Supplementary information, Data S1

Materials and Methods

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at Yuanxi Biotech Inc. Guangzhou. Healthy adult cynomolgus monkeys (*Macaca fascicularis*) were housed in cages at Yuanxi Biotech Inc. Guangzhou with Laboratory Animal Care accreditation. All methods including superovulation and surrogation were performed in accordance with the relevant guidelines and regulations.

Cas9/sgRNA injection into one-cell embryos

One-cell embryos were obtained as previously described (Tu et al., *Sci Rep*, 2016). Cas9 plasmid (MLM3613, Addgene) was used to express spCas9 nuclease under the control of the T7 promoter. The p-U6-sgRNA for HEK293 transfection and p-T7-sgRNA for *in vitro* transcription of sgRNA were gifts from Dr. Liangxue Lai at The Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences. Each zygote was injected with *Cas9* mRNA and sgRNAs (200 ng/μl each). The zygotes were cultured in embryo culture medium-9 (HECM-9) containing 15% fetal calf serum (SH30088.02, Hyclone Laboratories) at 6% CO_2 incubator. The pregnancy was diagnosed by ultrasonography at about 30-35 days after embryo transfer.

Off-target analysis

Potential off-target sites (OTS) of sgRNA guided Cas9 endonucleases for the *SHANK3* gene were predicted by a bioinformatics-based search tool (http://www.rgenome.net/cas-offinder/). Twenty OTS with up to 3 mismatches in the sgRNA targeted sequences were retrieved by a base-by-base alignment with the whole *Macaca fascicularis* genome (version: Mfa5.0). All the putative OTSs were PCR amplified from the genomic DNA of mutant offspring brains. The primer pairs used are listed in Supplementary information, Table S1. Hybridized duplex DNA was obtained by denaturation and renaturation in NEBuffer 2 (New England BioLabs) using a thermocycler and then subjected to T7EN1 cleavage assay. The samples with cleavage bands (marked with an asterisk) were subcloned into pEASY®-Blunt cloning vector (TransGen), followed by DNA sequencing. No authentic mutations were found in any clone by sequencing. We may miss some off-target sites, as the program we used has limitations.

Deep amplicon sequencing

Genomic DNA from various tissues of gene edited offspring (PFC, striatum, liver, kidney and gonad for *SHANK3*^{M1} and *SHANK3*^{M2}, blood, hair and placenta for *SHANK3*^{M3}) was used for PCR amplification with site specific primers (Supplementary information, Table S1). PCR products were then added with barcodes (Supplementary information, Table S1) in both ends and mixed in equal amount as a pool for each animal. The samples were purified and used for Illumina sequencing at

Novogene. Reads were analyzed for length changes (indels) by comparing extracted sequence with wild-type sequences. The ratio of reads with a specific indel to the total reads was analyzed. Rare reads (less than 1% of total reads) were filtered out.

Golgi Staining and Synaptic Spine Analysis

Half of the brain was removed from fetus and immediately frozen at −80°C. Brain blocks at a size of 1 cm^3 were first washed in PBS and then immersed into impregnation solution (solution A and B) for 6 weeks in the dark, followed by incubating in solution C for 3 days at room temperature, as described in the standard user manual (FD rapid Golgi Stain kit, FD Neuro Technologies). The brains were then sliced using a Vibratome (VT1000S; Leica) at a thickness of 120 μm for imaging. Images of cortical neurons were taken by bright-field microscopy (ECLIPSE Ni-E; Nikon). To quantify spine density, images of at least 10-15 neurons in the PFC for each brain were taken. The number and length of dendritic spines along a 100 μm dendrite were recorded by an experimenter blinded to the genotype.

Immunostaining and western blot analysis

For immunostaining, the brains of aborted fetus were removed and fixed for 48 h in 4% paraformaldehyde prepared in 1× PBS (Na_2HPO_4 8 mM, NaCl 136 mM, KH_2PO_4 2 mM, KCl 2.6 mM). Different brain regions including prefrontal cortex and striatum were dissected and paraffin embedded. Brain slices of 4 μm thickness were dewaxed in xylene, washed three times in 1× PBS before blocking with 5% goat

serum. The processed slices were then incubated with the primary antibodies at 4 °C overnight. The primary antibodies used in this study were listed in Supplementary information, Table S2. The samples were washed three times in 1× PBS and incubated for 1 h at 37 °C with HRP-conjugated secondary antibody, EnVision™ FLEX/HRP (K8000, ready-to-use, Dako) or fluorophore conjugated secondary antibodies, including Alexa Fluor 488 or 568-conjugated goat anti-mouse or anti-rabbit IgG (1:1000, Molecular Probes). After washing in PBS, 3, 3-diaminobenzidine (DAB) kit (ZLI-9017, ZSGB) was used for chemiluminescent detection. Hematoxylin or T3605 were used for nuclear staining. Images were acquired with a Nikon Eclipse Ci microscope. NeuN-positive and GFAP-positive cells in each cortical layer of the PFC were counted manually in 6 independent slides. The soma size of NeuN-positive neurons was analyzed by ImageJ.

For western analysis, tissues from different brain regions were dissected from aborted fetuses. The brain tissues were homogenized on ice in RIPA buffer (Hua Xing Bo Chuang) with proteinase inhibitor Set I. Protein concentration were examined by Bradford assay (Bio-Rad). Total proteins were separated by an acrylamide gel and then transferred to a PVDF membrane (0.45 μm, EMD Millipore). Membranes were blocked with milk and incubated with specific primary antibodies (Supplementary information, Table S2) at 4 °C overnight. A rabbit polyclonal antibody recognizing 1378-1731 aa of mouse Shank3 protein was generated by Beijing B and M Biotech. The membranes were washed in PBST buffer (0.05% Tween-20) and then incubated with HRP-conjugated secondary antibody (1:25000; Sigma) for 1 h at room

temperature. Specific bands were quantified by ImageJ and normalized to α-tubulin control.

Statistical analysis

Two-tailed Student's *t*-test (unpaired) was used to compare differences between age-matched control and mutant. Statistical analyses were performed with Excel. Values are represented in the text as mean ± SEM.

Accession number

Deep amplicon sequencing raw data were deposited to Sequence Reads Archive (https://submit.ncbi.nlm.nih.gov/subs/sra/) with accession number SRP111790.