

Motif V acts as a Regulator of Energy Transduction Between the Flavivirus NS3 ATPase and RNA Binding Cleft

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Figure S1

Figure S2

Table S1

Table S2

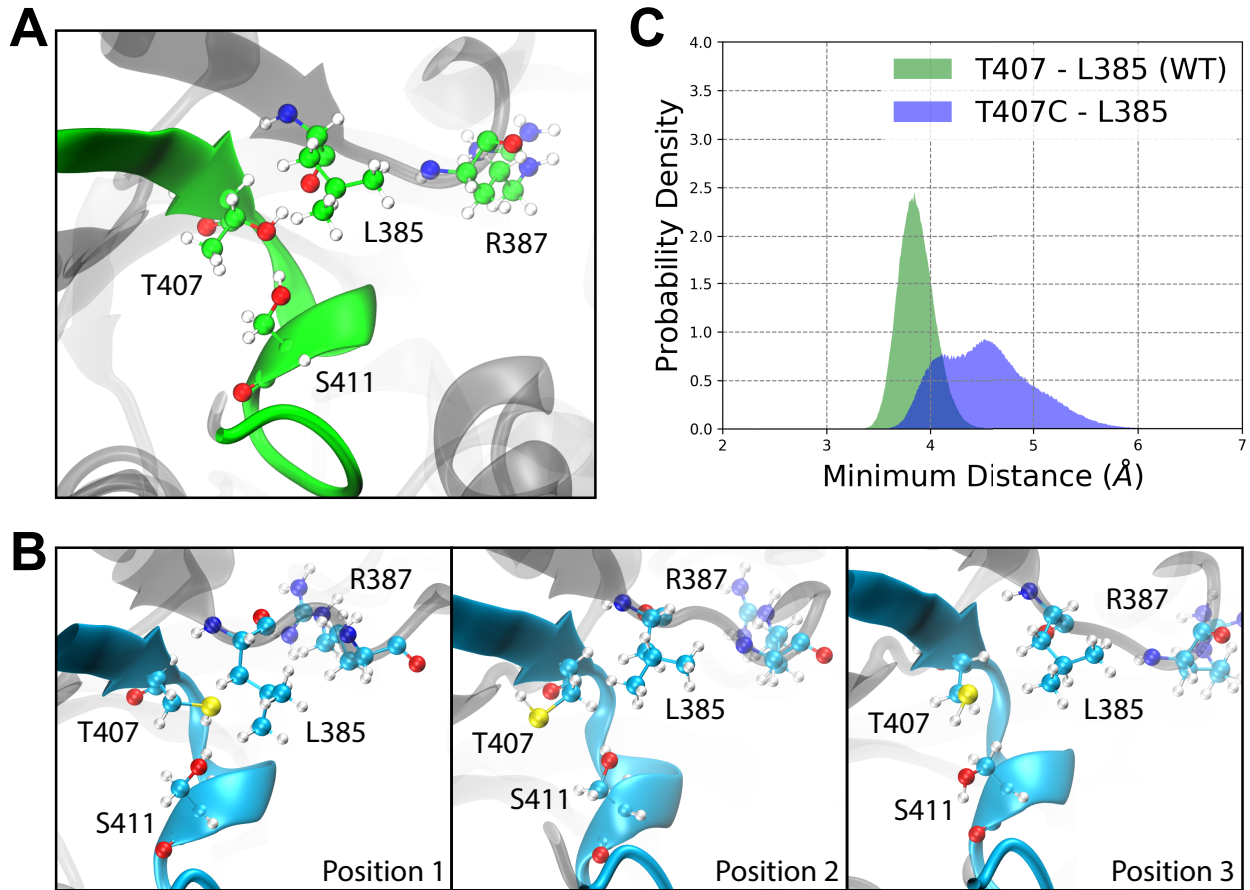


Figure S1. Methyl group in the T407 sidechain stabilizes T407 interactions with L385. **A)** The methyl group stabilizes the structure of T407 in the WT simulations. **B)** Without the methyl group, the sulfur group of T407C fluctuates frequently throughout the T407C simulations. **C)** The minimum distance was calculated between the L385 sidechain and either the hydroxyl group (threonine sidechain) or the thiol group (cysteine sidechain) at position 407 for each replicate of the WT and the T407C simulations. The probability density of the average minimum distance was then determined for WT and T407C simulations.

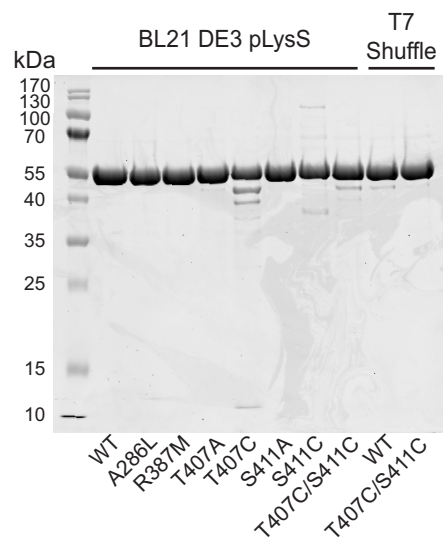


Figure S2. Confirmation purified NS3 helicase variants. A) Recombinant NS3 helicase variants were purified and confirmed on SDS-PAGE. We noted that T407C degraded over time but it was full-length when used in assays.

Virus	404		405		406		407		411													
	Number of Sequences	Consensus	F404(%)	Y404(%)	L404(%)	Consensus	V405(%)	L405(%)	I405(%)	Consensus	V406(%)	D406(%)	G406(%)	I406(%)	L406(%)	Consensus	T407(%)	A407(%)	S407(%)	Consensus	S411(%)	A411(%)
Karshi Virus	1	F	100.0	-	-	V	100.0	-	-	V	100.0	-	-	-	-	T	100.0	-	-	S	100.0	-
Powassan	22	F	100.0	-	-	V	100.0	-	-	V	100.0	-	-	-	-	T	100.0	-	-	S	100.0	-
MMLV	3	F	100.0	-	-	I	-	100.0	-	L	-	-	-	100.0	-	T	100.0	-	-	S	100.0	-
Rio Bravo	3	F	100.0	-	-	I	-	100.0	-	L	-	-	-	100.0	-	T	100.0	-	-	S	100.0	-
Modoc	3	F	100.0	-	-	I	-	100.0	-	L	-	-	-	100.0	-	T	100.0	-	-	S	100.0	-
Apol	2	F	100.0	-	-	I	-	100.0	-	L	-	-	-	100.0	-	T	100.0	-	-	S	100.0	-
JEV	321	F	100.0	-	-	V	100.0	-	-	I	0.9	-	-	99.1	-	T	100.0	-	-	S	100.0	-
Usutu	138	F	100.0	-	-	V	100.0	-	-	I	0.7	-	-	99.3	-	T	100.0	-	-	S	100.0	-
WNV	2008	F	100.0	-	-	V	100.0	-	-	I	0.6	-	-	99.4	-	T	100.0	-	-	S	100.0	-
Kunjin	44	F	100.0	-	-	V	100.0	-	-	V	100.0	-	-	-	-	T	100.0	-	-	S	100.0	-
Bagaza	12	F	100.0	-	-	V	100.0	-	-	I	-	-	-	100.0	-	T	100.0	-	-	S	100.0	-
Ilheus	1	F	100.0	-	-	V	100.0	-	-	I	-	-	-	100.0	-	T	100.0	-	-	S	100.0	-
Kedougou	1	F	100.0	-	-	V	100.0	-	-	I	-	-	-	100.0	-	T	100.0	-	-	S	100.0	-
Zika	659	F	100.0	-	-	V	100.0	-	-	I	85.6	-	-	14.4	-	T	100.0	-	-	S	100.0	-
Bussuquara	1	F	100.0	-	-	V	100.0	-	-	V	100.0	-	-	-	-	T	100.0	-	-	S	100.0	-
DENV1	1948	Y	0.1	99.9	-	V	100.0	-	-	V	99.9	0.1	-	0.1	-	T	100.0	-	-	S	100.0	-
DENV2	1524	F	100.0	-	-	V	100.0	-	-	V	99.8	0.1	-	0.1	-	T	100.0	-	-	S	100.0	-
DENV3	1112	F	100.0	-	-	V	99.9	0.1	-	V	99.9	0.1	-	0.1	-	T	99.8	0.2	-	S	100.0	-
DENV4	241	F	100.0	-	-	V	100.0	-	-	V	100.0	-	-	-	-	T	100.0	-	-	S	100.0	-
Kokobera	1	F	100.0	-	-	V	100.0	-	-	I	-	-	-	100.0	-	T	100.0	-	-	S	100.0	-
YFV	105	F	100.0	-	-	I	-	100.0	-	L	-	-	-	100.0	-	A	-	100.0	-	A	-	100.0
Sepik	1	F	100.0	-	-	I	-	100.0	-	L	-	-	-	100.0	-	A	-	100.0	-	A	-	100.0
Entebbe Bat	1	F	100.0	-	-	I	-	100.0	-	L	-	-	-	100.0	-	T	100.0	-	-	A	-	100.0
Cell fusing agent	35	L	-	100.0	-	V	100.0	-	-	I	-	-	-	100.0	-	S	-	-	100.0	S	100.0	-
Kamiti River	2	L	-	100.0	-	V	100.0	-	-	V	100.0	-	-	-	-	S	-	-	100.0	S	100.0	-
Gulex	32	F	100.0	-	-	I	-	100.0	-	V	100.0	-	-	-	-	S	-	-	100.0	S	100.0	-

Table S1. Flavivirus sequence variability at positions 404, 405, 406, 407 and 411. Flavivirus sequences were analyzed to determine the consensus sequence at motif V positions 404, 405, 406, 407 and 411. The percentage of finding the consensus or another residue instead of the consensus are reported for each position for each flavivirus.

Substrate Inhibition of ATPase Activity

NS3h variant	K_i (μM)
WT	888.2 ± 247.7
A286L	570.5 ± 377.4
R387M	745.7 ± 371.1
T407A	1442.1 ± 727.5
T407C	587.6 ± 177.8
S411A	695.5 ± 404.4
S411C	775.8 ± 166.9
T407C/S411C	1157.8 ± 480.1
WT reduced [†]	534.3 ± 110.5
WT oxidized [‡]	297.3 ± 133.0
T407C/S411C reduced [†]	729.0 ± 148.4
T407C/S411C oxidized [‡]	532.7 ± 137.3

Table S2. ATP substrate inhibition of ATPase activity. The ATPase activity exhibited substrate inhibition at high concentrations of ATP. As a result of substrate inhibition, the data was fit to the substrate inhibition equation. The inhibition constant (K_i) was reported for each NS3 helicase variant.