**Supporting information**

**­­Immunogenicity of outer membrane proteins VirB9-1 and VirB9-2, a novel nanovaccine against *Anaplasma marginale***

Liang Zhao1\*, Donna Mahony2\*, Antonino S. Cavallaro2, Bing Zhang3, Jun Zhang1, James R. Deringer4,Chun-Xia Zhao1, Wendy C. Brown4, Chengzhong Yu1, Neena Mitter2†, Anton P.J. Middelberg1†

1 Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, QLD 4072, Australia.

2 Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD 4072, Australia.

3 Animal Science, Queensland Department of Agriculture, Fisheries and Forestry, St Lucia, QLD 4072, Australia.

4 Department of Veterinary Microbiology and Pathology, Washington State University, College of Veterinary Medicine, P.O. Box 647040, Pullman, WA 99164-7040, USA.

\* These authors contributed equally.

† Corresponding authors: E-mail addresses: n.mitter@uq.edu.au (N. Mitter); a.middelberg@uq.edu.au (A. P. J. Middelberg).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Groups** | VirB9.1+Quil A | VirB9.1/SV-100 | VirB9.2+Quil A | VirB9.2/SV-100 | VirB9.1/9.2+Quil A | VirB9.1/9.2/SV-100 | SV-100 | Unimmunised |
| VirB9.1+Quil A |  | ns | \*\*\*\* | \*\*\*\* | ns | ns | \*\*\*\* | \*\*\*\* |
| VirB9.1/SV-100 | ns |  | \*\*\*\* | \*\*\*\* | ns | \* | \*\*\*\* | \*\*\*\* |
| VirB9.2+Quil A | \*\*\*\* | \*\*\*\* |  | ns | \*\*\*\* | \*\*\*\* | \* | \* |
| VirB9.2/SV-100 | \*\*\*\* | \*\*\*\* | ns |  | \*\*\*\* | \*\*\*\* | ns | ns |
| VirB9.1/9.2+Quil A | ns | ns | \*\*\*\* | \*\*\*\* |  | ns | \*\*\*\* | \*\*\*\* |
| VirB9.1/9.2/SV-100 | ns | \* | \*\*\*\* | \*\*\*\* | ns |  | \*\*\*\* | \*\*\*\* |
| SV-100 | \*\*\*\* | \*\*\*\* | \* | ns | \*\*\*\* | \*\*\*\* |  | ns |
| Unimmunised | \*\*\*\* | \*\*\*\* | \* | ns | \*\*\*\* | \*\*\*\* | ns |  |

**S1 Table.** Statistical analysis of ELISPOT results

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Groups** | VirB9.1+Quil A | VirB9.1/SV-100 | VirB9.2+Quil A | VirB9.2/SV-100 | VirB9.1/9.2+Quil A | VirB9.1/9.2/SV-100 | SV-100 | Unimmunised |
| VirB9.1+Quil A |  | ns | \*\*\*\* | \*\*\*\* | \*\*\*\* | \*\*\*\* | ns | ns |
| VirB9.1/SV-100 | ns |  | \*\*\*\* | \*\*\*\* | \*\*\*\* | \*\*\*\* | ns | ns |
| VirB9.2+Quil A | \*\*\*\* | \*\*\*\* |  | ns | ns | ns | \*\*\*\* | \*\*\*\* |
| VirB9.2/SV-100 | \*\*\*\* | \*\*\*\* | ns |  | ns | ns | \*\*\*\* | \*\*\*\* |
| VirB9.1/9.2+Quil A | \*\*\*\* | \*\*\*\* | ns | ns |  | ns | \*\*\*\* | \*\*\*\* |
| VirB9.1/9.2/SV-100 | \*\*\*\* | \*\*\*\* | ns | ns | ns |  | \*\*\*\* | \*\*\*\* |
| SV-100 | ns | ns | \*\*\*\* | \*\*\*\* | \*\*\*\* | \*\*\*\* |  | ns |
| Unimmunised | ns | ns | \*\*\*\* | \*\*\*\* | \*\*\*\* | \*\*\*\* | ns |  |

1. VirB9-1-specific ELISPOT assay results: \*, p < 0.05; \*\*\*\*, p < 0.0001; ns = not significant.
2. VirB9-2-specific ELISPOT assay results: \*\*\*\*, p < 0.0001; ns = not significant.