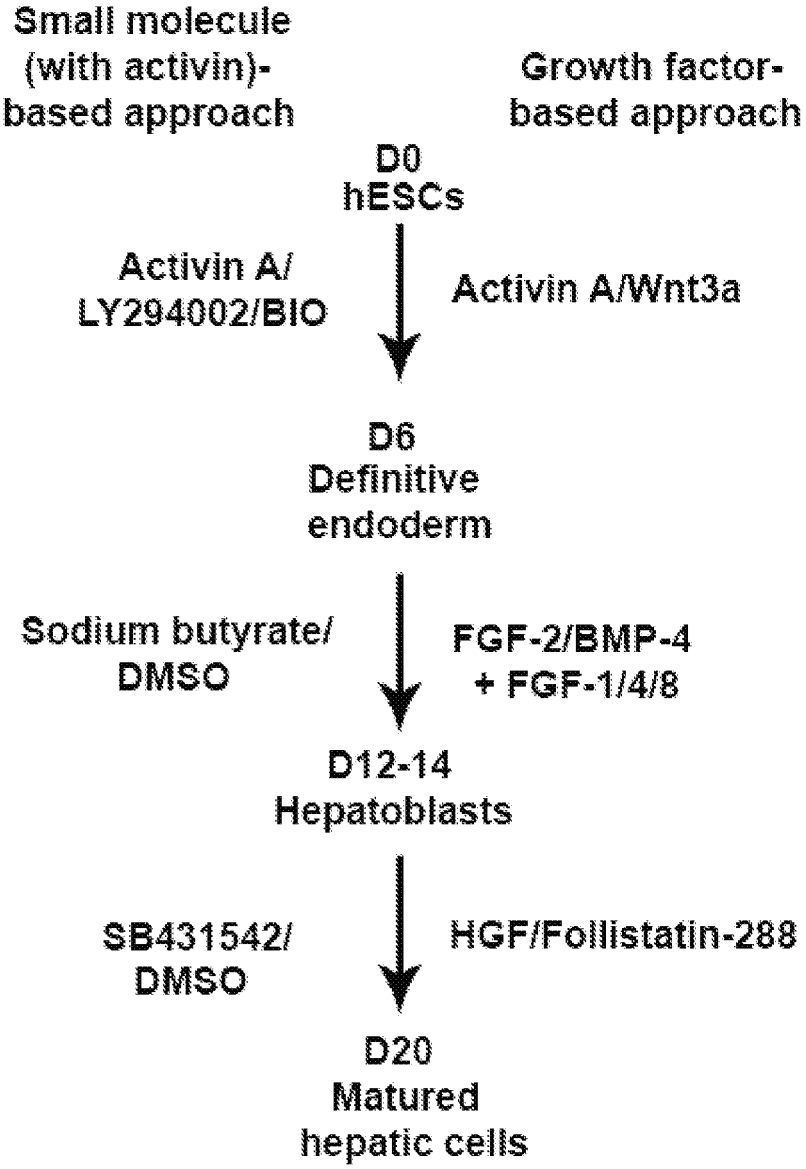

Small molecule (with activin)-based approach
Growth factor-based approach
D0
hESCs
Activin A/ LY294002/BIO
Activin A/Wnt3a
D6
Definitive endoderm
Sodium butyrate/ DMSO
FGF-2/BMP-4 + FGF-1/4/8
D12-14
Hepatoblasts
SB431542/ DMSO
HGF/Follistatin-288
D20
Matured hepatic cells

Figure 1

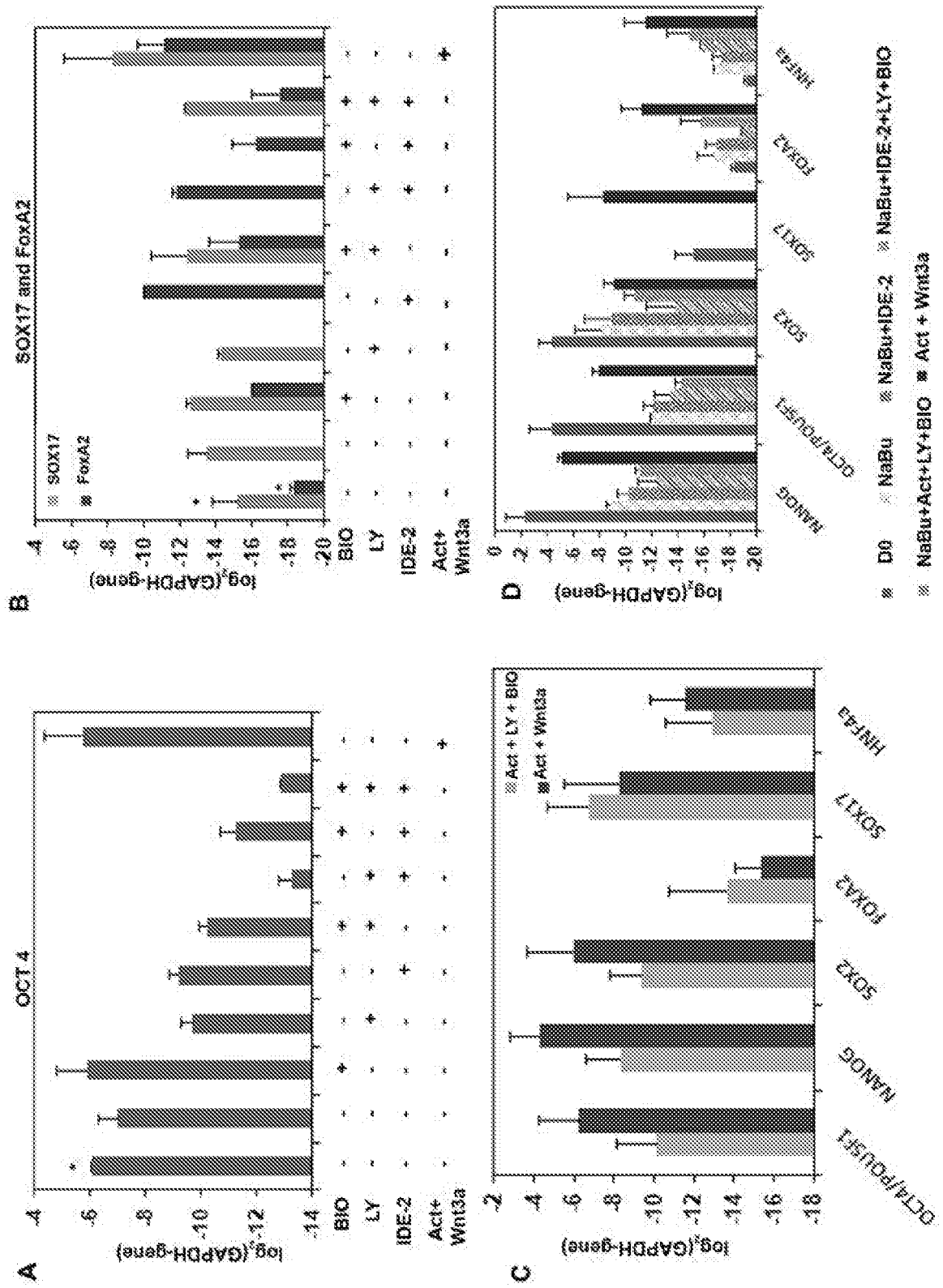

A
OCT 4
log2(GAPDH-gene)
BIO
LY
IDE-2
Act+ Wnt3a
B
SOX17 and FoxA2
SOX17
FoxA2
log2(GAPDH-gene)
C
Act + LY + BIO
Act + Wnt3a
OCT4/POU5F1
NANOG
SOX2
FOXA2
SOX17
HNF4A
D
log2(GAPDH-gene)
D0
NaBu
NaBu+IDE-2
NaBu+IDE-2+LY+BIO
NaBu+Act+LY+BIO
Act + Wnt3a

Figure 2

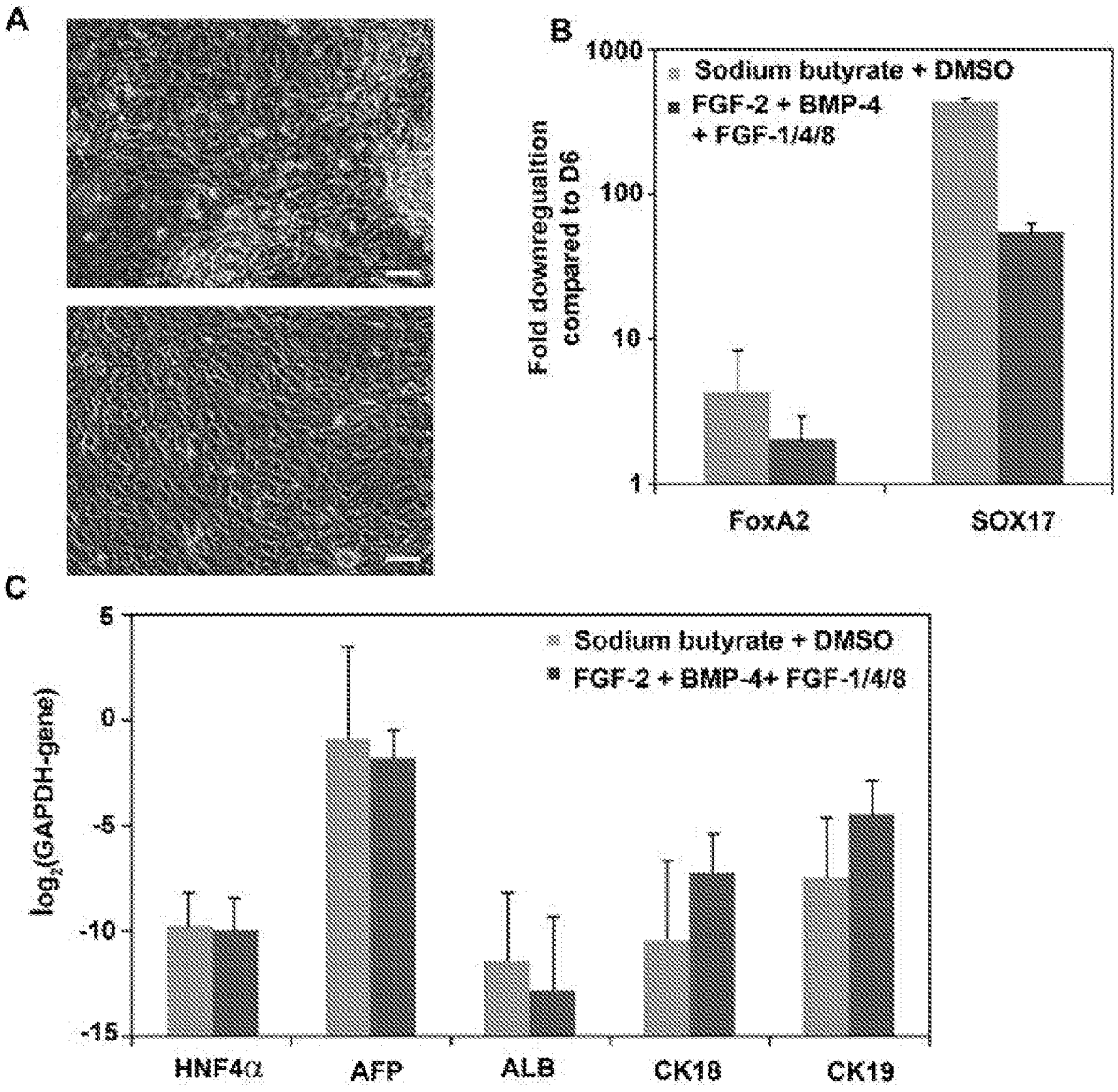

A
B
1000
100
10
1
Fold downregualtion compared to D6
Sodium butyrate + DMSO
FGF-2 + BMP-4 + FGF-1/4/8
FoxA2
SOX17
C
5
0
-5
-10
-15
log2(GAPDH-gene)
Sodium butyrate + DMSO
FGF-2 + BMP-4+ FGF-1/4/8
HNF4α
AFP
ALB
CK18
CK19

Figure 3

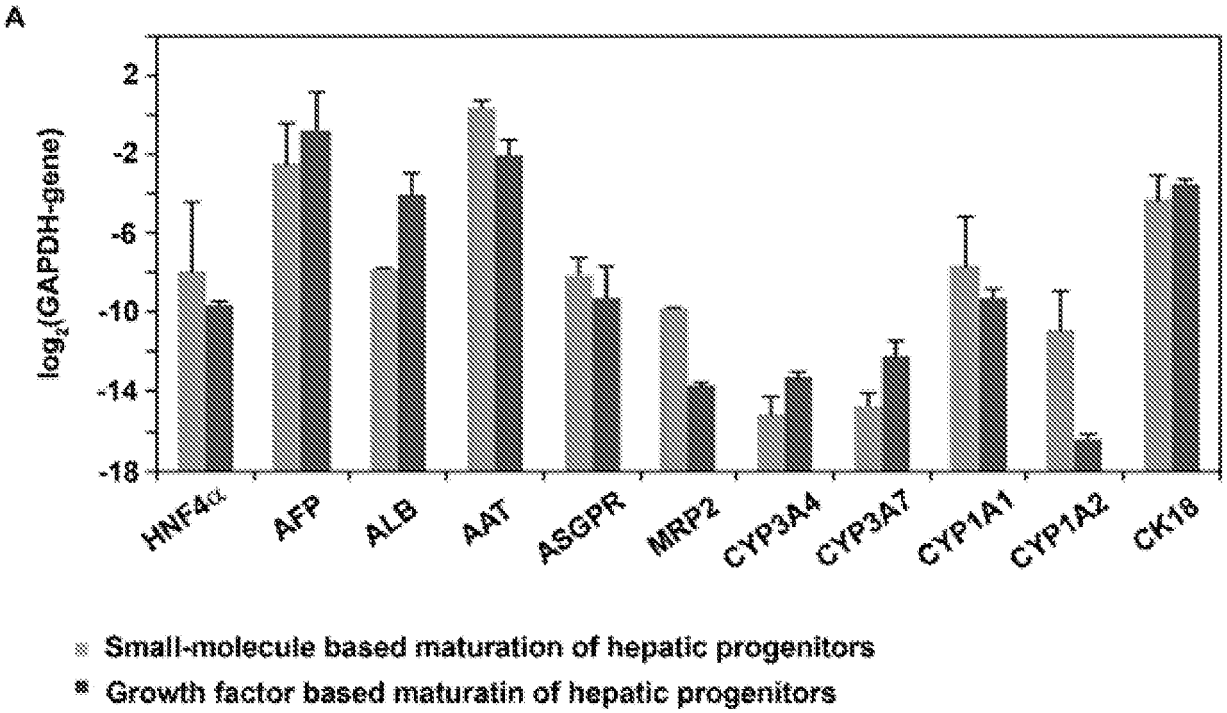

A
log2(GAPDH-gene)
2
-2
-6
-10
-14
-18
HNF4α
AFP
ALB
AAT
ASGPR
MRP2
CYP3A4
CYP3A7
CYP1A1
CYP1A2
CK18
Small-molecule based maturation of hepatic progenitors
Growth factor based maturatin of hepatic progenitors

Figure 4

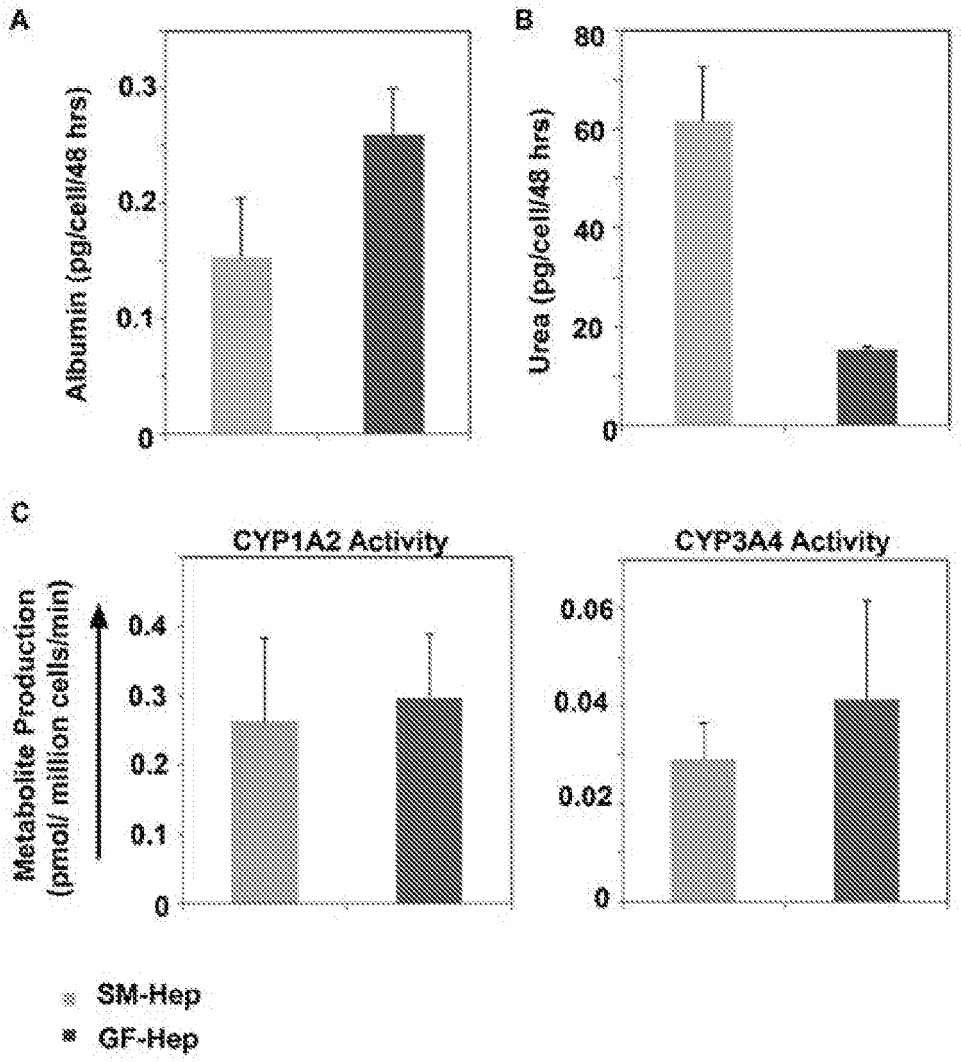

A
Albumin (pg/cell/48 hrs)
0.3
0.2
0.1
0
B
Urea (pg/cell/48 hrs)
80
60
40
20
0
C
Metabolite Production (pmol/ million cells/min)
CYP1A2 Activity
0.4
0.3
0.2
0.1
0
CYP3A4 Activity
0.06
0.04
0.02
0
SM-Hep
GF-Hep

Figure 5

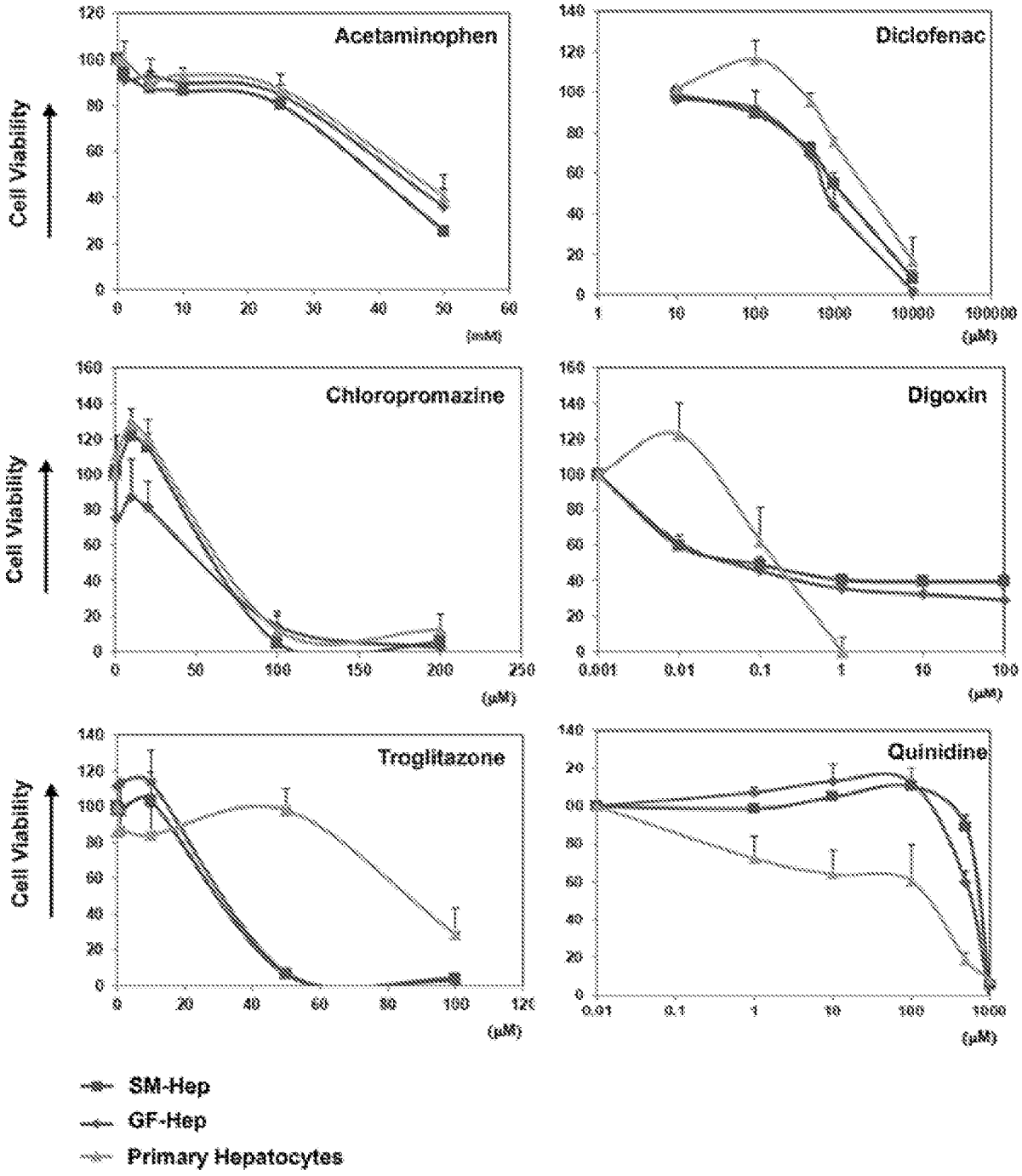

Acetaminophen
Diclofenac
Chloropromazine
Digoxin
Troglitazone
Quinidine
Cell Viability
(mM)
(μM)
SM-Hep
GF-Hep
Primary Hepatocytes

Figure 6

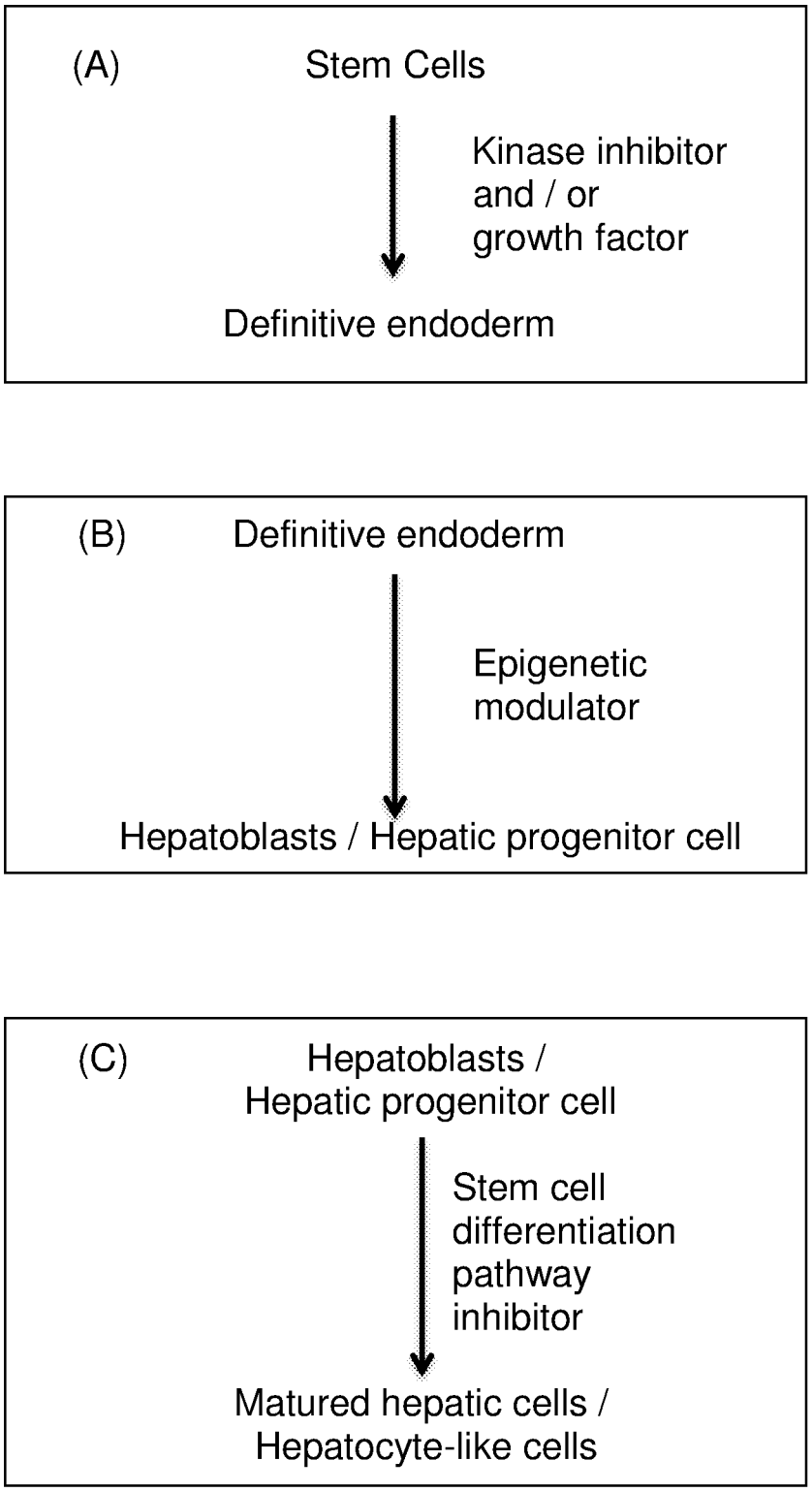

(A)
Stem Cells
Kinase inhibitor
and / or
growth factor
Definitive endoderm
(B)
Definitive endoderm
Epigenetic
modulator
Hepatoblasts / Hepatic progenitor cell
(C)
Hepatoblasts /
Hepatic progenitor cell
Stem cell
differentiation
pathway
inhibitor
Matured hepatic cells /
Hepatocyte-like cells

Figure 7

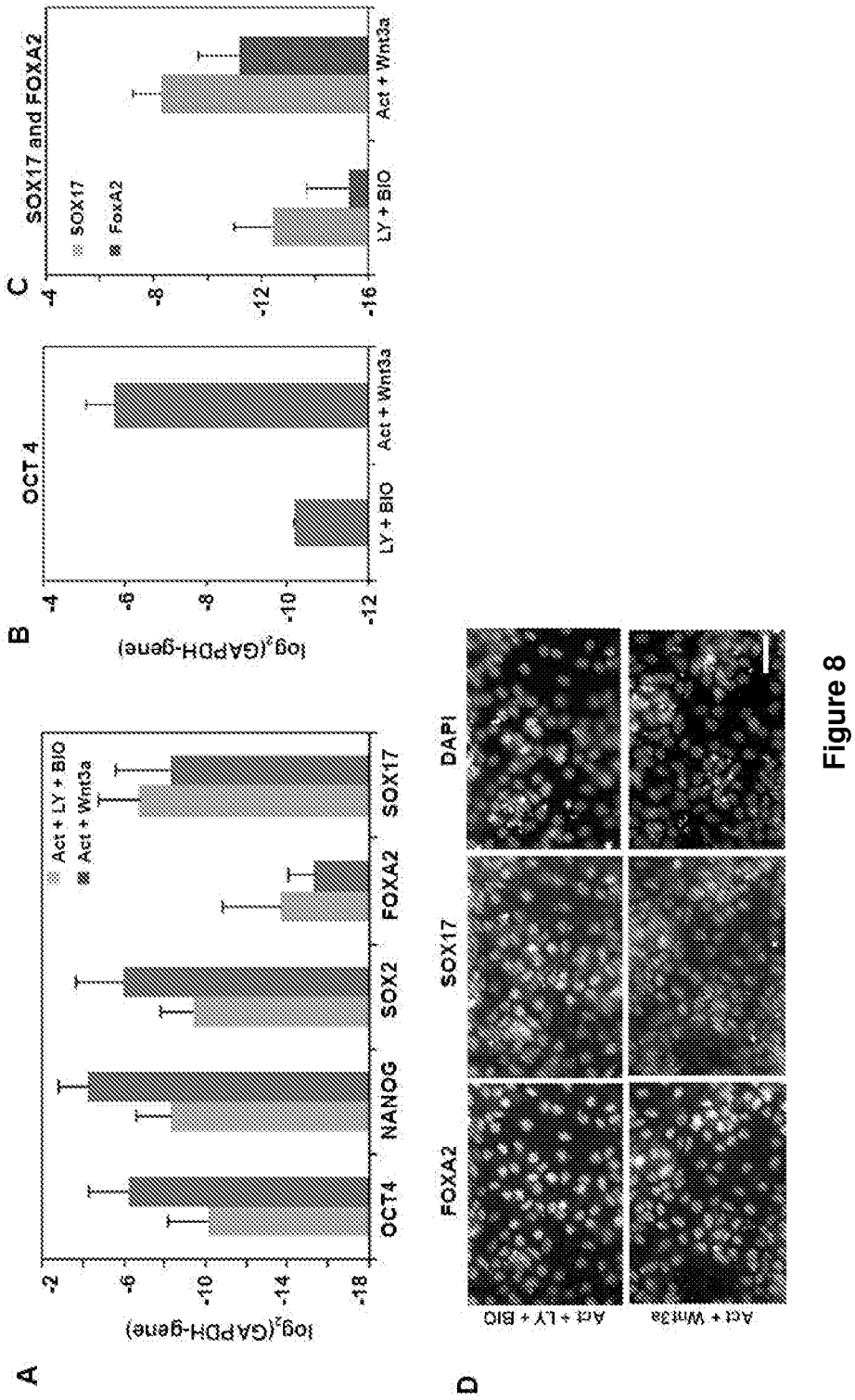

A
log2(GAPDH-gene)
-2
-6
-10
-14
-18
Act + LY + BIO
Act + Wnt3a
OCT4
NANOG
SOX2
FOXA2
SOX17
B
OCT 4
log2(GAPDH-gene)
-4
-6
-8
-10
-12
LY + BIO
Act + Wnt3a
C
SOX17 and FOXA2
SOX17
FoxA2
-4
-8
-12
-16
LY + BIO
Act + Wnt3a
D
DAPI
SOX17
FOXA2
Act + LY + BIO
Act + Wnt3a

Figure 8

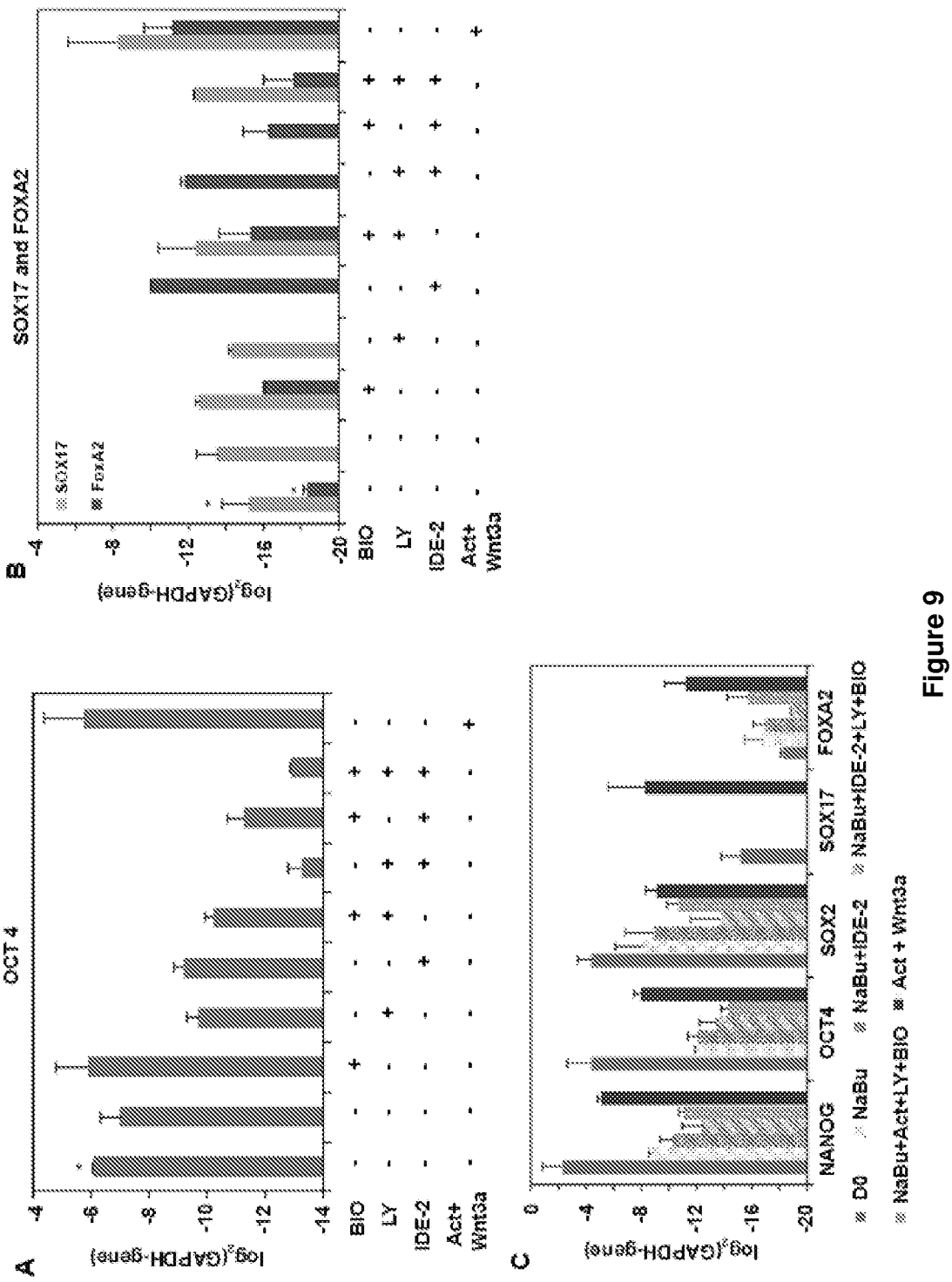

A
OCT 4
log2(GAPDH-gene)
-4
-6
-8
-10
-12
-14
BIO
LY
IDE-2
Act+ Wnt3a
B
SOX17 and FOXA2
SOX17
FoxA2
log2(GAPDH-gene)
-4
-8
-12
-16
-20
BIO
LY
IDE-2
Act+ Wnt3a
C
log2(GAPDH-gene)
0
-4
-8
-12
-16
-20
NANOG
OCT4
SOX2
SOX17
FOXA2
D0
NaBu
NaBu+IDE-2
NaBu+IDE-2+LY+BIO
NaBu+Act+LY+BIO
Act + Wnt3a

Figure 9

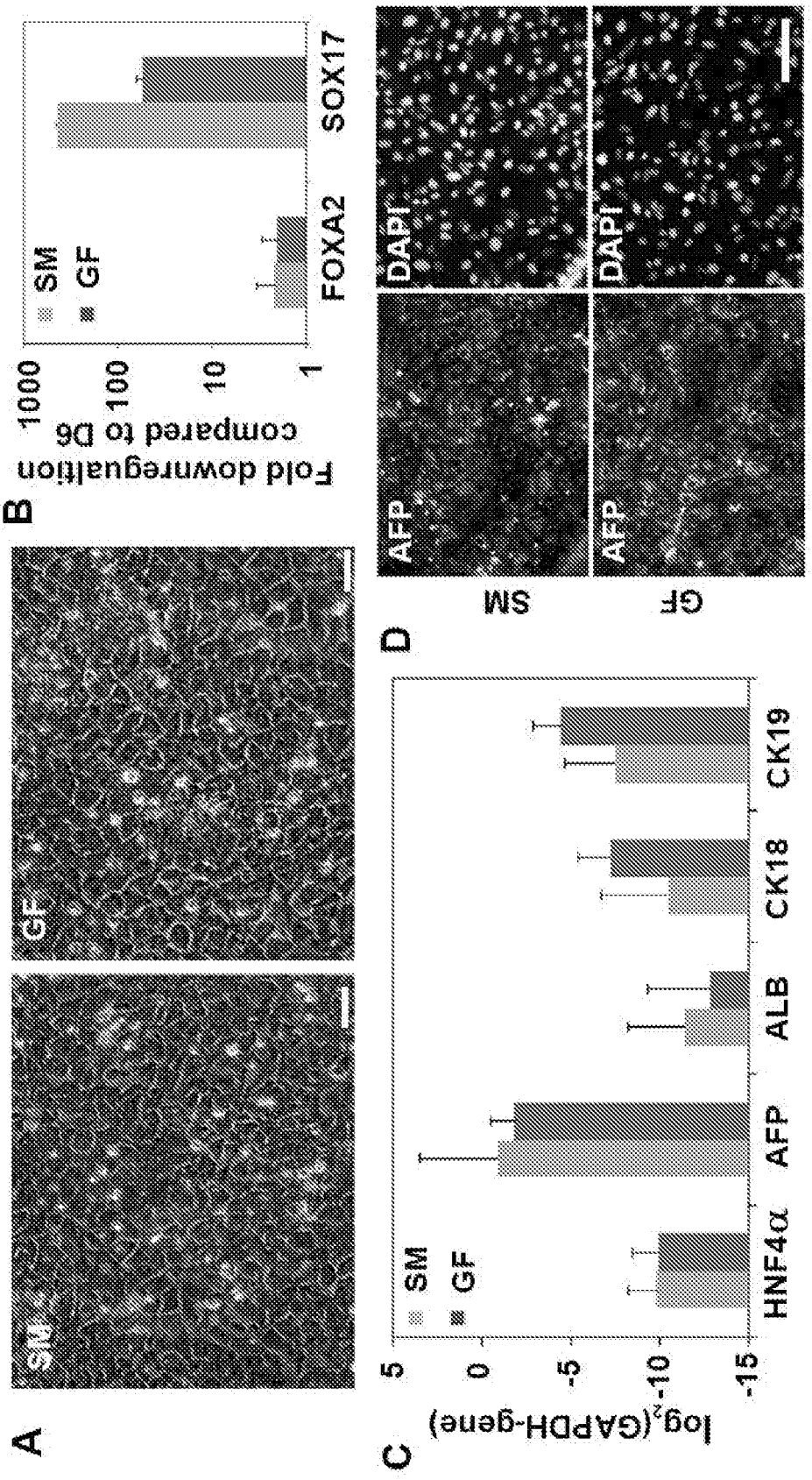

A
SM
GF
B
Fold downregulation compared to D6
1000
100
10
1
SM
GF
FOXA2
SOX17
C
log2(GAPDH-gene)
5
0
-5
-10
-15
SM
GF
HNF4α
AFP
ALB
CK18
CK19
D
SM
GF
AFP
DAPI
AFP
DAPI

Figure 10

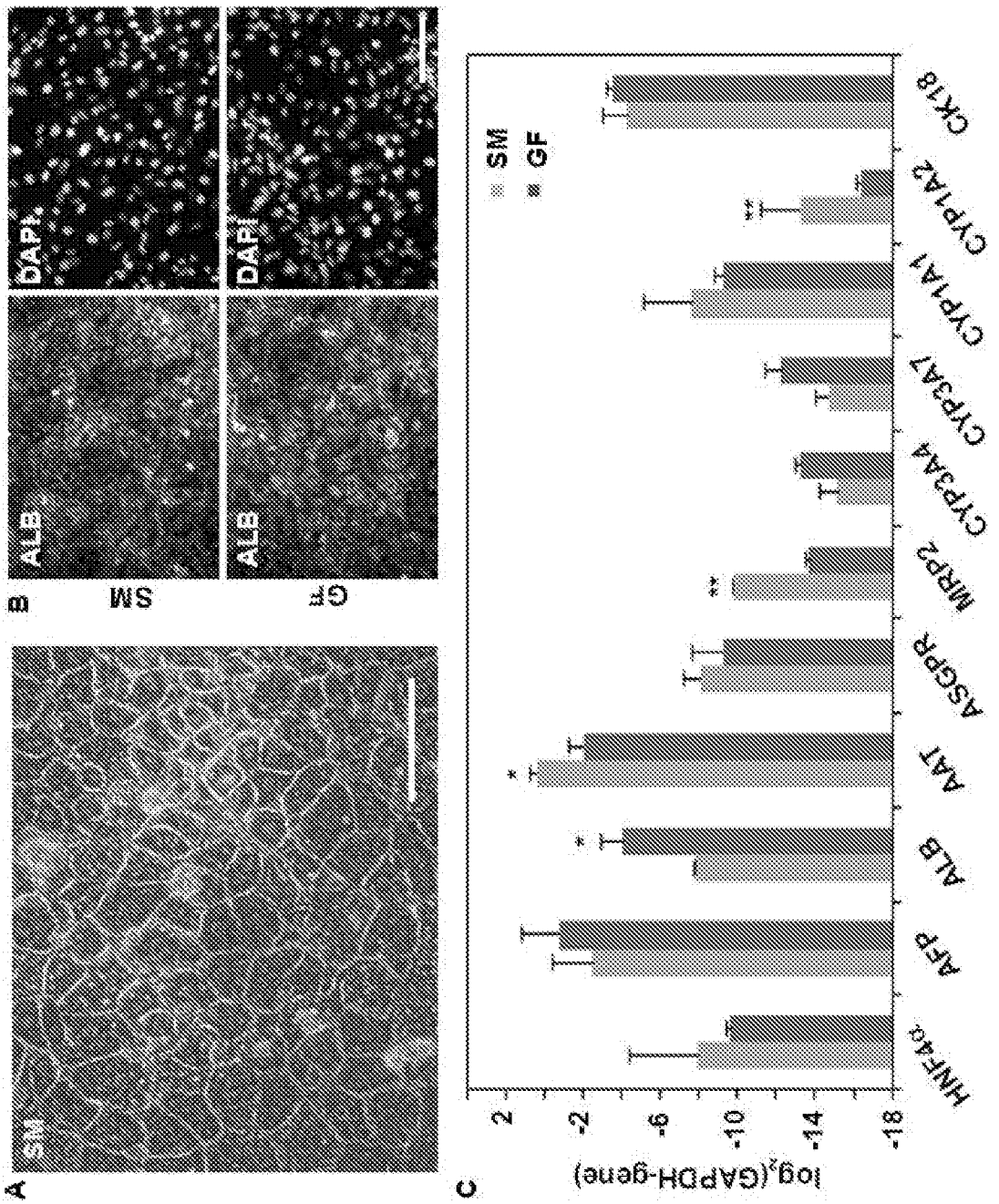

A
SM
B
SM
GF
DAPI
ALB
C
SM
GF
log2(GAPDH-gene)
2
-2
-6
-10
-14
-18
HNF4α
AFP
ALB
AAT
ASGPR
MRP2
CYP3A4
CYP3A7
CYP1A1
CYP1A2
CK18

Figure 11

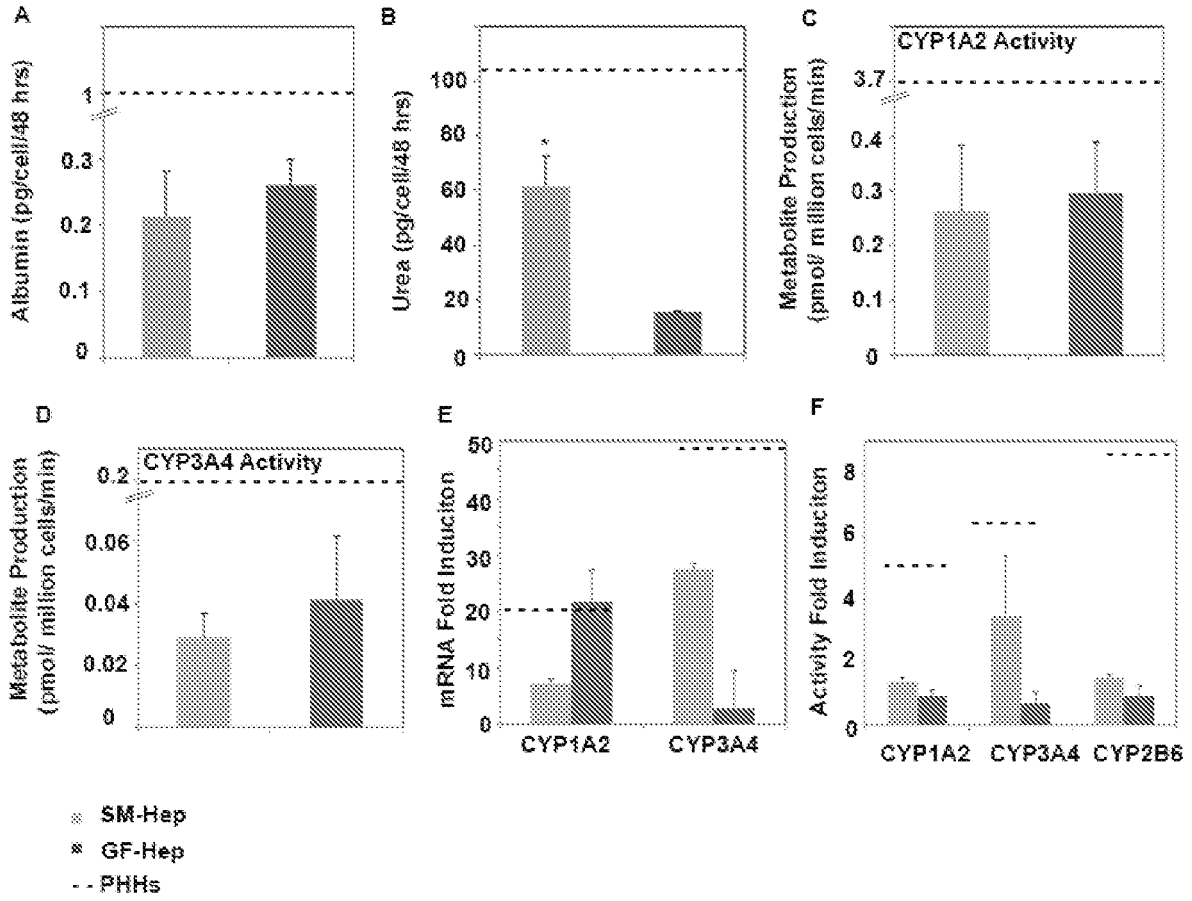

A
Albumin (pg/cell/48 hrs)
1
0.3
0.2
0.1
0
B
Urea (pg/cell/48 hrs)
100
80
60
40
20
0
C
CYP1A2 Activity
Metabolite Production (pmol/ million cells/min)
3.7
0.4
0.3
0.2
0.1
0
D
CYP3A4 Activity
Metabolite Production (pmol/ million cells/min)
0.2
0.06
0.04
0.02
0
E
mRNA Fold Induciton
50
40
30
20
10
0
CYP1A2
CYP3A4
F
Activity Fold Induciton
8
6
4
2
0
CYP1A2
CYP3A4
CYP2B6
SM-Hep
GF-Hep
PHHs

Figure 12

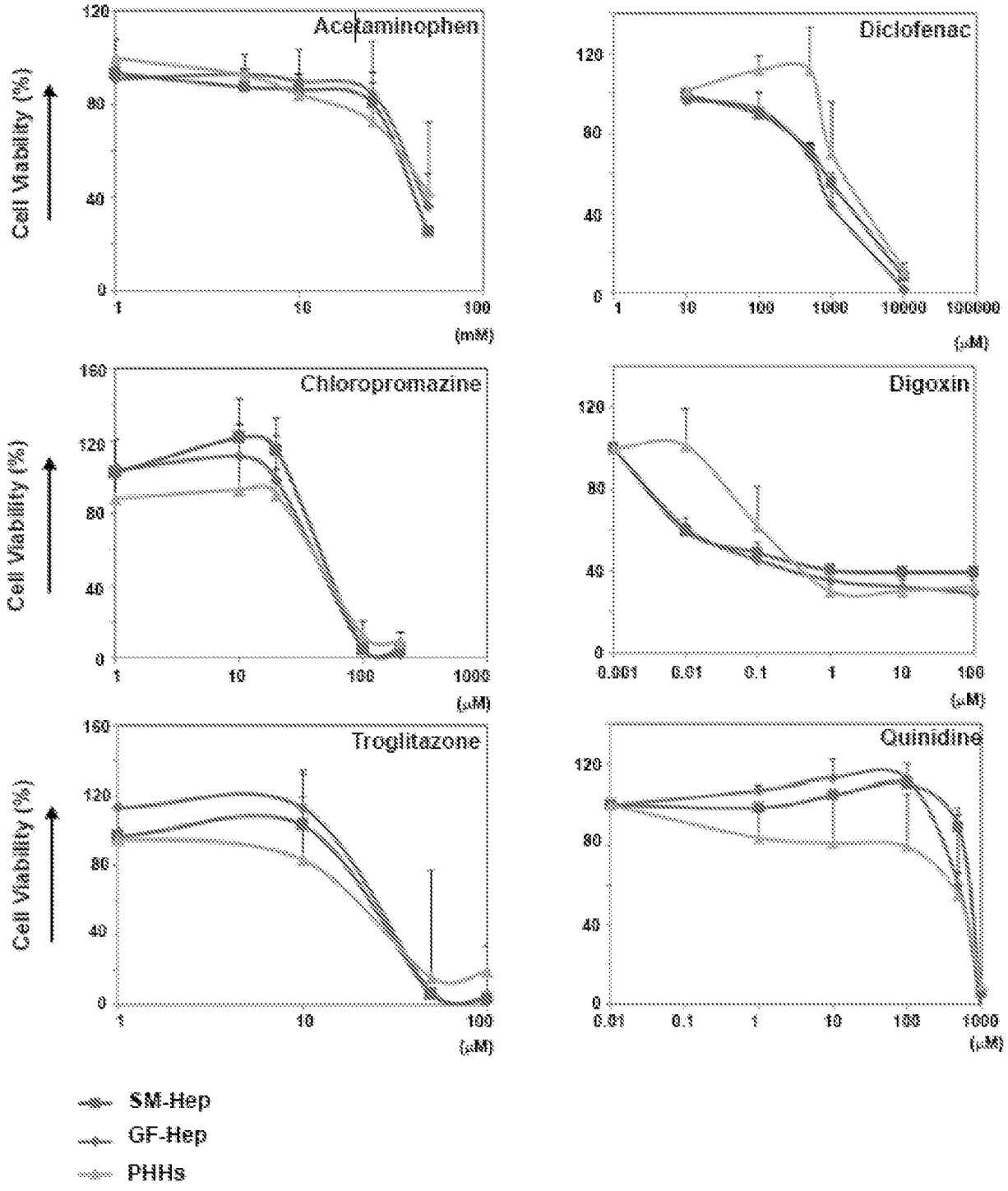

Acetaminophen
Diclofenac
Chloropromazine
Digoxin
Troglitazone
Quinidine
Cell Viability (%)
(mM)
(μM)
SM-Hep
GF-Hep
PHHs

Figure 13