

## Supplementary Data

**Table A1. Primers used in this study.**

Construct/Gene Name	Primer Name	5'-3' sequence
<b>Y2H</b>		
pGBKT7-Nsp9	BamHINsp9-F	TAATGGATCCAAGGAGCAGTGTTTAAACTG
	PstINsp9-R	TAATCTGCAGCTCATGATTGGATCTGAGT
pGADT7-N	EcoRIN-F	TAATGAATTCCCAAATAACAACGGCAAG
	BamHIN-R	TAATGGATCCTTACGCTGATGATGGCGT
pGBKT7-Nsp9 <sub>1-180</sub>	BamHINsp9-F	TAATGGATCCAAGGAGCAGTGTTTAAACTG
	PstINsp9-R1	TAATCTGCAGGCTTCCAGTGTCACTGGG
pGBKT7-Nsp9 <sub>181-452</sub>	BamHINsp9-F2	TAATGGATCCCAGTGCACGCGGCTGCC
	PstINsp9-R3	TAATCTGCAGGCCACCTCTCTTAGTTACTG
pGBKT7-Nsp9 <sub>453-646</sub>	BamHINsp9-F4	TAATGGATCCTGTCGTCTGGCGACCCG
	PstINsp9-R	TAATCTGCAGCTCATGATTGGATCTGAGT
pGBKT7-Nsp9 <sub>1-452</sub>	BamHINsp9-F	TAATGGATCCAAGGAGCAGTGTTTAAACTG
	PstINsp9-R3	TAATCTGCAGGCCACCTCTCTTAGTTACTG
pGBKT7-Nsp9 <sub>181-646</sub>	BamHINsp9-F2	TAATGGATCCCAGTGCACGCGGCTGCC
	PstINsp9-R	TAATCTGCAGCTCATGATTGGATCTGAGT
pGBKT7-Nsp9 <sub>453-551</sub>	BamHINsp9-F4	TAATGGATCCTGTCGTCTGGCGACCCG
	PstINsp9-R4	TAATCTGCAGGCGAGTCTGTTATGGCTGTC
pGBKT7-Nsp9 <sub>552-646</sub>	BamHINsp9-F5	TGCTGGATCCCATCATTTCTAGGCTGTAGG
	PstINsp9-R	TAATCTGCAGCTCATGATTGGATCTGAGT
pGBKT7-Nsp9 <sub>552-598</sub>	BamHINsp9-F5	TGCTGGATCCCATCATTTCTAGGCTGTAGG
	PstINsp9-R5	TAATCTGCAGGGTCCATGAGTATCGCAGCC
pGBKT7-Nsp9 <sub>599-646</sub>	BamHINsp9-F6	TTATGGATCCTGAGCTGTGCTTGTTTAGAG
	PstINsp9-R	TAATCTGCAGCTCATGATTGGATCTGAGT
<b>BiFC</b>		
pBiFC-N-VC155	EcoRIN-F	TAATGAATTCCCAAATAACAACGGCAAG
	XhoIN-R	TAATCTCGAGACGCTGATGATGGCGCT
pBiFC-Nsp9-VN	HindIIINSP9-F	TTGTAAGCTTGGAGCAGTGTTTAAACTG
	SalINSP9-R	TTGTGTCGACCTCATGATTGGATCTGAG
<b>Pull-down</b>		
pTriEx-Nsp9 <sub>NVSL97-7895</sub>	NcoINsp9-F	TTGTCCATGGGAGCAGTGTTTAAACTG
	XhoINsp9-R	TTGTCTCGAGCTCATGATTGGATCTGAG
pGEX-TEV-N <sub>NVSL97-7895</sub>	BamHIN-F	TAATGGATCCCCAAATAACAACGGCAAG
	EcoRIN-R	TAATGAATTCTTACGCTGATGATGGCGCT
pTriEx-Nsp9 <sub>TA-12</sub>	NcoINsp9-F	TCTTCCATGGCATTAAACTGCTAGCCGCC
	XhoINsp9-R	TTGTCTCGAGCTCATGATTGGATCTGAG
pGEX-TEV-N <sub>TA-12</sub>	BamHIN-F	TAATGGATCCCCAAATAACAACGGCAAG
	EcoRIN-R	TCGTGAATTCTCATGCTGAGGGTGTGCTG
pTriEx-Nsp9 <sub>O10u91</sub>	EcoRINsp9-F	TGGCGAATTCTACAGGCTTTAAACTGCTAG
	HindIIINsp9-R	GGCGAAGCTTTTCATTGTGACTTTTCAGT
pGEX-TEV-N <sub>O10u91</sub>	BamHIN-F	TAATGGATCCGCCGGTAAAAACCAGAGCC
	EcoRIN-R	TGATGAATTCTTAGCTTGCACCCTGACTGG
<b>CoIP</b>		
PTriEx-Nsp9 <sub>599-646(NVSL97-7895)</sub>	NcoINsp9His-F	TAGTCCATGGGAAGCTGTGCTTGTTTAGAG
	XhoINsp9-R	TTGTCTCGAG CTCATGATTGGATCTGAG

pTriEx-N-GFP <sub>(NVSL97-7895)</sub>	NcoIN-F	TAATCCATGGCACCAAATAACAACGGCAAGC
	BamHIN-R	TAATGGATCCCGCTGATGATGGCGCTGTG
PTriEx-Nsp9 <sub>596-643(TA-12)</sub>	NcoINsp9-F	TAGTCCATGGCAAGCTGTGCTTGTTTAGAG
	XhoINsp9-R	TGCTCTCGAGTCATGATTGGACCTGAGTTTC
pTriEx-N-GFP <sub>(TA-12)</sub>	NcoIN-F	TAATCCATGGCACCAAATAACAACGGCAAGC
	BamHIN-R	TAATGGATCCCTGCTGAGGGTGATGCTGTG
PTriEx-Nsp9 <sub>598-645 (Olot/91)</sub>	NcoINsp9-F	TTCTCCATGGCATGTGCTTGCATTGACCATGAC
	XhoINsp9-R	TGCCCTCGAGTTCATTGTGACTTTTCAGT
pTriEx-N-GFP <sub>(Olot/91)</sub>	NcoIN-F	TAGTCCATGGCCGGTAAAAACCAGAGCC
	BamHIN-R	TAATGGATCCCGCTTGCACCCTGACTGGCG
<b>Mutagenesis (Y2H)</b>		
pGBKT7-Nsp9 <sub>L603A</sub>	BamHIL603A-F	TAATGGATCCCTAGCTGTGCTTGTGCAGAGTATGATC
pGBKT7-Nsp9 <sub>E646A</sub>	PstIE646A-R	TCGTCTGCAGGTGCATGATTGGATCTGAG
pGBKT7-Nsp9 <sub>K640A</sub>	PstIK640A-R	TCGTCTGCAGGCTCATGATTGGATCTGAGTGCTTCCCAC
pGBKT7-Nsp9 <sub>E639A</sub>	PstIE639A-R	TCGTCTGCAGGCTCATGATTGGATCTGAGTTTTGCCACAT G
pGBKT7-Nsp9 <sub>S636A</sub>	Nsp9S636A-F	CGTTCTTCTTGGCCATGTGGGAAAAACTC
	Nsp9S636A-R	GAGTTTTTCCCACATGGCCAAGAAGAACG
pGBKT7-Nsp9 <sub>L635A</sub>	Nsp9L635A-F	CGCCGTTCTTCGCGTCCATGTGGGA
	Nsp9L635A-R	TCCCACATGGACGCGAAGAACGGCG
pGBKT7-Nsp9 <sub>E611A</sub>	Nsp9E611A-F	TCCCGAATGGTTTGCAGAGCTTGTAG
	Nsp9E611A-R	TACAAGCTCTGCAAACCATTTCGGGATC
pGBKT7-Nsp9 <sub>E608A</sub>	Nsp9E608A-F	TATGATCCCGCATGGTTTGAAGAGC
	Nsp9E608A-R	GCTCTTCAAACCATGCGGGATCATA
pGBKT7-Nsp9 <sub>V615A</sub>	Nsp9V615A-F	GCTTGTAGCTGGGATAGCGCAGTG
	Nsp9V615A-R	CACTGCGCTATCCCAGCTACAAGC
pGBKT7-Nsp9 <sub>F628A</sub>	Nsp9F628A-F	AAGGACGGCTACAGTGCTCCCGGCC
	Nsp9F628A-R	GGCCGGGAGCACTGTAGCCGTCCTT
pGBKT7-Nsp9 <sub>F633A</sub>	Nsp9F633A-F	CCC GCCGGCCTTCTTGTCCATGTG
	Nsp9F633A-R	CACATGGACAAGAAGGCCGGCGGG
pGBKT7-Nsp9 <sub>608/611A</sub>	Nsp9E608A-F	TCGTGGATCCCTTAGCTGTGCTTGTTTAGAGTATGATCCCGC ATGGTT
pGADT7-N <sub>R13A</sub>	NR13A-F	CAAAAGAAAAAGGCGGGGAATGGCCAG
	NR13A-R	CTGGCCATTCCCCGCTTTTTCTTTTG
pGADT7-N <sub>T81A</sub>	NT81A-F	TCGTGCATCCAGGCTGCCTTTAAC
	NT81A-R	GTTAAAGGCAGCCTGGATCGACGA
pGADT7-N Q85A	NQ85A-F	TGCCTTTAACGCGGGCGCTGGAAC
	NQ85A-R	GTTCCAGCGCCCGCGTTAAAGGCA
pGADT7-N T101A	NT101A-F	TCAGGTAGGATAAGTTACGCTGTGGAG
	NT101A-R	CTCCACAGCGTAACTTATCCTACCTGA
pGADT7-N P104A	NP104A-F	CTGTGGAGGCTAGTTTGGCCAGC
	NP104A-R	TGCGTCGGCAAACCTAGCCTCCACAG
<b>Mutagenesis(CoIP)</b>		
pTriEx-Nsp9 <sub>E608A</sub>	NcoINsp9-F	TCGTGGATCCCTTAGCTGTGCTTGTTTAGAGTATGATCCCGC ATGGTT
	XhoINsp9-R	TTGTCTCGAGCTCATGATTGGATCTGAG
pTriEx-Nsp9 <sub>E611A</sub>	Nsp9E611A-F	TCCCGAATGGTTTGCAGAGCTTGTAG
	Nsp9E611A-R	TACAAGCTCTGCAAACCATTTCGGGATC
pTriEx-Nsp9 <sub>E639A</sub>	NcoINsp9His-F	TAGTCCATGGGAAGCTGTGCTTGTTTAGAG

	XhoIE639A-R	TAGTCTCGAGCTCATGATTGGATCTGAGTTTTGCCAC
pTriEx-Nsp9 <sub>E646A</sub>	NcoINsp9His-F	TAGTCCATGGGAAGCTGTGCTTGTTTAGAG
	XhoIE646A-R	TAGTCTCGAGCGCATGATTGGATCTGAG
pTriEx-Nsp9 <sub>E608A/E611A</sub>	Nsp9E608A-F	TAGACCATGGGAAGCTGTGCTTGTTTAGAGTATGATCCCCG
		ATGGTTTGCAGAGC
	XhoINsp9-R	TTGTCTCGAG CTCATGATTGGATCTGAG
<b>Colocalization</b>		
PTriEx-mCherry-Nsp9	BamHINsp9-F	TAATGGATCCAAGGAGCAGTGTTTAAACTG
	XhoINsp9-R	TTGTCTCGAG CTCATGATTGGATCTGAG
PTriEx-mCherry-Nsp9 <sub>1-552</sub>	BamHINsp9-F	TAATGGATCCAAGGAGCAGTGTTTAAACTG
	XhoINsp9-R5	TACGCTCGAGTTACTCATGATTGGATCTGAG
PTriEx-mCherry-Nsp9 <sub>599-646</sub>	BamHINsp9-F	TGACGGATCCAGCTGTGCTTGTTTAGAG
	XhoINsp9-R	TTGTCTCGAG CTCATGATTGGATCTGAG
pTriEx-N-GFP	NcoIN-F	TAATCCATGGCACCAAATAACAACGGCAAGC
	BamHIN-R	TAATGGATCCCGCTGATGATGGCGCTGTG
pTriEx-mCherry-DHX9	BamHIDHX9-F	TCGCGGATCCATGGGTGACGTTAAAAATTTT
	HindIIIDHX9-R	TGACAAGCTTTTAATAGCCGCCACCTCCTC
<b>Over-expression</b>		
pTriEx-DHX9	BamHIDHX9r1-F	TCGCGGATCCATGGGTGACGTTAAAAATTTTC
	HindIIIDHX9r2-R	TGACAAGCTTTTAATAGCCGCCACCTCCTC
	EcoRIDHX9r1-R	GCCTCGATGAATTCAAAAGGAGTTTCC
	EcoRIDHX9r2-F	TCAGGAATTCATCGAGGCTCTACTTAAG

Note: Restriction enzyme sites are underlined.

**Table A2. Primers sets for quantification of gRNA and sgmRNAs.**

Primer name	Sequence (5'-3')	Remark
Leader-F	CACCTTGCTTCCGGAGTTG	gRNA and sgmRNA2-7
sgmRNA1-R	GAGAGACCGTGACTGAGACATC	gRNA
sgmRNA2-R	CAGCCAACCGGCGATTGTGAA	sgmRNA 2
sgmRNA3-R	GCAAAGCGGGCATAACCGTGT	sgmRNA 3
sgmRNA4-R	ACGAAGTCTGATGCTGCGGTG	sgmRNA 4
sgmRNA5-R	CTGGCGTTGACGAGCACAGCA	sgmRNA 5
sgmRNA6-R	CATCACTGGCGTGTAGGTAATGGA	sgmRNA 6
sgmRNA7-F	CCCGGGTTGAAAAGCCTCGTGT	total viral RNA
sgmRNA7-R	GGCTTCTCCGGGTTTTTCTTCCTA	sgmRNA 7 and total viral RNA
GAPDH-F	TGATGACATCAAGAAGGTGGTGAAG	internal control
GAPDH-R	TCCTTGAGGCCATGTGGGCCAT	internal control

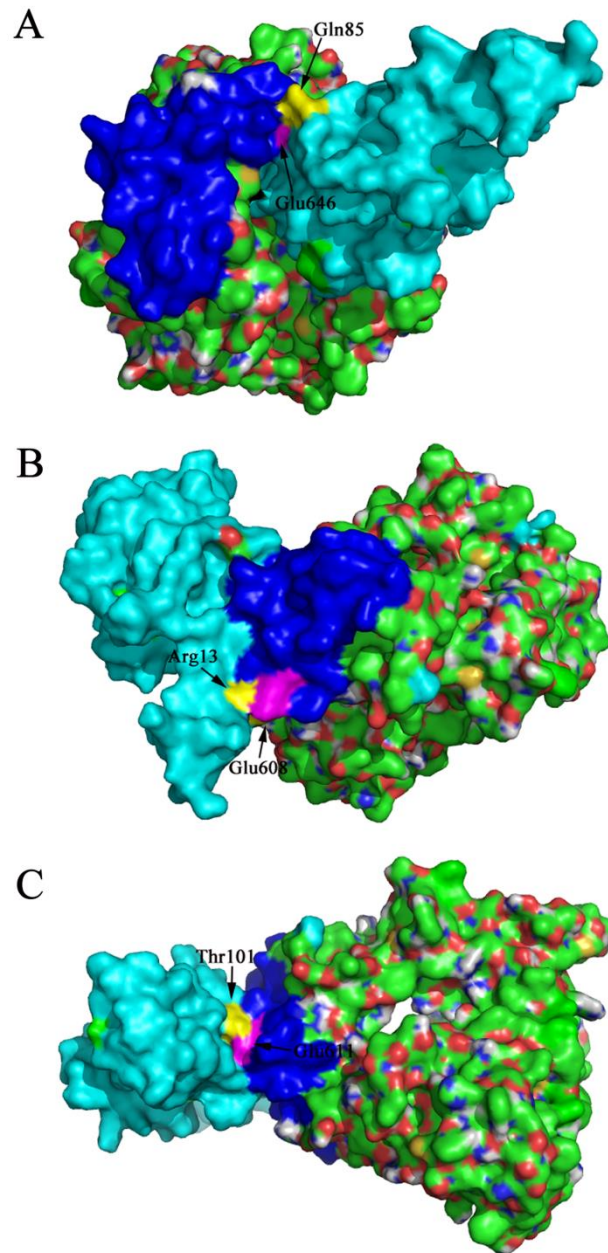
A

		1	50
NVSL 97-7895 NSP9	(1)	GAV	FKLLAASGLTRCGRGGLVVTETAVKIVKFNHRTFTLGPVNLKVA SEV
TA-12 NSP9	(1)	---	FKLLAASGLTRCGRGGLVVTETAVKIVKFNHRTFTLGPVNLKVA SEV
Olot/91 Nsp9	(1)	-TG	FKLLAASGLTRCGRGGLVVTETAVKIIKMH SRTFTFGPELDLKVTV SEV
Consensus	(1)		FKLLAASGLTRCGRGGLVVTETAVKIVKFNHRTFTLGPVNLKVA SEV
		51	100
NVSL 97-7895 NSP9	(51)	ELKDAVEHNOHPVARPVDGGVVLIRSAVPSLIDVLI S	-GADASPKLLARH
TA-12 NSP9	(48)	ELKDAVENNOHPVARPVDGGVVLIRSAVPSLIDVLI S	-GADASPKLLARH
Olot/91 Nsp9	(50)	EVKKSTECGHAVVAN-ICSGVVLNRPHPESLIDVLI K	PGGLDTTIGLQPGH
Consensus	(51)	ELKDAVENNOHPVARPVDGGVVLIRSAVPSLIDVLI S	GADASPKLLARH
		101	150
NVSL 97-7895 NSP9	(100)	GPGNTGIDGTLWDFEAEATKEEIALSAQIIQACDI	RRGDAPEI GLPYKLY
TA-12 NSP9	(97)	GPGNTGIDGTLWDFEAEATKEEIALSAQIIQACDI	RRGDAPEI GLPYKLY
Olot/91 Nsp9	(99)	GAGNMVGGSLWDFETAPTKAELELSKQIIQACEV	RRGDANLQGLPYKLY
Consensus	(101)	GPGNTGIDGTLWDFEAEATKEEIALSAQIIQACDI	RRGDAPEI GLPYKLY
		151	200
NVSL 97-7895 NSP9	(150)	PVRGNPERVKGVLQNTFRGDIPIYKTPSDTGS	PVHAAACLT PNATPVT DGR
TA-12 NSP9	(147)	PVRGNPERVKGVLQNTFRGDIPIYKTPSDTGS	PVHAAACLT PNATPVT DGR
Olot/91 Nsp9	(149)	PVRGDPERHKGRLINTRFGDLPYKTPQDTKSAI	HAAACCLHPGAPVSDGR
Consensus	(151)	PVRGNPERVKGVLQNTFRGDIPIYKTPSDTGS	PVHAAACLT PNATPVT DGR
		201	250
NVSL 97-7895 NSP9	(200)	SVLATTMPSGFELYVPTIPASVLDYLDSPDCPK	QKLTGHCEDAALRDLS
TA-12 NSP9	(197)	SVLATTMPSGFELYVPTIPASVLDYLDSPDCPK	QKLTGHCEDAALRDLS
Olot/91 Nsp9	(199)	STLGTTLQHGFEELYVPTIPYSVMHYLDSHP	DTPEPMFKHGTSKAAE DLQ
Consensus	(201)	SVLATTMPSGFELYVPTIPASVLDYLDSPDCPK	QKLTGHCEDAALRDLS
		251	300
NVSL 97-7895 NSP9	(250)	KYDLS TQGFVLPGLRLVRKYLFAHVGKCP	PVHRPSTY PAKNSMAGINGN
TA-12 NSP9	(247)	KYDLS TQGFVLPGLRLVRKYLFAHVGKCP	PVHRPSTY PAKNSMAGINGN
Olot/91 Nsp9	(249)	KYDLS TQGFVLPGLRLVRRFIFGHIGKAP	PIFLPSTY PAKNSMAGINGN
Consensus	(251)	KYDLS TQGFVLPGLRLVRKYLFAHVGKCP	PVHRPSTY PAKNSMAGINGN
		301	350
NVSL 97-7895 NSP9	(300)	RFPTKDIQSVPEIDVLCQA VRENWQVTPCTL	KKQYCGKKKTRT ILGTN
TA-12 NSP9	(297)	RFPTKDIQSVPEIDVLCQA VRENWQVTPCTL	KKQYCGKKKTRT ILGTN
Olot/91 Nsp9	(299)	RFPTKDIQSVPEIDEMCARAVKENWQVTP	CTLKKQYCSKPKTRT ILGTN
Consensus	(301)	RFPTKDIQSVPEIDVLCQA VRENWQVTPCTL	KKQYCGKKKTRT ILGTN
		351	400
NVSL 97-7895 NSP9	(350)	NFIALAHRAALSGVTCGFMKKA FNSPIALG	KNKFKELOTPVILGRLEADL
TA-12 NSP9	(347)	NFIALAHRAALSGVTCGFMKKA FNSPIALG	KNKFKELOTPVILGRLEADL
Olot/91 Nsp9	(349)	NFIALAHRSAALSGVTCGFMKKA FNSPIALG	KNKFKELOTPVILGRLEADL
Consensus	(351)	NFIALAHRAALSGVTCGFMKKA FNSPIALG	KNKFKELOTPVILGRLEADL
		401	450
NVSL 97-7895 NSP9	(400)	ASCDRSTPAI VRWFAANLYELACAEELP	SYV LNCCHDL LVTQSGAVTK
TA-12 NSP9	(397)	ASCDRSTPAI VRWFAANLYELACAEELP	SYV LNCCHDL LVTQSGAVTK
Olot/91 Nsp9	(399)	ASCDRSTPAI VRWFVANLYELACGEEY	LPSYV LNCCHDL LVTQSGAVTK
Consensus	(401)	ASCDRSTPAI VRWFAANLYELACAEELP	SYV LNCCHDL LVTQSGAVTK
		451	500
NVSL 97-7895 NSP9	(450)	RGGLSGDPI TSVSNTIYSLVIYAQHMVLS	YFKSGHPHGLLFLQDQLKFE
TA-12 NSP9	(447)	RGGLSGDPI TSVSNTIYSLVIYAQHMVLS	YFKSGHPHGLLFLQDQLKFE
Olot/91 Nsp9	(449)	RGGLSGDPI TSVSNTIYSLVIYAQHMVLS	ALKMGHEI GLKFLFLQDQLKFE
Consensus	(451)	RGGLSGDPI TSVSNTIYSLVIYAQHMVLS	YFKSGHPHGLLFLQDQLKFE
		501	550
NVSL 97-7895 NSP9	(500)	DMLKVQPLIVYSDDLVLVYAESPTMPNY	HWWVEHLN LMLGFTDPKKTAIT
TA-12 NSP9	(497)	DMLKVQPLIVYSDDLVLVYAESPTMPNY	HWWVEHLN LMLGFTDPKKTAIT
Olot/91 Nsp9	(499)	DLLEIQPLIVYSDDLVLVYAESPTMPNY	HWWVEHLN LMLGFTDPKKTAVIT
Consensus	(501)	DMLKVQPLIVYSDDLVLVYAESPTMPNY	HWWVEHLN LMLGFTDPKKTIT
		551	600
NVSL 97-7895 NSP9	(550)	DSPSFLGCRITNGRQLVLPNRDRI LAALA	YHMKASNVSEYYASAAA ILMDS
TA-12 NSP9	(547)	DSPSFLGCRITNGRQLVLPNRDRI LAALA	YHMKASNVSEYYASAAA ILMDS
Olot/91 Nsp9	(549)	DKPSFLGCRITNGRQLVLPNRDRI LAALA	YHMKASNVSEYYASAAA ILMDS
Consensus	(551)	DSPSFLGCRITNGRQLVLPNRDRI LAALA	YHMKASNVSEYYASAAA ILMDS
		601	647
NVSL 97-7895 NSP9	(600)	CACLEYDPEWFEE LVVGI AQCAR KDGY	SFPGPPFFLSMWEKLR SNHE
TA-12 NSP9	(597)	CACLEYDPEWFEE LVVGI AQCAR KDGY	SFPGPPFFLSMWEKLR SNHE
Olot/91 Nsp9	(599)	CACIDHDPEWFEE LVVGI AQCAR KDGY	SFPGPPFFLSMWEKLR SNHE
Consensus	(601)	CACLEYDPEWFEE LVVGI AQCAR KDGY	SFPGPPFFLSMWEKLR SNHE

B

		1	66
NVSL 97-7895 N	(1)	MPNNNGKQKKK	---RNGQPVNQLCQMLGKI I AQNQSRGKGF GKKIKNKNPEKPHFPLATEDD
TA-12 N	(1)	MPNNNGKQKKK	---RNGQPVNQLCQMLGKI I AQNQSRGKGF GKKIKNKNPEKPHFPLATEDD
Olot/91 N	(1)	MAGKNQSKKKKSAAPM	GNGQPVNQLCQMLGAMTKSQRQ---QPRGGQA KKKKPEKPHFPLAEDD
Consensus	(1)	MPNNNGKQKKK	KNGQPVNQLCQMLGKI I AQNQSRGKGF GKKIKNKNPEKPHFPLATEDD
		67	131
NVSL 97-7895 N	(63)	VRHHFTPSE RQLCLSSIQTAFNQAGTCS	LSDSGRISYTVFSLPTHTVRLIRVITAS SA
TA-12 N	(63)	VRHHFTPSE RQLCLSSIQTAFNQAGTCS	LSDSGRISYTVFSLPTHTVRLIRVITAS SA
Olot/91 N	(64)	IRHHLTQTE RSLCLSIQTAFNQAGTAS	LSSSGKVSQVEFMLEVAHTVRLIRVIT SASQAS
Consensus	(67)	VRHHFTPSE RQLCLSSIQTAFNQAGTCS	LSDSGRISYTVFSLPTHTVRLIRVITAS SA

Fig. A1. Alignment of Nsp9 (A) and N (B) protein sequences of three PRRSV strains.



**Fig. A2. The predicted binding models for N-Nsp9 interaction.**

The simulated binding of N to Nsp9 was performed using database PRISM 2.0. The three predicted interaction models (A, B, C) are shown in surface format. Nsp9<sub>599-646</sub> fragment is colored blue and N protein in cyan. Predicted interface residues involved in the N-Nsp9 interaction are indicated by arrows.