

1. Supplementary Tables

Supplementary Table S1. Coatings used for neural differentiation of BrainSpheres generated with different neural induction protocols.

Neural induction protocol	Differentiation medium	coating	Order number and supplier
2D-NIM	CINDA+	0.1 mg/mL PLO ¹ / 50 µg/mL laminin	PLO: #P4957, Sigma-Aldrich, St. Louis, MO, USA laminin: #LN521, Biolamina, Sundbyberg, Sweden
	Electro	0.1 % PEI ² / 50 µg/mL laminin	PEI: #181978, Sigma-Aldrich, St. Louis, MO, USA laminin: #LN521, Biolamina, Sundbyberg, Sweden
GNEIB	CINDA+	25 µg/mL laminin	laminin: #LN521, Biolamina, Sundbyberg, Sweden
	Electro	0.015 mg/mL PLO ¹ / 50 µg/mL laminin	PLO: #P4957, Sigma-Aldrich, St. Louis, MO, USA laminin: #LN521, Biolamina, Sundbyberg, Sweden
Stemdiff	CINDA+	50 µg/mL laminin	laminin: #LN521, Biolamina, Sundbyberg, Sweden
	Electro	0.1 mg/mL PLO ¹ / 50 µg/mL laminin	PLO: #P4957, Sigma-Aldrich, St. Louis, MO, USA laminin: #LN521, Biolamina, Sundbyberg, Sweden

¹PLO: poly-L-ornithine; ²PEI: polyethyleneimine

Supplementary Table S2. Information about used compounds and their solvents.

Compound	CASRN	Order number and supplier	solvent	effect
Bicuculline	485-49-4	#0130, Tocris, Bristol, UK	DMSO	GABA _A antagonist
Buspirone hydrochloride	33386-08-2	#HY-B1115, MedChemExpress, South Brunswick, NJ, USA	DMSO	5-HT _{1A} agonist
Carbaryl	63-25-2	#32055, Sigma-Aldrich, St. Louis, MO, USA	DMSO	inhibits acetylcholinesterase
DL-2-amino-5-phosphonovaleric acid (AP5)	76326-31-3	#SC-201503, Santa Cruz Biotechnology Inc., Dallas, TX, USA	H ₂ O	NMDA antagonist

Emamectin benzoate	155569-91-8	#31733, Merck, Darmstadt, Germany	DMSO	GABA _A and GABA _C agonist
Haloperidol	52-86-8	#HY-14538, MedChemExpress, South Brunswick, NJ, USA	DMSO	D2 antagonist
L-glutamate	6106-04-3	#49621, Sigma-Aldrich, St. Louis, MO, USA	H ₂ O	Neurotransmitter
NBQX disodium salt (NBQX)	479347-86-9	#SC-222048, Santa Cruz Biotechnology Inc., Dallas, TX, USA	DMSO	AMPA antagonist
Picrotoxin	124-87-8	#SC-202765, Santa Cruz Biotechnology Inc., Dallas, TX, USA	DMSO	GABA _A and GABA _C antagonist
Trimethyltin chloride (TMT)	1066-45-1	#146498, Merck, Darmstadt, Germany	H ₂ O	Increases glutamate release
γ-Aminobutyric acid (GABA)	56-12-2	#A5835, Sigma-Aldrich, St. Louis, MO, USA	H ₂ O	Neurotransmitter

Supplementary Table S3. Primer sequences.

Gene	Forward primer sequence (5' → 3')	Reverse primer sequence (5' → 3')
ACHE	GTCCAGACTAACGTACTGCT	ATGCGATACTGGGCCAAC
ACTB	CAGGAAGTCCCTTGCCATCC	ACCAAAAGCCTTCATACATCTCA
AQP4	TGGACAGAAGACATACTCATAAAGG	GGTGCCAGCATGAATCCC
CHAT	GCCCATAGTATTGCTTCATGC	ACCAGCTGAGGTTTGCAG
CHRNA4	CTCGCGGGCTCTAGATG	CCACAGGAGAAGACGAACC
DLG4	CCAGAAGAGTACAGCCGATTCT	CTTGGTCTTGTCGTAATCAAACAG
DRD2	ACGGCGAGCATCCTGAACTT	GCCGGGTTGGCAATGATGCA
EN1	CTGGGTGTACTGCACACGTTAT	TACTCGCTCTCGTCTTTGTCTT
FOXG1	TCCATCCGCCACAATCTGTC	CCCAGCGAGTTCTGAGTCAA
GABRA1	ACAACACTTACGCTCCAACA	TTTCGGGCTTGACCTCTTTAG
GAD1	GACCCCAATACCACTAACCTG	GCTGTTGGTCTTTGCAAG
GFAP	CACTGTGAGGCAGAAGCTC	CCTCCAGCGACTCAATCTTC
GLS	CAGTTCCAAGATCATTAACAGCA	CGATTGTGGGGTGTGTCT
GRIA1	CTTAATCGAGTTCTGCTACAAATCC	GTATGGCTTCGTTGATGGATTG
GRIN1	CTCCTGGAAGATTGAGCTCAA	GTGGATGGCTAACTAGGATGG
HOXA2	CACCACGTCTACGGGCAAGAAC	AGCTGCTGATGCTGACTTCTGA
HTR1A	TGTCGCTTCTCACAACCTCTC	CTTCTCCTCTTCTCTCTGCTC
LMX1A	CGGGTCATCTTGACAGGTTT	CAGAAGCAGCTCAGGTGGTA
MAP2	TGCCTCAGAACAGACTGTCAC	AAGGCTCAGCTGTAGAGGGA
S100B	CACATTCGCCGTCTCCATC	CACAAGCTGAAGAAATCCGAAC
SLC12A2	ACAAAGTTGAGGAAGAGGATGGC	CCTGATCTGCCGGTATGTCTTGG
SLC12A5	CCGATGACAATTACCCATGGA	CTGTCTACATCAGCTCCGTTG
SLC17A6	CCTCTACCCTAAATATGCTAATTCCA	TTGCTCCATATCCCATGACAT
SLC17A7	CAGAAAGCCCAGTTCAGC	ACAATAGCAAAGCCGAAACTC
SYN1	GTGCTGCTGGTCATCGAC	CCACAAGGTTGAGATCAGAGAA
TH	GGTCTCTAGATGGTGGATTTTG	TGCTAAACCTGCTCTTCTCC
TPH1	TGTCTCACATATTGAGTGCAGT	CCACAATTGGAAGATGTCTCC