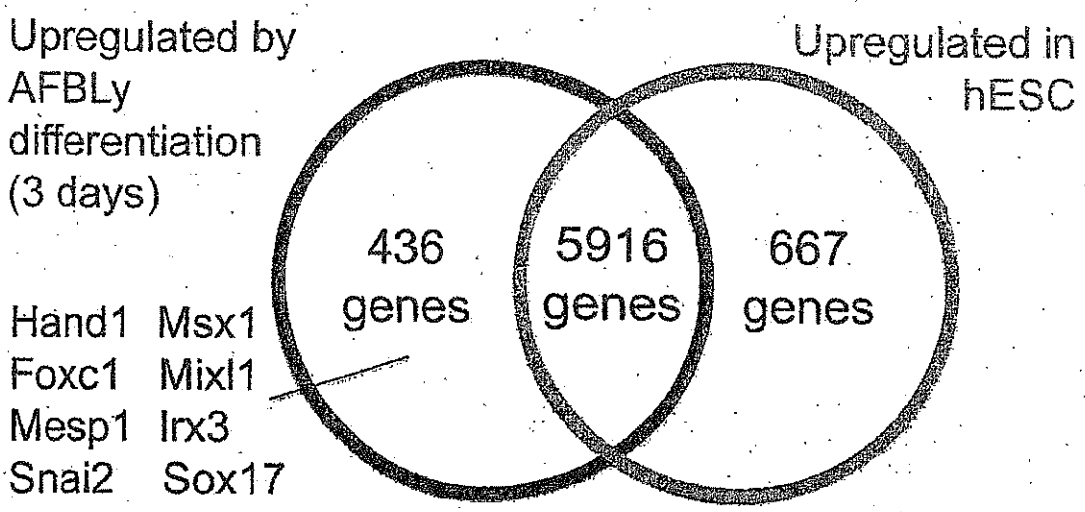
FIG. 1



GO functions:
Skeletal system development (P=3.3x10^{-10})
Mesoderm development (P=2.4x10^{-9})
Vasculature development (P=1.6x10^{-8})
Heart development (P=6.6x10^{-8})

FIG. 2

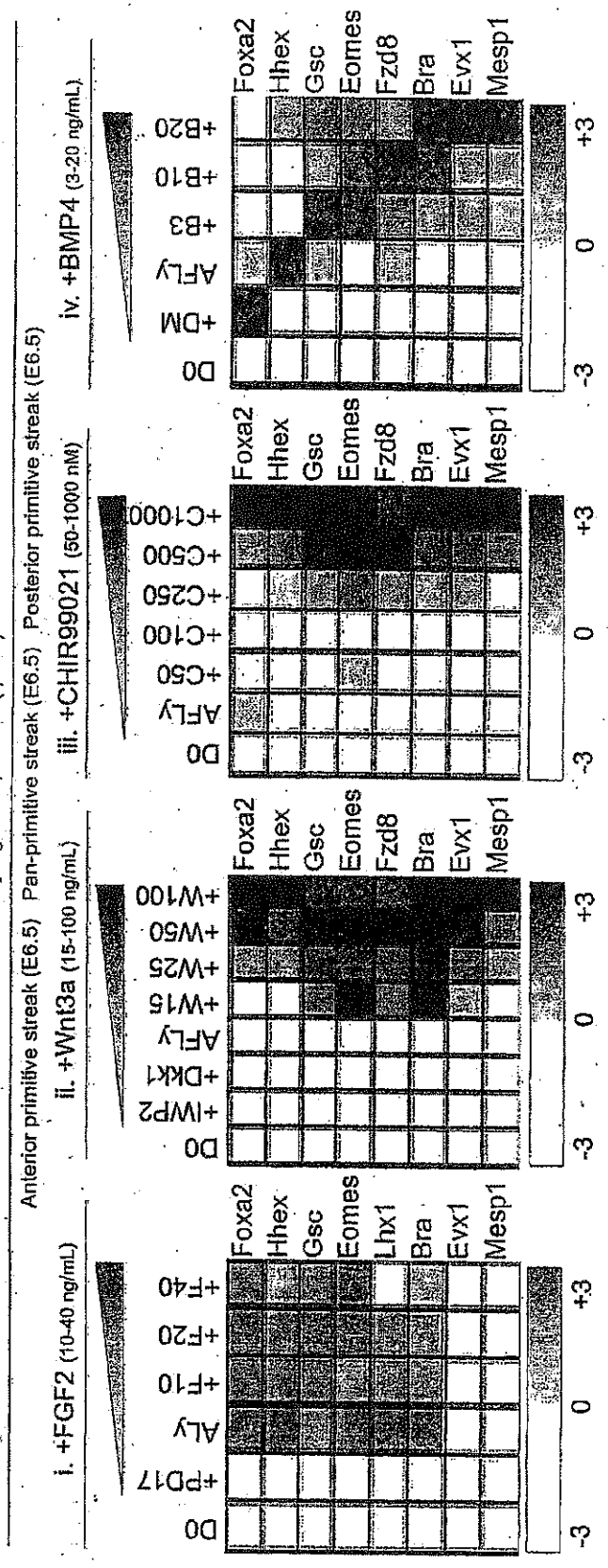

Day 1 gene expression (qPCR)
Anterior primitive streak (E6.5)
Pan-primitive streak (E6.5)
Posterior primitive streak (E6.5)
i. +FGF2 (10-40 ng/mL)
ii. +Wnt3a (15-100 ng/mL)
iii. +CHIR99021 (50-1000 nM)
iv. +BMP4 (3-20 ng/mL)
D0
+PD17
ALy
+F10
+F20
+F40
+IWP2
+DKK1
AFLy
+W15
+W25
+W50
+W100
+C50
+C100
+C250
+C500
+C1000
+DM
AFLy
+B3
+B10
+B20
Foxa2
Hhex
Gsc
Eomes
Lhx1
Bra
Evx1
Mesp1
Fzd8
-3
0
+3

FIG. 3

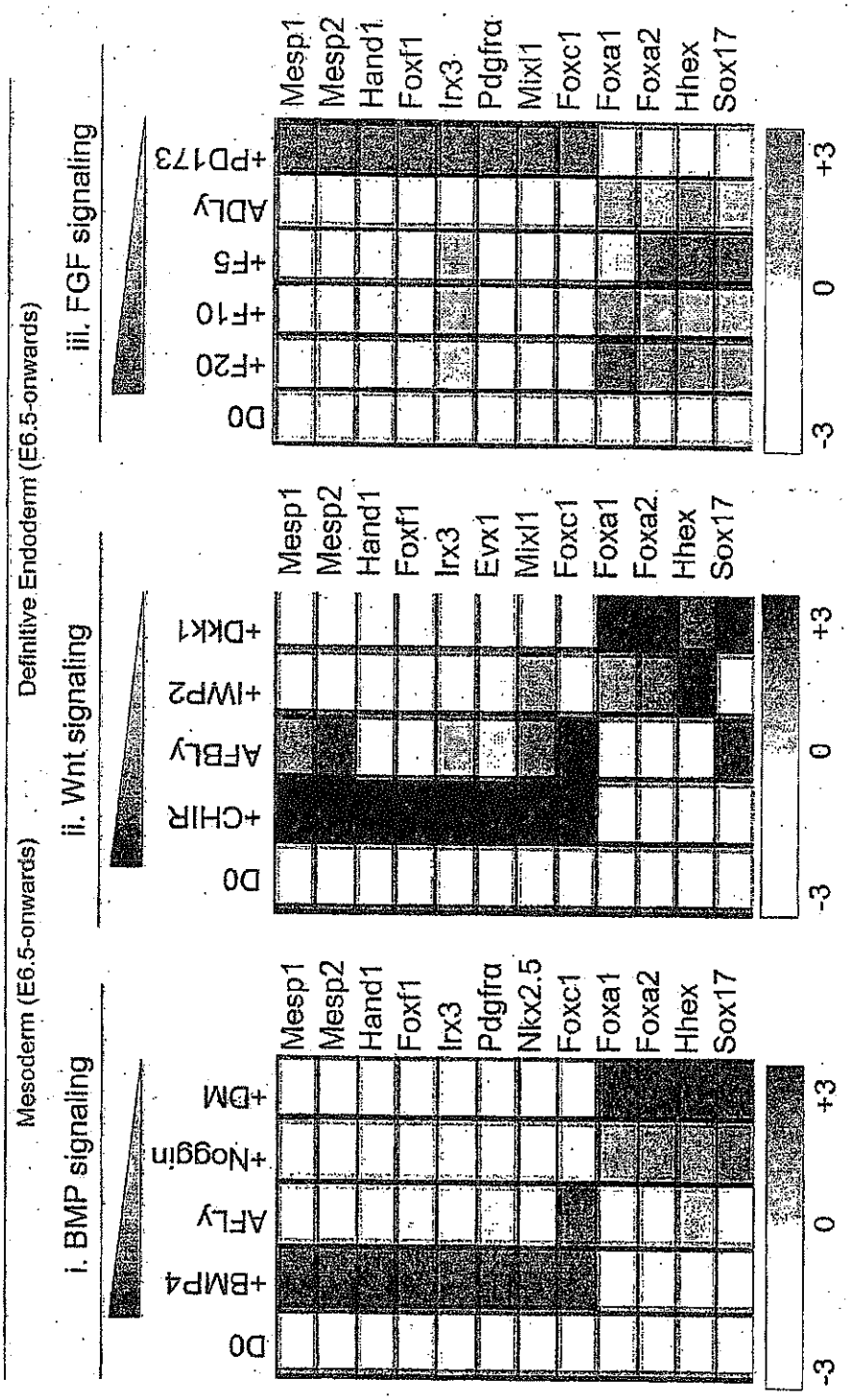
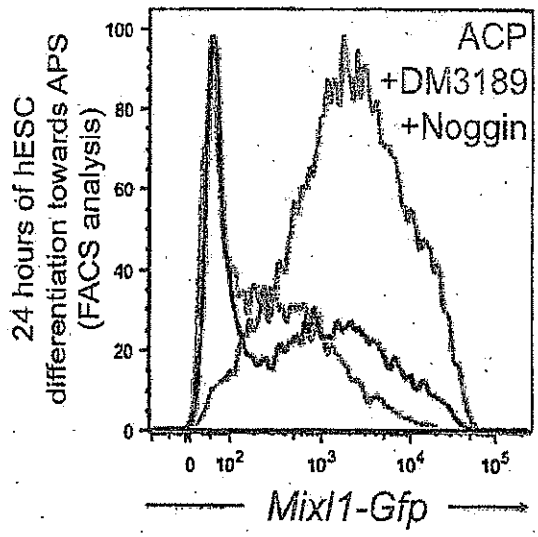

Day 3 gene expression (qPCR)
Mesoderm (E6.5-onwards)
Definitive Endoderm (E6.5-onwards)
i. BMP signaling
D0
+BMP4
AFLy
+Noggin
+DM
ii. Wnt signaling
D0
+CHIR
AFBLy
+IWP2
+Dkk1
iii. FGF signaling
D0
+F20
+F10
+F5
ADLy
+PD173
Mesp1
Mesp2
Hand1
Foxf1
Irx3
Pdgfra
Nkx2.5
Foxc1
Foxa1
Foxa2
Hhex
Sox17
Evx1
Mixl1
-3
0
+3

FIG. 4

i. BMP is essential for APS induction



ii. Signaling input truth table

iii. Current signaling paradigm

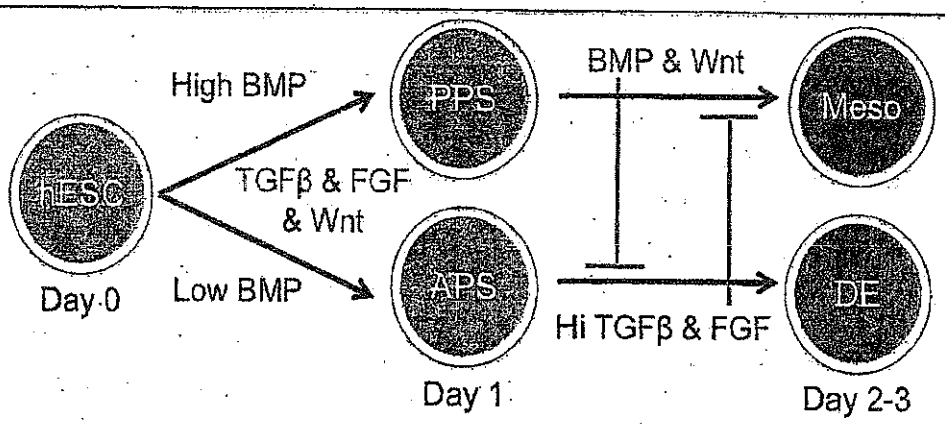


FIG. 5

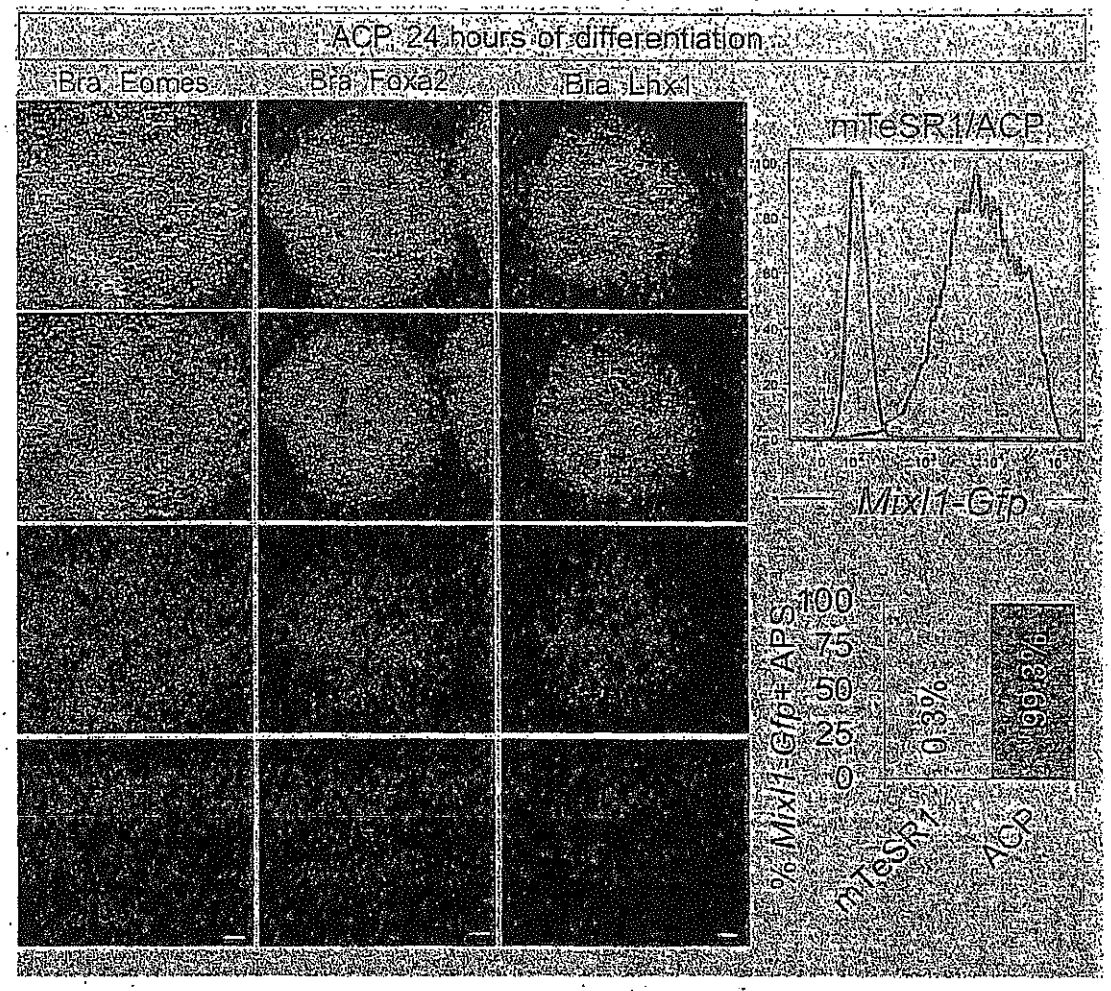

ACP, 24 hours of differentiation
Bra, Eomes
Bra, Foxa2
Bra, Lhx1
mTeSR1/ACP
Mixl1-Gfp
% Mixl1-Gfp+ APS
100
75
50
25
0
mTeSR1
ACP
0.3%
99.6%

FIG. 6

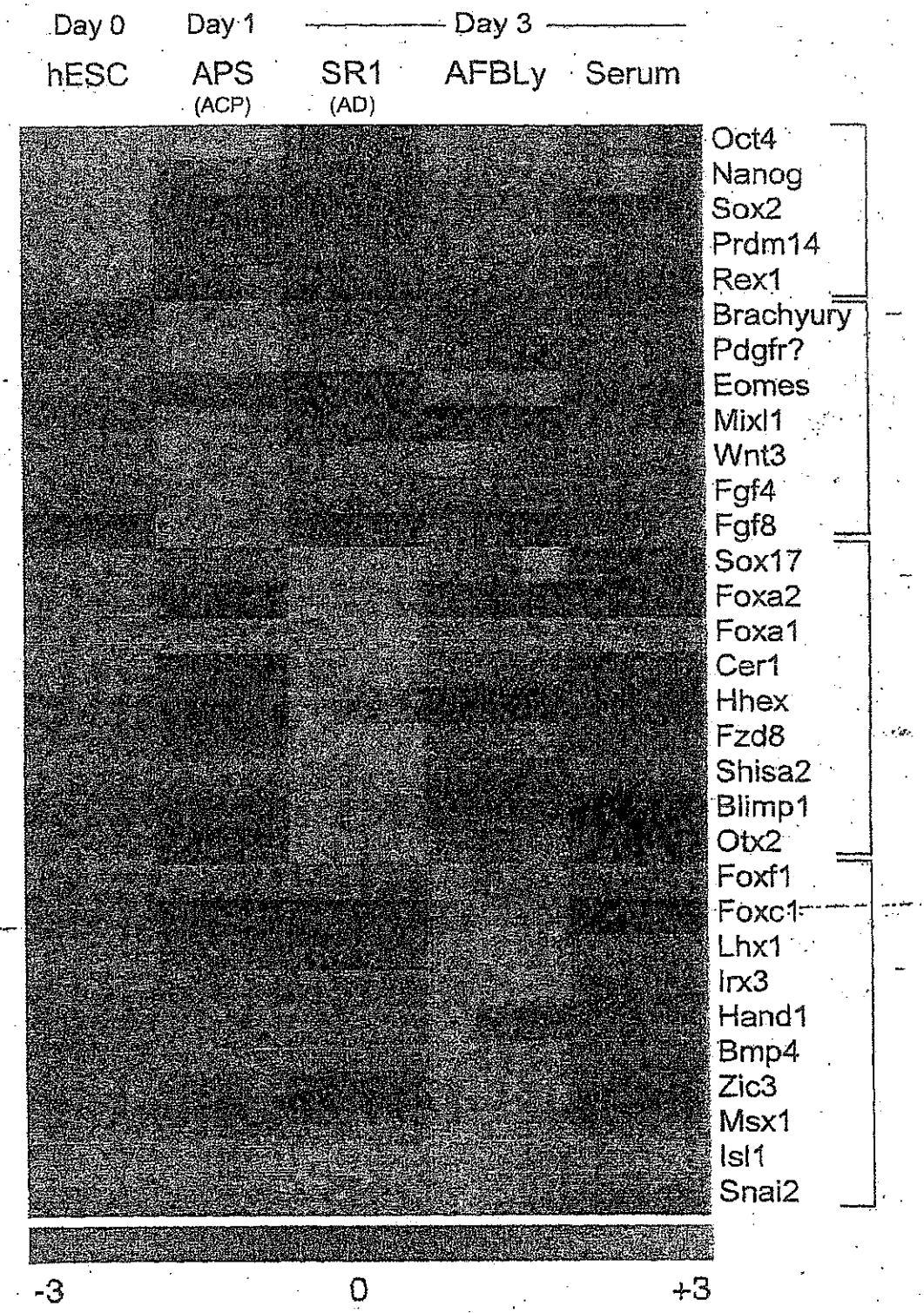
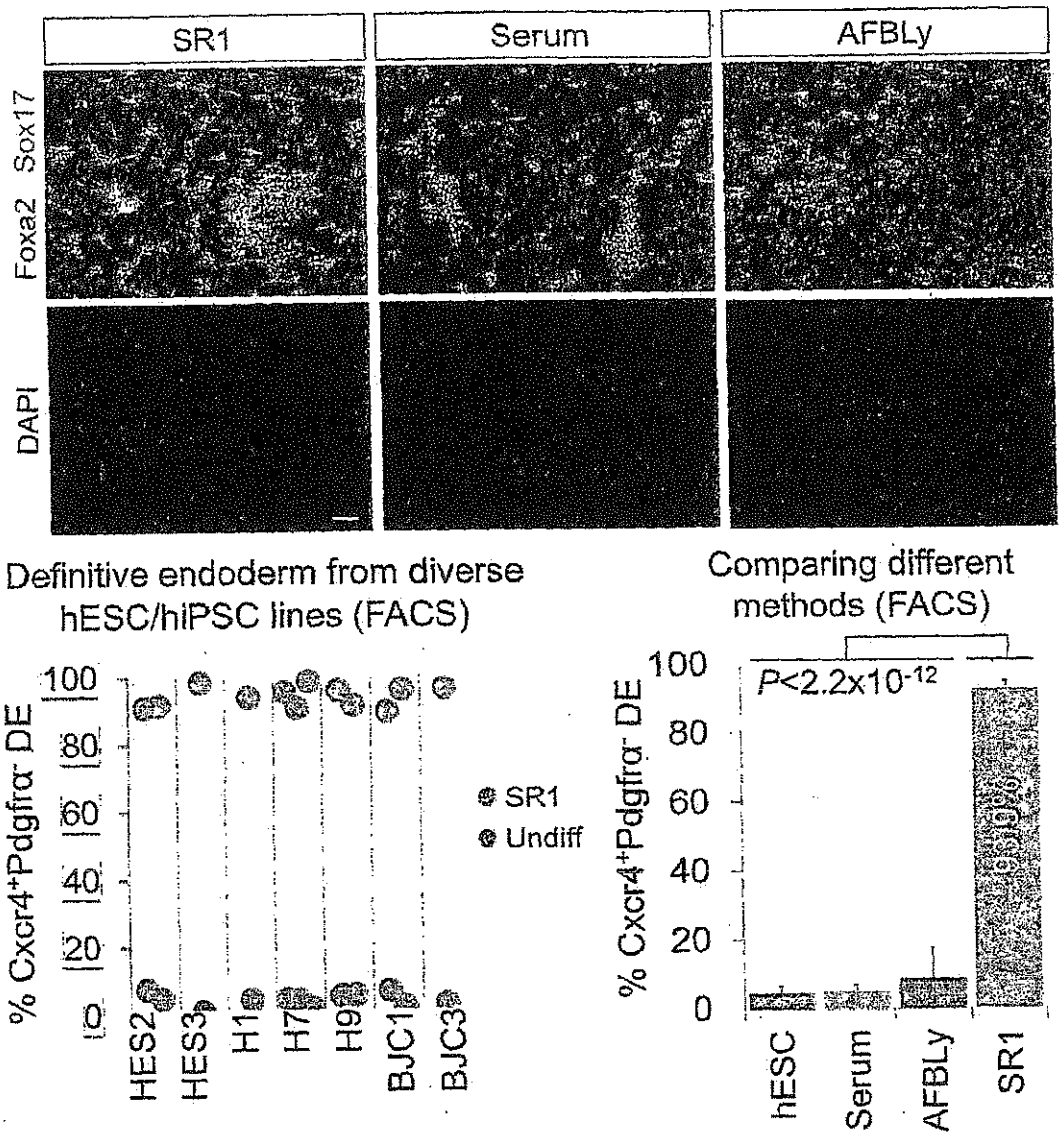

Day 0
Day 1
Day 3
hESC
APS
(ACP)
SR1
(AD)
AFBLy
Serum
Oct4
Nanog
Sox2
Prdm14
Rex1
Brachyury
Pdgfr?
Eomes
Mixl1
Wnt3
Fgf4
Fgf8
Sox17
Foxa2
Foxa1
Cer1
Hhex
Fzd8
Shisa2
Blimp1
Otx2
Foxf1
Foxc1
Lhx1
Irx3
Hand1
Bmp4
Zic3
Msx1
Isl1
Snai2
-3
0
+3

FIG. 7


SR1
Serum
AFBLy
Foxa2 Sox17
DAPI
Definitive endoderm from diverse hESC/hiPSC lines (FACS)
% Cxcr4+Pdgfrα- DE
100
80
60
40
20
0
HES2
HES3
H1
H7
H9
BJC1
BJC3
SR1
Undiff
Comparing different methods (FACS)
P<2.2x10-12
hESC
Serum
AFBLy
SR1

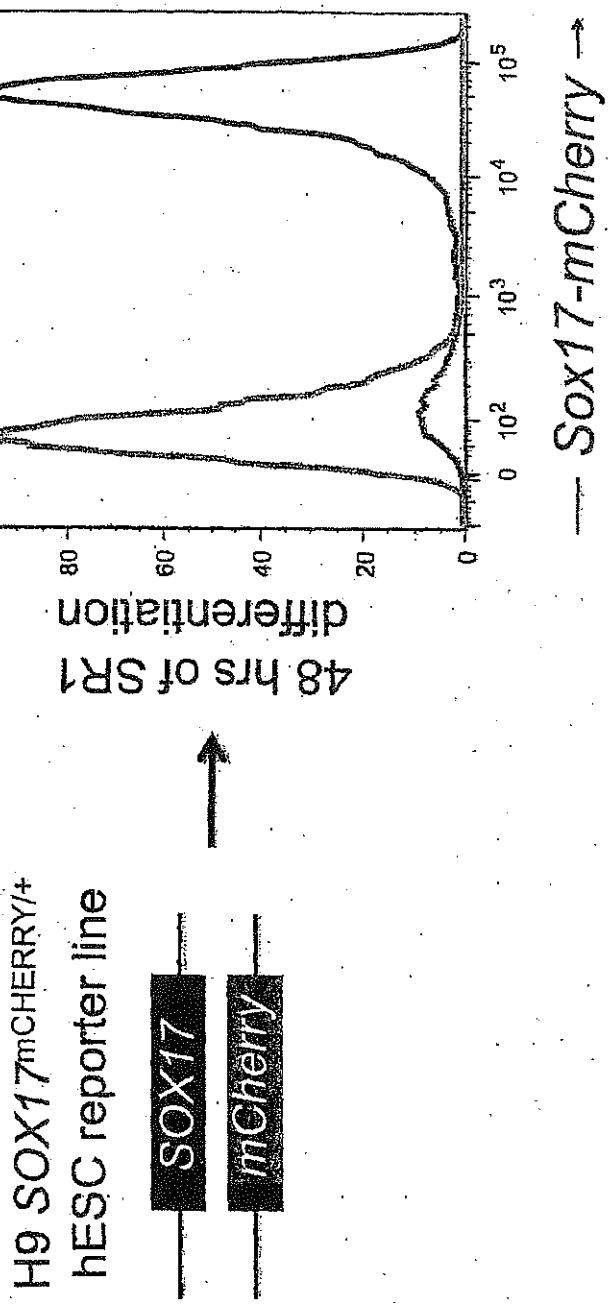

H9 SOX17mCHERRY/+
hESC reporter line
SOX17
mCherry
48 hrs of SR1
differentiation
Undifferentiated/SR1
1.79% vs. 90.6%
0
20
40
60
80
100
0
10^2
10^3
10^4
10^5
— Sox17-mCherry →

FIG. 8

FIG. 9

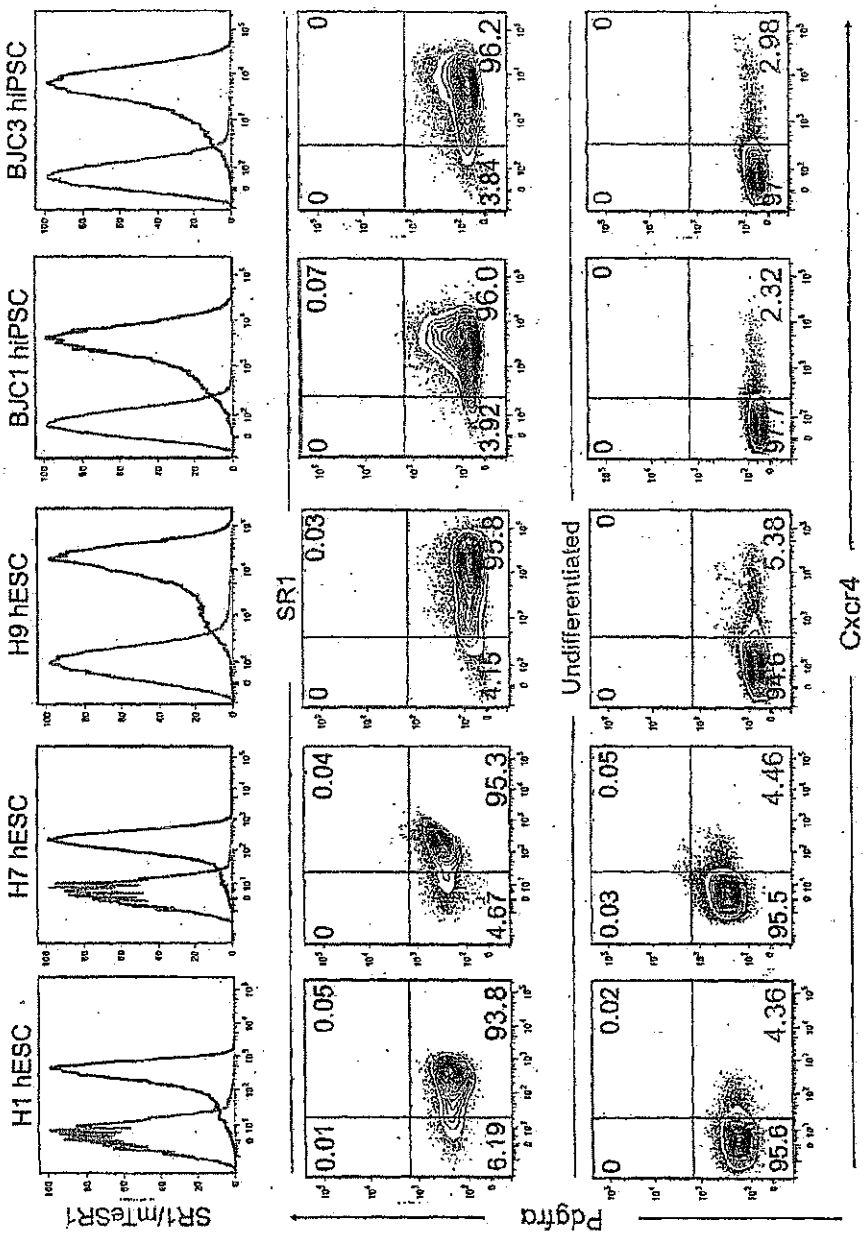

H1 hESC
H7 hESC
H9 hESC
BJC1 hiPSC
BJC3 hiPSC
SR1/mTeSR1
SR1
Undifferentiated
Pdgfra
Cxcr4
0.01
0.05
6.19
93.8
0.04
4.67
95.3
0.03
4.15
95.8
0.07
3.92
96.0
0.03
3.84
96.2
0.02
0.03
95.6
4.36
0.05
95.5
4.46
0.03
94.6
5.38
0.07
97.7
2.32
97
2.98

FIG. 10

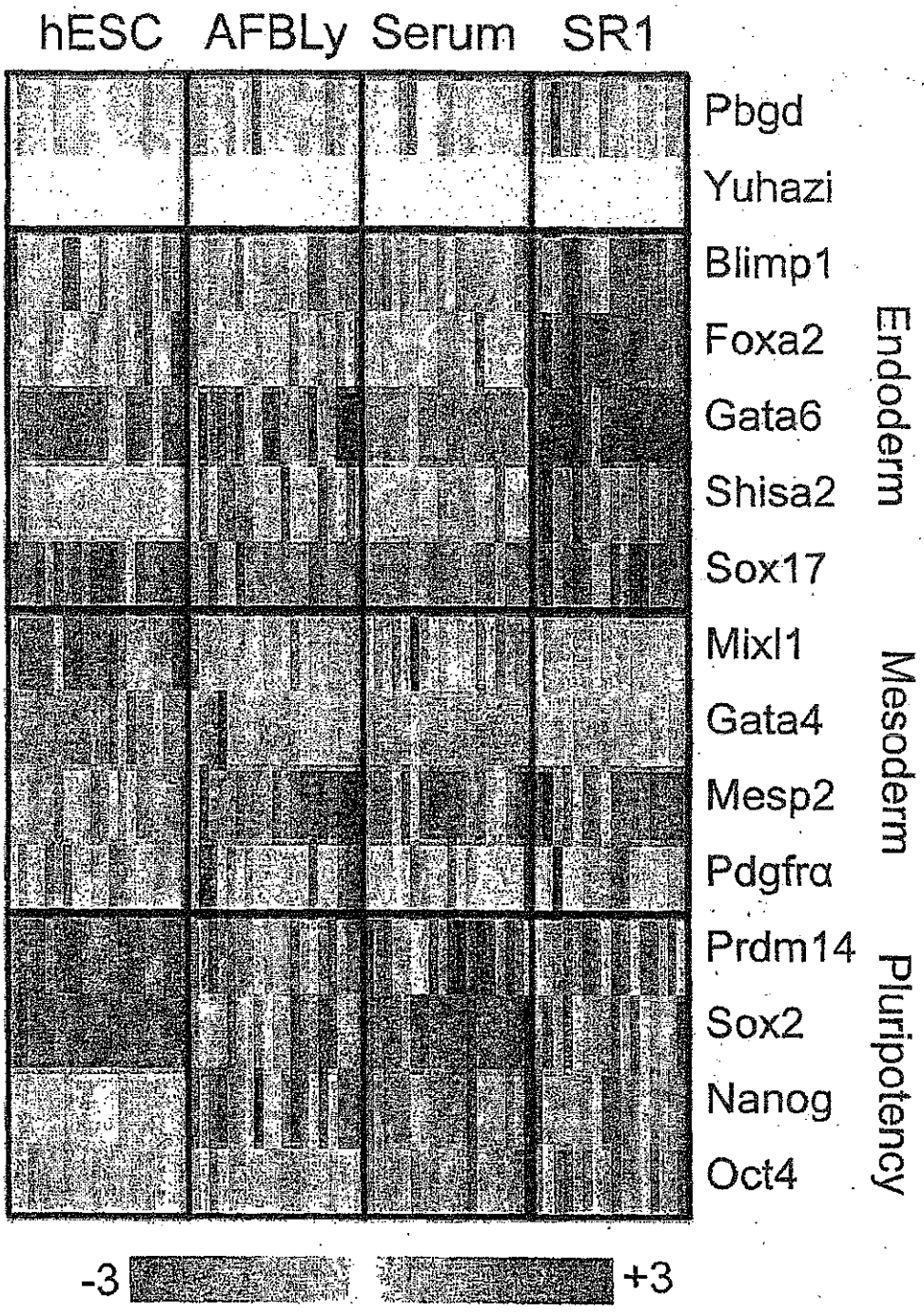

hESC
AFBLy
Serum
SR1
Pbgd
Yuhazi
Blimp1
Foxa2
Gata6
Shisa2
Sox17
Mixl1
Gata4
Mesp2
Pdgfrα
Prdm14
Sox2
Nanog
Oct4
Endoderm
Mesoderm
Pluripotency
-3
+3

FIG. 11

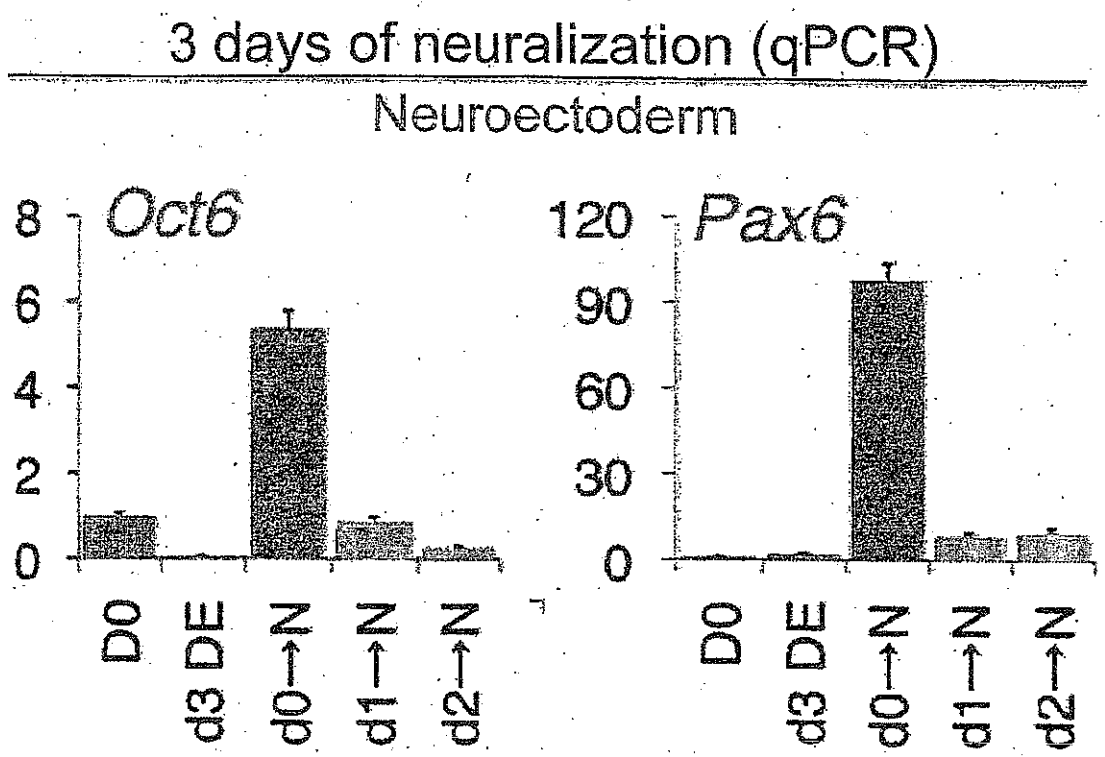
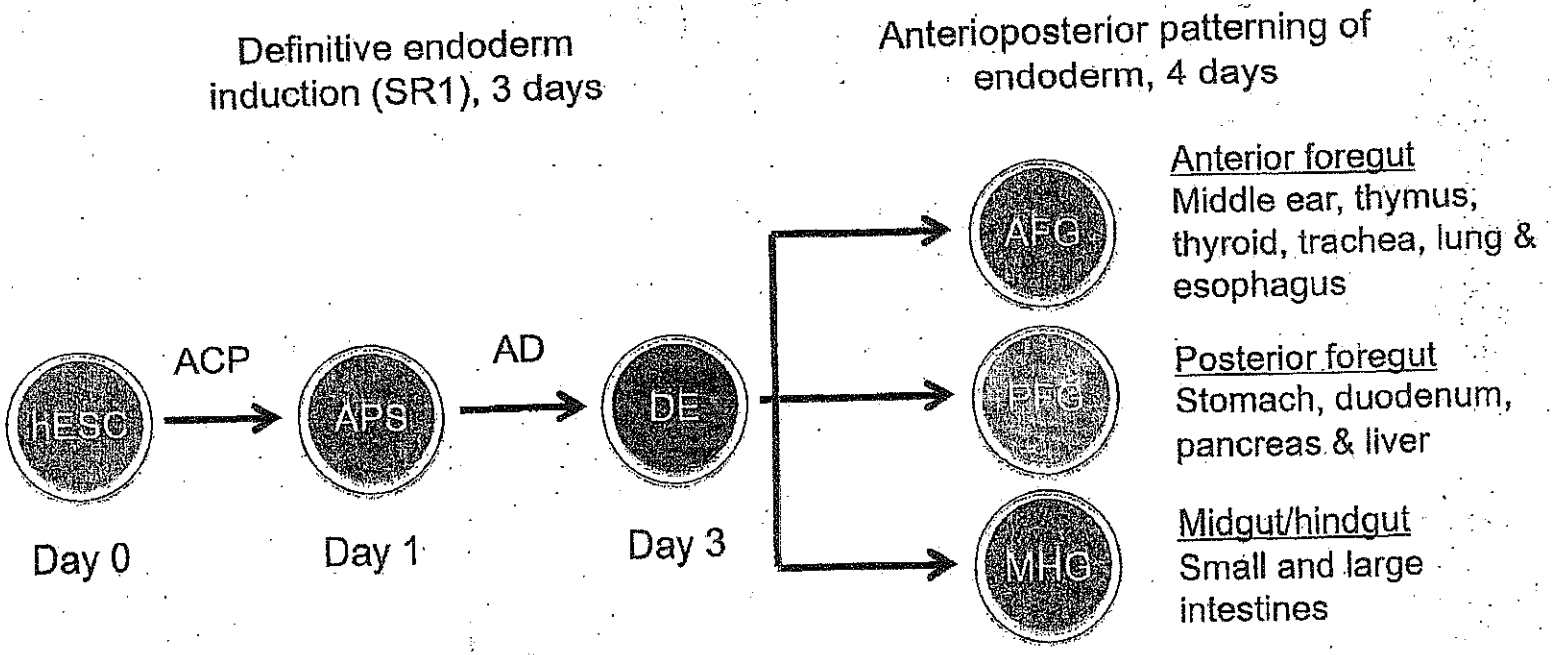

3 days of neuralization (qPCR)
Neuroectoderm
Oct6
8
6
4
2
0
D0
d3 DE
d0 → N
d1 → N
d2 → N
Pax6
120
90
60
30
0
D0
d3 DE
d0 → N
d1 → N
d2 → N

FIG. 12


Definitive endoderm induction (SR1), 3 days
Anterioposterior patterning of endoderm, 4 days
hESC
ACP
APS
AD
DE
AFG
PFG
MHG
Day 0
Day 1
Day 3
Anterior foregut
Middle ear, thymus, thyroid, trachea, lung & esophagus
Posterior foregut
Stomach, duodenum, pancreas & liver
Midgut/hindgut
Small and large intestines

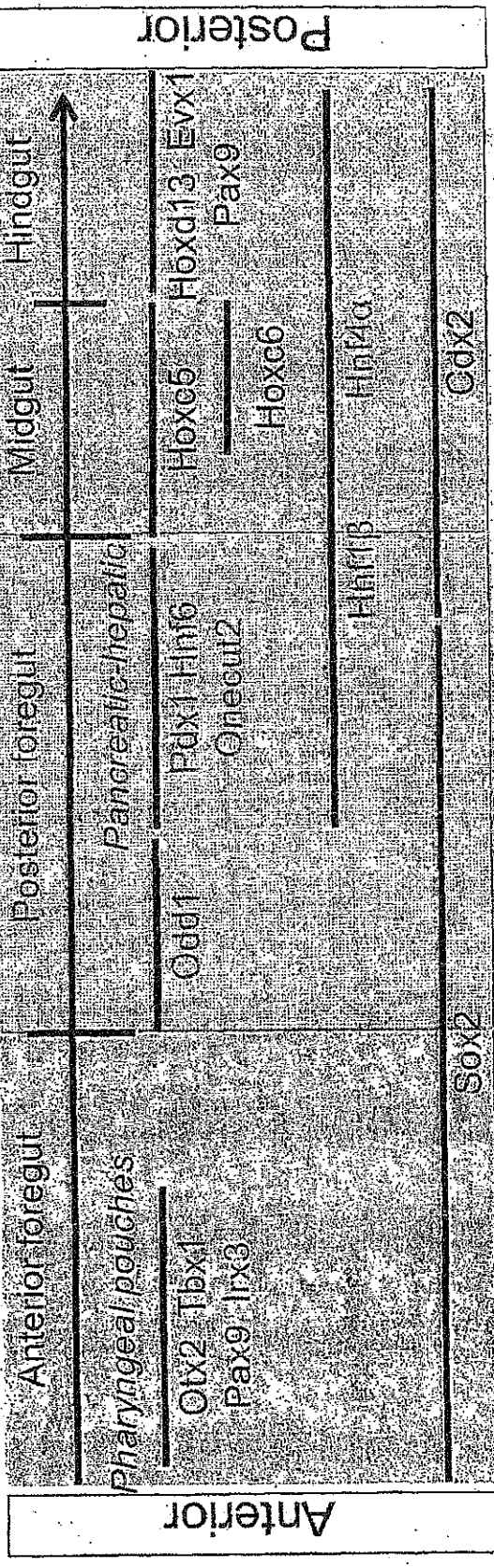

Anterior
Posterior
Anterior foregut
Posterior foregut
Midgut
Hindgut
Pharyngeal pouches
Pancreatic hepatic
Otx2
Tbx1
Pax9
Lhx3
Odd1
Pdx1
Hnf6
Onecut2
Hoxc5
Hoxd13
Evx1
Pax9
Hoxc6
Hnf1β
Hnf4α
Sox2
Cdx2

FIG. 13

FIG. 14

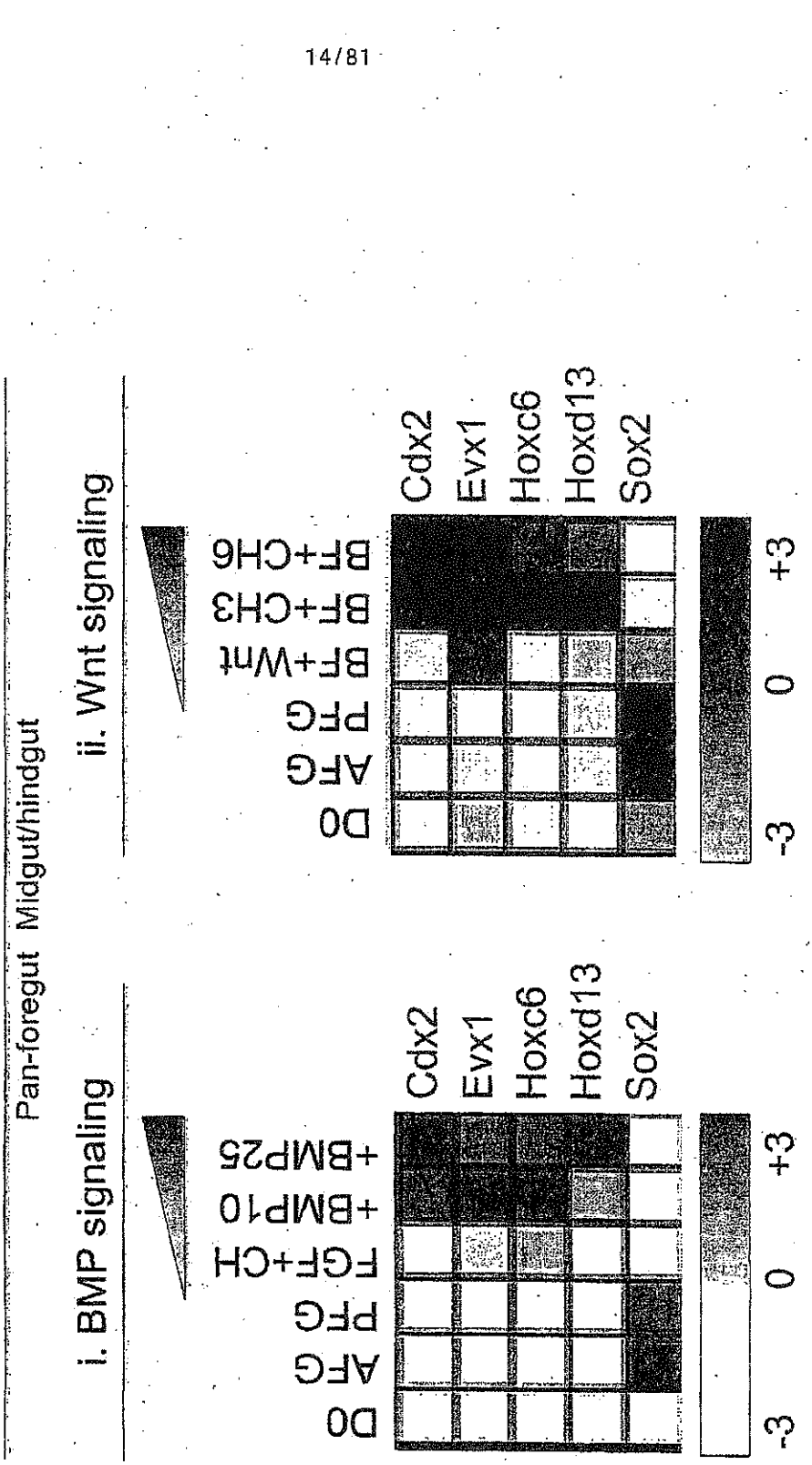

Day 7 gene expression (qPCR)
Pan-foregut Midgut/hindgut
i. BMP signaling
ii. Wnt signaling
D0
AFG
PFG
TGF+CH
+BMP10
+BMP25
BF+Wnt
BF+CH3
BF+CH6
Cdx2
Evx1
Hoxc6
Hoxd13
Sox2
-3
0
+3

FIG. 15

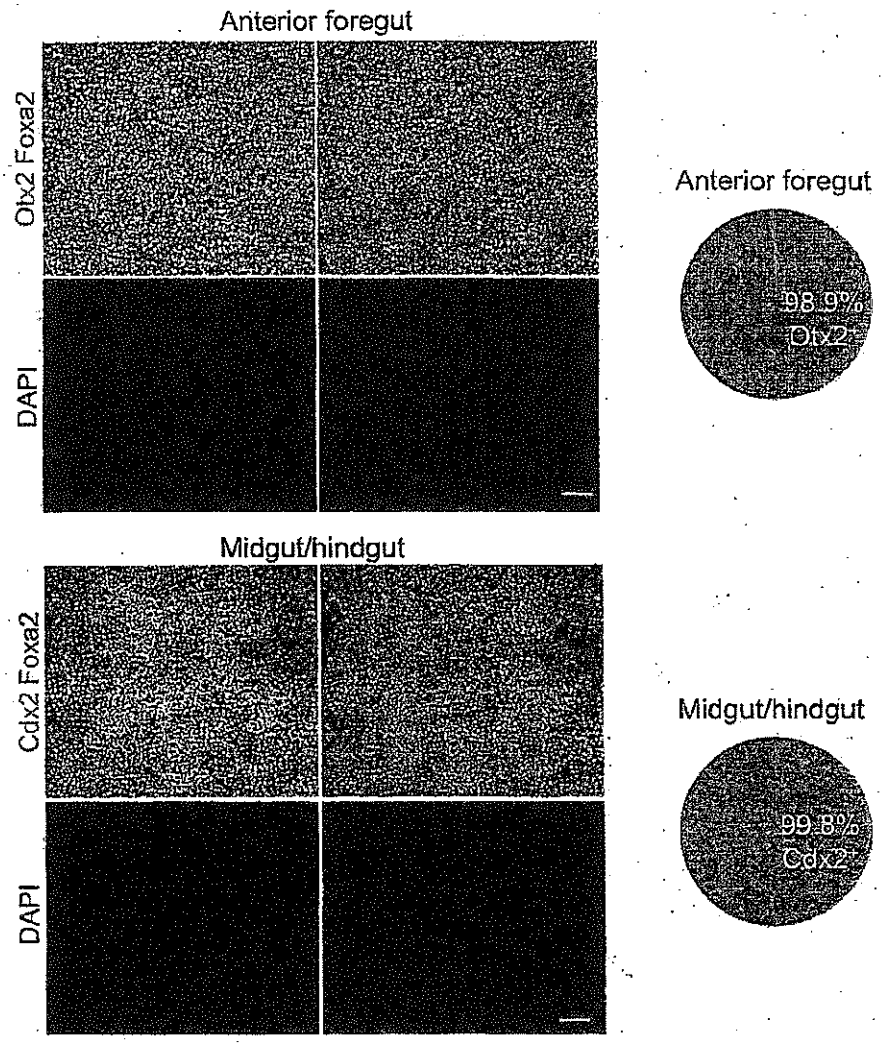

Anterior foregut
Otx2 Foxa2
DAPI
Anterior foregut
98.9%
Otx2+
Midgut/hindgut
Cdx2 Foxa2
DAPI
Midgut/hindgut
99.8%
Cdx2+

FIG. 16

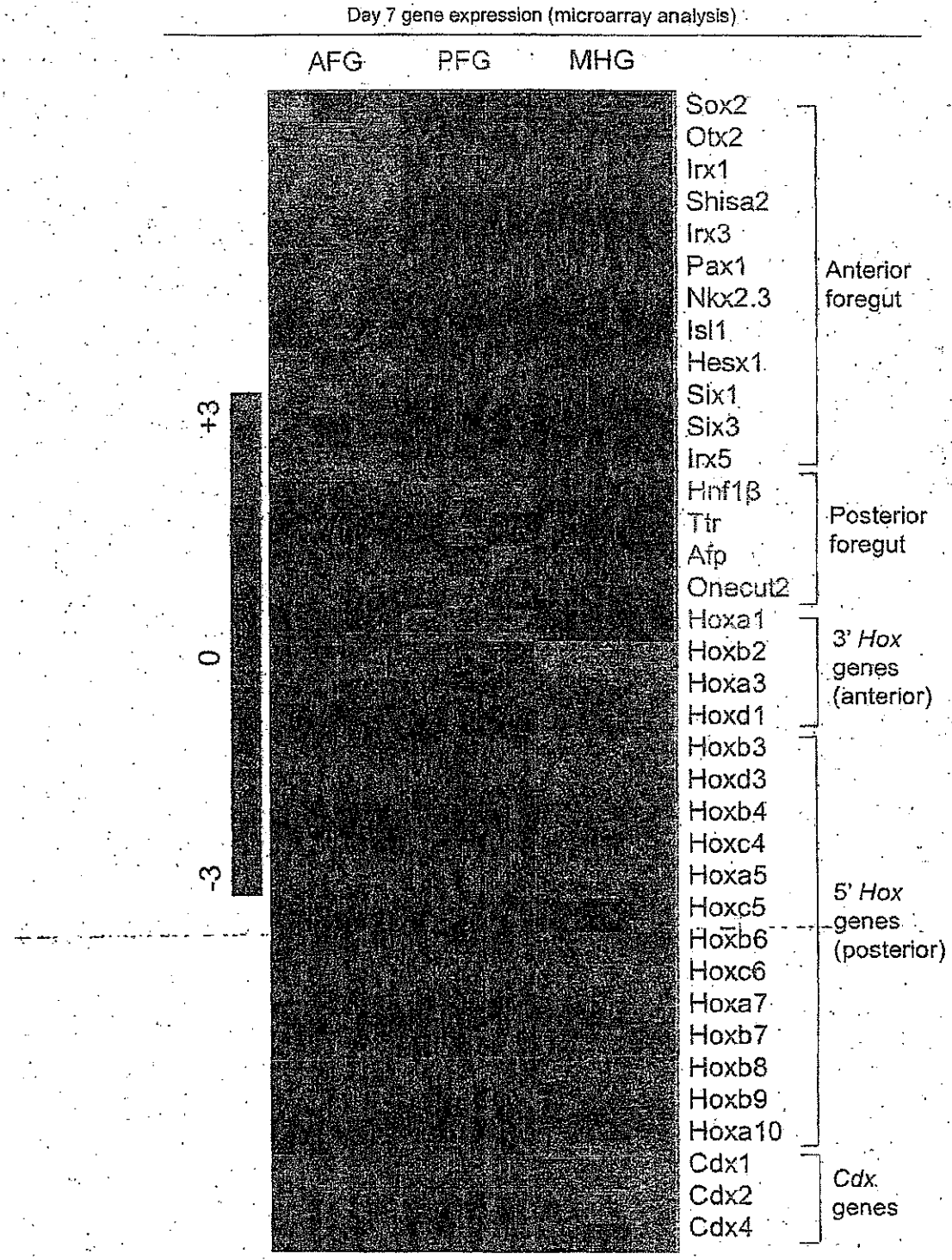

Day 7 gene expression (microarray analysis)
AFG
PFG
MHG
+3
0
-3
Sox2
Otx2
Irx1
Shisa2
Irx3
Pax1
Nkx2.3
Isl1
Hesx1
Six1
Six3
Irx5
Hnf1β
Ttr
Afp
Onecut2
Hoxa1
Hoxb2
Hoxa3
Hoxd1
Hoxb3
Hoxd3
Hoxb4
Hoxc4
Hoxa5
Hoxc5
Hoxb6
Hoxc6
Hoxa7
Hoxb7
Hoxb8
Hoxb9
Hoxa10
Cdx1
Cdx2
Cdx4
Anterior foregut
Posterior foregut
3' Hox genes (anterior)
5' Hox genes (posterior)
Cdx genes

FIG. 17

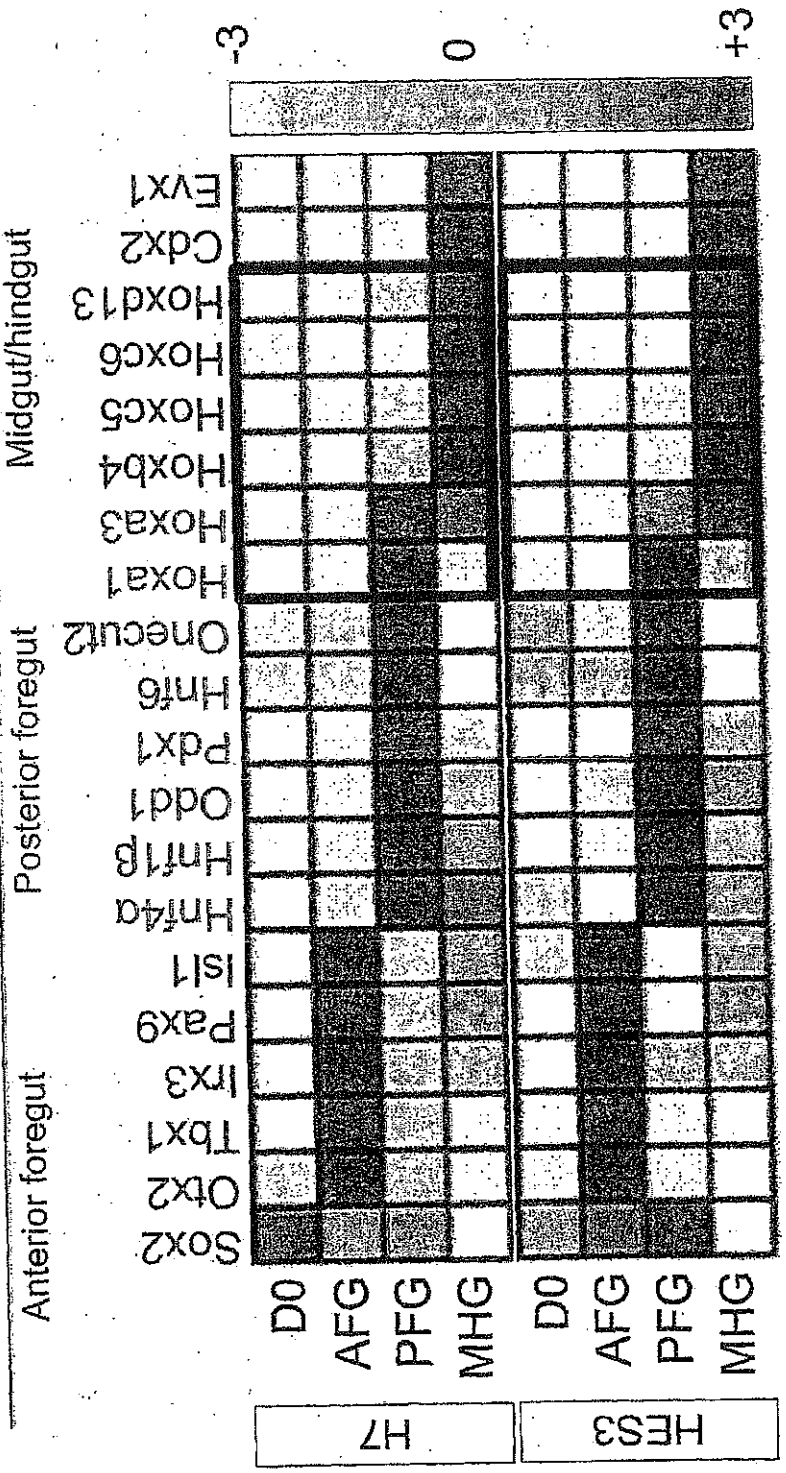

Day 7 gene expression (qPCR)
Anterior foregut
Posterior foregut
Midgut/hindgut
-3
0
+3
H7
HES3
D0
AFG
PFG
MHG
Sox2
Otx2
Tbx1
Irx3
Pax9
Isl1
Hnf4a
Hnf1β
Odd1
Pdx1
Hnf6
Onecut2
Hoxa1
Hoxa3
Hoxb4
Hoxc5
Hoxc6
Hoxd13
Cdx2
Evx1

FIG. 18

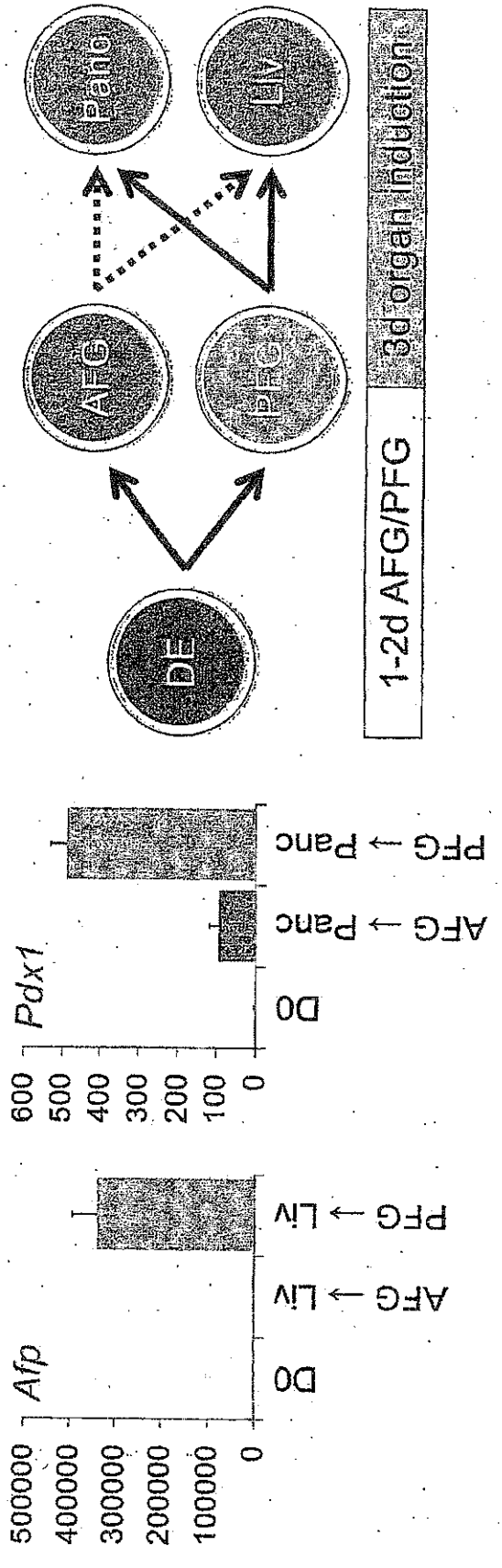

DE
AFG
PFG
Brain
Liv
1-2d AFG/PFG
3d organ induction
Afp
500000
400000
300000
200000
100000
0
D0
AFG → Liv
PFG → Liv
Pdx1
600
500
400
300
200
100
0
D0
AFG → Panc
PFG → Panc

FIG. 19

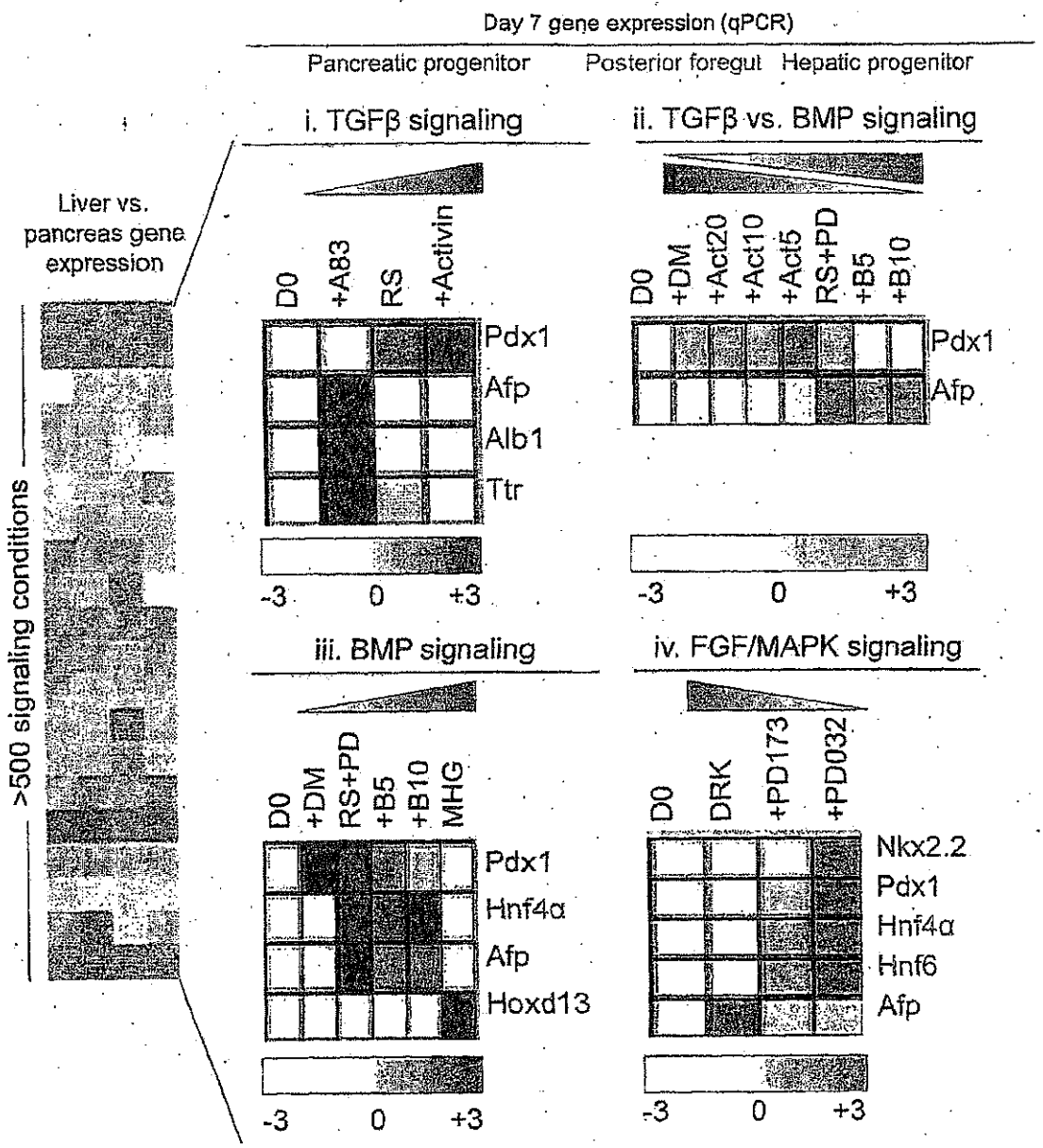
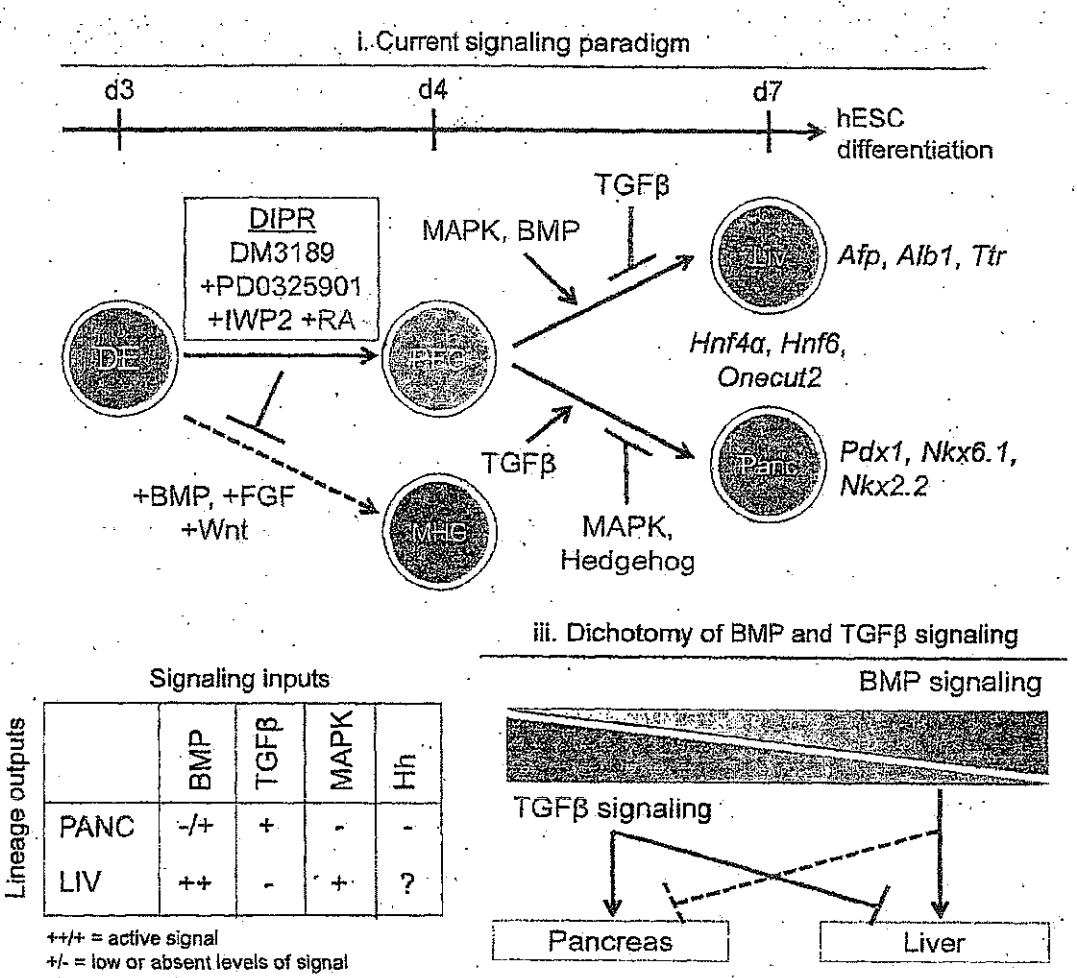

Day 7 gene expression (qPCR)
Pancreatic progenitor
Posterior foregut
Hepatic progenitor
Liver vs. pancreas gene expression
>500 signaling conditions
i. TGFβ signaling
D0
+A83
RS
+Activin
Pdx1
Afp
Alb1
Ttr
-3
0
+3
ii. TGFβ vs. BMP signaling
D0
+DM
+Act20
+Act10
+Act5
RS+PD
+B5
+B10
Pdx1
Afp
-3
0
+3
iii. BMP signaling
D0
+DM
RS+PD
+B5
+B10
MHG
Pdx1
Hnf4α
Afp
Hoxd13
-3
0
+3
iv. FGF/MAPK signaling
D0
DRK
+PD173
+PD032
Nkx2.2
Pdx1
Hnf4α
Hnf6
Afp
-3
0
+3

FIG. 20



Signaling inputs

Lineage outputs	BMP	TGFβ	MAPK	Hh
PANC	-/+	+	-	-
LIV	++	-	+	?

++/+ = active signal
+/- = low or absent levels of signal
- = absence of signal

FIG. 21

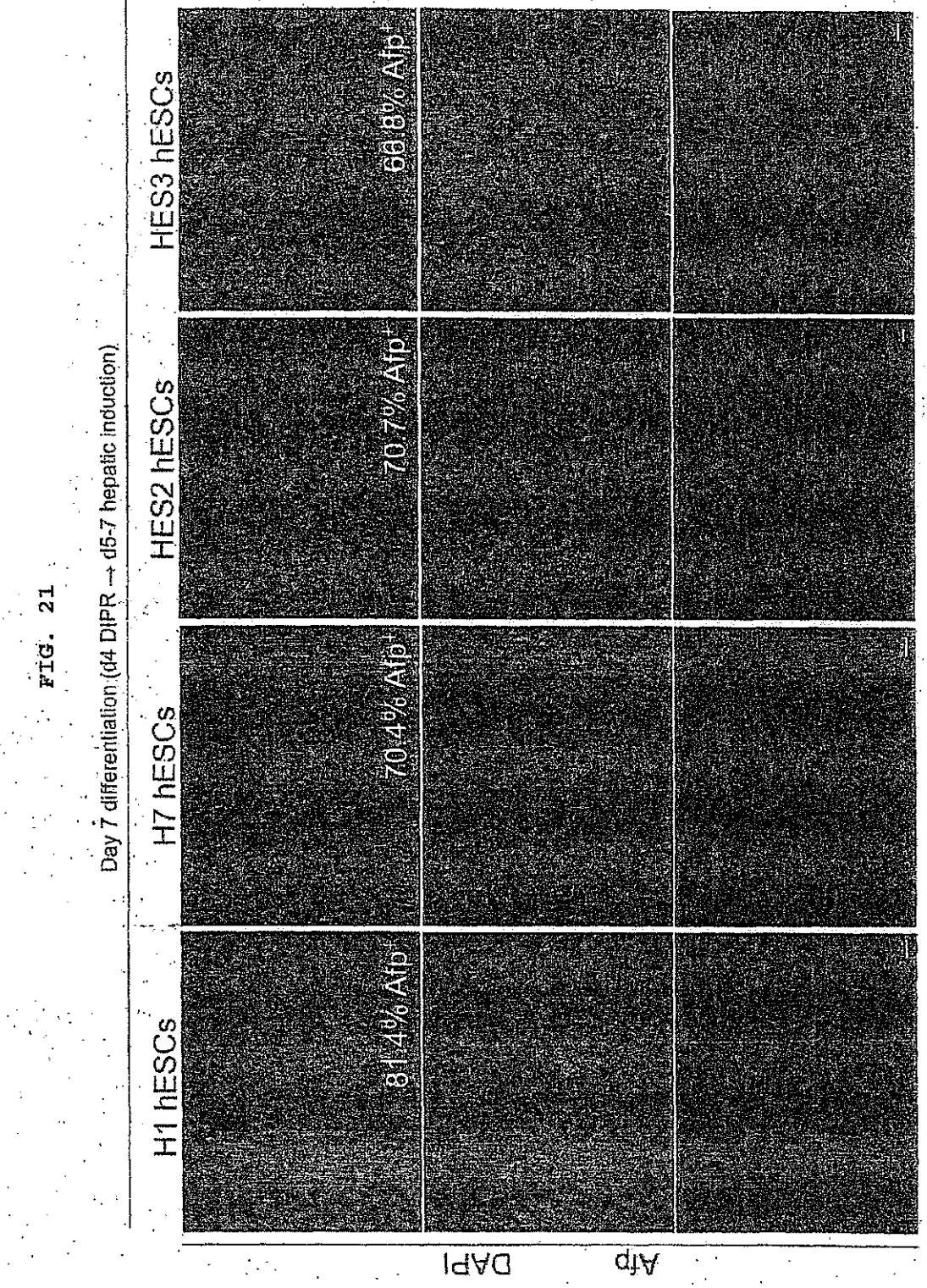

Day 7 differentiation (d4 DIPR → d5-7 hepatic induction)
H1 hESCs
H7 hESCs
HES2 hESCs
HES3 hESCs
81.4% Afp+
70.4% Afp+
70.7% Afp+
66.8% Afp+
Afp
DAPI

FIG. 22

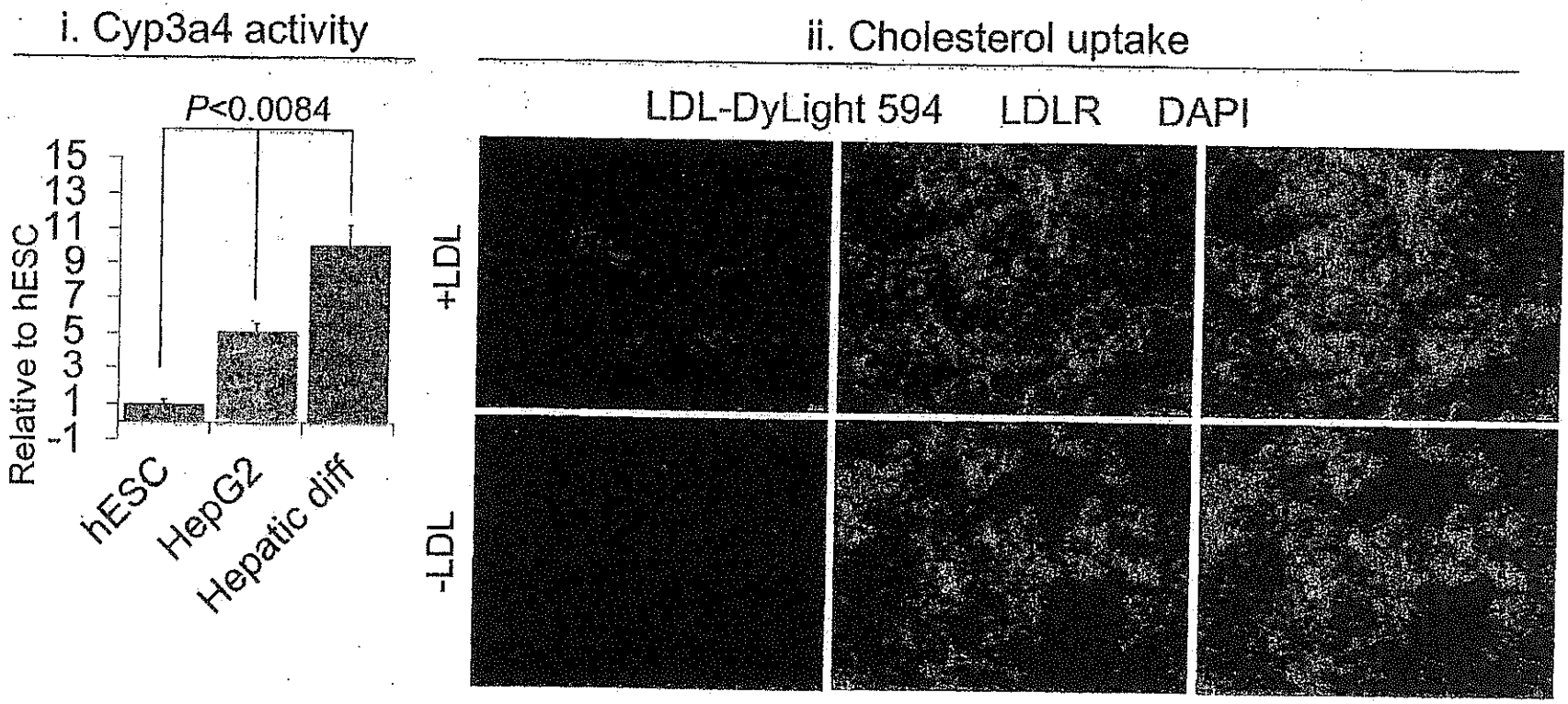

i. Cyp3a4 activity
P<0.0084
Relative to hESC
15
13
11
9
7
5
3
1
-1
hESC
HepG2
Hepatic diff
ii. Cholesterol uptake
LDL-DyLight 594
LDLR
DAPI
+LDL
-LDL

FIG. 23

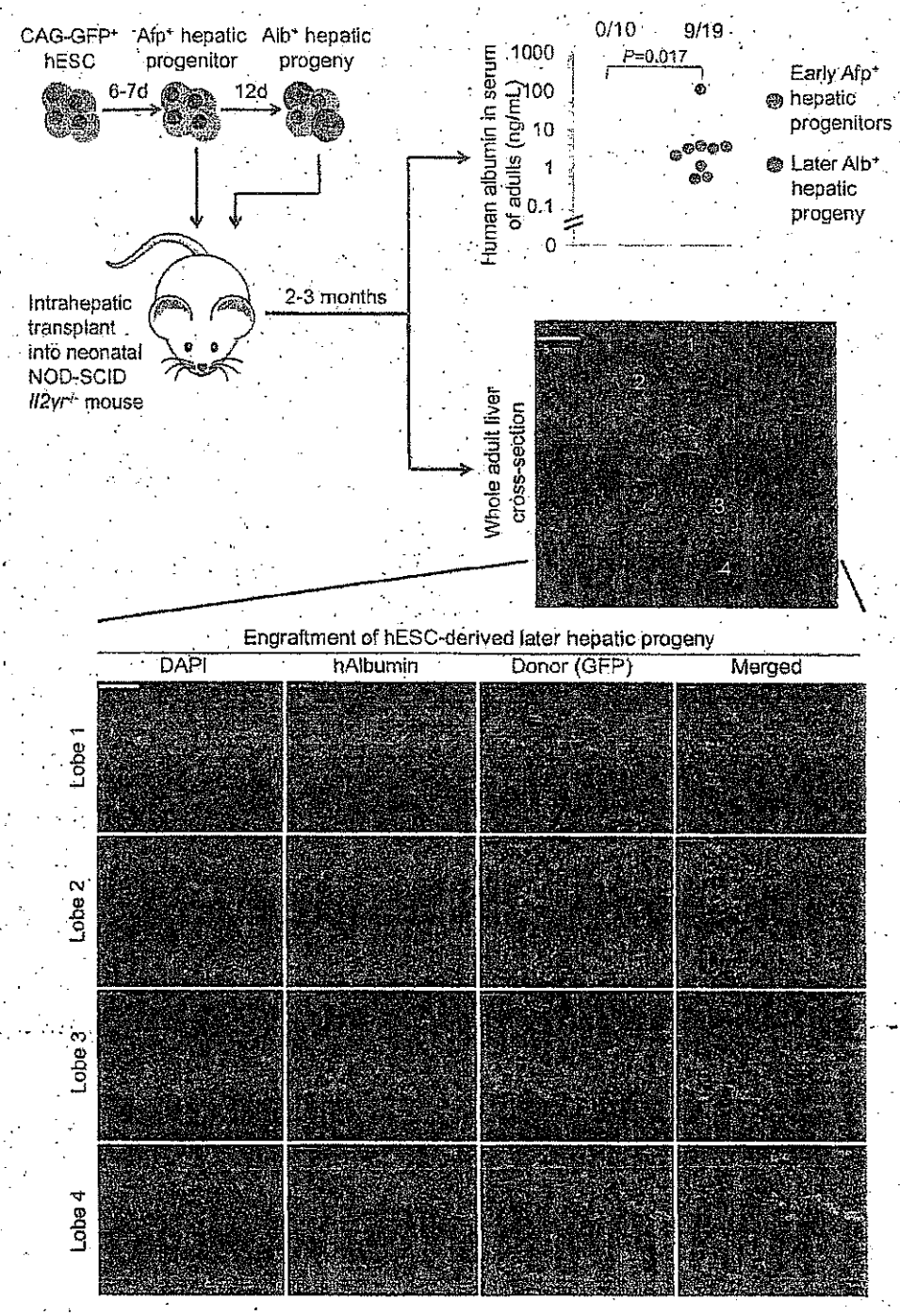

CAG-GFP+ hESC
Afp+ hepatic progenitor
Alb+ hepatic progeny
6-7d
12d
Intrahepatic transplant into neonatal NOD-SCID Il2rγ-/- mouse
2-3 months
Human albumin in serum of adults (ng/mL)
1000
100
10
1
0.1
0
0/10
9/19
P=0.017
Early Afp+ hepatic progenitors
Later Alb+ hepatic progeny
Whole adult liver cross-section
1
2
3
4
Engraftment of hESC-derived later hepatic progeny
DAPI
hAlbumin
Donor (GFP)
Merged
Lobe 1
Lobe 2
Lobe 3
Lobe 4

FIG. 24

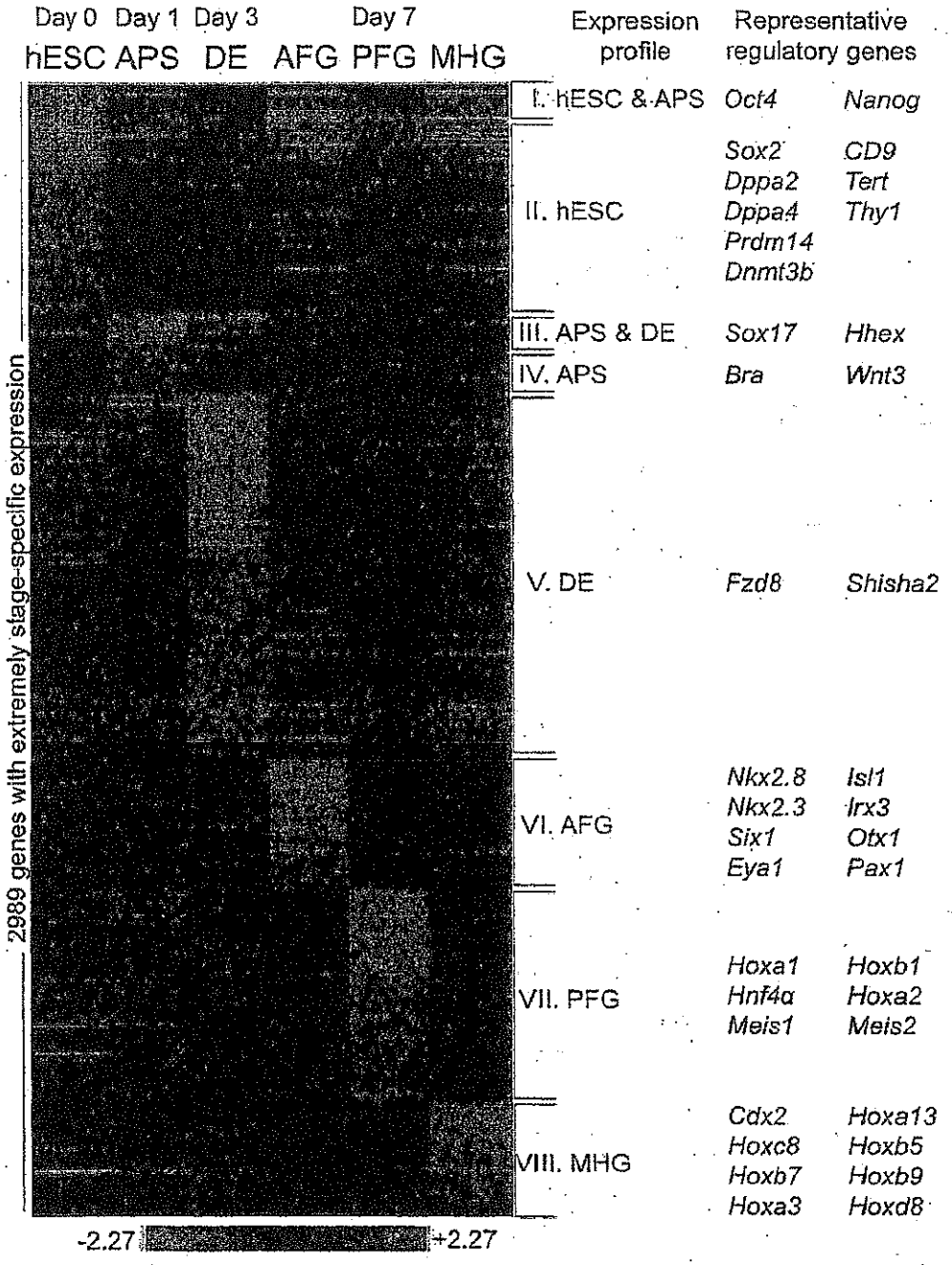

Day 0
Day 1
Day 3
Day 7
hESC
APS
DE
AFG
PFG
MHG
Expression profile
Representative regulatory genes
2989 genes with extremely stage-specific expression
I. hESC & APS
Oct4
Nanog
II. hESC
Sox2
CD9
Dppa2
Tert
Dppa4
Thy1
Prdm14
Dnmt3b
III. APS & DE
Sox17
Hhex
IV. APS
Bra
Wnt3
V. DE
Fzd8
Shisha2
VI. AFG
Nkx2.8
Isl1
Nkx2.3
Irx3
Six1
Otx1
Eya1
Pax1
VII. PFG
Hoxa1
Hoxb1
Hnf4α
Hoxa2
Meis1
Meis2
VIII. MHG
Cdx2
Hoxa13
Hoxc8
Hoxb5
Hoxb7
Hoxb9
Hoxa3
Hoxd8
-2.27
+2.27

FIG. 25

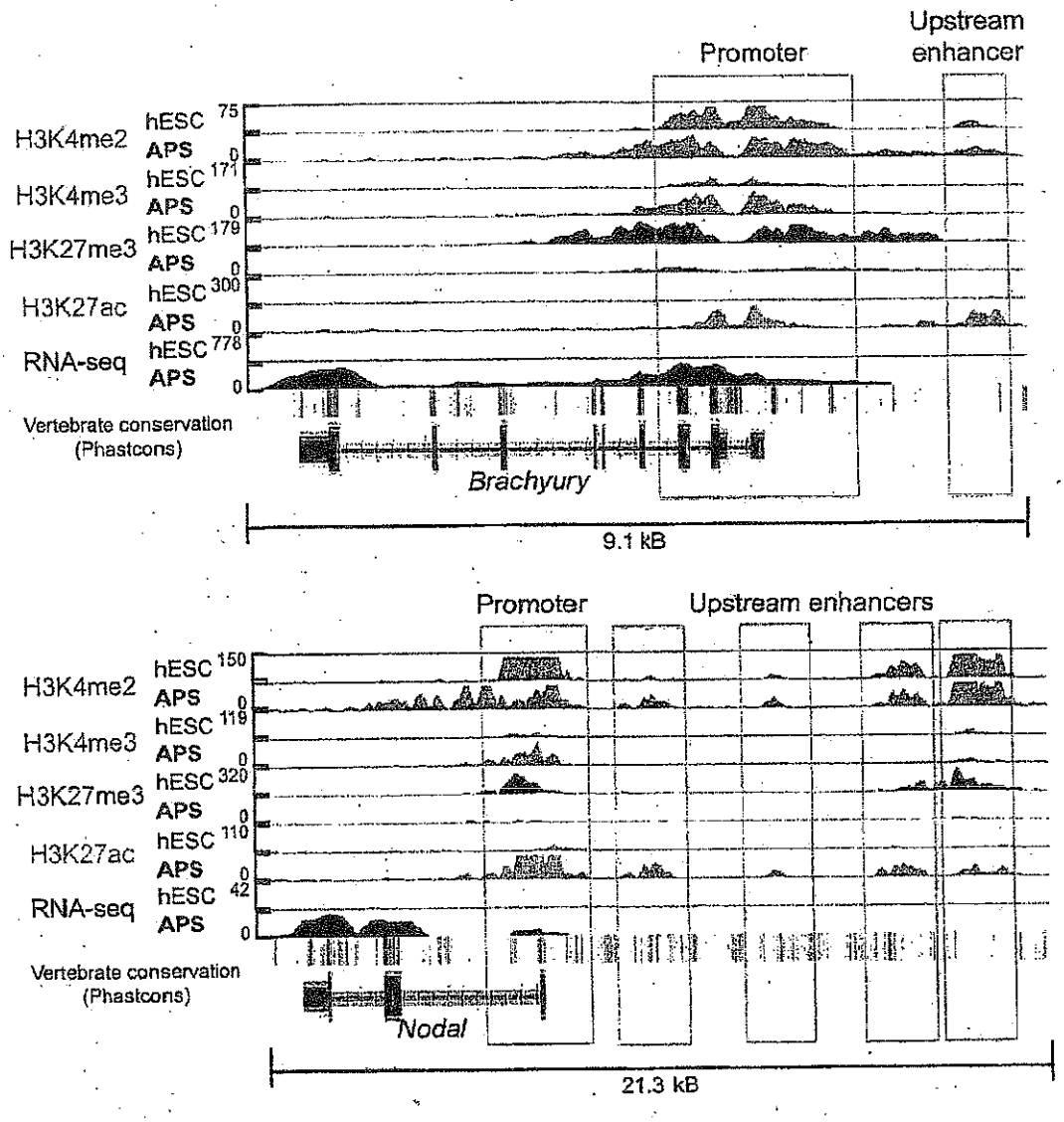

Promoter
Upstream enhancer
H3K4me2
hESC 75
APS 0
H3K4me3
hESC 171
APS 0
H3K27me3
hESC 179
APS 0
H3K27ac
hESC 300
APS 0
RNA-seq
hESC 778
APS 0
Vertebrate conservation (Phastcons)
Brachyury
9.1 kB
Promoter
Upstream enhancers
H3K4me2
hESC 150
APS 0
H3K4me3
hESC 119
APS 0
H3K27me3
hESC 320
APS 0
H3K27ac
hESC 110
APS 0
RNA-seq
hESC 42
APS 0
Vertebrate conservation (Phastcons)
Nodal
21.3 kB

FIG. 26

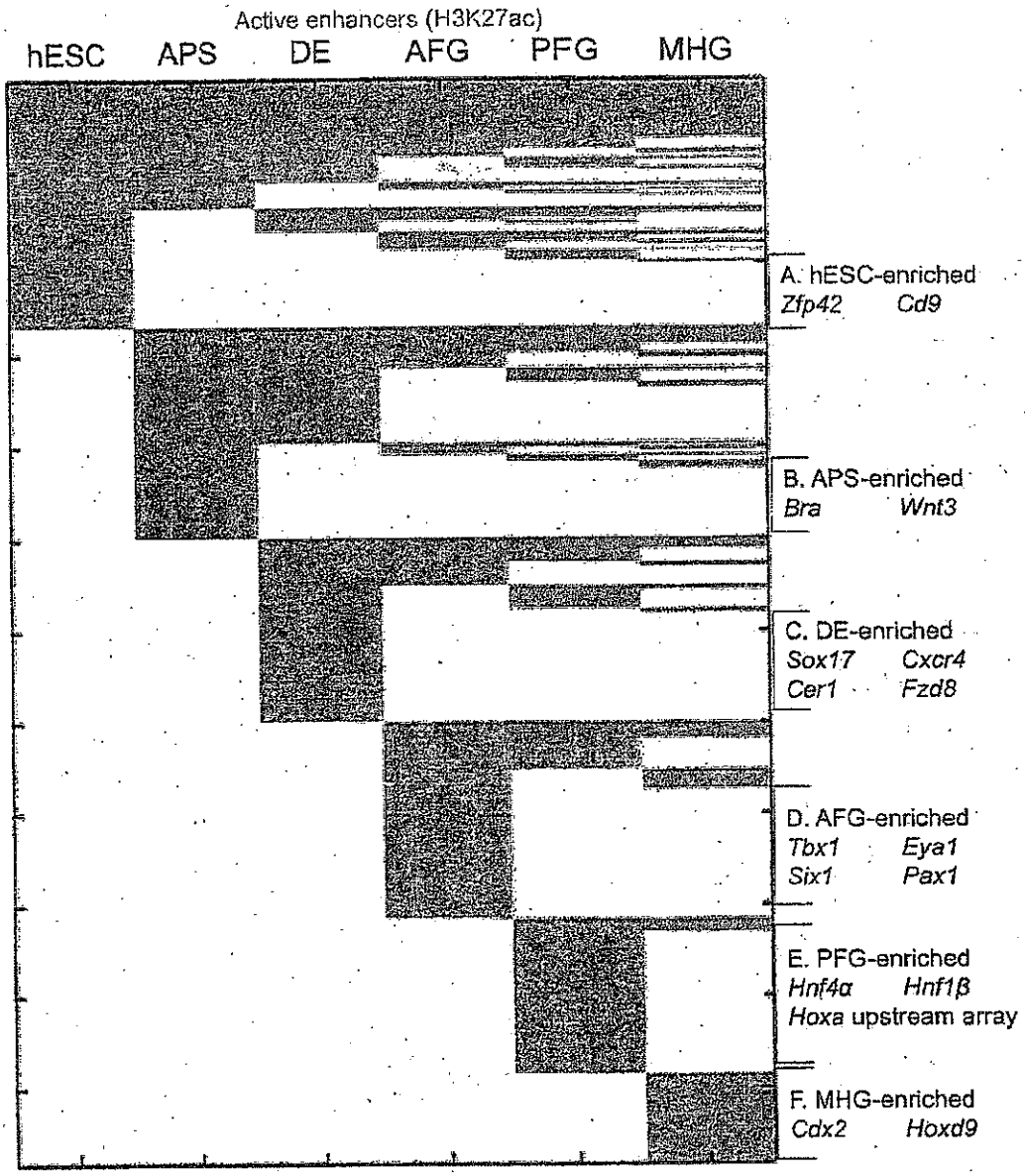

Active enhancers (H3K27ac)
hESC
APS
DE
AFG
PFG
MHG
A. hESC-enriched
Zfp42 Cd9
B. APS-enriched
Bra Wnt3
C. DE-enriched
Sox17 Cxcr4
Cer1 Fzd8
D. AFG-enriched
Tbx1 Eya1
Six1 Pax1
E. PFG-enriched
Hnf4α Hnf1β
Hoxa upstream array
F. MHG-enriched
Cdx2 Hoxd9

FIG. 27

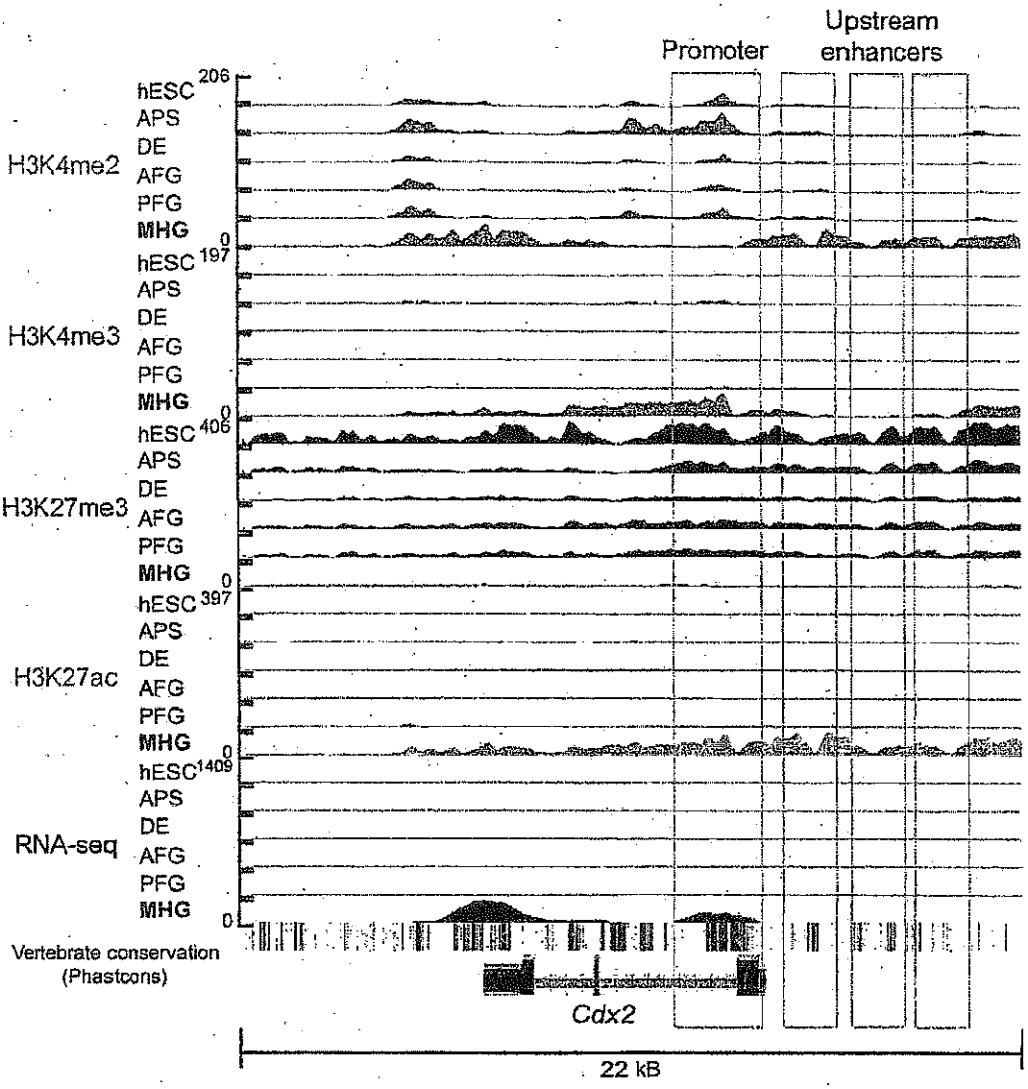

Promoter
Upstream enhancers
H3K4me2
H3K4me3
H3K27me3
H3K27ac
RNA-seq
hESC
APS
DE
AFG
PFG
MHG
206
197
406
397
1409
0
Vertebrate conservation (Phastcons)
Cdx2
22 kB

FIG. 28

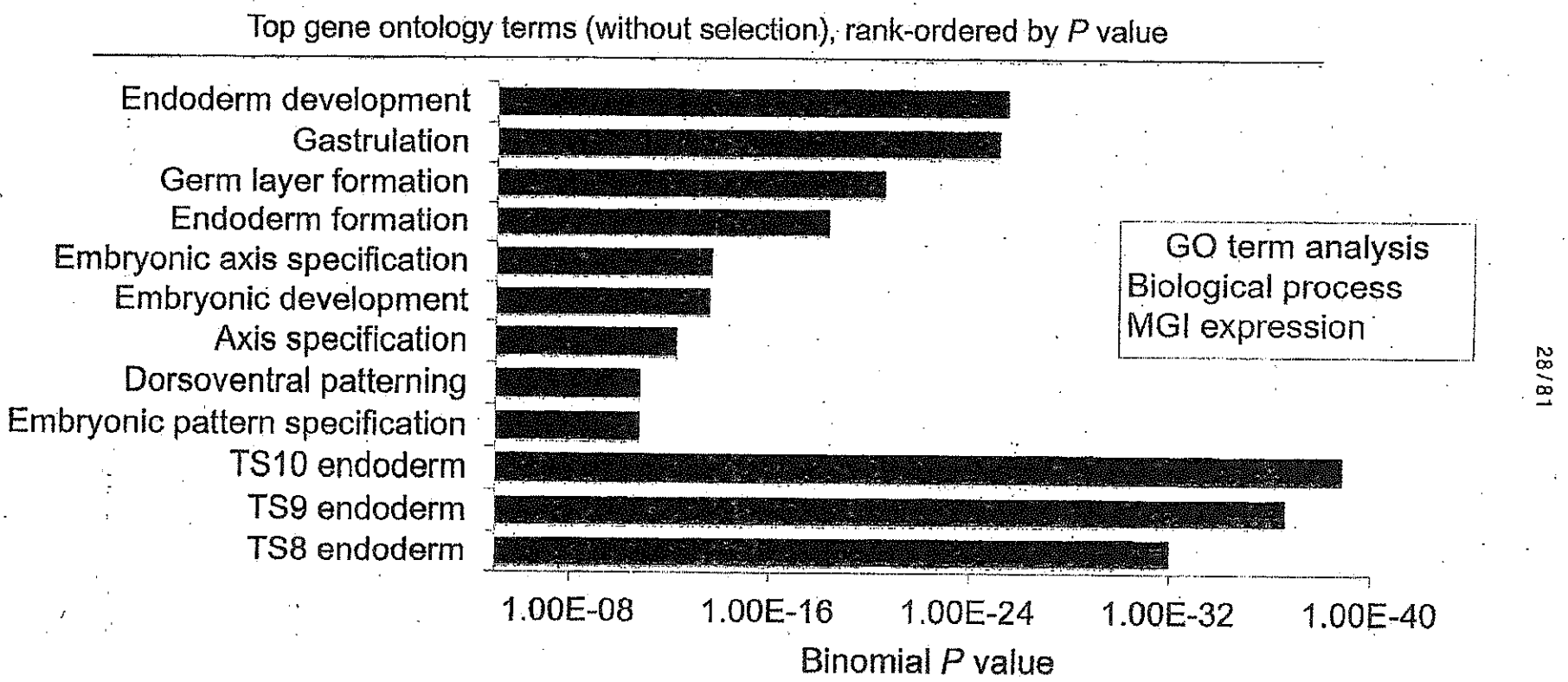

Top gene ontology terms (without selection), rank-ordered by P value
Endoderm development
Gastrulation
Germ layer formation
Endoderm formation
Embryonic axis specification
Embryonic development
Axis specification
Dorsoventral patterning
Embryonic pattern specification
TS10 endoderm
TS9 endoderm
TS8 endoderm
GO term analysis
Biological process
MGI expression
1.00E-08
1.00E-16
1.00E-24
1.00E-32
1.00E-40
Binomial P value

FIG. 29

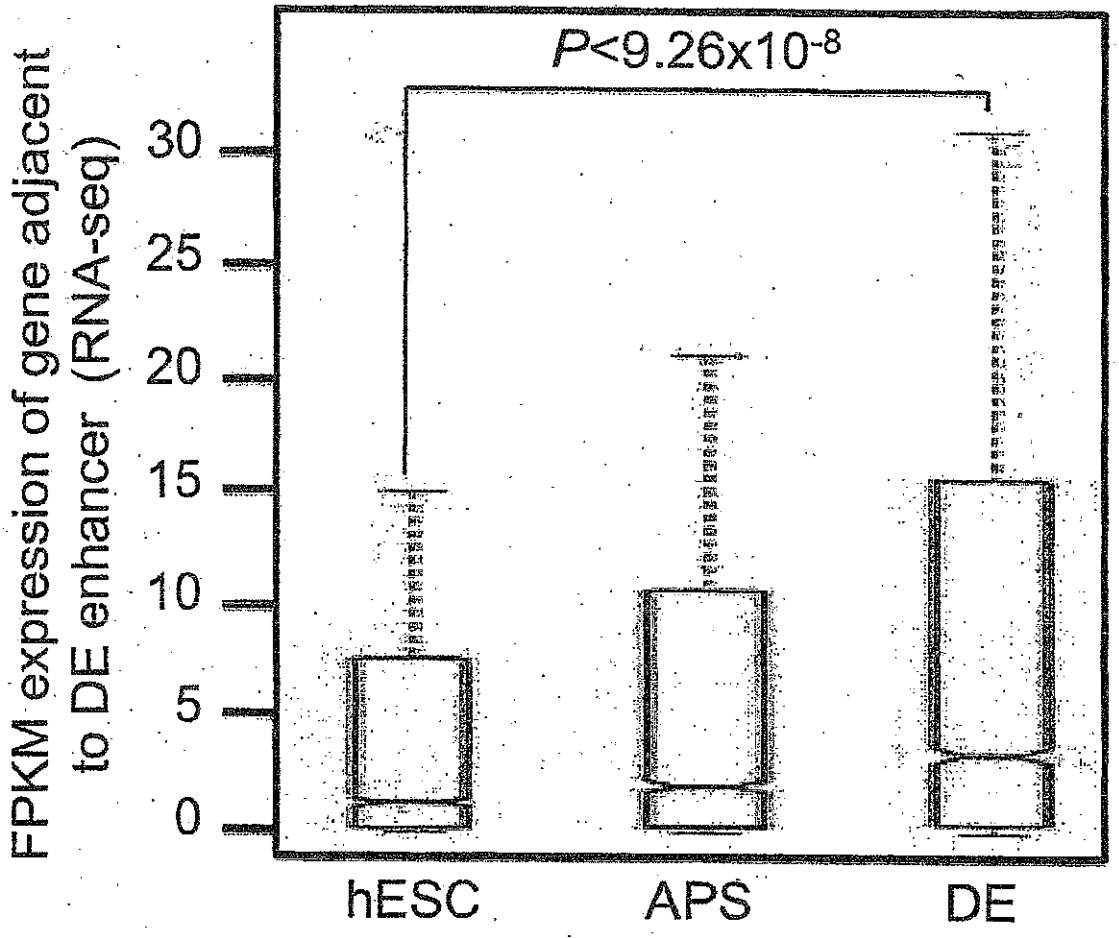

P<9.26x10-8
FPKM expression of gene adjacent to DE enhancer (RNA-seq)
30
25
20
15
10
5
0
hESC
APS
DE

FIG. 30

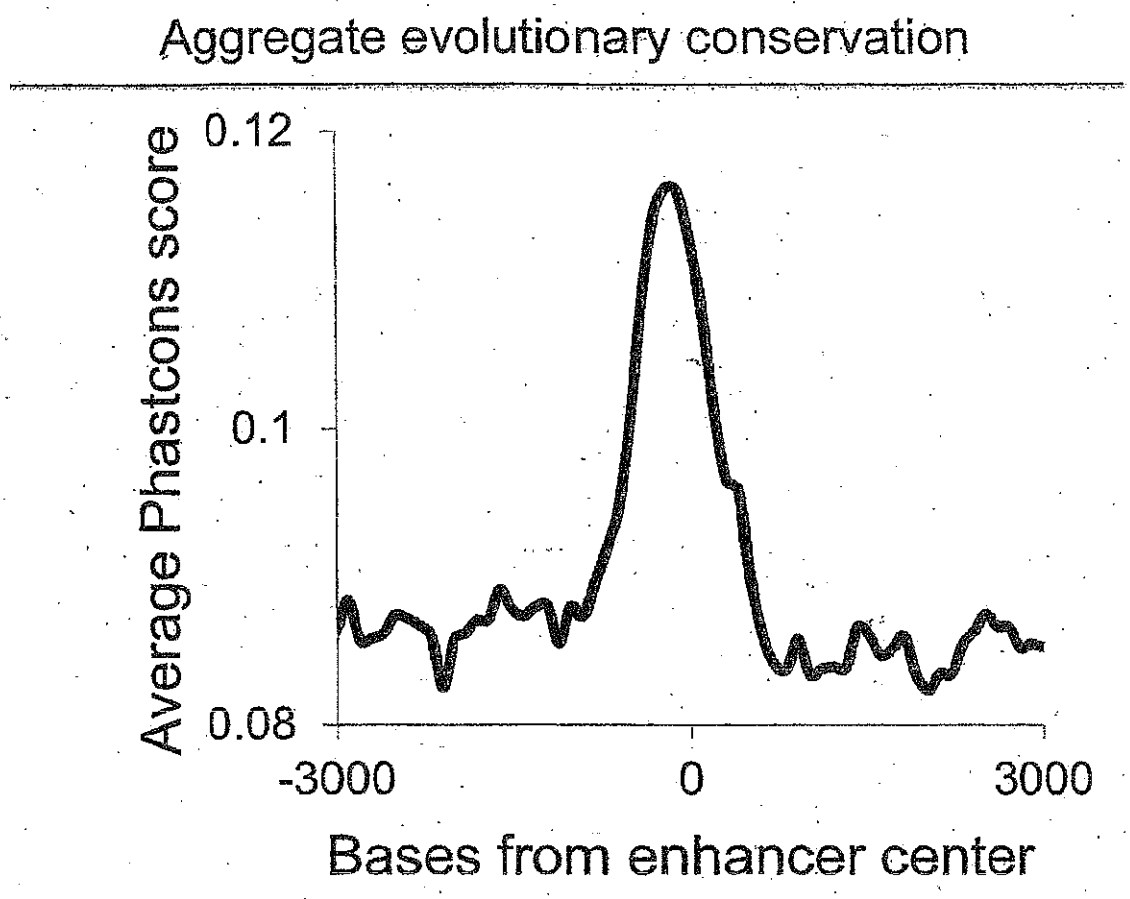

Aggregate evolutionary conservation
Average Phastcons score
0.12
0.1
0.08
-3000
0
3000
Bases from enhancer center

FIG. 31

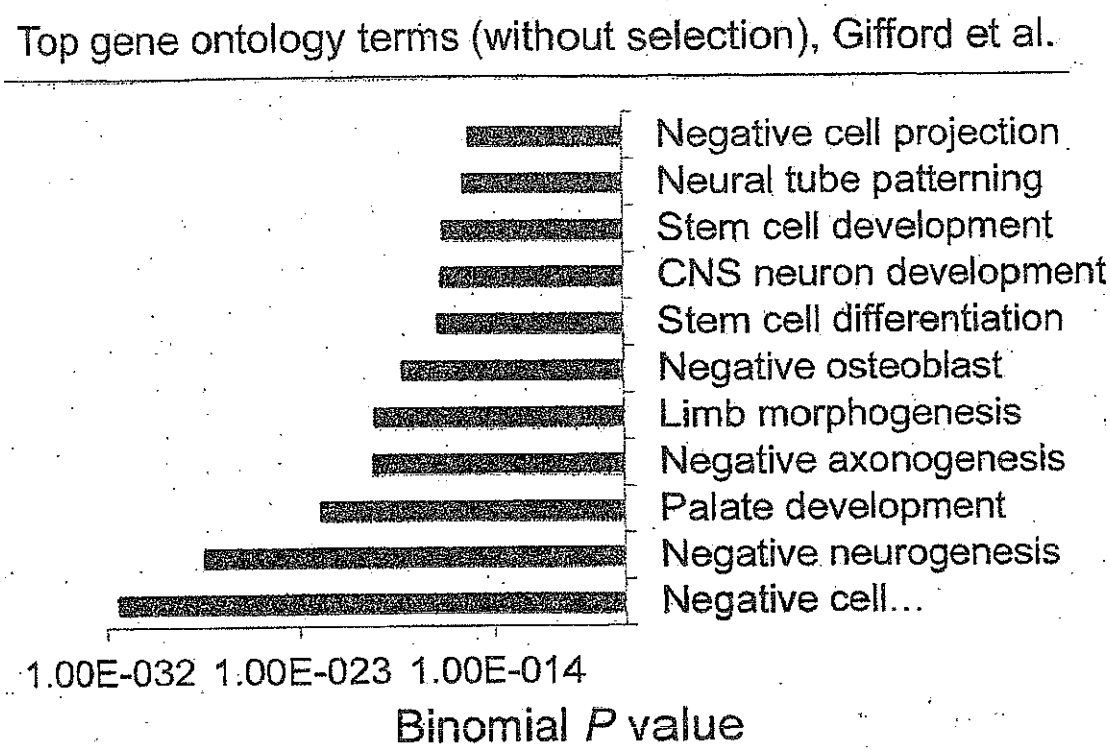

Top gene ontology terms (without selection), Gifford et al.
Negative cell projection
Neural tube patterning
Stem cell development
CNS neuron development
Stem cell differentiation
Negative osteoblast
Limb morphogenesis
Negative axonogenesis
Palate development
Negative neurogenesis
Negative cell...
1.00E-032
1.00E-023
1.00E-014
Binomial P value

FIG. 32

Within top 15 enriched motifs, rank-ordered by *P* value



Smad2/3
($P<10^{-197}$, $Q\approx0$)



Foxh1
($P<10^{-61}$, $Q\approx0$)



Eomes
($P<10^{-73}$, $Q\approx0$)



Foxa2
($P<10^{-59}$, $Q\approx0$)

FIG. 33

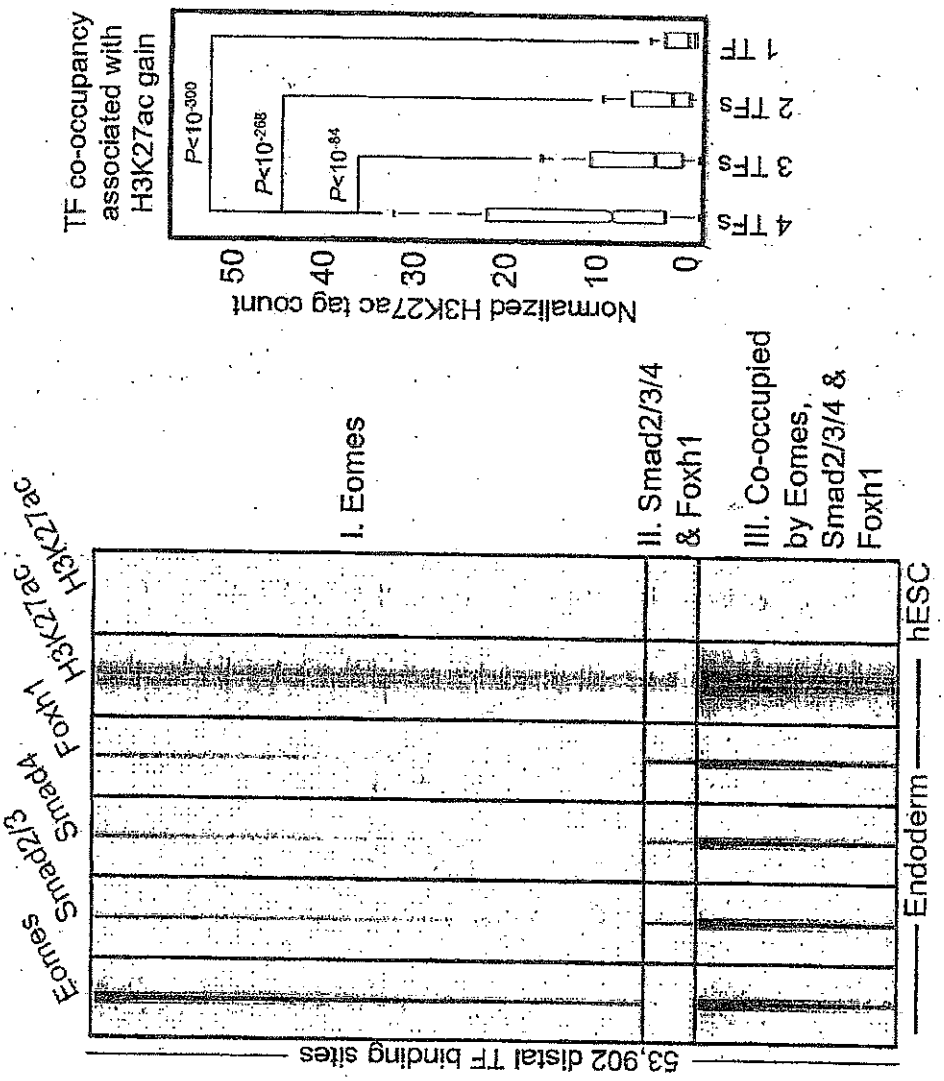

TF co-occupancy associated with H3K27ac gain
P<10-300
P<10-268
P<10-84
4 TFs
3 TFs
2 TFs
1 TF
Normalized H3K27ac tag count
0
10
20
30
40
50
53,902 distal TF binding sites
Eomes Smad2/3 Smad4 Foxh1 H3K27ac H3K27ac
I. Eomes
II. Smad2/3/4 & Foxh1
III. Co-occupied by Eomes, Smad2/3/4 & Foxh1
Endoderm
hESC

FIG. 34

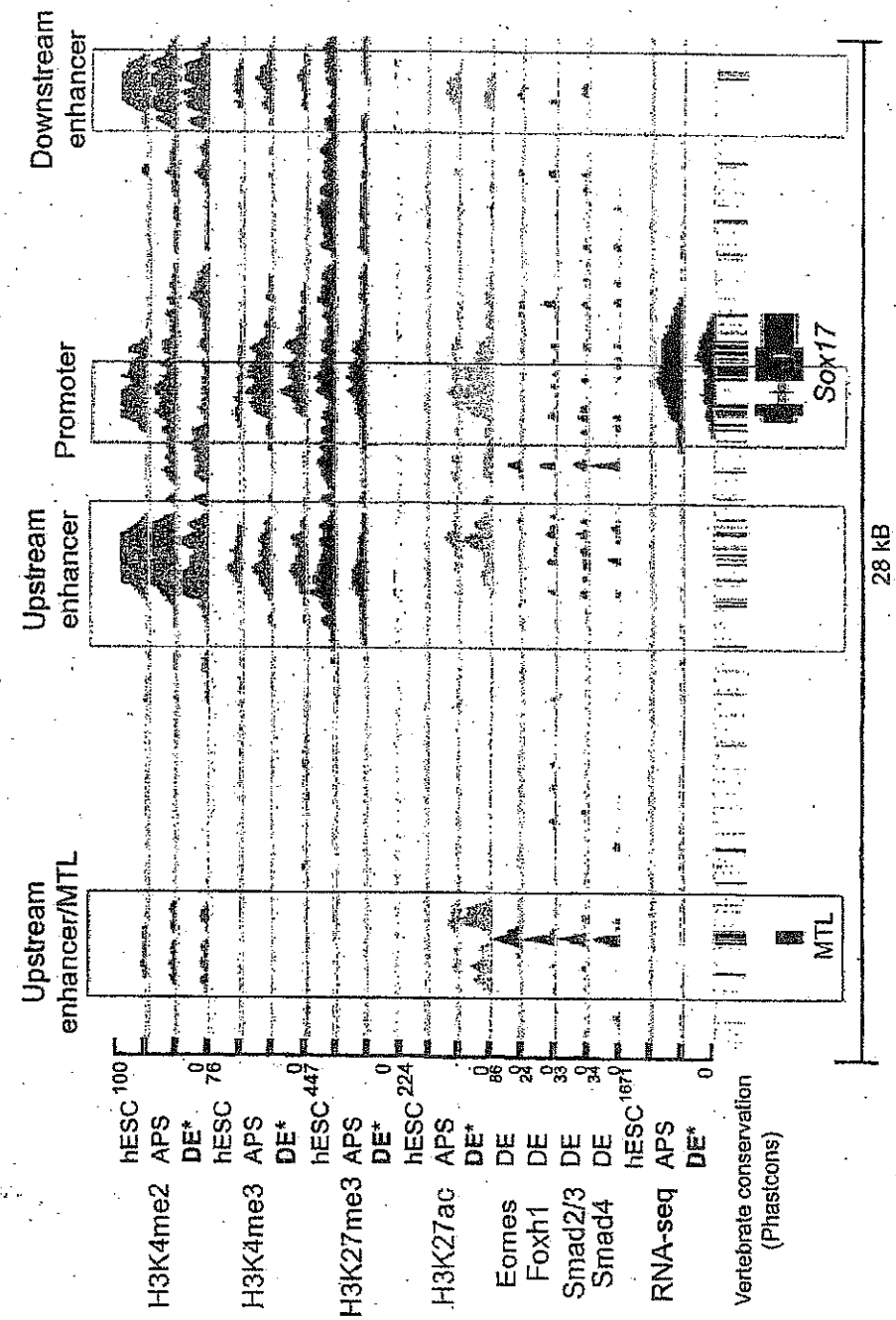
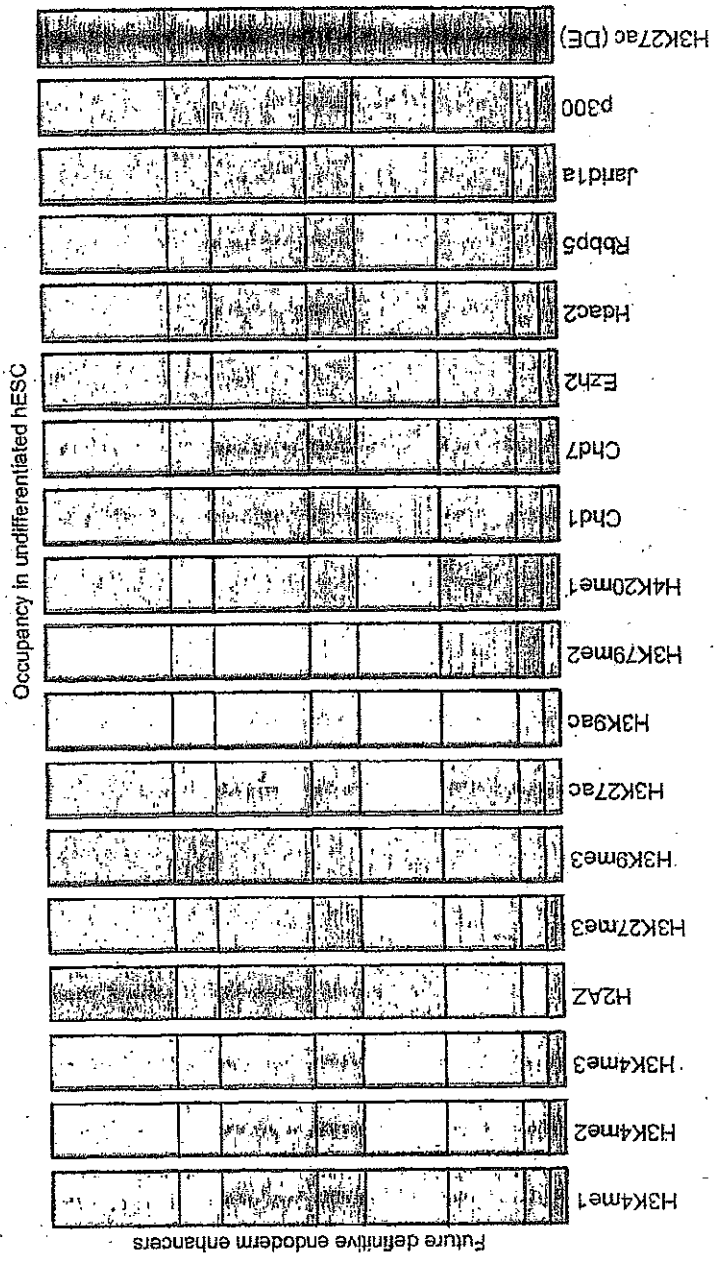

Upstream enhancer/MTL
Upstream enhancer
Promoter
Downstream enhancer
H3K4me2
H3K4me3
H3K27me3
H3K27ac
Eomes
Foxh1
Smad2/3
Smad4
RNA-seq
hESC
APS
DE*
DE
Vertebrate conservation (Phastcons)
MTL
Sox17
28 kB

FIG. 35


Occupancy in undifferentiated hESC
Future definitive endoderm enhancers
H3K4me1
H3K4me2
H3K4me3
H2AZ
H3K27me3
H3K9me3
H3K27ac
H3K9ac
H3K79me2
H4K20me1
Chd1
Chd7
Ezh2
Hdac2
Rbbp5
Jarid1a
p300
H3K27ac (DE)
Cluster 1: H2AZ alone
Cluster 2: H3K9me3 alone
Cluster 3: H2AZ and H3K4me1
Cluster 4: H3K4me1, with low H2AZ, H3K27me3 and p300 (poised)
Cluster 5: Largely barren (latent)
Cluster 6: Low H4K20me1
Cluster 7: H3K79me2 and H4K20me1
Cluster 8: H3K4me1, with low H2AZ, H3K27me3 and p300 (poised)

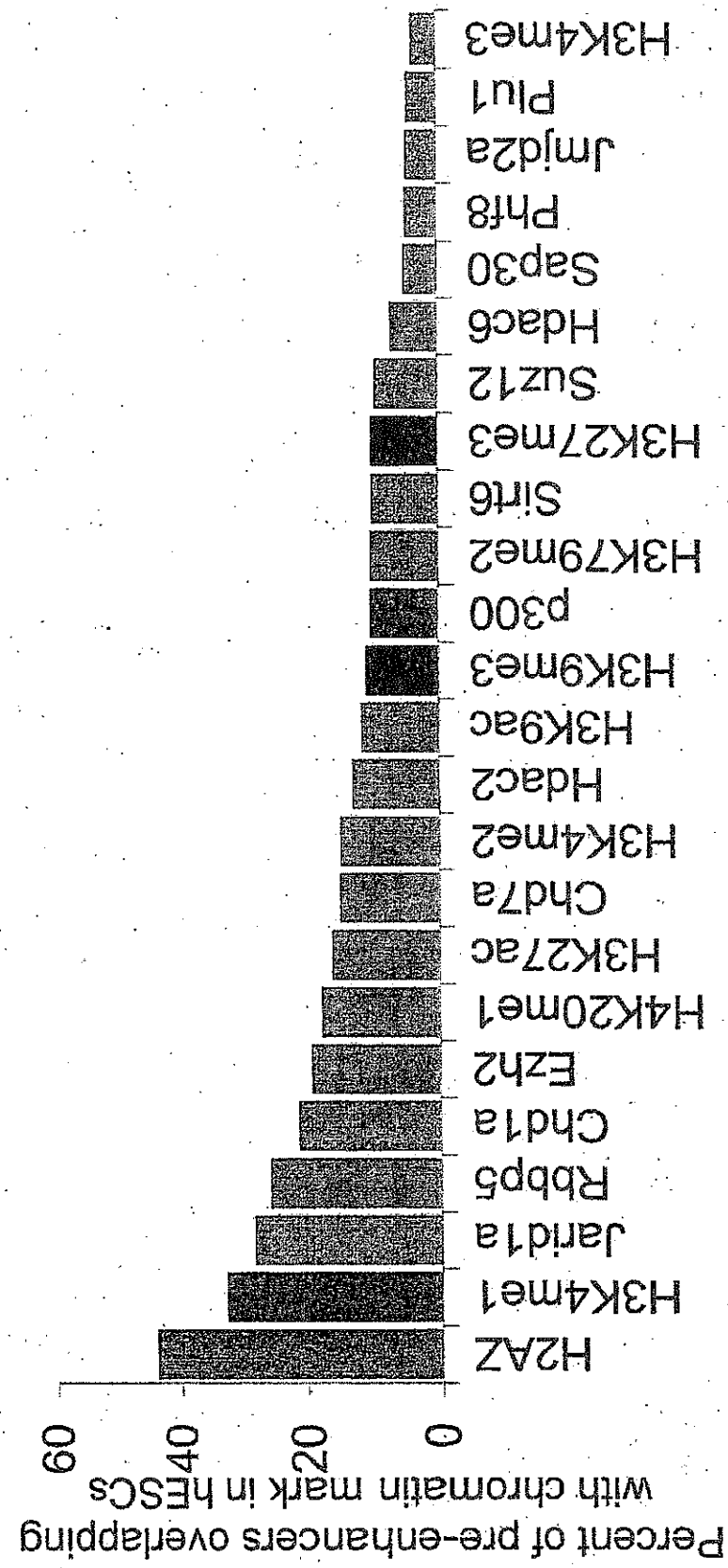

Percent of pre-enhancers overlapping with chromatin mark in hESCs
0
20
40
60
H2AZ
H3K4me1
Jarid1a
Rbbp5
Chd1a
Ezh2
H4K20me1
H3K27ac
Chd7a
H3K4me2
Hdac2
H3K9ac
H3K9me3
p300
H3K79me2
Sirt6
H3K27me3
Suz12
Hdac6
Sap30
Ph f8
Jmjd2a
Plu1
H3K4me3

FIG. 36

FIG. 37

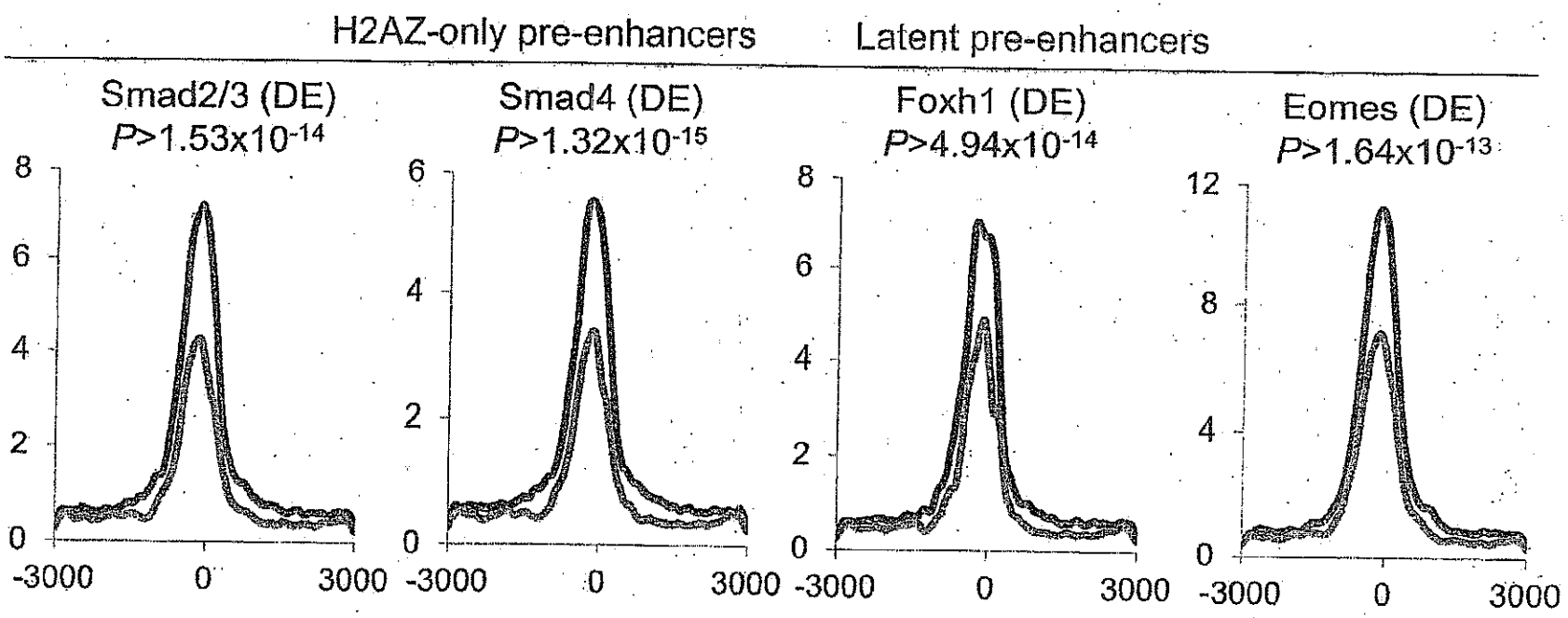

H2AZ-only pre-enhancers
Latent pre-enhancers
Smad2/3 (DE)
P>1.53x10-14
Smad4 (DE)
P>1.32x10-15
Foxh1 (DE)
P>4.94x10-14
Eomes (DE)
P>1.64x10-13
8
6
4
2
0
-3000
0
3000
12

FIG. 38

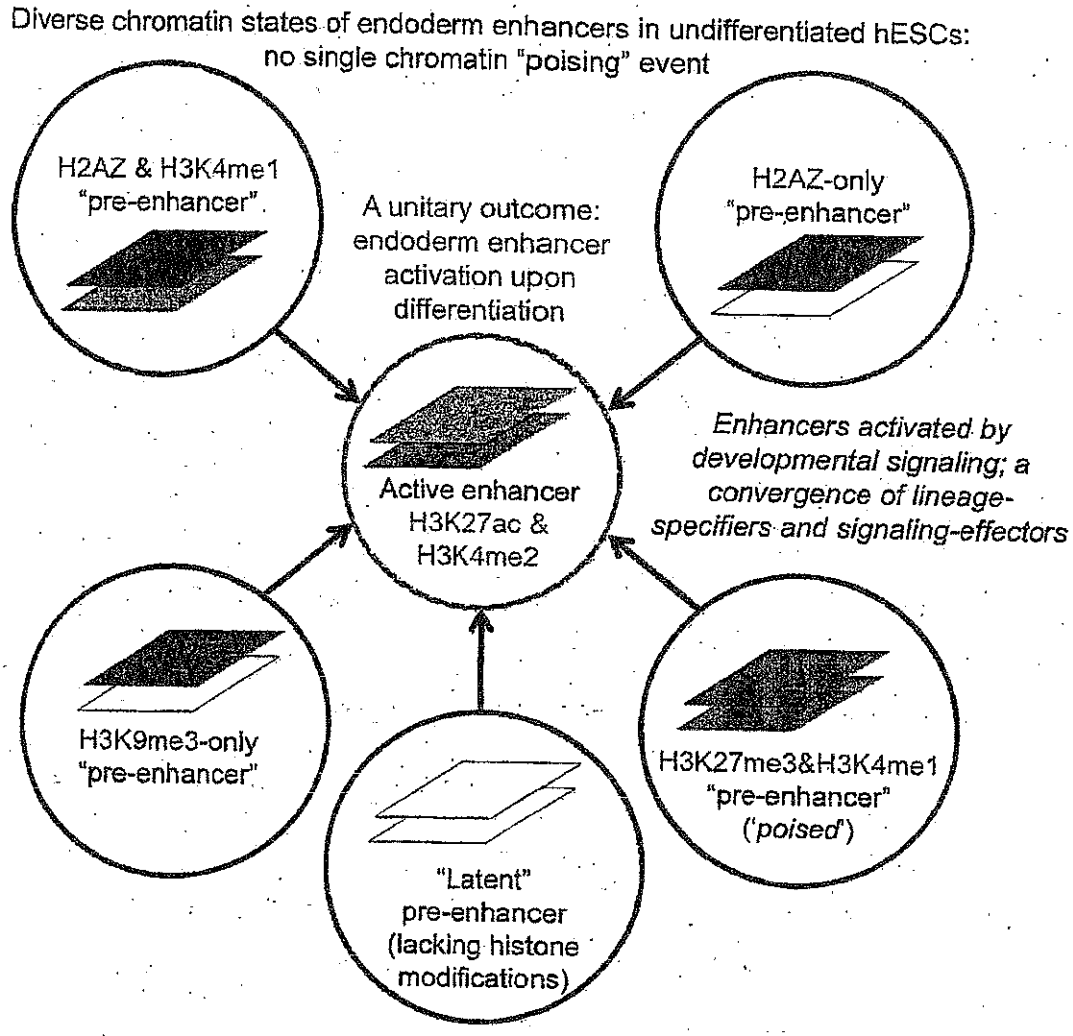

Diverse chromatin states of endoderm enhancers in undifferentiated hESCs:
no single chromatin "poising" event
H2AZ & H3K4me1
"pre-enhancer"
A unitary outcome:
endoderm enhancer
activation upon
differentiation
H2AZ-only
"pre-enhancer"
Active enhancer
H3K27ac &
H3K4me2
Enhancers activated by
developmental signaling; a
convergence of lineage-
specifiers and signaling-effectors
H3K9me3-only
"pre-enhancer"
"Latent"
pre-enhancer
(lacking histone
modifications)
H3K27me3&H3K4me1
"pre-enhancer"
('poised')

FIG. 39

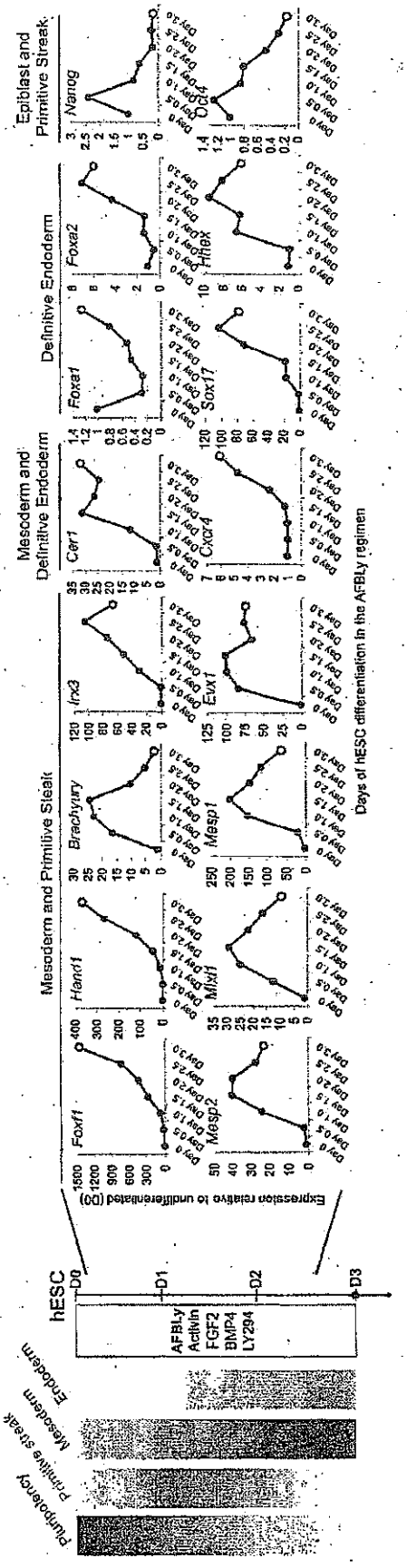
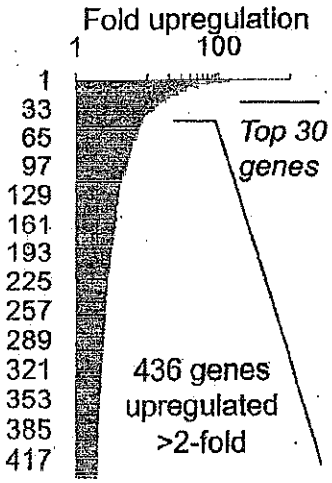

hESC
D0
D1
D2
D3
AFBLy
Activin
FGF2
BMP4
LY294
pluripotency
Primitive streak
Mesoderm
Endoderm
Expression relative to undifferentiated (D0)
Mesoderm and Primitive Streak
Mesoderm and Definitive Endoderm
Definitive Endoderm
Epiblast and Primitive Streak
Foxf1
Hand1
Brachyury
Irx3
Cer1
Foxa1
Foxa2
Nanog
Mesp2
Mixl1
Mesp1
Evx1
Cxcr4
Sox17
Hhex
Oct4
Days of hESC differentiation in the AFBLy regimen

FIG. 40



Top 30 genes upregulated by day 3 AFBLy differentiation versus undifferentiated hESCs (Touboul et al., 2010)

Rank	Gene name	Fold change	Rank	Gene name	Fold change	Rank	Gene name	Fold change
1	HAND1	146.30	11	GRP	35.63	21	BMP2	18.25
2	CER1	140.21	12	MFAP4	35.34	22	GSC	17.91
3	GRP	122.55	13	AGTRL1	34.41	23	SNAI2	17.43
4	LHX1	85.59	14	PTHR1	31.40	24	LZTS1	17.28
5	FOXC1	72.66	15	CXorf6	29.29	25	COL13A1	16.54
6	LEFTY2	53.34	16	CYP26A1	28.34	26	SOX17	14.74
7	MIXL1	52.05	17	IRX3	26.99	27	SMAD6	14.53
8	MESP1	44.61	18	EOMES	26.66	28	HAK	14.06
9	FGF17	38.37	19	TMOD1	22.22	29	NLF2	14.04
10	CYP26A1	35.91	20	FAM89A	19.65	30	PCDH17	13.48

Red = known mesodermal expression | Blue = definitive endoderm-exclusive

FIG. 41

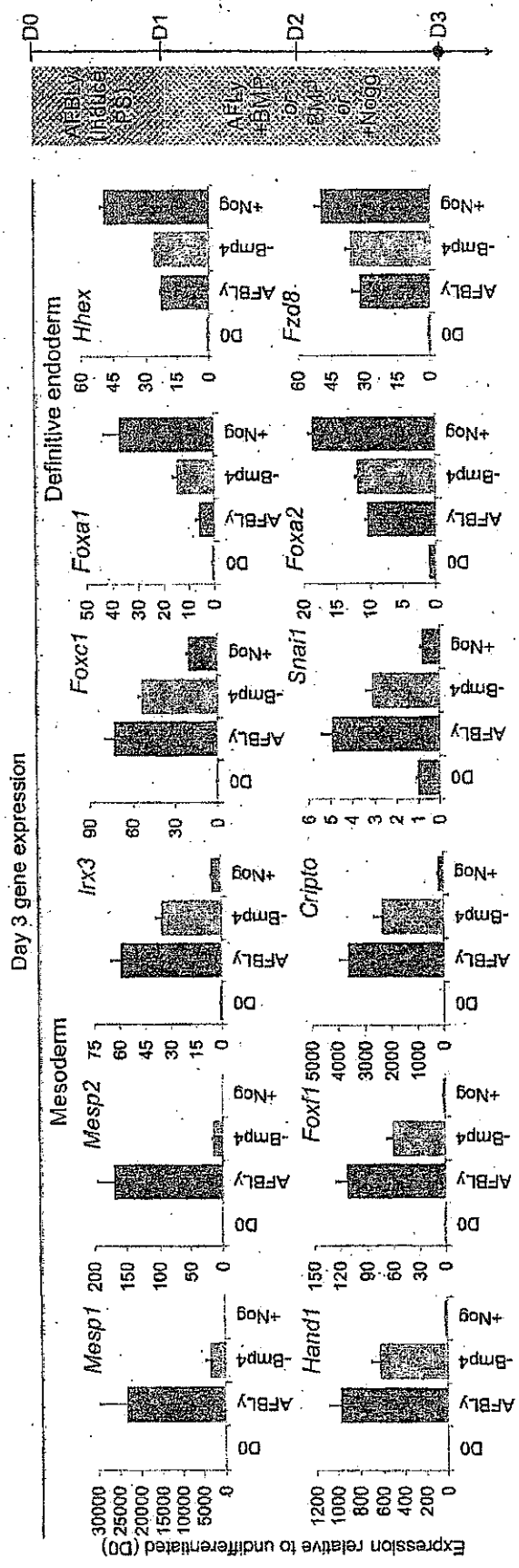

D0
D1
D2
D3
AFBLY
Undiff.
cells
AFBLY
+BMP4
or
-BMP4
+Noggin
Day 3 gene expression
Mesoderm
Definitive endoderm
Mesp1
Mesp2
Irx3
Foxc1
Foxa1
Hhex
Hand1
Foxf1
Cripto
Snai1
Foxa2
Fzd8
D0
AFBLy
-Bmp4
+Nog
Expression relative to undifferentiated (D0)

FIG. 42

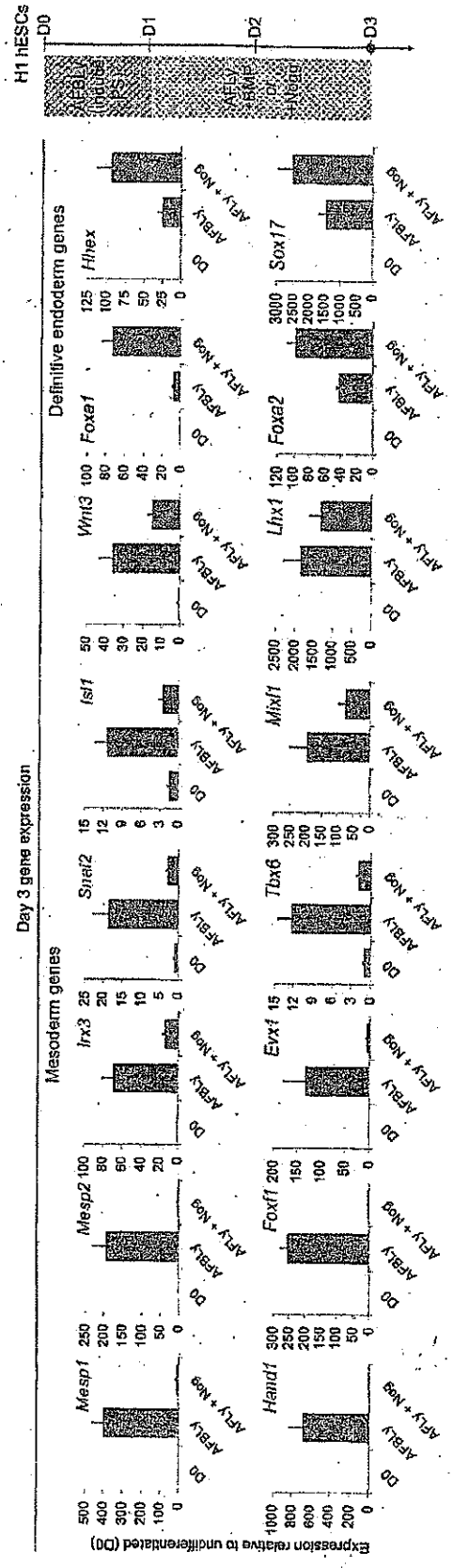

H1 hESCs
D0
D1
D2
D3
Day 3 gene expression
Mesoderm genes
Definitive endoderm genes
Expression relative to undifferentiated (D0)
Mesp1
Mesp2
Irx3
Snai2
Isl1
Wnt3
Foxa1
Hhex
Hand1
Foxf1
Evx1
Tbx6
Mixl1
Lhx1
Foxa2
Sox17
D0
AFBLY
AFLY + Nog

FIG. 43

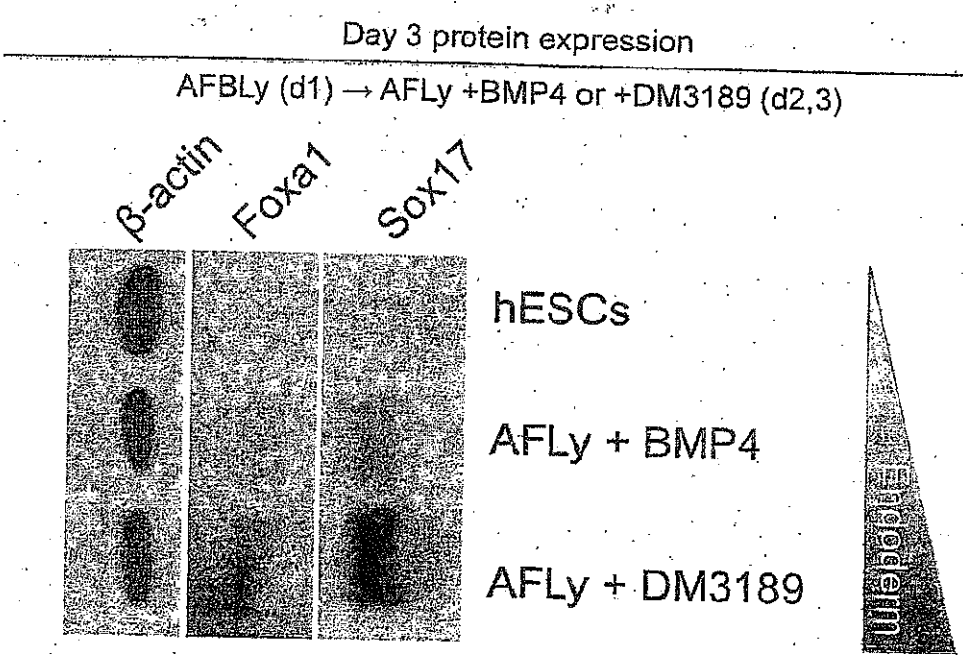

Day 3 protein expression
AFBLy (d1) → AFLy +BMP4 or +DM3189 (d2,3)
β-actin
Foxa1
Sox17
hESCs
AFLy + BMP4
AFLy + DM3189
Undifferentiated
Differentiated

FIG. 44

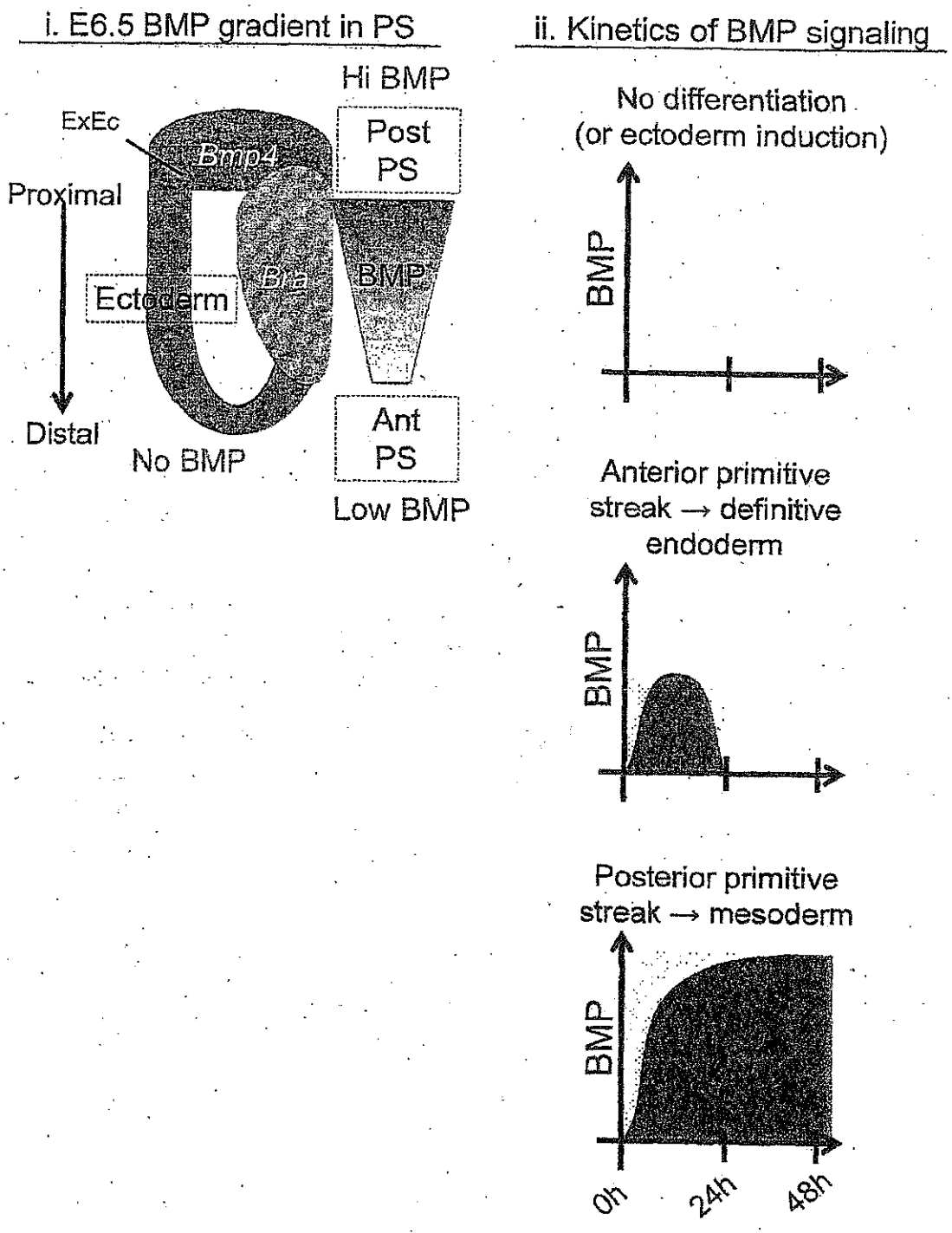
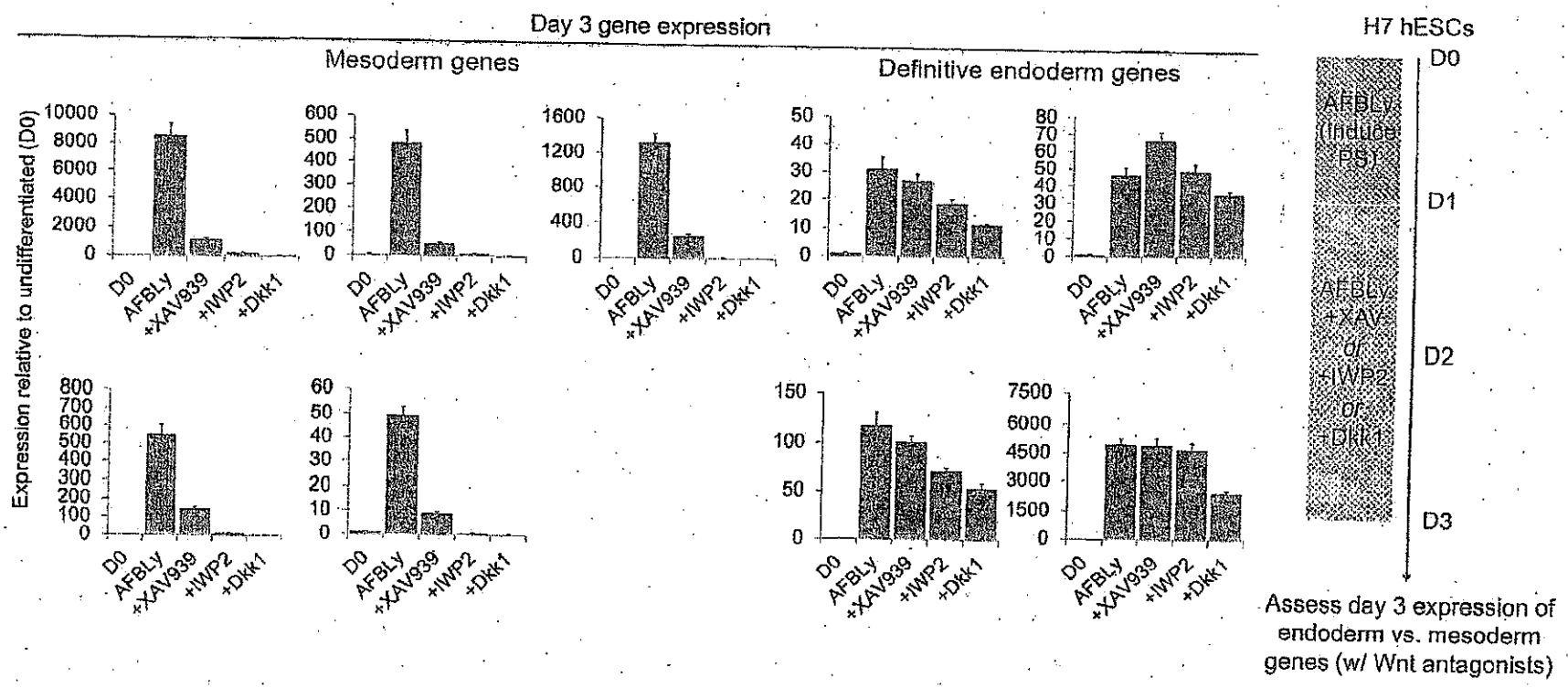

i. E6.5 BMP gradient in PS
Hi BMP
ExEc
Bmp4
Post PS
Proximal
Ectoderm
BMP
Distal
No BMP
Ant PS
Low BMP
ii. Kinetics of BMP signaling
No differentiation (or ectoderm induction)
BMP
Anterior primitive streak → definitive endoderm
BMP
Posterior primitive streak → mesoderm
BMP
0h
24h
48h

FIG. 45


Day 3 gene expression
Mesoderm genes
Definitive endoderm genes
Expression relative to undifferentiated (D0)
D0
AFBLy
+XAV939
+IWP2
+Dkk1
H7 hESCs
D0
AFBLy (Induce PS)
D1
AFBLy +XAV or +IWP2 or +Dkk1
D2
D3
Assess day 3 expression of endoderm vs. mesoderm genes (w/ Wnt antagonists)

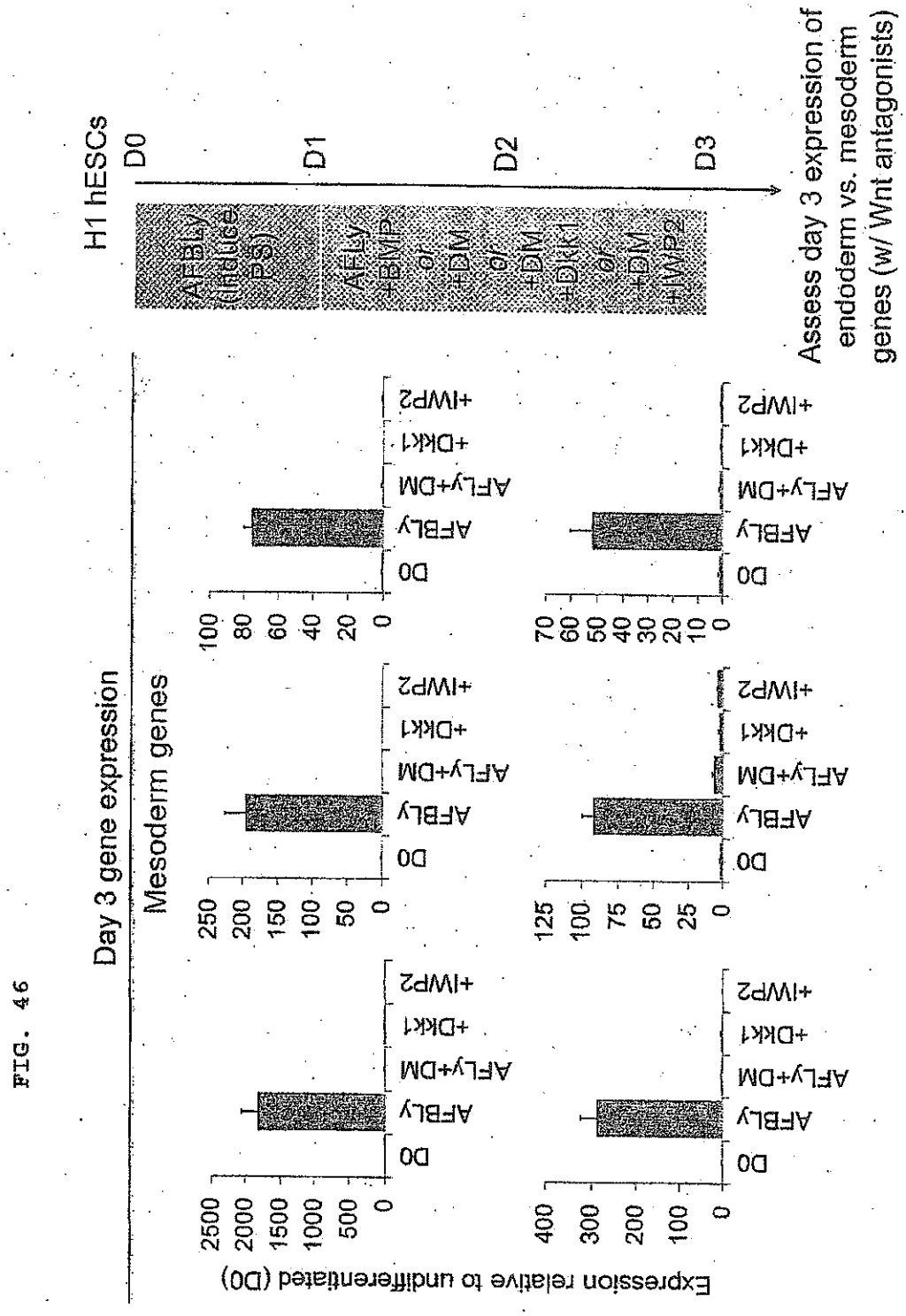
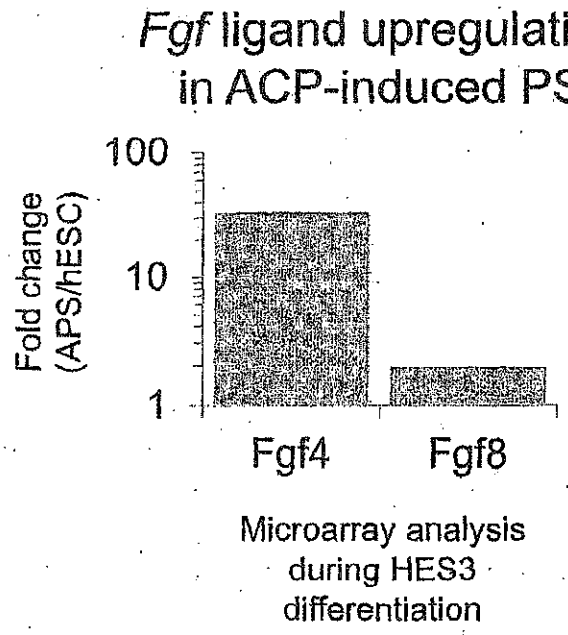

H1 hESCs
D0
D1
D2
D3
AFBLy (Induce PS)
AFLy +BMP or +DM
AFLy +DM +Dkk1 or +DM +IWP2
Assess day 3 expression of endoderm vs. mesoderm genes (w/ Wnt antagonists)
Day 3 gene expression
Mesoderm genes
Expression relative to undifferentiated (D0)
D0
AFBLy
AFLy+DM
+Dkk1
+IWP2

FIG. 46

FIG. 47


Fgf ligand upregulation
in ACP-induced PS
Fold change
(APS/hESC)
100
10
1
Fgf4
Fgf8
Microarray analysis
during HES3
differentiation

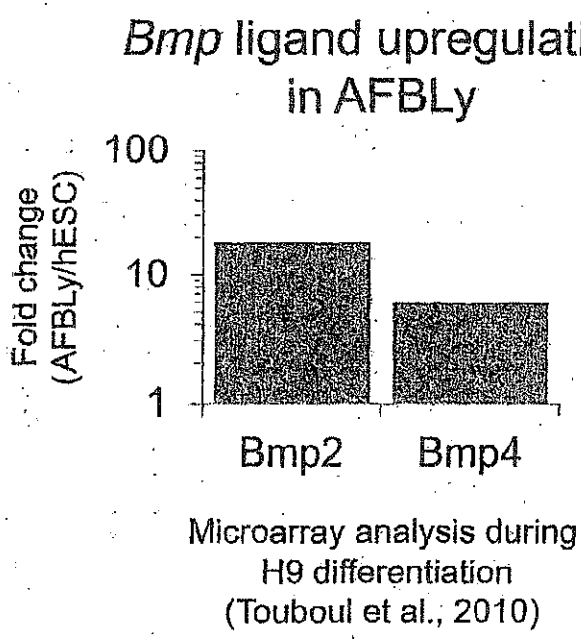

Bmp ligand upregulation
in AFBLy
Fold change
(AFBLy/hESC)
100
10
1
Bmp2
Bmp4
Microarray analysis during
H9 differentiation
(Touboul et al., 2010)

FIG. 48

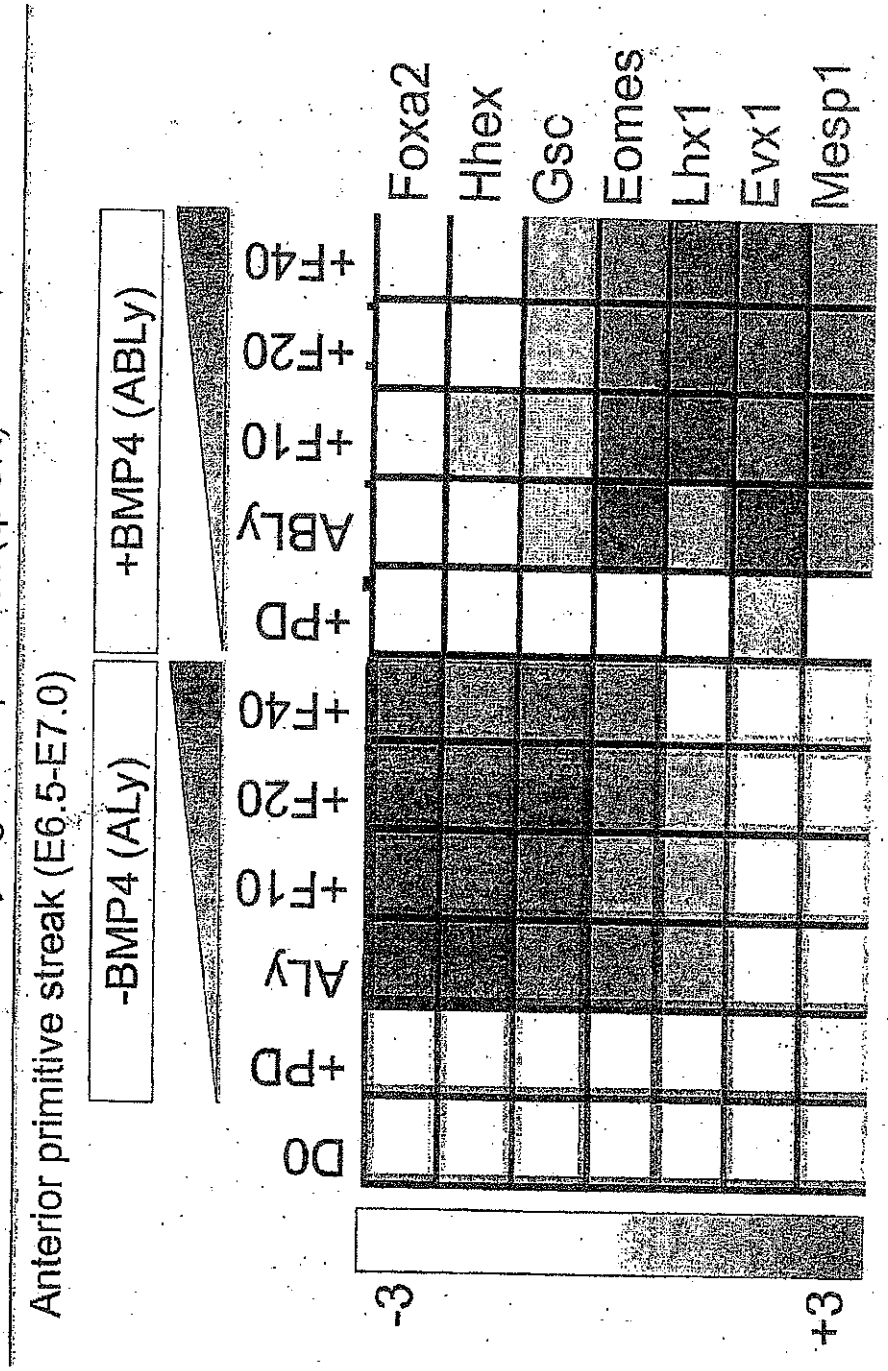

Day 1 gene expression (qPCR)
Anterior primitive streak (E6.5-E7.0)
-BMP4 (ALy)
+BMP4 (ABLy)
D0
+PD
ALy
+F10
+F20
+F40
ABLy
+PD
+F10
+F20
+F40
Foxa2
Hhex
Gsc
Eomes
Lhx1
Evx1
Mesp1
-3
+3

FIG. 49

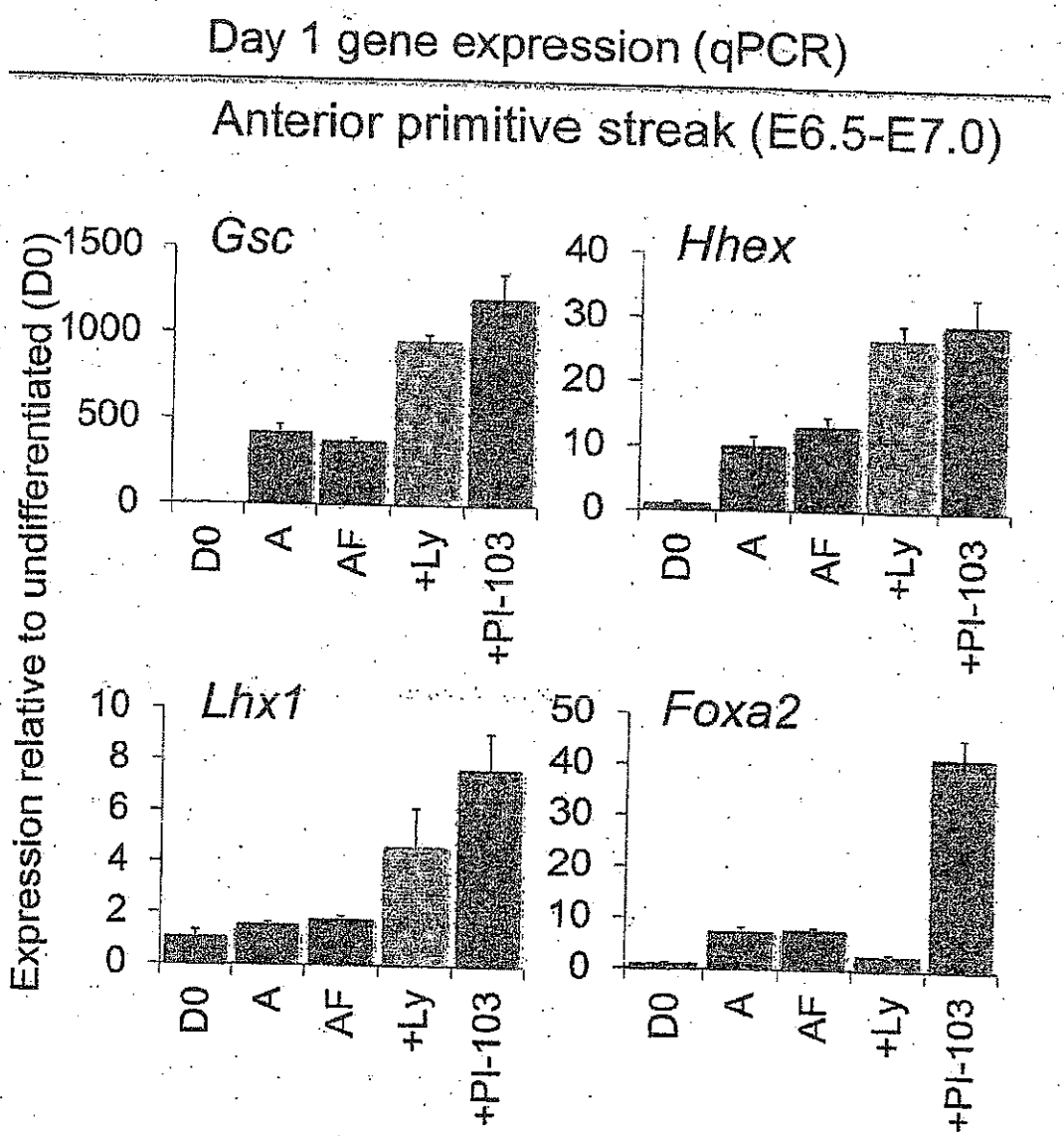
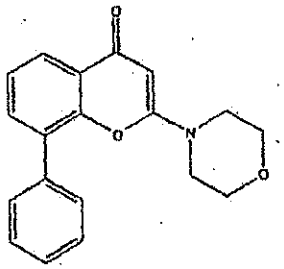

Day 1 gene expression (qPCR)
Anterior primitive streak (E6.5-E7.0)
Expression relative to undifferentiated (D0)
Gsc
Hhex
Lhx1
Foxa2
1500
1000
500
0
40
30
20
10
0
10
8
6
4
2
0
50
40
30
20
10
0
D0
A
AF
+Ly
+PI-103

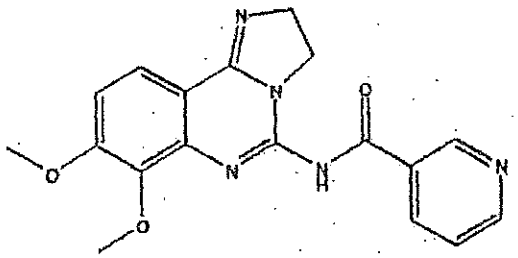
FIG. 50



LY294002

PI3K IC_{50} ≈ 1-2 μM

Also binds to and/or inhibits mTORC, CK2, GSK3β, PLK1, PIM1, PIM3, and HIPK2

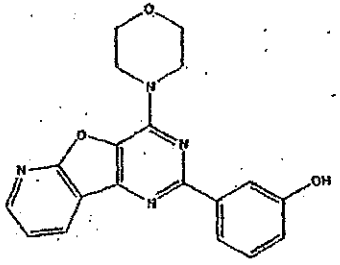


PIK-90

PI3K IC_{50} = 11-18 nM (p110α/p110γ)

Does not inhibit mTORC1, CK2, or GSK3β

Also weakly inhibits ATM (490 nM) and DNA-PK (64 nM)



PI-103

PI3K IC_{50} = 8 nM (p110α)

Does not inhibit CK2 or GSK3β

Also weakly inhibits ATR (850 nM), ATM (920 nM), DNA-PK (2 nM), mTORC1 (20 nM), mTORC2 (83 nM)

FIG. 51

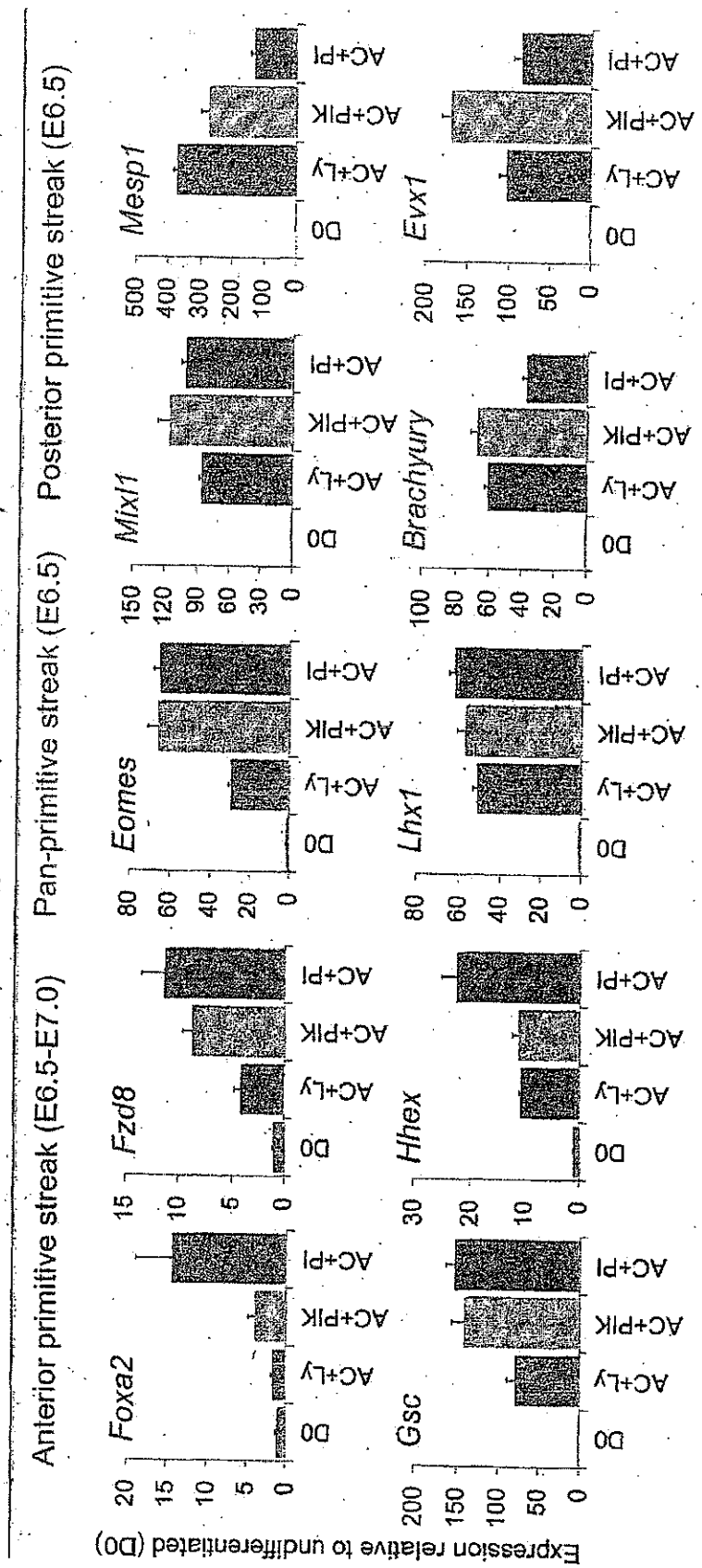

Day 1 gene expression (AC + PI3K inhibitor)
Anterior primitive streak (E6.5-E7.0)
Pan-primitive streak (E6.5)
Posterior primitive streak (E6.5)
Expression relative to undifferentiated (D0)
Foxa2
Fzd8
Eomes
Mixl1
Mesp1
Gsc
Hhex
Lhx1
Brachyury
Evx1
D0
AC+Ly
AC+PIK
AC+PI

FIG. 52

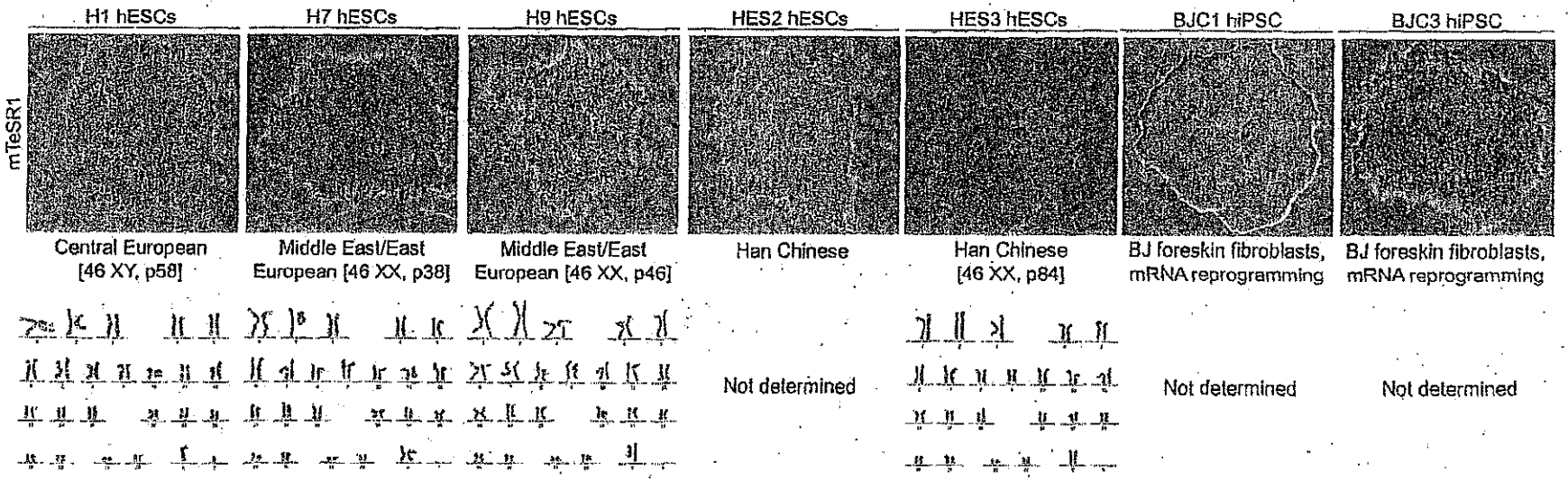

H1 hESCs
H7 hESCs
H9 hESCs
HES2 hESCs
HES3 hESCs
BJC1 hiPSC
BJC3 hiPSC
mTeSR1
Central European [46 XY, p58]
Middle East/East European [46 XX, p38]
Middle East/East European [46 XX, p46]
Han Chinese
Han Chinese [46 XX, p84]
BJ foreskin fibroblasts, mRNA reprogramming
BJ foreskin fibroblasts, mRNA reprogramming
Not determined
Not determined
Not determined

FIG. 53

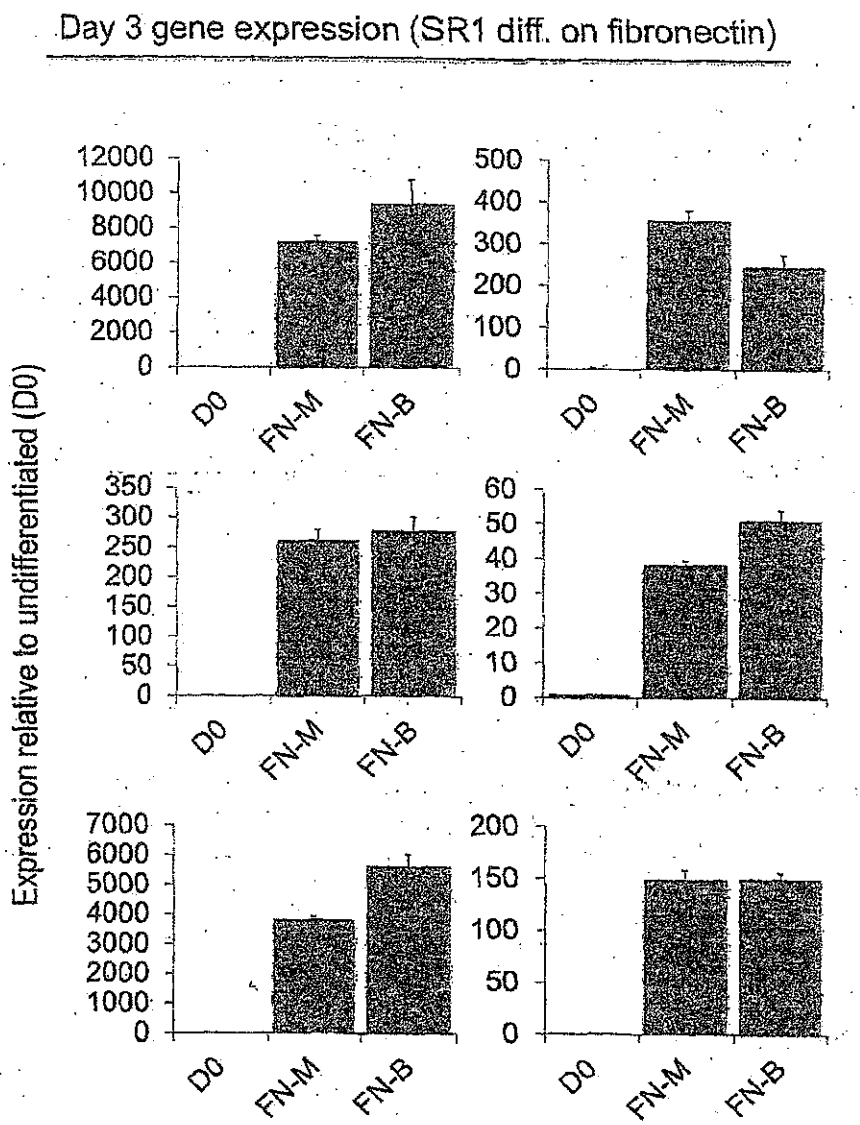

Day 3 gene expression (SR1 diff. on fibronectin)
Expression relative to undifferentiated (D0)
12000
10000
8000
6000
4000
2000
0
500
400
300
200
100
0
350
300
250
200
150
100
50
0
60
50
40
30
20
10
0
7000
6000
5000
4000
3000
2000
1000
0
200
150
100
50
0
D0
FN-M
FN-B

FIG. 54

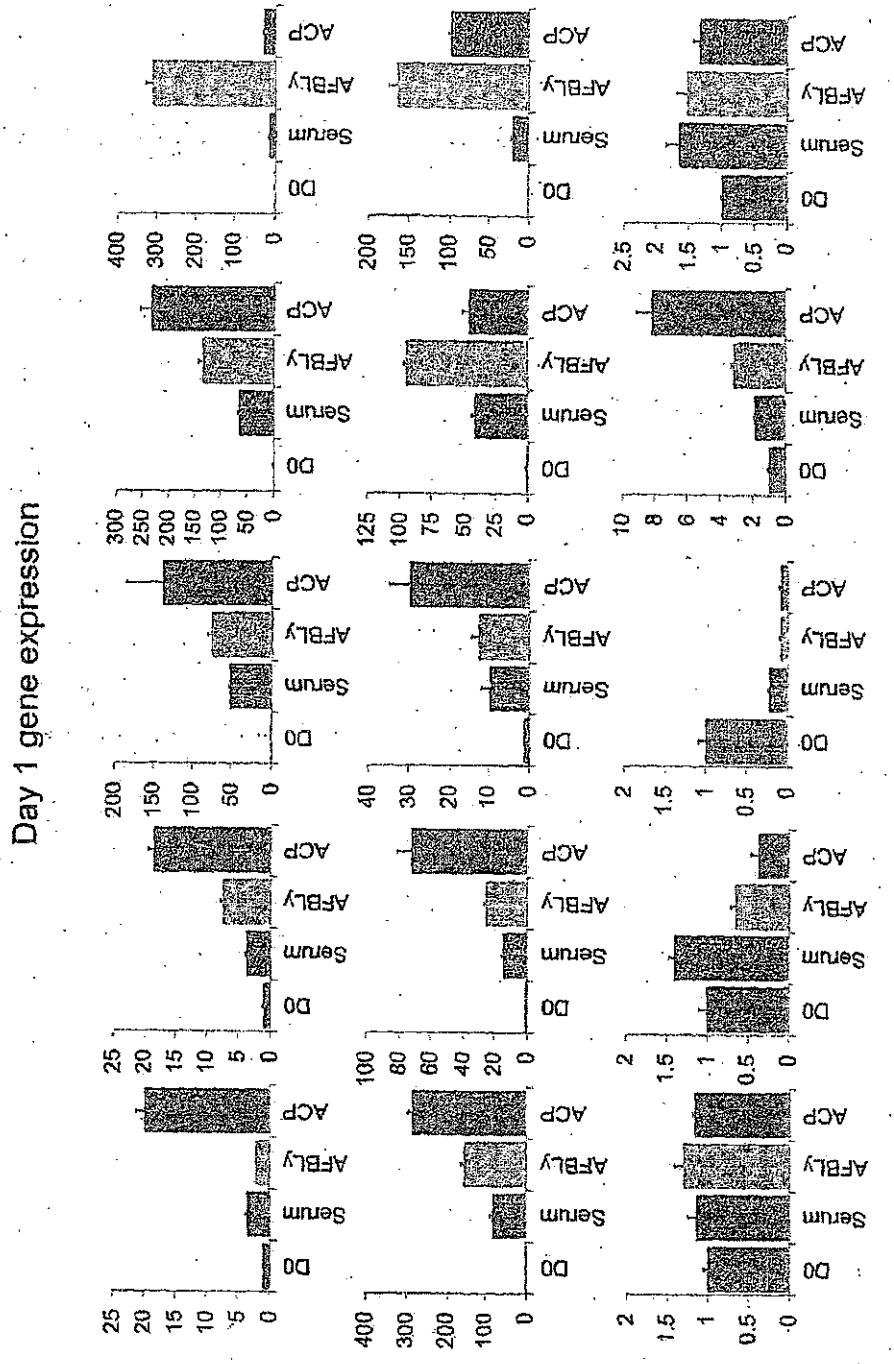
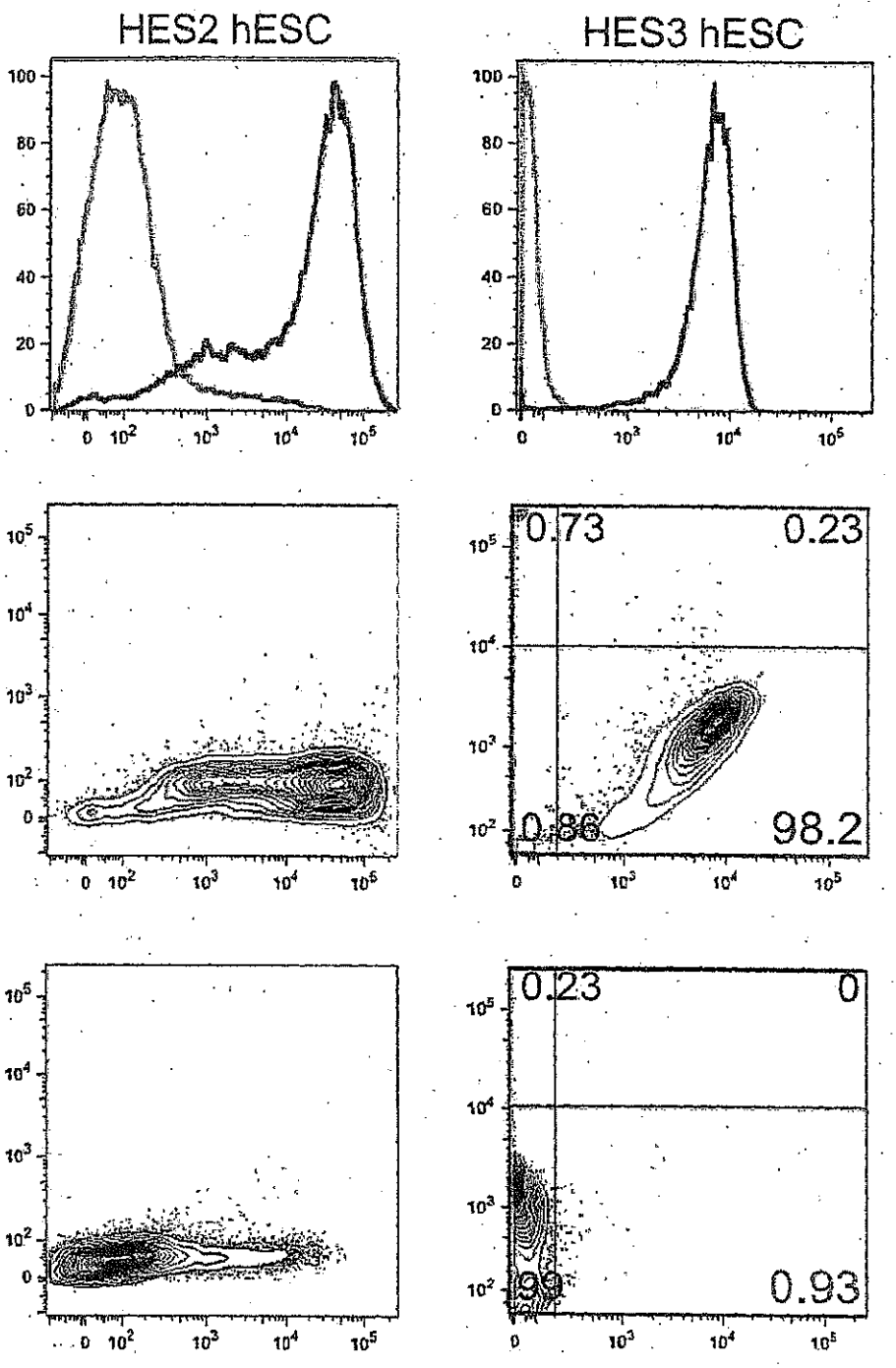


FIG. 55



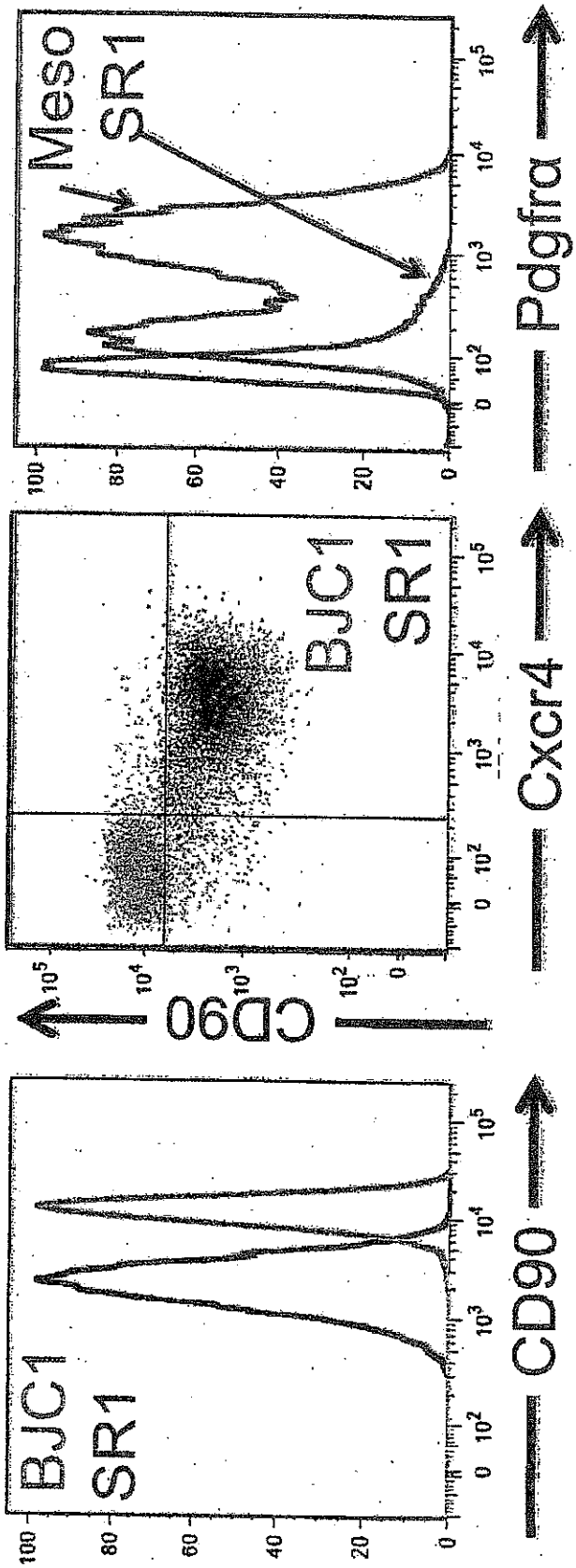


FIG. 56

FIG. 57

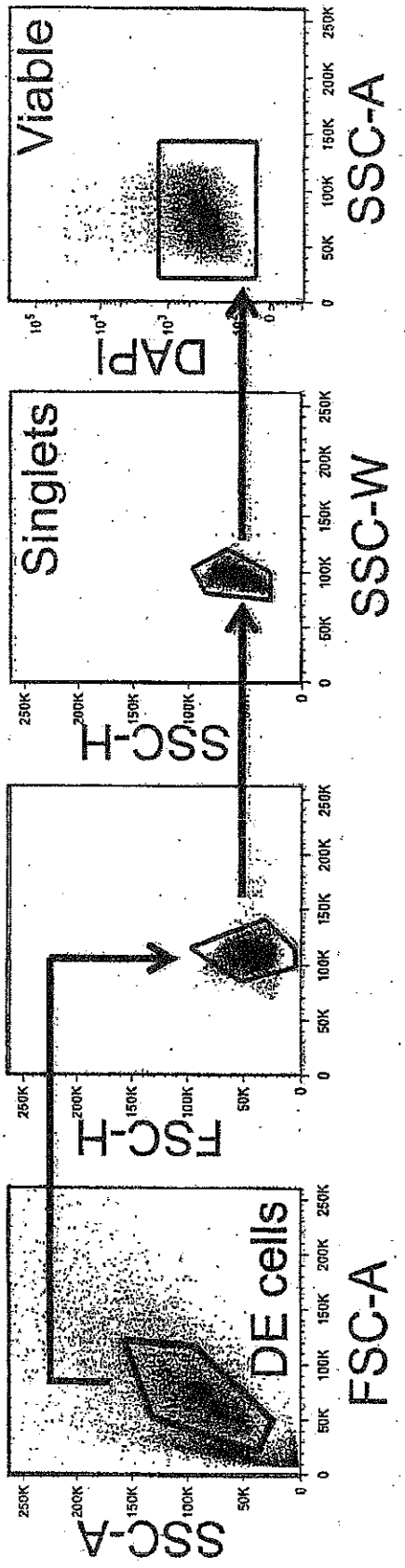


FIG. 58 (a), (b) and (c)

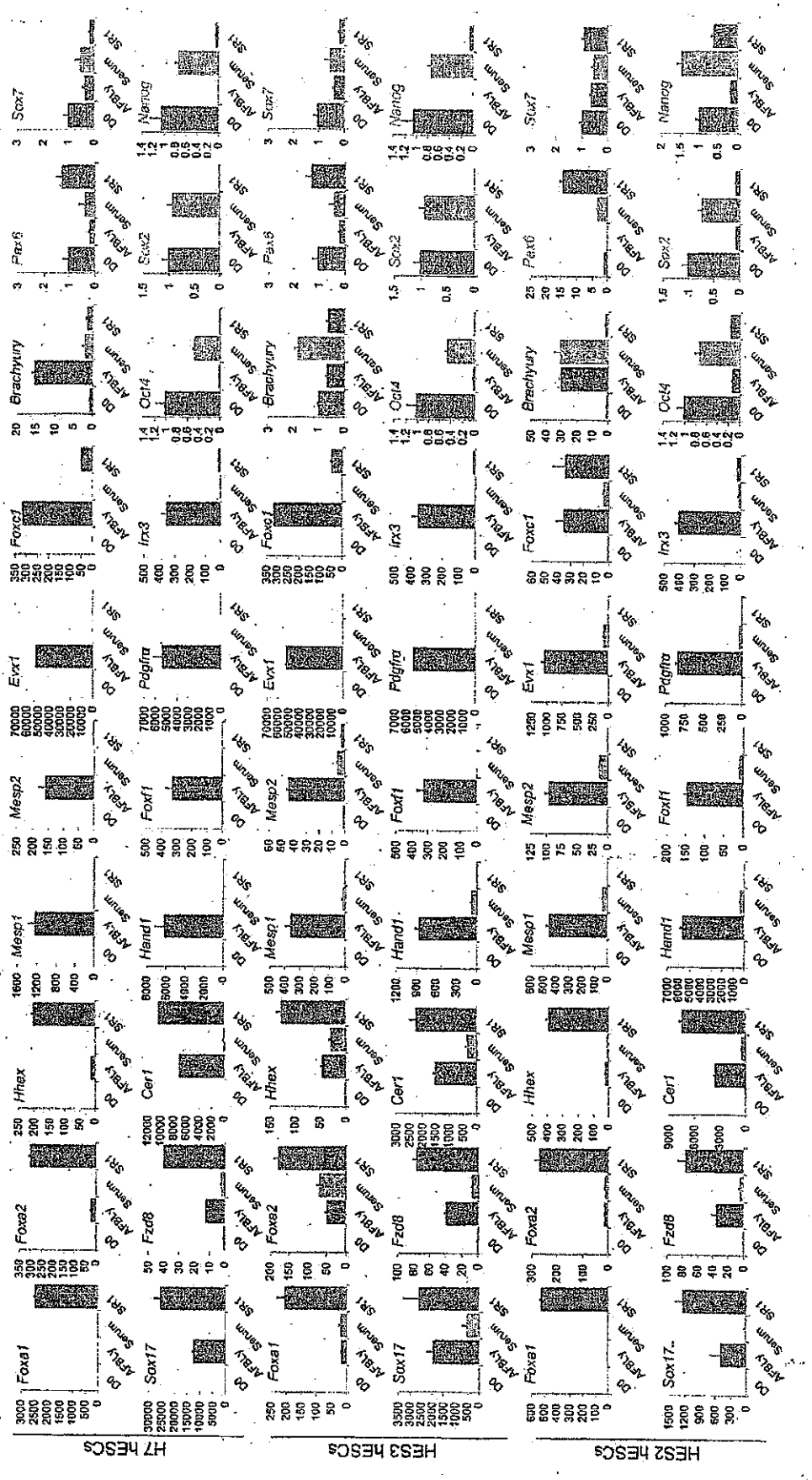


FIG. 58 (d), (e) and (f)

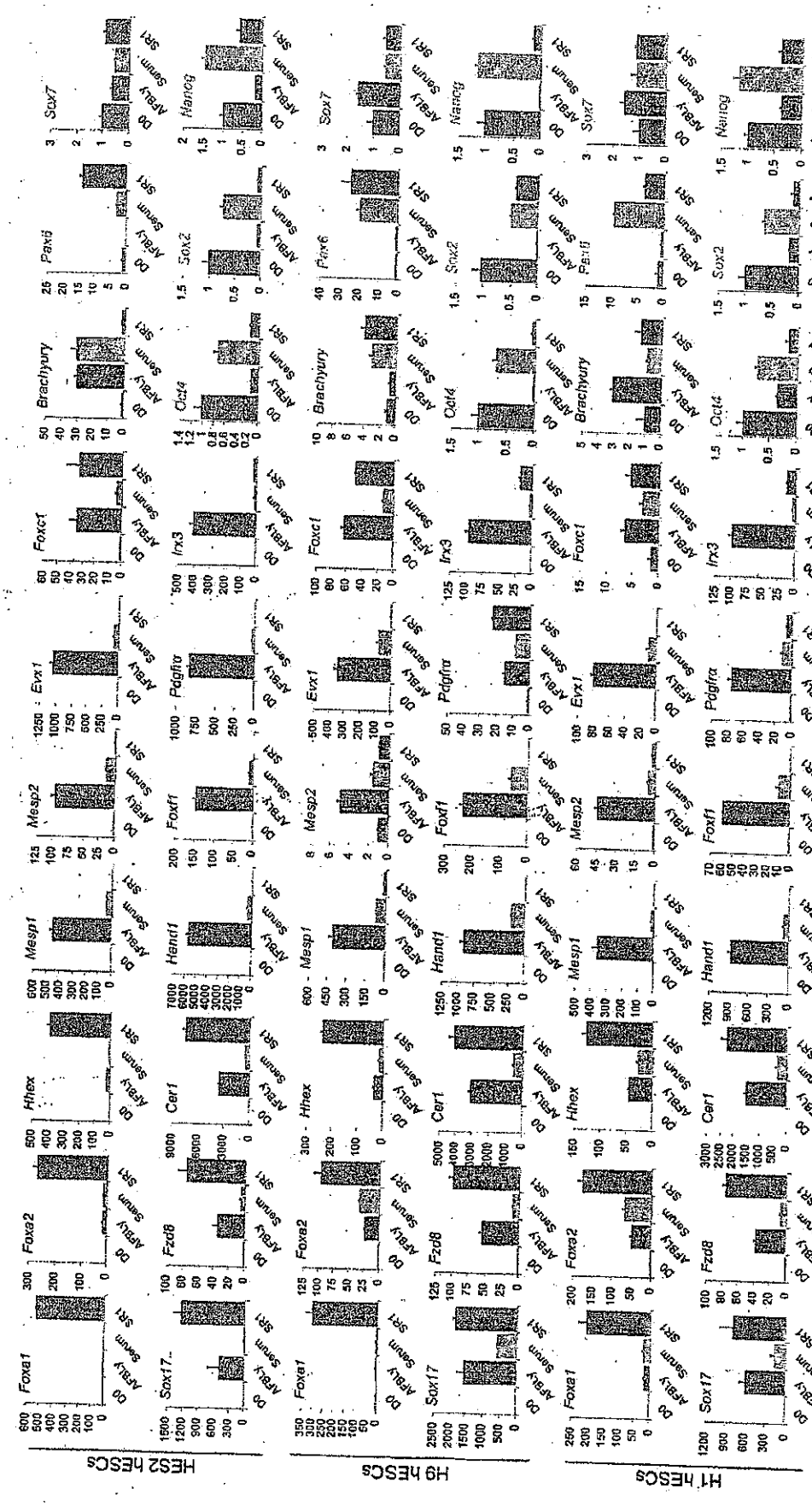
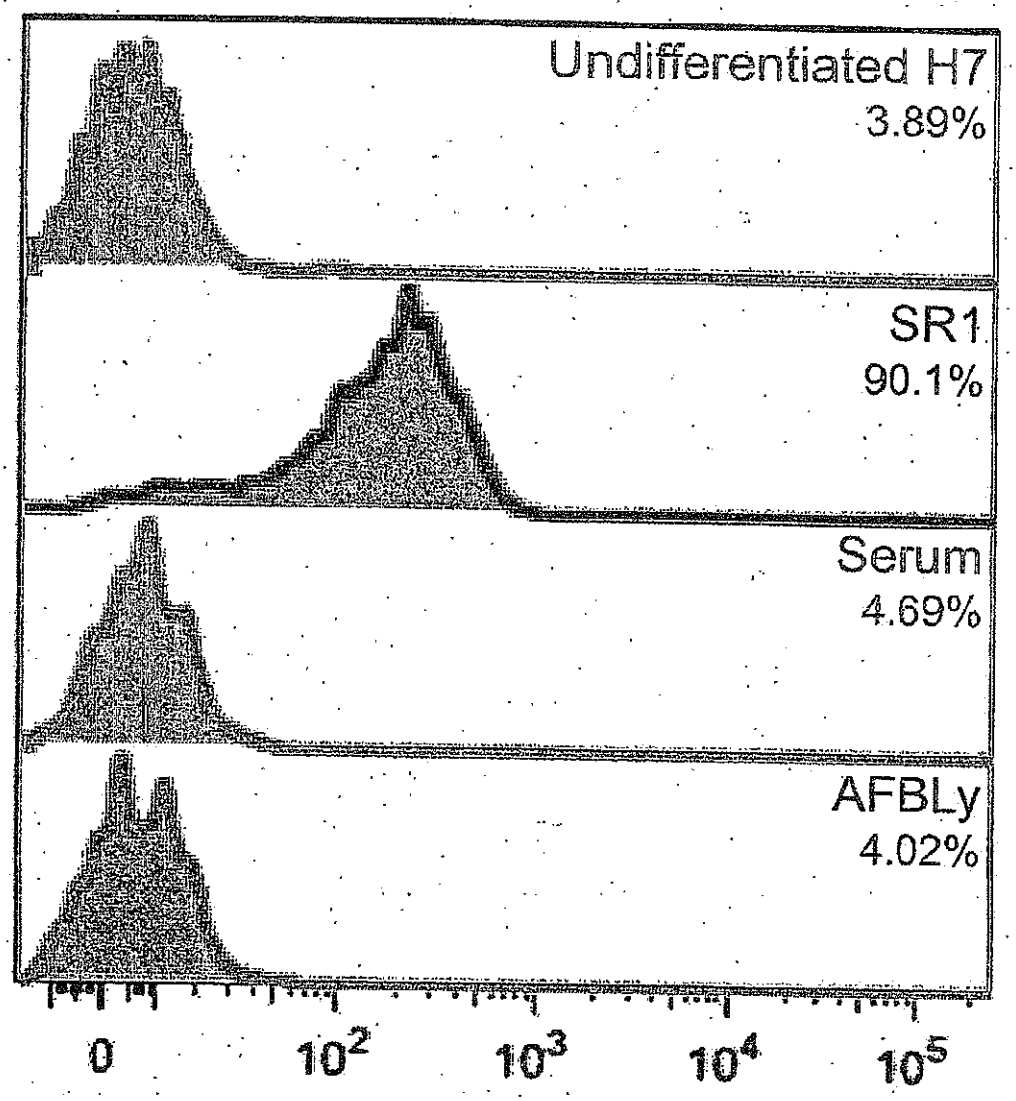


FIG. 59


Undifferentiated H7
3.89%
SR1
90.1%
Serum
4.69%
AFBLy
4.02%
0
10^2
10^3
10^4
10^5

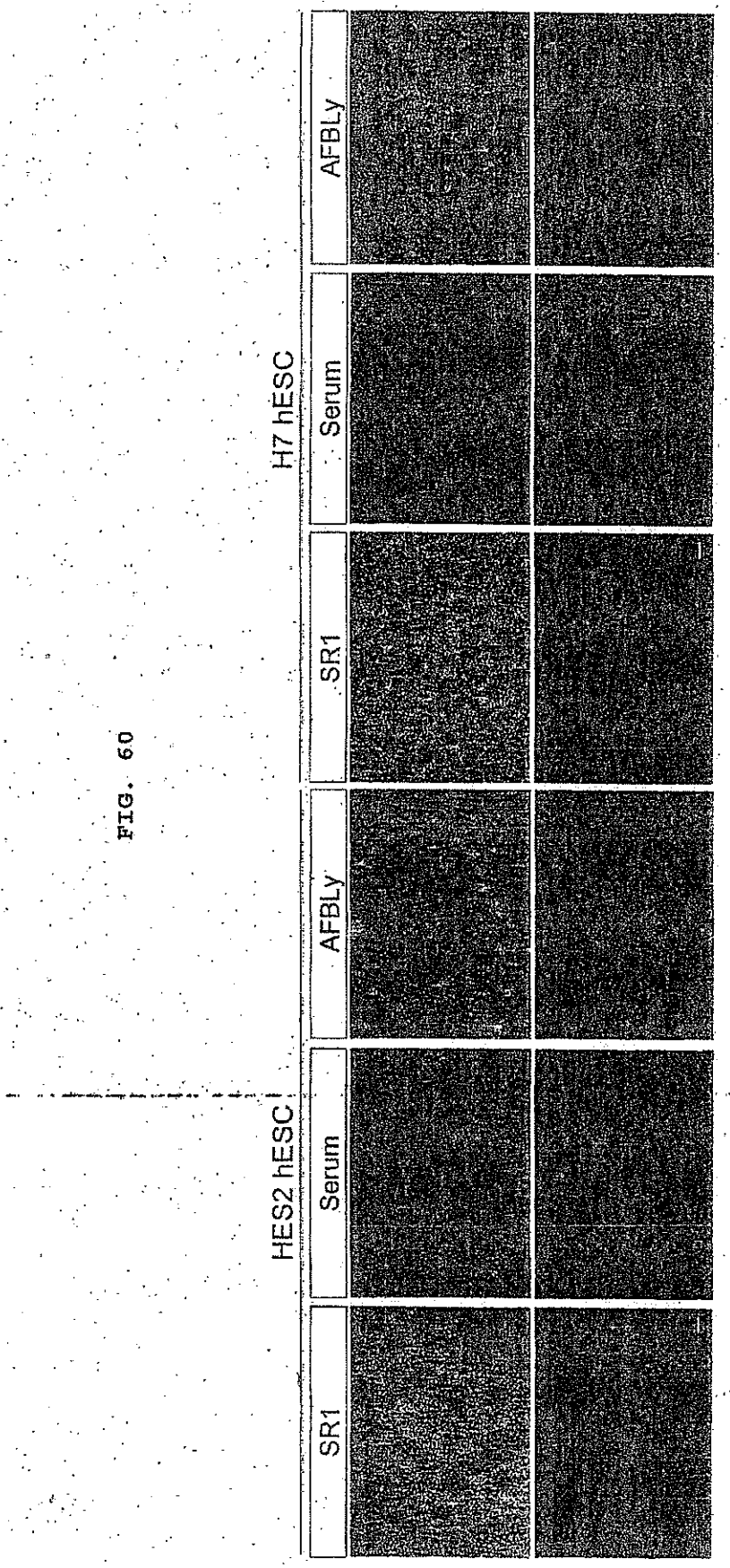


FIG. 60

FIG. 61

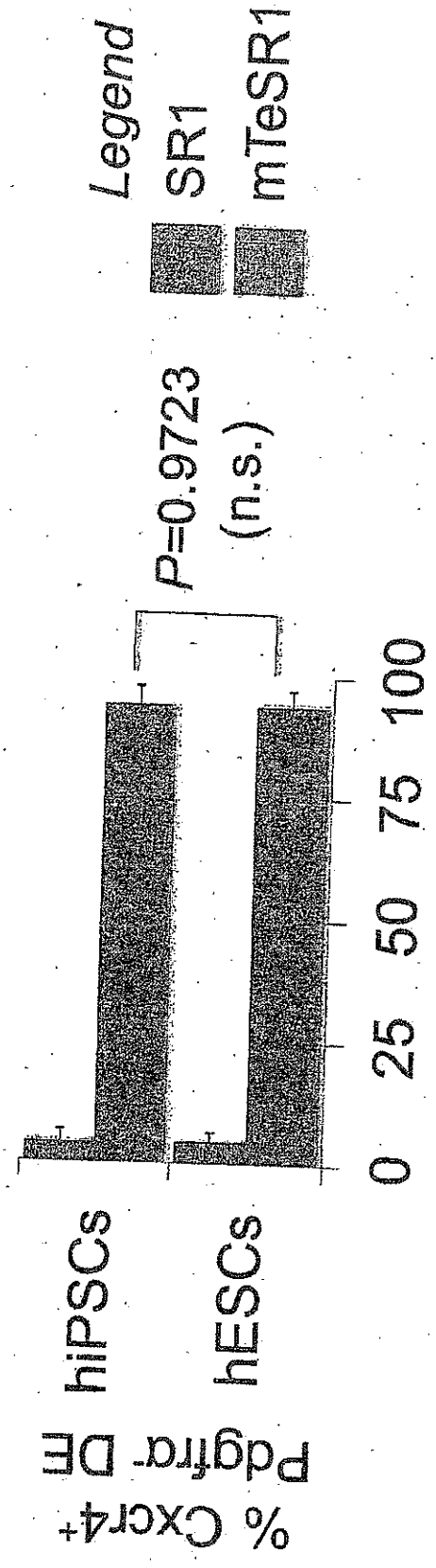

Legend
SR1
mTeSR1
P=0.9723
(n.s.)
0
25
50
75
100
hiPSCs
hESCs
% Cxcr4+
Pdgfra+ DE

FIG. 62

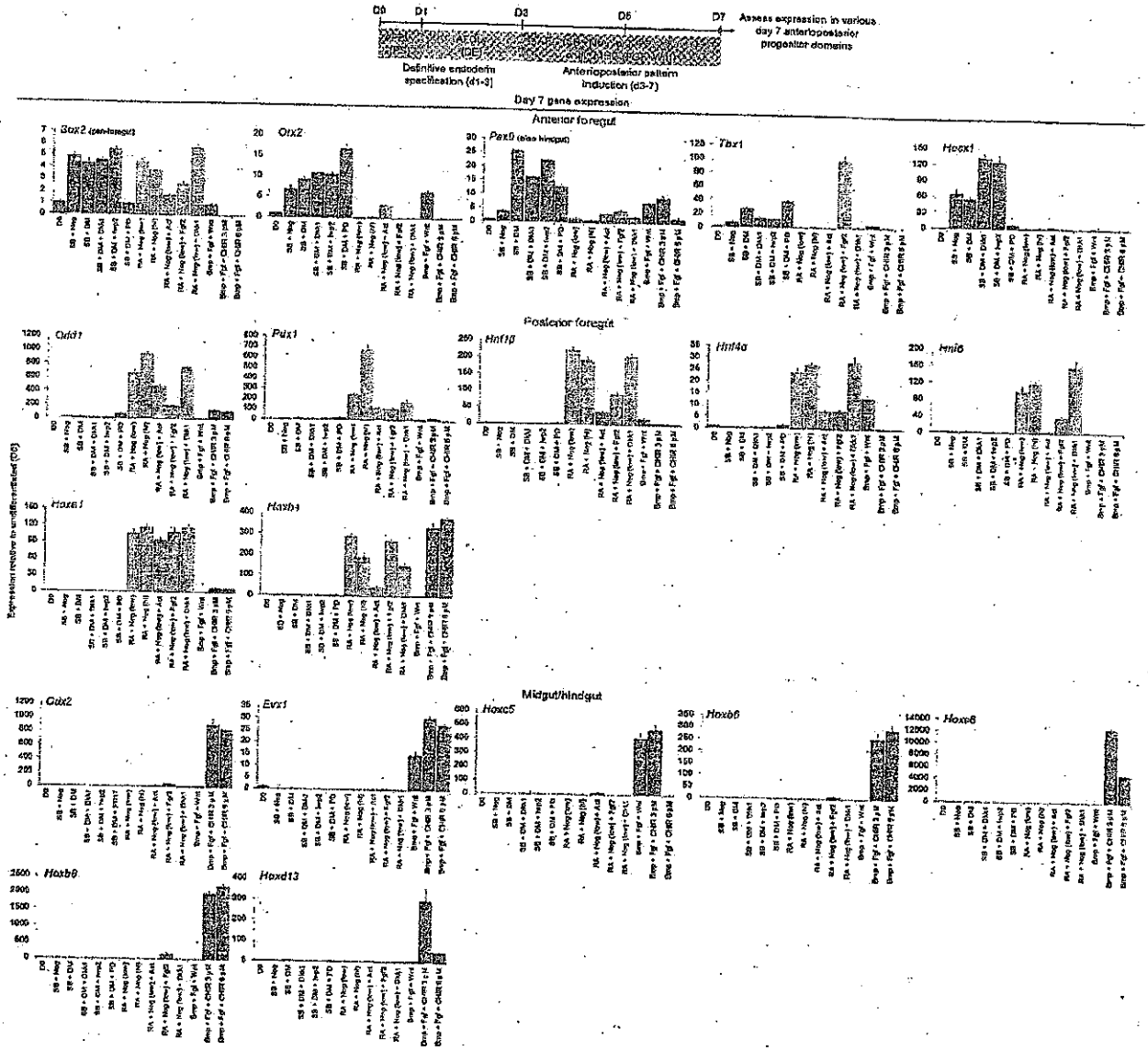

D0
D1
D3
D5
D7
Assess expression in various day 7 anteriroposterior progenitor domains
Definitive endoderm specification (d1-3)
Anteriroposterior pattern induction (d3-7)
Day 7 gene expression
Anterior foregut
Sox2 (pan-foregut)
Otx2
Pax9 (also hindgut)
Tbx1
Hoxc1
Posterior foregut
Cdx1
Pdx1
Hnf1β
Hnf4α
Hnf6
Hoxa1
Hoxb1
Midgut/hindgut
Cdx2
Evx1
Hoxc5
Hoxb6
Hoxb8
Hoxd13

FIG. 63

Day 7 gene expression (d4 DIPR → d5-7 conditions)

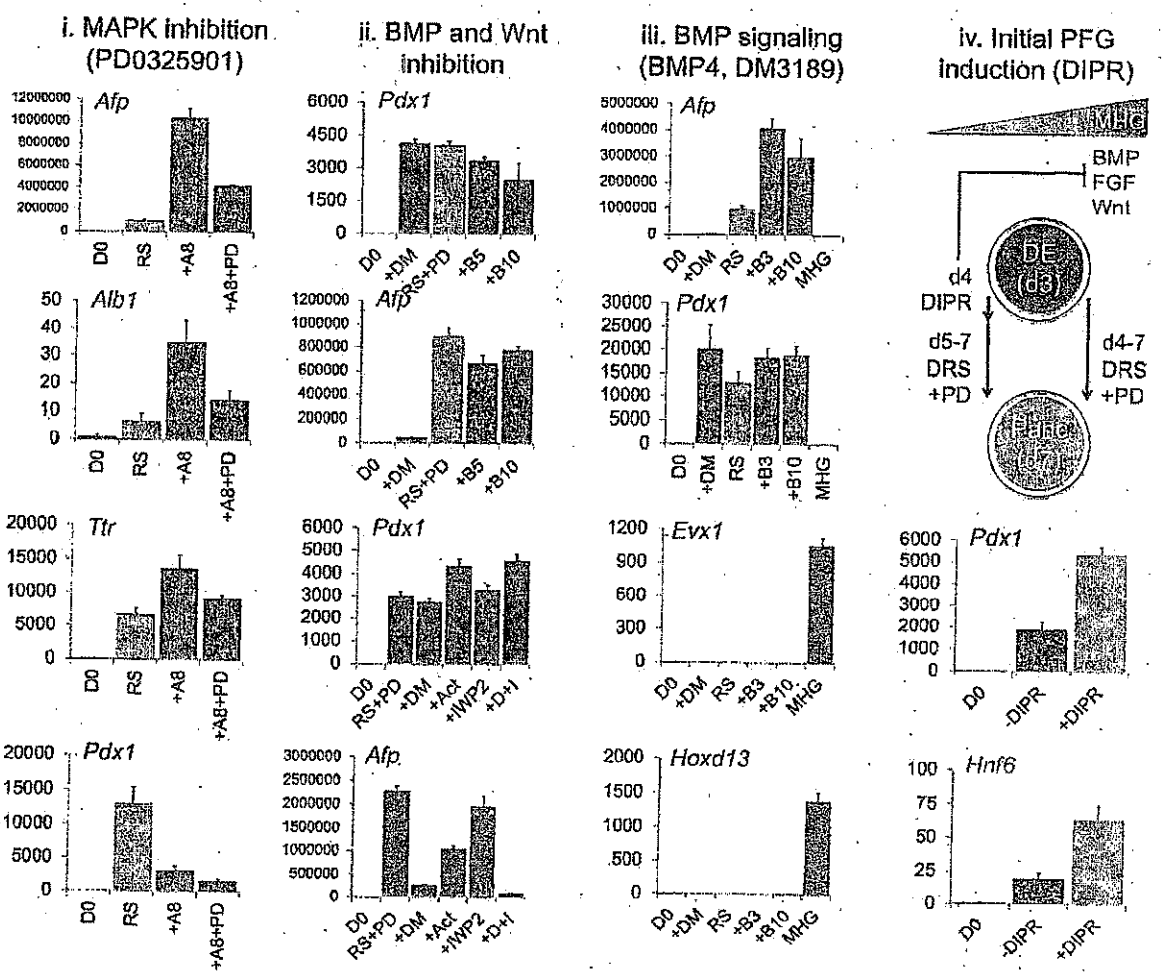


FIG. 64

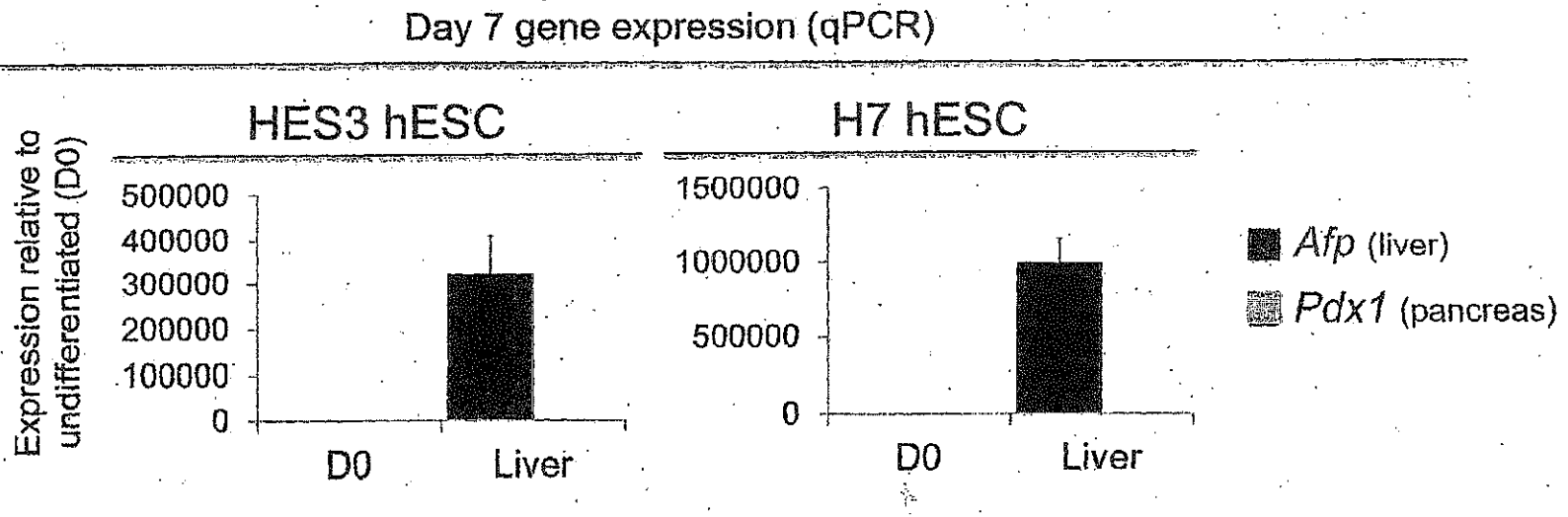

Day 7 gene expression (qPCR)
HES3 hESC
H7 hESC
Expression relative to undifferentiated (D0)
500000
400000
300000
200000
100000
0
1500000
1000000
500000
0
D0
Liver
D0
Liver
Afp (liver)
Pdx1 (pancreas)

FIG. 65

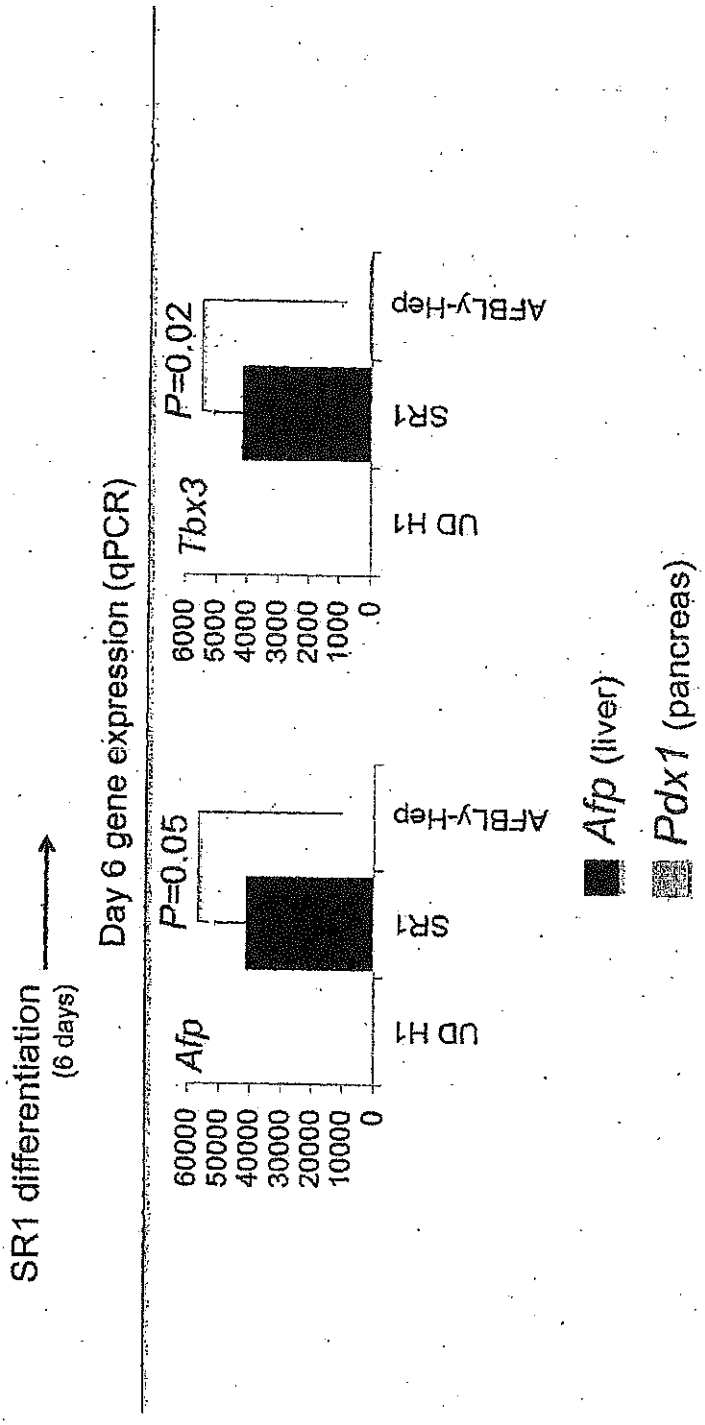

AFBLy-Hep
(25 days)
SR1 differentiation
(6 days)
Day 6 gene expression (qPCR)
Afp
P=0,05
60000
50000
40000
30000
20000
10000
0
UD H1
SR1
AFBLy-Hep
Tbx3
P=0,02
6000
5000
4000
3000
2000
1000
0
UD H1
SR1
AFBLy-Hep
Afp (liver)
Pdx1 (pancreas)

FIG. 66

Albumin



FIG. 67

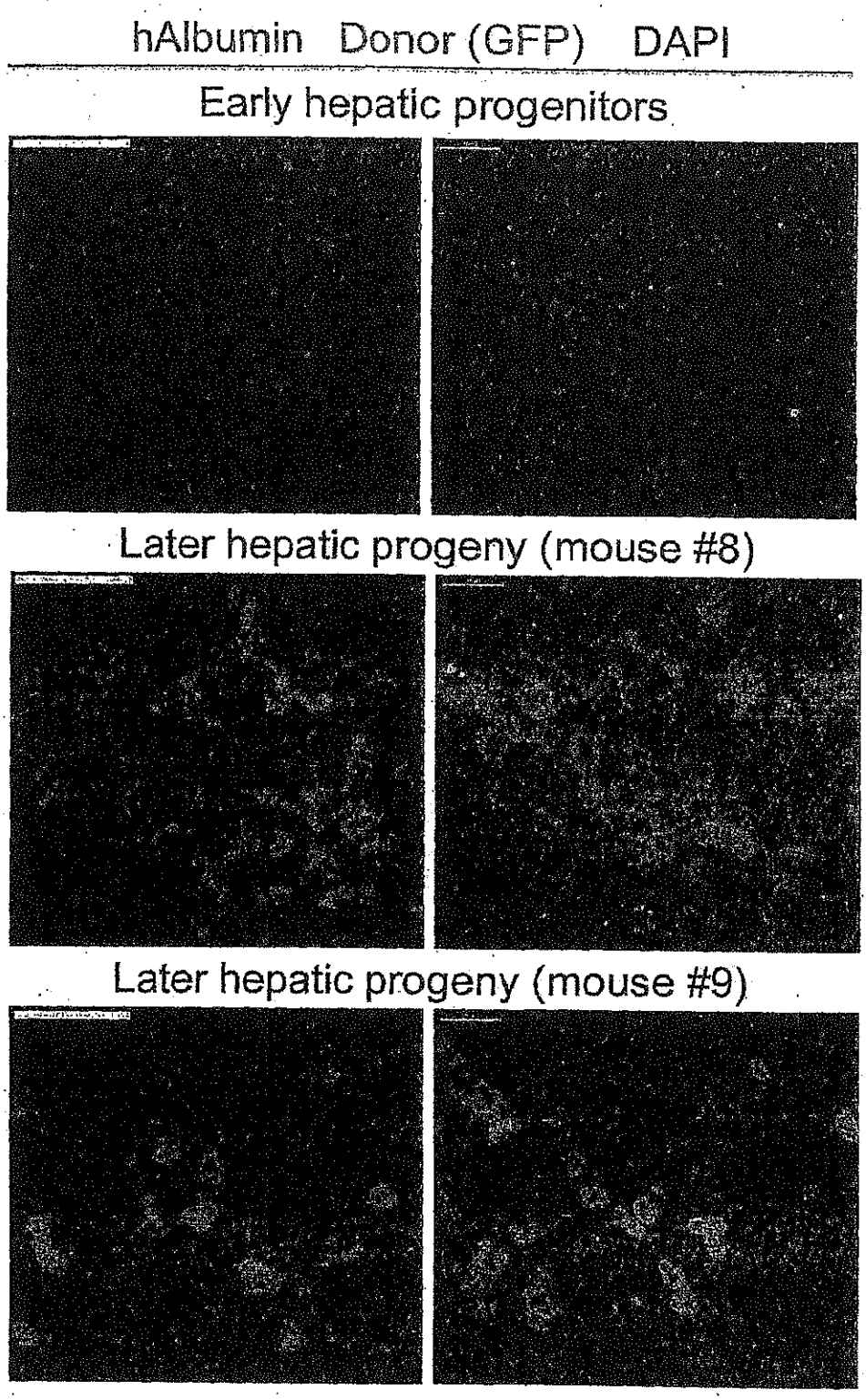

hAlbumin Donor (GFP) DAPI
Early hepatic progenitors
Later hepatic progeny (mouse #8)
Later hepatic progeny (mouse #9)

FIG. 68

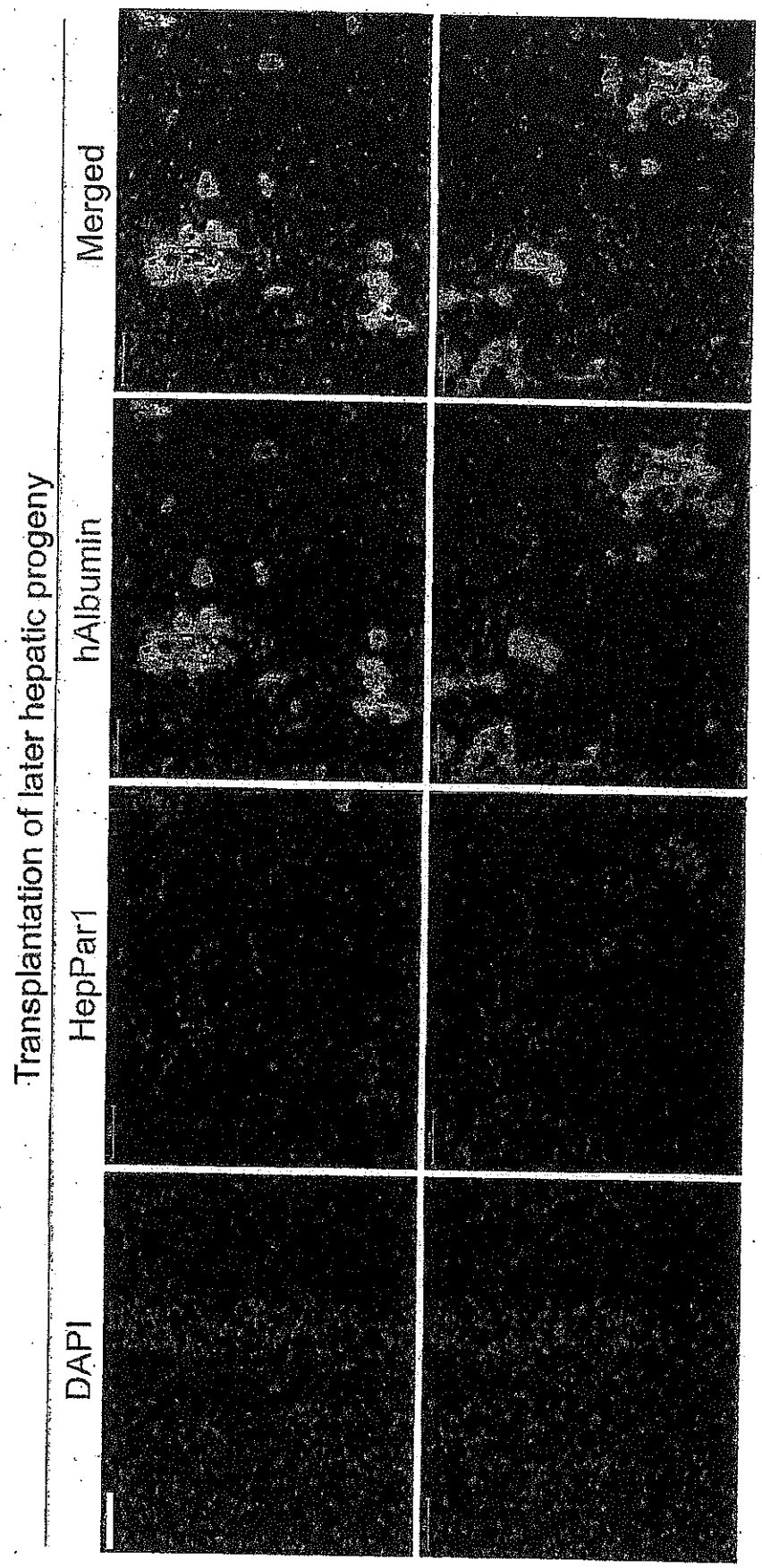

Transplantation of later hepatic progeny
DAPI
HepPar1
hAlbumin
Merged

FIG. 69

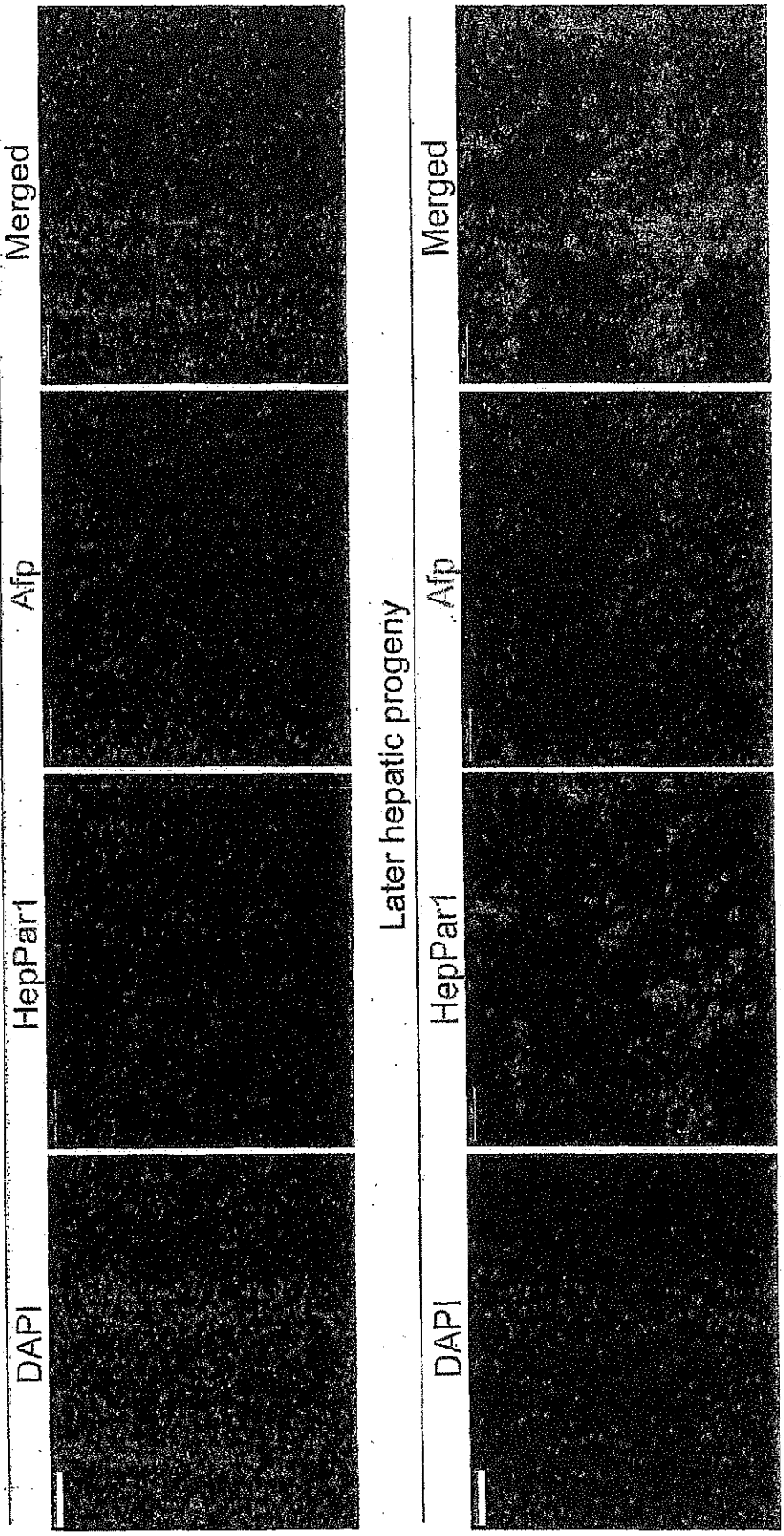

Early hepatic progenitors
DAPI
HepPar1
Afp
Merged
Later hepatic progeny
DAPI
HepPar1
Afp
Merged

FIG. 70

hESC line	H7 chIP-seq & RNA-seq # of Total Passed Illumina Reads (# of Uniquely Aligned Reads)						HES3 (# repli-cates)
	Input	**H3K4me3**	**H3K4me2**	**H3K27me3**	**H3K27ac**	**RNA-seq**	**Affymetrix microarray**
hESC	46,953,326 (37,999,327)	33,151,167 (23,968,294)	32,407,688 (28,071,539)	39,539,641 (28,812,536)	43,680,960 (37,421,478)	70,651,559 (54,097,899)	3
APS	40,668,117 (32,778,502)	33,621,173 (24,852,771)	35,277,317 (30,405,520)	36,807,047 (28,289,896)	37,109,028 (31,761,617)	90,049,689 (70,418,857)	3
DE	48,121,636 (38,684,983)	32,226,238 (24,024,660)	35,819,126 (30,360,291)	34,701,839 (27,362,400)	42,379,490 (36,162,419)	85,434,777 (68,458,887)	3
AFG	42,425,219 (34,203,212)	34,289,338 (25,343,250)	36,016,254 (31,503,417)	36,586,138 (28,987,197)	41,460,711 (36,427,381)	88,299,484 (71,416,623)	3
PFG	39,138,045 (31,423,936)	34,958,923 (26,369,516)	32,781,185 (28,808,105)	38,752,066 (31,017,154)	37,947,007 (33,321,267)	89,052,131 (73,263,188)	3
MHG	43,654,736 (35,469,473)	33,831,958 (25,238,641)	30,663,675 (26,708,061)	35,329,148 (28,295,115)	37,394,612 (32,219,198)	88,531,796 (68,860,031)	3

FIG. 71

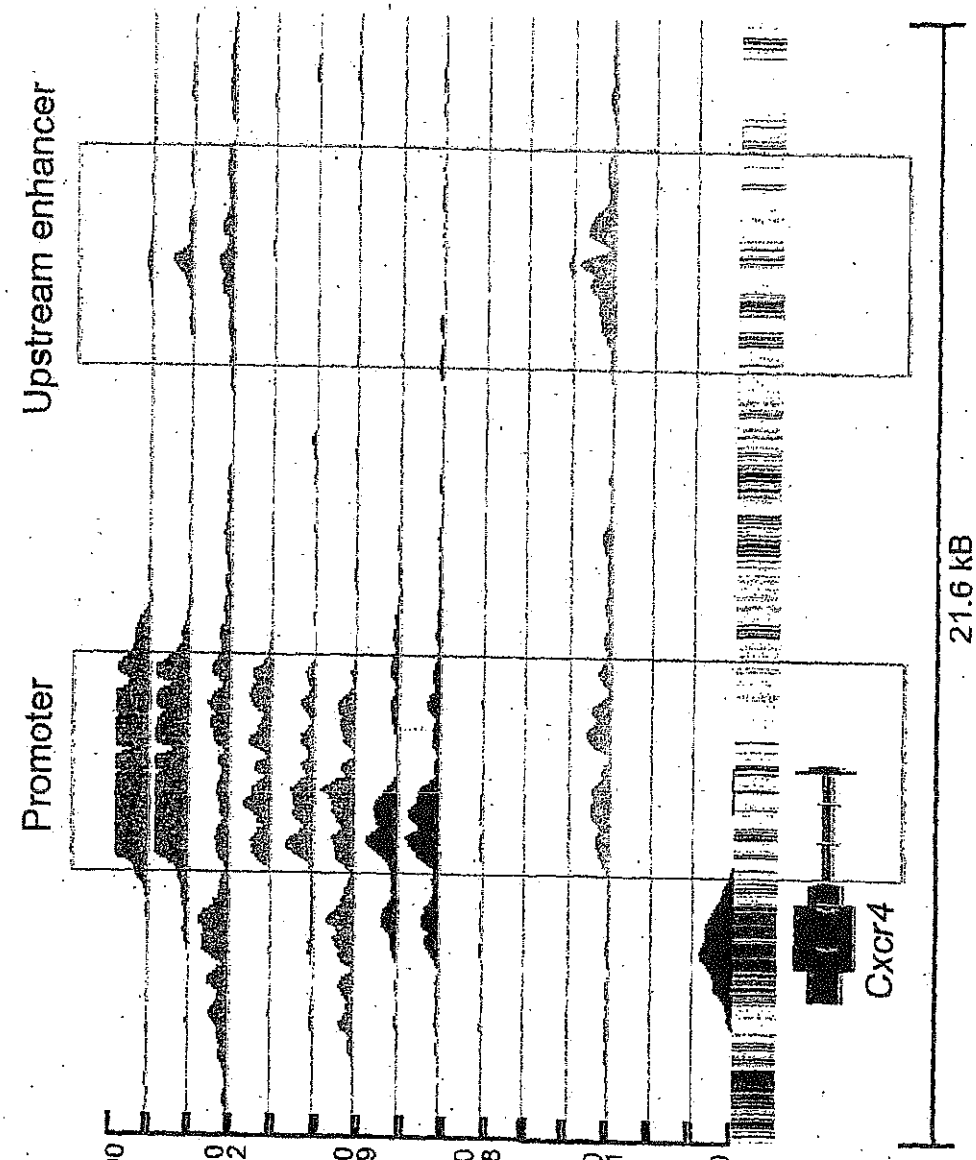

Promoter
Upstream enhancer
H3K4me2
H3K4me3
H3K27me3
H3K27ac
RNA-seq
hESC
APS
DE
100
62
149
458
3671
0
Vertebrate conservation
(Phastcons)
Cxcr4
21.6 kB

FIG. 72

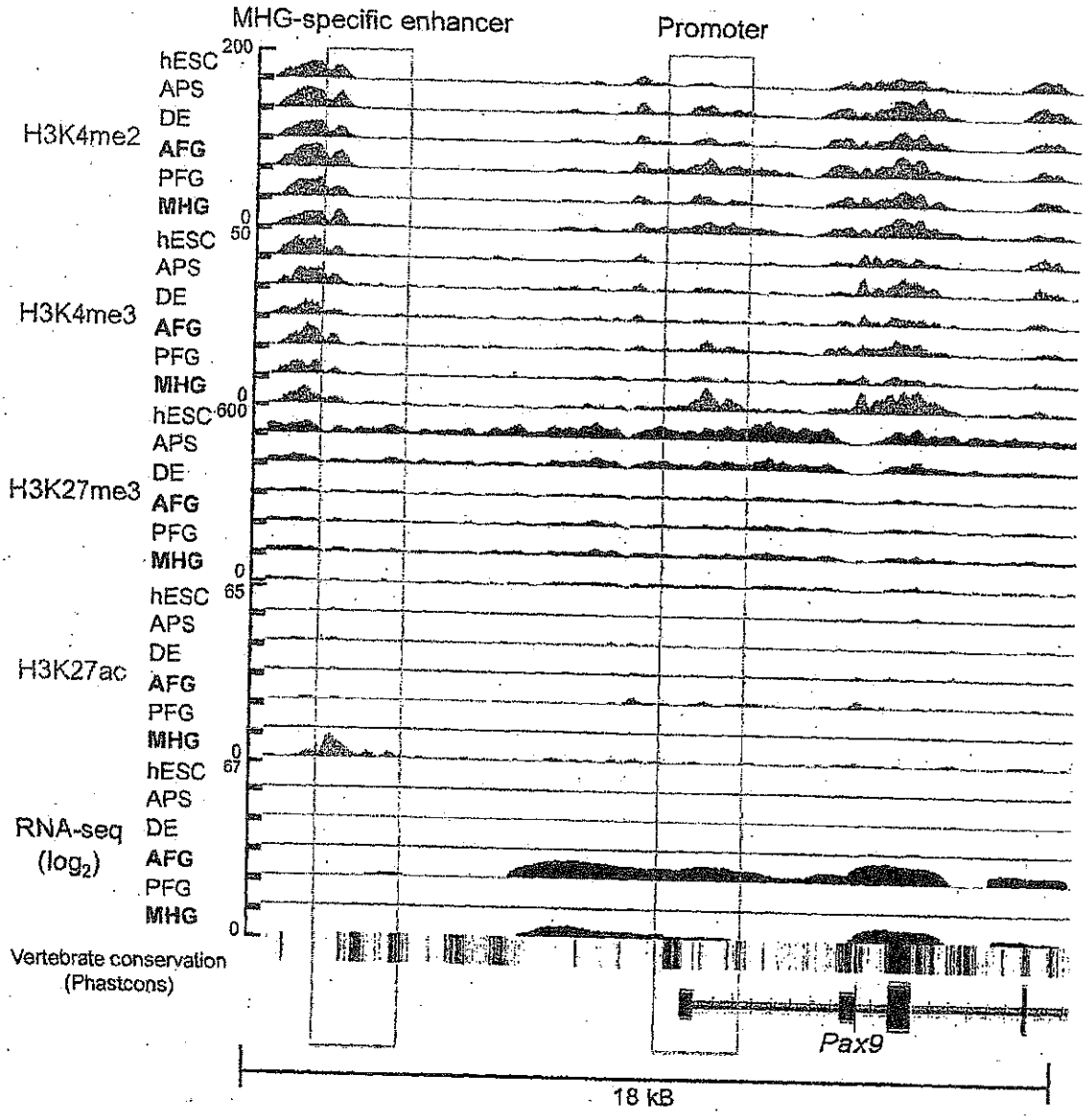

MHG-specific enhancer
Promoter
H3K4me2
H3K4me3
H3K27me3
H3K27ac
RNA-seq (log2)
hESC
APS
DE
AFG
PFG
MHG
200
0
50
0
600
0
65
0
67
0
Vertebrate conservation (Phastcons)
Pax9
18 kB

FIG. 73

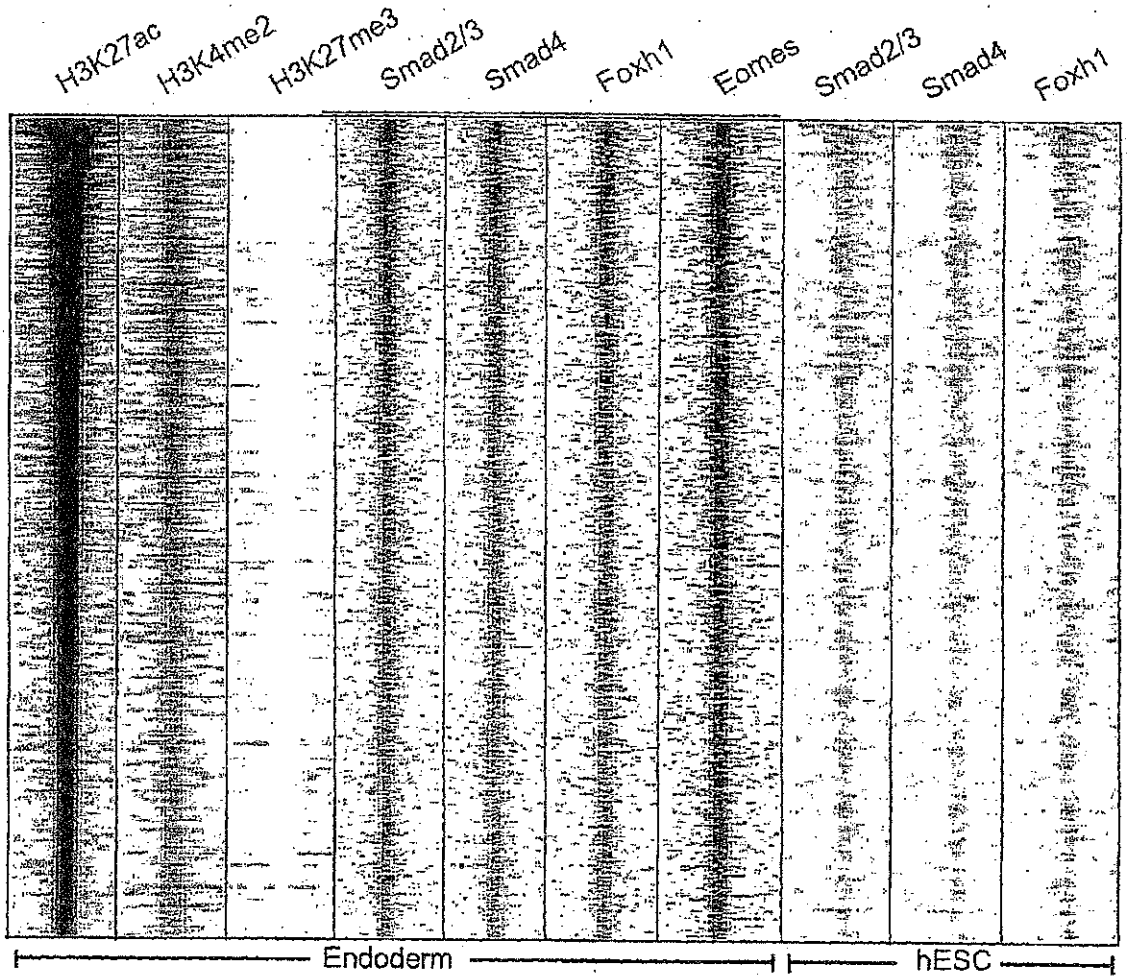

H3K27ac
H3K4me2
H3K27me3
Smad2/3
Smad4
Foxh1
Eomes
Smad2/3
Smad4
Foxh1
Endoderm
hESC

FIG. 74

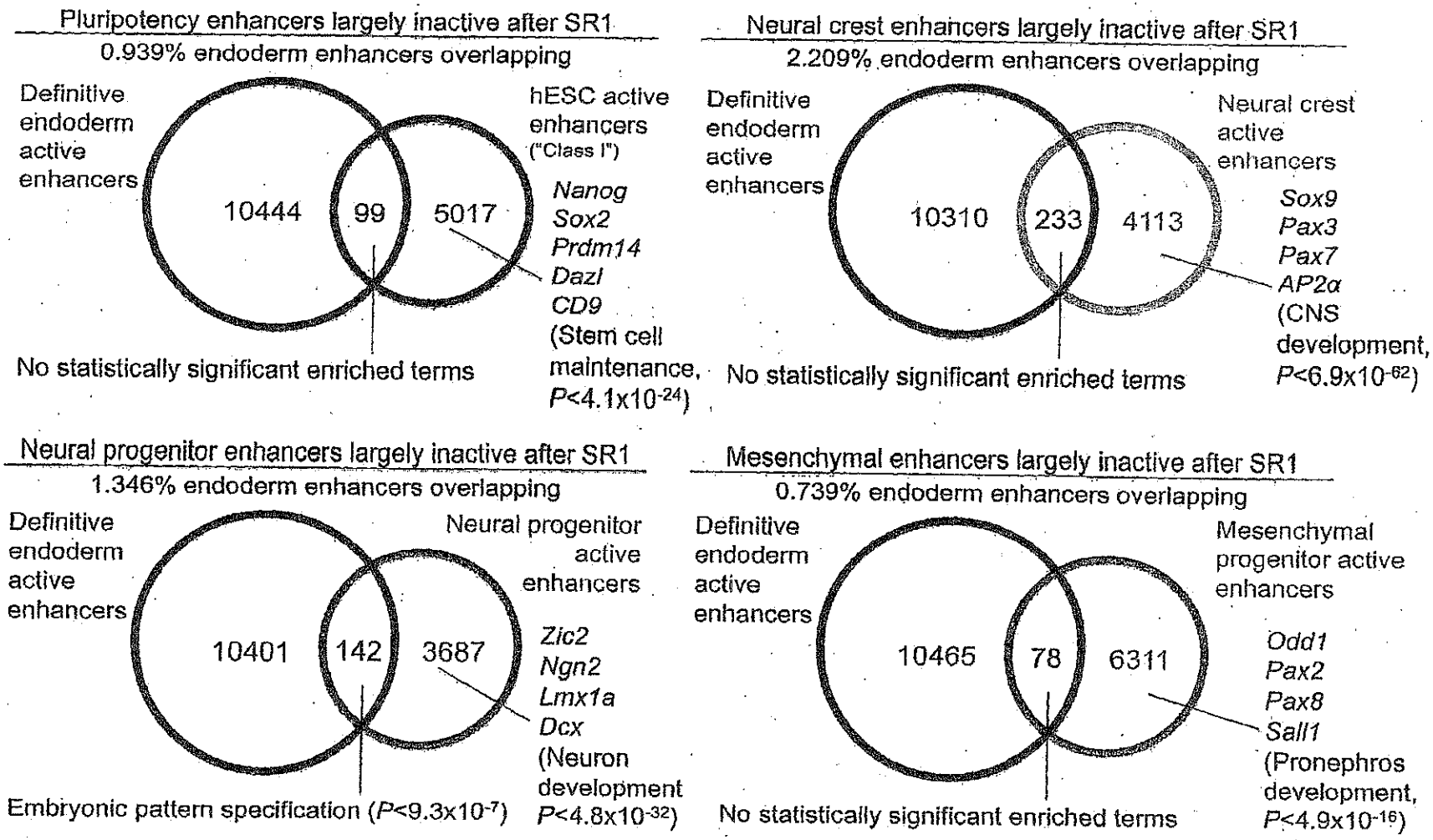
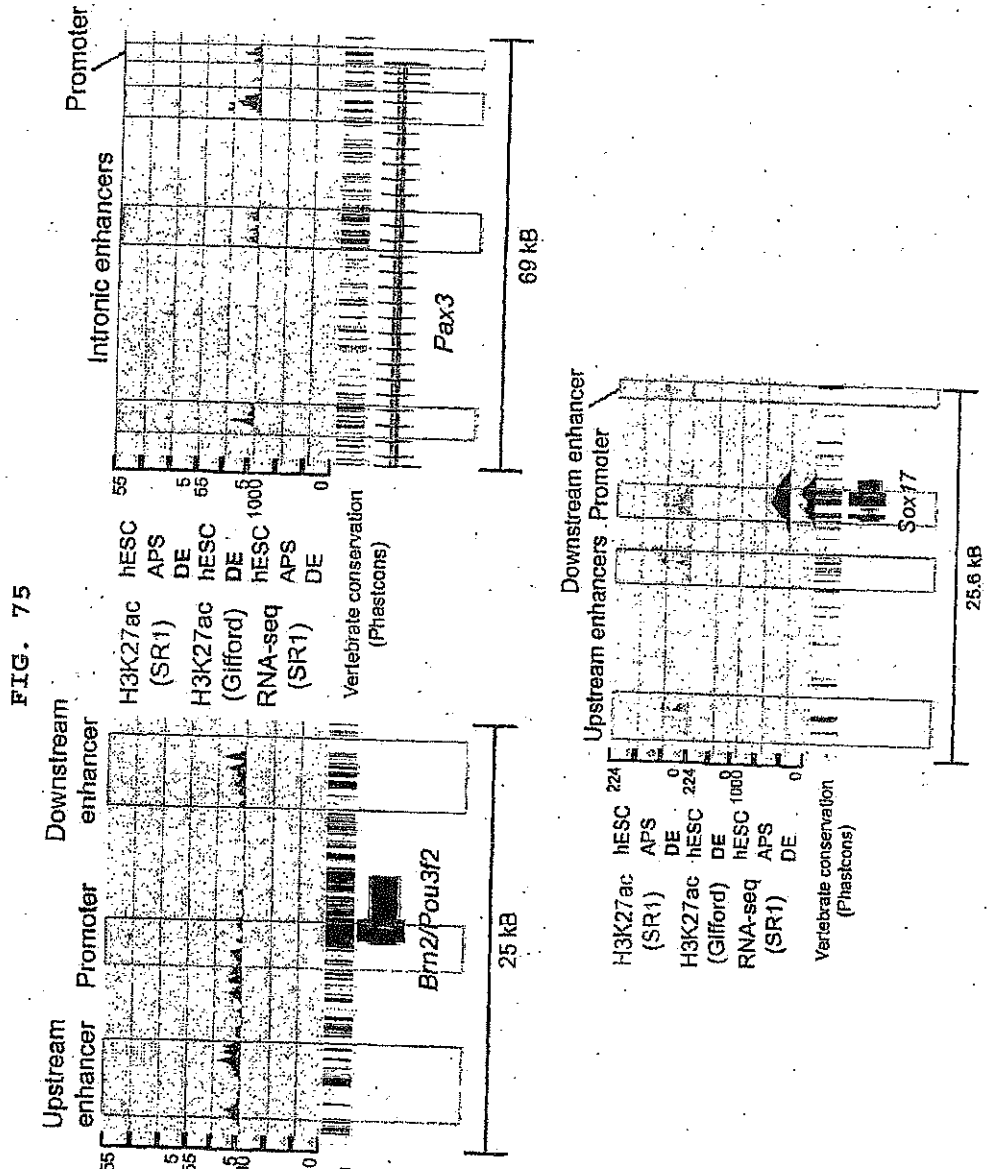

Pluripotency enhancers largely inactive after SR1
0.939% endoderm enhancers overlapping
Definitive endoderm active enhancers
hESC active enhancers ("Class I")
10444
99
5017
Nanog
Sox2
Prdm14
Dazl
CD9
(Stem cell maintenance, P<4.1x10-24)
No statistically significant enriched terms
Neural crest enhancers largely inactive after SR1
2.209% endoderm enhancers overlapping
Definitive endoderm active enhancers
Neural crest active enhancers
10310
233
4113
Sox9
Pax3
Pax7
AP2α
(CNS development, P<6.9x10-62)
No statistically significant enriched terms
Neural progenitor enhancers largely inactive after SR1
1.346% endoderm enhancers overlapping
Definitive endoderm active enhancers
Neural progenitor active enhancers
10401
142
3687
Zic2
Ngn2
Lmx1a
Dcx
(Neuron development P<4.8x10-32)
Embryonic pattern specification (P<9.3x10-7)
Mesenchymal enhancers largely inactive after SR1
0.739% endoderm enhancers overlapping
Definitive endoderm active enhancers
Mesenchymal progenitor active enhancers
10465
78
6311
Odd1
Pax2
Pax8
Sall1
(Pronephros development, P<4.9x10-16)
No statistically significant enriched terms

FIG. 75


Upstream enhancer
Promoter
Downstream enhancer
H3K27ac (SR1)
H3K27ac (Gifford)
RNA-seq (SR1)
hESC APS
hESC DE
Vertebrate conservation (Phastcons)
Brn2/Pou3f2
25 kB
Intronic enhancers
Promoter
Pax3
69 kB
Upstream enhancers
Promoter
Downstream enhancer
Sox17
25.6 kB

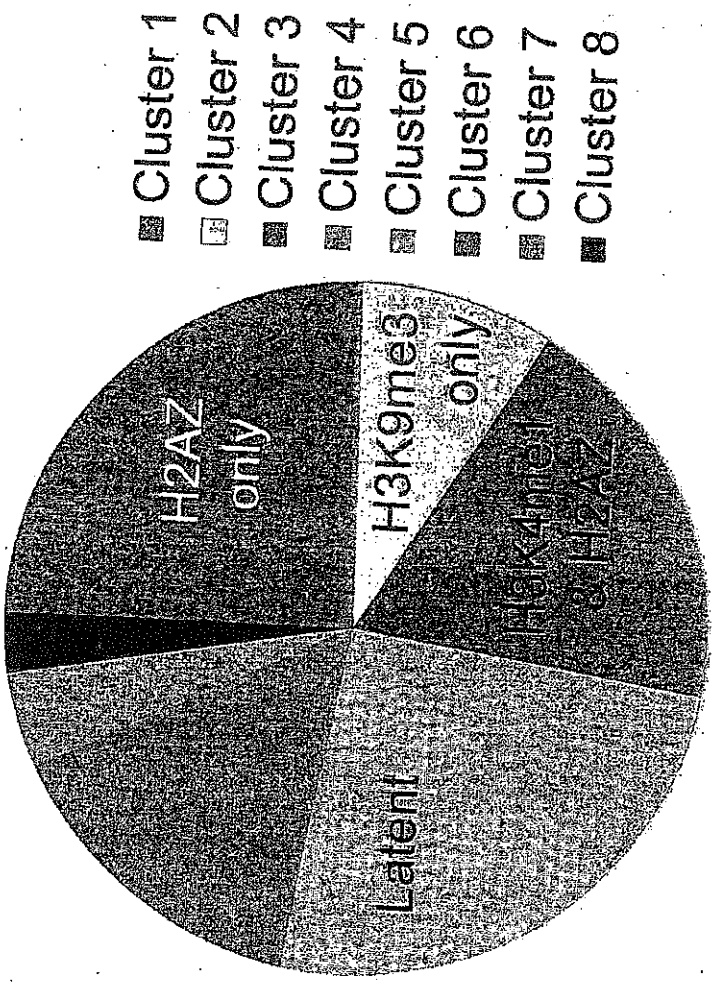
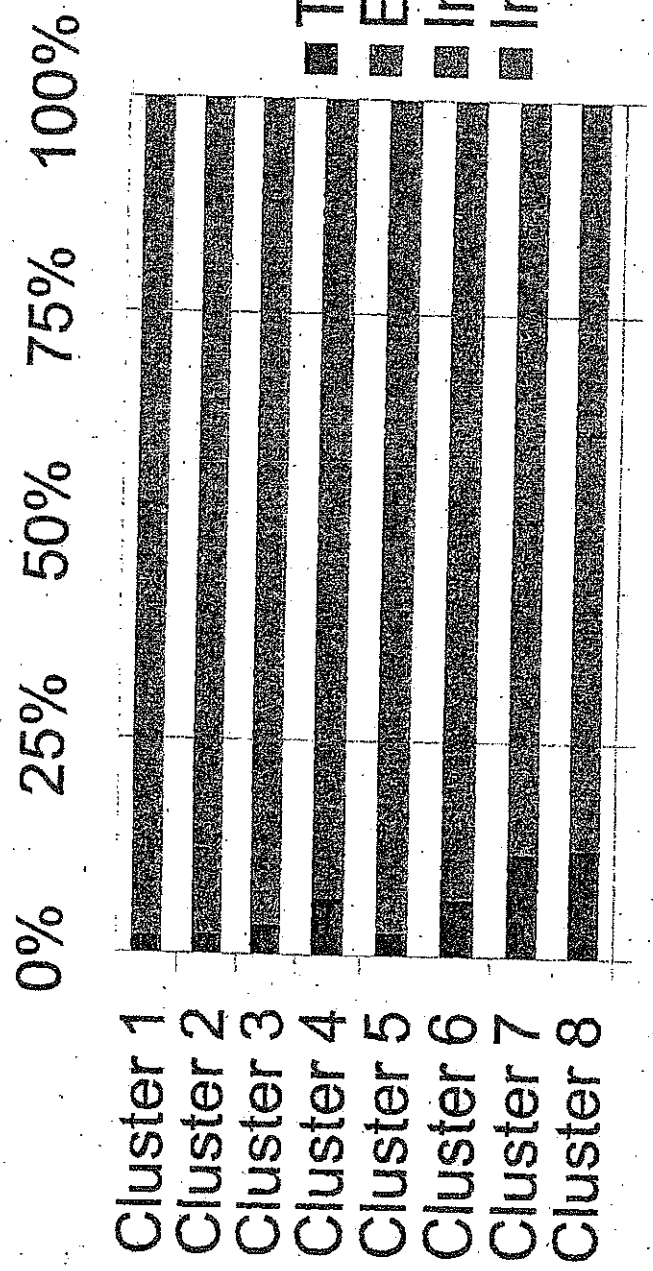

Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
H2AZ only
H3K9me3 only
H3K4me1 & H2AZ
Latent

FIG. 76

FIG. 77


0%
25%
50%
75%
100%
TSS proximal
Exons
Introns
Intergenic
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8

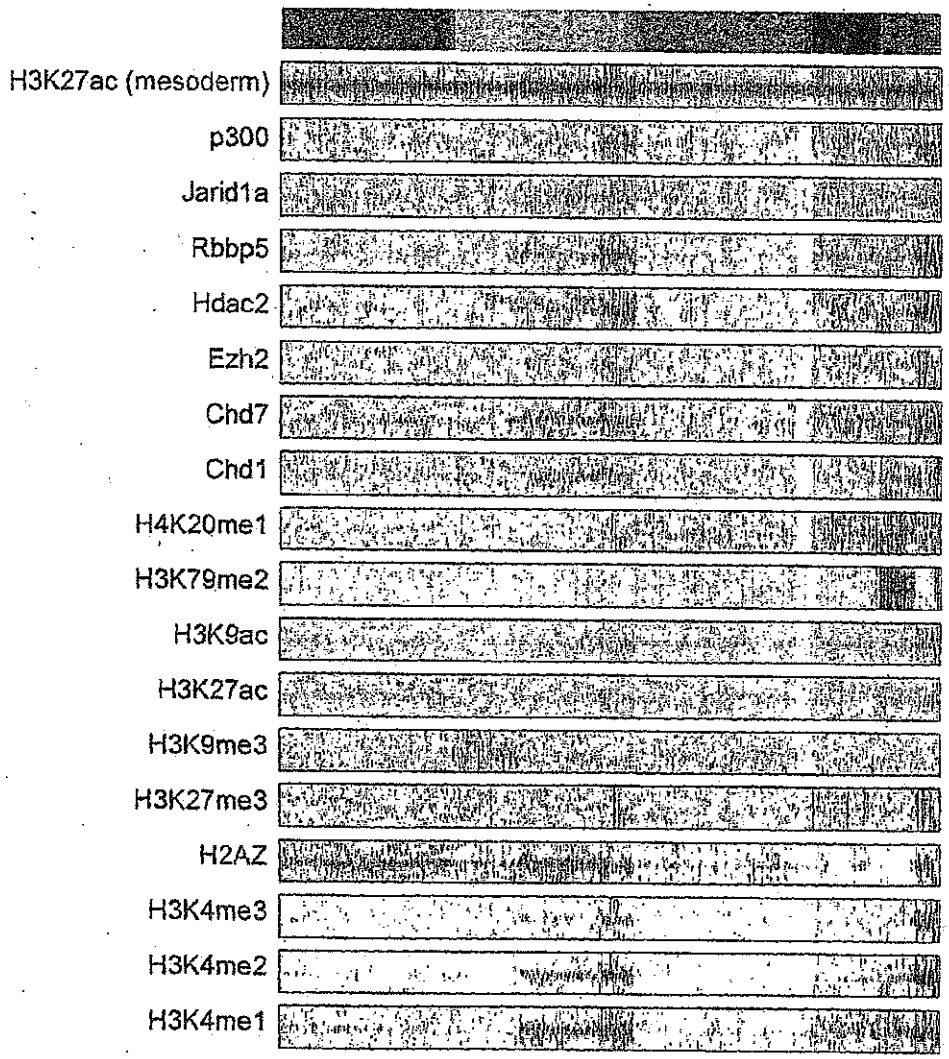

H3K27ac (mesoderm)
p300
Jarid1a
Rbbp5
Hdac2
Ezh2
Chd7
Chd1
H4K20me1
H3K79me2
H3K9ac
H3K27ac
H3K9me3
H3K27me3
H2AZ
H3K4me3
H3K4me2
H3K4me1

FIG. 78

FIG. 79

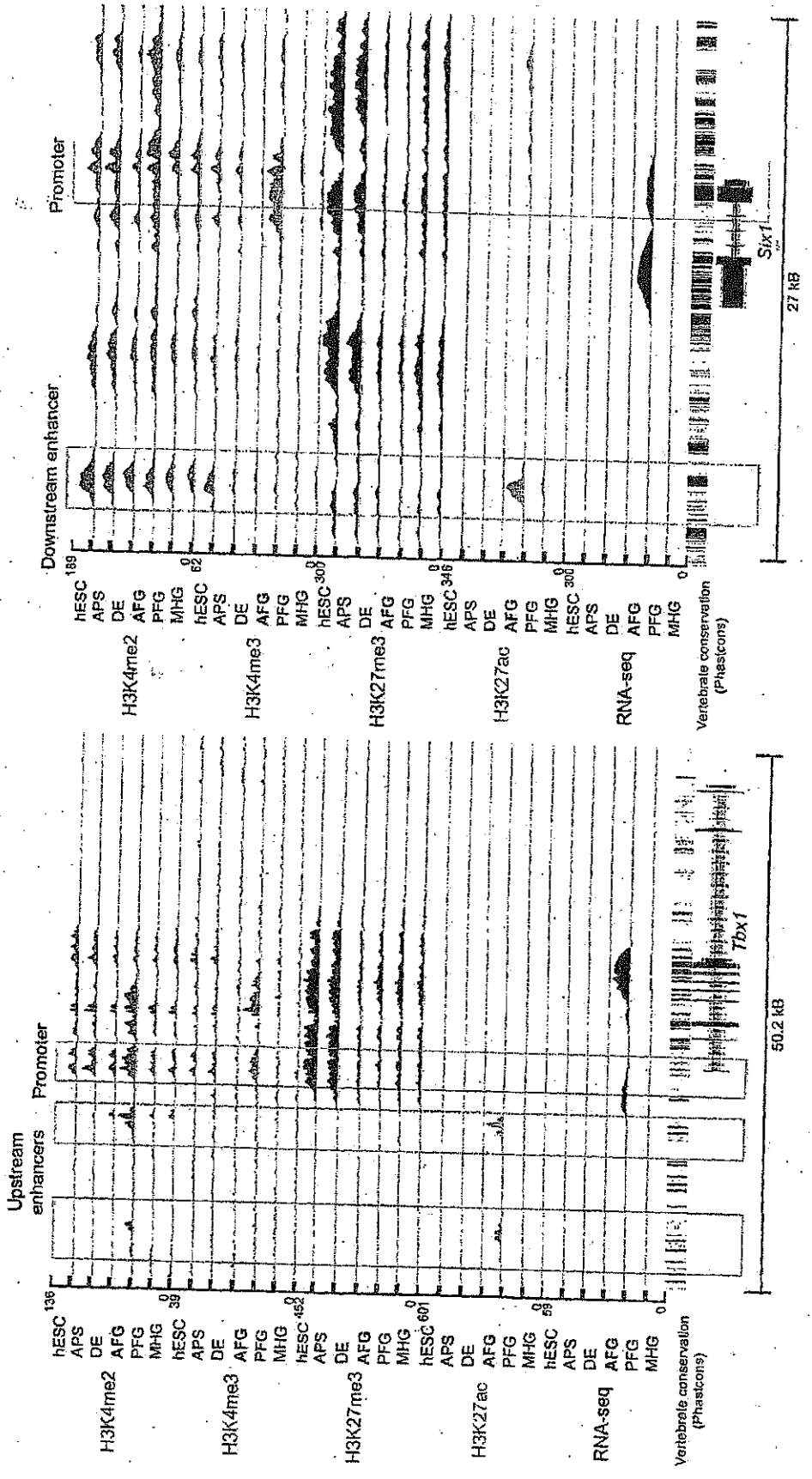
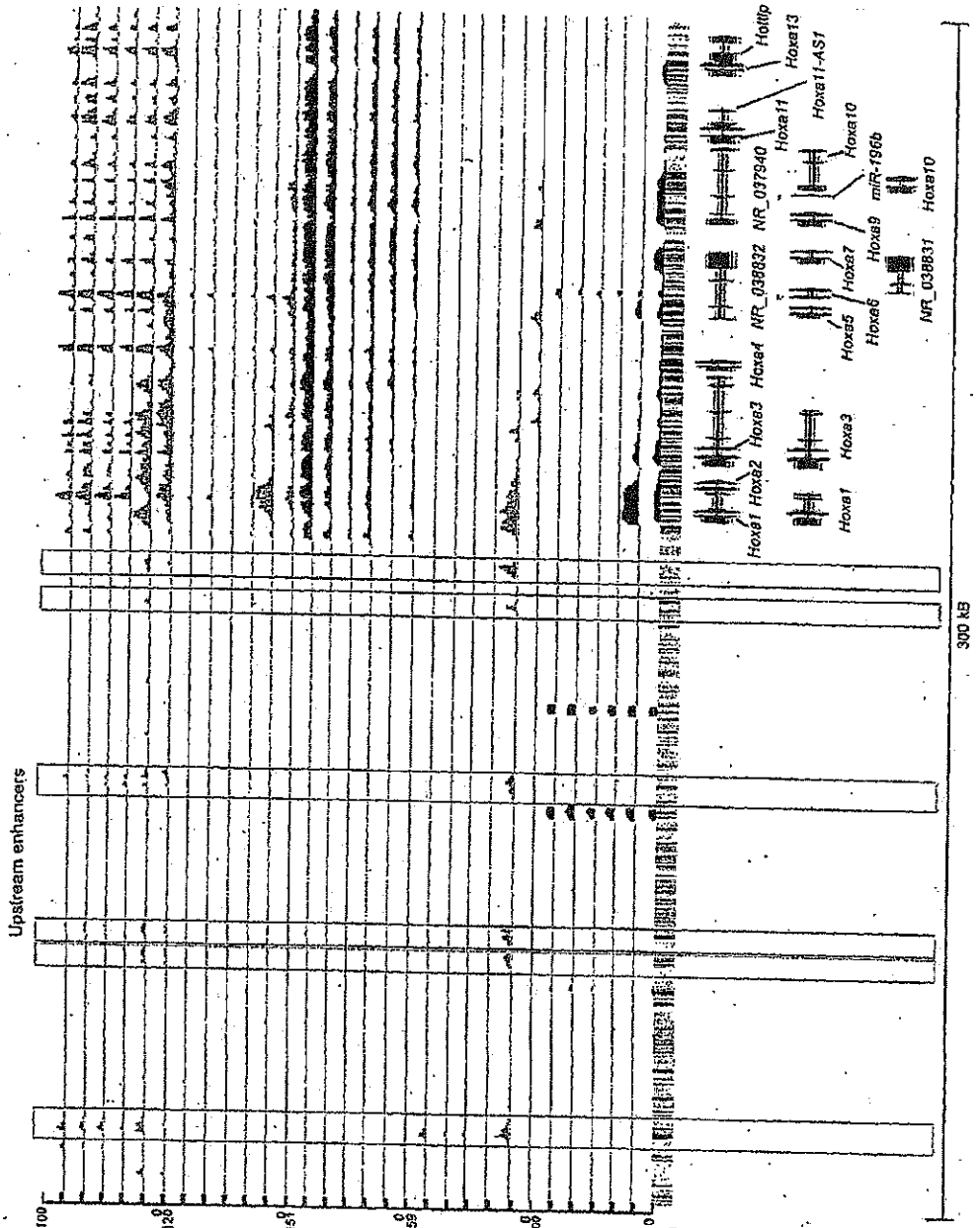

Upstream enhancers
Promoter
Downstream enhancer
Promoter
H3K4me2
H3K4me3
H3K27me3
H3K27ac
RNA-seq
hESC
APS
DE
AFG
PFG
MHG
Vertebrate conservation (Phastcons)
Tbx1
Six1
50.2 kB
27 kB

FIG. 80


Upstream enhancers
hESC
APS
DE
AFG
PFG
MHG
H3K4me2
H3K4me3
H3K27me3
H3K27ac
RNA-seq
Vertebrate conservation (Phastcons)
100
120
459
451
0
Hoxa1
Hoxa2
Hoxa3
Hoxa4
NR_038832
Hoxa5
Hoxa6
Hoxa7
Hoxa9
NR_037940
miR-196b
Hoxa10
Hoxa11
Hoxa11-AS1
Hoxa13
Hottip
NR_038831
300 kB