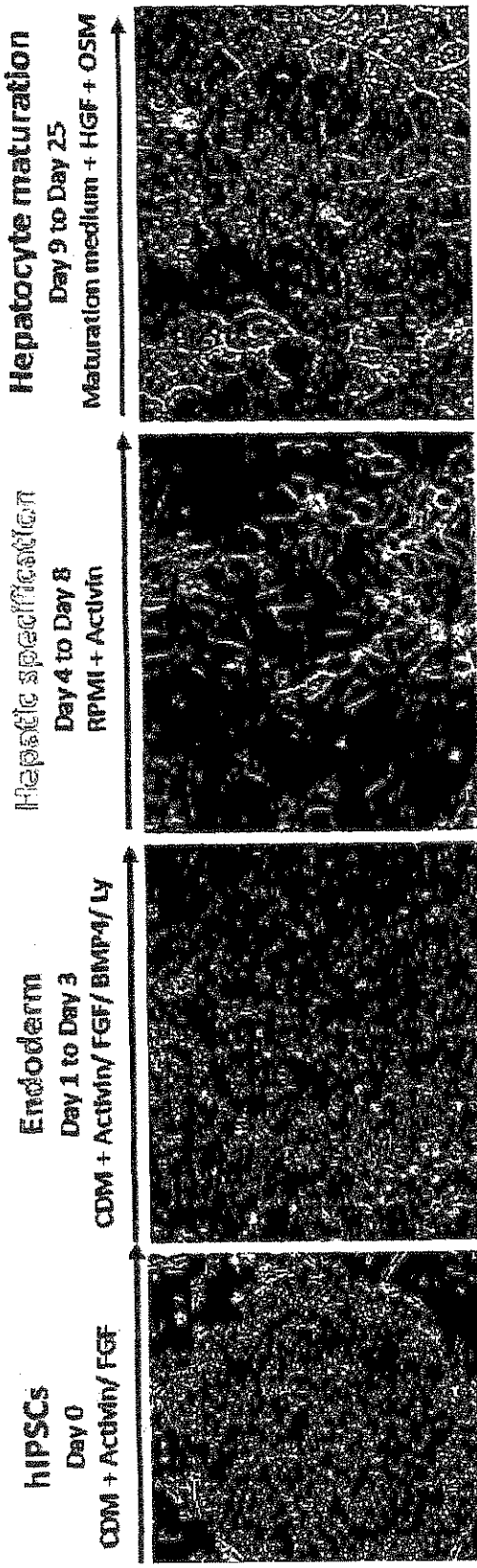

hIPSCs
Day 0
CDM + Activin/ FGF
Endoderm
Day 1 to Day 3
CDM + Activin/ FGF/ BMP4/ Ly
Hepatic specification
Day 4 to Day 8
RPMI + Activin
Hepatocyte maturation
Day 9 to Day 25
Maturation medium + HGF + OSM

Figure 1

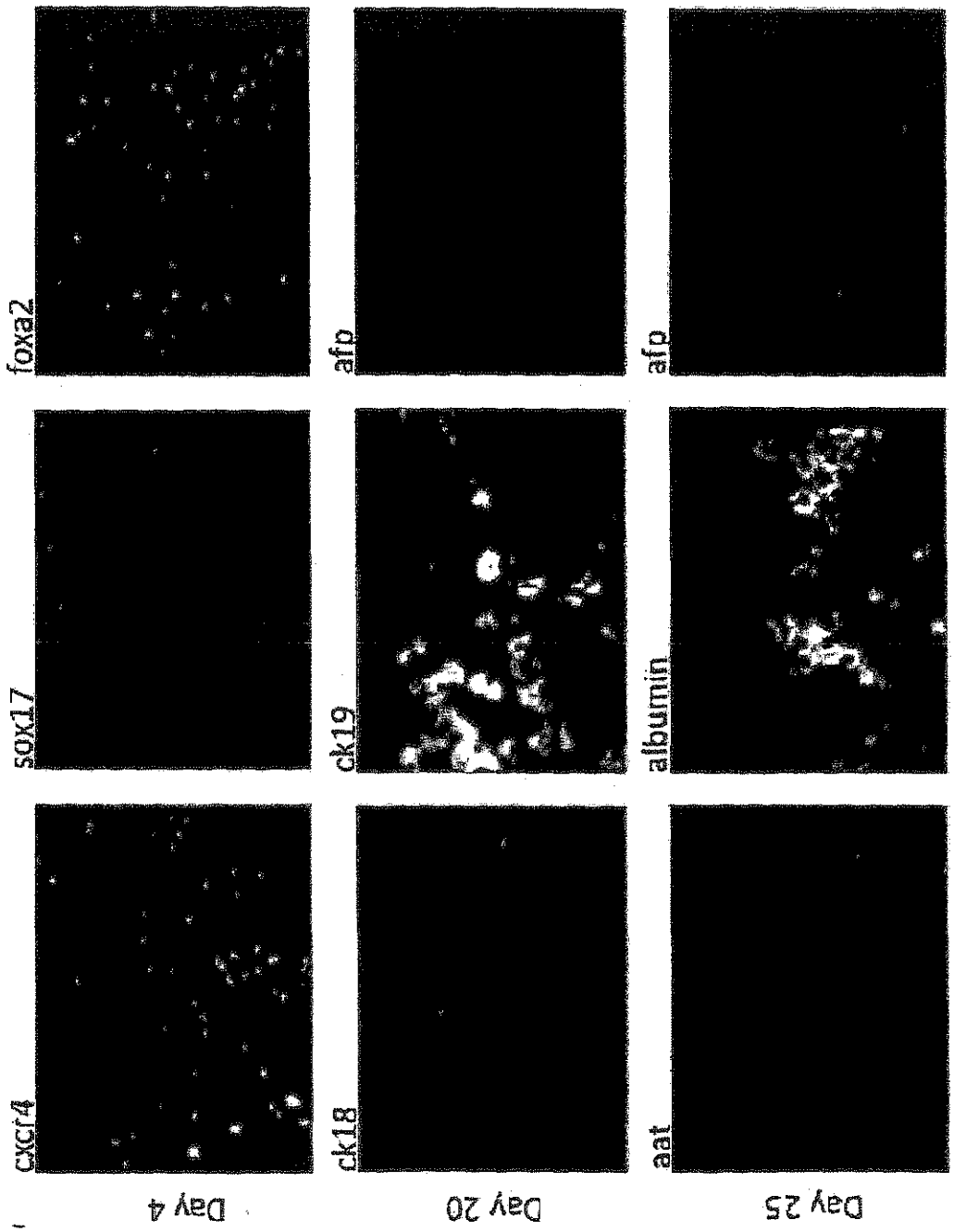
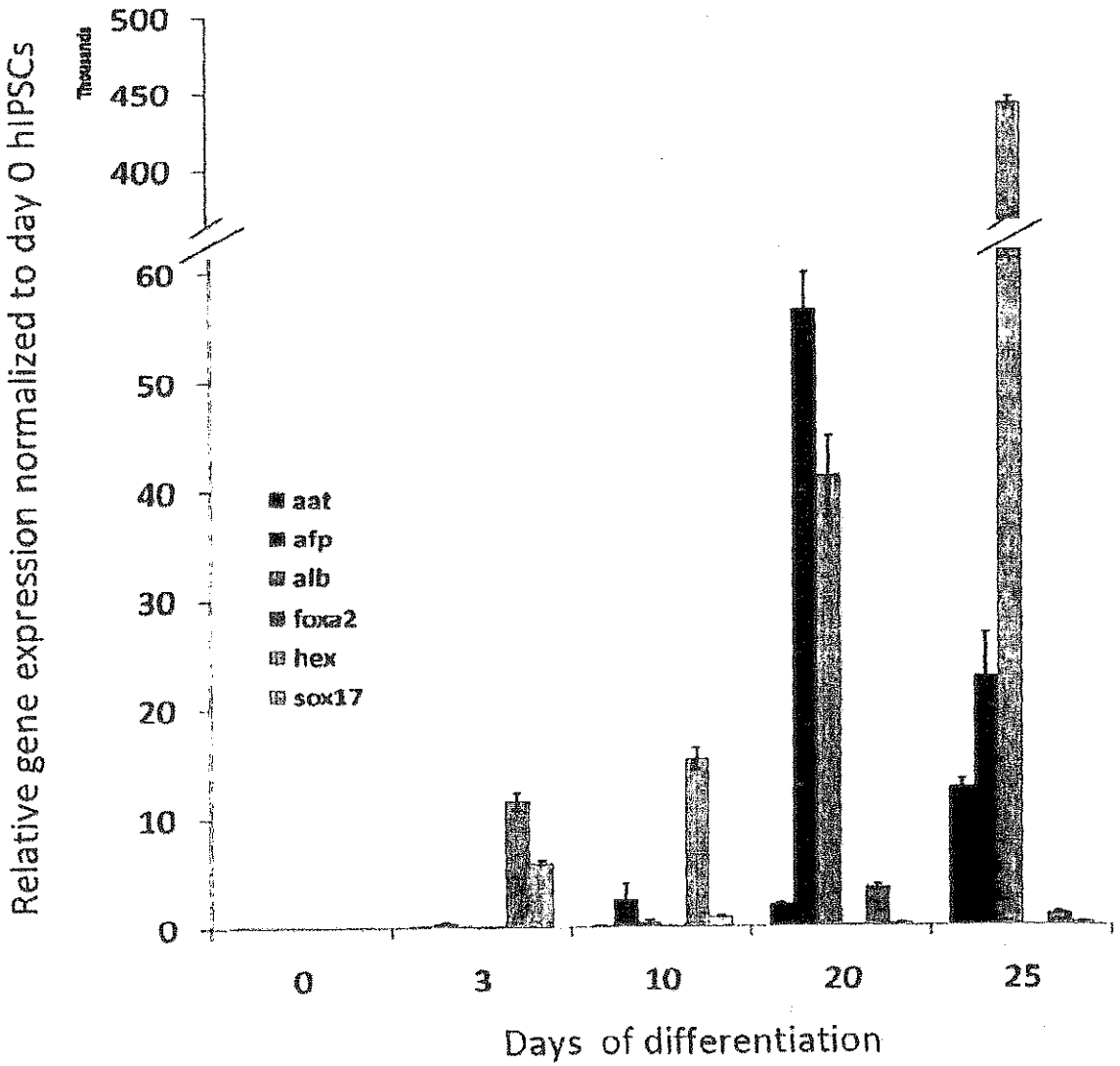
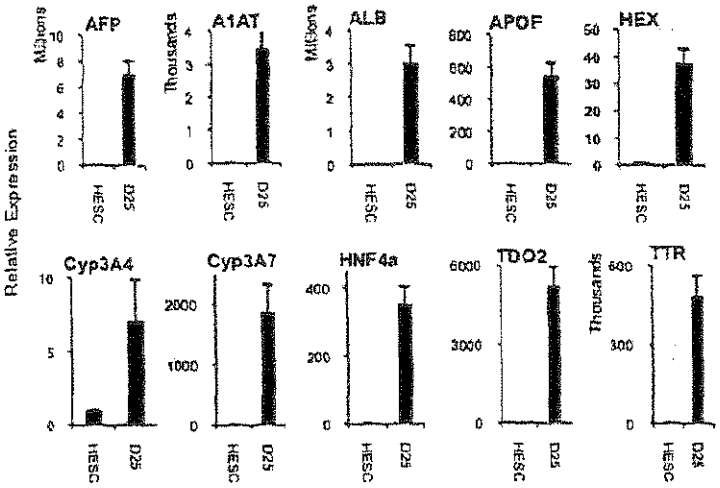

Day 4
Day 20
Day 25
cxcr4
sox17
foxa2
ck18
ck19
afp
aat
albumin
afp

Figure 2

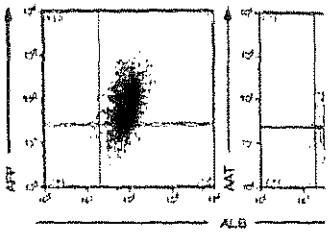
Figure 3


Relative gene expression normalized to day 0 hIPSCs
Thousands
500
450
400
60
50
40
30
20
10
0
aat
afp
alb
foxa2
hex
sox17
0
3
10
20
25
Days of differentiation

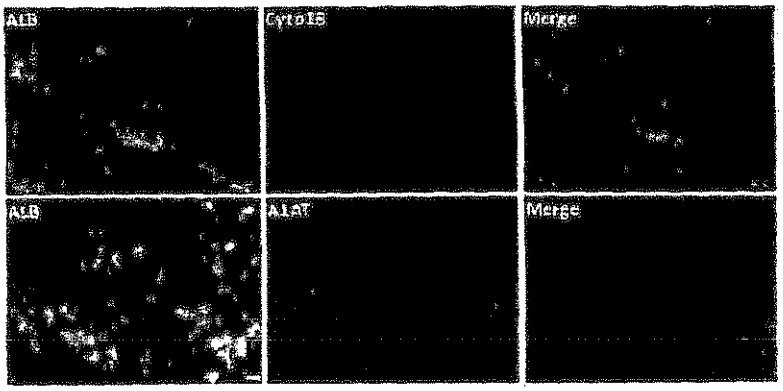
A


Relative Expression
AFP
Millions
A1AT
Thousands
ALB
Millions
APOF
HEX
Cyp3A4
Cyp3A7
HNF4a
TDO2
Thousands
TTR
HESC
D25

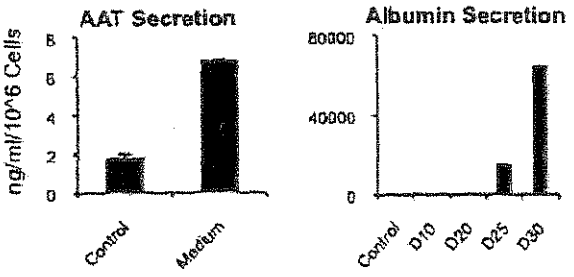
B


AFP
AAT
ALB

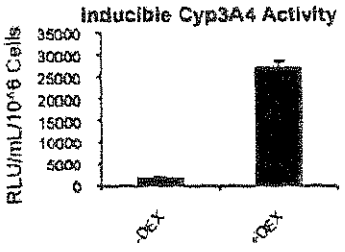
C


ALB
CytoIS
Merge
ALB
A1AT
Merge

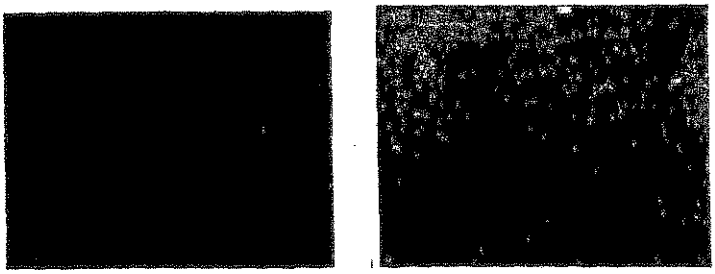
D


AAT Secretion
ng/ml/10^6 Cells
Control
Medium
Albumin Secretion
Control
D10
D20
D25
D30

E


Inducible Cyp3A4 Activity
RLU/mL/10^6 Cells
-DEX
+DEX

F



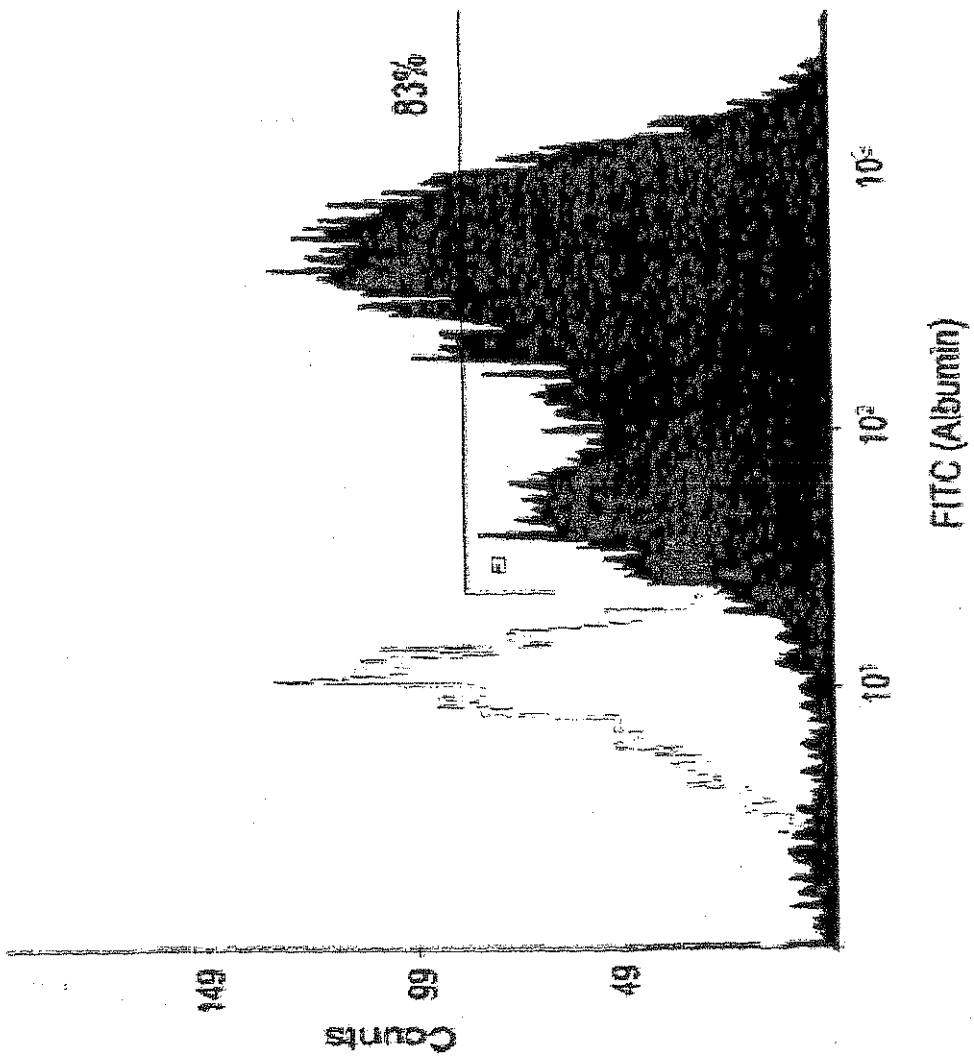


Figure 5

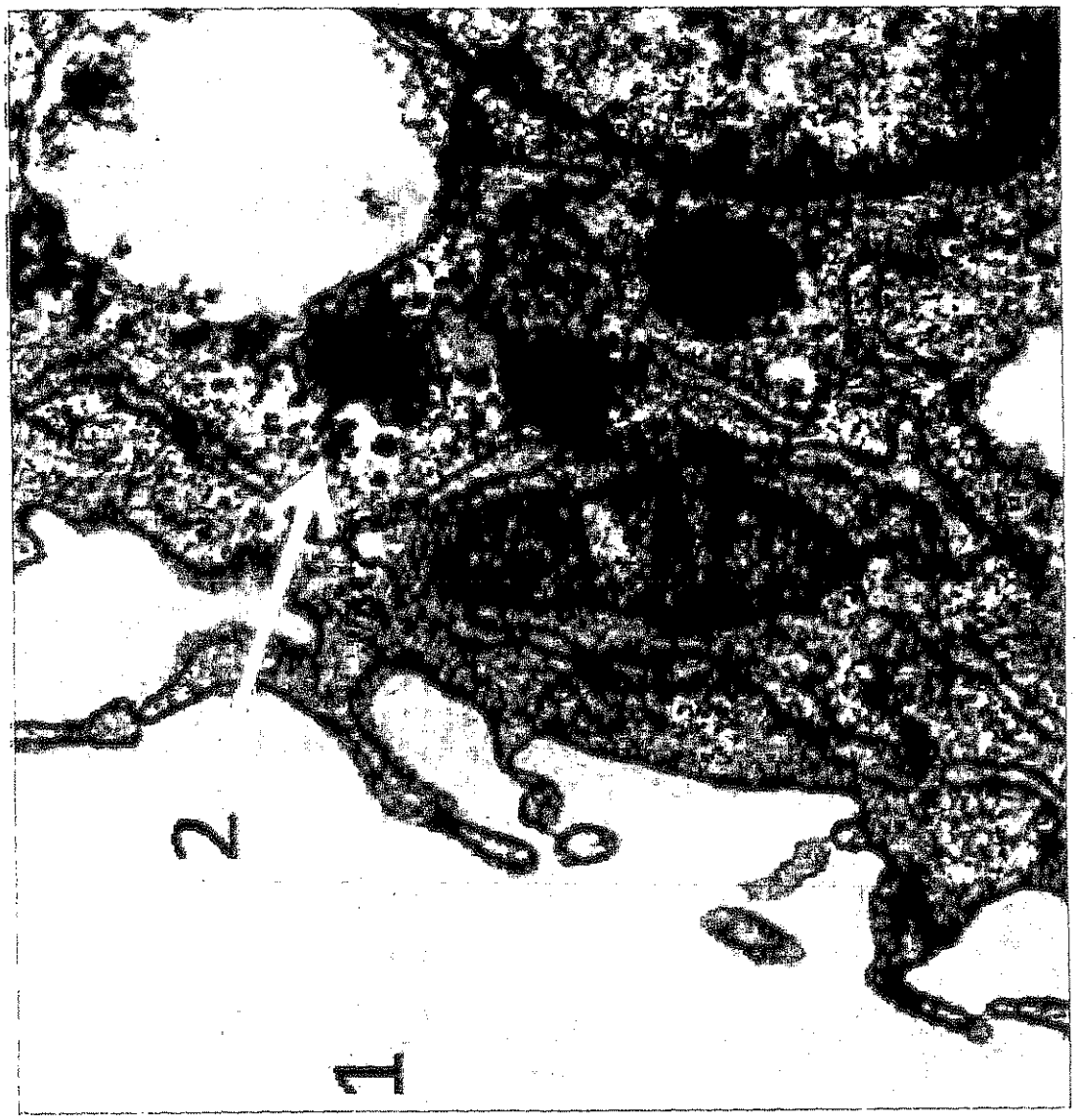


Figure 6

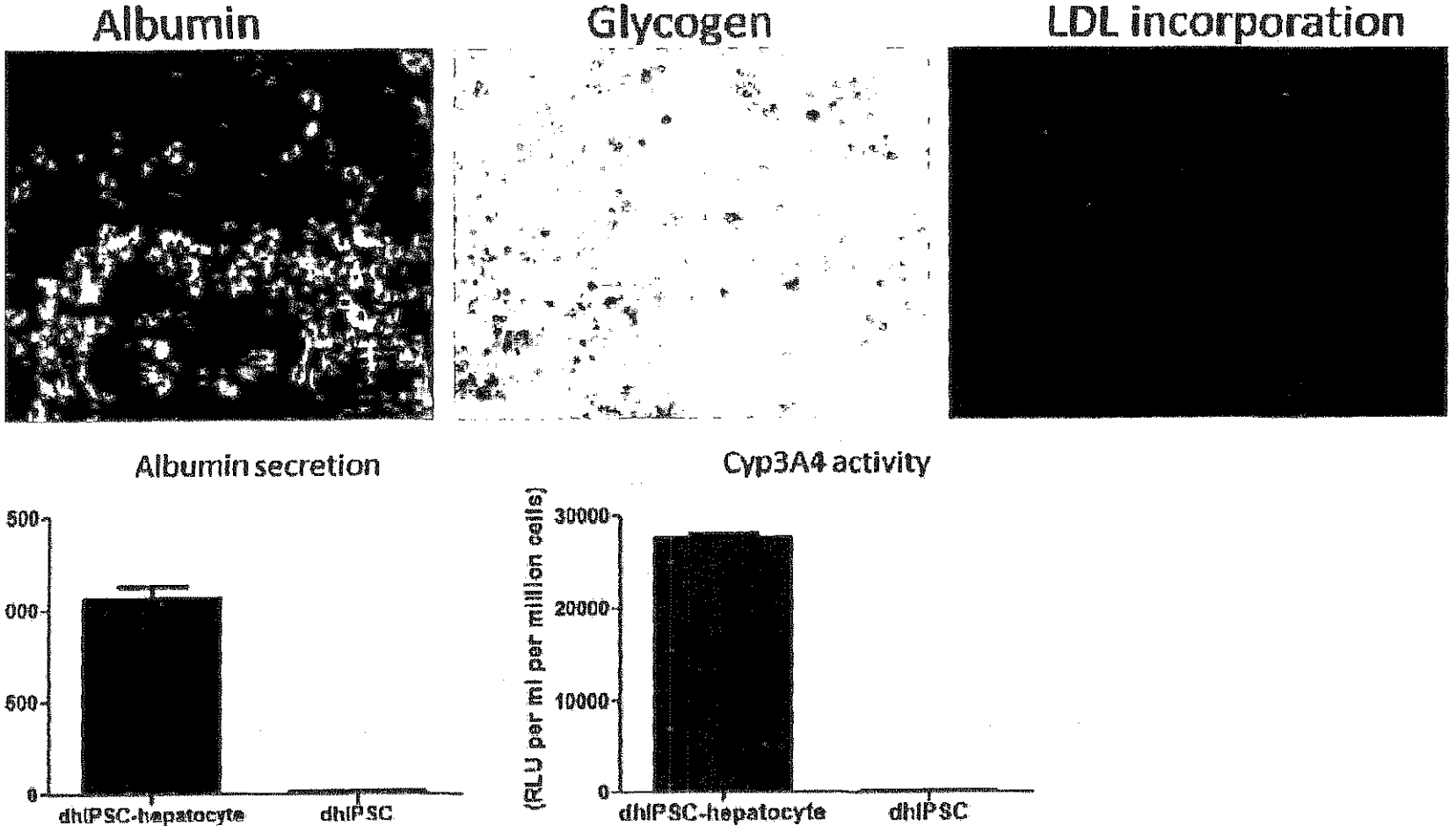

Albumin
Glycogen
LDL incorporation
Albumin secretion
500
000
500
0
dhIPSC-hepatocyte
dhIPSC
Cyp3A4 activity
(RLU per ml per million cells)
30000
20000
10000
0
dhIPSC-hepatocyte
dhIPSC

Figure 7

Figure 8

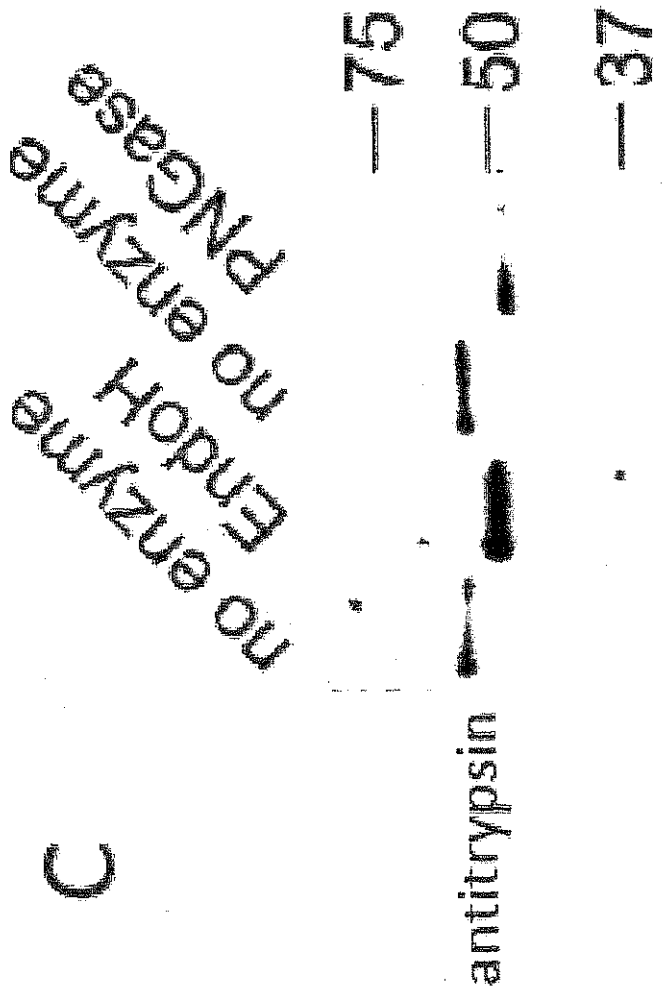


Figure 9

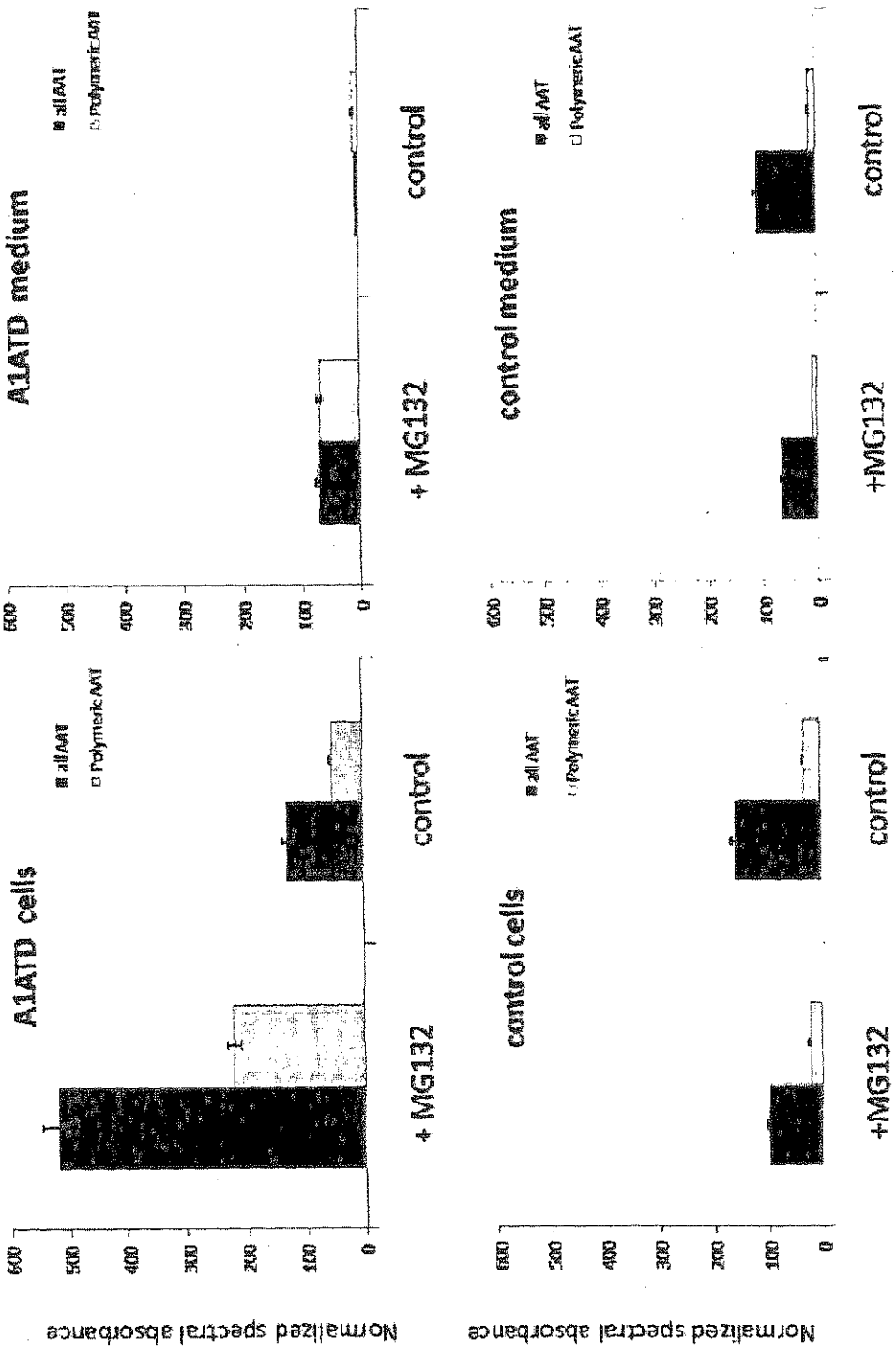
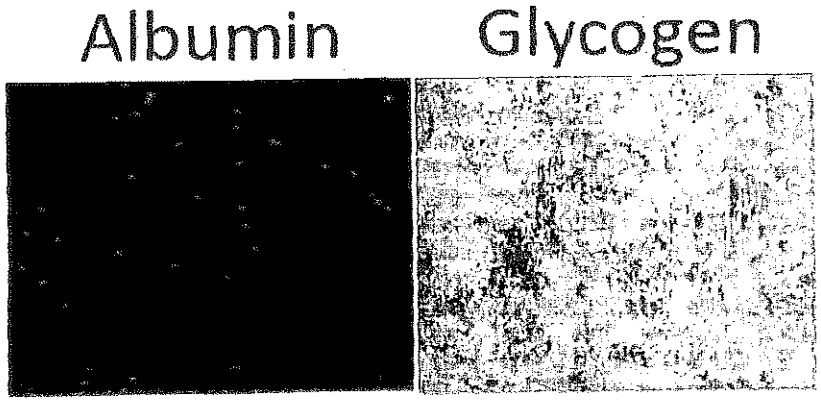
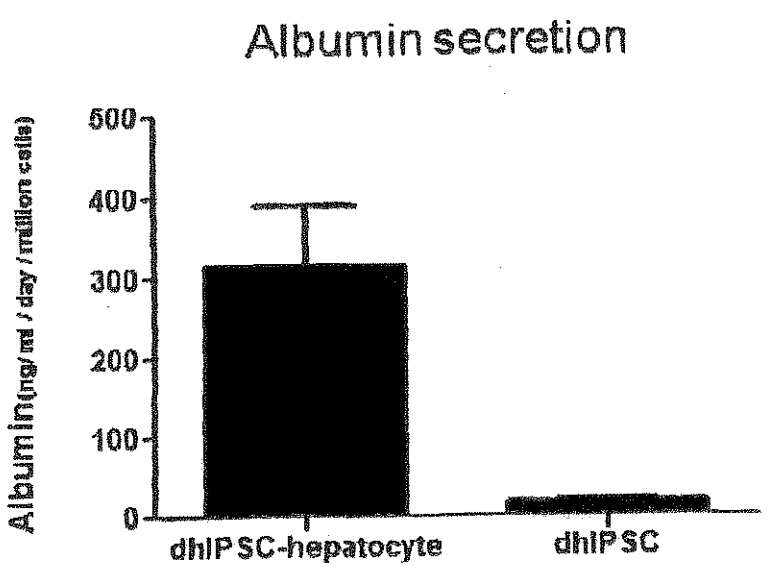

A1ATD cells
control cells
A1ATD medium
control medium
all AAT
Polymeric AAT
+ MG132
control
+MG132
Normalized spectral absorbance
0
100
200
300
400
500
600

Figure 10


Albumin
Glycogen


Albumin secretion
Albumin (ng/ml / day / million cells)
500
400
300
200
100
0
dhIPSC-hepatocyte
dhIPSC

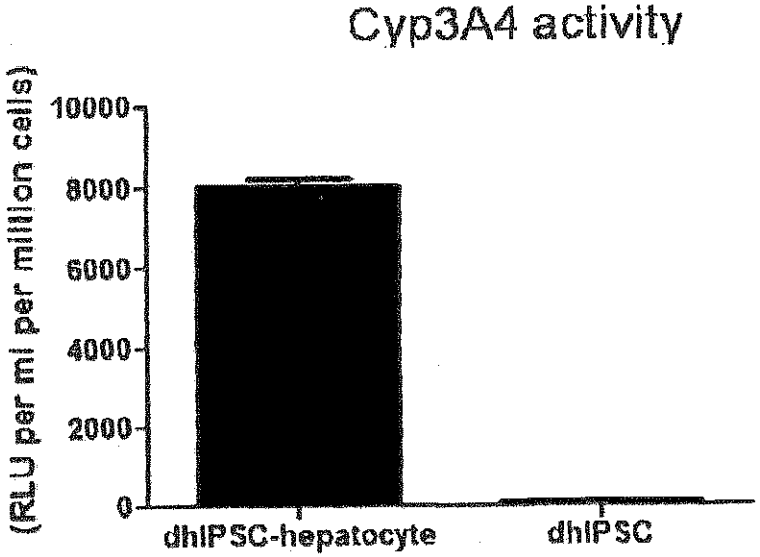

Cyp3A4 activity
(RLU per ml per million cells)
10000
8000
6000
4000
2000
0
dhIPSC-hepatocyte
dhIPSC

Figure 11

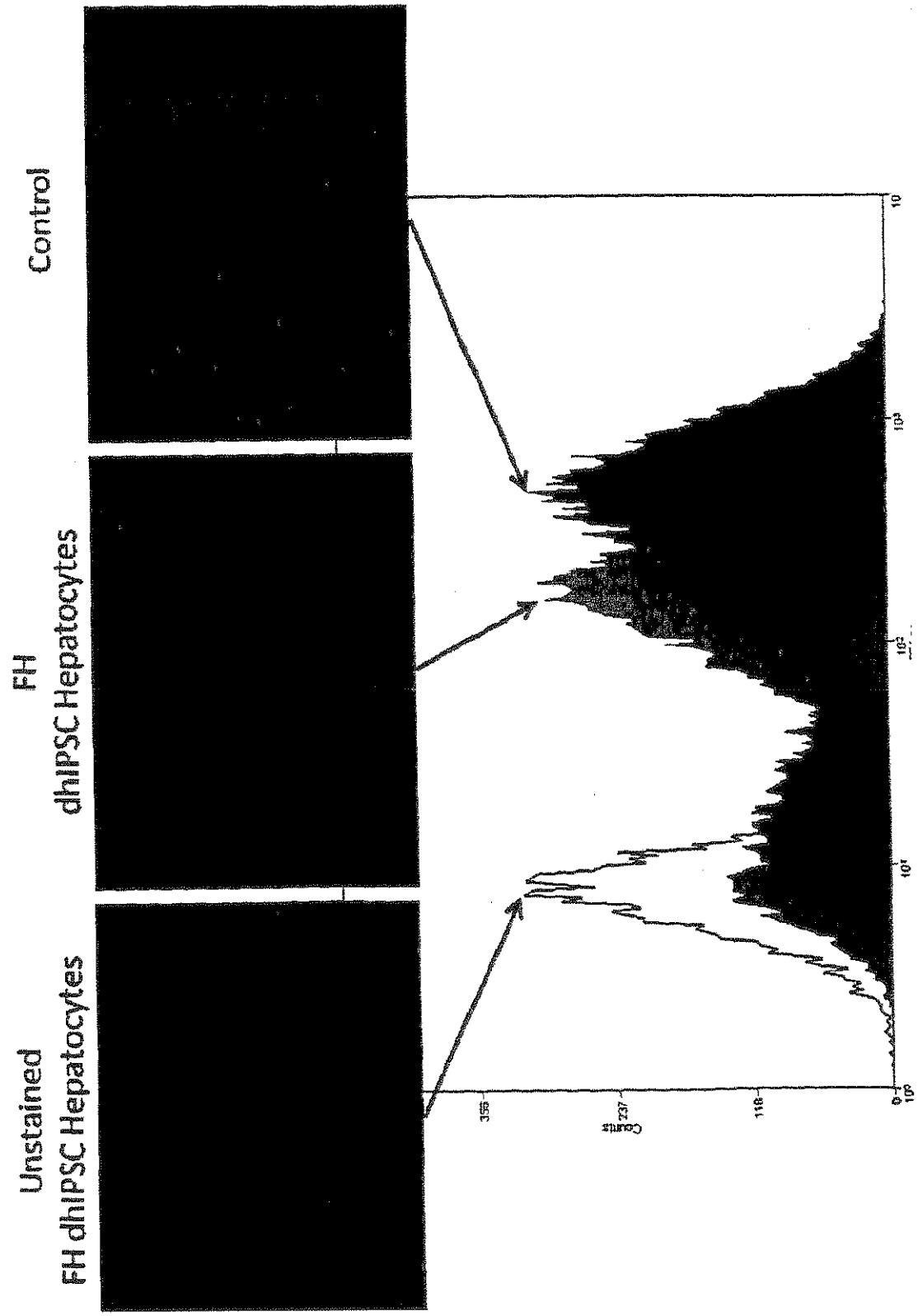
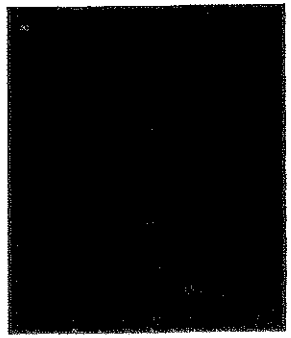
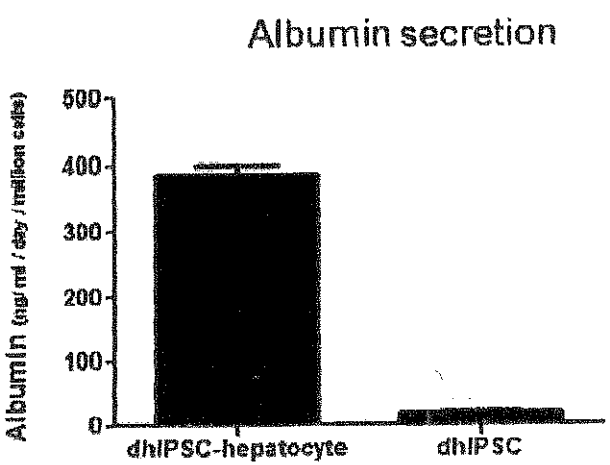

Unstained
FH dhIPSC Hepatocytes
FH
dhIPSC Hepatocytes
Control
Count
355
237
118
0
10⁰
10¹
10²
10³
10⁴

Figure 12


Albumin


Albumin secretion
Albumin (ng / ml / day / million cells)
500
400
300
200
100
0
dhIPSC-hepatocyte
dhIPSC

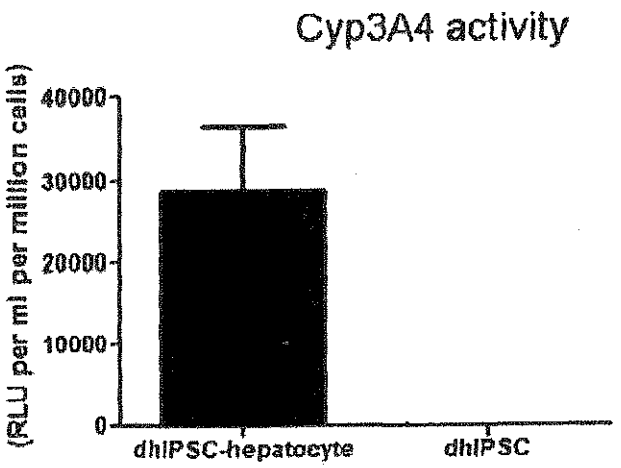

Cyp3A4 activity
(RLU per ml per million cells)
40000
30000
20000
10000
0
dhIPSC-hepatocyte
dhIPSC

Figure 13

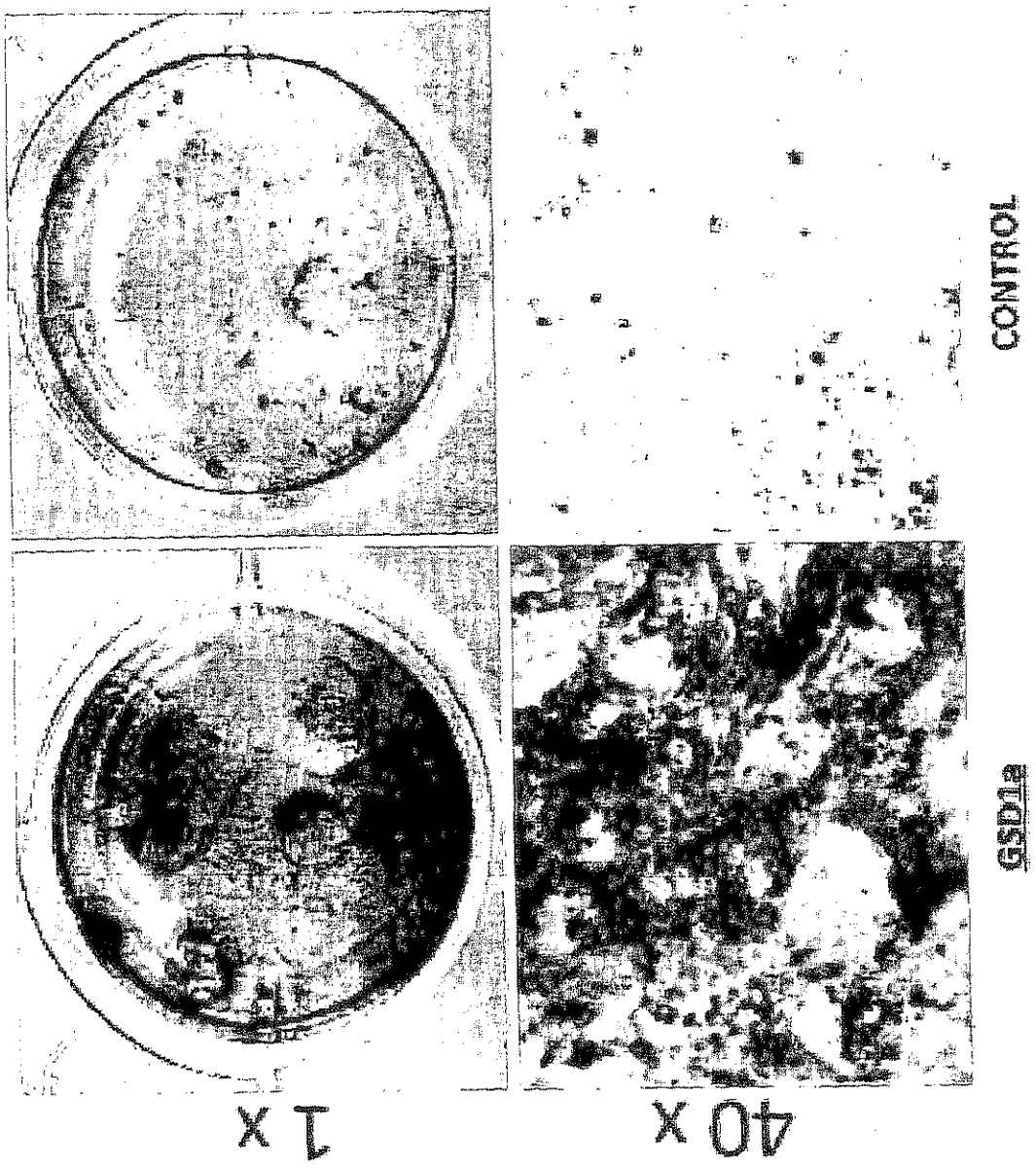


Figure 14

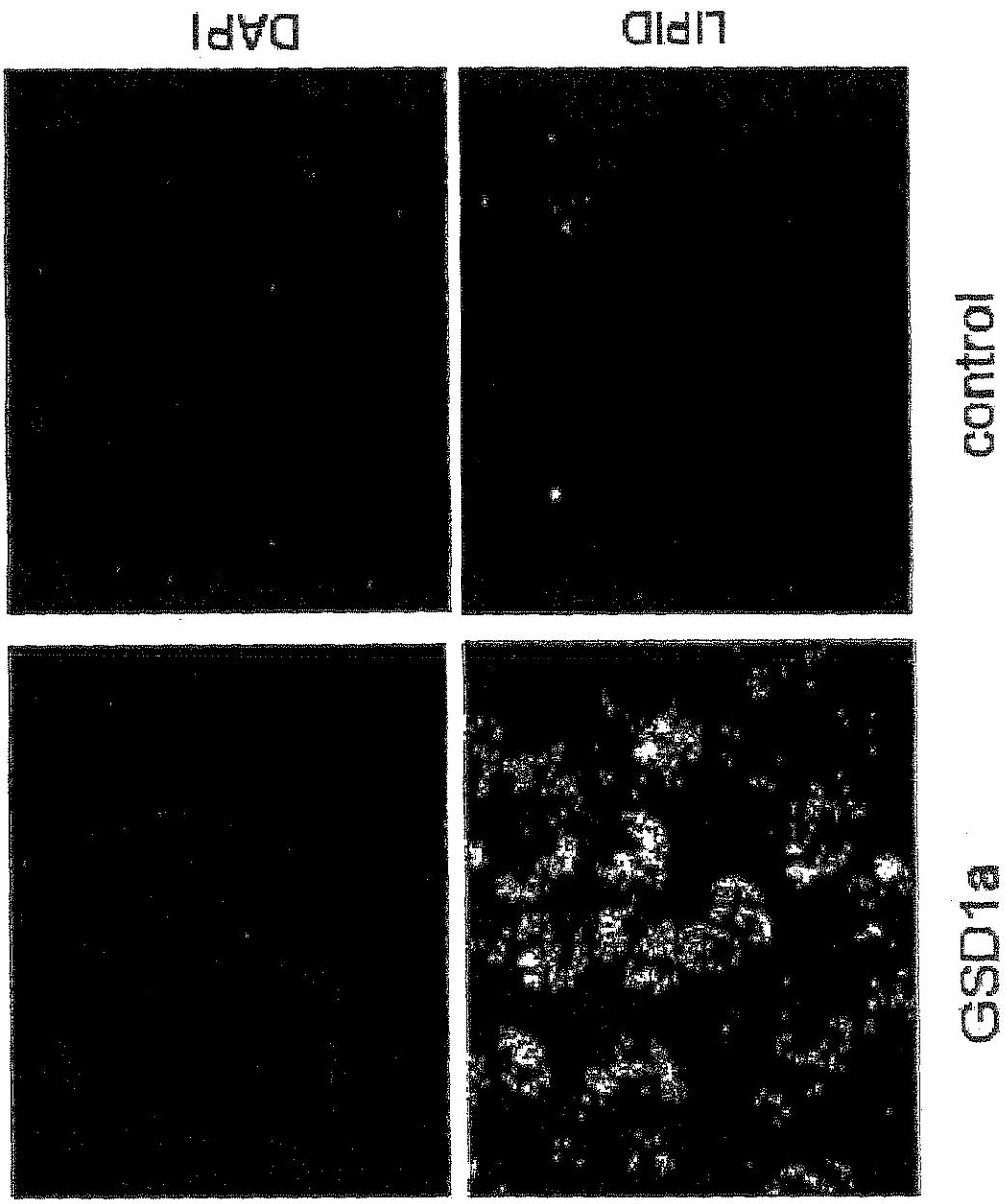
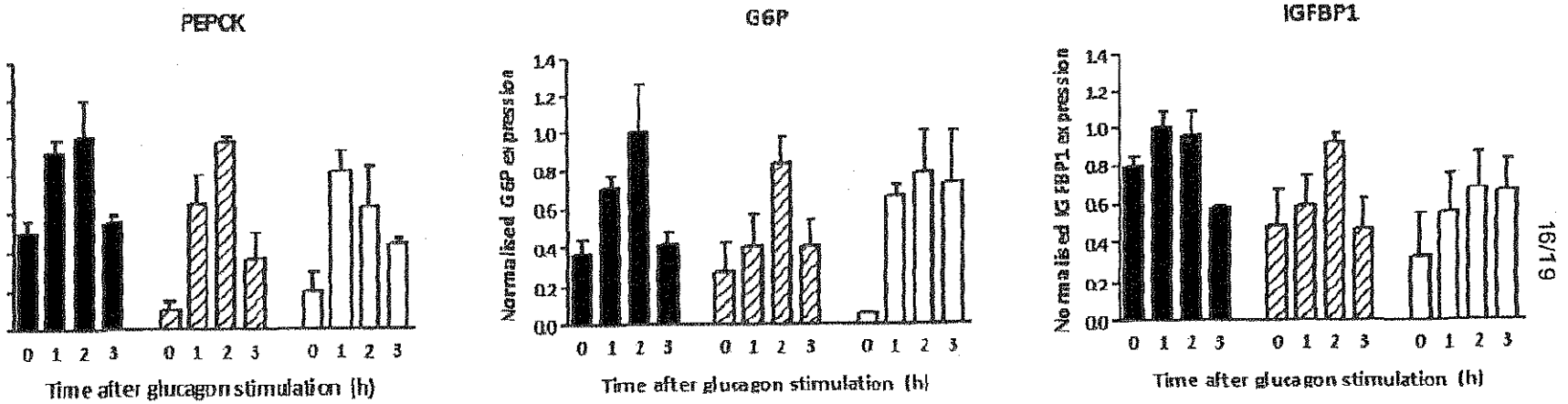


Figure 15



HepG2 (■), control hIPSC-hepatocytes (□) and GSD1a dhIPSC-hepatocytes (▨).

Figure 16

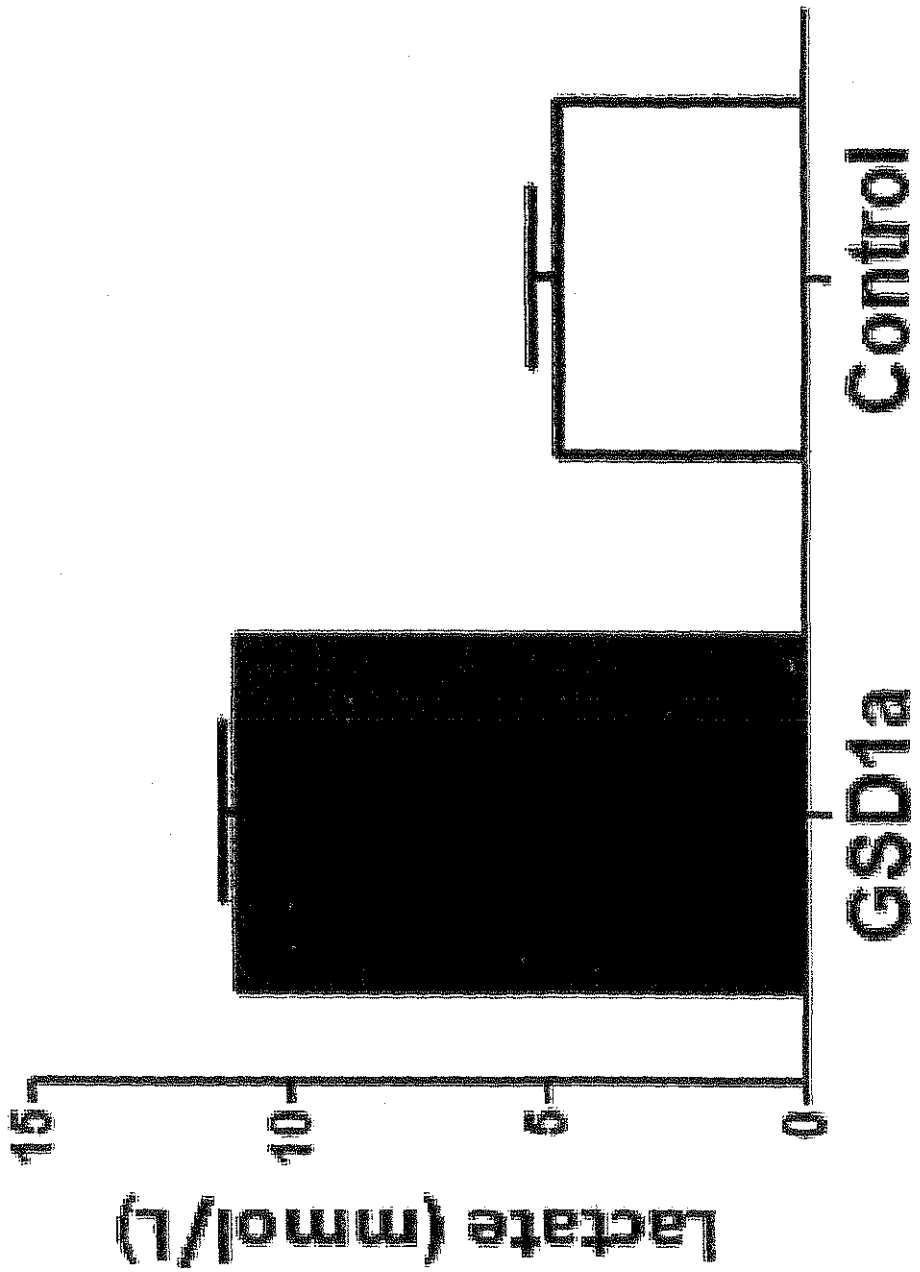


Figure 17

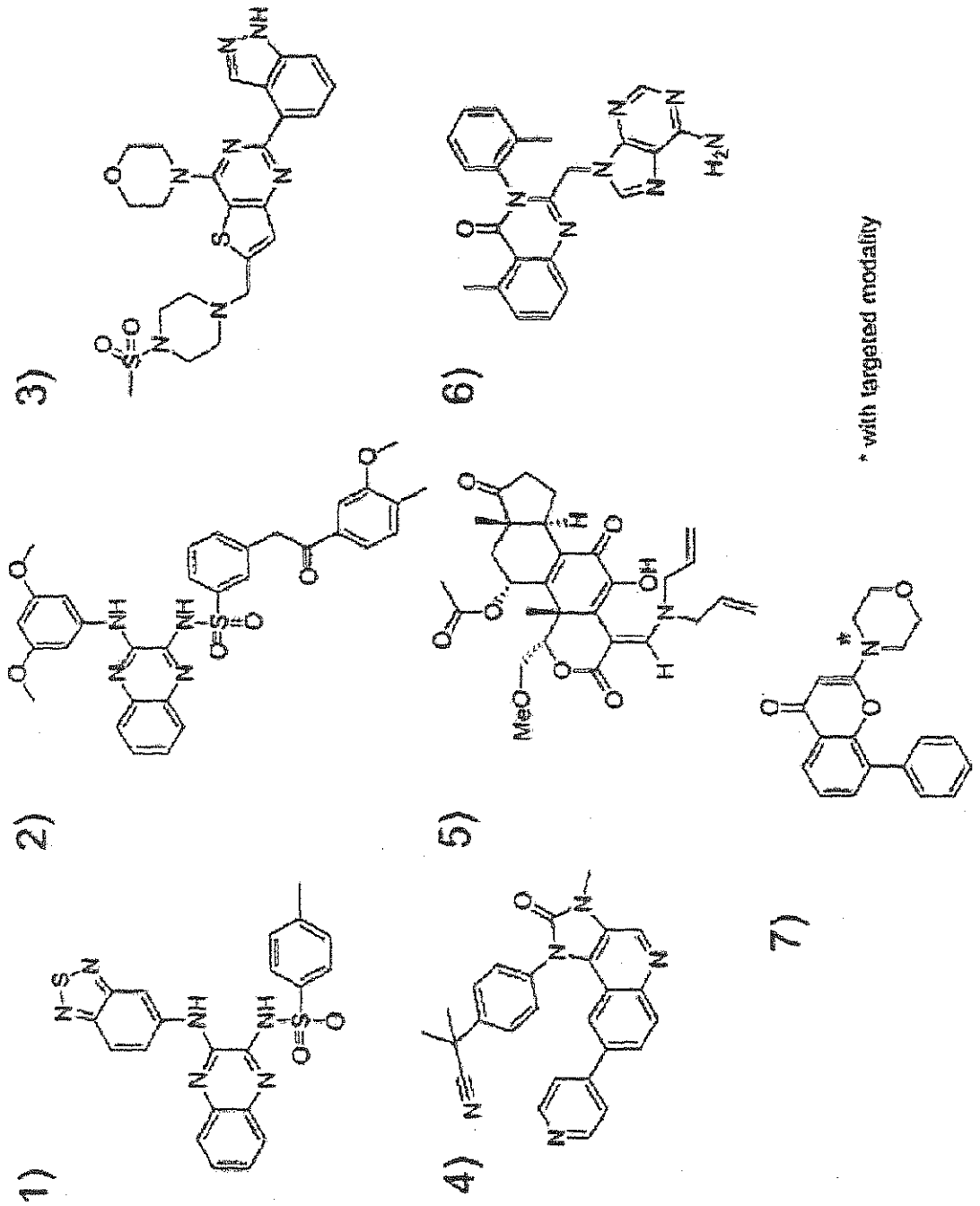


Figure 18

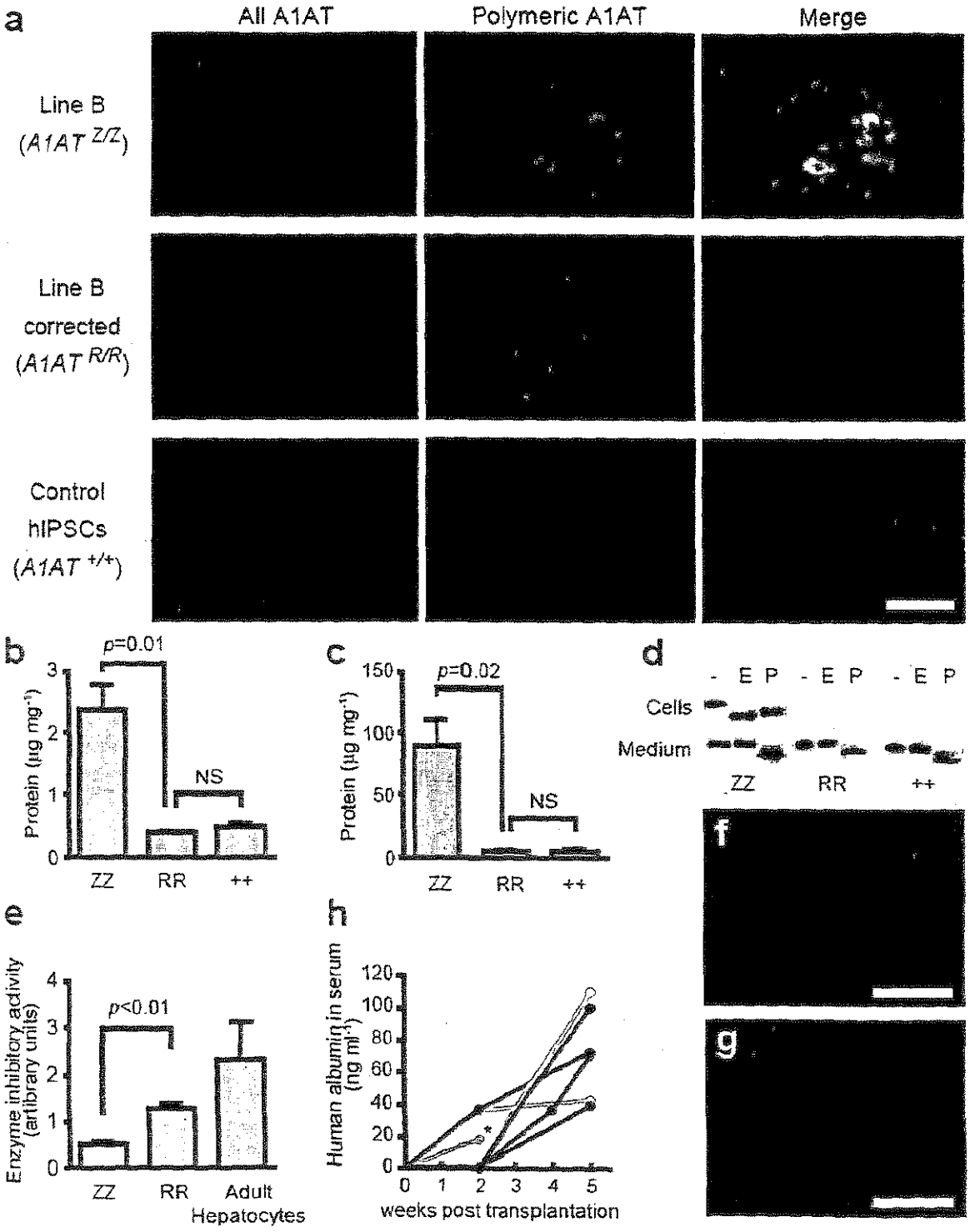

a
All A1AT
Polymeric A1AT
Merge
Line B
(A1AT Z/Z)
Line B
corrected
(A1AT R/R)
Control
hIPSCs
(A1AT +/+)
b
p=0.01
NS
Protein (µg mg-1)
ZZ
RR
++
c
p=0.02
NS
Protein (µg mg-1)
ZZ
RR
++
d
- E P - E P - E P
Cells
Medium
ZZ
RR
++
e
p<0.01
Enzyme inhibitory activity (arbitrary units)
ZZ
RR
Adult Hepatocytes
h
Human albumin in serum (ng ml-1)
weeks post transplantation
f
g

Figure 19