

FIG. 1A

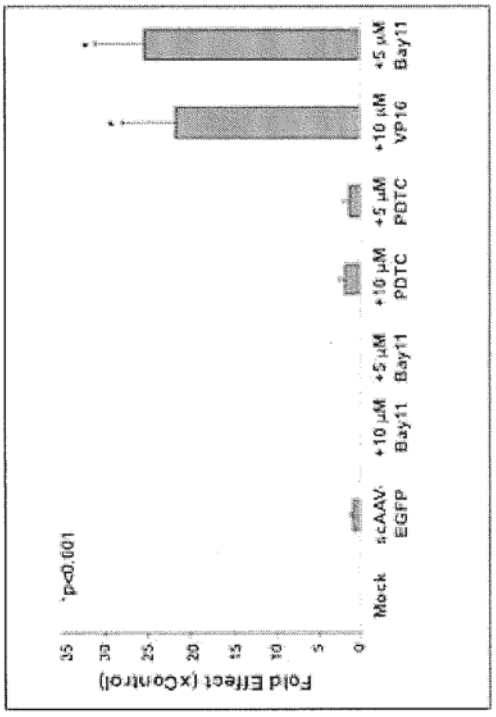


FIG. 1B

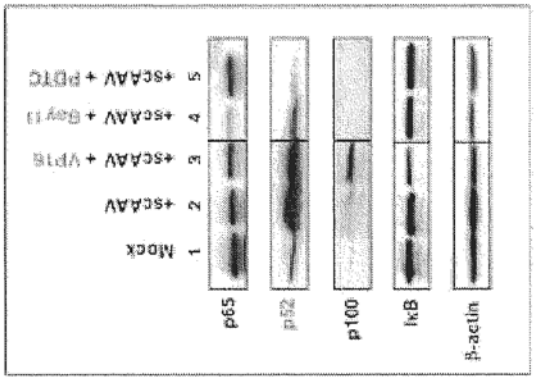


FIG. 1C

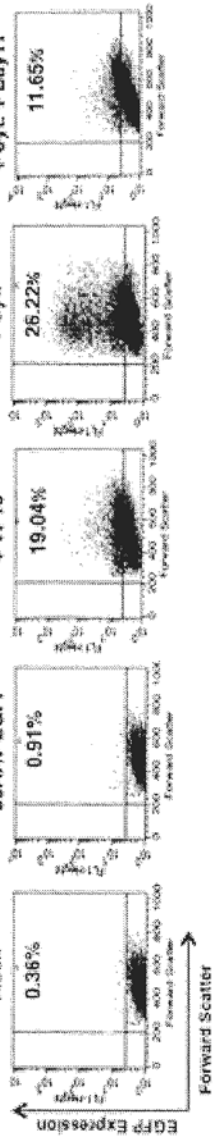

Mock
0.36%
scAAV-EGFP
0.91%
+ VP16
19.04%
+ Cyt.
26.22%
+ Cyt. + Bay11
11.65%
EGFP Expression
Forward Scatter

FIG. 2A

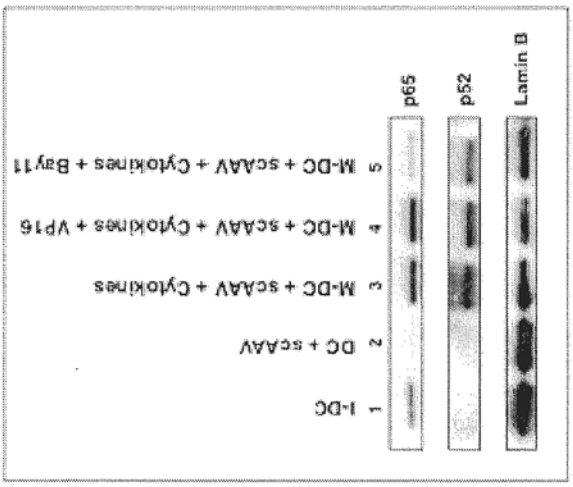

1 I-DC
2 DC + scAAV
3 M-DC + scAAV + Cytokines
4 M-DC + scAAV + Cytokines + VP16
5 M-DC + scAAV + Cytokines + Bay11
p65
p52
Lamin B

FIG. 2B

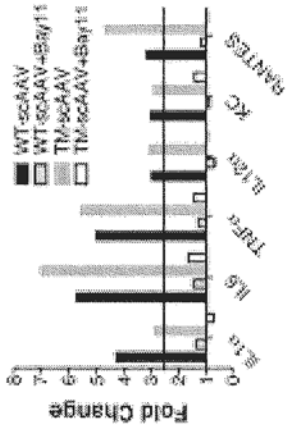

WT-scAAV
WT-scAAV+Bay11
TM-scAAV
TM-scAAV+Bay11
Fold change
0
1
2
3
4
5
6
7
8
IL1a
IL6
TNFa
IL10a
KC
CHANTES

FIG. 3A

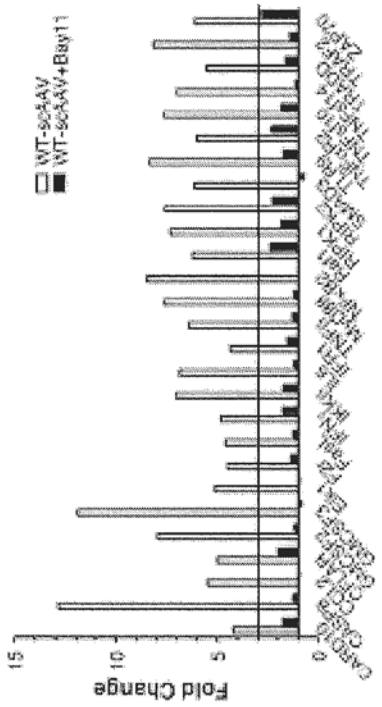

WT-scAAV
WT-scAAV+Bay11
Fold change
0
5
10
15

FIG. 3B

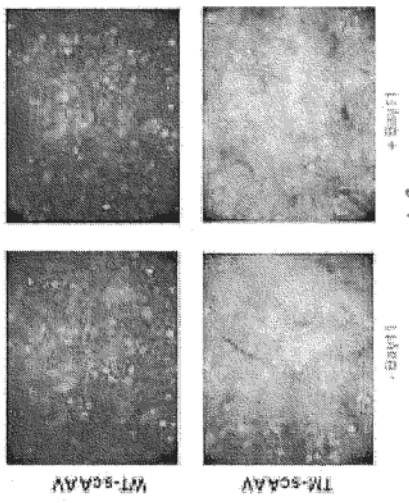

- Baf1
+ Baf1
TM-scAAV
WT-scAAV

FIG. 4A

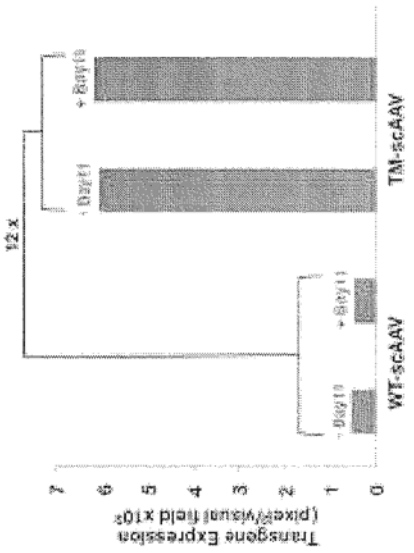
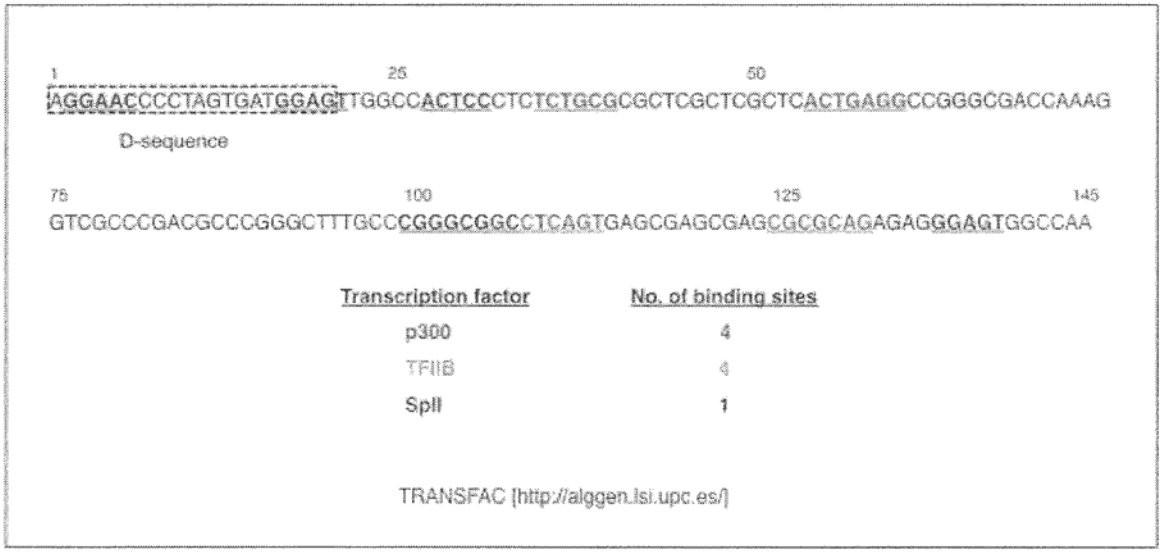

Transgene Expression
(pixel²/visual field x10⁵
0
1
2
3
4
5
6
7
- Baf1
+ Baf1
WT-scAAV
12 x
- Baf1
+ Baf1
TM-scAAV

FIG. 4B

FIG. 5



(SEQ ID NO: 22)

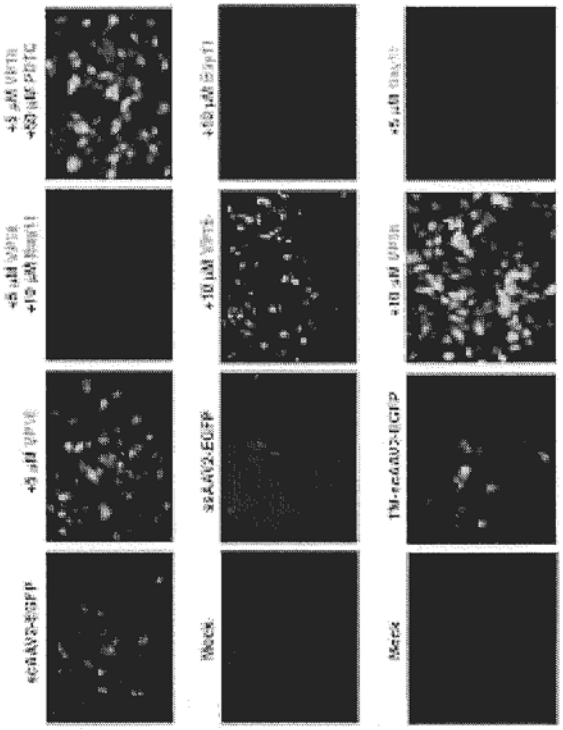


FIG. 6A

FIG. 6B

FIG. 6C

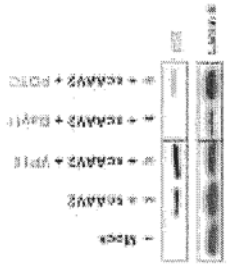


FIG. 6D FIG. 6E

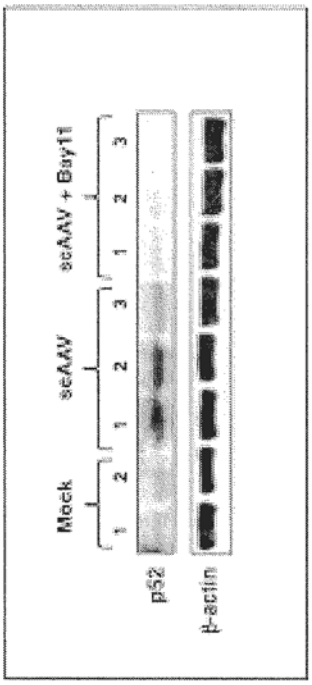


FIG. 7

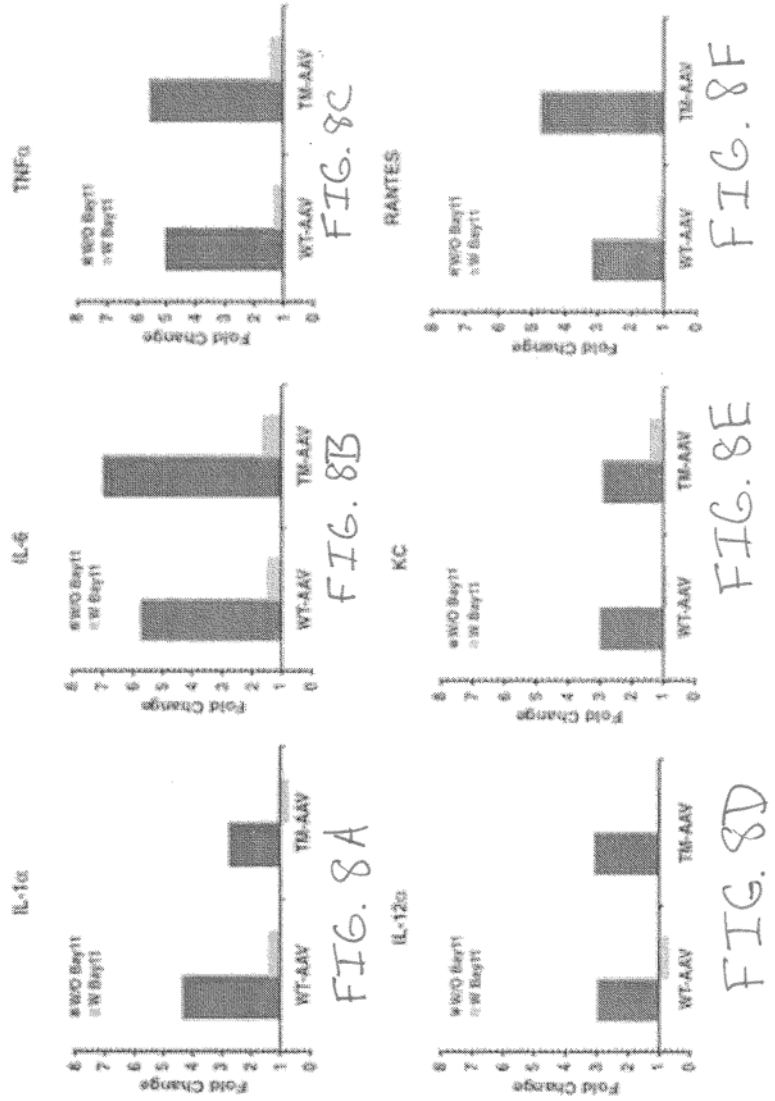


FIG. 8A

FIG. 8B

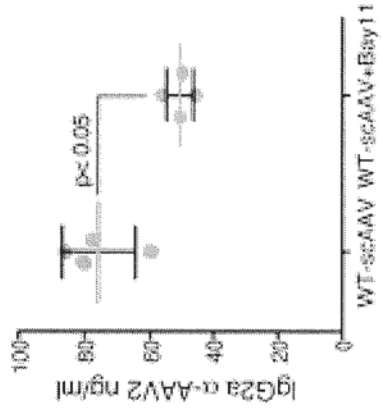
FIG. 8C

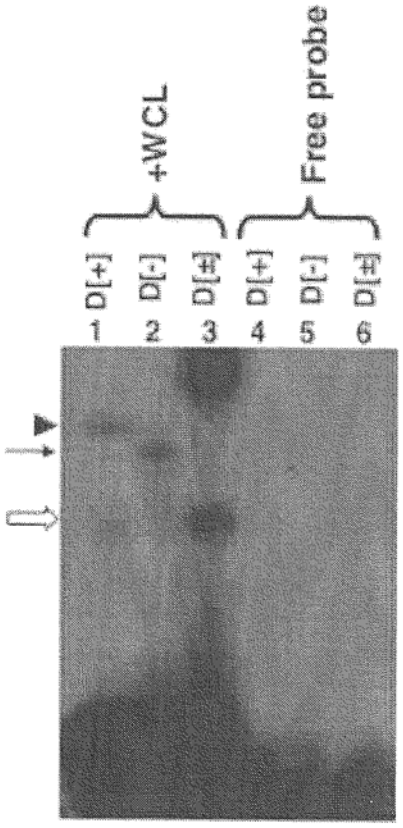
FIG. 8D

FIG. 8E

FIG. 8F

FIG. 9


IgG2a α-AAV2 ng/ml
100
80
60
40
20
0
p< 0.05
WT-scAAV
WT-scAAV+Bspy11



Strand	Sequence	
ssD[-]:	5'-CTC CA TCACTA GGGGT TCCT-3'	(SEQ ID NO: 23)
dsD[±]:	5'-CTC CA TCACTA GGGGT TCCT-3' 3'-GAGG TAGT GATCC CC AAGGA-5'	(SEQ ID NO: 24)
ssD[+]:	5'-GAGGTA GTGAT CC CCAAGGA-3'	(SEQ ID NO: 25)

FIG. 10

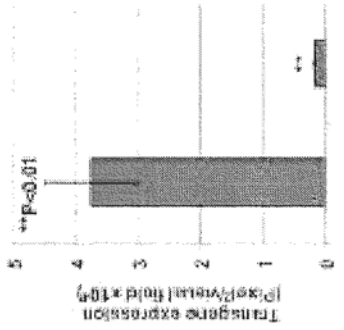


FIG. 11B

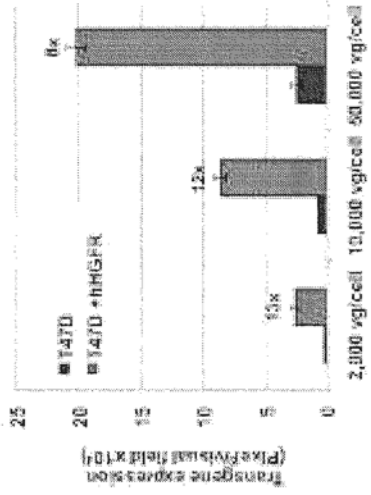
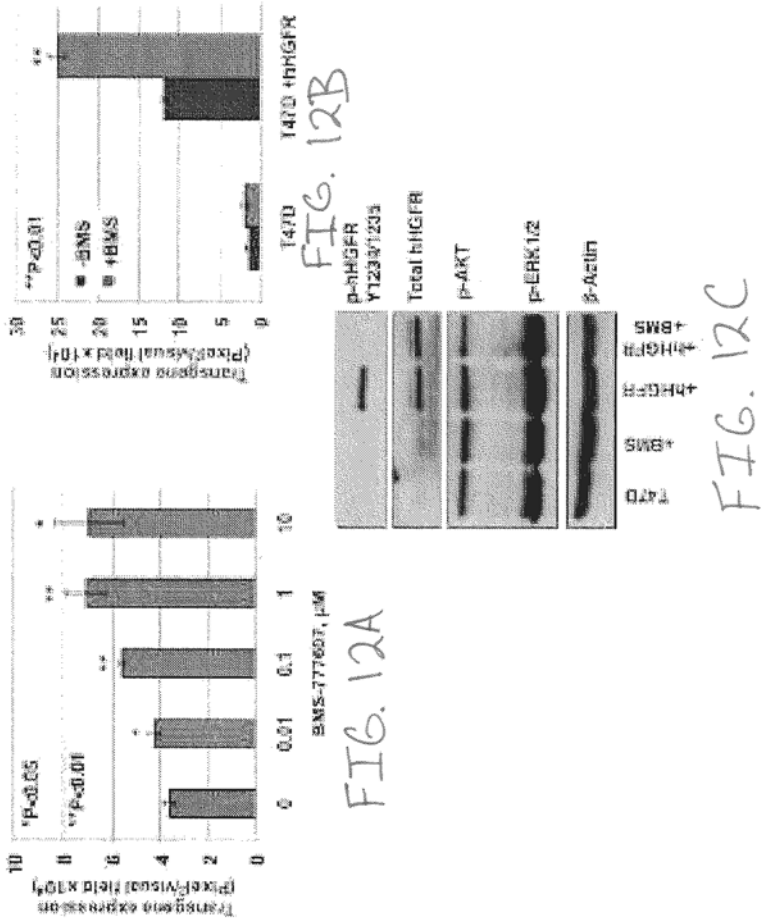


FIG. 11A


Transgene expression
(Pixel+/Visual field x 10⁴)
*P<0.05
**P<0.01
0
0.01
0.1
1
10
BMS-777607, μM
FIG. 12A
Transgene expression
(Pixel+/Visual field x 10⁴)
**P<0.01
-BMS
+BMS
T47D
T47D +hHGFR
FIG. 12B
p-hHGFR
Y1234/1235
Total hHGFR
p-AKT
p-ERK1/2
β-Actin
T47D
+BMS
+hHGFR
+hHGFR
+BMS
FIG. 12C

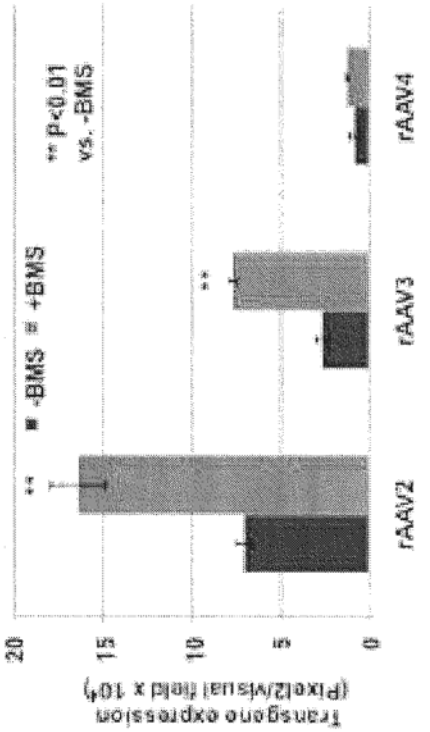


FIG. 13A

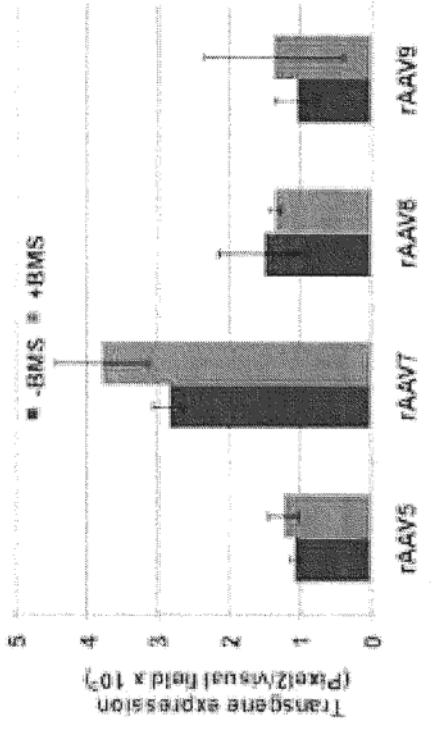


FIG. 13B

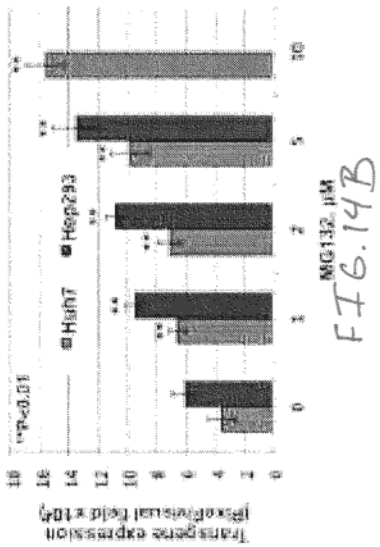


FIG. 14A

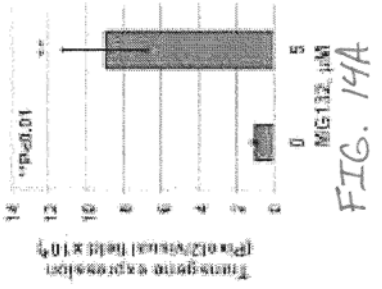


FIG. 14B

FIG. 14C

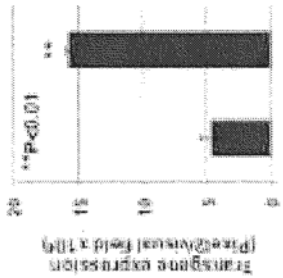


FIG. 14D

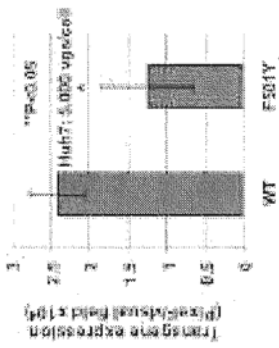


FIG. 15A

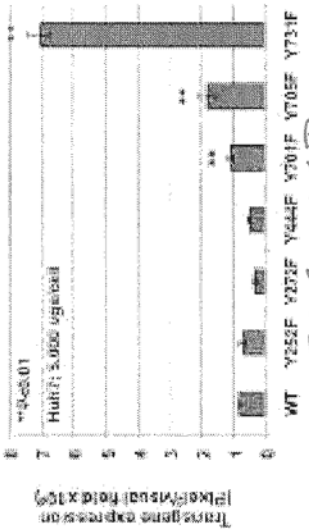


FIG. 15B

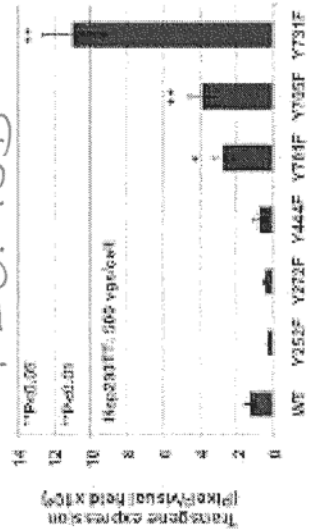


FIG. 15C

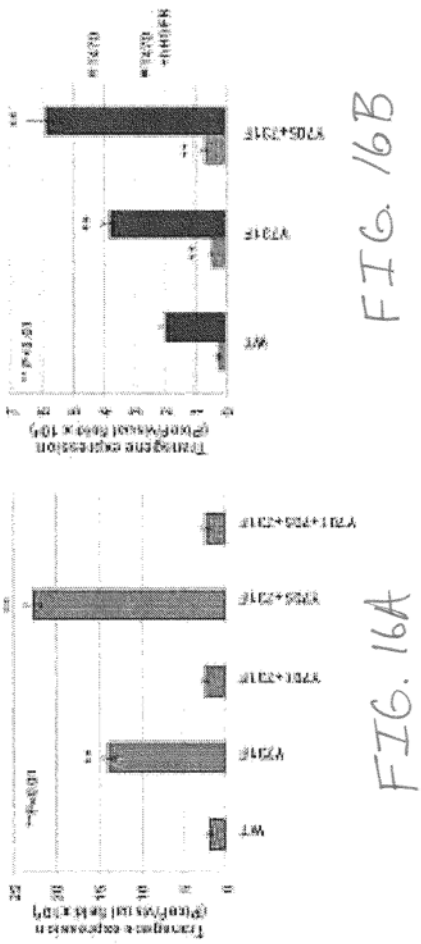


FIG. 16A

FIG. 16B

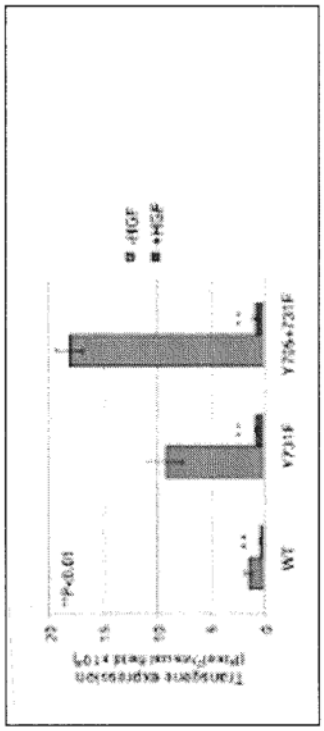


FIG. 16C

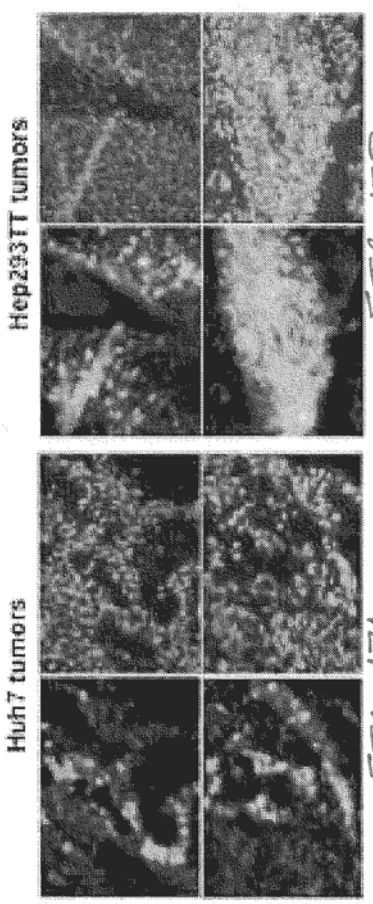


FIG. 17A

FIG. 17B

FIG. 17C

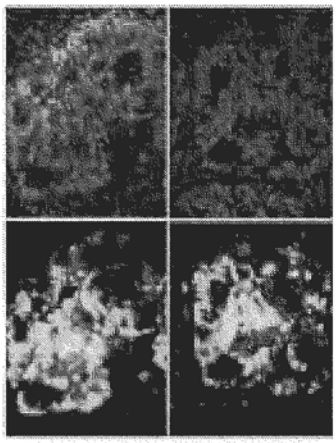


FIG. 17D

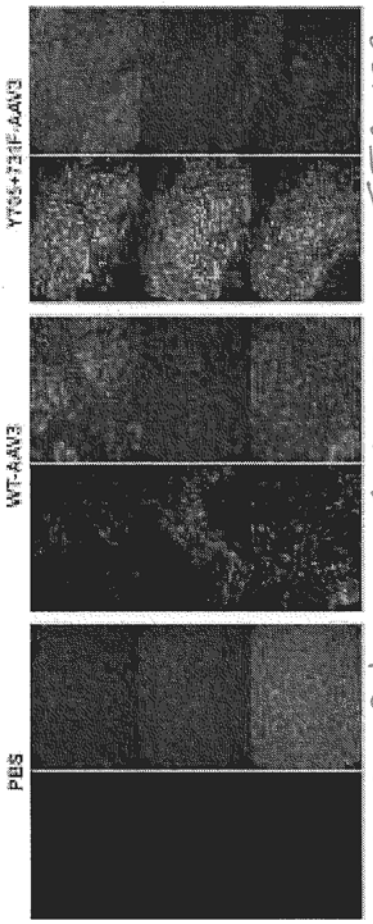


FIG. 18A FIG. 18B FIG. 18C

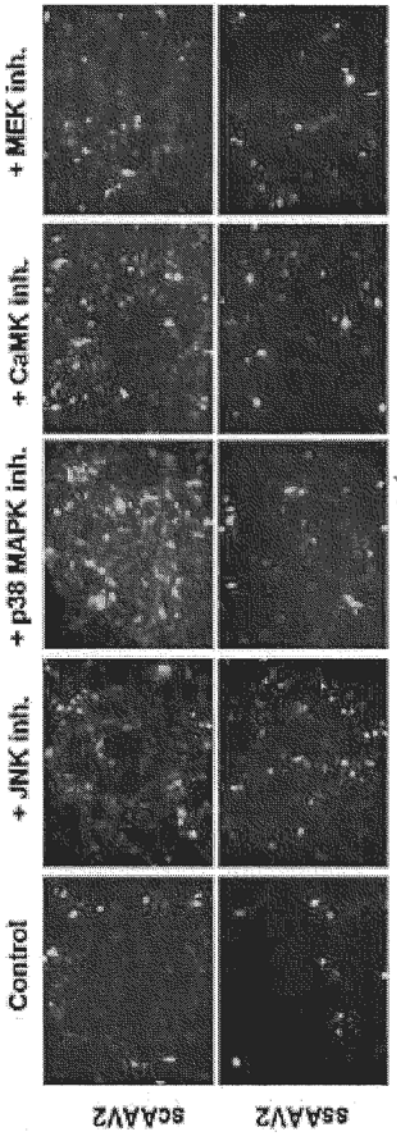


FIG. 19A

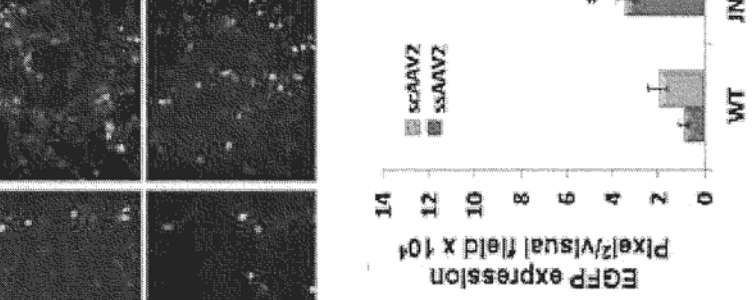


FIG. 19B

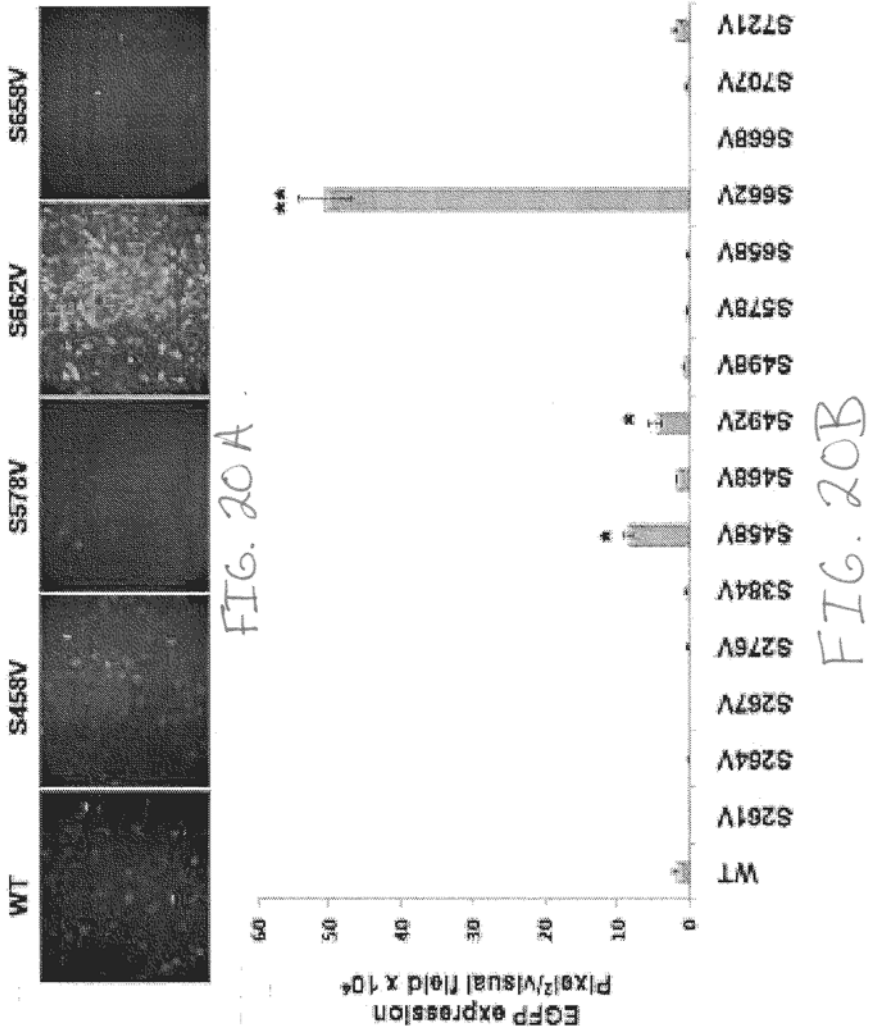


FIG. 20A

FIG. 20B

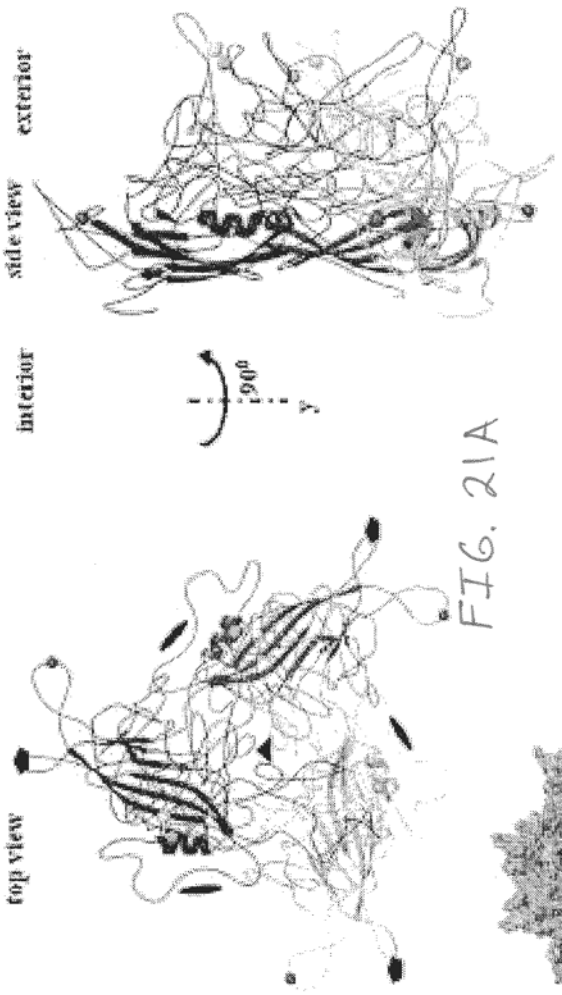


FIG. 21A

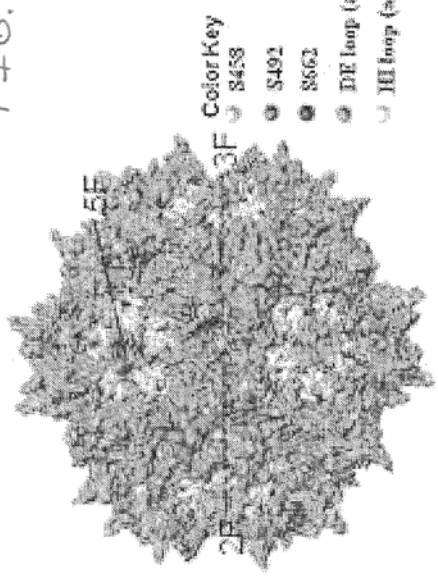


FIG. 21B

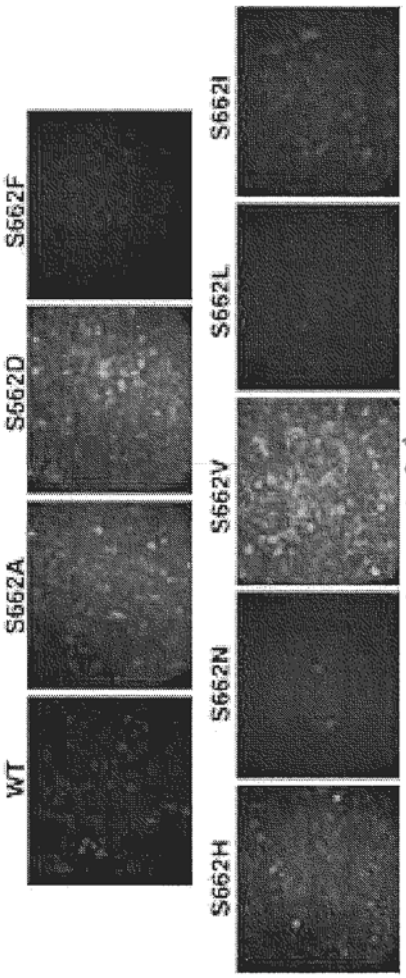


FIG. 22A

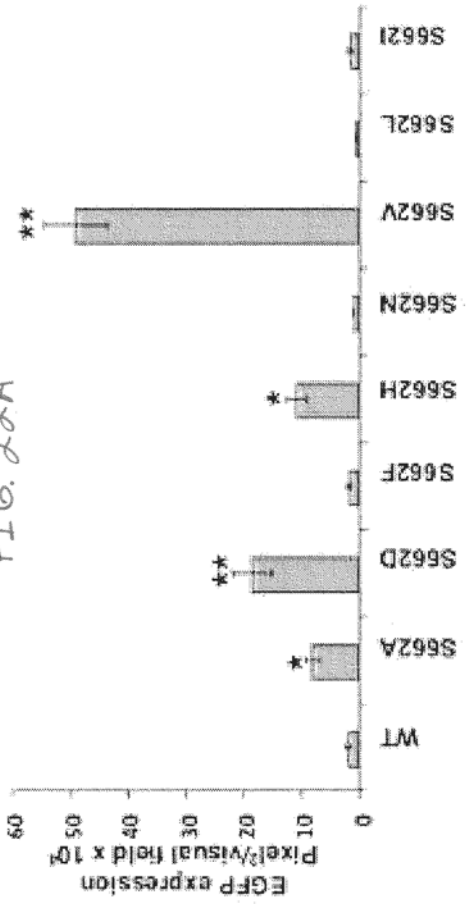


FIG. 22B

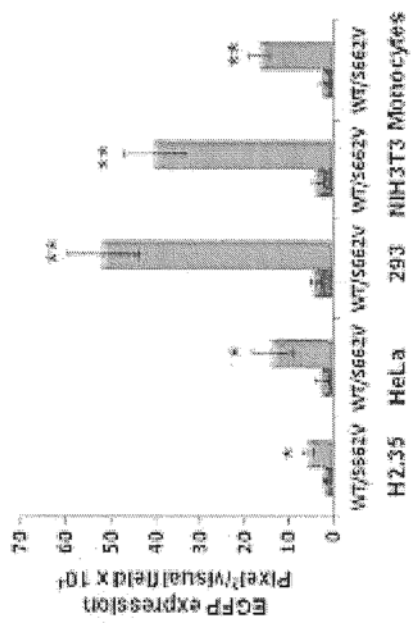


FIG. 23A

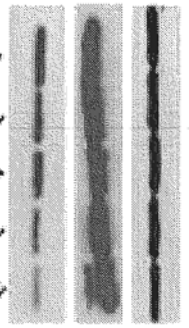


FIG. 23B

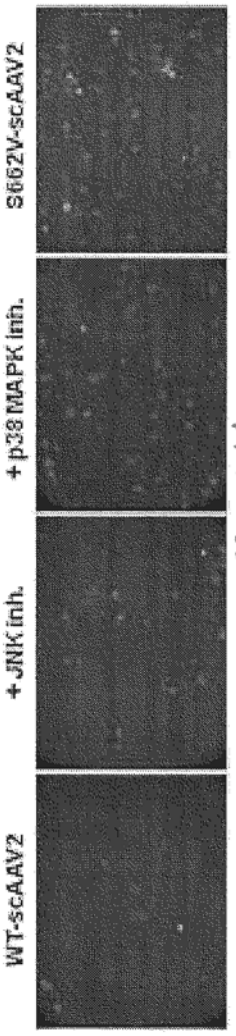


FIG. 24A

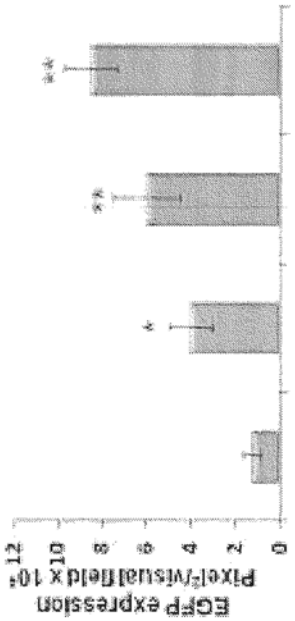


FIG. 24B

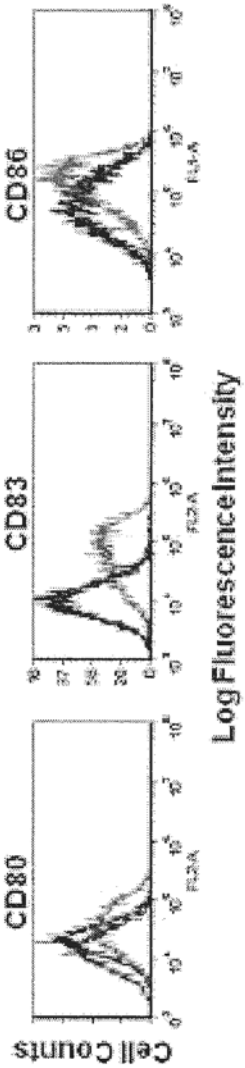


FIG. 24C

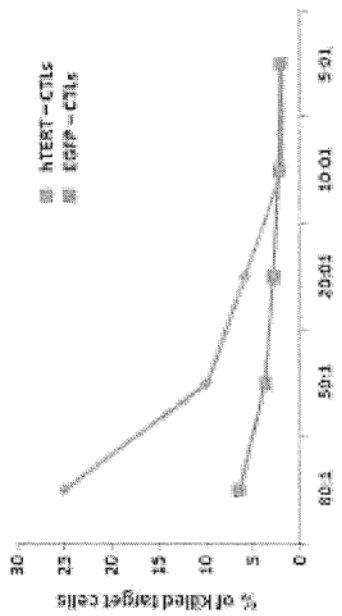

hTERT-CTLs
EGFP-CTLs
% of killed target cells
30
25
20
15
10
5
0
80:1
50:1
20:01
10:01
5:01
Effector/Target Cells Ratio

FIG. 25

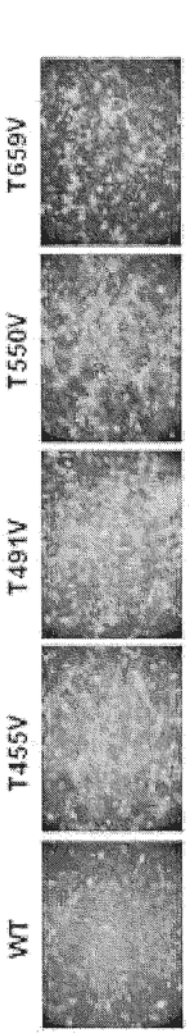


FIG. 26A

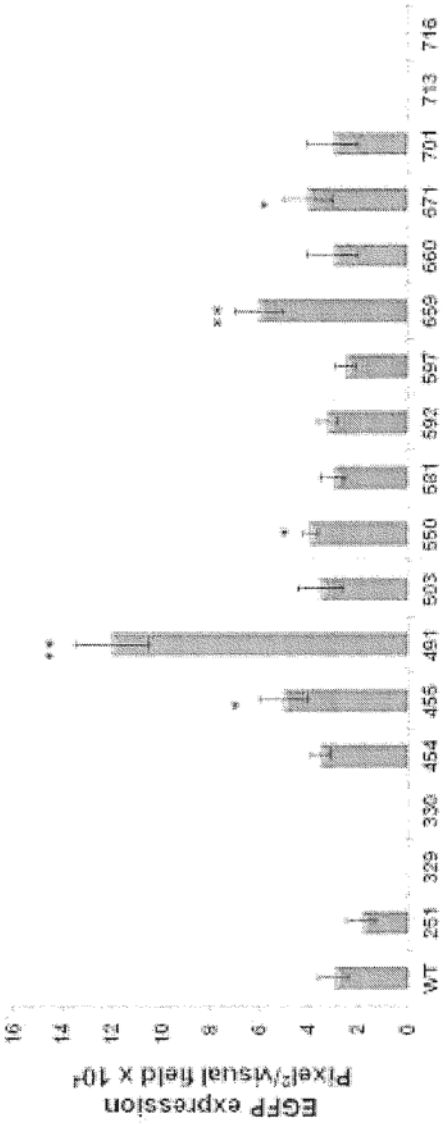


FIG. 26B

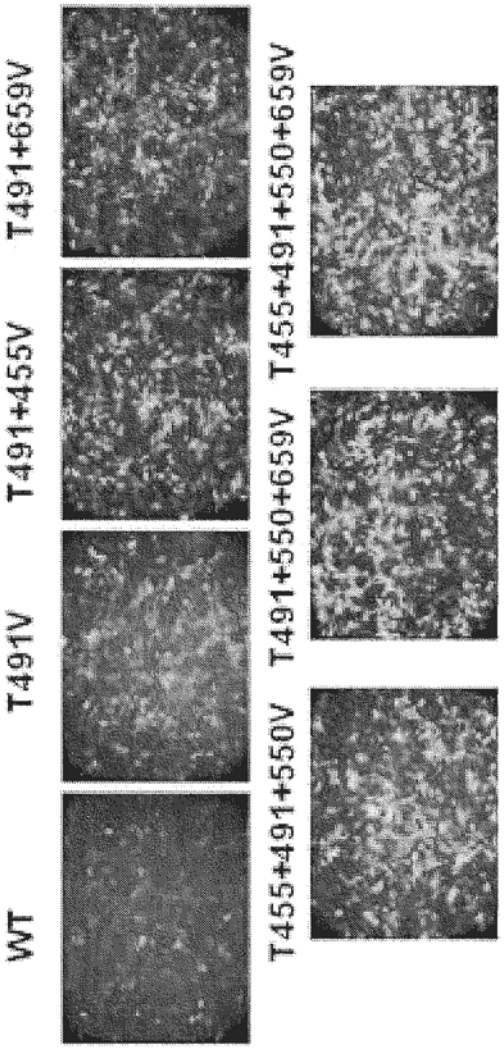


FIG. 27A

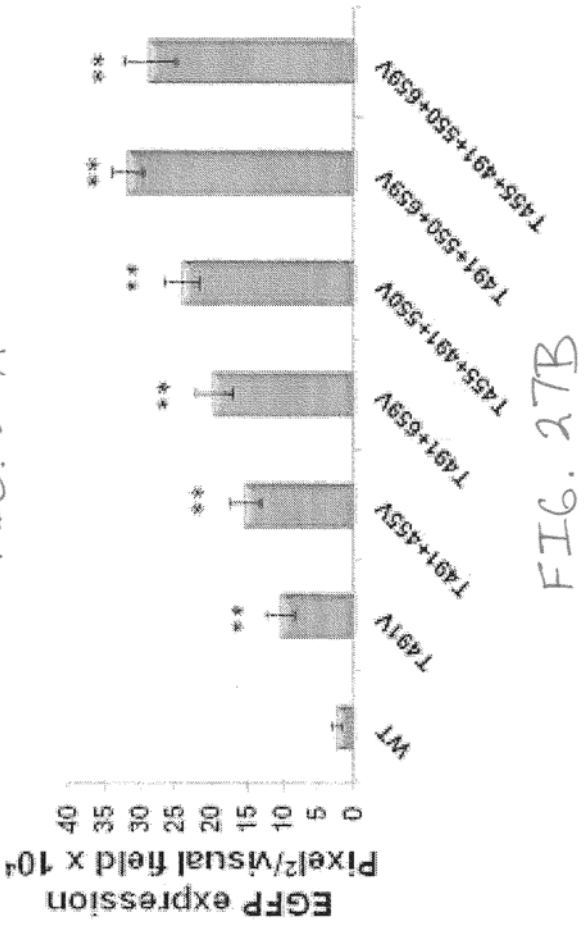


FIG. 27B

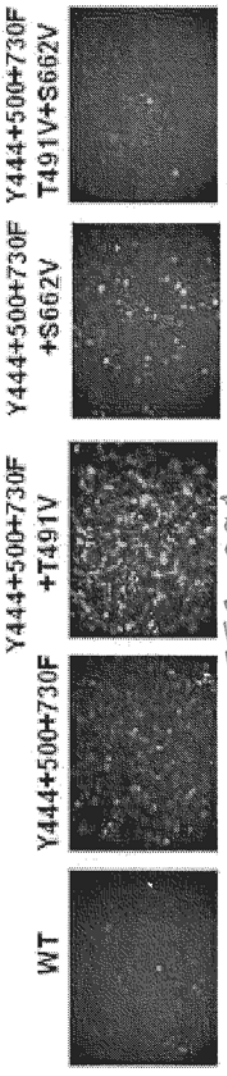


FIG. 28A

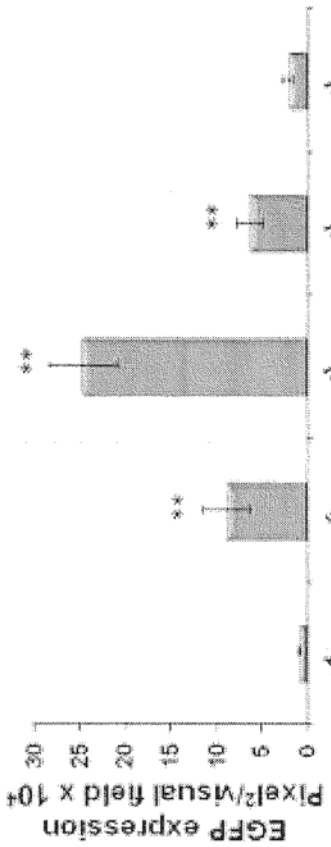


FIG. 28B

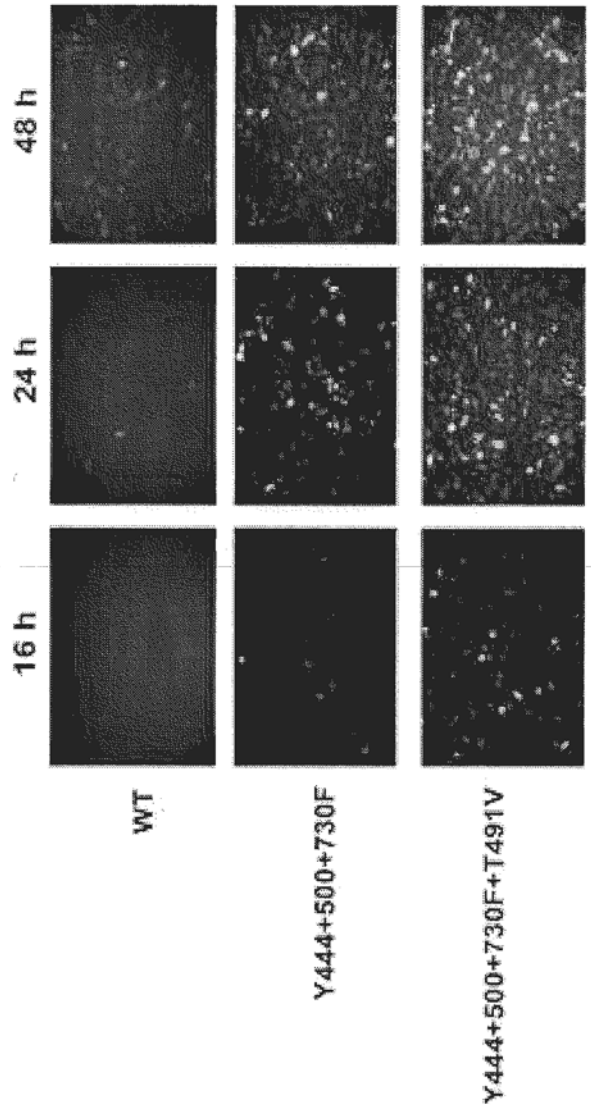


FIG. 29A

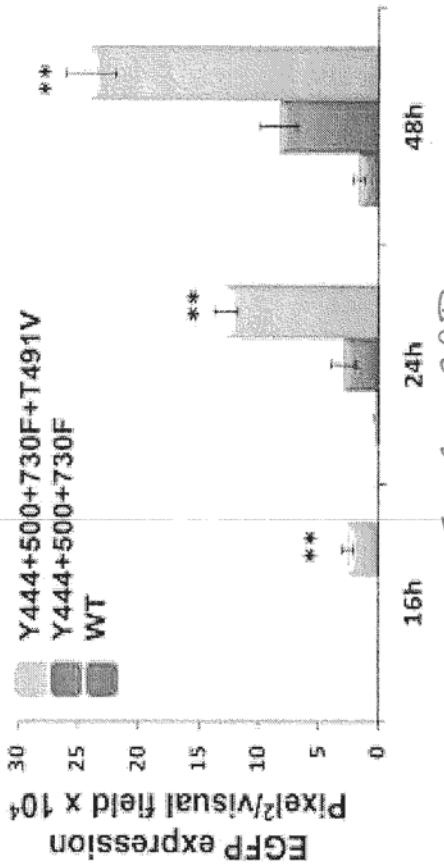


FIG. 29B

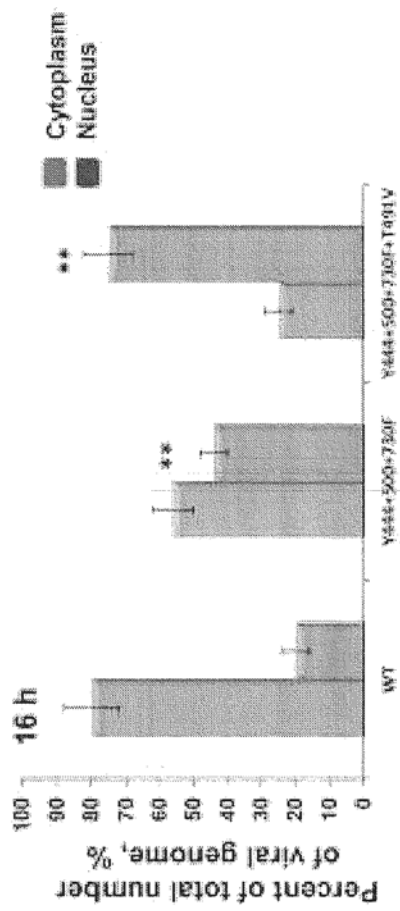


FIG. 30A

FIG. 30B

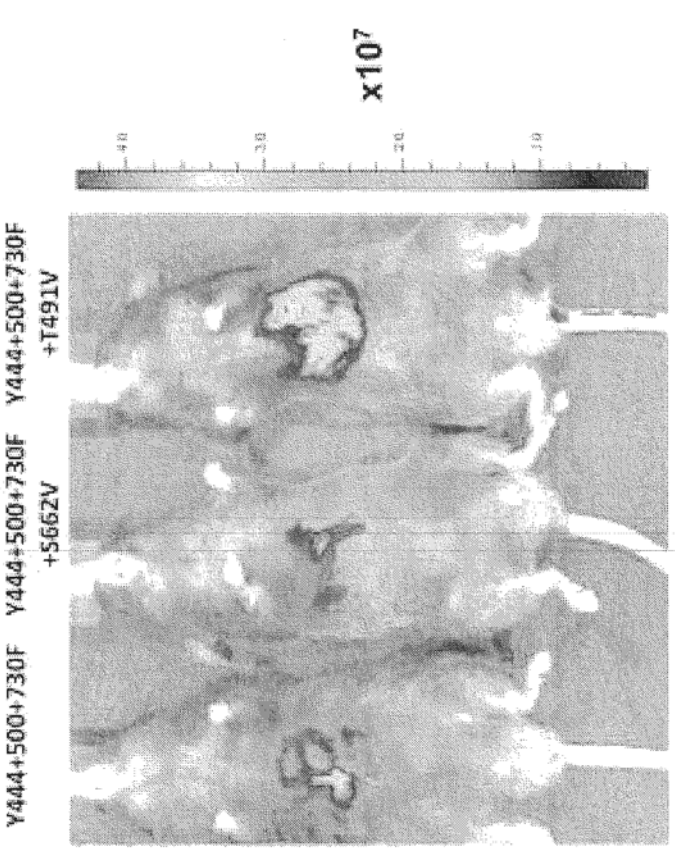


FIG. 31A

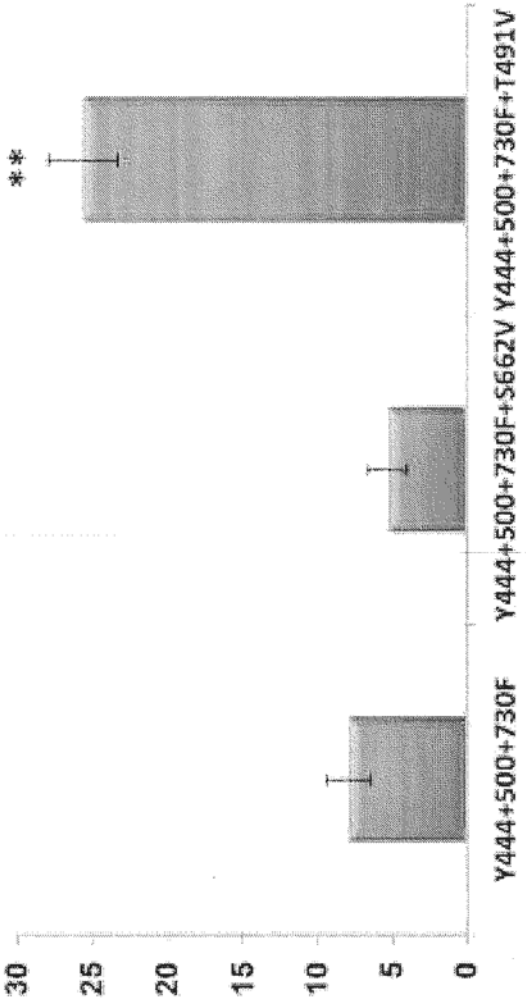


FIG. 31B

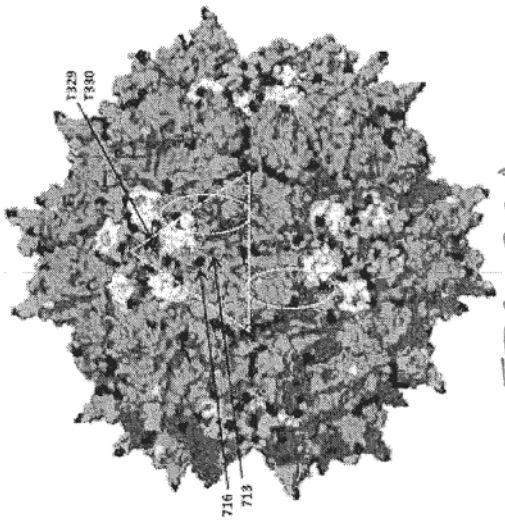


FIG. 32A

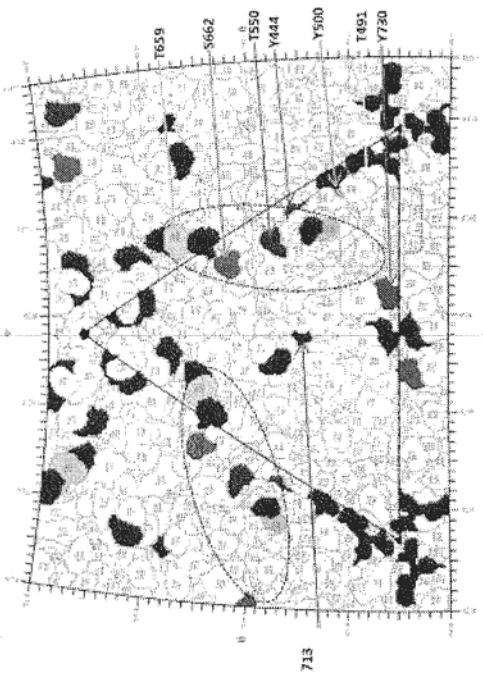
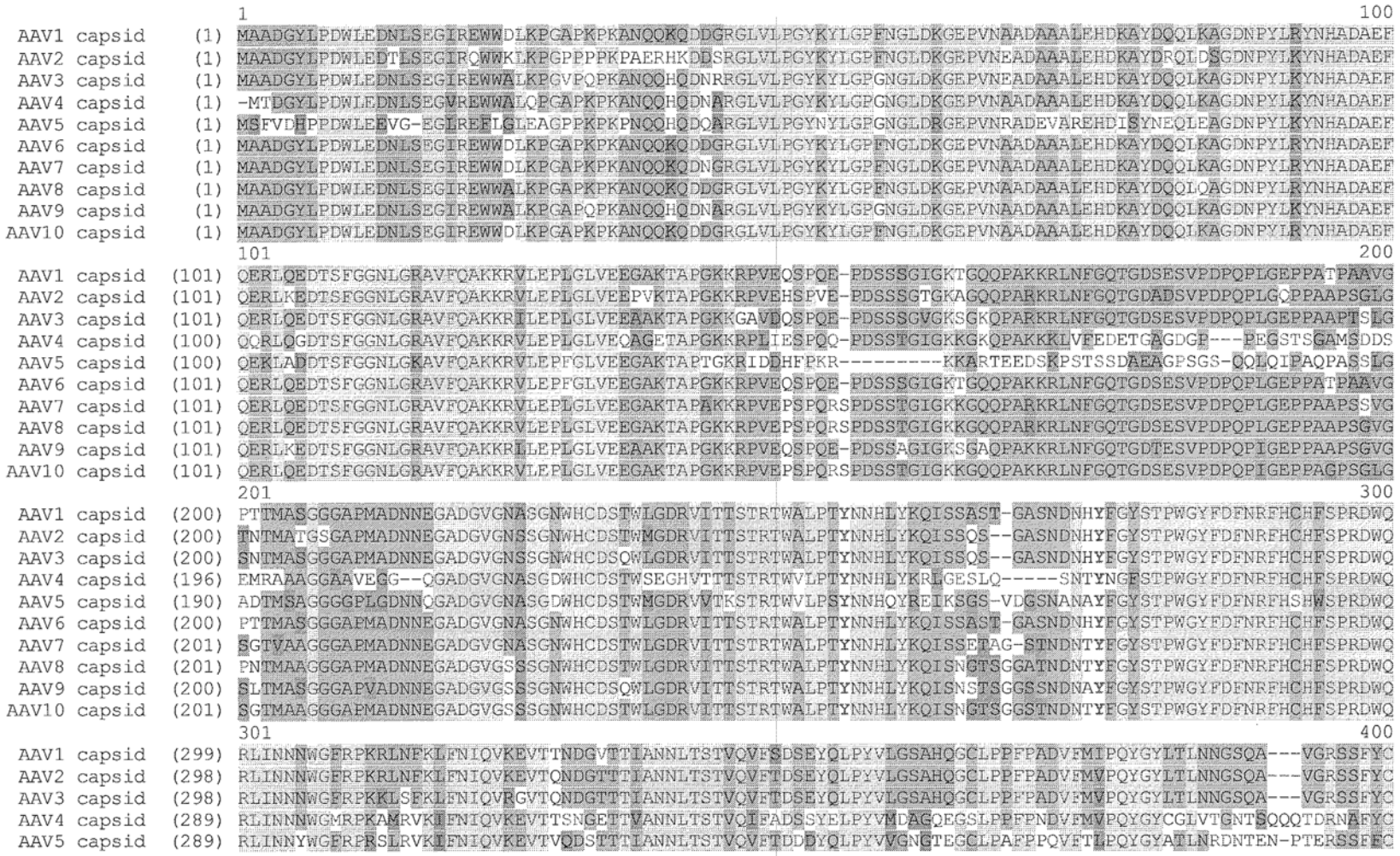


FIG. 32B

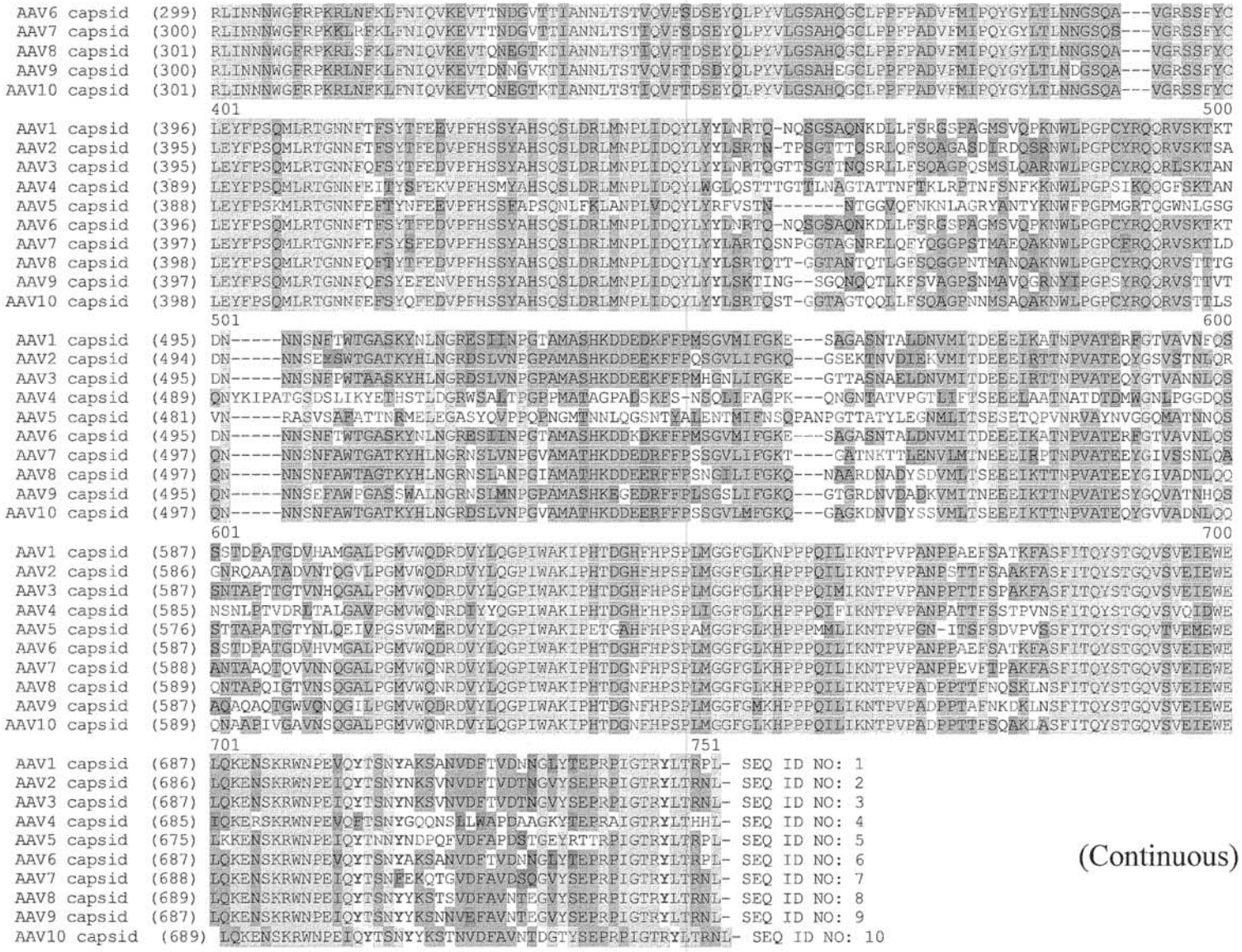
FIG. 33A

Amino acid alignment in AAV serotype capsids



(Continuous)

FIG. 33A (continued)



(Continuous)

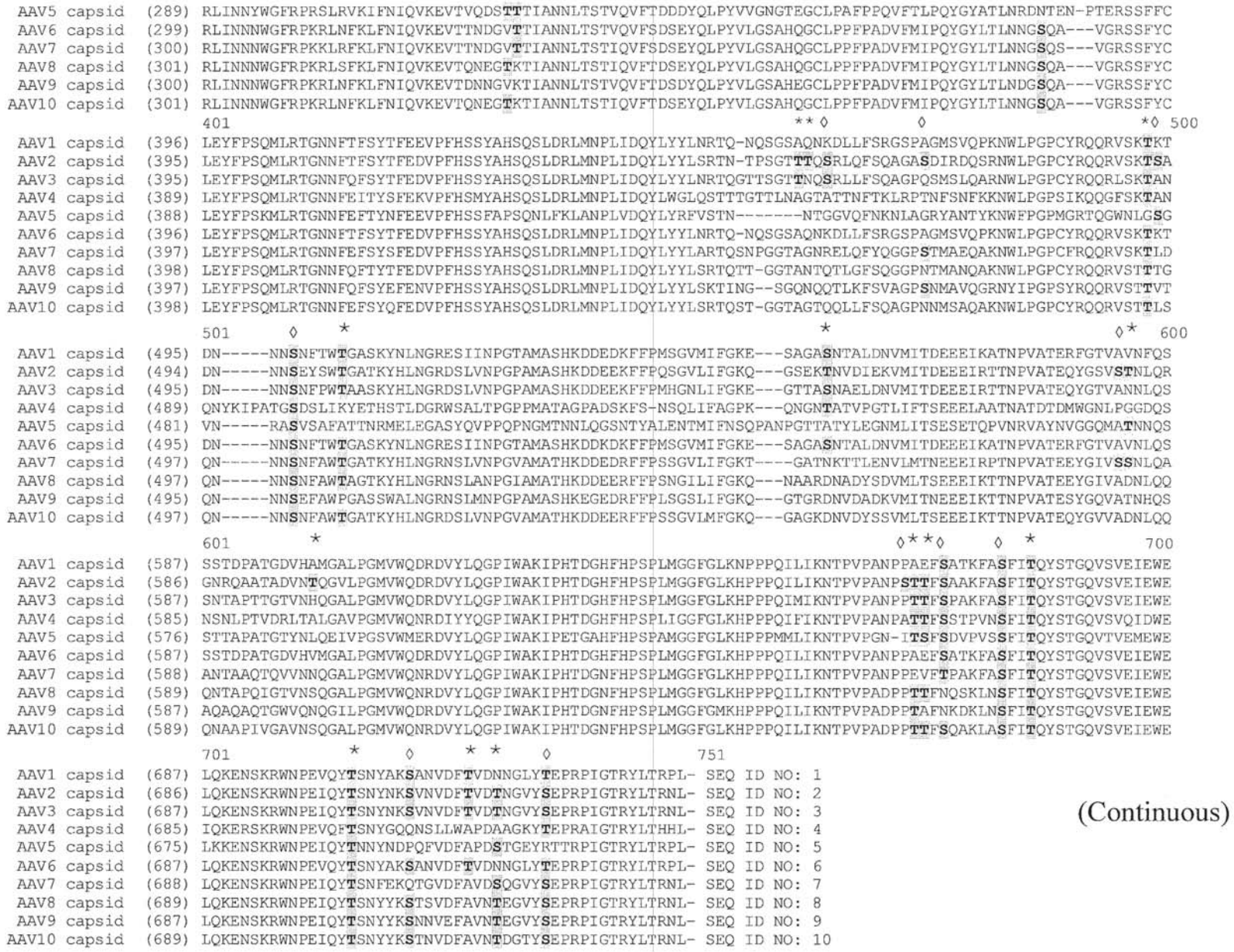
FIG. 33B

Serine and Threonine residue alignment in AAV serotype capsids



(Continuous)

FIG. 33B (Continued)



(Continuous)

FIG. 33C

Tyrosine residue alignment in AAV serotype capsids

```
                    1                                                                                                   100
AAV1 capsid     (1) MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEF
AAV2 capsid     (1) MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF
AAV3 capsid     (1) MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGYKYLGPGNGLDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
AAV4 capsid     (1) -MTDGYLPDWLEDNLSEGVREWWALQPGAPKPKANQQHQDNARGLVLPGYKYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
AAV5 capsid     (1) MSFVDHPPDWLEEVG-EGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEF
AAV6 capsid     (1) MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEF
AAV7 capsid     (1) MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEF
AAV8 capsid     (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEF
AAV9 capsid     (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
AAV10 capsid    (1) MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEF
                    101                                                                                                 200
AAV1 capsid   (101) QERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEQSPQE-PDSSSGIGKTGQQPAKKRLNFGQTGDSESVPDPQPLGEPPATPAAVG
AAV2 capsid   (101) QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVE-PDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLG
AAV3 capsid   (101) QERLQEDTSFGGNLGRAVFQAKKRILEPLGLVEEAAKTAPGKKGAVDQSPQE-PDSSSGVGKSGKQPARKRLNFGQTGDSESVPDPQPLGEPPAAPTSLG
AAV4 capsid   (100) QQRLQGDTSFGGNLGRAVFQAKKRVLEPLGLVEQAGETAPGKKRPLIESPQQ-PDSSTGIGKKGKQPAKKKLVFEDETGAGDGP---PEGSTSGAMSDDS
AAV5 capsid   (100) QEKLADDTSFGGNLGKAVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKR---------KKARTEEDSKPSTSSDAEAGPSGS-QQLQIPAQPASSLG
AAV6 capsid   (101) QERLQEDTSFGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQE-PDSSSGIGKTGQQPAKKRLNFGQTGDSESVPDPQPLGEPPATPAAVG
AAV7 capsid   (101) QERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPAKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVPDPQPLGEPPAAPSSVG
AAV8 capsid   (101) QERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVPDPQPLGEPPAAPSGVG
AAV9 capsid   (101) QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQE-PDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVG
AAV10 capsid  (101) QERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRLNFGQTGDSESVPDPQPIGEPPAGPSGLG
                    201                                  AAV2/252                  AAV2/272                       300
AAV1 capsid   (200) PTTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSAST-GASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQ
AAV2 capsid   (200) TNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQS--GASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQ
AAV3 capsid   (200) SNTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQS--GASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQ
AAV4 capsid   (196) EMRAAAGGAAVEGG--QGADGVGNASGDWHCDSTWSEGHVTTTSTRTWVLPTYNNHLYKRLGESLQ-----SNTYNGFSTPWGYFDFNRFHCHFSPRDWQ
AAV5 capsid   (190) ADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSGS-VDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQ
AAV6 capsid   (200) PTTMASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSAST-GASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQ
AAV7 capsid   (201) SGTVAAGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSETAG-STNDNTYFGYSTPWGYFDFNRFHCHFSPRDWQ
AAV8 capsid   (201) PNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDFNRFHCHFSPRDWQ
AAV9 capsid   (200) SLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQ
AAV10 capsid  (201) SGTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRFHCHFSPRDWQ
                    301                                                                                                 400
AAV1 capsid   (299) RLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQA---VGRSSFYC
AAV2 capsid   (298) RLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQA---VGRSSFYC
AAV3 capsid   (298) RLINNNWGFRPKKLSFKLFNIQVRGVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQA---VGRSSFYC
AAV4 capsid   (289) RLINNNWGMRPKAMRVKIFNIQVKEVTTSNGETTVANNLTSTVQIFADSSYELPYVMDAGQEGSLPPFPNDVFMVPQYGYCGLVTGNTSQQQTDRNAFYC
AAV5 capsid   (289) RLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTEN-PTERSSFFC
```

(Continuous)

FIG. 33C (Continued)



(Continuous)

Mutant	Packaging efficiency	Transduction efficiency
AAV2-S261V	~10-fold lower	-*
AAV2-S264V	No change	-*
AAV2-S267V	No DNAse I-resistant particles	-*
AAV2-S276V	~10-fold lower	-*
AAV2-S384V	~3-fold higher	~10-fold lower
AAV2-S458V	No change	~4-fold higher
AAV2-S468V	~5-fold higher	No change
AAV2-S492V	No change	~2-fold higher
AAV2-S498V	No change	~10-fold lower
AAV2-S578V	No change	~10-fold lower
AAV2-S658V	~10-fold lower	-*
AAV2-S662V	No change	~20-fold higher
AAV2-S668V	No DNAse I-resistant particles	-*
AAV2-S707V	No change	~10-fold lower
AAV2-S721V	No change	No change

Vector packaging and infectivity assays were performed at least twice for each of the mutant-AAV vectors. The packaging efficiency was determined by quantitative PCR analyses. The transduction efficiency was estimated by fluorescence intensity of HEK293 cells infected with an MOI of 1000 vgs/cell.

* No fluorescence was detected at the MOI tested.

FIG. 34

Mutant	Packaging efficiency	Transduction efficiency
AAV2-S662V	No change	~20-fold higher
AAV2-S662A	~5-fold higher	~3-fold higher
AAV2-S662D	No change	~8-fold higher
AAV2-S662F	~10-fold lower	No change
AAV2-S662H	No change	~4-fold higher
AAV2-S662N	~10-fold lower	No change
AAV2-S662L	~10-fold lower	No change
AAV2-S662I	~10-fold lower	No change

The packaging and infectivity assays were performed as described under the legend to Table 1.

V: valine; A: alanine; D: aspartic acid; F: phenylalanine; H: histidine; N: asparagine; L: leucine; and I: isoleucine.

FIG. 35

FIG. 36A

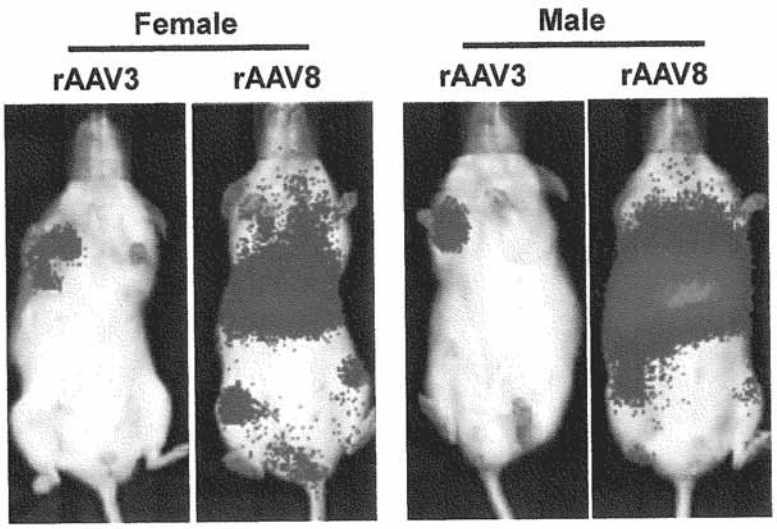


FIG. 36B

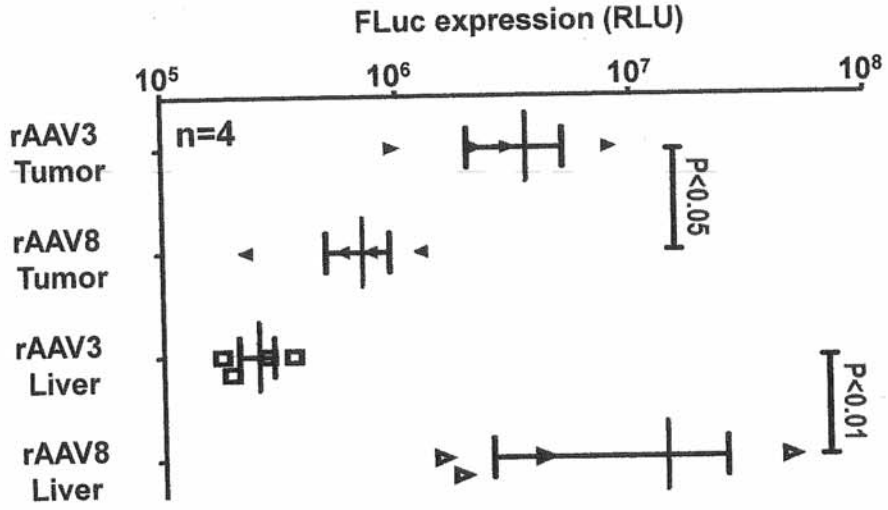


FIG. 36C

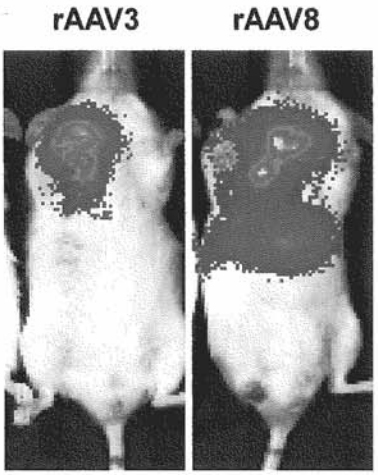


FIG. 36D

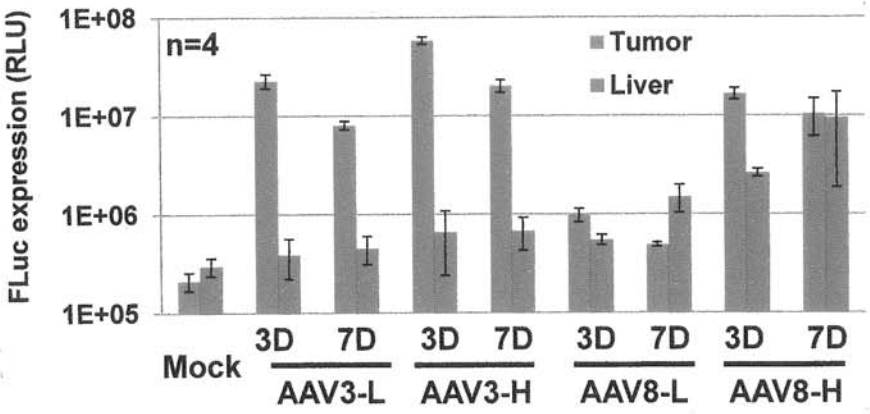


FIG. 36E

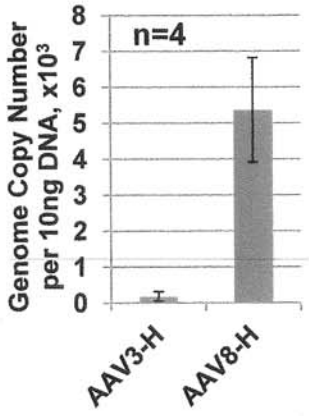


FIG. 37A

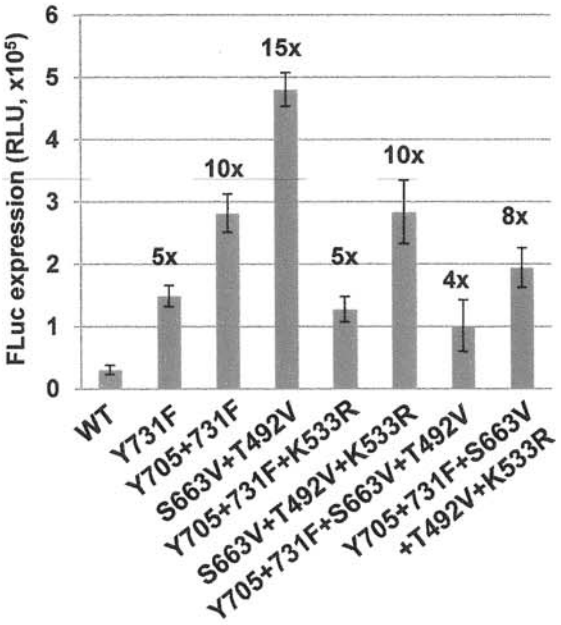
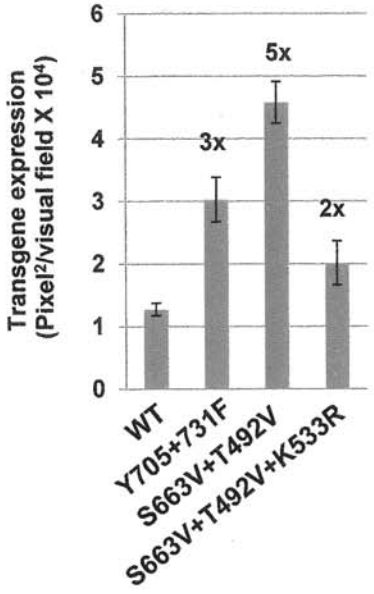


FIG. 37B



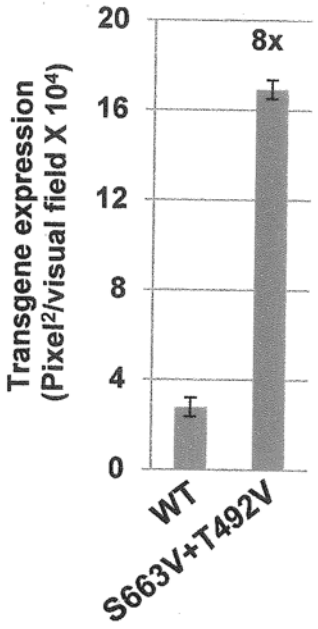


FIG. 37C

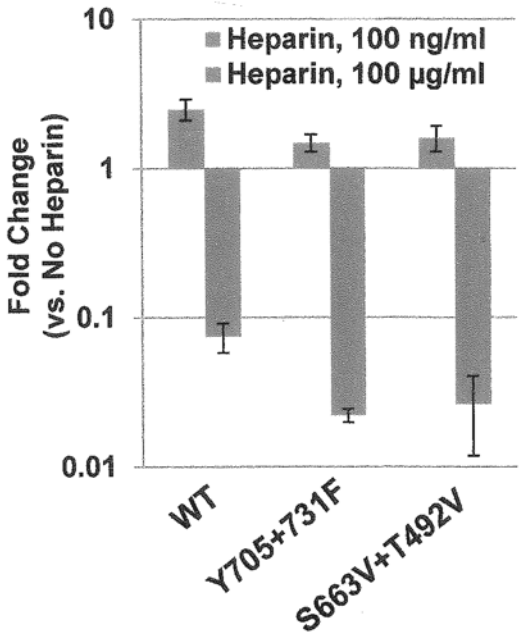


FIG. 37D

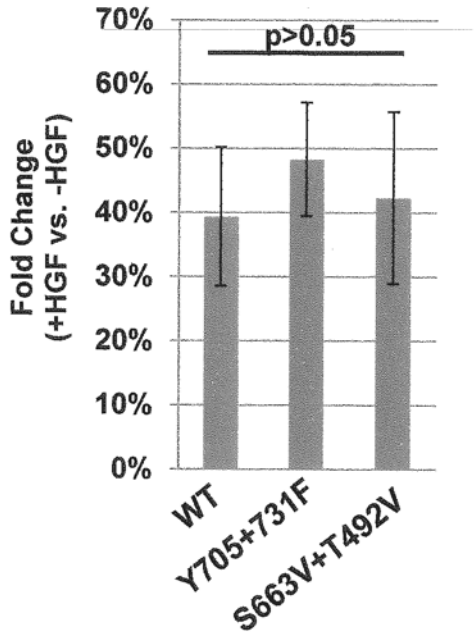


FIG. 37E

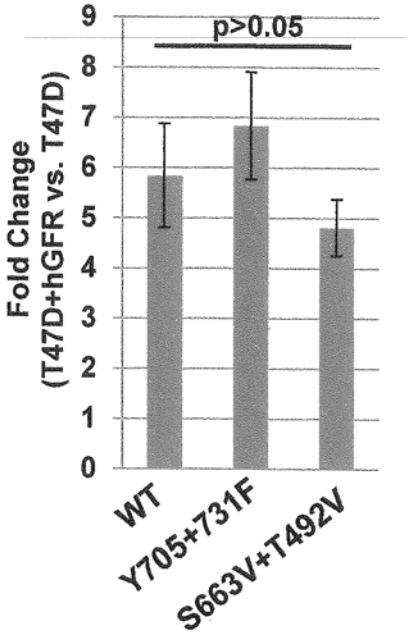


FIG. 37F

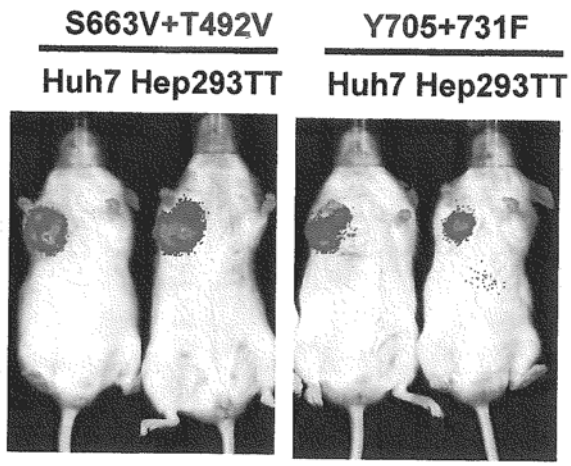


FIG. 38A

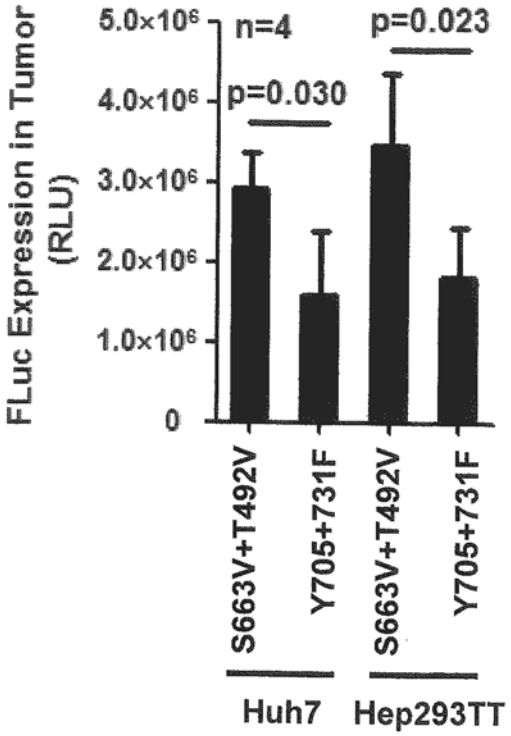


FIG. 38B

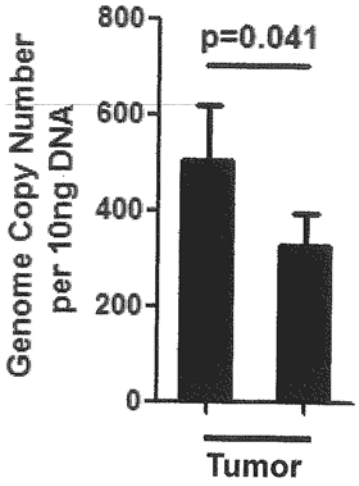


FIG. 38C

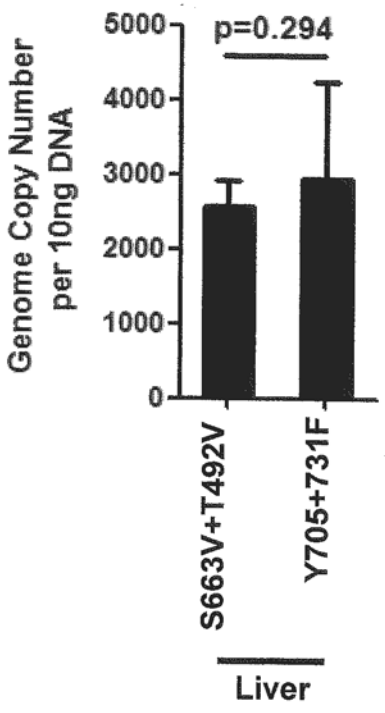
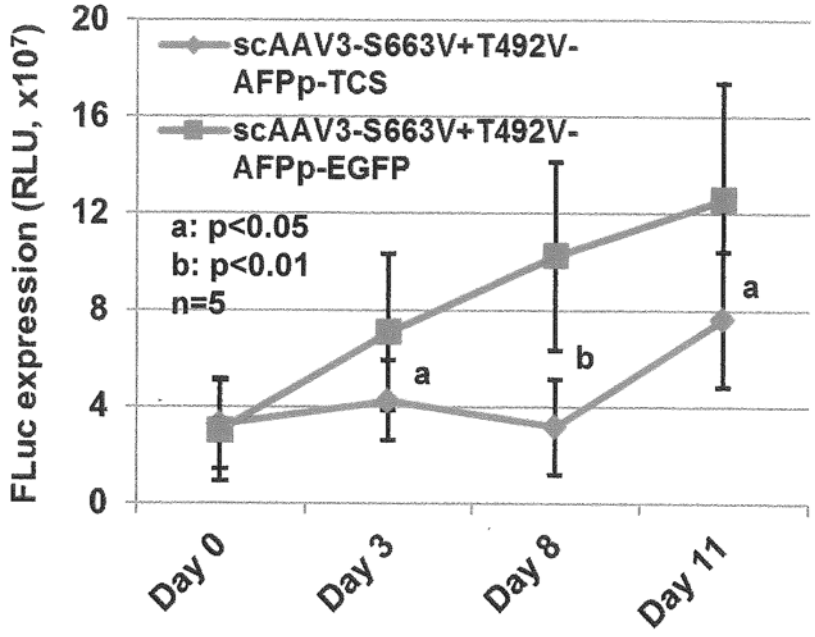


FIG. 38D

FIG. 39A



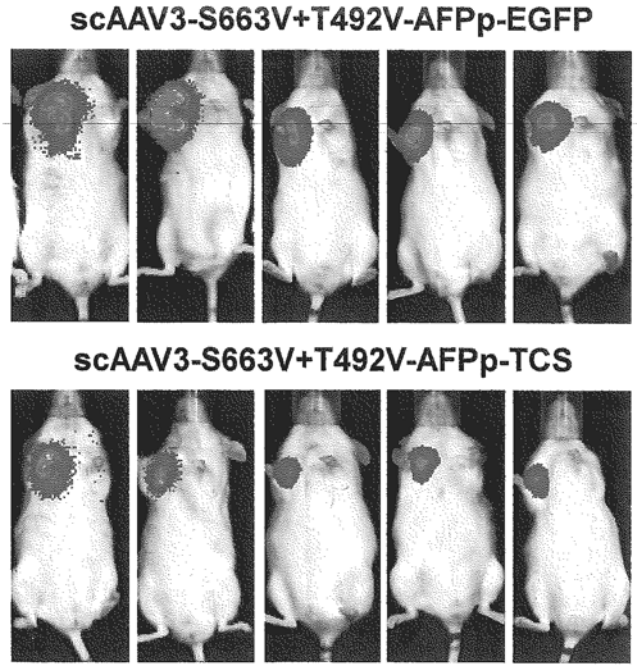


FIG. 39B

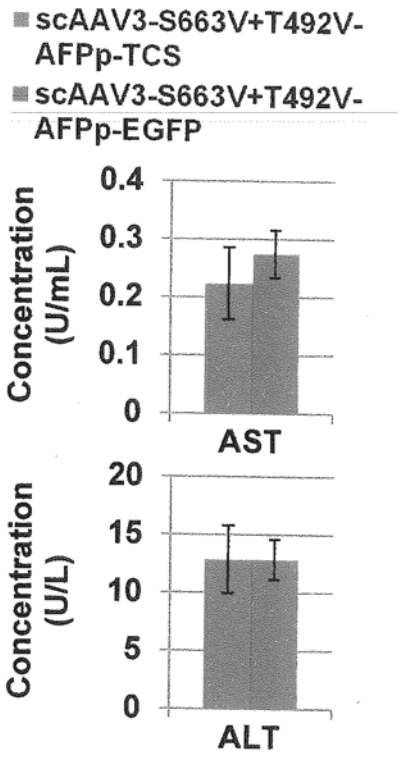


FIG. 39C

FIG. 40A

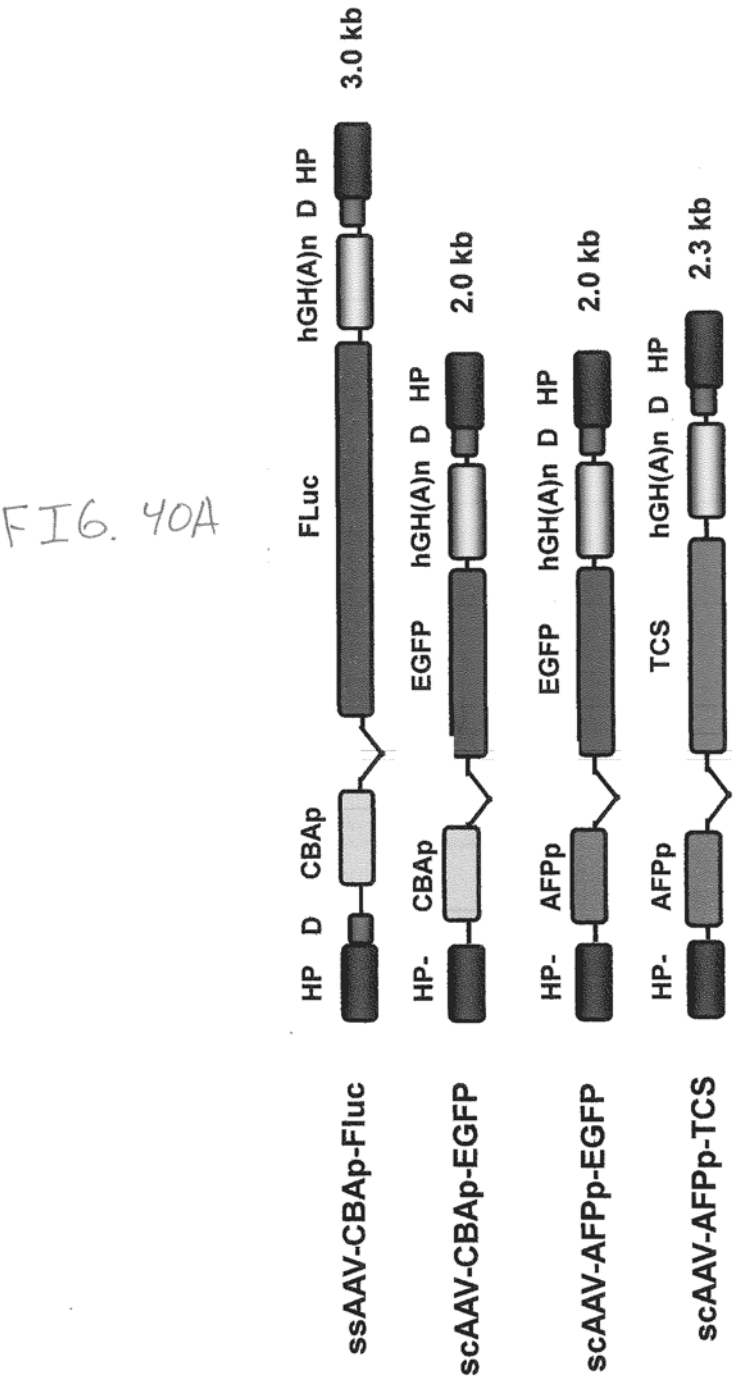

ssAAV-CBAp-Fluc
HP D CBAp FLuc hGH(A)n D HP
3.0 kb
scAAV-CBAp-EGFP
HP- CBAp EGFP hGH(A)n D HP
2.0 kb
scAAV-AFPp-EGFP
HP- AFPp EGFP hGH(A)n D HP
2.0 kb
scAAV-AFPp-TCS
HP- AFPp TCS hGH(A)n D HP
2.3 kb

FIG. 40B

ATGATCAGATTCTTAGTCCTCTCTTTGCTAATTCTCACCCTCTTCCTAACAACTCCTGCTGTGGAGGGCGATGTTAGCTTCCGTTTAT
CAGGTGCAACAAGCAGTTCCTATGGAGTTTTCATTTCAAATCTGAGAAAAGCTCTTCCAAATGAAAGGAAACTGTACGATATCCCTCT
GTTACGTTCCTCTCTTCCAGGTTCTCAACGCTACGCATTGATCCATCTCACAAATTACGCCGATGAAACCATTTCAGTGGCCATAGA
CGTAACGAACGTCTATATTATGGGATATCGCGCTGGCGATACATCCTATTTTTTCAACGAGGCTTCTGCAACAGAAGCTGCAAAATA
TGTATTCAAAGACGCTATGCGAAAAGTTACGCTTCCATATTCTGGCAATTACGAAAGGCTTCAAACTGCTGCAGGCAAAATAAGGGA
AAATATTCCGCTTGGACTCCCTGCTTTGGACAGTGCCATTACCACTTTGTTTTACTACAACGCCAATTCTGCTGCGTCGGCACTTAT
GGTACTCATTCAGTCGACGTCTGAGGCTGCGAGGTATAAATTTATTGAGCAACAAATTGGGAAGCGTGTTGACAAAACCTTCCTACC
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CTGCTGAATAGAAACAATATGGCAGCCATGGATGACGATGTTCCTATGACACAGAGCTTTGGATGTGGAAGTTATGCTATTTAG

(SEQ ID NO:26)

FIG. 40B

FIG. 40C

GAATTCATGATCAGATTCTTAGTCCTCTCTTTGCTAATTCTCACCCTCTTCCTAACAACTCCTGCTGTGGAGGGCGATGTTAGCTTCC
GTTTATCAGGTGCAACAAGCAGTTCCTATGGAGTTTTCATTTCAAATCTGAGAAAAGCTCTTCCAAATGAAAGGAAACTGTACGATAT
CCCTCTGTTACGTTCCTCTCTTCCAGGTTCTCAACGCTACGCATTGATCCATCTCACAAATTACGCCGATGAAACCATTTCAGTGGCC
ATAGACGTAACGAACGTCTATATTATGGGATATCGCGCTGGCGATACATCCTATTTTTTCAACGAGGCTTCTGCAACAGAAGCTGCA
AAATATGTATTCAAAGACGCTATGCGAAAAGTTACGCTTCCATATTCTGGCAATTACGAAAGGCTTCAAACTGCTGCAGGCAAAATAA
GGGAAAATATTCCGCTTGGACTCCCTGCTTTGGACAGTGCCATTACCACTTTGTTTTACTACAACGCCAATTCTGCTGCGTCGGCAC
TTATGGTACTCATTCAGTCGACGTCTGAGGCTGCGAGGTATAAATTTATTGAGCAACAAATTGGGAAGCGTGTTGACAAAACCTTCC
TACCAAGTTTAGCAATTATAAGTTTGGAAAATAGTTGGTCTGCTCTCTCCAAGCAAATTCAGATAGCGAGTACTAATAATGGACAGTTT
GAAAGTCCTGTTGTGCTTATAAATGCTCAAAACCAACGAGTCACGATAACCAATGTTGATGCTGGAGTTGTAACCTCCAACATCGCG
TTGCTGCTGAATAGAAACAATATGGCAGCCATGGATGACGATGTTCCTATGACACAGAGCTTTGGATGTGGAAGTTATGCTATT***CTC***
GAGGACTACAAGGATGACGATGACAAGGATTACAAAGACGACGATGATAAGGACTATAAGGATGATGACGACAAATAA

(SEQ ID NO:27)

FIG. 40C

Single Mutants		Double Mutants		Multiple Mutants	
Y252F	++	Y701+705F	++	Y701+705+731F	++
Y272F	+	Y701+731F	++	Y705+731F+S663V	++
Y444F	+	Y705+731F	++++	Y705+731F+T492V	+
F501Y	+	S663V+T492V	++++	Y705+731F+K533R	+++
Y701F	++			S663V5+T492V+K533R	++++
Y705F	+++			Y705+731F+S663V+T492V	+++
Y731F	+++			Y705+731F+S663V+T492V+K533R	++
S459V	+++				
S663V	+++				
T251V	+				
T492V	++				
K528E	-				
K528R	++				
K533E	-				
K533R	+++				
K545E	+++				
K545R	+++				

-: Greater than 10-fold reduction
+: Greater than 2-fold, but less than 10-fold reduction
++: Less than 2-fold increase
+++: Greater than 2-fold, but less than 10-fold increase
++++: Greater than 10-fold increase

FIG. 41