

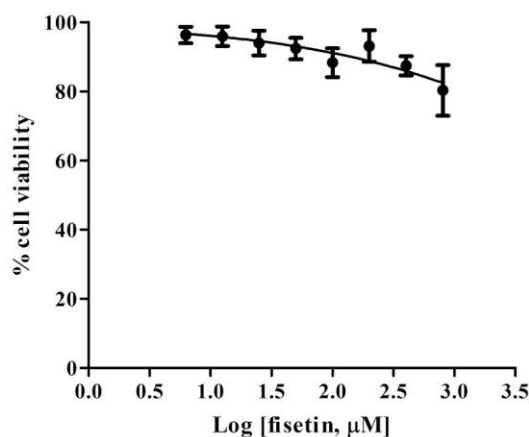
Supplementary Material

Supplemental Table S1: Forward and reverse primers used for qRT-PCR.

Gene	Primers
CHIKV-E1	Forward: 5'-TCG ACG CGC CCT CTT TAA-3' Reverse: 5'-ATC GAA TGC ACC GCA CAC T-3'
GAPDH	Forward: 5'-CCA GCA AGA GCA CAA GAG GAA-3' Reverse: 5'-ATG GTA CAT GAC AAG GTG CGG-3'
ISG-15	Forward: 5'-GGC TGG GAC CTG ACG GTG AAG-3' Reverse: 5'-GTC CGC CCG CCA GGC TCT GT-3'
MX-2	Forward: 5'-AAA CTG TTC AGA GCA CGA TTG AAG-3' Reverse: 5'-ACC ATC TGC TCC ATT CTG AAC TG-3'
OAS-3	Forward: 5'-CCG AAC TGT CCT GGG CCT GAT CC-3' Reverse: 5'-CCC ATT CCC CAG GTC CCA TGT GG-3'
PKR	Forward: 5'-ACG CTT TGG GGC TAA TTC TT-3' Reverse: 5'-TTC TCT GGG CTT TTC TTC CA-3'

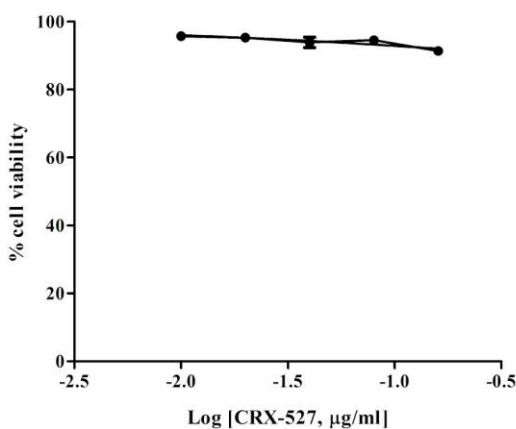
Supplementary Material

(A)



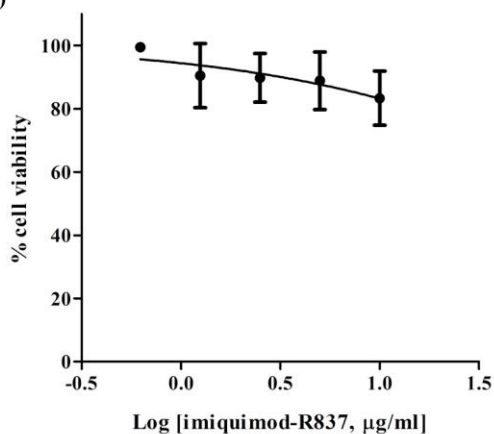
CC_{50} : 49.2 mM
MNTD: 1.22 mM

(B)



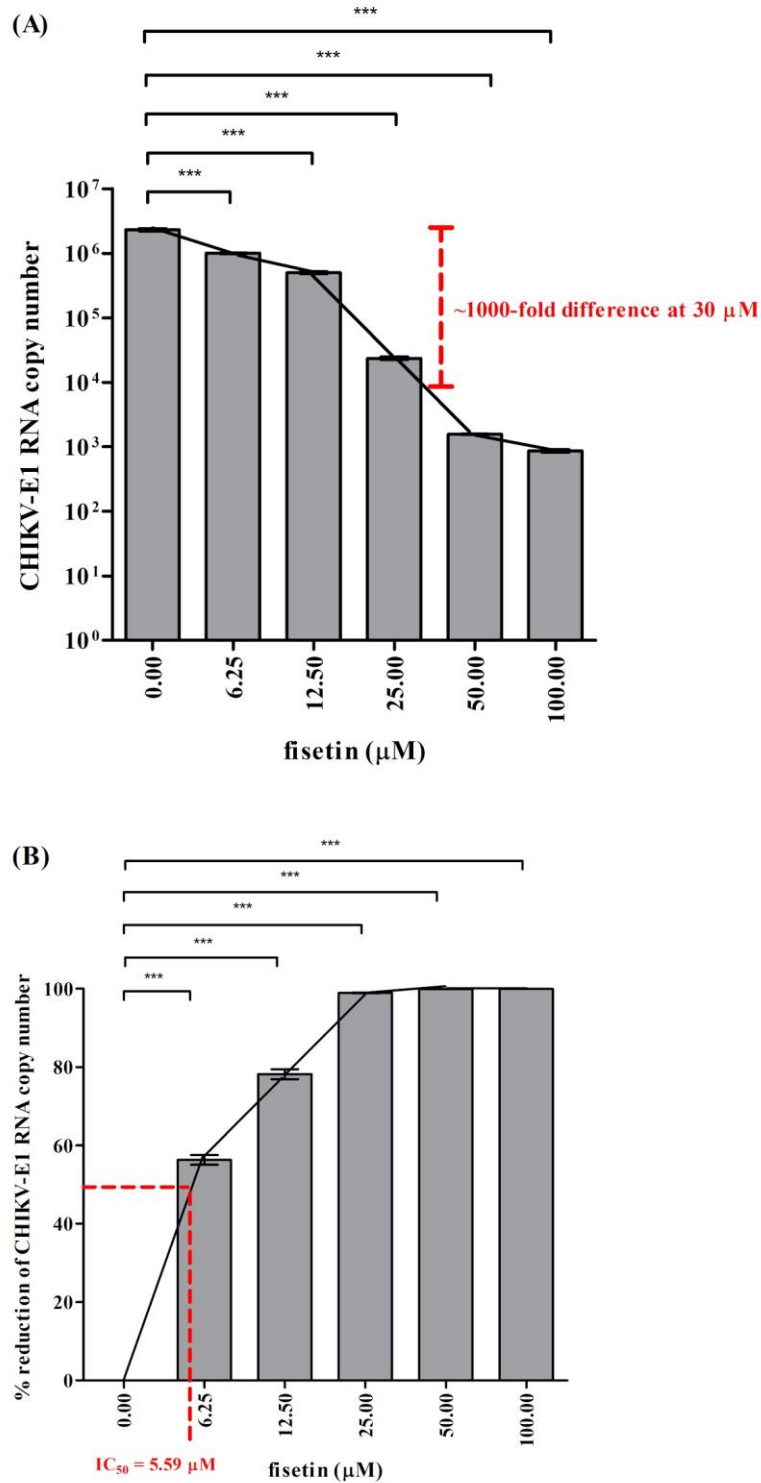
CC_{50} : 1.82 mg/ml
MNTD: 8.99 $\mu\text{g/ml}$

(C)



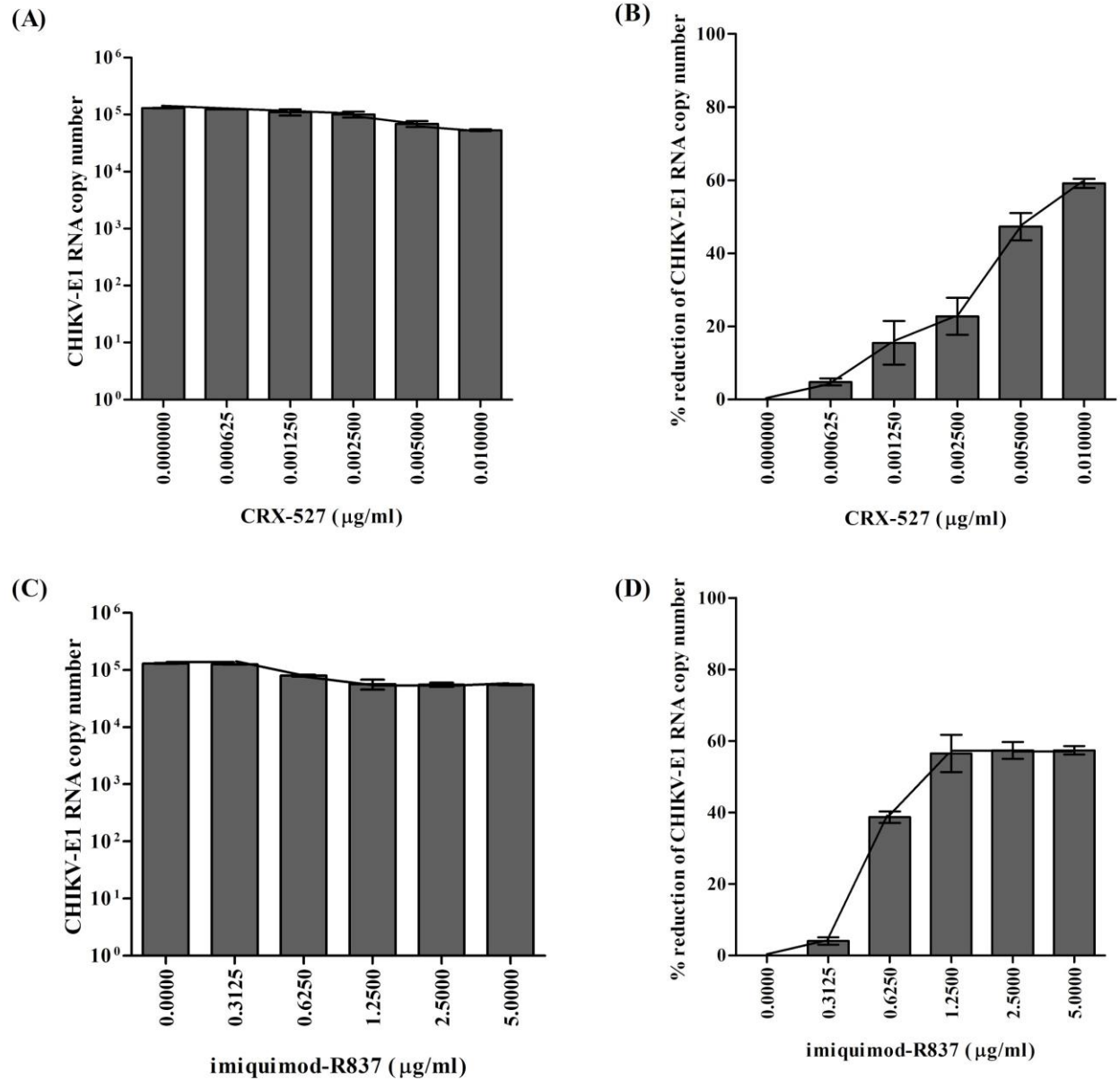
CC_{50} : 226.7 $\mu\text{g/ml}$
MNTD: 14.32 $\mu\text{g/ml}$

Supplemental Figure S1: Cytotoxicity assay of (A) fisetin, (B) CRX-527 and (C) imiquimod-R837 at 48 hpi on Huh7 cells. Data are representative of three independent experiments and values are expressed as mean \pm SD. The CC_{50} and MNTD were determined using non-linear regression curve analysis of GraphPad Prism 5.

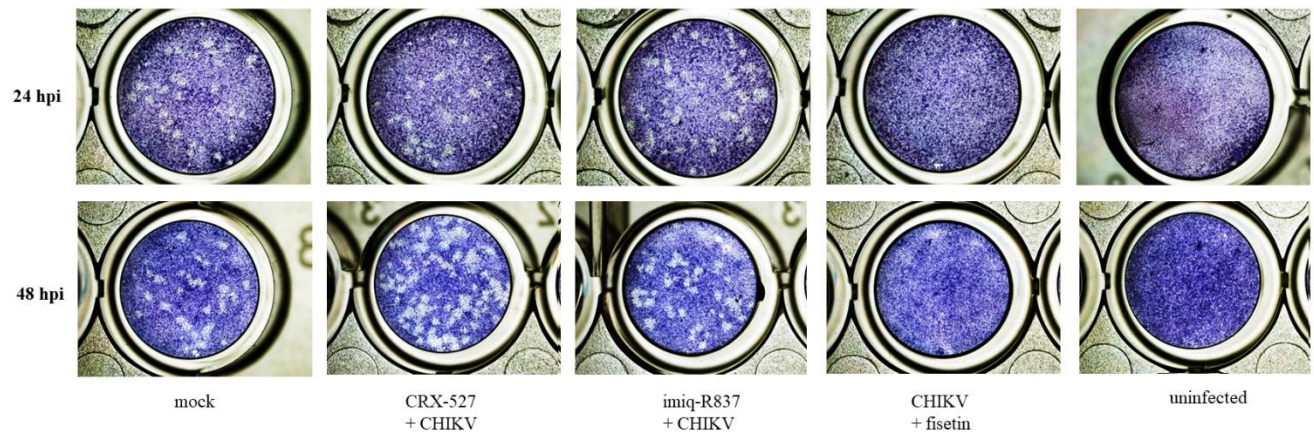


Supplemental Figure S2: Viral yield assay represented as (A) CHIKV-E1 RNA copy number and (B) percentage reduction of CHIKV-E1 RNA copy number, at 48 hpi of treatment with fisetin. Data are representative of three independent experiments and values are expressed as mean ± SD. One-way ANOVA ($p < 0.0001$) and Dunnett's multiple comparison post-test (* is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$) were performed by setting mock Huh7 cells as a control.

Supplementary Material

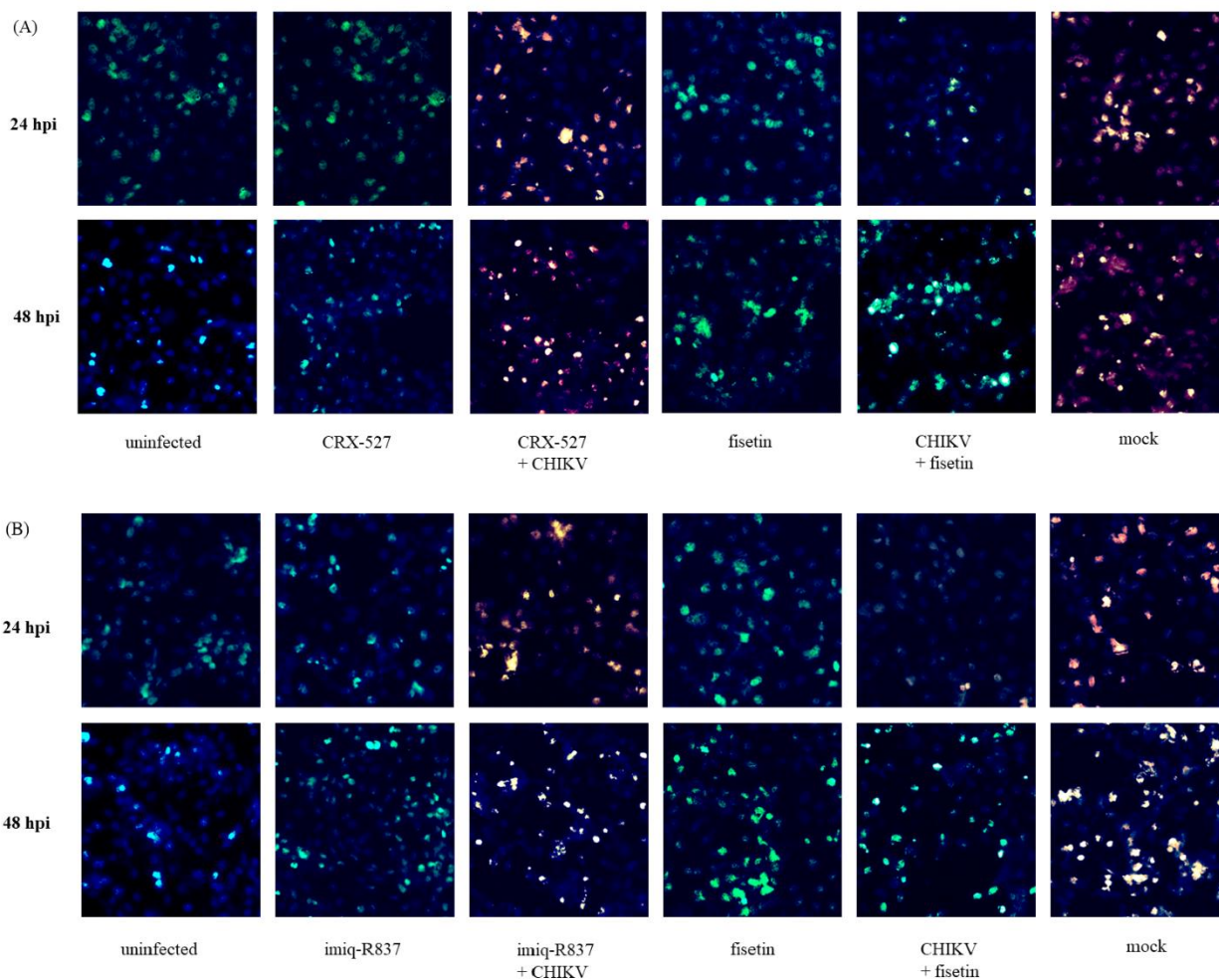


Supplemental Figure S3: Viral yield assay represented as (A) and (C) CHIKV-E1 RNA copy number and (B) and (D) percentage reduction of CHIKV-E1 RNA copy number, at 48 hpi of treatment with CRX-527 and imiquimod-R837. Data are representative of three independent experiments and values are expressed as mean \pm SD. One-way ANOVA ($p < 0.0001$) and Dunnett's multiple comparison post-test (* is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$) were performed by setting mock Huh7 cells as a control.



Supplemental Figure S4: CHIKV inhibitory potential of fisetin and the TLR agonists. Plaque count (pfu/ml) obtained from mock, agonist-treated, and fisetin-treated Huh7 cells (dilution 1:10). Data are representative of three independent experiments, and values are expressed as mean \pm SD. One-way ANOVA ($p < 0.0001$) and Dunnett's multiple comparison post-test (* is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$) were performed by setting mock Huh7 cells of respective time points as independent control.

Supplementary Material



Supplemental Figure S5: Endogenous TLR4 and TLR7 induction in Huh7 cells with respective treatments and CHIKV infection. Immunofluorescence was performed using anti-TLR4 or anti-TLR7 (green), anti-E2 (red) and DAPI, at 24 hpi and 48 hpi.