

Additional file 1:

Figures and Tables legends:

Figure S1. Effect of [E1-A66S and E1-D70N] mutations on CHIKV infectivity for cholesterol-depleted C6/36 cells.

Standard (blue bars) and cholesterol-depleted (yellow bars) C6/36 cells were infected with serial dilutions of LR-GFP-Cl#1-10660A. Results are normalized for 10^6 viral infections of standard C6/36 cells. Data indicate an average of three experiments $\pm$ standard deviation.

Table S1. Specific infectivity of *in vitro* transcribed RNA and titers after RNA electroporation for non-GFP expressing viruses with substitutions at E1-226.

a – amino acids at position E1-226.

b – time (h) required for visible plaques to develop.

c – Virus titers were determined by titration on Vero cells and expressed as $\text{Log}_{10}\text{TCID}_{50}/\text{mL}$.

ND- titer not determined.

Table S2. Specific infectivity of *in vitro* transcribed RNA and titers after RNA electroporation for GFP-expressing viruses with substitutions at E1-226.

a – amino acids at position E1-226.

b – time (h) required for visible plaques to develop.

c – Virus titers were determined by titration on Vero cells and expressed as $\text{Log}_{10}\text{TCID}_{50}/\text{mL}$.

ND- titer not determined.

Table S3. Stability of selected mutations at the position E1-226 in *Ae. albopictus* mosquitoes.

Time - time (h) required for visible plaques to develop.

N m - number of individual mosquitoes analyzed.

E1-226 – residue at E1-226 determined by sequencing of CHIKV RNA isolated from individual mosquito.

P/L means that CHIKV with leucine and proline at E1-226 are simultaneously present in this mosquito.

Table S4. Summary of adaptation experiment.

a – identified nucleotide mutations and genome location for each of plaque purified viruses.

b – corresponding amino acid substitutions.

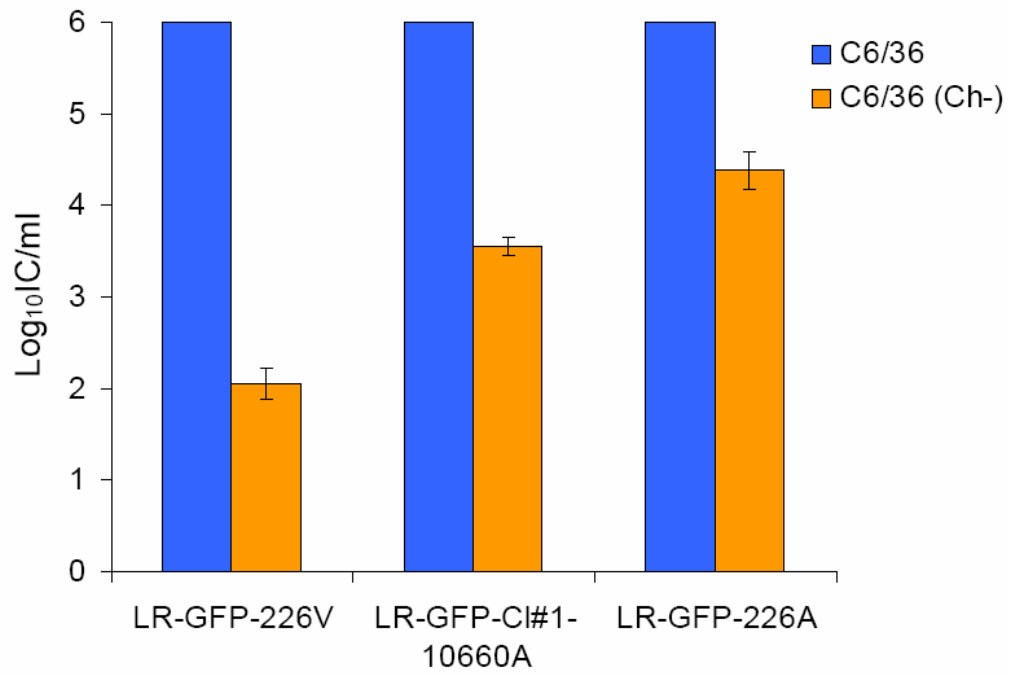


Figure S1. Effect of [E1-A66S and E1-D70N] mutations on CHIKV infectivity for cholesterol-depleted C6/36 cells.

Table S1. Specific infectivity of *in vitro* transcribed RNA and titers after RNA electroporation for non-GFP expressing viruses with substitutions at E1-226.

Template used for <i>in vitro</i> transcription.	E1-226 ^a	Time ^b	Specific infectivity (pfu/μg of RNA)	<u>Virus titer^c</u>	
				24 h	48 h
pLR-ApaI-226V	V	48	9.1 x 10 ⁵	6.95	7.52
pLR-226A	A	48	8.3x10 ⁵	6.52	7.52
pLR-226S	S	48	7.0x10 ⁵	7.95	7.95
pLR-226T	T	48	6.5x10 ⁵	7.95	7.95
pLR-226G	G	48	10.0x10 ⁵	6.52	7.52
pLR-226I	I	72	5.0x10 ⁵	6.52	6.52
pLR-226P	P	48	9.5x10 ⁵	7.52	6.52
pLR-226F	F	72	9.8x10 ⁵	6.95	6.52
pLR-226M	M	72	9.9x10 ⁵	7.52	7.52
pLR-226H	H	72	10.0x10 ⁵	6.95	6.95
pLR-226L	L	96	10.0x10 ⁵	6.95	6.95

Table S2. Specific infectivity of *in vitro* transcribed RNA and titers after RNA electroporation for GFP-expressing viruses with substitutions at E1-226.

Template used for <i>in vitro</i> transcription.	E1-226 ^a	Time ^b	Specific infectivity	Virus titer ^c	
				24h	48h
pLR-GFP-226V	V	48	4.8x10 ⁵	6.52	6.52
pLR-GFP-226A	A	48	3.3x10 ⁵	6.95	6.95
pLR-GFP-226S	S	48	7.0x10 ⁵	7.95	7.95
pLR-GFP-226T	T	48	6.5x10 ⁵	7.95	7.95
pLR-GFP-226G	G	48	10.0x10 ⁵	6.52	7.52
pLR-GFP-226I	I	72	5.0x10 ⁵	6.52	6.95
pLR-GFP-226P	P	48	7.2x10 ⁵	6.95	6.95
pLR-GFP-226F	F	72	5.0x10 ⁵	ND	6.95
pLR-GFP-226M	M	72	5.0x10 ⁵	ND	6.72
pLR-GFP-226H	H	72	5.0x10 ⁵	5.95	6.95
pLR-GFP-226L	L	96	3.0x10 ⁵	5.95	6.52

Table S3. Stability of selected mutations at the position E1-226 in *Ae. albopictus* mosquitoes.

Virus	Time	N m	E1-226
LR-GFP-226I	72	4	1-I; 2-I; 3-I; 4-I
LR-GFP-226F	72	3	1-F; 2-F; 3-F
LR-GFP-226M	72	3	1-M; 2-M; 3-M
LR-GFP-226H	72	3	1-H; 2-H; 3-H
LR-GFP-226L	96	4	1-L; 2-L/P; 3-L/P, 4-L

Table S4. Summary of adaptation experiment.

Clone #	Mutation (nt) ^a	Mutation (aa) ^b
1	10189 G→T 10201 G→A 10660 A→C	E1-A66S E1-D70N E1-R223R
2	10760 T→C	E1-V226A
3	10760 T→C 10337 A→C	E1-V226A E1-K114T
4	10760 T→C	E1-V226A
5	10189 G→T 10201 G→A 10660 A→C	E1-A66S E1-D70N E1-R223R
6	10760 T→C	E1-V226A
7	10189 G→T 10201 G→A 10660 A→C 10760 T→C	E1-A66S E1-D70N E1-R223R E1-V226A
8	10760 T→C	E1-V226A
9	10760 T→C	E1-V226A
10	10659 G→T 10970 G→A	E1-Q222H E1-G326D