FIG. 1

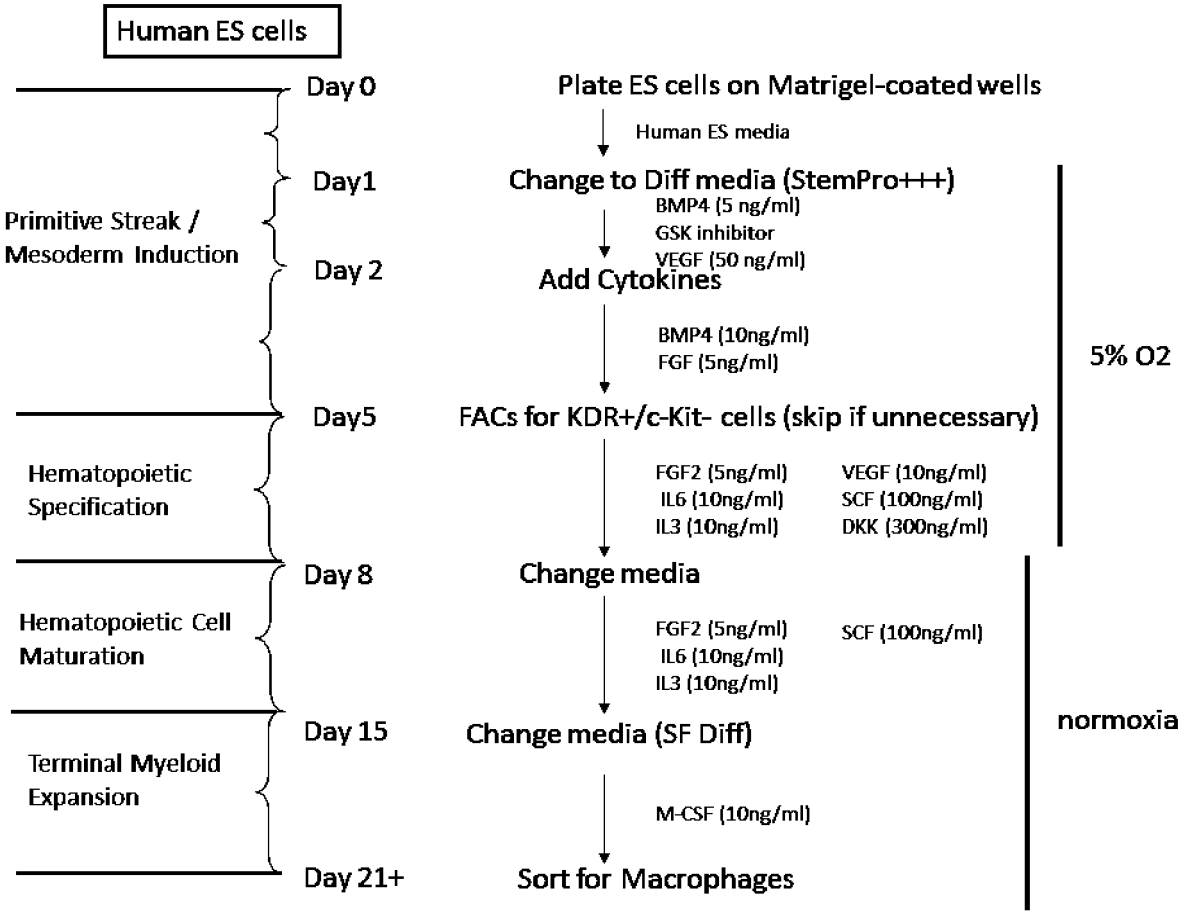

Human ES cells
Day 0
Plate ES cells on Matrigel-coated wells
Human ES media
Day1
Change to Diff media (StemPro+++)
BMP4 (5 ng/ml)
GSK inhibitor
VEGF (50 ng/ml)
Primitive Streak / Mesoderm Induction
Day 2
Add Cytokines
BMP4 (10ng/ml)
FGF (5ng/ml)
5% O2
Day5
FACs for KDR+/c-Kit- cells (skip if unnecessary)
Hematopoietic Specification
FGF2 (5ng/ml)
IL6 (10ng/ml)
IL3 (10ng/ml)
VEGF (10ng/ml)
SCF (100ng/ml)
DKK (300ng/ml)
Day 8
Change media
Hematopoietic Cell Maturation
FGF2 (5ng/ml)
IL6 (10ng/ml)
IL3 (10ng/ml)
SCF (100ng/ml)
Day 15
Change media (SF Diff)
normoxia
Terminal Myeloid Expansion
M-CSF (10ng/ml)
Day 21+
Sort for Macrophages

FIG. 2

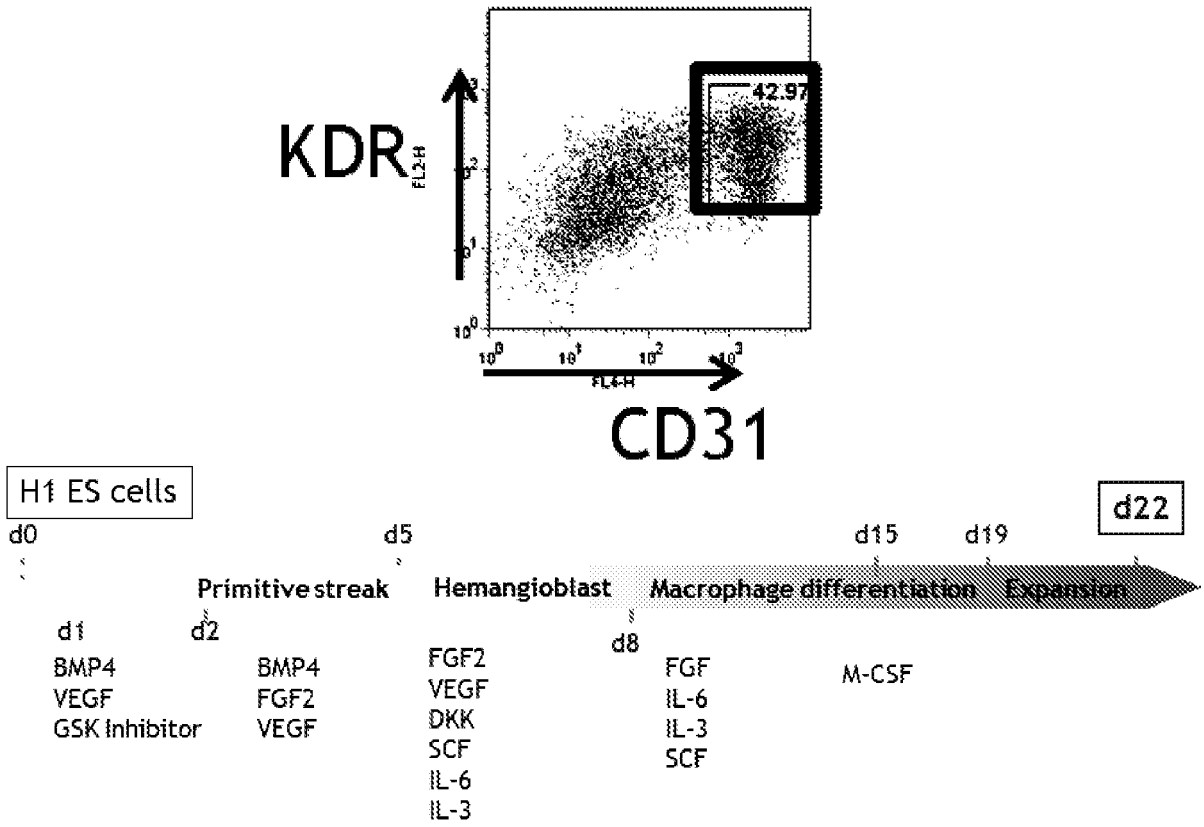
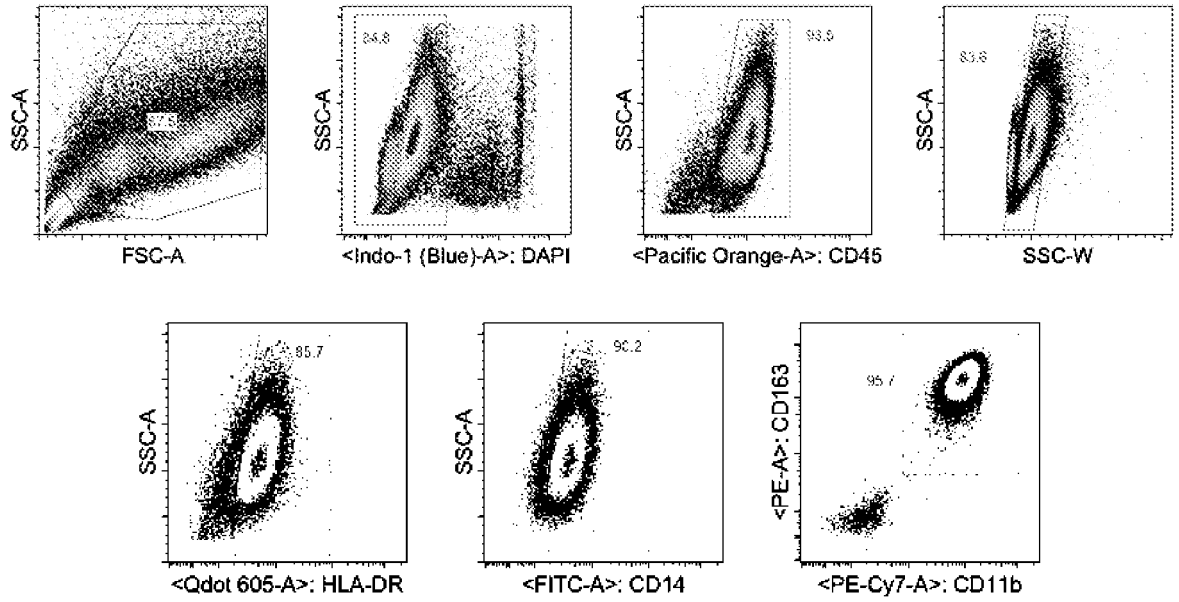

KDR
FL2-H
42.97
CD31
FL4-H
H1 ES cells
d0
d5
d15
d19
d22
Primitive streak
Hemangioblast
Macrophage differentiation
Expansion
d1
d2
d8
BMP4
VEGF
GSK Inhibitor
BMP4
FGF2
VEGF
FGF2
VEGF
DKK
SCF
IL-6
IL-3
FGF
IL-6
IL-3
SCF
M-CSF

FIG. 3

a)


SSC-A
FSC-A
SSC-A
<Indo-1 (Blue)-A>: DAPI
84.8
SSC-A
<Pacific Orange-A>: CD45
SSC-A
SSC-W
SSC-A
<Qdot 605-A>: HLA-DR
85.7
SSC-A
<FITC-A>: CD14
90.2
<PE-A>: CD163
<PE-Cy7-A>: CD11b
95.7

b)

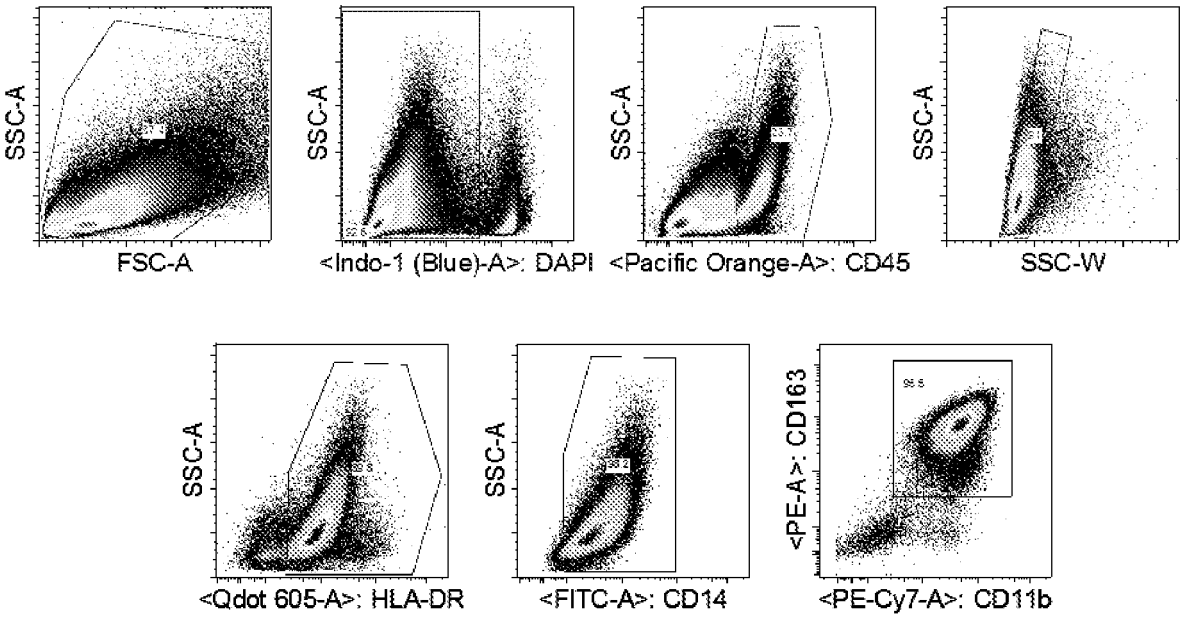

SSC-A
FSC-A
SSC-A
<Indo-1 (Blue)-A>: DAPI
SSC-A
<Pacific Orange-A>: CD45
SSC-A
SSC-W
SSC-A
<Qdot 605-A>: HLA-DR
SSC-A
<FITC-A>: CD14
<PE-A>: CD163
<PE-Cy7-A>: CD11b

FIG. 3

c)

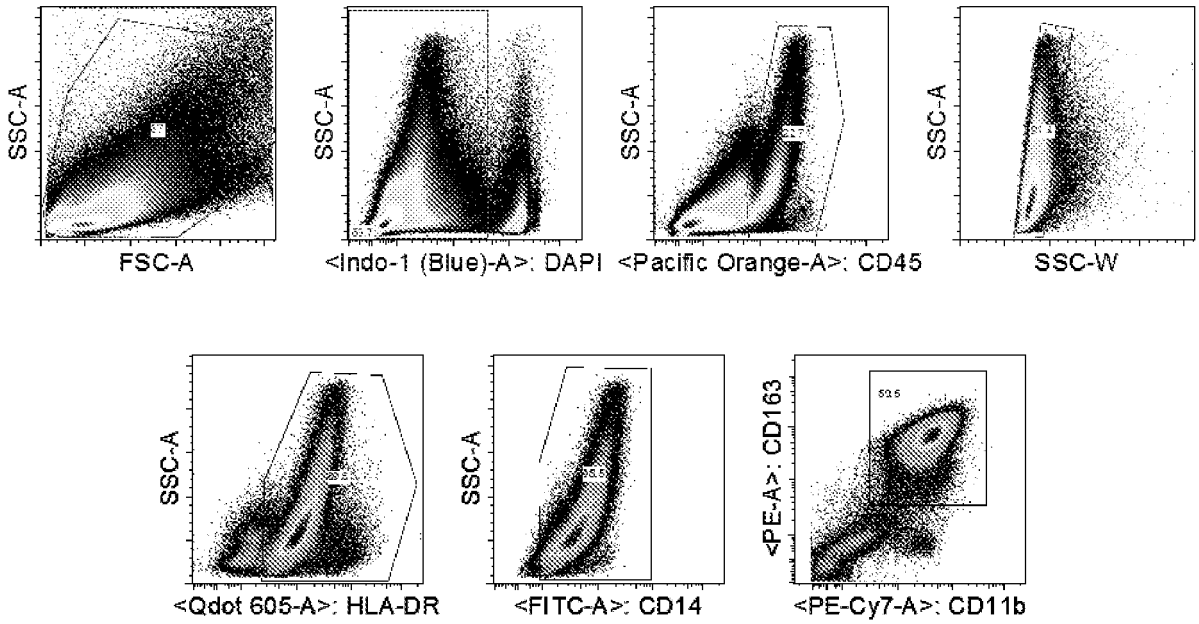

SSC-A
FSC-A
SSC-A
<Indo-1 (Blue)-A>: DAPI
SSC-A
<Pacific Orange-A>: CD45
SSC-A
SSC-W
SSC-A
<Qdot 605-A>: HLA-DR
SSC-A
<FITC-A>: CD14
<PE-A>: CD163
<PE-Cy7-A>: CD11b

FIG. 4

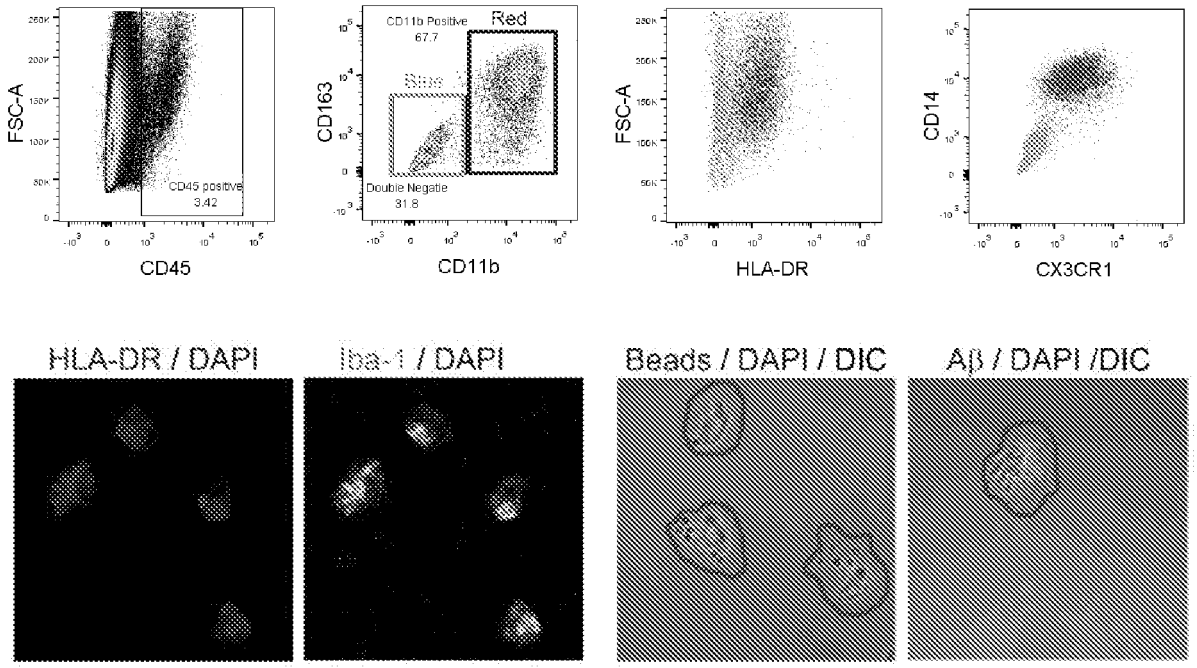
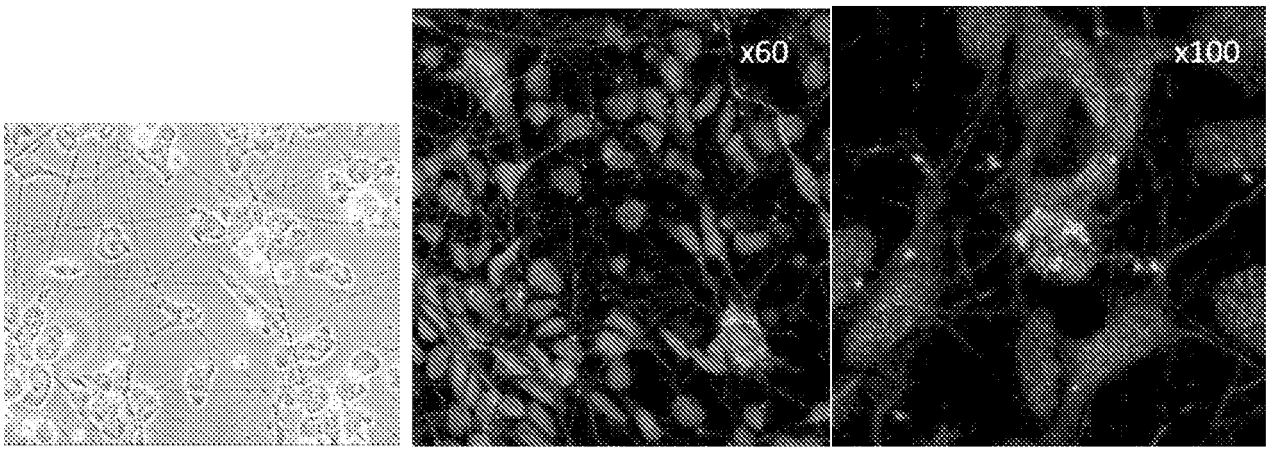

FSC-A
CD45
CD45 positive
3.42
CD163
CD11b
CD11b Positive
67.7
Red
Blue
Double Negatie
31.8
FSC-A
HLA-DR
CD14
CX3CR1
HLA-DR / DAPI
Iba-1 / DAPI
Beads / DAPI / DIC
Aβ / DAPI /DIC

FIG. 5



Neuron (Tuj-1)/ Macrophage (Iba1)/ Nuclei (Hoechst)

FIG.6

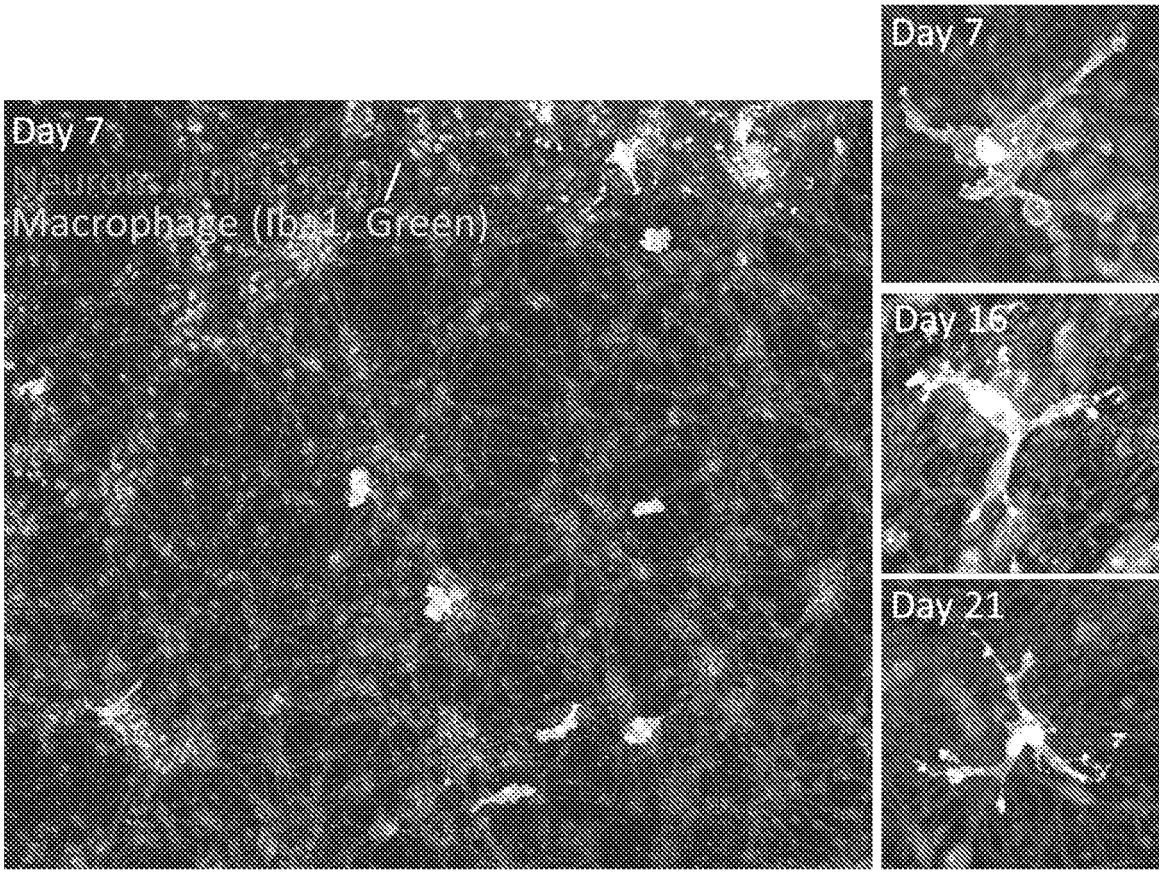


FIG. 7

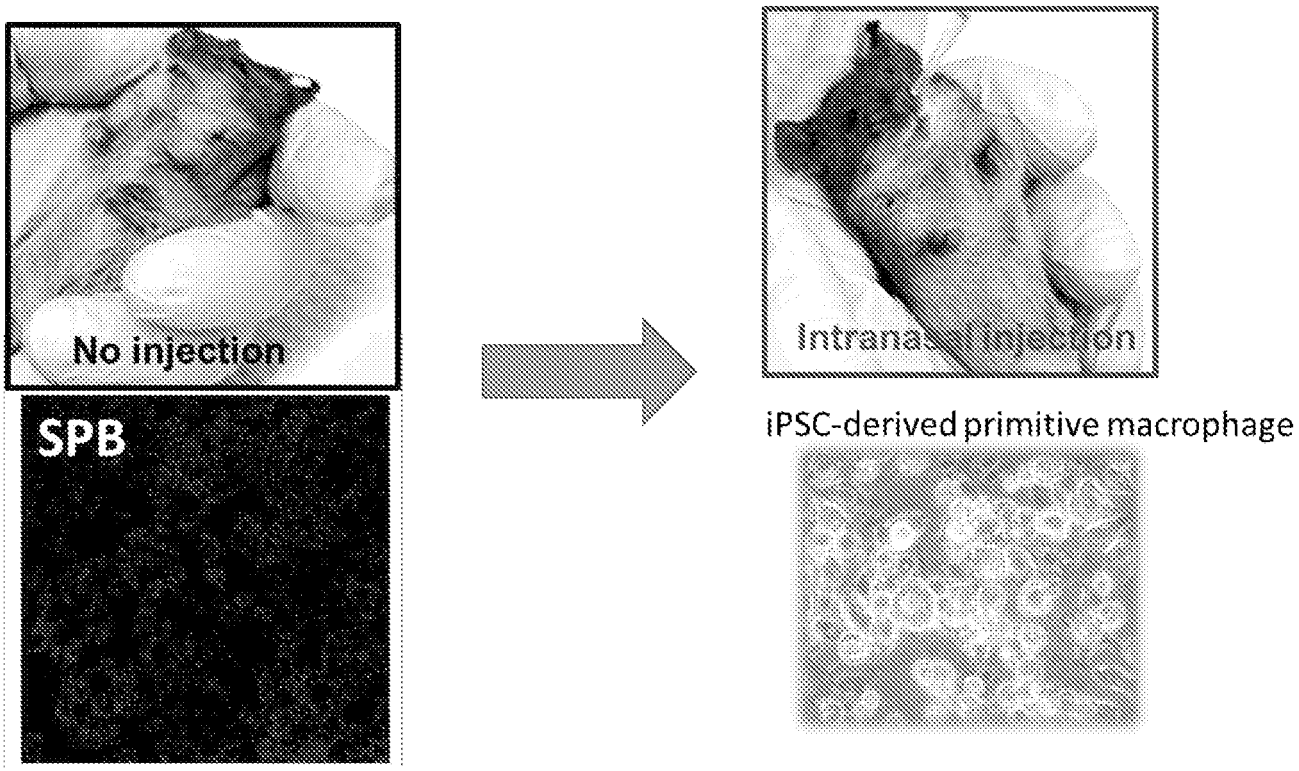

No injection
SPB
Intranasal injection
iPSC-derived primitive macrophage
Accumulation of Surfactant Associated Protein B (SPB)

FIG. 8a

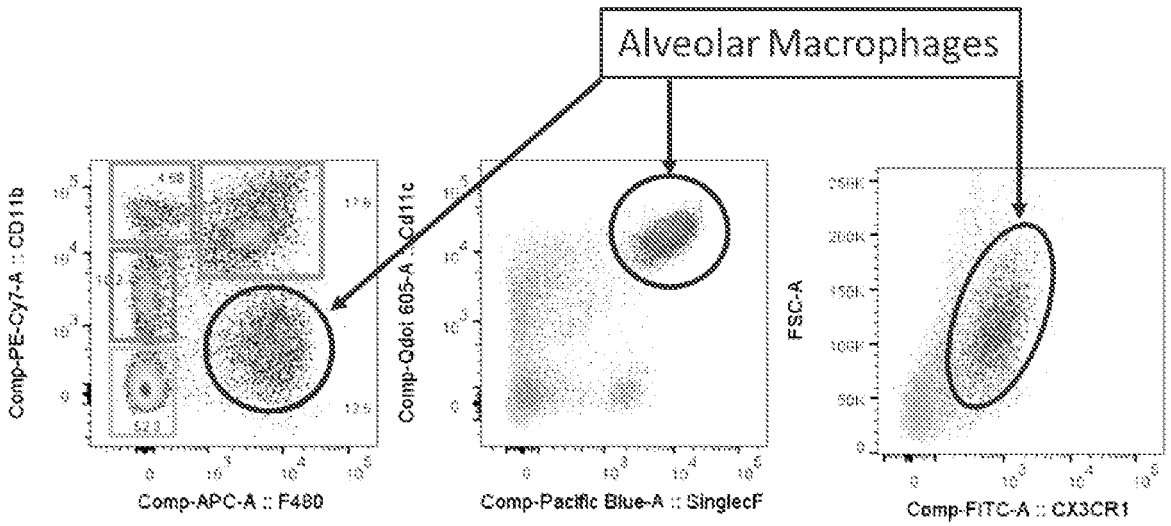

Alveolar Macrophages
Comp-PE-Cy7-A :: CD11b
Comp-APC-A :: F480
Comp-Qdot 605-A :: Cd11c
Comp-Pacific Blue-A :: SiglecF
FSC-A
Comp-FITC-A :: CX3CR1

FIG. 8b

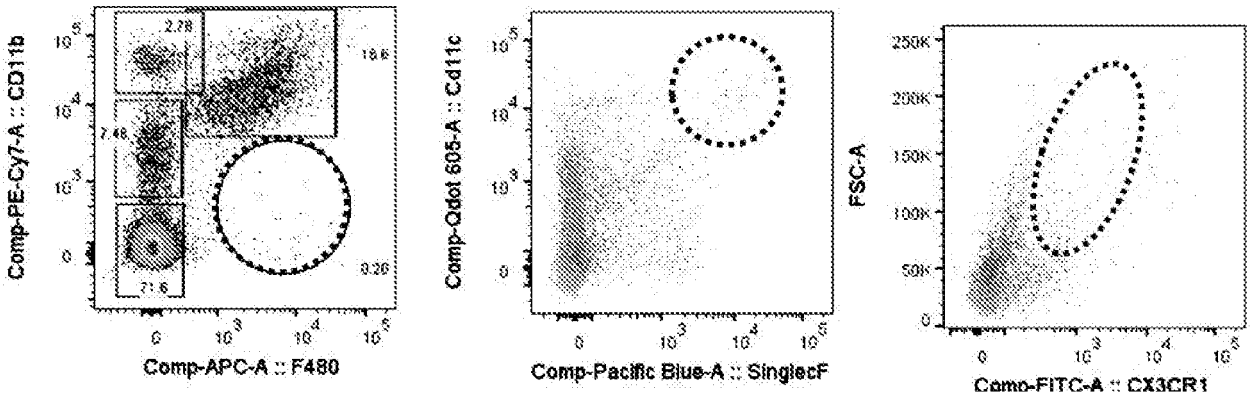

Comp-PE-Cy7-A :: CD11b
Comp-APC-A :: F480
2.28
18.6
7.48
0.26
71.6
Comp-Qdot 605-A :: Cd11c
Comp-Pacific Blue-A :: SiglecF
FSC-A
Comp-FITC-A :: CX3CR1

FIG.8c

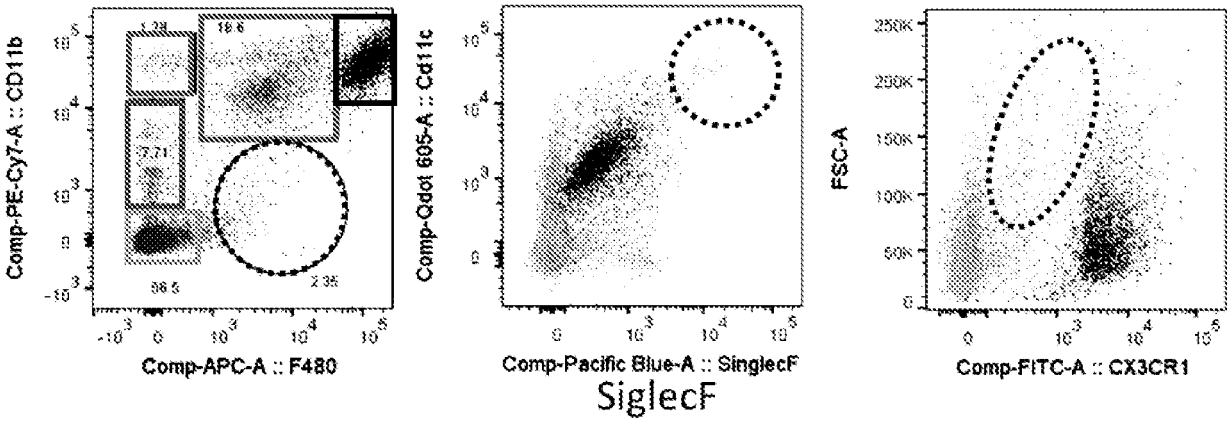
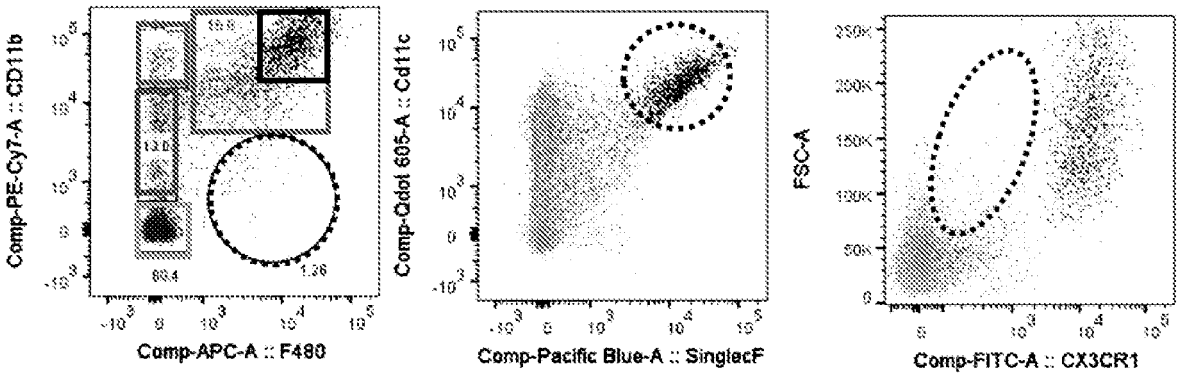

Comp-PE-Cy7-A :: CD11b
Comp-APC-A :: F480
Comp-Qdot 605-A :: Cd11c
Comp-Pacific Blue-A :: SiglecF
SiglecF
FSC-A
Comp-FITC-A :: CX3CR1

FIG. 9



SiglecF

FIG. 10

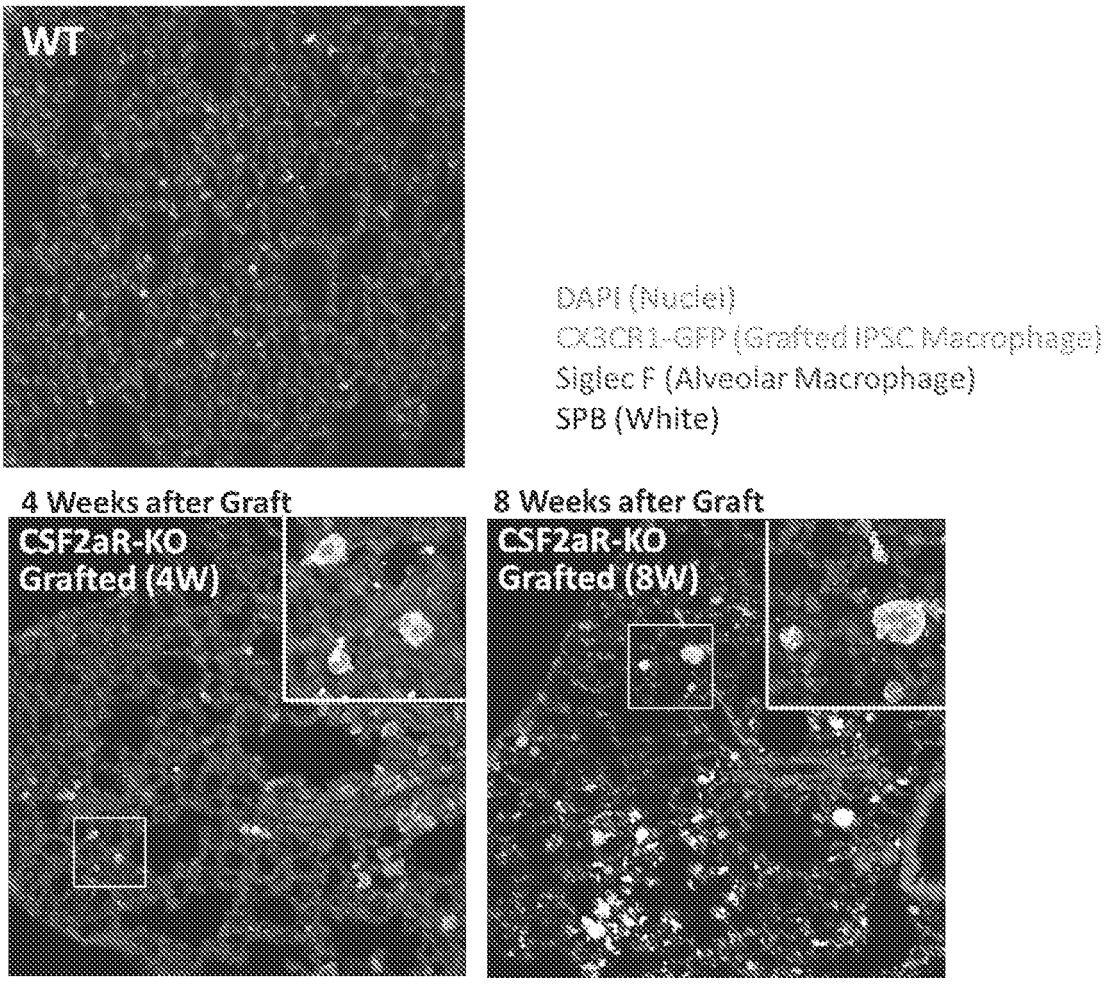

WT
DAPI (Nuclei)
CX3CR1-GFP (Grafted IPSC Macrophage)
Siglec F (Alveolar Macrophage)
SPB (White)
4 Weeks after Graft
CSF2aR-KO
Grafted (4W)
8 Weeks after Graft
CSF2aR-KO
Grafted (8W)

FIG. 11

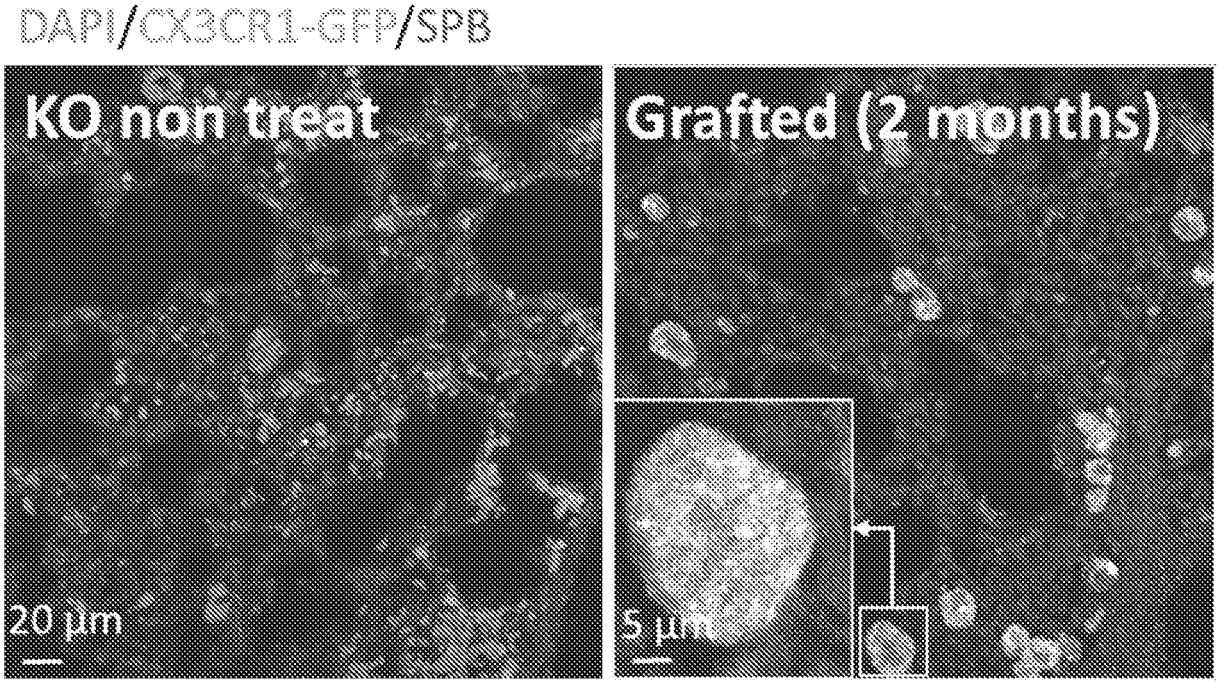


FIG. 12

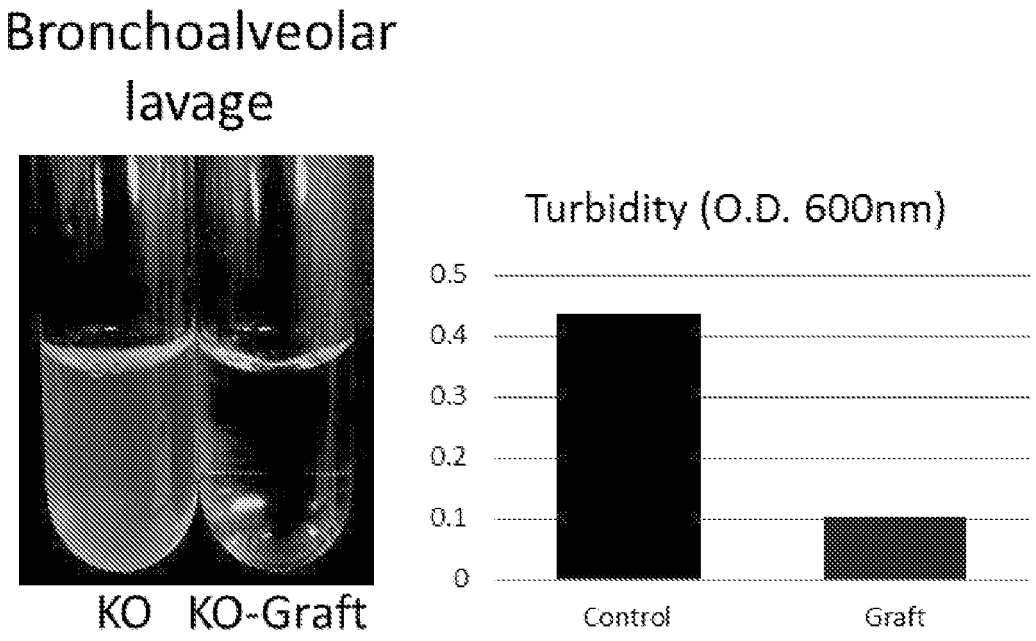
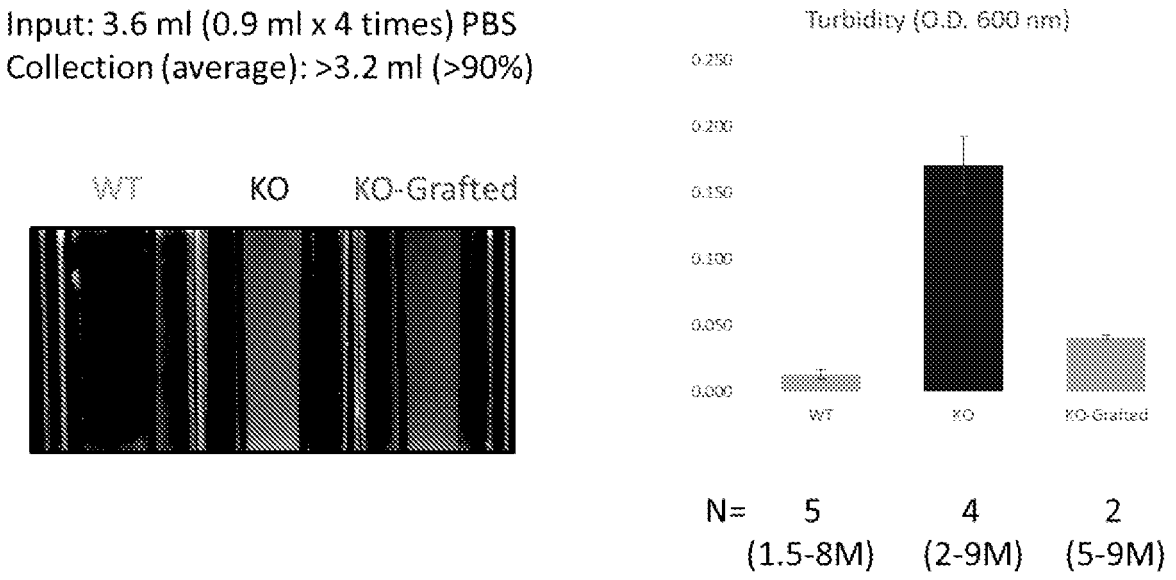

Bronchoalveolar lavage
KO
KO-Graft
Turbidity (O.D. 600nm)
0.5
0.4
0.3
0.2
0.1
0
Control
Graft

FIG. 13


Input: 3.6 ml (0.9 ml x 4 times) PBS
Collection (average): >3.2 ml (>90%)
WT
KO
KO-Grafted
Turbidity (O.D. 600 nm)
0.250
0.200
0.150
0.100
0.050
0.000
WT
KO
KO-Grafted
N= 5 (1.5-8M)
4 (2-9M)
2 (5-9M)