1/7

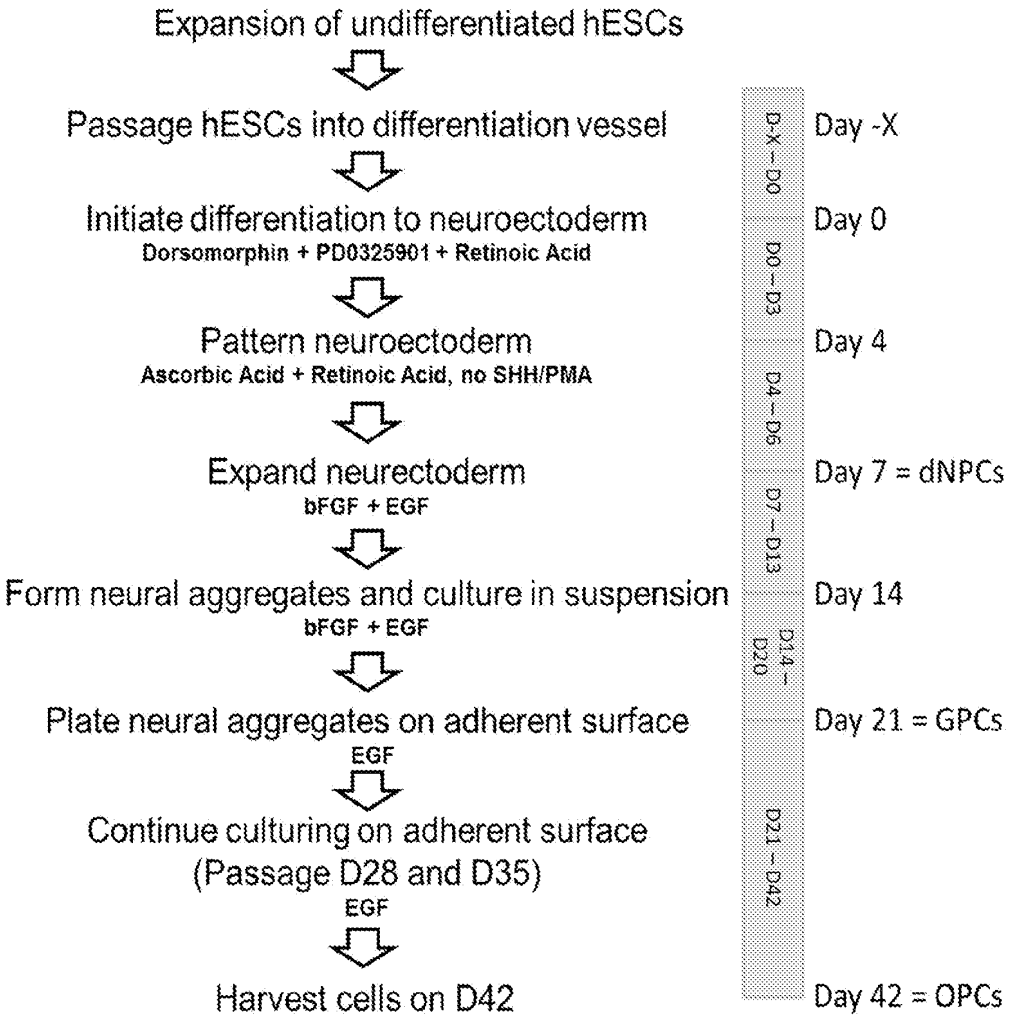

Expansion of undifferentiated hESCs
Passage hESCs into differentiation vessel
Initiate differentiation to neuroectoderm
Dorsomorphin + PD0325901 + Retinoic Acid
Pattern neuroectoderm
Ascorbic Acid + Retinoic Acid, no SHH/PMA
Expand neurectoderm
bFGF + EGF
Form neural aggregates and culture in suspension
bFGF + EGF
Plate neural aggregates on adherent surface
EGF
Continue culturing on adherent surface
(Passage D28 and D35)
EGF
Harvest cells on D42
D-X – D0
D0 – D3
D4 – D6
D7 – D13
D14 – D20
D21 – D42
Day -X
Day 0
Day 4
Day 7 = dNPCs
Day 14
Day 21 = GPCs
Day 42 = OPCs

FIG. 1

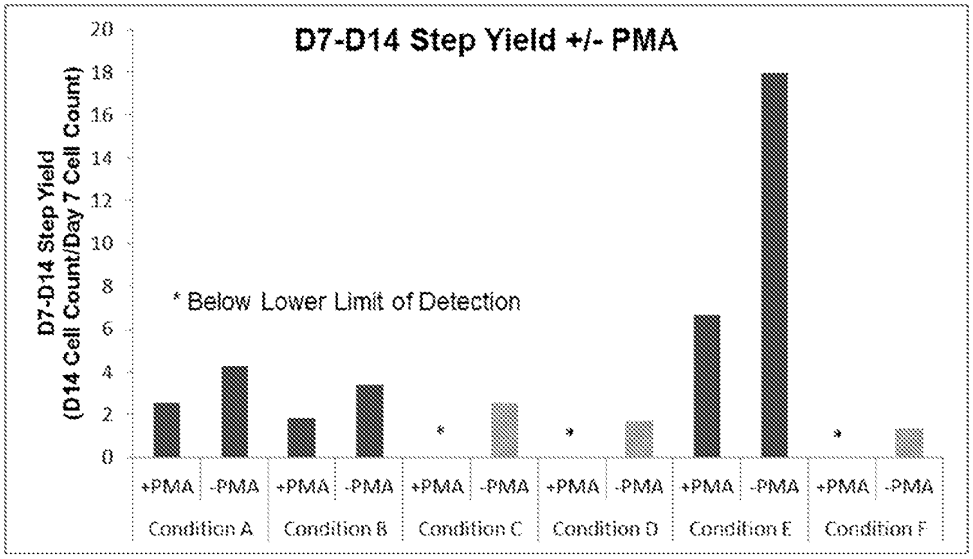

D7-D14 Step Yield +/- PMA
D7-D14 Step Yield
(D14 Cell Count/Day 7 Cell Count)
20
18
16
14
12
10
8
6
4
2
0
* Below Lower Limit of Detection
+PMA
-PMA
Condition A
Condition B
Condition C
Condition D
Condition E
Condition F

FIG. 2A

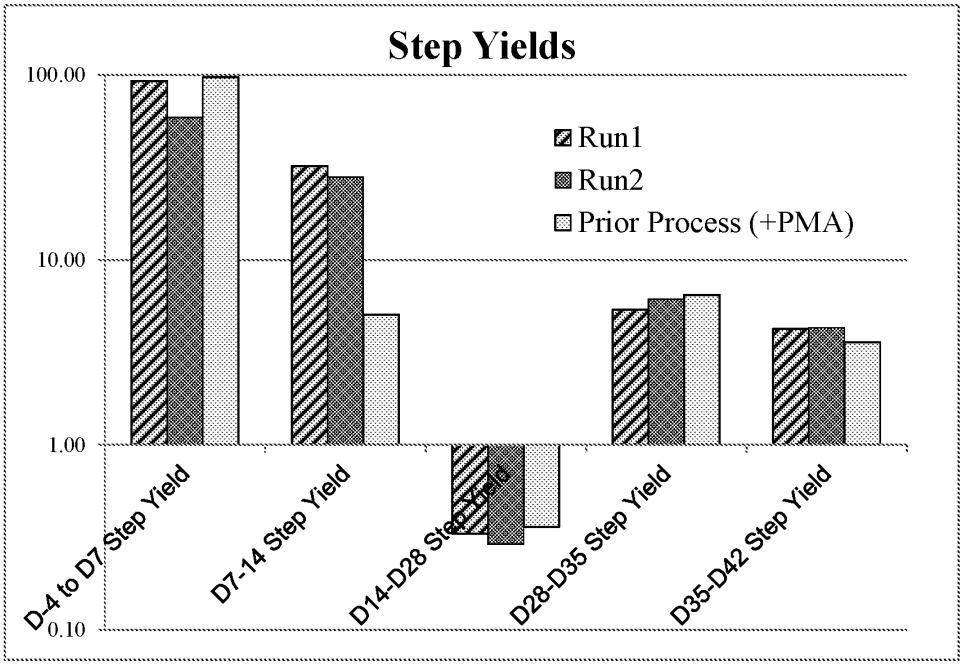

Step Yields
100.00
10.00
1.00
0.10
Run1
Run2
Prior Process (+PMA)
D-4 to D7 Step Yield
D7-14 Step Yield
D14-D28 Step Yield
D28-D35 Step Yield
D35-D42 Step Yield

FIG. 2B

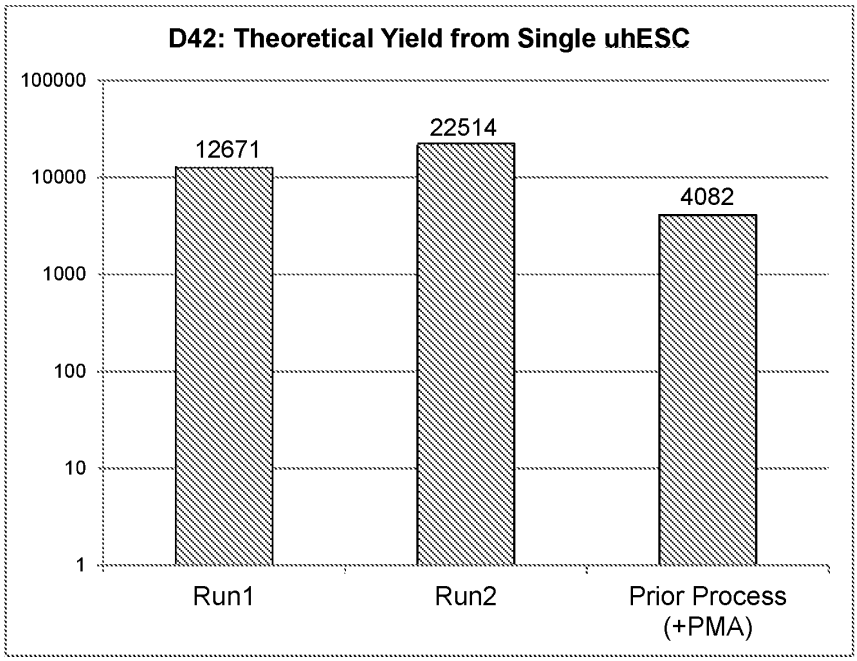

D42: Theoretical Yield from Single uhESC
100000
10000
1000
100
10
1
12671
22514
4082
Run1
Run2
Prior Process
(+PMA)

FIG. 2C

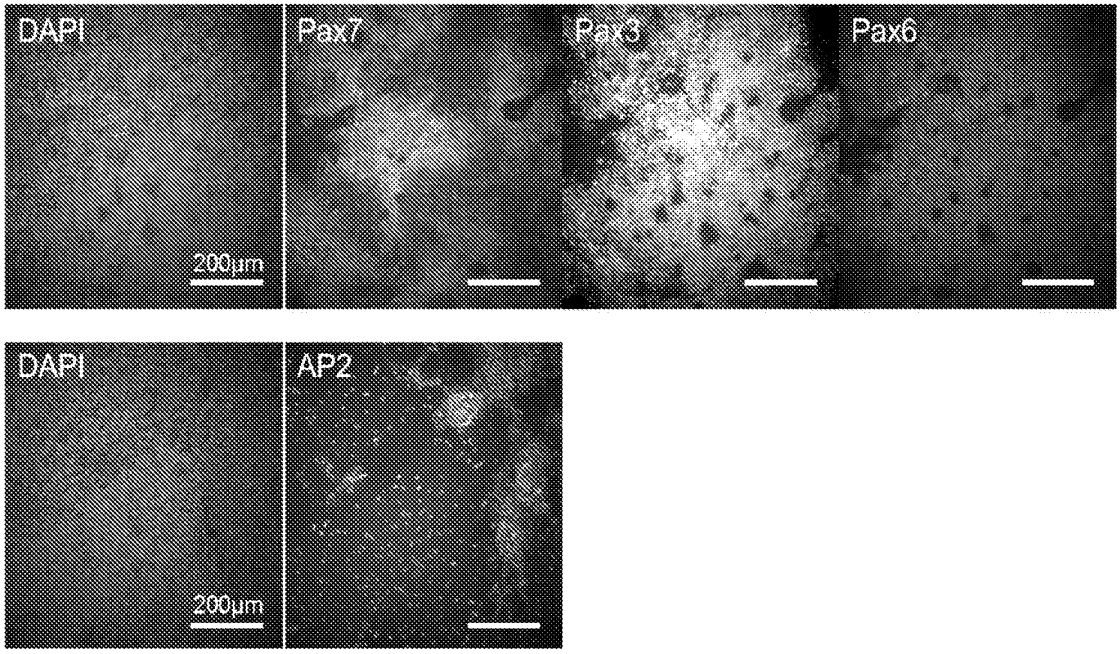


FIG. 3

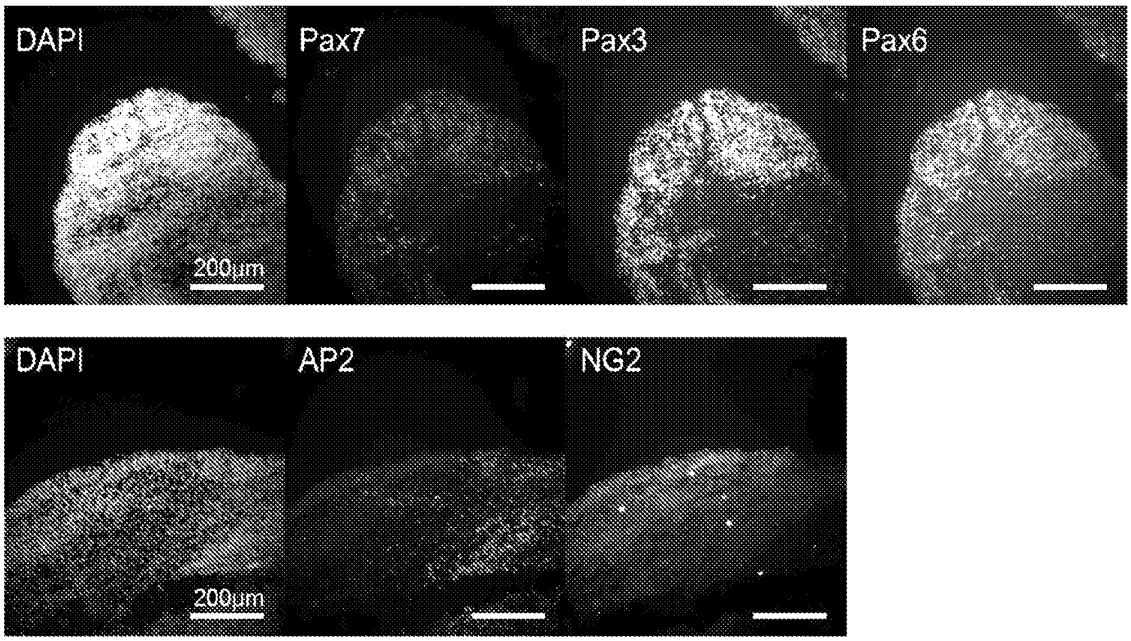


FIG. 4

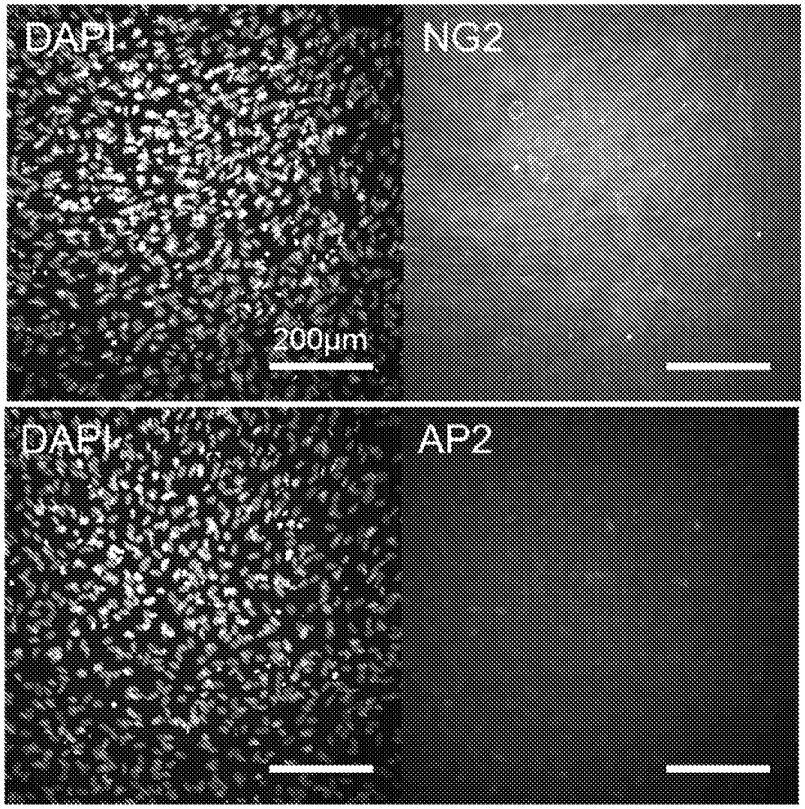


FIG. 5

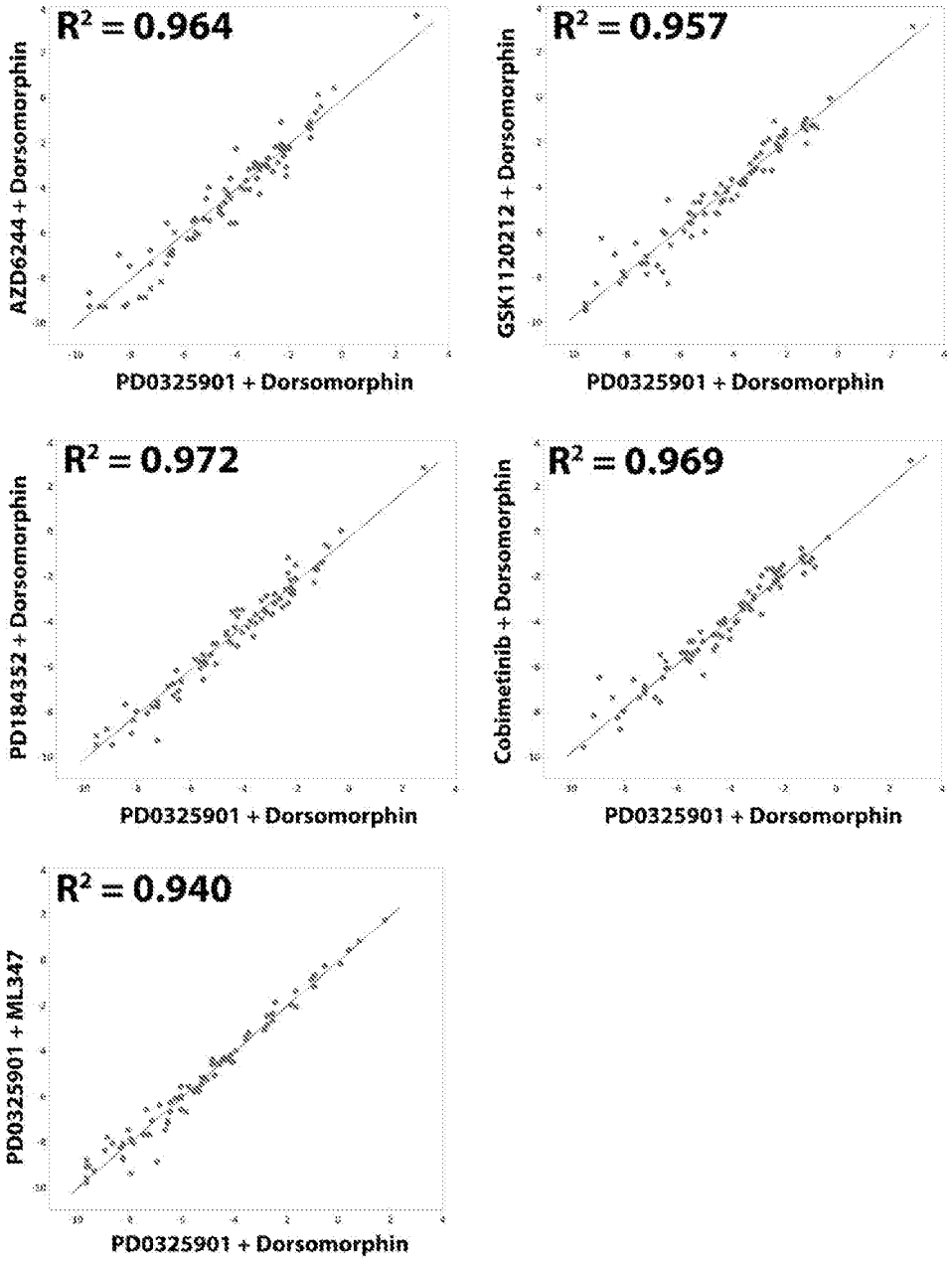

R² = 0.964
AZD6244 + Dorsomorphin
PD0325901 + Dorsomorphin
R² = 0.957
GSK1120212 + Dorsomorphin
PD0325901 + Dorsomorphin
R² = 0.972
PD184352 + Dorsomorphin
PD0325901 + Dorsomorphin
R² = 0.969
Cobimetinib + Dorsomorphin
PD0325901 + Dorsomorphin
R² = 0.940
PD0325901 + ML347
PD0325901 + Dorsomorphin

FIG. 6