

Supplemental Information

The viral capping enzyme nsP1: a novel target for the inhibition of chikungunya virus infection

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Supplemental Figure 1. Multiple sequence alignment of nsP1 amino acid sequences of alphaviruses.

A

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CHIKV  --MDPVYVDIDADSAFLKALQRAYPMFEVEPRQVT PNDHANARAFSHLAIKLIQEIDPD 58
ONNV   --MDSVYVDIDADSAFLKALQQAYPMFEVEPKQVT PNDHANARAFSHLAIKLIQEIDPD 58
RRV    ---MKVTVDVDEADSPFLKALQKAFPAFEVESQQVT PNDHANARAFSHLATKLIQEVPAN 57
SFV    --MAAKVHVDIEADSPFIKSLQKAFPSFEVESLQVT PNDHANARAFSHLATKLIQEETDKD 59
SINV   MEKPVVNVDPQSPFVVLQKSFQFEVVAQQVT PNDHANARAFSHLASKLIELEVPTT 60
WEEV   --MERIHVDLDADSPYVKSQRQTFPQFEIEARQVT DNDHANARAFSHVATKLIESEVDRD 58
EEEV   --MEKVHVDLDADSPFVKSLQRQCFPHFEIEATQVT DNDHANARAFSHLATKLIERGEVDTD 58
VEEV   --MEKVHVDIEEDSPFLRALQRSFQFEVEAKQVT DNDHANARAFSHLASKLIETEVDPS 58
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B

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899      MDPVYVDIDADSAFLKALQRAYPMFEVEPRQVT PNDHANARAFSHLAIKLIQEIDPDST
Venturini MDPVYVDIDADSAFLKALQRAYPMFEVEPRQVT PNDHANARAFSHLAIKLIQEIDPDST
Congo    MDPVYVDIDADSAFLKALQRAYPMFEVE SRQVT PNDHANARAFSHLAIKLIQEIDPDST
LS3      MDPVYVDIDADSAFLKALQRAYPMFEVEPRQVT PNDHANARAFSHLAIKLIQEIDPDST
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899      ILDIGSAPARRMMSDRKYHCVCPMRSAEDPERLANYARKLASAAGKVLDRNISGKIGDLQ
Venturini ILDIGSAPARRMMSDRKYHCVCPMRSAEDPERLANYARKLASAAGKVLDRNISGKIGDLQ
Congo    ILDIGSAPARRMMSDRKYHCVCPMRSAEDPERLANYARKLASAAGKVLDRNISGKIGDLQ
LS3      ILDIGSAPARRMMSDRKYHCVCPMRSAEDPERLANYARKLASAAGKVLDRNISGKIGDLQ
*****.*****

899      AVMAVPDTEPTPFCCLHTDVSCRQADVAIYQDVYAVHAPTSLYHQAIKGVVAVYVWVGFDT
Venturini AVMAVPD KETPFCCLHTDVSCRQADVAIYQDVYAVHAPTSLYHQAIKGV VAVYVWVGFDT
Congo    AVMAVPDTEPTPFCCLHTDVSCRQADVAIYQDVYA I HAPTSLYHQAIKGVVAVYVWVGFDT
LS3      AVMAVPD KETPFCCLHTDVSCRQADVAIYQDVYAVHAPTSLYHQAIKGVVAVYVWVGFDT
*****.*****

899      TPFMYNAMAGAYPSYSTNWADEQVLKAKNIGLCSTDLTEGRRGKLSIMRGKLLKPCDRVL
Venturini TPFMYNAMAGAYPSYSTNWADEQVLKAKNIGLCSTDLTEGRRGKLSIMRGKLLKPCDRVL
Congo    TPFMYNAMAGAYPSYSTNWADEQVLKAKNIGLCSTDLTEGRRGKLSIMRGKLLKPCDRVL
LS3      TPFMYNAMAGAYPSYSTNWADEQVLKAKNIGLCSTDLTEGRRGKLSIMRGKLLKPCDRVL
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899      FSVGSTLYPESRLLKSWHLPSVFHLKGKLSFTCRCDTVVSCEGYVVKRITMSPGLYGKT
Venturini FSVGSTLYPESRLLKSWHLPSVFHLKGKLSFTCRCDTVVSCEGYVVKRITMSPGLYGKT
Congo    FSVGSTLYPESRLLKSWHLPSVFHLKGKLSFTCRCDTVVSCEGYVVKRITMSPGLYGKT
LS3      FSVGSTLYPESRLLKSWHLPSVFHLKGKLSFTCRCDTVVSCEGYVVKRITMSPGLYGKT
*****

899      TGYAVTHHADGFLMCKTTDTVDGERVSFSVCTYVPATICDQMTGILATEVTPEDAQKLLV
Venturini TGYAVTHHADGFLMCKTTDTVDGERVSFSVCTYVPATICDQMTGILATEVTPEDAQKLLV
Congo    TGYAVTHHADGFLMCKTTDTVDGERVSFSVCTYVPATICDQMTGILATEVTPEDAQKLLV
LS3      TGYAVTHHADGFLMCKTTDTVDGERVSFSVCTYVPATICDQMTGILATEVTPEDAQKLLV
*****

899      GLNQRIVVNGRTQRNTNTMKNYLLPVVAQALSKWAKECRKDMEDEKLLGVRERTLTCCCL
Venturini GLNQRIVVNGRTQRNTNTMKNYLLPVVAQAF SKWAKECRKDMEDEKLLGVRERTLTCCCL
Congo    GLNQRIVVNGRTQRNTNTMKNYLLPVVAQAF SKWAKECRKDMEDEKLLGVRERTLTCCCL
LS3      GLNQRIVVNGRTQRNTNTMKNYLLPVVAQAF SKWAKECRKDMEDEKLLGVRERTLTCCCL
*****

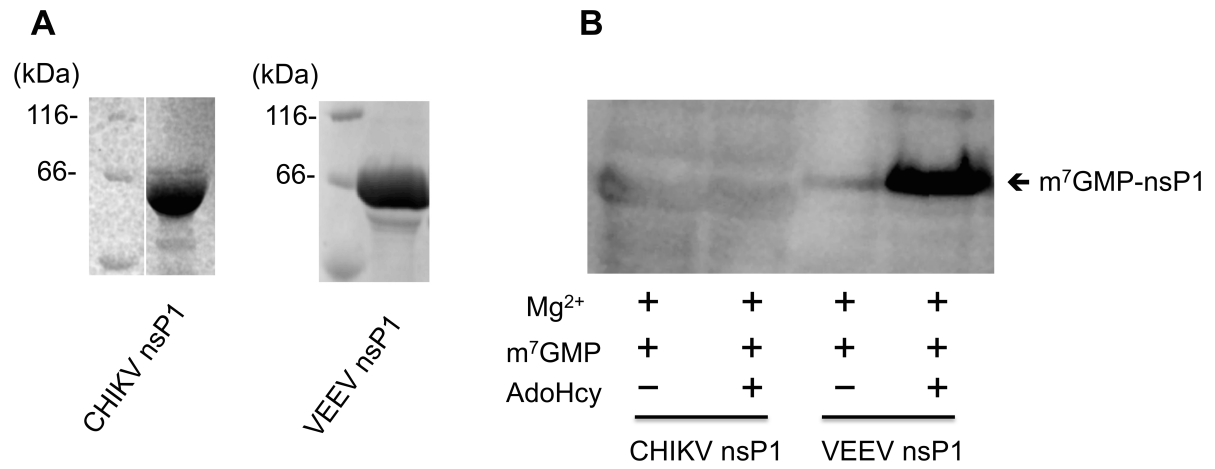
899      WAFKKQKTHTVYKRPDTSIQKVQAEFDSFVPSLWSSGLSIPLRTRIKWLLSKVPKTDL
Venturini WAFKKQKTHTVYKRPDTSIQKVQAEFDSFVPSLWSSGLSIPLRTRIKWLLSKVPKTDL
Congo    WAFKKQKTHTVYKRPDTSIQKVQAEFDSFVPSLWSSGLSIPLRTRIKWLLSKVPKTDL
LS3      WAFKKQKTHTVYKRPDTSIQKVQAEFDSFVPSLWSSGLSIPLRTRIKWLLSKVPKTDL
*****

899      IPYSGDAREARDAEKEAEEREAEELTREALPPLQAAQEDVQVEIDVEQLEDRAGA
Venturini IPYSGDAREARDAEKEAEEREAEELTREALPPLQAAQEDVQVEIDVEQLEDRAGA
Congo    IPYSGDAREARDAEKEAEEREAEELTRVALPPLQAAQEDVQVEIDVEQLEDRAGA
LS3      IPYSGDAREARDAEKEAEEREAEELTREALPPLQAAQEDVQVEIDVEQLEDRAGA
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A) The nsP1 amino acid sequences of the CHIKV strain 899 (ACV88658.1), ONNV (NP_041254.1), RRV (NP_062879.1), SFV (NP_463457.1), SINV (NP_062888.1), WEEV (NP_640330.1), EEV (NP_632021.2), and VEEV (AAY16003.1) were aligned using the ClustalW2 server (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) (1). Conserved amino acid sequences are shown in grey. The residues corresponding to P34 in CHIKV are highlighted in yellow as well as the corresponding D34 in the other alphaviruses. The conserved histidine of the methyltransferase motif is shown in green. B) The nsP1 sequences of the CHIKV strain 899 (ACV88658.1), clinical isolates Venturini (Italy 2008) and Congo 95 (2011) and CHIKV infectious clone LS3 (AIK21921.1) were aligned using the Muscle software (<http://www.ebi.ac.uk/Tools/msa/muscle/>). The residues corresponding to P34 in CHIKV 899 are highlighted in green. The residues of CHIKV Venturini, Congo and LS3 that differ from CHIKV 899 are highlighted in yellow, blue and pink, respectively.

Supplemental Figure 2. Production and functional comparison of CHIKV and VEEV nsP1.



A) Coomassie blue-stained SDS-PAGE gel showing the produced CHIKV and VEEV nsP1 proteins. B)

Western blotting showing the formation of m⁷GMP-nsP1 complex by CHIKV and VEEV nsP1.

Supplemental Table 1. Mutations identified in the MADTP-314-resistant CHIKV isolates.

Gene	WT codon	WT AA	Mutant codon			Mutant AA		
			c1	c2	c3	c1	c2	c3
nsP1	GAC	D ₉	GAC	GAC	TAC	D ₉	D ₉	Y ₉
	CAA	Q ₁₉	CAA	CAA	CGA	Q ₁₉	Q ₁₉	R ₁₉
	CCG	P ₃₄	TCG	TCG	TCG	S ₃₄	S ₃₄	S ₃₄
nsP3	GCC	A ₄₅₈	T/GCC	n.d.	n.d.	S/A ₄₅₈	n.d.	n.d.
E1	GTG	V ₆	GA/TG	n.d.	n.d.	E/V ₆	n.d.	n.d.

n.d.: not determined; c: independently selected resistant clone.

References

1. McWilliam H, et al. (2013) Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Res* 41:W597–600.