

A

Metadata						
Disease state	Number of participants	Biopsy type	Sex	Average age at harvest	MPS IIA Type	MPSIIIA patient genotypes
Neurotypical	5	Skin	2 x Female 3 x Male	7.7 years (from 4-12 years)	N/A	N/A
MPS IIIA	5	Skin	3 x Female 2 x Male	8.3 years (from 4-11 years)	Typical	c.733C>T; p.Arg245Cys / c.733C>T; p.Arg245Cys c.197C>G; p.Ser66Trp / c.1295_1303del; p.Tyr432_Arg435delinsCys c.734G>A; p.Arg245His / c.911G>T; p.Arg304Leu c.220C>T (p.Arg74Cys) / c.1080delC (p.Val361Serfs*52) c.734G>A; p.Arg245His / c.1416G>C; p.Gln472His

B

Culture details							
iPSC line	Reprogramming	Method	Split ratio	Reagents used	Passage confirmed loss of sendai virus	Passage frozen	Passage number at characterization
BIAD01	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	6	8	8
BIAD02	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	6	8	8
BIAD03	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	6	8	8
BIAD04	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	6	8	8
BIAD05	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	8	9	9
BIAD06	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	6	8	8
BIAD10	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	7	8	8
BIAD11	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	8	9	9
BIAD12	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	9	10	10
BIAD13	Sendai virus Non integrative	Feeder free	1:3-6	mTeSR™ Plus	8	9	9

C

Assay confirmation										
iPSC line	PCR analysis				Trilineage differentiation	Molecular characterization			Karyotyping	Manuscript figure reference
	CD44	OCT4	Nanog	SOX2		OCT4	KLF4	SOX2		
BIAD01	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD02	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD03	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD04	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD05	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD06	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD10	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD11	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD12	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10
BIAD13	✓	✓	✓	✓	Pass	✓	✓	✓	Normal	Fig 2, Supplementary Fig 7-10

Supplementary Figure 1 | iPSC line meta data

(A) iPSC line metadata, (B) cell culture details and (C) characterization details for all iPSC cell lines generated. See also Figures 1 and 2 and Supplementary Figures 7-10. MPS IIIA = mucopolysaccharidosis type IIIA.

A

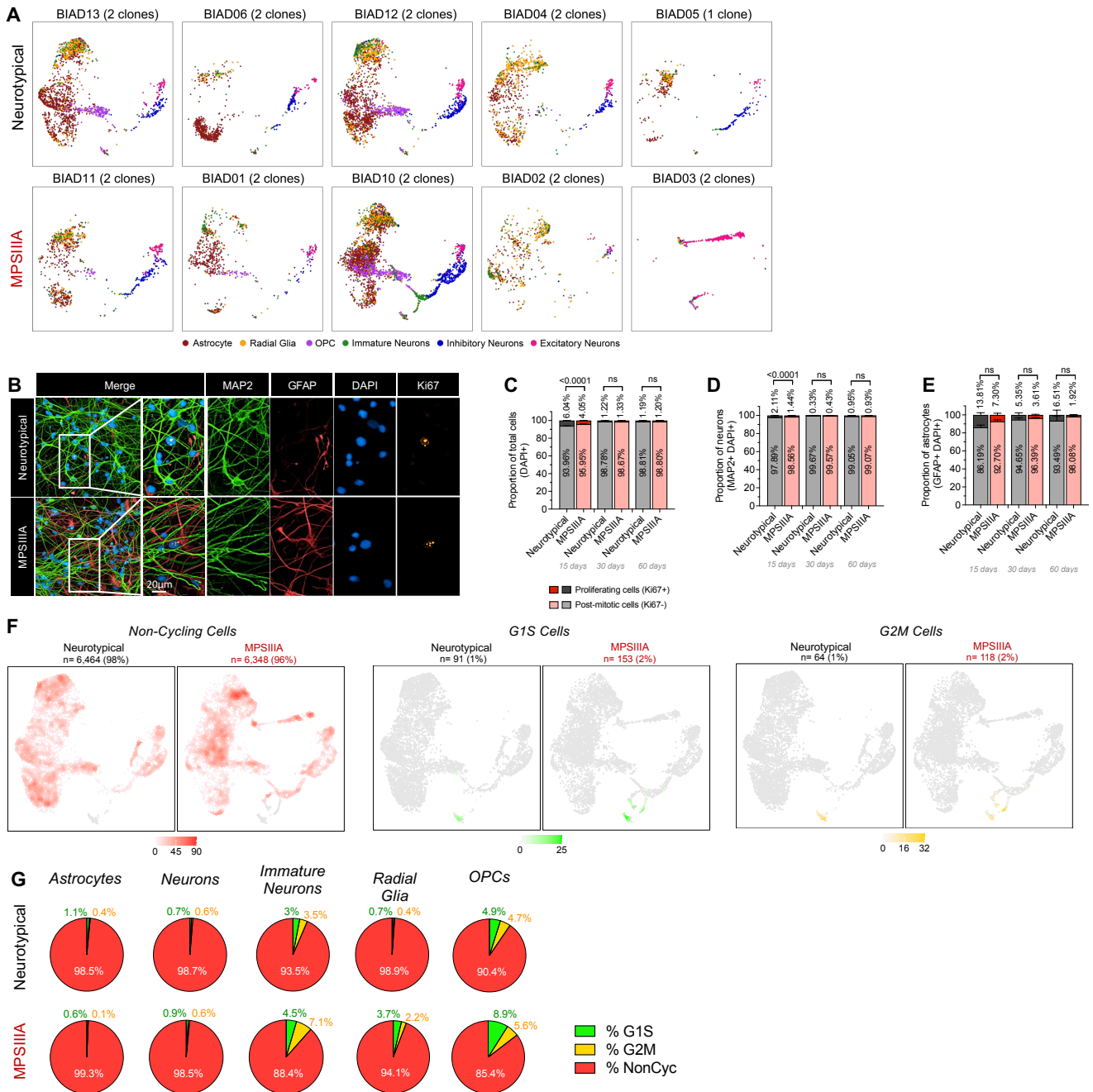
Phenotypic quality control of iPSCs (Figure 2A–G)			
Each point represents an individual patient (iPSC cell line)			
Cell line/patient	Type	Condition	Number of wells per cell line
BIAD04	iPSC	Neurotypical	3
BIAD05	iPSC	Neurotypical	3
BIAD06	iPSC	Neurotypical	3
BIAD12	iPSC	Neurotypical	3-6
BIAD13	iPSC	Neurotypical	3-6
WT	ESC	Neurotypical	3
BIAD01	iPSC	MPS IIIA	3
BIAD02	iPSC	MPS IIIA	3
BIAD03	iPSC	MPS IIIA	3
BIAD10	iPSC	MPS IIIA	2-6
BIAD11	iPSC	MPS IIIA	3-6
SGSHKO	ESC	MPS IIIA	3

B

Phenotypic quality control of NPCs (Figure 2H–P)				
Each point represents an NPC clone (2 NPC clones per patient)				
Patient	Clone	Derived from	Condition	Wells per clone
BIAD04	C1	iPSC	Neurotypical	3-6
BIAD04	C2	iPSC	Neurotypical	3-6
BIAD05	C1	iPSC	Neurotypical	3-6
BIAD06	C1	iPSC	Neurotypical	3-6
BIAD06	C2	iPSC	Neurotypical	3-6
BIAD12	C1	ESC	Neurotypical	3-6
BIAD12	C2	iPSC	Neurotypical	3-6
BIAD13	C1	iPSC	Neurotypical	3-6
BIAD13	C2	iPSC	Neurotypical	3-6
WT	C1	ESC	Neurotypical	3-6
BIAD01	C1	iPSC	MPS IIIA	3-6
BIAD01	C2	iPSC	MPS IIIA	3-6
BIAD02	C1	iPSC	MPS IIIA	3-6
BIAD02	C2	iPSC	MPS IIIA	3-6
BIAD03	C1	iPSC	MPS IIIA	3-6
BIAD03	C2	iPSC	MPS IIIA	3-6
BIAD10	C1	iPSC	MPS IIIA	3-6
BIAD10	C2	iPSC	MPS IIIA	3-6
BIAD11	C1	iPSC	MPS IIIA	3-6
BIAD11	C2	iPSC	MPS IIIA	3-6
SGSHKO	C1	ESC	MPS IIIA	3-6

Supplementary Figure 2 | Figure experimental breakdowns

Overview of patients, cell lines, replicates and data point breakdown used in each experiment in this study. Experimental summaries are split by iPSCs **(A)** and NPCs **(B)**. See also Figures 1 and 2. ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; MPS IIIA = mucopolysaccharidosis type IIIA; NPC = neural progenitor cell; WT = wild type; KO = knockout.



Supplementary Figure 3 | Characterisation of cell identity, differentiation and proliferation in Neurotypical and MPS IIIA iPSC-derived cultures

(A) UMAPs showing the distribution of various cell types among 5x Neurotypical donors and 5x MPS IIIA donors. All lines have 2 clones each except BIAD05, which contains one clone. **(B)** Representative images of immunofluorescence staining of Microtubule-Associated Protein 2 (MAP2), nuclear proliferation marker Ki67, Glial Fibrillary Acidic Protein (GFAP) and nuclear marker 4',6-diamidino-2-phenylindole (DAPI) at day 60, used to identify proliferating cell populations. Green indicates neuronal soma and dendrites (MAP2); red indicates astrocytes (GFAP); blue indicates nuclei (DAPI); yellow indicates proliferating cells (Ki67). **(C-E)** Stacked bar plots showing the proliferating and post-mitotic proportions of total cells (DAPI+), neurons (MAP2+ DAPI+) and astrocytes (GFAP+ DAPI+) at 15, 30 and 60 days. Data represents the mean $\pm$ SEM of playground wells from one independent neural differentiation (15 days: $n = 8$ wells for neurotypical and 10 wells for MPS IIIA; 30 days: $n = 12$ wells for neurotypical and 11 wells for MPS IIIA; 60 days: $n = 4$ wells for neurotypical and 4 wells for MPS IIIA), which was analysed using unpaired non-parametric two-tailed Mann-Whitney U tests. ns, not significant is defined as $P > 0.05$. **(F)** Cell cycle analysis shows that majority of cells in our 120-day cultures are not undergoing mitosis, indicative of 98% and 97% Non-cycling cells in Neurotypical and MPS IIIA. **(G)** Pie charts showing that the small percentage of dividing cells correspond to immature cells types. Green indicates G1S phase, yellow indicates G2M phase and red indicates non cycling. See also Figure 1. Source data are provided as a Source Data file. OPC = oligodendrocyte progenitor cell; MPS IIIA = mucopolysaccharidosis type IIIA; NonCyc = Non-cycling.

Whole-cell patch clamp summary			30-60 Days						90-120 Days						
Cell Line	Cell type	Genotype	n patched	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	n coverslips used	Avg neurons per coverslip	n patched	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	n coverslips used	Avg neurons per coverslip	Figures
H9 WT	ESC	Neurotypical	25	60	2	2020-12-07 2021-07-29	7	5-6	27	96	1	2021-07-29	5	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD04	iPSC	Neurotypical	35	55	2	2021-02-20 2022-01-26	6	5-6	34	87	2	2021-02-20 2022-01-26	6	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD06	iPSC	Neurotypical	17	67	1	2021-04-26	3	5-6	26	96	1	2021-04-26	4	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD12-cNP22	iPSC	Neurotypical	24	54	1	2022-06-03	4	5-6	29	97	1	2022-06-03	7	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD12-cNP24	iPSC	Neurotypical	25	54	1	2022-06-03	4	5-6	36	100	1	2022-06-03	6	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD13	iPSC	Neurotypical	23	56	1	2022-06-26	4	5-6	23	91	1	2022-06-26	5	5-6	Fig 3A-G, 4A-J, 5E-M
H9 SGSH-KO	ESC	MPSIIIA	25	63	2	2020-12-07 2021-07-29	6	5-6	35	97	1	2020-12-07 2021-07-29	6	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD01	iPSC	MPSIIIA	37	54	2	2021-02-20 2022-01-26	6	5-6	39	88	2	2021-02-20 2022-01-26	6	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD03	iPSC	MPSIIIA	15	68	1	2021-04-26	3	5-6	34	96	1	2021-04-26	5	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD02	iPSC	MPSIIIA	18	66	1	2021-06-25	4	5-6	19	90	2	2021-06-25 2021-09-23	4	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD10	iPSC	MPSIIIA	25	56	1	2022-06-26	4	5-6	35	98	2	2022-06-26 2022-06-30	6	5-6	Fig 3A-G, 4A-J, 5E-M
BIAD11	iPSC	MPSIIIA	23	56	1	2022-06-26	4	5-6	32	91	1	2022-06-26	4	5-6	Fig 3A-G, 4A-J, 5E-M
Neurotypical Playground	iPSC	Neurotypical	44	55	1	2023-01-15	8	5-6	124	101	2	2023-01-15 2023-12-05	12	5-6	Fig 3A-G, 4A-J, 5E-M
MPSIIIA Playground	iPSC	MPSIIIA	41	54	1	2023-01-15	7	5-6	127	102	2	2023-01-15 2023-12-05	13	5-6	Fig 3A-G, 4A-J, 5E-M
Total Neurotypical:			193	57.3	9		36		299	95.4	9		45		
Total MPSIIIA:			184	59.6	9		34		321	94.6	11		44		

Supplementary Figure 4 | Summary of all whole-cell patch clamped cells by cell line or playground model.
 Overview of the number of neurons whole-cell patch clamped from each individual cell line, their average days in culture, the number of independent neural differentiations and the neural differentiation date for each time point. ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; MPS IIIA = mucopolysaccharidosis type IIIA; WT = wild type; KO = knockout.

Multielectrode array summary for individual cell lines and playground (Figure 6)						
Cell Line	Type	Genotype	n wells	n electrodes	n independent neural differentiations	Neural differentiation batch date(s)
BIAD04-cNP10	iPSC	Neurotypical	30	480	3	2021-02-20, 2022-02-06, 2022-06-25
BIAD04-cNP11	iPSC	Neurotypical	9	144	1	2022-06-25
BIAD05-cNP23	iPSC	Neurotypical	12	192	1	2022-06-25
BIAD06-cNP08	iPSC	Neurotypical	19	304	2	2022-02-06, 2022-06-25
BIAD06-cNP09	iPSC	Neurotypical	9	144	1	2022-06-25
BIAD01-cNP06	iPSC	MPSIIIA	43	688	4	2021-02-20, 2022-02-06, 2022-02-06, 2022-06-25
BIAD01-cNP07	iPSC	MPSIIIA	10	160	1	2022-06-25
BIAD02-cNP14	iPSC	MPSIIIA	12	192	1	2022-06-25
BIAD02-cNP16	iPSC	MPSIIIA	36	576	3	2021-05-25, 2022-02-06, 2022-06-25
BIAD03-cNP12	iPSC	MPSIIIA	8	128	1	2022-06-25
BIAD03-cNP13	iPSC	MPSIIIA	12	192	2	2022-02-06, 2022-06-25
BIAD10-cNP25	iPSC	MPSIIIA	11	176	1	2022-06-25
BIAD10-cNP26	iPSC	MPSIIIA	11	176	1	2022-06-25
BIAD11-cNP30	iPSC	MPSIIIA	9	144	1	2022-06-25
BIAD11-cNP33	iPSC	MPSIIIA	9	144	1	2022-06-25
BIAD12-cNP22	iPSC	Neurotypical	8	128	1	2022-06-25
BIAD12-cNP24	iPSC	Neurotypical	9	144	1	2022-06-25
BIAD13-cNP18	iPSC	Neurotypical	10	160	1	2022-06-25
BIAD13-cNP20	iPSC	Neurotypical	8	128	1	2022-06-25
Neurotypical Playground	iPSC	Neurotypical	24	384	1	2023-06-06
MPSIIIA Playground	iPSC	MPSIIIA	24	384	1	2023-06-06
Total Neurotypical			138 wells	2,208 electrodes	13 independent neural differentiations	
Total MPSIIIA			185 wells	2,960 electrodes	17 independent neural differentiations	

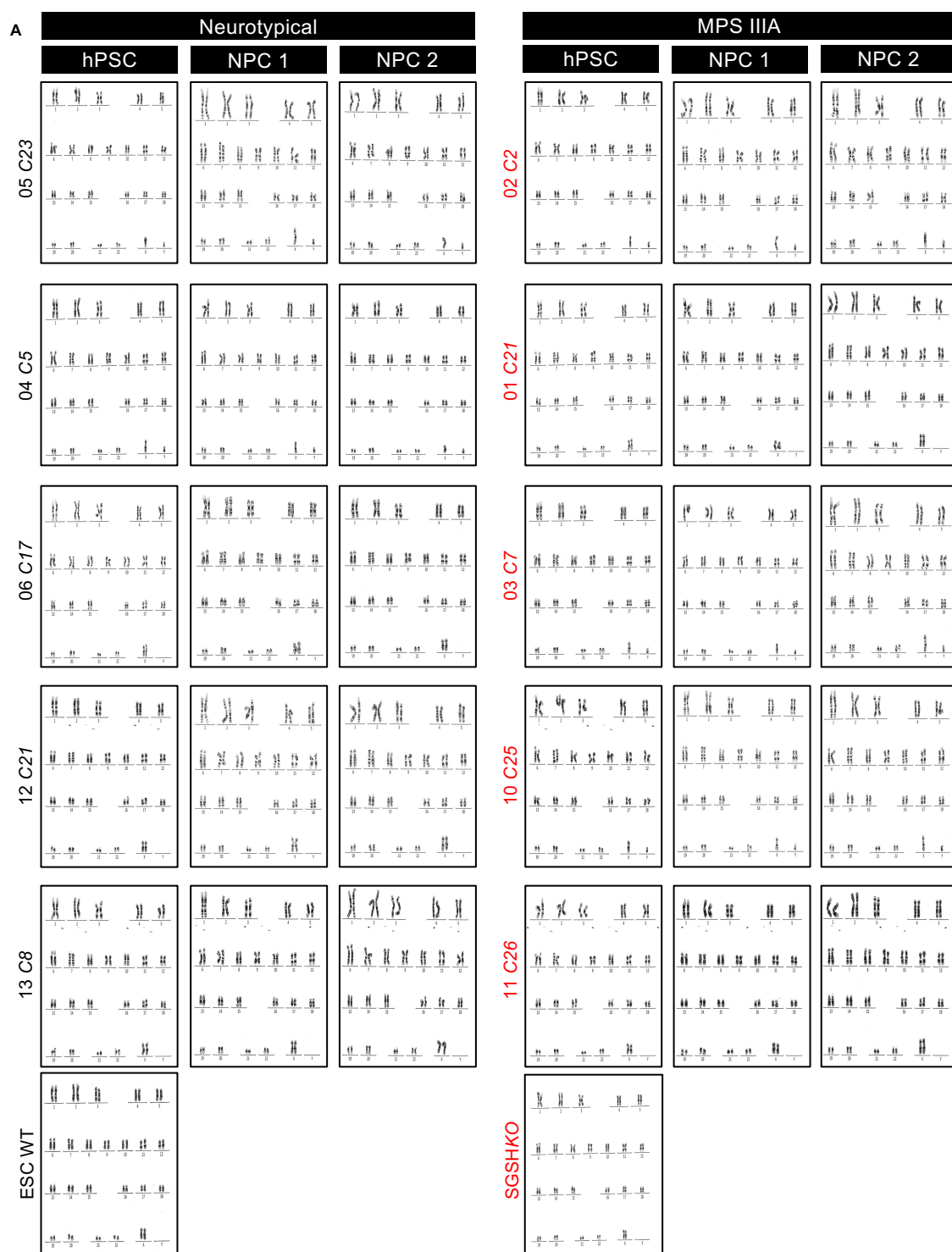
Supplementary Figure 5 | Summary of all multielectrode array recordings, wells, electrodes and experiments by cell line or playground model.

Overview of all the multielectrode array wells and electrodes recorded over 90 days of maturation in BrainPhys reduced neural maturation media. Data is broken down by individual patient cell line and clone, with the cell type and genotype listed. Summary of the number of independent neural differentiations (experiments), dates of neural differentiation (batches) and the associated figures for each cell line or playground model. Summary of the total number of wells, electrodes and independent neural differentiations per genotype. See also Figure 6. iPSC = induced pluripotent stem cell; MPS IIIA = mucopolysaccharidosis type IIIA.

Total neurons (MAP2+ DAPI+)	Maturation (Days <i>in vitro</i>)				
Cell Line	15	30	60	90	Figures
Neurotypical Playground	359,913	330,946	50,104	196,845	Fig 5A-D
MPSIIIA Playground	507,083	395,339	123,644	279,543	Fig 5A-D
Neurotypical Playground + cellular stress	-	149,777	-	-	Fig 5D
MPSIIIA Playground + cellular stress	-	172,295	-	-	Fig 5D
Neurons per timepoint	866,996	1,048,417	173,748	476,388	
Total	2,565,549 neurons				

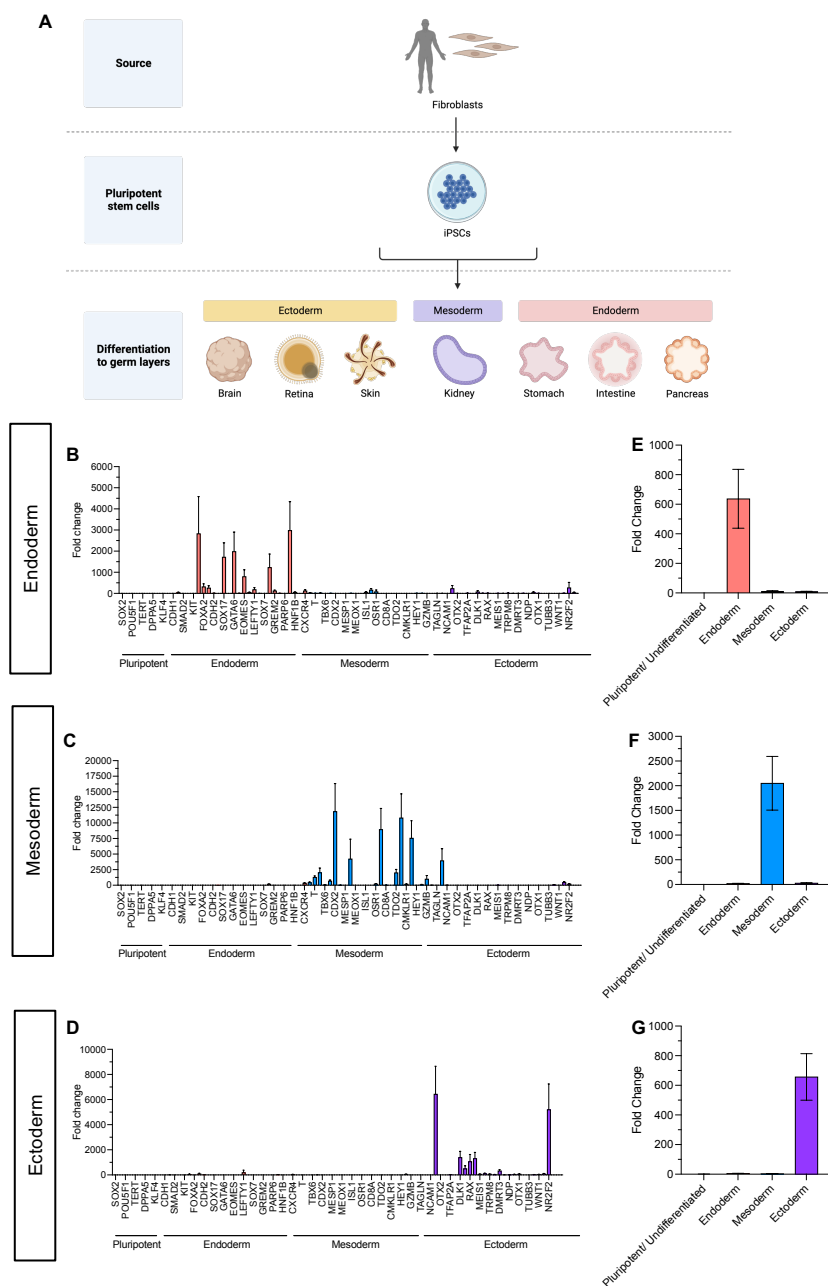
Number of wells imaged	Maturation (Days <i>In vitro</i>)				# of independent neural differentiations	Neural differentiation date(s)
Cell Line	15	30	60	90		
Neurotypical Playground	40	31	7	43	3	2023-01-15, 2023-09-05, 2025-03-18
MPSIIIA Playground	48	38	15	50	3	2023-01-15, 2023-09-05, 2025-03-18
Neurotypical Playground + Cellular stress	-	16	-	-	1	2023-01-15
MPSIIIA Playground + Cellular stress	-	16	-	-	1	2023-01-15
Wells imaged per timepoint	88	101	22	93	8 independent neural differentiations	
Total	304 wells imaged					

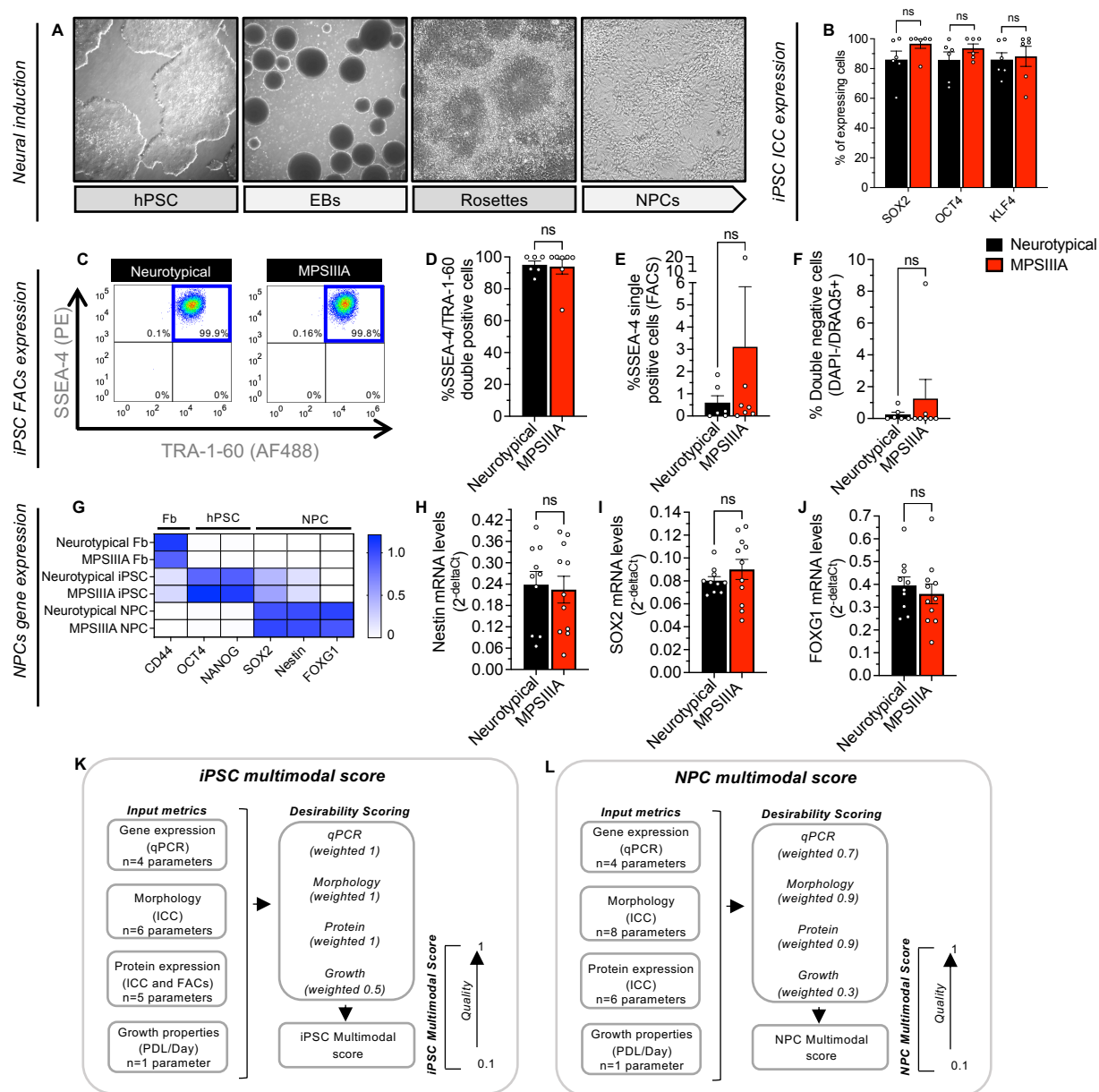
Supplementary Figure 6 | Analysis summary of high-content imaging from playground model.
 Overview of the number of neurons imaged from the respective number of wells using high-content microscopy. The number of neurons are defined as MAP2+ DAPI+ cells. Data is summarised per timepoint and total across all experiments. The number of independent neural differentiations (experiments) included for each cell line, the dates of neural differentiation and their associated figures are listed. See also Figure 5. MPS IIIA = mucopolysaccharidosis type IIIA.



Supplementary Figure 7 | iPSC and NPC karyotyping

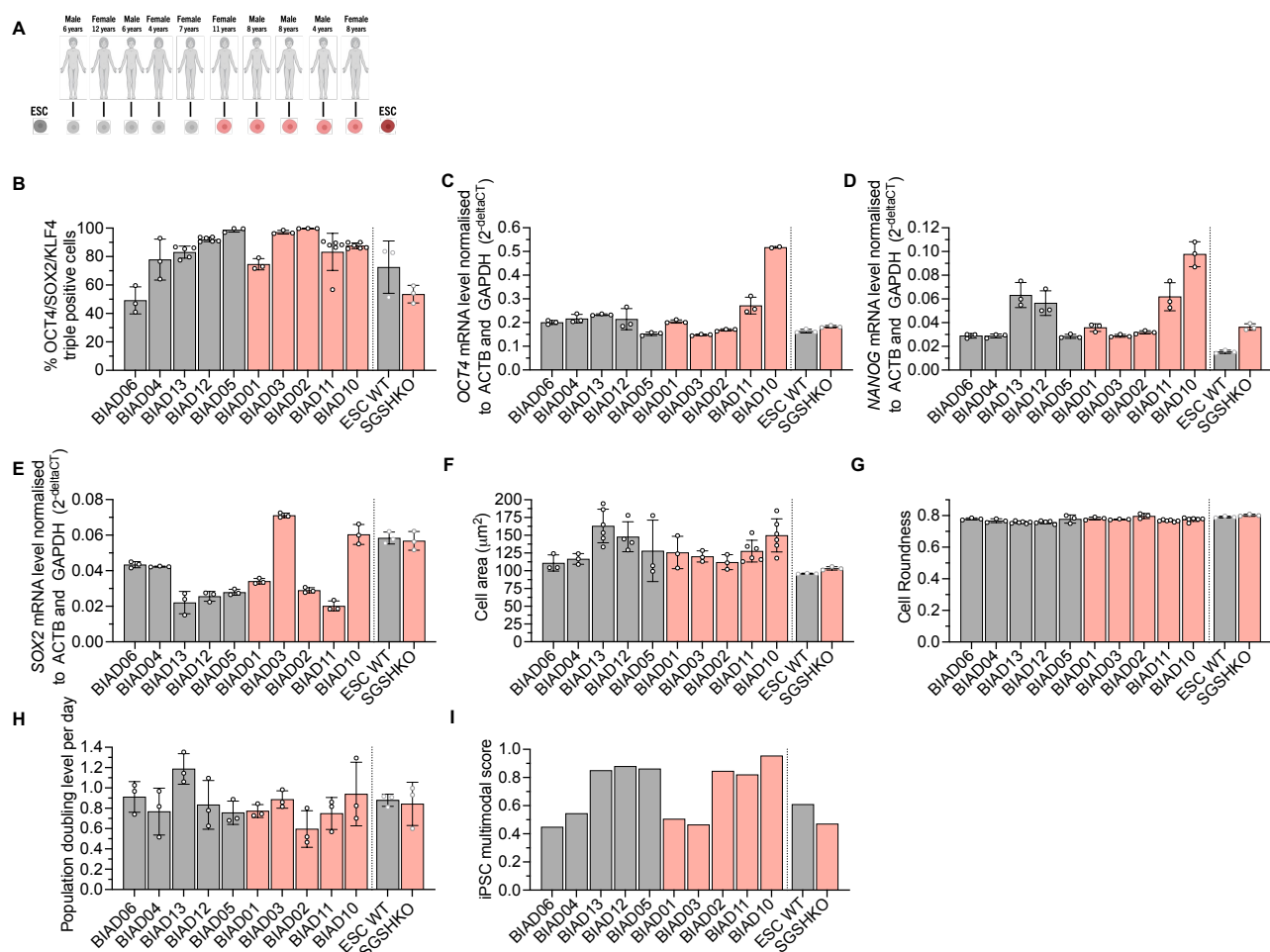
G-band karyotyping was performed for all hPSCs and NPCs used in this study. **(A)** Representative G-band karyotype analysis from 20 metaphases analysed for each hPSC and NPC line. See also Figure 2. ESC = embryonic stem cell; hPSC = human pluripotent stem cell; NPC = neural progenitor cell; MPS IIIA = mucopolysaccharidosis type IIIA; WT = wild type; SGSHKO = SGSH knockout.





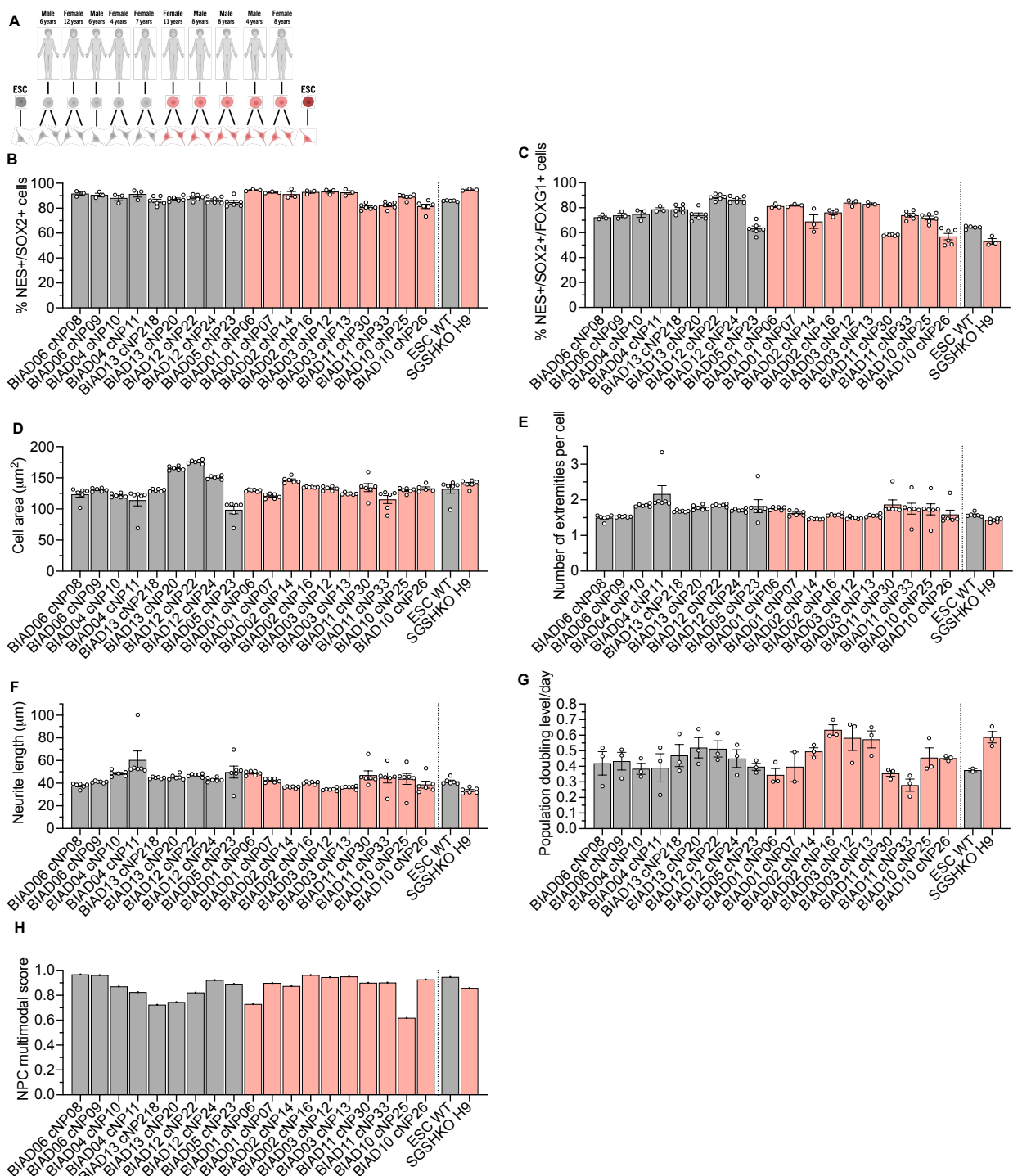
Supplementary Figure 9 | iPSC and NPC quality control

Quality control of hPSCs and NPCs to generate functional neural cultures. **(A)** Neural induction using SMAD inhibition of iPSCs to NPCs. Representative images of stages during hPSC neural induction when hPSCs undergo dual SMAD inhibition. **(B)** Quantification of immunofluorescence staining of iPSCs from Neurotypical and MPS IIIA patients for % of cells expressing SOX2, OCT4 and KLF4. **(C-F)** Expression of pluripotency surface markers SSEA-4 and TRA-1-60. **(C)** Representative density plots for expression of SSEA-4 and TRA-1-60 using flow cytometry on neurotypical and MPS IIIA iPSCs. Quantification of flow cytometry for **(D)** % of double positive (SSEA-4 and TRA-1-60) cells, **(E)** % of single positive SSEA-4 cells and **(F)** % of double negative (SSEA-4 and TRA-1-60) cells. **(B,D-F)** Each data point represents the mean of an individual patient. Bar graphs represent the means $\pm$ SEM of 6 neurotypical and 6 MPS IIIA hPSC lines. Black indicates neurotypical; red indicates MPS IIIA. Significance was determined using unpaired, two-tailed Mann-Whitney U tests. ns, not significant is defined as $P > 0.05$. **(G-J)** NPC gene expression of progenitor makers. **(G)** Heatmap to represent quantification of gene expression (qRT-PCR) for fibroblasts, human pluripotent stem cells, neural progenitor cells and neurons (y-axis) with respective cell type specific markers (X-axis) for neurotypical and MPS IIIA. Expression of progenitor and cortical genes **(H)** Nestin, **(I)** SOX2 and **(J)** FOXG1 in neurotypical and MPS IIIA. Each data point represents the mean of an individual NPC clone. Bar graphs represent the means $\pm$ SEM of 13 neurotypical and 13 MPS IIIA NPC lines. Black indicates neurotypical; red indicates MPS IIIA. Significance was determined using unpaired, two-tailed Mann-Whitney U tests. ns, not significant is defined as $P > 0.05$. **(K,L)** Schematic flow diagram of **(K)** iPSC and **(L)** NPC multimodal score criteria. See also Figure 2. Source data are provided as a Source Data file. EB = embryoid body; FACs = fluorescence-activated cell sorting; FB = fibroblast; hPSC = human pluripotent stem cell; ICC = immunocytochemistry; iPSC = induced pluripotent stem cell; MPS IIIA = mucopolysaccharidosis type IIIA; NPC = neural progenitor cell; qPCR = quantitative polymerase chain reaction.



Supplementary Figure 10 | Individual iPSC line phenotypic quality control.

Characterisation of pluripotency, morphology and proliferation in neurotypical and MPS IIIA iPSCs. **(A-H)** One iPSC clone was included per patient as well as an ESC control and ESC SGSH-KO line. Each data point represents the mean of a well. Bar graphs represent the means $\pm$ SD of 2-6 wells. Grey indicates wildtype or neurotypical; peach indicates SGSH knockout or MPS IIIA. **(A)** Schematic of representation of iPSCs used per donor. Created in BioRender. Bardy, C. (2026) <https://BioRender.com/d85fq44>; adapted by authors. **(B-D)** Relative mRNA expression of pluripotency genes SOX2 **(A)**, OCT4 **(B)** and NANOG **(C)**, each normalised to two housekeeping genes (ACTB and GAPDH). **(E)** Percentage of cells triple-positive for pluripotency markers OCT4, SOX2 and KLF4. **(F-G)** Quantification of cellular morphology, cell area **(F)** and cell roundness **(G)**. **(H)** The population doubling rate per day of iPSC lines, averaged across 3 passages for each line. **(I)** IPSC multimodal score. IPSC multimodal scores were used to determine overall quality of each iPSC line and were generated using a custom script taking into account gene expression, protein expression, growth rate and morphology. See also Figure 2. Source data are provided as a Source Data file. ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; WT = wild type; SGSHKO = SGSH knock out; MPS IIIA = mucopolysaccharidosis type IIIA.

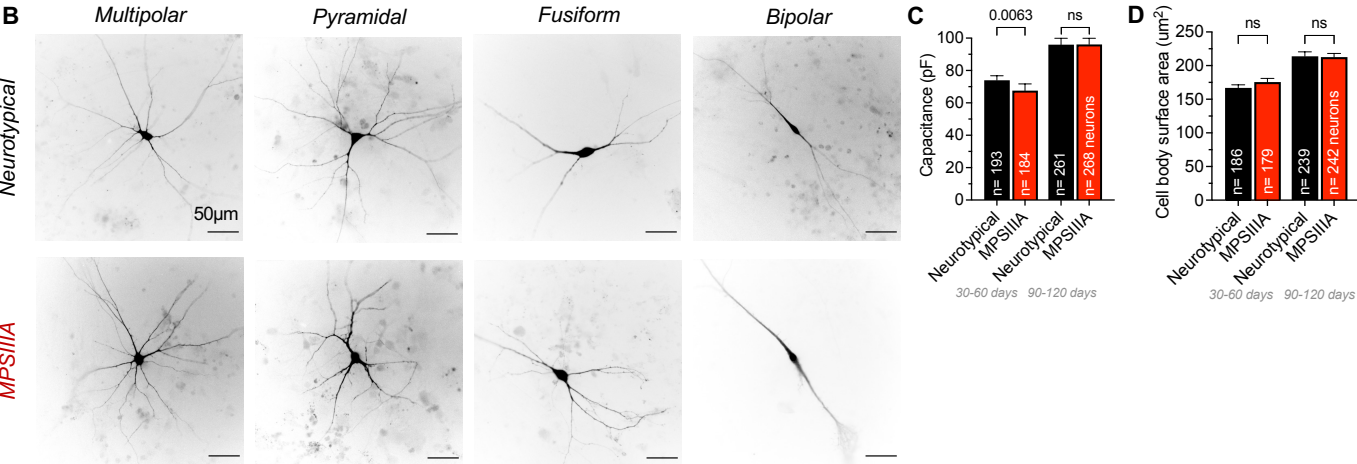


Supplementary Figure 11 | Individual NPC line phenotypic quality control.

Characterisation of cortical and progenitor markers, morphology and proliferation in Neurotypical and MPS IIIA NPCs. **(A-H)** Two NPC clones were included per patient (with the exception of BIAD05) as well as an ESC derived NPC wildtype control and SGSH-KO NPC line. Each data point represents the mean of a well. Bar graphs represent the means $\pm$ SD of 3-6 wells. Grey indicates wildtype or neurotypical; peach indicates SGSH knockout or MPS IIIA. **(A)** Schematic of representation of NPCs used per donor. *Created in BioRender. Bardy, C. (2026) <https://BioRender.com/d85fq44>*; adapted by authors. **(B-C)** Percentage of cells positive for cortical and progenitor markers **(B)** Nestin, SOX2 and FOXG1 and **(C)** Nestin, SOX2. **(D-F)** Cell size and complexity analysis of neurotypical and MPS IIIA NPCs showing, **(D)** cell area, **(E)** number of extremities per cell and **(F)** total neurite length. **(G)** The population doubling rate per day of NPC lines, averaged across 3 passages for each line. **(H)** NPC multimodal score. NPC multimodal scores were used to determine overall quality of each NPC line and were generated using a custom script taking into account gene expression, protein expression, growth rate and morphology. See also Figure 2. Source data are provided as a Source Data file. ESC = embryonic stem cell; NPC = neural progenitor cell; WT = wild type; SGSHKO = SGSH knock out; MPS IIIA = mucopolysaccharidosis type IIIA.

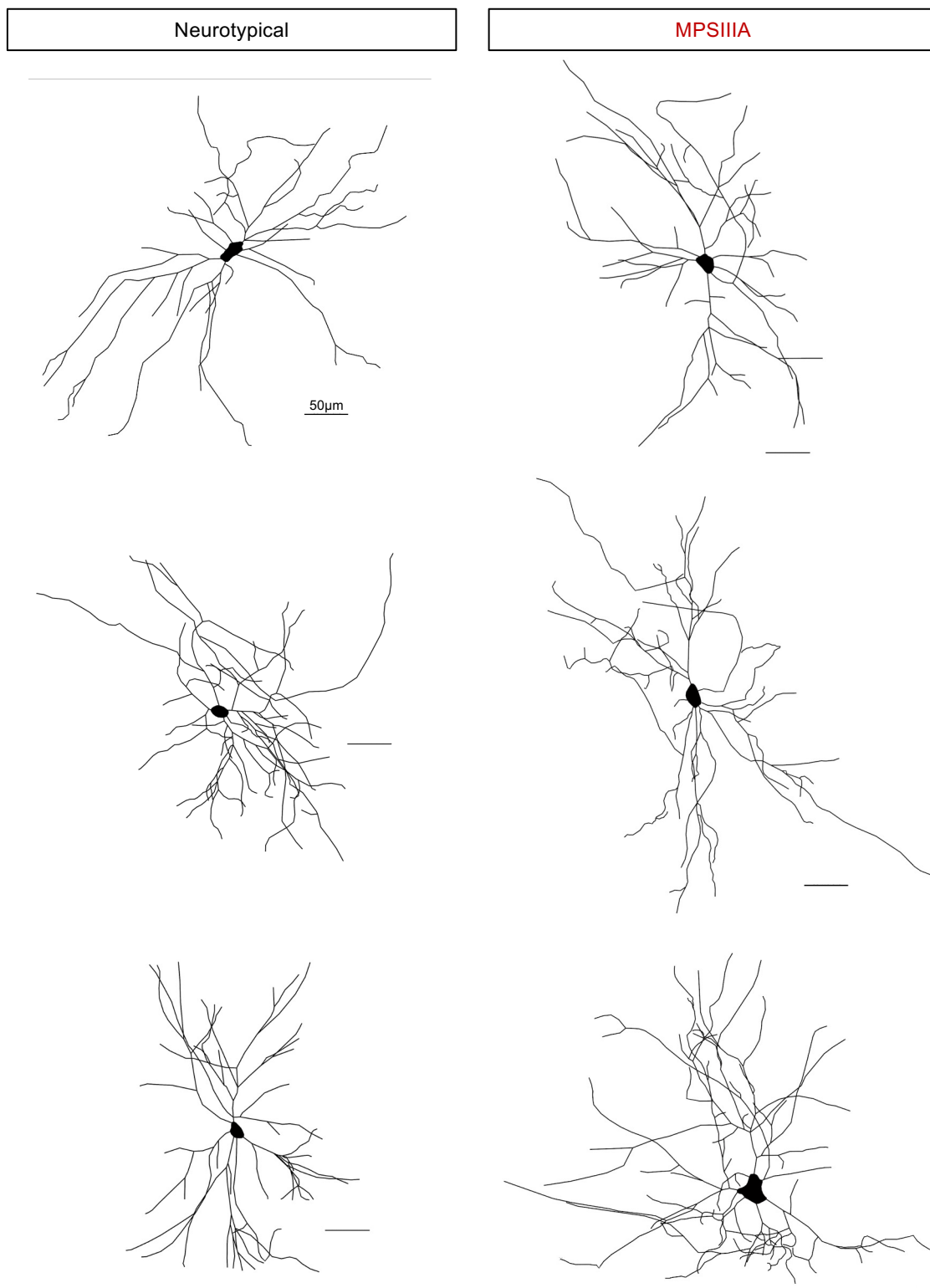
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Morphology analysis per cell line			30-60 Days				90-120 Days				
Cell Line	Cell type	Genotype	n neurons reconstructed	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	n neurons reconstructed	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	Figures
H9 WT	ESC	Neurotypical	22	49	2	2020-12-07 2021-07-29	18	98	2	2020-12-07 2021-07-29	Fig 3A-G
BIAD04	iPSC	Neurotypical	29	56	2	2021-02-20 2022-01-26	27	87	2	2021-02-20 2022-01-26	Fig 3A-G
BIAD06	iPSC	Neurotypical	16	67	1	2021-04-26	23	97	1	2021-04-26	Fig 3A-G
BIAD12-cNP22	iPSC	Neurotypical	25	54	1	2022-06-03	26	98	1	2022-06-03	Fig 3A-G
BIAD12-cNP24	iPSC	Neurotypical	25	55	1	2022-06-03	33	100	1	2022-06-03	Fig 3A-G
BIAD13	iPSC	Neurotypical	24	56	1	2022-06-26	24	91	1	2022-06-26	Fig 3A-G
H9 SGSH-KO	ESC	MPSIIIA	22	52	2	2020-12-07 2021-07-29	21	98	2	2020-12-07 2021-07-29	Fig 3A-G
BIAD01	iPSC	MPSIIIA	40	54	2	2021-02-20 2022-01-26	41	88	2	2021-02-20 2022-01-26	Fig 3A-G
BIAD03	iPSC	MPSIIIA	15	68	1	2021-04-26	25	96	1	2021-04-26	Fig 3A-G
BIAD02	iPSC	MPSIIIA	14	66	1	2021-06-25	14	90	1	2021-06-25	Fig 3A-G
BIAD10	iPSC	MPSIIIA	25	56	1	2022-06-26	36	98	1	2022-06-26	Fig 3A-G
BIAD11	iPSC	MPSIIIA	23	56	1	2022-06-26	21	91	1	2022-06-26	Fig 3A-G
Neurotypical Playground	iPSC	Neurotypical	45	55	1	2023-01-15	88	104	1	2023-01-15	Fig 3A-G
MPSIIIA Playground	iPSC	MPSIIIA	40	54	1	2023-01-15	84	104	1	2023-01-15	Fig 3A-G
Total Neurotypical:			186	56.0	9		239	96.4	9		
Total MPSIIIA:			179	58.0	9		242	95.0	9		



Supplementary Figure 12 | Summary of neurons from each cell line or playground model that were morphologically reconstructed

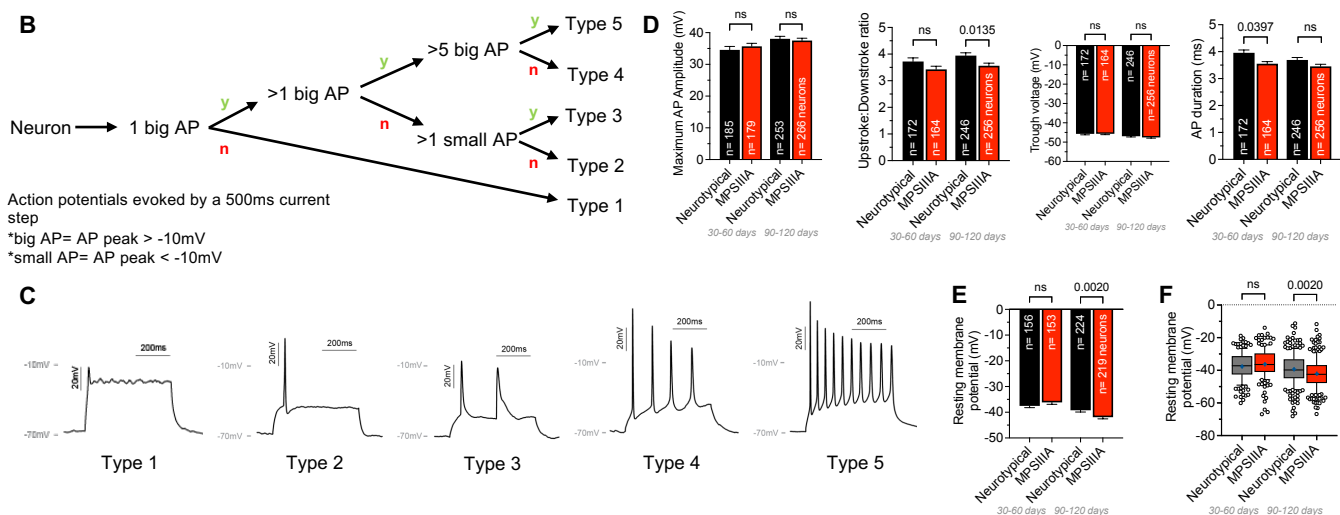
(A) Summary of the number of morphological reconstructions used in for analysis from each cell line or playground model at each whole-cell patch clamping time point. **(B)** Example rhodamine images of four morphological types from neurotypical and MPS IIIA that neurons that highlight similar neurite complexity and soma size. **(C)** Histograms displaying the mean $\pm$ SEM for capacitance. 30-60 days: $n = 193$ neurons in neurotypical and 184 neurons in MPS IIIA. 90-120 days: $n = 261$ neurons in neurotypical and 268 neurons in MPS IIIA. **(D)** Histograms displaying the mean $\pm$ SEM for cell body surface area. 30-60 days: $n = 186$ neurons in neurotypical and 179 neurons in MPS IIIA. 90-120 days: $n = 239$ neurons in neurotypical and 242 neurons in MPS IIIA. **(C-D)** Statistical analysis was completed using a Kruskal Wallis test with Dunn's correction for multiple comparisons. ns, not significant is defined as $P > 0.05$. Black indicates neurotypical; red indicates MPS IIIA. See also Figure 3. Source data are provided as a Source Data file. ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; MPSIIIA = mucopolysaccharidosis type IIIA; WT = wild type; KO = knockout; pF = picofarad.



Supplementary Figure 13 | Neurite complexity and soma size are comparable between neurotypical and MPS IIIA patient-derived neurons

Morphological reconstructions of rhodamine images taken during whole-cell patch clamping show that the neurite and soma complexity is similar between neurotypical and MPS IIIA iPSC-derived neurons. See also Figure 3. MPSIIIA = mucopolysaccharidosis type IIIA

Action potential analysis per cell line			30-60 Days					90-120 Days					
Cell Line	Cell type	Genotype	n neurons patched	n type 5	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	n neurons patched	n type 5	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	Figures
H9 WT	ESC	Neurotypical	25	12	60	2	2020-12-07 2021-07-29	26	24	96	2	2021-07-29	Fig 4A-E
BIAD04	iPSC	Neurotypical	32	24	56	2	2021-02-20 2022-01-26	33	20	87	2	2021-02-20 2022-01-26	Fig 4A-E
BIAD06	iPSC	Neurotypical	17	7	67	1	2021-04-26	25	18	96	1	2021-04-26	Fig 4A-E
BIAD12-cNP22	iPSC	Neurotypical	22	7	54	1	2022-06-03	22	11	96	1	2022-06-03	Fig 4A-E
BIAD12-cNP24	iPSC	Neurotypical	23	7	55	1	2022-06-03	31	16	99	1	2022-06-03	Fig 4A-E
BIAD13	iPSC	Neurotypical	23	19	56	1	2022-06-26	23	17	91	1	2022-06-26	Fig 4A-E
H9 SGSH-KO	ESC	MPSIIIA	24	16	65	2	2020-12-07 2021-07-29	35	26	97	2	2020-12-07 2021-07-29	Fig 4A-E
BIAD01	iPSC	MPSIIIA	36	14	54	2	2021-02-20 2022-01-26	39	24	88	2	2021-02-20 2022-01-26	Fig 4A-E
BIAD03	iPSC	MPSIIIA	15	7	68	1	2021-04-26	32	15	96	1	2021-04-26	Fig 4A-E
BIAD02	iPSC	MPSIIIA	18	8	66	1	2021-06-25	19	13	90	1	2021-06-25 2021-09-23	Fig 4A-E
BIAD10	iPSC	MPSIIIA	25	19	56	1	2022-06-26	33	26	99	1	2022-06-26 2022-06-30	Fig 4A-E
BIAD11	iPSC	MPSIIIA	23	17	56	1	2022-06-26	17	10	91	1	2022-06-26	Fig 4A-E
Neurotypical Playground	iPSC	Neurotypical	44	15	55	1	2023-01-15	86	60	103	2	2023-01-15 2023-12-05	Fig 4A-E
MPSIIIA Playground	iPSC	MPSIIIA	41	22	54	1	2023-01-15	83	73	105	2	2023-01-15 2023-12-05	Fig 4A-E
Total Neurotypical:			186	91.0	57.6	9		246.0	166	95.4	10		
Total MPSIIIA:			182	103.0	59.9	9		258.0	187	95.1	10		

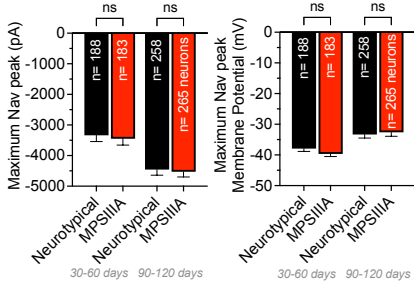


(A) Number of patched cells from each individual line or playground model included in the action potential analysis. **(B)** Decision tree of how neurons are classified into action potential types. Classification done using 500ms depolarising stimulus according to previous literature.⁶⁴ **(C)** Example traces of each respective action potential type. Type 1 is the least functional and Type 5 is the most functional. **(D)** Histograms displaying the mean $\pm$ SEM for capacitance for action potential kinetics parameters. 30-60 days: n= 172-185 neurons in neurotypical and 164-179 neurons in MPS IIIA. 90-120 days: n= 246-253 neurons in neurotypical and 266 neurons in MPS IIIA. **(E)** Resting membrane potential is similar over neurodevelopment between neurotypical and MPS IIIA neurons, with slightly greater hyperpolarisation seen at 90-120 days. **(F)** Box and whisker plot highlighting distribution of resting membrane potential data. The box represents the 25th to 75th percentiles, with the median located between these. The whiskers represent the 10th to 90th percentiles and the blue plus sign represents the mean of the data. Any data points present are those that fall outside the box and whisker ranges. 30-60 days: n= 156 neurons in neurotypical and 153 neurons in MPS IIIA. 90-120 days: n= 224 neurons in neurotypical and 219 neurons in MPS IIIA. **(D-F)** Statistical analysis was completed using a Kruskal Wallis test with Dunn's correction for multiple comparisons. ns, not significant is defined as $P > 0.05$. Black indicates neurotypical; red indicates MPS IIIA. See also Figure 4. Source data are provided as a Source Data file. AP = action potential; ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; MPSIIIA = mucopolysaccharidosis type IIIA; WT = wild type; KO = knockout; mV = millivolt; ms = millisecond.

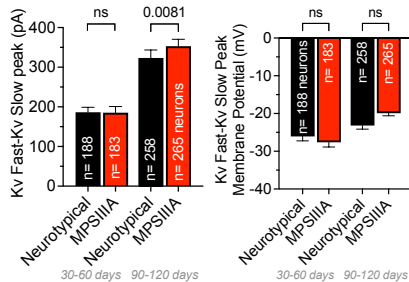
A

Nav/Kv channel analysis per cell line			30-60 Days				90-120 Days				
Cell Line	Cell type	Genotype	n neurons patched	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	n neurons patched	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	Figures
H9 WT	ESC	Neurotypical	24	60	2	2020-12-07 2021-07-29	27	96	2	2021-07-29	Fig 4F-J
BIAD04	iPSC	Neurotypical	33	55	2	2021-02-20 2022-01-26	34	87	2	2021-02-20 2022-01-26	Fig 4F-J
BIAD06	iPSC	Neurotypical	17	67	1	2021-04-26	25	96	1	2021-04-26	Fig 4F-J
BIAD12-cNP22	iPSC	Neurotypical	24	54	1	2022-06-03	25	95	1	2022-06-03	Fig 4F-J
BIAD12-cNP24	iPSC	Neurotypical	25	54	1	2022-06-03	32	98	1	2022-06-03	Fig 4F-J
BIAD13	iPSC	Neurotypical	23	56	1	2022-06-26	23	91	1	2022-06-26	Fig 4F-J
H9 SGSH-KO	ESC	MPSIIIA	24	65	2	2020-12-07 2021-07-29	35	97	2	2020-12-07 2021-07-29	Fig 4F-J
BIAD01	iPSC	MPSIIIA	37	54	2	2021-02-20 2022-01-26	38	88	2	2021-02-20 2022-01-26	Fig 4F-J
BIAD03	iPSC	MPSIIIA	15	68	1	2021-04-26	32	96	1	2021-04-26	Fig 4F-J
BIAD02	iPSC	MPSIIIA	18	66	1	2021-06-25	19	90	1	2021-06-25 2021-09-23	Fig 4F-J
BIAD10	iPSC	MPSIIIA	25	56	1	2022-06-26	33	99	1	2022-06-26 2022-06-30	Fig 4F-J
BIAD11	iPSC	MPSIIIA	23	56	1	2022-06-26	17	91	1	2022-06-26	Fig 4F-J
Neurotypical Playground	iPSC	Neurotypical	44	55	1	2023-01-15	85	103	2	2023-01-15 2023-12-05	Fig 4F-J
MPSIIIA Playground	iPSC	MPSIIIA	41	54	1	2023-01-15	83	105	2	2023-01-15 2023-12-05	Fig 4F-J
Total Neurotypical:			190	57.3	9		251	95.1	10		
Total MPSIIIA:			183	59.9	9		257	95.1	10		

B

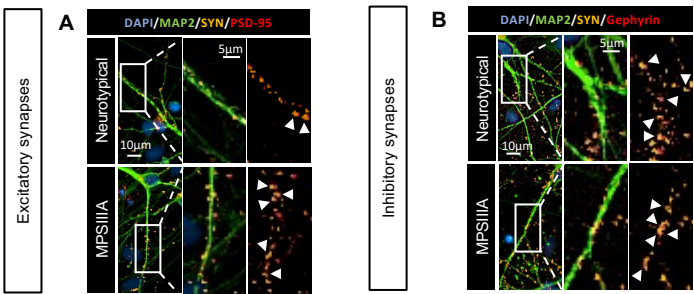


C



Supplementary Figure 15 | Voltage-gated sodium and potassium channel are unaltered in MPS IIIA patient-derived neurons.

(A) Number of patched cells from each individual line or playground model included in the voltage-gated sodium and potassium channel analysis. **(B)** Histograms displaying the mean $\pm$ SEM for voltage-gated sodium channel analysis. **(C)** Histograms displaying the mean $\pm$ SEM for voltage-gated potassium channel analysis. **(B-C)** 30-60 days: n= 188 neurons in neurotypical and 183 neurons in MPS IIIA. 90-120 days: n= 258 neurons in neurotypical and 265 neurons in MPS IIIA. Black indicates neurotypical; red indicates MPS IIIA. Statistical analysis was completed using a Kruskal Wallis test with Dunn's correction for multiple comparisons. ns, not significant is defined as $P > 0.05$. See also Figure 4. Source data are provided as a Source Data file. ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; MPSIIIA = mucopolysaccharidosis type IIIA; WT = wild type; KO = knockout; mV = millivolt; pA = picoampere; Nav = voltage-gated sodium channel; Kv = voltage-gated potassium channel.



Supplementary Figure 16 | Representative images illustrating synaptic co-localization in high-content imaging.

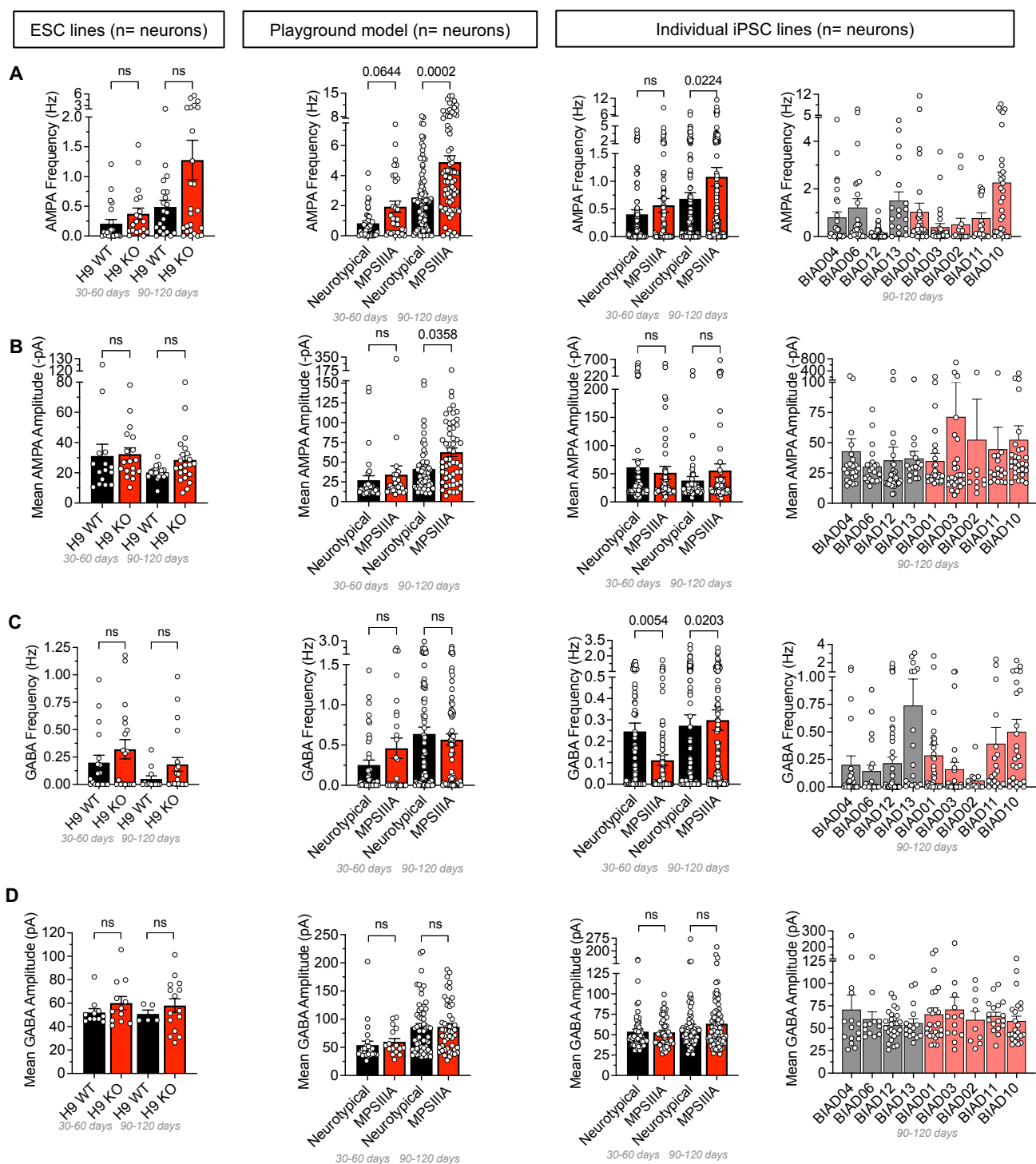
Neurotypical and MPS IIIA neural cultures were fluorescently stained with MAP2, Synapsin-I, PSD-95 and Gephyrin. **(A)** Representative images of immunofluorescence staining of Synapsin-I (SYN), Postsynaptic density protein 95 (PSD-95) at 60 days in culture (neurotypical and MPSIIIA groups) used to identify excitatory synapses. Blue indicates nuclei (DAPI); green indicates neuronal soma and dendrites (MAP2); yellow indicates pre-synaptic terminals (SYN); red indicates glutamatergic postsynaptic sites (PSD-95). **(B)** Representative images of immunofluorescence staining of Synapsin-I (SYN) and Gephyrin at 60 days in culture, used to identify inhibitory synapses. Blue indicates nuclei (DAPI); green indicates neuronal soma and dendrites (MAP2); yellow indicates pre-synaptic terminals (SYN); red indicates GABAergic postsynaptic sites (Gephyrin). White rectangles denote magnified regions, providing enhanced resolution of a subsection of the image. Additional panels display SYN+ PSD-95+ **(A)** or SYN+ Gephyrin+ **(B)** channels in the magnified region with and without MAP2 to better visualize synaptic puncta. The arrows **(A-B)** highlight co-localized synapses. See also Figure 5. MPSIIIA = mucopolysaccharidosis type IIIA.

AMPA analysis per cell line			30-60 Days				90-120 Days				
Cell Line	Cell type	Genotype	n neurons patched	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	n neurons patched	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	Figures
H9 WT	ESC	Neurotypical	21	60	2	2020-12-07 2021-07-29	21	96	2	2020-12-07 2021-07-29	Fig 5E-G,M
BIAD04	iPSC	Neurotypical	25	55	2	2021-02-20 2022-01-26	27	86	2	2021-02-20 2022-01-26	Fig 5E-G,M
BIAD06	iPSC	Neurotypical	17	67	1	2021-04-26	25	96	1	2021-04-26	Fig 5E-G,M
BIAD12-cNP22	iPSC	Neurotypical	16	53	1	2022-06-03	24	96	1	2022-06-03	Fig 5E-G,M
BIAD12-cNP24	iPSC	Neurotypical	19	54	1	2022-06-03	35	99	1	2022-06-03	Fig 5E-G,M
BIAD13	iPSC	Neurotypical	16	56	1	2022-06-26	18	91	1	2022-06-26	Fig 5E-G,M
H9 SGSH-KO	ESC	MPSIIIA	19	64	2	2020-12-07 2021-07-29	25	98	2	2020-12-07 2021-07-29	Fig 5E-G,M
BIAD01	iPSC	MPSIIIA	28	54	2	2021-02-20 2022-01-26	36	88	2	2021-02-20 2022-01-26	Fig 5E-G,M
BIAD03	iPSC	MPSIIIA	14	68	1	2021-04-26	28	97	1	2021-04-26	Fig 5E-G,M
BIAD02	iPSC	MPSIIIA	13	66	1	2021-06-25	16	90	1	2021-06-25	Fig 5E-G,M
BIAD10	iPSC	MPSIIIA	21	56	1	2022-06-26	31	98	1	2022-06-26	Fig 5E-G,M
BIAD11	iPSC	MPSIIIA	20	56	1	2022-06-26	20	90	1	2022-06-26	Fig 5E-G,M
Neurotypical Playground	iPSC	Neurotypical	40	55	1	2023-01-15	83	103	1	2023-01-15 2023-12-05	Fig 5E-G,M,O,R
MPSIIIA Playground	iPSC	MPSIIIA	31	54	1	2023-01-15	77	104	1	2023-01-15 2023-12-05	Fig 5E-G,M,O,R
Total Neurotypical:			154	57.1	9		233	95.3	9		
Total MPSIIIA:			146	59.7	9		233	95.0	9		

GABA analysis per cell line			30-60 Days				90-120 Days				
Cell Line	Cell type	Genotype	n neurons patched	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	n neurons patched	Avg days in BrainPhys medium	n independent neuronal differentiations	Neuronal differentiation dates	Figures
H9 WT	ESC	Neurotypical	18	60	2	2020-12-07 2021-07-29	12	94	2	2020-12-07 2021-07-29	Fig 5I-M
BIAD04	iPSC	Neurotypical	22	56	2	2021-02-20 2022-01-26	24	86	2	2021-02-20 2022-01-26	Fig 5I-M
BIAD06	iPSC	Neurotypical	13	67	1	2021-04-26	21	96	1	2021-04-26	Fig 5I-M
BIAD12-cNP22	iPSC	Neurotypical	14	54	1	2022-06-03	25	97	1	2022-06-03	Fig 5I-M
BIAD12-cNP24	iPSC	Neurotypical	19	54	1	2022-06-03	33	99	1	2022-06-03	Fig 5I-M
BIAD13	iPSC	Neurotypical	16	56	1	2022-06-26	17	91	1	2022-06-26	Fig 5I-M
H9 SGSH-KO	ESC	MPSIIIA	19	66	2	2020-12-07 2021-07-29	21	98	2	2020-12-07 2021-07-29	Fig 5I-M
BIAD01	iPSC	MPSIIIA	28	54	2	2021-02-20 2022-01-26	32	88	2	2021-02-20 2022-01-26	Fig 5I-M
BIAD03	iPSC	MPSIIIA	12	67	1	2021-04-26	25	97	1	2021-04-26	Fig 5I-M
BIAD02	iPSC	MPSIIIA	13	65	1	2021-06-25	13	90	1	2021-06-25	Fig 5I-M
BIAD10	iPSC	MPSIIIA	21	56	1	2022-06-26	26	96	1	2022-06-26	Fig 5I-M
BIAD11	iPSC	MPSIIIA	18	56	1	2022-06-26	19	90	1	2022-06-26	Fig 5I-M
Neurotypical Playground	iPSC	Neurotypical	38	55	1	2023-01-15	69	102	1	2023-01-15 2023-12-05	Fig 5I-M,P-R
MPSIIIA Playground	iPSC	MPSIIIA	28	54	1	2023-01-15	66	105	1	2023-01-15 2023-12-05	Fig 5I-M,P-R
Total Neurotypical:			140	57.4	9		201	95.0	9		
Total MPSIIIA:			139	59.7	9		202	94.9	9		

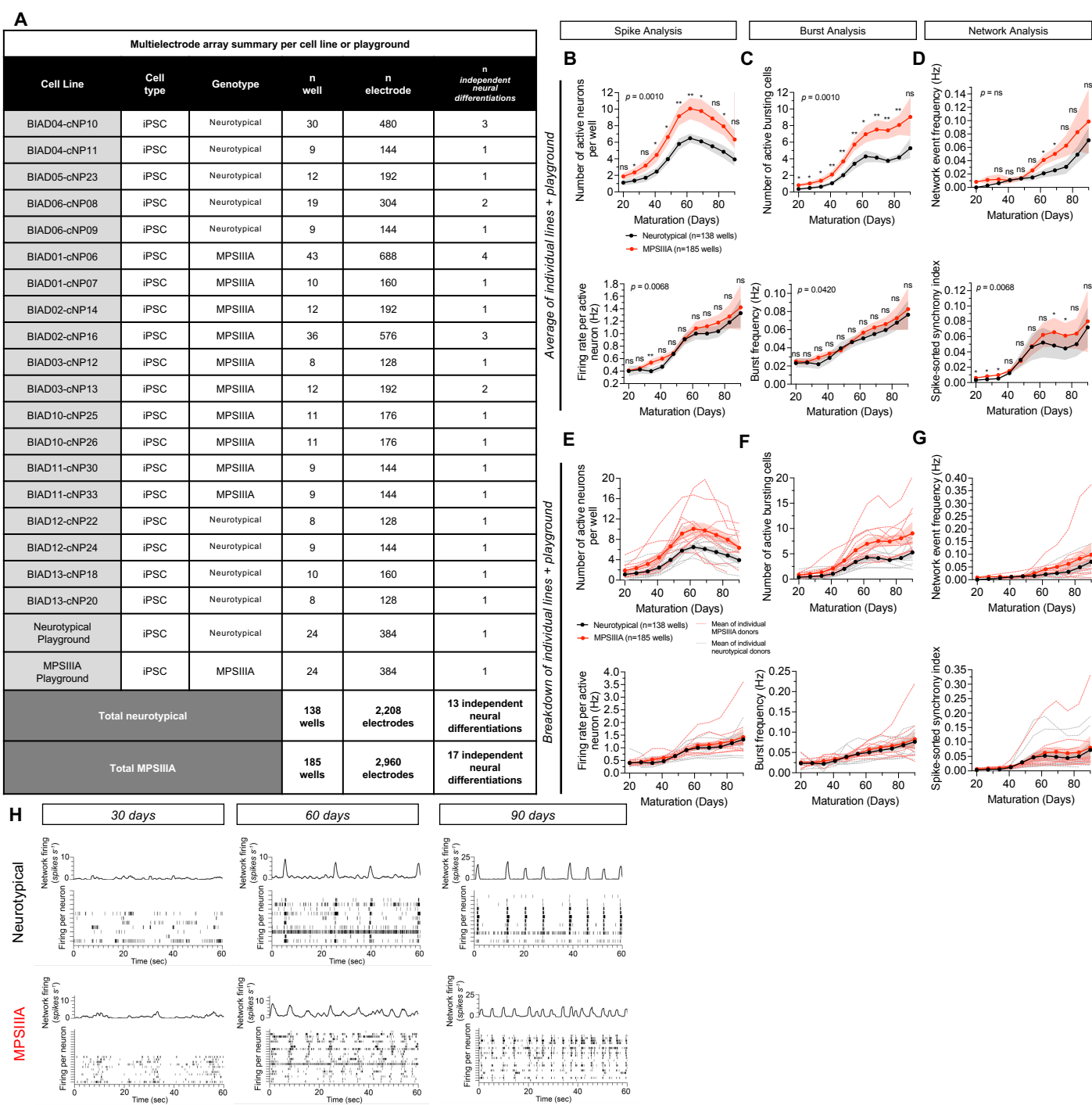
Supplementary Figure 17 | Summary of cells included in AMPA and GABA postsynaptic activity analysis

Number of patched cells included from each cell line or playground model in postsynaptic current analysis. See also Figure 5E-R. ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; MPSIIIA = mucopolysaccharidosis type IIIA; WT = wild type; KO = knockout.



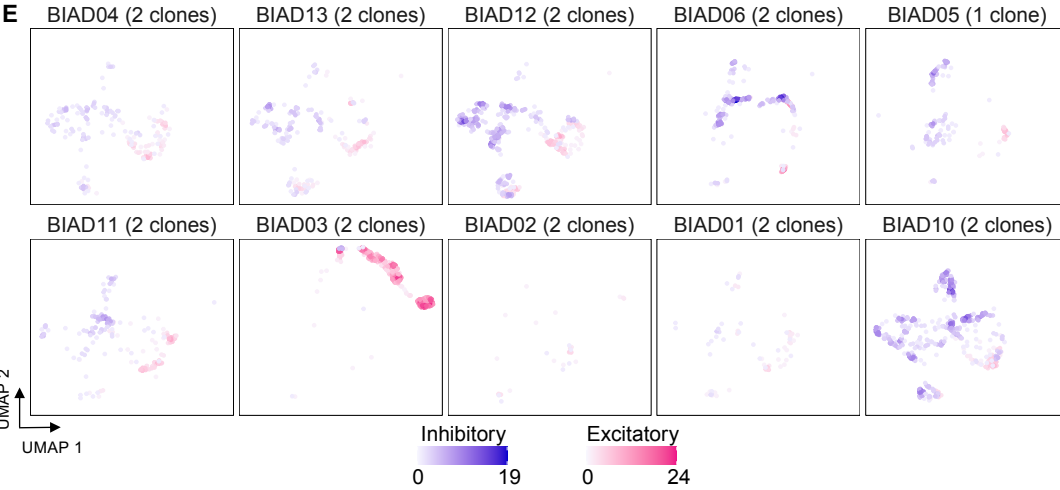
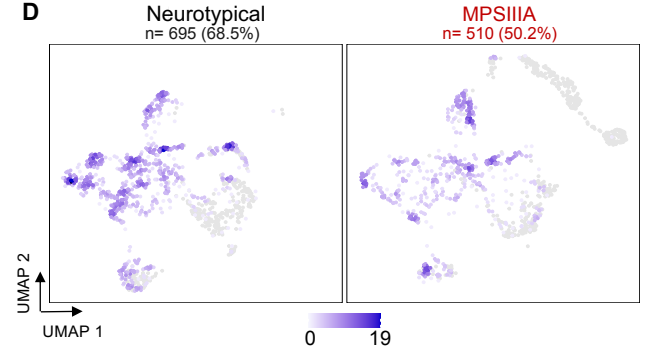
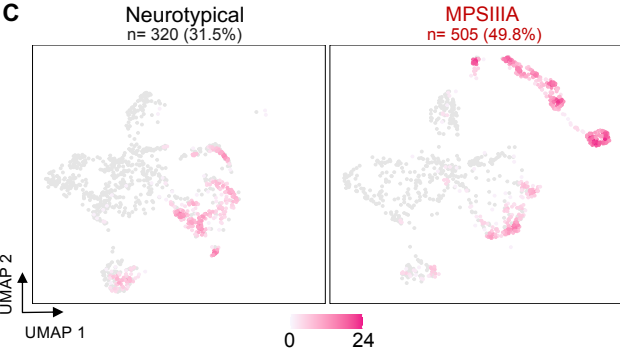
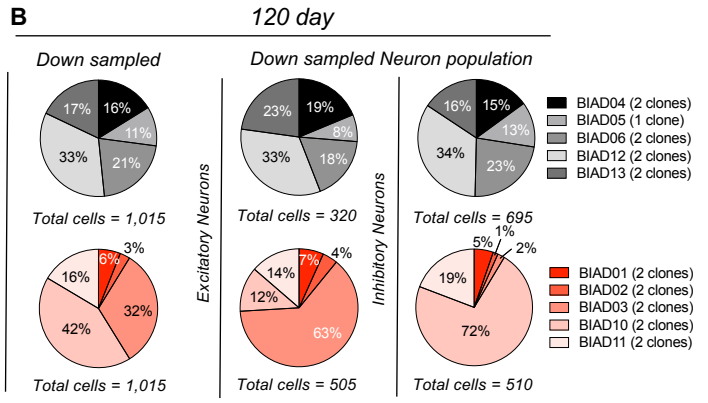
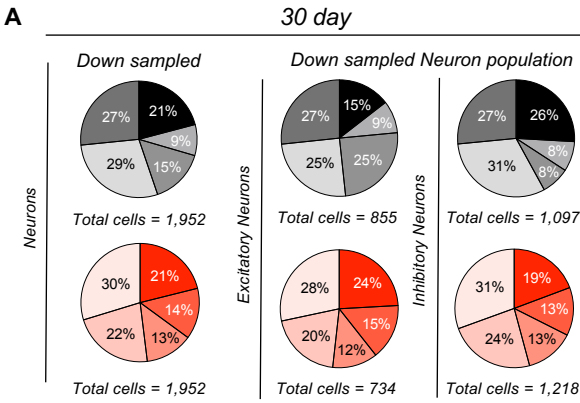
Supplementary Figure 18 | MPS IIIA patient-derived neurons have hyperactive excitatory postsynaptic communication that appears later in neurodevelopment

AMPA- and GABA-mediated spontaneous synaptic activity over neurodevelopment split into our embryonic wildtype/knockout lines, playground model and individual iPSC lines. **(A)** AMPA-mediated event frequency, showing that in each model type, MPS IIIA exhibits hyperactive excitatory postsynaptic communication later in neurodevelopment. **(B)** Mean AMPA event amplitude, showing that in each model type, MPS IIIA have larger AMPA events in the later stages of neurodevelopment. **(C)** GABA event frequency and **(D)** Mean GABA event amplitude highlight that MPS IIIA does not affect GABAergic communication. **(A-D)** Statistical analysis was completed using a Kruskal Wallis test with Dunn's correction for multiple comparisons. Each data point represents an individual neurons patched. Black indicates wildtype or neurotypical; red indicates SGSH knockout or MPSIIIA; grey indicates individual neurotypical lines; peach indicates individual MPSIIIA lines. ns, not significant is defined as $P > 0.05$. See also Figure 5E-M. Source data are provided as a Source Data file. ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; MPS IIIA = mucopolysaccharidosis type IIIA; WT = wild type; KO = knockout; pA = picoampere; Hz = hertz; AMPA = alpha-amino-3-hydroxy-5-methyl-4-isooxazole-propionic acid; GABA = gamma-aminobutyric acid.



Supplementary Figure 19 | Individual MPS IIIA patients display hyperactive neuronal network activity

MPS IIIA cultures show patient-specific hyperactivity in neuronal networks when compared to neurotypical controls. n = 138 wells from 10 lines and 13 independent neural differentiations in neurotypical (1-3 differentiations per line) and 185 wells from 11 lines and 17 independent neural differentiations in MPSIIIA (1-4 differentiations per line). **(A-C)** Analysis of single-cell spike sorted cortical neuronal electrophysiology data in neurotypical and MPS IIIA measured using multi-electrode array over 90 days of maturation in BrainPhys reduced neural maturation media. **(A)** Table represents the number of wells per condition included in multi-electrode array analysis. Analysis of **(B)** spike activity, **(C)** burst activity and **(D)** network activity in neurotypical (black) and MPS IIIA (red). Solid lines represent the averages of 10 neurotypical (black) and 11 MPS IIIA (red) cell lines and isogenic clones. The shaded area represents $\pm$ SEM. All recordings were binned for 7 days and smoothed with a two-point moving average algorithm. Each data point represents the average of cell lines per binned timepoint. Significance is based on averages for each cell line. Per-timepoint two-tailed non-parametric Mann-Whitney U tests were used to assess differences at individual timepoints, and a Wilcoxon test was used to determine overall trajectory differences between conditions. **(E-G)** Individual patient contributions to the average of each condition (neurotypical and MPS IIIA) for **(E)** spike activity, **(F)** burst activity and **(G)** network activity. Dashed lines represent the average of an individual cell line (neurotypical: grey; MPS IIIA: peach). **(H)** Representative raster plots depict 60 seconds of spontaneous firing activity per neuron in 6 individual wells over maturation. Each dash indicates an action potential. Network firing plots above the raster plots indicate synchronous firing events (arbitrary scale). ns, not significant is defined as $P > 0.05$. See also Figure 6 and Supplementary Figure 5. Source data are provided as a Source Data file. iPSC = induced pluripotent stem cell; MPSIIIA = mucopolysaccharidosis type IIIA; Hz = hertz.

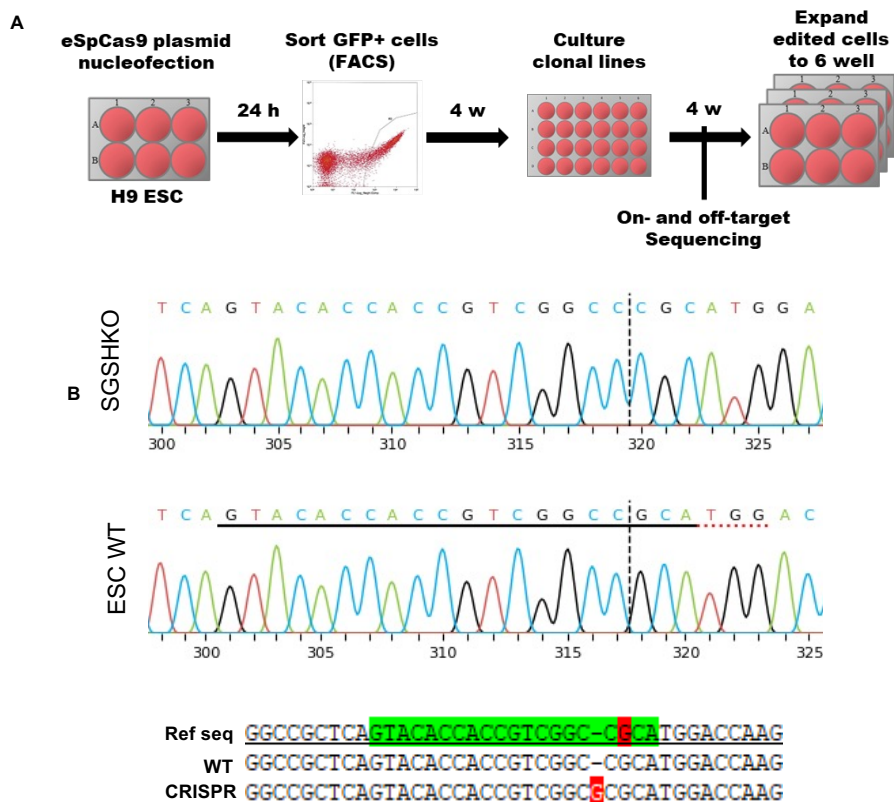


F

Excitatory neurons			Inhibitory neurons		
Cluster description	Top GOIDs	Top GOID Processes	Cluster description	Top GOIDs	Top GOID Processes
Cellular metabolism	GO:0002181, GO:0006119, GO:0042773, GO:0042775, GO:0009060	Cytoplasmic translation, Oxidative phosphorylation, ATP synthesis coupled electron transport, Mitochondrial ATP synthesis coupled electron transport, Aerobic respiration	Cellular metabolism	GO:0002181, GO:0006119, GO:0009060, GO:0015986, GO:0042773	Cytoplasmic translation, Oxidative phosphorylation, Aerobic respiration, Proton motive force-driven ATP synthesis, ATP synthesis coupled electron transport
Regulation and organisation	GO:0042274, GO:0042254, GO:0022613, GO:0042255, GO:0010257	Ribosomal small subunit biogenesis, Ribosome biogenesis, Ribonucleoprotein complex biogenesis, Ribosome assembly, NADH dehydrogenase complex assembly	Regulation and organisation	GO:0022613, GO:0007005, GO:0071826, GO:0010257, GO:0032981	Ribosomal small subunit biogenesis, Mitochondrion organisation, Protein-RNA complex organisation, NADH dehydrogenase complex assembly, Mitochondrial respiratory chain complex I assembly
Cellular differentiation and development	GO:0007409, GO:0010975, GO:0007411, GO:0097485, GO:0001764	Axonogenesis, Regulation of neuron projection development, Axon guidance, Neuron projection guidance, Neuron migration	Cellular differentiation and development	GO:0021762, GO:0048857, GO:0030901, GO:1901844, GO:1903867	Substantia nigra development, Neural nucleus development, Midbrain development, Regulation of cell communication by electrical coupling involved in cardiac conduction, Extraembryonic membrane development
Ions and transport	GO:1902600, GO:0140238, GO:0048488, GO:0036465, GO:1900242	Proton transmembrane transport, Presynaptic endocytosis, Synaptic vesicle endocytosis, Synaptic vesicle recycling, Regulation of synaptic vesicle endocytosis	Ions and transport	GO:1902600, GO:0072655, GO:0070972, GO:0006605, GO:0090151	Proton transmembrane transport, Establishment of protein localization to mitochondrion, Protein localization to endoplasmic reticulum, Protein targeting, Establishment of protein localization to mitochondrial membrane
Signalling pathways	GO:0042391, GO:1901798, GO:0032228, GO:1990089, GO:0050804	Regulation of membrane potential, Positive regulation of signal transduction by p53 class mediator, Regulation of synaptic transmission - GABAergic, Response to nerve growth factor, Modulation of chemical synaptic transmission	Signalling pathways	GO:0051881, GO:0016032, GO:1901798, GO:1990748, GO:0098754	Regulation of mitochondrial membrane potential, Viral process, Positive regulation of signal transduction by p53 class mediator, Cellular detoxification, Detoxification

Supplementary Figure 20 | Single-nuclei RNA sequencing analysis of 120-day playground cultures broken down by donor contribution.

Single-nuclei RNA sequencing of 30 and 120-day MPS IIIA and Neurotypical neural cultures highlighting donor proportions per cell type. **(A)** Pie charts showing the proportion of donors at 30 days when MPS IIIA and neurotypical neural cultures are filtered for neurons. Neurotypical and MPS IIIA genotypes were filtered for 'Neurons' based on cell type classification, then downsampled to have equal numbers of neurons. Pie charts show the proportions pre- and post-downsampling the neuronal populations and the donor contributions in excitatory and inhibitory neurons. **(B)** Pie charts showing the proportion of donors at 120-days MPS IIIA and neurotypical neural cultures are filtered for neurons. Neurotypical and MPS IIIA genotypes were filtered for 'Neurons' based on cell type classification, then downsampled to have equal numbers of neurons. Pie charts show the proportions pre- and post-downsampling the neuronal populations and the donor contributions in excitatory and inhibitory neurons. **(A-B)** Black and grey segments indicate neurotypical lines, and red/peach segments indicates MPS IIIA lines, as annotated in the colour key. **(C-D)** UMAP density plots of excitatory (pink) and inhibitory (blue) neuron distribution in neurotypical (n = 320 excitatory neurons and 695 inhibitory neurons) and MPS IIIA (n = 505 excitatory neurons and 520 inhibitory neurons). **(E)** UMAP density plots of excitatory (pink) and inhibitory (blue) neuron distribution in each donor for Neurotypical and MPS IIIA. **(F)** Top 5 GO IDs for each cluster of the chord plots in excitatory and inhibitory neurons. See also Figure 7. Source data are provided as a Source Data file. MPSIIIA = mucopolysaccharidosis type IIIA.



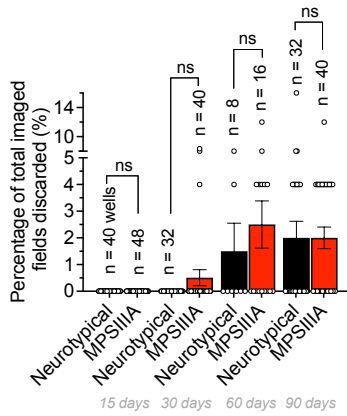
Supplementary Figure 21 | Generating SGSHKO ESC using CRISPR

(A,B) *SGSH* knockout (SGSHKO) ESC line was generated using CRISPR/Cas9 gene-editing of WA09 ESC cells. **(A)** Workflow for nucleofection and GFP sorting for positive cells. **(B)** The resulting insertion of a single nucleotide in exon 6 introducing a frame shift mutation in the *SGSH* gene. See also Figure 1. ESC = embryonic stem cell; CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats; FACS = fluorescence-activated cell sorting; SGSHKO = *SGSH* knock out; WT = wild type.

	Neurotypical Playground (Figure 4A-C)				MPSIIIA Playground (Figure 4A-C)			
Days in-vitro (DIV)	15	30	60	90	15	30	60	90
Total imaged regions (FOVs)	1,000	796	196	782	1,200	992	392	971
Total excluded image regions (FOVs)	0	0	3	16	0	5	10	20
% image regions discarded	0	0	1.53	2.05	0	0.50	2.55	2.06

Supplementary Figure 22 | Discarded data amounts per total number of imaged regions for synaptic phenotyping high content-imaging.

Overview of the number of fields of view (FOVs) imaged, number of FOVs excluded during quality control assessment and the percentage of fields excluded per well for neurotypical and MPS IIIA playgrounds at each timepoint. For synaptic imaging, cellular detachment and clumping can alter the accuracy of neuronal morphology analysis pipelines. Therefore, FOVs were assessed for detachment and clumping prior to being included in further analyses. FOVs that did not pass quality control assessment were excluded. See also Figure 5A-B, Supplementary Figure 23. For individual well data, please refer to Supplementary Information Tables 4 and 5.

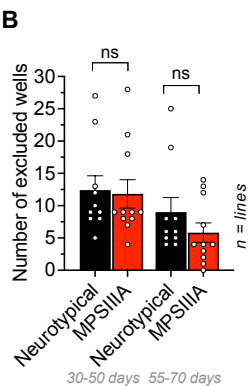
A

Supplementary Figure 23 | The total number of imaged regions discarded during quality control assessment is similar across conditions.

For synaptic imaging, cellular detachment and clumping can alter the accuracy of neuronal morphology analysis pipelines. Therefore, FOVs were assessed for detachment and clumping prior to being included in further analyses. FOVs that did not pass quality control assessment were excluded. The percentage of total imaged fields discarded during quality control assessment of synaptic high-content imaging does not significantly differ between conditions or treatment groups. **(A)** The mean percentage of total fields discarded from 15 to 90 days, showing that at each timepoint, there is no significant difference in the discarded data amounts between neurotypical (black) and MPS IIIA (red) groups. Histograms represent the means $\pm$ SEM. Each data point represents a well. 15 days: $n = 40$ wells in neurotypical and 48 wells in MPS IIIA; 30 days: $n = 32$ wells in neurotypical and 40 wells in MPS IIIA; 60 days: $n = 8$ wells in neurotypical and 15 in MPS IIIA; 90 days: $n = 32$ wells in neurotypical and 40 in MPS IIIA. Both neurotypical and MPS IIIA data are derived from 3 independent neural differentiations. Significance was determined using unpaired non-parametric two-tailed Mann-Whitney U tests. ns, not significant is defined as $P > 0.05$. See also Figures 5A-B and Supplementary Figure 6. Source data are provided as a Source Data file. MPS IIIA = mucopolysaccharidosis type IIIA.

A Multielectrode array summary of discarded wells for network frequency analyses of individual cell lines and playground

			Figure 6F	
Cell Line	Type	Genotype	30-50 days	55-70 days
			% wells and electrodes discarded	% wells and electrodes discarded
BIAD04-cNP10	iPSC	Neurotypical	89	82
BIAD04-cNP11	iPSC	Neurotypical	100	93
BIAD05-cNP23	iPSC	Neurotypical	97	69
BIAD06-cNP08	iPSC	Neurotypical	70	30
BIAD06-cNP09	iPSC	Neurotypical	56	41
BIAD01-cNP06	iPSC	MPSIIIA	49	28
BIAD01-cNP07	iPSC	MPSIIIA	90	67
BIAD02-cNP14	iPSC	MPSIIIA	36	0
BIAD02-cNP16	iPSC	MPSIIIA	78	40
BIAD03-cNP12	iPSC	MPSIIIA	88	54
BIAD03-cNP13	iPSC	MPSIIIA	75	11
BIAD10-cNP25	iPSC	MPSIIIA	70	39
BIAD10-cNP26	iPSC	MPSIIIA	82	24
BIAD11-cNP30	iPSC	MPSIIIA	93	41
BIAD11-cNP33	iPSC	MPSIIIA	96	19
BIAD12-cNP22	iPSC	Neurotypical	100	63
BIAD12-cNP24	iPSC	Neurotypical	100	48
BIAD13-cNP18	iPSC	Neurotypical	100	53
BIAD13-cNP20	iPSC	Neurotypical	97	75
Neurotypical Playground	iPSC	Neurotypical	97	79
MPSIIIA Playground	iPSC	MPSIIIA	75	53
Average % discarded			83	48



Supplementary Figure 24 | The number of inactive wells and electrodes discarded from multielectrode array network frequency metric analysis does not significantly differ between conditions.

Wells with a network event frequency of 0Hz were excluded from Network Event Frequency metric analysis as the absence of detectable network activity in these wells artificially lowered the average, masking true differences in network frequency between groups. The number of inactive wells discarded for Network Event Frequency metric analyses does not influence phenotypes. **(A)** Overview of the average percentage of multielectrode array wells and electrodes with a network event frequency of 0Hz, which were discarded from network event frequency metric analyses. Data is broken down by the individual patient cell line and clone, with the cell type and genotype listed for each associated figure and timepoint. **(B)** The mean number of wells discarded from Network Event Frequency metric analyses, showing that at each timepoint, there is no significant difference in the discarded data amounts between neurotypical (black) and MPS IIIA (red) groups. Histogram represents the means ± SEM. Each data point represents a cell line (neurotypical composed of 10 lines from 5 individual donors and 1 playground; MPS IIIA composed of 11 lines from 5 donors and 1 playground). 30-55 days: n = 10 neurotypical lines and 11 MPS IIIA lines; 55-70 days: n = 10 neurotypical lines and 11 MPS IIIA lines. Neurotypical line data are derived 13 independent neural differentiations (1-3 differentiations per line) and MPS IIIA line data are derived from 17 independent neural differentiations (1-4 differentiations per line). Significance was determined using unpaired non-parametric two-tailed Mann-Whitney U tests. ns, not significant is defined as $P > 0.05$. See also Figure 6F. Source data are provided as a Source Data file. MPSIIIA = mucopolysaccharidosis type IIIA.

Antibody	Source	Dilution
Chicken polyclonal anti-MAP2	Abcam (Cat# ab5392)	1:2000
Rabbit polyclonal anti-Synapsin I	Millipore (Cat# AB1543P)	1:1000
Rabbit polyclonal anti-Ki67	Abcam (Cat# ab15580)	1:500
Mouse monoclonal anti-PSD-95 (Clone 6G6-1C9)	Thermo Fisher Scientific (Cat# MA1-045)	1:500
Mouse monoclonal anti-Gephyrin (Clone mAb7a)	Synaptic Systems (Cat# 147021)	1:250
Mouse monoclonal anti-GFAP (Clone 2A5)	Abcam (Cat# ab4648)	1:300
Goat monoclonal anti-Oct4	Abcam (Cat# ab27985)	1:100
Mouse monoclonal anti-Nestin (Clone 10C2)	Abcam (Cat# ab22035)	1:200
Rabbit monoclonal recombinant anti-KLF4 (Clone EPR19590)	Abcam (Cat# ab215036)	1:100
Rabbit polyclonal anti-FOXP1	Abcam (Cat# ab18259)	1:400
Mouse monoclonal anti-human TRA-1-60 (Clone TRA-1-60R), Alexa 488 Conjugated	STEMCELL Technologies (Cat# 60064AD)	1:200
Mouse monoclonal anti-human SSEA-4 (Clone MC-813-70), PE conjugated	STEMCELL Technologies (Cat# 60062PE)	1:100
Mouse monoclonal anti-SOX2 (Clone 9-9-3)	Abcam (Cat# ab79351)	1:200
Goat polyclonal anti-SOX2	Abcam (Cat# ab110145)	1:100
Donkey anti Chicken AlexaFluor 488-conjugated	Jackson ImmunoResearch (703-545-155)	1:250
Donkey anti Rabbit AlexaFluor 568-conjugated	Abcam (Cat# AB175692)	1:250
Donkey anti Mouse AlexaFluor 647-conjugated	Abcam (Cat# AB150111)	1:250
Donkey anti Mouse AlexaFluor 568-conjugated	Abcam (Cat# ab175472)	1:250
Donkey anti Rabbit AlexaFluor 647-conjugated	Abcam (Cat# AB150063)	1:250
Donkey anti Mouse AlexaFluor 488-conjugated	Abcam (Cat# AB150109)	1:250
Donkey anti Goat AlexaFluor 568-conjugated	Abcam (Cat# AB175704)	1:250
Donkey anti Goat AlexaFluor 647-conjugated	Abcam (Cat# AB150131)	1:250

Supplementary Table 1: Antibodies

Table of antibodies and dilutions used throughout study.

Score type	Parameter	Weighting	Function
Morphology	Cell distance to nearest neighbour	1	d.low(cut1=0.005, cut2=0.5, des.min=0.1, des.max=1, scale=0.1)
Morphology	% of cell contact	1	d.high(cut1=20, cut2=65, des.min=0.1, des.max=1, scale=5)
Morphology	Cell Area	1	d.central(cut1=75, cut2=100, cut3=190, cut4=250, des.min=0.1, des.max=1, scale=0.5)
Morphology	Cell roundness	1	d.high(cut1=0.5, cut2=0.8, des.min=0.1, des.max=1, scale=0.1)
Morphology	Cytoplasm area	1	d.low(cut1=100, cut2=150, des.min=0.1, des.max=1, scale=10)
Morphology	Ratio of nucleus: cytoplasm	1	d.high(cut1=1, cut2=1.5, des.min=0.1, des.max=1, scale=0.25)
Gene expression	CD44 gene expression	2	d.low(cut1=0.0005, cut2=0.005, scale=1, des.min=0.1, des.max=1)
Gene expression	OCT4 gene expression	1	d.high(cut1=0.05, cut2=0.5, scale=1, des.min=0.1, des.max=1)
Gene expression	NANOG gene expression	1	d.high(cut1=0.01, cut2=0.05, scale=1, des.min=0.1, des.max=1)
Gene expression	SOX2 gene expression	1	d.high(cut1=0.01, cut2=0.05, scale=1, des.min=0.1, des.max=1)
Protein expression	% of KLF4+ only cells	1	d.low(data1\$KLF4_ICC, cut1=0.01, cut2=0.5, scale=0.1, des.min=0.1, des.max=1)
Protein expression	% of triple positive KLF4/OCT4/SOX2 cells (ICC)	1	d.high(cut1=75, cut2=90, scale=1, des.min=0.1, des.max=1)
Protein expression	% of SSEA4+ only cells	0.5	d.low(cut1=0.1, cut2=1, scale=1, des.min=0.1, des.max=1)
Protein expression	% of double positive SSEA4/TRA-1-60 cells (FACS)	1	d.high(cut1=95, cut2=99, scale=1, des.min=0.1, des.max=1)
Protein expression	% of double negative SSEA4/TRA-1-60 cells (FACS)	0.5	d.low(cut1=0, cut2=0.05, des.min=0.1, des.max=1)
Growth rate	Population doubling level/per day	1	d.central(cut1=0.5, cut2=0.75, cut3=1.25, cut4=1.5, des.min=0.1, des.max=1, scale=0.5)

Supplementary Table 2: iPSC multimodal scoring

Table of parameters and their weighting used to define iPSC multimodal score.

Score type	Parameter	Weighting	Function
Morphology	% of cells with 1-2 projections	1	d.high(data1\$X1.2.ext, cut1 = 50, cut2=85,scale=5,des.min=0.1,des.max=1)
Morphology	% of cells with more than 2 projections	1	d.low(data1\$X.multi.ext., cut1 = 15, cut2=30,scale=5,des.min=0.1,des.max=1)
Morphology	% of cells with 1-2 roots	1	d.high(data1\$X1.2.roots, cut1 = 50, cut2=85,des.min=0.1,des.max=1)
Morphology	% of cells with more than 2 roots	1	d.low(data1\$X.multi.root, cut1 = 10, cut2=25,des.min=0.1,des.max=1)
Morphology	Soma size (um)	0.5	d.central(data1\$Soma, cut1=50, cut2=100, cut3=165, cut4=200, des.min=0.1,des.max=1,scale=10)
Morphology	Neurite length (um)	1	d.central(data1\$Total.neurite, cut1=10, cut2=20, cut3=100, cut4=250, des.min=0.1,des.max=1,scale=10)
Morphology	Average number of roots	1	d.central(data1\$roots, cut1=0.5, cut2=1, cut3=2, cut4=3, des.min=0.1,des.max=1,scale=0.1)
Morphology	Average number of extremities	1	d.central(data1\$ext, cut1=0.5, cut2=1, cut3=2, cut4=3, des.min=0.1,des.max=1,scale=0.1)
Gene expression	PAX6 gene expression	0.3	d.high(data2\$PAX6, cut1 = 0, cut2=0.1,scale=1,des.min=0.1,des.max=1)
Gene expression	SOX2 gene expression	1	d.high(data2\$SOX2, cut1 = 0, cut2=0.1,scale=1,des.min=0.1,des.max=1)
Gene expression	Nestin gene expression	1	d.high(data2\$NES, cut1 = 0, cut2=0.1,scale=1,des.min=0.1,des.max=1)
Gene expression	FOXG1 gene expression	1	d.high(data2\$FOXG1, cut1 = 0, cut2=0.1,scale=1,des.min=0.1,des.max=1)
Protein expression	% of triple positive NES/SOX2/FOXG1 cells (ICC)	0.7	d.high(data3\$X..NES..SOX2..FOXG1, cut1 =20, cut2=65,scale=5,des.min=0.1,des.max=1)
Protein expression	% of double positive NES/SOX2 cells (ICC)	1	d.high(data3\$All..NES..SOX2, cut1 = 40, cut2=75,scale=5,des.min=0.1,des.max=1)
Protein expression	% DAPI only cells (Triple negative)	1	d.low(data3\$DAPI..only, cut1 = 1, cut2=2,scale=0.5,des.min=0.1,des.max=1)
Protein expression	% of Nestin+ cells	1	d.high(data3\$total..NES, cut1 = 70, cut2=90,scale=5,des.min=0.1,des.max=1)
Protein expression	% of SOX2+ cells	0.7	d.high(data3\$total..SOX, cut1 = 60, cut2=90,scale=5,des.min=0.1,des.max=1)
Protein expression	% of FOXG1+ cells	0.7	d.high(data3\$total..FOXG1, cut1 = 40, cut2=80,scale=5,des.min=0.1,des.max=1)
Growth rate	Population doubling level/per day	1	d.central(data4\$Growth.Rate, cut1=0.1, cut2=0.4, cut3=0.65, cut4=1, des.min=0.1,des.max=1,scale=0.1)

Supplementary Table 3: NPC multimodal scoring

Table of parameters and their weighting used to define NPC multimodal score.

15 days				30 days				60 days				90 days			
Well	Total FOVs	# FOVs excluded	% FOVs excluded	Well	Total FOVs	# FOVs excluded	% FOVs excluded	Well	Total FOVs	# FOVs excluded	% FOVs excluded	Well	Total FOVs	# FOVs excluded	% FOVs excluded
11_13	25	0	0	3_11	25	0	0	3_11	25	0	0	3_10	25	0	0
11_14	25	0	0	3_5	25	0	0	3_5	25	0	0	3_12	25	1	4
11_15	25	0	0	3_7	25	0	0	3_7	25	0	0	3_6	25	0	0
11_16	25	0	0	3_9	25	0	0	3_9	25	0	0	3_8	25	0	0
11_17	25	0	0	8_13	25	0	0	8_13	24	0	0	8_14	25	0	0
11_18	25	0	0	8_15	25	0	0	8_15	25	1	4	8_16	25	0	0
11_19	25	0	0	8_17	21	0	0	8_17	25	2	8	8_18	7	0	0
11_20	25	0	0	8_19	25	0	0	8_19	22	0	0	8_20	25	0	0
8_13	25	0	0	10_10	25	0	0					11_10	25	1	4
8_14	25	0	0	10_9	25	0	0					11_9	25	1	4
8_15	25	0	0	11_19	25	0	0					13_10	25	1	4
8_16	25	0	0	11_20	25	0	0					13_9	25	0	0
8_17	25	0	0	6_10	25	0	0					3_10	25	0	0
8_18	25	0	0	6_9	25	0	0					3_9	25	2	8
8_19	25	0	0	7_19	25	0	0					5_10	25	0	0
8_20	25	0	0	7_20	25	0	0					5_9	25	0	0
10_12	25	0	0	8_10	25	0	0					7_10	25	0	0
11_13	25	0	0	8_9	25	0	0					7_9	25	0	0
11_20	25	0	0	9_19	25	0	0					9_10	25	1	4
11_21	25	0	0	9_20	25	0	0					9_9	25	0	0
6_12	25	0	0	10_7	25	0	0					11_7	25	0	0
7_13	25	0	0	10_8	25	0	0					11_8	25	1	4
7_20	25	0	0	11_17	25	0	0					13_7	25	0	0
7_21	25	0	0	11_18	25	0	0					13_8	25	1	4
8_12	25	0	0	6_7	25	0	0					3_7	25	2	8
9_13	25	0	0	6_8	25	0	0					3_8	25	0	0
9_20	25	0	0	7_17	25	0	0					5_7	25	0	0
9_21	25	0	0	7_18	25	0	0					5_8	25	0	0
10_10	25	0	0	8_7	25	0	0	7_7	25	1	4				
10_11	25	0	0	8_8	25	0	0	7_8	25	0	0				
11_18	25	0	0	9_17	25	0	0	9_7	25	4	16				
11_19	25	0	0	9_18	25	0	0	9_8	25	0	0				
6_10	25	0	0												
6_11	25	0	0												
7_18	25	0	0												
7_19	25	0	0												
8_10	25	0	0												
8_11	25	0	0												
9_18	25	0	0												
9_19	25	0	0												

Supplementary Table 4: Discarded data amounts per total number of imaged regions of synaptic phenotyping high-content imaging for healthy playground.
 Table summarising the number of fields of view (FOVs) imaged, number of FOVs excluded during quality control assessment and the percentage of fields excluded per well for Healthy and MPSIIIA playgrounds at each timepoint. For synaptic imaging, cellular detachment and clumping can alter the accuracy of neuronal morphology analysis pipelines. Therefore, FOVs were assessed for detachment and clumping prior to being included in further analyses. FOVs that did not pass quality control assessment were excluded. See also Figure 4A-C and Supplementary Figure 15 and 20.

15 days				30 days				60 days				90 days			
Well	Total FOVs	# FOVs excluded	% FOVs excluded	Well	Total FOVs	# FOVs excluded	% FOVs excluded	Well	Total FOVs	# FOVs excluded	% FOVs excluded	Well	Total FOVs	# FOVs excluded	% FOVs excluded
3_13	25	0	0	3_13	25	0	0	3_13	25	1	4	3_14	25	1	4
3_14	25	0	0	3_15	25	0	0	3_15	25	0	0	3_16	25	0	0
3_15	25	0	0	3_17	25	0	0	3_17	25	1	4	3_18	25	1	4
3_16	25	0	0	3_19	25	0	0	3_19	25	0	0	3_20	25	0	0
3_17	25	0	0	4_13	25	0	0	4_13	25	1	4	4_14	25	1	4
3_18	25	0	0	4_15	25	0	0	4_15	25	2	8	4_16	25	1	4
3_19	25	0	0	4_17	25	0	0	4_17	25	0	0	4_18	25	1	4
3_20	25	0	0	4_19	25	0	0	4_19	25	0	0	4_20	25	0	0
4_13	25	0	0	8_11	22	0	0	8_11	17	0	0	8_10	25	0	0
4_14	25	0	0	8_5	25	0	0	8_5	25	0	0	8_12	7	0	0
4_15	25	0	0	8_7	21	0	0	8_7	25	0	0	8_6	25	1	4
4_16	25	0	0	8_9	25	0	0	8_9	25	1	4	8_8	14	0	0
4_17	25	0	0	9_11	25	1	4	9_11	25	0	0	9_10	25	0	0
4_18	25	0	0	9_5	25	2	8	9_5	25	3	12	9_12	25	0	0
4_19	25	0	0	9_7	25	0	0	9_7	25	1	4	9_6	25	1	4
4_20	25	0	0	9_9	25	0	0	9_9	25	0	0	9_8	25	0	0
6_13	25	0	0	10_17	25	0	0					10_7	25	1	4
6_14	25	0	0	10_18	25	0	0					10_8	25	0	0
6_15	25	0	0	11_7	25	0	0					12_7	25	0	0
6_16	25	0	0	11_8	25	0	0					12_8	25	1	4
6_17	25	0	0	6_17	25	0	0					14_7	25	1	4
6_18	25	0	0	6_18	25	0	0					14_8	25	0	0
6_19	25	0	0	7_7	25	0	0					4_7	25	0	0
6_20	25	0	0	7_8	25	0	0					4_8	25	1	4
10_13	25	0	0	8_17	25	0	0					6_7	25	0	0
10_20	25	0	0	8_18	25	0	0					6_8	25	1	4
10_21	25	0	0	9_7	25	0	0					8_7	25	1	4
11_12	25	0	0	9_8	24	2	8					8_8	25	0	0
6_13	25	0	0	10_19	25	0	0					10_10	25	0	0
6_20	25	0	0	10_20	25	0	0					10_9	25	0	0
6_21	25	0	0	11_10	25	0	0					12_10	25	0	0
7_12	25	0	0	11_9	25	0	0					12_9	25	1	4
8_13	25	0	0	6_19	25	0	0					14_10	25	3	12
8_20	25	0	0	6_20	25	0	0					14_9	25	0	0
8_21	25	0	0	7_10	25	0	0					4_10	25	0	0
9_12	25	0	0	7_9	25	0	0					4_9	25	0	0
10_18	25	0	0	8_19	25	0	0	6_10	25	1	4				
10_19	25	0	0	8_20	25	0	0	6_9	25	1	4				
11_10	25	0	0	9_10	25	0	0	8_10	25	1	4				
11_11	25	0	0	9_9	25	0	0	8_9	25	0	0				
6_18	25	0	0												
6_19	25	0	0												
7_10	25	0	0												
7_11	25	0	0												
8_18	25	0	0												
8_19	25	0	0												
9_10	25	0	0												
9_11	25	0	0												

Supplementary Table 5: Discarded data amounts per total number of imaged regions of synaptic phenotyping high-content imaging for MPSIIIA playground.

Table summarising the number of fields of view (FOVs) imaged, number of FOVs excluded during quality control assessment and the percentage of fields excluded per well for Healthy and MPSIIIA playgrounds at each timepoint. For synaptic imaging, cellular detachment and clumping can alter the accuracy of neuronal morphology analysis pipelines. Therefore, FOVs were assessed for detachment and clumping prior to being included in further analyses. FOVs that did not pass quality control assessment were excluded. See also Figure 4A-C and Supplementary Figure 23.

Antibodies		
Chicken polyclonal anti-MAP2	Abcam	Cat# ab5392, RRID: AB_2138153
Mouse monoclonal anti-PSD-95 (6G6-1C9)	Thermo Fisher Scientific	Cat# MA1-90314, RRID: AB_2537537
Rabbit polyclonal anti-Synapsin I	Millipore	Cat# AB1543P, RRID: AB_90757
Mouse monoclonal anti-Gephyrin (mAb7a)	Synaptic Systems	Cat# 147 021, RRID: AB_2232546
Goat monoclonal anti-Oct4	Abcam	Cat# ab27985, RRID: AB_776898
Mouse monoclonal anti-Nestin (clone 10C2)	Abcam	Cat# ab22035, RRID: AB_446723
Rabbit monoclonal recombinant anti-KLF4 (clone EPR19590)	Abcam	Cat# ab215036, RRID: AB_2933978
Rabbit polyclonal anti-FOXG1	Abcam	Cat# ab18259, RRID: AB_732415
Mouse monoclonal anti-human TRA-1-60 (Clone TRA-1-60R) Alexa Fluor 488-conjugated	STEMCELL Technologies	Cat# 60064AD, RRID:AB_2686905
Mouse monoclonal anti-human SSEA-4 (Clone MC-813-70) PE-conjugated	STEMCELL Technologies	Cat# 60062PE, RRID: AB_2721031
Mouse monoclonal anti-SOX2 (Clone 9-9-3)	Abcam	Cat# ab79351, RRID:AB_10710406
Goat polyclonal anti-SOX2	Abcam	Cat# ab110145, RRID:AB_10865544
Donkey anti Chicken AlexaFluor 488-conjugated	Jackson ImmunoResearch Labs	Cat# 703-545-155, RRID:AB_2340375
Donkey anti Rabbit AlexaFluor 568-conjugated	Abcam	Cat# ab175692, RRID:AB_3076303
Donkey anti Mouse AlexaFluor 647-conjugated	Abcam	Cat# ab150111, RRID:AB_2890625
Donkey anti Mouse AlexaFluor 568-conjugated	Abcam	Cat# ab175472, RRID:AB_2636996
Donkey anti Rabbit AlexaFluor 647-conjugated	Abcam	Cat# ab150063, RRID:AB_2687541
Donkey anti Mouse AlexaFluor 488-conjugated	Abcam	Cat# ab150109, RRID:AB_2571721
Donkey anti Goat AlexaFluor 568-conjugated	Abcam	Cat# ab175704, RRID:AB_2725786
Donkey anti Goat AlexaFluor 647-conjugated	Abcam	Cat# ab150131, RRID:AB_2732857
Virus vectors		
pCSC-Synapsin(0.5kb)-MCS-GFP (derivative of pCSC-SP-PW-EGFP)	Zabolocki et al.	Addgene plasmid # 12337, RRID: Addgene_12337
Cell lines		
Human ES (H9)	WiCell	Cat#:WA09
Healthy and MPSIIIA patient tissue samples	Women's and Children's Hospital, South Australia	N/A
Reagents		
DMEM/F12 with 15 mM HEPES	STEMCELL Technologies	Cat# 36254
N2 Supplement-A	STEMCELL Technologies	Cat# 07152
NeuroCult™ SM1 with vitamin A	STEMCELL Technologies	Cat# 05711
LDN193189 (Dihydrochloride)	STEMCELL Technologies	Cat# 72147
SB431542 (Hydrate)	STEMCELL Technologies	Cat# 72232
DMEM/F12 + GlutaMAX™ Basal Medium	Life Technologies	Cat# 10565018
bFGF (FGF2)	STEMCELL Technologies	Cat# 78134
Laminin (Natural, Mouse)	Life Technologies	Cat# 23017015
Ascorbic acid	STEMCELL Technologies	Cat# 72132
BrainPhys™ Basal Medium	STEMCELL Technologies	Cat# 05790
BrainPhys™ Imaging Optimized Medium	STEMCELL Technologies	Cat# 05796
NeuroCult™ SM1 without vitamin A	STEMCELL Technologies	Cat# 05731
Human Recombinant BDNF, ACF	STEMCELL Technologies	Cat# 78133
Human Recombinant GDNF, ACF	STEMCELL Technologies	Cat# 78139
Human Recombinant IGF	STEMCELL Technologies	Cat# 78142
N6,2-O-Dibutyryl adenosine 3,5-cyclic monophosphate sodium salt	Sigma-Aldrich	Cat# D0627
mTeSR™ Plus Basal Medium	STEMCELL Technologies	Cat# 05826
mTeSR™ Plus 5x Supplement	STEMCELL Technologies	Cat# 05827
Y-27632 ROCK inhibitor	STEMCELL Technologies	Cat# 72304
GlutaMAX™ Supplement	Thermo Fisher Scientific	Cat#: 35050061
Corning Matrigel matrix, hESC qualified	Bio-strategy	Cat# BDAA354277
Poly-L-ornithine hydrobromide	Sigma-Aldrich	Cat# P3655
Collagenase Type IV (1 mg/mL)	STEMCELL Technologies	Cat# 07909
Dispase (1 U/mL)	STEMCELL Technologies	Cat# 07923
CloneR™	STEMCELL Technologies	Cat# 05888
Donkey serum	Sigma-Aldrich	Cat# D9663
DAPI	Sigma-Aldrich	Cat# D9542
Biocytin	Sigma-Aldrich	Cat #B4261
Rhodamine B isothiocyanate–Dextran	Sigma-Aldrich	Cat# R8881
Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA)	Sigma-Aldrich	Cat# E3889
Guanosine 5'-triphosphate sodium salt hydrate (Na-GTP)	Sigma-Aldrich	Cat# G8877
Adenosine 5'-triphosphate magnesium salt (Mg-ATP)	Sigma-Aldrich	Cat# A9187
Accutase™	STEMCELL Technologies	Cat# 07920
TRIzol™ Reagent	Thermo Fisher Scientific	Cat# 15596026
DynaBeads MyOne Silane Beads	10x Genomics	Cat# 2000048
Tetrodotoxin	Abcam	Cat# ab120054
Commercial assays		
SuperScript™ IV VLO™ Master Mix	Thermo Fisher Scientific	Cat# 11756500
TRIzol™ Plus RNA Purification Kit	Thermo Fisher Scientific	Cat# 12183555
TaqMan™ Gene Expression Master Mix	Thermo Fisher Scientific	Cat# 4369016
Chromium Next GEM Single Cell 3' Reagent Kits v3.1	10X Genomics	Cat# CG000388 Rev B
Library Construction Kit	10X Genomics	Cat# 10000196
Chromium Next GEM Single Cell 3' v3.1 Gel Beads kit	10X Genomics	Cat# 2000164
SPRiSelect Reagent kit	Beckman Coulter	Cat# B23318
STEMdiff™ Trilineage Differentiation Kit	STEMCELL Technologies	Cat# 05230
Human Pluripotent Stem Cell Trilineage Differentiation qPCR Array	STEMCELL Technologies	Cat# 07515
CytoTune™-iPS 2.0 Sendai Reprogramming Kit	Thermo Fisher Scientific	Cat# A16518

Primers		
SOX2 TaqMan™	Thermo Fisher Scientific	Hs01053049_s1
SEV-KLF4 TaqMan™	Thermo Fisher Scientific	Mr04421256_mr
SEV-CMYC TaqMan™	Thermo Fisher Scientific	Mr04269876_mr
OCT4/POU5F1 TaqMan™	Thermo Fisher Scientific	Hs00999632_g1
ACTB TaqMan™	Thermo Fisher Scientific	Hs99999905_m1
GAPDH TaqMan™	Thermo Fisher Scientific	Hs99999905_m1
CD44 TaqMan™	Thermo Fisher Scientific	Hs00153304_m1
NES TaqMan™	Thermo Fisher Scientific	Hs00707120_s1
FOXG1 TaqMan™	Thermo Fisher Scientific	Hs01850784_s1
SEV TaqMan™	Thermo Fisher Scientific	Mr04269880_mr
SEV-KOS TaqMan™	Thermo Fisher Scientific	Mr04421257_mr
NANOG TaqMan™	Thermo Fisher Scientific	Hs02387400_g1
PAX6 TaqMan™	Thermo Fisher Scientific	Hs00240871_m1
SATB2 TaqMan™	Thermo Fisher Scientific	Hs00392652_m1
CTIP2 TaqMan™	Thermo Fisher Scientific	Hs01102259_m1
TUBB3 TaqMan™	Thermo Fisher Scientific	Hs00801390_s1
SGSH-KO target locus: CCCCTCCTGTGTTTCAGGAC		
SGSH-KO target locus: AACTCACAGACCTCAAGATCTCC		
Software		
ImageJ	NIH	https://imagej.nih.gov/ij/
Harmony 4.9	PerkinElmer	N/A
R	The R project	https://www.r-project.org/
R studio	RStudio: Integrated Development for R. RStudio	https://rstudio.com
Python v3.11.5	Anaconda	www.anaconda.com
Prism 10.1.1	GraphPad software, Inc	N/A
Cell Ranger 7.0.0	10X Genomics	https://www.10xgenomics.com/cn/support/software/cell-ranger
Seurat	Seurat v5	https://satijalab.org/seurat/
Demuxafy 2.0.1	Neavin et al 2024	Github link
PClamp software v10.7	Molecular Devices	N/A
NeuroLucida 360	MBF Bioscience	N/A
NeuroLucida Explorer	MBF Bioscience	N/A
Illustrator V26.5.2	Adobe	https://www.adobe.com/products/illustrator.html
AxiS Navigator v3.10.1.9	Axion Biosystems Inc	
Axion Data Export Tool v3.5.0	Axion Biosystems Inc	
Offline Sorter Application v4.5.0	Plexon Inc	
Neural Metric Tool v3.2.5	Axion Biosystems Inc	
Other		
Lumos MEA 48-well plates (16 electrodes)	TrendBio	M768-tMEA-48OPT-5

Supplementary Table 6: Key resource table

Table summarising all key biological, chemical, and computational resources used to conduct and analyze the experiments in this study. Resources are categorized by type (Antibodies, Virus vectors, Cell lines, Reagents, Commercial assays, Primers, Software, Other) for ease of reference. For each resource, the designation/name, description/target (where applicable), supplier/manufacturer, catalog number (Cat#), RRID (Research Resource Identifier, when assigned and available via <https://scicrunch.org/resources>), and relevant usage notes (e.g., dilution, concentration, or modifications) are provided. All commercial reagents, kits, and assays were used following the manufacturer's protocols unless specified otherwise in the Methods section.