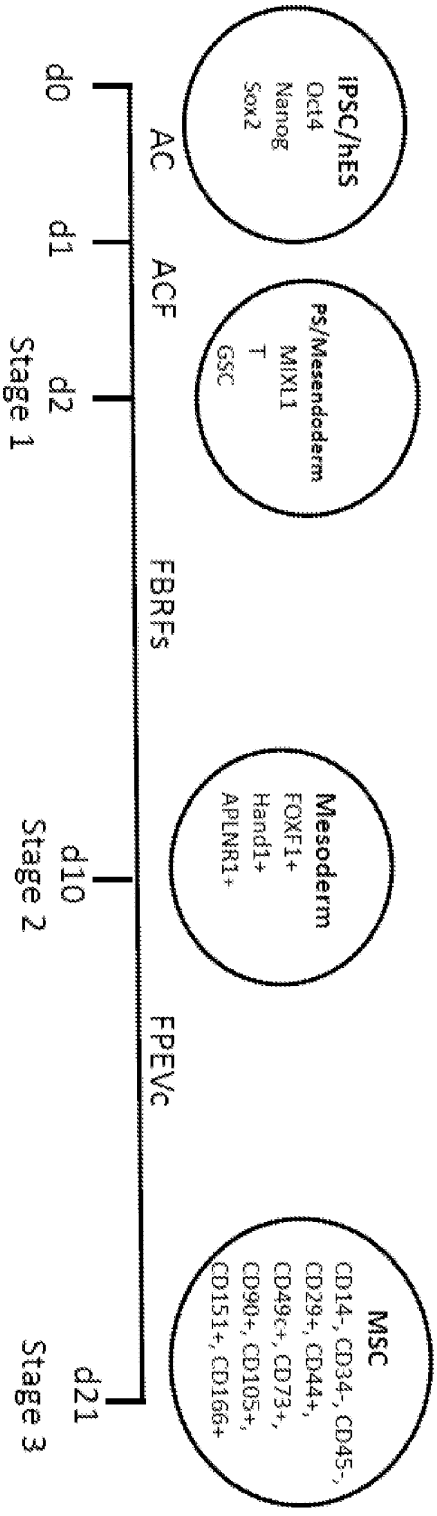

iPSC/hES
Oct4
Nanog
Sox2
PS/Mesendoderm
MIXL1
T
GSC
Mesoderm
FOXF1+
Hand1+
APLNR1+
MSC
CD14-, CD34-, CD45-,
CD29+, CD44+,
CD49c+, CD73+,
CD90+, CD105+,
CD151+, CD166+
d0
d1
d2
d10
d21
Stage 1
Stage 2
Stage 3
AC
ACF
FBRFs
FPEVc

Fig. 1

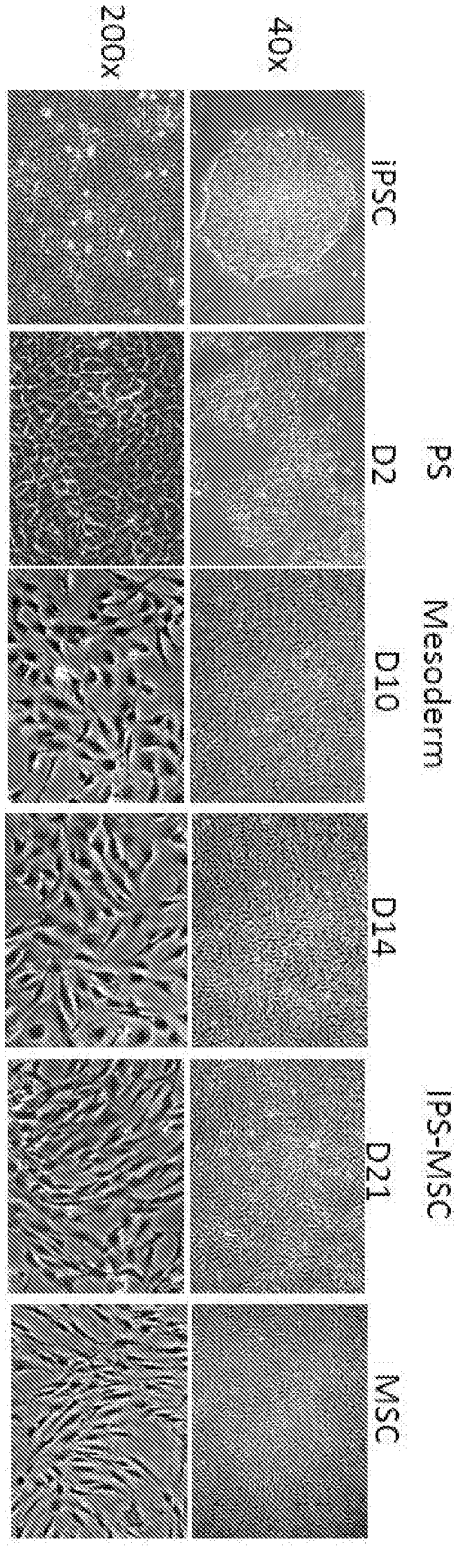


Fig. 2

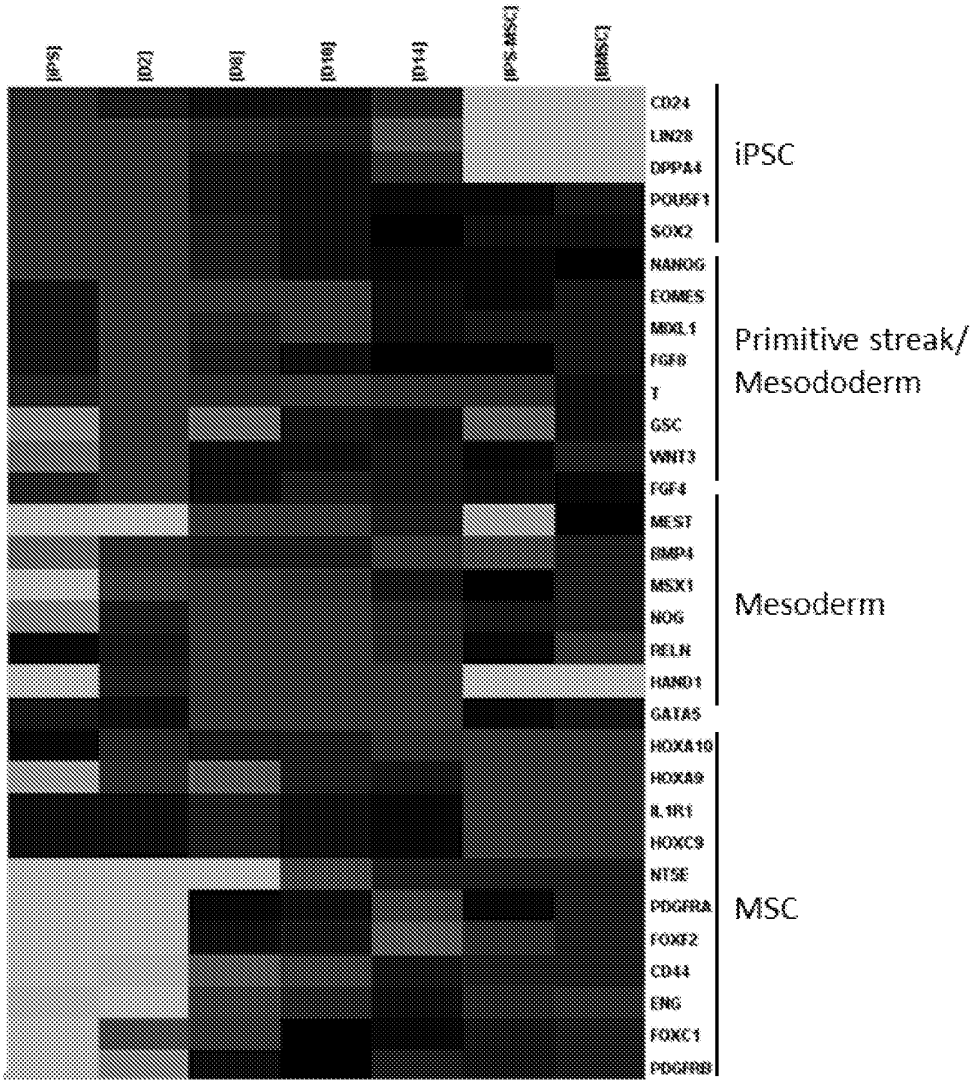

CD24
LIN28
DPPA4
POUSF1
SOX2
NANOG
EOMES
MIXL1
FGF8
T
GSC
WNT3
FGF4
MEST
BMP4
MSX1
NOG
DELN
HAND1
GATA5
HOXA10
HOXA9
IL1R1
HOXC9
NT5E
PDGFRA
FOXF2
CD44
ENG
FOXC1
PDGFRB
iPSC
Primitive streak/
Mesododerm
Mesoderm
MSC

Fig. 3

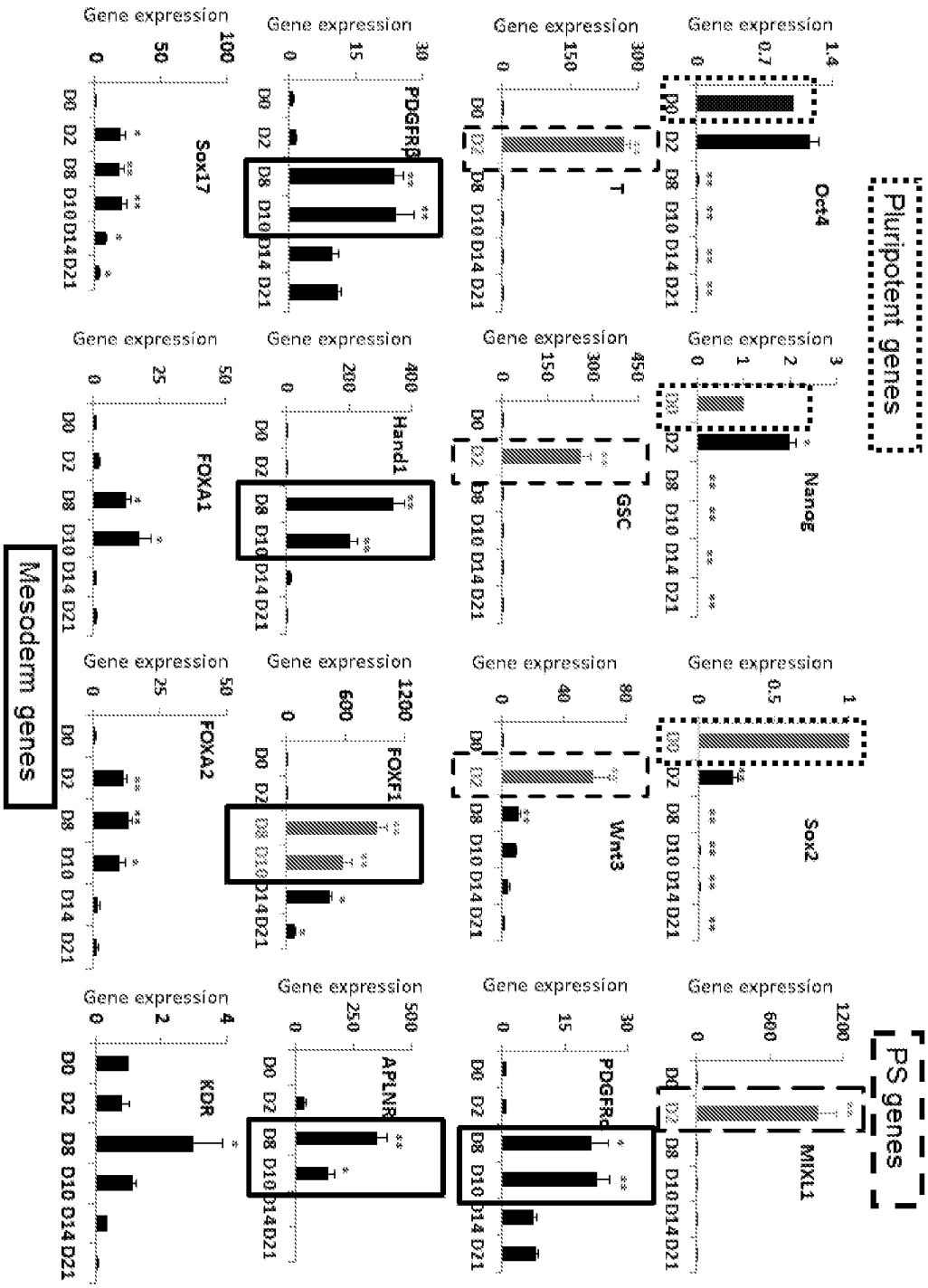

Pluripotent genes
Mesoderm genes
PS genes
Gene expression
Oct4
T
Nanog
GSC
Sox2
Wnt3
MIXL1
PDGFRα
Sox17
PDGFRβ
FOXA1
Hand1
FOXA2
FOXF1
KDR
APLNR
D0 D2 D8 D10 D14 D21

Fig. 4

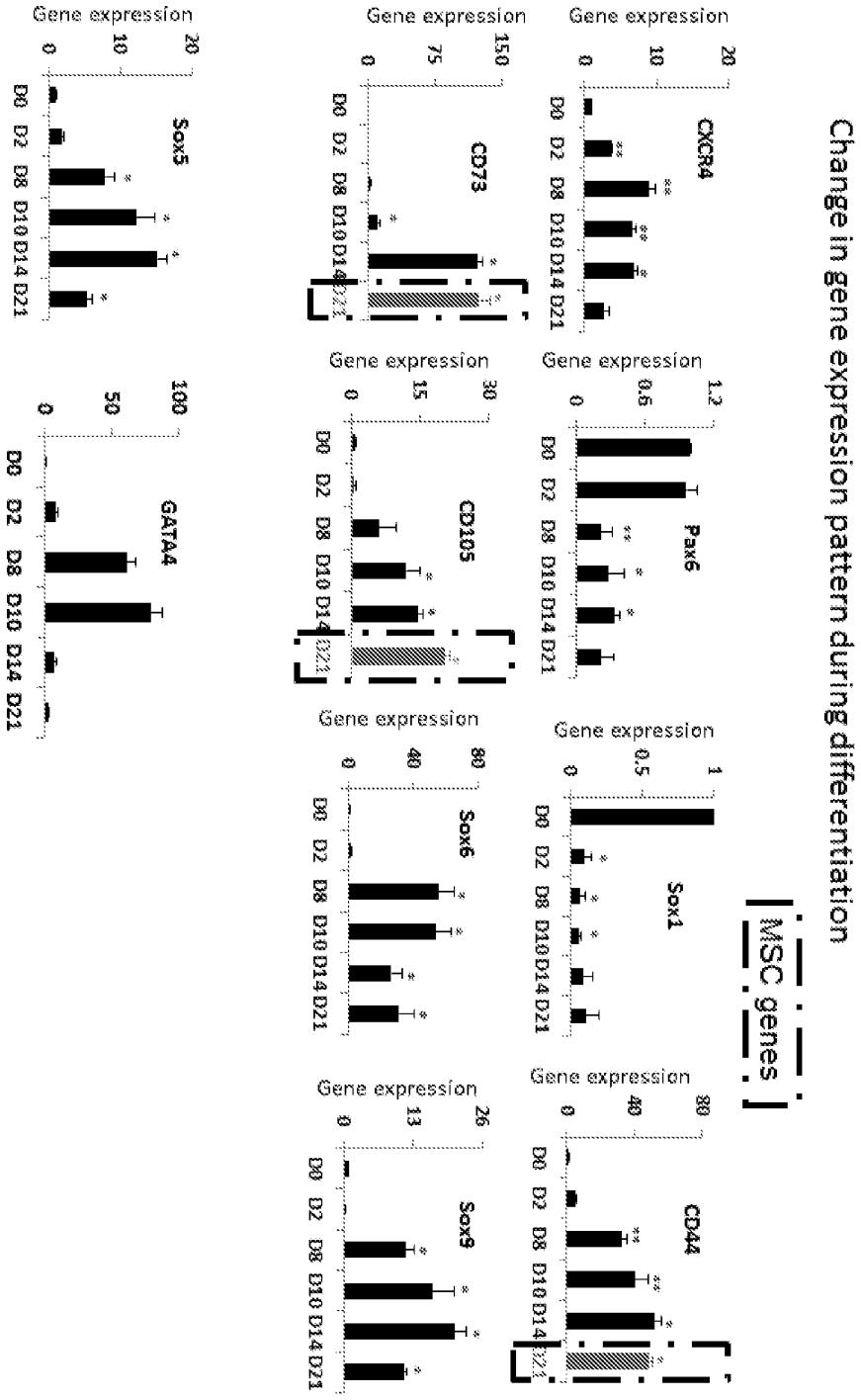

Change in gene expression pattern during differentiation
MSC genes
Gene expression
CXCR4
CD73
Pax6
CD105
Sox1
Sox6
CD44
Sox9
Sox5
GATA4
D0 D2 D8 D10 D14 D21

Fig. 5

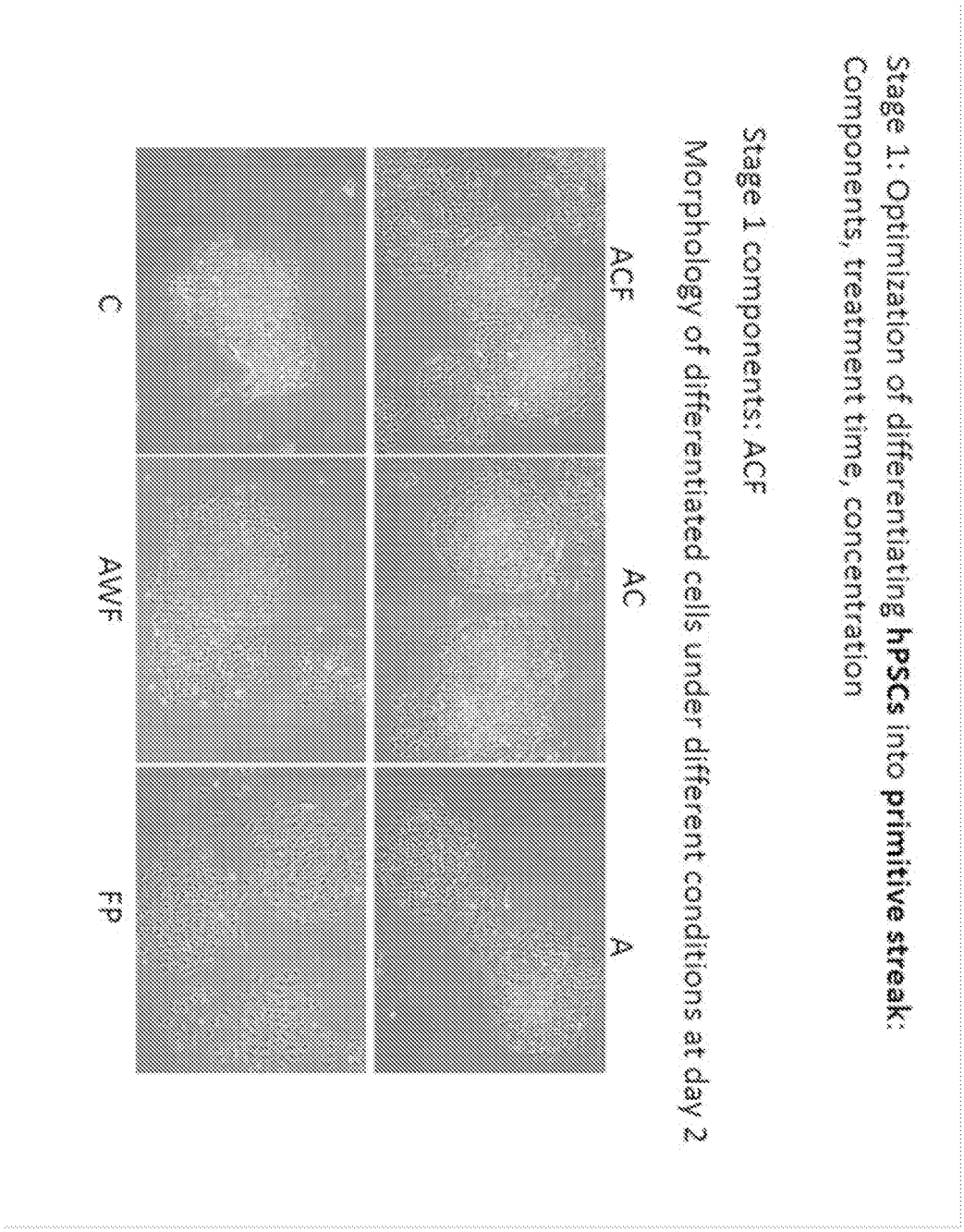

Stage 1: Optimization of differentiating hPSCs into primitive streak:
Components, treatment time, concentration
Stage 1 components: ACF
Morphology of differentiated cells under different conditions at day 2
ACF
AC
A
C
AWF
FP

Fig. 6

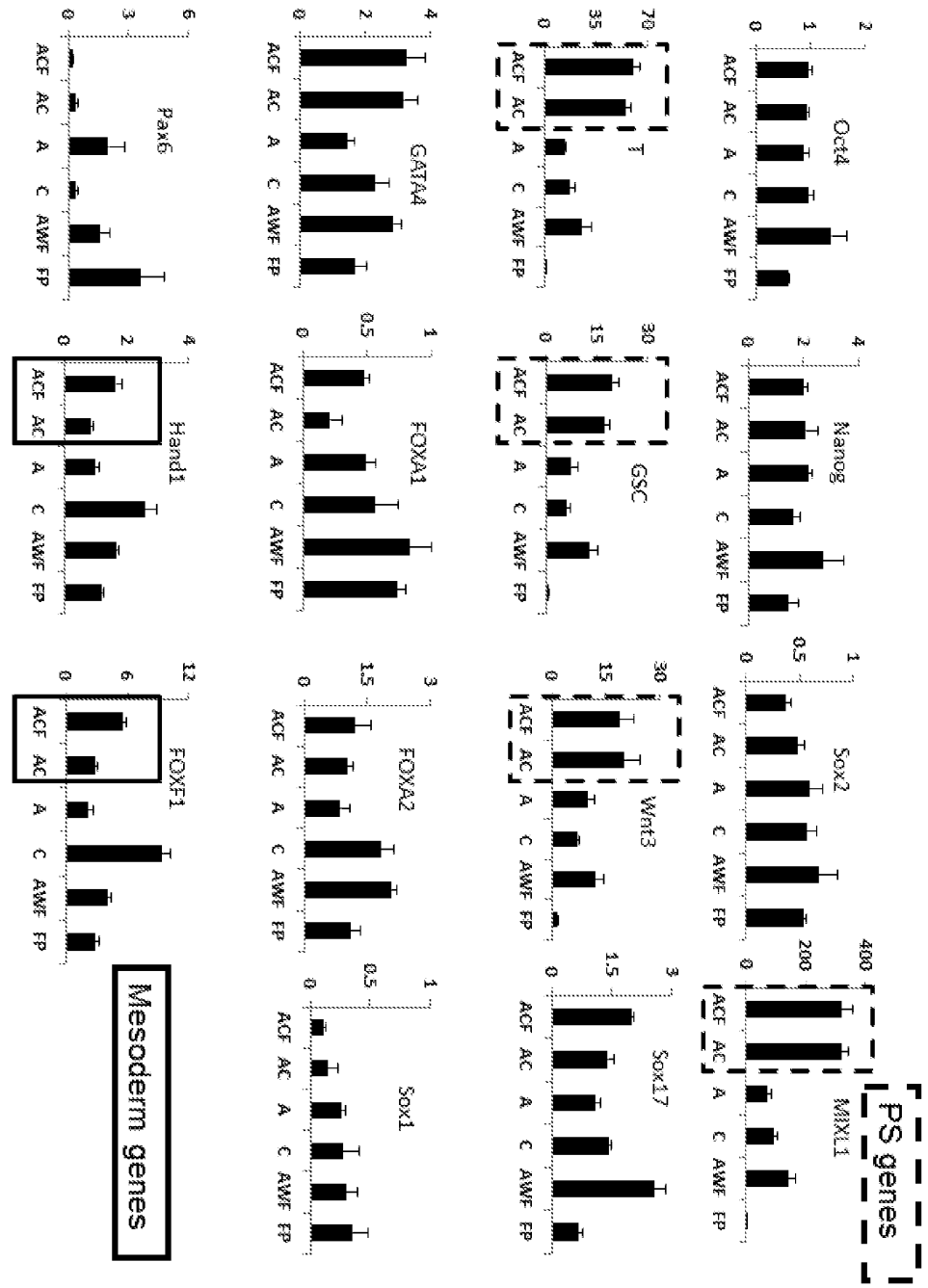

Stage 1: Optimization of differentiation condition at stage 1 (d2)
PS genes
Mesoderm genes
Oct4
Nanog
Sox2
MIXL1
T
GSC
Wnt3
Sox17
GATA4
FOXA1
FOXA2
Sox1
Pax6
Hand1
FOXF1
ACF
AC
A
C
AWF
FP

Fig. 7

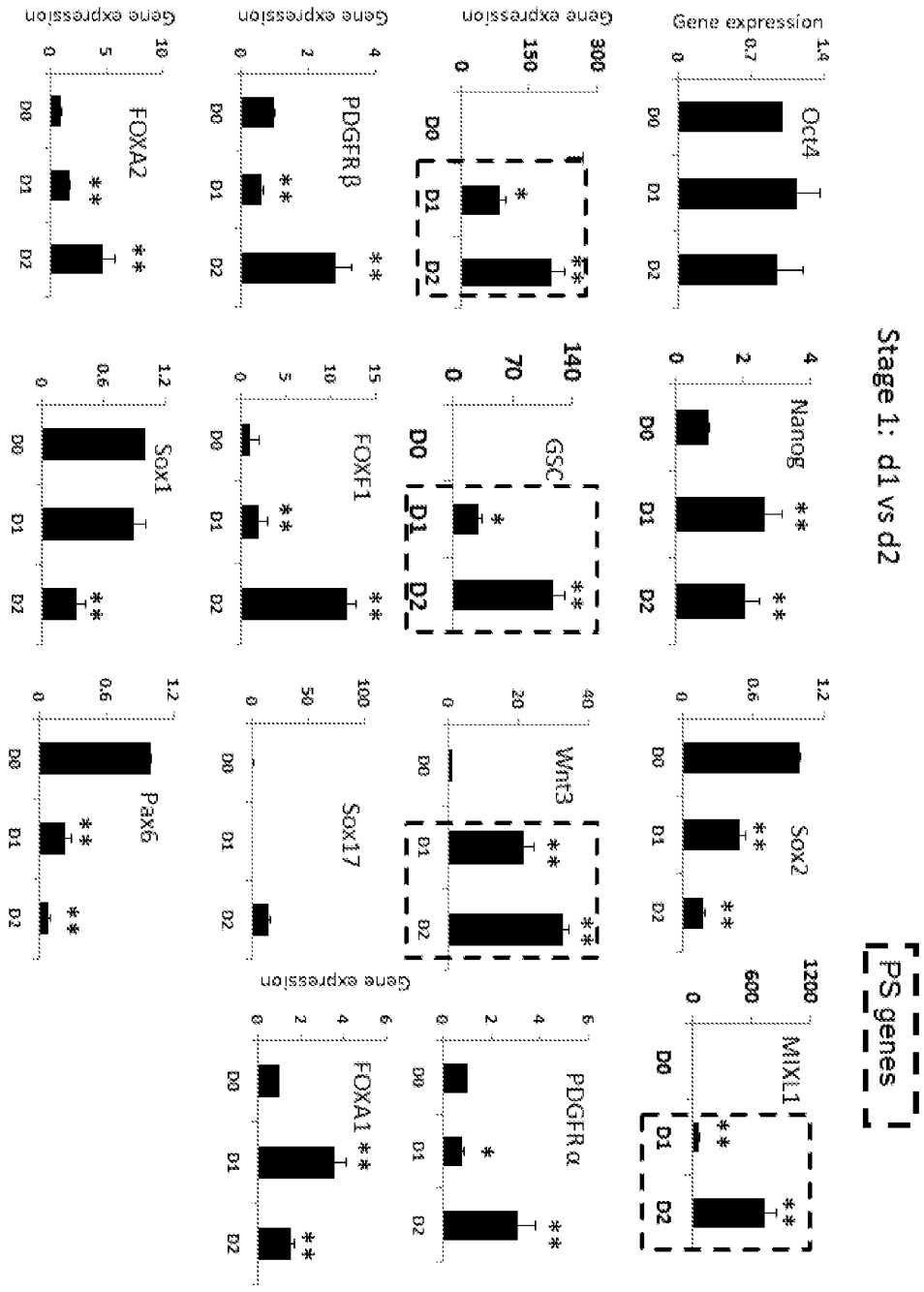

Stage 1: d1 vs d2
PS genes
Gene expression
Oct4
Nanog
GSC
Sox2
MIXL1
Sox1
FOXF1
Wnt3
PDGFRα
FOXA1
Sox17
Pax6
PDGFRβ
FOXA2
D0
D1
D2

Fig. 8

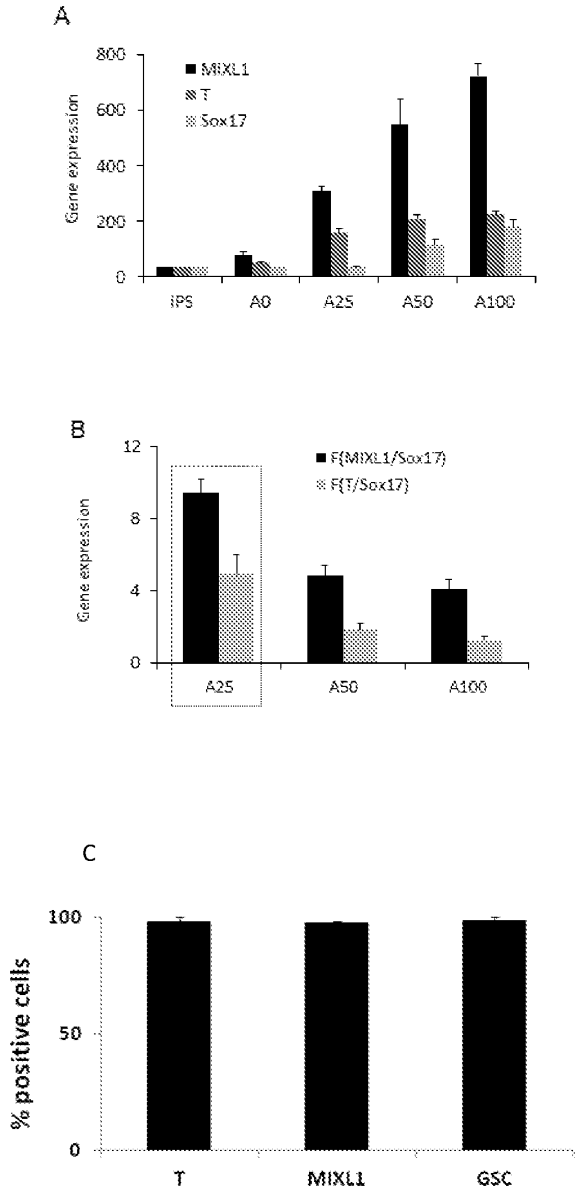
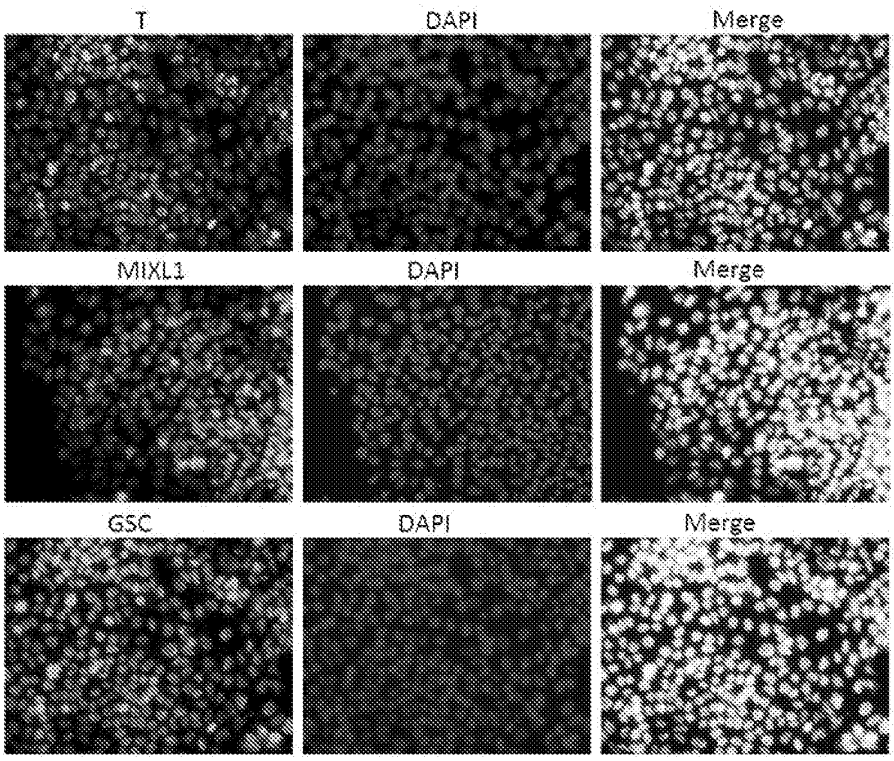

A
800
600
400
200
0
Gene expression
MIXL1
T
Sox17
IPS
A0
A25
A50
A100
B
12
8
4
0
Gene expression
F(MIXL1/Sox17)
F(T/Sox17)
A25
A50
A100
C
100
50
0
% positive cells
T
MIXL1
GSC

Fig. 9

D



E

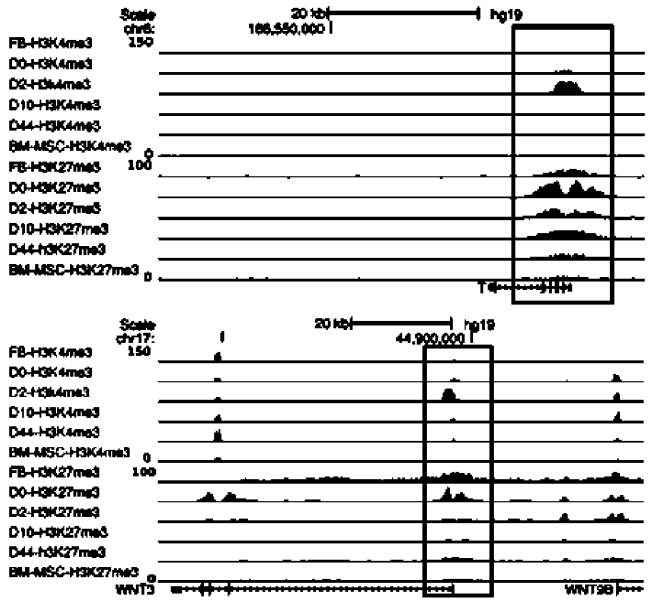
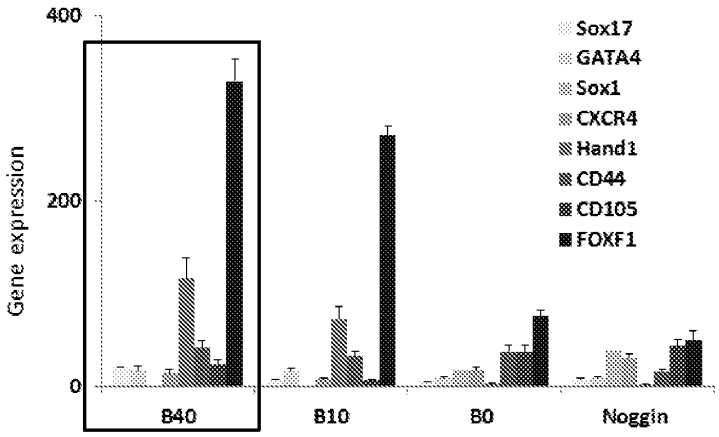
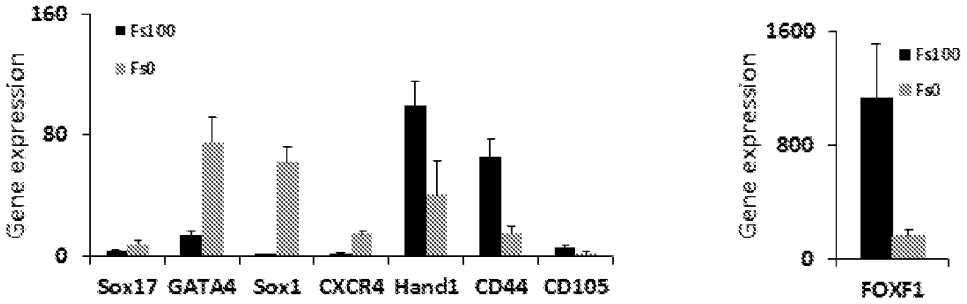


Fig. 9 (Continued)

A


400
200
0
Gene expression
Sox17
GATA4
Sox1
CXCR4
Hand1
CD44
CD105
FOXF1
B40
B10
B0
Noggin

B


160
80
0
Gene expression
Fs100
Fs0
Sox17
GATA4
Sox1
CXCR4
Hand1
CD44
CD105
1600
800
0
Gene expression
Fs100
Fs0
FOXF1

C

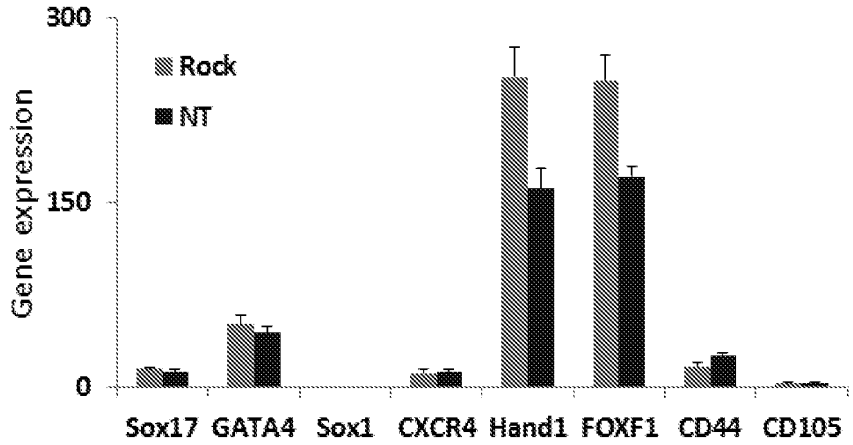

300
150
0
Gene expression
Rock
NT
Sox17
GATA4
Sox1
CXCR4
Hand1
FOXF1
CD44
CD105

Fig. 10

D

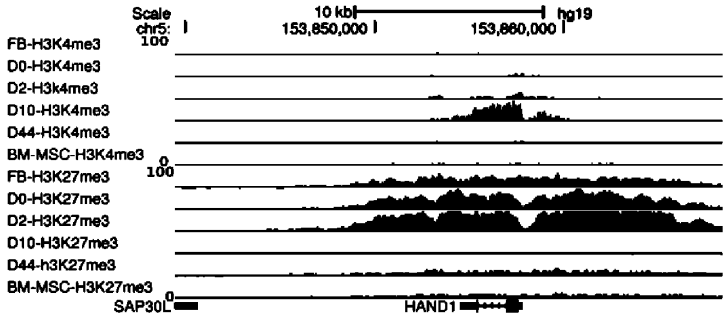


Fig. 10 (Continued)

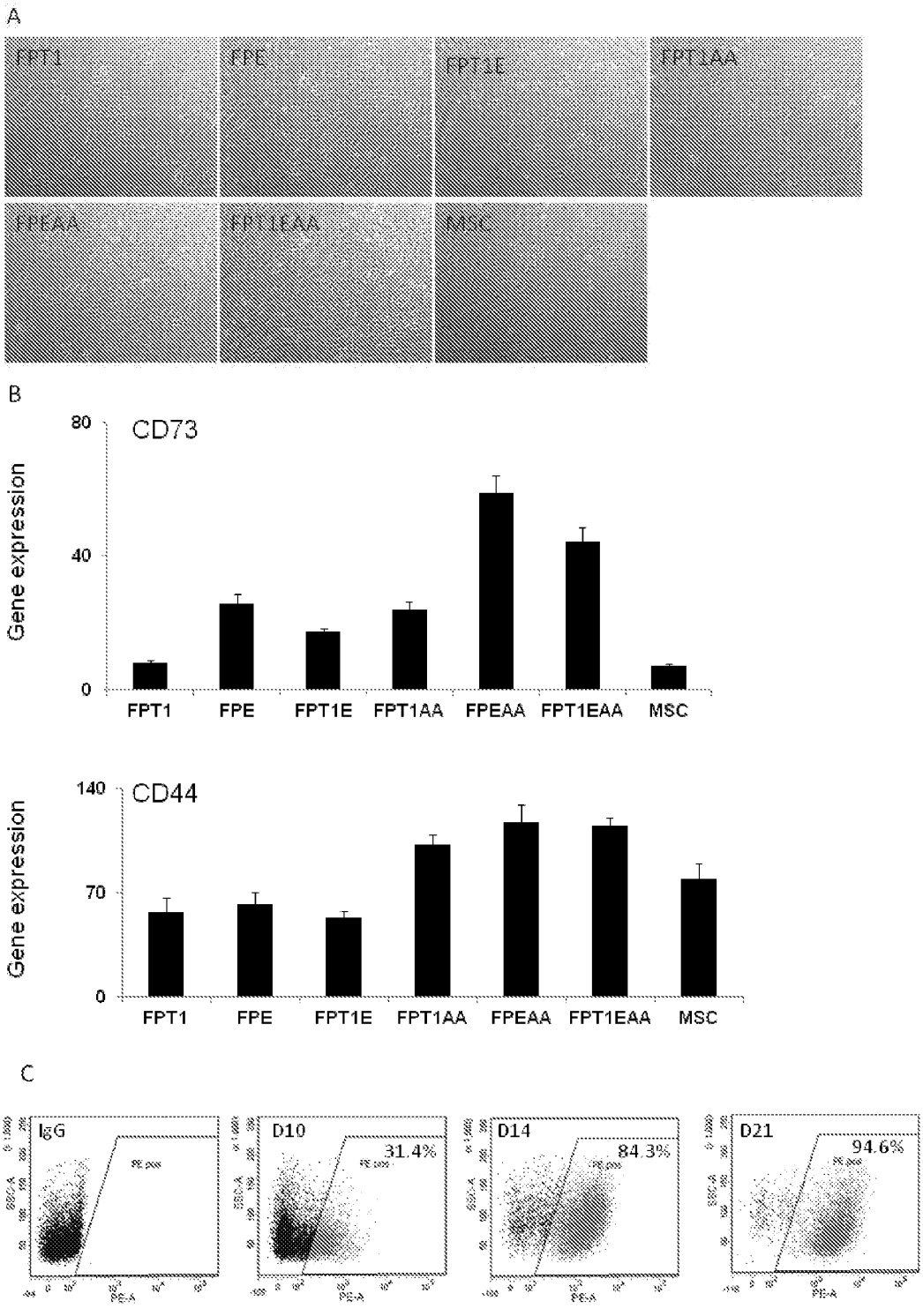
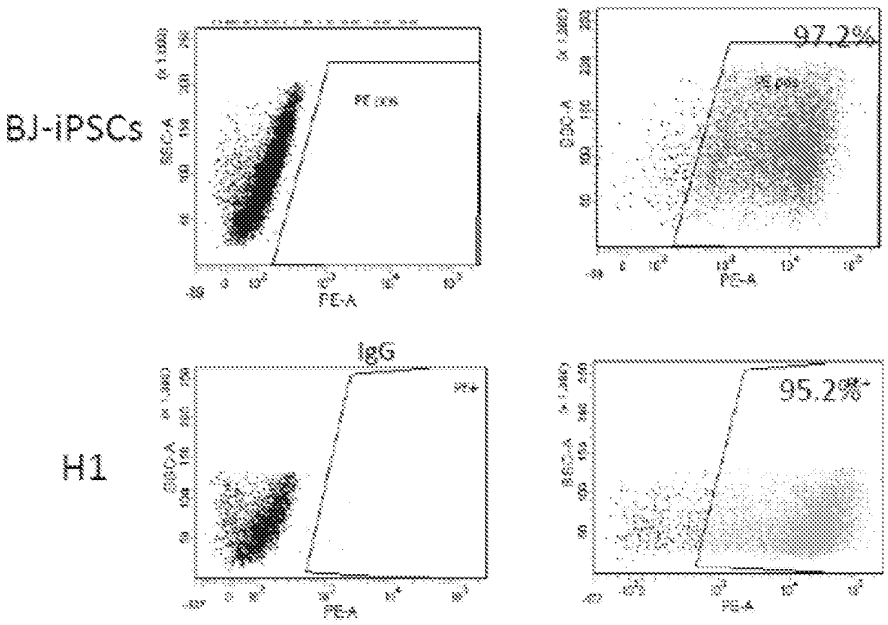

A
FPT1
FPE
FPT1E
FPT1AA
FPEAA
FPT1EAA
MSC
B
CD73
Gene expression
80
40
0
FPT1
FPE
FPT1E
FPT1AA
FPEAA
FPT1EAA
MSC
CD44
Gene expression
140
70
0
FPT1
FPE
FPT1E
FPT1AA
FPEAA
FPT1EAA
MSC
C
IgG
D10
31.4%
D14
84.3%
D21
94.6%

Fig. 11



D

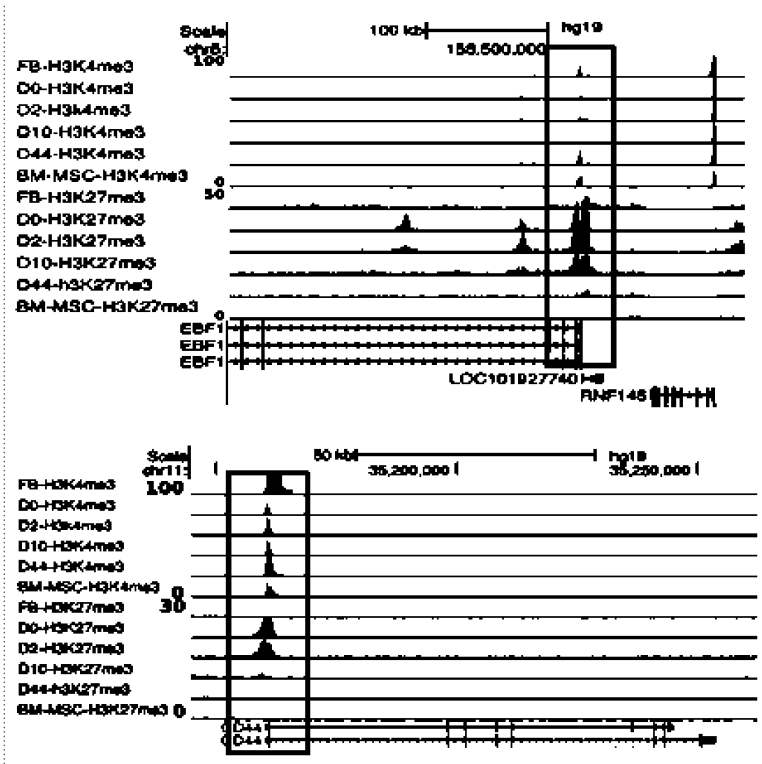


Fig. 11 (Continued)

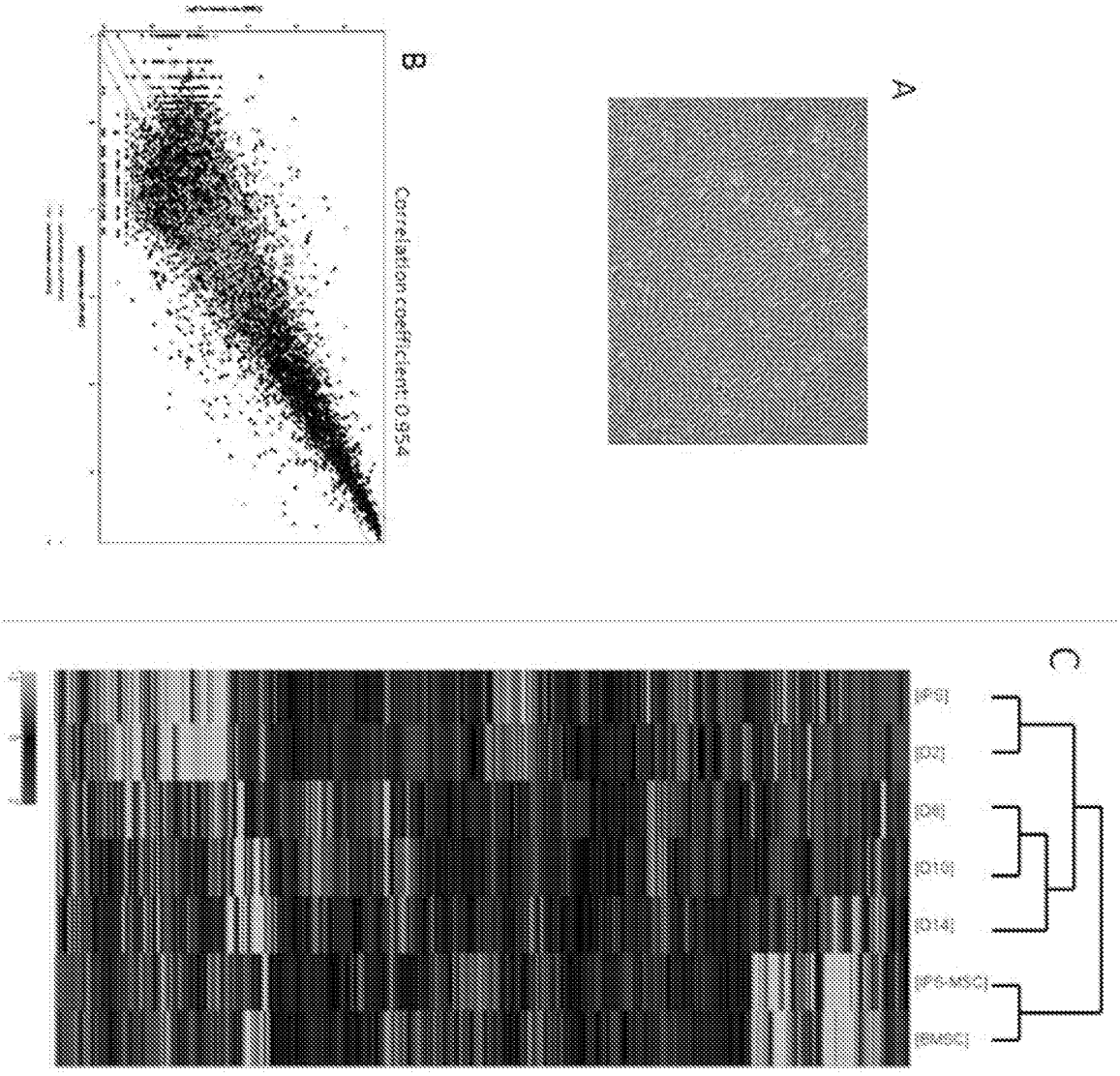


Fig. 12

D

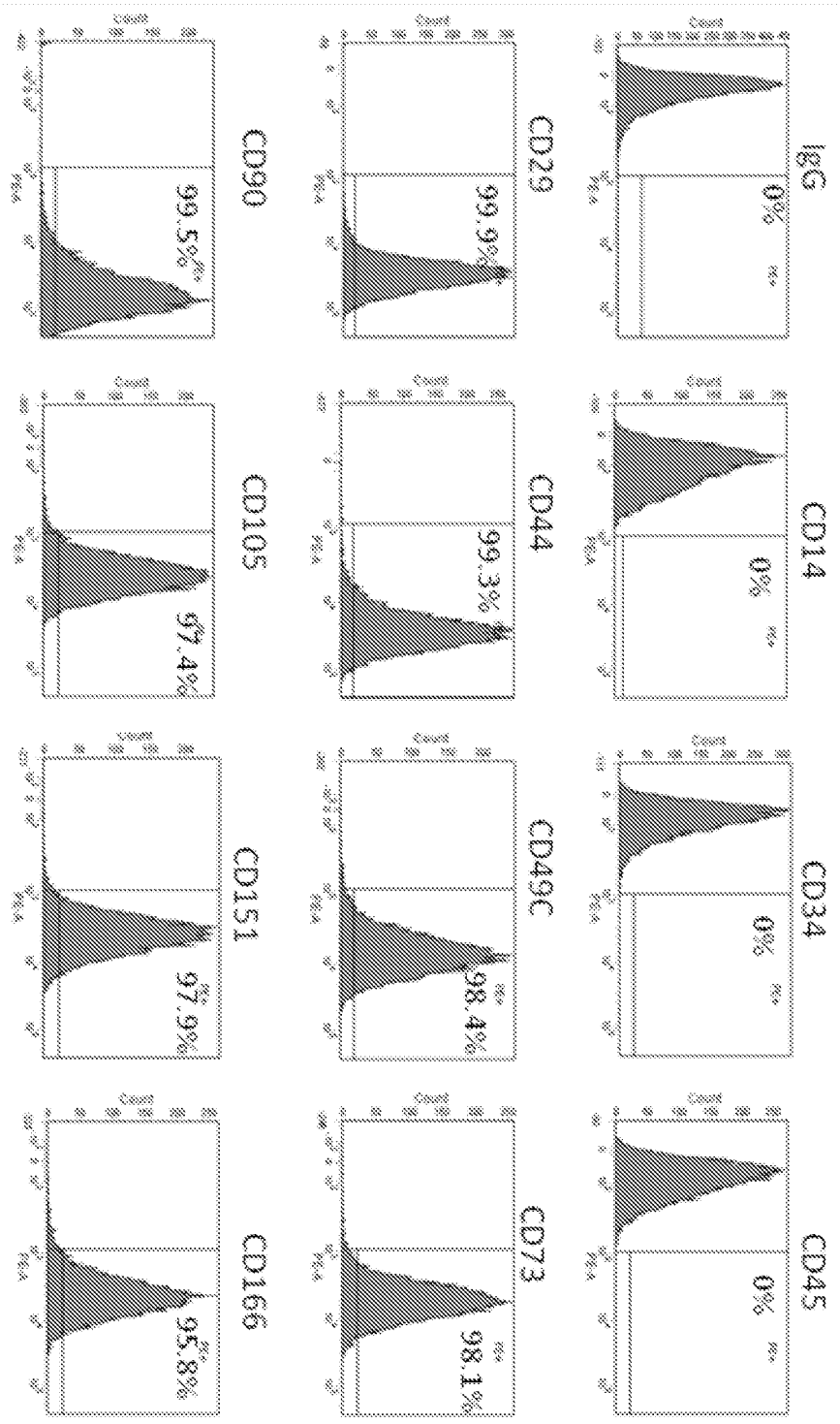
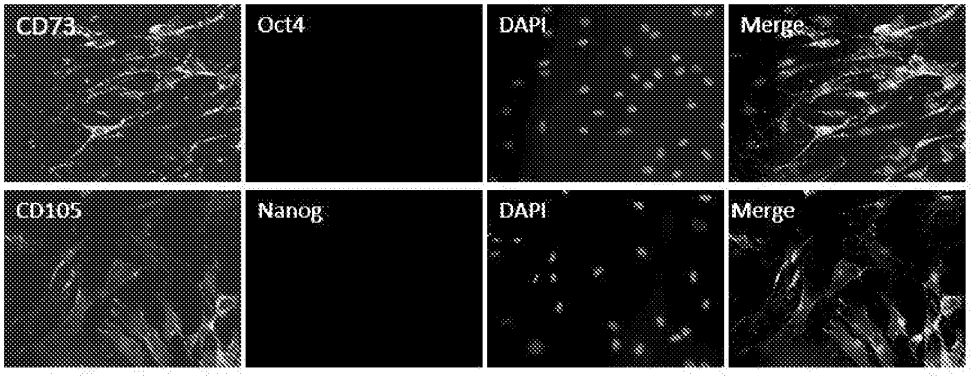


Fig. 12 (Continued)

E



F

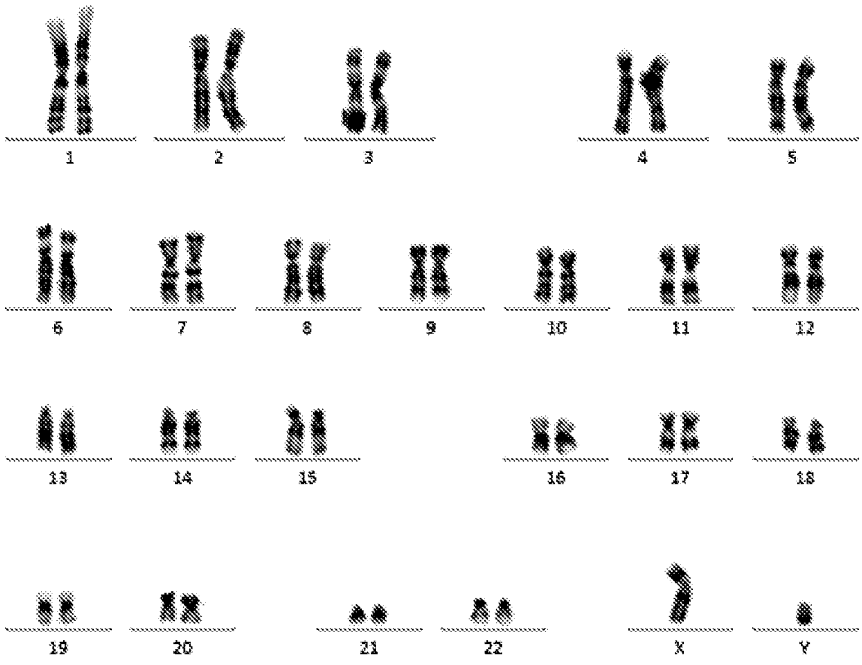
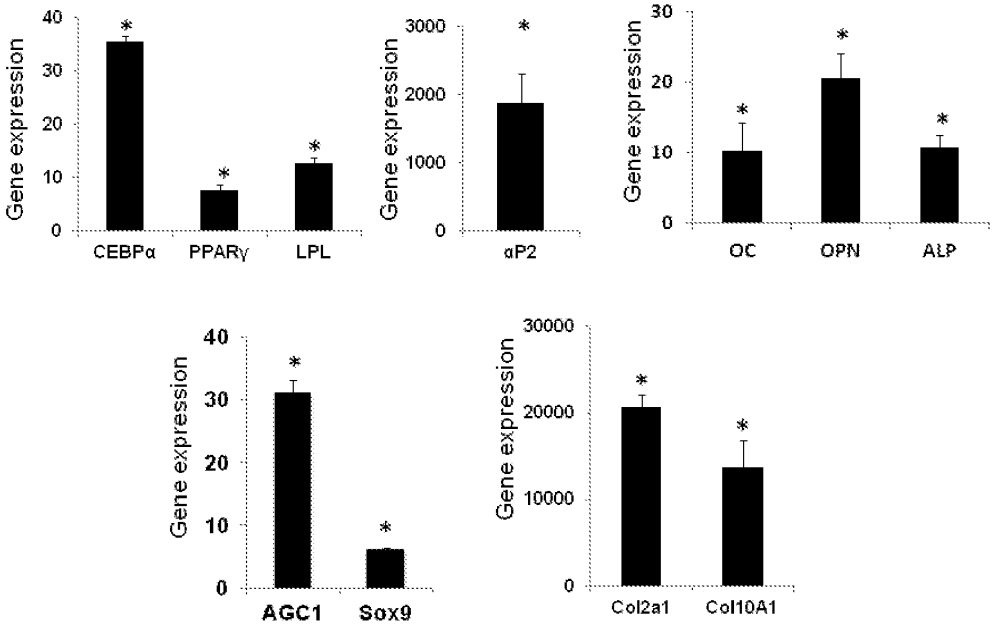


Fig. 12 (Continued)

G



H

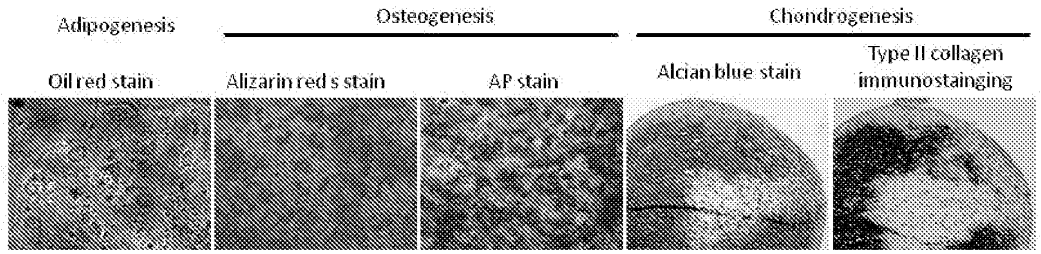


Fig. 12 (Continued)

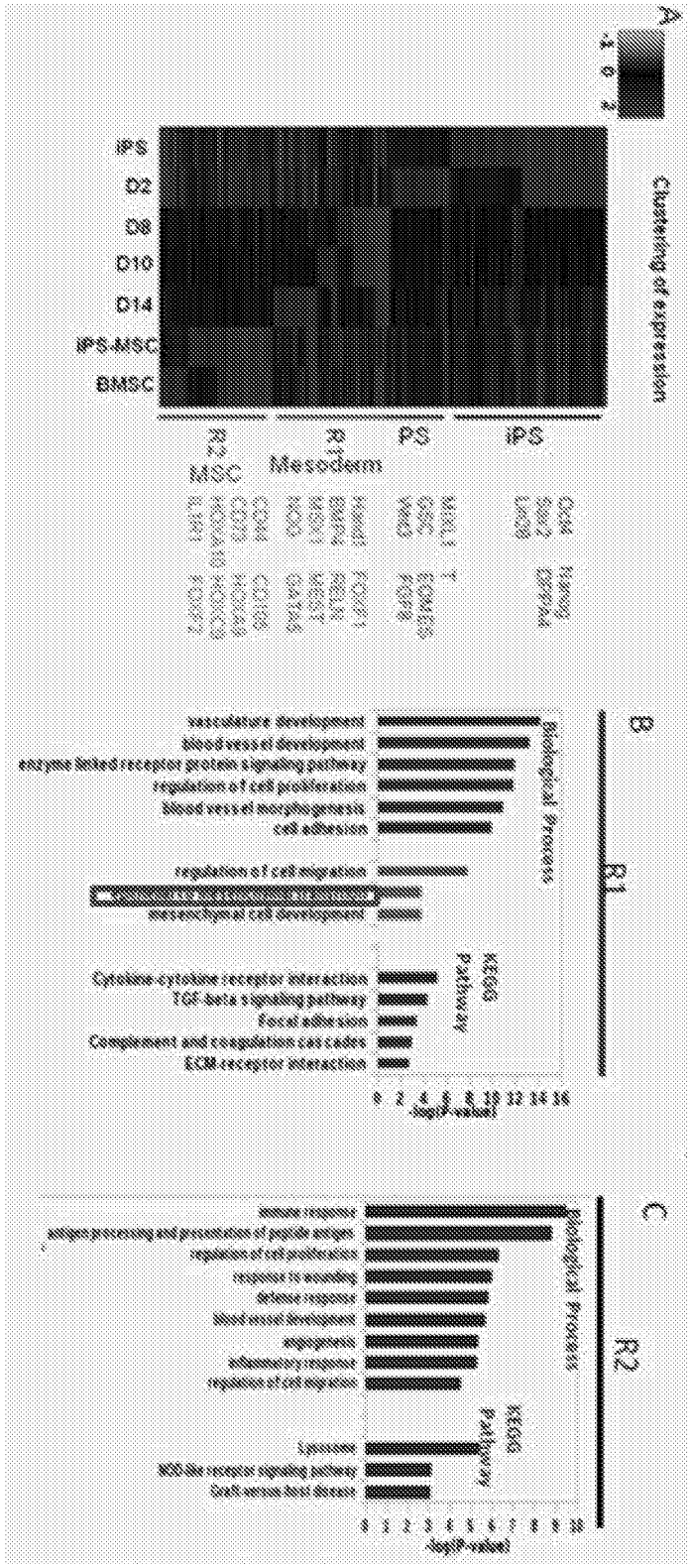

A
Clustering of expression
iPS
D2
D8
D10
D14
iPS-MSC
BMSC
R2
MSC
R1
Mesoderm
PS
iPS
B
R1
Biological Process
vasculature development
blood vessel development
enzyme linked receptor protein signaling pathway
regulation of cell proliferation
blood vessel morphogenesis
cell adhesion
regulation of cell migration
mesenchymal cell development
KEGG Pathway
Cytokine-cytokine receptor interaction
TGF-beta signaling pathway
Focal adhesion
Complement and coagulation cat cades
ECM-receptor interaction
C
R2
Biological Process
immune response
antigen processing and presentation of peptide antigen
regulation of cell proliferation
response to wounding
defense response
blood vessel development
angiogenesis
inflammatory response
regulation of cell migration
KEGG Pathway
Lysosome
NOD-like receptor signaling pathway
Graft-versus-host disease

Fig. 13

E

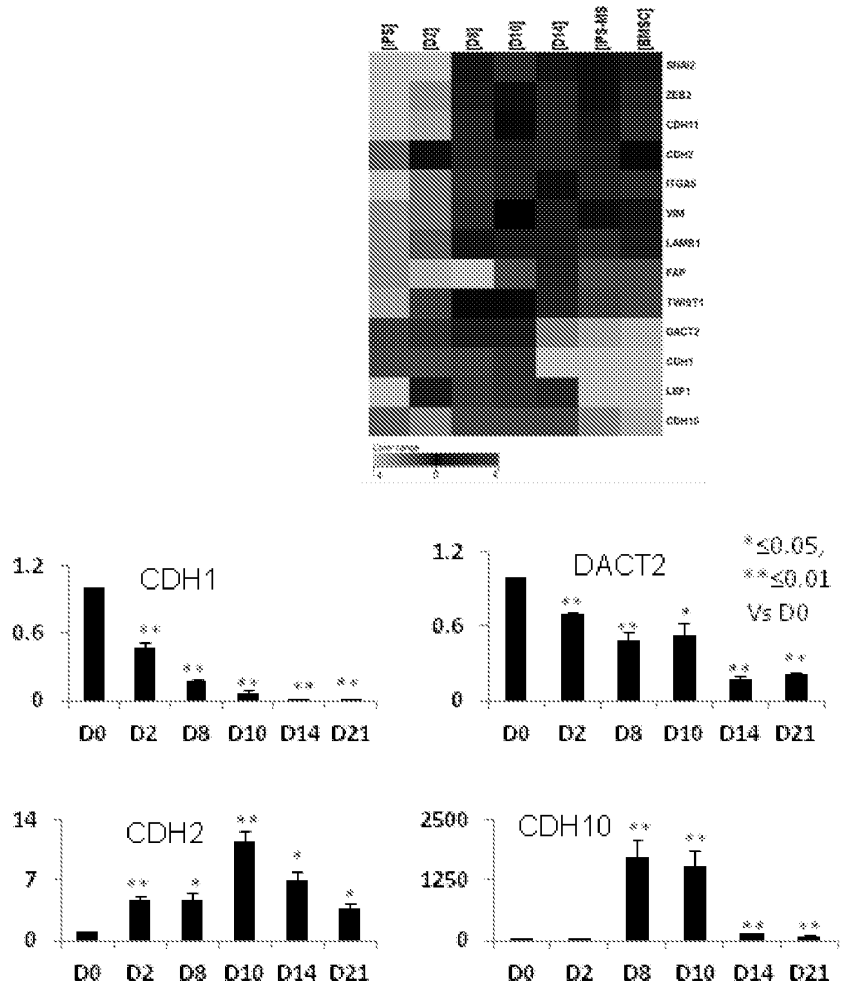


Fig. 13 (Continued)

F

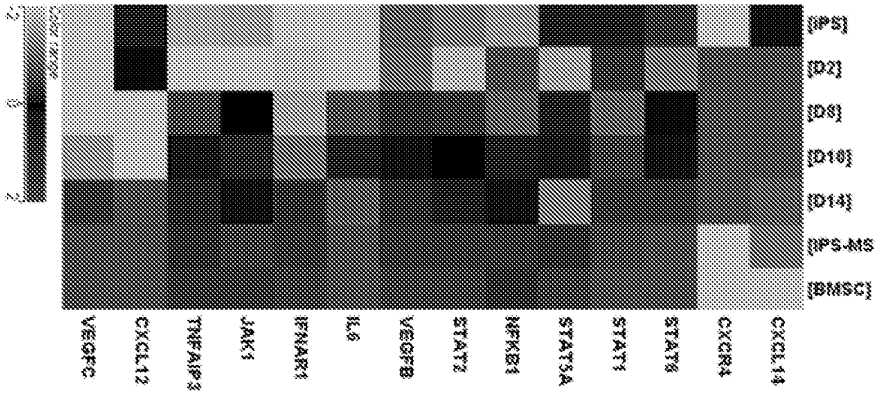


Fig. 13 (Continued)

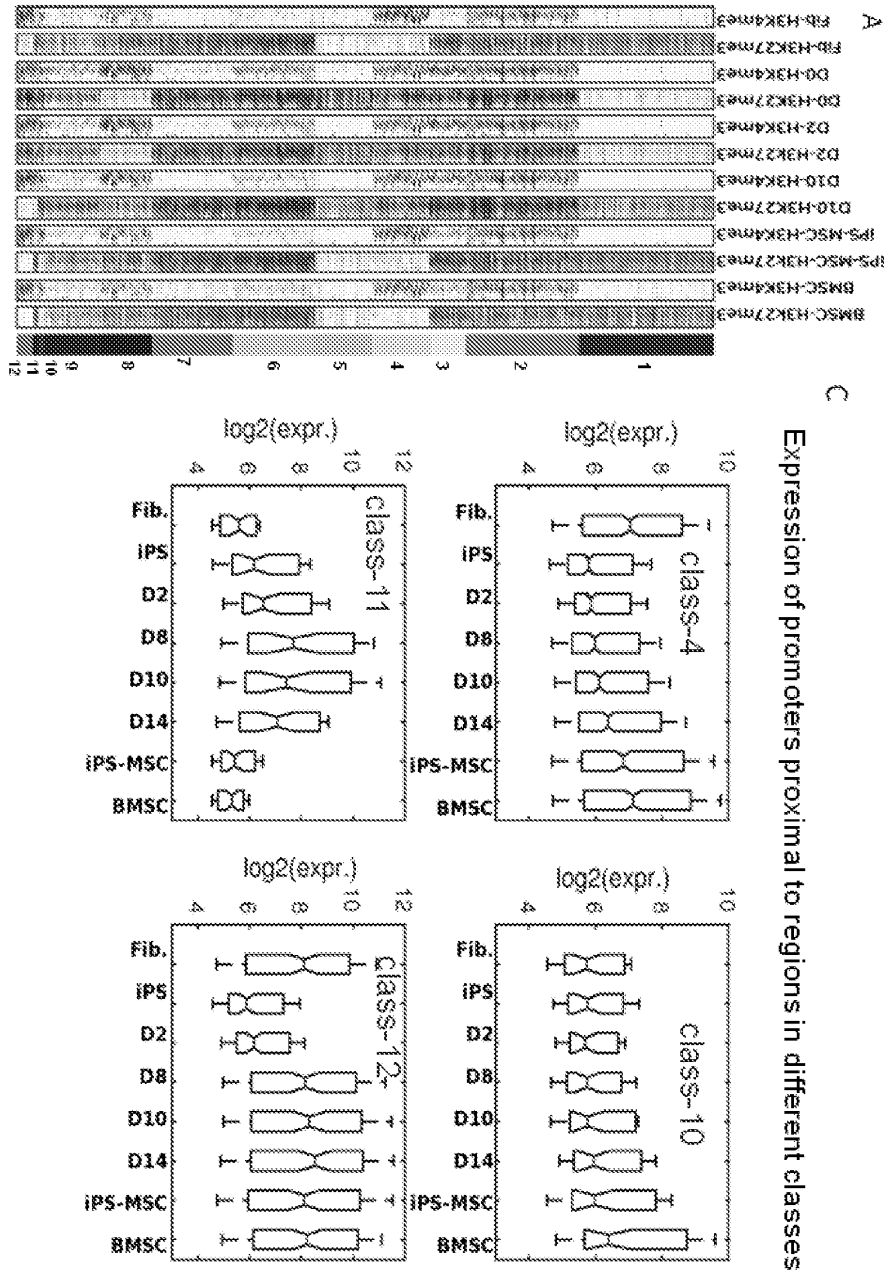

A
Fib-H3K4me3
Fib-H3K27me3
D0-H3K4me3
D0-H3K27me3
D2-H3K4me3
D2-H3K27me3
D10-H3K4me3
D10-H3K27me3
IPS-MSC-H3K4me3
IPS-MSC-H3K27me3
BMSC-H3K4me3
BMSC-H3K27me3
1 2 3 4 5 6 7 8 9 10 11 12
C
Expression of promoters proximal to regions in different classes
log2(expr.)
class-11
class-4
class-12
class-10
Fib.
iPS
D2
D8
D10
D14
iPS-MSC
BMSC

Fig. 14

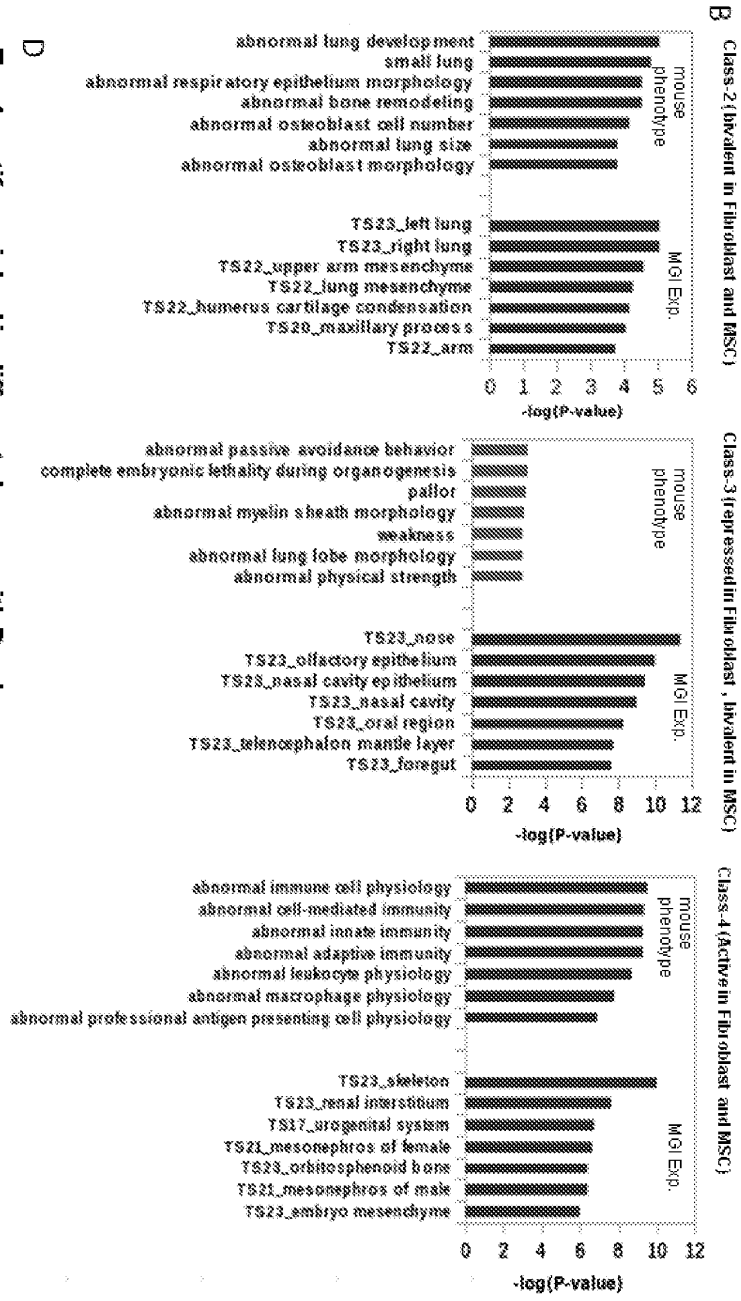


Fig. 14 (Continued)

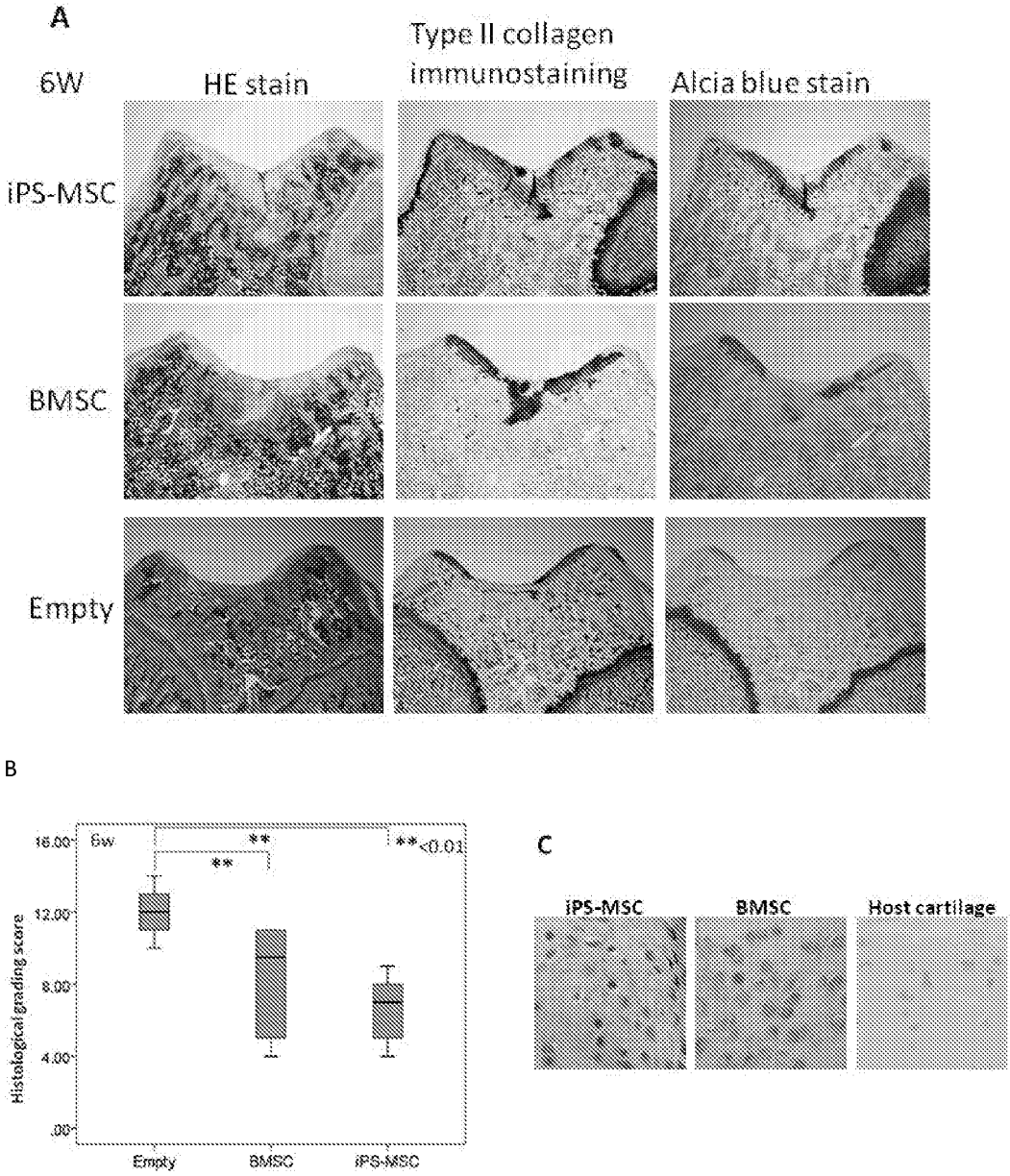

A
6W
HE stain
Type II collagen immunostaining
Alcia blue stain
iPS-MSC
BMSC
Empty
B
6w
**
**
**<0.01
16.00
12.00
8.00
4.00
.00
Histological grading score
Empty
BMSC
iPS-MSC
C
iPS-MSC
BMSC
Host cartilage

Fig. 15

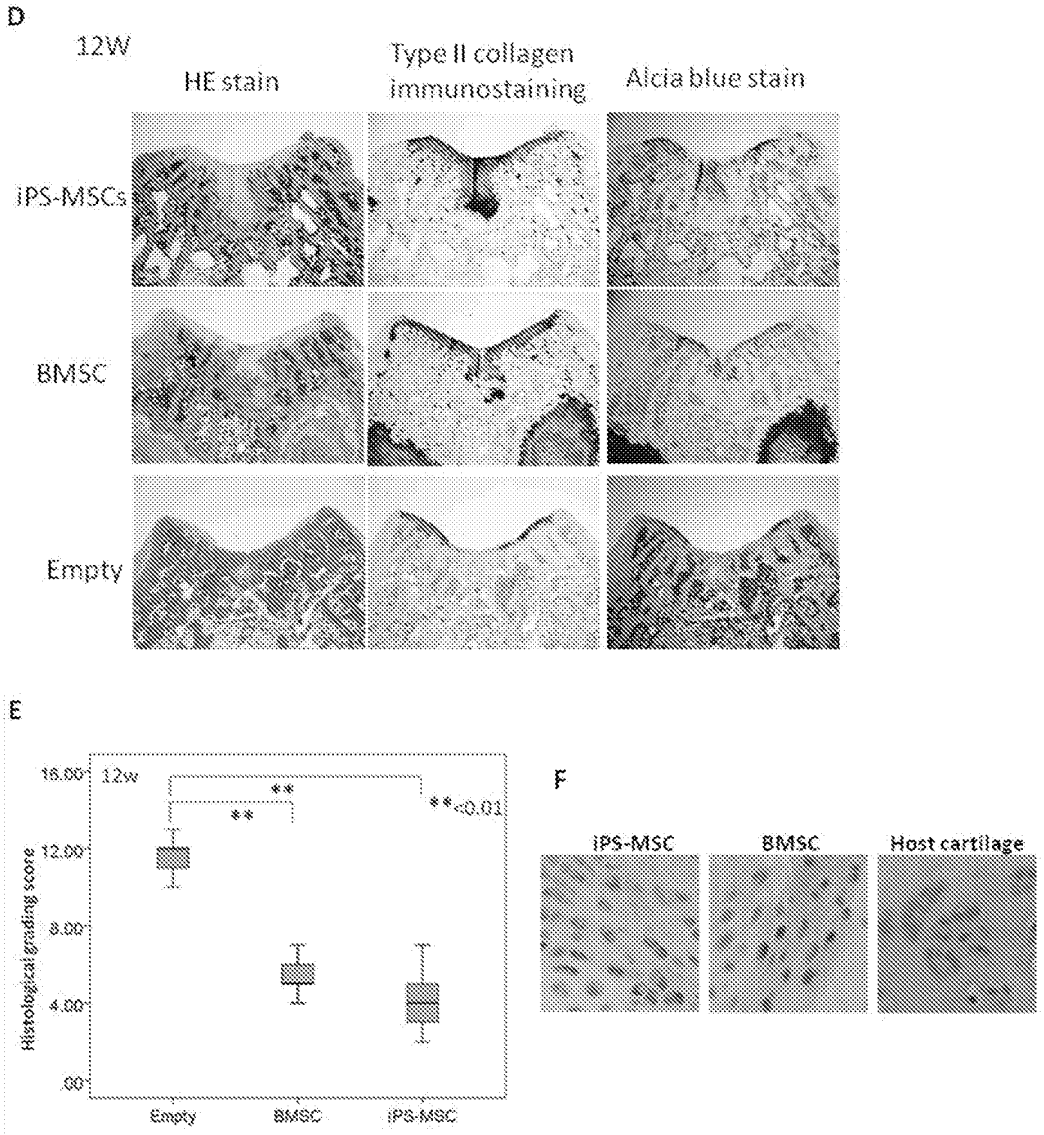
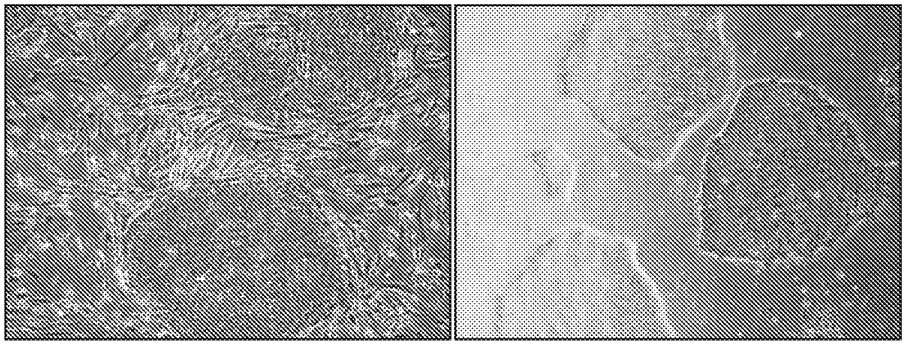


Fig. 15 (Continued)

A B



C

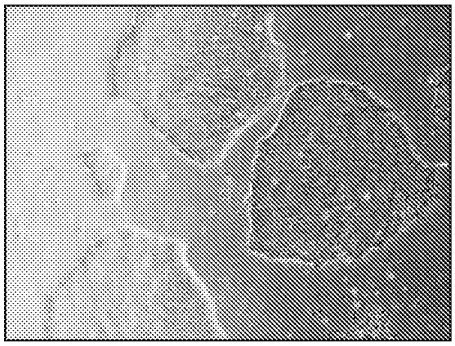


Fig. 16

D

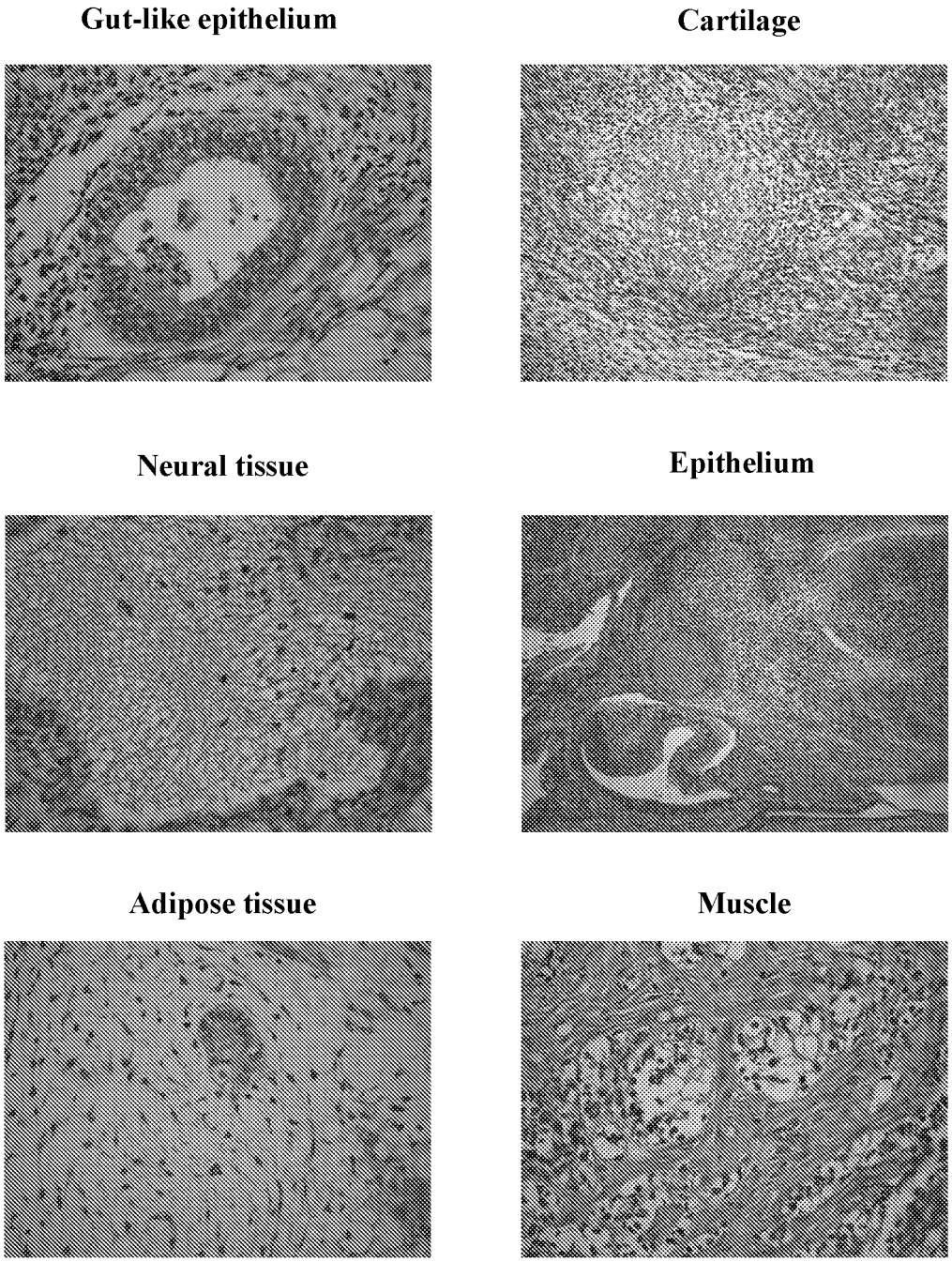


Fig. 16 (Continued)

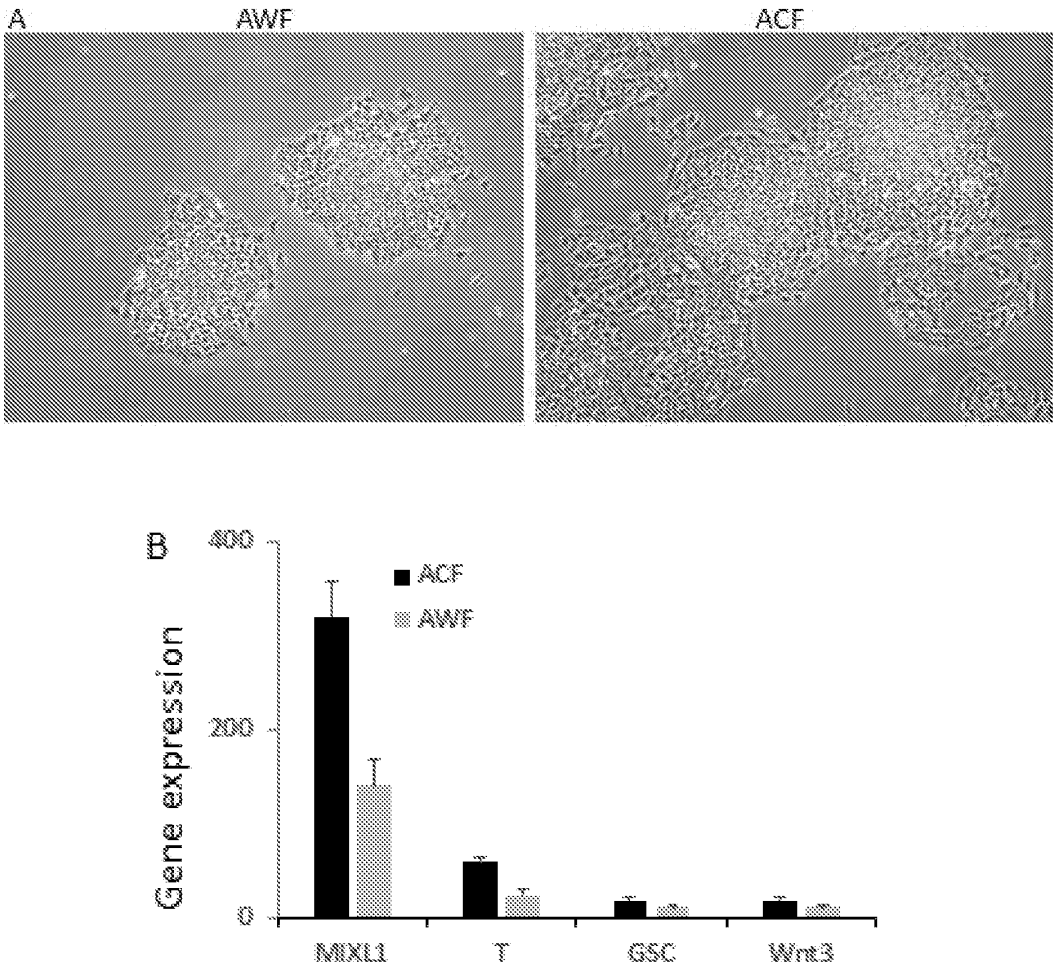


Fig. 17

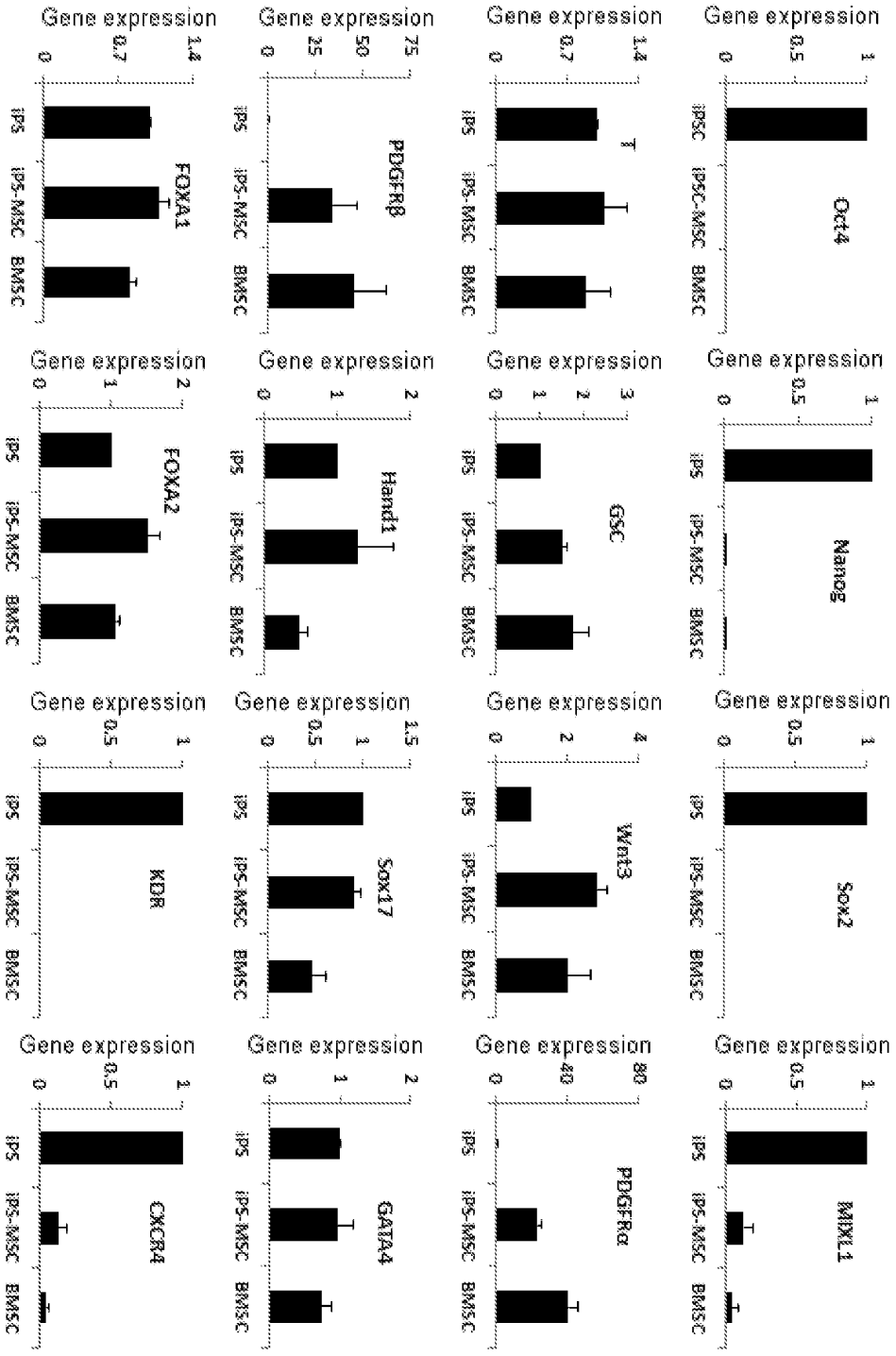

Gene expression
Oct4
Nanog
Sox2
MIXL1
T
GSC
Wnt3
PDGFRα
FOXA1
Hand1
Sox17
GATA4
PDGFRβ
FOXA2
KDR
CXCR4
iPS
iPS-MSC
BMSC

Fig. 18

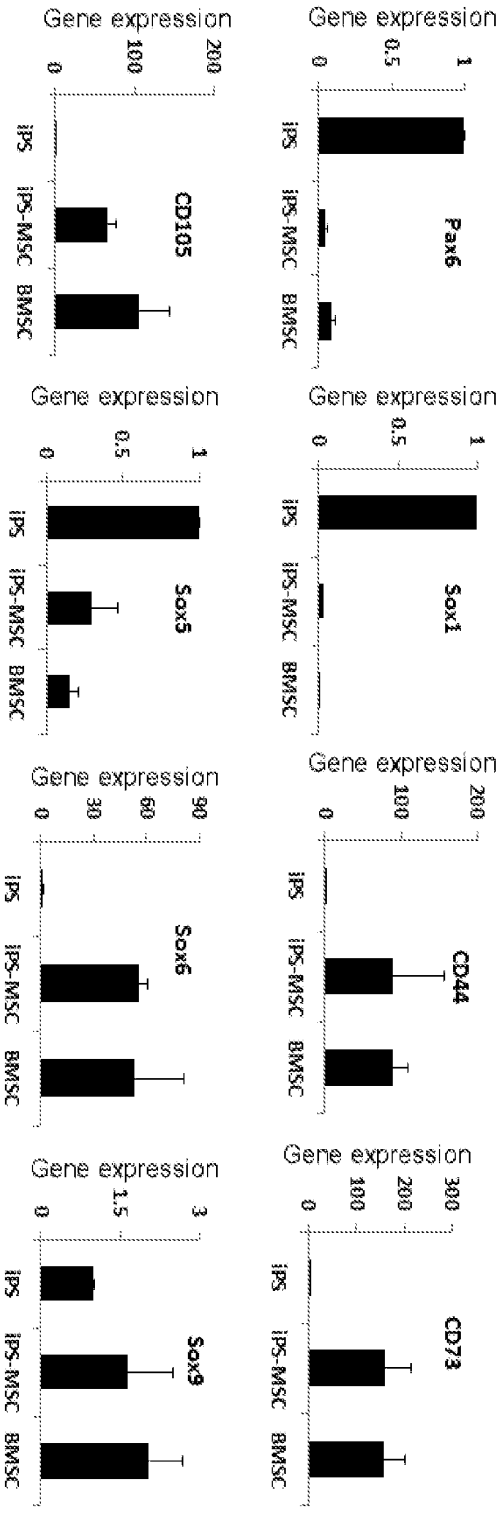


Fig. 18 (Continued)