Supporting Information

For

Structures and implications of the nuclease domain of human parvovirus B19 NS1 protein

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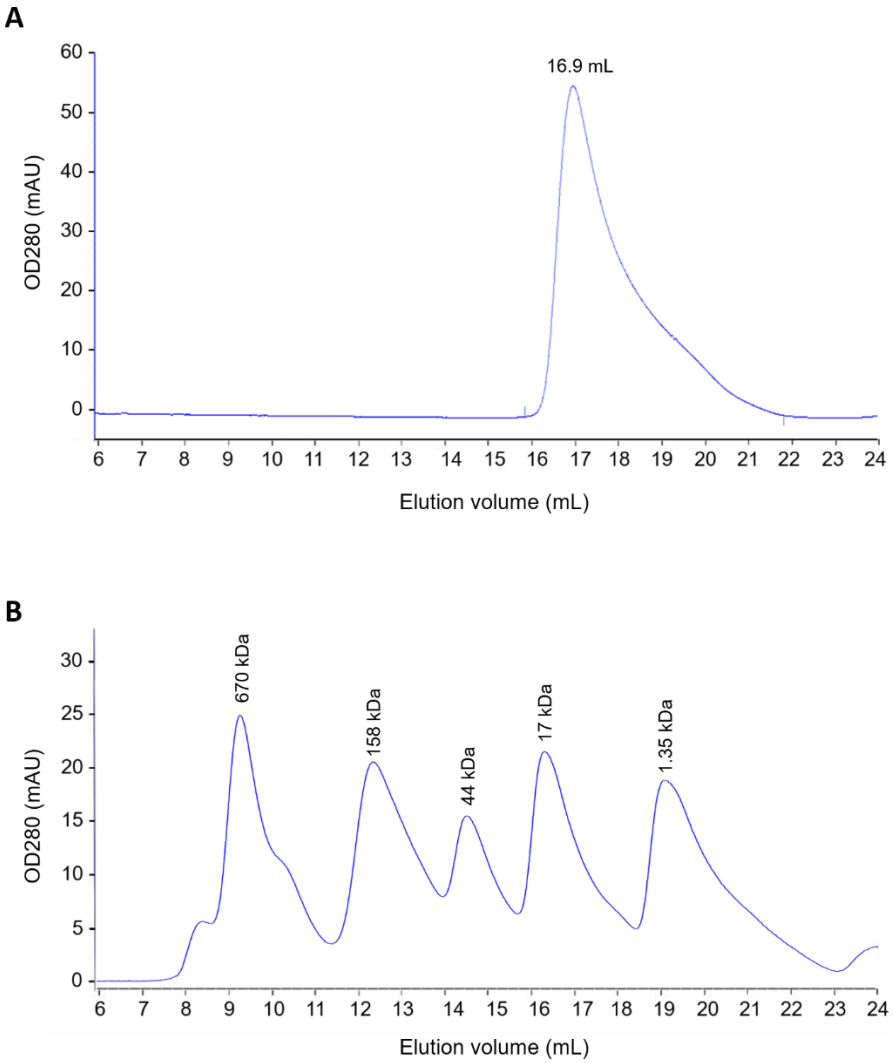
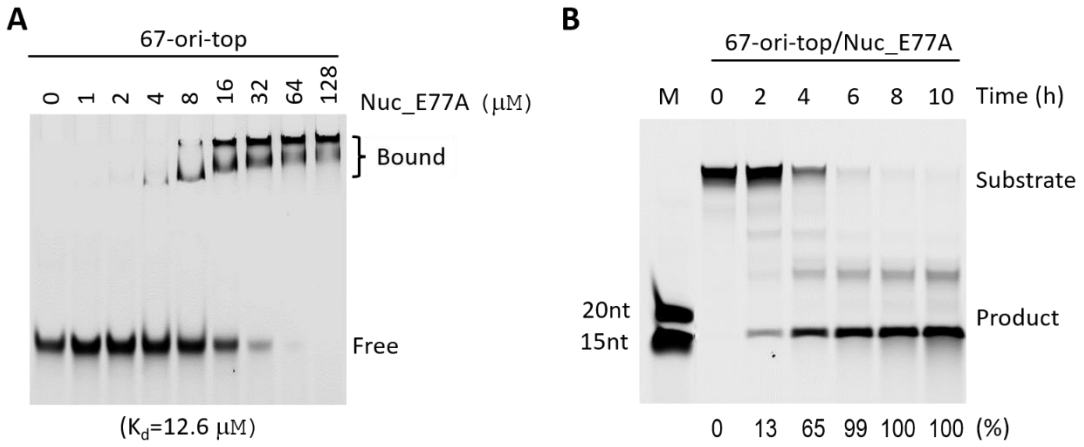
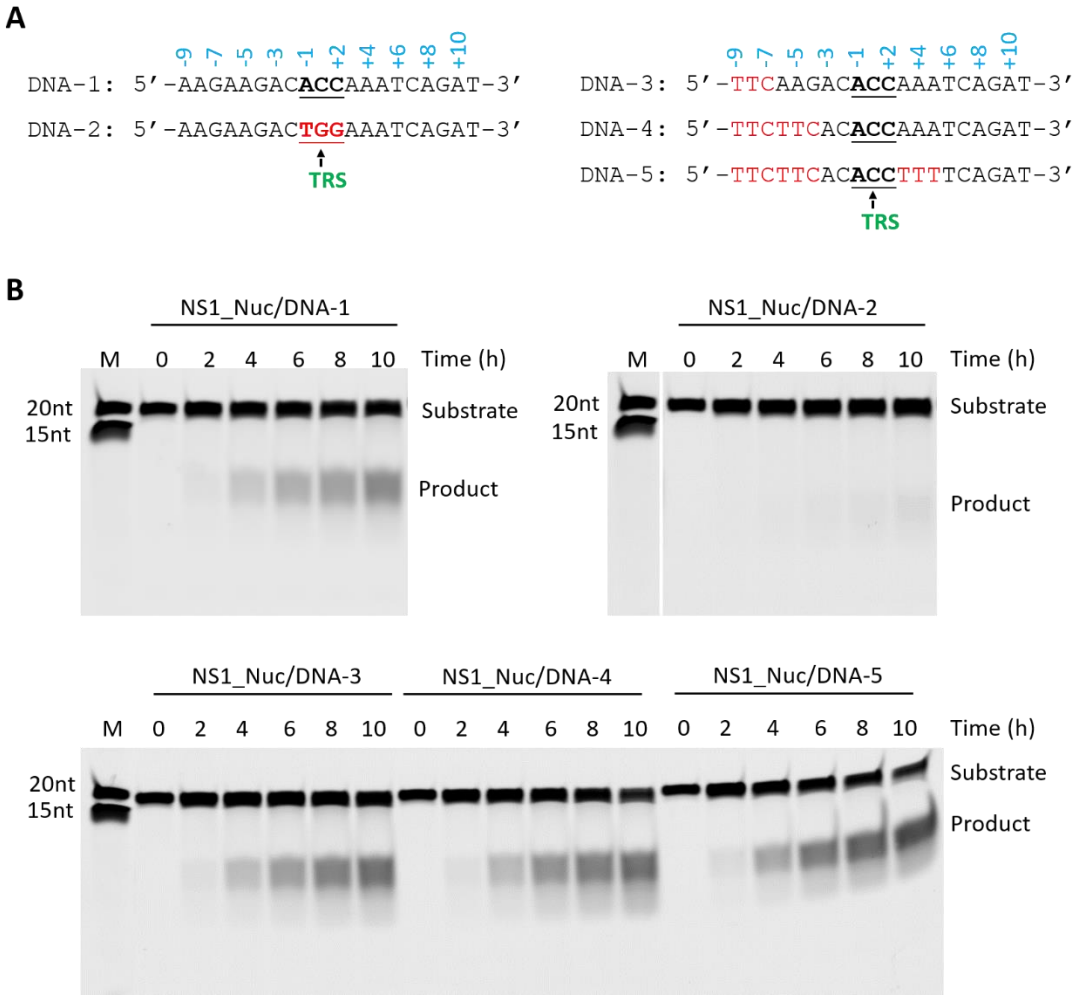


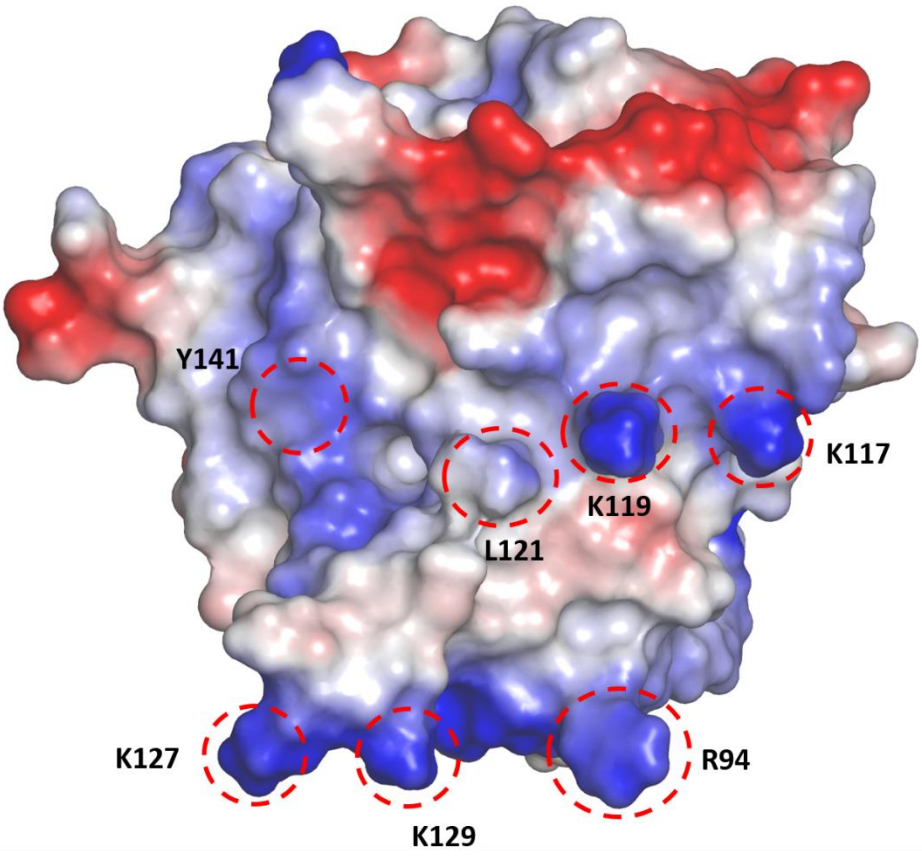
Supplementary Fig. S1. Size-exclusion analysis of (**A**) the NS1_Nuc protein and (**B**) the standard marker protein on Superdex 200 Increase 10/300 GL column.



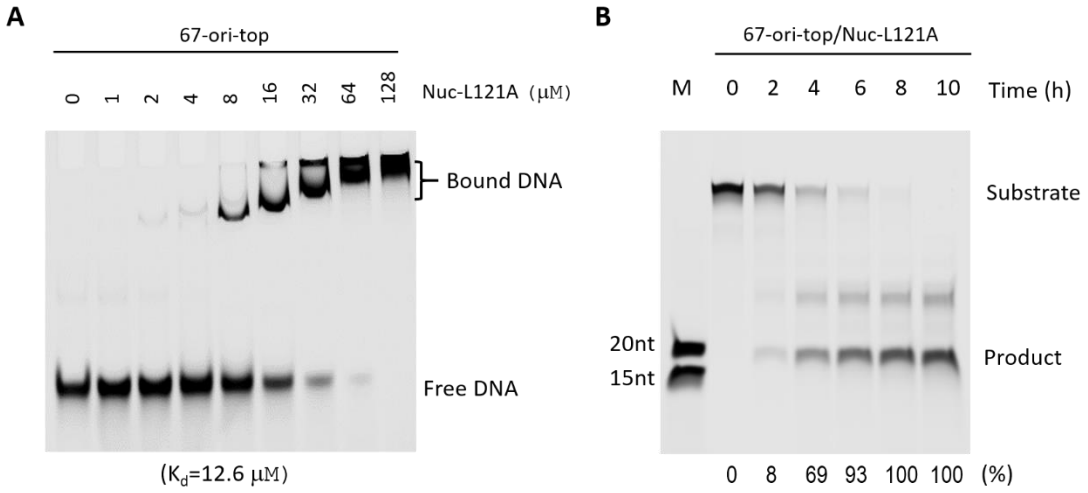
Supplementary Fig. S2. *In vitro* DNA binding (**A**) and cleavage (**B**) by E77A mutant of NS1_Nuc. The K_d value and substrate cleavage percentage derived from three independent experiments are listed at the bottom of the figures.



Supplementary Fig. S3. Impacts of DNA mutation on cleavage by NS1-Nuc. (**A**) Detailed sequence of the DNA variants. (**B**) *In vitro* DNA cleavage assays catalyzed by WT NS1_Nuc.



Supplementary Fig. S4. Surface presentation of NS1-Nuc. The residues mutated in this study are highlighted by red circles. The electrostatic surface is calculated by Pymol with the default settings.



Supplementary Fig. S5. *In vitro* DNA binding (A) and cleavage (B) by L121A mutant of NS1_Nuc. The K_d value and substrate cleavage percentage derived from three independent experiments are listed at the bottom of the figures.

Supplementary Table S1. Codon optimized cDNA sequence of NS1_Nuc (aa 2-176)

The optimized cDNA sequence of NS1_Nuc (from 5' to 3') [a,b]

GGATCC*GGCGGCGGT*GAACTGTTTCGCGGCGTGTTACAGGTGAGTAGCAATGTGCT
GGATTGTGCCAATGATAATTGGTGGTGTAGCCTGCTCGACCTGGATACCAGCGATT
GGGAACCGTTAACCCATACAAATCGCCTCATGGCCATTTATCTGTCAAGCGTTGCA
AGTAAACTGGATTTTACCGGCGGTCCGTTAGCAGGTTGTCTGTATTTTTTTCAGGT
GGAATGTAATAAATTTGAAGAAGGCTATCATATTCATGTGGTGATTGGCGGTCCGG
GTCTGAATCCGCGCAATCTGACCGTTTGTGTGGAAGGCTTATTTAATAATGTGCTG
TATCATTTAGTGACCGGCAATGTTAAACTGAAATTTCTGCCGGGTATGACCACCAA
AGGTAAATATTTTCGCGATGGCGAACAGTTTATTGAAAATTATCTGATGAAAAAAA
TTCCGCTGAATGTTGTTTGGTGTGTGACCAATATTGATGGCTATATTGATACCTGT
ATTAGCGCAACCTTTCGTCGCGGCGCCTGTCATGCCTAA***CTCGAG***

[a]: ***GGATCC*** and ***CTCGAG*** at the 5'-end and 3'-end are Bam HI and Xho I recognition sequence.

[b] ***GGCGGCGGT***, which codes for three Gly residues, was designed to enhance the cleavage efficiency of the UlP1 protease.

Supplementary Table S2. Primers used for NS1_Nuc mutant construction.

Name	Sequence (from 5’ to 3’)
NS1-Nuc_F	GGATCCGGCGGCGGTGAACTGTTTCGCGGCGTGTTACAGGTG
NS1-Nuc_R	CTCGAGTTAGGCATGACAGGCGCCGCGACGA
E77A_F	GTGGAATGTAATAAATTTGCAGAAGGCTATCATATT
E77A_R	ATGAATATGATAGCCTTCTGCAAATTTATTACATTC
R94A_F	CCGGGTCTGAATCCGGCAAATCTGACCGTTTGT
R94A_R	ACAAACGGTCAGATTTGCCGGATTCAGACCCGG
K117A_F	TTAGTGACCGGCAATGTTGCCCTGAAATTTCTGCCG
K117A_R	ACCCGGCAGAAATTTCAGGGCAACATTGCCGGTCAC
K119A_F	ACCGGCAATGTTAAACTGGCCTTTCTGCCGGGTATG
K119A_R	GGTCATACCCGGCAGAAAGGCCAGTTTAACATTGCC
K117/119A_F	GTGACCGGCAATGTTGCCCTGGCCTTTCTGCCGGGTATG
K117/119A_R	CATACCCGGCAGAAAGGCCAGGGCAACATTGCCGGTCAC
L121A_F	AATGTTAAACTGAAATTTGCACCGGGTATGACCACC
L121A_R	TTTGGTGGTCATACCCGGTGCAAATTTCAGTTTAAC
K127/129A_F	CCGGGTATGACCACCGCCGGTGCCTATTTTCGCGAT
K127/129A_R	GCCATCGCGAAAATAGGCACCGGCGGTGGTCATACC