Supplementary Material

**Supplementary Table 1.** Pro-inflammatory, anti-inflammatory, and housekeeping genes.

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Description** | **NCBI Reference Sequence** | **Efficiency**  **(%)\*** |
| **Pro-inflammatory** | | |  |
| COX2 | *Mus musculus* mRNA for prostaglandin synthase cyclooxygenase, 3'UTR | NM\_011198.3 | 90.54 |
| IFN-γ | *Mus musculus* interferon gamma (Ifng), mRNA | NM\_008337.3 | 96.12 |
| IL-12b | *Mus musculus* interleukin12b (IL12b), protein code | NC\_000077.6 | 100.00 |
| Il-17F | *Mus musculus* interleukin 17F (Il17f), mRNA | NM\_145856.2 | 100.00 |
| IL-1b | *Mus musculus* interleukin 1 beta (Il1b), mRNA | NM\_008361.3 | 100.00 |
| IL-2 | *Mus musculus* interleukin 2 (Il2), mRNA | NM\_008366.3 | 100.00 |
| IL-6 | *Mus musculus* interleukin 6 (Il6), mRNA | NM\_031168.1 | 111.35 |
| PGER2 | *Mus musculus* prostaglandin E receptor 2 (subtype EP2) (Ptger2), mRNA | NM\_008964.4 | 112.33 |
| PGER4 | *Mus musculus* prostaglandin E receptor 4 (subtype EP4) (Ptger4), transcript variant 2, mRNA | NM\_008965.1 | 107.19 |
| PGES2 | *Mus musculus* prostaglandin E synthase 2 (Ptges2), mRNA | NM\_133783.2 | 94.14 |
| TNF | *Mus musculus* tumor necrosis factor (Tnf), mRNA | NM\_013693.2 | 101.35 |
| **Anti-inflammatory** | | |  |
| CD39 | *Mus musculus* ecto-apyrase CD39 mRNA, complete cds | AF037366.1 | 102.79 |
| CD73 | *Mus musculus* 5' nucleotidase, ecto (Nt5e), mRNA | NM\_011851.4 | 109.17 |
| CTLA-4 | *Mus musculus* cytotoxic T-lymphocyte-associated protein 4 (Ctla4), mRNA | NM\_009843.3 | 111.48 |
| DAXX/FAS | Daxx Fas death domain-associated protein, protein code | NC\_000083.6 | 112.31 |
| FASL | *Mus musculus* Fas ligand (TNF superfamily, member 6) (Fasl), transcript variant 1, mRNA | NM\_010177.4 | 100.00 |
| EBI3 | *Mus musculus* Epstein-Barr virus induced gene 3 (Ebi3), mRNA | NM\_015766.2 | 87.41 |
| IL-12a | *Mus musculus* interleukin 12a (Il12a), transcript variant 2, mRNA | NM\_008351.2 | 119.25 |
| GITR V1 | *Mus musculus* tumor necrosis factor receptor superfamily, member 18 (Tnfrsf18), transcript variant 1, mRNA | NM\_009400.2 | 83.06 |
| GRANZYME a | *Mus musculus* granzyme A (Gzma), mRNA | NM\_010370.2 | 114.56 |
| GRANZYME b | *Mus musculus* granzyme B (Gzmb), mRNA | NM\_013542.2 | 108.88 |
| IDO1 | *Mus musculus* indoleamine 2,3-dioxygenase 1 (Ido1), mRNA | NM\_008324.1 | 100.00 |
| IL-4 | *Mus musculus* interleukin 4 (Il4), transcript variant 1, mRNA | NM\_021283.2 | 100.00 |
| IL-10 | *Mus musculus* interleukin 10 (Il10), mRNA | NM\_010548.2 | 100.00 |
| LAG-3 | *Mus musculus* lymphocyte-activation gene 3 (Lag3), mRNA | NM\_008479.2 | 85.88 |
| PD1 | *Mus musculus* programmed cell death 1 (Pdcd1), mRNA | NM\_008798.2 | 87.90 |
| PDL1 | *Mus musculus* CD274 antigen (Cd274), mRNA | NM\_021893.3 | 102.03 |
| PDL2 | *Mus musculus* programmed cell death 1 ligand 2 (Pdcd1lg2), mRNA | NM\_021396.2 | 94.96 |
| PRF1 | *Mus musculus* perforin 1 (pore forming protein), protein coding | NC\_000076.6 | 100.00 |
| TGF-β1 | *Mus musculus* transforming growth factor, beta 1 (Tgfb1), mRNA | NM\_011577.1 | 87.78 |
| TGF-β2 | *Mus musculus* transforming growth factor, beta 2 (Tgfb2), mRNA | NM\_009367.3 | 102.50 |
| TRAIL | TNF-related apoptosis-inducing ligand, protein code | NC\_000069.6 | 97.08 |
| VIP | *Mus musculus* vasoactive intestinal polypeptide (Vip), mRNA | NM\_011702.2 | 100.00 |
| Housekeeping gene | | |  |
| GAPDH | *Mus musculus* glyceraldehyde-3-phosphate dehydrogenase, protein coding | NC\_000072.6 | 102.31 |



**Supplementary Figure 1. Phenotypic characterization of cell populations.** A) Representative example of Treg (CD4+CD25+FOXP3+) and activated CD127+ T cells (CD4+CD25+FOXP3−CD127+). B) Representative example of proliferation of Tregs (CD4+CD25+FOXP3+). C) Representative example of naïve T cells (CD3+CD4+ CD62LhiCD44low), effector T cells (CD3+CD4+CD62LlowCD44hi), memory T cells (CD3+CD4+CD62LhiCD44hi), early activated CD4 (CD3+CD4+CD69+) or CD8 (CD3+CD8+CD69+) T cells, late activated CD4 (CD3+CD4+CD38+) or CD8 (CD3+CD8+CD38+) T cells, B cells (CD19+), plasma cells (CD19lowCD138hi), NK T cells (CD49b+CD3+), and monocytes (CD11b+F4/80+).



**Supplementary Figure 2.** Correlation between the levels of Treg cells and those of activated CD127+ cells from peritoneum. A) BALB/cAnN and B) C57BL/6 mice. In total, 24 infected mice of each strain (six for each time point) were analyzed. The Spearman’s correlation coefficient and *P*-value are shown on the plot.



**Supplementary Figure 3.** Parasite number in the site of infection (peritoneum) in susceptible, BALB/cAnN and non-susceptible, C57BL/6 mice on day 5, 30, 90, and 130 post-infection. Six infected mice and 6 controls were included for each time point analyzed. Significant differences were determined by the U Mann-Whitney test. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.005.

**Supplementary Table 2.** Percentage of immune CD4+ T cells and naïve-like cells in peritoneum, lymph nodes, and spleen of susceptible (BALB/cAnN) and non-susceptible (C57BL/6) mice.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Day post-infection** | | | | | |
| **Cell phenotypes** | **Uninfected controls** | **5** | **30** | **90** | **130** |
| **T CD4 cells** | **PERITONEUM** | | | | |
| BALB/cAnN | 8.47±5.80 | 11.76±4.97 | 10.79±4.57 | 13.85±3.54\*\* | 6.25±3.43 |
| C57BL/6J | 13.24±6.90 | 21.71±4.80\*\*\*b | 21.29±4.96\*\*c | 12.37±5.76 | 18.98±3.21\*\*c |
| **Naïve-like cellsΩ** |  |  |  |  |  |
| BALB/cAnN | 52.74±19.34 | 16.57±2.95\*\*\* | 29.14±9.16 | 36.15±13.69 | 6.38±2.42\*\*\* |
| C57BL/6J | 86.59±7.34 | 86.46±6.09c | 95.36±1.47\*\*\*c | 92.79±2.51\*b | 93.57±1.84a |
| **CD4 T cells** | **LYMPH NODES** | | | | |
| BALB/cAnN | 44.22±9.98 | 45.35±10.03 | 41.81±15.26 | 43.10±5.11 | 41.75±4.70 |
| C57BL/6J | 34.50±4.89 | 36.00±3.15 | 35.17±3.52 | 32.98±2.67c | 30.30±4.36\*b |
| **Naïve-like cellsΩ** |  |  |  |  |  |
| BALB/cAnN | 39.41±25.97 | 15.82±9.80 | 37.32±25.78 | 35.23±11.22 | 20.56±6.18 |
| C57BL/6J | 89.72±6.90 | 90.18±1.94a | 92.94±2.13b | 87.08±6.21b | 89.30±4.25\*a |
| **CD4 T cells** | **SPLEEN** | | | | |
| BALB/cAnN | 32.60±4.77 | 28.25±2.99\* | 25.60±1.50\*\*\* | 36.41±1.64\* | 30.27±3.54 |
| C57BL/6J | 24.60±5.24 | 16.69±2.67\*\*\*c | 25.32±4.82 | 29.62±4.03\*\*b | 18.16±4.83\*\*c |
| **Naïve-like cellsΩ** |  |  |  |  |  |
| BALB/cAnN | 35.52±11.87 | 22.66±9.81\* | 36.89±8.84 | 18.47±3.30\*\*\* | 21.54±4.18\*\*\* |
| C57BL/6J | 84.66±6.71 | 79.90±3.99b | 92.88±1.30\*\*\*b | 93.54±2.83\*\*b | 79.46±9.78a |

ΩNaïve-like lymphocytes (CD4+CD25-Foxp3-CD127+). Comparison at each time with non-infected controls. Six infected mice and 6 controls were included for each time point analyzed. \**P* < 0.05, \*\**P* < 0.001, \*\*\**P* < 0.0001. Comparison between mouse strains. a *P* < 0.05, b *P* < 0.001, and c *P* < 0.0001.



**Supplementary Figure 4.** Percentage of regulatory T cells in the peritoneum at the time of infection (day 0) in susceptible BALB/cAnN and non-susceptible C57BL/6 mice after either anti-CD25 (PC61) or isotype (YCATE) treatment. Four mice were used for each condition analyzed. Flow cytometry results are reported as a mean and standard deviation (bar graph). Significant differences in cell percentages as determined by the U Mann-Whitney test are shown as \* *P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.005.



**Supplementary Figure 5.** Percentage of regulatory T cells in the peritoneum on day 5, 15, and 30 after infection in susceptible BALB/cAnN and non-susceptible C57BL/6 mice treated with either anti-CD25 (PC61) or isotype (YCATE). Six infected mice for each time (days 5, 15, and 30) and each condition (isotype or depleted) were included, as well as six non-infected animals of each strain. Flow cytometry results are reported as a mean and standard deviation (bar graph). Significant differences in cell percentages as determined by the U Mann-Whitney test are shown as \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.005.



**Supplementary Figure 6.** Treg removal allows to mount an anti-tumor response in the non-susceptible strain. Melanoma B16 cells (2 105) were subcutaneously injected in C57BL/6 mice treated with either anti-CD25 (PC61) or isotype (YCATE) two days before tumor inoculation. Tumor size was measured as the product of the greatest longitudinal diameter (length) greatest transverse diameter (width). Six infected mice for each group were analyzed. Significant differences were determined by the Wilcoxon signed-rank test. \**P* < 0.05.





**Supplementary Figure 7.** Kinetic of immune populationsin susceptible BALB/cAnN and non-susceptible C57BL/6 mice treated with either anti-CD25 (PC61) or isotype (YCATE). A) Early activated CD4+ cells, B) late activated CD4+ cells, C) early activated CD8+, D) late activated CD8+, E) memory T cells, F) B cells, G) NKT cells. Six infected mice for each time (days 5, 15, and 30) and each condition (isotype or depleted) were included, as well as six non-infected animals of each strain. Flow cytometry results are reported as a mean and standard deviation (bar graph). Significant differences in cell percentages as determined by the U Mann-Whitney test are shown as \**P* < 0.05, \*\**P* < 0.01. Significant differences between strains were determined by the U Mann-Whitney test. Superscript letters indicate: a P < 0.05, b \*\*P < 0.01, and c \*\*P < 0.005.