Figure 1

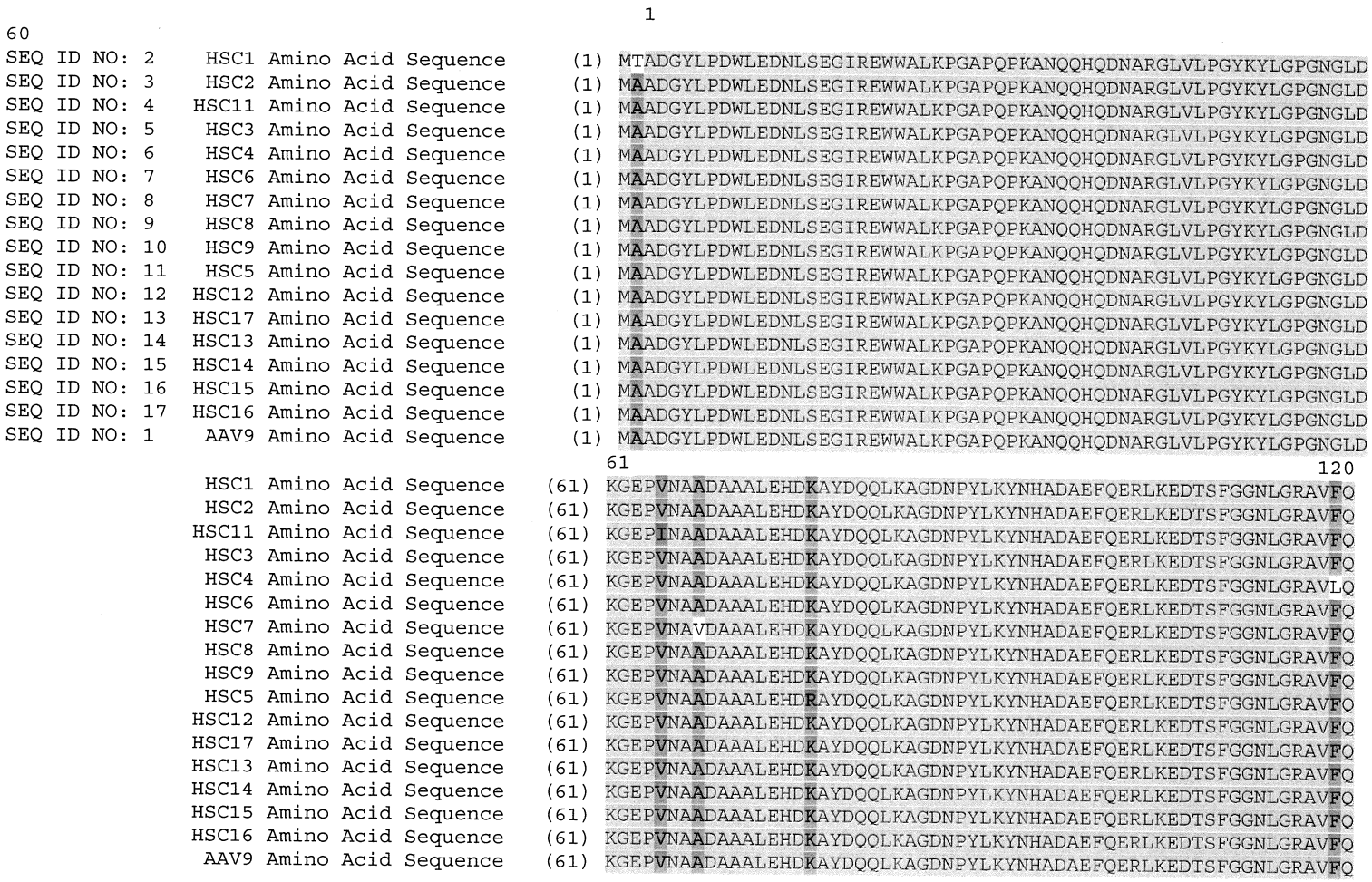

1
60
SEQ ID NO: 2 HSC1 Amino Acid Sequence (1) MTADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 3 HSC2 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 4 HSC11 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 5 HSC3 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 6 HSC4 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 7 HSC6 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 8 HSC7 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 9 HSC8 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 10 HSC9 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 11 HSC5 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 12 HSC12 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 13 HSC17 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 14 HSC13 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 15 HSC14 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 16 HSC15 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 17 HSC16 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
SEQ ID NO: 1 AAV9 Amino Acid Sequence (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD
61 120
HSC1 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC2 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC11 Amino Acid Sequence (61) KGEPINAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC3 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC4 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVLQ
HSC6 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC7 Amino Acid Sequence (61) KGEPVNAVDAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC8 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC9 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC5 Amino Acid Sequence (61) KGEPVNAADAAALEHDRAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC12 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC17 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC13 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC14 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC15 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
HSC16 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
AAV9 Amino Acid Sequence (61) KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ

Figure 1 (cont.)

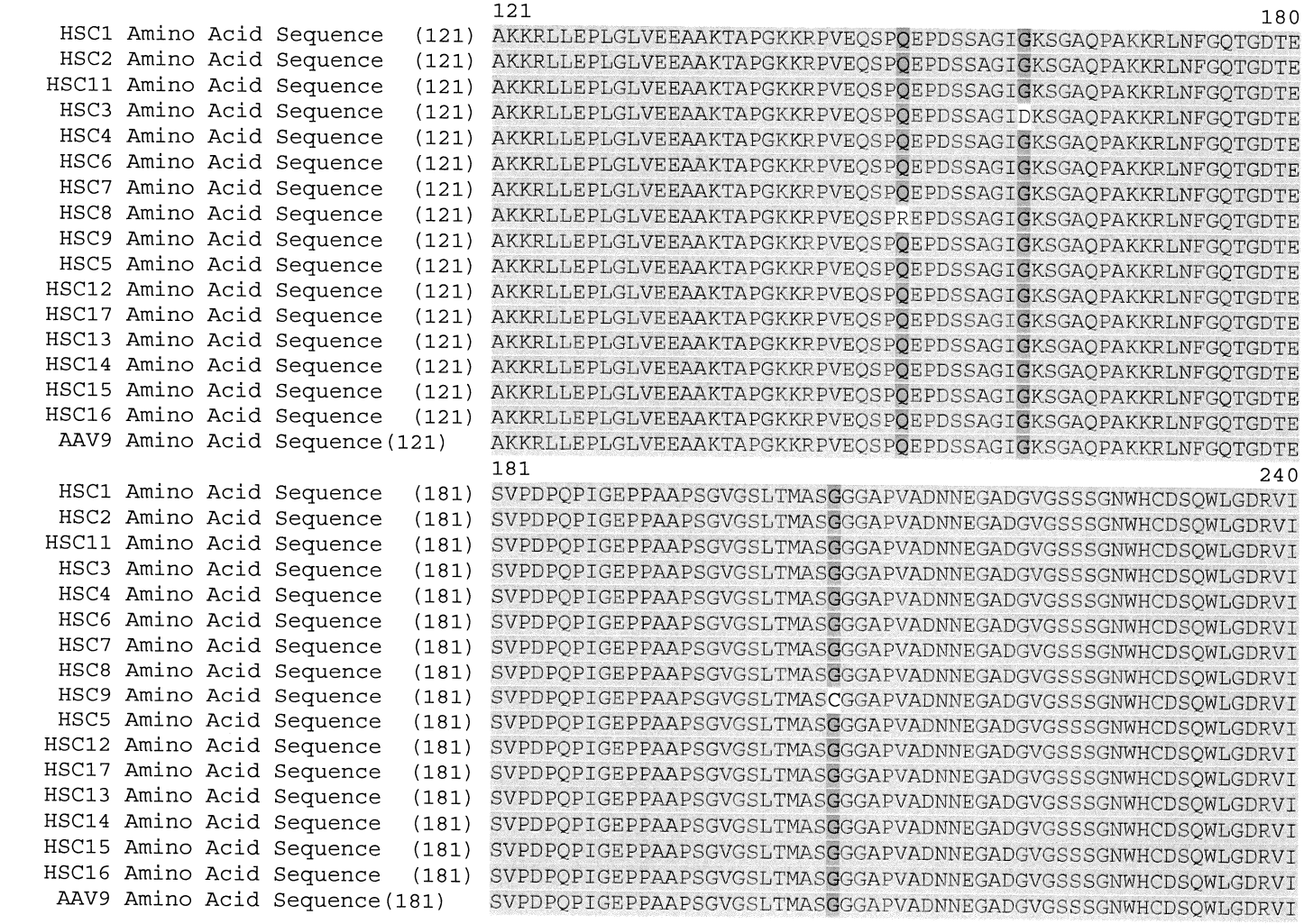


Figure 1 (cont.)

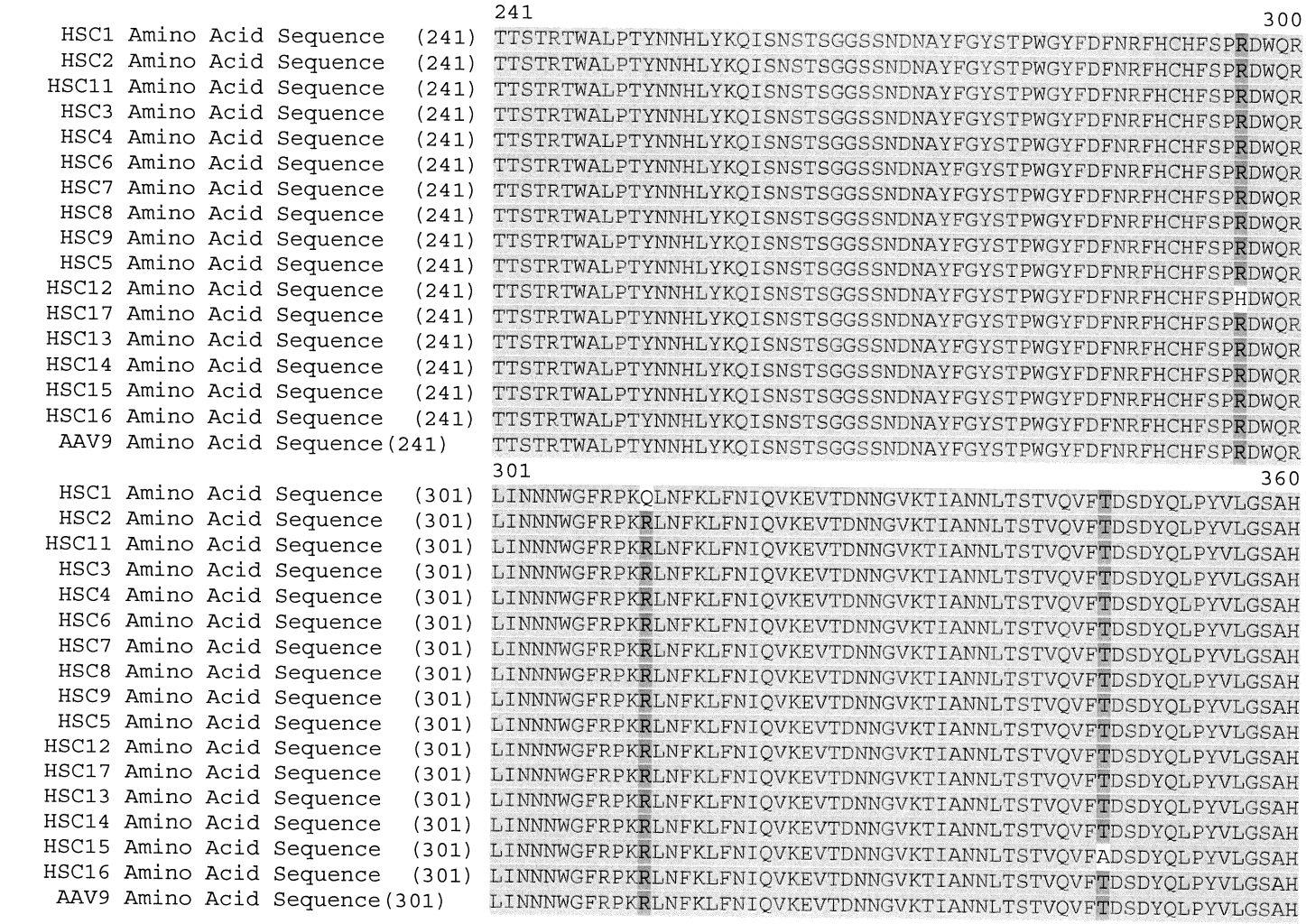


Figure 1 (cont.)

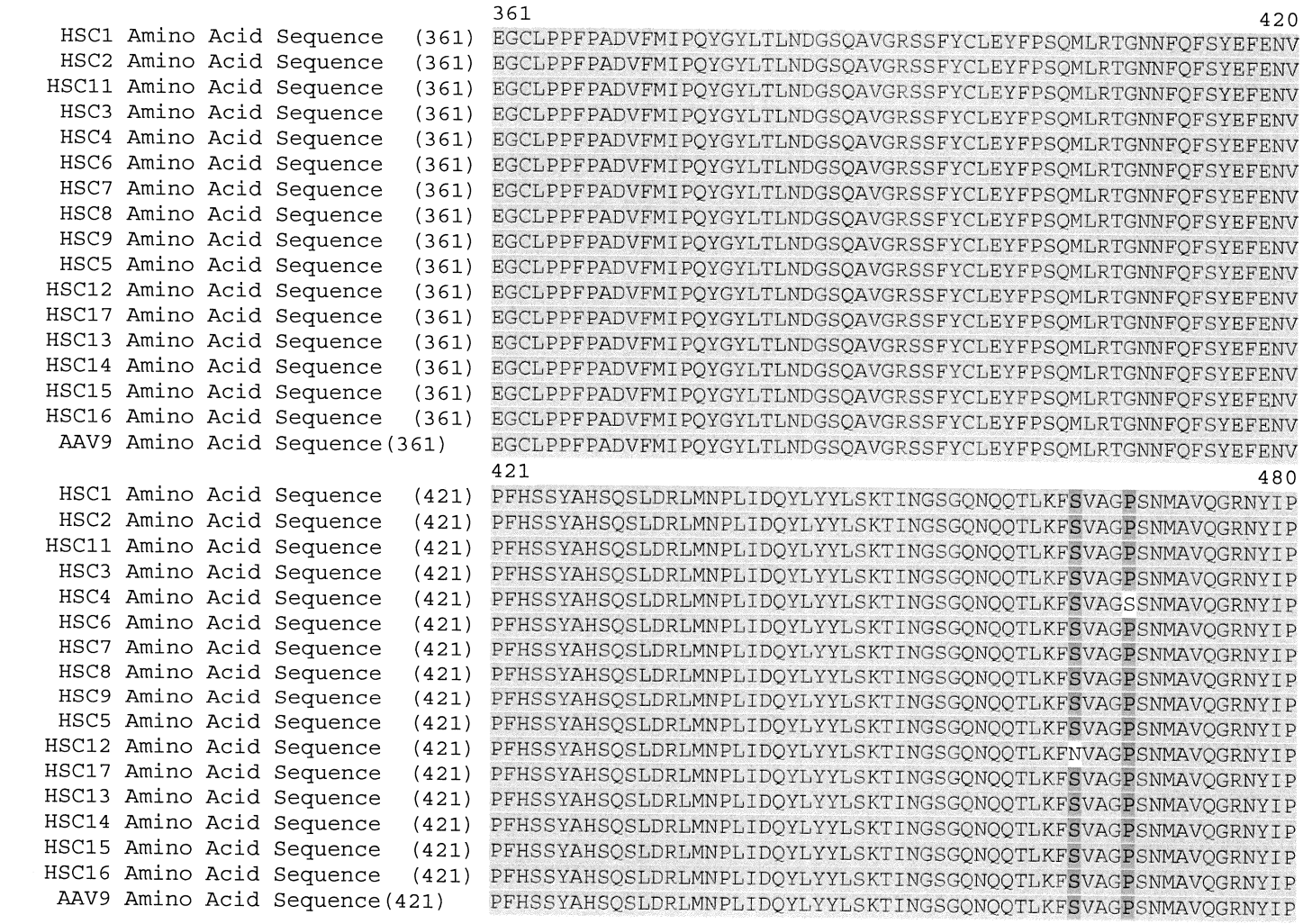


Figure 1 (cont.)

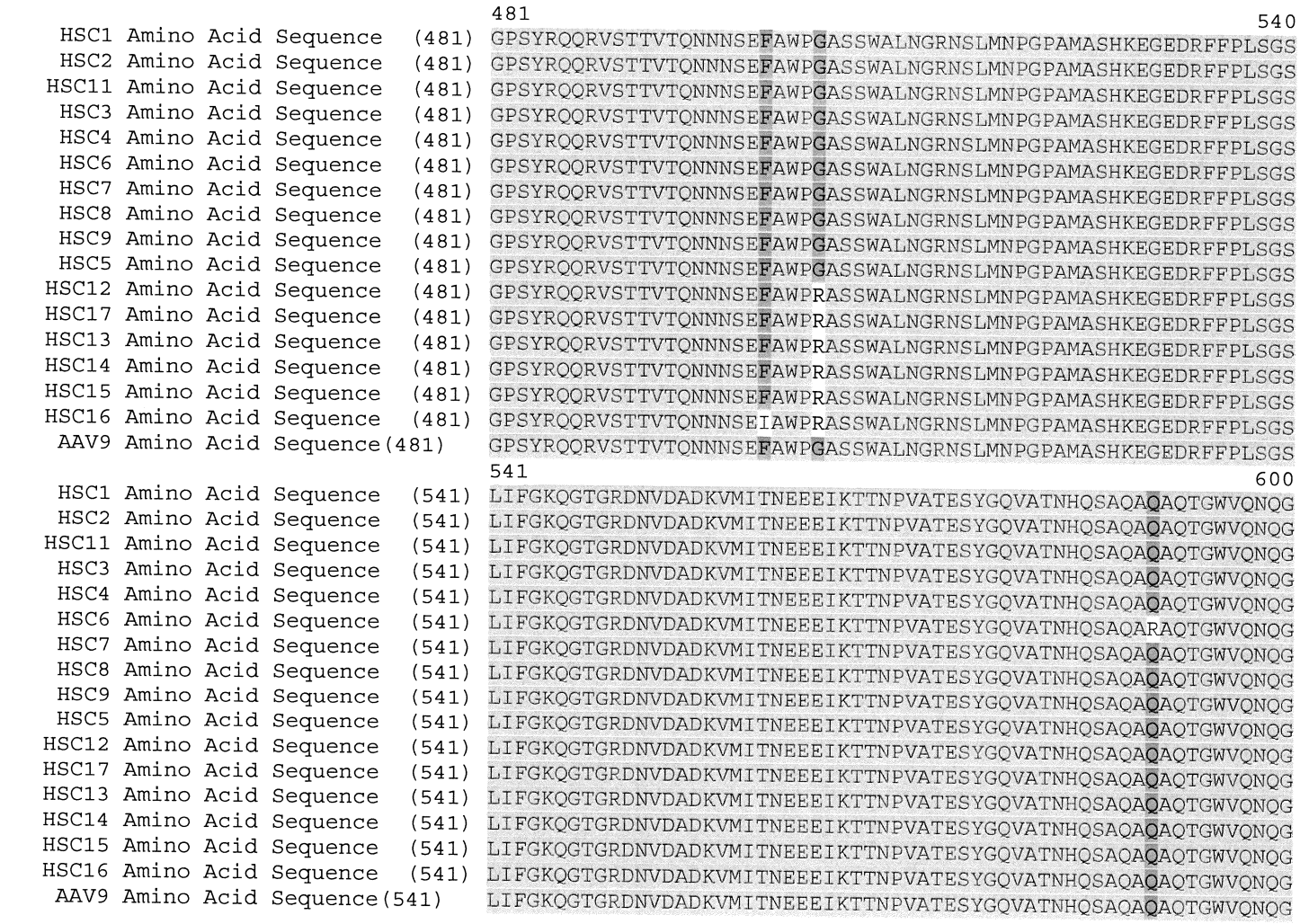


Figure 1 (cont.)

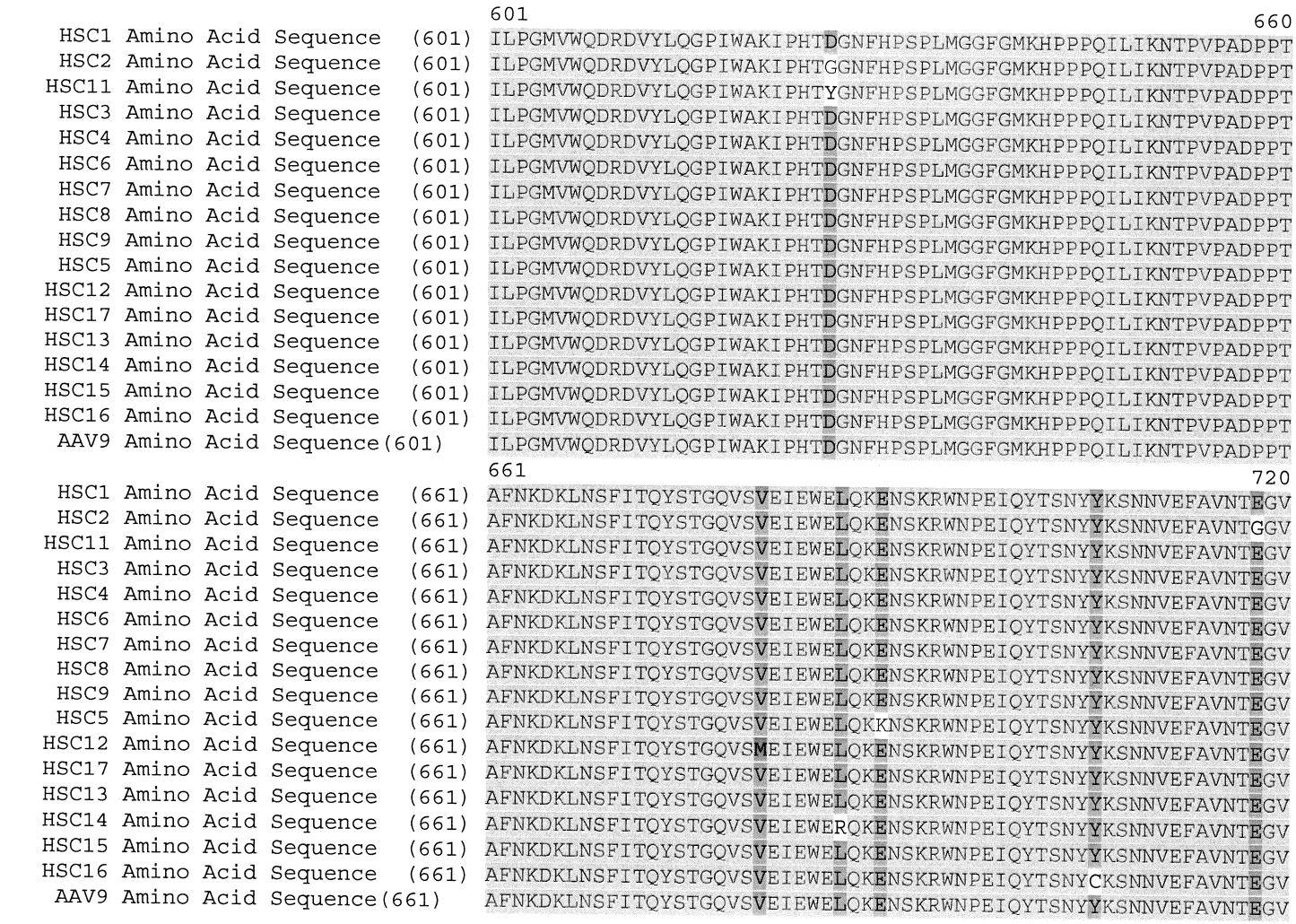


Figure 1 (cont.)

```
                                        721          736
  HSC1 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  HSC2 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
 HSC11 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  HSC3 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  HSC4 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  HSC6 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  HSC7 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  HSC8 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  HSC9 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  HSC5 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
 HSC12 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
 HSC17 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
 HSC13 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
 HSC14 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
 HSC15 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
 HSC16 Amino Acid Sequence  (721) YSEPRPIGTRYLTRNL
  AAV9 Amino Acid Sequence(721)   YSEPRPIGTRYLTRNL
```

Figure 2

Capsid	Base Change	AAV 9 hu. Isolate Amino Acid	Clone New Amino Acid	Difference in Amino Acids	Placement of Amino Acid (HVR = Hypervariable Region)
HSC1 SEQ ID NO: 2	G to A	GCT - Alanine	ACT - Threonine	Alanine is nonpolar & neutral; Threonine is polar & neutral	4 bases into capsid; 2nd amino acid; Not in HVR, VP1
HSC1 SEQ ID NO: 2	G to A	CGA - Arginine	CAA - Glutamine	Arginine is polar & strongly basic; Glutamine is polar & neutral	935 bases into the capsid; 312th amino acid; Not in HVR, VP3
HSC2 SEQ ID NO: 3	A to G	GGA - Glycine	GGG - Glycine	No Amino Acid Difference	1515 bases into the capsid; 505th amino acid; 1st amino acid after HVR 7, VP3
HSC2 SEQ ID NO: 3	A to G	GAC- Aspartic Acid	GGC - Glycine	Aspartic Acid is polar & acidic; Glycine is nonpolar & neutral	1877 bases into the capsid; 626th amino acid; Not in HVR, VP3
HSC2 SEQ ID NO: 3	A to G	GAA - Glutamic Acid	GGA - Glycine	Glutamic Acid is polar & acidic; Glycine is nonpolar & neutral	2153 bases into the capsid; 718th amino acid; In HVR 12 last amino acid, VP3
HSC3 SEQ ID NO: 5	G to A	GGC - Glycine	GAC - Aspartic Acid	Glycine is nonpolar & neutral; Aspartic Acid is polar and acidic	479 bases into the capsid; 160th amino acid; Not in HVR, VP1
HSC3 SEQ ID NO: 5	A to G	TCA - Serine	TCG - Serine	No Amino Acid Difference	1044 bases into the capsid, 348th amino acid; Not in HVR VP3
HSC4 SEQ ID NO: 6	T to C	TTC - Phenylalanine	CTC - Leucine	Phenylalanine is nonpolar & neutral; Leucine is nonpolar & neutral	355 bases into the capsid, 119th amino acid; Not in HVR; VP1
HSC4 SEQ ID NO: 6	C to T	CCC - Proline	TCC - Serine	Proline is nonpolar & neutral; Serine is polar & neutral	1402 bases into the capsid; 468th amino acid; In HVR 5, VP3
HSC4 SEQ ID NO: 6	G to A	CCG - Proline	CCA - Proline	No Amino Acid Difference	1809 bases into the capsid; 603rd amino acid; Not in HVR, VP3

Figure 2 (cont.)

Capsid	Base Change	AAV 9 hu. Isolate Amino Acid	Clone New Amino Acid	Difference in Amino Acids	Placement of Amino Acid (HVR = Hypervariable Region)
HSC6 SEQ ID NO: 8	A to G	CAG - Glutamine	CGG - Arginine	Glutamine is polar & neutral; Arginine is polar & strongly basic	1769 bases into the capsid; 590th amino acid; In HVR 10; VP3
HSC5 SEQ ID NO: 11	A to G	AAG - Lysine	AGG - Arginine	Lysine is polar & basic; Arginine is polar & strongly basic	230 bases into the capsid; 77th amino acid; Not in HVR, VP1
HSC5 SEQ ID NO: 11	C to T	CCC - Proline	CCT - Proline	No Amino Acid Difference	1404 bases into the capsid; 468th amino acid; In HVR 5, VP3
HSC5 SEQ ID NO: 11	G to A	GAA - Glutamic Acid	AAA - Lysine	Glutamic Acid is polar & acidic; Lysine is polar & basic	2068 bases into the capsid; 690th amino acid; Not in HVR, VP3
HSC5 SEQ ID NO: 11	T to C	AAT - Asparagine	AAC - Asparagine	No Amino Acid Difference	2148 bases into the capsid; 716th amino acid; In HVR 13, VP3
HSC11 SEQ ID NO: 5	G to A	GTC - Valine	ATC - Isoleucine	Isoleucine is nonpolar & neutral; valine is nonpolar & neutral	193 bases into the capsid; 65th amino acid; Not in HVR; VP1
HSC11 SEQ ID NO: 4	G to T	GAC- Aspartic Acid	TAC - Tyrosine	Aspartic Acid is polar & acidic; Tyrosine is polar & neutral	1876 bases into the capsid; 626th amino acid; Not in HVR; VP3
HSC7 SEQ ID NO: 8	C to T	GCA - Alanine	GTA - Valine	Alanine is nonpolar & neutral; Valine is nonpolar & neutral	203 bases into the capsid; 68th amino acid; Not in HVR; VP1
HSC7 SEQ ID NO: 8	C to T	TTC - Phenylalanine	TTT - Phenylalanine	No Amino Acid Difference	924 bases into the capsid; 308th amino acid; Not in HVR; VP3
HSC8 SEQ ID NO: 9	A to G	CAG - Glutamine	CGG - Arginine	Glutamine is polar & neutral; Arginine is polar & strongly basic	452 bases into the capsid; 151th amino acid; In HVR 1; VP1

Figure 2 (cont.)

Capsid	Base Change	AAV 9 hu. Isolate Amino Acid	Clone New Amino Acid	Difference in Amino Acids	Placement of Amino Acid (HVR = Hypervariable Region)
HSC8 SEQ ID NO: 9	G to A	GAG - Glutamine Acid	GAA - Glutamine Acid	No Amino Acid Difference	2058 bases into the capsid; 686th amino acid; Not in HVR; VP3
HSC9 SEQ ID NO: 10	T to C	TTT - Phenylalanine	TTC - Phenylalanine	No Amino Acid Difference	357 bases into the capsid; 119th amino acid; Not in HVR; VP1
HSC9 SEQ ID NO: 10	T to G	TGT - Cysteine	GGT - Glycine	Cysteine is polar & neutral; Glycine is nonpolar & neutral	616 bases into the capsid; 206th amino acid; Not in HVR; Very beginning of VP3
HSC12 SEQ ID NO: 12	A to T	GCA - Alanine	GCT - Alanine	No Amino Acid Difference	579 bases into capsid; 193rd amino acid into capsid, in the end of VP2 near HVR 2
HSC12 SEQ ID NO: 12	G to A	CGT - Arginine	CAT - Histidine	Arginine is polar and strongly basic; Histidine is polar and weakly basic	887 bases into capsid; 296th amino acid into capsid; in VP3, not in HVR
HSC12 SEQ ID NO: 12	G to A	AGT - Serine	AAT - Asparagine	Serine is polar and neutral; Asparagine is polar and neutral	1391 bases into capsid; 464th amino acid into capsid; in VP3 in HVR 5
HSC12 SEQ ID NO: 12	T to C	GCT - Alanine	GCC - Alanine	No Amino Acid Difference	1506 bases into capsid; 502nd amino acid into capsid; in VP3 in HVR 7
HSC12 SEQ ID NO: 12	G to A	GGA - Glycine	AGA - Arginine	Glycine is nonpolar and neutral; Arginine is polar and strongly basic	1513 bases into capsid; 505th amino acid into capsid; in VP3, right after HVR 7
HSC12 SEQ ID NO: 12	G to A	GTG - Valine	ATG - Methionine	Valine is nonpolar and neutral; Methionine is nonpolar and neutral	2041 bases into capsid; 681st amino acid into capsid; in VP3; not in HVR
HSC13 SEQ ID NO: 14	G to A	GGA - Glycine	AGA - Arginine	Glycine is nonpolar and neutral; Arginine is polar and strongly basic	1513 bases into capsid; 505th amino acid into capsid; in VP3, right after HVR 7

Figure 2 (cont.)

Capsid	Base Change	AAV 9 hu. Isolate Amino Acid	Clone New Amino Acid	Difference in Amino Acids	Placement of Amino Acid (HVR = Hypervariable Region)
HSC14 SEQ ID NO: 15	G to A	GGA - Glycine	AGA - Arginine	Glycine is nonpolar and neutral; Arginine is polar and strongly basic	1513 bases into capsid; 505th amino acid into capsid; in VP3, right after HVR 7
HSC14 SEQ ID NO: 15	T to G	CTG - Leucine	CGG - Arginine	Leucine is nonpolar and neutral; Arginine is polar and strongly basic	2060 bases into capsid; 687th amino acid into capsid; in VP3; Not in HVR.
HSC15 SEQ ID NO: 16	A to G	ACG - Threonine	GCG - Alanine	Threonine is polar and neutral; Arginine is polar and strongly basic	1036 bases into capsid; 346th amino acid into the capsid; In VP3; Not in HVR
HSC15 SEQ ID NO: 16	G to A	GGA - Glycine	AGA - Arginine	Glycine is nonpolar and neutral; Arginine is polar and strongly basic	1513 bases into capsid; 505th amino acid into capsid; in VP3, right after HVR 7
HSC16 SEQ ID NO: 17	T to A	TTT - Phenylalanine	ATT - Isoleucine	Phenylalanine is nonpolar and neutral; Isoleucine is nonpolar and neutral	1501 bases into capsid; 501st amino acid into capsid; In VP3, In HVR7
HSC16 SEQ ID NO: 17	G to A	GGA - Glycine	AGA - Arginine	Glycine is nonpolar and neutral; Arginine is polar and strongly basic	1513 bases into capsid; 505th amino acid into capsid; in VP3, right after HVR 7
HSC16 SEQ ID NO: 17	G to A	CCG - Proline	CCA - Proline	No Amino Acid Difference	1713 bases into capsid; 571st amino acid into capsid; In VP3 Not in HVR
HSC16 SEQ ID NO: 17	A to G	TAC - Tyrosine	TGC - Cysteine	Tyrosine is polar and neutral; Cysteine is polar and neutral	2117 bases into capsid; 706th amino acid into capsid; In VP3; In HVR 12 and is next to a YF mutation
HSC17 SEQ ID NO: 13	C to T	GAC - Aspartic Acid	GAT - Aspartic Acid	No Amino Acid Difference	180 bases into the capsid; 60th amino acid into capsid; In VP1; Not in HVR
HSC17 SEQ ID NO: 13	G to A	GGA - Glycine	AGA - Arginine	Glycine is nonpolar and neutral; Arginine is polar and strongly basic	1513 bases into capsid; 505th amino acid into capsid; in VP3, right after HVR 7

Figure 3

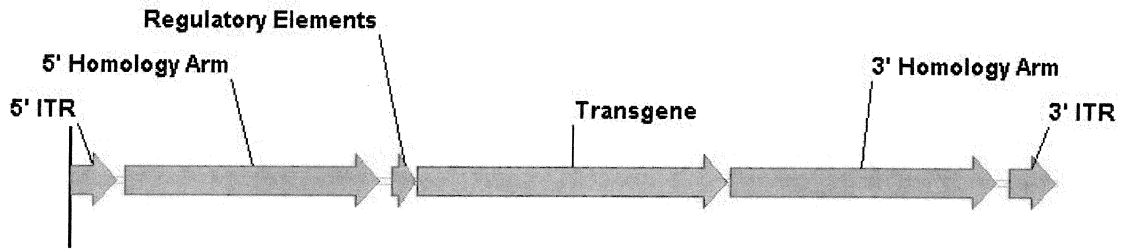
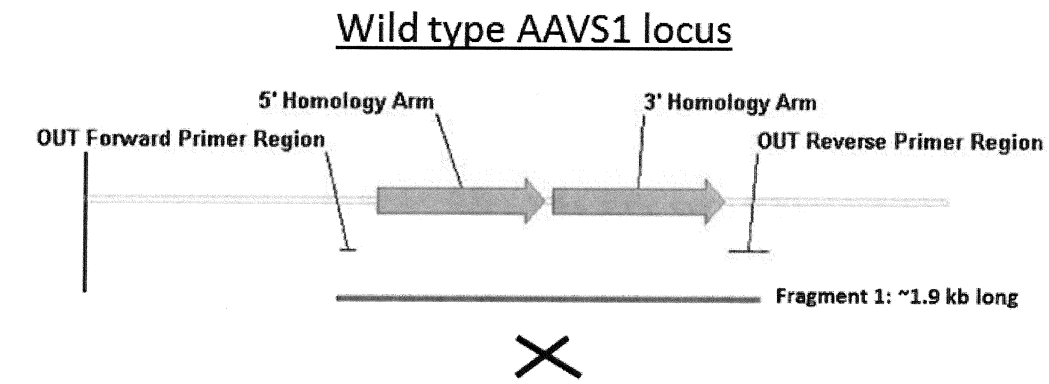

Regulatory Elements
5' Homology Arm
5' ITR
Transgene
3' Homology Arm
3' ITR

Figure 4A



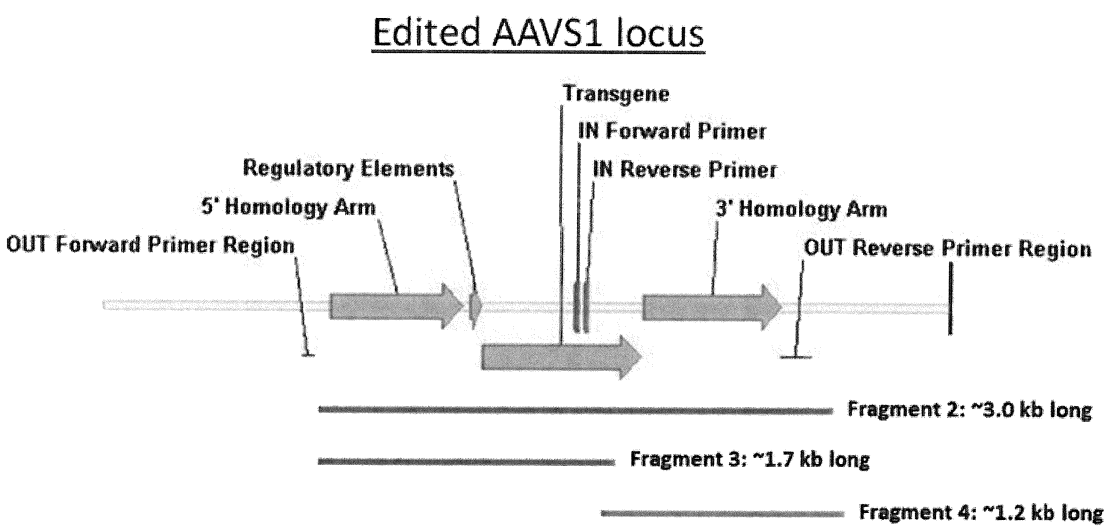
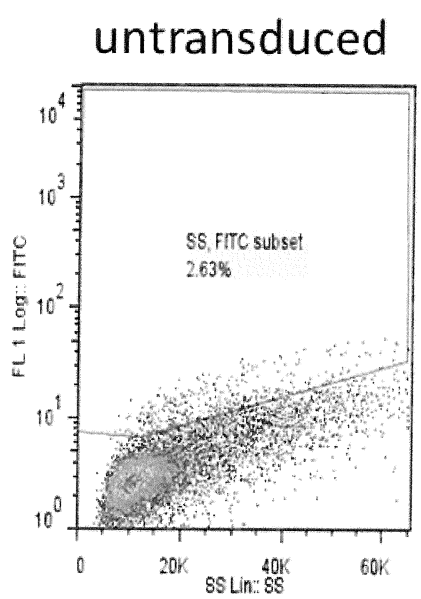


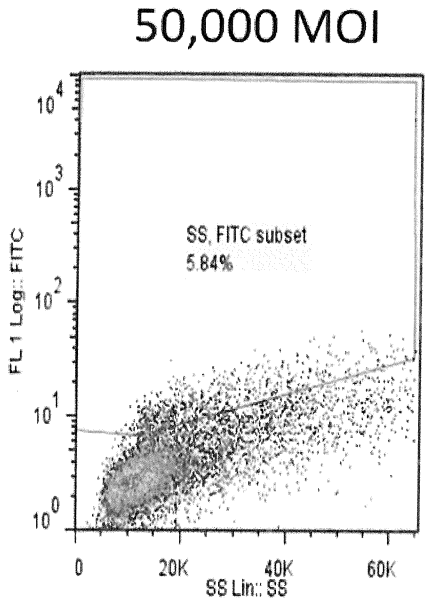
Figure 4B

Figure 5

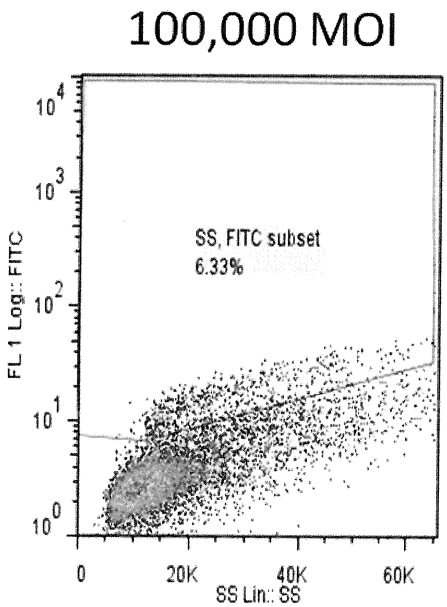
A.



B.



C.



D.

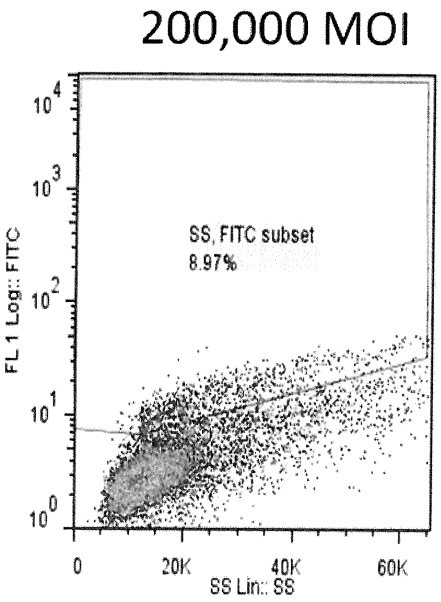


Figure 5 (cont.)

E.

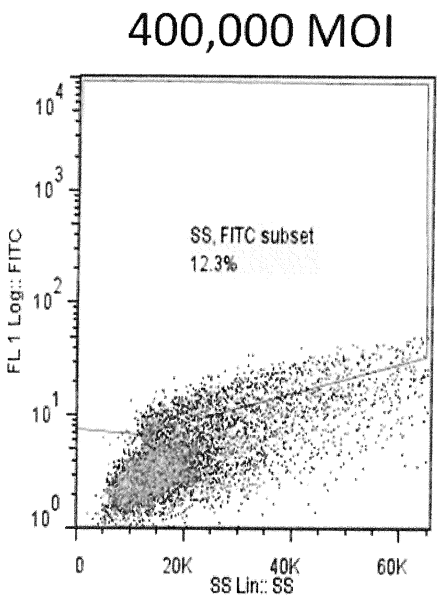
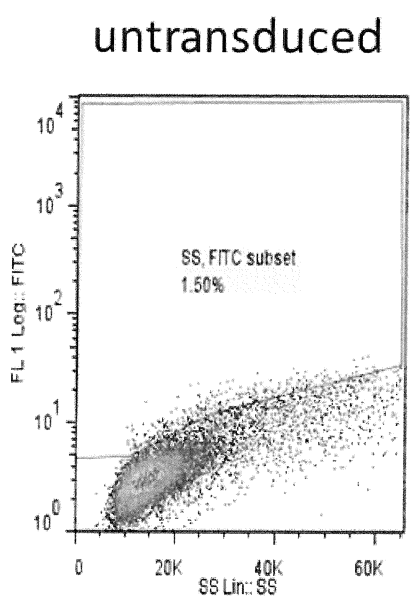
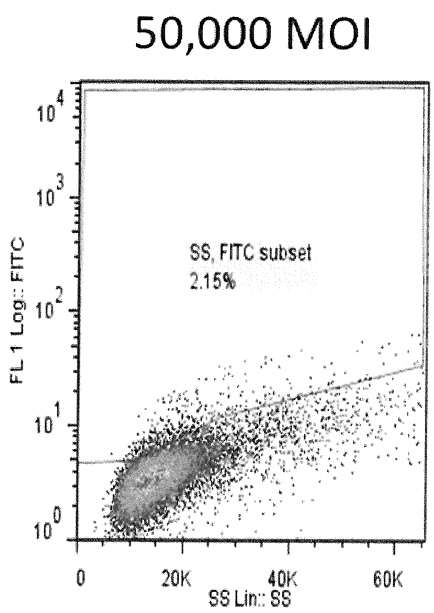


Figure 6

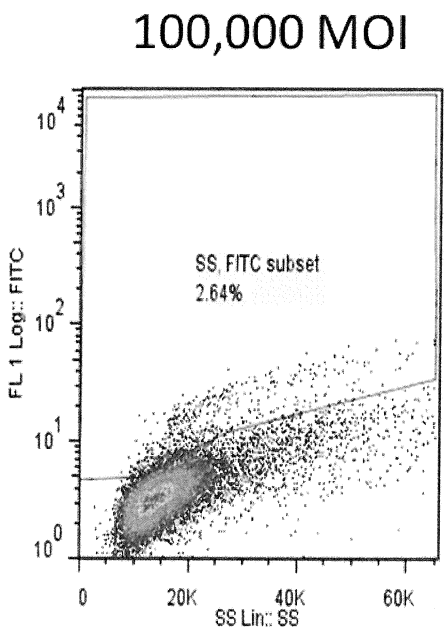
A.



B.



C.



D.

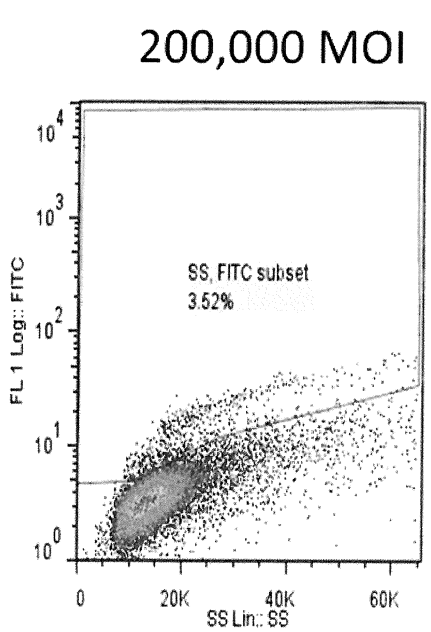


Figure 6 (cont.)

E.

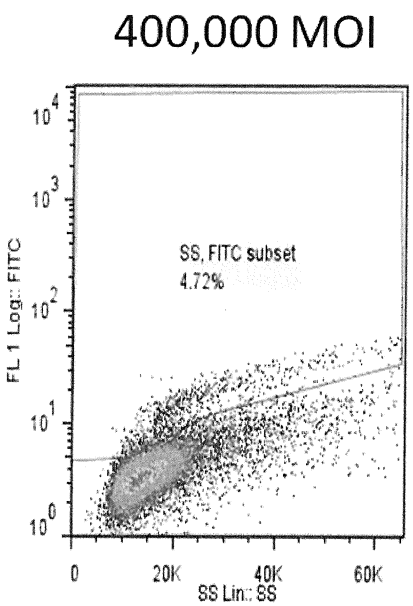
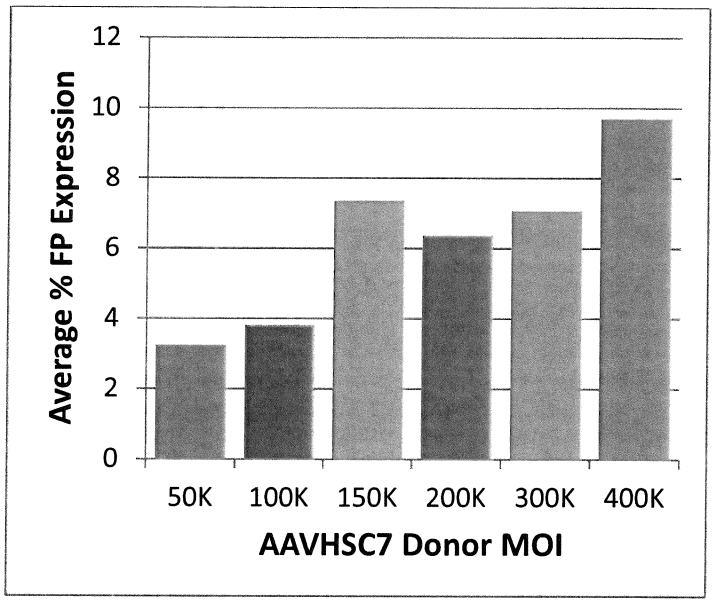


Figure 7

A.


Average % FP Expression
12
10
8
6
4
2
0
50K
100K
150K
200K
300K
400K
AAVHSC7 Donor MOI

B.

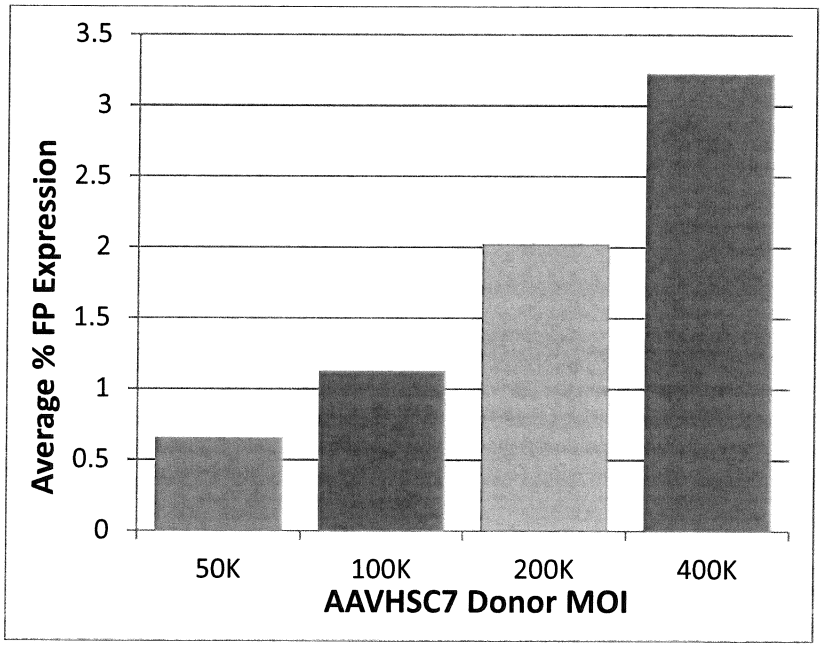
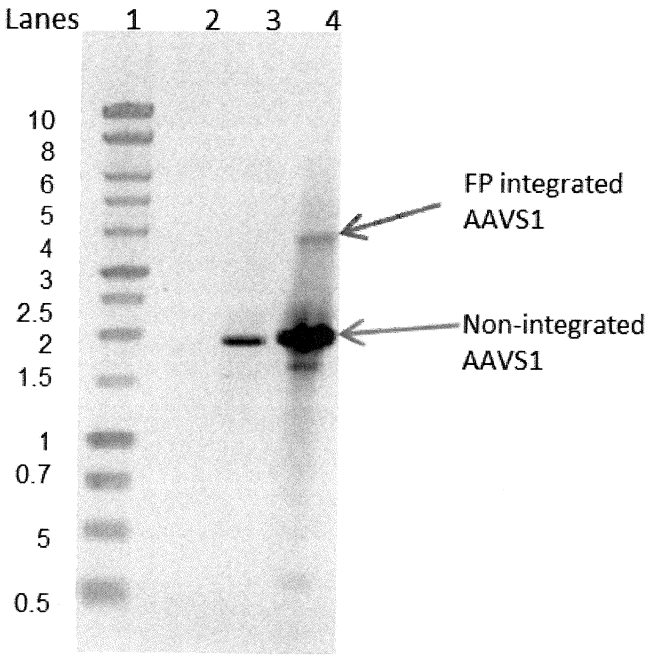

Average % FP Expression
3.5
3
2.5
2
1.5
1
0.5
0
50K
100K
200K
400K
AAVHSC7 Donor MOI

Figure 8

A.


Lanes 1 2 3 4
10
8
6
5
4
3
2.5
2
1.5
1
0.7
5
0.5
FP integrated AAVS1
Non-integrated AAVS1

B.

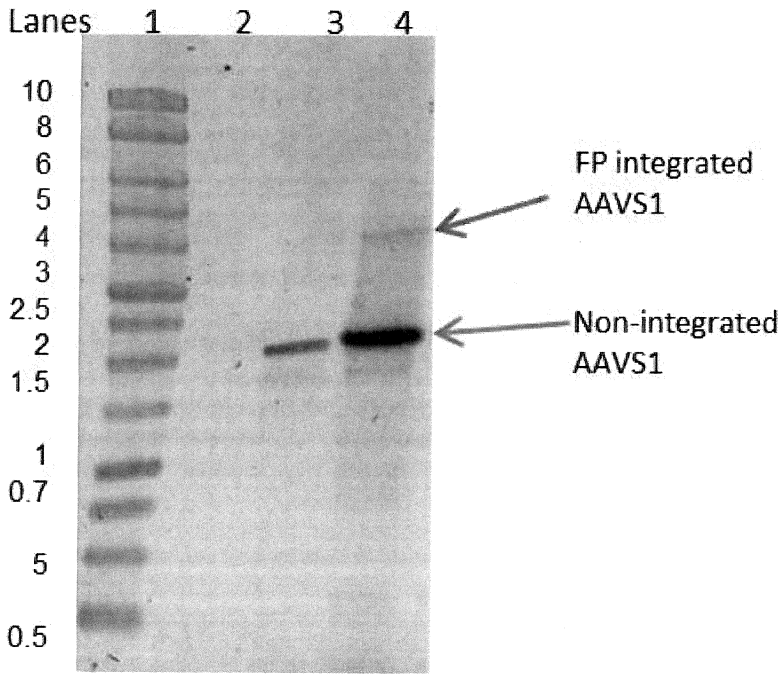
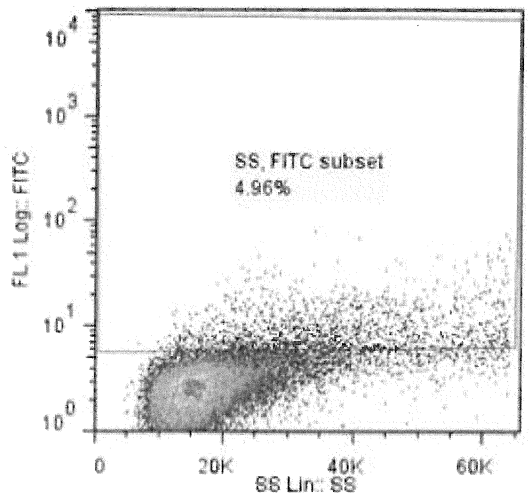
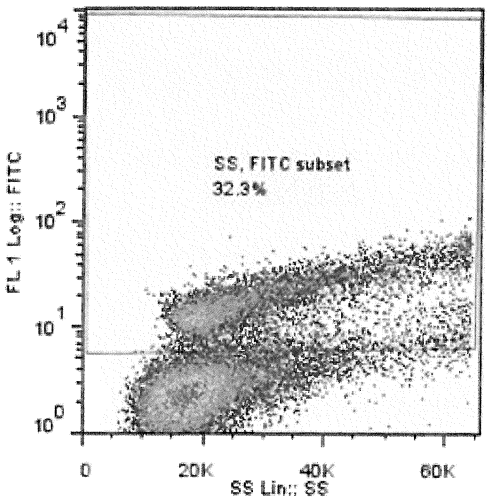

Lanes 1 2 3 4
10
8
6
5
4
3
2.5
2
1.5
1
0.7
5
0.5
FP integrated AAVS1
Non-integrated AAVS1

Figure 9

A.


SS, FITC subset
4.96%
FL 1 Log:: FITC
SS Lin:: SS
0
20K
40K
60K

B.


SS, FITC subset
32.3%
FL 1 Log:: FITC
SS Lin:: SS
0
20K
40K
60K

C.

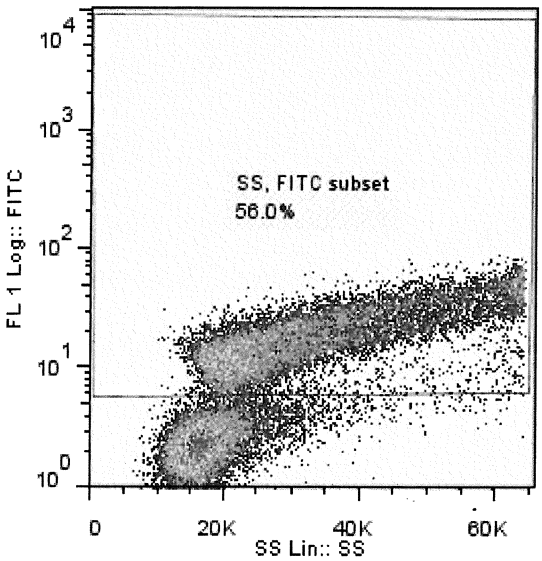
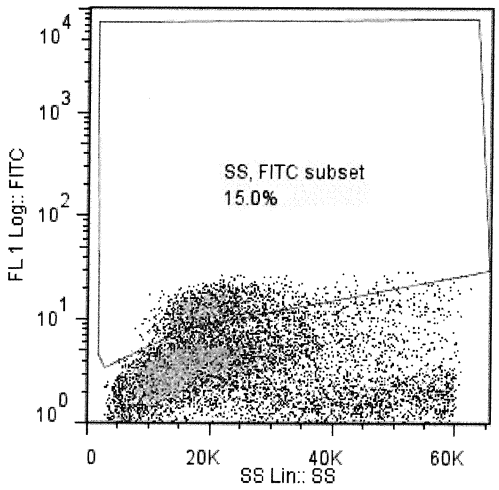
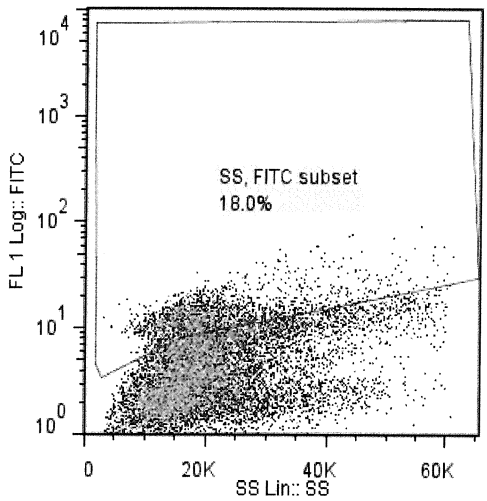

SS, FITC subset
56.0%
FL 1 Log:: FITC
SS Lin:: SS
0
20K
40K
60K

Figure 10

A.



B.



C.

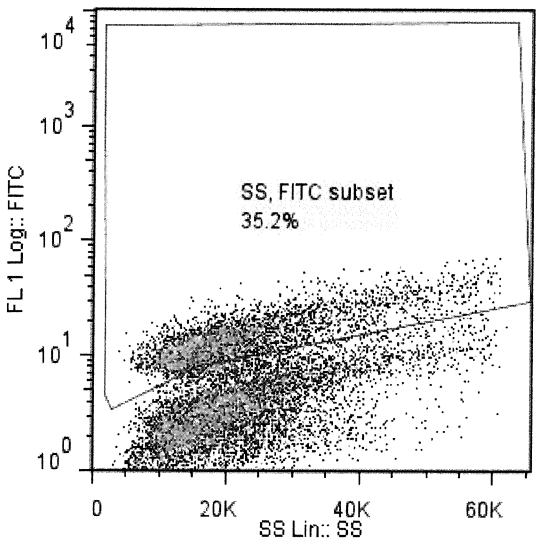
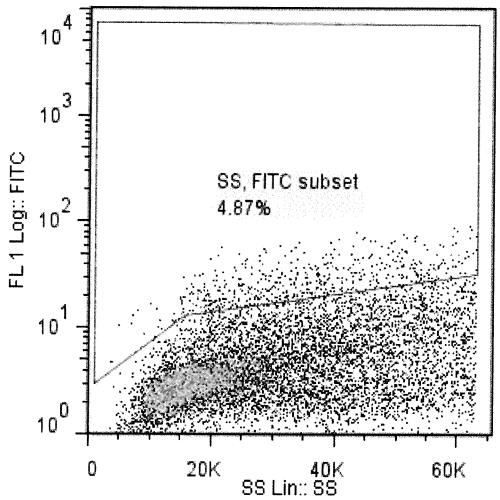
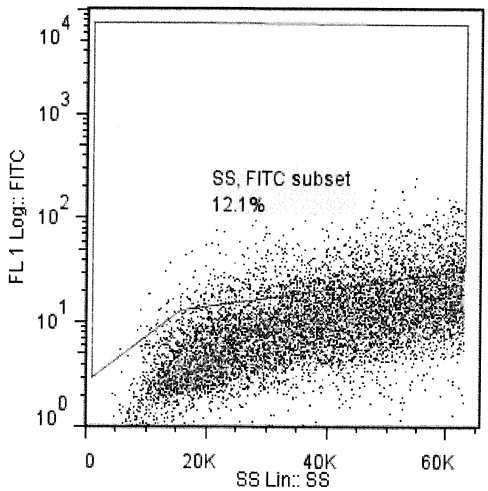


Figure 11

A.



B.



C.

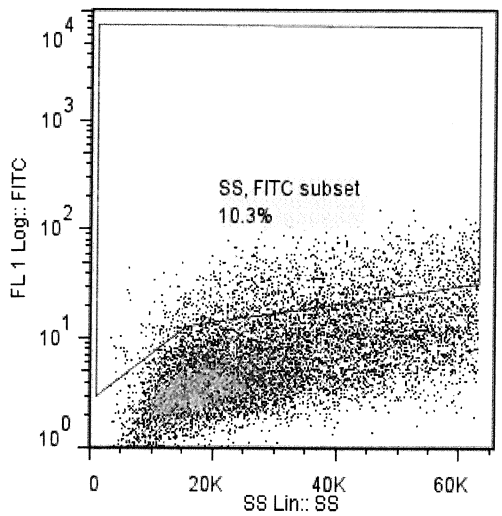


Figure 12

A.

% FP Positive Cells

	4 day	18 day	20 day	39 day
Untransduced	0	0	0	0
AAVHSC7 FP Donor	3	7.23	4.07	4.61
AAVHSC17 FP Donor	20.2	5.43	2.85	9.18

B.

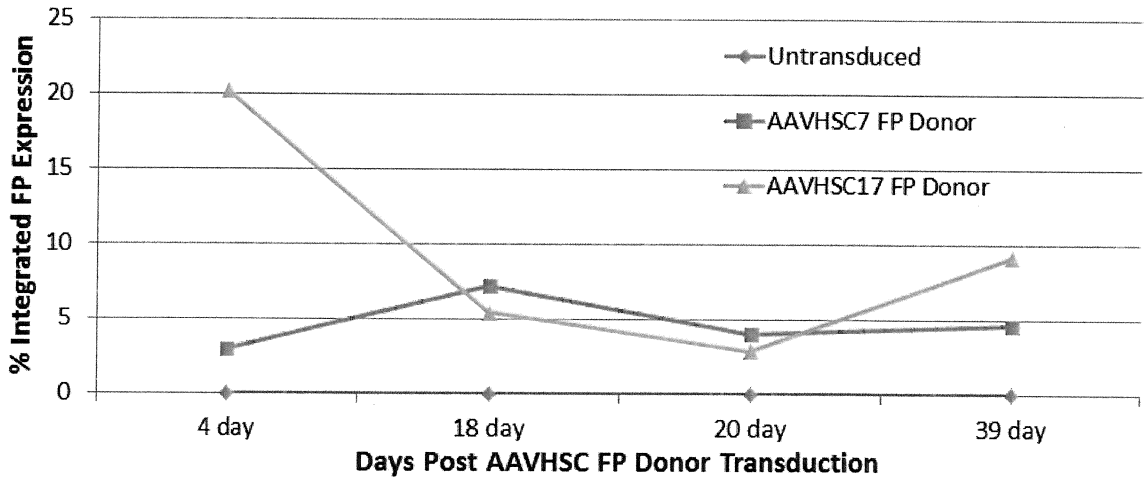


Figure 13

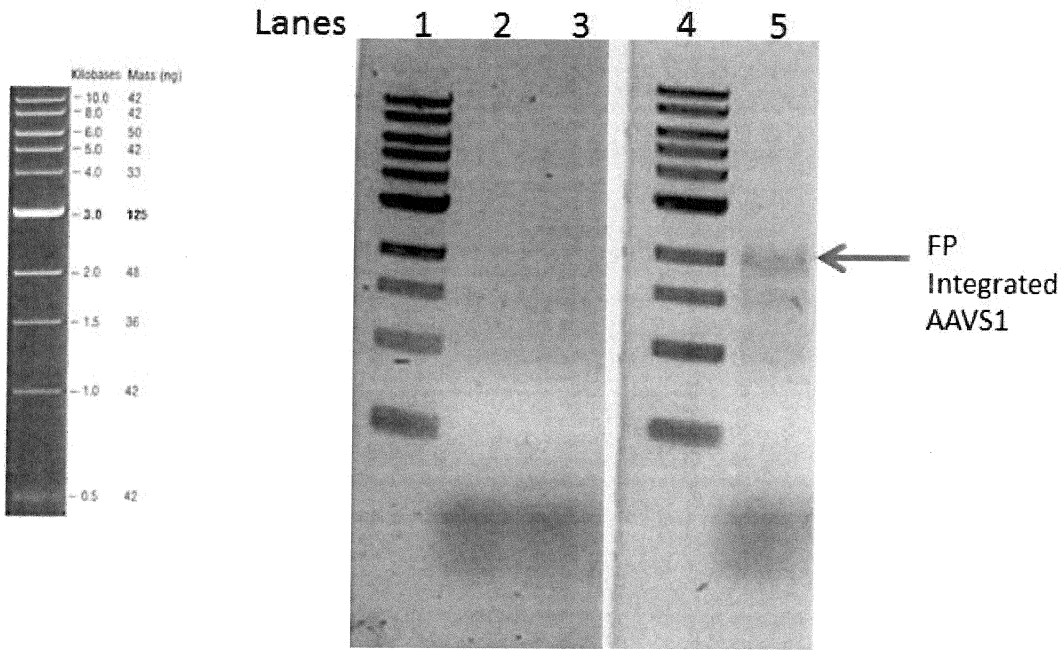

Lanes 1 2 3 4 5
Kilobases Mass (ng)
10.0 42
8.0 42
6.0 50
5.0 42
4.0 33
3.0 125
2.0 48
1.5 36
1.0 42
0.5 42
FP
Integrated
AAVS1

Figure 14

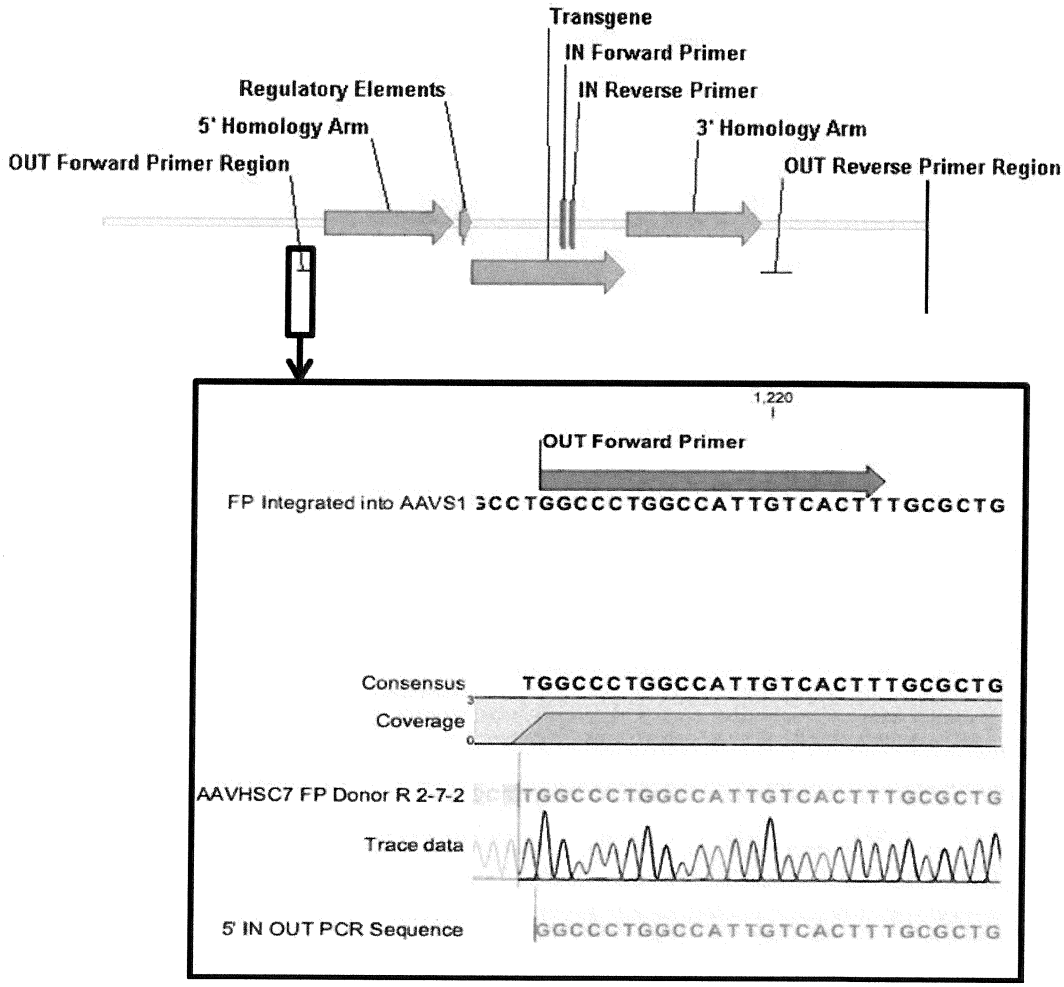

Transgene
IN Forward Primer
IN Reverse Primer
Regulatory Elements
5' Homology Arm
3' Homology Arm
OUT Forward Primer Region
OUT Reverse Primer Region
1,220
OUT Forward Primer
FP Integrated into AAVS1 3CCTGGCCCTGGCCATTGTCACTTTGCGCTG
Consensus
TGGCCCTGGCCATTGTCACTTTGCGCTG
Coverage
AAVHSC7 FP Donor R 2-7-2
TGGCCCTGGCCATTGTCACTTTGCGCTG
Trace data
5' IN OUT PCR Sequence
GGCCCTGGCCATTGTCACTTTGCGCTG

Figure 15

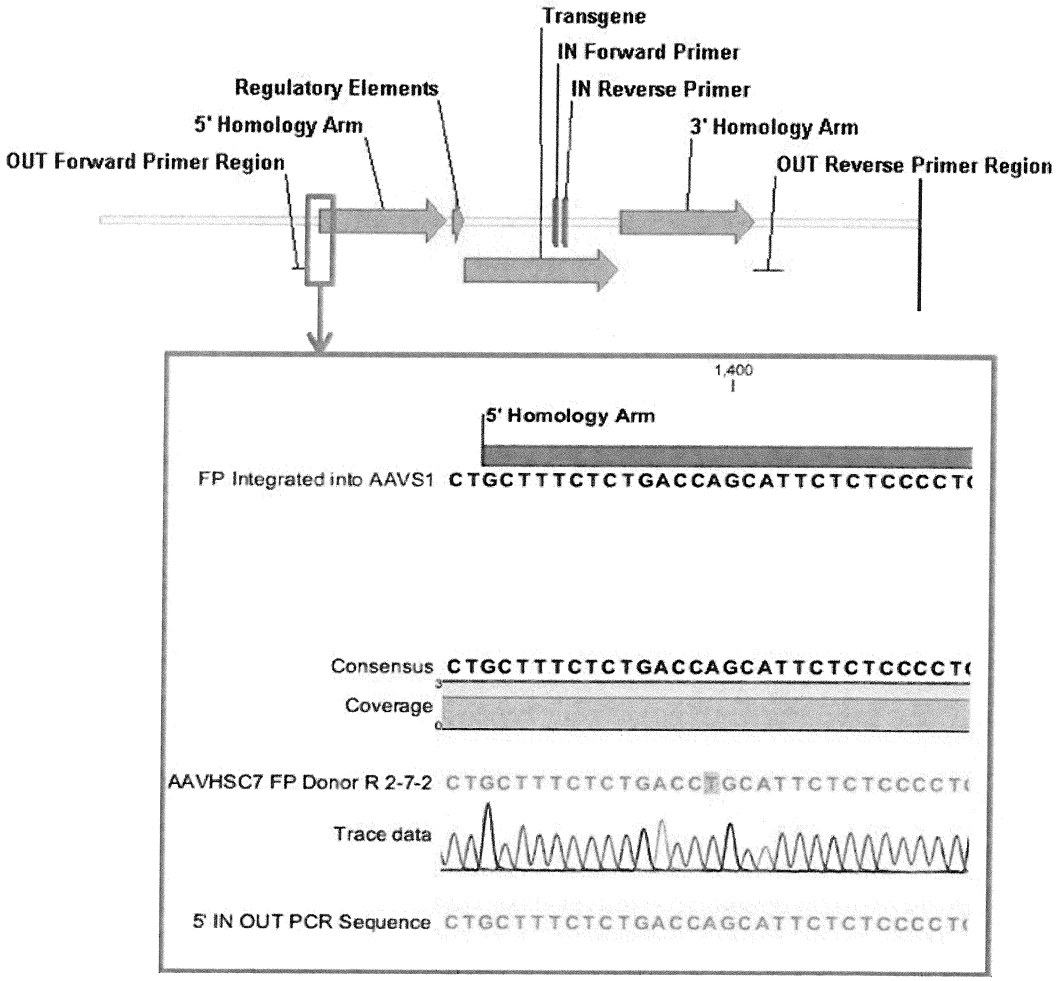

Transgene
IN Forward Primer
IN Reverse Primer
Regulatory Elements
5' Homology Arm
3' Homology Arm
OUT Forward Primer Region
OUT Reverse Primer Region
1,400
1
5' Homology Arm
FP Integrated into AAVS1 CTGCTTTCTCTGACCAGCATTCTCTCCCCTC
Consensus CTGCTTTCTCTGACCAGCATTCTCTCCCCTC
Coverage
AAVHSC7 FP Donor R 2-7-2 CTGCTTTCTCTGACCTGCATTCTCTCCCCTC
Trace data
5' IN OUT PCR Sequence CTGCTTTCTCTGACCAGCATTCTCTCCCCTC

Figure 16

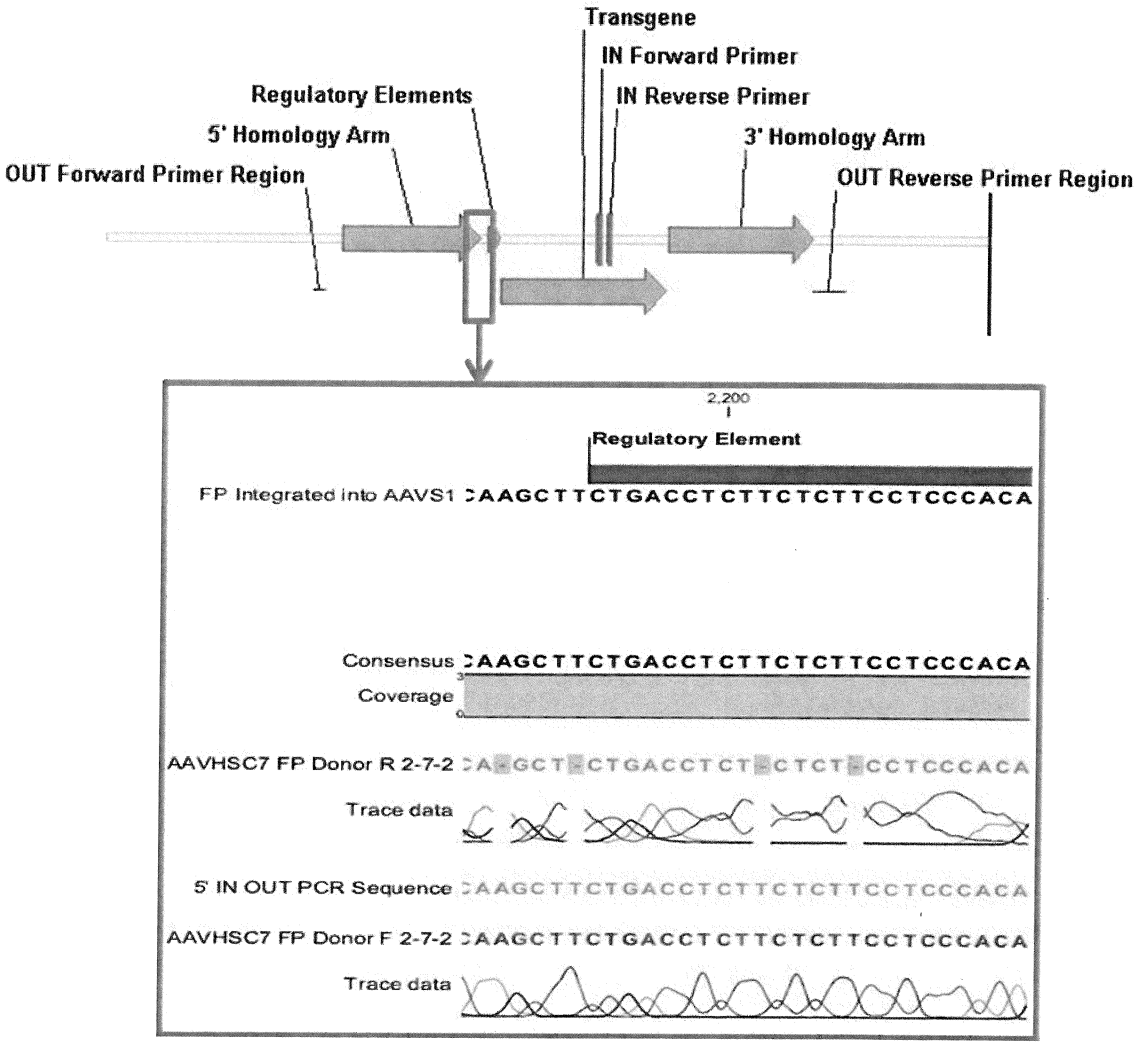

Transgene
IN Forward Primer
IN Reverse Primer
Regulatory Elements
5' Homology Arm
3' Homology Arm
OUT Forward Primer Region
OUT Reverse Primer Region
2,200
Regulatory Element
FP Integrated into AAVS1
Consensus
Coverage
AAVHSC7 FP Donor R 2-7-2
Trace data
5' IN OUT PCR Sequence
AAVHSC7 FP Donor F 2-7-2
Trace data

Figure 17

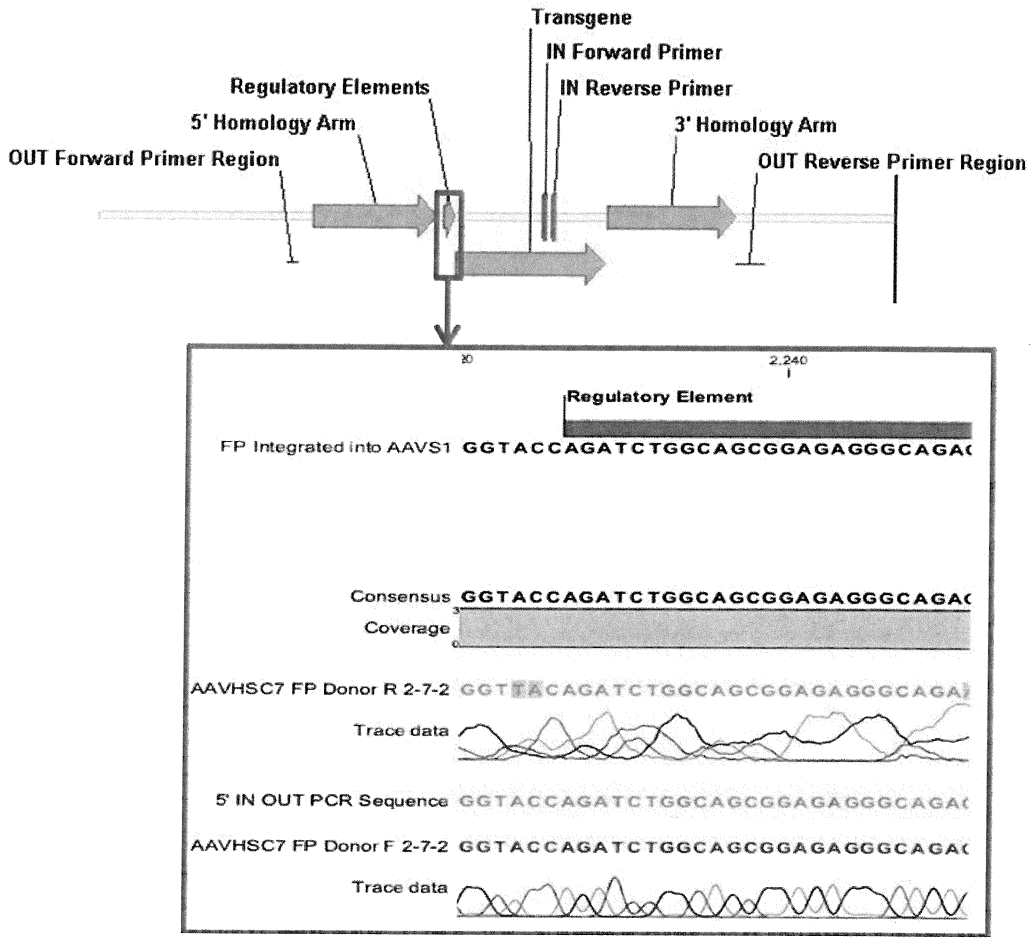

Transgene
IN Forward Primer
IN Reverse Primer
Regulatory Elements
5' Homology Arm
3' Homology Arm
OUT Forward Primer Region
OUT Reverse Primer Region
20
2,240
Regulatory Element
FP Integrated into AAVS1 GGTACCAGATCTGGCAGCGGAGAGGGCAGA
Consensus GGTACCAGATCTGGCAGCGGAGAGGGCAGA
Coverage
AAVHSC7 FP Donor R 2-7-2 GGTTACAGATCTGGCAGCGGAGAGGGCAGA
Trace data
5' IN OUT PCR Sequence GGTACCAGATCTGGCAGCGGAGAGGGCAGA
AAVHSC7 FP Donor F 2-7-2 GGTACCAGATCTGGCAGCGGAGAGGGCAGA
Trace data

Figure 18

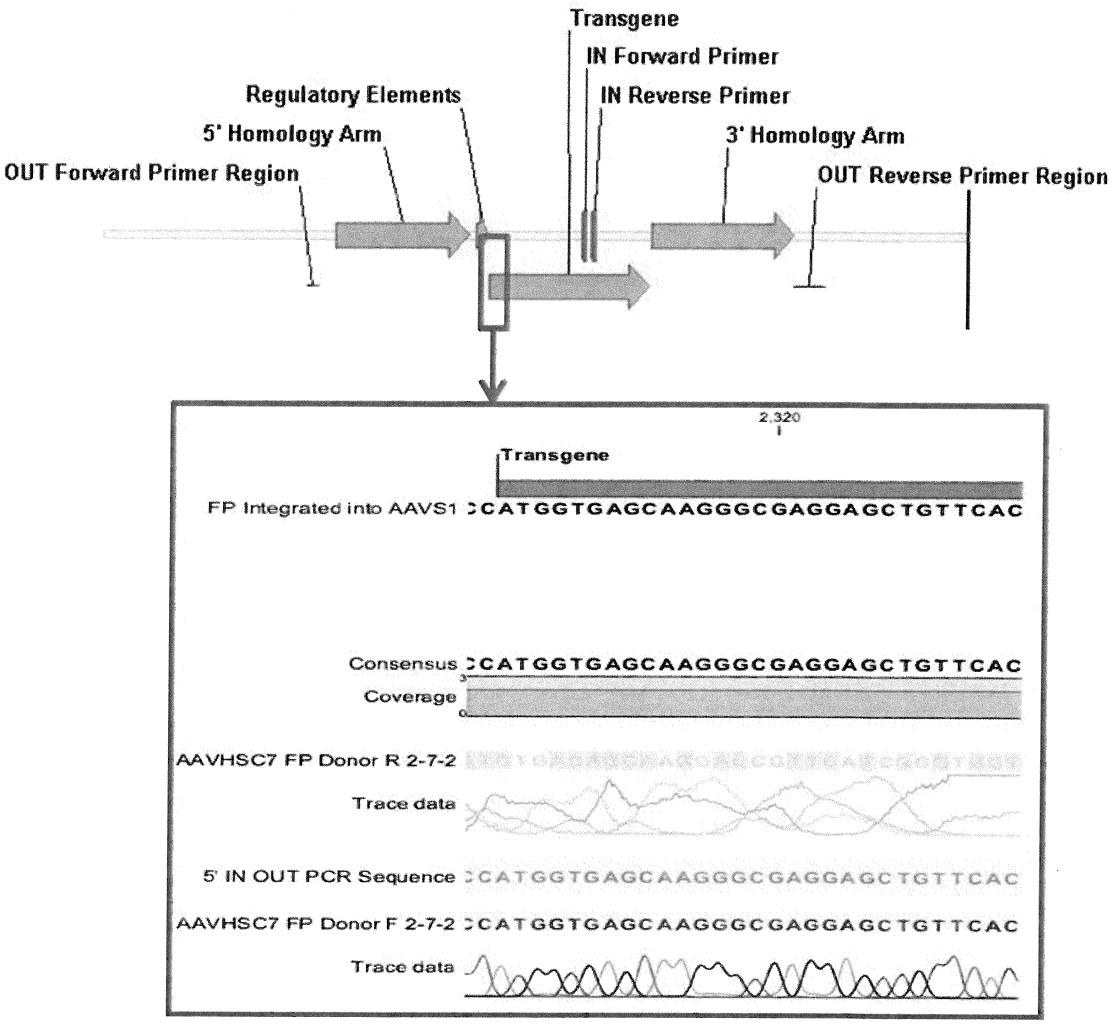

Transgene
IN Forward Primer
IN Reverse Primer
Regulatory Elements
5' Homology Arm
3' Homology Arm
OUT Forward Primer Region
OUT Reverse Primer Region
2,320
Transgene
FP Integrated into AAVS1 CATGGTGAGCAAGGGCGAGGAGCTGTTCAC
Consensus CATGGTGAGCAAGGGCGAGGAGCTGTTCAC
Coverage
AAVHSC7 FP Donor R 2-7-2
Trace data
5' IN OUT PCR Sequence CATGGTGAGCAAGGGCGAGGAGCTGTTCAC
AAVHSC7 FP Donor F 2-7-2 CATGGTGAGCAAGGGCGAGGAGCTGTTCAC
Trace data

Figure 19

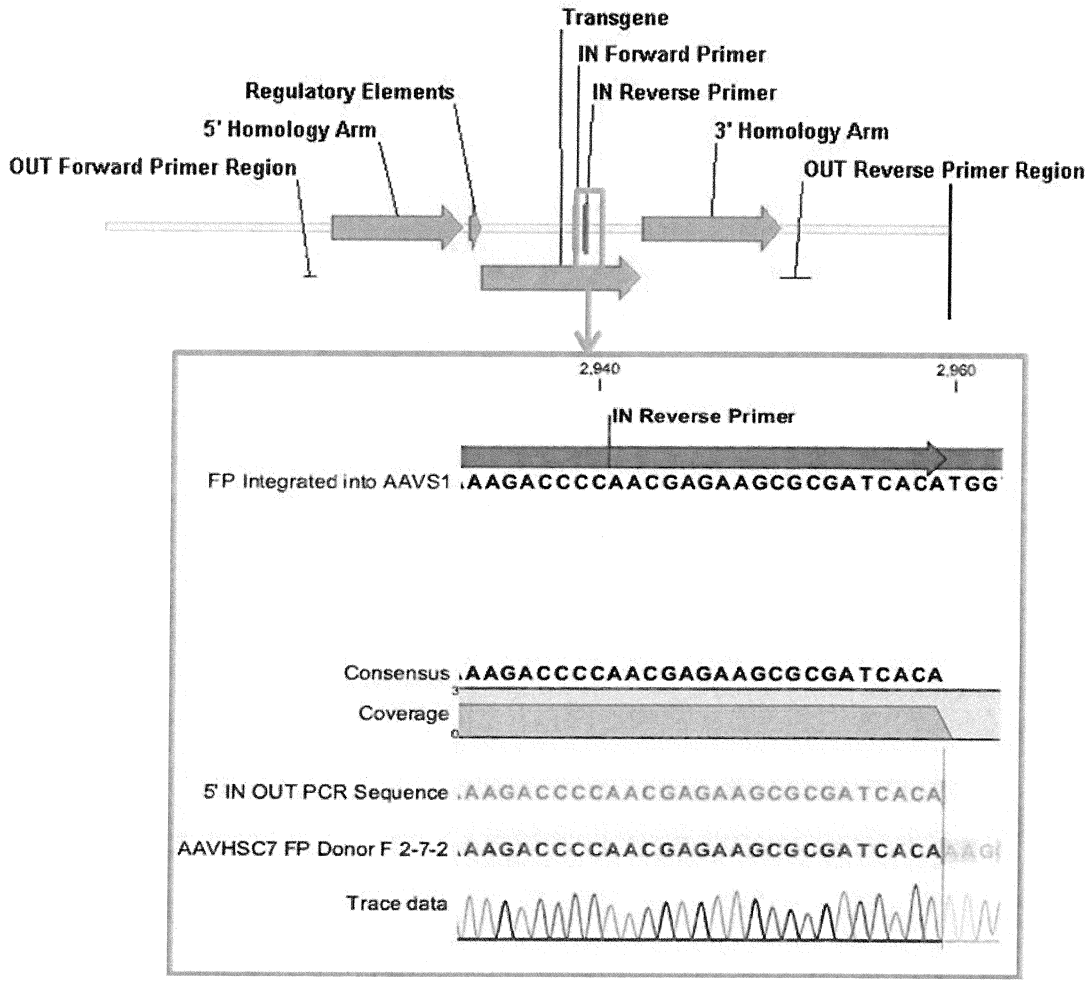

Transgene
IN Forward Primer
IN Reverse Primer
Regulatory Elements
5' Homology Arm
3' Homology Arm
OUT Forward Primer Region
OUT Reverse Primer Region
2,940
2,960
IN Reverse Primer
FP Integrated into AAVS1 AAGACCCCAACGAGAAGCGCGATCACATGG
Consensus AAGACCCCAACGAGAAGCGCGATCACA
Coverage
5' IN OUT PCR Sequence AAGACCCCAACGAGAAGCGCGATCACA
AAVHSC7 FP Donor F 2-7-2 AAGACCCCAACGAGAAGCGCGATCACA
Trace data

Figure 20

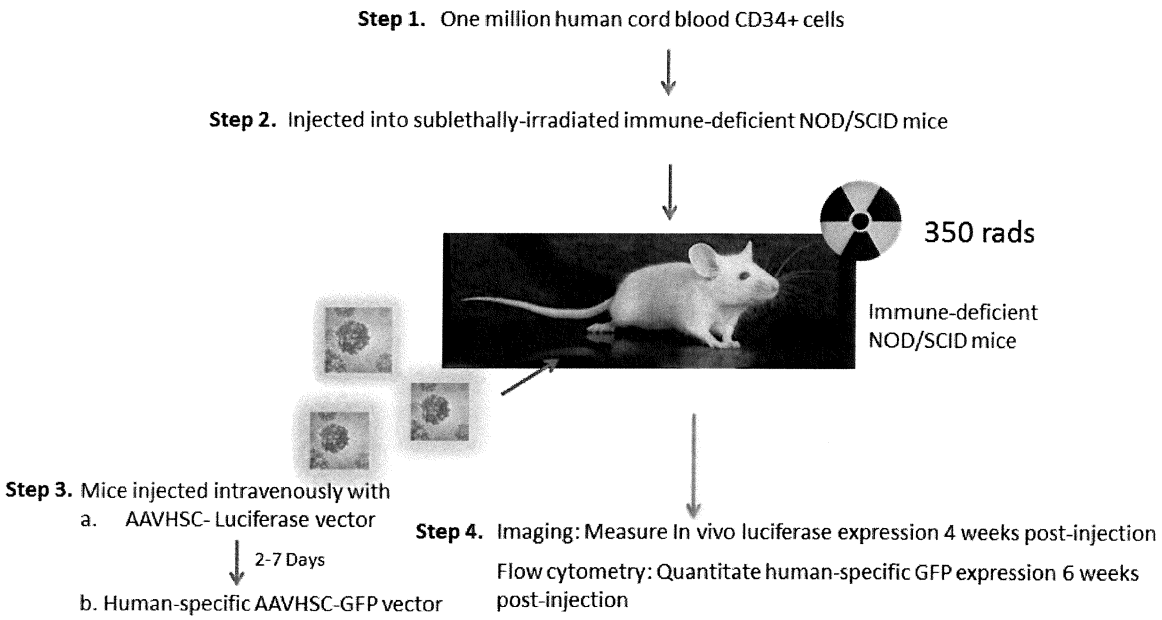
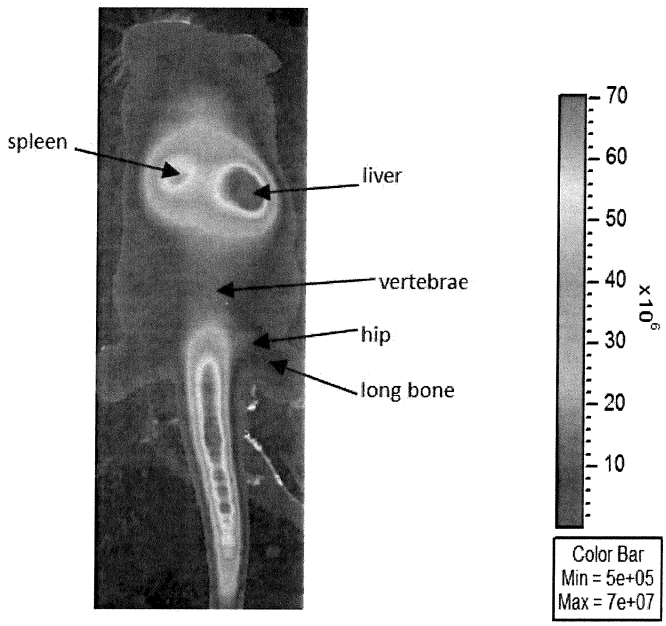

Step 1. One million human cord blood CD34+ cells
Step 2. Injected into sublethally-irradiated immune-deficient NOD/SCID mice
350 rads
Immune-deficient NOD/SCID mice
Step 3. Mice injected intravenously with
a. AAVHSC- Luciferase vector
2-7 Days
b. Human-specific AAVHSC-GFP vector
Step 4. Imaging: Measure In vivo luciferase expression 4 weeks post-injection
Flow cytometry: Quantitate human-specific GFP expression 6 weeks post-injection

Figure 21

A.


spleen
liver
vertebrae
hip
long bone
70
60
50
40
30
20
10
x10⁶
Color Bar
Min = 5e+05
Max = 7e+07

B.

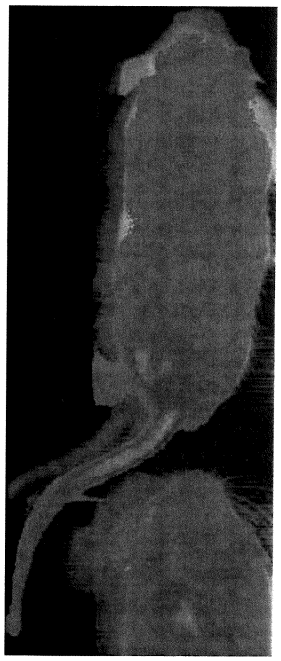
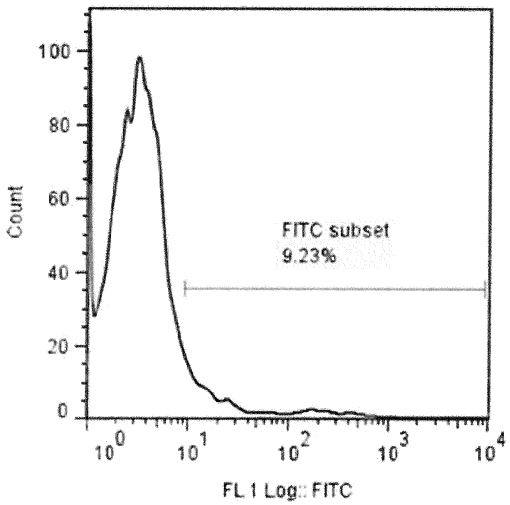
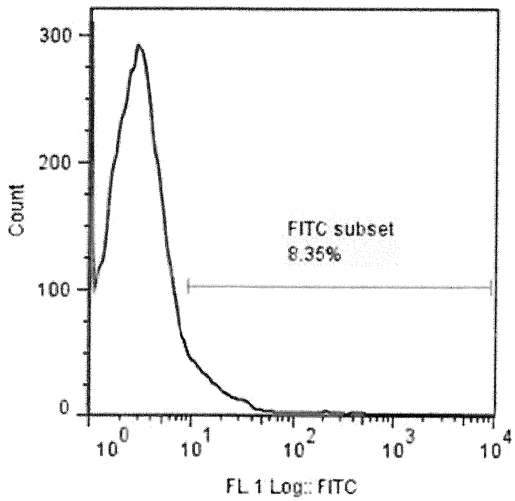


Figure 22

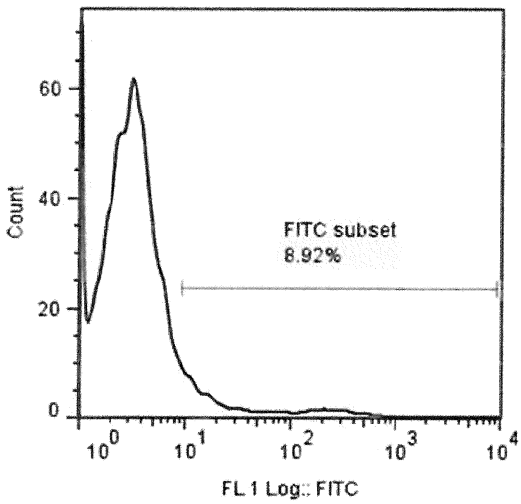
A.



B.



C.



D.

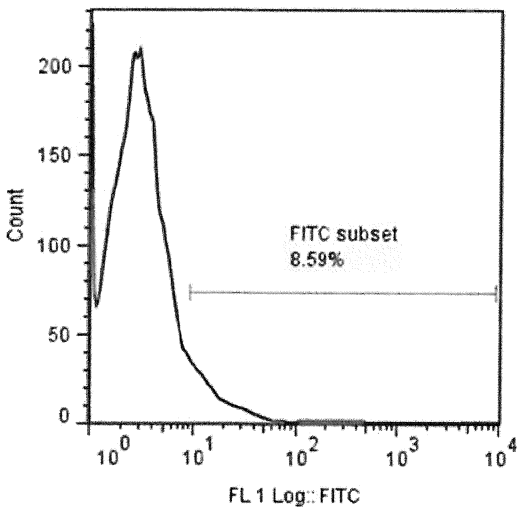
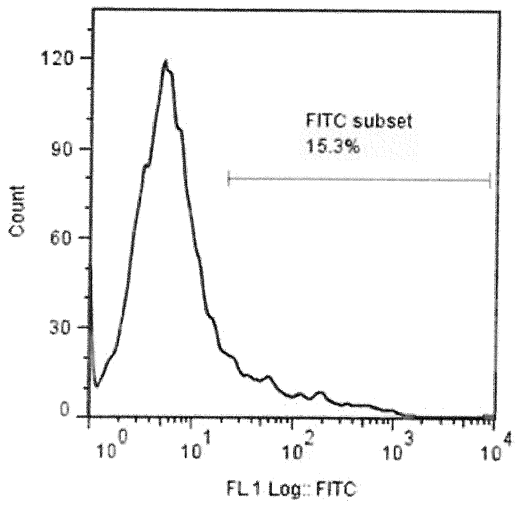
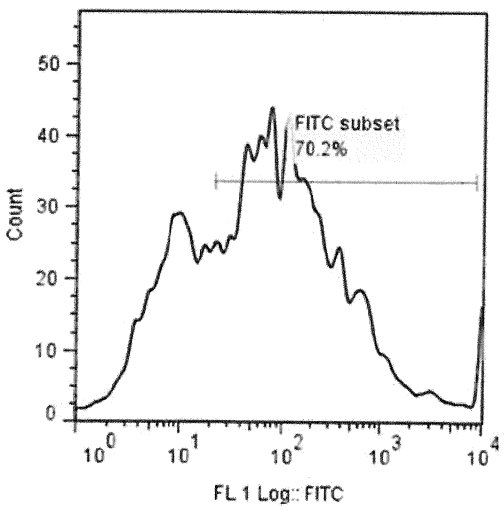


Figure 22 (cont.)

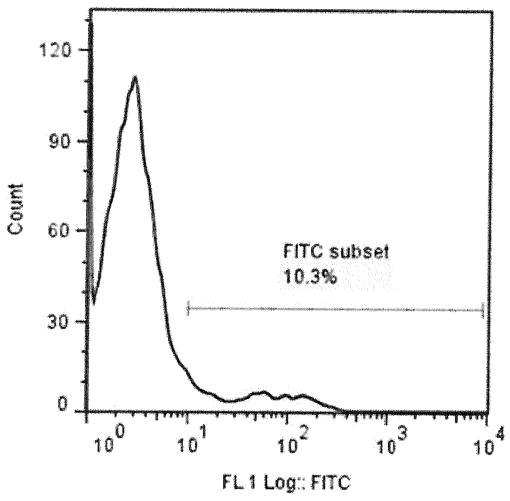
E.



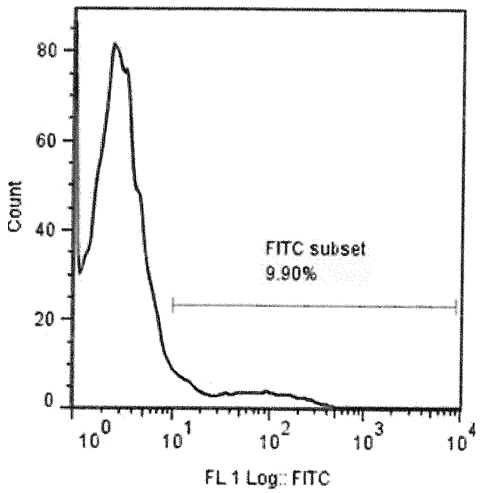
F.

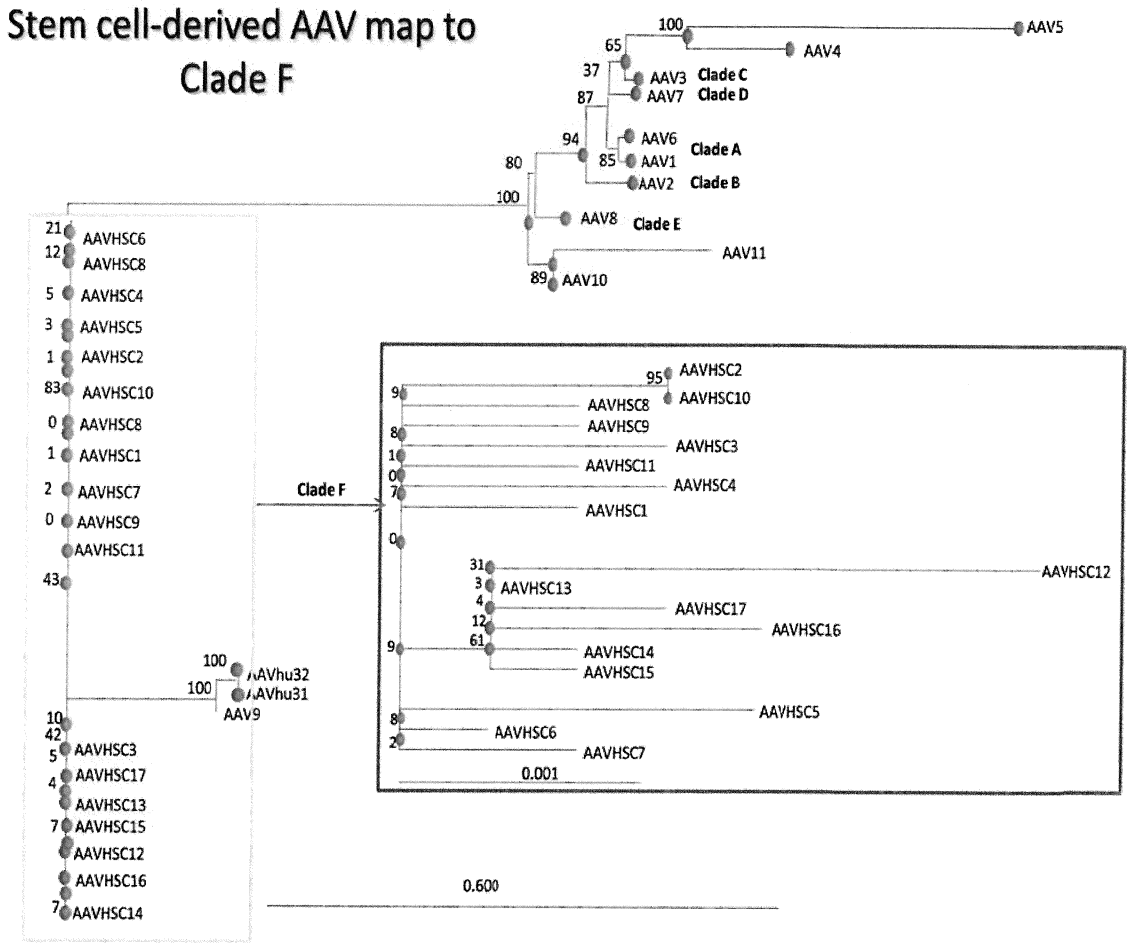


G.



H.





• Nucleotide homology

Figure 23

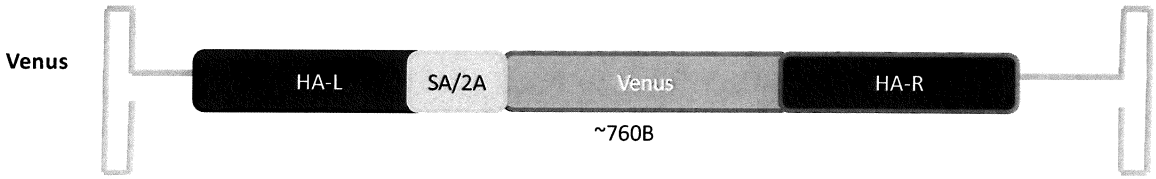


Figure 24

Schema: Targeted Integration of Promoterless Reporter into PPP1r12C INTRON (AAVS1)

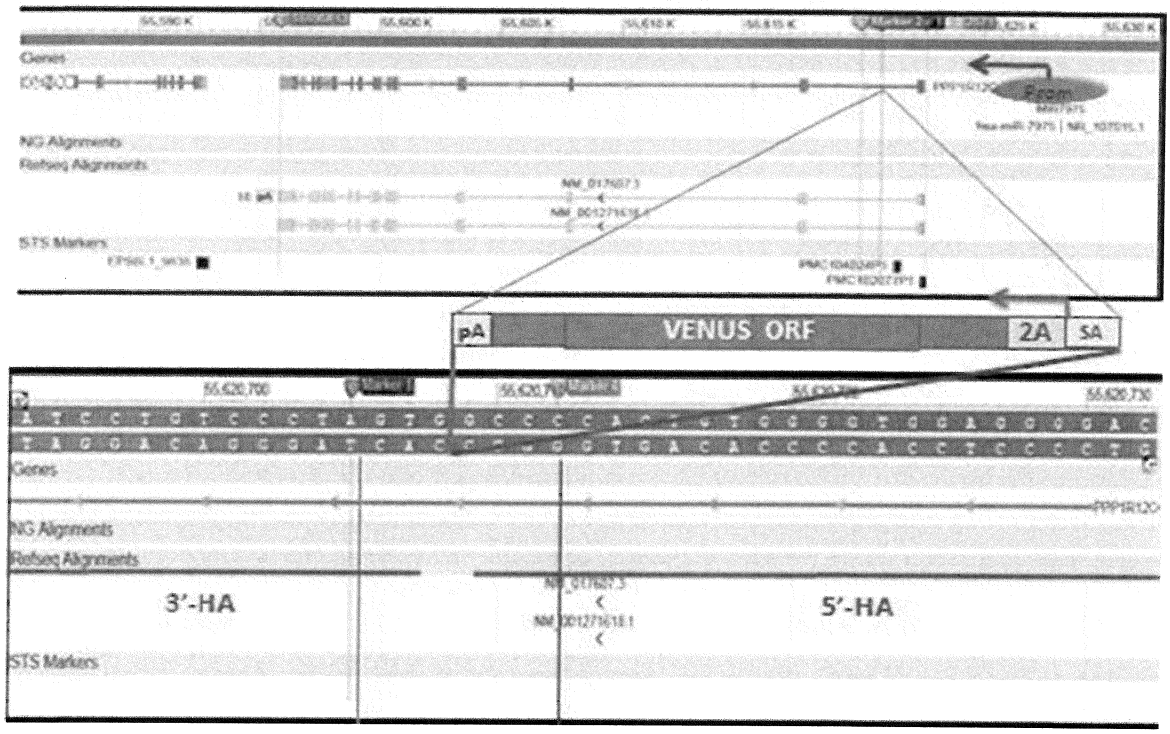


Figure 25

Figure 26A

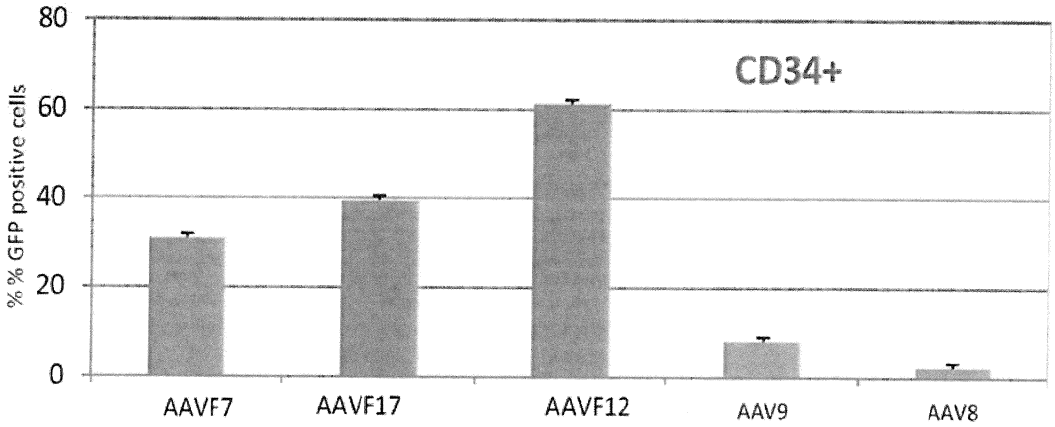


Figure 26B

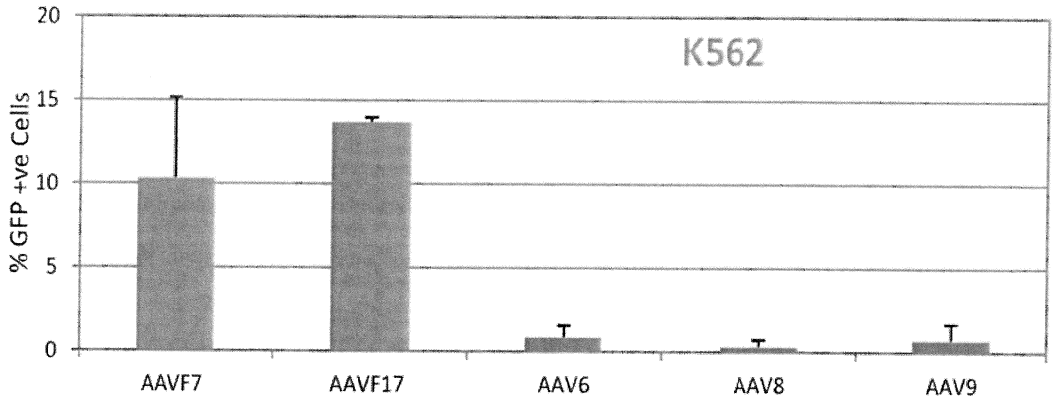
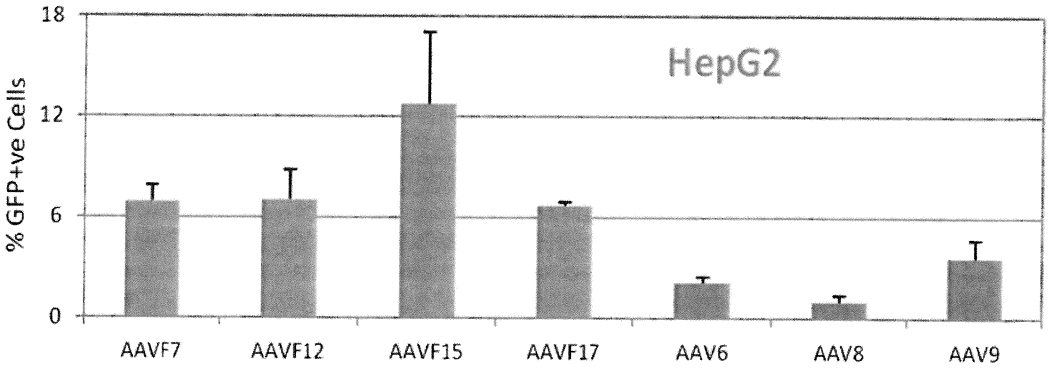
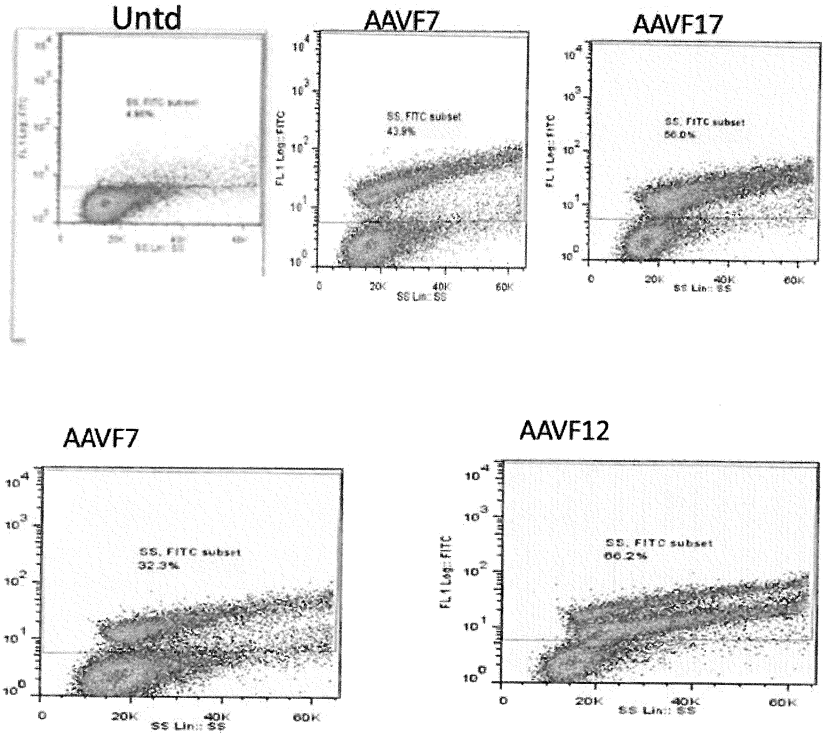


Figure 26C





- AAVF mediates efficient gene targeting in primary CD34+ cells
- High efficiency targeting is observed in human leukemic & liver cell lines
- AAVF editing does not require DNA synthesis

Figure 26D

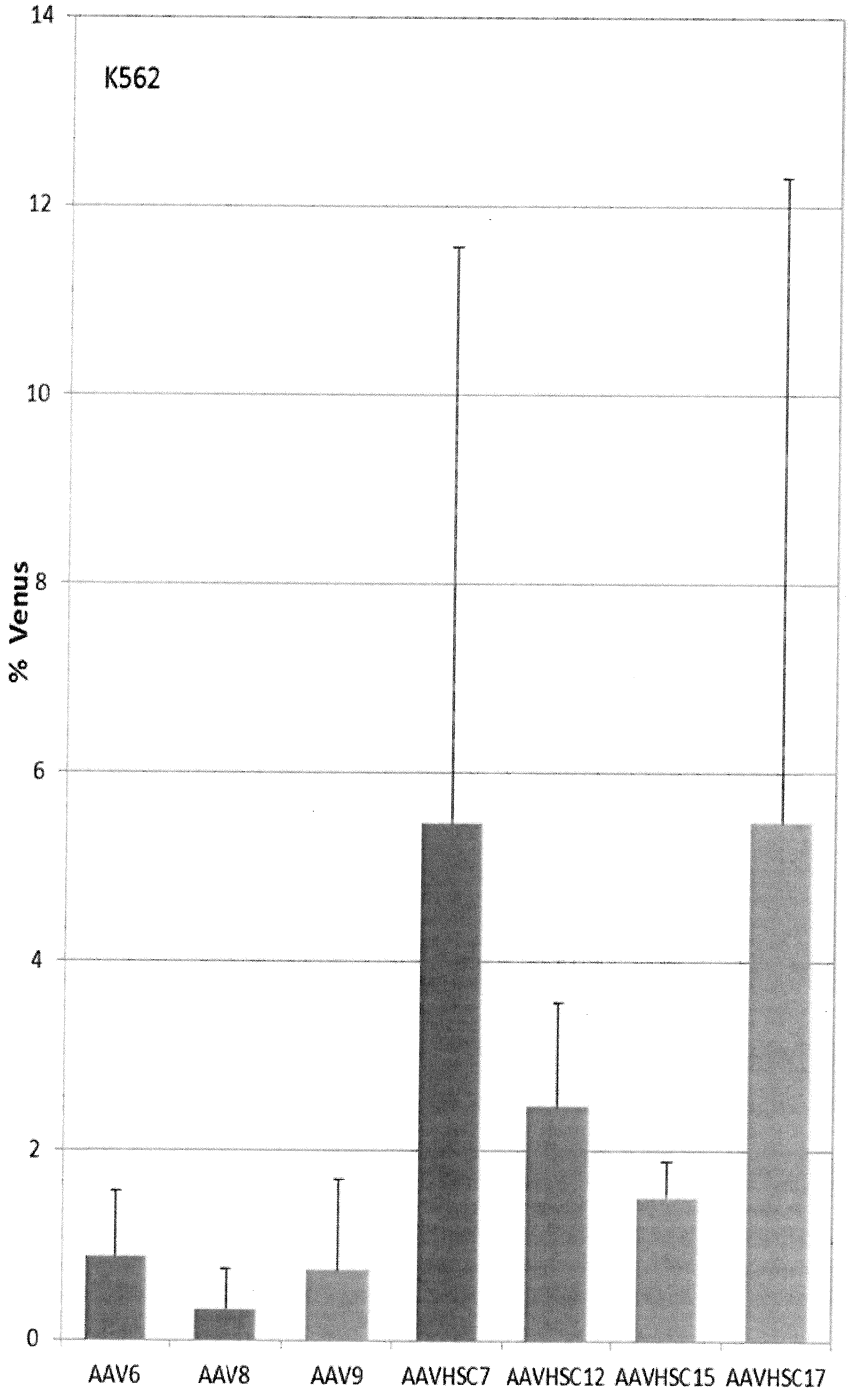

K562
% Venus
14
12
10
8
6
4
2
0
AAV6
AAV8
AAV9
AAVHSC7
AAVHSC12
AAVHSC15
AAVHSC17

Figure 26E

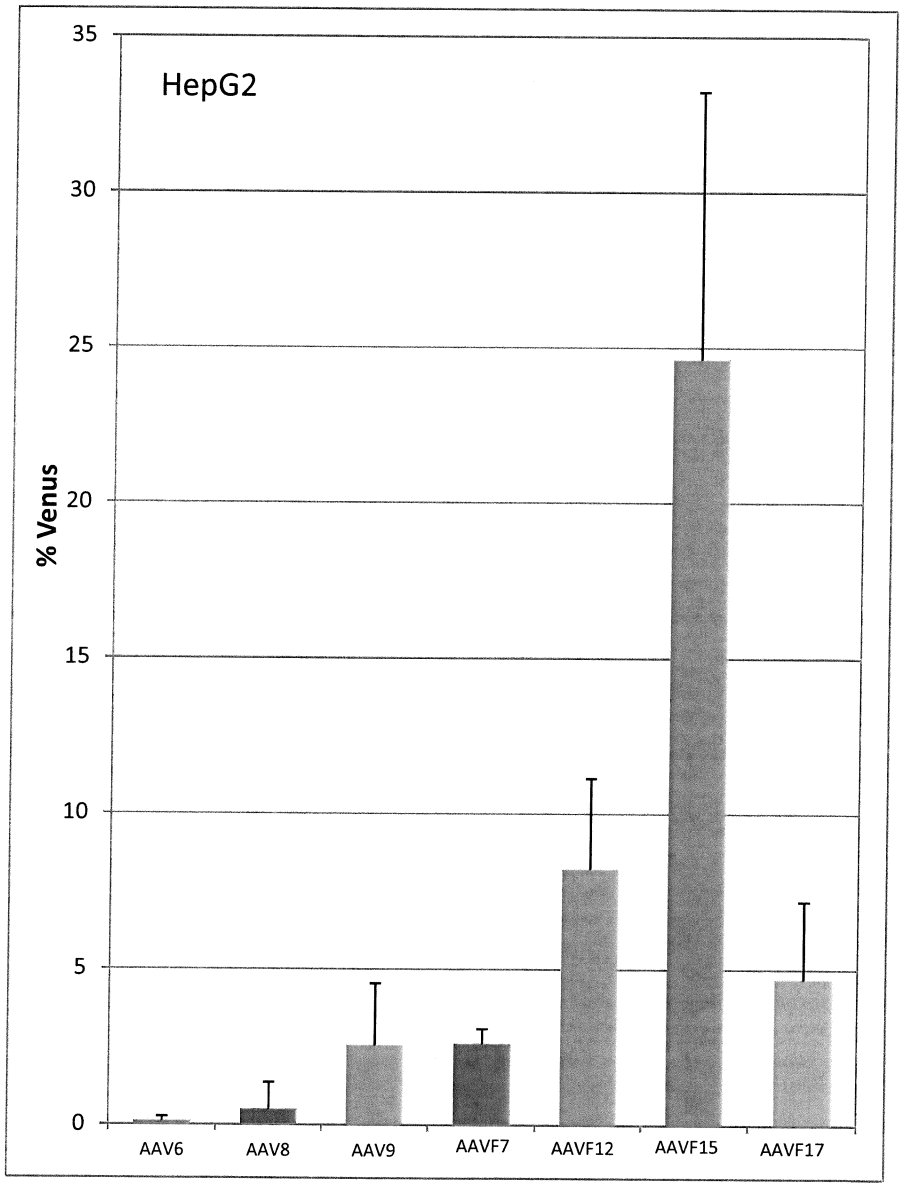

HepG2
% Venus
35
30
25
20
15
10
5
0
AAV6
AAV8
AAV9
AAVF7
AAVF12
AAVF15
AAVF17

Figure 26F

AAVF VECTORS TARGET INTEGRATION OF LARGE ORFS INTO THE AAVS1 LOCUS IN CD34+ CELLS

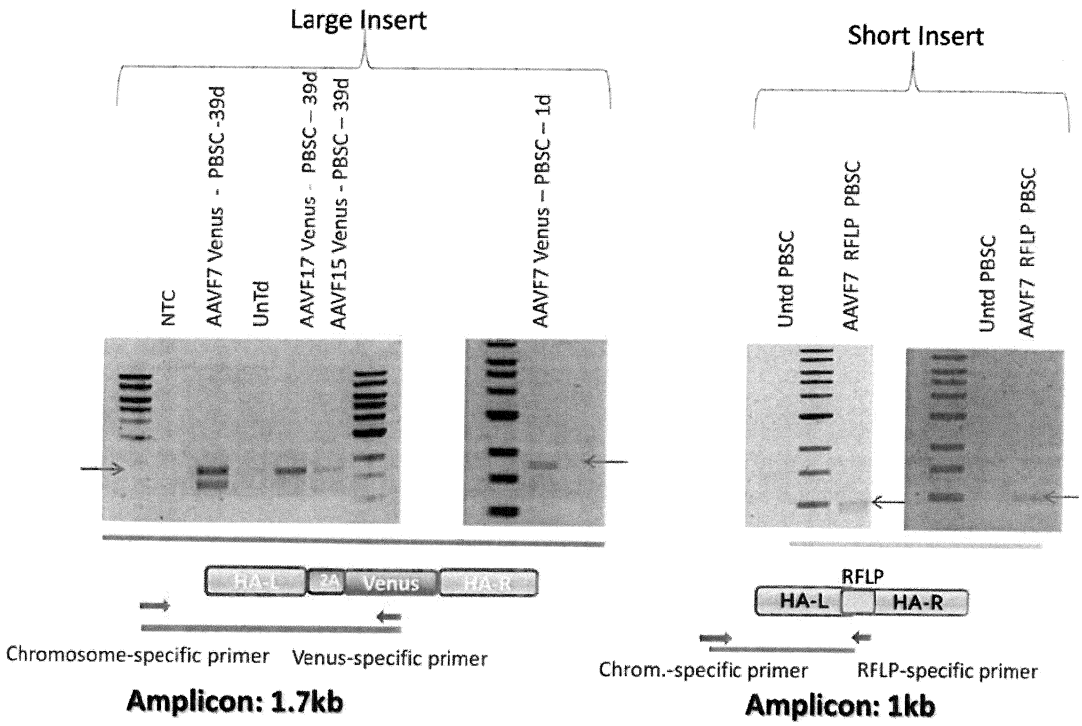
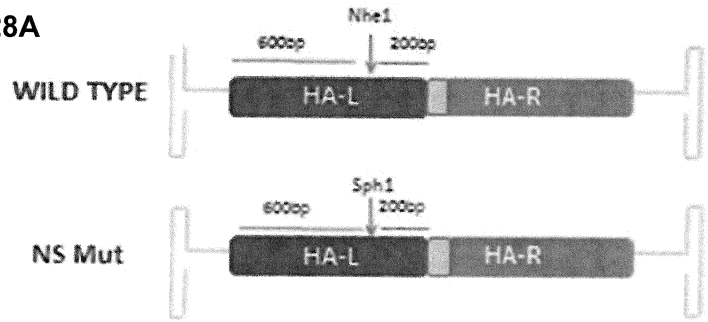


Figure 27

HA-L: 5'-Homology Arm
HA-R: 3'-Homology Arm
NTC: No Template Control
Untd: Untransduced Cells

AAVF VECTORS FOR NUCLEOTIDE SUBSTITUTION

Figure 28A



- Mutates TA to AT
- Changes an Nhe1 site to a Sph1 site

Nhe 1 ⟶ Sph 1

GCTAGC ⟶ GCATGC

Figure 28B

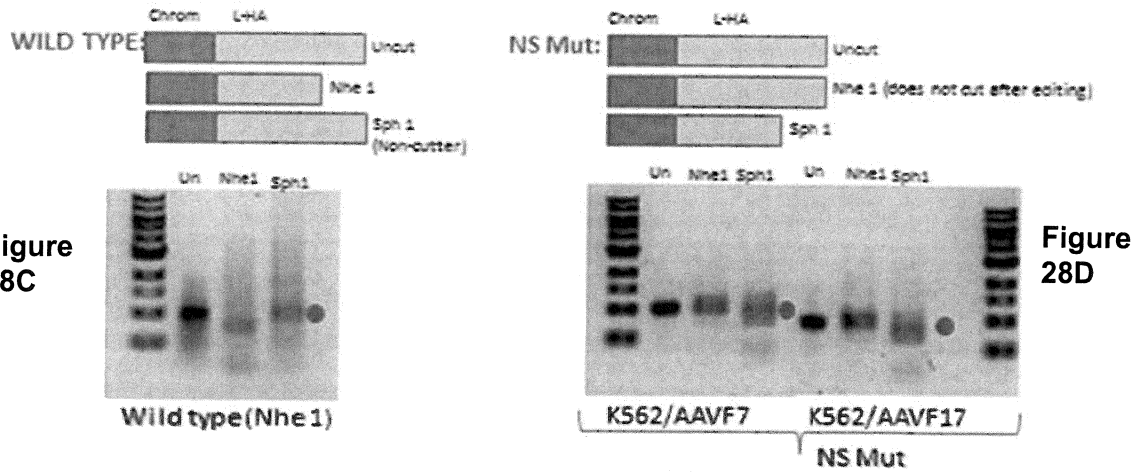


Figure 28C

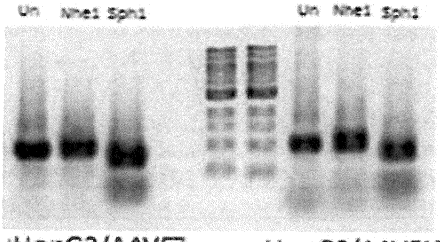
Wild type (Nhe 1)

Figure 28D

K562/AAVF7 K562/AAVF17

NS Mut

Figure 28E



HepG2/AAVF7 HepG2/AAVF17

NS Mut

RFLP: Nhe 1 cuts, Sph1 does not cut

NS Mut: Sph 1 cuts, Nhe 1 does not cut

AAVF MEDIATES PRECISE NUCLEOTIDE SUBSTITUTION

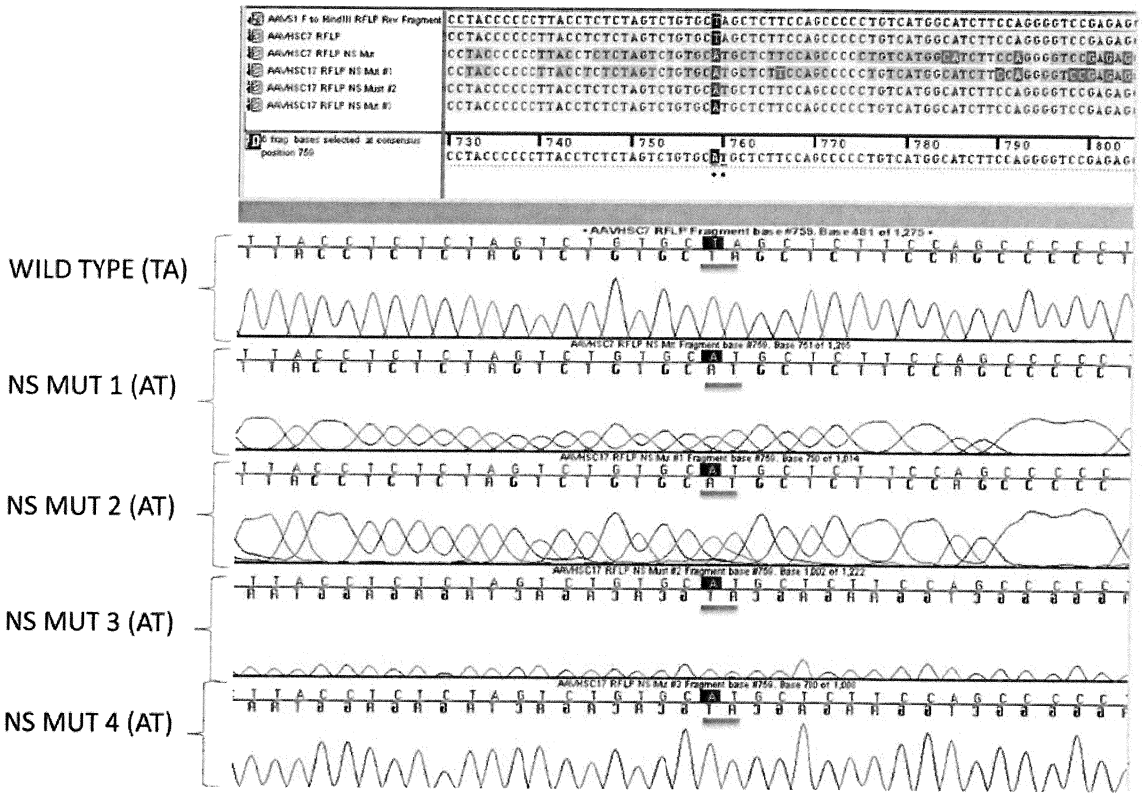
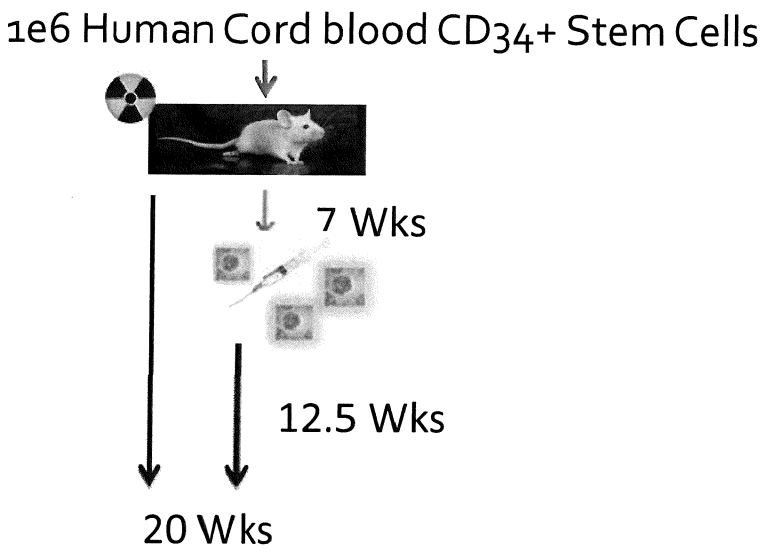


Figure 29

	GFP+ BrdU- (Non-dividing Cells)	GFP+BrdU+ (Dividing Cells)
AAVHSC7	**12**	14.6
AAVHSC17	**6.15**	6.9
AAV9	**4.31**	3.73
AAV8	**1.37**	0.839

Figure 30



Analysis of Human Blood Lineages in Marrow & Spleen

- Flow analysis
- Flow sort
- Venus, CD34+, Glyco A+ (erythroid)

Figure 31A

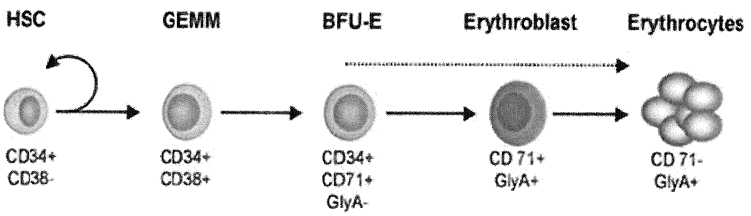


Figure 31B

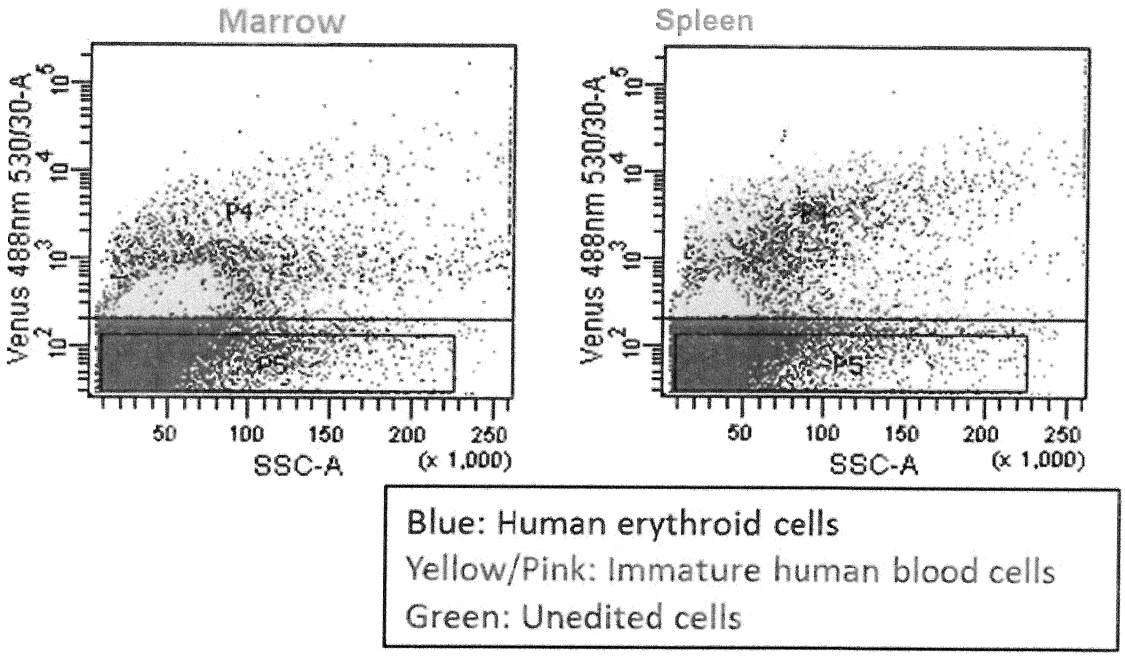

Marrow
Spleen
Venus 488nm 530/30-A
SSC-A
(x 1,000)
P4
P5
Blue: Human erythroid cells
Yellow/Pink: Immature human blood cells
Green: Unedited cells

Figure 31C

EXPT	1A	1B	2A	2A	2B	2B	3	3
	AAVF7	AAVF17	AAVF7	AAVF7	AAVF17	AAVF17	AAVF17	AAVF17
	CD45+VEN+^{4}	CD45+VEN+^{4}	CD45+VEN+^{4}	CD34+VEN+^{4}	CD45+VEN+^{4}	CD34+VEN+^{4}	GLYCO A+VEN+^{4}	CD34+VEN+^{4}
WKS POST TX TO VENUS	1	1	1	1	1	1	7	7
WKS POST VENUS TO HARVEST	5	5	6	6	6	6	12.5	12.5
WKS POST TX TO HARVEST	6	6	7	7	7	7	19	19
VBM	32.4	14.7	32.4	54.9	14.7	49.9	ND	ND
FBM	11.2	9.49	9.49	20.3	11.2	26.8	99.4	75.5
SPLN	15.9	24.8	15.9	71.1	24.8	82	99.2	58.1

Figure 32A

% Venus Positive	Mid-Term	Mid-Term	Mid-Term	Mid-Term	Long-Term	Long-Term
	AAVF7	AAVF7	AAVF17	AAVF17	AAVF17	AAVF17
Compartment	CD34+	CD45+	CD34+	CD45+	CD34+	GlycoA+
Vertebral Marrow	54.9	32.4	49.9	14.7	ND*	ND*
Femoral Marrow	20.3	9.49	26.8	11.2	75.5	99.4
Spleen	71.1	15.9	82	24.8	58.1	99.2

* ND: Not Done

Figure 32B

SEQUENCE ANALYSIS SHOWING CHROMOSOMAL INSERTION OF VENUS INTO INTRON 1 OF PPP1R12C BY AAVF VECTORS

ALIGNMENT OF AAV-TARGETED SEQUENCES IN AAVS1

```
» K562 AAVHSC7 #1      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» K562 AAVHSC7 #2      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» K562 AAVHSC7 #3      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» K562 AAVHSC7 #4      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» K562 AAVHSC7 #5      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC7 #1      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC7 #2      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC7 #3      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC7 #4      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC15 #1     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC15 #2     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC17 #1     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC17 #2     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC17 #3     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» PBSC AAVHSC17 #4     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
  HepG2 AAVHSC7 #1     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» AC010327 AAVS1       (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
  » Venus Donor        (1)
» Homology Arm 5'       (1)

» K562 AAVHSC7 #1      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» K562 AAVHSC7 #2      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» K562 AAVHSC7 #3      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» K562 AAVHSC7 #4      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» K562 AAVHSC7 #5      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC7 #1      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC7 #2      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC7 #3      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC7 #4      (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC15 #1     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC15 #2     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC17 #1     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC17 #2     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC17 #3     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» PBSC AAVHSC17 #4     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
  HepG2 AAVHSC7 #1     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» AC010327 AAVS1       (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
  » Venus Donor        (1)
» Homology Arm 5'       (1)

» K562 AAVHSC7 #1     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» K562 AAVHSC7 #2     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» K562 AAVHSC7 #3     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» K562 AAVHSC7 #4     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» K562 AAVHSC7 #5     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC7 #1     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC7 #2     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC7 #3     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC7 #4     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC15 #1    (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC15 #2    (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC17 #1    (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC17 #2    (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC17 #3    (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» PBSC AAVHSC17 #4    (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
  HepG2 AAVHSC7 #1     (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» AC010327 AAVS1       (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCAGCATTC
  » Venus Donor        (1)                                                  TGCTTTCTCTGACCTGCATTC
» Homology Arm 5'       (1)                                                  TGCTTTCTCTGACCTGCATTC
```

Figure 33

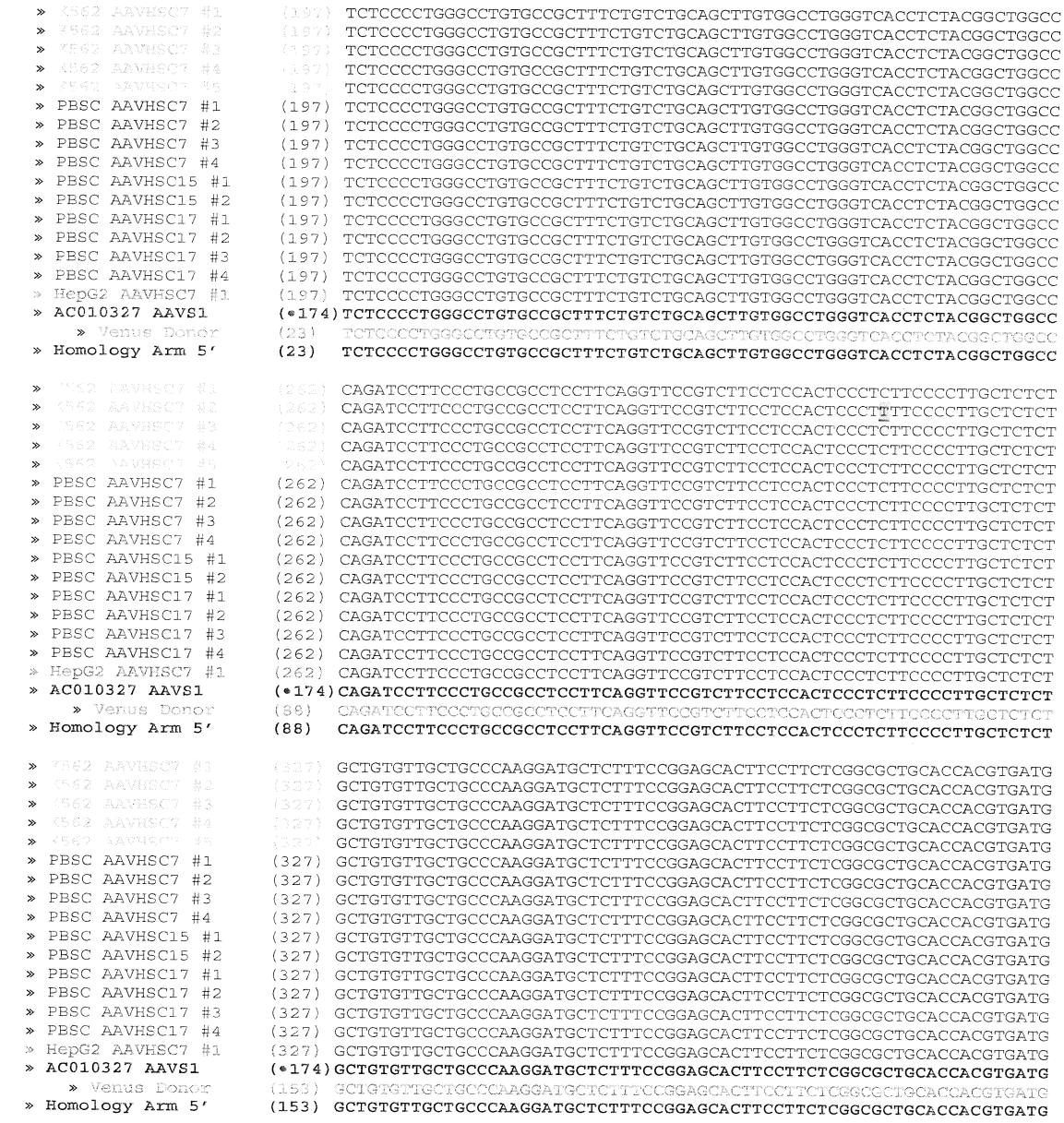


Figure 33 (cont.)

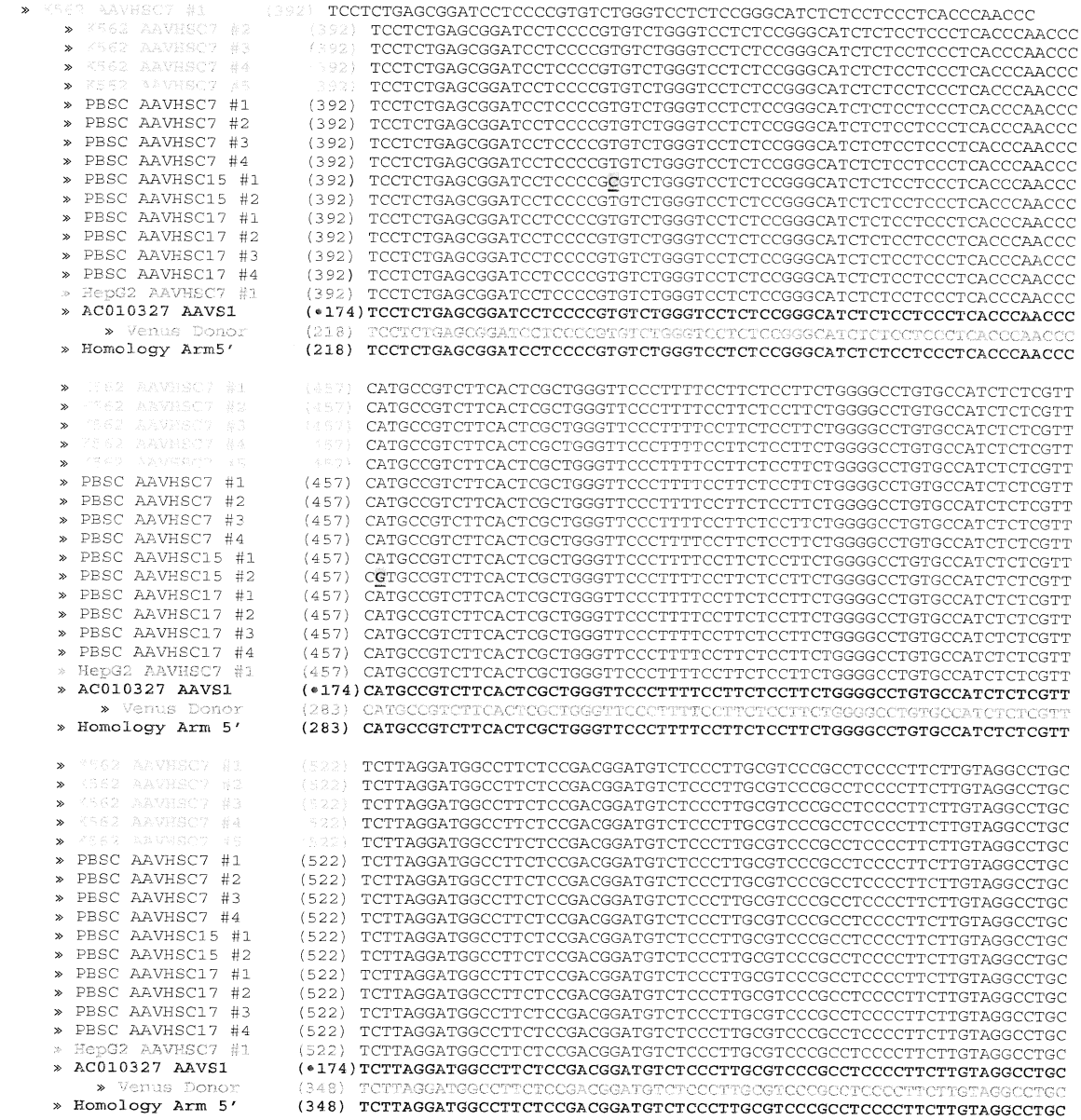


Figure 33 (cont.)

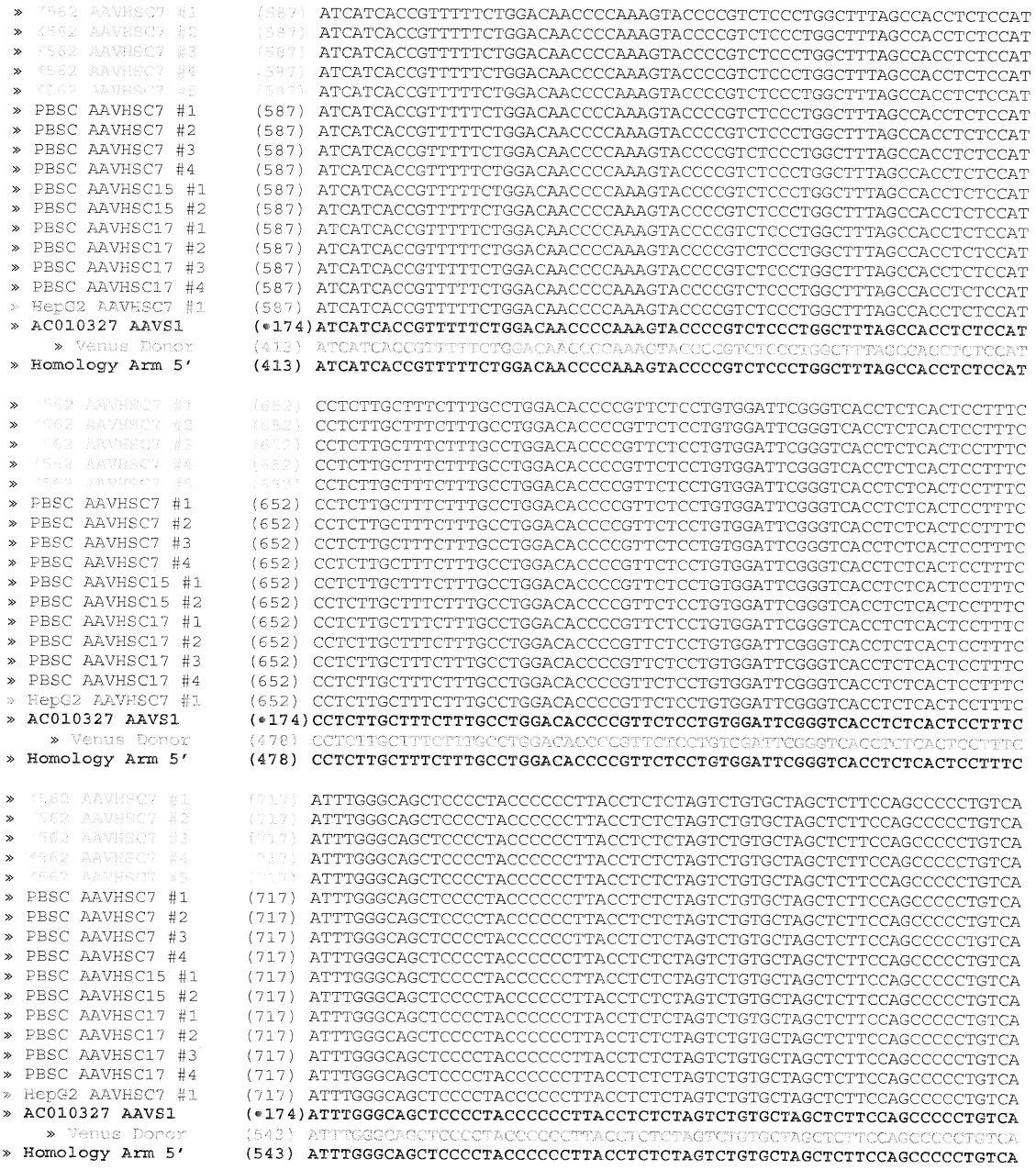


Figure 33 (cont.)

```
» K562 AAVHSC7 #1      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» K562 AAVHSC7 #2      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» K562 AAVHSC7 #3      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» K562 AAVHSC7 #4      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» K562 AAVHSC7 #5      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC7 #1      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC7 #2      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC7 #3      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC7 #4      (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC15 #1     (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC15 #2     (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC17 #1     (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC17 #2     (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC17 #3     (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» PBSC AAVHSC17 #4     (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» HepG2 AAVHSC7 #1     (782) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» AC010327 AAVS1      (•174)TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
    » Venus Donor      (608) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA
» Homology Arm 5'      (608) TGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGGCCCCTATGTCCA

» K562 AAVHSC7 #1      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» K562 AAVHSC7 #2      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» K562 AAVHSC7 #3      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» K562 AAVHSC7 #4      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» K562 AAVHSC7 #5      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC7 #1      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC7 #2      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC7 #3      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC7 #4      (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC15 #1     (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC15 #2     (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC17 #1     (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC17 #2     (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC17 #3     (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» PBSC AAVHSC17 #4     (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» HepG2 AAVHSC7 #1     (847) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» AC010327 AAVS1      (•174)CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
    » Venus Donor      (673) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT
» Homology Arm 5'      (673) CTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACCTTATATT

» K562 AAVHSC7 #1      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» K562 AAVHSC7 #2      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» K562 AAVHSC7 #3      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» K562 AAVHSC7 #4      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» K562 AAVHSC7 #5      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC7 #1      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC7 #2      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC7 #3      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC7 #4      (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC15 #1     (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC15 #2     (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC17 #1     (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC17 #2     (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC17 #3     (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» PBSC AAVHSC17 #4     (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» HepG2 AAVHSC7 #1     (912) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» AC010327 AAVS1      (•174)CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
    » Venus Donor      (738) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
» Homology Arm 5'      (738) CCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
```

Figure 33 (cont.)

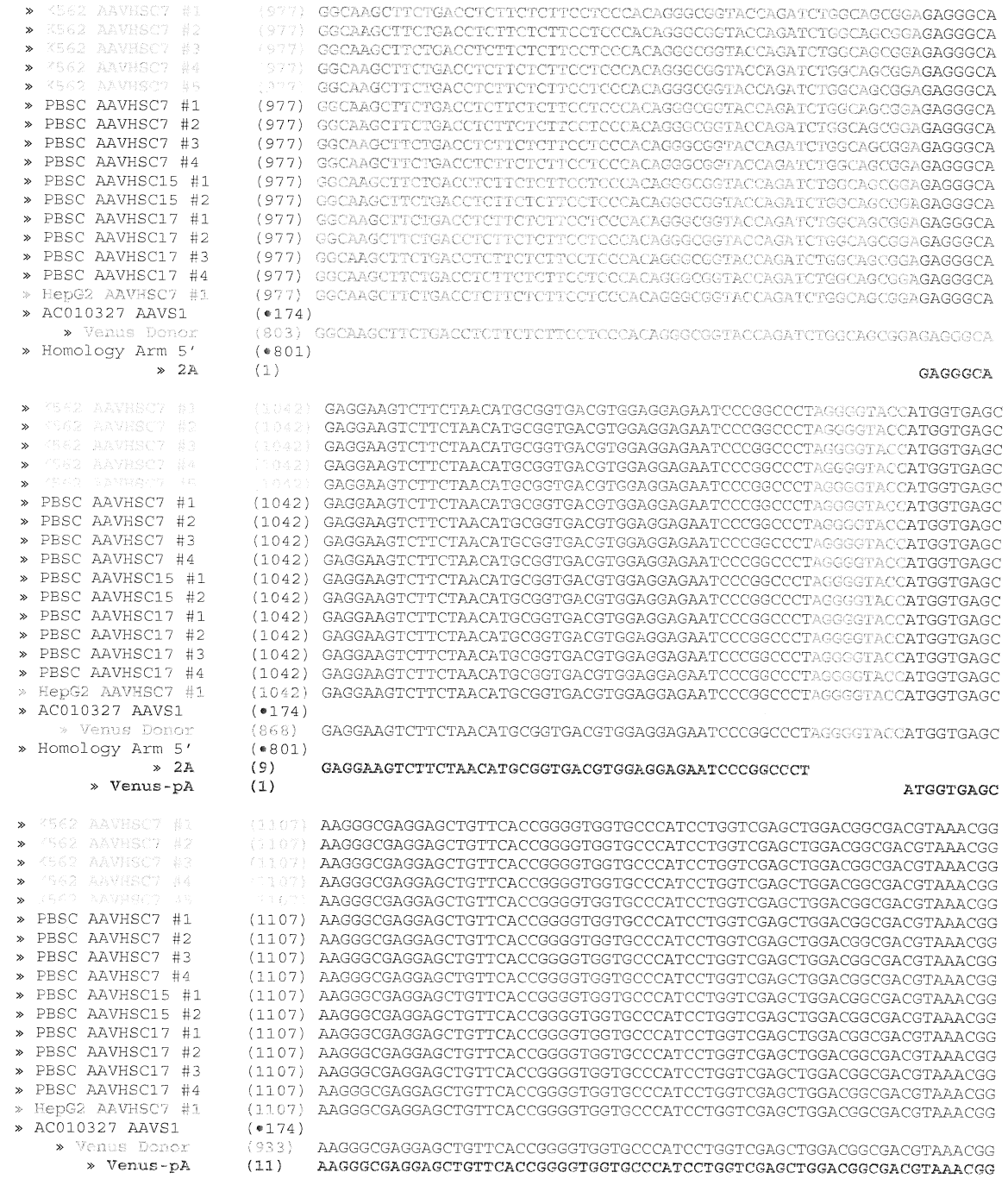


Figure 33 (cont.)

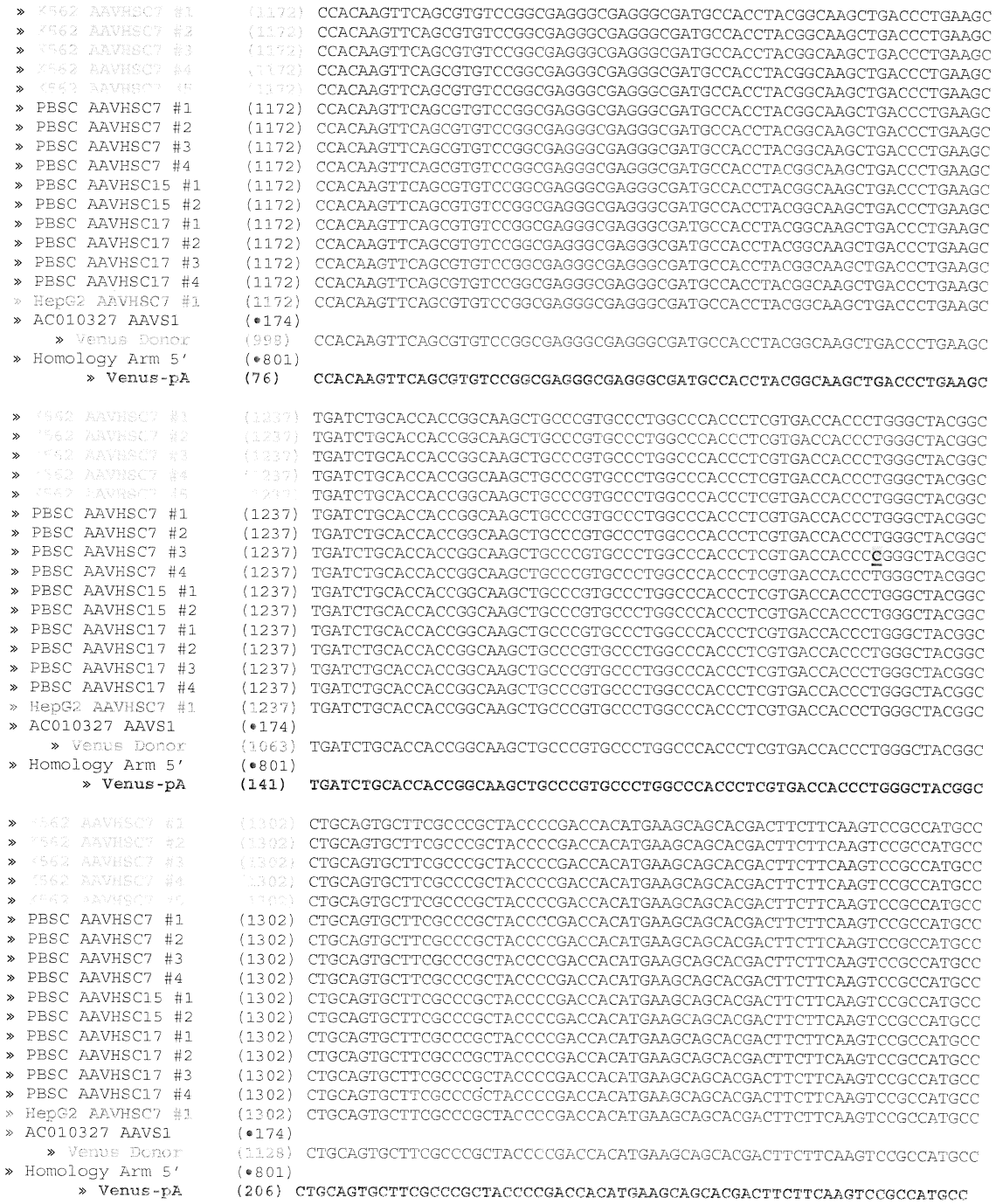


Figure 33 (cont.)

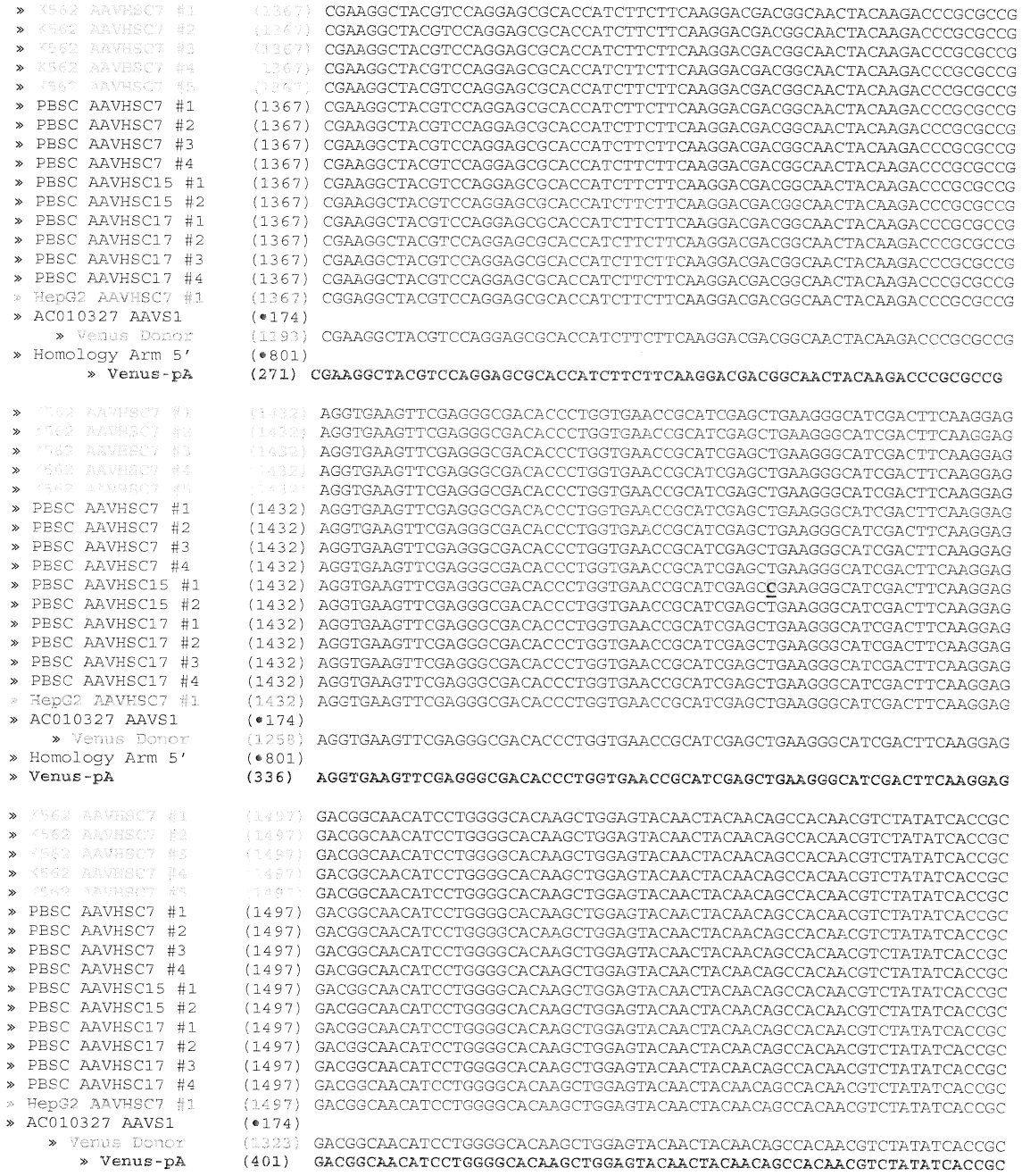


Figure 33 (cont.)

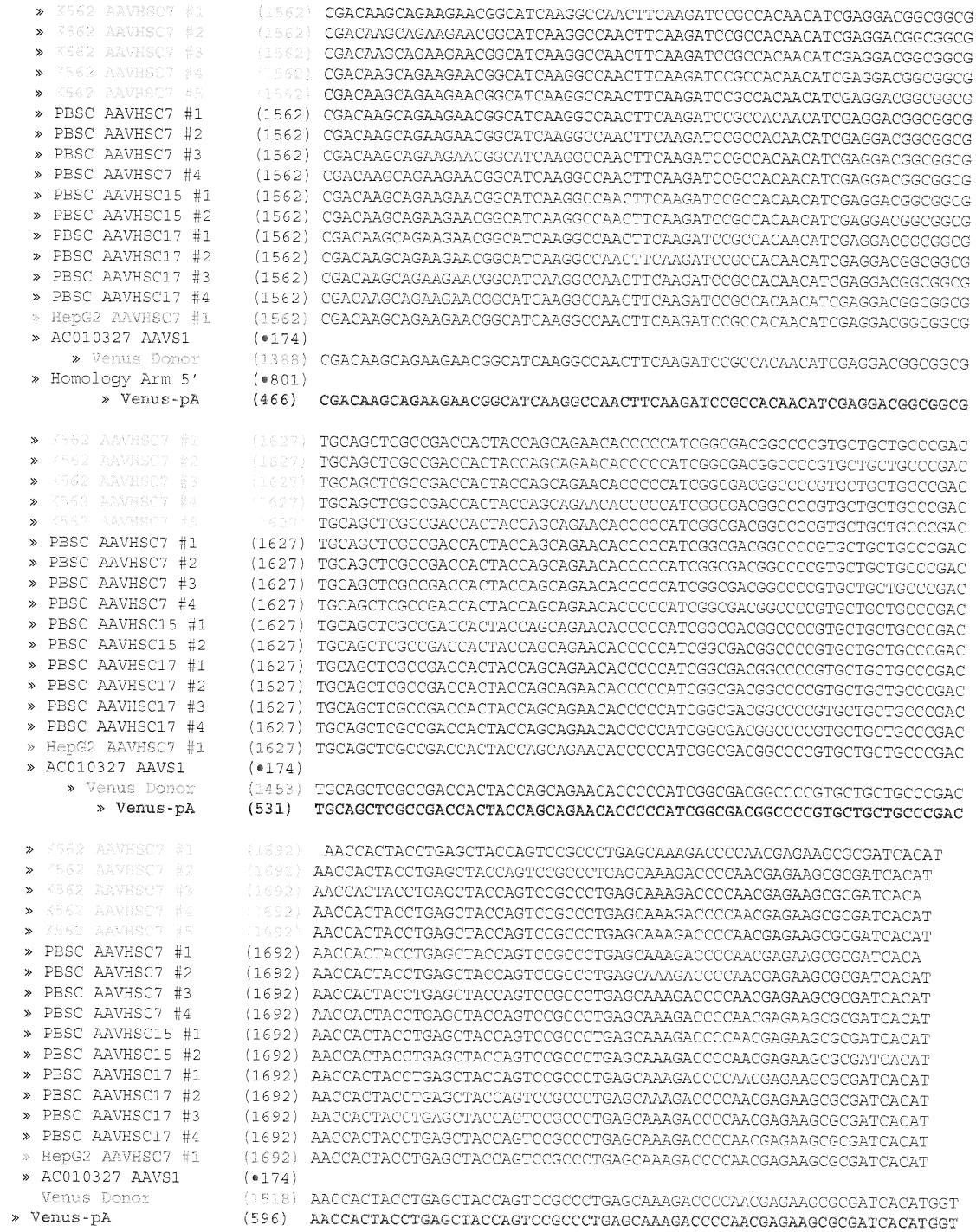


Figure 33 (cont.)

Sequence Analysis Showing Chromosomal Insertion of a Short Sequence Into Intron 1 of PPP1R12C by AAVF Vectors

```
                                    1                                                                   65
» AAVHSC7 RFLP PBSC #1      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» AAVHSC7 RFLP PBSC #2      (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» AAVHSC7 RFLP HepG2 #1     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» AAVHSC7 RFLP HepG2 #2     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» AAVHSC7 RFLP HepG2 #3     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» AAVHSC7 RFLP HepG2 #4     (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» RFLP Vector Sequence      (1)
» AC010327 AAVS1            (2) GGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAGTGCCCTTGCTGTGCCGCCG
» Homology Arm 5'           (1)
» Homology Arm 3'           (1)
                                   66                                                                  130
» AAVHSC7 RFLP PBSC #1     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» AAVHSC7 RFLP PBSC #2     (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» AAVHSC7 RFLP HepG2 #1    (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» AAVHSC7 RFLP HepG2 #2    (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» AAVHSC7 RFLP HepG2 #3    (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» AAVHSC7 RFLP HepG2 #4    (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» RFLP Vector Sequence     (1)
» AC010327 AAVS1           (67) GAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCGGACCACTTTGAGCTCTACTGGCTT
» Homology Arm 5'          (1)
» Homology Arm 3'          (1)
                                  131                                                                  195
» AAVHSC7 RFLP PBSC #1    (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» AAVHSC7 RFLP PBSC #2    (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» AAVHSC7 RFLP HepG2 #1   (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» AAVHSC7 RFLP HepG2 #2   (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» AAVHSC7 RFLP HepG2 #3   (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» AAVHSC7 RFLP HepG2 #4   (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACCTGCATTC
» RFLP Vector Sequence    (1)                                          TGCTTTCTCTGACCAGCATTC
» AC010327 AAVS1          (132) CTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAGGCAGGTCC
» Homology Arm 5'         (1)                                          TGCTTTCTCTGACCAGCATTC
» Homology Arm 3'         (1)
                                  196                                                                  260
» AAVHSC7 RFLP PBSC #1    (197) TCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCC
» AAVHSC7 RFLP PBSC #2    (197) TCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCC
» AAVHSC7 RFLP HepG2 #1   (197) TCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCC
» AAVHSC7 RFLP HepG2 #2   (197) TCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCC
» AAVHSC7 RFLP HepG2 #3   (197) TCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCC
» AAVHSC7 RFLP HepG2 #4   (197) TCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCC
» RFLP Vector Sequence    (23)  TCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCC
» AC010327 AAVS1          (•174)
» Homology Arm 5'         (23) TCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGCAGCTTGTGGCCTGGGTCACCTCTACGGCTGGCC
» Homology Arm 3'         (1)
```

Figure 34

```
                                    261                                                                 325
» AAVHSC7 RFLP PBSC #1      (262) CAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCT
» AAVHSC7 RFLP PBSC #2      (262) CAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCT
» AAVHSC7 RFLP HepG2 #1     (262) CAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCT
» AAVHSC7 RFLP HepG2 #2     (262) CAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCT
» AAVHSC7 RFLP HepG2 #3     (262) CAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCT
» AAVHSC7 RFLP HepG2 #4     (262) CAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCT
» RFLP Vector Sequence      (88)  CAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCT
» AC010327 AAVS1            (•174)
» Homology Arm 5′           (88)  CAGATCCTTCCCTGCCGCCTCCTTCAGGTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCT
» Homology Arm 3′           (1)
                                    326                                                                 390
» AAVHSC7 RFLP PBSC #1      (327) GCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATG
» AAVHSC7 RFLP PBSC #2      (327) GCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATG
» AAVHSC7 RFLP HepG2 #1     (327) GCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATG
» AAVHSC7 RFLP HepG2 #2     (327) GCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATG
» AAVHSC7 RFLP HepG2 #3     (327) GCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATG
» AAVHSC7 RFLP HepG2 #4     (327) GCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATG
» RFLP Vector Sequence      (153) GCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATG
» AC010327 AAVS1            (•174)
» Homology Arm 5′           (153) GCTGTGTTGCTGCCCAAGGATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATG
» Homology Arm 3′           (1)
                                    391                                                                 455
» AAVHSC7 RFLP PBSC #1      (392) TCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCC
» AAVHSC7 RFLP PBSC #2      (392) TCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCC
» AAVHSC7 RFLP HepG2 #1     (392) TCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCC
» AAVHSC7 RFLP HepG2 #2     (392) TCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCC
» AAVHSC7 RFLP HepG2 #3     (392) TCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCC
» AAVHSC7 RFLP HepG2 #4     (392) TCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCC
» RFLP Vector Sequence      (218) TCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCC
» AC010327 AAVS1            (•174)
» Homology Arm 5′           (218) TCCTCTGAGCGGATCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCC
» Homology Arm 3′           (1)
                                    456                                                                 520
» AAVHSC7 RFLP PBSC #1      (457) CATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTT
» AAVHSC7 RFLP PBSC #2      (457) CATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTT
» AAVHSC7 RFLP HepG2 #1     (457) CATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTT
» AAVHSC7 RFLP HepG2 #2     (457) CATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTT
» AAVHSC7 RFLP HepG2 #3     (457) CATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTT
» AAVHSC7 RFLP HepG2 #4     (457) CATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTT
» RFLP Vector Sequence      (283) CATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTT
» AC010327 AAVS1            (•174)
» Homology Arm 5′           (283) CATGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTT
» Homology Arm 3′           (1)
```

Figure 34 (cont.)

```
                                    521                                                                585
» AAVHSC7 RFLP PBSC #1      (522) TCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
» AAVHSC7 RFLP PBSC #2      (522) TCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
» AAVHSC7 RFLP HepG2 #1     (522) TCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
» AAVHSC7 RFLP HepG2 #2     (522) TCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
» AAVHSC7 RFLP HepG2 #3     (522) TCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
» AAVHSC7 RFLP HepG2 #4     (522) TCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
» RFLP Vector Sequence      (348) TCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
» AC010327 AAVS1            (•174)
» Homology Arm 5'           (348) TCTTAGGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
» Homology Arm 3'           (1)

                                    586                                                                650
» AAVHSC7 RFLP PBSC #1      (587) ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCAT
» AAVHSC7 RFLP PBSC #2      (587) ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCAT
» AAVHSC7 RFLP HepG2 #1     (587) ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCAT
» AAVHSC7 RFLP HepG2 #2     (587) ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCAT
» AAVHSC7 RFLP HepG2 #3     (587) ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCCGGCTTTAGCCACCTCTCCAT
» AAVHSC7 RFLP HepG2 #4     (587) ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCAT
» RFLP Vector Sequence      (413) ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCAT
» AC010327 AAVS1            (•174)
» Homology Arm 5'           (413) ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCAT
» Homology Arm 3'           (1)

                                    651                                                                715
» AAVHSC7 RFLP PBSC #1      (652) CCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCACTCCTTTC
» AAVHSC7 RFLP PBSC #2      (652) CCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCACTCCTTTC
» AAVHSC7 RFLP HepG2 #1     (652) CCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCCCTCACTCCTTTC
» AAVHSC7 RFLP HepG2 #2     (652) CCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCACTCCTTTC
» AAVHSC7 RFLP HepG2 #3     (652) CCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCACTCCTTTC
» AAVHSC7 RFLP HepG2 #4     (652) CCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCACTCCTTTC
» RFLP Vector Sequence      (478) CCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCACTCCTTTC
» AC010327 AAVS1            (•174)
» Homology Arm 5'           (478) CCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCACTCCTTTC
» Homology Arm 3'           (1)

                                    716                                                                780
» AAVHSC7 RFLP PBSC #1      (717) ATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCA
» AAVHSC7 RFLP PBSC #2      (717) ATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCA
» AAVHSC7 RFLP HepG2 #1     (717) ATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCA
» AAVHSC7 RFLP HepG2 #2     (717) ATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCA
» AAVHSC7 RFLP HepG2 #3     (717) ATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCA
» AAVHSC7 RFLP HepG2 #4     (717) ATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCA
» RFLP Vector Sequence      (543) ATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCA
» AC010327 AAVS1            (•174)
» Homology Arm 5'           (543) ATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCCCCTGTCA
» Homology Arm 3'           (1)
```

Figure 34 (cont.)

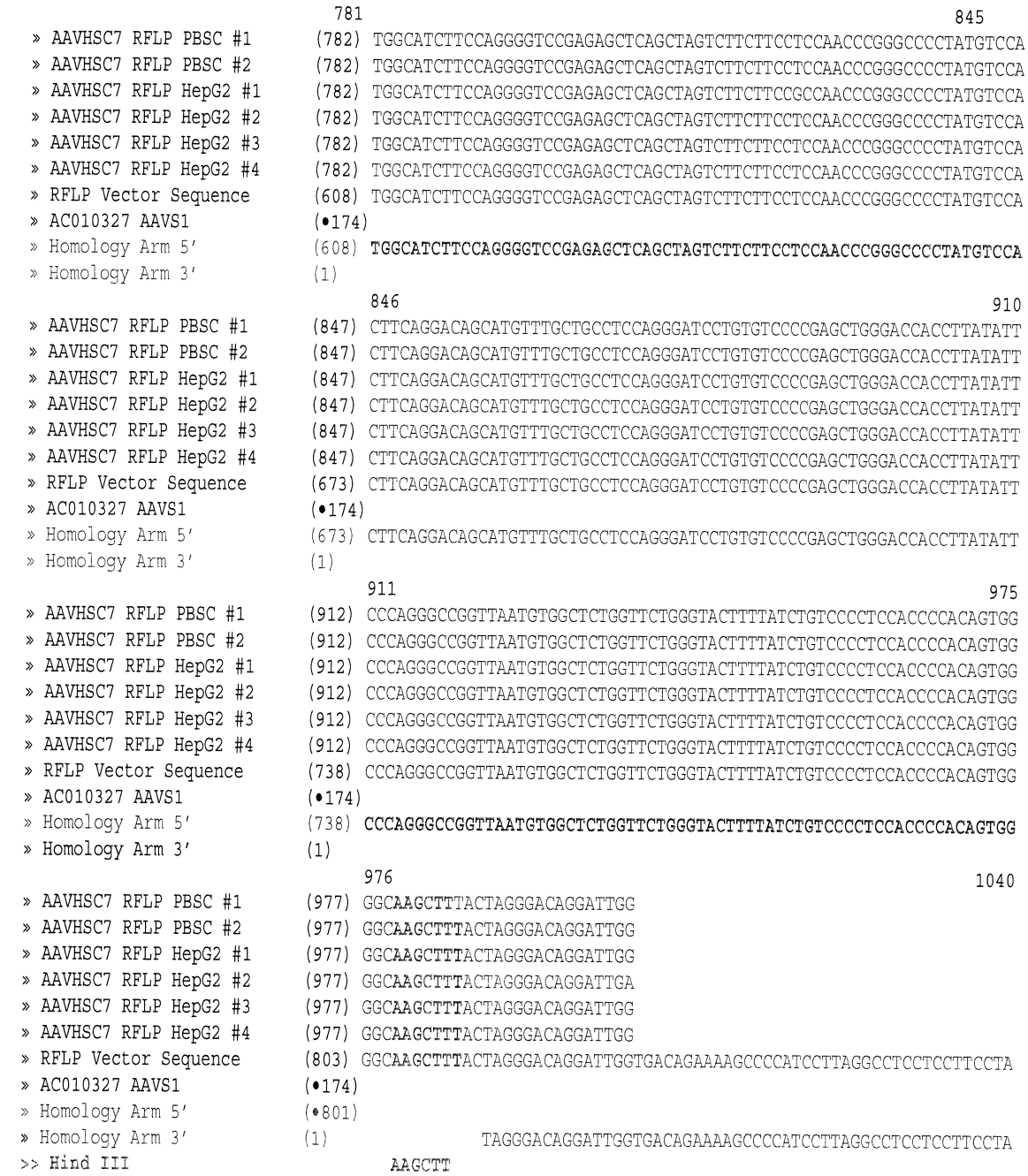


Figure 34 (cont.)

AAVF TARGETS INTEGRATION OF SMALL INSERTS INTO AAVS1 - CD34+ CELLS

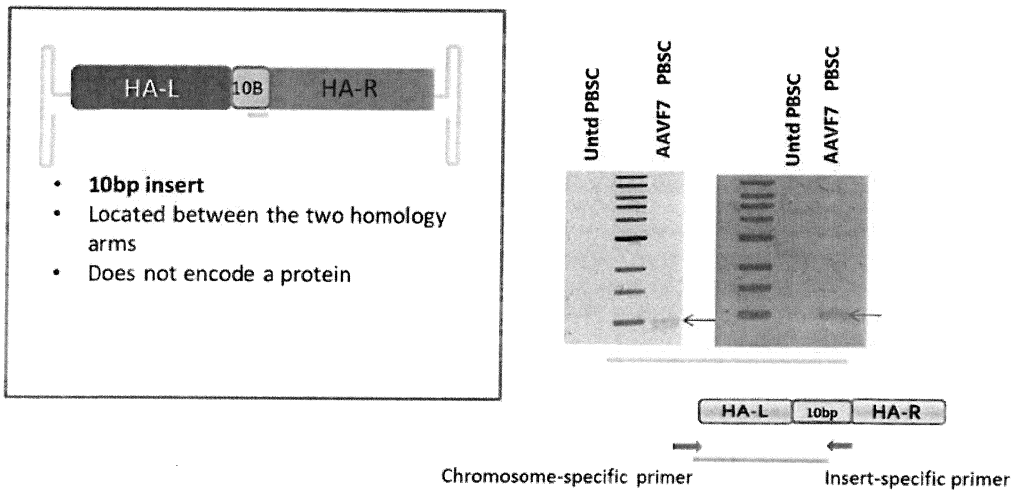
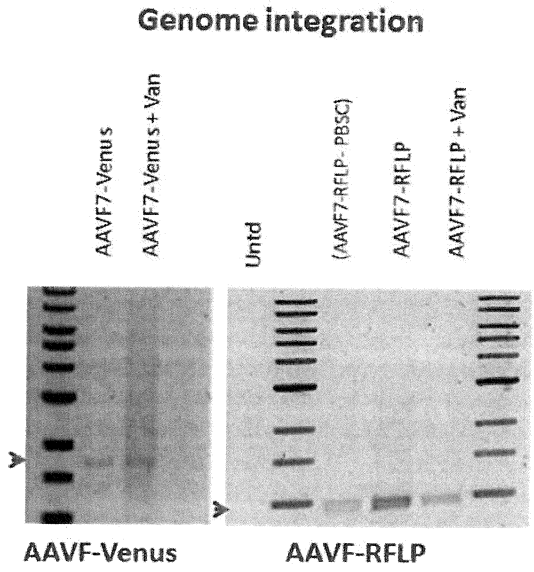


Figure 35

Figure 36

GFP Expression (% Cells)

	24H	48H
AAVF7	18.5	15.2
AAVF17	31.6	26.4
AAV6	0	ND
AAV8	0	ND
AAV9	2.81	ND



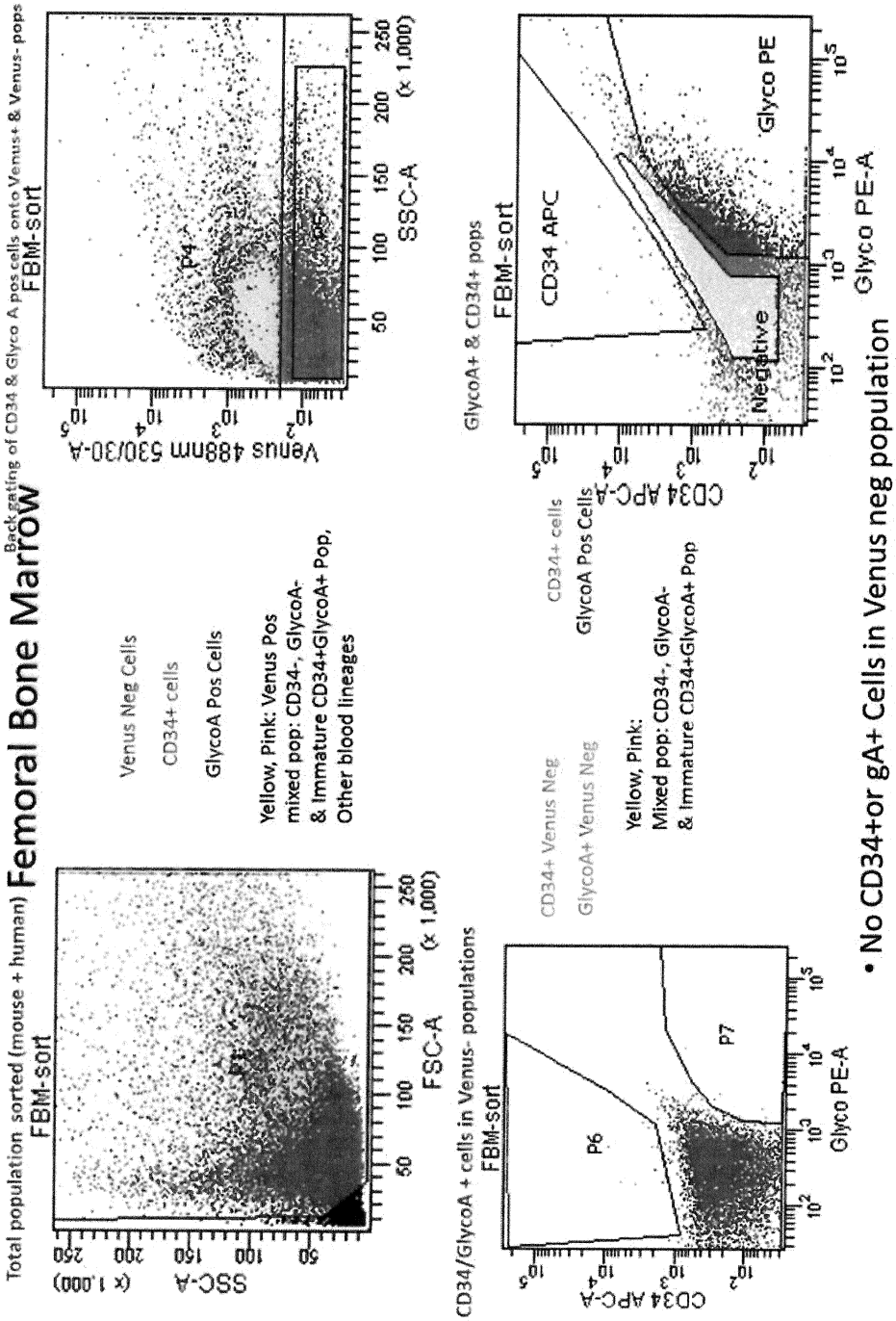

Femoral Bone Marrow
Total population sorted (mouse + human)
FBM-sort
SSC-A (x 1,000)
FSC-A (x 1,000)
P1
Venus Neg Cells
CD34+ cells
GlycoA Pos Cells
Yellow, Pink: Venus Pos
mixed pop: CD34-, GlycoA-
& Immature CD34+GlycoA+ Pop,
Other blood lineages
Back gating of CD34 & Glyco A pos cells onto Venus+ & Venus- pops
FBM-sort
Venus 488nm 530/30-A
SSC-A (x 1,000)
P4
P5
CD34/GlycoA + cells in Venus- populations
FBM-sort
CD34 APC-A
Glyco PE-A
P6
P7
CD34+ Venus Neg
GlycoA+ Venus Neg
CD34+ cells
GlycoA Pos Cells
Yellow, Pink:
Mixed pop: CD34-, GlycoA-
& Immature CD34+GlycoA+ Pop
GlycoA+ & CD34+ pops
FBM-sort
CD34 APC
Glyco PE
Negative
CD34 APC-A
Glyco PE-A
• No CD34+or gA+ Cells in Venus neg population
• Majority of gA+ cells express Venus

Figure 37

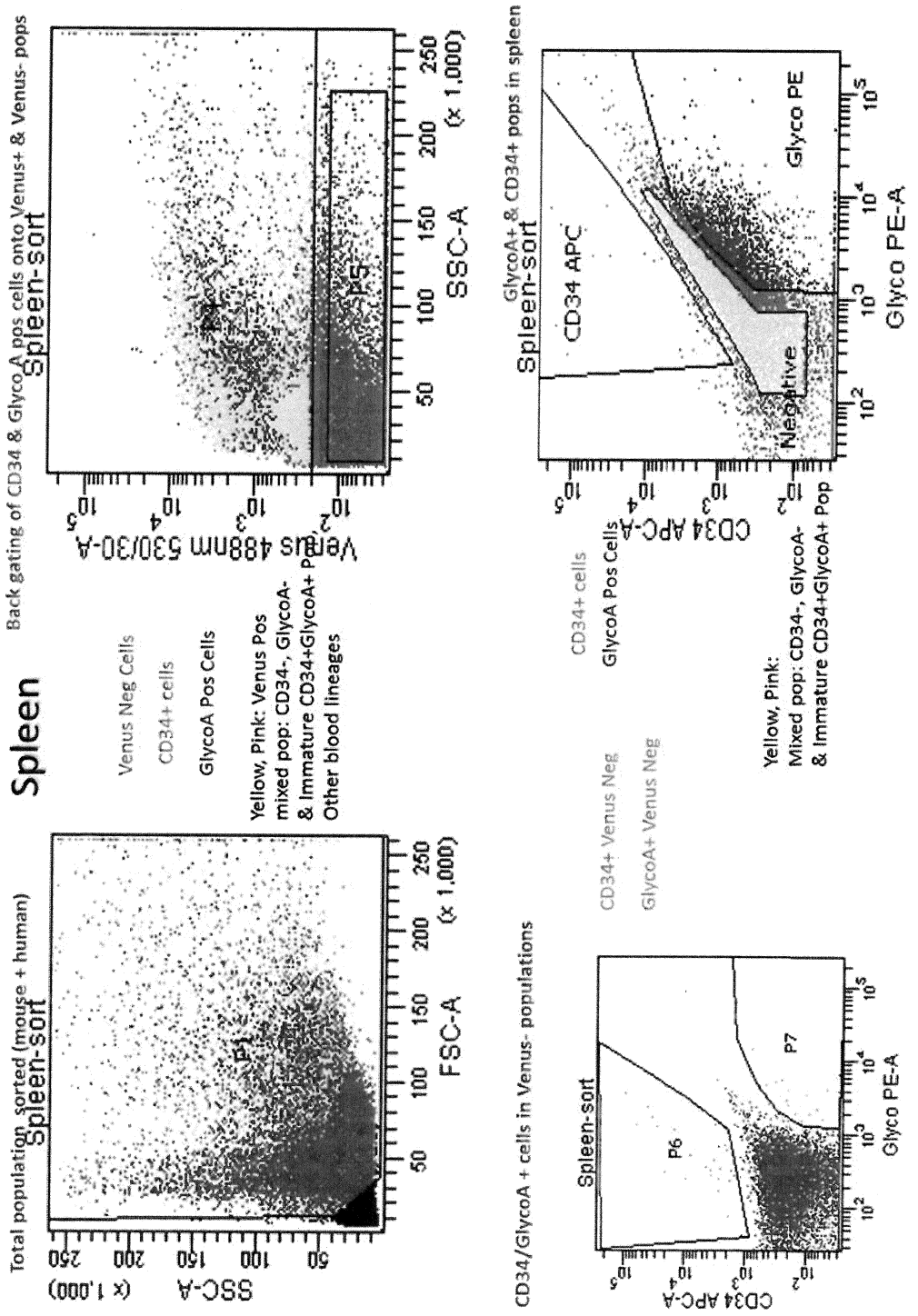

Spleen
Total population sorted (mouse + human)
Spleen-sort
SSC-A (x 1,000)
FSC-A (x 1,000)
Venus Neg Cells
CD34+ cells
GlycoA Pos Cells
Yellow, Pink: Venus Pos
mixed pop: CD34-, GlycoA-
& Immature CD34+GlycoA+ pops
Other blood lineages
Back gating of CD34 & GlycoA pos cells onto Venus+ & Venus- pops
Spleen-sort
Venus 488nm 530/30-A
SSC-A (x 1,000)
CD34/GlycoA + cells in Venus- populations
Spleen-sort
CD34+ Venus Neg
GlycoA+ Venus Neg
CD34 APC-A
Glyco PE-A
GlycoA+ & CD34+ pops in spleen
Spleen-sort
CD34 APC
Glyco PE
Negative
CD34+ cells
GlycoA Pos Cells
Yellow, Pink:
Mixed pop: CD34-, GlycoA-
& Immature CD34+GlycoA+ Pop

Figure 38

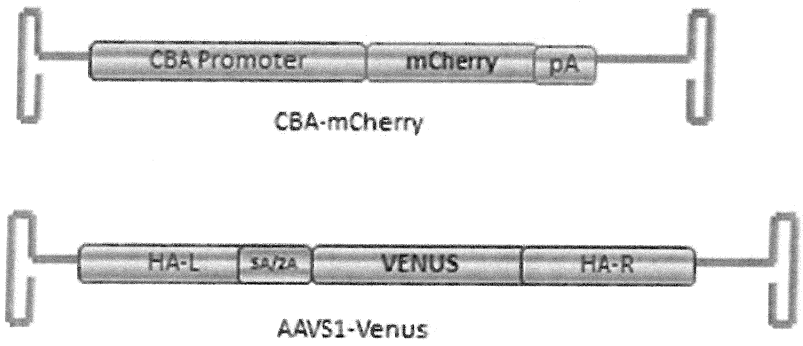

CBA Promoter
mCherry
pA
CBA-mCherry
HA-L
SA/2A
VENUS
HA-R
AAVS1-Venus

Figure 39

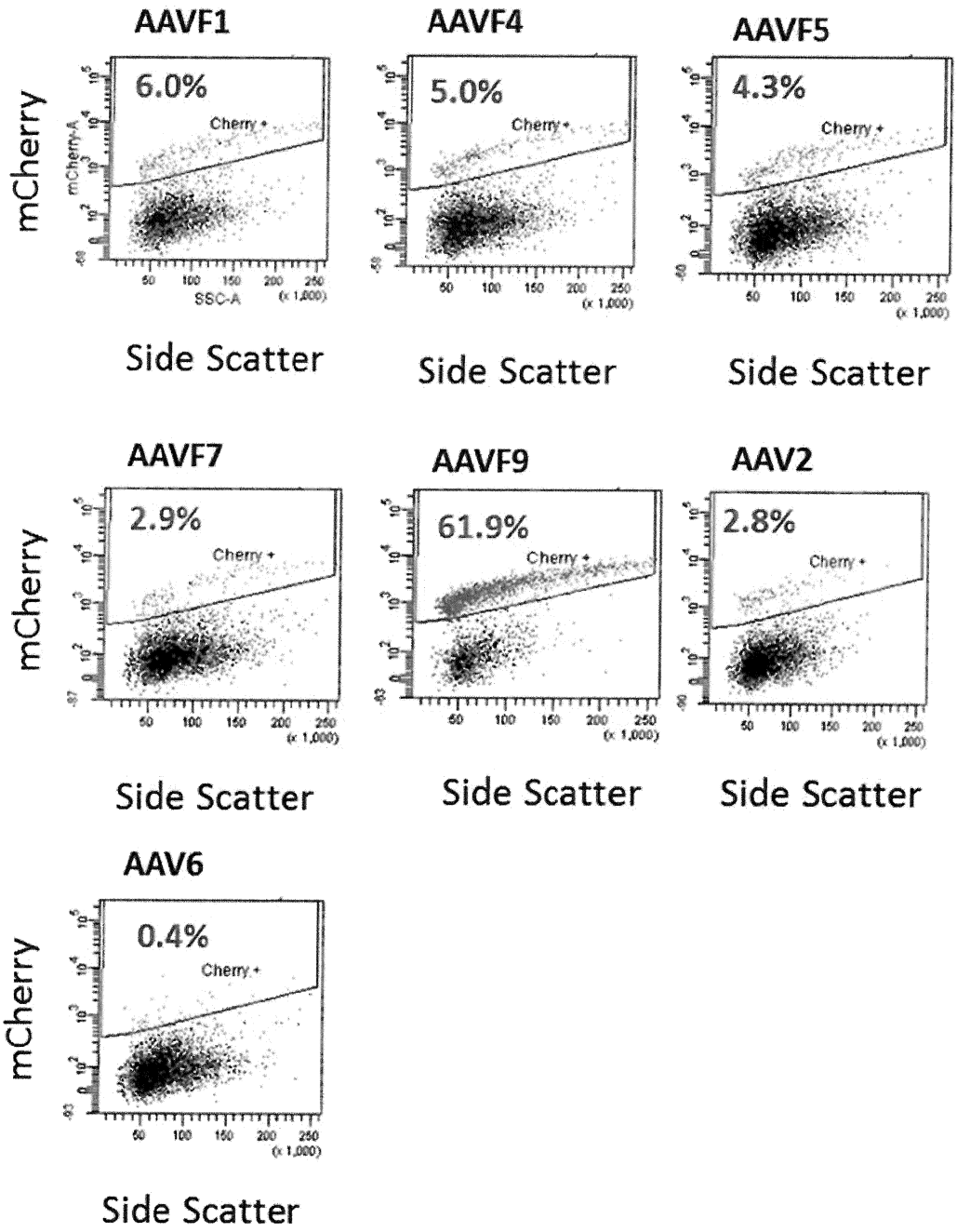

AAVF1
6.0%
Cherry +
mCherry-A
SSC-A
(x 1,000)
mCherry
Side Scatter
AAVF4
5.0%
Cherry +
Side Scatter
AAVF5
4.3%
Cherry +
Side Scatter
AAVF7
2.9%
Cherry +
mCherry
Side Scatter
AAVF9
61.9%
Cherry +
Side Scatter
AAV2
2.8%
Cherry +
Side Scatter
AAV6
0.4%
Cherry +
mCherry
Side Scatter

Figure 40A

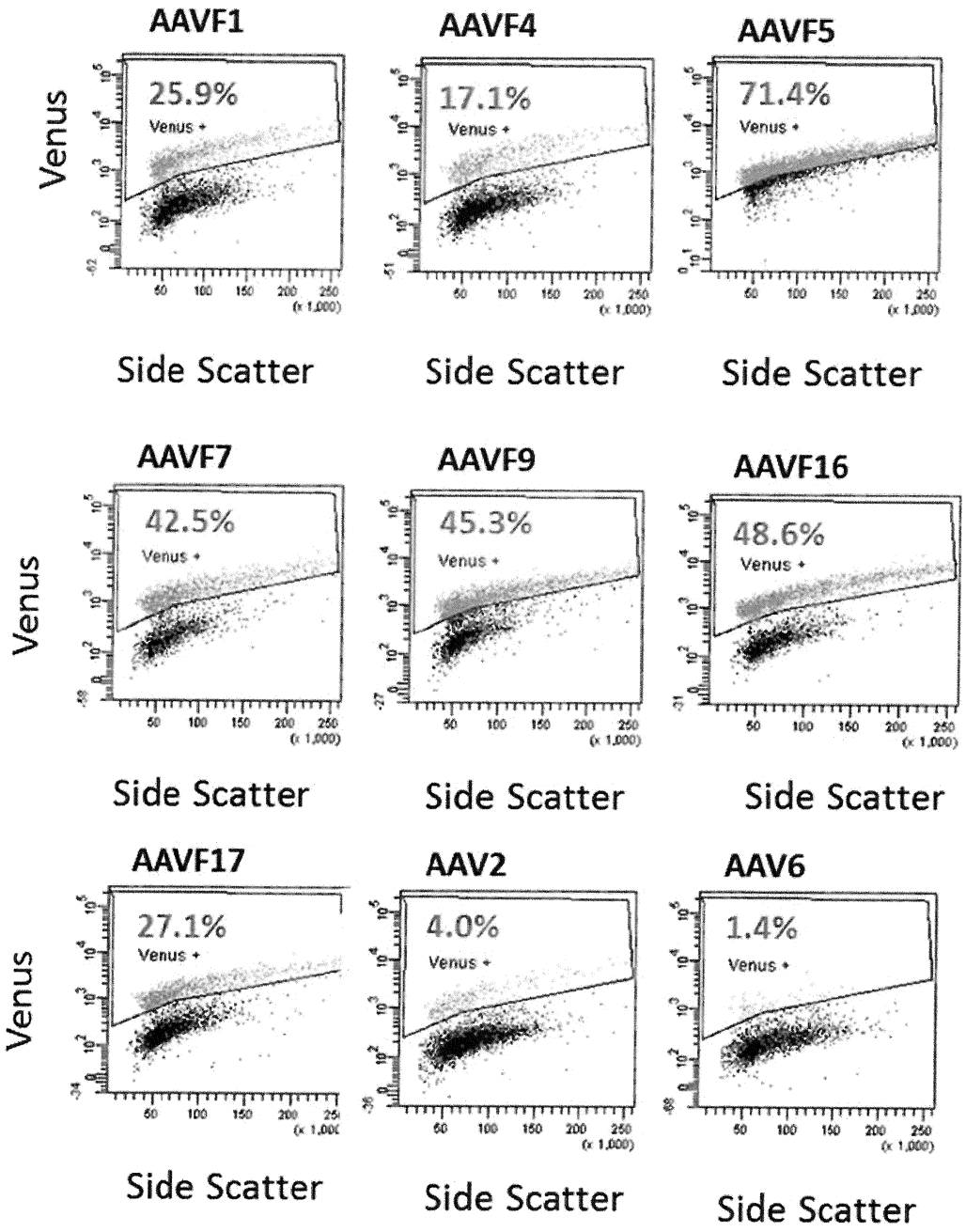

AAVF1
25.9%
Venus +
AAVF4
17.1%
Venus +
AAVF5
71.4%
Venus +
Venus
Side Scatter
AAVF7
42.5%
Venus +
AAVF9
45.3%
Venus +
AAVF16
48.6%
Venus +
Venus
Side Scatter
AAVF17
27.1%
Venus +
AAV2
4.0%
Venus +
AAV6
1.4%
Venus +
Venus
Side Scatter

Figure 40B

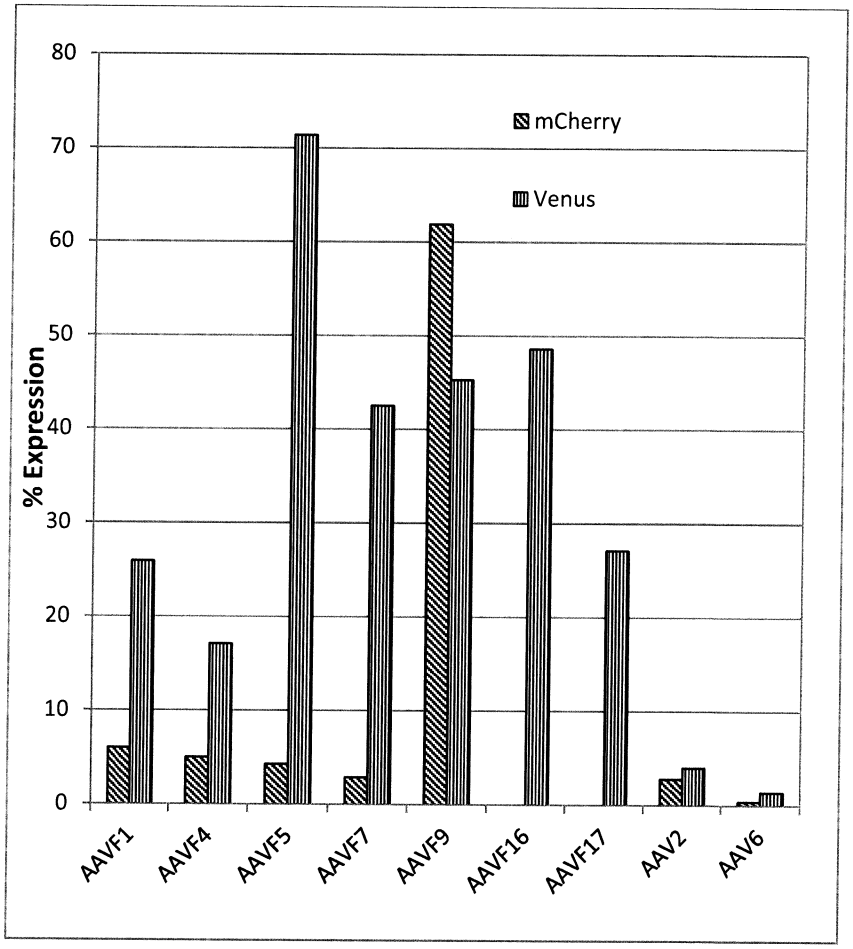

80
70
60
50
40
30
20
10
0
% Expression
mCherry
Venus
AAVF1
AAVF4
AAVF5
AAVF7
AAVF9
AAVF16
AAVF17
AAV2
AAV6

Figure 40C

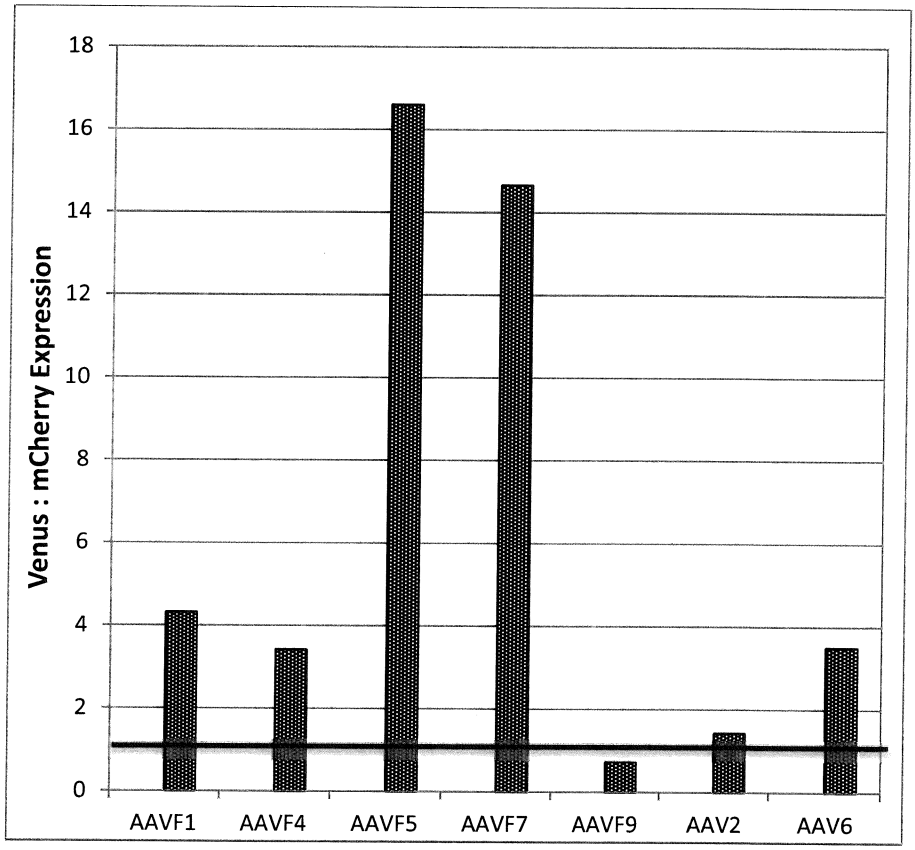

Venus : mCherry Expression
18
16
14
12
10
8
6
4
2
0
AAVF1
AAVF4
AAVF5
AAVF7
AAVF9
AAV2
AAV6

Figure 40D