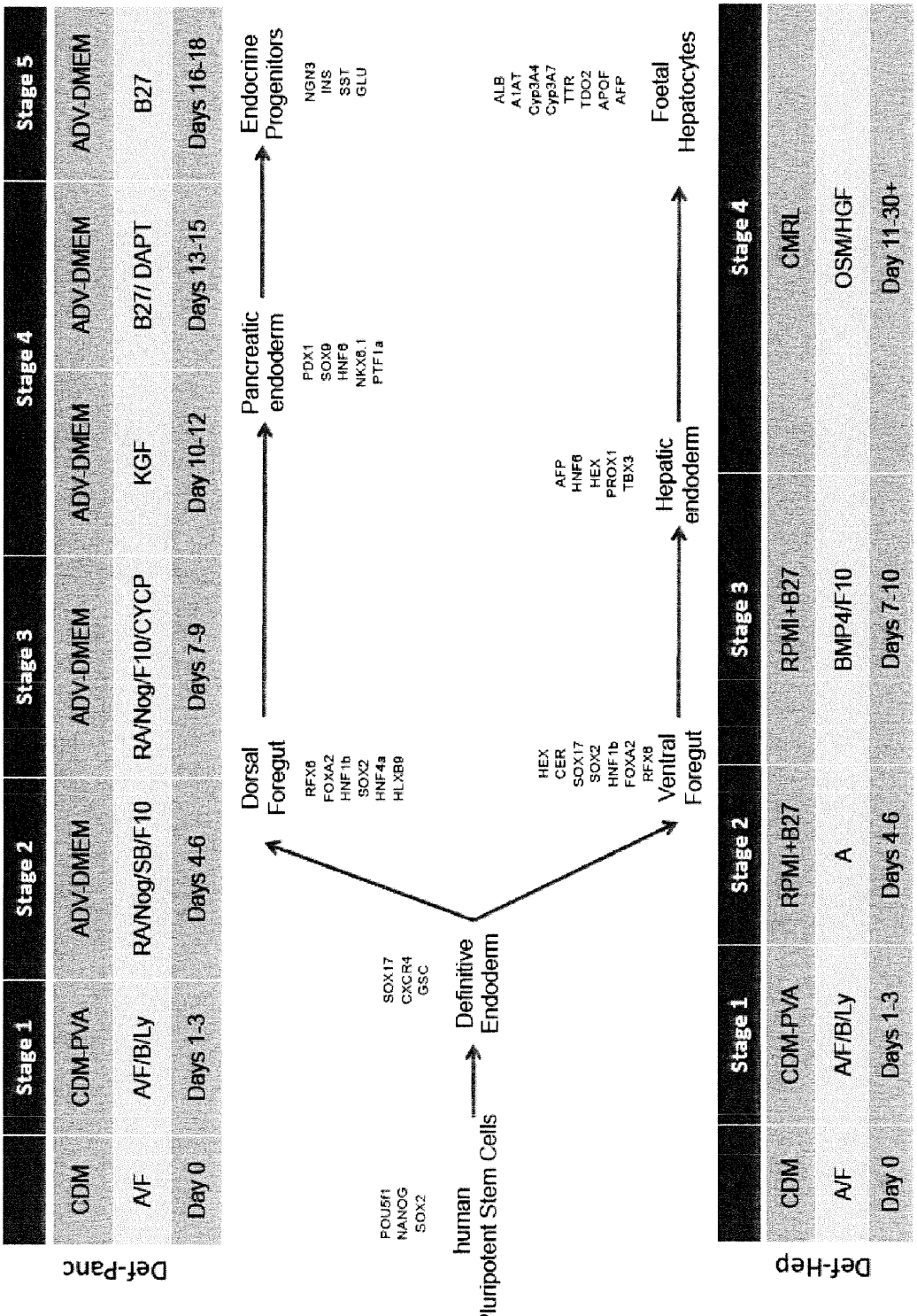

Def-Panc
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
CDM
CDM-PVA
ADV-DMEM
ADV-DMEM
ADV-DMEM
ADV-DMEM
A/F
A/F/B/Ly
RA/Nog/SB/F10
RA/Nog/F10/CYCP
KGF
B27/ DAPT
B27
Day 0
Days 1-3
Days 4-6
Days 7-9
Day 10-12
Days 13-15
Days 16-18
POU5f1
NANOG
SOX2
human
Pluripotent Stem Cells
SOX17
CXCR4
GSC
Definitive
Endoderm
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

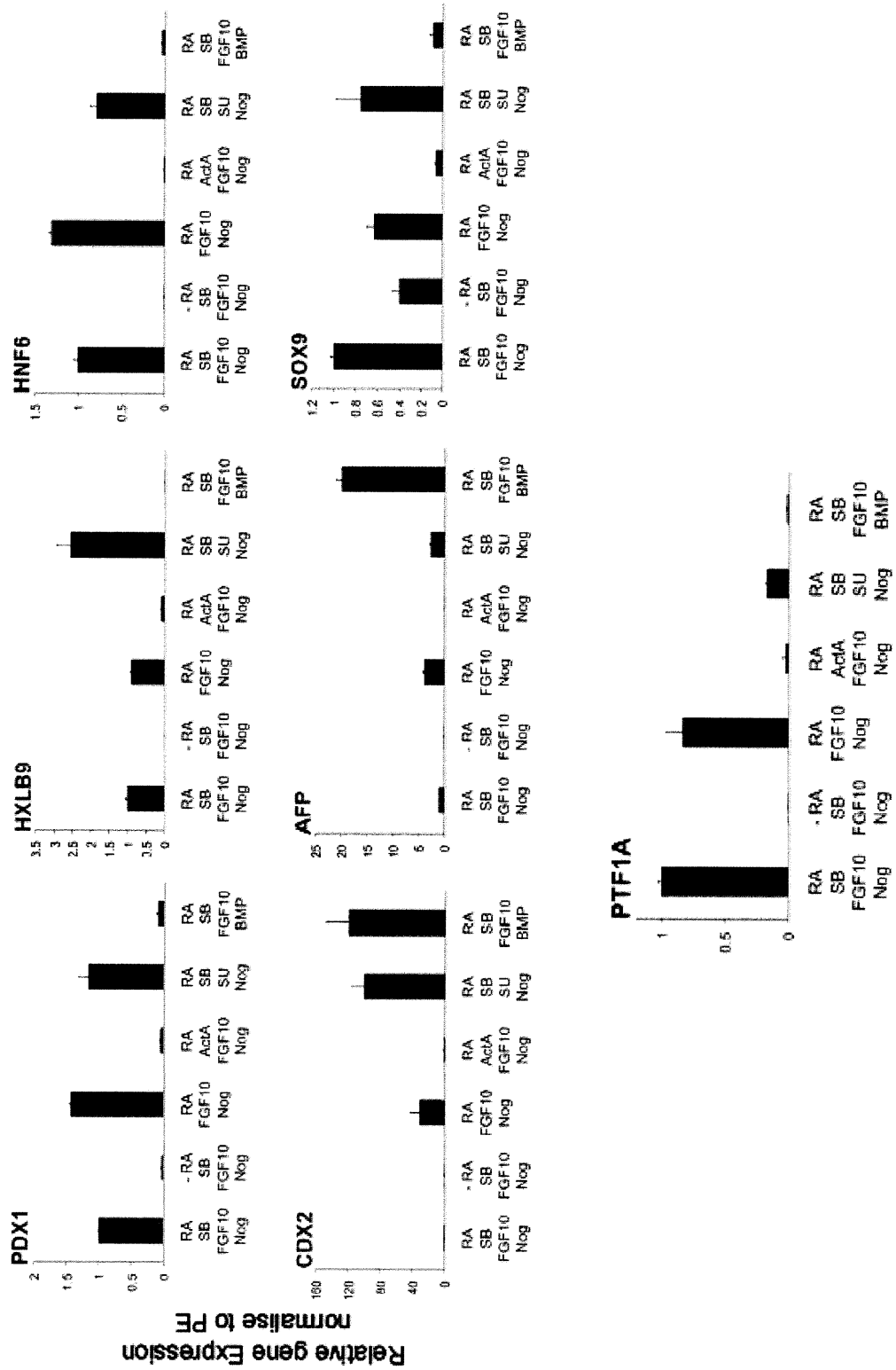

PDX1
HXLB9
HNF6
CDX2
AFP
SOX9
PTF1A
Relative gene Expression
normalise to PE
RA SB FGF10 Nog
- RA SB FGF10 FGF10 Nog
RA FGF10 Nog
RA ActA FGF10 Nog
RA SB SU Nog
RA SB FGF10 BMP

Figure 2

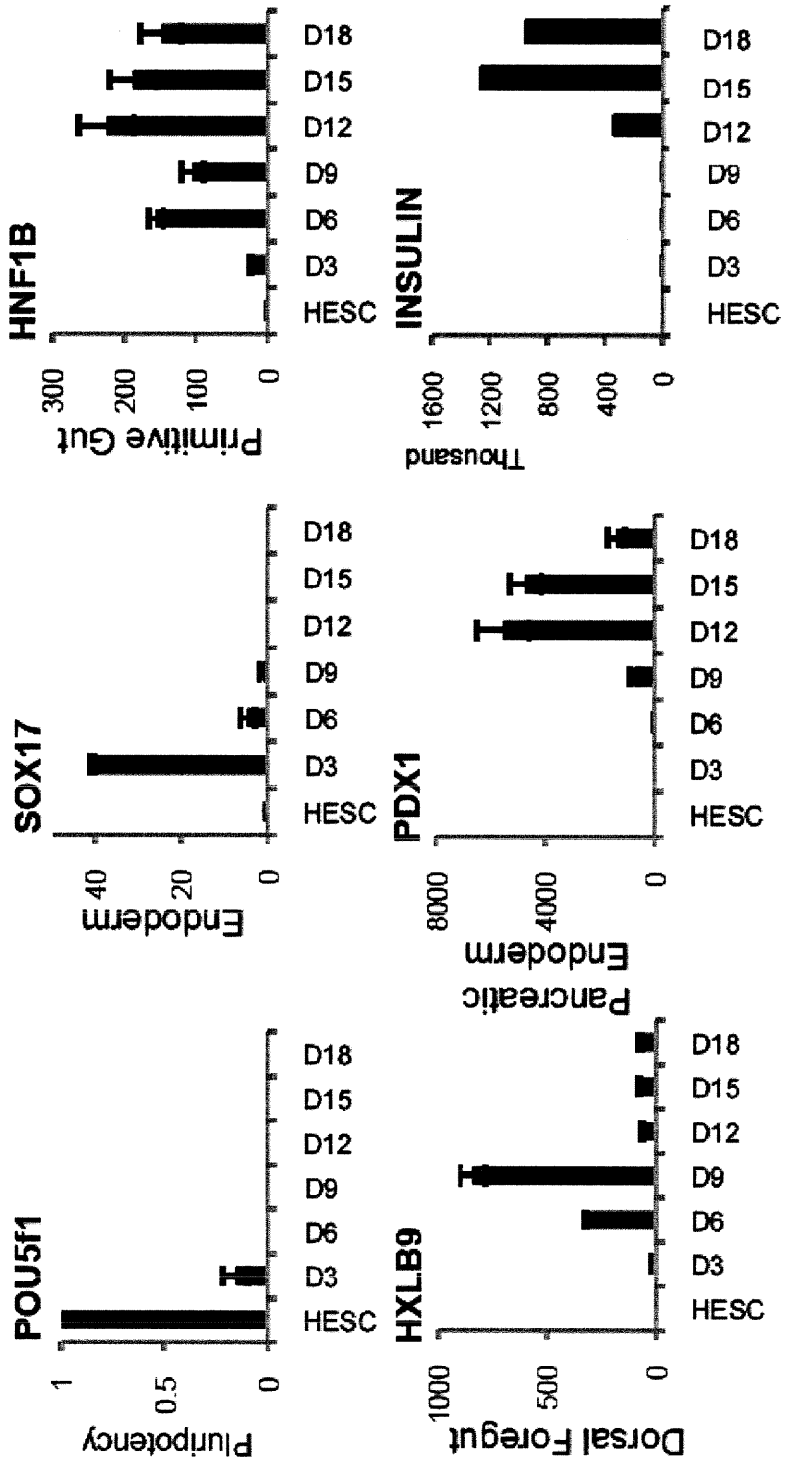

Relative gene Expression
normalise to hESCs
Pluripotency
POU5f1
Endoderm
SOX17
Pancreatic Endoderm
PDX1
Dorsal Foregut
HXLB9
Primitive Gut
HNF1B
Thousand
INSULIN
HESC
D3
D6
D9
D12
D15
D18

Figure 3

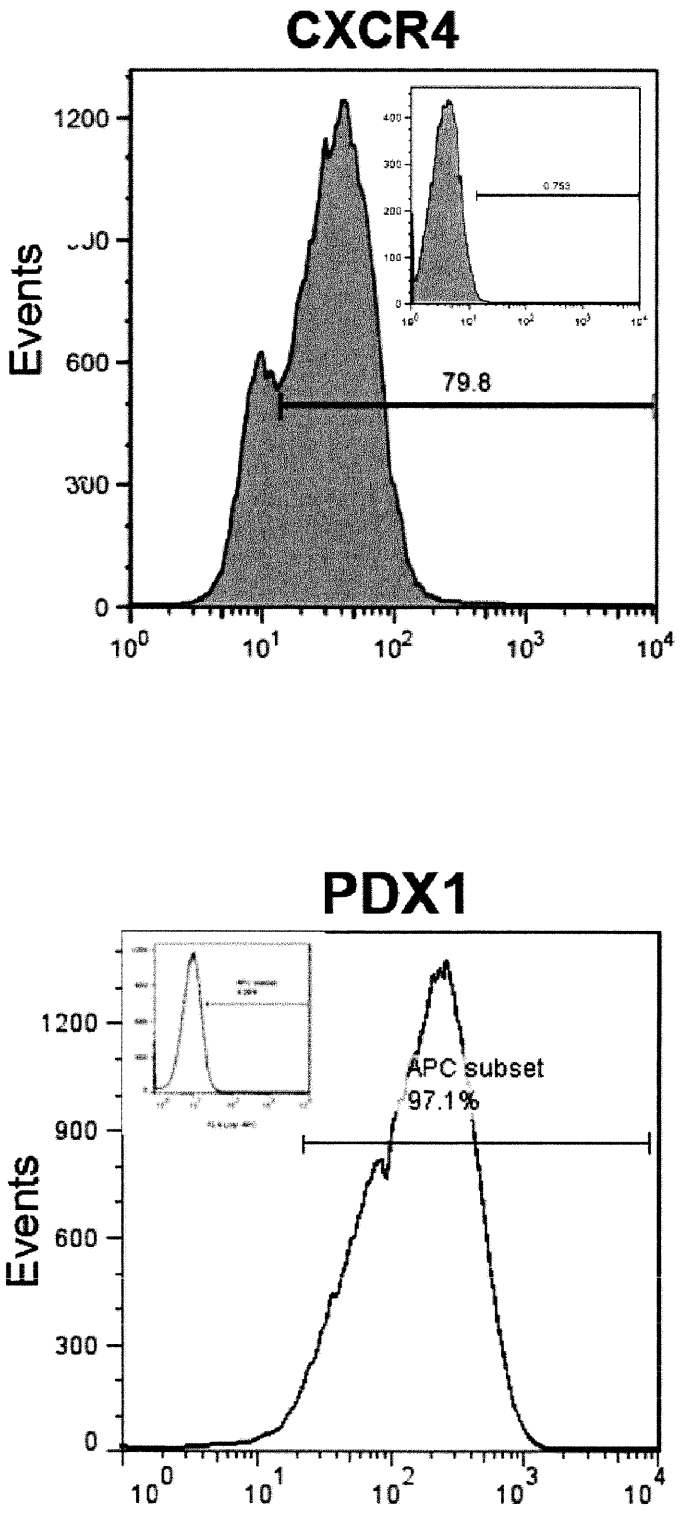

CXCR4
Events
1200
600
300
0
79.8
10^0
10^1
10^2
10^3
10^4
PDX1
Events
1200
900
600
300
0
APC subset
97.1%
10^0
10^1
10^2
10^3
10^4

Figure 4

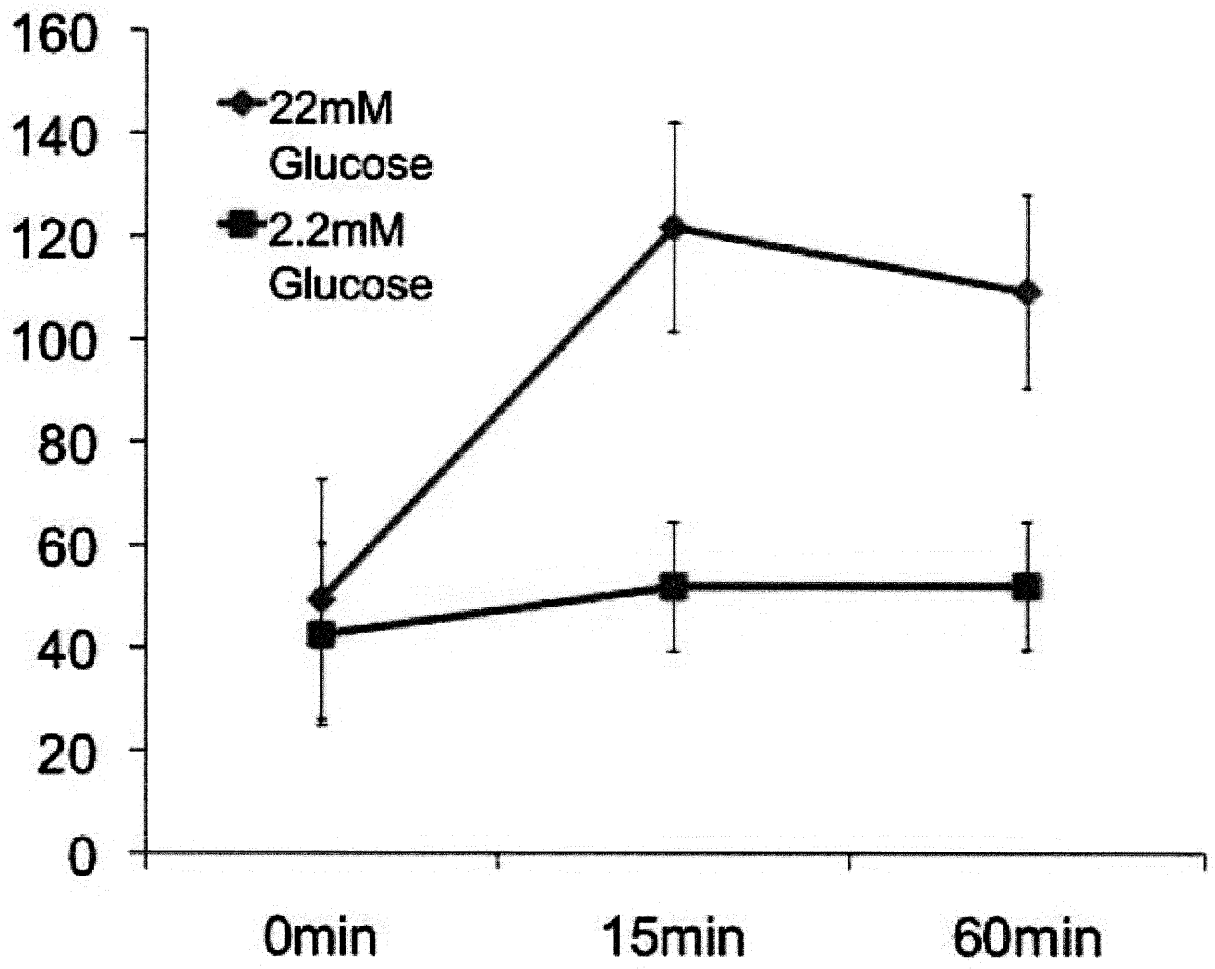

160
140
120
100
80
60
40
20
0
22mM Glucose
2.2mM Glucose
0min
15min
60min

Figure 5

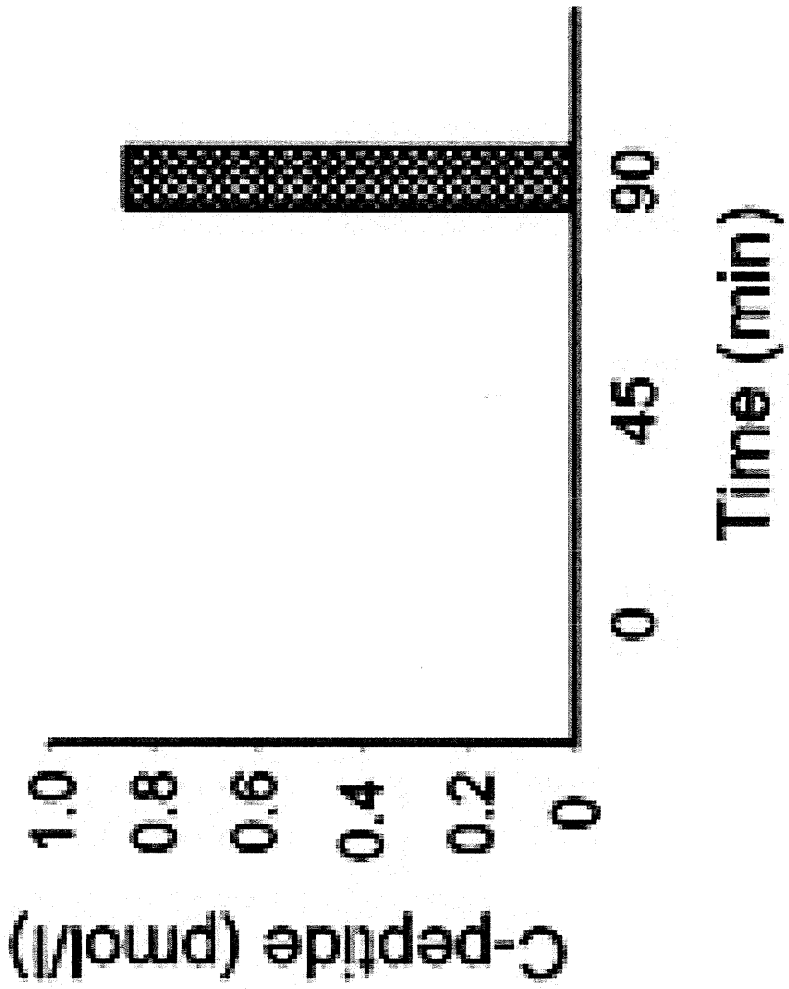


Figure 6

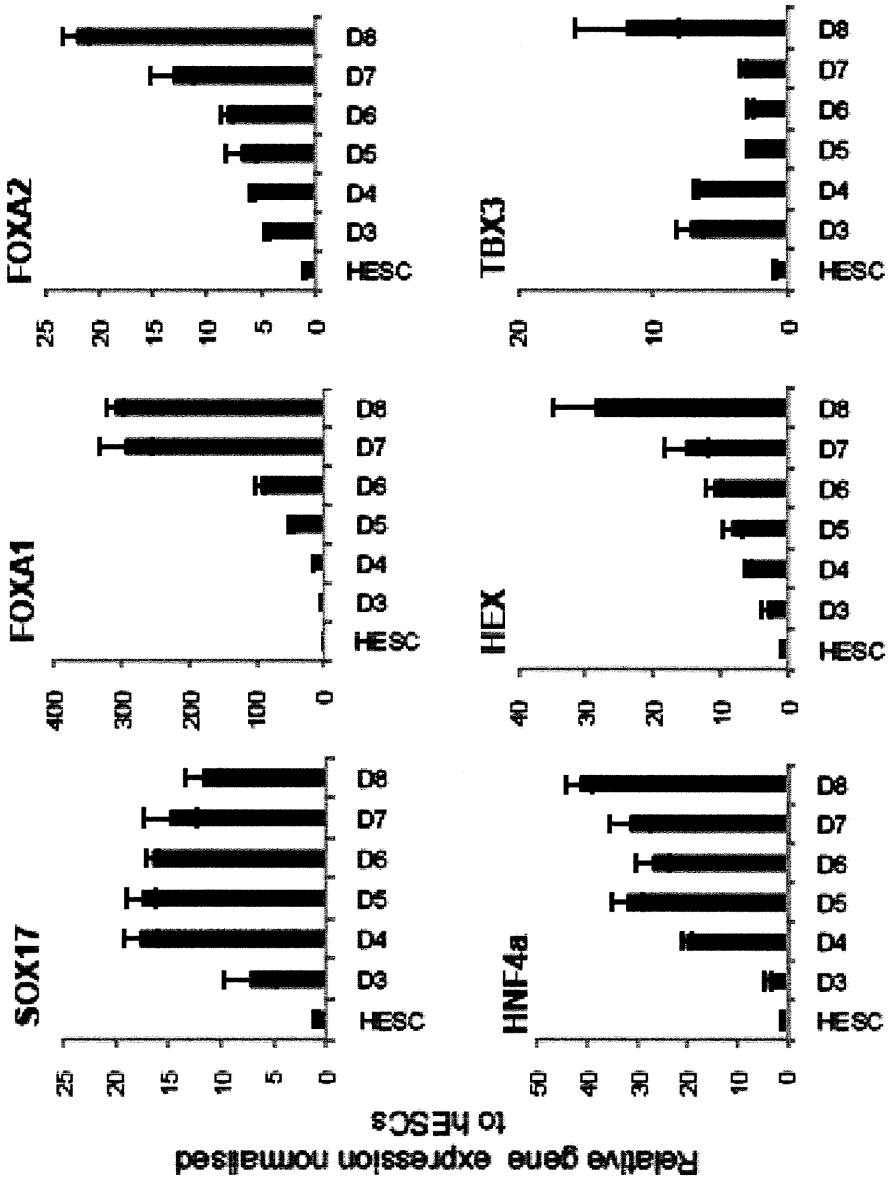

SOX17
FOXA1
FOXA2
HNF4a
HEX
TBX3
HESC
D3
D4
D5
D6
D7
D8
Relative gene expression normalised to hESCs

Figure 7

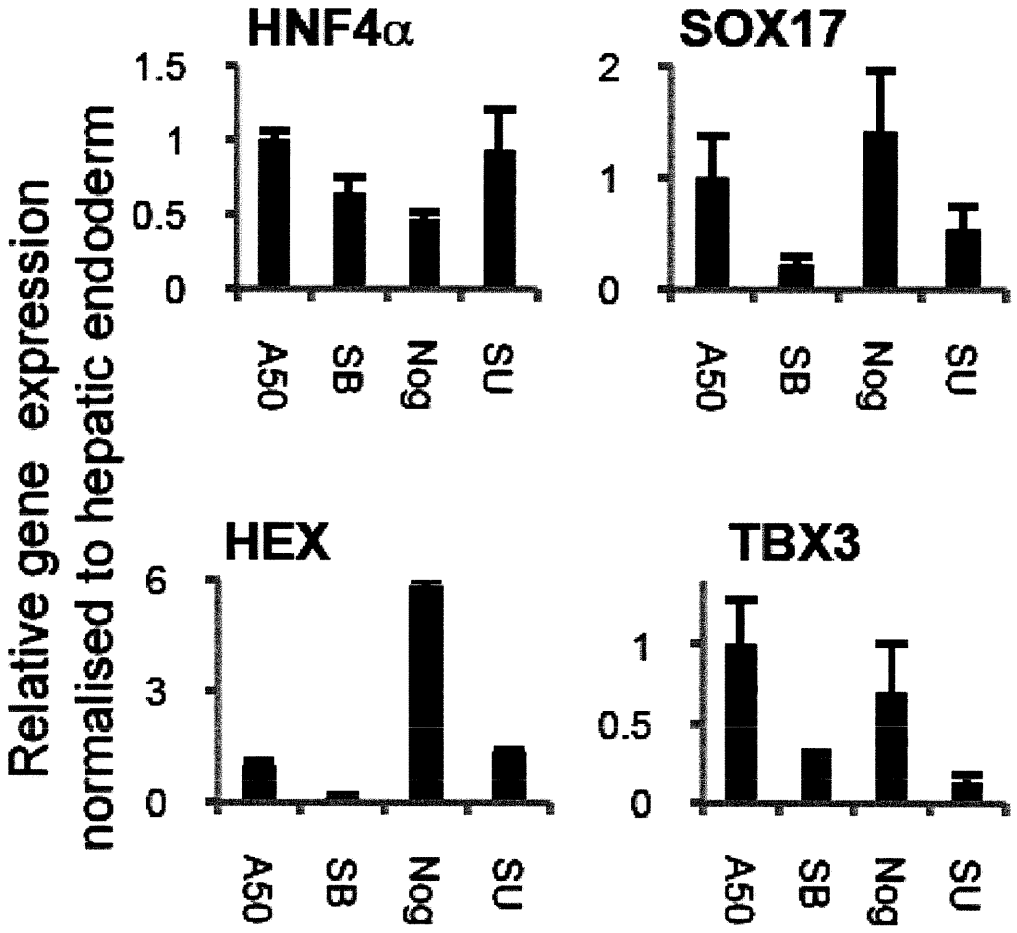

HNF4α
SOX17
HEX
TBX3
Relative gene expression normalised to hepatic endoderm
1.5
1
0.5
0
2
1
0
6
3
0
1
0.5
0
A50
SB
Nog
SU

Figure 8

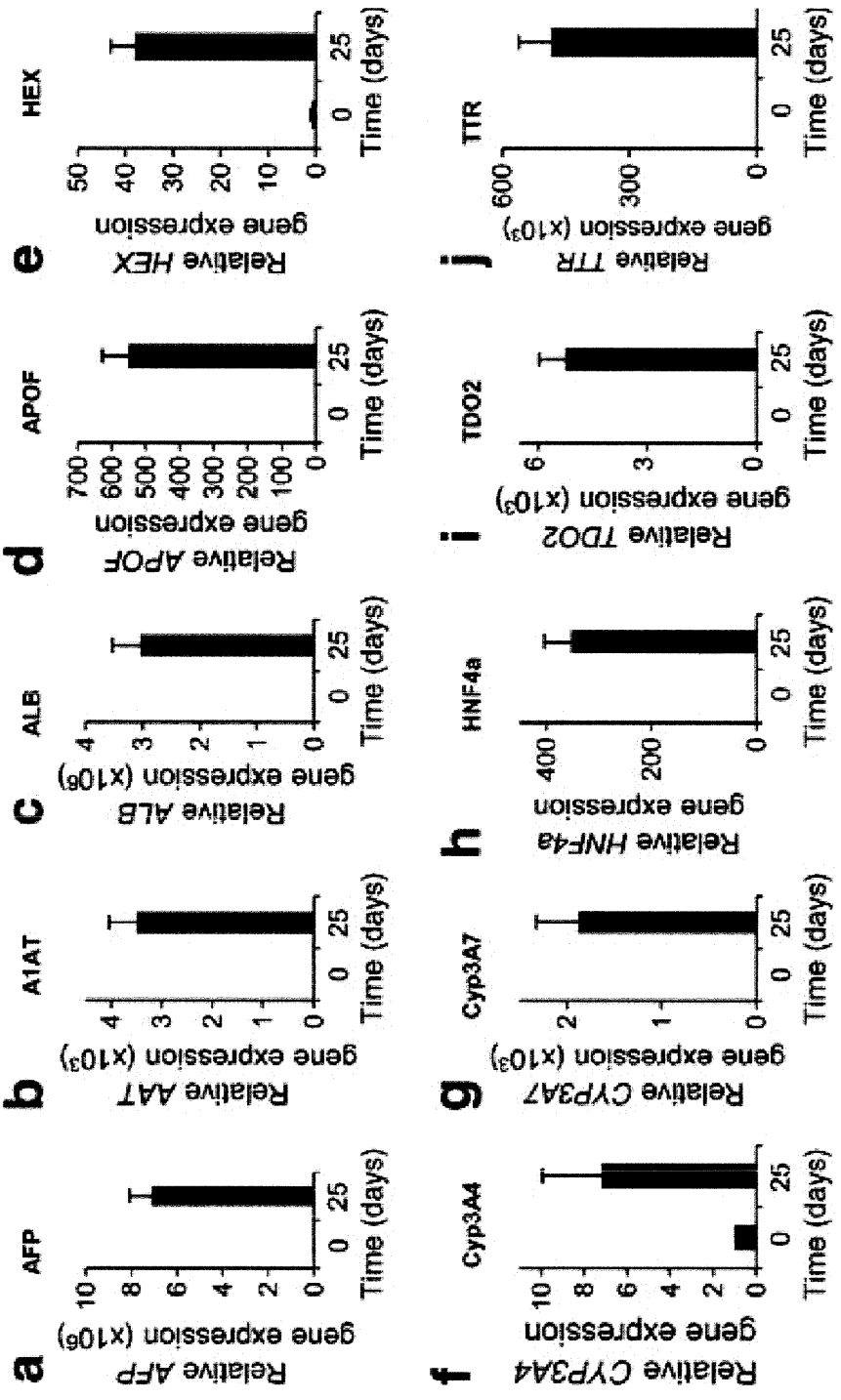

a
AFP
Relative AFP gene expression (x10⁶)
Time (days)
b
A1AT
Relative AAT gene expression (x10³)
Time (days)
c
ALB
Relative ALB gene expression (x10⁶)
Time (days)
d
APOF
Relative APOF gene expression
Time (days)
e
HEX
Relative HEX gene expression
Time (days)
f
Cyp3A4
Relative CYP3A4 gene expression
Time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

Figure 9

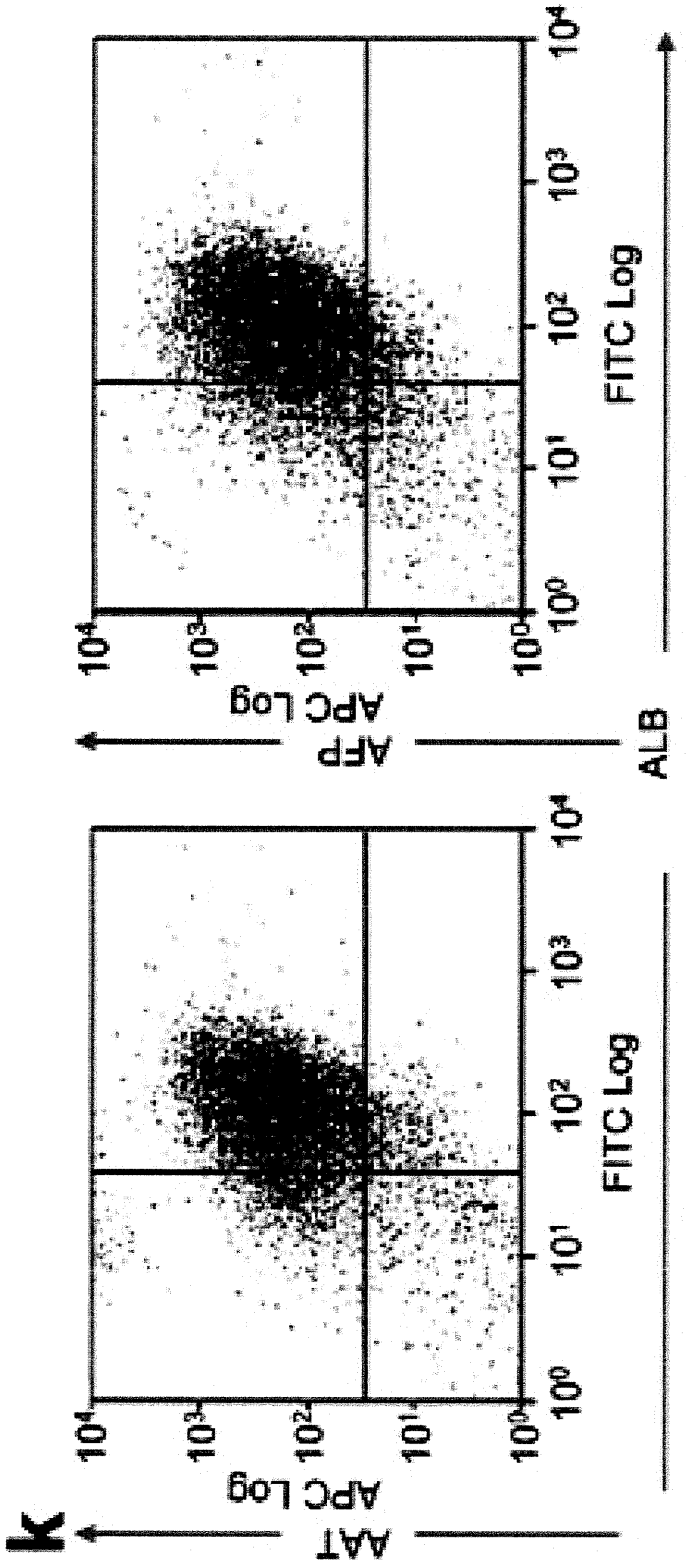

K
AAT
APC Log
FITC Log
ALB
AFP
APC Log
FITC Log
10^0
10^1
10^2
10^3
10^4

Figure 10

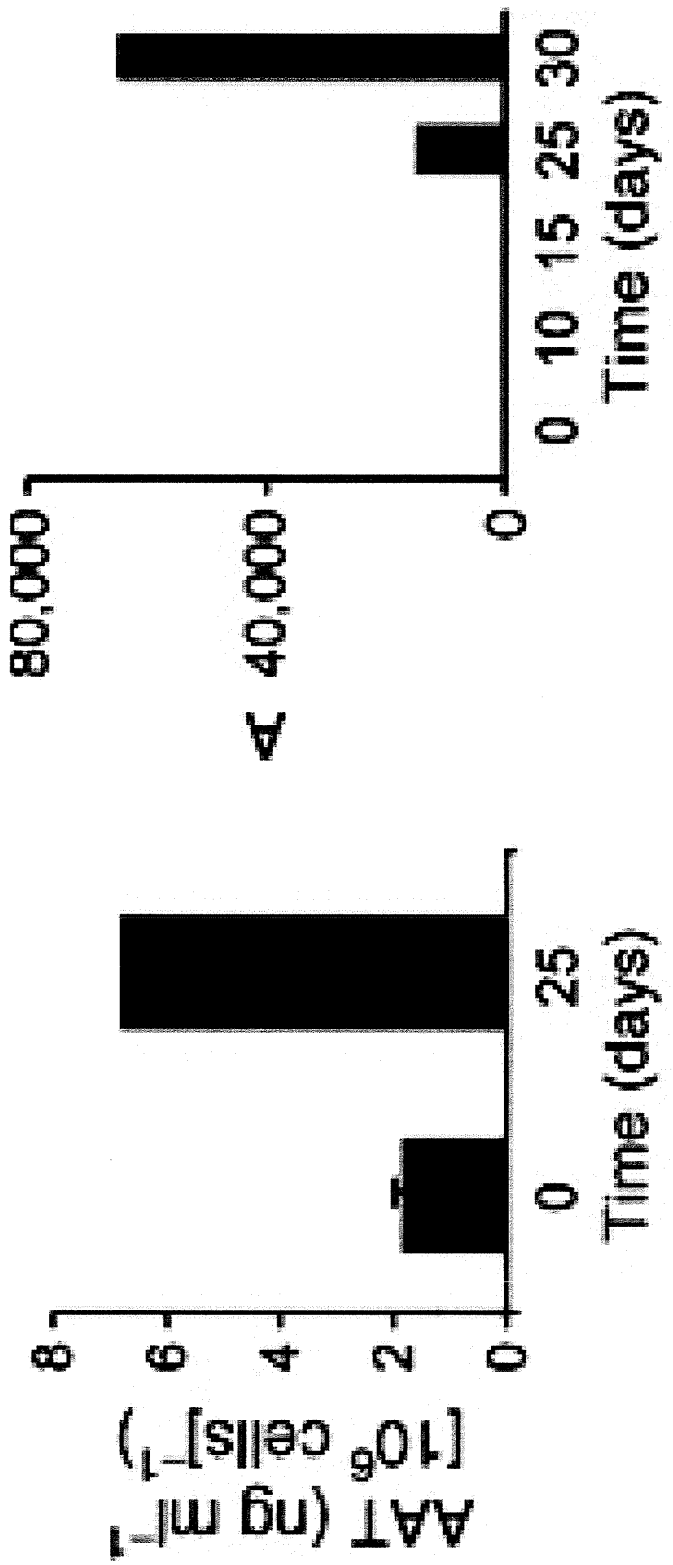

AAT (ng ml⁻¹)
[10⁶ cells]⁻¹)
8
6
4
2
0
0
25
Time (days)
A
80,000
40,000
0
0
10
15
25
30
Time (days)

Figure 11

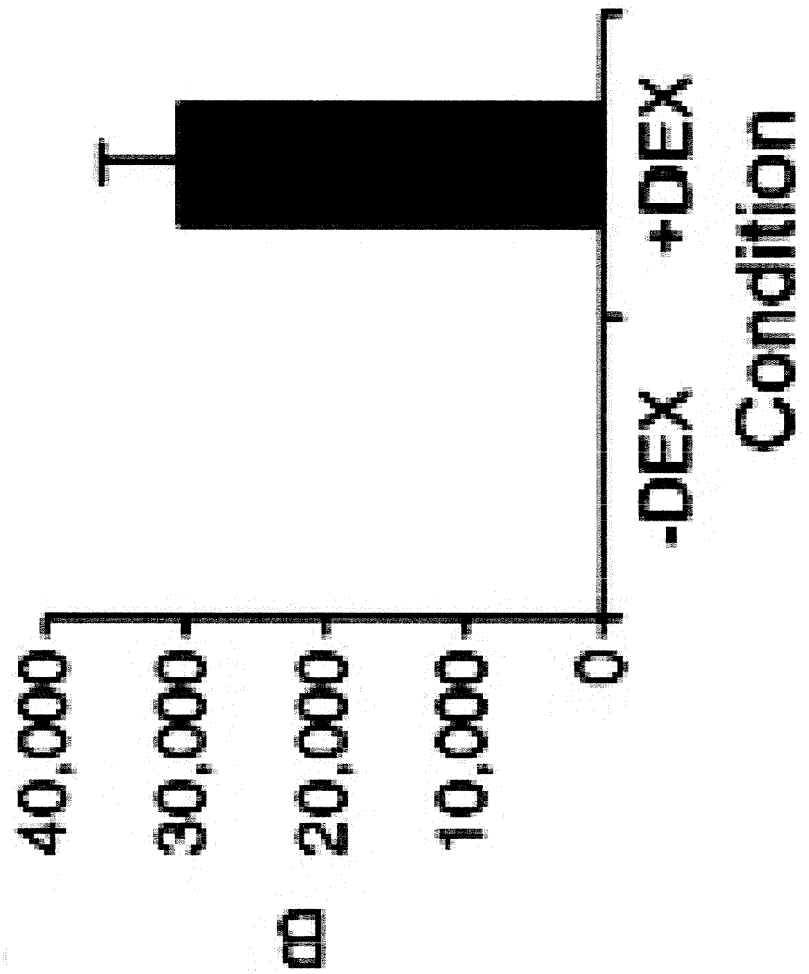


Figure 12

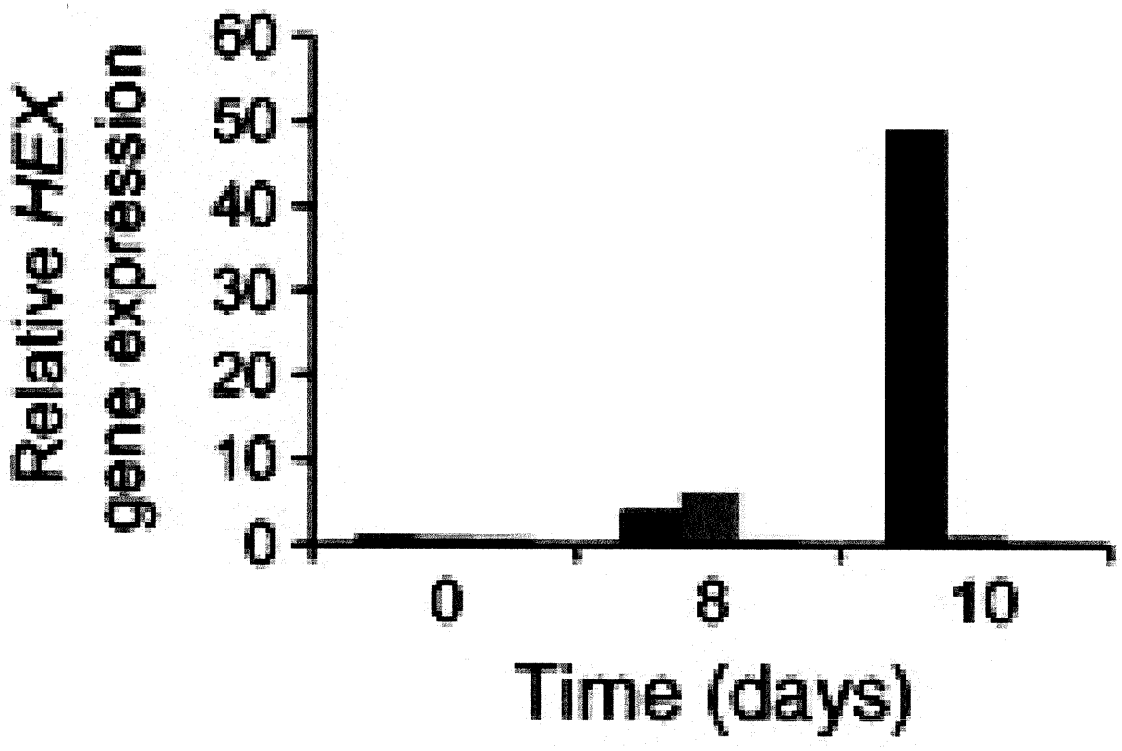

Relative HEX gene expression
60
50
40
30
20
10
0
0
8
10
Time (days)

Figure 13

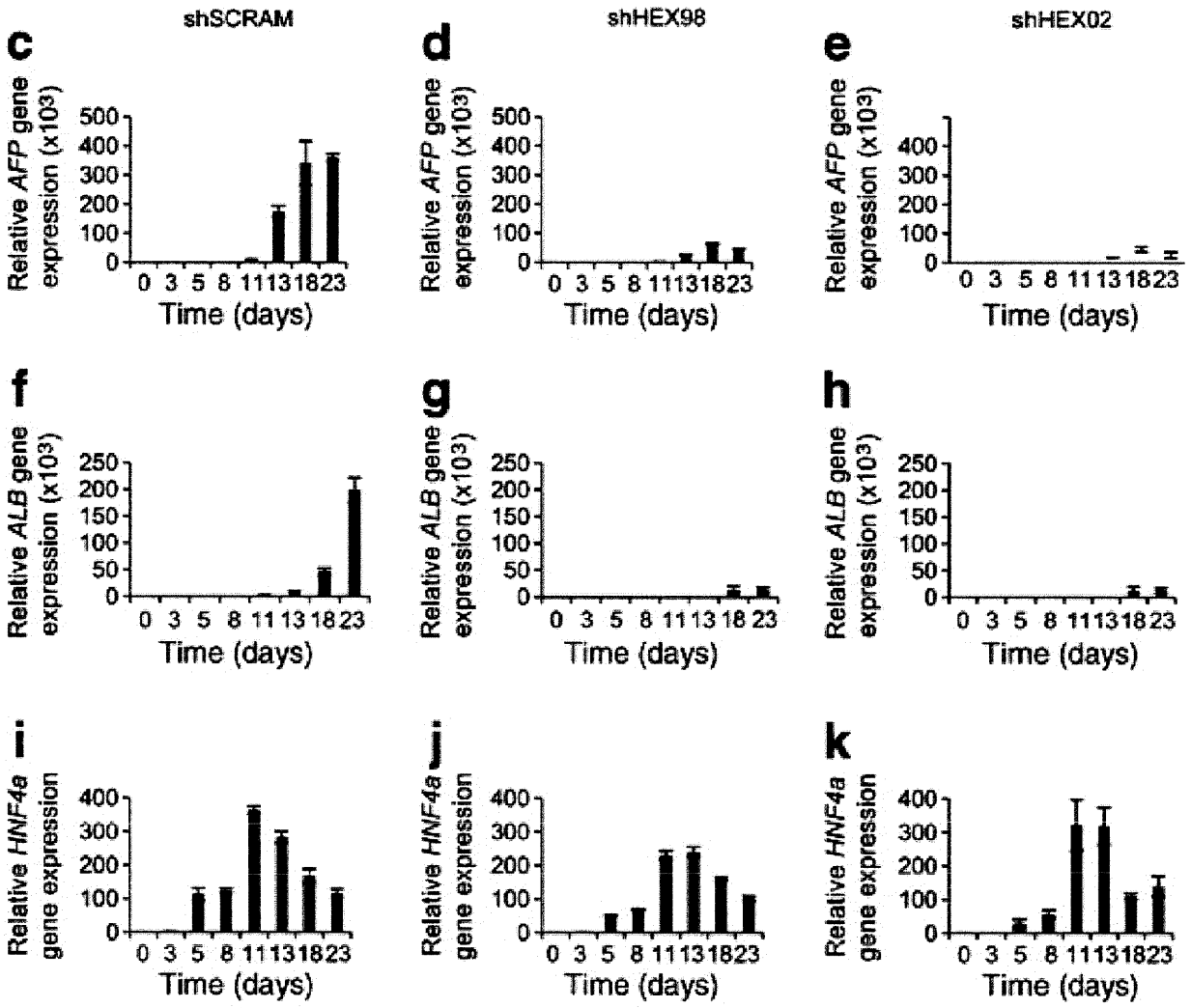

shSCRAM
shHEX98
shHEX02
c
d
e
f
g
h
i
k
Relative AFP gene expression (x10³)
Relative ALB gene expression (x10³)
Relative HNF4a gene expression
Time (days)
0 3 5 8 11 13 18 23

Figure 14

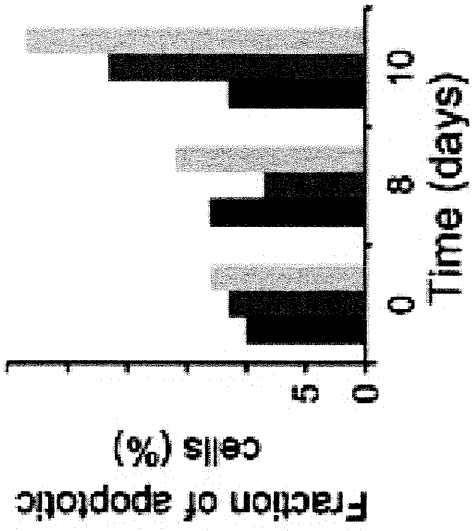


Figure 15

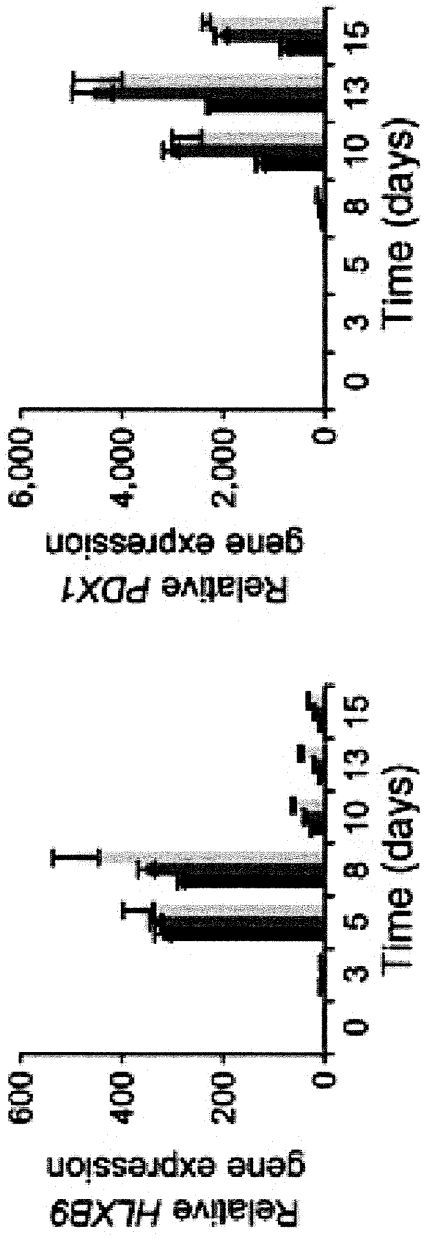


Figure 16

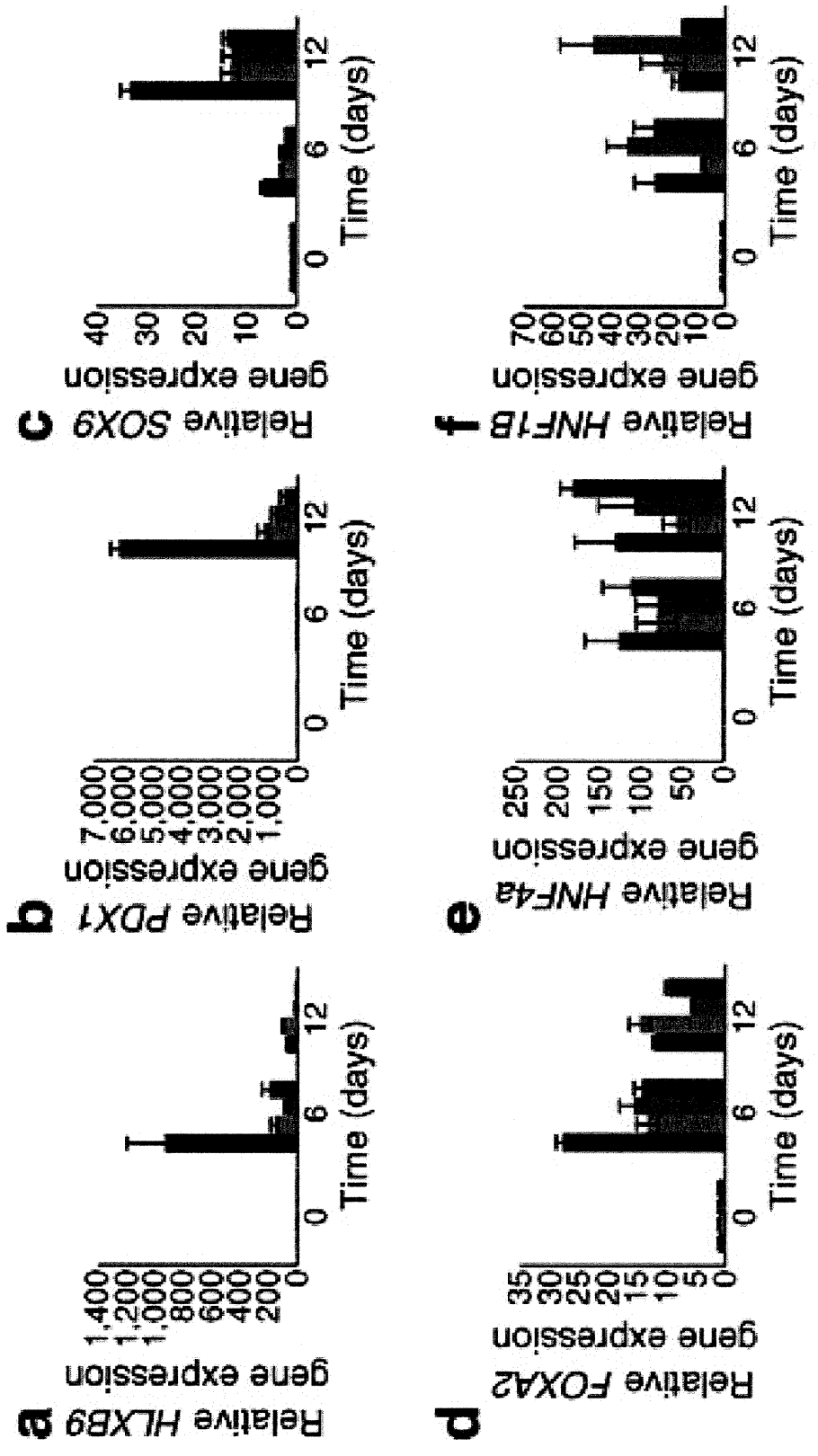

a
Relative HLXB9 gene expression
1,400
1,200
1,000
800
600
400
200
0
0
6
12
Time (days)
b
Relative PDX1 gene expression
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
0
6
12
Time (days)
c
Relative SOX9 gene expression
40
30
20
10
0
0
6
12
Time (days)
d
Relative FOXA2 gene expression
35
30
25
20
15
10
5
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

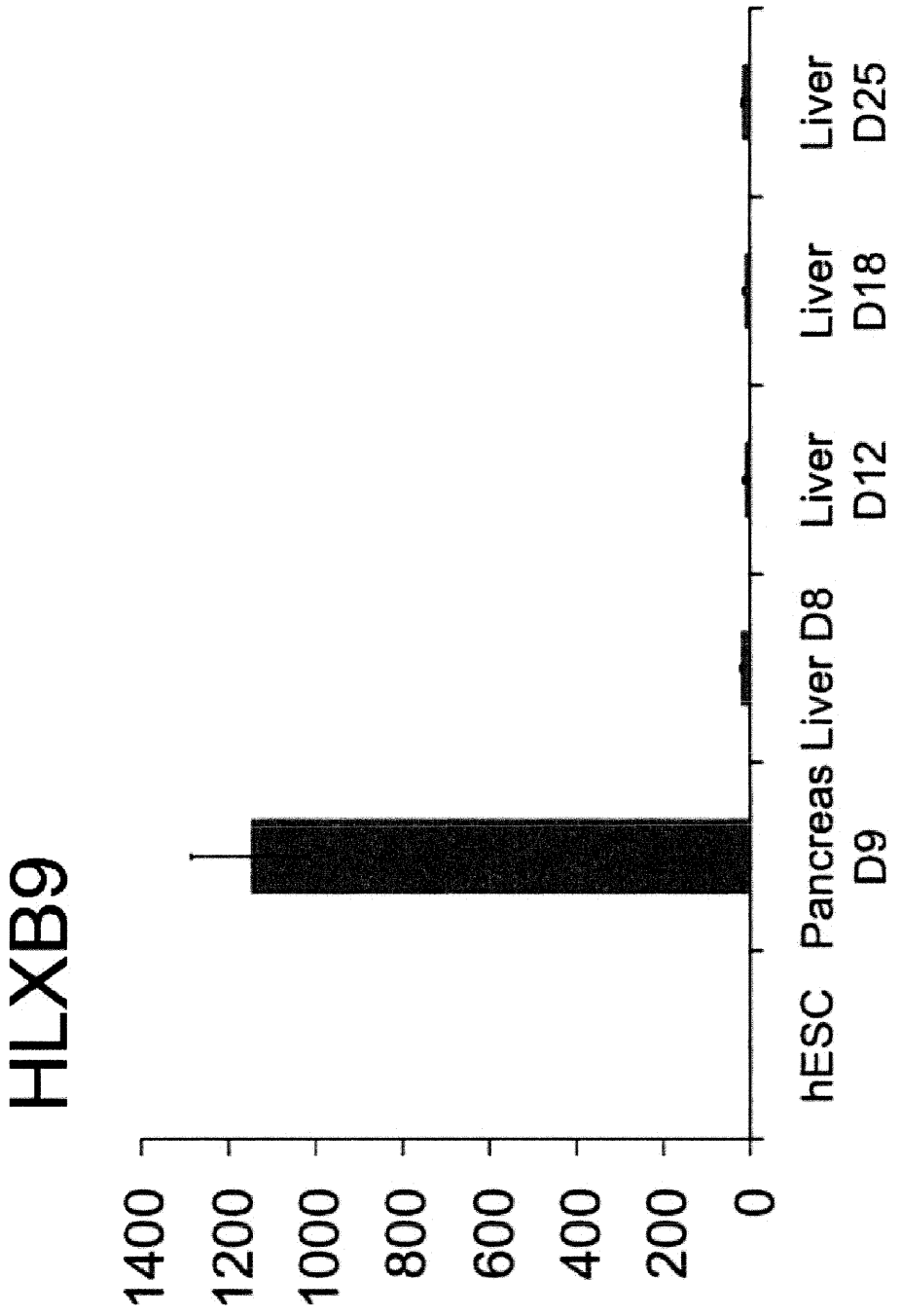

HLXB9
1400
1200
1000
800
600
400
200
0
hESC
Pancreas D9
Liver D8
Liver D12
Liver D18
Liver D25

Figure 18