

A conserved arginine in NS5 binds genomic 3'stem-loop RNA for primer-independent initiation of flavivirus RNA-replication

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Supplemental Material

Supplementary Figure 1

A

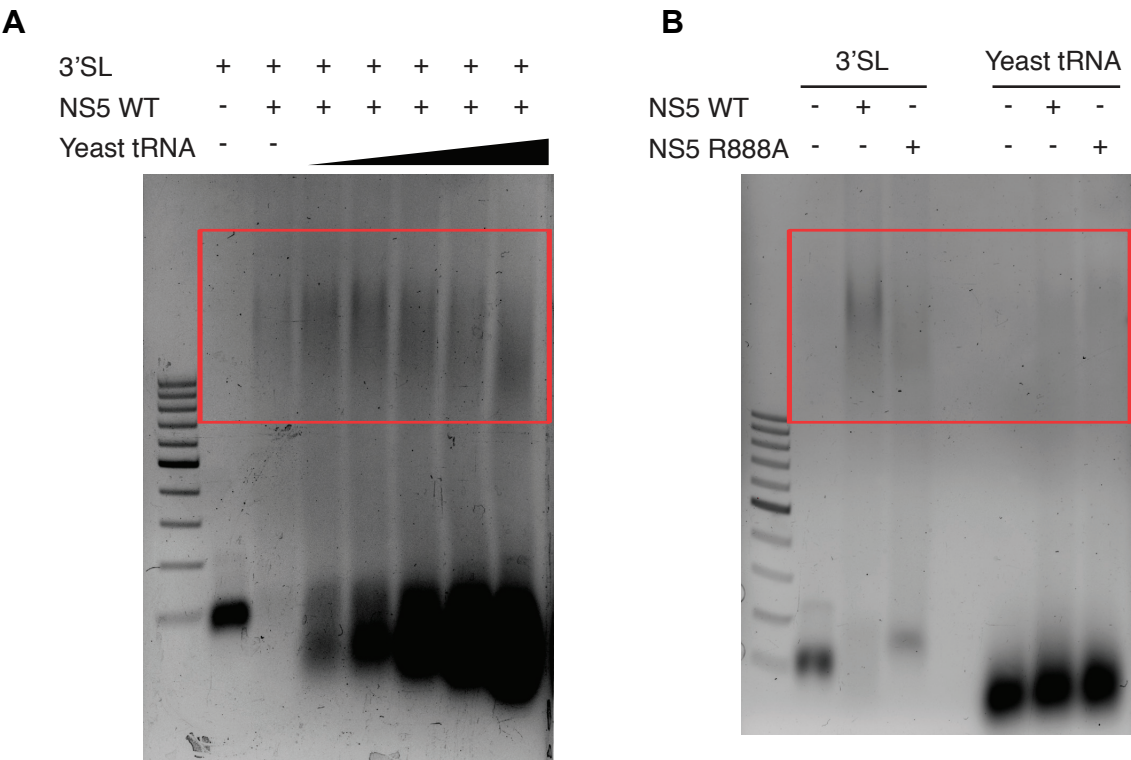
		top-loop	side-loop
DENV2	AGATCCT-GCTGTCT--CCTCAGCA--TCATTCCA	GGCACGAACGCCAG-AAAAT	GG-----AATGGTGCT--G--T-TGAA--TCAACAGGTTCT
DENV1	AGATCCT-GCTGTCT--CTACAGCA--TCATTCCAGGCACAGAACGCCAG-AAAAT	GG-----AATGGTGCT--G--T-TGAA--TCAACAGGTTCT	
DENV3	AGATCCT-GCTGTCT--CCTCAGCA--TCATTCCAGGCACAGAACGCCAG-AAAAT	GG-----AATGGTGCT--G--T-TGAA--TCAACAGGTTCT	
DENV4	AGATCCT-GCTGTCT--CTGCAACA--TCAATCCAGGCACAGAGCGCCGC-GAGAT	GG-----ATTGGTGTT--G--T-TGAT--CCAACAGGTTCT	
ZIKV_MR766	AGACTCCATGAGTTT--CCACCACGCTGGCCGCCAGGCACAGATCGCCGA-ACAGC	GG-C--GGCCGGTGTG--G--G-GAAA--TCCATGGTTTCT	
ZIKV_H/PF/2013	AGACTCCATGAGTTT--CCACCACGCTGGCCGCCAGGCACAGATCGCCGA-ATAGC	GG-C--GGCCGGTGTG--G--G-GAAA--TCCATGGTGCT-	
ZIKV_Paraiba01	AGACTCCATGAGTTT--CCACCACGCTGGCCGCCAGGCACAGATCGCCGA-ATAGC	GG-C--GGCCGGTGTG--G--G-GAAA--TCCATGGTGCT-	
YFV	AGTGGTTCTCTGCTT--TTCTCCAGAGGTCTGTGAGCAGATTGCTCA-AGAAT-AA	GCAGACCTTTTGA--T--GAC--A--AACACAAAACCA	
WNV	GGATCTTCTGCTCTG-----CACAAACGACACACGGCAGTGCGCCGA-CATAG	GT---GGCTGGTGGT--G---CTAG--AACACAGGATCT	
MVEV	AGATCTTCTGCTCTA--TTCCAACATCAGTCACAAGGCACCGAGCGCCGA-ACACT	GT---GACTGATGGG--G--GAGAAG--ACCACAGGATCT	
TBEV	GGTTCTTGTCTCCCTGAGCCACCATCA---CCCAGACACAGGTAGTCTG-ACAAG	GA--GGTGATGTGTGAC--T--CGGAAA--AACACCCGCT--	
JEV	AGATCTTCTGCTCTA--TCTCAACATCAGTCTACTAGGCACAGAGCGCCGA-AGTAT	GT---AGCTGGTGGT--G--AGGAAG--AACAC-----	
USUV_Vienna-2001	AGATCTTCTGCTCTA--TTCCAACATCAACCACAAGGCACAGAGCGCCGA-AAAAT	GT---GGCTGGTGGG--G--AACTAG--ACCACAGGATCT	
BCV	AAAGGTTTCATTA-----A-CTACCTGAGTGAAATCGGGAGGTACTCCGA-AGAAG	A-----GA-----AGGCA-----TTTGCCTT	
ENTV	AGACCCCCCGCGCCCATAAACCAATAAA-----ACAG-----C-ATATT	G-----ACACCTGG-----GAAAAG--ACCGGAGACTCT	
MLLV	TGGATATATACT-----CCAGCC-AG--AAAAGACTCAGATTGTCTC-ATGACTT	--TCTGACTGGC-G---T--ACATA--GCCATCCGCT--	
PPBV	AAAGGTTTCATTA-----A-CTACTTGAGTGAAATCGGAAGGTATCCGA-AAAAG	A-----GA-----GGGCG-----TTTGTCTT	
RBV	AAAATTACGTCA-----A-CTACCTTGAGCAATGGGACGCTATGAATG-TGATG	A-----TG-----TCTCA-----TTCCAAC	
YOKV	-----TCGGTTCCGGAGAGCTCCGGAGG--CCAGGGCGCGCTTTGCCCG	-TAGTTTATAACTGGCCTTCGGGGATCGAAGGAGTTGCCAAACACT--	
APOIV	AGCATTACTCTG-----A-CTACTTGAGCAATATGGGTAGGTACCAAGA-AGGA	A-C-----GG-----AAAG-----TTCCACAT	
JUTV	AAAACCTCAGGG-----A-TTACCTTCAAGTGATGGGCAGGTTTGTTC	A-AAAAC-A-----GCCTTCAG-----CCACC-----TTCTCCAT	
MODV	TGATTAGCCATG-----G--TCGCAC-AG--ATCAAGCTCAGATTGCTTACATG-TAA	--TCTGTGTGGTCA--T--GAATA--TGACCTCCGCT--	

B

		838				888
DENV2	VESWEEIP	Y	LGKREDQWCGSLIGLTSRATWAKNIQTAINQVRS	LIGN-EEYTDYMP	SMK	R FRREEEEAGVLW--
DENV1	ISSWEDVP	Y	LGKREDQWCGSLIGLTSRATWATNIQVAINQVRR	LIGN-ENYLDYMT	SMK	R FKNESDPEGALW--
DENV3	VTTWENV	Y	LGKREDQWCGSLIGLTSRATWAQNIPTAIQVRS	LIGN-EEFLDYMP	SMK	R FRKEEELEGAIW--
DENV4	VHSWEDIP	Y	LGKREDLWCGSLIGLSSRATWAKNIHTAITQVRN	LIGK-EEYVDYMP	VMK	R YSAPSESEGLV--
ZIKV_MR766	VTKWTDIP	Y	LGKREDLWCGSLIGHRPRTTWAENIKDVTNVMVR	RIIGDEEKYMDYL	STQV	R YLGEESTPGVL--
ZIKV_H/PF/2013	VTKWTDIP	Y	LGKREDLWCGSLIGHRPRTTWAENIKDVTNVMVR	RIIGDEEKYMDYL	STQV	R YLGEESTPGVL--
ZIKV_Paraiba01	VTKWTDIP	Y	LGKREDLWCGSLIGHRPRTTWAENIKDVTNVMVR	RIIGDEEKYMDYL	STQV	R YLGEESTPGVL--
YFV	VKKWRDVP	Y	LTKRQDKLCSLIGMTNRATWASHIHLVIHRI	RTLIGQ-EKYDYL	TVMD	R YSVADADLQLGELI-
WNV	VERWSDVP	Y	SGKREDIWCGSLIGTRTRATWAENIHVAINQVRS	VIGE-EKYVDYM	SSLR	R YEDTIVVEDTVL--
MVEV	VSDWTEVP	Y	VGKREDIWCGSLIGTRTRATWAENIYAAINQVRS	VIGK-EKYVDYV	QSLR	R YEETHVSEDRVL--
TBEV	VMIEWRDP	Y	LPKAQDMLCSSLVGRRERAEWAKNIWGA	EKVRKMIGP-EKFKDY	LSCMD	R HDLHWELRLESSII
JEV	ITSWTDVP	Y	VGKREDIWCGSLIGTRSRATWAENIYAAINQVRA	VIGK-ENYVDYMT	SLR	R YEDVLIQEDRVI--
USUV_Vienna-2001	VQSWTDIP	Y	TGKREDIWCGSLIGTRTRATWAENIYAAINQVRA	IIGQ-EKYRDYML	SLR	R YEEVNVQEDRVL--
BCV	INDWKNI	Y	LPRGQDISCGSLVGTGKRAQWAE	LIPGATLKVRNLMGN-ERFINYL	SEIG	R YSEEE--KAFALY-
ENTV	LEDWKEIP	Y	LNKSQDVRCGSHIGCSQRKSWADALPVGAV	MKVRNLFPG-EKFSNYM	DCIG	R YHVGH--QDFCLY-
MLLV	VREWNAIP	Y	LPRREDINCGSLIGTSKRSTWATLVP	PGAVMKVRNLFPG-EKFSNYM	DCIG	R YHVGH--QDFCLY-
PPBV	INDWKDVP	Y	LPRGQDISCGSLVGTGKRAQWAE	LIPGATLKVRNLMGN-ERFINYL	SEIG	R YSEKE--RAFVLY-
RBV	ITTWKNVA	Y	LPRGQDMCCGSLIGSSKRATWAKLIP	GAVEKVRGMIGN-ENYVNYL	LGAMG	R YECDD--VSFQLY-
YOKV	IGKWQDIP	Y	ISKSQDVRCGSMIGTSKRSSWAEALPHTVQ	KVRGIVGTQERYRDYLETQN		R FRTIVQHLVGDIL-
APOIV	VAEWKNIP	Y	LQRNQDLSCSSLIDNPTRAQWAKLLKGAV	MKVREMIGR-EHYSYDLS	NMG	R YQEGT--EEFHMW-
JUTV	IDQWSNIP	Y	LPRKVDKKCGSLIGMKNRIEAKLLPGAVL	KVRNVFGR-ENFRDYLQ	VMG	R FVQKQPSATFSMY-
MODV	VERWSEVP	Y	LPRNQDKSCGSLIGTTARA	EAKLLPGAVEKVRNIFGK-QRFRNYL	LRNMG	R YESQE-EAPFSMY-

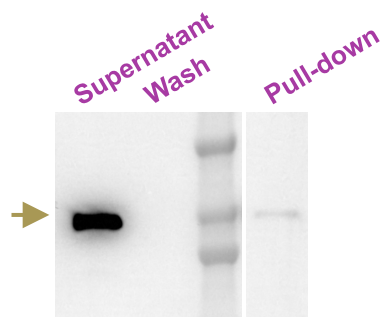
Supplementary Figure 1. Multiple sequence alignment of the (A) 3'SL and (B) NS5 C-terminal sequences of flaviviruses. The residues Y838 and R888 are highlighted in bold in (B). GenBank accession numbers for the relevant sequences are: DENV1 (EU081230), DENV2 (EU081177), DENV3 (EU081190), DENV4 (GQ398256), ZIKV_MR766 (LC002520.1), ZIKV_H/PF/2013 (KJ776791.2), ZIKV_Paraiba01/2015 (KX280026.1), Yellow Fever virus (YFV; X15062), West Nile virus (WNV; M12294), Murray Valley Encephalitis virus (MVE; AF161266), Tick-Borne Encephalitis Virus (TBEV; U27495), Japanese Encephalitis virus (JEV; M55506), Usutu virus (USUV_Vienna-2001; AY453411), Batu Cave Virus, strain P70-1459 (BCV; KJ469370.1), Entebbe bat virus, strain UGIL-30 (ENTV; NC_008718.1), Montana myotis leukoencephalitis virus, stain UNKNOWN-NC_004119 (MLLV; NC_004119.1), Phnom Penh bat virus, strain 30834_A38 (PPBV; NC_034007.1), Rio Bravo virus, strain RIMAR (RBV; NC_003675.1), Yokose virus, strain Oita 36 (YOKV; NC_005039.1), Apoi virus, strain ApMAR (APOIV; NC_003676.1), Jutiapa virus, strain JG-128 (JUTV; NC_026620.1), Modoc virus, strain M544 (MODV; NC_003635.1).

Supplementary Figure 2



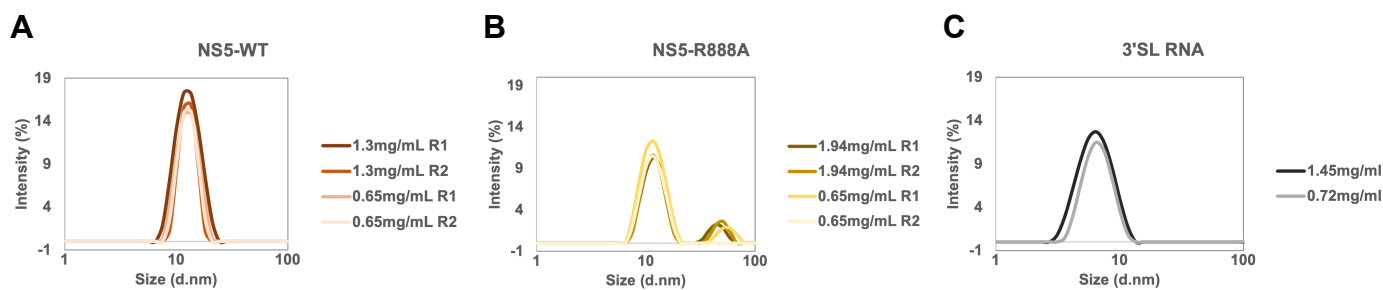
Supplementary Figure 2. 3' SL binds specifically to wild type (WT) DENV2 NS5. (A) 3'SL was incubated with DENV2 NS5 in the absence and presence of increasing amounts of yeast tRNA (0.1, 0.5, 1, 2 and 5ug). (B) 3'SL or yeast tRNA was incubated with wild type and R888A DENV2 NS5. In both panels, the reactions were resolved by agarose gel electrophoresis and the RNA was detected using GelRed. An interaction between the RNA and NS5 protein will result in a shift in the RNA from the bottom of the gel to the top (area boxed in red)

Supplementary Figure 3



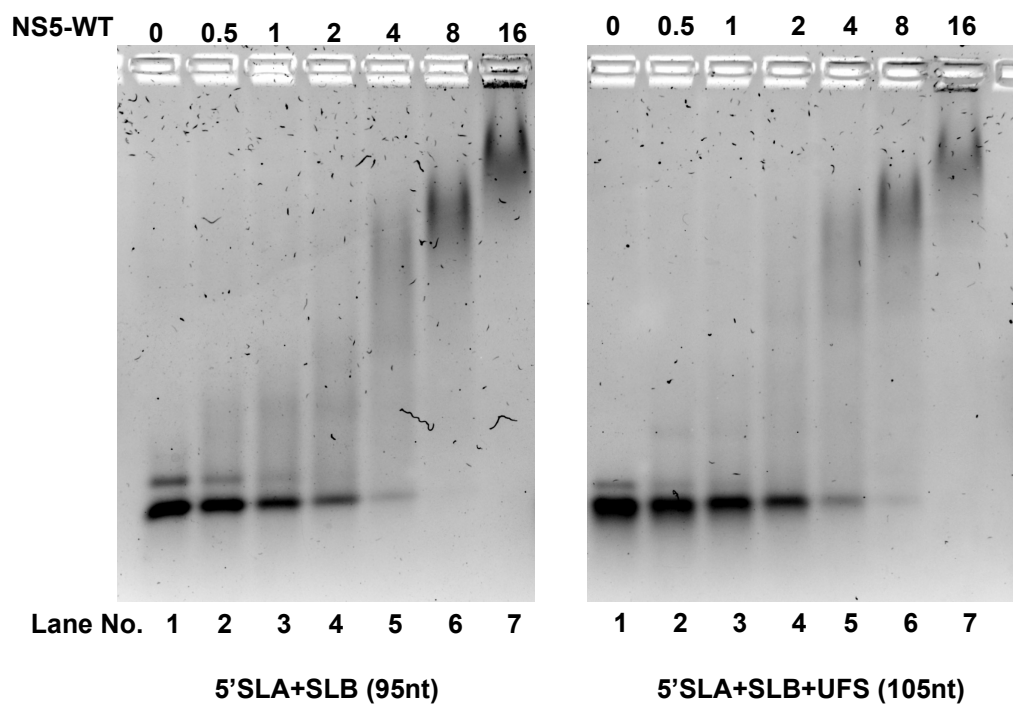
Supplementary Figure 3. Western blot analysis of various samples (supernatant from centrifugation following the incubation of NS5 R888A protein with beads; wash of beads after centrifugation and removal of supernatant; pull-down) from NS5 R888A mutant and biotinylated 5'SLA RNA Co-IP experiment. NS5 was detected by 5M1 antibody.

Supplementary Figure 4



Supplementary Figure 4. Intensity-based size distribution profiles from Dynamic Light Scattering (DLS) experiments showing **[A]** WT NS5 at two different concentrations and each with two technical repeats; **[B]** NS5 R888A mutant at two different concentrations and each with two technical repeats and **[C]** 3'SL RNA at two different concentrations. X-axis represents hydrodynamic diameter of NS5 or 3'SL RNA molecules. Concentrations of NS5 or 3'SL RNA are noted in the legend of each sub-figure.

Supplementary Figure 5



Supplementary Figure 5. RNA electrophoretic mobility shift assay (REMSA) of 5' SLA+SLB or 5'SLA+SLB+UFS with WT NS5 that was analyzed by agarose gel electrophoresis and visualized by GelRed staining. Lane 1 in each gel represents 5' SLA+SLB or 5'SLA+SLB+UFS RNA alone in binding buffer. Lanes 2-7 represents binding reactions of NS5 with RNA at molar ratio of 0:1, 0.5:1, 1:1, 2:1, 4:1, 8:1 and 16:1 as indicated at the top of each lane.