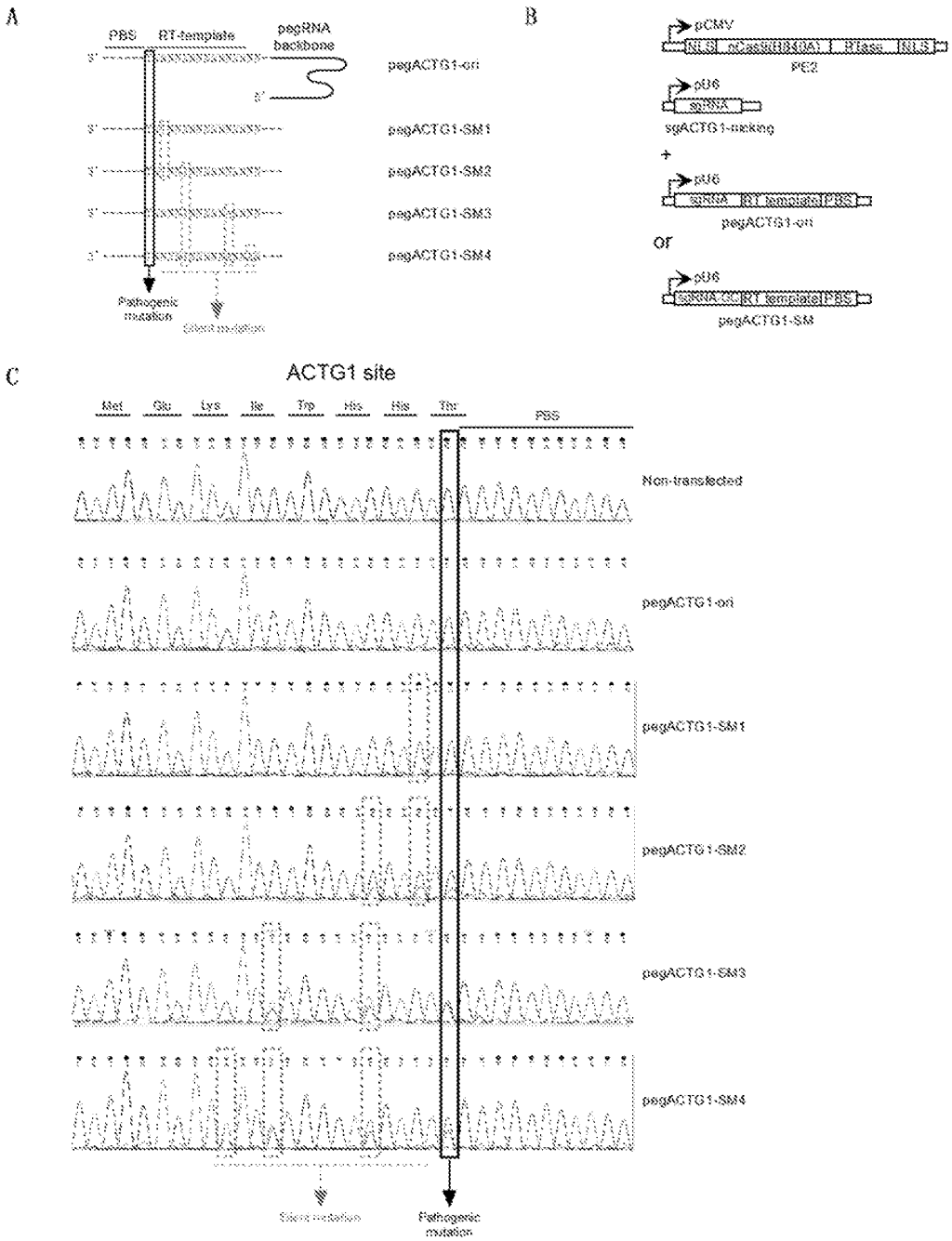

A
PBS
RT-template
pegRNA backbone
pegACTG1-on
pegACTG1-SM1
pegACTG1-SM2
pegACTG1-SM3
pegACTG1-SM4
Pathogenic mutation
Silent mutation
B
pCMV
PE2
pU6
sgRNA
sgACTG1-nicking
+
pegACTG1-on
or
pegACTG1-SM
C
ACTG1 site
Met
Glu
Lys
Ile
Trp
His
His
Thr
PBS
Non-translected
pegACTG1-on
pegACTG1-SM1
pegACTG1-SM2
pegACTG1-SM3
pegACTG1-SM4
Silent mutation
Pathogenic mutation

FIG. 1

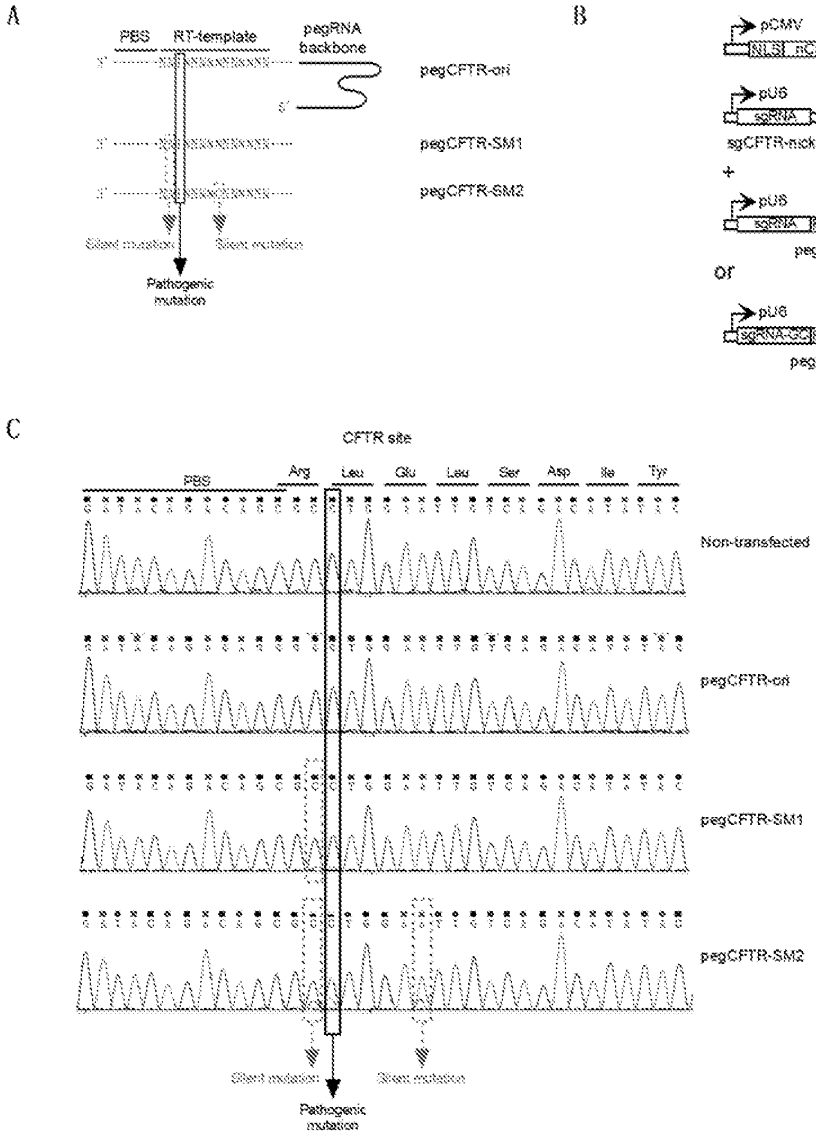

A
PBS
RT-template
pegRNA backbone
pegCFTR-ori
pegCFTR-SM1
pegCFTR-SM2
Silent mutation
Silent mutation
Pathogenic mutation
B
pCMV
NLS
nCas9(H840A)
RTase
NLS
PE2
pU6
sgRNA
sgCFTR-nicking
+
pU6
sgRNA
RT template
PBS
pegCFTR-ori
or
pU6
sgRNA-GC
RT template
PBS
pegCFTR-SM
C
CFTR site
PBS
Arg
Leu
Glu
Leu
Ser
Asp
Ile
Tyr
Non-transfected
pegCFTR-ori
pegCFTR-SM1
pegCFTR-SM2
Silent mutation
Silent mutation
Pathogenic mutation

FIG. 2

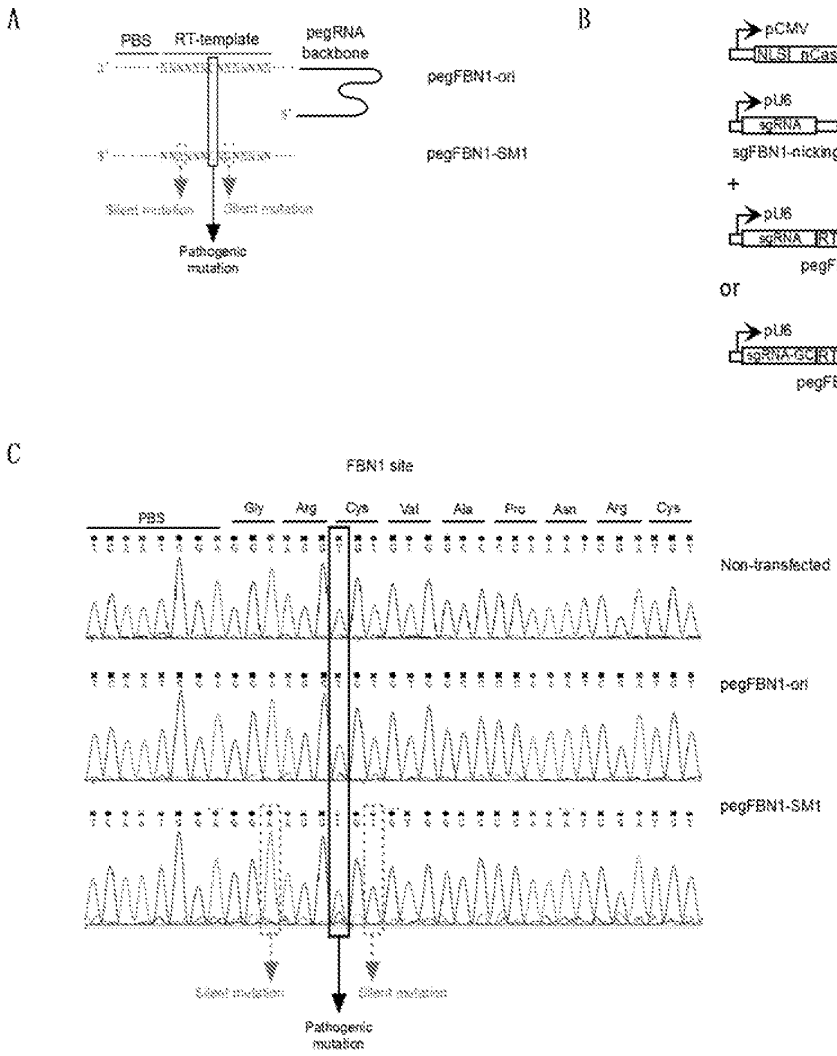

A
PBS
RT-template
pegRNA backbone
pegFBN1-ori
pegFBN1-SM1
Silent mutation
Silent mutation
Pathogenic mutation
B
pCMV
NLS nCas9(H840A) RTase NLS
PE2
pU6
sgRNA
sgFBN1-nicking
+
pU6
sgRNA RT template PBS
pegFBN1-ori
or
pU6
sgRNA GC RT template PBS
pegFBN1-SM
C
FBN1 site
PBS
Gly
Arg
Cys
Val
Ala
Pro
Asn
Arg
Cys
Non-transfected
pegFBN1-ori
pegFBN1-SM1
Silent mutation
Silent mutation
Pathogenic mutation

FIG. 3

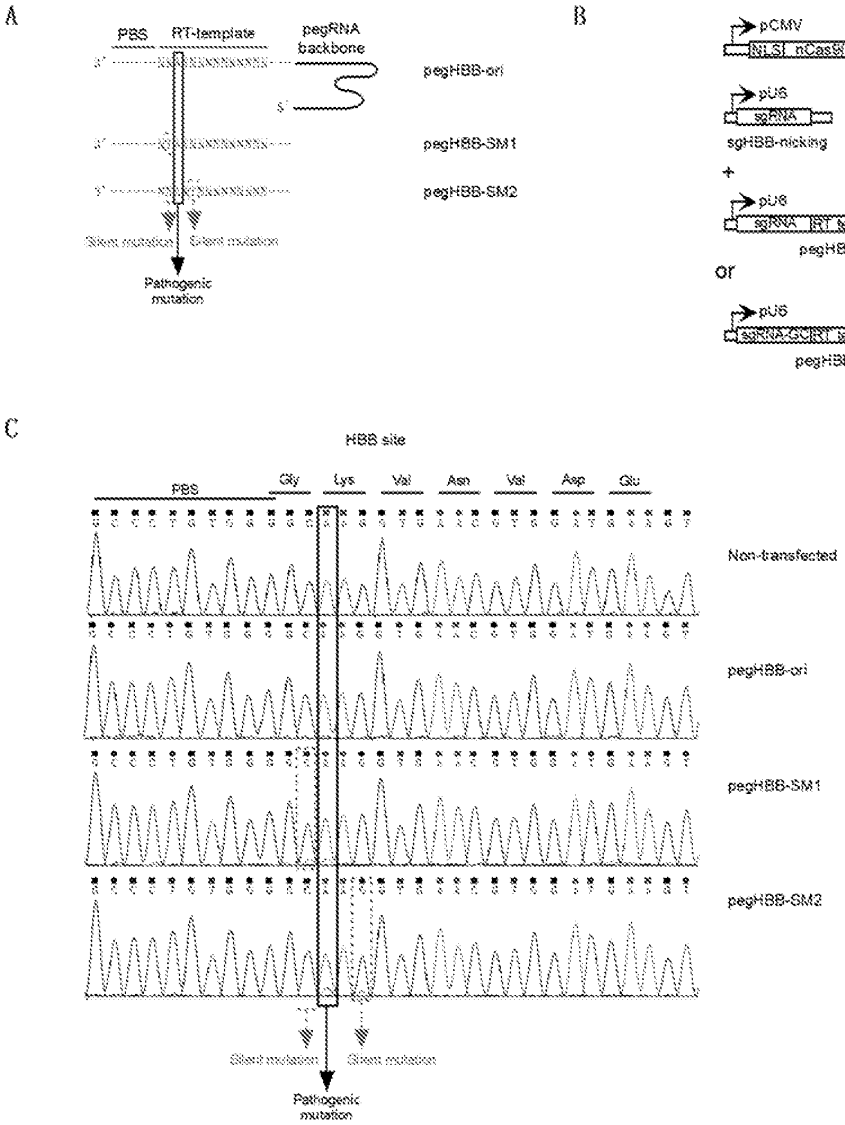

A
PBS
RT-template
pegRNA backbone
pegHBB-ori
pegHBB-SM1
pegHBB-SM2
Silent mutation
Silent mutation
Pathogenic mutation
B
pCMV
NLS nCas9(H840A) RTase NLS
PE2
pU6
sgRNA
sgHBB-nicking
+
pU6
sgRNA RT template PBS
pegHBB-ori
or
pU6
sgRNA 3'RT template PBS
pegHBB-SM
C
HBB site
PBS
Gly
Lys
Val
Asn
Val
Asp
Glu
Non-transfected
pegHBB-ori
pegHBB-SM1
pegHBB-SM2
Silent mutation
Silent mutation
Pathogenic mutation

FIG. 4

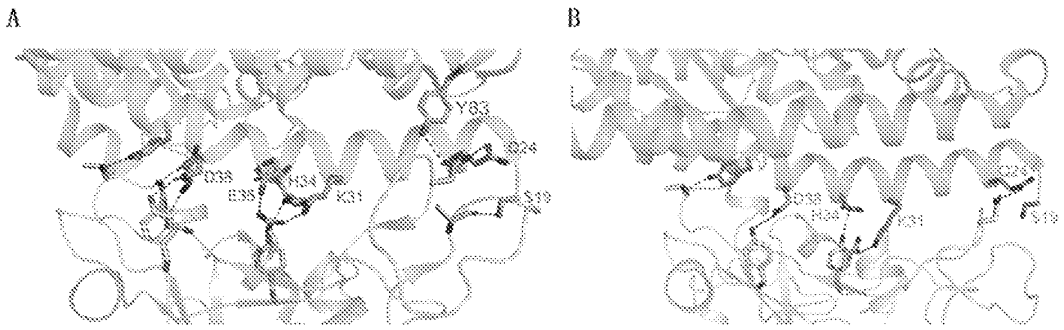

A
B
Y83
Q24
D38
E35
H34
K31
S19

FIG. 5

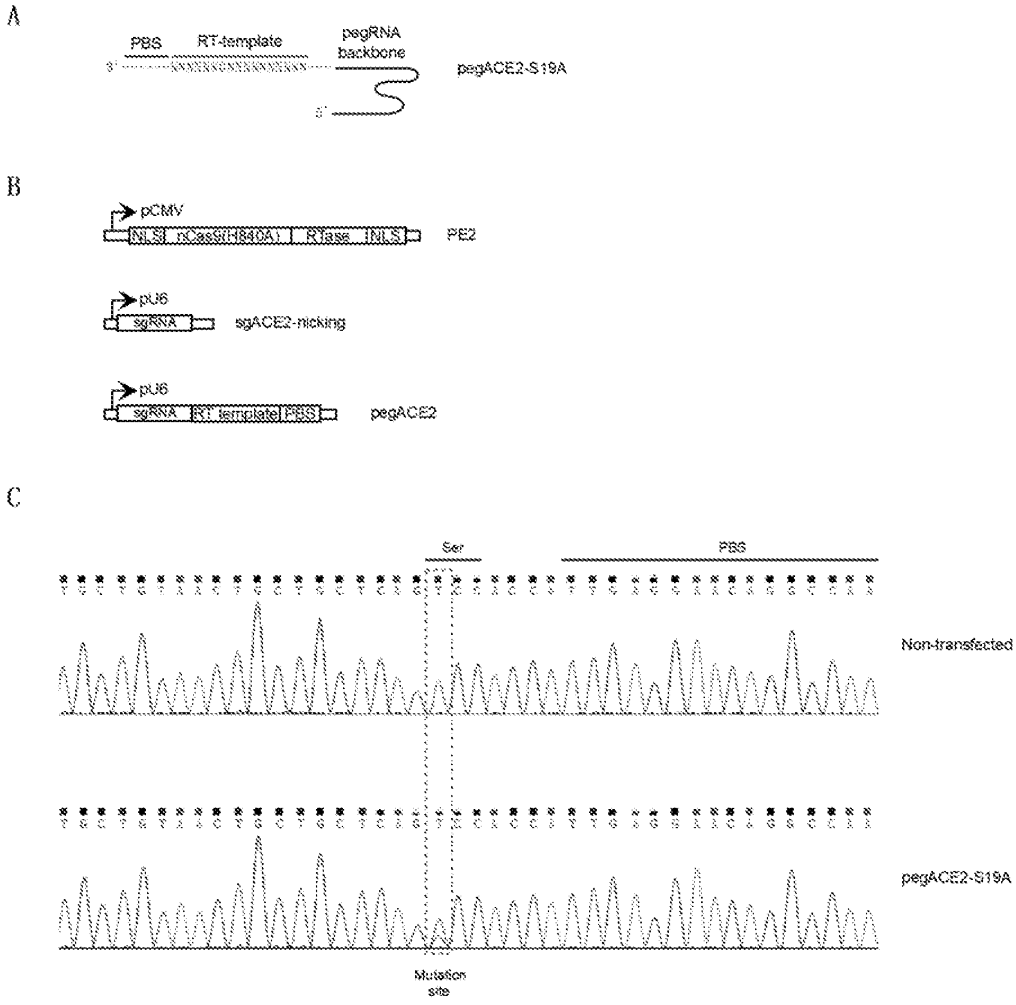

A
PBS
RT-template
pegRNA backbone
pegACE2-S19A
B
pCMV
NLS nCas9(H840A) RTase (N)LS
PE2
pU6
sgRNA
sgACE2-nicking
pU6
sgRNA RT template PBS
pegACE2
C
Ser
PBS
Non-transfected
pegACE2-S19A
Mutation site

FIG. 6

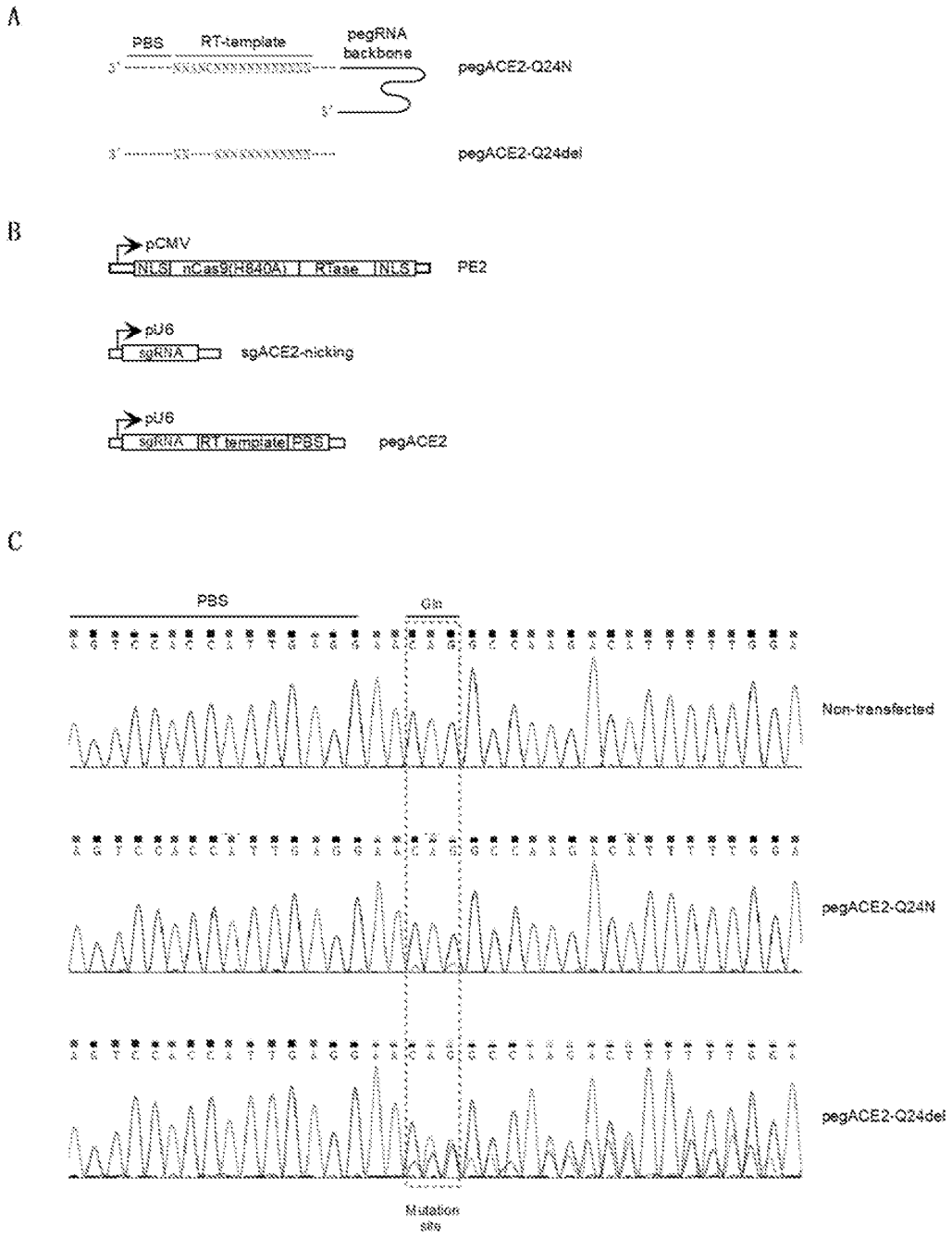

A
PBS
RT-template
pegRNA backbone
pegACE2-Q24N
pegACE2-Q24del
B
pCMV
NLS nCas9(H840A) RTase NLS
PE2
pU6
sgRNA
sgACE2-nicking
pU6
sgRNA RT template PBS
pegACE2
C
PBS
Gln
Non-transfected
pegACE2-Q24N
pegACE2-Q24del
Mutation site

FIG. 7

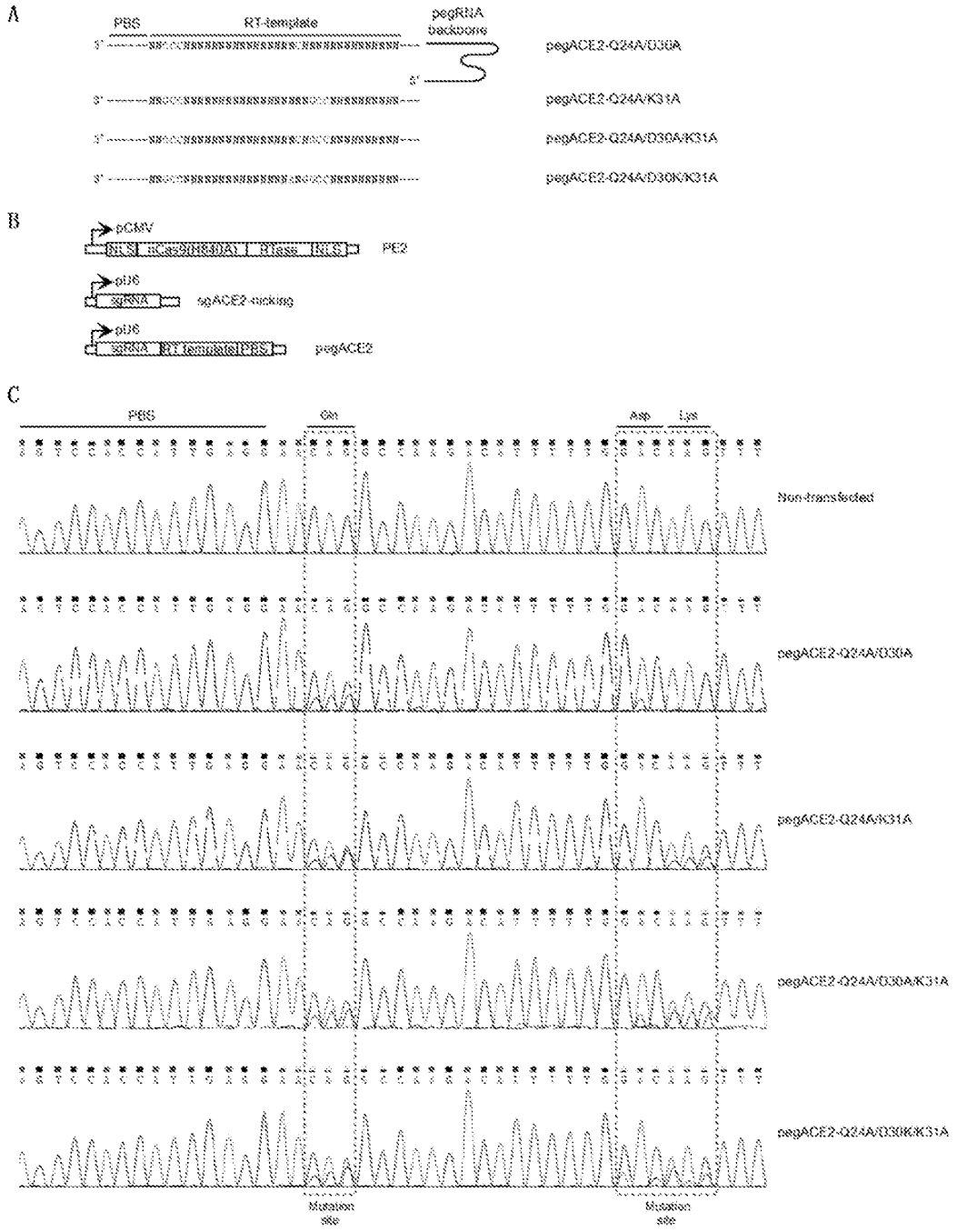

A
PBS
RT-template
pegRNA backbone
pegACE2-Q24A/D30A
pegACE2-Q24A/K31A
pegACE2-Q24A/D30A/K31A
pegACE2-Q24A/D30K/K31A
B
pCMV
PE2
pU6
sgACE2-nicking
pegACE2
C
PBS
Gln
Asp
Lys
Non-transfected
pegACE2-Q24A/D30A
pegACE2-Q24A/K31A
pegACE2-Q24A/D30A/K31A
pegACE2-Q24A/D30K/K31A
Mutation site
Mutation site

FIG. 8

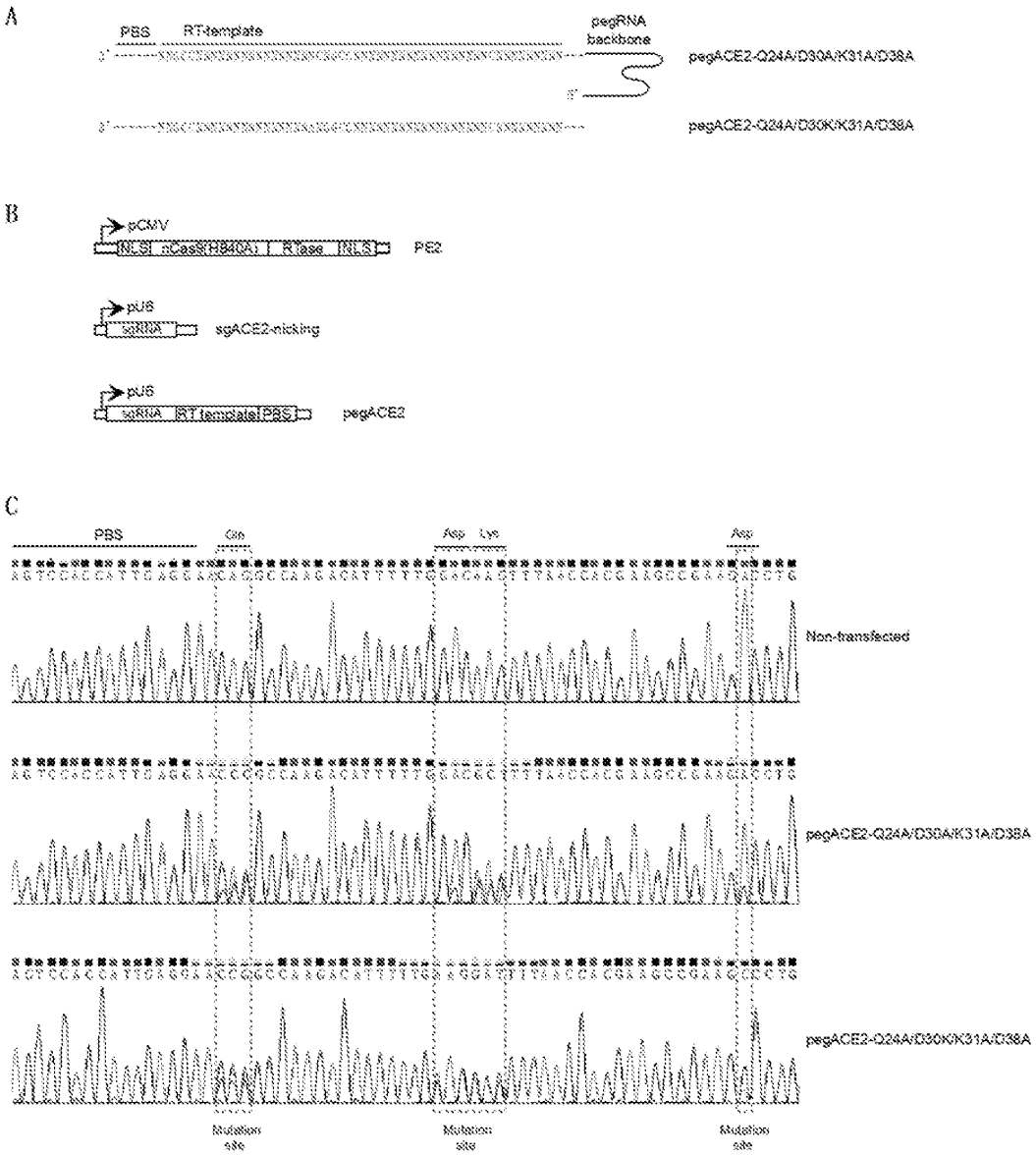

A
PBS
RT-template
pegRNA backbone
pegACE2-Q24A/D30A/K31A/D38A
pegACE2-Q24A/D30K/K31A/D38A
B
pCMV
NLS nCas9(H840A) RTase NLS
PE2
pU6
sgRNA
sgACE2-nicking
pU6
sgRNA RT template PBS
pegACE2
C
PBS
Gln
Asp Lys
Asp
Non-transfected
pegACE2-Q24A/D30A/K31A/D38A
pegACE2-Q24A/D30K/K31A/D38A
Mutation site
Mutation site
Mutation site

FIG. 9

A

ACE2 mutant	K_{on}	K_{off}	K_D	Relative Affinity (%)
WT	5.36E+04	1.07E-02	1.99E-07	100%
S19A	4.39E+04	2.28E-02	5.19E-07	38.46%
Q24A	4.92E+04	3.28E-02	6.66E-07	29.93%
Q24del	4.12E+04	1.71E-02	4.16E-07	48.00%
Q24A/D30K/K31A	1.54E+04	5.12E-01	N.B.	N.B.
Q24A/D30A/K31A	2.95E+03	4.25E-01	N.B.	N.B.

B

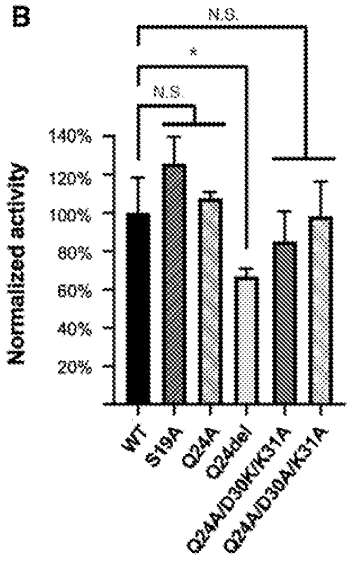


FIG. 10

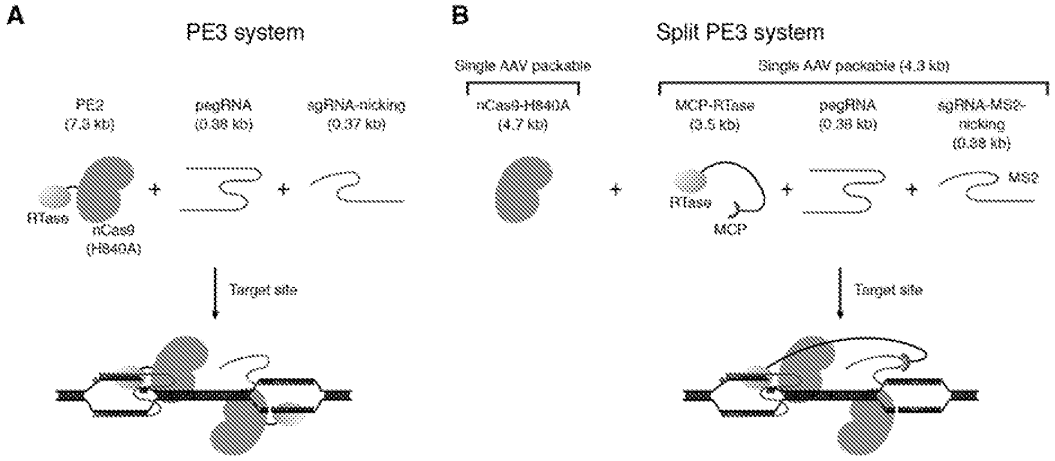


FIG. 11

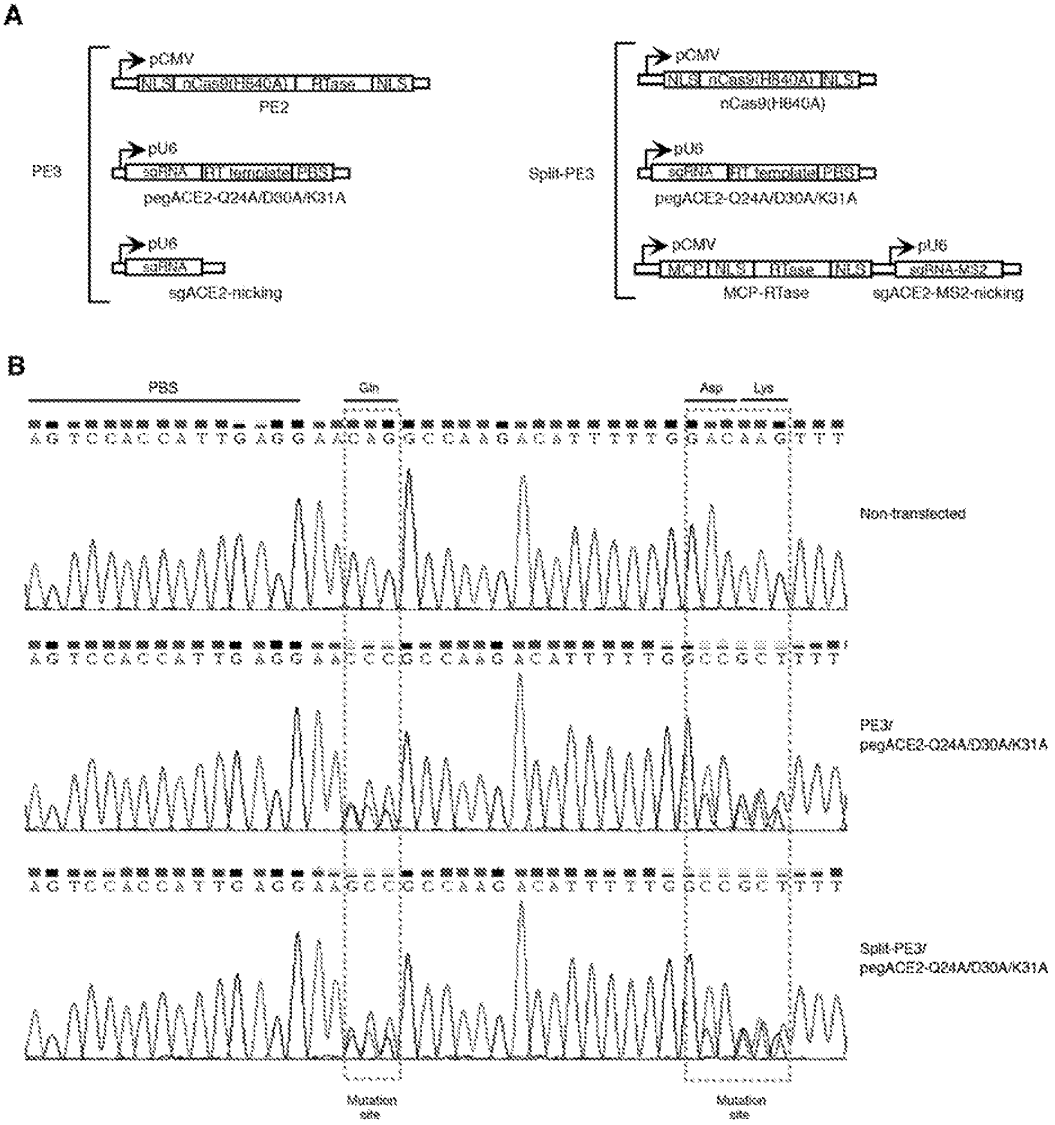

A
PE3
pCMV
NLS nCas9(H840A) RTase NLS
PE2
pU6
spRNA RT template PBS
pegACE2-Q24A/D30A/K31A
pU6
spRNA
sgACE2-nicking
Split-PE3
pCMV
NLS nCas9(H840A) NLS
nCas9(H840A)
pU6
spRNA RT template PBS
pegACE2-Q24A/D30A/K31A
pCMV
MCP NLS RTase NLS
pU6
spRNA-MS2
MCP-RTase
sgACE2-MS2-nicking
B
PBS
Gln
Asp
Lys
Non-transfected
PE3/
pegACE2-Q24A/D30A/K31A
Split-PE3/
pegACE2-Q24A/D30A/K31A
Mutation site
Mutation site

FIG. 12

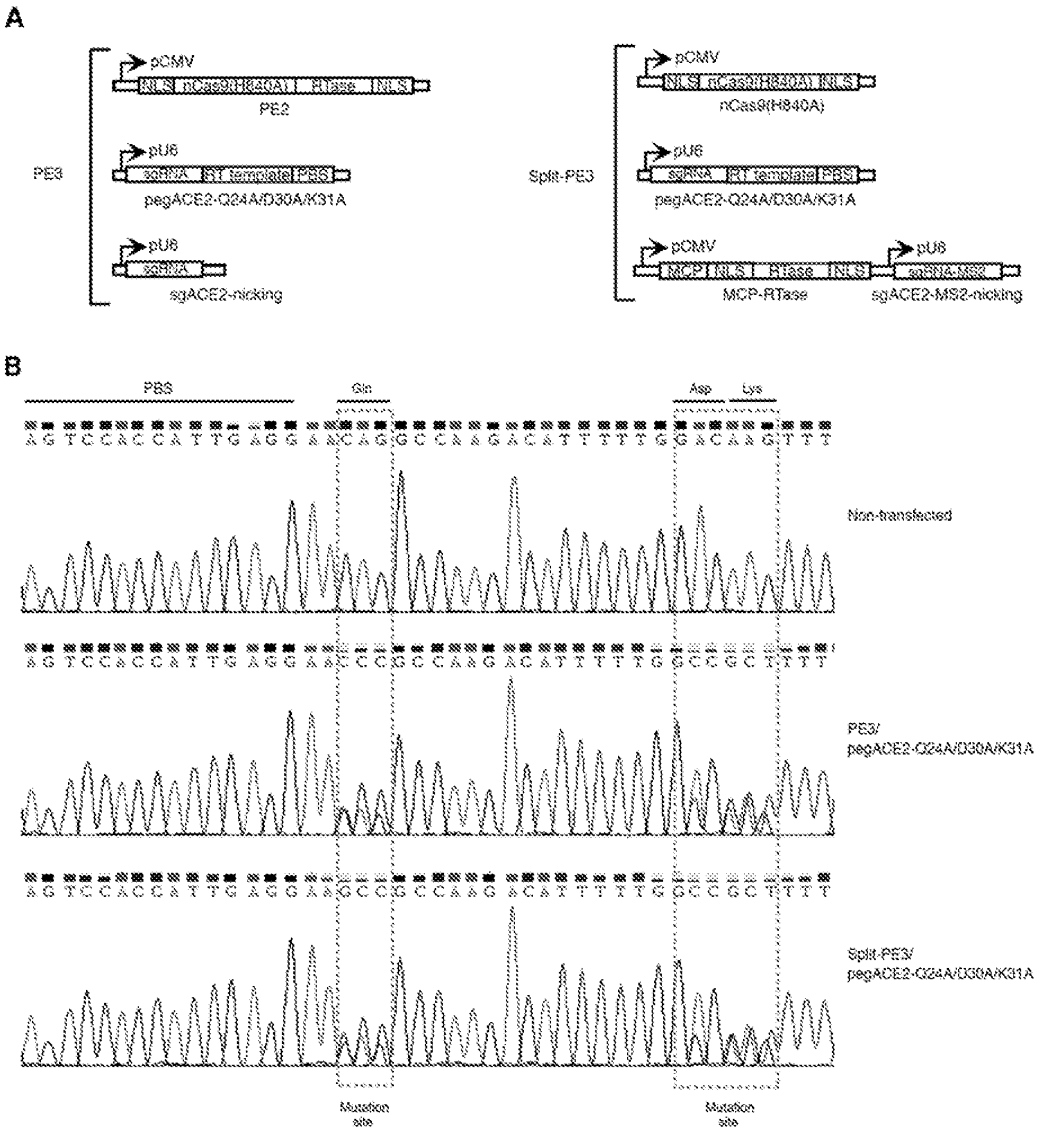

A
PE3
pCMV
NLS nCas9(H840A) RTase NLS
PE2
pU6
sgRNA RT template PBS
pegACE2-Q24A/D30A/K31A
pU6
sgRNA
sgACE2-nicking
Split-PE3
pCMV
NLS nCas9(H840A) NLS
nCas9(H840A)
pU6
sgRNA RT template PBS
pegACE2-Q24A/D30A/K31A
pCMV
MCP NLS RTase NLS
MCP-RTase
pU6
sgRNA MS2
sgACE2-MS2-nicking
B
PBS
Gln
Asp
Lys
Non-transfected
PE3/
pegACE2-Q24A/D30A/K31A
Split-PE3/
pegACE2-Q24A/D30A/K31A
Mutation site
Mutation site

FIG. 13

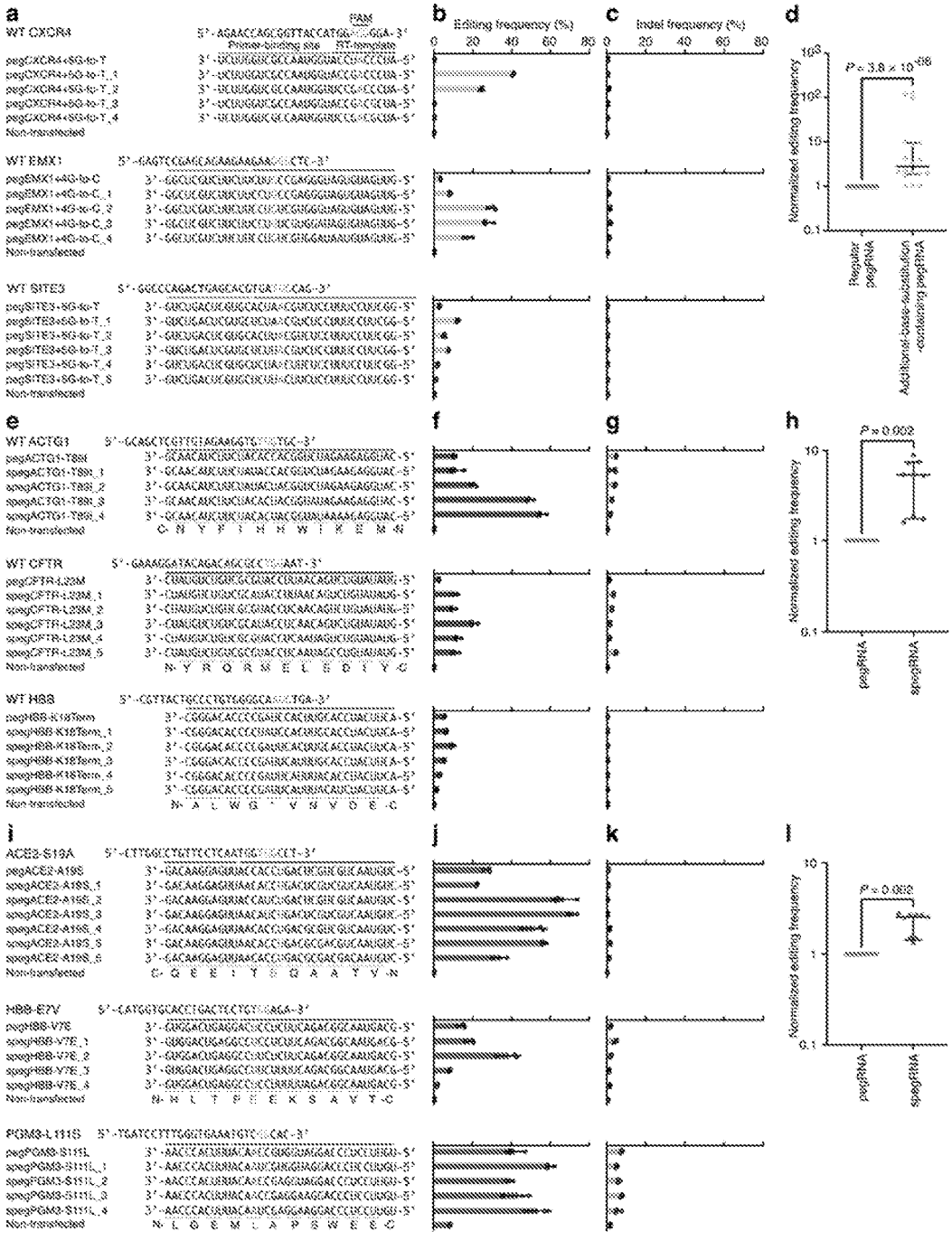


FIG. 14

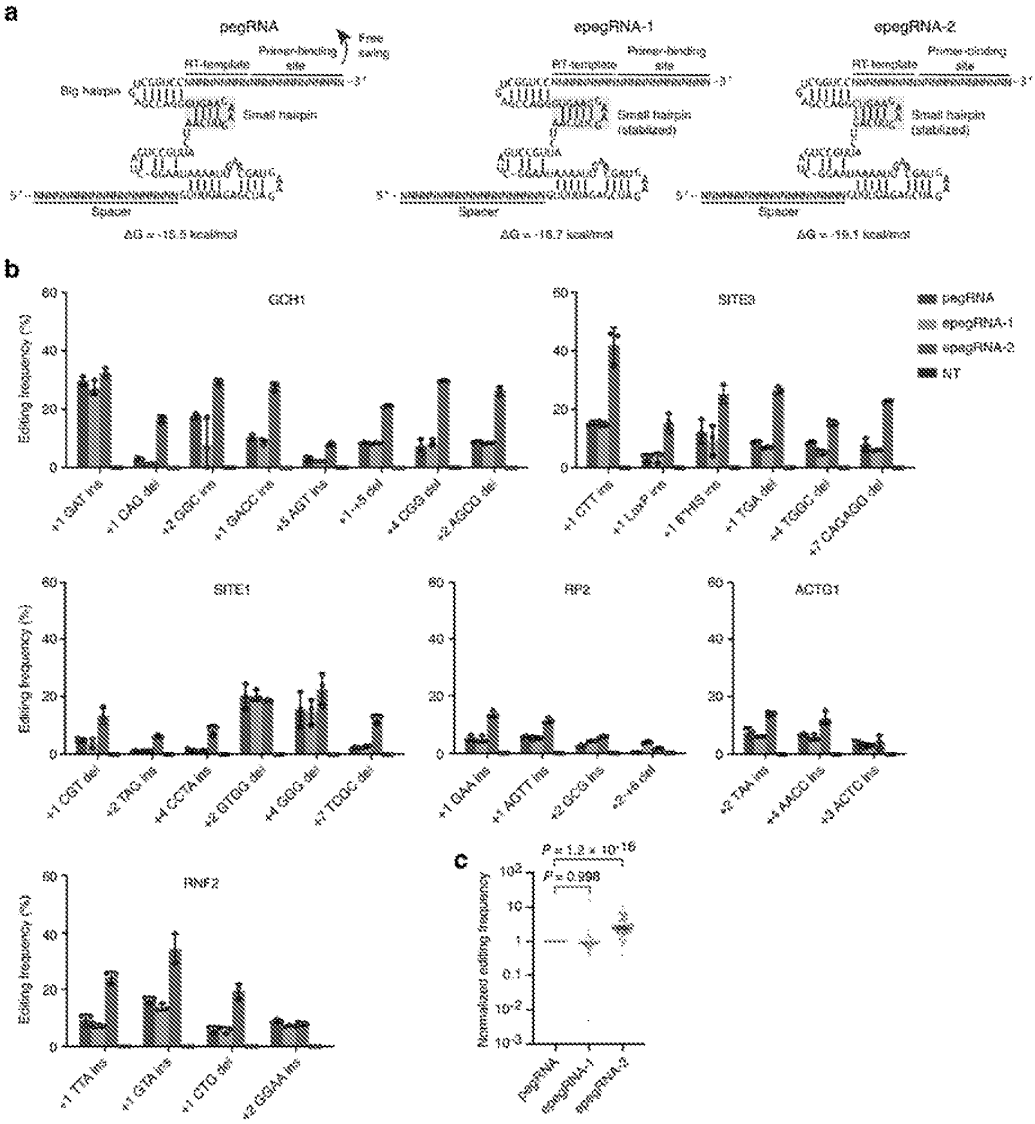

a
pegRNA
epegRNA-1
epegRNA-2
Primer-binding site
RT-template
Free swing
Big hairpin
Small hairpin
Small hairpin (stabilized)
Spacer
ΔG = -16.5 kcal/mol
ΔG = -16.7 kcal/mol
ΔG = -16.1 kcal/mol
b
GCH1
SITE3
SITE1
RP2
ACTG1
RNF2
pegRNA
epegRNA-1
epegRNA-2
NT
Editing frequency (%)
+1 GAT ins
+1 CAG del
+2 GGC ins
+1 GACC ins
+5 AGT ins
+1~15 del
+4 CGG del
+2 AGCG del
+1 CTT ins
+1 CAAP ins
+1 ATHS ins
+1 TGA del
+4 TGGC del
+7 CACAGD del
+1 CGT del
+2 TAG ins
+4 CCTA ins
+2 GTGG del
+4 GGG del
+7 TCGC del
+1 GAA ins
+1 AGTT ins
+2 GC-G ins
+2~16 del
+2 TAA ins
+4 AACC ins
+3 ACTC ins
+1 TTA ins
+1 GTA ins
+1 CTG del
+2 GGAA ins
c
P = 1.2 × 10-16
P = 0.998
Relative editing frequency
pegRNA
epegRNA-1
epegRNA-2

FIG. 15

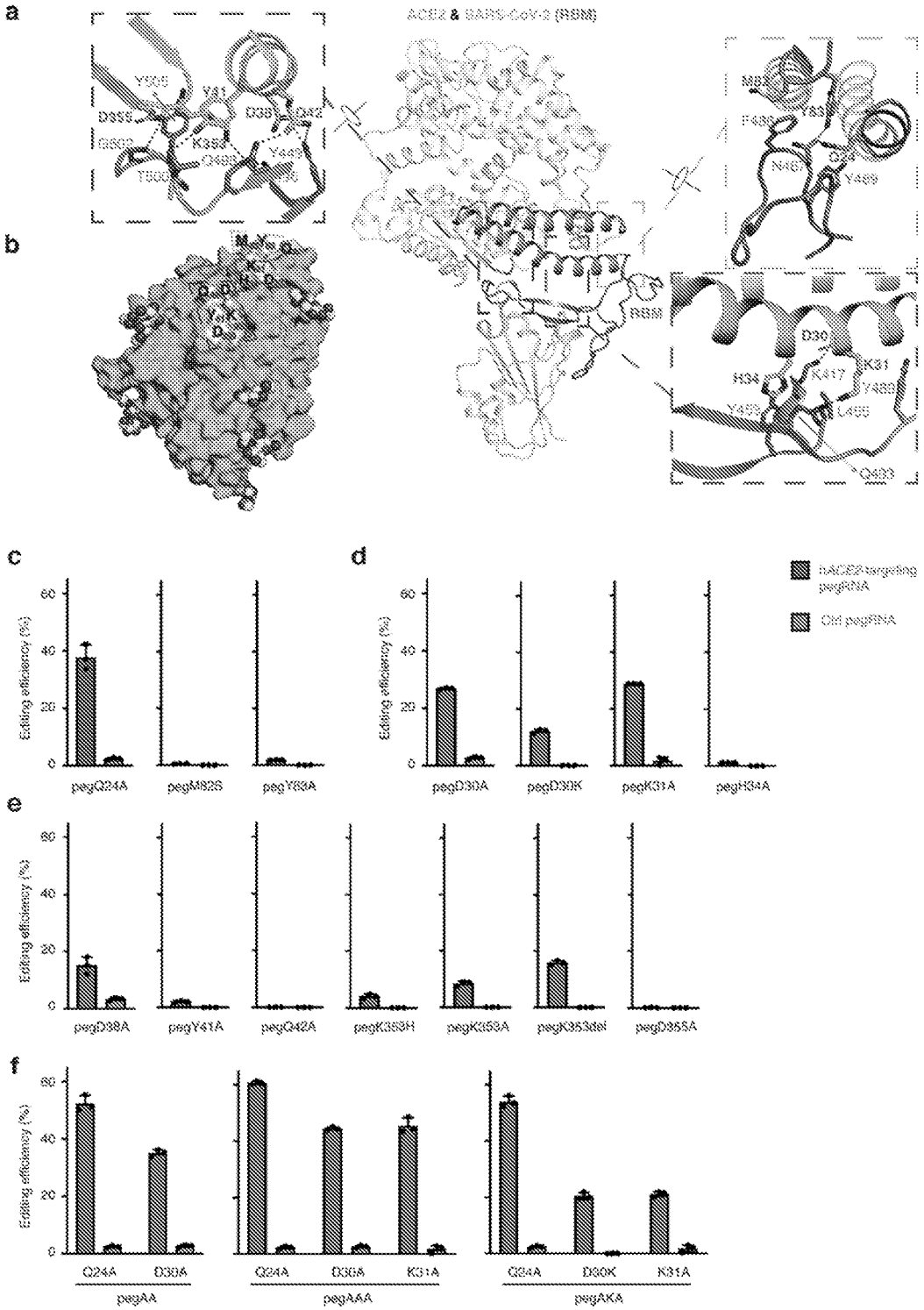

a
ACE2 & SARS-CoV-2 (RBM)
b
RBM
c
d
hACE2-targeting pegRNA
Old pegRNA
Editing efficiency (%)
pegQ24A
pegM82S
pegY83A
pegD30A
pegD30K
pegK31A
pegH34A
e
pegD38A
pegY41A
pegQ42A
pegK353H
pegK353A
pegK353del
pegD355A
f
Q24A
D30A
pegAA
Q24A
D30A
K31A
pegAAA
Q24A
D30K
K31A
pegAKA

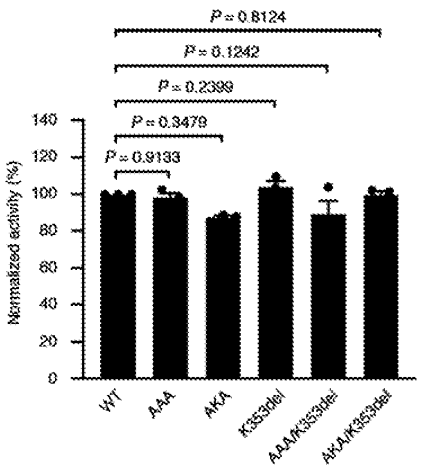
FIG. 16

a

With WT RBD

ACE2 mutants	k_{on} ($M^{-1}S^{-1}$)	k_{off} (S^{-1})	K_D (M^{-1})	Relative activity
WT	5.4×10^{5}	0.011	2.0×10^{-7}	100%
Q24A	4.9×10^{5}	0.033	6.7×10^{-7}	29.9%
Q24A/D30A	4.1×10^{5}	0.056	1.4×10^{-6}	14.8%
AAA	/	/	N.B.	N.B.
AKA	/	/	N.B.	N.B.
K353del	/	/	N.B.	N.B.
AAA/K353del	/	/	N.B.	N.B.
AKA/K353del	/	/	N.B.	N.B.

b



c

With B.1.1.7 RBD

ACE2 mutants	k_{on} ($M^{-1}S^{-1}$)	k_{off} (S^{-1})	K_D (M^{-1})	Relative activity
WT	4.2×10^{5}	0.002	4.6×10^{-9}	100%
AAA	4.6×10^{4}	0.030	6.5×10^{-7}	6.9%
AKA	1.2×10^{5}	0.053	4.2×10^{-7}	0.1%
K353del	/	/	N.B.	N.B.
AAA/K353del	/	/	N.B.	N.B.
AKA/K353del	/	/	N.B.	N.B.

With B.1.351 RBD

ACE2 mutants	k_{on} ($M^{-1}S^{-1}$)	k_{off} (S^{-1})	K_D (M^{-1})	Relative activity
WT	5.3×10^{5}	0.005	1.0×10^{-7}	100%
K353del	/	/	N.B.	N.B.
AAA/K353del	/	/	N.B.	N.B.
AKA/K353del	/	/	N.B.	N.B.

With P.1 RBD

ACE2 mutants	k_{on} ($M^{-1}S^{-1}$)	k_{off} (S^{-1})	K_D (M^{-1})	Relative activity
WT	4.5×10^{5}	0.004	8.0×10^{-9}	100%
K353del	/	/	N.B.	N.B.
AAA/K353del	/	/	N.B.	N.B.
AKA/K353del	/	/	N.B.	N.B.

FIG. 17

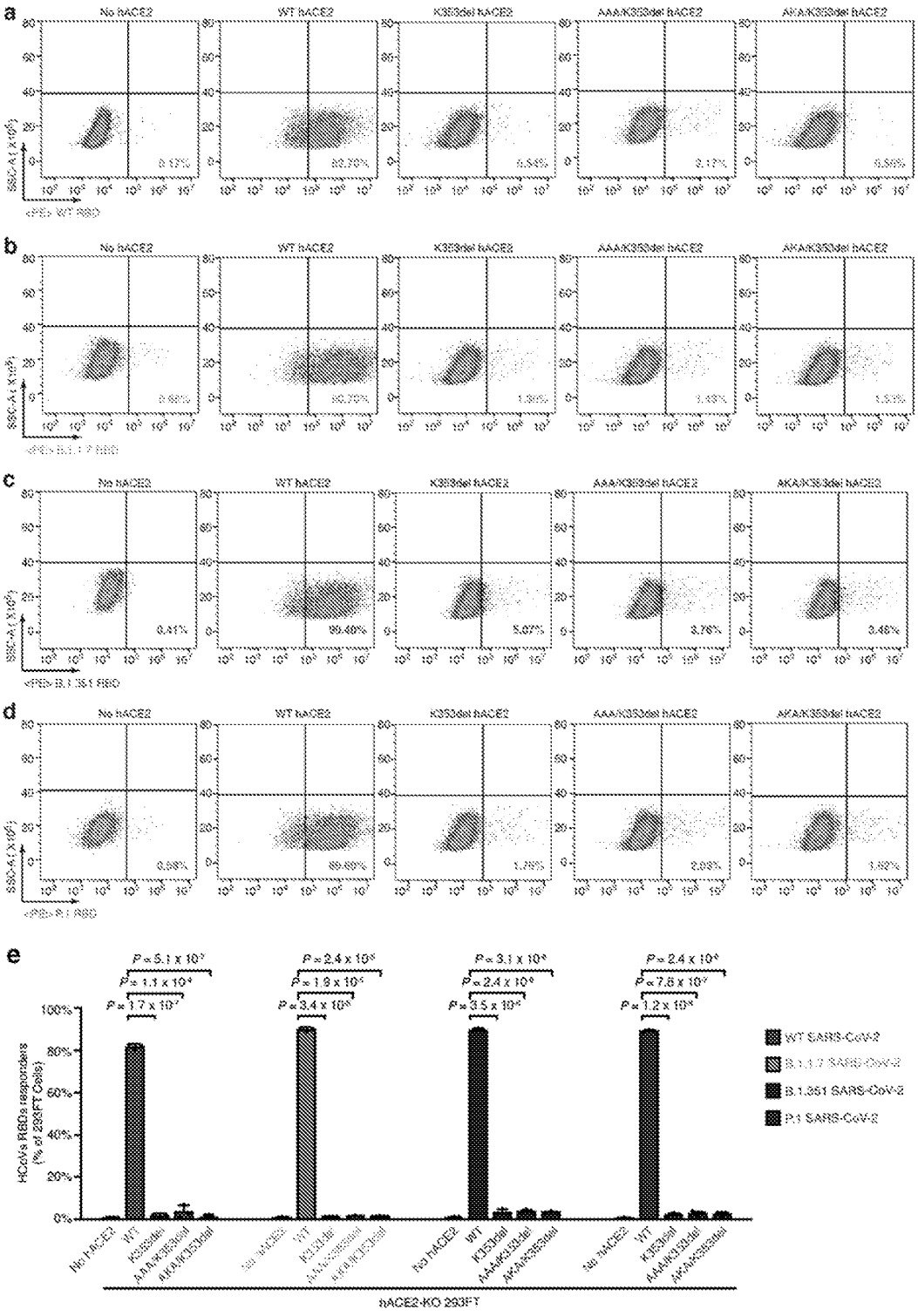

a
No hACE2
WT hACE2
K353del hACE2
AAA/K353del hACE2
AKA/K353del hACE2
SSC-A (X10⁴)
<PE> WT RBD
0.17%
80.70%
0.54%
0.17%
0.59%
b
No hACE2
WT hACE2
K353del hACE2
AAA/K353del hACE2
AKA/K353del hACE2
SSC-A (X10⁴)
<PE> B.1.1.7 RBD
0.66%
80.70%
1.36%
1.43%
1.51%
c
No hACE2
WT hACE2
K353del hACE2
AAA/K353del hACE2
AKA/K353del hACE2
SSC-A (X10⁴)
<PE> B.1.351 RBD
0.41%
89.46%
5.07%
3.76%
3.46%
d
No hACE2
WT hACE2
K353del hACE2
AAA/K353del hACE2
AKA/K353del hACE2
SSC-A (X10⁴)
<PE> P.1 RBD
0.58%
89.69%
1.76%
2.09%
1.92%
e
P = 5.1 x 10⁻⁷
P = 1.1 x 10⁻⁶
P = 1.7 x 10⁻⁷
P = 2.4 x 10⁻⁶
P = 1.9 x 10⁻⁵
P = 3.4 x 10⁻⁶
P = 3.1 x 10⁻⁶
P = 2.4 x 10⁻⁶
P = 3.5 x 10⁻⁶
P = 2.4 x 10⁻⁶
P = 7.8 x 10⁻⁷
P = 1.2 x 10⁻⁶
HCoVs RBDs responders (% of 293FT Cells)
100%
80%
60%
40%
20%
0%
No hACE2
WT
K353del
AAA/K353del
AKA/K353del
hACE2-KO 293FT
WT SARS-CoV-2
B.1.1.7 SARS-CoV-2
B.1.351 SARS-CoV-2
P.1 SARS-CoV-2

FIG. 18

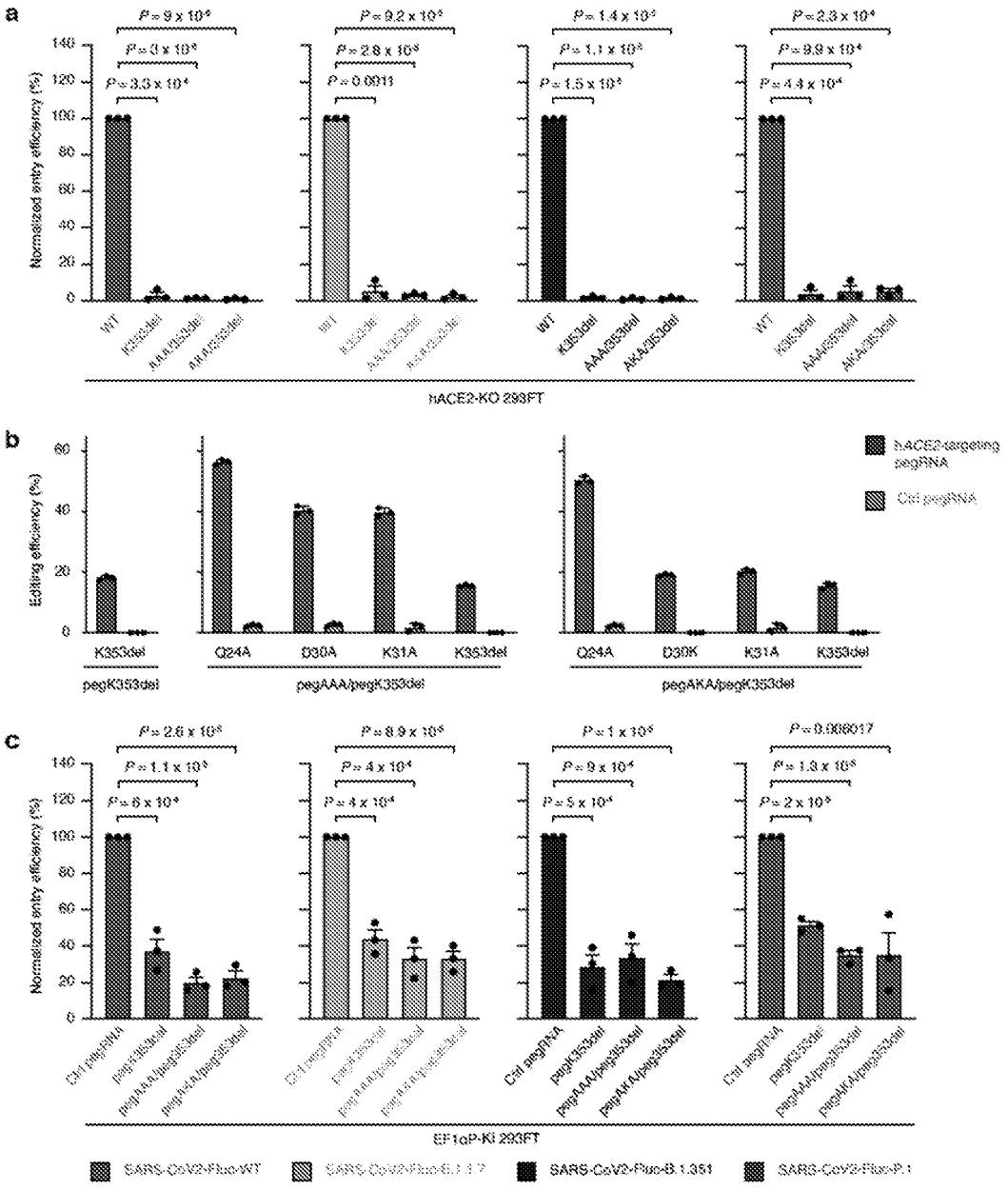

a
Normalized entry efficiency (%)
WT
K353del
AAA/353del
AKA/353del
hACE2-KO 293FT
b
Editing efficiency (%)
hACE2-targeting pegRNA
Ctrl pegRNA
K353del
Q24A
D30A
K31A
pegK353del
pegAAA/pegK353del
D30K
pegAKA/pegK353del
c
Normalized entry efficiency (%)
Ctrl pegRNA
pegK353del
pegAAA/pegK353del
pegAKA/pegK353del
EF1αP-KI 293FT
SARS-CoV2-Fluc-WT
SARS-CoV2-Fluc-B.1.351

FIG. 19

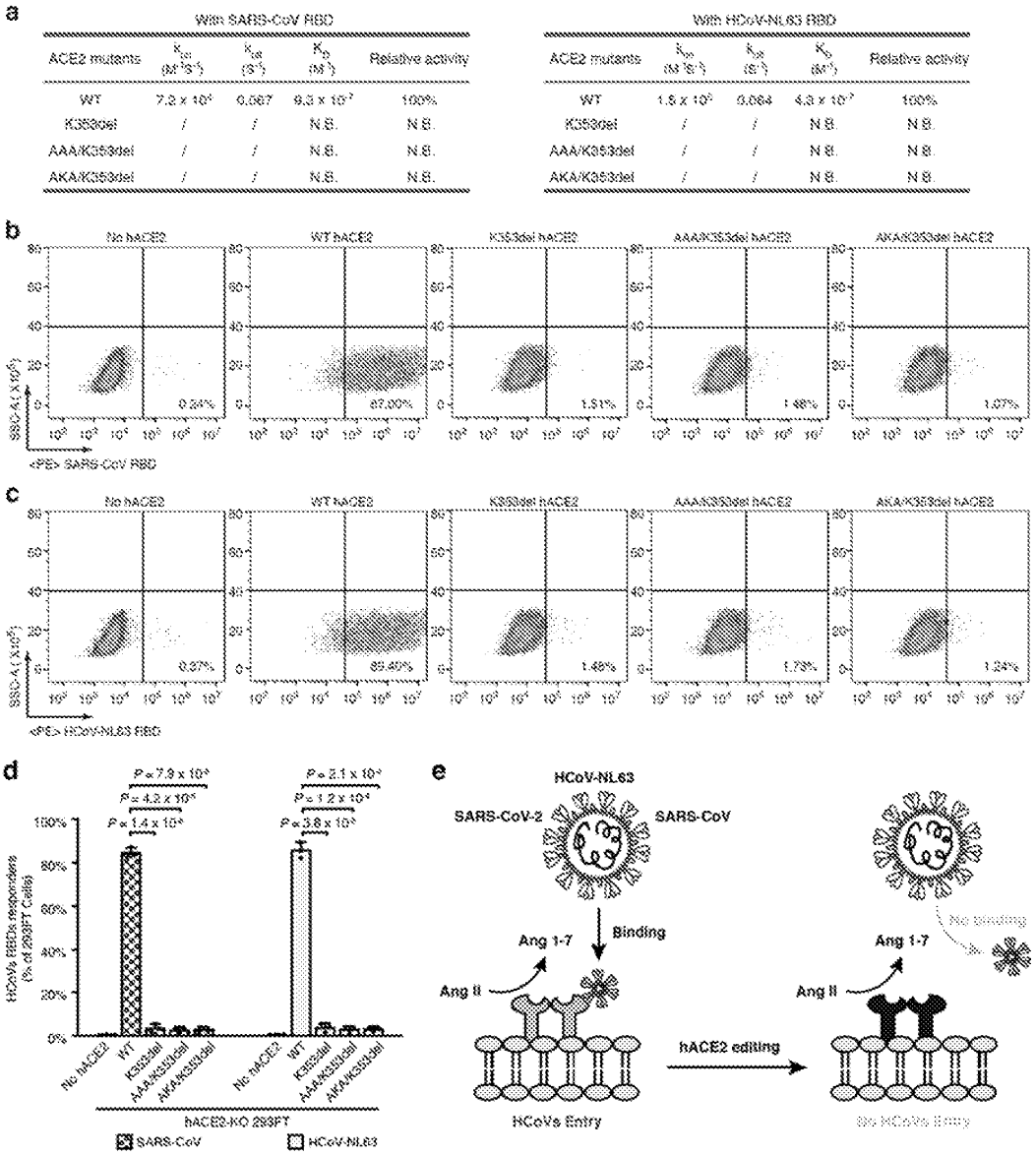
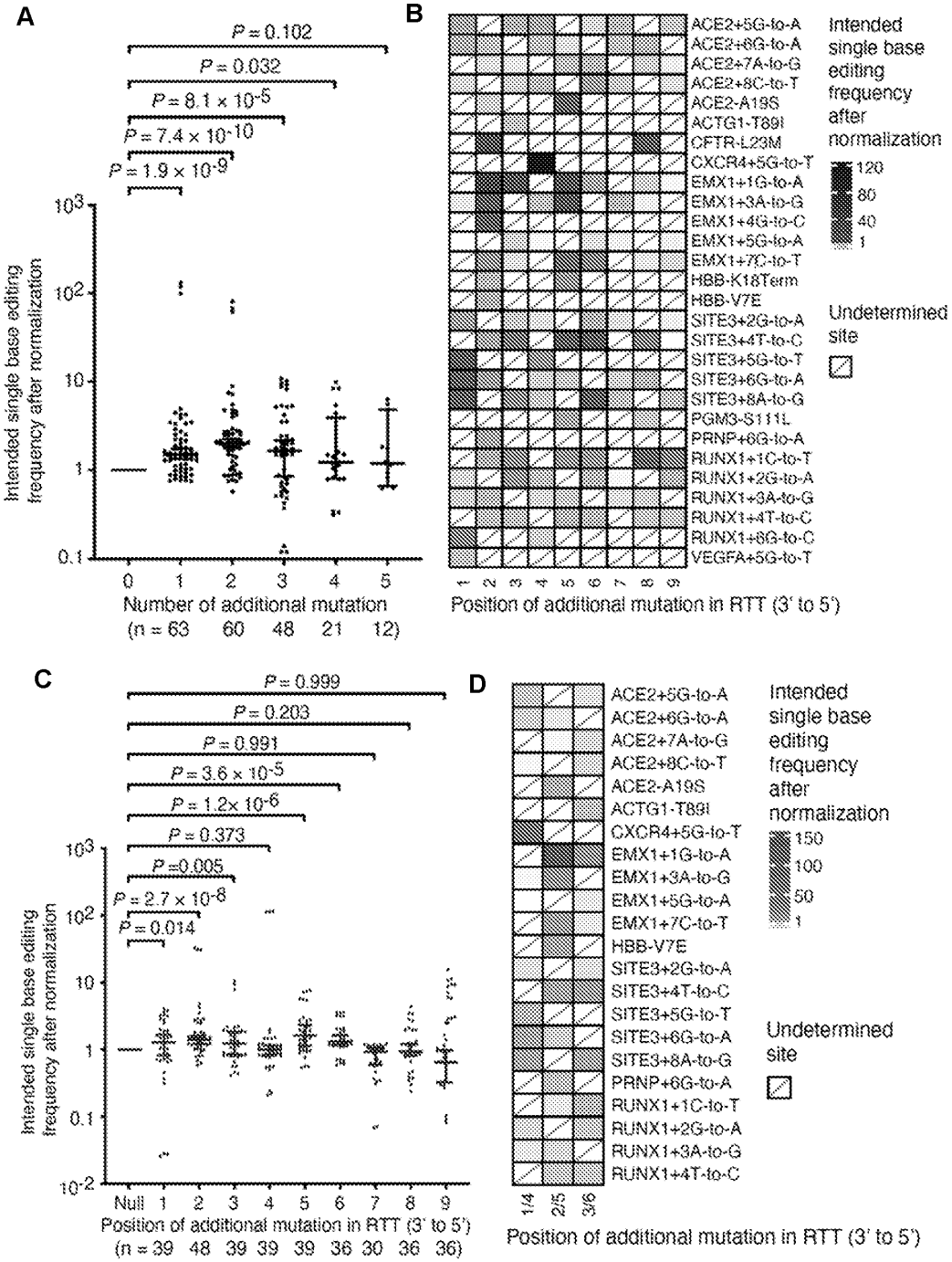

a
With SARS-CoV RBD
ACE2 mutants
Relative activity
WT
7.2 x 10⁵
0.067
9.3 x 10⁻⁸
100%
K353del
AAA/K353del
AKA/K353del
N.B.
With HCoV-NL63 RBD
1.5 x 10⁵
0.064
4.3 x 10⁻⁷
b
No hACE2
WT hACE2
K353del hACE2
AAA/K353del hACE2
AKA/K353del hACE2
0.24%
87.00%
1.51%
1.48%
1.07%
SSC-A (X10⁵)
<PE> SARS-CoV RBD
c
0.07%
85.40%
1.46%
1.79%
1.24%
<PE> HCoV-NL63 RBD
d
P = 7.9 x 10⁻⁵
P = 4.2 x 10⁻⁵
P = 1.4 x 10⁻⁴
P = 2.1 x 10⁻⁵
P = 1.2 x 10⁻⁴
P = 3.8 x 10⁻⁵
HCoVs RBDs responders (% of 293FT Cells)
100%
80%
60%
40%
20%
0%
No hACE2
WT
K353del
AAA/K353del
AKA/K353del
hACE2-KO 293FT
SARS-CoV
HCoV-NL63
e
HCoV-NL63
SARS-CoV-2
SARS-CoV
Ang 1-7
Binding
Ang II
hACE2 editing
HCoVs Entry
No binding
No HCoVs Entry

FIG. 20


A
P = 0.102
P = 0.032
P = 8.1 × 10-5
P = 7.4 × 10-10
P = 1.9 × 10-9
Intended single base editing frequency after normalization
Number of additional mutation
(n = 63 60 48 21 12)
B
ACE2+5G-to-A
ACE2+6G-to-A
ACE2+7A-to-G
ACE2+8C-to-T
ACE2-A19S
ACTG1-T89I
CFTR-L23M
CXCR4+5G-to-T
EMX1+1G-to-A
EMX1+3A-to-G
EMX1+4G-to-C
EMX1+5G-to-A
EMX1+7C-to-T
HBB-K18Term
HBB-V7E
SITE3+2G-to-A
SITE3+4T-to-C
SITE3+5G-to-T
SITE3+6G-to-A
SITE3+8A-to-G
PGM3-S111L
PRNP+6G-to-A
RUNX1+1C-to-T
RUNX1+2G-to-A
RUNX1+3A-to-G
RUNX1+4T-to-C
RUNX1+6G-to-C
VEGFA+5G-to-T
Intended single base editing frequency after normalization
Undetermined site
Position of additional mutation in RTT (3' to 5')
C
P = 0.999
P = 0.203
P = 0.991
P = 3.6 × 10-5
P = 1.2× 10-6
P = 0.373
P =0.005
P = 2.7 × 10-8
P = 0.014
Intended single base editing frequency after normalization
Position of additional mutation in RTT (3' to 5')
(n = 39 48 39 39 39 36 30 36 36)
D
ACE2+5G-to-A
ACE2+6G-to-A
ACE2+7A-to-G
ACE2+8C-to-T
ACE2-A19S
ACTG1-T89I
CXCR4+5G-to-T
EMX1+1G-to-A
EMX1+3A-to-G
EMX1+5G-to-A
EMX1+7C-to-T
HBB-V7E
SITE3+2G-to-A
SITE3+4T-to-C
SITE3+5G-to-T
SITE3+6G-to-A
SITE3+8A-to-G
PRNP+6G-to-A
RUNX1+1C-to-T
RUNX1+2G-to-A
RUNX1+3A-to-G
RUNX1+4T-to-C
Intended single base editing frequency after normalization
Undetermined site
Position of additional mutation in RTT (3' to 5')

(cont'd)

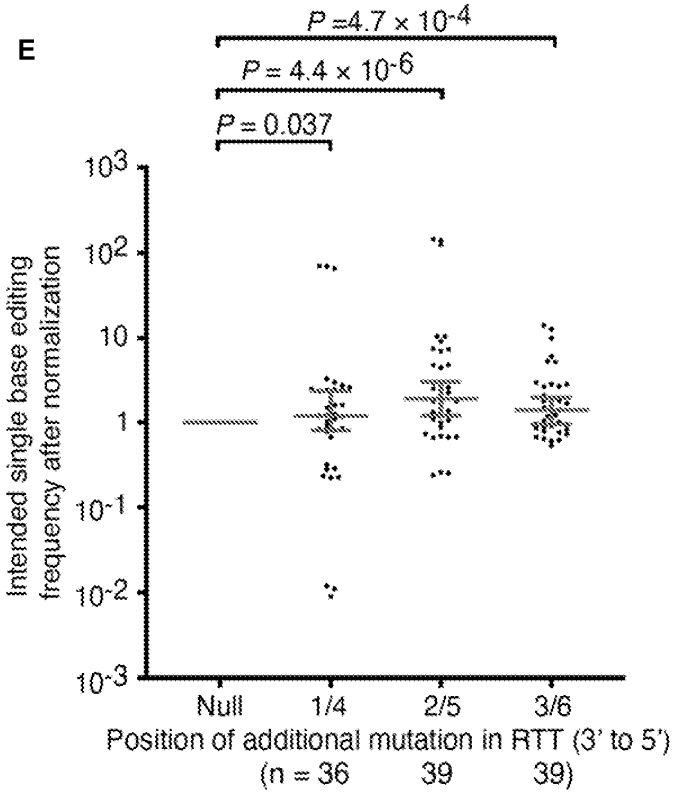

E
P =4.7 × 10-4
P = 4.4 × 10-6
P = 0.037
Intended single base editing frequency after normalization
10³
10²
10
1
10-1
10-2
10-3
Null
1/4
2/5
3/6
Position of additional mutation in RTT (3' to 5')
(n = 36 39 39)

FIG. 21