

## Supporting Materials

**TABLE 1.** Transcription profiles of selected genes at defined time points after primary and secondary immunizations as determined by both microarray and real-time RT-PCR. Numbers indicate the fold of change compared with the non-infected controls, except IBV spike gene, where the fold change is compared with d5. -, down-regulated compared to control; ND, not detectable. Di, i-day after primary immunization. Note: D22 and D24 mean 1 and 3 days after the secondary immunization.

| Gene name                             | Reference Gene | Methods  | D1    | D3     | D5    | D8    | D12   | D21   | D22   | D24   |
|---------------------------------------|----------------|----------|-------|--------|-------|-------|-------|-------|-------|-------|
| Toll like receptor 2 type 1 precursor | Bu2004623      | Array    | 2.91  | 4.17   | 1.53  | 1.02  | 1.39  | -1.51 | -1.67 | -1.69 |
|                                       |                | Real PCR | 11.51 | 12.88  | 5.47  | 4.14  | -4.39 | -1.1  | -1.1  | -4.39 |
| Fas antigen                           | BU300974       | Array    | 3.45  | 1.19   | 2.07  | 1.23  | -1.15 | 1.08  | 1.11  | 1.25  |
|                                       |                | Real PCR | 11.12 | 5.83   | 11.48 | 4.13  | -3.57 | 2.98  | 4.51  | -2.27 |
| Mx                                    | BU457953       | Array    | 18.36 | 4.05   | 2.69  | 1.12  | -1.33 | 1.03  | 1.66  | -1.7  |
|                                       |                | Real PCR | 65.17 | 15.88  | 19.11 | 6.47  | 4.68  | 3.95  | 3.81  | -3.85 |
| Interferon regulatory factor 1        | AI980049       | Array    | 4.32  | 2.01   | 2.42  | 1.05  | 1.32  | 1.06  | 1.25  | -1.1  |
|                                       |                | Real PCR | 5.6   | 3.12   | 3.07  | -1.11 | -14.3 | -1.01 | 1.32  | -1.02 |
| Interleukin 1 beta                    | BG625387       | Array    | 10.8  | 5.47   | 2.13  | 1.5   | 1.47  | 2.54  | 1.68  | 1.6   |
|                                       |                | Real PCR | 10.43 | 6.34   | -1.89 | ND    | ND    | 2.05  | -2.78 | -1.12 |
| Suppressor of cytokine signaling 3    | AI981631       | Array    | 8.27  | 2.84   | 2.1   | 1.1   | 1.12  | -1.03 | -1.11 | -1.02 |
|                                       |                | Real PCR | 28.18 | 10.04  | 1.86  | 1.96  | 1.01  | 1.1   | 1.99  | 1.95  |
| Osteopontin                           | BG625306       | Array    | 5.25  | 1.58   | 1.95  | -2.18 | 1.24  | -1.69 | -1.52 | 1.19  |
|                                       |                | Real PCR | 33.21 | 2.07   | 3.04  | -2.63 | ND    | -1.61 | -1.61 | 1.03  |
| Cytochrome C                          | BF845754       | Array    | 1.45  | -28.82 | -7.41 | -71   | -1.41 | -1.95 | -2.01 | -1.05 |
|                                       |                | Real PCR | 2.18  | 2.46   | 3.6   | 2.94  | 1.46  | 1.13  | 1.87  | 1.64  |
| Ig light chain precursor              | M24403         | Array    | -1.64 | -1.21  | 2.1   | 2.06  | -9.07 | 1.34  | 1.63  | 1.54  |
|                                       |                | Real PCR | -1.01 | 1.79   | 2.36  | 4.88  | 0.21  | 3.42  | 7.47  | 1.92  |
| Embryo fibroblast protein 1 precursor | AI980458       | Array    | 18.66 | 6.53   | 5.61  | 1.25  | 2.68  | 3.84  | 1.04  | 1.65  |
|                                       |                | Real PCR | 9.48  | 1.99   | 4.89  | 1.25  | 0.98  | 1.38  | 1.13  | 1.45  |
| Ig mu chain IgM                       | AI980934       | Array    | -1.28 | -1.08  | 1.16  | 1.28  | 1.72  | 1     | 1.11  | 1.03  |
|                                       |                | Real PCR | 0.91  | 0.71   | 1.82  | 1.1   | 0.81  | 0.81  | 1.31  | 1.39  |
| Interleukin 6                         | AI982185       | Array    | -1.18 | -1.09  | 1.04  | 1.12  | -1.15 | -1.01 | 1.03  | 1.11  |
|                                       |                | Real PCR | -1.88 | -1.82  | -0.3  | -1.79 | -1.58 | -2.24 | -1.7  | -1.8  |
| Transforming growth factor beta       | BU107503       | Array    | -1.28 | -1.2   | -2.35 | -2.18 | -3.6  | -1.22 | -1.14 | -1.36 |
|                                       |                | Real PCR | -1.4  | -1.32  | -2.41 | -1.24 | -1.31 | -1.36 | 1.62  | -1.41 |
| Ig alpha chain                        | S40610.1       | Real PCR | -1.21 | 4.2    | 1.48  | -27.3 | -17.9 | ND    | -4.51 | ND    |
| IBV spike protein                     | AY851295       | Real PCR | 7.39  | 2.51   | 1     | ND    | ND    | ND    | ND    | ND    |

**TABLE 2.** Numbers of host genes that were significantly changed at different time points after the primary and secondary immunization. Di, i-day after primary immunization. D22 and D24, 1 and 3 days after the secondary immunization.

| <b>Time points</b>       | <b>D1</b> | <b>D3</b> | <b>D5</b> | <b>D8</b> | <b>D12</b> | <b>D21</b> | <b>D22</b> | <b>D24</b> |
|--------------------------|-----------|-----------|-----------|-----------|------------|------------|------------|------------|
| No. of genes increased   | 701       | 700       | 1101      | 312       | 870        | 542        | 22         | 132        |
| Percent of increase (%)  | 59.91     | 57.66     | 51.23     | 48.90     | 37.15      | 82.75      | 31.43      | 76.30      |
| No. of genes decreased   | 469       | 514       | 1048      | 326       | 1472       | 113        | 48         | 41         |
| Percent of decrease (%)  | 40.09     | 42.34     | 48.77     | 51.10     | 62.85      | 17.25      | 68.57      | 23.70      |
| Total No. of alteration  | 1170      | 1214      | 2149      | 638       | 2342       | 655        | 70         | 173        |
| Alteration in genome (%) | 7.42      | 7.70      | 13.63     | 4.05      | 14.85      | 4.15       | 0.44       | 1.10       |

**TABLE 3. Log<sub>2</sub> fold changes of selected gene transcription levels used in clustering pathway analyses in Figures 2 and 3. D<sub>i</sub>, i-day after primary immunization. D22 and D24 mean 1 and 3 days after the secondary immunization.**

| Gene ID                              | Gene Name    | D1    | D3    | D5    | D8    | D12   | D21   | D22   | D24   |
|--------------------------------------|--------------|-------|-------|-------|-------|-------|-------|-------|-------|
| <b>Innate Immunity</b>               |              |       |       |       |       |       |       |       |       |
| <b>Virus Recognition</b>             |              |       |       |       |       |       |       |       |       |
| BU200466                             | Type 1 TLR2  | 1.54  | 2.06  | 0.61  | 0.02  | 0.47  | -0.60 | -0.74 | -0.75 |
| BU471724                             | TLR6         | 0.34  | 0.51  | 1.81  | 0.15  | 0.19  | 0.44  | 0.03  | -0.04 |
| BU242827                             | TLR3         | 2.02  | 0.71  | 0.45  | -0.35 | -0.14 | -0.25 | -0.33 | -0.79 |
| BU435893                             | TLR7         | 1.94  | 2.35  | 1.60  | 0.56  | 0.95  | -0.20 | -0.55 | 0.00  |
| BU382078                             | RIG-1        | 3.03  | 2.61  | 2.77  | 1.82  | -0.50 | 0.49  | 0.58  | 0.98  |
| <b>Cytokine IL and IFN responses</b> |              |       |       |       |       |       |       |       |       |
| BG625387                             | IL-1beta     | 3.43  | 2.45  | 1.09  | 0.58  | 0.56  | 1.34  | 0.75  | 0.68  |
| BU324362                             | IL-4R        | 0.07  | 0.78  | 0.04  | 0.35  | 1.02  | 0.06  | 0.13  | 0.01  |
| BU322177                             | IL10R        | 2.78  | 1.24  | 0.74  | 0.24  | -1.19 | 0.19  | 0.37  | 0.19  |
| BU468733                             | γc           | 1.28  | 2.02  | 2.14  | 1.51  | -0.89 | 0.08  | 0.05  | 0.99  |
| BU457953                             | Mx           | 4.20  | 2.02  | 1.43  | 0.17  | -0.42 | 0.04  | 0.73  | -0.77 |
| BU425330                             | VLIG-1       | 4.34  | 2.41  | 3.14  | 0.44  | 0.29  | -0.12 | -0.28 | -0.45 |
| AI980049                             | IRF-1        | 2.11  | 1.01  | 1.27  | 0.07  | 0.41  | 0.08  | 0.32  | -0.13 |
| <b>Complement</b>                    |              |       |       |       |       |       |       |       |       |
| BU247397                             | C1q          | 1.59  | 1.31  | 1.29  | 0.66  | -0.43 | -0.47 | -0.24 | -0.35 |
| BU344075                             | C1s          | 3.11  | 2.65  | 3.08  | 0.86  | -0.31 | 1.25  | 0.70  | 0.99  |
| BU476741                             | C3a          | 2.25  | 2.47  | 2.05  | 1.06  | 1.23  | 0.02  | 0.05  | 0.18  |
| BU375371                             | C4           | 0.40  | 1.02  | 0.82  | 1.57  | -0.89 | -0.45 | 0.28  | 0.56  |
| AI981748                             | H Factor     | -1.06 | -0.84 | -1.02 | -0.36 | -0.16 | -0.09 | 0.07  | -0.21 |
| <b>Monocyte and macrophage</b>       |              |       |       |       |       |       |       |       |       |
| AI981644                             | Spi-1/PU.1   | 1.41  | 1.08  | 1.09  | 0.59  | 1.20  | 0.25  | 0.12  | 0.32  |
| BU298713                             | GMF-beta     | 1.22  | 1.04  | 1.35  | 0.83  | 0.71  | 0.89  | 0.33  | 0.49  |
| AI981893                             | M-CSFR       | 1.79  | 1.48  | 0.87  | 0.22  | -0.12 | -0.23 | -0.12 | -0.18 |
| AI980743                             | Cathepsin S  | 1.12  | 1.27  | 1.01  | 0.61  | -0.70 | 0.22  | 0.27  | 0.12  |
| <b>Inflammation</b>                  |              |       |       |       |       |       |       |       |       |
| AJ446979                             | ASHI         | 1.18  | 1.45  | 1.58  | 1.05  | -0.40 | -0.06 | -0.14 | 0.23  |
| AJ394103                             | p47-phox     | 1.00  | 1.50  | 1.40  | 1.53  | 1.43  | 0.62  | 0.66  | 0.71  |
| BU419337                             | p40-phox     | 2.06  | 1.52  | 0.93  | 0.23  | 0.68  | -0.42 | -0.59 | -0.23 |
| BU133108                             | BPI          | 4.69  | 2.01  | 2.29  | 0.46  | 0.58  | 0.29  | -0.34 | 0.09  |
| BU376038                             | HMT          | -0.33 | -1.03 | -2.39 | -2.37 | -0.76 | -1.12 | -0.41 | -1.17 |
| AI979826                             | HMG1         | -0.06 | 1.29  | 1.61  | 0.69  | -2.02 | 0.45  | 0.11  | 0.20  |
| AI981624                             | P2X7         | 2.42  | 1.20  | 1.62  | 0.70  | 0.89  | 0.20  | 0.30  | 0.37  |
| <b>Apoptosis related genes</b>       |              |       |       |       |       |       |       |       |       |
| BU377655                             | CD82         | 1.00  | 1.13  | 1.28  | 0.83  | 0.05  | 1.97  | 0.06  | 1.38  |
| AI981754                             | STAT-1       | 2.57  | 1.77  | 1.89  | 0.49  | -0.59 | 0.32  | 0.31  | 0.01  |
| BU419580                             | STAT-4       | 1.75  | 1.33  | 1.56  | 0.51  | -0.32 | -0.24 | -0.23 | 0.16  |
| BU241925                             | CARD9        | 1.61  | 1.86  | 1.28  | 0.46  | -0.64 | -0.12 | -0.18 | -0.01 |
| BU288166                             | CARMA1       | 0.48  | 1.06  | 1.24  | 0.29  | 1.54  | -0.31 | -0.46 | 0.05  |
| BU114693                             | Caspase-3    | 1.50  | 0.49  | 0.85  | 0.28  | -0.23 | -0.04 | -0.24 | 0.17  |
| BU121710                             | Caspase-6    | 1.44  | 1.38  | 0.57  | 0.41  | 0.23  | -0.02 | 0.11  | -0.08 |
| BF845754                             | Cytochrome c | 0.53  | -4.85 | -2.89 | -6.15 | -0.50 | -0.96 | -1.01 | -0.07 |
| <b>Adaptive Immunity</b>             |              |       |       |       |       |       |       |       |       |

| <b>T cell signaling</b>       |             |       |       |       |       |       |       |       |       |
|-------------------------------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
| BG625359                      | L-plastin   | 3.51  | 3.28  | 3.24  | 1.87  | 0.73  | 0.85  | 0.87  | 1.13  |
| BU376258                      | MMP3        | 7.36  | 5.57  | 4.81  | 4.00  | 2.64  | 1.24  | 0.68  | 0.46  |
|                               | Tyrosine    |       |       |       |       |       |       |       |       |
| AI979785                      | kinase      | 1.47  | 1.09  | 1.40  | 0.55  | 0.47  | 0.58  | 0.41  | 0.48  |
| BU374370                      | GRAP        | 0.94  | 1.27  | 1.51  | 1.20  | -0.57 | 0.01  | -0.06 | 0.52  |
| AI980859                      | ZAP-70      | 1.27  | 1.31  | 2.03  | 1.14  | 0.41  | -0.10 | 0.12  | 0.52  |
| BG625390                      | TCR zeta    | 1.33  | 1.93  | 2.11  | 1.58  | 1.43  | -0.21 | 0.42  | 0.87  |
| BG625372                      | TCR beta    | 2.01  | 2.49  | 3.01  | 2.74  | 2.64  | 0.82  | 0.97  | 1.76  |
|                               | CD3         |       |       |       |       |       |       |       |       |
| BU492201                      | delta/gamma | 1.84  | 2.27  | 3.01  | 2.79  | 1.19  | 0.94  | 1.06  | 1.70  |
| AI980528                      | CD3 epsilon | 1.62  | 1.83  | 2.80  | 1.79  | 0.46  | 0.67  | 0.64  | 1.33  |
| AI981465                      | CD83        | 2.13  | 0.88  | 1.01  | 0.65  | 0.22  | 0.57  | 0.55  | 0.32  |
| BU245144                      | CD80        | 1.41  | 0.48  | 1.47  | 0.00  | 0.12  | 1.97  | 0.43  | 0.84  |
|                               | MHC II-M    |       |       |       |       |       |       |       |       |
| BU294829                      | beta        | 1.61  | 1.39  | 1.01  | 0.86  | -0.61 | 0.20  | 0.02  | 0.08  |
|                               | MHC II      |       |       |       |       |       |       |       |       |
| BU119964                      | invariant   | 1.16  | 1.24  | 1.32  | 0.97  | -0.66 | 0.32  | 0.38  | 0.58  |
| <b>Th1 response effectors</b> |             |       |       |       |       |       |       |       |       |
| BG625689                      | MHC I B-F   | 0.75  | 1.21  | 0.83  | 0.91  | -0.92 | -0.40 | 0.48  | 0.67  |
| BU233555                      | Ikaros      | 0.62  | 1.33  | 1.97  | 0.56  | 0.22  | 0.00  | -0.43 | 0.20  |
| AI980159                      | CD8 alpha   | 0.06  | 0.56  | 1.19  | 1.65  | 0.25  | -0.40 | -0.14 | 0.31  |
| BG625508                      | Granzyme A  | 2.20  | 1.03  | 4.18  | 2.69  | 2.05  | -0.21 | -0.19 | -0.31 |
| BU299622                      | Granzyme K  | 1.02  | 0.70  | 2.09  | 2.28  | 1.23  | 0.46  | 0.56  | 0.92  |
| BU447879                      | Porimin     | 2.49  | 1.16  | 2.46  | 0.38  | -0.77 | 1.78  | 0.83  | 0.31  |
| AI981685                      | Fas         | 2.17  | 0.44  | 1.07  | 0.48  | 0.33  | 0.09  | 0.25  | 0.17  |
| BU212557                      | Fas L       | 1.25  | 0.25  | 1.18  | 0.44  | 0.04  | 0.32  | 0.17  | 0.12  |
| <b>Th1 molecular markers</b>  |             |       |       |       |       |       |       |       |       |
| BU242857                      | WC1.1       | 2.42  | 2.15  | 1.21  | 0.11  | -1.45 | -0.44 | -0.22 | -0.08 |
| BG625445                      | Rac2        | 1.84  | 1.71  | 2.15  | 1.82  | 0.51  | 0.61  | 0.69  | 1.22  |
| BU207995                      | Tim3        | 1.34  | 1.28  | 1.33  | 1.22  | 1.23  | -0.03 | -0.01 | 0.29  |
| AW773238                      | Mg11        | 2.93  | 1.70  | 2.07  | 0.92  | 0.55  | 0.59  | 0.51  | 0.68  |
| <b>B cell signaling</b>       |             |       |       |       |       |       |       |       |       |
| AI981631                      | SOCS-3      | 3.05  | 1.50  | 1.07  | 0.14  | 0.16  | -0.04 | -0.15 | -0.03 |
| BU294989                      | PBEF        | 2.48  | 1.18  | 1.32  | -0.17 | 1.02  | 0.26  | 0.29  | 0.17  |
| BU249559                      | SAMSN-1     | 1.86  | 1.61  | 2.47  | 0.75  | 0.78  | 0.30  | -0.21 | 0.67  |
| BU481163                      | ChB1        | 1.71  | 1.96  | 1.64  | 0.65  | -0.09 | -0.17 | -0.15 | 0.30  |
| BU249956                      | EZH2        | 1.07  | 1.51  | 2.71  | 0.92  | -0.10 | 1.44  | 0.20  | 0.34  |
| BU483469                      | CD138       | 2.15  | 1.19  | 2.07  | 0.13  | -0.55 | 1.78  | 0.14  | 0.39  |
| BF845749                      | BCAP        | 0.46  | 1.46  | 2.33  | 1.36  | -1.43 | 2.67  | 0.07  | 1.58  |
| <b>Ig molecules</b>           |             |       |       |       |       |       |       |       |       |
| BG625356                      | Ig gamma    | -1.07 | 0.23  | 2.35  | 2.73  | 0.02  | 2.35  | 2.31  | 2.96  |
| BG625386                      | Ig mu       | -1.11 | -0.63 | 0.95  | 0.79  | -3.08 | -0.22 | 0.52  | -0.52 |
| AW773313                      | Ig J        | -1.49 | -1.03 | 1.31  | 0.97  | 0.51  | 0.33  | 0.35  | -0.09 |
| <b>Regulatory factors</b>     |             |       |       |       |       |       |       |       |       |
| BU107503                      | TGF-beta    | -0.35 | -0.27 | -1.23 | -1.13 | -1.85 | -0.28 | -0.19 | -0.44 |
| BU108381                      | TGFR        | -0.92 | -1.16 | -1.78 | -1.17 | 0.37  | -0.30 | -0.05 | -0.34 |