**Supplementary Table 1**

Genotypic differences between NY99 infectious clone and WNV strain 3356 infectious clone

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Nucleotide Position** | **WNV NY99 Clone Nucleotide** | **WNV 3356 Clone Nucleotide** | **Viral Protein Position** | **WNV NY99 Clone Residue** | **WNV 3356 Clone Residue** |
| 625 | C | U | prM-54 | P | S |
| 1428 | U | C | E-154 | N | N |
| 1599 | C | U | E-211 | F | F |
| 1855 | C | U | E-297 | L | L |
| 1974 | C | U | E-336 | C | C |
| 2321 | C | U | E-452 | S | L |
| 2562 | G | A | NS1-31 | R | R |
| 3707 | U | C | NS2A-61 | V | A |
| 3840 | U | C | NS2A-105 | I | I |
| 3880 | U | C | NS2A-119 | Y | H |
| 4195 | G | A | NS2A-224 | A | T |
| 4257 | A | G | NS2B-12 | L | L |
| 4922 | G | A | NS3-104 | R | K |
| 5865 | C | U | NS3-418 | F | F |
| 7015 | U | C | NS4B-34 | L | L |
| 7029 | U | G | NS4B-38 | P | P |
| 7797 | G | A | NS5-39 | A | A |
| 7826 | U | C | NS5-49 | V | A |
| 8811 | U | C | NS5-377 | N | N |
| 8859 | C | A | NS5-393 | P | P |
| 8862 | A | G | NS5-394 | R | R |
| 8880 | A | G | NS5-400 | E | E |
| 8958 | A | G | NS5-426 | E | E |
| 9123 | C | U | NS5-481 | L | L |
| 9660 | C | U | NS5-660 | L | L |
| 10613 | C | U | 3’ UTR | - | - |
| 10851 | A | G | 3’ UTR | - | - |

Amino acid residues in blue text indicate non-synonymous substitutions.

**Supplementary Table 2**

Comparison of NY99ic and NS5 mutants at 36 hpi in A549 cell culture supernatant.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Low detection** | **No change** | **Statistical change** |
| **Pro-Inflammatory** | IL-1β | IFN-γ | IL-6 |
| IL-12 p70 | IFN-α2 | TNF-α |
|  | IFN-β |  |
| **Anti-Inflammatory/****Regulatory** | IL-1ra | IL-4 |  |
| IL-10 | IL-5 |  |
| IL-15 | IL-9 |  |
| IL-17 | IL-13 |  |
| **Growth/Differentiation** | PDGF | IL-2 | G-CSF |
|  | IL-7 |  |
|  | FGF |  |
|  | VEGF |  |
|  | GM-CSF |  |
| **Chemokines** |  | CXCL8 (IL-8) | CXCL10 (IP-10) |
|  | CCL11 (Eotaxin) | CCL2 (MCP-1) |
|  | MIP-1α | CCL5 (RANTES) |
|  | MIP-1β |  |

Low detection refers to cytokines that were detected in quantities < 1 pg/mL and were not detected in all replicates tested. Significance comparing the mutants and mock to NY99ic was tested using a Kruskal-Wallis test with Dunn’s multiple comparisons.

IL = interleukin, IFN = interferon, TNF = tumor necrosis factor, PDGF = platelet-derived growth factor, FGF = fibroblast growth factor, VEGF = vascular endothelial growth factor, GM-CSF = granulocyte-macrophage colony-stimulating factor, G-CSF = granulocyte colony-stimulating factor, CXCL and CCL = chemokine ligand, MIP = macrophage inflammatory protein, IP = interferon gamma-induced protein, MCP = monocyte chemoattractant protein, RANTES = regulated on activation, normal T cell expressed and secreted