Figure 1

A

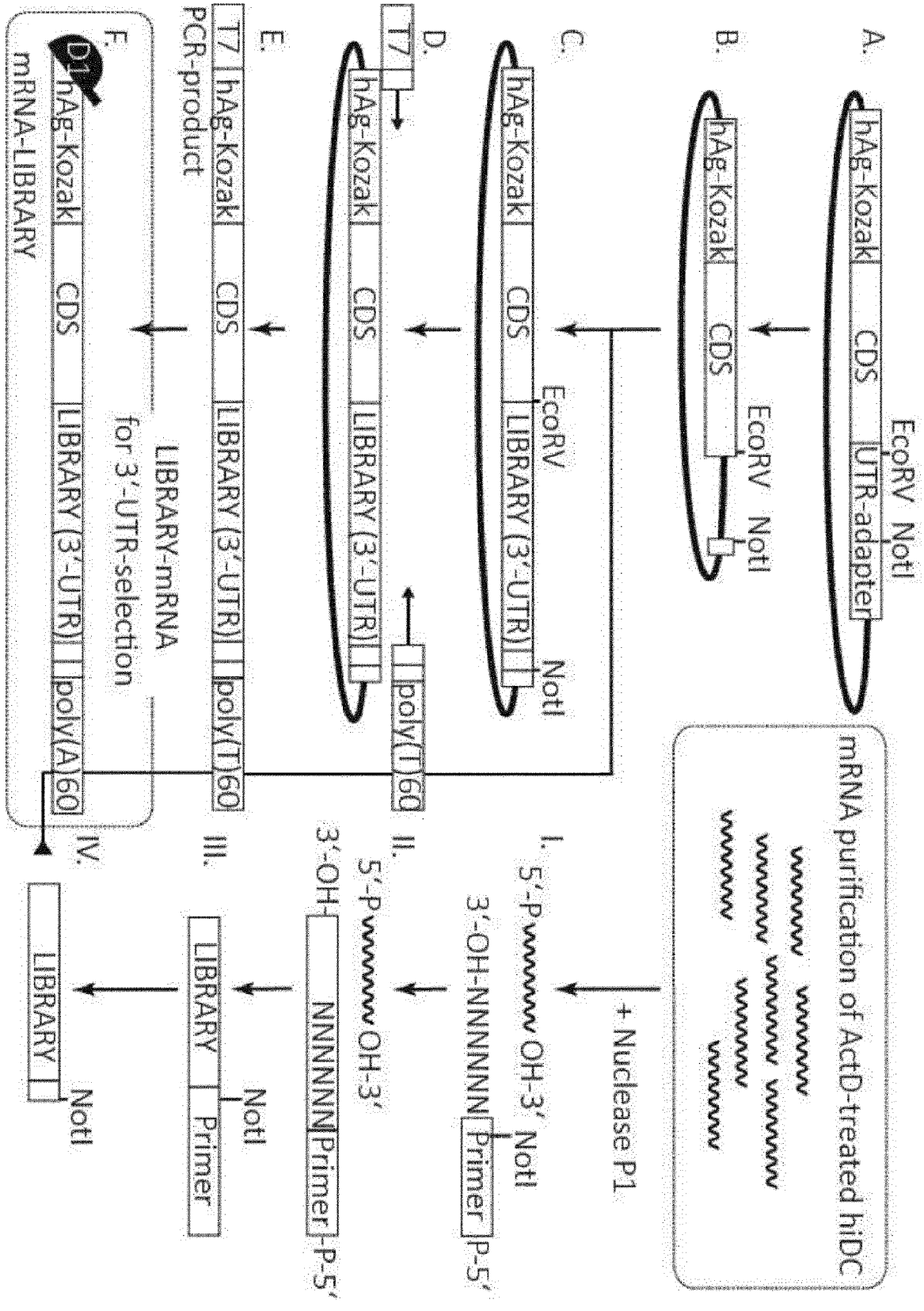

A.
hAg-Kozak
CDS
UTR-adapter
EcoRV
NotI
B.
hAg-Kozak
CDS
EcoRV
NotI
mRNA purification of ActD-treated hiDC
+ Nuclease P1
C.
hAg-Kozak
CDS
LIBRARY (3'-UTR)
EcoRV
NotI
I.
5'-P
OH-3'
NotI
3'-OH-NNNNNN
Primer
P-5'
D.
T7
hAg-Kozak
CDS
LIBRARY (3'-UTR)
poly(T)60
II.
5'-P
OH-3'
3'-OH-
NNNNNN
Primer
P-5'
E.
T7
hAg-Kozak
CDS
LIBRARY (3'-UTR)
poly(T)60
PCR-product
III.
LIBRARY
Primer
NotI
F.
D1
hAg-Kozak
CDS
LIBRARY (3'-UTR)
poly(A)60
LIBRARY-mRNA
for 3'-UTR-selection
IV.
LIBRARY
NotI
mRNA-LIBRARY

Figure 1

B

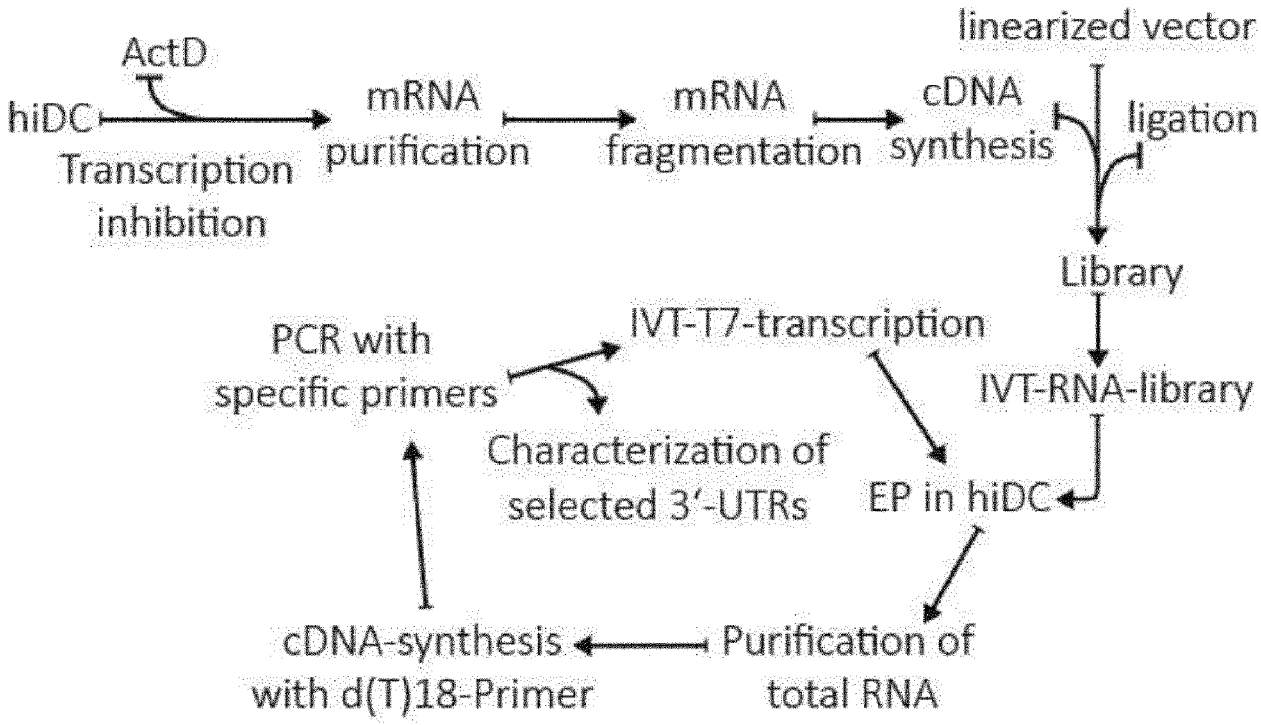

ActD
hiDC
Transcription inhibition
mRNA purification
mRNA fragmentation
cDNA synthesis
linearized vector
ligation
Library
IVT-RNA-library
EP in hiDC
IVT-T7-transcription
PCR with specific primers
Characterization of selected 3'-UTRs
cDNA-synthesis with d(T)18-Primer
Purification of total RNA

Figure 2

A

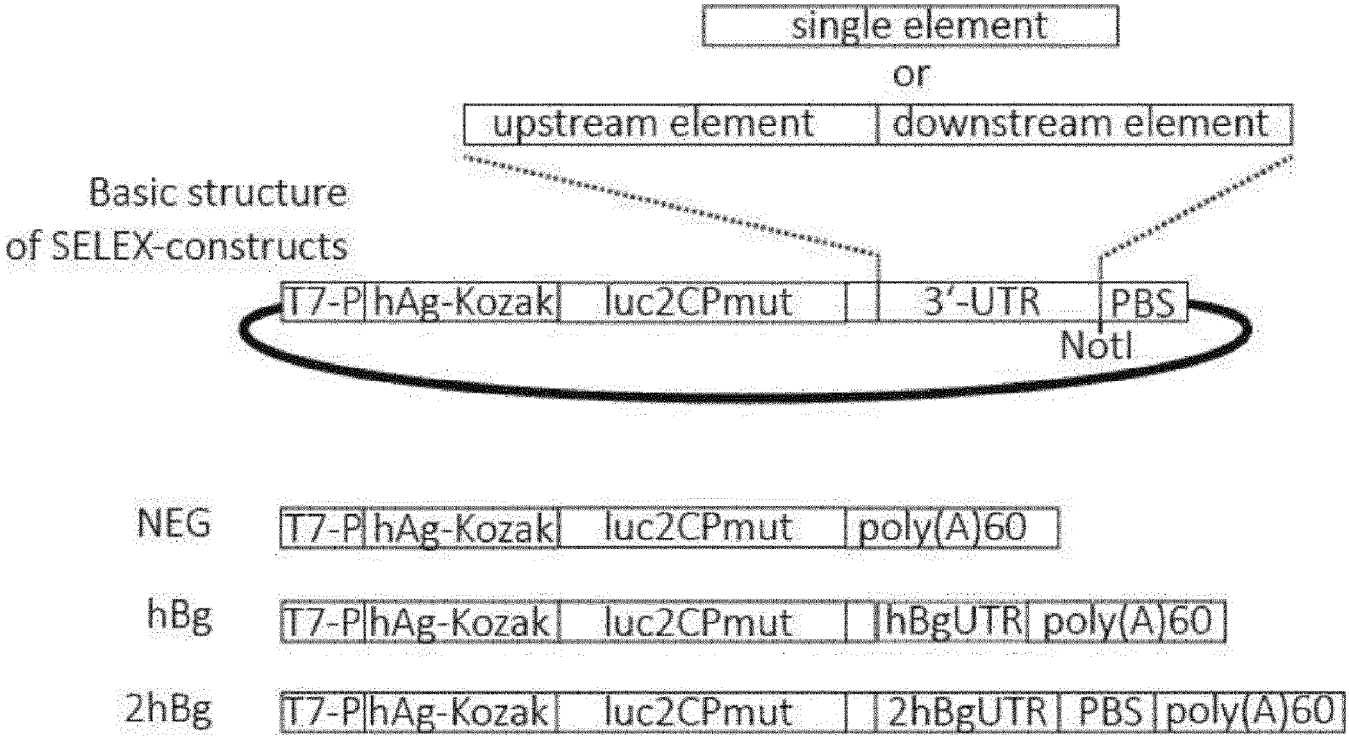
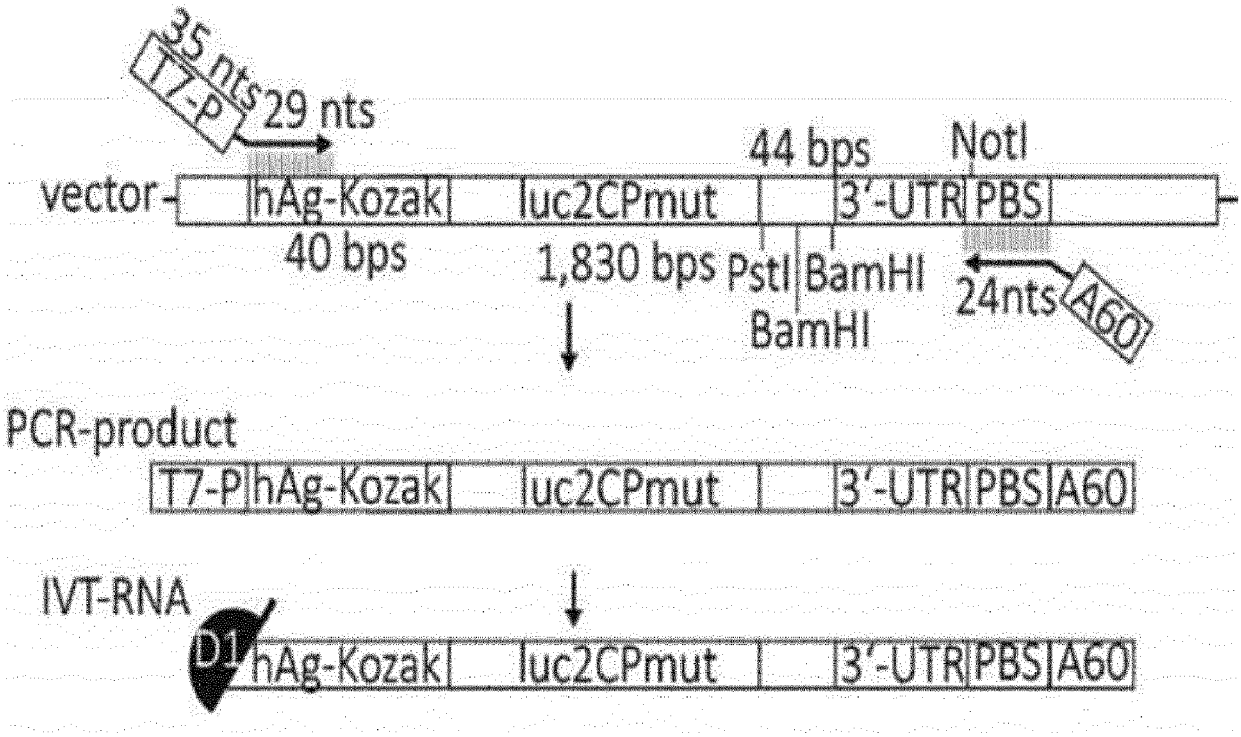

single element
or
upstream element
downstream element
Basic structure
of SELEX-constructs
T7-P
hAg-Kozak
luc2CPmut
3'-UTR
PBS
NotI
NEG
T7-P
hAg-Kozak
luc2CPmut
poly(A)60
hBg
T7-P
hAg-Kozak
luc2CPmut
hBgUTR
poly(A)60
2hBg
T7-P
hAg-Kozak
luc2CPmut
2hBgUTR
PBS
poly(A)60

Figure 2

B



PBS:	Primer Binding Site
T7-P:	T7 promotor
LIB:	Library 3'-UTRs

3'-UTR:	MT-RNR1	AES	hBg
(bps)	142	136	142

Figure 3

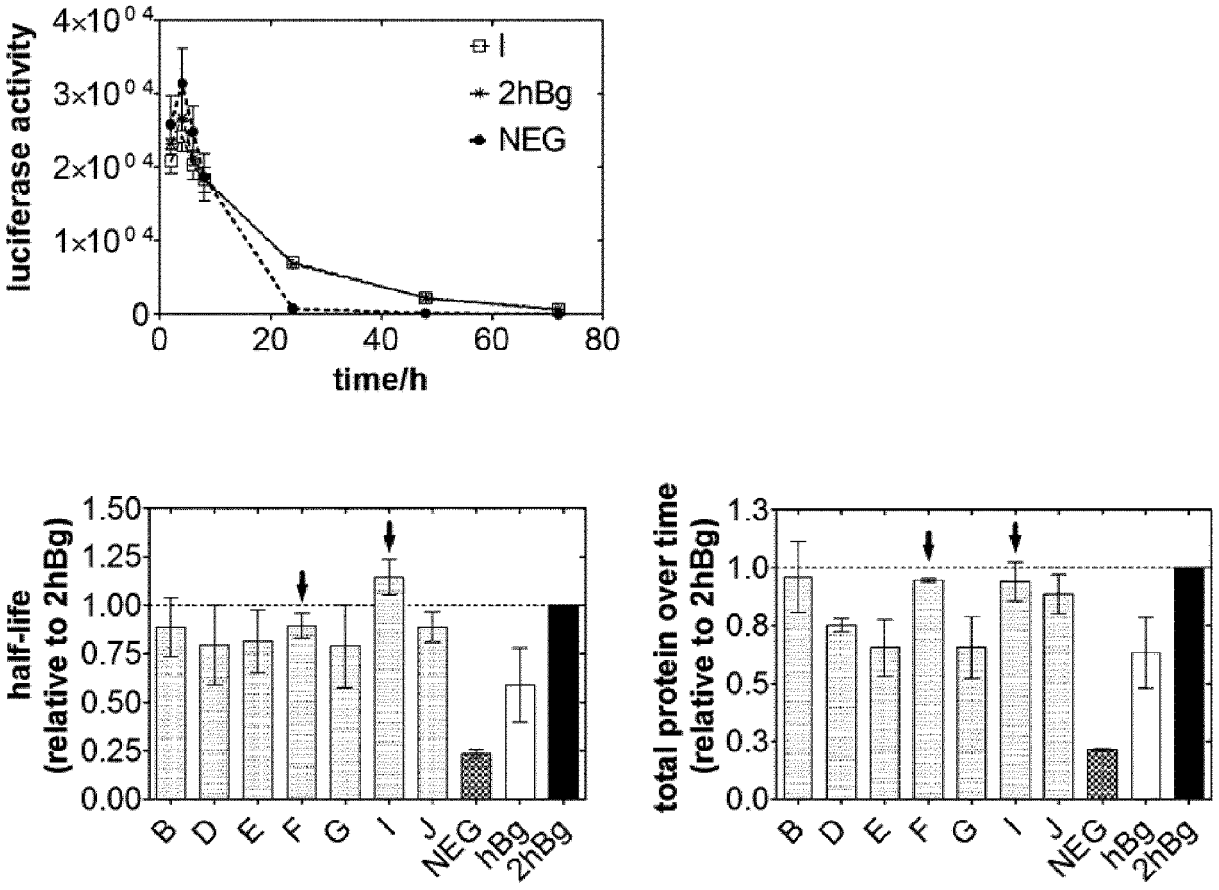

luciferase activity
4×10^04
3×10^04
2×10^04
1×10^04
0
0
20
40
60
80
time/h
I
2hBg
NEG
half-life (relative to 2hBg)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
B D E F G I J NEG hBg 2hBg
total protein over time (relative to 2hBg)
1.3
1.0
0.8
0.5
0.3
0.0
B D E F G I J NEG hBg 2hBg

Figure 4

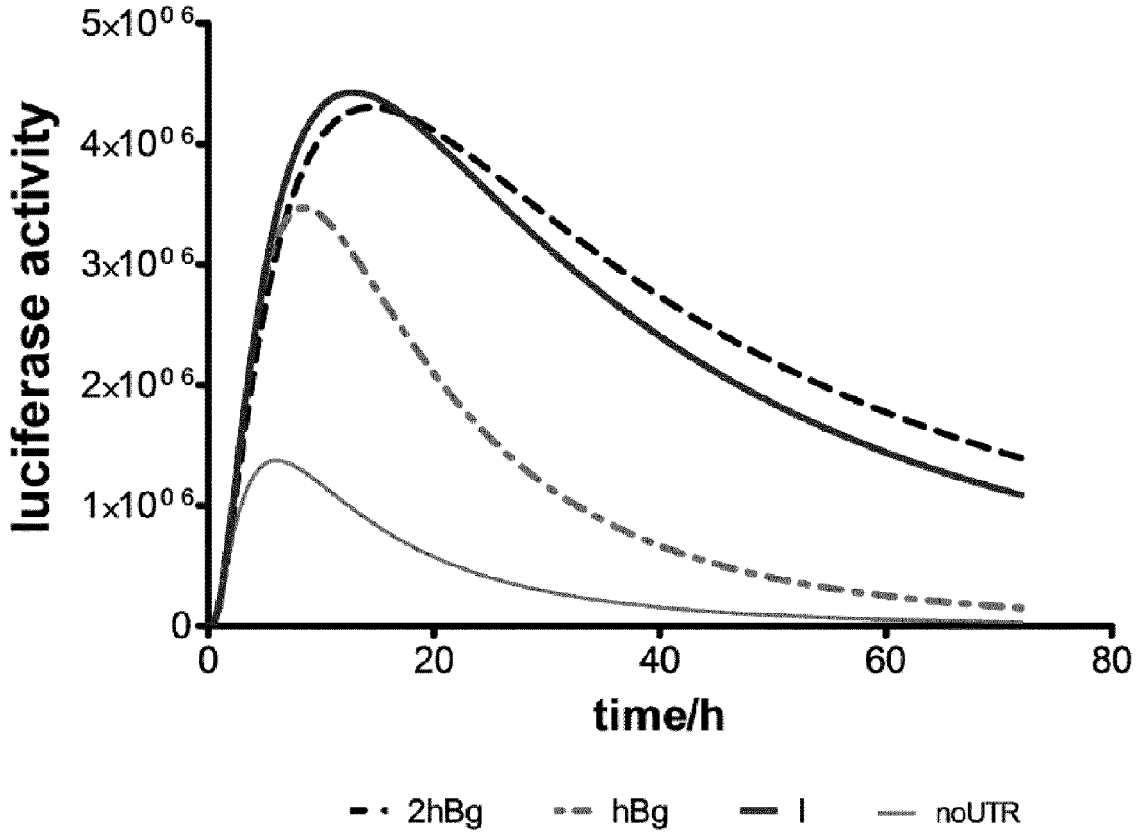

5x10^06
4x10^06
3x10^06
2x10^06
1x10^06
0
luciferase activity
0
20
40
60
80
time/h
2hBg
hBg
I
noUTR

Figure 5

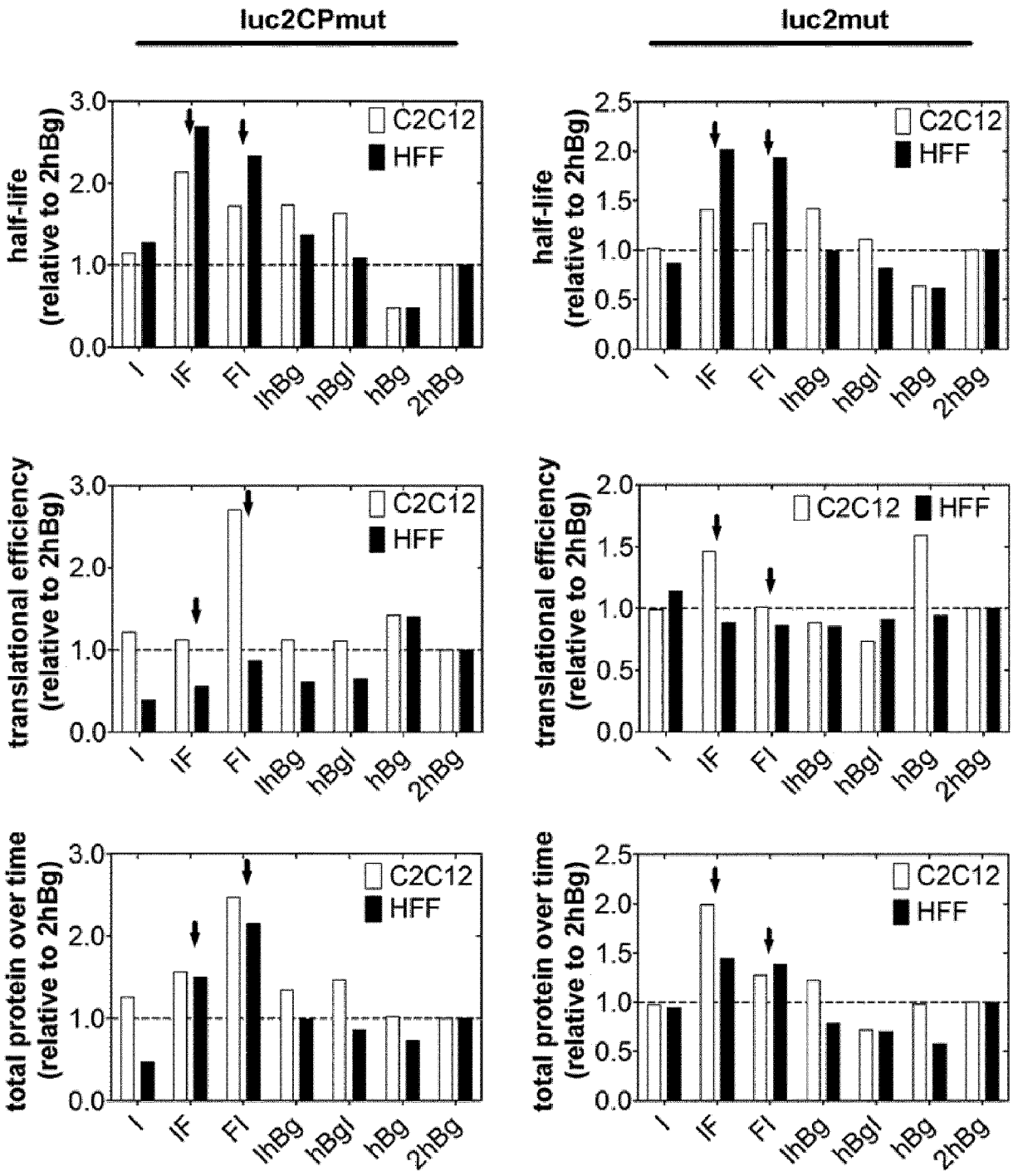

luc2CPmut
luc2mut
half-life (relative to 2hBg)
translational efficiency (relative to 2hBg)
total protein over time (relative to 2hBg)
C2C12
HFF
I
IF
FI
IhBg
hBgI
hBg
2hBg

Figure 6

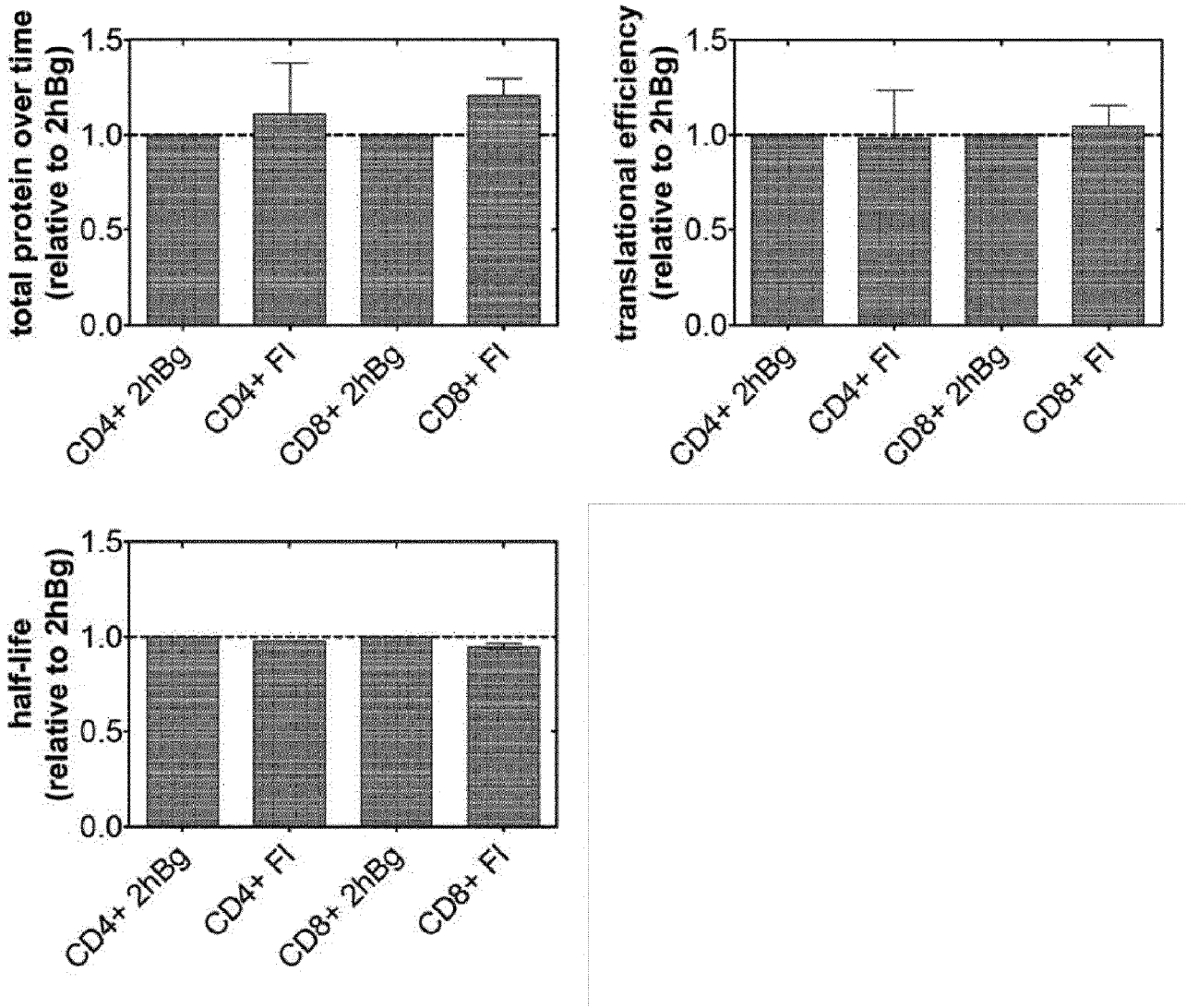
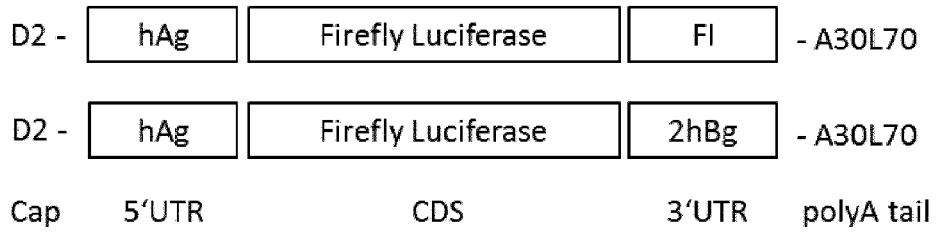

total protein over time (relative to 2hBg)
translational efficiency (relative to 2hBg)
half-life (relative to 2hBg)
1.5
1.0
0.5
0.0
CD4+ 2hBg
CD4+ FI
CD8+ 2hBg
CD8+ FI

Figure 7

A



B

	RNA integrity	
	unmodified	m1Y modified
D-2-hAg-Luc-F-I-UTR-[A30-L-A70]	81	84
D-2-hAg-Luc-2hBgUTR-[A30-L-A70]	82	84

Figure 8

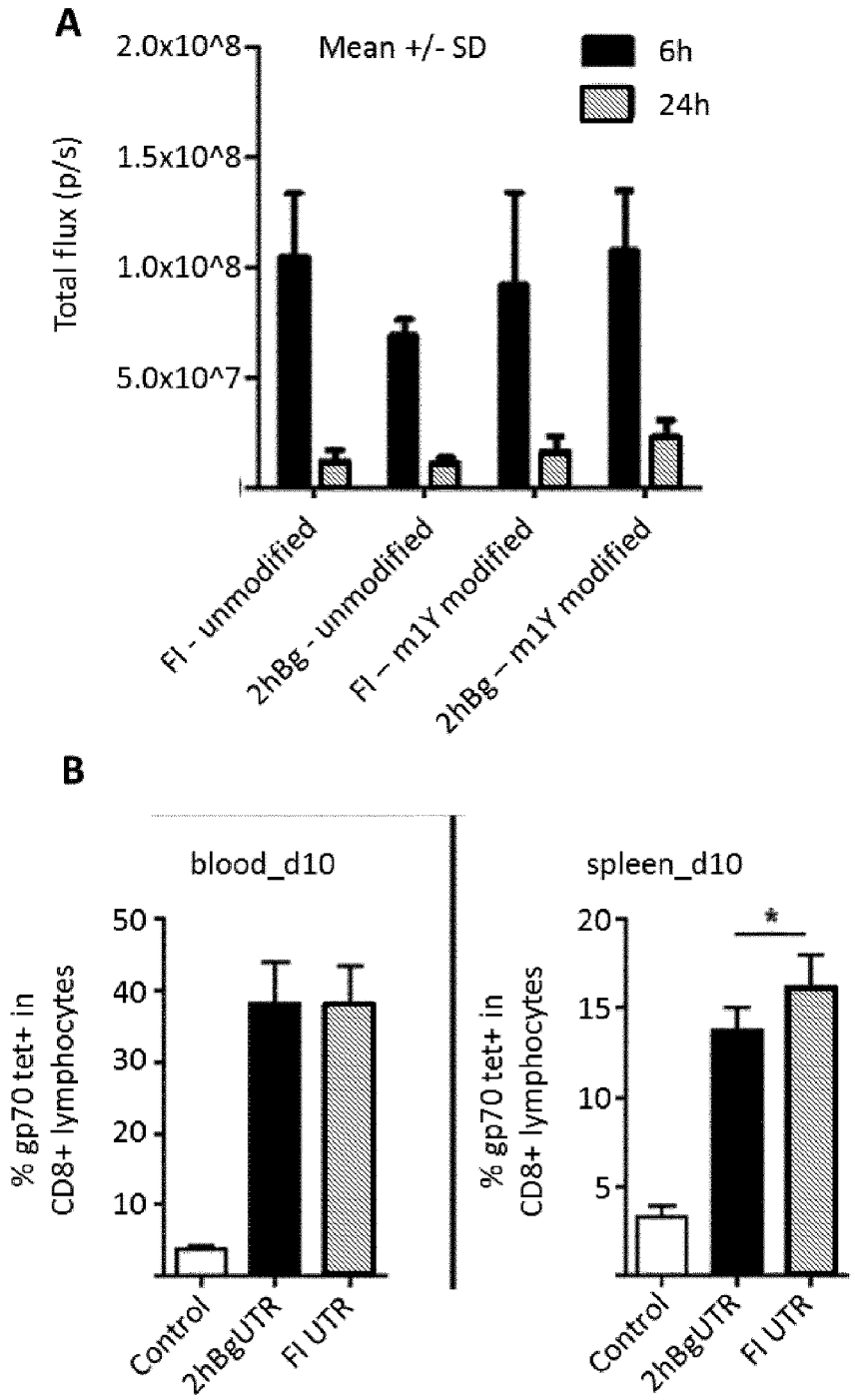

A
Mean +/- SD
6h
24h
Total flux (p/s)
2.0x10^8
1.5x10^8
1.0x10^8
5.0x10^7
Fl - unmodified
2hBg - unmodified
Fl – m1Y modified
2hBg – m1Y modified
B
blood_d10
spleen_d10
% gp70 tet+ in CD8+ lymphocytes
50
40
30
20
10
20
15
10
5
*
Control
2hBgUTR
Fl UTR

Figure 9

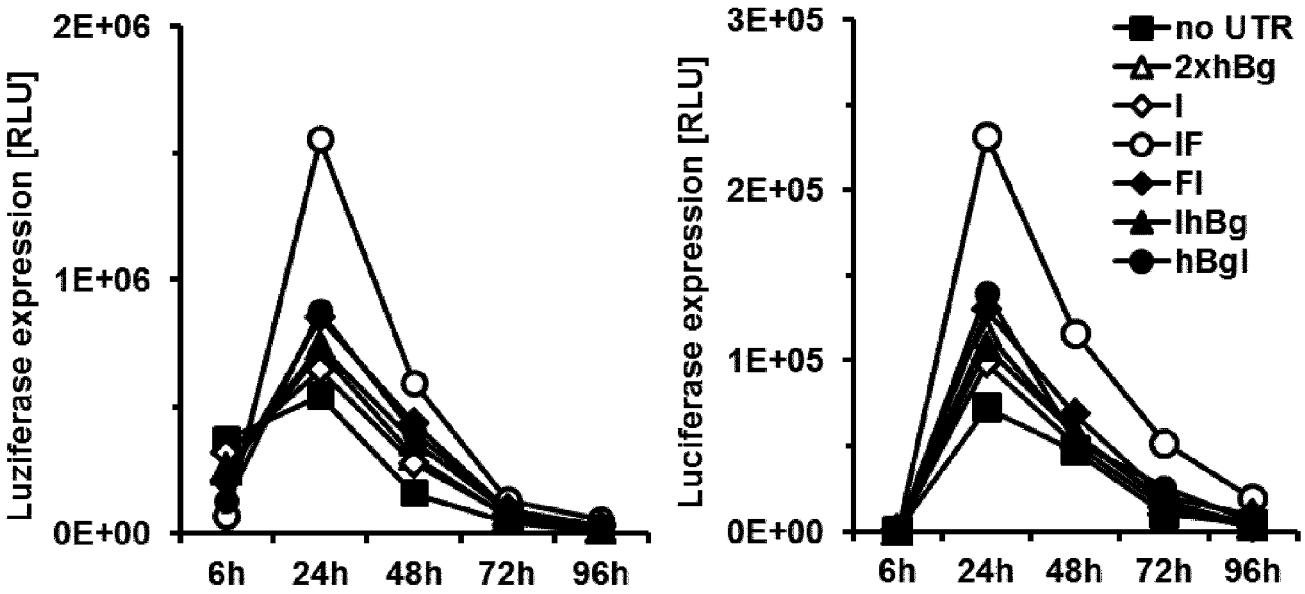

Luziferase expression [RLU]
2E+06
1E+06
0E+00
6h
24h
48h
72h
96h
Luciferase expression [RLU]
3E+05
2E+05
1E+05
0E+00
no UTR
2xhBg
I
IF
FI
IhBg
hBgI

Figure 10

CUGGUACUGCAUGCACGCAAUGCUAGCUGCCCC**UUUCCCGU**CCUGGGUACCCCGAGU
CUCCCCCGACCUCGGGUCCCAGGUAUGCUCCCACCUCCACCUGCCCCACUCACCACC
UCUGCUAGUUCCAGACACCUCCcaagcacgcagcaaugcagcucaaaacgcuuagcc
uagccacaccccc**acgggaaa**cagcagugauuaaccuuuagcaauaaacgaaaguuu
aacuaagcuauacuaaccccaggguuggucaauuucgugccagccacacc

Figure 11

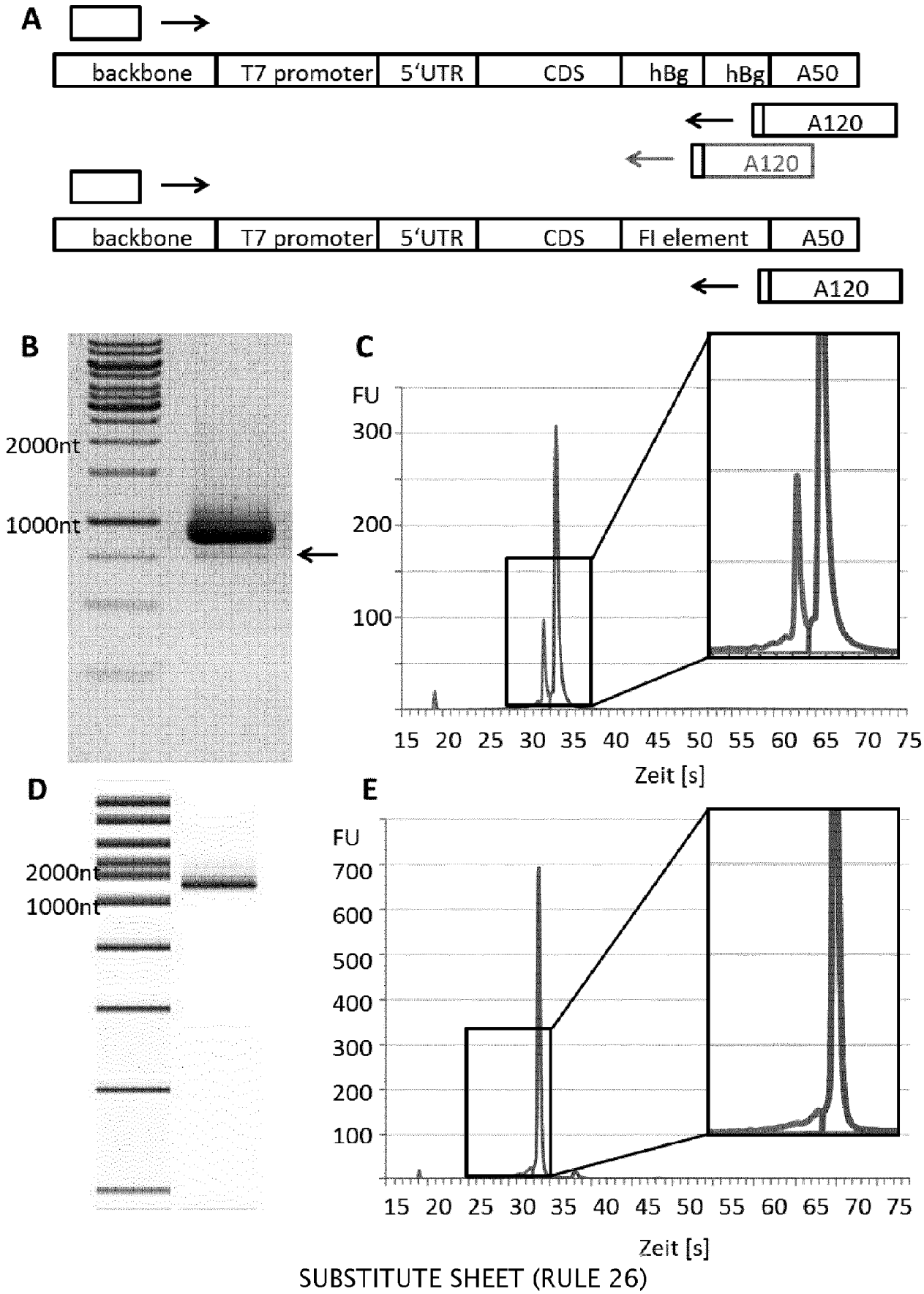
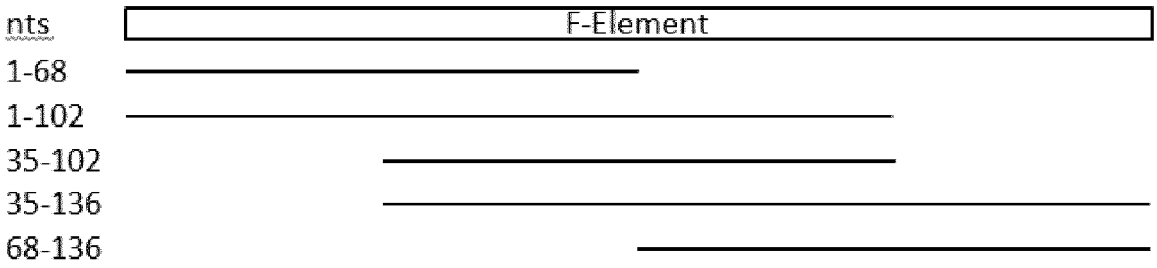

A
backbone
T7 promoter
5'UTR
CDS
hBg
hBg
A50
A120
A120
backbone
T7 promoter
5'UTR
CDS
FI element
A50
A120
B
2000nt
1000nt
C
FU
300
200
100
15 20 25 30 35 40 45 50 55 60 65 70 75
Zeit [s]
D
2000nt
1000nt
E
FU
700
600
500
400
300
200
100
15 20 25 30 35 40 45 50 55 60 65 70 75
Zeit [s]

Figure 12 A


nts
F-Element
1-68
1-102
35-102
35-136
68-136

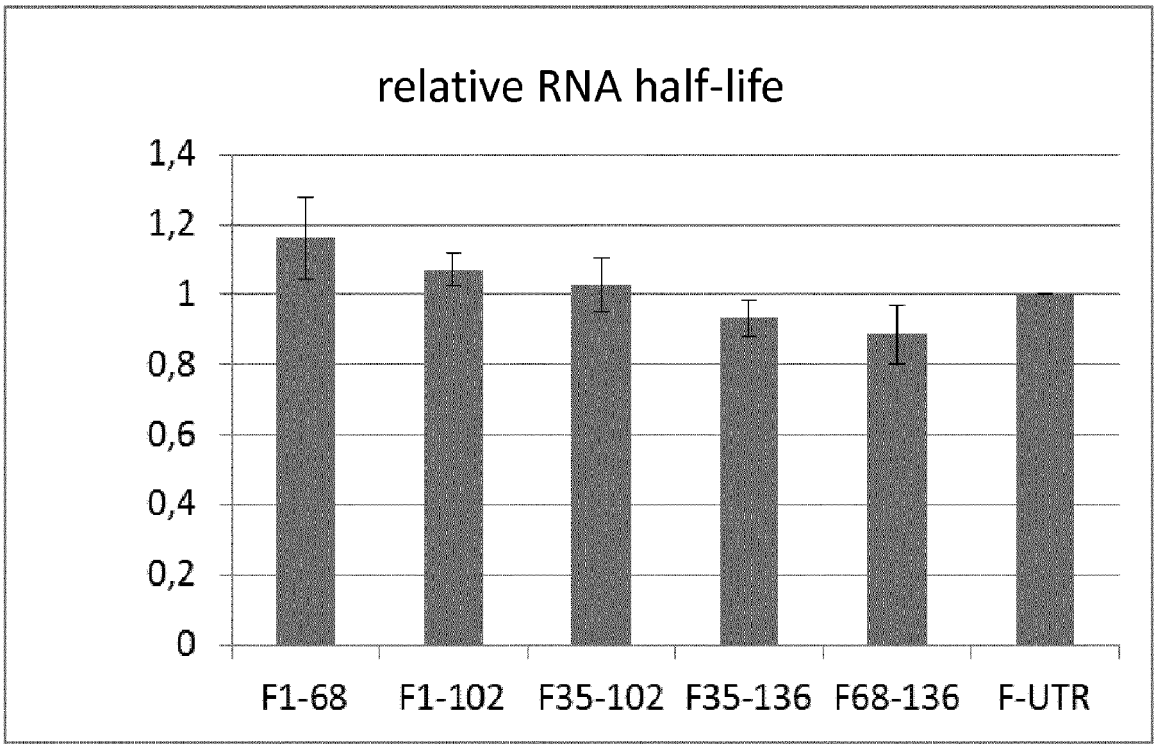
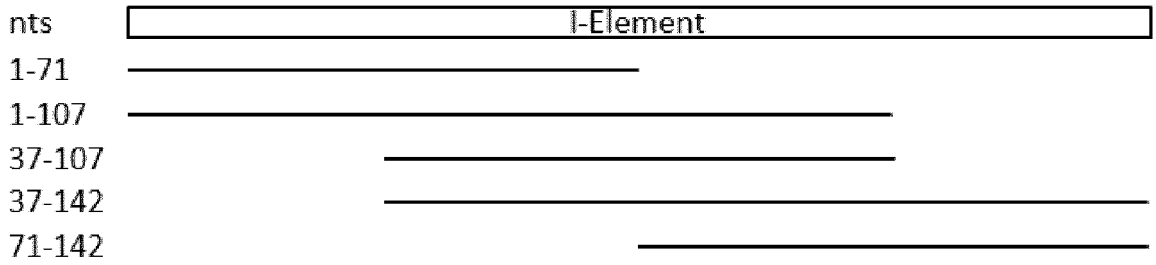

relative RNA half-life
1,4
1,2
1
0,8
0,6
0,4
0,2
0
F1-68
F1-102
F35-102
F35-136
F68-136
F-UTR

Figure 12 B


nts
I-Element
1-71
1-107
37-107
37-142
71-142

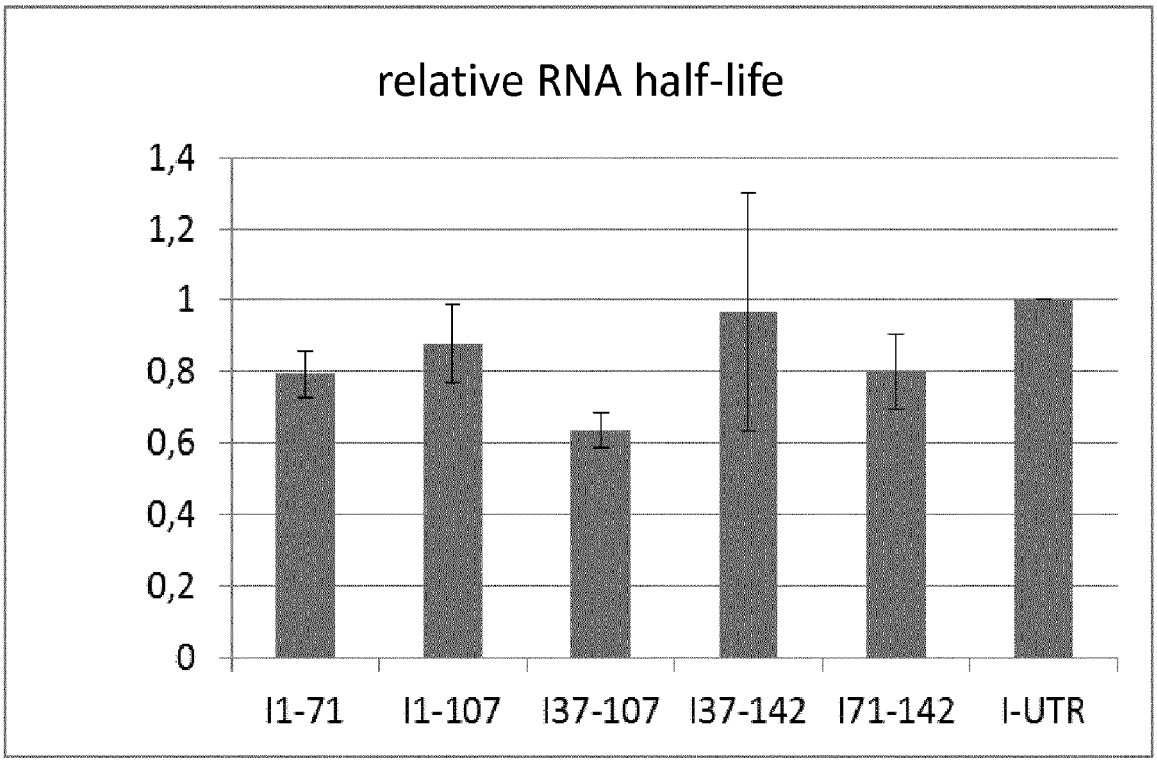
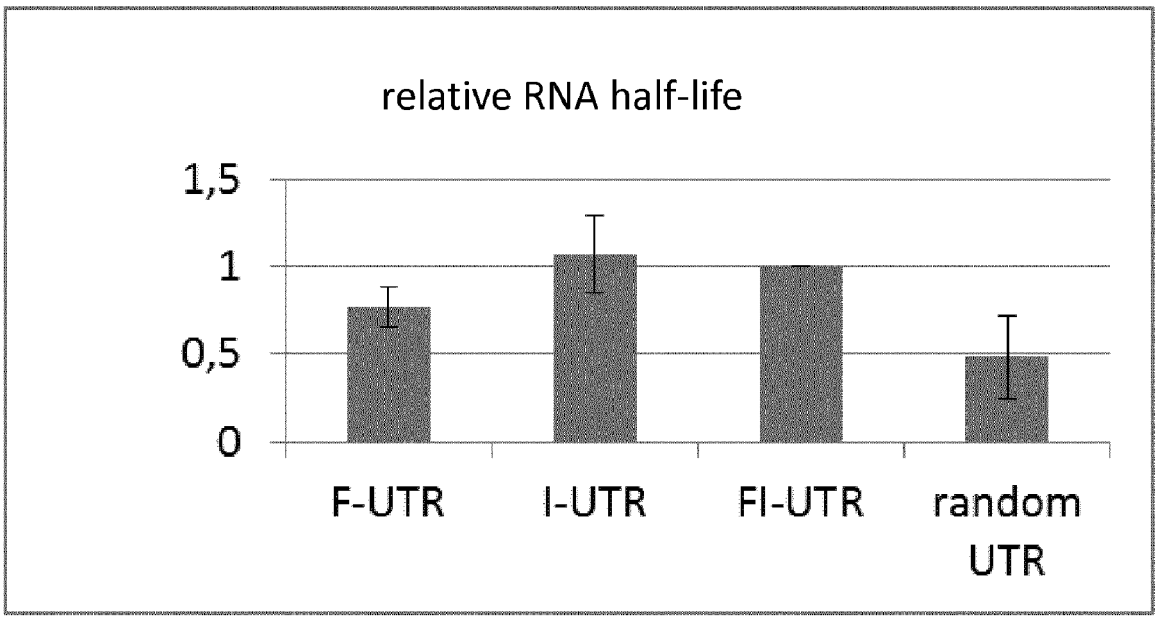

relative RNA half-life
1,4
1,2
1
0,8
0,6
0,4
0,2
0
I1-71
I1-107
I37-107
I37-142
I71-142
I-UTR

Figure 13


relative RNA half-life
1,5
1
0,5
0
F-UTR
I-UTR
FI-UTR
random UTR

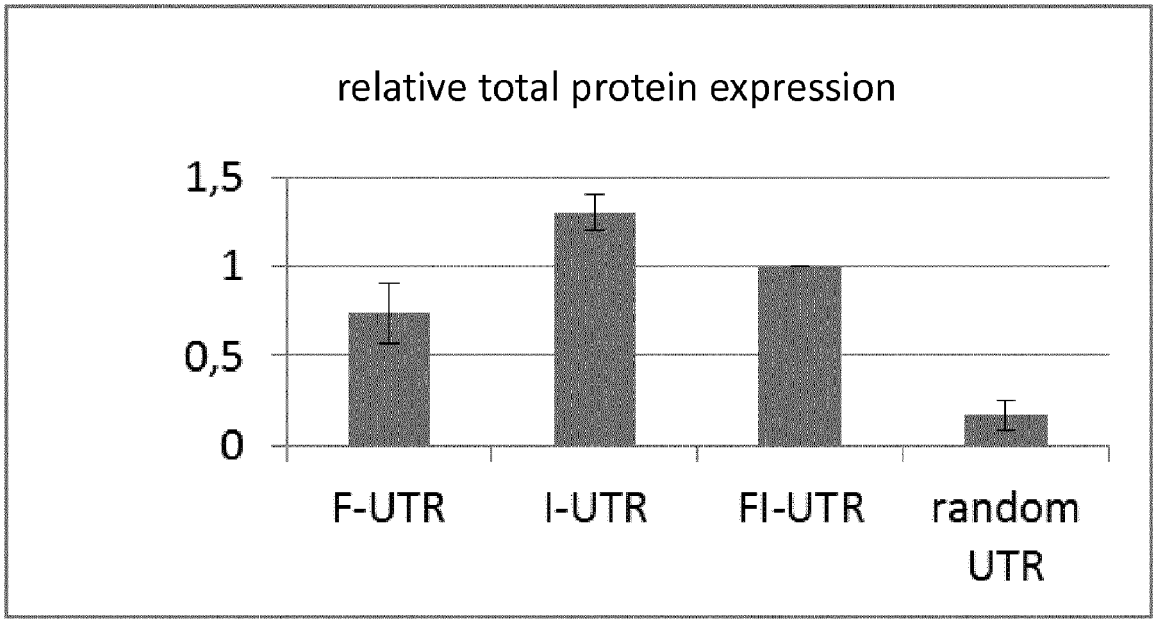
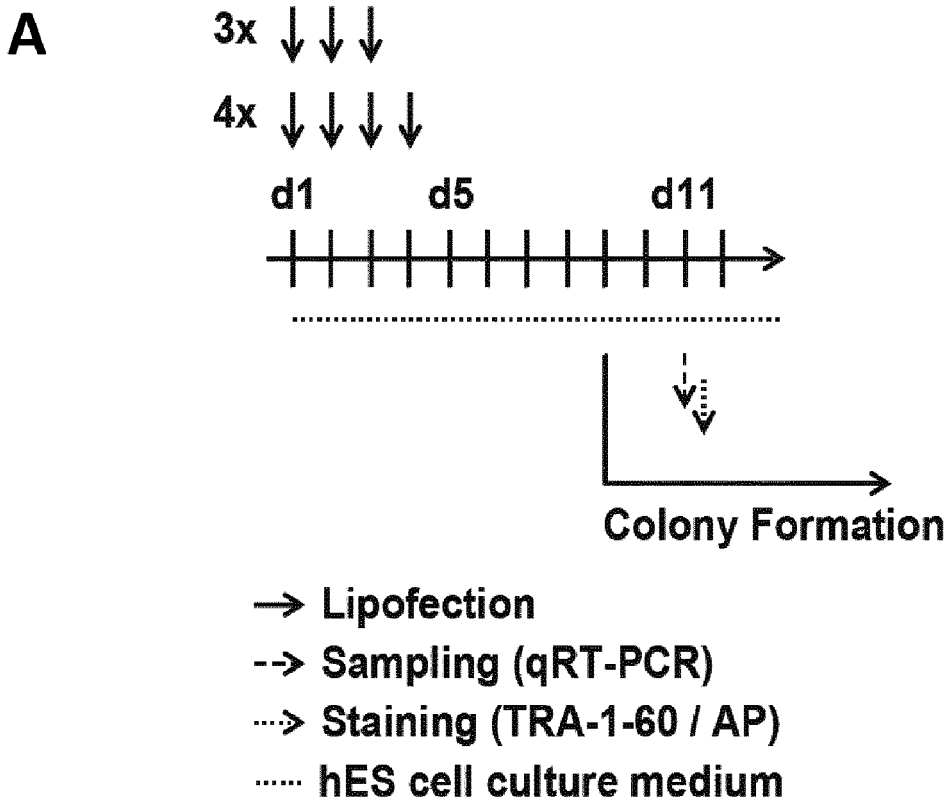

relative total protein expression
1,5
1
0,5
0
F-UTR
I-UTR
FI-UTR
random UTR

Figure 14


A
3x
4x
d1
d5
d11
Colony Formation
→ Lipofection
--> Sampling (qRT-PCR)
···> Staining (TRA-1-60 / AP)
····· hES cell culture medium

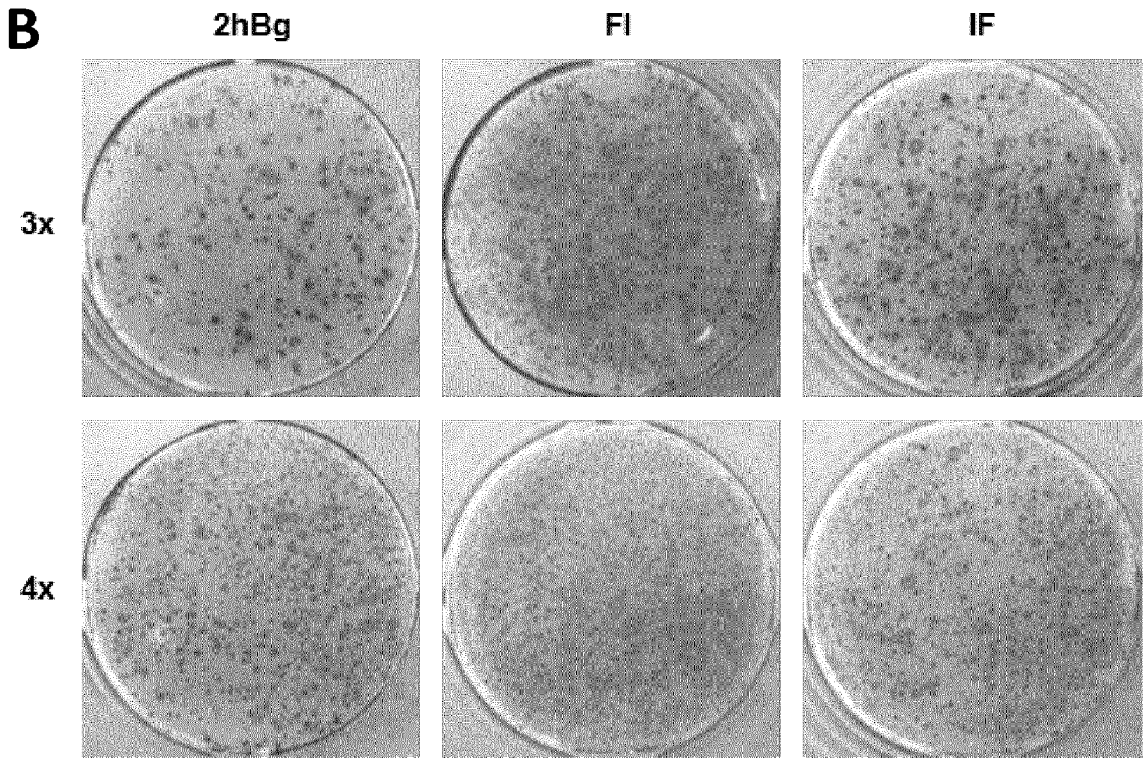

B
2hBg
FI
IF
3x
4x

Figure 14

C

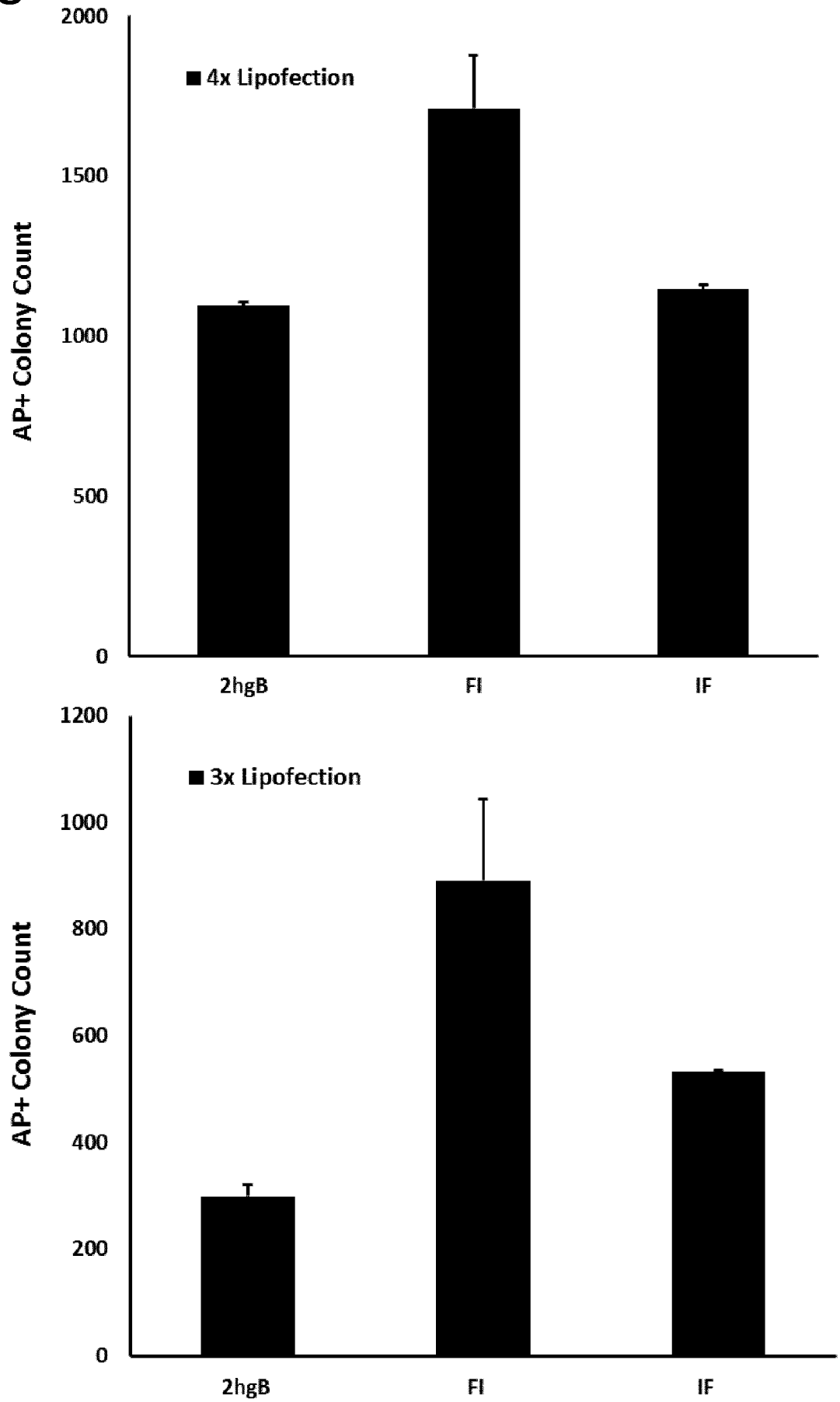
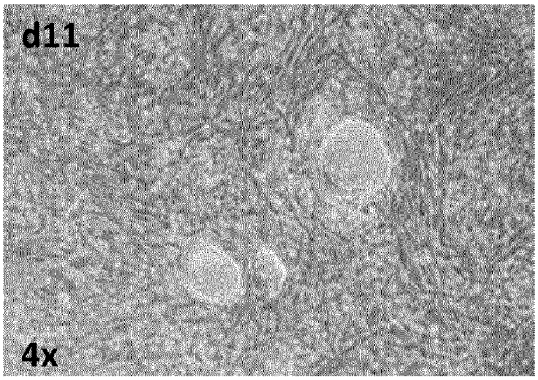
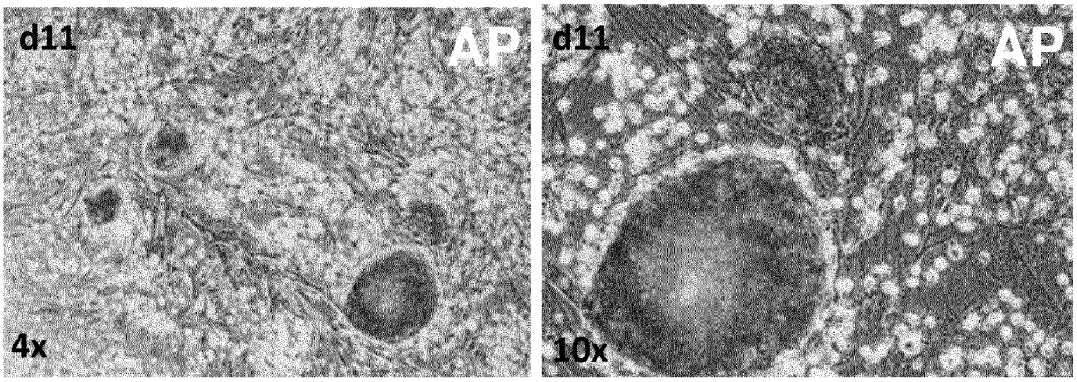

4x Lipofection
AP+ Colony Count
2000
1500
1000
500
0
2hgB
FI
IF
3x Lipofection
AP+ Colony Count
1200
1000
800
600
400
200
0
2hgB
FI
IF

Figure 14

D



E



F

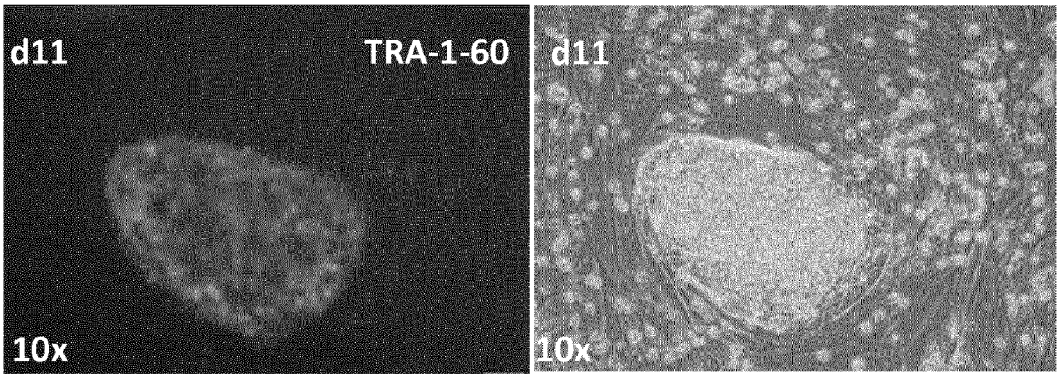
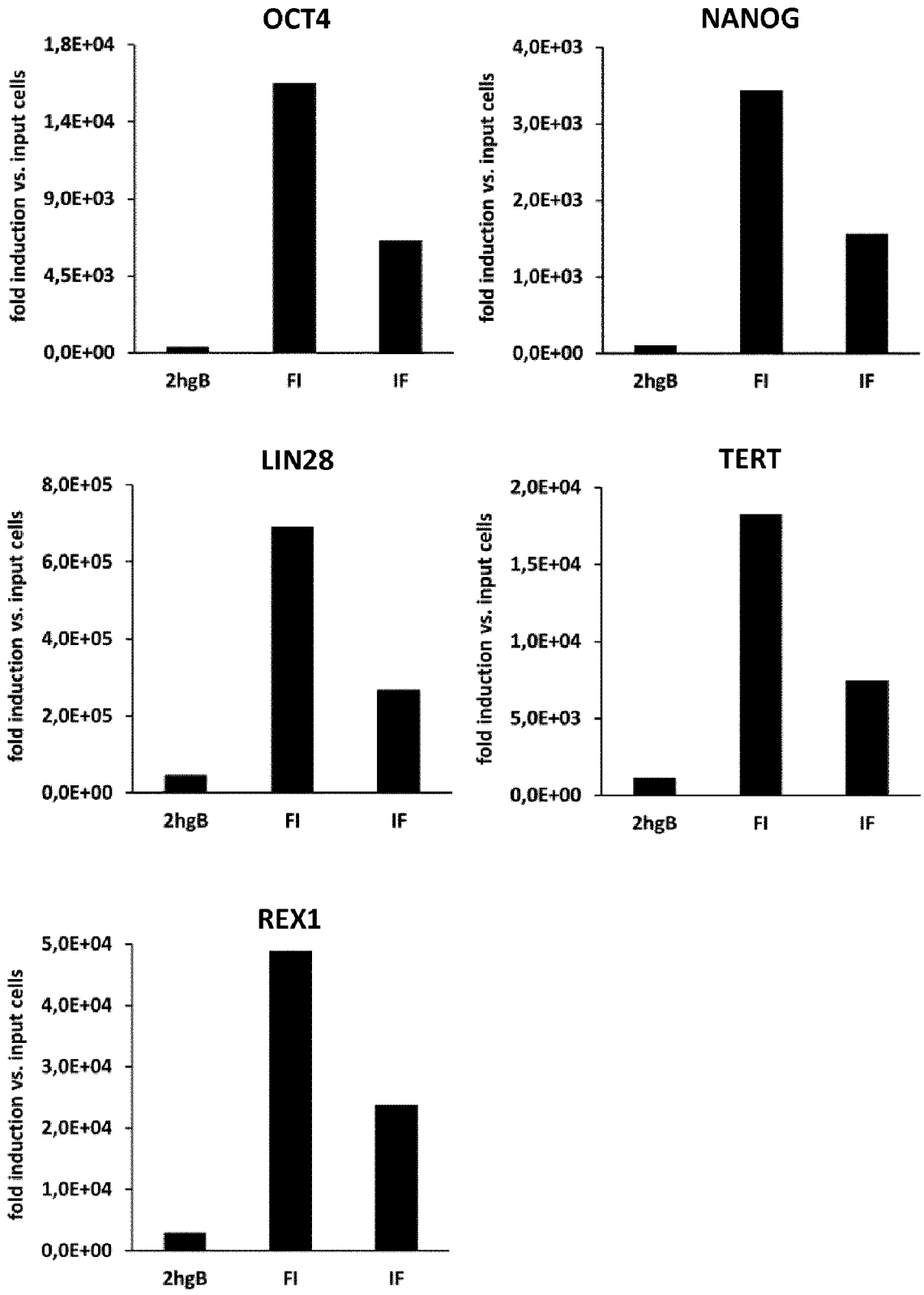


Figure 14 G


OCT4
fold induction vs. input cells
1,8E+04
1,4E+04
9,0E+03
4,5E+03
0,0E+00
2hgB
FI
IF
NANOG
fold induction vs. input cells
4,0E+03
3,0E+03
2,0E+03
1,0E+03
0,0E+00
2hgB
FI
IF
LIN28
fold induction vs. input cells
8,0E+05
6,0E+05
4,0E+05
2,0E+05
0,0E+00
2hgB
FI
IF
TERT
fold induction vs. input cells
2,0E+04
1,5E+04
1,0E+04
5,0E+03
0,0E+00
2hgB
FI
IF
REX1
fold induction vs. input cells
5,0E+04
4,0E+04
3,0E+04
2,0E+04
1,0E+04
0,0E+00
2hgB
FI
IF