Supporting Information

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SI Materials and Methods

Genetic Association Study Populations. Only Caucasian subjects of self-reported European ancestry were included to avoid genetic stratification and to reduce heterogeneity. Three clinical samples were used for clinical genetic study of PIK3CD and PIK3R3. The principal family sample was ascertained as part of the Clinical Brain Disorders Branch sibling study (CDBD SS). DNA was available from 445 probands, 400 siblings of probands, 612 parents, and 488 unrelated controls. All probands met *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) criteria for a broad diagnosis category consisting of schizophrenia, schizoaffective disorder, simple schizophrenia, psychosis not otherwise specified (NOS), delusional disorder, schizotypal, and schizoid or paranoid personality disorder. Control subjects were ascertained from the National Institute of Mental Health (NIMH) normal volunteer office and required absence of diagnosis of a psychiatric disorder, extended to include first-degree relatives. For family-based association analysis, we examined (n = 356) families with a single affected proband. A partially independent case–control analysis was used, comprising 445 unrelated probands and 488 unrelated healthy controls. Inclusion criteria for all participants included self-identification as Caucasian (mostly European ancestry), age between 18–60 y, and IQ scores above 70 (for probands, premorbid IQ). All subjects gave written informed consent. A second smaller independent sample for follow-up investigation to confirm family-based association with schizophrenia was obtained from the NIMH Genetics Initiative (NIMH-GI) consisting of n = 50 African-American families (GI-AA).

Human Brain Tissue Collection, Quality Control, RNA Extraction, and Reverse Transcription. Diagnoses were determined by independent reviews of clinical records and family interviews by two psychiatrists using DSM-IV criteria. Inpatient and outpatient clinical records were reviewed for every subject. Macro- and microscopic neuropathological examinations and toxicology screening were performed on all cases before inclusion in the study. RNA quality was assessed with high-resolution capillary electrophoresis (Agilent Technologies), as described previously (1). The different genotype groups in this cohort did not differ on any of the measured variables that potentially affect gene expression in human postmortem brain [i.e., age, postmortem interval (PMI), pH, and RNA integrity number (RIN)].

B Lymphoblast Culture and Epstein–Barr Virus (EBV) Transformation. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. B lymphocytes in the mononuclear cell preparation were transformed by infection with EBV as previously described (2). Transformed B lymphoblasts were grown in RPMI-1640 medium (Gibco) containing L-glutamine (2 mmol/L) (Gibco), 10% FBS (10 ml/100 ml) (Cambrex), 100 mg/mL streptomycin, and 100 units/mL penicillin (Gibco) in an incubator (95% air/5% CO_2 at 37 °C). The same batch of medium and neuregulin 1 (NRG1)α was used for all experiments. Cells obtained from patients and controls were similar in regard to passage number.

RNA Extraction and Reverse Transcription. Total RNA was extracted from B lymphoblasts as described previously (3). Hippocampal and dorsolateral prefrontal cortical gray matter (DLPFC) brain tissue was stored at –80 °C and total RNA was extracted from 300 mg of tissue, using TRIZOL Reagent (Life Technologies). The yield of total RNA was determined by absorbance at 260 nm. RNA quality was assessed with high-resolution capillary electrophoresis on an Agilent Bioanalyzer 2100 (Agilent Technologies). Approximately 700 ng of RNA was applied to an RNA 6000 Nano Lab Chip without prior heating. RIN, obtained from the entire Agilent electrophoretic trace using the RIN software algorithm, was used for the assessment of RNA quality (scale 1–10, with 1 being the lowest and 10 being the highest RNA quality). Total RNA (3 μg) was used in 50 μL of reverse transcriptase reaction to synthesize cDNA, by using a SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen), according to the manufacturer's protocol. To control for potential variability between reverse transcriptase reactions, a total of three sequential reactions were performed (3 μg total RNA each) and the products pooled.

Quantitative Real-Time RT-PCR. Gene expression levels were measured by quantitative real-time RT-PCR, using an ABI Prism 7900 sequence detection system with 384-well format (Applied Biosystems). Briefly, each 20-μL reaction contained 900 nM of each primer, 250 nM of probe and Taqman Universal PCR Mastermix (Applied Biosystems) containing Hot Goldstar DNA Polymerase, dNTPs with dUTP, uracil-*N*-glycosylase, passive reference, and 200 ng of cDNA template. PCR cycle parameters were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 59 °C or 60 °C for 1 min. PCR data were acquired from the Sequence Detector Software (SDS version 2.0; Applied Biosystems) and quantified by a standard curve method. In each experiment the R^2 of the curve was more than 0.99 and controls constituting no-template cDNA 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. quantity of RNA. For each target isoform, in each brain region, all samples were measured with constant reaction conditions and their Ct values were in the linear range of the standard curve. All measurements were performed in triplicates for each mRNA and expression level was calculated as an average of the triplicates. Experimental measurements with a >20% variance from the mean of the triplicate samples were omitted. Primer and probe designs for JM-a and CYT-1 variant isoforms of ErbB4 were as described previously (4, 5) Taqman assay on demand sets were purchased from Applied Biosystems: hCG2012284 was used for total ErbB4; Hs00908671 and Mm00435674 for PIK3CD; Hs00177524 for PIK3R3; and Hs01001599, ErbB2, and Hs00176538 for ErbB3 (all other assay IDs available on request). All assays were exon spanning and should not detect genomic DNA. Our primary data analysis is based on normalization of mRNA transcripts to the geometric mean of the quantity of three endogenous control genes purchased from Applied Biosystems, Assays-on-Demand: porphobilinogen deaminase (PBGD), glyceraldehyde-3-phosphate dehydrogenase (GADPH), and B-Actin (ACTBH): Hs00609297, Hs99999905, and Hs99999903, respectively.

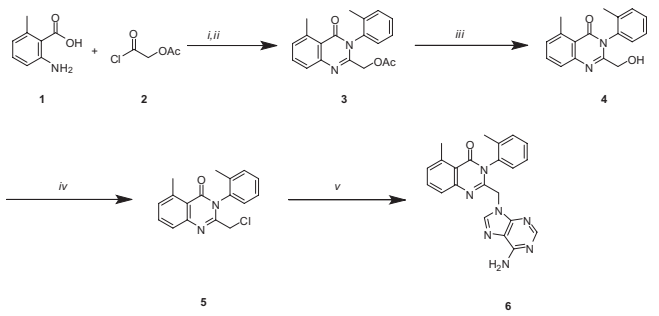
ErbB4, PIK3CD, and PIK3R3 Genotype Determination. Three intronic SNPs in the ErbB4 gene were genotyped (rs7598440, rs839523, and rs707284) that have previously been associated with schizophrenia risk and with brain expression of Erbb4 CYT-1 (4, 6). Nineteen SNPs were spanning a 92.72-kb region [chromosome (chr)1: 9,618,018–9,710,740] encompassing PIK3CD (77.17-kb gene; chr1: 9,634,390–9,711,563). For PIK3CD Tag SNPs were elected from the international HapMAP I project (http://www.hapmap.org),

using the HAPLOVIEW program (3.31; http://www.broad.mit.edu/mpg/haploview) "Tagger" algorithm (aggressive tagging using two marker haplotypes). SNPs located in functional domains such as promoters, coding exons (including synonymous SNPs), 5′- and 3′-untranslated regions, and conserved noncoding sequences were also selected. Genotyping was performed using the Taqman 5′-exonuclease allelic discrimination assay (details available on request). Genotype reproducibility was routinely assessed by regenotyping all samples for selected SNPs and was generally >99%. Overall genotyping failure rate was <1%. Linkage disequilibrium (LD) between SNPs was determined using the program LDMAX/GOLD (7). The program SNPHAP written by David Clayton (University of Cambridge, Cambridge, UK) (version 1.0) was used to calculate haplotype frequencies and to assign diplotypes to individuals (8). For ErbB4, individuals were divided according to diplotype into three groups: risk haplotype (hap) homozygotes (AGG/AGG), risk hap carrier (AGG/nonrisk), and nonrisk/nonrisk (all other diplotypes).

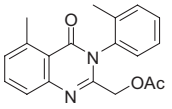
Flow Cytometric Analysis of Intracellular [PI(3,4,5)P3] in Human LCLs. Intracellular staining was used to determine relative [PI(3,4,5)P3] concentrations at the single-cell level, using the Cytofix/Cytoperm kit (BD Biosciences). Cells were stimulated with either NRG1α (296-HR), a 65-aa residue recombinant protein from the epidermal growth factor (EGF) domain (177–241) (100 ng/mL; R&D Systems), or CD19/B-cell receptor (BCR) cross-linking in a 5% CO_2 incubator at 37 °C. For the CD19/BCR cross-linking, cells were incubated with mouse monoclonal anti-human IgM antibody (BD Biosciences) and mouse monoclonal anti-CD19 antibody (BD Biosciences) followed by incubation with goat anti-mouse antibody (Pierce). The reaction was terminated at 5, 10, 15, and 30 min by fixing cells with Phosflow Fix Buffer I (BD Biosciences) for 10 min at 37 °C. Baseline represented 0 time point in the absence of NRG1α stimulation. Cells were washed with Phosflow Perm/Wash Buffer I (BD Biosciences) and permeabilized in Phosflow Perm/Wash Buffer I and stained with biotin-conjugated anti-[PI(3,4,5)P3] antibody (Echelon Biosciences) for 1 h at room temperature. After washing twice with Phosflow Perm/Wash Buffer I, cells were incubated with phycoerythrin-conjugated avidin (BD Biosciences). After washing with Phosflow Perm/Wash Buffer I, cells were analyzed using FACScan (BD Biosciences). CellQuest software (BD Biosciences) was used to acquire and quantify the fluorescence signal intensities. Data are presented as Sum Delta [PI(3,4,5)P3] calculated as the sum of ratios {[geometric mean of fluorescent intensity level (GMF) – baseline GMF]/baseline GMF} over five consecutive time points (0, 5, 10, 15, and 30 min).

Western Blot Analysis of PIK3CD in Human LCLs. B lymphoblasts were lysed in TNESV buffer (50 mM Tris·HCl, pH 7.4, 100 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, 1 mM Na3VO4, and protease inhibitor mixture) and incubated for 20 min on ice. Following centrifugation at 14,000 × *g* for 10 min, the supernatants were collected. Fifty micrograms of protein was denatured in 4XNuPAGELDS sample buffer at 95 °C for 5 min. Samples were separated by gel electrophoresis using NuPAGE 4–12% bis-Tris gels, transferred to nitrocellulose membranes, then probed with the primary antibodies 1:200 of PI3Kinase p110δ (Abcam; ab32401) at 4 °C overnight and 1:10,000 of anti-B-actin-HRP (Sigma; A3854) at room temperature for 1 h, and then incubated with 1:2,000 goat anti-rabbit IgG-HRP (Santa Cruz Biotechnology; sc-2004). SC-348 (Santa Cruz Biotechnology) was used for selected ErbB4 analysis. Protein bands were detected by the ECL Western blotting analysis system (Amersham Biosciences; RPN2109) and exposed to Kodak scientific imaging film. Protein bands were imaged and the relative optical density of each band was measured using National Institutes of Health (NIH) Image software.

NCGC00168114 (IC87114) Synthesis. Synthesis of NCGC00168114 (IC87114) (**6**):

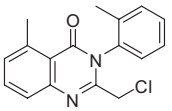


Scheme S1. Reagents and conditions: (*i*) Py, $P(OPh)_3$, room temperature (r.t.) 1 h; (*ii*) 2-methylaniline, MW 230 ° C, 10 min; (*iii*) LiOH, THF/MeOH/H2O, r.t. 4 h; (*iv*) $POCl_3$, MW 150 ° C, 10 min; and (*v*) adenine, DMA, Cs_2CO_3, 60 ° C, 1.5 h.



Scheme S2. Compound **3**:(5-methyl-4-oxo-3-*O*-tolyl-3,4-dihydroquinazolin-2-yl)methyl acetate.

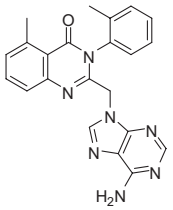
To a solution of anthranilic acid **1** (0.605 g, 4.0 mmol) in anhydrous pyridine (15 mL) were added **2** (0.820 g, 6.0 mmol) and triphenyl phosphite (1.490 g, 4.8 mmol). After stirring at room temperature for 1 h, 2-methylaniline (0.857 g, 8.0 mmol) was added and the mixture was heated in a microwave for 10 min at 230 °C. The reaction mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography eluting with 20% ethyl acetate in hexanes to give **3** (0.803 g, 62.3%) as a white solid: 1HNMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.09 (s, 3 H), 2.19 (s, 3 H), 2.84 (s, 3 H), 4.64 (s, 1 H), 4.65 (s, 1 H), 7.19–7.28 (m, 2 H), 7.33–7.44 (m, 3 H), 7.55–7.66 (m, 2 H); 13CNMR (100 MHz, CHLOROFORM-*d*) δ ppm 17.51, 20.51, 22.97, 62.75, 119.64, 125.90, 127.54, 128.20, 129.91, 130.13, 131.50, 133.74, 134.92, 136.17, 141.70, 148.75, 150.24, 161.81, 169.82.



Scheme S3. Compound **5**:2-(chloromethyl)-5-methyl-3-*O*-tolylquinazolin-4-one.

To a solution of **3** (0.700 g, 2.17 mmol) in THF (30 mL) were added MeOH (15 mL), water (15 mL), and LiOH (0.520 g, 21.71 mmol). After stirring at room temperature for 4 h, the reaction mixture was evaporated under reduced pressure and the residue was dissolved in dichloromethane (DCM) (50 mL) and washed with water (2 × 30 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give **4** (0.499 g, 82%), which was used without further purification in the next step. Alcohol **4** (502 mg, 1.79 mmol) was dissolved in acetonitrile (10 mL). To this solution was added POCl3 (5 mL) and the mixture was heated in a microwave at 150 °C for 10 min. The reaction mixture was concentrated in vacuo and the residue was dissolved in 30 mL of DCM. The organic layer was washed with an ice cold saturated NaHCO3

aqueous solution (2 × 20 mL) and dried over $MgSO_4$. The crude product was purified by silica gel column chromatography, eluting with ethyl acetate/hexanes (1:9, vol/vol) to yield **5** (0.343 g, 64%) as a yellowish solid: 1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.17 (s, 3 H), 2.84 (s, 3 H), 4.11 (d, J = 11.7 Hz, 1 H), 4.28 (d, J = 11.7 Hz, 1 H), 7.19–7.49 (m, 5 H), 7.57–7.71 (m, 2 H); 13C NMR (100 MHz, CHLOROFORM-*d*) δ ppm 17.79, 22.94, 43.41, 119.66, 125.92, 127.37, 128.72, 129.89, 130.54, 131.46, 133.86, 135.33, 136.21, 141.81, 148.69, 151.20, 161.86.



Scheme S4. Compound **6**:2-((6-amino-9H-purin-9-yl)methyl)-5-methyl-3-*O*-tolylquinazolin-4(3H)-one.

To a solution of adenine (543 mg, 4.0 mmol) in DMA (100 mL) were added **5** (300 mg, 1.0 mmol) and Cs2CO3 (3.25 g, 10.0 mmol) and the mixture was heated in an oil bath at 60 °C for 1.5 h. After cooling to room temperature, the reaction mixture was passed through a plug of silica gel and the plug was washed with 200 mL of ethyl acetate/MeOH (1:1, vol/vol). Solvent was removed in vacuo and the residue was purified by HPLC to give the desired **6** (121 mg, 30%) as a solid: 1HNMR (400 MHz, DMSO-*d*6) δ ppm 2.18 (s, 3 H), 2.73 (s, 3 H), 4.76 (d, J = 17.4 Hz, 1 H), 5.09 (d, J = 17.4 Hz, 1 H), 7.10–7.28 (m, 4 H), 7.40–7.55 (m, 4 H), 7.60 (t, J = 7.7 Hz, 1 H), 8.01 (s, 1 H), 8.03 (s, 1 H); 13CNMR (100 MHz, DMSO-*d*6) δ ppm 17.06, 22.50, 44.49, 118.14, 118.87, 125.43, 127.63, 128.61, 129.64, 129.76, 131.36, 133.91, 135.02, 135.76, 140.52, 141.79, 148.12, 149.70, 151.01, 152.46, 155.81, 160.89; HPLC: tR = 4.01 min, UV254 = 100%; HRMS (ESI): *m/z* calculated for C22H19N7O [M+1]+ 398.1724, found 398.1727.

All commercially available reagents and solvents were purchased and used without further purification. All microwave reactions were carried out in sealed microwave vials equipped with magnetic stir bars and heated in a Biotage Initiator Microwave Synthesizer. HPLC purification was performed using a Waters semipreparative HPLC equipped with a Phenomenex Luna C18 reverse phase (5 μm, 30 × 75 mm) column having a flow rate of 45 mL/min. The mobile phase was a mixture of acetonitrile and H_2O, each containing 0.1% trifluoroacetic acid. During purification, a gradient of 20–80% acetonitrile over 8 min was used with fraction collection triggered by UV detection (220 nM). Pure fractions were passed through a PL-HCO3 MP SPE column (Varian) to remove trifluoroacetic acid and concentrated under vacuum on a lyophilizer.

Passive Blood–Brain Barrier Permeability Analysis of IC87114. A combination of two descriptors representing the lipophilicity (as measured by octanol-buffer partitioning, logD 7.4) and relative hydrophobicity [as measured by aqueous two-phase partitioning (ATPPS), N(CH2)] of organic compounds was used to determine whether NCGC00168114 (IC87114) permeates the blood–brain barrier. Assays were performed using the Millenium Pharmaceuticals passive blood–brain barrier permeability assay (Analiza). The probability of a compound to cross the blood–brain barrier through passive transport was calculated with the equation Ln[P(CNS = "+")/(1 − P(CNS = "+"))] = −7.90 + 24.91*nlogD* − 1.10*nlogD**N*(CH2). The results are presented as the ratio of the compound concentration in the brain to compound concentration in the blood (C_{brain}/C_{blood}). Values greater than 2 can be interpreted as CNS positive and values less than 0.1 (<0.1) can be interpreted as CNS negative.

Rat Haloperidol Treatment Study. All animal procedures were performed in accordance with the National Institutes of Health guidelines for use and care of laboratory animals. Male Sprague–Dawley rats (weight 250 g) were on a 12-h light/dark cycle (lights on/off 0600 hours/1800 hours) in a temperature-controlled environment and with access to food and water. Rats were randomly assigned to drug treatment groups (eight per dose) and administered i.p. injections of haloperidol (0.08 or 0.6 $mg \cdot kg^{-1} \cdot d^{-1}$) or vehicle (0.02% lactic acid) once daily for 28 d. Haloperidol (Research Biochemicals) (20 mg/mL) was prepared in 1% lactic acid, diluted with water, and neutralized with 1 M NaOH to obtain pH 5.3. Rats were euthanized 7 h after the last injection. Brains were dissected and frozen at −80 °C. RNA from hippocampus and surrounding cortex was extracted using standard procedures.

IC87114 Pretreatment in a Mouse Amphetamine Model of Psychosis. All mice were group housed (four per cage) in a climate-controlled animal facility (22 ± 2 °C) and maintained on a 12-h light/dark cycle, with free access to food and water. Testing was conducted in male mice, at ages 2–3 mo, during the light phase of the circadian cycle. Mice were handled by the experimenter on alternate days during the week preceding the tests. At least 1 h before any test manipulation, mice were habituated in a room adjacent to the testing room. For examination of the effects of IC87114 on locomotor activity, mice were tested on day 1 in an experimental apparatus consisting of four Plexiglas Digiscan automated open fields (Accuscan; 42 × 42 × 30-cm dimensions). One red light (5 ± 2 lux) was placed overhead, evenly illuminating each open field. Each apparatus contained photobeam sensors to measure the exploratory and locomotor activity of the mice. During the first 10-min session mice were placed in the empty open field and allowed to explore the arena. Immediately after, mice were removed from the field and given an injection of either 0.1 mg/kg IC87114 or vehicle. Mice were placed back in the same open field for an additional 75 min. All sessions were videotaped. IC87114 was dissolved in 0.25% DMSO in physiological saline (vehicle) and injected i.p. in a volume of 10 mL/kg of body weight. "Vehicle-treated" mice were injected with the same volume of 0.25% DMSO in physiological saline. On day 3, mice were tested in the same experimental apparatus and conditions as on day 1. Thirty minutes before the start of the first 10-min session the mice were injected with either IC87114 (0.1 mg/kg) or vehicle (the same as the treatment received on day 1). During the first 10-min session mice were placed in the same empty open field. Immediately after, mice were removed from the field and given an i.p. injection of D-amphetamine sulfate (0.75 mg/kg or 1.5 mg/kg; Sigma-Aldrich). Mice were placed back in the same open field and allowed to explore for an additional 75 min. Amphetamine was dissolved in physiological saline and injected in a volume of 10 mL/kg.

IC87114 Pretreatment in the Rat Neonatal Ventral Hippocampal Lesion (NVHL) Model of Schizophrenia. Timed pregnant female Sprague–Dawley rats were obtained at gestation day 15 (Charles River), upon which they were individually housed and maintained in a standard 12-h light/dark schedule with ad libitum access to standard rat chow. Male pups at 7–8 d of age (P7–8) were subjected to bilateral NVHL or sham procedure (SHAM), as previously described (9). Briefly, pups were anesthetized via hypothermia, followed by bilateral cannula insertion into the ventral hippocampus (3 mm posterior to bregma, ±3.5 mm lateral to midline, and 5 mm ventral to skull surface). SHAM animals received cannula placement alone, whereas NVHL animals received bilateral infusions of a 10-μg/μL Ibotenic acid solution (0.3 μL/2 min). Cannula remained in place for a total of 5 min, to allow for diffusion of toxin from the tip. Animals were weaned and group housed (two to three animals

per cage) postoperatively at P21 and subjected to prepulse inhibition (PPI) testing at P57–60. Startle chambers (SR-LAB; San Diego Instruments) were used, consisting of a Plexiglass enclosure (8 inches in length, inner diameter of 3.5 inches) mounted on a 12.5 × 25.5-cm Plexiglass frame, within a sound-attenuating enclosure. A piezoelectric accelerometer mounted below the Plexiglass enclosure transduced motion within the cylinder. Acoustic stimuli were delivered via a speaker mounted 24 cm above the animal. Thirty minutes before testing, animals were given i.p. injections of either IC87114 (0.1 mg/kg) or vehicle (0.50% DMSO in saline). Animals were randomly assigned to either drug or vehicle conditions for the first PPI session, and the treatment was reversed in a second test session 1 wk later. Immediately preceding testing, all animals were given injections of the dopamine agonist apomorphine (0.1 mg/kg), a dose that does not disrupt baseline PPI (10) but has been shown to exacerbate the PPI deficit in NVHL animals (10). Upon being placed in the startle chamber, animals experienced a 5-min acclimation period consisting of a constant 65-dB background noise. Animals then experienced the following trial types: pulse alone (120 dB, 40-ms noise burst), prepulse (5, 10, or 15 dB above background noise) plus pulse (stimuli separated by 100 ms), no stimulus (65 dB background noise), and prepulse alone (5, 10, or 15 dB above background noise). Trials were presented in a pseudorandom order, with a variable intertrial interval of 15 ± 5 s. Each session lasted ∼30 min. Startle boxes were cleaned with a disinfectant and calibrated in between testing sessions.

Following completion of behavioral testing, animals were anesthetized with isoflurane (5% in oxygen) and decapitated, and brains were removed, postfixed in 4% paraformaldehyde for 24–48 h, and stored in sucrose solution until sectioning. For confirmation of lesion status, 50-μm serial sections running the rostrocaudal extent of the hippocampus were cut, mounted, and Nissl stained, using neutral red.

IC87114 and AKT Signaling in Vivo. All mice were group housed (five per cage) in a climate-controlled animal facility (22 ± 2 °C) and maintained on a 12-h light/dark cycle, with free access to food and water. On the day of experimentation, during the light phase of the circadian cycle, mice were weighed and then placed in individual cages with free access to food and water. IC87114 was dissolved in 0.25% DMSO in physiological saline and administered by i.p. injection of 0.1 mg/kg. For vehicle-treated mice, a 0.25% DMSO solution in physiological saline was prepared and an equivalent volume to that given the IC87114-treated group was administered by i.p. injection. After 45–60 min mice were decapitated and the brains were immediately removed from the skull. The prefrontal cortex and striatum were dissected out bilaterally and placed in precooled centrifuge tubes in a bath of dry ice within 4 min of decapitation. Brain tissue was then stored at −80 °C. Right hemistriata were thawed and 250 μL of Tissue Lysis Buffer [T-Per (Thermo Scientific), 1 M DTT (Sigma), and Halt Protease and Phosphotase Inhibitor Mixture (HHPIC) (Thermo Scientific)] was added to each sample. Samples were sonicated for 10 s and centrifuged at 4 °C. Supernatant was stored at −80 °C. Protein concentrations were determined by Bradford assay (Bio-Rad). Protein was size fractionated on NuPAGE 10% Bis-Tris Gels (Invitrogen) and transferred to nitrocellulose membranes (Invitrogen). Membranes were probed with primary antibodies against pAkt-Thr308 (C31E5E; Cell Signaling), total Akt1 (07-416; Millipore), and β-Actin (A3854; Sigma) and then goat anti-rabbit secondary IgG-HRP (Santa Cruz). Membranes were exposed to ECL Plus (GE Healthcare) and Kodak scientific imaging film. Protein bands were imaged and the relative optical density of each band was measured using NIH Image software.

Statistical Analyses. Multiple linear regression (within SPSS version 15.0) was used to examine effects of diagnosis and ErbB4 diplotype on quantitative traits relating to gene expression and NRG1-stimulated intracellular [PI(3,4,5)P3] production. ErbB4 CYT-1 expression and PIK3CD expression were included as additional predictor variables when PIK3CD gene expression and [PI(3,4,5)P3] production were examined, respectively. Correlations of mRNA levels in the human postmortem brain with antipsychotic medication (chlorpromazine equivalents, CPZ) were investigated in the schizophrenic cohort, using Spearman's correlations. Association between ErbB4 diplotypes and PIK3CD/PIK3R3 expression traits in the human brain was examined in controls using ANCOVA with age, pH, and PMI as covariates. Primary planned comparisons between diagnostic groups were made using ANCOVA with diagnosis as the independent variable and age, pH, and PMI as covariates. Analysis of the effects of race was restricted to African-American and Caucasian individuals due to the small sample size in other ethnic groups. Genotype groups did not differ on any of the demographic variables. In the rat brain, effects of antipsychotic medication on gene expression were examined using ANOVA with drug dose as an independent factor. Hardy–Weinberg equilibrium was tested with Fisher's exact test. Clinical genetic association was conducted using unconditional logistic regression and the family-based association test (FBAT). Pharmacological treatment effects in rodents were analyzed by ANOVA (rat IC87114 NVHL study) and repeated-measures ANOVA (IC87114 mouse study). Significant interactions were explored by a posthoc *t* test. All experimental and statistical analyses were conducted blind to diagnosis or treatment.

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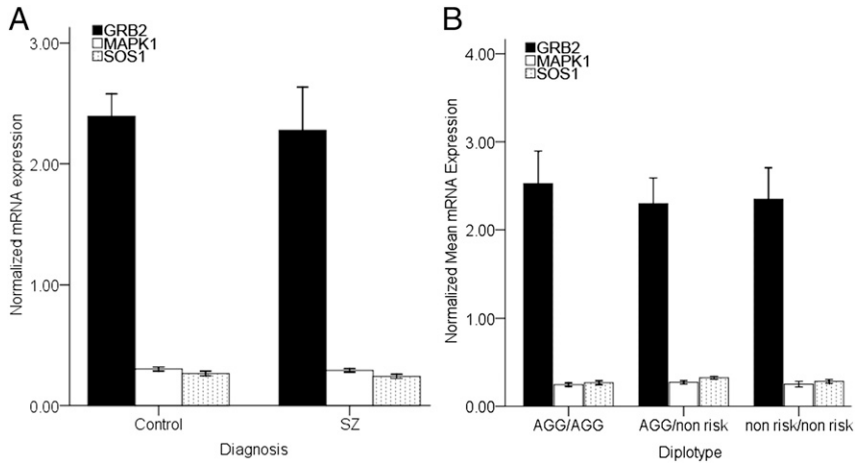

Fig. S1. Real-time quantitative RT-PCR analysis of downstream MAPK pathway genes in LCLs in normal individuals and patients with schizophrenia. No association between schizophrenia (*A*) or ErbB4 risk haplotype (*B*) and MAPK1, SOS1, and GRB2 expression. AGG/AGG, n = 13; AGG/nonrisk, n = 28; nonrisk/nonrisk, n = 14. (Mean ± SEM, univariate ANOVA.)

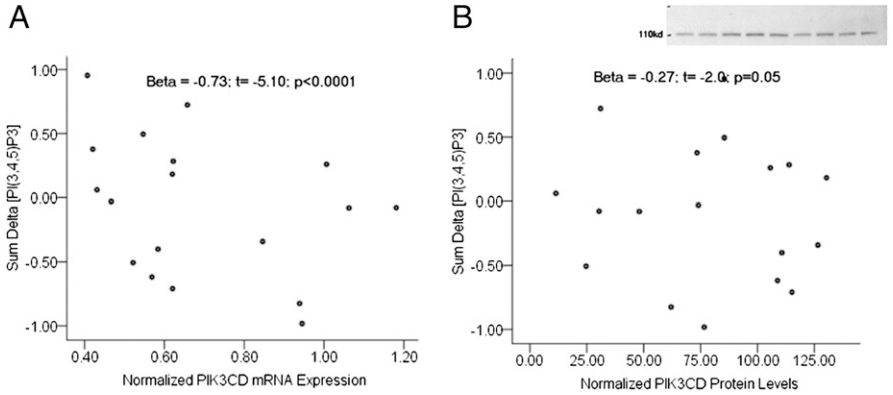

Fig. S2. (*A* and *B*) Significant inverse linear relationship between (*A*) PIK3CD mRNA and (*B*) protein expression and NRG1-stimulated [PI(3,4,5)P3] production in human LCLs derived from patients with schizophrenia. β-values are derived from the multiple-regression model. (*B*, *Inset*) Representative Western Blot analysis of PI3KCD showing a single 110-kd band in nine separate individuals.

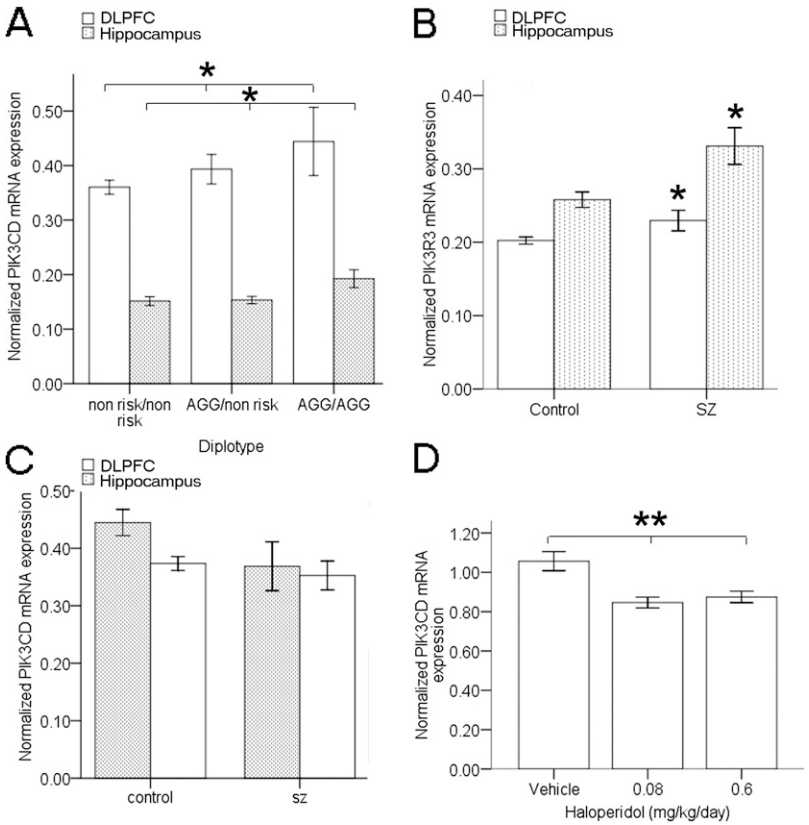

Fig. S3. (*A*) An ErbB4 risk-associated haplotype predicts expression of PIK3CD in the human hippocampus [*$F(2, 53) = 2.37$; $P = 0.04$] and dorsolateral prefrontal cortex [*DLPFC; $F(2, 65) = 2.27$, $P = 0.05$] of normal subjects (hippocampus, $n = 4$ AGG/AGG, $n = 24$ AGG/nonrisk, $n = 27$ nonrisk/nonrisk; DLPFC, $n = 5$ AGG/AGG, $n = 30$ AGG/nonrisk, $n = 32$ nonrisk/nonrisk), mean ± SEM, one-tailed univariate ANOVA. (*B*) PIK3R3 mRNA is increased in both brain regions in schizophrenia, $*P < 0.05$. (*C*) PIK3CD mRNA is not changed in either brain region in schizophrenia (DLPFC, $n = 72$ controls and 31 patients; hippocampus, $n = 69$ vs. 31. (*D*) Chronic administration of haloperidol (0.08 or 0.6 mg/kg/d) decreases expression of PIK3CD in rat hippocampus ($n = 24$). (Mean ± SEM, ANOVA, $**P < 0.001$.)

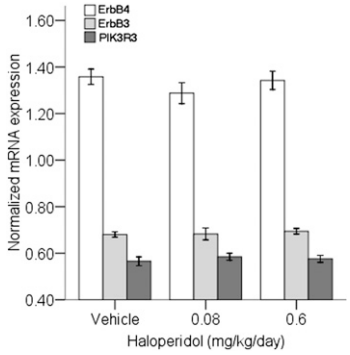

Fig. S4. ErbB4, ErbB3, and PIK3R3 expression is unaltered in the hippocampus of rats treated with haloperidol (0.08 or 0.6 mg/kg/d). (Mean ± SEM, ANOVA, $n = 22$.)

Table S1. Single-marker results for CBDB family-based, NIMHGI-AA family-based, and CBDB case–control cohorts

	Location, bp, UCSC Build 36			CBDB SS study, 356 families				NIMHGI-AA, 50 families					Case–control, 445 patients and 488 controls			
SNP rs	(HG18)	Alleles	MAF	*P* value	Association	Risk	MAF	*P* value	Association	Risk	MAF controls	MAF cases	Genotype	OR	95% CI	*P* value
rs4846053	9,618,018	G/C	0.24	0.387			0.53	0.39			0.25	0.25				0.76
rs7518602	9,633,931	C/T	0.38	0.203			0.85	0.37			0.41	0.39				0.17
rs7518793	9,634,074	C/T	0.21	0.589			0.29	0.63			0.2	0.21				0.79
rs7516214	9,634,324	A/G	0.37	0.658			0.65	0.55			0.41	0.38				0.16
rs6540991	**9,640,674**	**T/C**	**0.31**	***0.050***	**Positive**	**T**	**0.72**	***0.001***	**Positive**	**T**	**0.34**	**0.32**	**T/C**	**0.7**	**0.51–0.96**	***0.02***
rs11802023	9,655,498	C/T	0.08	0.503			0.09	0.32			0.09	0.08				0.28
rs12567553	9,658,431	A/G	0.12	0.655			0.56	0.04	Positive	A	0.15	0.13				0.2
rs9430635	9,661,373	C/G	0.48	0.065			0.47	0.851			0.46	0.46				0.16
rs6660363	9,663,780	A/G	0.48	0.044	Positive	A	0.31	0.127			0.46	0.47				0.36
rs4601595	9,673,413	G/T	0.49	0.050	Positive	G	0.29	0.08			0.45	0.47				0.73
rs11801864	9,677,860	G/A	0.04	0.257			0.23	0.08			0.04	0.04				0.9
rs6541017	9,694,151	A/G	0.17	0.143			0.18	0.02	Positive	A	0.15	0.15				0.73
rs9430220	**9,702,458**	**T/C**	**0.24**	***0.009***	**Positive**	**T**	**0.6**	***0.05***	**Positive**	**T**	**0.24**	**0.21**	**C/C**	**0.46**	**0.22–0.94**	***0.03***
rs11589267	9,705,143	T/C	0.45	0.162			0.11	0.1			0.46	0.45	C/T	1.48	1.05–2.07	0.02
rs10864435	9,705,353	C/T	0.08	0.480			0.33	0.976			0.09	0.08				0.28
rs11121484	9,707,010	C/T	0.11	0.208			0.47	0.97			0.1	0.11				0.99
rs12037599	9,709,432	G/C	0.04	*0.050*	Positive	G	0.28	0.791			0.05	0.04				0.29
rs1135427	9,710,427	T/G	0.45	*0.030*	Positive	T	0.75	0.154			0.44	0.45	T/G	1.46	1.04–2.04	*0.02*
rs1141402	9,710,740	G/A	0.05	*0.021*	Positive	G	0.25	0.871			0.05	0.04				0.48

CBDB SS, Clinical Brain Disorders Branch sibling study families; NIMHGI-AA, NIMH genetics initiative African-American families. Alleles represent major/minor according to CEU(C) HapMap Phase III (rel 1). Minor allele frequency (MAF) is set in SS Caucasian controls. MAF in family samples is from parents. Association risk is positive when one allele is overtransmitted. Entries in boldface type denote consistent replication and directionality across all three study samples. The empirical *P* value for association significance was calculated using permutation testing. Case–control dataset is representative of 445 probands and 488 healthy controls. Italics denotes consistent replication and directionality across all three study samples.

Table S2. FBAT association results for markers in PIK3R3 in CBDB SS family sample

Marker	Allele	*Z*	*P* value
rs11584814	1	0.412	0.726444
rs7538978	1	0.138	0.824373
rs1707322	1	0.137	0.838496
rs6429593	1	0.412	0.691358
rs1707321	1	−0.38	0.669643
rs1707337	1	0.062	0.923153
rs1707317	1	0.062	0.873852
rs785471	1	−0.308	0.752941
rs785470	1	0.062	0.900505
rs785468	1	0.138	0.843552
rs785464	1	−0.238	0.75
rs1613296	1	0.062	0.901007
rs1768802	1	0.062	0.912225
rs12143865	1	−0.371	0.852243
rs785483	1	0.215	0.756144
rs12090501	1	0.254	0.802
rs785505	1	0.18	0.881523
rs1707304	1	−0.102	0.885308
rs1355641	1	0.487	0.607903
rs1707302	1	0.013	0.973126
rs1473840	1	0.013	0.965445
rs1416706	1	0.088	0.979679
rs9429095	1	0.013	0.983041
rs2486445	1	−0.323	0.740634
rs785475	1	0.358	0.628125