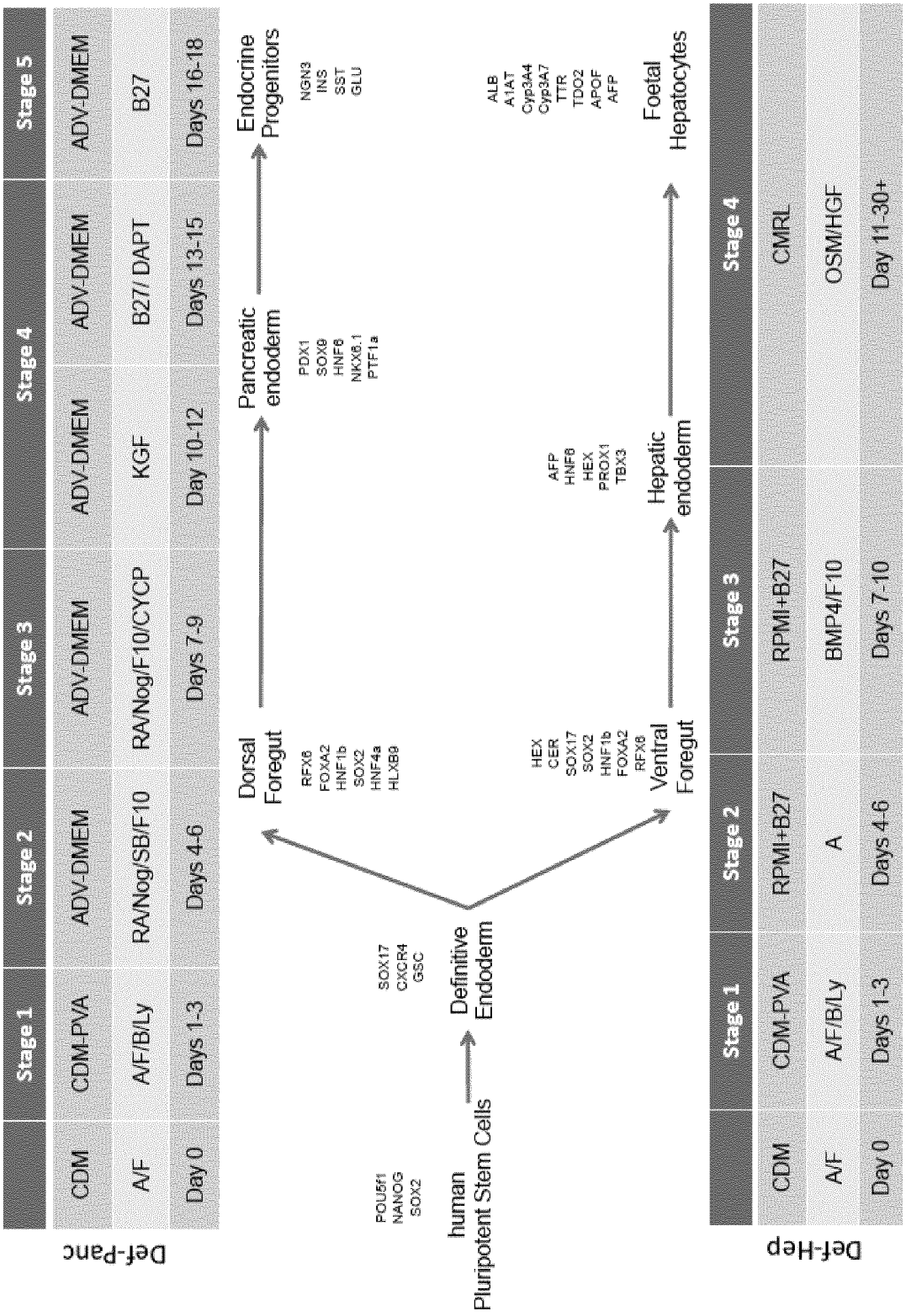

Def-Panc
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
CDM
CDM-PVA
ADV-DMEM
ADV-DMEM
ADV-DMEM
ADV-DMEM
A/F
A/F/B/Ly
RA/Nog/SB/F10
RA/Nog/F10/CYCP
KGF
B27/ DAPT
B27
Day 0
Days 1-3
Days 4-6
Days 7-9
Day 10-12
Days 13-15
Days 16-18
POU5f1
NANOG
SOX2
human
Pluripotent Stem Cells
SOX17
CXCR4
GSC
Definitive
Endoderm
Dorsal
Foregut
RFX6
FOXA2
HNF1b
SOX2
HNF4a
HLXB9
Pancreatic
endoderm
PDX1
SOX9
HNF6
NKX6.1
PTF1a
Endocrine
Progenitors
NGN3
INS
SST
GLU
HEX
CER
SOX17
SOX2
HNF1b
FOXA2
RFX6
Ventral
Foregut
AFP
HNF6
HEX
PROX1
TBX3
Hepatic
endoderm
ALB
A1AT
Cyp3A4
Cyp3A7
TTR
TDO2
APOF
AFP
Foetal
Hepatocytes
Def-Hep
Stage 1
Stage 2
Stage 3
Stage 4
CDM
CDM-PVA
RPMI+B27
RPMI+B27
CMRL
A/F
A/F/B/Ly
A
BMP4/F10
OSM/HGF
Day 0
Days 1-3
Days 4-6
Days 7-10
Day 11-30+

Figure 1

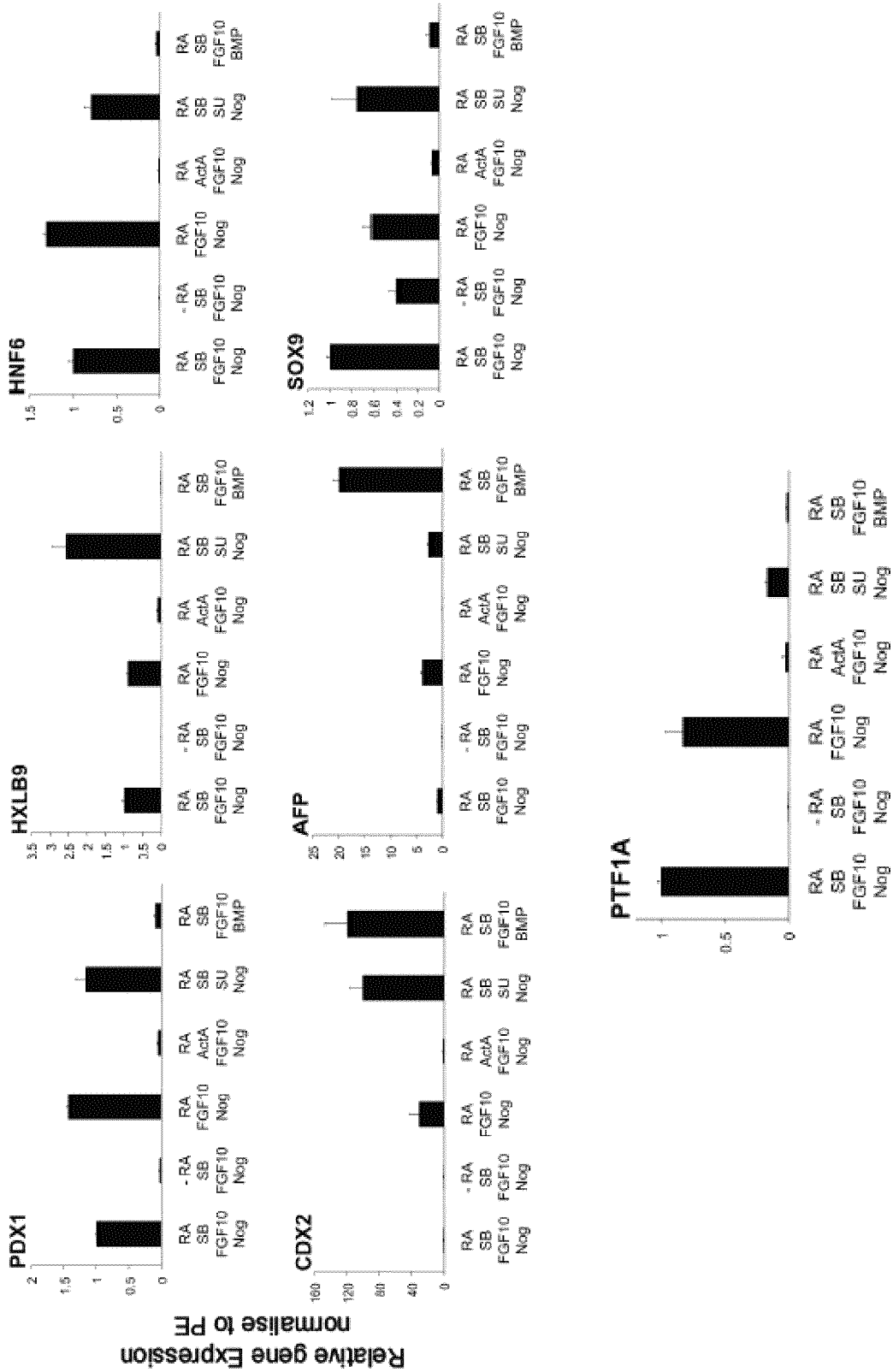

Relative gene Expression
normalise to PE
PDX1
HXLB9
CDX2
AFP
HNF6
SOX9
PTF1A
RA SB FGF10 Nog
- RA SB FGF10 Nog
RA FGF10 Nog
RA ActA FGF10 Nog
RA SB SU Nog
RA SB FGF10 BMP

Figure 2

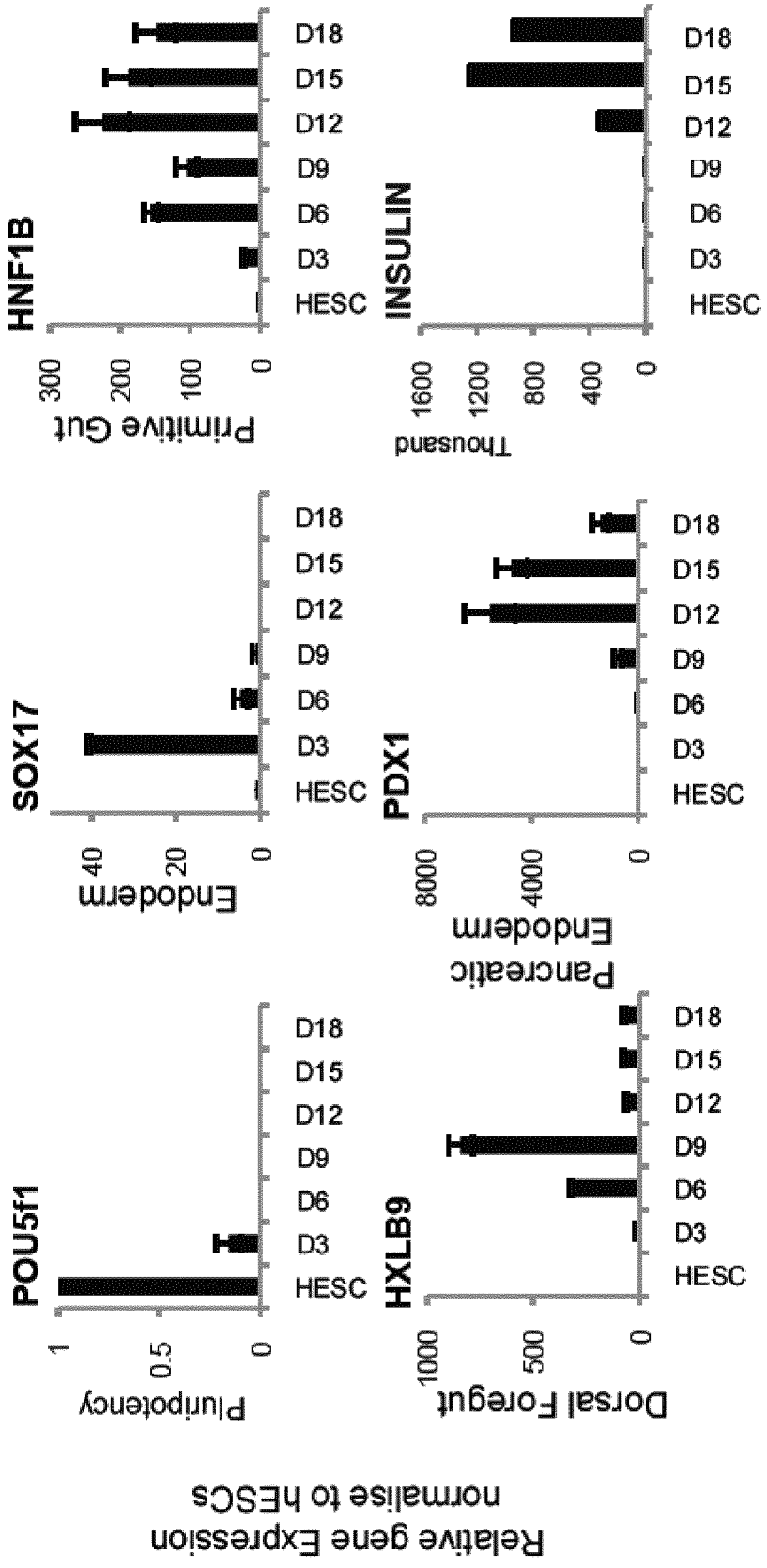
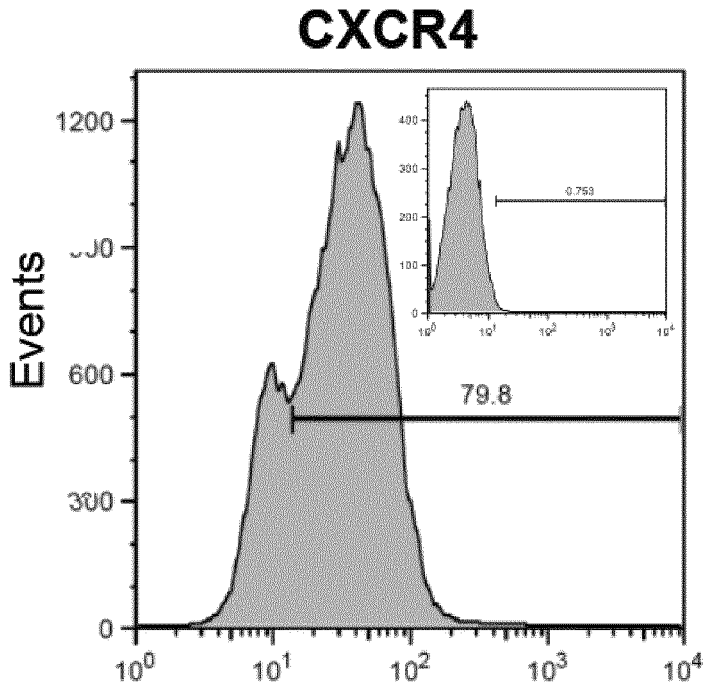

Relative gene Expression
normalise to hESCs
Pluripotency
POU5f1
1
0.5
0
HESC
D3
D6
D9
D12
D15
D18
Endoderm
SOX17
40
20
0
Pancreatic Endoderm
PDX1
8000
4000
0
Dorsal Foregut
HXLB9
1000
500
0
Thousand
INSULIN
1600
1200
800
400
0
Primitive Gut
HNF1B
300
200
100
0

Figure 3


CXCR4
Events
1200
600
300
0
79.8
0.759
10^0
10^1
10^2
10^3
10^4

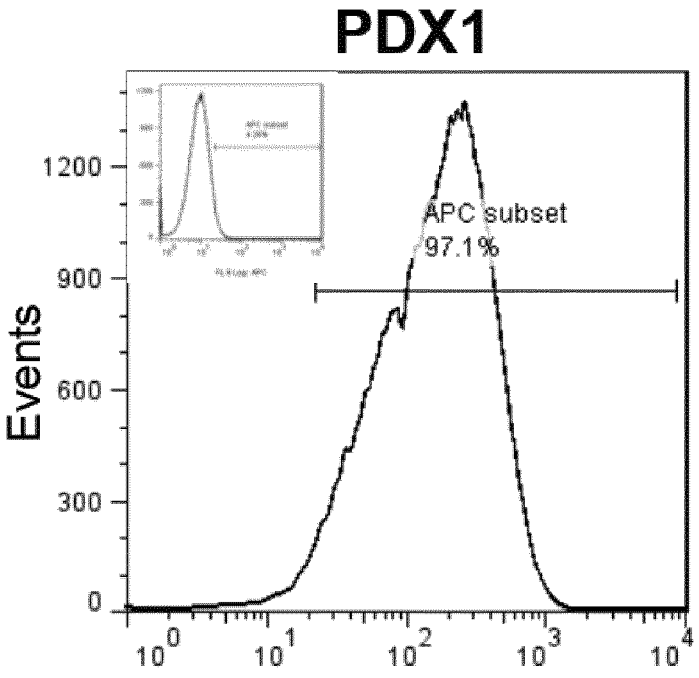

PDX1
Events
1200
900
600
300
0
APC subset
97.1%
10^0
10^1
10^2
10^3
10^4

Figure 4

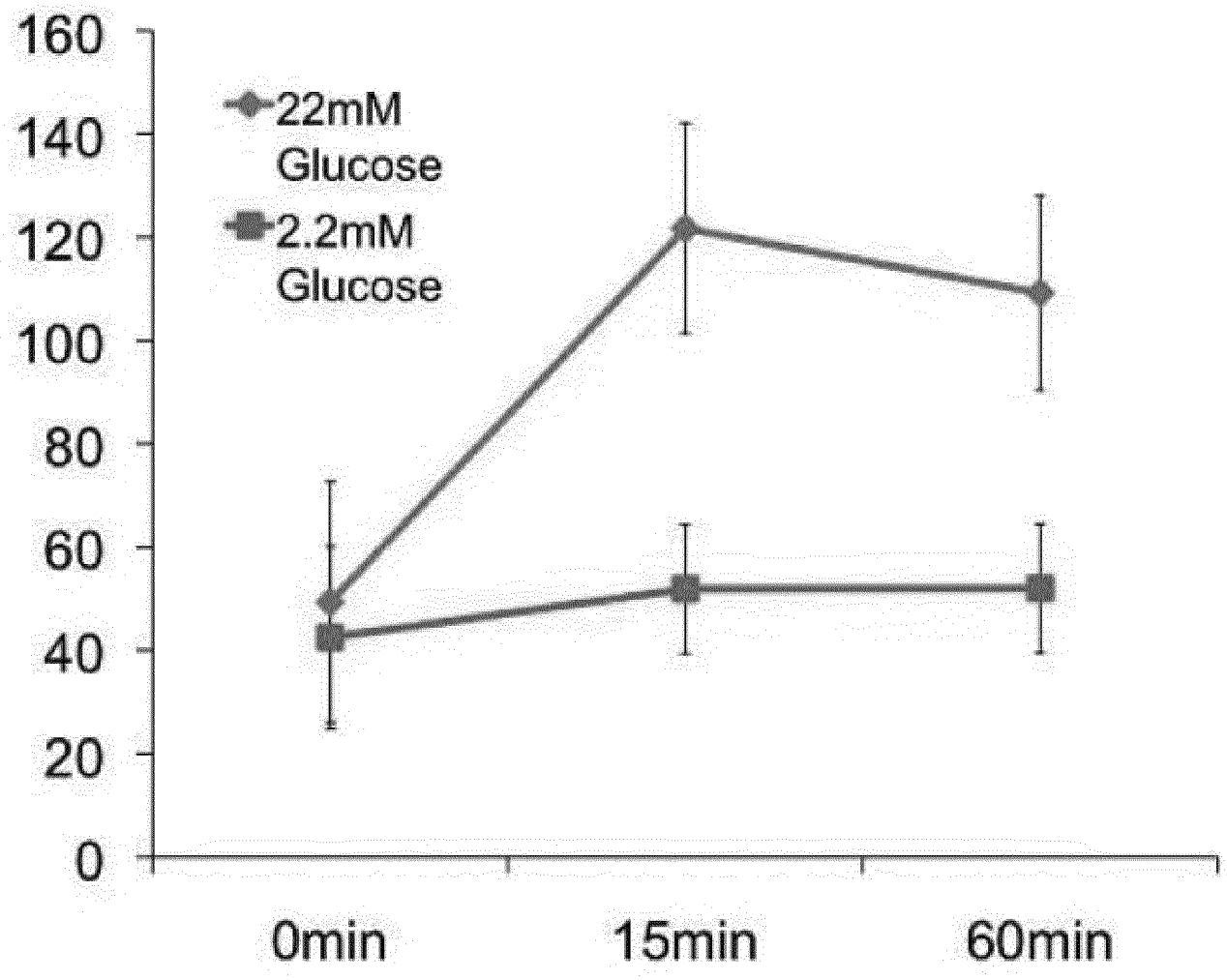

160
140
120
100
80
60
40
20
0
22mM Glucose
2.2mM Glucose
0min
15min
60min

Figure 5

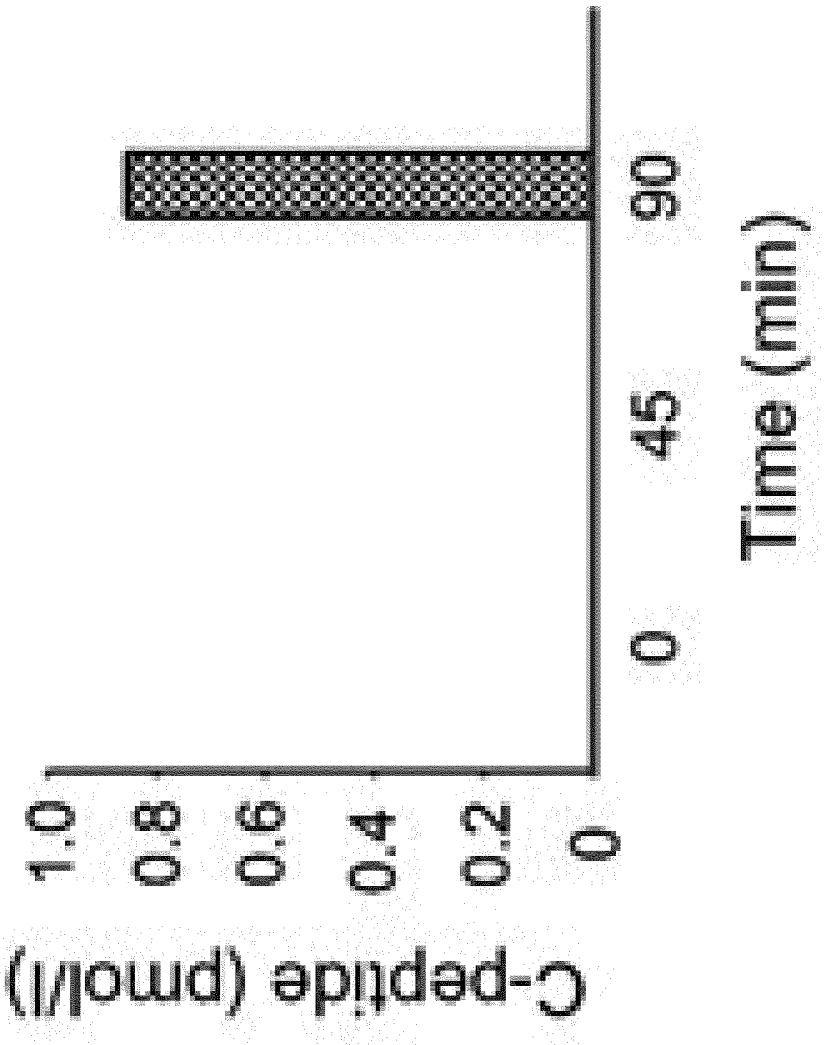


Figure 6

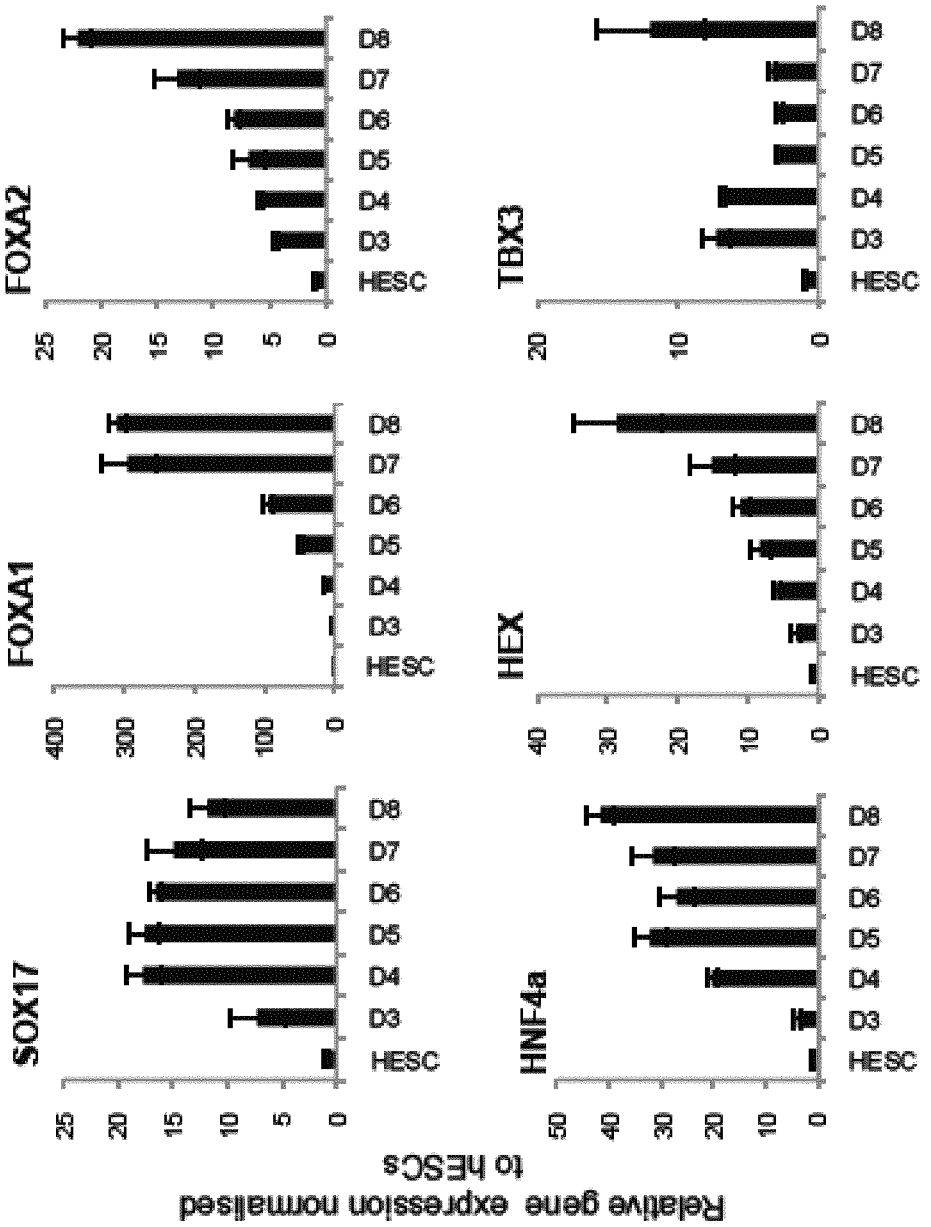

FOXA2
TBX3
FOXA1
HEX
SOX17
HNF4a
D8
D7
D6
D5
D4
D3
HESC
Relative gene expression normalised to hESCs

Figure 7

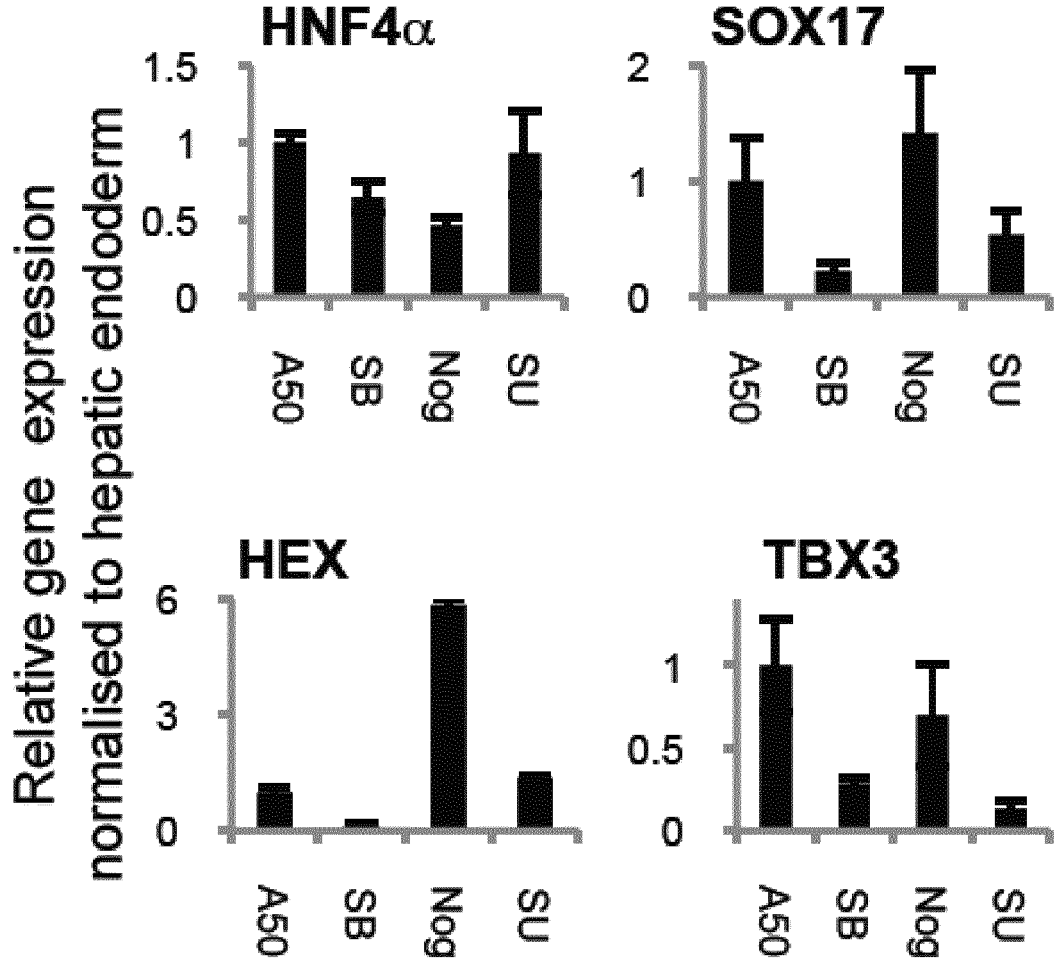

HNF4α
SOX17
HEX
TBX3
Relative gene expression normalised to hepatic endoderm
1.5
1
0.5
0
2
1
0
6
3
0
1
0.5
0
A50
SB
Nog
SU

Figure 8

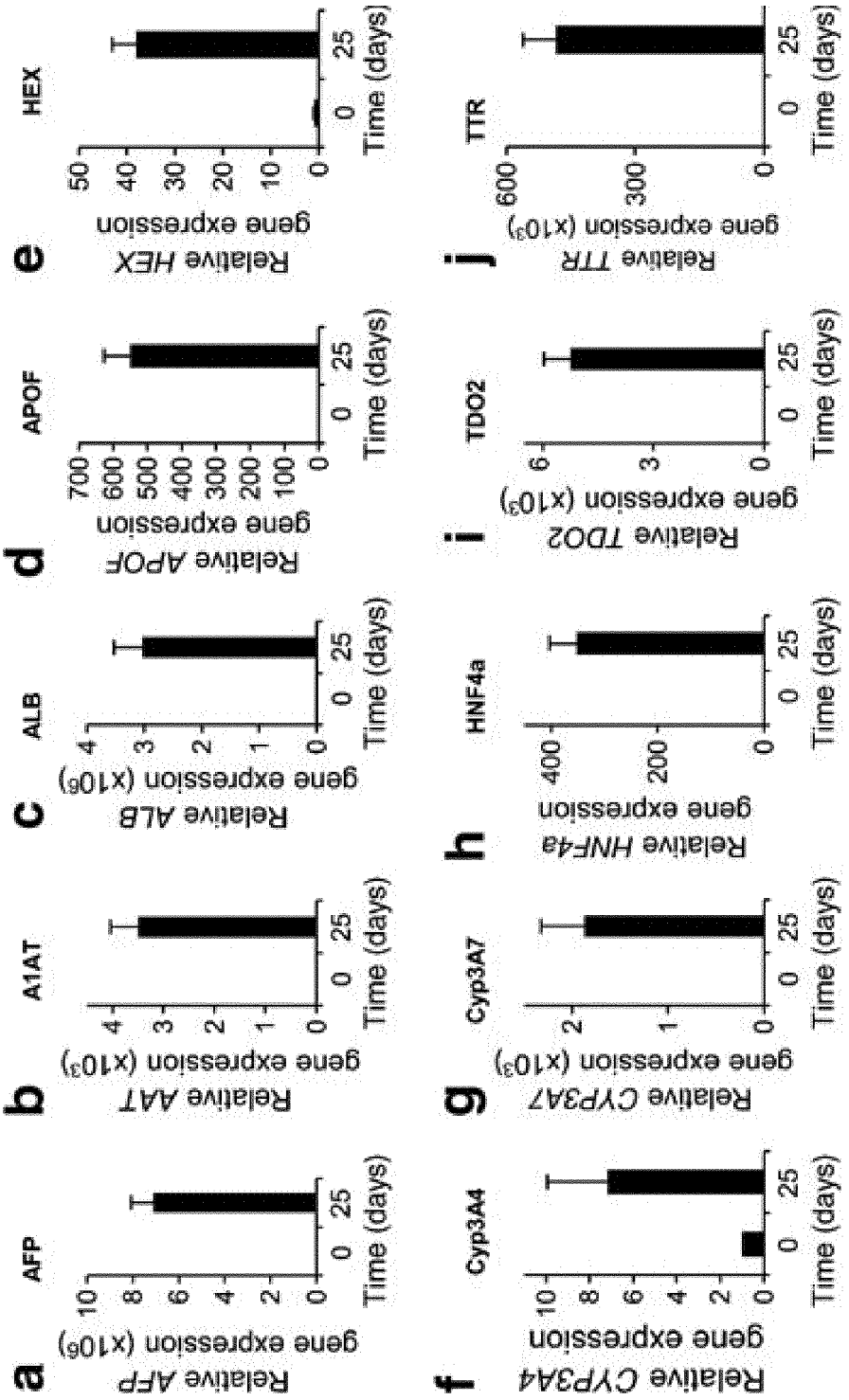

a
Relative AFP gene expression (x10⁶)
AFP
Time (days)
b
Relative AAT gene expression (x10³)
A1AT
Time (days)
c
Relative ALB gene expression (x10⁶)
ALB
Time (days)
d
Relative APOF gene expression
APOF
Time (days)
e
Relative HEX gene expression
HEX
Time (days)
f
Relative CYP3A4 gene expression
Cyp3A4
Time (days)
g
Relative CYP3A7 gene expression (x10³)
Cyp3A7
Time (days)
h
Relative HNF4a gene expression
HNF4a
Time (days)
i
Relative TDO2 gene expression (x10³)
TDO2
Time (days)
j
Relative TTR gene expression (x10³)
TTR
Time (days)

Figure 9

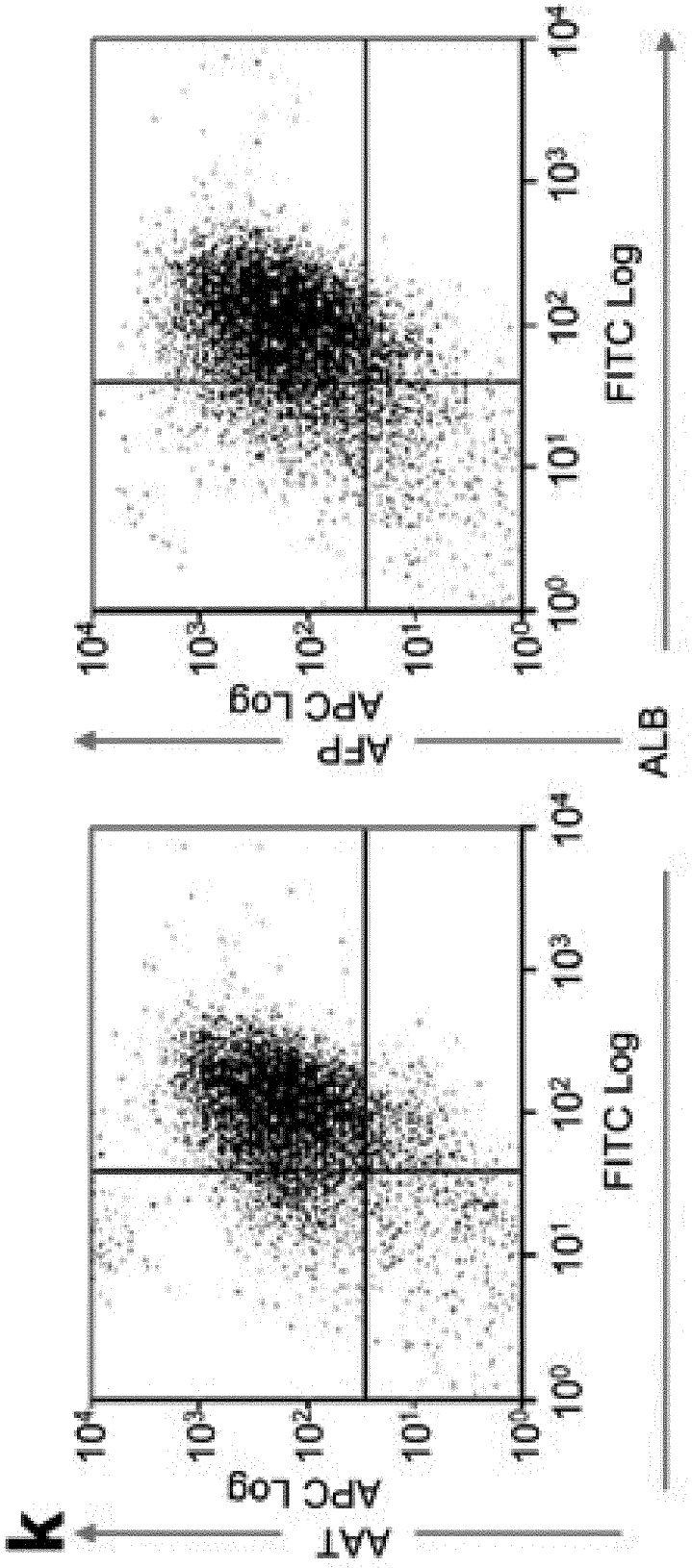


Figure 10

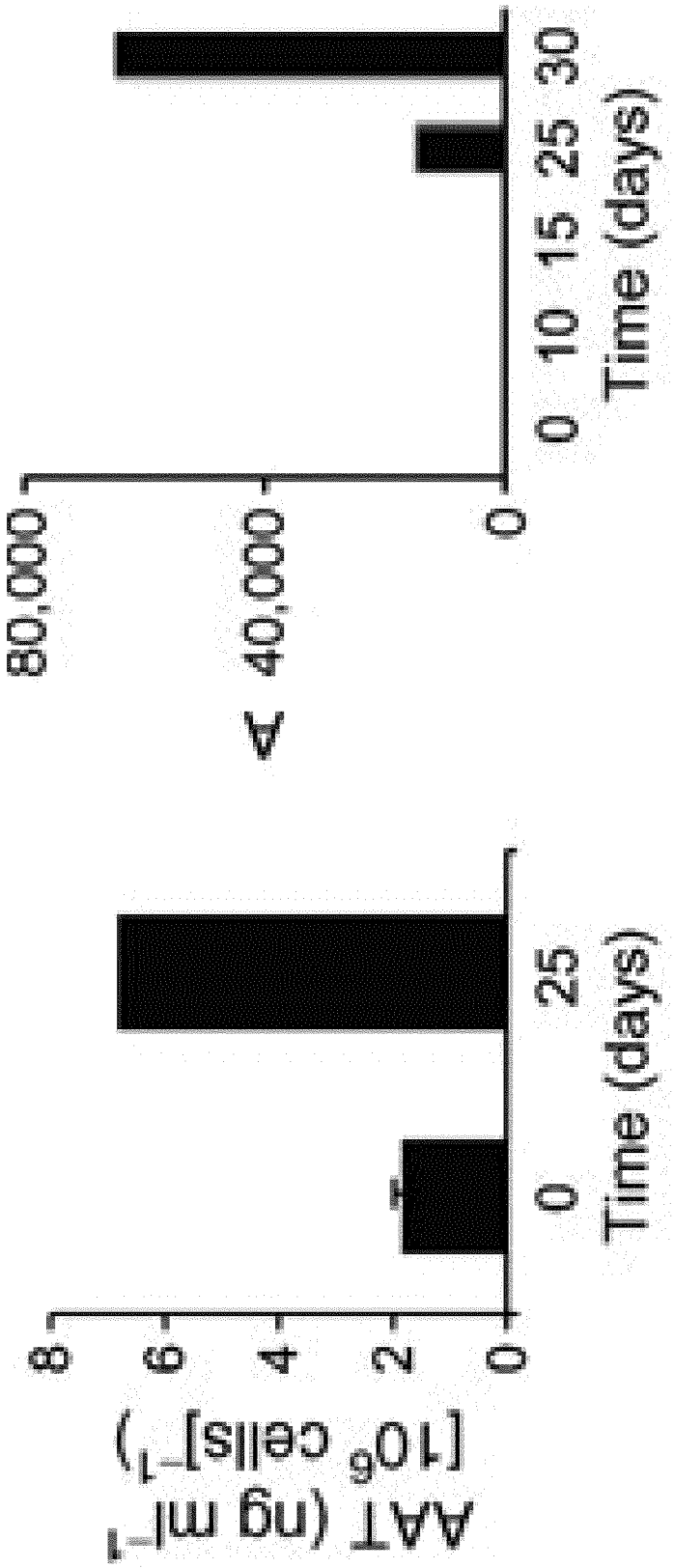

AAT (ng ml^{-1} [10^6 cells]$^{-1}$)
0
2
4
6
8
0
25
Time (days)
A
0
40,000
80,000
0
10
15
25
30
Time (days)

Figure 11

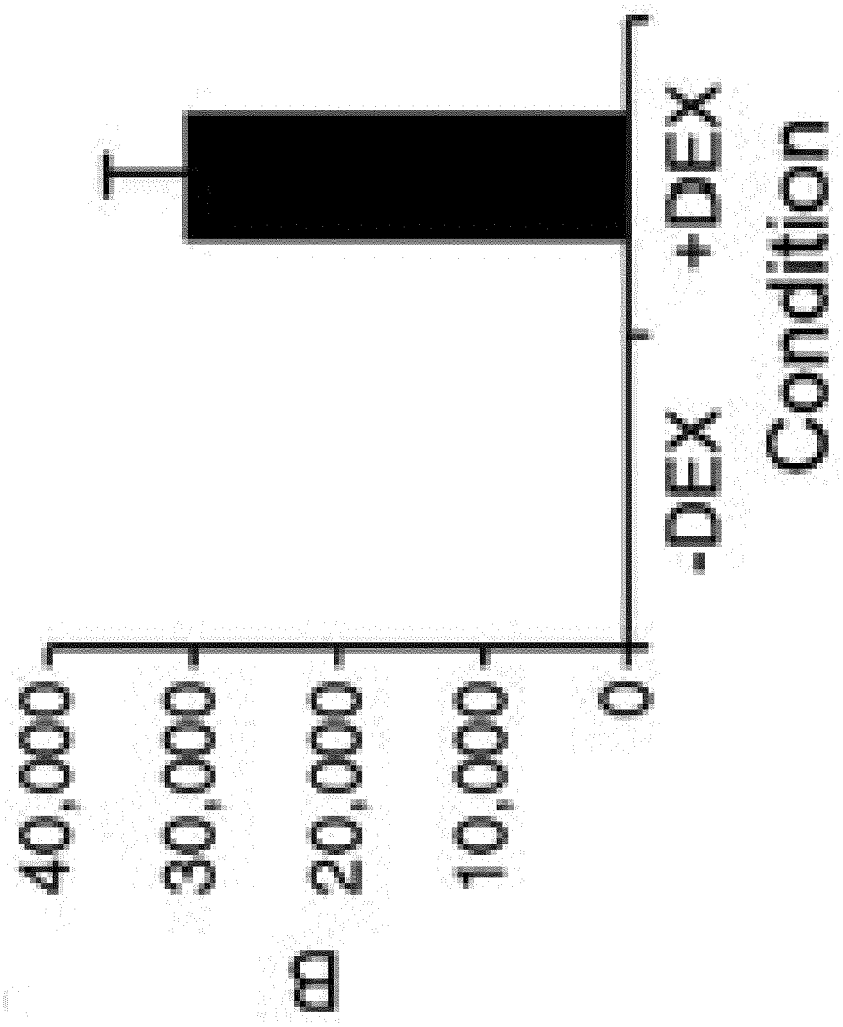


Figure 12

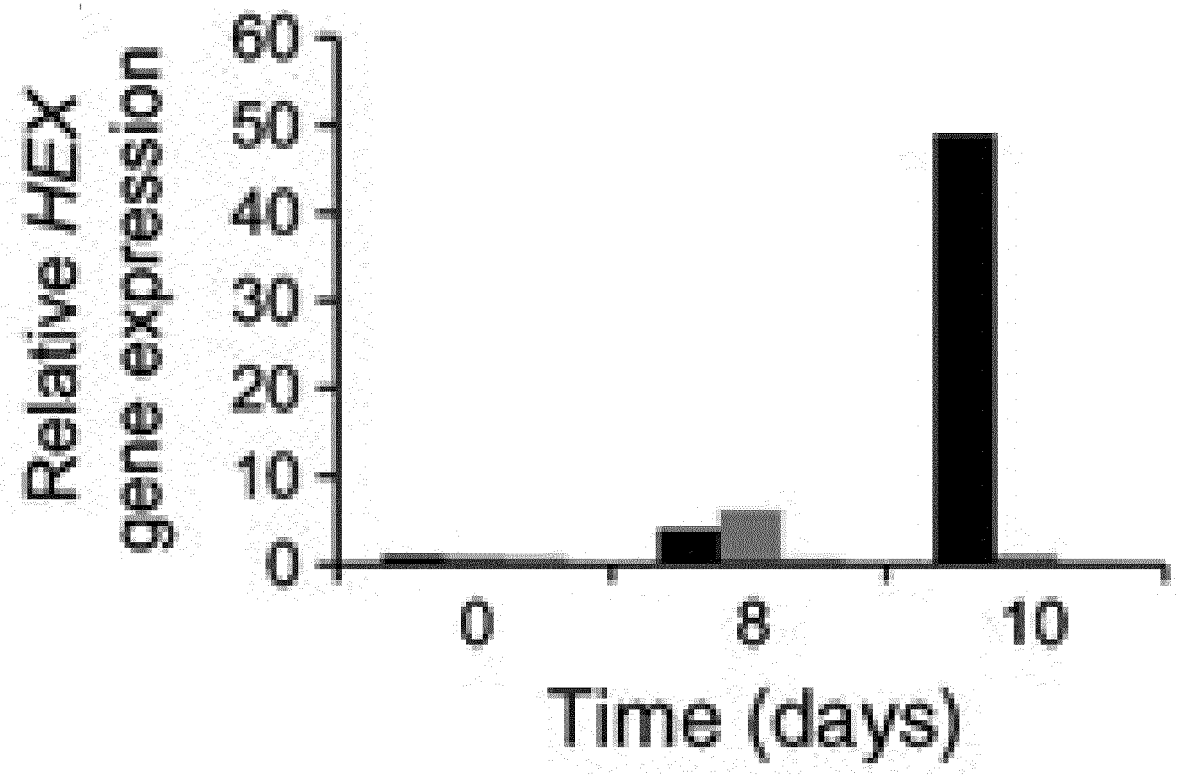

Relative HEX gene expression
60
50
40
30
20
10
0
0
8
10
Time (days)

Figure 13

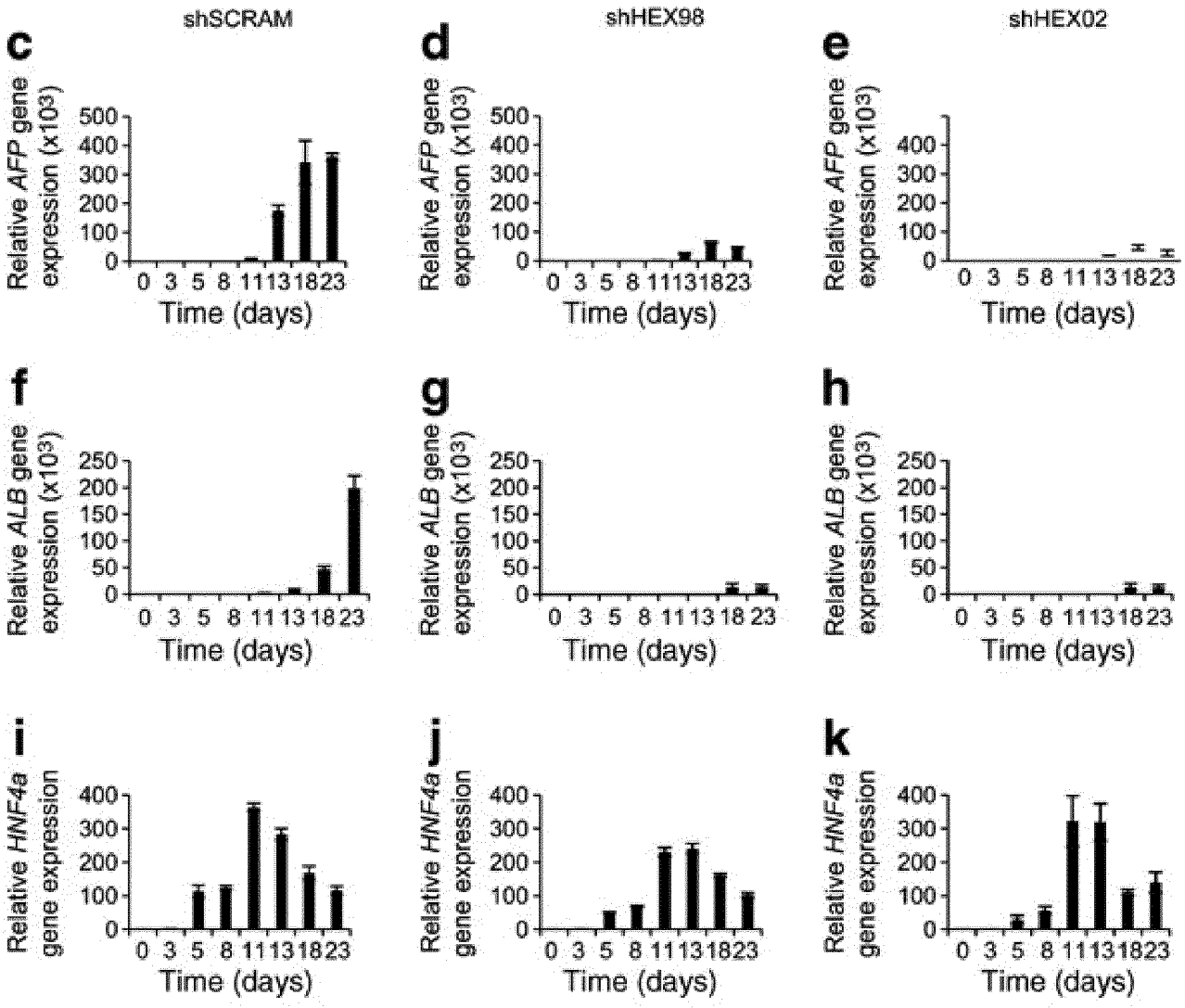

shSCRAM
shHEX98
shHEX02
c
d
e
f
g
h
i
j
k
Relative AFP gene expression (x10³)
Relative ALB gene expression (x10³)
Relative HNF4a gene expression
Time (days)
0 3 5 8 11 13 18 23

Figure 14

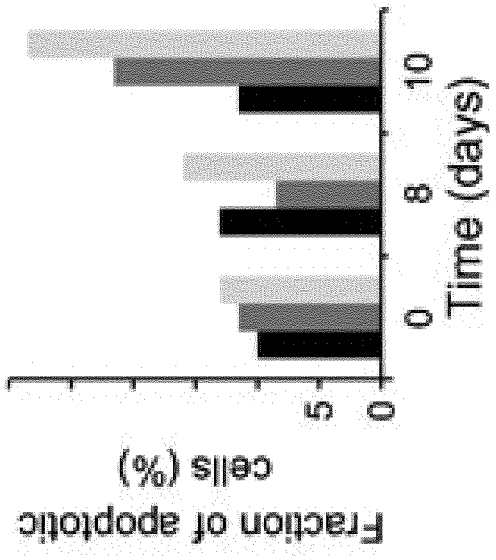


Figure 15

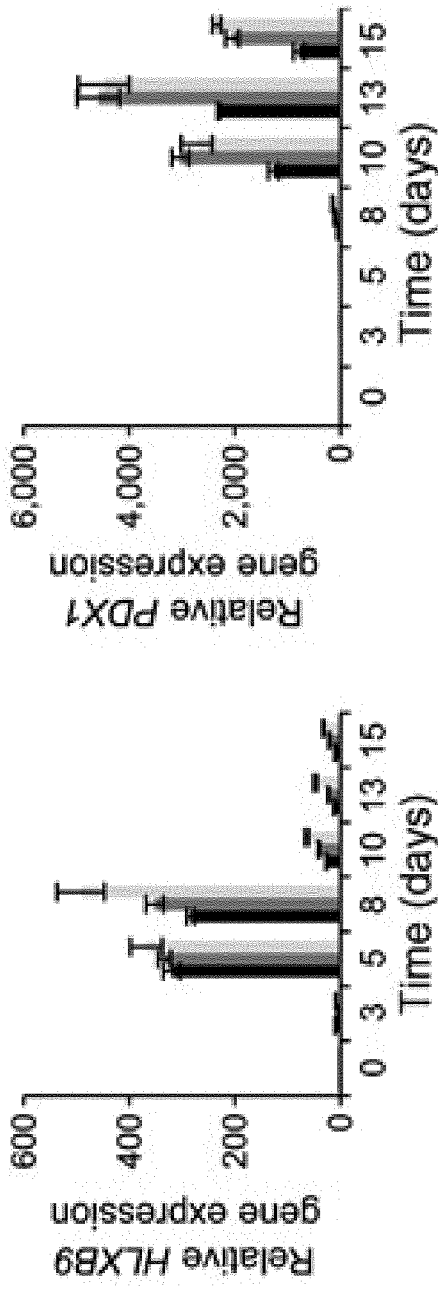


Figure 16

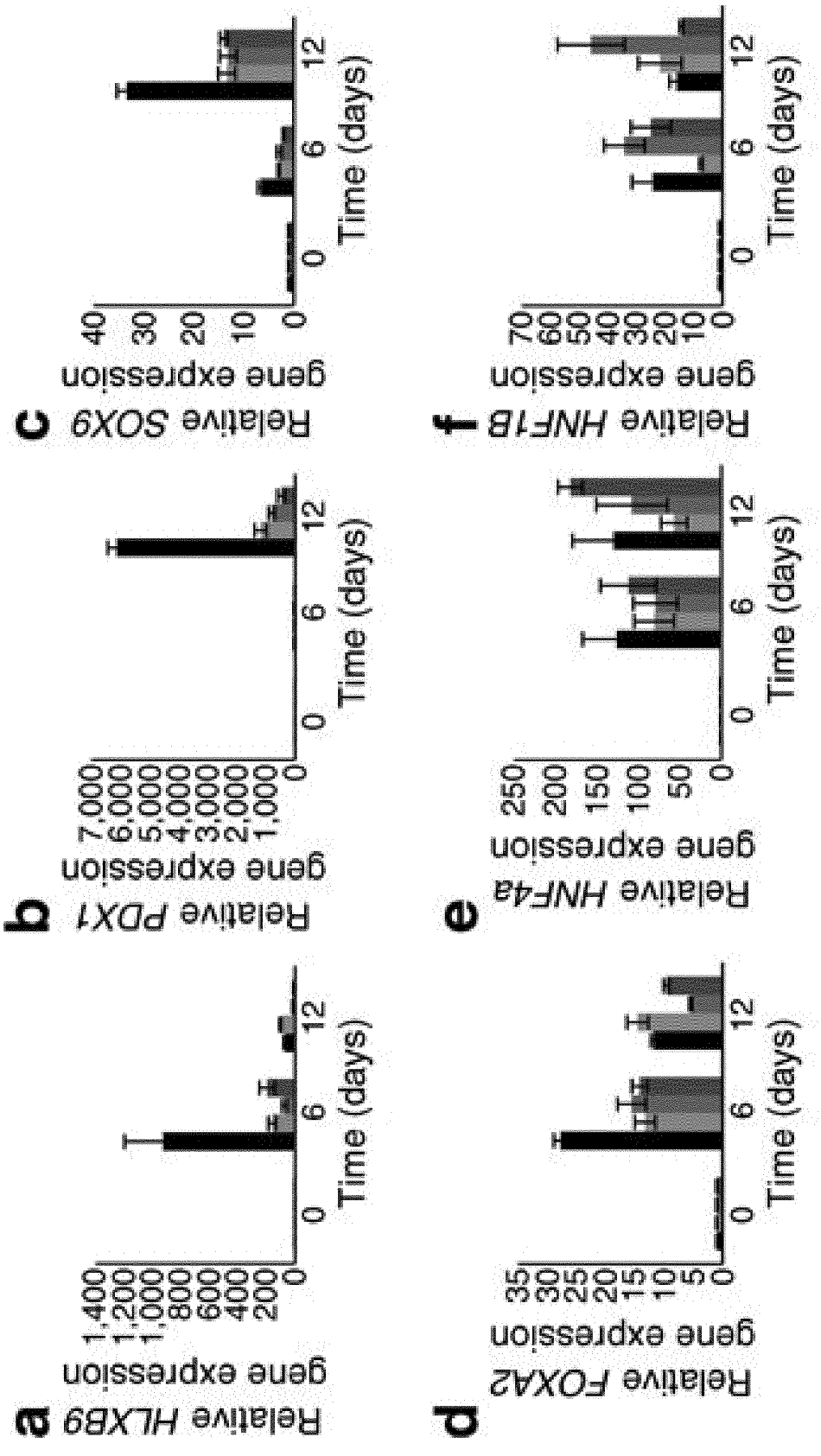

a
Relative HLXB9 gene expression
1,400
1,200
1,000
800
600
400
200
0
0
6
12
Time (days)
b
Relative PDX1 gene expression
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
0
6
12
Time (days)
c
Relative SOX9 gene expression
40
30
20
10
0
0
6
12
Time (days)
d
Relative FOXA2 gene expression
35
30
25
20
15
10
5
0
0
6
12
Time (days)
e
Relative HNF4a gene expression
250
200
150
100
50
0
0
6
12
Time (days)
f
Relative HNF1B gene expression
70
60
50
40
30
20
10
0
0
6
12
Time (days)

Figure 17

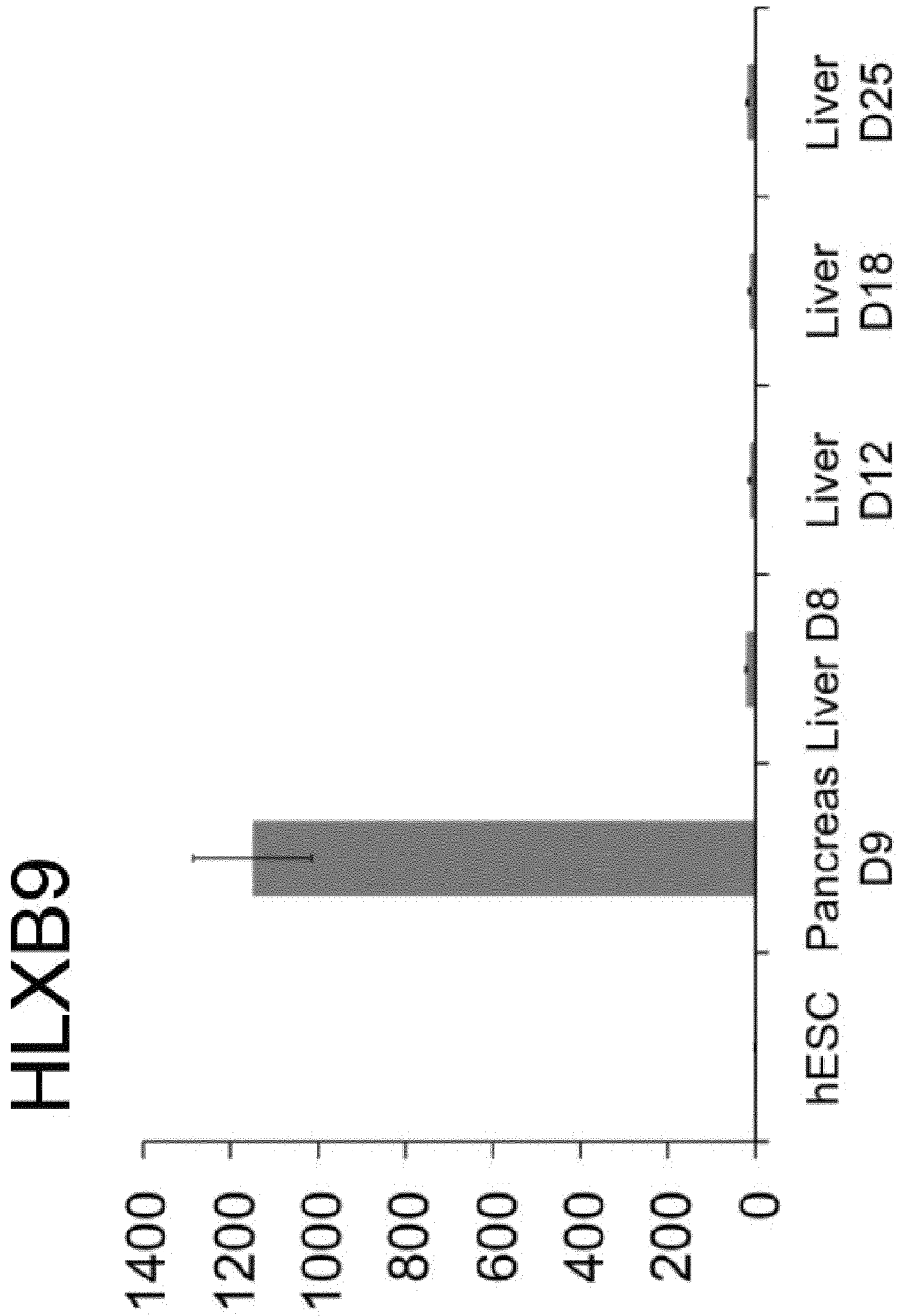

HLXB9
1400
1200
1000
800
600
400
200
0
hESC
Pancreas Liver D8 D9
Liver D12
Liver D18
Liver D25

Figure 18