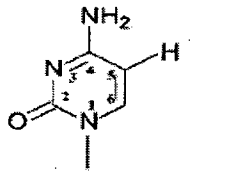
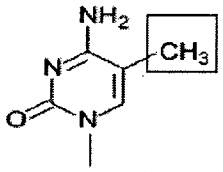
A



Cytosine
(within CG context)



5-methylcytosine

B

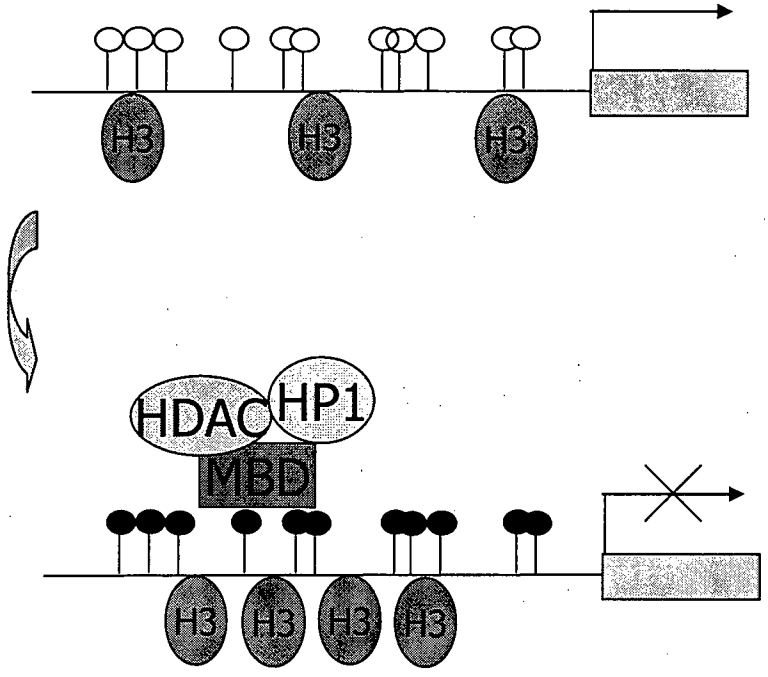


Figure 1

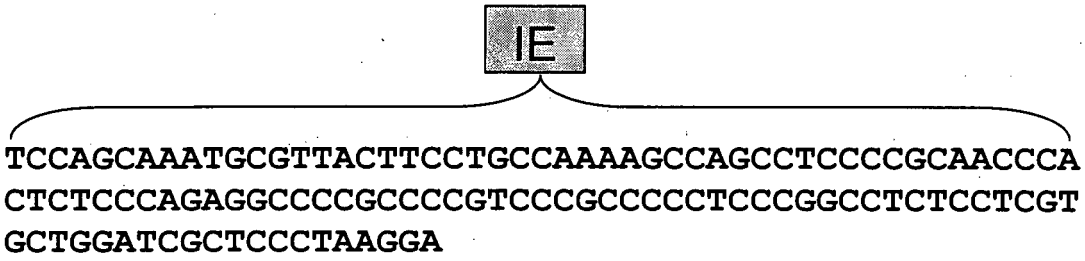


Figure 2

A

TTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTA**CG**GGGTCATTAGTTCATA
GCCCATATATGGAGTTC**CGCG**TTACATAACTTA**CG**GTAAATGGCC**CG**CCTGGCTGAC**CG**
CCCAA**CG**ACCCC**CG**CCCATTGA**CG**TCAATAATGA**CG**TATGTTCCCATAGTAA**CG**CCAAT
AGGGACTTTCCATTGA**CG**TCAATGGGTGGAGTATTTA**CG**GTAAACTGCCCACTTGGCAG
TACATCAAGTGTATCATATGCCAAGTA**CG**CCCCCTATTGA**CG**TCAATGA**CG**GTAAATGG
CC**CG**CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACAT
CTA**CG**TATTAGTCAT**CG**CTATTA<u>**CTCGAG**</u>TGATG**CG**GTTTTGGCAGTACATCAATGGG**C**
GTGGATAG**CG**GTTTGACTCA**CG**GGGATTTCCAAGTCTCCACCCCATTGA**CG**TCAATGGG
AGTTTGTTTTGGCACCAAAATCAA**CG**GGACTTTCCAAAATGT**CG**TAACAACTC**CG**CCCC
ATTGA**CG**CAAATGGG**CG**GTAGG**CG**TGTA**CG**GTGGGAGGTCTATATAAGCAGAGCTC

B

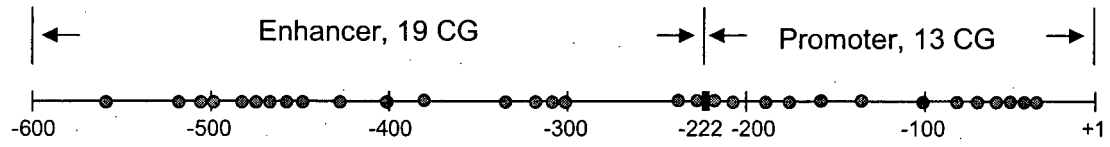


Figure 3

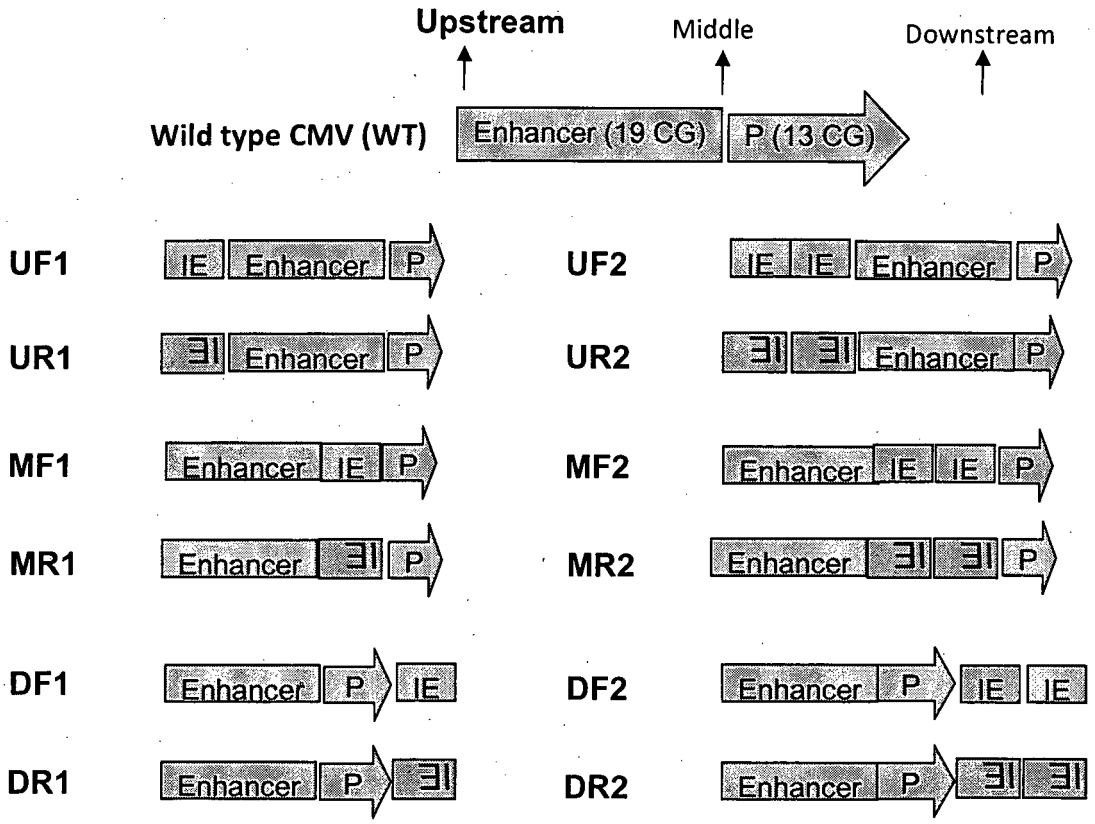

Upstream
Middle
Downstream
Wild type CMV (WT)
Enhancer (19 CG)
P (13 CG)
UF1
IE
Enhancer
P
UF2
IE
IE
Enhancer
P
UR1
∃I
Enhancer
P
UR2
∃I
∃I
Enhancer
P
MF1
Enhancer
IE
P
MF2
Enhancer
IE
IE
P
MR1
Enhancer
∃I
P
MR2
Enhancer
∃I
∃I
P
DF1
Enhancer
P
IE
DF2
Enhancer
P
IE
IE
DR1
Enhancer
P
∃I
DR2
Enhancer
P
∃I
∃I

Figure 4

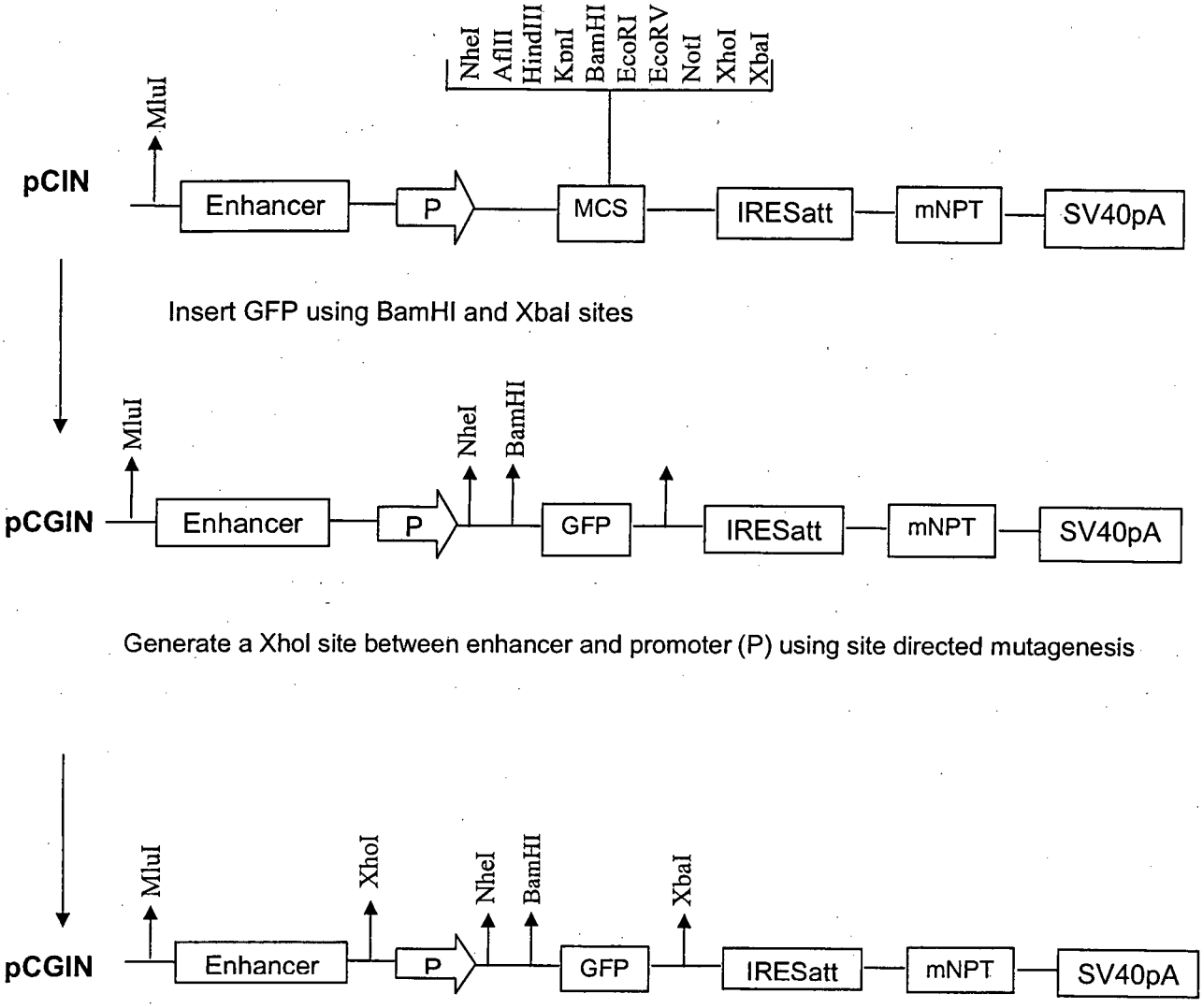

NheI
AflII
HindIII
KpnI
BamHI
EcoRI
EcoRV
NotI
XhoI
XbaI
MluI
pCIN
Enhancer
P
MCS
IRESatt
mNPT
SV40pA
Insert GFP using BamHI and XbaI sites
MluI
NheI
BamHI
pCGIN
Enhancer
P
GFP
IRESatt
mNPT
SV40pA
Generate a XhoI site between enhancer and promoter (P) using site directed mutagenesis
MluI
XhoI
NheI
BamHI
XbaI
pCGIN
Enhancer
P
GFP
IRESatt
mNPT
SV40pA

Figure 5

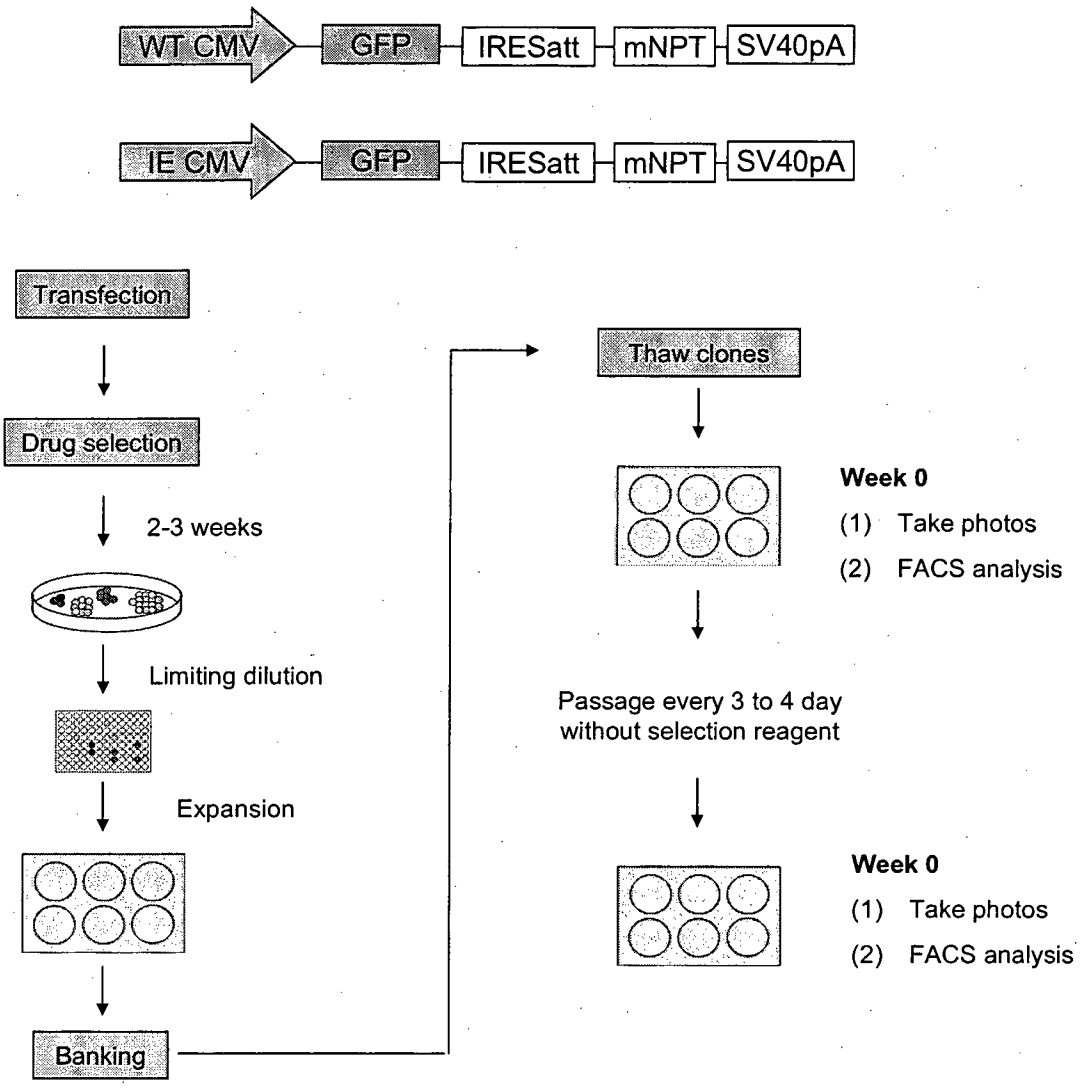
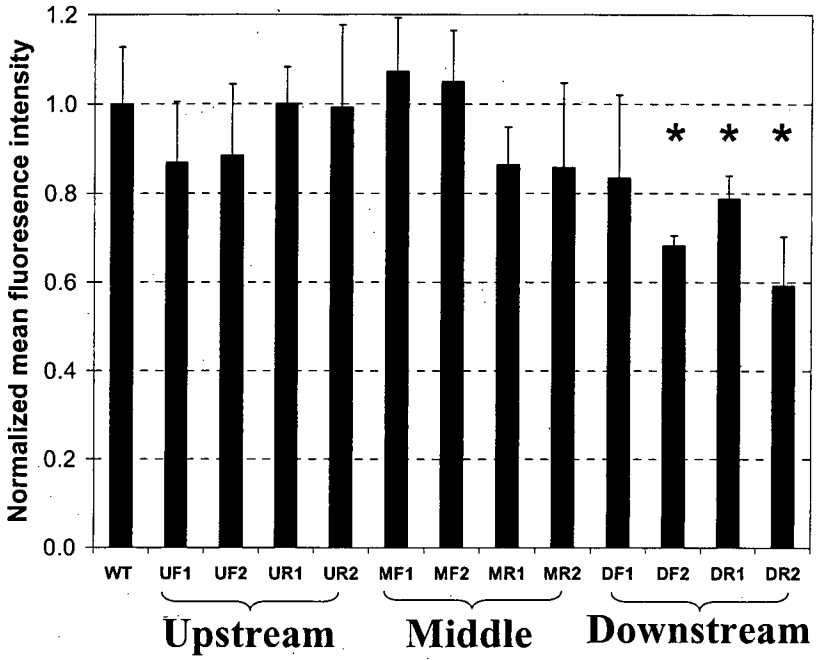

WT CMV
GFP
IRESatt
mNPT
SV40pA
IE CMV
GFP
IRESatt
mNPT
SV40pA
Transfection
Drug selection
2-3 weeks
Limiting dilution
Expansion
Banking
Thaw clones
Week 0
(1) Take photos
(2) FACS analysis
Passage every 3 to 4 day
without selection reagent
Week 0
(1) Take photos
(2) FACS analysis

Figure 6

A


Normalized mean fluoresence intensity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WT UF1 UF2 UR1 UR2 MF1 MF2 MR1 MR2 DF1 DF2 DR1 DR2
Upstream
Middle
Downstream

B

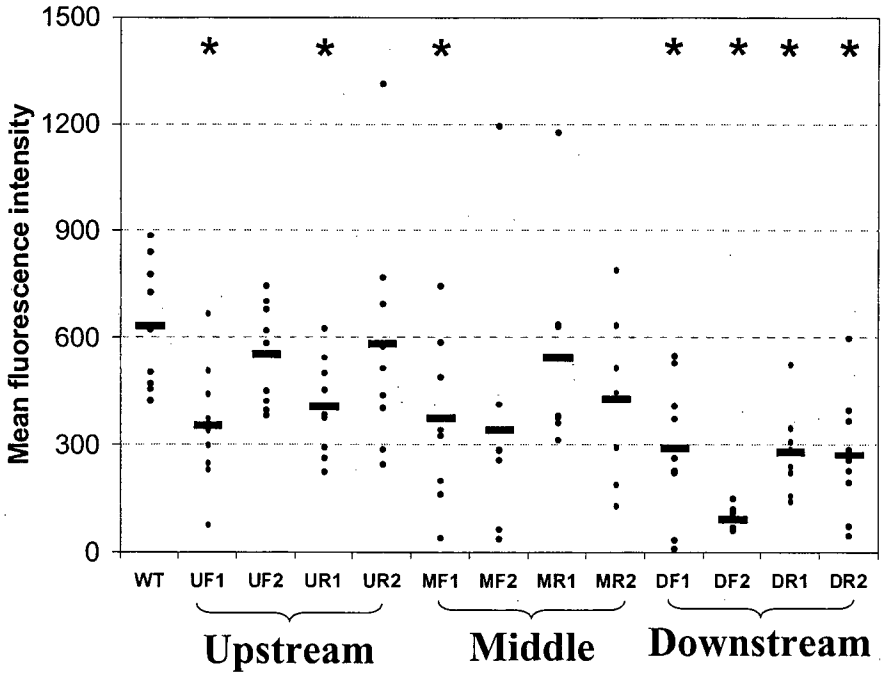
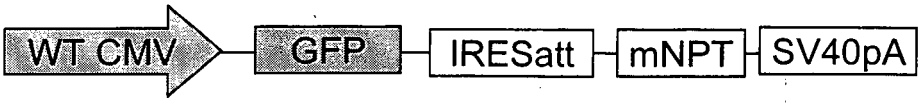

Mean fluorescence intensity
1500
1200
900
600
300
0
WT UF1 UF2 UR1 UR2 MF1 MF2 MR1 MR2 DF1 DF2 DR1 DR2
Upstream
Middle
Downstream

Figure 7

A



B

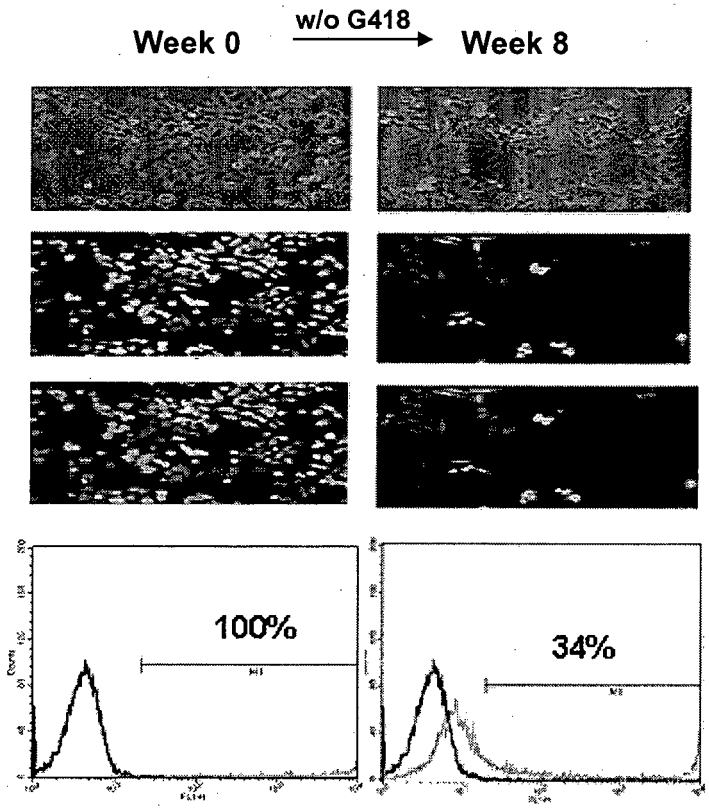


Figure 8

A


IE CMV (MF2)
Enhancer
IE
IE
mP
GFP
IRESatt
mNPT
SV40pA

B

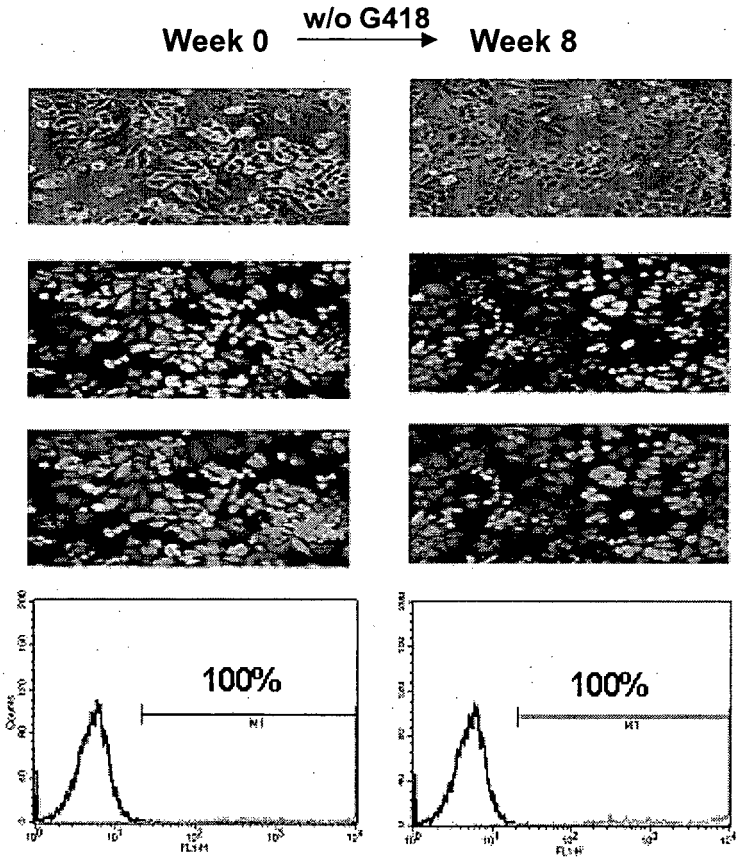

Week 0
w/o G418
Week 8
100%
100%

Figure 9

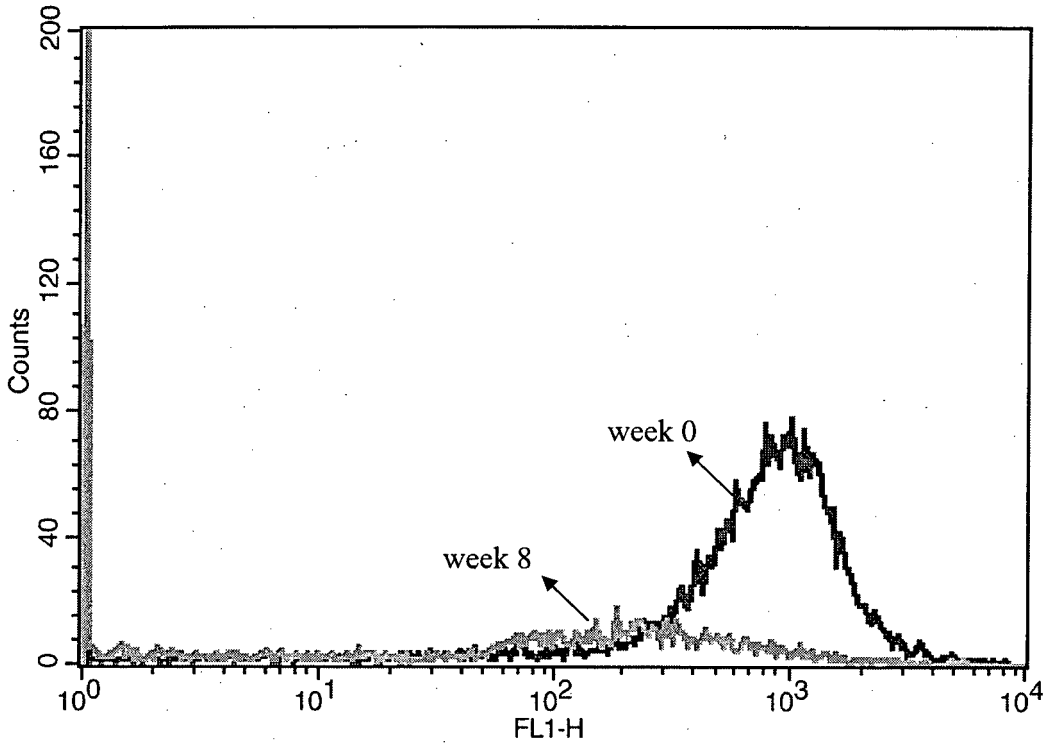


Figure 10

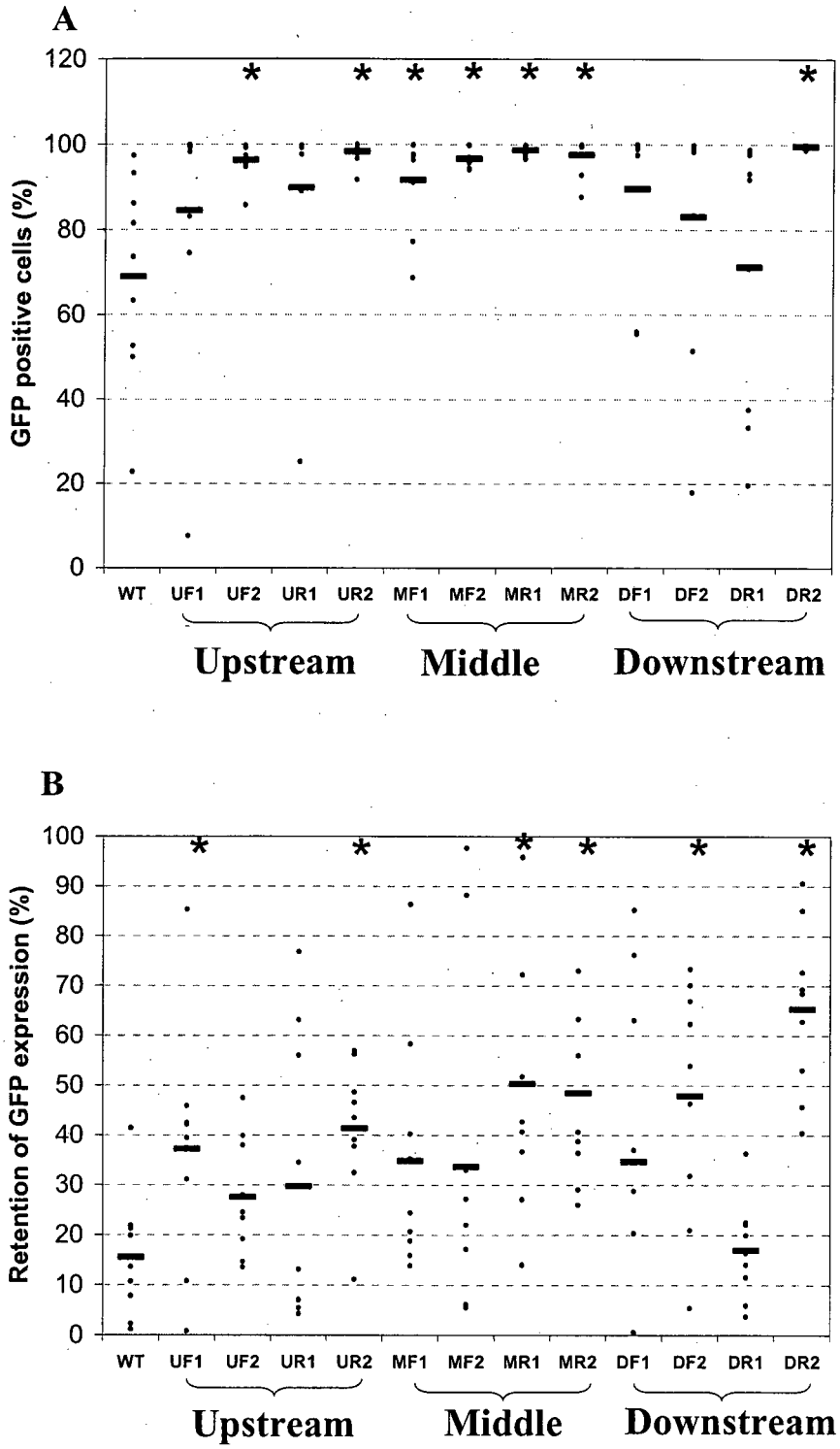

A
GFP positive cells (%)
120
100
80
60
40
20
0
WT
UF1
UF2
UR1
UR2
MF1
MF2
MR1
MR2
DF1
DF2
DR1
DR2
Upstream
Middle
Downstream
B
Retention of GFP expression (%)
100
90
80
70
60
50
40
30
20
10
0
WT
UF1
UF2
UR1
UR2
MF1
MF2
MR1
MR2
DF1
DF2
DR1
DR2
Upstream
Middle
Downstream

Figure 11

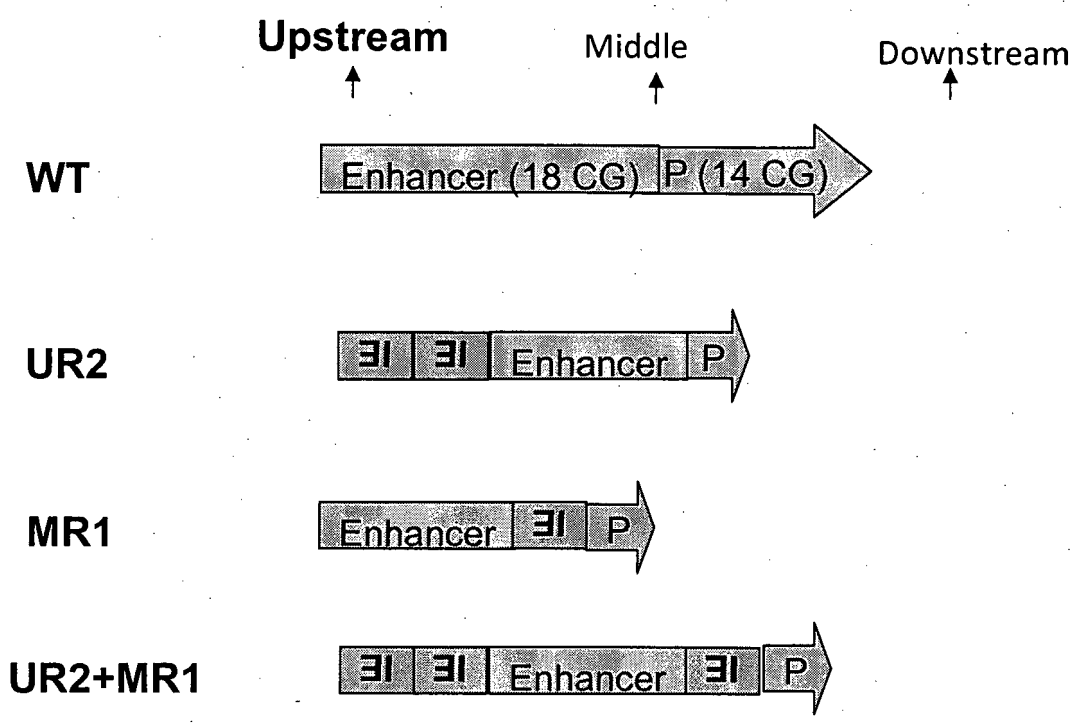

Upstream
Middle
Downstream
WT
Enhancer (18 CG)
P (14 CG)
UR2
Enhancer
P
MR1
Enhancer
P
UR2+MR1
Enhancer
P

Figure 12

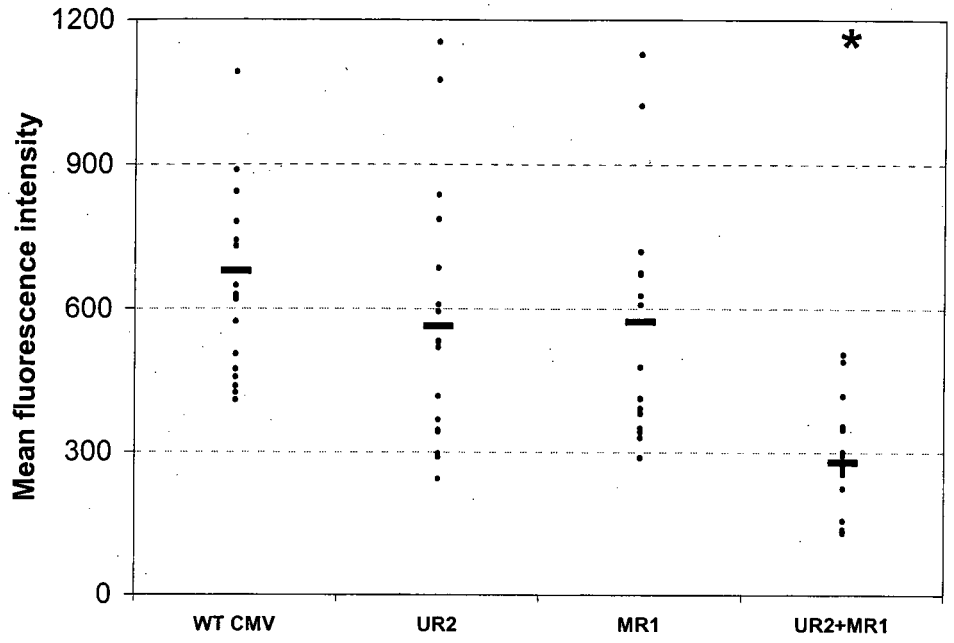


Figure 13

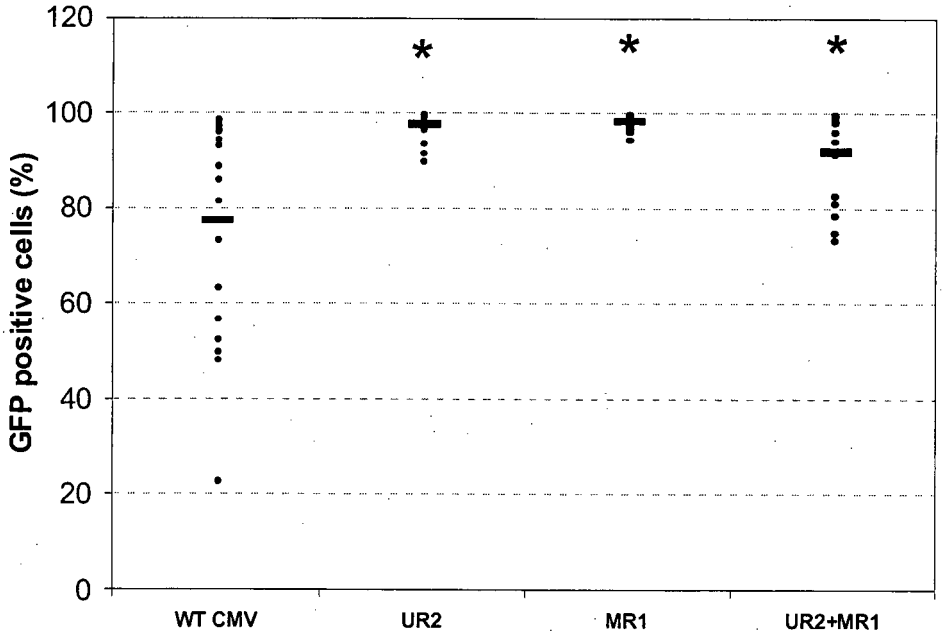


Figure 14

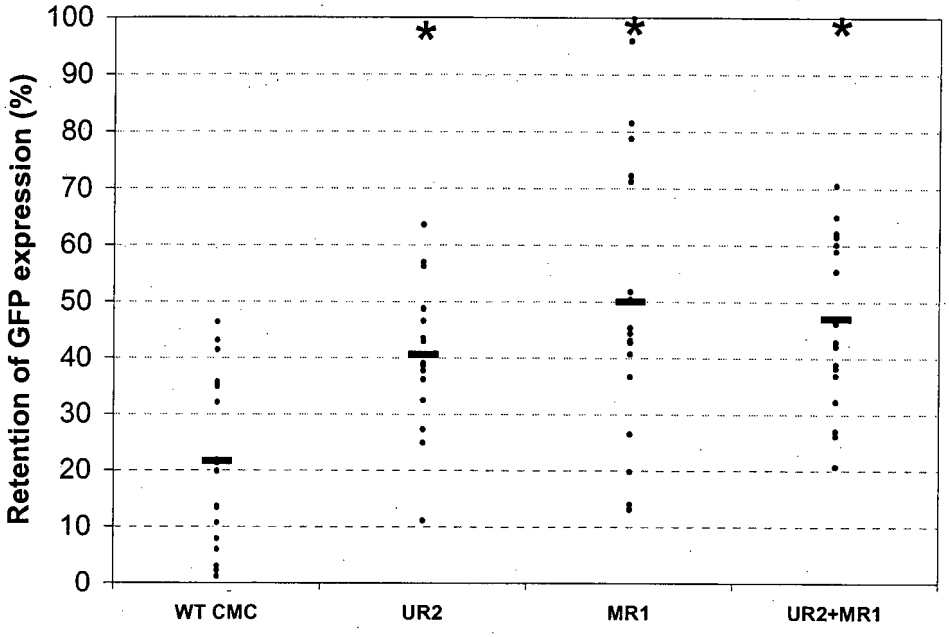

Retention of GFP expression (%)
100
90
80
70
60
50
40
30
20
10
0
*
*
*
WT CMC
UR2
MR1
UR2+MR1

Figure 15

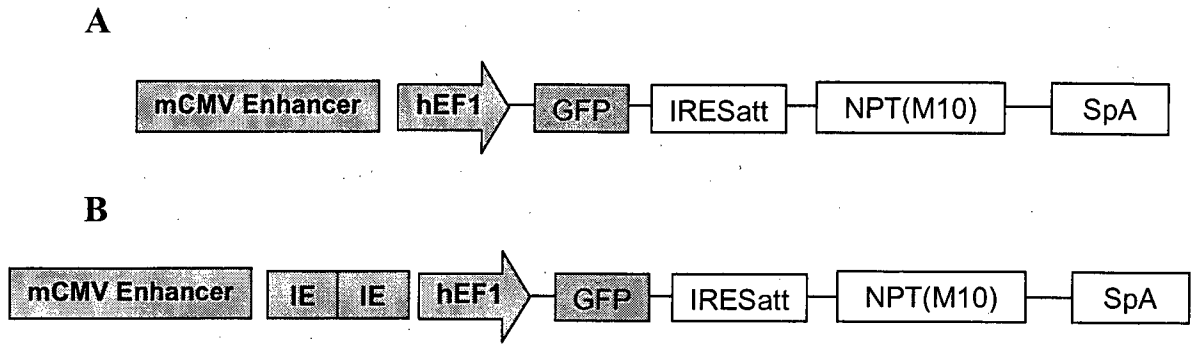


Figure 16

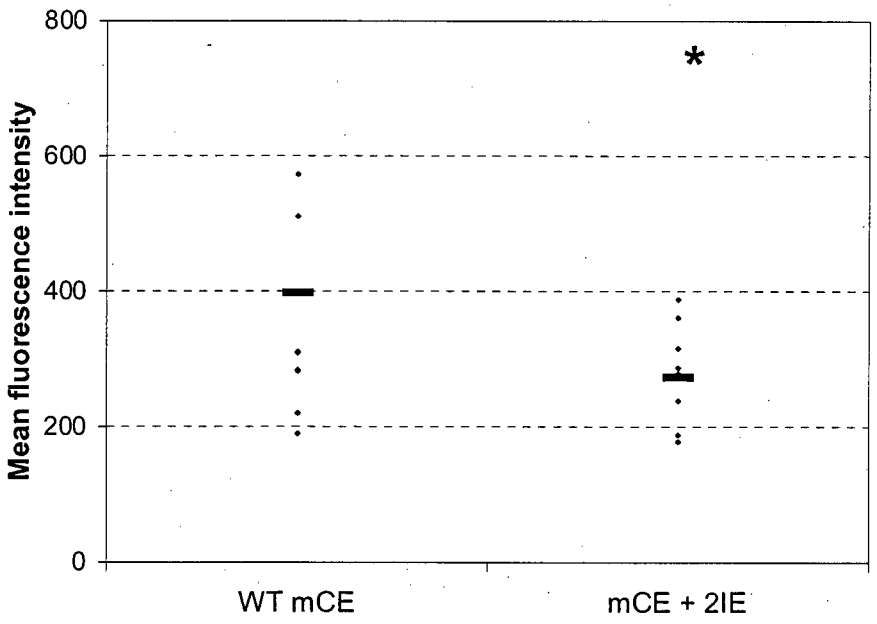


Figure 17

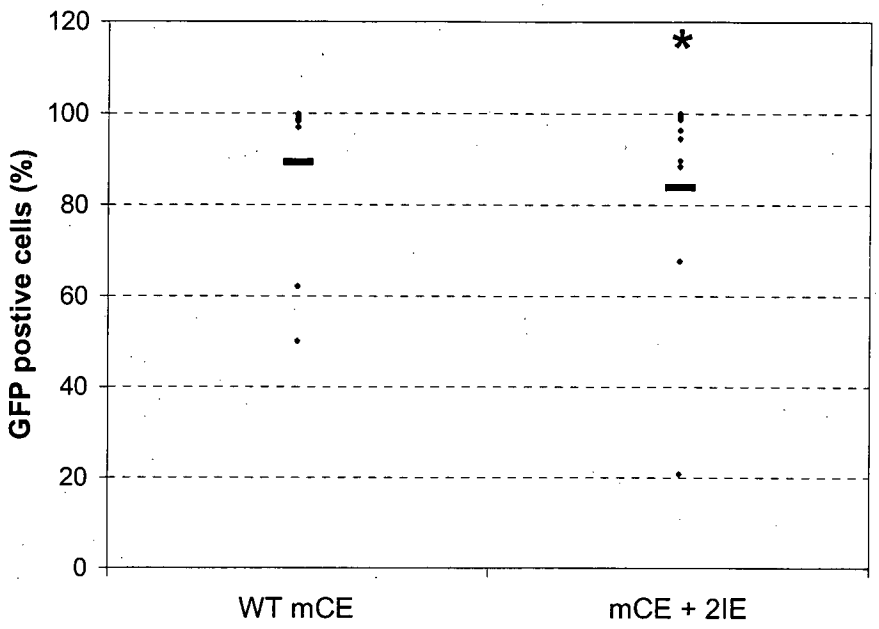


Figure 18

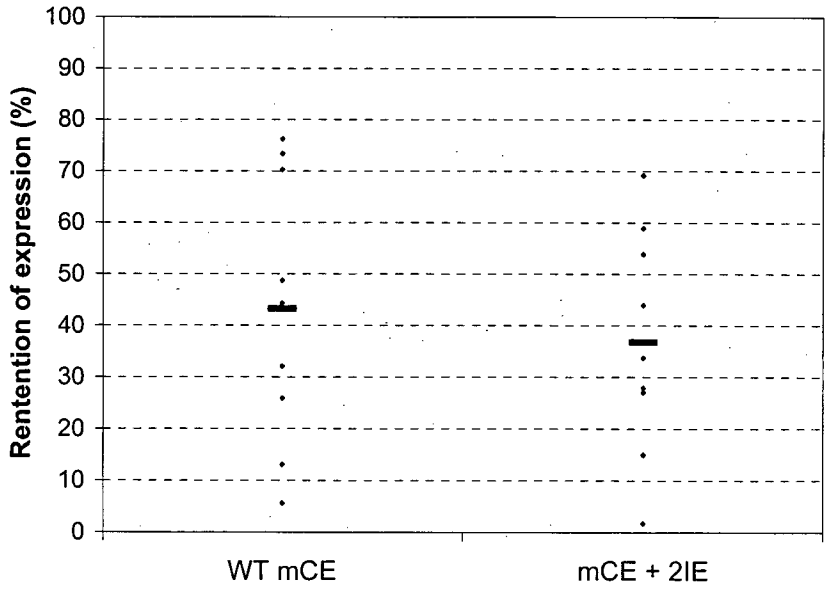


Figure 19

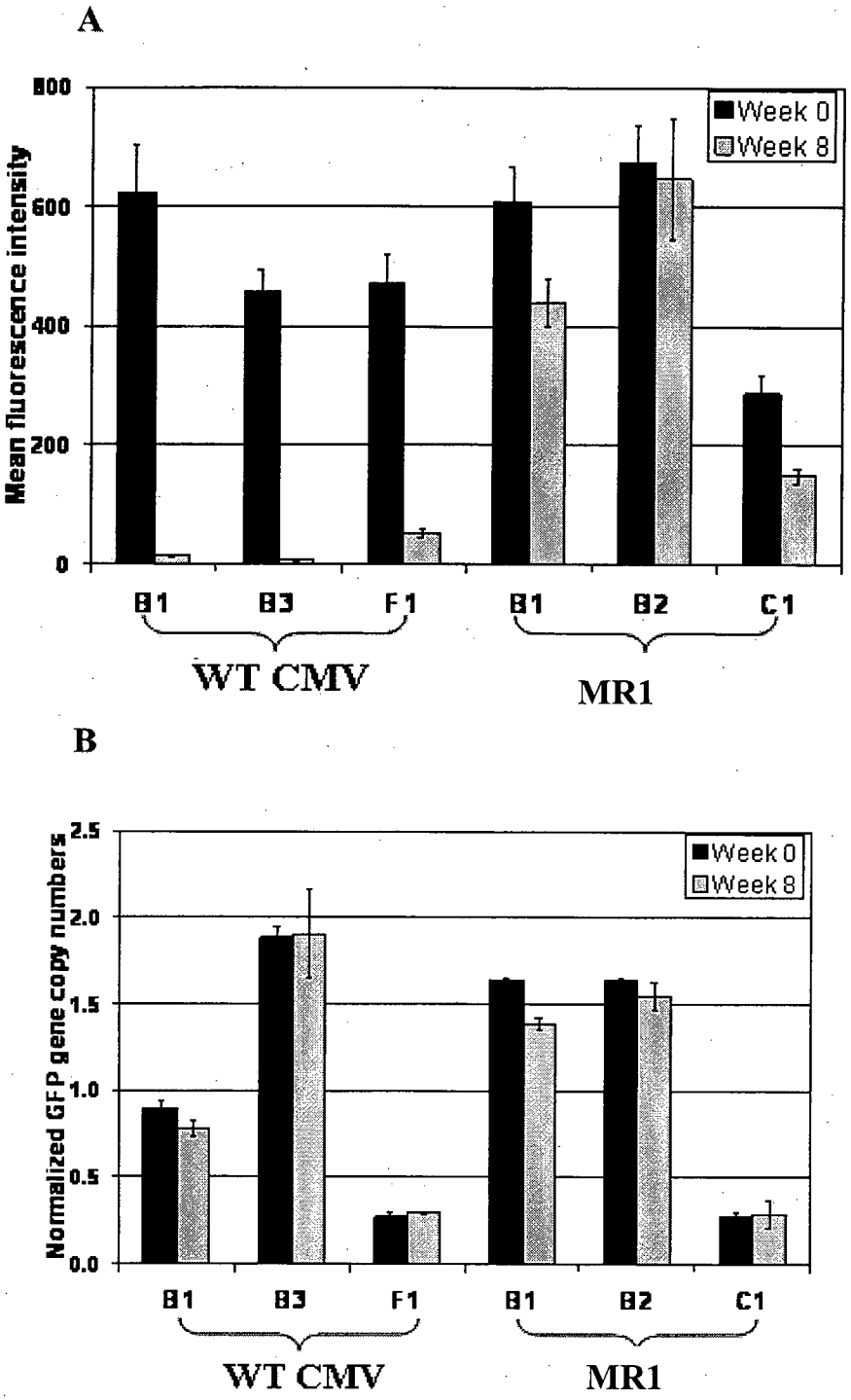

A
800
600
400
200
0
Mean fluorescence intensity
Week 0
Week 8
B1
B3
F1
B1
B2
C1
WT CMV
MR1
B
2.5
2.0
1.5
1.0
0.5
0.0
Normalized GFP gene copy numbers
Week 0
Week 8
B1
B3
F1
B1
B2
C1
WT CMV
MR1

Figure 20

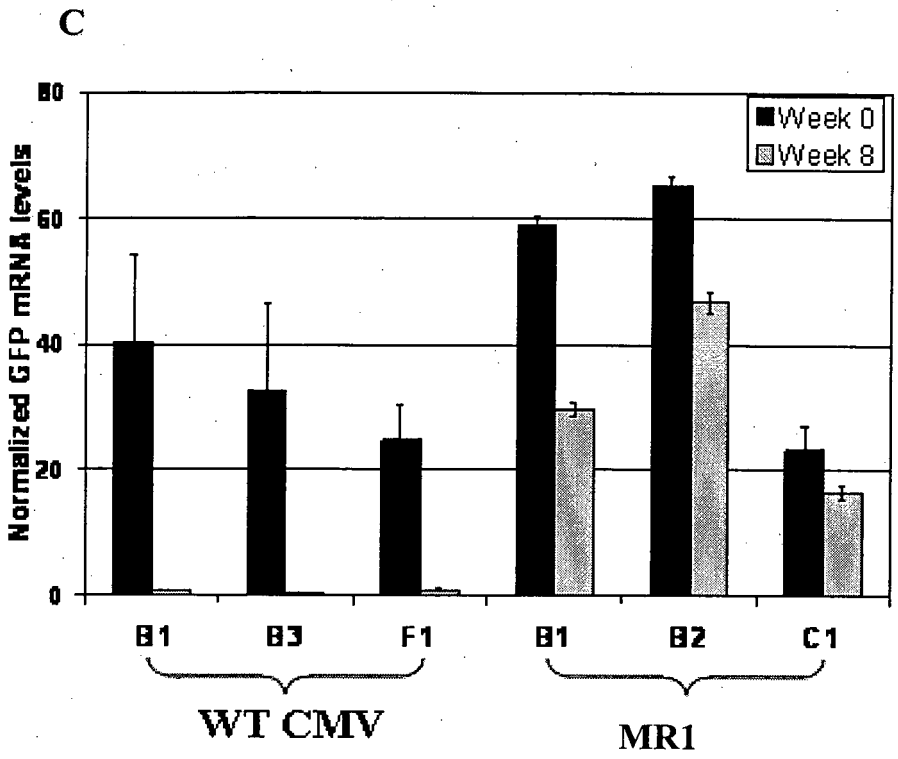

C
Normalized GFP mRNA levels
80
60
40
20
0
Week 0
Week 8
B1
B3
F1
B1
B2
C1
WT CMV
MR1

Figure 20