

Supplementary Materials

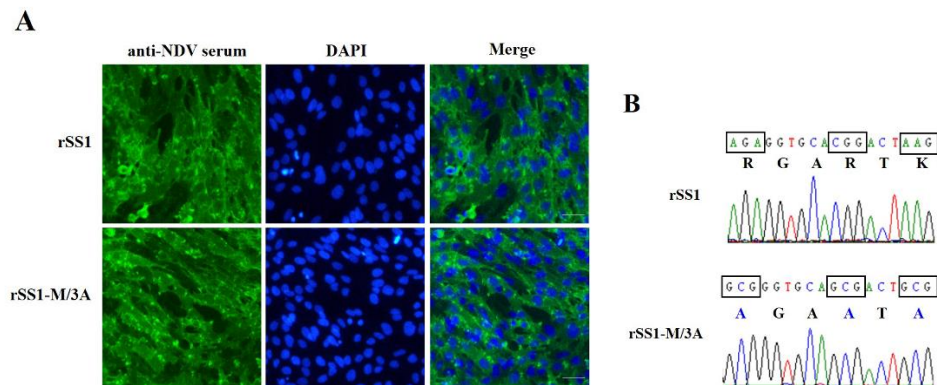


Figure S1. Identification of the rescued virus rSS1-M/3A by IFA and DNA sequencing. (A) Characterization of rSS1-M/3A in virus-infected DF-1 cells by IFA. (B) Nucleotide sequence analysis of the M/3A mutations introduced in the rSS1-M/3A M gene after serially passed five times in 10-day-old SPF chicken eggs.

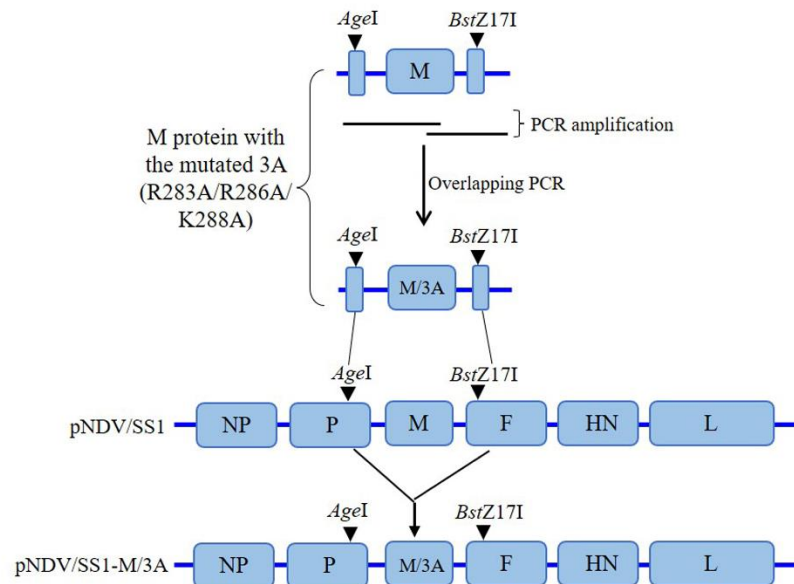


Figure S2. Schematic diagram of the mutation strategy for the construction of the full-length cDNA clone pNDV/SS1-M/3A.

Table S1. The information of primers used for the construction of recombinant plasmids.

Plasmid	Sense Primer (5'→3')	Anti-Sense Primer (5'→3')	Restriction Sites ^a
pCMV-HA-M(264-292)	<u>AATTC</u> GGCTCAATCTATCTGTTGG GCTCAGTGATGTGCTCGGGCCCTC TGTGCTTGTGAAGGCGAGAGGTG CACGGACTAAGCTACTTGCTCCTT C	<u>TCGAG</u> AAGGAGCAAGTAGCTTAGTC CGTGACCTCTCGCCTTCACAAGCA CAGAGGGCCCCGAGCACATCACTGA GCCCAACAGATAGATTGAGCCG	<i>EcoR</i> I / <i>Xho</i> I
pCMV-HA-M(289-313)	<u>AATTC</u> GGCTACTTGCTCCTTTCTTC TCTAGCAGTGGGACAGCCTGCTA TCCTATAGCAAATGCCTCTCCCCA GGTTGCCAAGTC	<u>TCGAG</u> ACTTGGCAACCTGGGGAGAG GCATTTGCTATAGGATAGCAGGCTG TCCCACTGCTAGAGAAGAAAGGAG CAAGTAGCCG	<i>EcoR</i> I / <i>Xho</i> I
pCMV-HA-M(264-280)	<u>AATTC</u> GGCTCAATCTATCTGTTGG GCTCAGTGATGTGCTCGGGCCCTC TGTGCTTGTGTC	<u>TCGAG</u> ACACAAGCACAGAGGGCCC GAGCACATCACTGAGCCCAACAGA TAGATTGAGCCG	<i>EcoR</i> I / <i>Xho</i> I
pCMV-HA-M(281-288)	<u>AATTC</u> GGAAGGCGAGAGGTGCAC GGACTAAGTC	<u>TCGAG</u> ACTTAGTCCGTGCACCTCTCG CCTTCCG	<i>EcoR</i> I / <i>Xho</i> I
pCMV-HA-M/mt1	<u>AATTC</u> GGCTCAATCTATCTGTTGG GCTCAGTGATGTGCTCGGGCCCTC TGTGCTTGTGGCGGCGAGAGGTG CACGGACTAAGCTACTTGCTCCTT TCTTCTCTAGCAGTGGGACAGCCT GCTATCCTATAGCAAATGCCTCTC CCCAGGTTGCCAAGTC	<u>TCGAG</u> ACTTGGCAACCTGGGGAGAG GCATTTGCTATAGGATAGCAGGCTG TCCCACTGCTAGAGAAGAAAGGAG CAAGTAGCTTAGTCCGTGCACCTCT CGCCGCCACAAGCACAGAGGGCCC GAGCACATCACTGAGCCCAACAGA TAGATTGAGCCG	<i>EcoR</i> I / <i>Xho</i> I
pCMV-HA-M/mt2	<u>AATTC</u> GGCTCAATCTATCTGTTGG GCTCAGTGATGTGCTCGGGCCCTC TGTGCTTGTGAAGGCGGCGGGTG CACGGACTAAGCTACTTGCTCCTT TCTTCTCTAGCAGTGGGACAGCCT GCTATCCTATAGCAAATGCCTCTC CCCAGGTTGCCAAGTC	<u>TCGAG</u> ACTTGGCAACCTGGGGAGAG GCATTTGCTATAGGATAGCAGGCTG TCCCACTGCTAGAGAAGAAAGGAG CAAGTAGCTTAGTCCGTGCACCCGC CGCCTTCACAAGCACAGAGGGCCC GAGCACATCACTGAGCCCAACAGA TAGATTGAGCCG	<i>EcoR</i> I / <i>Xho</i> I
pCMV-HA-M/mt3	<u>AATTC</u> GGCTCAATCTATCTGTTGG GCTCAGTGATGTGCTCGGGCCCTC TGTGCTTGTGAAGGCGAGAGCGG CACGGACTAAGCTACTTGCTCCTT TCTTCTCTAGCAGTGGGACAGCCT GCTATCCTATAGCAAATGCCTCTC CCCAGGTTGCCAAGTC	<u>TCGAG</u> ACTTGGCAACCTGGGGAGAG GCATTTGCTATAGGATAGCAGGCTG TCCCACTGCTAGAGAAGAAAGGAG CAAGTAGCTTAGTCCGTGCCGCTCT CGCCTTCACAAGCACAGAGGGCCC GAGCACATCACTGAGCCCAACAGA TAGATTGAGCCG	<i>EcoR</i> I / <i>Xho</i> I
pCMV-HA-M/mt4	<u>AATTC</u> GGCTCAATCTATCTGTTGG GCTCAGTGATGTGCTCGGGCCCTC TGTGCTTGTGAAGGCGAGAGGTG CAGCGACTAAGCTACTTGCTCCTT TCTTCTCTAGCAGTGGGACAGCCT GCTATCCTATAGCAAATGCCTCTC CCCAGGTTGCCAAGTC	<u>TCGAG</u> ACTTGGCAACCTGGGGAGAG GCATTTGCTATAGGATAGCAGGCTG TCCCACTGCTAGAGAAGAAAGGAG CAAGTAGCTTAGTCCGTGCACCTCT CGCCTTCACAAGCACAGAGGGCCC GAGCACATCACTGAGCCCAACAGA TAGATTGAGCCG	<i>EcoR</i> I / <i>Xho</i> I
pCMV-HA-M/mt5	<u>AATTC</u> GGCTCAATCTATCTGTTGG GCTCAGTGATGTGCTCGGGCCCTC TGTGCTTGTGAAGGCGAGAGGTG CACGGGCGAAGCTACTTGCTCCTT TCTTCTCTAGCAGTGGGACAGCCT GCTATCCTATAGCAAATGCCTCTC CCCAGGTTGCCAAGTC	<u>TCGAG</u> ACTTGGCAACCTGGGGAGAG GCATTTGCTATAGGATAGCAGGCTG TCCCACTGCTAGAGAAGAAAGGAG CAAGTAGCTTCGCCCCTGCACCTCT CGCCTTCACAAGCACAGAGGGCCC GAGCACATCACTGAGCCCAACAGA TAGATTGAGCCG	<i>EcoR</i> I / <i>Xho</i> I

pCMV-HA-M/mt6	<u>AATTC</u> GGCTCAATCTATCTGTTGG <u>TCGAG</u> ACTTGGCAACCTGGGGAGAG	<i>EcoR I /XhoI I</i>
	GCTCAGTGATGTGCTCGGGCCCTC GCATTTGCTATAGGATAGCAGGCTG	
	TGTGCTTGTGAAGGCGAGAGGTG TCCCACTGCTAGAGAAGAAAGGAG	
	CACGGA CTGCGCTACTTGCTCCTT CAAGTAGCGCAGTCCGTGCACCTCT	
pCMV-HA-M/mt7	TCTTCTCTAGCAGTGGGACAGCCT CGCCTTCACAAGCACAGAGGGCCC	<i>EcoR I /XhoI I</i>
	GCTATCCTATAGCAAATGCCTCTC GAGCACATCACTGAGCCCAACAGA	
	CCCAGGTTGCCAAGTC TAGATTGAGCCG	
	<u>AATTC</u> GGCTCAATCTATCTGTTGG <u>TCGAG</u> ACTTGGCAACCTGGGGAGAG	
pCMV-HA-M/3A	GCTCAGTGATGTGCTCGGGCCCTC GCATTTGCTATAGGATAGCAGGCTG	<i>EcoR I</i>
	TGTGCTTGTGAAGGCGGCGGGTG TCCCACTGCTAGAGAAGAAAGGAG	
	CAGCGACTGCGCTACTTGCTCCTT CAAGTAGCGCAGTCGCTGCACCCGC	
	TCTTCTCTAGCAGTGGGACAGCCT CGCCTTCACAAGCACAGAGGGCCC	
pNDV/SS1-M/3A	GCTATCCTATAGCAAATGCCTCTC GAGCACATCACTGAGCCCAACAGA	<i>XhoI I</i>
	CCCAGGTTGCCAAGTC TAGATTGAGCCG	
	TTGA <u>AATTC</u> GGATGGACTCATCCAG CGCAGTCGCTGCACCCGCCGCCTTC	
	GAC ACAAGCACAGAGGGCCC	
pNDV/SS1-M/3A	AAGGCGGCGGGTGCAGCGACTGC GC <u>ACTTCGAG</u> TTATTTCTGAAAGG	<i>Age I</i>
	GCTACTTGCTCCTTTCTTCTCTAGC	
	ACA <u>ACCGGT</u> GCAGCACCTTCTGA CGCAGTCGCTGCACCCGCCGCCTTC	
	ATTG ACAAGCACAGAGGGCCC	
	AAGGCGGCGGGTGCAGCGACTGC GTG <u>GATAC</u> ATTGACTGCCTTATCTC	<i>BstZ17 I</i>
	GCTACTTGCTCCTTTCTTCTCTAGC CTG	

^a: Restriction sites are given in italics and underlines.