Figure 1.

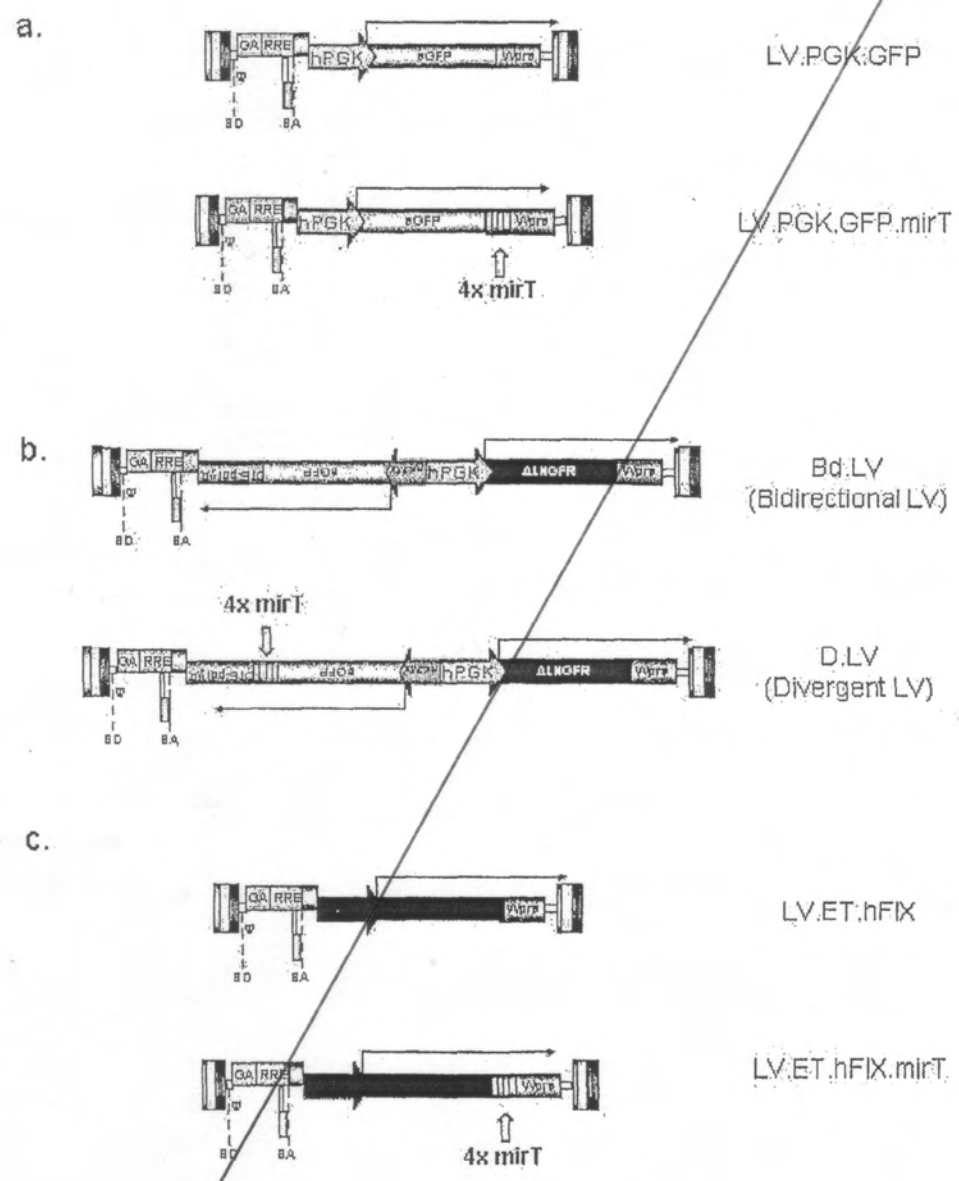
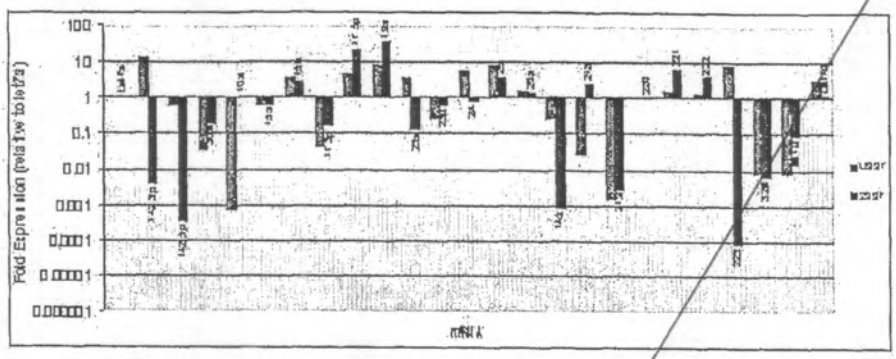
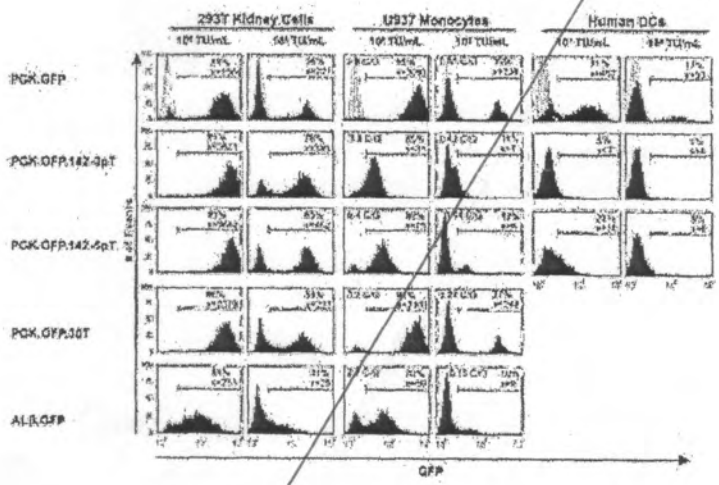

a.
GA
RRE
hPGK
eGFP
WPRE
BD
BA
LV.PGK.GFP
LV.PGK.GFP.mirT
4x mirT
b.
hPGK
ΔLNGFR
Bd.LV
(Bidirectional LV)
4x mirT
D.LV
(Divergent LV)
c.
LV.ET.hFIX
LV.ET.hFIX.mirT

Figure 2.

a.


Fold Expression (relative to let-7a)
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
miRNA

b.


293T Kidney Cells
U937 Monocytes
Human DCs
PGK.GFP
PGK.GFP.142-3pT
PGK.GFP.142-5pT
PGK.GFP.3GT
ALB.GFP
GFP

c.

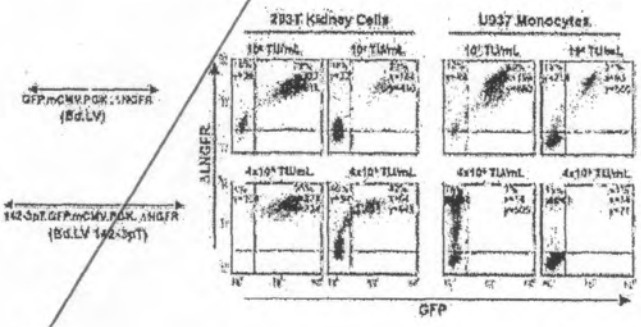

293T Kidney Cells
U937 Monocytes
GFP.mCMV.PGK.ΔNGFR
(Bd.LV)
142-3pT.GFP.mCMV.PGK.ΔNGFR
(Bd.LV 142-3pT)
ΔLNGFR
GFP

Figure 2.

d.

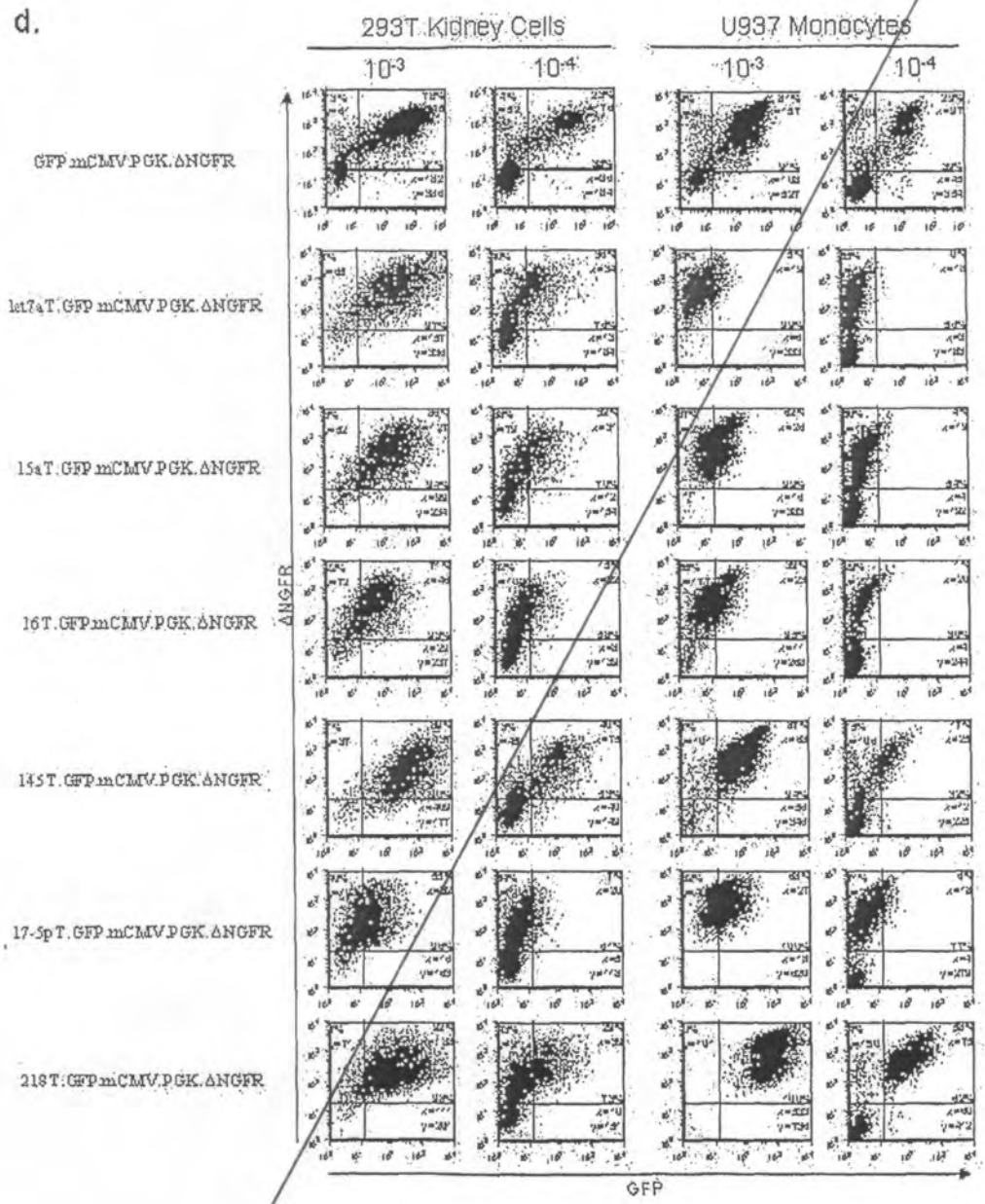

293T Kidney Cells
U937 Monocytes
10-3
10-4
10-3
10-4
GFP.mCMV.PGK.ΔNGFR
let7aT.GFP.mCMV.PGK.ΔNGFR
15aT.GFP.mCMV.PGK.ΔNGFR
16T.GFP.mCMV.PGK.ΔNGFR
145T.GFP.mCMV.PGK.ΔNGFR
17-5pT.GFP.mCMV.PGK.ΔNGFR
218T.GFP.mCMV.PGK.ΔNGFR
ΔNGFR
GFP

Figure 3.

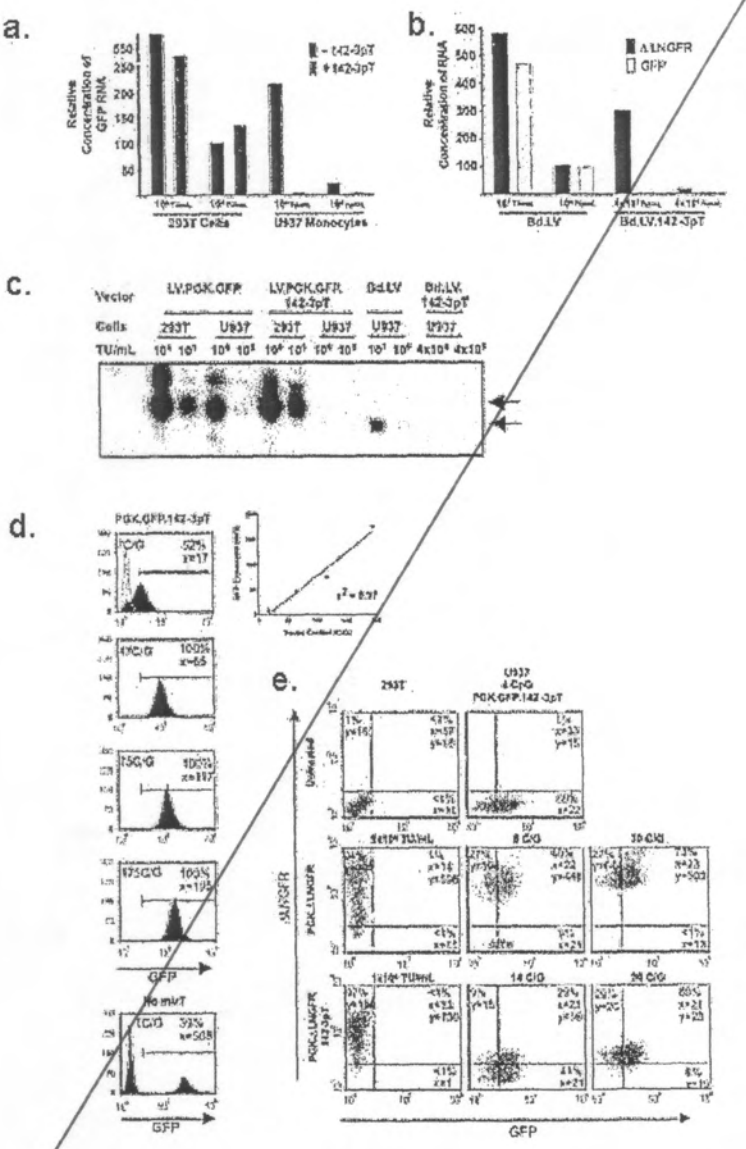

a.
b.
c.
d.
e.
293T Cells
U937 Monocytes
Bd.LV
Bd.LV.142-3pT
Vector
LV.PGK.GFP
LV.PGK.GFP 142-3pT
Bd.LV
Bd.LV. 142-3pT
Cells
293T
U937
TU/mL
PGK.GFP.142-3pT
No mvT
GFP

Figure 4.

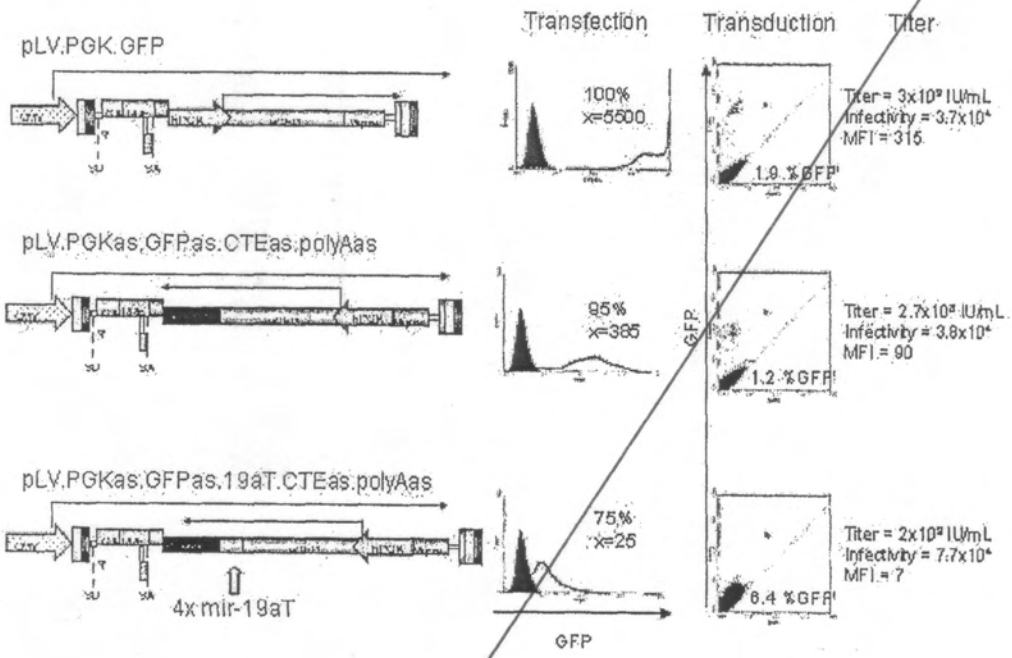

Transfection
Transduction
Titer
pLV.PGK.GFP
100%
x=5500
1.9 %GFP
Titer = 3x10⁸ IU/mL
Infectivity = 3.7x10⁴
MFI = 315
pLV.PGKas.GFPas.CTEas.polyAas
95%
x=385
1.2 %GFP
Titer = 2.7x10⁸ IU/mL
Infectivity = 3.8x10⁴
MFI = 90
pLV.PGKas.GFPas.19aT.CTEas.polyAas
4x mir-19aT
75%
x=25
6.4 %GFP
Titer = 2x10⁸ IU/mL
Infectivity = 7.7x10⁴
MFI = 7
GFP
GFP

Figure 5.

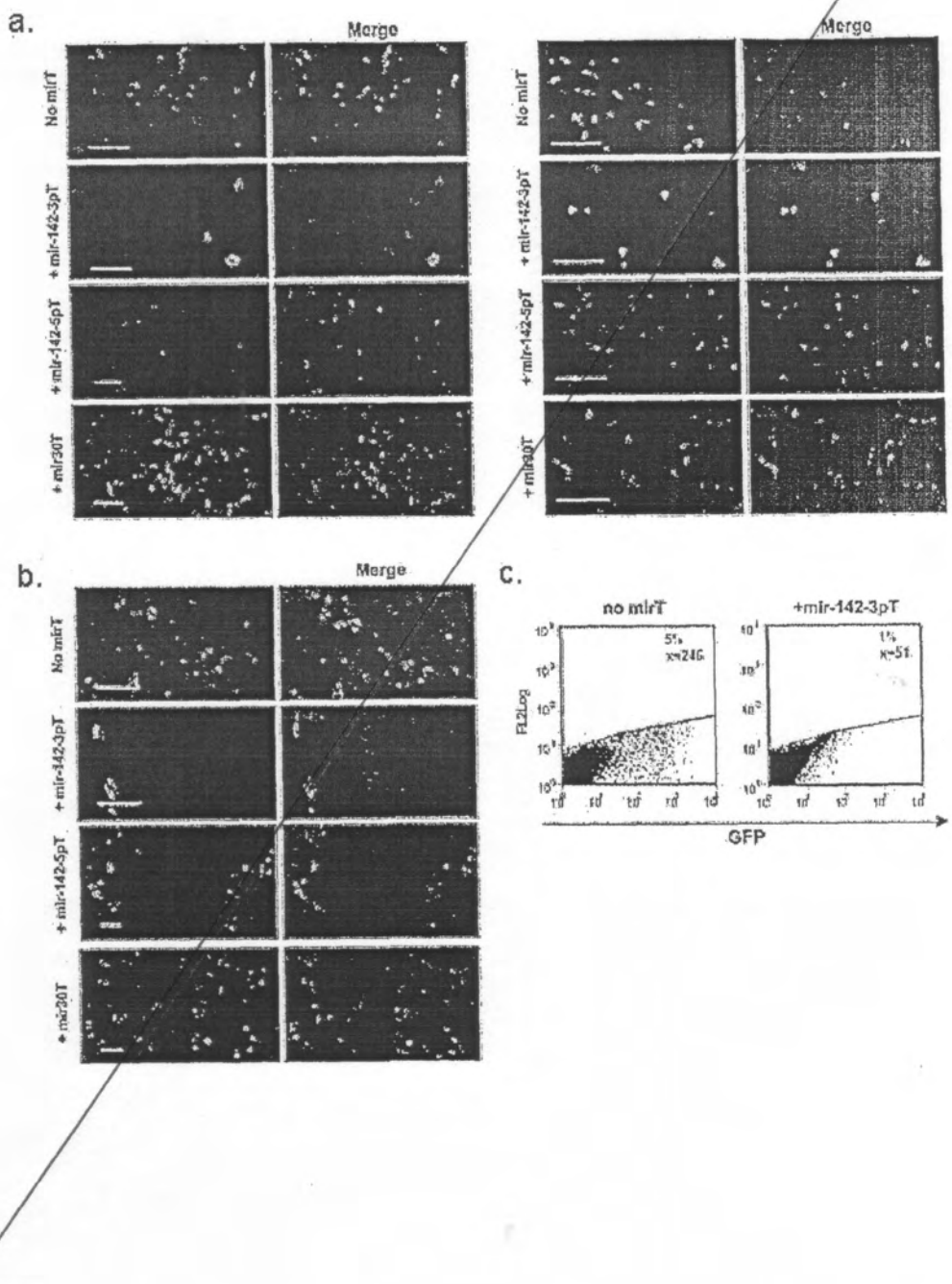
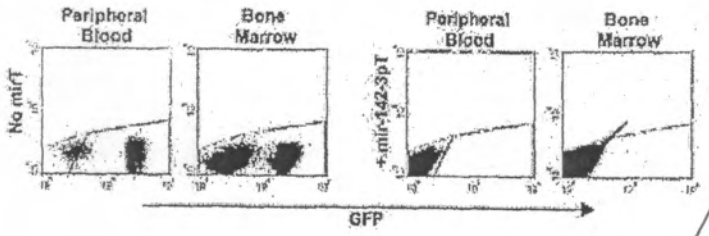

a.
Merge
No mirT
+ mir-142-3pT
+ mir-142-5pT
+ mir30T
Merge
No mirT
+ mir-142-3pT
+ mir-142-5pT
+ mir30T
b.
Merge
No mirT
+ mir-142-3pT
+ mir-142-5pT
+ mir30T
c.
no mirT
+mir-142-3pT
FL2Log
GFP

Figure 6

a.


Peripheral Blood
Bone Marrow
Peripheral Blood
Bone Marrow
No mirT
+ mir-142-3pT
GFP

b.

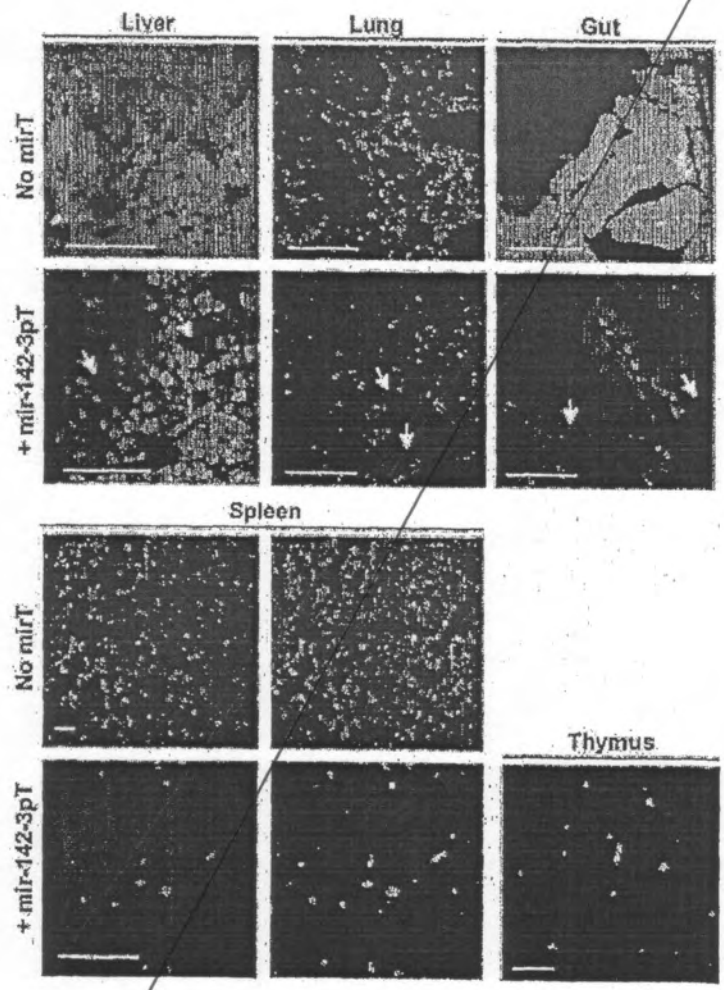

Liver
Lung
Gut
No mirT
+ mir-142-3pT
Spleen
No mirT
Thymus
+ mir-142-3pT

Figure 7.

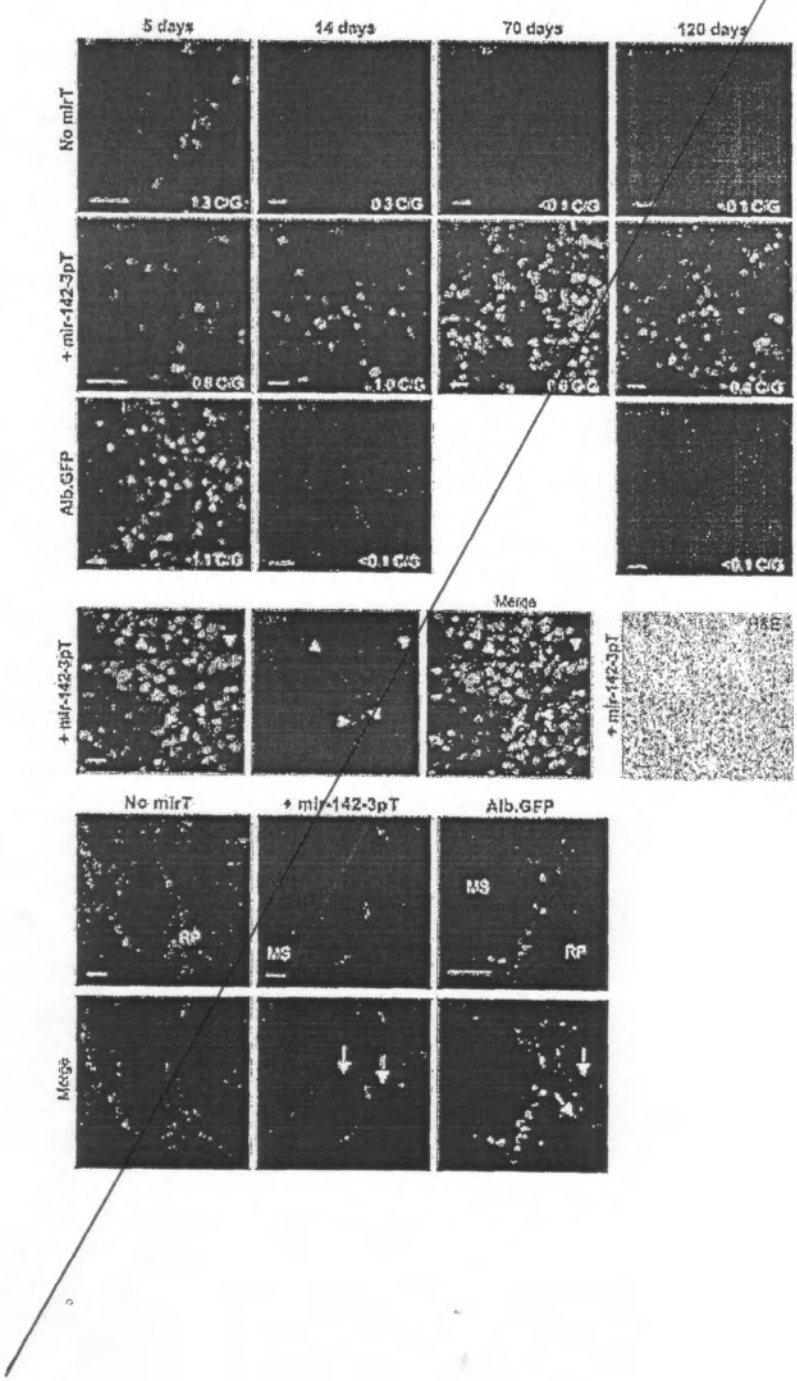
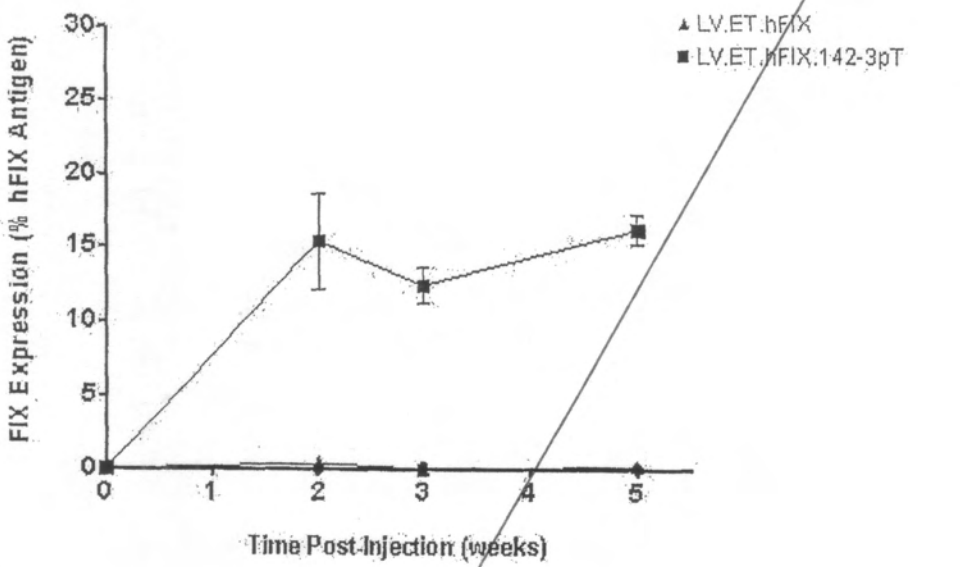

a.
5 days
14 days
70 days
120 days
No mirT
+ mir-142-3pT
Alb.GFP
b.
Merge
+ mir-142-3pT
DSE
d.
No mirT
+ mir-142-3pT
Alb.GFP
MS
RP
Merge

Figure 8.


LV.ET.hFIX
LV.ET.hFIX.142-3pT
FIX Expression (% hFIX Antigen)
30
25
20
15
10
5
0
0
1
2
3
4
5
Time Post-Injection (weeks)

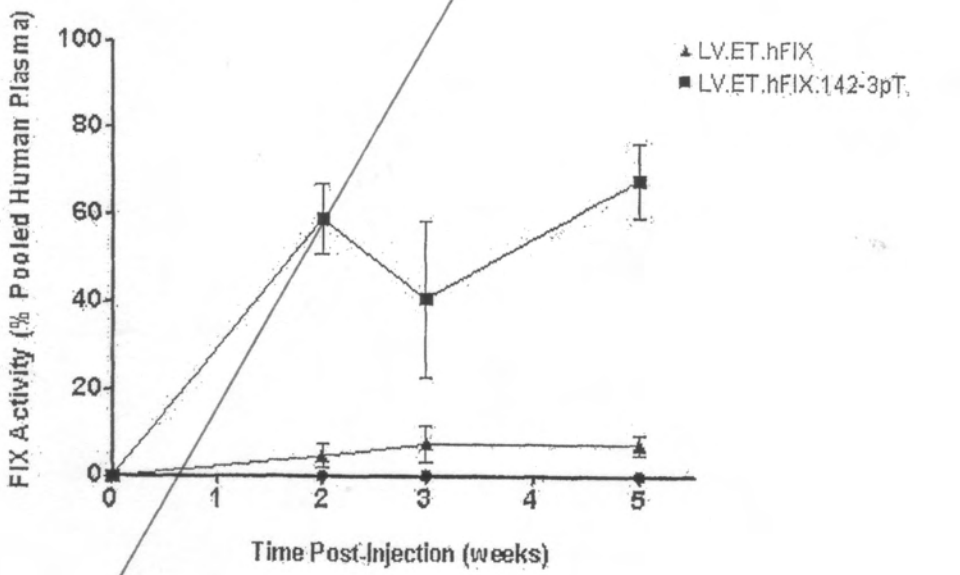
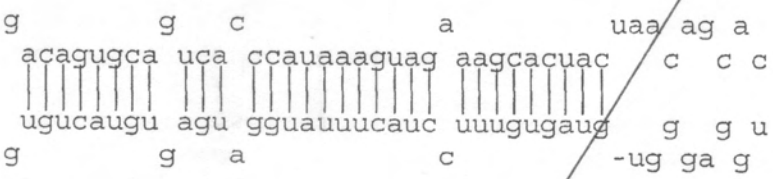

LV.ET.hFIX
LV.ET.hFIX.142-3pT
FIX Activity (% Pooled Human Plasma)
100
80
60
40
20
0
0
1
2
3
4
5
Time Post-Injection (weeks)

Figure 9

A. Mature hsa-mir-142 stem loop sequence.



B. 1x.mir-142as.Target

TCCATAAAGTAGGAAACACTACA

Figure 1.

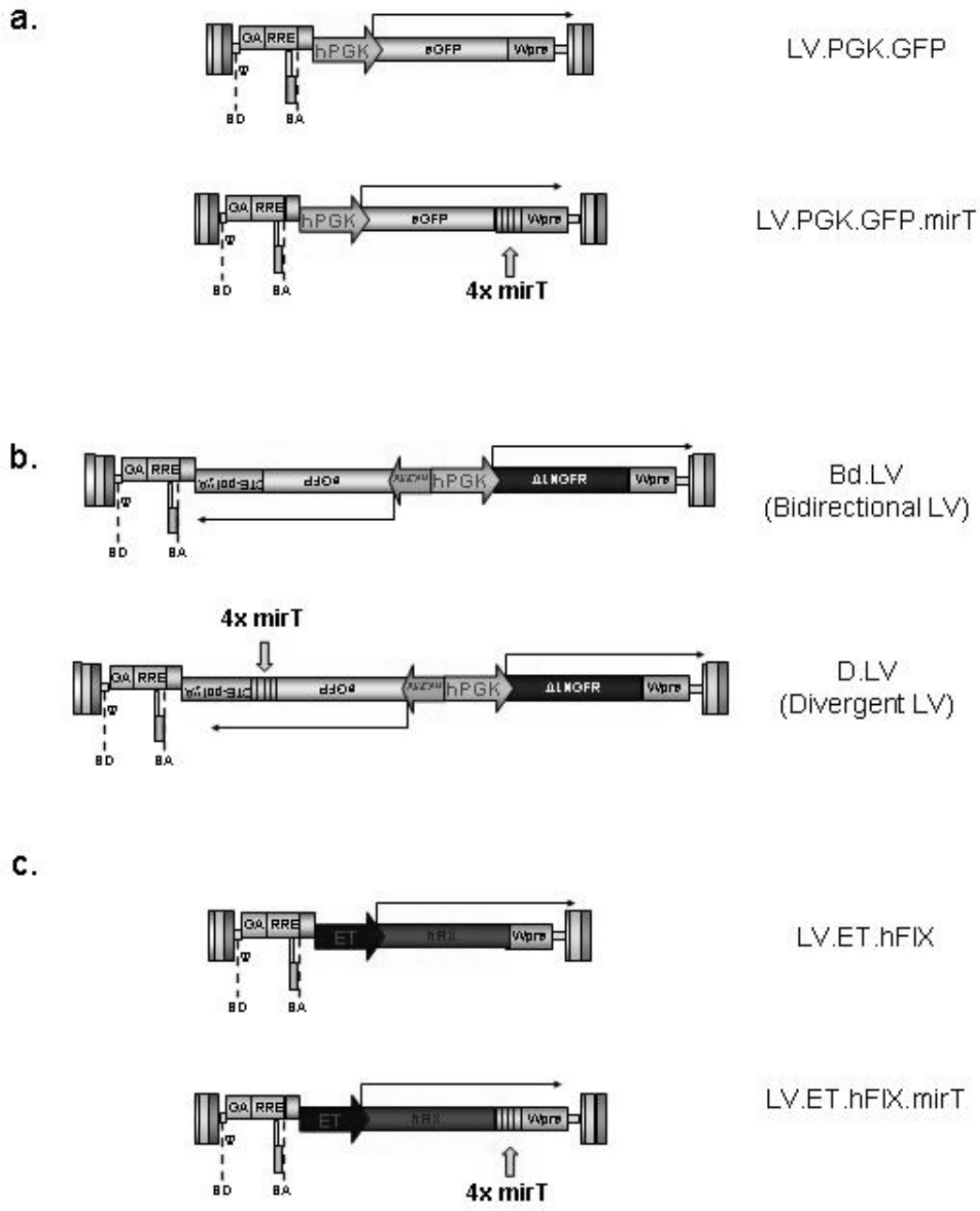
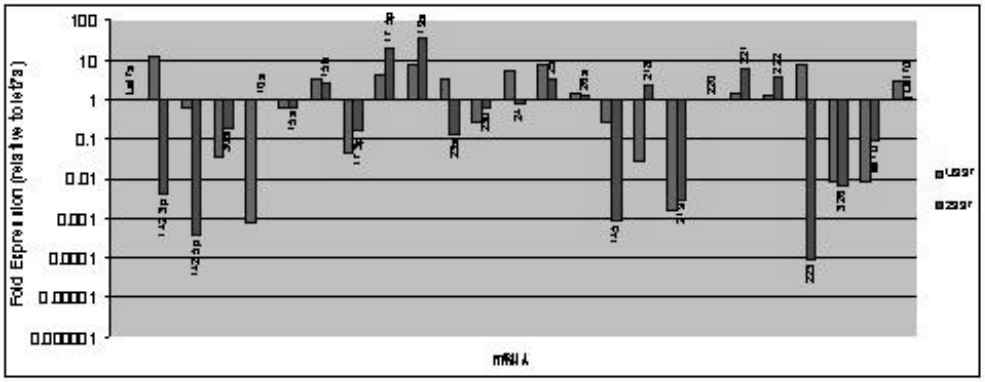
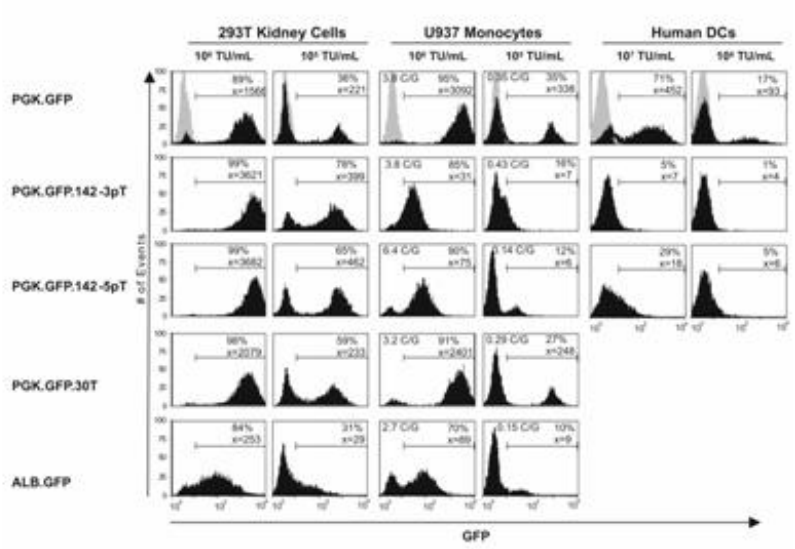

a.
GA
RRE
hPGK
eGFP
Wpre
BD
BA
LV.PGK.GFP
LV.PGK.GFP.mirT
4x mirT
b.
ΔLNGFR
Bd.LV
(Bidirectional LV)
D.LV
(Divergent LV)
c.
ET
LV.ET.hFIX
LV.ET.hFIX.mirT

Figure 2.

a.


100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
Fold Expression (relative to let7a)
miRNA

b.


293T Kidney Cells
U937 Monocytes
Human DCs
10⁶ TU/mL
10⁵ TU/mL
10⁷ TU/mL
10⁴ TU/mL
PGK.GFP
PGK.GFP.142-3pT
PGK.GFP.142-5pT
PGK.GFP.30T
ALB.GFP
# of Events
GFP

c.

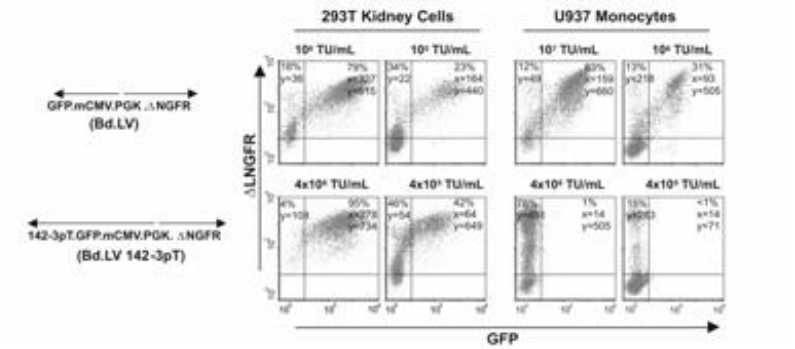

293T Kidney Cells
U937 Monocytes
10⁶ TU/mL
10⁵ TU/mL
10⁷ TU/mL
10⁴ TU/mL
4x10⁵ TU/mL
4x10⁴ TU/mL
GFP.mCMV.PGK .ΔNGFR
(Bd.LV)
142-3pT.GFP.mCMV.PGK. .ΔNGFR
(Bd.LV 142-3pT)
ΔLNGFR
GFP

Figure 2.

d.

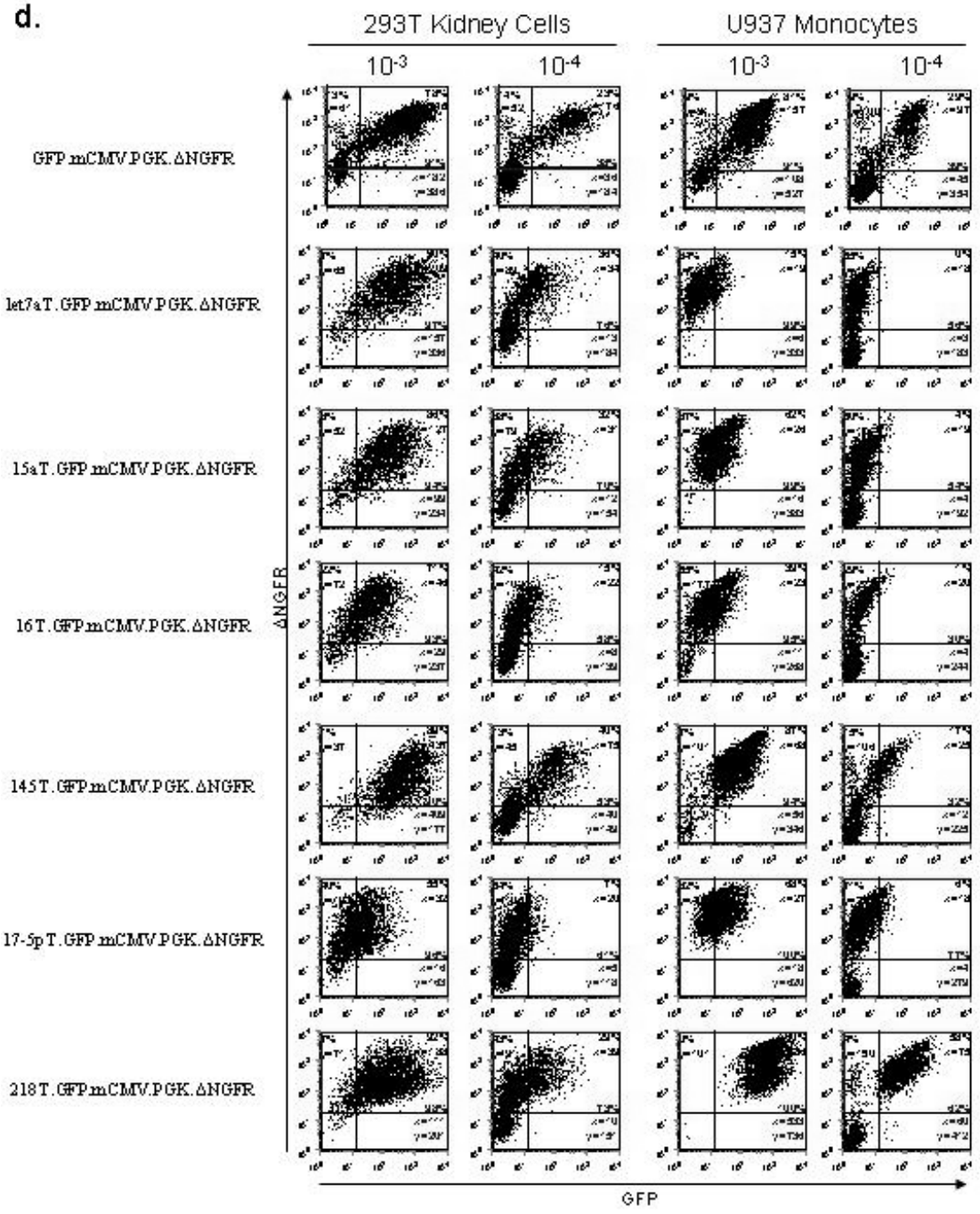

293T Kidney Cells
U937 Monocytes
10-3
10-4
10-3
10-4
GFP.mCMV.PGK.ΔNGFR
let7aT.GFP.mCMV.PGK.ΔNGFR
15aT.GFP.mCMV.PGK.ΔNGFR
16T.GFP.mCMV.PGK.ΔNGFR
145T.GFP.mCMV.PGK.ΔNGFR
17-5pT.GFP.mCMV.PGK.ΔNGFR
218T.GFP.mCMV.PGK.ΔNGFR
ΔNGFR
GFP

Figure 3.

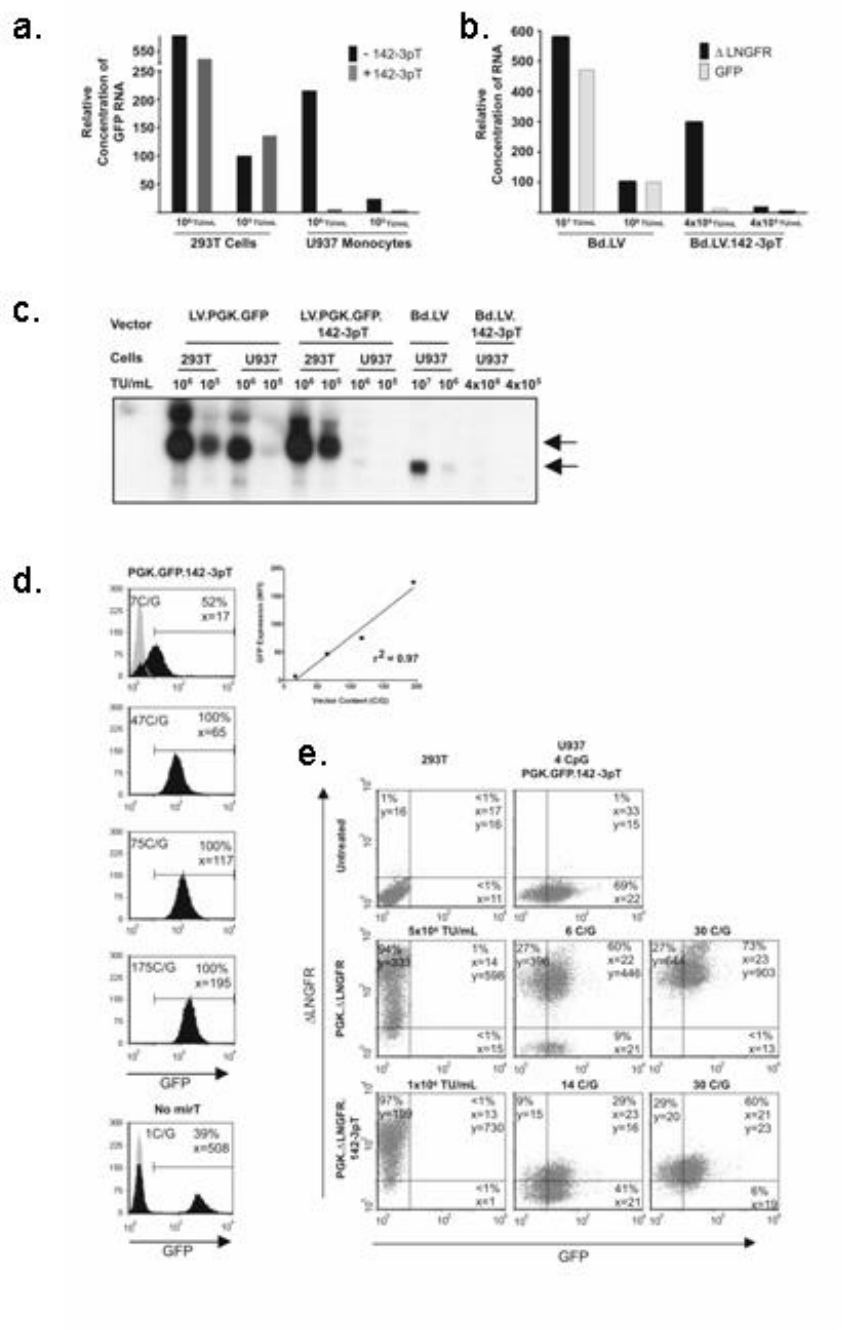

a.
Relative Concentration of GFP RNA
- 142-3pT
+ 142-3pT
293T Cells
U937 Monocytes
b.
Relative Concentration of RNA
ΔLNGFR
GFP
Bd.LV
Bd.LV.142-3pT
c.
Vector
LV.PGK.GFP
LV.PGK.GFP. 142-3pT
Bd.LV
Bd.LV. 142-3pT
Cells
293T
U937
TU/mL
d.
PGK.GFP.142-3pT
7C/G
52%
x=17
47C/G
100%
x=65
75C/G
100%
x=117
175C/G
100%
x=195
GFP
No mir T
1C/G
39%
x=508
e.
293T
U937
4 CpG
PGK.GFP.142-3pT
Untreated
5x10⁵ TU/mL
6 C/G
30 C/G
1x10⁶ TU/mL
14 C/G
30 C/G
ΔLNGFR
PGK-ΔLNGFR
PGK-ΔLNGFR. 142-3pT
GFP

Figure 4.

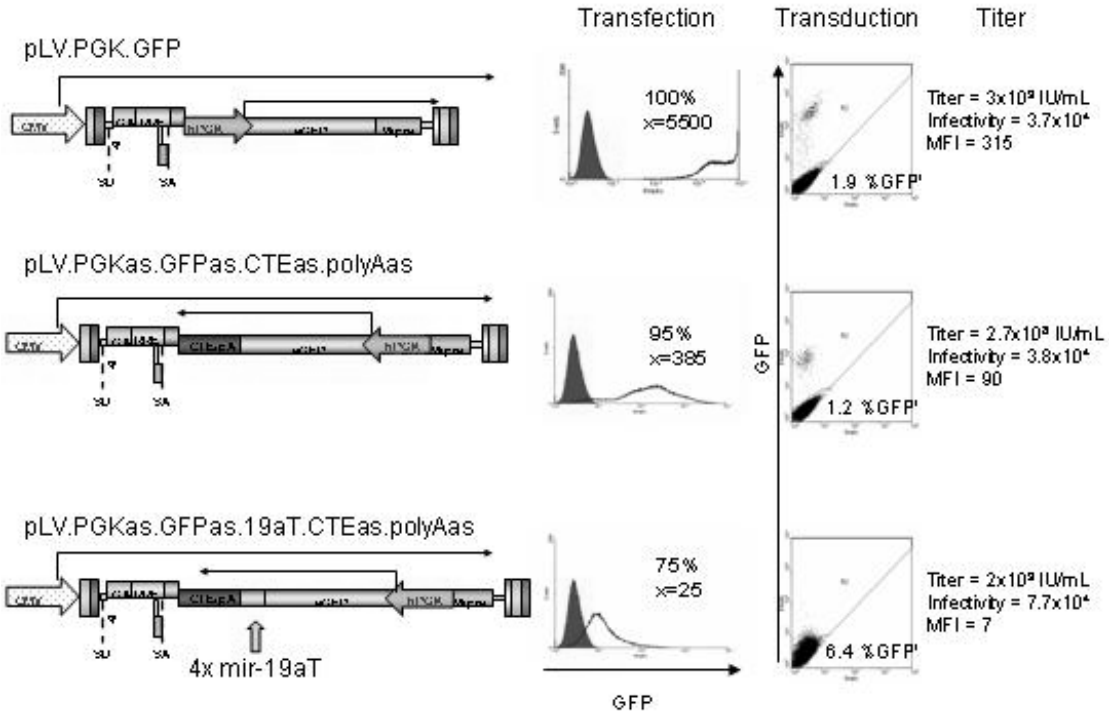

Transfection
Transduction
Titer
pLV.PGK.GFP
100%
x=5500
1.9 %GFP
Titer = 3x10⁸ IU/mL
Infectivity = 3.7x10⁴
MFI = 315
pLV.PGKas.GFPas.CTEas.polyAas
95%
x=385
1.2 %GFP
Titer = 2.7x10⁸ IU/mL
Infectivity = 3.8x10⁴
MFI = 90
pLV.PGKas.GFPas.19aT.CTEas.polyAas
4x mir-19aT
75%
x=25
6.4 %GFP
Titer = 2x10⁸ IU/mL
Infectivity = 7.7x10⁴
MFI = 7
GFP
GFP

Figure 5.

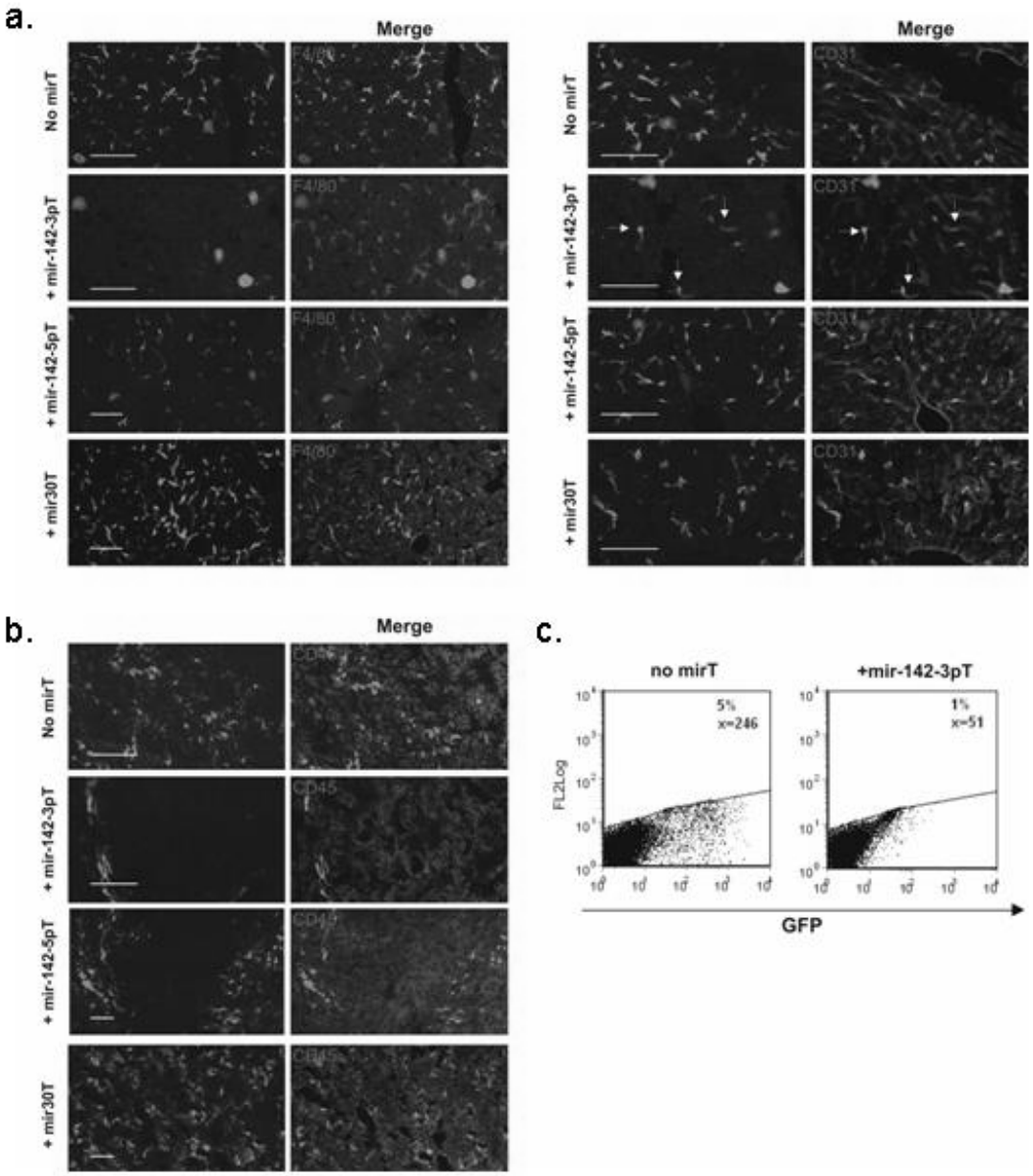

a.
Merge
F4/80
CD31
No mirT
+ mir-142-3pT
+ mir-142-5pT
+ mir30T
b.
Merge
CD45
c.
no mirT
+mir-142-3pT
5%
x=246
1%
x=51
FL2Log
GFP

Figure 6

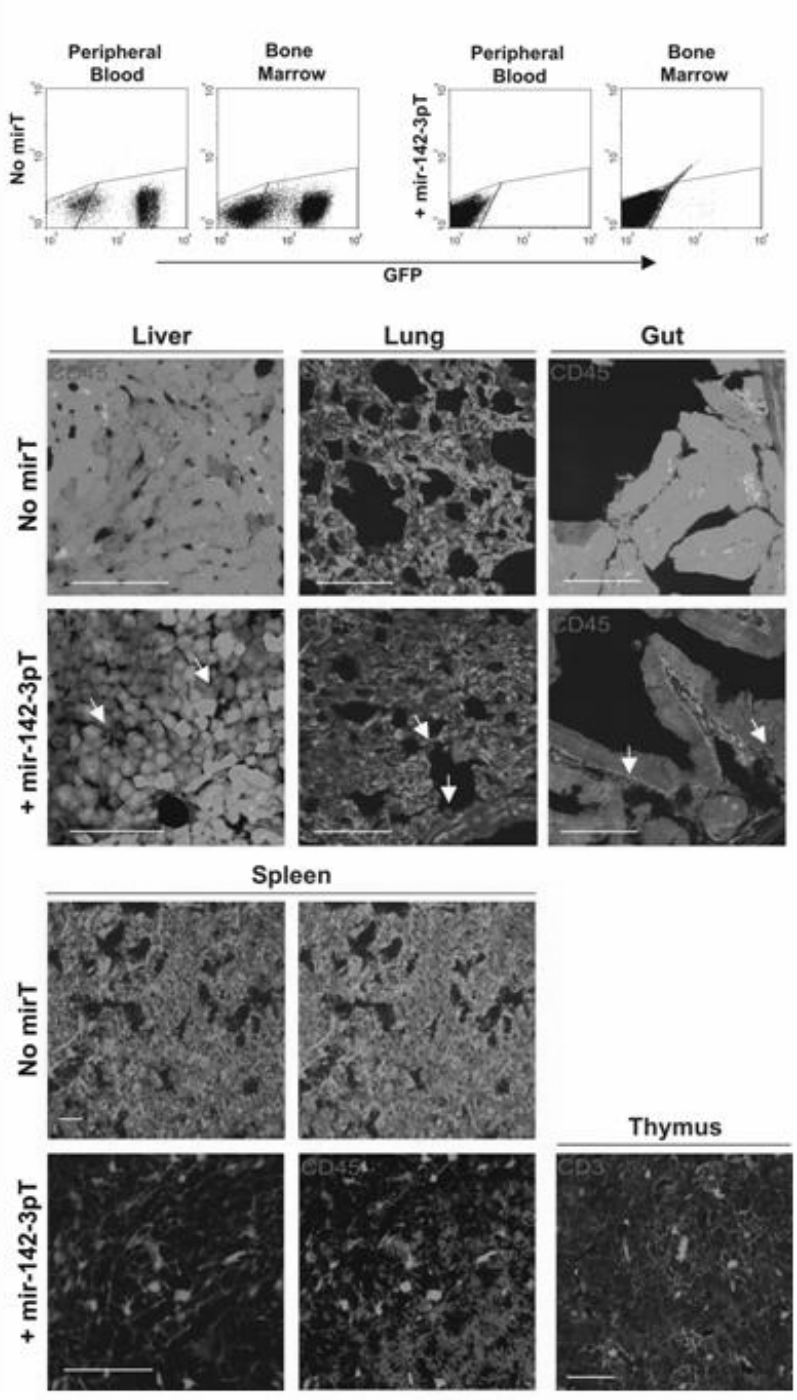
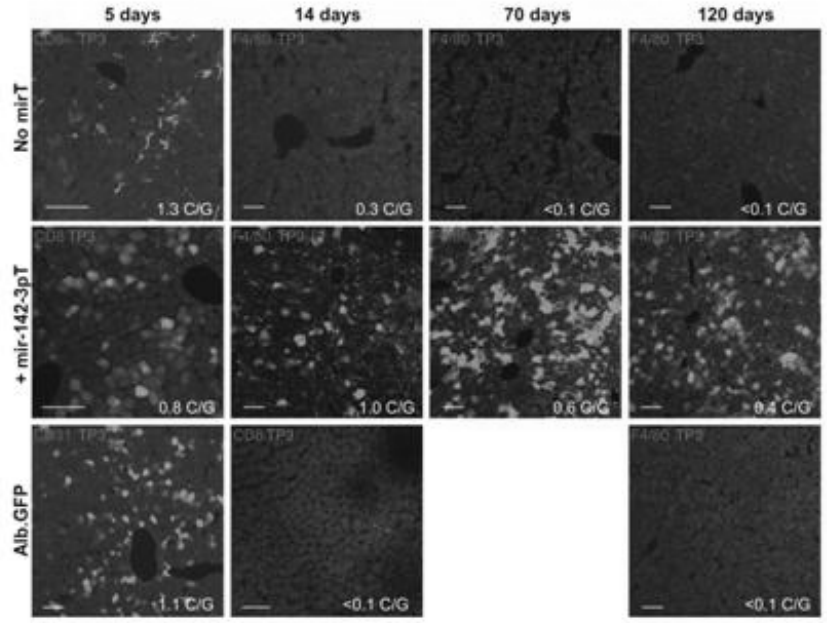
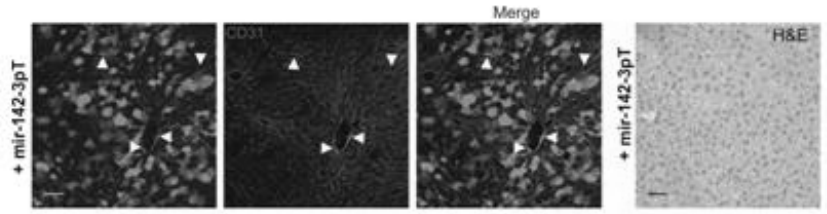

a.
Peripheral Blood
Bone Marrow
Peripheral Blood
Bone Marrow
No mirT
+ mir-142-3pT
GFP
b.
Liver
Lung
Gut
No mirT
+ mir-142-3pT
CD45
Spleen
Thymus
CD3

Figure 7.

a.


5 days
14 days
70 days
120 days
No mirT
+ mir-142-3pT
Alb.GFP
1.3 C/G
0.3 C/G
<0.1 C/G
<0.1 C/G
0.8 C/G
1.0 C/G
0.6 C/G
0.4 C/G
1.1 C/G
<0.1 C/G
<0.1 C/G

b.


Merge
+ mir-142-3pT
CD31
H&E
+ mir-142-3pT

d.

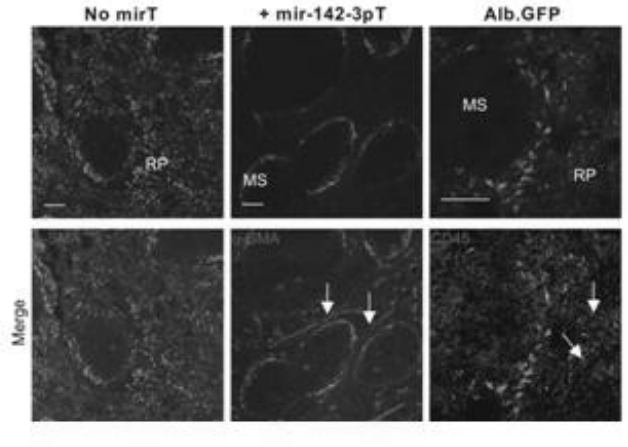
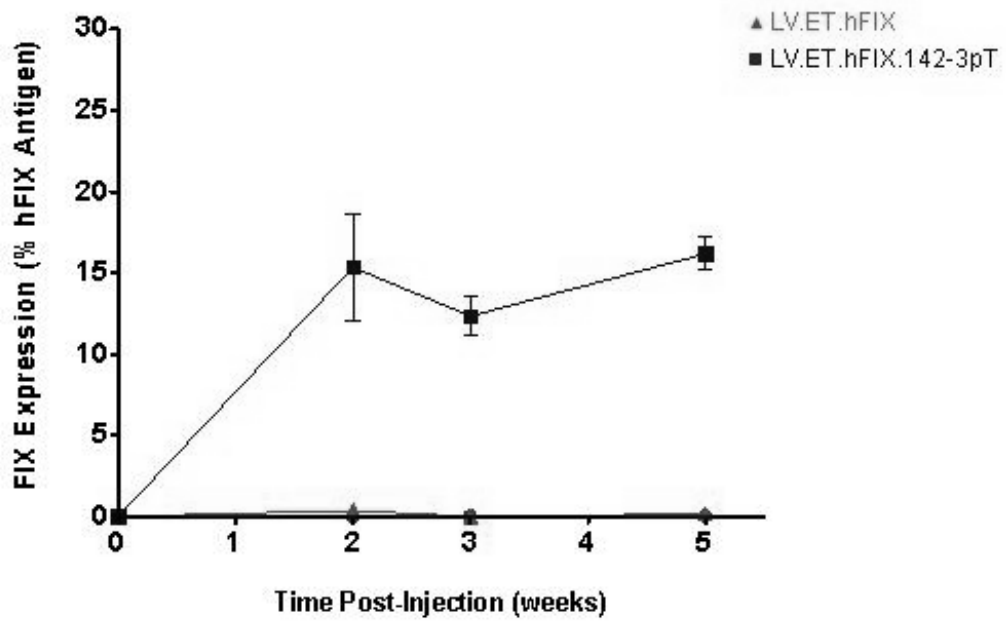

No mirT
+ mir-142-3pT
Alb.GFP
RP
MS
MS
RP
Merge

Figure 8.


LV.ET.hFIX
LV.ET.hFIX.142-3pT
FIX Expression (% hFIX Antigen)
30
25
20
15
10
5
0
0
1
2
3
4
5
Time Post-Injection (weeks)

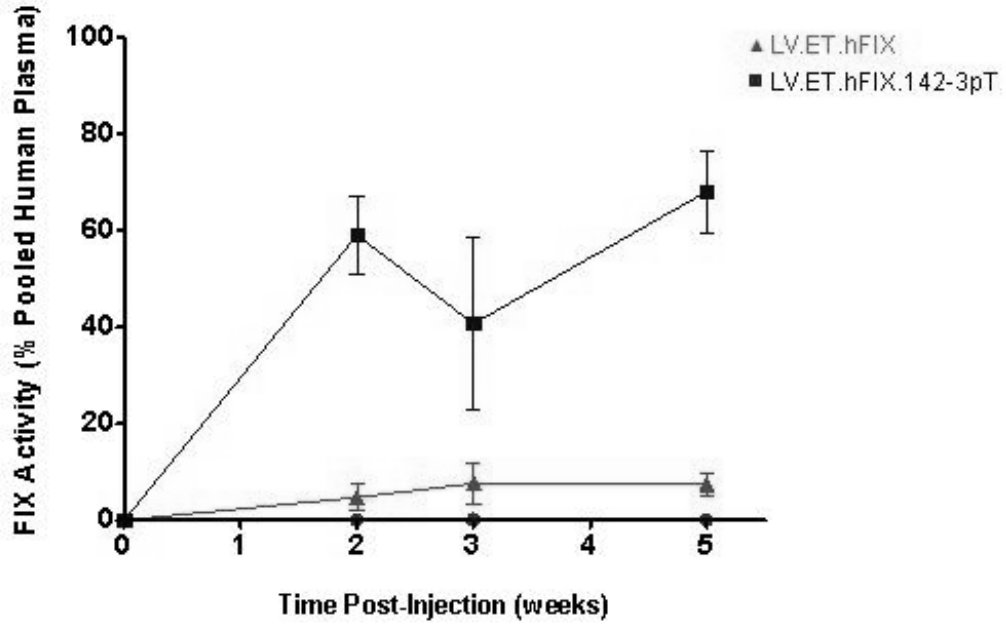
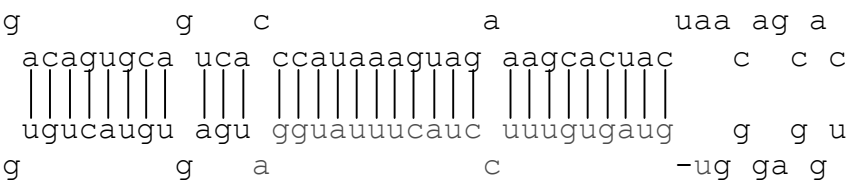

LV.ET.hFIX
LV.ET.hFIX.142-3pT
FIX Activity (% Pooled Human Plasma)
100
80
60
40
20
0
0
1
2
3
4
5
Time Post-Injection (weeks)

Figure 9

A. Mature hsa-mir-142 stem loop sequence.



B. 1x.mir-142as.Target
TCCATAAAGTAGGAAACACTACA