Fig. 1

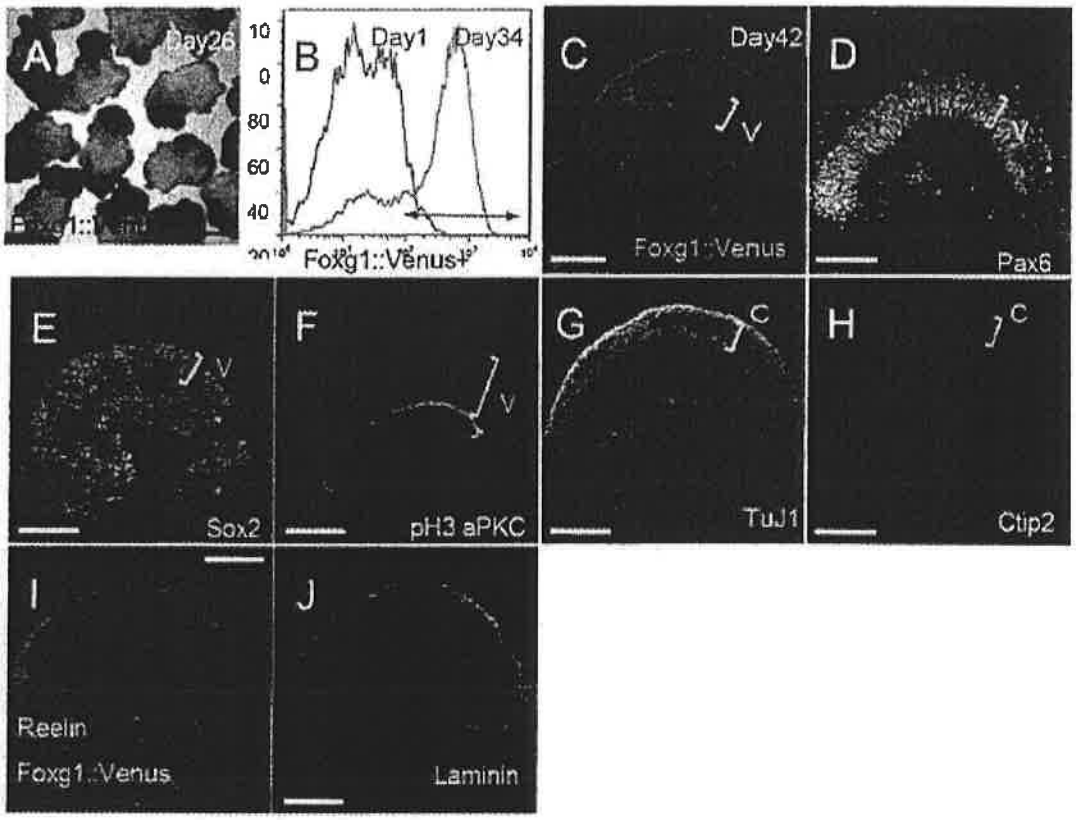

A
Day26
B
Day1
Day34
10
0
80
60
40
Foxg1::Venus
C
Day42
V
Foxg1::Venus
D
V
Pax6
E
V
Sox2
F
V
pH3 aPKC
G
C
TuJ1
H
C
Ctip2
I
Reelin
Foxg1::Venus
J
Laminin

Fig. 2

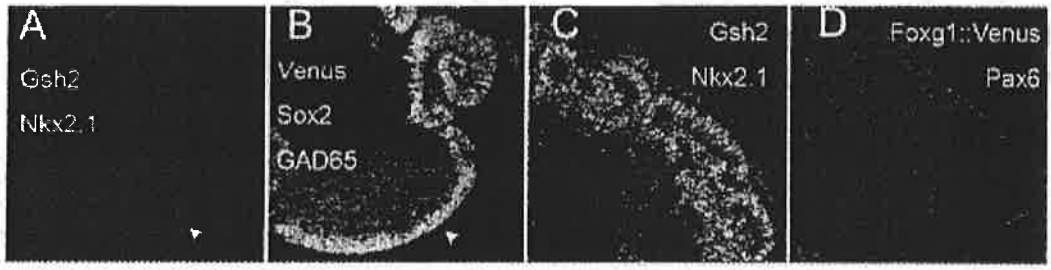

A
Gsh2
Nkx2.1
B
Venus
Sox2
GAD65
C
Gsh2
Nkx2.1
D
Foxg1::Venus
Pax6

Fig. 3

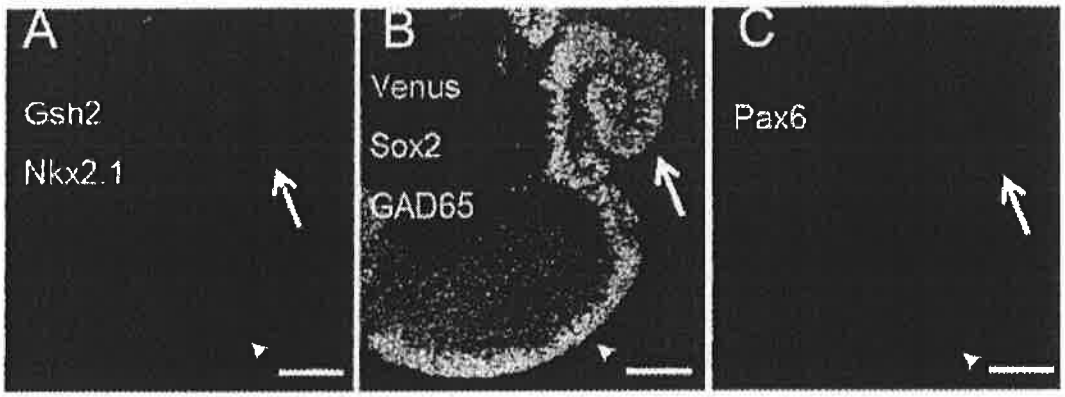


Fig. 4

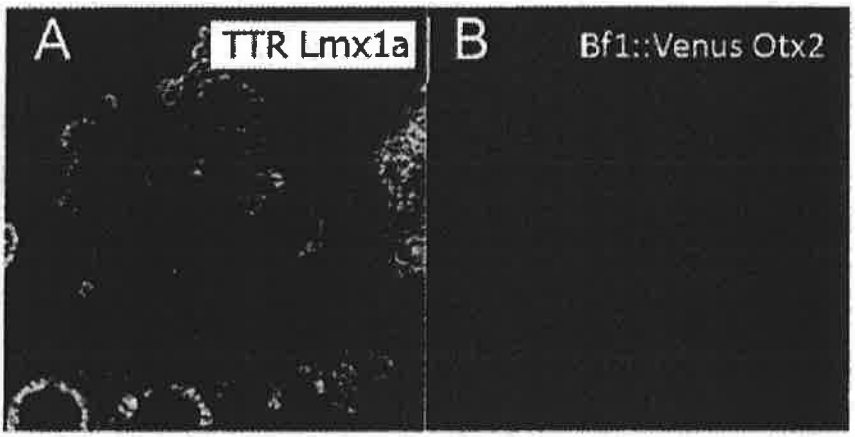


Fig. 5

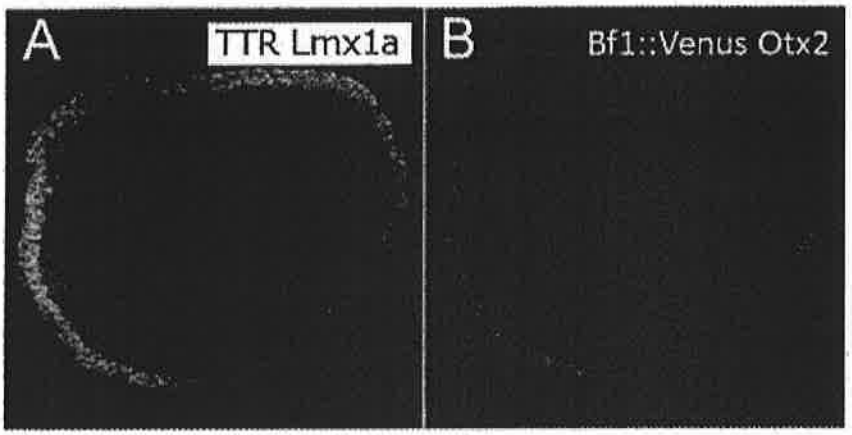


Fig. 6

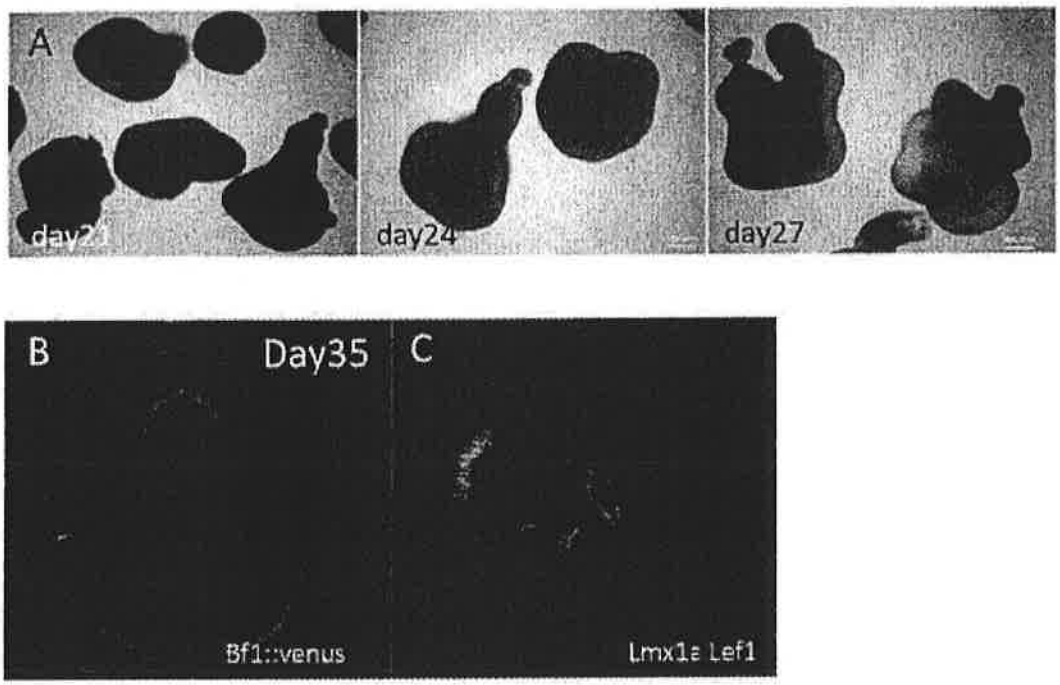


Fig. 7

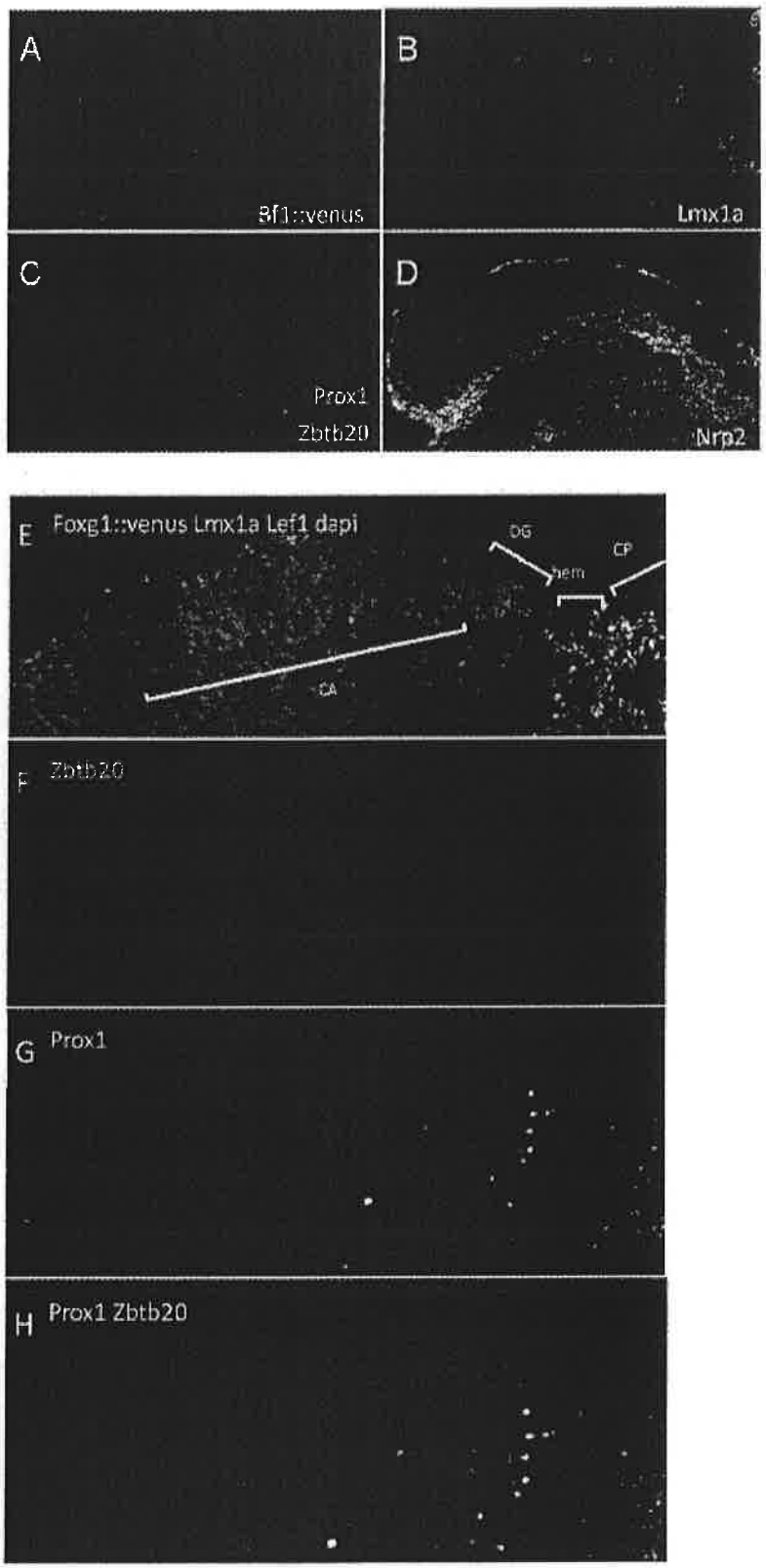

A
Bf1::venus
B
Lmx1a
C
Prox1
Zbtb20
D
Nrp2
E
Foxg1::venus Lmx1a Lef1 dapi
DG
hem
CP
CA
F
Zbtb20
G
Prox1
H
Prox1 Zbtb20

Fig. 8

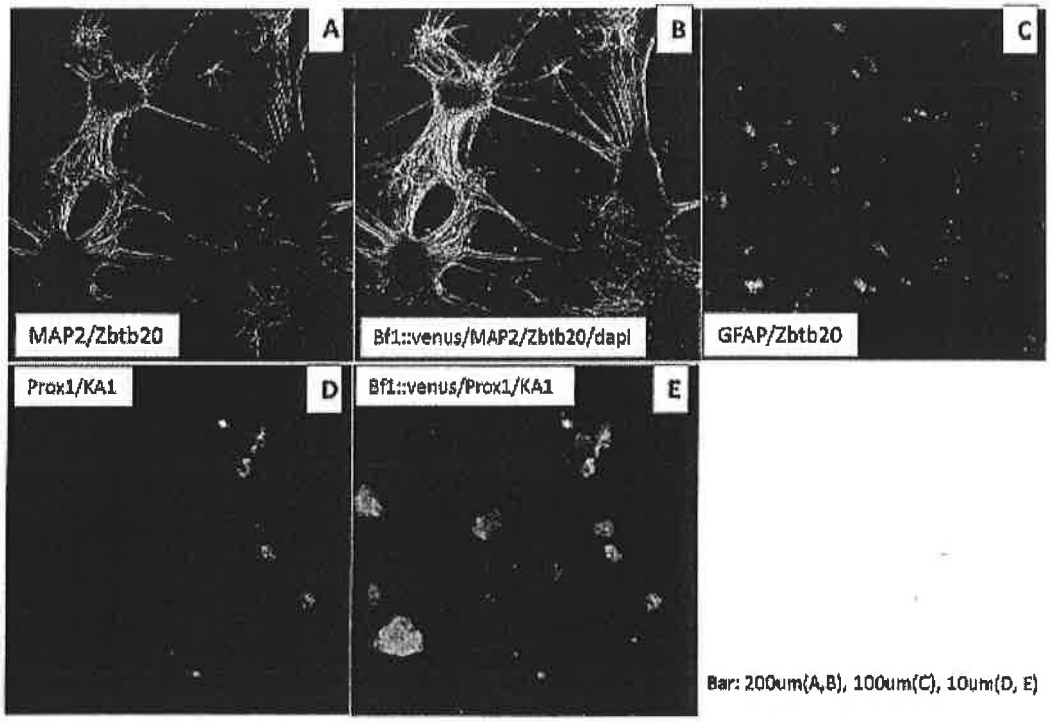

A
MAP2/Zbtb20
B
Bf1::venus/MAP2/Zbtb20/dapi
C
GFAP/Zbtb20
Prox1/KA1
D
Bf1::venus/Prox1/KA1
E
Bar: 200um(A,B), 100um(C), 10um(D, E)

Fig. 9

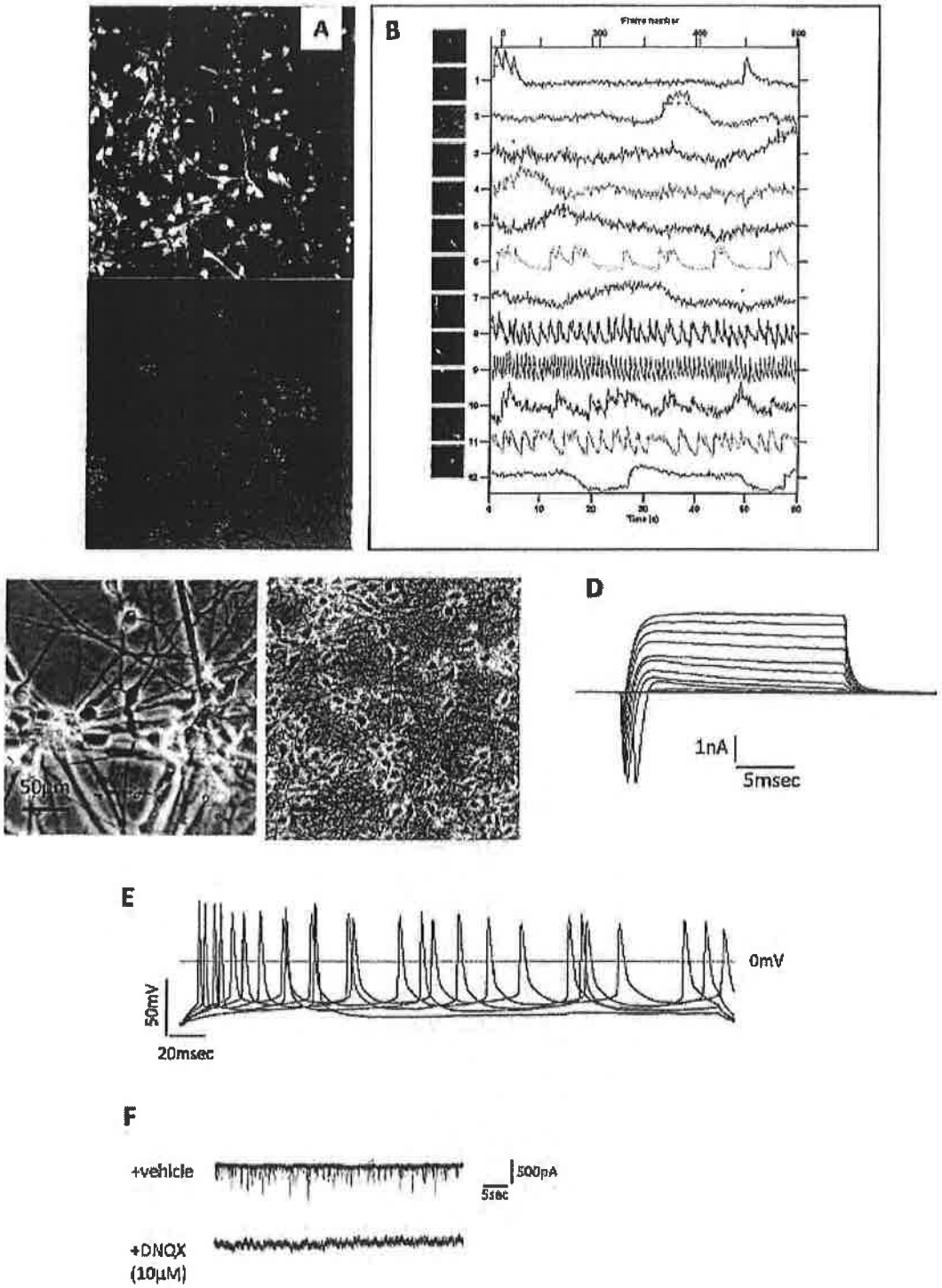

A
B
50μm
D
1nA
5msec
E
50mV
20msec
0mV
F
+vehicle
500pA
5sec
+DNQX
(10μM)

Fig. 10

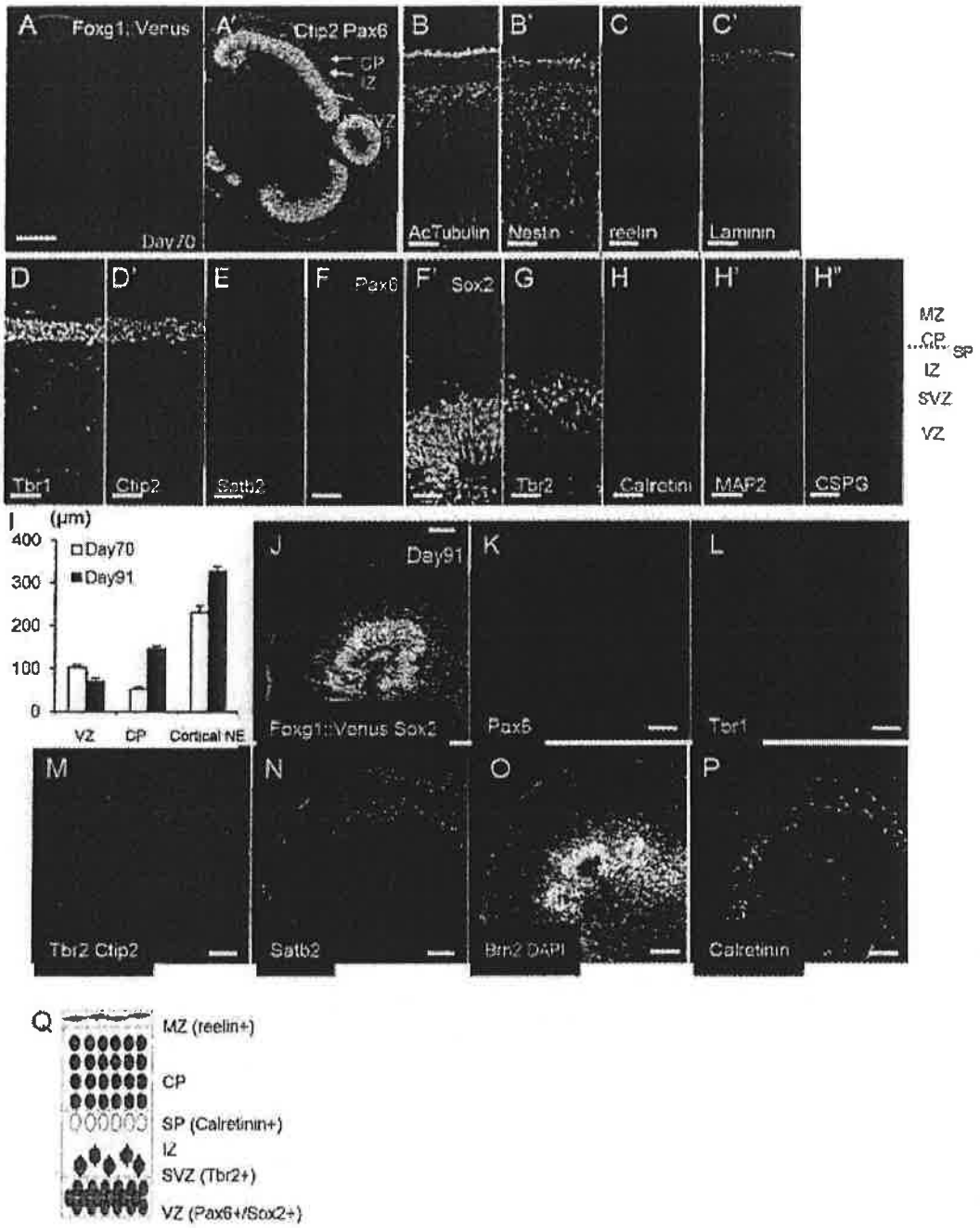
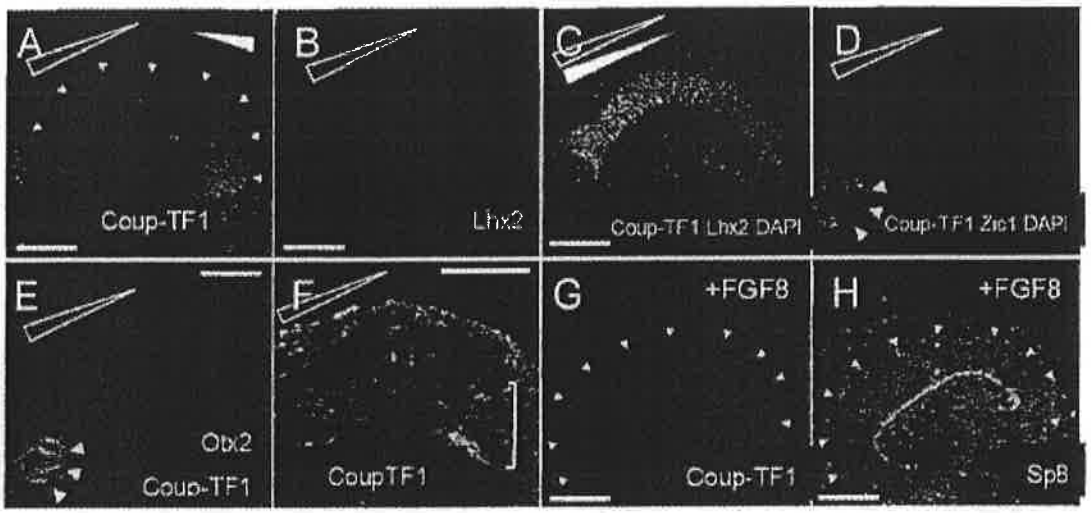

A Foxg1::Venus
A' Ctip2 Pax6
CP
IZ
VZ
Day70
B
B'
C
C'
AcTubulin
Nestin
reelin
Laminin
D
D'
E
F Pax6
F' Sox2
G
H
H'
H"
Tbr1
Ctip2
Satb2
Tbr2
Calretini
MAP2
CSPG
MZ
CP
SP
IZ
SVZ
VZ
I (μm)
400
300
200
100
0
Day70
Day91
VZ
CP
Cortical NE
J
Day91
Foxg1::Venus Sox2
K
Pax6
L
Tbr1
M
Tbr2 Ctip2
N
Satb2
O
Brn2 DAPI
P
Calretinin
Q
MZ (reelin+)
CP
SP (Calretinin+)
IZ
SVZ (Tbr2+)
VZ (Pax6+/Sox2+)

Fig. 11


A
Coup-TF1
B
Lhx2
C
Coup-TF1 Lhx2 DAPI
D
Coup-TF1 Zic1 DAPI
E
Otx2
Coup-TF1
F
CoupTF1
G
+FGF8
Coup-TF1
H
+FGF8
Sp8

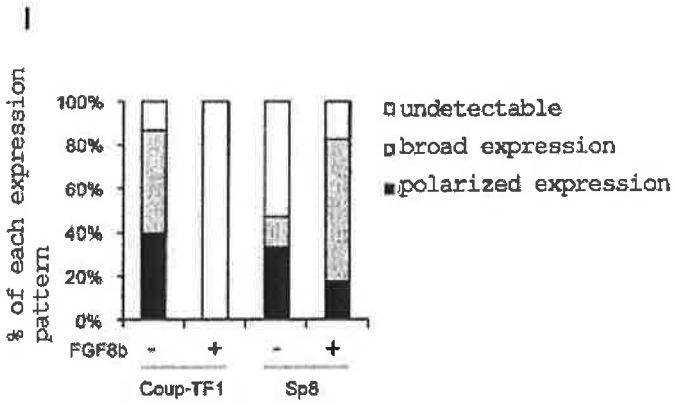

I
% of each expression pattern
100%
80%
60%
40%
20%
0%
FGF8b - + - +
Coup-TF1
Sp8
undetectable
broad expression
polarized expression

Fig. 12

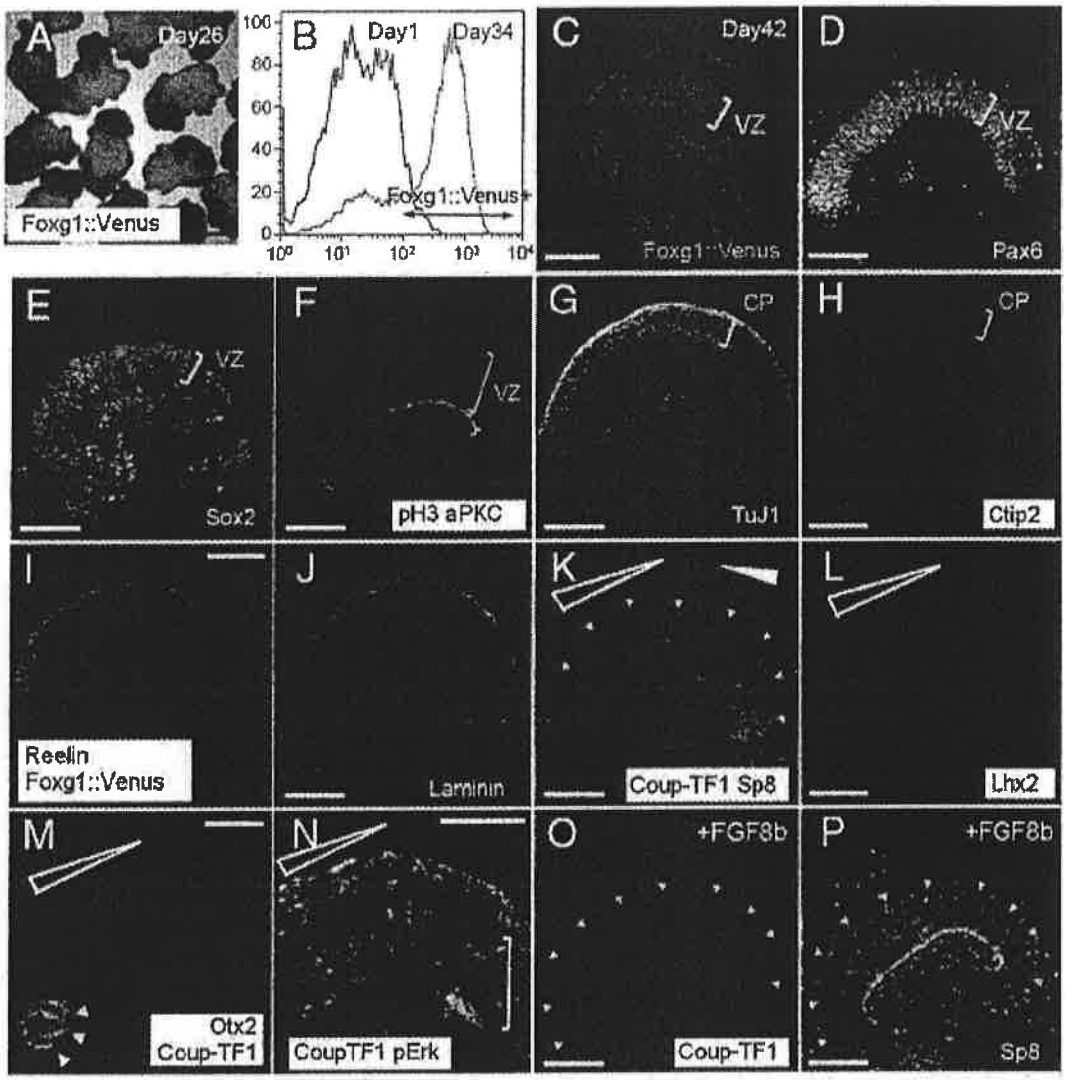

A
Day26
Foxg1::Venus
B
Day1
Day34
Foxg1::Venus+
100
80
60
40
20
0
10⁰
10¹
10²
10³
10⁴
C
Day42
VZ
Foxg1::Venus
D
VZ
Pax6
E
VZ
Sox2
F
VZ
pH3 aPKC
G
CP
TuJ1
H
CP
Ctip2
I
Reelin
Foxg1::Venus
J
Laminin
K
Coup-TF1 Sp8
L
Lhx2
M
Otx2
Coup-TF1
N
CoupTF1 pErk
O
+FGF8b
Coup-TF1
P
+FGF8b
Sp8

Fig. 13

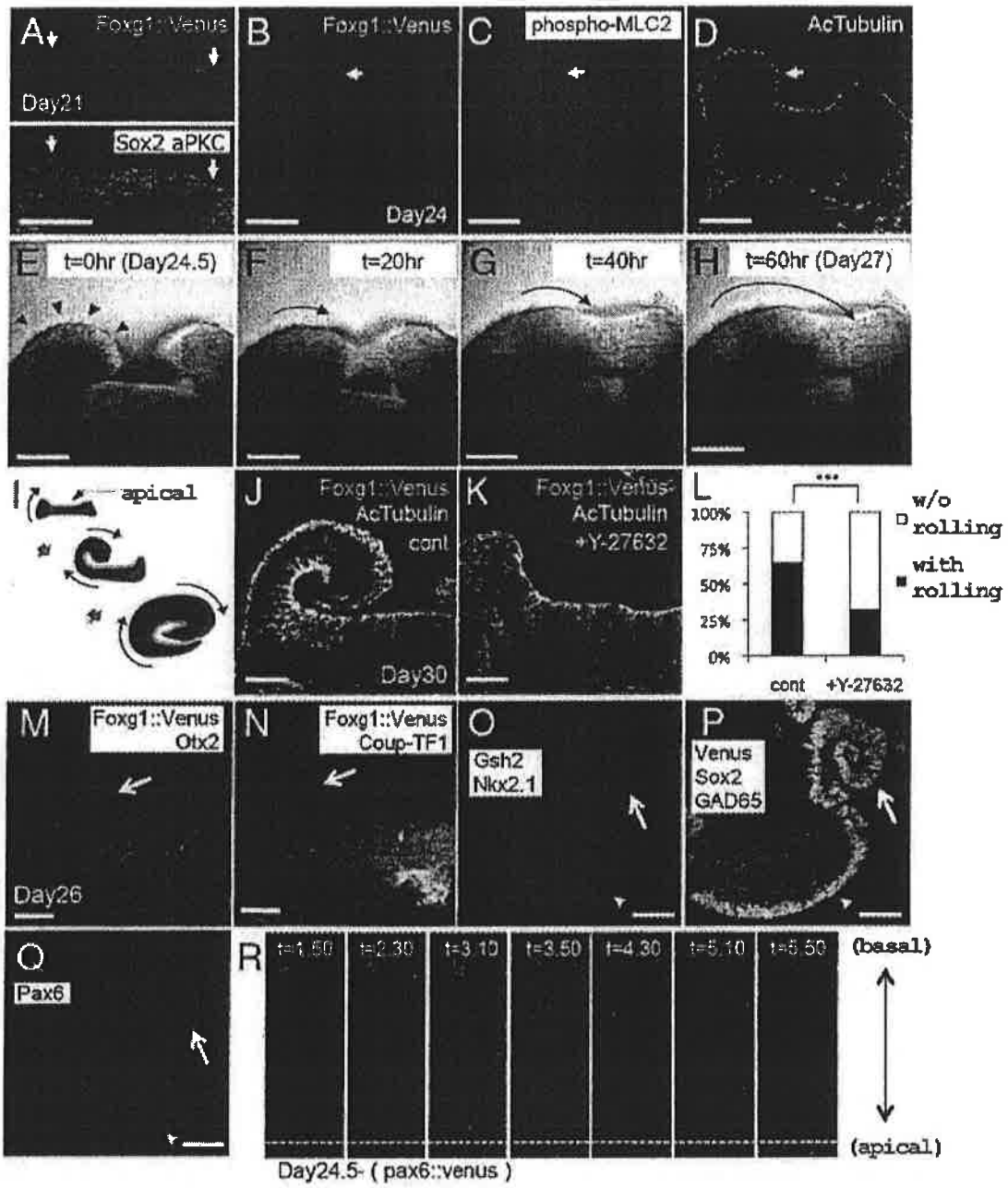

A
Foxg1::Venus
Day21
Sox2 aPKC
B
Foxg1::Venus
Day24
C
phospho-MLC2
D
AcTubulin
E
t=0hr (Day24.5)
F
t=20hr
G
t=40hr
H
t=60hr (Day27)
I
apical
J
Foxg1::Venus
AcTubulin
cont
Day30
K
Foxg1::Venus
AcTubulin
+Y-27632
L
***
100%
75%
50%
25%
0%
w/o rolling
with rolling
cont
+Y-27632
M
Foxg1::Venus
Otx2
Day26
N
Foxg1::Venus
Coup-TF1
O
Gsh2
Nkx2.1
P
Venus
Sox2
GAD65
Q
Pax6
R
t=1.50
t=2.30
t=3.10
t=3.50
t=4.30
t=5.10
t=5.50
(basal)
(apical)
Day24.5- (pax6::venus)

Fig. 14

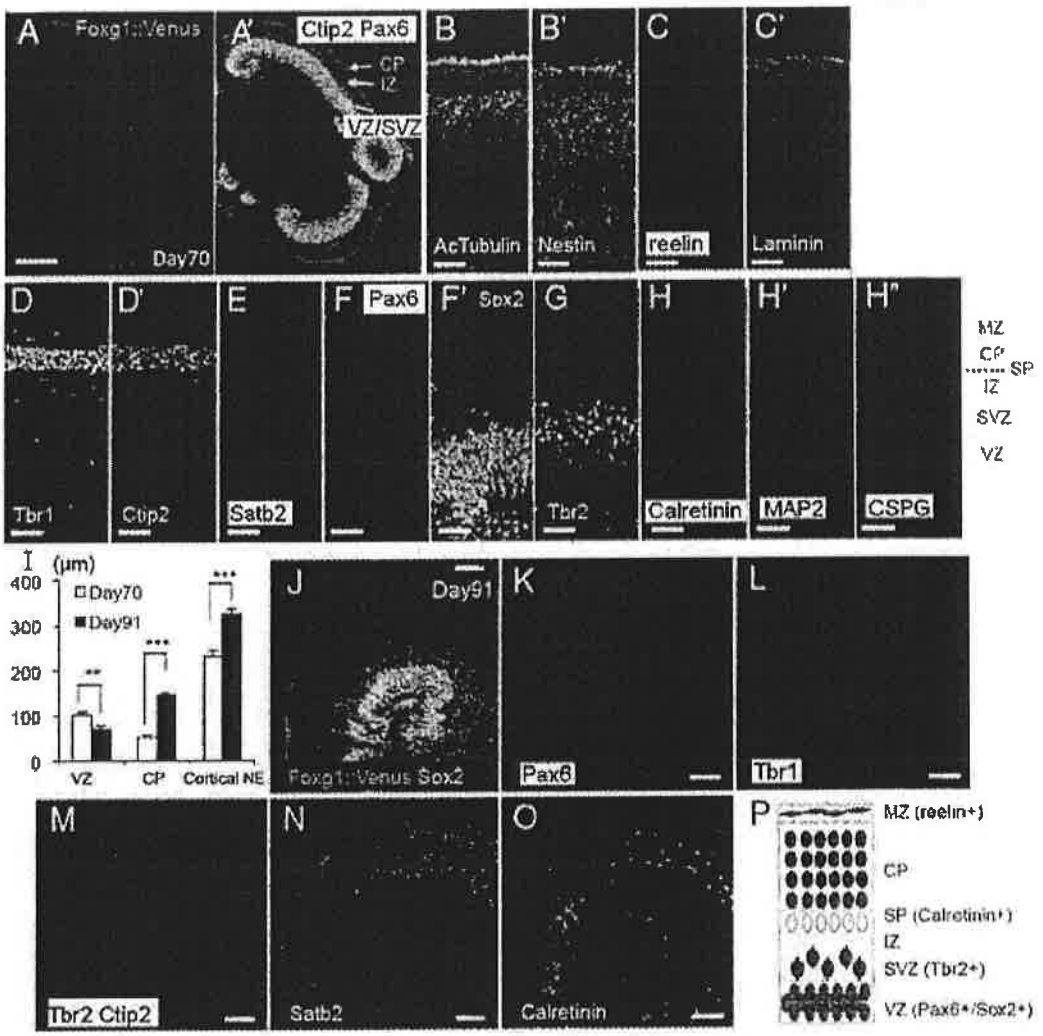

A
Foxg1::Venus
Day70
A'
Ctip2 Pax6
CP
IZ
VZ/SVZ
B
AcTubulin
B'
Nestin
C
reelin
C'
Laminin
D
Tbr1
D'
Ctip2
E
Satb2
F
Pax6
F'
Sox2
G
Tbr2
H
Calretinin
H'
MAP2
H"
CSPG
MZ
CP
SP
IZ
SVZ
VZ
I
(μm)
400
300
200
100
0
Day70
Day91
**
***
***
VZ
CP
Cortical NE
J
Day91
Foxg1::Venus Sox2
K
Pax6
L
Tbr1
M
Tbr2 Ctip2
N
Satb2
O
Calretinin
P
MZ (reelin+)
CP
SP (Calretinin+)
IZ
SVZ (Tbr2+)
VZ (Pax6+/Sox2+)

Fig. 15

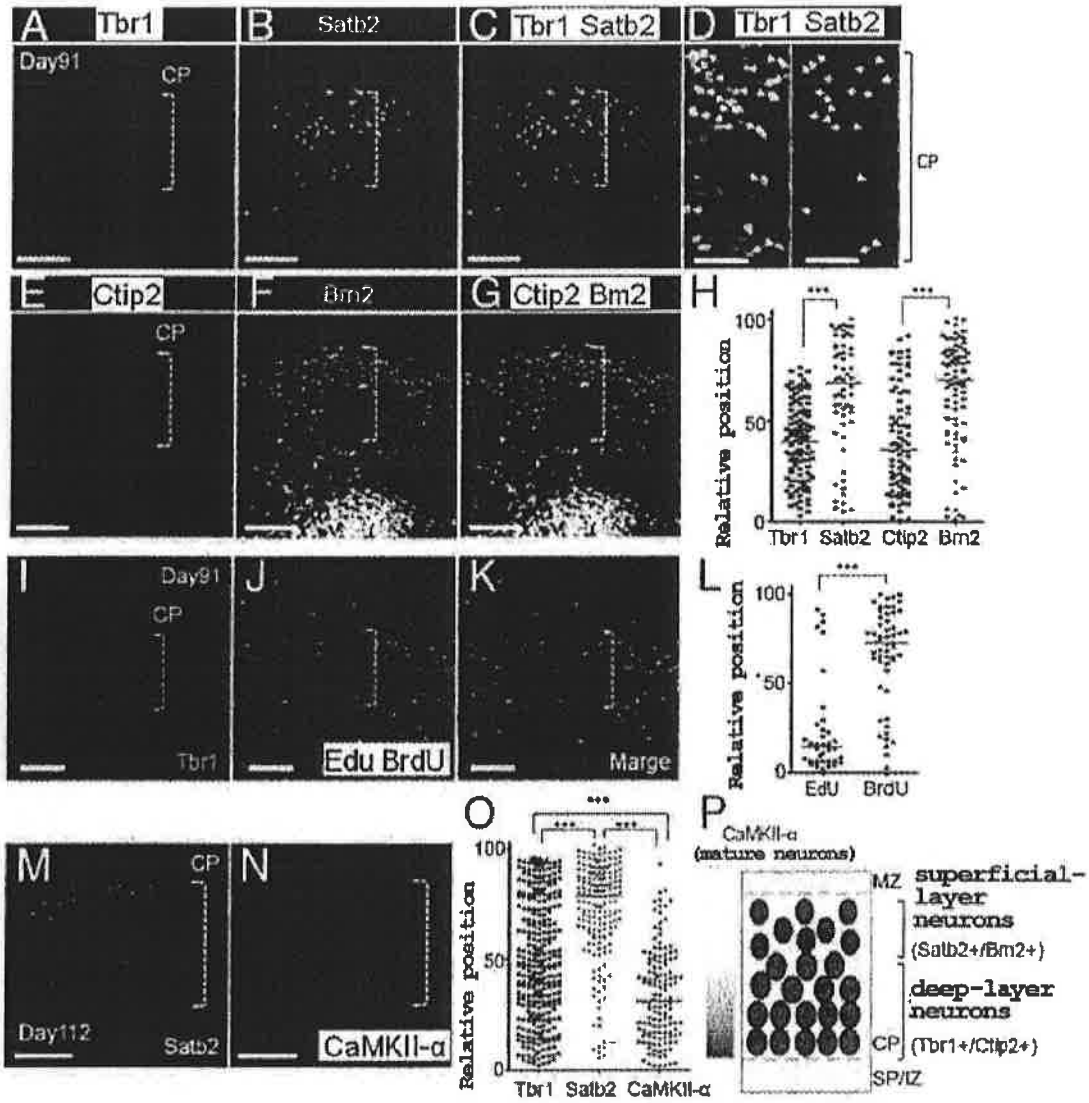

A Tbr1
Day91
CP
B Satb2
C Tbr1 Satb2
D Tbr1 Satb2
CP
E Ctip2
CP
F Bm2
G Ctip2 Bm2
H
Relative position
100
50
0
Tbr1 Satb2 Ctip2 Bm2
I Day91
CP
Tbr1
J
Edu BrdU
K
Marge
L
Relative position
100
50
0
EdU BrdU
M
CP
Day112
Satb2
N
CaMKII-α
O
Relative position
100
50
0
Tbr1 Satb2 CaMKII-α
P
CaMKII-α
(mature neurons)
MZ
superficial-layer neurons
(Satb2+/Bm2+)
deep-layer neurons
CP
(Tbr1+/Ctip2+)
SP/IZ

Fig. 16

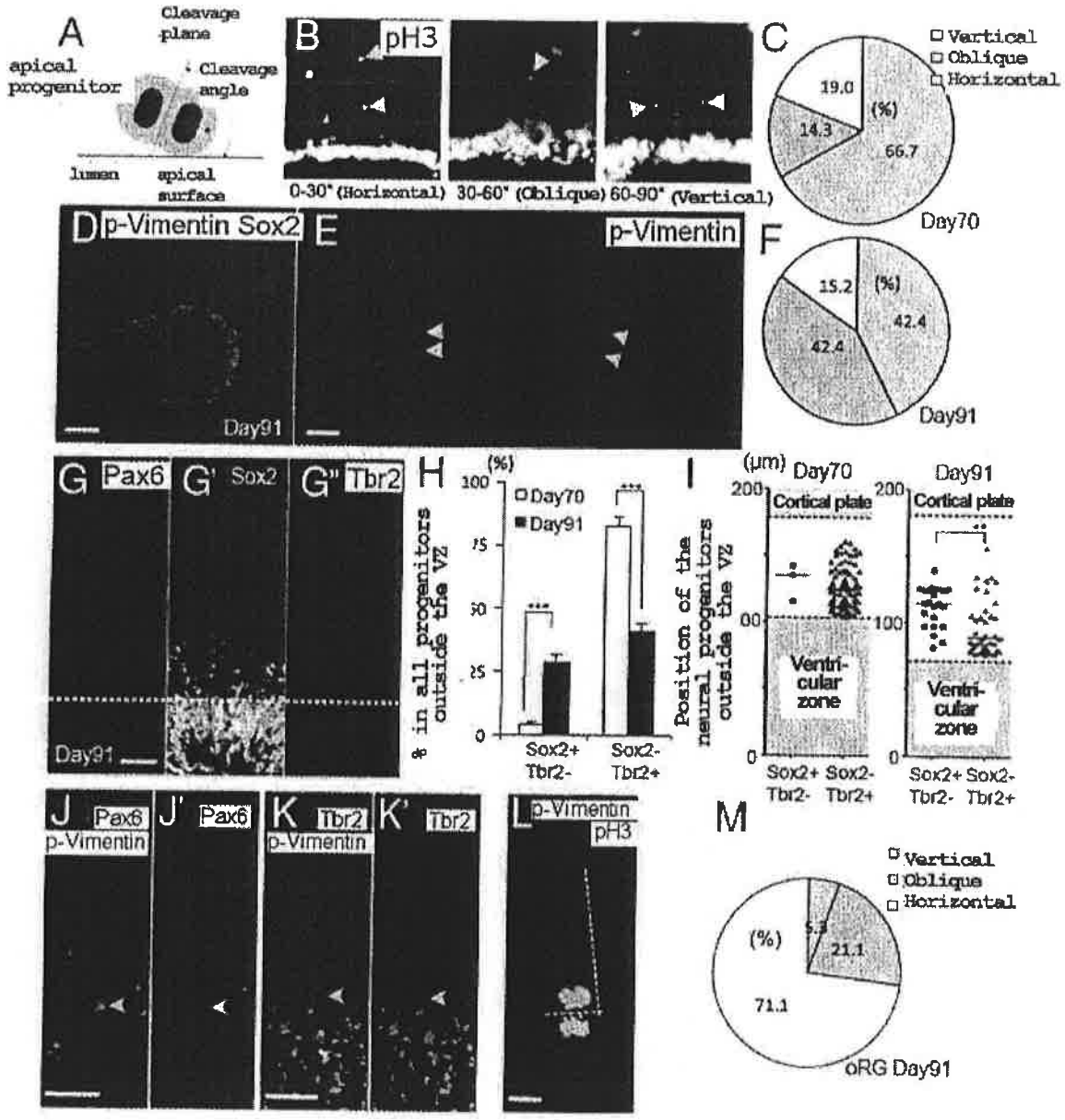

A
Cleavage plane
apical progenitor
Cleavage angle
lumen
apical surface
B
pH3
0-30° (Horizontal)
30-60° (Oblique)
60-90° (Vertical)
C
Vertical
Oblique
Horizontal
19.0
(%)
14.3
66.7
Day70
D
p-Vimentin Sox2
Day91
E
p-Vimentin
F
15.2
(%)
42.4
42.4
Day91
G
Pax6
G'
Sox2
G"
Tbr2
Day91
H
(%)
Day70
Day91
% in all progenitors outside the VZ
Sox2+ Tbr2-
Sox2- Tbr2+
I
(μm)
Day70
Day91
Cortical plate
Ventri-cular zone
Position of the neural progenitors outside the VZ
Sox2+ Tbr2-
Sox2- Tbr2+
J
Pax6
p-Vimentin
J'
Pax6
K
Tbr2
p-Vimentin
K'
Tbr2
L
p-Vimentin
pH3
M
Vertical
Oblique
Horizontal
(%)
21.1
71.1
oRG Day91

Fig. 17

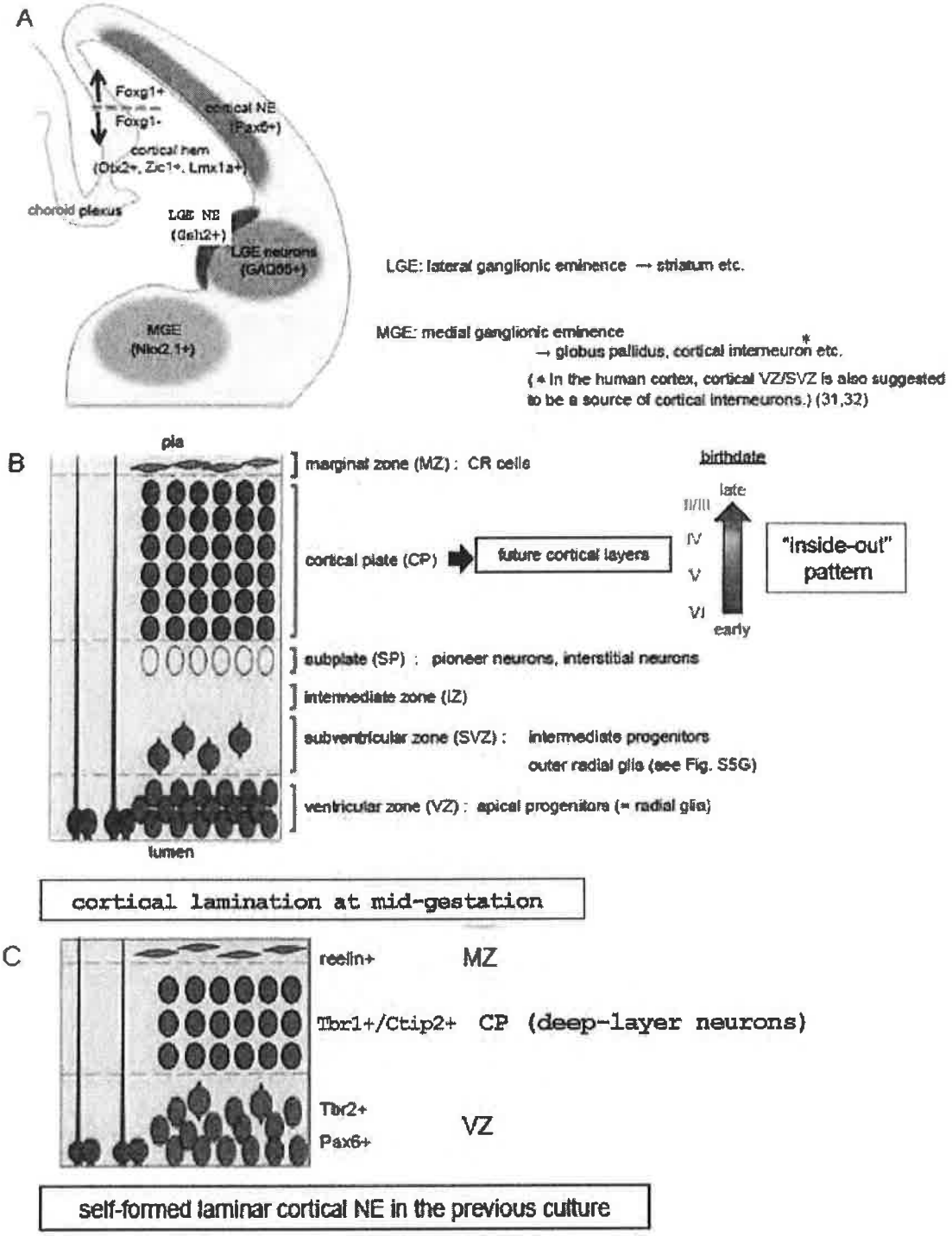

A
Foxg1+
Foxg1-
cortical NE
(Pax6+)
cortical hem
(Otx2+, Zic1+, Lmx1a+)
choroid plexus
LGE NE
(Dlx2+)
LGE neurons
(GAD65+)
MGE
(Nkx2.1+)
LGE: lateral ganglionic eminence → striatum etc.
MGE: medial ganglionic eminence
→ globus pallidus, cortical interneuron* etc.
(* In the human cortex, cortical VZ/SVZ is also suggested to be a source of cortical interneurons.) (31,32)
B
pia
marginal zone (MZ) : CR cells
cortical plate (CP)
future cortical layers
birthdate
late
II/III
IV
V
VI
early
"inside-out" pattern
subplate (SP) : pioneer neurons, interstitial neurons
intermediate zone (IZ)
subventricular zone (SVZ) : intermediate progenitors
outer radial glia (see Fig. S5G)
ventricular zone (VZ) : apical progenitors (= radial glia)
lumen
cortical lamination at mid-gestation
C
reelin+
MZ
Tbr1+/Ctip2+
CP (deep-layer neurons)
Tbr2+
Pax6+
VZ
self-formed laminar cortical NE in the previous culture

Fig. 18

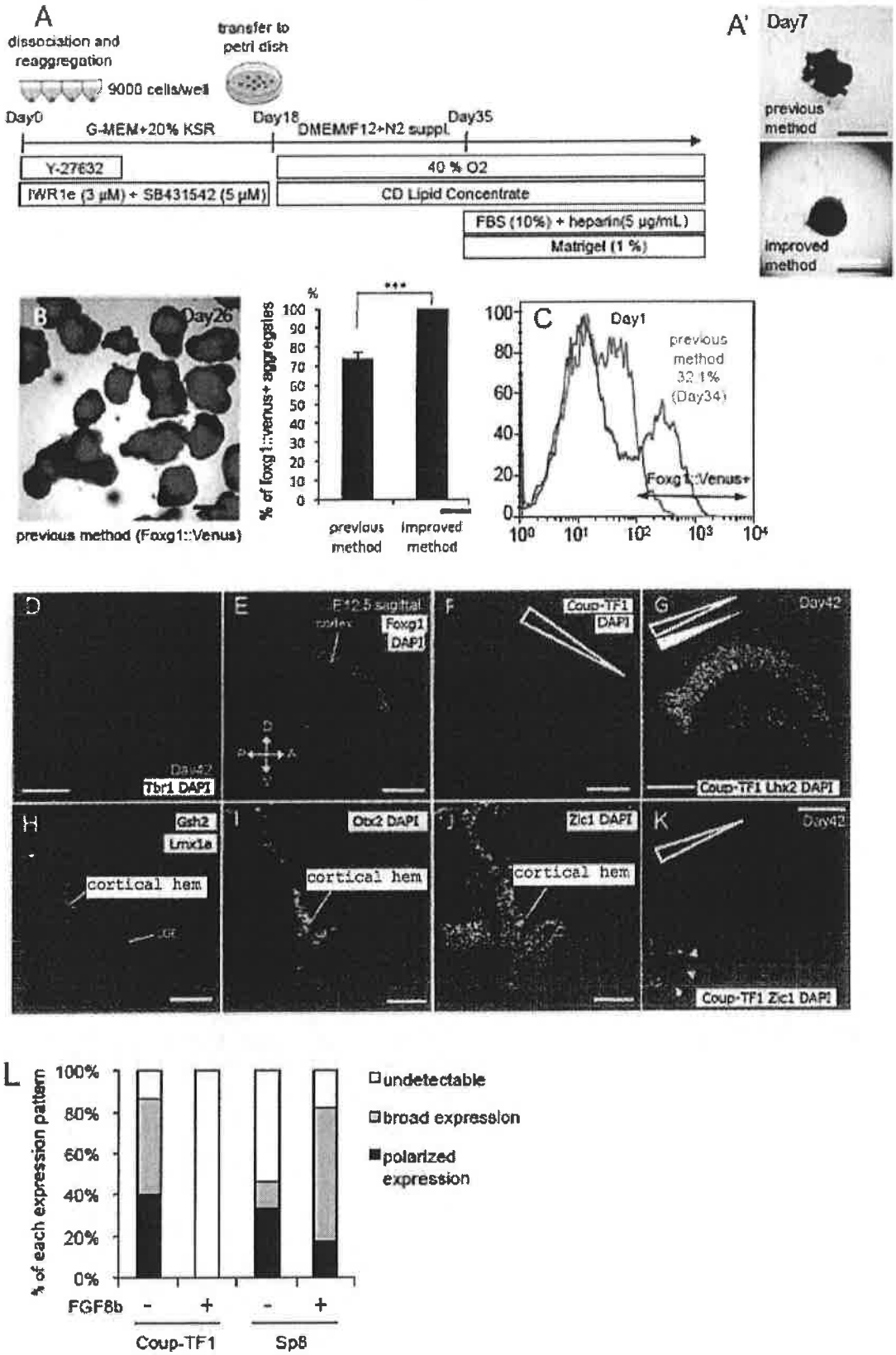

A
dissociation and reaggregation
transfer to petri dish
9000 cells/well
Day0
Day18
Day35
G-MEM+20% KSR
DMEM/F12+N2 suppl.
Y-27632
IWR1e (3 μM) + SB431542 (5 μM)
40 % O2
CD Lipid Concentrate
FBS (10%) + heparin(5 μg/mL)
Matrigel (1 %)
A'
Day7
previous method
improved method
B
Day26
previous method (Foxg1::Venus)
% of foxg1::venus+ aggregates
previous method
improved method
C
Day1
previous method
32.1%
(Day34)
Foxg1::Venus+
D
Tbr1 DAPI
E
E12.5 sagittal
Foxg1
DAPI
F
Coup-TF1
DAPI
G
Day42
Coup-TF1 Lhx2 DAPI
H
Gsh2
Lmx1a
cortical hem
I
Otx2 DAPI
cortical hem
J
Zic1 DAPI
cortical hem
K
Day42
Coup-TF1 Zic1 DAPI
L
% of each expression pattern
undetectable
broad expression
polarized expression
FGF8b
Coup-TF1
Sp8

Fig. 19

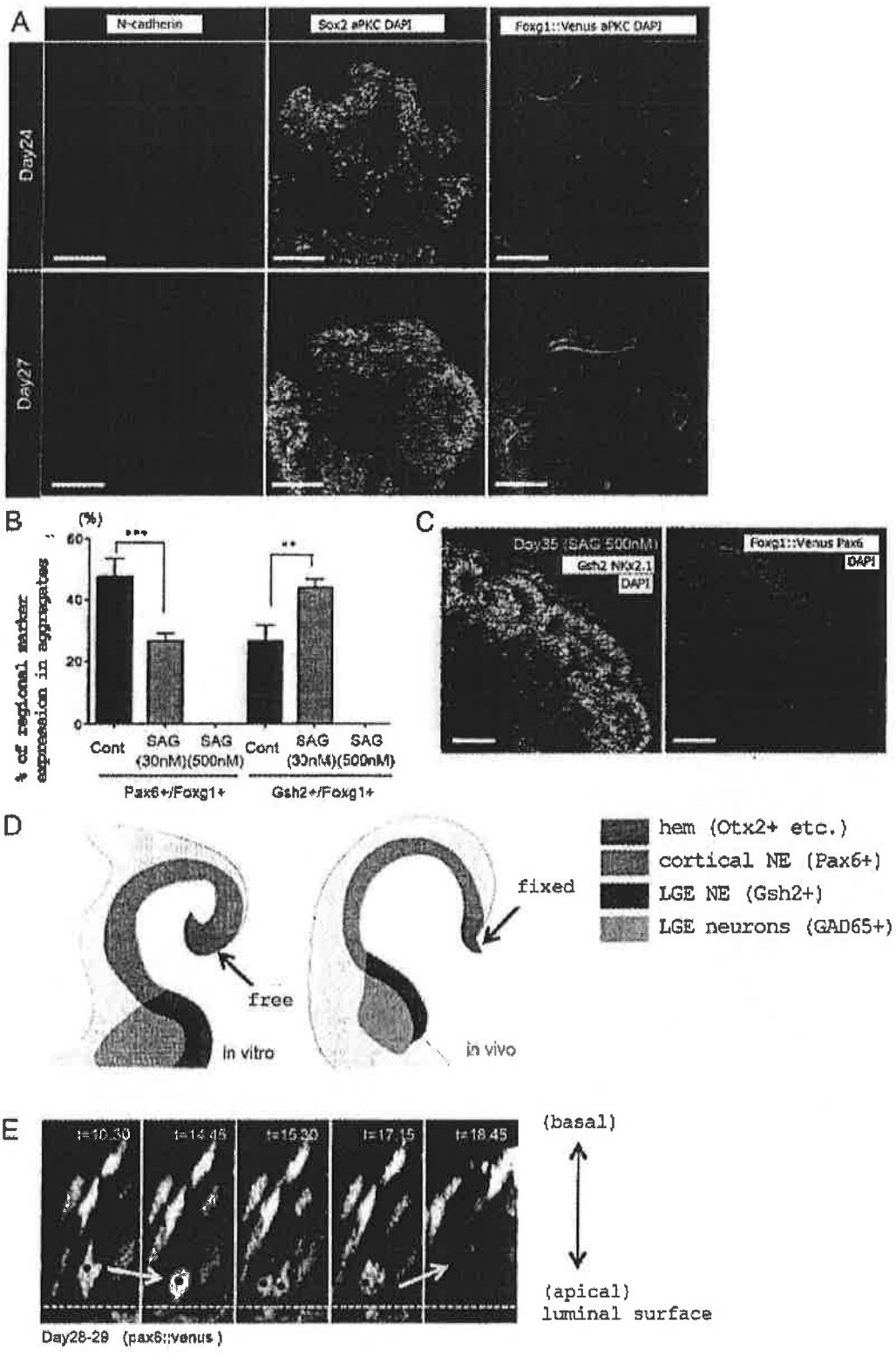

A
N-cadherin
Sox2 aPKC DAPI
Foxg1::Venus aPKC DAPI
Day24
Day27
B
(%)
% of regional marker expression in aggregates
Cont
SAG (30nM)
SAG (500nM)
Cont
SAG (30nM)
SAG (500nM)
Pax6+/Foxg1+
Gsh2+/Foxg1+
C
Day35 (SAG 500nM)
Gsh2 NKx2.1
DAPI
Foxg1::Venus Pax6
DAPI
D
free
in vitro
fixed
in vivo
hem (Otx2+ etc.)
cortical NE (Pax6+)
LGE NE (Gsh2+)
LGE neurons (GAD65+)
E
t=10.30
t=14.45
t=15.30
t=17.15
t=18.45
Day28-29 (pax6::venus)
(basal)
(apical)
luminal surface

Fig. 20

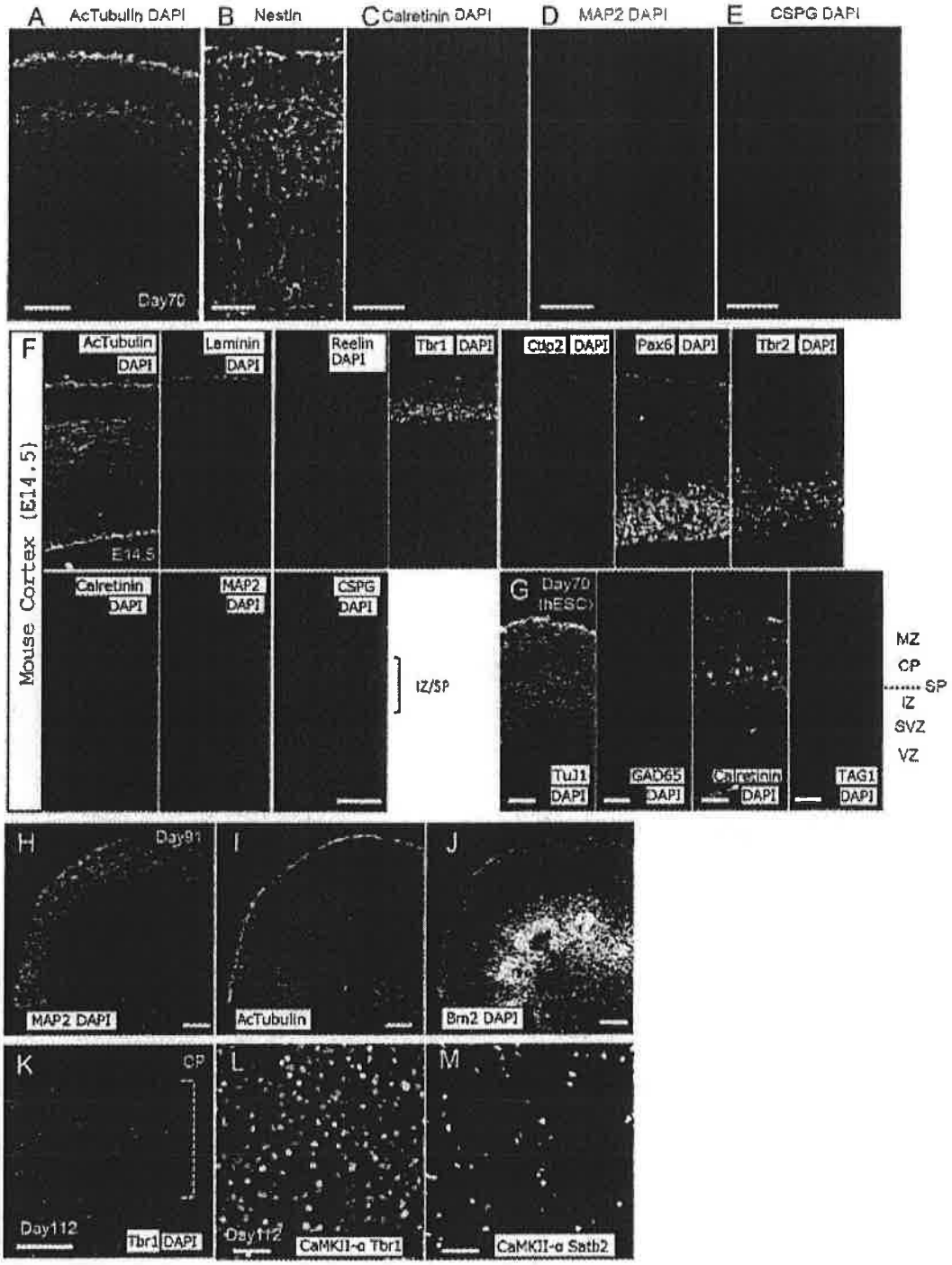
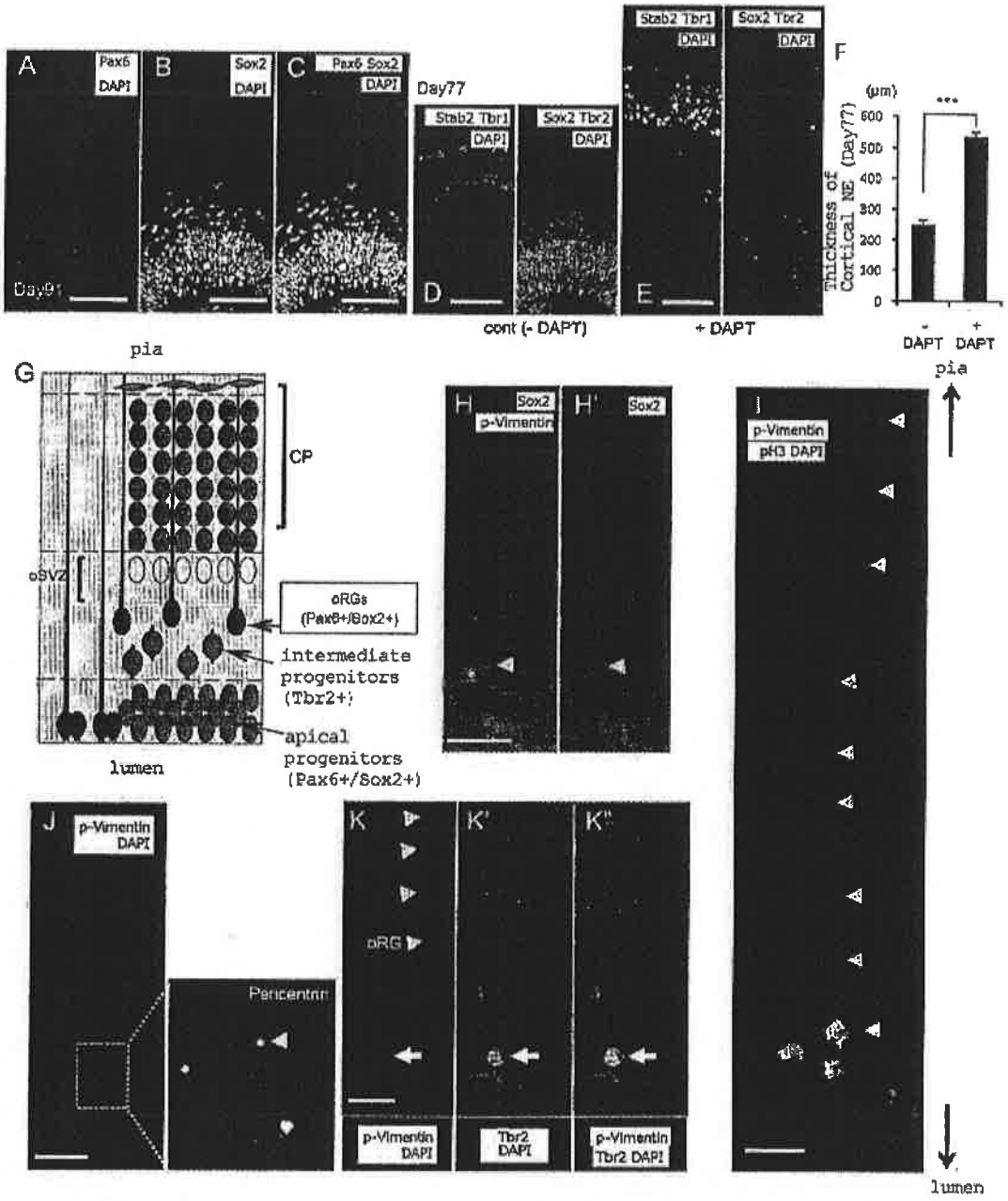

A AcTubulin DAPI
B Nestin
C Calretinin DAPI
D MAP2 DAPI
E CSPG DAPI
Day70
F
Mouse Cortex (E14.5)
AcTubulin DAPI
Laminin DAPI
Reelin DAPI
Tbr1 DAPI
Ctip2 DAPI
Pax6 DAPI
Tbr2 DAPI
E14.5
Calretinin DAPI
MAP2 DAPI
CSPG DAPI
IZ/SP
G Day70 (hESC)
TuJ1 DAPI
GAD65 DAPI
Calretinin DAPI
TAG1 DAPI
MZ
CP
SP
IZ
SVZ
VZ
H Day91
MAP2 DAPI
I
AcTubulin
J
Brn2 DAPI
K CP
Day112
Tbr1 DAPI
L
Day112
CaMKII-α Tbr1
M
CaMKII-α Satb2

Fig. 21


A
Pax6
DAPI
B
Sox2
DAPI
C
Pax6 Sox2
DAPI
Day91
Day77
Stab2 Tbr1
DAPI
Sox2 Tbr2
DAPI
D
E
cont (- DAPT)
+ DAPT
F
(μm)
600
500
400
300
200
100
0
Thickness of Cortical NE (Day77)
DAPT
DAPT
pia
G
CP
oSVZ
oRGs
(Pax6+/Sox2+)
intermediate progenitors
(Tbr2+)
apical progenitors
(Pax6+/Sox2+)
lumen
H
Sox2
p-Vimentin
H'
Sox2
I
p-Vimentin
pH3 DAPI
lumen
J
p-Vimentin
DAPI
Pericentrin
K
oRG
K'
K''
p-Vimentin
DAPI
Tbr2
DAPI
p-Vimentin
Tbr2 DAPI