Supplementary Information

SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients

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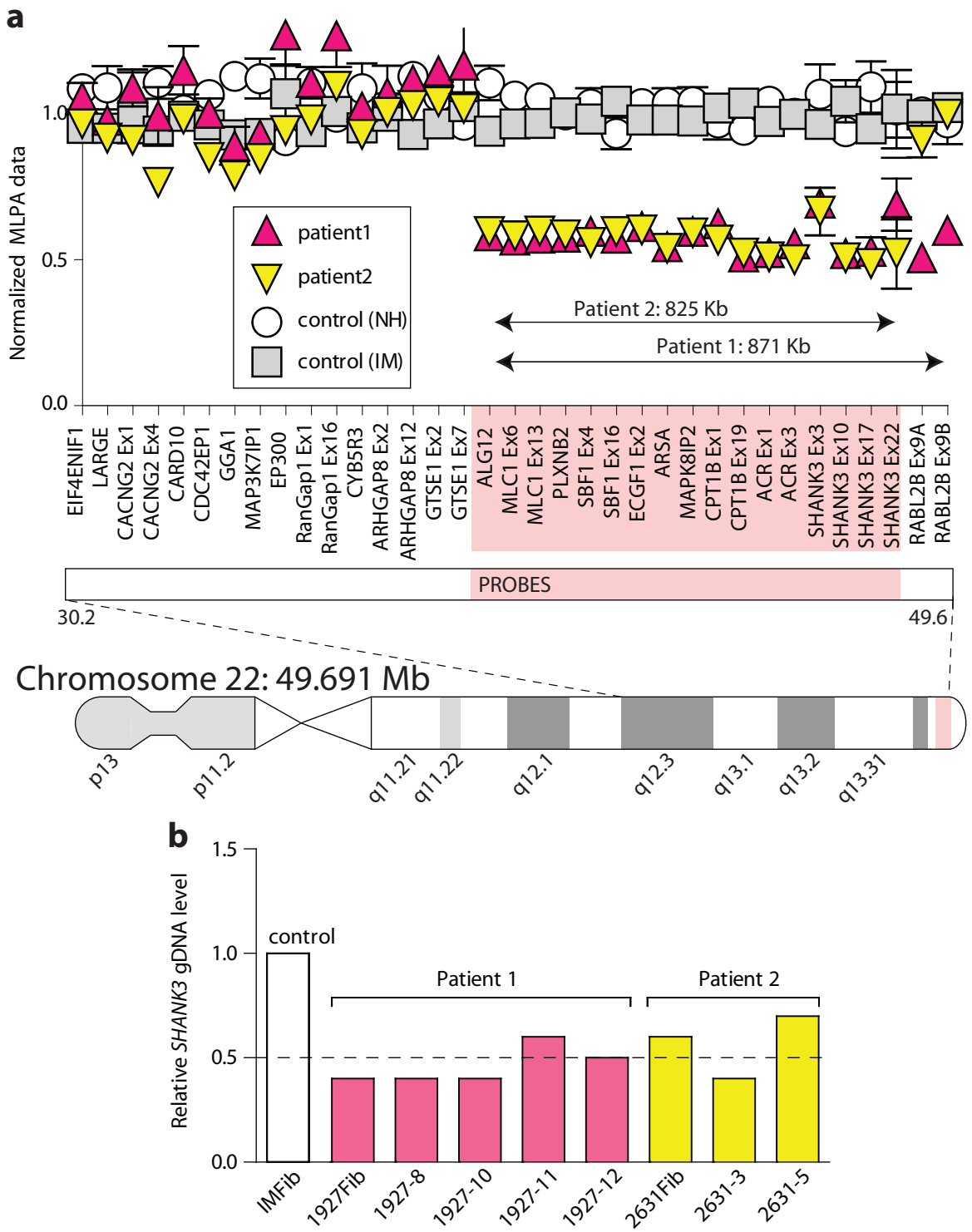
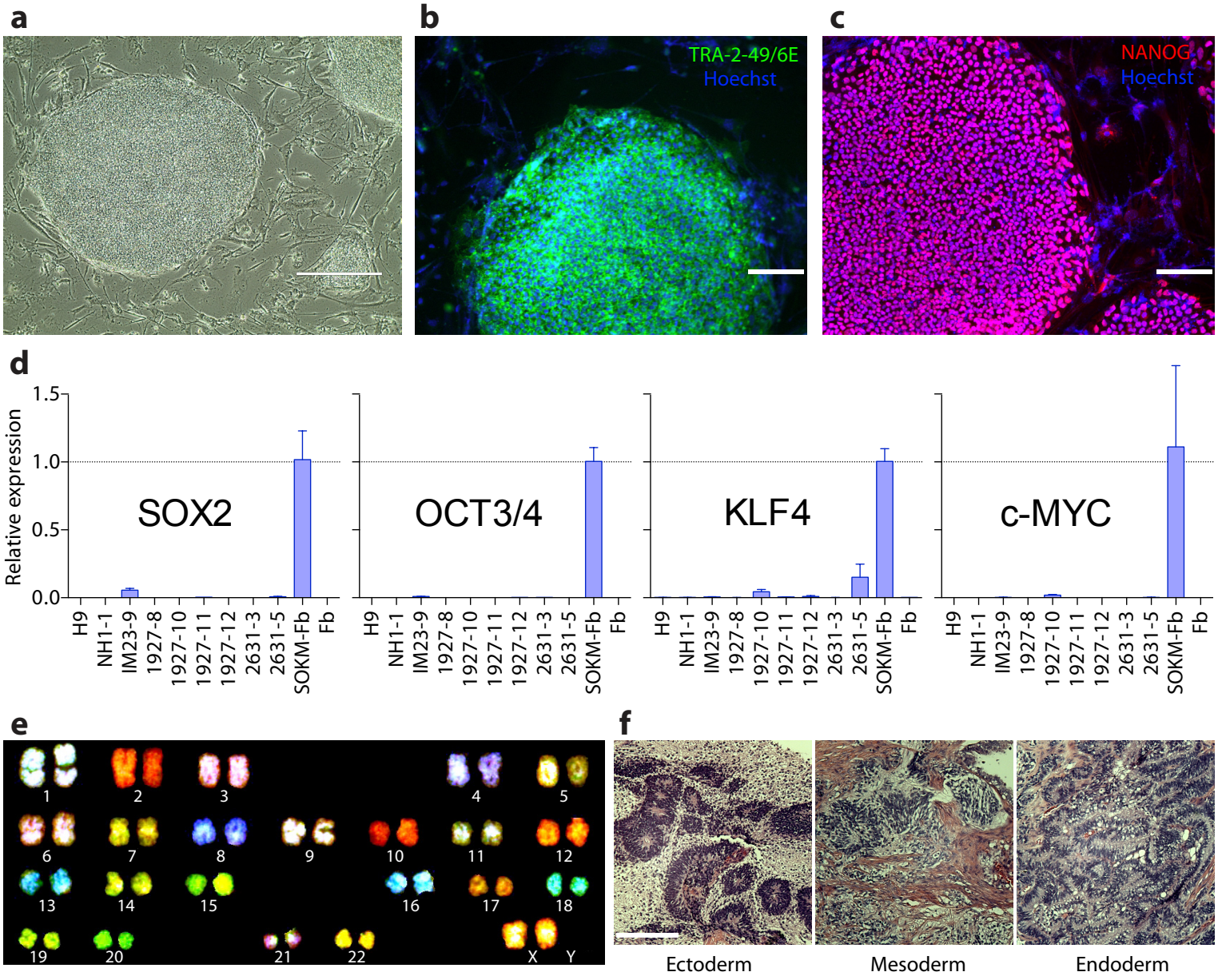
Supplementary Figure 1



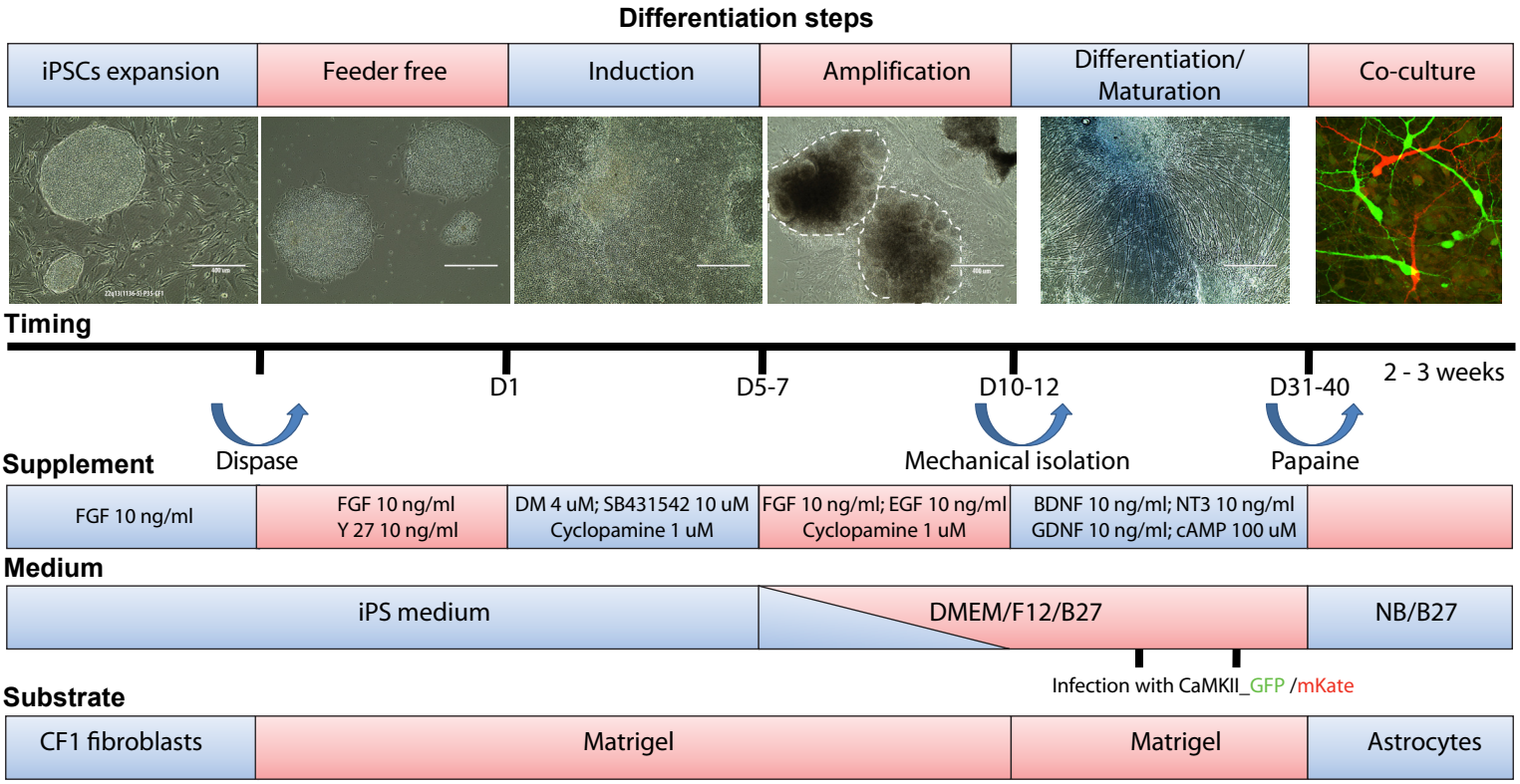
Verification of deletions in cells from PMDS patients. a, Multiplex Ligation Probe Amplification Assay (MLPA) on patient and control gDNA demonstrates the haploinsufficiency of genes from patients with 22q13 deletions, top. Graphical representation of Chromosome 22 and the region covered by the MLPA probes, bottom. The minimum deleted region is shown in red. MLPA was performed following the manufacturer's protocol (SALSA MLPA kit P188-B1 22q13; MRC-Holland, Amsterdam, Netherlands). Data were acquired using a capillary sequencer and normalized by dividing the peak area of each probe's amplification product by the total area of the reference probes (n = 3 - 10 gDNA extracts). Data presented as means ± s.e.m. Probe positions are based on the NCBI36/hg18 genome assembly (http://genome.ucsc.edu/). **b**, Reduced level of *SHANK3* gDNA detected in patients' fibroblasts and iPS cells using qPCR with SHANK3ex22 primers (normalized to 18S).

Supplementary Figure 2



Characterization of iPS cells. a, Representative images of PMDS iPS cell colonies (1927-8; scale bar, 400 μm). **b-c**, Representative images of PMDS iPS cell colony immunostained with Tra-2-49/6E (**b**; 2631-5; scale bar, 200 μm) and Nanog (**c**; 1927-10; scale bar, 200 μm). **d**, qRT-PCR detection of expression of exogenous transcription factors (SOX2, OCT3/4, KLF4, and c-MYC) introduced during reprogramming. Negative control, H9–ESC line; positive control, SOMK-Fb–IMR90 human fibroblasts (ATCC: CCL-186) infected with the four transcription factors. Normalized to GAPDH and expression levels in SOMK-Fb. Data presented as means ± s.d. Representative image of spectral karyogram derived from PMDS iPS cells (1927-8). **f**, Representative images of teratoma sections formed 3-8 weeks after injection of iPS cells into a kidney capsule of SCID mice (2631-3; Scale bar, 200 μm).

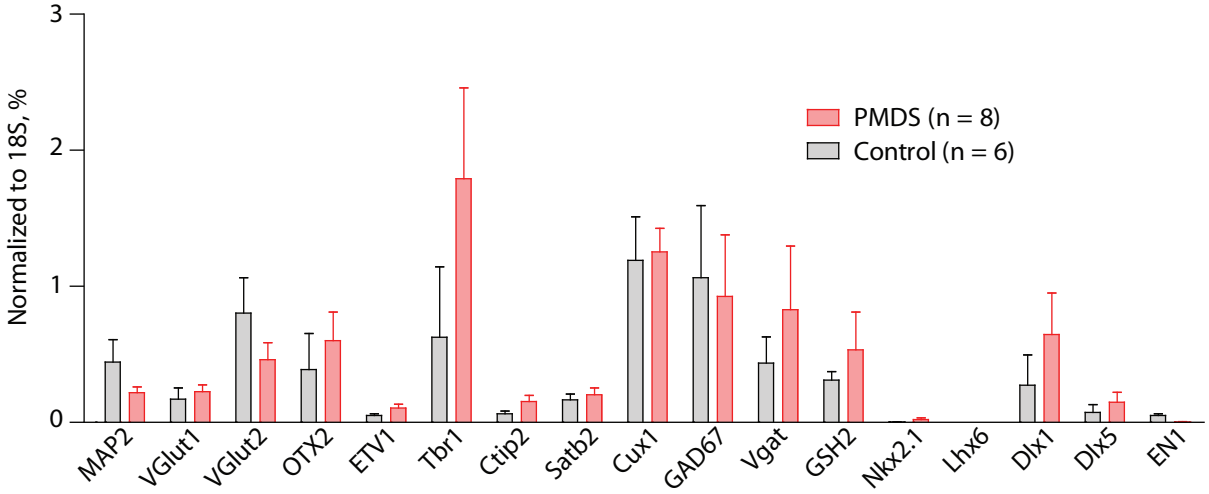
Supplementary Figure 3



Neural differentiation protocol. We used a combination of two previously published protocols (Chambers et al., 2009; Gaspard et al., 2010). The protocol developed by Chambers et al., was chosen based on its effectiveness in converting iPS cells into largely FoxG1/Pax6-positive telencephalic neuronal precursors. A cyclopamine treatment step was introduced based on the protocol by Gaspard et al., where authors used cyclopamine to efficiently convert mouse ES cells into cortical excitatory neurons.

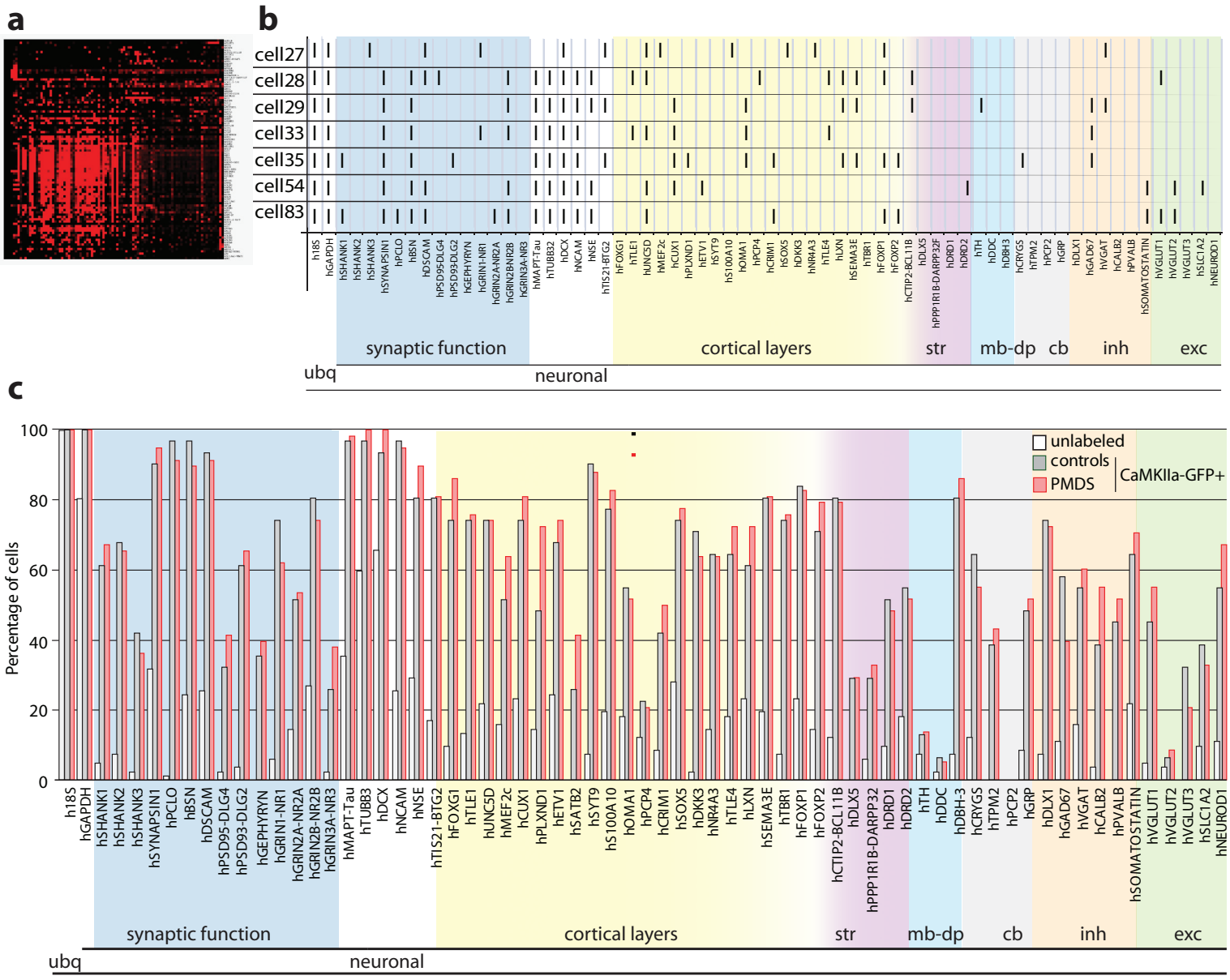
iPS cells were cultured in iPS-medium supplemented with bFGF (R&D Systems) on top of CF1 feeder cells and used for neural differentiation between passages 20 and 60. iPS cell colonies were then re-plated onto 2 cm dishes pre-coated with matrigel (BD, 1:20 in iPS media) and allowed to reach approximately 60–80% confluence in standard iPS medium. On day 1 (D1), growth factors were withdrawn and neuroectodermal fate was induced by a 5-7-day-long incubation in iPS-medium containing inhibitors of SMAD signaling, SB431542 (Tocris), and Dorsomorphin (Tocris), in the presence of cyclopamine (Calbiochem). On day 5-7, medium was changed to that containing a mixture of iPS and neuronal medium (DMEM/F12, 2% B27 (Chen et al., 2008), 1% penicillin-streptomycin) at the following ratios (iPS/neuronal) for each day: D1, 75/25; D2, 50/50; D3, 25/75; and D4: 0/100, and supplemented with bFGF and EGF (R&D Systems), and cyclopamine. On day 10-12, clusters with rosettes were mechanically isolated and transferred onto 2 cm dishes pre-coated with matrigel (1:20 in neuronal medium) and cultured in neuronal medium supplemented with cAMP (Tocris), BDNF (R&D Systems), NT3 (R&D Systems), and GDNF (R&D Systems) for the next 21-28 days. During this time, cells were infected with lentiviruses carrying GFP or mKate under the CaMKIIα promoter (constructs were made by in-frame substitution of GFP or mKate for ChR2 in Addgene plasmid 20944 (Zhang et al., 2007)). On day 31–40, PMDS and control cells expressing different fluorescent proteins were co-plated together at 2x105 cells/well in 24-well plates on the bed of neonatal rat cortical astrocytes grown on 15 mm glass cover slips in NB medium (NB medium, 10888-022, Gibco; 2% B27; 1% L-glutamine).

Supplementary Figure 4



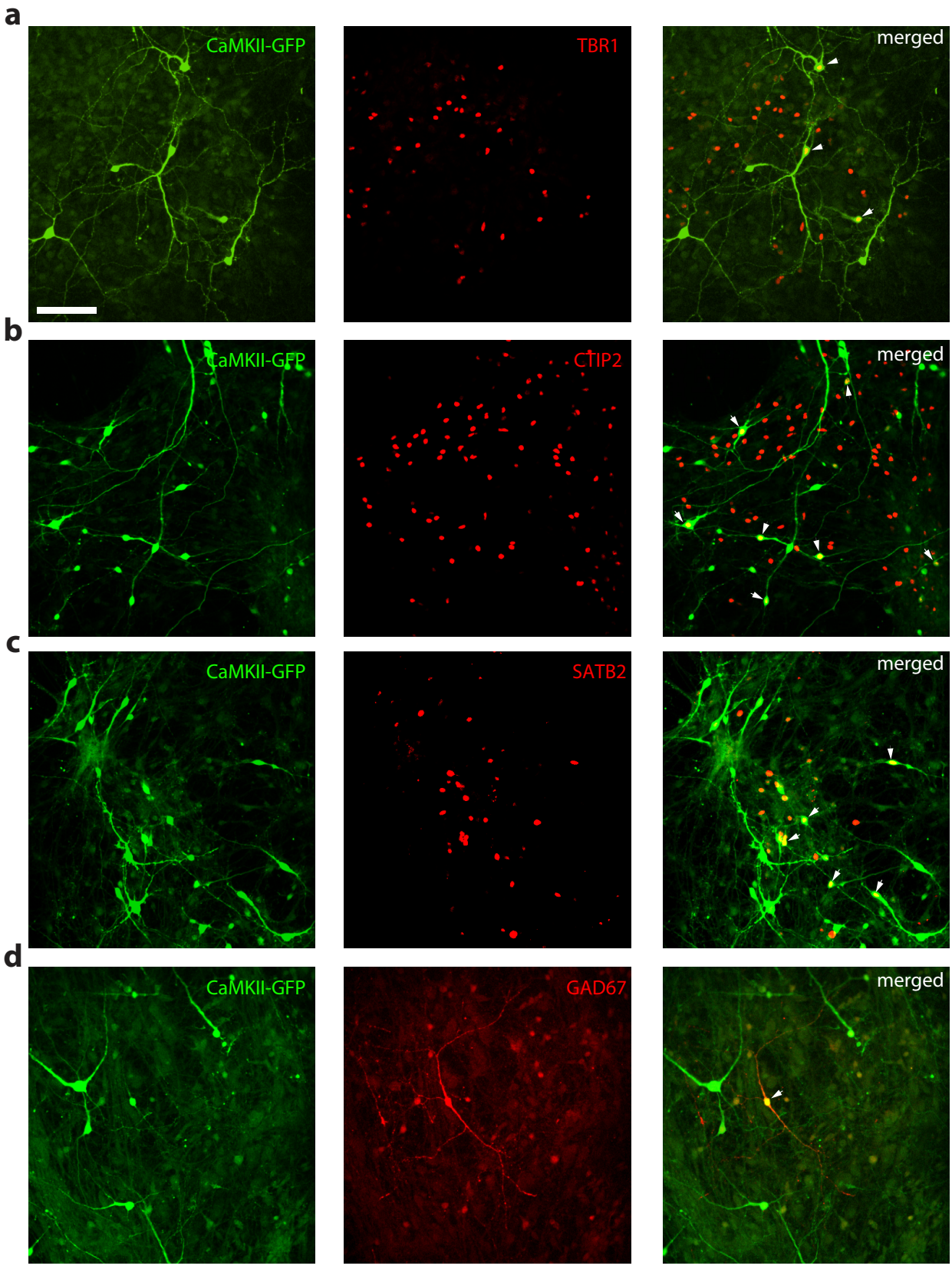
Expression of neuronal cell markers. mRNA expression level of different cell identity markers was assessed by qRT-PCR in cultures (D30 – D35) of PMDS (n = 8 biological replicates) and control (n = 6 biological replicates) iPS cell-derived neurons. Data are presented as means ± s.e.m. There were no statistically significant differences between PMDS and control group means as determined by Student's t-test, $p > 0.05$.

Supplementary Figure 5



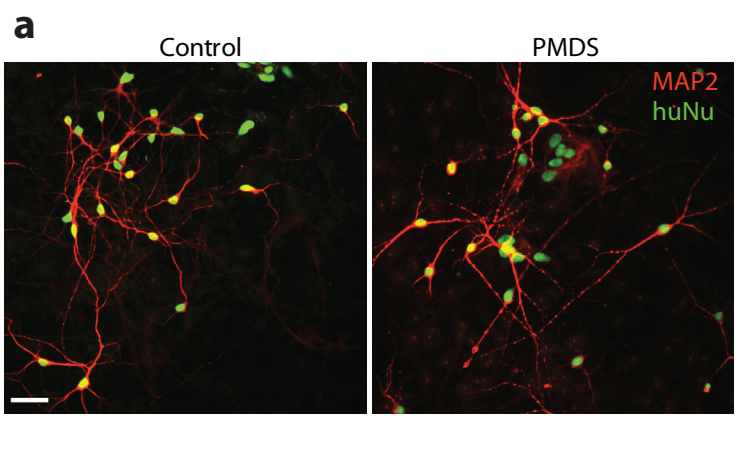
Characterization of single cell gene expression profiles. a, Heat map generated using Fluidigm Real-Time PCR Analysis 3.02 software, visualizing the expression of 96 selected genes (y-axis) in 96 single cells (x-axis). **b**, Representative analysis of the expression of selected genes in single cells, binarized based on the defined threshold for gene expression. **c**, Proportion of single cells expressing selected genes, collected either by random FACS clone sorting (unlabeled, n = 90 cells from 1 line, D35) or based on CaMKIIα-GFP expression (CaMKIIα-GFP+, n = 32 cells from 3 lines (control) and n = 58 cells from 5 lines (PMDS), D30-D40). Proportion of single cells expressing SHANKs is also presented in Fig. 1b. Genes were grouped according to their apparent identities. Abbreviations: ubiquitously expressed genes (Ubq), genes preferentially expressed in excitatory (exc), inhibitory (inh), cerebellar (cb), midbrain dopaminergic (mb-dp), and striatal (str) neurons. There were no statistically significant differences between PMDS and control cells as determined by Fisher's exact test, $p > 0.05$. Significance tested with Fisher's exact test.

Supplementary Figure 6



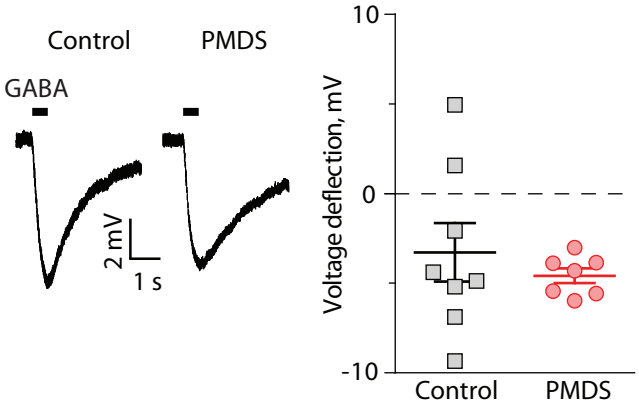
Characterization of iPSC-derived neurons expressing CaMKII-GFP. a-d, Representative images of control and PMDS iPSC-derived neurons immunostained with antibodies against GFP (green) and (**a**) TBR1, (**b**) CTIP2, (**c**) SATB2, and (**d**) GAD67 (red). Scale bar = 200 μm.

Supplementary Figure 7



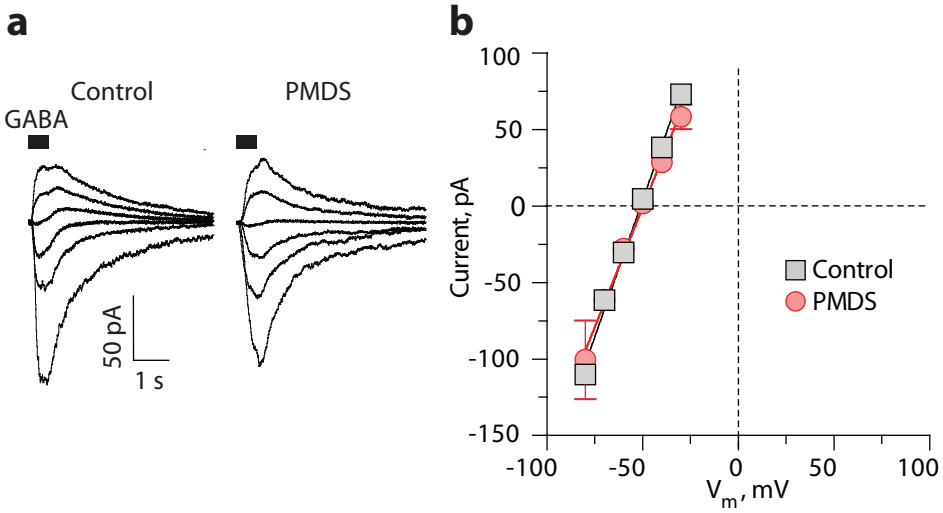
Map2 expression in cultures in iPS cell-derived neurons. a, Representative images of control and PMDS neurons immunostained with antibodies against MAP2 and human-specific nuclei. **b**, Proportion of neurons (MAP2 possitive cells) among control (n = 8 cover slips (26452 cells)) and PMDS (n = 7 (19704)) cells plated on astrocytes. Data presented as means ± s.e.m.; * p = 0.02 by Mann Whitney test.

Supplementary Figure 8



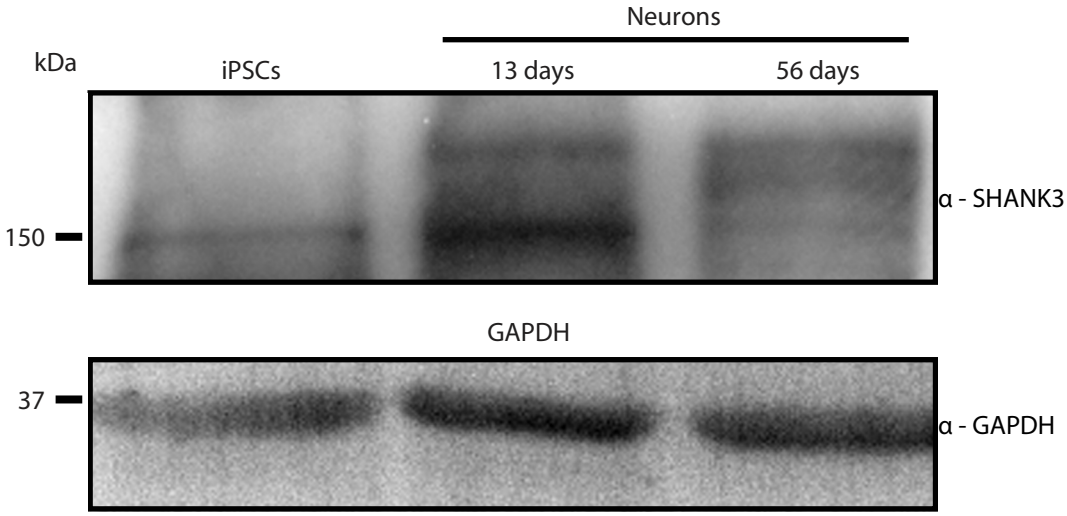
Voltage deflections induced by GABA application. Voltage deflections were measured in response to focal applications of 100 µM GABA in cell-attached current-clamp mode from control (n = 8 cells) and PMDS (n = 7 cells) neurons. Data presented as means ± s.e.m. This approach allows to detect a significant fraction of changes in membrane potential without perturbing the cytoplasmic composition (Mason et al., 2005, Biophys J; Perkins 2006, J Neurosci Methods; Kirmse et al., 2010, J Neurosci). Recordings were performed using the following solutions (in mM): extracellular, 140 NaCl, 2.5 KCl, 2.5 CaCl2, 2 MgCl2, 1 NaH2PO4, 20 glucose, 10 HEPES, pH 7.4; intracellular, 135 CsMeS, 5 CsCl, 10 HEPES, 0.5 EGTA, 1 MgCl2, 4 Mg2ATP, 0.4 NaGTP, 5 QX-314, pH 7.4. R_{pip} = 5 - 7 MΩ, R_{seal} = 2.8 ± 0.3 GΩ.

Supplementary Figure 9



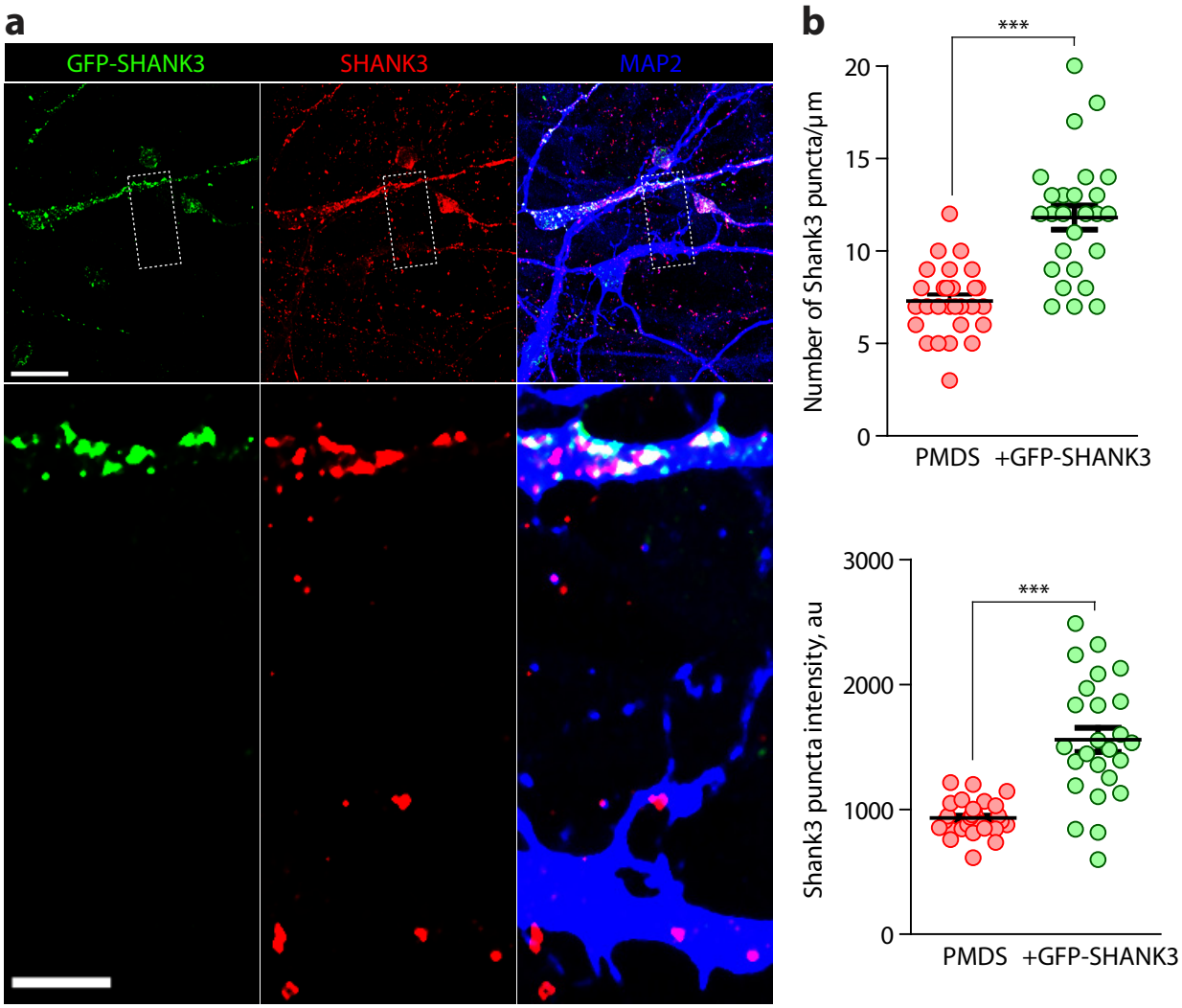
Currents induced by GABA application. a, Representative current traces measured in control and PMDS neurons at different holding potentials (-80 - -30, Δ10 mV) in response to focal applications of 100 µM GABA. **b,** Peak current IV-relationships (n = 6 control and 6 PMDS cells). Data presented as means ± s.e.m.

Supplementary Figure 10



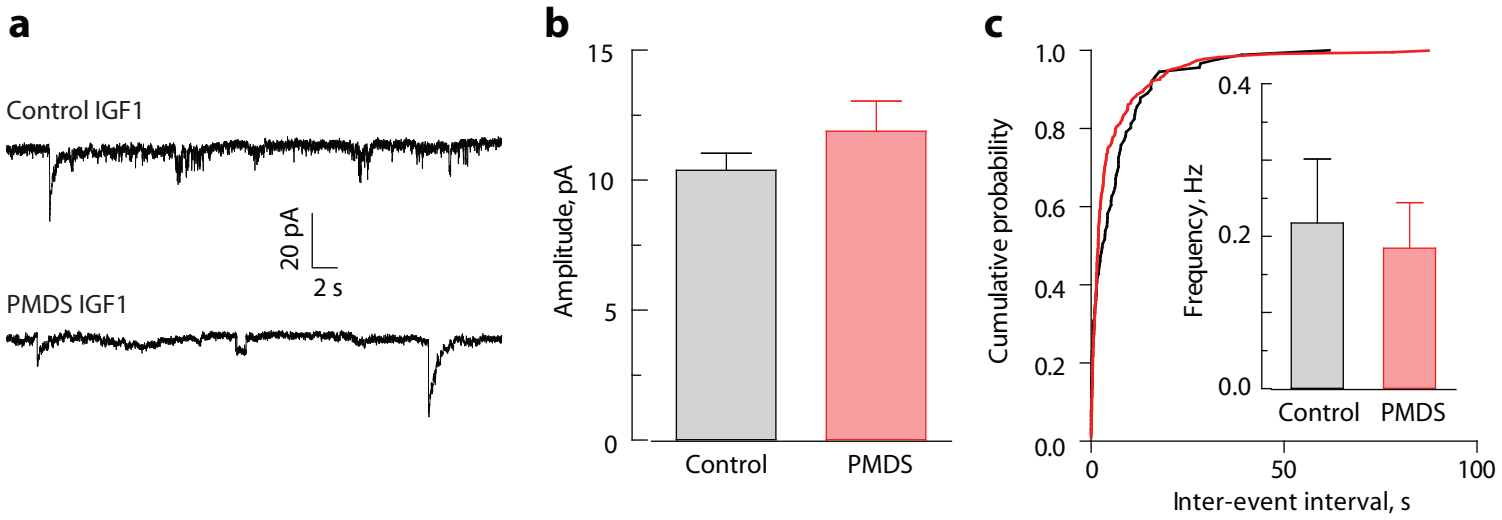
Expression of SHANK3 proteins at different stages of differentiation. Western blot analysis using protein lysates extracted from control iPS cells and iPS cell-derived neurons using anti-SHANK3 antibody. GAPDH was used as a loading control.

Supplementary Figure 11

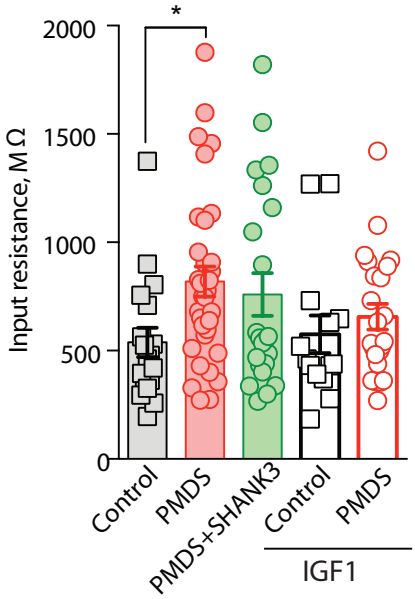


Characterization of SHANK3 expression in infected PMDS neurons. a, Representative images of PMDS iPS cell-derived neurons infected with GFP-SHANK3 lentivirus and immunostained with antibodies against GFP, SHANK3, and MAP2. Scale bars = 20 (top) and 5 µm (bottom). **b**, Quantification of the number (left) and intensity (right) of SHANK3-positive puncta on neigboring uninfected (n = 28 cells) and GFP-SHANK3 infected (n = 26 cells) PMDS iPS cell-derived neurons. Data presented as means ± s.e.m.; *** $p < 0.001$ by Sudent's t-test

Supplementary Figure 12



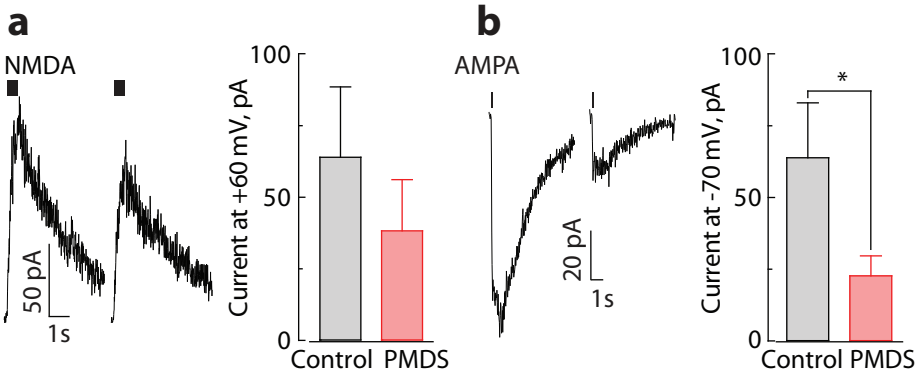
Characterization of inhibitory synaptic transmission after IGF1 treatment. a, Representative traces of spontaneous IPSCs recorded from co-cultured control and PMDS neurons at -70 mV in the presence of 10 μM NBQX and 50 μM APV. **b-c**, Quantification of the amplitude (b) and frequency (c) of spontaneous IPSCs (n = 4 control and 8 PMDS cells). Data presented as means ± s.e.m.



Supplementary Figure 13

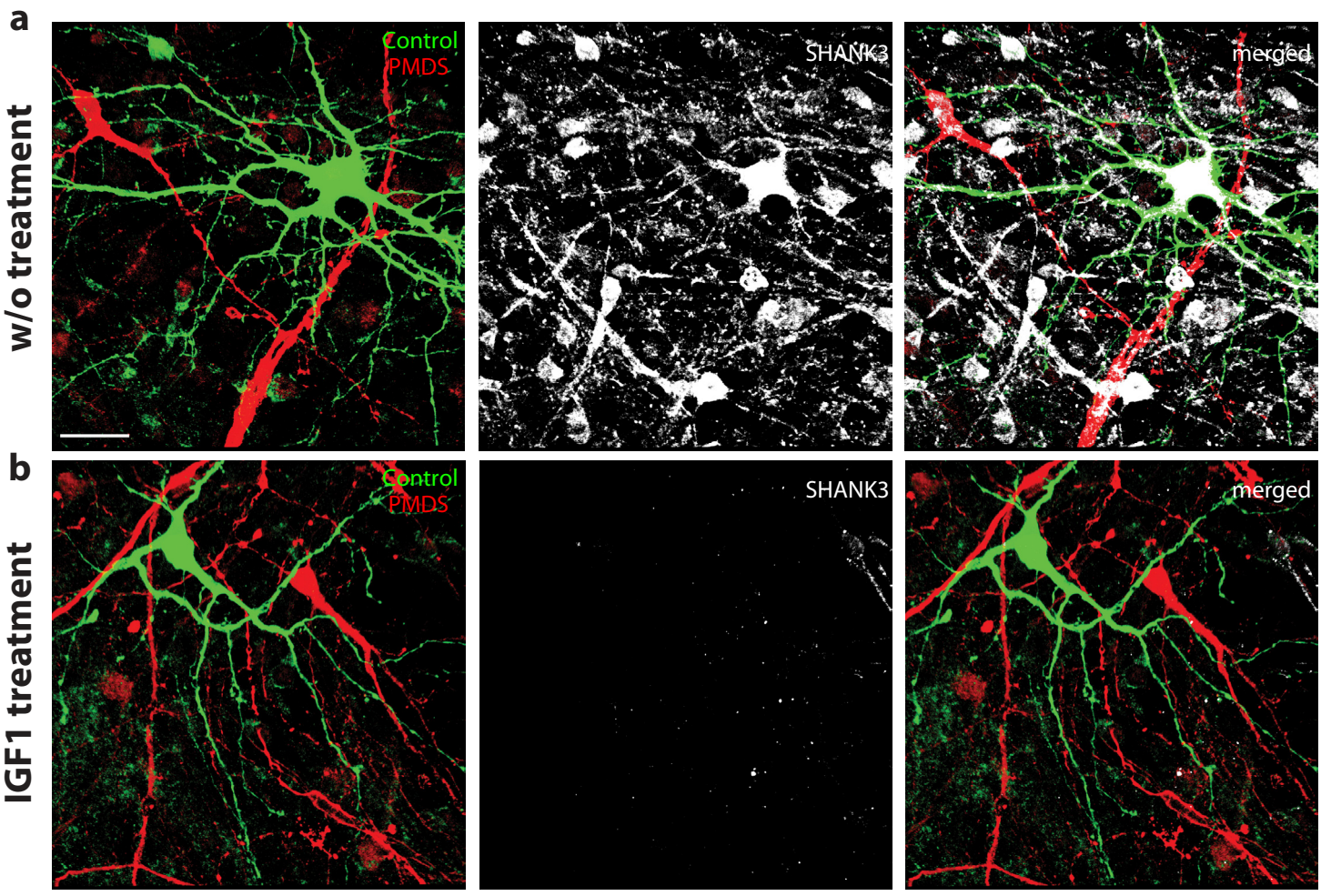
Measurements of input resistance. Input resistance was calculated from the current deflections measured in response to a 5 mV hyperpolarizing voltage pulse from Vhold = -70 mV (w/o: n = 18 control and 33 PMDS cells; PMDS+SHANK3, n = 22 cells; IGF1, n = 14 control and 23 PMDS cells) in Cs-containing intracellular solution. Data presented as means ± s.e.m.; * $p < 0.05$, by Kruskal-Wallis test.

Supplementary Figure 14



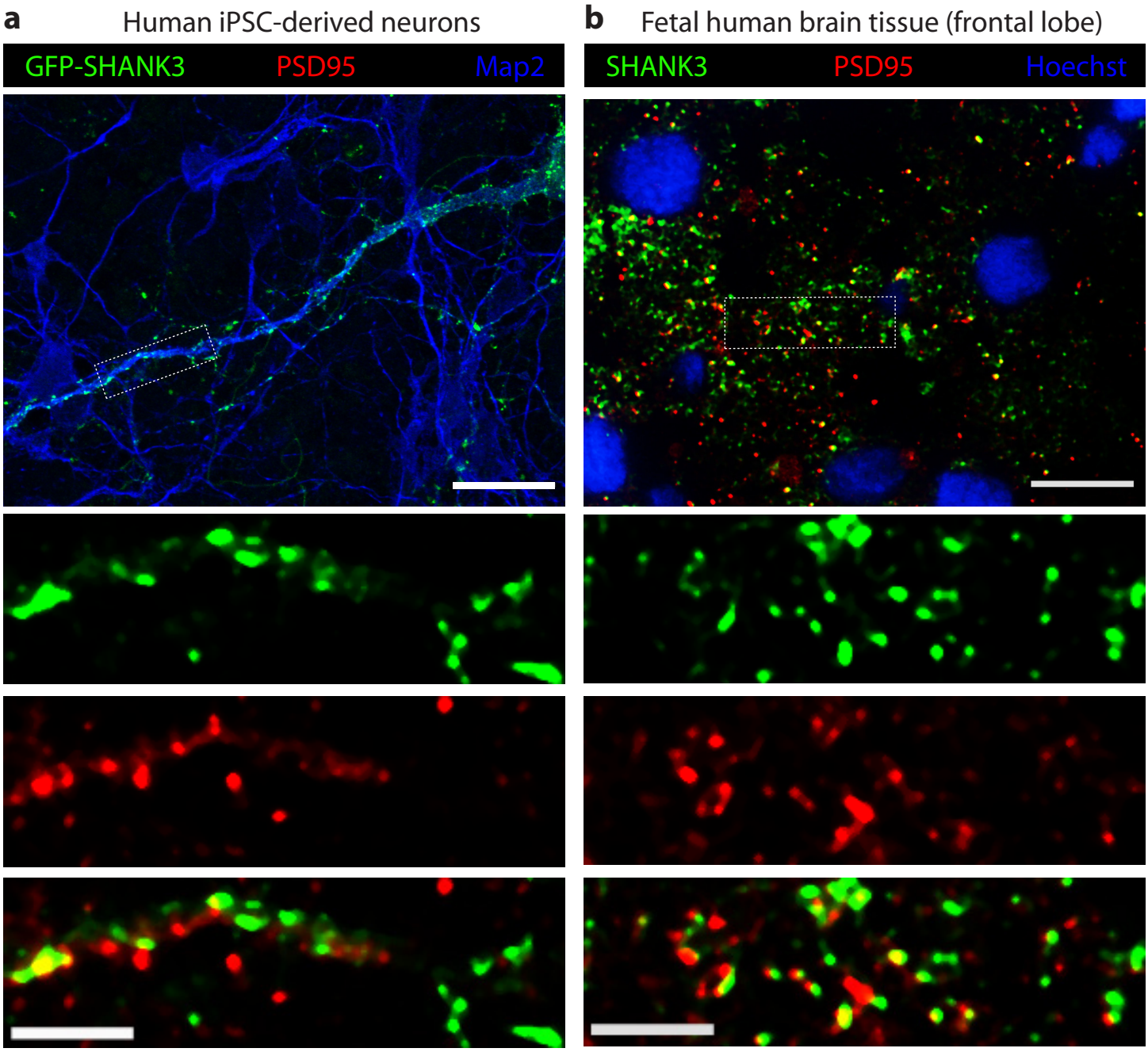
Currents induced by NMDA and AMPA applications after IGF1 treatment. a, NMDA (n = 8 control and 7 PMDS cells) and **b**, AMPA (n = 7 control and 11 PMDS cells) receptor currents induced by focal applications of 100 μM NMDA (**a**) and 200 μM AMPA (**b**) in control and PMDS neurons at +60 and -70 mV, respectively. Representative current traces are shown on left; quantification is shown on right. Data presented as means ± s.e.m.; * $p < 0.05$, by the Mann-Whitney test.

Supplementary Figure 15



Characterization of SHANK3 expression after IGF1 treatment. a-b, Representative images of co-cultured control (green) and PMDS (red) neurons in untreated (**a**) and IGF1-treated (**b**) cultures, immunostained with anti-GFP, mKate, and SHANK3 antibodies. Scale bar = 20 µm. **c**, Quantification of SHANK3 expression in the soma and processes (w/o, n = 23 control and 26 PMDS cells; IGF1, n = 19 control and 28 PMDS cells). Data presented as means ± s.e.m.; **p < 0.01, ***p < 0.001, by one-way ANOVA with Bonferroni's test.

Supplementary Figure 16



Localization of SHANK3 and PSD95 proteins in human neurons. a, Representative images of a PMDS iPS cell-derived neuron infected with GFP-SHANK3 and immunostained with antibodies against GFP, PSD95, and MAP2. **b**, Representative images of a normal fetal human brain tissue (frontal lobe, 37 PGW, Biochain) immunostained with antibodies against SHANK3, PSD95, and Hoechst. Scale bars = 20 (top) and 5 (bottom) μm.

Supplementary Table 1

Characterization of PMDS patients

	Patient 1 (1927)	Patient 2 (2631)
Gender	Female	Male
Age	9 years	6 years
Developmental Milestones	First word between 2 and 3 years of age. First phrase between 4 and 5 years. First steps at 21 months	First word between 12 and 18 months. Has not acquired phrase language. First steps at 19 months
Language	Severely impaired with poor articulation, often stereotyped/repetitive, uses some words and verbs, no history of language regression	Severely impaired, uses fewer than 5 words total, some language regression between 12 and 18 months
Intellectual disability	FSIQ - 42, NVIQ- 44, VIQ - 44	Intellectually disabled based on parent's report and ADI-R
Autistic Measures	ADI-R: Met full Autism criteria on all domains; ADOS: Met Autism Spectrum range on all domains; SRS: Met full Autism Range	ADI-R: Met full Autism criteria on all domains; ADOS: Met full Autism range on all domains; SRS: Met full Autism Range
Other clinical features	No history of seizures (has not undergone EEG), reduced pain sensitivity, abnormal gait, no formal psychiatric diagnosis, but possible manic, depressive, and anxiety-related symptoms reported	Epileptiform discharges detected by EEG, but no history of clinical seizure, reduced pain sensitivity, abnormal gait
Treatment	Risperidone for mood symptoms; Speech therapy, physical therapy, occupational therapy, and behavior therapy	Oxcarbazepine for seizure prophylaxis; Speech therapy, physical therapy, occupational therapy, behavior therapy, and hippotherapy

FSIQ - Full scale Intelligence Quotient, NVIQ – Nonverbal Intelligence Quotient, VIQ – Verbal Intelligence Quotient per Stanford-Binet, 5th ed.

ADI-R – Autism Diagnostic Interview- Revised, ADOS – Autism Diagnostic Observation Schedule, SRS – Social Responsiveness Scale

Supplementary Table 2

Summary of the Multiplex Ligation-dependent Probe Amplification (MLPA) assay

	Last probe before deletion	First probe within deletion	Last probe within deletion	First probe after deletion	Minimum deletion size	Maximum possible deletion size
					871 Kb	4.5 Mb
					Genes within deletion	Genes within deletion
Patient 1	6097-L06370 GTSE1 exon7	6733-L14002 ALG12 exon10	6088-L5543 RABL2B exon9	N/A	*ALG12, CRELD2, PIM3, IL17REL, TTLL8, MLC1, MOV10L1, PANX2, TRABD, SELO, TUBGCP6, HDAC10, MAPK12, MAPK11, PLXNB2, FAM116B, SAPS2, SBF1, ADM2, MIOX, LMF2, NCAPH2, SCO2, TYMP, ODF3B, KLHDC7B, CPT1B, CHKB, MAPK8IP2, ARSA, SHANK3, ACR, RABL2B*	*GTSE1_ex7, TRMU, CELSR1, GRAMDD4, CERK, FAM19A5, BRD1, ZBED4, ALG12* through *RABL2B*
Patient 2	6097-L06370 GTSE1 exon7	6733-L14002 ALG12	6786-L6378 SHANK3 exon17 and ambiguous amplification of 6787-L6379 SHANK3exon 22	67343-L5558 RABL2B	*ALG12, CRELD2, PIM3, IL17REL, TTLL8, MLC1, MOV10L1, PANX2, TRABD, SELO, TUBGCP6, HDAC10, MAPK12, MAPK11, PLXNB2, FAM116B, SAPS2, SBF1, ADM2, MIOX, LMF2, NCAPH2, SCO2, TYMP, ODF3B, KLHDC7B, CPT1B, CHKB, MAPK8IP2, ARSA, SHANK3*	*GTSE1_ex7, TRMU, CELSR1, GRAMDD4, CERK, FAM19A5, BRD1, ZBED4, ALG12* through *SHANK3*

Genomic DNA from all fibroblasts and iPS lines was assayed using MLPA kit P188-B1 22q13 according to the manufacturer's protocol (MRC-Holland, Amsterdam, Netherlands). Minimum deletion size was determined by calculating the distance in base pairs between the unamplified probes. Maximum possible deletion size was determined by calculating the distance between the amplified probes flanking the 22q13 deletion. Probe positions are based on the NCBI36/hg18 genome assembly (http://genome.ucsc.edu/)

Supplementary Table 3

Summary of performed experiments

Assays \ Lines		Controls			Patient1				Patient2	
		IM23-9	H9	NH1-1	1927-12	1927-11	1927-10	1927-8	2631-3	2631-5
Characterization of iPSCs										
ESC-like morphology		+	+	+	+	+	+	+	+	+
SKY		+	+	+	+	+	+	+	+	+
teratoma formation assay		+	+	+	+	+			+	+
ICC										
Nanog		+	+	+	+	+	+	+	+	+
Tra2-49/6E		+	+	+	+	+	+	+	+	+
qRT-PCR										
Endog. genes		+	+	+	+	+	+	+	+	+
Exog. transc. factors		+	+	+	+	+	+	+	+	+
Characterization of neurons										
qRT-PCR		+	+	+	+		+	+	+	+
Single cell qRT-PCR		+	+	+	+	+	+	+	+	+
ICC (Map2, huNu)		+	+		+	+			+	+
ICC (Tbr1, Ctip2, Satb2, GAD)		+	+		+	+			+	+
Western blot		+	+		+				+	
Electrophysiology (intrinsic)		+	+	+	+	+	+	+	+	+
Characterization of synaptic properties: electrophysiology										
separate culture	w/o	+	+	+		+	+	+	+	+
co-culture EPSCs	w/o	+	+		+	+			+	+
	IGF1	+	+		+	+			+	
IPSCs	w/o	+	+		+	+			+	
	IGF1	+	+		+				+	
AMPA application	w/o	+	+		+	+			+	
	IGF1	+	+		+	+			+	
NMDA application	w/o	+	+	+	+	+	+	+	+	+
	IGF1	+	+		+	+			+	
GABA application	w/o	+	+		+	+			+	
Shank3 rescue	w/o				+				+	
ICC										
Synapsin1-Homer1	w/o	+	+		+	+			+	
	TSA	+			+	+			+	
	VPA	+			+	+			+	
	Nif	+							+	
	IGF1	+	+		+	+			+	
	IGF2	+	+		+	+			+	
Shank3	w/o	+	+		+	+		+	+	
	IGF1		+		+	+			+	
PSD-95	w/o		+		+				+	
	IGF1		+		+				+	

(+) - lines that were used for indicated experiments

Supplementary Table 4

Characterization of functional properties of PMDS and control iPSC-derived neurons cultured separately without astrocytes

Intrinsic properties									
	control				PMDS				Statistics
	Mean	SEM	N, cells	N, lines	Mean	SEM	N, cells	N, lines	P value
Number of APs at threshold	3.6	0.7	22	3	2.4	0.3	38	5	
AP_{Thr}, mV	-44.4	1.4	22	3	-44.5	1.0	38	5	
AP_{ampl}, mV	60.8	2.5	22	3	58.4	1.9	38	5	
AP $width_{Thr}$, ms	6.9	0.3	22	3	6.9	0.4	38	5	
AP $width_{Half}$, ms	2.9	0.1	22	3	2.9	0.2	38	5	
AP dV/dt, mV/ms	46.2	2.7	22	3	47.3	2.7	38	5	
RMP, mV	-53.4	1.9	22	3	-50.7	1.7	38	5	
R_{in}, GΩ	1.60	0.15	22	3	2.75	0.25	38	5	***, 0.0003
C_m, pF	34.9	3.2	22	3	27.4	2.6	38	5	
τ_m, ms	55.6	6.4	22	3	67.0	7.9	38	5	
Synaptic properties									
Amplitude, pA	19.4	2.7	19	3	10.0	0.8	26	5	***, 0.0002
Frequency, Hz	0.24	0.05	19	3	0.10	0.02	26	5	***, 0.001

Abbreviations: AP - action potential; AP_{Thr} – AP threshold, measured from the phase-plane plot; AP_{ampl} – AP amplitude, calculated as a difference between the threshold and the peak of AP; AP $width_{Th}$ – AP width measured at the threshold; AP $width_{Half}$ – AP width measured at the half of AP amplitude; AP dV/dt - the rate of the AP rise, calculated for the first AP measured in response to increasing current injections; RMP – resting membrane potential, measured right after brake-in into the cell and corrected for the liquid junction potential (-22 mV); R_{in} – input resistance, measured from the linear voltage deflection in response to negative and positive current injections around V_{hold} = -60 – -75 mV, C_m - membrane capacitance, calculated as the ratio of τ_{on} and Rin. Significance tested with the Mann-Whitney test.

Spontaneous synaptic activity was recorded for 3 min at -70 mV. EPSCs were detected using a template created in Clampfit 10 by averaging at least 50 events recorded in the presence of 50 μM picrotoxin.

iPSC-derived neurons were recorded using the following extracellular and intracellular solutions (in mM): 140 NaCl, 2.5 KCl, 2.5 CaCl2, 2 MgCl2, 1 NaH2PO4, 20 Glucose, 10 HEPES, pH 7.4 (extracellular); 120 KGluc, 20 KCl, 4 NaCl, 4 Mg2ATP, 0.3 NaGTP, 10 Na2PCr, 0.5 EGTA, 10 HEPES, pH 7.25 (intracellular)

Supplementary Table 5

List of primers used for qRT-PCR

Symbol	Forward primer	Reverse primer	Accession number[1]
18S	GATGGGCGGCGGAAAATAG	GCGTGGATTCTGCATAATGGT	11968182a1
GAPDH	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT	7669492a3
OCT3/4 exogenous	CCCCAGGGCCCCATTTTGGTACC	TTATCGTCGACCACTGTGCTGCTG	
SOX2 exog	GGCACCCCTGGCATGGCTCTTGGCTC	TTATCGTCGACCACTGTGCTGCTG	
MYC exog	CTGAAGAGGACTTGTTGCGGAAAC	TTATCGTCGACCACTGTGCTGCTG	
KLF4 exog	CCCACACAGGTGAGAAACCTTACC	TTATCGTCGACCACTGTGCTGCTG	
OCT3/4	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC	
SOX2	GGGAAATGGGAGGGGTGCAAAAGAGG	TTGCGTGAGTGTGGATGGGATTGGTG	
MYC	GCGTCCTGGGAAGGGAGATCCGGAGC	TTGAGGGGCATCGTCGCGGGAGGCTG	
KLF	CCCACACAGGTGAGAAACCTTACC	GTAGTGCTTTCTGGCTGGGCTC	
NANOG	CAGTCTGGACACTGGCTGAA	CTCGCTGATTAGGCTCCAAC	
LIN28	GAAATCCACAGCCCTACCCT	CTCTGCCTGCTCCTCAAAAC	
REX1	CAGATCCTAAACAGCTCGCAGAAT	GCGTACGCAAATTAAAGTCCAGA	
SHANK1	AGTTCCGATACAAGACCCGAG	CCGAGCTGCACATACTCCA	11968152a2
SHANK2	TGAAGGAGTCTCAACAGGGAC	CCTGGTGACCGTAGGGAAG	19743794a3
SHANK3ex2-3	AGGACGCGCTCAACTATGG	CTCGCCGCTTGTATCGAAACT	122937240b1
SHANK3ex8-9	GTCCTGCTCTTCCGTGGAG	TGGGTCTTGATAACCTCTGCAA	122937240b3
SHANK3ex22	GGAGAGCGGGGAACTCACT	CTGTCCGAGGACTGCTTCAG	13359173a1
SYNAPSIN1	TGAAGCCGGATTTTGTGCTGA	GACCAAACTGCGGTAGTCTCC	9924097a2
PCLO	CAGACACTTTCAGGTCAGAGC	AGGCATCATACTAGACTTGTGCT	6433936a2
BSN	CCACATCACCCTACTCCGTC	TTGCAGACCTTGTTGTGACAC	4508019a1
DSCAM	TTTTACGGGAGCCCTATACAGT	GCAACATTGCCTCTCATGGTTT	3169768a1
PSD95-DLG4	TCACAACCTCTTATTCCCAGCA	CATGGCTGTGGGGTAGTCG	1527215a1
PSD93-DLG2	GGCCTGGGATTCAGTATTGCT	CCCGCAAGATACAATCATTGACC	4557527a2
GEPHYRYN	TGCCATTGACCTTTTACGTGAT	ACAGCAGGACTGGTGTAGAAT	10880983a1
GRIN1	AGGAACCCCTCGGACAAGTT	CCGCACTCTCGTAGTTGTG	11496971a1

GRIN2A	GGGCTGGGACATGCAGAAT	CGTCTTTGGAACAGTAGAGCAA	4504125a3
GRIN2B	GTAGCCATGAATGAGACCGAC	GGATCGGGGTGAGAGTCTGT	4504127a1
GRIN3A	GACGCCCTCCTATTTGCCG	CCACGGTATGGCACACACT	18916849a1
MAPT-Tau	TACAAACCAGTTGACCTGAGCA	ATGGATGTTGCCTAATGAGCC	8400715a3
TUBB3	CGGTGGTGGAACCCTACAAC	AGGTGGTGACTCCGCTCAT	5174737a1
DCX	CCTTGGCTAGCAGCAACAGT	CCACTGCGGATGATGGTAA	
NCAM	ACATCACCTGCTACTTCCTGA	CTTGGACTCATCTTTCGAGAAGG	10834990a1
NSE	GGAGTTGGATGGGACTGAGAA	CTGAGCAATGTGGCGATACAG	5803011a3
TIS21-BTG2	CAGAGCACTACAAACACCACTG	CTGAGTCCGATCTGGCTGG	5802988a1
FOXG1	GCCACAATCTGTCCCTCAACA	CGGGTCCAGCATCCAGTAG	32307177a3
TLE1	AAGTTCACTATCCCGGAGTCC	TCTGTCTTTTCACTTGCCAGTTT	21541824a1
UNC5D	AAGCCCTTCCCGAATCCATC	AGTGCAATAGGGTTGCTCTTG	18254472a1
MEF2c	ATGCCATCAGTGAATCAAAGGAT	CTGGTAAAGTAGGAGTTGCTACG	298698a1
CUX1	GCTCTCATCGGCCAATCACT	TCTATGGCCTGCTCCACGT	
PLXND1	CATGGAGATGGCCTGTGACTA	GGAAGGGCGGAAACTGGTC	3327054a1
ETV1	CTGGATGACCCGGCAAATTCT	CCTCTTCAGGCTCAATCAGTTT	1045061a3
SATB2	TCTCCCCCTCAGTTATGTGAC	AGGCAAGTCTTCCAACTTTGAA	5689405a3
SYT9	TGGCAGACGACTGAAGAAGAG	GGATTTGGTCAATGTTCTCGGG	28376627a2
S100A10	GGACCAGTGTAGAGATGGCAAA	ATGGTGAGGCCCGCAATTA	
OMA1	TAGGCAGGGGCATAAGGAAAT	CTCAAACCAAGGAATAGCTTCCA	21686999a3
PCP4	GCTGGGCCAACCAATGGAA	CACGTTCTGTCTCTGGTGCAT	5453858a1
CRIM1	GCGTTTGCGAAGATGAGAACT	TGGTGTTACATTCACATTTCCCA	10092639a1
SOX5	CAGAGTGGCGAGTCCTTGTC	TTTCTTCCGGCTCGTTTTTGA	23308715a3
DKK3	TGGGGTCACTGCACCAAAAT	GAAGGTCGGCTTGCACACATA	27735014a1
NR4A3	CTGAGCATGTGCAACAATTCTAC	ACAGCTCCAAAAAGGCTGATTC	1311505a2
TLE4	ACAAGCAGGCAGAGATTGTCA	TCCATGTGATAAATGCTGGGC	6330948a1
LXN	AACGGGACAAGAAACTGCAC	CTAGCGGTTCCTTCATGGACT	21359933a3
SEMA3E	ATTGTTTGCTGGACTCTACAGT	CTTTCAACAGACGCTCATCGT	6912650a3
TBR1	GCCTTTCTCCTTCTATCATGCTC	GTCAGTGGTCGAGATAATGGGA	5730081a1
FOXP1	AGACAAAAAGTAACGGTTCAGCC	CGCACTCTAGTAAGTGGTTGC	21750965a3
FOXP2	TTTCTAAAGAACGCGAACGTCT	GCAATATGCACTTACAGGTTTGG	21518701a2
CTIP2-BCL11B	TGGGTGCCTGCTATGACAAG	GGCTCGGACACTTTCCTGAG	12597635a1

DLX5	AGCTCCTACCACCAGTACGG	GTTTGCCATTCACCATTCTCAC	4885187a3
PPP1R1B-DARPP32	AGTCTGCTGGGCAAAAGACAA	AGGCTCACTTAGTGCTGGGT	21735492a2
DRD1	AGGGACTTCTCTGTTCGTATCC	GTCGGAACCTGATAACGGCAG	4503383a1
DRD2	CAACGGGTCAGACGGGAAG	CTCCAGGTAGACAACCCAGG	17986270a3
TH	GCCCTACCAAGACCAGACGTA	CGTGAGGCATAGCTCCTGA	37127a2
DDC	ACTGGCTCGGGAAGATGCT	CCGATGGATCACTTTGGTCC	4503281a1
DBH	CTGAAGCCCAATATCCCCGAA	GTAGCACCAGTACGTGGTCTC	18426906a3
CRYGS	TGACTGTGATTGCGACTGTG	GCAAAGTTGGGCCTTTCATAAAC	8922120a1
TPM2	CTGAGACCCGAGCAGAGTTTG	TGAATCTCGACGTTCTCCTCC	4507649a1
PCP2	AGAGGCCAGCAGAAAAGTGACT	GTGGCTCAGCAGATTGAAGAA	
GRP	GTGGGGCACTTAATGGGGAAA	CTATGAGACCCAGCAAATTCCTT	31542860a1
DLX1	CCATGCCAGAAAGTCTCAACA	GGCCCAAACTCCATAAACACC	31418473a3
GAD67	GCCAGACAAGCAGTATGATGT	CCAGTTCCAGGCATTTGTTGAT	4503873a2
VGAT	CCGAGTGGTGAACGTAGCG	GTGGCGATAATGGACCAGGAC	17999520a3
CALB2	TCAGAGATGTCCCGACTCCTG	GCCGCTTCTATCCTTGTCGTAA	4502543a1
PVALB	GCTGAACGCTGAGGACATCAA	TCACATCATCCGCACTCTTTTTC	4506335a1
SOMATOST	GCTGCTGTCTGAACCCAAC	CGTTCTCGGGGTGCCATAG	4507243a1
VGLUT1	CGACGACAGCCTTTTGTGGT	GCCGTAGACGTAGAAAACAGAG	9945322a2
VGLUT2	GGGAGACAATCGAGCTGACG	CAGCGGATACCGAAGGAGATG	9966811a1
VGLUT3	AAACCGGAAATTCAGACAGCA	CCAAAGACCCTGTTAGCAGCA	21322234a2
SLC1A2	AAGTGCGAATGCCAGACAGTC	CAGGATGACACCAAACACCG	4759124a1
NEUROD1	ATGACCAAATCGTACAGCGAG	GTTCATGGCTTCGAGGTCGT	4505377a1

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