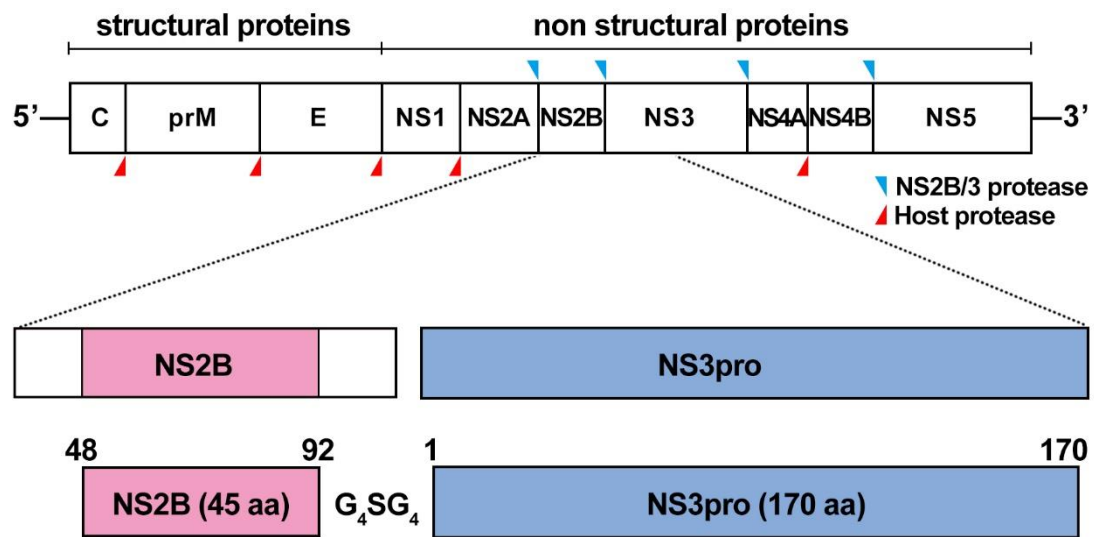




Supplementary information, Figure S1A Genomic organization of ZIKV polyprotein and the construct used. The cleavage sites by the NS2B/NS3 protease are indicated by blue arrows and those cleaved by host protease are labeled with red arrows. The ZIKV NS2B-NS3pro construct was generated by fusing residues 48-92 of NS2B to residues 1-170 of NS3 protease via a glycine-serine linker (GGGGSGGGG).

		1	10	20	30	40	50
ZIKV	NS2B	SWPPSEVLTAVGLIC	ALAGGFAKADIE	MAGPMAAVGLLIV	SYVVS	GKSVDMY	IERAGDI
JEV	NS2B	GWPATEFTSAVGLMF	AIVGGLAELDIES	MSIPFMLAGLM	AVSYVVS	GKATDMW	LDRAADI
WNV	NS2B	GWPATEVMTAVGLMF	AIVGGLAELDID	SMAPMTIAGLMF	AVFVIS	GKSTDMW	IERTADI
DENV1	NS2B	SWPLNEGIMAGIVS	ILLSSLLKNDVP	LACPLIAGGMLI	ACYVIS	GSSADLS	LEKAAEV
DENV2	NS2B	SWPLNEGIMAVGMV	SILLSSLLKNDIP	MTGPLVAGGLIT	VCYVLT	GRSADL	ELERAADV
DENV3	NS2B	SWPPSEVLTAVGLIC	ALAGGFAKADIE	MAGPMAAVGLLIV	SYVVS	GKSVDMY	IERAGDI
DENV4	NS2B	SWPLNEGIMAVGLV	SLGSA	LLKNDVP	LACGPMVAG	GLLAAV	YVMSGSSADLSLEKAANV

		60	70	80	90	100	110
ZIKV	NS2B	TWEKDAEVTGNSPRLDV	ALDES	GDFSLVEDDGP	PMREIILKVV	LMTICGN	NEI
JEV	NS2B	SWEMDAAITGSSRRLDV	KLDDDGDFHL	IDDPGV	PKVWVLRMS	CIGLAAL	TPWAIVPAAF
WNV	NS2B	TWESDAEITGSSSERVD	RLDDDGDFH	LDNDP	APWKIWMLRMA	CLASAYT	TPWAILPSVI
DENV1	NS2B	SWEQDAEHS	GASHSILVEV	QDDGTMKIK	DEERDDTLT	ILLKATLL	AVSGVYFMSIPATLF
DENV2	NS2B	RWEEQAEISGSSPILS	ITISEDGSM	IKNEEE	EQTLTILIR	TGLLVISGLF	EVSIPTAA
DENV3	NS2B	TWEKDAEVTGNSPRLDV	ALDES	GDFSLVEDDGP	PMREIILKVV	LMTICGN	NEI
DENV4	NS2B	QWDEMA	ITGSSPIIEVK	QDE	GSF	SIR	VEETNMITLLV

		120	130
ZIKV	NS2B	AWYVYVTKKR	
JEV	NS2B	GYWLTLLKTKR	
WNV	NS2B	GFWITLQYTKR	
DENV1	NS2B	VWYFWQKKQR	
DENV2	NS2B	AWYLWEVKKQR	
DENV3	NS2B	AWYVYVTKKR	
DENV4	NS2B	LWYIWQVKTQR	

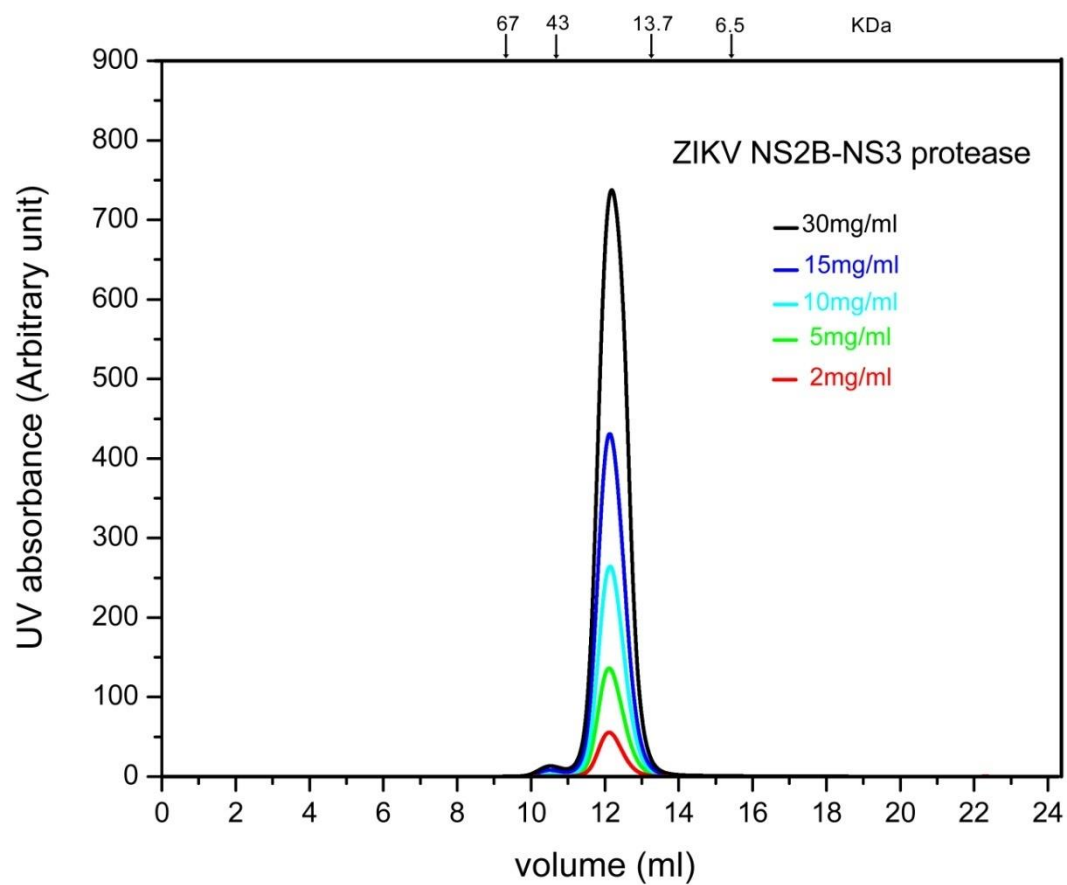
		1	10	20	30	40	50	60
ZIKV	NS3pro	SGALWDVPAPKEV	KKGETTDGVYRVM	T	RRLLG	STQVGVGV	QEGVFHTMWHV	TGKSALRS
JEV	NS3pro	GGVFWDTSPSPKPCS	KGDDTTTGVYRIM	A	RGLIG	TYQAGVGVM	YENVFHTLWHT	TRGAAIMS
WNV	NS3pro	GGLVWDTPSPKEYK	KGDDTTTGVYRIM	T	RGLIG	SYQAGAGVM	VEGVFHTLWHT	TGKAALMS
DENV1	NS3pro	SGVLWDTPSPPEVER	AVLDNGIYRIL	Q	RGLIG	RSQVGVGV	QDGVFHTMWHV	TRGAVLMY
DENV2	NS3pro	AGVLWDVPSPPPV	GKAELEDGAYRIK	Q	KGILG	YSQIGAGVYK	EGTFHTMWHV	TRGAVLMH
DENV3	NS3pro	SGALWDVPAPKEV	KKGETTDGVYRVM	T	RRLLG	STQVGVGV	QEGVFHTMWHV	TGKSALRS
DENV4	NS3pro	SGALWDVPSPAATQ	KATLSEGVYRIM	Q	RGLIG	KTQVGVGI	HMEGVFHTMWHV	TRGSVICH

		70	80	90	100	110	120
ZIKV	NS3pro	GEGRLDPYWG	VDVKQDLVS	YCGFPWKLD	AAWDGH	SEVQLLAVPP	GERARNIQTLPCTIFKTKD
JEV	NS3pro	GEGKLT	PYWGSVKEDRIA	YGGPWRFD	RKWN	GTDDVQVIVVE	PGKAAVNIQTKPGVFRTP
WNV	NS3pro	GEGRLDPYWG	SVKEDRLCY	GGFPWKLO	HKWNGH	DEVQMI	VEPGKNVKNVQTKPGVFKTPE
DENV1	NS3pro	QGKRLEPS	WASVKKDLIS	YCGGWRFQ	GSWNT	GEEVQVIAVE	PGKNPKNVQTPGTFKTP
DENV2	NS3pro	KGKRIEPS	WADVKKDLIS	YCGGWKLE	GEWKE	GEEVQLALE	PGKNPRAVQTKPGFKTNT
DENV3	NS3pro	GEGRLDPYWG	VDVKQDLVS	YCGFPWKLD	AAWDGH	SEVQLLAVPP	GERARNIQTLPCTIFKTKD
DENV4	NS3pro	ETGRLEPS	WADVVRNDMIS	YCGGWRLG	DKWKE	EDVQVLAIE	PGKNPKHVQTKPGFKTTLT

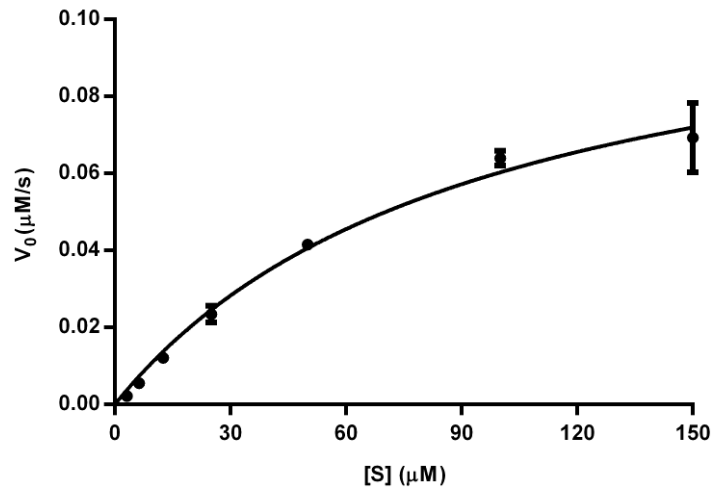
		130	140	150	160	170	180
ZIKV	NS3pro	GDIGAV	ALDYF	AGTSGSPI	LDK	CGR	VIGLYNGV
JEV	NS3pro	GEVGAV	SLDYF	RGTS	SGSPI	LD	SN
WNV	NS3pro	GEIGAV	TLDYF	TGTS	SGSPI	VD	KN
DENV1	NS3pro	GEVGAV	ALDYF	AGTSGSPI	IVN	REGK	IV
DENV2	NS3pro	GTIGAV	SLDYF	RGTS	SGSPI	VD	KN
DENV3	NS3pro	GDIGAV	ALDYF	AGTSGSPI	LDK	CGR	VIGLYNGV
DENV4	NS3pro	GEIGAV	TLDYF	AGTSGSPI	IN	KN	KV

ZIKV	NS3pro	PSMLKK
JEV	NS3pro	PNMLKK
WNV	NS3pro	PEMLRK
DENV1	NS3pro	DEVFKK
DENV2	NS3pro	DDIFRK
DENV3	NS3pro	PSMLKK
DENV4	NS3pro	EDIFRK

Supplementary information, Figure S1B Multiple sequence alignment of the NS2B-NS3pro from ZIKV, JEV, WNV and DENV 1-4 generated by ESPript 3.0. The residues involved in inhibitor binding are highlighted with blue triangles. The catalytic triad residues are labeled with asterisks. The residues included in NS2B-NS3pro construct are marked with a blue line and the missing residues in the structure are marked by a blue dashed line. The relevant Genbank sequence accession numbers are: ZIKV (KU312312), JEV (JX131374.1), WNV (DQ318019.1), DENV-1 (KJ189353.1), DENV-2 (KC762672.1), DENV-3 (KJ737430.1), and DENV-4 (JX024757.1).

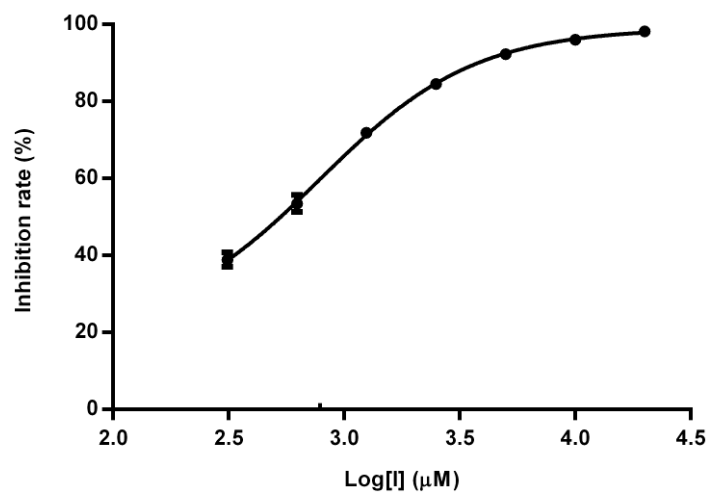


Supplementary information, Figure S1C The monomeric ZIKV NS2B-NS3pro. The overlaid size exclusion chromatograms of ZIKV NS2-NS3pro at various concentrations are indicated.



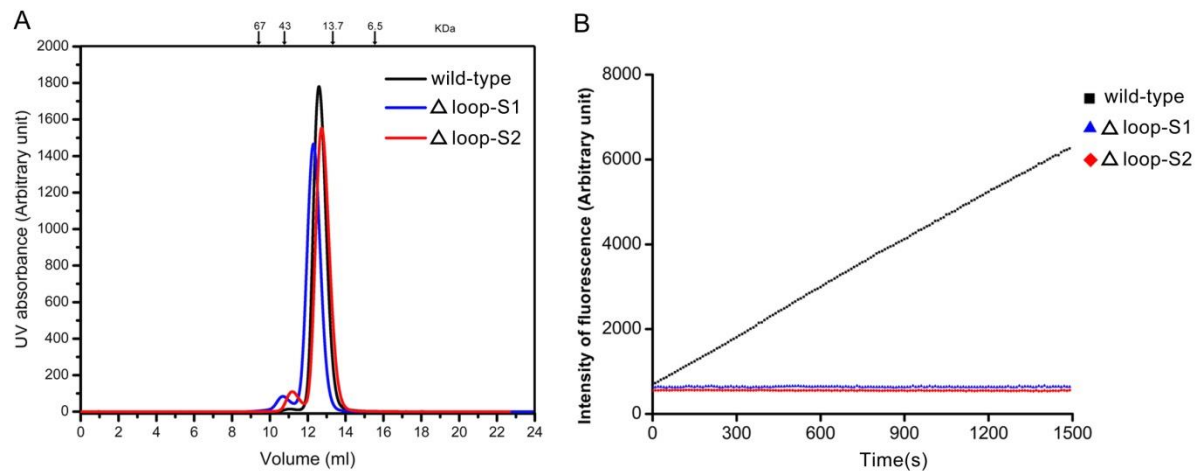
Supplementary information, Figure S1D Enzymatic Activity of ZIKV NS2B-NS3pro.

The initial rate constant V_0 over the concentration of fluorescence-labeled substrate (AC-LKRR-AMC) is plotted.



Supplementary information, Figure S1E Enzymatic inhibition of ZIKV NS2B-NS3pro

by aprotinin. Percent inhibition of the initial rate constant over $\text{Log}[I]$ is plotted.



Supplementary information, Figure S1F The gel filtration profile and enzymatic activity ZIKV NS2B-NS3pro WT, Δ loop-S1₁₅₂₋₁₆₇ and Δ loop-S2₆₉₋₈₇. (A) The size-exclusion gel filtration profile and (B) enzymatic activity ZIKV NS2B-NS3pro wild-type (black), Δ loop-S1₁₅₂₋₁₆₇ (blue) and Δ loop-S2₆₉₋₈₇ (red).