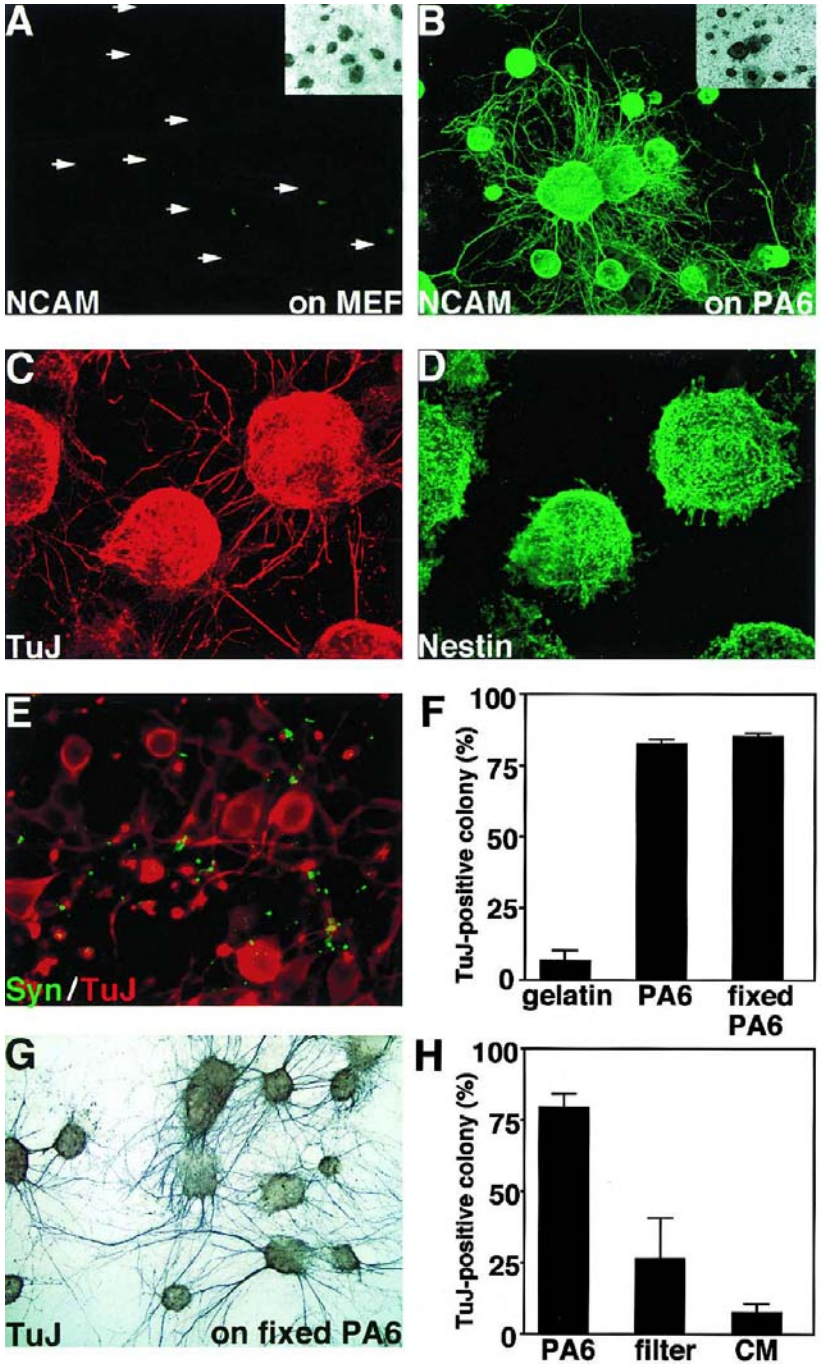
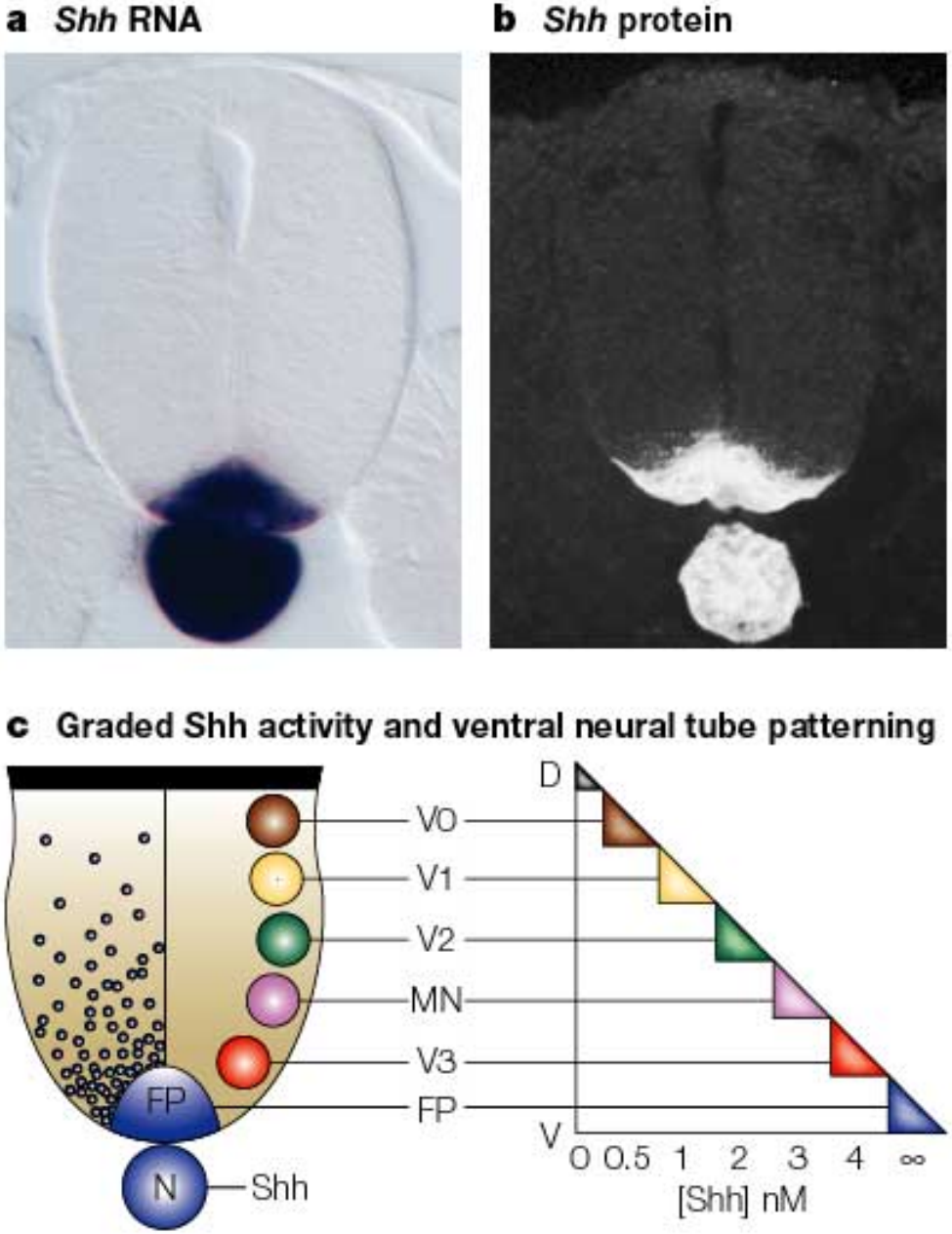


Neural-Inducing Factors Produced by PA6 Stromal Cells

(A and B) NCAM staining of ES cell colonies grown from a single cell for 8 days on MEF and PA6 feeder cells, respectively. Inset, phase contrast view.
(C and D) PA6-induced ES colonies double-stained with TuJ and nestin antibodies, respectively.
(E) The presynapse-specific marker synaptophysin (Syn, green) was detected on the induced neurons (TuJ, red).
(F) Percentages of TuJ-positive ES cell colonies on gelatin, PA6, and PFA-fixed PA6 after 8 days of induction.
(G) TuJ staining of ES cells cultured on PFA-fixed PA6 for 8 days.
(H) PA6 cells induce the neuronal marker TuJ in cocultured ES cells even when separated by a filter membrane (filter). The medium conditioned with PA6 for 3 days (CM) did not induce significant neural differentiation in ES cells cultured on gelatin. Arrows, negative colonies in immunostaining.
Kawasaki et al., 2000



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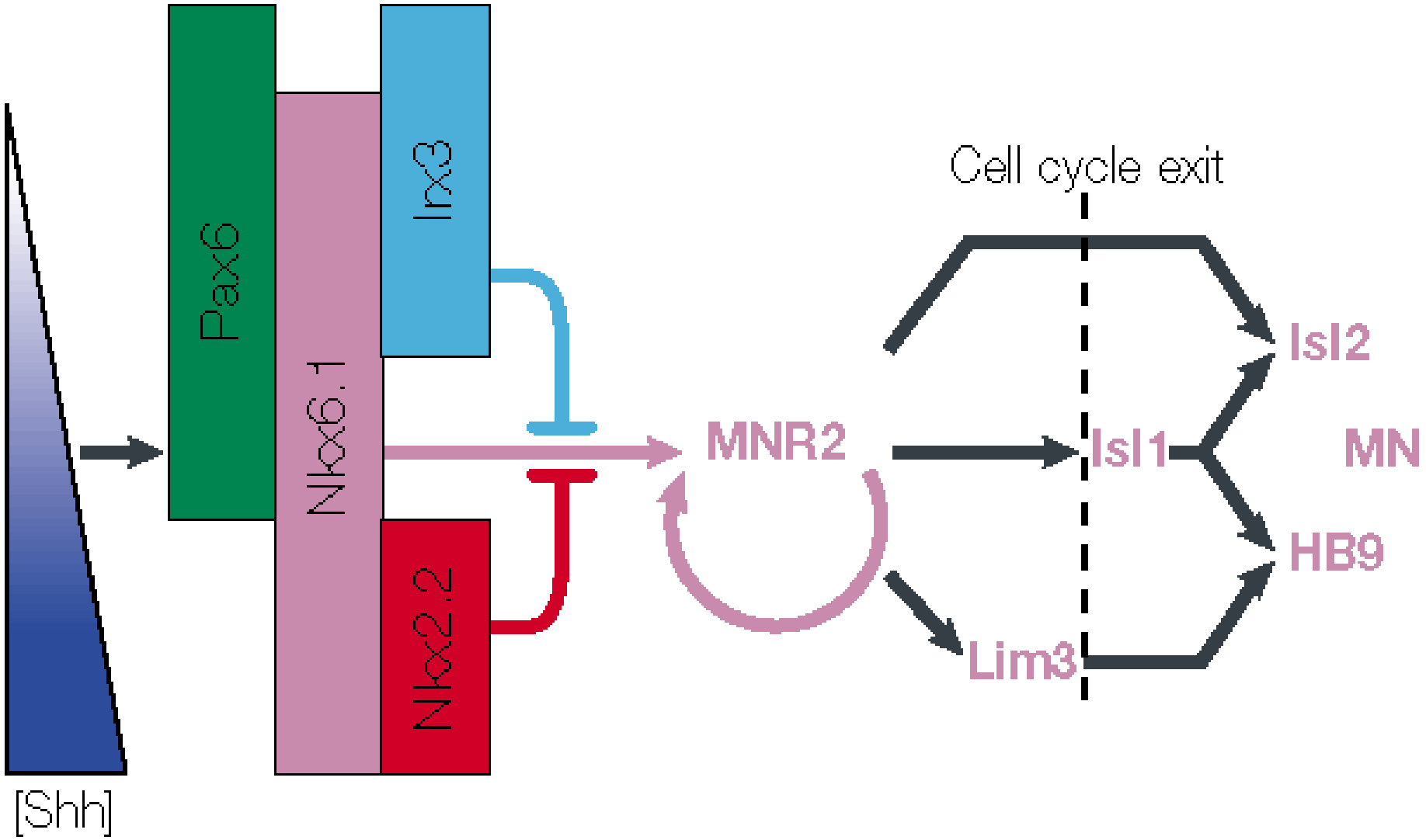
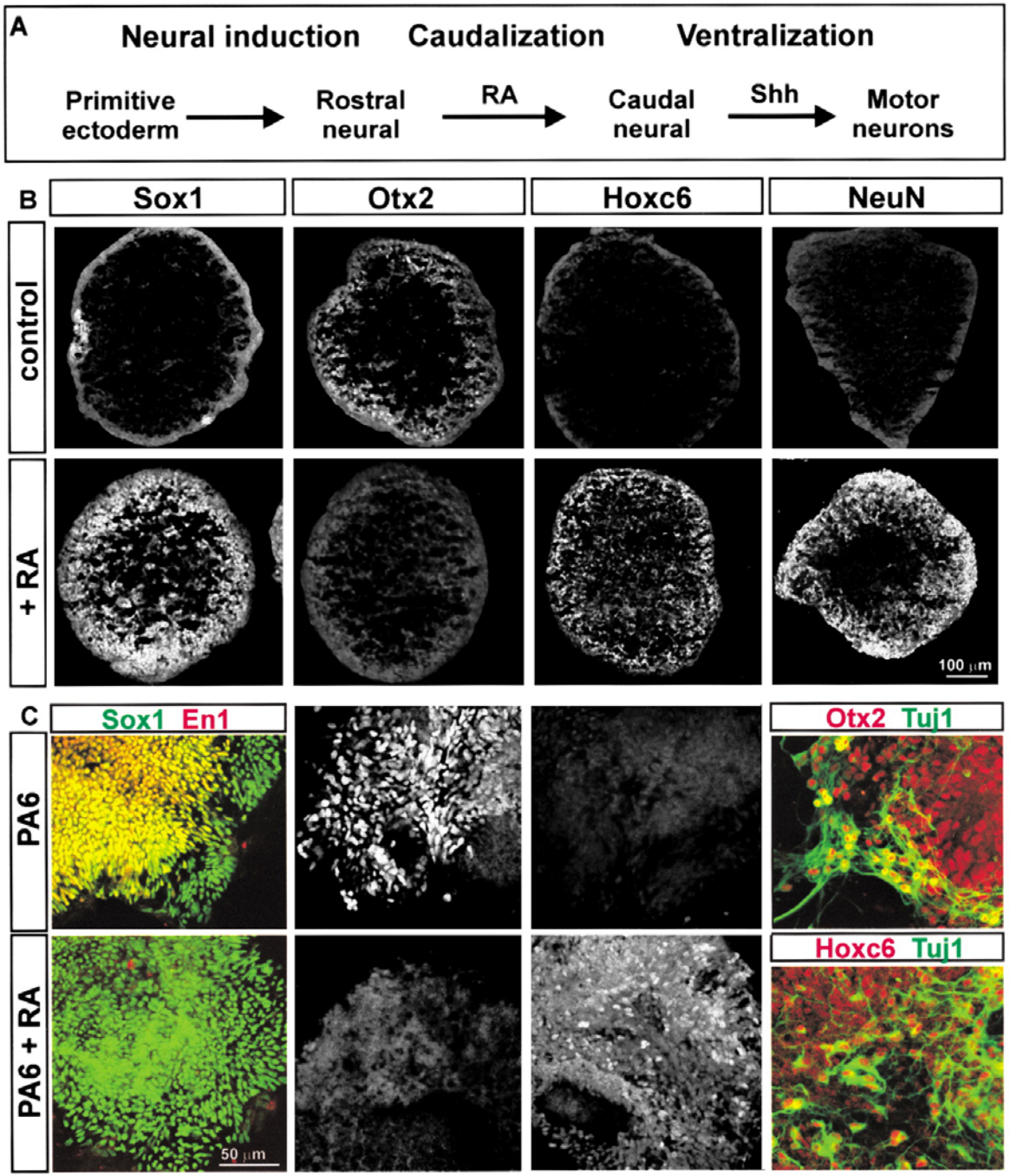

Pax6
Nkx6.1
Irx3
Nkx2.2
[Shh]
Cell cycle exit
MNR2
Isl1
Lim3
Isl2
HB9
MN



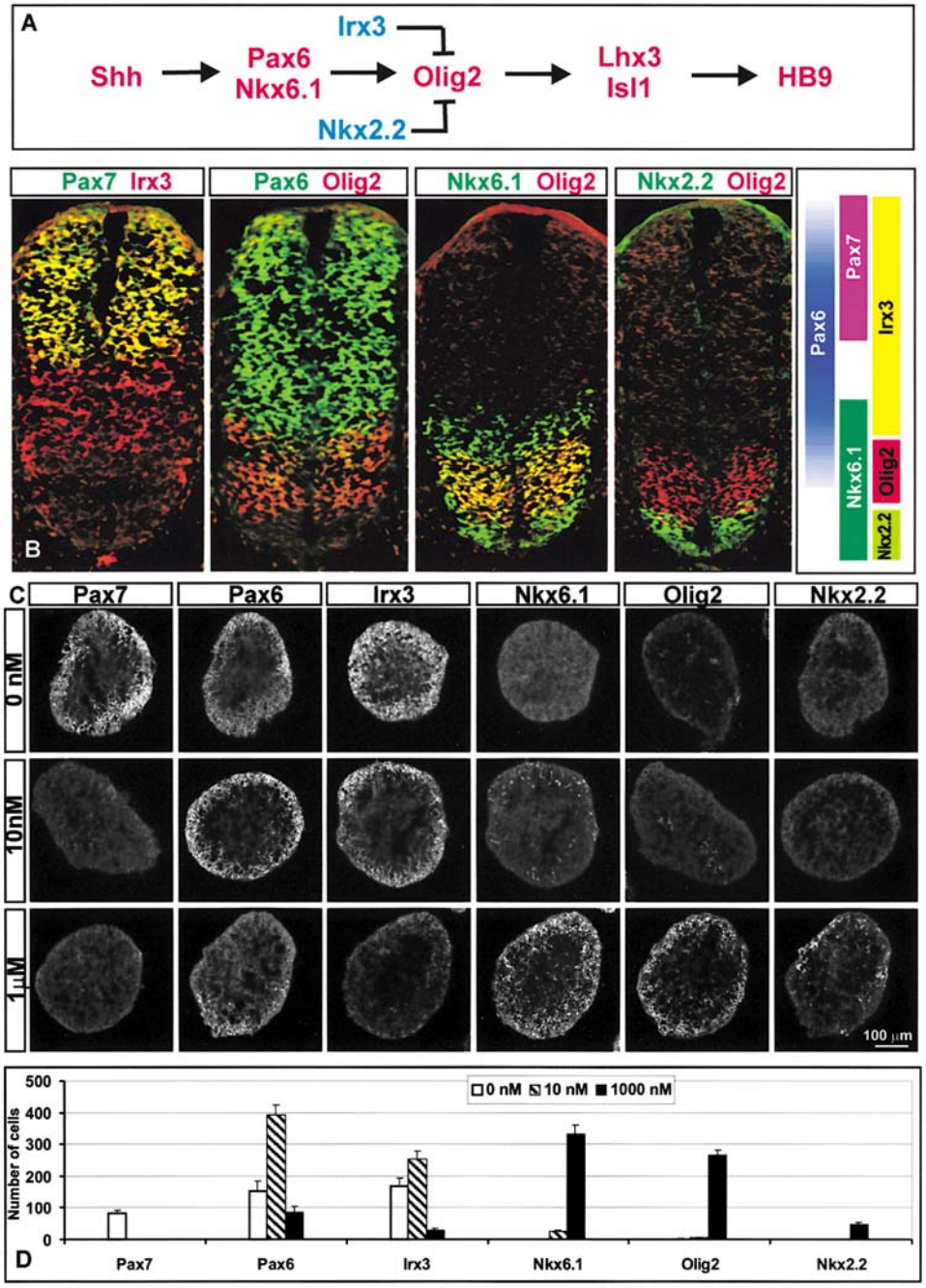
Sox1 – Panneural progenitor marker

Otx2 – Midbrain marker (w/En1)

Hoxc6 – Spinal cord marker

NeuN – Neuronal marker

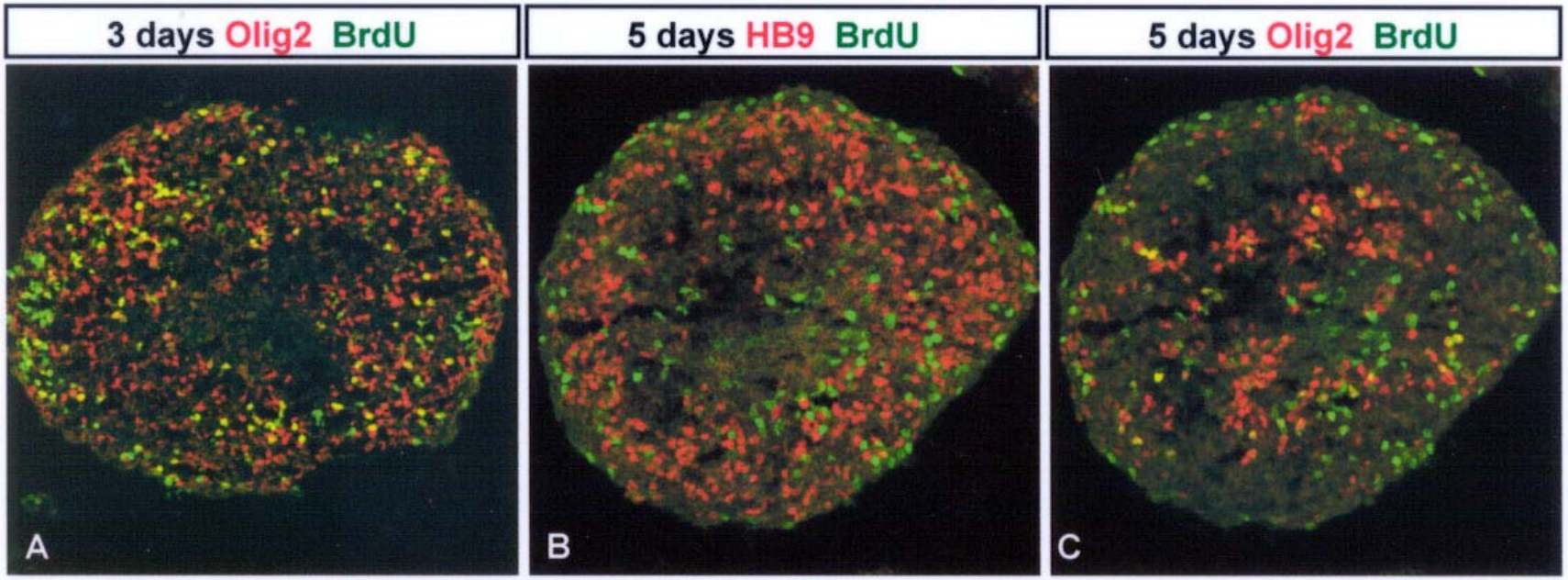
TuJ1 – Neural specific beta tubulin

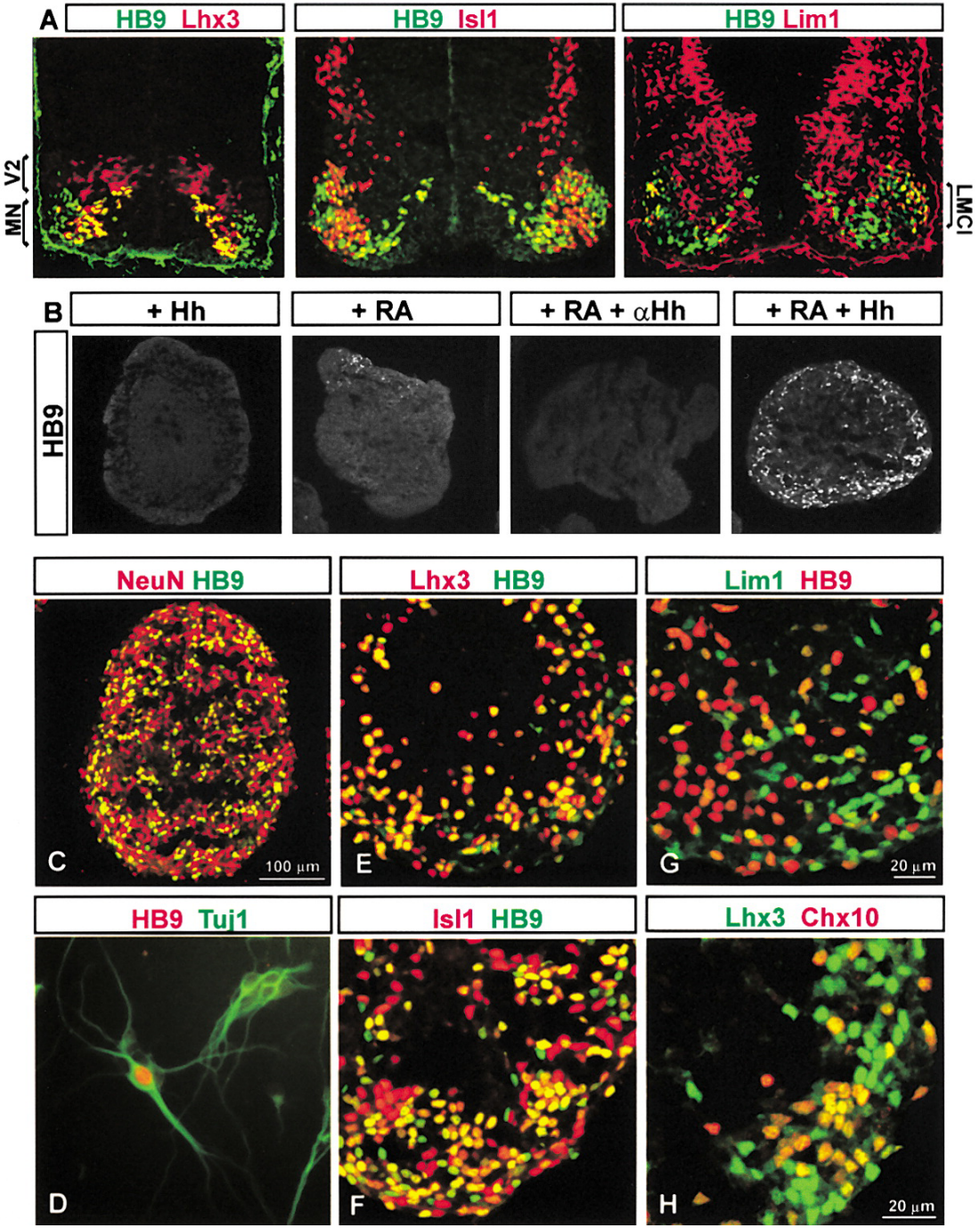


Endogenous expression patterns

Increasing Hh-Ag1.3

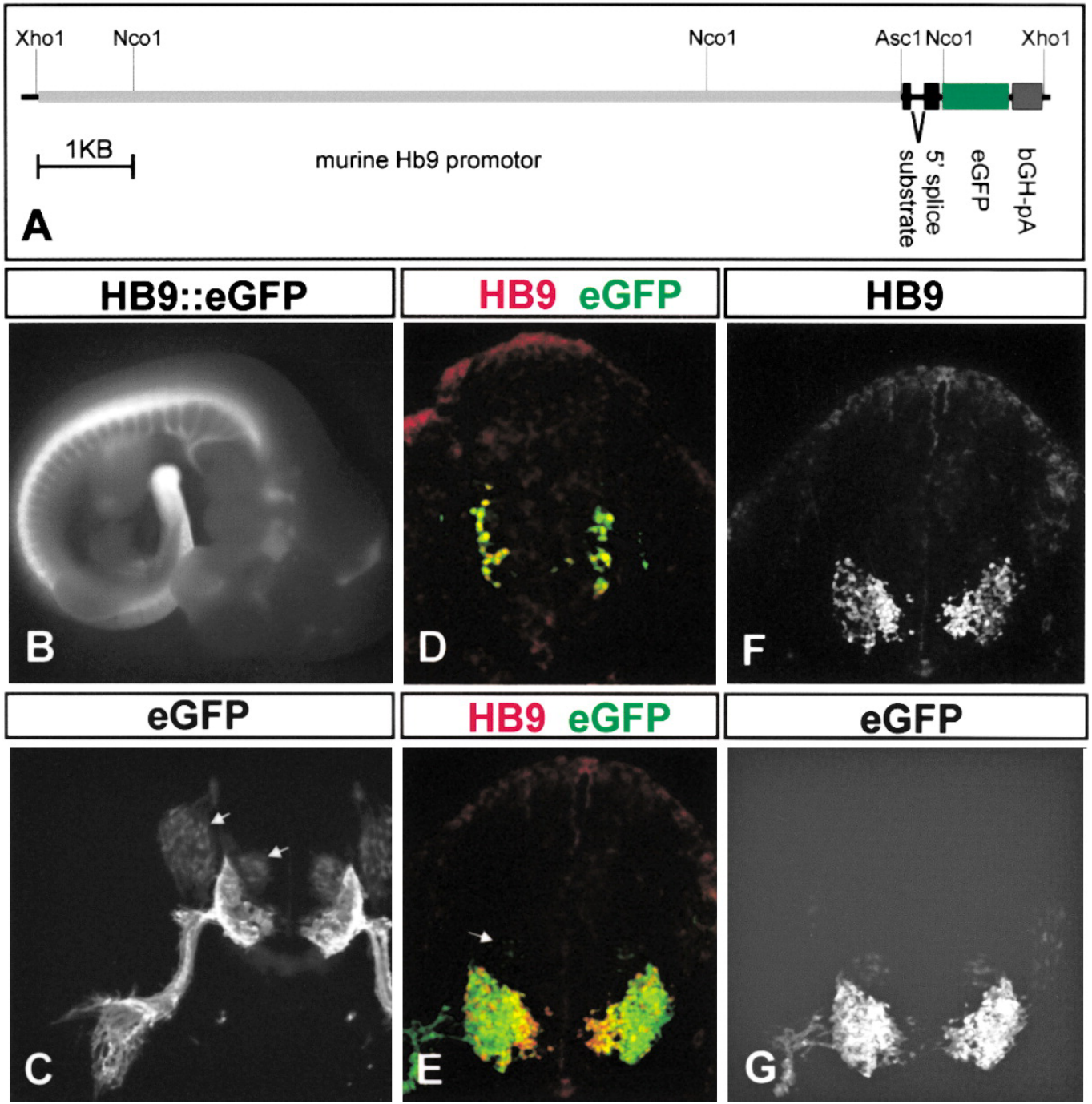
(RA present in all)

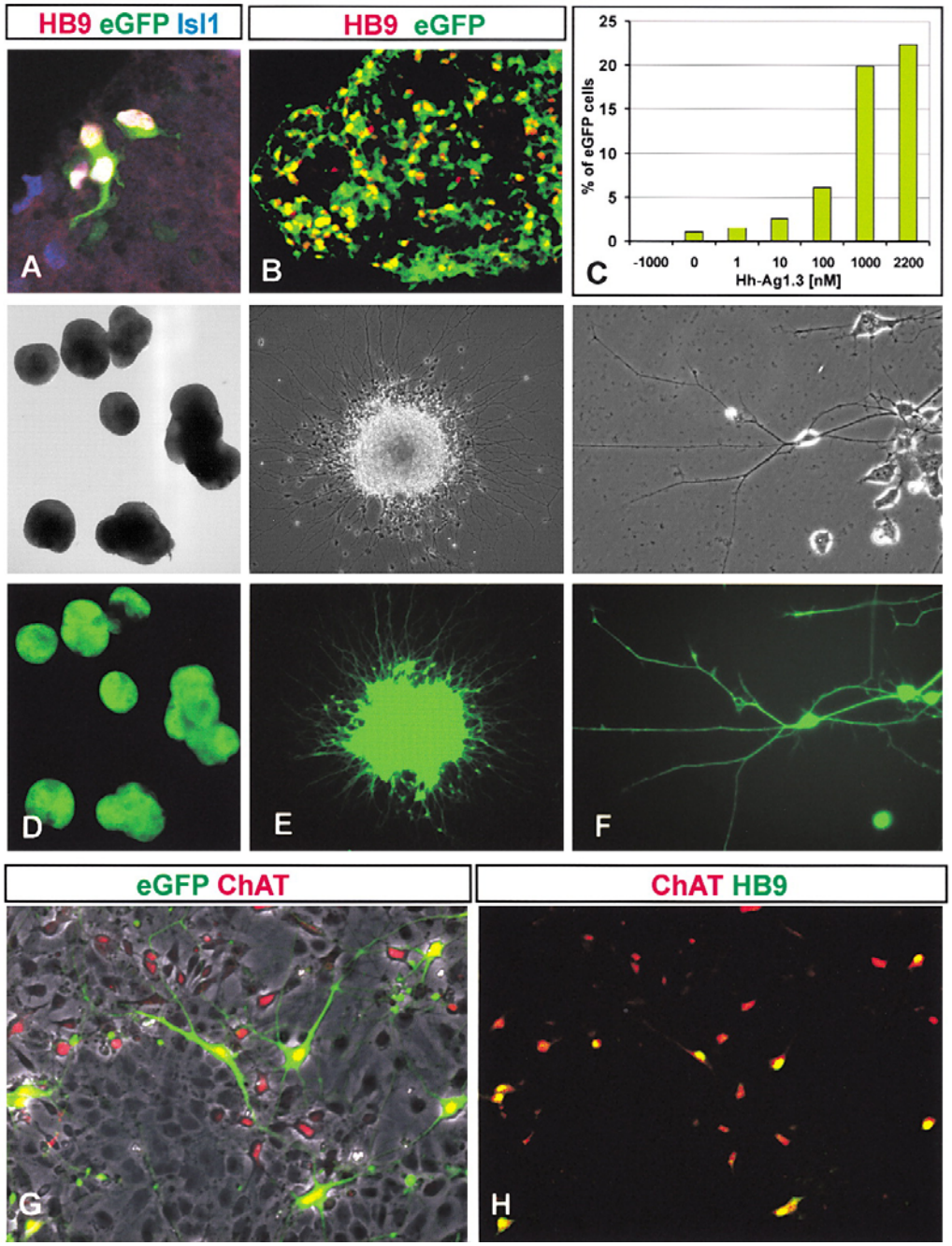

3 days Olig2 BrdU
5 days HB9 BrdU
5 days Olig2 BrdU
A
B
C

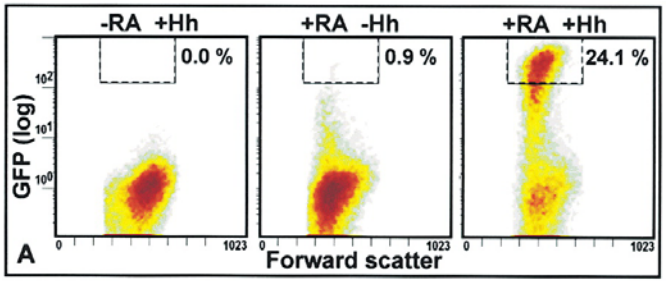


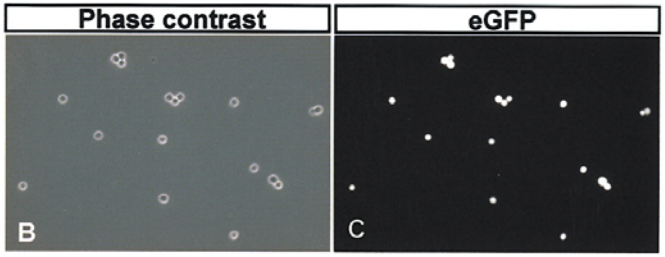
HB9 – MN marker

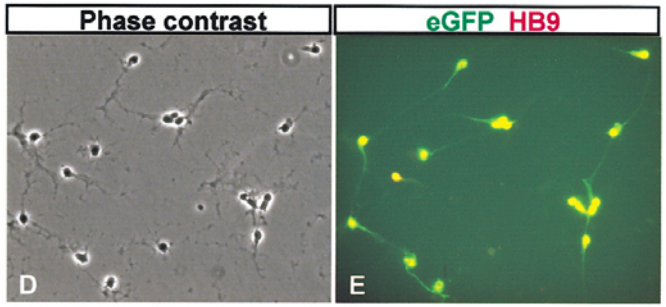
Lhx3/Isl1/Lim1 – expressed by subsets of MNs

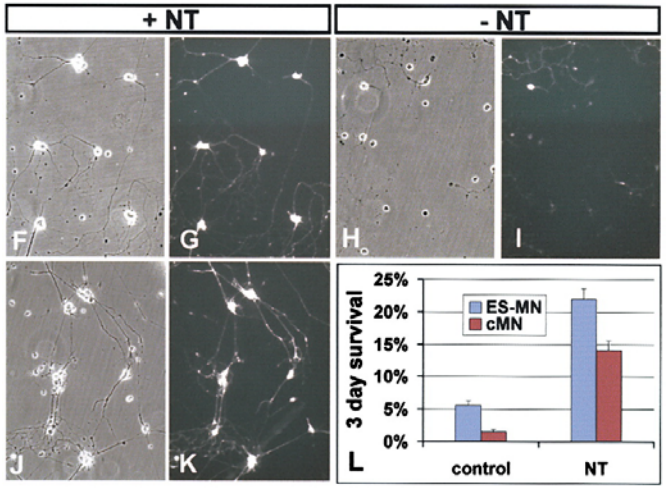

Xho1
Nco1
Nco1
Asc1
Nco1
Xho1
1KB
murine Hb9 promotor
5' splice substrate
eGFP
bGH-pA
A
HB9::eGFP
HB9 eGFP
HB9
B
D
F
eGFP
HB9 eGFP
eGFP
C
E
G

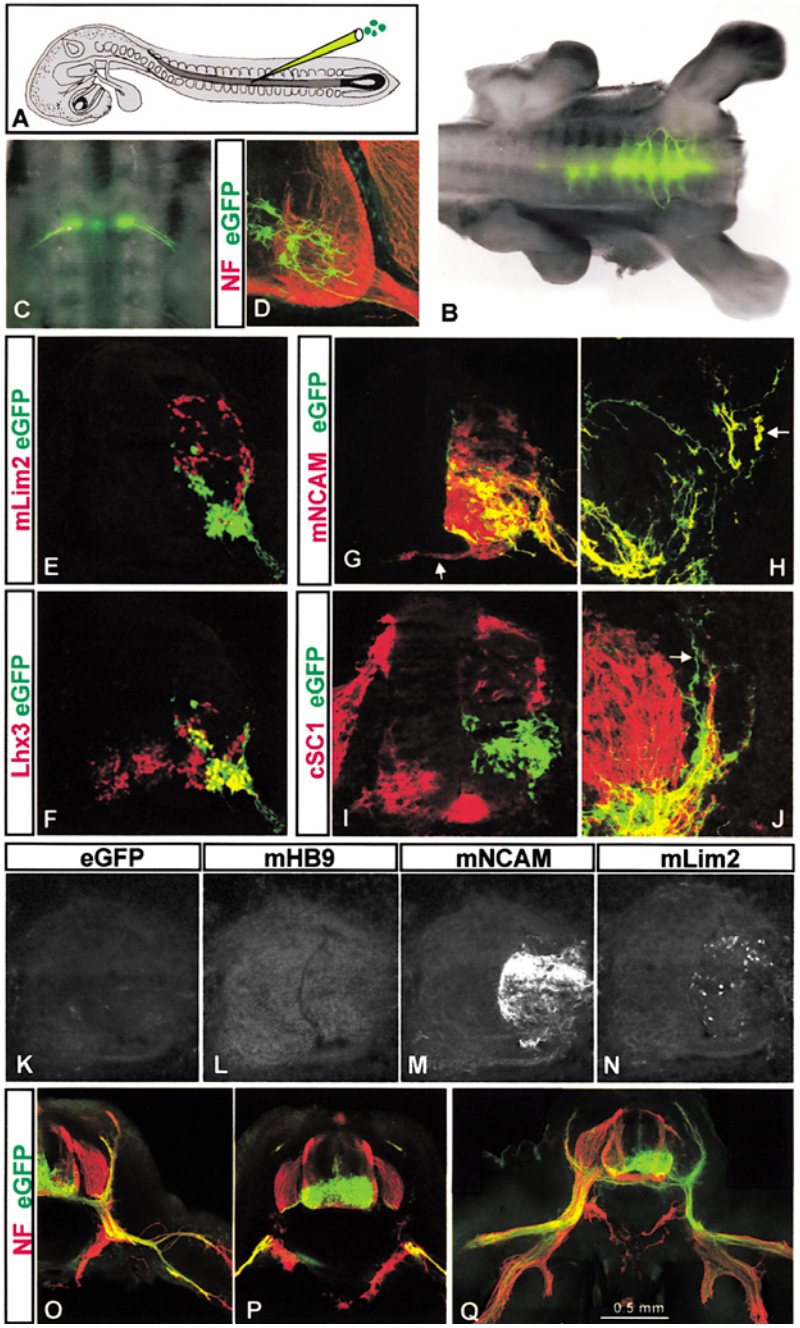

HB9 eGFP Isl1
HB9 eGFP
A
B
C
% of eGFP cells
25
20
15
10
5
0
-1000
0
1
10
100
1000
2200
Hh-Ag1.3 [nM]
D
E
F
eGFP ChAT
ChAT HB9
G
H


-RA +Hh
+RA -Hh
+RA +Hh
0.0 %
0.9 %
24.1 %
GFP (log)
Forward scatter
A


Phase contrast
eGFP
B
C


Phase contrast
eGFP HB9
D
E


+ NT
- NT
F
G
H
I
J
K
3 day survival
25%
20%
15%
10%
5%
0%
ES-MN
cMN
control
NT
L



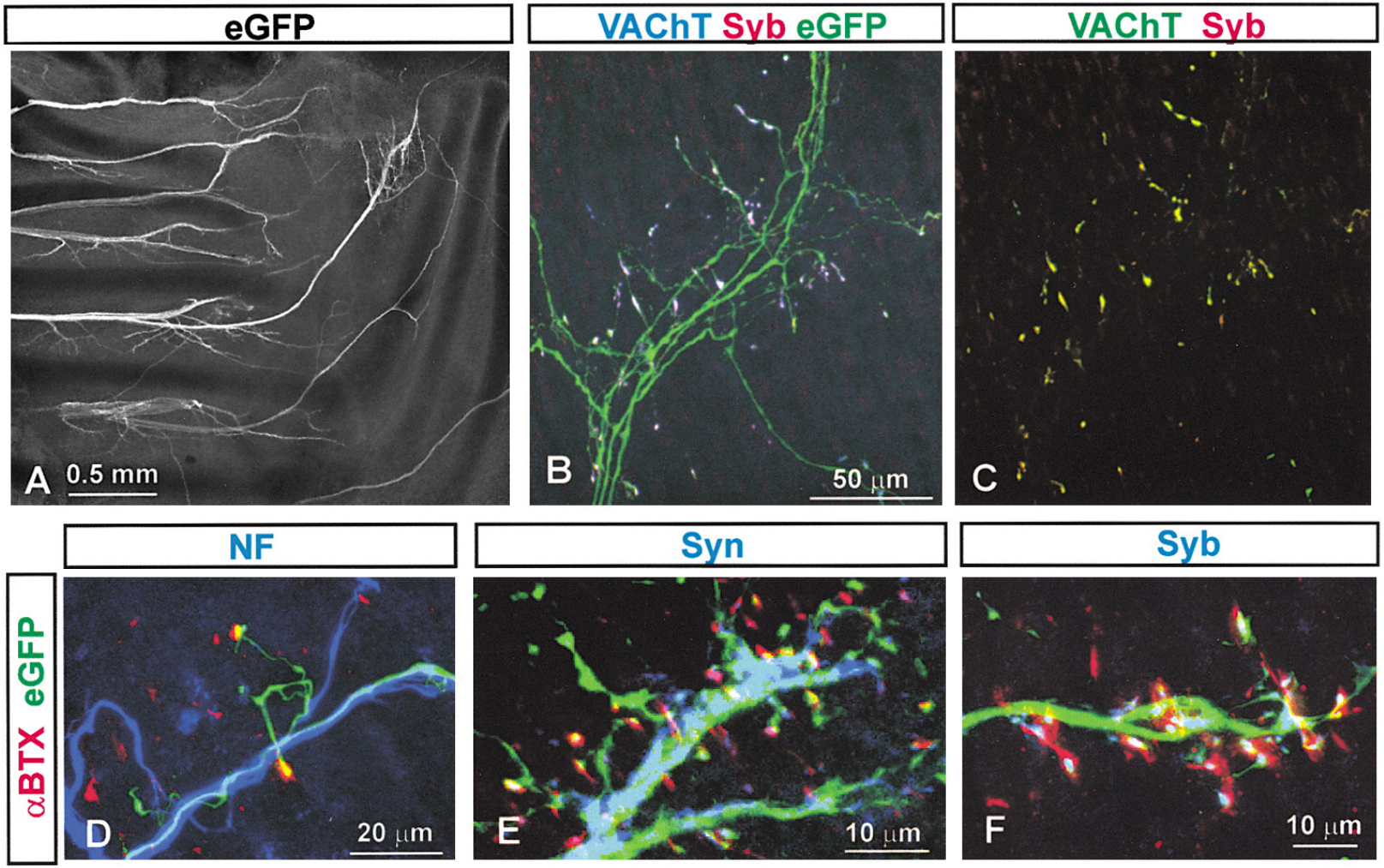
NF – Neurofilament expression

mLim2 – mouse specific interneuron marker

Lhx3 – MN marker

mNCAM – mouse spec. interneuron marker

cSC1 – Chick spec. MN marker



VAChT/Syb/Syn – nerve terminal differentiation markers

aBTX – motor axon terminal marker