BRAIN
ORIGINAL ARTICLE

Regulatory variants at 2q33.1 confer schizophrenia risk by modulating distal gene *TYW5* expression

Shiwu Li,[1,2,†] Jiao Li,[1,2,†] Jiewei Liu,[1,†] Junyang Wang,[1,2] Xiaoyan Li,[1,2] Yongxia Huo,[1] Yifan Li,[1,2] Yixing Liu,[1,2] Ming Li,[1,2,3] Xiao Xiao[1] and Xiong-Jian Luo[1,2,3,4]

[†]**These authors contributed equally to this work.**

Genome-wide association studies have shown that genetic variants at 2q33.1 are strongly associated with schizophrenia. However, potential causal variants in this locus and their roles in schizophrenia remain unknown. Here, we identified two functional variants (rs796364 and rs281759) that disrupt CTCF, RAD21 and FOXP2 binding at 2q33.1. We systematically investigated the regulatory mechanisms of these two variants with serial experiments, including reporter gene assays and electrophoretic mobility shift assay.

Intriguingly, these two single nucleotide polymorphisms physically interacted with *TYW5* and showed the most significant associations with *TYW5* expression in human brain. Consistently, CRISPR-Cas9-mediated genome editing confirmed the regulatory effect of the two single nucleotide polymorphisms on *TYW5* expression. Additionally, expression analysis indicated that *TYW5* was significantly upregulated in brains of schizophrenia cases compared with controls, suggesting that rs796364 and rs281759 might confer schizophrenia risk by modulating *TYW5* expression.

We over-expressed *TYW5* in mouse neural stem cells and rat primary neurons to mimic its upregulation in schizophrenia and found significant alterations in the proliferation and differentiation of neural stem cells, as well as dendritic spine density following *TYW5* overexpression, indicating its important roles in neurodevelopment and spine morphogenesis.

Furthermore, we independently confirmed the association between rs796364 and schizophrenia in a Chinese cohort of 8202 subjects. Finally, transcriptome analysis revealed that *TYW5* affected schizophrenia-associated pathways. These lines of evidence consistently revealed that rs796364 and rs281759 might contribute to schizophrenia risk by regulating the expression of *TYW5*, a gene whose expression dysregulation affects two important schizophrenia pathophysiological processes (i.e. neurodevelopment and dendritic spine formation).

1 Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
2 Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650204, China
3 KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
4 Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming, Yunnan 650223, China

Received March 01, 2021. Revised August 05, 2021. Accepted August 13, 2021. Advance access publication September 28, 2021

Correspondence to: Xiong-Jian Luo
Key Laboratory of Animal Models and Human Disease Mechanisms
Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650204, China
E-mail: luoxiongjian@mail.kiz.ac.cn

Keywords: schizophrenia; genome-wide association studies; functional genomics; rs796364; rs281759; *TYW5*

Abbreviations: EMSA = electrophoretic mobility shift assay; eQTL = expression quantitative trait loci; GWAS = genome-wide association studies; LD = linkage disequilibrium; NSC = neural stem cell; SNP = single nucleotide polymorphism; TWAS = transcriptome-wide association study

Introduction

Schizophrenia is a severe and chronic psychiatric disorder with a lifetime prevalence of about 1%. It is characterized by the presence of delusions, hallucinations, lack of motivation, alogia, avolition and cognitive impairments.[1,2] Twin studies estimated that schizophrenia has a heritability (broad-sense heritability, total phenotypic variance explained by genetic variation or genes) of around 79–81%,[3,4] indicating the dominant role of genetic factors in schizophrenia pathogenesis. Genetic studies, especially recent genome-wide association studies (GWAS), have made considerable advances in identifying risk loci and dissecting the genetic architecture of schizophrenia.[5–12] For example, the Psychiatric Genomics Consortium (PGC) and other research groups have carried out large-scale GWAS and reported multiple robustly-associated loci for schizophrenia in the past decade.[5–10] In addition, Lam *et al.*[10] conducted a meta-analysis (56 418 schizophrenia cases and 78 818 controls) by combining results from populations with European and East Asian ancestries and identified 176 schizophrenia-associated loci. Although these GWAS have provided important insights into the genetic aetiology of schizophrenia, the genetic and pathogenic mechanisms of the most reported risk loci in schizophrenia remain elusive. Considering the vast majority of schizophrenia risk variants identified by GWAS are located in non-coding regions, it is likely that these variants contribute to schizophrenia susceptibility by regulating gene expression rather than altering protein structure or function.[6] Therefore, identifying the functional (or potential causal) variants at the reported schizophrenia risk loci and elucidating their regulatory mechanisms will provide important insights into the genetic mechanisms of this disease.

Genetic variants at 2q33.1 (the index variant is chr2_200825237_I, a single base insertion first reported by the PGC in 2014) are strongly associated with schizophrenia.[6] For each locus identified by GWAS, a representative variant or single nucleotide polymorphism (SNP; in most cases, the variant with the smallest *P*-value in the locus was selected as the index variant) showed robust associations with schizophrenia (Supplementary Table 1),[6,8,10] indicating that the locus may harbour authentic risk variants for schizophrenia. However, due to the complexity of linkage disequilibrium (LD, the non-random association between genetic variants at two or more loci; the SNP with the smallest association *P*-value is not necessarily the causal variant) and gene regulation (genetic variants may regulate distal genes by affecting 3D chromosomal structure),[13,14] pinpointing causal variants in risk loci remains a major challenge in the post-GWAS era. Functional genomics analyses are believed to promote the identification of functional variants.[15–17] By integrating chromatin immunoprecipitation sequencing (ChIP-Seq) and position weight matrix data, we identified 132 functional SNPs from the reported schizophrenia risk loci in our previous study,[16] including two functional SNPs (rs796364 and rs281759) at the 2q33.1 locus (Fig. 1). Of note, among the 132 SNPs that disrupted transcription factor binding (identified in our previous study),[16] rs796364 ($P = 9.41 \times 10^{-17}$) and rs281759 ($P = 9.25 \times 10^{-16}$) showed the second and third most associations with schizophrenia in a recent GWAS (Figs 1 and 2A),[8] strongly suggesting that the two SNPs are true risk variants for schizophrenia. Moreover, we explored the associations between these two SNPs and schizophrenia in East Asian populations and found that they were also associated with schizophrenia in these populations (rs796364, $P = 5.66 \times 10^{-6}$; rs281759, $P = 2.88 \times 10^{-6}$).[10] Finally, we demonstrated previously that rs796364 and rs281759 are significantly associated with the expression of *TYW5* and *FTCDNL1* in multiple human brain tissues,[16] suggesting their potential functional consequences. These lines of evidence suggest that rs796364 and rs281759 are potential causal variants for schizophrenia; we therefore focused on these two SNPs in this study.

Of note, our functional genomics study showed that rs796364 disrupted CTCF and RAD21 binding, while rs281759 disrupted FOXP2 binding (Fig. 2B and C).[16] The distance between rs796364 (located in the promoter of *FTCDNL1*) and rs281759 (located in the intron 1 of *C2orf69*) was 71 600 bp, and these two functional SNPs were in high LD ($r^2 = 0.97$). ChIP-Seq, DNase-Seq and histone modification data (from ENCODE) indicated that these two functional SNPs were located in actively transcribed genomic regions (i.e. regulatory elements) of human neuroblastoma cell lines, with CTCF, RAD21 and FOXP2 binding (Fig. 2D and E).[22,23] It is clear that these functional SNPs could provide pivotal genetic insights into the aetiology of schizophrenia. Nevertheless, their regulatory mechanisms and the corresponding biological implications of this locus in schizophrenia remain unknown.

To elucidate how these SNPs exert their regulatory effects, we conducted serial experiments. We first validated the regulatory effects of the SNPs with reporter gene assays, and our electrophoretic mobility shift assay (EMSA) showed that these two SNPs affected the binding affinity of transcription factors. We then performed expression quantitative trait loci (eQTL) analysis to identify the potential target gene (or genes) regulated by these two SNPs and found that they showed the most significant associations with *TYW5* expression in human brain. Further integrative analyses that involved integrating GWAS associations and brain eQTL data indicated that *TYW5* is a schizophrenia risk gene. Consistent with eQTL and integrative analyses, we found that *TYW5* was significantly dysregulated in the brains of patients with schizophrenia compared with controls. Therefore, these two SNPs may confer risk of schizophrenia by modulating *TYW5* expression. We also mimicked the effects of *TYW5* dysregulation in schizophrenia and found that *TYW5* affected the proliferation and differentiation of neural stem cells (NSCs), indicating pivotal roles for this gene in neurodevelopment. Moreover, we found that *TYW5* affected the density and ratio of dendritic spines, further supporting its vital roles in spine morphogenesis and maturity. Finally, we showed

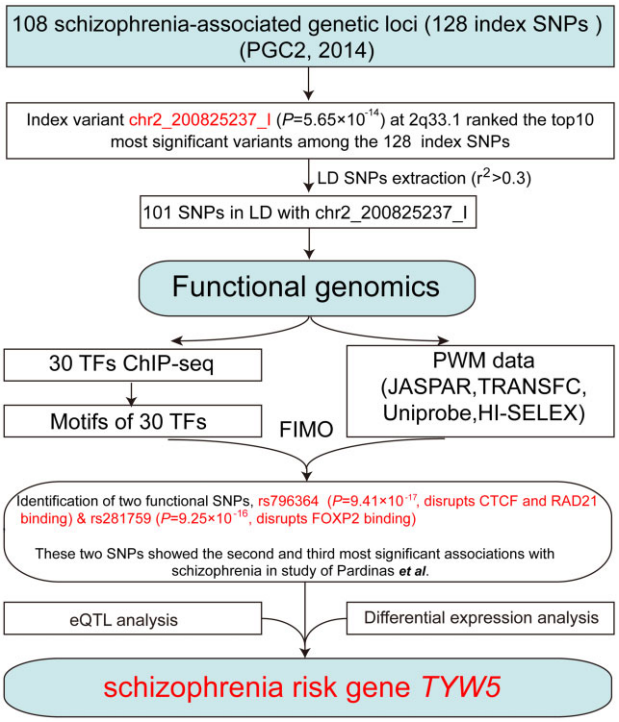

Figure 1 Flow chart of the functional genomics analysis of the 2q33.1 locus. The 2q33.1 locus (index variant chr2_200825237_I) was ranked among the top 10 most significant risk loci for schizophrenia ($P = 5.65 \times 10^{-14}$).[6] Our previous functional genomics identified two functional SNPs (rs796364 and rs281759) at 2q33.1,[16] and they ranked as the second ($P_{rs796364} = 9.41 \times 10^{-17}$) and third most significant associations ($P_{rs281759} = 9.25 \times 10^{-16}$) with schizophrenia in a study by Pardiñas *et al.*[8] Briefly, SNPs in LD ($r^2 > 0.3$) with the index variant (chr2_200825237_I) were extracted using genotype data (Europeans) from the 1000 Genomes Project.[18] The DNA binding motifs of the included transcription factors were then derived and compared with the position weight matrices (from the databases JASPAR, TRANSFC, Uniprobe and Hi-SELEX) and those that were best matched were used for the subsequent analysis. FIMO software was used to determine whether the test SNP was located in the binding motif and if different alleles of this SNP disrupted transcription factor binding. Two functional SNPs (rs796364 and rs281759) were identified at the 2q33.1 locus and these two SNPs disrupted CTCF, RAD21 and FOXP2 binding. Furthermore, brain eQTL data (CMC and LIBD2) were used to identify the potential target genes of rs796364 and rs281759,[19,20] and expression data from the CMC (279 controls and 258 schizophrenia cases) and PsychEncode (936 controls and 559 schizophrenia cases) were used to investigate whether the target gene of rs796364 and rs281759 was dysregulated in schizophrenia.[19,21] These data suggested that rs796364 and rs281759 may confer schizophrenia risk by regulating TYW5 expression.

that rs796364 was significantly associated with schizophrenia in Chinese populations (with the same risk allele as in Europeans), suggesting that these two SNPs are likely to be authentic risk variants for schizophrenia. In summary, our study demonstrates that functional genetic variants rs796364 and rs281759 (at 2q33.1) may confer risk of schizophrenia by affecting transcription factor binding and regulating the expression of TYW5, a novel schizophrenia risk gene with important roles in neurodevelopment and dendritic morphogenesis.

Materials and methods

Functional genomics analysis

Details of the functional genomics approach were described in our previous study.[16] Briefly, by integrating ChIP-Seq and position weight matrix data, we identified 132 functional SNPs that disrupted the binding of 21 distinct transcription factors. In this study, we focused on the 2q33.1 risk locus (the functional SNPs in this locus are rs796364 and rs281759). The flow chart of the functional genomics for the 2q33.1 locus is provided in Fig. 1.

Linkage disequilibrium analysis

We used a well-established LD analysis database, SNiPA, which calculates LD-values using data from the 1000 Genomes Project.[18,24] The main parameters used were as follows: GRCh37 genome build, 1000 genome phase3 v5 and European populations. For further detailed information, please refer to the original SNiPA publication[24] and the SNiPA website (https://snipa.helmholtz-muenchen.de/snipa3/).

Expression quantitative trait loci analysis

To explore the potential target genes of the identified functional SNPs, we utilized two human brain eQTL datasets, CMC (CommonMind Consortium) and LIBD2 (Lieber Institute for Brain Development, Phase II),[19,20] to perform eQTL analysis. Both eQTL datasets used tissues from human dorsolateral prefrontal cortex. The CMC dataset contains RNA sequencing (RNA-seq) data from 258 schizophrenia cases and 279 controls. Detailed information about sample collection, RNA-seq data processing and eQTL calculation is provided in the original paper.[19] The LIBD2 dataset contained brain eQTL analysis data for 412 samples (175 schizophrenia cases and 237 healthy controls). Gene expression levels were quantified with RNA-seq and detailed information can be found in the original paper.[20]

Transcriptome-wide association study

To identify genes whose expression changes are associated with schizophrenia, we performed a transcriptome-wide association study (TWAS) analysis using FUSION software.[25] Four predictive models, including LASSO, GBLUP, Elastic Net and BSLM, were used. Genome-wide association data and two SNP-expression weights (from the dorsolateral prefrontal cortex; i.e. CMC and LIBD2) were used for the TWAS analysis.[10,19,20] Bonferroni correction was applied to correct the TWAS *P*-values. Details about the TWAS analysis is provided in the original paper[25] and on the FUSION website (http://gusevlab.org/projects/fusion/).

TYW5 expression analysis in brains of schizophrenia cases and controls

We used expression data from PsychEncode (http://resource.psychencode.org/) to explore whether TYW5 expression was dysregulated in the brains of schizophrenia cases compared with controls.[21] Briefly, expression data (from the brain tissues) from 559 schizophrenia cases and 936 controls were measured using RNA-seq. Detailed information about PsychEncode is given in the original study.[21]

Cell culture

Cell lines (HEK293T, SH-SY5Y and SK-N-SH) used in this study were kindly provided by Dr Ming Li's lab (Kunming Institute of Zoology, Chinese Academy of Sciences; all cells were originally from ATCC) and cultured as previously described.[16,17] The procedures for isolation and culture of mouse NSCs (embryonic Day 14) and rat cortical neurons (embryonic Day 18) have also been previously described,[26,27] and detailed information about cell culture is listed in the Supplementary material. All cells were cultured at

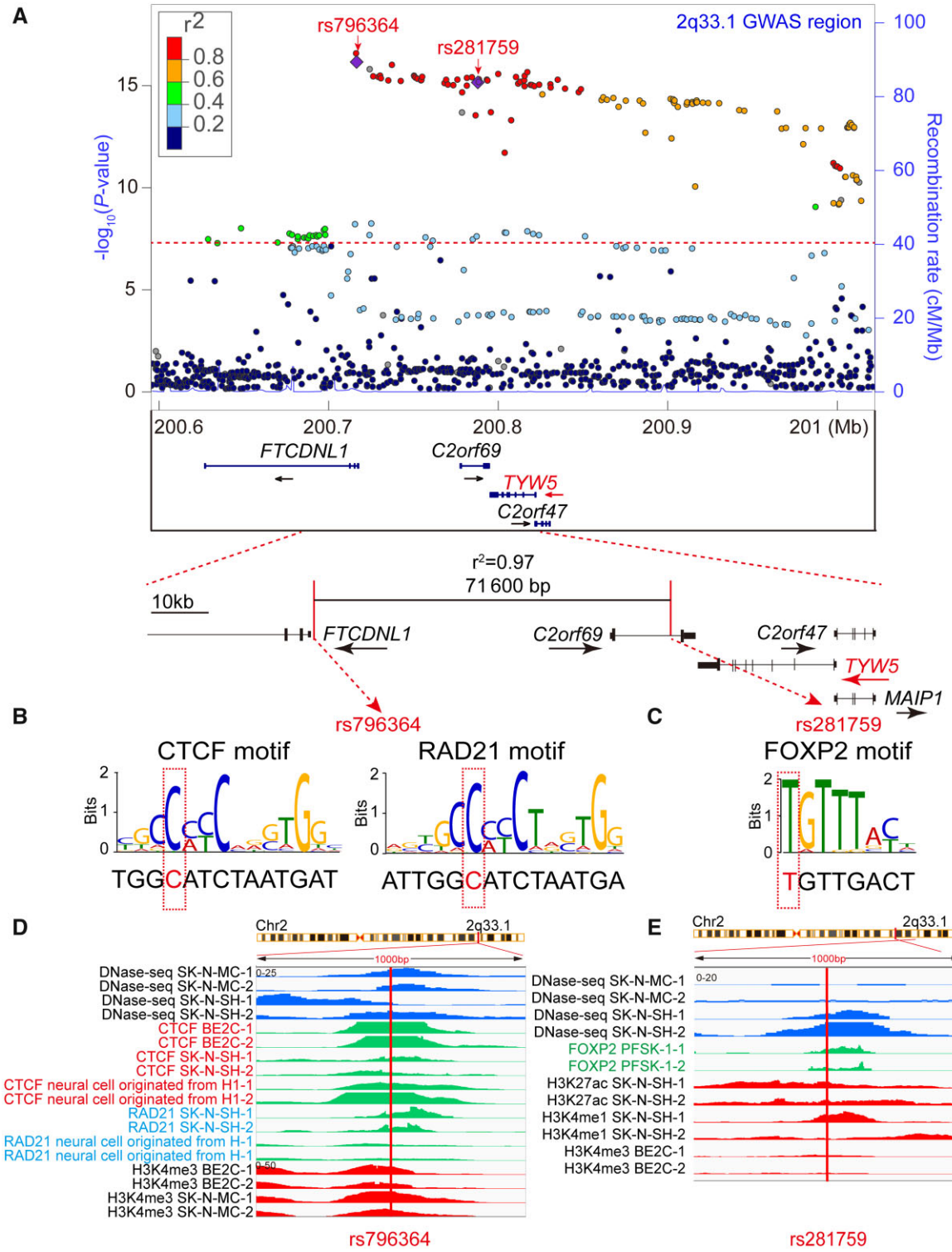

Figure 2 Functional genomics identified two functional SNPs (rs796364 and rs281759) at 2q33.1. (**A**) The SNPs rs796364 ($P = 9.25 \times 10^{-16}$) and rs281759 ($P = 9.41 \times 10^{-17}$) were significantly associated with schizophrenia (data from PGC + CLOZUK).[8] Of note, these two SNPs are in high LD ($r^2 = 0.97$) and both SNPs are located downstream of *TYW5*. The distance between these two SNPs is 71 600 bp. (**B** and **C**) Functional genomics showed that rs796364 and rs281759 disrupt transcription factor binding: rs796364 disrupts CTCF and RAD21 binding (**B**); and rs281759 disrupts FOXP2 binding (**C**). (**D** and **E**) The SNPs rs796364 and rs281759 are located in genomics regions according to signals from DNase-Seq, ChIP-Seq and histone modifications in human neuroblastoma cell lines, indicating that they are located in actively transcribed (i.e. regulatory elements) regions (with corresponding transcription factor binding).

37°C with 95% air and 5% CO_2. Mycoplasma tests were performed periodically using specific PCR primers, and no mycoplasma contamination was detected in the cells used in this study.

Reporter gene assays (enhancer assay)

Reporter gene assays were conducted as previously described.[16,17] Briefly, about 500 bp of genomic DNA containing rs796364 or rs281759 were amplified using PCR and inserted into a pGL3-promoter (Promega, E1761) vector using specific primers (Supplementary Table 2). The pGL3-promoter (which contains promoter sequences but lacks enhancer sequences, thus resulting in moderate luciferase expression) vector can be used to test the enhancer (or repressor) effect of the target DNA sequence. If the target DNA sequence is inserted into the pGL3-promoter vector and the inserted sequences have enhancer activity, the luciferase activity will significantly increase compared with the pGL3-promoter vector alone (Supplementary Fig. 1). The constructed vectors were then used to transform DH5α-competent cells. Vectors containing the alternative allele were generated by PCR-mediated point mutation, with the use of Golden Star T6 Super PCR mix (TSINGKE, TSE101), corresponding mutation primers (Supplementary Table 2) and DpnI (NEB, R0176S). All constructed vectors were verified by Sanger sequencing. Detailed information about the reporter gene assays is provided in the Supplementary material.

Electrophoretic mobility shift assay

EMSA was used to investigate the interaction between DNA sequences (containing the test SNP) and binding proteins.[28,29] We conducted EMSA as previously described.[28] Briefly, 36 bp DNA probes containing the test SNPs were synthesized (Supplementary Table 3) and labelled with biotin (using a Biotin labelling kit, Beyotime, GS008). The labelled probes were then incubated with nuclear protein extracts (from SH-SY5Y cells; using a Nuclear and cytoplasmic protein extraction kit, Beyotime, P0028). A Chemiluminescent EMSA kit (Beyotime, GS009) was used to detect whether the probes interacted with nuclear extracts. The results of EMSA were quantified with ImageJ software (https://imagej.nih.gov/ij/) by scanning the grey value of each target band. The relative grey value was defined as follows: (grey value of target band) / (total grey value of the same lane).

Chromatin interaction analysis

We explored the long-range chromatin interaction between two regulatory variants (rs794364 and rs281759) and *TYW5* using the chromatin interaction data from 3DIV (a 3D-genome interaction viewer and database).[30] Interaction data from human brain tissues and neuroblastoma cell lines were selected and visualized with 3DIV.

Knockout of genomic regions containing rs796364 and rs281759

We knocked out about 300 bp of genomic DNA containing rs796364 or rs281759 using the CRISPR-cas9 system as previously described.[17] Briefly, two single-guide (sg)RNAs (one located upstream and another downstream of the target SNP) were designed using the CRISPR sgRNA design tool (https://zlab.bio/guide-design-resources) (Supplementary Table 4). These sgRNAs were then inserted into PX458M and EZ-guide XH vectors to assemble a knockout vector (PX458M-sgRNA1/2, with a two sgRNA insertion) by using two restriction enzymes, XhoI (Thermo Scientific, FD0694) and HindIII (Thermo Scientific, FD0504). For the HEK293T cell knockout assay, HEK293T cells were placed into 12-well plates (2.0×10^5/ml) containing 1 ml culture medium and transfected with 1.5 μg knockout vectors (PX458M-sgRNA1/2) or control plasmids (PX458M) using polyethylenimine. At 72 h post-transfection, the cells were collected for total RNA and DNA extraction. For knockout of rs796364 and rs281759 in the SH-SY5Y and SK-N-SH cell lines, we inserted sgRNAs into lentiCRISPR v2 (Addgene, 52961), and the lentiviruses (expressing CAS9 and sgRNA1/2) packaged with pMD2G (Addgene, 12259) and psPAX2 (Addgene, 12260) were used to co-infect the two cell lines. The infected cells were cultured with 1 μg/ml puromycin for 12 days to select the stably infected cells. Genomic DNA was extracted to verify the knockout efficiency (using PCR), and total RNA was used to detect *TYW5* gene expression [using quantitative PCR (qPCR)]. As suggested by Vandesompele *et al.*,[31] different internal control genes were used for the HEK293T (*ACTB* and *GAPDH*), SK-N-SH and SH-SY5Y (*ACTB* and *B2M*) cell lines. The geometric averages of the two internal control genes were used to normalize the qPCR data, and the expression level was determined using the $2^{-\Delta\Delta Ct}$ approach.[32] The primers used for PCR and qPCR in this study are provided in Supplementary Tables 4 and 5.

Knockdown of CTCF, RAD21 and FOXP2

To test whether the transcription factors CTCF, RAD21 and FOXP2 regulate *TYW5* gene expression, they were knocked down using short hairpin (sh)RNAs designed using the ThermoFisher shRNA design tool (http://rnaidesigner.thermofisher.com/rnaiexpress/). The targeting sequences were as follows: *CTCF*-shRNA, 5′-GCGAAAGCAGCATTCCTATAT-3′, *RAD21*-shRNA, 5′-GCCATTACTTTACCTGAAGAA-3′ and *FOXP2*-shRNA, 5′-GCAAACAAGTGGATTGAAATC-3′. The synthesized shRNA oligos (Supplementary Table 6) were inserted into the pLKO.1-EGFP-Puro vector at two restriction sites (AgeI and EcoRI), and the recombinant plasmids were verified by Sanger sequencing. Lentiviral viruses produced by control plasmids and target shRNA vectors were used to infect the HEK293T, SH-SY5Y and SK-N-SH cell lines. After a 72-h infection period, 1–2 μg/ml puromycin was used to kill the uninfected cells. Quantitative PCR was used to quantify the expression of the target genes (i.e. *CTCF*, *RAD21*, *FOXP2* and *TYW5*) in the control and transcription factor-knockdown cells. The qPCR primers used are listed in Supplementary Table 5.

TYW5 overexpression in mouse neural stem cells

Considering *TYW5* was upregulated in schizophrenia cases, we evaluated the effect of *TYW5* overexpression on neurodevelopment using mouse NSCs. Briefly, the lentiviruses (produced by PCDH-*TYW5*-6×*his* and control vectors) were used to infect mouse NSCs, and puromycin (1–2 μg/ml, increased gradually for a week) was used to select NSCs stably expressing the human *TYW5* gene. Reverse transcription (RT)-PCR and western blot were used to validate the overexpression of *TYW5*. The primers used for RT-PCR are listed in Supplementary Table 5. The antibodies used for the western blot were as follows: primary antibodies were mouse anti-his (1:500, Proteintech, 66005-1-Ig) and mouse anti-beta actin antibody (1:1000, Proteintech, 66009-1-Ig); the secondary antibodies were HRP-labelled goat anti-mouse (1:1000, Beyotime, A0216).

Proliferation assays

To investigate whether *TYW5* overexpression affected the proliferation of NSCs, we used a cell counting kit-8 (CCK-8) assay and performed EdU (5-ethynyl-2′-deoxyuridine) incorporation assays to test proliferation capabilities. For the CCK-8 assay, 1×10^4 cells were plated into 96-well plates (pre-coated with 5 μg/ml laminin).

After culturing for 8, 42 and 66 h, the absorbance at 450 nm was measured according to the manufacturer's instructions. For the EdU assay, 1×10^5 NSCs were plated into 48-well plates (pre-coated with 5 μg/ml laminin). After culturing for 48 h, 20 μM EdU (RIBO-Bio, C00054) was added to label the dividing cells. After incubation for 45 min, a Cell-light EdU Apollo567 *in vitro* kit (RIBO-Bio, C10310-1) was used to detect EdU-labelled cells. Briefly, cells were washed with PBS, fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.3% PBST for 20 min and then incubated with 100 μl 1 × Apollo® staining reaction solution for 1 h. A laser scanning confocal microscope (OLYMPUS, FV1000) was used to acquire images. Twenty-five images in each group (five images in each well) were used to analyse the ratio of EdU-positive cells (scaled to DAPI) using ImageJ software.

Differentiation of neural stem cells

To investigate whether TYW5 overexpression affected the differentiation of NSCs, we conducted a differentiation assay as previously described.[26] In brief, 3×10^5 cells (per well) in 24-well plates and 2×10^6 cells (per well) in 6-well plates pre-coated with 5 μg/ml laminin were cultured in proliferation media for 12 h. The proliferation media were then replaced with differentiation media (DMEM/F12, containing 1% penicillin/streptavidin, 1% N2 supplement, 1% B27 supplement and 2 μg/ml heparin). Three days post-differentiation, immunofluorescence staining was carried out to measure the proportions of astrocytes (GFAP-positive) and neurons (MAP2-positive) in all cells, and qPCR was performed to detect the expression of *Gfap*, *Map2*, *Tubb3* (expressing TUJ1 protein, a marker for newly generated immature post-mitotic neurons) and *Cldn11* (expressing O4 protein, a marker for oligodendroglia cells). The primary antibodies used were rabbit anti-GFAP (1:1000, Sigma, G9269) and rabbit anti-MAP2 (1:200, Millipore, AB5622). The secondary antibodies were goat anti-rabbit 555 (1:500, Invitrogen, A32732). The images were captured by laser scanning confocal microscopes (Olympus, FV1000) with the same scanning parameters. Fifteen images in each group (five images from each biological replicate) were used to analyse the ratio of GFAP- and MAP2-positive cells (scaled to DAPI) using ImageJ software. The primers used for qPCR are provided in Supplementary Table 5. Western blot was also used to detect the GFAP and MAP2 protein levels at three different time points (differentiation of mouse NSCs for 24, 48 and 72 h). The primary antibodies were rabbit anti-GFAP (1:1000, Sigma, G9269), rabbit anti-MAP2 (1:500, Millipore, AB5622) and mouse anti-GAPDH (1:2000, Proteintech, 60004-1-Ig). Secondary antibodies were HRP-labelled goat anti-rabbit (1:1000, Beyotime, A0208) and HRP-labelled goat anti-mouse (1:1000, Beyotime, A0216).

Morphological analysis of rat cortical neurons

Previous studies have shown dysregulation of dendritic spines in schizophrenia.[33–35] To investigate whether TYW5 overexpression affected dendritic spines, we carried out morphological analysis of dendritic spines as previously described.[27] Briefly, after culturing the isolated rat primary neurons for 14–15 days *in vitro*, 3 μg pCAG-TYW5-1 × *flag* vectors and 1 μg Venus plasmids (expressing green-fluorescent protein, GFP) were co-transfected into rat cortical neurons using Lipofectamine 3000 (Invitrogen, L3000015). At 72 h post-transfection, immunofluorescence staining was performed. The primary antibodies were rabbit anti-flag antibody (1:150, Sigma, F7425-2MG) and chicken anti-GFP antibody (1:1000, Abcam, ab13970). Secondary antibodies were goat anti-rabbit 555 (1:500, Invitrogen, A32732) and donkey anti-chicken Cy™2 (1:200, Jackson Immuno Research, 703-225-155). A laser scanning confocal microscope (Leica, TCS SP8 X) was used to take images of neurons co-expressing GFP and TYW5-flag fusion protein, using the same scanning parameters. Images of at least 20 independent neurons from each group were captured for morphological analyses. Two secondary or tertiary dendrites of each neuron (at least a total length of 100 μm) were selected to quantify spine numbers and classify spines (thin, long thin, stubby, mushroom, filopodia and branched).[36,37] ImageJ software was used for the analysis.[36] The data were compared between two different groups using a two-tailed Student's *t*-test.[37,38]

Transcriptome analysis

To explore the genes and biological processes regulated by TYW5, we conducted RNA-seq in control mouse NSCs and mouse NSCs stably overexpressing TYW5. RNA-seq was performed by ANOROAD Co. (Beijing), using a HiSeq/X-ten Illumina platform. Transcriptome analysis was performed as follows: first, clean reads were mapped to the mouse genome (GRCm38) using bowtie2 (v2.3.5) with default parameters.[39,40] The sequence alignment map (SAM) files were then converted to BAM and sorted by SAMtools (v1.9).[41] HTSeq (v0.12.4) was used to quantify the read counts for each BAM file.[42] Differentially expressed genes were identified using the DEseq2 package in R (v3.5.1),[43] and *P*-values were corrected using the Benjamini-Hochberg method. The thresholds for differentially expressed genes were set to $|\log_2(\text{foldchange})| > 0.5$ and $P_{adj} < 0.01$. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed with DAVID (v6.8) (https://david.ncifcrf.gov).[44]

Data availability

The data that support the findings of this study are available in Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC), GTEx, LIBD, CMC, ENCODE, 3DIV, PsychEncode, PROMO, AliBaba2.1, Brennand Lab RNA-seq, CoDEx Viewer, UCSC Cell Browser and GEO (GSE106589). These data were derived from the following resources available in the public domain: PGC, https://www.med.unc.edu/pgc/; GTEx, https://www.gtexportal.org/home/index.html; LIBD, http://eqtl.brainseq.org/; ENCODE, https://www.encodeproject.org/; 3DIV, http://kobic.kr/3div/; PsychEncode, http://resource.psychencode.org/; PROMO software website, http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3; AliBaba2.1 website, http://gene-regulation.com/pub/programs/alibaba2/index.html;

Brennand Lab RNA-seq website, https://schroden.shinyapps.io/BrennandLab-ExpressionApp-limited/; CoDEx Viewer website, http://solo.bmap.ucla.edu/shiny/webapp/; UCSC Cell Browser, https://cells.ucsc.edu/; GEO (GSE106589), https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE106589.

Results

Reporter gene assays validated the regulatory effects of rs796364 and rs281759

Dual-luciferase reporter gene assays can be used to evaluate the regulatory effect of target DNA sequences.[45] By inserting target DNA sequences into the regulatory region (promoter or enhancer) of the reporter gene (firefly luciferase) vector, the regulatory effect of target DNA sequences can be detected by measuring the activity of firefly luciferase activity. Renilla luciferase was used to control the transfection efficiency and the normalized ratio (i.e. firefly luciferase/renilla luciferase) reflects the regulatory effect of the target DNA sequences (Supplementary Fig. 1). To validate the regulatory effects of rs796364 and rs281759, we conducted reporter

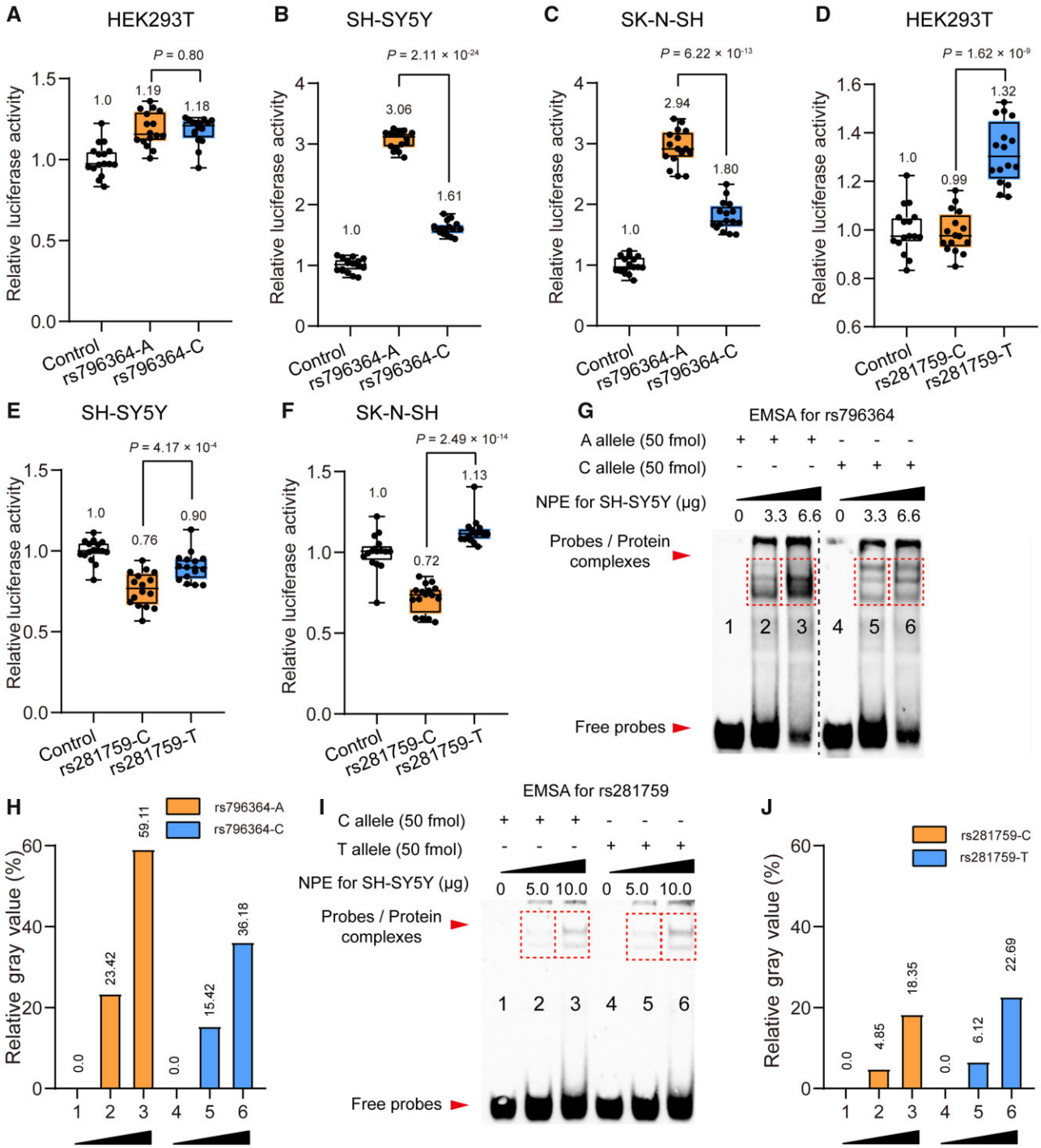

Figure 3 Reporter gene assays and EMSA validated the regulatory effects of rs796364 and rs281759. (**A**–**F**) Reporter gene assays (enhancer assay) showed that different alleles of rs796364 and rs281759 significantly affected luciferase activity. Compared with the C allele, the A allele of rs796364 conferred significantly higher luciferase activity in SH-SY5Y and SK-N-SH cells. For rs281759, the T allele exhibited significantly higher activity compared with the C allele in all three tested cells lines. As these two SNPs were cloned into the pGL3-promoter, these assays reflect the regulatory (i.e. enhancer) effect of the inserted fragments on the luciferase promoter. (**G**–**J**) rs796364 and rs281759 affected the binding affinity of nuclear protein extracts. The quantification data (grey values, arbitrary units) of the probes/protein complexes were first quantified using ImageJ. The obtained values were then normalized to the total quantity of probes. The quantification data for **G** is shown in **H**, and those for **I** are shown in **J**. For rs796364, the A allele showed stronger binding compared with the C allele (**G** and **H**). For rs281759, the T allele showed stronger binding compared with the C allele (**I** and **J**). Data represent the mean ± SD. $n = 16$. Two-tailed Student's *t*-test was used to test for a significant difference.

gene assays in neuroblastoma cell lines from human brain (SK-N-SH and SH-SY5Y) and HEK293T (a cell line from human kidney). For rs796364, the A and C allele of rs796364 did not show a significant difference in luciferase activity in HEK293T (Fig. 3A). However, the A allele conferred significantly higher luciferase activity than the C allele in SK-N-SH and SH-SY5Y cells (Fig. 3B and C), suggesting a cell-type specific regulatory effect of rs796364 (i.e. rs796364 has a regulatory effect in neuroblastoma cell lines but not in

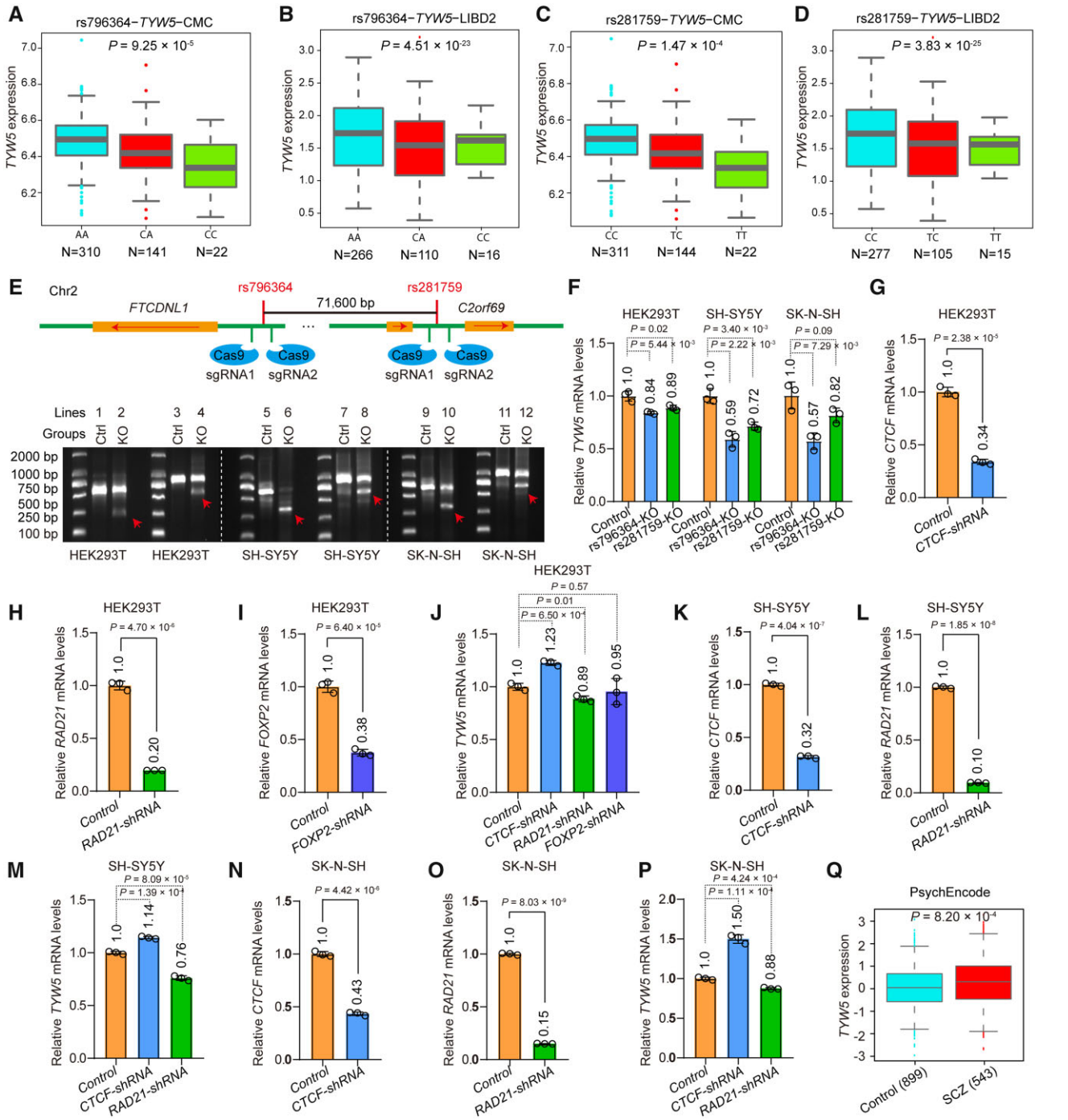

Figure 4 Rs796364 and rs281759 are associated with TYW5 expression in the human brain. (**A**–**D**) Brain eQTL analysis (CMC and LIBD2) showed that rs796364 and rs281759 were significantly associated with TYW5 expression in the human brain.[19,20] (**E** and **F**) Knockout of genomic sequences (about 300 bp) containing rs796364 or rs281759 resulted in significant downregulation of *TYW5* expression, indicating that the genomic regions surrounding rs796364 and rs281759 act as enhancers to regulate *TYW5* expression. (**G**–**P**) Transcription factor (CTCF, RAD21 and FOXP2) knockdown altered *TYW5* gene expression, indicating that *TYW5* gene expression was regulated by CTCF, RAD21 and FOXP2. CTCF, RAD21 and FOXP2 were significantly knocked-down in HEK293T (**G**–**I**), SH-SY5Y (**K** and **L**) and SK-N-SH cell lines (**N** and **O**). (**J**, **M** and **P**) Quantitative PCR showed that *TYW5* expression was significantly altered in cells with knocked-down transcription factors. (**Q**) *TYW5* expression was significantly upregulated in brains of schizophrenia cases compared with controls. Data represent mean ± SD. $n = 3$ for **F**–**P**. *ACTB* and *GAPDH* were used as endogenous reference genes for HEK293T, and *ACTB* and *B2M* were used as endogenous reference genes for SH-SY5Y and SK-N-SH. Two-tailed Student's *t*-test was used to test for a significant difference.

HEK293T). For rs281759, the T allele exhibited significantly higher luciferase activity than the C allele in all three tested cell lines (Fig. 3D–F). These results confirmed the regulatory effects of rs796364 and rs281759, suggesting that these two functional SNPs regulate the expression level of their target gene (or genes) by modulating the activity of enhancer elements.

EMSA showed that rs796364 and rs281759 affected the binding affinities of transcription factors

Electrophoretic mobility shift assay is widely used to evaluate the interaction between target DNA sequences and transcription factors.[28,29] If a target DNA sequence binds specific transcription factor(s), the protein-probe complexes migrate more slowly than the corresponding free probes during electrophoresis. To investigate whether rs796364 and rs281759 affect the binding of transcription factors, we conducted EMSA. The EMSA results indicated that these two SNPs significantly altered binding affinities to nuclear proteins extracted from SH-SY5Y cells (which contained abundant proteins and transcription factors; Fig. 3G–J). The A allele of rs796364 (Fig. 3G and H) and the T allele of rs281759 (Fig. 3I and J) showed a higher affinity to transcription factors than the C allele. Further bioinformatic analysis suggested that different alleles of these two SNPs affect the binding of several transcription factors (Supplementary Fig. 2 and Supplementary Table 7). These results indicated that both of these two functional SNPs (rs796364 and rs281759) may affect transcription factor binding. Interestingly, the results of the reporter gene assay and EMSA showed that the alleles associated with higher affinity (rs796364-A and rs281759-T) to nuclear protein extractions exhibited higher luciferase activity.

Rs796364 and rs281759 showed the most significant associations with *TYW5* expression in human brain

Our reporter gene assays and EMSA verified the regulatory effects of rs796364 and rs281759, indicating that these two SNPs might confer schizophrenia risk by regulating gene expression. To identify the potential target gene (or genes) regulated by these two SNPs, we examined their correlations with the expression of genes in the CMC and LIBD2 brain eQTL datasets.[19,20] We found that these two SNPs were significantly associated with mRNA levels of four genes (within 1 Mb distance) in the CMC dataset, including *C2orf47*, *FTCDNL1*, *SATB2* and *TYW5* (Supplementary Fig. 3A and B). Intriguingly, these two SNPs showed the most significant associations with *TYW5* expression in both the CMC ($P_{rs796364} = 9.25 \times 10^{-5}$, $P_{rs281759} = 1.47 \times 10^{-4}$) and LIBD2 ($P_{rs796364} = 4.51 \times 10^{-23}$, $P_{rs281759} = 3.83 \times 10^{-25}$) datasets (Fig. 4A–D), implying that they likely conferred schizophrenia risk by regulating *TYW5* expression.

CRISPR-Cas9-mediated genome editing validated the regulatory effects of rs796364 and rs281759 on *TYW5*

As our eQTL analysis suggested that these two functional SNPs regulate *TYW5* expression, we sought to validate whether they indeed exerted regulatory effects on this gene. Considering that both SNPs were located downstream of *TYW5* (Fig. 2A) and reporter gene assays revealed strong enhancer activity of the genomic sequences containing rs796364 in neuroblastoma cells (Fig. 3B and C), it appeared likely that rs796364 and rs281759 regulate *TYW5* by affecting enhancer activity. We thus knocked out genomic sequences of about 300 bp surrounding these two SNPs in three cell lines (HEK293T, SH-SY5Y and SK-N-SH) and found that deletion of these two SNPs resulted in significant downregulation of *TYW5* in all three cell lines ($P < 0.05$; Fig. 4E and F and Supplementary Fig. 4). These data indicated that these two SNPs are located in the enhancer elements of *TYW5*, and it is likely that they regulate *TYW5* expression by altering enhancer activity. Interestingly, chromatin interaction analyses in the human neuroblastoma cell line SK-N-DZ and brain tissues (using 3DIV) showed that these two SNPs physically interact with the *TYW5* gene in the dorsolateral prefrontal cortex, hippocampus and SK-N-DZ cell line (Supplementary Figs 5–8),[30] suggesting that rs796364 and rs281759 may regulate *TYW5* expression by affecting chromatin interactions.

Knock-down of corresponding transcription factors affected *TYW5* expression

Our functional genomics analysis revealed that rs796364 and rs281759 disrupted the binding of CTCF, RAD21 and FOXP2, providing molecular explanations for their regulatory impact on *TYW5* expression. To examine the regulatory effects of these transcription factors on *TYW5* expression, we knocked down *CTCF*, *RAD21* and *FOXP2* expression in HEK293T, SH-SY5Y and SK-N-SH cells (using shRNAs). The qPCR results showed that RAD21 knockdown resulted in significant downregulation of *TYW5* ($P < 0.05$), while CTCF knockdown led to significant upregulation of *TYW5* ($P < 0.05$) in all three tested cell lines (Fig. 4G–P). As *FOXP2* expression in SH-SY5Y and SK-N-SH cell lines was not detected by RT-PCR (Supplementary Fig. 9), we did not knock-down *FOXP2* in the SH-SY5Y and SK-N-SH cell lines. Taken together, these results indicated that rs796364 and rs281759 regulate *TYW5* expression by affecting transcription factor binding affinity.

Dysregulation of *TYW5* in schizophrenia cases

We provided convergent lines of evidence (including functional genomics, eQTL analysis, Cas9-mediated genome editing and transcription factor knockdown), suggesting that the functional SNPs rs796364 and rs281759 might confer schizophrenia risk by regulating *TYW5* expression. It was therefore necessary to examine whether expression perturbation of *TYW5* was involved in schizophrenia. We thus compared *TYW5* expression in brains of schizophrenia cases versus that in controls using expression data from PsychEncode.[21] Consistent with our hypothesis above, we found that *TYW5* expression was significantly upregulated in brains of schizophrenia cases compared with controls ($P = 8.20 \times 10^{-4}$; Fig. 4Q), indicating a potentially pivotal role of *TYW5* dysregulation in schizophrenia.

Overexpression of *TYW5* inhibits proliferation of mouse neural stem cells

Appropriate proliferation and differentiation of NSCs is necessary for neurodevelopment,[46] and previous studies have shown that dysfunction of neurodevelopment might play important roles in the pathogenesis of schizophrenia.[47,48] To characterize the roles of *TYW5* in schizophrenia, we utilized the NSCs model (a model widely used to investigate the role of schizophrenia risk genes in neurodevelopment).[49,50] Of note, the neurodevelopmental hypothesis of schizophrenia posits that schizophrenia risk genes may confer risk of this disorder by affecting brain development.[47,48] As *TYW5* was significantly upregulated in schizophrenia patients, we conducted overexpression experiments to mimic the effects of *TYW5* upregulation. Cells from the ventricular zone of embryonic mouse brains (embryonic Day 14) were isolated and the identity of these cells was validated with three well-characterized NSC markers (SOX2, NESTIN and PAX6) (Supplementary Fig. 10). The NSCs were then infected with the packaged viral particles, and puromycin (1 μg/ml) was added to select the stably infected cells for 2 weeks. Reverse transcription-PCR and western blot confirmed stable overexpression of human *TYW5* in the infected mouse NSCs (Fig. 5A and B). We then conducted EdU incorporation and CCK-8 assays to assess the effect of *TYW5* overexpression on the proliferation of these cells. Both assays showed that *TYW5* overexpression significantly inhibited the proliferation of mouse NSCs (Fig. 5C–E). Taken

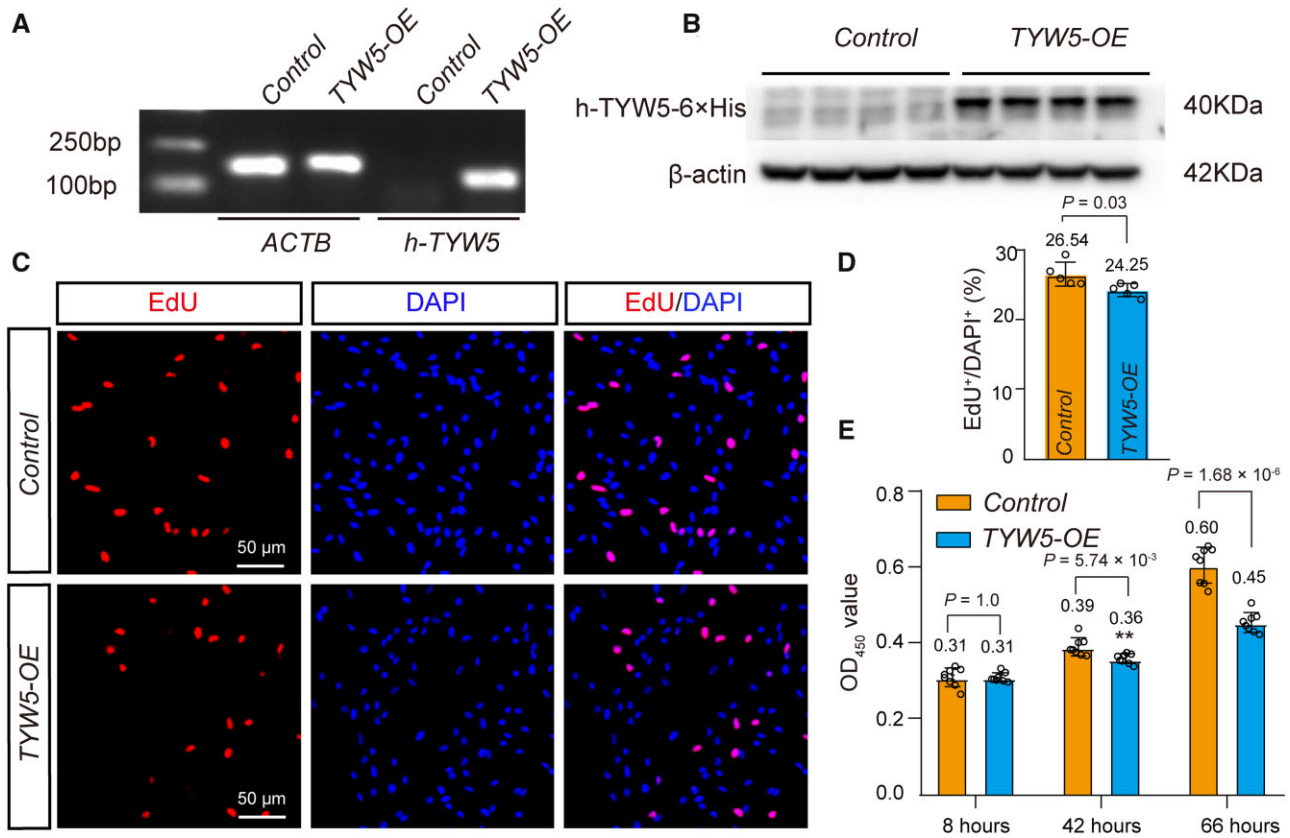

Figure 5 TYW5 overexpression significantly inhibited proliferation of mouse neural stem cells. (**A** and **B**) Validation of TYW5 overexpression in mouse NSCs with RT-PCR (**B**) and western blot (**C**). To distinguish the endogenous and overexpressed TYW5 (both can express TYW5), we ligated a 6 × His tag to TYW5 to detect if the constructed overexpression vector can express TYW5 successfully in NSCs using antibodies that can recognize the His tag. (**C–E**) EdU incorporation assays and CCK-8 assays showed that *TYW5* overexpression significantly inhibited proliferation of mouse NSCs. (**C** and **D**) Compared with controls, the ratio of EdU-positive cells was significantly decreased in *TYW5* overexpression groups. (**C**) Representative immunofluorescence images for EdU staining; the statistical analysis result for **C** is shown in **D**. (**E**) A CCK-8 assay showed that the OD_{450} values of the *TYW5* overexpression groups were significantly decreased compared with the controls. Two-tailed Student's *t*-test was used to test for a significant difference. Data represent mean ± SD, $n = 5$ for **D**, $n = 8$ for **E**.

together, *TYW5* overexpression affected proliferation of NSCs and thereby modulated neurodevelopment.

Overexpression of *TYW5* affects differentiation of mouse neural stem cells

In addition to proliferation, differentiation is also important for neurodevelopment.[46] We thus sought to investigate whether *TYW5* played a role in neuronal differentiation. Neural stem cells can differentiate into glia cells and neurons, two major cell types in the human brain. The markers for astrocytes (GFAP), mature neurons (MAP2), immature post-mitotic neurons (TUJ1 encoded by *Tubb3*) and oligodendrocytes can be labelled with O4 (encoded by *Cldn11*). These cell types are generated during the differentiation of NSCs, and the markers can be used to investigate the differentiation process.[51,52] After 3 days of NSC differentiation, GFAP- and MAP2-positive cells were counted. We found that the ratio of GFAP- (a marker for glia cells, but not MAP2, a marker for neurons) positive cells was significantly decreased in cells overexpressing *TYW5* compared with control cells ($P < 0.05$; Fig. 6A–D), indicating that *TYW5* overexpression impaired the differentiation of glia cells. In addition, we also measured the mRNA level of *Gfap*, *Map2*, *Tubb3* and *Cldn11* in *TYW5* overexpression and control cells using *Actb* and *Ywhaz* as endogenous reference genes (Supplementary Table 5). We found that the expression of *Tubb3* and *Cldn11* (but not *Gfap* and *Map2*) were significantly downregulated in *TYW5* overexpression cells compared with the controls ($P < 0.05$; Supplementary Fig. 11). To validate the effect of *TYW5* overexpression on the differentiation of NSCs, we used western blotting to detect GFAP and MAP2 protein expression after 24, 48, and 72 h of NSC differentiation. After 24 h of differentiation, GFAP was significantly downregulated in the *TYW5* overexpressing groups compared with the controls, and after 48 h, MAP2 was significantly upregulated in the *TYW5* overexpressing groups compared with the controls. However, GFAP and MAP2 did not show significant differences after 72 h of NSC differentiation (Fig. 6E–H). A possible explanation for this is the degradation of GFAP and MAP2 proteins (Fig. 6E). These data demonstrated that *TYW5* overexpression may affect the differentiation of mouse NSCs.

TYW5 regulates schizophrenia-related pathways

Our data above show that *TYW5* overexpression affected the proliferation and differentiation of NSCs. Nevertheless, the underlying biological processes and signalling pathways remain unclear. We thus used NSCs that stably overexpress *TYW5* and control NSCs (cultured in proliferation medium) to perform transcriptome analysis through RNA-seq. A total of 304 differentially expressed genes (183 upregulated, 121 downregulated) were identified (Fig. 7A and B and Supplementary Table 8). GO analysis showed that these genes were significantly enriched in multiple essential physiological processes, including DNA replication, cell adhesion and the

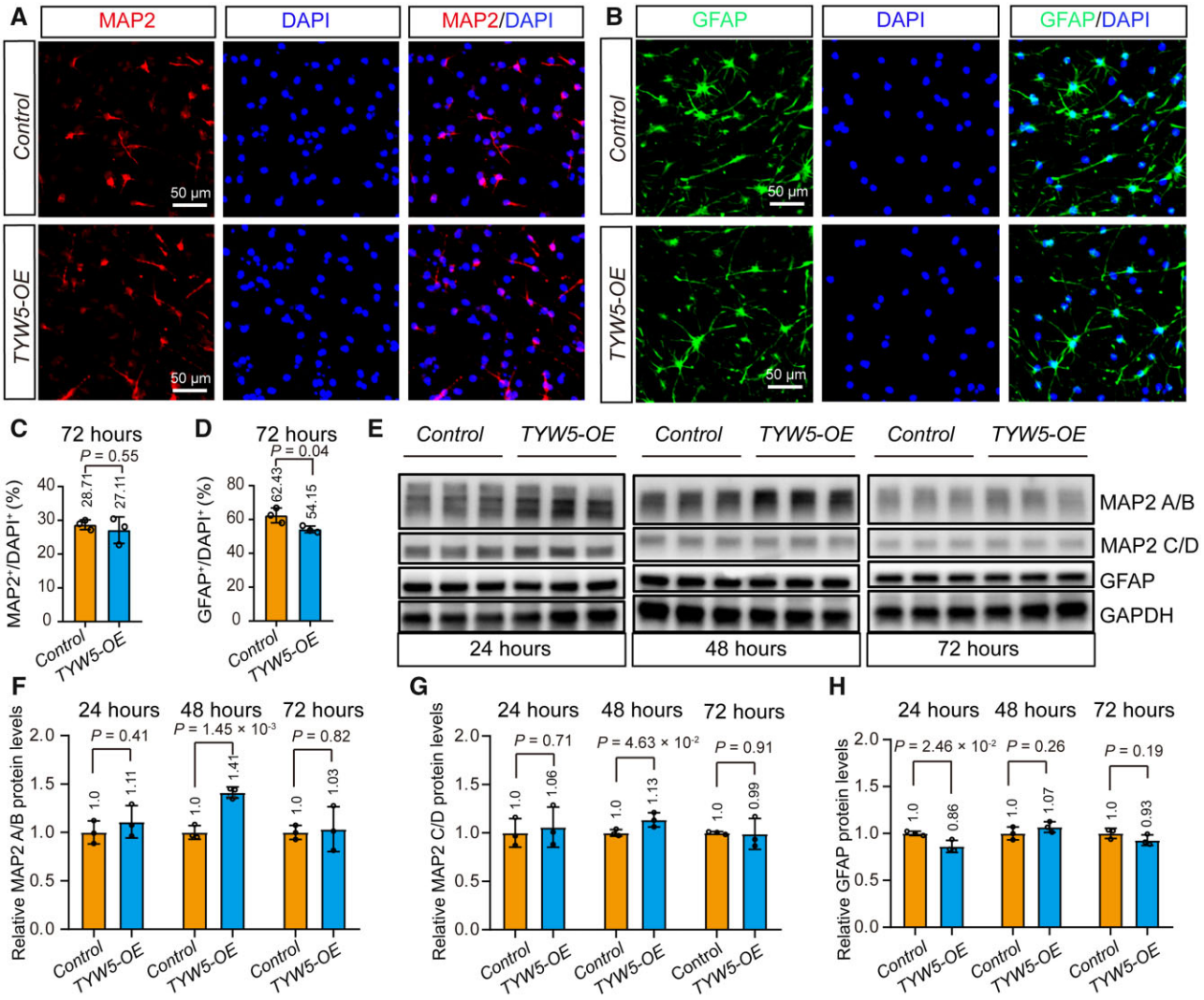

Figure 6 TYW5 overexpression significantly affected differentiation of mouse neural stem cells. (**A** and **B**) Representative immunofluorescence images for GFAP (a marker for astrocyte cells) and MAP2 (a marker for mature neurons) staining. (**C**) Compared with the controls, the ratio of GFAP-positive cells were significantly decreased in TYW5 overexpression groups, indicating that TYW5 overexpression impaired the differentiation of NSCs into glia cells. (**D**) In contrast, the ratio of MAP2-positive cells remained unchanged. (**C**) The quantification data for **A**. (**D**) The quantification data for **B**. (**E–H**) Western blot results for GFAP and MAP2 expression in TYW5 overexpression and control groups. For MAP2, two bands (MAP2 A/B, molecular weight: 270–300 kDa; and MAP2 C/D, molecular weight: 70–75 kDa) were detected. (**F–H**) The quantification data of MAP2 A/B, MAP2 C/D and GFAP protein expression for **E**. Two-tailed Student's *t*-test was used to test for a significant difference. Data represent mean ± SD, $n = 3$ for **C**, **D** and **F–H**).

cell cycle ($P_{Benjamini} < 0.05$; Fig. 7C). Further KEGG analysis indicated that the differentially expressed genes were significantly enriched in schizophrenia-associated signalling pathways, including extracellular matrix-receptor interaction, focal adhesion and PI3K-Akt ($P_{Benjamini} < 0.05$; Fig. 7D).[53–55] Of note, both GO and KEGG analyses indicated that the differentially expressed genes showed the most significant enrichment in DNA replication-related pathways. Collectively, these results suggested that *TYW5* may contribute to schizophrenia susceptibility by regulating extracellular matrix-receptor interaction, focal adhesion and PI3K-Akt pathways.[53–55]

Overexpression of TYW5 affects the morphology and density of dendritic spines

In addition to the abnormality of neurodevelopment, the density of dendritic spines was also significantly reduced in schizophrenia cases,[33–35] indicating that the formation of dendritic spines may also participate in schizophrenia pathogenesis. We thus investigated the effects of *TYW5* overexpression on dendritic spine formation using rat primary cortical neurons and found that *TYW5* overexpression affected dendritic spine density (Fig. 8A–C). The density and ratio of stubby spines was significantly decreased, while the density and ratio of thin spines was significantly increased in *TYW5*-overexpressing neurons compared with the controls ($P < 0.05$; Fig. 8A–C). However, the density and ratio of mushroom spines, other spines (including long thin, filopodia and branched spines) and total spines were not affected ($P > 0.05$). We further analysed the long thin, filopodia and branched spines and found that the density and ratio of branched spines was significantly decreased in *TYW5*-overexpressing neurons compared with the controls ($P < 0.05$; Supplementary Fig. 12E and F). Taken together, these results showed that overexpression of *TYW5* could affect the formation of dendritic spines on rat neurons, proving that *TYW5* has potentially important roles in the formation of dendritic spines.

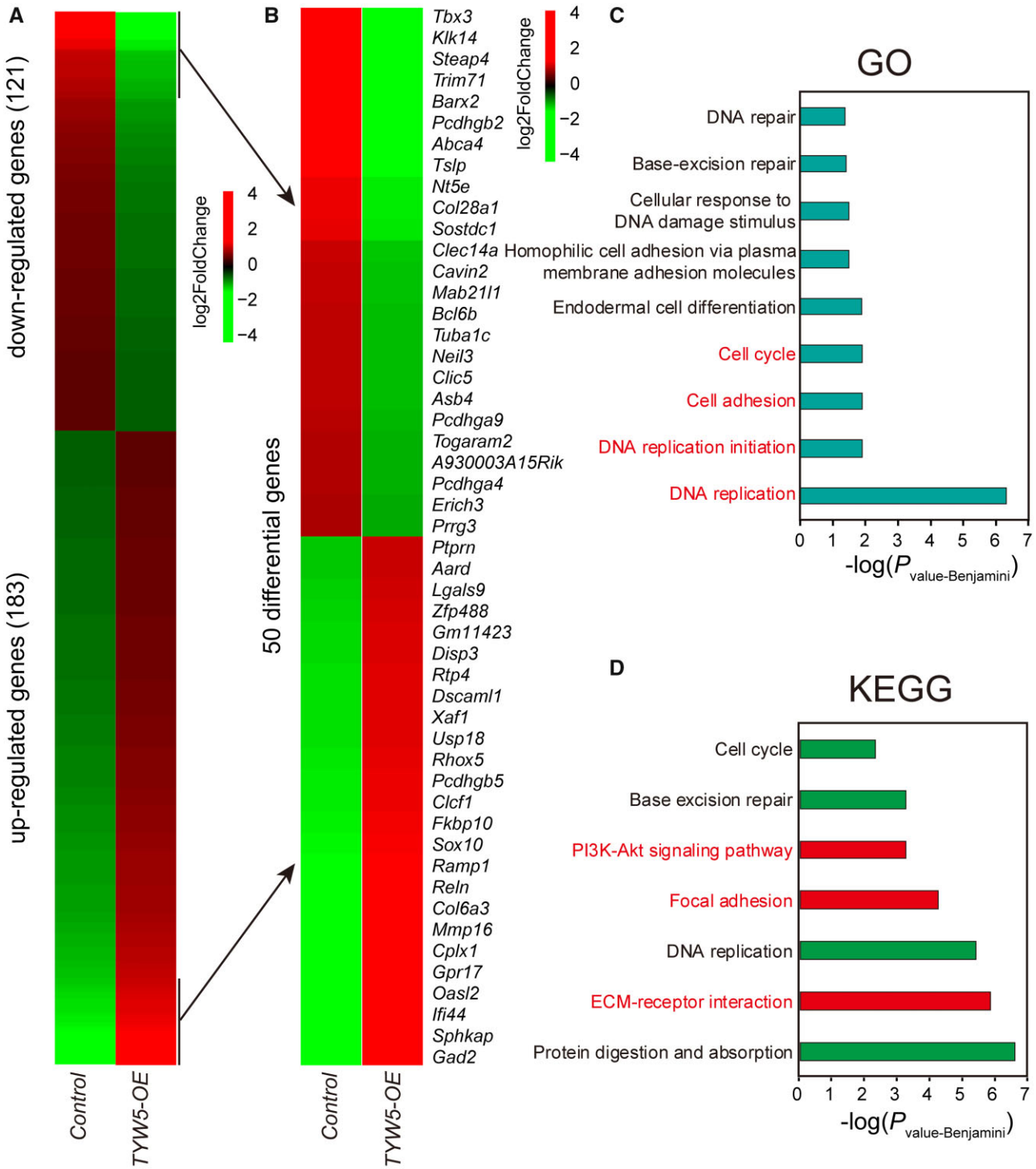

Figure 7 TYW5 regulates schizophrenia-associated pathways. (**A**) Heat map for the 304 (183 upregulated and 121 downregulated) differentially expressed genes (|fold change| > 0.5 and $P_{adj} < 0.01$) in the TYW5 overexpression group compared with the control group. (**B**) Heat map for the top 25 up or downregulated genes. (**C**) GO analysis showed that the differentially expressed genes showed the most significant enrichment in DNA replication. (**D**) KEGG enrichment analysis showed that the differentially expressed genes showed significant enrichment in schizophrenia-associated pathways, including extracellular matrix-receptor interaction, focal adhesion and PI3K-Akt signalling pathways.

Discussion

Identifying the functional (or causal) risk variants at the reported risk loci and elucidating their roles in schizophrenia is an important and necessary step towards understanding the genetic mechanisms and pathogenesis of schizophrenia in the post-GWAS era. Although recent studies have demonstrated the potential regulatory mechanisms of some schizophrenia risk variants,[16,21,56,57] the functional variants and their roles in schizophrenia for most of the GWAS risk loci remain largely unknown. In this study, we systematically characterized the regulatory mechanisms of risk variants rs796364 and rs281759, two functional SNPs identified in our previous functional genomics study.[16] We have provided convergent lines of evidence to support the regulatory

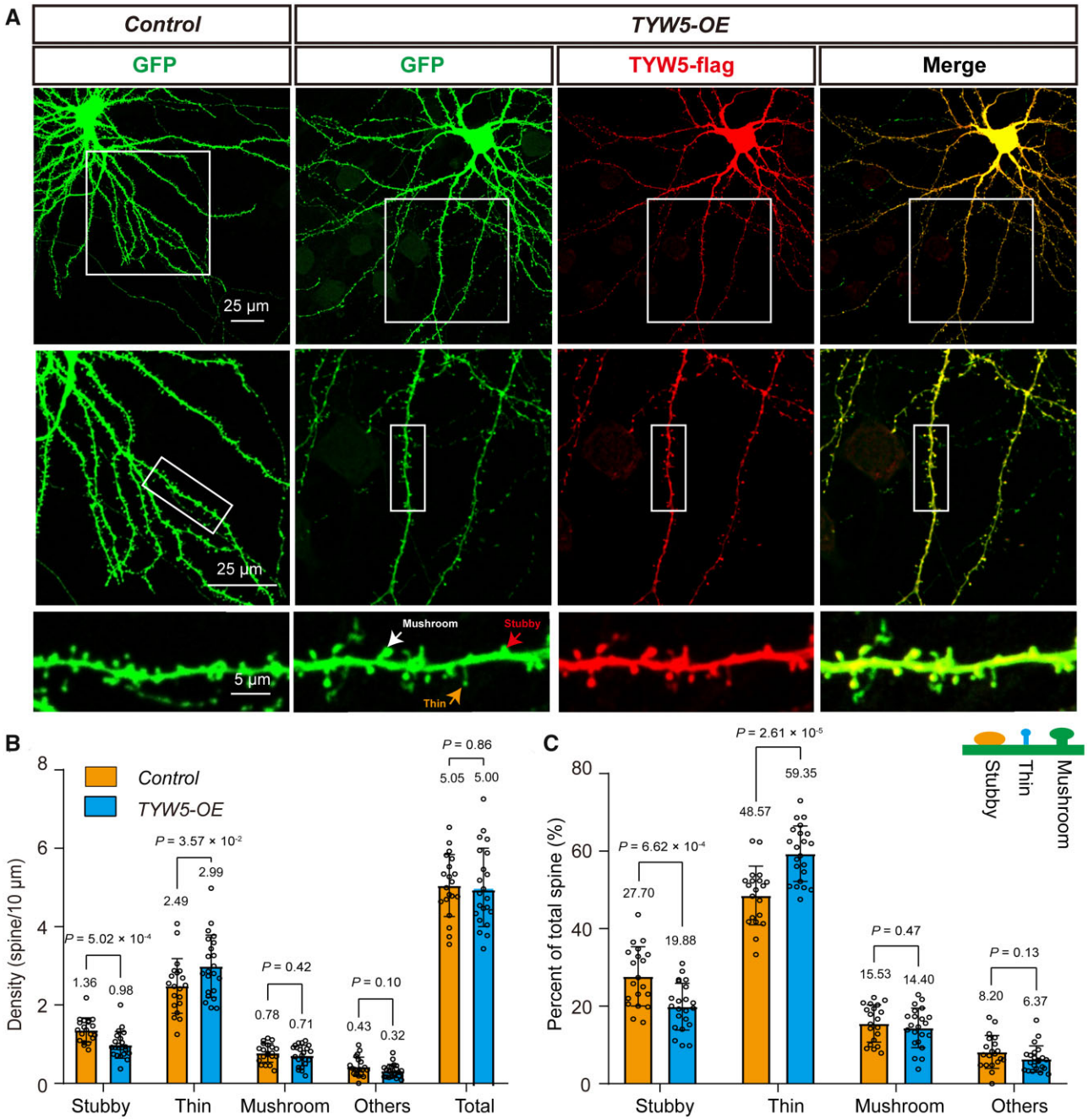

Figure 8 TYW5 overexpression affected dendritic spine density. (**A**) Representative immunofluorescence images for GFP (visualizing neuronal morphologies with green) and TYW5-1 × flag (indicating overexpression with red) in rat primary cortical neurons. The arrows of different colours point to different types of spines: stubby (green), thin (orange), long thin (blue) and mushroom (white). (**B** and **C**) Compared with the control, the density and ratio of the stubby spines was significantly decreased in *TYW5*-overexpressing neurons. However, the density and ratio of thin spines was significantly increased in *TYW5*-overexpressing neurons. (**B**) The quantification data for the density of stubby spines in **A**. (**C**) The quantification data for the ratio of stubby spines in **A**. Two-tailed Student's *t*-test was used to test for a significant difference. Data represent mean ± SD, the numbers of neurons used for this quantitative analysis in the control and experimental groups were $n = 20$ and $n = 22$.

effects of rs796364 and rs281759. First, we validated the regulatory effects of these two functional SNPs with reporter gene assays. Second, our EMSA results showed that they could affect the binding affinity of transcription factors. Third, PAINTOR fine-mapping suggested that rs796364 is a potential causal variant (posterior probability of causality 0.66) (Supplementary Table 9).[58] Fourth, we conducted eQTL analysis and found the potential target gene regulated by these two SNPs, *TYW5*, whose mRNA expression was significantly associated with these two SNPs in human brain. Fifth, our integrative TWAS analyses showed that *TYW5* is a schizophrenia risk gene whose change in expression might play a role in this disorder [TWAS $P_{(CMC)} = 1.34 \times 10^{-11}$, TWAS $P_{(LIBD2)} = 1.50 \times 10^{-12}$; GWAS associations and two brain eQTL datasets (CMC and LIBD2) were used for TWAS integrative analyses; Supplementary Table 10].[10,19,20] Furthermore, we confirmed the regulatory effect of rs796364 and rs281759 on *TYW5* expression using CRISPR-Cas9-mediated genome editing and defined the transcription factors facilitating such a regulatory effect. Therefore, rs796364 and

rs281759 likely confer schizophrenia risk by affecting *TYW5* expression. Consistent with this, we found that these two regulatory variants physically interacted with *TYW5* in the dorsolateral prefrontal cortex, hippocampus and neuroblastoma cells of human brain. These data indicate that these two functional SNPs regulate *TYW5* expression by altering transcription factor binding and long-range chromatin interaction. Intriguingly, expression analysis showed that *TYW5* was significantly upregulated in brains of schizophrenia cases (Fig. 4Q), highlighting its pivotal roles in schizophrenia pathogenesis.

In addition, we also independently confirmed that rs796364 was significantly associated with schizophrenia in non-Europeans (i.e. a Chinese population; $P = 9.68 \times 10^{-4}$; data from a recent study in our lab),[59] with the same risk allele as in Europeans. A meta-analysis (a total of 59 911 cases and 83 527 controls) using published data indicated that rs796364 was strongly associated with schizophrenia ($P = 1.14 \times 10^{-19}$). This successful genetic validation in an independent population provided robust evidence for the involvement of these two regulatory variants in schizophrenia and further supported the potential functional consequences of this SNP. Considering that cognitive impairment is a core symptom of schizophrenia and previous studies have shown that schizophrenia risk variants are also associated with cognitive performance,[1,2,60,61] if rs796364 represents an authentic risk variant for schizophrenia, it may also be associated with cognitive performance. Examination of the association between rs796364 and cognitive performance using published data showed that rs796364 was also associated with cognitive performances ($P_{Intelligence} = 3.91 \times 10^{-4}$, $P_{RT} = 1.42 \times 10^{-3}$ and $P_{VNR} = 9.00 \times 10^{-3}$), and the risk allele predicted poorer cognitive performance.[62,63] This successful validation in genetic independent populations and its association with cognitive performance provides extra support for the involvement of rs796364 in schizophrenia.

We noted that rs796364 is located in the promoter region of *FTCDNL1* (Fig. 2). We thus explored *FTCDNL1* expression in different cell types of human brain. *FTCDNL1* expression in different neural cells was much lower than that of *TYW5* (Supplementary Fig. 13A and B). We also examined *FTCDNL1* and *TYW5* expression in the Cortical development expression viewer and UCSC Cell browser and observed similar results (Supplementary Figs 14 and 15A and B).[64–66] Intriguingly, *TYW5* has a higher expression level in neural progenitor cells and 6-week-old forebrain neurons (derived from human-induced pluripotent stem cells of normal subject; Supplementary Fig. 16). Of note, our RT-PCR showed no obvious expression of *FTCDNL1* in HEK293T, SK-N-SH and SH-SY5Y cell lines (Supplementary Fig. 9). Finally, spatio-temporal expression data showed that *TYW5* is stably and highly expressed in different regions of human brain over the whole life (Supplementary Fig. 17),[67,68] implying the pivotal role of *TYW5* in human brain. These expression data suggest that rs796364 and rs281759 might confer schizophrenia risk by modulating *TYW5* (i.e. compared with *FTCDNL1*, it is more likely that *TYW5* represents the potential risk gene at this locus).

In addition to elucidating the regulatory mechanisms of rs796364 and rs281759, we also provided further evidence to support the involvement of *TYW5* in schizophrenia pathogenesis. *TYW5* was upregulated in brains of schizophrenia cases and overexpression of *TYW5* affected the proliferation and differentiation of NSCs, indicating that *TYW5* has a role in neurodevelopment. Intriguingly, we also found that *TYW5* regulated the density of dendritic spines, an important neuronal structure that is frequently found to be dysregulated in schizophrenics.[33–35] We also checked the associations between differentially expressed genes (between *TYW5*-overexpression and control NSCs) and schizophrenia (detailed information is provided in the Supplementary material).[8] The differentially expressed genes (including *Snap91, Ndrg4, Ier3* and *Ip6k3*) showed significant associations with schizophrenia, suggesting that *TYW5* may confer risk of schizophrenia by regulating these genes. Moreover, our transcriptome analysis showed that *TYW5* regulated schizophrenia-associated pathways, including extracellular matrix-receptor interaction, focal adhesion and the PI3K-Akt signalling pathway.[53–55] Interestingly, a previous TWAS (which used foetal brain eQTL) also suggested that *TYW5* was a schizophrenia risk gene (TWAS $P = 4.16 \times 10^{-9}$) whose expression changes in early brain development might play a role in schizophrenia.[69] Finally, the exploration of association between *TYW5* and other psychiatric disorders (including bipolar disorder, depression and attention deficit and hyperactivity) showed suggestive associations between *TYW5* and bipolar disorder (rs73066802, $P = 2.61 \times 10^{-5}$; Supplementary Fig. 18).[70–72] These consistent and convergent lines of evidence demonstrated that *TYW5* might confer schizophrenia risk by affecting neurodevelopment and schizophrenia-associated pathways.

TYW5 encodes TRNA-YW synthesizing protein 5 [a Jumonji C (JmjC)-domain-containing protein, JMJD], a tRNA modification enzyme which forms the OHyW nucleoside by carbon hydroxylation using 2-oxoglutarate (2-OG) and Fe^{2+} ion as cofactors.[73,74] JMJD proteins usually act as epigenetic regulators. However, TYW5 acts as a RNA hydroxylase.[73,74] It is involved in the wybutosine biosynthesis pathway, with six enzymes (including TRMT5 and TYW1-5) working sequentially to transfer the original nucleoside in position 37 of tRNAPhe into hydroxy wybutosine (OHyW).[73,75] By homo-dimerization, a large and positively-charged patch is formed for tRNA binding. *TYW5* expression is significantly decreased under iron depletion.[76] Interestingly, the levels of trace element iron in blood from schizophrenia cases were lower than those of normal people.[77] To date, the function of *TYW5* remains largely unknown and no study has investigated its role in brain development and schizophrenia. Interestingly, we found that TYW5 is mainly located in the cytoplasm and nucleus of five different cell-types (HEK293T, SH-SY5Y, SK-N-SH, mouse NSCs and rat neurons) (Supplementary Fig. 19–22), and for the first time we showed its pivotal role in neurodevelopment and spine morphogenesis (i.e. dysregulation of *TYW5* affected the proliferation and differentiation of NSCs and spine density in neurons). Although the pathophysiology of schizophrenia remains elusive, accumulating data support the involvement of aberrant neurodevelopment.[47,48] Our findings highlight *TYW5* as an important gene in appropriate neurodevelopment and schizophrenia susceptibility.

Despite the above implications of this study for the genetic basis of schizophrenia, there are several limitations to be acknowledged. First, although we showed that rs796364 and rs281759 could regulate *TYW5* expression (likely by affecting the binding of CTCF, RAD21 and FOXP2), the exact mechanisms still need to be elucidated, probably through precise single-base editing (using CRISPR-Cas9 system) of these SNPs. Second, in addition to *TYW5*, chromatin interaction analysis also showed interactions between rs796364 and rs281759 and other genes (including *C2orf69* and *MAIP1*). Moreover, gene expression analysis showed significant downregulation of *FTCDNL1* in the brains of schizophrenia cases compared with controls ($P = 5.92 \times 10^{-4}$, FDR 6.31×10^{-3}) in PsychENCODE.[21] Therefore, we cannot exclude the possibility that these two SNPs may confer schizophrenia risk by modulating genes other than *TYW5*. Third, although PAINTOR fine-mapping supported the causality of rs796364, we noticed that another SNP, rs2949006, also had high posterior probability (0.838) for causality (Supplementary Table 9). Expression QTL analysis showed that rs2949006 was significantly associated with *FTCDNL1* ($P = 9.31 \times 10^{-14}$) and *TYW5* expression ($P = 2.52 \times 10^{-8}$) in human brain.[19] The strong association between rs2949006 and *FTCDNL1*

suggested that *FTCDNL1* may also be a potential risk gene for schizophrenia. Furthermore, *FTCDNL1* was significantly downregulated in schizophrenia cases compared with the controls, further supporting the potential role of *FTCDNL1* in schizophrenia. These data suggest that risk variants in this region may confer risk of schizophrenia by modulating *FTCDNL1*. More work is needed to investigate whether *FTCDNL1* is an authentic risk gene for schizophrenia. Fourth, our results showed that *TYW5* overexpression affected the proliferation and differentiation of NSCs and the formation of dendritic spines *in vitro*. Relevant physiological processes linking these alterations to schizophrenia pathogenesis remain to be illuminated using animal models. Fifth, we used the mouse NSC model to explore the role of *TYW5* in neurodevelopment. The rationale behind the use of NSCs are as follows: (i) the neurodevelopmental hypothesis of schizophrenia posits that schizophrenia risk genes may confer risk of this disorder by affecting brain development.[47,48] Consistent with this hypothesis, previous studies have indicated the pivotal roles of schizophrenia risk genes in early neurodevelopment.[49,50] Thus, we think that mouse NSCs represent a feasible model to explore the function of schizophrenia risk genes; (ii) compared with human NSCs, culturing (and manipulating) of mouse NSCs is much easier, and the growth rate of mouse NSCs is faster; (iii) immature/mature neurons may also be a good model. Nevertheless, isolating and culturing immature/mature neurons currently remains challenging. More work (including the use of human NSCs and immature/mature neurons) is needed to elucidate the role of *TYW5* in schizophrenia pathogenesis. Sixth, considering the polygenicity nature of schizophrenia,[78] the clinical relevance of this study is limited due to the tiny influence of each individual risk locus on the risk of schizophrenia. Further work is needed to explore the potential clinical relevance of this study in schizophrenia. Finally, while our study provides insights into the biological relevance of *TYW5* in schizophrenia, further characterization of its functionality and underlying mechanisms by which this gene participate in the disorder is needed.

In summary, we systematically characterized the regulatory mechanisms of rs796364 and rs281759. We showed that these two functional SNPs confer schizophrenia risk by regulating the expression of *TYW5*, a previous uncharacterized gene with important roles in neurodevelopment and spine morphogenesis (Supplementary Fig. 23).

Acknowledgements

One of the Brain eQTL datasets used in this study was generated as part of the CommonMind Consortium, supported by funding from Takeda Pharmaceuticals Company Limited, F. Hoffman-La Roche Ltd and NIH grants R01MH085542, R01MH093725, P50MH066392, P50MH080405, R01MH097276, RO1-MH-075916, P50M096891, P50MH084053S1, R37MH057881 and R37MH057881S1, HHSN271201300031C, AG02219, AG05138 and MH06692. Brain tissue for the study was obtained from the following brain bank collections: the Mount Sinai NIH Brain and Tissue Repository, the University of Pennsylvania Alzheimer's Disease Core Center, the University of Pittsburgh NeuroBioBank and Brain and Tissue Repositories and the NIMH Human Brain Collection Core. CMC Leadership: Pamela Sklar, Joseph Buxbaum (Icahn School of Medicine at Mount Sinai), Bernie Devlin, David Lewis (University of Pittsburgh), Raquel Gur, Chang-Gyu Hahn (University of Pennsylvania), Keisuke Hirai, HiroyoshiToyoshiba (Takeda Pharmaceuticals Company Limited), Enrico Domenici, Laurent Essioux (F. Hoffman-La Roche Ltd), Lara Mangravite, Mette Peters (Sage Bionetworks), Thomas Lehner, Barbara Lipska (NIMH). The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. We thank Miss Qian Li for her technical support.

Funding

This study was equally supported by the National Nature Science Foundation of China (82171511) (to S.W.L.) and (U2102205 and 31970561) (to X.-J.L.) and the Key Research Project of Yunnan Province (202101AS070055). It was also supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDPB17), the Innovative Research Team of Science and Technology department of Yunnan Province (2019HC004), the Distinguished Young Scientists grant of the Yunnan Province (202001AV070006) (to X.-J.L.), the Western Light Innovative Research Team of Chinese Academy of Sciences (to X.-J.L.) and the Western Light Program of Chinese Academy of Sciences (to J.W.L).

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Mueser KT, McGurk SR. Schizophrenia. *Lancet*. 2004;363(9426):2063–2072.
2. Tandon R, Gaebel W, Barch DM, et al. Definition and description of schizophrenia in the DSM-5. *Schizophr Res*. 2013;150(1):3–10.
3. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: Evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry*. 2003;60(12):1187–1192.
4. Hilker R, Helenius D, Fagerlund B, et al. Heritability of schizophrenia and schizophrenia spectrum based on the Nationwide Danish Twin Register. *Biol Psychiatry*. 2018;83(6):492–498.
5. Ripke S, O'Dushlaine C, Chambert K, et al.; Wellcome Trust Case Control Consortium 2. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat Genet*. 2013;45(10):1150–1159.
6. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*. 2014;511(7510):421–427.
7. Li Z, Chen J, Yu H, et al. Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. *Nat Genet*. 2017;49(11):1576–1583.
8. Pardiñas AF, Holmans P, Pocklington AJ, et al.; CRESTAR Consortium. Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. *Nat Genet*. 2018;50(3):381–389.
9. Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. Genome-wide association study identifies five new schizophrenia loci. *Nat Genet*. 2011;43:969–976.
10. Lam M, Chen C-Y, Li Z, et al. Genetic REsearch on schizophreniA neTwork-China and the Netherlands (GREAT-CN). Comparative genetic architectures of schizophrenia in East Asian and European populations. *Nat Genet*. 2019;51(12):1670–1678.
11. Yue WH, Wang HF, Sun LD, et al. Genome-wide association study identifies a susceptibility locus for schizophrenia in Han Chinese at 11p11.2. *Nat Genet*. 2011;43(12):1228–1231.
12. Shi Y, Li Z, Xu Q, et al. Common variants on 8p12 and 1q24.2 confer risk of schizophrenia. *Nat Genet*. 2011;43(12):1224–1227.

13. Mah W, Won H. The three-dimensional landscape of the genome in human brain tissue unveils regulatory mechanisms leading to schizophrenia risk. *Schizophr Res*. 2020;217:17–25.
14. Won H, de la Torre-Ubieta L, Stein JL, et al. Chromosome conformation elucidates regulatory relationships in developing human brain. *Nature*. 2016;538(7626):523–527.
15. Whitington T, Gao P, Song W, et al. Gene regulatory mechanisms underpinning prostate cancer susceptibility. *Nat Genet*. 2016;48(4):387–397.
16. Huo Y, Li S, Liu J, et al. Functional genomics reveal gene regulatory mechanisms underlying schizophrenia risk. *Nat Commun*. 2019;10(1):670.
17. Li S, Li Y, Li X, et al. Regulatory mechanisms of major depressive disorder risk variants. *Mol Psychiatry*. 2020;25(9):1926–1945.
18. Auton A, Brooks LD, Durbin RM, et al.; 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*. 2015;526(7571):68–74.
19. Fromer M, Roussos P, Sieberts SK, et al. Gene expression elucidates functional impact of polygenic risk for schizophrenia. *Nat Neurosci*. 2016;19(11):1442–1453.
20. Collado-Torres L, Burke EE, Peterson A, et al.; BrainSeq Consortium. Regional heterogeneity in gene expression, regulation, and coherence in the frontal cortex and hippocampus across development and schizophrenia. *Neuron*. 2019;103(2):203–16.e8.
21. Wang D, Liu S, Warrell J, et al. Comprehensive functional genomic resource and integrative model for the human brain. *Science*. 2018;362(6420):eaat8464.
22. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489(7414):57–74.
23. Davis CA, Hitz BC, Sloan CA, et al. The encyclopedia of DNA elements (ENCODE): Data portal update. *Nucl Acids Res*. 2018;46(D1):D794–D801.
24. Arnold M, Raffler J, Pfeufer A, et al. SNiPA: An interactive, genetic variant-centered annotation browser. *Bioinformatics*. 2015;31(8):1334–1336.
25. Gusev A, Ko A, Shi H, et al. Integrative approaches for large-scale transcriptome-wide association studies. *Nat Genet*. 2016;48(3):245–252.
26. Zhang Y, Li S, Li X, et al. Convergent lines of evidence support NOTCH4 as a schizophrenia risk gene. *J Med Genet*. 2021;58(10):666–678.
27. Yang Z, Zhou D, Li H, et al. The genome-wide risk alleles for psychiatric disorders at 3p21.1 show convergent effects on mRNA expression, cognitive function, and mushroom dendritic spine. *Mol Psychiatry*. 2020;25(1):48–66.
28. Miller DE, Patel ZH, Lu X, et al. Screening for functional noncoding genetic variants using electrophoretic mobility shift assay (EMSA) and DNA-affinity precipitation assay (DAPA). *J Vis Exp*. 2016;(114):54093.
29. Hellman LM, Fried MG. Electrophoretic mobility shift assay (EMSA) for detecting protein-nucleic acid interactions. *Nat Protocol*. 2007;2(8):1849–1861.
30. Yang D, Jang I, Choi J, et al. 3DIV: A 3D-genome interaction viewer and database. *Nucl Acids Res*. 2018;46(D1):D52–D57.
31. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. 2002;3(7):Research0034.
32. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*. 2001;25(4):402–408.
33. Glausier JR, Lewis DA. Dendritic spine pathology in schizophrenia. *Neuroscience*. 2013;251:90–107.
34. Garey LJ, Ong WY, Patel TS, et al. Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J Neurol Neurosurg Psychiatry*. 1998;65(4):446–453.
35. Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry*. 2000;57(1):65–73.
36. Srivastava DP, Woolfrey KM, Penzes P. Analysis of dendritic spine morphology in cultured CNS neurons. *J Vis Exp*. 2011;53:e2794.
37. Dumitriu D, Rodriguez A, Morrison J. High-throughput, detailed, cell-specific neuroanatomy of dendritic spines using microinjection and confocal microscopy. *Nat Protocols*. 2011;6(9):1391–1411.
38. Zagrebelsky M, Holz A, Dechant G, et al. The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. *J Neurosci*. 2005;25(43):9989–9999.
39. Langmead B, Wilks C, Antonescu V, et al. Scaling read aligners to hundreds of threads on general-purpose processors. *Bioinformatics*. 2019;35(3):421–432.
40. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012;9(4):357–359.
41. Li H, Handsaker B, Wysoker A, et al.; 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAMtools. *Bioinformatics*. 2009;25(16):2078–2079.
42. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2015;31(2):166–169.
43. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
44. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucl Acids Res*. 2009;37(1):1–13.
45. Sherf B, Navarro SL, Hannah R, Wood K. Dual-luciferase TM reporter assay: An advanced co-reporter technology integrating firefly and renilla luciferase assays. *Promega Notes*. 1996;57:2–9.
46. Götz M, Huttner WB. The cell biology of neurogenesis. *Nat Rev Mol Cell Biol*. 2005;6(10):777–788.
47. Insel TR. Rethinking schizophrenia. *Nature*. 2010;468(7321):187–193.
48. Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry*. 1987;44(7):660–669.
49. Mao Y, Ge X, Frank CL, et al. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. *Cell*. 2009;136(6):1017–1031.
50. Kim JY, Liu CY, Zhang F, et al. Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. *Cell*. 2012;148(5):1051–1064.
51. Kirschenbaum B, Nedergaard M, Preuss A, et al. In vitro neuronal production and differentiation by precursor cells derived from the adult human forebrain. *Cereb Cortex*. 1994;4(6):576–589.
52. Ying Z, Gonzalez-Martinez J, Tilelli C, et al. Expression of neural stem cell surface marker CD133 in balloon cells of human focal cortical dysplasia. *Epilepsia*. 2005;46(11):1716–1723.
53. Pantazopoulos H, Markota M, Jaquet F, et al. Aggrecan and chondroitin-6-sulfate abnormalities in schizophrenia and bipolar disorder: A postmortem study on the amygdala. *Transl Psychiatry*. 2015;5(1):e496.
54. Fan Y, Abrahamsen G, Mills R, et al. Focal adhesion dynamics are altered in schizophrenia. *Biol Psychiatry*. 2013;74(6):418–426.
55. Zheng W, Wang H, Zeng Z, et al. The possible role of the Akt signaling pathway in schizophrenia. *Brain Res*. 2012;1470:145–158.

56. Schrode N, Ho SM, Yamamuro K, et al. Synergistic effects of common schizophrenia risk variants. *Nat Genet*. 2019;51(10): 1475–1485.
57. Chang H, Cai X, Li H-J, et al. Functional genomics identify a regulatory risk variation rs4420550 in the 16p11.2 schizophrenia-associated locus. *Biol Psychiatry*. 2021;89(3):246–255.
58. Kichaev G, Yang WY, Lindstrom S, et al. Integrating functional data to prioritize causal variants in statistical fine-mapping studies. *PLoS Genet*. 2014;10(10):e1004722.
59. Liu J, Li S, Li X, et al. Genome-wide association study followed by trans-ancestry meta-analysis identify 17 new risk loci for schizophrenia. *BMC Med*. 2021;19(1):177.
60. Sharma T, Antonova L. Cognitive function in schizophrenia. Deficits, functional consequences, and future treatment. *Psychiatr Clin North Am*. 2003;26(1):25–40.
61. Kuperberg G, Heckers S. Schizophrenia and cognitive function. *Curr Opin Neurobiol*. 2000;10(2):205–210.
62. Savage JE, Jansen PR, Stringer S, et al. Genome-wide association meta-analysis in 269,867 individuals identifies new genetic and functional links to intelligence. *Nat Genet*. 2018;50(7):912–919.
63. Davies G, Marioni RE, Liewald DC, et al. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N=112 151). *Mol Psychiatry*. 2016;21(6):758–767.
64. Polioudakis D, de la Torre-Ubieta L, Langerman J, et al. A single-cell transcriptomic atlas of human neocortical development during mid-gestation. *Neuron*. 2019;103(5):785–801.e8.
65. Speir ML, Bhaduri A, Markov NS, et al. UCSC Cell Browser: visualize your single-cell data. *Bioinformatics*. 2021;37(23): 4578–4580.
66. Nowakowski TJ, Bhaduri A, Pollen AA, et al. Spatiotemporal gene expression trajectories reveal developmental hierarchies of the human cortex. *Science*. 2017;358(6368):1318–1323.
67. Kang HJ, Kawasawa YI, Cheng F, et al. Spatio-temporal transcriptome of the human brain. *Nature*. 2011;478(7370):483–489.
68. Pletikos M, Sousa AM, Sedmak G, et al. Temporal specification and bilaterality of human neocortical topographic gene expression. *Neuron*. 2014;81(2):321–332.
69. Walker RL, Ramaswami G, Hartl C, et al. Genetic control of expression and splicing in developing human brain informs disease mechanisms. *Cell*. 2019;179(3):750–71.e22.
70. Stahl EA, Breen G, Forstner AJ, et al.; Bipolar Disorder Working Group of the Psychiatric Genomics Consortium. Genome-wide association study identifies 30 loci associated with bipolar disorder. *Nat Genet*. 2019;51(5):793–803.
71. Wray NR, Ripke S, Mattheisen M, et al.; Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nat Genet*. 2018;50(5):668–681.
72. Demontis D, Walters RK, Martin J, et al.; 23andMe Research Team. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. *Nat Genet*. 2019; 51(1):63–75.
73. Noma A, Ishitani R, Kato M, et al. Expanding role of the jumonji C domain as an RNA hydroxylase. *J Biol Chem*. 2010;285(45): 34503–34507.
74. Kato M, Araiso Y, Noma A, et al. Crystal structure of a novel JmjC-domain-containing protein, TYW5, involved in tRNA modification. *Nucl Acids Res*. 2011;39(4):1576–1585.
75. Noma A, Kirino Y, Ikeuchi Y, et al. Biosynthesis of wybutosine, a hyper-modified nucleoside in eukaryotic phenylalanine tRNA. *EMBO J*. 2006;25(10):2142–2154.
76. Key J, Sen NE, Arsović A, et al. Systematic surveys of iron homeostasis mechanisms reveal ferritin superfamily and nucleotide surveillance regulation to be modified by PINK1 absence. *Cells*. 2020;9(10):2229.
77. Saghazadeh A, Mahmoudi M, Shahrokhi S, et al. Trace elements in schizophrenia: A systematic review and meta-analysis of 39 studies (N5151 participants). *Nutr Rev*. 2020;78(4):278–303.
78. Purcell SM, Wray NR, Stone JL, et al.; International Schizophrenia Consortium. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460(7256):748–752.