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Supplementary Table 1. Clinical data of patient-derived iPSC lines used in this study.

ID	Race/ Ethnicity	Scan Age, years	Total Brain Volume	ADOS[b] or PL-ADOS Age[c]	ADOS Total Score or PL-ADOS P/F[d]	Age at Early IQ[e]	Early IQ Non Verbal Score	Age at Biopsy	ADOS[c] Total Score at Biopsy	IQ[e] at Biopsy	Vineland ABC[f] at Biopsy	iPS lines	condition
T3T3G	Caucasian Hispanic	2.52	1271	2.45^{1}	19	2.45^{M}	86	5	16^{1}	75^{WP}	62	Able 3-12	ASD
												Able 3-6	ASD
T7D4N	Caucasian	4.5	1458	4.06^{PL}	F	4.07^{L}	68	18	13^{3}	50^{WA}	60	Acai 1-1	ASD
												Acai 3-2	ASD
Q7N2P	Caucasian	3.2	1338	2.82^{PL}	F	4.84^{L}	108	16	20^{3}	49^{WI}	58	Aero 3-13	ASD
												Aero 3-14	ASD
Z8H4V	Caucasian	2.98	1374	6.41^{1}	19	6.24^{L}	49	20	20^{4}	53^{WA}	34	Ahoy 5-13	ASD
												Ahoy 5-19	ASD
P6G8M	Caucasian Hispanic	3.62	1515	3.67^{2}	21	3.67^{L}	100	16	18^{4}	78^{WI}	71	Apex 5-15	ASD
												Apex 5-4	ASD
B6P8N	Caucasian Hispanic	4.05	1409	3.77^{PL}	F	5.68^{L}	106	16	13^{3}	84^{WI}	71	Aqua 3-9	ASD
												Aqua 3-3	ASD
N4H6M	Caucasian	3.41	1257	3.4^{1}	21	3.4^{M}	71	7	18^{1}	71^{WA}	67	Arch 1-1	ASD
												Arch 1-2	ASD
X6D9M	Caucasian/ African American	4.21	1361	4.23^{2}	17	4.23^{M}	65	8	16^{3}	83^{WI}	34	Avid 5-20	ASD
												Avid 5-5	ASD
T2V2V	Caucasian	3.58	1389	n/a	n/a	3.48^{M}	128	9	n/a	111^{WI}	101	Cent 3-3	control
												Cent 3-6	control
Q5E3S	Caucasian	2.57	1184	n/a	n/a	4.76^{P}	n/a	17	n/a	116^{WA}	90	Chap 1-2	control
												Chap 1-5	control
M5W3M	Caucasian	2.70	1182	n/a	n/a	2.8^{M}	109	8	n/a	130^{WI}	102	Clay 1-2	control
												Clay 1-3	control
K854F	Caucasian	3.41	1206	n/a	n/a	3.43^{P}	n/a	17	n/a	125^{WA}	106	Clue 4-7	control
K5C3V	Caucasian	2.43	1225	n/a	n/a	2.44^{M}	117	6	n/a	111^{WI}	96	Cove 3-1	control
												Cove 3-7	control

[a]All cases were male. ASD cases met or exceeded cutoffs for a diagnostic classification of ASD on the ADOS/PL-ADOS and DSM-IV.

[b]Abbreviations: ADOS, Autism Diagostic Observation Schedule; PL-ADOS, Pre-Linguistic Autism Diagnostic Observation Schedule

[c]ADOS Module denoted in superscript: *PL*, PL-ADOS; *1*, Pre-verbal/Single Words; *2*, Phrase Speech; *3*, Fluent Speech - Child/Adolescent; *4*, Fluent Speech - Adolescent/Adult.

[d]ADOS Social + Communication Total (diagnostic cutoff = 7 for *autism spectrum*, 10 to 12 for *autism* depending on Module administered.

[e]IQ Measures denoted in superscript: L = Leiter International Performance Scale; M = Mullen Scales of Early Learning; P = Peabody Picture Vocabulary Scale; WP = Wechsler Primary and Preschool Scale of Intelligence; WI = Wechsler Intelligence Scale for Children; WA = Wechsler Abbreviated Scale of Intelligence.

[f]Vineland Adaptive Behavior Scales and Vineland Adaptive Behavior Scales, Second Edition, Adaptive Behavior Composite (SS=100, SD=15).

Supplementary Methods

Electrophysiological recordings. NSC-derived neurons were matured on coverslips coated with poly-ornithine (10 µg/ml, Life Technologies) and laminin (5 µg/ml, Life Technologies) for the indicated time. Electrophysiological recordings were performed at room temperature. Neurons on coverslips were transferred to a recording chamber in standard recording medium, containing (in mM) 10 HEPES, 4 KCl, 2 CaCl2, 1 MgCl2, 139 NaCl, 10 D-glucose (310 mOsm, pH 7.4). The recording micropipette (tip resistance 10–15MΩ) was filled with intracellular solution composed of (in mM) 130 K-gluconate, 6 KCl, 4 NaCl, 10 Na-HEPES, 0.2 K-EGTA, 0.3 GTP, 2 Mg-ATP, 0.2 cAMP, 10 Dglucose, 0.15% biocytin and 0.06% rhodamine. The pH and osmolarity of the internal solution were brought close to physiological conditions (pH 7.3, 290–300 mOsmol). Signals were amplified with a Multiclamp700B amplifier and recorded with Clampex 10.2 software (Axon Instruments). Data were acquired at a sampling rate of 20 kHz and analyzed using Clampfit-10 and the software package Matlab (2014b, The MathWorks Inc., Natick, MA, 2000). The sodium and potassium currents were acquired in voltage clamp mode. Cells were held at −60 mV, and voltage steps of 400 ms were made in the range of −100 mV to 80 mV. Currents were generally normalized by the cell capacitance. For current clamp recordings, cells were typically held in current clamp mode near -60 mV with a steady holding current, and current injections were given starting 12 pA below the steady holding current, in 3-pA steps 400 ms in duration.

Immunofluorescence staining and analysis of cerebral organoids

Residual Matrigel was manually removed from organoids, and three washes with PBS were performed using transwell inserts. Organoids were then fixed in 4% PFA in 0.1 M Phopshate buffer (PB pH 7.2-7.5) for 45 - 60 minutes at 4°C and washed three times in PBS, cryoprotected in 30% sucrose and embedded in TFM (tissue freezing medium; GeneralData) for subsequent cryosectioning. Twenty-µm sections were cut on a microtome, mounted on Superfrost plus slides (Thermo Scientific, Menzel-Glaser), dried at 37ºC and subjected to immunofluorescence staining. For immunofluorescence, sections were rehydrated, rinsed in PBS for 5 min, permeabilized in 0.25% Triton X-100 in PBS, and blocked in Blocking solution (3% normal donkey serum in PBST) in a humidified chamber for 1 hr at room temperature (RT). Slides were then incubated with the appropriate primary antibodies diluted in blocking solution at 4°C overnight. The next day, sections were washed three times (five min each) in PBST, incubated with appropriate fluorophore-conjugated secondary antibodies diluted in blocking solution at RT for 2 hrs, washed once in PBS, counter stained with DAPI for 10 min, rinsed twice in PBS and mounted with Shandon Immu-Mount (Thermo Scientific, 9990412). The antibodies used are listed in the following ‘Antibodies’ section.

For the assessment of cortical plate (CP)-like regions, cerebral organoids were immunostained for SOX2 and doublecortin (DCX) at day 35 and images were acquired using a confocal microscope system (Zeiss LSM780). Ventricular zone (VZ)-like regions were defined by SOX2 immunoreactivity and the outer layer was defined as the area from outside the VZ to the nearest pial surface. For each ventricular structure, three to four sets of cell quantifications were performed within the boundaries of a right angle fan area pointing to the nearest pial surface, segmented into subfields of 22.5° (Extended Data Fig. 6g). Cell numbers were plotted as percentage of DCX^+ cells per $DAPI^+$ as well as the relative ratio between DCX^+ and $SOX2^+$ cells. Two to three organoid replicates with 2-3 ‘VZ-like’ regions per cerebral organoid were quantified for every patient line using three ASD and three control individuals (n_{total}= 15 organoids or 43 ‘VZ-like’ regions; see Supplementary Table 7 for details).

Antibodies. All antibodies used in this study were commercially available antibodies. FACS and flow cytometry were performed with the following antibodies: APC-conjugated PSA-NCAM (Miltenyi Biotec, cat.no. 130-093-273, 1:50) and APC-conjugated IgM Isotype control (Miltenyi Biotec, cat.no. 130-093-176, 1:50). Validations and references at https://www.miltenyibiotec.com/US-en/products/macs-flow-cytometry/antibodies/primary-antibodies/anti-psa-ncam-antibodies-human-mouse-rat-2-2b-1-11.html#apc:for-100-tests.
All antibodies supplied in the BD Stemflow™ Human Neural Cell Sorting Kit (BD Biosciences, cat.no. 562271, 1:20). Validations and references at http://www.bdbiosciences.com/us/applications/research/stem-cell-research/stem-cell-kits-and-cocktails/human/human-neural-cell-sorting-kit/p/562271. FBXO2 (abcam, cat.no. ab133717, 1:100). Validations at https://www.abcam.com/fbxo2-antibody-epr73282-ab133717-references.html. 647-conjugated SOX2 (BD Biosciences, cat.no. 562139, 1:25) and 647-conjugated IgG1, k Isotype Control (BD Biosciences, cat.no. 562139, 1:25). Validations and references at https://www.bdbiosciences.com/eu/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/alexa-fluor-647-mouse-anti-sox2-o30-678/p/562139. 488-conjugated SSEA-4 (BioLegend, cat.no. 330411, 1:20) and 488-conjugated IgG3, k Isotype Control (BioLegend, cat.no. 401323, 1:25). Validations and references at https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-ssea-4-antibody-4825. 647-conjugated TRA1-81 (BioLegend, cat.no. 330705, 1:20) and 647-conjugated IgM, k Isotype Control (BioLegend, cat.no. 401618, 1:200). Validations and references at https://www.biolegend.com/fr-ch/products/alexa-fluor-647-anti-human-tra-1-81-antibody-4827.

The following antibodies were used for immunofluorescence: MAP2AB (Abcam, cat.non. ab5392, RRID: AB_2138153, 1:500), Validations and references at https://www.abcam.com/map2-antibody-ab5392.html. PSA-NCAM (EMD Millipore, cat.non. MAB5324, RRID: AB_95211, 1:500). Validations and references at http://www.emdmillipore.com/US/en/product/Anti-Polysialic-Acid-NCAM-Antibody-clone-2-2B,MM_NF-MAB5324. Synapsin I (EMD Millipore, cat.non. 574778, RRID: AB_2200121, 1:200). Validation and references at http://www.emdmillipore.com/US/en/product/Anti-Synapsin-I-Rabbit-pAb,EMD_BIO-574778.
Mouse anti-TUJ1/TUBB3 (BioLegend, cat.non. 801201, RRID: AB_2313773, 1:1000). Validations and references at https://www.biolegend.com/fr-ch/products/purified-anti-tubulin-beta-3-tubb3-antibody-11580. Rabbit anti-TUJ1 (BioLegend, cat.no. 802001, RRID: AB_2564645, 1:2000). Validations and references at https://www.biolegend.com/fr-ch/products/purified-anti-tubulin-beta-3-tubb3-antibody-11579. VGLUT1 (Synaptic Systems, cat.no. 135302, RRID: AB_887877, 1:1000). Validations and references at https://www.sysy.com/products/vglut1/facts-135302.php. Chicken anti-GFP (Aves Labs, cat.no. GFP-1020, RRID: AB_10000240, 1:1000). Validations and references at http://www.aveslab.com/products/epitope-tag-and-gfp-antibodies/anti-gfp-green-fluorescent-protein-antibodies-2/. Rabbit anti-GFP (Thermo Fisher Scientific, cat.no. A-6455, RRID: AB_221570, 1:1000). Validations and references at https://www.thermofisher.com/antibody/product/GFP-Tag-Antibody-Polyclonal/A-6455. SMI312 (BioLegend, cat.no. 837904, RRID: AB_2566782, 1:1000). Validations and references at https://www.biolegend.com/en-us/products/purified-anti-neurofilament-marker-pan-axonal-cocktail-12811. DCX (Santa Cruz, cat.no. sc-8066, RRID: AB_2088494, 1:500). Validations and references at https://www.scbt.com/scbt/product/doublecortin-antibody-c-18. FOXG1 (Abcam, cat.no. ab18259, RRID: AB_732415, 1:500). Validations and references at https://www.abcam.com/foxg1-antibody-chip-grade-ab18259-references.html. PAX6 (BioLegend, cat.no.901301, RRID: AB_2565003, 1:400). Validations and references at

https://www.biolegend.com/en-us/products/purified-anti-pax-6-antibody-11511. NESTIN (MilliporeSigma, cat.no. MAB5326, RRID: AB_2251134, 1:500). Validations and references at http://www.emdmillipore.com/US/en/product/Anti-Nestin-Antibody-clone-10C2,MM_NF-MAB5326. TBR1 (Abcam, cat.no. ab31940, RRID:AB_2200219, 1:1000). Validations and references at https://www.abcam.com/tbr1-antibody-ab31940.html. SOX2 (Cell Signaling, cat.no. 3579, RRID: AB_2195767, 1:500). Validations and references at https://www.cellsignal.com/products/primary-antibodies/sox2-d6d9-xp-rabbit-mab/3579. BrdU (Accurate Chemical, cat.no. OBT0030, RRID: AB_2313756, 1:250). Validations and references at http://antibodyregistry.org/search.php?q=AB_2313756. PHF1 (generously provided by Dr. Peter Davies, RRID: AB_2315150, 1: 250) is specific towards phosphorylated human tau (hTau) and was used as published previously[1].

Comparative gene network analysis. To contrast the gene networks of NSC-derived neurons (65 time series samples) with the RNA-seq timeline of iPSC-iNs (52 time series samples), we first matched Gene IDs between the two sets and used the normalized expression values described above. We next constructed the adjacency matrix, calculated the topological overlap and used the Dynamic Hybrid Tree Cut algorithm to define gene modules using the parameters described in the methods section for each network independently. To obtain moderately large and distinct modules for the iPSC-iN network, we used the following parameters: deepSplit=0 and minClusterSize=30. Gene set enrichment analysis was performed using a hypergeometric test and the GeneOverlap package (bioconductor.org) was used to plot the results. In addition, the alternative WGCNA cluster dendrogram for the NSC-N network (Extended Fig. 4d) was constructed by excluding lowly expressed genes, resulting in 17,082 genes for network construction, in order to test robustness of the initial network assignment in Fig. 1a. The adjacency matrix was then constructed by raising the co-expression measure (0.5 + 0.5 X correlation matrix) to the power of $\beta = 12$. Using the Hybrid Adaptive Tree Cut algorithm[2], we defined modules as branches resulting from the tree cutting of the hierarchical clustering tree using the following parameters: cutHeight=0.99, deepSplit=1 and minClusterSize=30. Preservation statistics and permutation Z statistics were computed using the function 'modulePreservation'[3] implemented in the WGCNA package. Both network preservation and quality statistics were calculated for the NSC-N network in Figure 1a considering the alternative network in Extended Data Fig. 4d as test network.

Enrichment of peaks in genomic regions and elements. Enrichment of DA peaks within epigenetically annotated regions or TM-related regions was calculated using the Genomic Association Test (GAT)[4], which estimates the significance of overlap between multiple sets of genomic intervals. GAT computes the overlap between intervals $Iseg_{1..n}$ and $Iannot_{1..n}$ within the genomic workspace W by using the number of nucleotides overlapping: $|Iseg_{1..n} \cap Iannot_{1..n} \cap W|$. A subsequent step creates randomized intervals in the genome with the same size distribution of $Iseg_{1..n}$ ($n_{simulations}$=10,000). The overlap between each simulated set and the annotation intervals is recorded as expected overlap. The fold enrichment is then calculated as the ratio of observed and expected overlap and an empirical P-value is associated with it. The Benjamini-Hochberg method was further used for multiple testing correction to control the false discovery rate (FDR). To test for DA peak enrichment in gene-distal regions, we generated windows encompassing 50kbp upstream and downstream of RefSeq annotated transcription start sites (TSS) from TM1-

3 genes. Any DA peak that was annotated by HOMER as a promoter (-1000bp, +100bp) was discarded from the analysis, as described previously[5]. The remaining DA peak intervals were then used to estimate whether the observed overlap between DA peaks and gene-distal regions of TM genes was larger or smaller than expected by chance.

Chromatin state definitions from the imputed ENCODE 25-state model were acquired from human embryonic stem cell-derived neuronal progenitors (E009) for hg38 tracks and downloaded from the Roadmaps Epigenomics project (https://egg2.wustl.edu/roadmap/data/byFileType/chromhmmSegmentations/ChmmModels/imputed12marks/jointModel/final/). The chromatin annotations and associated color codes were adapted from the Roadmaps Epigenomics project[6,7] obtained from (https://egg2.wustl.edu/roadmap/web_portal_/imputed.html#chr_imp) and are as follows:

MNEMONIC	DESCRIPTION	COLOR NAME	COLOR CODE
TssA	Active TSS	Red	255,0,0
PromU	Promoter Upstream TSS	Orange Red	255,69,0
PromD1	Promoter Downstream TSS 1	Orange Red	255,69,0
PromD2	Promoter Downstream TSS 2	Orange Red	255,69,0
Tx5’	Transcribed - 5' preferential	Green	0,128,0
Tx	Strong transcription	Green	0,128,0
Tx3’	Transcribed - 3' preferential	Green	0,128,0
TxWk	Weak transcription	Lighter Green	0,150,0
TxReg	Transcribed & regulatory (Prom/Enh)	Electric Lime	194,225,5
TxEnh5’	Transcribed 5' preferential and Enh	Electric Lime	194,225,5
TxEnh3’	Transcribed 3' preferential and Enh	Electric Lime	194,225,5
TxEnhW	Transcribed and Weak Enhancer	Electric Lime	194,225,5
EnhA1	Active Enhancer 1	Orange	255,195,77
EnhA2	Active Enhancer 2	Orange	255,195,77
EnhAF	Active Enhancer Flank	Orange	255,195,77
EnhW1	Weak Enhancer 1	Yellow	255,255,0
EnhW2	Weak Enhancer 2	Yellow	255,255,0
EnhAc	Primary H3K27ac possible Enhancer	Yellow	255,255,0
DNase	Primary DNase	Lemon	255,255,102
ZNF/Rpts	ZNF genes & repeats	Aquamarine	102,205,170
Het	Heterochromatin	Light Purple	138,145,208
PromP	Poised Promoter	Pink	230,184,183
PromBiv	Bivalent Promoter	Dark Purple	112,48,160
ReprPC	Repressed Polycomb	Gray	128,128,128
Quies	Quiescent/Low	White	255,255,255

Regional classification analysis using the BrainSpan transcriptome. Transcriptome correlation analysis was performed using RPKM values of NSC-derived neurons (n_{ASD}=8, $n_{control}$=5) as well as iPSC-iNs at 14 days of differentiation (n_{ASD}=8, $n_{control}$=5). The BrainSpan dataset was used to classify neuronal cultures as corresponding to a particular brain region (n=524 samples; brainspan.org/static/download; RNAseq Gencode v10). Gene IDs were matched between our set and the BrainSpan dataset, and genes with a standard deviation of less than 1 across the entire BrainSpan dataset were removed. We computed a Pearson correlation matrix between log transformed expression values (log2RPKMs=log2(RPKM+1)) of NSC-derived neurons or IPSC-iNs and each of the BrainSpan samples. The average maximum correlation coefficient was 0.76 for NSC-derived neurons and 0.73 for iPSC-iNs. We calculated the mean correlation value (of each of our samples with all 524 BrainSpan samples) to then define a sample-specific correlation threshold above the 97.72nd percentile (two standard deviations above mean). Here, each sample overlaps with more than one sample in the BrainSpan dataset, resulting in a final BrainSpan subset with region classes represented by a range of 10-27 samples. Representations of the regional enrichments are then plotted as relative percentage per region among the samples above correlation threshold.

Retro- and lentiviral design and production. Cloning of the Moloney Murine Leukemia Virus (MMLV)-based retroviral plasmids was performed as previously described[8]. Briefly, the sequence of the membrane localization domain from Lck (LckN) was linked to the eGFP sequence and cloned into the retroviral backbone resulting in pRV-CAG:LckN-eGFP (CAG:LckN-eGFP). For the Wnt reporter constructs pRV-M50:d2eGFP and pRV-M51:d2eGFP, the CAG promoter along with the GFP sequence was exchanged for the TCF/LEF or mutant TCF/LEF driven sequence of d2eGFP cloned from the LV-TOP-dGFP or LV-FOP-dGFP reporter[9] (addgene #14715 and #14885). To assess maturational progression, we cloned a retroviral reporter using a published sequence from the human DCX promoter[10] into the retroviral backbone by replacing the CAG promoter, which resulted in the plasmid pRV-hDCX:eGFP (hDCX:eGFP).
For lentiviral constructs, a human cDNA sequence for Ngn2 and the sequence for eGFP were linked by a 2A sequence and cloned into the tet-inducible pLV-XTP backbone (Clonetech). To allow constitutive expression of the reverse tetracycline-controlled transcriptional activator (rtTA) in iPSCs, the human EF1a promoter described in [1] was replaced with the human UbC promoter, resulting in pLV-X-UbC:TetOn (UtO). For monitoring nestin promoter activity during iN conversion, the eGFP sequence in pLV-X-Ngn2-2A-eGFP (N2AG) was first exchanged for a monomeric red fluorescence protein (mRFP) to create pLV-X-Ngn2-2A-mRFP (N2AR). The 2A-linked N2AR sequence along with the TRE-promoter sequence was then transferred into the CX-nestinGFPas[11,12] backbone (addgene #32401). A synapsin promoter-driven lentiviral vector expressing GFP (hsyn:LckN-GFP) was modified based on a construct recently described to label mature neurons[13]. For controlled overexpression of FBXO2 or FBXO2, FBXO44 and CEND1 in control NSCs, human cDNA sequences for these three genes as well as the sequence for eGFP were linked by 2A sequences and cloned into the tet-inducible pLV-XTP backbone (Clonetech). The sequence UbC:TetOn from the above-described UtO vector was then cloned in antisense orientation into the same backbone, resulting in the construct pLV-X-UbC:TetOn-TRE:FbxO2-2A-eGFP (UF-GFP) and pLV-X-UbC:TetOn-TRE:Cend1-2A-FbxO2-2A-FbxO44-2A-eGFP (UFFC-

GFP). As control, we designed the same construct but lacking the TM1 genes; thus it only contained the GFP sequence, resulting in pLV-X-UbC:TetOn-TRE:eGFP (UGFP).
Lenti- and retroviral particles were produced in HEK-293T cells. Briefly, retroviral vector plasmids were co-transfected with packaging plasmids pCMV-VSV-G and pCMV-Gag-Pol, whereas lentiviral vector plasmids were co-transfected with the plasmids psPAX2 and pMD2.G. After three days, viral particles were enriched by centrifugation and stored at high concentration at -80ºC before usage.

Quantitative RT-PCR (qRT-PCR). RNA was isolated using TRIzol reagent (Life Technologies) and subsequently reversibly transcribed using SuperScript III Reverse Transcriptase (Life Technologies). qRT-PCR was performed in technical triplicates using SYBRgreen Real-Time Mastermix (BioRad). Fold changes of mRNA levels were calculated with the delta-delta-Ct method using the Bio-Rad CFX Manager 3.1 (BioRad) and Microsoft Excel. The following primer sequences were used: hGAPDH (fw: GGTCACCAGGGCTGCTTTTA, rv: GGATCTCGCTCCTGGAAGATG), hSOX2 (fw: TTCACATGTCCCAGCACTACCAGA, rv: TCACATGTGTGAGAGGGGCAGTGTGC), hNESTIN (fw: CTCCAGAAACTCAAGCACC, rv: TCCTGATTCTCCTCTTCCA), hPAX6 (fw: TGGTATTCTCTCCCCCTCCT, rv: TAAGGATGTTGAACGGGCAG), hTUBB-3 (fw: TCCGCTCAGGGGCCTTTGGAC, rv: GCTCCGCCCCCTCCGTGTAG), hNANOG (fw: TGATTTGTGGGCCTGAAGAAAA, rv: GAGGCATCTCAGCAGAAGACA), hEMX2 (fw: GCTTCTAAGGCTGGAACACG; rv: CCAGCTTCTGCCTTTTGAAC), hPAX6 (fw: GTGTCTACCAACCAATTCCACAAC; rv: CCCAACATGGAGCCAGATG), hFBXO2 (fw: CAACCTTCTGCGTAACCCGT; rv: CTTTGCGACACCACTCAAAGG), hRASD2 (fw: CACAGACTCTGGGAGGCTCG; rv: ATCATGGCTCGGGGCTGG), hINSM2 (fw: AAAAGTTTCGTCGCCAAGCC; rv: ATCTGCTGTAGGGAAGTGCG) and hISLR2 (fw: GACCGTGTGTCTGCTTGAGA; rv: CCGCTTTTACGCCCTGCT). The following primers were used for the IPSC transgene assays (Extended Data Fig. 1a): hcMYC-S1011 (fw: CAACAACCGAAAATGCACCAGCCCCAG), hKLF4-S1128 (fw: ACGATCGTGGCCCCGGAAAAGGACC), hSOX2-S691 (fw: GGCACCCCTGGCATGGCTCTTGGCTC), hOCT3/4-S944 (fw: CCCCAGGGCCCCATTTTG GTACC), the reverse primer pMX-L3205 (rv: CCCTTTTTCTGGAGACTAAATAAA) and hACTB (fw: CTATCCCTGTACGCCTCTGG; rv: CCATCTCTTGCTCGAGTCC) as previously described[14].

Reporter assays and flow cytometry. Human patient-derived NSCs were infected with retroviral reporter constructs (see Retro- and lentiviral design for details). Two days after infection, NSCs were analyzed by flow cytometry and subsequently re-plated onto coverslips coated with poly-ornithine (10 µg/ml, Life Technologies) and laminin (5 µg/ml, Life Technologies) for neuronal differentiation. Sequential analysis of reporter activities was performed at indicated time points in combination with PSA-NCAM staining to identify neuronal populations. Each line was normalized to the clonal progeny of their infected progenitors. The experiments were performed with one clone per line (eight ASD and five control lines; Supplementary Table 7), with 50,000 cells analyzed for every flow cytometry measurement. For the assessment of Wnt signaling, cells were stimulated with recombinant human Wnt3a (R&D systems) 20 hrs prior FACS and the signal of RV-M50:d2eGFP was normalized to a similar designed reporter containing mutated TCF/LEF

binding sites (RV-M51:d2eGFP). Antibodies and their respective concentrations used for FACS are listed in the 'Antibodies' section.

TM1 gene overexpression in control NSCs. Human control patient-derived NSCs were infected with the above-mentioned constructs UF-GFP, UFFC-GFP or UGFP (see Retro- and lentiviral design). One day after infection, expression of transgenes was controlled by using different concentrations of doxycycline (FFC-GFP_{low} 200 ng/ml, FFC-GFP_{edium} 400 ng/ml, FFC-GFP_{high} 1 µg/ml: Sigma Aldrich) for one day prior to re-plating them onto coverslips or dishes coated with poly-ornithine (10 µg/ml, Life Technologies) and laminin (5 µg/ml, Life Technologies) at a density of $5x10^4$ cells/cm^2. Following neuronal differentiation, cell fate commitment was analyzed after four days of differentiation by flow cytometry as described below. Morphological assessments were performed after four days of differentiation following the above-mentioned paradigm in FFC-GFP_{high} conditions as well as for overexpressing FBXO2-GFP alone (1 µg/ml doxycycline: Sigma Aldrich).

PSA-NCAM FACS assays. Neuronal cell fate commitment (GFP^+/PSA-$NCAM^+$) was quantified by flow cytometry. Briefly, NSCs were infected with viral vectors (see Retro- and lentiviral design for details) and stained for PSA-NCAM on the days indicated. As neuronal cells express this marker, we calculated commitment efficiencies as relative numbers of GFP^+ cells expressing PSA-NCAM. A similar approach was used for assessing neuronal fate commitment of iPSC-iNs at day 4. To compare the values of NSC-derived neurons to those of iPSC-iNs, we calculated the fold enrichment as the ratio between GFP^+/PSA-$NCAM^+$ ratios from each line to the mean of GFP^+/PSA-$NCAM^+$ ratios from all five control lines (control NSC-derived neurons or iPSC-iNs at day 4 respectively). The experiments were performed with one clone per line (eight ASD and five control lines; Supplementary Table 7), with three to five technical replicates per line, resulting in n_{total}=62 measurements with 50,000 cells analyzed for every flow cytometry replicate.

BrdU proliferation assay. To assess proliferation in transdifferentiating neurons, converting cells were treated with 5-bromo-2'-deoxyuridine (BrdU) throughout the entire culture period at a concentration of 10 µM. Cells were fixed with 4% PFA for 20 min at RT, incubated with 2N HCl for 30 min at 37 ºC, and subsequently rinsed with 0.1M Borate buffer for 10 min and washed four times with PBS. The following staining procedures were performed as described above. Stereological counts of cells incorporating BrdU were performed seven days after transdifferentiation of iPSC-iNs (n=4 cell lines) using the Cell Counter Plugin of the Fiji package of ImageJ (NIH).

Supplementary References

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