FIG. 1

Polynucleotide cassette constructs

Cassette No.	First Enhancer	Promoter	Intron	5`UTR		Gene	Second Enhancer	RNA Export	polyA
1	EF1α	EF1a		TPL	eMLP	Coding Sequence	Full EES	HPRE	HGH
2		EF1a	EF1α	UTR2		Coding Sequence	410-564 EES	WPRE	HGH
3	EF1α	EF1a	EF1α	UTR2		Coding Sequence	511-810 EES		BGH
4	EF1α	EF1a	EF1α	UTR1		Coding Sequence	511-810 EES		βGlobin
5	CMV	GAPDH		UTR1		Coding Sequence	511-810 EES	HPRE	HGH
6	CMV	GAPDH		TPL	eMLP	Coding Sequence	410-564 EES	WPRE	βGlobin
7	CMV	Actin	Actin	UTR1		Coding Sequence	410-564 EES	HPRE	BGH
8	CMV	Actin	Actin	TPL	eMLP	Coding Sequence	410-564 EES		βGlobin
9	CMV	EF1a	EF1α	UTR1		Coding Sequence	410-564 EES		HGH
10	CMV	EF1a	EF1α	UTR2		Coding Sequence	511-810 EES	WPRE	BGH
11	CMV	CMV		TPL	eMLP	Coding Sequence	Full EES		HGH
12	CMV	CMV		TPL	eMLP	Coding Sequence	410-564 EES	HPRE	BGH
13	CMV	Actin			eMLP	Coding Sequence	511-810 EES	HPRE	βGlobin
14	CMV	CMV	CMVc	UTR1		Coding Sequence	Full EES	WPRE	βGlobin
15	CMV	CMV		UTR2		Coding Sequence	Full EES	HPRE	βGlobin
16	CMV	CMV		UTR1		Coding Sequence	Full EES	WPRE	BGH
17	CMV	GAPDH	CMVc			Coding Sequence	511-810 EES	WPRE	HGH
18	CMV	Actin			eMLP	Coding Sequence	Full EES		BGH
Base plasmid	CMV	EF1α	EF1α			Coding Sequence			Rb Globin

FIG. 2

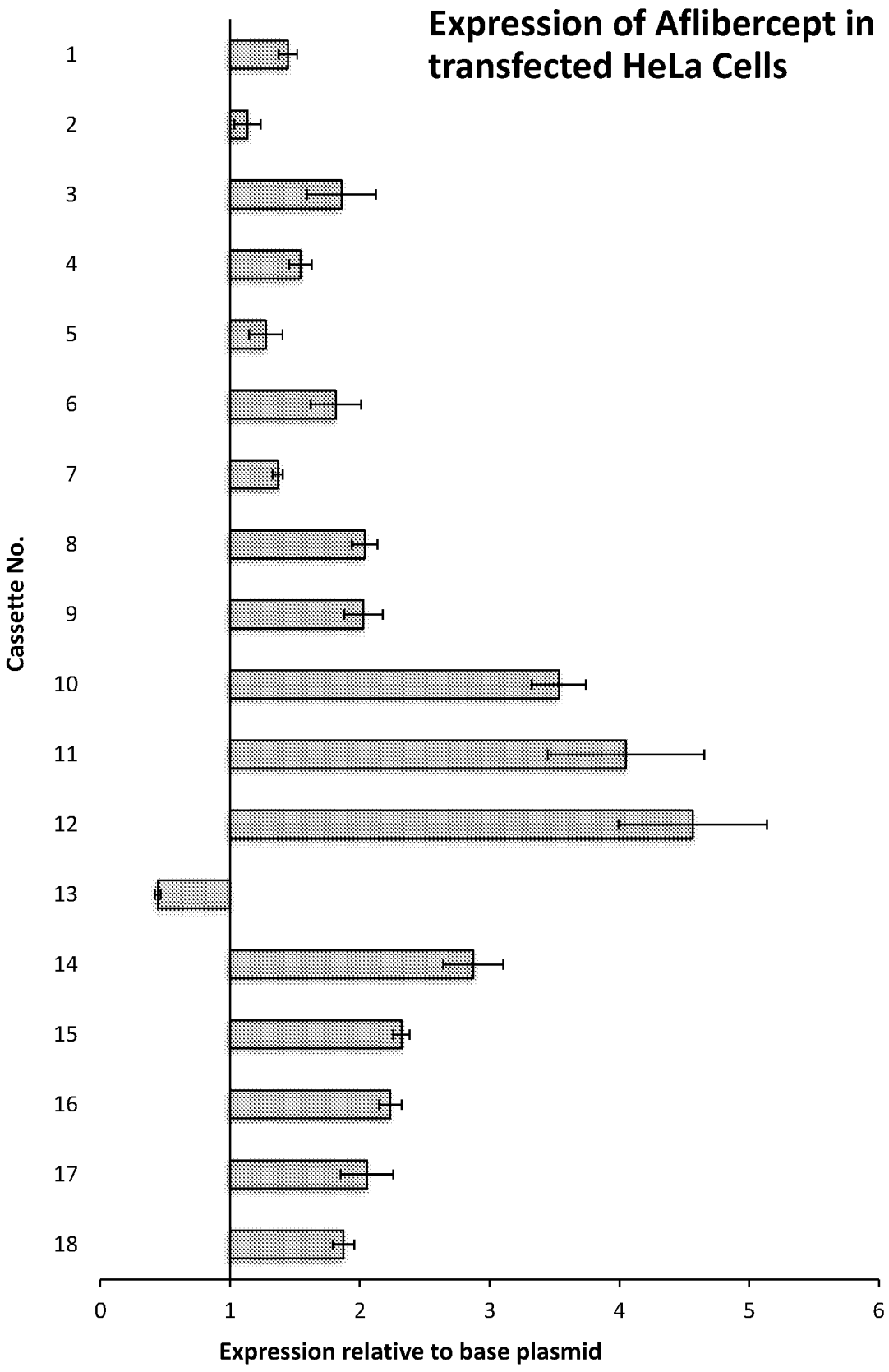

Expression of Aflibercept in transfected HeLa Cells
Cassette No.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
0
1
2
3
4
5
6
Expression relative to base plasmid

FIG. 3

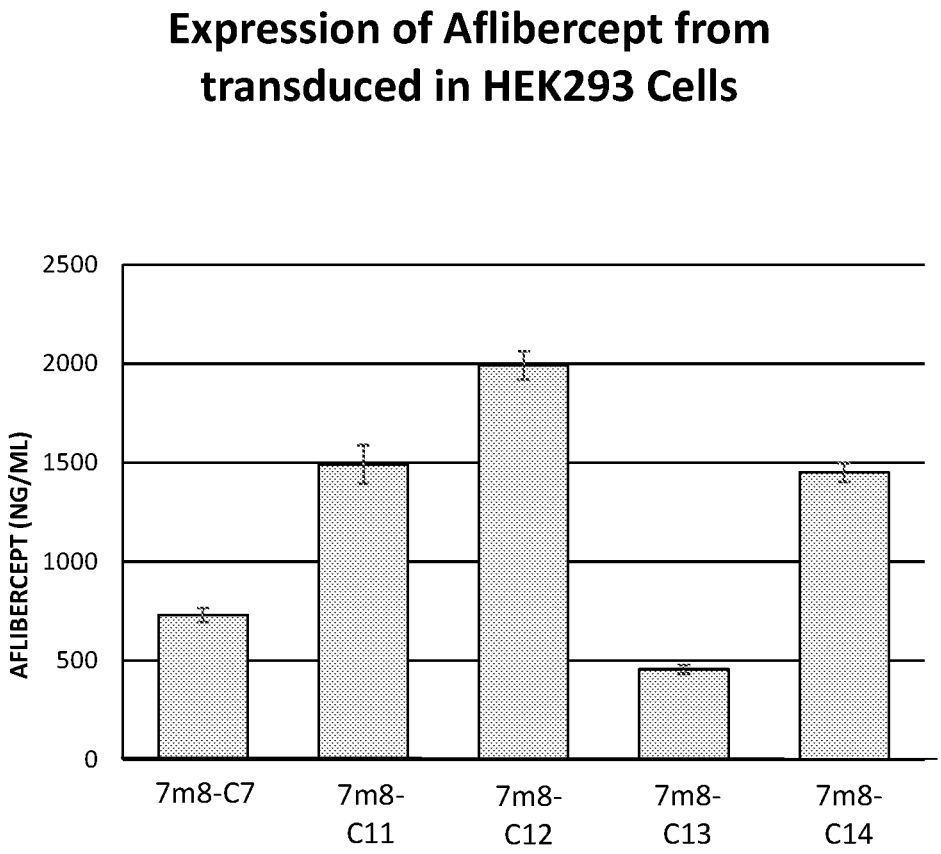

Expression of Aflibercept from transduced in HEK293 Cells
AFLIBERCEPT (NG/ML)
2500
2000
1500
1000
500
0
7m8-C7
7m8-C11
7m8-C12
7m8-C13
7m8-C14

FIG. 4A

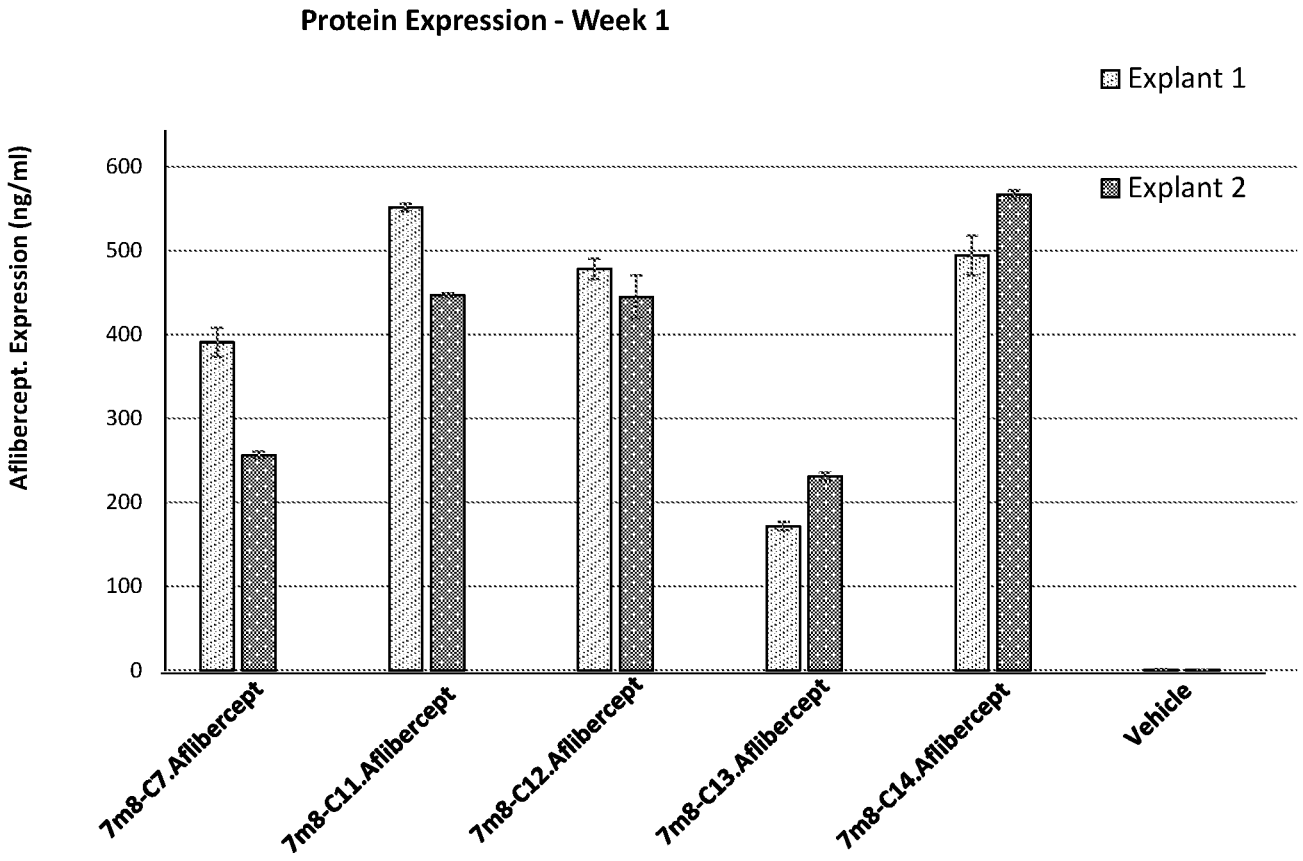

Protein Expression - Week 1
Explant 1
Explant 2
Aflibercept. Expression (ng/ml)
600
500
400
300
200
100
0
7m8-C7.Aflibercept
7m8-C11.Aflibercept
7m8-C12.Aflibercept
7m8-C13.Aflibercept
7m8-C14.Aflibercept
Vehicle

FIG. 4B

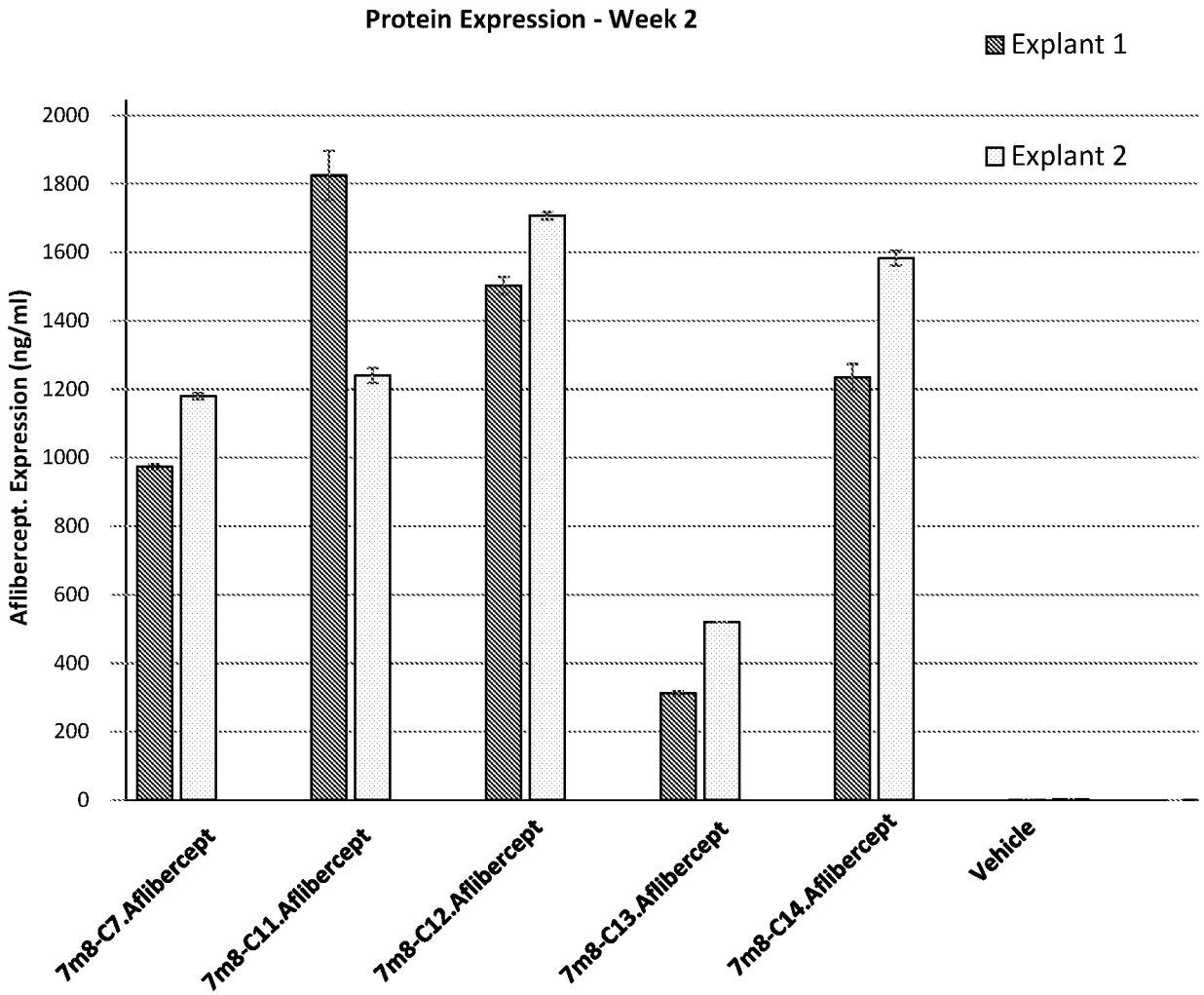

Protein Expression - Week 2
Explant 1
Explant 2
Aflibercept. Expression (ng/ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
7m8-C7.Aflibercept
7m8-C11.Aflibercept
7m8-C12.Aflibercept
7m8-C13.Aflibercept
7m8-C14.Aflibercept
Vehicle

FIG. 5

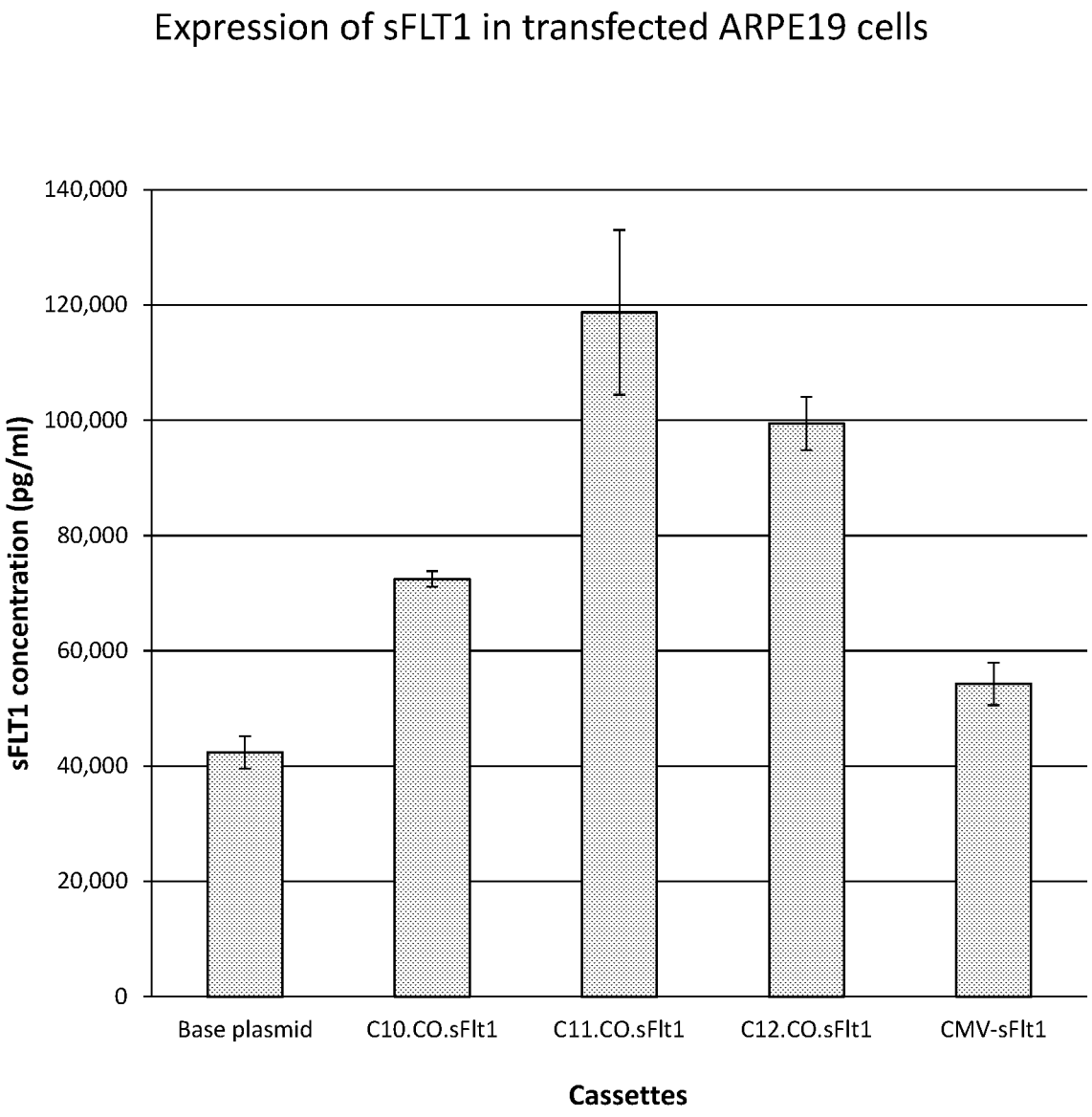

Expression of sFLT1 in transfected ARPE19 cells
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
sFLT1 concentration (pg/ml)
Base plasmid
C10.CO.sFlt1
C11.CO.sFlt1
C12.CO.sFlt1
CMV-sFlt1
Cassettes

FIG. 6

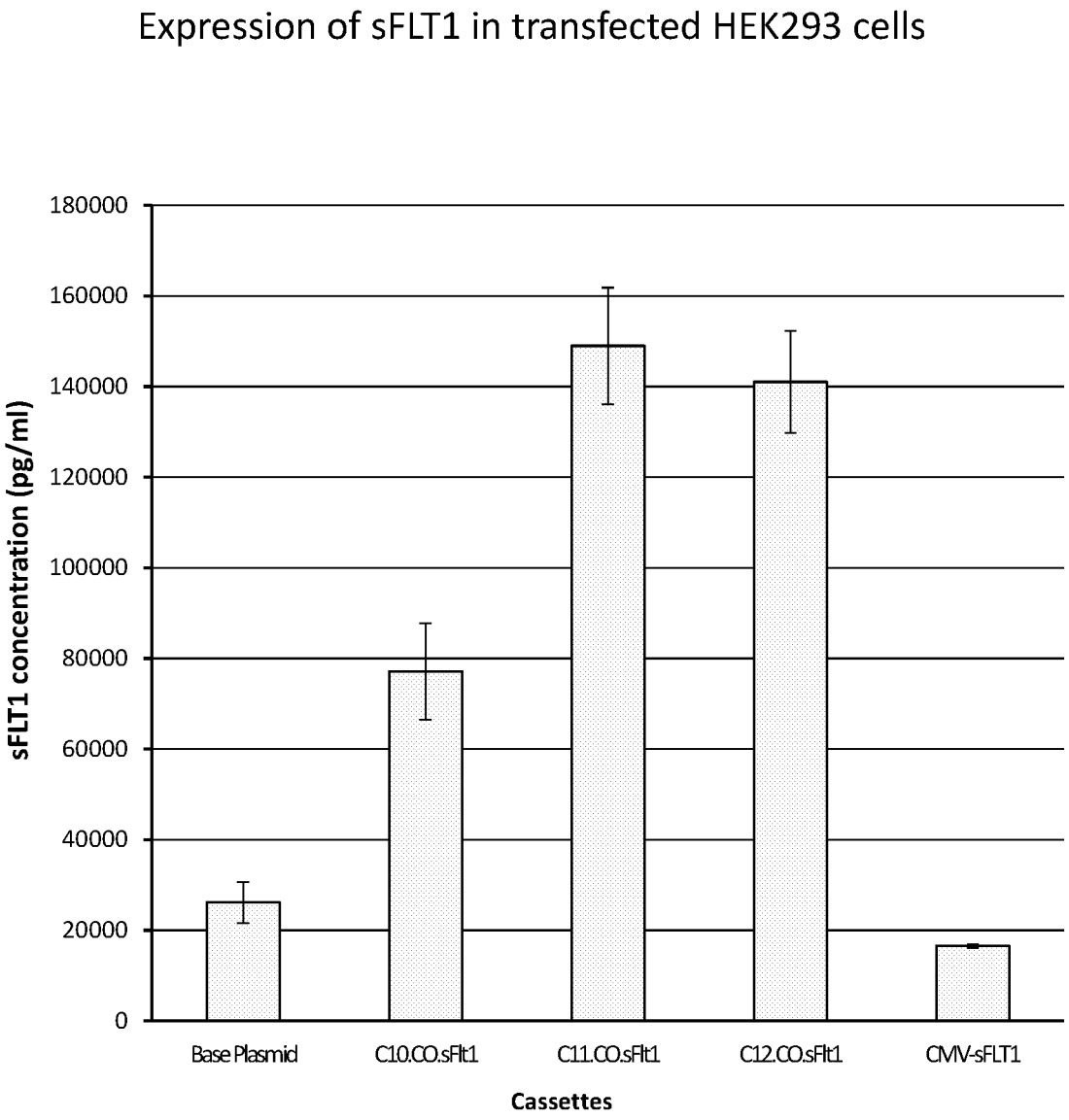

Expression of sFLT1 in transfected HEK293 cells
sFLT1 concentration (pg/ml)
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Base Plasmid
C10.CO.sFlt1
C11.CO.sFlt1
C12.CO.sFlt1
CMV-sFLT1
Cassettes

FIG. 7

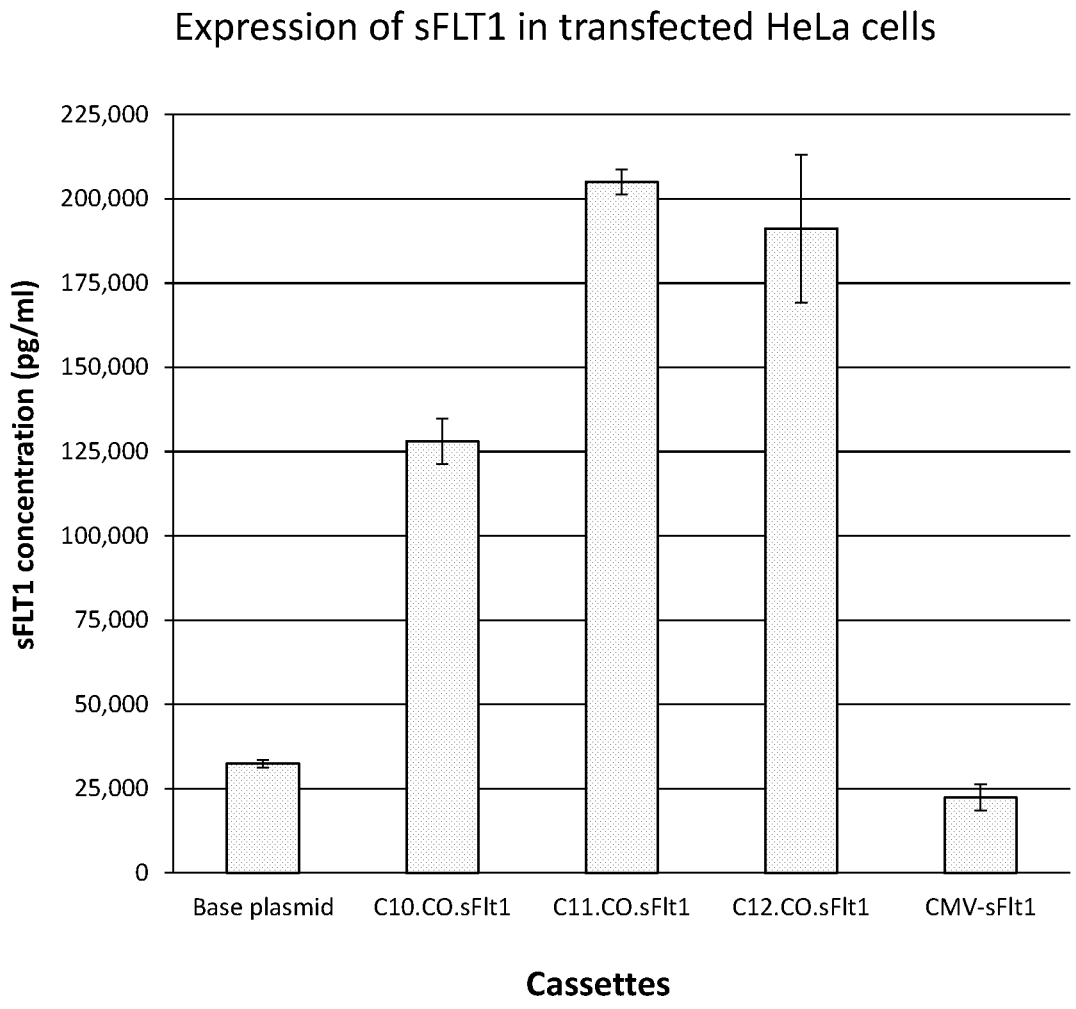

Expression of sFLT1 in transfected HeLa cells
sFLT1 concentration (pg/ml)
225,000
200,000
175,000
150,000
125,000
100,000
75,000
50,000
25,000
0
Base plasmid
C10.CO.sFlt1
C11.CO.sFlt1
C12.CO.sFlt1
CMV-sFlt1
Cassettes

FIG. 8

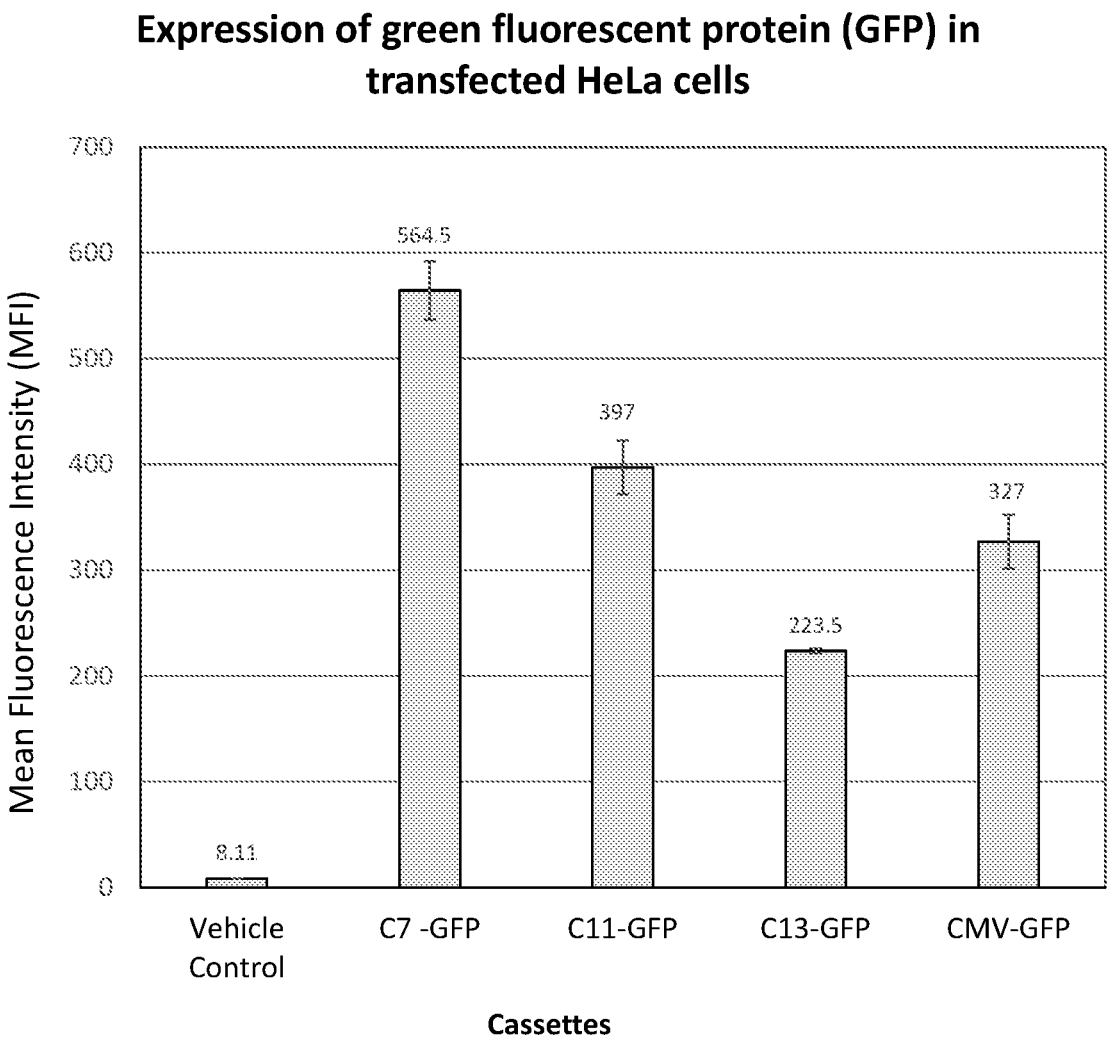
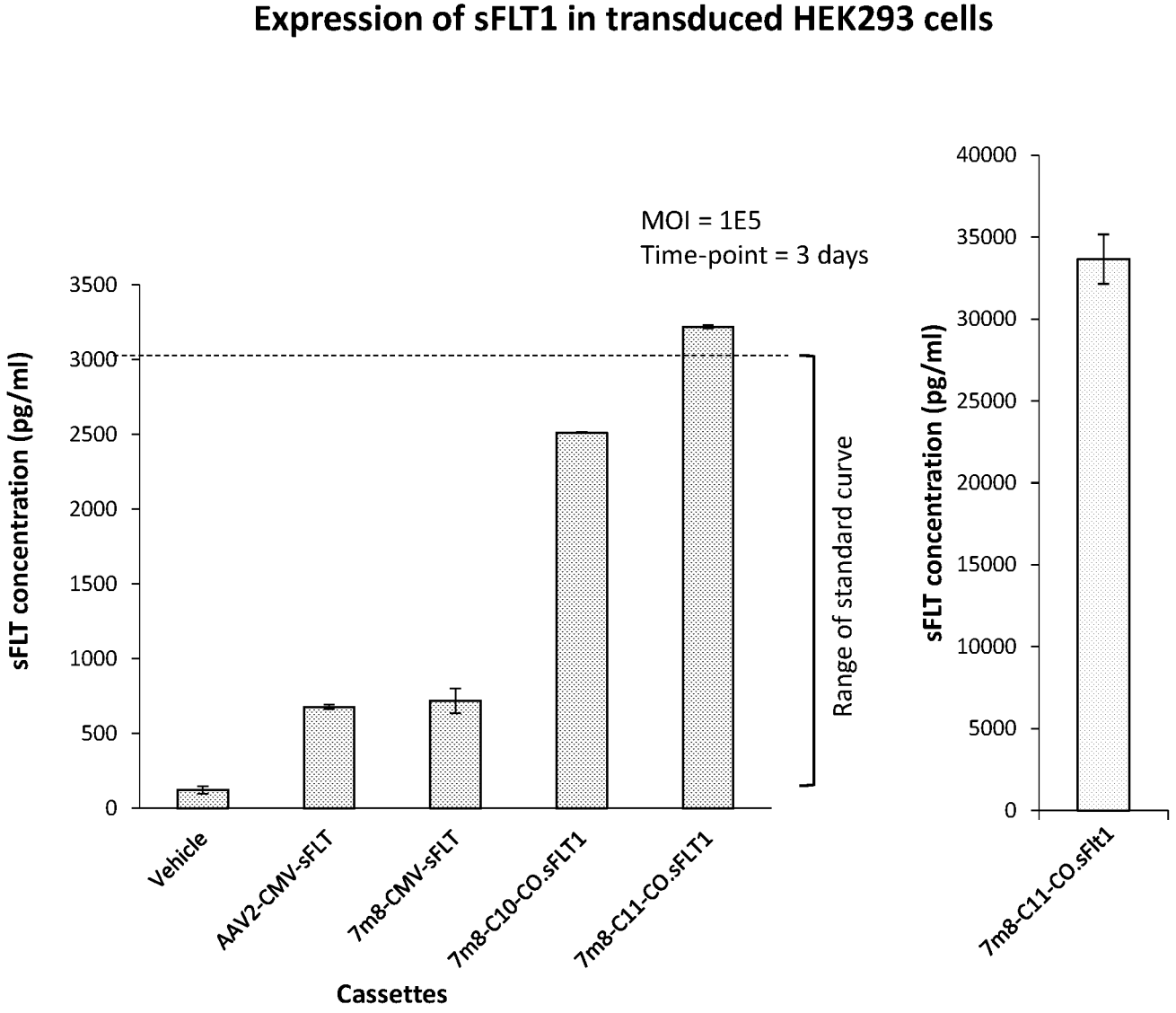

Expression of green fluorescent protein (GFP) in transfected HeLa cells
Mean Fluorescence Intensity (MFI)
700
600
500
400
300
200
100
0
8.11
564.5
397
223.5
327
Vehicle Control
C7 -GFP
C11-GFP
C13-GFP
CMV-GFP
Cassettes

FIG. 9


Expression of sFLT1 in transduced HEK293 cells
MOI = 1E5
Time-point = 3 days
sFLT concentration (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
Range of standard curve
Vehicle
AAV2-CMV-sFLT
7m8-CMV-sFLT
7m8-C10-CO.sFLT1
7m8-C11-CO.sFLT1
Cassettes
40000
35000
30000
25000
20000
15000
10000
5000
0
sFLT concentration (pg/ml)
7m8-C11-CO.sFlt1

Expression of sFLT1 from transduced pig retinal explants

FIG. 10A

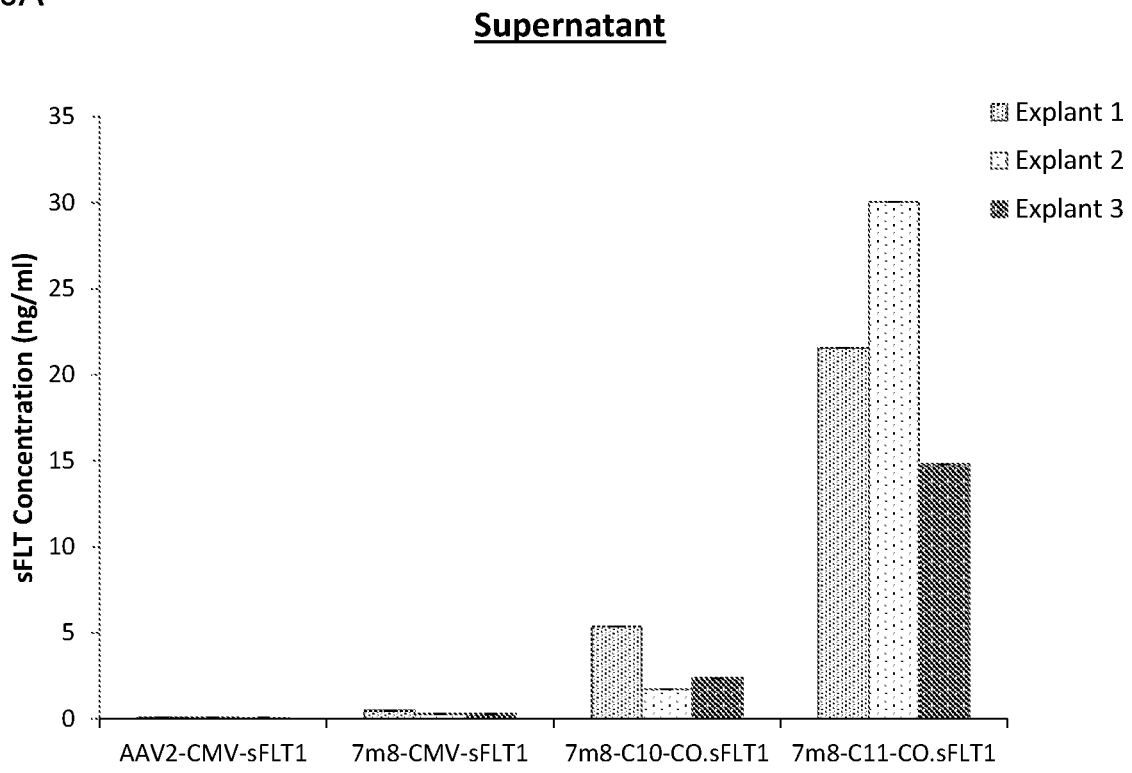
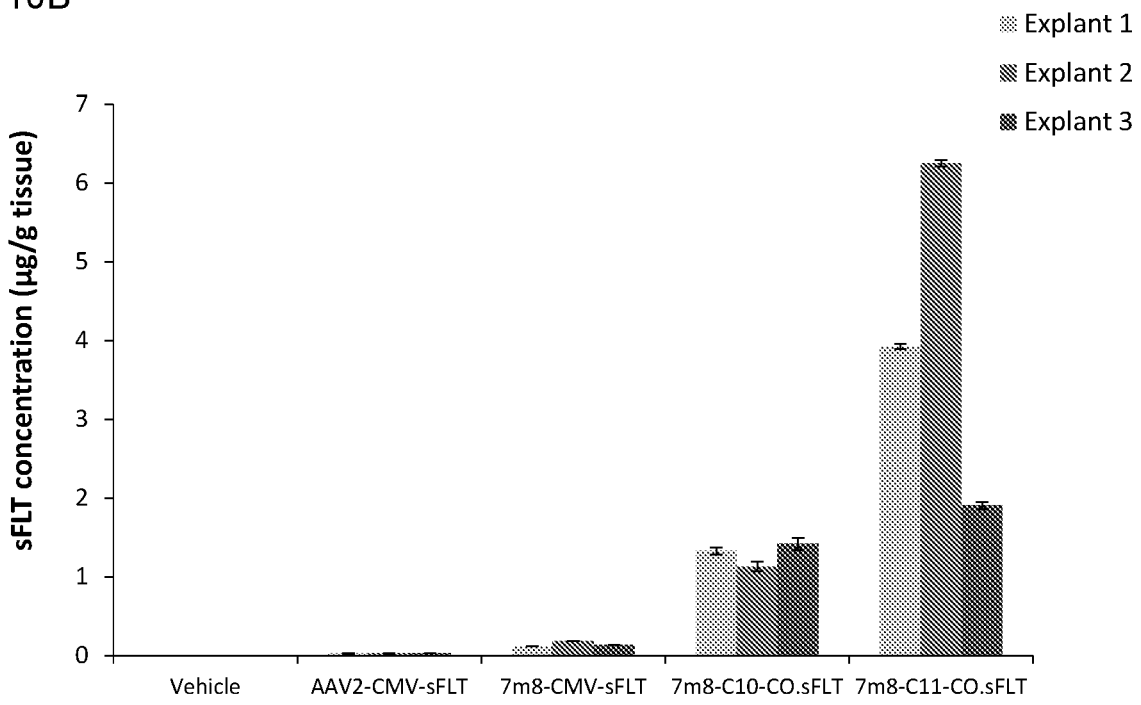


FIG. 10B



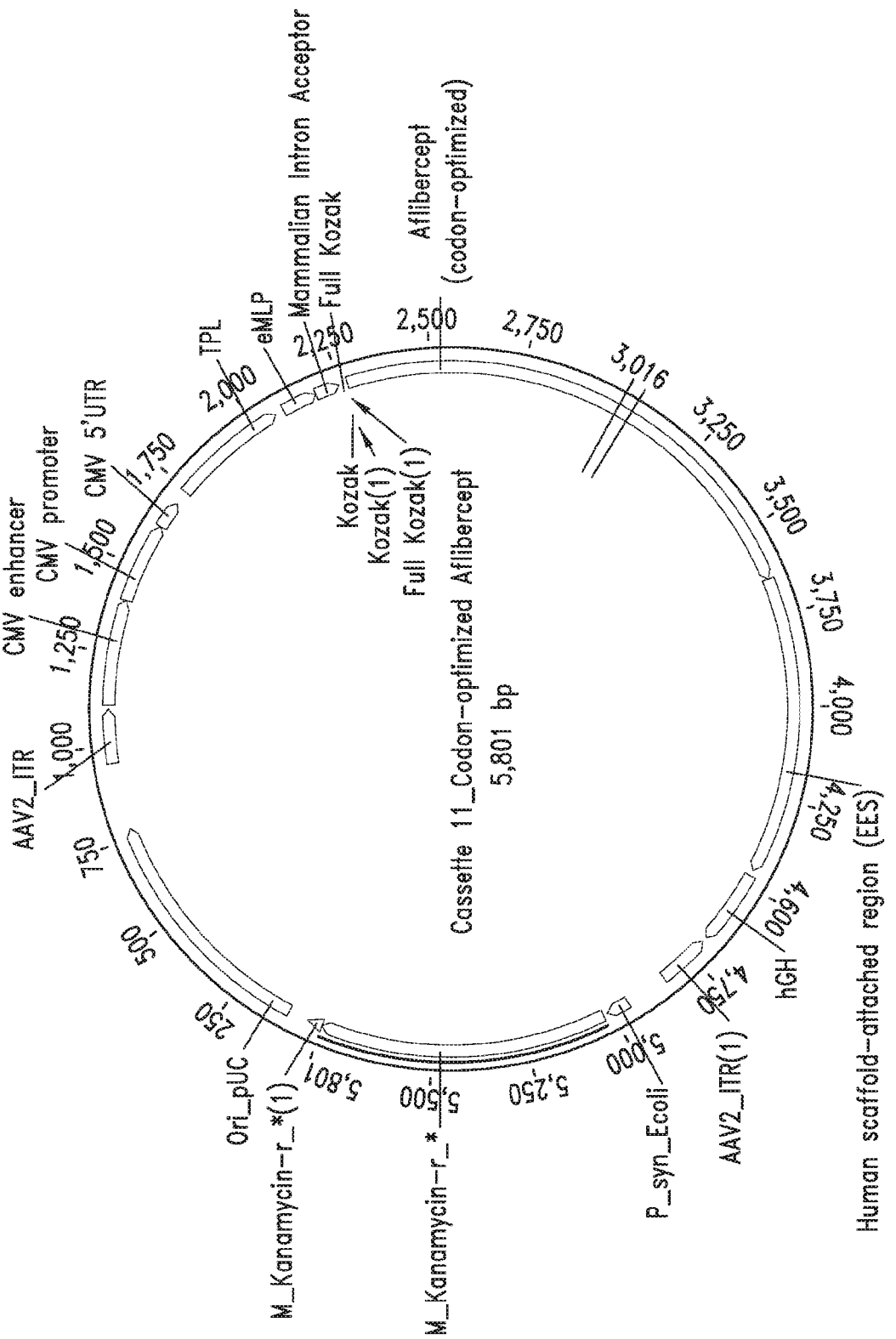

Cassette 11_Codon-optimized Aflibercept
5,801 bp
Kozak
Kozak(1)
Full Kozak(1)
Aflibercept (codon-optimized)
Mammalian Intron Acceptor
Full Kozak
eMLP
TPL
CMV 5'UTR
CMV promoter
CMV enhancer
AAV2_ITR
Ori_pUC
M_Kanamycin-r_*(1)
M_Kanamycin-r_*
P_syn_Ecoli
AAV2_ITR(1)
hGH
Human scaffold-attached region (EES)

FIG. 11