

Supplementary materials for

Evaluation of Immune Responses in mice to Recombinant *Bacillus subtilis* Displaying Newcastle Disease Virus *HN* Protein Truncations

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Table S1. List of strains, vaccines, cell lines, plasmids, and primer sequences.

Strains, Vaccines, Cell lines, Plasmids, and Primer Sequences	Description	Source or Restriction Site
Strains		
<i>Escherichia coli</i> (<i>E.coli</i>)	Competent cell strains	TIANGEN, Beijing, China
DH5α and BL21		
<i>B. subtilis</i> 168	Genbank accession: AL009126.3	Our lab
<i>B. subtilis</i> RH	<i>B. subtilis</i> 168 <i>amyE::cotB-HNJD</i>	This work
Vaccines		
Newcastle disease vaccine	Live (Strain LaSota)	HARVAC, Harbin, China
	Live (Strain CS2)	EBVAC, Hangzhou, China
	Inactivated (Strain La Sota)	Yebio, Qingdao, China
Cell lines		
Chicken embryo fibroblast (CEF)	UMNSAH/DF-1	SUNNCELL, Wuhan, China
Plasmids		
pUCm-T	T-A cloning vector	Sangon, Shanghai, China
pET-32a- <i>HNJD</i>	Prokaryotic expression vector	This work
pDG364	<i>E. coli-B. subtilis</i> shuttle vector	Our lab
pDG364- <i>cotB</i>	pDG364 derivative carrying <i>cotB</i> gene	Our previous work
pDG364- <i>cotB-HNJD</i>	pDG364 derivative carrying the fusion <i>cotB-HNJD</i> gene	This work
Primer Sequences		
<i>HNJD</i> -F1	5'-CGCGGATCCATGGTGATATACAAGCGATACAATGAC-3'	<i>BamH</i> I
<i>HNJD</i> -R1	5'-CCGGAATTCTTAAGCAATGCTGAGACAATAGGT-3'	<i>EcoR</i> I
<i>HNJD</i> -F2	5'-CCCAAGCTTATGGTGATATACAAGCGATACAATGAC-3'	<i>Hind</i> III
<i>HNJD</i> -R2	5'-CCGGAATTCTTAAGCAATGCTGAGACAATAGGT-3'	<i>EcoR</i> I
<i>cotB</i> -F	5'-CGGGATCCACGGATTAGGCCGTTTGTCC-3'	<i>BamH</i> I
<i>cotB</i> -R	5'-GGGAAGCTTGGATGATTGATCATCTGAAG-3'	<i>Hind</i> III
<i>amyE</i> -F	5'-CCAATGAGGTTAAGAGTATTCC-3'	Null
<i>amyE</i> -R	5'-CGAGAAGCTATCACCGCCAGC-3'	Null

Table S2. Sequences of primers used for real-time quantitative PCR.

Gene	Primer Sequences (5'-3')		Accession Number
β -actin	F: GCTCTTTTCCAGCCTTCCTT	R: GATGTCAACGTCACACTT	NM_007393.5
IL-1 β	F: ATGAAAGACGGCACCAC	R: GCTTGCTCTGCTTGAG	NM_008361.3
IL-6	F: TGCAAGAGACTTCCATCCAGT	R: GTGAAGTAGGGAAGGCCG	NM_031168.1
IL-10	F: GGTTGCCAAGCCTTATCGGA	R: ACCTGCTCCACTGCCTTGCT	NM_010548.2
IFN- γ	F: TCAAGTGGCATAGATGTGGAAGAA	R: TGGCTCTGCAGGATTTTCATG	NM_008337.4
TNF- α	F: ACGGCATGGATCTCAAAGAC	R: AGATAGCAAATCGGCTGACG	NM_001278601.1

Table S3. *HNJD* gene sequences and sites of optimization.

Original <i>HNJD</i>	Optimized <i>HNJD</i>
*ATG GTGATATACAAGCGATACAATGACACATG CCCAGATGAGCAAGACTACCAGATTCGAATGG CCAAGTCTTCGTATAAGCCTGGACGGTTTGGT GGGAAACGCATACAGCAGGCTATCTTATCTATC AAGGTGTCAACATCCTTAGGCGAAGACCCGGT ACTGACTGTACCGCCCAACACAGTCACACTCA TGGGGGCCGAAGGCA GAATTCACAGTAGG GACATCTCATTTCTTGTATCAACGAGGGTCATC ATACTTCTCTCCCGCGTTATTATATCCTATGACA GTCAGCAACAAAACAGCCACTCTTCATAGTCC TTATACATTCAATGCCTTCACTCGGCCAGGTAG TATCCCTTGCCAGGCTTCAGCAAGATGCCCCA ACTCGTGTGTTACTGGAGTCTATACAGATCCAT ATCCCCTAATCTTCTATAGAAACCACACCTTGC GAGGGGTATTTCGGGACAATGCTTGATGGTGTA CAAGCAAGACTTAACCTGCGTCTGCAGTATT CGATAGCACATCCCGCAGTCGCATTACACGAG TGAGTTCAAGCAGTACCAAAGCAGCATACACA ACATCAACTTGTTTTAAAGTGGTCAAGACTAA TAAGACCTATTGTCTCAGCATTGCTTAA	ATG GTGATATACAAGCGATACAATGACACATG CCCAGATGAGCAAGACTACCAGATTCGAATGG CCAAGTCTTCGTATAAGCCTGGACGGTTTGGT GGGAAACGCATACAGCAGGCTATCTTATCTATC AAGGTGTCAACATCCTTAGGCGAAGACCCGGT ACTGACTGTACCGCCCAACACAGTCACACTCA TGGGGGCCGAAGGCA GGATTCACAGTAGG GACATCTCATTTCTTGTATCAACGAGGGTCATC ATACTTCTCTCCCGCGTTATTATATCCTATGACA GTCAGCAACAAAACAGCCACTCTTCATAGTCC TTATACATTCAATGCCTTCACTCGGCCAGGTAG TATCCCTTGCCAGGCTTCAGCAAGATGCCCCA ACTCGTGTGTTACTGGAGTCTATACAGATCCAT ATCCCCTAATCTTCTATAGAAACCACACCTTGC GAGGGGTATTTCGGGACAATGCTTGATGGTGTA CAAGCAAGACTTAACCTGCGTCTGCAGTATT CGATAGCACATCCCGCAGTCGCATTACACGAG TGAGTTCAAGCAGTACCAAAGCAGCATACACA ACATCAACTTGTTTTAAAGTGGTCAAGACTAA TAAGACCTATTGTCTCAGCATTGCTTAA

*The yellow-highlighted sequences indicate the optimized sites, while the red-highlighted sequences denote the start codons.