

Supplementary materials

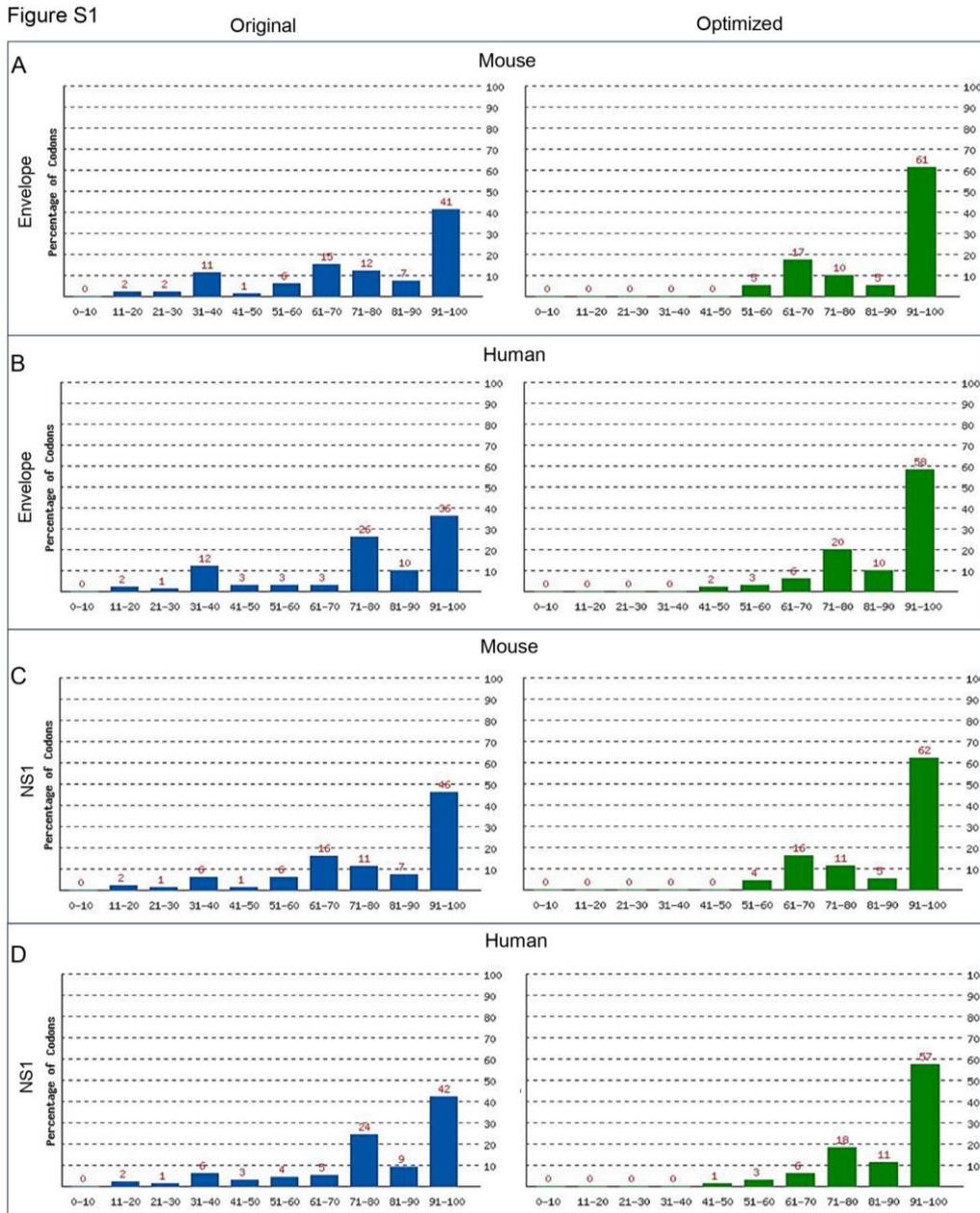


Figure S1. Optimized codon frequency of the sequences that encode the E and NS1 proteins. The percentage of codons of the *e* (A, B) and *ns1* (C, D) genes that are commonly used in mouse (A, C) and human (B, D) cells is presented. The bars indicate the percentage of codons distributed according to their usage, before (original; blue) and after (optimized; green) optimization. The value of 100 is set for the codon with the highest usage frequency for a given amino acid in the desired expression organism.

Figure S2

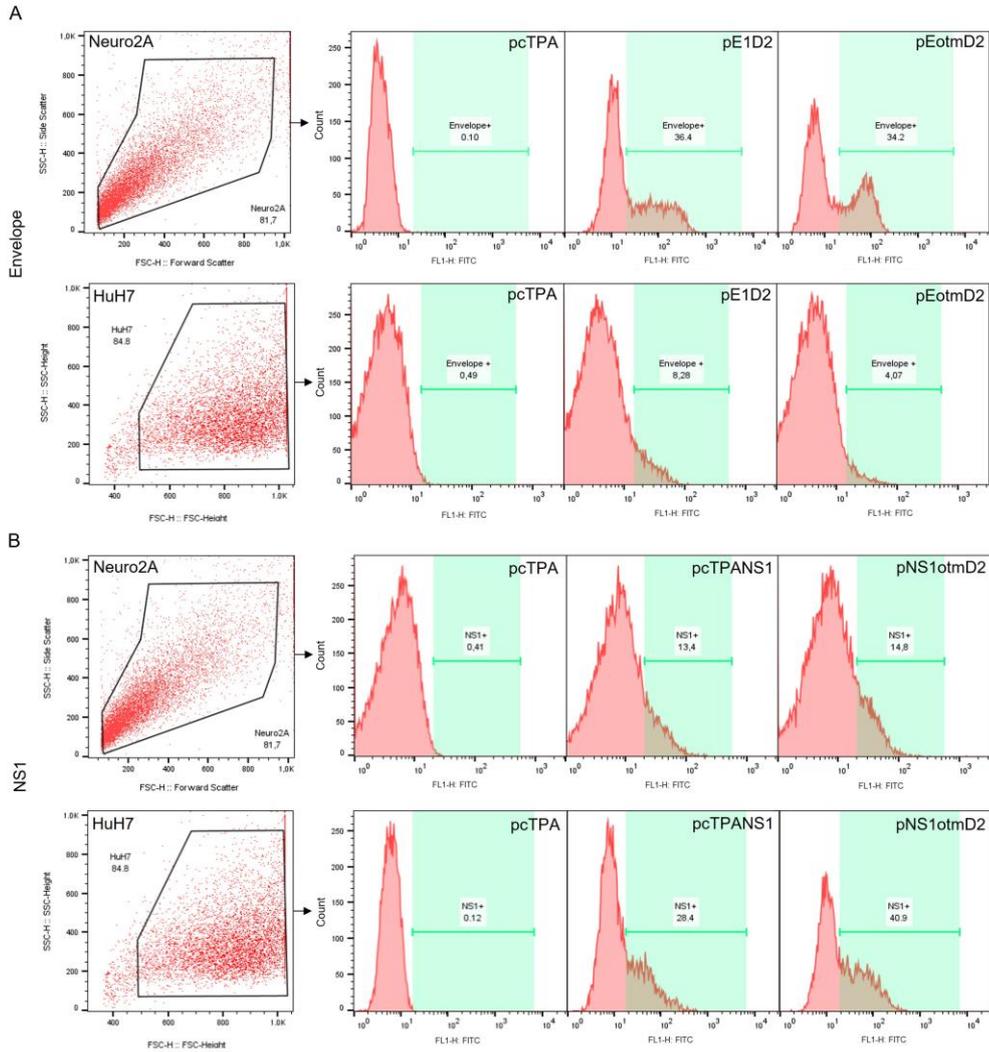


Figure S2. Flow cytometry analysis of Neuro2A and HuH7 cells transfected with the DNA vaccines to quantify expression of E and NS1 proteins. Representative flow cytometry dot plots and histograms displaying (A) E- and (B) NS1-positive frequencies in Neuro2A and HuH7 cells transfected with the DNA vaccines pE1D2, pEotmD2, pcTPANS1, pNS1otmD2 or the control pcTPA plasmid. After the 24h-transfection, cells were harvested and stained with specific antibodies for E and NS1 detection followed by incubation with goat anti-mouse IgG conjugated to Alexa Fluor 488. Gates for selection of populations containing E or NS1-positive Neuro2A and HuH7 cells were determined on histograms from the negative control cells transfected with pcTPA and incubated with the specific E and NS1 primary and secondary antibodies.