<table>
<thead>
<tr>
<th>Antigen</th>
<th>Adjuvant/Carrier</th>
<th>Ag delivery</th>
<th>Mouse strain</th>
<th>Challenge</th>
<th>Immune responses</th>
<th>Brain cyst load</th>
<th>Survival</th>
<th>Conclusions or suggestions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC3</td>
<td>-/pseudorabies virus (PRV)</td>
<td>i.m</td>
<td>BALB/c</td>
<td>1×10² Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response</td>
<td>Increased survival rate (50%, 28-day post challenge, p &lt; 0.05)</td>
<td>NR</td>
<td>These results suggested that expression of protective Ag of <em>T. gondii</em> in PRV is a novel approach towards the development of a vaccine against toxoplasmosis.</td>
<td>[23]</td>
</tr>
<tr>
<td>MIC3</td>
<td>-/baculovirus (bv)</td>
<td>i.m</td>
<td>BALB/c</td>
<td>1×10⁵ Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response (p &lt; 0.01)</td>
<td>Prolonged survival time (p &lt; 0.05)</td>
<td>NR</td>
<td>These results suggest that an excellent vector-mediated vaccine strategy might be used to develop a new generation of vaccines against <em>T. gondii</em> infection.</td>
<td>[5]</td>
</tr>
<tr>
<td>MIC3</td>
<td>-/S. typhimurium strain SV4089 Dam− and PhoP− mutant</td>
<td>Orally</td>
<td>ICR</td>
<td>5×10² Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response (p &lt; 0.05)</td>
<td>Prolonged survival time (11 days compared with 6 days in control, p &lt; 0.05)</td>
<td>NR</td>
<td>This study preliminarily shows that attenuated <em>S. typhimurium</em> strain (Dam− and PhoP−) could be utilized as an oral delivery vector for recombinant eukaryotic expression plasmids as DNA vaccines for prevention from toxoplasmosis.</td>
<td>[24]</td>
</tr>
<tr>
<td>MIC8</td>
<td>-/VLP recombinant baculovirus (rBV) influenza matrix protein 1 (M1)</td>
<td>i.m, i.n</td>
<td>BALB/c, i.n</td>
<td>1×10⁶ Tachyzoites, RH strain, orally</td>
<td>i.m mice group showed higher levels of <em>T. gondii</em>-specific IgG antibody response compared to i.m mice group (p &lt; 0.01).</td>
<td>Increased survival rate i.m group: 100%, 16-day post challenge i.n group: 60%, 16-day post challenge</td>
<td>NR</td>
<td>Our study shows the effective protection against <em>T. gondii</em> infection provided by VLPs containing MIC8 of <em>T. gondii</em>, thus indicating a potential <em>T. gondii</em> vaccine candidate.</td>
<td>[25]</td>
</tr>
</tbody>
</table>
### Supplementary Table 5. Continued

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Adjuvant/Carrier</th>
<th>Ag delivery</th>
<th>Mouse strain</th>
<th>Challenge</th>
<th>Immune responses</th>
<th>Brain cyst load</th>
<th>Survival</th>
<th>Conclusions or suggestions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC3+SA1/-/pseudorabies virus (PRV)</td>
<td>i.m</td>
<td>BALB/c</td>
<td>1 × 10⁶</td>
<td>Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response</td>
<td>NR</td>
<td>Increased survival rate (66.7%, 28-day post challenge, p &lt; 0.05)</td>
<td>These results suggested that expression of protective antigens of <em>T. gondii</em> in PRV is a novel approach towards the development of a vaccine against toxoplasmosis.</td>
<td>[23]</td>
</tr>
<tr>
<td>-/baculovirus (bv)</td>
<td>i.m</td>
<td>BALB/c</td>
<td>1 × 10³</td>
<td>Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response</td>
<td>NR</td>
<td>Increased survival rate (50%, 22-day post challenge, p &lt; 0.05)</td>
<td>These results suggest that an excellent vector-mediated vaccine cocktail strategy might be used to develop a new generation of vaccines against <em>T. gondii</em> infection.</td>
<td>[5]</td>
</tr>
<tr>
<td>-/S. typhimurium strain SV4089</td>
<td>Orally</td>
<td>ICR</td>
<td>5 × 10⁶</td>
<td>Tachyzoites, RH strain, i.p</td>
<td>Induced a strong IgG antibody response</td>
<td>NR</td>
<td>Increased survival rate (p &lt; 0.05)</td>
<td>The current study shows that the oral multi-antigenic DNA vaccine, ZJ111/pSA1-MIC3, produces partial protection against <em>T. gondii</em> challenge.</td>
<td>[24]</td>
</tr>
<tr>
<td>DAM− and PhoP− mutant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encoding</td>
<td>Ubiquitin/Ad</td>
<td>DNA vaccines</td>
<td>BALB/c</td>
<td>Acute: 1 × 10³ tachyzoites, RH strain (genotype II), i.p</td>
<td>Induced a strong IgG antibody response in both p-MAS and p-UMAS immunized mice (especially in the p-UMAS group), compared to control groups.</td>
<td>Reduced (p &lt; 0.01). The brain cyst burden was 50% lower in p-MAS group (833 ± 116), compared with the control groups p-UMAS (570 ± 98) Ad-UMAS (469 ± 103)</td>
<td>Distinct humoral and cellular immunity induced by immunization with DNA vaccine and recombinant Ad vaccine encoding ubiquitin conjugated multistage Ag of <em>T. gondii</em>. The DNA vaccine had the advantage of inducing a stronger humoral response, whereas the Ad-vectorized vaccine improved the cellular immune response.</td>
<td>[26]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-MAS or p-UMAS plasmid, 100 μg each)</td>
<td></td>
<td></td>
<td>Splenocyte proliferation in both p-MAS and p-UMAS immunized mice (a further 30% increase in latter group)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.m or recombinant Ad vaccine (Ad-UMAS virus, 3 × 10⁶ PFU each), i.m or the combination of DNA vaccine (p-UMAS, 100 μg each) and recombinant Ad vaccine (Ad-UMAS virus, 3 × 10⁶ PFU each), i.g via oral gavage</td>
<td></td>
<td></td>
<td>Levels of an IgG1 and IgG2a in p-MAS and p-UMAS immunized mice (predominance of IgG2a over IgG1), compared to control groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DNA vaccine or Ad vaccine</td>
<td></td>
<td></td>
<td>Percentages of CD4⁺ T and CD8⁺ cells in p-MAS and p-UMAS groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significantly higher levels of IFN-γ and IL-2 secretion and increased splenocyte proliferation in Ad-UMAS immunized mice compared with p-UMAS group (p &lt; 0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Percentages of CD8⁺ T cells in immunized with Ad-UMAS compared with p-UMAS group (p &lt; 0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Supplementary Table 5. Continued

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Adjuvant/Carrier</th>
<th>Ag delivery</th>
<th>Mouse strain</th>
<th>Challenge</th>
<th>Immune responses</th>
<th>Brain cyst load</th>
<th>Survival</th>
<th>Conclusions or suggestions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encoding</td>
<td>Ubiquitin/Ad</td>
<td>i.m, i.n, s.c, i.o, i.v</td>
<td>BALB/c</td>
<td>Acute: 1×10⁶ tachyzoites, RH strain (type I), i.p</td>
<td>Levels of <em>T. gondii</em>-specific IgG antibodies in the five Ad-UMAS immunization routes, compared to the controls (p &lt; 0.05).</td>
<td>Reduced (p &lt; 0.05)</td>
<td>Increased survival rate i.m, i.o, and i.n vaccinated groups: 50% survival rate 28 days after challenge i.v and s.c vaccinated groups: 40% survival rate 28 days after challenge. All the control mice died within 8 days.</td>
<td>[27]</td>
<td></td>
</tr>
<tr>
<td>Ad-UMAS</td>
<td>ROP18(96–396), SAG3(13–144), MIC6(339–347), GRA7(18–224), MA61(60–125), BAG1(156–211), and SPA1(142–200)</td>
<td></td>
<td></td>
<td></td>
<td>Highest titer of IgG antibody was observed by i.m route and followed by s.c, i.n, i.o and i.v. IgG subtypes in the five Ad-UMAS immunization routes, compared to the controls (p &lt; 0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significantly higher values of IgG2a in i.m and s.c vaccination groups, compared with other vaccination routes. Significantly higher values of IgA in i.n and i.o vaccination groups, compared with other vaccination routes.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Percentages of CD4⁺ and CD8⁺ T cells in the five Ad-UMAS immunization routes, compared to the controls (p &lt; 0.05).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significantly higher percentages of CD4⁺ and CD8⁺ T cells in i.n and i.o vaccination groups, compared with other vaccination routes.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IFN-γ and IL-2 in the five Ad-UMAS immunization routes, compared to the controls (p &lt; 0.05).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significantly higher secretion of IFN-γ and IL-2 in i.n and i.o vaccination groups, compared with other vaccination routes.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lymphocyte proliferation ability in the five Ad-UMAS immunization routes, compared to the controls (p &lt; 0.05).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significantly higher lymphocyte proliferation ability in i.n and i.o vaccination groups, compared with other vaccination routes.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MIC, microneme proteins; i.m, intramuscular; IFN-γ, interferon-γ; IL, interleukin; i.p, intraperitoneal; i.n, intranasal; NR, Not reported; s.c, subcutaneous; i.o, intraoral; i.v, intravenous.