Supporting Information

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SI Materials and Methods

Methylation-Sensitive Restriction Endonuclease Quantitative PCR Assay. The Methyl Profiler enzyme kit and *SNRPN* primer set (SA biosciences) were used according to the manufacturer's instructions to assay methylation at the PWS-IC. Genomic DNAs were digested overnight with methyl-sensitive, methyl-dependent, or both enzyme mixes. A mock-digested sample was set up in parallel. Enzymes were inactivated, and the digested samples were used to seed quantitative PCR (qPCR) reactions using Sybr green MasterMix (Applied Biosystems) and *SNRPN* methyl profiler primers. Changes in critical threshold (ΔCT) values obtained from the qPCR reaction were analyzed using the methyl profiler data analysis template and reported as percent methylation plus or minus the SD of three replicates. No intermediate methylation was observed in any of the fibroblast or iPSC lines with this assay. The *SNRPN* primer set assays seven different CpG sites in the PWS-IC.

qRT-PCR. RNA extraction was performed using RNAzol (Tel-test, Inc.) according to the manufacturer's instructions. First-strand cDNA was generated using SuperScript II (Invitrogen) according to the manufacturer's instructions. qRT-PCR was performed in triplicate on three independently cultured iPSC isolates and three independently established iPSC-derived neuron cultures using inventoried gene expression assays for exons 9 and 10 of *UBE3A* according to the manufacturer's instructions (TaqMan GeX assay; Applied Biosystems). A predesigned assay for *GAPDH* (Applied Biosystems) was used as an endogenous control. Fold change was calculated as $-2^{\Delta\Delta CT}$.

For measurement of pluripotency gene and lineage marker expression, RNA was extracted from pools of three separate iPSC cultures and three separate 14-d EB cultures representative of each iPSC line, as described above. First-strand cDNA was generated as described above and subjected to qRT-PCR using the TaqMan Human Stem Cell Pluripotency Array (Applied Biosystems). CT values were normalized to those of 18S rRNA using RQ manager software (Applied Biosystems), generating a ΔCT value for each gene on the array, which indicates the relative expression levels. Expression levels as a percentage of *GAPDH* expression are reported.

Antibodies. Antibodies used include the following. The nuclear markers used were rabbit anti-NANOG (1:100; Abcam) and mouse anti-PAX6 (1:20; Developmental Studies Hybridoma Bank, developed under the auspices of the National Institute of Child Health and Human Development and maintained by the Department of Biology, University of Iowa). The cytoplasmic markers used were mouse anti-UBE3A (1:500; Becton Dickinson), rabbit anti-MAP2 (1:200; Millipore), mouse anti-TUJ1 (1:200; Covance), rabbit anti-Synapsin I (1:200; Millipore), mouse anti-PanNav (Pan-sodium channel, 1:200; Sigma), rabbit anti-S100β (1:200; Abcam), and rabbit anti-GAD65/67 (1:2,000; Sigma). The cell surface markers used were mouse anti-SSEA4 (1:20; Developmental Studies Hybridoma Bank), mouse anti-TRA1-60 (1:200; Santa Cruz), and mouse anti-TRA1-81 (1:200; Santa Cruz). The secondary antibodies used were AlexaFluor goat anti-rabbit 488 and 594 and AlexaFluor goat anti-mouse 488 and 594, which were all used at a concentration of 1:200 and obtained from Molecular Probes.

Northern and Southern Blot Analysis. Northern blot analysis of snoRNAs was carried out as described previously (1). Oligonucleotides complementary to *SNORD116* (5′-AGAGTTTTCACTCATTTTGTTCAGC-3′), *SNORD115* (5′-CCTCAGCGTAATCCTATTGAGCATGA-3′), and 5.8SrRNA (5′-TCCTGCAATTCACATTAATTCTCGCAGCTAGC-3′) were end-labeled with γ-^{32}P ATP using T4 Polynucleotide kinase (New England Biolabs). Blots were generated by loading 10 mg of total RNA from iPSCs and iPSC-derived neuron cultures onto an 8% (vol/vol) denaturing urea-polyacrylamide gel (Sequagel; National Diagnostics) run in 0.5× TBE (Tris, Borate, EDTA buffer).

Southern blot analysis of genomic DNAs was performed using standard protocols. DNA was cut with EcoRI, EcoRI plus HpaII, EcoRI plus SacII, or EcoRI plus NotI. The probe used was a 1.05-kb PCR fragment adjacent to the CpG island at the 5′-end of *UBE3A* that has been published previously (2). The probe was labeled using α-^{32}P dCTP and the RadPrime labeling kit (Invitrogen). The blot was hybridized using ExpressHyb (Clontech) and washed using standard methods. This combination of digests and probe detects methylation at three different CpG residues at the 5′-end of *UBE3A*.

1. Cavaillé J, et al. (2000) Identification of brain-specific and imprinted small nucleolar RNA genes exhibiting an unusual genomic organization. *Proc Natl Acad Sci USA* 97: 14311–14316.

2. Jiang YH, et al. (2004) A mixed epigenetic/genetic model for oligogenic inheritance of autism with a limited role for UBE3A. *Am J Med Genet A* 131:1–10.

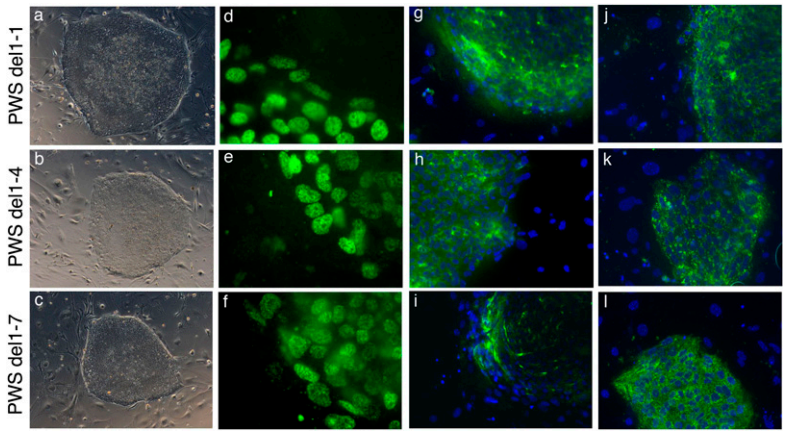


Fig. S1. Marker analysis of PWS iPSCs. Phase-contrast images of PWS iPSCs show hESC-like morphology (*a–c*). Immunocytochemistry for pluripotency markers on representative iPSC lines shows expression of NANOG (*d–f*), TRA1-81 (*g–i*), and SSEA4 (*j–l*).

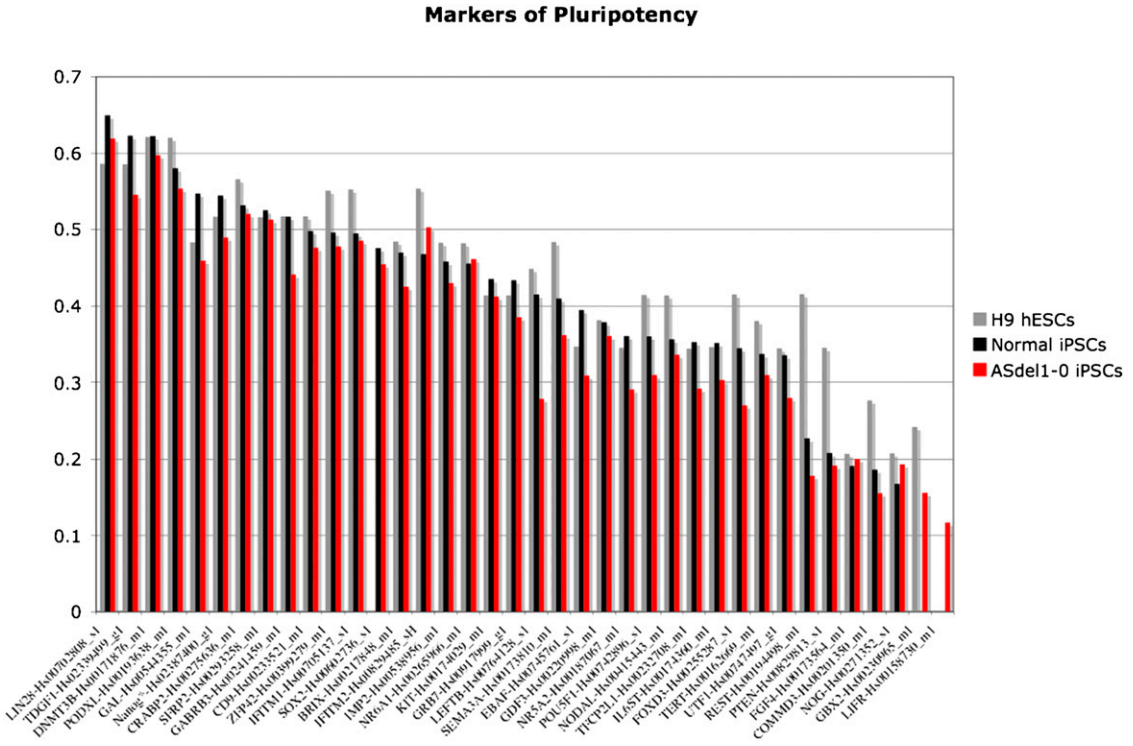


Fig. S2. AS and normal iPSCs express genes associated with pluripotency at levels similar to those observed in hESCs. qRT-PCR was used to quantify gene expression from transcripts associated with the pluripotent state in AS and normal iPSCs. Expression levels were compared with those obtained from H9 (WA09) hESCs. Expression levels relative to *GAPDH* are shown. Human stem cell pluripotency arrays were used to perform the qRT-PCR assay.

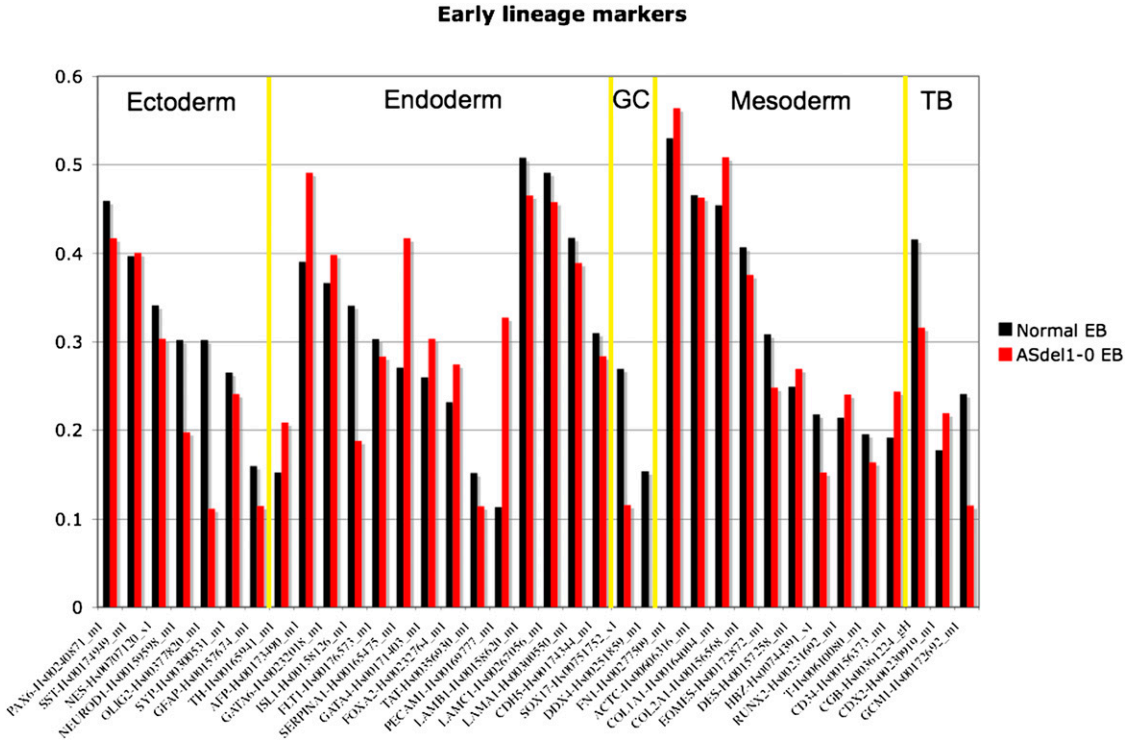
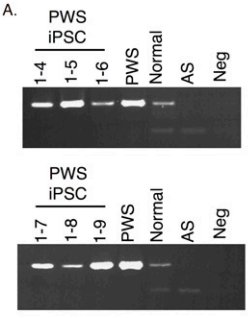


Fig. S3. Fourteen-day-old EBs derived from AS and normal iPSCs express lineage markers. qRT-PCR was used to quantify gene expression from lineage markers representative of the endoderm, ectoderm, and mesoderm germ layers as well as from markers representing the trophoblast (TB) and germ cell (GC) lineages. Expression levels relative to *GAPDH* are shown. Human stem cell pluripotency arrays were used to perform the qRT-PCR assay.



B.

Cell line	Percent methylation
Normal fibroblast	58.77±1.49
Normal iPS 1-0	60.06±2.41
Normal iPS 1-1	50.00±0.00
AS 1-0 fibroblast	0.21 ±0.08
AS iPS del 1-0	0.17±0.14
AS iPS del 1-1	1.10±0.85
AS iPS del 2-0	0.33±0.17
AS iPS del 2-3	0.38±0.32
PWS fibroblast	95.17±3.53
PWS iPS del 1-1	96.92 ±0.50
PWS iPS del 1-2	97.68 ±0.88
PWS iPS del 1-3	99.66 ±0.57
PWS iPS del 1-4	98.45 ±0.44
PWS iPS del 1-5	98.49 ±1.36
PWS iPS del 1-6	98.34 ±0.76
PWS iPS del 1-7	99.03±1.24
PWS iPS del 1-8	98.81±1.03
PWS iPS del 1-9	95.15 ±0.82

Fig. S4. Methylation analysis of PWS-IC in AS, normal, and PWS iPSC lines. (*A*) Methylation-specific PCR analysis of genomic DNA from six additional PWS iPSC lines. Lines 1-1 through 1-3 are shown in Fig. 2. As in Fig. 2, primers specific for the methylated allele amplify a band that is 174 bp, whereas primers specific for the unmethylated allele amplify a 100-bp product. Neg, negative. (*B*) Methyl profiler assay was used to quantify DNA methylation at the PWS-IC in normal, AS, and PWS iPSC lines. Data are expressed as percent methylation plus or minus the SD, which is the average and SD from three replicates.

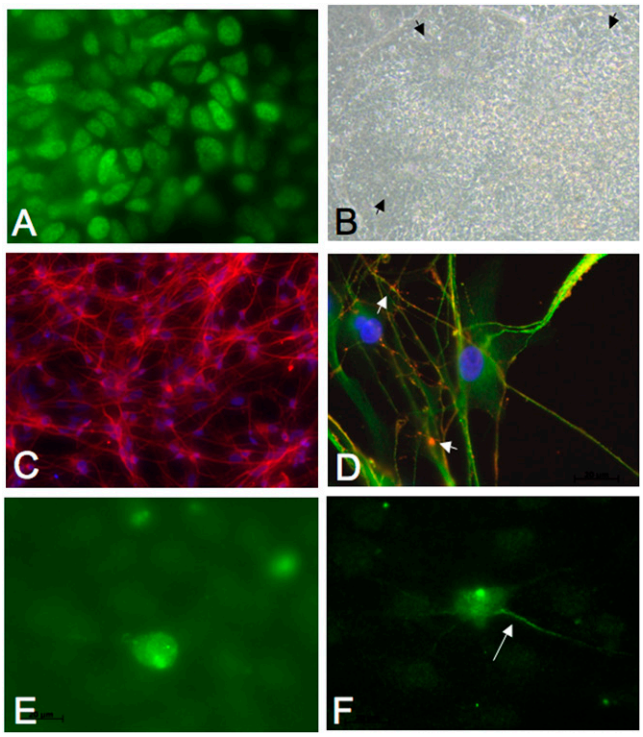


Fig. S5. In vitro maturation of AS iPSC-derived neurons. Differentiating iPSC-derived neural epithelial cells express PAX6 (*A*) and form neural rosettes (*B*, arrows). iPSC-derived neurons express MAP2 (*C*, in red) and show Synapsin I (SYN1)-positive synaptic puncta (*D*, in red, arrows) after 6 wk of development. SYN1-positive neurons are counterstained with an antibody against βIII-TUBULIN (green). DAPI is used to visualize the nuclei and is shown in blue in both images. Ten-week-old iPSC-derived neuron cultures have both immature and mature neurons. Immunocytochemistry for PanNav, an antibody that recognizes voltage-gated sodium channels, shows lack of localization in the axonal initial segment (AIS) in most neurons (*E*) but proper mature localization in the AIS in some neurons (*F*). The arrow in *F* points to proper localization of sodium channels at the AIS.

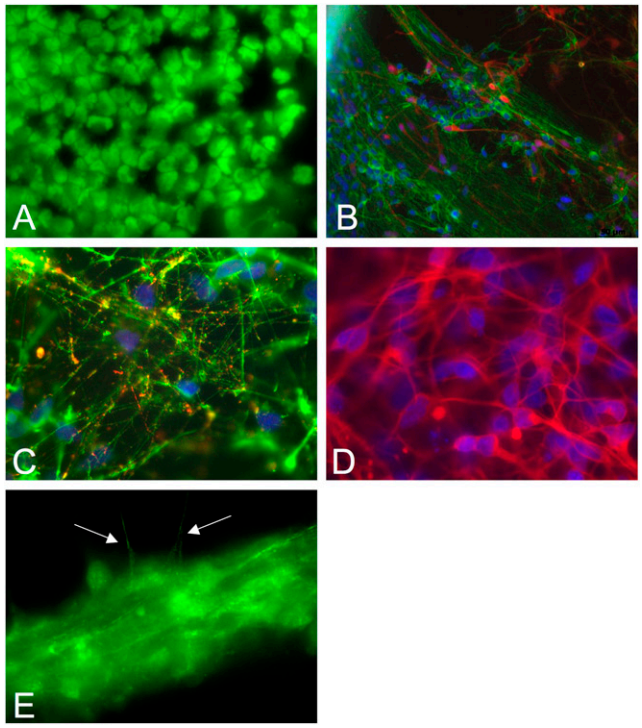


Fig. S6. In vitro maturation of normal iPSC-derived neurons. (*A*) Differentiating iPSC-derived neural epithelial cells express PAX6 after 2 wk of in vitro differentiation. (*B*) Astrocytes that stain positively with an antibody against S100β were observed after 6 wk of differentiation. βIII-TUBULIN-positive neurons are shown in green, and DAPI is used to visualize the nuclei. (*C*) Normal iPSC-derived neurons also show Synapsin I (SYN1)-positive synaptic puncta (in red) after 6 wk of development. SYN1-positive neurons are counterstained with an antibody against βIII-TUBULIN (green). DAPI is used to visualize the nuclei. (*D*) Ten-week-old iPSCs stain positively with an antibody against MAP2. (*E*) Ten-week-old iPSC-derived neuron cultures have both immature and mature neurons. Immunocytochemistry for PanNav, an antibody that recognizes voltage-gated sodium channels, shows lack of localization in the axonal initial segment (AIS) in most neurons but proper mature localization in the AIS in some neurons (arrows).

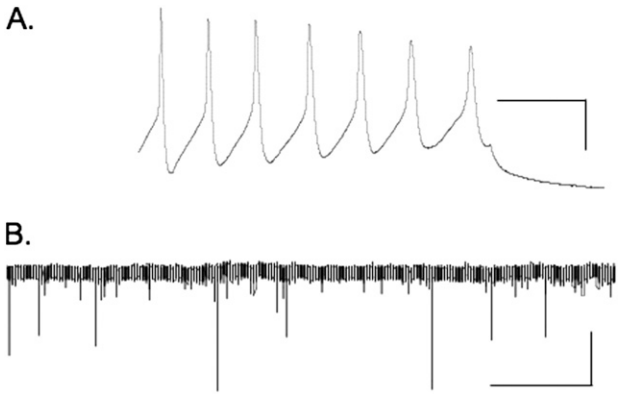


Fig. S7. Electrophysiological recording from a mature normal iPSC-derived neuron. (*A*) Train of action potentials evoked by 50-pA depolarizing current injection in a 10-wk-old normal iPSC-derived neuron. (Calibration bars: 20 mV, 0.1 s.) (*B*) Spontaneous EPSCs recorded from the same neuron. (Calibration bars: 10 pA, 10 s.)

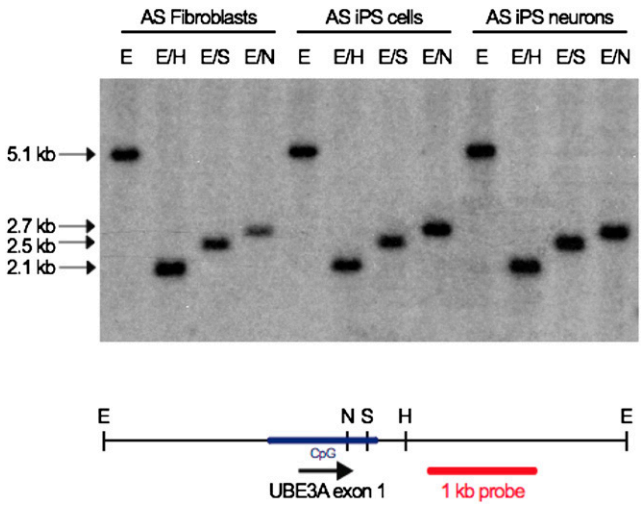


Fig. S8. CpG island at the *UBE3A* promoter remains unmethylated during reprogramming and during neural differentiation. Southern blot analysis was used to assay genomic DNA from AS fibroblasts, iPSCs, and iPSC-derived neurons digested with *Eco*RI (E) and *Eco*RI along with methylation-sensitive restriction enzymes *Hpa*II (E/H), *Sac*II (E/S), and *Not*I (E/N). The 5-kb *Eco*RI fragment is completely digested to the 2.1-, 2.5-, and 2.7-kb digestion products in the E/H, E/S, and E/N lanes, respectively, indicating that the *UBE3A* promoter is completely unmethylated in all cell types.