Figures

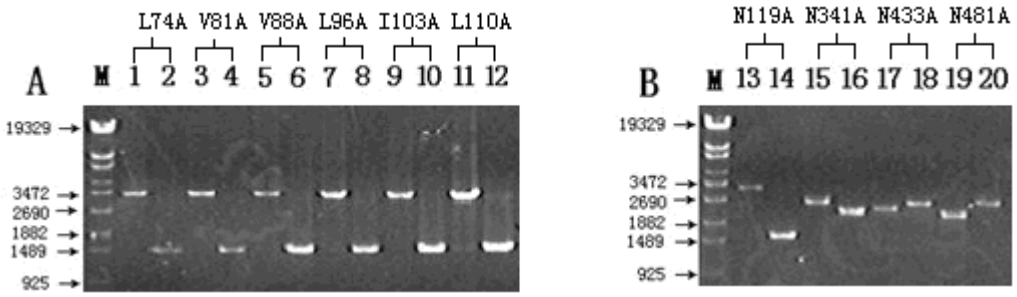


Fig. 1. A The agarose electrophoresis of PCR products of HRs-mutants. **B** PCR products of four glycosylation mutations. There was a short homologous sequence from reverse-complement mutagenesis primers every two products. The two products were transformed into TG1 cells to generate a complete plasmid containing the mutated HN gene. Product 1 and product 2 were used to get L74A as shown, 3 and 4 for V81A, 5 and 6 for V88A, and so on. M represented λ-EcoT14 digest DNA Marker (Takara Biotechnology Co. Ltd., Dalian, China). 1, L74A-P1; 2, L74A-P2；3, V81A-P1；4, V81A-P2；5, V88A-P1；6, V88A-P2；7, L96A-P1；8, L96A-P2；9, I103A-P1；10, I103A-P2；11, L110A-P1；12, L110A-P2; 13, N119A-P1; 14, N119A-P2; 15, N341A-P1; 16, N341A-P2; 17, N433A-P1; 18 N433A-P2; 19, N481A-P1; 20, N481A-P2

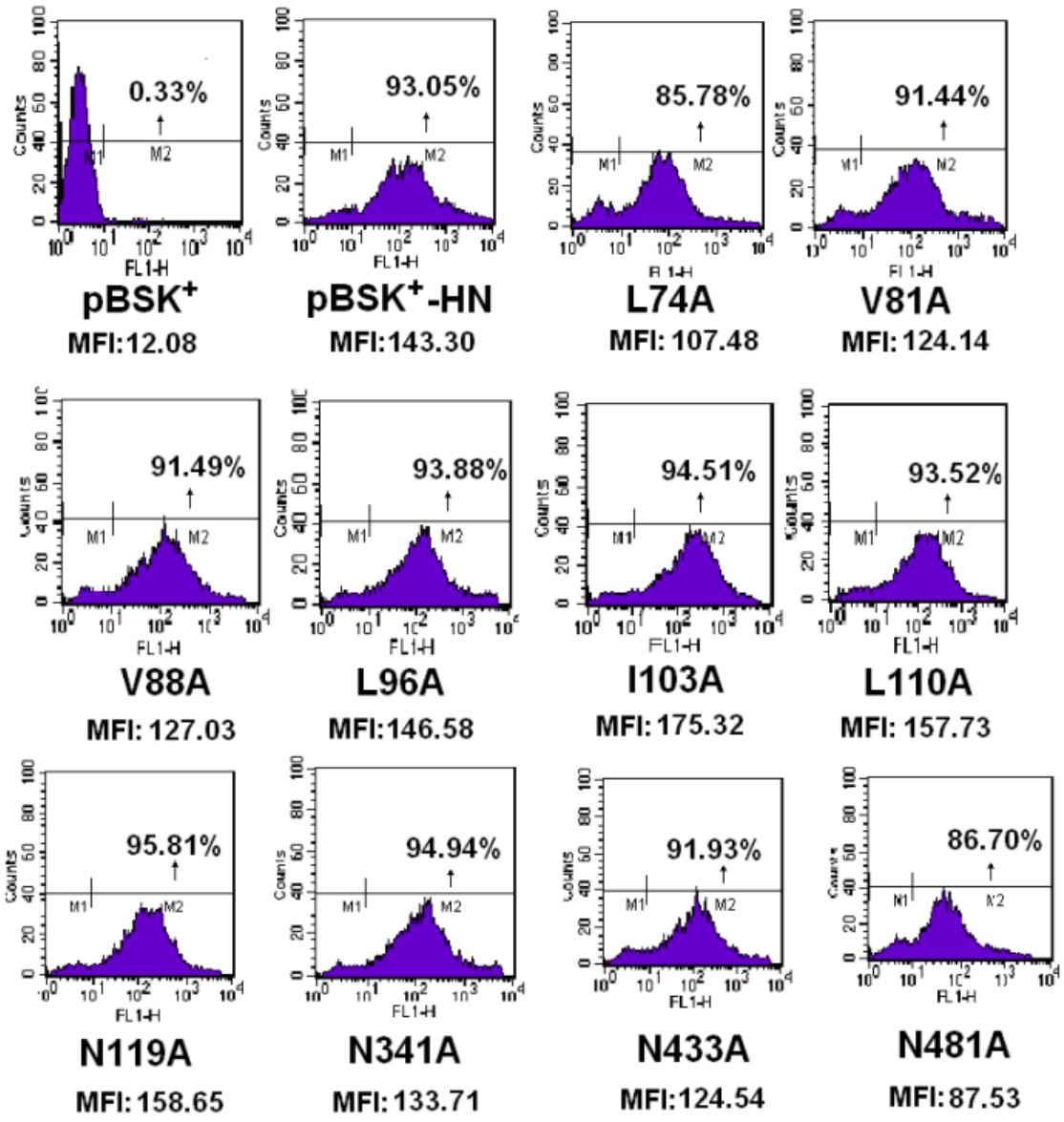


Fig. 2. Flow cytometry analyses of the levels of cell surface expression of mutant proteins. The x and y axes of the histograms represent fluorescein isothiocyanate and cell counts, respectively. M2 refers to the percentage of the number of positive cells with respect to the total cell count in each sample. The cell surface expression efficiency of each sample was assessed by median fluorescence intensity (MFI).

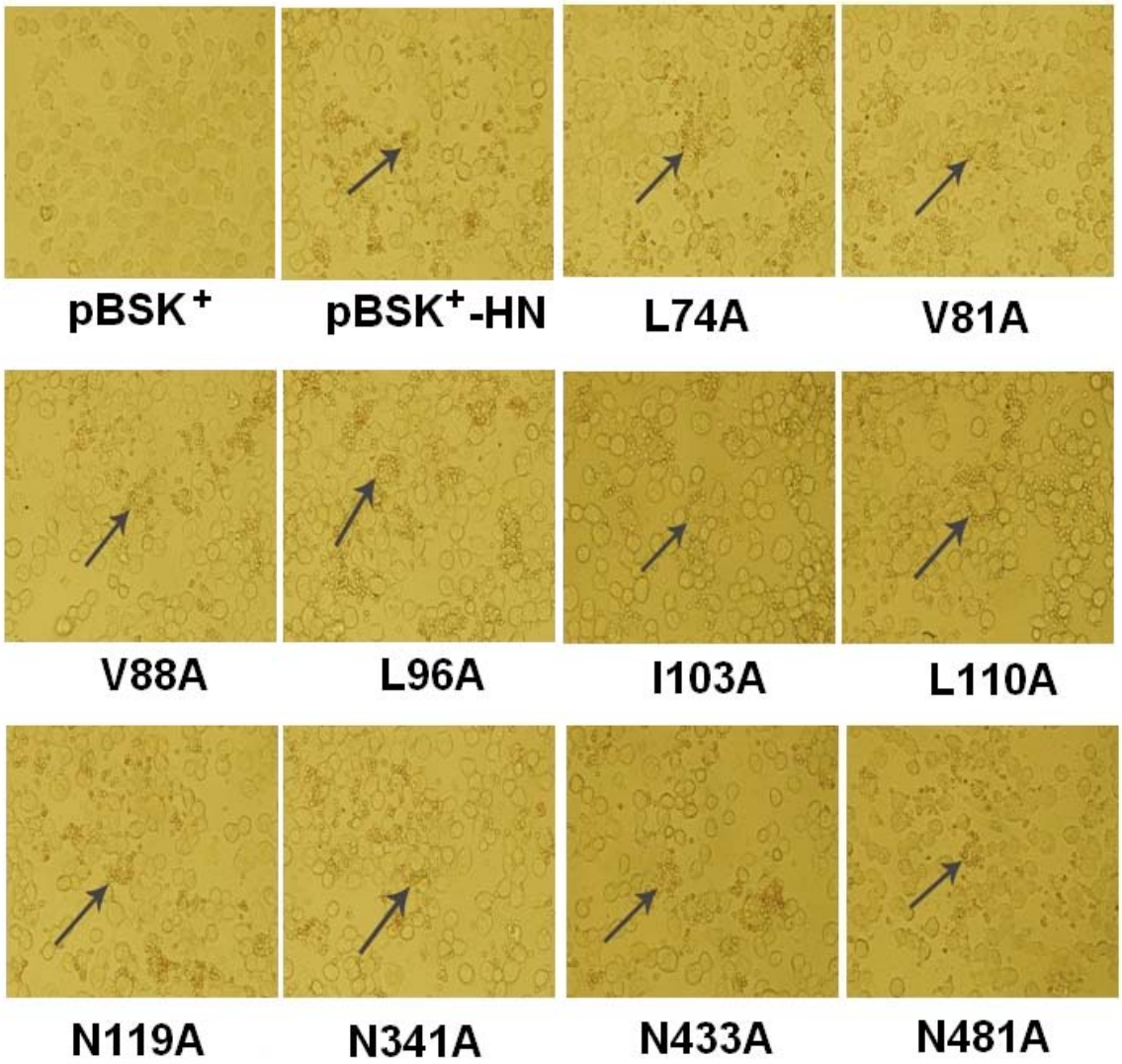
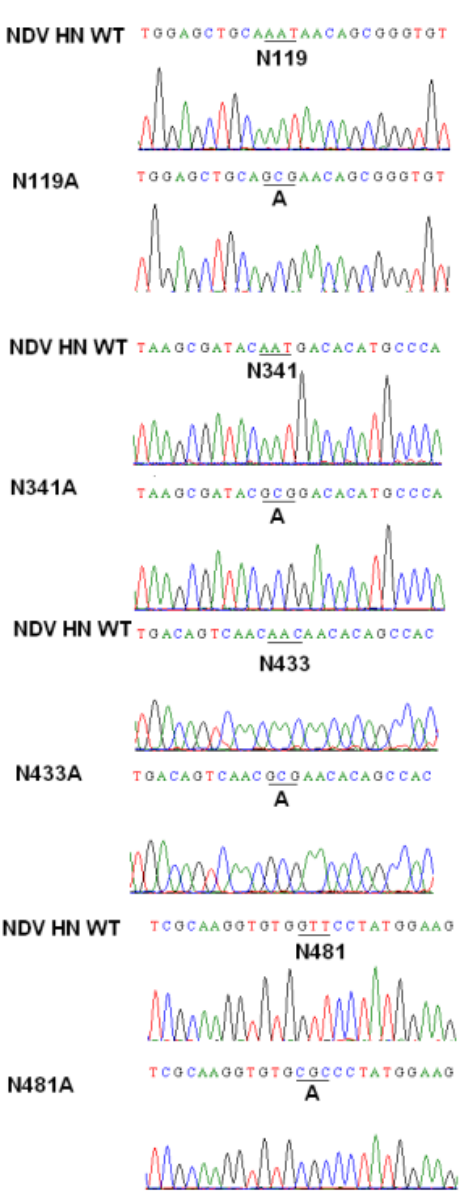


Fig. 3. Hemadsorption (HAD) activity was determined using guinea pig erythrocytes as described in Materials and Methods. Arrows indicate hemadsorbing spots.



	CSE (%)	HAD (%)	NA (%)	Fusion (%)
NDV HN WT	100.0±10.9	100.0±9.2	100.0±9.6	100.0±5.5
N119A	111.7±10.1[a]	97.2±3.5[a]	98.6±11.2[a]	98.4±7.0[a]
NDV HN WT	100.0±10.9	100.0±9.2	100.0±9.6	100.0±5.5
N341A	101.9±5.7[a]	112.1±6.9[a]	84.1±6.5[a]	88.6±4.9[a]
NDV HN WT	100.0±10.9	100.0±9.2	100.0±9.6	100.0±5.5
N433A	85.7±3.5[a]	112.6±3.6[a]	70.0±8.2[c]	109.3±4.4[a]
NDV HN WT	100.0±10.9	100.0±9.2	100.0±9.6	100.0±5.5
N481A	57.5±6.2[c]	33.4±3.9[c]	6.5±2.7[c]	66.2±2.3[c]

Fig. 4. The sequencing results and functions of mutants in the carbohydrate addition sites. NDV HN WT represents wide-type (wt) HN protein of NDV. The results are expressed as mean±the standard deviation for three independent experiments, a, P>0.05; b, P<0.05; c, P<0.01

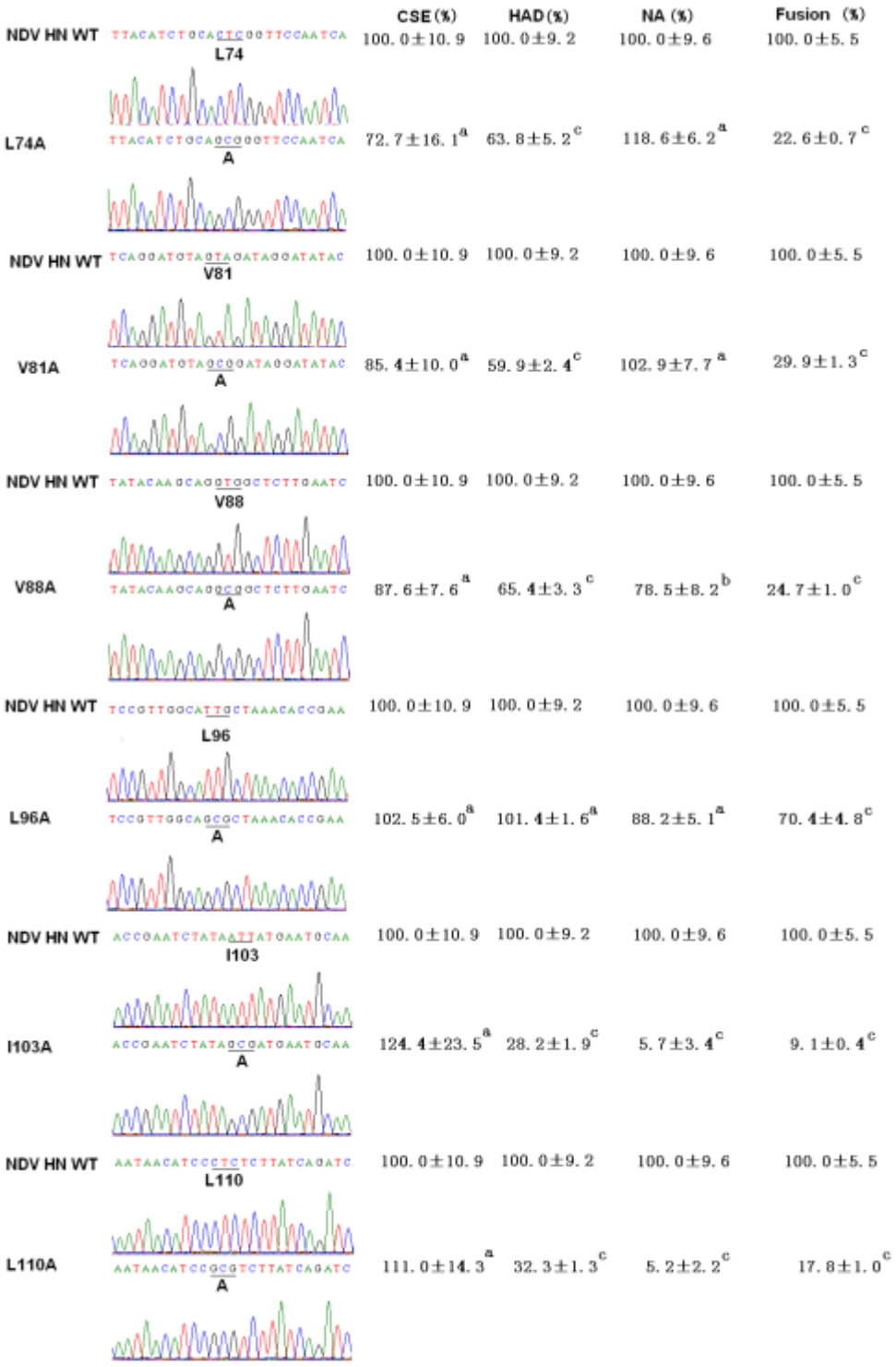


Fig. 5. The sequencing results and functions of mutants in the heptad repeat regions (HRs). The experiment was performed and the data were expressed as described in the legend to Fig. 4.