SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Sequencing of viral RNA from rhesus macaques after infection with CHIKV-LR and treatment with CHK-152 + CHK-166 MAbs. Rhesus macaques were inoculated with 10^7 PFU of CHIKV-LR (WT) via a subcutaneous route and then treated intravenously at days 1 and 3 after infection with anti-CHIKV MAbs (CHK-152 + CHK-166). At day 7, spleen (**A, C**) and tissue from the right quadriceps (**B, D**) or right toe (**B, D**) was harvested and consensus sequencing of the E2 (**A, B**) or E1 (**C, D**) structural genes was performed using overlapping primers. The data were aligned using Geneious software and compared to the CHIKV-LR (2006 OPY-1) infectious clone sequence. Each five-digit number corresponds to an individual animal and the appended number indicates sequence from an independent clone. The areas of possible escape mutation (E2-D59 and E1-K61) are bracketed. No consistent mutations were detected.

Figure S2. Antibody resistant mutations are retained in C6/36 *Ae. albopictus* cell cultures. C6/36 *Ae. albopictus* cells were infected with Vero cell-derived WT or mutant CHIKV (E1-K61T, E2-D59N, or E1-K61T + E2-D59N) and cell supernatants was collected at 72 hours later. Consensus sequencing of the E2-E1 structural genes was performed using overlapping primers and the data was aligned using Geneious software. The E2-D59N (GAU → AAU) (**A**) and E1-K61T (AAG → ACG) (**B**) sequences were preserved in single and double mutant viruses. The mutated nucleotide in each structural gene is highlighted in red (**A**) or blue (**B**). The results are from samples harvested from three independent cultures.

Figure S3. Antibody resistant mutations in CHIKV are retained in Vero cell cultures. Vero cells were infected with C6/36-derived WT or mutant CHIKV (E1-K61T, E2-D59N, or E1-K61T + E2-D59N) and cell supernatant was collected at 36 hours later. Consensus sequencing of the E2-E1 structural genes was performed using overlapping primers and the data was aligned using Geneious software. The E2-D59N (GAU → AAU) (**A**) and E1-

K61T (AAG → ACG) (**B**) sequences were preserved in the single and double mutant viruses. The mutated nucleotide in each structural gene is highlighted in red (**A**) or blue (**B**). The results are from samples harvested from three independent cultures.

Figure S4. Antibody resistant mutations in CHIKV are retained in mosquitoes. *Ae. albopictus* mosquitos were fed blood meals containing BHK21 cell-derived WT or CHIKV mutant viruses (E1-K61T, E2-D59N, or E1-K61T + E2-D59N) and RNA was isolated from whole bodies after 14 days. Consensus sequencing of the E2-E1 structural genes was performed using overlapping primers and the data was aligned using Geneious software. The E2-D59N (AAU → GAU) (**A**) and E1-K61T (AAG → ACG) (**B**) mutations were retained in mosquitoes infected with each of the mutant viruses. The mutated nucleotide in each structural gene is highlighted in red (**A**) and blue (**B**). The results are from samples harvested from three to four independent mosquitoes.

Figure S5. **Antibody resistant mutations in CHIKV are retained in *Ifnar1*$^{-/-}$ mice.** Six to eight week-old *Ifnar1*$^{-/-}$ mice were passively transferred saline or 50 μg of CHK-152, CHK-166, or CHK-152 + CHK-166 via an intraperitoneal injection one day before subcutaneous infection with 10 FFU of CHIKV WT, CHIKV E2-D59N, CHIKV E1-K61T, or CHIKV E2-D59N + E1-K61T. Three days later, muscle was harvested and consensus sequencing of the E2-E1 structural genes was performed using overlapping primers. The data was aligned using Geneious software. The E2-D59N (AAU → GAU) (**A**) and E1-K61T (AAG → ACG) (**B**) mutations were retained in *Ifnar1*$^{-/-}$ mice infected with each of the mutant viruses regardless of whether the selecting MAb was absent or present. The mutated nucleotide in each structural gene is highlighted in red (**A**) and blue (**B**). The results are from samples harvested from two to three independent mice.

Figure S6. Mutant nucleotide sequences are maintained following CHIKV infection in *Rag1*$^{-/-}$ mice. *Rag1*$^{-/-}$ mice were infected in the left footpad with 10^3 FFU of C6/36-derived CHIKV-WT or CHIKV E1-K61T + E2-D59N. Twenty-eight days after infection, RNA was

harvested from serum. The E2-E1 structural genes were sequenced using overlapping primers and aligned using Geneious software. The E2-D59N (**A**) and E1-K61T (**B**) substitutions were preserved in the double mutant virus. The mutated nucleotide is highlighted in red (**A**) or blue (**B**).

Figure S7. Mutant nucleotide sequences are maintained following infection in WT mice at day 28 after infection. Three week-old WT C57BL/6 mice (n = 4 each) were infected with 10^3 FFU of CHIKV WT, CHIKV E1-K61T, CHIKV E2-D59N, or CHIKV E1-K61T + E2-D59N via a subcutaneous route. At day 28, the right ankles were harvested and RNA was purified. The E2-E1 structural genes were sequenced using overlapping primers and aligned using Geneious software. The E2-D59N (**A**) and E1-K61T (**B**) mutations were preserved in animals infected with the corresponding viruses. The mutant nucleotides are highlighted in red or blue.

Figure S8. Fitness comparison of CHIKV-WT and CHIKV E1-K61T + E2-D59N. Three week-old WT C57BL/6 mice were infected with 10^3 FFU of CHIKV WT and CHIKV E1-K61T + E2-D59N in a ratio of 1:1, 5:1, or 1:5 (n = 4 for each group) via a subcutaneous route. At day 28, the right ankles were harvested and RNA was purified. The E2-E1 structural genes were sequenced using overlapping primers and aligned using Geneious software. The E2-D59N (**A**) and E1-K61T (**B**) mutations were present in tissue from all animals regardless of the input ratio. The mutant nucleotides are highlighted in red or blue. **C**. A single sample from one animal showed evidence of a minority population of E1 that corresponded to CHIKV-WT strain. The sequence tracing shows a mixture (M) of nucleotides C and A at position 10,175, with the mutant nucleotide being dominant. Of note, at position 10.176 a mixture (R) of nucleotides G and A also were present in this sample although this did not result in an amino acid change.

Table S1. Primers used for sequencing, amplification, and mutagenesis of the CHIKV E2-E1 genes

Sequencing Primers	5'-Sequence-3'
Primer 1: 8248F	GTCTTAGGAGGAGCTAATGAAGGAG
Primer 2: 8573F	CCACAAGACCATACTTAGCTCACTGTCC
Primer 3: 8912F	CATGTACGCACCCATTTCACC
Primer 4: 9224F	CGGTCACCAATCACAAAAAGT
Primer 5: 9500F	CCGTGCCGACTGAAGGG
Primer 6: 9802F	GCTAAAGCGGCCACATACC
Primer 7: 10101F	CACTTTGGAGCCAACACTATCG
Primer 8: 10389F	GCTCCGCGTCCTTTACCA
Primer 9: 10676F	CGGTACACGTGCCATACTCTCAGG
Primer 10: 11017F	GCTGAGATAGAAGTTGAAGGGA

PCR Amplification Primers	5'-Sequence-3'
Sense: 8018F	AACTGGCCTTTAAGCGGTCATC
Antisense: 8930R	TGAAATGGGTGCGTACATGAGTG
Sense: 8365F	GTTATGTGCCTGTTGGCAAACAC
Antisense: 9348R	AGGCACCCTGCATGTTACATTTG
Sense: 9205F	GTTGATCAATGTCATGCCGCGG
Antisense: 10112R	GGCTCCAAAGTGACTGACAGTAG
Sense: 9993F	GTACGAACACGTAACAGTGATCCC
Antisense: 10899R	CTCGCACGACATGTCCGTTAAAG
Sense: 10781F	CAAACCCGGTAAGAGCGGTGAA
Antisense: 11715R	CGGCTGCTTTTAGGAAGCTTAAG
Sense: 8248F	GTCTTAGGAGGAGCTAATGAAGGAGCCCGT
Antisense: 11359R	GTGTGTCTCTTAGGGGACACATATACCTTCATACTT

Mutagenesis Primers	5'-Sequence-3'
E1-K61T - Sense: 10161F	GTCTCCGTACGTGACGTGCTGCGGTACAG
E1-K61T - Antisense: 10189R	CTGTACCGCAGCACGTCACGTACGGAGAC
E2-D59N - Sense: 8698F	CAAATCGGAATAAAGACGAATGACAGCCACGATTGGA
E2-D59N - Antisense: 8734R	TCCAATCGTGGCTGTCATTCGTCTTTATTCCGATTTG

Nucleotide sequences are based on CHKV-LR 2006_OPY1 (GenBank: DQ443544.2).