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Supplemental Information

Abnormal Neural Progenitor Cells Differentiated from Induced Pluripotent Stem Cells Partially Mimicked Development of *TSC2* Neurological Abnormalities

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Supplemental information

Supplemental Figures and Legends:

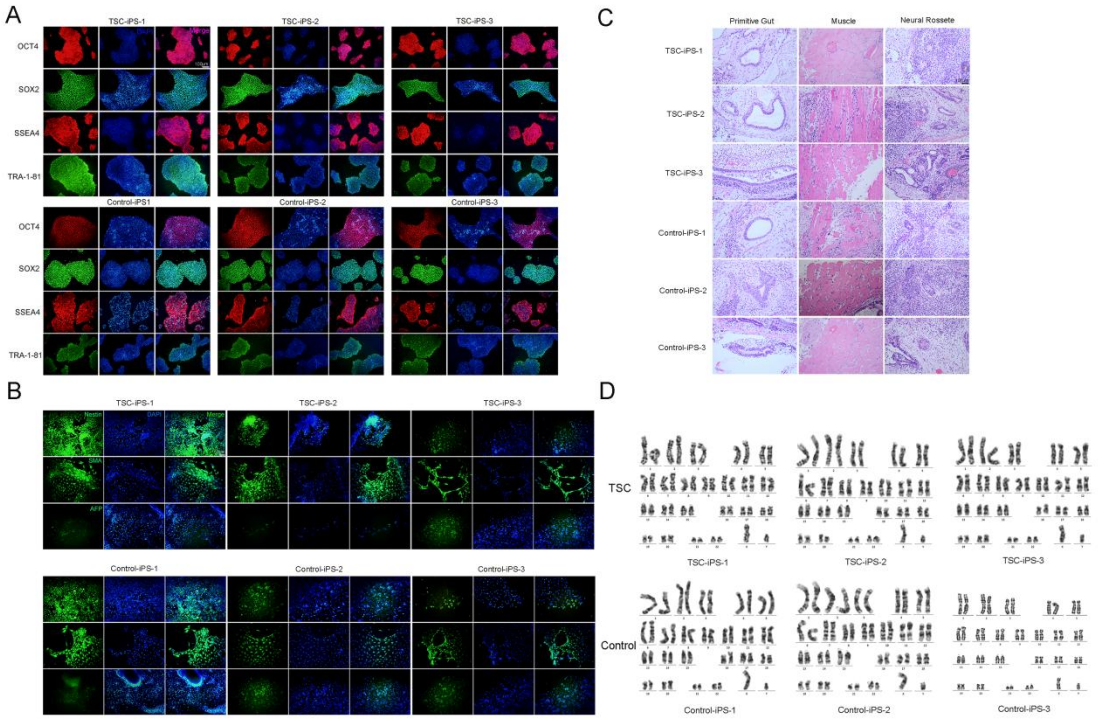

Fig. S1 Identification of induced pluripotent stem cells (iPSCs) from TSC patient and unaffected controls.

(A)iPSCs derived from TSC patient and unaffected controls expressed TRA-1-80, SOX2, SSEA4, and OCT4 as demonstrated by immunofluorescence staining. Scale bar: 100 µm. (B)Immunofluorescence for the endoderm (alpha-fetoprotein, AFP), mesoderm (smooth muscle actin, SMA), and ectoderm (NESTIN) markers in TSC and unaffected iPSC lines. Scale bar: 50 µm. (C) Teratomas of iPSCs derived from TSC patient and unaffected controls showed typical tissues from the three germ layers. Scale bar: 100 µm. (D) Karyotype analysis of iPSC lines derived from TSC patient and unaffected controls showed a normal karyotype. The images of TSC-iPS-1 were the same images of Figure 2C,D,E,F.

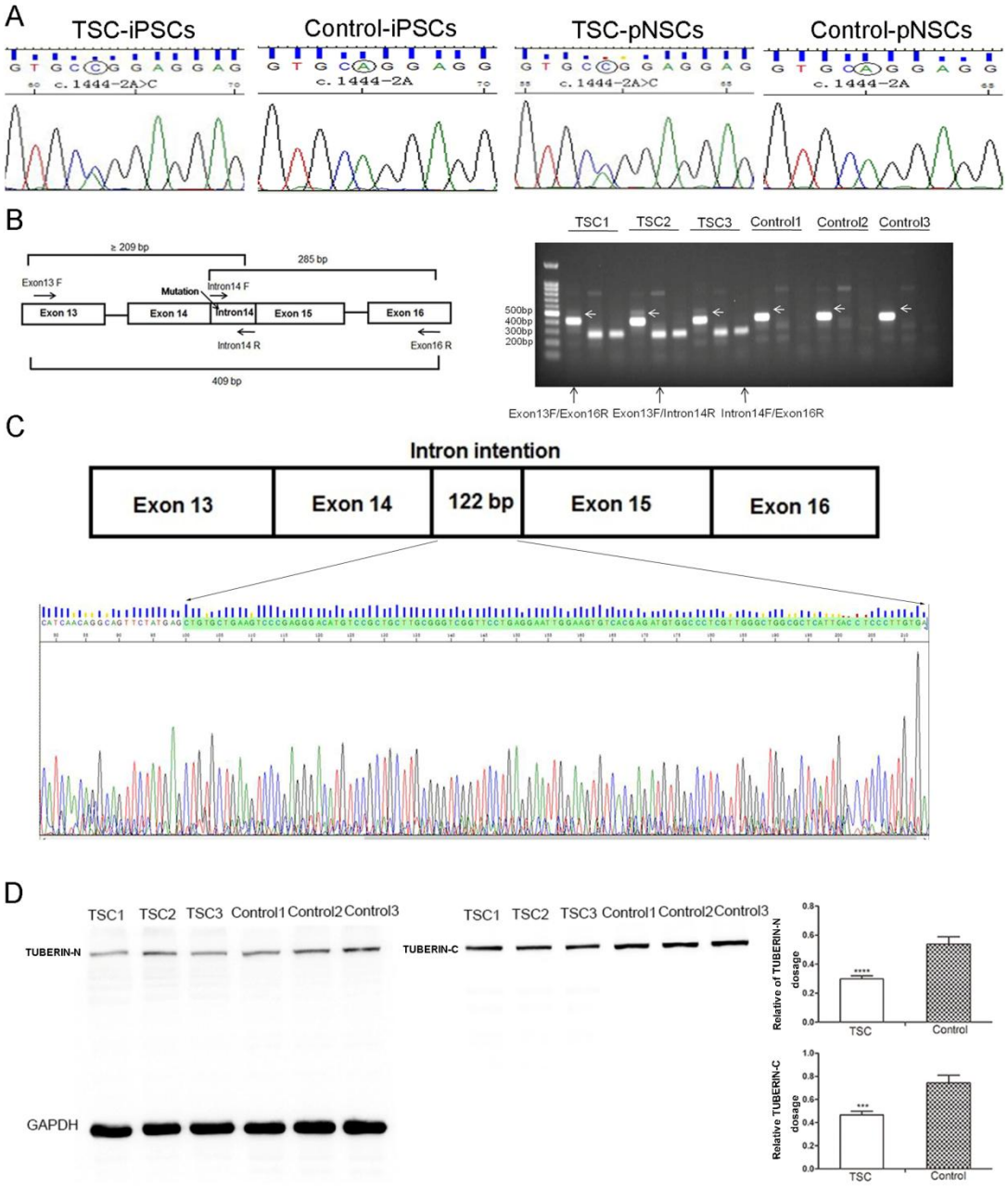

Fig. S2 Detection of *TSC1/TSC2* gene mutation, aberrant mRNA transcripts, and TUBERIN expression in TSC-pNSCs.

(A)*TSC1/TSC2* gene sequencing of a TSC patient and unaffected control-specific iPSCs (TSC1 AND Control1 lines) and pNSCs revealed a c.1444-2A>C mutation in TSC-iPSCs and pNSCs. Unaffected control-iPSCs and pNSCs did not have any detectable mutations. (B) and (C) Primer sets used in this study. The set Exon13F/Exon16 was used to amplify both the classical form and the corresponding splice variant, whereas the sets Exon13F/Intron14R and Intron14F/Exon16R were used for the specific amplification of the splice variants. RT-PCR amplification and cDNA sequence chromatograms obtained by Sanger sequencing of *TSC2* using the primer set Exon13F/Intron14R showed that the *TSC2* mRNA sequence was lengthened by 122 bp in the affected individual compared with that in normal controls. (D) Expression of TUBERIN in TSC-pNSCs and control-pNSCs detected by western blotting. Expression levels were normalized to that of glyceraldehyde-3-phosphate (GAPDH) (n = 5 independent

experiments, mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001; Student's t test).

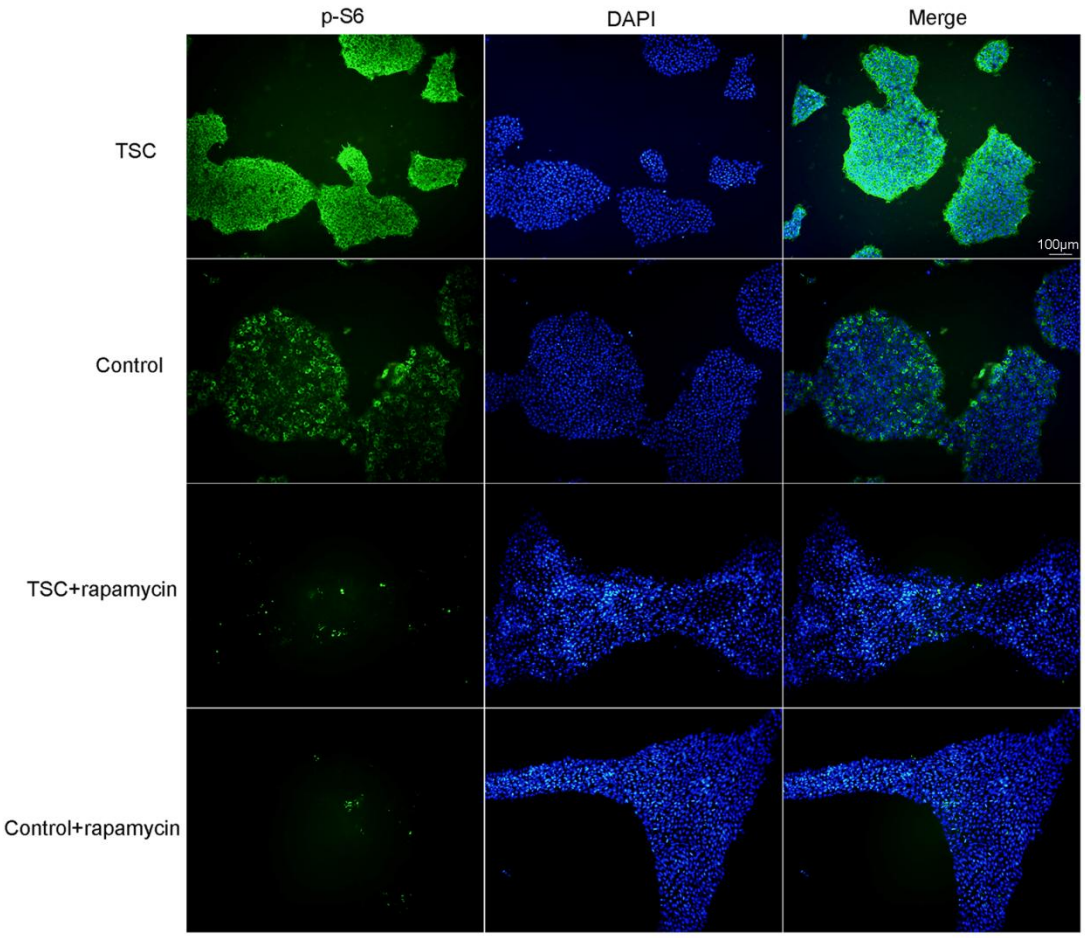

Fig.S3 mTOR pathway activation of TSC, unaffected controls, and rapamycin-treated iPSCs.

TSC-iPSCs had an increased expression of pS6 compared with that of unaffected control-iPSCs. TSC-iPSCs and unaffected control-iPSCs treated with rapamycin did not express pS6. Scale bar: 100 μm.

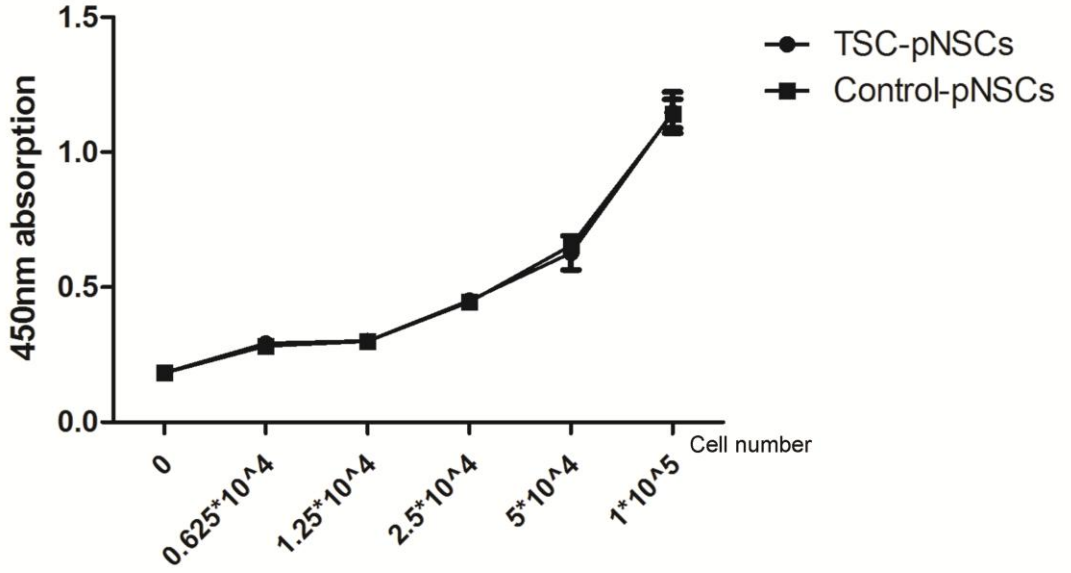

Fig. S4 CCK8 standard curves for TSC and unaffected control pNSCs.

The standard curve of CCK8 for TSC-pNSCs was consistent with that for unaffected control-pNSCs(TSC1 AND Control1 lines).

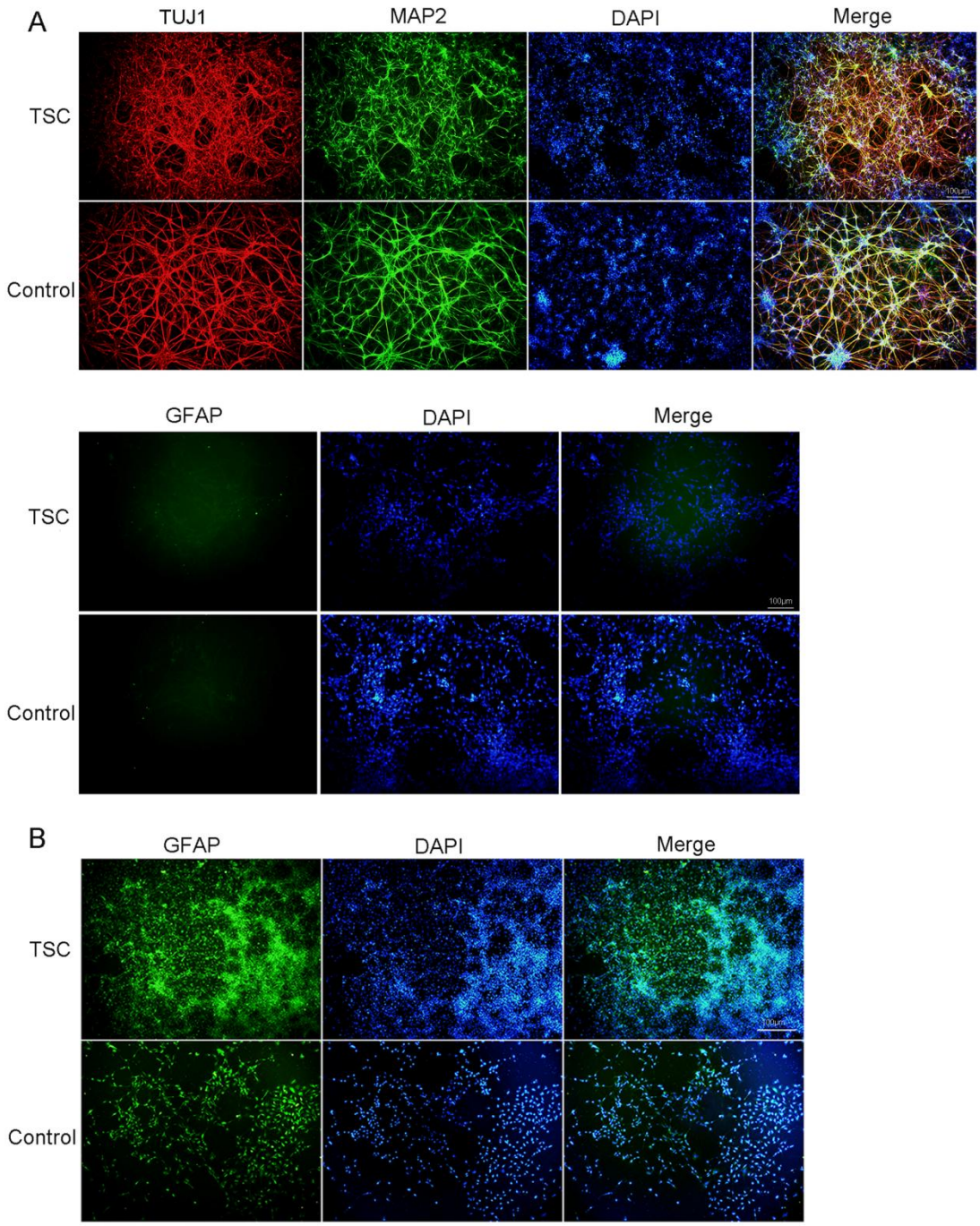

Fig. S5. iPSCs can differentiate into neurons and astrocytes through neural progenitor cells.

(A)Neurons derived from TSC and unaffected control-pNSCs expressed TUJ1 and MAP2, but were devoid of GFAP by immunohistochemistry analysis. (B) Astrocytes derived from TSC and unaffected control-pNSCs expressed GFAP. Scale bar: 100 μm.

Experimental procedures

Mutation analysis

DNA was extracted from the TSC and unaffected control iPSCs and pNSCs (TSC1 and Control1) using a Blood/Cell/Tissue DNA Extraction Kit (Tiangen, Beijing, China). The subsequent procedures are described in the experimental procedures section of the manuscript.

Identification of abnormal TSC2 splicing

Total RNA was extracted from cells using RNAiso (Takara Bio, Otsu, Japan) according to the manufacturer's protocol and cDNA was prepared using PrimeScript™ RT Master Mix (Takara Bio). RT-PCR was performed using Tli RNaseH Plus SYBR® Premix Ex Taq (Takara Bio) and the following primer sets: Set 1: Exon13F: ATAGAGCGCAGTCCATCCAC; Exon 16R: AGGACGGCTGTCTTCACATC; Set 2: Exon13F: ATAGAGCGCAGTCCATCCAC; Intron14R: CACAAGGGAGGCCAATGAG; Set 3: Exon 16R: AGGACGGCTGTCTTCACATC; Intron 14F: CTCATTGGCCTCCCTTGTG. The cDNA sequences with RT-PCR products were verified by Sanger sequencing.

Immunoblotting

Western blotting of TSC patient-specific and unaffected controls' pNSCs was performed as described previously. The primary antibodies used were rabbit anti-Tuberin-N, anti-Tuberin-C, and anti-GAPDH (all from Cell Signaling, cat#3635s, 4308s, 2118s, Danvers, MA, USA). Reactive proteins were visualized using an ImageQuant™ LAS 4000 mini system (GE Healthcare, Little Chalfont, UK).

Immunofluorescence

Immunofluorescence staining of neurons and astrocytes was performed as described in the experimental procedures section of the manuscript. The primary antibodies used were as follows: rabbit anti-β-III-Tubulin (TUJ1; Abcam, cat#ab18207, Cambridge, UK); rabbit anti-MAP2 (Abcam, cat#ab11267, Cambridge, UK), rabbit anti-GFAP (Abcam, cat#ab7260, Cambridge, UK).

CCK8

For CCK8 detection, pNSCs from the TSC patient and unaffected controls were plated in a 96-well plate at a density of 0.625×10^4, 1.25×10^4, 2.5×10^4, 5×10^4, or 1×10^5 cells/100 µL/well in a humidified incubator containing 5% CO_2 for 6 h. Ten microliters of CCK8 was added to the 100 µL of cultured cells. After 2 h of incubation at 37°C in a humidified incubator containing 5% CO_2, the absorbance was detected at a wavelength of 450 nm.