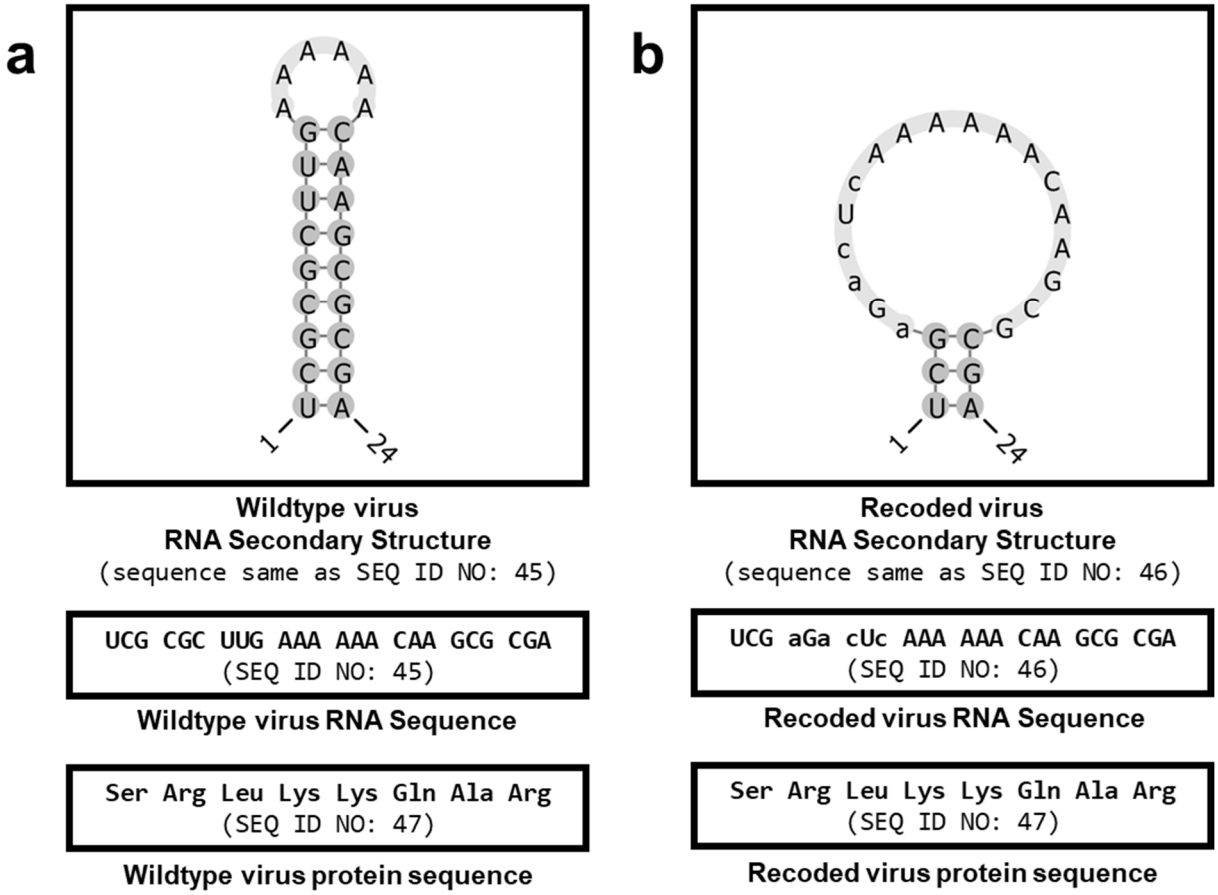

a
b
Wildtype virus
RNA Secondary Structure
(sequence same as SEQ ID NO: 45)
UCG CGC UUG AAA AAA CAA GCG CGA
(SEQ ID NO: 45)
Wildtype virus RNA Sequence
Ser Arg Leu Lys Lys Gln Ala Arg
(SEQ ID NO: 47)
Wildtype virus protein sequence
Recoded virus
RNA Secondary Structure
(sequence same as SEQ ID NO: 46)
UCG aGa cUc AAA AAA CAA GCG CGA
(SEQ ID NO: 46)
Recoded virus RNA Sequence
Ser Arg Leu Lys Lys Gln Ala Arg
(SEQ ID NO: 47)
Recoded virus protein sequence

Figure 1

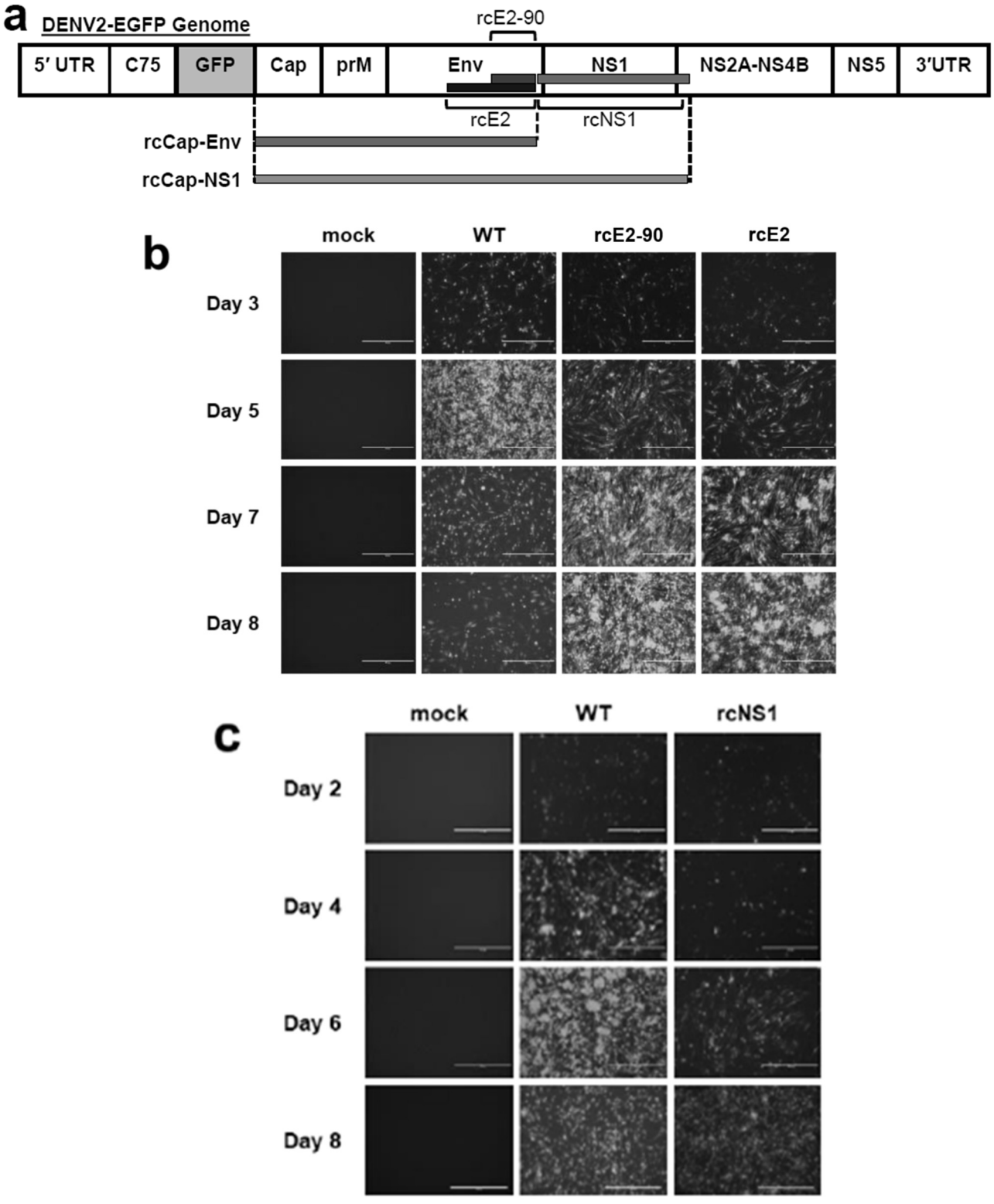

a
DENV2-EGFP Genome
rcE2-90
5′ UTR
C75
GFP
Cap
prM
Env
NS1
NS2A-NS4B
NS5
3′UTR
rcE2
rcNS1
rcCap-Env
rcCap-NS1
b
mock
WT
rcE2-90
rcE2
Day 3
Day 5
Day 7
Day 8
c
mock
WT
rcNS1
Day 2
Day 4
Day 6
Day 8

Figure 2

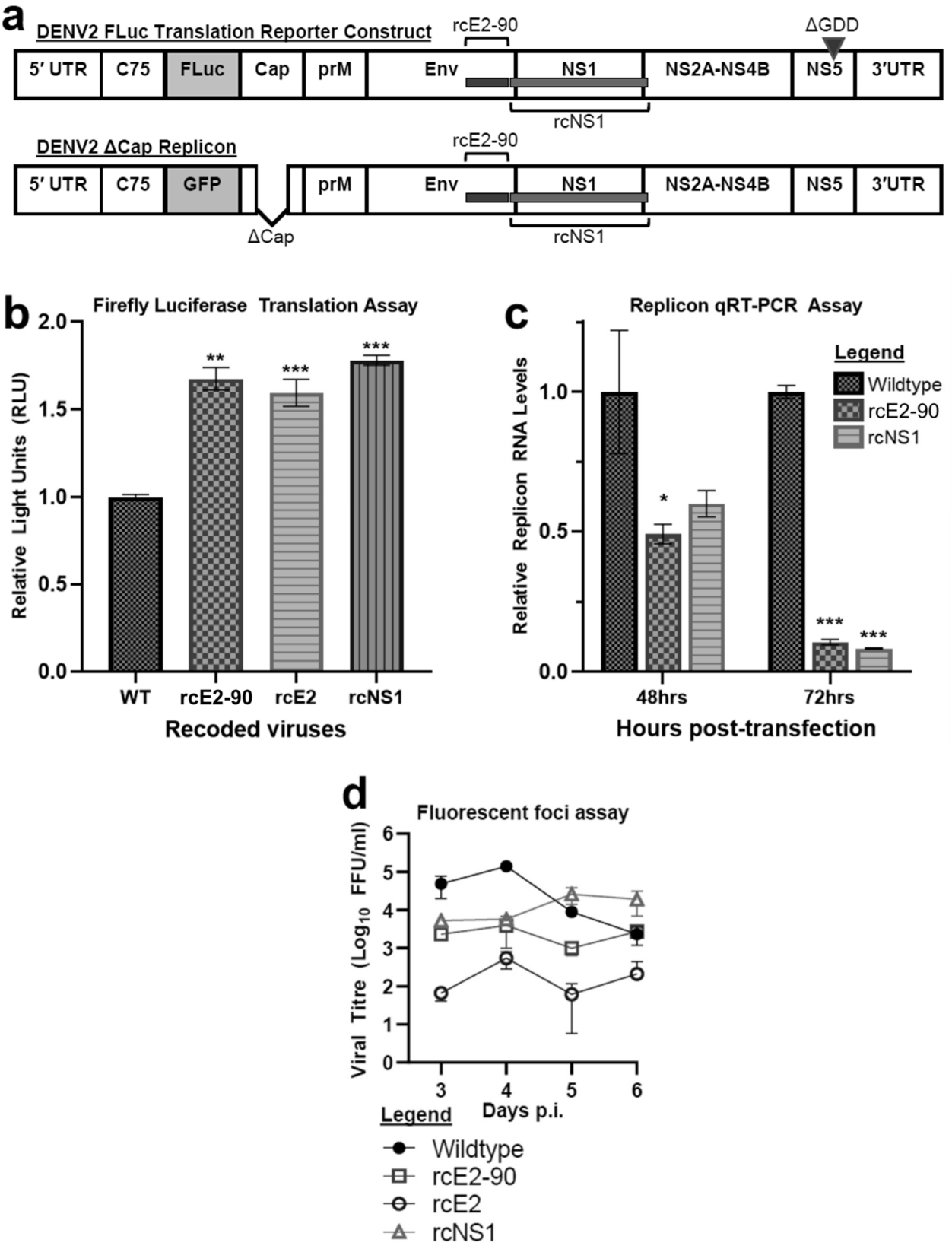

a
DENV2 FLuc Translation Reporter Construct
rcE2-90
ΔGDD
5′ UTR
C75
FLuc
Cap
prM
Env
NS1
NS2A-NS4B
NS5
3′UTR
rcNS1
DENV2 ΔCap Replicon
GFP
ΔCap
b
Firefly Luciferase Translation Assay
Relative Light Units (RLU)
WT
rcE2-90
rcE2
rcNS1
Recoded viruses
c
Replicon qRT-PCR Assay
Relative Replicon RNA Levels
Legend
Wildtype
rcE2-90
rcNS1
48hrs
72hrs
Hours post-transfection
d
Fluorescent foci assay
Viral Titre (Log10 FFU/ml)
Days p.i.
Legend
Wildtype
rcE2-90
rcE2
rcNS1

Figure 3

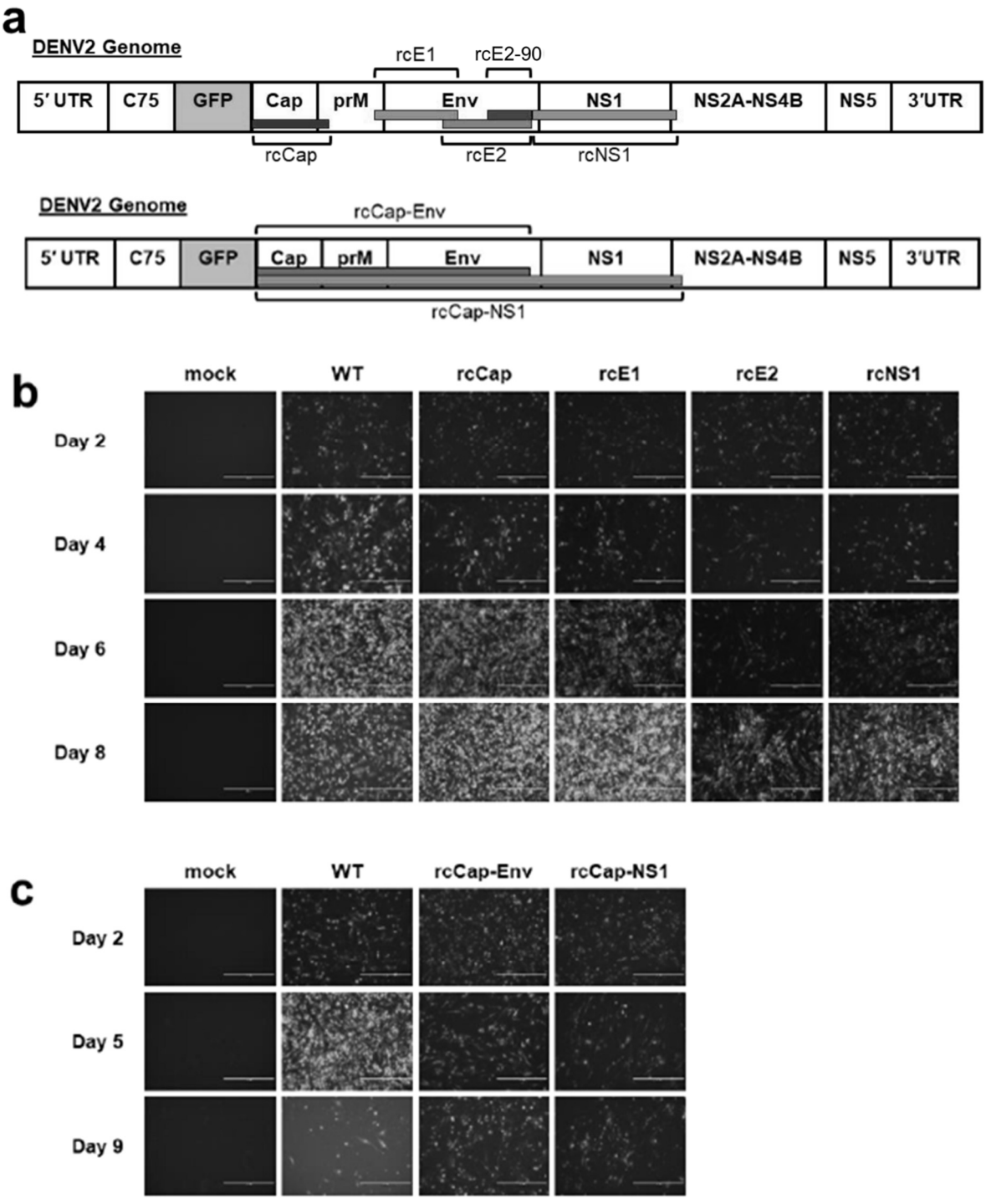

a
DENV2 Genome
rcE1
rcE2-90
5′ UTR
C75
GFP
Cap
prM
Env
NS1
NS2A-NS4B
NS5
3′UTR
rcCap
rcE2
rcNS1
DENV2 Genome
rcCap-Env
5′ UTR
C75
GFP
Cap
prM
Env
NS1
NS2A-NS4B
NS5
3′UTR
rcCap-NS1
b
mock
WT
rcCap
rcE1
rcE2
rcNS1
Day 2
Day 4
Day 6
Day 8
c
mock
WT
rcCap-Env
rcCap-NS1
Day 2
Day 5
Day 9

Figure 4

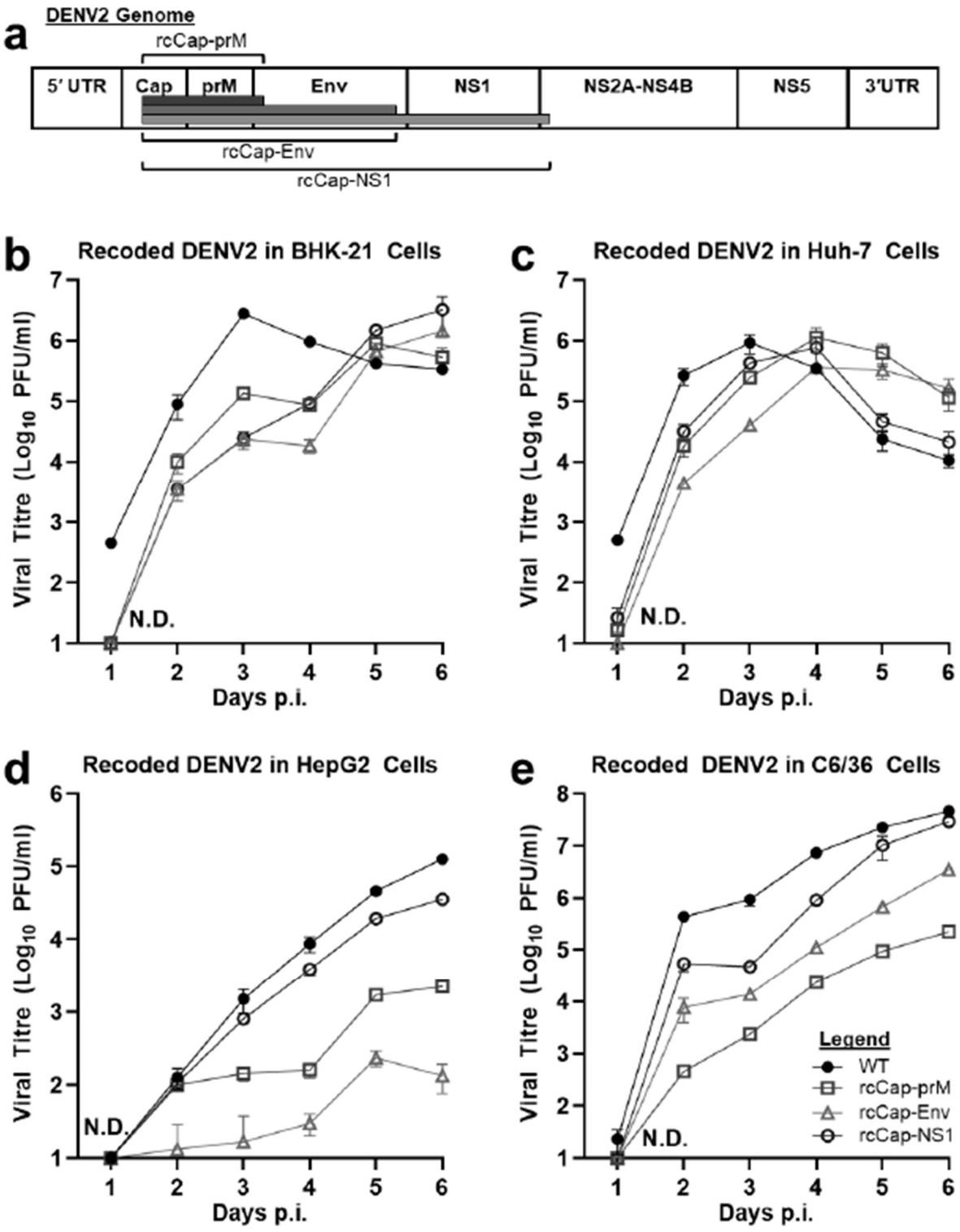

a
DENV2 Genome
rcCap-prM
5′ UTR
Cap
prM
Env
NS1
NS2A-NS4B
NS5
3′UTR
rcCap-Env
rcCap-NS1
b
Recoded DENV2 in BHK-21 Cells
c
Recoded DENV2 in Huh-7 Cells
d
Recoded DENV2 in HepG2 Cells
e
Recoded DENV2 in C6/36 Cells
Viral Titre (Log10 PFU/ml)
Days p.i.
N.D.
Legend
WT
rcCap-prM
rcCap-Env
rcCap-NS1

Figure 5

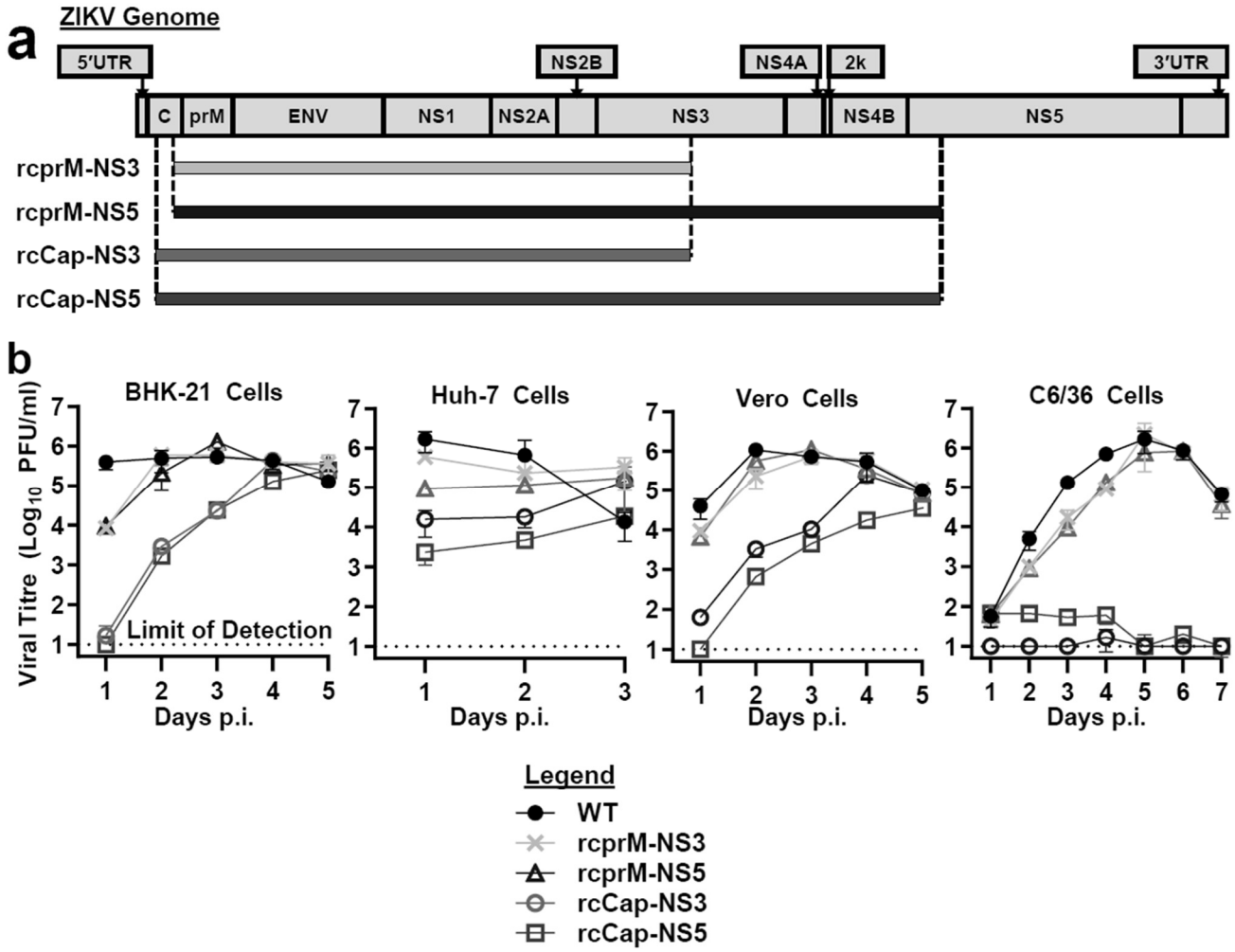

a
ZIKV Genome
5′UTR
C
prM
ENV
NS1
NS2A
NS2B
NS3
NS4A
2k
NS4B
NS5
3′UTR
rcprM-NS3
rcprM-NS5
rcCap-NS3
rcCap-NS5
b
BHK-21 Cells
Huh-7 Cells
Vero Cells
C6/36 Cells
Viral Titre (Log10 PFU/ml)
Limit of Detection
Days p.i.
Legend
WT
rcprM-NS3
rcprM-NS5
rcCap-NS3
rcCap-NS5

Figure 6

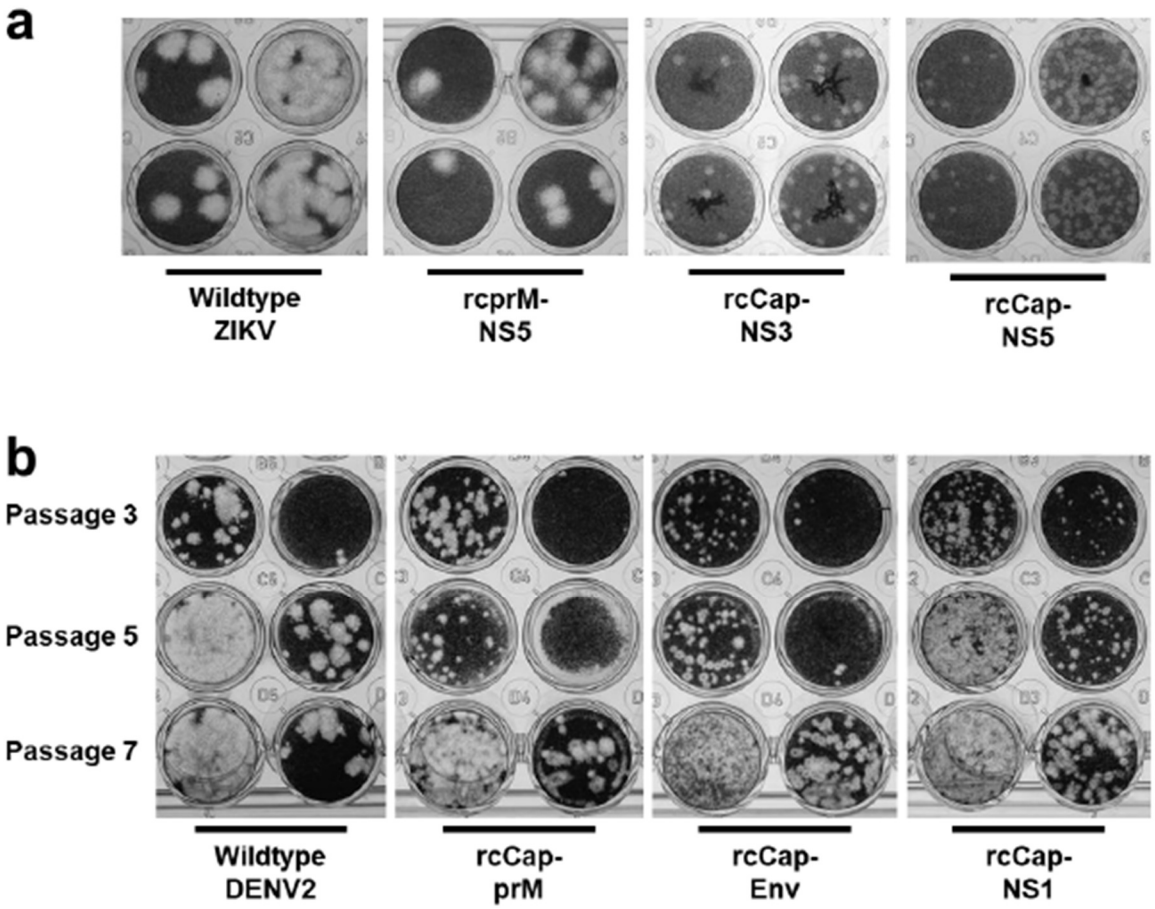

a
Wildtype ZIKV
rcprM-NS5
rcCap-NS3
rcCap-NS5
b
Passage 3
Passage 5
Passage 7
Wildtype DENV2
rcCap-prM
rcCap-Env
rcCap-NS1
c
Viral Plaque Size (mm²)
Passage number
Legend
Wildtype
rcCap-prM
rcCap-Env
rcCap-NS1
***
***
***
*
***
***

Figure 7

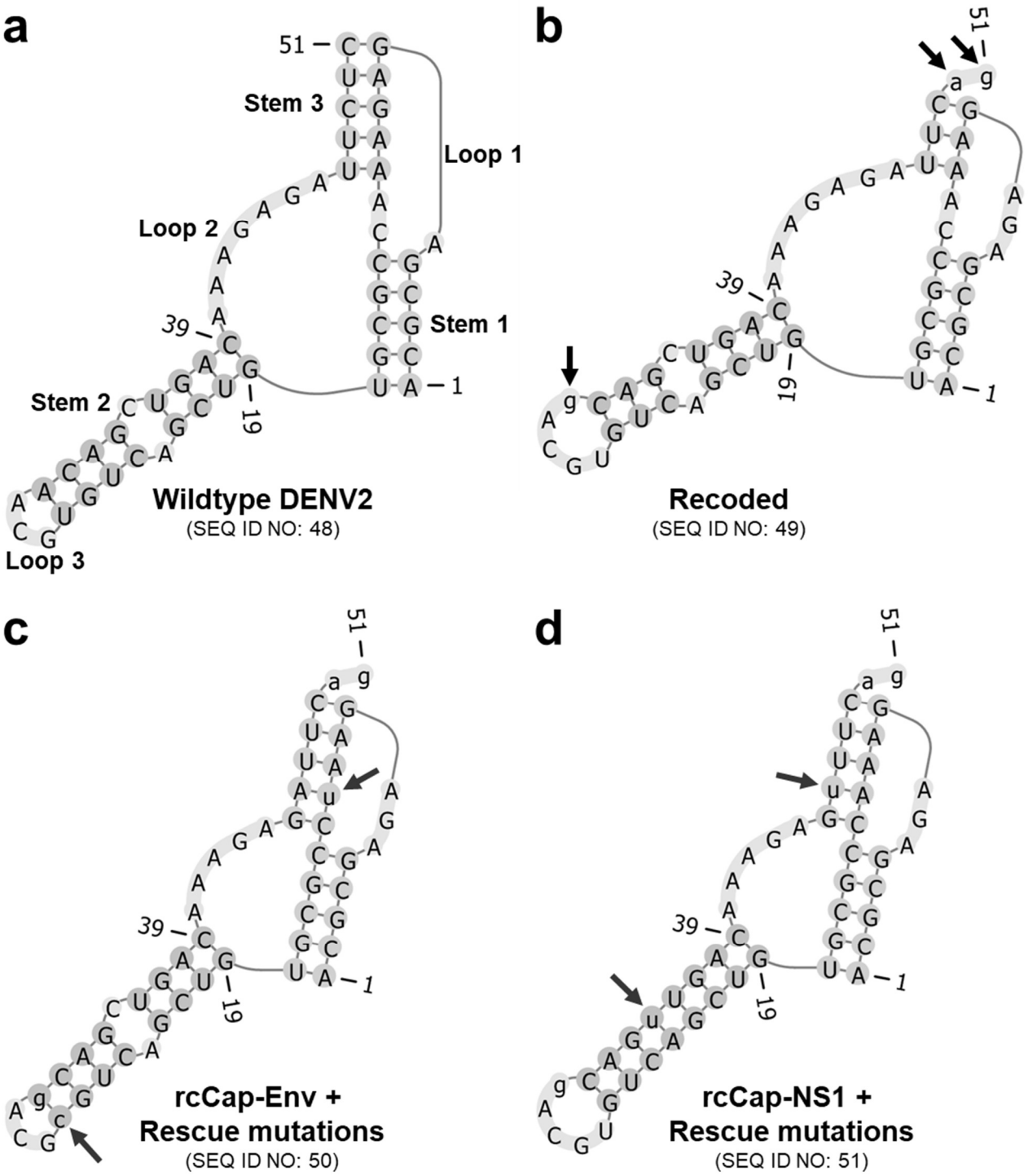

a
Stem 3
Loop 1
Loop 2
Stem 1
Stem 2
Loop 3
Wildtype DENV2
(SEQ ID NO: 48)
b
Recoded
(SEQ ID NO: 49)
c
rcCap-Env +
Rescue mutations
(SEQ ID NO: 50)
d
rcCap-NS1 +
Rescue mutations
(SEQ ID NO: 51)

Figure 8

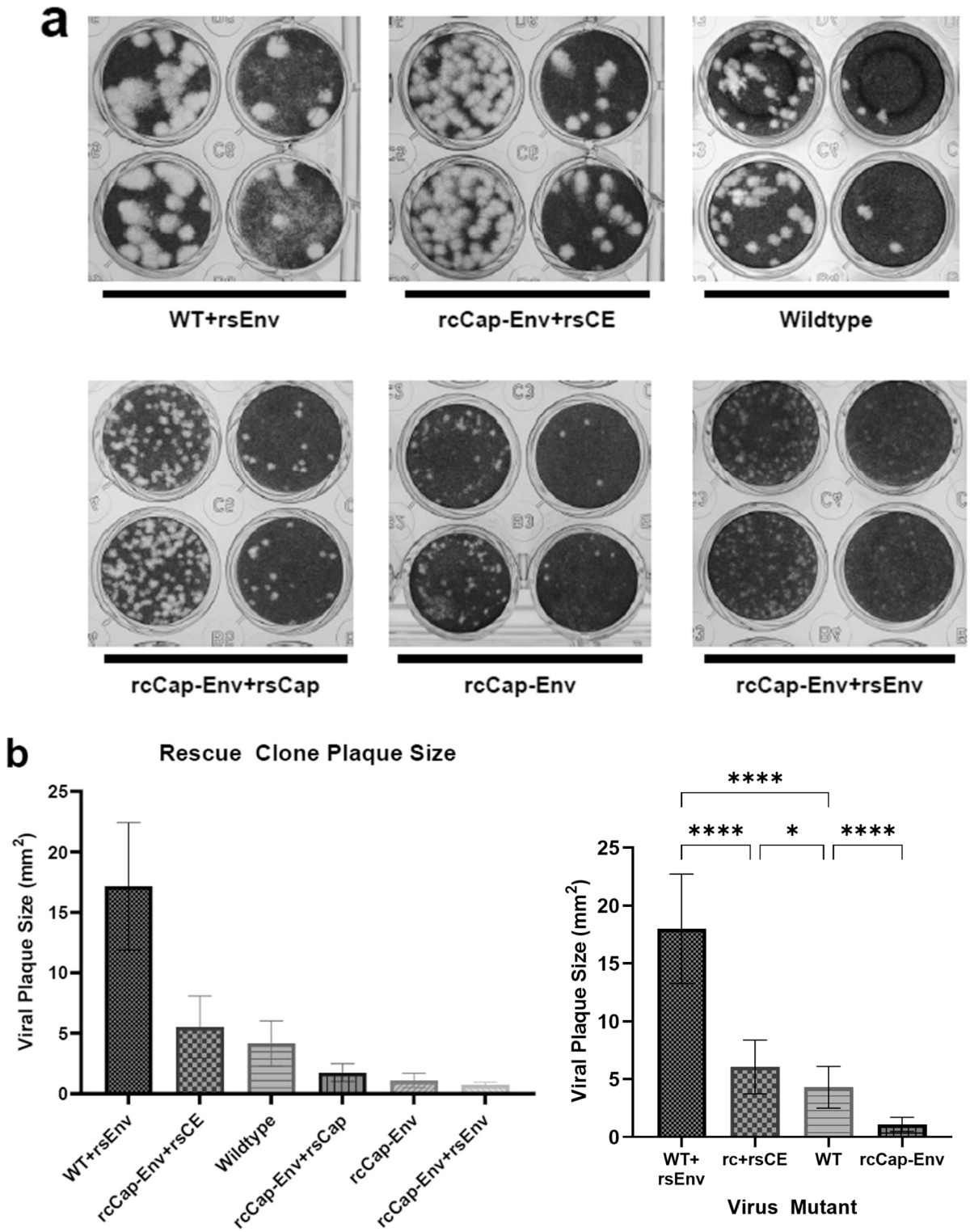


Figure 9(a)-(b) (continued)

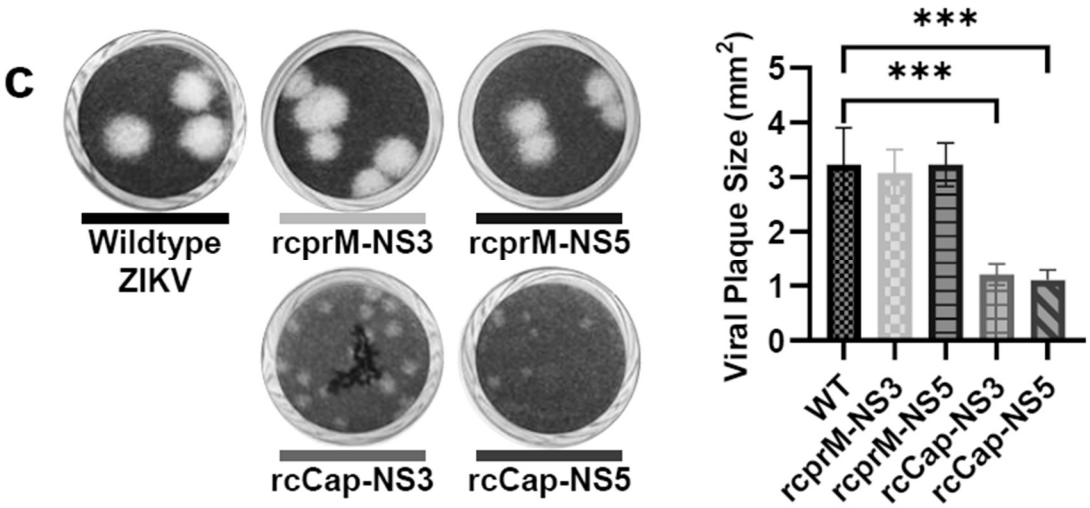


Figure 9(c) (end)

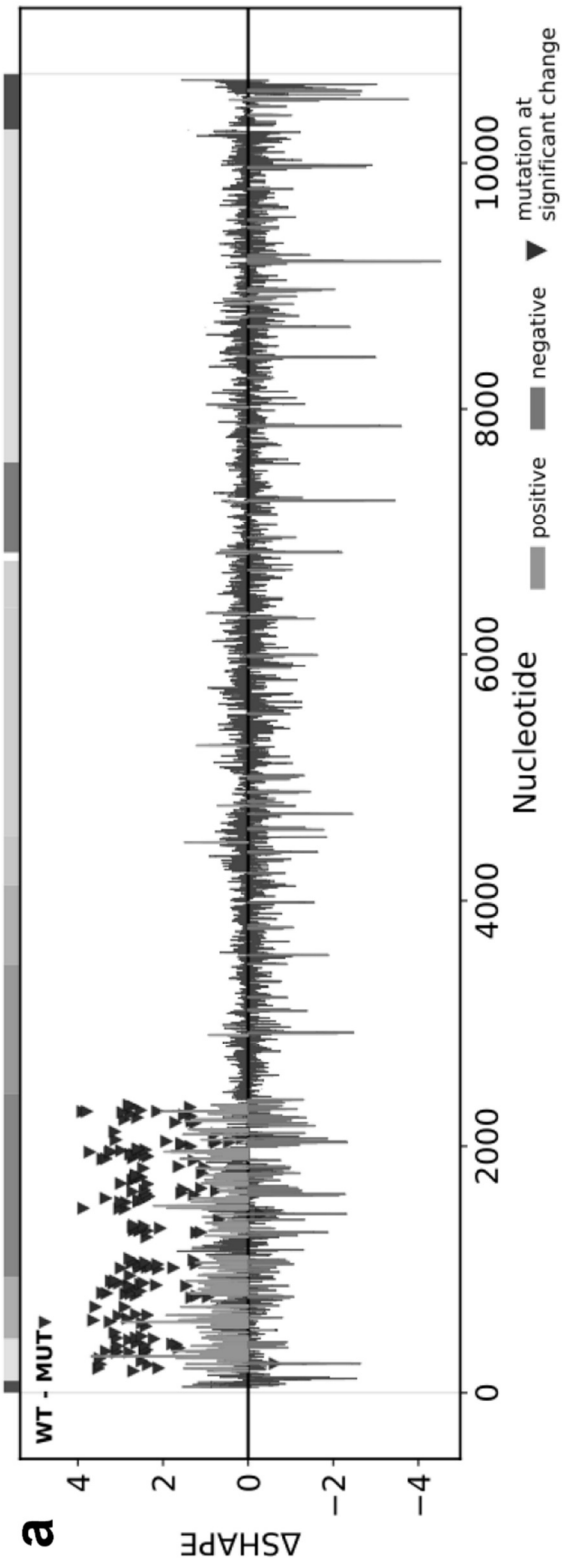


Figure 10(a) (continued)

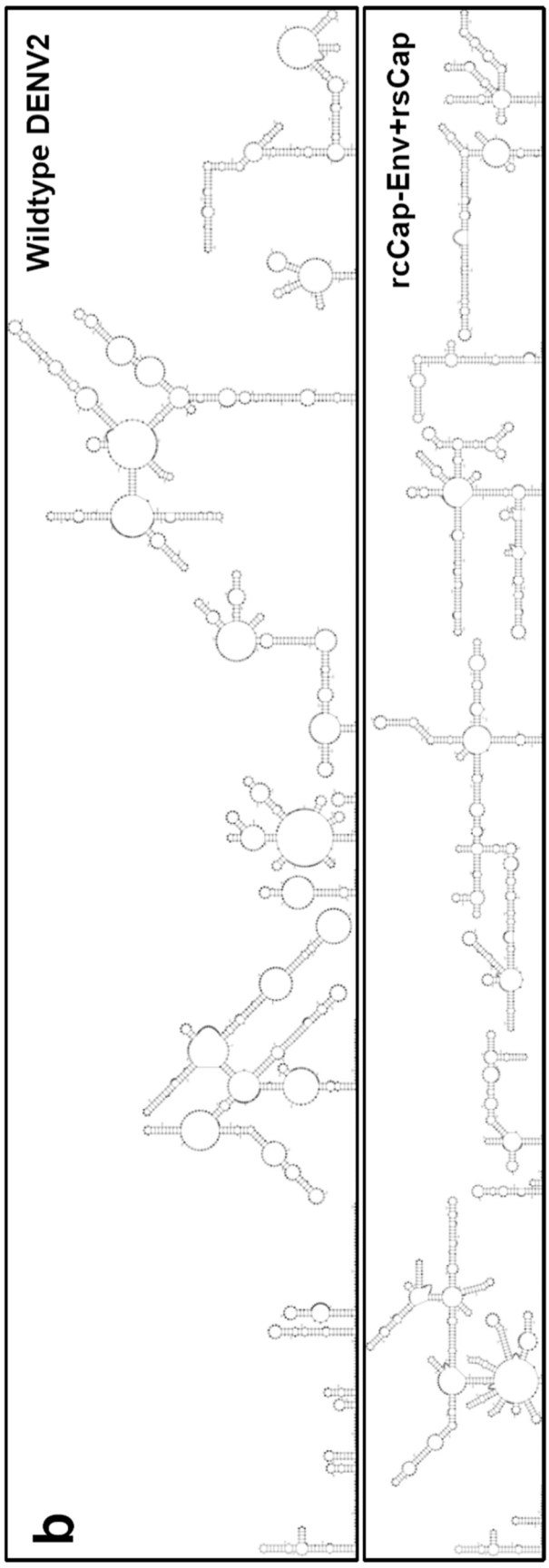


Figure 10(b) (continued)

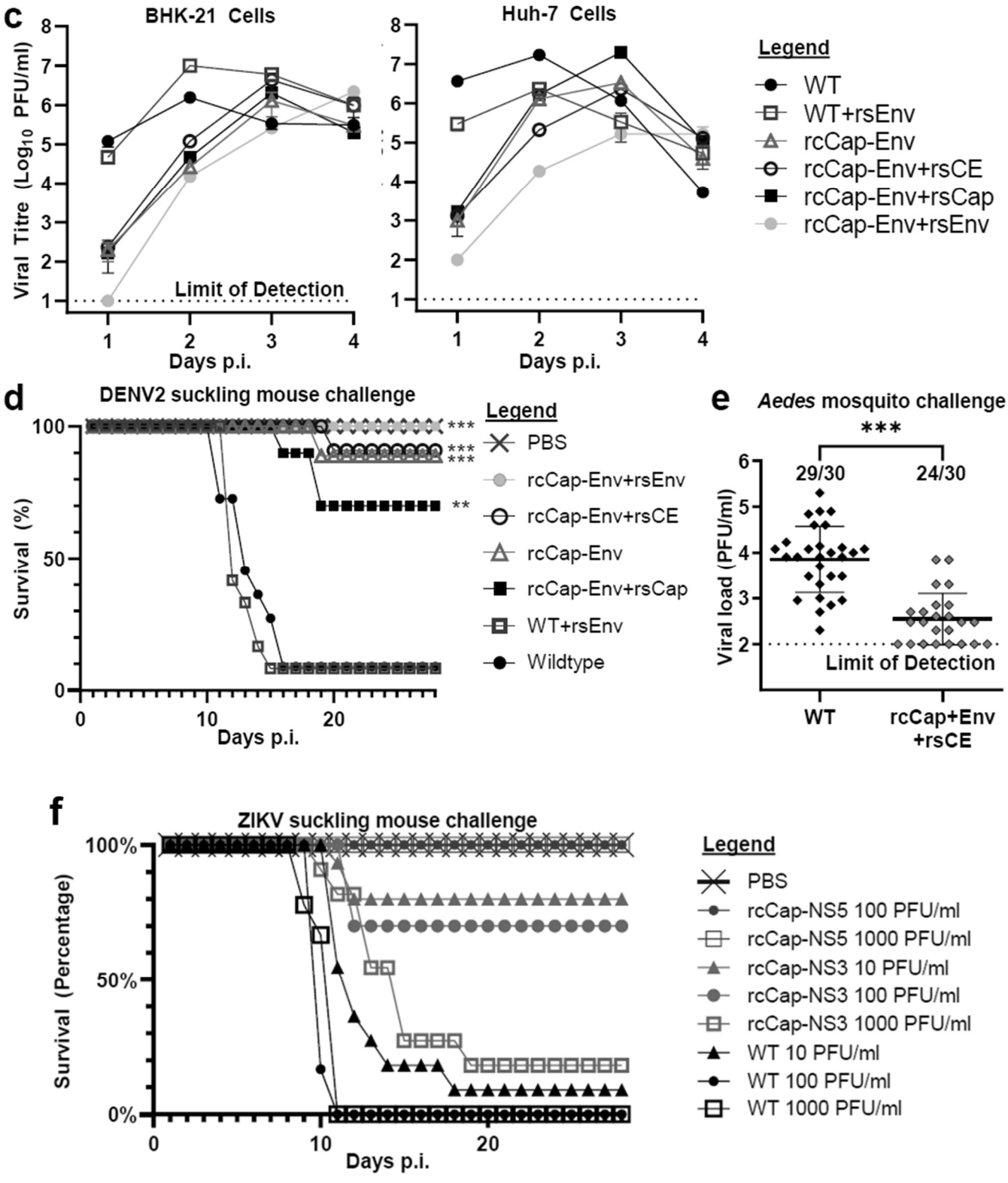


Figure 10(c)-(f) (end)

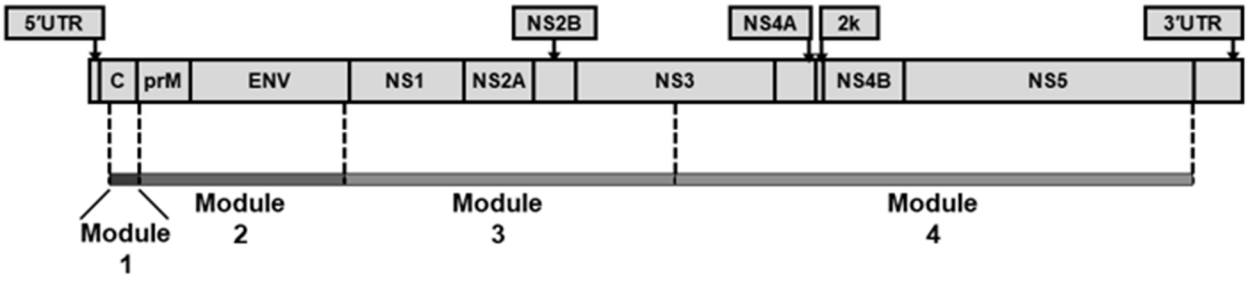

5'UTR
NS2B
NS4A
2k
3'UTR
C
prM
ENV
NS1
NS2A
NS3
NS4B
NS5
Module 1
Module 2
Module 3
Module 4

Figure 11

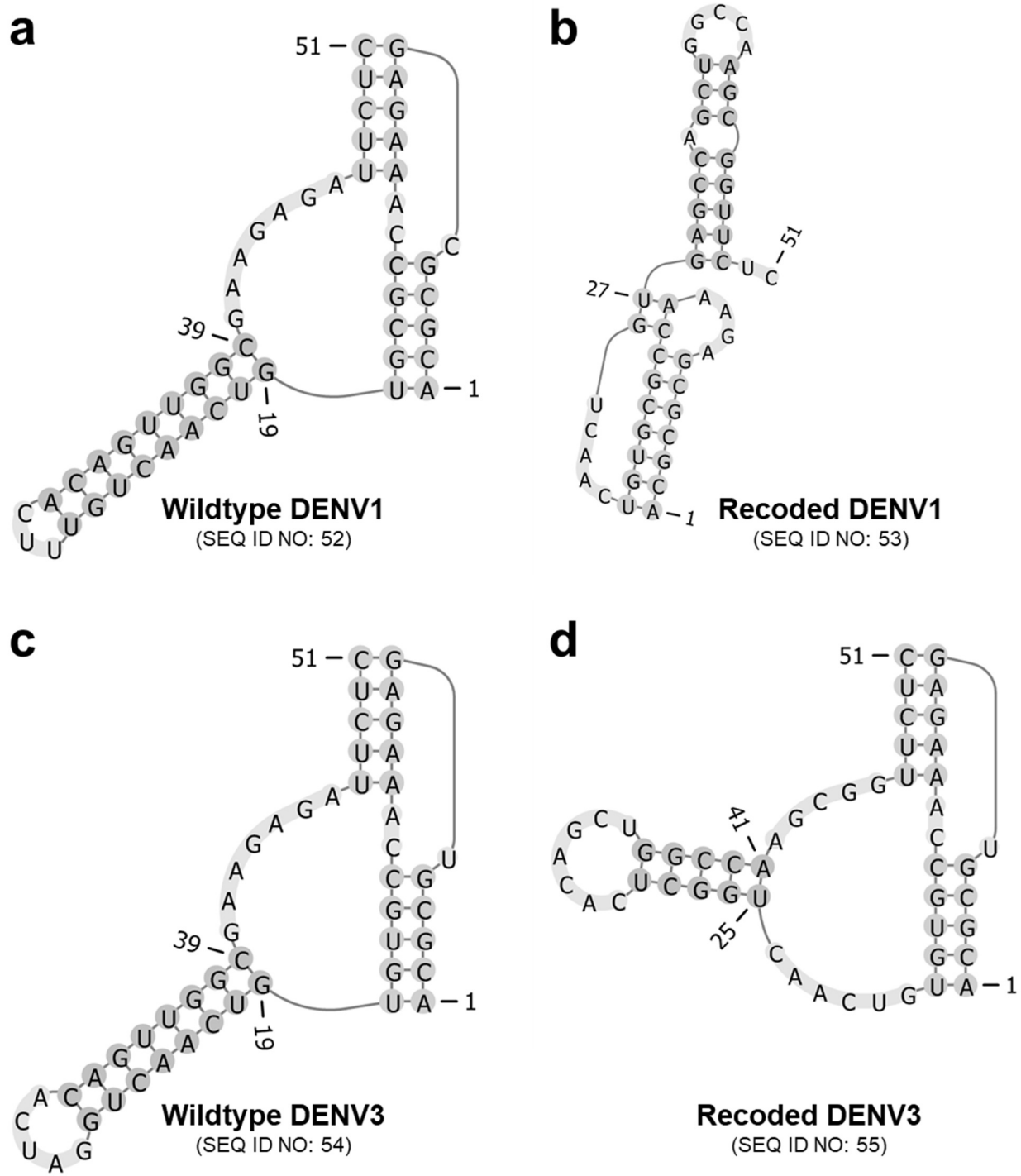

a
Wildtype DENV1
(SEQ ID NO: 52)
b
Recoded DENV1
(SEQ ID NO: 53)
c
Wildtype DENV3
(SEQ ID NO: 54)
d
Recoded DENV3
(SEQ ID NO: 55)

Figure 12

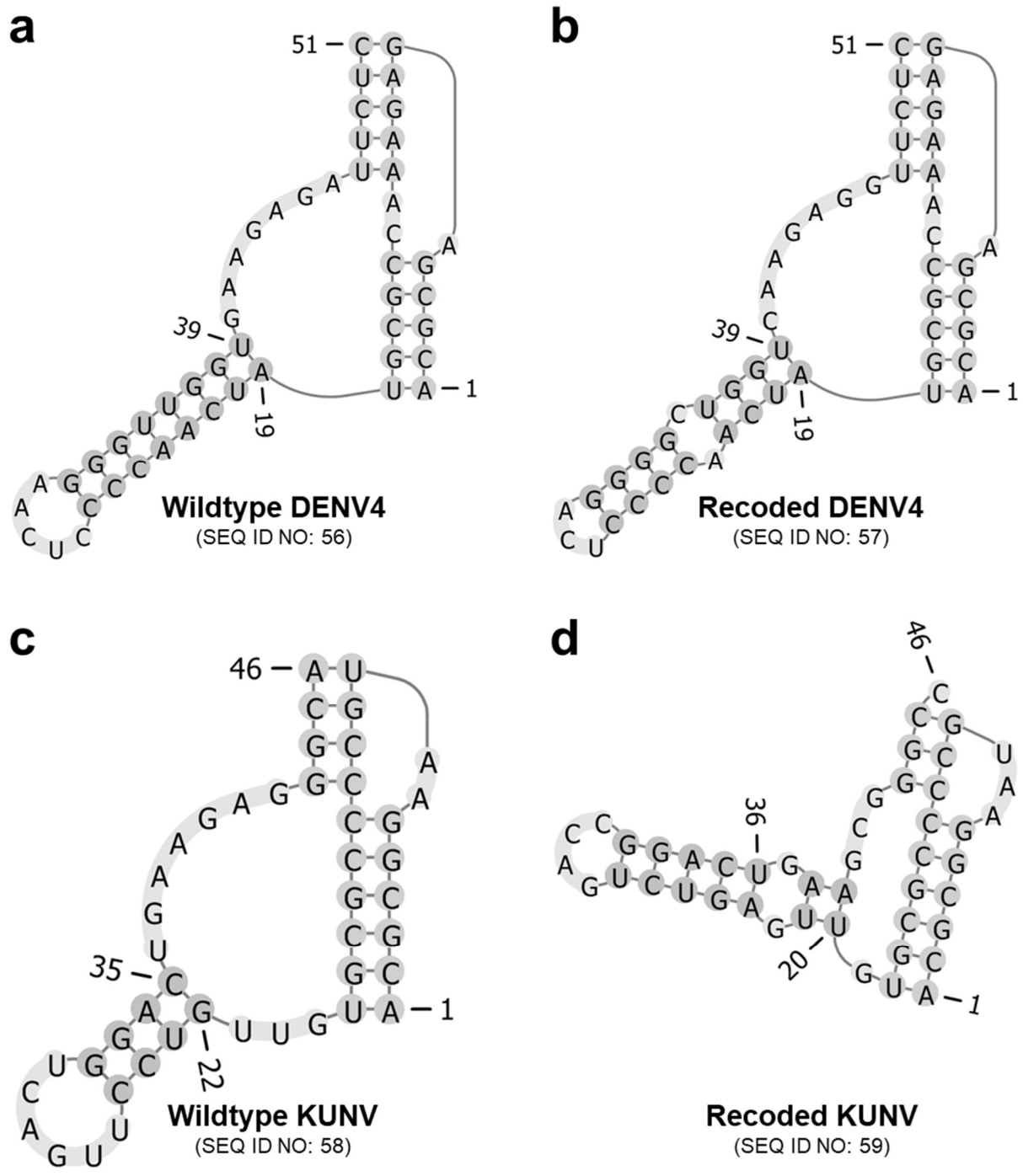

a
Wildtype DENV4
(SEQ ID NO: 56)
b
Recoded DENV4
(SEQ ID NO: 57)
c
Wildtype KUNV
(SEQ ID NO: 58)
d
Recoded KUNV
(SEQ ID NO: 59)

Figure 13

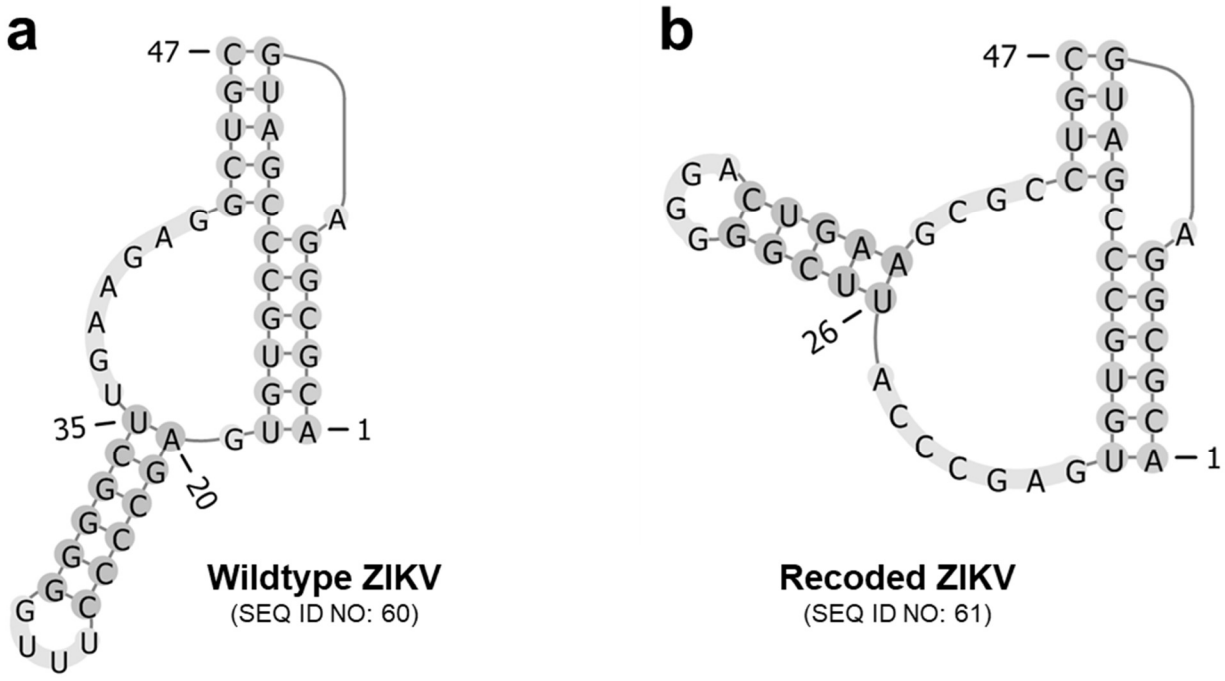

a
47
35
20
1
Wildtype ZIKV
(SEQ ID NO: 60)
b
47
26
1
Recoded ZIKV
(SEQ ID NO: 61)

Figure 14

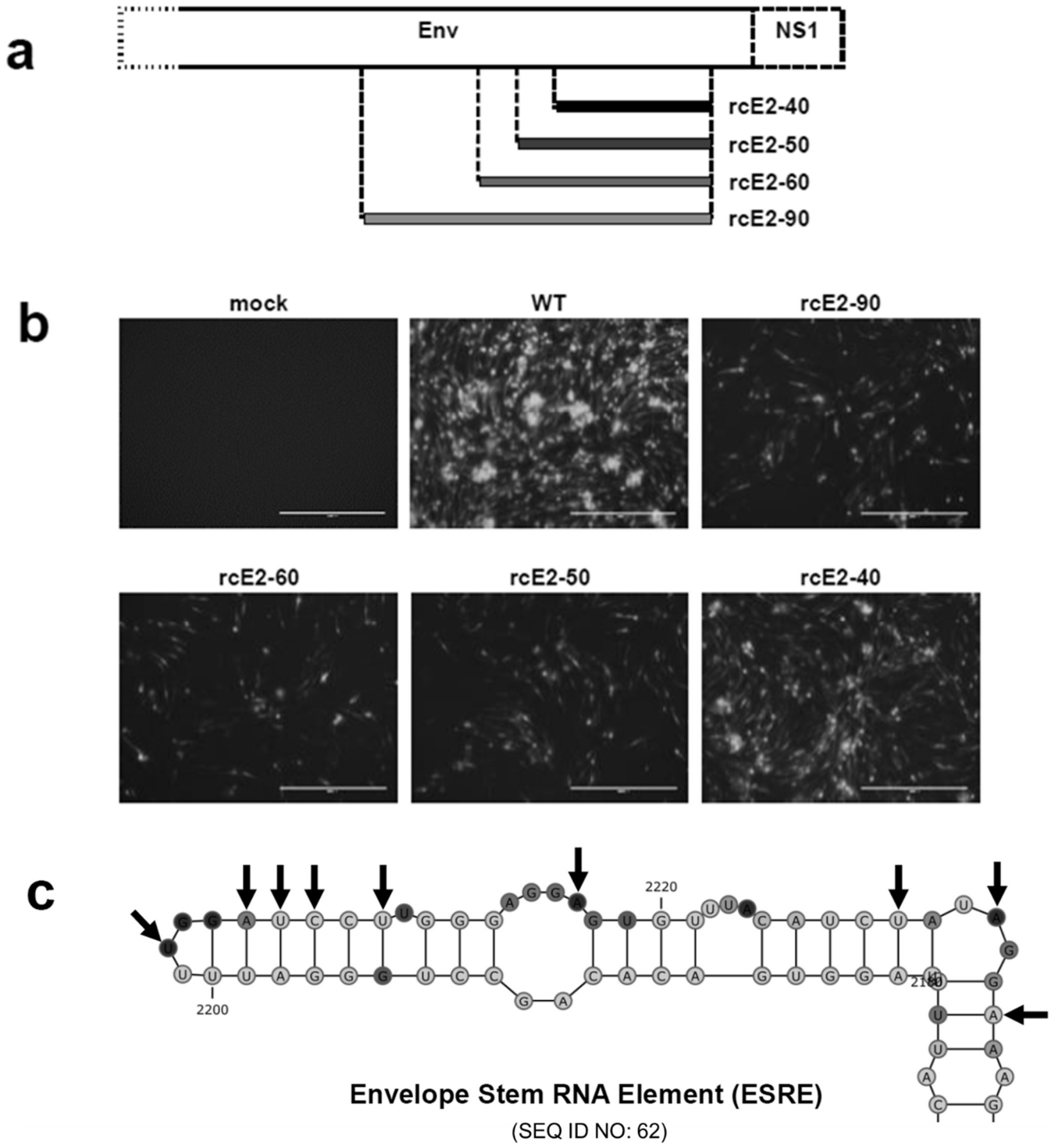

a
Env
NS1
rcE2-40
rcE2-50
rcE2-60
rcE2-90
b
mock
WT
rcE2-90
rcE2-60
rcE2-50
rcE2-40
c
2200
2220
Envelope Stem RNA Element (ESRE)
(SEQ ID NO: 62)

Figure 15

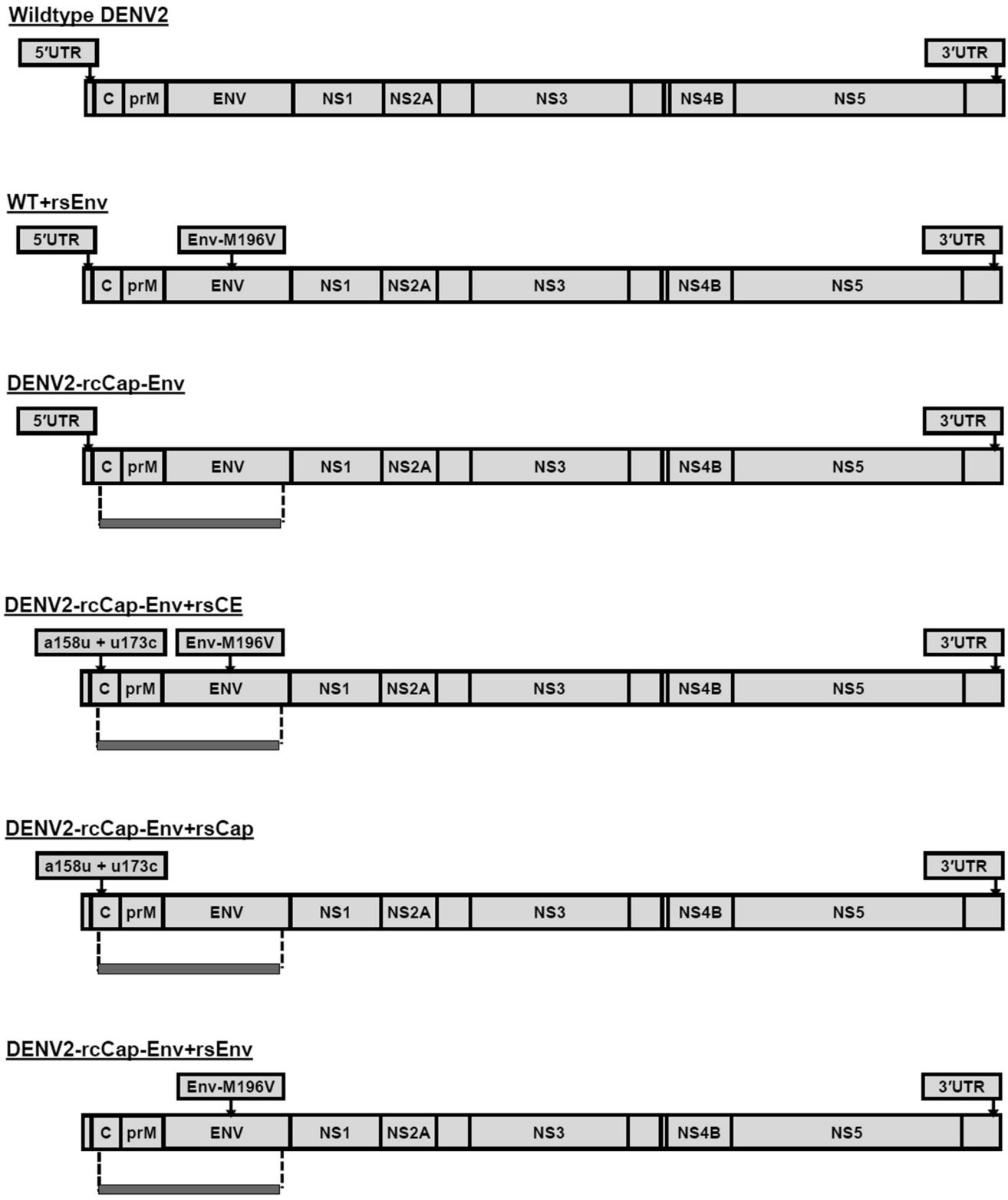

Wildtype DENV2
5′UTR
3′UTR
C
prM
ENV
NS1
NS2A
NS3
NS4B
NS5
WT+rsEnv
5′UTR
Env-M196V
3′UTR
C
prM
ENV
NS1
NS2A
NS3
NS4B
NS5
DENV2-rcCap-Env
5′UTR
3′UTR
C
prM
ENV
NS1
NS2A
NS3
NS4B
NS5
DENV2-rcCap-Env+rsCE
a158u + u173c
Env-M196V
3′UTR
C
prM
ENV
NS1
NS2A
NS3
NS4B
NS5
DENV2-rcCap-Env+rsCap
a158u + u173c
3′UTR
C
prM
ENV
NS1
NS2A
NS3
NS4B
NS5
DENV2-rcCap-Env+rsEnv
Env-M196V
3′UTR
C
prM
ENV
NS1
NS2A
NS3
NS4B
NS5

Figure 16

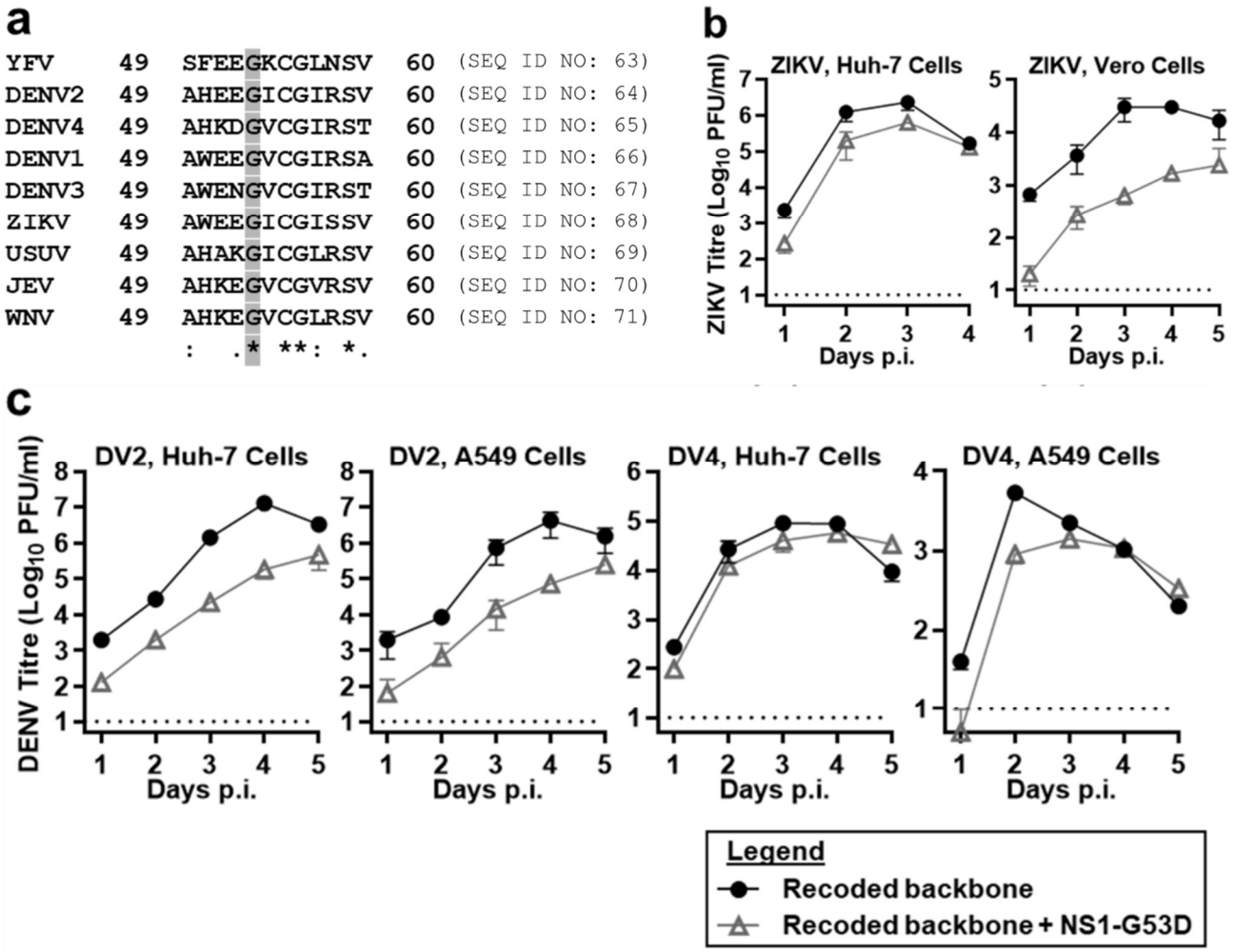

a
YFV 49 SFEEGKCGLNSV 60 (SEQ ID NO: 63)
DENV2 49 AHEEGICGIRSV 60 (SEQ ID NO: 64)
DENV4 49 AHKDGVCGIRST 60 (SEQ ID NO: 65)
DENV1 49 AWEEGVCGIRSA 60 (SEQ ID NO: 66)
DENV3 49 AWENGVCGIRST 60 (SEQ ID NO: 67)
ZIKV 49 AWEEGICGISSV 60 (SEQ ID NO: 68)
USUV 49 AHAKGICGLRSV 60 (SEQ ID NO: 69)
JEV 49 AHKEGVCGVRSV 60 (SEQ ID NO: 70)
WNV 49 AHKEGVCGLRSV 60 (SEQ ID NO: 71)
b
ZIKV, Huh-7 Cells
ZIKV, Vero Cells
ZIKV Titre (Log10 PFU/ml)
Days p.i.
c
DV2, Huh-7 Cells
DV2, A549 Cells
DV4, Huh-7 Cells
DV4, A549 Cells
DENV Titre (Log10 PFU/ml)
Days p.i.
Legend
Recoded backbone
Recoded backbone + NS1-G53D

Figure 17

a

Vaccine interferon response (Gene Log_2 Fold-change)	Wildtype ZIKV control	Recoded ZIKV +NS1-G53D
IFNB1	2.71	5.41
IRF7	1.22	3.75
IL6	0.38	2.92
CXCL10	0.75	2.59
C19orf66	0.59	2.61
IFIT1	2.45	5.06
IFIT2	2.42	4.57
IFI6	1.1	3.79
MX1	1.46	5.06
OAS1	0.55	2.23
OAS3	0.6	2.54

b

Vaccine interferon response (Gene Log_2 Fold-change)	Wildtype DENV4 control	Recoded DENV4 +NS1-G53D
IFNB1	3.11	3.51
IRF7	0.96	1.75
IL6	2.36	3.02
CXCL10	1.47	1.52
C19orf66	4.41	4.75
IFIT1	4.32	5.03
IFIT2	4.32	4.14
IFI6	5.40	5.92
MX1	2.42	2.55
OAS1	1.02	1.63
OAS3	3.11	3.51

Figure 18

a

Primary codon	Primary coding space	Phenotype
ATG	Methionine	Attenuated

Secondary codons	Secondary coding space	Secondary phenotype
ATT	Isoleucine	Lethal
ATC	Isoleucine	Lethal
ATA	Isoleucine	Lethal
ACG	Threonine	Lethal
AAG	Lysine	Attenuated
AGG	Arginine	Attenuated
GTG	Valine	Lethal
TTG	Leucine	Lethal
CTG	Leucine	Lethal

b

Primary codon	Primary coding space	Phenotype
TCA	Serine	Attenuated

Secondary codons	Secondary coding space	Secondary phenotype
TCG	Serine	Attenuated
TCT	Serine	Attenuated
TCC	Serine	Attenuated
TAA	Stop	Lethal
TGA	Stop	Lethal
TTA	Leucine	Lethal
CCA	Proline	Lethal
ACA	Threonine	Lethal
GCA	Alanine	Attenuated

Figure 19