

Table S1. Cytokine expression of dendritic cells after stimulation with OMVs purified from *B. melitensis* 16M and *B. melitensis* VTRM1. The cytokine expression is reported as fold change in mRNA expression

| OMVs from <i>B. melitensis</i> VTRM1 |                                   | OMVs from <i>B. melitensis</i> 16M |                                  |         |                   |
|--------------------------------------|-----------------------------------|------------------------------------|----------------------------------|---------|-------------------|
| Time (h)                             | Cytokine fold change IFN $\gamma$ | Time (h)                           | Cytokine foldchange IFN $\gamma$ | P value | P value per group |
| 1                                    | 11.27                             | 1                                  | 0.4467                           | 0.05    | 0.05              |
| 3                                    | 6.111                             | 3                                  | 1.007                            | Ns      |                   |
| 6                                    | 0.7433                            | 6                                  | 1.537                            | Ns      |                   |
| 12                                   | 7.453                             | 12                                 | 5.18                             | Ns      |                   |
| Time (h)                             | Cytokine fold change IL-2         | Time (h)                           | Cytokine fold change IL-2        | P value | P value per group |
| 1                                    | 19.12                             | 1                                  | 0.16                             | 0.05    | Ns                |
| 3                                    | 7.653                             | 3                                  | 1.49                             | Ns      |                   |
| 6                                    | 1.243                             | 6                                  | 4.52                             | Ns      |                   |
| 12                                   | 8.463                             | 12                                 | 8.733                            | Ns      |                   |
| Time (h)                             | Cytokine fold change IL-6         | Time (h)                           | Cytokine fold change IL-6        | P value | P value per group |
| 1                                    | 61.37                             | 1                                  | 0.3567                           | 0.001   | Ns                |
| 3                                    | 7.253                             | 3                                  | 5.753                            | Ns      |                   |
| 6                                    | 4.633                             | 6                                  | 12.54                            | Ns      |                   |
| 12                                   | 5.267                             | 12                                 | 67.37                            | Ns      |                   |
| Time (h)                             | Cytokine fold change IL-12        | Time (h)                           | Cytokine fold change IL-12       | P value | P value per group |
| 1                                    | 53.83                             | 1                                  | 0.2767                           | 0.001   | 0.05              |
| 3                                    | 5.6                               | 3                                  | 1.653                            | Ns      |                   |
| 6                                    | 1.277                             | 6                                  | 6.087                            | Ns      |                   |
| 12                                   | 1.16                              | 12                                 | 9.6                              | Ns      |                   |
| Time (h)                             | Cytokine fold change IL-4         | Time (h)                           | Cytokine fold change IL-4        | P value | P value per group |
| 1                                    | 1.572                             | 1                                  | 0.1867                           | Ns      | Ns                |
| 3                                    | 3.343                             | 3                                  | 0.6933                           | Ns      |                   |
| 6                                    | 1.353                             | 6                                  | 3.197                            | Ns      |                   |
| 12                                   | 0.1433                            | 12                                 | 13.38                            | Ns      |                   |
| Time (h)                             | Cytokine fold change IL-10        | Time (h)                           | Cytokine fold change IL-10       | P value | P value per group |
| 1                                    | 2.36                              | 1                                  | 1.007                            | Ns      | Ns                |
| 3                                    | 9.457                             | 3                                  | 4.62                             | Ns      |                   |
| 6                                    | 4.33                              | 6                                  | 25.71                            | Ns      |                   |
| 12                                   | 3.193                             | 12                                 | 41.54                            | Ns      |                   |

| Time (h) | Cytokine fold change IL-17        | Time (h) | Cytokine fold change IL-17        | P value | P value per group |
|----------|-----------------------------------|----------|-----------------------------------|---------|-------------------|
| 1        | 11.27                             | 1        | 0.16                              | 0.05    | Ns                |
| 3        | 6.09                              | 3        | 0.44                              | Ns      |                   |
| 6        | 0.07667                           | 6        | 2.24                              | Ns      |                   |
| 12       | 7.453                             | 12       | 14.54                             | Ns      |                   |
| Time (h) | Cytokine fold change TNF $\alpha$ | Time (h) | Cytokine fold change TNF $\alpha$ | P value | P value per group |
| 1        | 71.5                              | 1        | 0.8109                            | 0.05    | 0.001             |
| 3        | 100.7                             | 3        | 2.778                             | 0.05    |                   |
| 6        | 56                                | 6        | 11.2                              | 0.05    |                   |
| 12       | 59.33                             | 12       | 19.17                             | Ns      |                   |
| Time (h) | Cytokine fold change TGF $\beta$  | Time (h) | Cytokine fold change TGF $\beta$  | P value | P value per group |
| 1        | 26.5                              | 1        | 0.1754                            | 0.01    | Ns                |
| 3        | 4.145                             | 3        | 0.04006                           | Ns      |                   |
| 6        | 3.295                             | 6        | 4.862                             | Ns      |                   |
| 12       | 13.71                             | 12       | 10.74                             | Ns      |                   |
| Time (h) | Cytokine fold change IL-23        | Time (h) | Cytokine fold change IL-23        | P value | P value per group |
| 1        | 18.96                             | 1        | 0.0003104                         | 0.05    | Ns                |
| 3        | 0.1574                            | 3        | 0.001534                          | Ns      |                   |
| 6        | 0.06513                           | 6        | 0.1167                            | Ns      |                   |
| 12       | 1.736                             | 12       | 0.2051                            | Ns      |                   |

Table S2. *B. melitensis* 16M OMVs proteins identified by 1D-SDS-PAGE coupled to LC-MS/MS

| Gel section     | Protein  | Gene          | Accession number (a.a) | Score (%)* | Coverage (%)** |
|-----------------|--|---------------|------------------------|------------|----------------|
| 1               | Bacterial surface antigen                              | <i>omp1</i>   | YP_221860              | 71         | 4              |
|                 | Iron-Regulated outer membrane protein FRPB             | <i>bhuA</i>   | NP_541082              | 100        | 13             |
|                 | Metal chelate outer membrane receptor                  | ND            | NP_539574              | 90         | 3              |
| 2               | Sugar binding protein                                  | <i>sp39</i>   | NP_541568              | 90         | 7              |
|                 | Outer surface protein                                  | ND            | YP_223106              | 91         | 18             |
|                 | D-Ribose binding periplasmic protein precursor         | <i>rbsB</i>   | NP_541413              | 99         | 50             |
|                 | Hypothetical protein BMEI0542                          | ND            | NP_539459              | 99         | 42             |
| 3               | 25 kDa outer-membrane immunogenic protein precursor    | <i>omp2b</i>  | NP_539924              | 99         | 28             |
|                 | 25 kDa outer-membrane immunogenic protein precursor    | <i>omp25</i>  | NP_540166              | 100        | 19             |
|                 | 25 kDa outer-membrane immunogenic protein precursor    | <i>omp25c</i> | NP_540746              | 96         | 18             |
|                 | 25 kDa outer-membrane immunogenic protein precursor    | <i>omp25d</i> | NP_540747              | 99         | 50             |
|                 | 31 kDa outer-membrane immunogenic protein precursor    | <i>omp31</i>  | NP_541822              | 99         | 32             |
|                 | BP26   | <i>bp26</i>   | ABC17803               | 91         | 10             |
|                 | Protein YBIS precursor                                 | ND            | AAL52550.              | 100        | 22             |
|                 | OmpA family protein                                    | <i>ompA</i>   | NP_698208              | 99         | 17             |
| 4               | Ribosome recycling factor                              | <i>frr</i>    | NP_698164              | 100        | 38             |
|                 | 22 kDa outer-membrane immunogenic protein precursor    | <i>omp22</i>  | NP_539634              | 100        | 43             |
|                 | DNA starvation/stationary phase protection protein Dps | <i>dps</i>    | NP_699124              | 100        | 25             |
|                 | Hypothetical lipoprotein                               | ND            | NP_539702              | 90         | 14             |
| 5               | Hypothetical Membrane Associated Protein (BMEII0692)   | <i>ialB</i>   | NP_541670              | 90         | 14             |
|                 | Invasion protein B                                     | <i>invB</i>   | YP_221125              | 53         | 12             |
|                 | Peptidoglycan-associated lipoprotein                   | <i>omp16</i>  | NP_539257              | 99         | 40             |
|                 | Chain A , -Cu-Zn superoxide dismutase                  | <i>sodC</i>   | 2AQM_A                 | 100        | 48             |
|                 | Co-chaperonin GroES                                    | <i>groES</i>  | NP_542025              | 99         | 42             |
| Thioredoxin C-1 | <i>trxC</i>  | NP_540939     | 98                     | 16         |                |
| 6               | Outer membrane lipoprotein                             | <i>omp19</i>  | NP_539053              | 97         | 26             |
|                 | DNA-binding protein HU                                 | <i>hu</i>     | YP_221813              | 95         | 20             |
|                 | Hypothetical lipoprotein                               | ND            | YP_221486              | 100        | 43             |
|                 | Hypothetical protein BMEI0287                          | ND            | NP_539204              | 100        | 50             |

ND. None designated

\* The percentage of detection is the value assigned on the identity of a protein. \*\*The percentage of coverage corresponds to the length of the sequence covering by the peptides obtained in the LC-MS/MS for this sequence.

Table S3. *In silico* analyses of proteins and annotation of identified vesicular proteins based on hypothetical function by software database (ProLinks and Gene Ontology).

| Identified protein                                     | Antigenic region  | I. P. | *Signal peptide | Related to (with ProLinks) | GO terms (Process or function)   |
|--|---|-------|-----------------|----------------------------|--|
| Bacterial surface antigen                              | [ +7.627]:SGRRLRDVI; [ +5.862]:ETLRRFYYN; [ +4.107]:LSAGFDVFR                     | 5.37  | Yes             | Membrane proteins          | Membrane, transmembrane, outer membrane  |
| Iron-Regulated outer membrane protein FRPB             | [ +6.969]:PGKTFGARI; [ +3.994]:AGASRTWLG [ +4.461]:APGRTFTFQ                      | 5.54  | Yes             | Membrane proteins          | Membrane, transmembrane, outer membrane  |
| Metal chelate outer membrane receptor                  | [ +6.223]:RTASATSGS; [ +6.164]:LQGSFNFAL [ +5.277]:QYDATAPAF; [ +8.431]:TIDYDETGR | 5.00  | No              | Membrane proteins          | Receptor, transporter, membrane  |
| Sugar binding protein                                  | [ +5.349]:IESPAFQSA; [ +11.014]:AIYDVVTRQ   | 4.97  | Yes             | Sugar transport            | Sugar transport, periplasmic   |
| Outer surface protein                                  | [ +5.263]:GAGIGAAYV; [ +4.550]:YGYDEQDRQ [ +4.643]:VINQYQGA                       | 5.28  | Yes             |                            | Membrane, transmembrane, outer membrane  |
| D-Ribose binding periplasmic protein precursor         | [ +10.821]:AANIPVFLI; [ +4.572]:ANIPVFLIN [ +2.382]:ANWDRTOGH                     | 5.60  | No              | Sugar transport            | D-ribose transport, outer membrane-bounded periplasmic space, sugar binding                                      |
| Hypothetical protein BMEI0542                          | [ +3.159]:SKVEWTDPF; [ +3.446]:DINDRWEIF [ +4.666]:DIGGFGAGT                      | 4.83  | Yes             |                            |  |
| 25 kDa outer-membrane immunogenic protein precursor    | [ +5.837]:GVDSKLRWS; [ +3.644]:DFSVVVDGDL   | 4.72  | Yes             | Membrane proteins          | Membrane, transmembrane, outer membrane  |
| 25 kDa outer-membrane immunogenic protein precursor    | [ +3.213]:WAGGYTGLY; [ +12.005]:NYDLAGTTV   | 8.58  | Yes             |                            | Membrane, transmembrane, outer membrane  |
| 25 kDa outer-membrane immunogenic protein precursor    | [ +2.813]:ATGANAAADA; [ +2.005]:APVVVAPTF   | 4.78  | Yes             | Membrane proteins          | Membrane, transmembrane, outer membrane  |
| 25 kDa outer-membrane immunogenic protein precursor    | [ +4.891]:EPAPIAIAP; [ +4.062]:NNLKSRDF   | 9.28  | Yes             | Membrane proteins          | Membrane, transmembrane, outer membrane  |
| 31 kDa outer-membrane immunogenic protein precursor    | [ +5.082]:KHPFSSFDK; [ +4.300]:RLGYTATER  | 5.21  | Yes             | Membrane proteins          | Membrane, transmembrane, outer membrane  |
| BP26   | [ +1.069]:TMLAAAPDN**   | 7.93  | Yes             |                            | Periplasmic space  |
| Precursor YBIS protein                                 | [ +2.858]:KRQWPRWP; [ +8.867]:ALYIFKDGK   | 8.49  | No              |                            |  |
| OmpA family protein                                    | [ +4.455]:GAGIGALGG; [ +2.212]:DTDQDQVKS  | 9.55  | Yes             |                            | Outer membrane component, transmembrane  |
| Hypothetical lipoprotein                               | [ +6.979]: RGGWFNNQ; [ +7.882]: DAIHFIGWY [ +5.847]:ADAIHFIGW                     | 10.48 | Yes             |                            | Lytic endotransglycosylase activity  |
| Ribosome recycling factor                              | [ +4.144]:RIAARHVRR   | 5.98  | No              | Translation                | Translation, translation termination   |
| Hypothetical membrane-associated protein(BMEII0692)    | [ +2.166]: MLASATMPA; [ +2.363]: PNPIPVALT  | 9.00  | No              |                            |  |
| 22 kDa outer membrane protein precursor                | [ +7.382]: QVNGIEQRN  | 8.59  | No              | Membrane proteins          | Membrane, transmembrane, outer membrane  |
| DNA starvation/stationary phase protection protein Dps | [ +7.137]: ITKQAHWNL; [ +8.278]: RLKPYPTDI [ +4.828]: LKPYPTDIY                   | 5.25  | No              | Aminoacid transport        | Metal ion binding, redox activity, ferric iron binding   |
| Peptidoglycan associated lipoprotein                   | [ +8.084]: ASKKNLNPN; [ +3.394]: RTISYGNER  | 9.92  | Yes             | Toluene transport          | Membrane, transmembrane, outer membrane  |
| Invasion protein B                                     | [ +4.700]:KIDDTAGPN; [ +2.813]:QETYQDWTV  | 8.98  | Yes             |                            | Membrane   |
| Outer membrane lipoprotein                             | [ +5.631]: ASAPDLTPG; [ +4.541]: ISKASLLSL [ +4.923] : LLSLAAAGI                  | 8.91  | Yes             |                            | Membrane, transmembrane, outer membrane  |
| Chain A, Cu-Zn superoxide dismutase                    | [ +1.343]: TGPGKEVGT**  | 6.11  | No              |                            | Ion binding, redox activity  |
| Thioredoxin C-1  | [ +10.205]:WAEWCGPK; [ +3.554]:MFKDGELA   | 4.53  | No              | A transketolase            | Cellular protein metabolic process, generation of precursor metabolites and energy, nucleotide metabolic process |
| Co-chaperonin GroES                                    | [ +4.231]:VRRVESEAK   | 5.41  | No              |                            | Protein folding, chaperone binding, chaperone regulator activity   |
| DNA binding protein HU                                 | [ +1.540]:AEKGGLTKA**   | 9.77  | No              | The primosome              | DNA binding  |
| Hypothetical lipoprotein                               | [ +2.847]:AGGAIIGGI   | 10.50 | Yes             |                            |  |
| Hypothetical protein BMEI0287                          | [ +2.729]:RTAGYGVGG   | 9.02  | Yes             |                            |  |

I. P. Isoelectric point.

\*Signal P: used for the identification of the signal peptide into the protein sequence.

\*\* Unique antigenic region into the sequence.