

Features	Nucleotides
AAV2-ITR	1-140
LoxP	156-189
SV40 pA	330-196
Repressor Cloning Site 1	351-345
EF1-MTLV prom	901-533
Transcription Blocker	913-1091
Promoter Cloning Site 2	1092-1098
Gene of Interest Cloning Site 3	1098-1104
SV40 pA	1113-1247
LoxP	1248-1281
AAV2-ITR	1305-1444

FIGURE 1

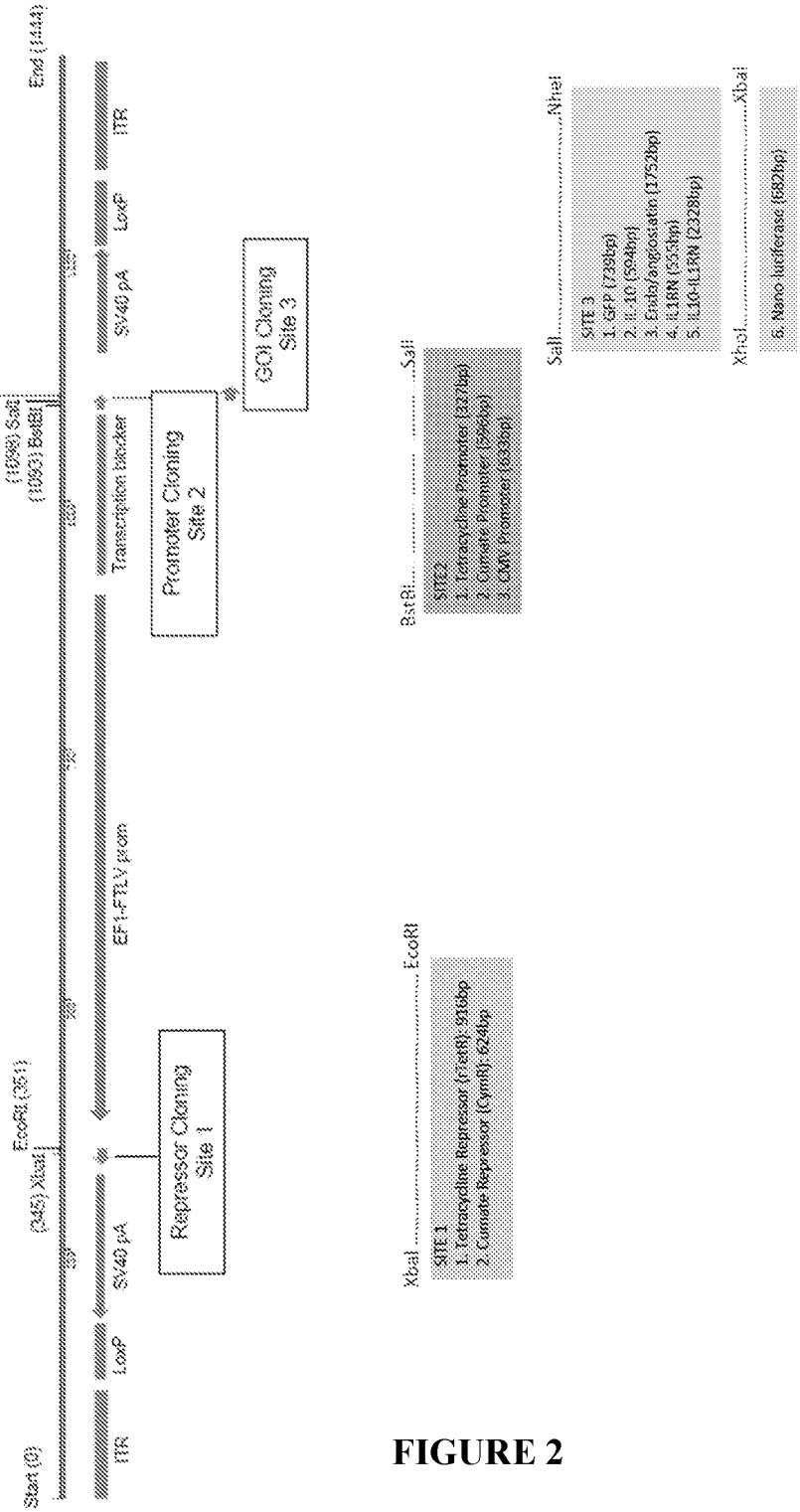

Start (0)
ITR
LoxP
SV40 pA
Repressor Cloning Site 1
EcoRI (351)
(345) XbaI
EF1-FTLV prom
Transcription blocker
Promoter Cloning Site 2
(11104) NheI
(10998) SalI
(10993) BstBI
SV40 pA
LoxP
ITR
GOI Cloning Site 3
End (1444)
XbaI
EcoRI
SITE 1
1. Tetracycline Repressor (rTetR) 516bp
2. Cumate Repressor (CymR) 624bp
BstBI
SalI
SITE 2
1. Tetracycline Promoter (327bp)
2. Cumate Promoter (594bp)
3. CMV Promoter (1338bp)
SalI
NheI
SITE 3
1. GFP (738bp)
2. IL-10 (594bp)
3. Endostatin (1752bp)
4. L18N (555bp)
5. IL6-L18N (2328bp)
XhoI
XbaI
6. Nano-luciferase (682bp)

FIGURE 2

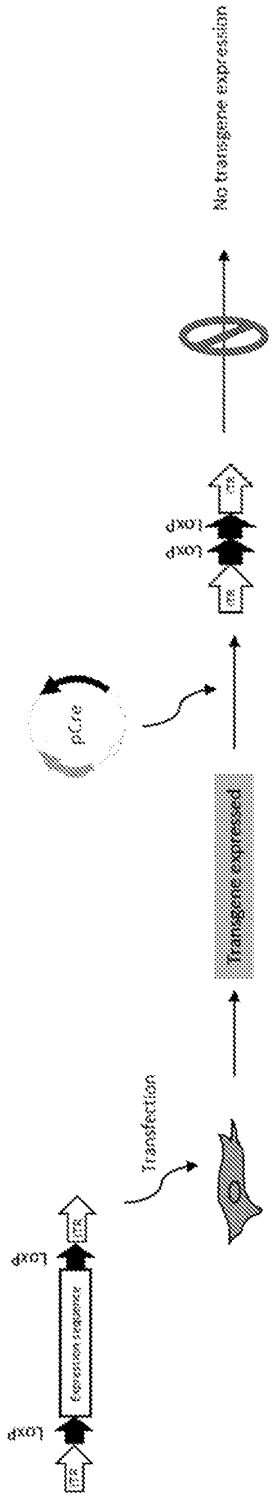

No transgene expression
loxP
loxP
pCre
Transgene expressed
Transfection
loxP
loxP

FIGURE 3

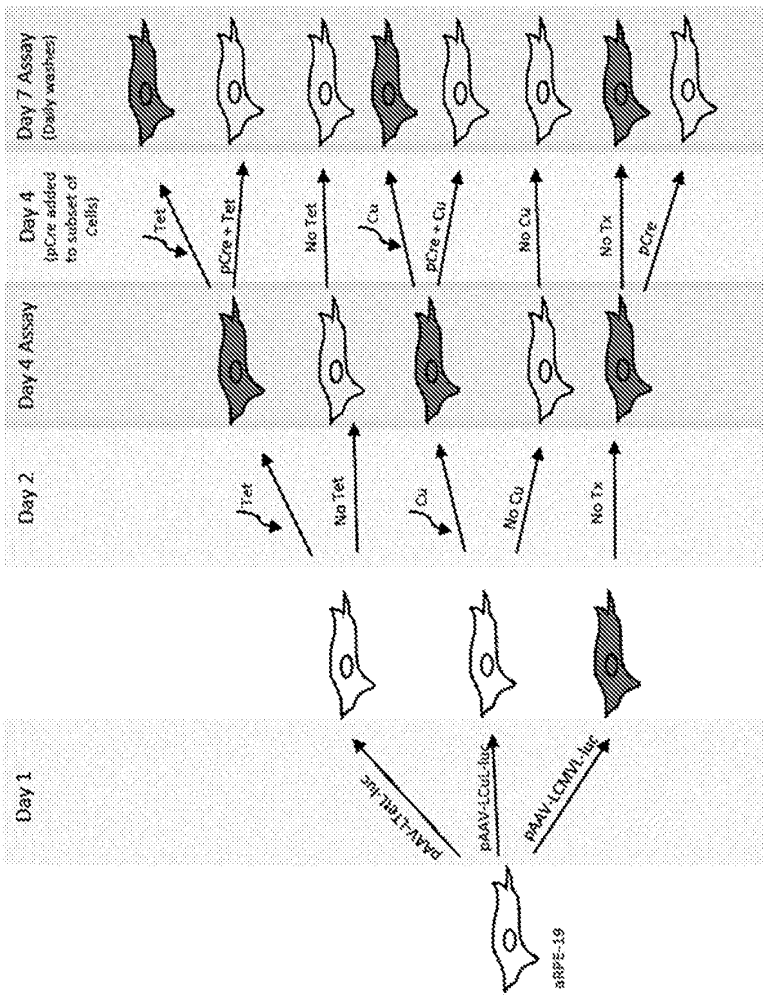

Day 7 Assay
(Daily washes)
Day 4
(pCre added
to subset of
Cells)
Day 4 Assay
Day 2
Day 1
Tet
pCre + Tet
No Tet
Cu
pCre + Cu
No Cu
No Tx
pCre
pAAV-LTetL-luc
pAAV-LCuL-RIG
pAAV-LEMVL-luc
aRPE-19

FIGURE 4

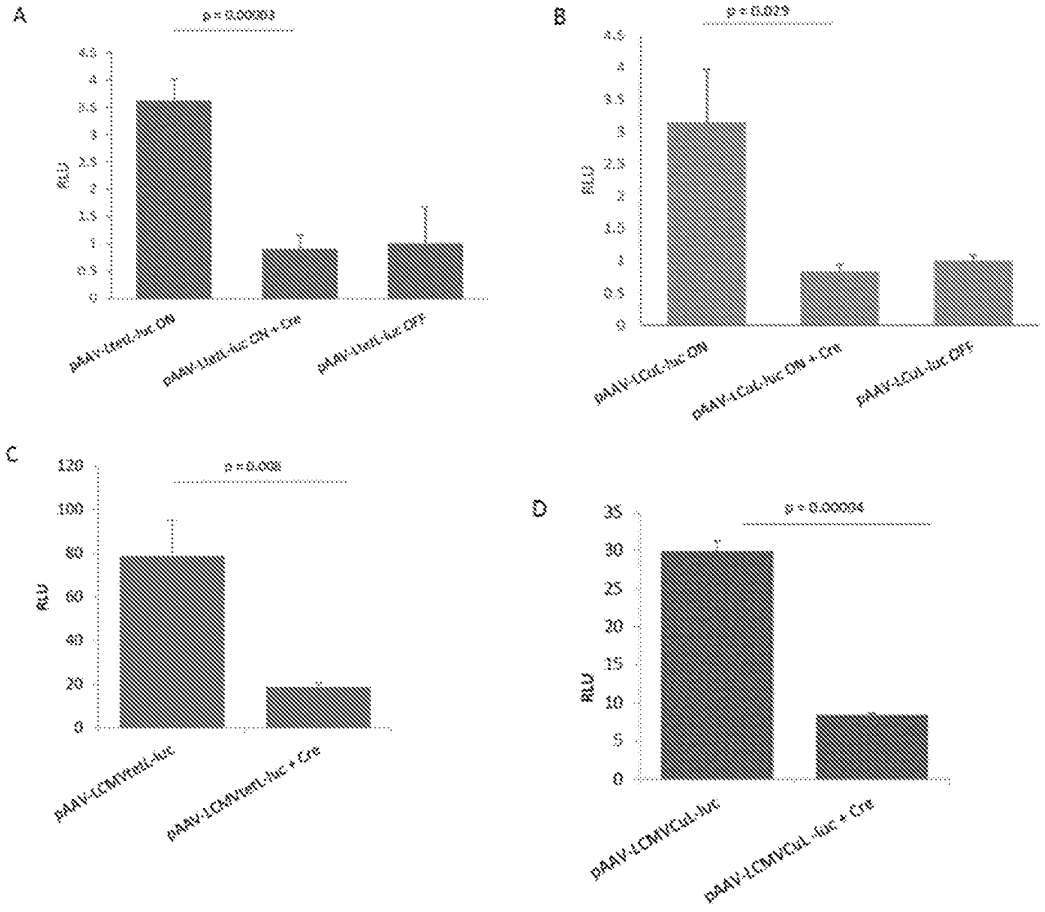

A
p = 0.00003
RLU
pAAV-LTetL-luc ON
pAAV-LTetL-luc ON + Cre
pAAV-LTetL-luc OFF
B
p = 0.029
RLU
pAAV-LCaL-luc ON
pAAV-LCaL-luc ON + Cre
pAAV-LCaL-luc OFF
C
p = 0.008
RLU
pAAV-LCMVTetL-luc
pAAV-LCMVTetL-luc + Cre
D
p = 0.00004
RLU
pAAV-LCMVCaL-luc
pAAV-LCMVCaL -luc + Cre

FIGURE 5

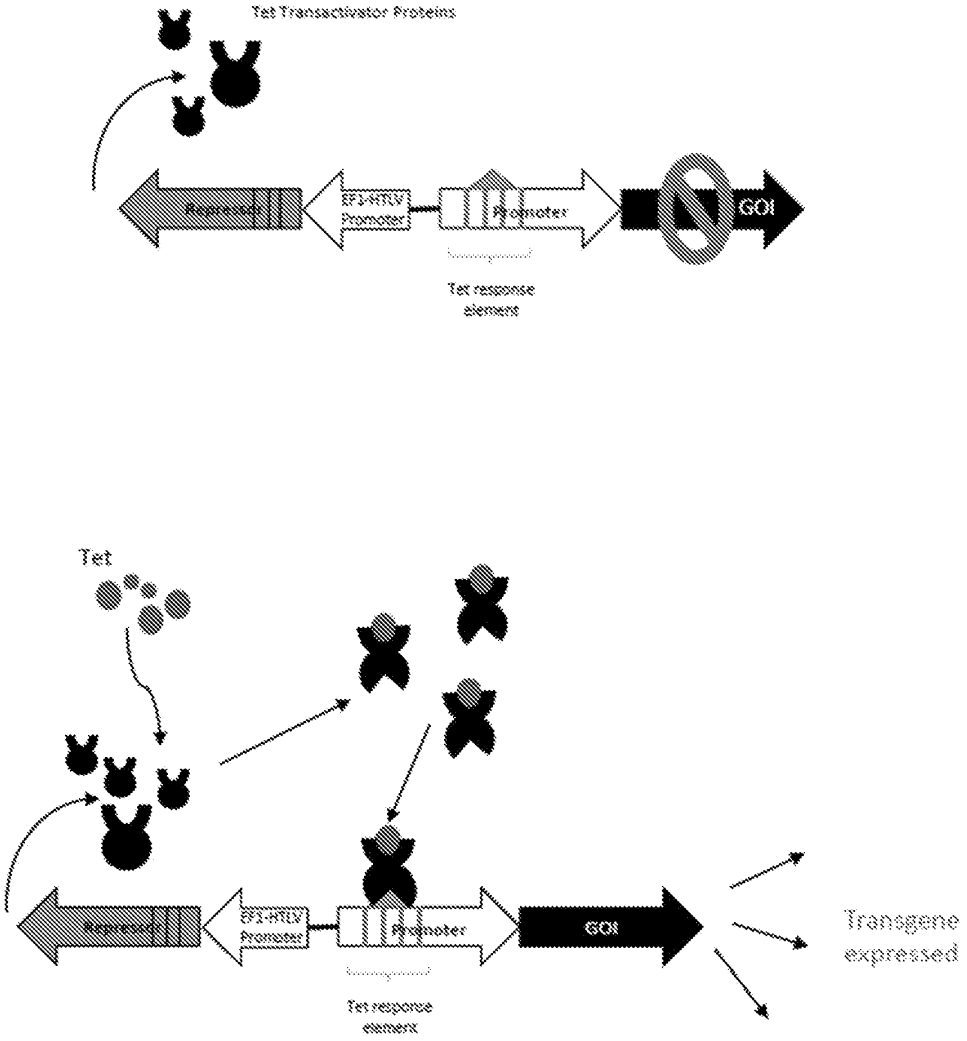

Tet Transactivator Proteins
Reverse
EF1-HTLV Promoter
Promoter
GOI
Tet response element
Tet
Reverse
EF1-HTLV Promoter
Promoter
GOI
Tet response element
Transgene expressed

FIGURE 6A

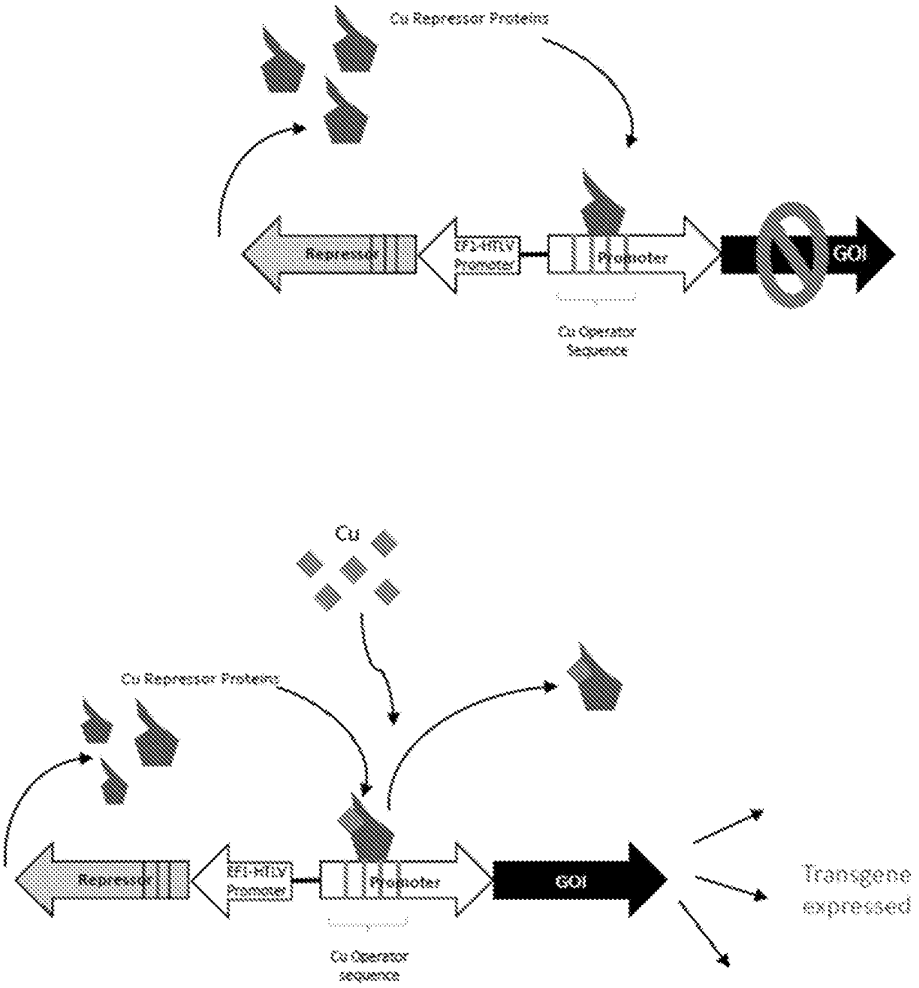

Cu Repressor Proteins
Repressor
EF1-HTLV Promoter
Promoter
GOI
Cu Operator Sequence
Cu
Cu Repressor Proteins
Repressor
EF1-HTLV Promoter
Promoter
GOI
Cu Operator sequence
Transgene expressed

FIGURE 6B

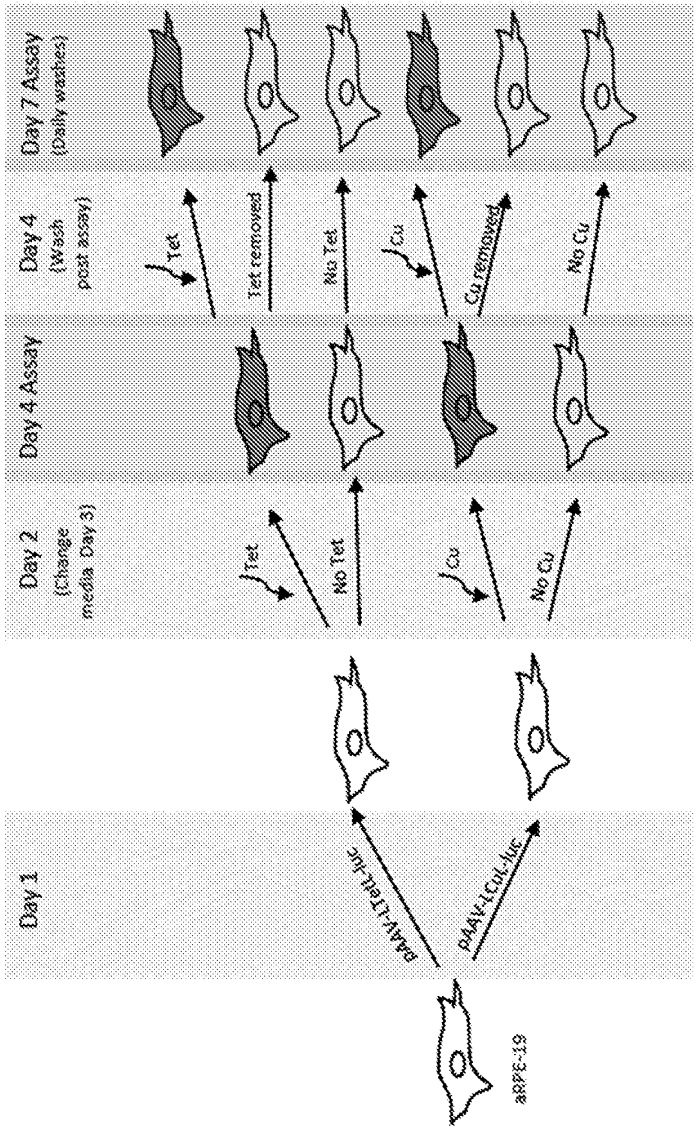
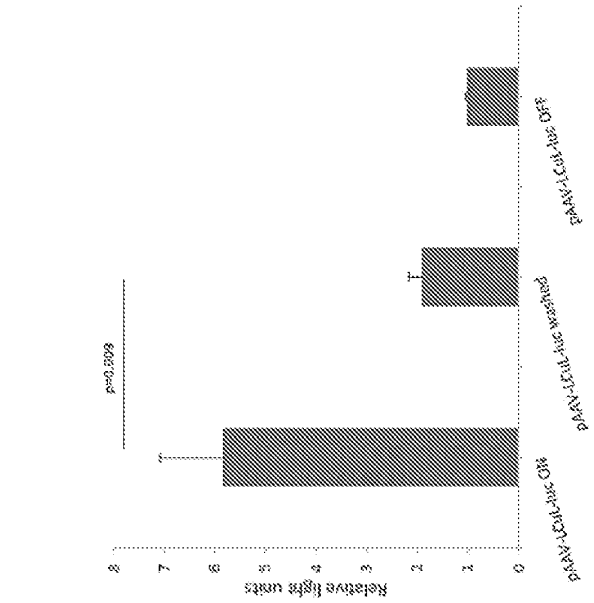

Day 1
pAAV-LTetL-luc
pAAV-1Cu1-AsC
aRPE-19
Day 2
(Change
media Day 3)
Tet
No Tet
Cu
No Cu
Day 4 Assay
Day 4
(Wash
post assay)
Tet
Tet removed
No Tet
Cu
Cu removed
No Cu
Day 7 Assay
(Daily washes)

FIGURE 7


B
Relative light units
0
1
2
3
4
5
6
7
8
p=0.009
pAAV-LCtrL-luc ON
pAAV-LCtrL-luc washed
pAAV-LCtrL-luc OFF

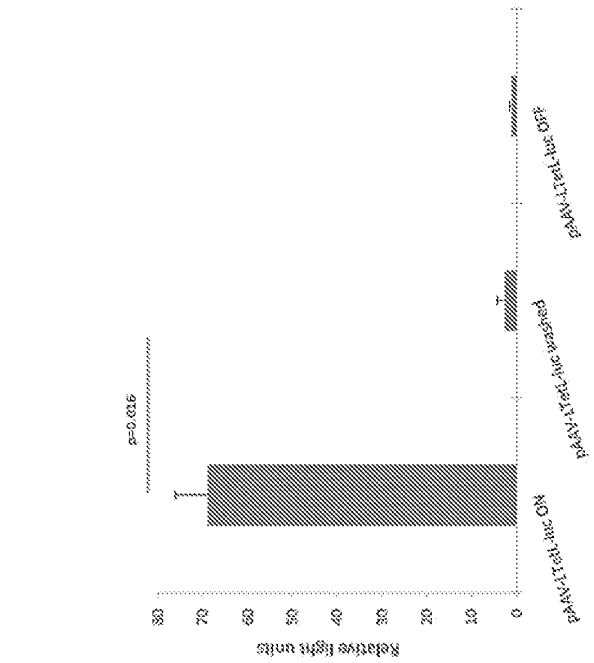

A
Relative light units
0
10
20
30
40
50
60
70
80
p=0.016
pAAV-LTetL-luc ON
pAAV-LTetL-luc washed
pAAV-LTetL-luc OFF

FIGURE 8

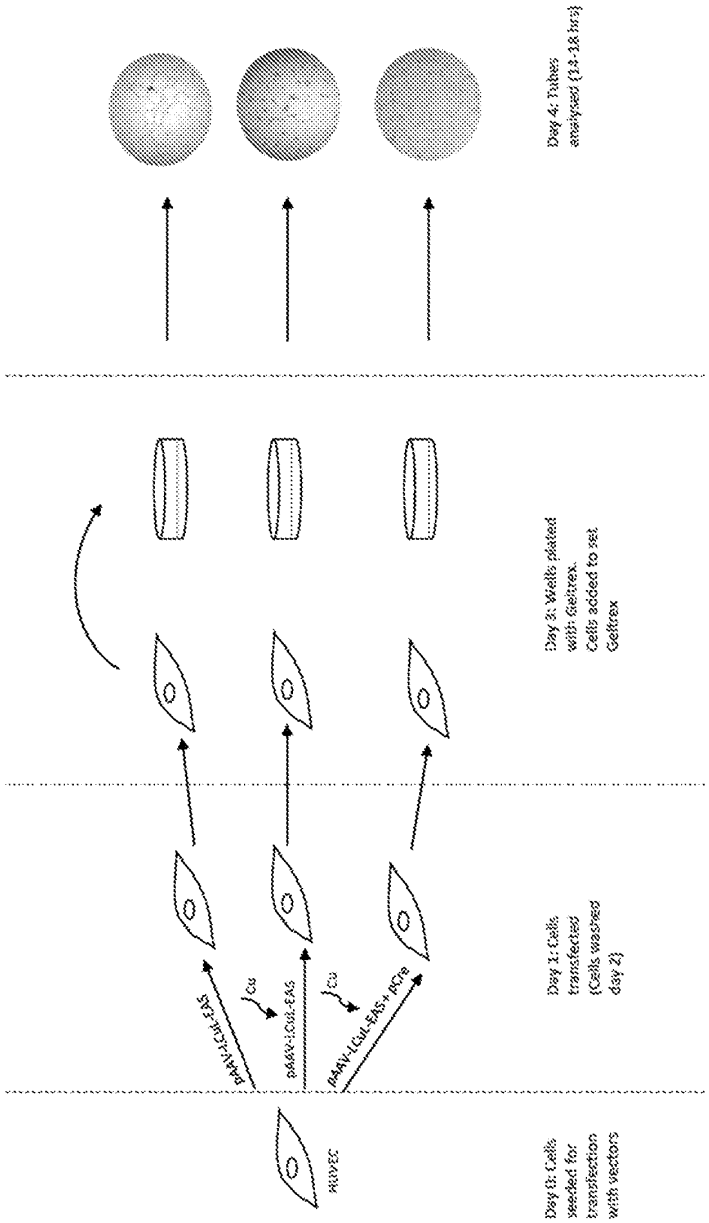

Day 4: Tubes analysed (13-18 hrs)
Day 3: Wells plated with Geltrex. Cells added to set Geltrex
Day 1: Cells transfected (Cells washed day 2)
Day 0: Cells seeded for transfection with vectors
Cu
Cu
wuvec

FIGURE 9

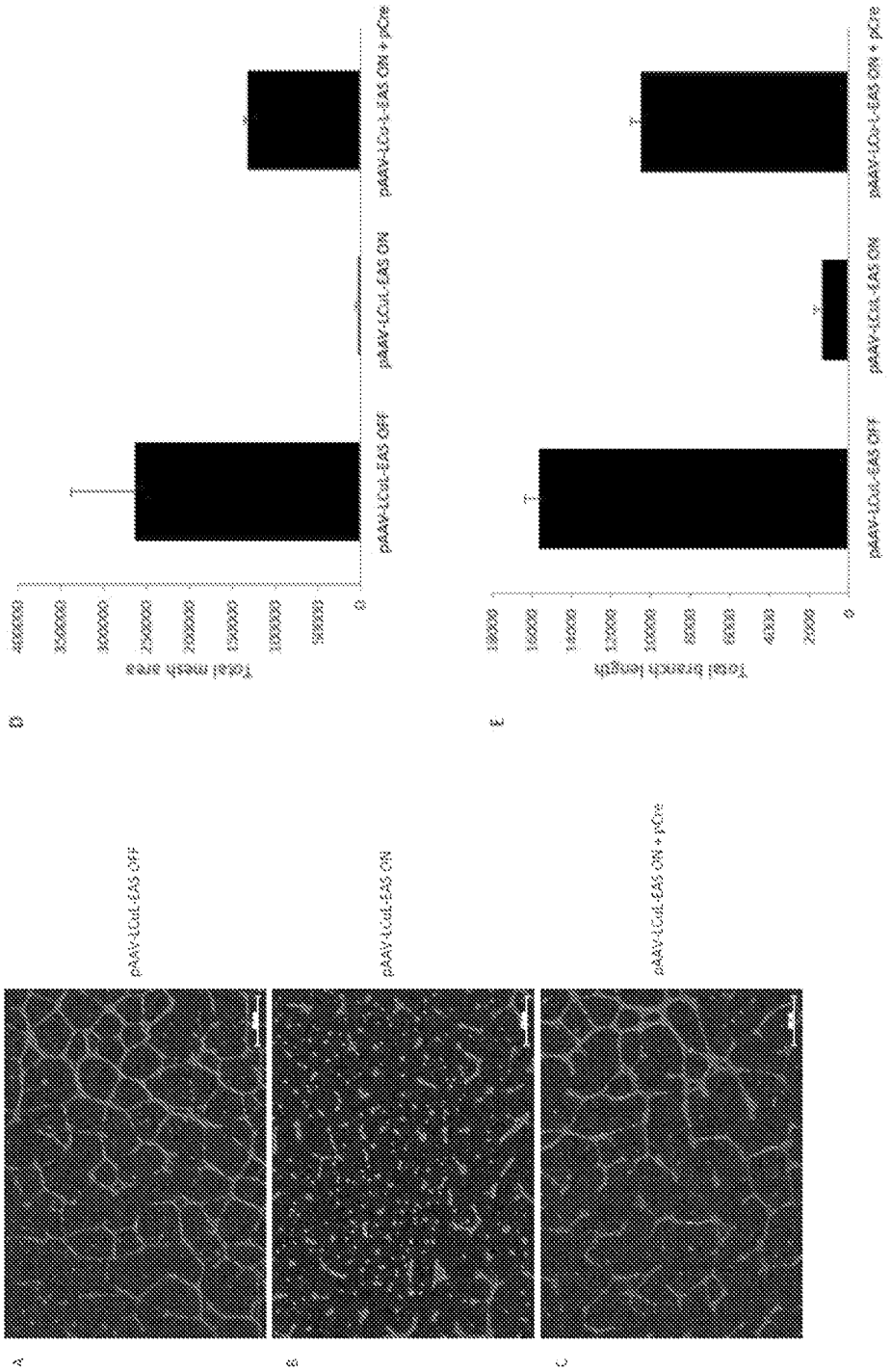

pAAV-UCoL-EAS OFF
pAAV-UCoL-EAS ON
pAAV-UCoL-EAS ON + pCre
Total mesh area
Total branch length
A
B
C

FIGURE 10

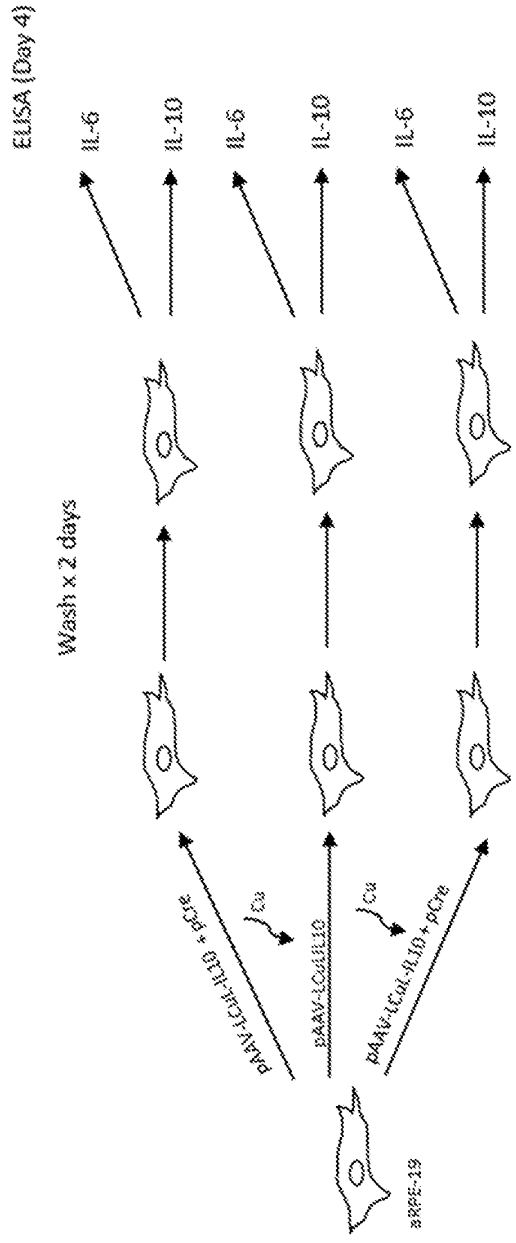

ELISA (Day 4)
IL-6
IL-10
IL-6
IL-10
IL-6
IL-10
Wash x 2 days
Ca
Ca
ARPE-19

FIGURE 11

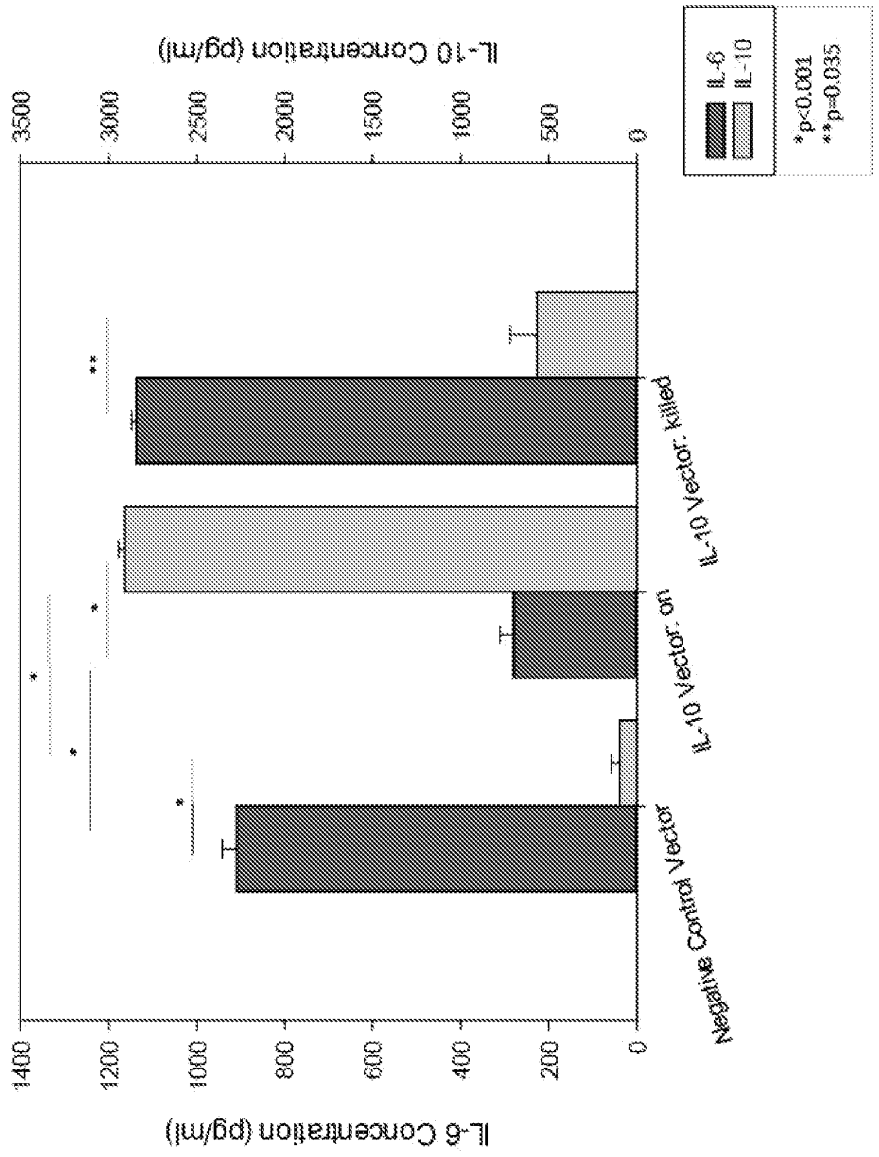

IL-6 Concentration (pg/ml)
1400
1200
1000
800
600
400
200
0
IL-10 Concentration (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
Negative Control Vector
IL-10 Vector: on
IL-10 Vector: killed
IL-6
IL-10
*p<0.001
**p=0.035

FIGURE 12