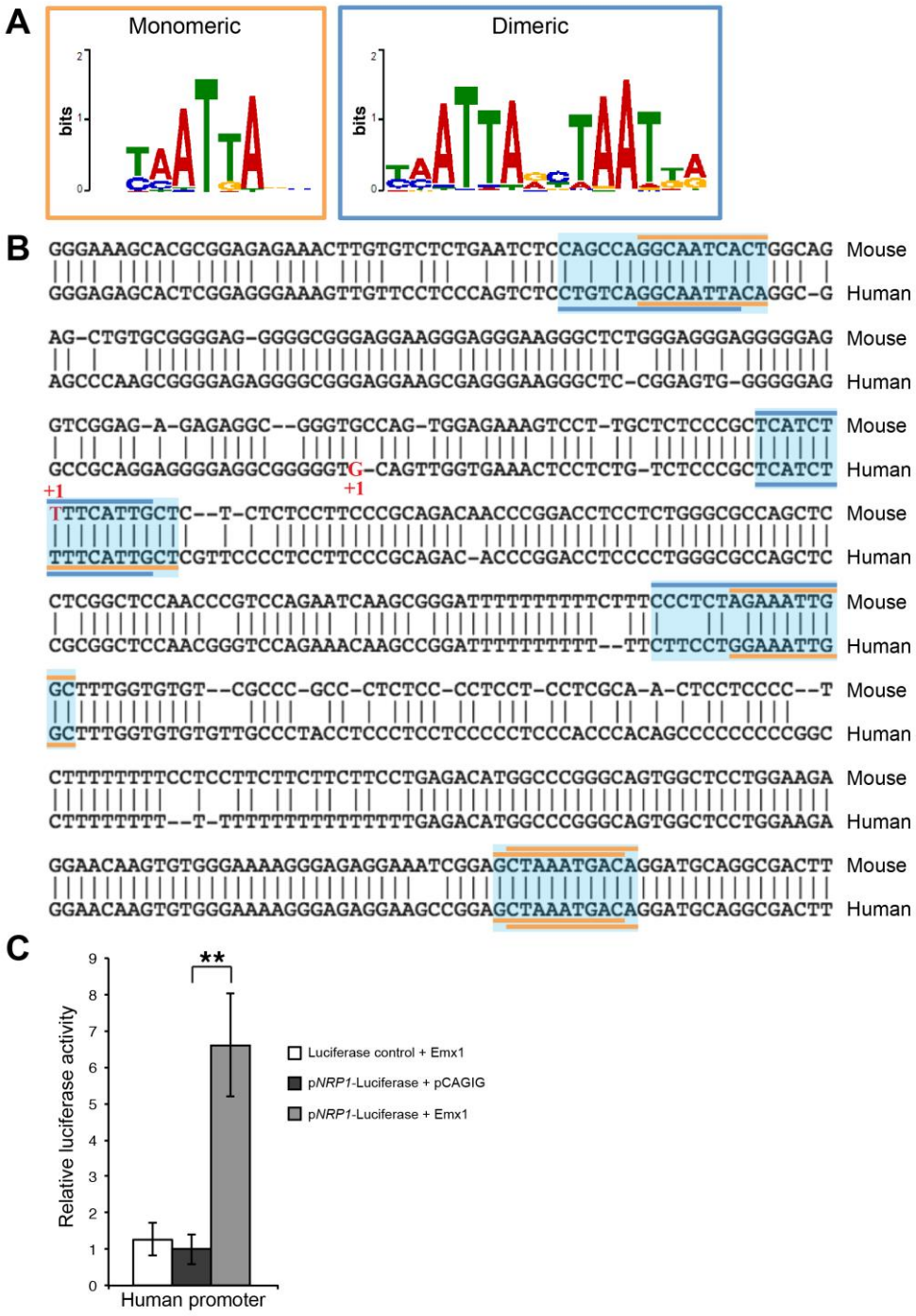

Figure S1: EMX1 over-expression up-regulates human *NRP1* promoter activity.

Monomeric and dimeric consensus EMX1 binding sites represented as position weight matrices (EMX1_DBD and EMX1_DBD_2 respectively from Jolma et al., 2013) were used to identify putative EMX1 binding sites within the mouse and human *Nrp1* promoter and untranslated regions (A). In a 643 base pair region of the mouse

Nrp1 locus (chr8:130,882,800-130,883,258 obtained from NCBI37/mm9) that is conserved in humans (chr10:33,623,535-33,623,944 obtained from GRCh37/hg19) with 83% identity, four highly conserved sequences (shaded blue) with EMX1 binding sites were identified (B). Either monomeric (orange lines) or dimeric (blue lines) consensus EMX1 binding sites were detected at identical positions in the mouse and human promoters ($p<0.01$). Nucleotides in red, denoted +1, represent the respective transcriptional start sites. Dual-luciferase reporter assays demonstrate significant activation of the human *NRP1* promoter with EMX1 over-expression, normalized to over-expression of the empty pCAG-IRES-GFP control (C). ** $p<10^{-6}$ for two-sided Student's *t*-test. Results are mean±s.d. from $n=3$ independent experiments.

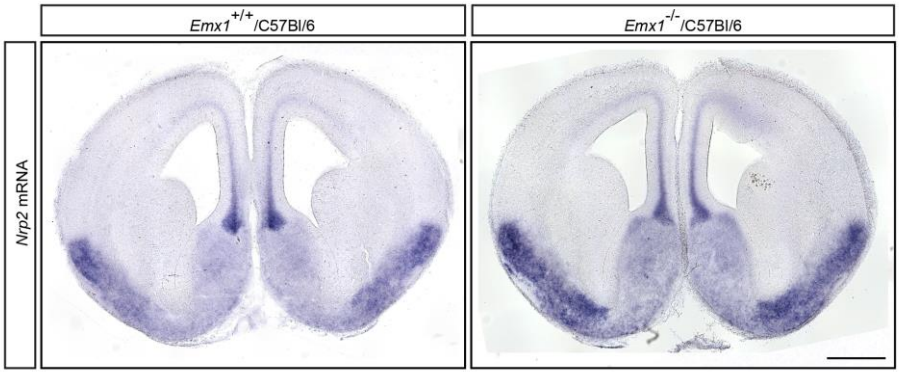

Figure S2: *Nrp2* mRNA is expressed in the anterior cingulate cortex of *Emx1*$^{+/+}$/C57Bl/6 and *Emx1*$^{-/-}$/C57Bl/6 mice. *Nrp2 in situ* hybridization in coronal brain sections from E15 *Emx1*$^{+/+}$/C57Bl/6 and *Emx1*$^{-/-}$/C57Bl/6 mice (minimum *n*=3 per genotype). Scale bar = 500 μm.

Table S1: Cloning primers for generating mouse *Nrp1* promoter constructs.

Name (see Fig. 7 for schematic)	Forward primer	Reverse primer
pNrp1	5'-TATAACTAGTCTGGCACTAGGAGACCCTTG-3'	5'-TATACTCGAGCCCCTCTCCATCCTCCTTC-3'
prom Δ*s1*	5'-TATAACTAGTCTGGCACTAGGAGACCCTTG-3'	5'-TATACTCGAGTTTCTCTCCGCGTGCTTTC-3'
prom	5'-TATAACTAGTCTGGCACTAGGAGACCCTTG-3'	5'-TATACTCGAGAAGATGAGCGGGAGAGCAAG-3'
5' UTR +*s1*	5'-TATACTCGAGTTTCTCTCCGCGTGCTTTC-3'	5'-TATACTCGAGCCCCTCTCCATCCTCCTTC-3'
5' UTR	5'-TATAACTAGTAAGGGAGGGAAGGGCTCTG-3'	5'-TATACTCGAGCCCCTCTCCATCCTCCTTC-3'
5' UTR Δ*s2*	5'-TATAACTAGTCAACCCGTCCAGAATCAAGC-3'	5'-TATACTCGAGCCCCTCTCCATCCTCCTTC-3'
5' UTR Δ*s2,s3*	5'-TATAACTAGTCTTCCTGAGACATGGCCC-3'	5'-TATACTCGAGCCCCTCTCCATCCTCCTTC-3'
5' UTR Δ*s2,s3,s4*	5'-TATAACTAGTCTCGCGAATTCAGGCATTG-3'	5'-TATACTCGAGCCCCTCTCCATCCTCCTTC-3'

Supplementary Materials and Methods

Immunohistochemistry

All incubations were performed at room temperature on a rotator. Sections were washed in PBS for 30 minutes, after which they were incubated in blocking solution (2% v/v normal goat serum (Vector Laboratories) or normal donkey serum (Jackson Laboratories); 0.2% v/v Triton X-100, in PBS) for 2 hours. They were then incubated overnight with the primary antibody diluted in blocking solution. Primary antibodies and the concentrations used were: mouse anti-Neurofilament (145 kDa; 1:50000 for floating immunohistochemistry and 1:500 for immunofluorescence; EMD Millipore Cat# MAB1621), mouse anti-GAP43 (1:100000; EMD Millipore Cat# AB5220), rabbit anti-NRP1 (1:50000; a gift from Prof. David Ginty, Johns Hopkins University, MD, USA), rabbit anti-EMX1 (provided by Prof. Giorgio Corte, University of Genova Medical School, Genova, Italy), rabbit anti-DsRed (1:500; Clontech Cat# 632496; for amplification of tdTomato fluorescence signal following *Nrp1 in situ* hybridization) and rabbit anti-GFP (1:500; Invitrogen Cat# A6455).

Sections were washed in PBS for 1 hour before incubating with secondary antibody for 1 hour. Secondary antibodies used for fluorescence analysis were either donkey anti-rabbit Alexa Fluor 546 (1:500; Invitrogen Cat# A-10040) or donkey anti-mouse Alexa Fluor 488 (1:500; Invitrogen Cat# A-21202). Biotinylated dextran amine was fluorescently labeled with Streptavidin, Alexa Fluor 555 conjugated (1:500; Invitrogen Cat# S-32355). Sections stained for fluorescence were coverslipped using ProLong Gold antifade reagent (Invitrogen). Secondary antibodies for chromogenic immunohistochemistry analysis were biotinylated goat anti-rabbit (1:500; Vector Laboratories Cat# BA-1000), biotinylated donkey anti-mouse (1:500; Jackson Laboratories Cat# 715-065-150) or biotinylated donkey anti-goat (1:500;

Jackson Laboratories Cat# 705-065-003), diluted in PBS containing 0.2% v/v Triton X-100. Sections were washed for 1 hour in PBS and then incubated for 1 hour in an avidin-biotin complex solution (0.2% v/v Triton X-100, avidin (A) [1:500] and biotin (B) [1:500] (VECTASTAIN Elite ABC kit, Vector Laboratories) in PBS). After a 30-minute wash in PBS, sections were transferred to a nickel-3'3'-diaminobenzidine tetrahydrochloride (DAB) color reaction solution (95 mM $NiSO_4$, 175 mM CH3COONa.3H_2O, 0.56 mM DAB (Sigma-Aldrich), 0.0025% v/v hydrogen peroxide solution). Sections were observed until staining was visible. The color reaction was stopped by returning the sections to PBS. Sections were mounted in serial order onto gelatinized glass slides and dried at room temperature. They were then dehydrated through an ethanol series (70% v/v, 95% v/v, and 2x 100%) and clearing agents (3x Histo-clear; National Diagnostics) for at least 1 minute each. Coverslips were added using DPX neutral mounting medium (Ajax Finechem).

Extended protocol for iontophoretic injection of anterograde tracer

Adult mice were anesthetized with 3% isoflurane in oxygen (flowrate of 1 L/minute) and maintained under anesthesia with 1% isoflurane in oxygen (1 L/minute). The anesthetized mouse was placed in a stereotactic frame (David Kopf Instruments) and a small incision was made to expose the skull. A small hole was made through the skull using a 27.5 gauge needle, through which the biotinylated dextran amine was iontophoretically injected. A retention current was applied when inserting and removing the glass micropipette from the injection site, with the micropipette left in place for 2 minutes before and after the injection. All animals were perfused 4 days after tracer injection.

Cloning of DNA constructs for luciferase assays

Emx1 mouse cDNA (NCBI Reference Sequence: NM_010131.2) was obtained from OriGene Technologies and fused at its C-terminus to a HA-tag epitope. The HA-tagged *Emx1* construct was re-cloned into pCAG-IRES-GFP at the EcoRI and NotI restriction sites and verified by sequencing. The pSGG_prom firefly luciferase construct driven by ~1kb of the human *NRP1* promoter was obtained from SwitchGear Genomics. The empty pSGG_prom control vector was constructed by removing the *NRP1* promoter at the MluI and BglII restriction sites and ligating the digested ends using the Quick Blunting and Quick Ligation Kits (New England Biolabs). Primers used to clone the mouse *Nrp1* promoter and 5' UTR regions are listed in supplementary material Table S1. Forward primers were designed to contain a SpeI restriction site and the reverse primers contained an XhoI restriction site. The *Nrp1* promoter constructs was cloned to drive firefly luciferase expression from the pGL4.23 plasmid (Promega) using the NheI and XhoI restriction sites.

Validation of antibodies for chromatin immunoprecipitation

We tested the following three commercial antibodies as described below: Santa Cruz Biotechnology Cat# sc-28220; Abcam Cat# ab82901; Abcam Cat#32925. Firstly, antibodies were tested for immunohistochemistry and immunofluorescence on brain sections of *Emx1*/C57Bl/6 wildtype and knockout mice. Secondly, we over-expressed a HA-tagged EMX1 construct in HEK293T cells and performed chromatin immunoprecipitation (ChIP) with 5 µg of antibody and 200 µg Pierce Protein G Magnetic Beads (Thermo Scientific). Following ChIP, the antibodies and proteins bound to the magnetic beads were denatured for western blot, with blots stained using an anti-HA tag antibody (Cell Signaling Technology Cat# 2367). All three antibodies failed both tests in all instances.

Supplementary References

Jolma, A., Yan, J., Whitington, T., Toivonen, J., Nitta, K. R., Rastas, P., Morgunova, E., Enge, M., Taipale, M., Wei, G. et al. (2013). DNA-binding specificities of human transcription factors. *Cell* **152**, 327-339.