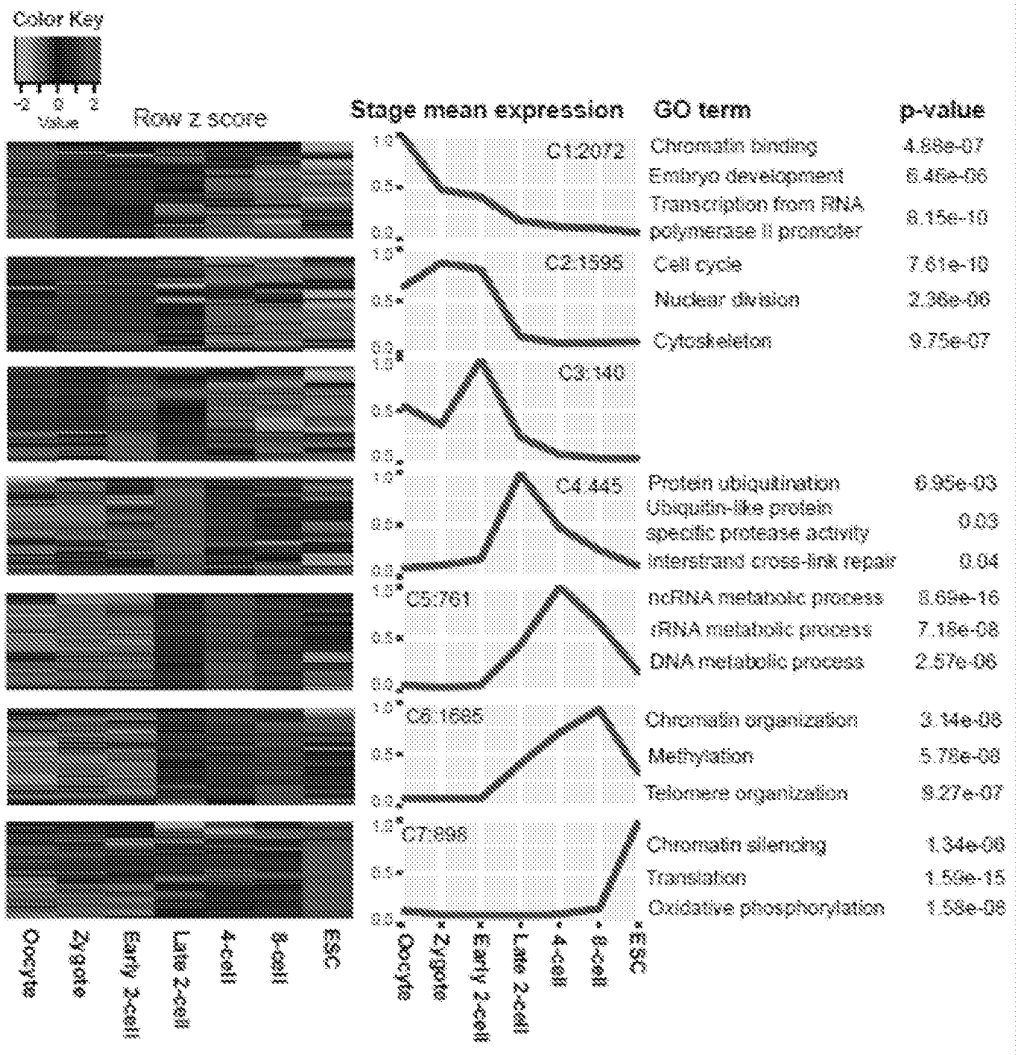
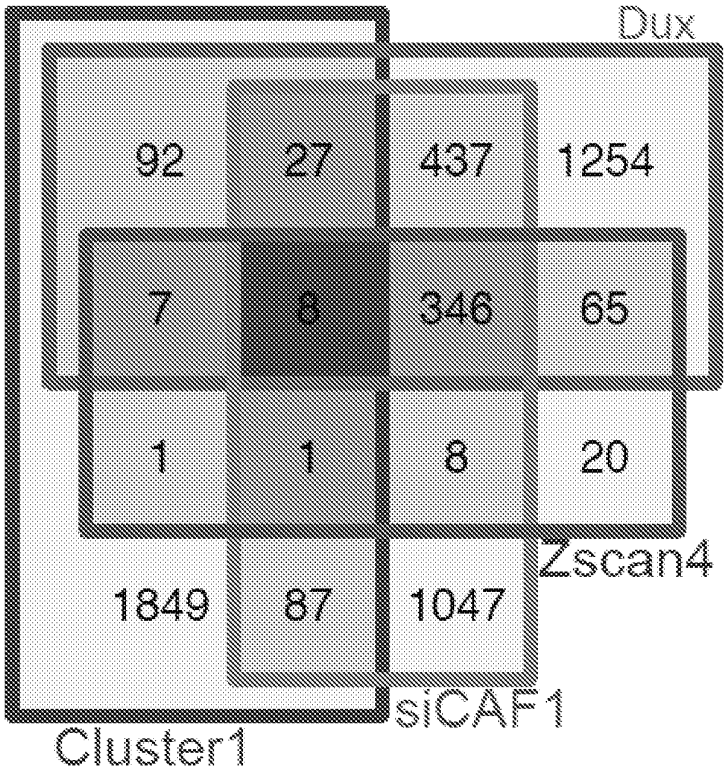

Color Key
-2
0
2
Value
Row z score
Stage mean expression
GO term
p-value
C1:2072
Chromatin binding 4.88e-07
Embryo development 6.46e-06
Transcription from RNA polymerase II promoter 8.15e-10
C2:1595
Cell cycle 7.61e-10
Nuclear division 2.36e-06
Cytoskeleton 9.75e-07
C3:140
C4:445
Protein ubiquitination 6.95e-03
Ubiquitin-like protein specific protease activity 0.03
Interstrand cross-link repair 0.04
C5:761
ncRNA metabolic process 8.69e-16
rRNA metabolic process 7.18e-08
DNA metabolic process 2.57e-06
C6:1685
Chromatin organization 3.14e-08
Methylation 5.78e-08
Telomere organization 9.27e-07
C7:896
Chromatin silencing 1.34e-08
Translation 1.59e-15
Oxidative phosphorylation 1.58e-08
Oocyte
Zygote
Early 2-cell
Late 2-cell
4-cell
8-cell
ESC

Figure 1a



Nelfa, Sh3kbp1, Trim75, Abhd3, Strip2, Gm839, Slc25a31 and Galnt3

Figure 1b

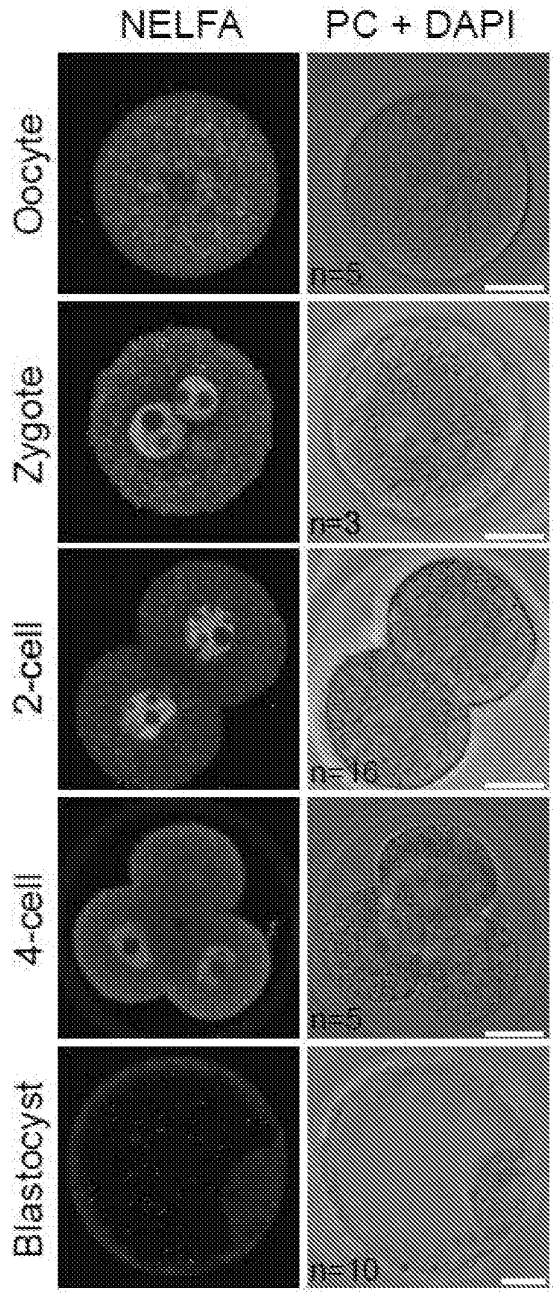

NELFA
PC + DAPI
Oocyte
n=5
Zygote
n=3
2-cell
n=16
4-cell
n=5
Blastocyst
n=10

Figure 1c

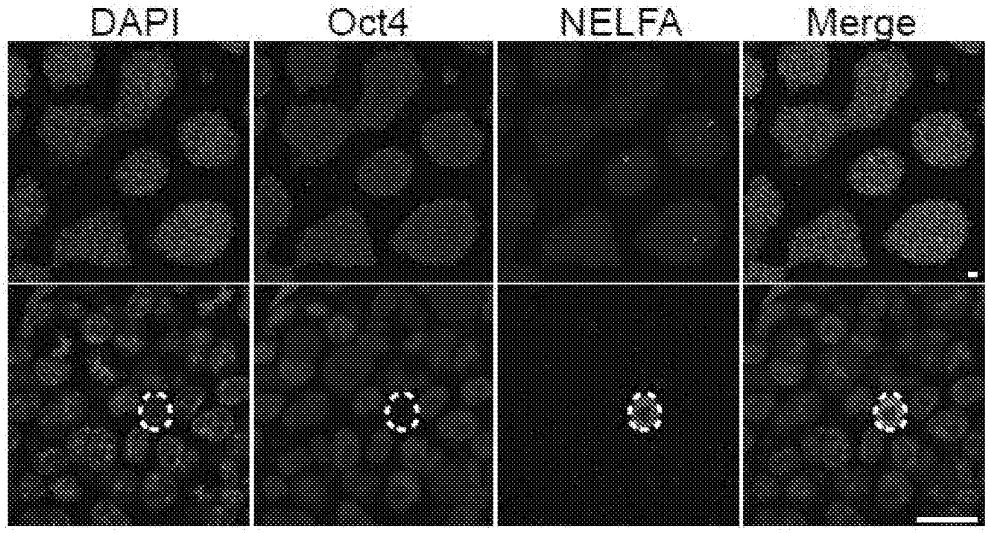


Figure 1d

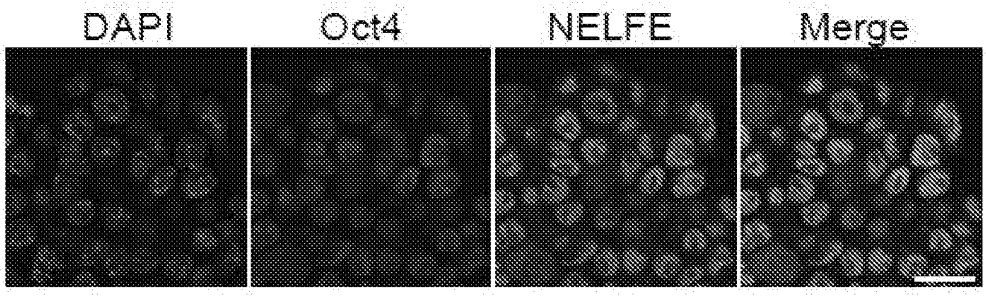


Figure 1e

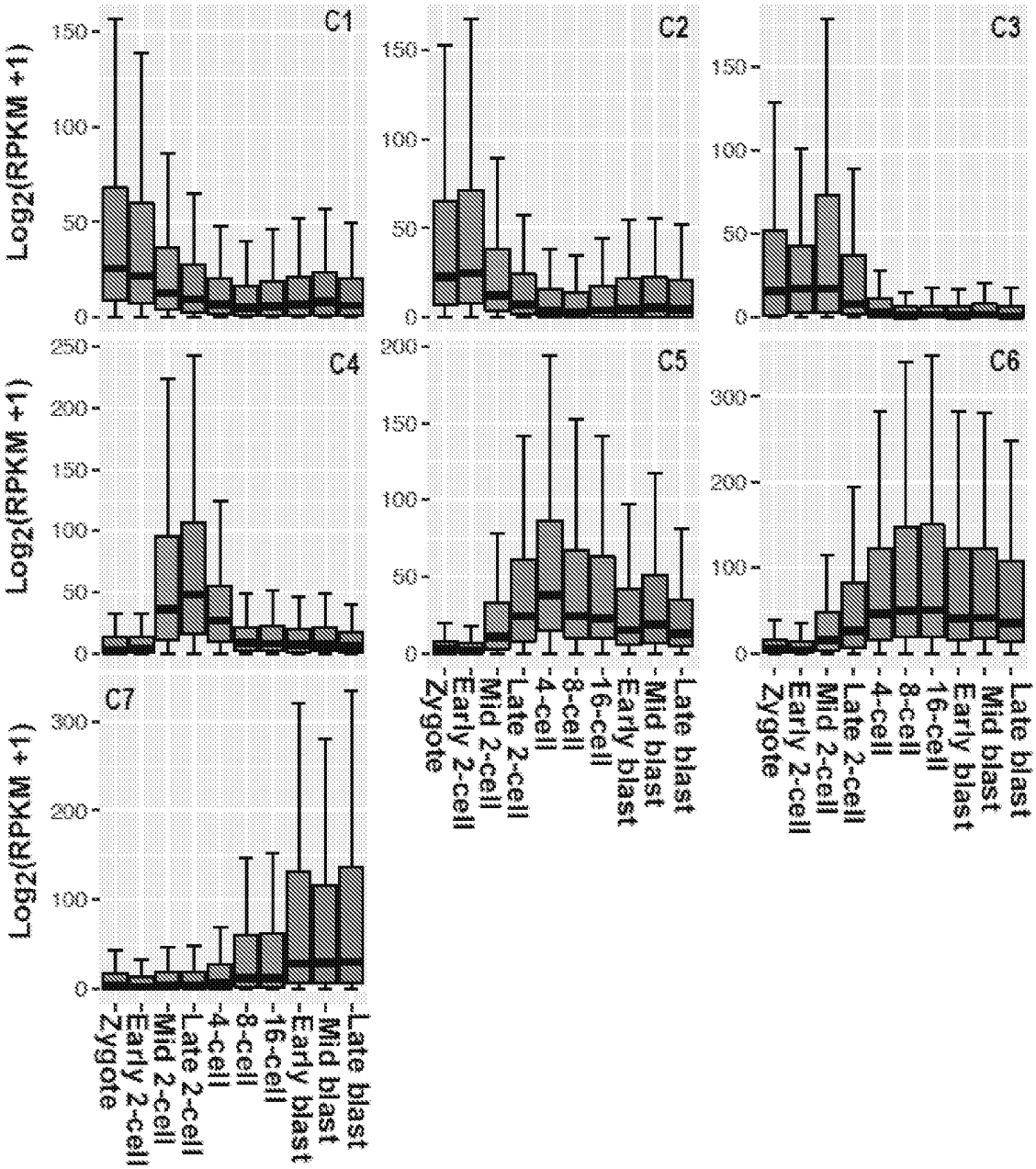

C1
C2
C3
C4
C5
C6
C7
Log2(RPKM +1)
Zygote
Early 2-cell
Mid 2-cell
Late 2-cell
4-cell
8-cell
16-cell
Early blast
Mid blast
Late blast

Figure 1f

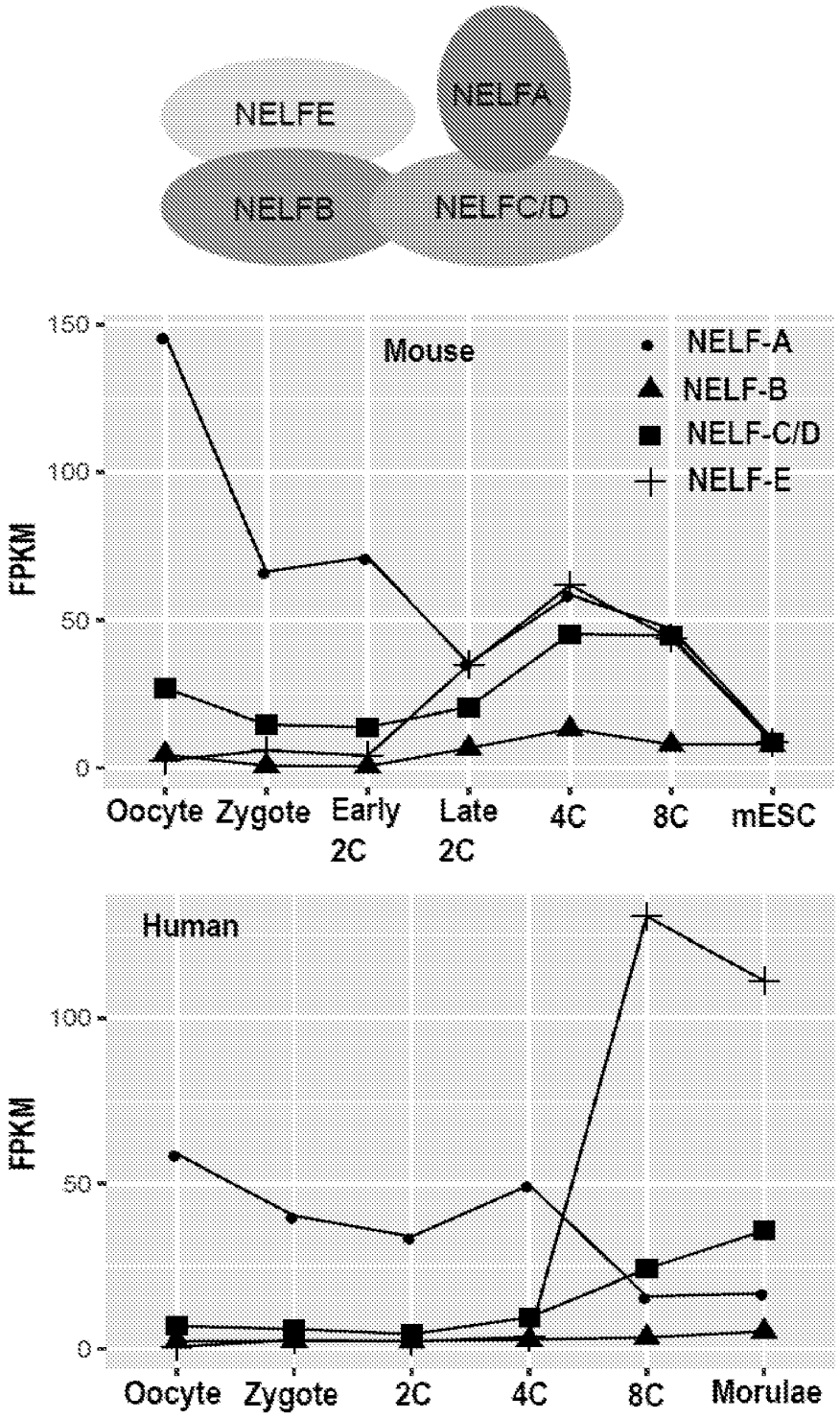

NELFE
NELFA
NELFB
NELFC/D
Mouse
NELF-A
NELF-B
NELF-C/D
NELF-E
FPKM
150
100
50
0
Oocyte
Zygote
Early 2C
Late 2C
4C
8C
mESC
Human
FPKM
100
50
0
Oocyte
Zygote
2C
4C
8C
Morulae

Figure 1g

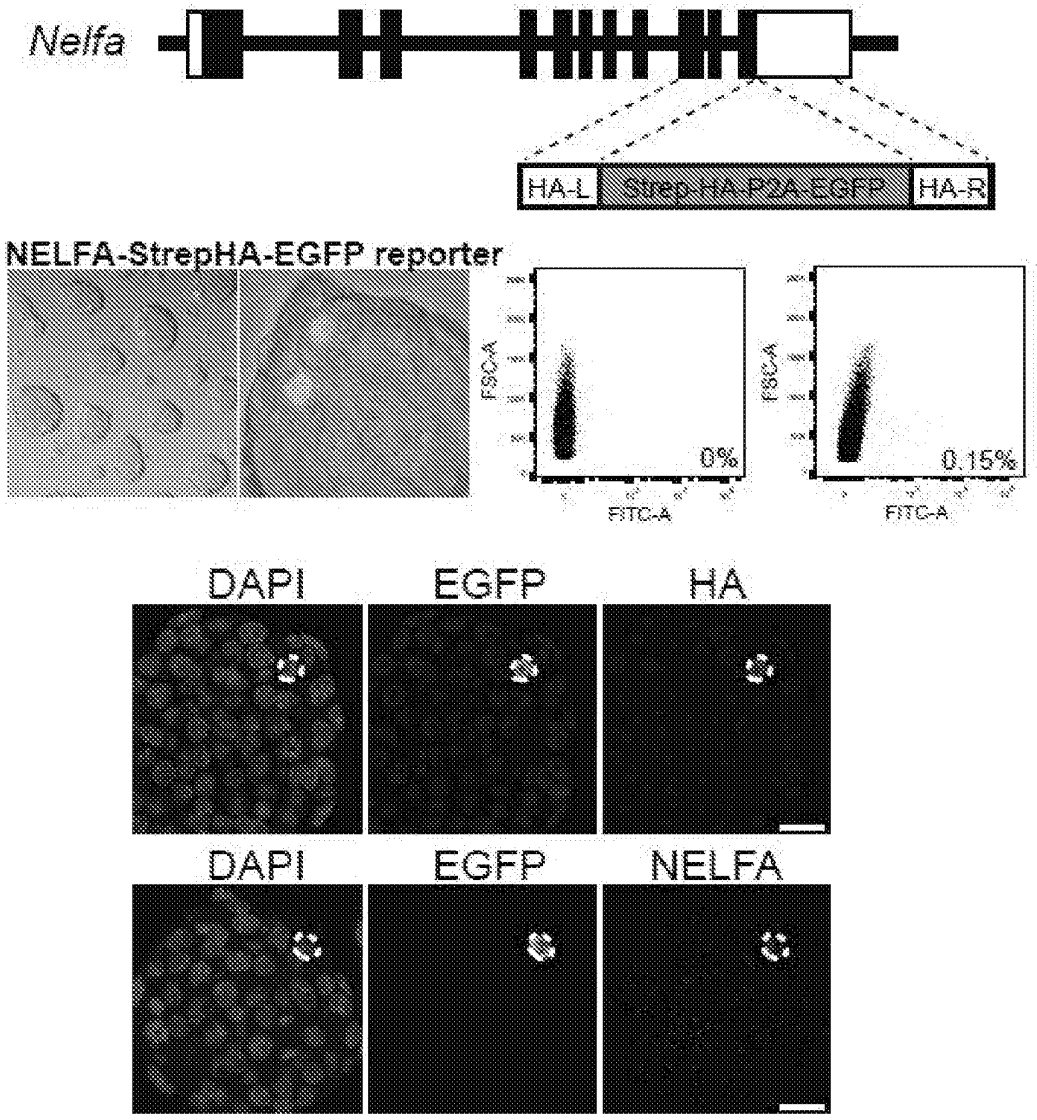

Nelfa
HA-L
Strep-HA-P2A-EGFP
HA-R
NELFA-StrepHA-EGFP reporter
FSC-A
FITC-A
0%
0.15%
DAPI
EGFP
HA
DAPI
EGFP
NELFA

Figure 2a

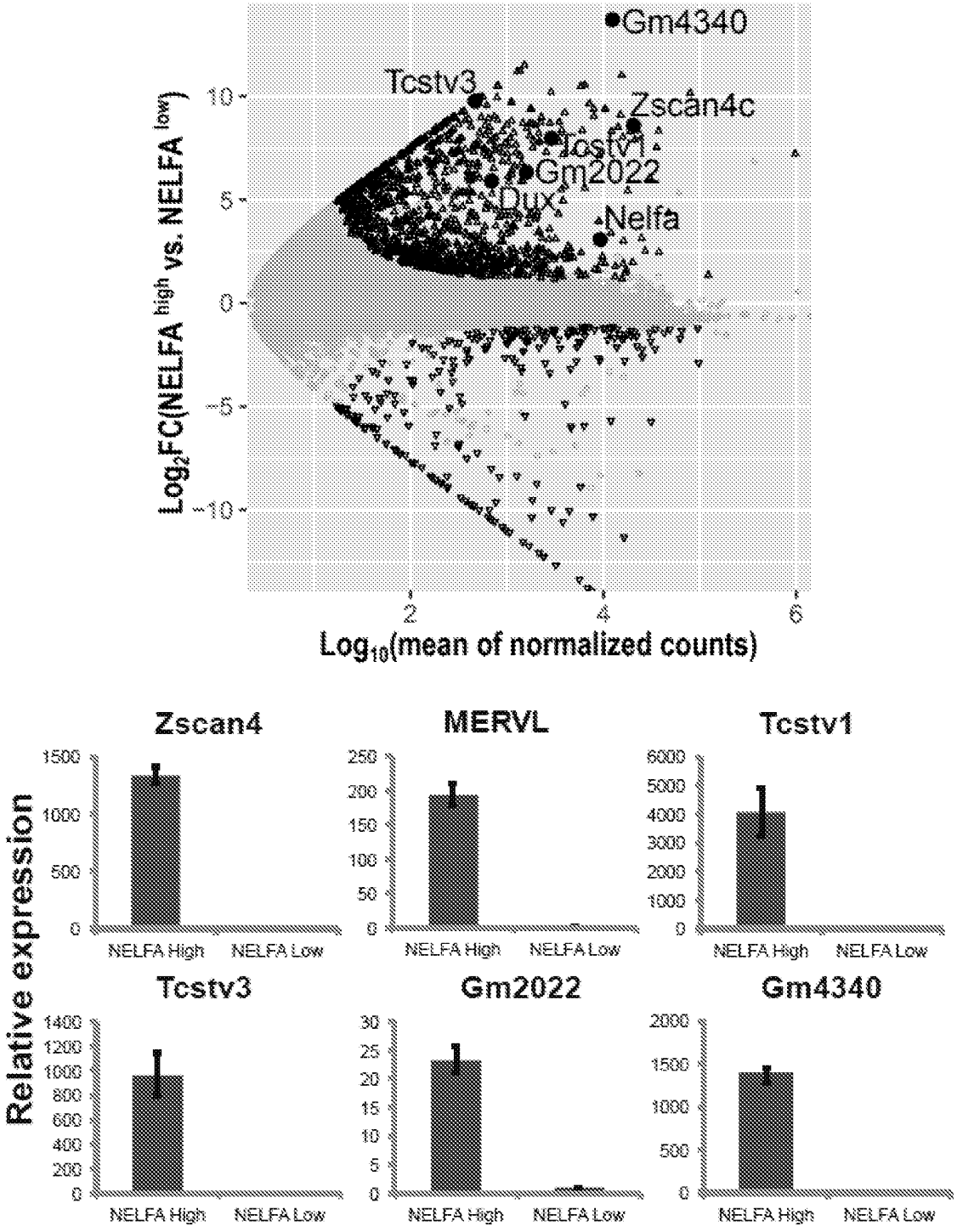

Gm4340
Tcstv3
Zscan4c
Tcstv1
Gm2022
Dux
Nelfa
Log2FC(NELFA high vs. NELFA low)
Log10(mean of normalized counts)
Zscan4
MERVL
Tcstv1
Tcstv3
Gm2022
Gm4340
Relative expression
NELFA High
NELFA Low

Figure 2b

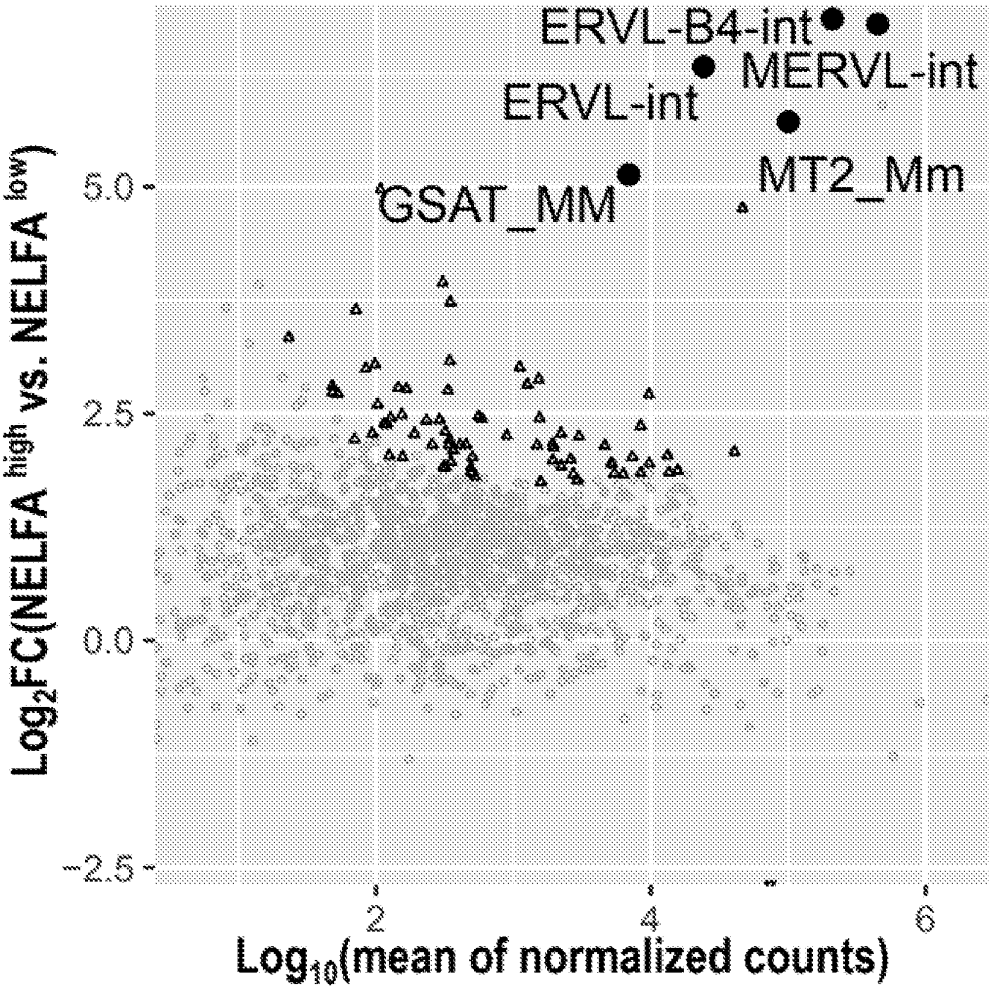

ERVL-B4-int
MERVL-int
ERVL-int
MT2_Mm
GSAT_MM
5.0
2.5
0.0
−2.5
2
4
6
Log2FC(NELFA high vs. NELFA low)
Log10(mean of normalized counts)

Figure 2c

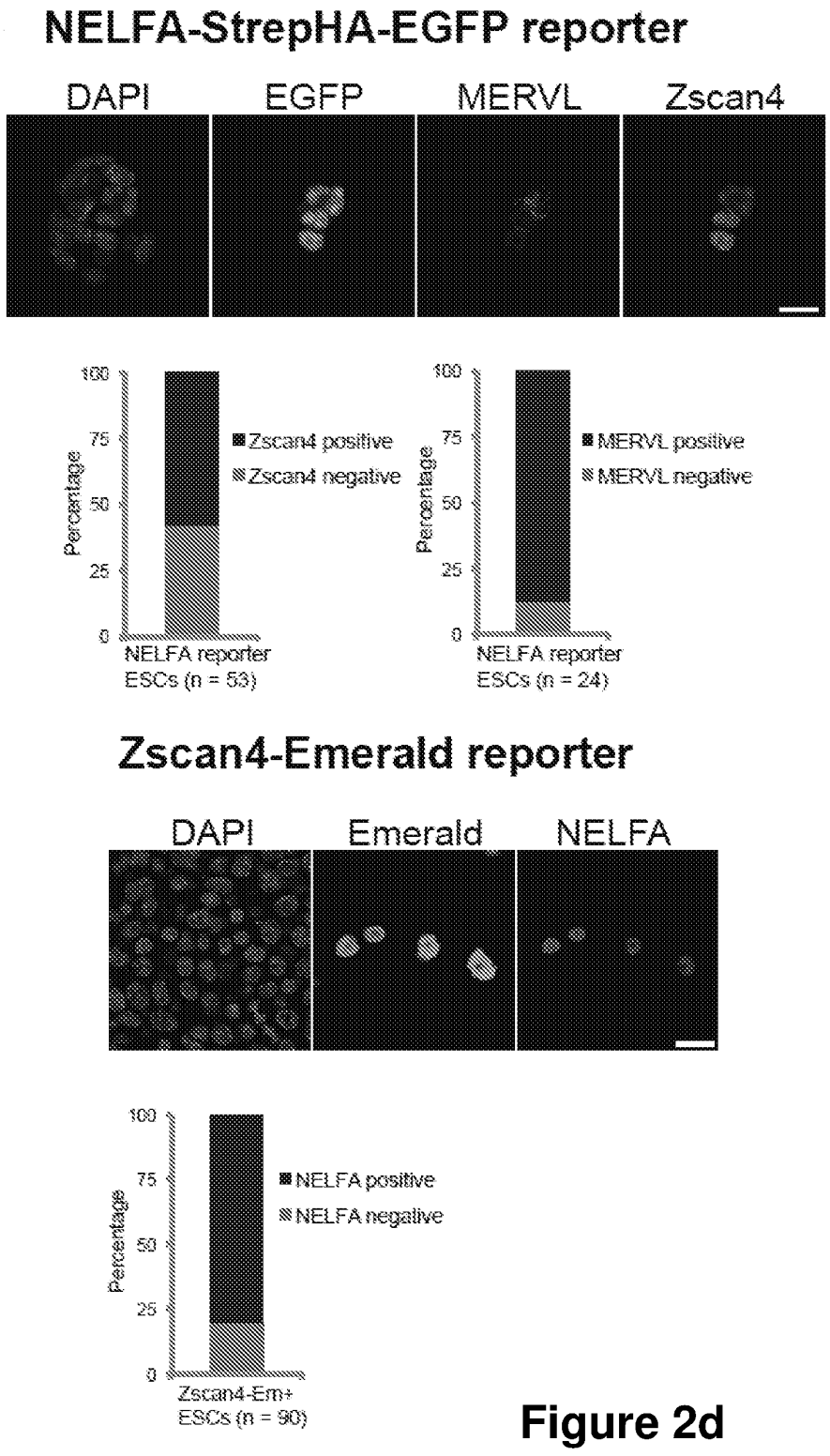

NELFA-StrepHA-EGFP reporter
DAPI
EGFP
MERVL
Zscan4
Percentage
100
75
50
25
0
Zscan4 positive
Zscan4 negative
NELFA reporter
ESCs (n = 53)
MERVL positive
MERVL negative
NELFA reporter
ESCs (n = 24)
Zscan4-Emerald reporter
DAPI
Emerald
NELFA
NELFA positive
NELFA negative
Zscan4-Em+
ESCs (n = 90)

Figure 2d

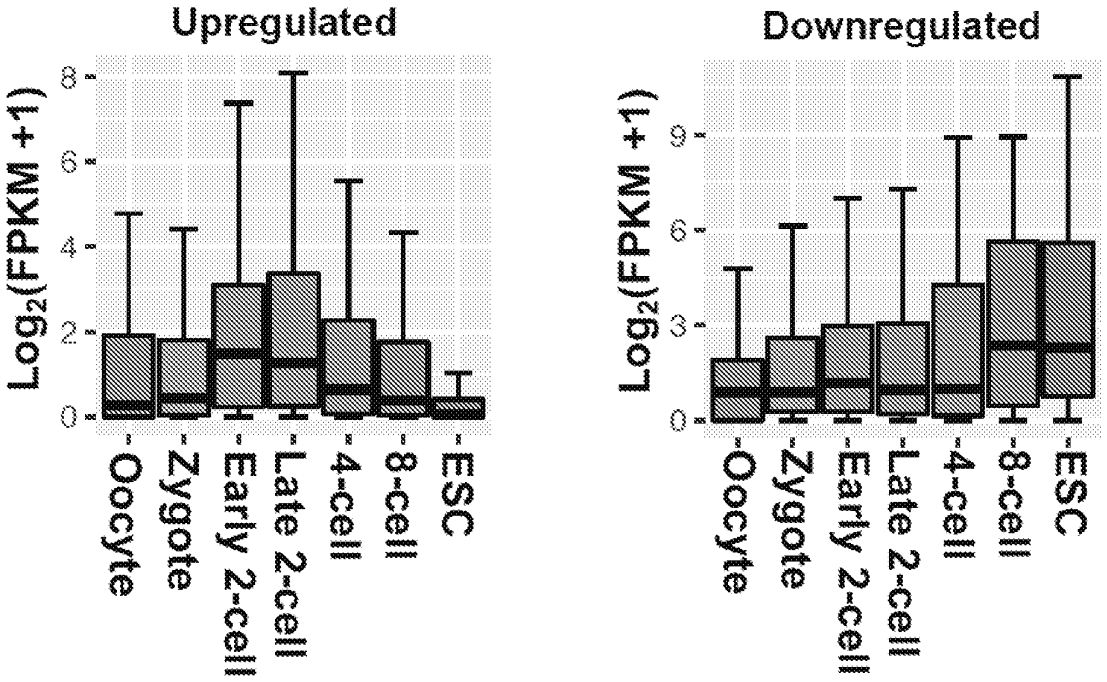


Figure 2e

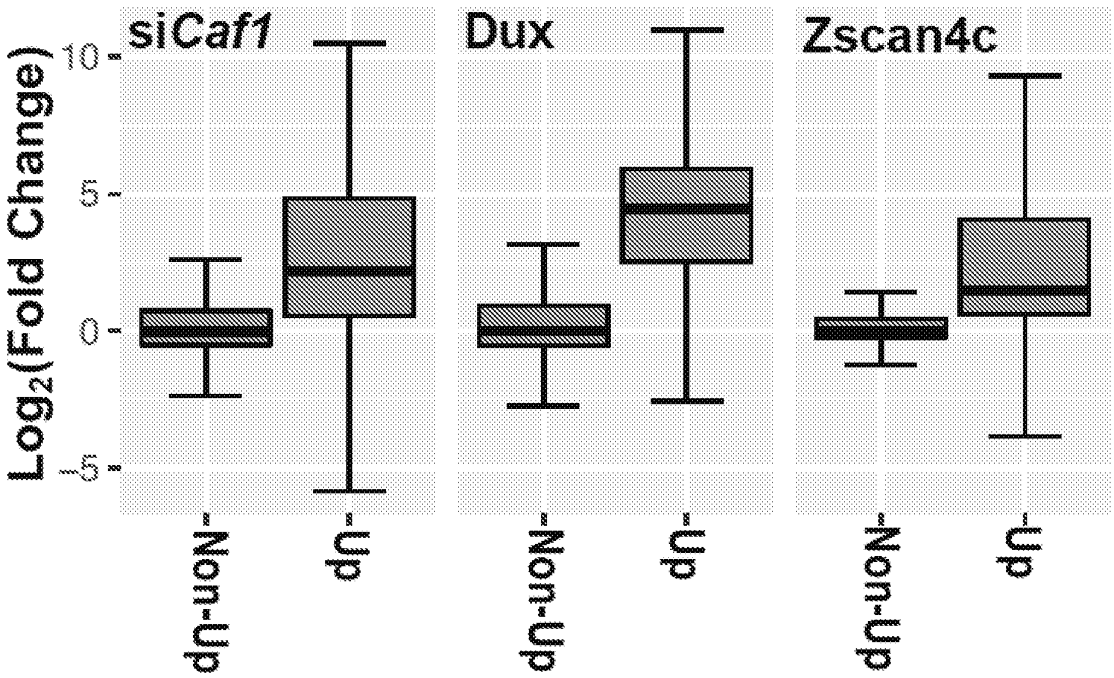


Figure 2f

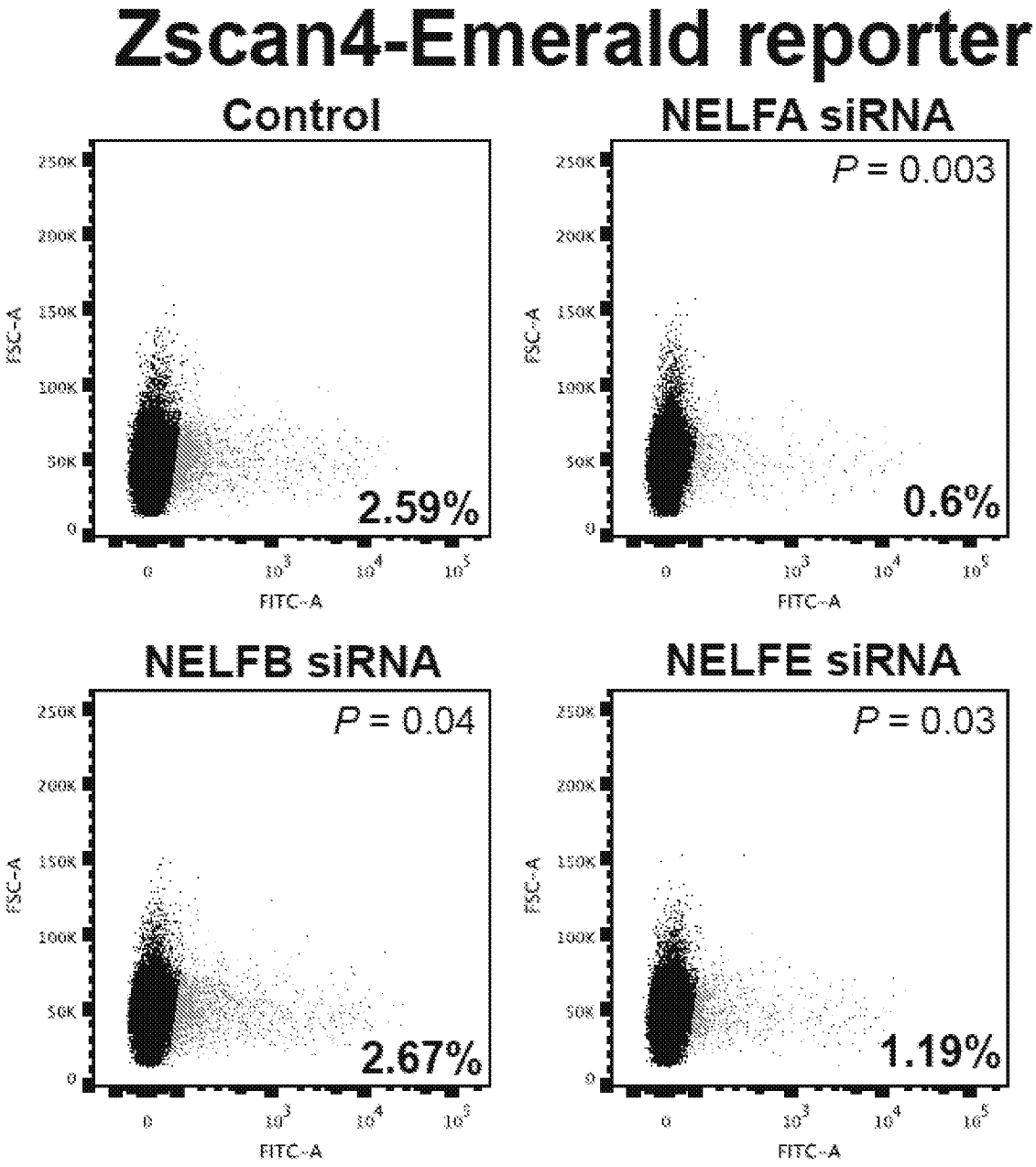


Figure 3a (cont.)

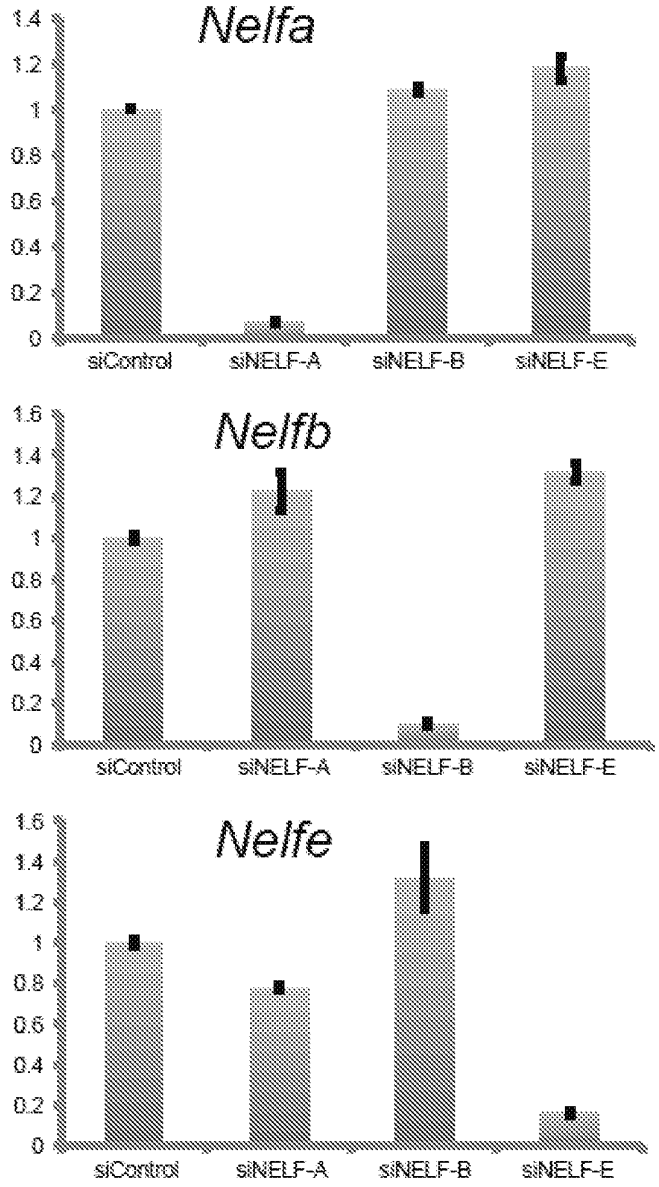

Nelfa
1.4
1.2
1
0.8
0.6
0.4
0.2
0
siControl
siNELF-A
siNELF-B
siNELF-E
Nelfb
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
siControl
siNELF-A
siNELF-B
siNELF-E
Nelfe
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
siControl
siNELF-A
siNELF-B
siNELF-E

Figure 3a

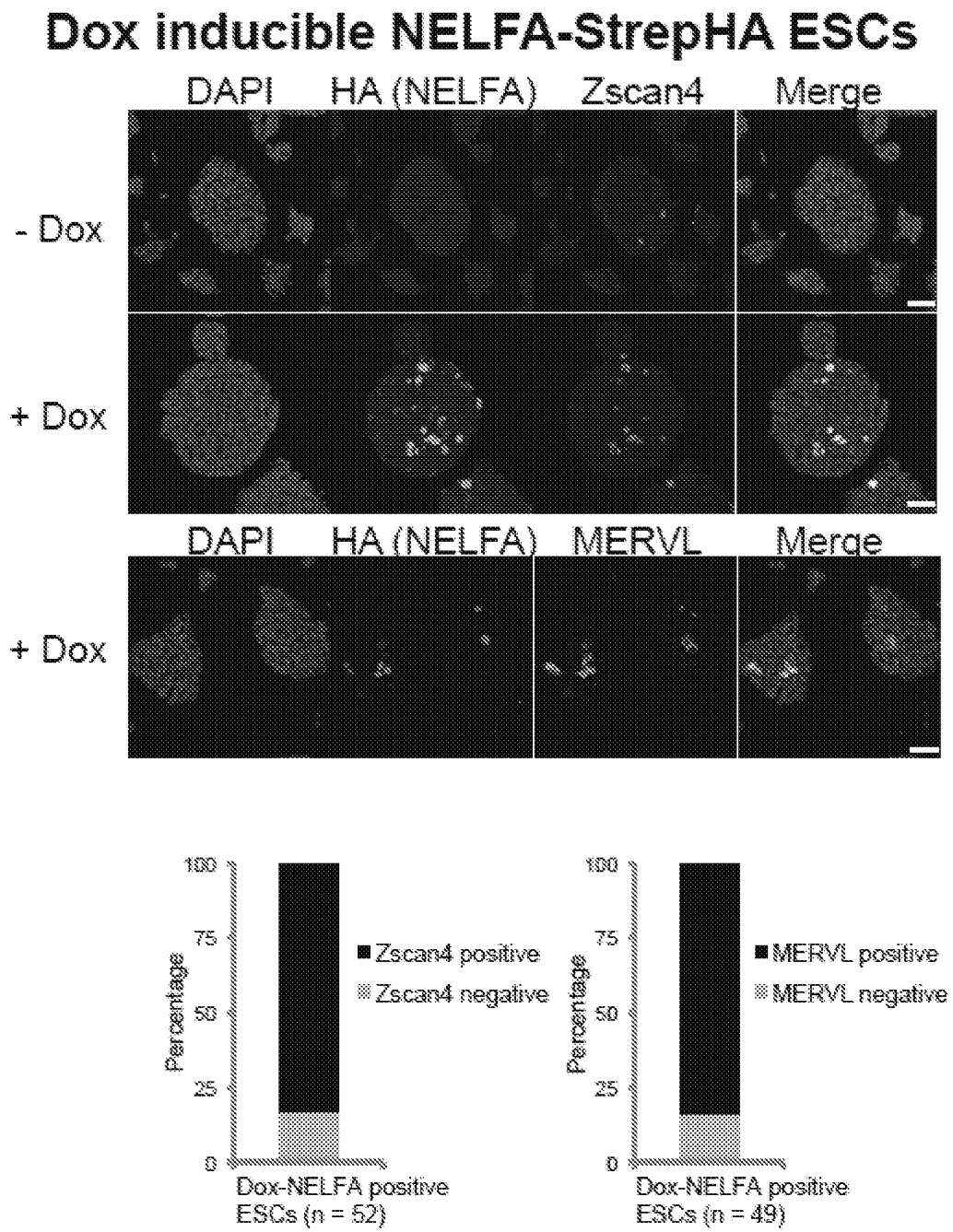

Dox inducible NELFA-StrepHA ESCs
DAPI
HA (NELFA)
Zscan4
Merge
- Dox
+ Dox
DAPI
HA (NELFA)
MERVL
Merge
+ Dox
100
75
50
25
0
Percentage
Zscan4 positive
Zscan4 negative
Dox-NELFA positive ESCs (n = 52)
100
75
50
25
0
Percentage
MERVL positive
MERVL negative
Dox-NELFA positive ESCs (n = 49)

Figure 3b

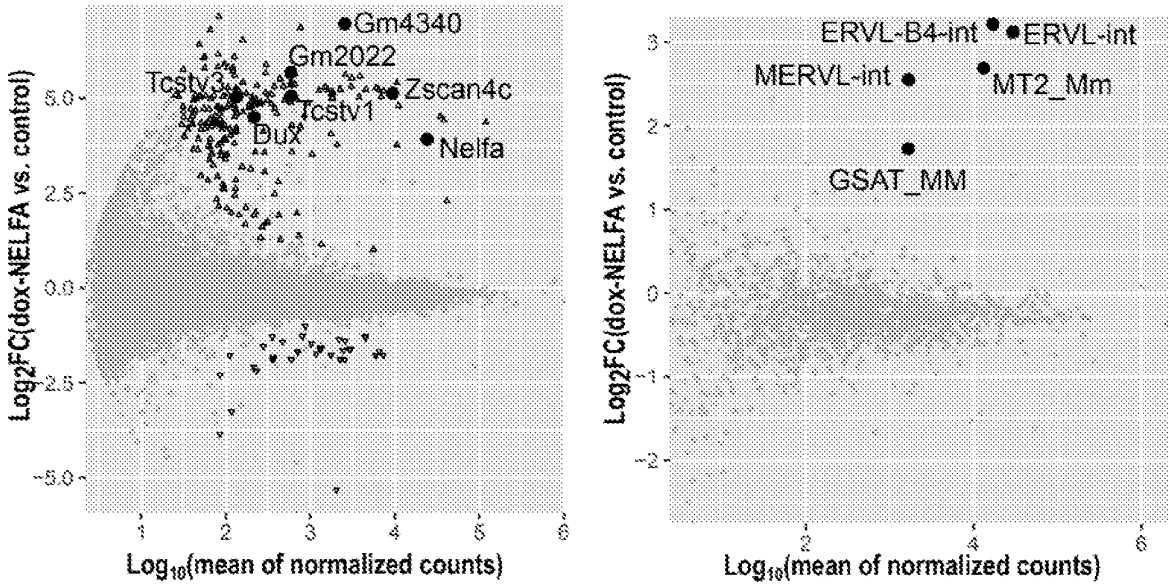


Figure 3c

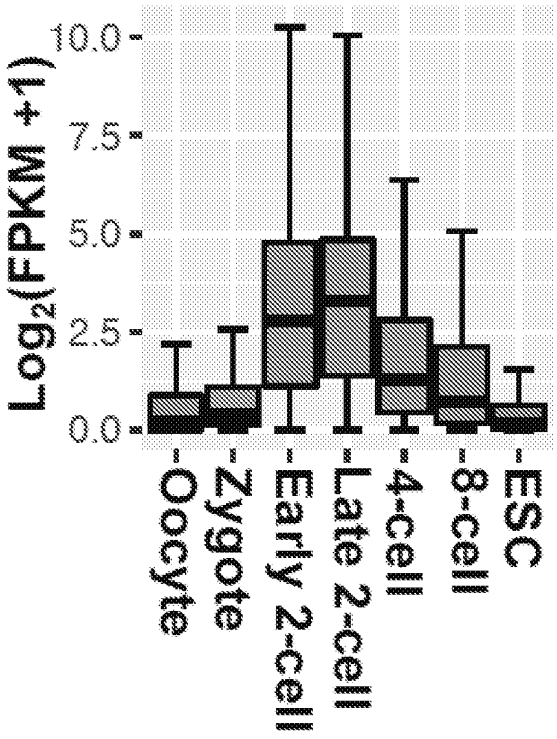


Figure 3d

Known motif: GATA3



Dataset	p-value
NELFA ^{high}	p=1e-04
NELFA (+Dox)	p=1e-05
DUX-ChIP-seq	p=1e-3140

De novo motif: DUX



Dataset	p-value
NELFA ^{high}	p=1e-20
NELFA (+Dox)	p=1e-23
DUX-ChIP-seq	p=1e-8233

Figure 3e

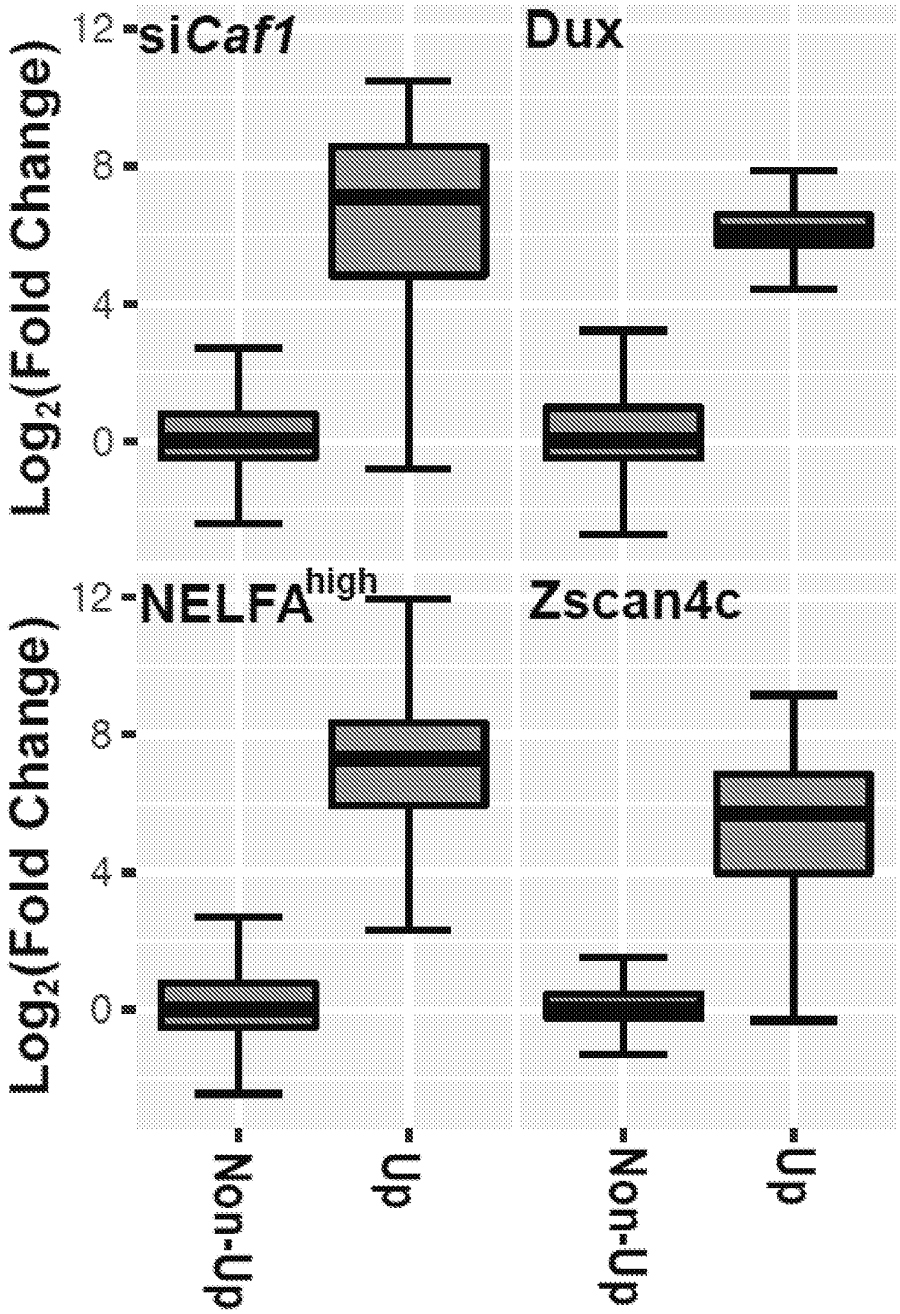

siCaf1
Dux
NELFA^high
Zscan4c
Log2(Fold Change)
12
8
4
0
Non-Up
Up

Figure 3f

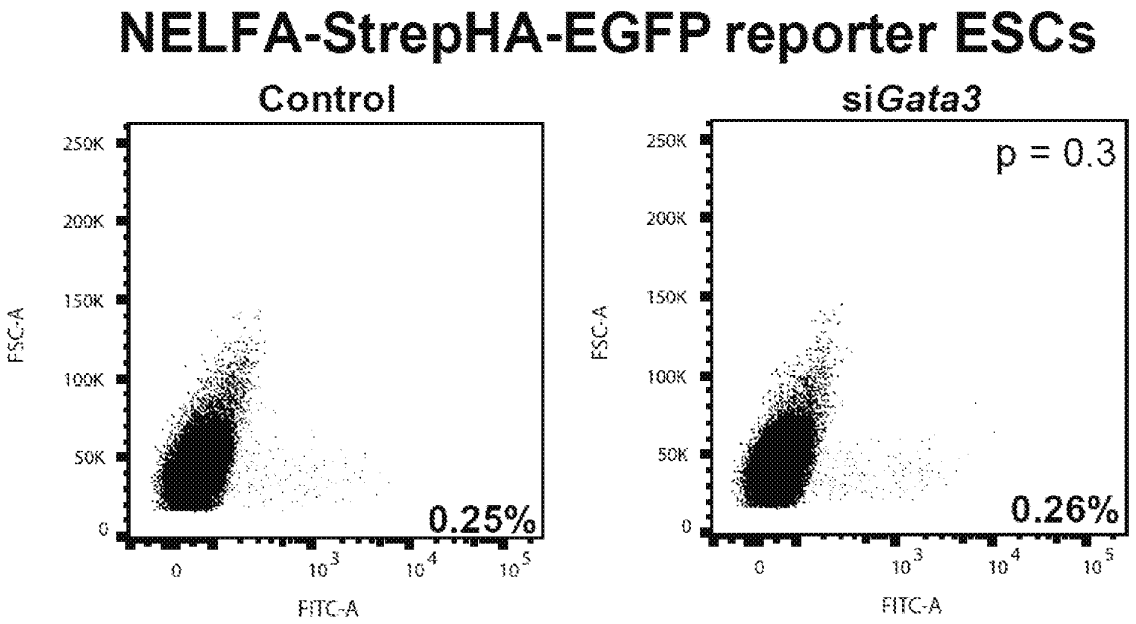


Figure 3g

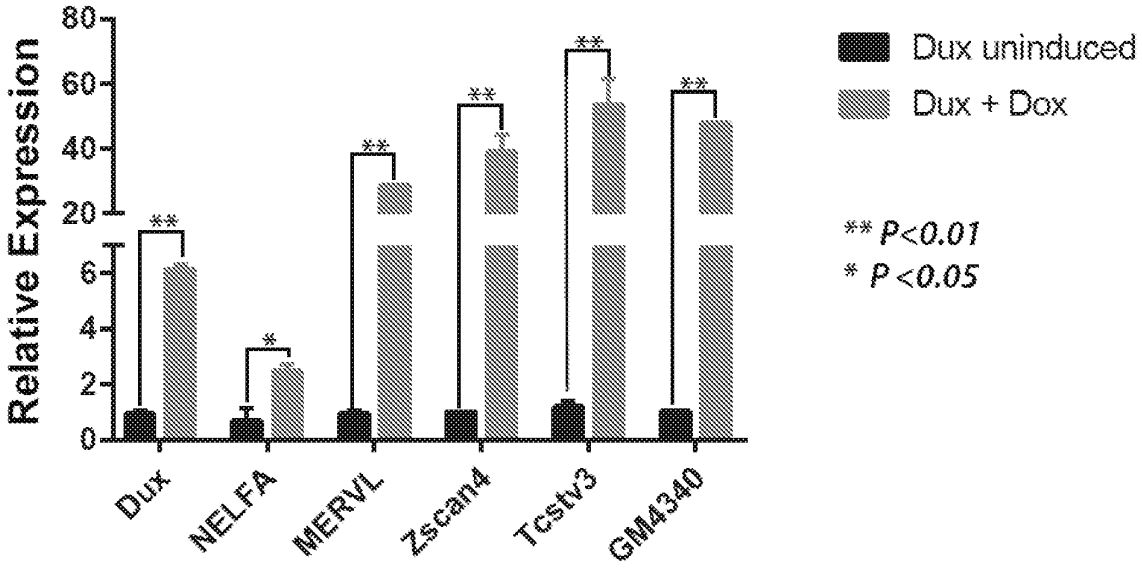


Figure 3h

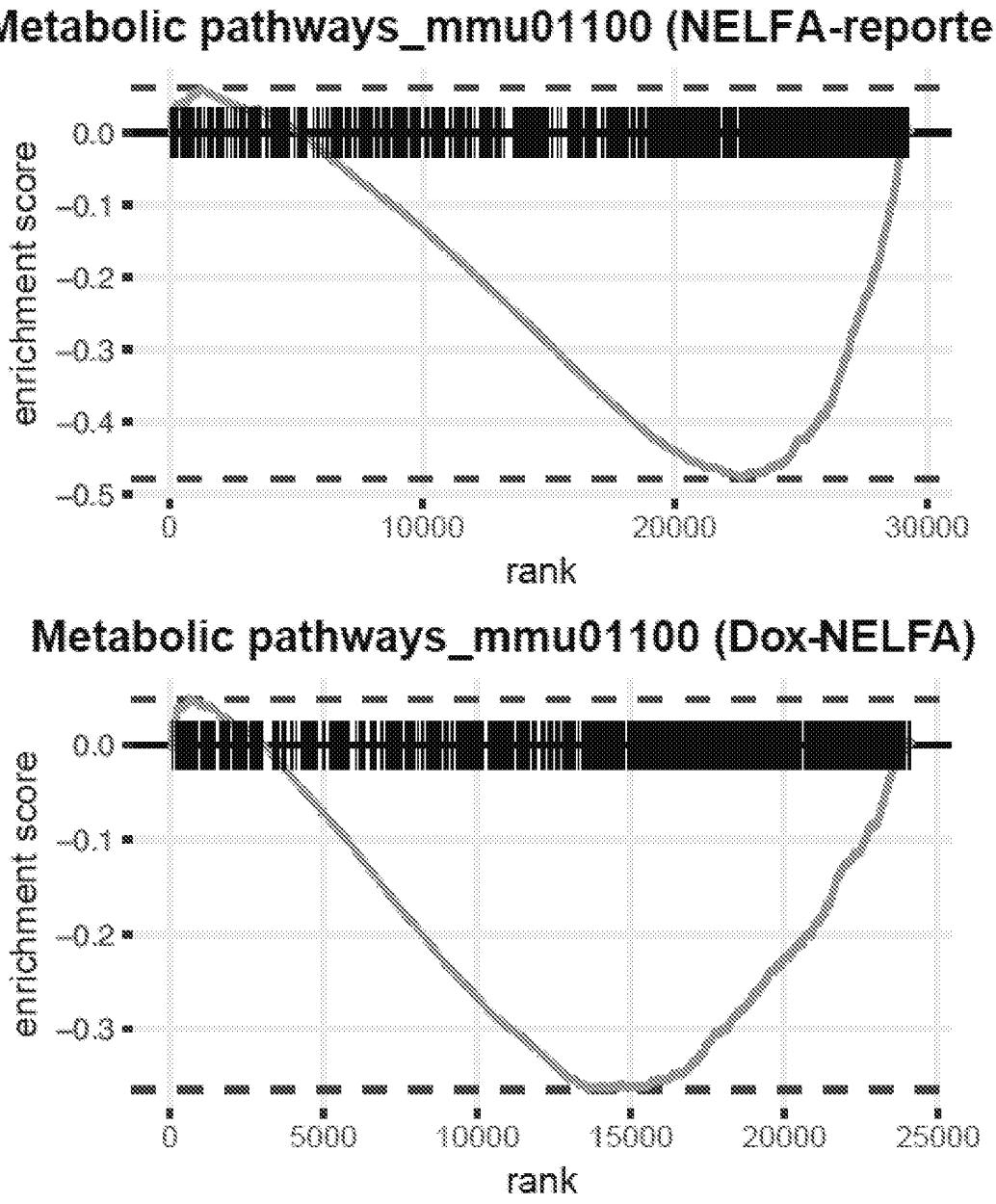

Metabolic pathways_mmu01100 (NELFA-reporter)
enrichment score
0.0
−0.1
−0.2
−0.3
−0.4
−0.5
0
10000
20000
30000
rank
Metabolic pathways_mmu01100 (Dox-NELFA)
enrichment score
0.0
−0.1
−0.2
−0.3
0
5000
10000
15000
20000
25000
rank

Figure 4a

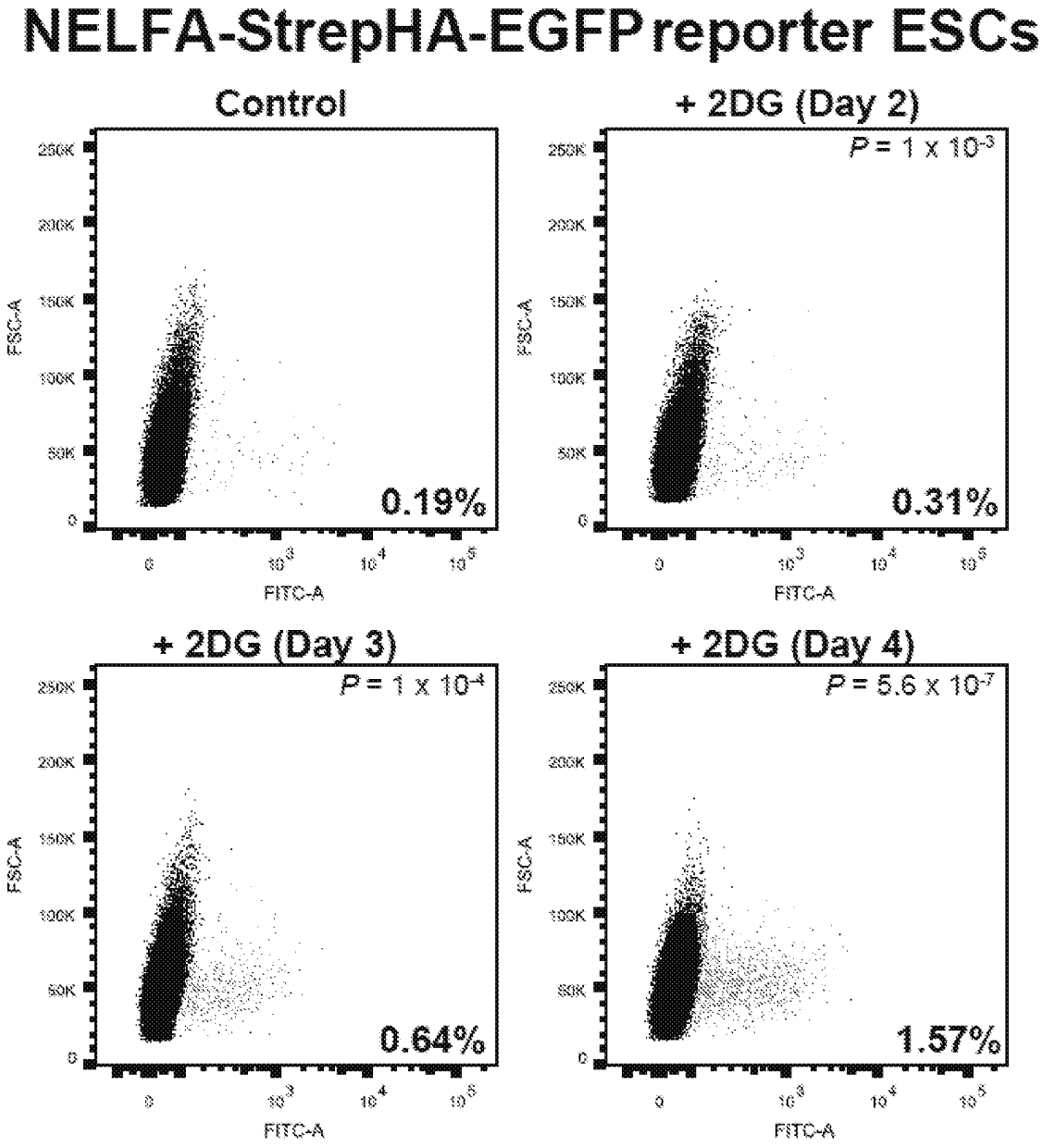

NELFA-StrepHA-EGFP reporter ESCs
Control
0.19%
+ 2DG (Day 2)
P = 1 x 10-3
0.31%
+ 2DG (Day 3)
P = 1 x 10-4
0.64%
+ 2DG (Day 4)
P = 5.6 x 10-7
1.57%
FSC-A
FITC-A
250K
200K
150K
100K
50K
0
10^3
10^4
10^5

Figure 4b

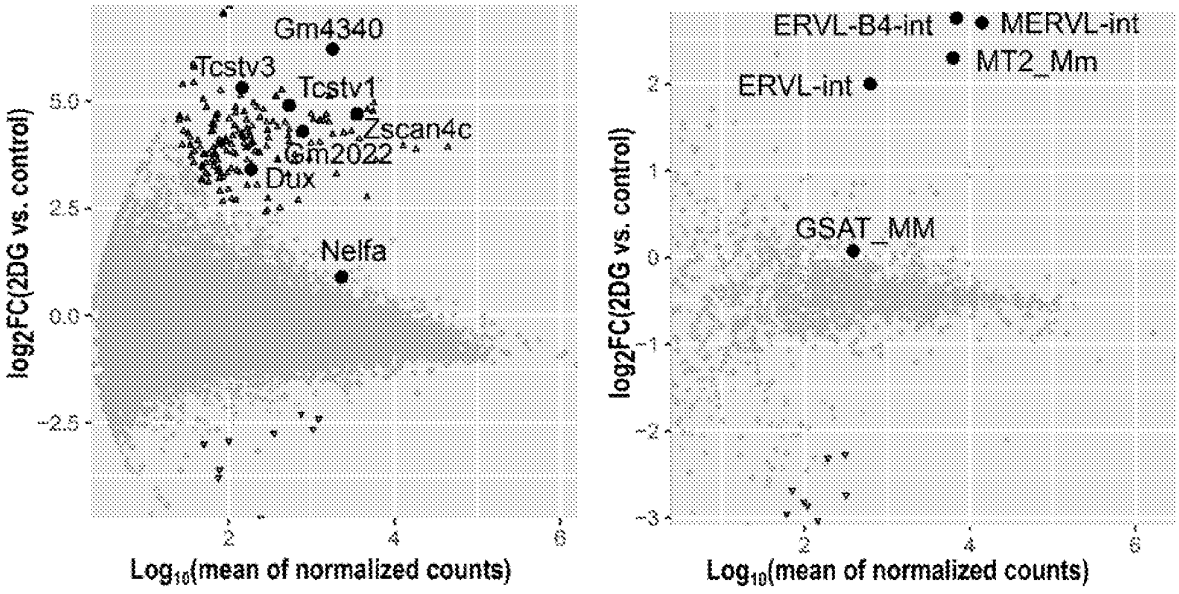


Figure 4c

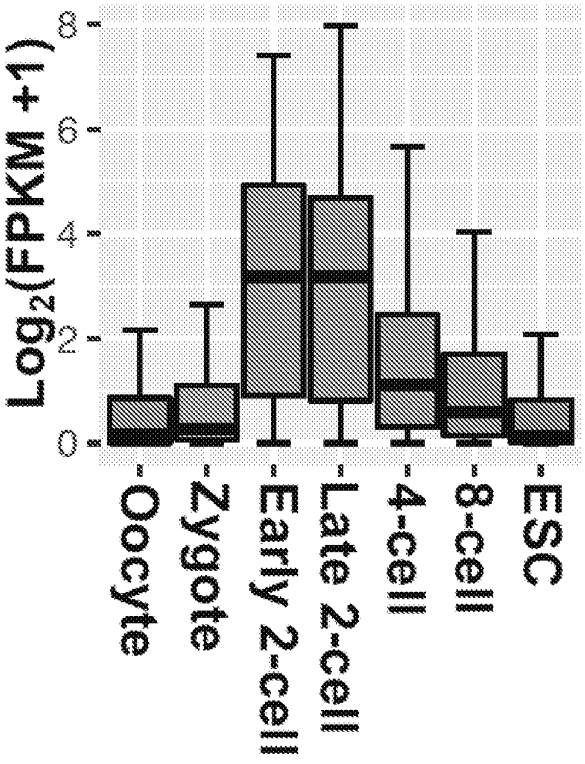


Figure 4d

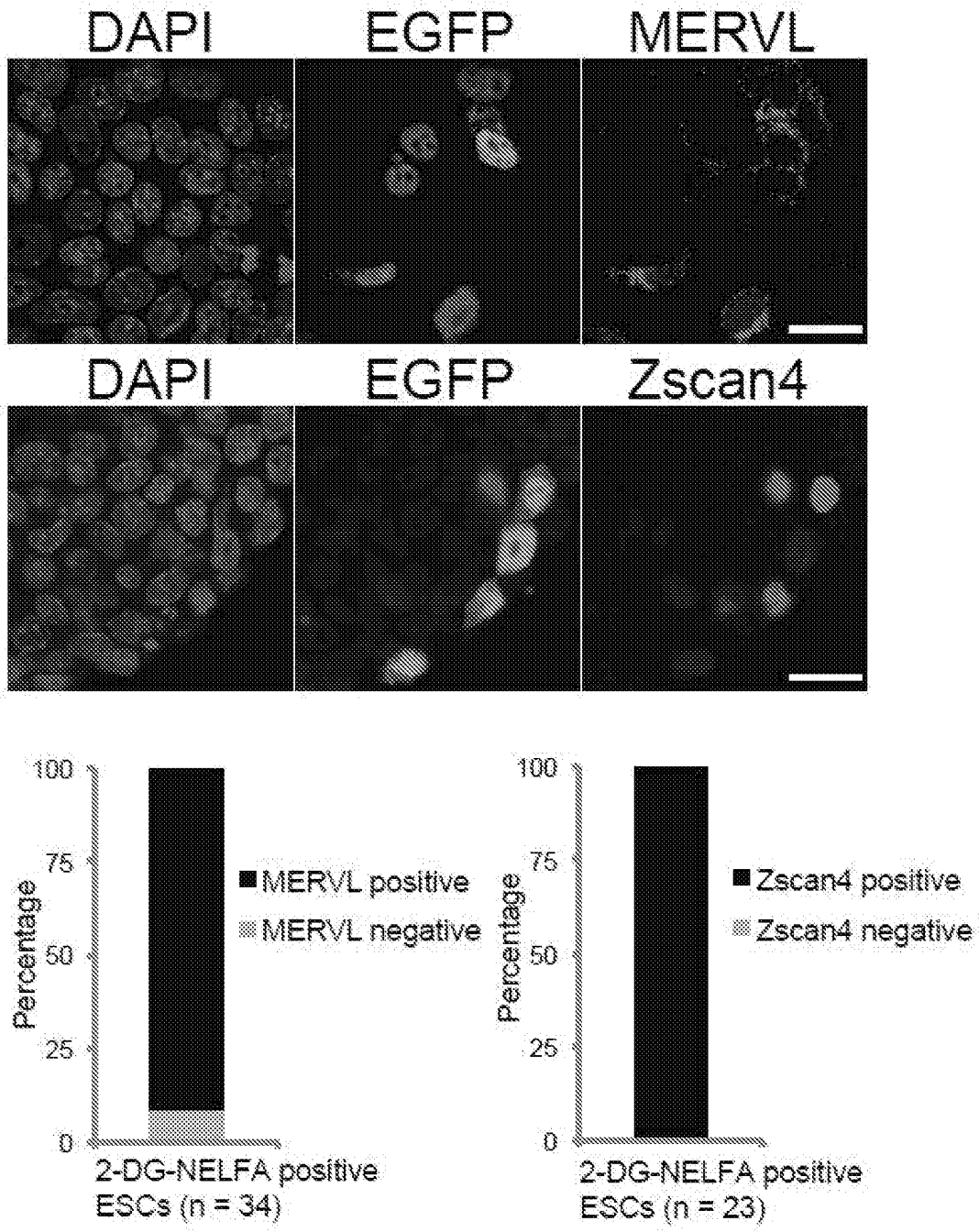

DAPI
EGFP
MERVL
DAPI
EGFP
Zscan4
100
75
50
25
0
Percentage
MERVL positive
MERVL negative
2-DG-NELFA positive ESCs (n = 34)
100
75
50
25
0
Percentage
Zscan4 positive
Zscan4 negative
2-DG-NELFA positive ESCs (n = 23)

Figure 4e

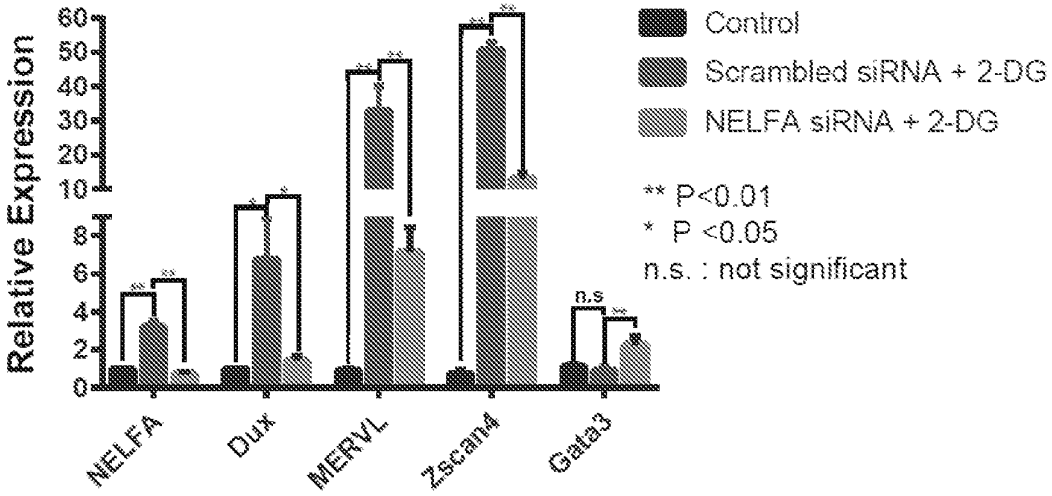


Figure 4f

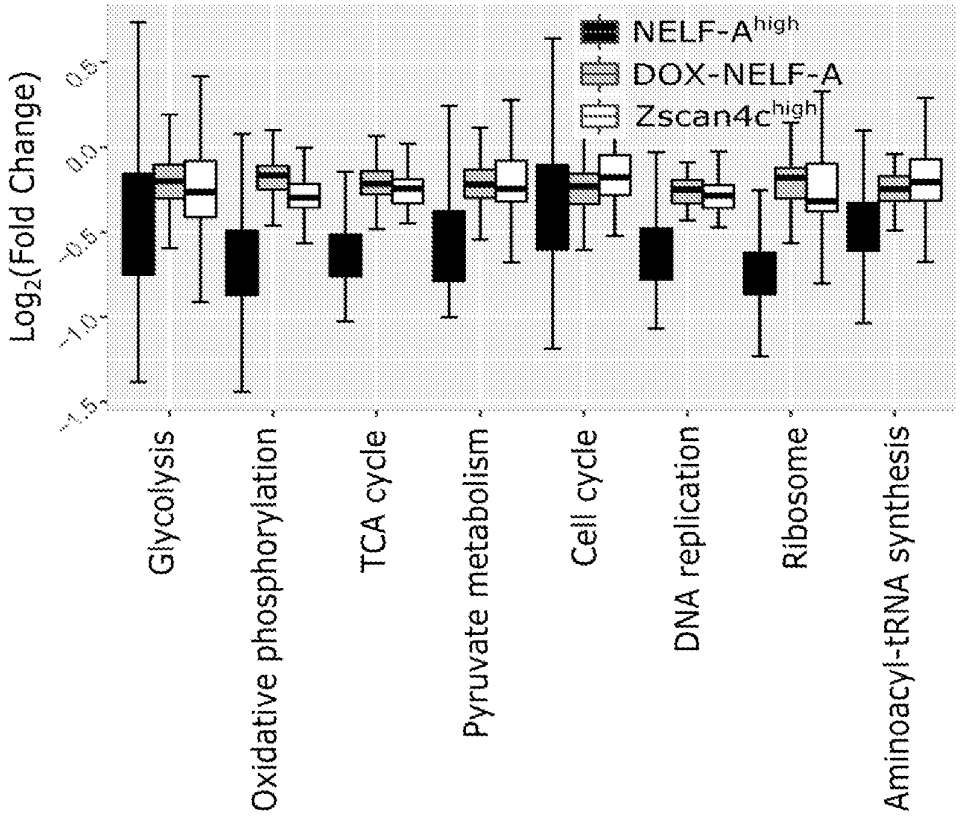


Figure 4g

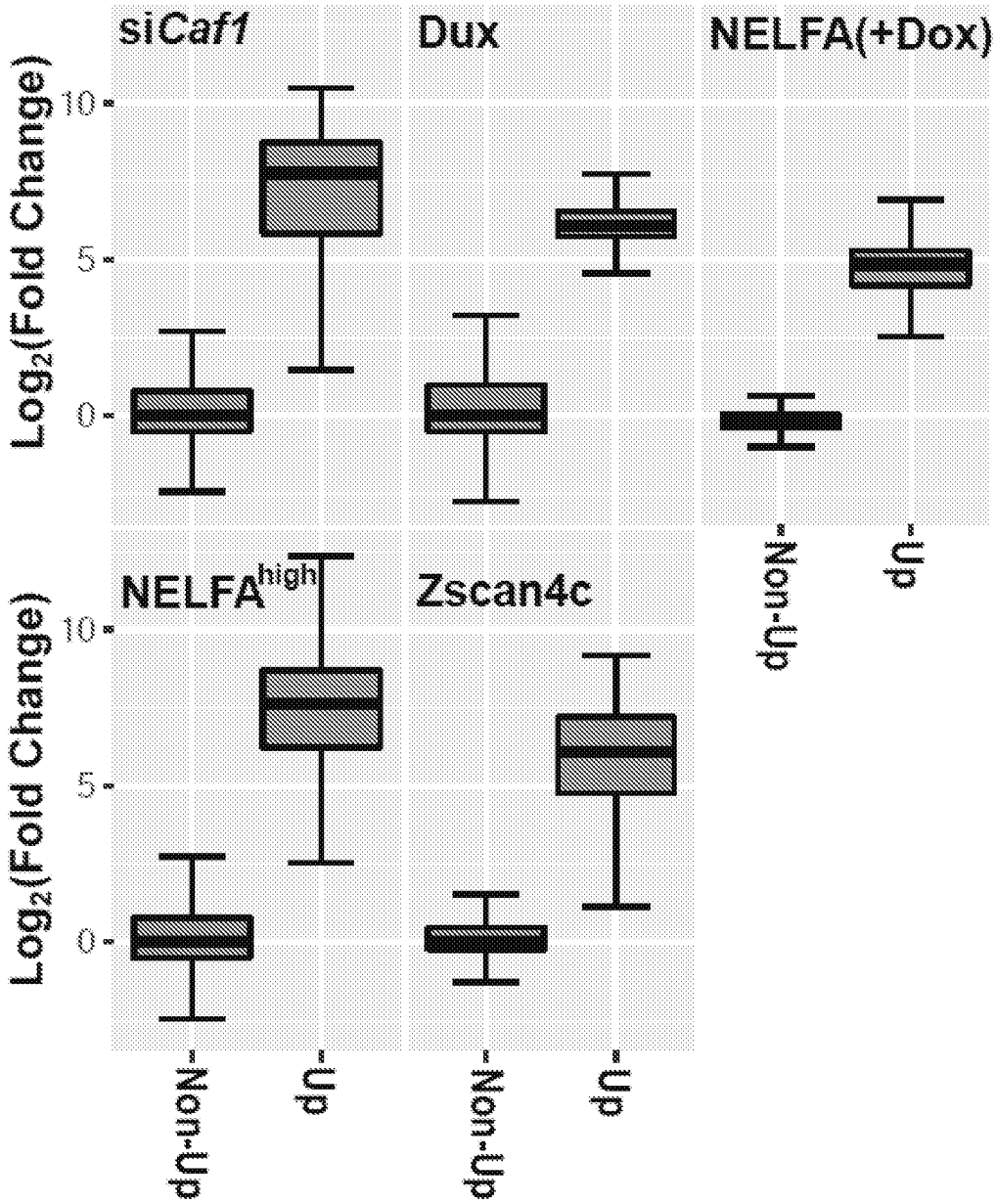

siCaf1
Dux
NELFA(+Dox)
NELFA^high
Zscan4c
Log2(Fold Change)
10
5
0
Non-Up
Up

Figure 4h

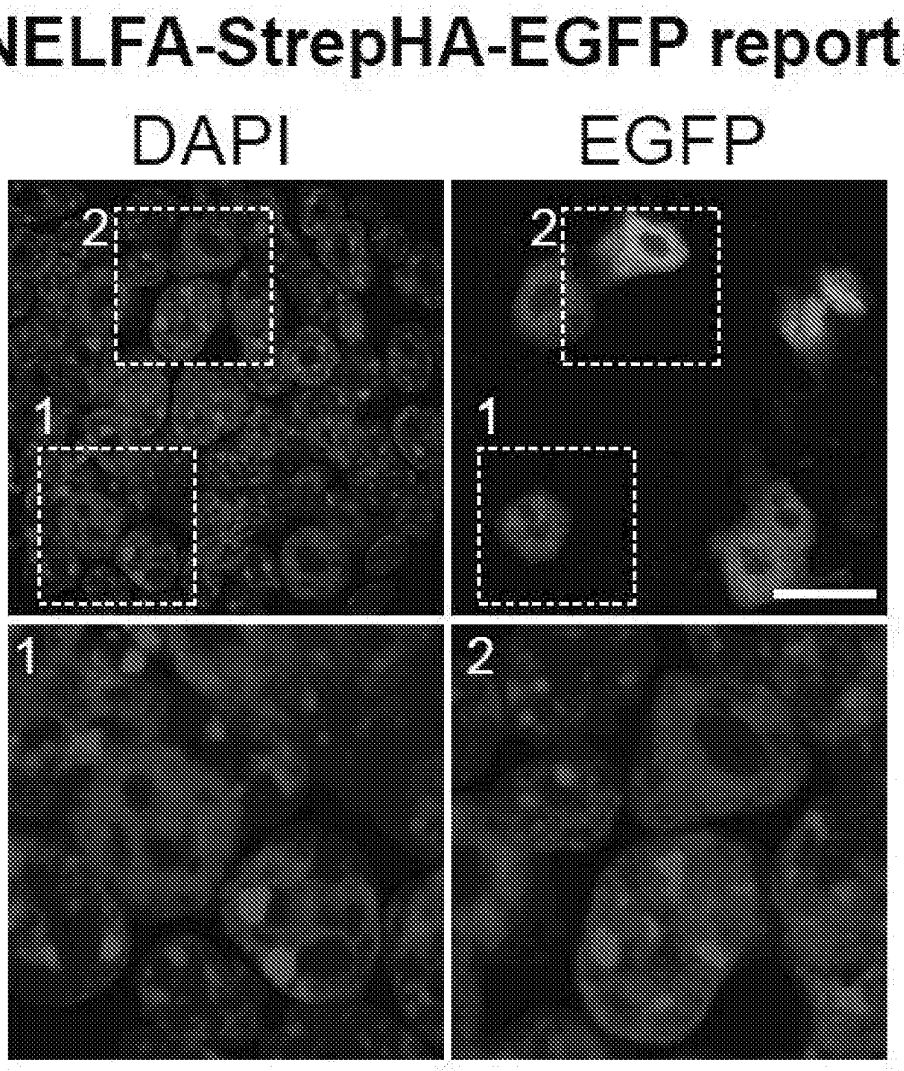


Figure 5a

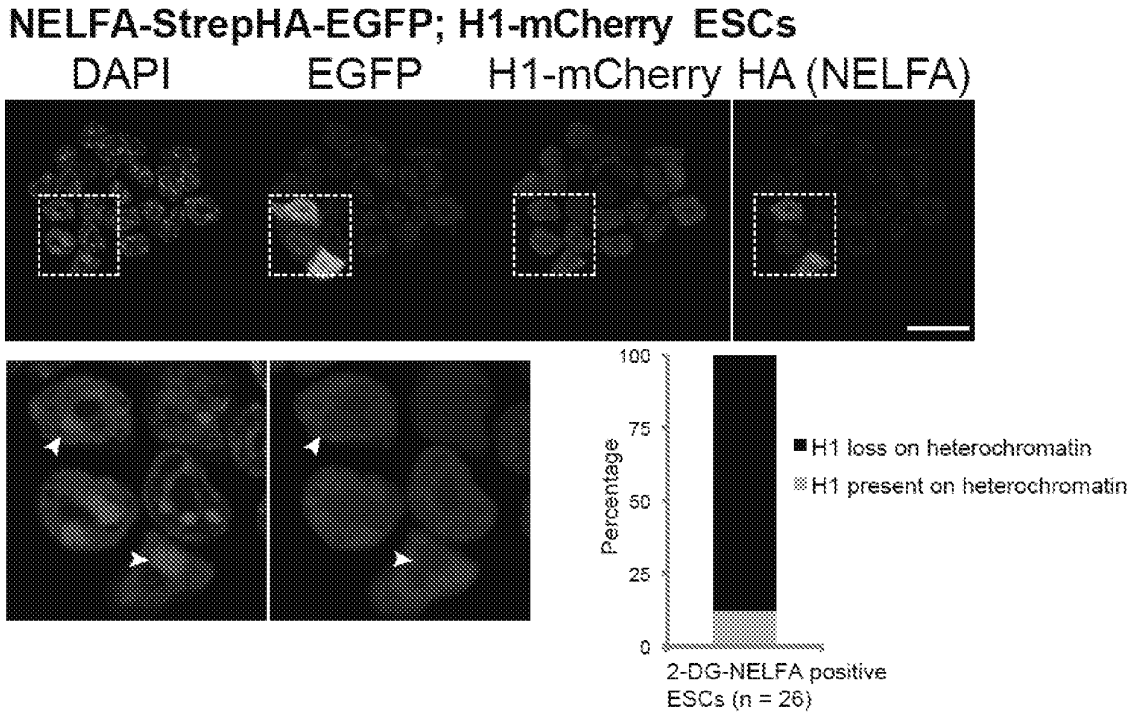


Figure 5b

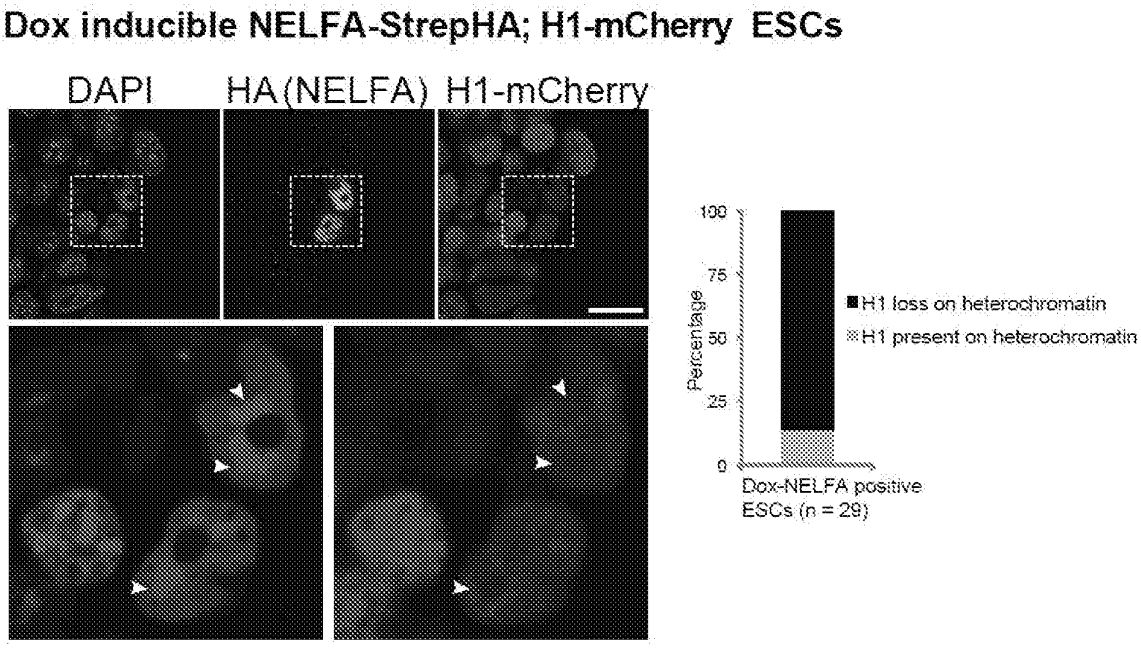


Figure 5c

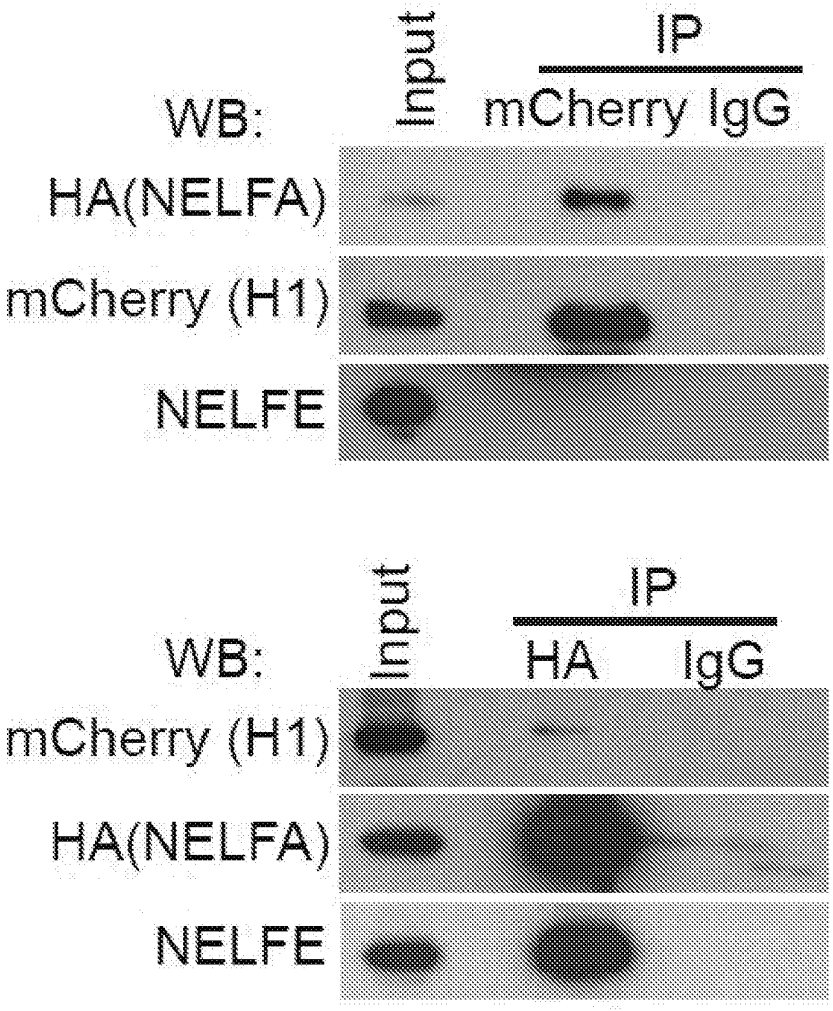

Input
IP
WB:
mCherry IgG
HA(NELFA)
mCherry (H1)
NELFE
Input
IP
WB:
HA IgG
mCherry (H1)
HA(NELFA)
NELFE

Figure 5d

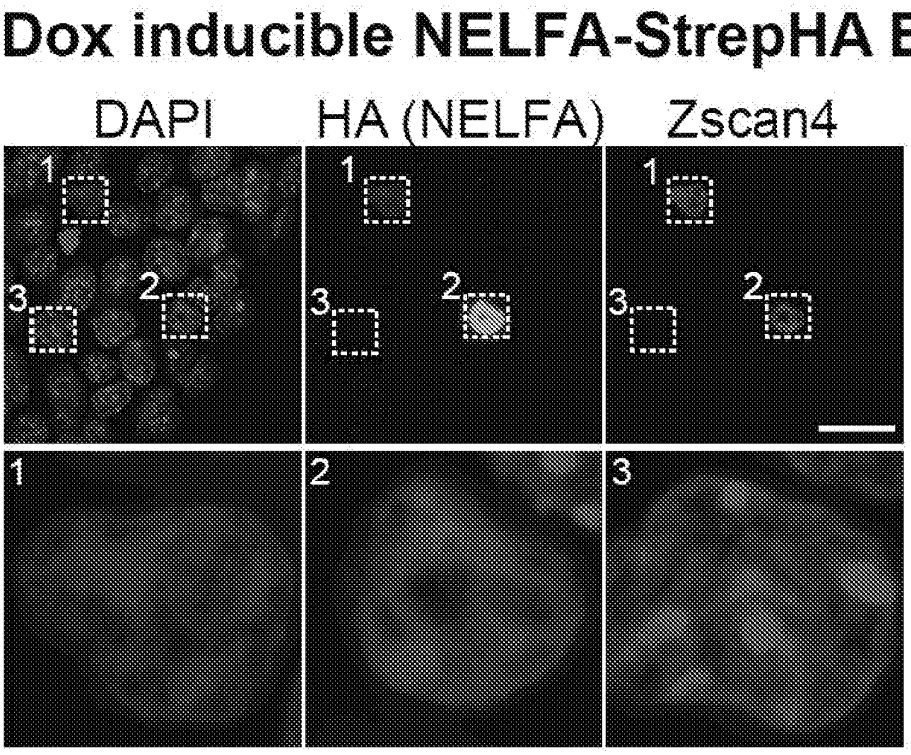


Figure 5e

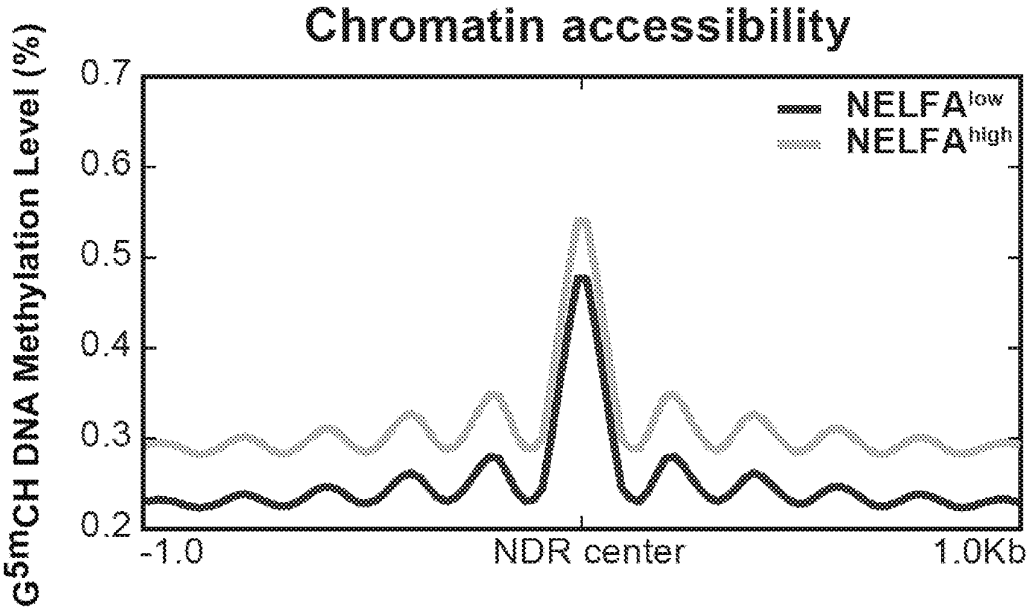


Figure 5f

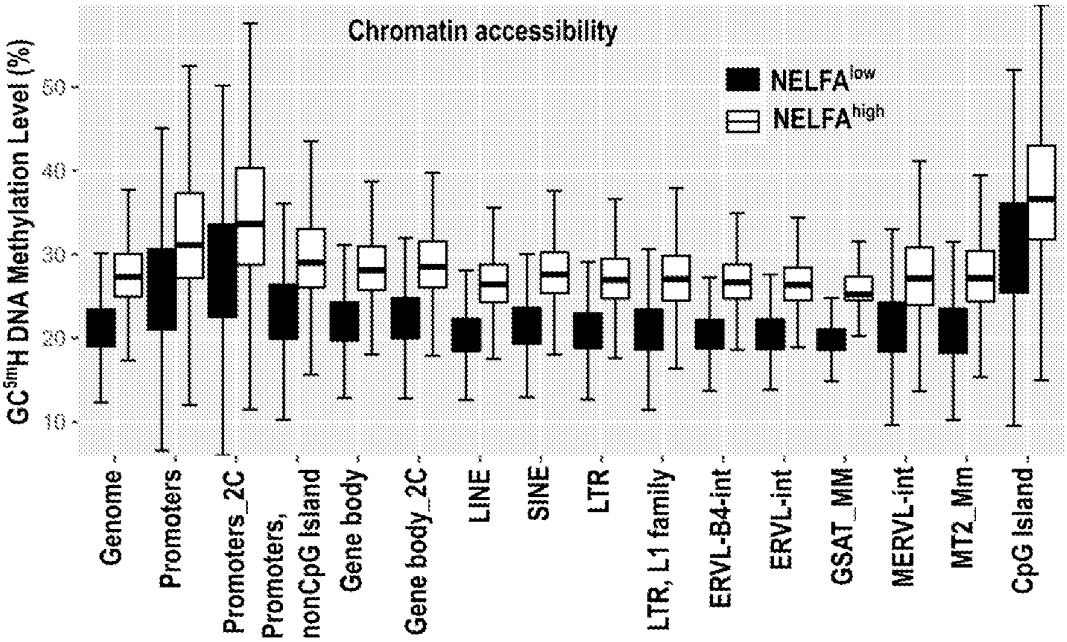


Figure 5g

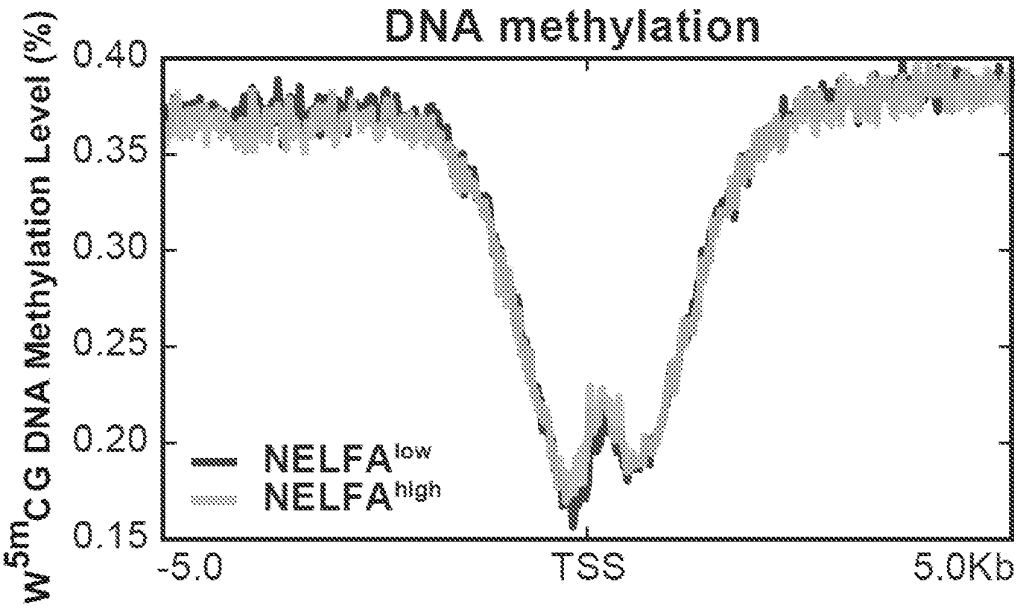


Figure 5h

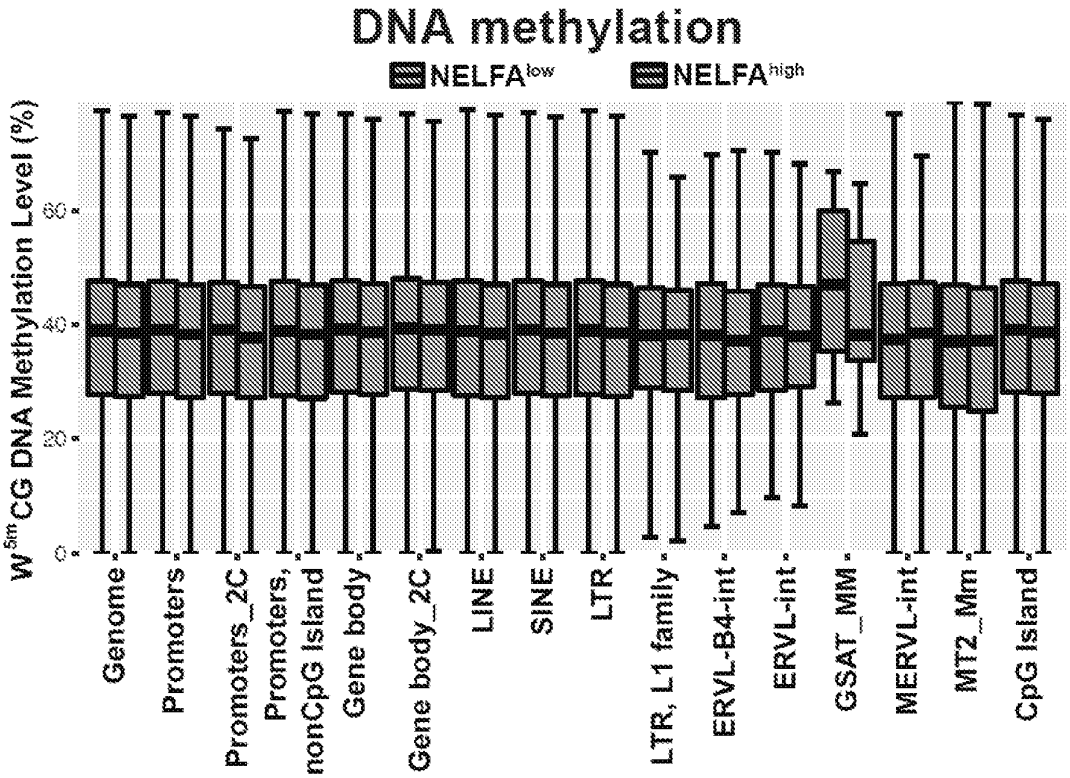
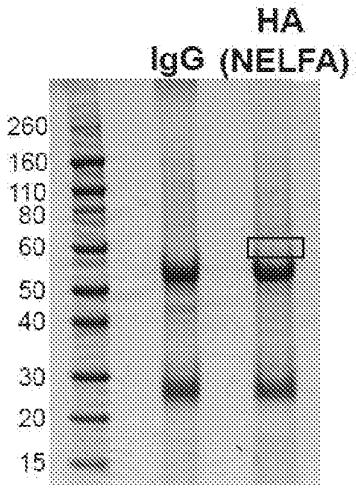


Figure 5i



MSETAPAAPAAPAPAEKTPVKKKARKAAGGAKRKTSGPPVSELITKAVAASKERSGVSLAALKKA
LAAAGYDVEKNNSRIKLGLKSLVSRGTLVQTKGTGASGSFKLNKKAASGEAKPKAKRAGAAKARK
PAGAAKKPKKAAGTATAKKSTKKTPKKAKKPAAAAGAKRAKSPKKAKATKAKKAPKSPAKAKTVK
PKAAKPKTSKPPAAKPKETAAKRRGDSGSGSGVSKGEELFTGVVPILVELDGDVNGHKFSVSGE
GEGDATYGKLTLKFICTTGKLPVPWPTLVTTFSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQER
TIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNG
IKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFV
TAAGITHGMDELYK

Sequence	Andromeda Score
ALAAAGYDVEK	93.111
GEEDNMAIIK	112.84
GTNFPSDGPVMQK	162
HPADIPDYLK	182.98
KPVQLPGAYNVNIK	148.13
KTSGPPVSELITK	81.619
LDITSHNEDYTIVEQYER	159.69
LSFPEGFK	100.19
MYPEDGALK	63.565
MYPEDGALKGEIK	104.24
PVQLPGAYNVNIK	47.198
SETAPAAPAAPAPAEK	74.76
TSGPPVSELITK	134.57
VHMEGSVNGHEFEIEGEGEGRPYEGTQTAK	95.212
VMNFEDGGVVTVTQDSSLQDGEFIYK	72.227

Figure 5j

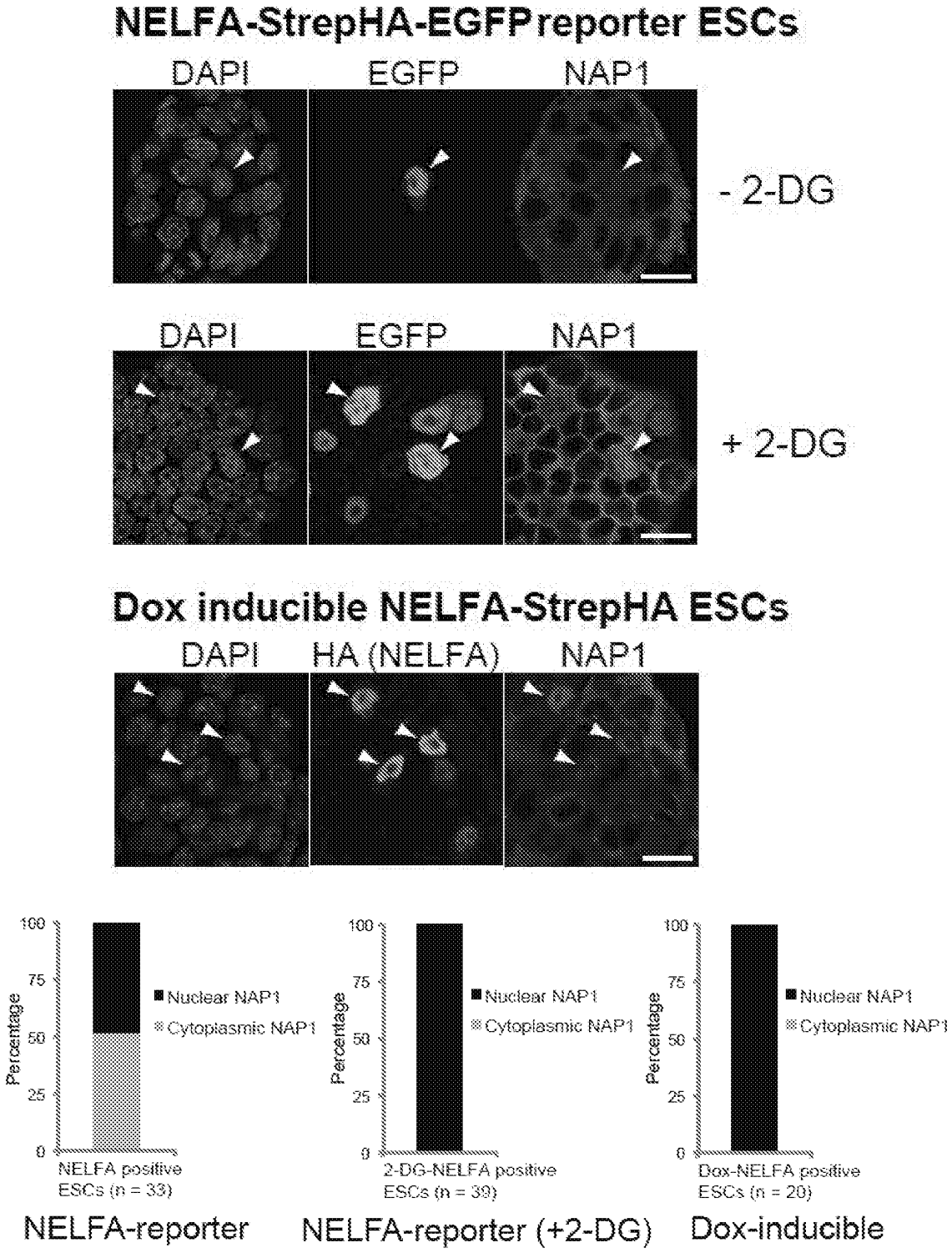

NELFA-StrepHA-EGFP reporter ESCs
DAPI
EGFP
NAP1
- 2-DG
DAPI
EGFP
NAP1
+ 2-DG
Dox inducible NELFA-StrepHA ESCs
DAPI
HA (NELFA)
NAP1
Percentage
100
75
50
25
0
Nuclear NAP1
Cytoplasmic NAP1
NELFA positive ESCs (n = 33)
2-DG-NELFA positive ESCs (n = 39)
Dox-NELFA positive ESCs (n = 20)
NELFA-reporter
NELFA-reporter (+2-DG)
Dox-inducible

Figure 6a

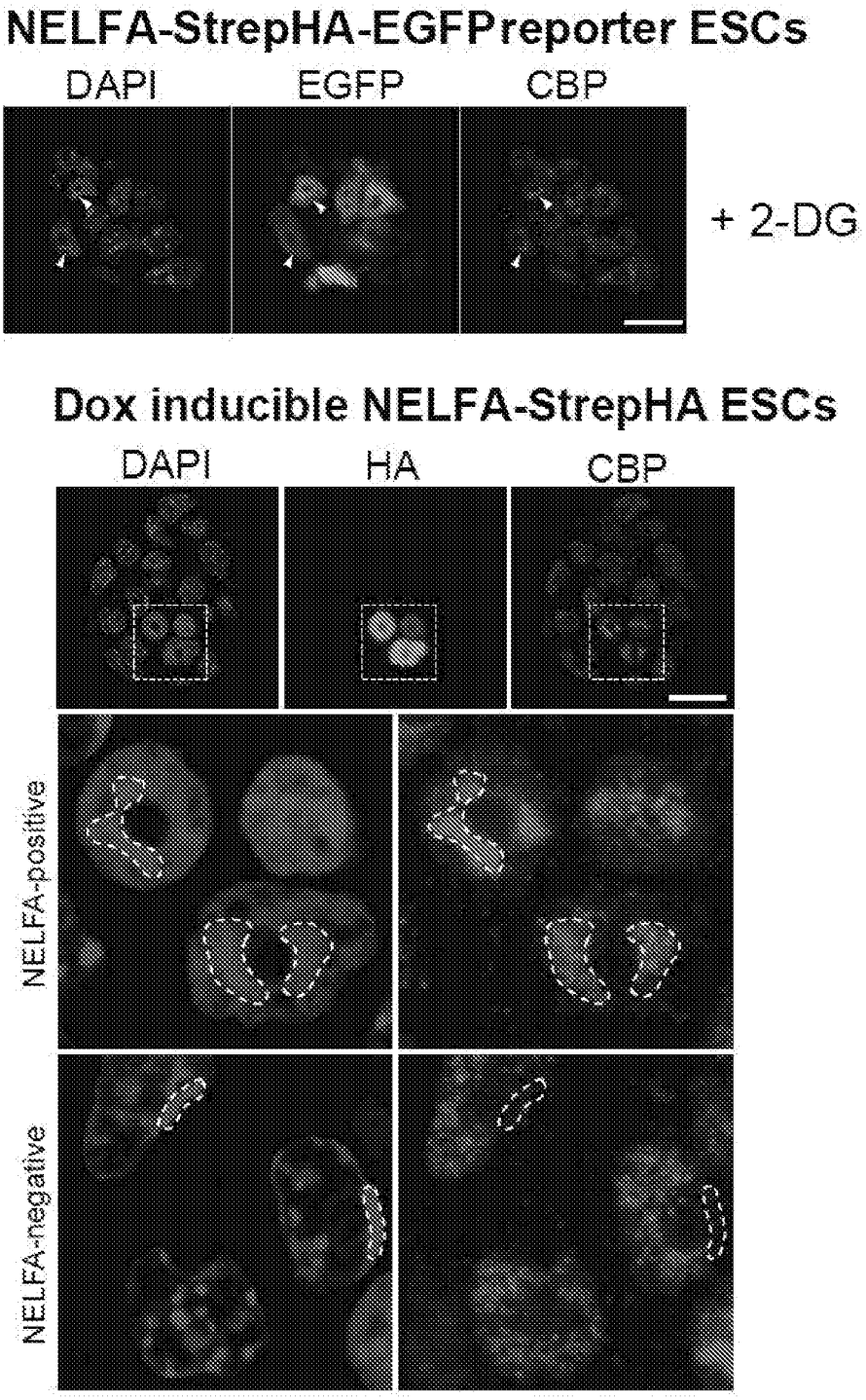


Figure 6b (cont.)

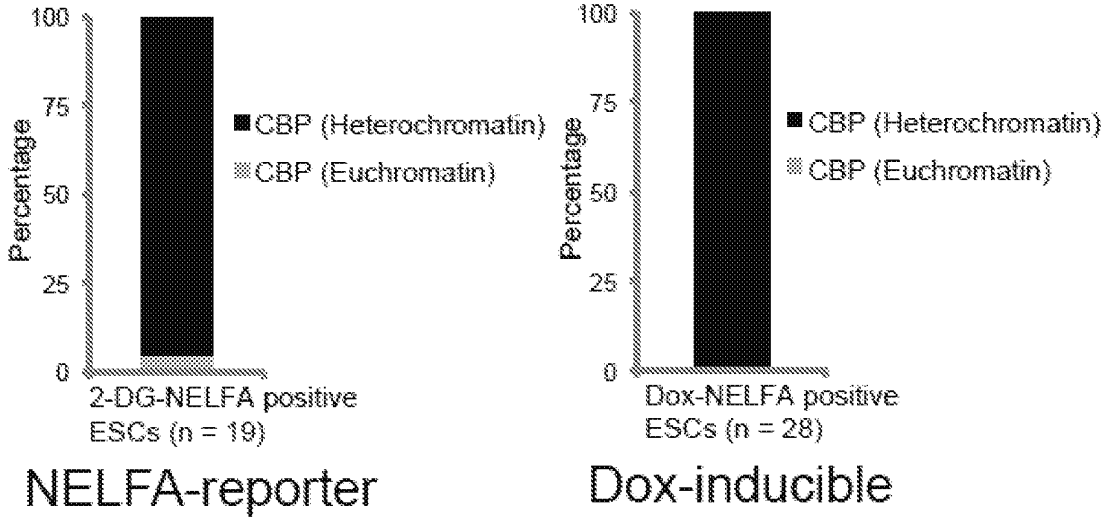


Figure 6b

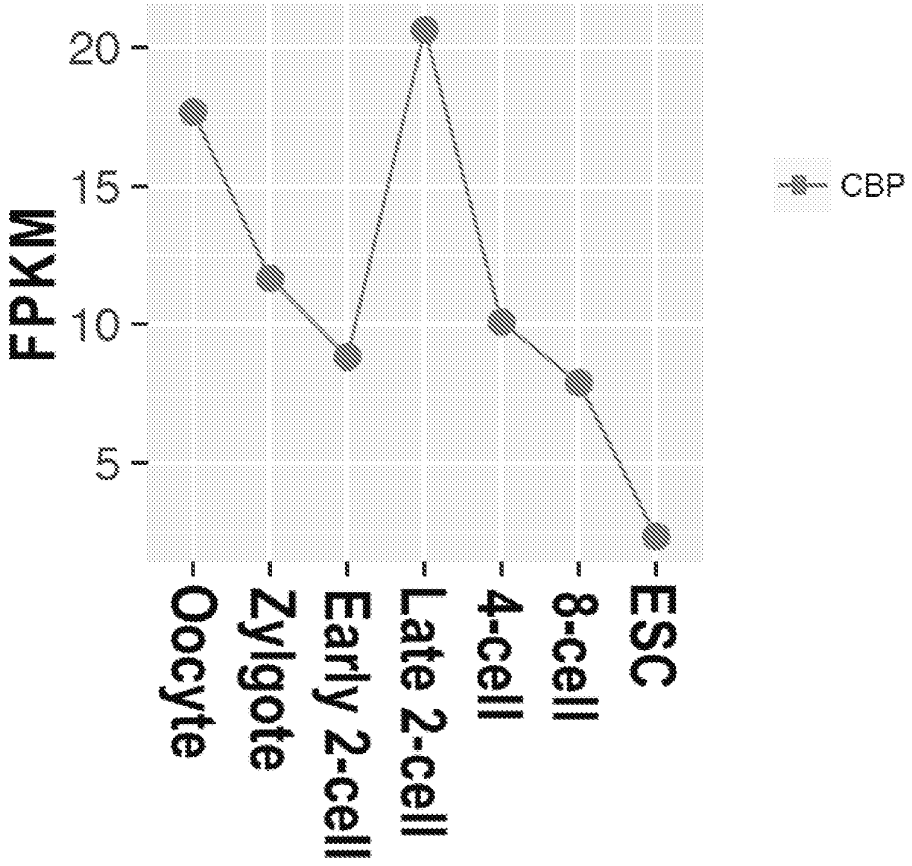


Figure 6c

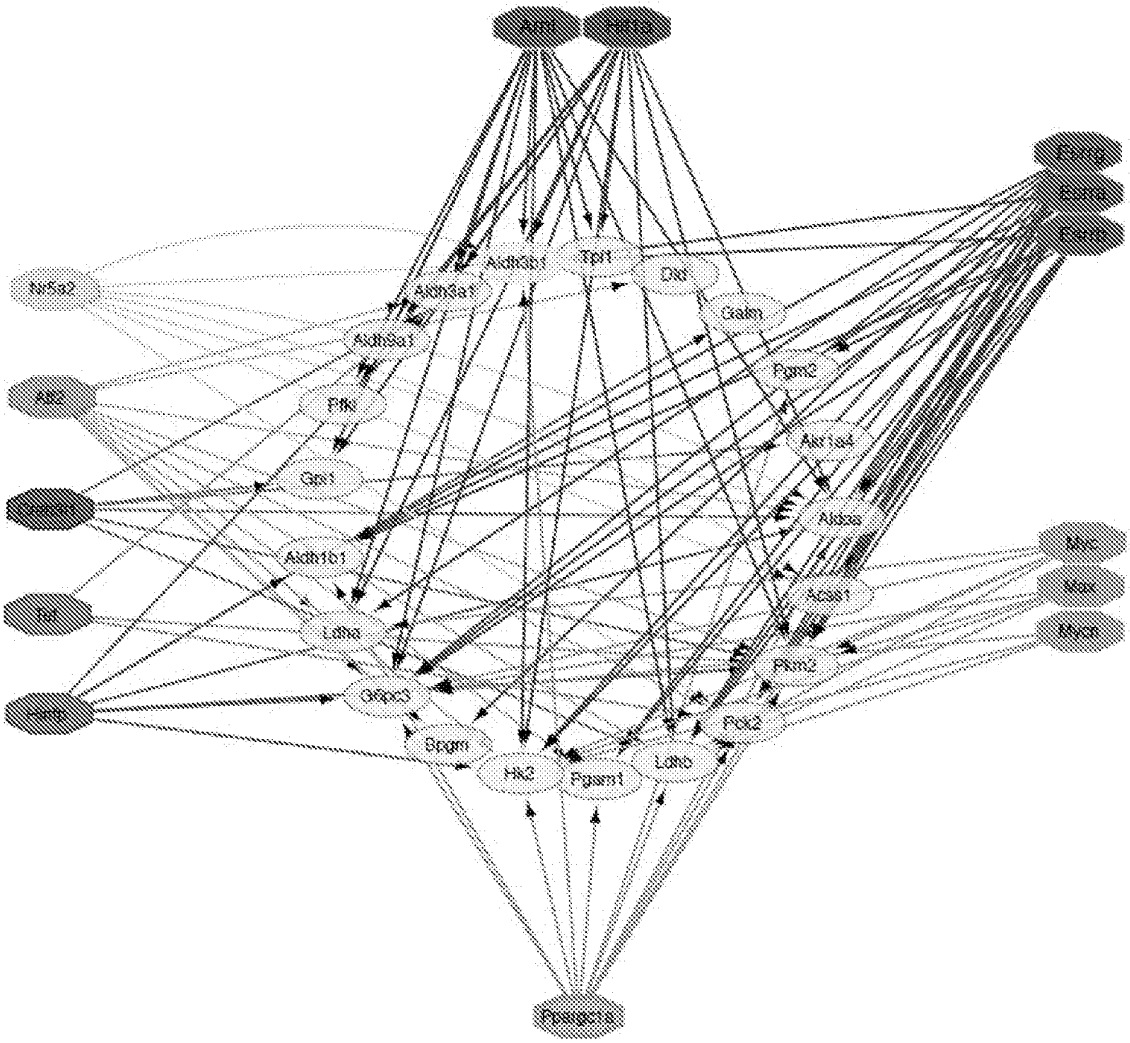

Aldh3a1
Tpt1
Dld
Galm
Fpm2
Aldh3a1
Fbp1
Aldh1a1
Gpi1
Aldh1b1
Ldhb
Gfpt2
Bpgm
Hk2
Fgam1
Ldhb
Pgam1
Aldh3a1
Aldoa
Acsl1
Pkm2
Pck2

Figure 7a

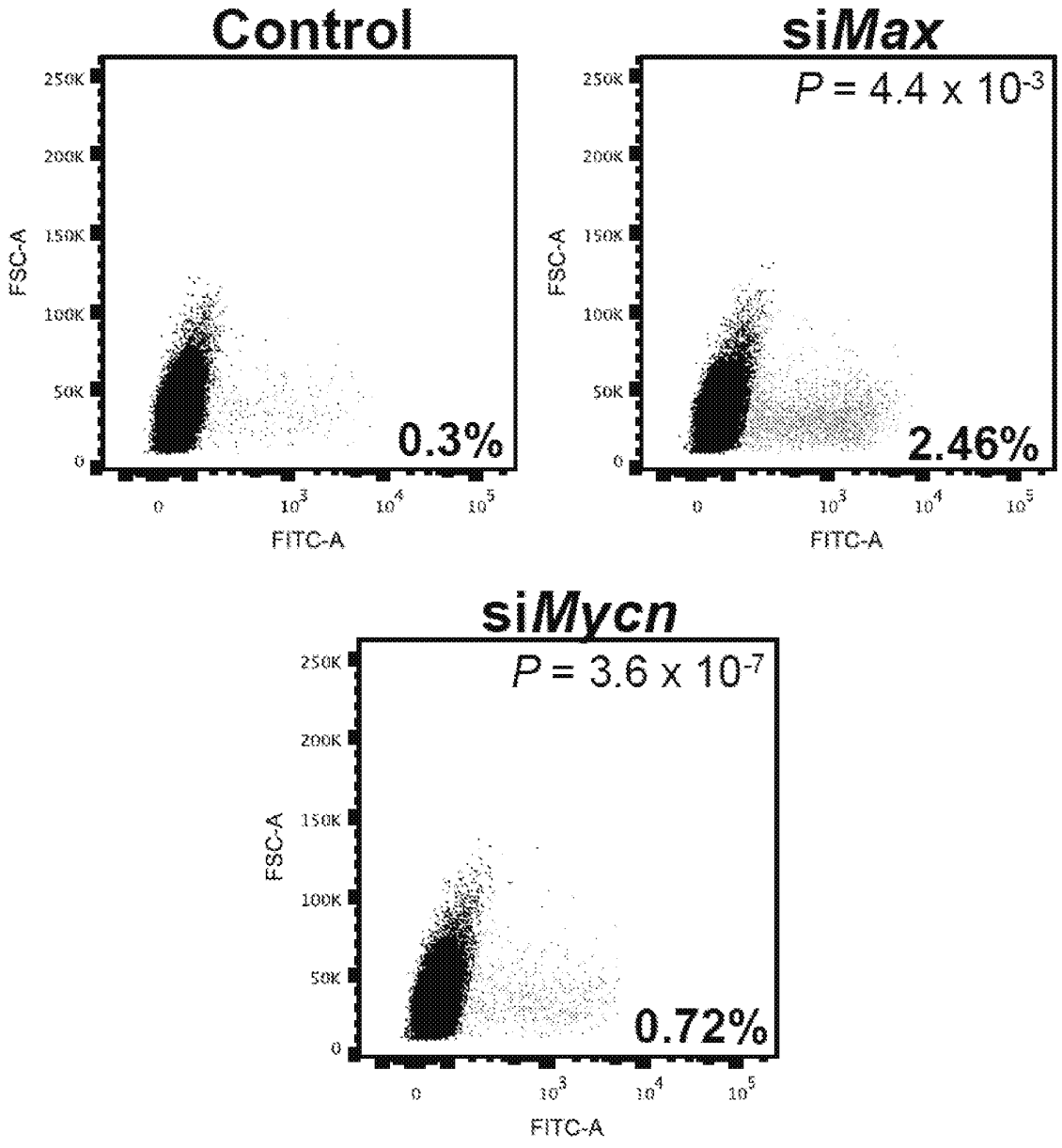


Figure 7b (cont.)

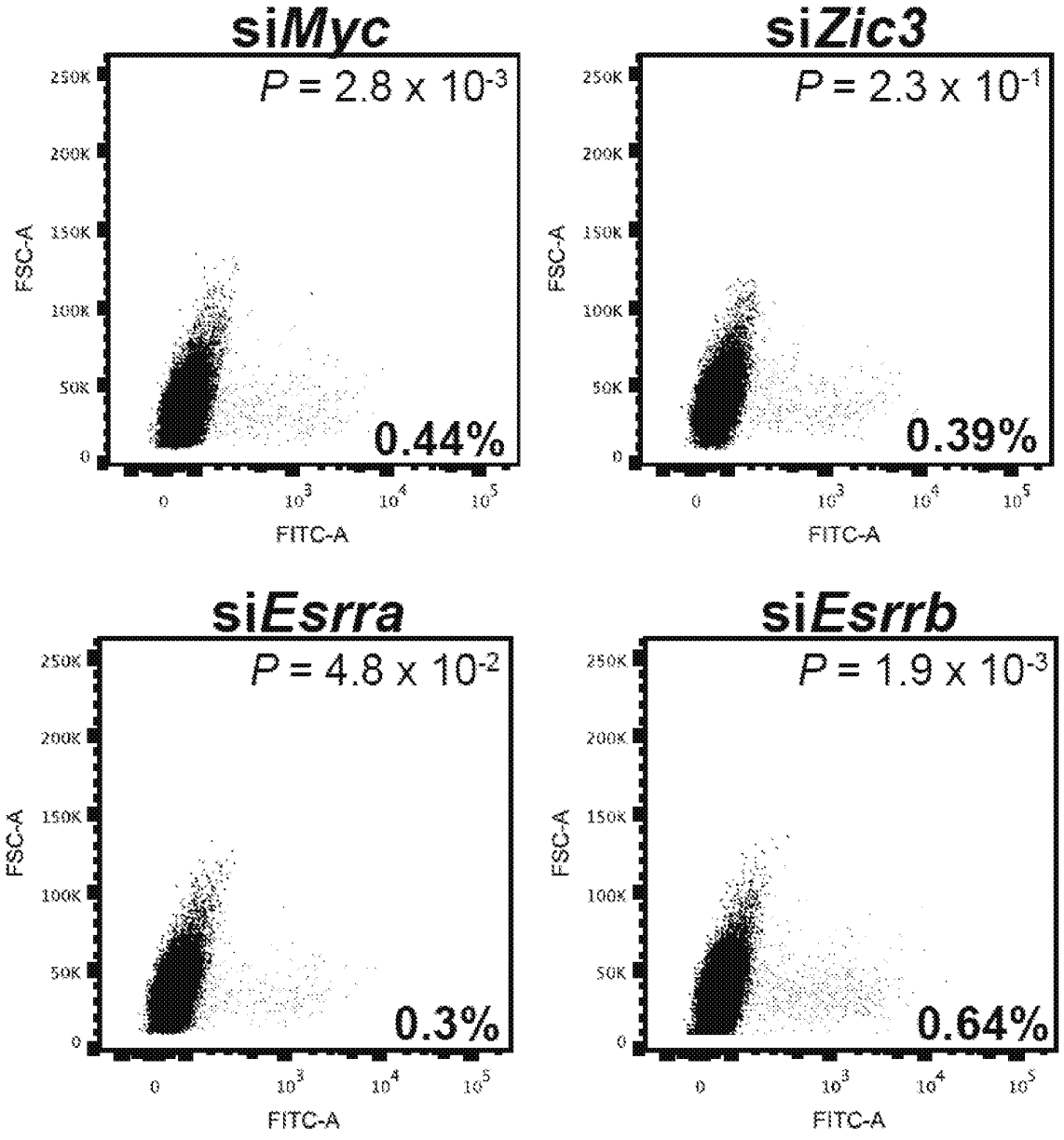


Figure 7b (cont.)

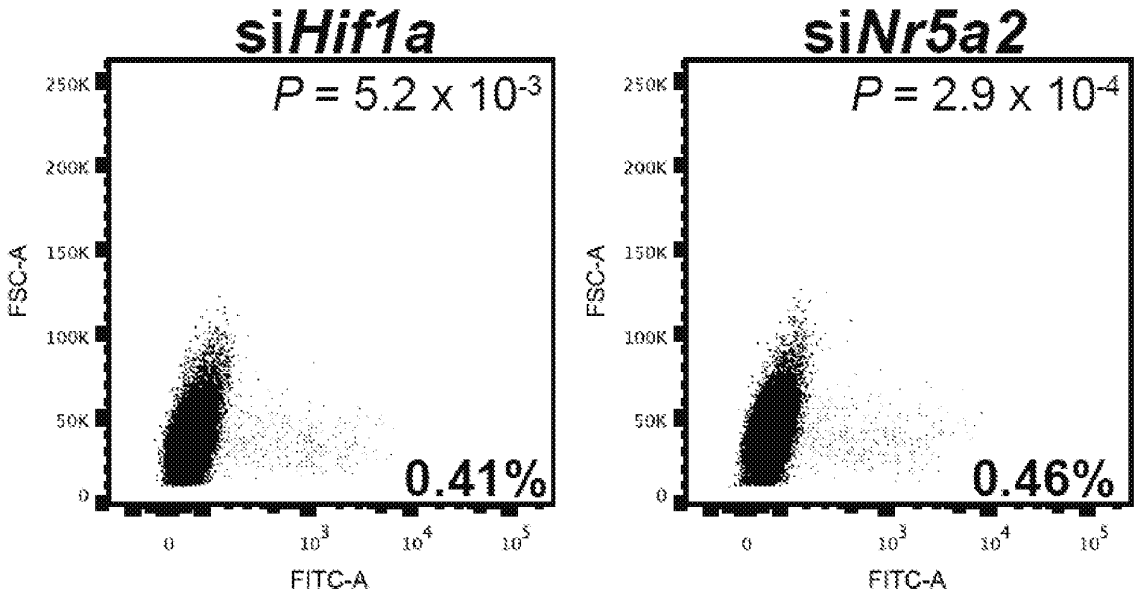
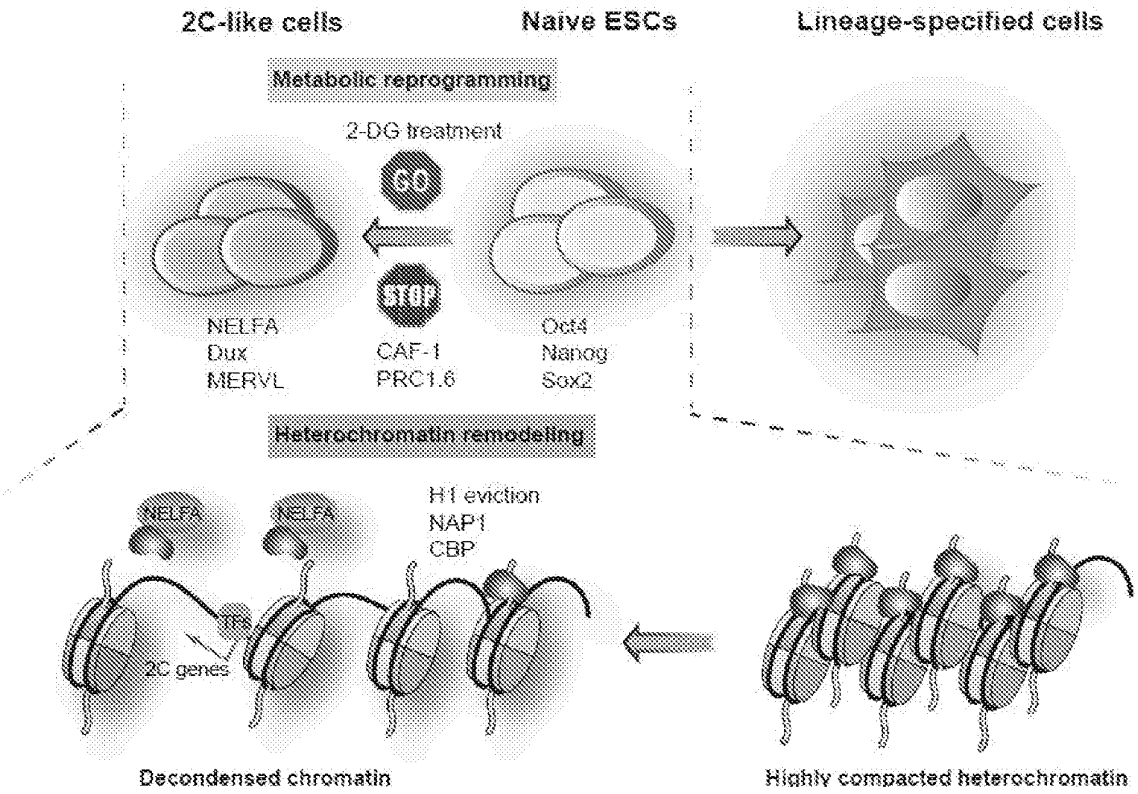

siHif1a
P = 5.2 x 10-3
0.41%
siNr5a2
P = 2.9 x 10-4
0.46%
FSC-A
FITC-A
250K
200K
150K
100K
50K
0
10^3
10^4
10^5

Figure 7b


2C-like cells
Naive ESCs
Lineage-specified cells
Metabolic reprogramming
2-DG treatment
GO
STOP
NELFA
Dux
MERVL
CAF-1
PRC1.6
Oct4
Nanog
Sox2
Heterochromatin remodeling
H1 eviction
NAP1
CBP
NELFA
NELFA
TFs
2C genes
Decondensed chromatin
Highly compacted heterochromatin

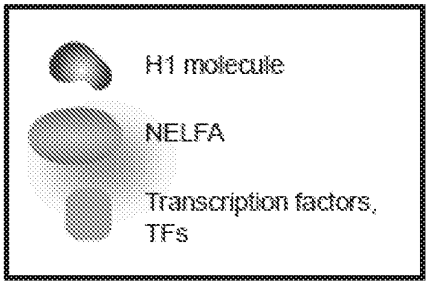

H1 molecule
NELFA
Transcription factors,
TFs

Figure 7c

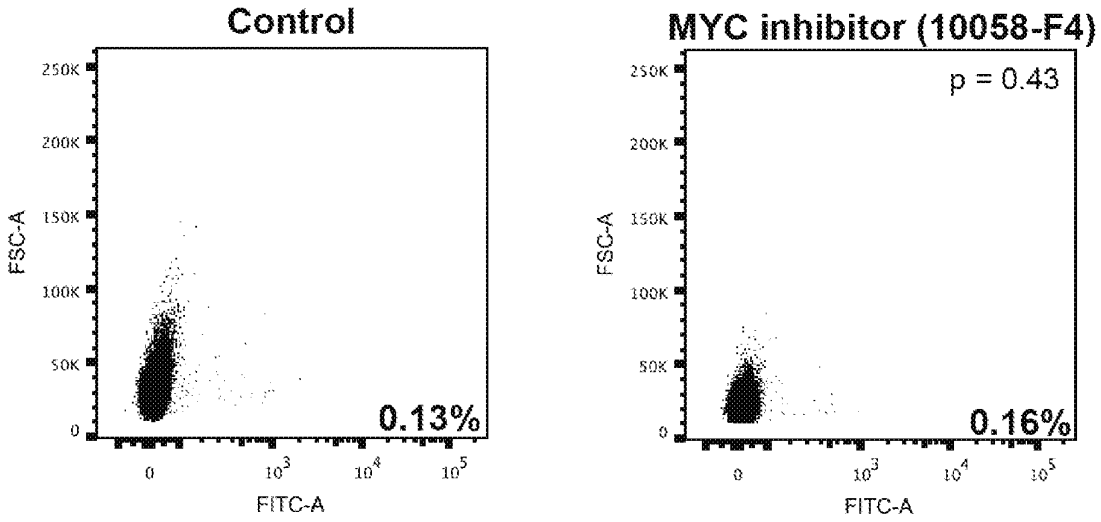


Figure 7d

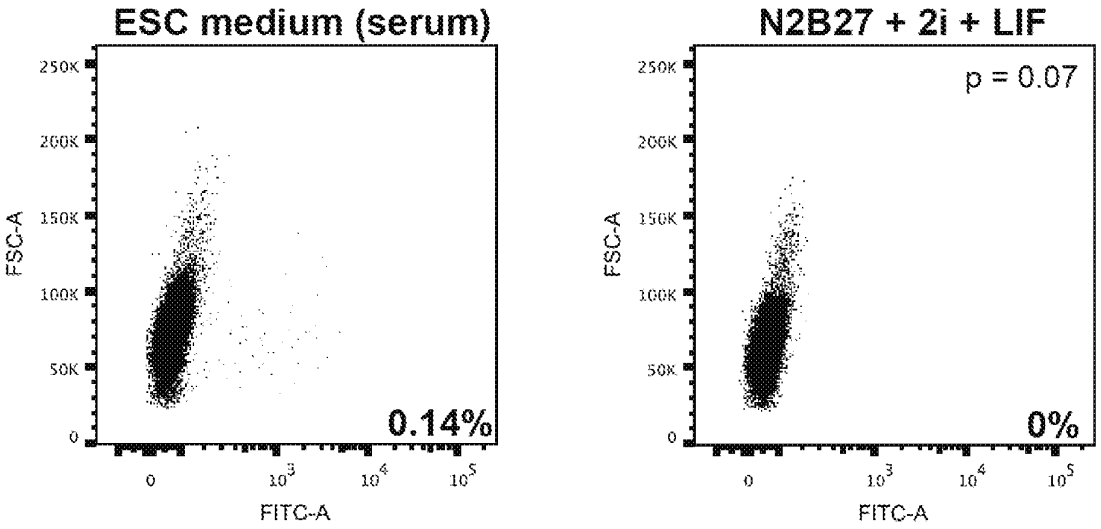


Figure 7e (cont.)

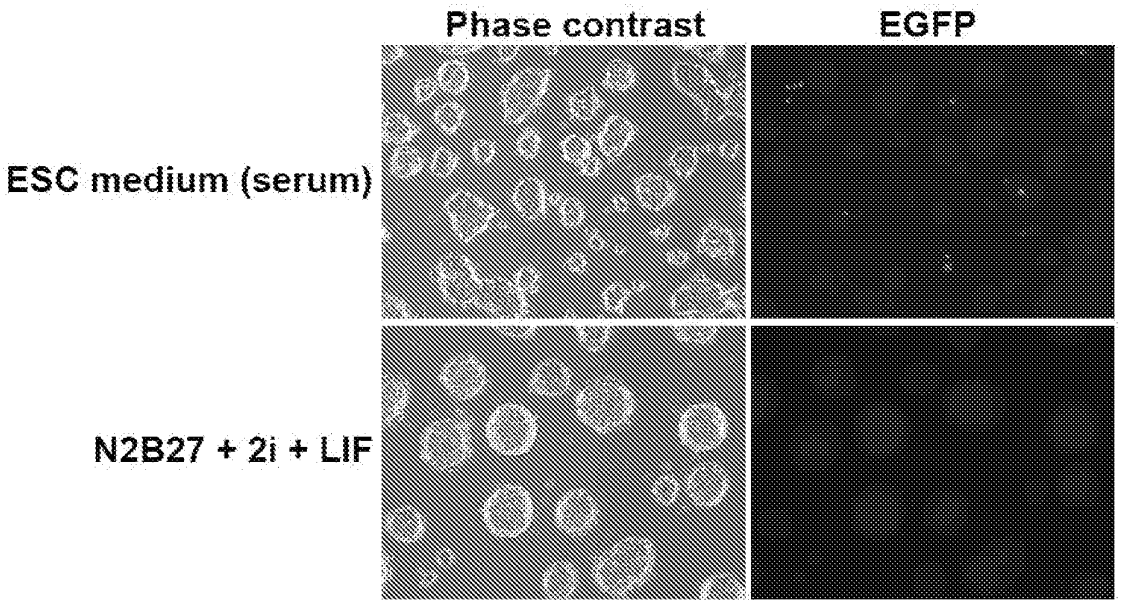


Figure 7e

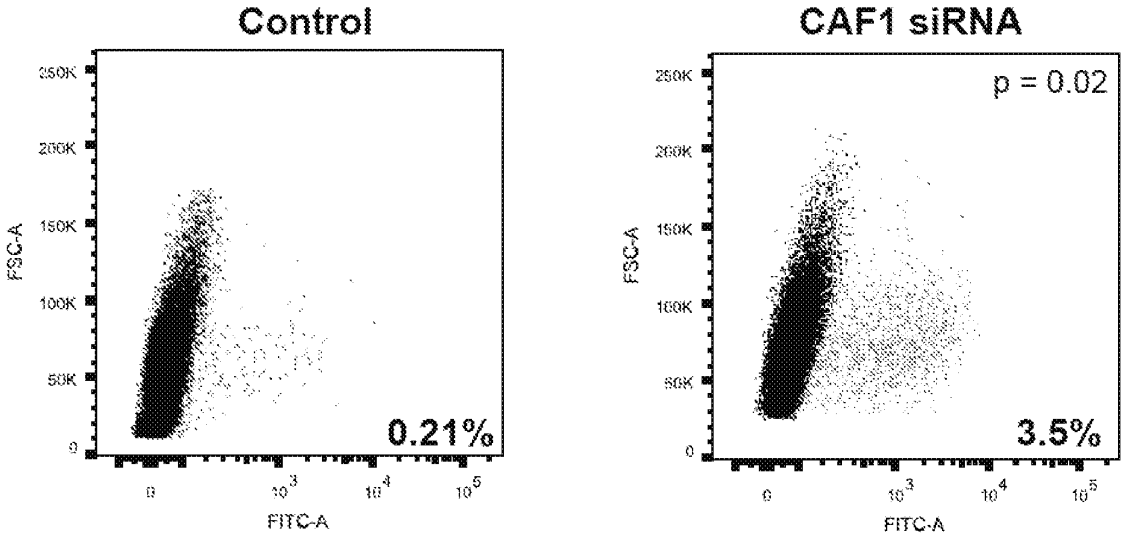


Figure 7f