Supporting Information

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SI Materials and Methods

Volumetric Measurement of the Hippocampus. Mice were perfused through the heart with PBS followed by formaline and fixed for 24 h. Brain tissue was subsequently embedded in celloidin. Sections were taken at ∼40-μm thickness using a sliding microtome and stained using cresyl violet. Images of serial sections through the entire hippocampus from postnatal day 0 (P0) mice were analyzed in ImageJ (National Institutes of Health). The interval between adjacent sections on each slide was 120 μm. The volume was determined by the Cavalieri method. Borders of the hippocampus, excluding the subiculum but including the fimbria and dentate gyrus, were traced manually, followed by manual tracing of the dentate gyrus alone. Finally, areas of the hippocampus proper and the granule cell layer of the dentate gyrus were measured bilaterally for all cases.

BrdU Labeling Analysis. We injected pregnant mice intraperitoneally with BrdU (100 mg/kg). Cryostat sections were prepared as described above. For the quantification of the number of proliferating cells, serial sections were photographed and six equivalent sections of *Df1*/+ or *Dgcr8*$^{+/-}$ and control mice were used to count BrdU-positive cells in the dentate ventricular zone, fimbriodentate junction, and dentate gyrus.

Immunohistochemistry of Tissue Sections. Immunohistochemical staining was performed with primary antibodies for 24 h at 4 °C after blocking for 30 min at room temperature with 5% (vol/vol) donkey serum (Chemicon). Cryostat sections were incubated for 1 h at room temperature with secondary antibodies and fluorescent Nissl (Molecular Probes). The primary antibodies used were anti-BrdU (BU1/75; Oxford Biotechnology), anti-Prox1 (Covance), anti-Nestin (Chemicon), anti-Gad67 (Chemicon), and anti-Lhx6 (AVIVA Systems Biology). The distribution of Lhx6-positive or Gad67-positive cells was estimated as previously described (1). Slides were examined with a confocal laser scanning microscope (FV-300; Olympus).

In Situ Hybridization. In situ hybridization experiments were performed using digoxigenin riboprobes on 12-μm frozen sections. Slides were fixed in 4% paraformaldehyde (PFA) and 0.2% glutaraldehyde for 10 min and treated with proteinase K (10 μg/mL) for 3 min. Slides were incubated with hybridization buffer for 2 h at 70 °C, followed by overnight incubation with a digoxigenin-labeled probe at 70 °C. Six washes were performed as follows: 50% formamide, 6× SSC at 70 °C for 15 min three times, and 50% formamide, 2.4× SSC at 65 °C for 15 min three times. Slides were then incubated with horseradish alkaline phosphatase-conjugated anti-digoxigenin and nitroblue tetrazolium/5-bromo-4-chloro-indolyl phosphate for signal detection. The probes used were as follows: *Wnt3a* (GenBank accession no. BC152754), *Lef1* (GenBank accession no. BC057543), *Cxcl12* (GenBank accession no. BC046827), and *Cxcr*4 (GenBank accession no. BC031665).

Chemotaxis Assay. Hippocampal dentate gyri were removed from pups of *Df1*/+ and control mice at P0 and dissociated using a Neural Tissue Dissociation Kit (Papain) (Miltenyi Biotec). The migration of dentate gyrus (DG)-derived cells was studied using a cell-culture polyethylene terephthalate (PET) membrane insert (Becton Dickinson) containing 8.0-μm pores, coated with 20 μg/mL laminin. For chemotaxis, increasing concentrations of Cxcl12 (Peprotech) ranging from 1 ng/mL to 1 μg/mL were added to the bottom compartment of the chemotaxis chamber, and 10^6 cells per mL in Neurobasal medium (Gibco) with 2% B27 supplement, 0.5% glucose, and 2 mM L-glutamine was added to the upper compartment of each well. The chemotactic responses of hippocampal DG-derived cells were determined after 24 h by counting migrating cells in five high-powered fields.

Hippocampal Dentate Ventricular Zone and Medial Ganglionic Eminence Explant Coculture. Dentate ventricular zone (VZ) and medial ganglionic eminence (MGE) explants were dissected out from organotypic slices of embryonic day 17.5 (E17.5) and E13.5 *Df1*/+ and control mouse embryos, respectively. The explants were confronted with 293T cell aggregates expressing DsRed (Mock) or DsRed and Cxcl12 and were cultured in Matrigel (BD Biosciences) diluted 1:1 with Neurobasal medium (Invitrogen) supplemented with 2% B27 supplement (Gibco). For quantification, the distance migrated by the 20 farthest cells from the explants was quantified.

Cloning and Production of Lentiviral Vectors. CMV-immediate early gene enhancer (CMV-IE)/chicken β-actin promoter, internal ribosome entry sites (IRES)-EGFP, and *Dgcr8*, *Gnb1l*, or *Zdhhc8* were introduced into pLenti6.4 lentiviral vector (Invitrogen), and lentiviruses were produced as previously described (2, 3). Briefly, 293T cells were transfected using Lipofectamine 2000 (Invitrogen) with the lentiviral vector and two helper, Δ8.9 and VSVG, plasmids. After 48 h, the supernatants were spun at 83,000 × *g* for 1.5 h, and the pellet was resuspended in 100 μL of PBS.

Stripe Choice Assay. 293T cells were plated in a four-cell chamber slide coated with Matrigel (BD Biosciences) and transfected with DsRed and Cxcl12 expression vectors. After an overnight incubation, transfected cells were removed with a pipette tip (one line every 2–3 mm to make stripes). Nontransfected 293T cells were plated on top and allowed to attach to the empty stripes for 2 h. After the unattached cells were washed out with PBS, lentivirus-infected dissociated MGE-derived cells from E13.5 *Df1*/+ and control embryos were added on top. The distribution of the MGE-derived cells was identified by the fluorescence of lentivirus-mediated EGFP expression 48 h after plating.

Slice Culture. Brain slice cultures were prepared from E13.5 embryonic mouse telencephalon. The 300-μm coronal cortical sections were prepared by cutting on a vibrating microtome (VT1000S; Leica). Slices were cultured on Millicell-CM (Biopore PICMORG50; Millipore) in organ tissue dishes containing 1.2 mL of medium (Neurobasal/B-27 with glutamine, 5% FCS, and 1% penicillin/streptomycin; Life Technologies). Slices were allowed to recover for 1–2 h before lentiviral injection. Analysis was performed after a 3-d culture at 37 °C with 5% CO_2.

Neural Progenitor Cell Culture. Neural progenitor cell cultures were established from the MGE of E13.5 mouse embryos. Tissues were dissociated by trituration with a fire-polished Pasteur pipette. Cells were cultured in Neurobasal/B-27 with glutamine, 5% FCS, and 1% penicillin/streptomycin (Life Technologies).

Ca Imaging. Neuronal progenitors were prepared from the MGE at E13.5 and plated onto Matrigel-coated glass-bottom dishes. All measurements were performed 3 d after preparation. For measurement of intracellular calcium, primary cultured neuronal progenitors were loaded with 10 μM Rhod3 acetoxymethyl ester and 2.5 mM probenecid (Rhod3 Imaging Kit; Molecular Probes)

at 37 °C for 1 h according to the manufacturer's instructions. Cells were incubated for 30 min at 37 °C before adding Cxcl12 (R&D Systems), and then fluorescent images were captured through a Zeiss AxioCam MRm CCD camera and processed using ImageJ (National Institutes of Health).

MicroRNA Real-Time RT-PCR. Total cellular RNA, including microRNA (miRNA), was extracted from cells using an miRNeasy Mini Kit (QIAGEN). Total RNA (200–500 ng) was reverse-transcribed with an miScript II RT Kit (QIAGEN). Real-time RT-PCR for the quantification of a subset of miRNAs (miR-200a and miR-224) was carried out with miScript Primer Assays and an miScript SYBR Green PCR Kit (QIAGEN). Values were normalized to SNORD 96A.

miRNA Knockdown Studies. Fluorescein 5′-isothiocyanate–labeled miRCURY LNA miRNA Power Inhibitors (Exiqon) were obtained to inhibit miR-200a, miR-224, or a negative control with no known mouse sequence homology (negative control A). LNA Power Inhibitors were transfected using a NEPA21 electroporator (Nepa Gene). Dissociated mouse MGE-derived neural progenitors were centrifuged at 90 × *g* for 5 min at 4 °C and resuspended in a 100-μL mixture of Opti-MEM (Invitrogen) and 150 pmol LNA Power Inhibitor. Two types of electric pulses were applied to the mixture. Poring pulse condition: 275 V; pulse length, 0.5 ms; two pulses; interval between the pulses, 50 ms; decay, 10%; rate with + polarity. Transfer pulse condition: 20 V; pulse length, 50 ms; five pulses; interval between the pulses, 50 ms; decay, 40%; rate with +/− polarity. After the electroporation, cells were immediately seeded onto a Matrigel-coated slide chamber (Nunc). Three days after the electroporation, cells were fixed for immunocytochemistry.

Real-Time RT-PCR. Total cellular RNA was extracted from cells using an RNeasy Mini Kit (QIAGEN). Total RNA was reverse-transcribed with a PrimeScript First-Strand cDNA Synthesis Kit (Takara). Intron-spanning Taqman probes were designed using the Roche Universal Probe Library method. Amplifications were run in a LightCycler 480 system (Roche). All of the data were analyzed by using β-actin levels as reference.

The following primers were used: *Cxcr4*, 5′-gtctatgtgggcgtctggat-3′ and 5′-acgtcggcaaagatgaagtc-3′, probe: human 63; *Neuregulin1 type I*, 5′-ggaagggcaagaagaaggac-3′ and 5′-cctggcttttcatctctttca-3′, probe: human 107; *Neuregulin1 type III*, 5′-caggaactcagccacaaaca-3′ and 5′-cagtcgtggatgtagatgtgg-3′, probe: human 68; *Neuregulin3*, 5′-aggaagccagcctatcaagc-3′ and 5′-ttcctatgcaacatcccactc-3′, probe: human 26; *ErbB4*, 5′-ctgggggagccttctgat-3′ and 5′-ctgttcctgcgcacactg-3′, probe: human 26.

Subjects and Clinical Assessment. Eighteen subjects with schizophrenia and 17 age-, sex-, education-, and smoking habit-matched normal controls were recruited from our pool of patients and controls who we previously described (4). Patients were recruited from the outpatient psychiatric clinics of the Johns Hopkins Medical Institutions. Diagnosis was performed according to criteria of the Diagnostic and Statistical Manual of Mental Disorders–Fourth Edition (DSM-IV) (American Psychiatric Association). Normal controls were recruited from the general population through flyers posted at the Johns Hopkins Hospital and an ad hoc advertisement placed in a local magazine. All subjects were administered the Structured Clinical Interview for DSM-IV Axis I Disorders–Clinician Version. All patients were assessed with the Scales for the Assessment of Positive and Negative Symptoms by a study psychiatrist who specializes in schizophrenia. Subjects were excluded from the study if they had a history of traumatic brain injury with loss of consciousness for >1 h, a history of drug abuse within 6 mo of the study, drug dependence within 12 mo of the study, or a history of untreated major medical illnesses. The study was approved by the Johns Hopkins Institutional Review Board, and all subjects gave written consent for their participation.

Microarray Analysis. Total RNA from the frontal cortex and striatum was extracted using the RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. The quality of RNA was 10 in RNA integrity number score, which was assessed using a Bioanalyzer RNA 6000 Nano Chip (Agilent Technologies). Fragmented biotin-labeled cRNA was hybridized to Affymetrix U133Plus2.0 according to the manufacturer's protocols. Hybridization, washing, and scanning were conducted according to the manufacturer's instructions. Data analysis was performed using Partek Genomics Suite software (version 6.5). Raw intensities were normalized using gcrma. All microarray procedures were carried out at the Microarray Core Facility of The Johns Hopkins University.

1. Wang Y, et al. (2011) CXCR4 and CXCR7 have distinct functions in regulating interneuron migration. *Neuron* 69(1):61–76.
2. Niwa H, Yamamura K, Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 108(2):193–199.
3. Kimoto S, et al. (2012) Selective overexpression of Comt in prefrontal cortex rescues schizophrenia-like phenotypes in a mouse model of 22q11 deletion syndrome. *Transcult Psychiatry* 2:e146.
4. Ishizuka K, et al. (2010) Negative symptoms of schizophrenia correlate with impairment on the University of Pennsylvania smell identification test. *Neurosci Res* 66(1):106–110.

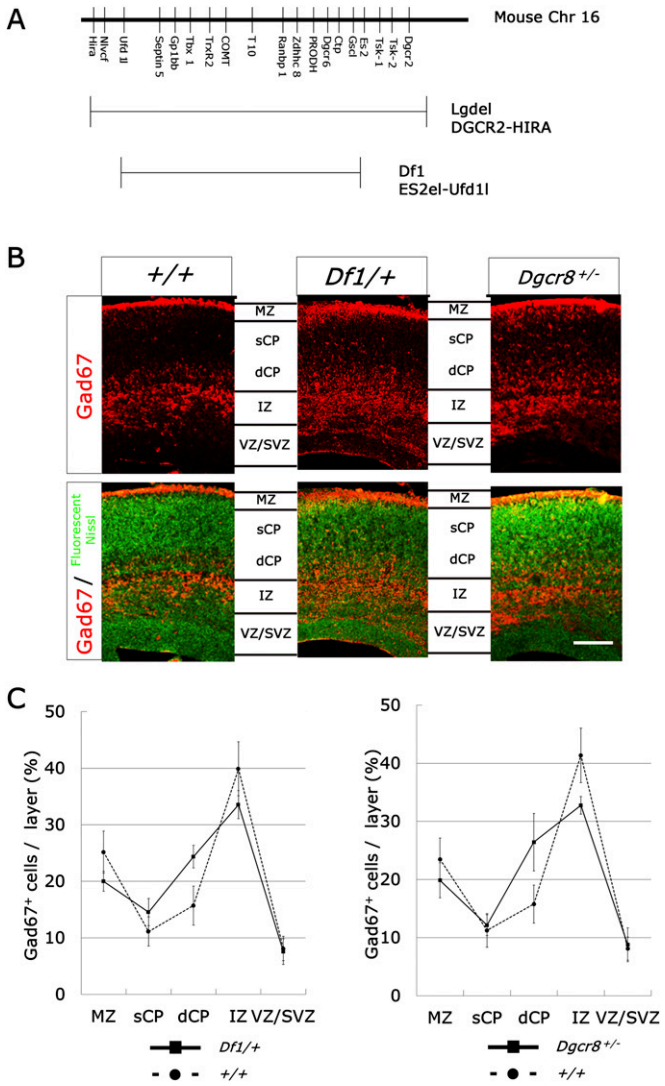

Fig. S1. Abnormal distribution of Gad67^{+} cells in the cortices of *Df1*/+ and *Dgcr8*$^{+/-}$ mice. (*A*) A partial map of the mouse 22q11-related region on chromosome 16. The line below the map indicates the deleted region in *Df1*/+ mice. (*B* and *C*) Immunofluorescence for Gad67 (red; *A*) and fluorescent Nissl (green) of coronal sections of E18.5 *Df1*/+, *Dgcr8*$^{+/-}$, and control cerebral cortices (*B*). Quantification of the distribution of marker-positive cells per layer (*C*). Values are mean ± SD. dCP, deep cortical plate; IZ, intermediate zone; MZ, marginal zone; sCP, superficial cortical plate; VZ/SVZ, ventricular zone/subventricular zone. (Scale bar, 200 μm.)

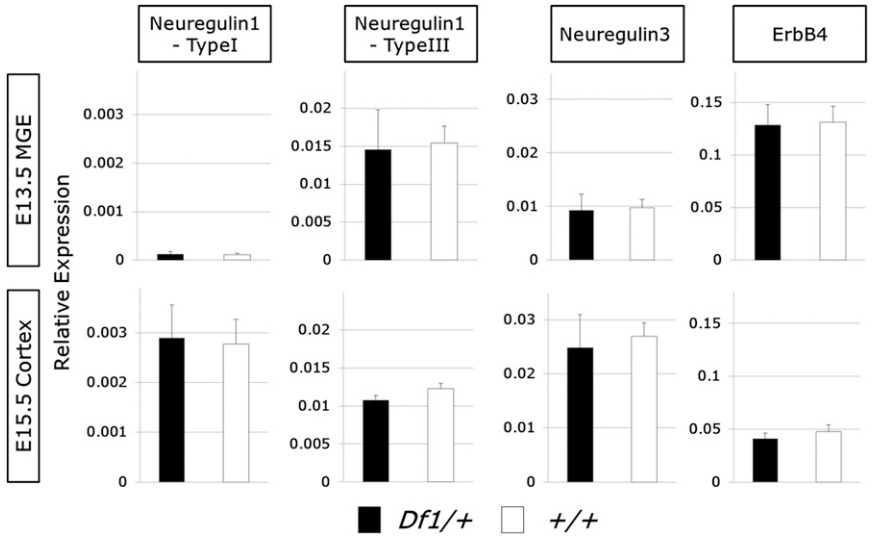

Fig. S2. No abnormalities in the expression of Neuregulin/ErbB4 signaling-related genes in *Df1*/+ mice. Quantitative real-time RT-PCR of Neuregulin1, Neuregulin3, and ErbB4 in E13.5 *Df1*/+ MGE and E15.5 *Df1*/+ cortex. Values have been normalized to β-actin abundance.

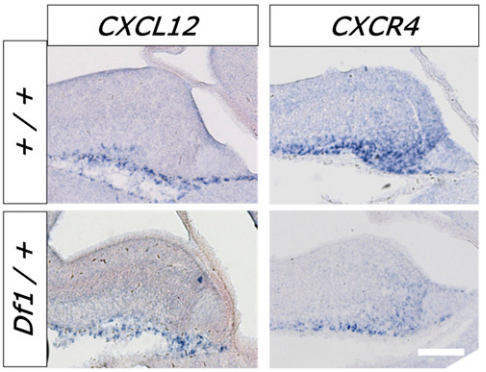

Fig. S3. Microdeletion of the 22q11-related region reduced Cxcr4 expression in the hippocampus. In situ hybridization of Cxcl12 and Cxcr4 in the hippocampus of *Df1*/+ and control mice at E15.5. (Scale bar, 200 μm.)

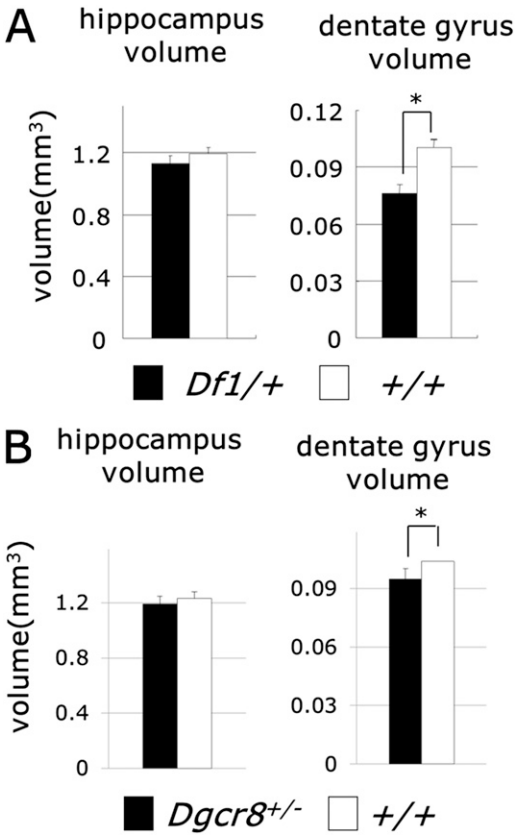

Fig. S4. Reduced volume of the hippocampal dentate gyrus in *Df1*/+ and *Dgcr8*$^{+/-}$ mice. The reduction in the volume of the dentate gyrus, but not hippocampus proper, of P0 *Df1*/+ mice was measured by stereology. Data are shown as mean ± SD. *P = 0.0069 (*A*), *P = 0.036 (*B*).

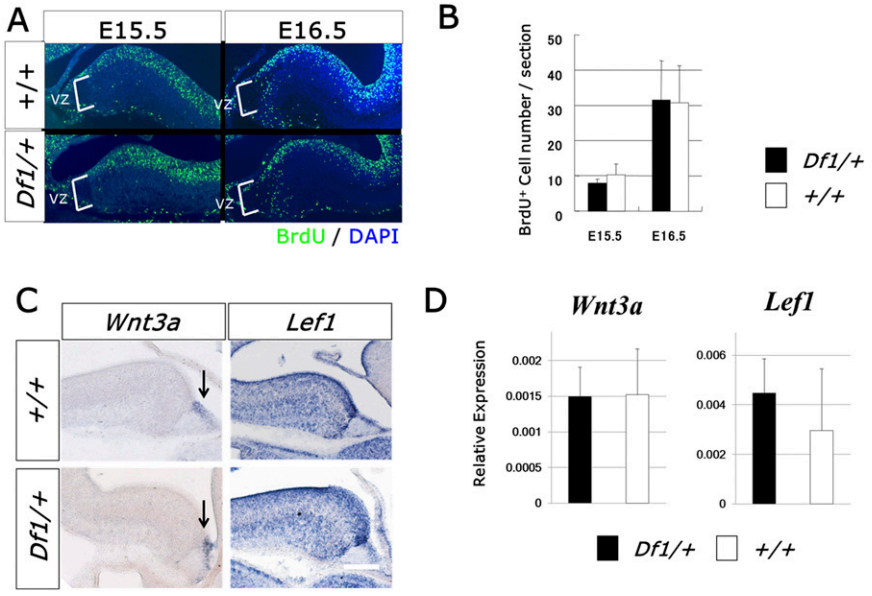

Fig. S5. Intact proliferation of dentate progenitors in the embryonic hippocampus of *Df1*/+ mice. (*A*) Representative images of sections processed for fluorescent immunostaining of BrdU. BrdU was administered 2 h before sacrifice at E15.5 or E16.5. (*B*) The number of BrdU+ cells in the dentate ventricular zone of *Df1*/+ and control mice. BrdU was administered 2 h before sacrifice at E15.5 or E16.5. Data are shown as mean ± SD. (*C*) In situ hybridization of Wnt3a and Lef1 in the hippocampus of *Df1*/+ and control mice at E15.5. Arrows indicate the sites of Wnt3a expression. (Scale bar, 200 μm.) (*D*) Quantitative real-time RT-PCR of Wnt3a and Lef1 in the E15.5 *Df1*/+ hippocampus. Data are shown as mean ± SD. Values have been normalized to β-actin abundance.

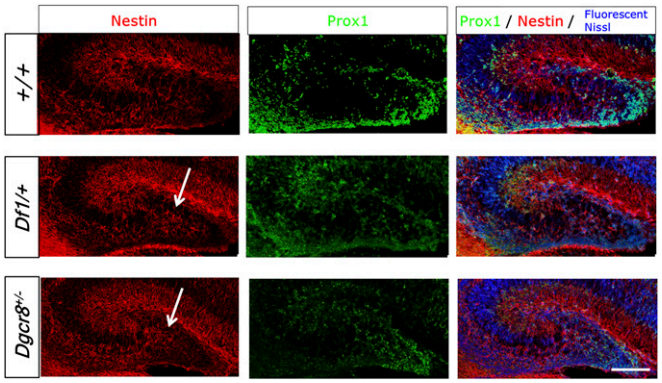

Fig. S6. Abnormal distribution of dentate progenitors in the embryonic hippocampus of *Df1*/+ and *Dgcr8*$^{+/-}$ mice. Immunofluorescence for Nestin (red), Prox1 (green), and fluorescent Nissl (blue) of the hippocampus of E18.5 *Df1*/+, *Dgcr8*$^{+/-}$, and control mice. Arrows indicate ectopic Nestin-positive cells. (Scale bar, 200 μm.)

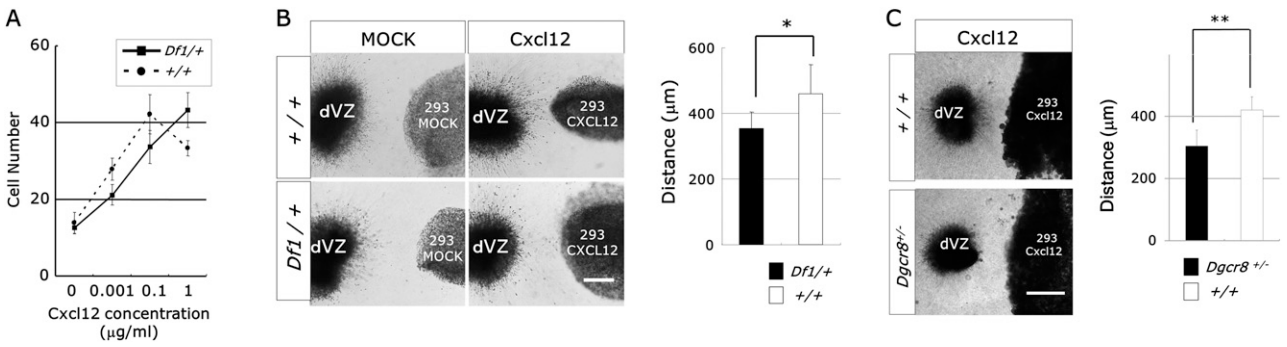

Fig. S7. *Df1*/+ and *Dgcr8*$^{+/-}$ hippocampal dentate precursor cells exhibit decreased responses to Cxcl12. (*A*) Chemotactic response of P0 DG-derived cells from *Df1*/+ and control mice to increasing concentrations of Cxcl12 in vitro. Data are shown as mean ± SD. (*B* and *C*) Dentate ventricular zone (dVZ) explants from the hippocampus of E17.5 *Df1*/+ (*B*), *Dgcr8*$^{+/-}$ (*C*), and control embryos were cultured in Matrigel adjacent to Cxcl12-expressing 293T cell aggregates for 100 h (*Left*). (Scale bars, 400 μm.) The distance migrated by the farthest 20 dVZ-derived cells was measured (*Right*). Values are mean ± SD. $*P = 0.041$ (*B*), $**P = 0.039$ (*C*).

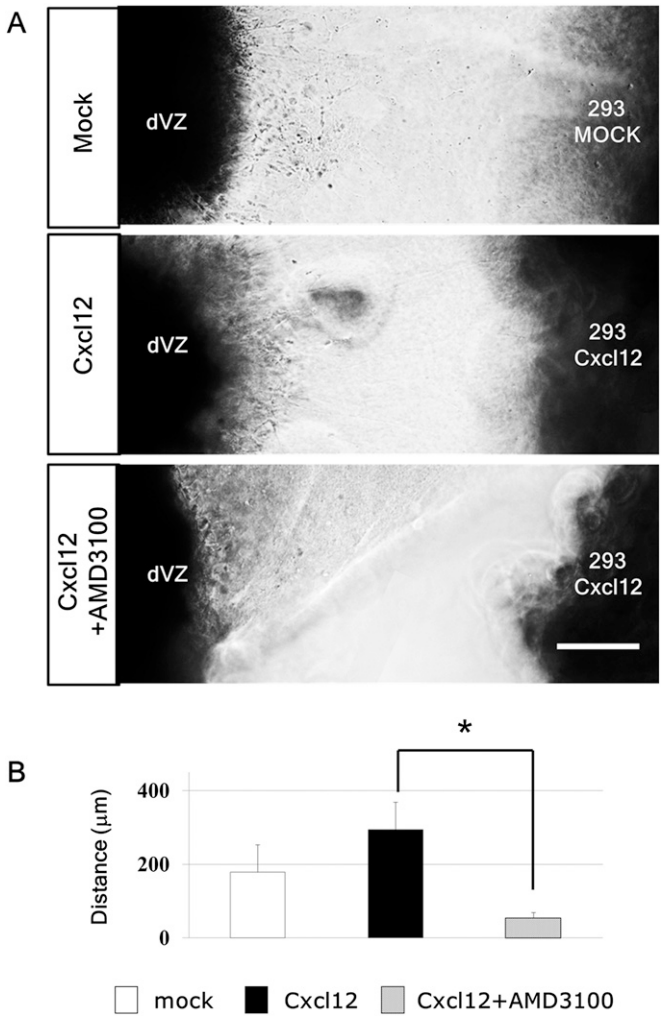

Fig. S8. Cxcl12-dependent migration of dentate ventricular zone-derived cells. dVZ explants from the hippocampus of E17.5 wild-type embryos were cultured in Matrigel adjacent to Cxcl12-expressing 293T cell aggregates for 100 h with or without a Cxcr4 inhibitor, AMD3100 (30 μM) (*A*). (Scale bar, 400 μm.) The distance migrated by the farthest 20 dVZ-derived cells was measured (*B*). Values are mean ± SD. **P* = 0.0022.

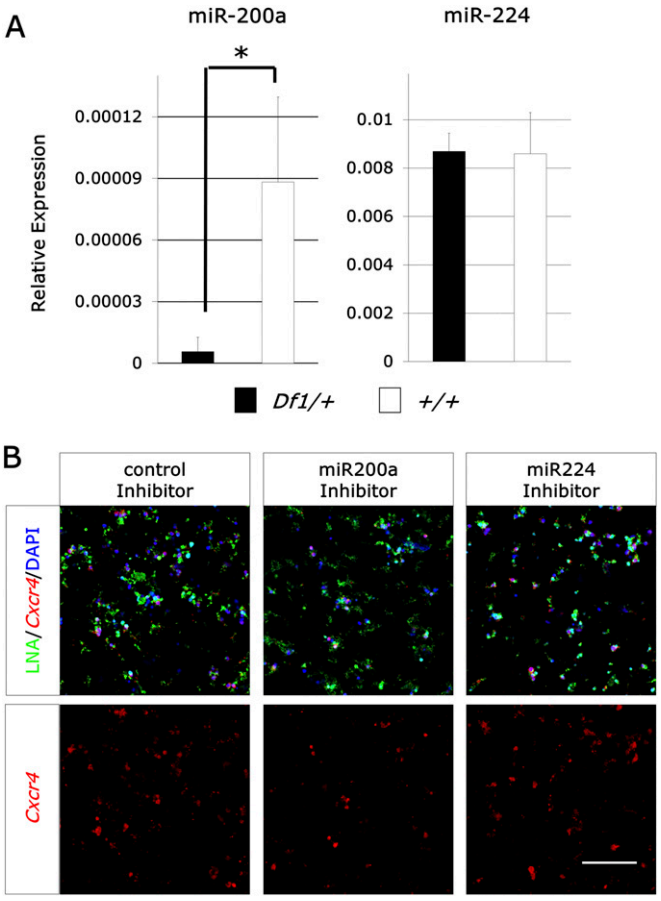

Fig. S9. miRNA abnormality in *Df1*/+ MGE and its effects on Cxcr4 expression. (*A*) Quantitative real-time RT-PCR analysis of miR-200a and miR-224 in E13.5 *Df1*/+ and control MGE. Values have been normalized to SNORD 96A abundance. Values are mean ± SD. **P* = 0.027. (*B*) Inhibition of miR-200a but not miR-224 decreased Cxcr4 expression in MGE-derived neural progenitors. MGE-derived neural progenitors were isolated from E13.5 mouse embryos and transfected with miRNA inhibitors. Immunofluorescent staining was performed 3 d after transfection. Representative images are shown. (Scale bar, 100 μm.)