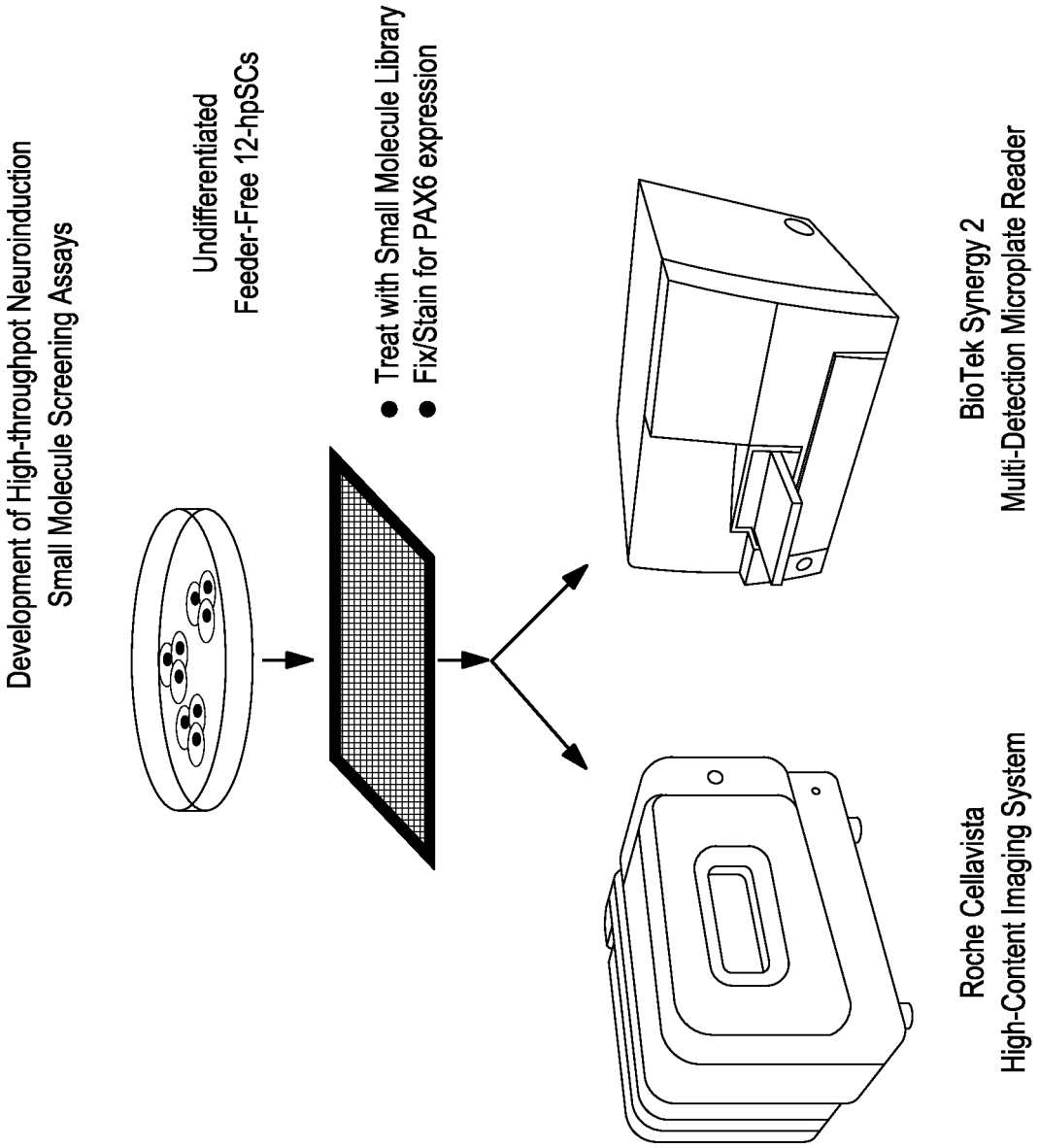

Development of High-throughpot Neuroinduction
Small Molecule Screening Assays
Undifferentiated
Feeder-Free 12-hpSCs
● Treat with Small Molecule Library
● Fix/Stain for PAX6 expression
BioTek Synergy 2
Multi-Detection Microplate Reader
Roche Cellavista
High-Content Imaging System

FIG. 1

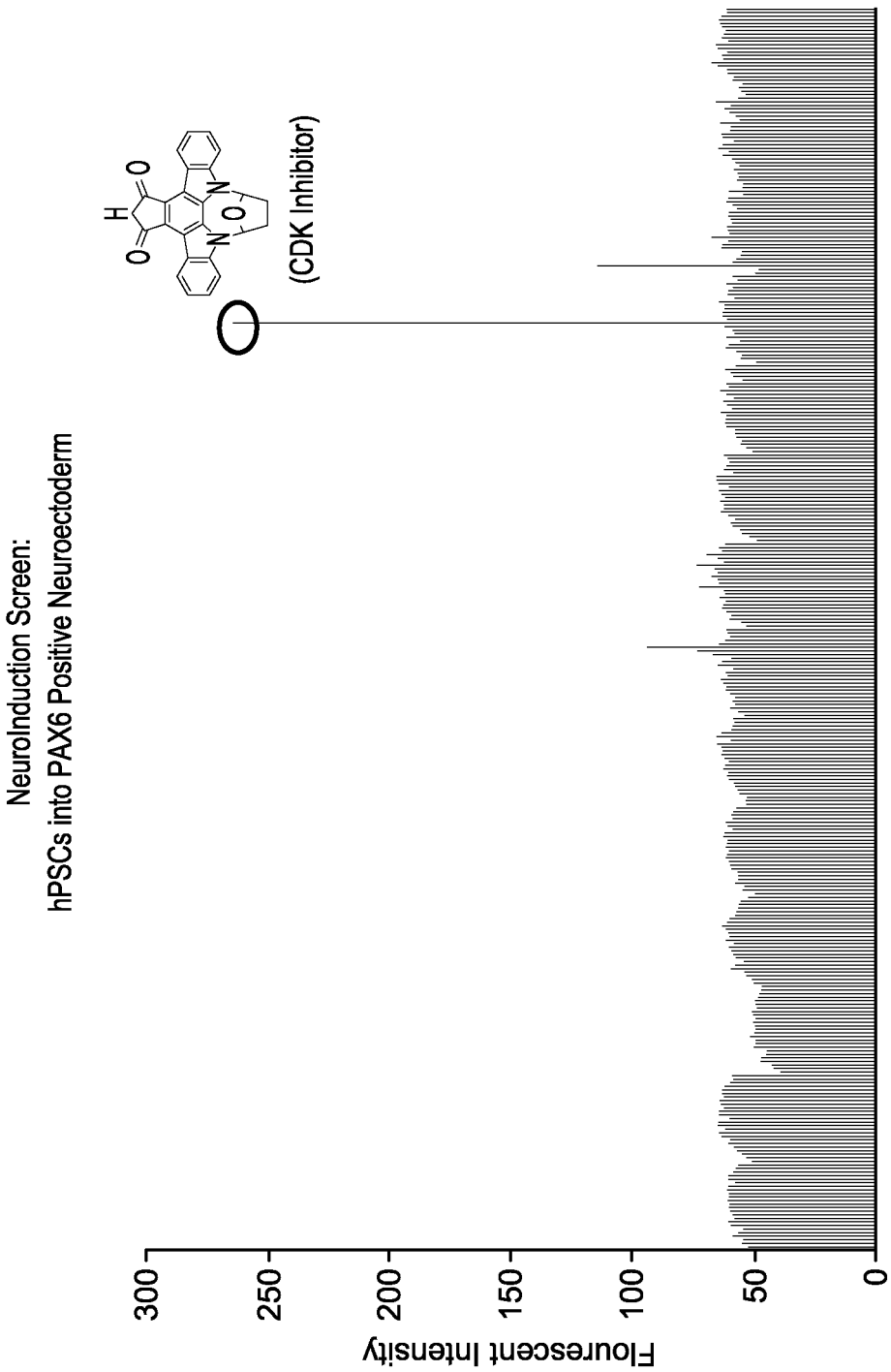

NeuroInduction Screen:
hPSCs into PAX6 Positive Neuroectoderm
(CDK Inhibitor)
Fluorescent Intensity
300
250
200
150
100
50
0
Tocriscreen Compound Library
(1120 Biologically Active Compounds)

FIG. 2

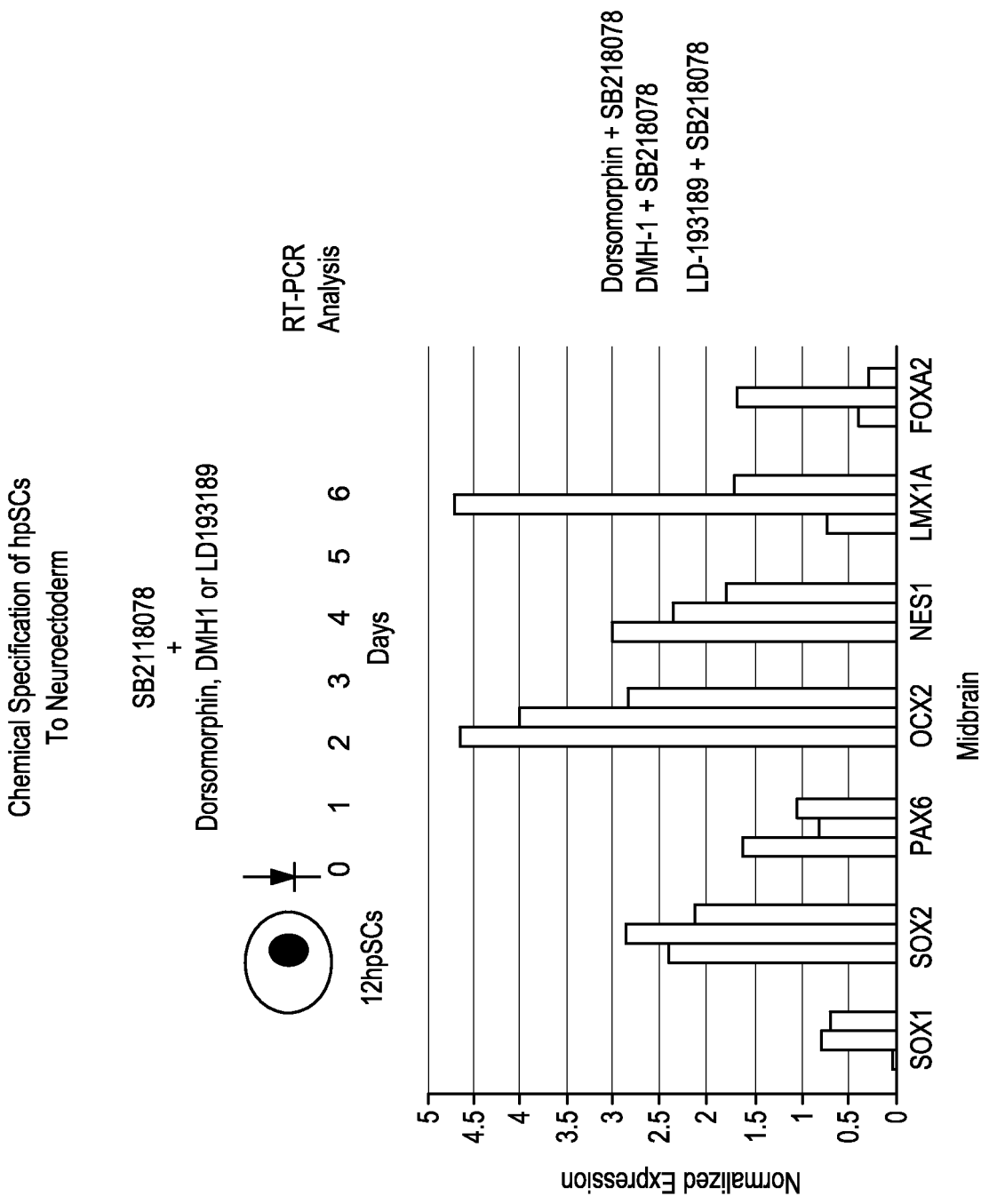

Chemical Specification of hpSCs
To Neuroectoderm
SB2118078
+
Dorsomorphin, DMH1 or LD193189
12hpSCs
0 1 2 3 4 5 6
Days
RT-PCR
Analysis
Dorsomorphin + SB218078
DMH-1 + SB218078
LD-193189 + SB218078
Normalized Expression
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5
SOX1 SOX2 PAX6 OCX2 NES1 LMX1A FOXA2
Midbrain

FIG. 3

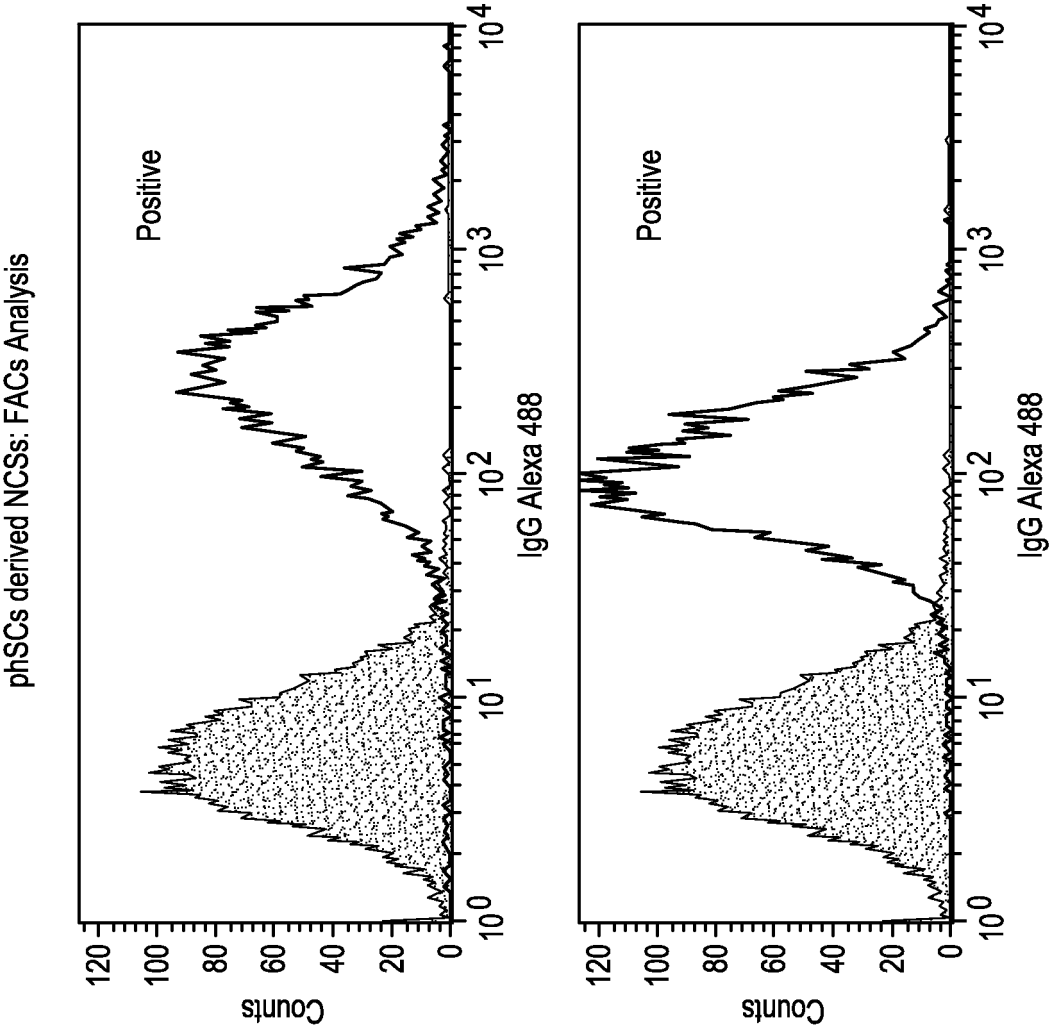

phSCs derived NCSs: FACs Analysis
Positive
Counts
IgG Alexa 488
Positive
Counts
IgG Alexa 488

FIG. 4

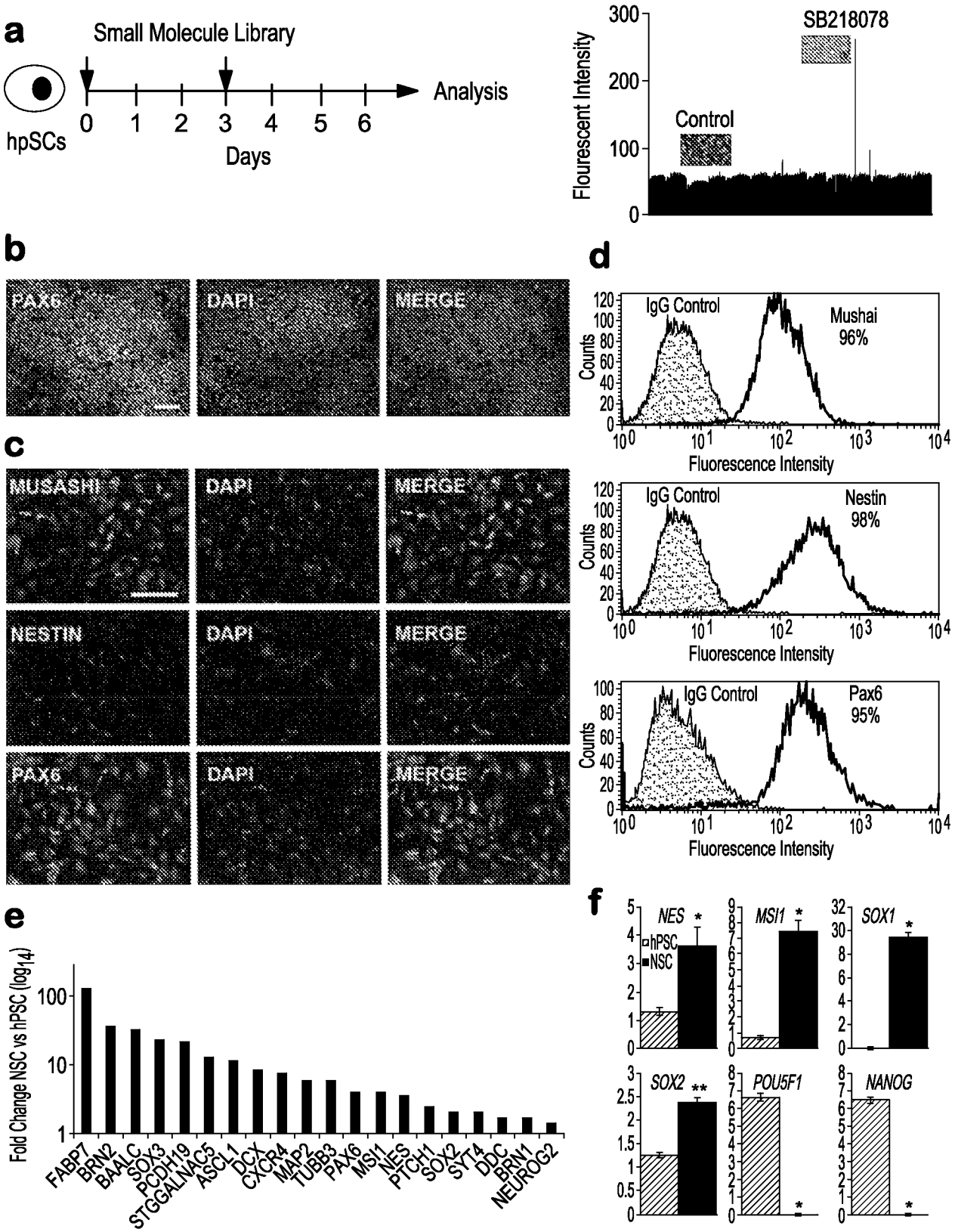

a
Small Molecule Library
hpSCs
0 1 2 3 4 5 6
Days
Analysis
Flourescent Intensity
300
200
100
0
SB218078
Control
b
PAX6
DAPI
MERGE
c
MUSASHI
DAPI
MERGE
NESTIN
DAPI
MERGE
PAX6
DAPI
MERGE
d
IgG Control
Mushai
96%
Counts
Fluorescence Intensity
IgG Control
Nestin
98%
Counts
Fluorescence Intensity
IgG Control
Pax6
95%
Counts
Fluorescence Intensity
e
Fold Change NSC vs hPSC (log14)
100
10
1
FABP7
BRN2
BAALC
SOX3
PCDH19
STGGALNAC5
ASCL1
DCX
CXCR4
MAP2
TUBB3
PAX6
MSI1
NES
PTCH1
SOX2
SYT4
DDC
BRN1
NEUROG2
f
NES
hPSC
NSC
MSI1
SOX1
SOX2
POU5F1
NANOG

FIG. 5

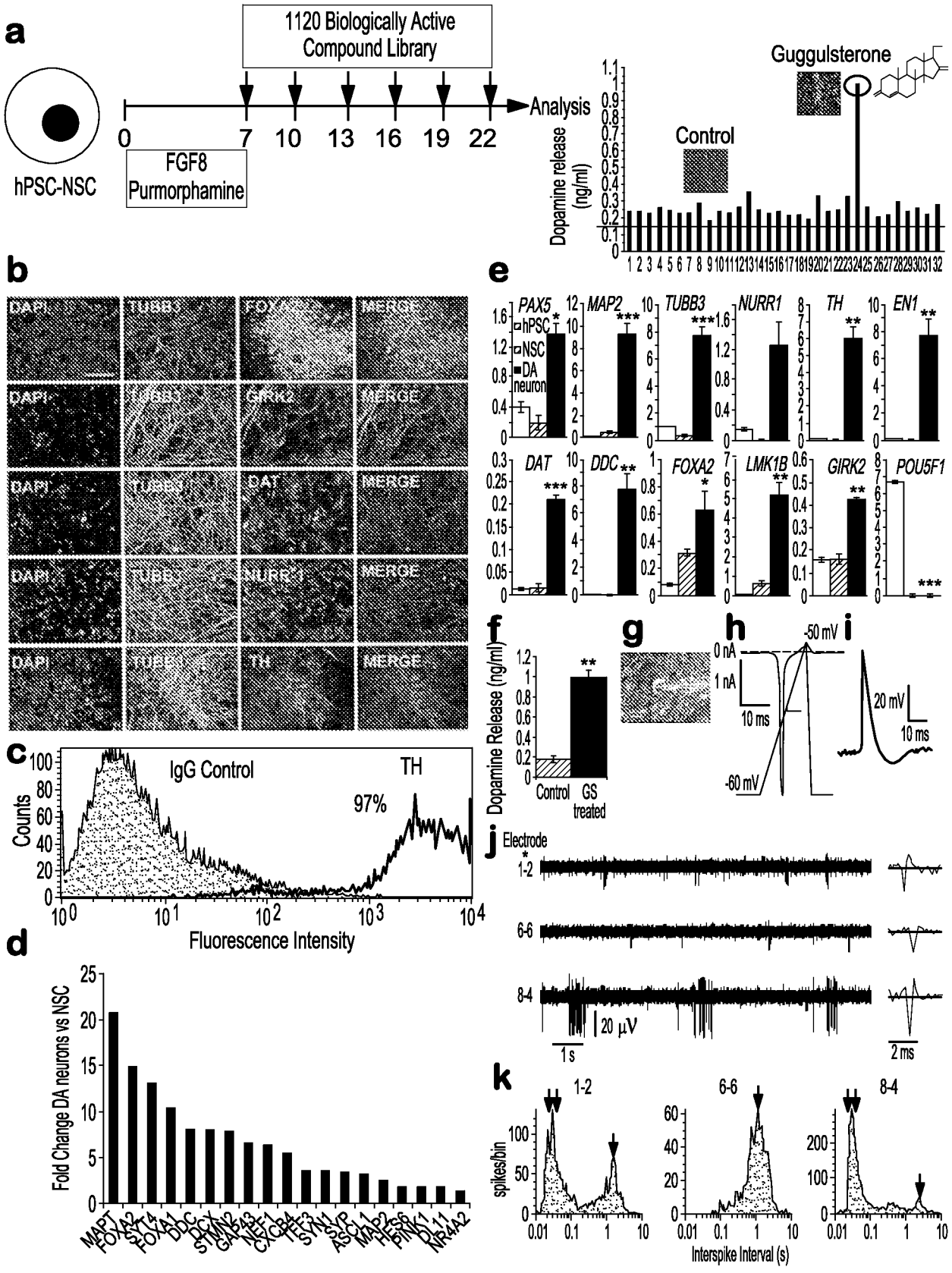

a
1120 Biologically Active Compound Library
hPSC-NSC
0
7
10
13
16
19
22
Analysis
FGF8
Purmorphamine
Guggulsterone
Control
Dopamine release (ng/ml)
b
DAPI
TUBB3
FOXA2
MERGE
GIRK2
DAT
NURR1
TH
c
IgG Control
TH
97%
Counts
Fluorescence Intensity
d
Fold Change DA neurons vs NSC
MAPT
FOXA2
SYT4
FOXA1
DDC
DCX
STMN2
GAP43
NEF1
CXCR4
TEF3
SYN1
SYP
ASCL1
MAP2
HES6
PINK1
DLL1
NR4A2
e
PAX5
MAP2
TUBB3
NURR1
TH
EN1
hPSC
NSC
DA neuron
DAT
DDC
FOXA2
LMK1B
GIRK2
POU5F1
f
Dopamine Release (ng/ml)
Control
GS treated
g
h
-50 mV
0 nA
1 nA
10 ms
-60 mV
i
20 mV
10 ms
j
Electrode
1-2
6-6
8-4
20 μV
1 s
2 ms
k
1-2
6-6
8-4
spikes/bin
Interspike Interval (s)

FIG. 6