24 MAR 2006

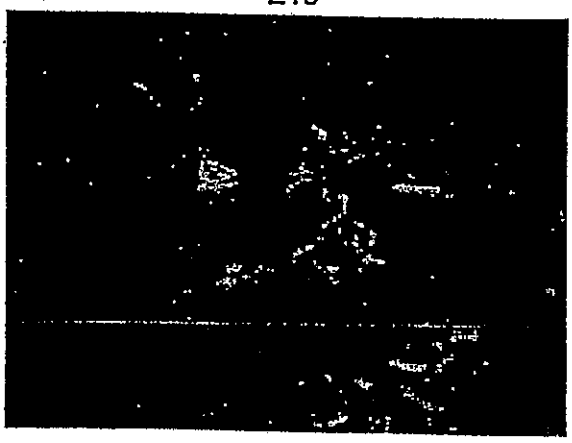


FIG. 1A

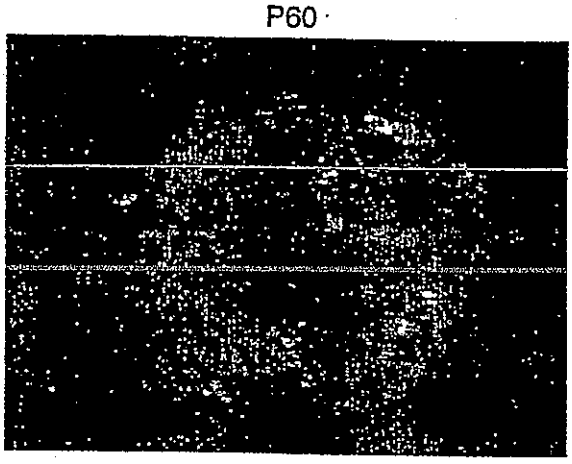
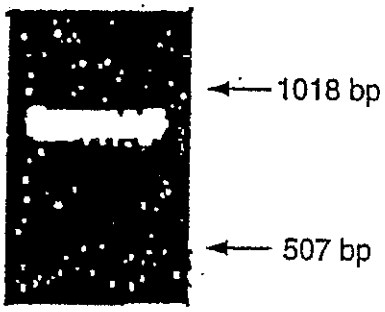


FIG. 1B



FORWARD PRIMER [GCGGGGCGGTGCGTGACTAC]
REVERSE PRIMER [GGGTGGTGAGGGTTGAGGTTTGTG]

FIG. 2

2 4 MAR 2006

NESTIN POSITIVE CELLS PROLIFERATE AROUND ISLETS IN VITRO

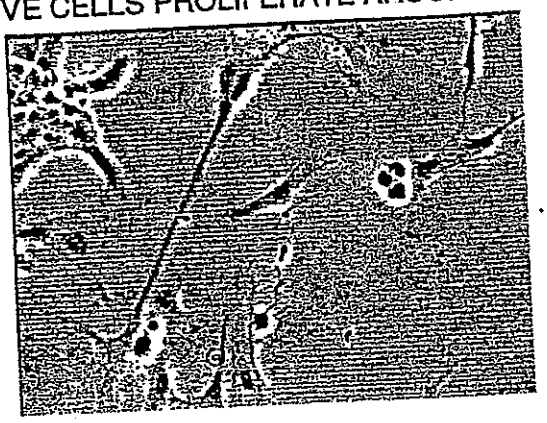


FIG. 3

100x



FIG. 4A

200x

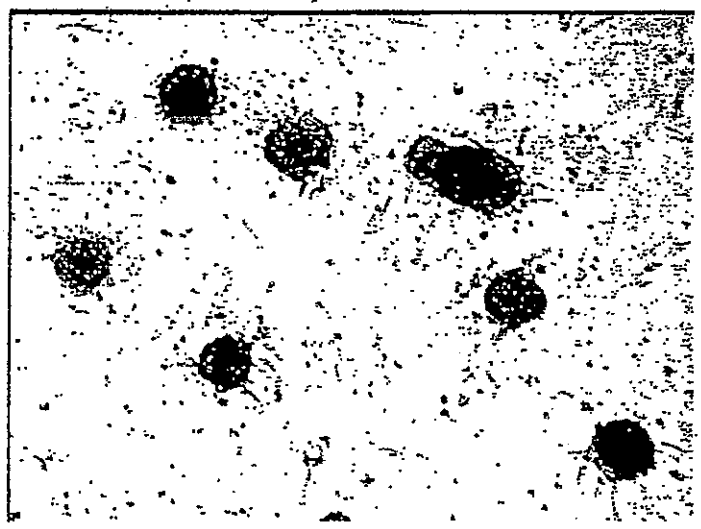


FIG. 4B

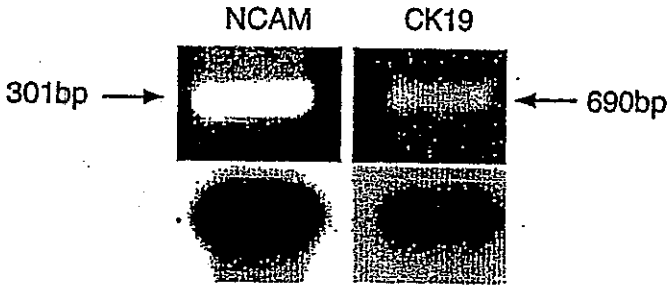


FIG. 5

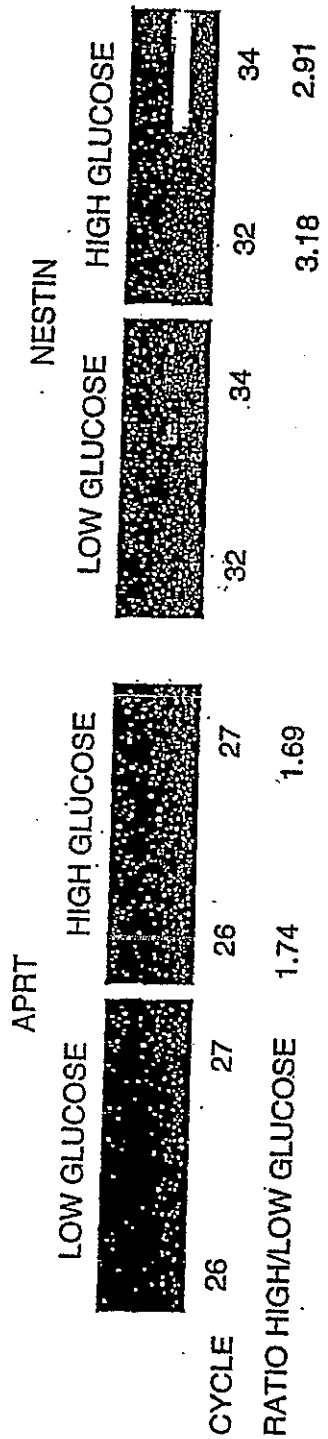

APRT
LOW GLUCOSE
HIGH GLUCOSE
CYCLE 26 27 26 27
RATIO HIGH/LOW GLUCOSE 1.74 1.69
NESTIN
LOW GLUCOSE
HIGH GLUCOSE
32 34 32 34
3.18 2.91

FIG. 6

Nestin Amino Acid Sequence:
"MEGCMGEESFQMWELNRRLEAYLGRVKALEEQNELLSAGLGGLR
RQSADTSWRAHADDELAALRALVDQRWREKHAAEVARDNLAEELEGVAGRCEQLRL
ARERTTEEVARNRRAVEAEKCARAWLSSQGAELERELEALRVAHEEERVGLNAQAAC
APRLPAPPRPPAPAPEVEELARRLGEAWRGAVRGYQERVAHMETSLDQTRERLARAVQ
GAR
EVRLELQQLQAERGGLLERRAALEQRLEGRWQERLRATEKFQLAVEALEQEKQGLQSQ
IAQVLEGRQQLAHLKMSLSLEVATYRTLLEAENSRLQTPGGGSKTSLSFQDPKLELQF
PRTPEGRRLGSLLPVLSPTSLPSPLPATLETPVPAFLKNQEFLQARTPTLASTPIPPT
PQAPSPAVDAEIRAQDAPLSLLQTQGGRKQAPEPLRAEARVAIPASVLPGPEEPGGQR
QEASTGQSPEDHASLAPPLSPDHSSLEAKDGESGGSRVFSICRGEGEGQIWGLVEKET
AIEGKVVSSLQQEIWEEEDLNRKEIQDSQVPLEKETLKSLGEEIQESLKTLENQSHET
LERENQECPRSLEEDLETLKSLEKENKRAIKGCGGSETSRKRGCRQLKPTGKEDTQTL
QSLQKENQELMKSLEGNLETFLFPGTENQELVSSLQENLESLTALEKENQEPLRSPEV
GDEEALRPLTKENQEPLRSLEDENKEAFRSLEKENQEPLKTLEEEDQSIVRPLETENH
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RNLEEEENLGKGEYQESLRSLEEEGQELPQSADVQRWEDTVEKDQELAQESPPGMAGV
ENKDEAELNLREQDGFTGKEEVVEQGELNATEEVWFPGEGHPENPEPKEQRGLVEGAS
VKGGAEGLQDPEGQSQQVGTPGLQAPQGLPEAIEPLVEDDVAPGGDQASPEVMLGSEP
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LGSEEAEEDVPPVLVSPSPTYTPILEDAPGLQPQAEGSQEASWGVQGRAEAGKVESEQ
EELGSGEIPEGLQEEGEESREESEEDELGETLPDSTPLGFYLRSPTSPRWTPLESRGH
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VPGEVAEPLGQVPQLLLDPAAWDRDGESDGFADEEESGEEGEEDQEEGREPGAGRWGP
GSSVGSLQALSSSQRGEFLESDSVSVSVPWDDSLRGAVAGAPKTALETESQDSAEPSG
SEEESDPVSLEREDKVPGPLEIPSGMEDAGPGADIIGVNGQGPNLEGKSQHVNGGVMN
GLEQSEESGARNALVSEGDRGSPFQEEEGSALKRSSAGAPVHLGQGQFLKFTQREGDR
ESWSSGED"

Nestin Nucleotide Sequence:
BASE COUNT 1238 a 1176 c 1676 g 764 t ORIGIN 1
atggagggct gcatggggga ggagtcgttt cagatgtggg agctcaatcg gcgcctggag 61
gcctacctgg gccgggtcaa ggcgctggag gagcagaatg agctgctcag cgccggactc 121
ggggggctcc ggcgacaatc cgcggacacc tcctggcggg cgcatgccga cgacgagctg 181
gcggccctgc gtgcgctcgt tgaccaacgc tggcgggaga agcacgcggc cgaggtggcg 241
cgcgacaacc tggctgaaga gctggagggc gtggcaggcc gatgcgagca gctgcggctg 301
gccccgggagc ggacgacgga ggaggtagcc cgcaaccggc gcgccgtcga ggcagagaaa
361 tgcgcccggg cctggctgag tagccagggg gcagagctgg agcgcgagct agaggctcta
421 cgcgtggcgc acgaggagga gcgcgtcggt ctgaacgcgc aggctgcctg tgccccccgc

FIG. 7A

481 ctgcccgcgc cgccccggcc tcccgcgccg gccccggagg tagaggagct ggcaaggcga
541 ctgggcgagg cgtggcgcgg ggcagtgcgc ggctaccagg agcgcgtggc acacatggag
601 acgtcgctgg accagacccg cgagcgcctg gcccgggcgg tgcagggtgc ccgcgaggtc
661 cgcctggagc tgcagcagct ccaggctgag cgcggaggcc tcctggagcg cagggcagcg
721 ttggaacaga ggttggaggg ccgctggcag gagcggctgc gggctactga aaagttccag
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901 tacaggaccc tcctggaggc tgagaactcc cggctgcaaa cacctggcgg tggctccaag
961 acttccctca gctttcagga ccccaagctg gagctgcaat tccctaggac cccagagggc
1021 cggcgtcttg gatctttgct cccagtcctg agcccaactt ccctcccctc acccttgcct
1081 gctacccttg agacacctgt gccagccttt cttaagaacc aagaattcct ccaggcccgt
1141 acccctacct tggccagcac ccccatcccc cccacacctc aggcaccctc tcctgctgta
1201 gatgcagaga tcagagccca ggatgctcct ctctctctgc tccagacaca gggtgggagg
1261 aaacaggctc cagagccccc gcgggctgaa gccagggtgg ccattcctgc cagcgtcctg
1321 cctggaccag aggagcctgg gggccagcgg caagaggcca gtacaggcca
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agggggcaat 2581 gaatcctcta gaaaaggaaa ttcaagaacc actggagtct gtggaagtga
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ggaccaagaa 2881 ctggctcagg aaagccctcc tgggatggct ggagtggaaa ataaggatga
ggcagagctg 2941 aatctaaggg agcaggatgg cttcactggg aaggaggagg tggtagagca
gggagagctg 3001 aatgccacag aggaggtctg gttcccaggc gaggggcacc

FIG. 7B

cagagaaccc tgagcccaaa 3061 gagcagagag gcctggttga gggagccagt
gtgaagggag gggctgaggg cctccaggac 3121 cctgaagggc aatcacaaca
ggtggggacc ccaggcctcc aggctcccca ggggctgcca 3181 gaggcgatag agcccctggt
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gcctgccatg ggtgagtctg ctgcgggagc tgagccaggc 3301 ctggggcagg gggtgggagg
gctgggggac ccaggccatc tgaccaggga agaggtgatg 3361 gaaccacccc
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FIG. 7C

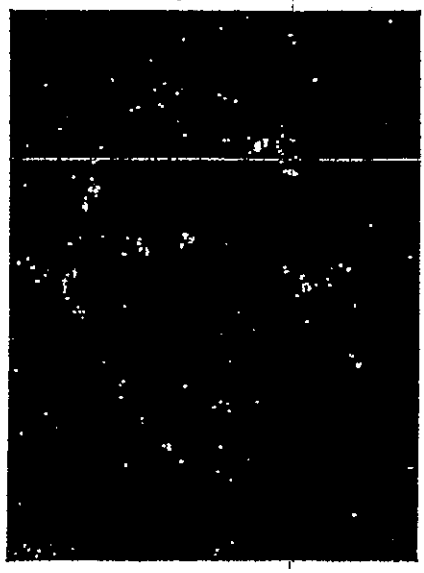


FIG. 8B

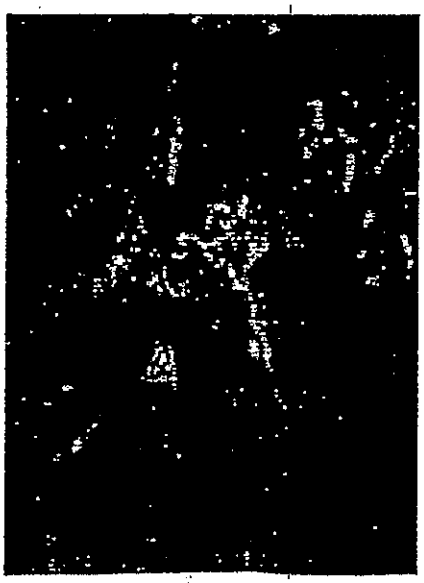


FIG. 8A

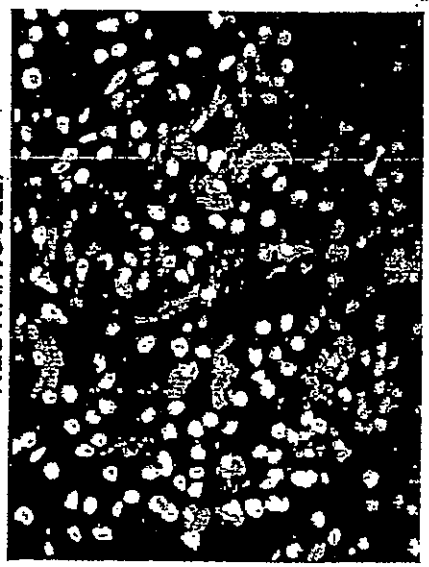


FIG. 8D

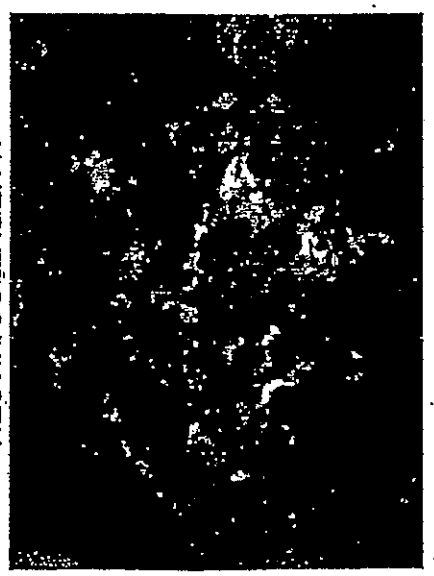


FIG. 8C

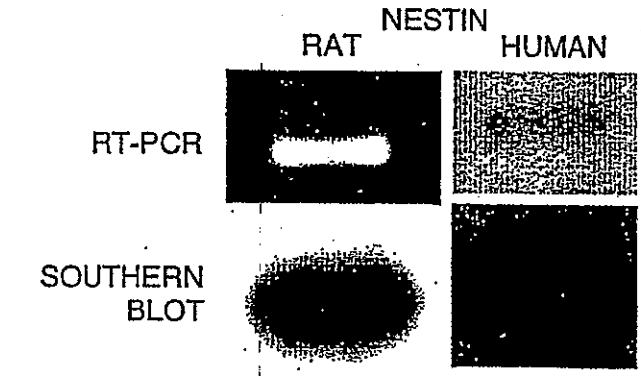


FIG. 8E

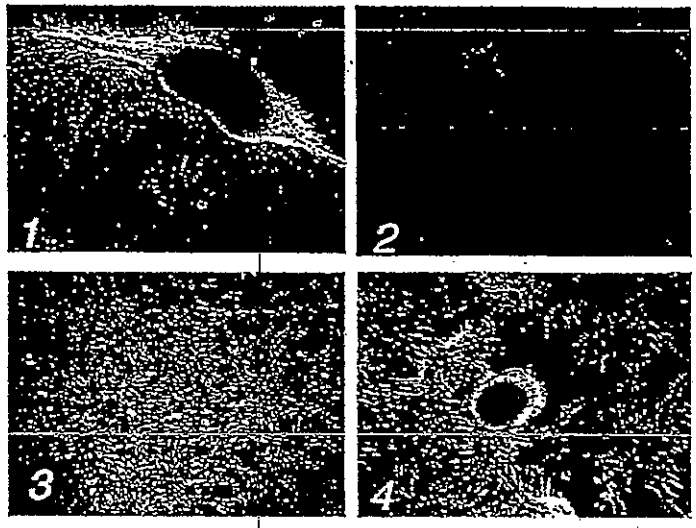


FIG. 9A

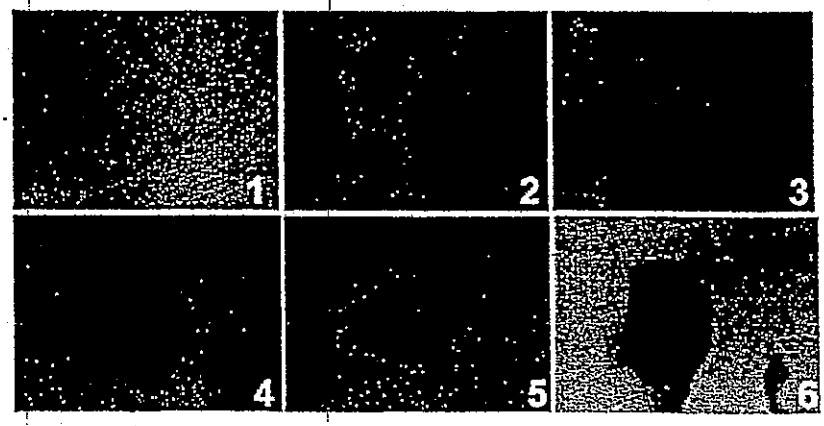


FIG. 9B

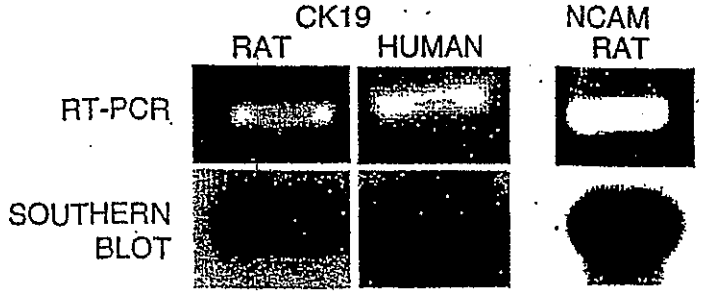


FIG. 9C

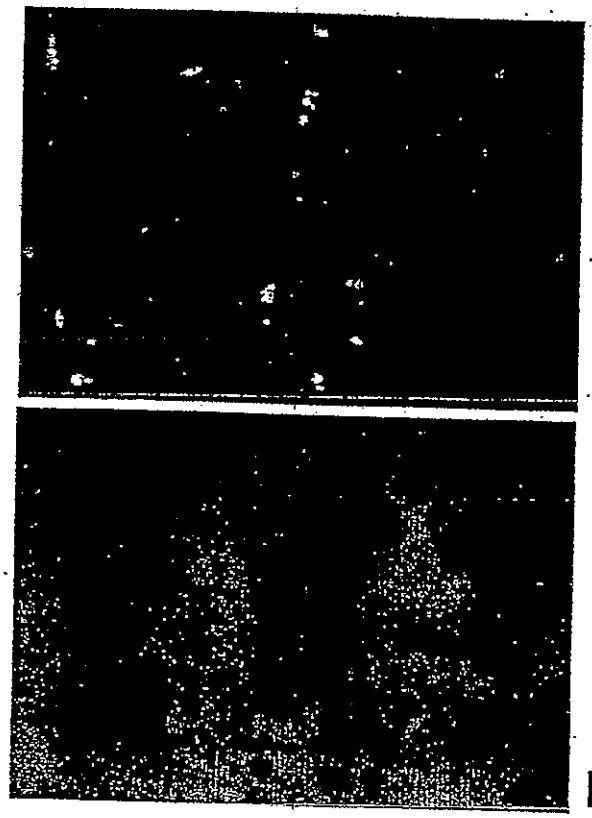


FIG. 10A

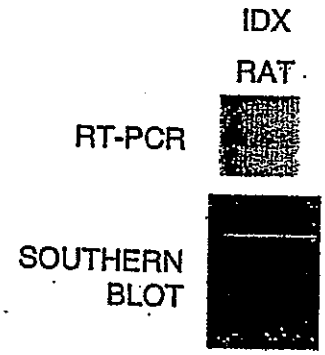


FIG. 10B

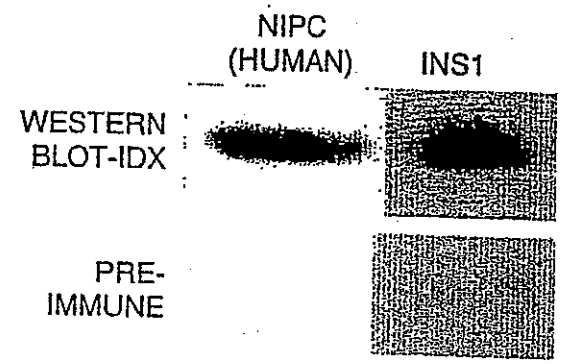


FIG. 10C

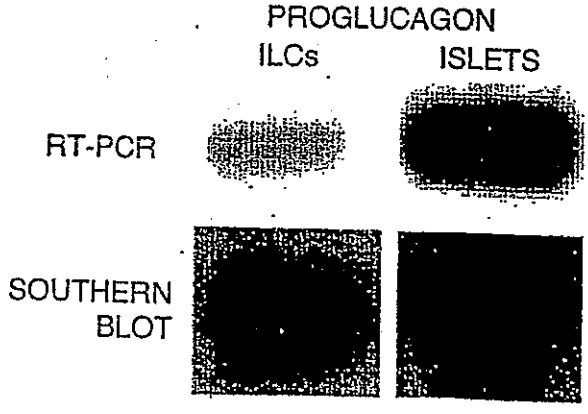


FIG. 10D

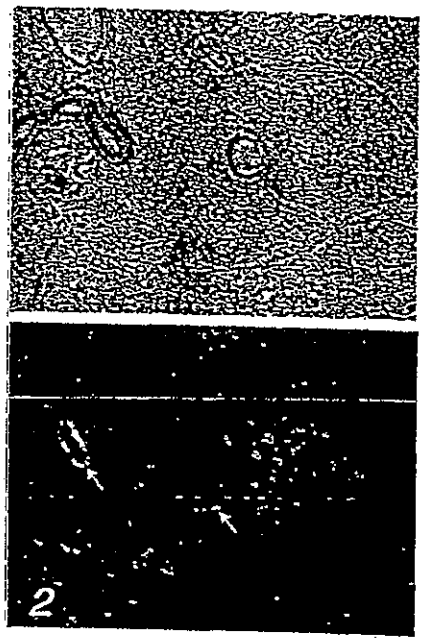


FIG. 11A

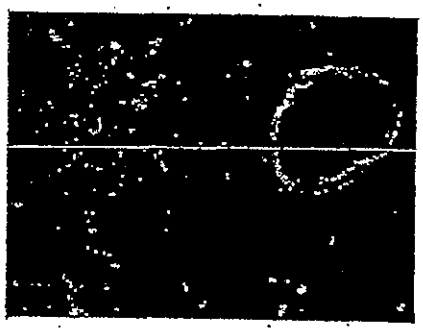


FIG. 11B

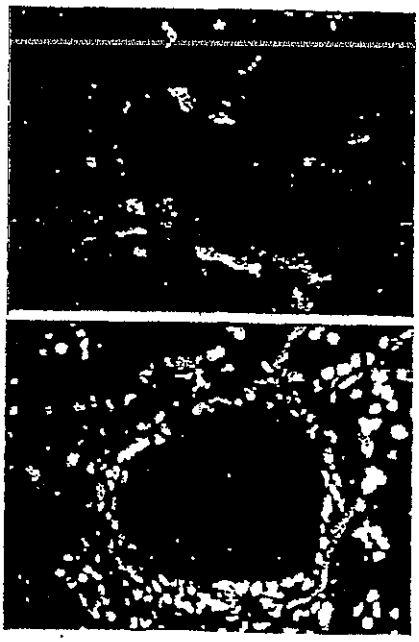


FIG. 11C

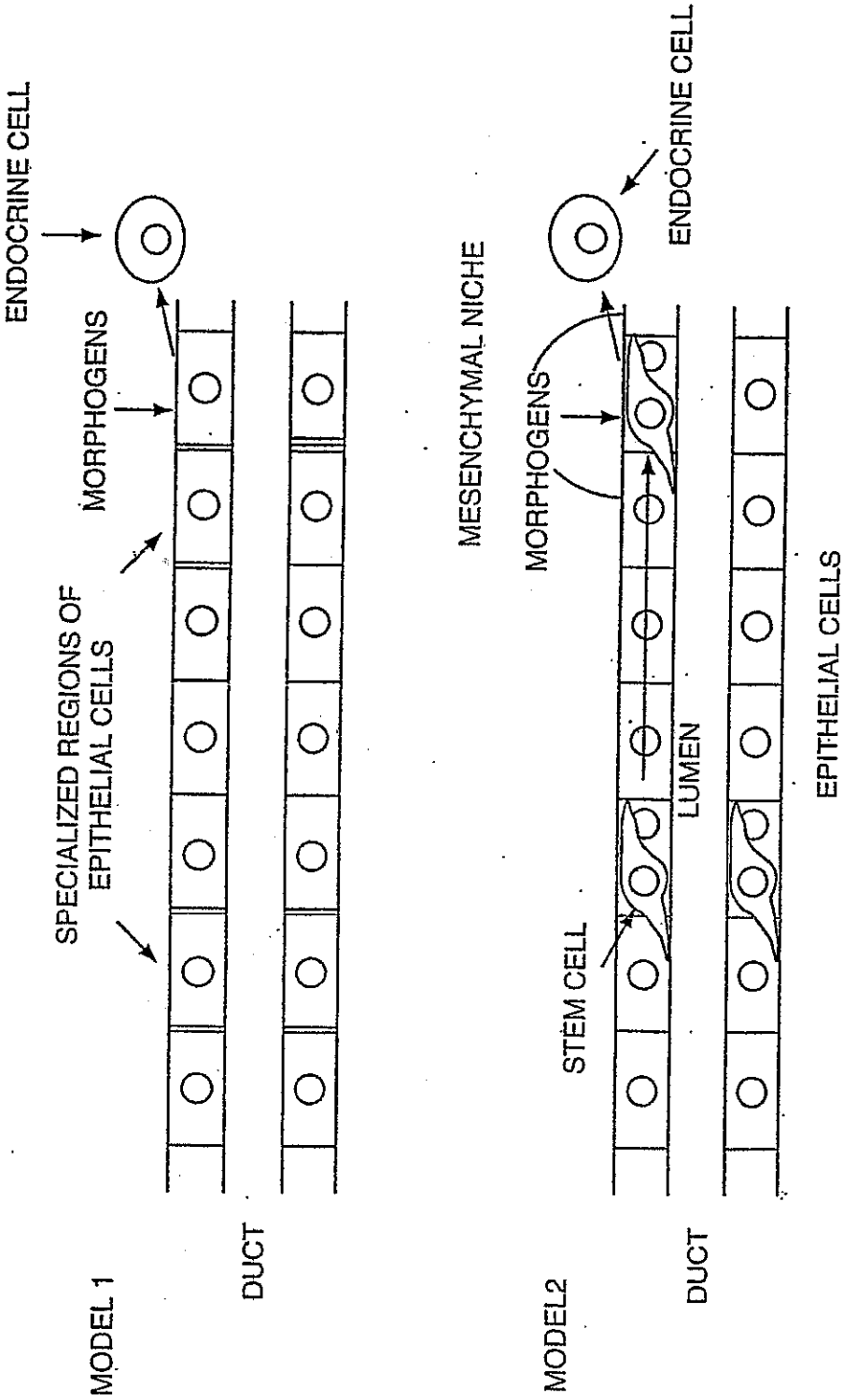

ENDOCRINE CELL
MORPHOGENS
SPECIALIZED REGIONS OF EPITHELIAL CELLS
MODEL 1
DUCT
MESENCHYMAL NICHE
ENDOCRINE CELL
MORPHOGENS
STEM CELL
LUMEN
EPITHELIAL CELLS
MODEL2
DUCT

FIG. 12

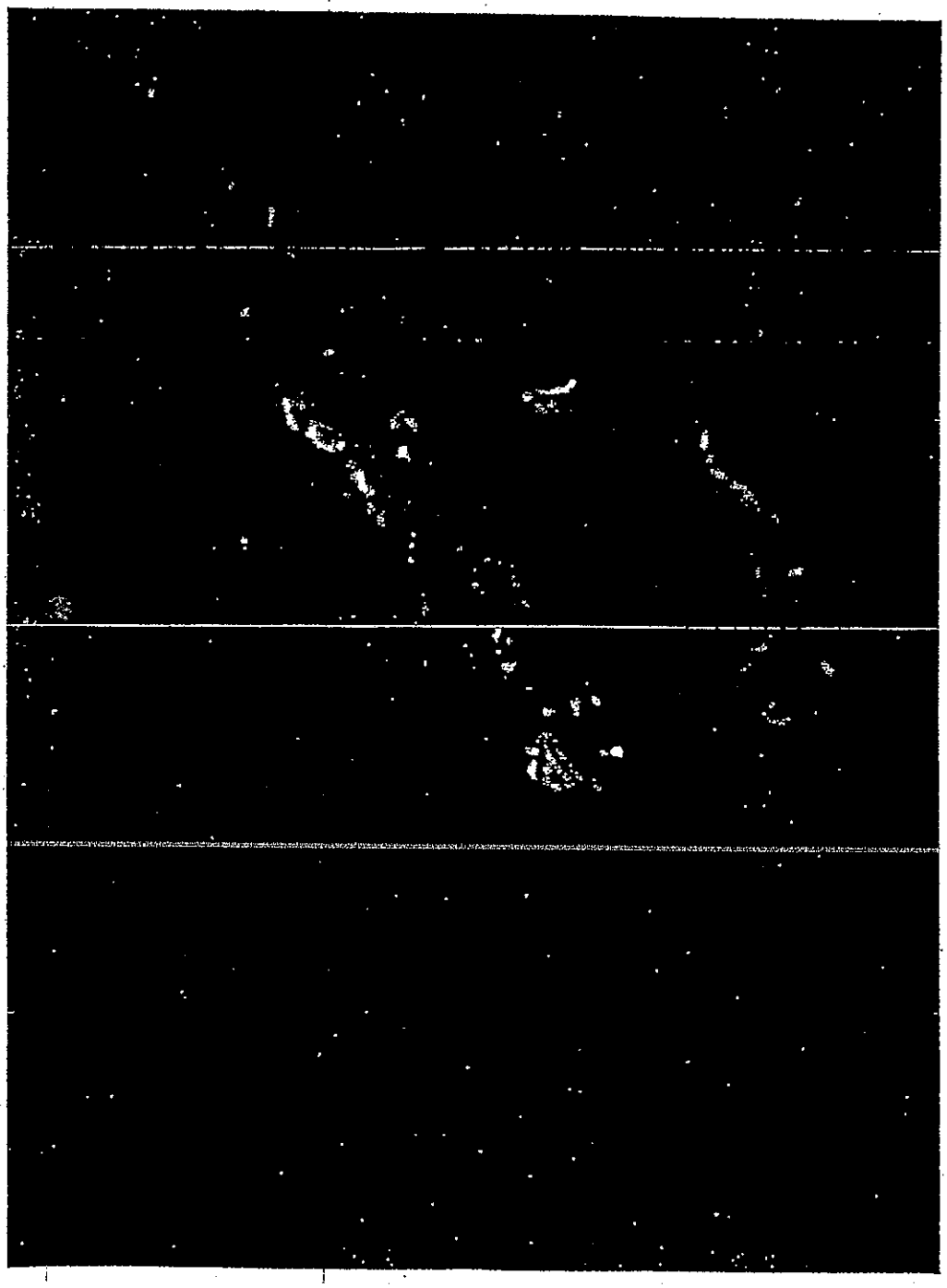


FIG. 13A

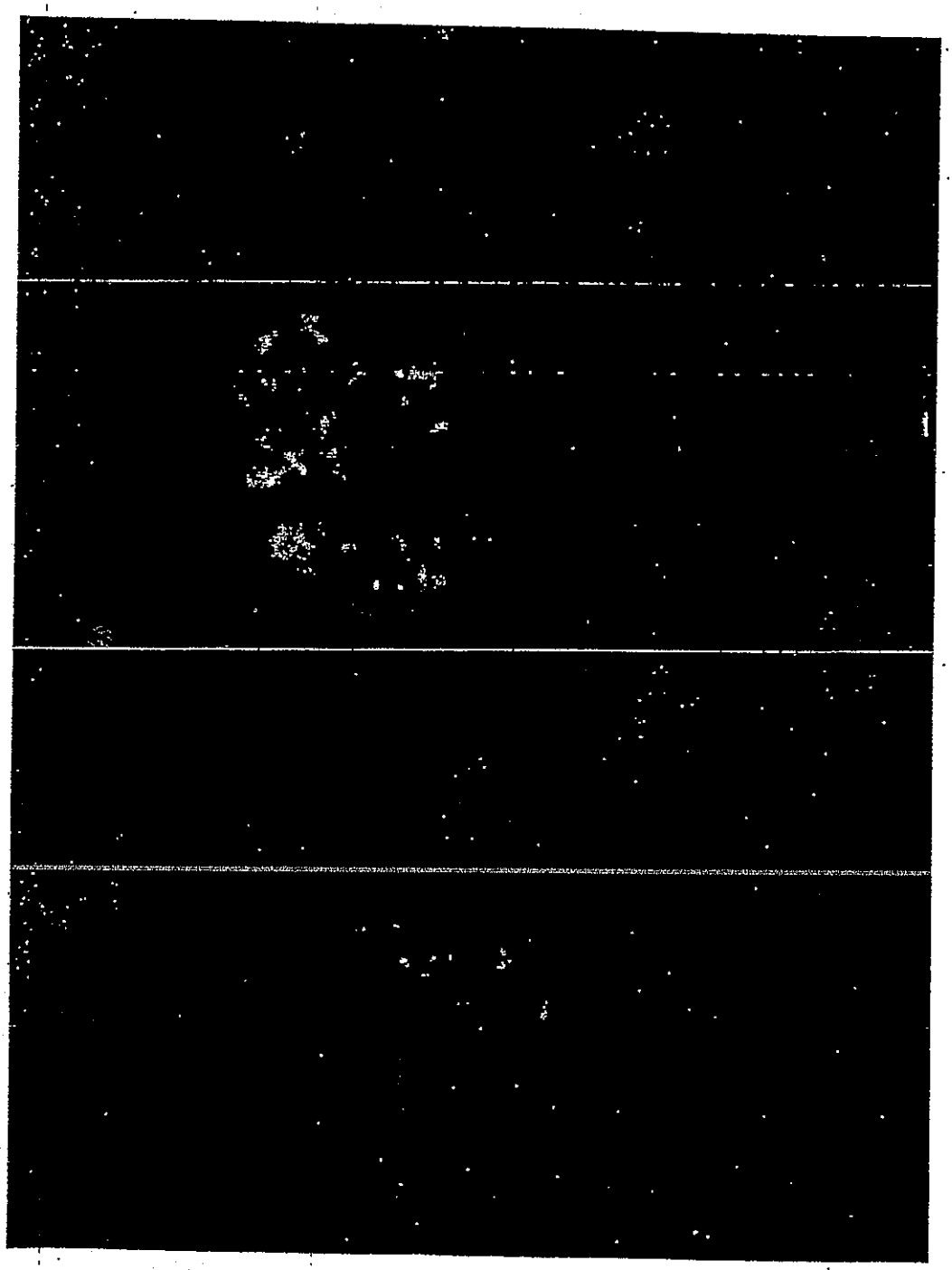


FIG. 13B

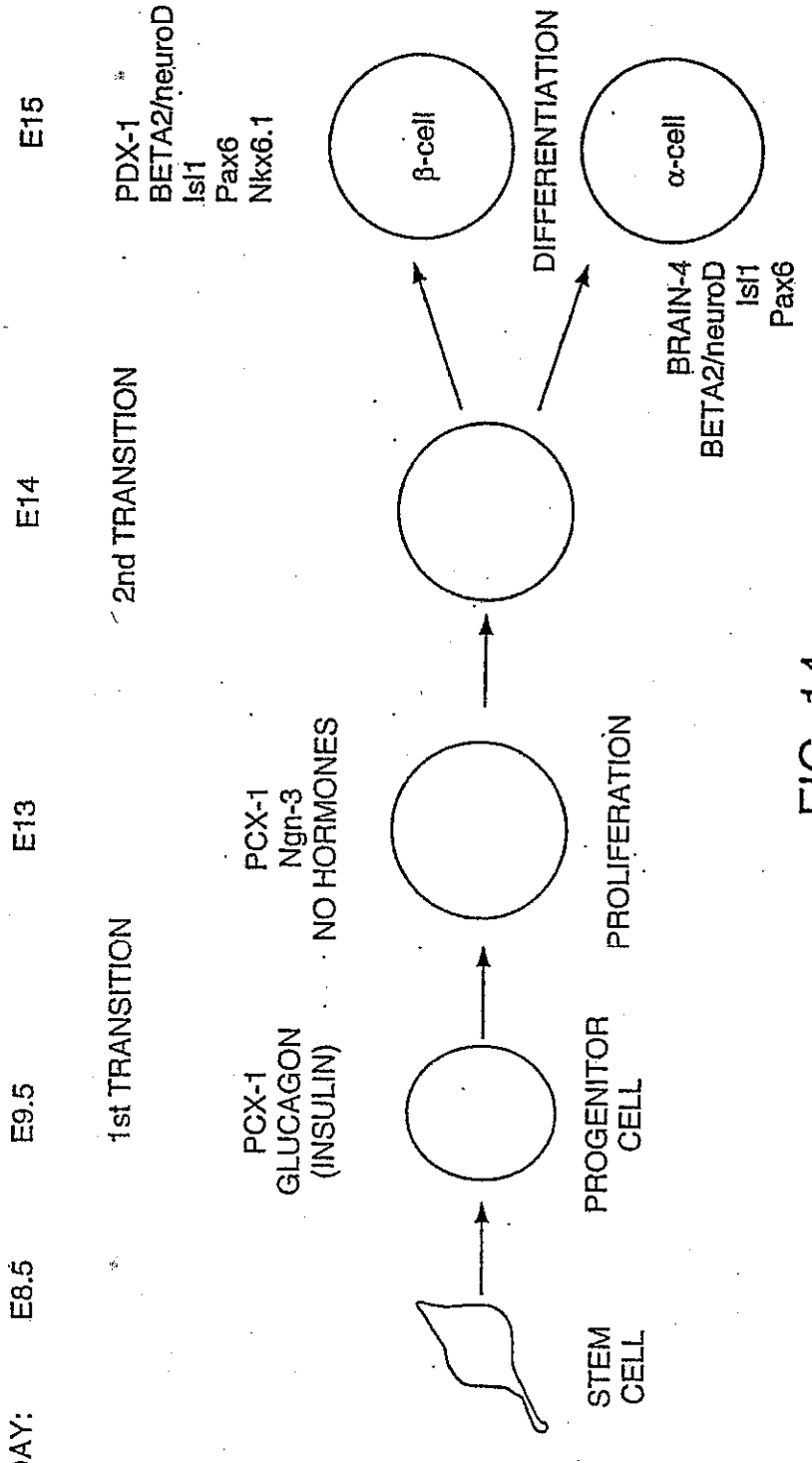

DAY:
E8.5
E9.5
E13
E14
E15
1st TRANSITION
2nd TRANSITION
PCX-1
GLUCAGON
(INSULIN)
PCX-1
Ngn-3
NO HORMONES
PDX-1
BETA2/neuroD
Isl1
Pax6
Nkx6.1
STEM
CELL
PROGENITOR
CELL
PROLIFERATION
DIFFERENTIATION
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α-cell
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BETA2/neuroD
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Pax6

FIG. 14

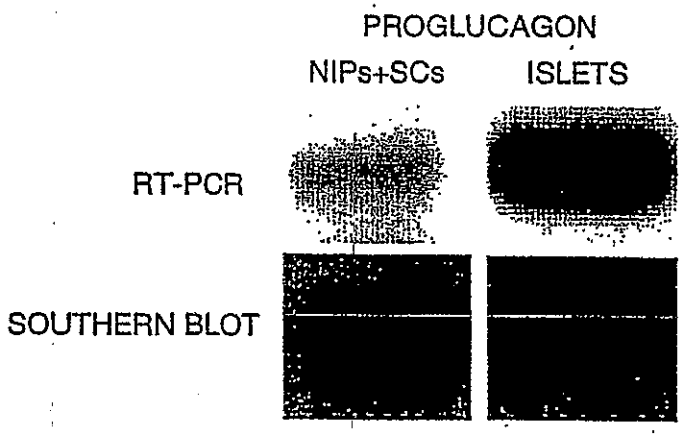


FIG. 15A

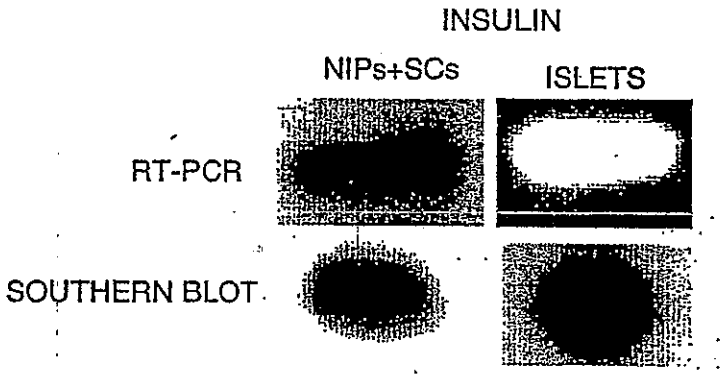


FIG. 15B

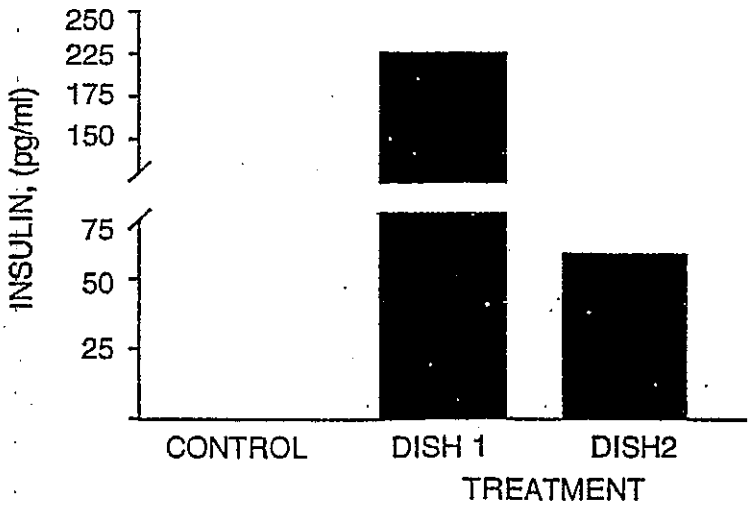


FIG. 15C

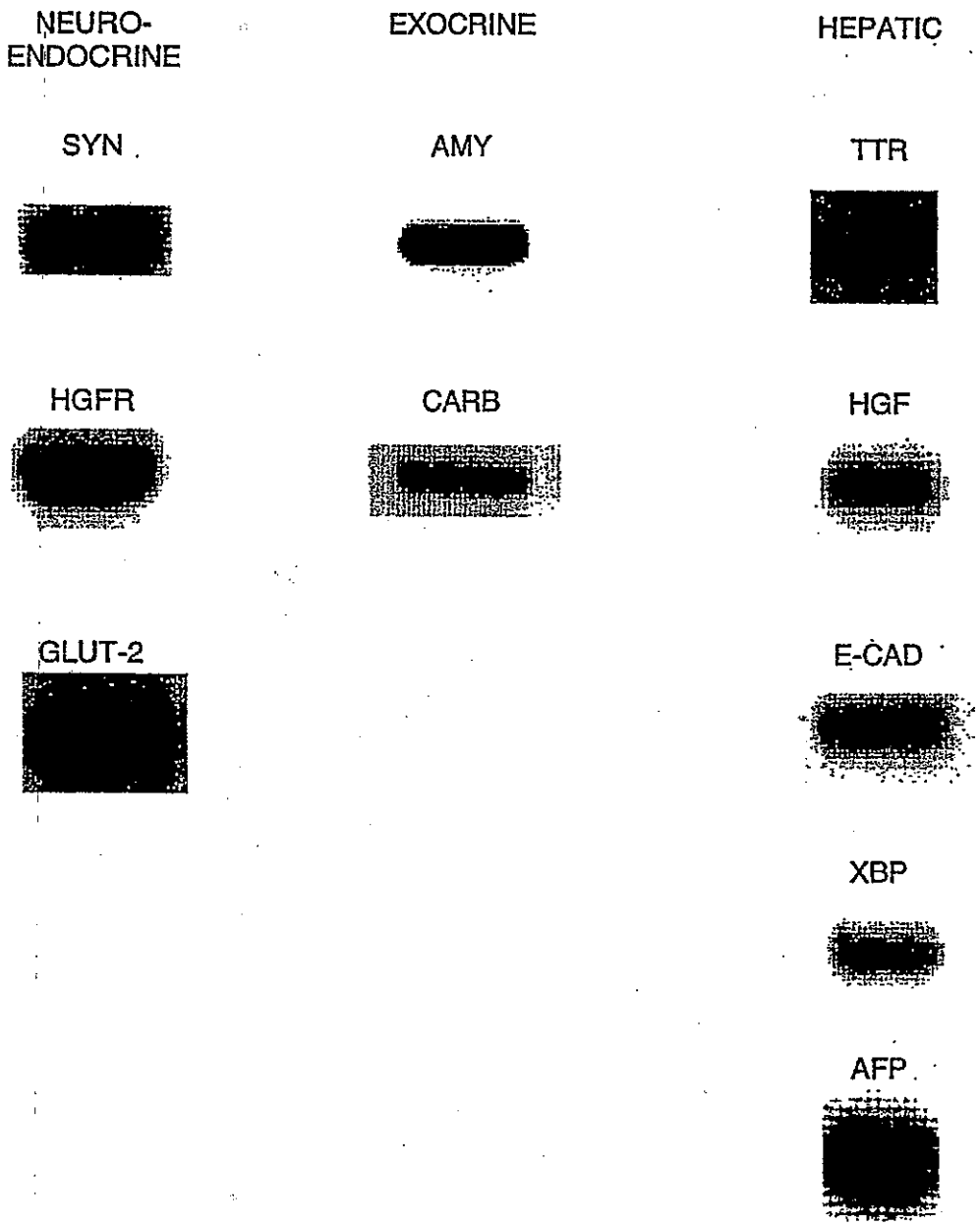

NEURO-ENDOCRINE
EXOCRINE
HEPATIC
SYN
AMY
TTR
HGFR
CARB
HGF
GLUT-2
E-CAD
XBP
AFP

FIG. 16

- ABCG2 (Bcrp1) ATP-binding cassette: SP stem cell marker
- Oct3/4: Pou/homeodomain transcription factor (ES cell marker)
- GLP-1 receptor: 7 MS GPCR
- Latrophilin (type 2): 7 MS GPCR
- Hes-1: bHLH transcription factor
- Nestin: intermediate filament protein
- Integrinα6 β1: laminen receptor (CD20)
- C-kit (CD117): stem cell factor receptor
- MDR-1: multidrug resistance transporter
- SST-R, 2, 3, 4: somatostatin receptors
- SUR-1: sulfonylurea receptor
- Kir 6.2: inward rectifying K^+ channel subunit with SUR-1
- Negative for CD34, CD45, CD133, MHC I & II

FIG. 17

A

TTTAGGAACGCACCGTGCACATGCTTGGTGGTCTTGTTAAGTGGAAACTGCTGCTTTAGAGTTTGTTTGG
AAGGTCCGGGTGACTCATCCCAACATTTACATCCTTAATTGTTAAAGCGCTGCCTCCGAGCGCACGCATC
CTGAGATCCTGAGCCTTTGGTTAAGACCGAGCTCTATTAAGCTGAAAAGATAAAAACTCTCCAGATGTCT
TCCAGTAATGTCGAAGTTTTTATCCCAGTGTCACAAGGAAACACCAATGGCTTCCCCGCGACAGCTTCCA
ATGACCTGAAGGCATTTACTGAAGGAGCTGTGTTAAGTTTTCATAACATCTGCTATCGAGTAAAACTGAA
GAGTGGCTTTCTACCTTGTCGAAAACCAGTTGAGAAAGAAATATTATCGAATATCAATGGGATCATGAAA
CCTGGTCTCAACGCCATCCTGGGACCCACAGGTGGAGGCAAATCTTCGTTATTAGATGTCTTAGCTGCAA
GGAAAGATCCAAGTGGATTATCTGGAGATGTTCTGATAAATGGAGCACCGCGACCTGCCAATTTCAAATG
TAATTCAGGTTACGTGGTACAAGATGATGTTGTGATGGGCACTCTGACGGTGAGAGAAAACTTACAGTTC
TCAGCAGCTCTTCGGCTTGCAACAACTATGACGAATCATGAAAAAAACGAACGGATTAACAGGGTCATTC
AAGAGTTAGGTCTGGATAAAGTGGCAGACTCCAAGGTTGGAACTCAGTTTATCCGTGGTGTGTCTGGAGG
AGAAAGAAAAAGGACTAGTATAGGAATGGAGCTTATCACTGATCCTTCCATCTTGTTCTTGGATGAGCCT
ACAACTGGCTTAGACTCAAGCACAGCAAATGCTGTCCTTTTGCTCCTGAAAAGGATGTCTAAGCAGGGAC
GAACAATCATCTTCTCCATTCATCAGCCTCGATATTCCATCTTCAAGTTGTTTGATAGCCTCACCTTATT
GGCCTCAGGAAGACTTATGTTCCACGGGCCTGCTCAGGAGGCCTTGGGATACTTTGAATCAGCTGGTTAT
CACTGTGAGGCCTATAATAACCCTGCAGACTTCTTCTTGGACATCATTAATGGAGATTCCACTGCTGTGG
CATTAAACAGAGAAGAAGACTTTAAAGCCACAGAGATCATAGAGCCTTCCAAGCAGGATAAGCCACTCAT
AGAAAAATTAGCGGAGATTTATGTCAACTCCTCCTTCTACAAAGAGACAAAAGCTGAATTACATCAACTT
TCCGGGGGTGAGAAGAAGAAGAAGATCACAGTCTTCAAGGAGATCAGCTACACCACCTCCTTCTGTCATC
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CATTGTCACAGTCGTACTGGGACTGGTTATAGGTGCCATTTACTTTGGGCTAAAAAATGATTCTACTGGA
ATCCAGAACAGAGCTGGGGTTCTCTTCTTCCTGACGACCAACCAGTGTTTCAGCAGTGTTTCAGCCGTGG
AACTCTTTGTGGTAGAGAAGAAGCTCTTCATACATGAATACATCAGCGGATACTACAGAGTGTCATCTTA
TTTCCTTGGAAAACTGTTATCTGATTTATTACCCATGAGGATGTTACCAAGTATTATATTTACCTGTATA
GTGTACTTCATGTTAGGATTGAAGCCAAAGGCAGATGCCTTCTTCGTTATGATGTTTACCCTTATGATGG
TGGCTTATTCAGCCAGTTCCATGGCACTGGCCATAGCAGCAGGTCAGAGTGTGGTTTCTGTAGCAACACT
TCTCATGACCATCTGTTTTGTGTTTATGATGATTTTTTCAGGTCTGTTGGTCAATCTCACAACCATTGCA
TCTTGGCTGTCATGGCTTCAGTACTTCAGCATTCCACGATATGGATTTACGGCTTTGCAGCATAATGAAT
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ATTCTGGAAACTCCTGACAAGTTATTACTGTCTCTGGCATTTGTTTCCTCATCTTTAAAATGAATAGGTA
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ATTAATGCTATACTGGAAATGTAAAATTGAAAATATGTTGGAAAAAAGATTCTGTCTTATAGGGTAAAAA
AAGCCACCGTGATAGAAAAAAAATCTTTTTGATAAGCACATTAAAGTTAATAGAACTT

FIG. 18 A

B

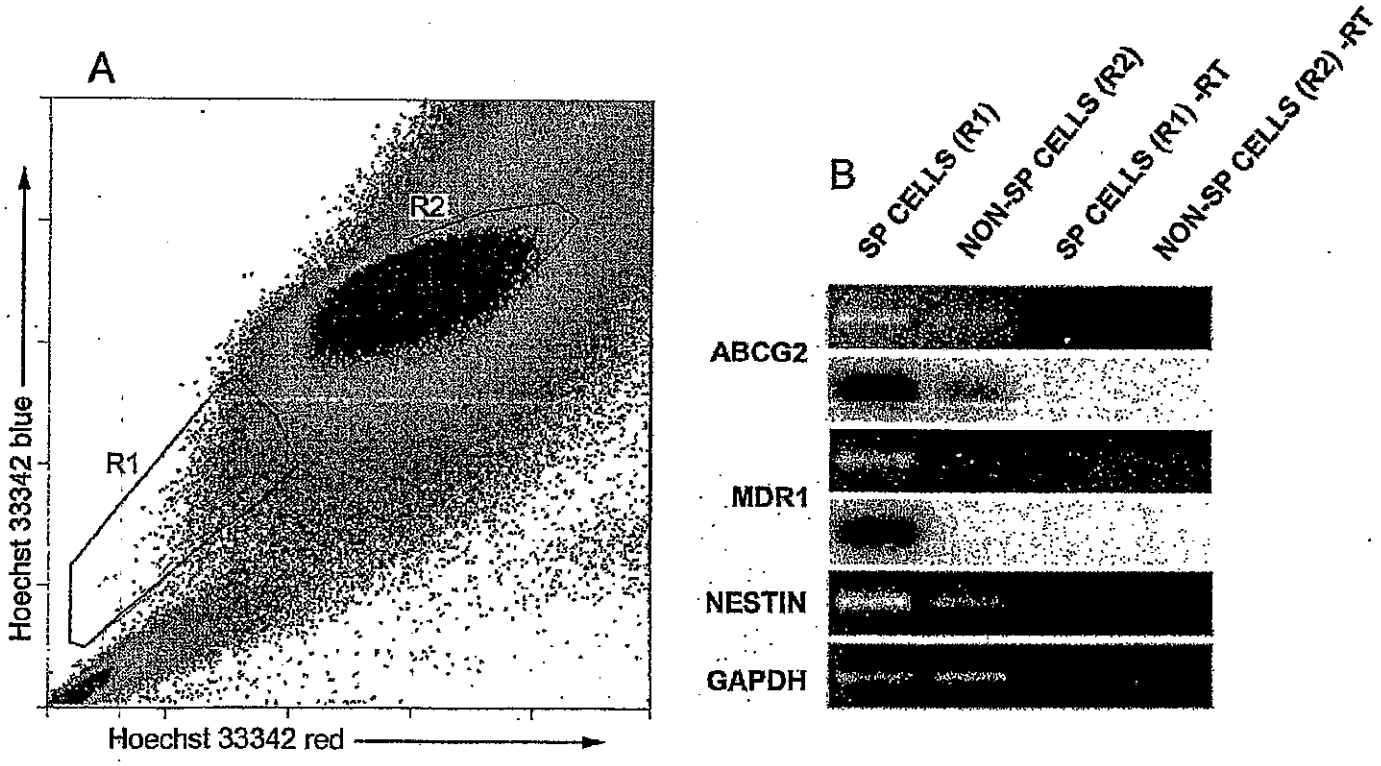
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INRVIQELGLDKVADSKVGTQFIRGVSGGERKRTSIGMELITDPSILFLDEPTTGLDS
STANAVLLLLKRMSKQGRTIIFSIHQPRYSIFKLFDSLTLLASGRLMFHGPAQEALGY
FESAGYHCEAYNNPADFFLDIINGDSTAVALNREEDFKATEIIEPSKQDKPLIEKLAE
IYVNSSFYKETKAELHQLSGGEKKKKITVFKEISYTTSFCHQLRWVSKRSFKNLLGNP
QASIAQIIVTVVLGLVIGAIYFGLKNDSTGIQNRAGVLFFLTTNQCFSSVSAVELFVV
EKKLFIHEYISGYYRVSSYFLGKLLSDLLPMRMLPSIIFTCIVYFMLGLKPKADAFFV
MMFTLMMVAYSASSMALAIAAGQSVVSVATLLMTICFVFMMIFSGLLVNLTTIASWLS
WLQYFSIPRYGFTALQHNEFLGQNFCPGLNATGNNPCNYATCTGEEYLVKQGIDLSPW
GLWKNHVALACMIVIFLTIAYLKLLFLKKYS

FIG. 18B

A
+RT
-RT
ABCG2
B
Hoechst 33342 blue
Hoechst 33342 red
2.1%
C
+ Verapamil
Hoechst 33342 blue
0.1%

Figure 19

FIG. 20


A
R1
R2
Hoechst 33342 blue
Hoechst 33342 red
B
SP CELLS (R1)
NON-SP CELLS (R2)
SP CELLS (R1) -RT
NON-SP CELLS (R2) -RT
ABCG2
MDR1
NESTIN
GAPDH

A.

atggccggcgccccggcccgctgcgccttgcgctgctgctcgggatggtgggcagggccggcccccgccccccagggtgccactg
tgtccctctgggagacggtgcagaaatggcgagaataccgacgccagtgccagcgctccctgactgaggatccacctcctgccacagact
tgttctgcaaccggaccttcgatgaatacgcctgctggccagatggggagccaggctcgttcgtgaatgtcagctgccccctggtacctgccc
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cagcagcatgaagcccctcaagtgtcccaccagcagcctgagcagtggagccacggcgggcagcagcatgtacacagccacttgccag
gcctcctgcagctgagactccagcgcctgccctccctggggtccttgctgcaggccgggtggccaatccaggagaagcagcctcctaattt
gatcacagtggcgagaggagaggaaaaacgatcgctgtgaaaatgaggaggattgcttcttgtgaaaccacaggcccttggggttccccc
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agcgaaatcatggttgtagtgatgttg

B

MAGAPGPLRLALLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSLTEDPPPA
TDLFCNRTFDEYACWPDGEPGSFVNVSCPWYLPWASSVPQGHVYRFCTAEGLWLQKD
NSSLPWRDLSECEESKRGERSSPEEQLLFLYIIYTVGYALSFSALVIASAILLGFRHLHCTR
NYIHLNLFASFILRALSVFIKDAALKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQ
YCVAANYYWLLVEGVYLYTLLAFSVFSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYE
DEGCWTRNSNMNYWLIIRLPILFGIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLAKST
LTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAILYCFVNNEVQLEFR
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FIG. 21

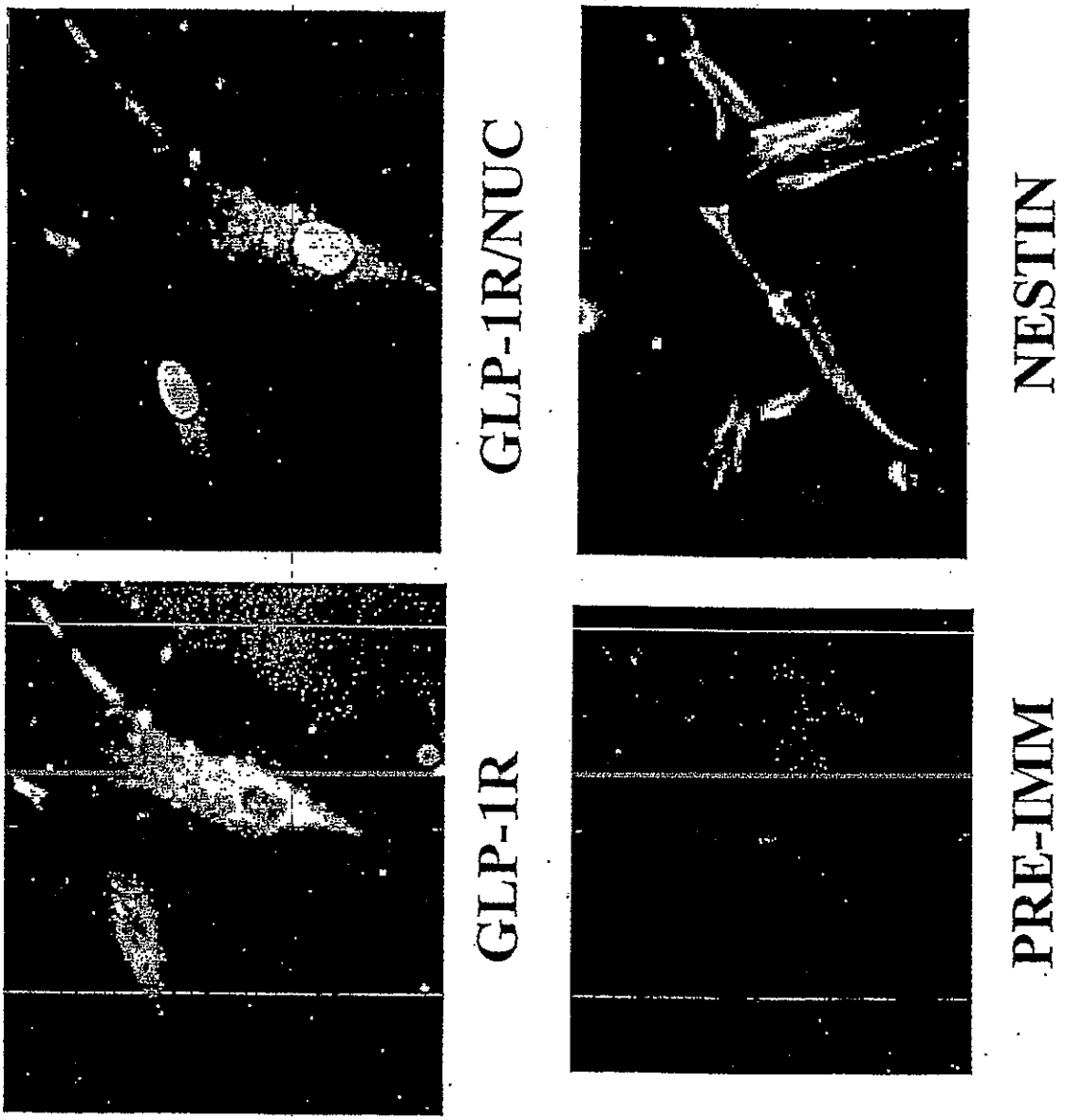


FIG. 22A

24 MAR 2006

B

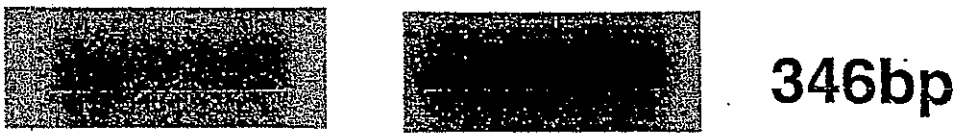


FIG. 22B

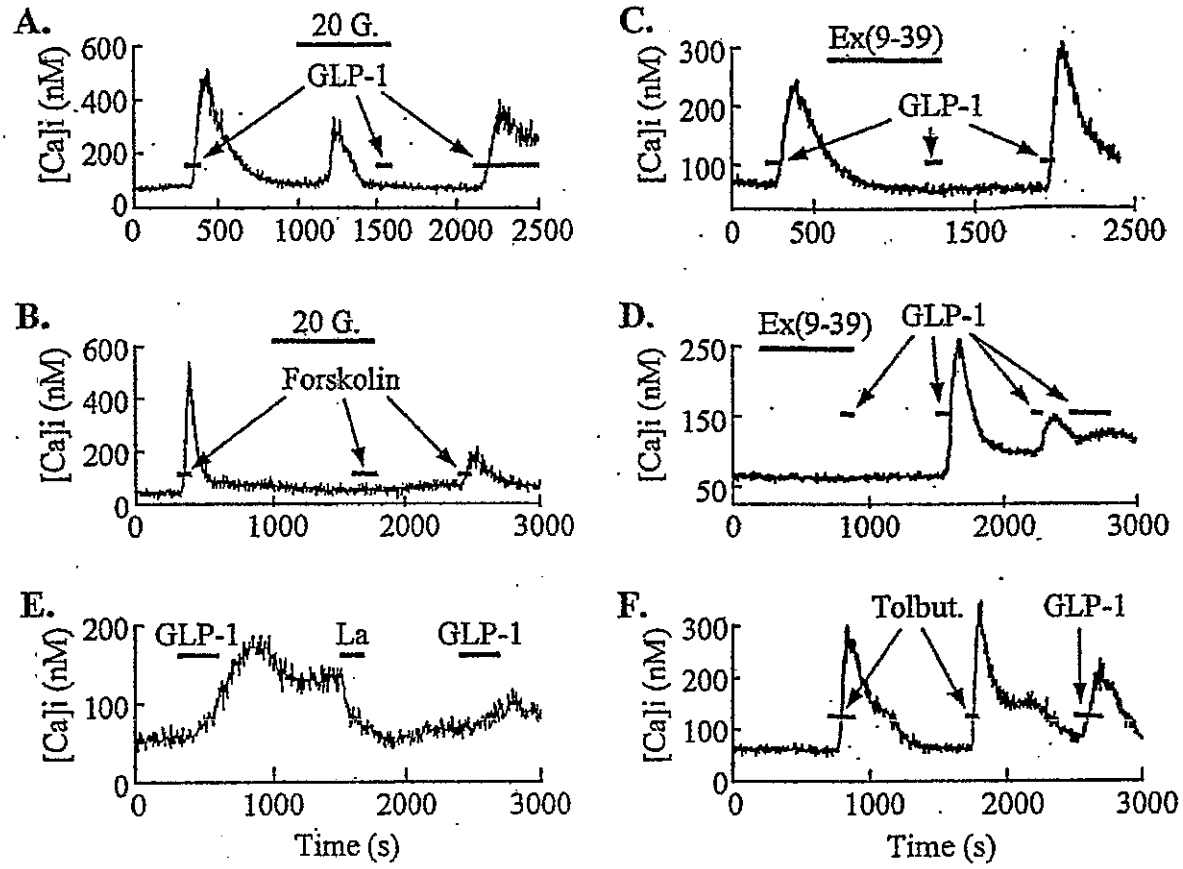


FIG. 23

Figure . GLP-1(7-36)amide and Tolbutamide stimulate $[Ca^{2+}]_i$ influx in stem cells.

(A) Fura 2 loaded cells bathed in 5.6 mM glucose show a $[Ca^{2+}]_i$ increase in response to 10 nM GLP-1. Increasing the extracellular glucose to 20 mM (20 G) also caused an increase of $[Ca^{2+}]_i$ but application of GLP-1 in 20 mM glucose failed to produce a $[Ca^{2+}]_i$ response. A third application of GLP-1 on returning to 5.6 mM glucose produced a $[Ca^{2+}]_i$ response. **(B)** The glucose-dependent effects of GLP-1 were reproduced by 10 mM forskolin, suggesting that $[Ca^{2+}]_i$ elevation is cAMP-mediated. **(C)** The GLP-1 mediated increase of $[Ca^{2+}]_i$ was reversibly inhibited by 10 nM exendin (9-39). This effect is not due to receptor desensitization **(D)** as application of GLP-1 in the presence of exendin (9-39) failed to produce a response whereas subsequent applications of GLP-1 after washout of exendin produced repeated $[Ca^{2+}]_i$ elevations. **(E)** The GLP-1-mediated increase of $[Ca^{2+}]_i$ is inhibited by 0.5 mM extracellular La^{3+}, suggesting that GLP-1 stimulates Ca^{2+} influx. **(F)** Stem cells bathed in 5.6 mM glucose were stimulated with 100 μM tolbutamide (Tolbut.) and respond to repeated applications with increases in $[Ca^{2+}]_i$. Application of 10 nM GLP-1 also stimulates an increase of $[Ca^{2+}]_i$ suggesting that GLP-1 acts by depolarizing the cells.

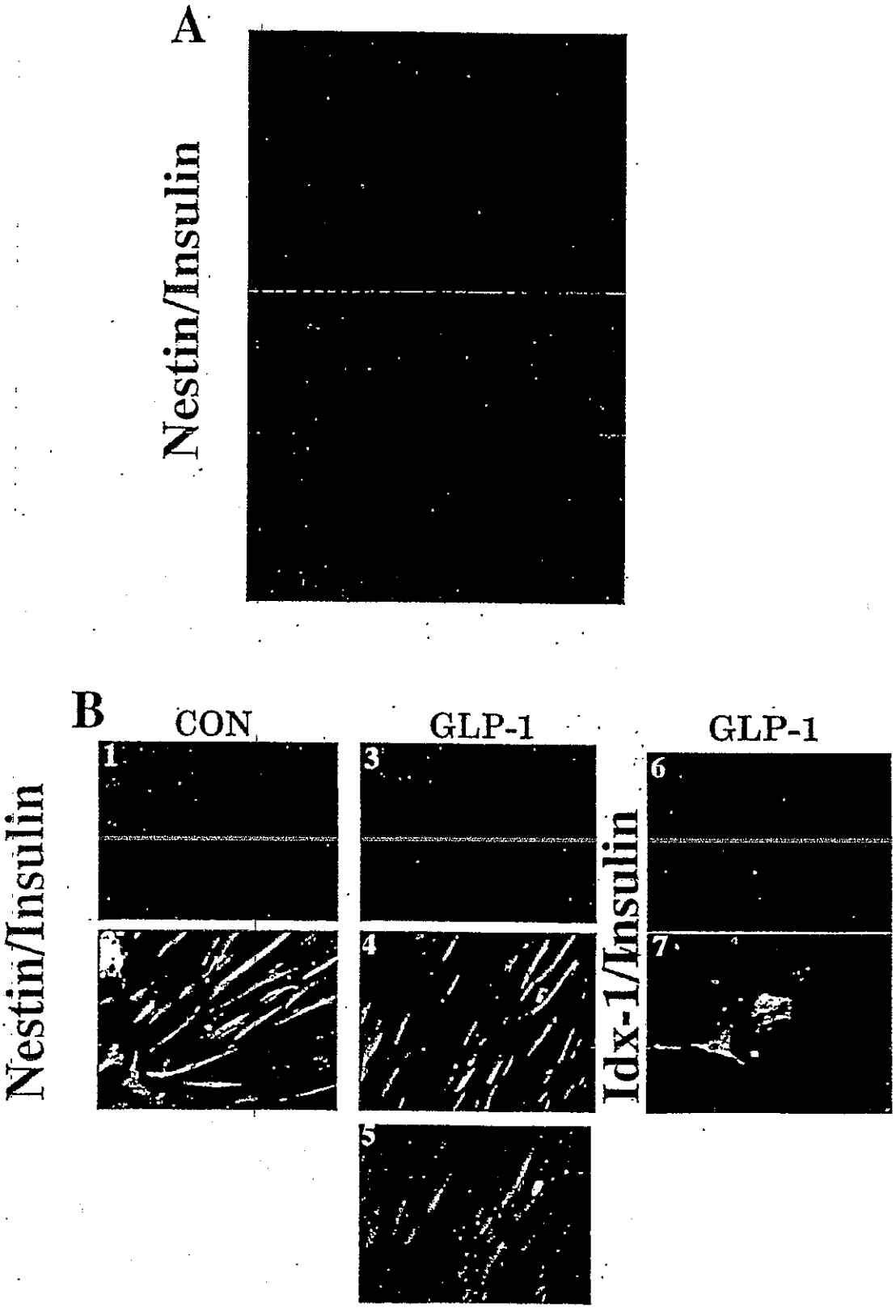

A
Nestin/Insulin
B
CON
GLP-1
GLP-1
Nestin/Insulin
Idx-1/Insulin
1
3
6
4
7
5

FIG. 24

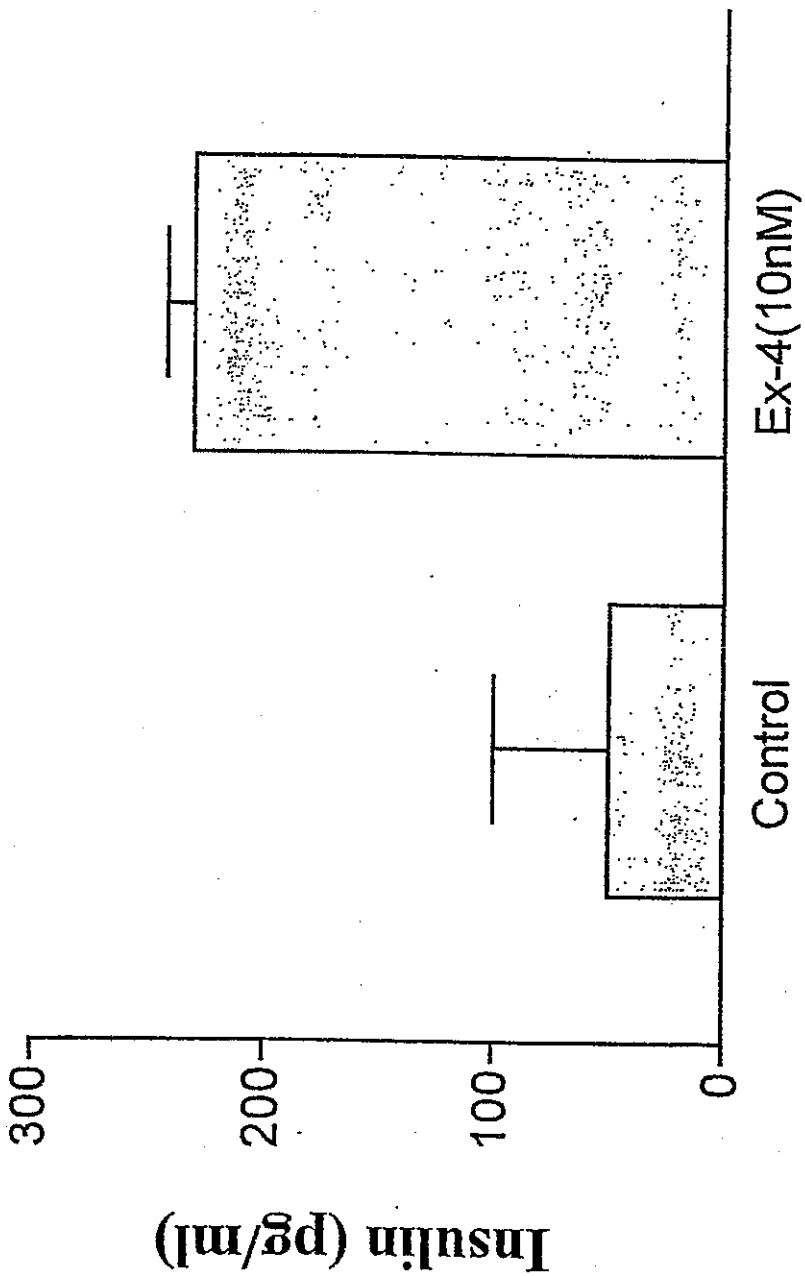


FIG. 25

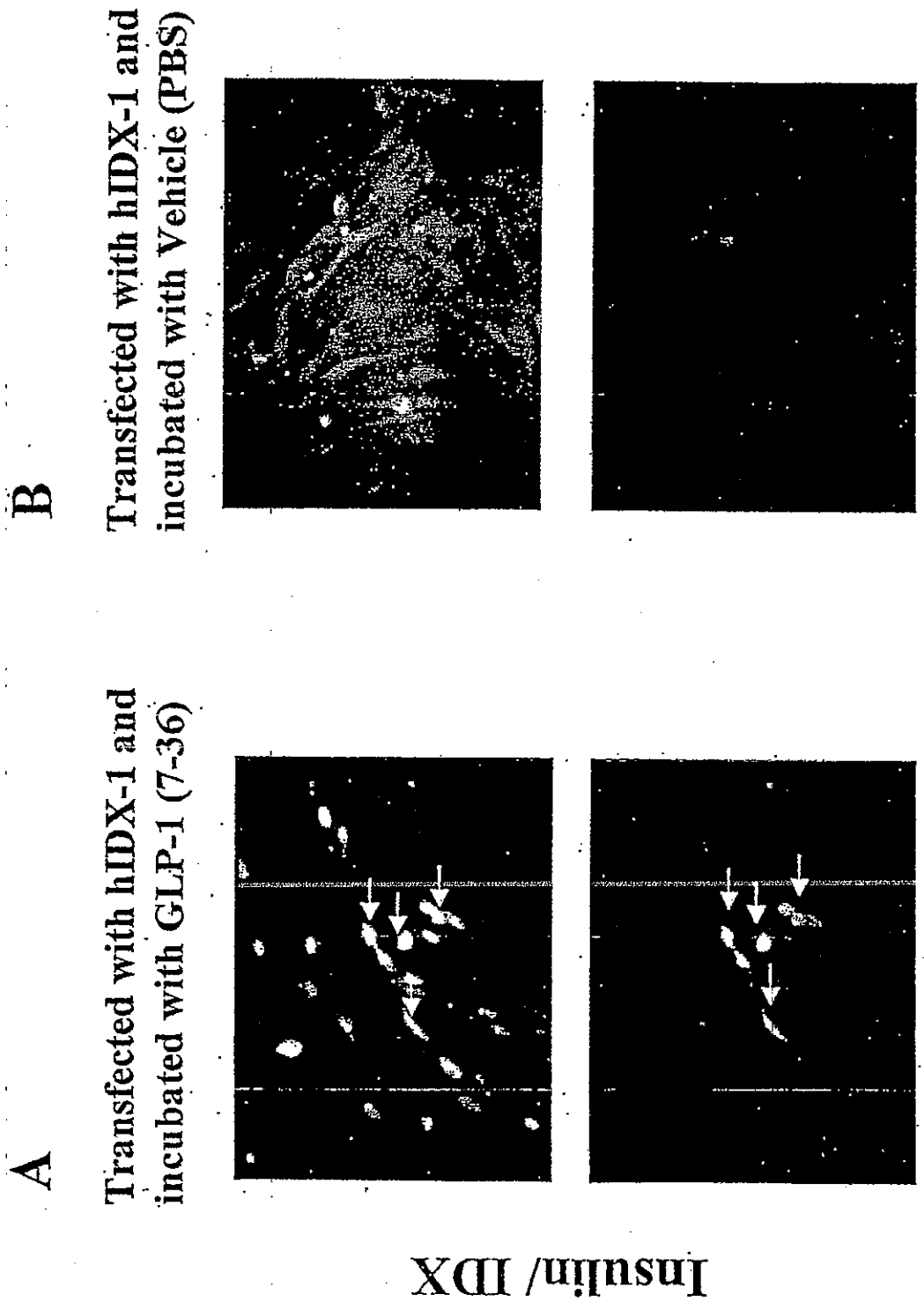

A
Transfected with hIDX-1 and incubated with GLP-1 (7-36)
B
Transfected with hIDX-1 and incubated with Vehicle (PBS)
Insulin/ IDX

FIG. 26