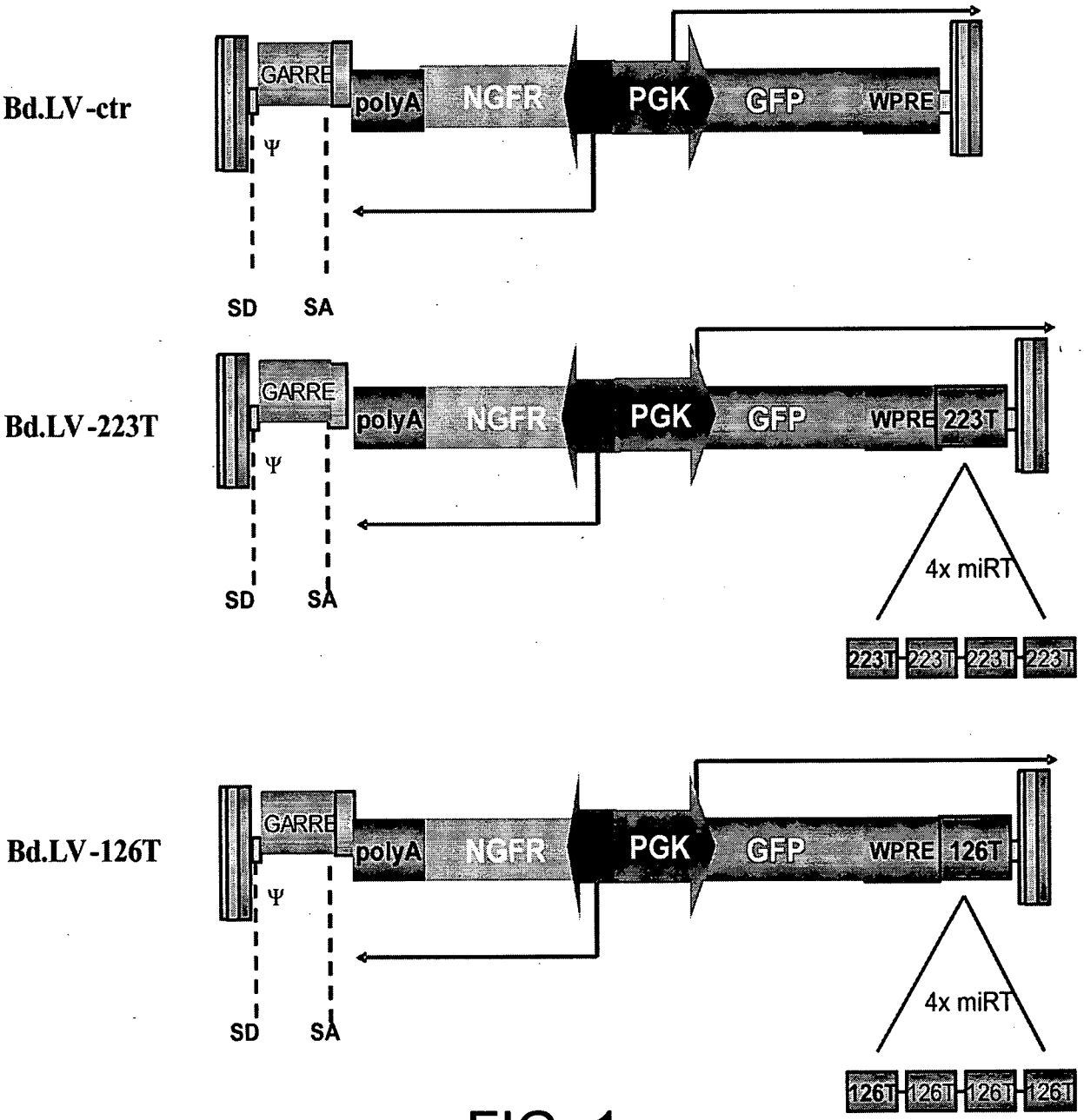

Bd.LV-ctr
GARRE
polyA
NGFR
PGK
GFP
WPRE
Ψ
SD
SA
Bd.LV-223T
GARRE
polyA
NGFR
PGK
GFP
WPRE
223T
Ψ
SD
SA
4x miRT
223T
223T
223T
223T
Bd.LV-126T
GARRE
polyA
NGFR
PGK
GFP
WPRE
126T
Ψ
SD
SA
4x miRT
126T
126T
126T
126T

FIG. 1

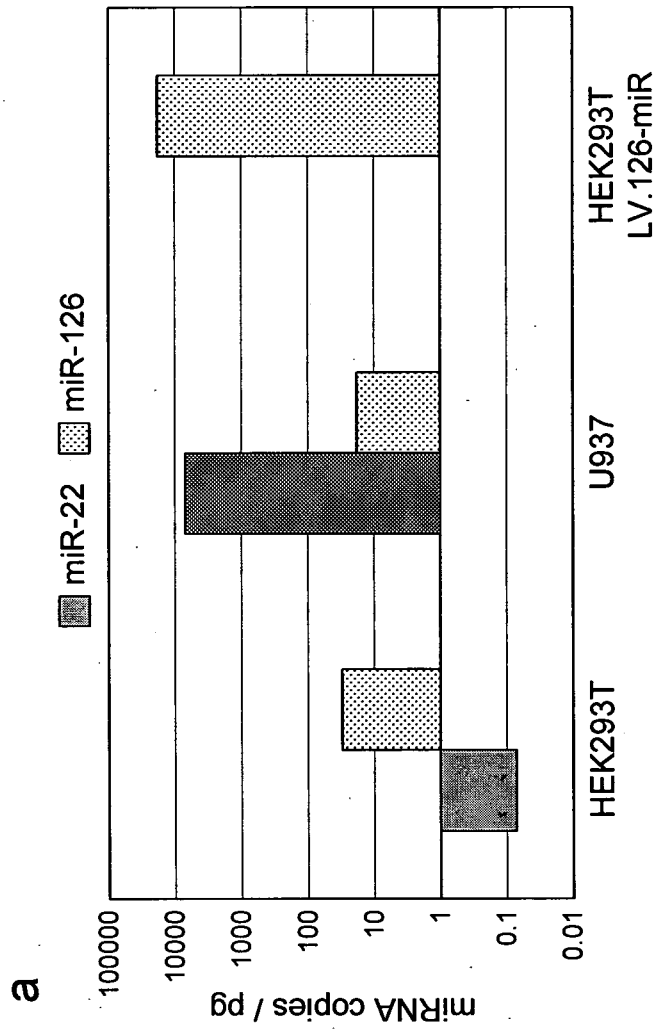

a
miRNA copies / pg
100000
10000
1000
100
10
1
0.1
0.01
HEK293T
U937
HEK293T
LV.126-miR
miR-22
miR-126

FIG. 2

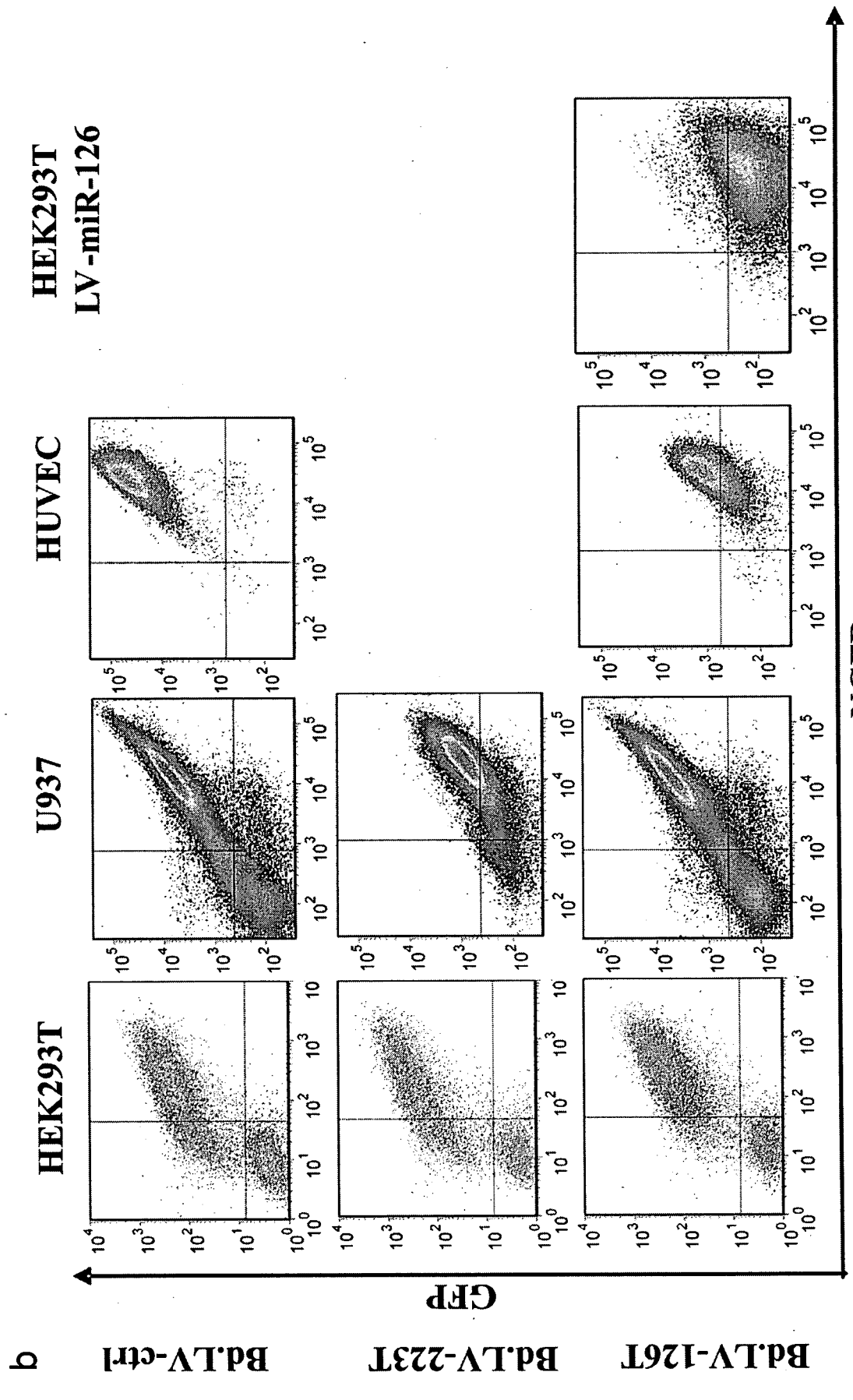

b
Bd.LV-ctrl
Bd.LV-223T
Bd.LV-126T
GFP
NGFR
HEK293T
U937
HUVEC
HEK293T
LV-miR-126

FIG. 2 CONT'D

c

RNA Fold repression

$\Delta CT =$ GFP mRNA - NGFR mRNA

$\Delta\Delta CT =$ miRT ΔCT - ctr ΔCT

$FR =$ $2^{\wedge -\Delta\Delta CT}$

Protein Fold repression

$$TGR = \frac{\text{NGFR MFI}}{\text{GFP MFI}}$$

$$FR = \frac{\text{TGR Bd.LV-miRT}}{\text{TGR Bd.LV-ctr}}$$

d

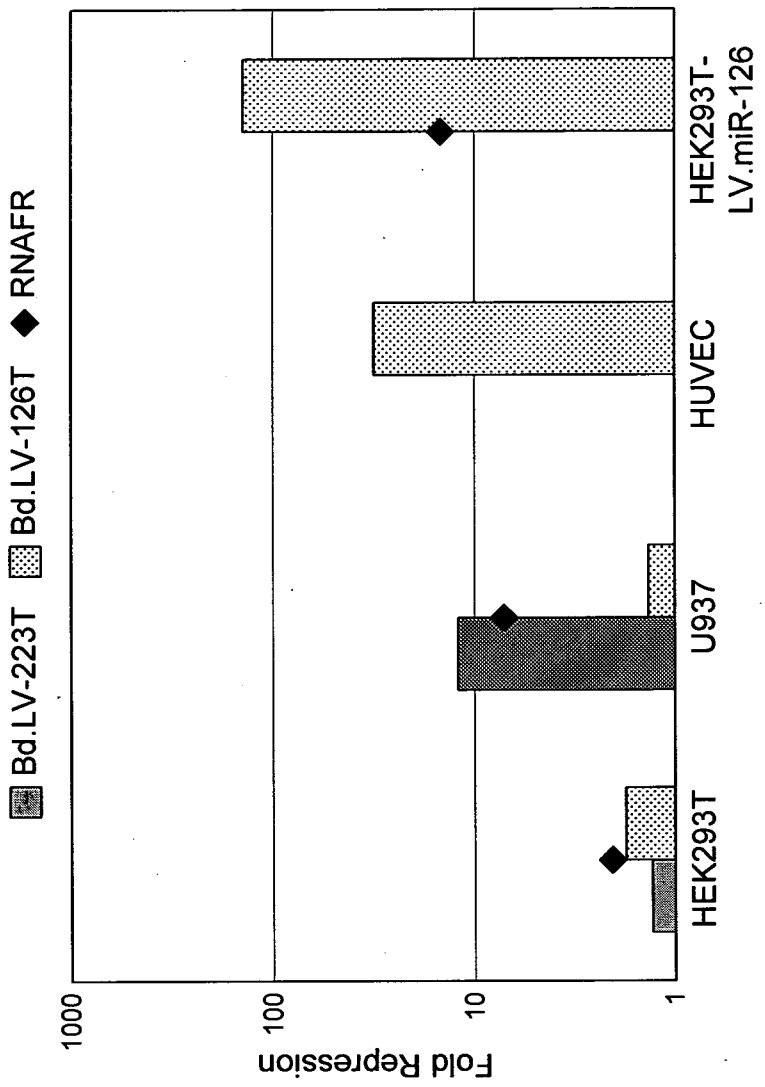


FIG. 2 CONT'D

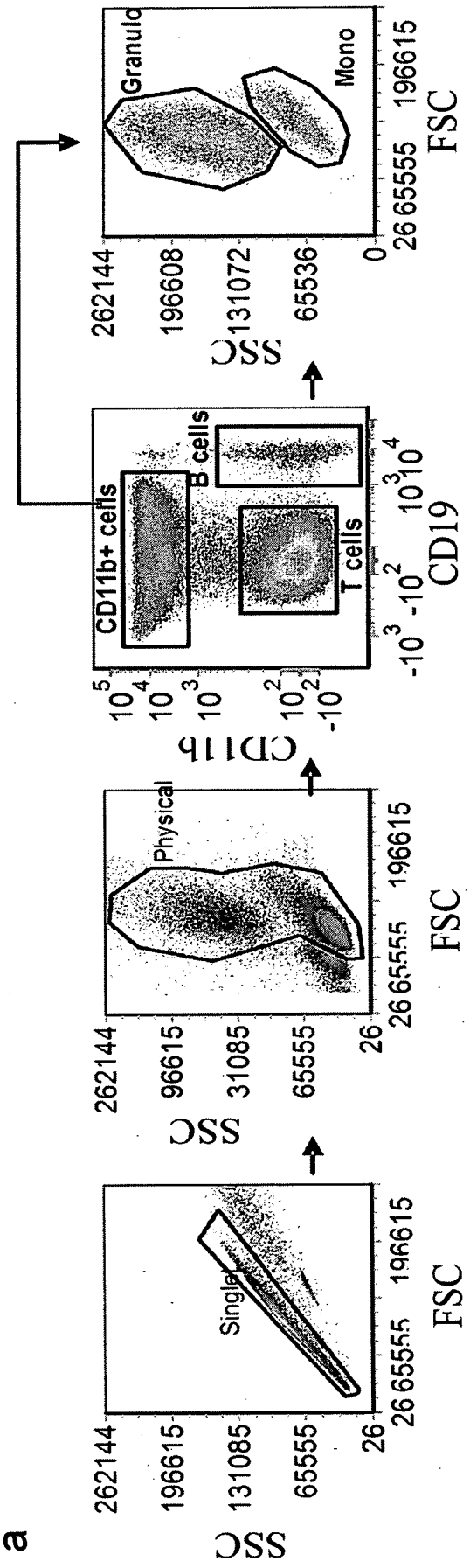

a
SSC
FSC
Singlet
Physical
CD11b
CD19
CD11b+ cells
B cells
T cells
Granulo
Mono

FIG. 3

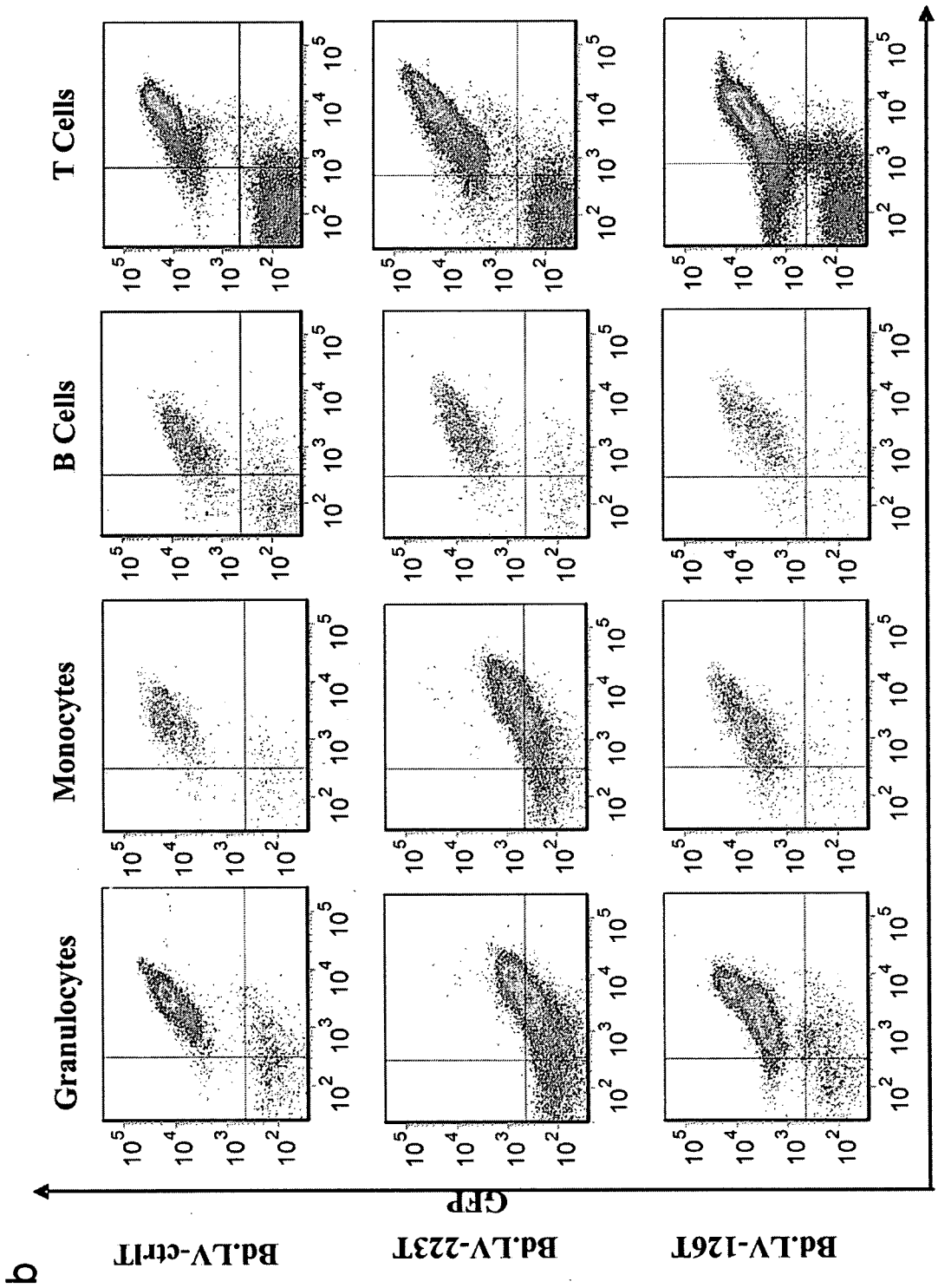

b
Bd.LV-ctrlT
Bd.LV-223T
Bd.LV-126T
GFP
NGFR
Granulocytes
Monocytes
B Cells
T Cells

FIG. 3 CONT'D

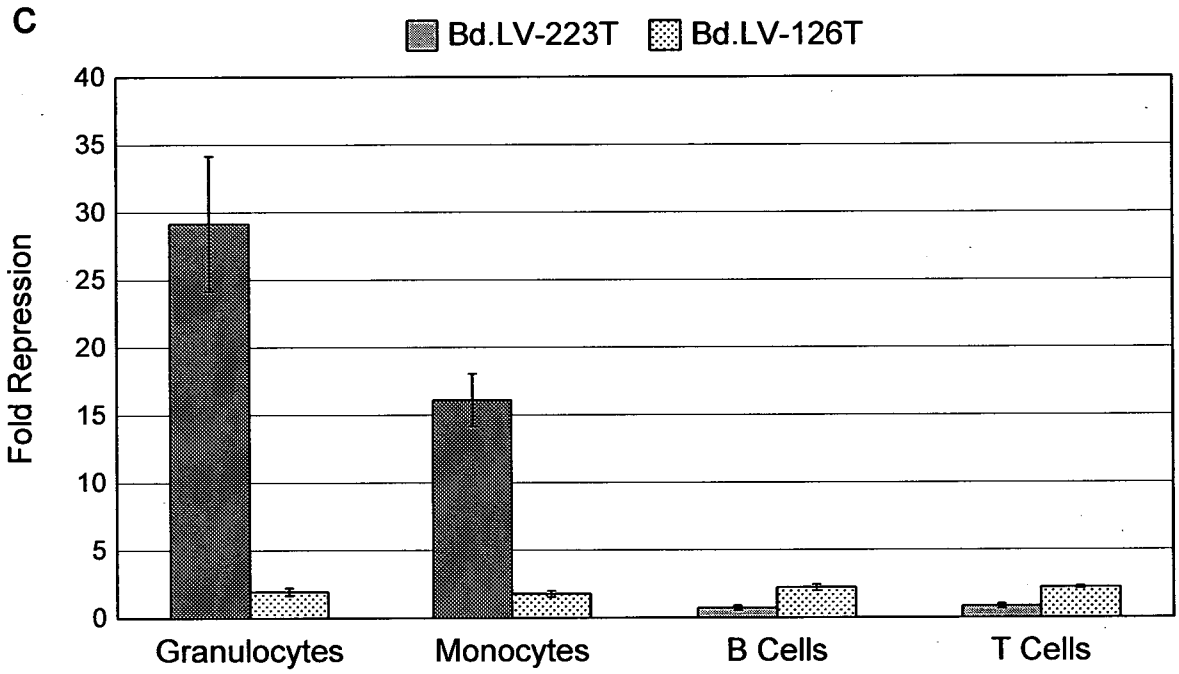

C
Bd.LV-223T
Bd.LV-126T
Fold Repression
40
35
30
25
20
15
10
5
0
Granulocytes
Monocytes
B Cells
T Cells

FIG. 3 CONT'D

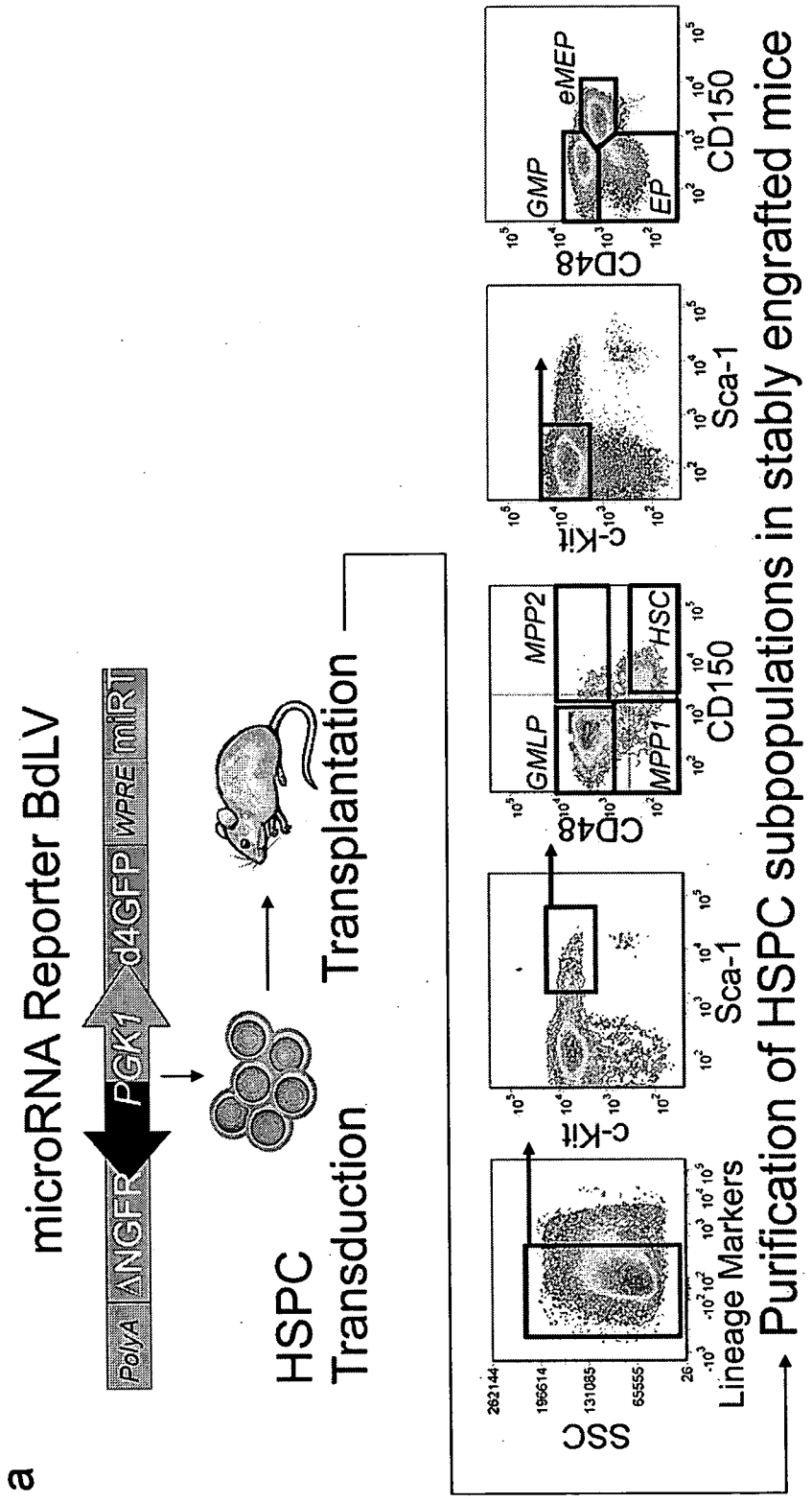

a
microRNA Reporter BdLV
PolyA
ΔNGFR
PGK1
d4GFP
WPRE
miR1
HSPC
Transduction
Transplantation
Purification of HSPC subpopulations in stably engrafted mice
SSC
Lineage Markers
c-Kit
Sca-1
CD48
CD150
GMLP
MPP2
MPP1
HSC
GMP
eMEP
EP

FIG. 4

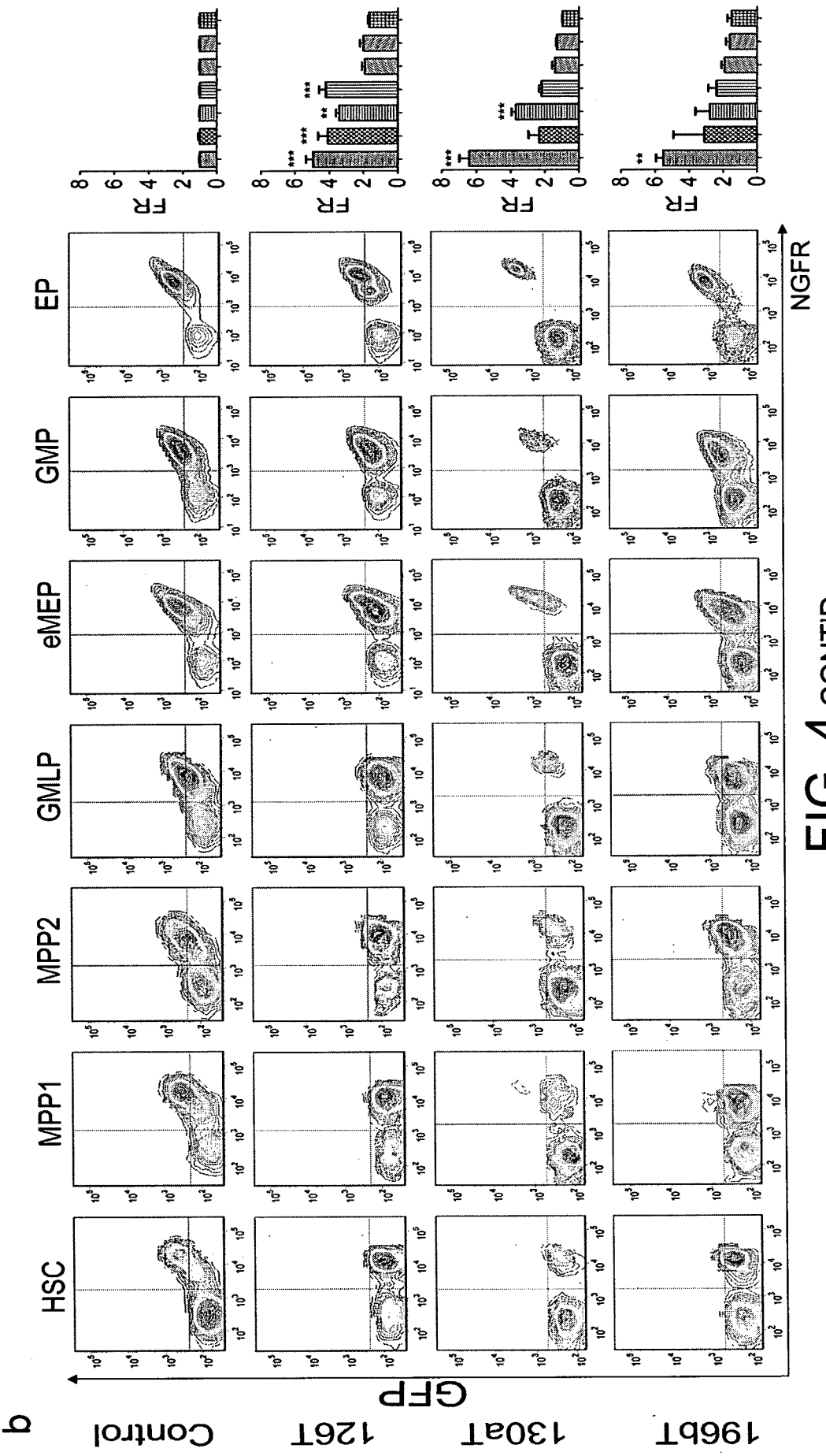

HSC
MPP1
MPP2
GMLP
eMEP
GMP
EP
FR
GFP
NGFR
Control
126T
130aT
196bT

FIG. 4 CONT'D

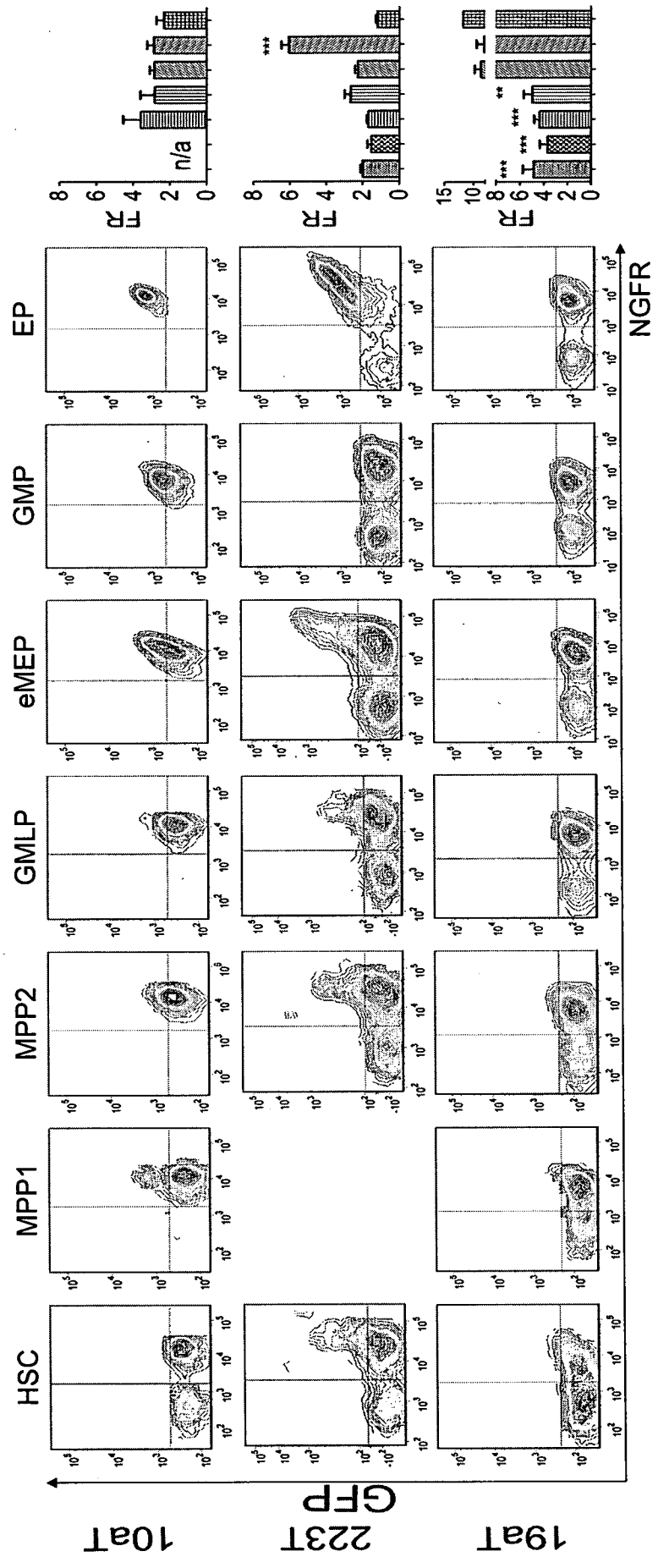


FIG. 4 CONT'D

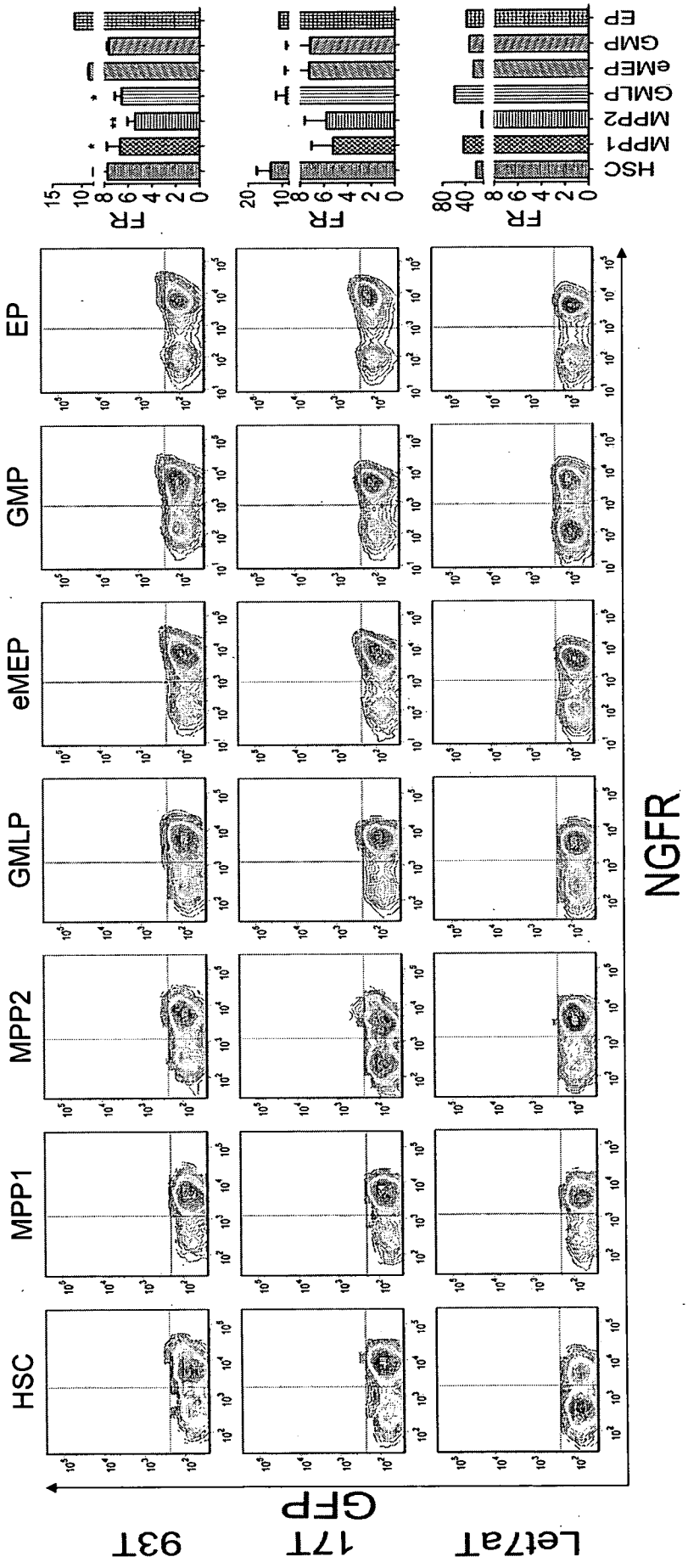


FIG. 4 CONT'D

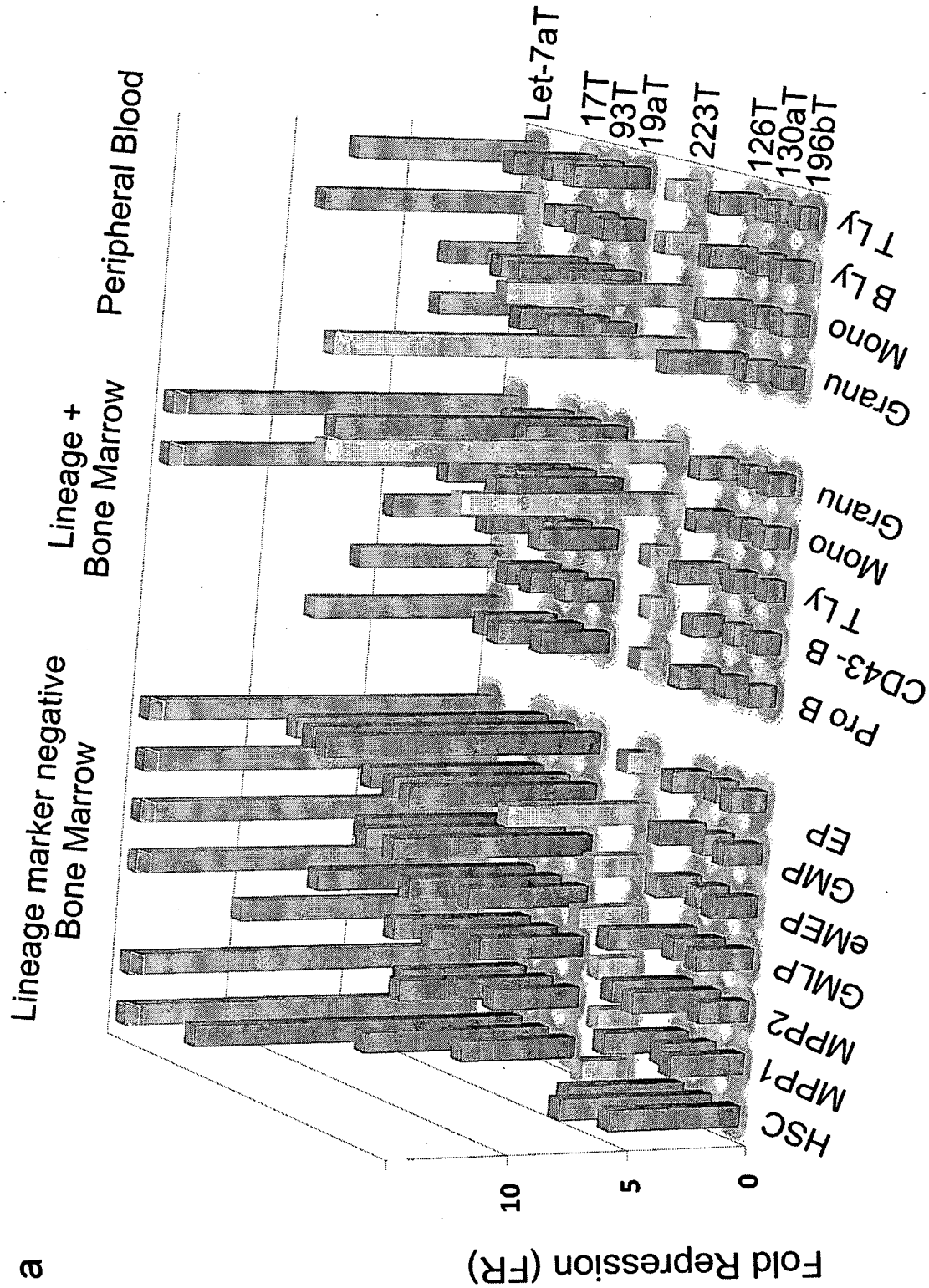

a
Fold Repression (FR)
0
5
10
Lineage marker negative Bone Marrow
HSC
MPP1
MPP2
GMLP
eMEP
GMP
EP
Lineage + Bone Marrow
Pro B
CD43- B
T Ly
Mono
Granu
Peripheral Blood
Granu
Mono
B Ly
T Ly
Let-7aT
17T
93T
19aT
223T
126T
130aT
196bT

FIG. 5

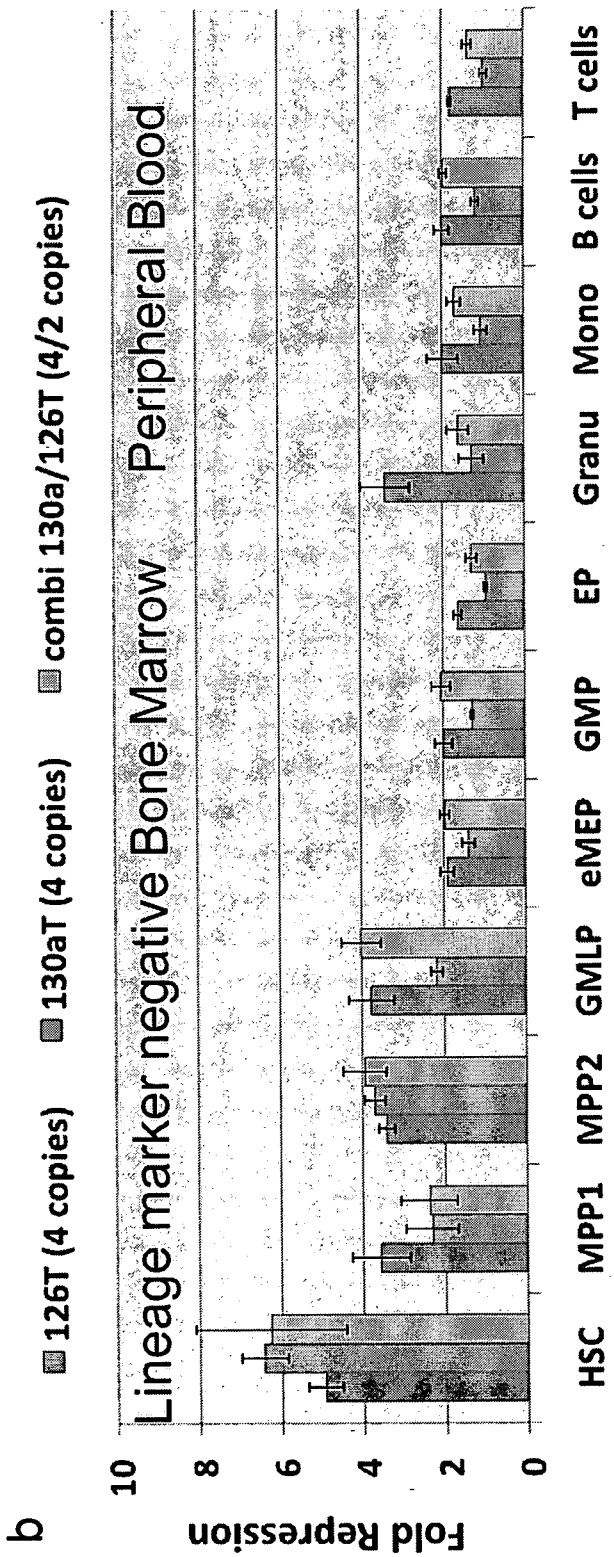

b
Fold Repression
10
8
6
4
2
0
Lineage marker negative Bone Marrow Peripheral Blood
126T (4 copies)
130aT (4 copies)
combi 130a/126T (4/2 copies)
HSC
MPP1
MPP2
GMLP
eMEP
GMP
EP
Granu
Mono
B cells
T cells

FIG. 5 CONT'D

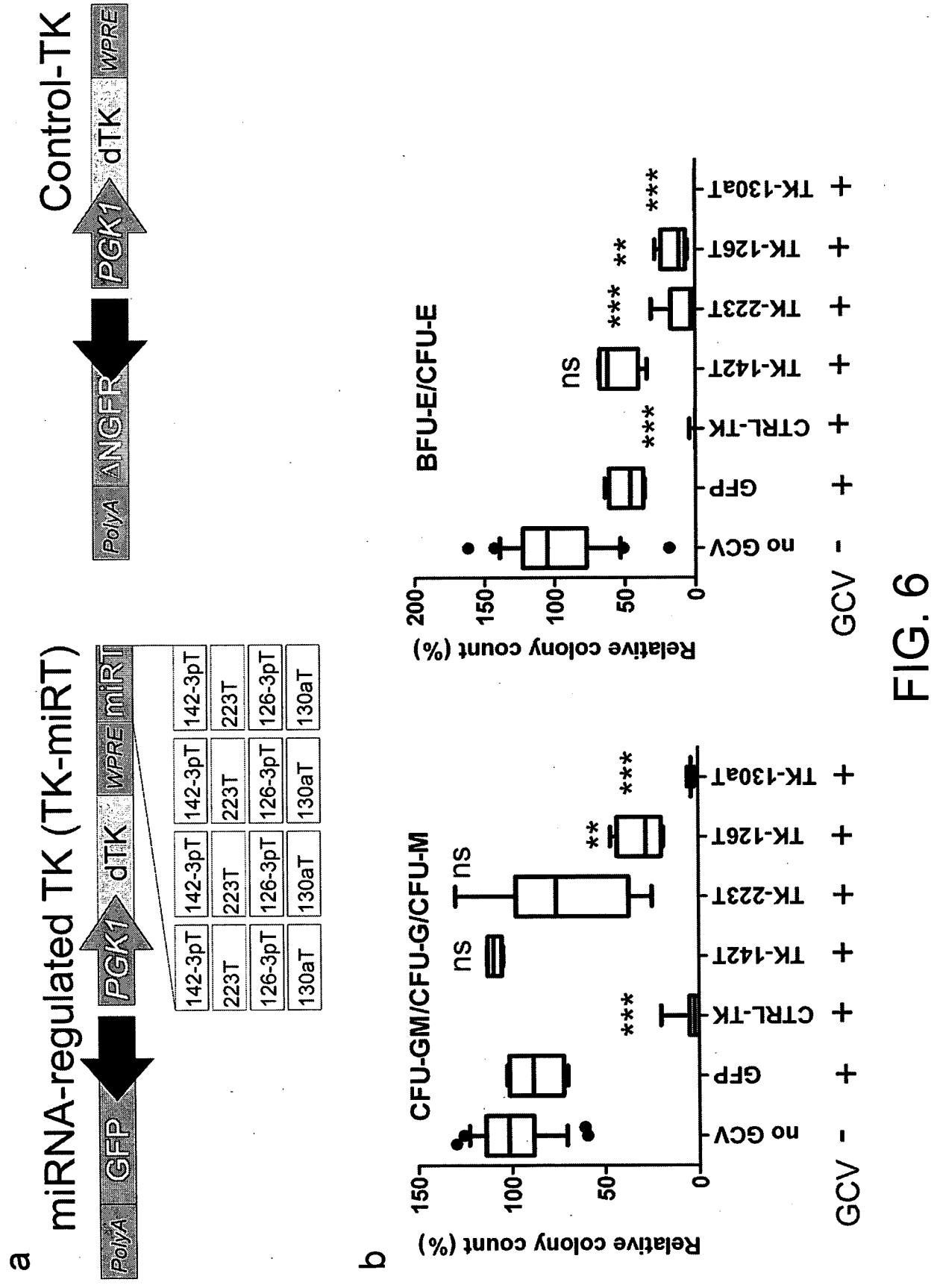

a
miRNA-regulated TK (TK-miRT)
PolyA*
GFP
PGK1
dTK
WPRE
miRT
142-3pT
223T
126-3pT
130aT
Control-TK
PolyA
ΔNGFR
PGK1
dTK
WPRE
b
CFU-GM/CFU-G/CFU-M
Relative colony count (%)
150
100
50
0
ns
ns
**
***
***
GCV
-
+
no GCV
GFP
CTRL-TK
TK-142T
TK-223T
TK-126T
TK-130aT
BFU-E/CFU-E
Relative colony count (%)
200
150
100
50
0
ns
***
***
**
***
GCV
-
+
no GCV
GFP
CTRL-TK
TK-142T
TK-223T
TK-126T
TK-130aT

FIG. 6

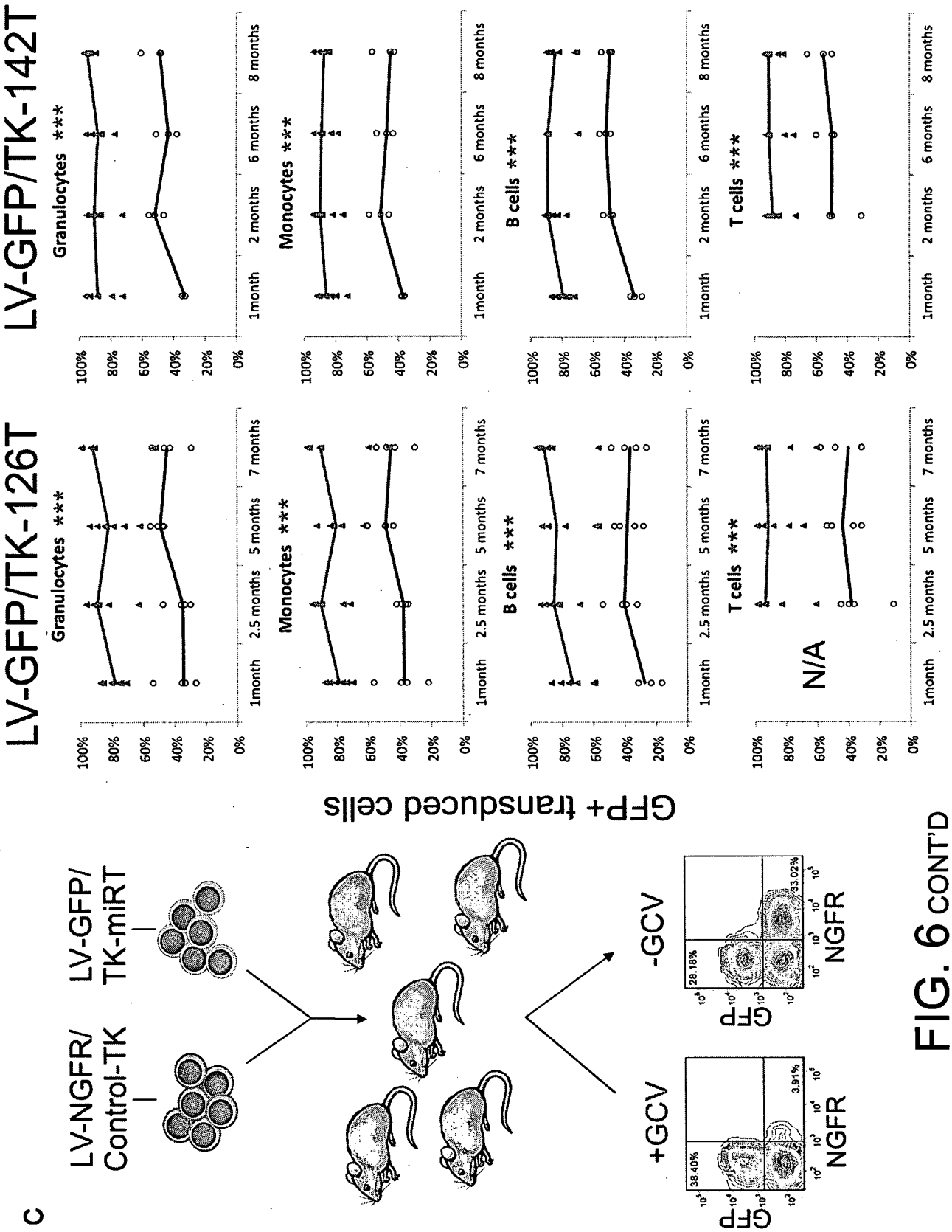

c
LV-NGFR/ Control-TK
LV-GFP/ TK-miRT
+GCV
-GCV
GFP
NGFR
38.40%
3.91%
28.18%
33.02%
N/A
LV-GFP/TK-126T
LV-GFP/TK-142T
Granulocytes ***
Monocytes ***
B cells ***
T cells ***
GFP+ transduced cells
100%
80%
60%
40%
20%
0%
1month
2.5 months
5 months
7 months
2 months
6 months
8 months

FIG. 6 CONT'D

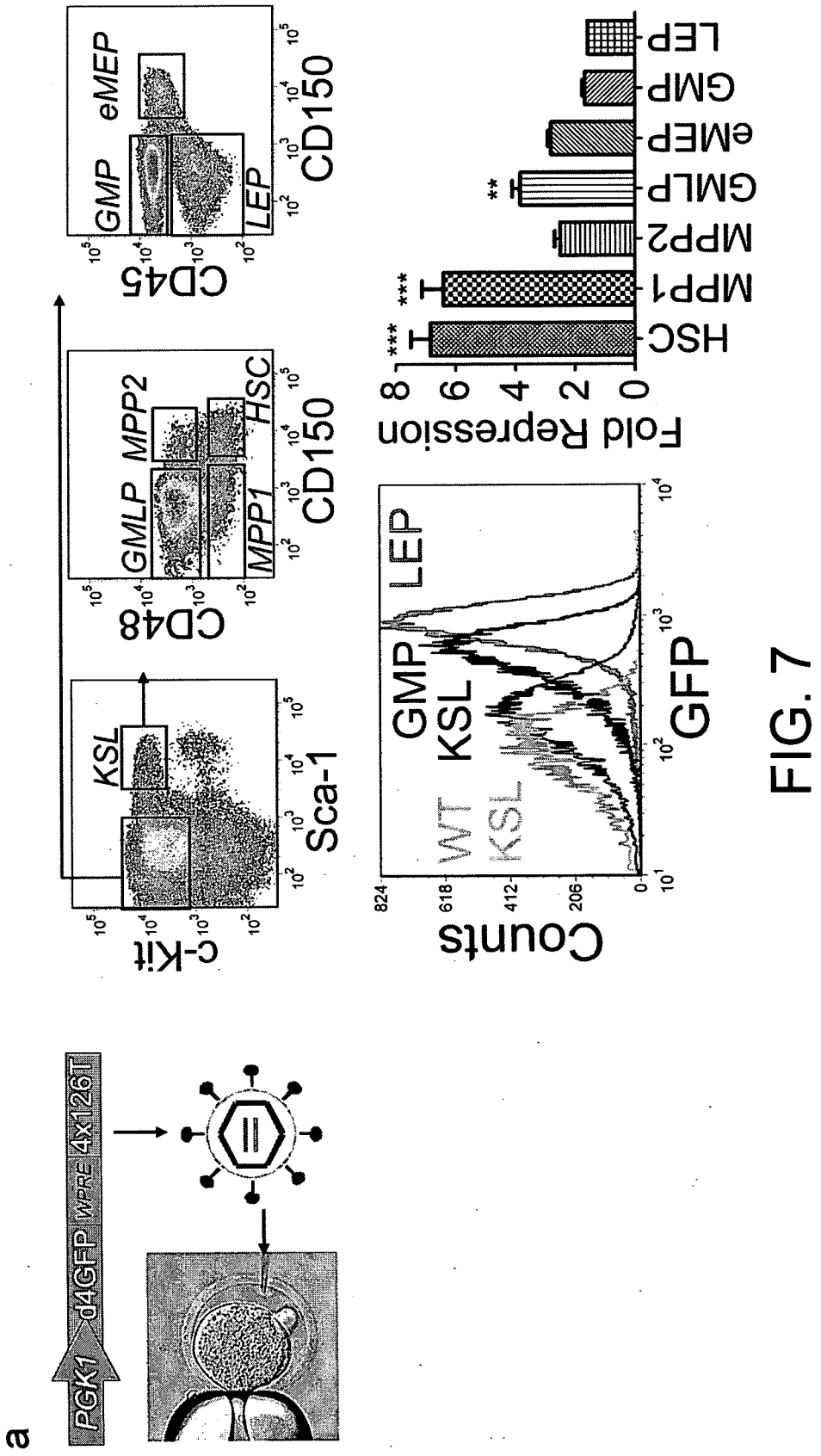

a
PGK1
d4GFP
WPRE
4x126T
c-Kit
Sca-1
KSL
CD48
CD150
GMLP
MPP2
MPP1
HSC
CD45
CD150
GMP
eMEP
LEP
Fold Repression
HSC
MPP1
MPP2
GMLP
eMEP
GMP
LEP
Counts
GFP
GMP
LEP
KSL
WT
KSL

FIG. 7

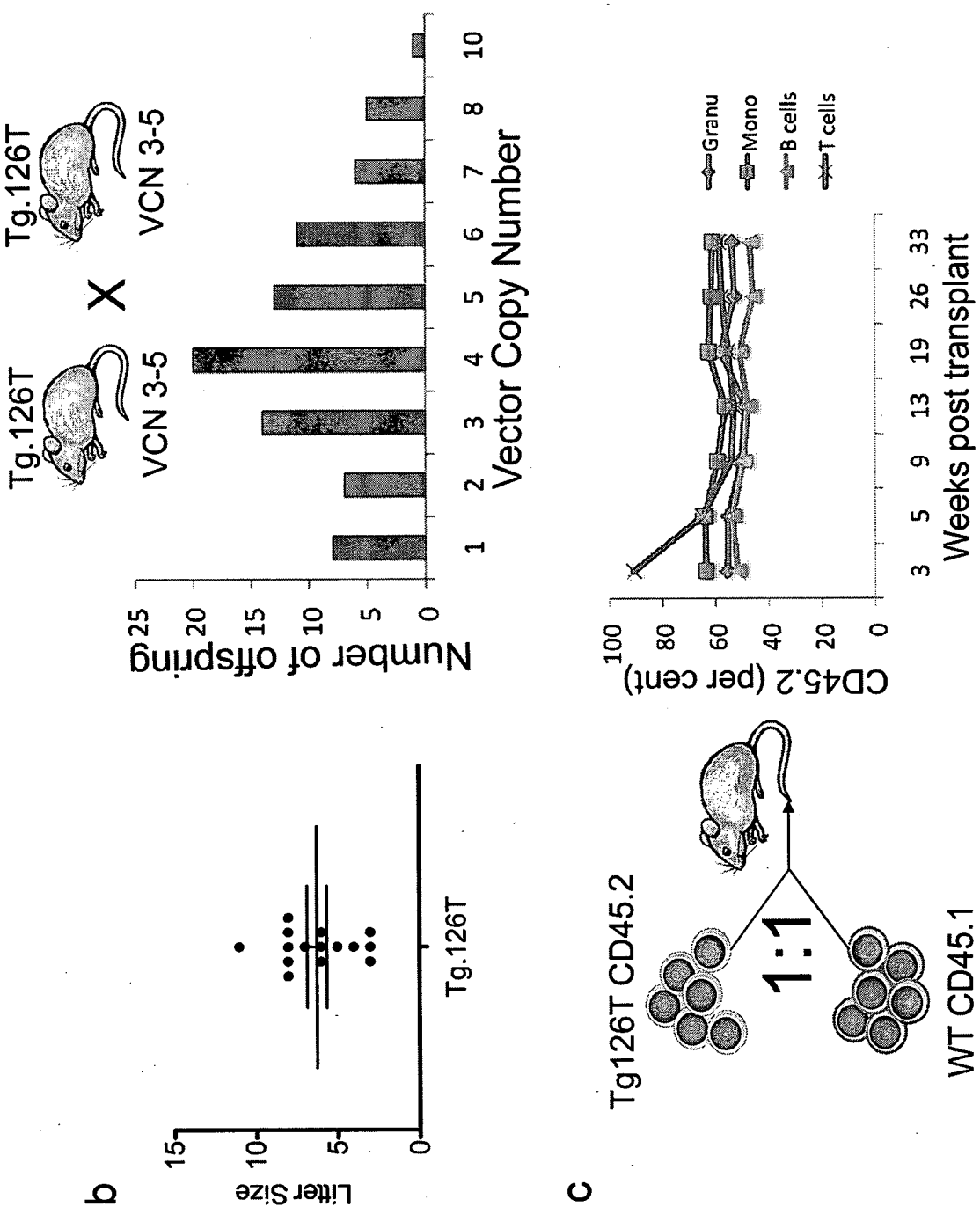

Tg.126T
VCN 3-5
X
Tg.126T
VCN 3-5
Number of offspring
Vector Copy Number
b
Litter Size
Tg.126T
c
Tg126T CD45.2
1:1
WT CD45.1
CD45.2 (per cent)
Weeks post transplant
Granu
Mono
B cells
T cells

FIG. 7 CONT'D

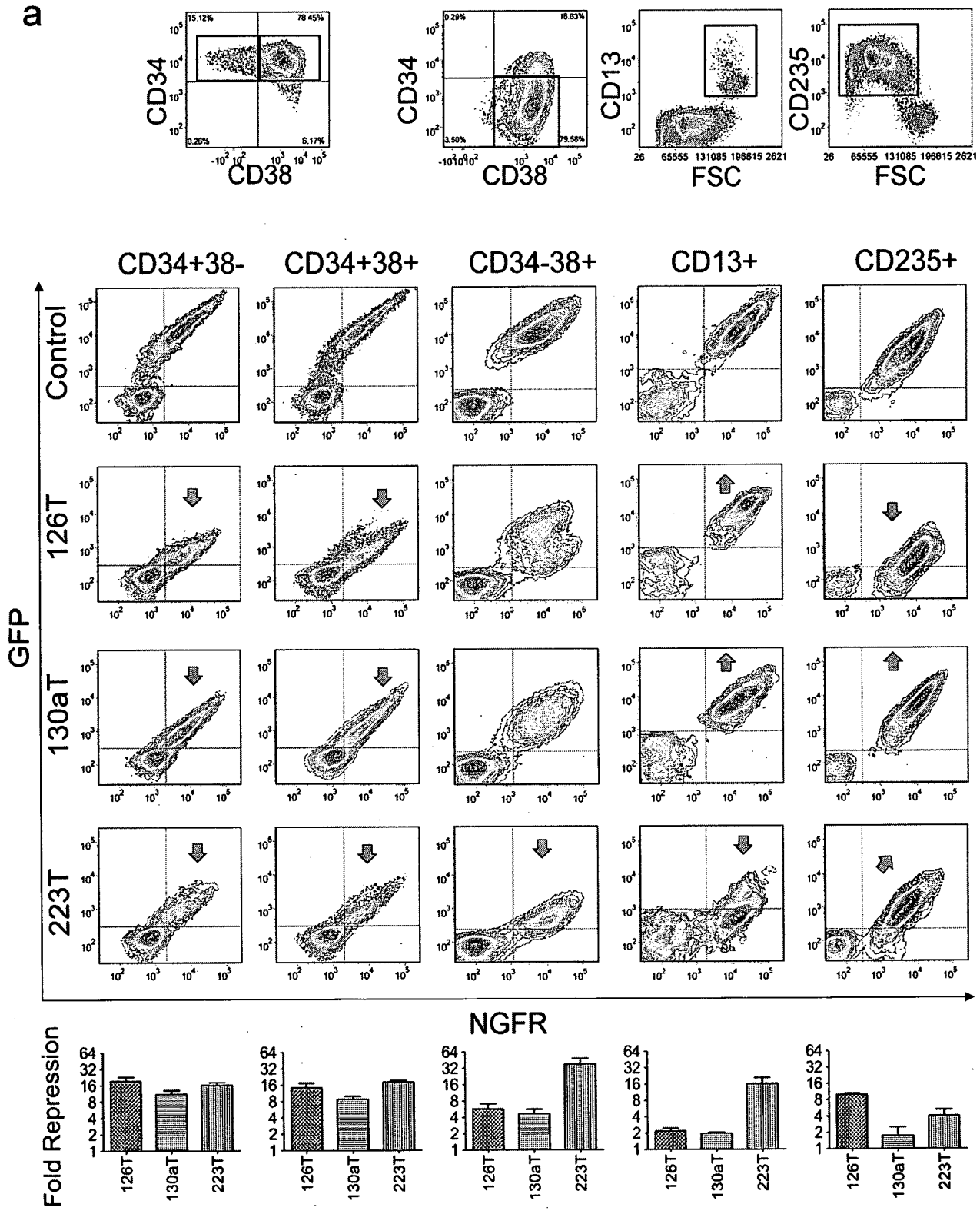

a
CD34
CD38
CD13
FSC
CD235
CD34+38-
CD34+38+
CD34-38+
CD13+
CD235+
Control
126T
130aT
223T
GFP
NGFR
Fold Repression
64
32
16
8
4
2
1

FIG. 8

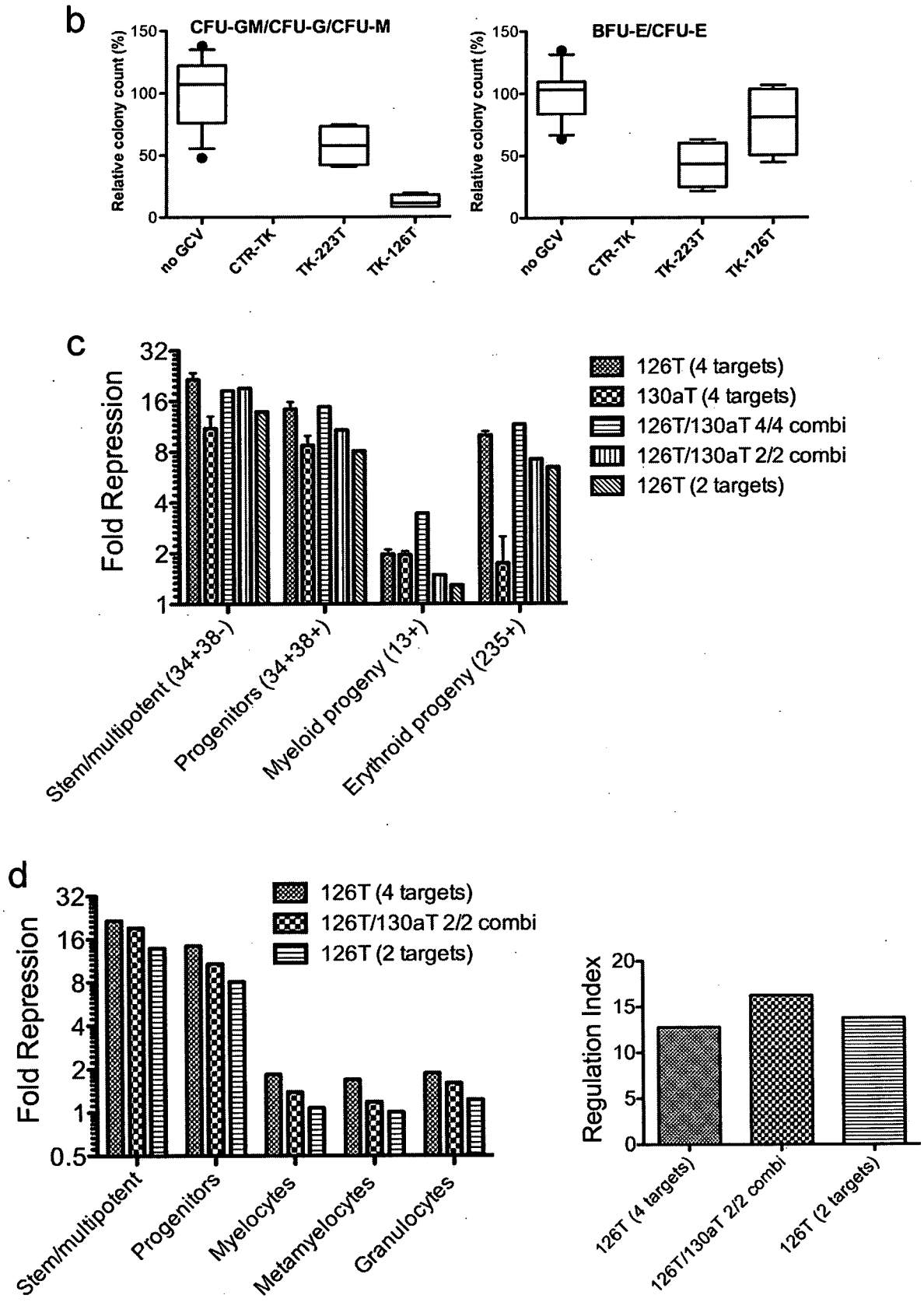

b
CFU-GM/CFU-G/CFU-M
BFU-E/CFU-E
Relative colony count (%)
150
100
50
0
no GCV
CTR-TK
TK-223T
TK-126T
c
Fold Repression
32
16
8
4
2
1
126T (4 targets)
130aT (4 targets)
126T/130aT 4/4 combi
126T/130aT 2/2 combi
126T (2 targets)
Stem/multipotent (34+38-)
Progenitors (34+38+)
Myeloid progeny (13+)
Erythroid progeny (235+)
d
Fold Repression
32
16
8
4
2
1
0.5
126T (4 targets)
126T/130aT 2/2 combi
126T (2 targets)
Stem/multipotent
Progenitors
Myelocytes
Metamyelocytes
Granulocytes
Regulation Index
20
15
10
5
0
126T (4 targets)
126T/130aT 2/2 combi
126T (2 targets)

FIG. 8 CONT'D

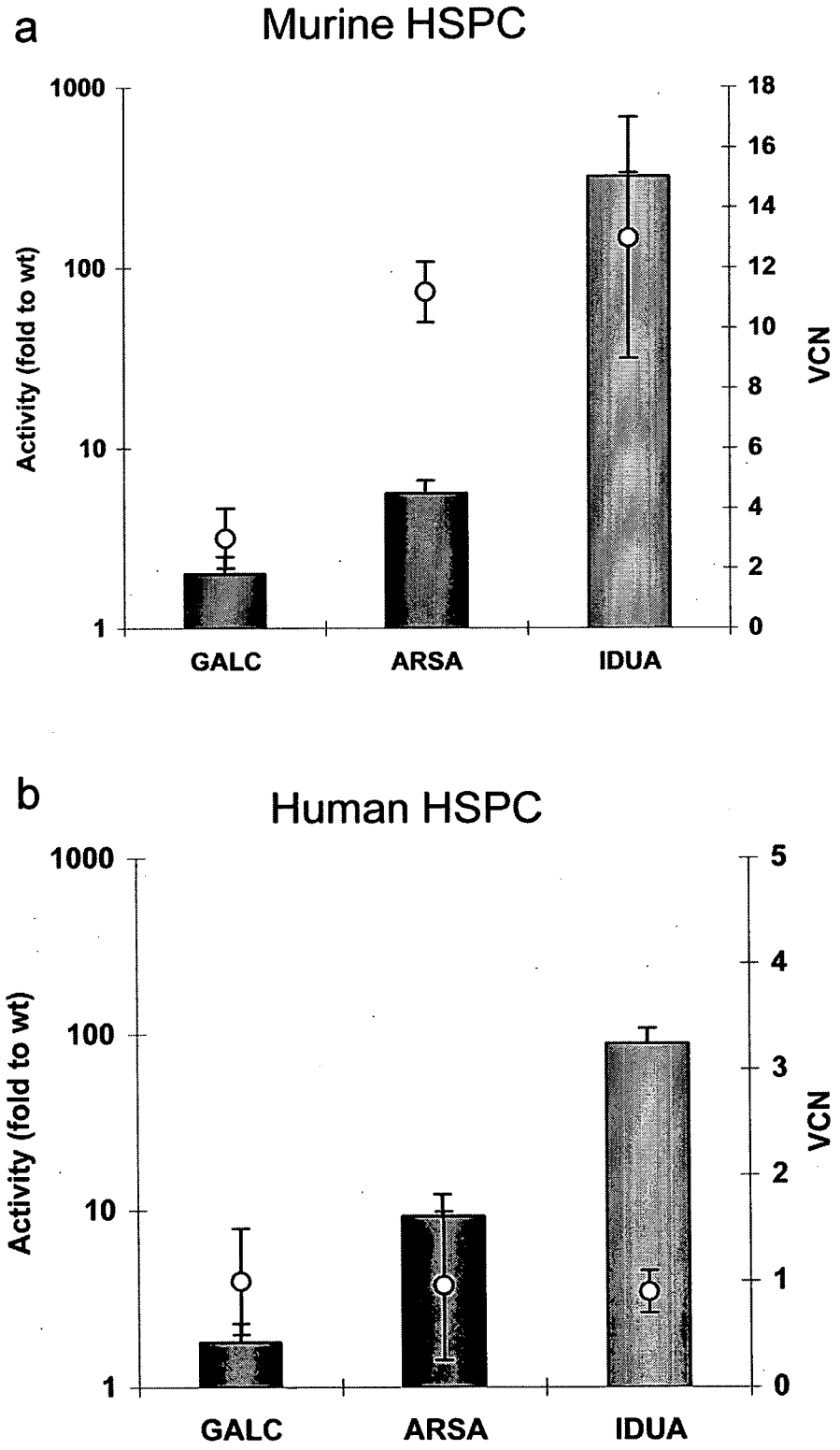

a
Murine HSPC
Activity (fold to wt)
1000
100
10
1
VCN
18
16
14
12
10
8
6
4
2
0
GALC
ARSA
IDUA
b
Human HSPC
Activity (fold to wt)
1000
100
10
1
VCN
5
4
3
2
1
0
GALC
ARSA
IDUA

FIG. 9

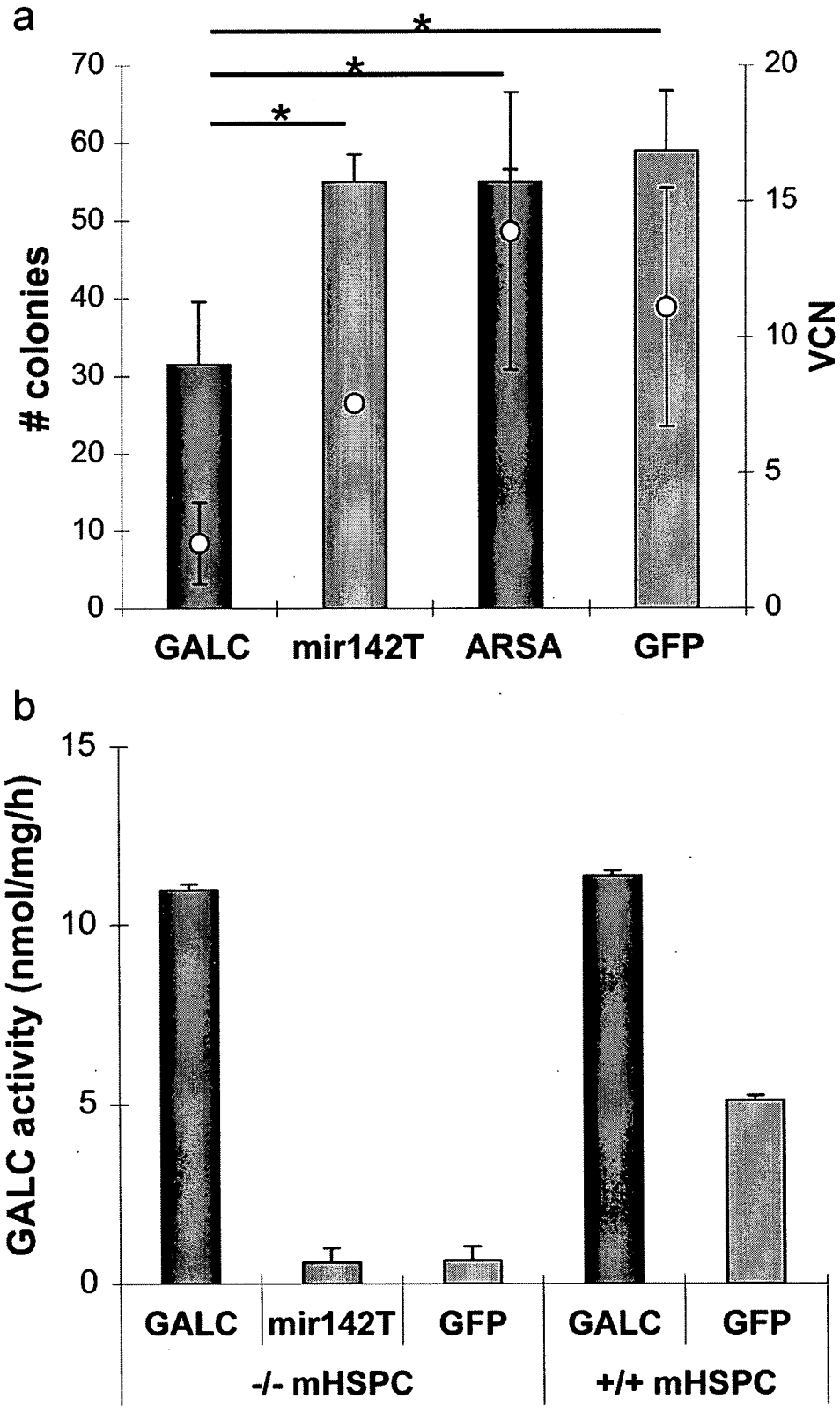

a
*
*
*
70
60
50
40
30
20
10
0
colonies
20
15
10
5
0
VCN
GALC
mir142T
ARSA
GFP
b
15
10
5
0
GALC activity (nmol/mg/h)
GALC
mir142T
GFP
GALC
GFP
-/- mHSPC
+/+ mHSPC

FIG. 10

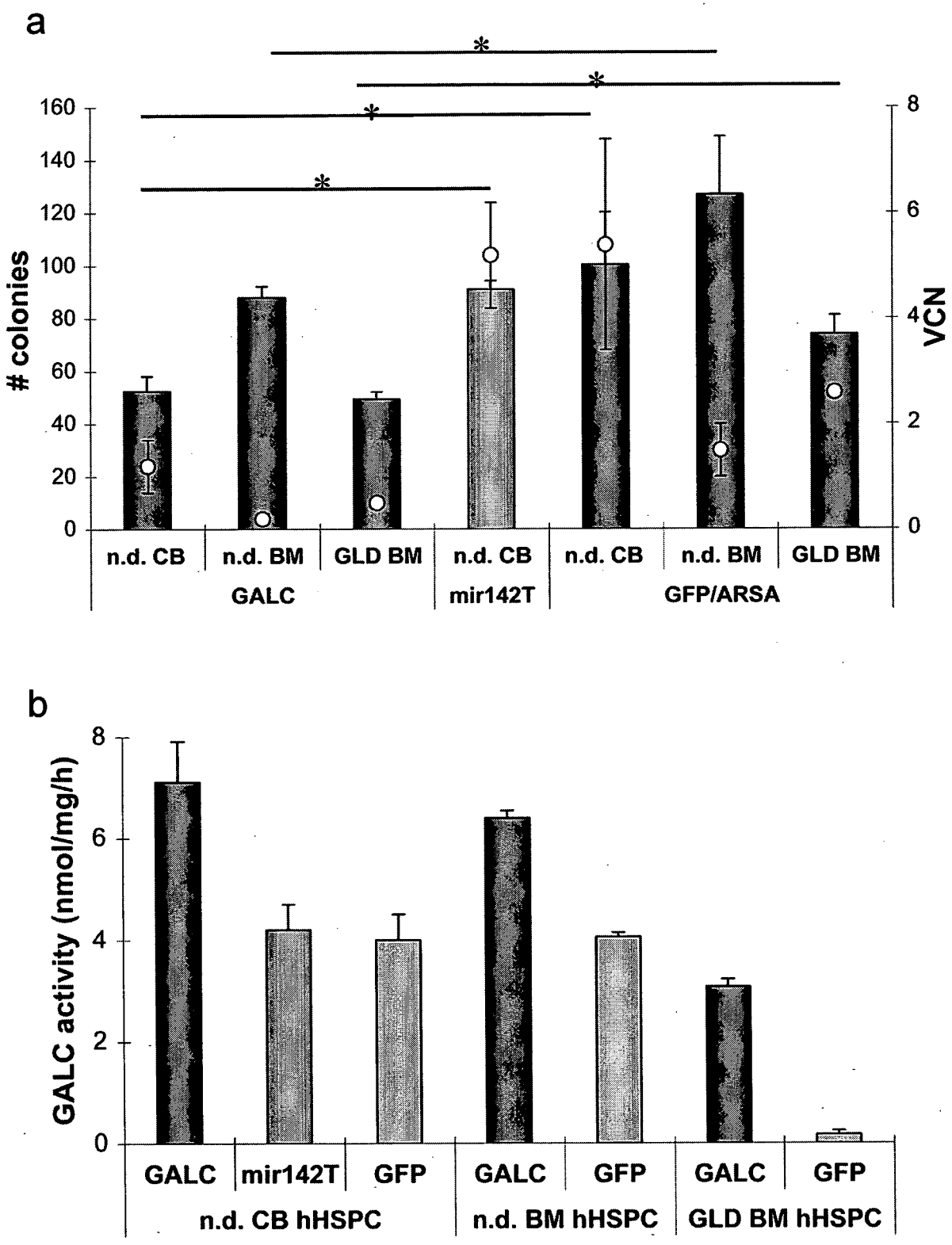
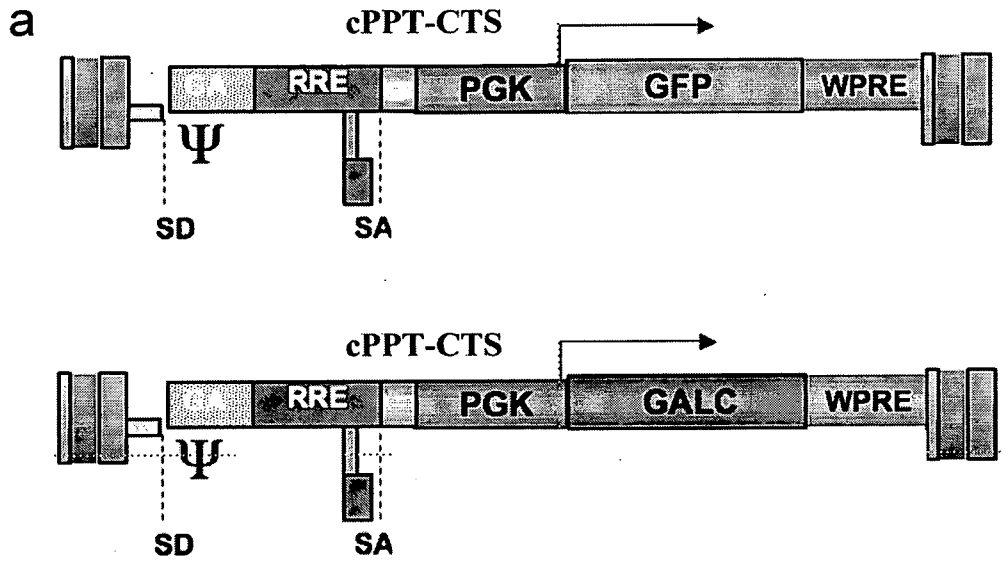
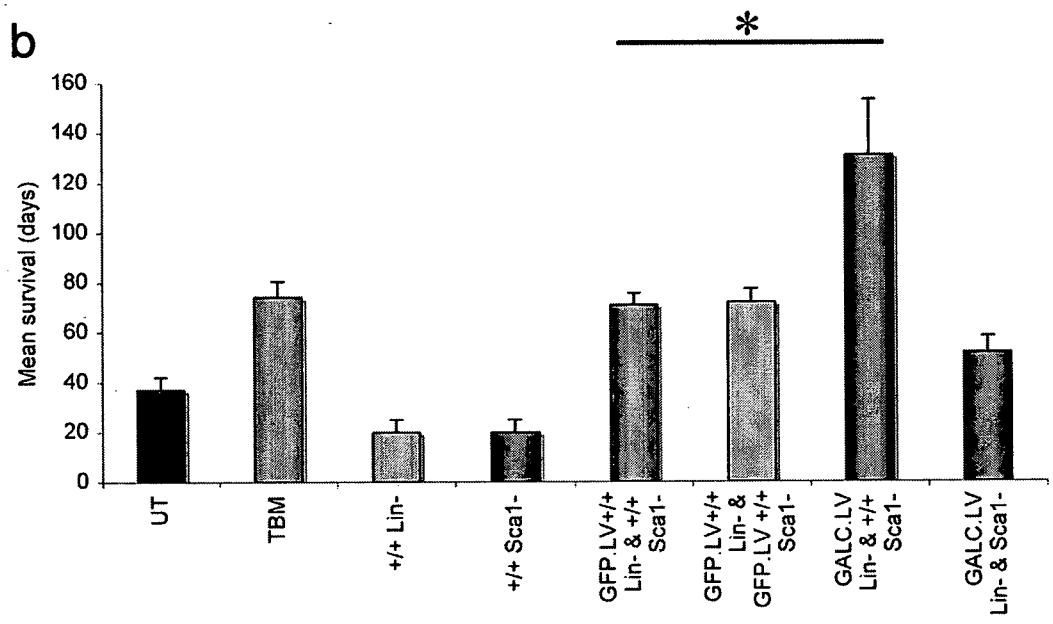

a
colonies
VCN
160
140
120
100
80
60
40
20
0
8
6
4
2
0
*
*
*
*
n.d. CB
n.d. BM
GLD BM
n.d. CB
n.d. CB
n.d. BM
GLD BM
GALC
mir142T
GFP/ARSA
b
GALC activity (nmol/mg/h)
8
6
4
2
0
GALC
mir142T
GFP
GALC
GFP
GALC
GFP
n.d. CB hHSPC
n.d. BM hHSPC
GLD BM hHSPC

FIG. 11


a
cPPT-CTS
RRE
PGK
GFP
WPRE
Ψ
SD
SA
cPPT-CTS
RRE
PGK
GALC
WPRE
Ψ
SD
SA


b
*
Mean survival (days)
160
140
120
100
80
60
40
20
0
UT
TBM
+/+ Lin-
+/+ Sca1-
GFP.LV+/+ Lin- & +/+ Sca1-
GFP.LV+/+ Lin- & GFP.LV +/+ Sca1-
GALC.LV Lin- & +/+ Sca1-
GALC.LV Lin- & Sca1-

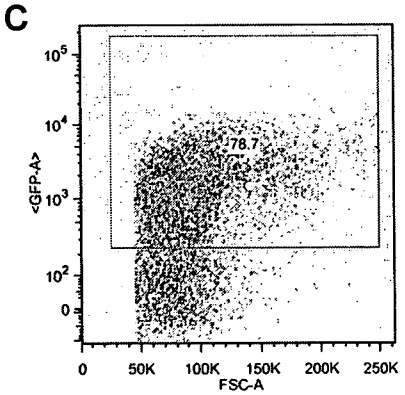

c
<GFP-A>
78.7
FSC-A
0
50K
100K
150K
200K
250K

FIG. 12

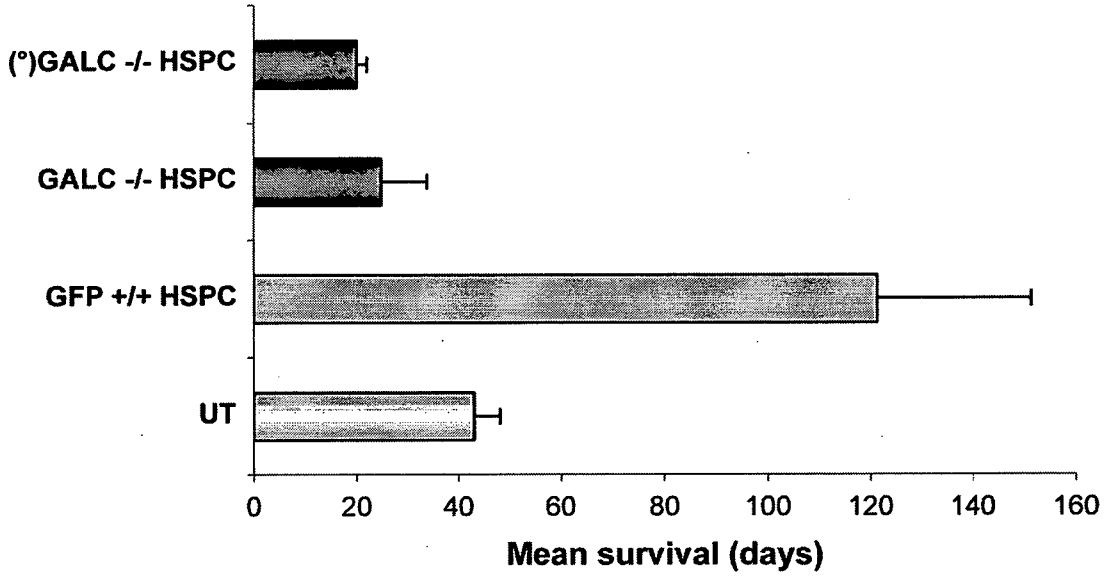
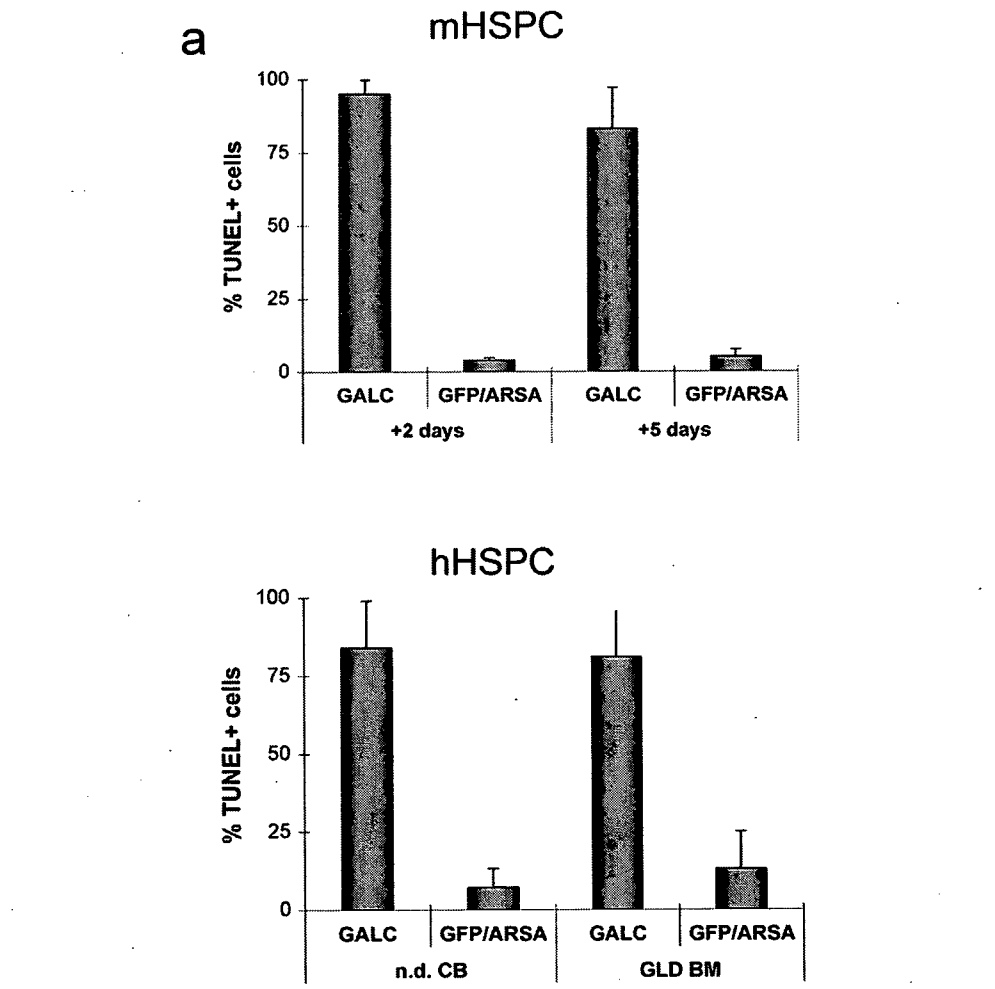


FIG. 13

Transplanted cells	Host	VCN (20 days)	VCN (≥120 days)
(°)GALC -/- HSPC (§)	+/-	0.01±0	-
GALC -/- HSPC (§)	-/-	0.02±0	-
GALC -/- HSPC (§)	-/-	2.3±1	2±0.5

Table 2


a
mHSPC
% TUNEL+ cells
100
75
50
25
0
GALC
GFP/ARSA
GALC
GFP/ARSA
+2 days
+5 days
hHSPC
% TUNEL+ cells
100
75
50
25
0
GALC
GFP/ARSA
GALC
GFP/ARSA
n.d. CB
GLD BM

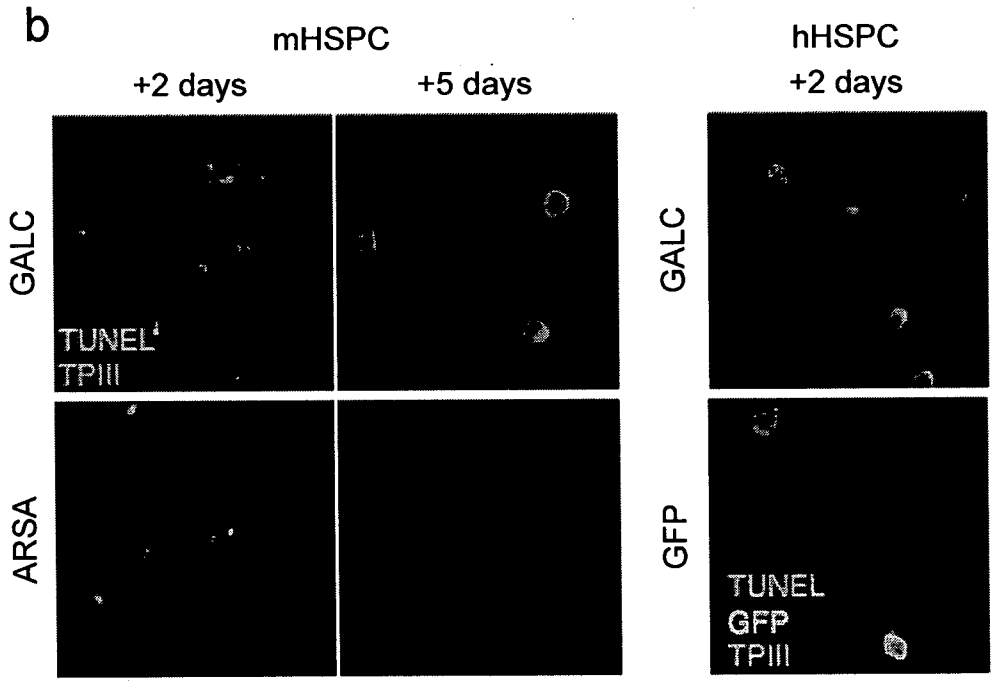

b
mHSPC
+2 days
+5 days
hHSPC
+2 days
GALC
TUNEL
TPIII
ARSA
GALC
GFP
TUNEL
GFP
TPIII

FIG. 13 CONT'D

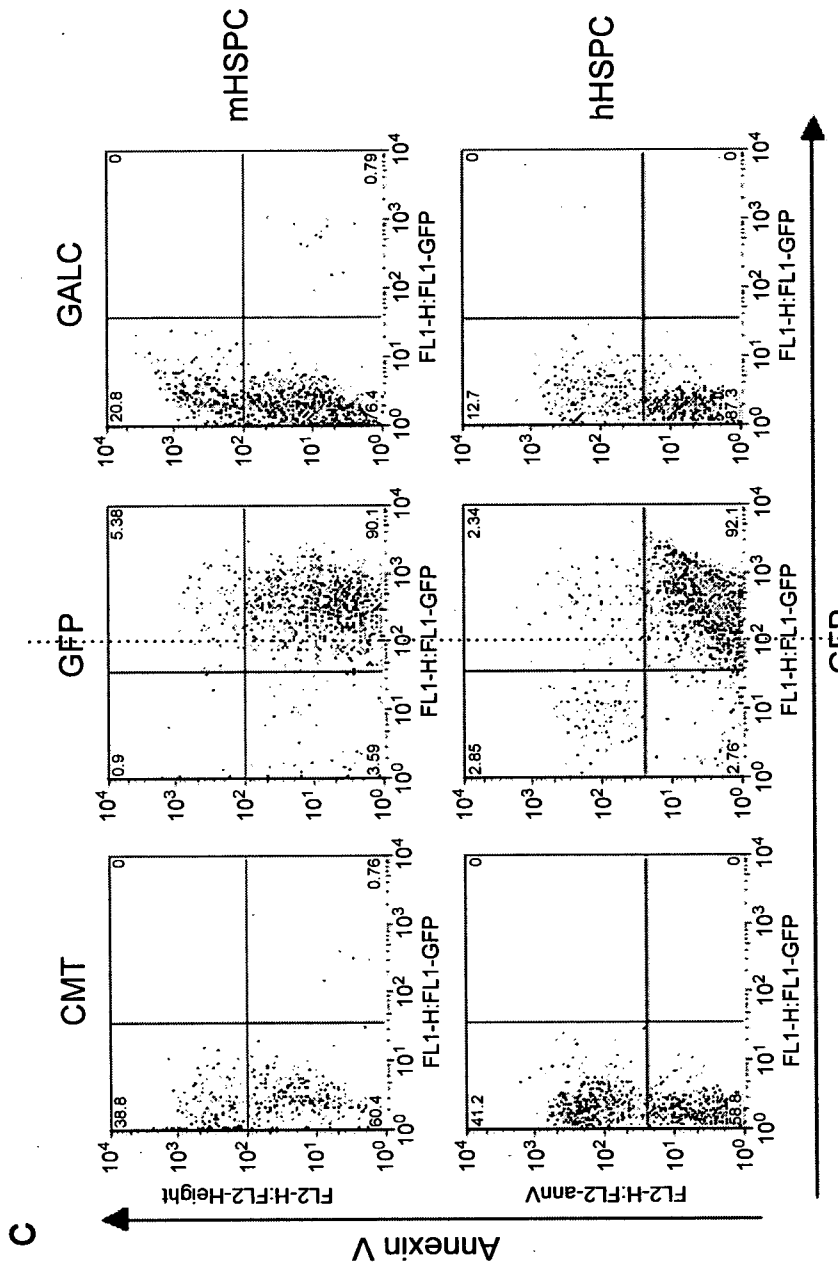

C
Annexin V
FL2-H:FL2-Height
FL2-H:FL2-annV
GFP
FL1-H:FL1-GFP
CMT
GALC
mHSPC
hHSPC
38.8
60.4
0.76
41.2
58.8
0.9
3.59
5.38
90.1
2.85
2.76
2.34
92.1
20.8
76.4
0.79
12.7
87.3

FIG. 14

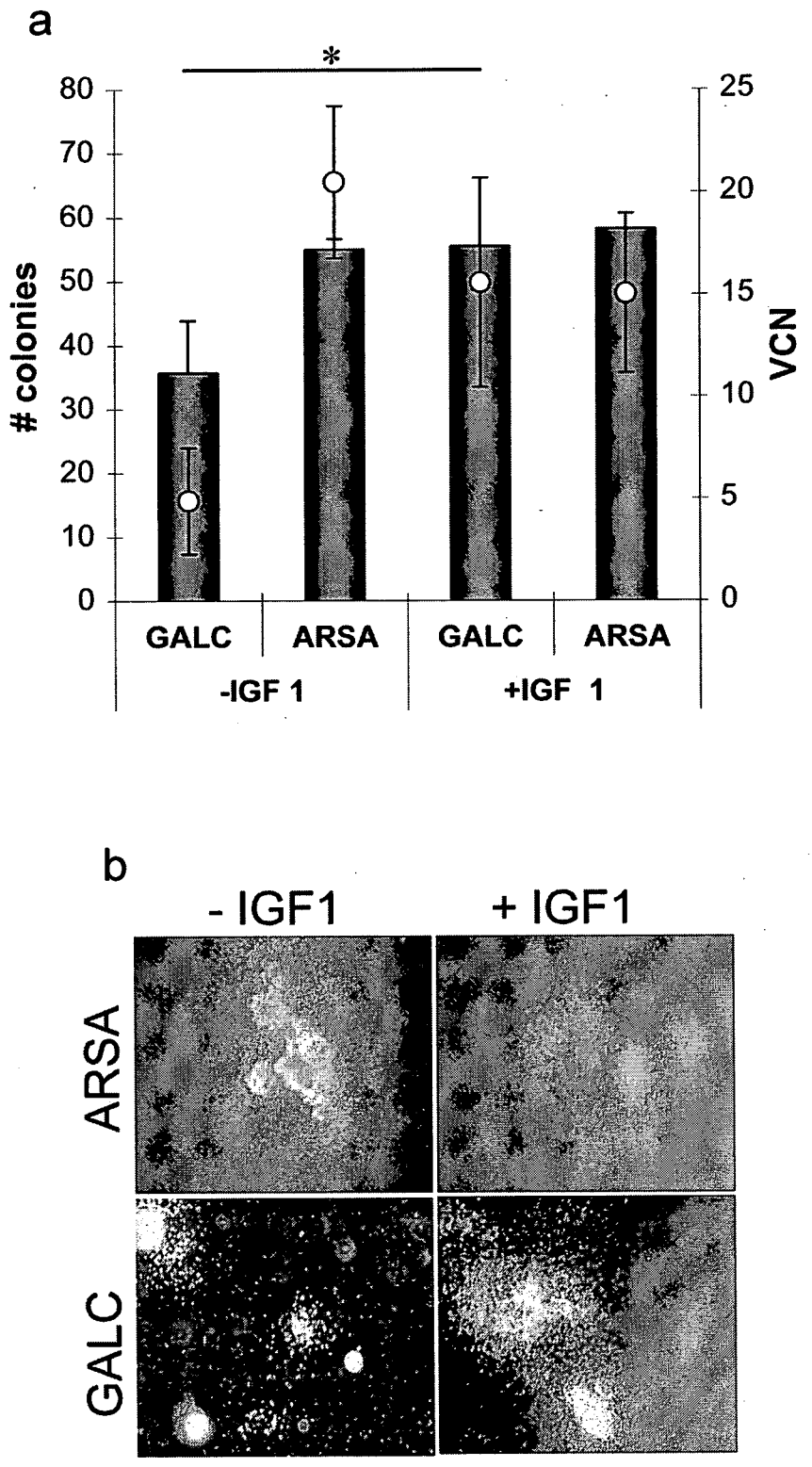

a
*
80
70
60
50
40
30
20
10
0
colonies
25
20
15
10
5
0
VCN
GALC
ARSA
GALC
ARSA
-IGF 1
+IGF 1
b
- IGF1
+ IGF1
ARSA
GALC

FIG. 14 CONT'D

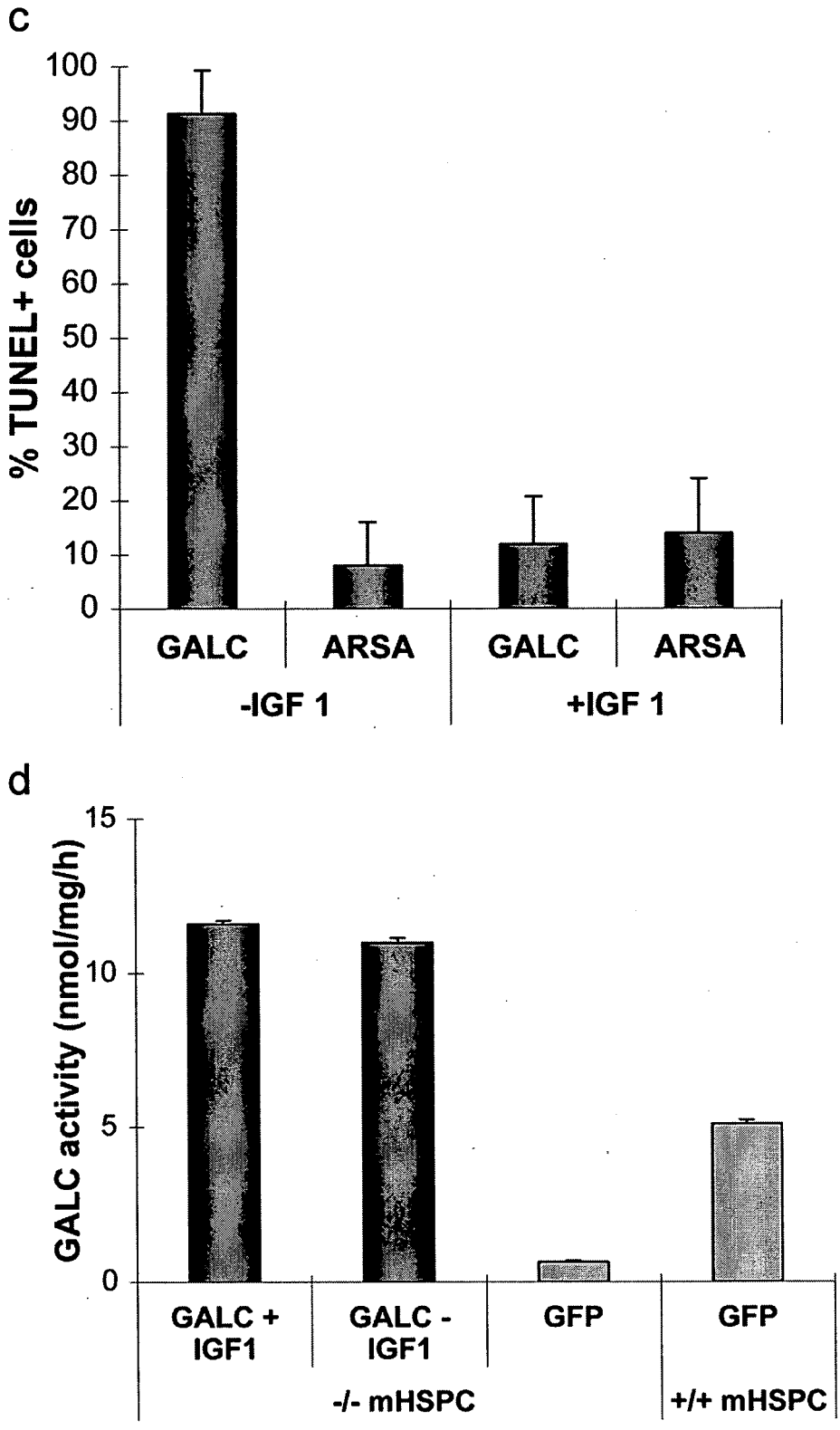

c
% TUNEL+ cells
100
90
80
70
60
50
40
30
20
10
0
GALC
ARSA
GALC
ARSA
-IGF 1
+IGF 1
d
GALC activity (nmol/mg/h)
15
10
5
0
GALC + IGF1
GALC - IGF1
GFP
GFP
-/- mHSPC
+/+ mHSPC

FIG. 15

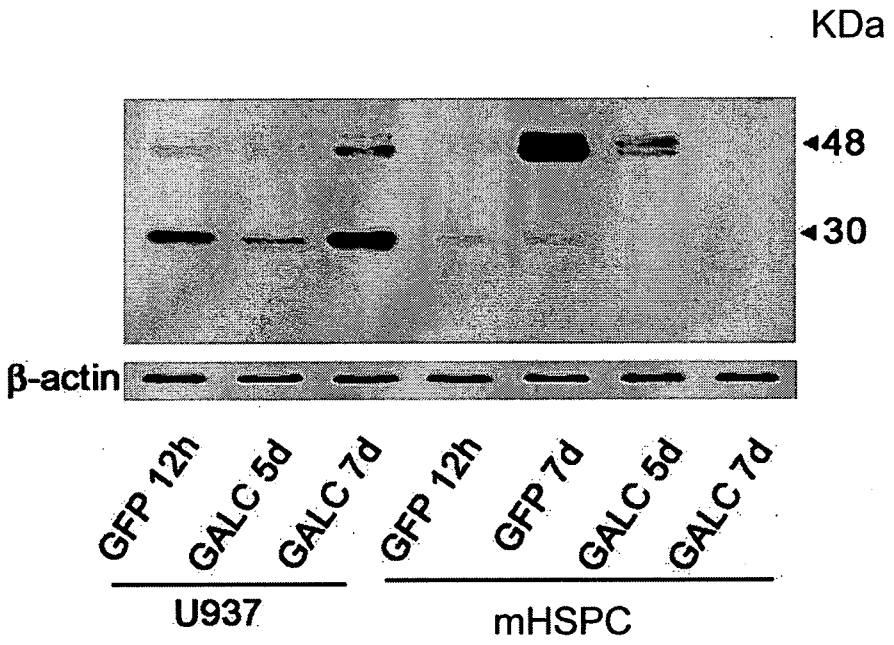

KDa
48
30
β-actin
GFP 12h
GALC 5d
GALC 7d
GFP 12h
GFP 7d
GALC 5d
GALC 7d
U937
mHSPC

FIG. 16

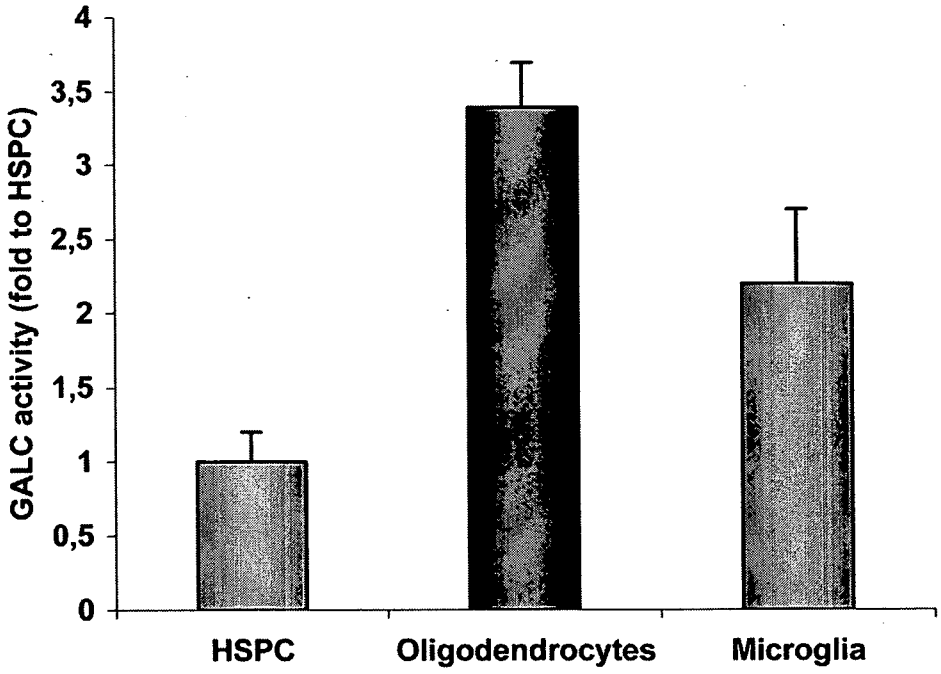

GALC activity (fold to HSPC)
4
3,5
3
2,5
2
1,5
1
0,5
0
HSPC
Oligodendrocytes
Microglia

FIG. 17

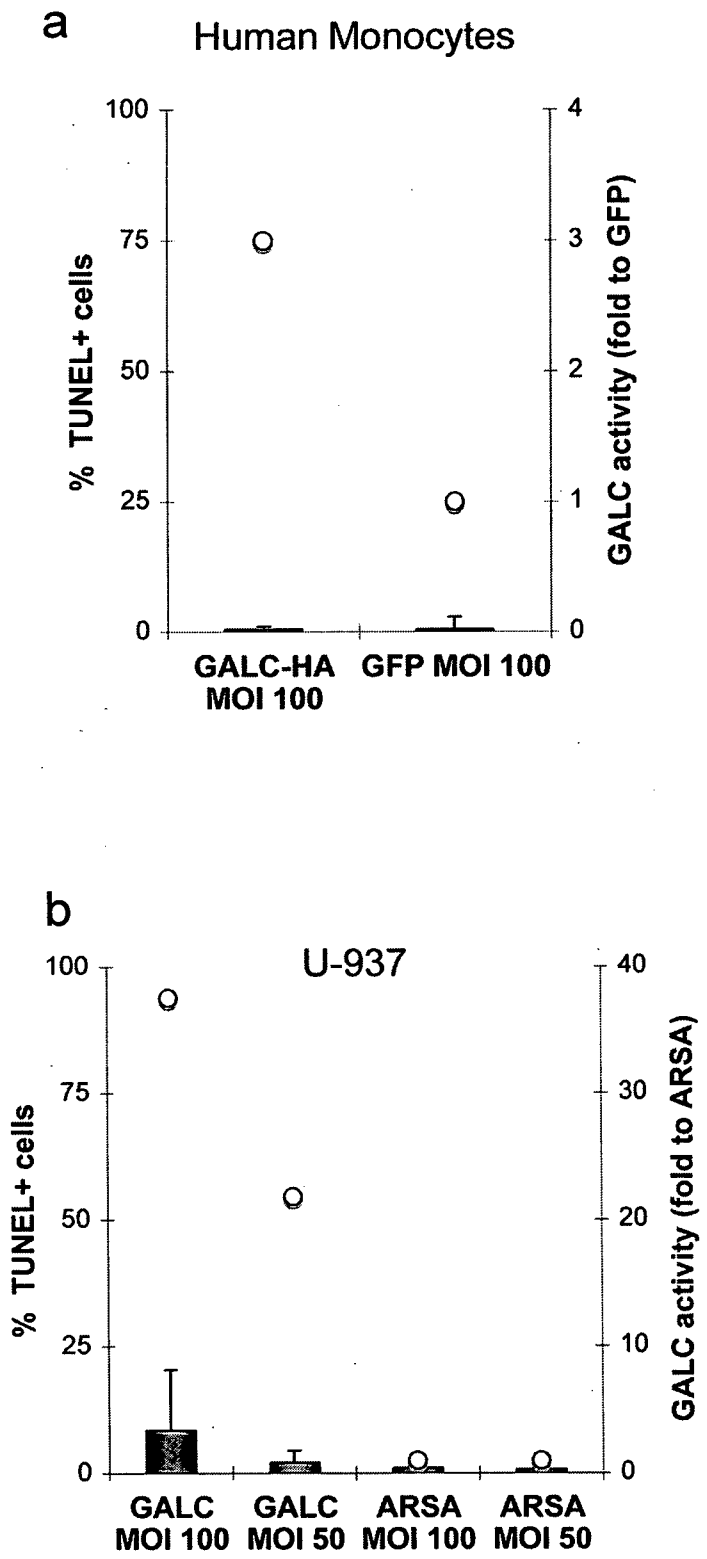
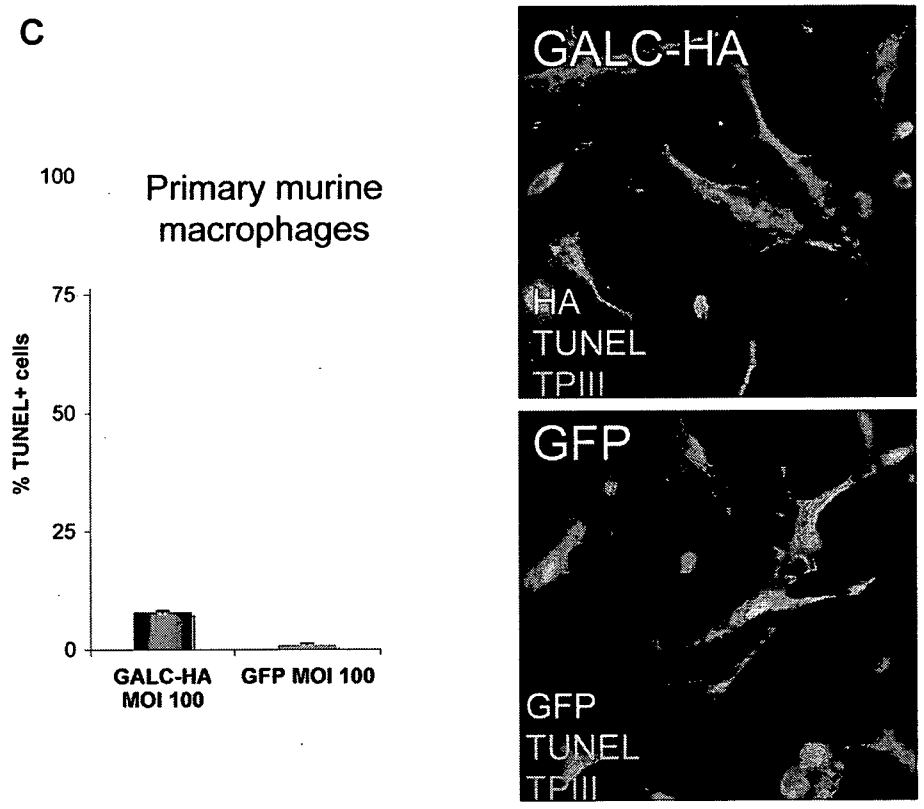

a
Human Monocytes
% TUNEL+ cells
GALC activity (fold to GFP)
100
75
50
25
0
4
3
2
1
0
GALC-HA MOI 100
GFP MOI 100
b
U-937
% TUNEL+ cells
GALC activity (fold to ARSA)
100
75
50
25
0
40
30
20
10
0
GALC MOI 100
GALC MOI 50
ARSA MOI 100
ARSA MOI 50

FIG. 18


c
Primary murine macrophages
% TUNEL+ cells
100
75
50
25
0
GALC-HA MOI 100
GFP MOI 100
GALC-HA
HA
TUNEL
TPIII
GFP
GFP
TUNEL
TPIII

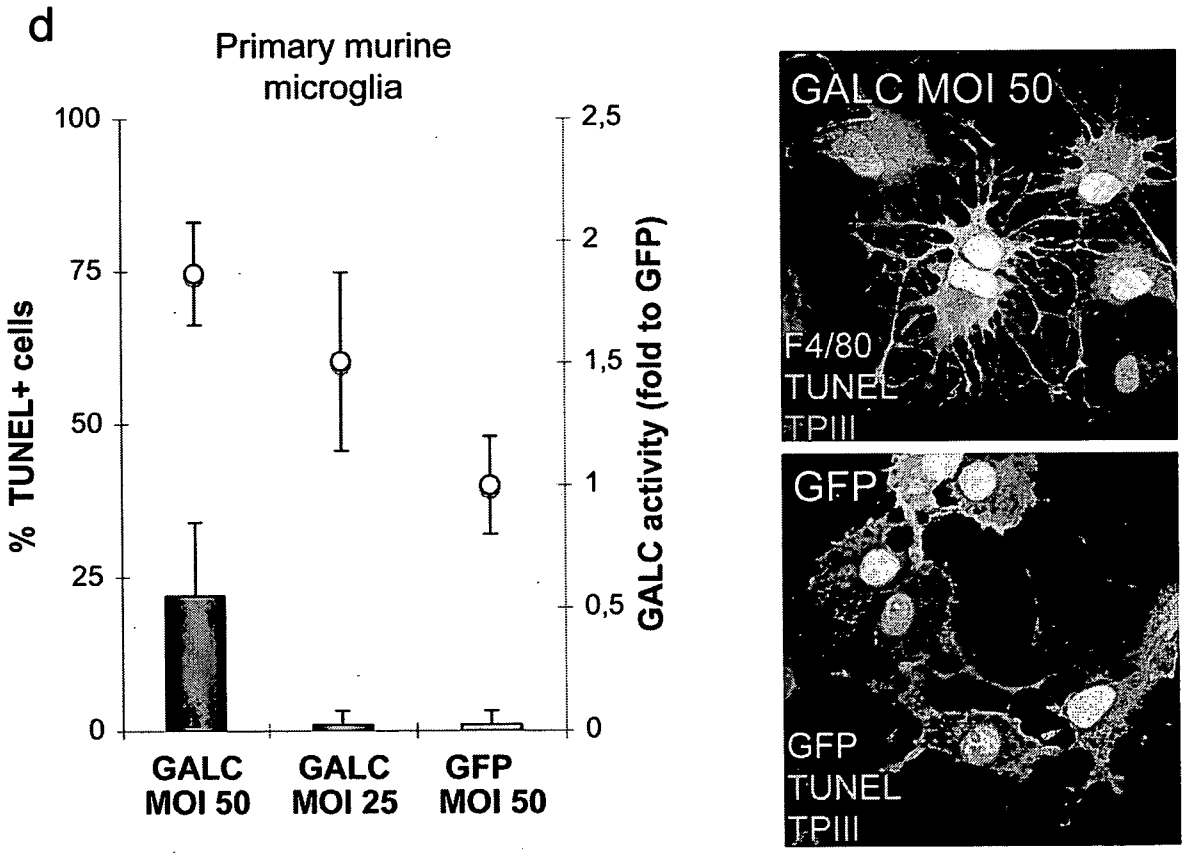

d
Primary murine microglia
% TUNEL+ cells
GALC activity (fold to GFP)
100
75
50
25
0
2,5
2
1,5
1
0,5
0
GALC MOI 50
GALC MOI 25
GFP MOI 50
GALC MOI 50
F4/80
TUNEL
TPIII
GFP
GFP
TUNEL
TPIII

FIG. 18 CONT'D

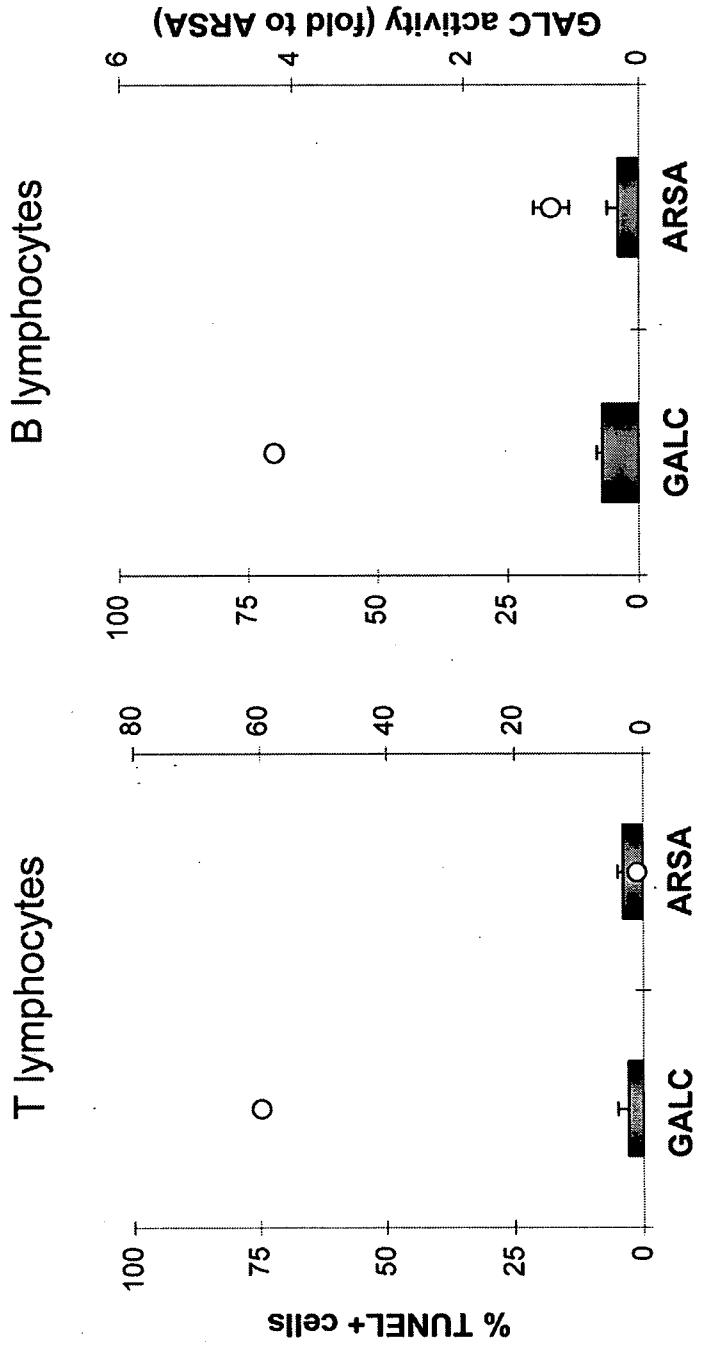

% TUNEL+ cells
100
75
50
25
0
GALC
ARSA
T lymphocytes
80
60
40
20
0
GALC
ARSA
B lymphocytes
GALC activity (fold to ARSA)
6
4
2
0

FIG. 19

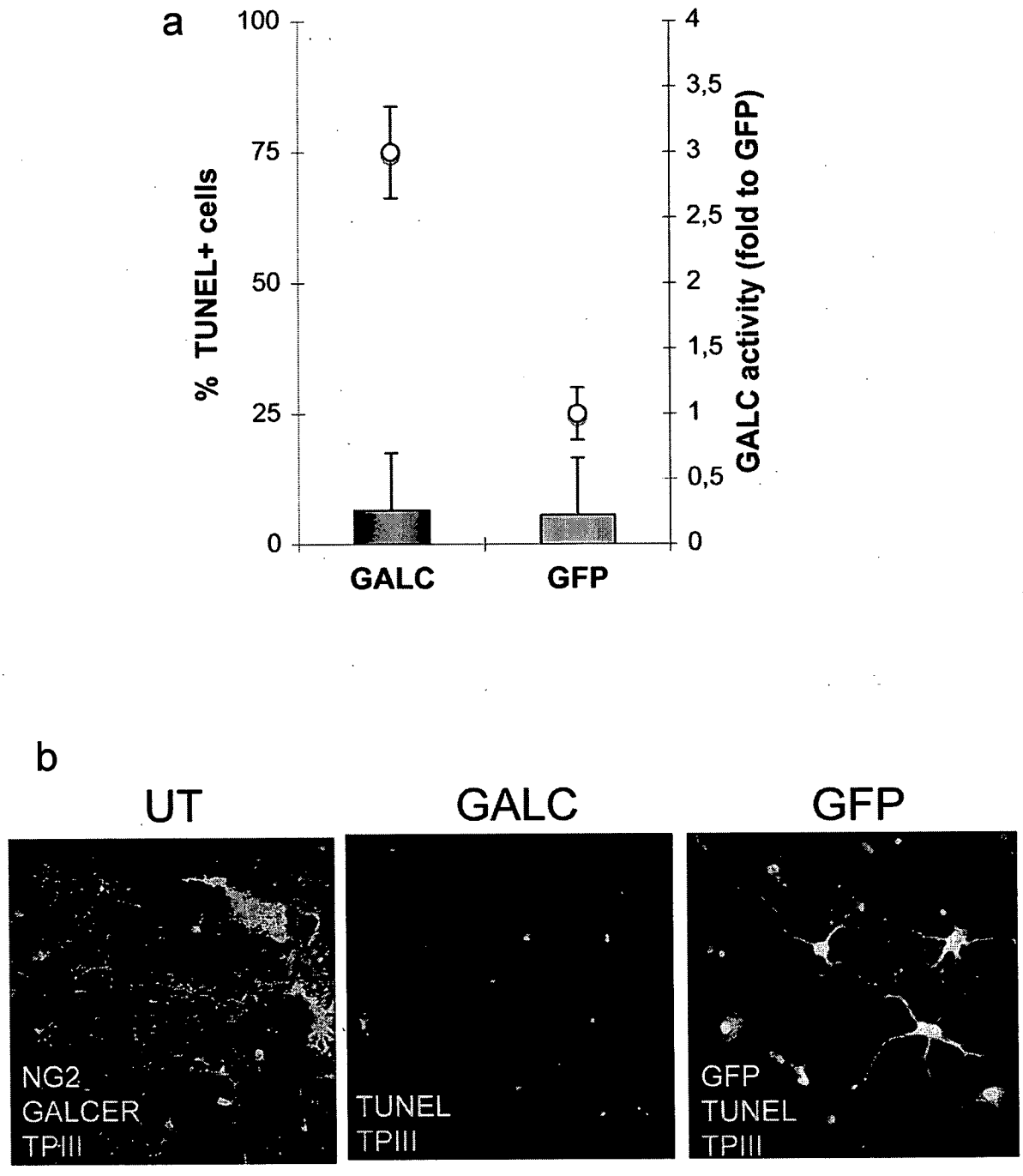
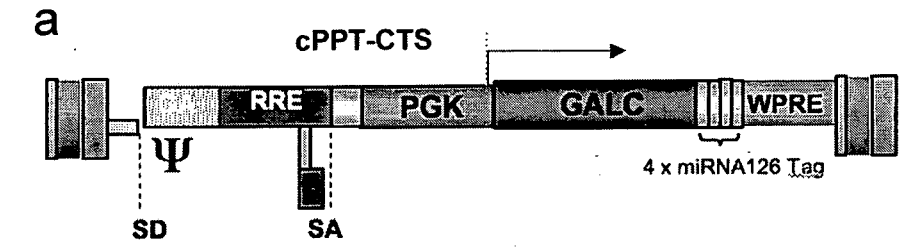
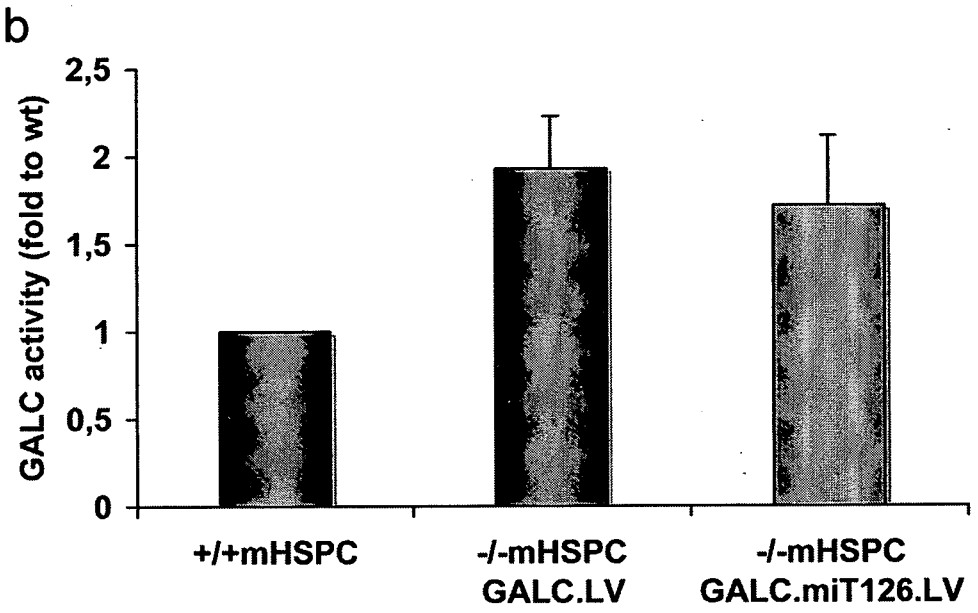

a
100
75
50
25
0
% TUNEL+ cells
4
3,5
3
2,5
2
1,5
1
0,5
0
GALC activity (fold to GFP)
GALC
GFP
b
UT
GALC
GFP
NG2
GALCER
TPIII
TUNEL
TPIII
GFP
TUNEL
TPIII

FIG. 20


a
cPPT-CTS
RRE
PGK
GALC
WPRE
Ψ
4 x miRNA126 Tag
SD
SA


b
GALC activity (fold to wt)
2,5
2
1,5
1
0,5
0
+/+mHSPC
-/-mHSPC GALC.LV
-/-mHSPC GALC.miT126.LV

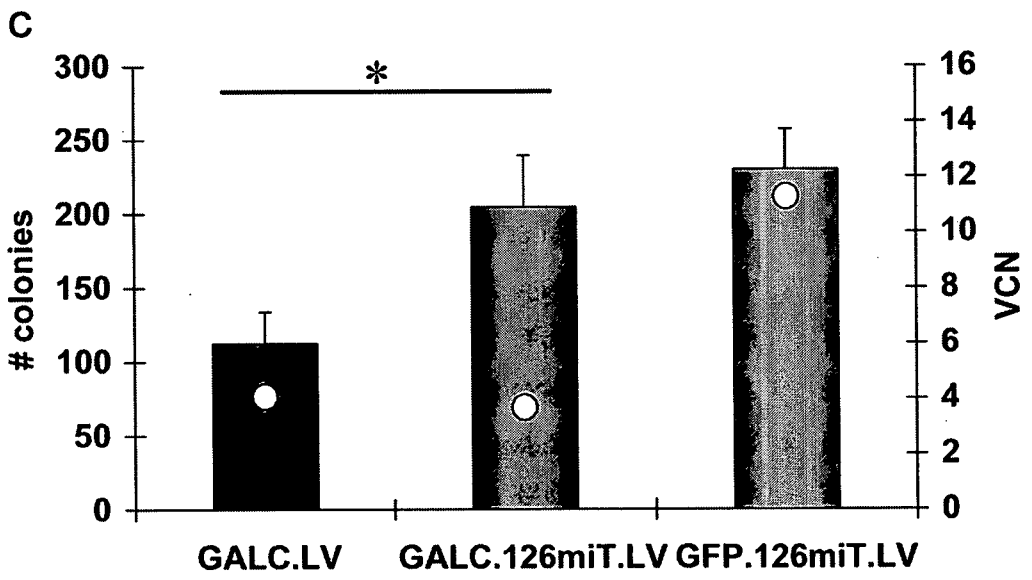

c
*
colonies
300
250
200
150
100
50
0
VCN
16
14
12
10
8
6
4
2
0
GALC.LV
GALC.126miT.LV
GFP.126miT.LV

FIG. 21

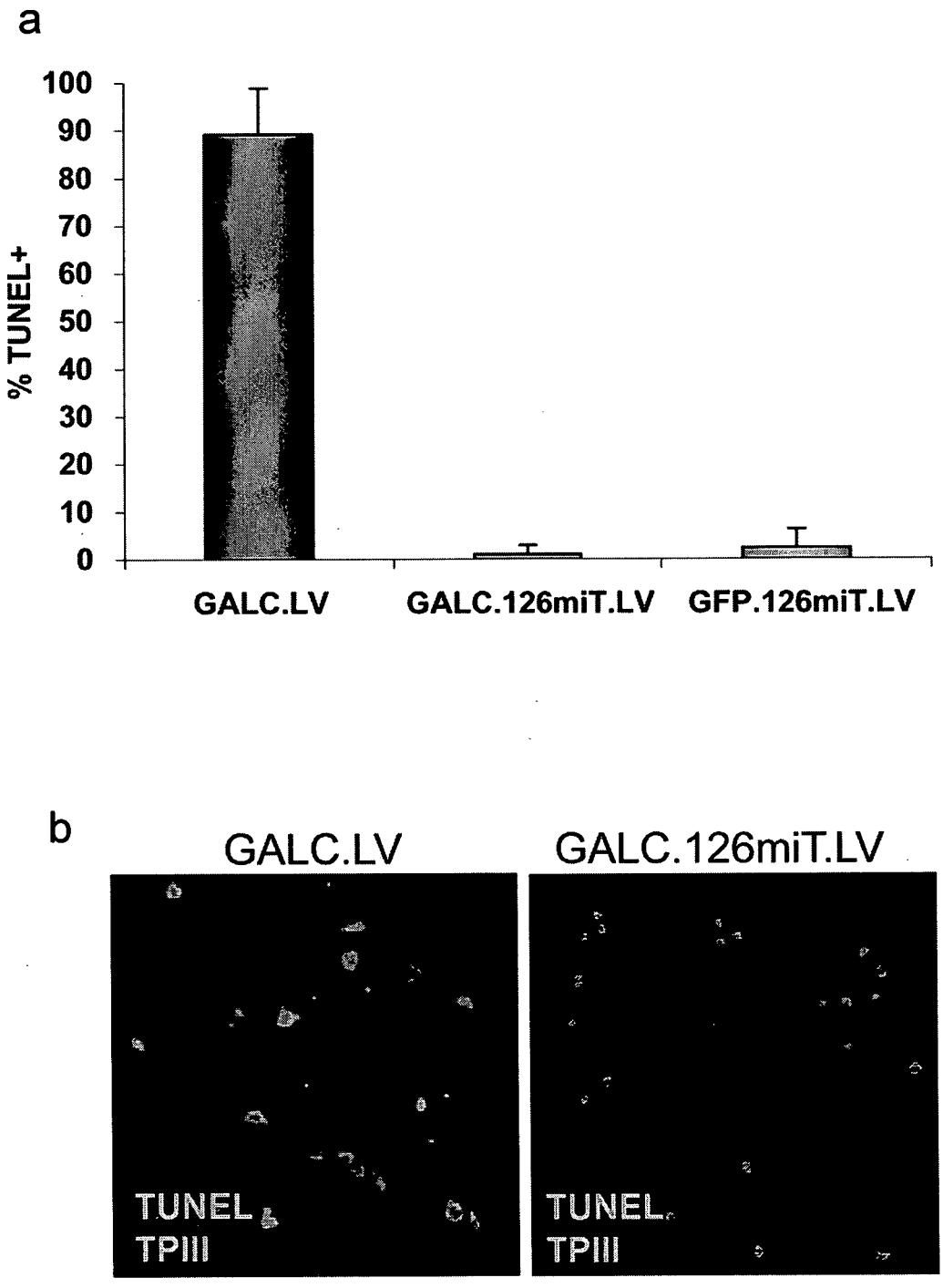

a
100
90
80
70
60
50
40
30
20
10
0
% TUNEL+
GALC.LV
GALC.126miT.LV
GFP.126miT.LV
b
GALC.LV
GALC.126miT.LV
TUNEL
TPIII
TUNEL
TPIII

FIG. 22

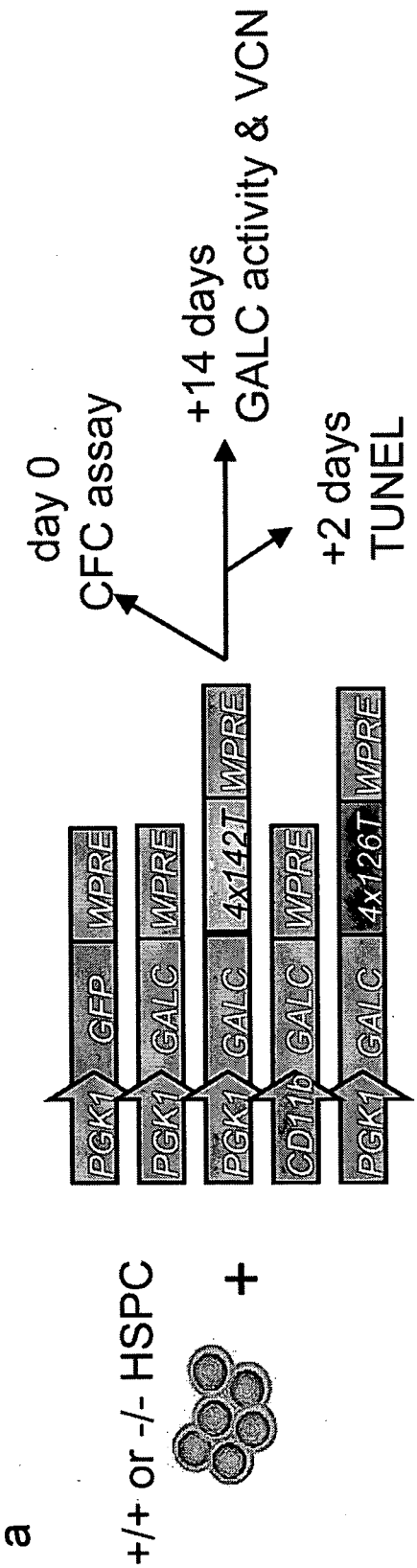

a
+/+ or -/- HSPC
+
day 0
CFC assay
+14 days
GALC activity & VCN
+2 days
TUNEL
PGK1
GFP
WPRE
PGK1
GALC
WPRE
PGK1
GALC
4x142T
WPRE
CD11b
GALC
WPRE
PGK1
GALC
4x126T
WPRE

FIG. 23

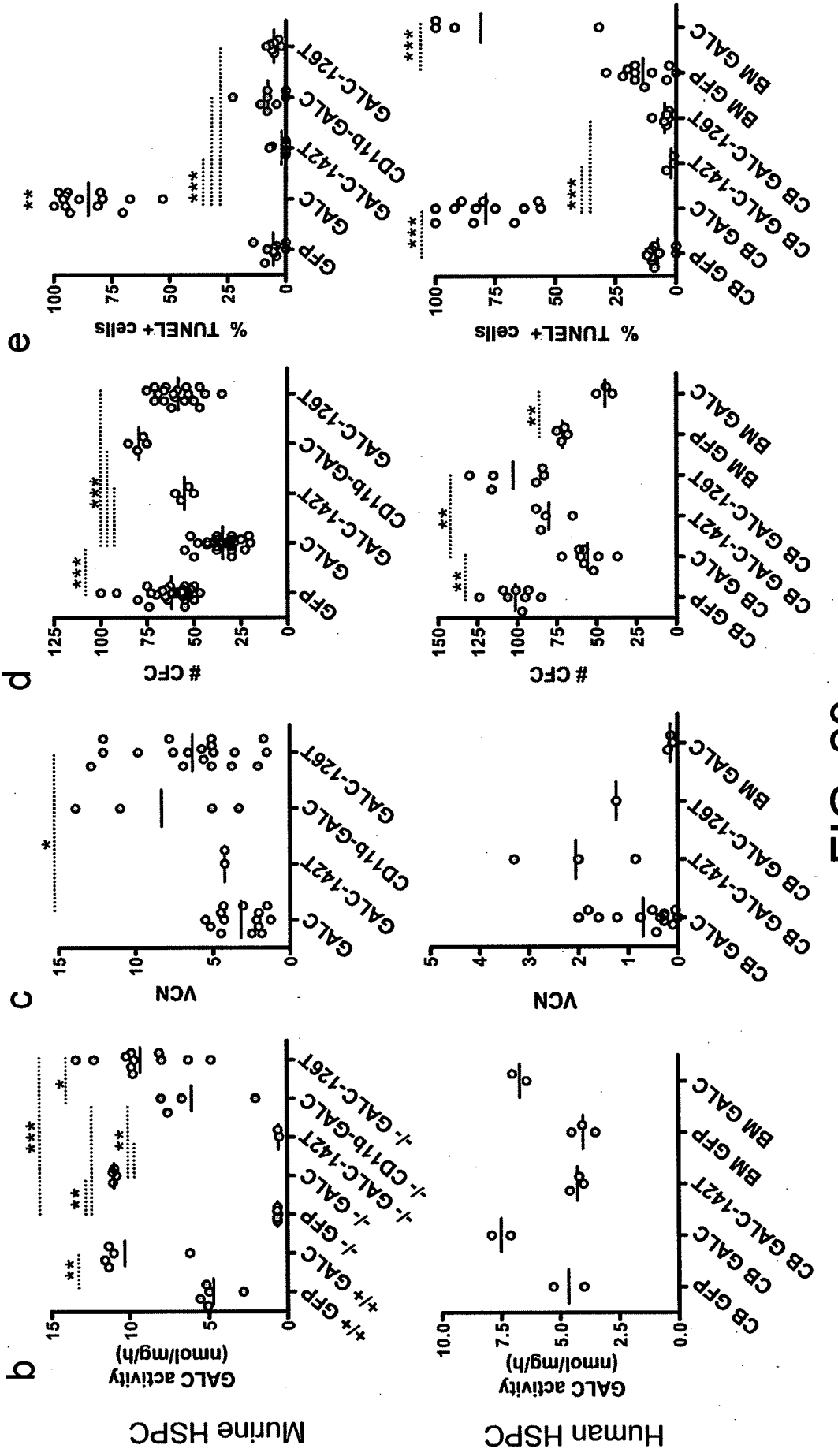

Murine HSPC
Human HSPC
b
GALC activity (nmol/mg/h)
+/+ GFP
+/+ GALC
-/- GFP
-/- GALC
-/- CD11b-GALC
-/- GALC-142T
-/- GALC-126T
c
VCN
GFP
GALC
GALC-142T
CD11b-GALC
GALC-126T
CB GFP
CB GALC
CB GALC-142T
CB GALC-126T
BM GFP
BM GALC
d
CFC
e
% TUNEL+ cells

FIG. 23 CONT'D

f



FIG. 23 CONT'D

a

Transplanted HSPC		*Recipient*	*Survival*	*Donor cell engraftment*
Genotype	*Vector*	*Genotype*	*Days*	*%GFP+ cells / VCN*
n.a.	none	-/- (*)	51±7	n.a.
+/+	GFP LV	-/-	63±22	68±16%
-/- (§)	GALC LV	-/-	21±6	0.01±0
-/-	GALC-126T LV	-/-	90±39	5±2.3

FIG. 24

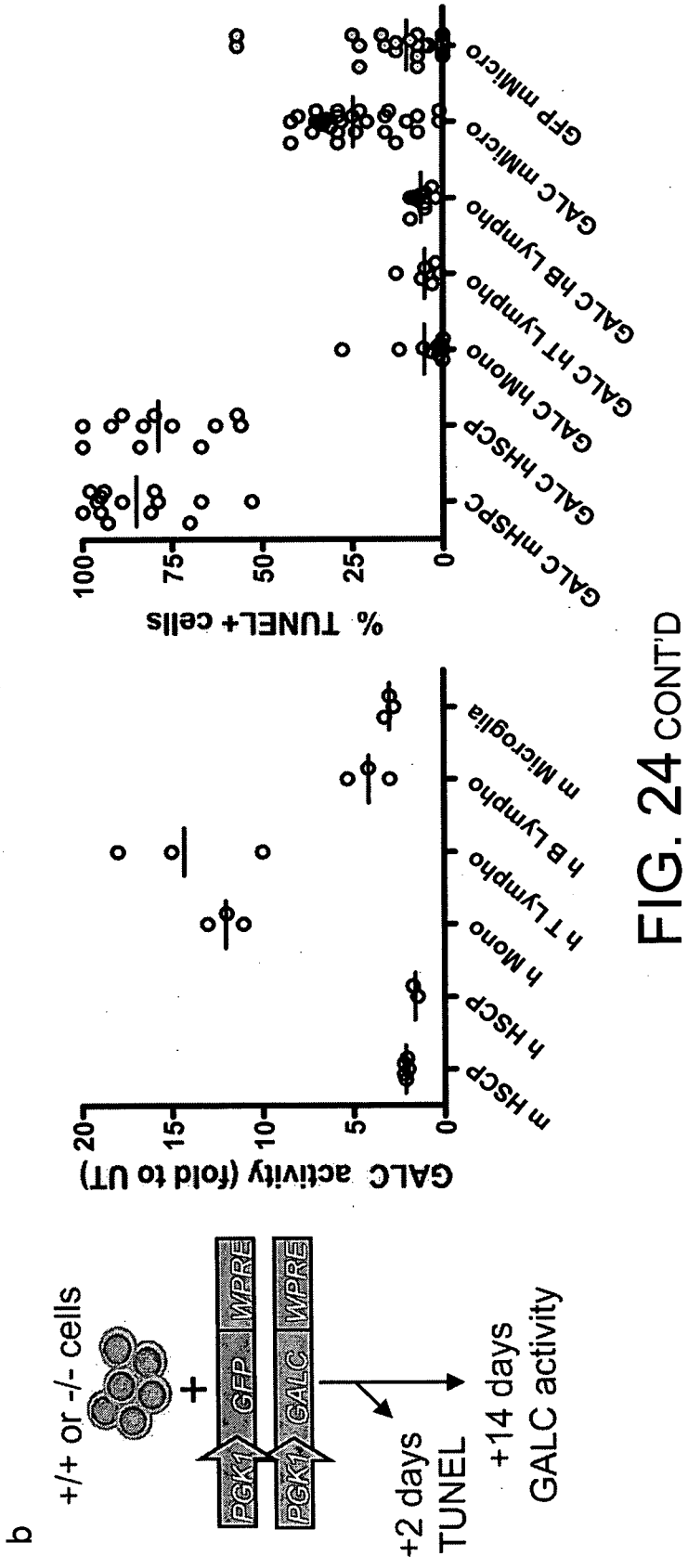

b
+/+ or -/- cells
+
PGK1
GFP
WPRE
PGK1
GALC
WPRE
+2 days
TUNEL
+14 days
GALC activity
GALC activity (fold to UT)
20
15
10
5
0
m HSCP
h HSCP
h Mono
h T Lympho
h B Lympho
m Microglia
% TUNEL+ cells
100
75
50
25
0
GALC mHSPC
GALC hHSCP
GALC hMono
GALC hT Lympho
GALC hB Lympho
GALC mMicro
GFP mMicro

FIG. 24 CONT'D

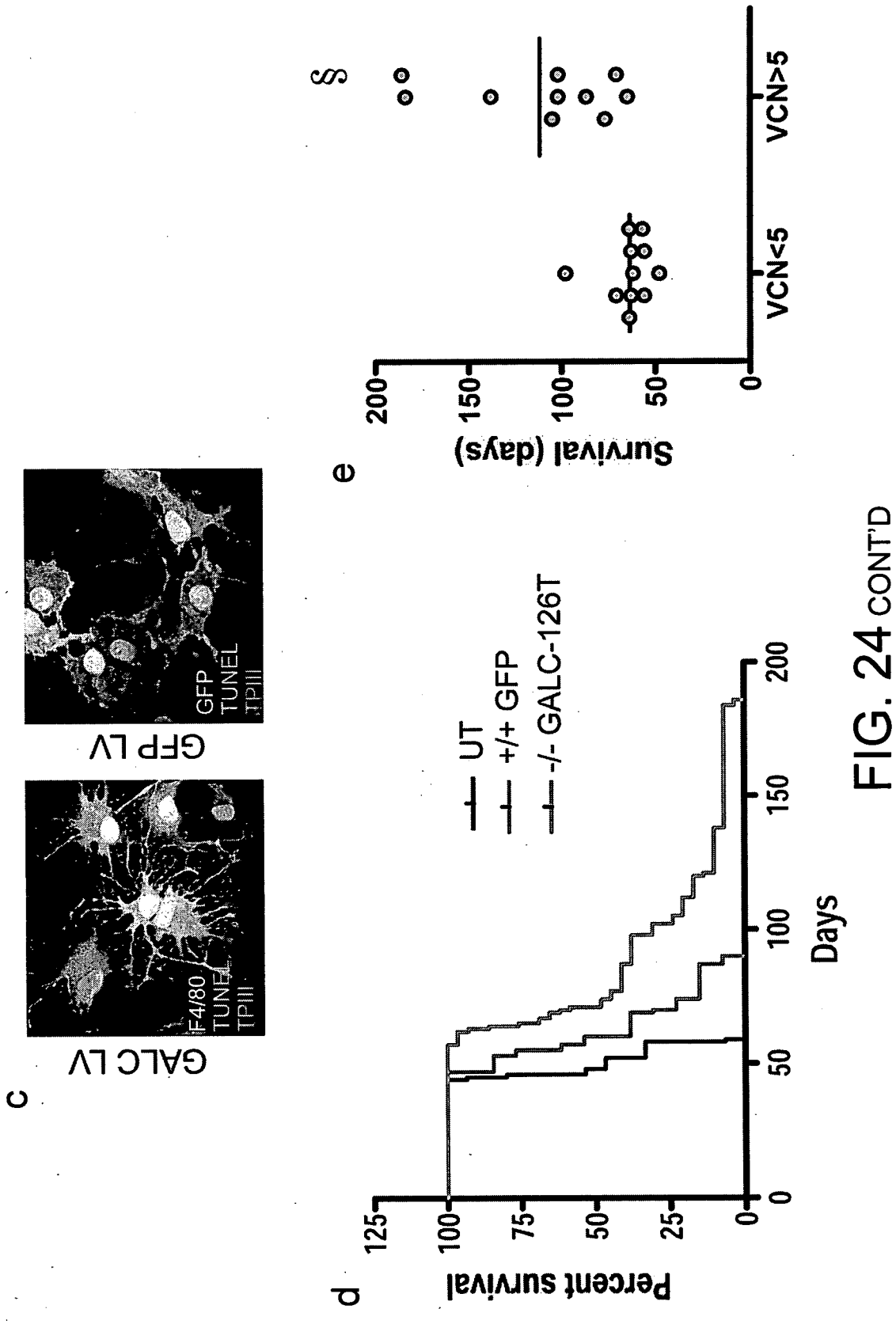

c
GALC LV
F4/80
TUNEL
TPIII
GFP LV
GFP
TUNEL
TPIII
d
Percent survival
125
100
75
50
25
0
0
50
100
150
200
Days
UT
+/+ GFP
-/- GALC-126T
e
Survival (days)
200
150
100
50
0
VCN<5
VCN>5
§

FIG. 24 CONT'D