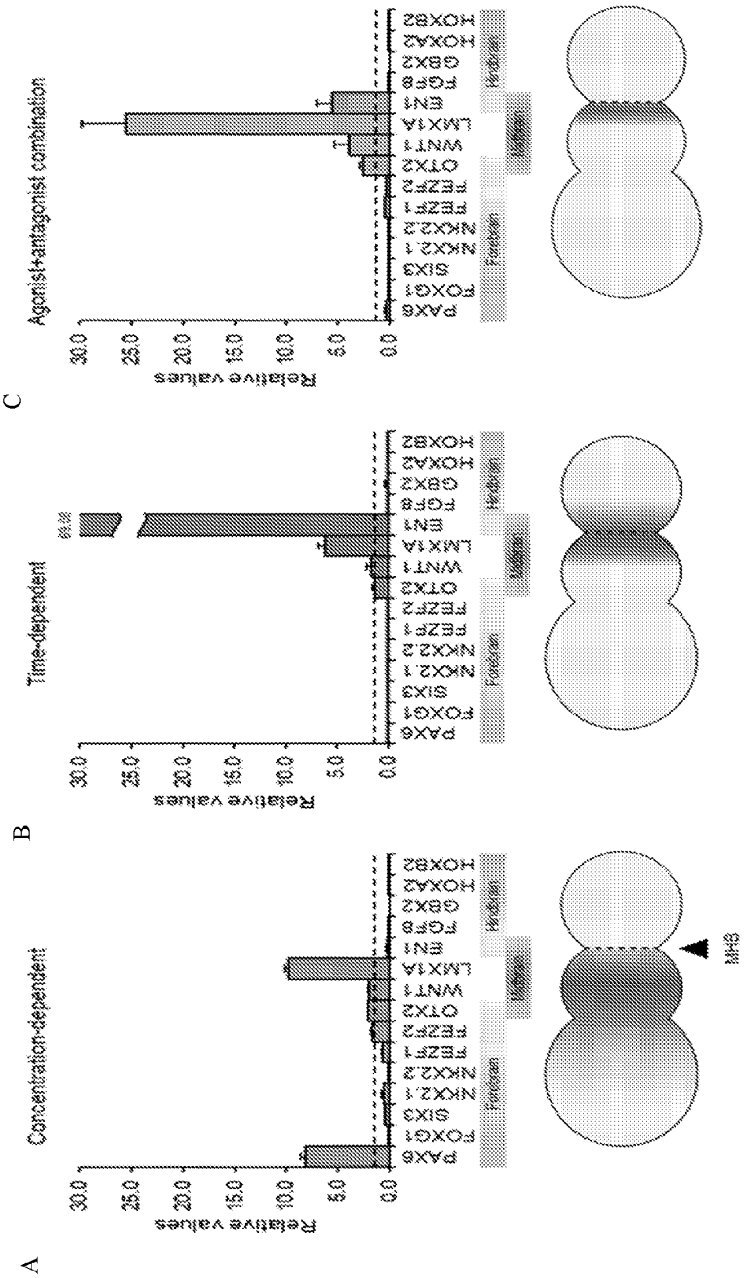

A
Concentration-dependent
B
Time-dependent
C
Agonist+antagonist combination
Relative values
30.0
25.0
20.0
15.0
10.0
5.0
0.0
PAX6
FOXG1
SIX3
NKX2.1
NKX2.2
FEZF1
FEZF2
OTX2
WNT1
LMX1A
EN1
FGF8
GBX2
HOXA2
HOXB2
Forebrain
Midbrain
Hindbrain
MHB

Figure 1

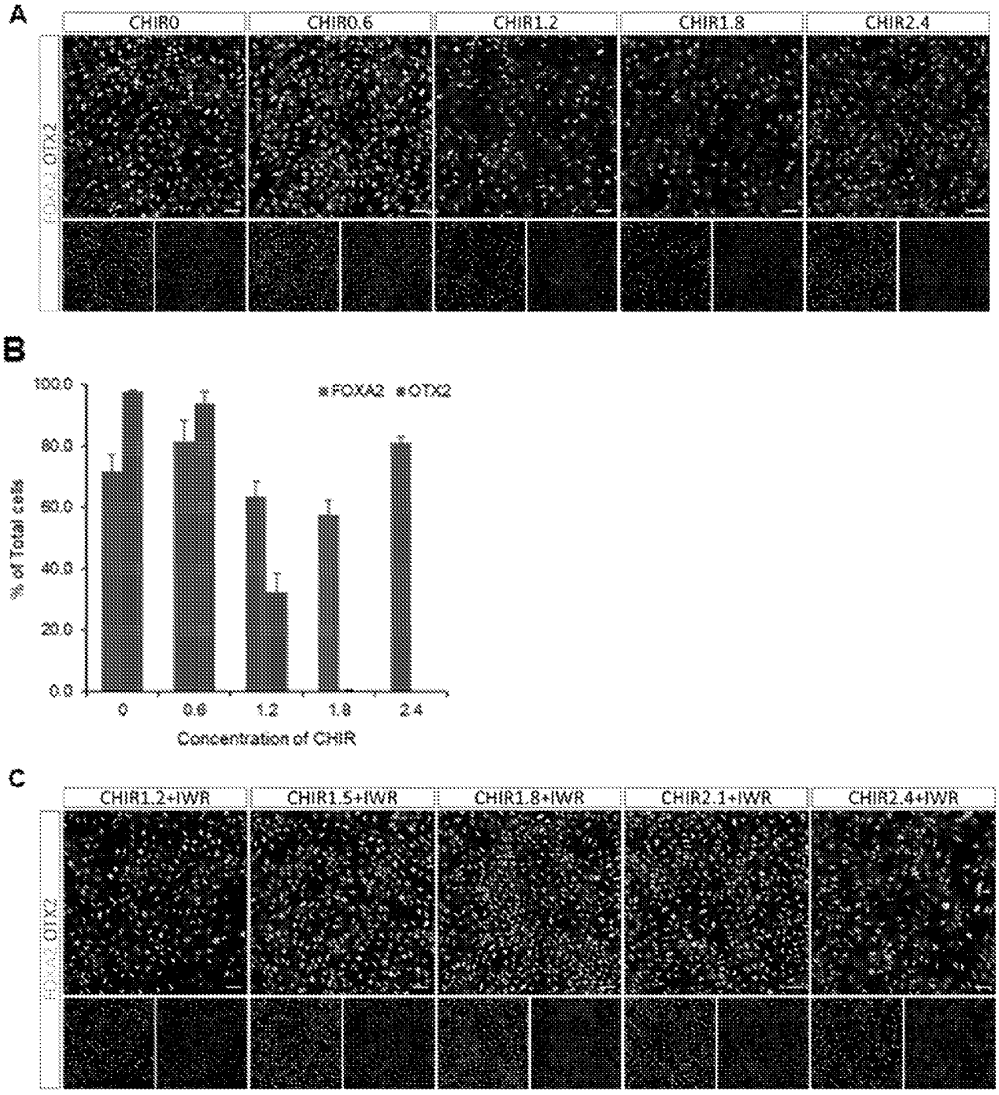

A
CHIR0
CHIR0.6
CHIR1.2
CHIR1.8
CHIR2.4
FOXA2 OTX2
B
FOXA2 OTX2
% of Total cells
100.0
80.0
60.0
40.0
20.0
0.0
0
0.6
1.2
1.8
2.4
Concentration of CHIR
C
CHIR1.2+IWR
CHIR1.5+IWR
CHIR1.8+IWR
CHIR2.1+IWR
CHIR2.4+IWR
FOXA2 OTX2

Figure 2

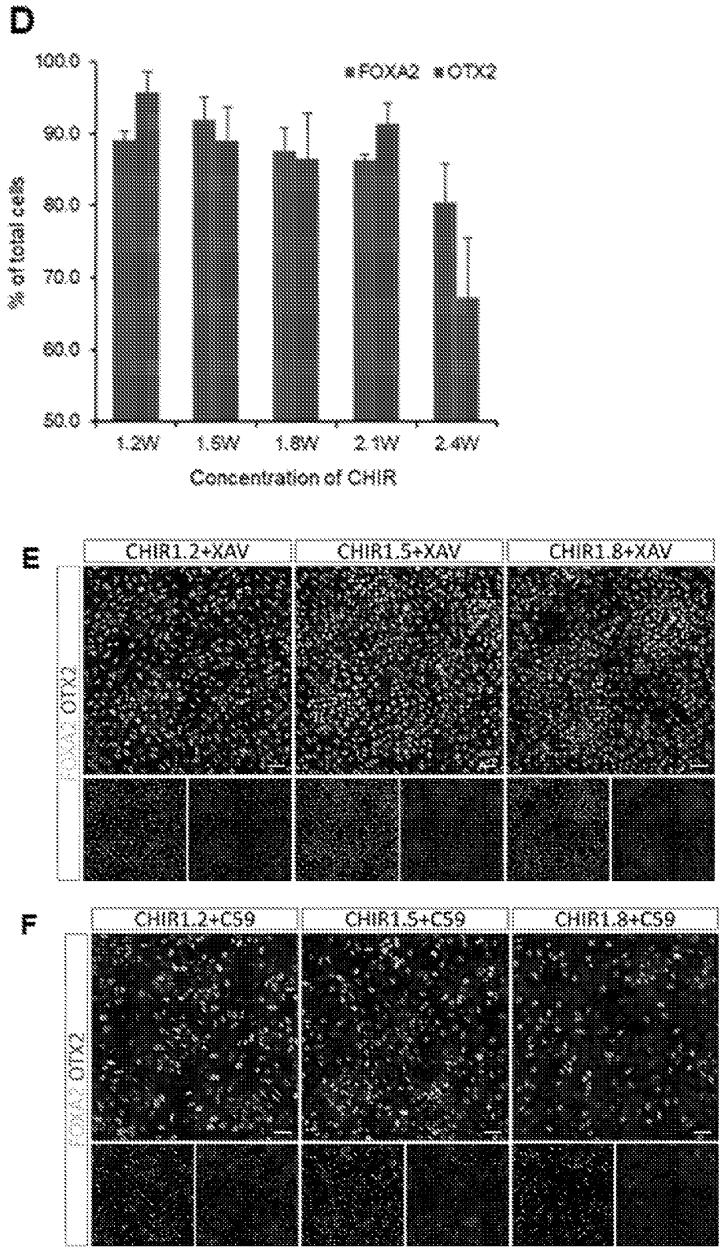


Figure 2 (continued)

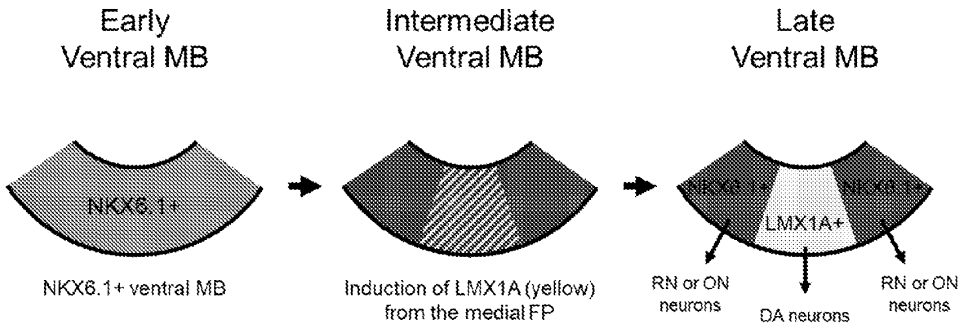

Early
Ventral MB
Intermediate
Ventral MB
Late
Ventral MB
NKX6.1+
LMX1A+
NKX6.1+ ventral MB
Induction of LMX1A (yellow)
from the medial FP
RN or ON
neurons
DA neurons
RN or ON
neurons

Figure 3

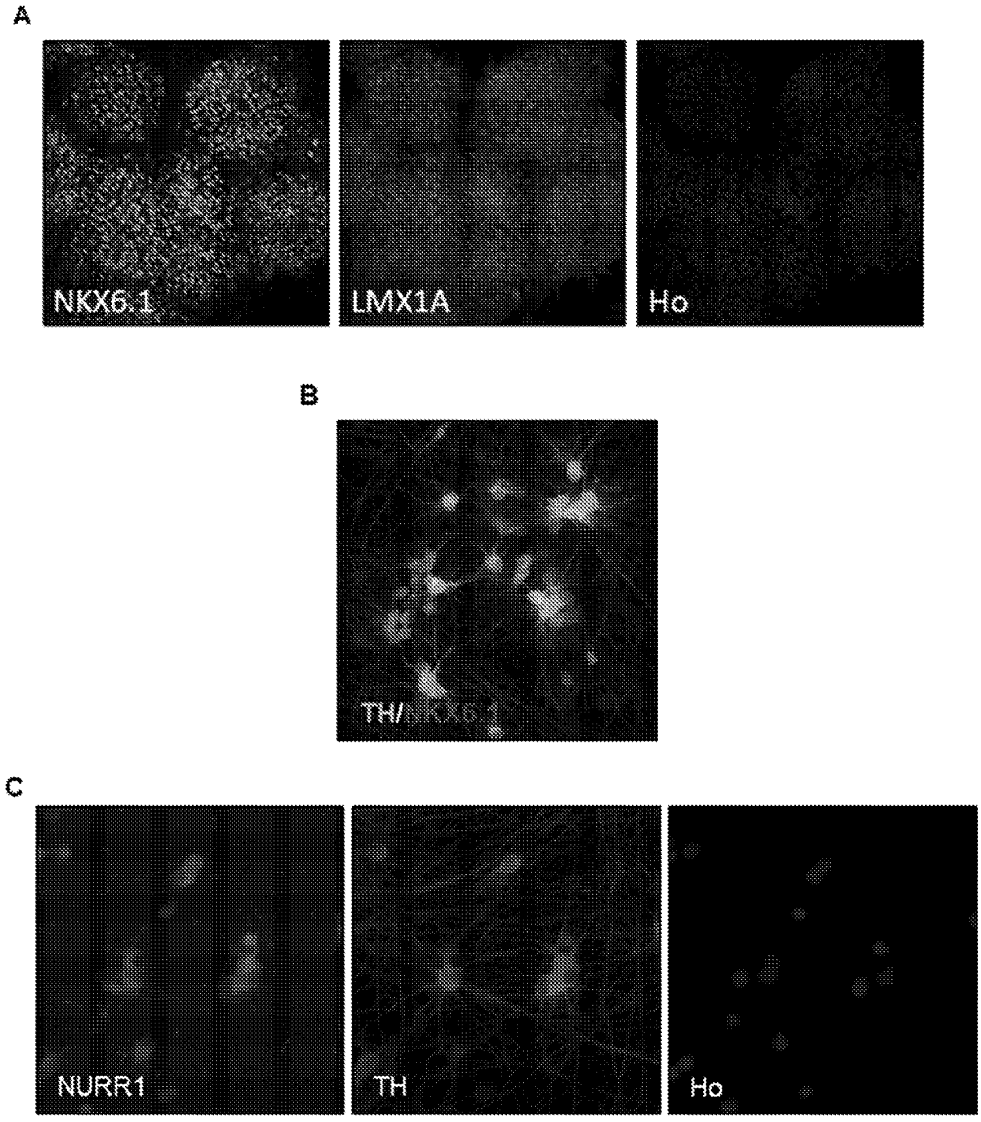


Figure 4

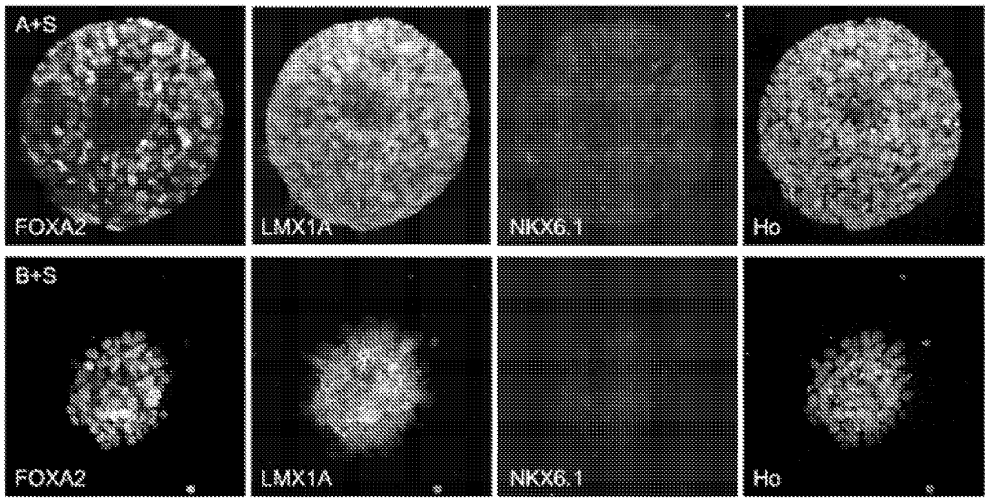


Figure 5

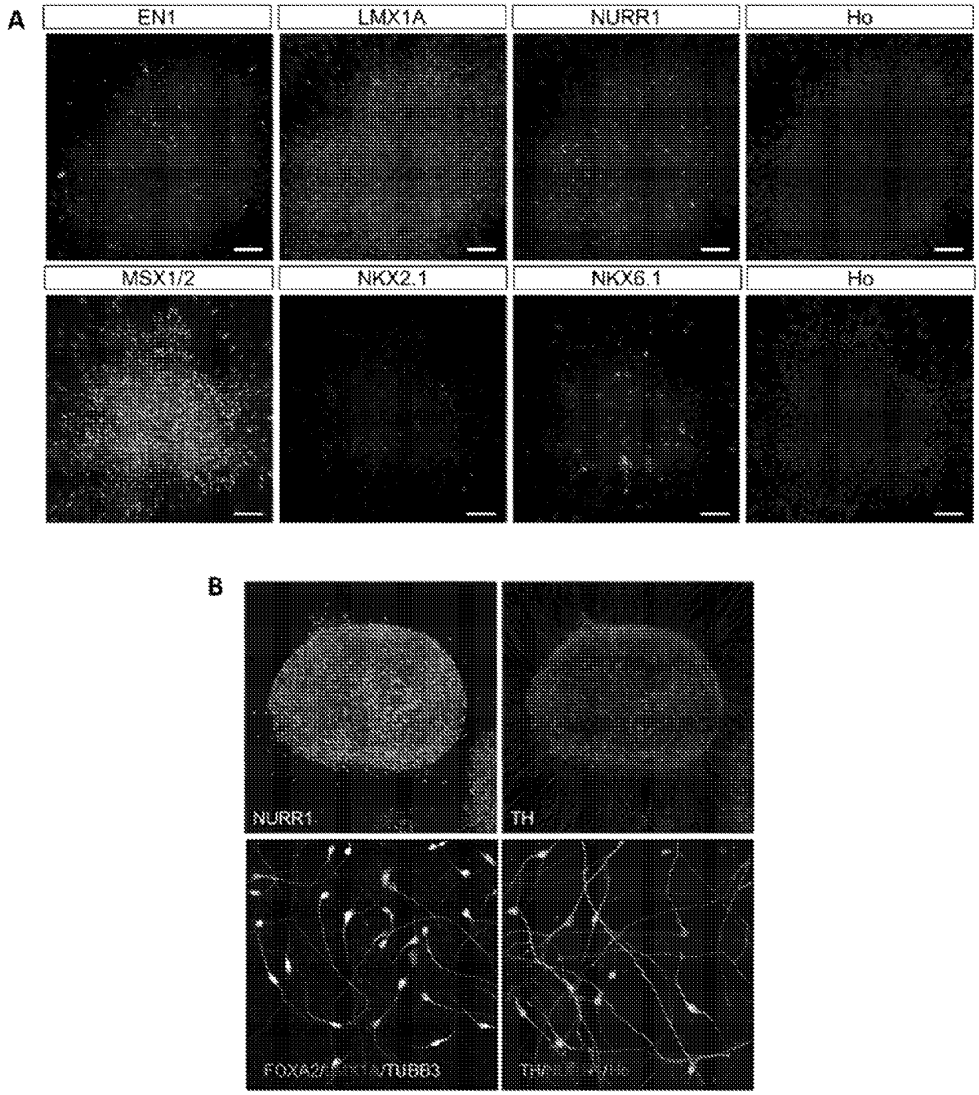


Figure 6

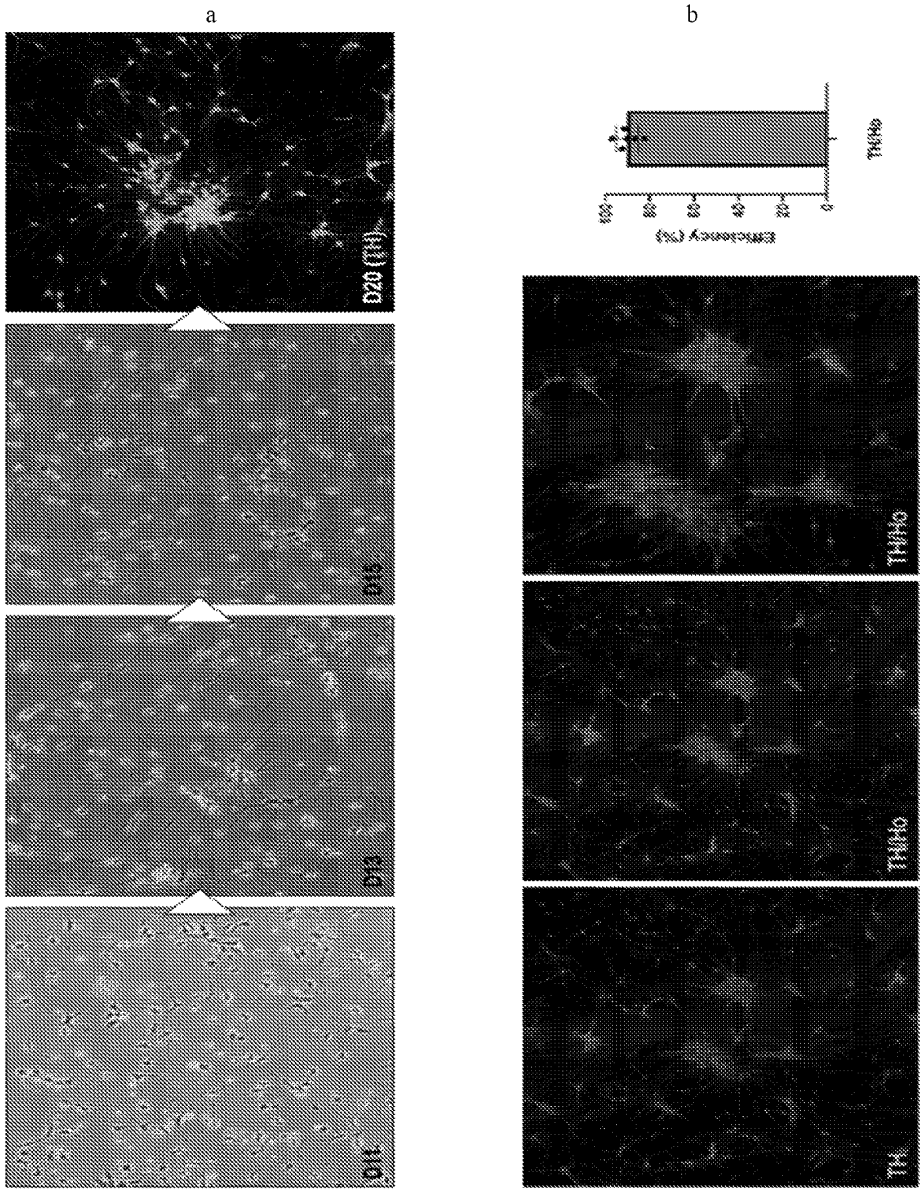


Figure 7

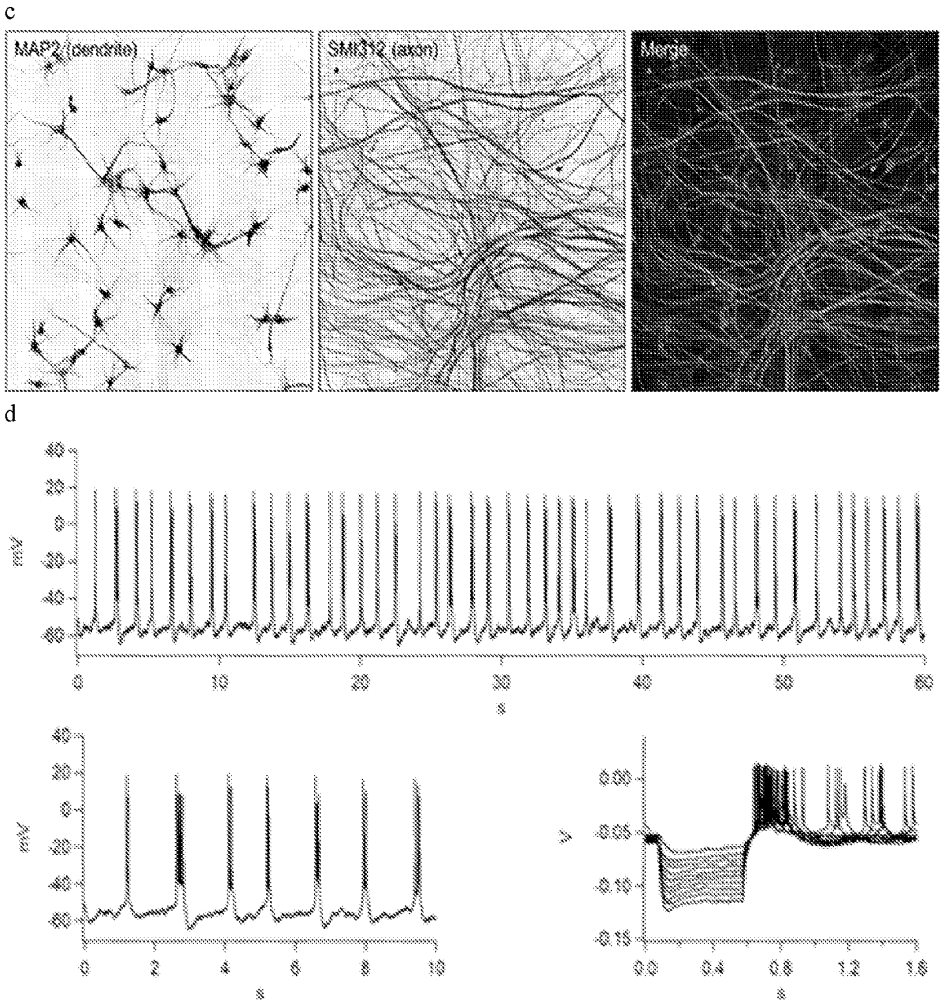


Figure 7 (continued)

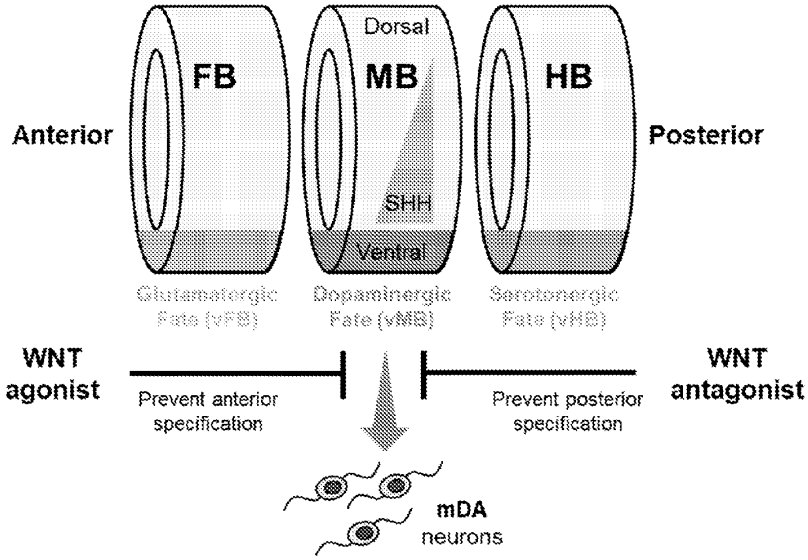

Dorsal
FB
MB
HB
Anterior
Posterior
SHH
Ventral
Glutamatergic Fate (vFB)
Dopaminergic Fate (vMB)
Serotonergic Fate (vHB)
WNT agonist
Prevent anterior specification
Prevent posterior specification
WNT antagonist
mDA neurons

Figure 8

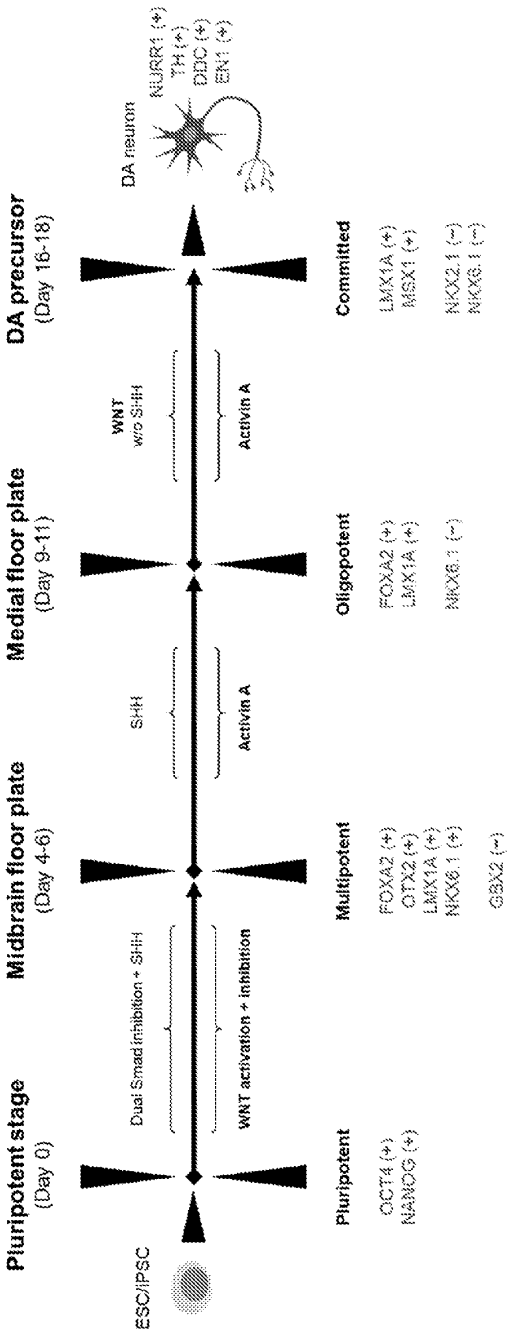

Pluripotent stage
(Day 0)
Midbrain floor plate
(Day 4-6)
Medial floor plate
(Day 9-11)
DA precursor
(Day 16-18)
ESC/iPSC
Dual Smad inhibition + SHH
WNT activation + inhibition
SHH
Activin A
WNT
w/o SHH
Activin A
DA neuron
NURR1 (+)
TH (+)
DDC (+)
EN1 (+)
Pluripotent
OCT4 (+)
NANOG (+)
Multipotent
FOXA2 (+)
OTX2 (+)
LMX1A (+)
NKX6.1 (+)
GBX2 (−)
Oligopotent
FOXA2 (+)
LMX1A (+)
NKX6.1 (−)
Committed
LMX1A (+)
MSX1 (+)
NKX2.1 (−)
NKX6.1 (−)

Figure 9