Figure 1

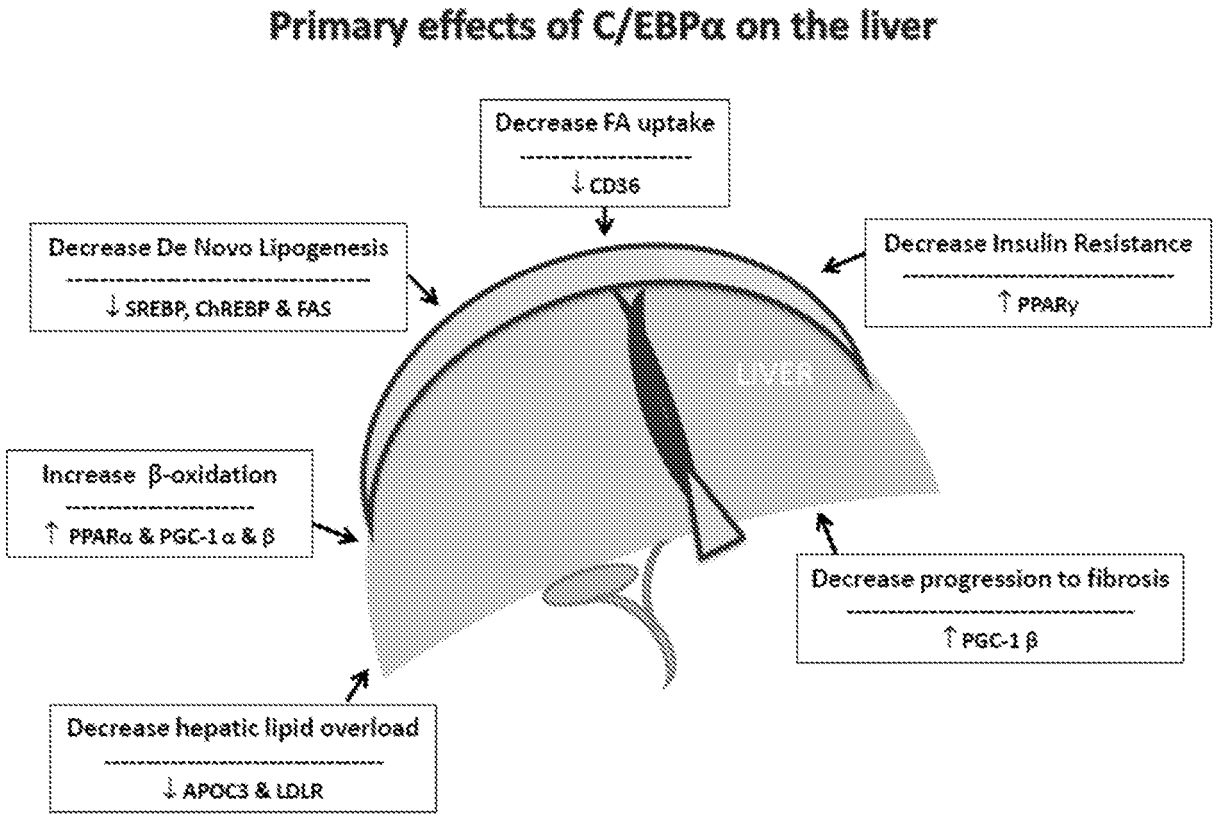

Primary effects of C/EBPα on the liver
Decrease FA uptake
↓ CD36
Decrease De Novo Lipogenesis
↓ SREBP, ChREBP & FAS
Decrease Insulin Resistance
↑ PPARγ
LIVER
Increase β-oxidation
↑ PPARα & PGC-1 α & β
Decrease progression to fibrosis
↑ PGC-1 β
Decrease hepatic lipid overload
↓ APOC3 & LDLR

Figure 2

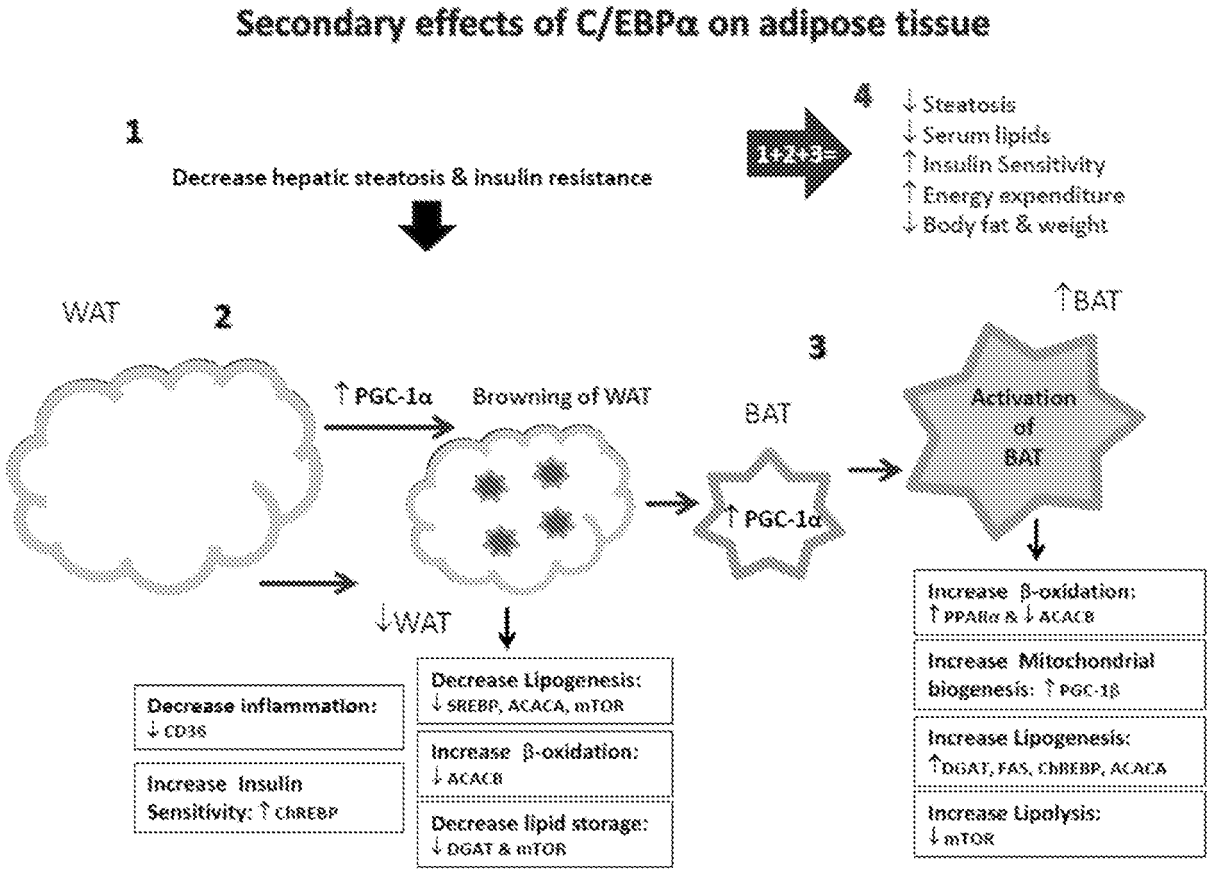

Secondary effects of C/EBPα on adipose tissue
1
Decrease hepatic steatosis & insulin resistance
4
↓ Steatosis
↓ Serum lipids
↑ Insulin Sensitivity
↑ Energy expenditure
↓ Body fat & weight
WAT
2
↑ PGC-1α
Browning of WAT
3
BAT
↑ PGC-1α
Activation of BAT
↑BAT
↓WAT
Decrease inflammation: ↓ CD36
Increase Insulin Sensitivity: ↑ ChREBP
Decrease Lipogenesis: ↓ SREBP, ACACA, mTOR
Increase β-oxidation: ↓ ACAC8
Decrease lipid storage: ↓ DGAT & mTOR
Increase β-oxidation: ↑ PPARα & ↓ ACAC8
Increase Mitochondrial biogenesis: ↑ PGC-1β
Increase Lipogenesis: ↑DGAT, FAS, ChREBP, ACACA
Increase Lipolysis: ↓ mTOR

Figure 3

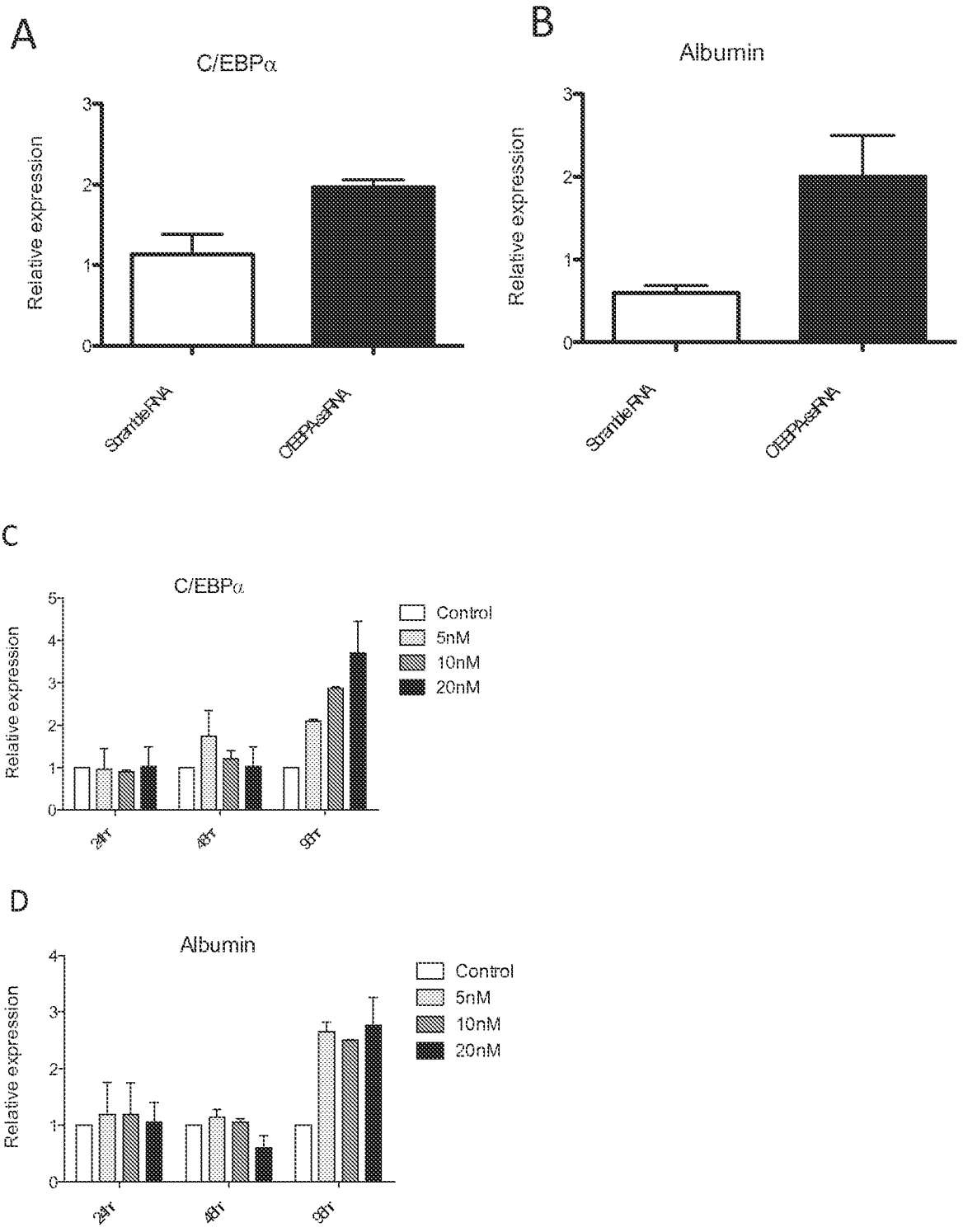

A
C/EBPα
Relative expression
0
1
2
3
StranteRNA
CEBPAsaRNA
B
Albumin
Relative expression
0
1
2
3
StranteRNA
CEBPAsaRNA
C
C/EBPα
Relative expression
0
1
2
3
4
5
24hr
48hr
96hr
Control
5nM
10nM
20nM
D
Albumin
Relative expression
0
1
2
3
4
24hr
48hr
96hr
Control
5nM
10nM
20nM

FIGURE 3, cont.

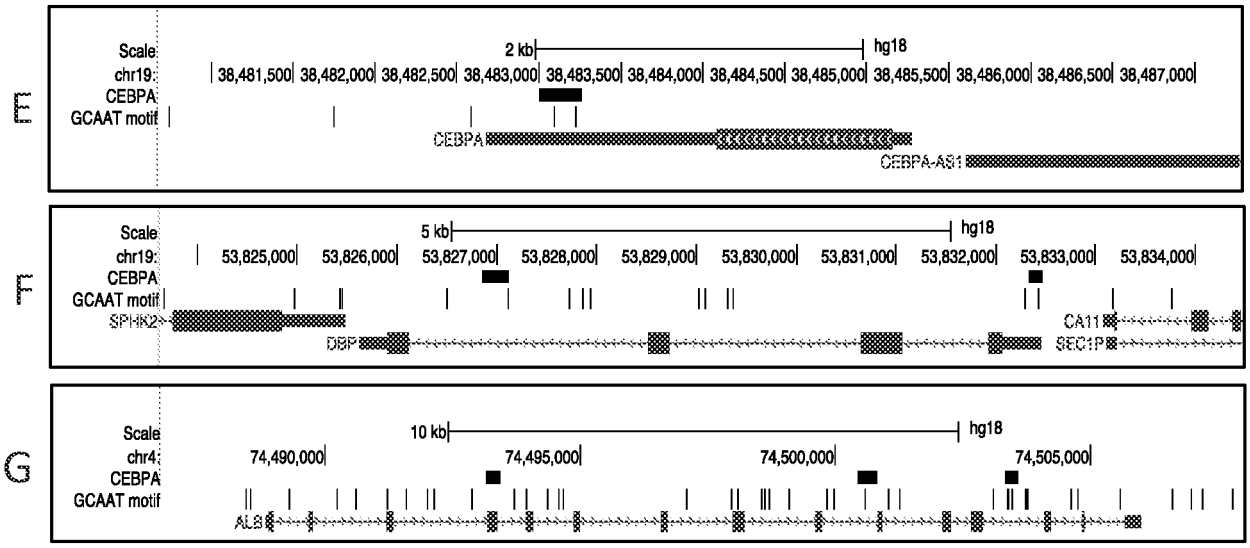


Figure 4

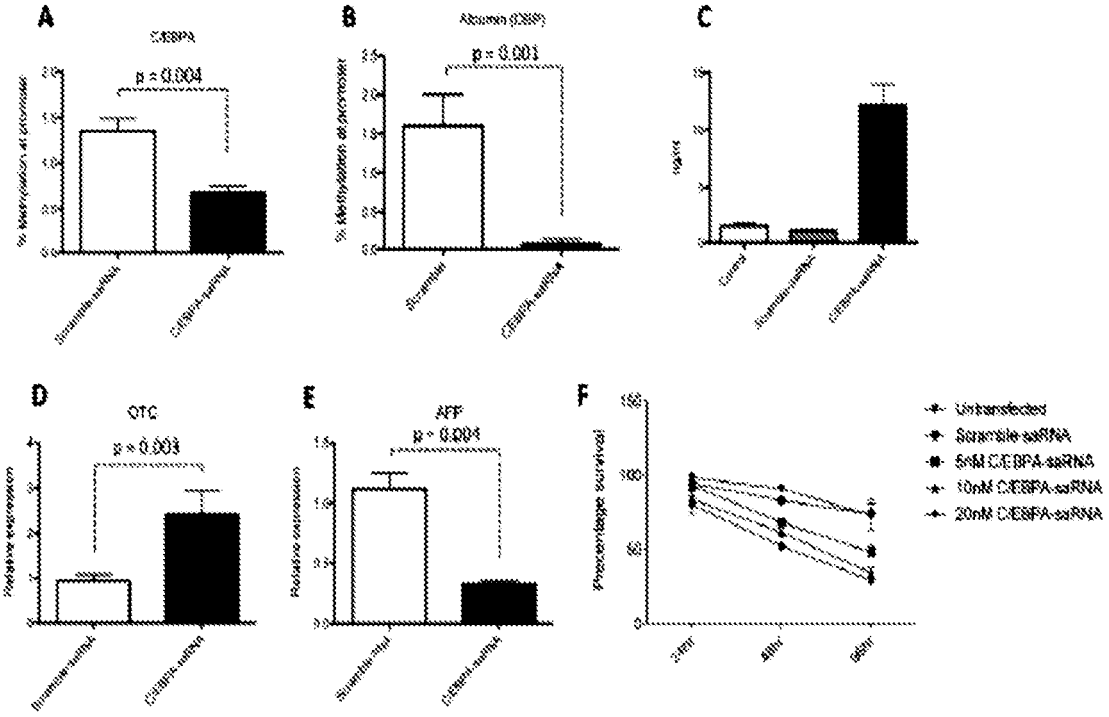

A
p = 0.004
B
p = 0.001
C
D
OTC
p = 0.003
E
AFP
p = 0.004
F
Untransfected
Scramble siRNA
5nM C/EBPA-siRNA
10nM C/EBPA-siRNA
20nM C/EBPA-siRNA

Figure 5

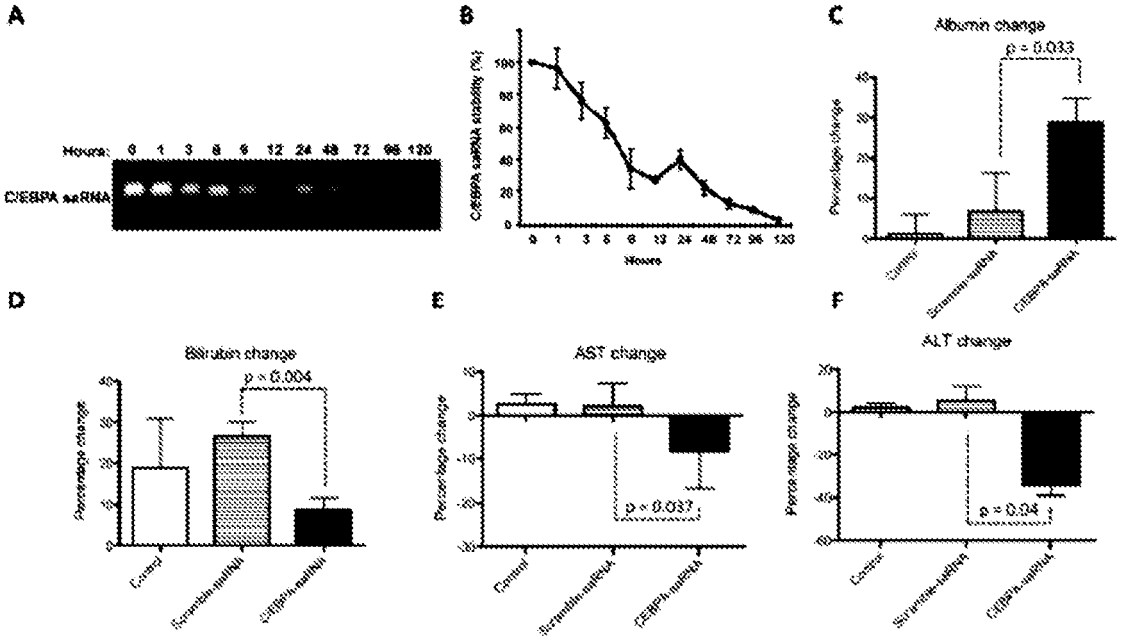

A
Hours: 0 1 3 6 9 12 24 48 72 96 120
C/EBPA saRNA
B
C/EBPA saRNA stability (%)
Hours
C
Albumin change
p = 0.033
Percentage change
Control
Scramble-saRNA
C/EBPA-saRNA
D
Bilirubin change
p = 0.004
Percentage change
Control
Scramble-saRNA
C/EBPA-saRNA
E
AST change
p = 0.037
Percentage change
Control
Scramble-saRNA
C/EBPA-saRNA
F
ALT change
p = 0.04
Percentage change
Control
Scramble-saRNA
C/EBPA-saRNA

Figure 6

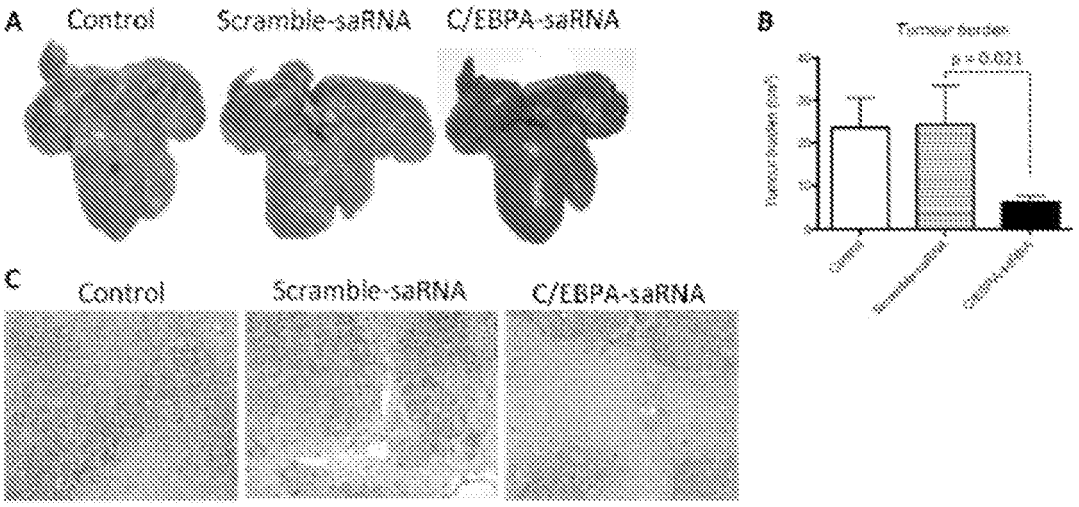

A
Control
Scramble-saRNA
C/EBPA-saRNA
B
p = 0.021
C
Control
Scramble-saRNA
C/EBPA-saRNA

Figure 7

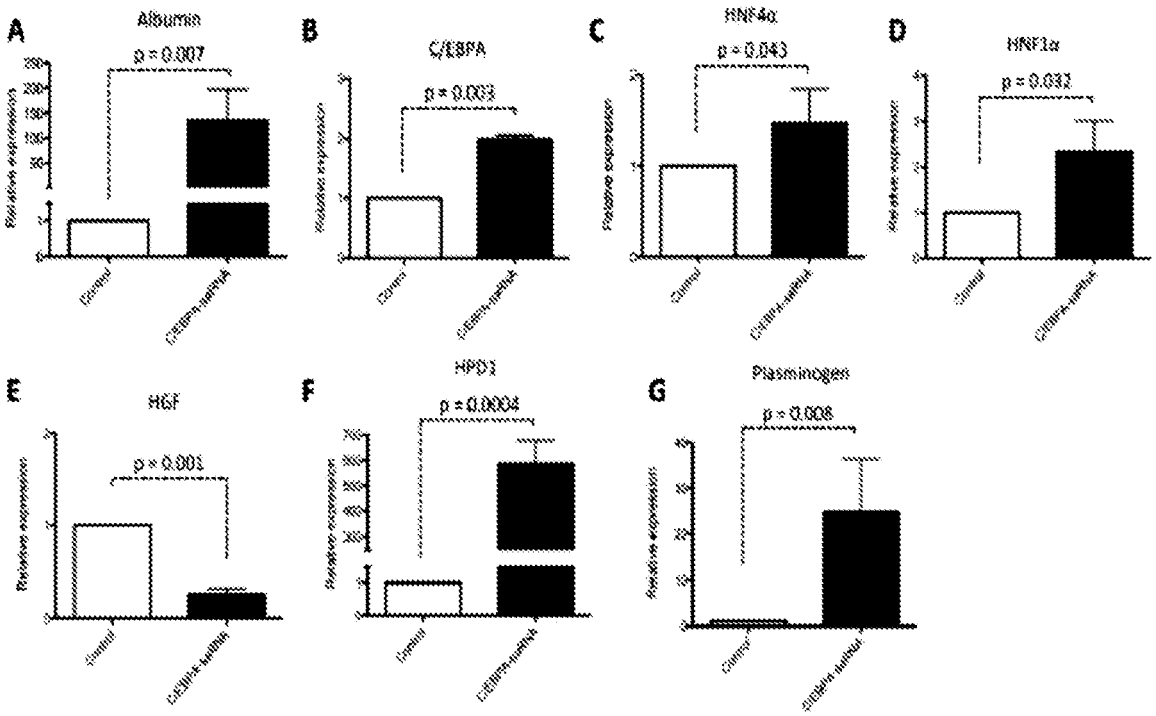

A
Albumin
p = 0.007
B
C/EBPA
p = 0.003
C
HNF4α
p = 0.043
D
HNF1α
p = 0.032
E
HGF
p = 0.001
F
HPD1
p = 0.0004
G
Plasminogen
p = 0.008
Control
C/EBPA-mRNA

Figure 8

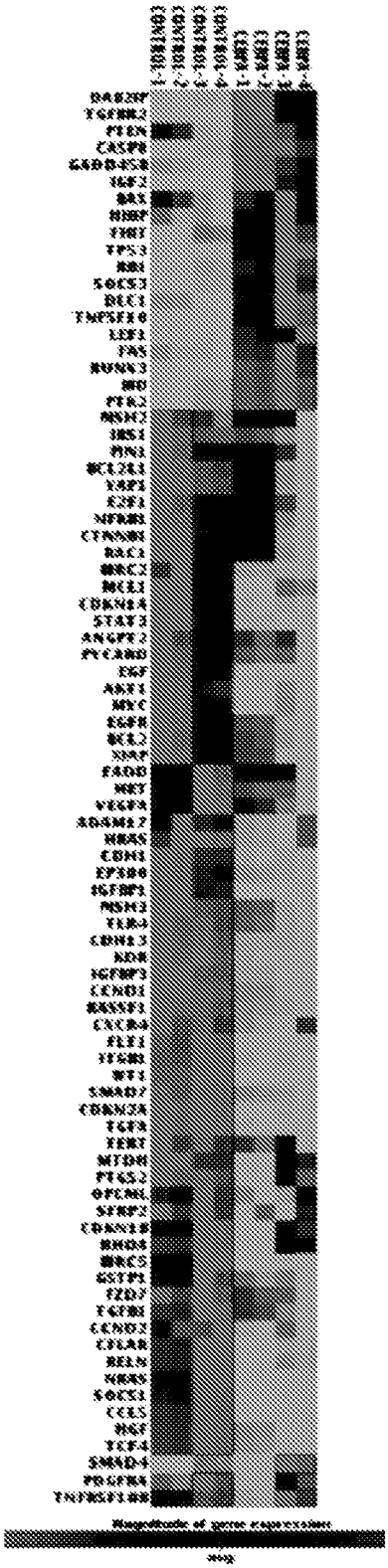
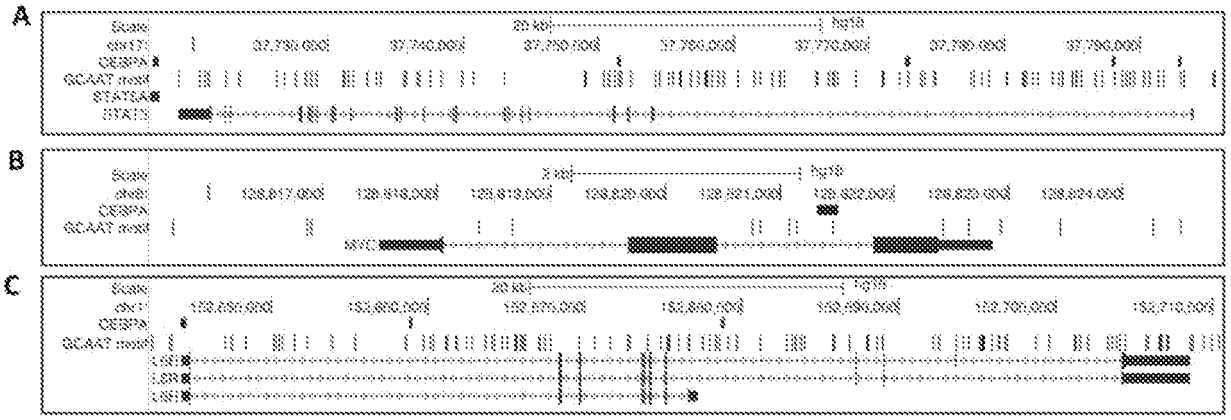


Figure 9


A
B
C

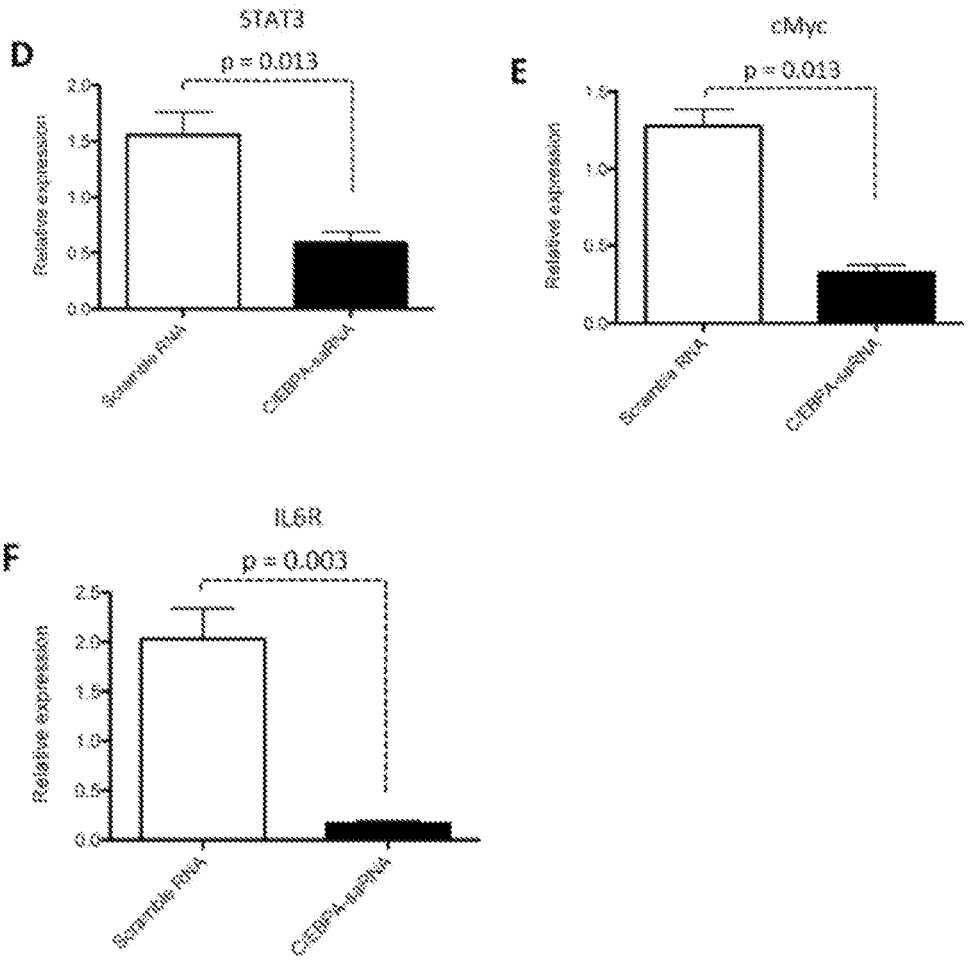

D
STAT3
p = 0.013
Relative expression
Scramble RNA
C/EBPA-siRNA
E
cMyc
p = 0.013
Relative expression
Scramble RNA
C/EBPA-siRNA
F
IL6R
p = 0.003
Relative expression
Scramble RNA
C/EBPA-siRNA

Figure 10

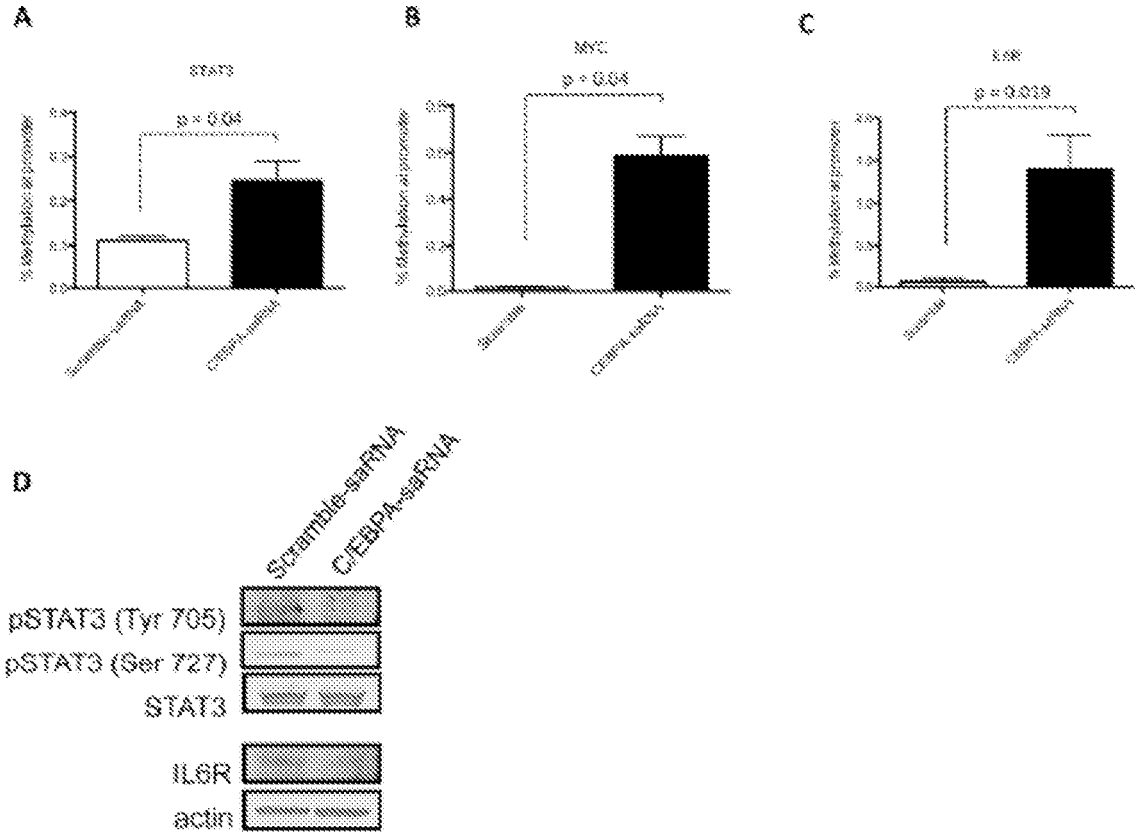
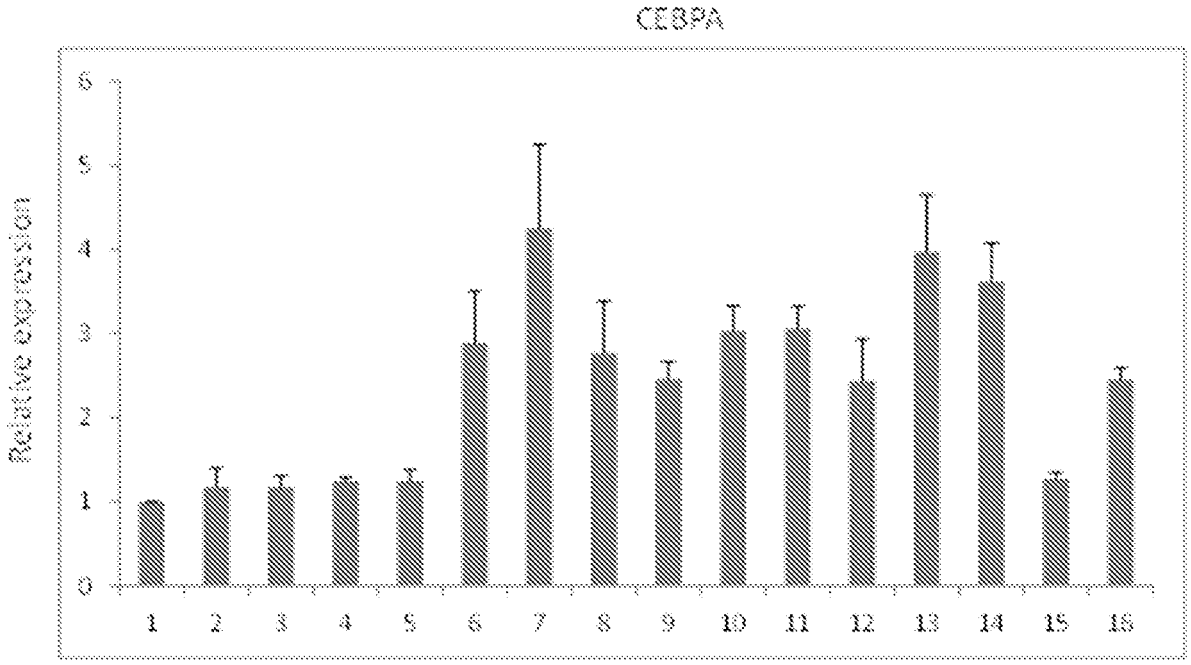

A
B
C
D
p = 0.04
p = 0.04
p = 0.029
Scramble-saRNA
C/EBPA-saRNA
pSTAT3 (Tyr 705)
pSTAT3 (Ser 727)
STAT3
IL6R
actin

Figure 11



1. control. No saRNA
2. saRNA AW1
3. dendrimer1; sample 2-2
4. dendrimer1; sample 2-1
5. dendrimer2
6. saRNA AW1/dendrimer1; sample 2-2, complex 1:1
7. saRNA AW1/dendrimer1; sample 2-2, complex 1:2
8. saRNA AW1/dendrimer1; sample 2-2, complex 1:3
9. saRNA AW1/dendrimer1; sample 2-1, complex 1:1
10. saRNA AW1/dendrimer1; sample 2-1, complex 1:2
11. saRNA AW1/dendrimer1; sample 2-1, complex 1:3
12. saRNA AW1/dendrimer2, complex 1:1
13. saRNA AW1/dendrimer2, complex 1:2
14. saRNA AW1/dendrimer2, complex 1:3
15. Nanofectamine
16. saRNA AW1/nanofectamine

Figure 12

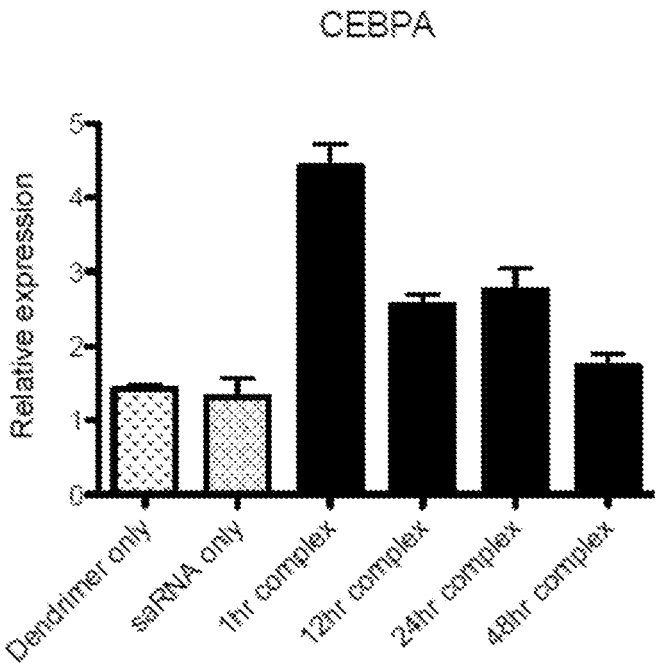

CEBPA
Relative expression
0
1
2
3
4
5
Dendrimer only
saRNA only
1hr complex
12hr complex
24hr complex
48hr complex

Figure 13

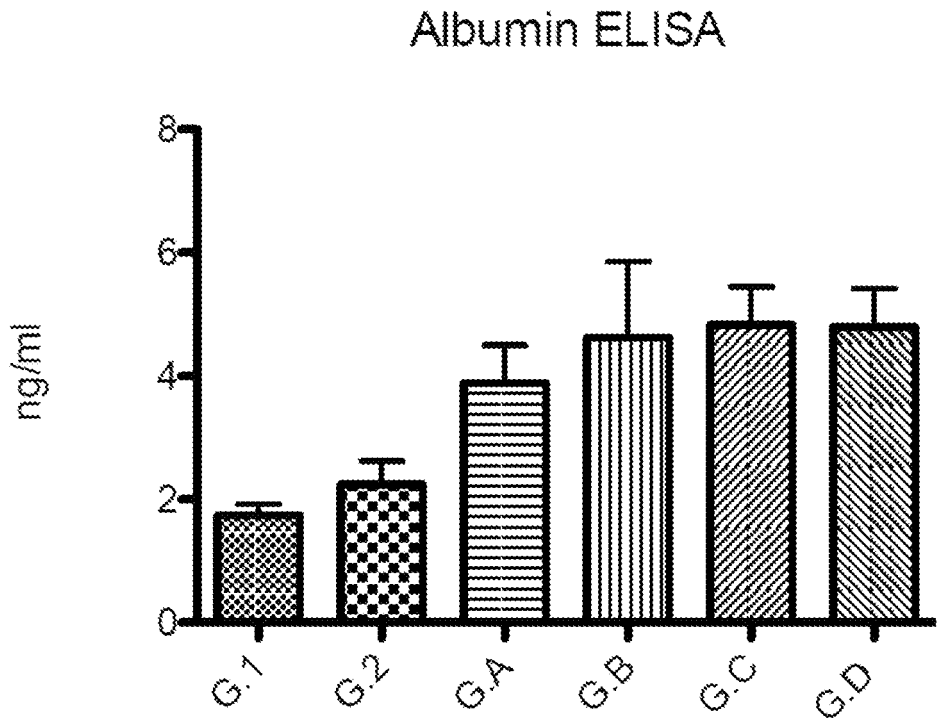


Figure 14

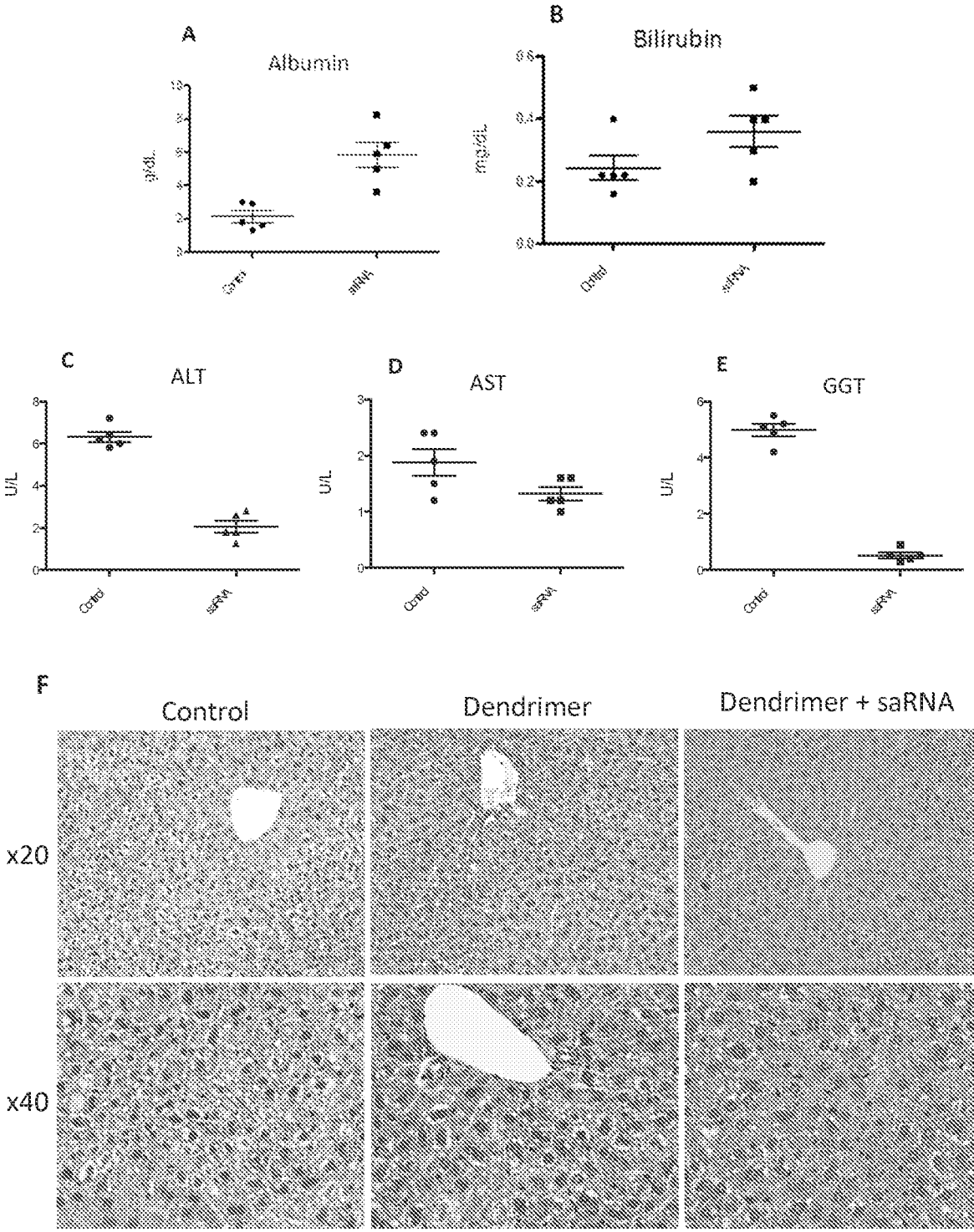

A
Albumin
g/dL
Control
siRNA
B
Bilirubin
mg/dL
Control
siRNA
C
ALT
U/L
Control
siRNA
D
AST
U/L
Control
siRNA
E
GGT
U/L
Control
siRNA
F
Control
Dendrimer
Dendrimer + saRNA
x20
x40

Figure 15

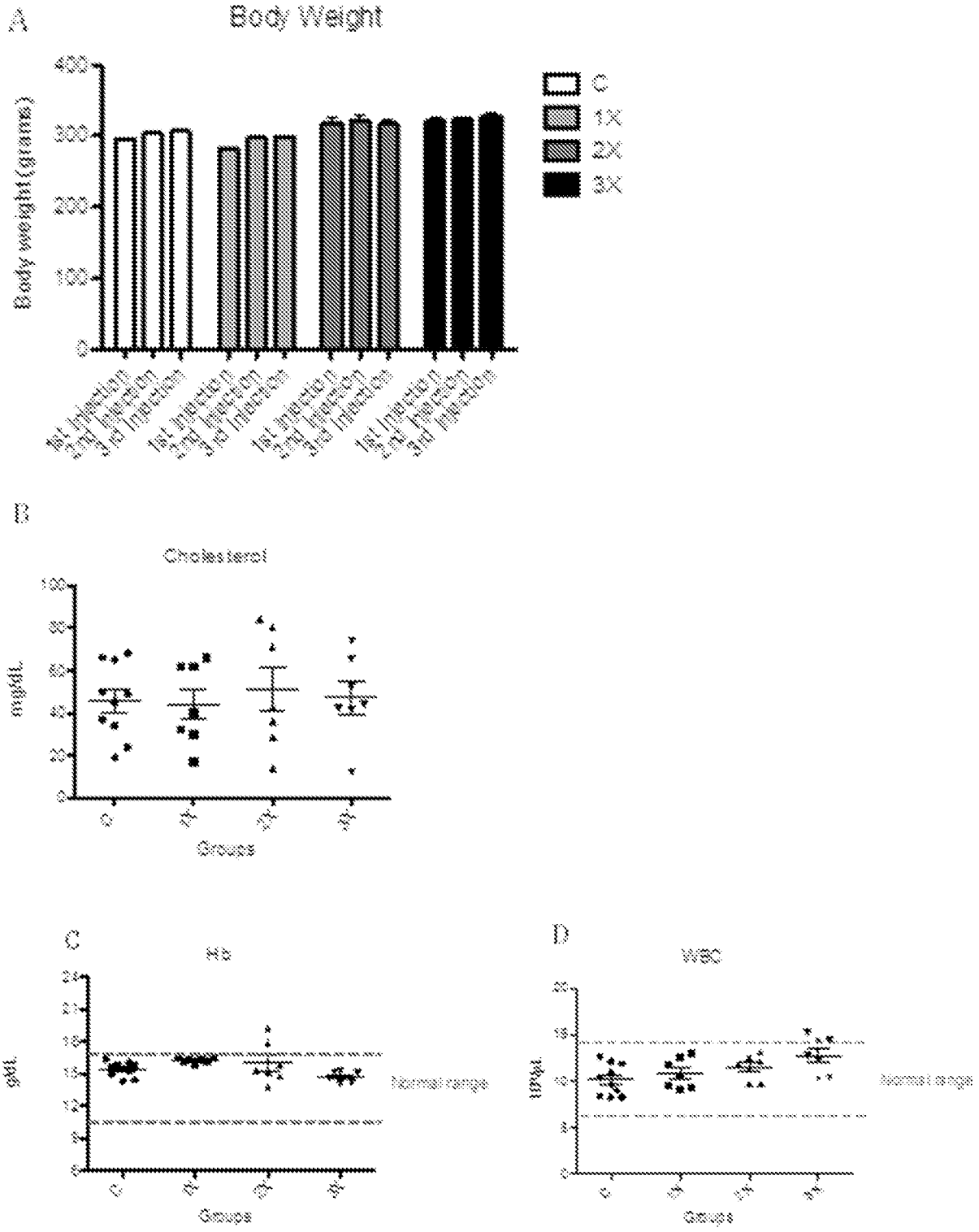

A
Body Weight
Body weight (grams)
400
300
200
100
0
C
1X
2X
3X
1st injection
2nd injection
3rd injection
B
Cholesterol
mg/dL
Groups
C
Hb
g/dL
Normal range
Groups
D
WBC
10^3/µL
Normal range
Groups

Figure 15, cont.

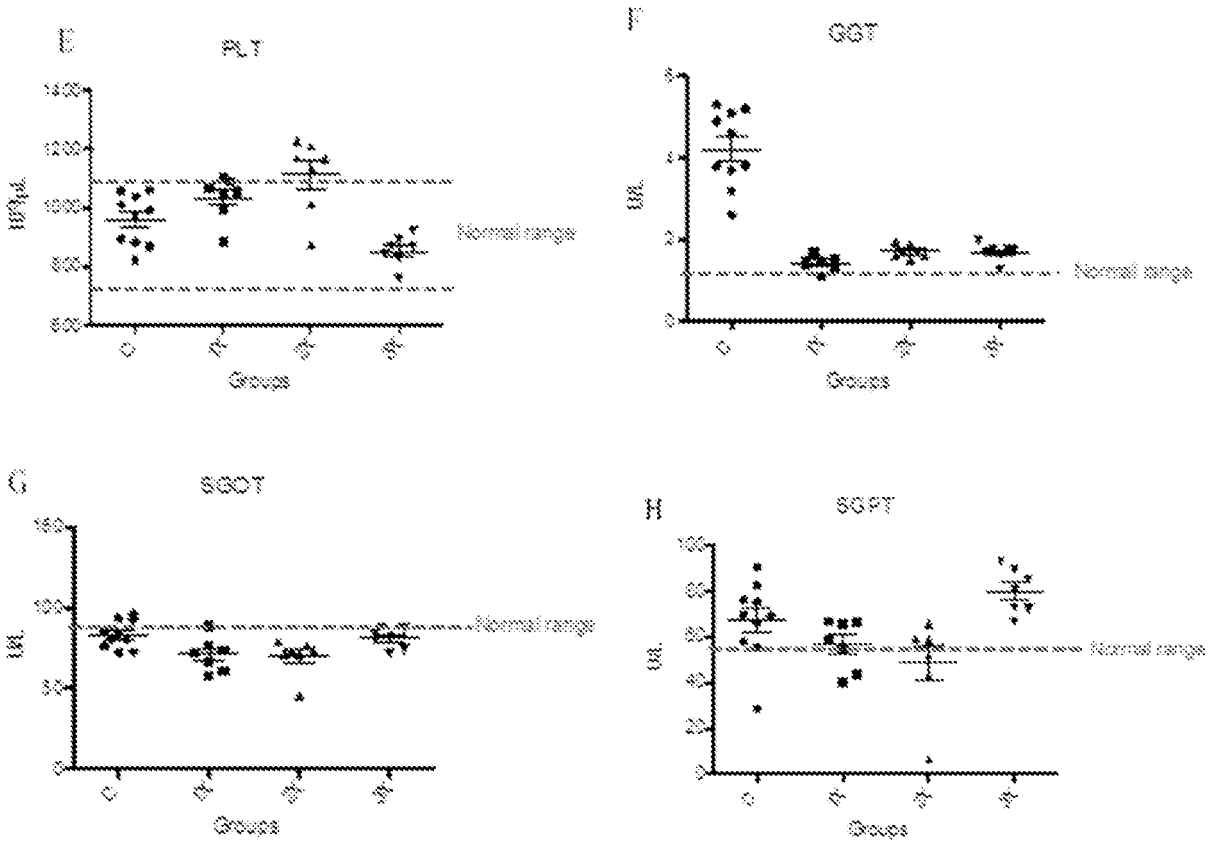
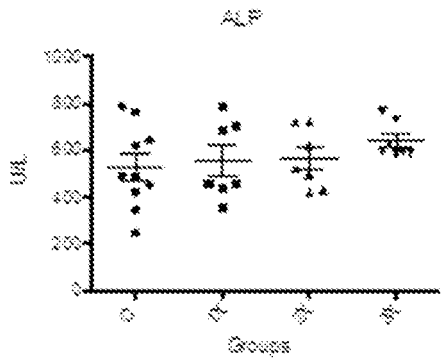
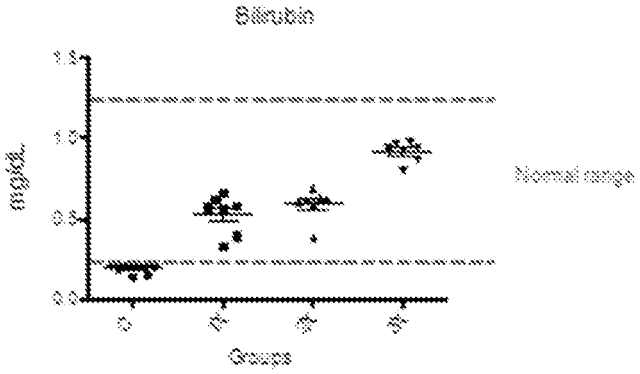


Figure 15, cont.

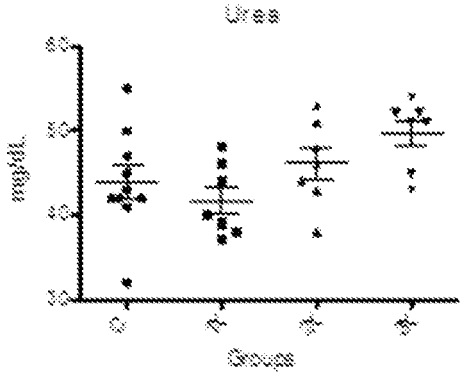
I



J



K



L

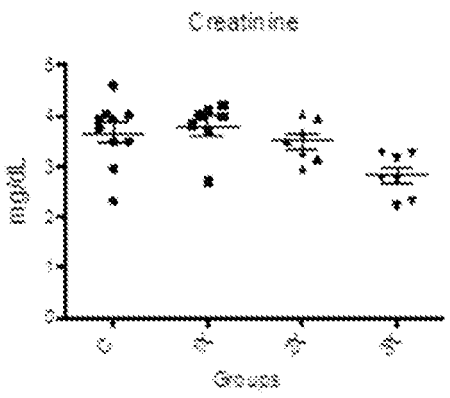


Figure 16

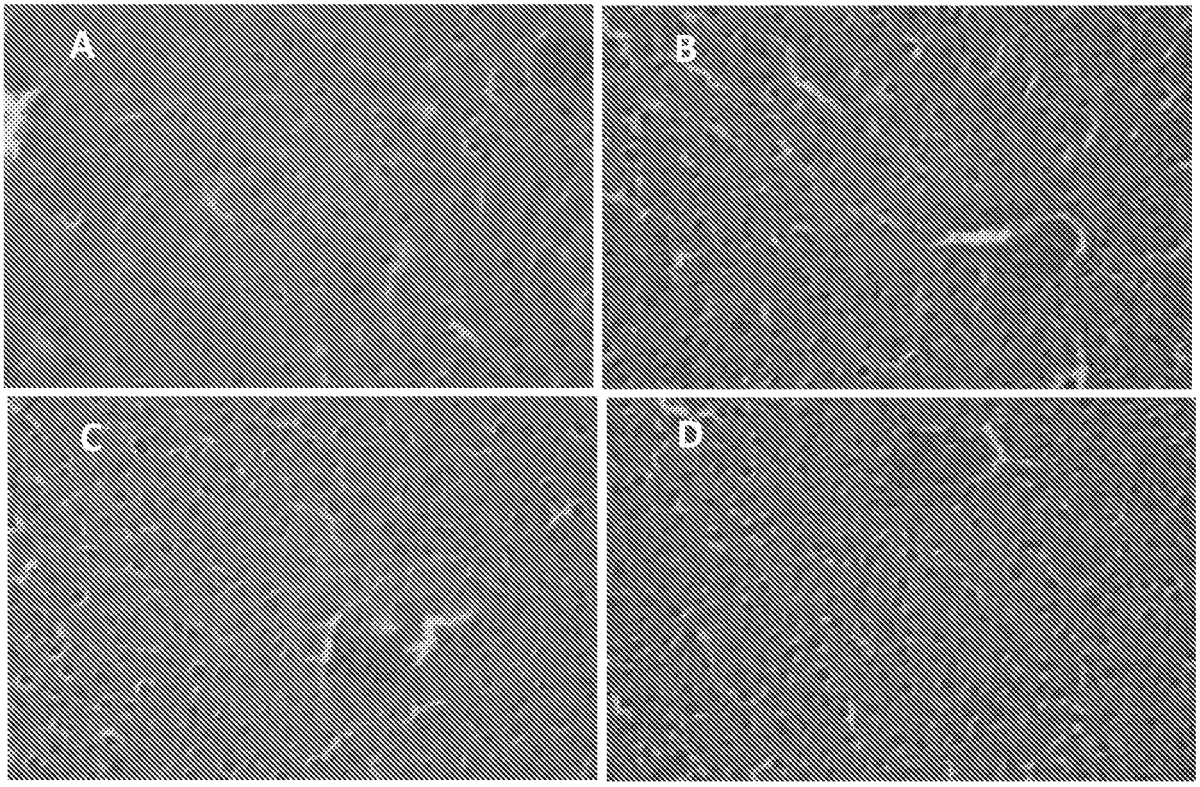


Figure 17

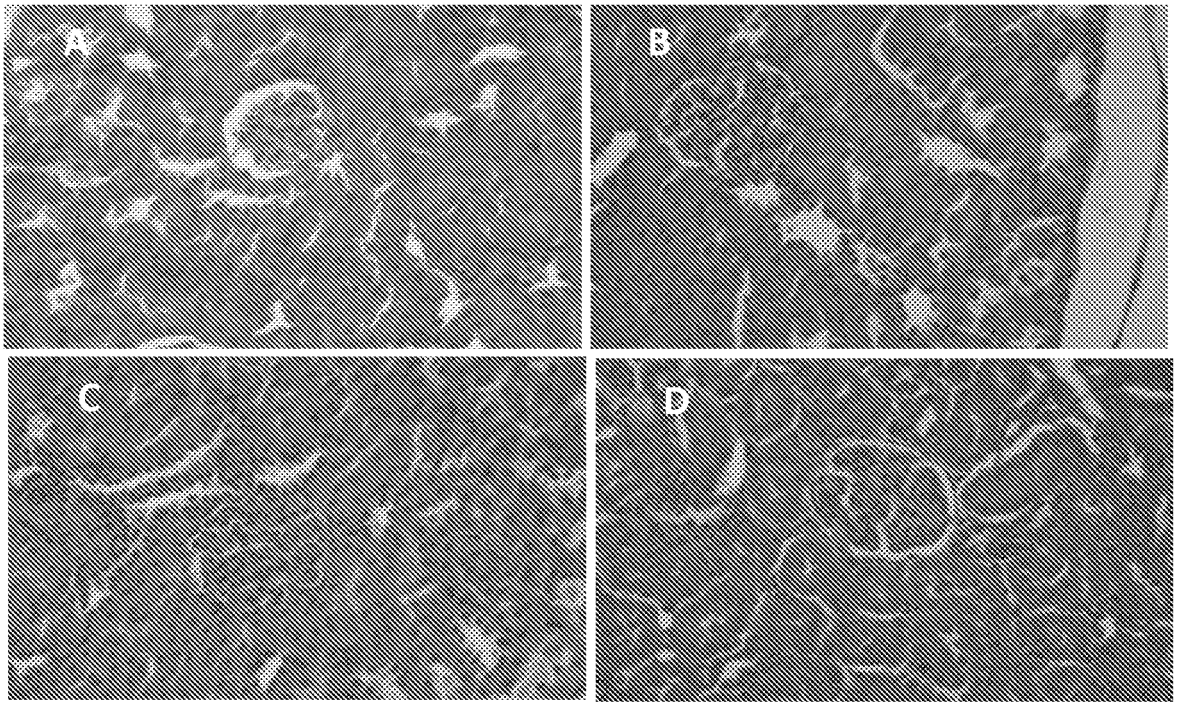


Figure 18

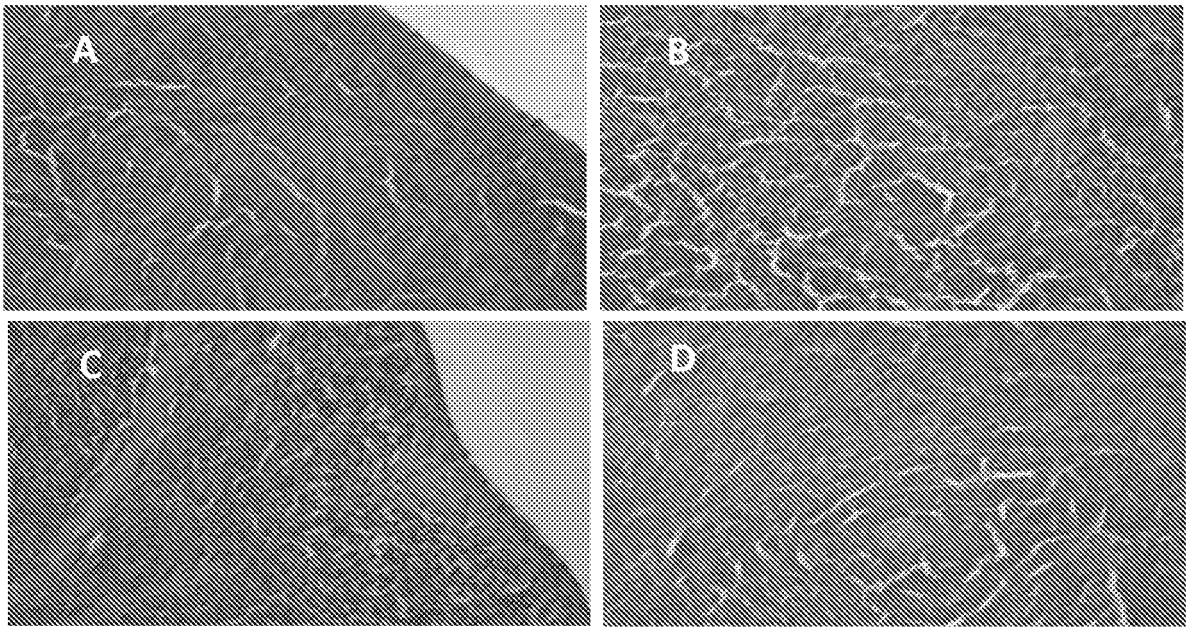


Figure 19

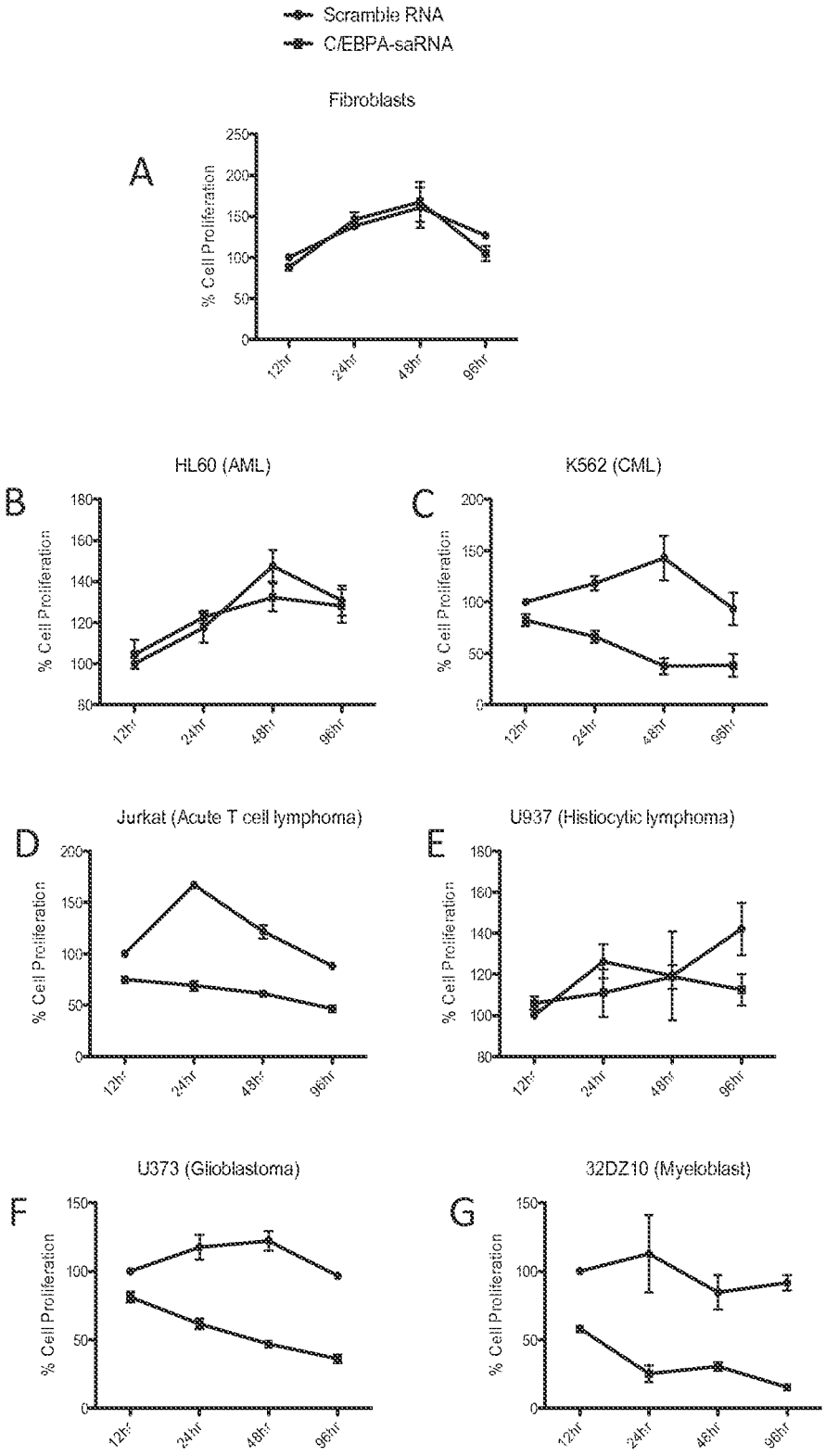
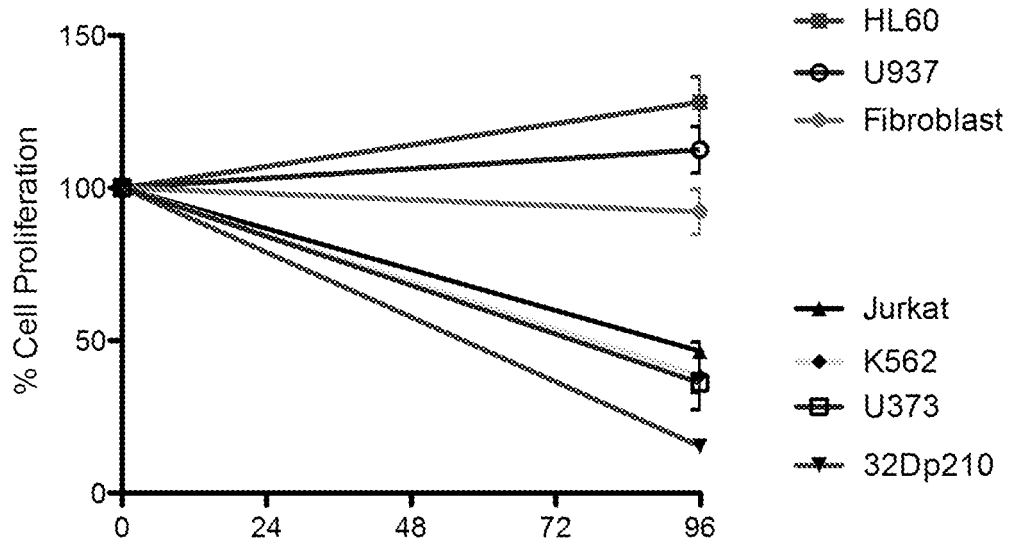

Scramble RNA
C/EBPA-saRNA
Fibroblasts
A
% Cell Proliferation
12hr
24hr
48hr
96hr
HL60 (AML)
B
K562 (CML)
C
Jurkat (Acute T cell lymphoma)
D
U937 (Histiocytic lymphoma)
E
U373 (Glioblastoma)
F
32DZ10 (Myeloblast)
G

Figure 20



Cell line	% Cell death
HL60 (AML)	0%
U937 (Histiocytic lymphoma)	0%
Fibroblast	10%
Jurkat (Acute T cell lymphoma)	54%
K562 (CML)	62%
U373 (Glioblastoma)	64%
32Dp210 (Myeloid leukemia)	85%

Figure 21

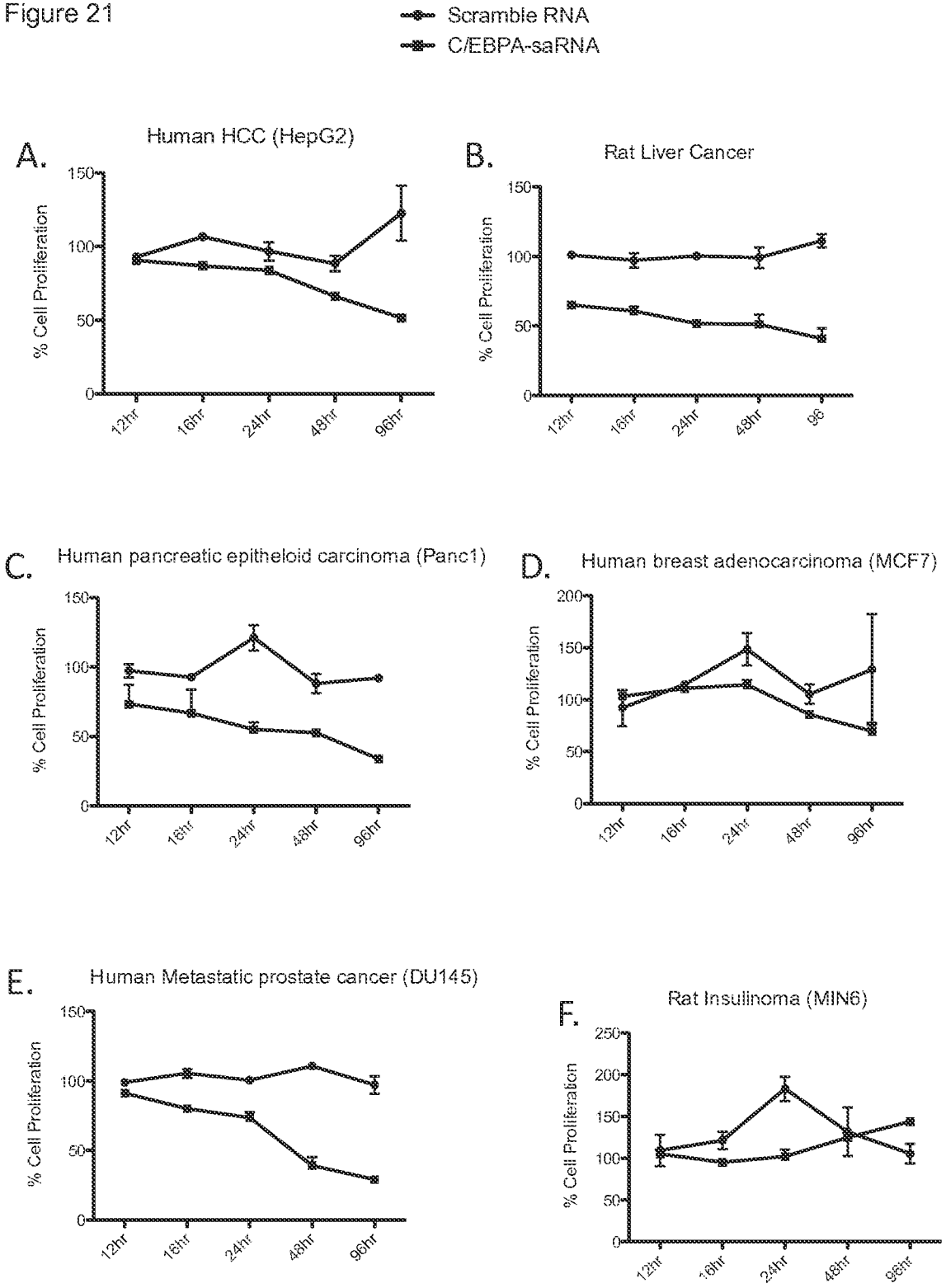
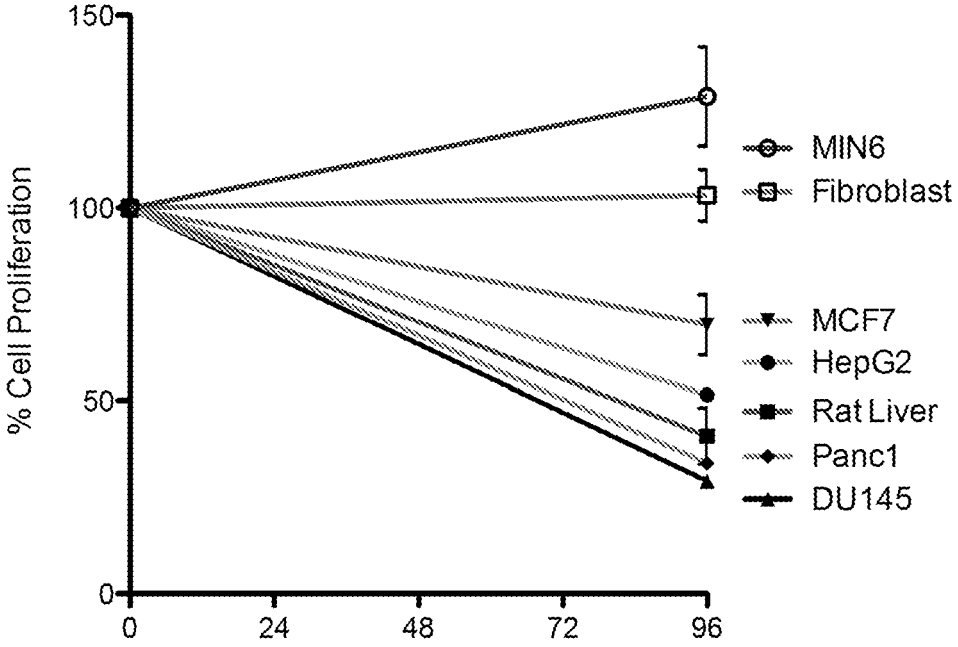

Scramble RNA
C/EBPA-saRNA
A.
Human HCC (HepG2)
B.
Rat Liver Cancer
C.
Human pancreatic epitheloid carcinoma (Panc1)
D.
Human breast adenocarcinoma (MCF7)
E.
Human Metastatic prostate cancer (DU145)
F.
Rat Insulinoma (MIN6)
% Cell Proliferation
12hr
16hr
24hr
48hr
96hr

Figure 22



Cell line	% Cell death
MIN6 (Rat Insulinoma)	0%
Fibroblast	0%
MCF7 (ER +ve Human breast cancer)	31%
HepG2 (Human HCC)	50%
Rat Liver	59%
Panc1 (Human Pancreatic Carcinoma)	66%
DU145 (Human metastatic Prostate Cancer)	71%

Figure 23

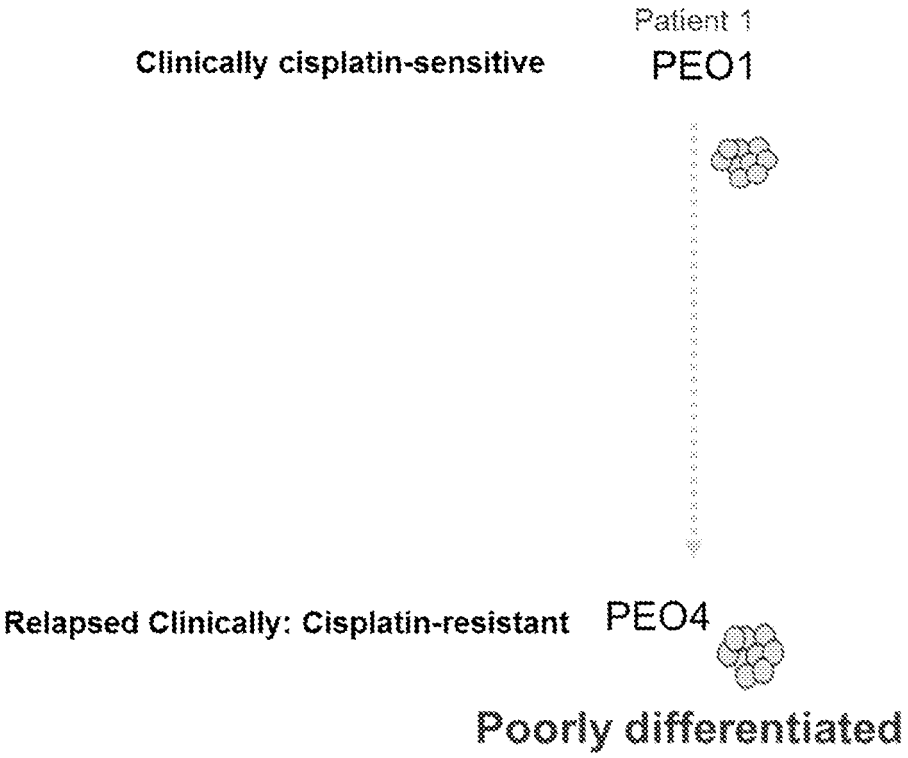
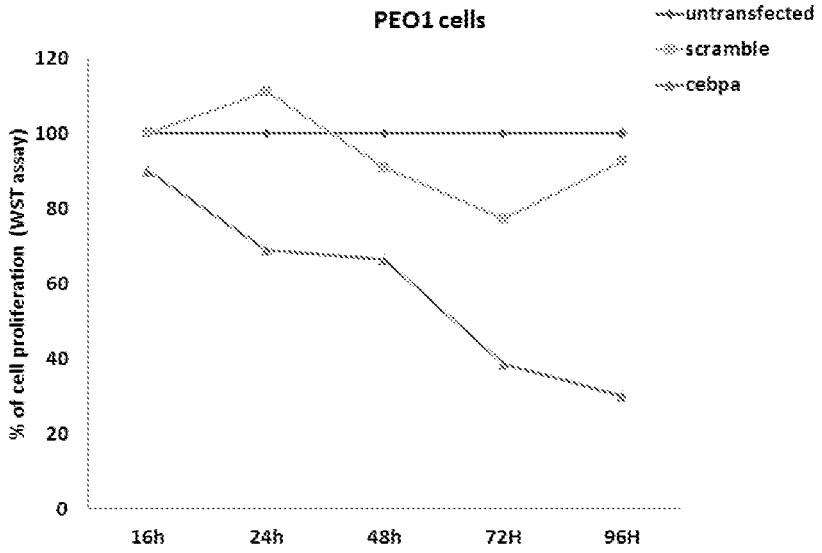
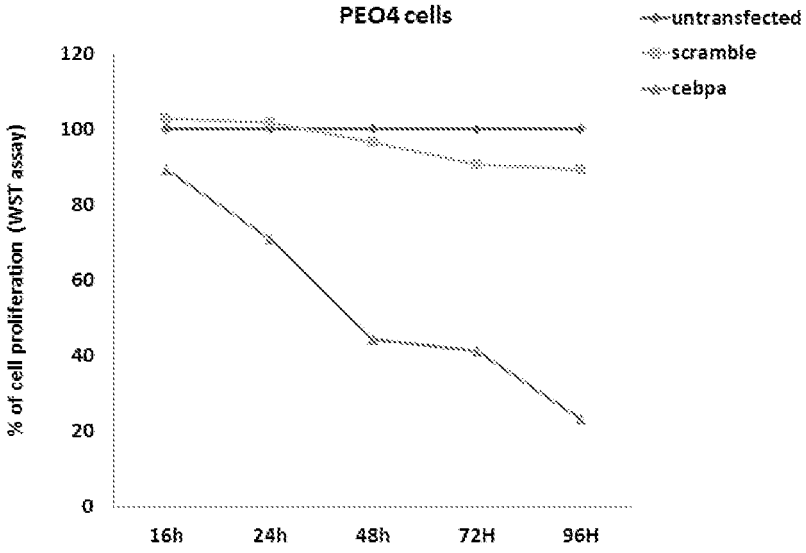

Patient 1
Clinically cisplatin-sensitive
PEO1
Relapsed Clinically: Cisplatin-resistant
PEO4
Poorly differentiated

Figure 24

A.



B.



C.

	16h	24h	48h	72H	96H
PEO1-untransfected	100	100	100	100	100
PEO1-scramble	100.0966	111.1156	90.91157	77.33881	92.50884
PEO1-cebpa	90.10618	68.90823	66.48472	38.74842	30.1099

	16h	24h	48h	72H	96H
PEO4-untransfected	100	100	100	100	100
PEO4-scramble	103.0379	101.7525	96.5309	90.68987	89.43513
PEO4-cebpa	89.39099	70.96851	44.38783	41.31064	23.28988

Figure 25

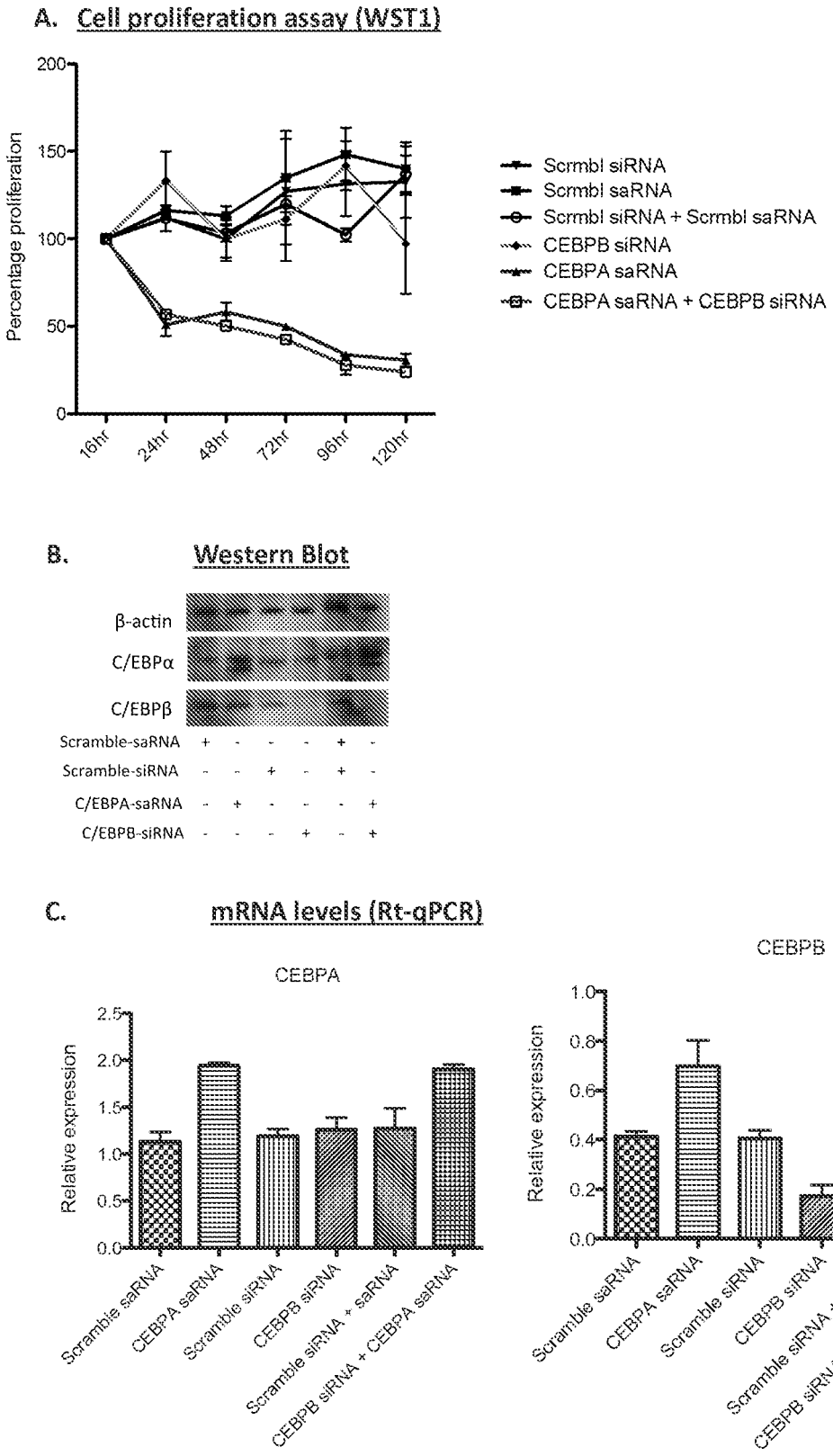
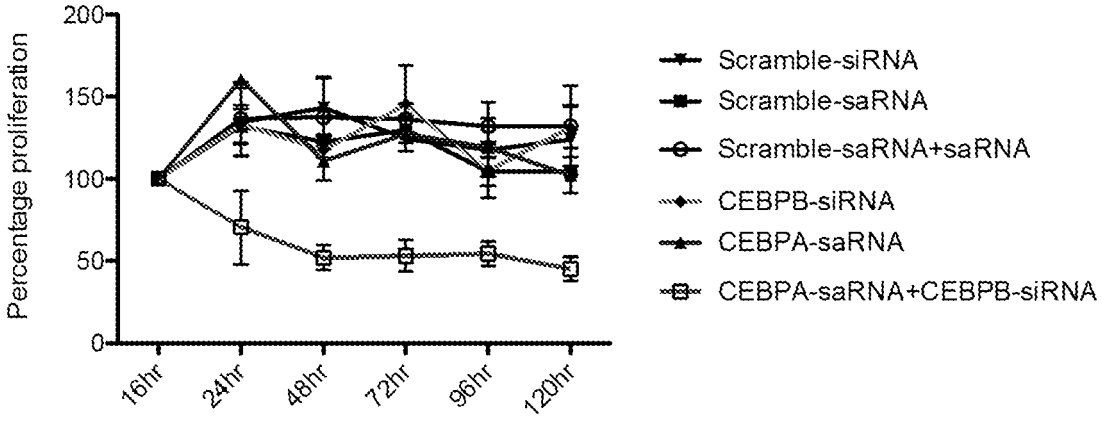
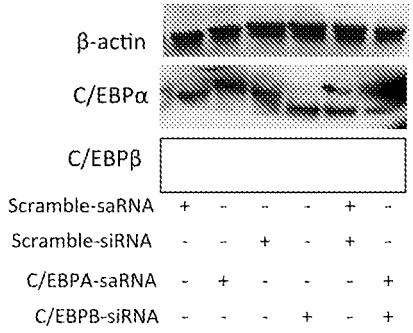

A. Cell proliferation assay (WST1)
Percentage proliferation
200
150
100
50
0
16hr
24hr
48hr
72hr
96hr
120hr
Scrmbl siRNA
Scrmbl saRNA
Scrmbl siRNA + Scrmbl saRNA
CEBPB siRNA
CEBPA saRNA
CEBPA saRNA + CEBPB siRNA
B. Western Blot
β-actin
C/EBPα
C/EBPβ
Scramble-saRNA + - - - + -
Scramble-siRNA - - + - + -
C/EBPA-saRNA - + - - - +
C/EBPB-siRNA - - - + - +
C. mRNA levels (Rt-qPCR)
CEBPA
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Scramble saRNA
CEBPA saRNA
Scramble siRNA
CEBPB siRNA
Scramble siRNA + saRNA
CEBPB siRNA + CEBPA saRNA
CEBPB
Relative expression
1.0
0.8
0.6
0.4
0.2
0.0
Scramble saRNA
CEBPA saRNA
Scramble siRNA
CEBPB siRNA
Scramble siRNA + saRNA
CEBPB siRNA + CEBPA saRNA

Figure 26

A. Cell proliferation assay (WST1)



B. Western Blot



C. mRNA levels (Rt-qPCR)

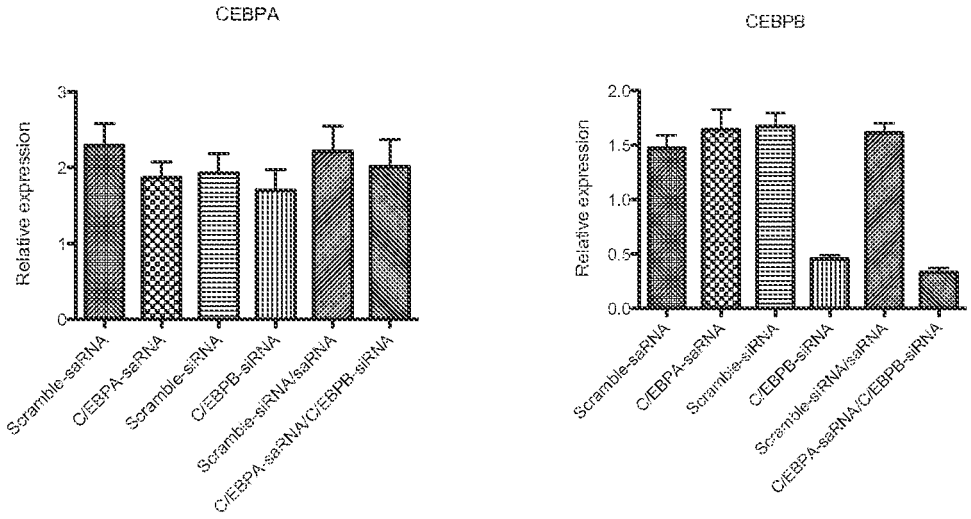


Figure 27

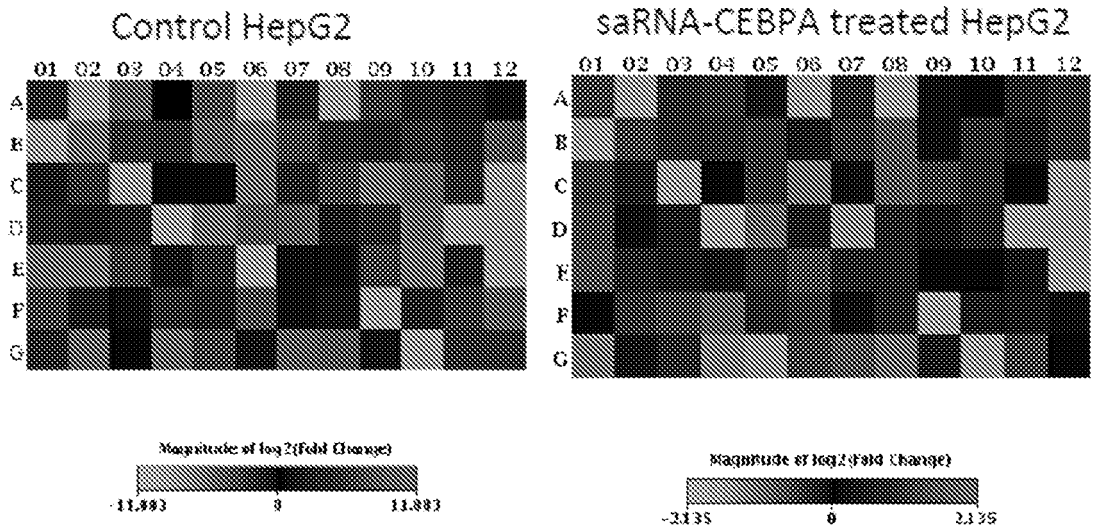

Control HepG2
01 02 03 04 05 06 07 08 09 10 11 12
A
B
C
D
E
F
G
saRNA-CEBPA treated HepG2
01 02 03 04 05 06 07 08 09 10 11 12
A
B
C
D
E
F
G

Figure 28

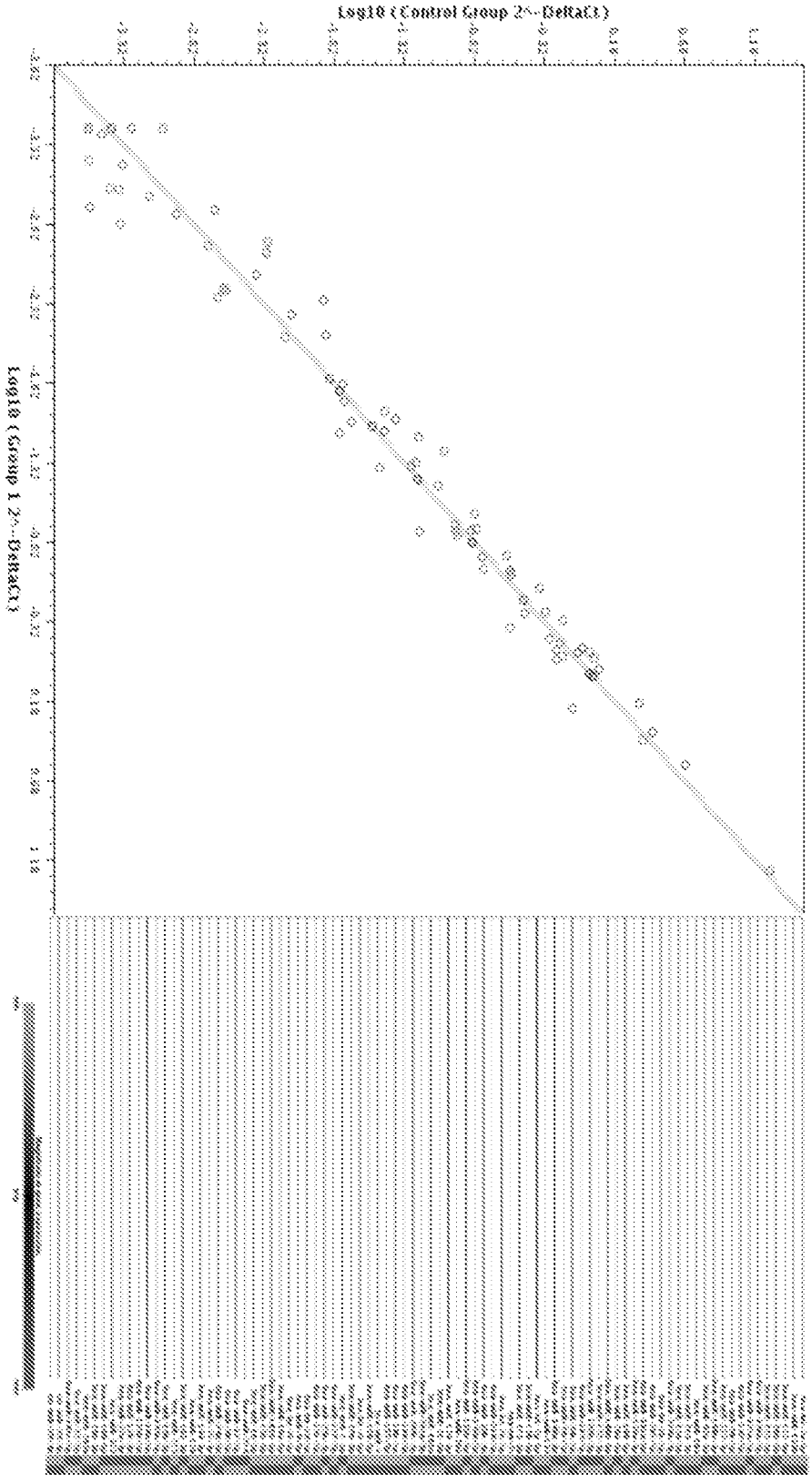


Figure 29

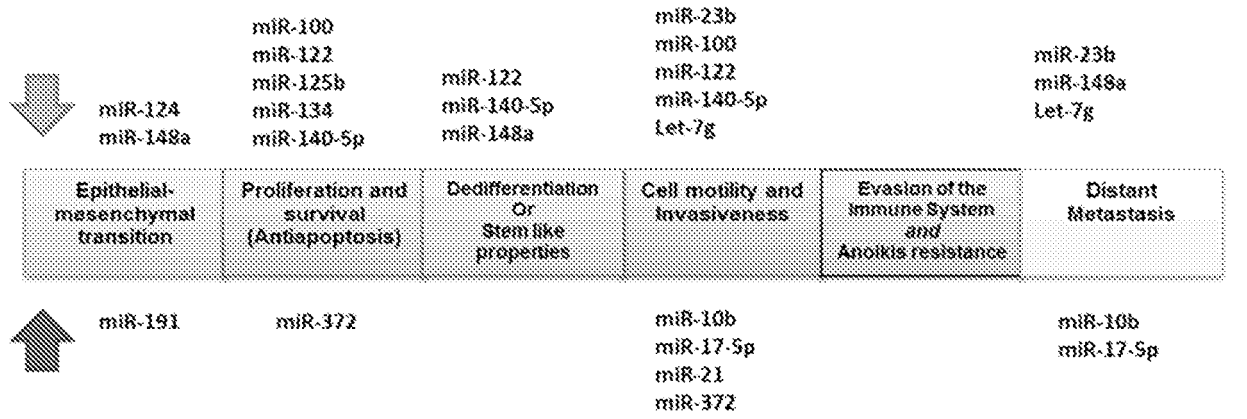

miR-124
miR-148a
miR-100
miR-122
miR-125b
miR-134
miR-140-5p
miR-122
miR-140-5p
miR-148a
miR-23b
miR-100
miR-122
miR-140-5p
Let-7g
miR-23b
miR-148a
Let-7g
Epithelial-mesenchymal transition
Proliferation and survival (Antiapoptosis)
Dedifferentiation Or Stem like properties
Cell motility and Invasiveness
Evasion of the Immune System and Anoikis resistance
Distant Metastasis
miR-191
miR-372
miR-10b
miR-17-5p
miR-21
miR-372
miR-10b
miR-17-5p

Figure 30

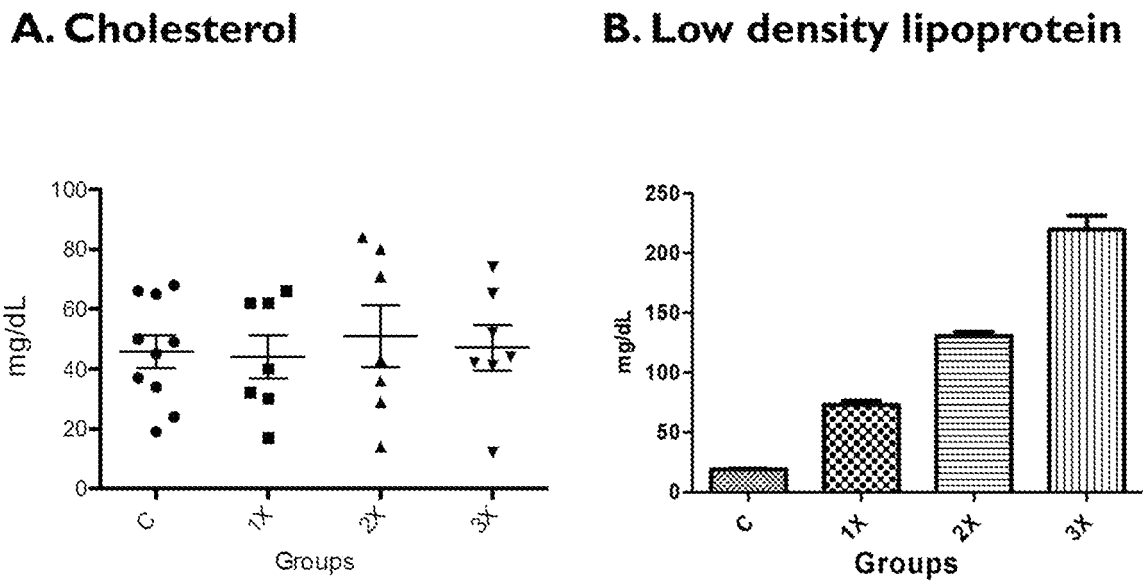
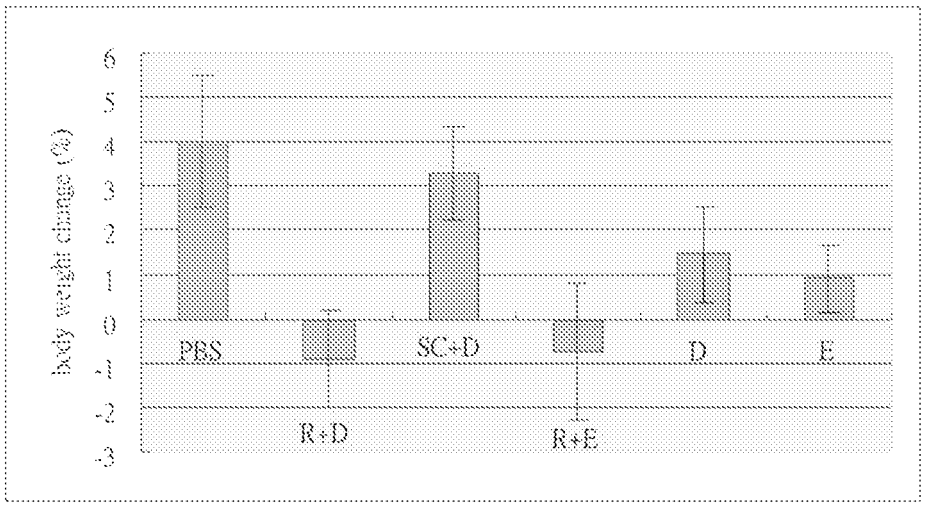

A. Cholesterol
B. Low density lipoprotein
mg/dL
100
80
60
40
20
0
C
1X
2X
3X
Groups
mg/dL
250
200
150
100
50
0
C
1X
2X
3X
Groups

Figure 31

A



R: RNA
SC: Scrambled RNA
D: Dendrimer
E: Ezetimibe

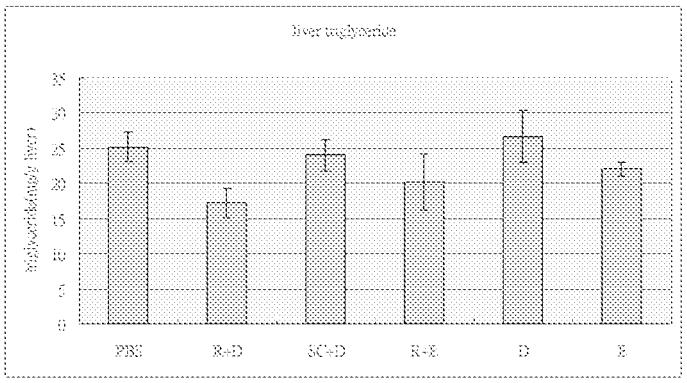
B



Obese animal Normal animal

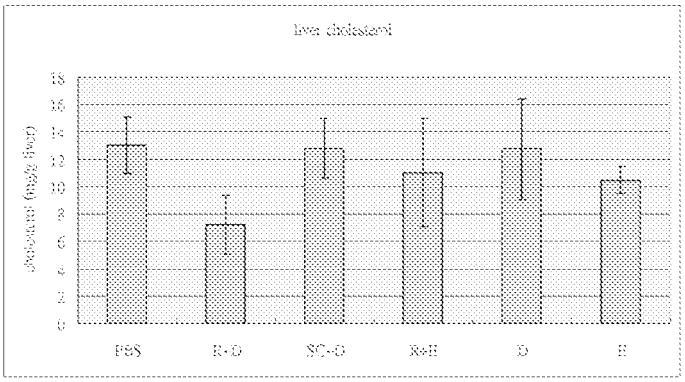
Figure 32

A



PBS vs R+D: p= 0.001
PBS vs Sc+D: p= 0.446
PBS vs R+E: p= 0.055
PBS vs D: p= 0.504
PBS vs E: p= 0.039
R+D vs R+E: p= 0.220
R+D vs E: p= 0.008

B



PBS vs R+D: p= 8.832E-06
PBS vs Sc+D: p= 0.886
PBS vs R+E: p= 0.032
PBS vs D: p= 0.665
PBS vs E: p= 0.007
R+D vs R+E: p= 0.002
R+D vs E: p= 0.004

Figure 32, cont.

C

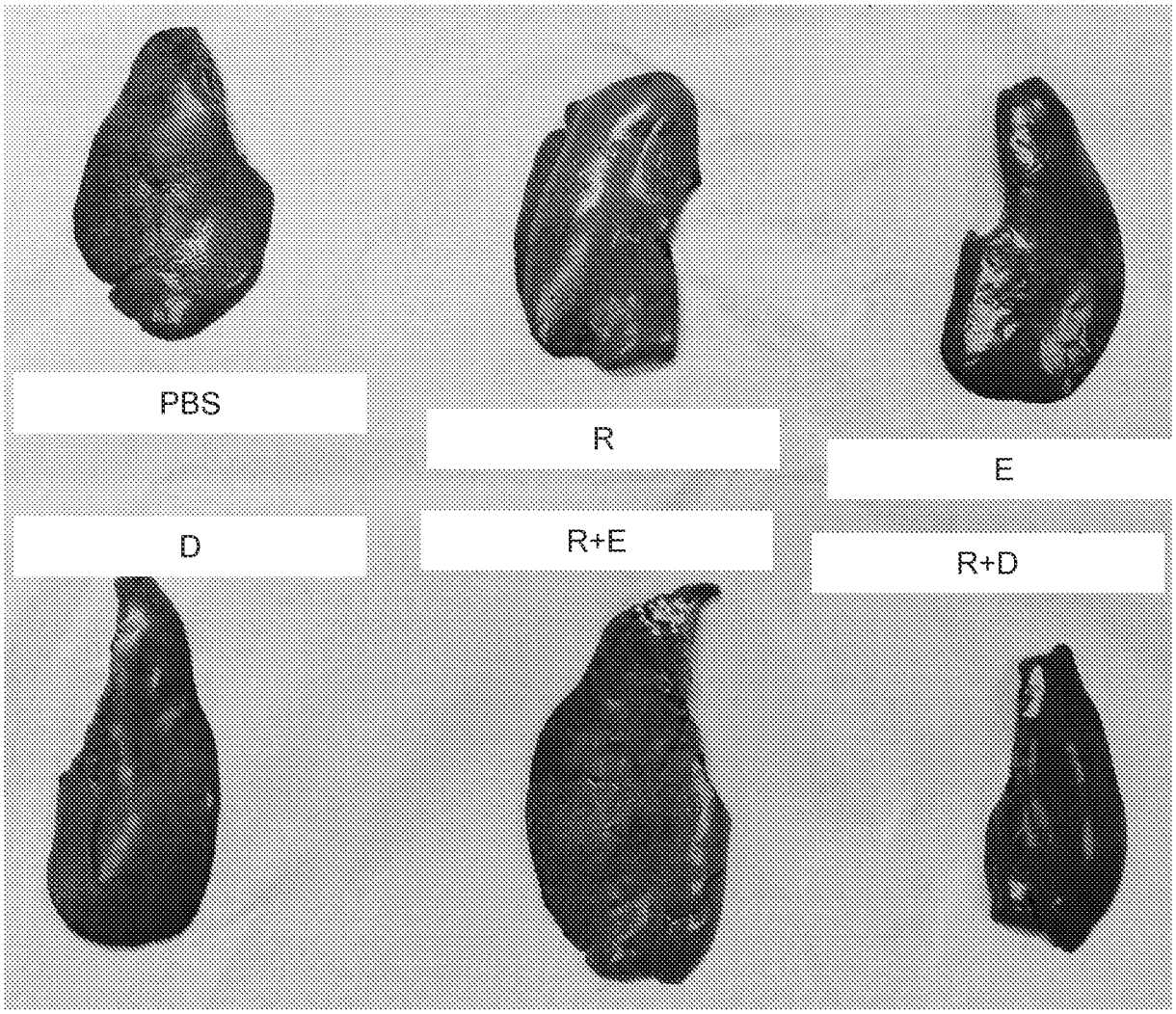
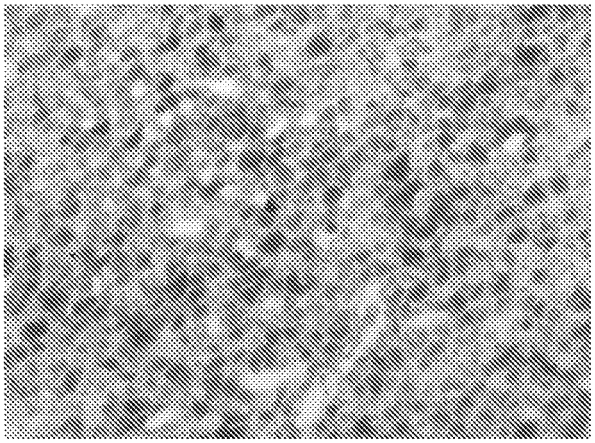
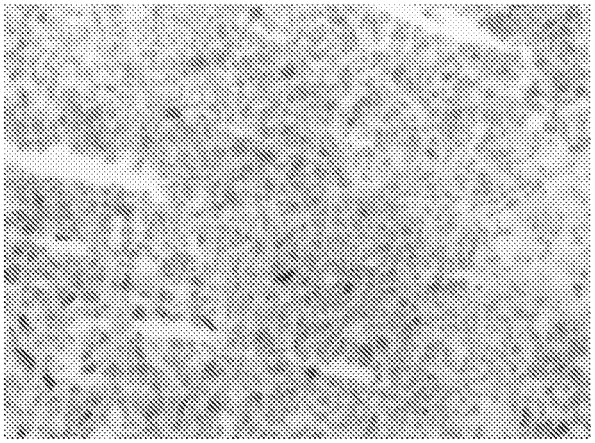


Figure 32, cont.

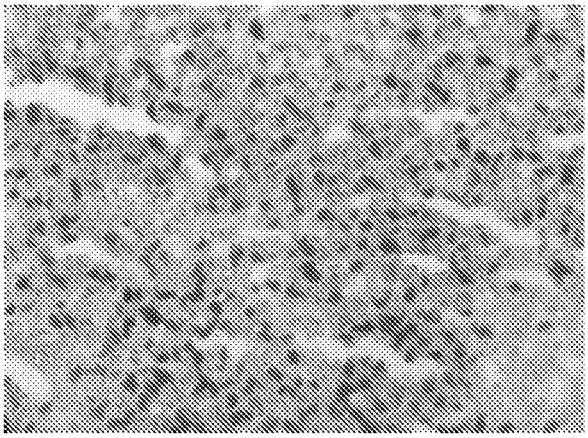
D



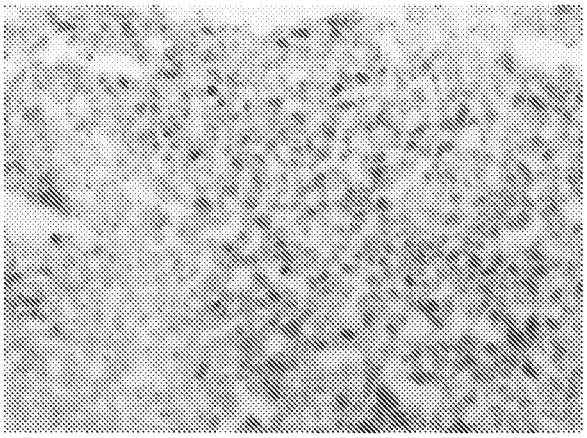
PBS



R+D



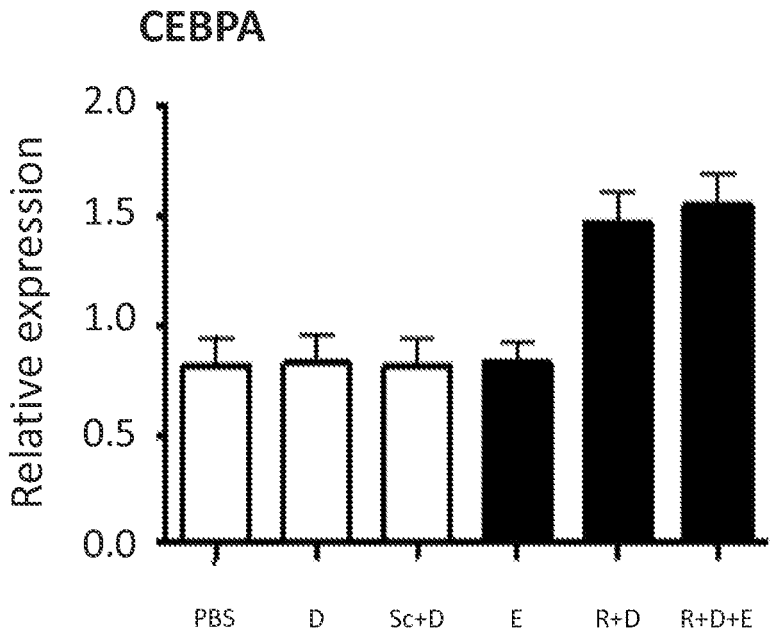
Sc+D



E

Figure 33

A


CEBPA
Relative expression
2.0
1.5
1.0
0.5
0.0
PBS
D
Sc+D
E
R+D
R+D+E

B

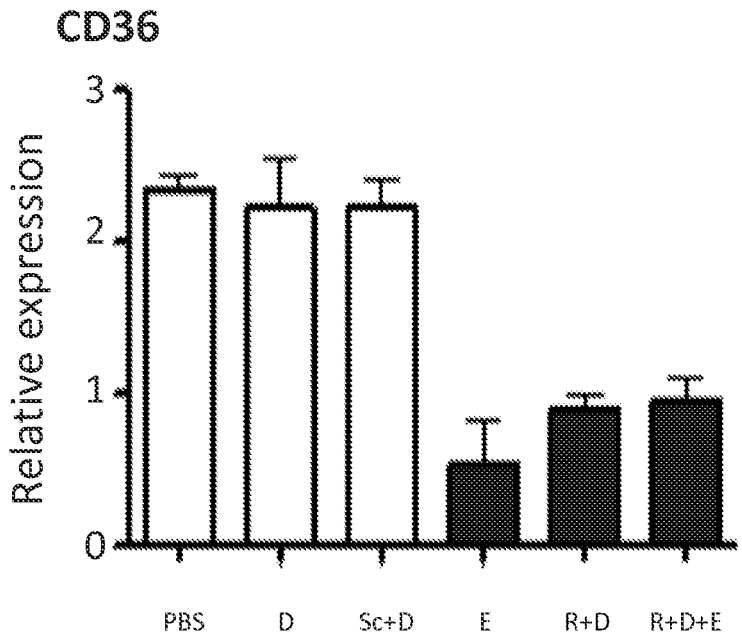
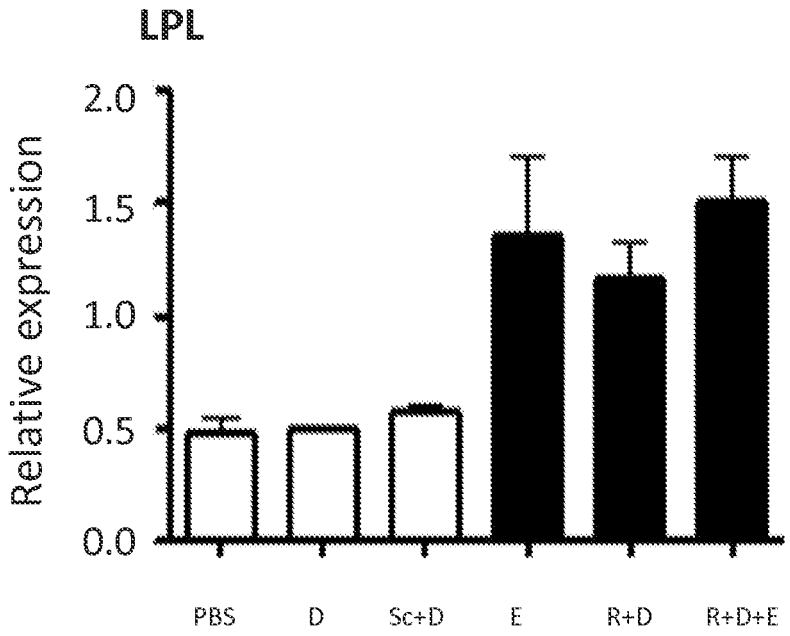

CD36
Relative expression
3
2
1
0
PBS
D
Sc+D
E
R+D
R+D+E

Figure 33, cont.

C



D

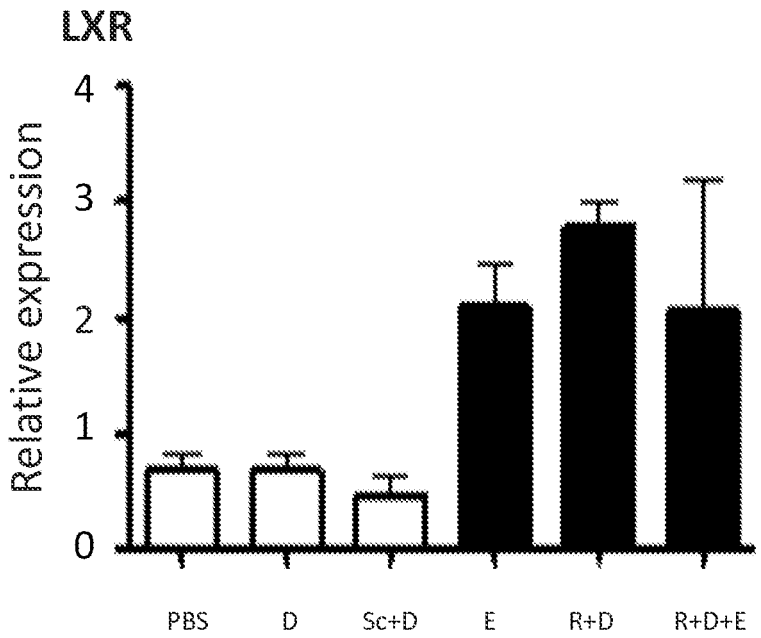


Figure 34

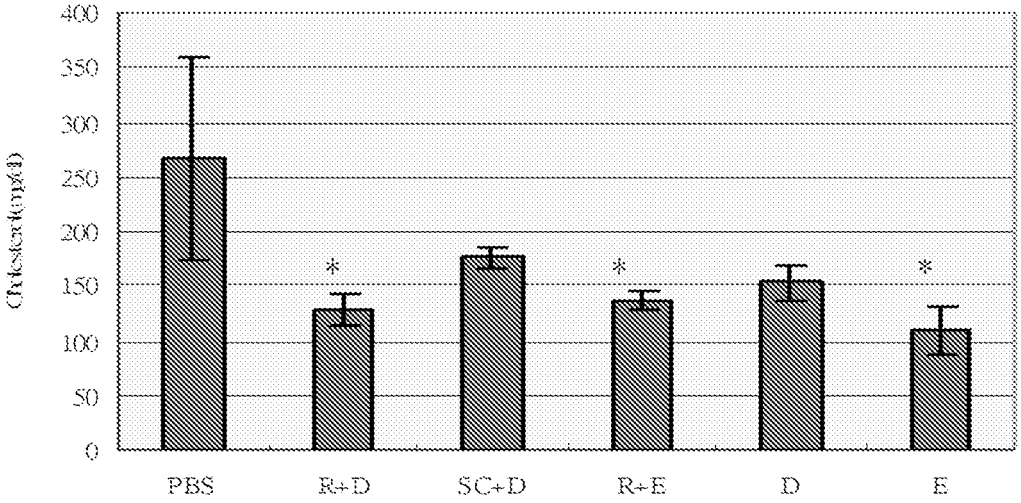


Figure 35

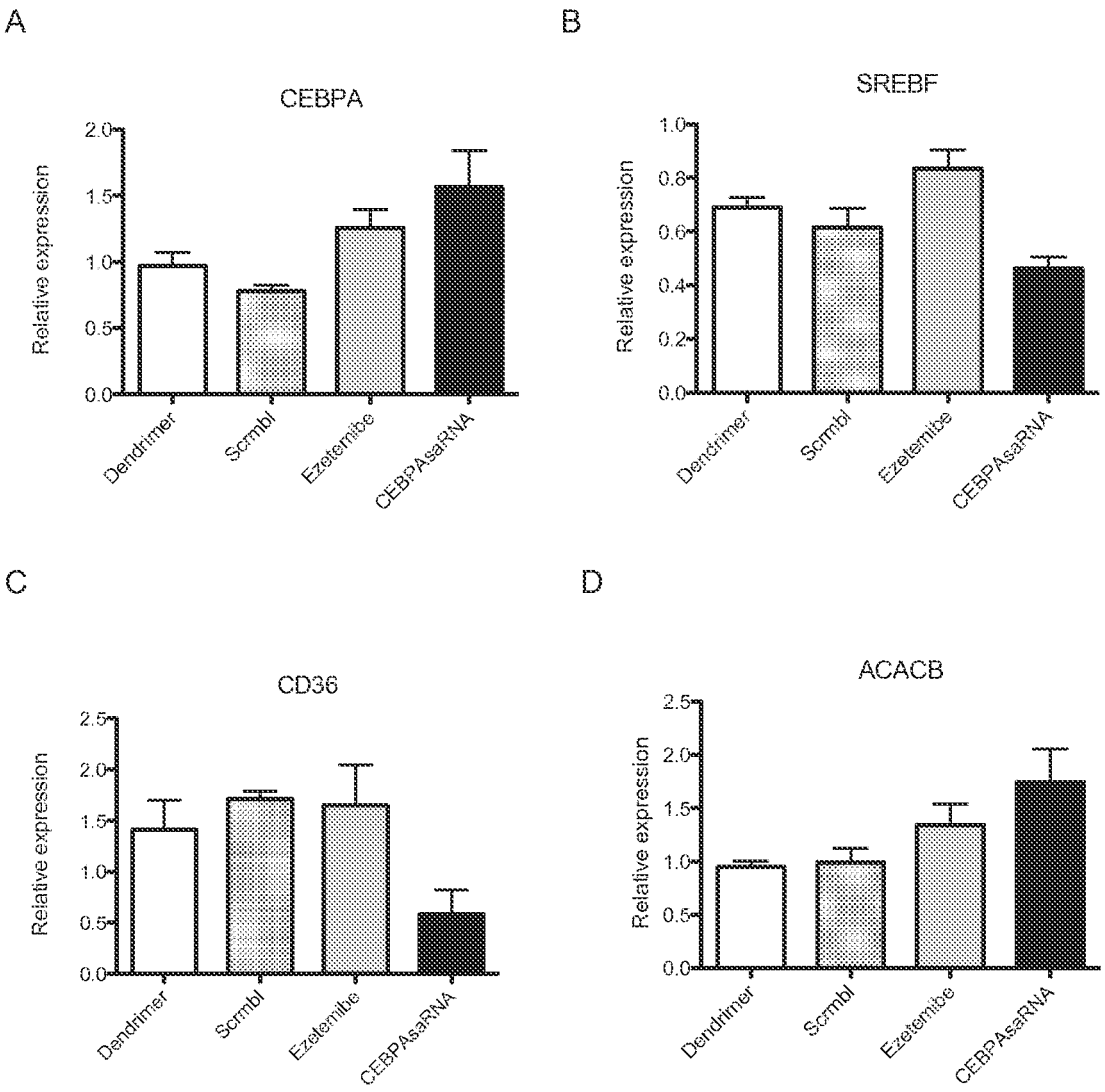
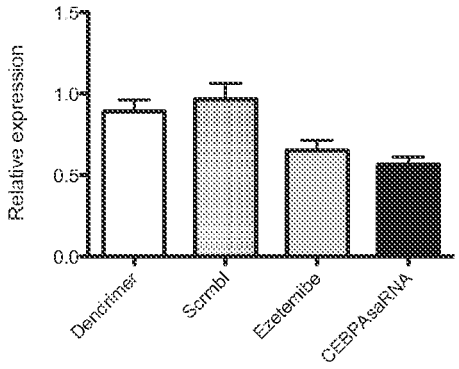
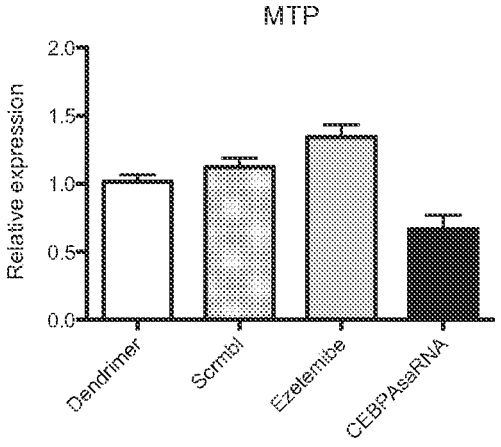

A
CEBPA
Relative expression
2.0
1.5
1.0
0.5
0.0
Dendrimer
Scrmbl
Ezetemibe
CEBPAsaRNA
B
SREBF
Relative expression
1.0
0.8
0.6
0.4
0.2
0.0
Dendrimer
Scrmbl
Ezetemibe
CEBPAsaRNA
C
CD36
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Dendrimer
Scrmbl
Ezetemibe
CEBPAsaRNA
D
ACACB
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Dendrimer
Scrmbl
Ezetemibe
CEBPAsaRNA

Figure 35, cont

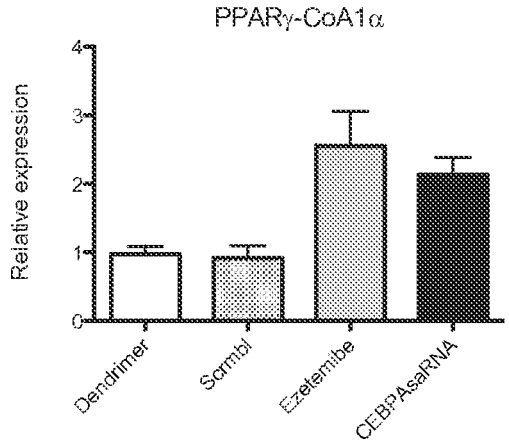
E



F



G



H

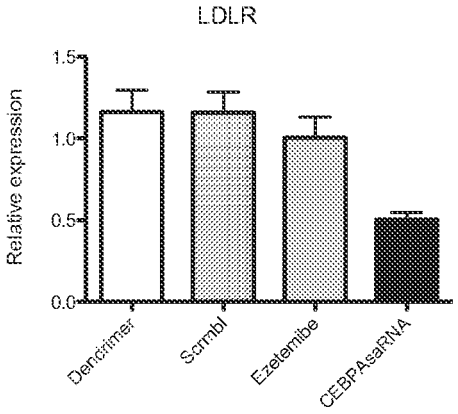
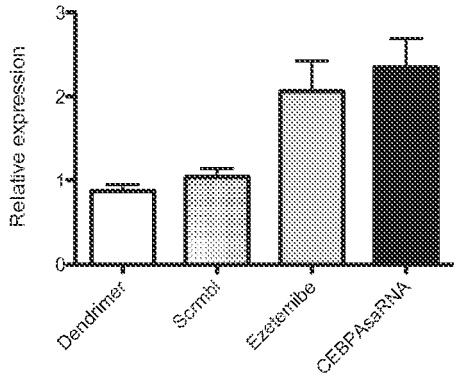
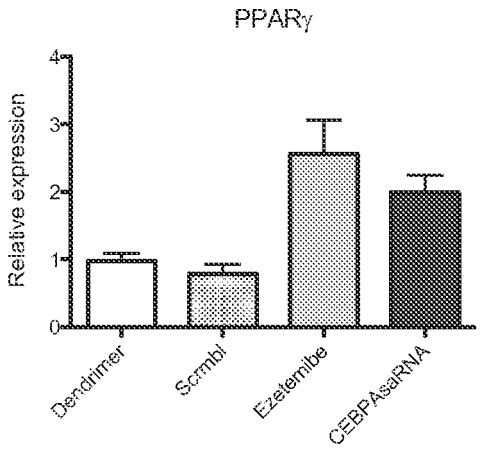


Figure 35, cont.

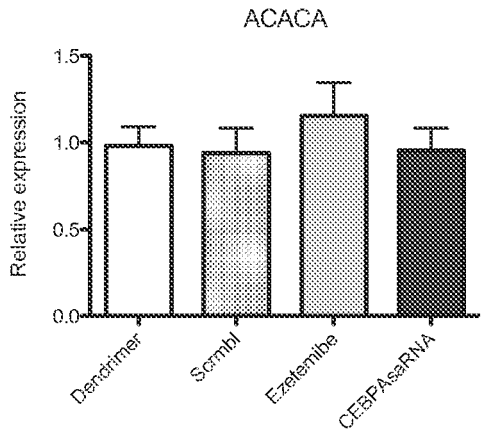
I



J



K



L

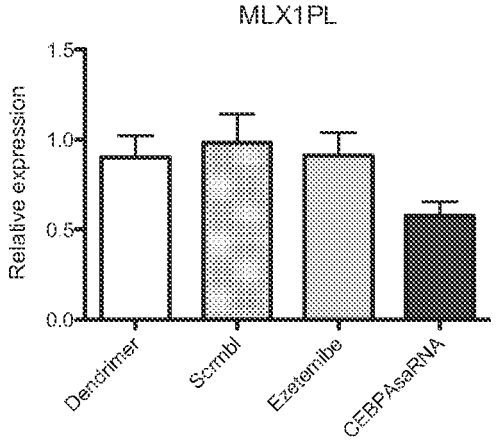
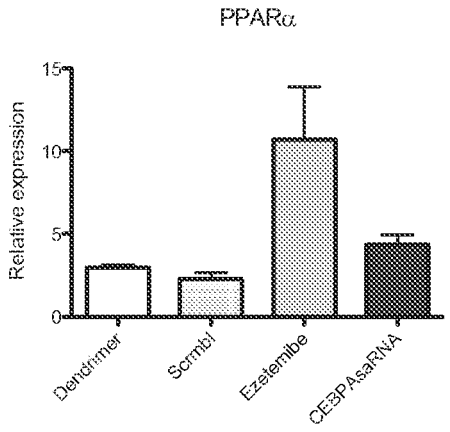
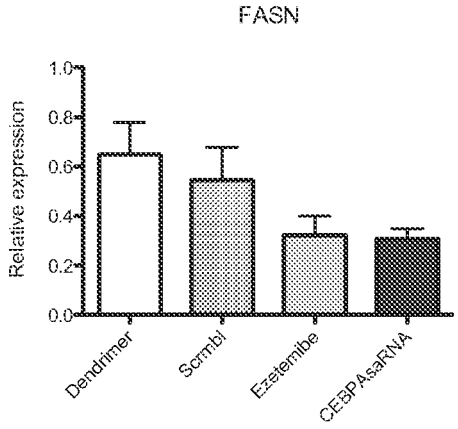


Figure 35, cont.

M



N



O

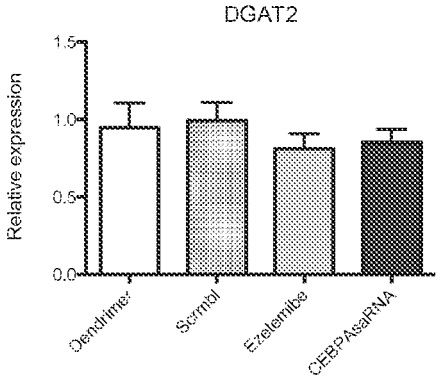


Figure 36

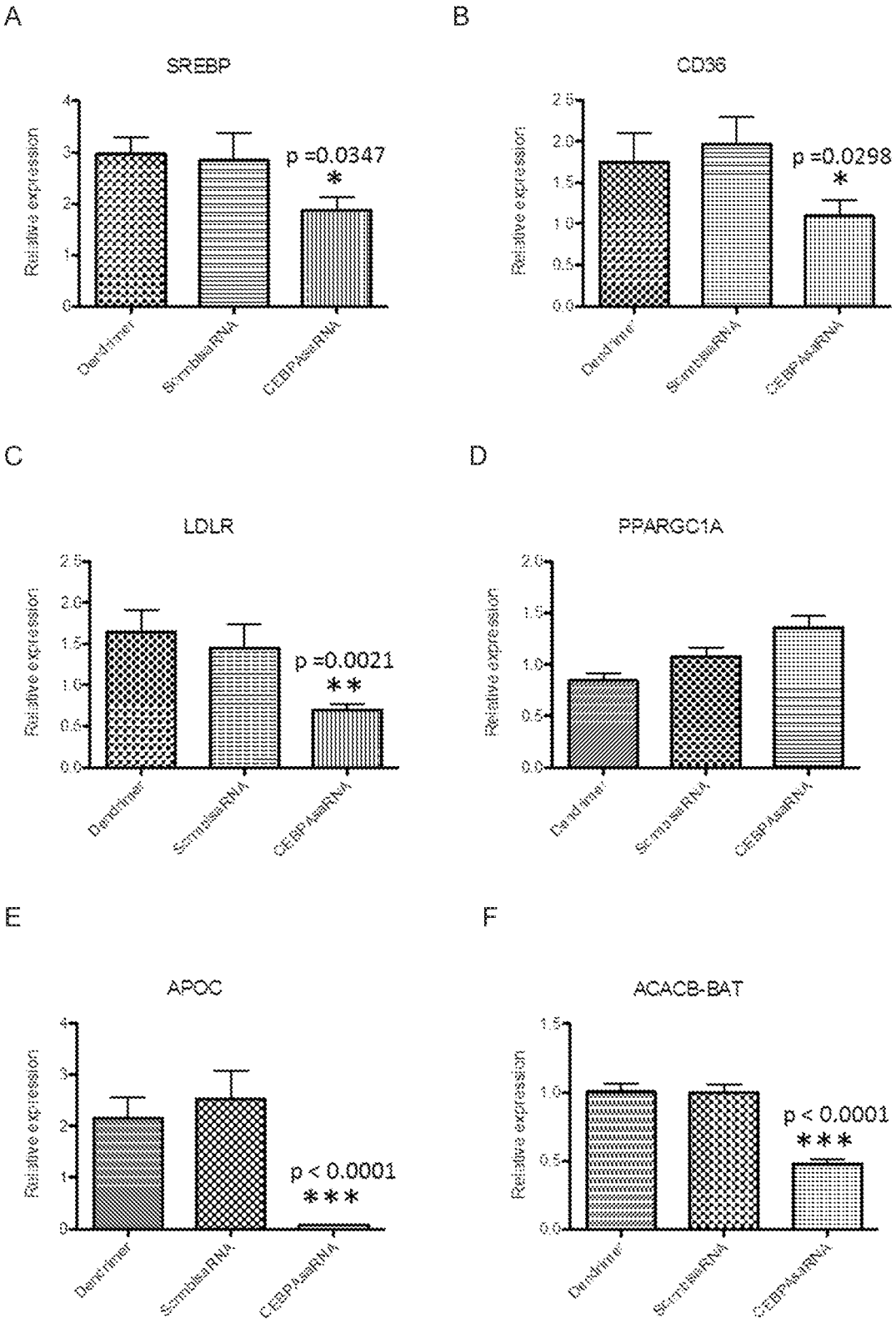
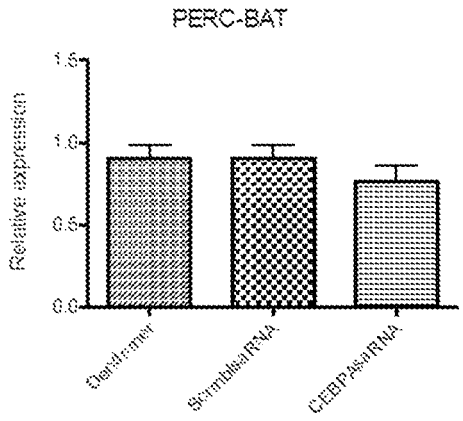
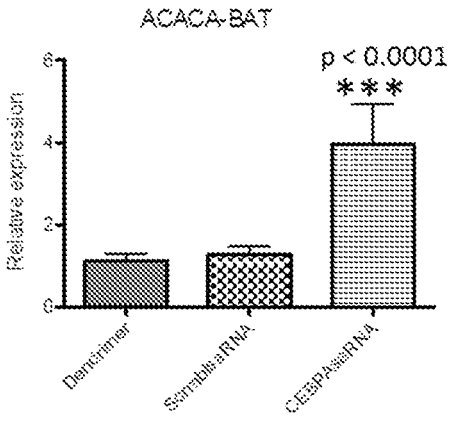

A
SREBP
Relative expression
p =0.0347
*
Dendrimer
ScrambledRNA
CEBPAsiRNA
B
CD36
Relative expression
p =0.0298
*
Dendrimer
ScrambledRNA
CEBPAsiRNA
C
LDLR
Relative expression
p =0.0021
**
Dendrimer
ScrambledRNA
CEBPAsiRNA
D
PPARGC1A
Relative expression
Dendrimer
ScrambledRNA
CEBPAsiRNA
E
APOC
Relative expression
p < 0.0001
***
Dendrimer
ScrambledRNA
CEBPAsiRNA
F
ACACB-BAT
Relative expression
p < 0.0001
***
Dendrimer
ScrambledRNA
CEBPAsiRNA

Figure 36, cont.

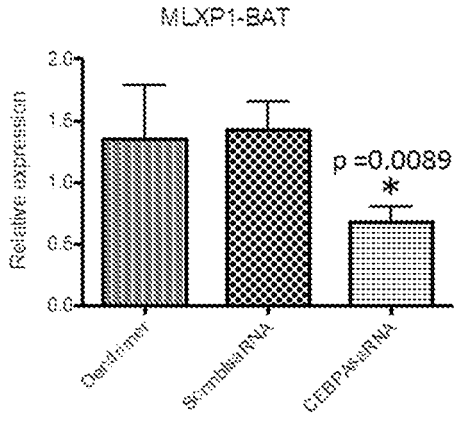
G



H



I



J

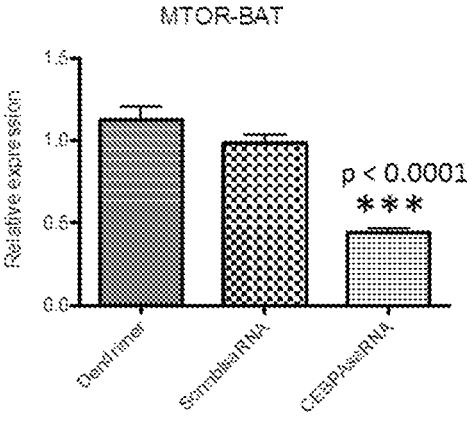
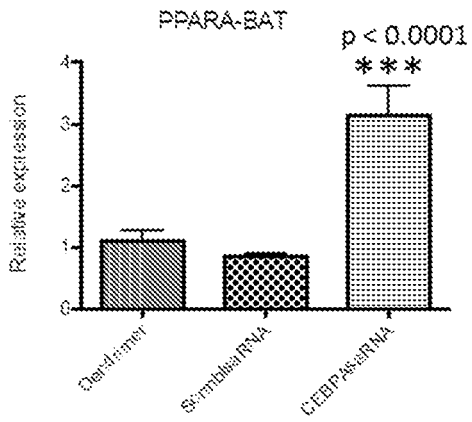
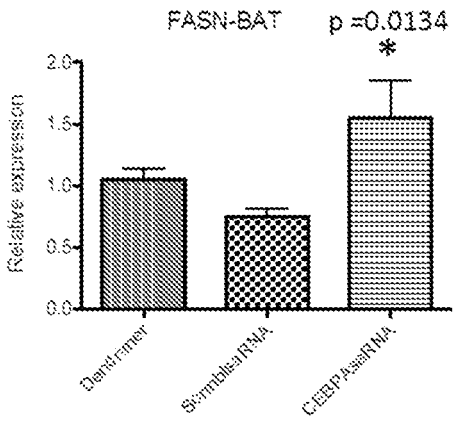


Figure 36, cont.

K



L



M

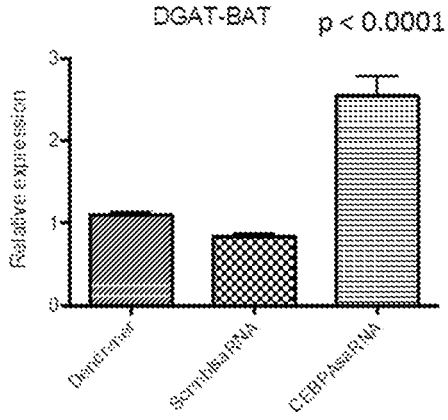


Figure 37

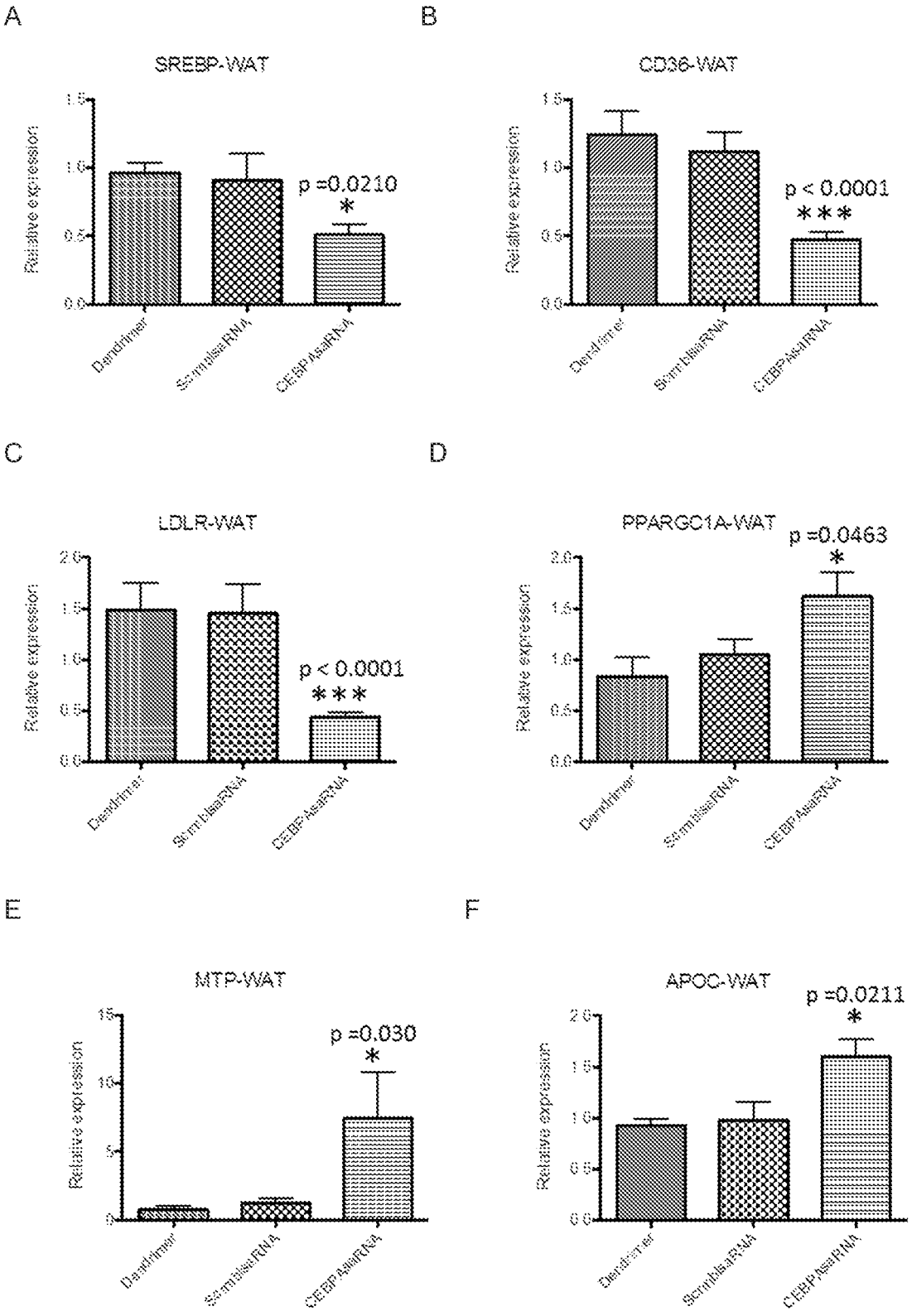
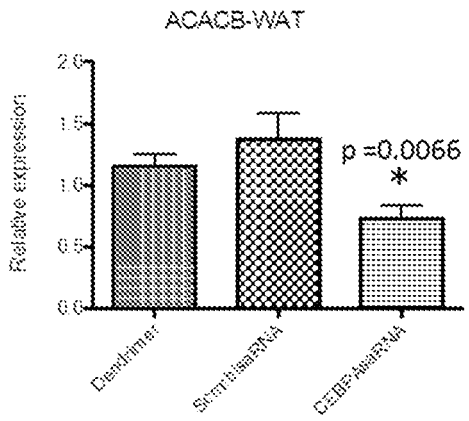
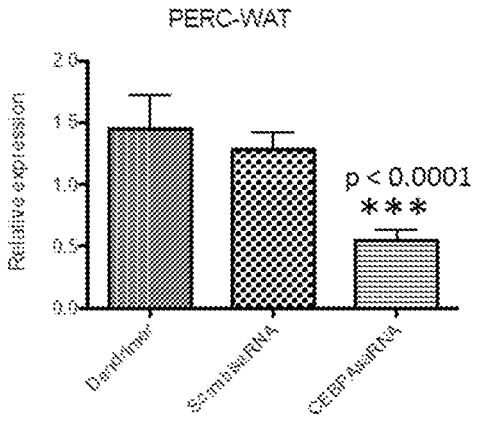

A
SREBP-WAT
Relative expression
p =0.0210
*
Dendrimer
ScrmbldsiRNA
CEBPAsiRNA
B
CD36-WAT
Relative expression
p < 0.0001
***
Dendrimer
ScrmbldsiRNA
CEBPAsiRNA
C
LDLR-WAT
Relative expression
p < 0.0001
***
Dendrimer
ScrmbldsiRNA
CEBPAsiRNA
D
PPARGC1A-WAT
Relative expression
p =0.0463
*
Dendrimer
ScrmbldsiRNA
CEBPAsiRNA
E
MTP-WAT
Relative expression
p =0.030
*
Dendrimer
ScrmbldsiRNA
CEBPAsiRNA
F
APOC-WAT
Relative expression
p =0.0211
*
Dendrimer
ScrmbldsiRNA
CEBPAsiRNA

Figure 37, cont.

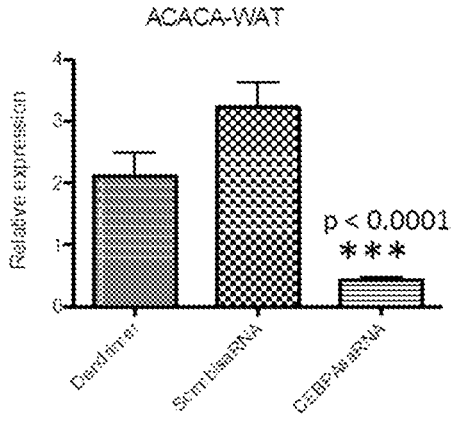
G



H



I



J

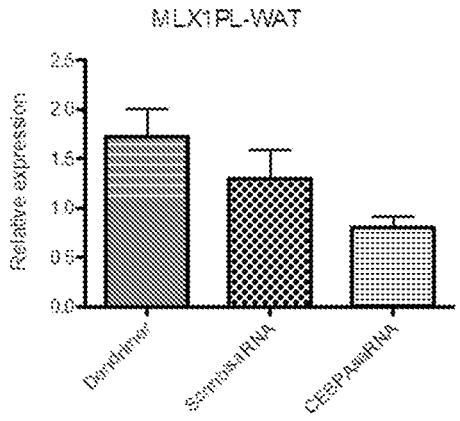
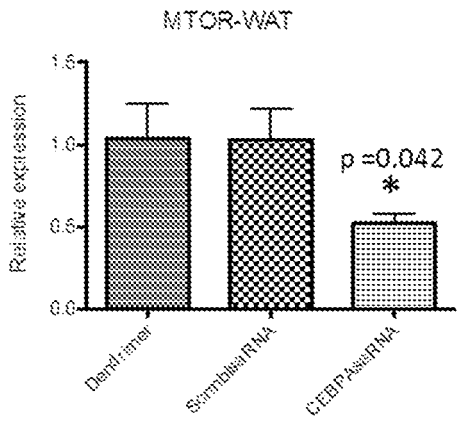
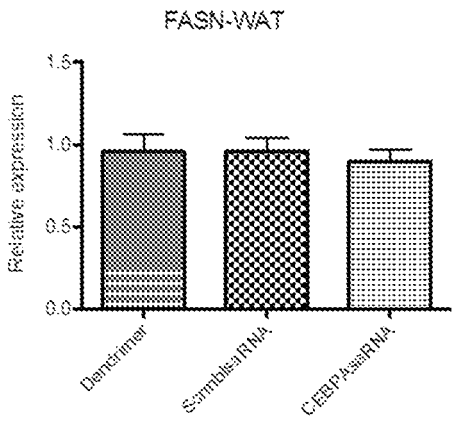


Figure 37, cont.

K



L



M

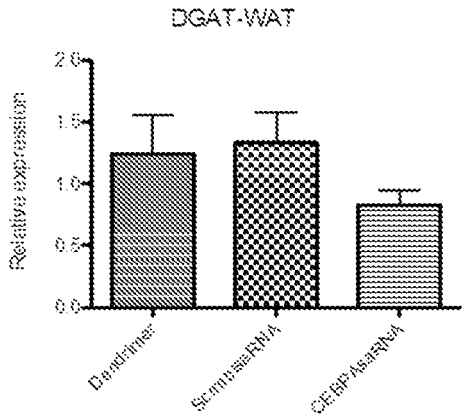
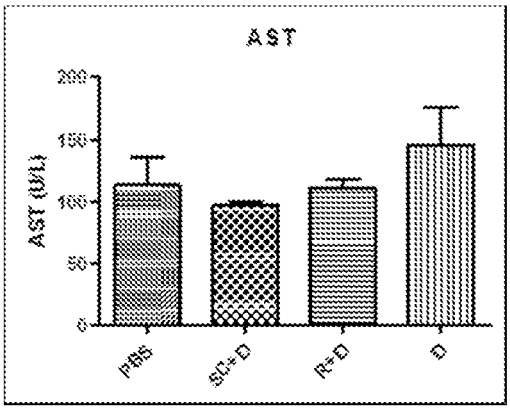
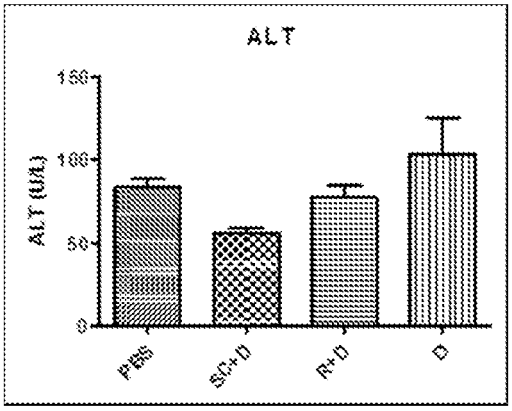


Figure 38

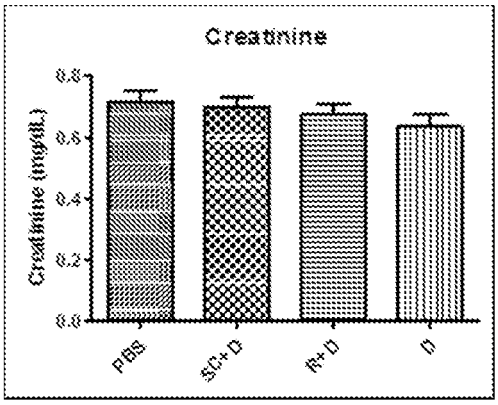
A



B



C



D

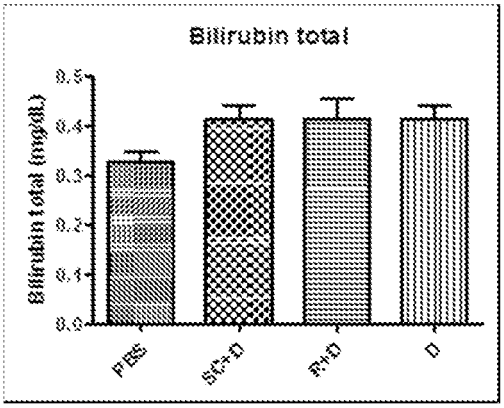
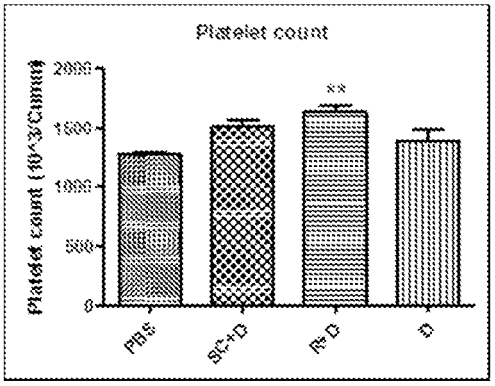
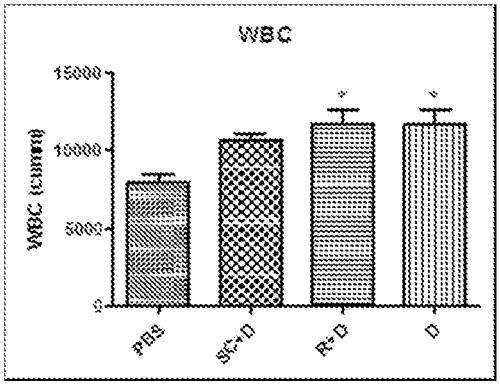


Figure 38, cont.

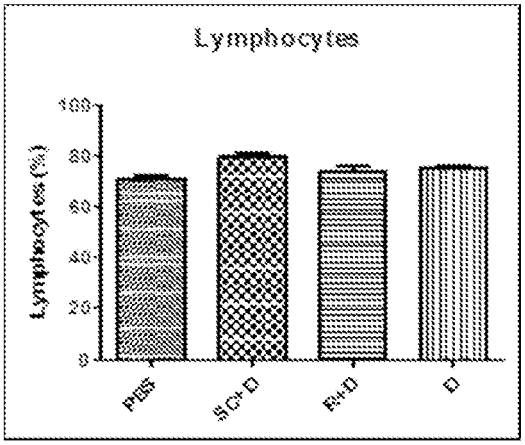
E



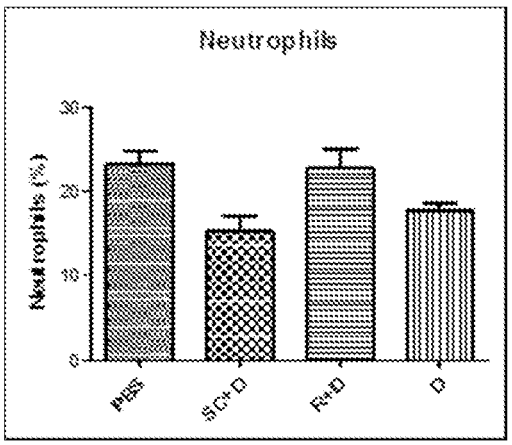
F



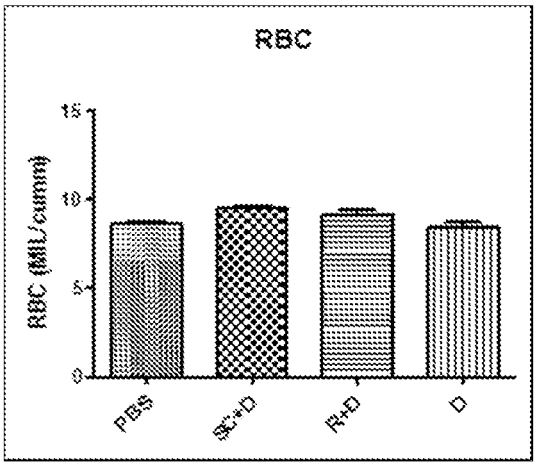
G



H



I



J

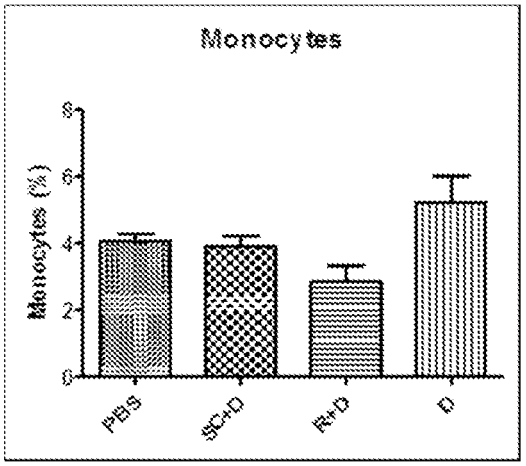
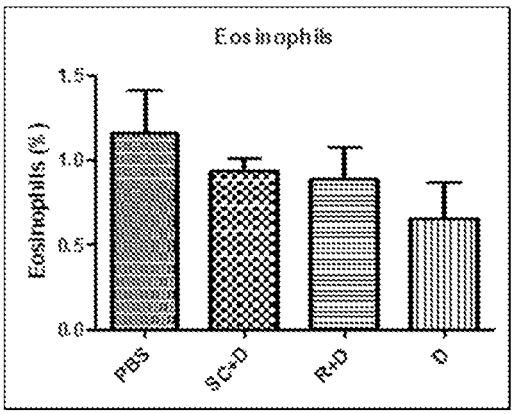
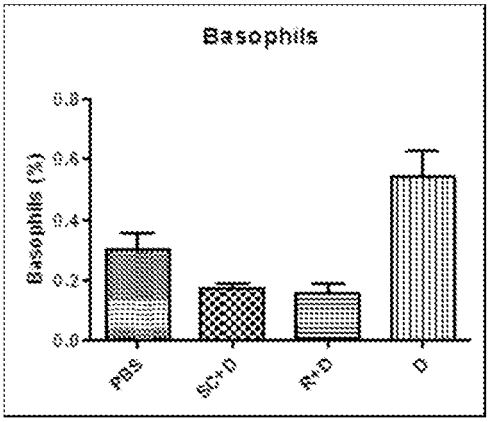


Figure 38, cont.

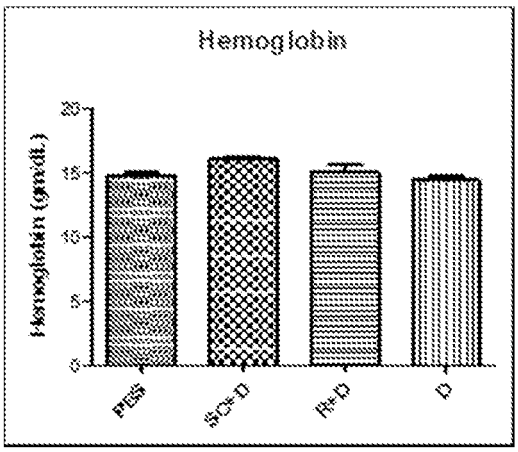
K



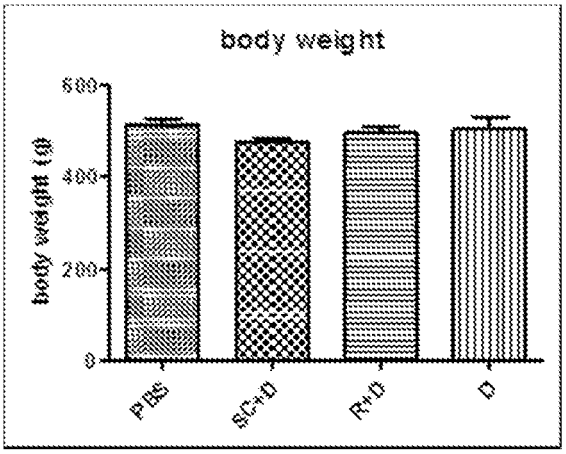
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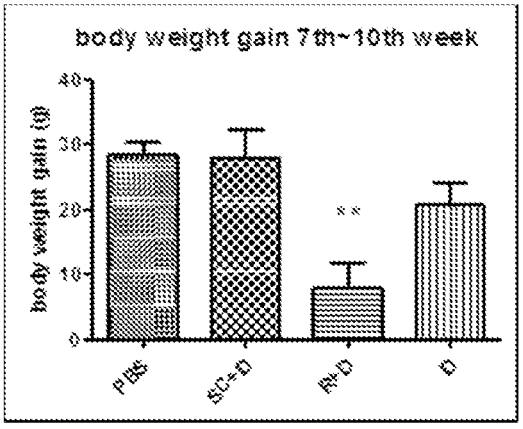
M



N



O



P

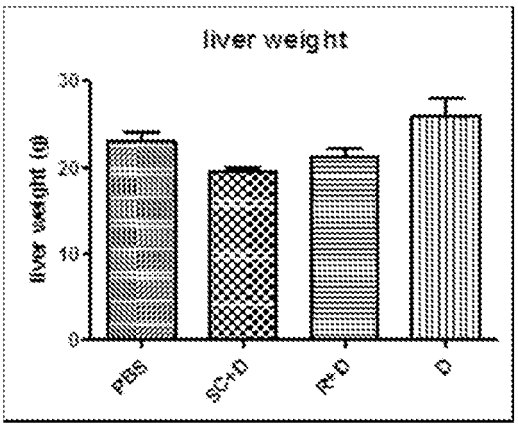
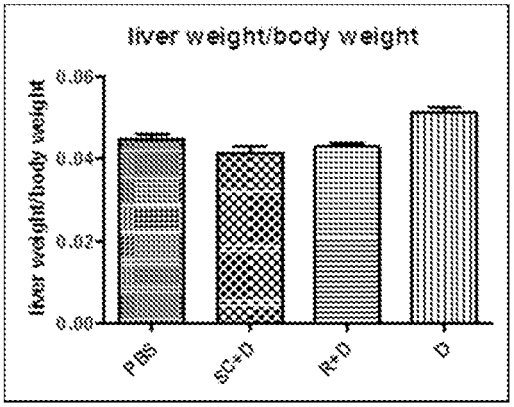
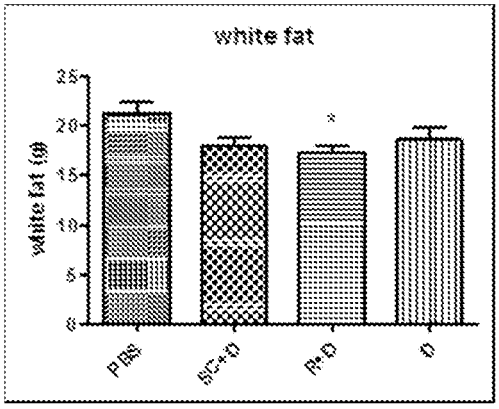


Figure 38, cont.

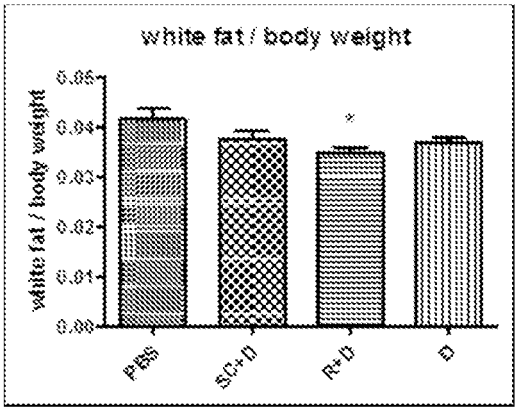
Q



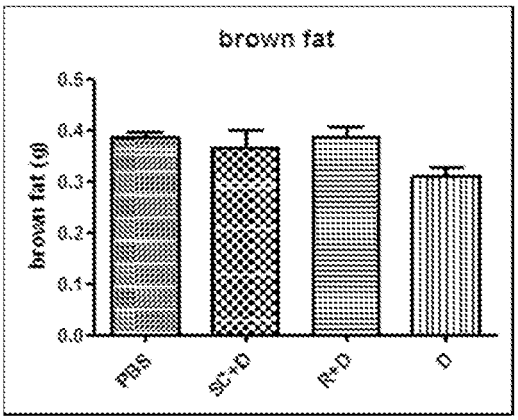
R



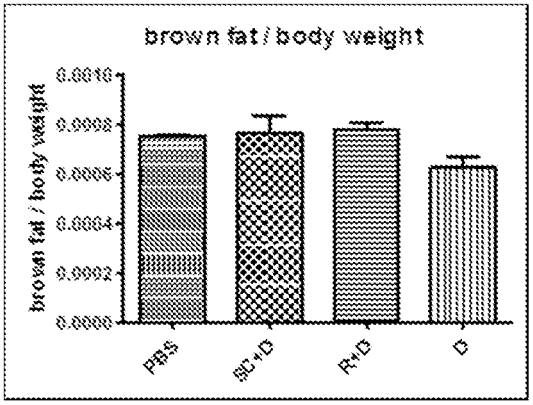
S



T



U



V

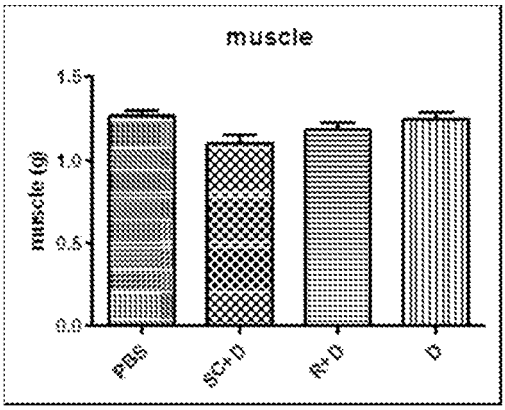


Figure 38, cont.

W

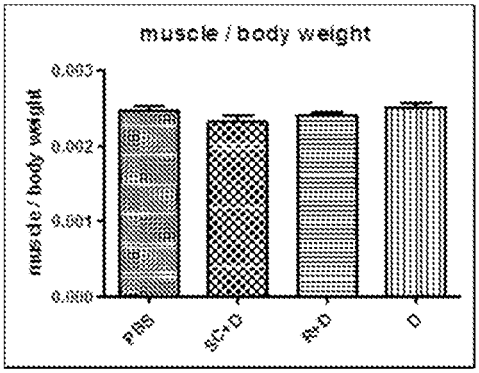
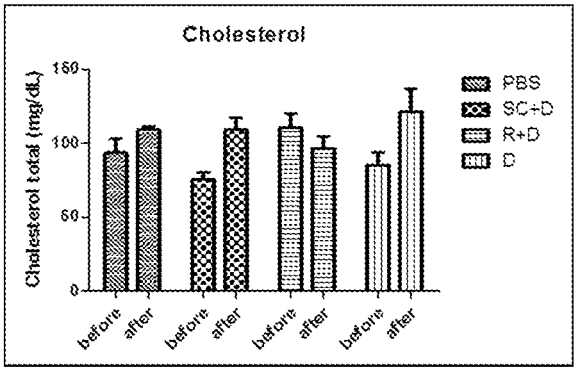


Figure 39

A


Cholesterol
Cholesterol total (mg/dL)
150
100
50
0
before
after
before
after
before
after
before
after
PBS
SC+D
R+D
D

B

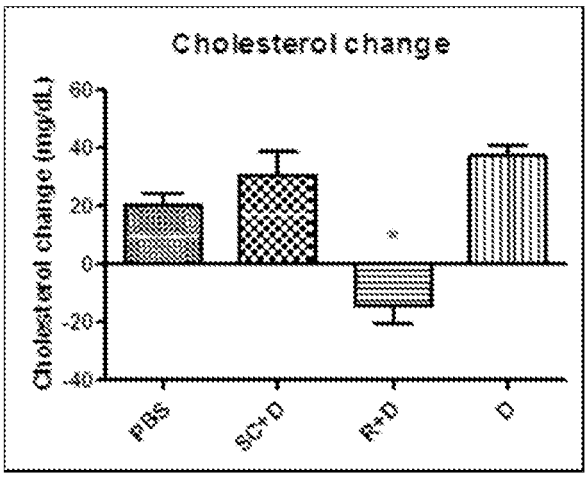
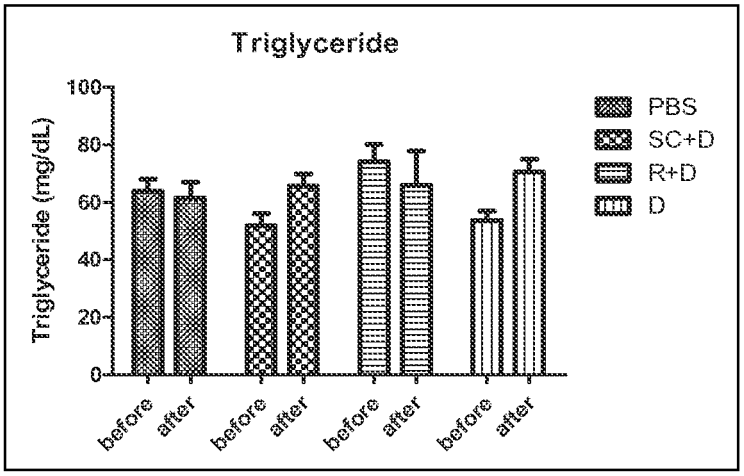
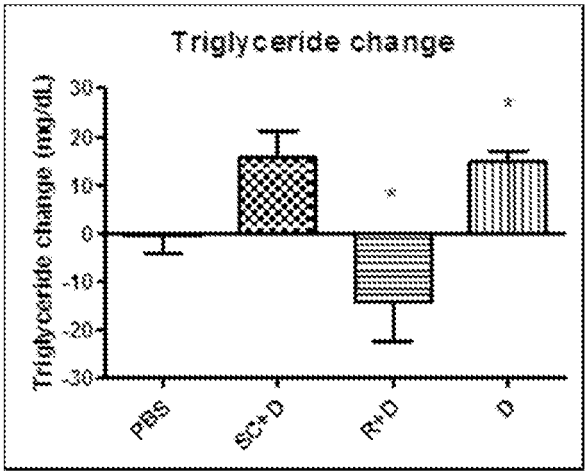

Cholesterol change
Cholesterol change (mg/dL)
60
40
20
0
-20
-40
*
PBS
SC+D
R+D
D

Figure 39, cont.

C



D



E

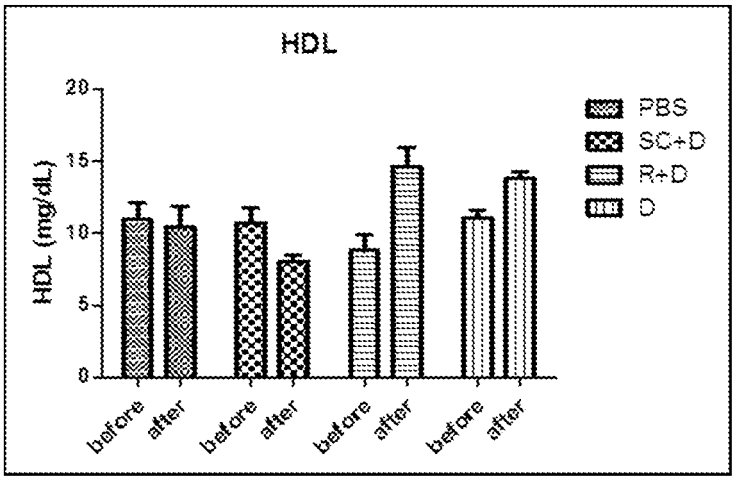
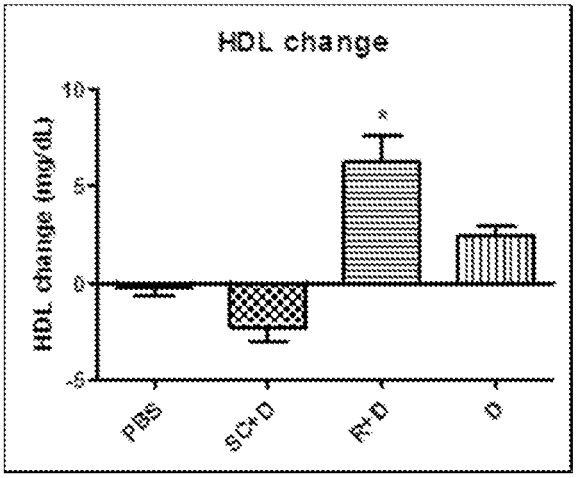
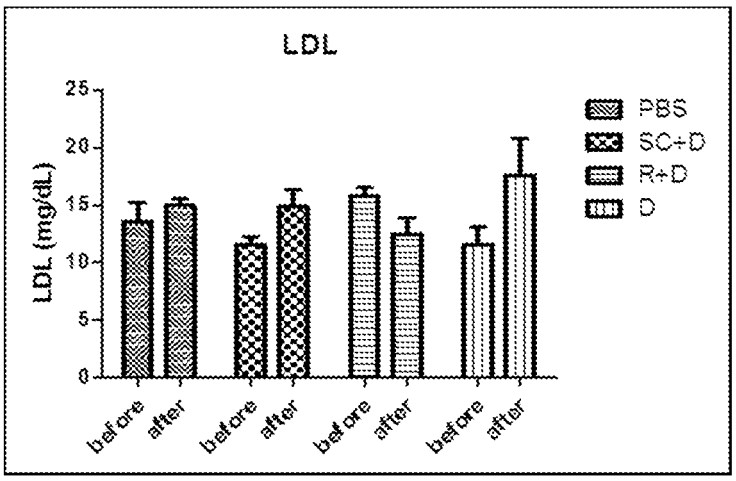


Figure 39, cont.

F



G



H

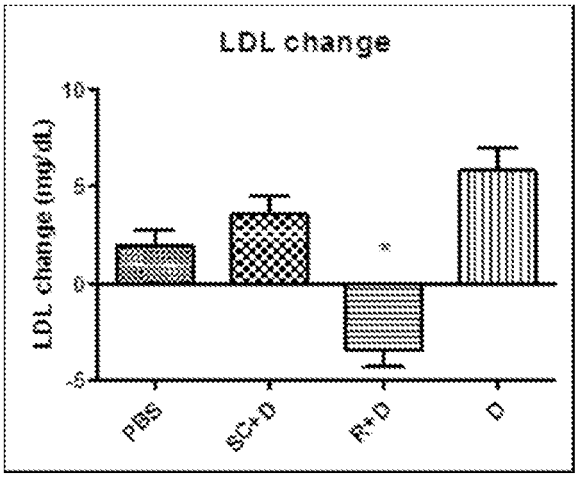
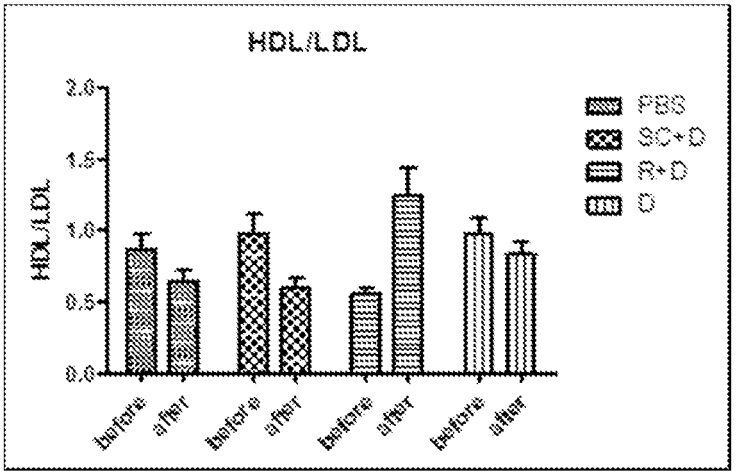


Figure 39 cont.

I



J

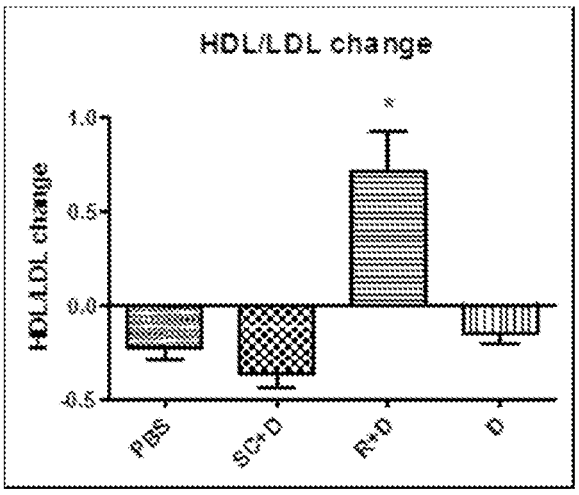
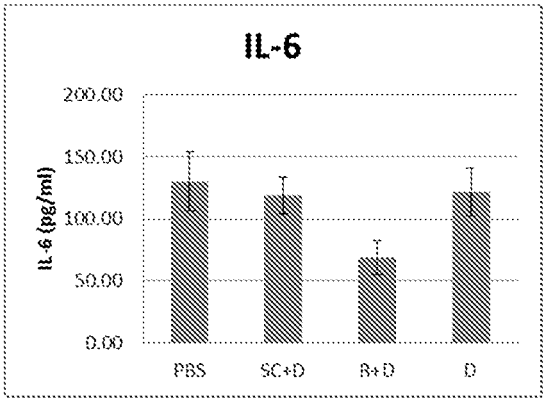
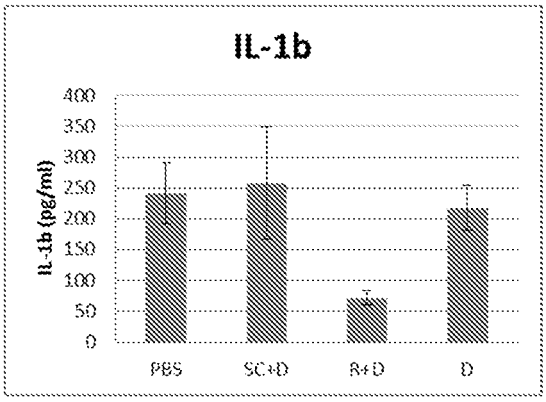


Figure 40

A



B



C

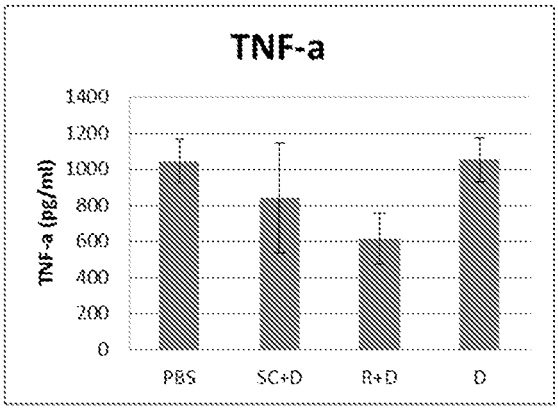


Figure 41

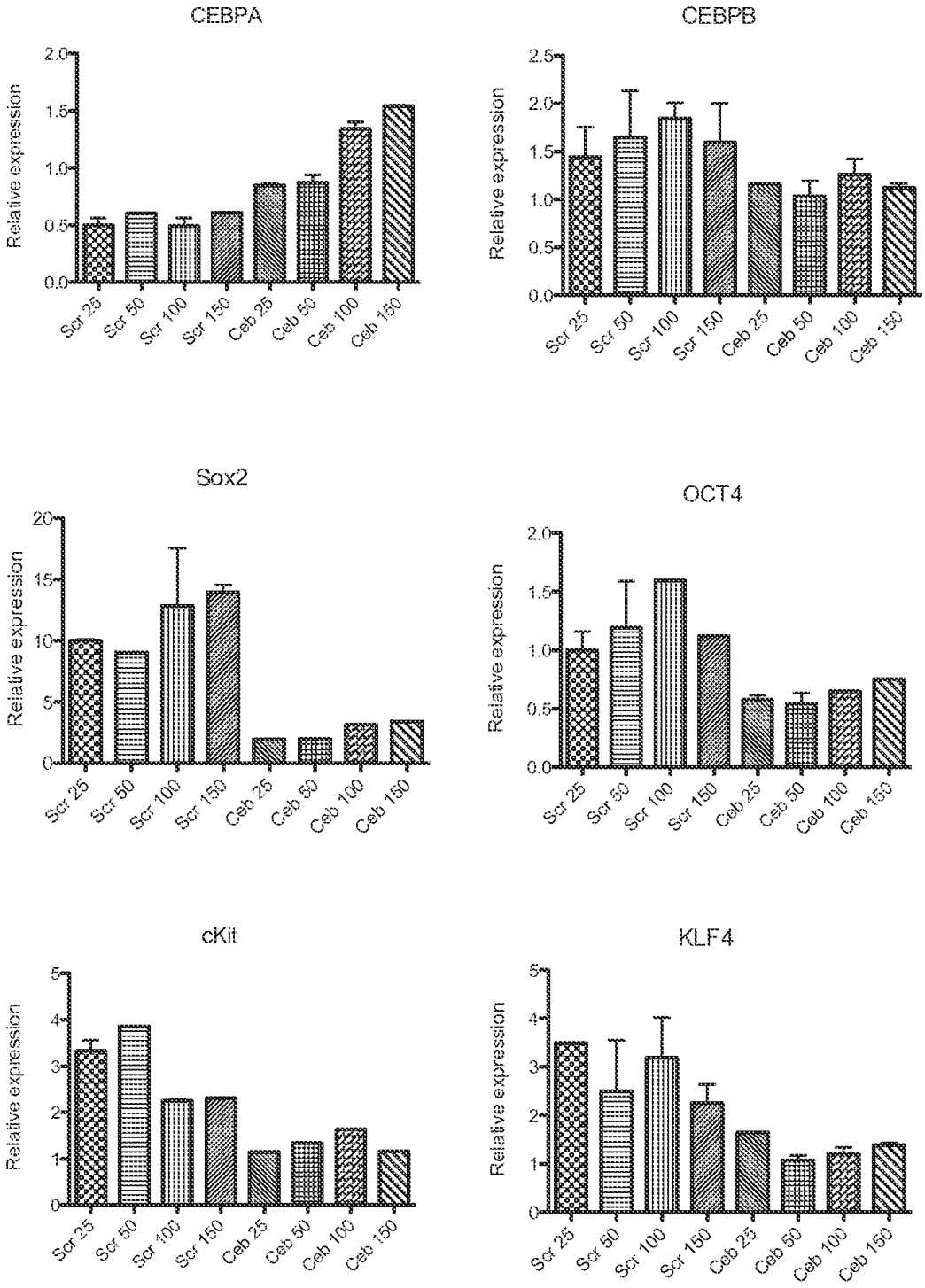

CEBPA
Relative expression
2.0
1.5
1.0
0.5
0.0
Scr 25
Scr 50
Scr 100
Scr 150
Ceb 25
Ceb 50
Ceb 100
Ceb 150
CEBPB
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Scr 25
Scr 50
Scr 100
Scr 150
Ceb 25
Ceb 50
Ceb 100
Ceb 150
Sox2
Relative expression
20
15
10
5
0
Scr 25
Scr 50
Scr 100
Scr 150
Ceb 25
Ceb 50
Ceb 100
Ceb 150
OCT4
Relative expression
2.0
1.5
1.0
0.5
0.0
Scr 25
Scr 50
Scr 100
Scr 150
Ceb 25
Ceb 50
Ceb 100
Ceb 150
cKit
Relative expression
5
4
3
2
1
0
Scr 25
Scr 50
Scr 100
Scr 150
Ceb 25
Ceb 50
Ceb 100
Ceb 150
KLF4
Relative expression
5
4
3
2
1
0
Scr 25
Scr 50
Scr 100
Scr 150
Ceb 25
Ceb 50
Ceb 100
Ceb 150

Figure 42

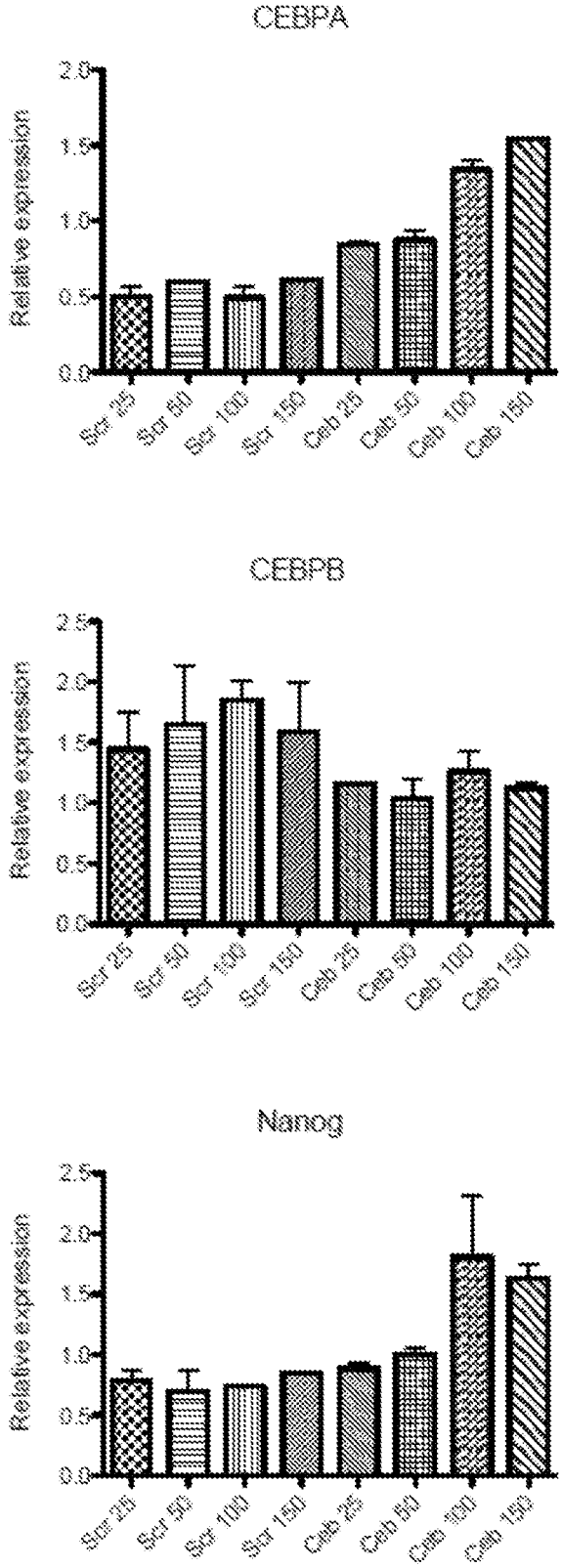

CEBPA
Relative expression
2.0
1.5
1.0
0.5
0.0
Scr 25
Scr 50
Scr 100
Scr 150
Celb 25
Celb 50
Celb 100
Celb 150
CEBPB
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Scr 25
Scr 50
Scr 100
Scr 150
Celb 25
Celb 50
Celb 100
Celb 150
Nanog
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Scr 25
Scr 50
Scr 100
Scr 150
Celb 25
Celb 50
Celb 100
Celb 150

Figure 43

A

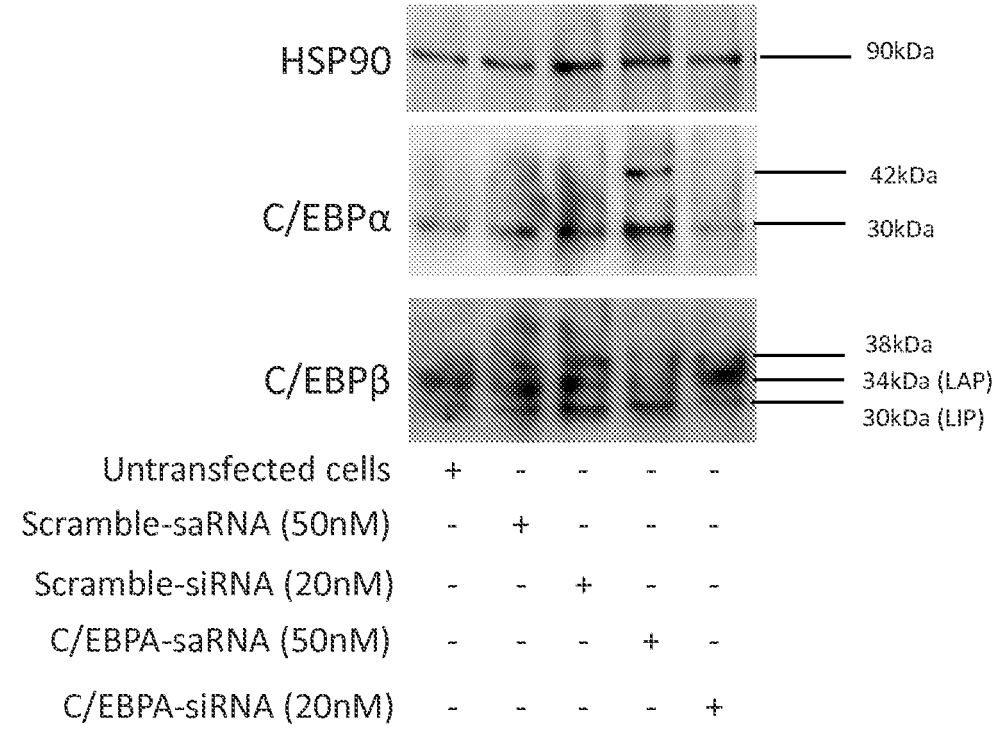

HSP90
90kDa
C/EBPα
42kDa
30kDa
C/EBPβ
38kDa
34kDa (LAP)
30kDa (LIP)
Untransfected cells + - - - -
Scramble-saRNA (50nM) - + - - -
Scramble-siRNA (20nM) - - + - -
C/EBPA-saRNA (50nM) - - - + -
C/EBPA-siRNA (20nM) - - - - +

Figure 43, cont.

B:

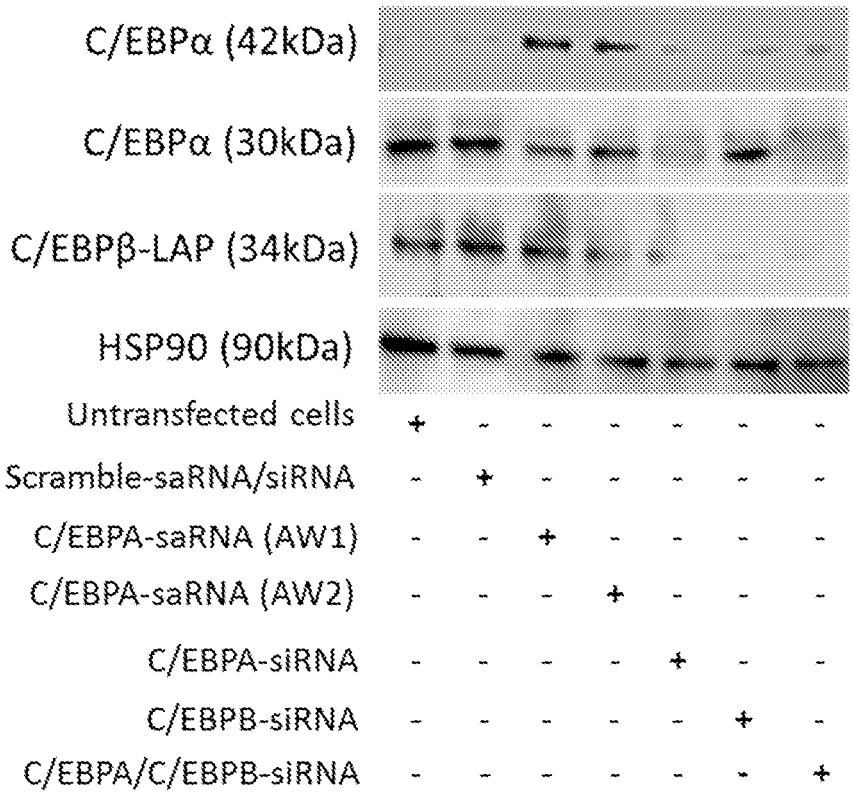
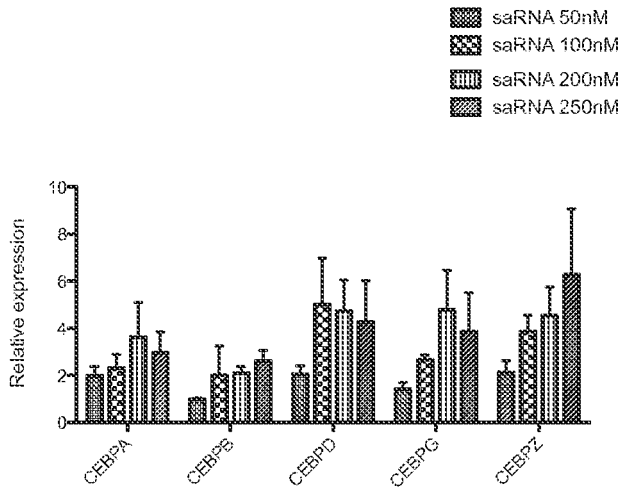
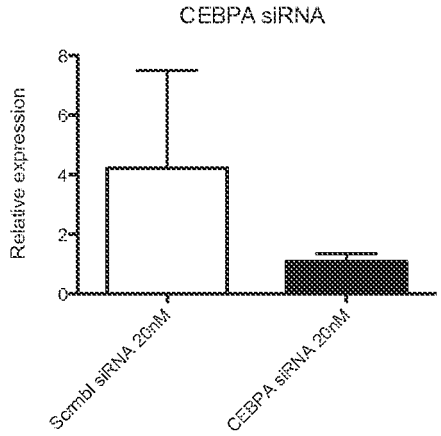


Figure 44

A



B



C

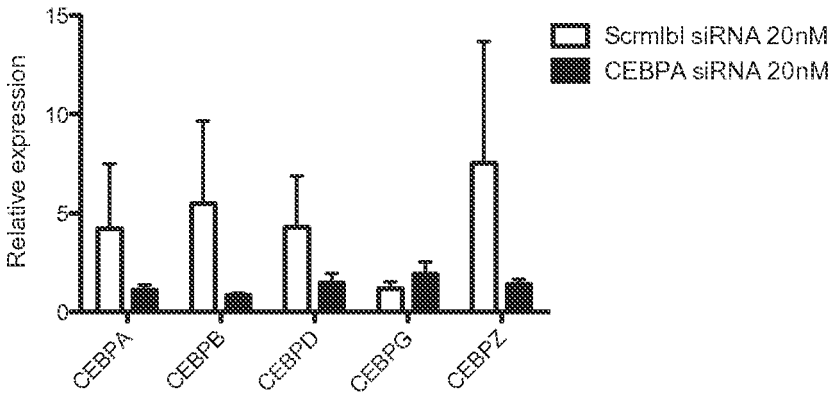


Figure 45

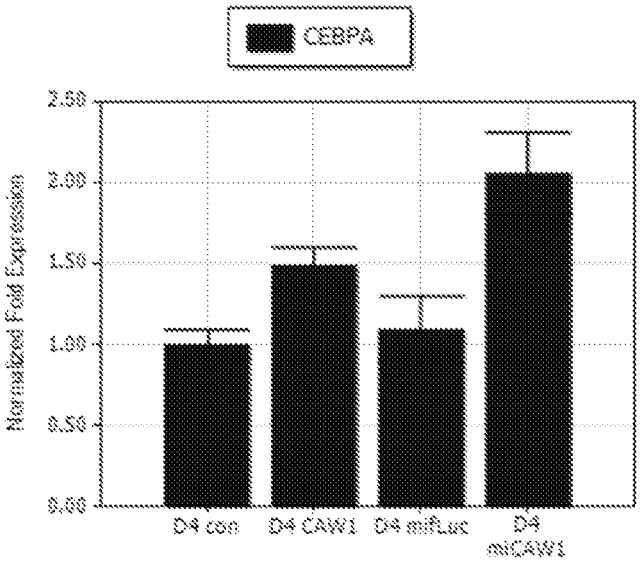

CEBPA
2.50
2.00
1.50
1.00
0.50
0.00
Normalized Fold Expression
D4 con
D4 CAW1
D4 miRLuc
D4 miCAW1

Figure 46

CEBPA-AW1 miRNA insert sequence

[redacted]ttgtttgaatgaggcttcagtactttacagaatcgttgcctgcacatcttgg aaacacttgctgggattacttcttcaggttaacccaacagaaggctcgagaaggtatatt**t** **gctgttgacagtgagcg**cggcggtcattgtcactggtcatagtgaagccacagatgtatg accagtgacaatgaccgcct**tgcctactgcctcgga**attcaaggggctactttaggagca attatcttgtttactaaaactgaatacttgctatctctttgatacatt[redacted]	[redacted] **miR-30 5′ flank** Passenger strand miR-30 loop Guide strand **miR-30 3′ flank** AscI

Common miR-30 context miR-30 loop

UG UUGA A C A

shRNA-mir: 5′- **CUG CAGUG GCG** GGCGGUCAUUGUCACUGGUCAUA---GUG A

3′- **GGC GUCAU CGU** CCGCCAGUAACAGUGACCAGUAU CAC G

A UCC C U GUAGA C

Predicted small RNA

Figure 47

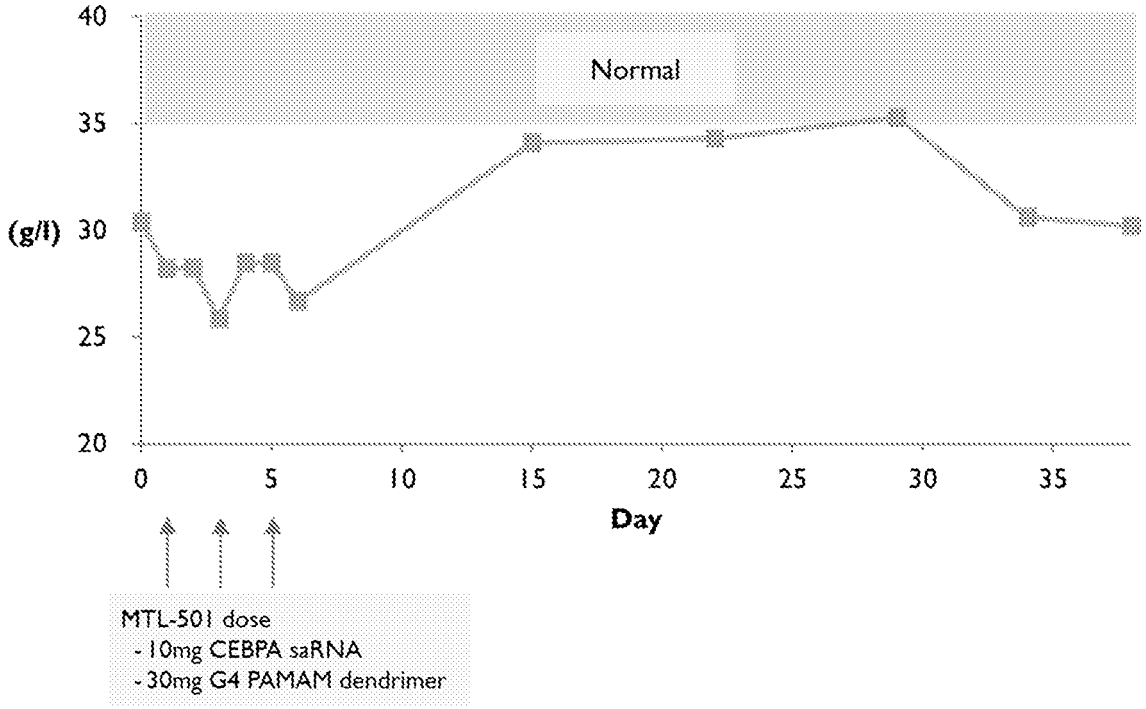

40
35
30
25
20
(g/l)
Normal
0
5
10
15
20
25
30
35
Day
MTL-501 dose
- 10mg CEBPA saRNA
- 30mg G4 PAMAM dendrimer

Figure 48

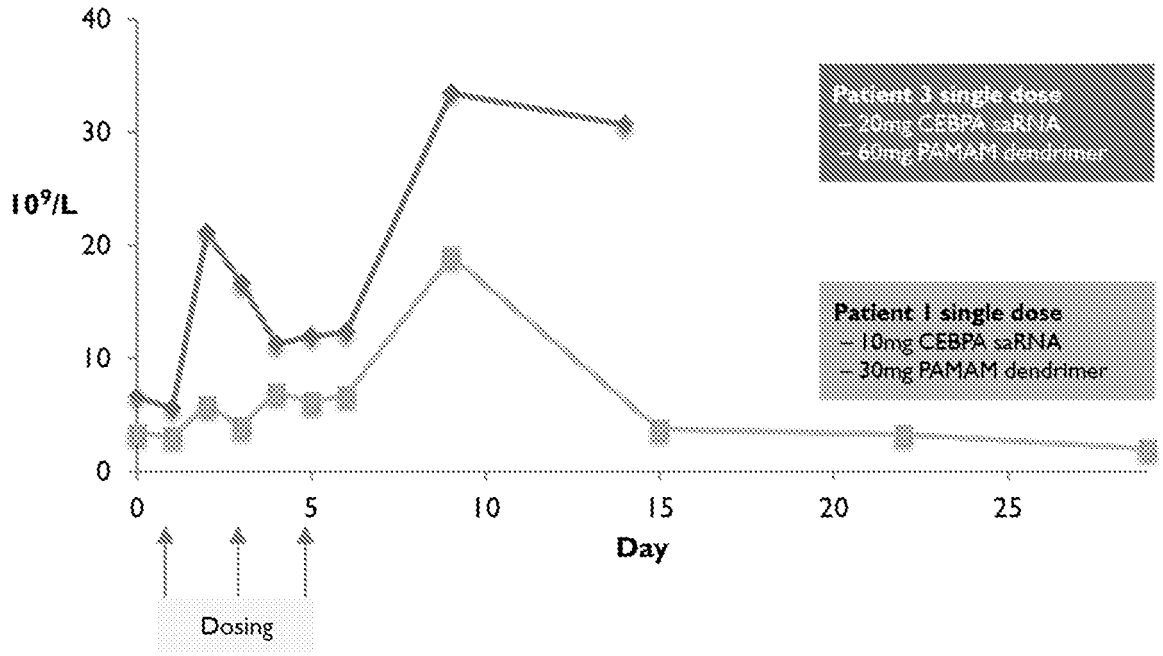
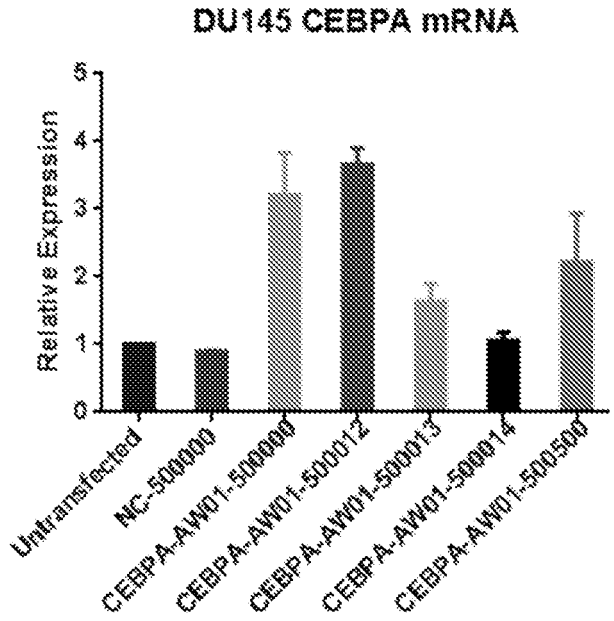

40
30
20
10
0
10^9/L
0
5
10
15
20
25
Day
Dosing
Patient 3 single dose
– 20mg CEBPA saRNA
– 60mg PAMAM dendrimer
Patient 1 single dose
– 10mg CEBPA saRNA
– 30mg PAMAM dendrimer

Figure 49

A


DU145 CEBPA mRNA
Relative Expression
0
1
2
3
4
5
Untransfected
NC-500990
CEBPA-AW01-500990
CEBPA-AW01-500012
CEBPA-AW01-500013
CEBPA-AW01-500014
CEBPA-AW01-500500

B

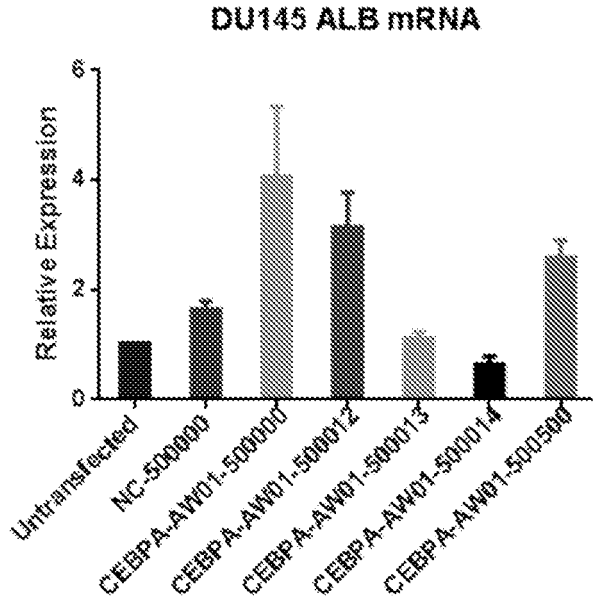
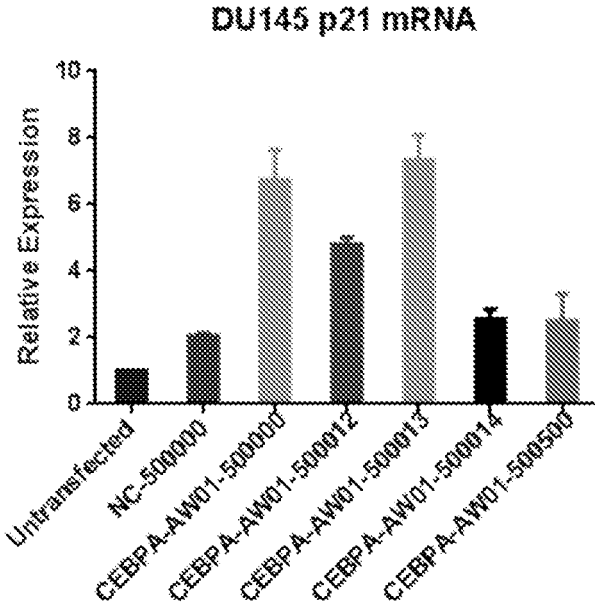

DU145 ALB mRNA
Relative Expression
0
2
4
6
Untransfected
NC-500990
CEBPA-AW01-500000
CEBPA-AW01-500012
CEBPA-AW01-500013
CEBPA-AW01-500014
CEBPA-AW01-500500

Figure 49, cont.

C



D

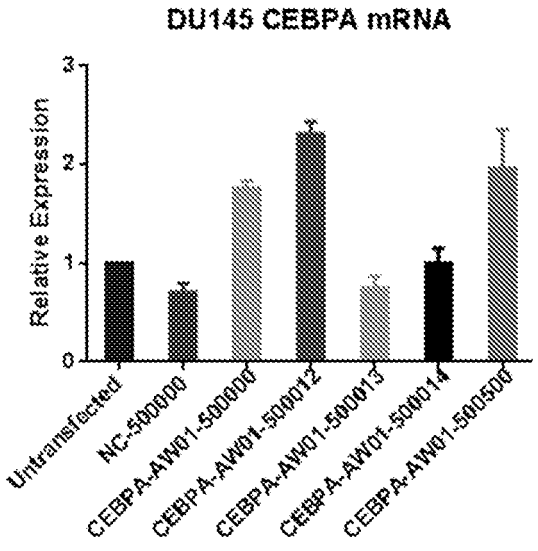
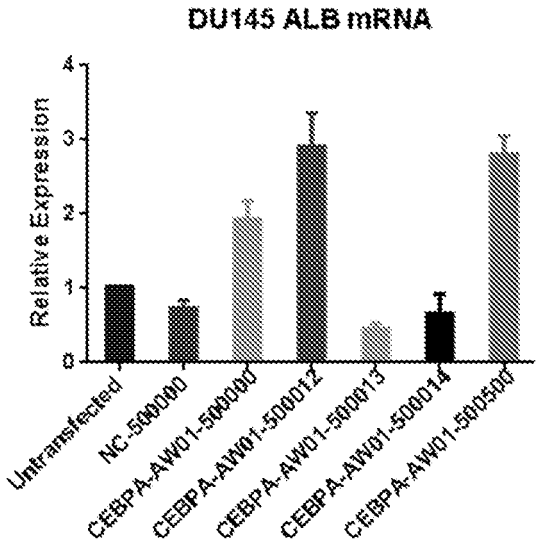


Figure 49, cont.

E



F

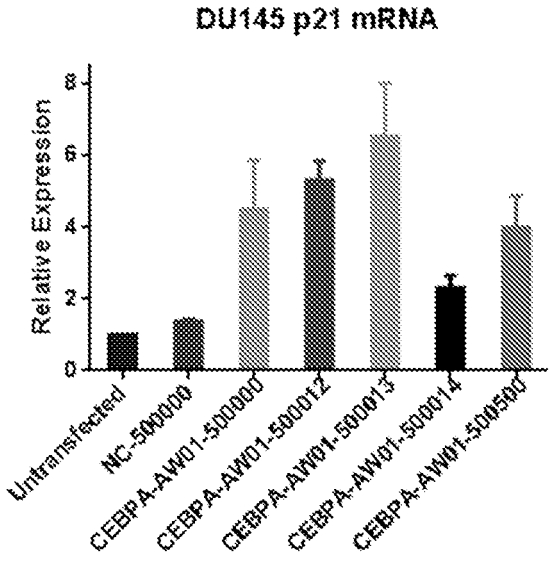
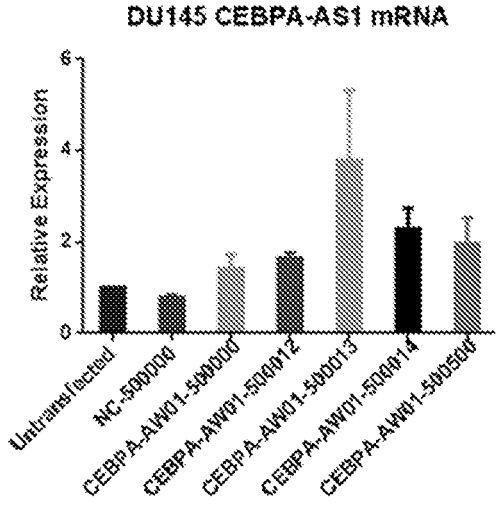
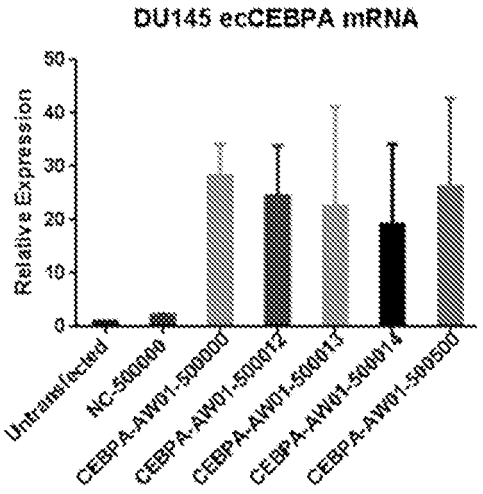


Figure 50

A



B



C

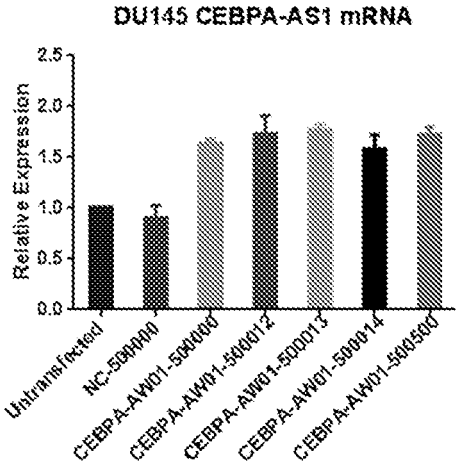
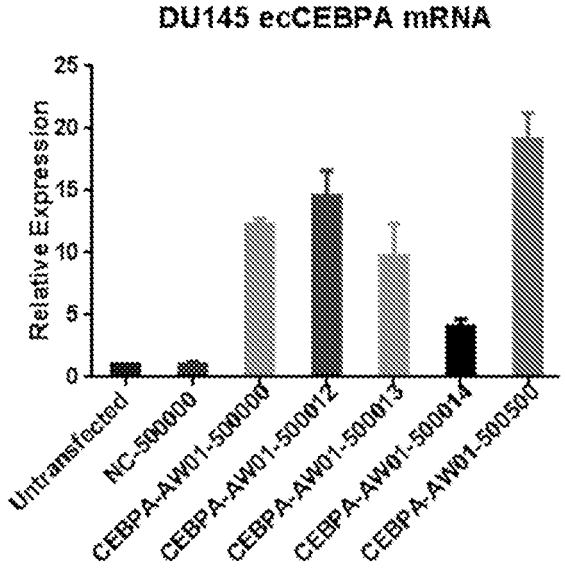


Figure 50, cont.

D



E

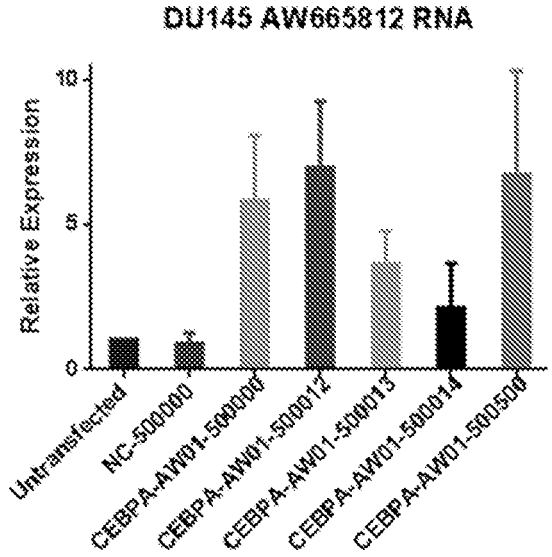


Figure 51

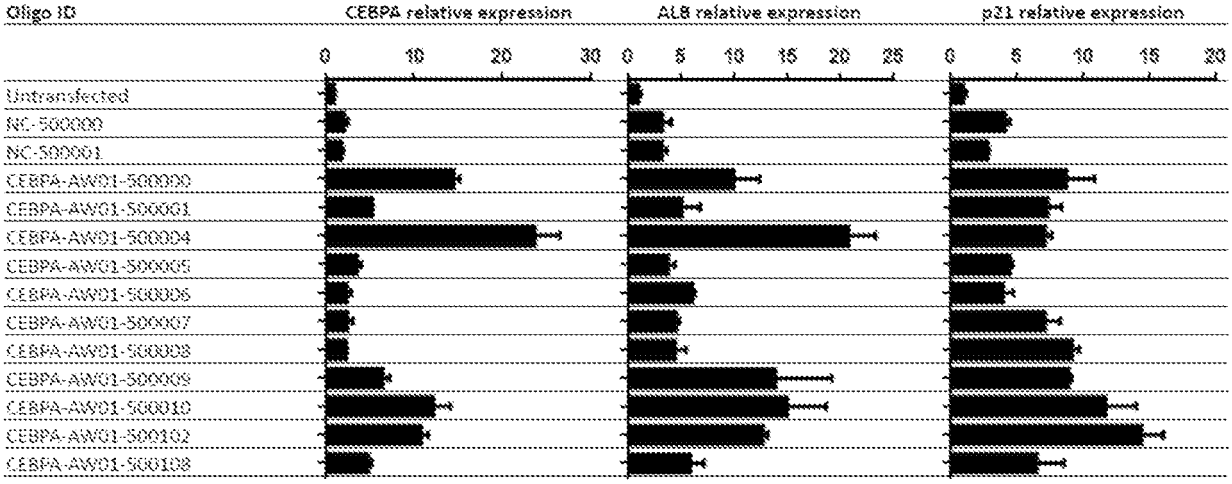

Oligo ID
CEBPA relative expression
ALB relative expression
p21 relative expression
0
10
20
30
0
5
10
15
20
25
0
5
10
15
20
Untransfected
NC-500000
NC-500001
CEBPA-AW01-500000
CEBPA-AW01-500001
CEBPA-AW01-500004
CEBPA-AW01-500005
CEBPA-AW01-500006
CEBPA-AW01-500007
CEBPA-AW01-500008
CEBPA-AW01-500009
CEBPA-AW01-500010
CEBPA-AW01-500102
CEBPA-AW01-500108

Figure 52

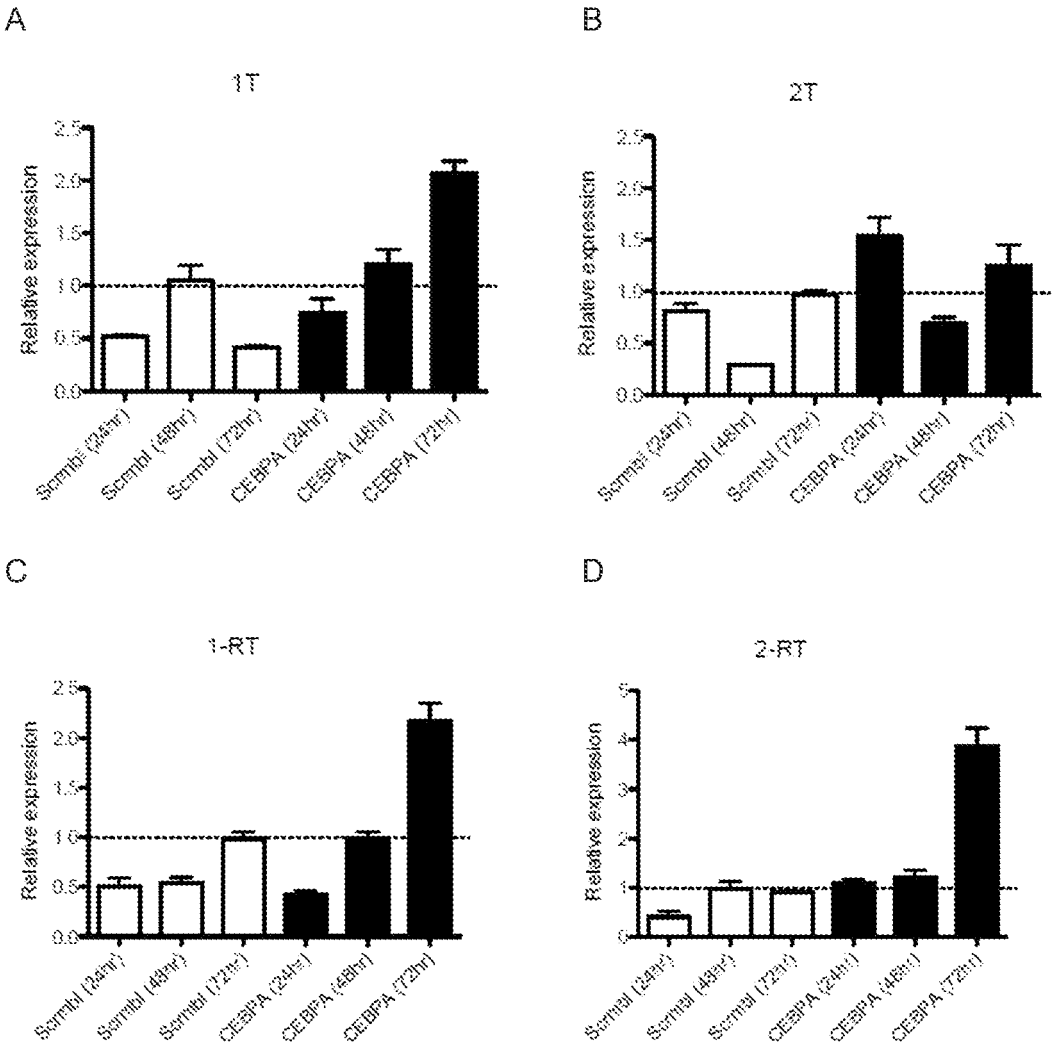

A
1T
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Scrmbl (24hr)
Scrmbl (48hr)
Scrmbl (72hr)
CEBPA (24hr)
CEBPA (48hr)
CEBPA (72hr)
B
2T
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Scrmbl (24hr)
Scrmbl (48hr)
Scrmbl (72hr)
CEBPA (24hr)
CEBPA (48hr)
CEBPA (72hr)
C
1-RT
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
Scrmbl (24hr)
Scrmbl (48hr)
Scrmbl (72hr)
CEBPA (24hr)
CEBPA (48hr)
CEBPA (72hr)
D
2-RT
Relative expression
5
4
3
2
1
0
Scrmbl (24hr)
Scrmbl (48hr)
Scrmbl (72hr)
CEBPA (24hr)
CEBPA (48hr)
CEBPA (72hr)

Figure 53

A

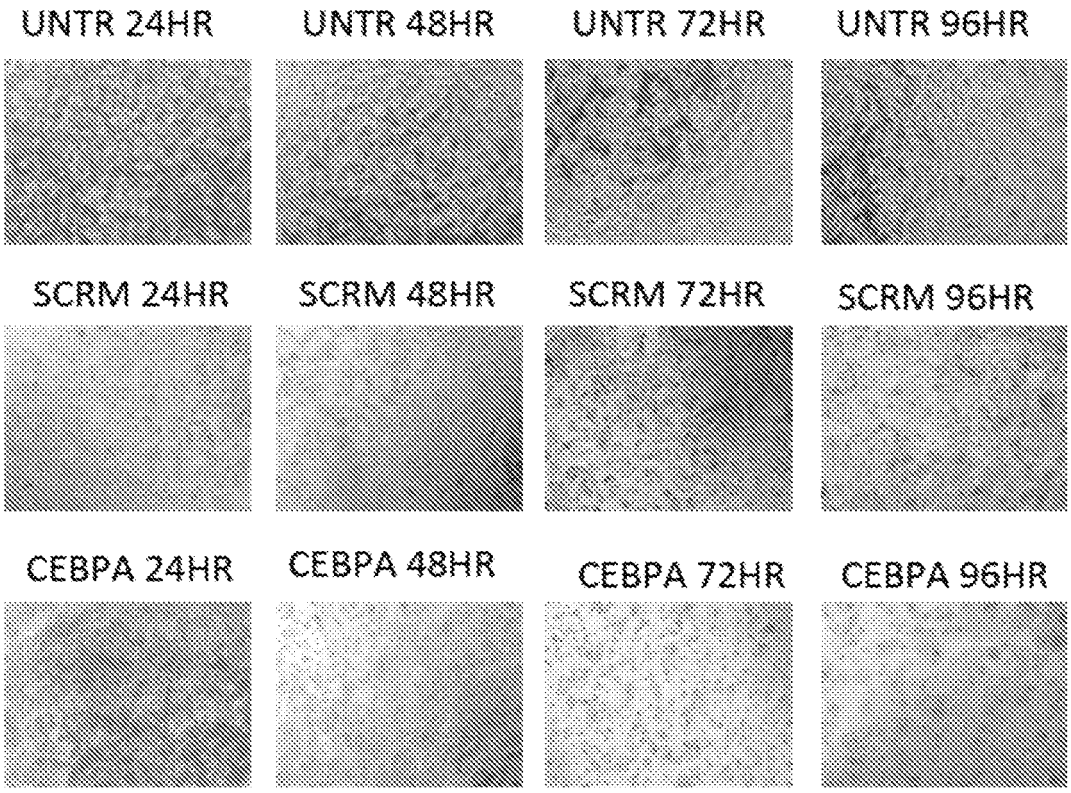
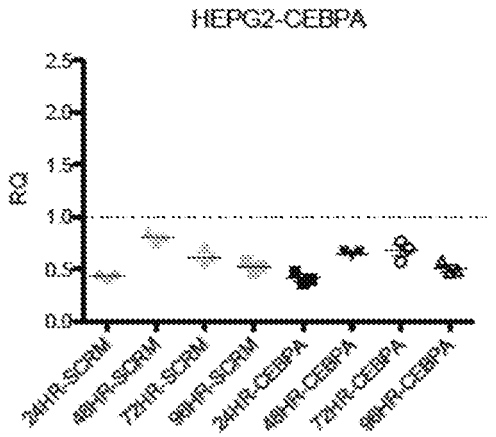
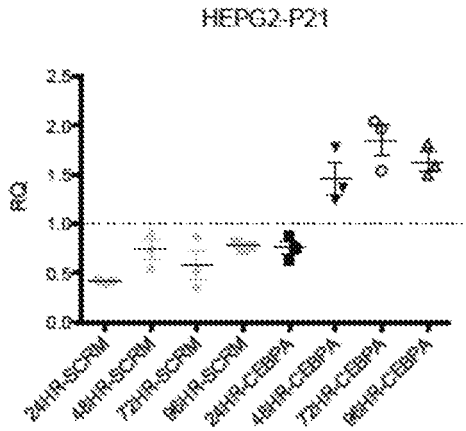

UNTR 24HR
UNTR 48HR
UNTR 72HR
UNTR 96HR
SCRM 24HR
SCRM 48HR
SCRM 72HR
SCRM 96HR
CEBPA 24HR
CEBPA 48HR
CEBPA 72HR
CEBPA 96HR

Figure 53

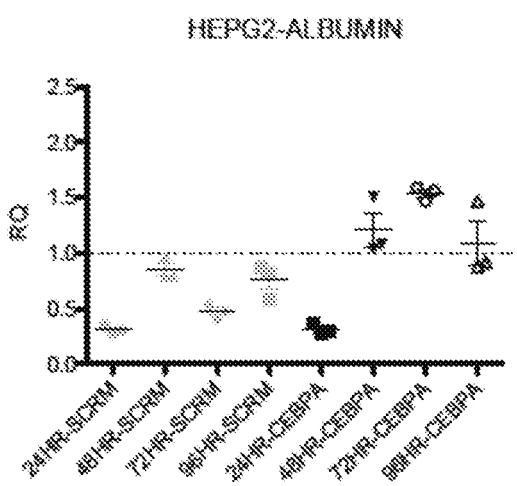
B



C



D



E

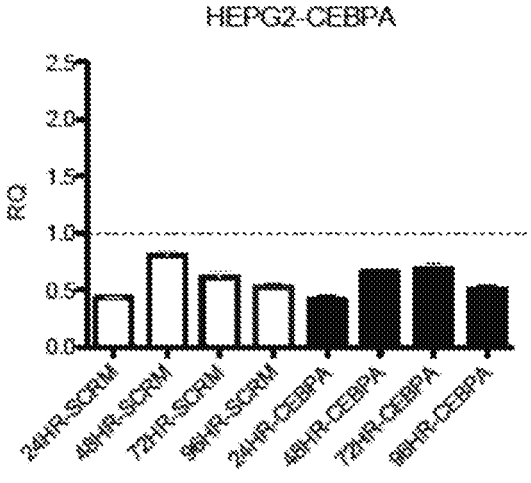
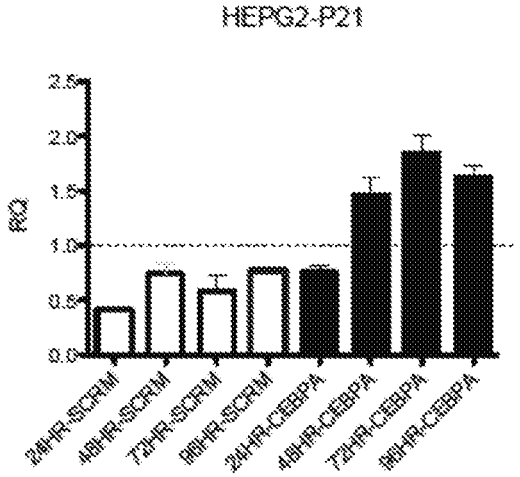


Figure 53, cont.

F



G

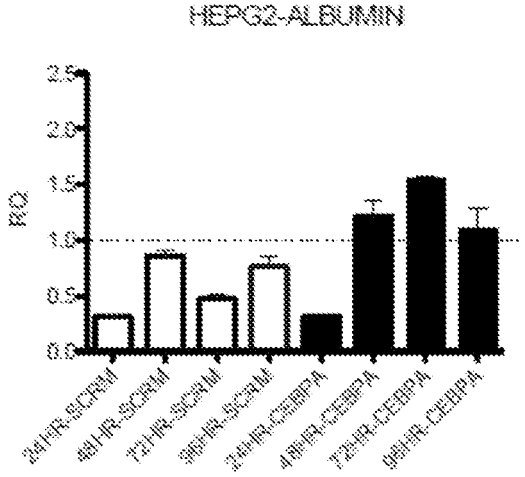


Figure 54

A

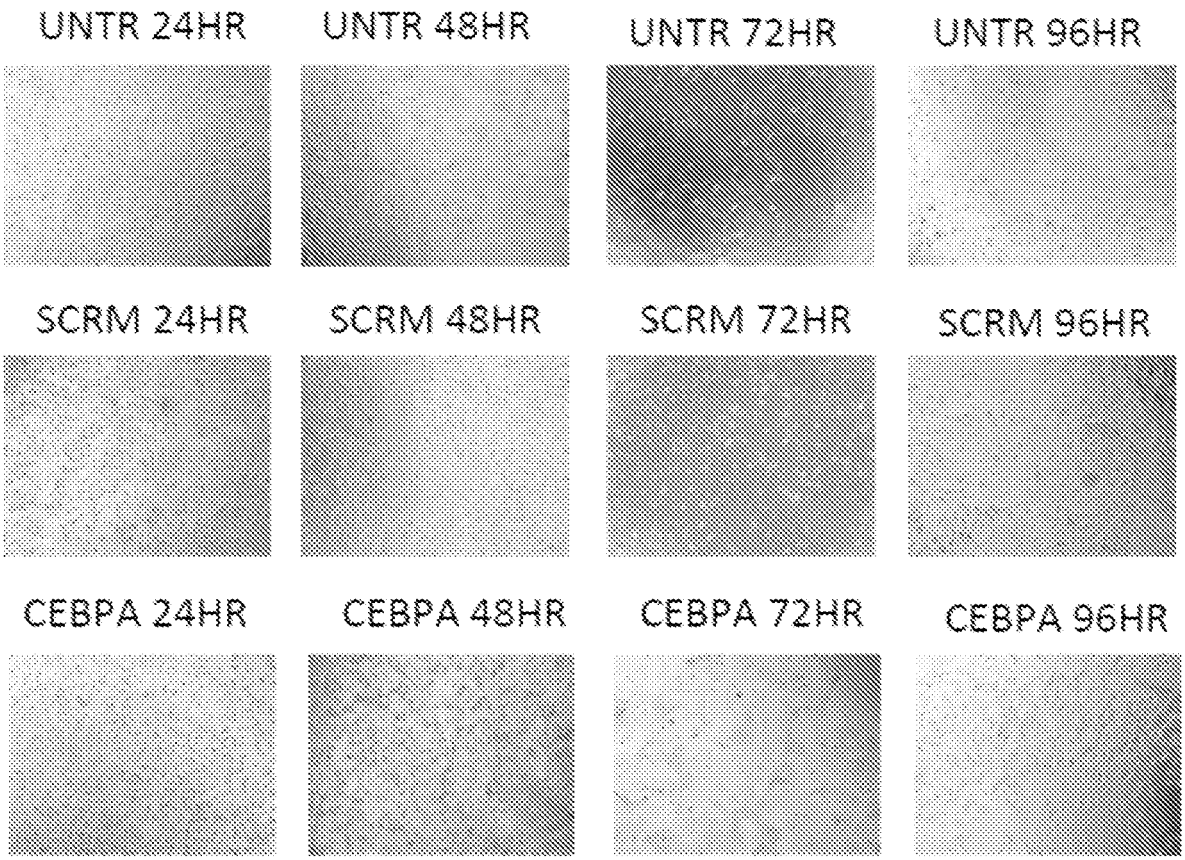

UNTR 24HR
UNTR 48HR
UNTR 72HR
UNTR 96HR
SCRM 24HR
SCRM 48HR
SCRM 72HR
SCRM 96HR
CEBPA 24HR
CEBPA 48HR
CEBPA 72HR
CEBPA 96HR

Figure 54, cont.

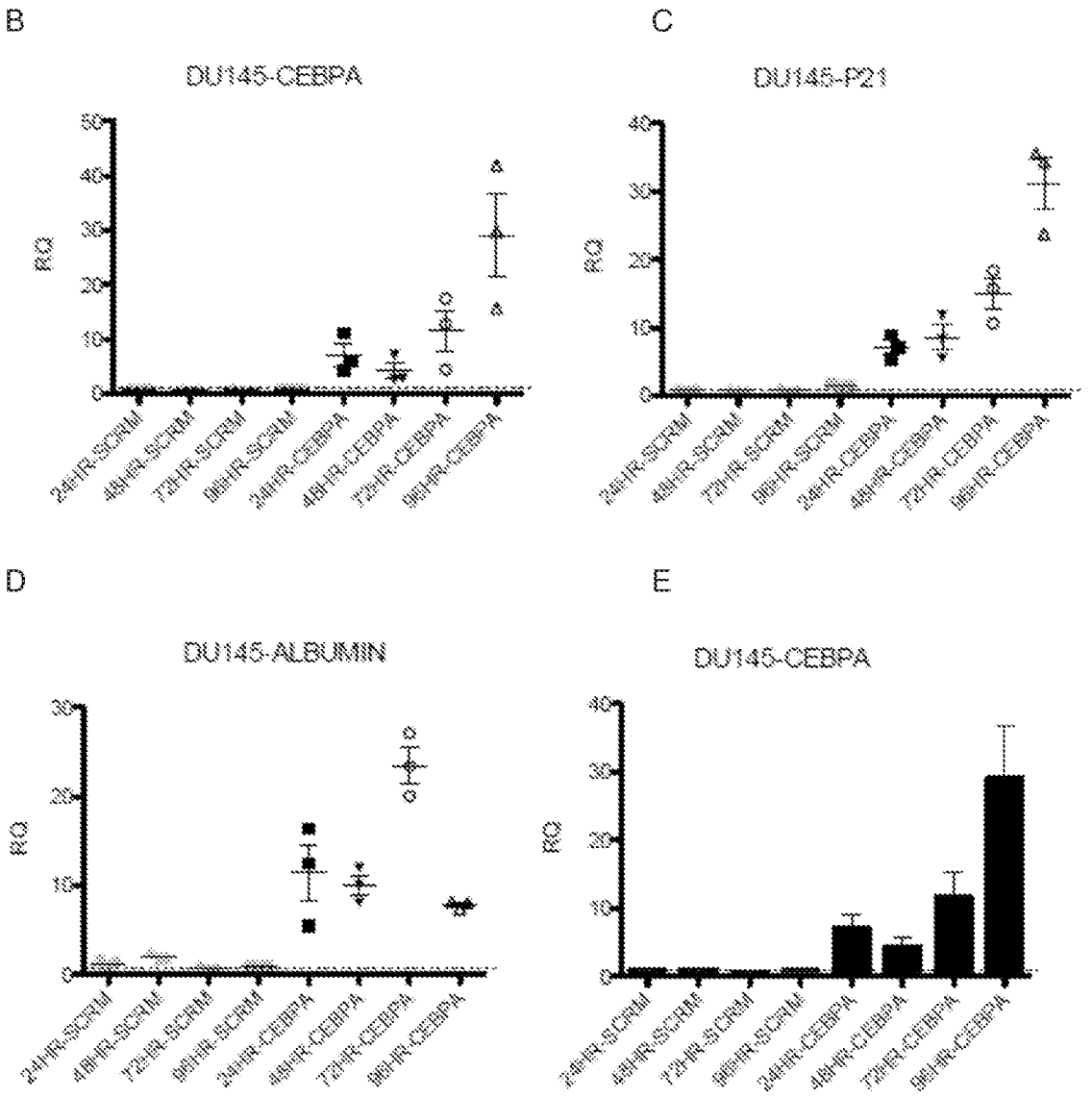
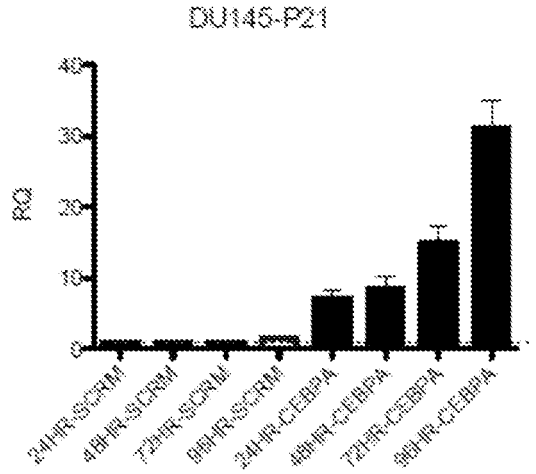


Figure 54, cont.

F



G

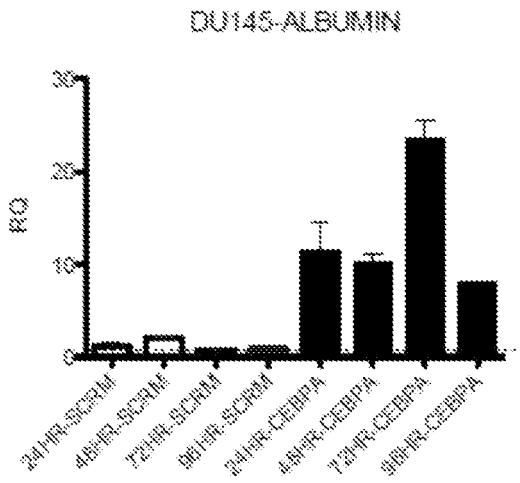


Figure 55

A

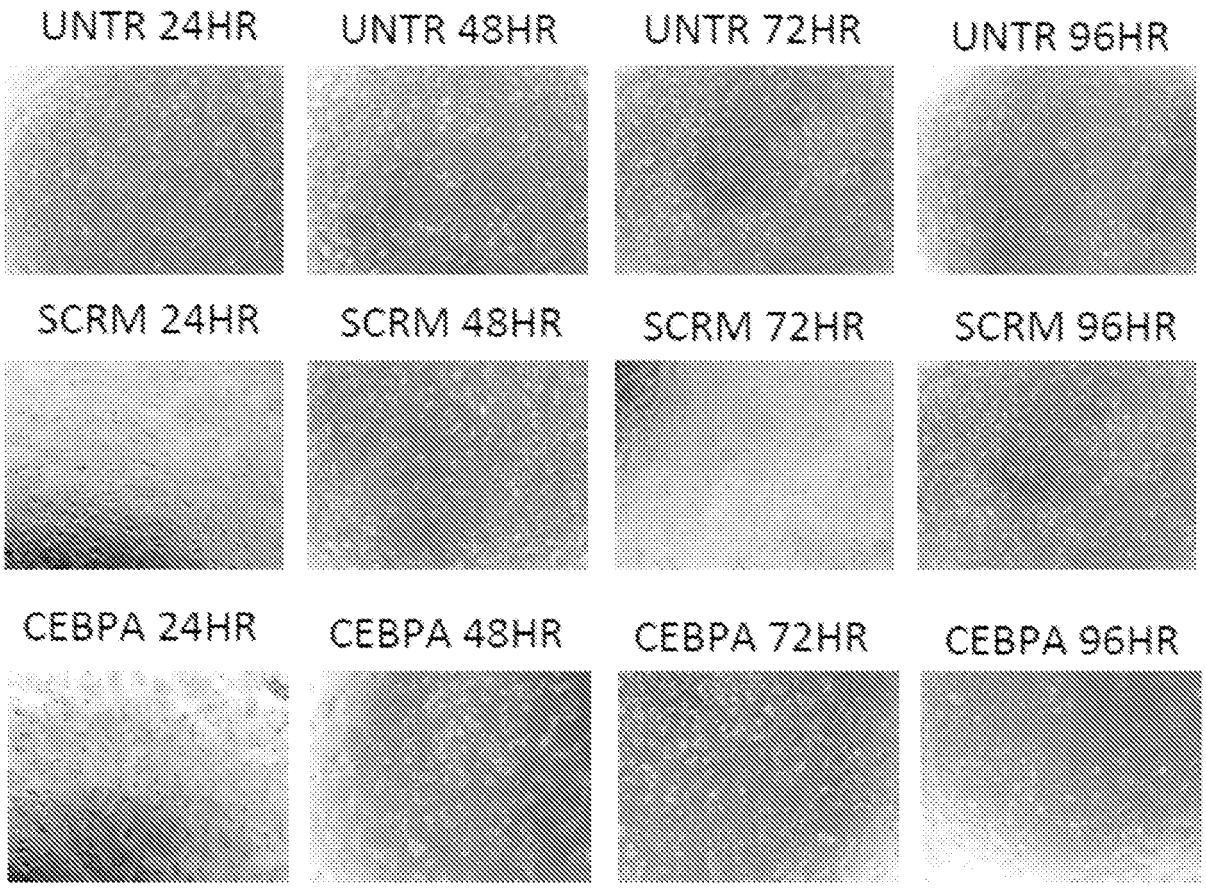

UNTR 24HR
UNTR 48HR
UNTR 72HR
UNTR 96HR
SCRM 24HR
SCRM 48HR
SCRM 72HR
SCRM 96HR
CEBPA 24HR
CEBPA 48HR
CEBPA 72HR
CEBPA 96HR

Figure 55, cont.

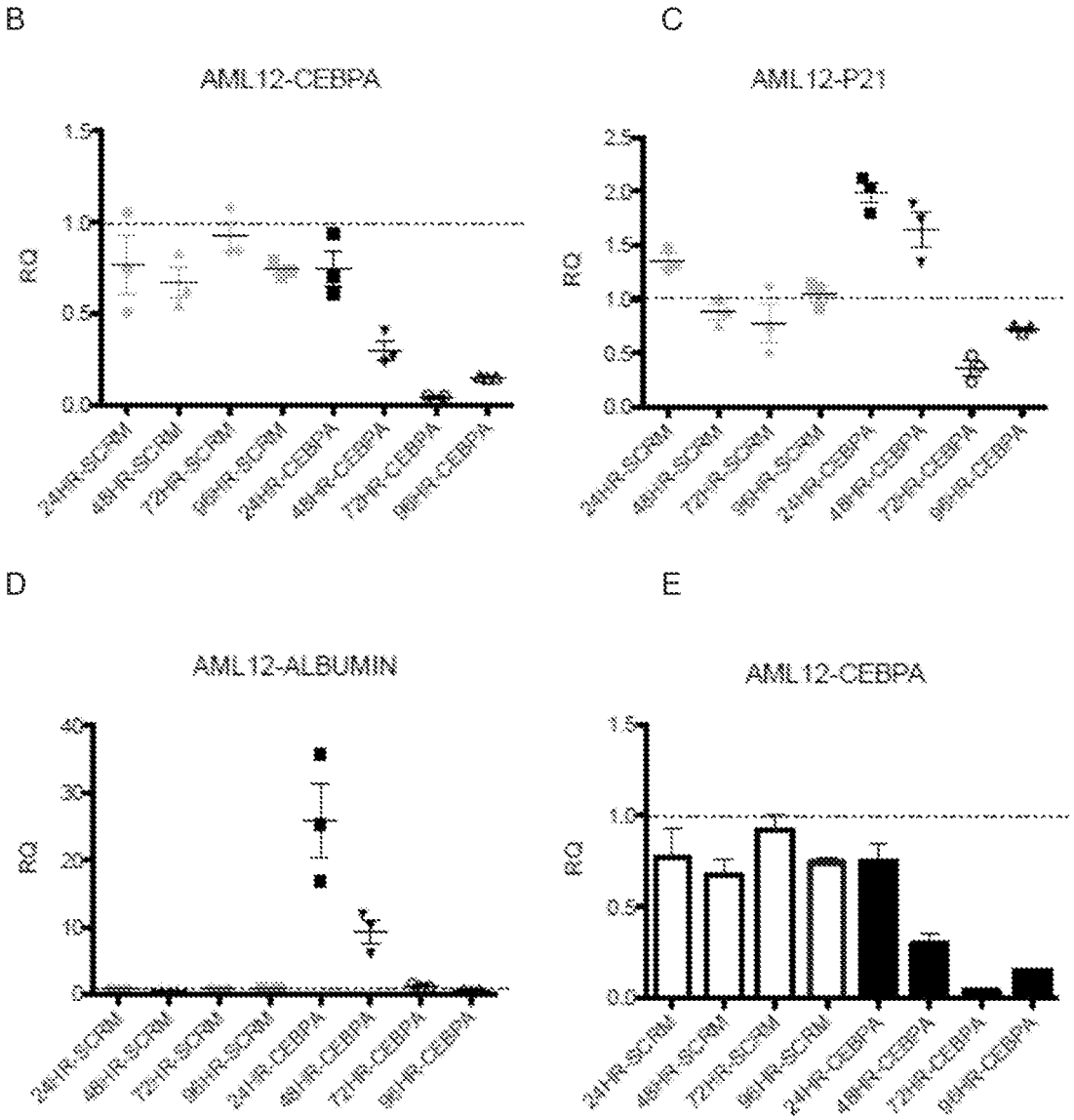
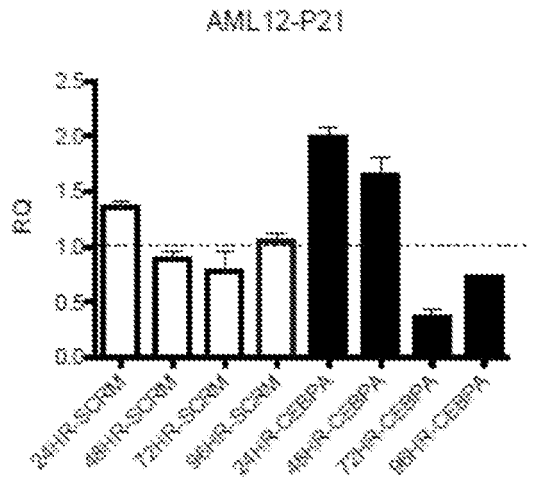


Figure 55, cont.

F



G

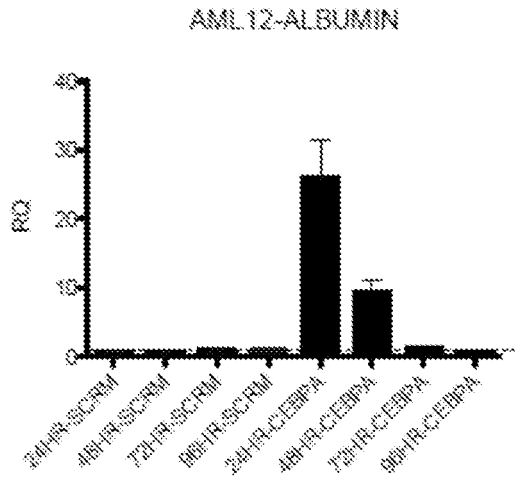


Figure 56

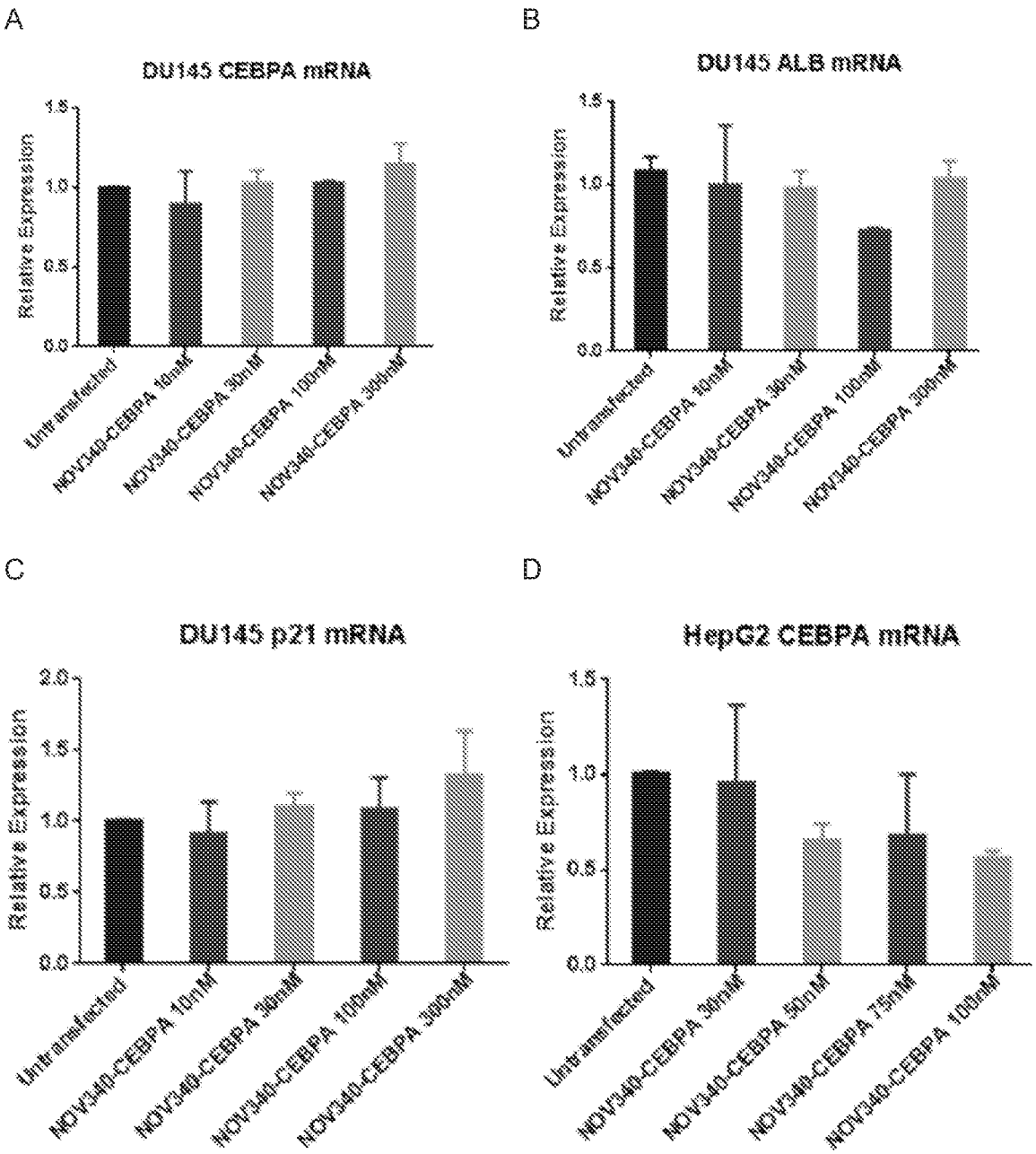
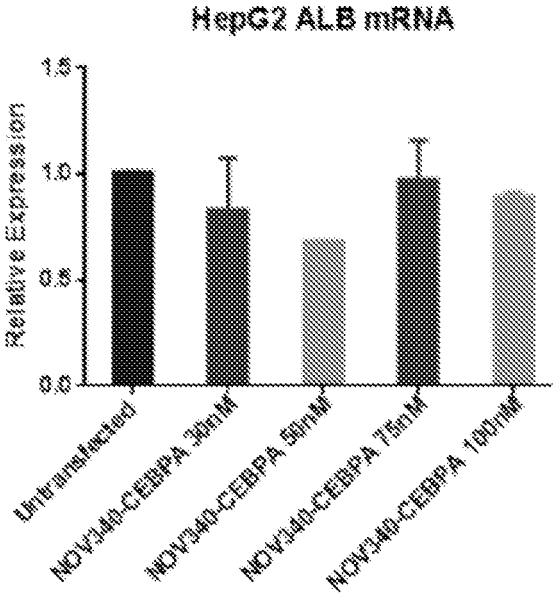

A
DU145 CEBPA mRNA
Relative Expression
1.5
1.0
0.5
0.0
Untransfected
NOV340-CEBPA 10nM
NOV340-CEBPA 30nM
NOV340-CEBPA 100nM
NOV340-CEBPA 300nM
B
DU145 ALB mRNA
Relative Expression
1.5
1.0
0.5
0.0
Untransfected
NOV340-CEBPA 10nM
NOV340-CEBPA 30nM
NOV340-CEBPA 100nM
NOV340-CEBPA 300nM
C
DU145 p21 mRNA
Relative Expression
2.0
1.5
1.0
0.5
0.0
Untransfected
NOV340-CEBPA 10nM
NOV340-CEBPA 30nM
NOV340-CEBPA 100nM
NOV340-CEBPA 300nM
D
HepG2 CEBPA mRNA
Relative Expression
1.5
1.0
0.5
0.0
Untransfected
NOV340-CEBPA 30nM
NOV340-CEBPA 50nM
NOV340-CEBPA 75nM
NOV340-CEBPA 100nM

Figure 56, cont.

E



F

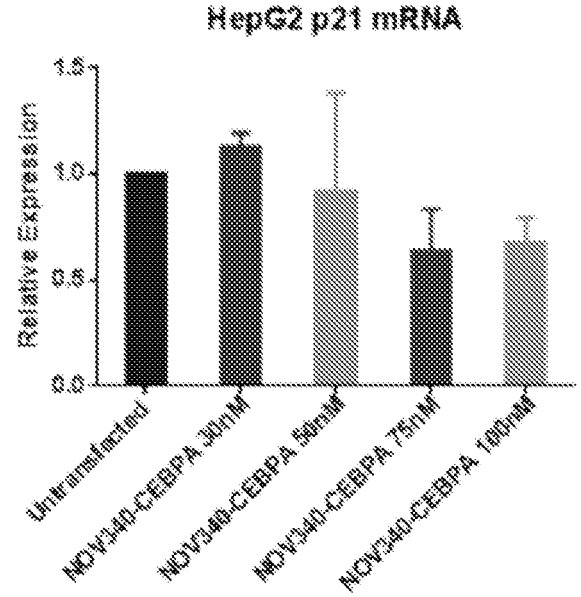
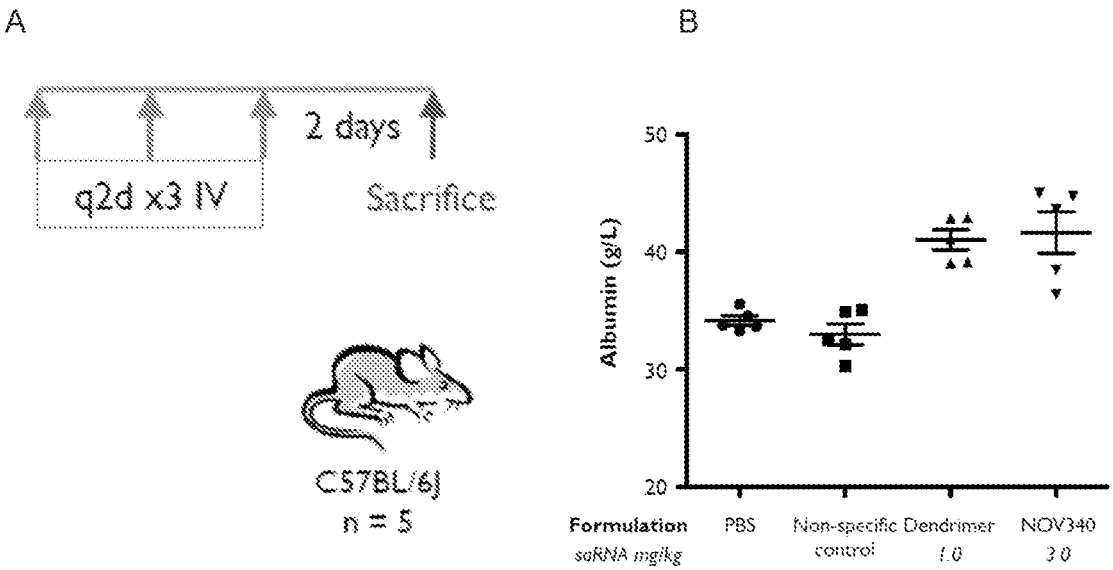


Figure 57


A
2 days
q2d x3 IV
Sacrifice
C57BL/6J
n = 5
B
Albumin (g/L)
50
40
30
20
Formulation
saRNA mg/kg
PBS
Non-specific control
Dendrimer 1.0
NOV340 3.0

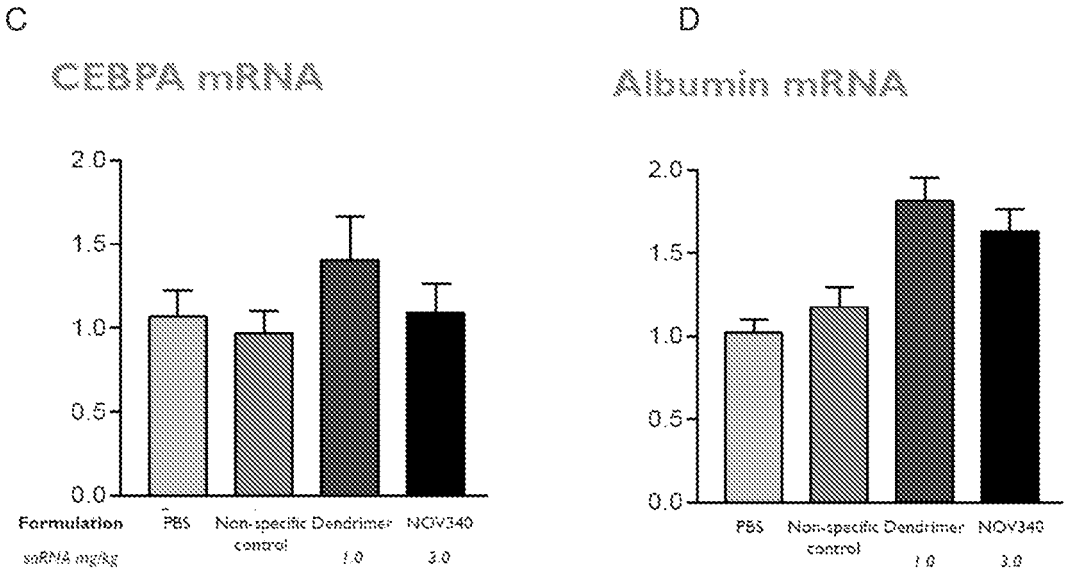
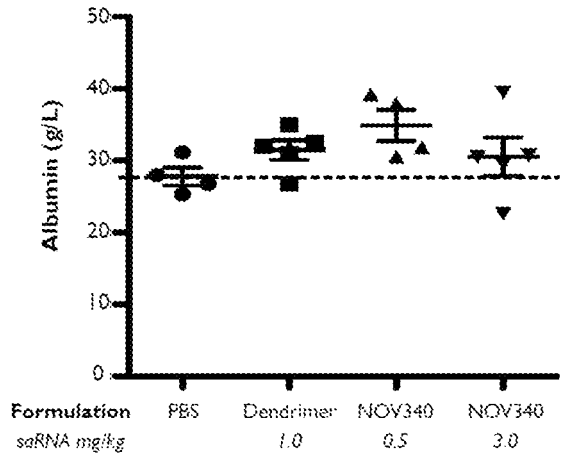
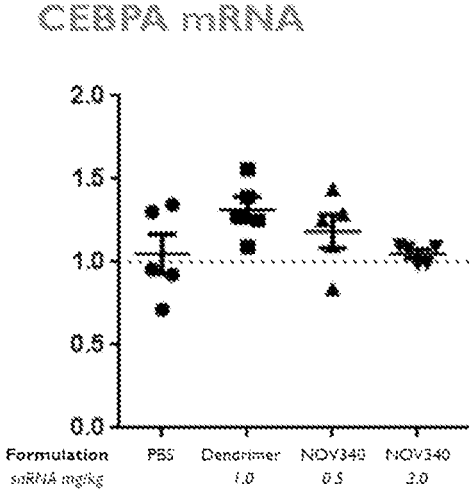

C
CEBPA mRNA
2.0
1.5
1.0
0.5
0.0
Formulation
saRNA mg/kg
PBS
Non-specific control
Dendrimer 1.0
NOV340 3.0
D
Albumin mRNA
2.0
1.5
1.0
0.5
0.0
PBS
Non-specific control
Dendrimer 1.0
NOV340 3.0

Figure 57, cont.

E



F



G

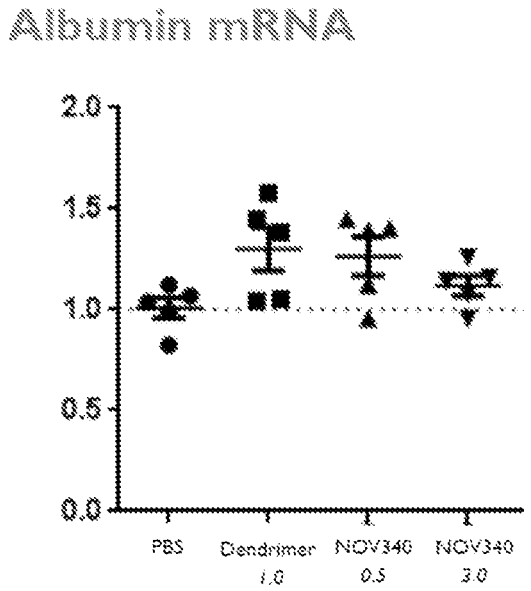
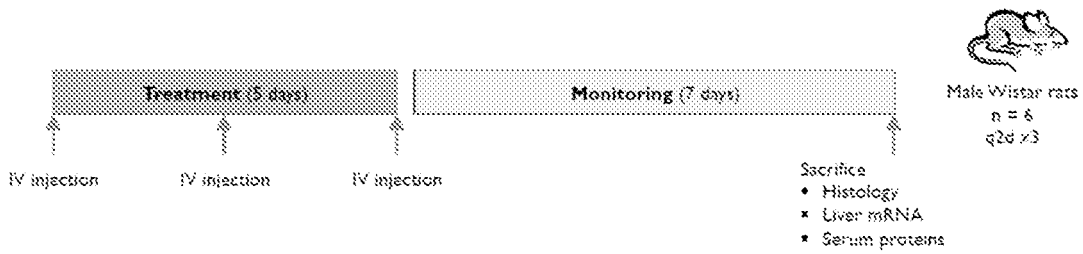
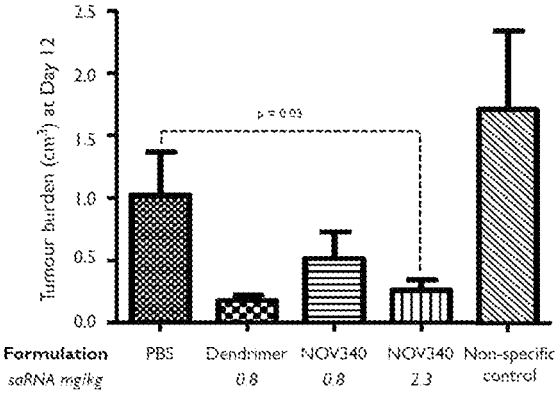


Figure 58

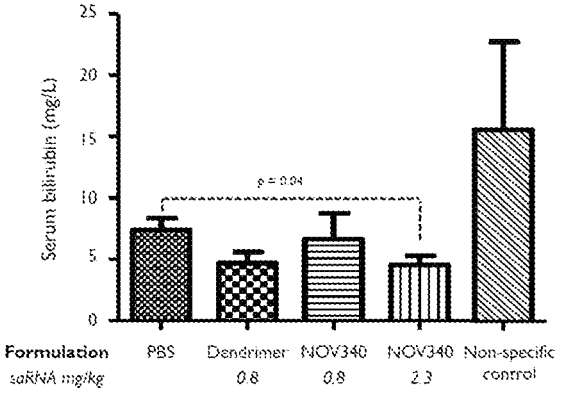
A



B



C



D

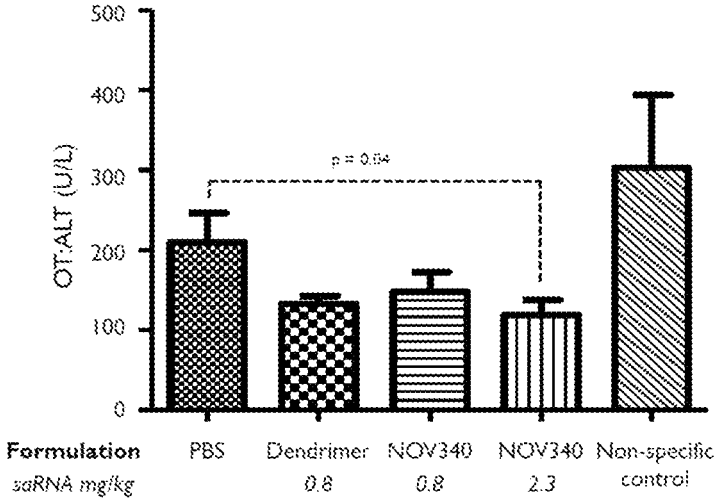
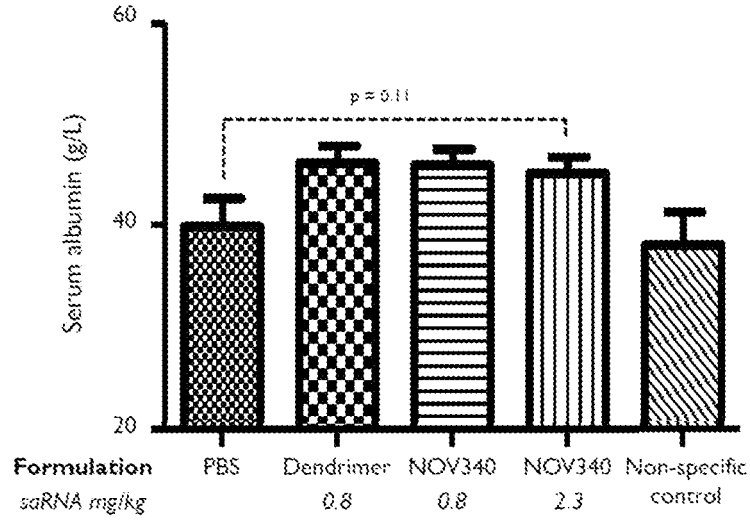


Figure 58, cont.

E



F

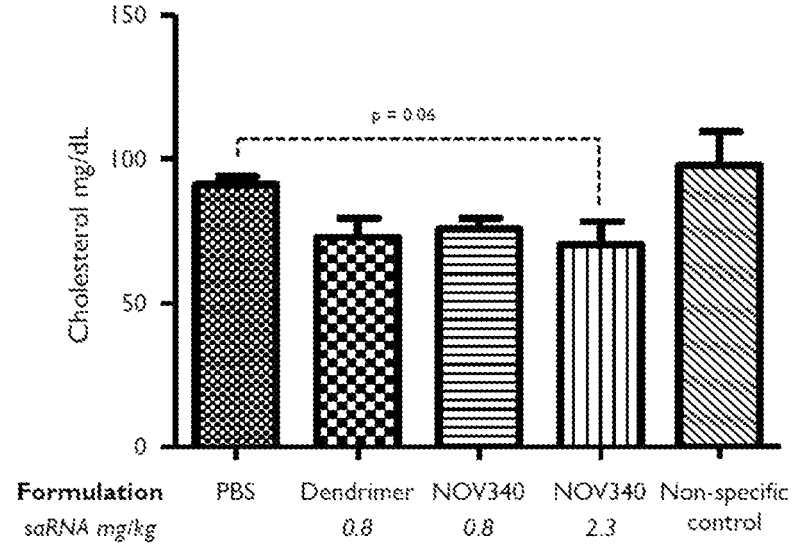


Figure 59

A

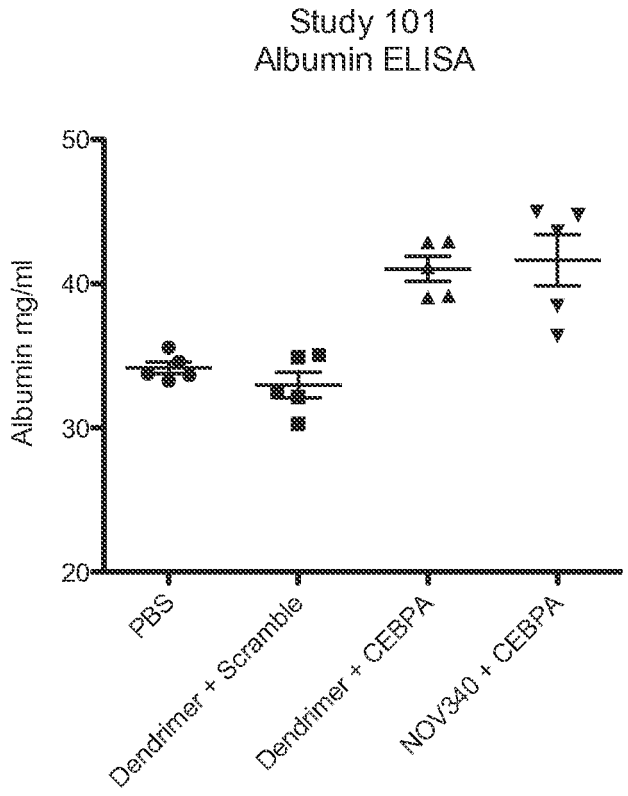
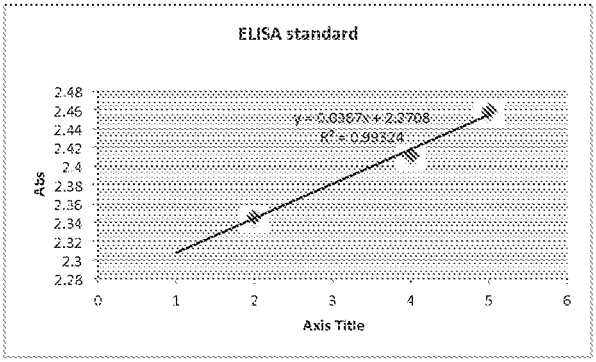

Study 101
Albumin ELISA
Albumin mg/ml
50
40
30
20
PBS
Dendrimer + Scramble
Dendrimer + CEBPA
NOV340 + CEBPA

Figure 59, cont.

B

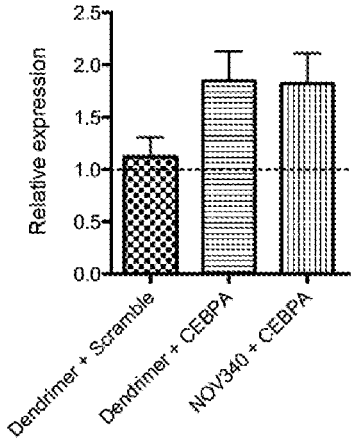
Standard	Abs avg		Abs(-bckgrnd)
0mg	0.7205	0mg	
11.4mg	3.0665	11.4mg	2.346
32mg	3.133	32mg	2.4125
45mg	3.1785	45mg	2.458

Sample	Abs avg	Abs(-bckgrnd)	mg/ml
1_1	3.897	3.1765	34.54986376
1_2	3.863	3.1425	33.25286104
1_3	3.8756	3.1551	33.73351499
1_4	3.873	3.1525	33.63433243
1_5	3.923	3.2025	35.54168937
5_1	3.452	2.7315	17.57438692
5_2	3.2265	2.506	8.972207084
5_3	2.948	2.2275	-1.651771117
5_4	3.7915	3.071	30.5253406
5_5	2.7855	2.065	-7.850681199
6_1	1.147	0.4265	-70.35476839
6_2	3.254	2.5335	10.02125341
6_3	3.9275	3.207	35.7133515
6_4	3.193	2.4725	7.694277929
6_5	2.9985	2.278	0.274659401



C

CEBPA mRNA
With outliers omitted (3 data points)



D

Albumin mRNA
With outliers omitted (2 data points)

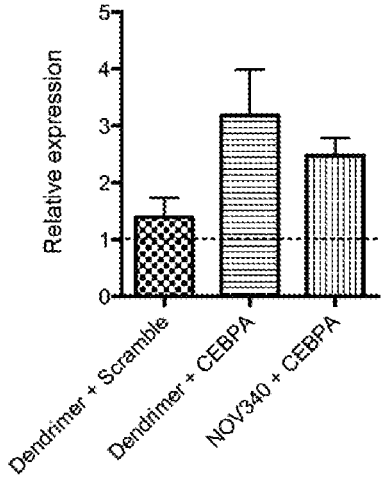
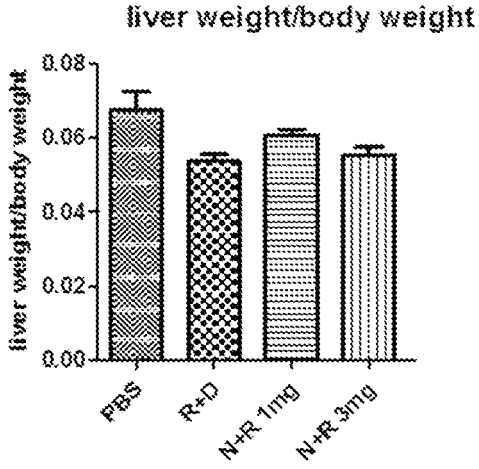


Figure 60

A


liver weight/body weight
liver weight/body weight
0.08
0.06
0.04
0.02
0.00
PBS
R+D
N+R 1mg
N+R 3mg

B

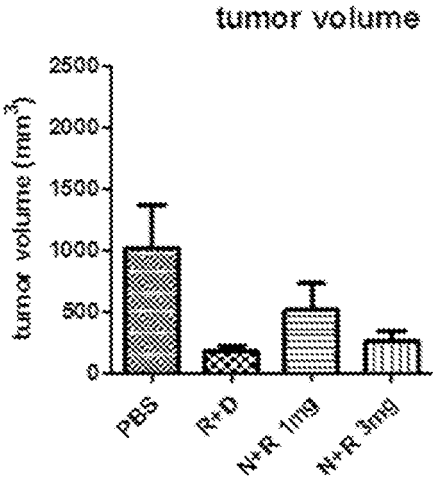
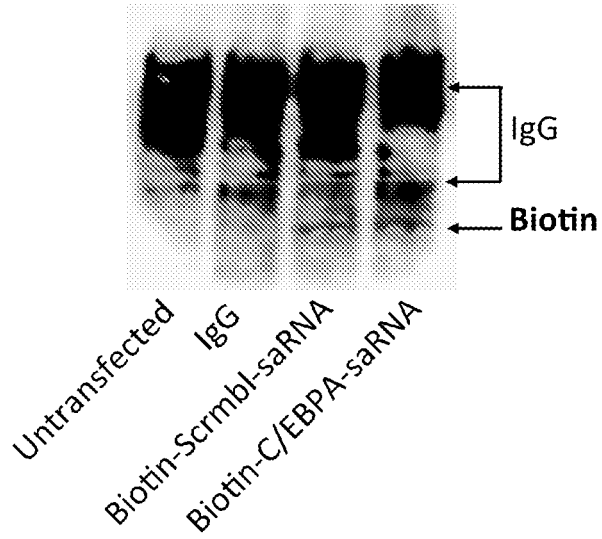

tumor volume
tumor volume (mm3)
2500
2000
1500
1000
500
0
PBS
R+D
N+R 1mg
N+R 3mg

Figure 61

A


IgG
Biotin
Untransfected
IgG
Biotin-ScrmbI-saRNA
Biotin-C/EBPA-saRNA

B

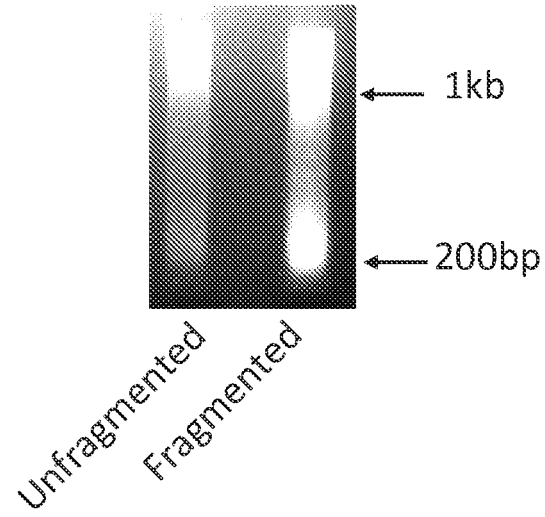
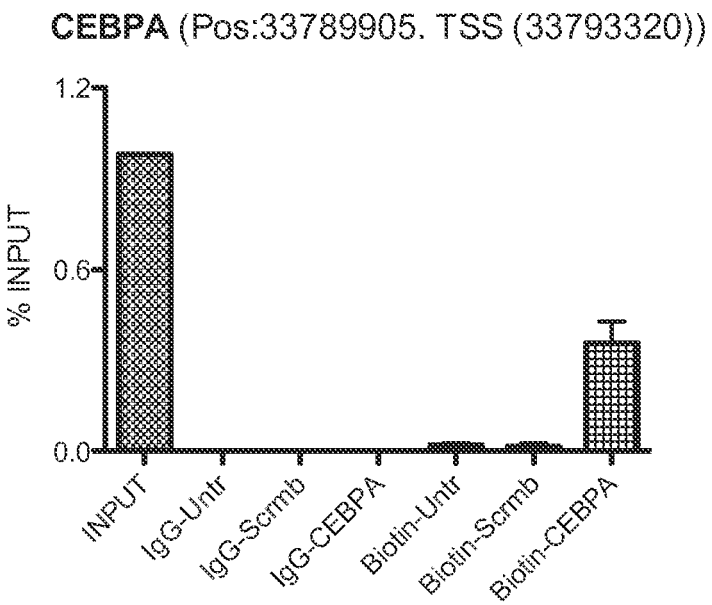

1kb
200bp
Unfragmented
Fragmented

Figure 62

A


CEBPA (Pos:33789905. TSS (33793320))
% INPUT
1.2
0.6
0.0
INPUT
IgG-Untr
IgG-Scrmb
IgG-CEBPA
Biotin-Untr
Biotin-Scrmb
Biotin-CEBPA

B

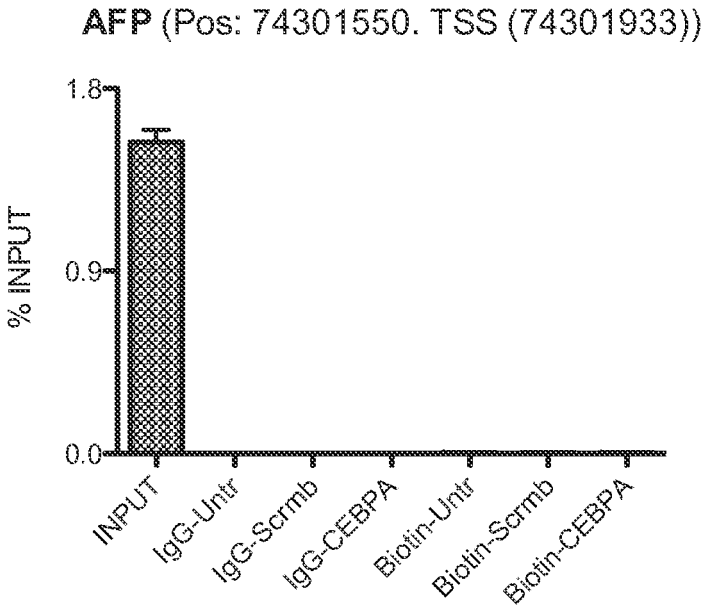
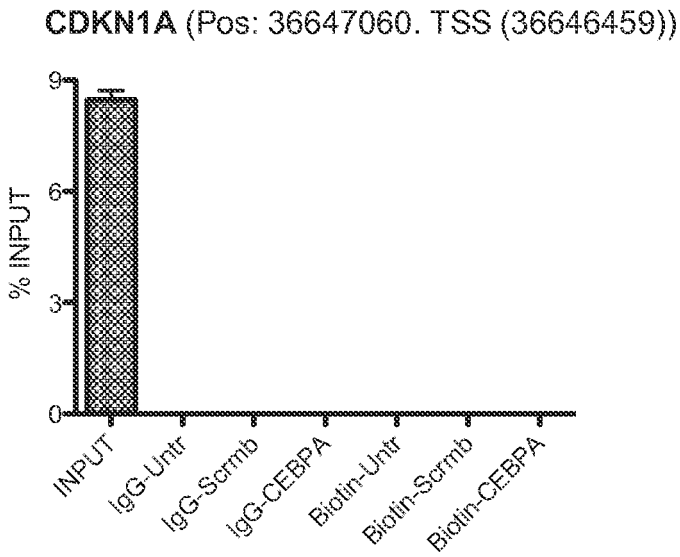

AFP (Pos: 74301550. TSS (74301933))
% INPUT
1.8
0.9
0.0
INPUT
IgG-Untr
IgG-Scrmb
IgG-CEBPA
Biotin-Untr
Biotin-Scrmb
Biotin-CEBPA

Figure 62, cont.

C



D

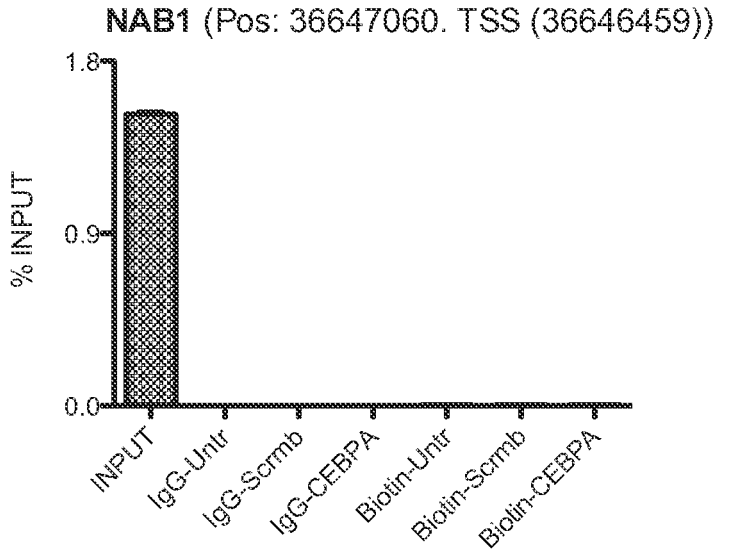


Figure 62, cont.

E

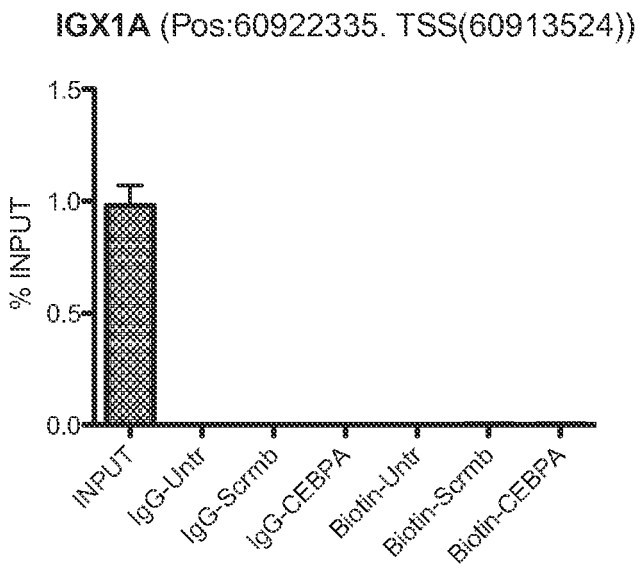
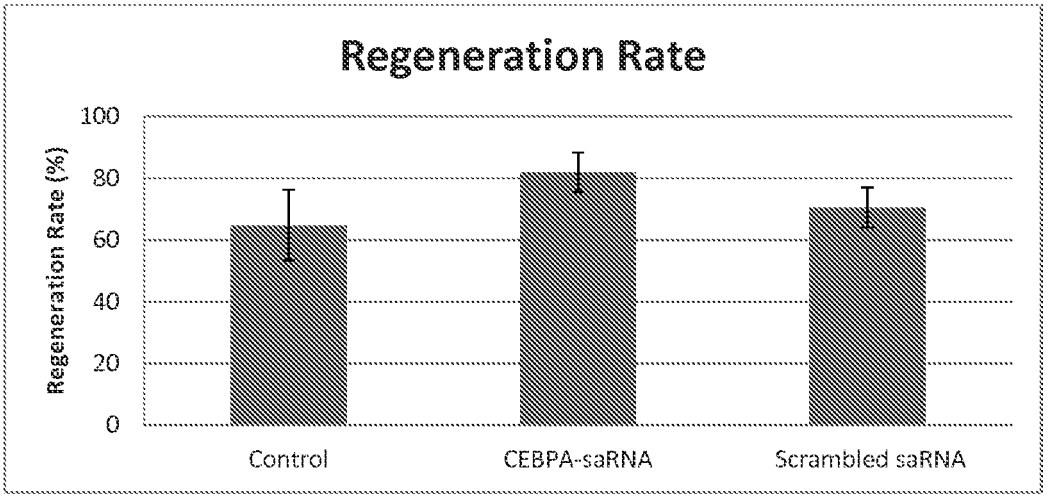


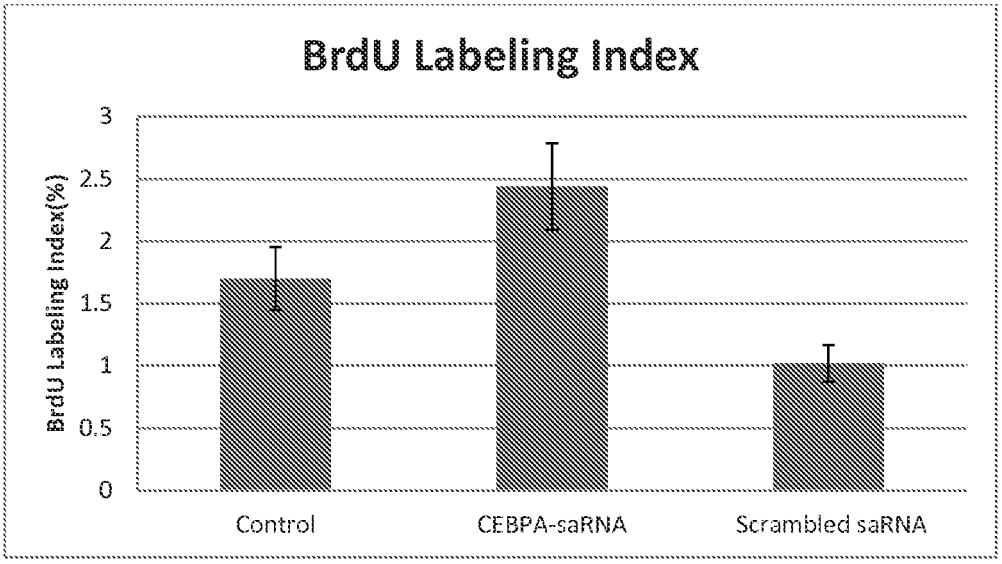
Figure 63

A



Regeneration Rate	Control	CEBPA-saRNA	Scrambled saRNA
mean	64.79021	81.9510952	70.57962819
SD	11.48042	6.386366856	6.505185255

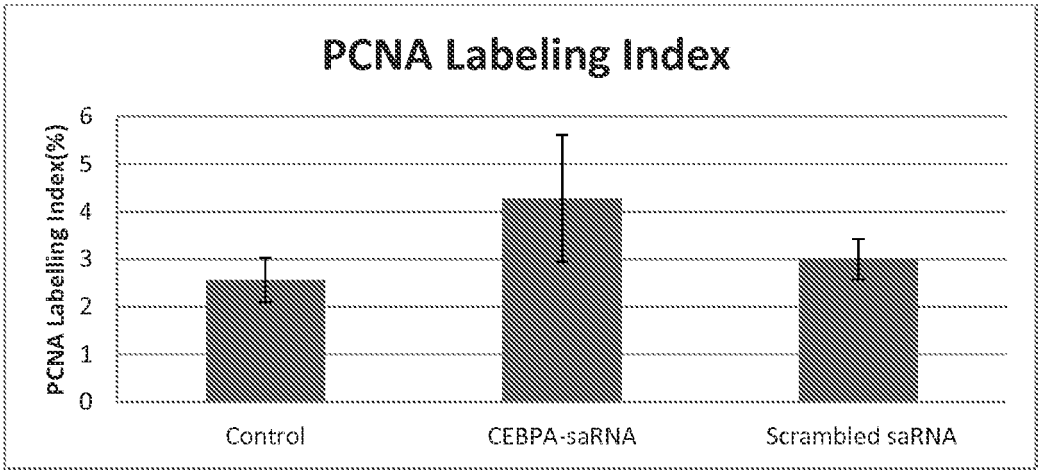
B



BrdU Labeling Index	Control	CEBPA-saRNA	Scrambled saRNA
mean	1.7	2.44	1.02
SD	0.252982	0.347051069	0.14832397

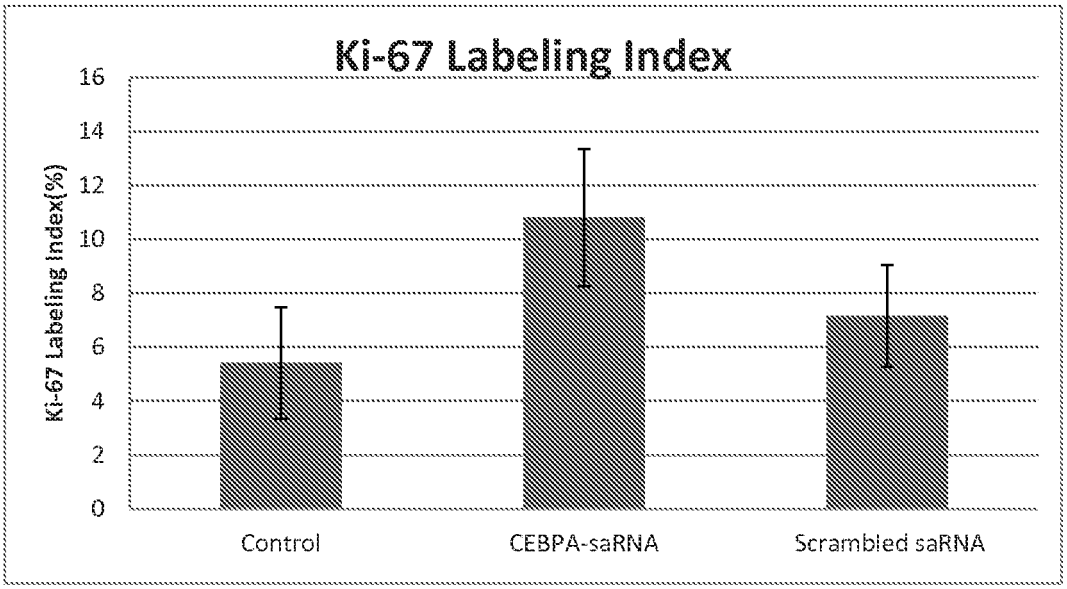
Figure 63, cont.

C



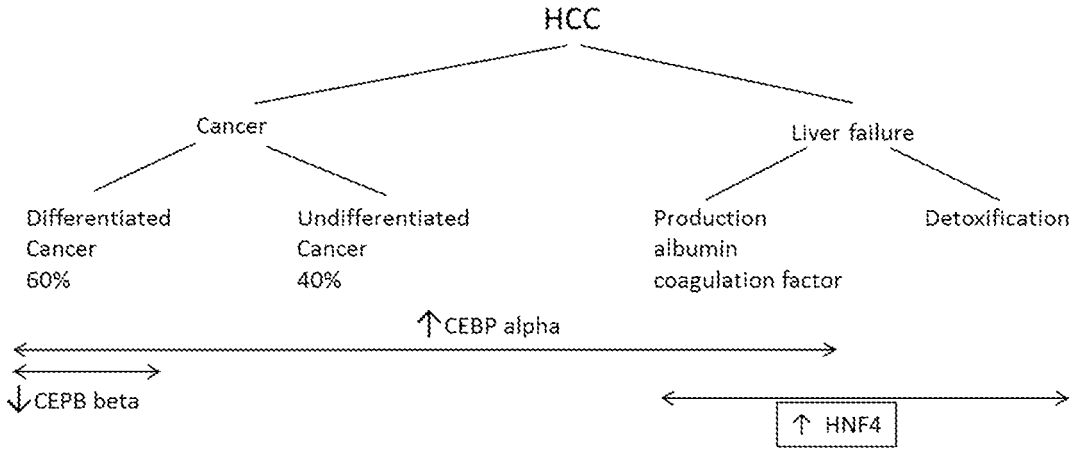
PCNA Labelling Inde	Control	CEBPA-saRNA	Scrambled saRNA
mean	2.56	4.28	3
SD	0.463033	1.333999833	0.424264069

D



Ki-67 Labeling Index	Control	CEBPA-saRNA	Scrambled saRNA
mean	5.42	10.81	7.16
SD	2.067269	2.54490777	1.895521037

Figure 64



Child A Undifferentiated (~ 10%) treat with CEPA alpha

All Child A, B, C, undifferentiated (40%) treat with CEPA + HNF4

All HCC (100%) treat with CEPA + Beta + HNF4

Fatty liver treat with CEBPA

liver failure treat with HNF4

Figure 65

A

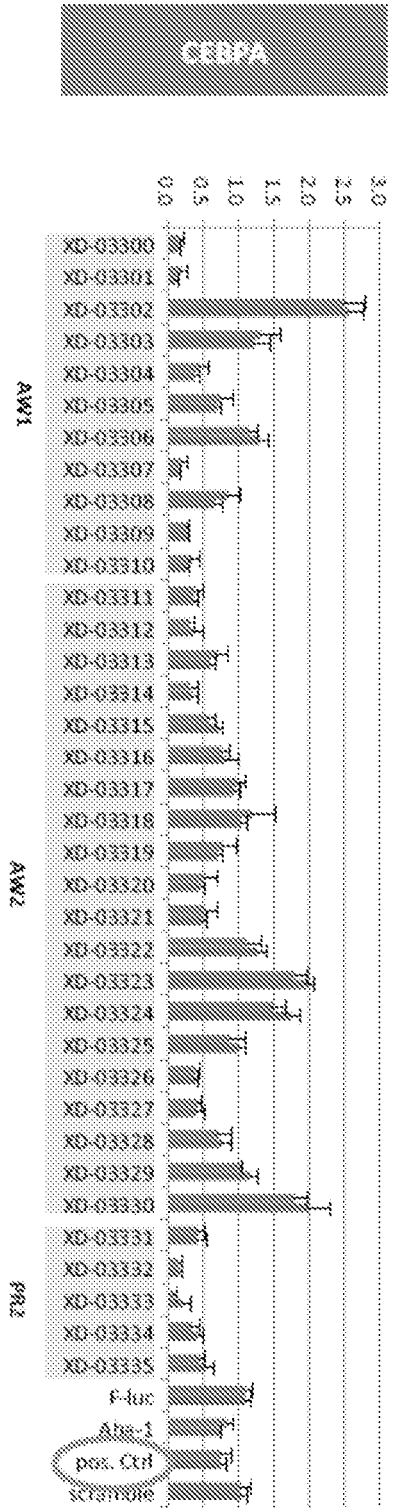

CEBPA
0.0
0.5
1.0
1.5
2.0
2.5
3.0
AW1
AW2
PB2
XD-03300
XD-03301
XD-03302
XD-03303
XD-03304
XD-03305
XD-03306
XD-03307
XD-03308
XD-03309
XD-03310
XD-03311
XD-03312
XD-03313
XD-03314
XD-03315
XD-03316
XD-03317
XD-03318
XD-03319
XD-03320
XD-03321
XD-03322
XD-03323
XD-03324
XD-03325
XD-03326
XD-03327
XD-03328
XD-03329
XD-03330
XD-03331
XD-03332
XD-03333
XD-03334
XD-03335
F-luc
Abs-1
pos. Ctrl
scramble

Figure 65, cont.

B

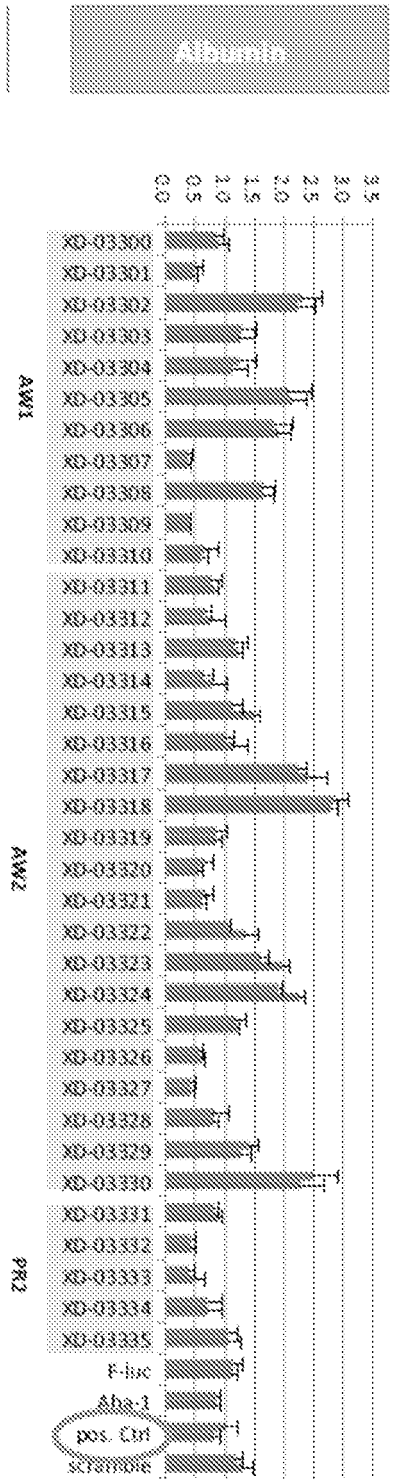


Figure 65, cont.

C

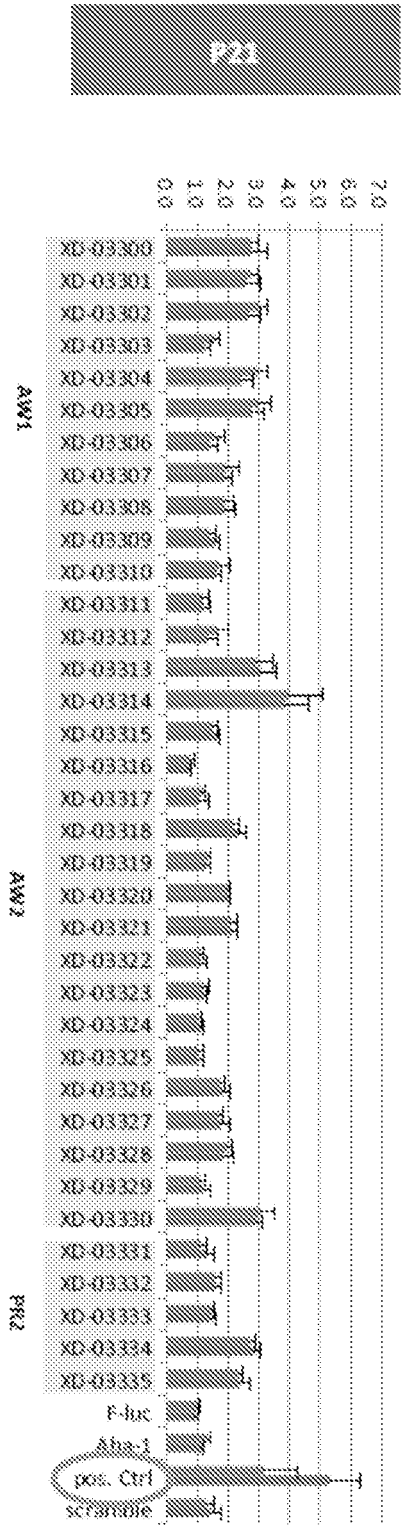


Figure 65, cont.

D

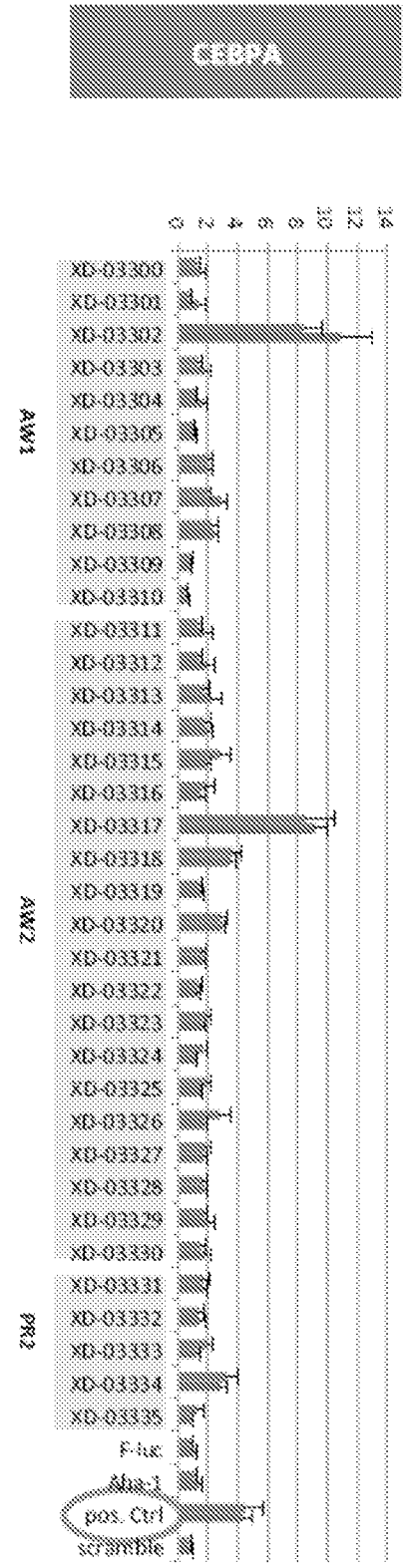


Figure 65, cont.

E

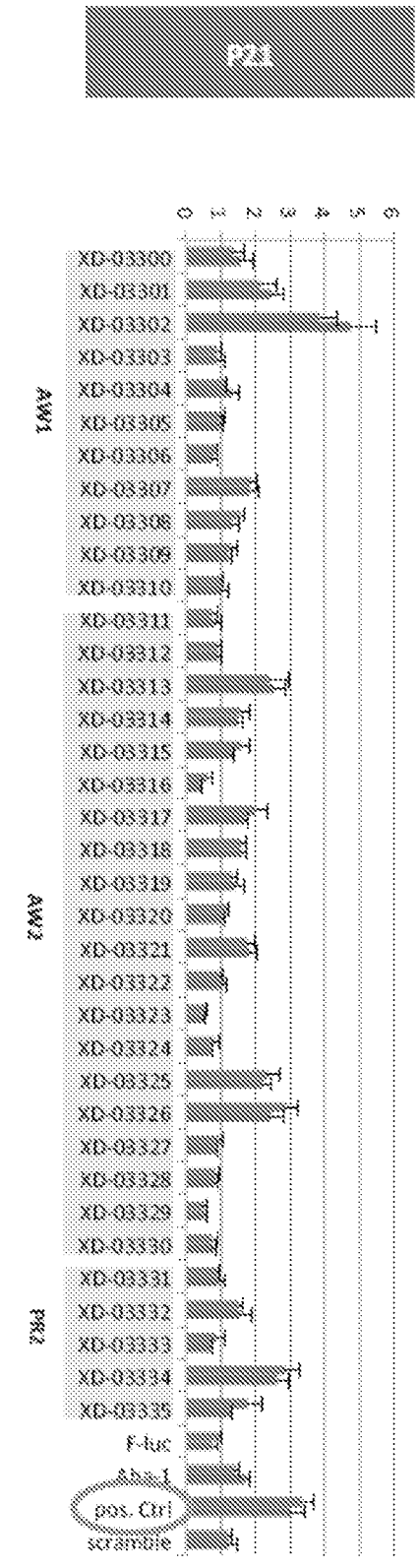
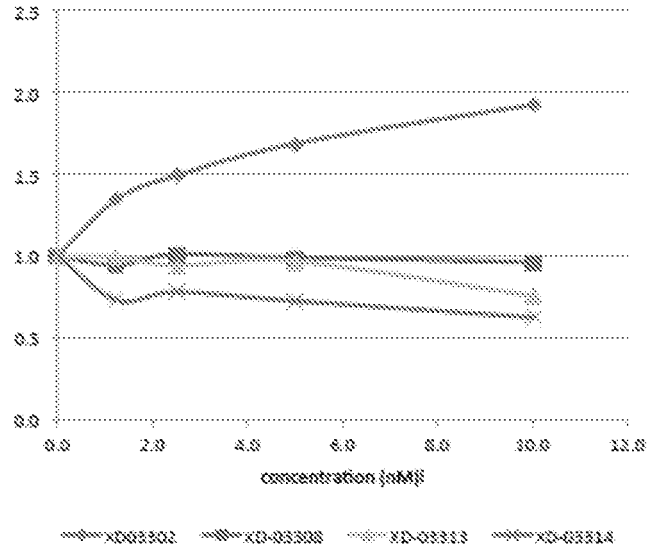


Figure 66

A


2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
concentration (nM)
XD-03302
XD-03308
XD-03313
XD-03314

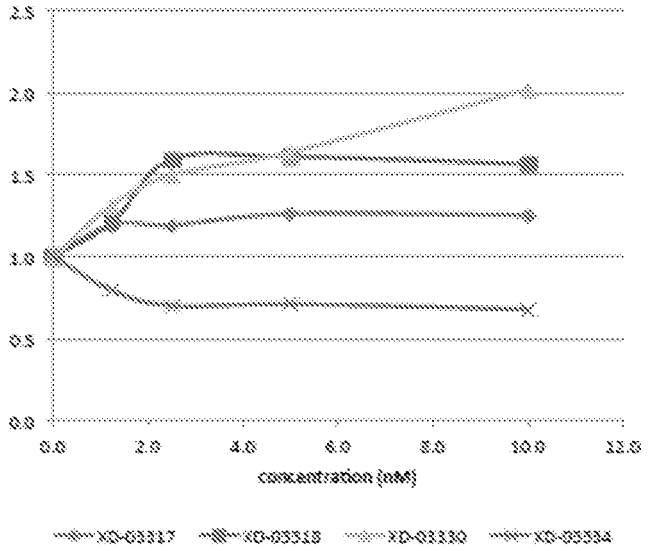
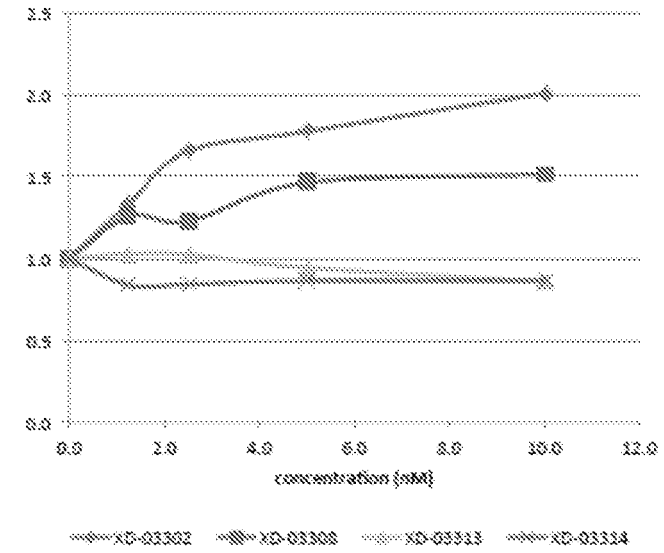

2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
concentration (nM)
XD-03317
XD-03318
XD-03330
XD-03334

Figure 66, cont.

B



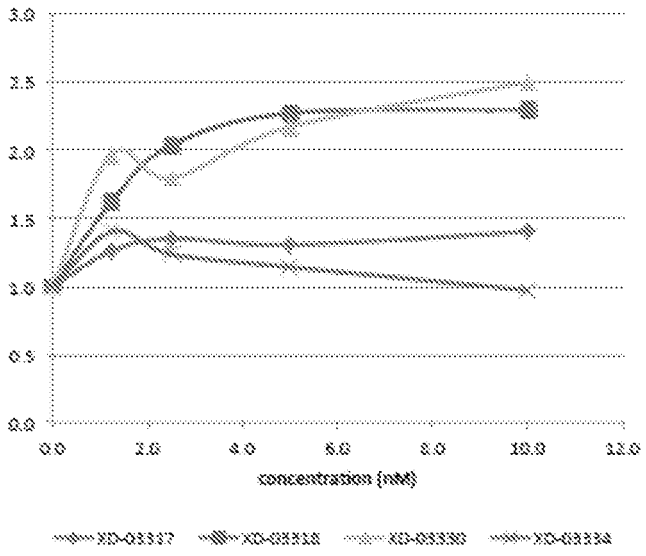
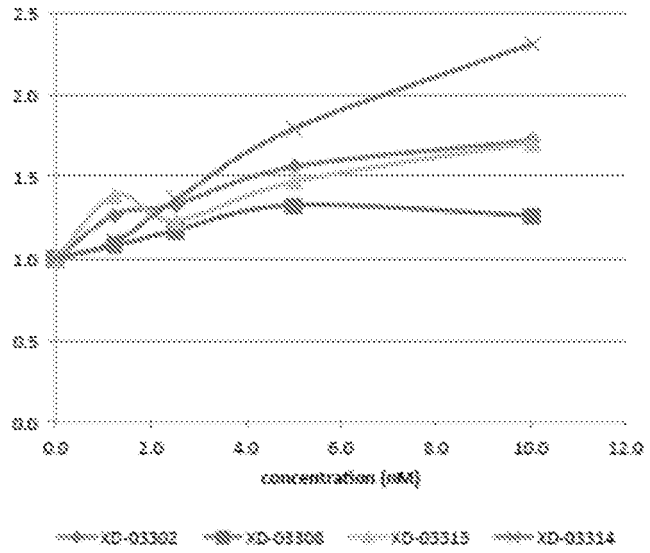


Figure 66, cont.

C



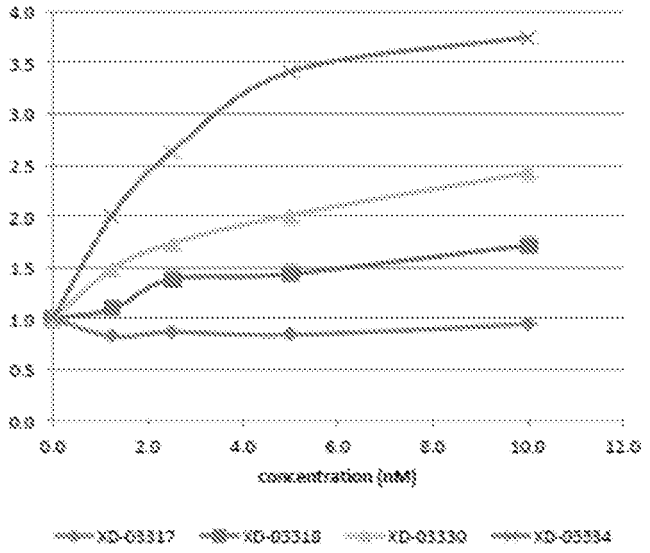
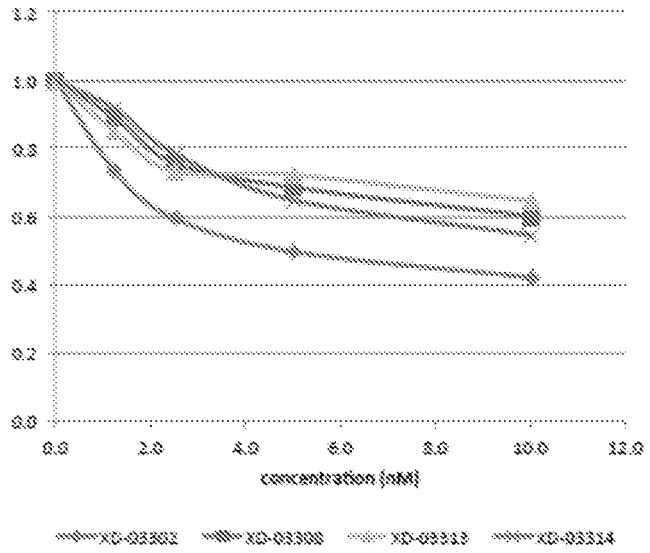


Figure 66, cont.

D



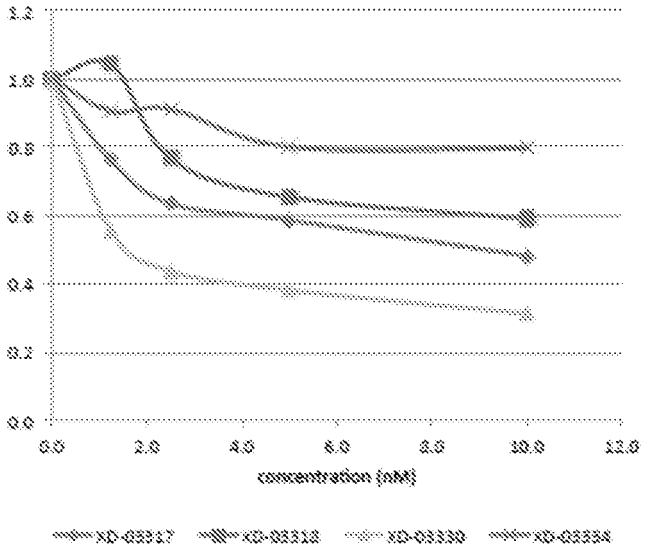
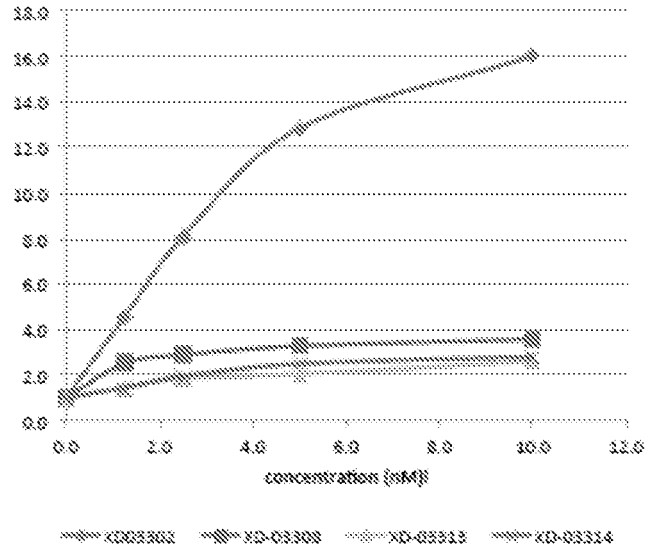


Figure 66, cont.

E



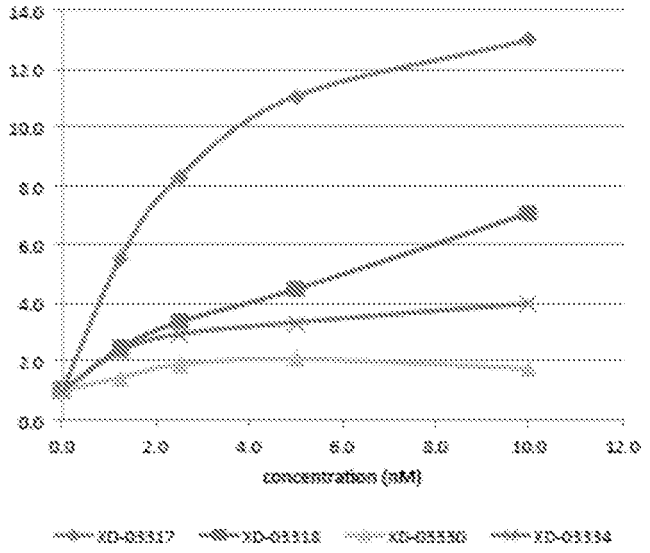
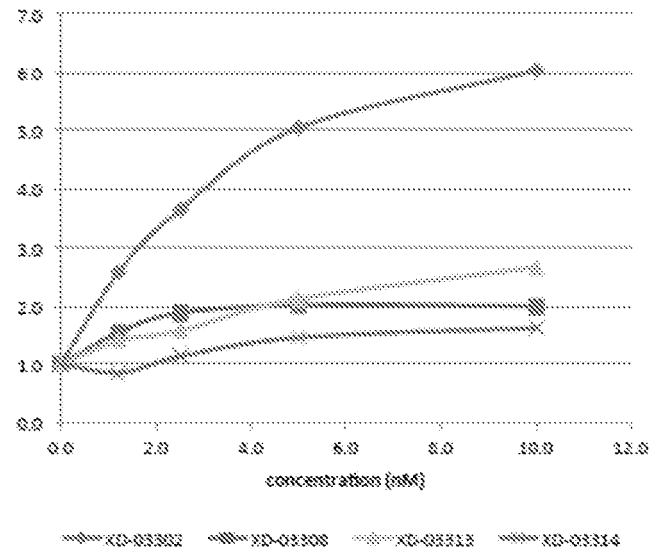


Figure 66, cont.

F



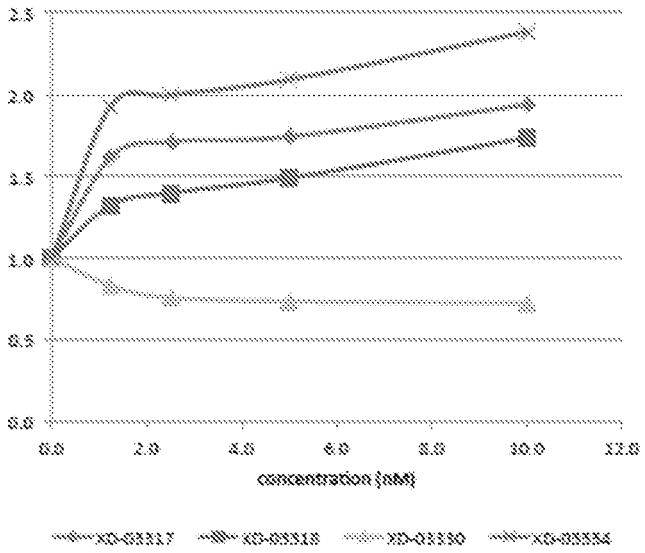
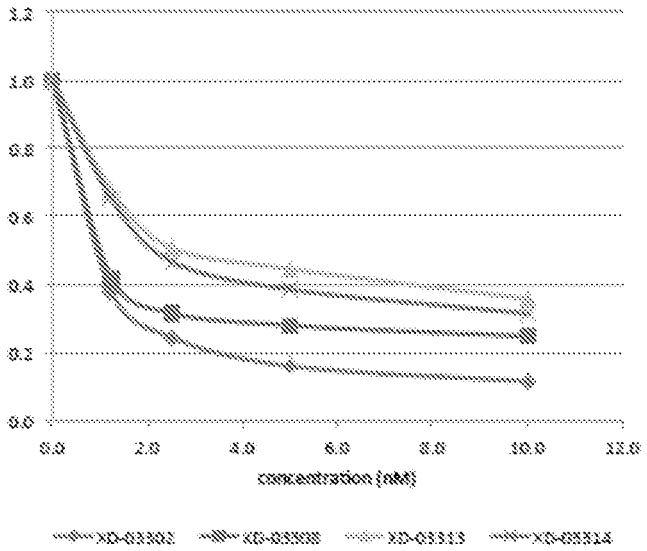


Figure 66, cont.

G



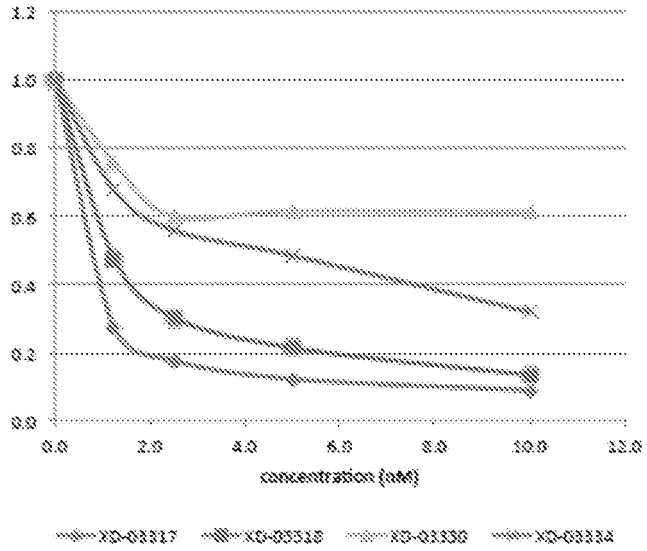


Figure 67

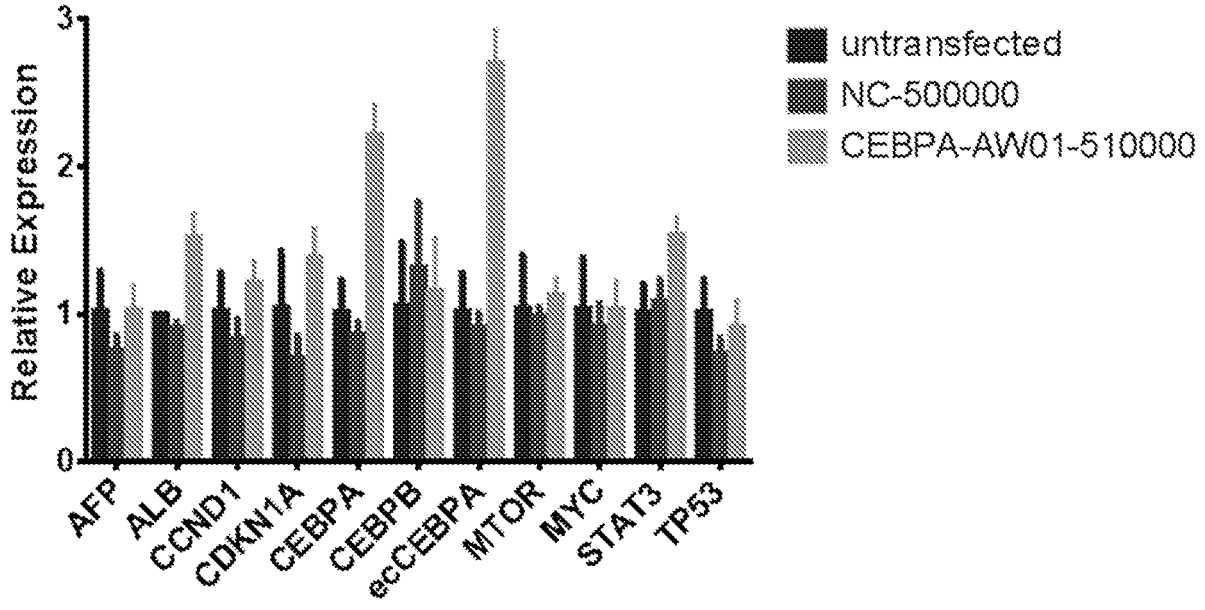
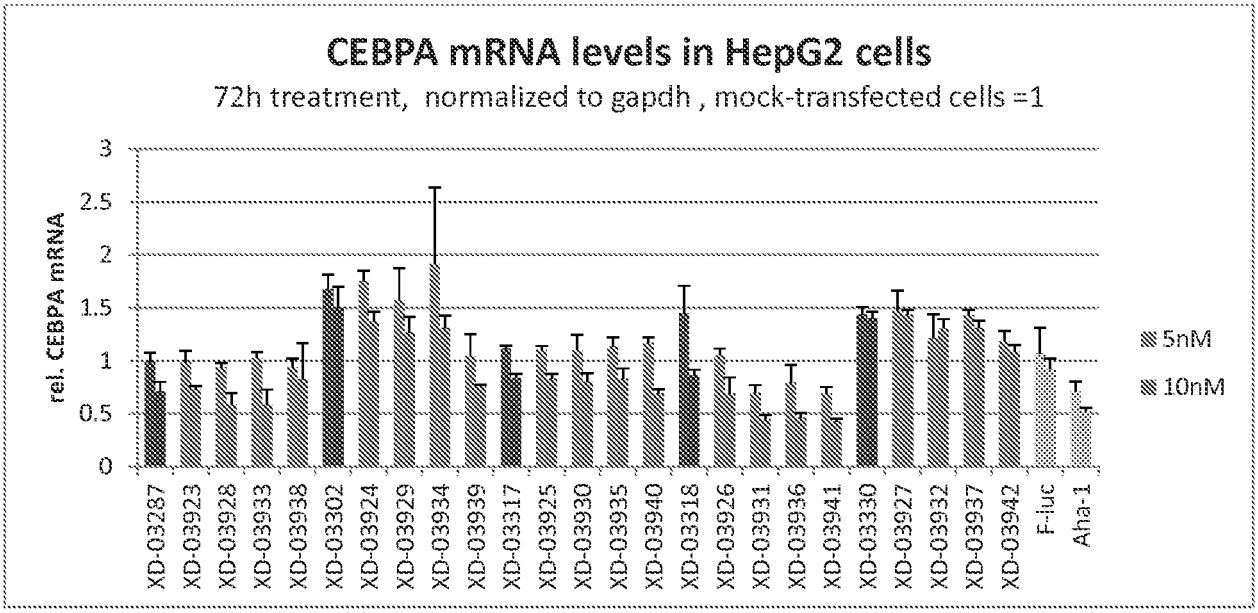

Relative Expression
3
2
1
0
AFP
ALB
CCND1
CDKN1A
CEBPA
CEBPB
ecCEBPA
MTOR
MYC
STAT3
TP53
untransfected
NC-500000
CEBPA-AW01-510000

Figure 68

A


CEBPA mRNA levels in HepG2 cells
72h treatment, normalized to gapdh , mock-transfected cells =1
rel. CEBPA mRNA
3
2.5
2
1.5
1
0.5
0
5nM
10nM
XD-03287
XD-03923
XD-03928
XD-03933
XD-03938
XD-03302
XD-03924
XD-03929
XD-03934
XD-03939
XD-03317
XD-03925
XD-03930
XD-03935
XD-03940
XD-03318
XD-03926
XD-03931
XD-03936
XD-03941
XD-03330
XD-03927
XD-03932
XD-03937
XD-03942
F-luc
Aha-1

B

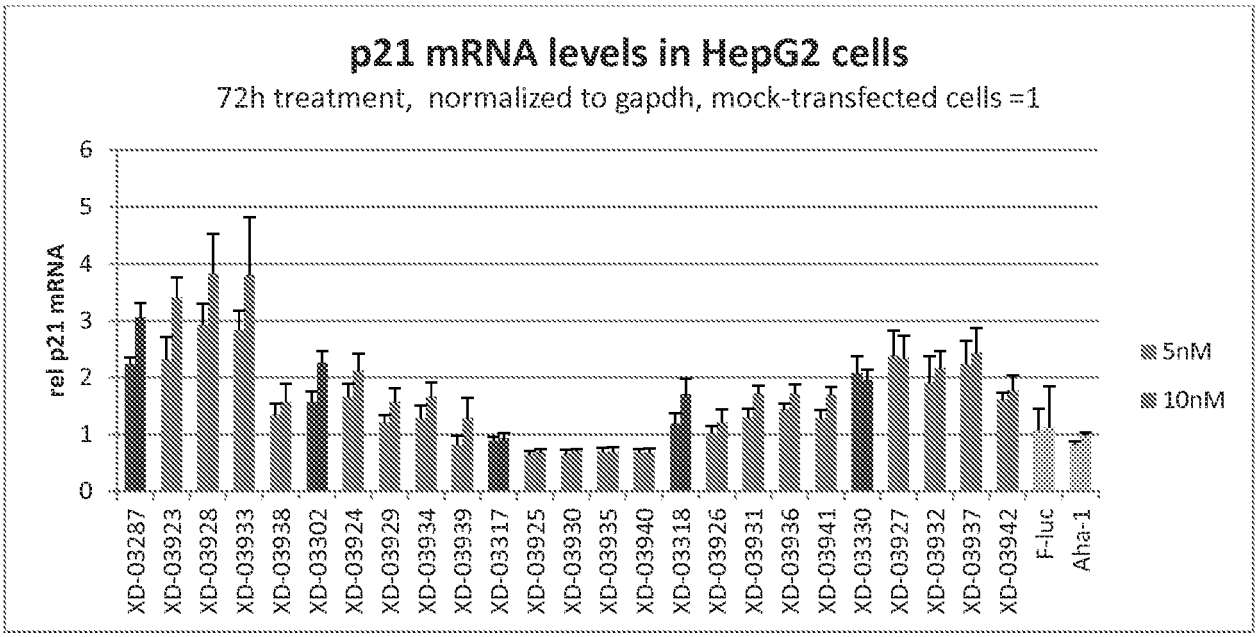

p21 mRNA levels in HepG2 cells
72h treatment, normalized to gapdh, mock-transfected cells =1
rel p21 mRNA
6
5
4
3
2
1
0
5nM
10nM
XD-03287
XD-03923
XD-03928
XD-03933
XD-03938
XD-03302
XD-03924
XD-03929
XD-03934
XD-03939
XD-03317
XD-03925
XD-03930
XD-03935
XD-03940
XD-03318
XD-03926
XD-03931
XD-03936
XD-03941
XD-03330
XD-03927
XD-03932
XD-03937
XD-03942
F-luc
Aha-1

Figure 68, cont.

C

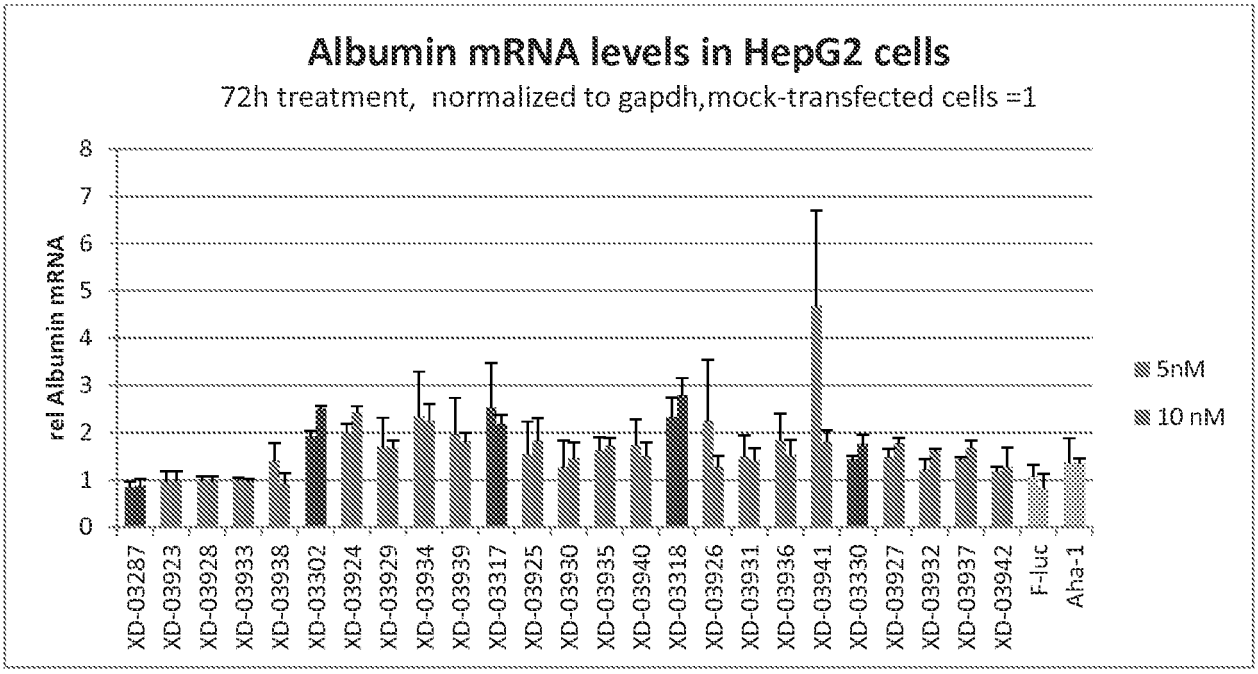
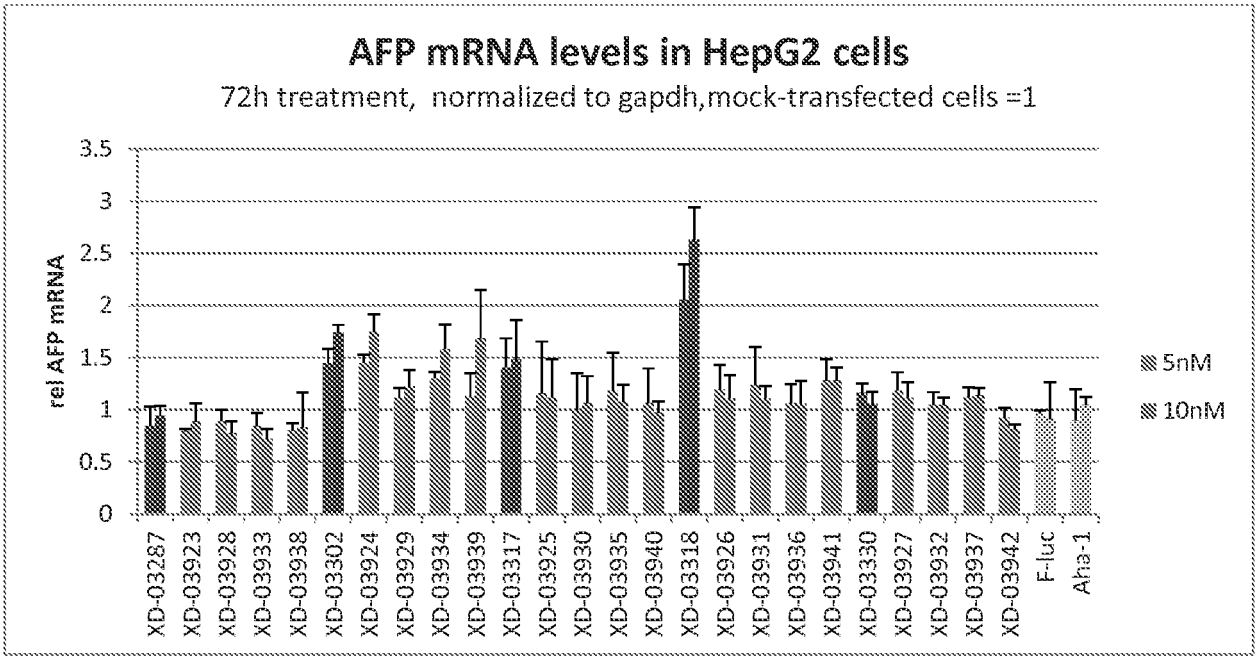


Figure 68, cont.

D



E

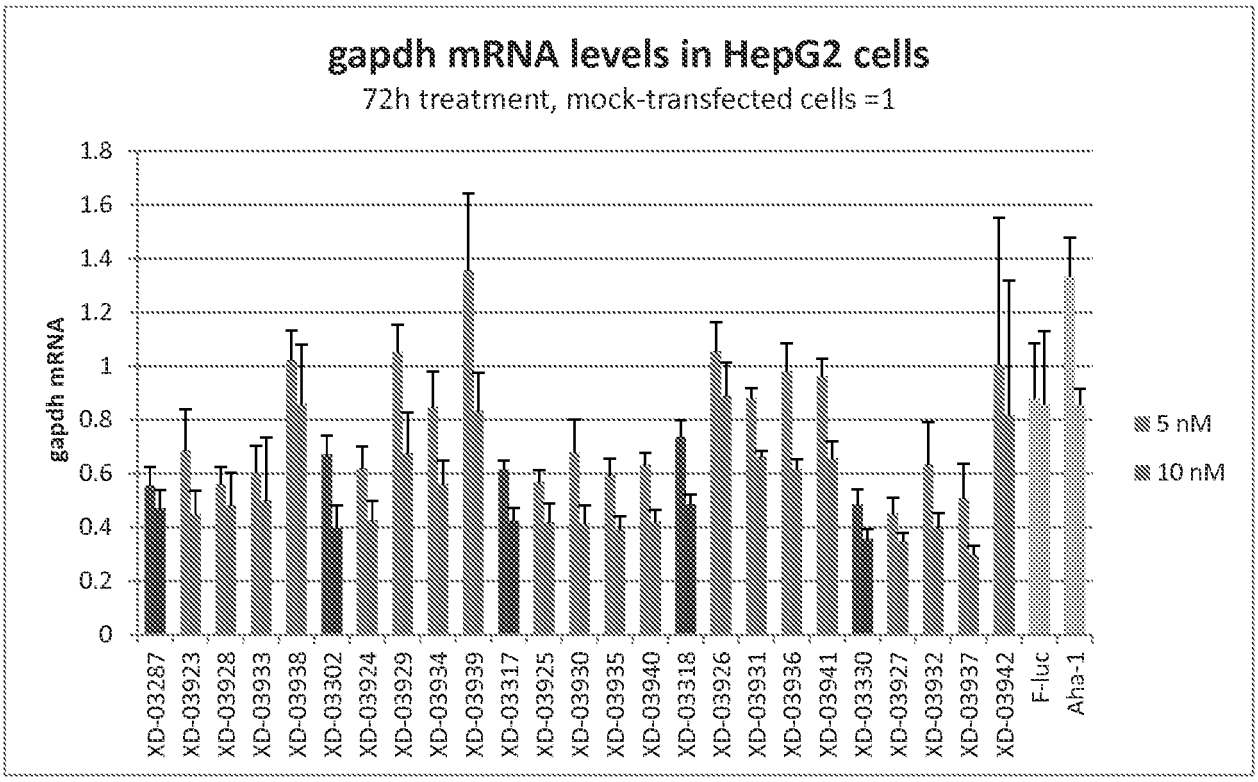
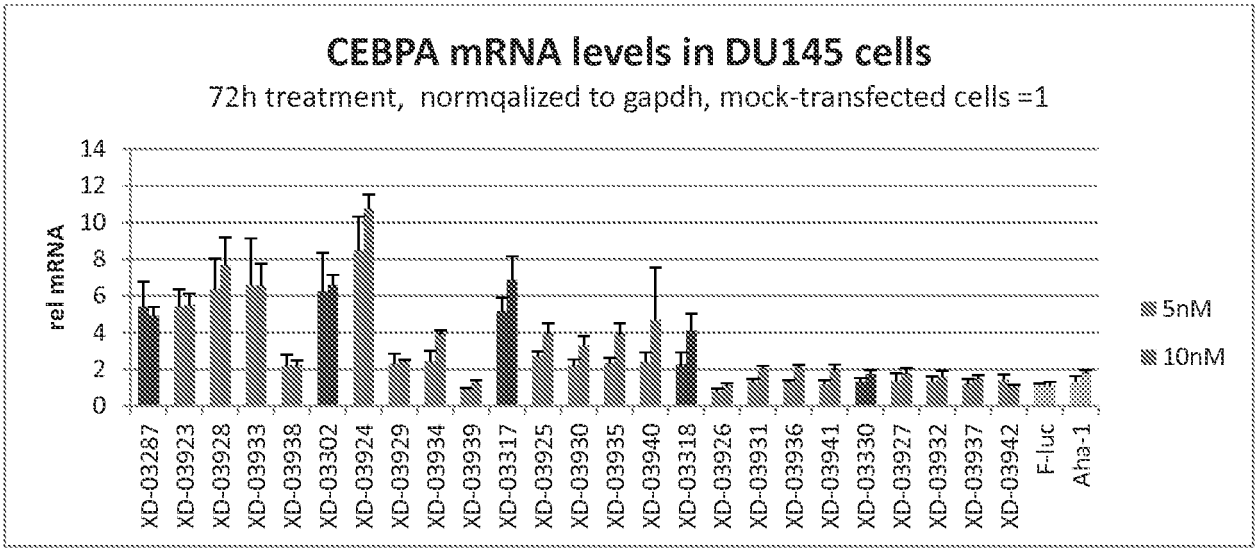


Figure 69

A


CEBPA mRNA levels in DU145 cells
72h treatment, normqalized to gapdh, mock-transfected cells =1
rel mRNA
14
12
10
8
6
4
2
0
XD-03287
XD-03923
XD-03928
XD-03933
XD-03938
XD-03302
XD-03924
XD-03929
XD-03934
XD-03939
XD-03317
XD-03925
XD-03930
XD-03935
XD-03940
XD-03318
XD-03926
XD-03931
XD-03936
XD-03941
XD-03330
XD-03927
XD-03932
XD-03937
XD-03942
F-luc
Aha-1
5nM
10nM

B

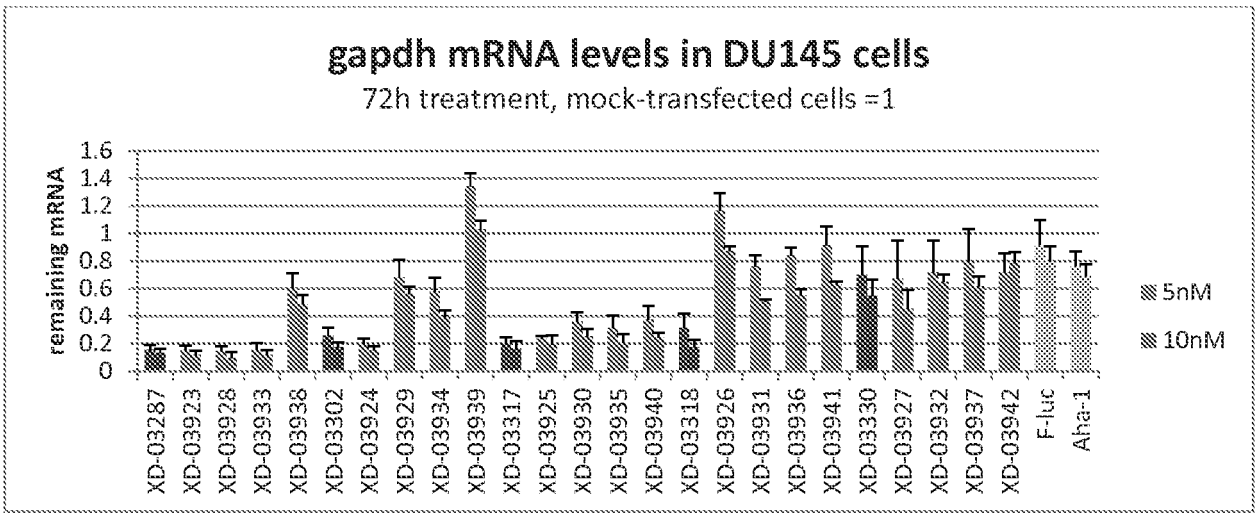

gapdh mRNA levels in DU145 cells
72h treatment, mock-transfected cells =1
remaining mRNA
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
XD-03287
XD-03923
XD-03928
XD-03933
XD-03938
XD-03302
XD-03924
XD-03929
XD-03934
XD-03939
XD-03317
XD-03925
XD-03930
XD-03935
XD-03940
XD-03318
XD-03926
XD-03931
XD-03936
XD-03941
XD-03330
XD-03927
XD-03932
XD-03937
XD-03942
F-luc
Aha-1
5nM
10nM

Figure 69, cont.

C

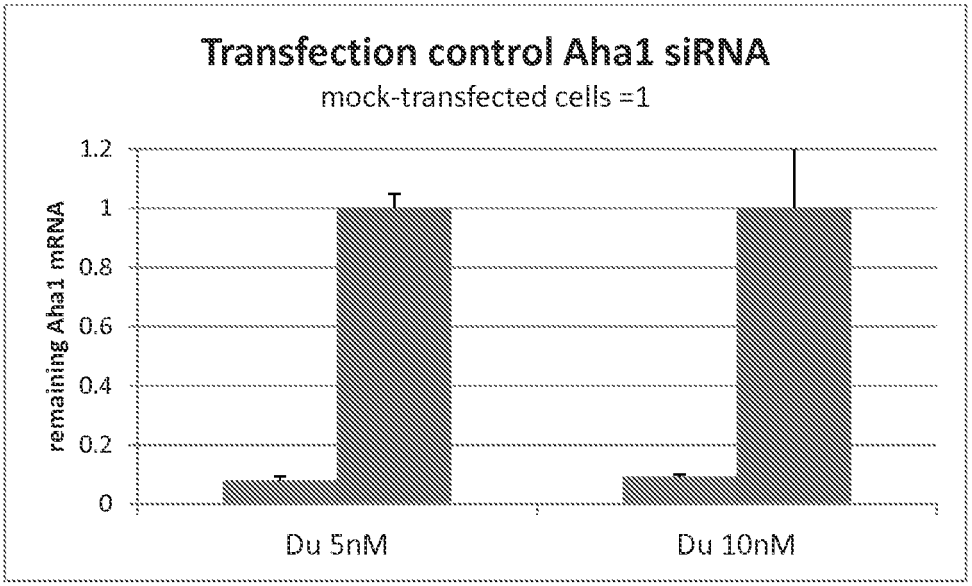


Figure 70

A

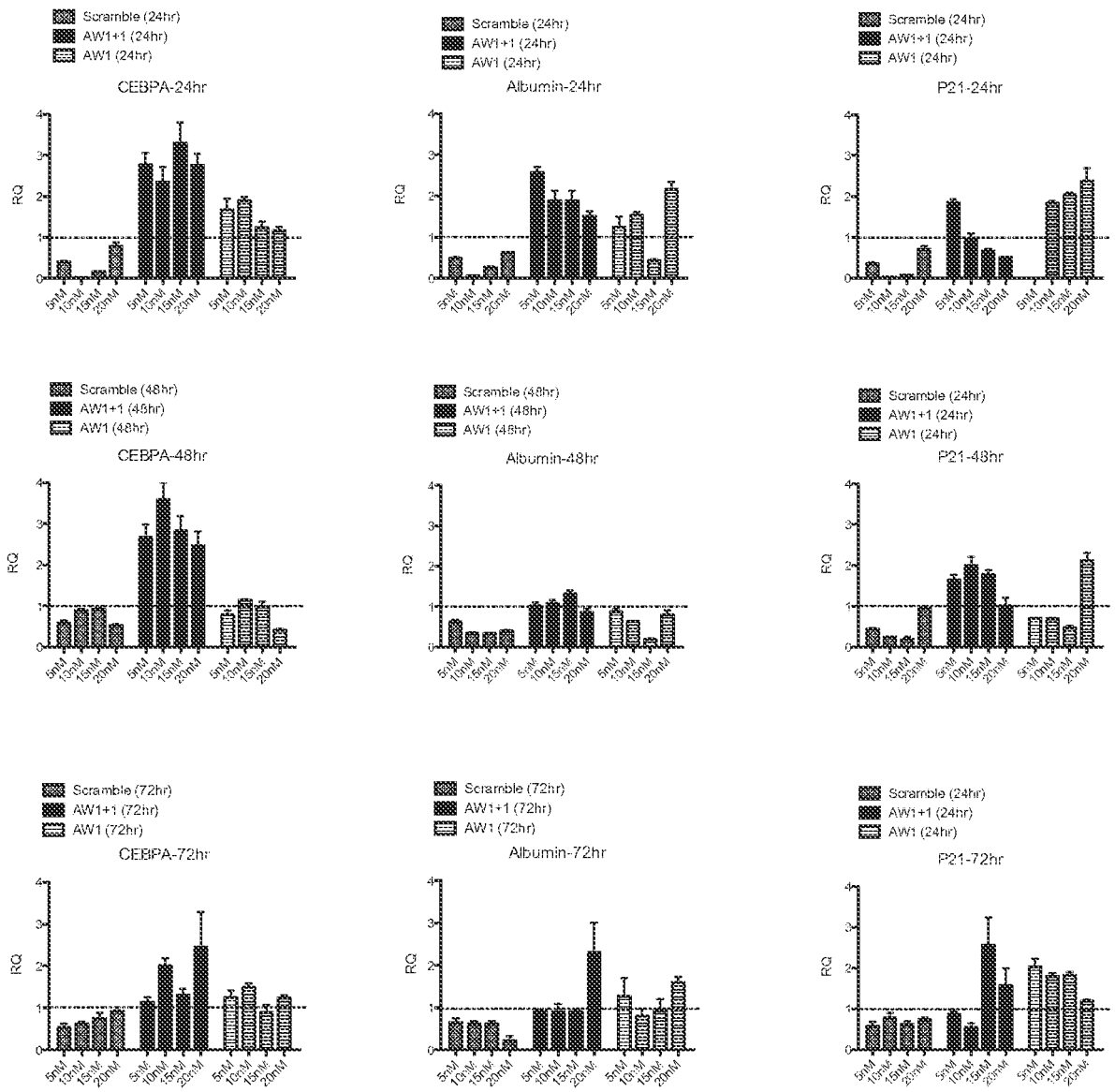

Scramble (24hr)
AW1+1 (24hr)
AW1 (24hr)
CEBPA-24hr
Albumin-24hr
P21-24hr
Scramble (48hr)
AW1+1 (48hr)
AW1 (48hr)
CEBPA-48hr
Albumin-48hr
P21-48hr
Scramble (72hr)
AW1+1 (72hr)
AW1 (72hr)
CEBPA-72hr
Albumin-72hr
P21-72hr
RQ
5nM
10nM
15nM
20nM

Figure 70, cont.

B

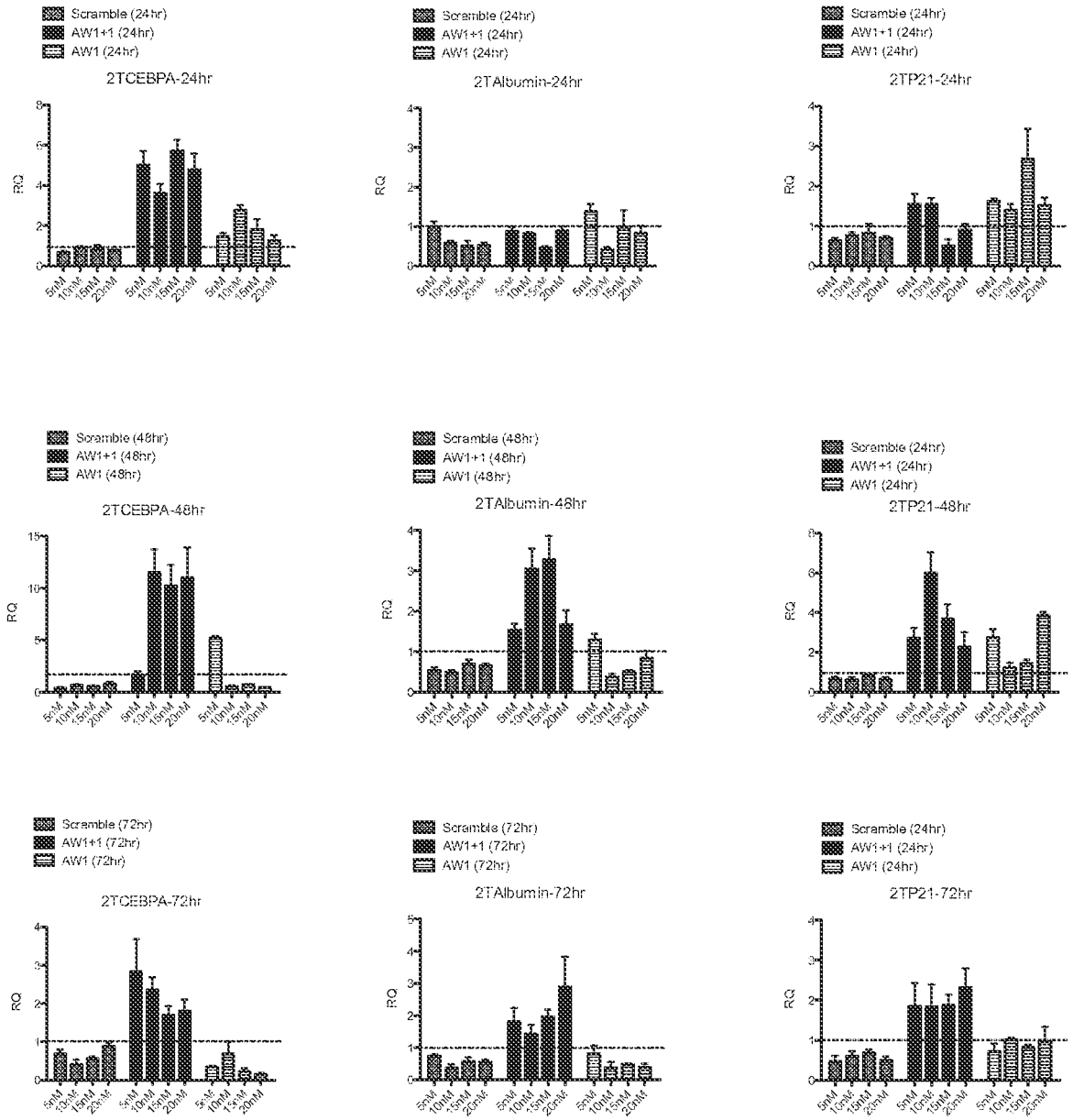


Figure 71

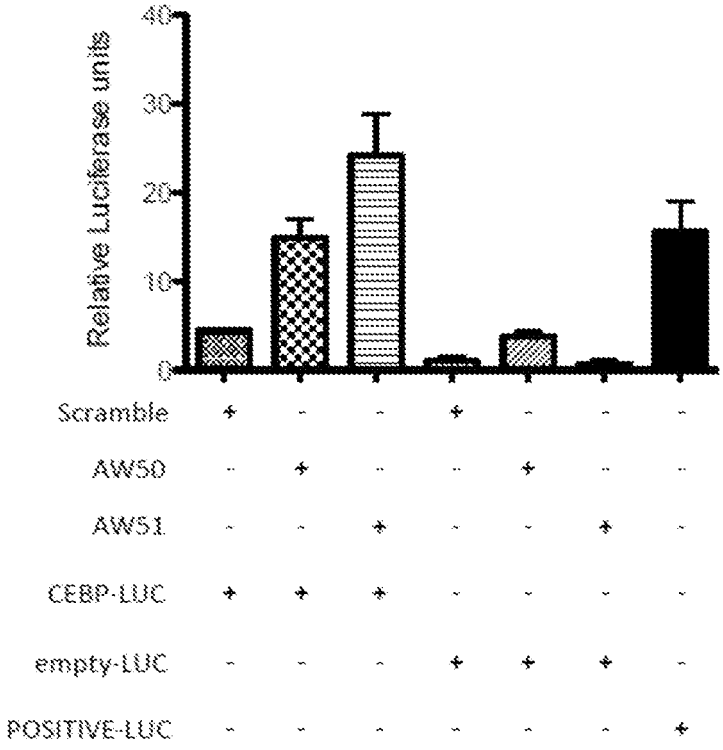

Relative Luciferase units
40
30
20
10
0
Scramble + - - + - - -
AW50 - + - - + - -
AW51 - - + - - + -
CEBP-LUC + + + - - - -
empty-LUC - - - + + + -
POSITIVE-LUC - - - - - - +

Figure 72

A

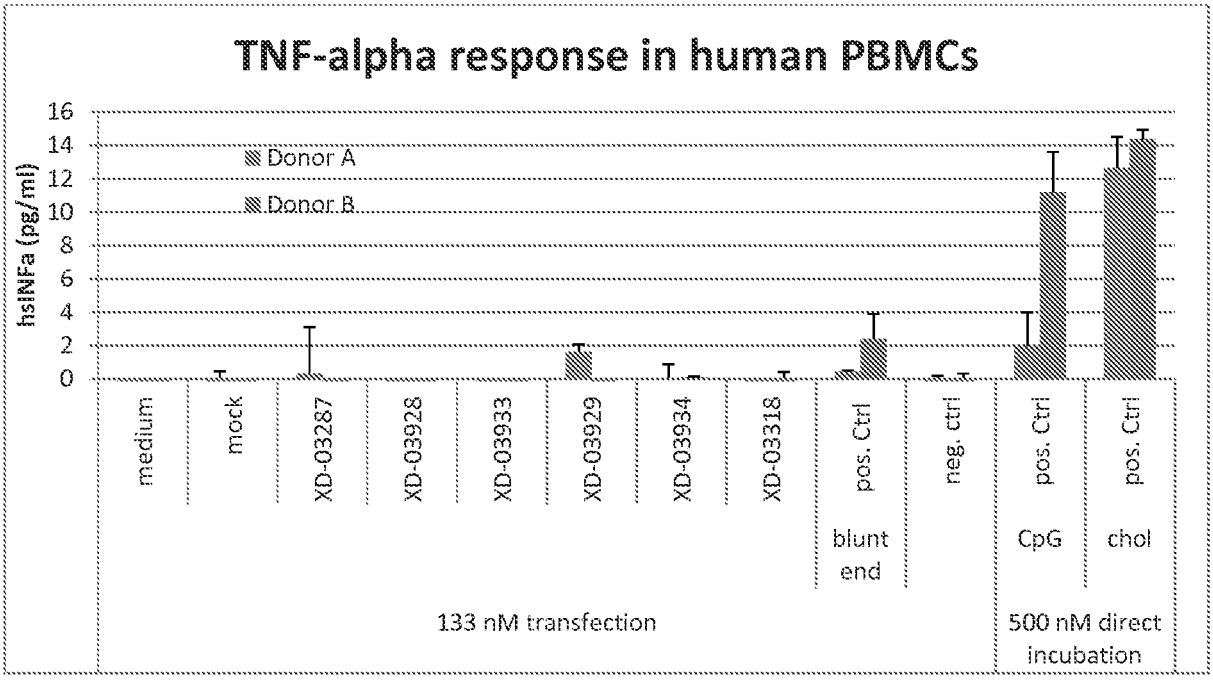

TNF-alpha response in human PBMCs
hsINFa (pg/ml)
16
14
12
10
8
6
4
2
0
Donor A
Donor B
medium
mock
XD-03287
XD-03928
XD-03933
XD-03929
XD-03934
XD-03318
pos. Ctrl
blunt end
neg. ctrl
pos. Ctrl
CpG
pos. Ctrl
chol
133 nM transfection
500 nM direct incubation

Figure 72, cont.

B

