**Data Supplement**

**Supplementary Methods**

**Clinical characterization and sample preparation of human postmortem samples**

Clinical characterization, diagnoses, macro- and microscopic neuropathological examinations, dissection, RNA extraction and quality control measures (RNA integrity number, pH) were performed on all cases using a standardized paradigm. Toxicological analysis was performed on every case. The UMD cases were handled in a standardized manner by the same team of investigators (http://medschool.umaryland.edu/btbank/family/). Postmortem tissue homogenates of prefrontal cortex grey matter (dorsolateral prefrontal cortex, BA46/9) in postnatal samples and corresponding region of prefrontal cortex in fetal samples were obtained for all subjects, as previously described (32). In fetal brain the prefrontal cortex consisted of a wedge of frontal cortex dissected from the dorsal convexity of the frontal lobes, midway between the frontal pole and the anterior temporal pole, under visual guidance using a dental drill. All brain dissections from both sites were conducted by the same neuropathologist (TM Hyde; see 32). Total RNA isolation, assessment of RNA concentration and quality and cDNA synthesis was carried out as previously described (34).

**Human postmortem samples and rs6994992 genotype**

Genotype reproducibility was routinely assessed by re-genotyping all samples and was generally >99%. 34 fetal samples (MAF (T) = 0.47; C/C: 3 female, 5 male; 7 African American, 1 Caucasian; C/T: 10 female, 10 male; 18 African American, 2 Caucasian; T/T: 4 female, 2 male; 6 African American) and 23 postnatal (0-3 years) samples (MAF (T) =0.39; C/C: 3 female, 4 male; 2 African American, 5 Caucasian; C/T: 2 female, 12 male; 9 African American, 5 Caucasian; T/T: 1 female, 1 male; 2 Caucasian) were used for analysis.

**Quantitative real-time PCR**

Real-time PCR data were acquired from the Sequence Detector Software (Applied Biosystems) and quantified using a standard curve method. In each experiment the $R^2$ value of the curve was more than 0.99 and controls comprising no-template cDNA or no reverse transcriptase step resulted in no detectable signal. SDS software plotted real-time fluorescence intensity and selected the threshold within the exponential phase of the amplicon profiles. The software plotted a standard curve of the cycles at threshold (Ct) vs known quantity of RNA. For each target isoform all samples were measured with constant reaction conditions and their Ct values were in the linear range of the standard curve. For postnatal NRG1-IVNV expression analysis, normalized Ct values were used to calculate ΔCt and $^{2-\Delta\Delta}$Ct. Standard curve methods could not be applied to this analysis due to lack of detectable expression in samples above the age of 3 years. All measurements were performed in triplicates for each mRNA and expression level calculated as an average of the triplicates. Experimental measurements with a >20% variance from the mean of the triplicate samples were omitted.

**Synthesis and confirmation of NRG1 isoform DNA constructs**

To generate full-length NRG1-IV-1β c-Myc tagged constructs, we PCR-amplified the NRG1-IV coding region from a brain cDNA clone (N65-22) (19) containing the NRG1-IV-β1a sequence (Genbank accession EF372273) using the forward primer E187 senseW and the reverse primer Atail-asW. To generate NRG1-IVNV c-Myc tagged constructs, we PCR-amplified a DNA fragment from a cDNA clone (N54-1; Genbank accession EF372275; Table S2) containing the Fetal F coding sequence using primers E187 senseW and Atail-asW. The PCR products were digested with SalI and EcoRI, and subsequently cloned into SalI-EcoRI-digested pMyc-GW1-2b vectors, generating an N-terminally c-Myc tagged NRG1-IV-1β expression plasmid (pMyc-GW1-N65-22) and an N-terminally c-Myc tagged NRG1-IVNV expression plasmid (pMyc-GW1-N54-1), respectively.

To generate full-length NRG1-I-β1 c-Myc tagged constructs, we PCR amplified the HRG-β1 coding region from human fetal brain, Marathon-Ready cDNA library (Clontech, Mountain View, CA) using primers AP1 and Atail_anti1 (Table S2). The first round of PCR product was diluted to 1:100, and then used as a template in the second round of PCR with the primer pair E592_s1 and E13_as3. The PCR product was analyzed on a 1% agarose gel. The PCR fragment of expected size was gel excised and cloned into TOPO XL PCR Cloning Vector (Invitrogen, Carlsbad, CA). To generate an NRG1-I-β1 expression plasmid with a c-Myc tag at the N-terminus, we PCR-amplified HRG-β1 coding sequence by a forward primer HRG-β1s and a reverse primer HRG-β1anti. The PCR product was digested with SalI and EcoRI, and subsequently cloned into a SalI-EcoRI-digested pMyc-GW1-2b vector, generating an N-terminally c-Myc tagged NRG1-I-β1 expression plasmid (pMyc-GW1-HRG-b1W). PCR reactions were performed as follows: 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 55°C for 2 min, and 68°C for 3 min. After the last cycle, extension was conducted at 68°C for 10 min. All the coding regions of the above expression plasmids were sequenced multiple times by the BigDye terminator kit to confirm sequence identity. All primer sequences are listed in Table S2.

**Assessment of NRG1 isoform sensitivity to proteolytic processing in HEK293 cells**

HEK293 cells (ATTC, Manassas, VA) were cultured in DMEM/10% FBS and maintained at 37°C/5% $CO_2$. Once confluent, cells were transfected with controlled amounts of DNA (1.8μg) encoding either c-Myc tagged NRG1-I β1, NRG1-IV-β1a, NRG1-IVNV or pGW1 vector containing no inserted transcript (empty vector) using Lipofectamine™ 2000 (Invitrogen). 24-30 hour after transfection, the cells were serum starved for 12 hours in OPTIMEM-1 (Invitrogen). The medium then removed from the cells and replaced with fresh OPTIMEM alone or OPTIMEM containing 100nM PMA (Calbiochem, La Jolla, CA). The cells were incubated at 37°C/5% $CO_2$ for 45 minutes; the conditioned medium was then collected and concentrated using Centricon Plus centrifugal filters (Millipore).

Western blot analysis for the detection of c-Myc was carried out on 50µl of conditioned medium from each treatment group. Samples were size fractioned by electrophoresis using 4-12% Bis-Tris gels (Invitrogen), transferred to nitrocellulose membrane (Invitrogen) and non-specific antibody binding blocked by 1 hour incubation of the membrane in Tris buffered saline supplemented with 0.1% Tween-20 (TBST) and 5% non-fat milk (BioRad). Primary antibody incubation was performed overnight at 4°C using an antibody raised against human c-Myc (Calbiochem). After extensive washes in TBST, membranes were incubated with an HRP-conjugated secondary antibody (Santa Cruz). Antibody binding was detected using ECL Plus (Amersham) and exposure to autoradiography film (Kodak).

**Culture, transfection and immunostaining of hippocampal neurons and HEK293 cells**

Dissociated rat hippocampal neuronal cultures were prepared from embryonic day 18 Sprague Dawley rat embryos. In brief, pooled hippocampi were dissociated initially with trypsin at 37°C for 10 minutes, then secondly by manual trituration in DMEM. The resultant dissociated cell number was counted using the trypan blue exclusion method, resuspended in DMEM and plated at a density of $5x10^4$ cells/ 12mm poly-D-lysine/laminin coated glass cover slips (BD Biosystems) and maintained at 37°C/5% $CO_2$. The following day the medium was changed to Neurobasal medium supplemented with B27 (Invitrogen). Neurons were transfected at 10 days in vitro (DIV) with pGW1 vectors encoding c-Myc tagged NRG1-I-β1, NRG1-IV-β1 or NRG1-IVNV (1.8µg) using Lipofectamine 2000. HEK293 cells were plated at a density of $1x10^5$ cells/well in LabTek chamber slides (Nunc) and maintained at 37°C/5% $CO_2$ overnight. The following day HEK293 cells were transfected with pGW1 vectors encoding c-Myc tagged NRG1- IV-β1 or NRG1-IVNV using Lipofectamine 2000. At 72hours (hippocampal neurons, DIV 13) or 48 hours (HEK293 cells) after transfection cells were fixed for 15 minutes with 4% paraformaldehyde/4% sucrose in PBS at room temperature. Cells were then washed four times in PBS and incubated overnight at 4°C with an antibody against c-Myc (Calbiochem) diluted in GDB buffer (0.18% Gelatin, 0.7% TritonX-100, 0.8M NaCl,

30mM Phosphate buffer, pH7.4). The cells were then washed four times with PBS and incubated with an Alexa-488 conjugated secondary antibody (Invitrogen) in GDB for 1 hour at room temperature. Following another series of washes in PBS, the coverslips or slides were mounted using either Vectashield Mounting medium (Vector Laboratories) or ProLong Gold Antifade reagent with DAPI (Invitrogen). Fluorescent images were obtained using a Ziess 510 Meta confocal microscope at 25X and 40X magnification using oil immersion objectives. Visualization of approximately 20-30 cells across separate cultures was used for assessment.

## Supplementary Results

### Ct (threshold cycle) values of NRG1 isoforms during fetal and postnatal development

Ct values for NRG1 isoforms during fetal development were 19-24 (NRG1-I), 21-23 (NRG1-II), 22-25 (NRG1-III), 28-30 (NRG1-IV), and 25-27 (NRG1-IVNV). Ct values for NRG1 isoforms across postnatal development were 20-30 (NRG1-I), 22-30 (NRG1-II), 19-31 (NRG1-III), and 27-31 (NRG1-IVNV).

### Temporal dynamics of NRG1 isoform expression in human prefrontal cortex throughout the postnatal lifespan and aging

*Post hoc* analysis of expression trajectories of NRG1-I, II and III isoforms across postnatal development, using ANOVA controlling for sex, race, pH, PMI and RNA Integrity Number, confirmed a main effect of age on NRG1-II ($F(67,121)=3.12$; $p<0.0001$) and NRG1-III expression ($F(67,121)=7.58$; $p<0.0001$). No main effects of sex ($p>0.8$), race ($p>0.9$) or interactions between age x sex and age x race were observed ($p>0.1$). No main effects of PMI or pH were observed ($p>0.1$). Main effects of RNA Integrity Number on both NRG1-II and NRG1-III expression ($p<0.05$) were observed.

**Table S1. Specifications of custom qPCR assay designed to detect NRG1-IVNV**

| | Exon location | Sequence |
|---|---|---|
| Forward primer | **E130 (EGFc)** | **5' GGGGAGTGCTTCATGGT 3'** |
| Reverse primer | **E103 (TMc)** | **5' ATGCAGATGCCGGTTATGGT 3'** |
| TaqMan® Probe | **Spans E130 and E103** | **5' FAM-TACTTGTGCAGAGGCGGAGG-MGB 3'** |

**Table S2. Primers used for the cloning of c-Myc-tagged expression vectors**

| Primer name | Primer sequence |
|---|---|
| E187 (senseW) | **TCTAGAGTCGACGGGAAAGGACGCGCGGGCCGAGTT** |
| Atail-asW | **ATCAATGAATTCTTATACAGCAATAGGGTCTTGGTTAGCAAT** |
| AP1 | **CCATCCTAATACGACTCACTATAGGGC** |
| Atail_anti1 | **AATACTGTAAAAAATTGCTACATATGG** |
| E592_s1 | **TCTAGAGTCGACTCCGAGCGCAAAGAAGGCAGAGGCAAA** |
| E13_as3 | **GTATGTGTTTATTTAGGTTTTATACAGCAAT** |
| HRG-β1s | **TCTAGAGTCGACTCCGAGCGCAAAGAAGGCAGAGGCAAA** |
| HRG-β1anti | **ATCAATGAATTCTTATACAGCAATAGGGTCTTGGTTAGCAAT** |

**Figure S1. Quantitative real-time PCR assay design to detect NRG1-IV and NRG1-IVNV transcripts in the human brain.** Exonic organization of NRG1-IV and NRG1-IVNV transcripts (fetal variants E and F) is shown. The exon nomenclature is stated in the top row, with the corresponding functional domain specified underneath. The location of transcript specific real-time PCR forward and reverse primers is depicted by right or left pointing arrows, respectively. The location of the exon spanning, transcript specific, internal TaqMan probe is denoted by solid red lines (the adjoining dashed red line depicts the intronic sequence not detected). Note that the assay design is identical for fetal E and F transcripts; therefore, this assay will detect expression levels of both variants herein collectively named NRG1-IVNV. Exons and primer or probe lengths are not drawn to scale. Abbreviations: Ig, immunoglobulin; s, spacer; EGFc, epidermal growth factor-like domain; TMc, transmembrane domain.

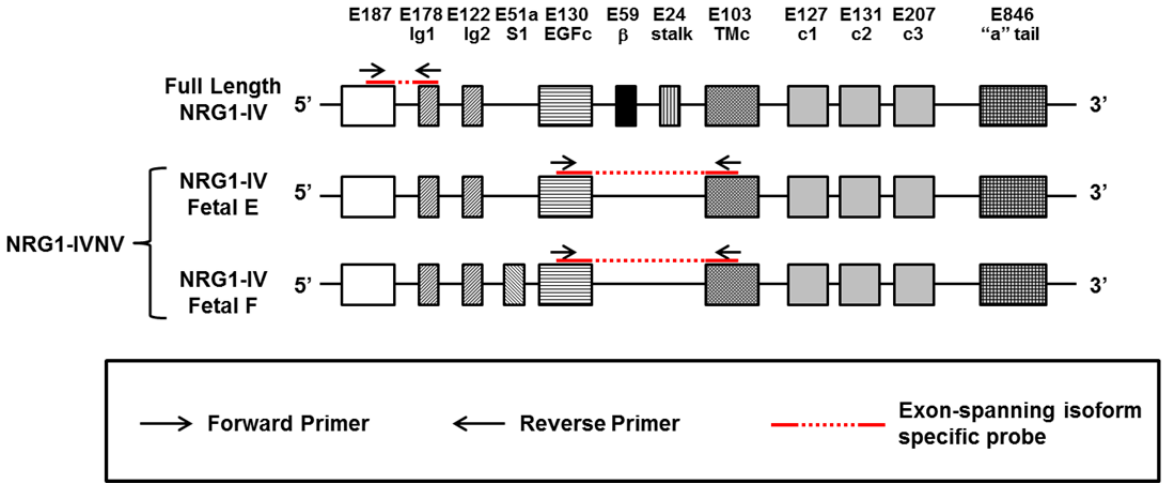

**Figure S2. Cellular distribution of NRG1-IV-β1a and NRG1-IVNV in transiently transfected HEK293 cells.** HEK293 cells transiently expressing *N*-terminal c-Myc tagged human NRG1-IV-β1a (Panels A-C) or NRG1-IVNV constructs (Panels D-F) were stained with a c-Myc antibody to determine subcellular protein distribution (Panels A, D) and mounted onto slides with medium containing a stain to detect the nuclear marker DAPI (Panels B, E). Merged images of both cellular markers show NRG1-IV-β1a proteins are expressed predominantly in a cell membrane bound pattern with little overlap with DAPI (Panel C), whereas NRG1-IVNV expression is more diffuse with a high level of colocalization with DAPI expression, confirming nuclear enrichment of expression (Panel F). Scale bar represents 20μM.

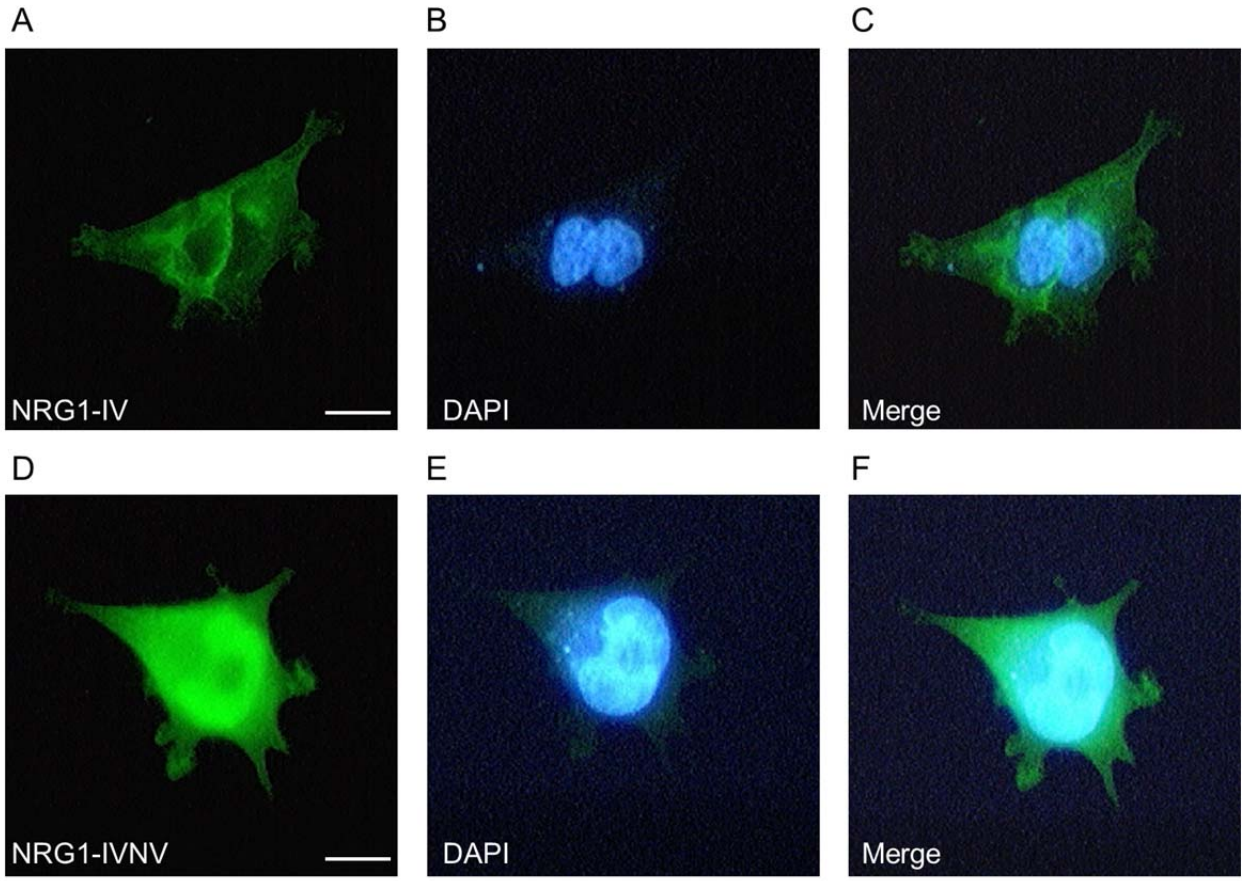

**Figure S3. Proteolytic processing of NRG1-IV-β1a and NRG1-IVNV in transiently transfected HEK293 cells.** HEK293 cells transiently expressing *N*-terminus c-Myc tagged human NRG1-IV-β1a or NRG1-IVNV (fetal variants E and F) constructs were assessed for protein translation from whole cell lysate samples (Panel A) and proteolytic processing of the extracellular domain from conditioned media samples (Panel B). Expression of NRG1-IV-β1a (IV) resulted in a protein of 66kDa (lanes 3 and 7, Panel A), whereas NRG1-IVNV E (FV E) and F (FV F) resulted in protein products of 29–31kDa (lanes 4–5 and 8–9, Panel A). Treatment of HEK293 cells expressing NRG1-IV-β1a with PMA (+) induced the release of a 30kDa fragment in the conditioned media (lane 6, Panel B); whereas no protein products were identified in the conditioned media of PMA treated HEK293 cells expressing NRG1-IVNV (lanes 7 and 8, Panel B). Untransfected HEK cells (HEK) and empty vectors (CON) were used as negative controls.