Supplementary Methods

*In situ* hybridization

Adult or postnatal day 11 Sprague Dawley rats were perfused for 20 minutes with 4% paraformaldehyde in 0.1M sodium borate buffer, pH 9.5. Brains were postfixed in the perfusate for 3-4 days at 4°C, cryoprotected in 15% sucrose/0.1M phosphate buffer pH 7.2 and then frozen on dry ice and stored at −70°C. Sagittal sections (30 μm) were cut on a sliding microtome and mounted on gelatin and poly-L-lysine coated slides. The pre- and post-hybridization procedures were performed as described in Simmons et al., 1989, except for the inclusion of a 30 minute post-fixation step of the tissue sections using 10% buffered formalin followed by four 5 minute washes in 0.05 M KPBS (0.15 M NaCl, 0.04 M KH₂PO₄, 0.01 M K₂HPO₄) prior to the pre-hybridization steps. Transcriptions were performed using 125 Ci ³³P-UTP (2000-4000 Ci/mmole, either NEN or ICN). After hybridization, the sections were defatted in xylene, rinsed first in 100% ethanol, then 95% ethanol, air dried and dipped in NTB2 emulsion (Kodak) diluted 1:1 with water. The slides were exposed for 2-5 weeks and developed in Kodak D-19 developer. For the ErbB4 exon 2 hybridizations, tissues were prepared from either postnatal day 12 (nestin-cre; F/−) or (hGFAP-cre; F/−) conditional knockout and control mice. All images were captured with a Hamamatsu Orca ER CCD camera using darkfield microscopy on an Olympus BX-51 microscope at 1.25 X magnification. The relative levels of expression were determined by reviewing multiple sections for each probe. We performed these analyses using sections prepared from between 2-5 rats per probe.

All constructs were generated by RT-PCR from rat brain cDNA, except for the ErbB4 murine exon-2 specific construct, as described below: Amplified products were subcloned into EcoRI/BamH1 cut pBluescript SK(−), unless otherwise noted.
**EGFR**- spans sequence #2909-3790 in accession# M37394, the rat EGFR cDNA sequence. Amplified products were subcloned into the EcoRV/SmaI site of pBluescript KS(+). After linearization with Not I, antisense transcripts were produced using T3 RNA polymerase.

**ErbB2**- spans sequence #3184-3896 in accession# AY116182, the rat ErbB2 cDNA sequence. Amplified products were subcloned into the SrfI site of pCRScript. After linearization with Xho I, antisense transcripts were generated using T3 RNA polymerase.

**ErbB3**- spans sequence #3276-4158 in accession # NM_017218, the rat ErbB3 cDNA sequence. Amplified products were subcloned into EcoRV/SmaI cut pBluescript (KS+). After linearization with Not I, antisense transcripts were produced with T3 RNA polymerase.

**ErbB4**- spans sequence #1009-1931 in accession # NM_021687, the rat ErbB4 cDNA sequence. Amplified products were subcloned into the SrfI site of pCRScript. Linearization with Not I permits the production of antisense RNAs using T7 RNA polymerase.

**ErbB4 (exon-2 specific)**- corresponds to sequence #114-267 in accession # NM_021687, which contains all of exon 2. Note that this construct was amplified from mouse brain cDNA and subcloned into pGEM-T. Linearization with Nco I permits antisense production using SP6 RNA polymerase.

**NRG1 (Ig-Types I/II)**- spans sequence #345-845 in accession # NM_031588, one of the rat neuregulin-1 Ig-domain-containing isoforms. Linearization with Spe I permits production of antisense RNAs with T7 RNA polymerase.

**NRG1 (SMDF-Type III)**- spans sequence #555-1321 in accession #AF194438, a rat neuregulin-1 SMDF isoform. Linearization with EcoRI permits production of antisense RNAs with T3 RNA polymerase.
NRG2- spans sequence #586-1336 in accession #D89996, the rat NTAK-a2 cDNA sequence. Linearization with EcoRI permits production of antisense RNAs with T3 RNA polymerase.

NRG3- spans the rat equivalent of sequence #940-1503 in accession #NM_008734, the murine NRG3 cDNA sequence. Amplified products were subcloned into pCR2.1. Linearization with HindIII permits antisense production with T7 RNA polymerase.

EGF- spans sequence #2651-3554 in accession #NM_012842, the rat EGF cDNA sequence. Linearization with BamH1 permits antisense production with T7 RNA polymerase.

Epiregulin- spans sequence #63-684 in accession #AF074952, the rat epiregulin cDNA sequence. Linearization with Not I permits antisense production with T7 RNA polymerase.

Betacellulin- spans sequence 187-492 in accession #NM_022256, the rat betacellulin cDNA sequence. Linearization with EcoRI permits antisense production with T3 RNA polymerase.

TGF-α- spans sequence #179-892 in accession #M31076, the rat TGF-a cDNA sequence. Linearization with BamH1 permits antisense production with T7 RNA polymerase.

HB-EGF- spans sequence #76-647 in accession #L05489, the rat HB-EGF cDNA sequence. Generated by PCR amplification using an antisense primer containing a T7 promoter sequence (AATTGTAATACGACTCACTATAGGCGC), that permitted antisense RNA production.
**Antibodies and histology**

The following primary antibodies were used: an affinity-purified polyclonal anti-ErbB4 antibody #0616 (affinity purification accomplished using reagents from Sterogene, C. Lai); monoclonal anti-ErbB4 antibody (Ab-1, Neomarkers), anti-NeuN (Chemicon), TuJ1 (Berkley Antibodies), polyclonal class-III β-tubulin (Covance), monoclonal anti- PSA-NCAM (gift from Dr. G. Rougon, Univ. Aix-Marseille II, France), monoclonal anti-dlx-2 (gift from Dr. D. Eisenstat, Univ. Manitoba), anti-BrdU (Becton-Dickenson), monoclonal anti-SSEA1/LeX (Developmental Hybridoma Bank, Iowa), anti-GABA (Chemicon), anti-GAD65 (Chemicon), anti-calbindin (Chemicon), anti-calretinin (Chemicon), anti-tyrosine hydroxylase (Chemicon), and anti-GFAP sera (Dako and Chemicon).

Wholemount brains, sections of brain or cultured neural cells were immunolabeled with various primary antibodies as described earlier. Immunoreactivity was detected with biotin, Cy2 or Cy3 conjugated anti-mouse or anti-rabbit secondary antibodies (Jackson Immunoresearch). Tissue sections were processed for electron microscopy as described in Doetsch et al., 1997, and cellular organization of the RMS was compared between controls and ErbB4 mutants (n=3).

**Construction of full-length Neuregulin 1 cDNA clones and cell transfections**

Full-length murine NRG1 cDNA clones were assembled and inserted into a modified version of pEGFP (Clontech) to replace the existing EGFP coding region. A full-length murine NRG1 type III cDNA was obtained by screening an adult murine brain λZAP cDNA library. The type III cDNA was amplified in 2 parts and inserted into this modified vector at the Sal I site (part of a newly inserted polylinker). The full-length murine NRG1 type I sequence was assembled by using the shared 3′ region of the type III cDNA, which encodes the transmembrane and cytoplasmic tail regions (the long NRG1 type “a” tail), and generating a type I-specific 5′ region by amplifying from adult murine brain cDNA. Both of these clones contain a beta-1 type of EGF-like domain. The nucleotide sequences of the full-length clones were determined to verify that PCR-introduced mutations were not present. The open reading frames encoded by these 2 cDNA clones have been previously used to generate transgenic mice.
that have produced neuregulin type I and type III proteins\(^ {18}\). The GenBank accession numbers are AY648975 ("type III") and AY648976 ("type I"). These plasmids were transfected into COS cells using the transfection reagent Effectene (Qiagen).

**Mice**

All knockout mice analyzed were floxed ("F")/– with regard to ErbB4 genotype. The null ("–") ErbB4 allele has been previously described\(^ {25}\). The mutant genotypes utilized were nestin-cre; ErbB4 (F/–) and hGFAP-cre; ErbB4 (F/–). Although grossly normal in appearance, the nestin-cre ErbB4 (F/–) mice have been reported to exhibit reduced body weight as weanlings, and have deficits in spontaneous motor activity and grip strength\(^ {24}\). Our yield of conditional knockout nestin-cre; ErbB4 (F/–) or hGFAP-cre; ErbB4 (F/–) animals at the weaning stage was lower than the expected Mendelian ratios (14% or 20%, respectively, vs. 25%), suggesting some loss either in utero or during the early postnatal period. These animals appeared to be in good health with no obvious selective loss of conditional knockout mice compared to littermate controls post-weaning. Volumetric measurements indicate that there is approximately a 14% reduction in the volume of olfactory bulb (estimated average volume: hGFAP-cre; F/+ = 6.08 ± 0.24 mm\(^3\); hGFAP-cre; F/– = 4.64 ± 0.7 mm\(^3\), n=10). Genotypes of the nestin-cre and GFAP-cre mice were assessed by PCR as described earlier\(^ {26}, 28\). For PCR analyses of the ErbB4 alleles, the following set of primers were used: (#1) 5’-TAT TGT GTT CAT CTA TCA TCA TTG CAA CCC AG-3’, (#2) 5’-CAA ATG CTC TCT CTG TTC TTT GTG TCT G-3’, (#3) 5’-TTC TGC CAA GGT ATG TTA TTA TCA GAA GC-3’. Primers 1 and 2 amplify the wild type allele (~ 320 bp) as well as the Floxed allele (~ 370 bp). Primers 2 and 3 amplify the null allele (~ 225 bp).

**In vitro SVZ explant migration assays**

The brains from adult mice were dissected and placed in ice cold Basal Medium (Invitrogen/Gibco). The SVZ from the anterior horn region of the lateral ventricles was excised and cut into 100 m thick pieces using a McIlwain tissue chopper. Explants were mixed with gel matrix (Collaborative) and plated onto a
glass coverslip in the center of a 35 mm petri dish and maintained in NB (Neurobasal)/B27 medium (Gibco) for 36 hours. The extent and pattern of neuronal migration away from these explants were analyzed using a Zeiss Axiovert microscope equipped with 37°C microincubator stage and the ORCA CCD camera [Hamamatsu]. Neuronal chain formation and isolated cell scattering around the explants were evaluated using the NIH Image.1 program.

**Analysis of BrdU positive cell distribution in distinct layers of the olfactory bulb**

Following the 30 day survival period after BrdU injections, mice were perfused intracardially with 4% paraformaldehyde, brains were cryoprotected through graded solutions of sucrose (10-30%), cut sagittally at 50 m, and immunolabeled with anti-BrdU (Becton-Dickenson). The distribution of BrdU-positive cells in distinct layers of the olfactory bulb was quantified and comparisons were made between control and ErbB4 mutant groups (n=5).

**Analysis of chemotropic or guidance role of NRG1**

To determine if NRG1–type I or type III isoforms provided a permissive substratum for migration, neuroblasts emigrating from SVZ explants were given a choice of normal or NRG1-expressing substrates. COS cells were co–transfected with NRG1 isoforms and EGFP plasmid DNA (pEGFP-N; Clontech) as described in Gongidi et al., 2004. Transfected and mock-transfected cells were plated on adjacent sides of 500µm wide parafilm barriers on collagen coated glass coverslips. After one hour, the barriers were removed and non-adherent cells were washed away in MEM (Gibco) to generate stripes of mock and NRG1–expressing cells. SVZ explants (from 2 week old mice) were labeled with CMTMR for 15 minutes, washed 4 times with MEM/5% horse serum, and then placed between these cell strips to evaluate any preference for a neuregulin-rich cellular substratum by neuroblasts migrating out of the SVZ explants. The percentage of CMTMR–labeled cells emigrating from the explants onto the respective substrates were quantified and used as index of substrate preference.
Analysis of process arborization in olfactory glomeruli

In order to quantify changes in calbindin positive processes within the glomeruli of ErbB4 conditional null and control mice, images of single glomerulus were captured using a Zeiss Pascal confocal microscope. For each glomerulus, the total area and the area occupied by the calbindin positive processes within the glomerulus was measured using the MetaMorph image analysis software. A process arborization index was calculated as area occupied by the calbindin positive processes/ total area. To quantify branch points of individual, optical stacks (0.4µm interval) through single calbindin positive cells within the periglomerular layer were captured at high magnification (63X oil objective) using a Zeiss LSM510 confocal microscope. The multi-layer confocal images were deconvoluted to create three-dimensional images of the interneurons. All branches of the processes of individual CB+ cells were then counted. Quantitative analysis was based on 6 animals (10 glomeruli/animal) each for cre; F/-, and cre; F/+ groups.