Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell–derived cortical interneurons from subjects with schizophrenia

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We generated cortical interneurons (cINs) from induced pluripotent stem cells derived from 14 healthy controls and 14 subjects with schizophrenia. Both healthy control cINs and schizophrenia cINs were authentic, fired spontaneously, received functional excitatory inputs from host neurons, and induced GABA-mediated inhibition in host neurons in vivo. However, schizophrenia cINs had dysregulated expression of protocadherin genes, which lie within documented schizophrenia loci. Mice lacking protocadherin-α showed defective arborization and synaptic density of prefrontal cortex cINs and behavioral abnormalities. Schizophrenia cINs similarly showed defects in synaptic density and arborization that were reversed by inhibitors of protein kinase C, a downstream kinase in the protocadherin pathway. These findings reveal an intrinsic abnormality in schizophrenia cINs in the absence of any circuit-driven pathology. They also demonstrate the utility of homogenous and functional populations of a relevant neuronal subtype for probing pathogenesis mechanisms during development.

Schizophrenia is a devastating disease affecting 1% of the adult population worldwide. It is a highly heritable neurodevelopmental disorder characterized by positive symptoms (for example, hallucinations and delusions), negative symptoms (for example, apathy and anhedonia) and cognitive symptoms (for example, impairments of memory, executive functions and attention). Cognitive and negative symptoms persist even with antipsychotic treatment, preventing normal functioning of subjects. Given that schizophrenia is the seventh most costly medical disorder in our society, it is imperative to unravel its pathogenic mechanisms to open the way to a deeper understanding of schizophrenia pathogenic mechanisms. However, deciphering how these risk loci affect gene expression and result in schizophrenia pathogenesis is not a trivial task. Recent studies have made progress in this regard and shown correlations between risk locus genotypes and gene expression levels (for example, FURIN and C4A). However, whether the observed manner of gene expression changes cause schizophrenia-relevant neuroanatomic and behavioral deficits in animal models needs to be determined. One caveat of current expression quantitative trait loci (eQTL) studies is that most of the attempts to connect gene expression levels with risk loci are based on transcriptome studies carried out on postmortem tissues, which typically involve subjects with chronic illness and years of exposure to psychotropic

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medications. Such studies cannot address pathogenic mechanisms operational during development. Thus, disease-specific developmental brain tissue will be needed to unravel the role of these risk genes during critical developmental periods. Induced pluripotent stem cell (iPSC) technology7 allows the generation of such subject-specific developmental brain tissues with the same genetic makeup as the subject brains.

Schizophrenia modeling using iPSCs has begun8, and a steady stream of studies have reported schizophrenia-specific transcriptome aberrations and cellular phenotypes in iPSC-derived neural tissues9–11. It will be important to sort out causal abnormalities versus changes in effectors downstream of causal abnormalities. Correlation with genetic risk loci will provide a pathway for identifying causal abnormalities. In addition, animal model studies can help determine causal abnormalities that can recapitulate schizophrenia-like histological and behavioral abnormalities. Using iPSC-derived neural tissues for studying schizophrenia pathology is challenging because of the heterogeneity and stochasticity of iPSC-derived tissues, which can lead to inconsistency due to the changing composition of neuronal subtypes within culture. Single-cell RNA sequencing (RNA-seq) can be used to address this problem. However, owing to the more limited sequencing depth inherent to single-cell RNA-seq, it is not as easy to identify differences in low-abundance genes, which could have critical roles. Thus, generation of a homogeneous and consistent population of neural subtypes is critical for successful disease modeling using iPSCs.

Cortical GABAergic interneurons (cINs) are one of the most consistently affected neuronal types in schizophrenia, especially medial ganglionic eminence (MGE)–derived cINs expressing parvalbumin (PV+) or somatostatin (SST+), as has been shown in many postmortem studies12–14. In line with these findings, altered GABA neurotransmission in schizophrenia has been suggested to disrupt cortical gamma oscillations and thus cause cognitive deficits in subjects15. Accordingly, GABA receptor agonists restore gamma band activity in schizophrenia subjects, accompanied by some degree of improved cognitive functions16. Furthermore, developmental cIN hypofunction leads to a schizophrenia-like phenotype, including deficits in dopaminergic systems in adult mice17, suggesting a role of impaired cIN development in schizophrenia pathogenesis.

In this study, we used homogeneous populations of cINs derived from iPSCs to analyze cIN-intrinsic deficits in schizophrenia and found that the protocadherin pathway is significantly altered in schizophrenia cINs early in development. cINs in the prefrontal cortex (PFC) of Pcdha knockout (KO) mice showed arborization and synaptic deficits. In line with this, knockdown (KD) of protocadherins in healthy control cINs led to significant reduction in arborization. Developmental schizophrenia cINs displayed arborization deficits in cell culture and synaptic deficits in vivo after transplantation into mouse brains. These deficits were also observed in cINs in schizophrenia postmortem PFCs. Inhibitors of protein kinase C (PKC), a downstream kinase in the protocadherin signaling pathway, normalized the arborization deficits observed in schizophrenia cINs. This study reveals intrinsic deficits in schizophrenia cINs in the absence of any circuit-mediated deficits and demonstrates the utility of iPSC-derived homogeneous neural subtypes to illuminate the mechanistic basis of schizophrenia pathogenesis and to identify potential new therapeutic targets.

Results
Generation of homogeneous populations of cINs from iPSCs. As the first step to study the schizophrenia pathogenic mechanisms in disease-relevant developmental tissues, we generated iPSCs from healthy control and schizophrenia fibroblasts using footprint-free modified RNA methods18. Footprint-free reprogramming is even more important for studying schizophrenia when multiple single nucleotide polymorphisms (SNPs) with small effects work together to lead to pathogenesis. We chose Caucasian male subjects to reduce variation caused by ethnicity and gender. Furthermore, we chose subjects who required clozapine treatment to narrow down the subject group to those with more severe cases of the disease (Fig. 1a). iPSCs reprogrammed with modified RNA transfection19 showed human pluripotent stem cell–like morphology and expressed human pluripotent stem cell markers (Fig. 1b).

To obtain a homogeneous population of developmental cINs from generated iPSCs, we differentiated the iPSCs using the protocol we optimized previously20 with a slight modification (Fig. 1c). This protocol generated a homogeneous population of developmental cINs from multiple iPSC lines. The homogeneity was extensively analyzed by expression of neuronal marker β-tubulin III, MGE-derived cIN marker SOX621, and GABA-synthesizing enzyme glutamate decarboxylase 1 (GAD1) (Fig. 1d,e and Supplementary Fig. 1). Caudal ganglionic eminence (CGE)-derived cINs or non-cIN neural subtypes were observed only rarely regardless of their disease status (Supplementary Fig. 2). We further analyzed the phenotypes of the generated cINs by quantitative real-time PCR (rtPCR) analysis, comparing them with undifferentiated iPSCs and induced glutamatergic neurons22. We observed that cIN-specific genes (GAD1, LHx6, Nkx2.1 and SOX6) are highly expressed in cIN groups but not in the other cell groups. However, there was minimal expression of non-cIN markers (NANOG, PAX6, TBR1 and SLC17A7 (Vglut1)) in generated cINs (Fig. 1f), confirming the identity and homogeneity of the generated cells. The homogeneity of differentiated progeny is critical for the reliable transcriptome analysis of developing cINs.

Neuronal properties of cINs transplanted into mouse brains. Previous studies on cIN induction from human PSCs have focused on human embryonic stem cell–derived cINs, and thus it was unclear whether cINs that are derived from iPSCs, which have gone through the reprogramming process from skin cells, are authentic and functional, and thus are suitable for disease modeling. To address this question, we performed extensive electrophysiological analysis after grafting these cells into mouse brains. Healthy control and schizophrenia cINs were transduced with lentivirus expressing channelrhodopsin 2 (ChR2) H134R–green fluorescent protein (GFP) fusion protein under the control of the synapsin promoter (LV-Syn-ChR2 H134R–GFP) to permit optogenetic study as well as the identification of grafted cells in brain slices. Infected cINs were then transplanted into the cerebral cortices of Nod Scid mice, followed by electrophysiological analysis 7 months after transplantation (Fig. 2a). In GFP+ grafted cells, blue light illumination induced typical ChR2-mediated currents (Fig. 2b) and evoked action-potential firings (Supplementary Fig. 3a). The passive membrane properties of schizophrenia cINs, including membrane resistance (Rm) and membrane capacitance (Cm), were similar to those in the healthy control group (Supplementary Fig. 3b,c). When depolarizing voltage pulses were applied, grafted cINs showed rapidly desensitizing inward currents and sustained outward currents (Fig. 2c), indicating the expression of voltage-gated Na+ and K+ channels. There was no difference between groups in the voltage-dependent Na+ current density (Supplementary Fig. 3d) and the K+ current density (Supplementary Fig. 3e).

In current-clamp mode, the injection of depolarizing currents induced action-potential firings in all human cINs examined (Fig. 2d). Nonaccommodating repetitive action-potential firings were observed in >75% of the grafted cINs in both groups, whereas grafted cells in the remaining proportion displayed single or burst action-potential firing patterns. The frequency of action-potential firings induced by current injection was comparable between groups (Fig. 2e) with no significant difference in afterhyperpolarization, action potential threshold and action potential half-width (Supplementary Fig. 3f–h). However, the resting membrane potential of the cINs was significantly more depolarized in the schizophrenia group than in
Schizophrenia and healthy control iPSCs efficiently generate homogeneous population of cINs. The lines used in each experiment are summarized in Supplementary Table 3. a, Table of subjects analyzed in pilot study. HC, healthy control; SCZ, subject with schizophrenia. b, Immunocytochemistry analysis of human PSC markers OCT4 and TRA-1-60 in generated iPSCs (scale bar, 200 μm). c, Differentiation scheme of cINs from human iPSCs. SRM, serum replacement medium; LDN, 100 nM LDN193189; SB, 10 μM SB431542; SAG, 0.1 μM Smoothened agonist; IWP2, 5 μM inhibitor of Wnt production-2. d, Immunocytochemistry analysis for expression of β-tubulin III (β-Tub), Sox6 and GAD1 in generated cINs after 8 weeks’ differentiation (scale bar, 50 μm). Differentiation was repeated at least three times with comparable results. e, Cell counting analysis after 8 weeks’ differentiation. Data are presented as mean ± s.e.m. from three independent differentiations (n = 3 differentiations). There were no significant differences among different lines based on one-way ANOVA (β-Tubulin III P = 0.2626, Sox6 P = 0.3802, and GAD1 P = 0.5072). f, rtPCR analysis of different cell types (H9 human ES cell, n = 3 batches; human iPSC, n = 6 batches from two iPSC lines; healthy control cIN, n = 9 batches from three lines; schizophrenia cIN, n = 9 batches from three lines; glutamatergic neuron, n = 6 batches from two lines). Results were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression levels and presented as mean ± s.e.m. See also Supplementary Figs. 1 and 2.
the healthy control group (Supplementary Fig. 3i). At resting membrane potential, spontaneous action potential firings were observed in >80% of human cINs in both groups (Fig. 2f and Supplementary Fig. 3j), with comparable spontaneous action potential frequency between groups (Supplementary Fig. 3k), suggesting that most grafted cINs generate tonic spontaneous firings in both the schizophrenia and healthy control groups. Neuronal properties of iPSC-derived cINs were also confirmed in vitro (Supplementary Fig. 4). These results demonstrate that healthy control and schizophrenia cINs develop into functional cINs whose neuronal properties are similar to those of endogenous interneurons.

We next analyzed the synaptic properties of grafted human cINs to determine whether they integrate into adult brain circuitry and receive excitatory synaptic inputs from host cortical neurons. In acute cortical slices, all 26 GFP+ grafted interneurons showed spontaneous excitatory postsynaptic currents (sEPSCs) at −85 mV in voltage-clamp mode (Fig. 2g). These currents were inhibited completely by NBQX, a glutamate receptor antagonist, in all three grafted cells examined, indicating that they were mediated by the excitatory neurotransmitter glutamate. There was no significant difference in the frequency or amplitude of sEPSCs between the healthy control and schizophrenia groups (Supplementary Fig. 5a–c). These results suggest that human cINs from both healthy control and schizophrenia groups have functional postsynaptic machinery to receive excitatory synaptic inputs from host glutamatergic cortical neurons.

We also examined whether grafted human cINs had presynaptic mechanisms for the release of GABA, which induces inhibitory postsynaptic responses in host cortical neurons. To this end, we used optogenetic approaches to selectively stimulate grafted cINs expressing ChR2-GFP and record postsynaptic responses in GFP-host cortical neurons (Fig. 2h,i). Short pulses of blue light illumination induced postsynaptic currents at 0 mV in voltage-clamp mode; these currents were inhibited completely by SR95531, a GABA_A receptor antagonist (Fig. 2j), indicating that they were mediated by inhibitory neurotransmitter GABA. Proportions of host neurons with inhibitory postsynaptic currents were comparable between groups (Fig. 2k,l), suggesting that the majority of recorded host neurons received inhibitory inputs from grafted cINs. The peak amplitude of inhibitory postsynaptic currents induced by photostimulation was not significantly different between groups (Fig. 2k,l and Supplementary Fig. 5d). Our power analysis indicates that we would need to record from 196 host neurons to achieve 80% power for detecting a 36.5 pA mean difference with standard deviation of 57.2 pA and intracluster correlation of 0.2, indicating the large sample size needed to detect mild phenotypes of schizophrenia. These results demonstrate that grafted cINs have presynaptic machinery for GABA release and inhibit host cortical neurons.

**PCDHA2 expression is altered in schizophrenia cINs.** Given the evidence that iPSC-derived healthy control and schizophrenia cINs are authentic and fully functional interneurons, we next investigated whether there were any schizophrenia cIN-specific abnormalities in gene expression during development. We performed RNA-seq analysis, comparing transcriptomes of healthy control cINs to schizophrenia cINs after 8 weeks’ differentiation. A total of 273 million reads were obtained from eight lines (four healthy control and four schizophrenia) with three independent differentiations (Supplementary Fig. 6a). The total number of genes were similar among different RNA-seq reactions (Supplementary Fig. 6b), suggesting that the read depth from each RNA-seq was sufficient to identify most of the genes expressed. When we performed a pair-wise comparison, the average r^2 value for biological replicates was −0.95, showing high correlation that reflects the homogeneous and efficient cIN generation from iPSCs (Supplementary Fig. 6c). Along the same line, the average r^2 between individuals in this study was −0.93, as compared to −0.82 for postmortem tissues (Supplementary Fig. 6c), stressing the consistency of iPSC-derived cIN samples. Principal component analysis showed that biological replicates from the same individual tend to cluster together (Supplementary Fig. 6d), whereas there is no clear separation based on diagnostic group, indicating the subtle nature of schizophrenia phenotype (Supplementary Fig. 6d,e). Neuronal markers (MAP2 and DCX) and cIN markers (GAD1, VGAT, SST and Lhax) were highly expressed, whereas the expression of nonrelevant markers such as GFAP (astrocyte marker), MBP (oligodendrocyte marker), Vglut2 (glutamatergic neuron marker), CoupTFII (CGE-derived cIN marker), Olig2 (early MGE progenitor marker), ChAT (cholinergic neuron marker), TH (dopaminergic neuron marker) and TPH2 (serotonergic neuron marker) were low (Fig. 3a and Supplementary Fig. 7a). Notably, expression of those markers did not differ between healthy control and schizophrenia samples (Supplementary Fig. 7b–f). Although the global gene expression pattern did not show a clear separation between healthy control and schizophrenia samples, select genes did exhibit significant differences in expression between schizophrenia and healthy control samples, such as **PCDHA2** (Fig. 3b and Supplementary Fig. 6f); this was confirmed by RTPCR analysis (Fig. 3c).

To test whether **PCDHA2** expression was also altered in other neuronal subtypes, we generated glutamatergic neurons using the
Ngn2 induction protocol with a slight modification (Fig. 3d). Glutamatergic neurons were efficiently generated from both healthy control and schizophrenia iPSCs (Fig. 3e). rtPCR analysis showed no significant change in PCDHA2 expression in schizophrenia glutamatergic neurons (Fig. 3f). Further studies could reveal whether this observation applies to all developmental glutamatergic neurons or just the induced glutamatergic neurons analyzed here.

Multiple protocadherins affected in schizophrenia cINs. Considering the heterogeneity of schizophrenia etiology, we expanded the sample size and performed RNA-seq analysis on a larger cohort with 14 healthy control lines and 14 schizophrenia lines after 8 weeks’ differentiation (Fig. 4a). Again, the same subject selection criteria were employed with Caucasian males who required clozapine treatment. In addition to PCDHA2, additional
Fig. 3 | PCDHA2 expression is significantly altered in developing schizophrenia cINs. The lines used in each experiment are summarized in Supplementary Table 3. The breakdown data are summarized in Supplementary Tables 4 and 5. a, RNA-seq transcriptome profiling of cINs derived from four healthy control (HC) versus four schizophrenia (SCZ) iPSCs in three independent differentiations ($n=12$ differentiations). Gene expression is shown as RPKM. Differential expression was analyzed in R by the Voom function in Limma with adjusted multiple testing. Center and error bars show mean ± s.e.m. Adj. $P$, adjusted $P$ value. b, PCDHA2 expression is significantly decreased in schizophrenia cINs. Data were collected by RNA-seq transcriptome profiling of cINs derived from four healthy control versus four schizophrenia iPSCs in three independent differentiations ($n=12$ differentiations). Differential expression was analyzed in R by the Voom function in Limma with adjusted multiple testing. Center and error bars show mean ± s.e.m. c, Quantitative rtPCR analysis of PCDHA2 expression in cINs derived from four healthy control versus four schizophrenia iPSCs in three independent differentiations ($n=12$ differentiations). Data were normalized by GAPDH expression level. Center and error bars show mean ± s.e.m. d, Differentiation scheme for glutamatergic neurons. Human iPSCs were plated with lentivirus expressing inducible Ngn2-puroR and treated with doxycycline (DOX) until day 10. Cells were treated with puromycin on day 2 and were used for analysis on day 14. e, Immunocytochemistry analysis of iPSC-derived glutamatergic neurons (scale bar, 20 μm). This analysis was repeated in two batches of differentiation with similar results. f, Quantitative rtPCR analysis of PCDHA2 expression in glutamatergic neurons derived from four healthy control versus four schizophrenia iPSCs in two independent differentiations ($n=8$ differentiations). PCDHA2 expression level was normalized using that of GAPDH. Center and error bars show mean ± s.e.m. See also Supplementary Figs. 6 and 7. For detailed statistics information, see Supplementary Table 15.

PCDHA genes are downregulated in schizophrenia cINs, including PCDHA3, PCDHA6 and PCDHA8 (Fig. 4b). rtPCR analysis confirmed significant decreases of these PCDHA family members in schizophrenia cINs (Fig. 4c), whereas expression of PCDHA3, PCDHA6 and PCDHA8, like that of PCDHA2, was not altered in schizophrenia glutamatergic neurons (Supplementary Fig. 8a).

Publicly available eQTL databases (https://www.gtexportal.org and https://www.synapse.org/cmc) showed that there are many PCDHA2 eQTL SNPs with high schizophrenia association (Supplementary Fig. 8b), suggesting a potential role of schizophrenia risk loci in regulating expression of PCDHA2. We selected rs7445192 for genotype-gene expression correlation analysis owing to its high correlation with schizophrenia with a higher odds ratio and significant eQTL status for PCDHA2 (Supplementary Fig. 8c).

There was a trend of decreasing PCDHA2 expression in iPSC-derived cINs with more alternate alleles of rs7445192 (Fig. 4d), although this did not reach statistical significance. Power analysis showed that 256 samples would be needed to reach statistical significance. Searches in the CommonMind portal showed that PCDHA2 has 533 eQTL SNPs and PCDHA3 has 0 eQTL SNPs. PCDHA6 has 88 eQTL SNPs, but rs7445192 was not one of them (Supplementary Fig. 8d). Notably, we did not see genotype-expression level correlation in these two genes without a previous eQTL connection with this SNP (Fig. 4d). PCDHA8 has 800 eQTL SNPs, including rs7445192, and also shows the tendency of decreasing expression by more alternate alleles (Fig. 4d), although again this finding did not reach statistical significance. Power analysis showed that 226 samples would be needed to reach statistical significance.
Multiple PCDHA family members are affected in developing schizophrenia cINs. The lines used in each experiment are summarized in Supplementary Table 3. The breakdown data are summarized in Supplementary Table 6. a, Table of subjects in the expanded cohort. b, Changes in multiple PCDHA gene expression, analyzed by RNA-seq of cINs derived from 14 healthy control versus 14 schizophrenia iPSCs in two independent differentiations (n = 28 differentiations). Expression level was shown as transcripts per kilobase million (TPM). Differentially expressed genes were analyzed by DESeq2 or Sleuth using Wald test for significance testing. Center and error bars show mean ± s.e.m. c, Correlation between genotype and gene expression level. R designates reference allele (Guanine) and A designates alternate allele (Adenosine) of rs7445192. Gene expression levels were normalized by β-actin and grouped by genotype. The difference between the RR group and the AA group was analyzed by two-tailed unpaired t-test (n = 8 for RR and n = 8 for AA). PCDHA2 P = 0.5102, PCDHA3 P = 0.009, PCDHA8 P = 0.0065, PCDHA6 P = 0.0234. Center and error bars show mean ± s.e.m.
d, Heatmap depicting expression changes of protocadherin genes in schizophrenia cINs. Blue color depicts downregulation in schizophrenia cINs and red depicts upregulation. Numbers in colored box indicate expression change in log2 fold change. e, eQTL diagram of different protocadherin genes showing presence of multiple eQTLs for protocadherin family members within schizophrenia risk loci (Chrs: 14,023,664–140,222,664). Red dots are significant cis-eQTLs for the given gene at false discovery rate (FDR) < 5%. More detailed information on this analysis is available in the GTEx portal (https://gtexportal.org/home/). See also Supplementary Fig. 8. For detailed statistics information, see Supplementary Table 16.
In addition to PCDHA members, the majority of other protocadherin members were also downregulated in schizophrenia cINs (Fig. 4e). RT-PCR analysis confirmed significant decreases of all PCDHA and PCDHG family members in schizophrenia cINs, using primers against the PCDHA-constant region (CR) and PCDHG-CR (Supplementary Fig. 8e). However, expression of these genes was not altered in schizophrenia glutamatergic neurons (Supplementary Fig. 8f). The publicly available eQTL databases showed that other highly affected protocadherins also had eQTL within the schizophrenia risk loci (Chr5: 140023664–140222664) a little bit further from the area where most of their eQTLs reside (Fig. 4f and Supplementary Fig. 8g). This suggests that this schizophrenia risk locus may be related to the regulation of multiple protocadherin family members in addition to PCDHA members.

**Altered cINs and prepulse inhibition in Pedha KO mice.** Having observed downregulation of multiple protocadherin family members, including PCDHA and PCDHG, in schizophrenia cINs, we examined the effect of protocadherin hypofunction on cIN development using a mouse model with a Pedha null mutation (Δα/Δα)20, in which all members of the Pedha family are knocked out. Pedha is necessary for proper Pedha localization and function21–22 (but not vice versa). As such, Pedha KO mice show severe lethal phenotypes, unlike Pedha KO mice, which show mild phenotypes. Thus, we used the partially compromised protocadherin function in Pedha KO mice to model the protocadherin hypofunction seen in schizophrenia cINs. We analyzed the arborization of PFC cINs within 150 μm from the cell bodies and found a mild but significant arborization deficit in Pedha KO cINs, shown by significant decreases in neurite number from soma, total branch number and total neurite length (Fig. 5a and Supplementary Fig. 9a). We also observed deficits in inhibitory synapse formation of PFC cINs in Pedha KO mice by analyzing the number of VGAT+ puncta on PV+ cINs that were juxtaposed with gephyrin+ puncta (Fig. 5b and Supplementary Fig. 9b). However, there was no significant difference in the excitatory synapses that cINs receive between Pedha KO mice and littermate wild-type controls (Fig. 5c and Supplementary Fig. 9c). These results suggest the importance of protocadherin pathways in the normal development of cINs in the PFC.

Previous studies have shown that Pedha KO mice harbor projection and connection deficits in different brain regions and display deficits in contextual learning and working memory23, which are relevant to the schizophrenia clinical phenotype. We tested whether Pedha KO mice show deficits in prepulse inhibition (PPI), which occur in many schizophrenia subjects and can be attenuated by antipsychotic treatment24. Pedha CR KO mice25, which have nonfunctional Pedha expression, showed significant deficits in PPI compared to their wild-type littermates (Fig. 5d). Pedha3 hypomorphic mice (ΔBneo/ΔBneo)24 also showed significant PPI deficits (Fig. 5e), suggesting the importance of the protocadherin pathway for normal sensorimotor gating.

**Deficits in arborization and synapses in schizophrenia cINs.** Having observed cIN deficits by protocadherin hypofunction during cIN development, we tested whether schizophrenia cINs also demonstrate similar phenotypic deficits. To analyze arborization, we injected healthy control and schizophrenia cINs with a limiting titer of lentivirus expressing GFP under the ubiquitin promoter (LV-Ubi-GFP) to label cells only scarcely. We then traced and analyzed GFP+ cells. There was a mild but significant decrease in neurite number from soma, total branch numbers and total neurite lengths in schizophrenia cINs compared to healthy control cINs (Fig. 6a). Linear regression analysis showed that the expression levels of PCDHA2, PCDHA3, PCDHA6 and PCDHA8 were weakly or mildly correlated with arborization (Supplementary Fig. 10a). In the excitatory glutamatergic neuronal context, protocadherin CR inhibits focal adhesion kinase (FAK) and, in turn, PKCα, which otherwise inhibits arborization by inactivating MARCKS via phosphorylation21,22. Thus, we tested whether treating schizophrenia cINs with PKC inhibitors can reverse this deficit through protocadherin hypofunction. Treatment with PKC inhibitor GO6893 reversed the arborization deficit in the schizophrenia cINs, whereas it did not produce a significant difference in healthy control cINs (Fig. 6b and Supplementary Fig. 10b). To more directly test the effect of lower protocadherin expression on cINs, we performed gene KD experiments against the PCDHA-CR or the PCDHG-CR that knock down entire PCDHA or PCDHG families (Supplementary Fig. 10c). One-month-old cINs were transfected with negative-control short interfering RNA (siRNA), PCDHA siRNA, PCDHG siRNA, or PCDHA siRNA plus PCDHG siRNA, infected with limiting titer of LV-Ubi-GFP virus, and traced at 7d after transfection. There was a mild but significant decrease in arborization of healthy control cINs by PCDHA KD or PCDHG KD, and to a lesser degree in schizophrenia cINs (Fig. 6c and Supplementary Fig. 10d).

To study healthy control and schizophrenia cINs in an in vivo circuit environment, we transplanted 8-week-old cINs to the cerebral cortices of Nod Scid mice after labeling them with LV-Syn-Chir2 H134R–GFP and analyzed their phenotypes (Fig. 7a). One of the characteristic behaviors of developing cINs is robust migration, even in transplanted adult brains; this migration comparable to their extensive migration during early development25. DISCO+ clearing26 and light sheet microscopy showed robust migration of grafted cINs (Fig. 7b) up to 2,000 μm from the injection site in the adult mouse cortex (Fig. 7c,d). We further characterized cINs in the brain environment with immunocytochemistry. Consistent with their MGE-derived phenotype, many grafted cINs express either MEF2C, a marker enriched in PV+ interneuron precursors in mice27, or SST (Fig. 7e), regardless of their disease status. We also observed that a small proportion of cINs started to express PV, regardless of their disease status (Supplementary Fig. 11a). Overall, these results suggest that there was no difference in cIN fate determination between healthy control and schizophrenia populations. Synapse analysis showed a mild but significant deficit in inhibitory synapse formation by schizophrenia cINs compared to healthy control cINs (Fig. 7f and Supplementary Fig. 11b). The excitatory synapse density did not differ between grafted healthy control cINs and schizophrenia cINs (Fig. 7g and Supplementary Fig. 11c). Linear regression analysis showed that the expression levels of PCDHA2, PCDHA3, PCDHA6 and PCDHA8 were weakly or mildly correlated with inhibitory synapse formation (Supplementary Fig. 11d). These results suggest that schizophrenia cINs have an intrinsic deficit in the formation of inhibitory synapses in the absence of a schizophrenia circuit environment.

**cIN deficits in schizophrenia subject postmortem brains.** Having observed developmental schizophrenia cIN-intrinsic phenotypic deficits, we sought to explore whether the observed developmental deficits continue to be present in adult postmortem brains. Thus, we analyzed layer 3 PV+ cINs from eight healthy control and eight schizophrenia postmortem PFCs (Fig. 8a). Arborization analysis showed a mild but significant deficit in schizophrenia postmortem cINs that was indicated by significant decreases in neurite number from soma, total branch number and total neurite length (Fig. 8b and Supplementary Fig. 12a). This was consistent with the phenotypes observed in developmental cINs. A mild but significant deficit in inhibitory synapse formation was also observed in postmortem schizophrenia cINs in the PFC (Fig. 8c and Supplementary Fig. 12b), also consistent with results from developmental cINs. However, unlike in the developmental schizophrenia cINs, excitatory synapse formation was significantly reduced in postmortem schizophrenia cINs (Fig. 8d and Supplementary Fig. 12c), possibly owing to deficits in glutamatergic neurons.
**Discussion**

In this study, we find that the expression of protocadherins, genes within well-documented schizophrenia risk loci, are significantly downregulated in schizophrenia cINs during development and that their altered expression led to disease-relevant phenotypes in KO mice and human schizophrenia cINs. The discovery of altered...
expression of genes within schizophrenia risk loci is important as it may shed light on the causal pathophysiology of schizophrenia. The presence of many significant eQTL SNPs for PCDHA members within schizophrenia risk loci suggests that this particular schizophrenia risk locus may regulate PCDHA gene expression. The modest change of PCDHA gene expression we observed is in agreement with the previous large-scale eQTL studies for schizophrenia risk loci. Also, it is consistent with the notion that small changes in many genes may epistatically cause the disease phenotype, stressing the importance of studying ‘pathway’ changes instead of ‘single-gene’ changes to better understand schizophrenia pathogenesis. Notably, the direction of expression alterations by risk alleles is different depending on the brain regions examined, stressing the importance of using disease-relevant and homogeneous cell populations to understand the effect of the risk genotype.

Fig. 6 | Developing schizophrenia cINs show arborization deficit relevant to protocadherin dysregulation in vitro. The lines used in each experiment are summarized in Supplementary Table 3. The breakdown data for each line are summarized in Supplementary Tables 9–11. a, Arborization analysis of healthy control versus schizophrenia cINs infected with a limiting titer of GFP-expressing lentivirus. Images were analyzed using ImageJ with the Neuron J plugin. (n = 180 neurons). Center and error bars show mean ± s.e.m. b, Arborization analysis of PKC inhibitor–treated healthy control versus schizophrenia cINs. cINs infected with a limiting titer of GFP-expressing lentivirus were treated with PKC inhibitor GO6893 for 12 d and analyzed using ImageJ with the Neuron J plugin (n = 160 neurons). Center and error bars show mean ± s.e.m. VEH, vehicle-treated samples. c, Arborization analysis of siRNA transfected healthy control versus schizophrenia cINs (two independent differentiations). cINs infected with a limiting titer of GFP-expressing lentivirus were transfected with siRNA against PCDHA, PCDHG, or a mixture of the two (PCDHA+G) and analyzed using ImageJ with the Neuron J plugin (n = 60 neurons). Center and error bars show mean ± s.e.m. Negative cont., negative control. *P < 0.05 compared to the negative control. See also Supplementary Fig. 10. For detailed statistics information, see Supplementary Tables 17 and 18.
Fig. 7 | Developing schizophrenia cINs show synaptic deficit in vivo after transplantation into mice brains. The lines used in each experiment are summarized in Supplementary Table 3. The breakdown data for each line are summarized in Supplementary Table 12. a, Schematic diagram of transplantation analysis of healthy control versus schizophrenia cINs. 3D, three-dimensional. b, 3D lightsheet microscopy images of an iDISCO+–cleared mouse cortex transplanted with iPSC-derived human cINs, stained with human cytoplasm antibody. Major grid in reference frame is 300 µm. This result is from a single whole-brain staining. c, d, Recognition of grafted neurons by Imaris software, green dots. Cells in front look bigger with bigger green dots, and the cells in back look smaller with smaller green dots. Graphs depict location of grafted cells along the x, y and z axes and distance from the injection site. This result is from a single whole-brain staining. e, Immunocytochemistry analysis of transplanted cINs 7 months after grafting (scale bar, 50 µm). Left, MEF2C+ neurons among human NCAM+ grafted neurons (P = 0.7737, chi-squared test). Right, SST+ neurons among human NCAM+ grafted neurons (P = 0.6575, chi-squared test). White arrowheads indicate double-positive neurons. f, Inhibitory synapse analysis on transplanted GFP+ cINs 4 months after grafting. The data are presented as number of inhibitory synapses (juxtaposed GFP+VGAT+ puncta and gephyrin+ puncta) per 100 µm² of GFP+ cINs. Center and error bars show mean ± s.e.m. (n = 14 images (116 µm x 116 µm) per group). g, Excitatory synapse analysis on transplanted GFP+ cINs 4 months after grafting. The data are presented as the number of excitatory synapses (juxtaposed GFP+PSD95+ puncta and Vglut1+ puncta) per 100 µm² of GFP+ cINs. Center and error bars show mean ± s.e.m. (n = 14 images (116 µm x 116 µm) per group). See also Supplementary Fig. 11. For detailed statistics information, see Supplementary Table 17.
Protocadherins are a family of cell surface proteins that have important roles during brain development. They are transferred between the cell surface and intracellular organelles, and cell surface localization of PCDHA is facilitated by heterodimer formation with PCDHG. Once PCDHA is on the cell surface, often times as a heterodimer with PCDHG, it inhibits the FAK-PKC pathway, thereby facilitating neurite arborization, thereby facilitating neuro-synthesis. Consistent with this inference, we observed that Pcdha deficiency leads to an arborization defect in cINs in Pchda-null mice PFC and in schizophrenia cINs transplanted into mouse cortex. Downregulation only in PCDHA members probably does not generate protocadherin-related phenotypes in schizophrenia cINs, considering the mild phenotype observed in Pchda KO mice, unlike the severe lethal phenotypes of Pcdhg KO mice. However, downregulation of many members of the protocadherin family, including both PCDHA and PCDHG, may explain the observed mild phenotype in schizophrenia cINs. A modesty abnormal phenotype in schizophrenia cINs is in line with the overall mild pathology of schizophrenia observed in postmortem studies.

Schizophrenia is a uniquely human disease that is not easy to model behaviorally in animals, but there are animal models in which environmental and genetic risk factors generate schizophrenia-like behaviors. Schizophrenia subjects display sensory motor gating deficits, cognitive deficits and anhedonia, and these deficits can be seen in animal models of schizophrenia. Pchda-null mutant mice have working memory deficits. In this study, we observed an inhibitory presynaptic deficit in cINs both in the Pchda-null mice PFC and in schizophrenia cINs transplanted into mouse cortex. Downregulation only in PCDHA members probably does not generate protocadherin-related phenotypes in schizophrenia cINs, considering the mild phenotype observed in Pchda KO mice, unlike the severe lethal phenotypes of Pcdhg KO mice. However, downregulation of many members of the protocadherin family, including both PCDHA and PCDHG, may explain the observed mild phenotype in schizophrenia cINs. A modestly abnormal phenotype in schizophrenia cINs is in line with the overall mild pathology of schizophrenia observed in postmortem studies.

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that Pedha-null mutant mice and Pedha-hypomorphic mice display deficits in PPI, indicating that reduced Pedha expression leads to impaired sensory motor gating. This behavioral deficit is consistent with the schizophrenia-like histological deficits that we observed in PFC cINs in Pedha-null mice and suggests the role of reduced PCDHA expression in schizophrenia pathogenesis.

Previously, it was not clear whether cIN hypofunction in schizophrenia PFC was due to intrinsic defects in cINs and/or a result of hypofunction of glutamatergic input into cINs. In this study, using a homogeneous population of cINs without any schizophrenia glutamatergic input, we observed cIN-intrinsic abnormalities in vitro and in vivo. These findings demonstrate the potential of homogeneous iPSC-derived neural subtypes for unraveling disease mechanisms. In this example, the mixed cell populations used in most previous iPSC disease modeling studies10 would not have allowed detection of cell type–specific pathological changes of minor populations. We also obtained a homogeneous population of glutamatergic neurons using Ngn2-based induction, a well-accepted protocol in the field19, although the induced glutamatergic neurons may not be identical to in vivo glutamatergic neurons. In addition to the homogeneity and consistency of generated cINs, the opportunity to perform extensive electrophysiological characterization gave us confidence about their authenticity and functionality. In this study, inhibitory postsynaptic currents in host cortical neurons induced by stimulation of grafted cINs were not significantly different between groups, although significant differences were detected in synapse analysis. This could be due to the small sample size of our electrophysiological experiments and/or compensatory mechanisms20 in human cINs transplanted into normal mice brains. In the future, further technical developments that allow high-throughput analysis, such as the patch clamp array, will be pivotal to obtaining enough power to electrophysiologically study mild schizophrenia phenotypes. iPSC-derived human disease tissues can be used to screen chemicals to correct identified cellular phenotypes, thus providing unprecedented opportunities to develop mechanistic treatments. This is especially important considering that the complex genetics of schizophrenia, with over a hundred risk alleles already identified that each have only a modest effect, are difficult to replicate in experimental animals. We are not surprised that many effective treatments developed in animal models have failed in human clinical trials21,22. In our study, we have observed that treatment with a PKC inhibitor corrects the abnormal cellular phenotype in schizophrenia cINs. In this regard, it is notable that a PKC inhibitor was successfully used to treat acute mania in bipolar disorders in small-scale clinical trials23,24, as bipolar disorder shows substantial clinical and genetic overlap with schizophrenia25.

Reduction in synapse density in both excitatory synapses and inhibitory synapses has been reported in schizophrenia postmortem studies26,27, and the decrease in the number of inhibitory synapses in developmental schizophrenia cINs is in line with that observation. However, we observed fewer excitatory synapses in postmortem cINs, but not in developmental cINs transplanted into the mouse cortex. This can be explained if the decrease seen in human postmortem brains is due to the reduction in excitatory output from glutamatergic neurons rather than the intrinsic deficit of interneurons in receiving excitatory input. In addition, whereas numerous postmortem studies confirmed reduced GAD expression in schizophrenia interneurons, we did not see such deficits in these developmental cIN populations. This discrepancy could be a consequence of comparing cells in culture with cells in vivo, or may have resulted from differences in interneuron age or subtypes. Also, it could be due to the fact that GAD expression is regulated in an activity-dependent manner28. Thus, rather than being an intrinsic deficit of schizophrenia interneurons, the reduction in GAD expression could have appeared only in the circuit environment with defective schizophrenia glutamatergic neurons.

In summary, the discovery of disrupted gene expression within schizophrenia risk loci, along with the animal and cell model effects, suggests the possibility of a causal relationship between protocadherins and schizophrenia. In addition, our study demonstrates the power of using homogeneous and functional populations of a specific neuronal subtype known to be affected in schizophrenia to probe the pathogenesis of schizophrenia, which may provide a pathway to identify more effective therapies and preventive interventions.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41593-018-0313-z.

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Author contributions

Z.S., H.N., P.N., W.B.K., J.T.C., R.H.S., B.M.C., T.H., E.F., T.Y., S.G., K.C.E., A.A.M., P.K.S., J.-H.C. and S.C. designed the experiments. Z.S., H.N., P.N., C.N., S.E.C., E.N., J.T., T.P., J.M.P., K.Z. and J.J.P. performed analysis of iPSC-derived interneurons. W.B.K., A.A.M., P.K.S. and J.-H.C. performed electrophysiological studies. T.A.L., H.S.X., C.Y. and W.H. did RNA-seq analysis. T.H., E.F. and T.Y. generated PCDHA KO mice and performed behavioral analysis of PCDHA KO mice. R.E.S., S.G. and K.C.E. performed genotype analysis. I.M.E. performed confocal analysis. H.-Y.K. performed statistical analysis. H.N., J.T.C., T.A.L., H.S.X., W.H., T.Y., E.F., T.Y., S.G., K.C.E., A.A.M., P.K.S., J.-H.C. and S.C. wrote the manuscript. D.R.W., R.E.S., K.F.B., J.A., D.O., B.M.C., D.L.M., J.L.R. and R.H.P. provided subject cell lines and reviewed data interpretation and manuscript contents. T.H., T.Y., J.-H.C. and S.C. supported this study financially.

Competing interests

T.A.L. and H.S.X. were employees of Pfizer, Inc. at the time this work was performed.

Additional information

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Generation of induced pluripotent stem cells. These study protocols were approved by the McLean Hospital/Partners Healthcare Institutional Review Board and New York Medical College Institutional Review Board. All procedures were performed in accordance with the Institutional Review Boards guidelines and all human samples were obtained with informed consents. We have complied with all relevant ethical regulations. Human fibroblasts were obtained from the laboratories of B.M. Cohen (McLean Hospital), D.R. Weinberger (Lieber Institute for Brain Development) and J.L. Rapoport (National Institute of Mental Health). Skin biopsies were performed on healthy volunteers. The skin tissue was cut out under local anesthesia (2% lidocaine with or without epinephrine), washed with PBS three times and placed in 35-mm culture dishes. One drop of DMEM with Glutamax with 10% FBS, 2% l-glutamine, 1% penicillin-streptomycin solution and 1% amphotericin and gentamycin solution was added on each piece of skin and put in the incubator (37 °C, 5% CO2). The skin samples in the culture dish were changed with fresh medium every other day to induce fibroblast cell growth as described7. Human fibroblasts were reprogrammed using modified RNA methods by Cellular Reprogramming, Inc. (San Diego, CA). Each fibroblast line was plated in 6-well plates without feeders at three different plating densities and subjected to mRNA reprogramming. Nascent colonies were bulk passaged from the most productive well to establish passage 1 (P1) iPSC cultures on Laminin-521 (BioLamina) in Nutristem XF medium (Biological Industries) and expanded in the same culture system until at least passage 3 before being characterized by 4′,6-diamidino-2-phenylindole (DAPI)/OCT4/TRA-1-60 immunostaining and frozen down for storage.

Differentiation of iPSCs into cINs. Thawed human iPSCs were maintained on Matrigel (BD) coated plates with Essential 8 (E8) medium (Invitrogen). For differentiation, iPSCs were trypsinized and grown as floating spheres in low adherent flasks in KSR medium (DMEM, 15% knockout serum replacement, 2 mM L-glutamine and 0.1 mM non-essential amino acids and 4 mM HEPES) from day 0 to day 14. For neuroectoderm induction, cells were treated with LDN193189 (100 nM, Stemgent) from day 0 to day 14 and SB431542 (10 μg, Tocris Cookson) from day 0 to day 7. For MGE phenotype induction, medium was supplemented with IWP2 (5 μM, EMD Millipore) from day 0 to day 7 and SAG (0.1 μM, EMD Millipore) from day 0 to day 21. From day 14, cells were grown in N2AA medium (DMEM:F12 with N2-supplement (1:200, Invitrogen) and 200 μM ascorbic acid (Sigma)). FGF2 (100 ng/mL, Peprotech) was added from day 14 to day 21 to induce MGE phenotype at the expense of the CGE phenotype1. N2AA medium was supplemented with 10 ng/ml glial cell-derived neurotrophic factor (GDNF, Peprotech) and 10 ng/ml brain-derived neurotrophic factor (BDNF, Peprotech) from day 21. At day 5 of differentiation, cIN spheres were trypsinized in the presence of 0.1% trachelase (Sigma) and then plated on polyornithine (15 mg/ml); Sigma) - and fibronectin (1 mg/ml); Sigma)-coated plates in B27G medium (DMEM:F12 medium with B27 supplement (1:100, Invitrogen), 10 ng/ml GDNF and 10 ng/ml BDNF). Cell lines differentiated for each experiment are summarized in Supplementary Table 3.

siRNA transfection. At day 28 of differentiation, cIN spheres were trypsinized and then plated onto polyornithine- and fibronectin-coated plates in B27G medium. At day 2 after plating, cINs were transfected with negative-control siRNA (Ribobio), PCDHA siRNA (siG80120120039, Ribobio), PCDH8 siRNA (siB18042313250, Ribobio) and PCDHA plus PCDH8 siRNA (Ribobio) at a final concentration of 25 nM by using Lipofectamine RNAiMAX reagent (Life Technologies) according to the manufacturer’s protocol. At 6–8h after siRNA transfection, the cells were infected with a LV-Ubi-GFP virus, followed by arborization analysis 7 d after transfection.

Differentiation of iPSCs into glutamatergic neurons. Human iPSC cells were seeded at a density of 105 cells per well on Matrigel-coated 6-well plates with E8 medium, 5 μM Rock inhibitor Y-27632 (Selleck Chemicals), lentinivirus that inductively expressed Ngn2 and constitutively expressed puromycin resistance gene (packaged using PLV_TRET_hNgn2_UBC_Puro plasmid from Addgene, plasmid 61474 (Addgene)), 2 μg/mL doxycycline and 2 μg/mL polybrene. After overnight incubation (day 1), the medium was changed with N2AA medium (DMEM:F12 with N2 supplement (1:200), 200 μM ascorbic acid, 10 ng/mL GDNF and 10 ng/mL BDNF) with 2 μg/mL doxycycline. On day 2, 1 μg/mL puromycin was added to the culture. On day 3, the medium was changed with B27AAGB medium (DMEM:F12 with B27 supplement (1:100), 200 μM ascorbic acid, 10 ng/mL GDNF and 10 ng/mL BDNF). Santa Fe glia (Molecular Devices) were added on day 6. On days 11–14, the medium was replaced with B27AAGB medium without doxycycline until the neurons were harvested for RNA preparation or fixed for immunocytochemistry on day 14 of differentiation.

Immunocytochemistry, cell counting and arborization analysis. Cells were fixed in 4% paraformaldehyde for 10 min. The fixed cells were blocked in PBS with 10% normal serum and 0.1% Triton X-100 for 10 min at room temperature. Cells were incubated with the primary antibody in PBS with 2% normal serum overnight at 4°C. After being washed in PBS, the cells were incubated with the fluorescently labeled secondary antibody in PBS with 2% normal serum for 1 h at room temperature. Cell nuclei were also counterstained with DAPI (Invitrogen, 1:10,000). We used antibodies to GAD65 (AB1511, Millipore), glutamate (G-6642, Sigma Aldrich), PV (PV 2150, Swant), and PS-47693, ThermoFisher), PSD95 (3459P, Cell Signaling), Vglut1 (73-066, UC Davis), VGAT (147 011, Synaptic Systems), SOX6 (AB8508, Millipore), b-tubulin III (80021, Covance), OCT4 (CA0-A3, Cell Signaling), TRA-1-60 (ab16288, Abcam), GAP43 (sc-106, Abcam), NCAM (sc-6332, Santa Cruz Biotechnology), Cell Signaling, SST (sc-7819, Santa Cruz Biotech), human cytoplasm (AB-121-U-050, StemCells), VIP (2007, Immunostar), CCK (C2581, Sigma Aldrich), calretinin (CG1, Swant), COPUITHI (HP-H7147-00, Perseus Proteomics), OLG2 (AB9610, Millipore), ChAT (AB114P, Chemicon), TH (P40101-150, Pel-Freez) and 5-HT (20080, Immunostar). Information on antibodies is summarized in Supplementary Table 8. After being washed in PBS, coverslips were mounted onto slide glasses using Fluoromount-G (Southern Biotech). Fluorescent images were taken using the EVOS FL Auto microscope (Life Technologies) or Olympus DSU Spinning Disc Confocal on an IX71 inverted microscope (Olympus).

Whole-cell patch-clamp recordings in brain slices. For electrophysiological studies, human iPSNs derived from human iPSCs were infected with LV-Syn-R (Invitrogen) and 300 ng of the total RNA was used for cDNA synthesis using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) primer Gene Link, Recombinant Human hTau Protein (BioLamina) in Nutristem XF medium (Biological Industries) and expanded in the same culture system until at least passage 3 before being characterized by 4′,6-diamidino-2-phenylindole (DAPI)/OCT4/TRA-1-60 immunostaining and frozen down for storage.

RNA preparation and real-time PCR. RNA samples were isolated using a Trizol reagent (Invitrogen) and 300 ng of the total RNA was used for cDNA synthesis using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) primer Gene Link, Recombinant Human hTau Protein (BioLamina) in Nutristem XF medium (Biological Industries) and expanded in the same culture system until at least passage 3 before being characterized by 4′,6-diamidino-2-phenylindole (DAPI)/OCT4/TRA-1-60 immunostaining and frozen down for storage.

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was calculated from the equation $\tau_c = (1/|I_c| + 1) + (1/|I_c|)$ and was then used to calculate $C_m$ from the equation $C_m = (|R_c| + |R_s|)/|R_s|$. To compare membrane excitability of grafted human cINs between groups (Fig. 2a–d), the membrane potential was held at approximately –85 mV in current-clamp mode, and square pulses of depolarizing currents (10–90 pA with increments of 10 pA, 0.5–s duration) were applied. The number of action-potential firings were counted for each current pulse in each grafted cell. The first action potential induced by depolarizing current injection near threshold was used to calculate action potential half-width in Supplementary Fig. 3f–h. Spontaneous action potential firings were counted for each current pulse in each grafted cell. The first action potential induced by depolarizing current was used to analyze action potential duration (APD). $\tau_c$ was used to calculate $\tau = I_R[I_c]$. $\tau = I_R[I_c]$ and was then followed by a series of exponential decay components ($\tau_1$, $\tau_2$, $\tau_3$, and $\tau_4$).

Electrophysiological analysis of iPSC-derived cINs in vitro. Differentiation, human cINs were used for electrophysiological experiments. The intracellular solution containing 120 mM K-gluconate, 9 mM NaHPO$_4$, 10 mM dextrose, 1 mM MgCl$_2$, 2 mM CaCl$_2$ and 10 mM HEPES at pH 7.4 was used to fill the patch pipettes. To record the action potentials in grafted neurons, we performed whole-cell patch-clamp experiments as described.

Transplantation of human cINs, immunohistochemistry and synapse analysis. All animal protocols were approved by the Institutional Animal Care and Use Committee at McLean Hospital, New York Medical College, and University of California Riverside. Procedures were carried out in accordance with the approved guidelines. We have complied with all relevant ethical regulations. The cIN spheres were trypsinized on day 45 of differentiation, cells were trypsinized and resuspended in Transplantation medium (HBSS, 4.5 mg ml$^{-1}$ sucrose, 0.1 ng ml$^{-1}$ GFP to facilitate optogenetic study and then treated with CultureOne (Invitrogen, 1:100) for 1 week. At 8 weeks after differentiation, cells were trypsinized and resuspended in Transplantation medium (HBSS, 4.5 mg ml$^{-1}$ sucrose, 0.1 ng ml$^{-1}$ GFP). Samples were incubated with secondary antibody in PBS with 2% normal serum for 1 h at room temperature. The antibodies used are summarized in Supplementary Table 1.
total area of 13,611 µm² for schizophrenia cINs. Total counted colocalized puncta numbers were 1,286 for healthy control cINs and 1,104 for schizophrenia cINs. For excitatory synaptic analysis, we analyzed a total of 614 neurite segments with a total area of 12,134 µm² for healthy control cINs and a total of 669 neurite segments with a total area of 11,413 µm² for schizophrenia cINs. Total counted colocalized puncta numbers were 3,542 for healthy control cINs and 4,410 for schizophrenia cINs. The experimenters were blind to sample identification during data collection.

**tissue clearing according to the iDISCO**

To visualize the cINs, numbers were 3,542 for healthy control cINs and 4,410 for schizophrenia cINs. The area of 11,732 µm² for healthy control cINs and 1,104 for schizophrenia cINs. For excitatory synaptic analysis, we analyzed a total of 9,051 neurite segments with a total area of 111,301 µm² for healthy control postmortem tissue and a total of 4,209 neurite segments with a total area of 31,165.3 µm² for schizophrenia postmortem tissue. Total counted colocalized puncta numbers were 14,562 for healthy control postmortem tissue and 13,113 for schizophrenia postmortem tissue. For excitatory synaptic analysis, we analyzed a total of 7,471 neurite segments with a total area of 57,218 µm² for healthy control postmortem tissue and a total of 7,644 neurite segments with a total area of 68,563 µm² for schizophrenia postmortem tissue. Total counted colocalized puncta numbers were 10,777 for healthy control postmortem tissue and 9,664 for schizophrenia postmortem tissue. The experimenters were blind to sample identification during data collection.

**Histological analysis of wild-type and PcdhaΔαCR/ΔαΔα mice.** Wild-type and PcdhaΔαCR/ΔαΔα mice were generated as described and the mice were perfused at P40 for histological analysis. The mouse brains were post-fixed using a 4% paraformaldehyde solution overnight and then washed in a 30% sucrose solution for 2 days. Coronal sections (40-µm thick) were cut by a microtome. Brain sections were blocked and permeabilized in PBS with 10% normal serum and 0.1% Triton X-100 for 30 min at room temperature. For amplification of signals, the sections were incubated with the biotinylated rabbit antibody for 2 h at room temperature, followed by amplification steps as follows: incubation with Alexa647-streptavidin (1:2,000, Life Technologies) for 2 h at room temperature, then with biotinylated antibody to streptavidin (1:2,000, Vector) for 2 h at room temperature, and then with Alexa647-streptavidin for 2 h at room temperature. Z-stack images were obtained using an Olympus DSU Spinning Disc Confocal on an IX81 inverted microscope. Arborization of wild-type versus PcdhaΔαCR/ΔαΔα PV cINs was analyzed using Neuron J to obtain parameters such as neurite length, neurite number from the soma, and branch number within 150µm of the cell body. The experimenters were blind to the sample identification during data collection.

**Synapse analysis** was performed using triple-stained wild-type or KO mice brain sections (PV, gephyrin and VGAT; or PV, VGlut1 and PSD95) as described above. For inhibitory synaptic analysis, we analyzed a total of 5,497 neurite segments with a total area of 55,186 µm² for wild-type cINs and a total of 4,733 neurite segments with a total area of 57,671 µm² for PcdhaΔαCR/ΔαΔα cINs. Total counted colocalized puncta numbers were 20,393 for wild-type and 12,697 for PcdhaΔαCR/ΔαΔα mice. For excitatory synaptic analysis, total counted colocalized neurite segments with a total area of 30,394 µm² for wild-type cINs and a total of 3,648 neurite segments with a total area of 44,121 µm² for PcdhaΔαCR/ΔαΔα cINs. Total counted colocalized puncta numbers were 9,655 for wild-type and 19,125 for PcdhaΔαCR/ΔαΔα mice. The experimenters were blind to sample identification during data collection.

**Histological analysis of human postmortem tissue.** Formalin-fixed PFC of human postmortem tissues from eight healthy control and eight schizophrenia subjects was obtained from McLean Hospital, the University of Maryland Brain and Tissue Bank, the University of Pittsburgh, and the Human Brain and Spinal Fluid Resource Center (Fig. 8a). The tissue samples were placed in a 30% sucrose solution for 2 days. Coronal sections (40-µm thick) were cut using a cryostat (Leica). The brain sections were blocked in PBS with 10% normal serum and 0.1% Triton X-100 for 10 min at room temperature and then incubated with primary antibody in PBS with 2% normal serum overnight at 4°C. After being washed with PBS, the sections were incubated with the secondary antibody in PBS with 2% normal serum for 1 h at room temperature. The sections were incubated in a solution containing 0.01 M CuSO4 (Sigma) and 0.05 M ammonium acetate (Sigma) for 2 min at room temperature and then washed with PBS five times. For arborization analysis, Z-stack images of layer 3 PV⁺ neurons were taken using the EVOS FL Auto microscope (Life Technologies) and analyzed using Neuron J to obtain parameters such as neurite length, neurite number from the soma, and branch number within 150µm of the cell body. The experimenters were blind to sample identification during data collection.

For synaptic analysis, synaptic puncta images of layer 3 PV⁺ neurons were taken using a LSM 710 confocal microscope (Carl Zeiss) using sequential acquisition of separate channels with the 100x objective. For inhibitory synaptic analysis, we analyzed a total of 7,471 neurite segments with a total area of 57,218 µm² for healthy control postmortem tissue and a total of 7,644 neurite segments with a total area of 68,563 µm² for schizophrenia postmortem tissue. Total counted colocalized puncta numbers were 14,562 for healthy control postmortem tissue and 13,113 for schizophrenia postmortem tissue. For excitatory synaptic analysis, we analyzed a total of 9,051 neurite segments with a total area of 111,301 µm² for healthy control postmortem tissue and a total of 4,209 neurite segments with a total area of 31,165.3 µm² for schizophrenia postmortem tissue. Total counted colocalized puncta numbers were 27,086 for healthy control postmortem tissue and 4,822 for schizophrenia postmortem tissue. The experimenters were blind to sample identification during data collection.

**Statistical analysis.** All statistical analyses were performed using GraphPad Prism7 (GraphPad Software), SPSS (version 16; SPSS) and SAS statistical software (9.4, SAS Institute). We tested normality of data distribution as summarized in Supplementary Tables 15–18. For cell counting analysis (Fig. 1 and Supplementary Fig. 1), one-way analysis of variance (ANOVA) was used to analyze the differences among group means of individual cell lines. For in vitro electrophysiology data (Supplementary Fig. 4), Welch’s t-test was used to compare the mean difference between groups. For mice behavioral assessments (Fig. 5d, e), the data were analyzed by two-tailed unpaired t-test. For rPCR analysis of siRNA-mediated gene KD (Supplementary Fig. 10c), the were analyzed by two-tailed unpaired t-test.

For phenotype analysis such as in vivo electrophysiology data (Fig. 2), Supplementary Figs. 3 and 5 and Supplementary Table 15), gene expression analysis (Figs. 3 and 4, Supplementary Fig. 8 and Supplementary Table 16), synaptic analysis (Figs. 5b, c, 7b and 8c and Supplementary Table 17) and arborization analysis (Figs. 5a, 6a and 8b and Supplementary Table 17), the linear mixed-effect model with random intercept was used for the continuous data and the linear mixed-effect model with random log-linear interaction was used for categorical data to compare the mean difference between groups. Mixed model methods were chosen to handle clustering and covariance among correlated samples. Some of the data were log-transformed to deal with skewedness as indicated in Supplementary Tables 15–18. Power analysis (Fig. 2i) was performed using the two-tailed t-test to compare two means in a cluster design to incorporate the clustering of samples within the same line. For arborization analysis after GO6893 treatment (Fig. 6b and Supplementary Table 17), the two-level hierarchical mixed-effects models were used by incorporating the two random effects (that is, the second effect is for observations with and without GO6893 nested within the first effect, same batch of cells) to account for correlation among the paired samples in each cell line. For arborization analysis after siRNA transfection (Fig. 6c and Supplementary Table 18), the two-level hierarchical linear mixed-effect model for the continuous data and the two-level hierarchical mixed-effect log-linear model for the count data were used. Dunnnett’s test was used to compare each of the groups with a negative-control siRNA control group as a post hoc analysis with an adjustment for multiple comparisons. All other comparisons were two-sided. A significance level of 5% was used for all analyses. More information on statistical analysis can be found in the Life Sciences Reporting Summary.

**Study design.** No statistical method was used to predetermine sample sizes. However, the sample size we used in this study is similar to the largest one among previous publications. The sample size was adequate in identifying the disruption of protocadherin expression in schizophrenia cINs. No data were excluded from analysis. Experimental cohorts were chosen based on our selection criteria (Caucasian male subjects treated with clozapine versus age- and gender-matched Caucasian male healthy controls) without randomization to reduce variation caused by age, ethnicity and gender. Blinding was used during cell counting, arborization analysis and synaptic analysis.

**Accession codes.** The RNA-seq data were deposited at GEO under accession numbers GSE118313 and GSE121376.
Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The RNA-seq data were deposited at GEO with accession numbers GSE118313 and GSE121376. The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Reporting Summary

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- **n/a** Confirmed
- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- [ ] An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [ ] The statistical test(s) used AND whether they are one- or two-sided
  - *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
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- [ ] A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- [ ] For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - *Give P values as exact values whenever suitable.*
- [ ] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- [ ] Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- [ ] Clearly defined error bars
  - *State explicitly what error bars represent (e.g. SD, SE, CI)*

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Software and code

Policy information about availability of computer code

**Data collection**

All the traced neuronal images were taken using the EVOS FL Auto microscope (Life Technologies, Carlsbad, CA). ImageJ software (Version 1.51p, NIH, Bethesda, MD) was used to count the cell number using the multi point function. ImageJ software version 1.51 (NIH) with the Neuronl plugin was used to obtain parameters of arborization. For synapse analysis, images were processed using Imaris software (Version 9.2, Bitplane, Switzerland). RNA-seq of HC and SCZ cINs were collected on Illumina NextSeq 550 system. Raw sequence reads were de-multiplexed and trimmed for adapters by using the Illumina bcl2fastq conversion software (v2.19). Whole-cell patch-clamp recordings were performed using a Multiclamp 700B amplifier, a Digidata 1550A or 1320A digitizer and Clampex 10 software (Molecular Devices, Sunnyvale, CA).

**Data analysis**

All statistical analyses were performed using GraphPad Prism7 (GraphPad Software, La Jolla, CA), SPSS (version 16; SPSS Inc., Chicago, IL) and SAS statistical software (9.4, SAS Institute, Cary, NC). Offline analysis of electrophysiological data was performed using the Clampfit 9 program (Molecular Devices). FASTQ files were aligned to the human genome (assembly hg19) using STAR and GENCODE v19 transcriptome annotation. Read pairs aligned to gene features were counted and summarized as RPKM values at the gene-level using featureCount, taking into account the strandness of the reads and all transcript variants of each gene in the GENCODE annotation. Differentially expressed genes were determined using the voom-limma package in the Bioconductor R software.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A description of any restrictions on data availability

The RNA-seq data were deposited at the GEO <https://www.ncbi.nlm.nih.gov/geo/> and the accession numbers are GSE118313 and GSE121376.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical method was used to pre-determine the sample sizes. However, the samples size we used in this study is similar to the largest one among previous publications (Topol et al., 2016, Cell Reports 15, 1024–1036). This sample size was adequate to identify the disruption of expression of protocadherin family members in schizophrenia interneurons. |
| Data exclusions | No data was excluded. |
| Replication | All attempts in replication were successful. |
| Randomization | Experimental cohorts were chosen based on our selection criteria (Caucasian male patients treated with Clozapine vs. age- and gender-matched Caucasian male healthy controls) without randomization to reduce variation caused by age, ethnicity and gender. |
| Blinding | Blinding was used during cell counting, arborization analysis and synapse analysis. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
| --- | --- |
| x | Unique biological materials |
| x | Antibodies |
| x | Eukaryotic cell lines |
| x | Palaeontology |
| x | Animals and other organisms |
| x | Human research participants |

Methods

| n/a | Involved in the study |
| --- | --- |
| x | ChIP-seq |
| x | Flow cytometry |
| x | MRI-based neuroimaging |

Antibodies

| Antibodies used |
| --- |
| anti-GAD1 (Millipore, AB1511, 1:1,000), anti-Glutamate (Sigma Aldrich, G-6642, 1:15,000), anti-PV (Swant, PV25 , 1:5,000/Thermo Fisher, PAS-47693, 1:200), anti-PSD95 (Cell Signaling, 3450P, 1:1,000), anti-Vglut1 (UC Davis, 73-066, 1:1,000, lot#437-4VA-6), anti-VGAT (Synaptic Systems, 131 003, 1:1,000, lot#D1816), anti-Gephyrin (Synatic Systems, 147 011, 1:1,000), anti-Sox6 (Millipore, AB5805, 1:1,000, lot#2921391), anti-beta-tubulin III (Covance, MMS-435P, 1:2,000), anti-Oct4 (Cell Signaling, 82405, 1:400,lot#15), anti-Tra-1-60 (Abcam, ab16288, 1:500), anti-GFP (Abcam, ab13970, 1:1,000), anti-NCAM (Santa Cruz, SC-106, 1:1,000,lot#E7475) anti-MEF2C (Cell Signaling, 5030S, 1:1,000, lot#2), anti-SST (Santa Cruz, SC-7819, 1:1,000, lot#541213), anti-human Cytoplasm (StemCells Inc, SC121, 1:1,000), anti-VIP (Immunostar, 20080, 1:1,000), anti-CCK (Sigma Aldrich, C2581, 1:1,000, lot#9884760V), anti-Calretinin (Swant, CG1, 1:1,000), anti-COUPTFII (Perseus Proteomics, PP-H7147-00, 1:1,000, lot#A-2), anti-Olig2 (Millipore, AB9610, 1:1,000), anti-GFAP(UC Davis, 75240, 1:1,000), anti-ChAT (Chemico, AB144, 1:1,000, lot#210421C3), anti-TH (Pel-Freez, P40101, 1:1,000 ), and anti-5-HT (Immunostar, 20080, 1:1,000) |
### Validation

The antibodies were validated by the manufacturers as below:

- **anti-GAD1**: [http://www.emdmillipore.com/US/en/product/Anti-Glutamate-Decarboxylase-65-67-Antibody,MM_NF-AB1511?ReferrerURL=https%3A%2F%2Fwww.google.com%2F&bn=1](http://www.emdmillipore.com/US/en/product/Anti-Glutamate-Decarboxylase-65-67-Antibody,MM_NF-AB1511?ReferrerURL=https%3A%2F%2Fwww.google.com%2F&bn=1)
- **anti-Glutamate**: [https://www.sigmaaldrich.com/catalog/product/sigma/g5642?lang=en&region=US](https://www.sigmaaldrich.com/catalog/product/sigma/g5642?lang=en&region=US), [http://www.biocompare.com/Product-Reviews/185132-Excellent-Rabbit-anti-Parvalbumin-antibody-for-immunohistochemical-staining-of-mouse-brain-slices](http://www.biocompare.com/Product-Reviews/185132-Excellent-Rabbit-anti-Parvalbumin-antibody-for-immunohistochemical-staining-of-mouse-brain-slices)
- **anti-PV**: [https://www.cellsignal.com/products/primary-antibodies/psd95-d27e11-xp-rabbit-mab/3450?&print=true](https://www.cellsignal.com/products/primary-antibodies/psd95-d27e11-xp-rabbit-mab/3450?&print=true)
- **anti-PSD95**: [https://www.cellsignal.com/products/primary-antibodies/psd95-d27e11-xp-rabbit-mab/3450?&print=true](https://www.cellsignal.com/products/primary-antibodies/psd95-d27e11-xp-rabbit-mab/3450?&print=true)
- **anti-Vglut1**: [https://www.labome.com/product/Neuromab/73-066.html](https://www.labome.com/product/Neuromab/73-066.html)
- **anti-VGAT**: [https://www.sysy.com/products/vgat/facts-131003.php](https://www.sysy.com/products/vgat/facts-131003.php)
- **anti-Gephyrin**: [https://www.sysy.com/products/gephyrin/facts-147011.php](https://www.sysy.com/products/gephyrin/facts-147011.php)
- **anti-Sox6**: [http://www.emdmillipore.com/US/en/product/Anti-Sox6-Antibody,MM_NF-AB5805?ReferrerURL=https%3A%2F%2Fwww.google.com%2F](http://www.emdmillipore.com/US/en/product/Anti-Sox6-Antibody,MM_NF-AB5805?ReferrerURL=https%3A%2F%2Fwww.google.com%2F)
- **anti-beta-tubulin III**: [https://www.biolegend.com/en-us/products/purified-anti-tubulin-beta-3-tubb3-antibody-11580](https://www.biolegend.com/en-us/products/purified-anti-tubulin-beta-3-tubb3-antibody-11580)
- **anti-Oct4**: [https://www.biocompare.com/tr/a-1-60-r-antibody-tra-1-60-ab16288.html](https://www.biocompare.com/tr/a-1-60-r-antibody-tra-1-60-ab16288.html)
- **anti-GFP**: [https://www.abcam.com/gfp-antibody-ab13970.html](https://www.abcam.com/gfp-antibody-ab13970.html)
- **anti-NCAM**: [https://www.biocompare.com/9776-Antibodies/249615-NCAM-ERIC-1/](https://www.biocompare.com/9776-Antibodies/249615-NCAM-ERIC-1/)
- **anti-MEF2C**: [https://www.cellsignal.com/products/primary-antibodies/mef2c-d80c1-xp-rabbit-mab/5030](https://www.cellsignal.com/products/primary-antibodies/mef2c-d80c1-xp-rabbit-mab/5030)
- **anti-SST**: [https://www.labome.com/product/Pel-Freez/P40101-0.html](https://www.labome.com/product/Pel-Freez/P40101-0.html)
- **anti-OLIG2**: [https://www.emdmillipore.com/US/en/product/Anti-Olig-2-Antibody,MM_NF-AB9610](https://www.emdmillipore.com/US/en/product/Anti-Olig-2-Antibody,MM_NF-AB9610)
- **anti-ChAT**: [https://www.cellsignal.com/products/primary-antibodies/choline-acetyltransferase-antibody/](https://www.cellsignal.com/products/primary-antibodies/choline-acetyltransferase-antibody/)
- **anti-TH**: [https://www.labome.com/product/antibodycatalog/5-h-5-HT-serotonin-rabbit-antibody/](https://www.labome.com/product/antibodycatalog/5-h-5-HT-serotonin-rabbit-antibody/)

### Eukaryotic cell lines

Human fibroblasts were obtained from the laboratories of Dr. Bruce Cohen (McLean Hospital), Dr. Daniel Weinberger (Lieber Institute for Brain Development), and Dr. Judith Rapoport (National Institute of Mental Health). Skin biopsies were performed on HC and SCZ patients. These study protocols were approved by the McLean Hospital/Partners Healthcare Institutional Review Board and New York Medical College Institutional Review Board. All procedures were performed in accordance with the Institutional Review Board’s guidelines and all human samples were obtained with informed consents. Human fibroblasts were reprogrammed using modified RNA methods by Cellular Reprogramming, Inc. (San Diego, CA). Established iPSC cultures on laminin-521 (BioLamina, Sweden) in Nutristem XF media (Biological Industries, Israel) and expanded in the same culture system.

### Authentication

iPSC lines were validated by immunocytochemistry as described in Methods section.

### Mycoplasma contamination

All cell lines are routinely tested for mycoplasma contamination. Cell lines used in this study were verified to be mycoplasma free before undertaking any experiment with them.

### Commonly misidentified lines

No commonly misidentified cell line was used.

### Animals and other organisms

Human fibroblasts were obtained from the laboratories of Dr. Bruce Cohen (McLean Hospital), Dr. Daniel Weinberger (Lieber Institute for Brain Development), and Dr. Judith Rapoport (National Institute of Mental Health). Skin biopsies were performed on HC and SCZ patients. These study protocols were approved by the McLean Hospital/Partners Healthcare Institutional Review Board and New York Medical College Institutional Review Board. All procedures were performed in accordance with the Institutional Review Board’s guidelines and all human samples were obtained with informed consents. Human fibroblasts were reprogrammed using modified RNA methods by Cellular Reprogramming, Inc. (San Diego, CA). Established iPSC cultures on laminin-521 (BioLamina, Sweden) in Nutristem XF media (Biological Industries, Israel) and expanded in the same culture system.

#### Laboratory animals

We used 5-7 weeks old male and female Nod Scid mice for transplantation. We used 10-13 weeks old male PCDHA deltaCR/deltaCR mice and 10-13 weeks old male PCDHA deltaBneo/deltaBneo mice for behavior testing. We used 40 days old male PCDHA deltaAlpha/deltaAlpha mice for immunohistochemistry.

#### Wild animals

No wild animal was used.

#### Field-collected samples

No field-collected sample was used.

### Human research participants

SCZ patients were Caucasian males treated with Clozapine with age from 21 - 51 years old. Age and gender-matched healthy controls were also selected.
Recruitment

The selection criteria for SCZ patients were Caucasian males treated with Clozapine. Age and gender-matched healthy controls were also selected. These selection criteria were to narrow down the samples to more severe cases of diseases for stronger phenotype. Thus, it is likely that our results may be more biased for the case of more severe form of disease.