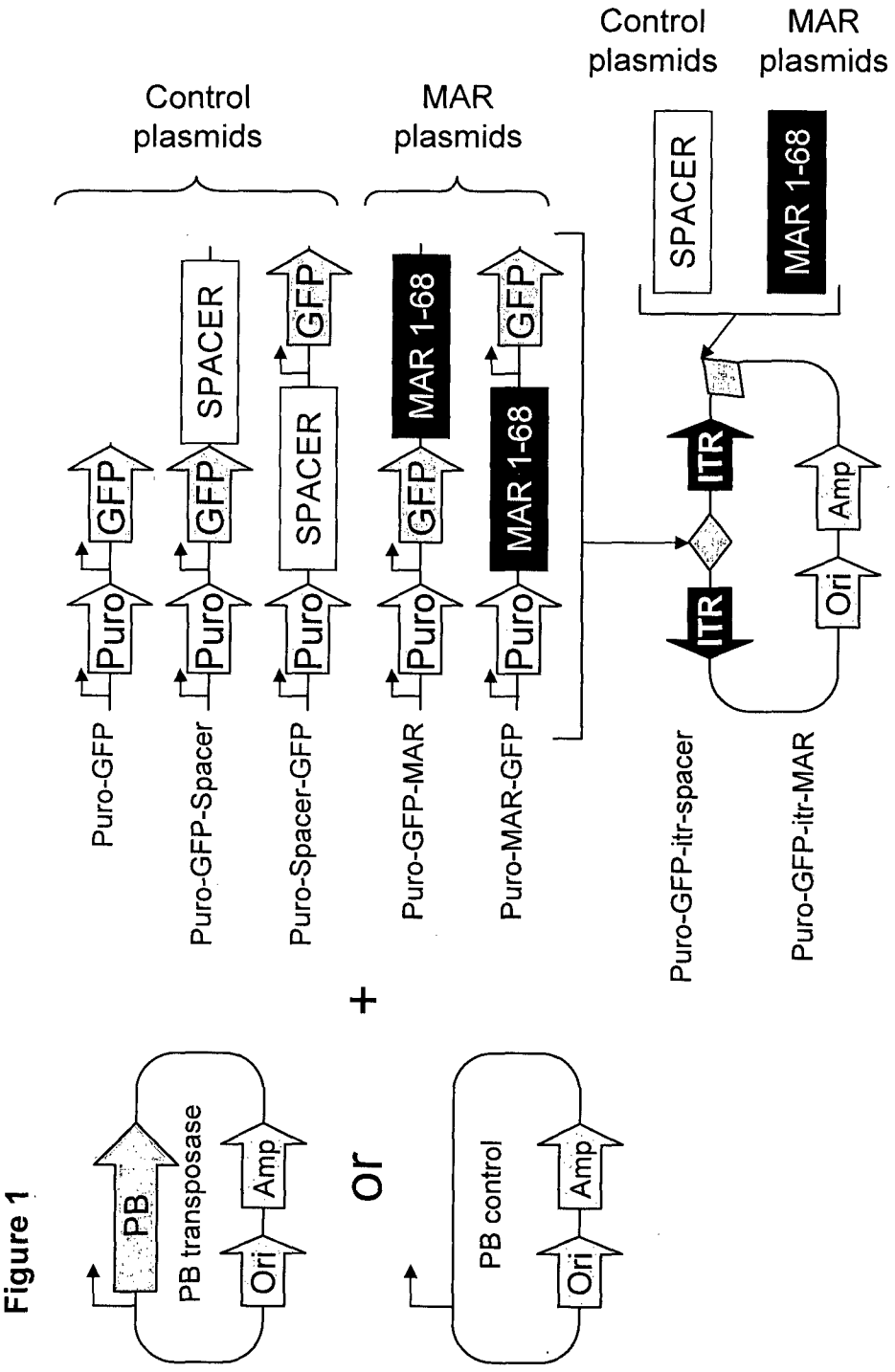

Control plasmids
MAR plasmids
Puro
GFP
SPACER
MAR 1-68
Puro-GFP
Puro-GFP-Spacer
Puro-Spacer-GFP
Puro-GFP-MAR
Puro-MAR-GFP
ITR
Amp
Ori
Puro-GFP-itr-spacer
Puro-GFP-itr-MAR
+
or
PB
PB transposase
PB control

Figure 1

Figure 2

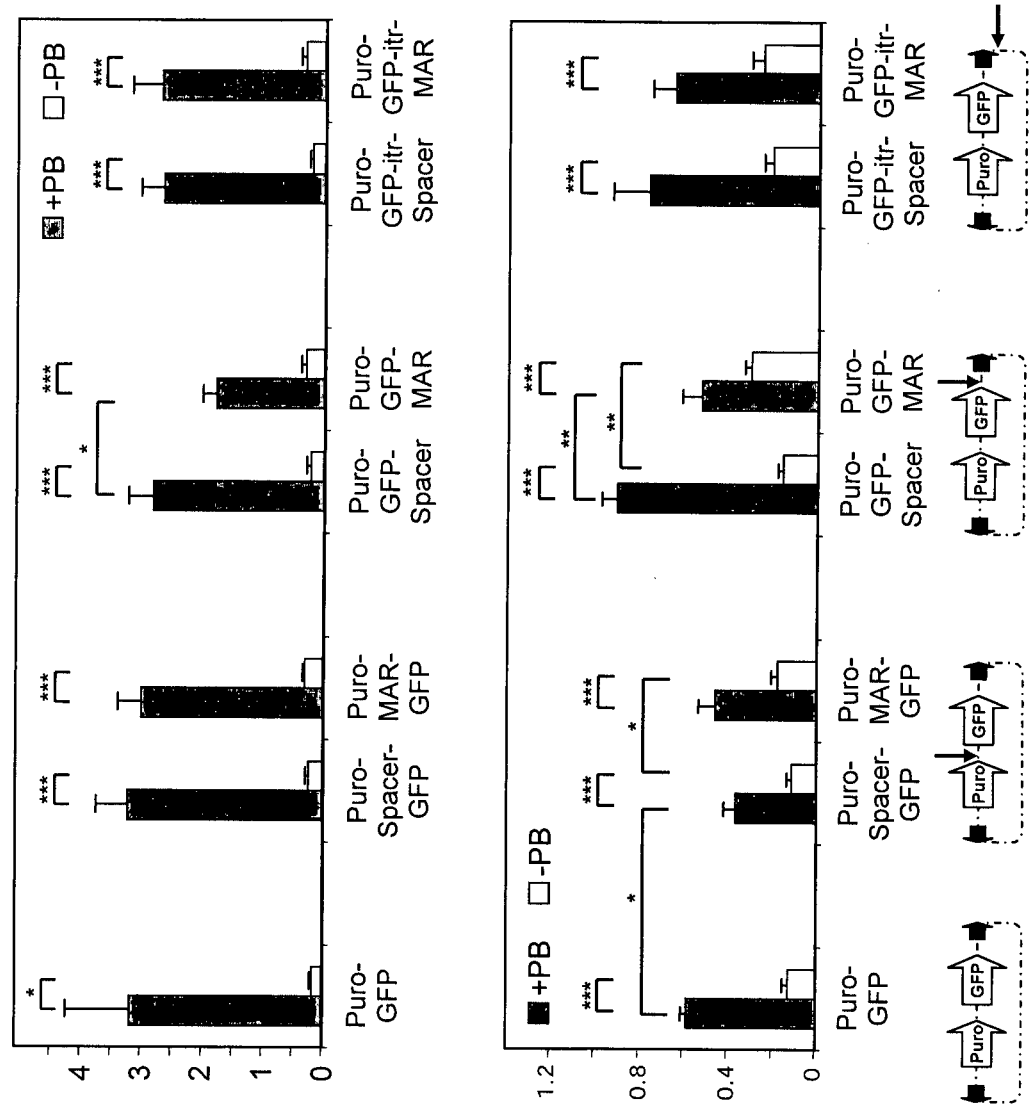

A
% GFP positive cells
0
1
2
3
4
+PB
-PB
Puro-GFP
Puro-Spacer-GFP
Puro-MAR-GFP
Puro-GFP-Spacer
Puro-GFP-MAR
Puro-GFP-itr-Spacer
Puro-GFP-itr-MAR
B
% Puromycin-resistant
0
0.4
0.8
1.2
+PB
-PB
Puro-GFP
Puro-Spacer-GFP
Puro-MAR-GFP
Puro-GFP-Spacer
Puro-GFP-MAR
Puro-GFP-itr-Spacer
Puro-GFP-itr-MAR
Puro
GFP

Figure 3

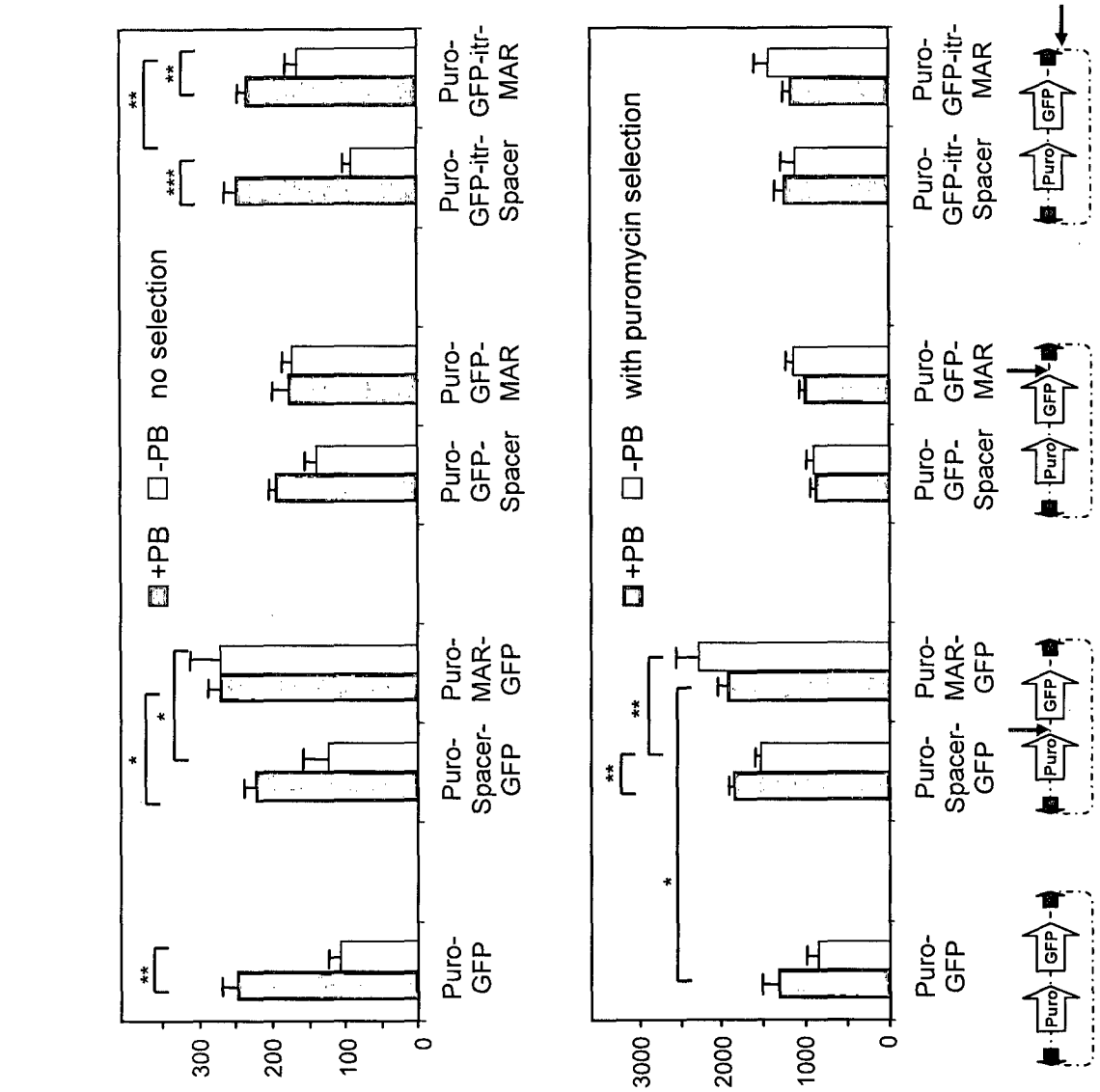

A
Mean GFP fluorescence (RLU)
300
200
100
0
+PB
-PB
no selection
**
*
*
***
**
**
Puro-GFP
Puro-Spacer-GFP
Puro-MAR-GFP
Puro-GFP-Spacer
Puro-GFP-MAR
Puro-GFP-itr-Spacer
Puro-GFP-itr-MAR
B
Mean GFP fluorescence (RLU)
3000
2000
1000
0
+PB
-PB
with puromycin selection
**
*
**
Puro-GFP
Puro-Spacer-GFP
Puro-MAR-GFP
Puro-GFP-Spacer
Puro-GFP-MAR
Puro-GFP-itr-Spacer
Puro-GFP-itr-MAR
Puro
GFP

Figure 4

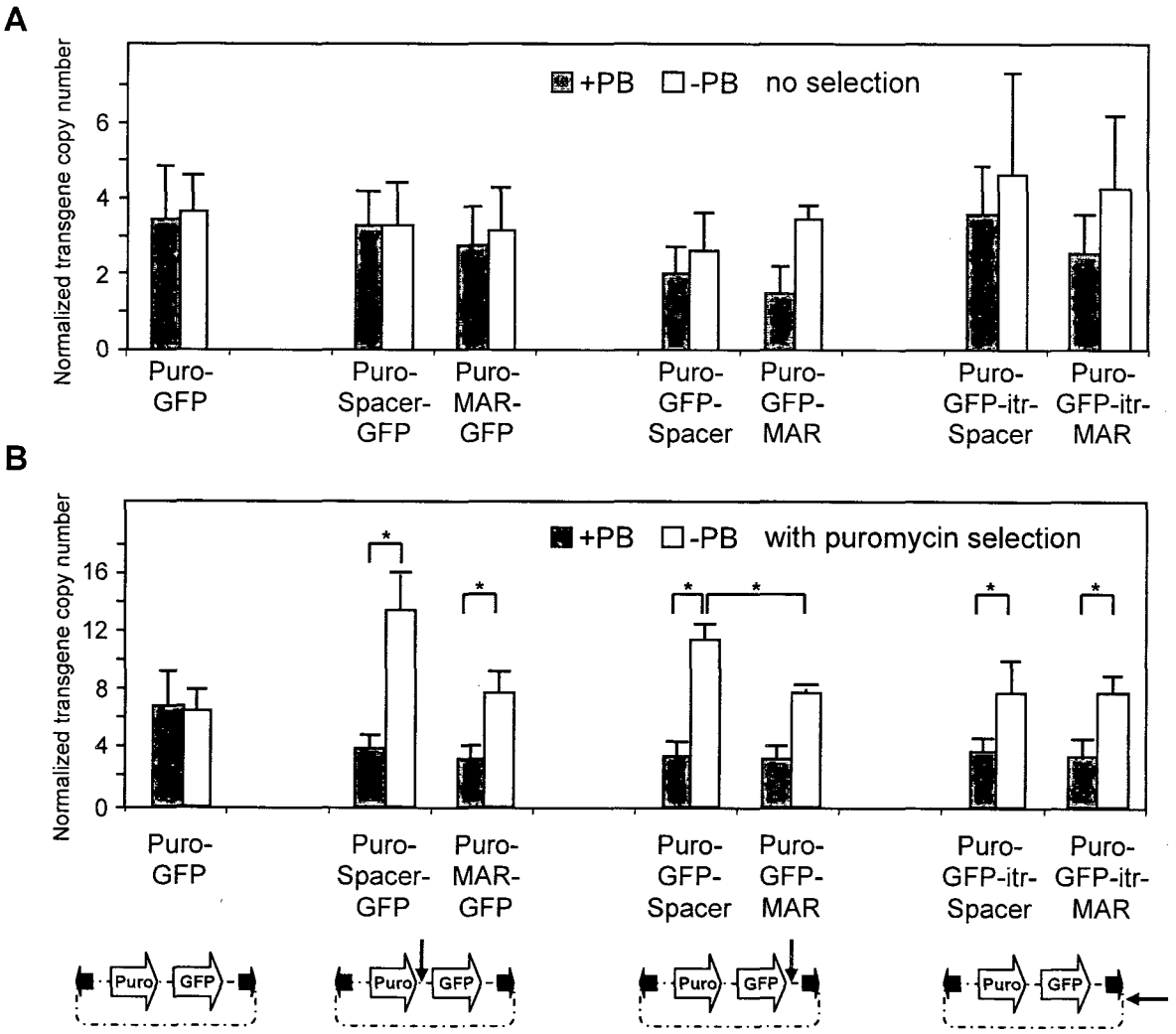

A
Normalized transgene copy number
+PB -PB no selection
0
2
4
6
Puro-GFP
Puro-Spacer-GFP
Puro-MAR-GFP
Puro-GFP-Spacer
Puro-GFP-MAR
Puro-GFP-itr-Spacer
Puro-GFP-itr-MAR
B
Normalized transgene copy number
+PB -PB with puromycin selection
0
4
8
12
16
*
Puro-GFP
Puro-Spacer-GFP
Puro-MAR-GFP
Puro-GFP-Spacer
Puro-GFP-MAR
Puro-GFP-itr-Spacer
Puro-GFP-itr-MAR
Puro
GFP

Figure 5

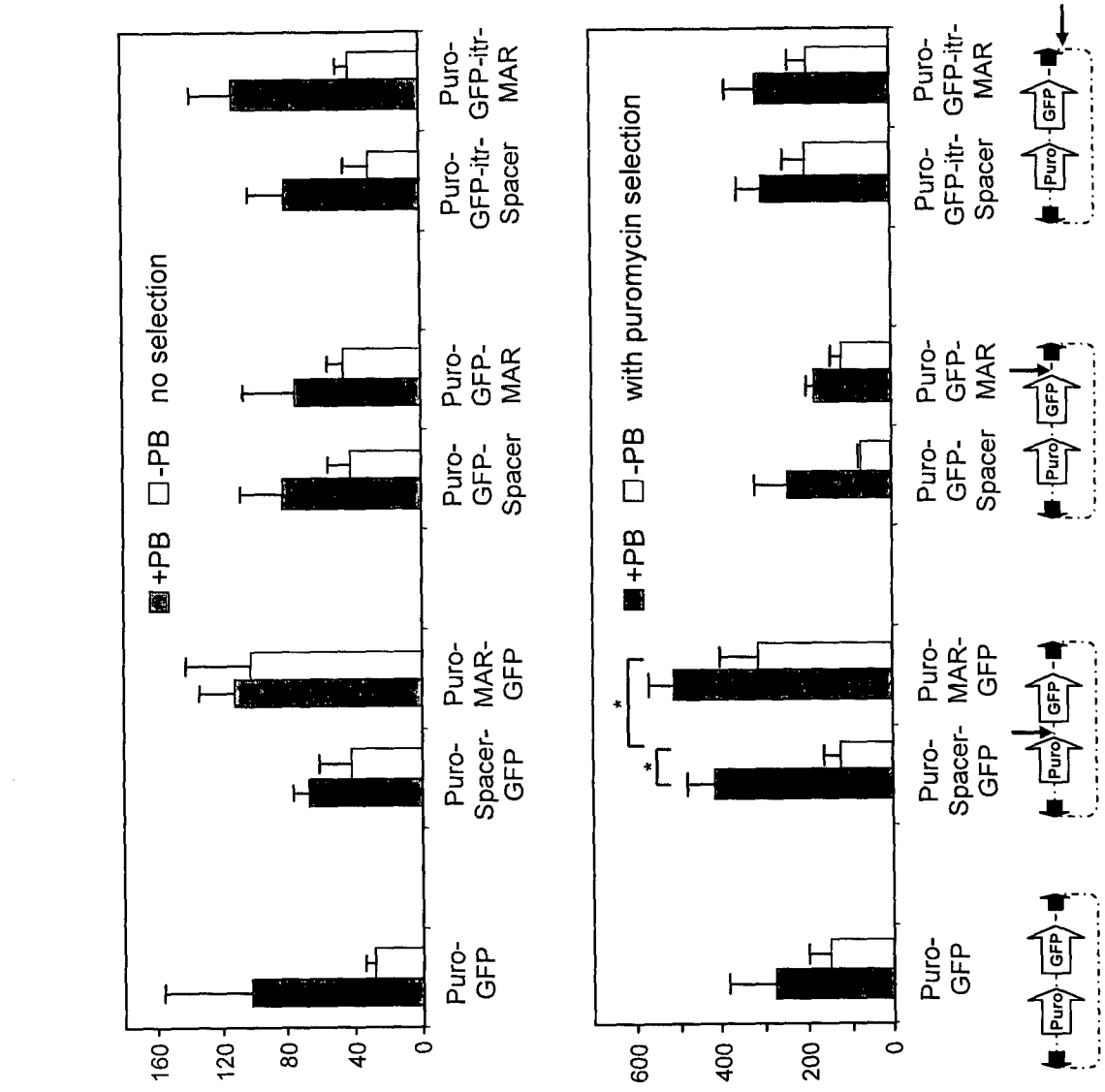

A
GFP fluorescence per transgene
160
120
80
40
0
+PB
-PB
no selection
Puro-GFP
Puro-Spacer-GFP
Puro-MAR-GFP
Puro-GFP-Spacer
Puro-GFP-MAR
Puro-GFP-itr-Spacer
Puro-GFP-itr-MAR
B
GFP fluorescence per transgene
600
400
200
0
+PB
-PB
with puromycin selection
*
*
Puro-GFP
Puro-Spacer-GFP
Puro-MAR-GFP
Puro-GFP-Spacer
Puro-GFP-MAR
Puro-GFP-itr-Spacer
Puro-GFP-itr-MAR
Puro
GFP

Figure 6

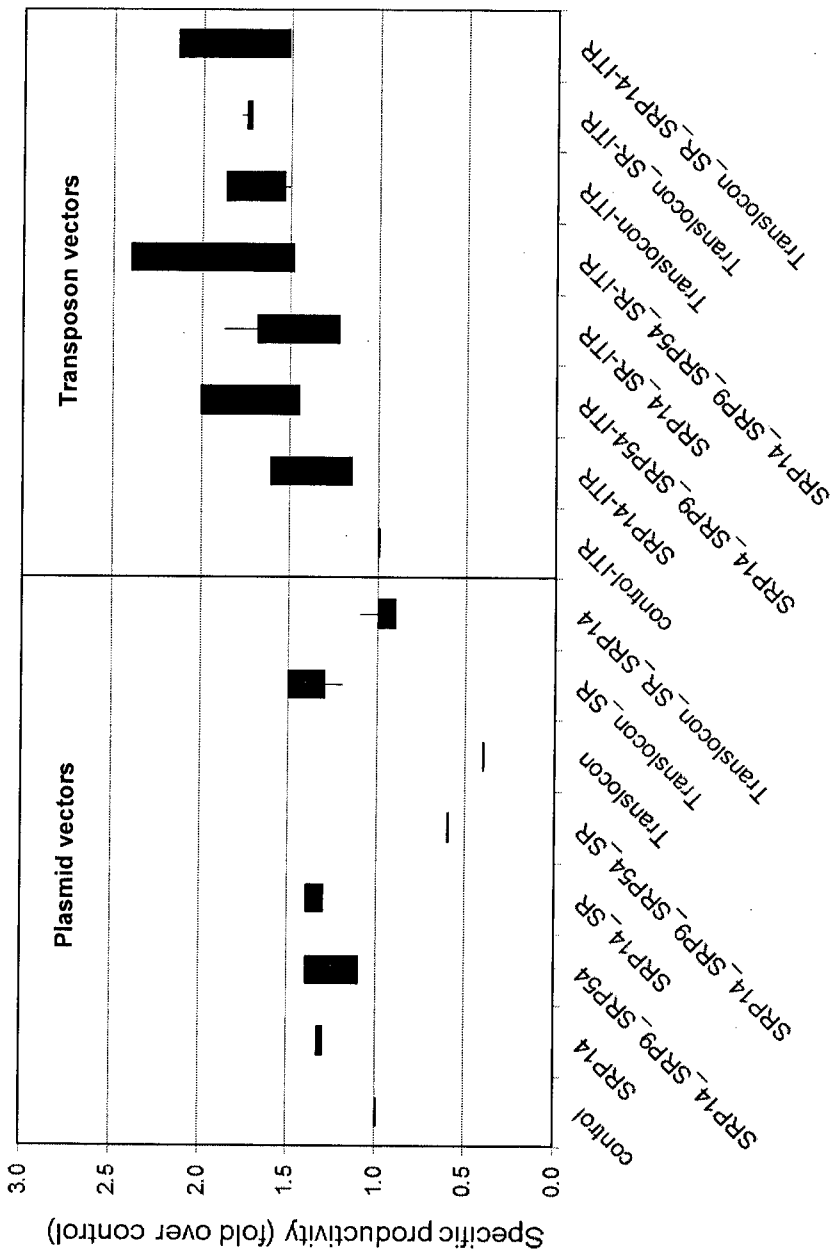

Specific productivity (fold over control)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Plasmid vectors
Transposon vectors
control
SRP14
SRP14_SRP9_SRP54
SRP14_SR
SRP14_SRP9_SRP54_SR
Transiocon
Transiocon_SR
Transiocon_SR_SRP14
control-ITR
SRP14-ITR
SRP14_SRP9_SRP54-ITR
SRP14_SR-ITR
SRP14_SRP9_SRP54_SR-ITR
Transiocon-ITR
Transiocon_SR-ITR
Transiocon_SR_SRP14-ITR

Figure 7

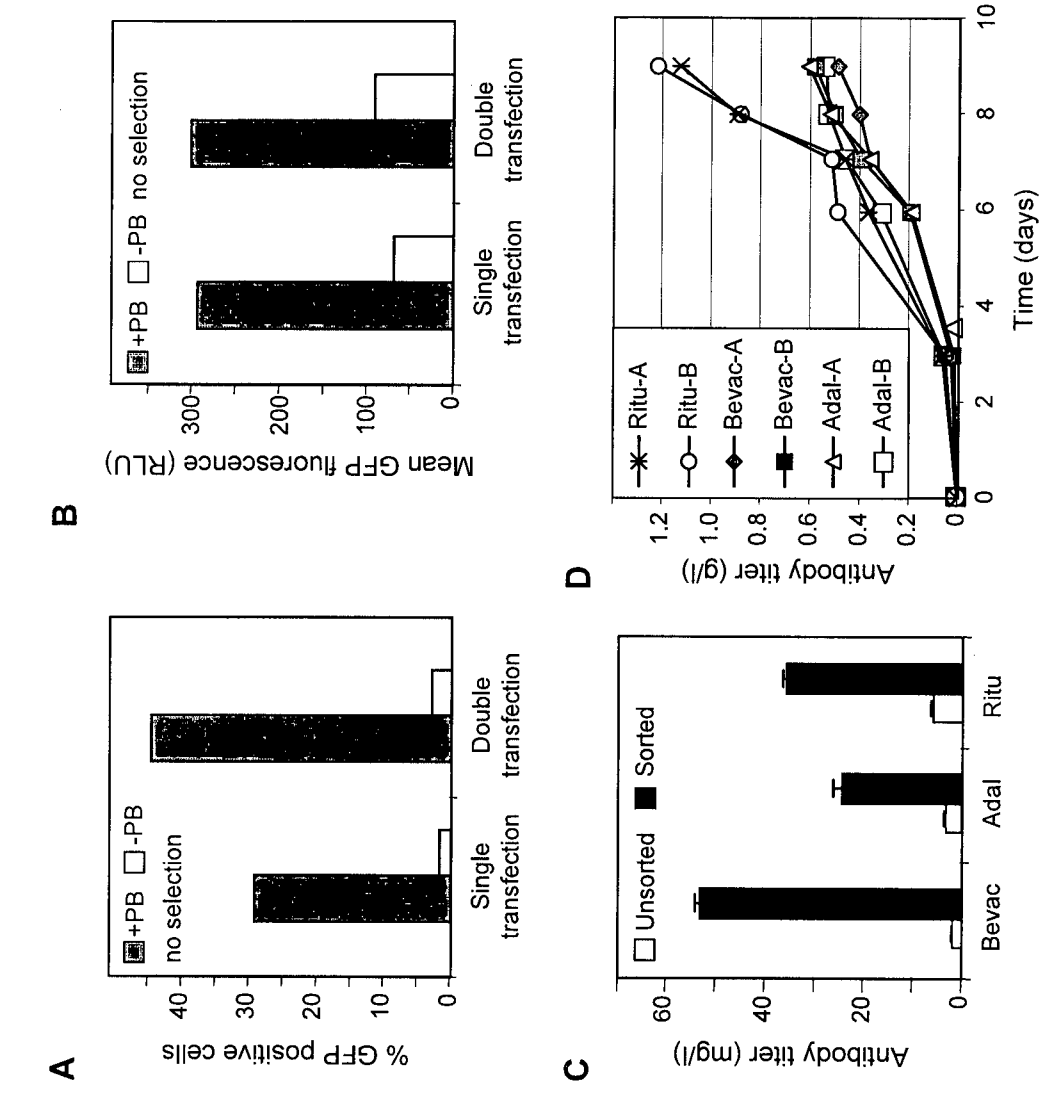

A
+PB -PB
no selection
% GFP positive cells
0
10
20
30
40
Single transfection
Double transfection
B
+PB -PB no selection
Mean GFP fluorescence (RLU)
0
100
200
300
Single transfection
Double transfection
C
Unsorted
Sorted
Antibody titer (mg/l)
0
20
40
60
Bevac
Adal
Ritu
D
Ritu-A
Ritu-B
Bevac-A
Bevac-B
Adal-A
Adal-B
Antibody titer (g/l)
0
0.2
0.4
0.6
0.8
1.0
1.2
0
2
4
6
8
10
Time (days)

Figure 8

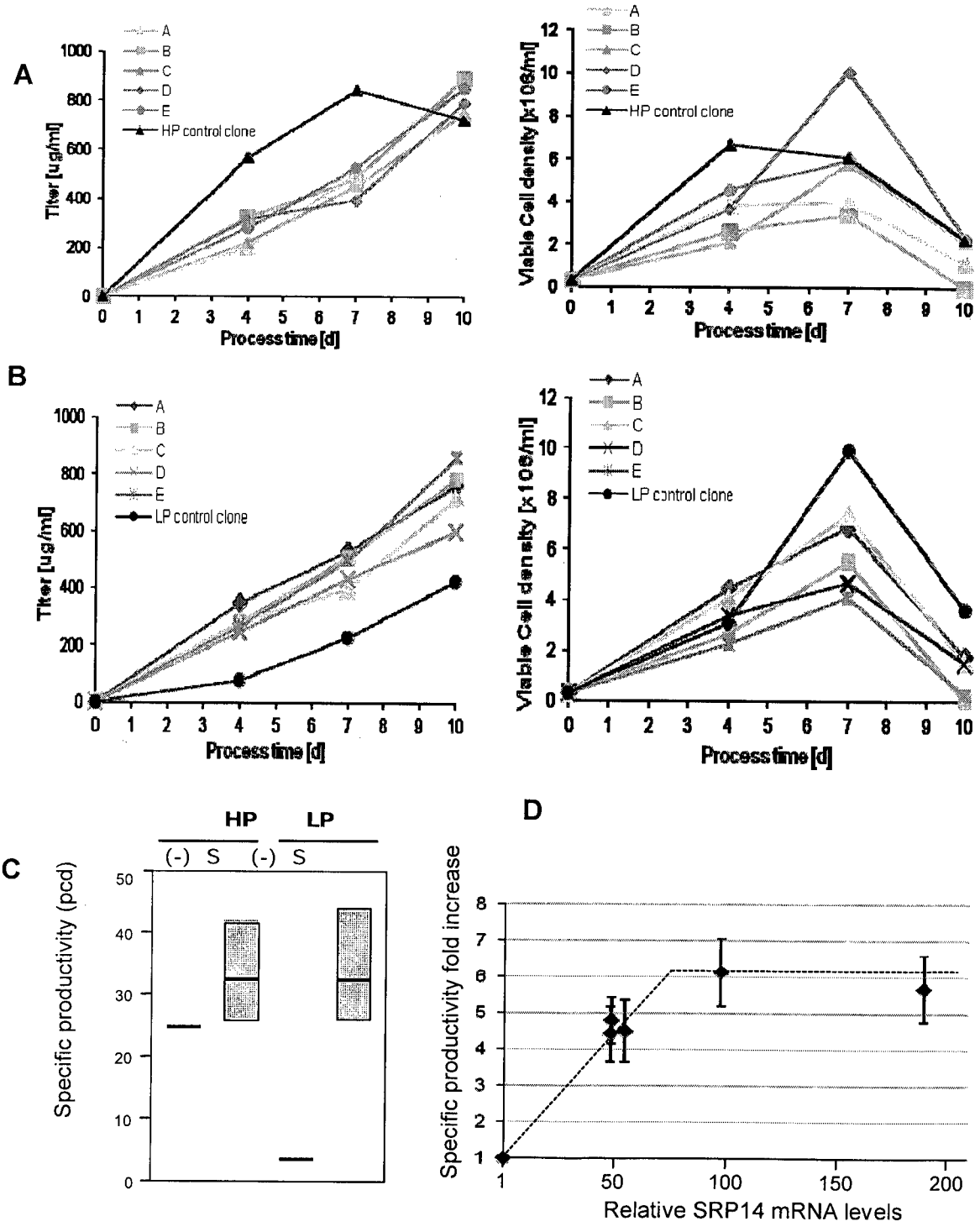

A
B
C
D
E
HP control clone
LP control clone
Titer [ug/ml]
Viable Cell density [x10E6/ml]
Process time [d]
HP
LP
(-) S (-) S
Specific productivity (pcd)
Specific productivity fold increase
Relative SRP14 mRNA levels

Figure 9

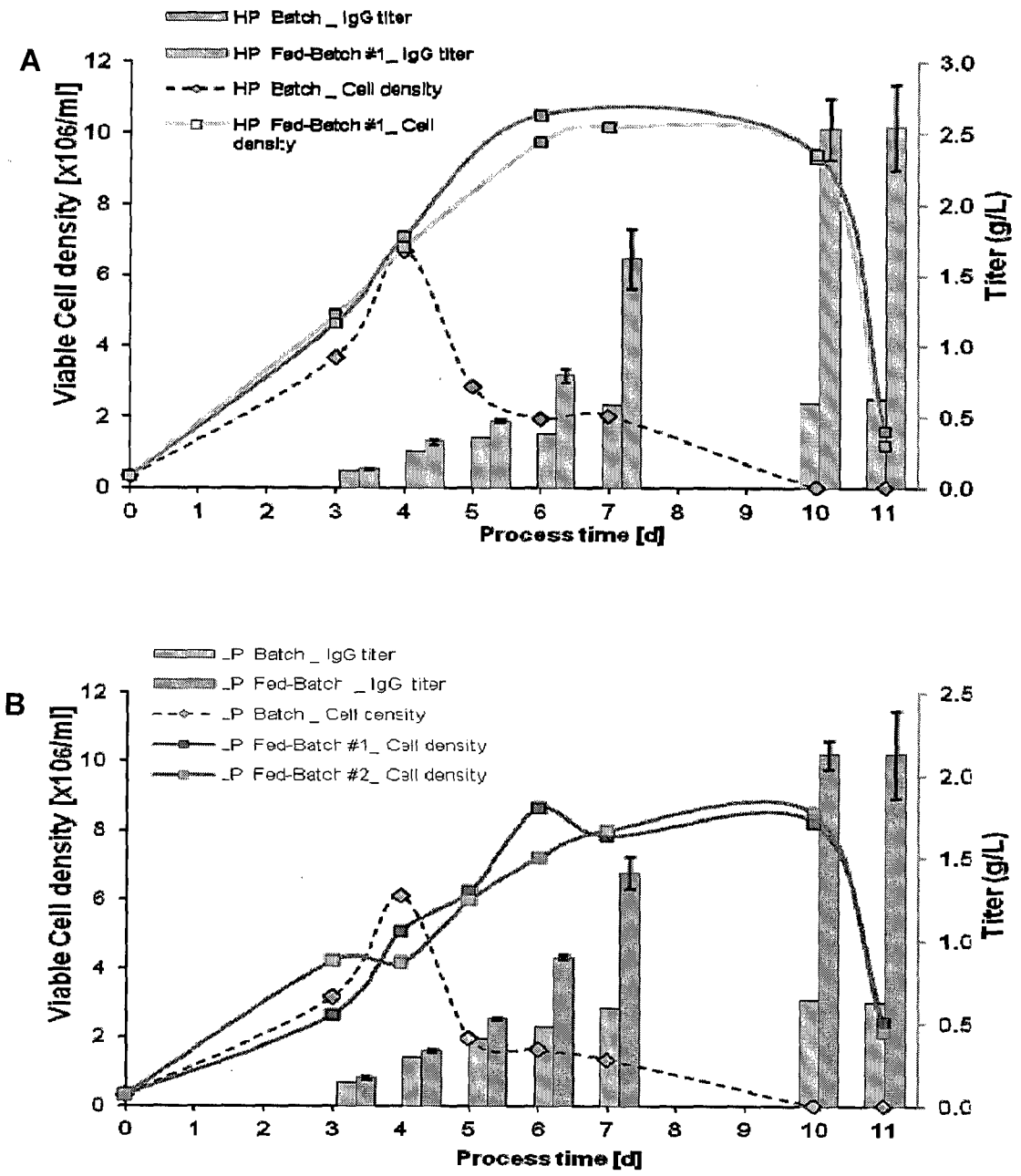

A
HP Batch _ IgG titer
HP Fed-Batch #1_ IgG titer
HP Batch _ Cell density
HP Fed-Batch #1_ Cell density
Viable Cell density [x10e6/ml]
Titer (g/L)
Process time [d]
B
_P Batch _ IgG titer
_P Fed-Batch _ IgG titer
_P Batch _ Cell density
P Fed-Batch #1 Cell density
P Fed-Batch #2 Cell density
Viable Cell density [x10e6/ml]
Titer (g/L)
Process time [d]

Figure 10

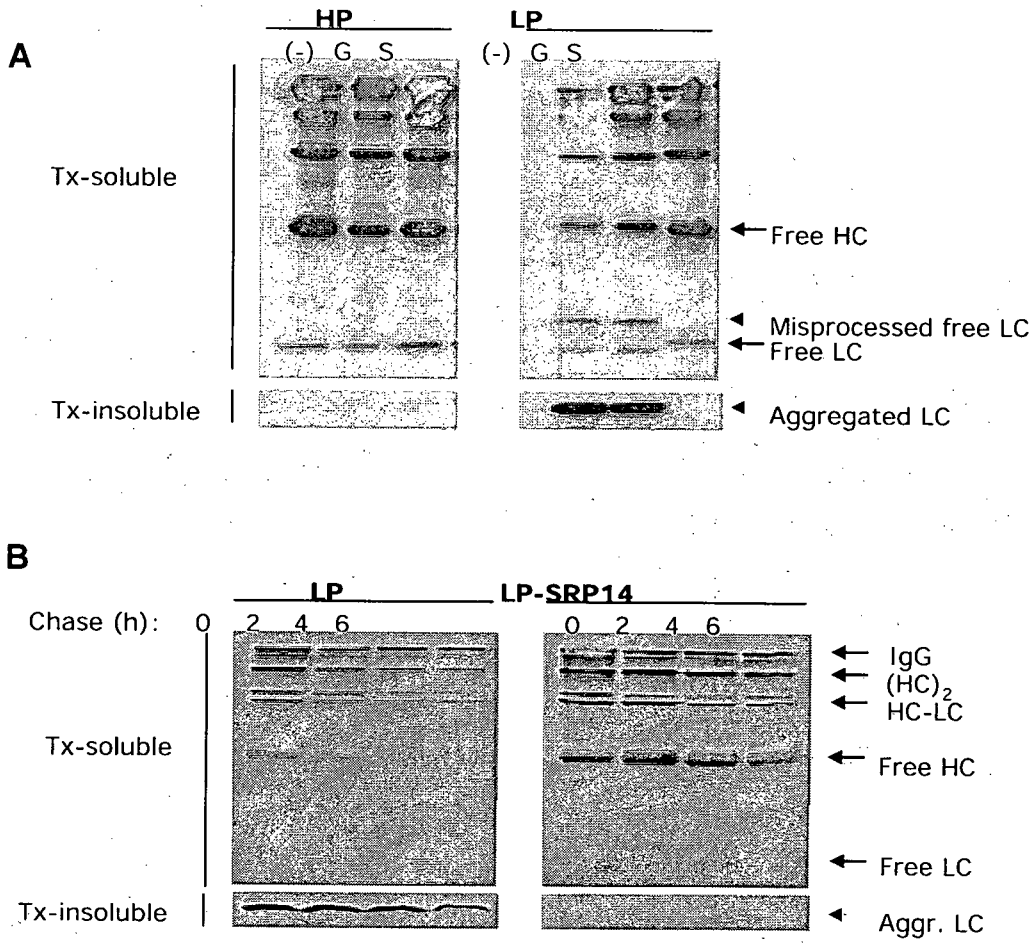

A
HP
LP
(-) G S
(-) G S
Tx-soluble
Tx-insoluble
Free HC
Misprocessed free LC
Free LC
Aggregated LC
B
LP
LP-SRP14
Chase (h): 0 2 4 6
0 2 4 6
Tx-soluble
Tx-insoluble
IgG
(HC)2
HC-LC
Free HC
Free LC
Aggr. LC

Figure 11

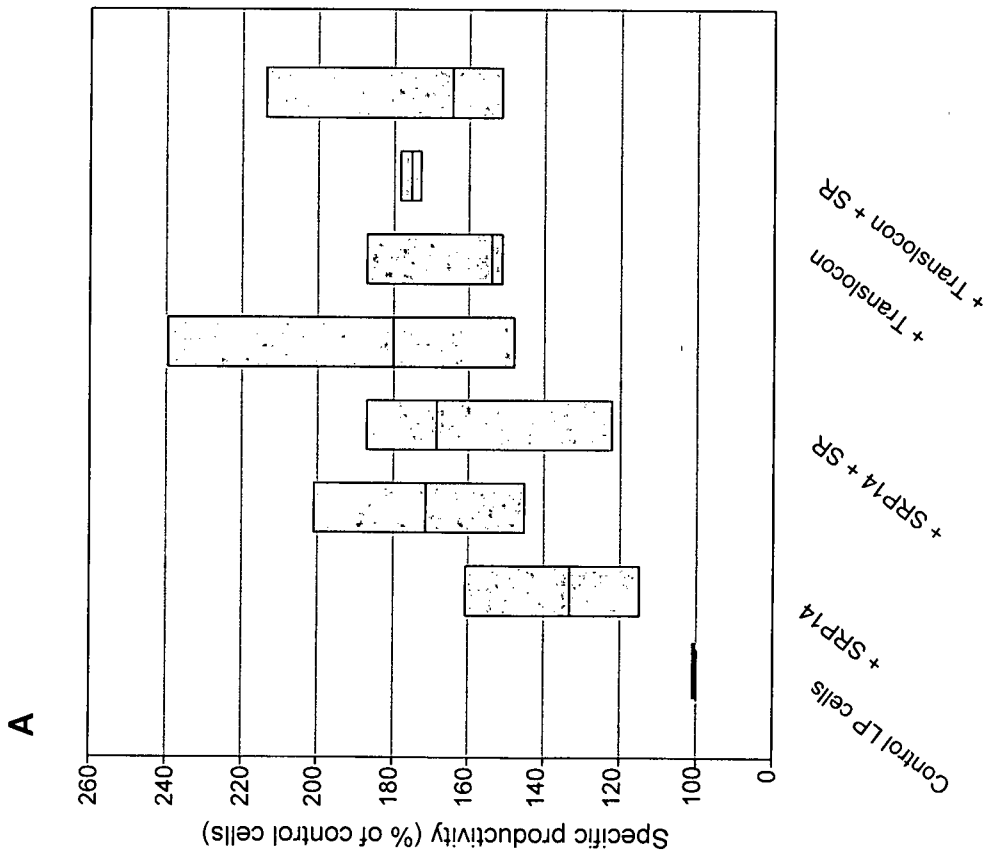

A
Specific productivity (% of control cells)
260
240
220
200
180
160
140
120
100
0
Control LP cells
+ SRP14
+ SRP14 + SR
+ Transiocon
+ Transiocon + SR
B
Specific productivity (% of control cells)
80
60
40
20
0
SRP14-LP subclone
SRP14-LP + SR
SRP14-LP + Transiocon
SRP14-LP + SR + Transiocon

Figure 12

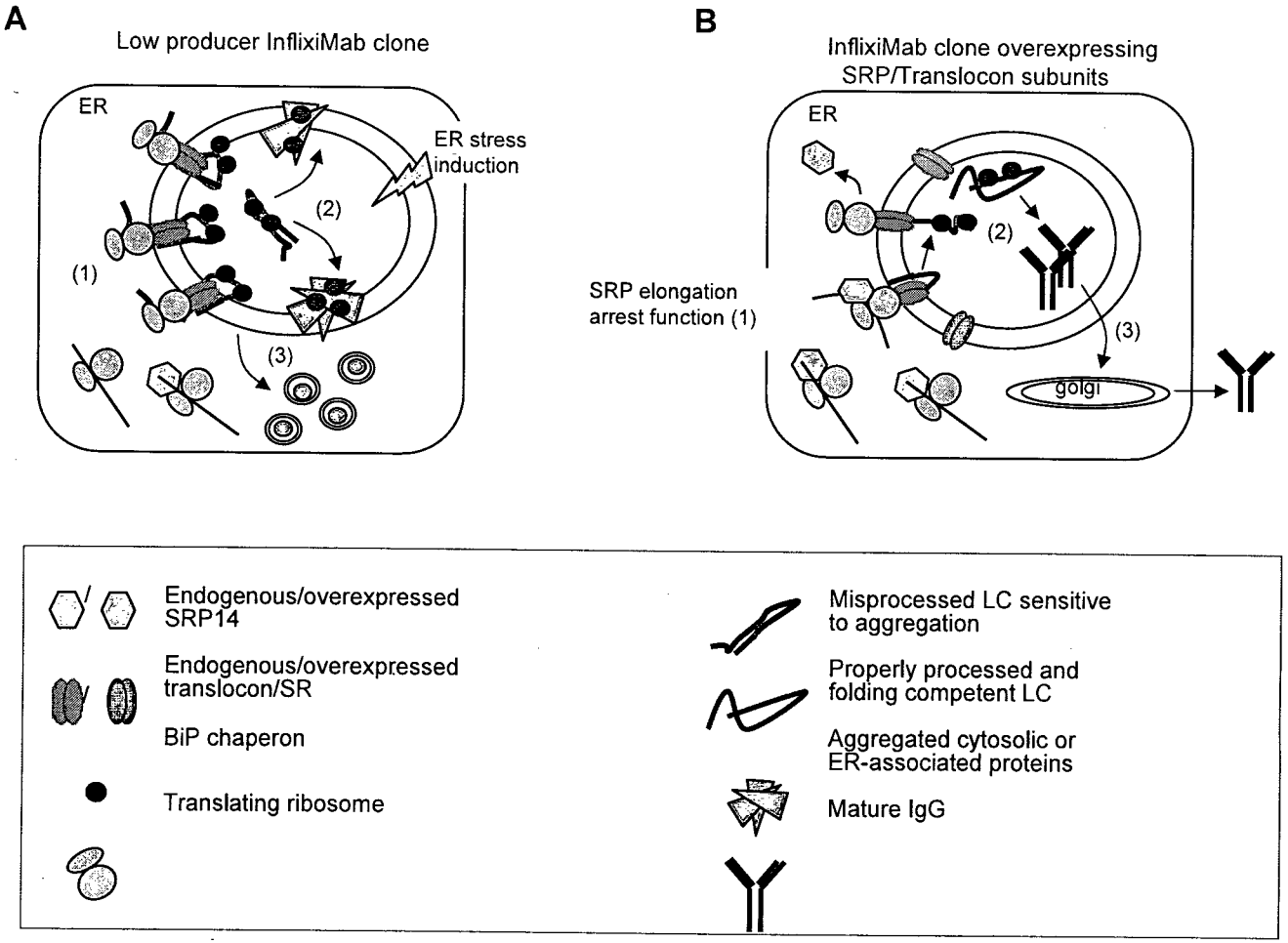

A
Low producer InflixiMab clone
ER
ER stress induction
(1)
(2)
(3)
B
InflixiMab clone overexpressing SRP/Translocon subunits
ER
SRP elongation arrest function (1)
(2)
(3)
golgi
Endogenous/overexpressed SRP14
Endogenous/overexpressed translocon/SR
BiP chaperon
Translating ribosome
Misprocessed LC sensitive to aggregation
Properly processed and folding competent LC
Aggregated cytosolic or ER-associated proteins
Mature IgG

Figure 13

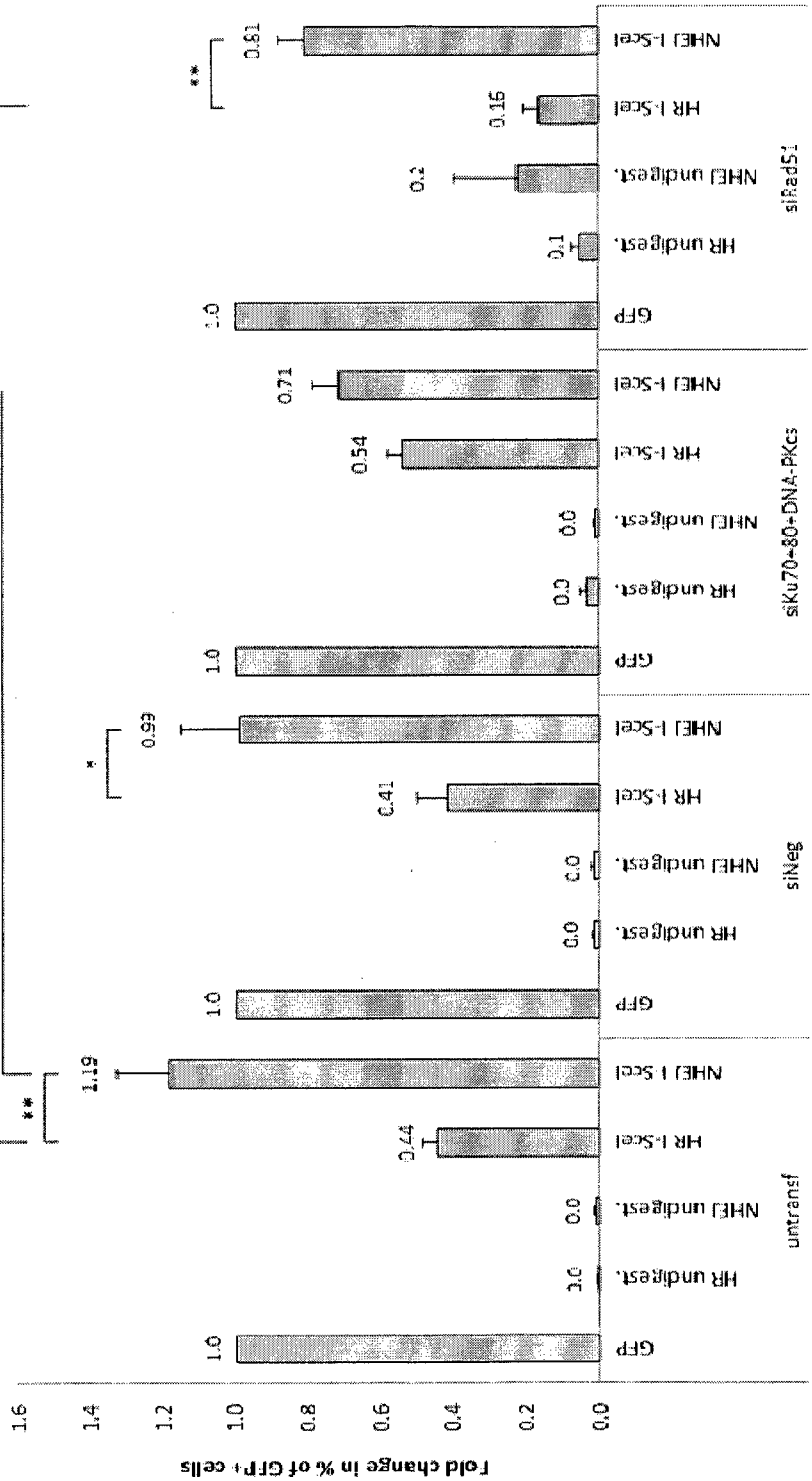

Fold change in % of GFP+ cells
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
untransf
siNeg
siKu70-80+DNA-PKcs
siRad51
GFP
HR undigest.
NHEJ undigest.
HR I-SceI
NHEJ I-SceI
1.0
3.0
0.0
0.44
1.19
0.41
0.99
0.54
0.71
0.2
0.1
0.15
0.81
*
**

Figure 14

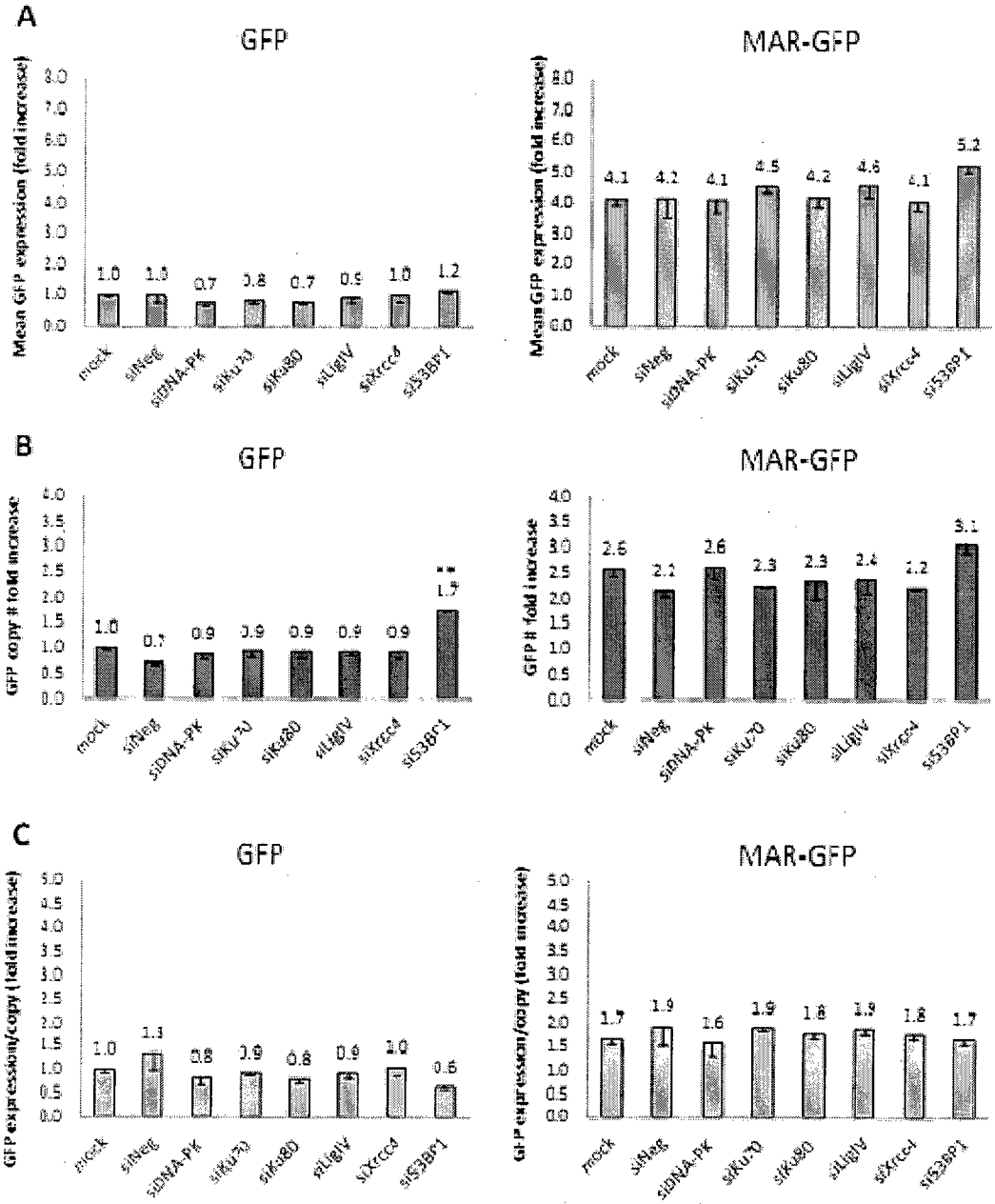

A
GFP
MAR-GFP
Mean GFP expression (fold increase)
1.0 1.3 0.7 0.8 0.7 0.9 1.0 1.2
4.1 4.2 4.1 4.5 4.2 4.6 4.1 5.2
B
GFP
MAR-GFP
GFP copy # fold increase
GFP # fold increase
1.0 0.7 0.9 0.9 0.9 0.9 0.9 1.7 **
2.6 2.1 2.6 2.3 2.3 2.4 2.2 3.1
C
GFP
MAR-GFP
GFP expression/copy (fold increase)
1.0 1.3 0.8 0.9 0.8 0.9 1.0 0.6
1.7 1.9 1.6 1.9 1.8 1.9 1.8 1.7
mock
siNeg
siDNA-PK
siKu70
siKu80
siLigIV
siXrcc4
si53BP1

Figure 15

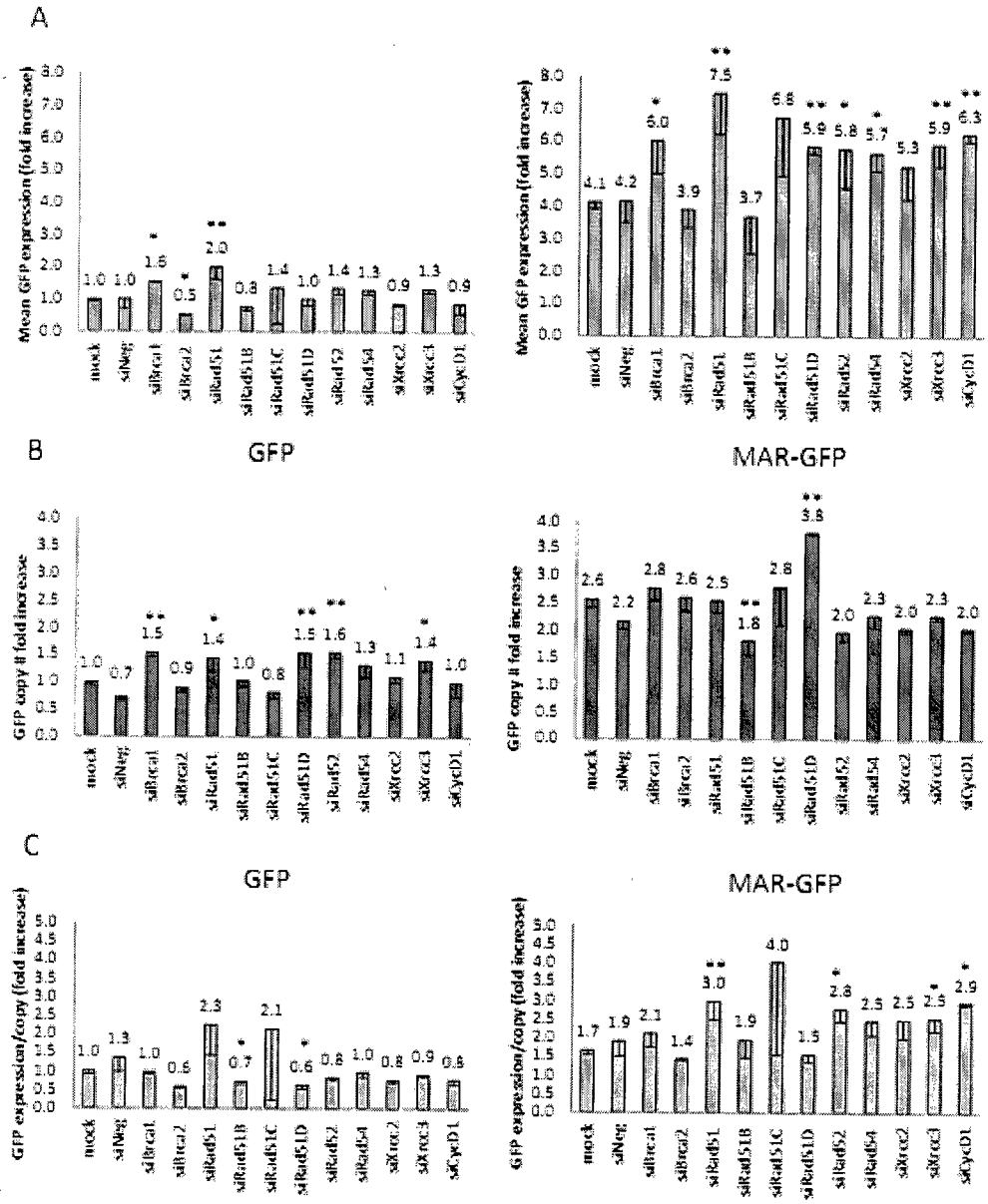

A
Mean GFP expression (fold increase)
mock
siNeg
siBrca1
siBrca2
siRad51
siRad51B
siRad51C
siRad51D
siRad52
siRad54
siXrcc2
siXrcc3
siCycD1
B
GFP
MAR-GFP
GFP copy # fold increase
C
GFP
MAR-GFP
GFP expression/copy (fold increase)

Figure 16A

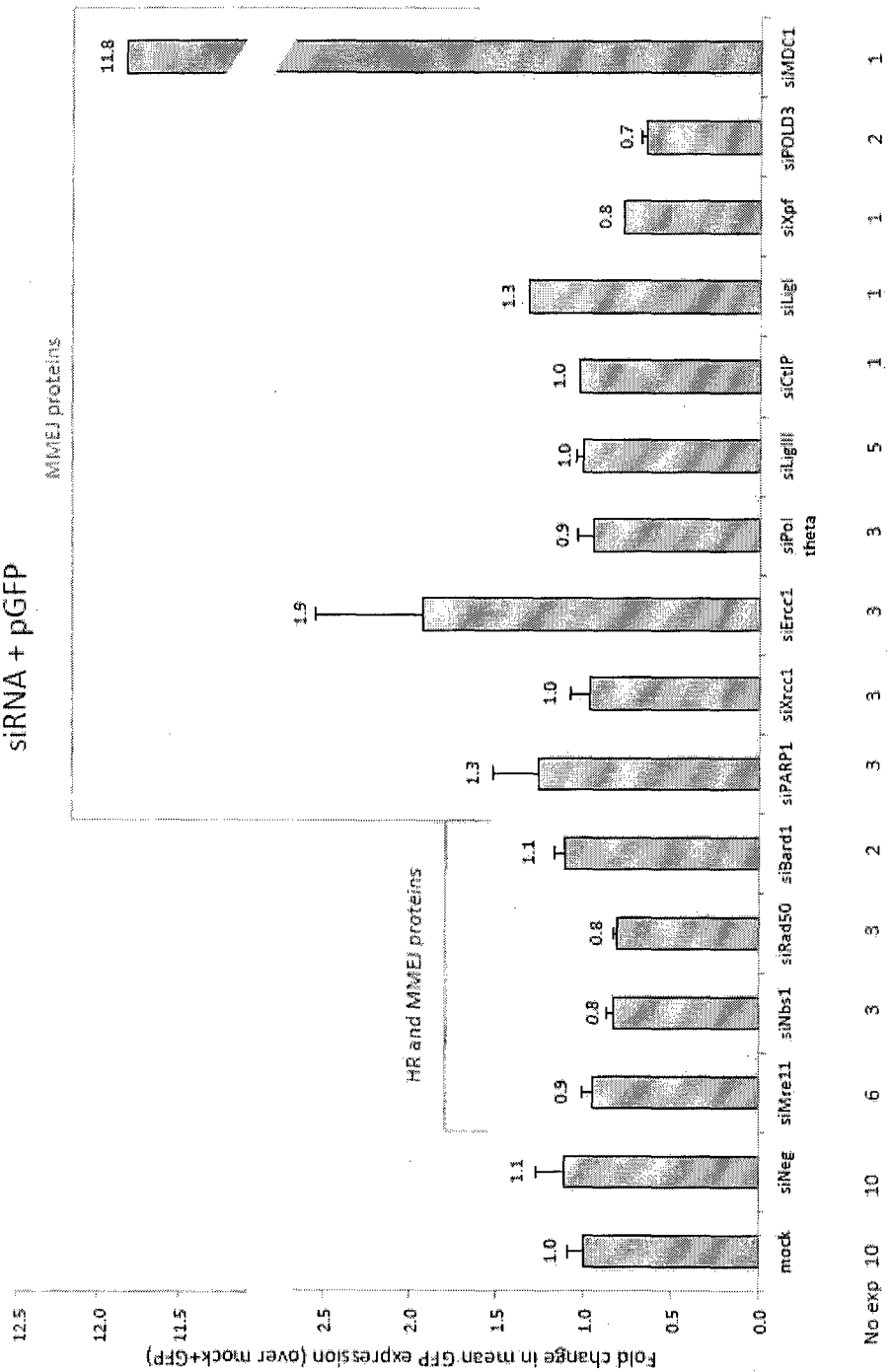

siRNA + pGFP
Fold change in mean GFP expression (over mock+GFP)
12.5
12.0
11.5
2.5
2.0
1.5
1.0
0.5
0.0
MMEJ proteins
HR and MMEJ proteins
1.0
1.1
0.9
0.8
0.8
1.1
1.3
1.0
1.9
0.9
1.0
1.0
1.3
0.8
0.7
11.8
mock
siNeg
siMre11
siNbs1
siRad50
siBard1
siPARP1
siXrcc1
siErcc1
siPol theta
siLigIII
siCtIP
siLigI
siXpf
siPOLD3
siMDC1
No exp 10 10 6 3 3 2 3 3 3 3 5 1 1 1 2 1

Figure 16B

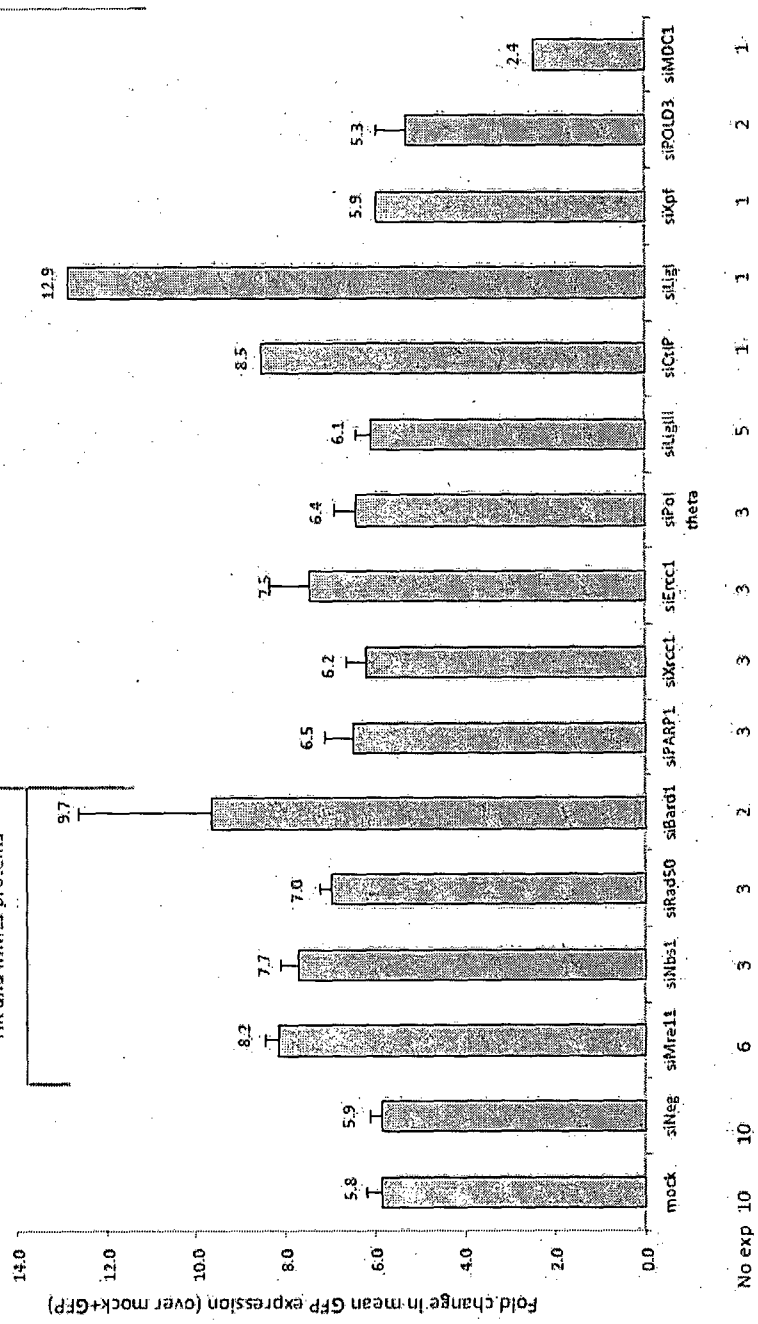

siRNA + pMAR-GFP
HR and MMEJ proteins
MMEJ proteins
Fold change in mean GFP expression (over mock+GFP)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
5.8
5.9
8.2
7.7
7.0
9.7
6.5
6.2
7.5
6.4
6.1
8.5
12.9
5.9
5.3
2.4
mock
siNeg
siMre11
siNbs1
siRad50
siBard1
siPARP1
siXrcc1
siErcc1
siPol theta
siLigIII
siCtIP
siLigI
siXpf
siPOLD3
siMDC1
No exp
10
10
6
3
3
2
3
3
3
3
5
1
1
1
2
1

Figure 17

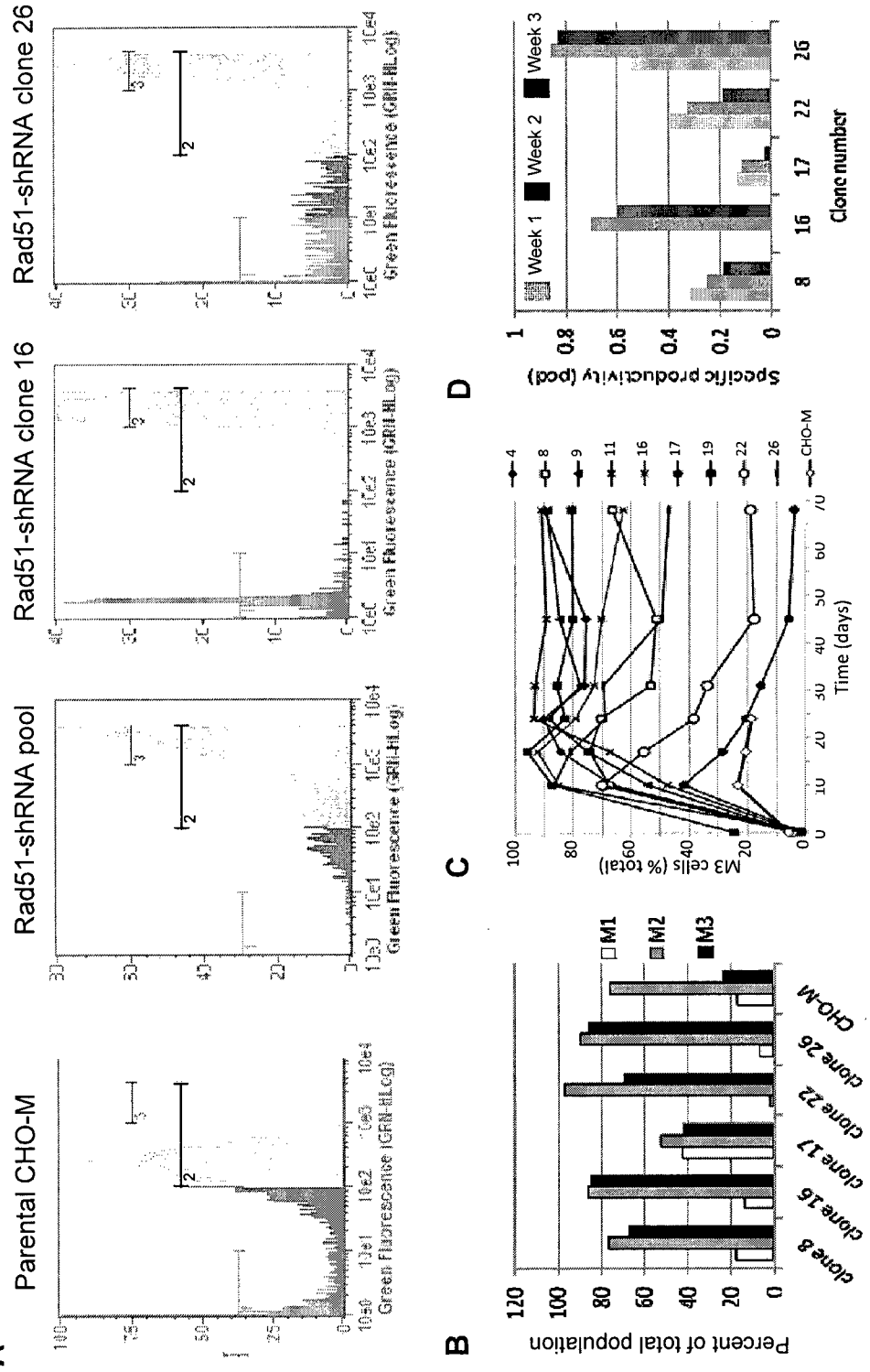

A
Parental CHO-M
Rad51-shRNA pool
Rad51-shRNA clone 16
Rad51-shRNA clone 26
Green Fluorescence (GRN-HLog)
B
Percent of total population
M1
M2
M3
clone 8
clone 16
clone 17
clone 22
clone 26
CHO-M
C
M3 cells (% total)
Time (days)
4
8
9
11
16
17
19
22
26
CHO-M
D
Specific productivity (pcd)
Week 1
Week 2
Week 3
Clone number

Figure 18A

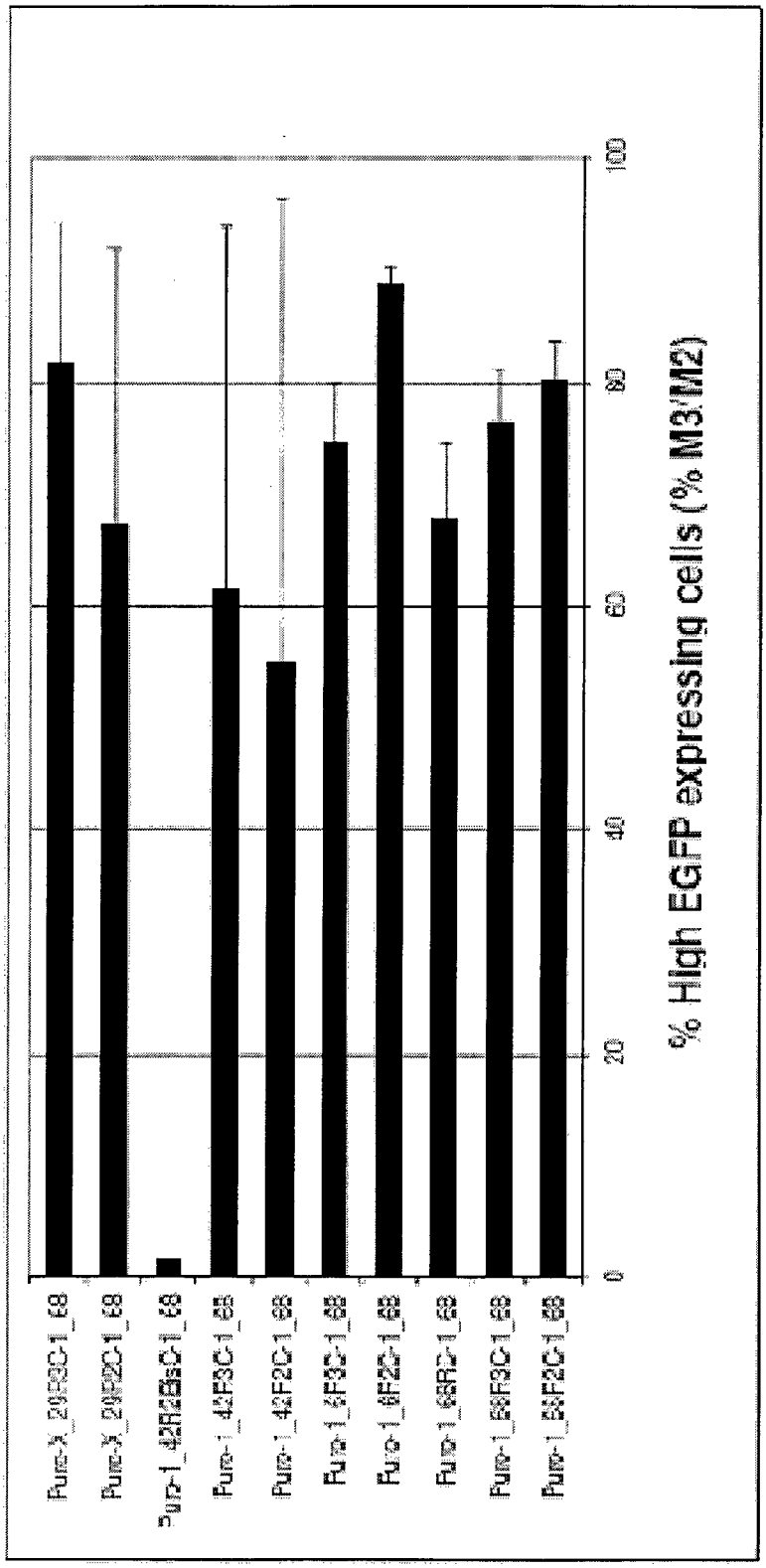
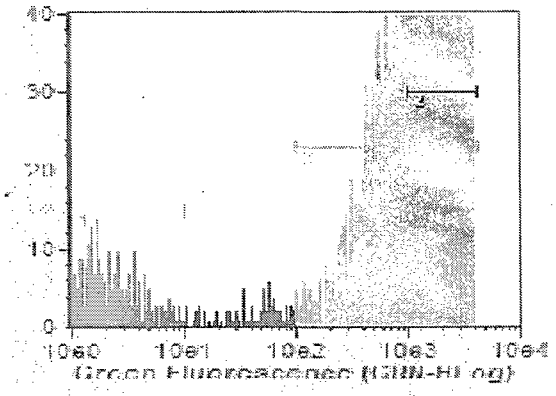
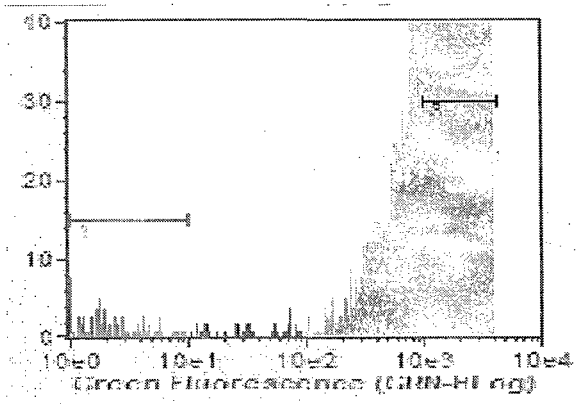


Figure 18B

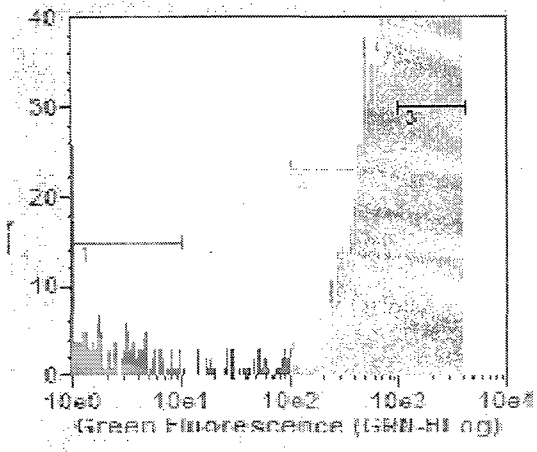
SGE 1 (1_68R)



SGE 1 (1_6R2)



SGE 1 (1_68R2)



SGE 1 (X_29R3)

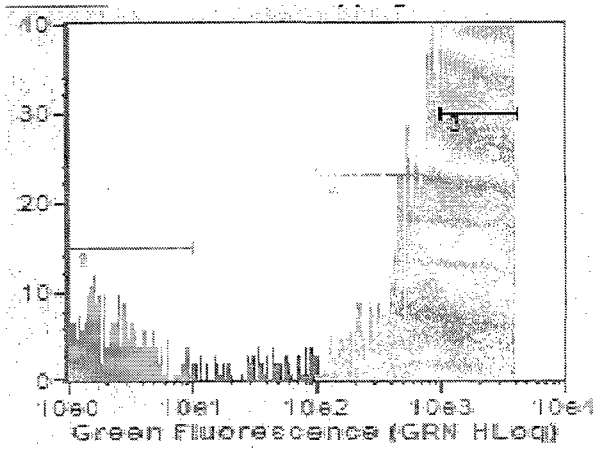
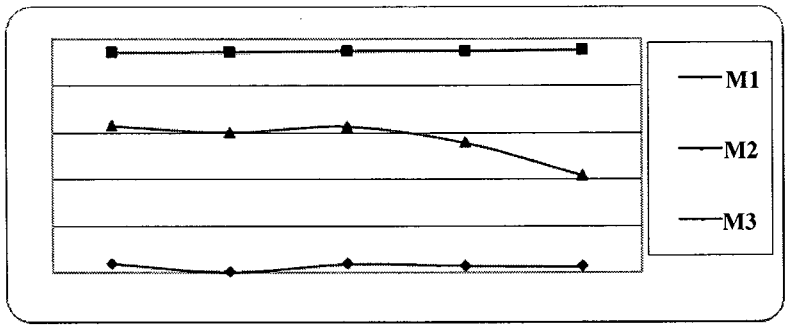
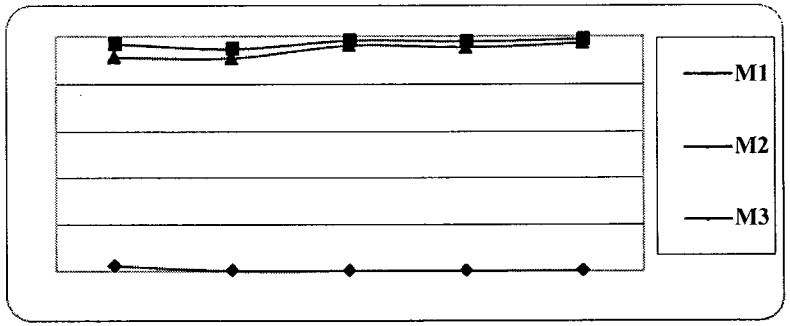


Figure 19

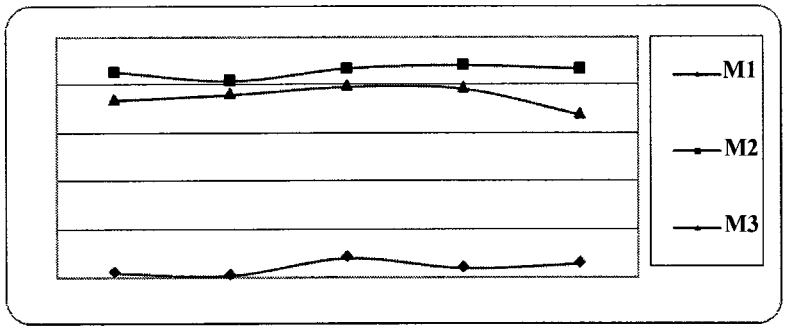
pSV puro < 1_68R2C



pSV puro < 1_6R2C



pSV puro < X_29R3C



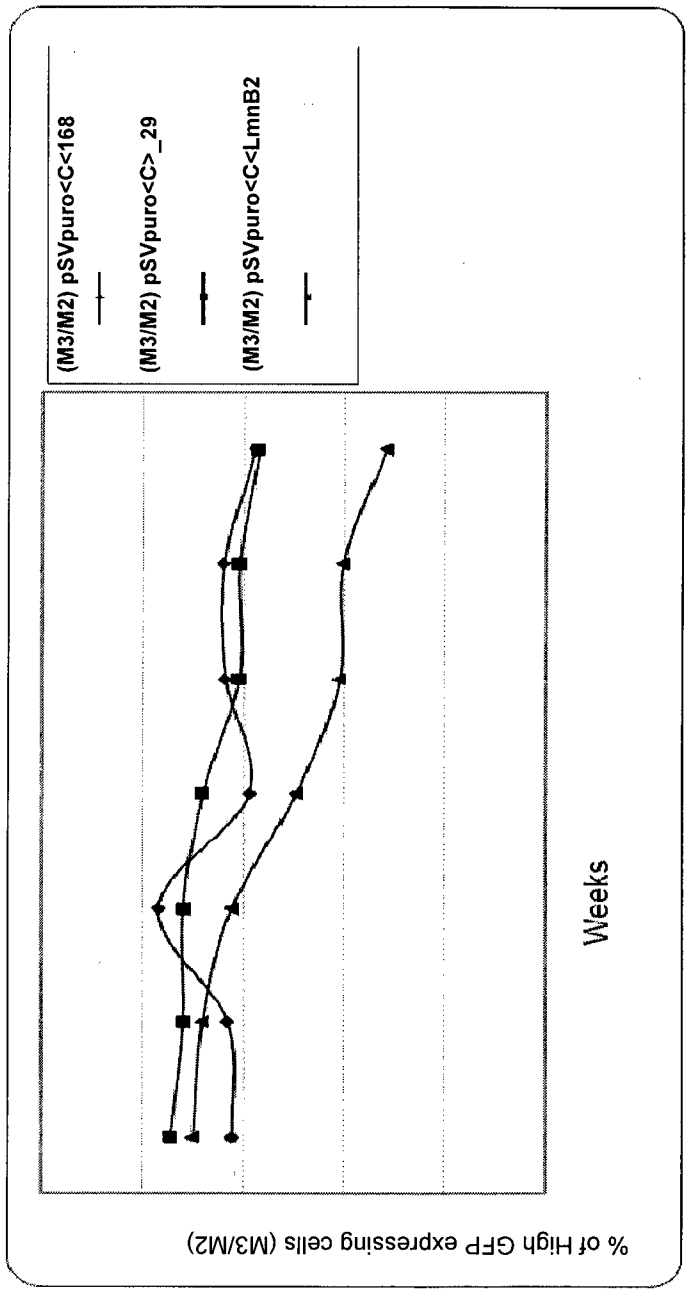

% of High GFP expressing cells (M3/M2)
Weeks
(M3/M2) pSVpuro<C<168
(M3/M2) pSVpuro<C>_29
(M3/M2) pSVpuro<C<LmnB2

Figure 20A

Figure 20B

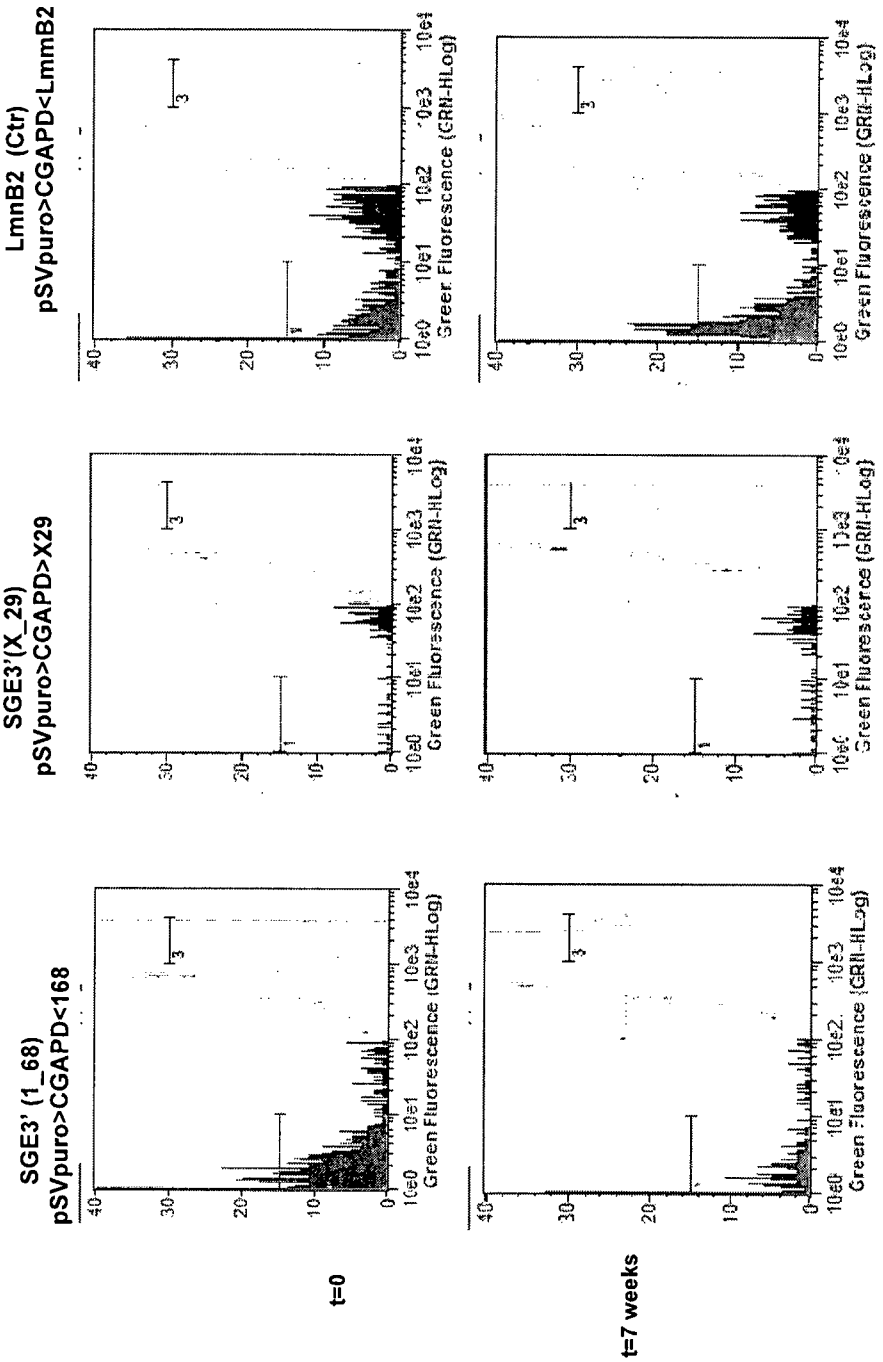

GFP expression and stability (2 months)
SGE3' (1_68)
pSVpuro>CGAPD<168
SGE3'(X_29)
pSVpuro>CGAPD>X29
LmnB2 (Ctr)
pSVpuro>CGAPD<LmmB2
t=0
t=7 weeks
Green Fluorescence (GRN-HLog)

Figure 21

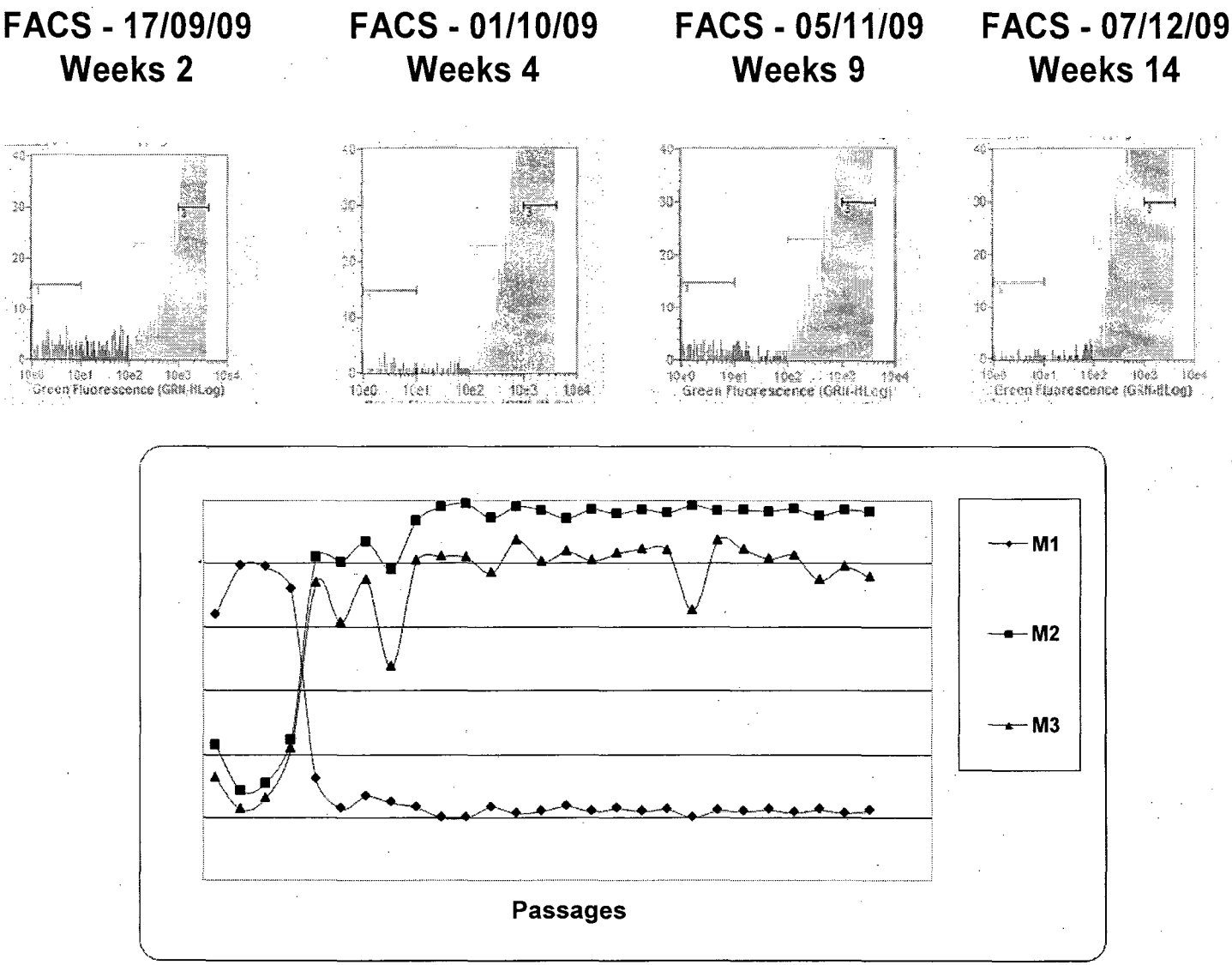
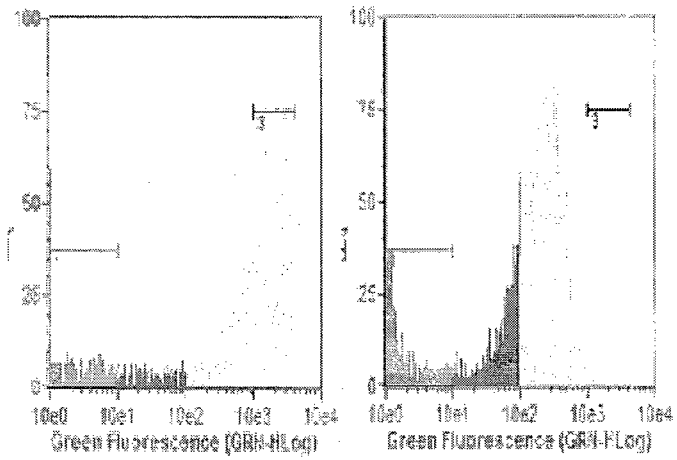

FACS - 17/09/09
Weeks 2
FACS - 01/10/09
Weeks 4
FACS - 05/11/09
Weeks 9
FACS - 07/12/09
Weeks 14
Green Fluorescence (GRN-HLog)
M1
M2
M3
Passages

Figure 22

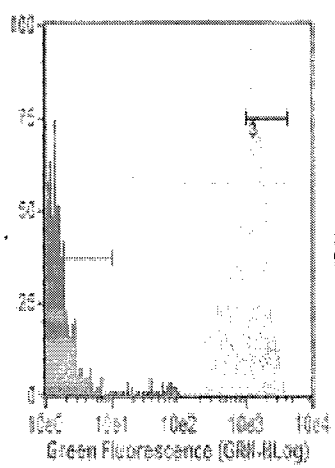
Puro_CGAPD_GFP_gastrin_X29



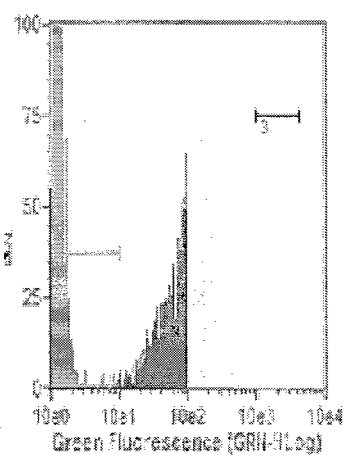
High GFP

Low GFP

Puro_1_68R2_CGAPD_GFP_gastrin_168



High GFP



Low GFP