
1: TOF MS ES+
TIC
1.45e7
21.81
37.20
40.71
42.22
43.64
44.87
46.16
47.27
100
%
0
7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00 37.50 40.00 42.50 45.00 47.50 50.00 52.50 55.00 57.50
Time

FIG. 1A

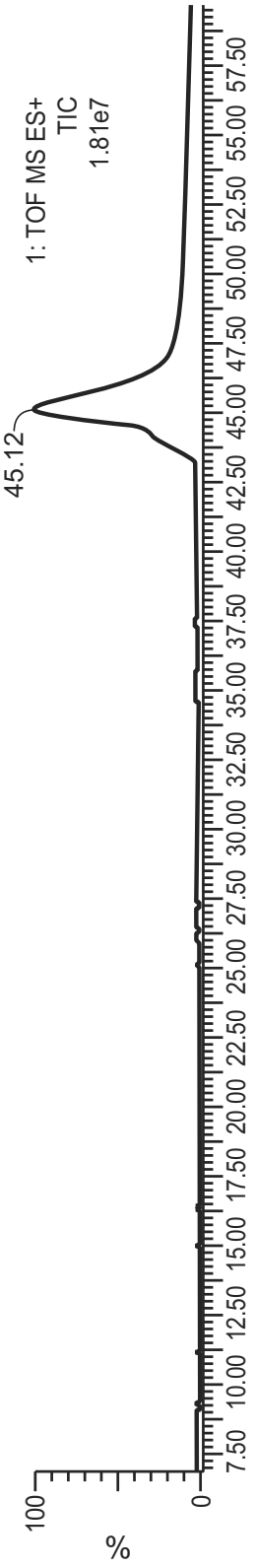

1: TOF MS ES+
TIC
1.81e7
45.12
100
%
0
7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00 37.50 40.00 42.50 45.00 47.50 50.00 52.50 55.00 57.50
Time

FIG. 1B

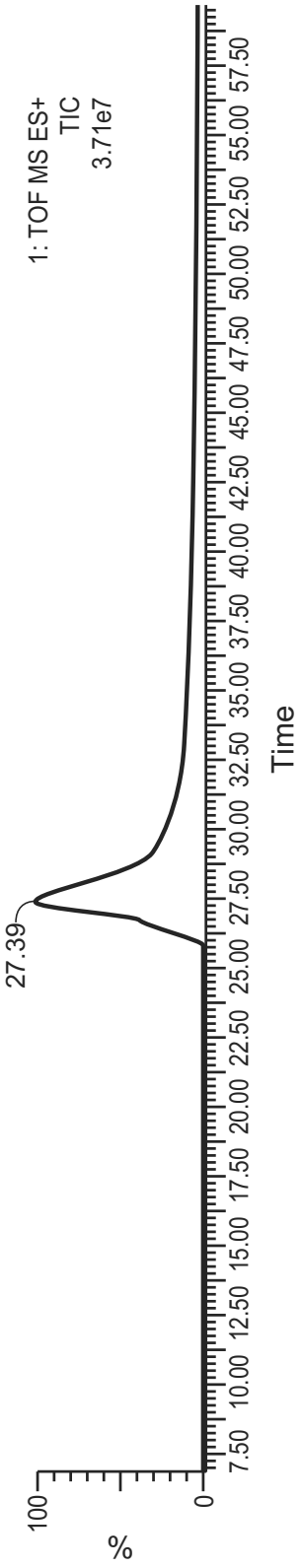

1: TOF MS ES+
TIC
3.71e7
27.39
100
%
0
7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00 37.50 40.00 42.50 45.00 47.50 50.00 52.50 55.00 57.50
Time

FIG. 1C

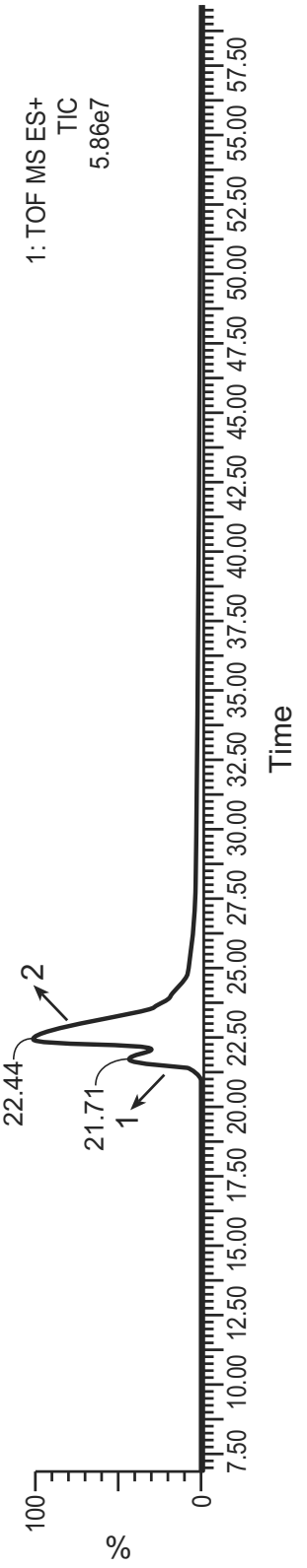

1: TOF MS ES+
TIC
5.86e7
22.44
2
21.71
1
100
%
0
7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00 37.50 40.00 42.50 45.00 47.50 50.00 52.50 55.00 57.50
Time

FIG. 1D

FIG. 2A

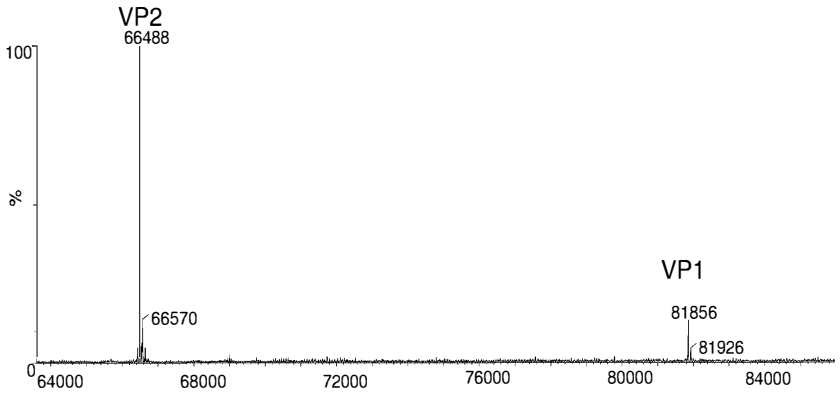
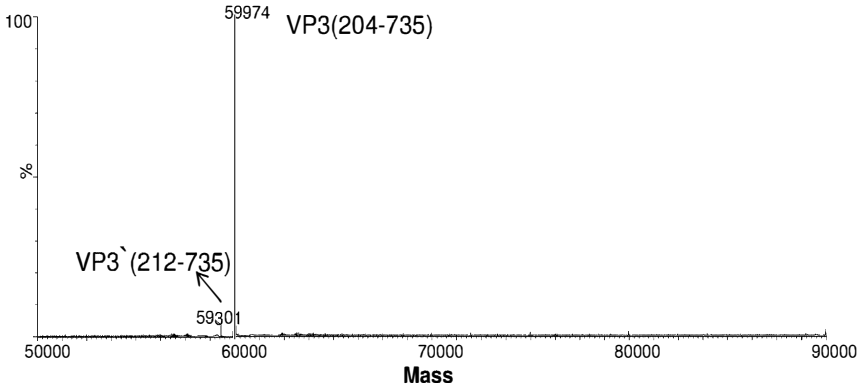

VP2
66488
100
%
VP1
66570
81856
81926
0
64000
68000
72000
76000
80000
84000

FIG. 2B


59974
VP3(204-735)
100
%
VP3`(212-735)
59301
50000
60000
70000
80000
90000
Mass

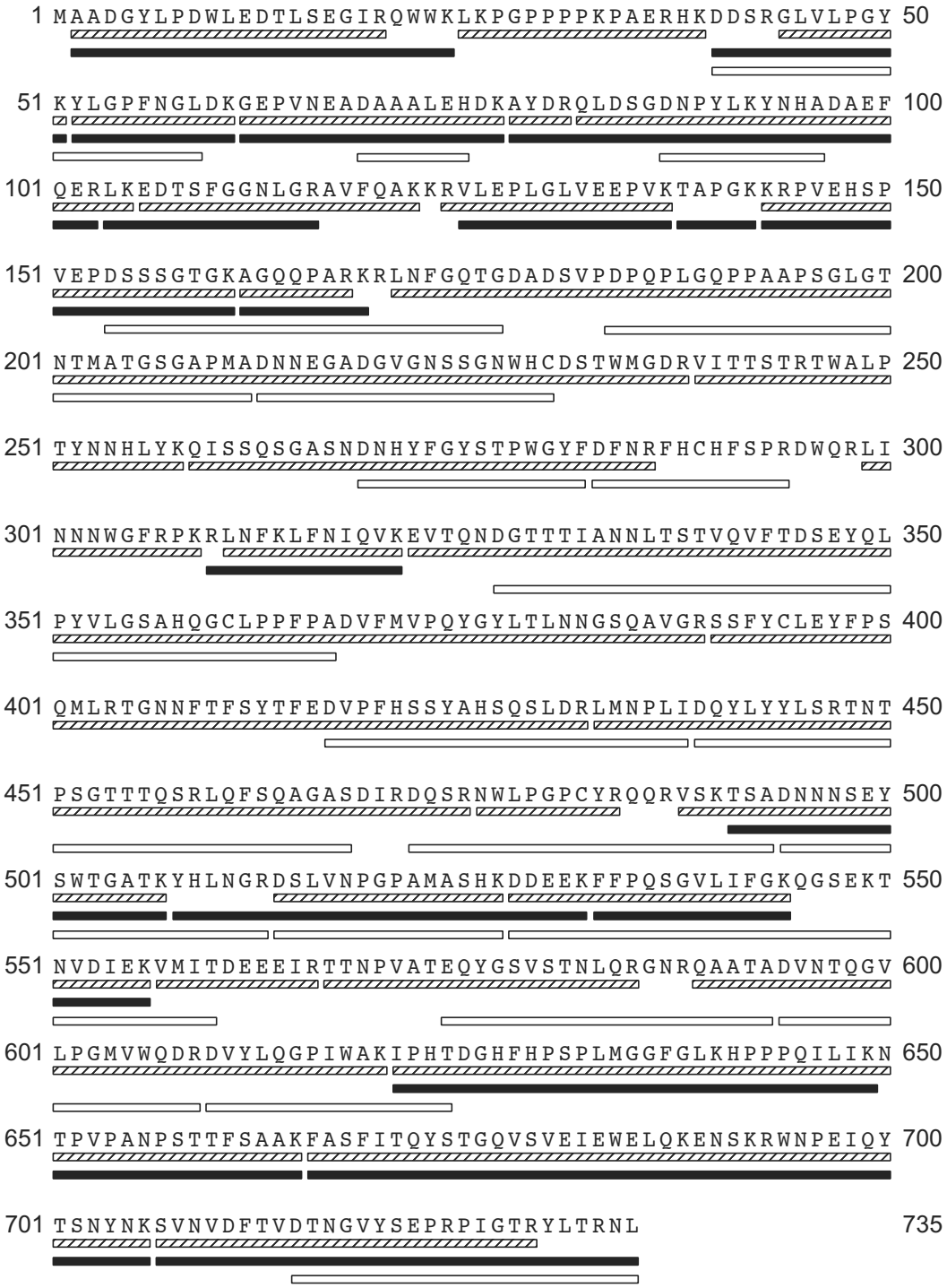

1 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY 50
51 KYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF 100
101 QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP 150
151 VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGT 200
201 NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALP 250
251 TYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI 300
301 NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQL 350
351 PYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPS 400
401 QMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNT 450
451 PSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEY 500
501 SWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKT 550
551 NVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV 600
601 LPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKN 650
651 TPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQY 700
701 TSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL 735

FIG. 3

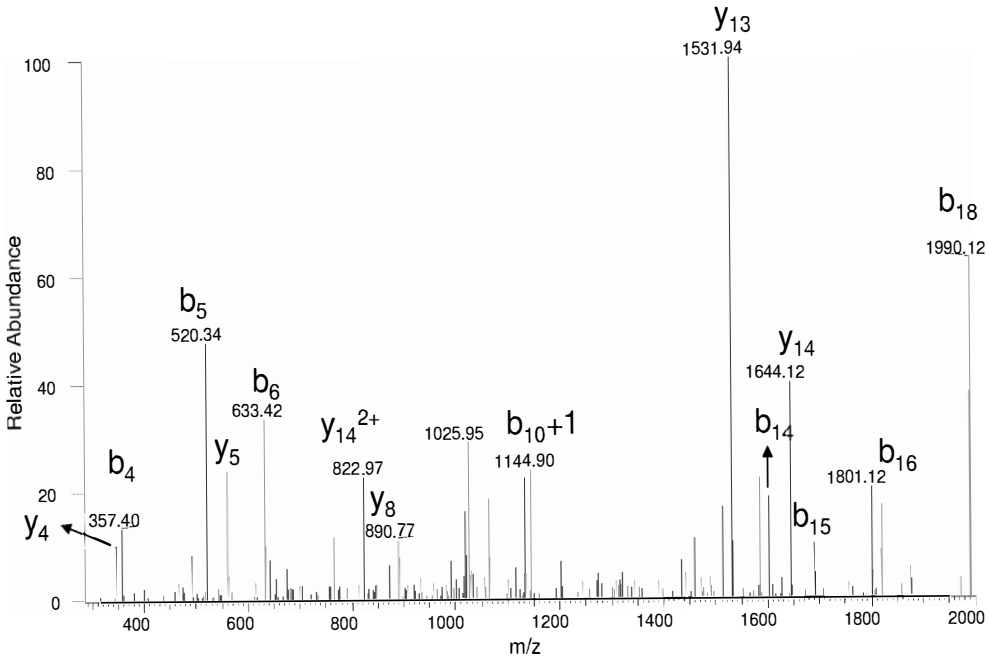

y13
1531.94
b18
1990.12
b5
520.34
y14
1644.12
b6
633.42
y14 2+
822.97
1025.95
b10+1
1144.90
b14
b16
1801.12
b4
y5
y8
890.77
y4
357.40
b15
Relative Abundance
m/z
100
80
60
40
20
0
400
600
800
1000
1200
1400
1600
1800
2000

FIG. 4A

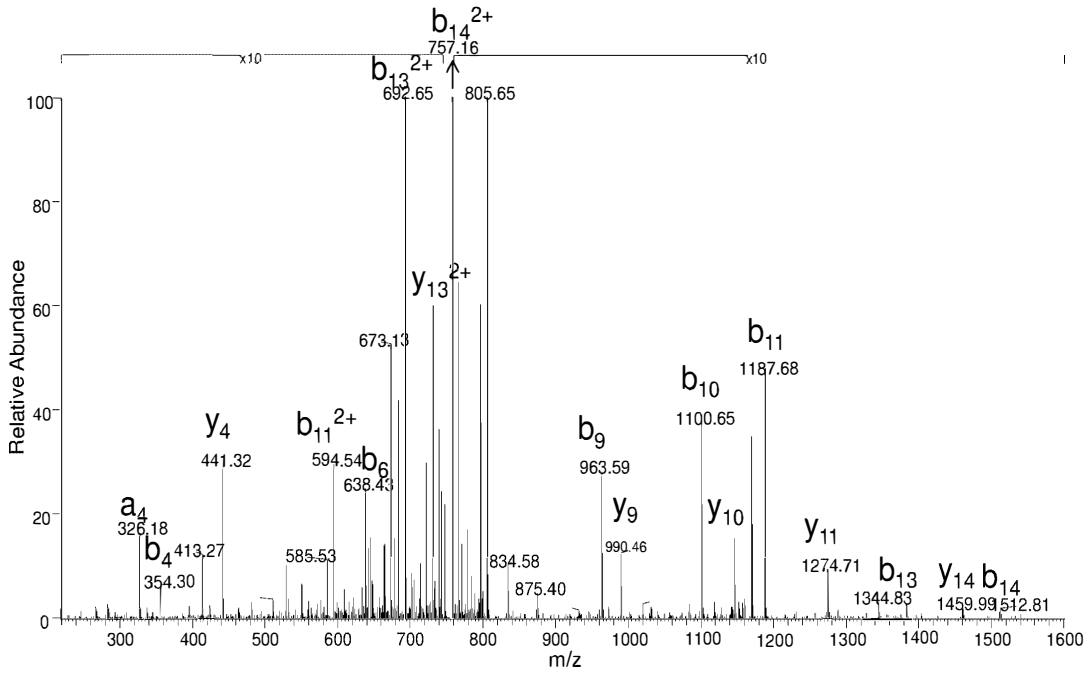

b14 2+
757.16
x10
x10
b13 2+
692.65
805.65
100
80
60
40
20
0
Relative Abundance
y13 2+
673.13
b11
1187.68
b10
1100.65
y4
441.32
b11 2+
594.54
b6
638.43
b9
963.59
a4
326.18
b4
413.27
354.30
585.53
y9
990.46
y10
834.58
875.40
y11
1274.71
b13
1344.83
y14
1459.99
b14
1512.81
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
m/z

FIG. 4B

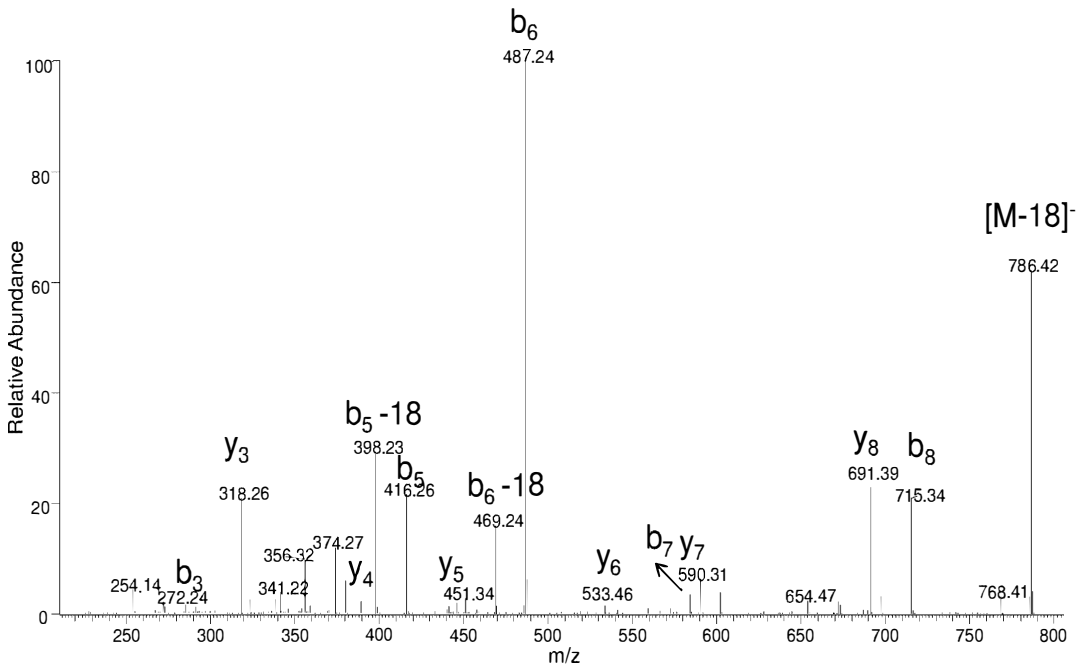

b6
487.24
[M-18]-
786.42
b5 -18
398.23
y3
318.26
b5
416.26
b6 -18
469.24
y8
691.39
b8
715.34
b7 y7
590.31
y6
533.46
y5
451.34
y4
374.27
356.32
341.22
b3
272.24
254.14
654.47
768.41
Relative Abundance
m/z
0
20
40
60
80
100
250
300
350
400
450
500
550
600
650
700
750
800

FIG. 4C

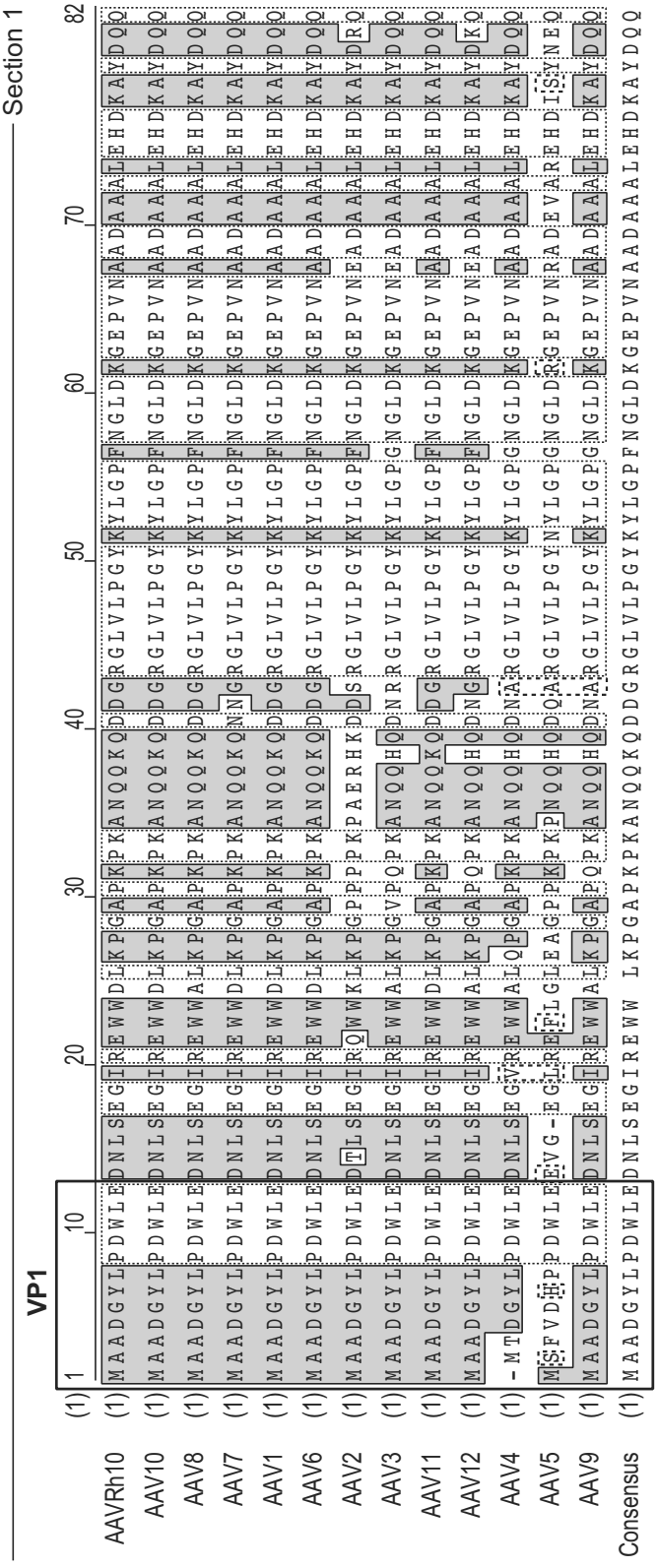

Section 1
VP1
AAVRh10
AAV10
AAV8
AAV7
AAV1
AAV6
AAV2
AAV3
AAV11
AAV12
AAV4
AAV5
AAV9
Consensus

FIG. 5A

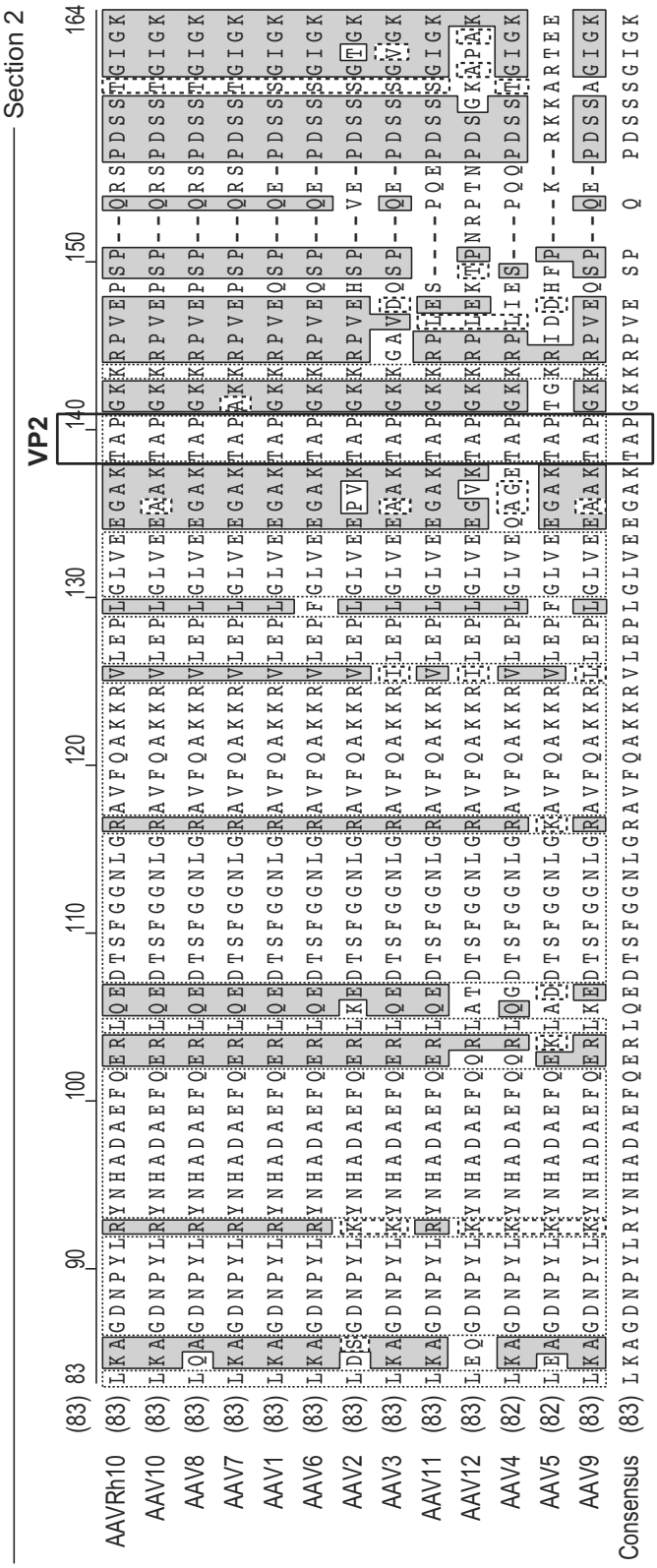

Section 2
VP2
AAVRh10
AAV10
AAV8
AAV7
AAV1
AAV6
AAV2
AAV3
AAV11
AAV12
AAV4
AAV5
AAV9
Consensus

FIG. 5B

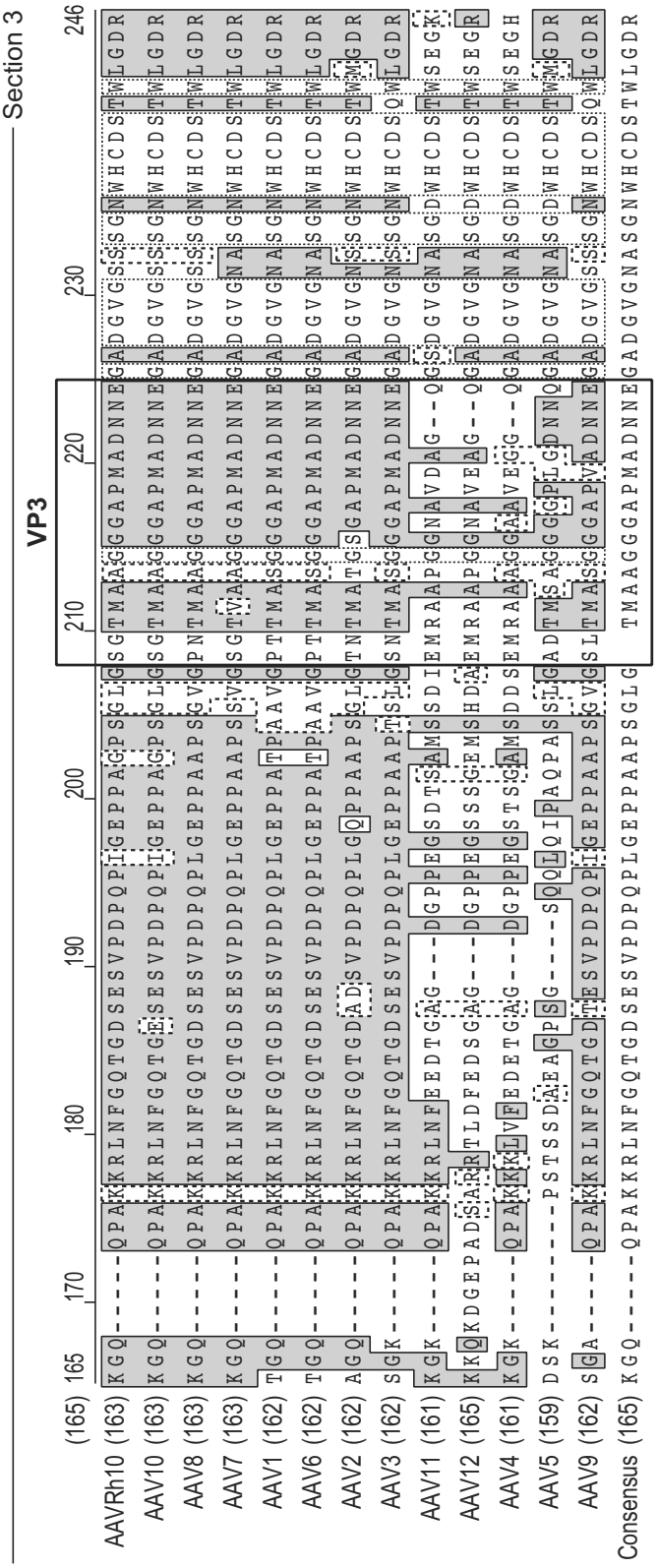

Section 3
VP3
(165) 165 170 180 190 200 210 220 230 246
AAVRh10 (163)
AAV10 (163)
AAV8 (163)
AAV7 (163)
AAV1 (162)
AAV6 (162)
AAV2 (162)
AAV3 (162)
AAV11 (161)
AAV12 (165)
AAV4 (161)
AAV5 (159)
AAV9 (162)
Consensus (165)

FIG. 5C

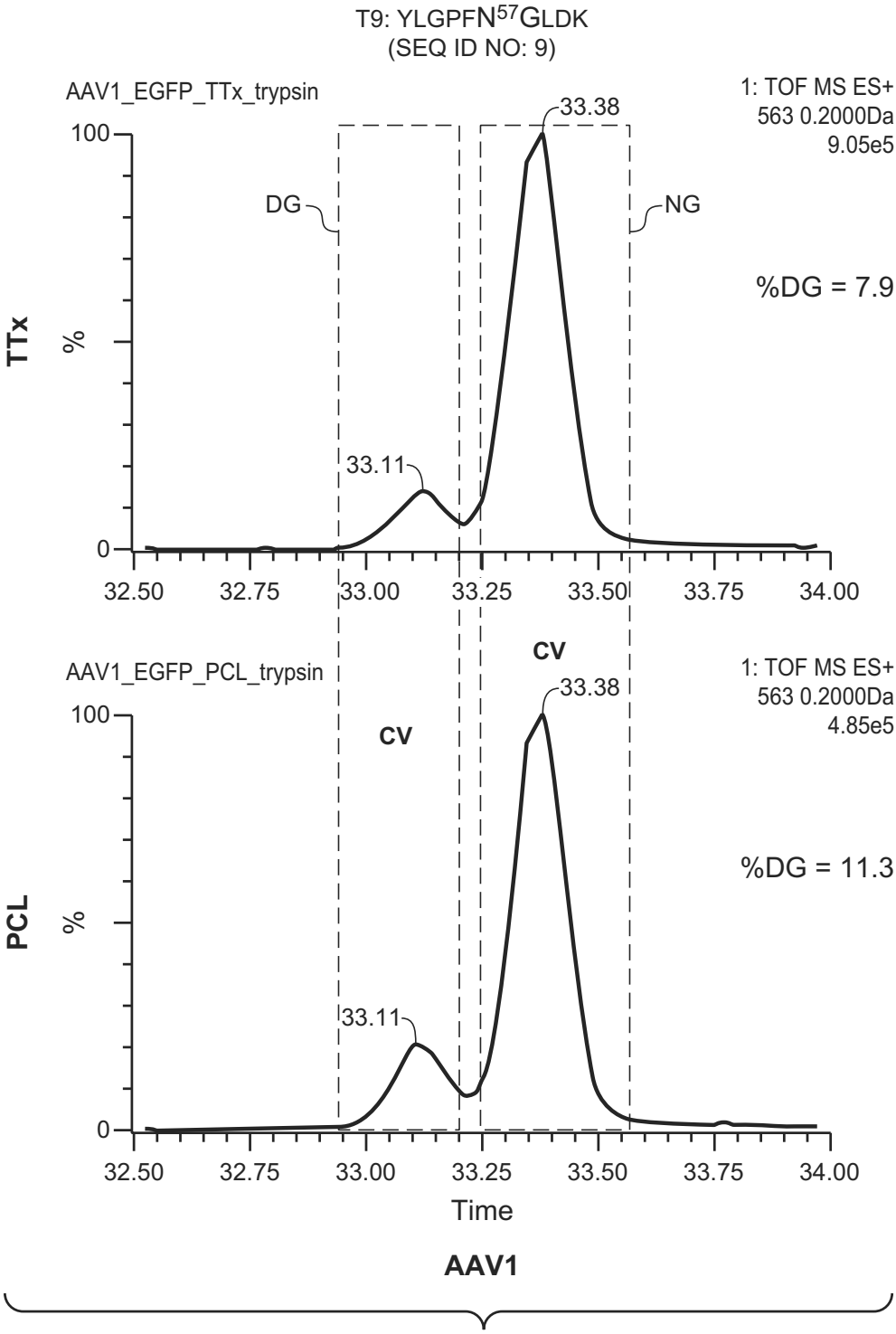

T9: YLGPFN57GLDK
(SEQ ID NO: 9)
AAV1_EGFP_TTx_trypsin
1: TOF MS ES+
563 0.2000Da
9.05e5
33.38
DG
NG
%DG = 7.9
TTx
%
33.11
100
0
32.50
32.75
33.00
33.25
33.50
33.75
34.00
CV
AAV1_EGFP_PCL_trypsin
1: TOF MS ES+
563 0.2000Da
4.85e5
33.38
CV
%DG = 11.3
PCL
33.11
Time
AAV1

FIG. 6A

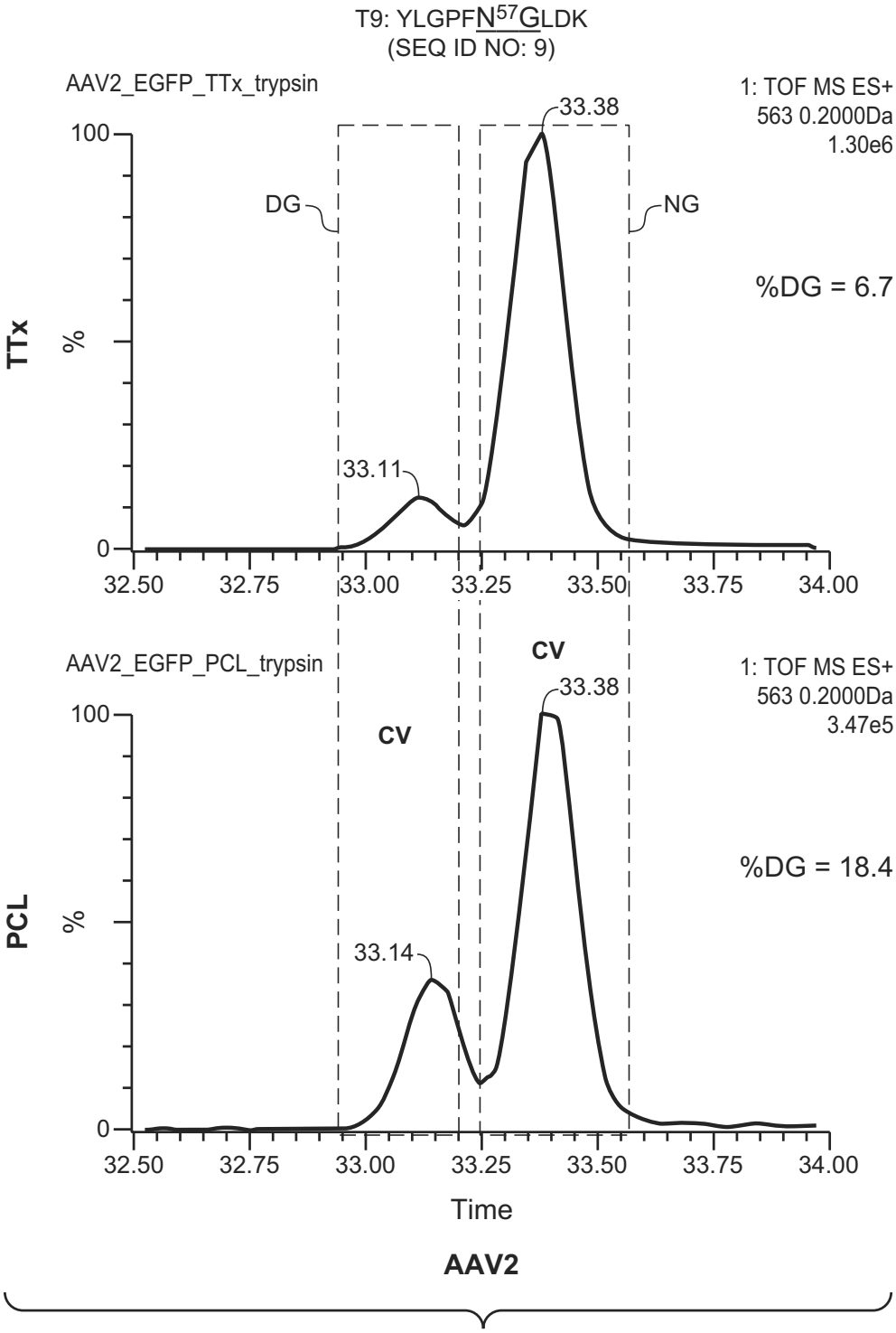

T9: YLGPFN57GLDK
(SEQ ID NO: 9)
AAV2_EGFP_TTx_trypsin
1: TOF MS ES+
563 0.2000Da
1.30e6
33.38
DG
NG
%DG = 6.7
TTx
%
33.11
100
0
32.50
32.75
33.00
33.25
33.50
33.75
34.00
AAV2_EGFP_PCL_trypsin
CV
1: TOF MS ES+
563 0.2000Da
3.47e5
33.38
CV
%DG = 18.4
PCL
%
33.14
Time
AAV2

FIG. 6B

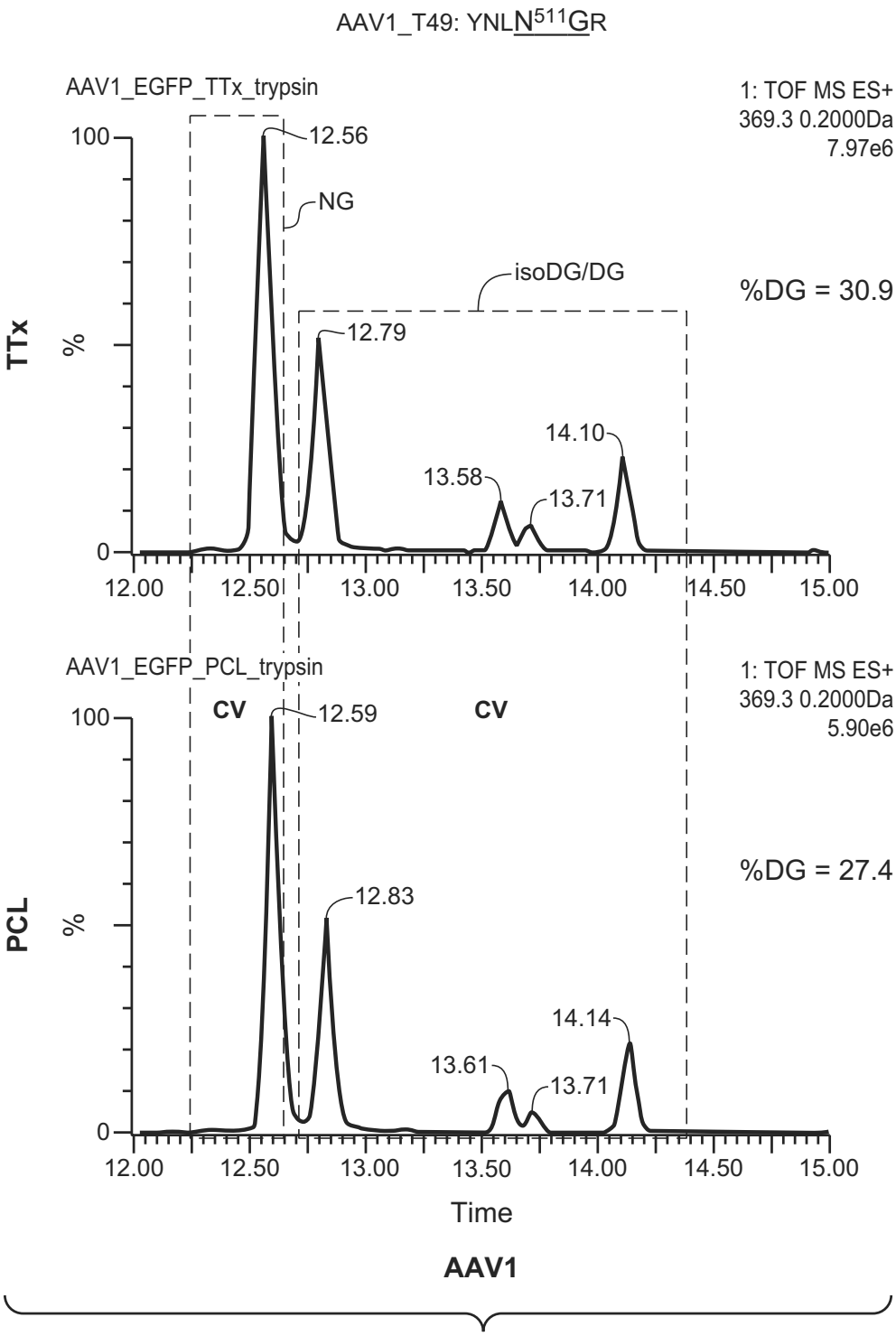

AAV1_T49: YNLN511GR
AAV1_EGFP_TTx_trypsin
1: TOF MS ES+
369.3 0.2000Da
7.97e6
100
%
0
12.56
NG
isoDG/DG
%DG = 30.9
12.79
14.10
13.58
13.71
12.00
12.50
13.00
13.50
14.00
14.50
15.00
TTx
AAV1_EGFP_PCL_trypsin
1: TOF MS ES+
369.3 0.2000Da
5.90e6
CV
CV
12.59
%DG = 27.4
12.83
14.14
13.61
13.71
PCL
Time
AAV1

FIG. 7A

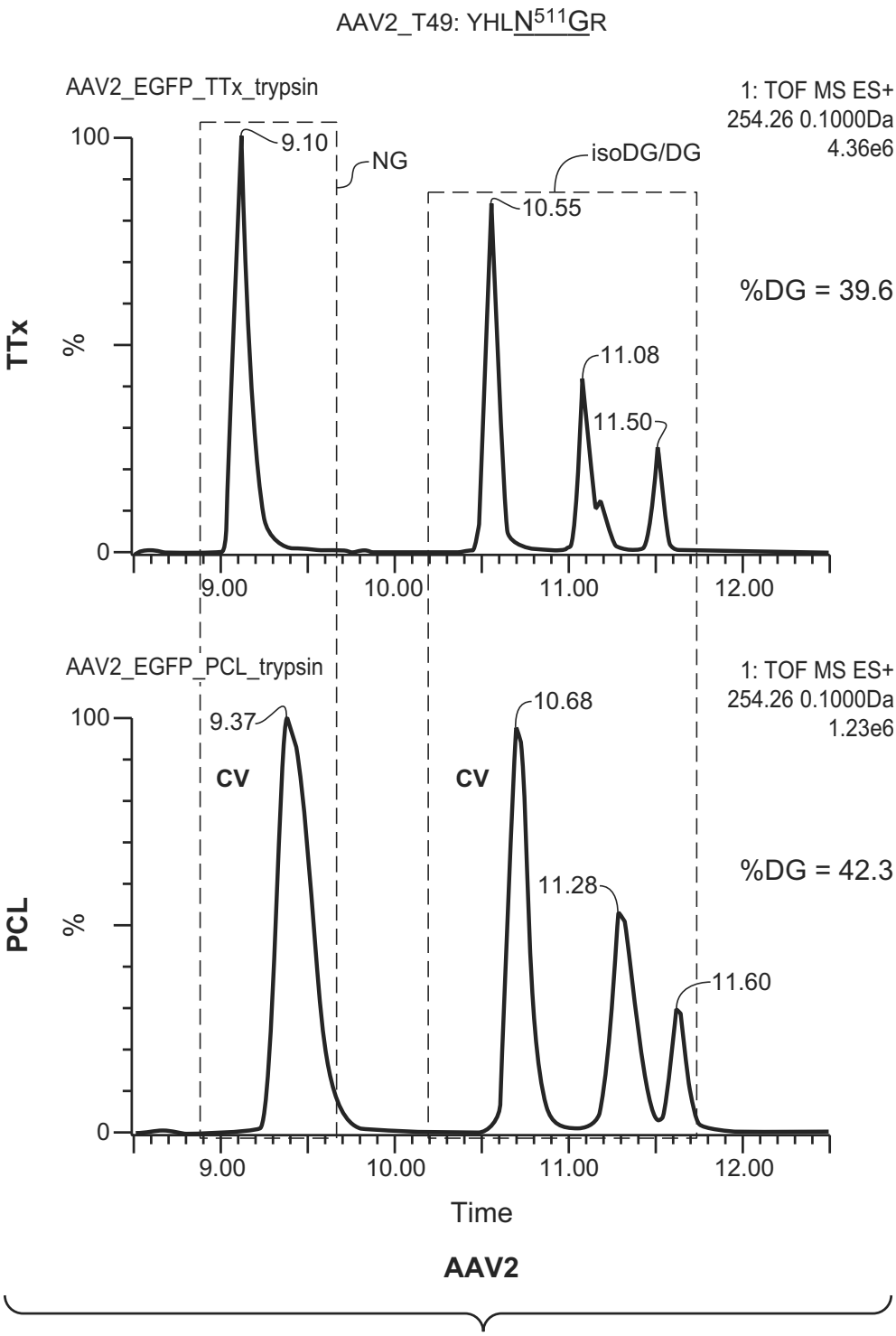

AAV2_T49: YHLN511GR
AAV2_EGFP_TTx_trypsin
1: TOF MS ES+
254.26 0.1000Da
4.36e6
100
%
0
9.10
NG
isoDG/DG
10.55
%DG = 39.6
11.08
11.50
TTx
9.00
10.00
11.00
12.00
AAV2_EGFP_PCL_trypsin
1: TOF MS ES+
254.26 0.1000Da
1.23e6
100
%
0
9.37
CV
CV
10.68
11.28
11.60
%DG = 42.3
PCL
9.00
10.00
11.00
12.00
Time
AAV2

FIG. 7B

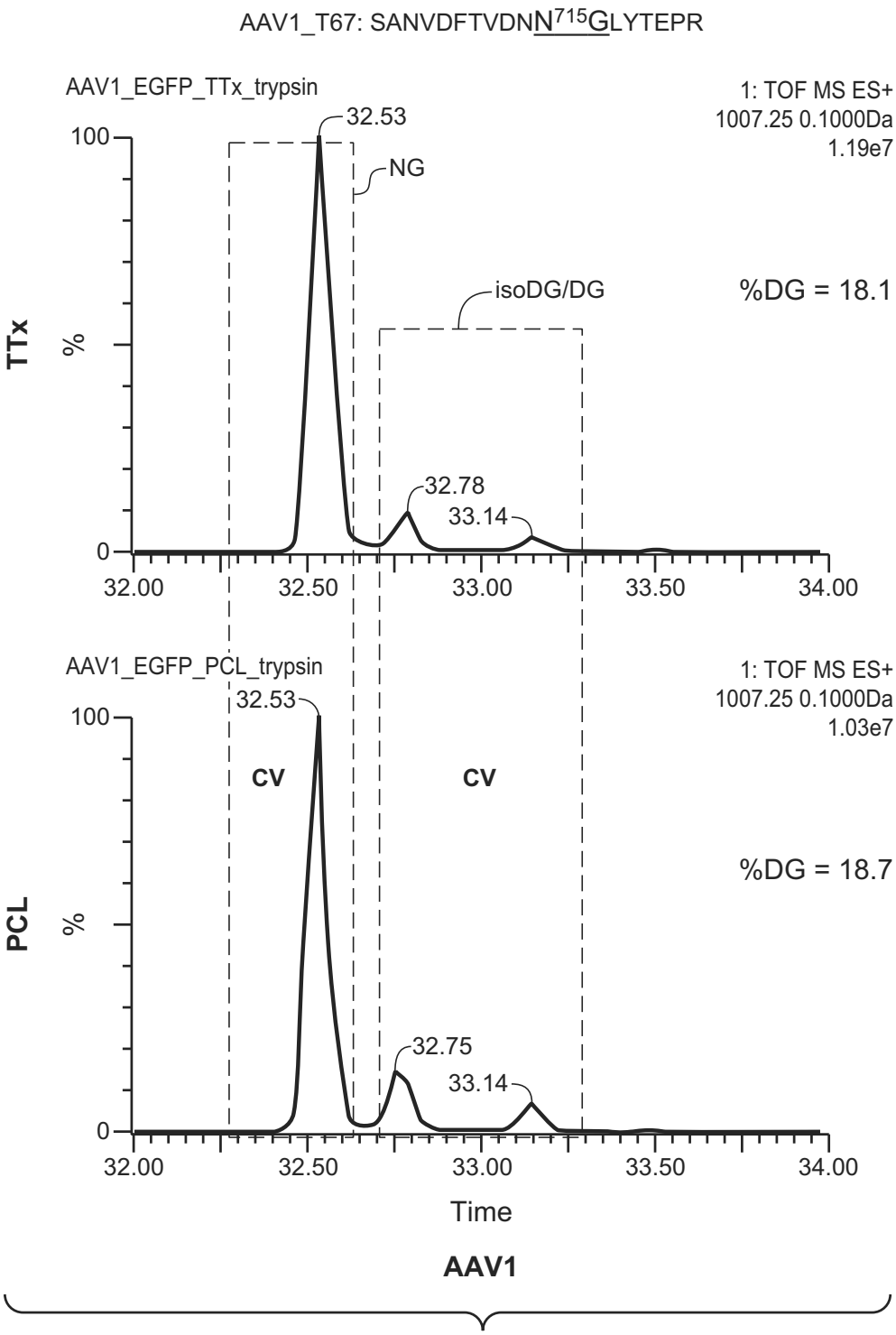

AAV1_T67: SANVDFTVDNN715GLYTEPR
AAV1_EGFP_TTx_trypsin
1: TOF MS ES+
1007.25 0.1000Da
1.19e7
32.53
NG
isoDG/DG
%DG = 18.1
TTx
%
100
0
32.78
33.14
32.00
32.50
33.00
33.50
34.00
AAV1_EGFP_PCL_trypsin
1: TOF MS ES+
1007.25 0.1000Da
1.03e7
32.53
CV
CV
%DG = 18.7
PCL
%
100
0
32.75
33.14
32.00
32.50
33.00
33.50
34.00
Time
AAV1

FIG. 8A

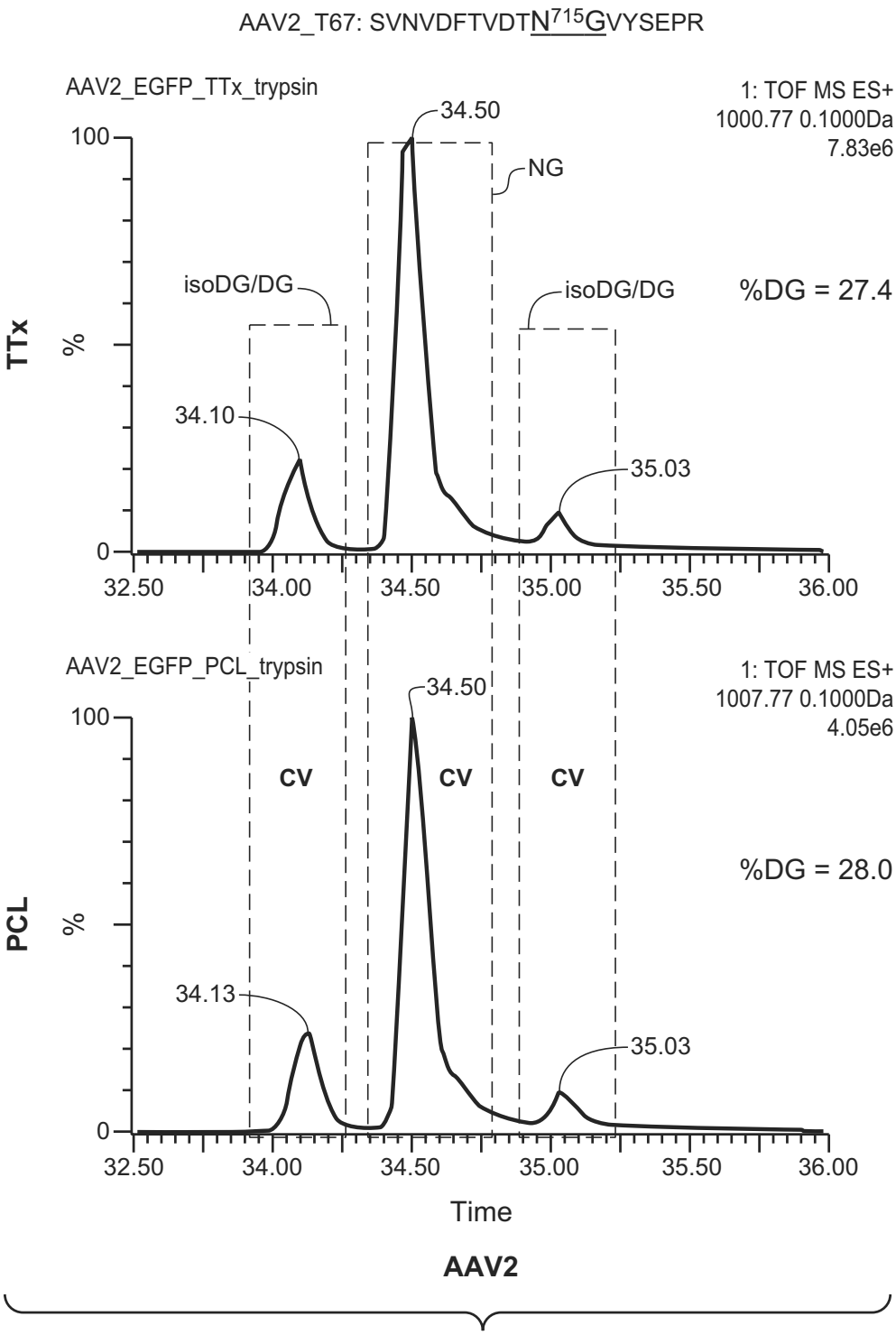
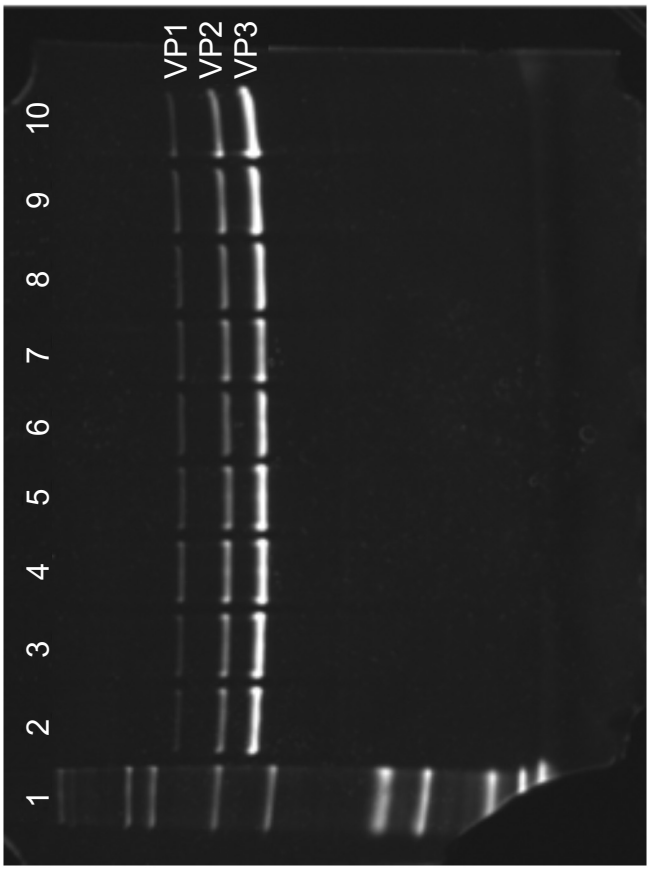

AAV2_T67: SVNVDFTVDTN715GVYSEPR
AAV2_EGFP_TTx_trypsin
1: TOF MS ES+
1000.77 0.1000Da
7.83e6
34.50
NG
isoDG/DG
isoDG/DG
%DG = 27.4
34.10
35.03
TTx
%
100
0
32.50
34.00
34.50
35.00
35.50
36.00
AAV2_EGFP_PCL_trypsin
1: TOF MS ES+
1007.77 0.1000Da
4.05e6
34.50
CV
CV
CV
%DG = 28.0
34.13
35.03
PCL
%
100
0
32.50
34.00
34.50
35.00
35.50
36.00
Time
AAV2

FIG. 8B



Deacetylation AAV5 Mutant Vectors

Lane	Sample
1	Mark12 Marker
2	deAC-AAV5 (S2G)/CBA-eGFP X17052C
3	deAC-AAV5 (S2L)/CBA-eGFP X17052D
4	deAC-AAV5 (S2GVP3)/CBA-eGFP X17058D
5	deAC-AAV5 (S2LVP3)/CBA-eGFP X17065A
6	deAC-AAV5 (S2PVP1VP3)/CBA-eGFP X17065B
7	deAC-AAV5 (S2GVP1VP3)/CBA-eGFP X17065C
8	deAC-AAV5 (S2PVP3)/CBA-eGFP X17065D
9	deAC-AAV5 (S2P)/CBA-eGFP X17065E
10	deAC-AAV5 (S2LVP1VP3)/CBA-eGFP X17072B

(~1E10 VGs/Lane)

FIG. 9

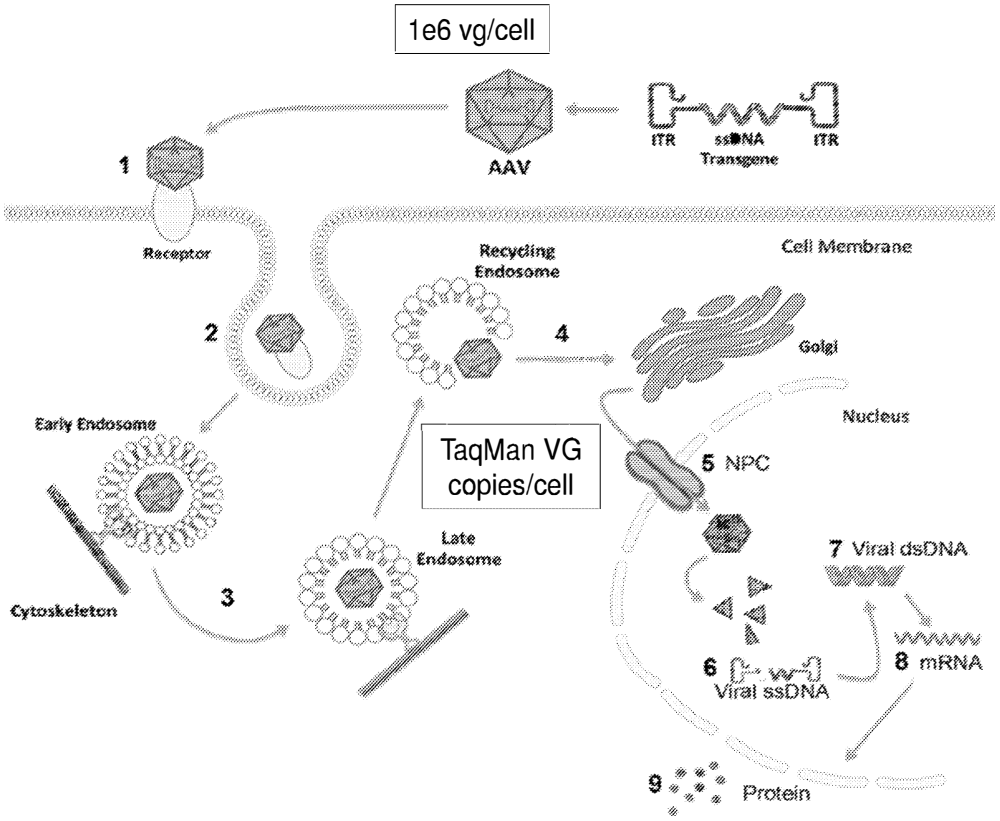

1e6 vg/cell
AAV
ITR
ssDNA
Transgene
ITR
1
Receptor
Cell Membrane
Recycling Endosome
2
4
Golgi
Early Endosome
Nucleus
TaqMan VG copies/cell
5 NPC
Late Endosome
7 Viral dsDNA
Cytoskeleton
3
6 Viral ssDNA
8 mRNA
9 Protein
293
HeLa
HuH7
eGFP ELISA

FIG. 10

FIG. 11

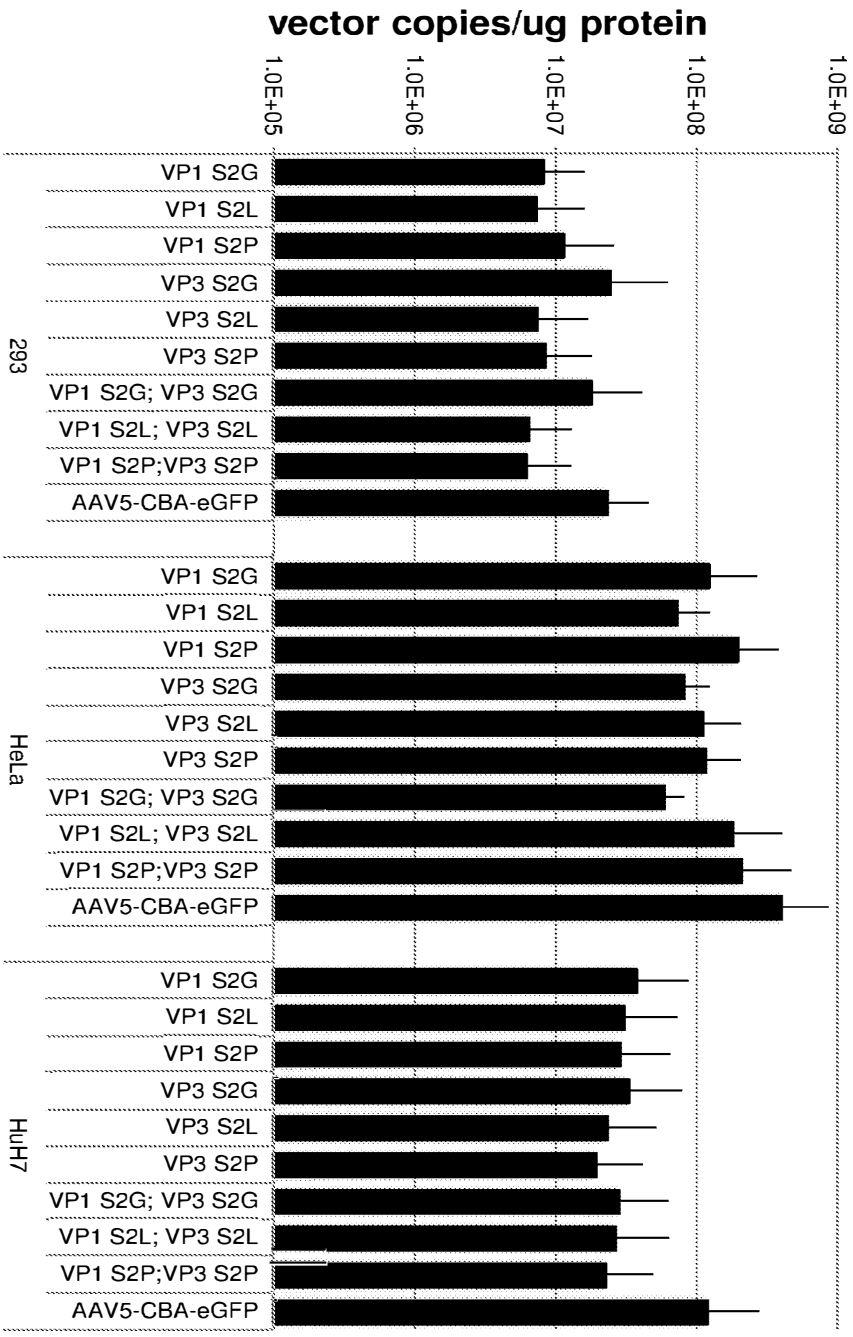
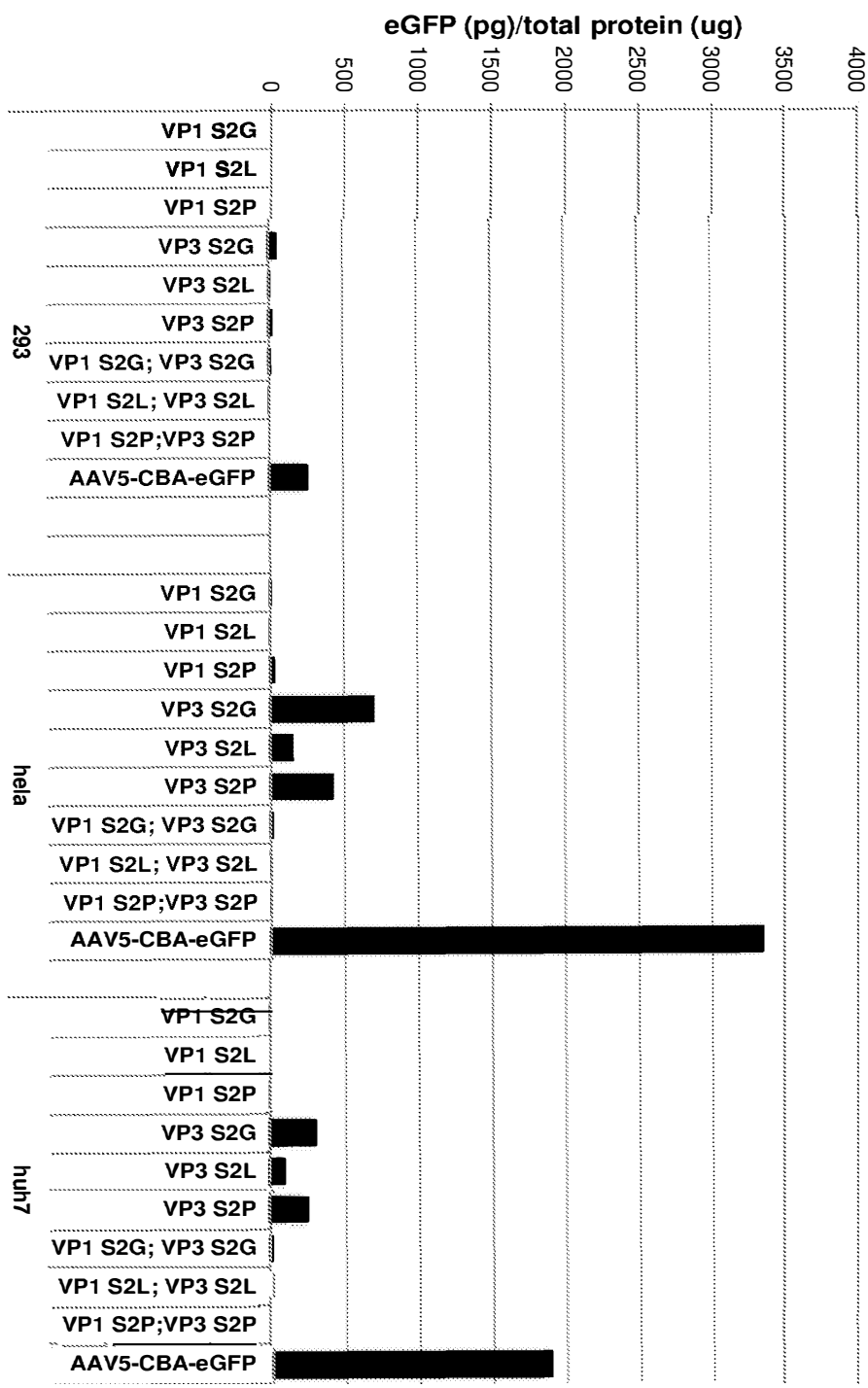

vector copies/ug protein
1.0E+05
1.0E+06
1.0E+07
1.0E+08
1.0E+09
293
VP1 S2G
VP1 S2L
VP1 S2P
VP3 S2G
VP3 S2L
VP3 S2P
VP1 S2G; VP3 S2G
VP1 S2L; VP3 S2L
VP1 S2P;VP3 S2P
AAV5-CBA-eGFP
HeLa
VP1 S2G
VP1 S2L
VP1 S2P
VP3 S2G
VP3 S2L
VP3 S2P
VP1 S2G; VP3 S2G
VP1 S2L; VP3 S2L
VP1 S2P;VP3 S2P
AAV5-CBA-eGFP
HuH7
VP1 S2G
VP1 S2L
VP1 S2P
VP3 S2G
VP3 S2L
VP3 S2P
VP1 S2G; VP3 S2G
VP1 S2L; VP3 S2L
VP1 S2P;VP3 S2P
AAV5-CBA-eGFP

FIG. 12


eGFP (pg)/total protein (ug)
0
500
1000
1500
2000
2500
3000
3500
4000
293
VP1 S2G
VP1 S2L
VP1 S2P
VP3 S2G
VP3 S2L
VP3 S2P
VP1 S2G; VP3 S2G
VP1 S2L; VP3 S2L
VP1 S2P;VP3 S2P
AAV5-CBA-eGFP
hela
VP1 S2G
VP1 S2L
VP1 S2P
VP3 S2G
VP3 S2L
VP3 S2P
VP1 S2G; VP3 S2G
VP1 S2L; VP3 S2L
VP1 S2P;VP3 S2P
AAV5-CBA-eGFP
huh7
VP1 S2G
VP1 S2L
VP1 S2P
VP3 S2G
VP3 S2L
VP3 S2P
VP1 S2G; VP3 S2G
VP1 S2L; VP3 S2L
VP1 S2P;VP3 S2P
AAV5-CBA-eGFP

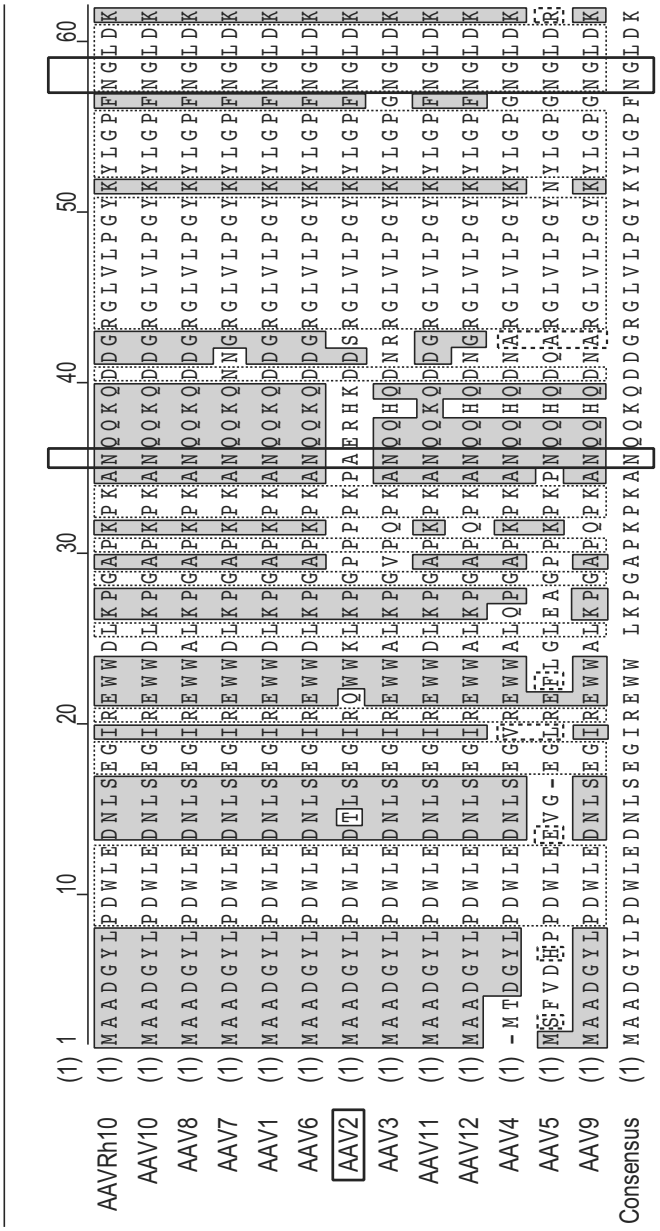

AAVRh10
AAV10
AAV8
AAV7
AAV1
AAV6
AAV2
AAV3
AAV11
AAV12
AAV4
AAV5
AAV9
Consensus

FIG. 13

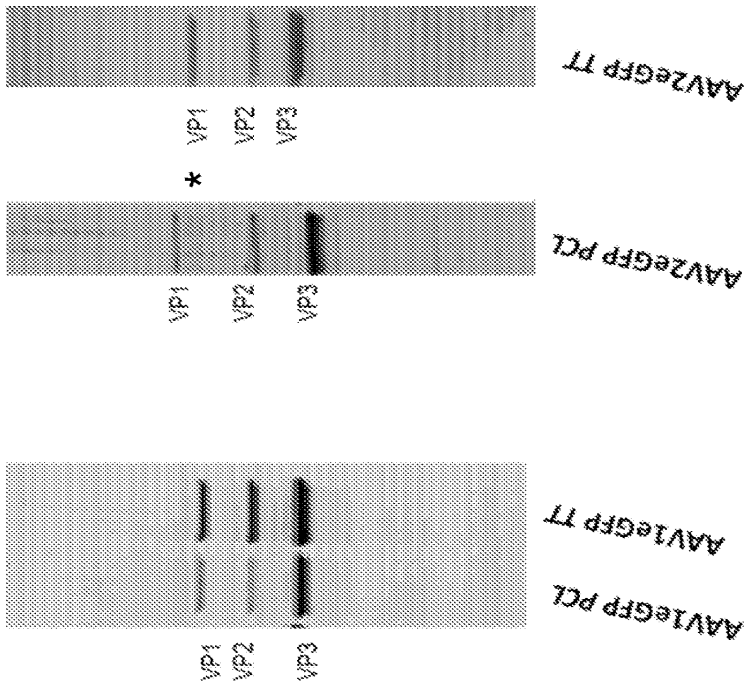

AAV2eGFP TT
VP1
VP2
VP3
*
AAV2eGFP PCL
VP1
VP2
VP3
AAV1eGFP TT
AAV1eGFP PCL
VP1
VP2
VP3

FIG. 14

Deamidation of N57(G)

	% Deamidation on N57	replicate
AAV2(A3SN)	17.8	1
AAV2(S68D)	1.1	1
AAV2 control	5.7	2

AAV2(A3SN) has been stored at 4C for 1 months

FIG. 15

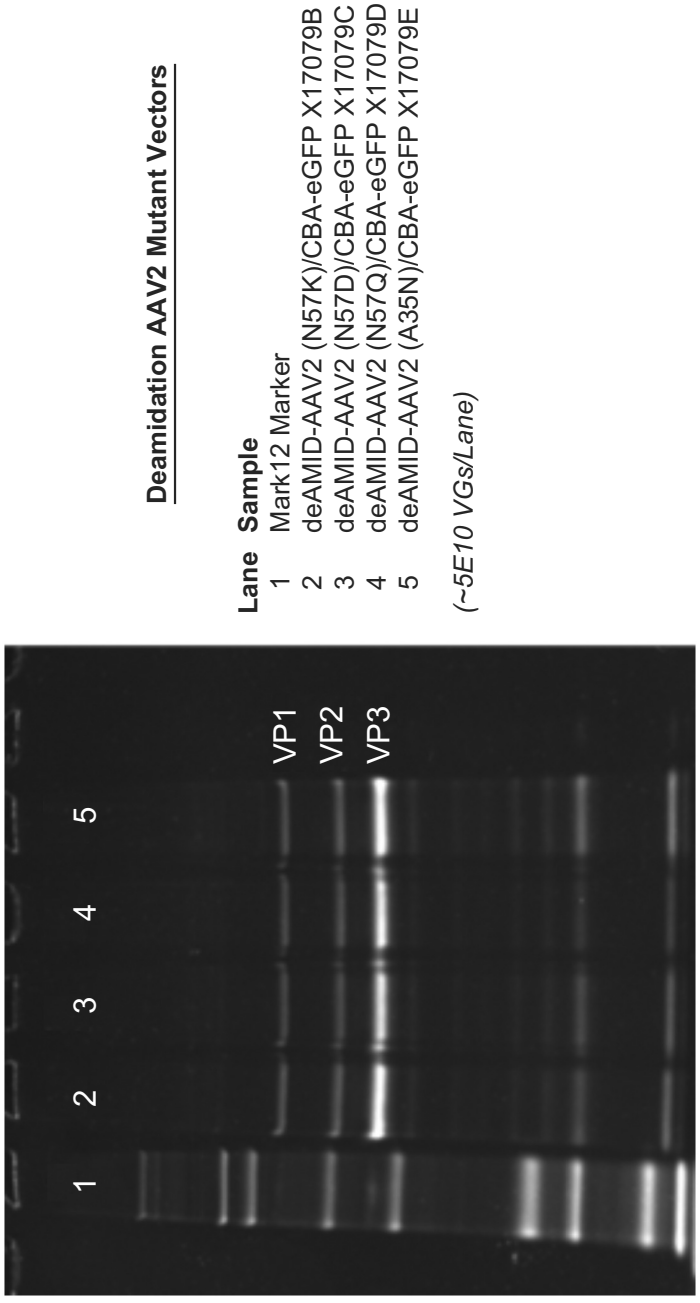

Deamidation AAV2 Mutant Vectors
Lane Sample
1 Mark12 Marker
2 deAMID-AAV2 (N57K)/CBA-eGFP X17079B
3 deAMID-AAV2 (N57D)/CBA-eGFP X17079C
4 deAMID-AAV2 (N57Q)/CBA-eGFP X17079D
5 deAMID-AAV2 (A35N)/CBA-eGFP X17079E
(~5E10 VGs/Lane)
1
2
3
4
5
VP1
VP2
VP3

FIG. 16

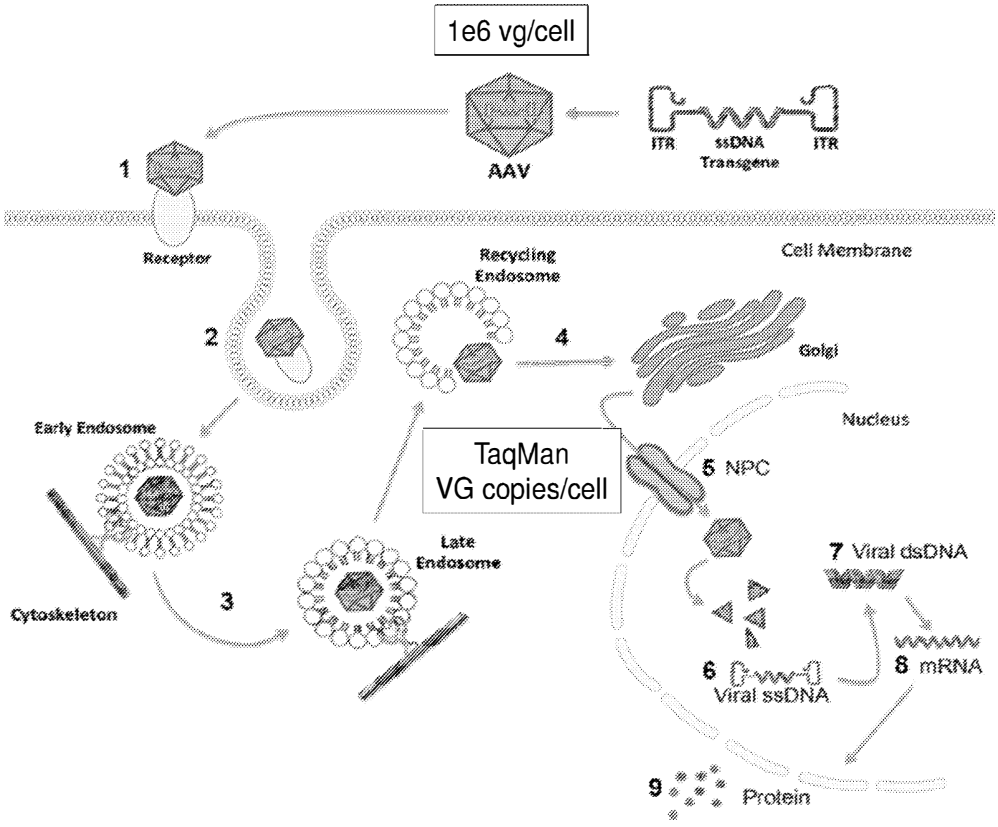
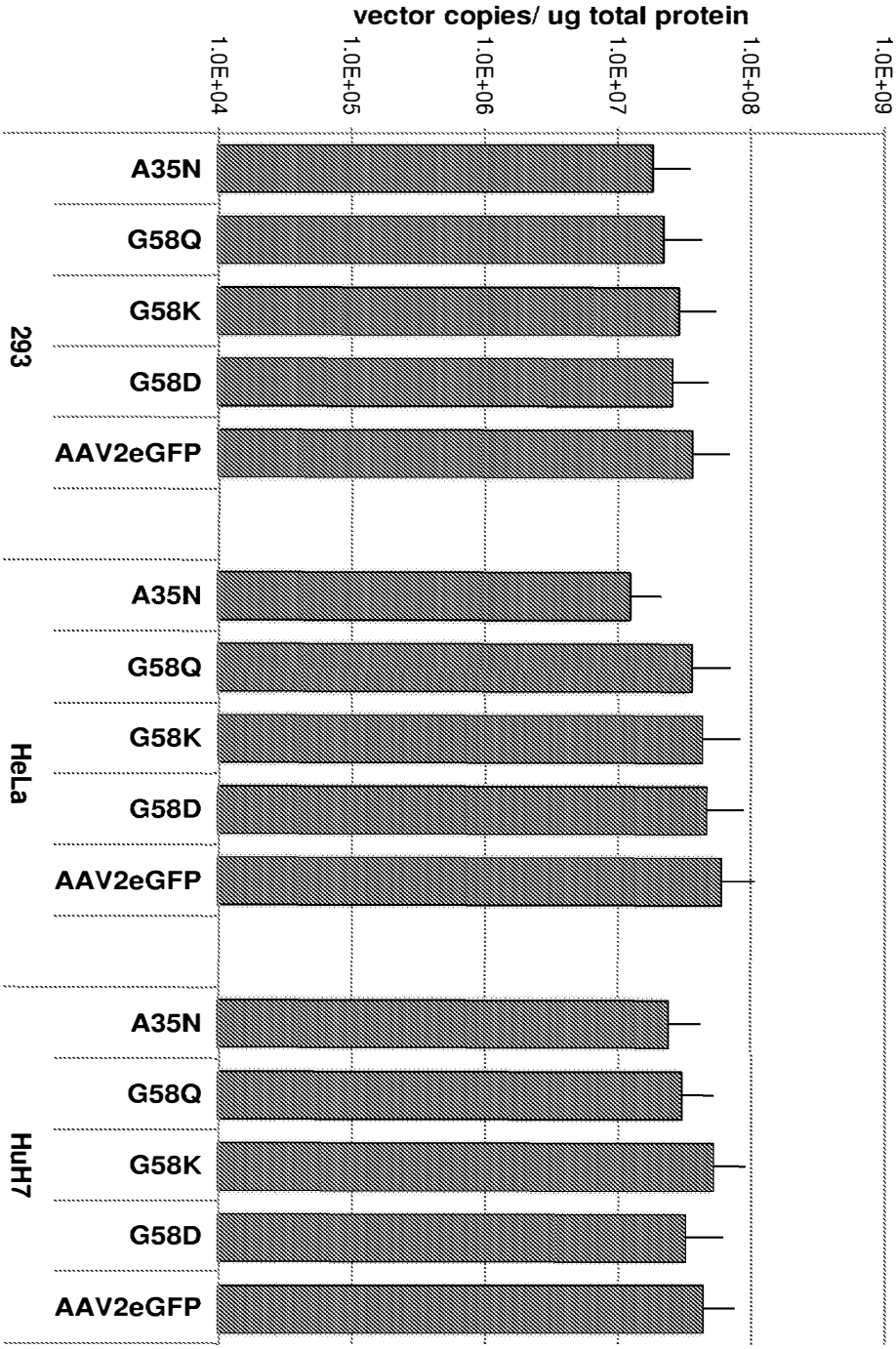

1e6 vg/cell
AAV
ITR
ssDNA
Transgene
ITR
1
Receptor
2
Recycling
Endosome
Cell Membrane
4
Golgi
Nucleus
Early Endosome
TaqMan
VG copies/cell
5 NPC
Late
Endosome
7 Viral dsDNA
Cytoskeleton
3
6
Viral ssDNA
8 mRNA
9
Protein
293
HeLa
HuH7
eGFP ELISA

FIG. 17

FIG. 18


vector copies/ ug total protein
1.0E+04
1.0E+05
1.0E+06
1.0E+07
1.0E+08
1.0E+09
293
A35N
G58Q
G58K
G58D
AAV2eGFP
HeLa
A35N
G58Q
G58K
G58D
AAV2eGFP
HuH7
A35N
G58Q
G58K
G58D
AAV2eGFP

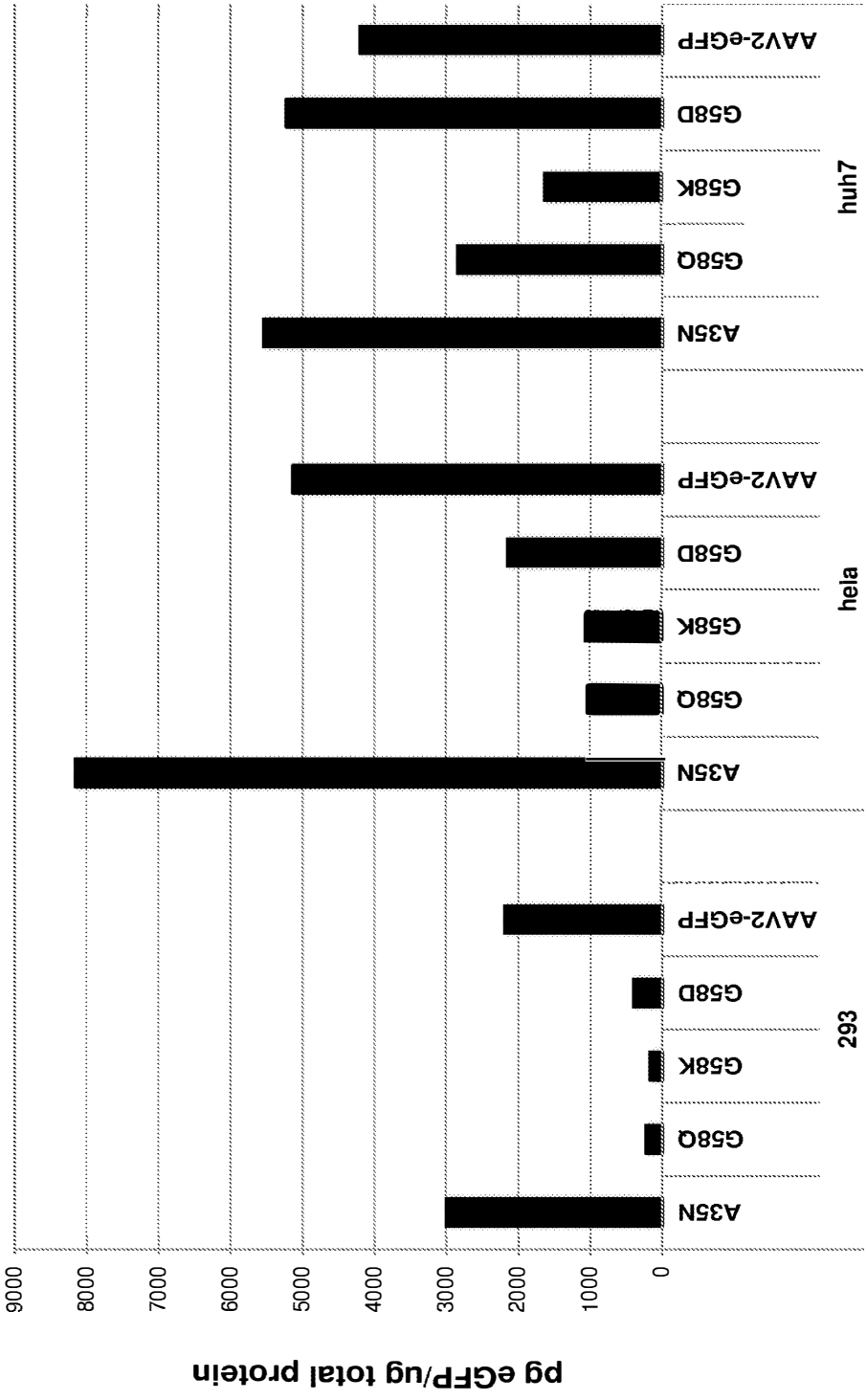

pg eGFP/ug total protein
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
293
A35N
G58Q
G58K
G58D
AAV2-eGFP
hela
A35N
G58Q
G58K
G58D
AAV2-eGFP
huh7
A35N
G58Q
G58K
G58D
AAV2-eGFP

FIG. 19