
<td>5×10^5 Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response (p&lt;0.05) ↑ CD4+ and CD8+ T lymphocytes (especially CD8+, p&lt;0.05) ↓ CD4+/CD8 ratio (p&lt;0.05)</td>
<td>No significant difference in terms of IgG responses between the groups immunized with pSCA-MIC3 and pcDNA-MIC3 (p&gt;0.05) ↑ Splenocyte proliferation p&lt;0.05</td>
<td>However, the mean lymphocyte SI of the pSCA-MIC3 vaccinated group was higher than pcDNA-MIC3 vaccinated group, but this was not statistically significant (p&gt;0.05). ↑ IFN-γ significantly (especially in the mice immunized with pSCA-MIC3) ↓ IL-4 (p&lt;0.05) No significant difference in terms of IFN-γ and IL-4 responses between the pSCA-MIC3 group and pcDNA-MIC3 group (p&gt;0.05)</td>
<td>Increased survival time (p&lt;0.05)</td>
<td>[3]</td>
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<td></td>
<td></td>
<td>i.m</td>
<td>BALB/c</td>
<td>1×10^4 Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response (p&lt;0.05) No significant difference in terms of IgG responses between the groups immunized with pSCA-MIC3 and pcDNA-MIC3 (p&gt;0.05) ↑ Splenocyte proliferation p&lt;0.05</td>
<td>NR</td>
<td>Increased survival rate (p&lt;0.05)</td>
<td>The findings demonstrated that like conventional DNA vaccine pcDNA-MIC3, suicidal DNA vaccine pSCA-MIC3 also provided favorable efficacy, but it could improve the biosafety of conventional vaccines. This result suggested that suicidal DNA vaccine pSCA-MIC3 is a potential candidate vaccine against toxoplasmosis.</td>
<td>[4]</td>
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(Continued to the next page)
Antigen | Adjuvant or carrier | Ag delivery | Mouse strain | Challenge | Immune responses | Brain cyst load | Survival | Conclusions or suggestions | Reference
--- | --- | --- | --- | --- | --- | --- | --- | --- | ---
- | i.m | BALB/c | 1×10^3 | Tachyzoites, RH strain, i.p | Induced a strong IgG antibody response (p<0.01) |↑| Prolonged survival time (p<0.05) | - | [5]
- | i.m | ICR | 1×10^3 | Tachyzoites, RH strain, i.p | Induced a strong IgG antibody response with predominance of IgG2α over IgG1 (p<0.05) |↑| Prolonged survival time (14 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. | [6]
- | i.m | BALB/c | 1×10^4 | Tachyzoites, RH strain, i.p |↑| Prolonged survival time (13 days compared with 6 days in control, p<0.05) | These results indicated DNA vaccine encoded MIC3 gene of *T. gondii* capable to induced partially protection against toxoplasmosis. | [7]
- | i.m | BALB/c | 1×10^2 | Tachyzoites, RH strain, i.p |↑| Prolonged survival time (11 days compared with 7 days in control) | These results demonstrate that TgMIC3 could elicit some protection against toxoplasmosis. | [8]
- | i.m | BALB/c | 1×10^2 (high dose) and 1×10^1 (low dose) tachyzoites, RH strain, i.p |↑| Prolonged survival time (13 days compared with 6-7 days in controls, p<0.05) | High dose: increased survival time (14 days compared with 6-7 days in controls, p<0.05) | The present study indicates that MIC3 showed the potential as target for vaccine investigation against toxoplasmosis. | [9]
- | i.m | BALB/c | 1×10^3 | Tachyzoites, RH strain, i.p | Induced a strong IgG and IgA antibodies responses |↓| Prolonged survival time in mice (death within 11 days, p<0.05) | Although this vaccine elicited humoral and celluar immune response and prolonged the life of mice which infected with the RH tachyzoites, they can’t protect mice from death or unhealthy. So it is a long way for us to explore an authentic vaccine against this parasite. | [10]

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### Supplementary Table 1. Continued

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| MIC8    | -                   | i.m         | Kunming      | 1 × 10⁷; T. gondii RH strain, i.p | Induced a strong IgG antibody response (p < 0.05)  
- Proliferation SI (1.39 ± 0.13, p < 0.05)  
- IFN-γ (484.67 ± 25.58 pg/mL, p < 0.05), IL-2 (359.33 ± 61.76 pg/mL, p < 0.05), IL-4 (189.00 ± 18.33 pg/mL, p < 0.05) and IL-10 (404.33 ± 67.87 pg/mL, p < 0.05) | Reduced (63.82%, p < 0.05) | Increased survival time (10.3 ± 0.9 days, p < 0.05)  
Control mice were died within 5 days. | These data demonstrate that the *T. gondii* MIC8 is a potential vaccine candidate against toxoplasmosis. | [14] |
| mIL-15 and mIL-21 | -                   | i.m         | Kunming      | Acute: 1 × 10⁷ tachyzoites, RH strain, i.p and 80 cysts PRU strain, orally  
Chronic: 20 cysts PRU strain, orally  
| Induced a strong IgG antibody response (p < 0.05)  
- Proliferation SI (2.37 ± 0.14, p < 0.05)  
- IFN-γ (306.84 ± 46.42 pg/mL, p < 0.05), IL-2 (495.73 ± 46.81 pg/mL, p < 0.05), IL-12 (317.08 ± 37.41 pg/mL, p < 0.05), IL-4 (168.78 ± 22.61 pg/mL, p < 0.05) and IL-10 (151.75 ± 28.28 pg/mL, p < 0.05)  
- Percentages of CD4⁺ T and CD8⁺ T cells (p < 0.05) | Reduced (83.82%, p < 0.05) | Increased survival time (1 × 10⁷ tachyzoites, RH strain, i.p  
16.2 ± 1.30 days, p < 0.05  
80 Cysts PRU strain, orally: 44.8 ± 4.45 days, p < 0.05 | The present study demonstrates, for the first time, a synergistic effect of rIL-15 and rIL-21 genes in augmenting the efficacy of TgMIC8 DNA vaccine through induction of strong humoral and cellular immune responses which were protective against *T. gondii* challenge. | [15] |
| MIC11   | -                   | i.m         | BALB/c       | 1 × 10⁷; T. gondii, RH strain, i.p | Induced a strong IgG antibody response (p < 0.05)  
- Splenocyte proliferation (p < 0.05)  
- IFN-γ, IL-2, and IL-12 (p < 0.05)  
- Similar levels of IL-4 between mice vaccinated with pcDNA/MIC11 and control groups (p > 0.05) | NR | Increased survival rate (17%, 15-day post challenge, p < 0.05)  
Control mice were died within 8-10 days. | These data suggest that *T. gondii* MIC11 is a reasonable vaccine candidate deserving further studies, and pcDNA/MIC11 is a potential strategy for the control of toxoplasmosis. | [16] |
| MIC13   | -                   | i.m         | Kunming      | Acute: 1 × 10⁷ tachyzoites, RH strain, i.p  
Chronic: 10 tissue cysts PRU strain, orally | Induced a strong IgG antibody response (p < 0.05)  
- Proliferation SI (1.74 ± 0.05, p < 0.05)  
- IFN-γ (342.0 ± 20.2 pg/mL, p < 0.05), IL-2 (228.3 ± 15 pg/mL, p < 0.05), IL-4 (185.8 ± 8.2 pg/mL, p < 0.05) and IL-10 (358.3 ± 33.8 pg/mL, p < 0.05) | Reduced (57.14%, p < 0.05) | Increased survival time (21.3 ± 11.3 days, p < 0.05)  
Control mice were died within 10 days. | *T. gondii* MIC13 is a potential vaccine candidate, worth being included in future vaccine development against acute and chronic *T. gondii* infection. | [17] |

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### Supplementary Table 1. Continued

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<tr>
<td>PLP1</td>
<td>pLL-18</td>
<td>i.m</td>
<td>Kunming</td>
<td>$1 \times 10^3$ Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response ($p &lt; 0.05$) † Proliferation SI (4.51 ± 0.68 and 7.95 ± 0.87 in mice immunized with pVAX-TgPLP1 and pVAX-TgPLP1+pVAX-IL-18, respectively, $p &lt; 0.05$) † IFN-γ, IL-2, IL-4, and IL-10 ($p &lt; 0.05$)</td>
<td>NR</td>
<td>Increased survival time pVAX-TgPLP1: 11.3 ± 0.9 days, $p &lt; 0.05$ pVAX-TgPLP1+pVAX-IL-18: 12.7 ± 1.2 days, $p &lt; 0.05$</td>
<td>This study demonstrated, for the first time, that TgPLP1 induced a strong protective humoral and cellular response against T. gondii, indicating that it is a potential vaccine candidate against toxoplasmosis, worth further development. The murine IL-18 enhanced such immune protection. Further studies are warranted to evaluate the immune efficacy of this DNA vaccine construct in other animal host species against toxoplasmosis.</td>
<td>[18]</td>
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<tr>
<td>-</td>
<td>i.m</td>
<td>Kunming</td>
<td>20 and 80 cysts of strain PRU, i.g</td>
<td>Induced a strong IgG antibody response ($p &lt; 0.05$) † Proliferation SI (4.20 ± 0.27, $p &lt; 0.05$) † IFN-γ (471.5 ± 28.9 pg/mL, $p &lt; 0.05$), IL-2 (206.3 ± 28.2 pg/mL, $p &lt; 0.05$), IL-12 (130.3 ± 17.7 pg/mL, $p &lt; 0.05$), IL-4 (118.5 ± 6.4 pg/mL, $p &lt; 0.05$), and IL-10 (67.3 ± 2.9 pg/mL, $p &lt; 0.05$)</td>
<td>Reduced (43.99%, $p &lt; 0.05$)</td>
<td>Increased survival time (p &lt; 0.05)</td>
<td>Immunization with the recombinant plasmid DNA encoding T. gondii TgPLP1 offers protective efficacy, and this is a promising vaccine candidate against chronic toxoplasmosis.</td>
<td>[13]</td>
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<td>M2AP</td>
<td>Gold particles</td>
<td>Gene gun into abdomen</td>
<td>BALB/c (H-2$^d$) and C57BL/6 (H-2$^b$)</td>
<td>20 Cysts of the T. gondii Beverley strain, orally</td>
<td>Induce the production of specific antibodies † IFN-γ</td>
<td>NR</td>
<td>BALB/c: increased survival rate (20%, 30-day post challenge, non-significant) C57BL/6: none of the mice from pM2AP or control groups survived the infection.</td>
<td>-</td>
<td>[1]</td>
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<tr>
<td>AMA1</td>
<td>Gold particles</td>
<td>Gene gun into abdomen</td>
<td>BALB/c (H-2$^d$) and C57BL/6 (H-2$^b$)</td>
<td>20 Cysts of the T. gondii Beverley strain, orally</td>
<td>Induce the production of specific antibodies † IFN-γ</td>
<td>NR</td>
<td>BALB/c: increased survival rate (60%, 30-day post challenge, p=0.0058) C57BL/6: increased survival rate (37.5%, 30-day post challenge, p=0.0038)</td>
<td>The AMA1 gene appears to generate a strong specific immune response and also provides effective protection against toxoplasmosis.</td>
<td>[1]</td>
</tr>
<tr>
<td>SPATR</td>
<td>-</td>
<td>i.m</td>
<td>BALB/c</td>
<td>$1 \times 10^2$ Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response ($p &lt; 0.05$) † Both IgG1 and IgG2a with the predominance of IgG2a over IgG1 ($p &lt; 0.05$) † Proliferation SI (1.24 ± 0.14, $p &lt; 0.05$) † Elicited both Th1/Th2 type response † IFN-γ (672.87 ± 8.35 pg/mL, $p &lt; 0.05$), IL-2 (367.93 ± 10.30 pg/mL, $p &lt; 0.05$), IL-4 (212.7 ± 42 pg/mL, $p &lt; 0.05$), and IL-10 (261.8 ± 10.03 pg/mL, $p &lt; 0.05$)</td>
<td>NR</td>
<td>Increased survival time (15.7 ± 1.42 days, p &lt; 0.05) Control mice were died within 7 days.</td>
<td>The current study indicated that pVAX1-TgSPATR induce a TgSPATR specific immune response and might be a promising vaccine candidate against toxoplasmosis. To the best of our knowledge, this is the first report to evaluate the immunoprotective value of TgSPATR against T. gondii.</td>
<td>[19]</td>
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</table>

MIC, microneme proteins; IFN-γ, interferon-γ; NR, not reported; pGM-CSF, plasmid encoding granulocyte-macrophage colony-stimulating factor; i.m, intramuscular; IL, interleukin; PBS, phosphate-buffered saline; i.p, intraperitoneal; i.n, intranasal; SI, stimulation index.