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Supplemental Information

Evaluating Synthetic Activation and Repression of Neuropsychiatric-Related Genes in hiPSC-Derived NPCs, Neurons, and Astrocytes

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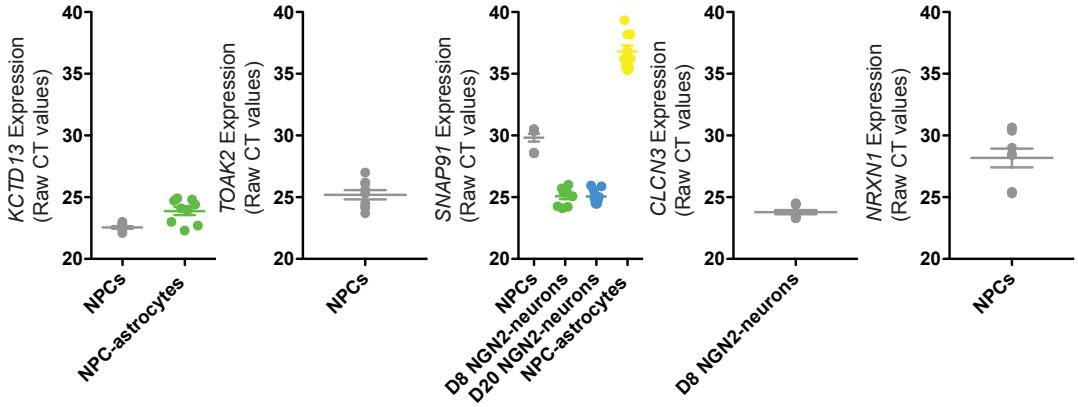
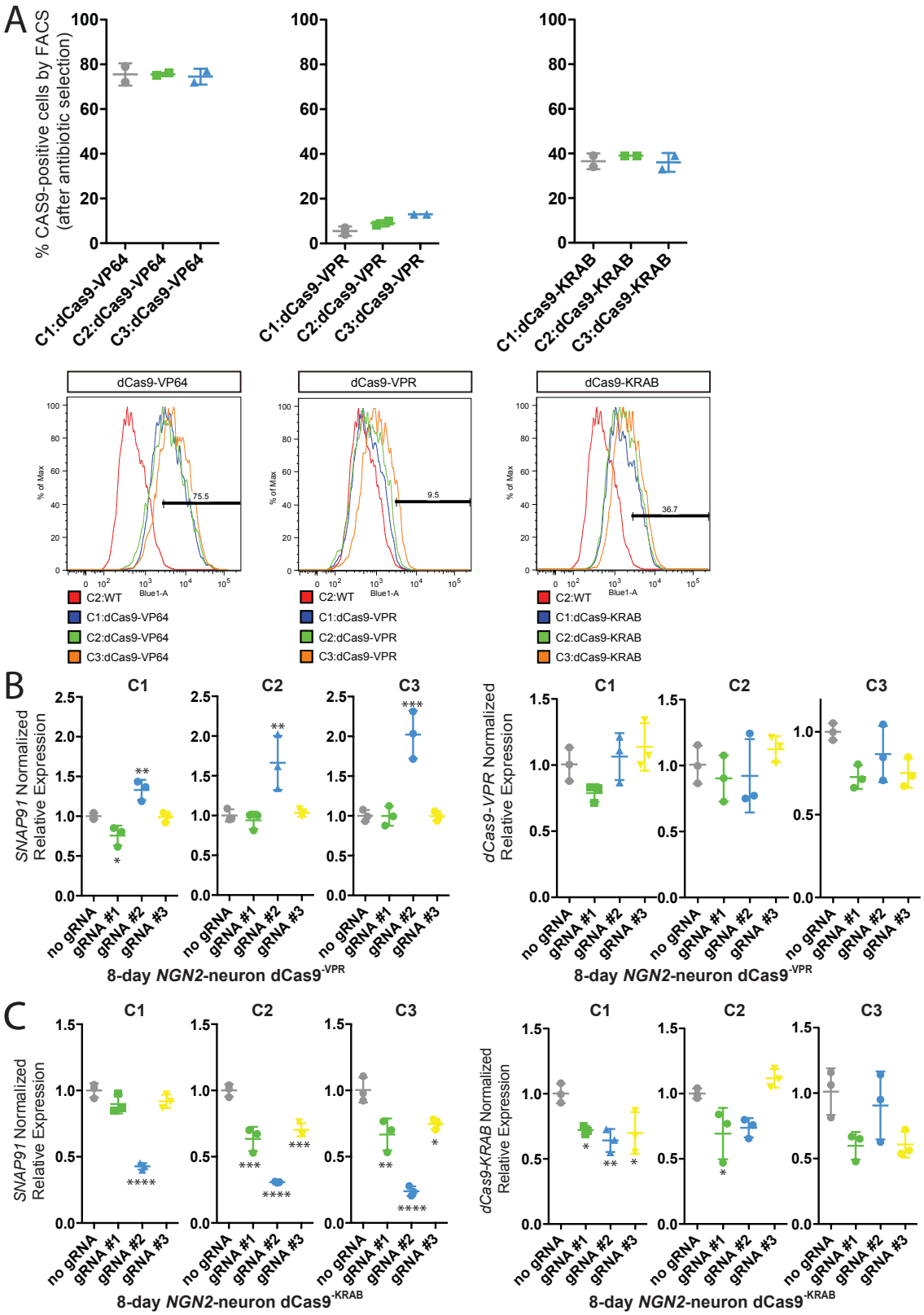
SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES

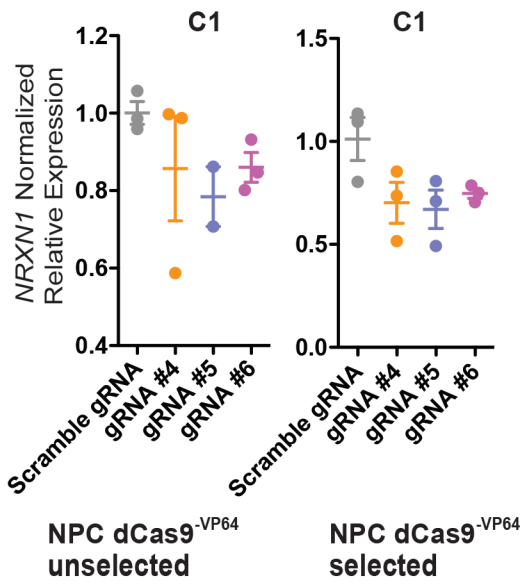


SI Figure 1. Baseline cell-type specific expression of SZ genes. Raw CT scores indicating baseline *KCTD13, TOAK2, NRXN1, SNAP91* and *CLCN3* mRNA levels in NPCs (grey), 8-day *NGN2*-neurons (green), 20-day *NGN2*-neurons (blue) and NPC-astrocytes (yellow), as appropriate for the cell types evaluated for each gene. Data are presented as mean ± s.e.m (bar graph) from at least 3 independent biological replicates. Each biological replicate is depicted by a circle.

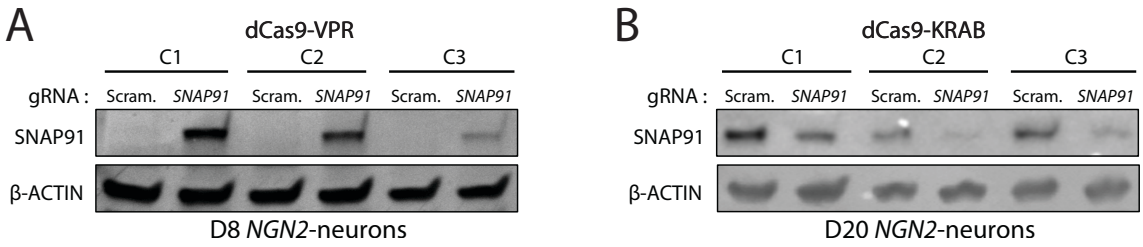


SI Figure 2. Evaluation of dCas9 expression and protein levels. **A.** FACS analysis of

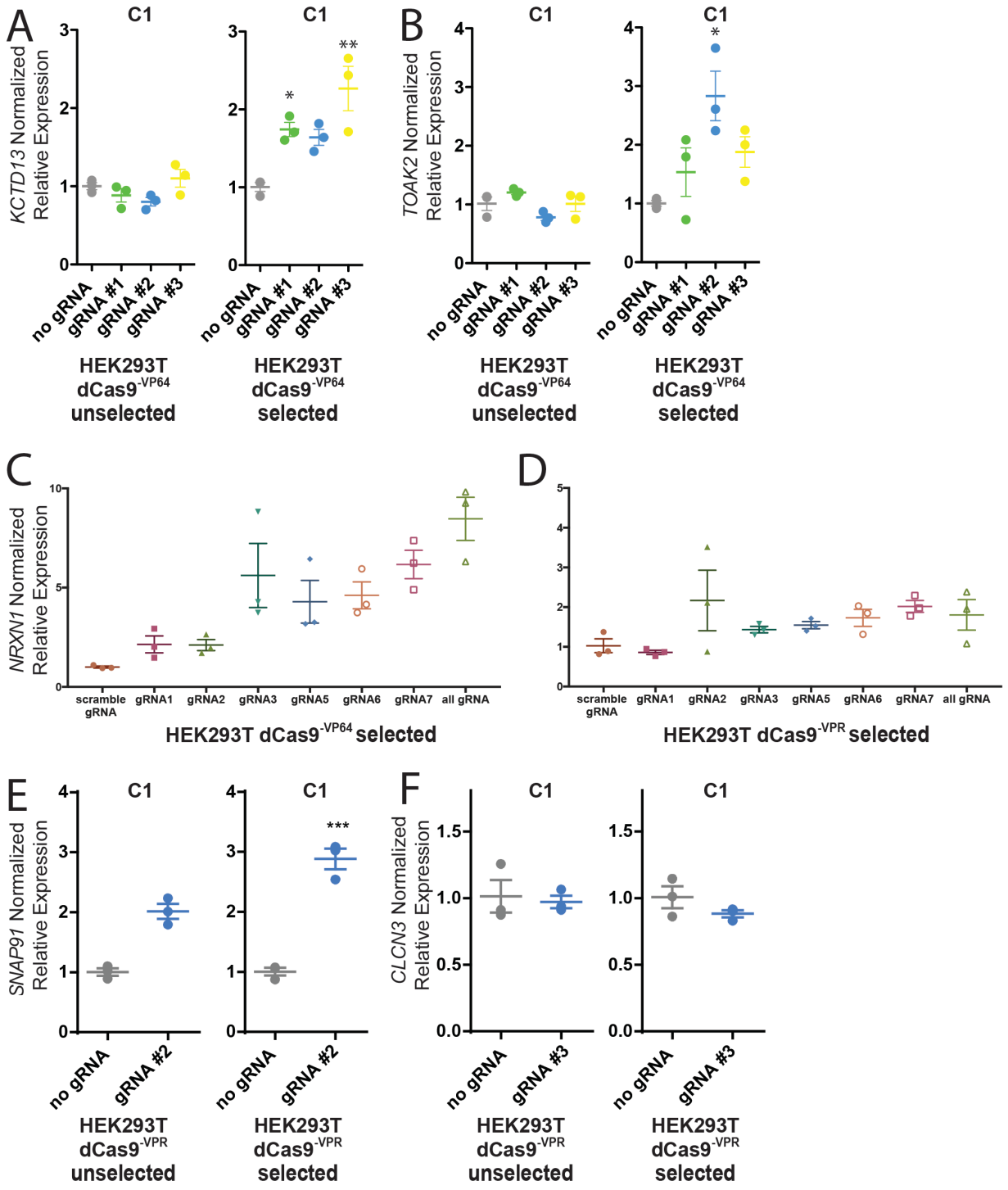
Cas9 protein in antibiotic selected dCas9$^{-VP16,}$ dCas9$^{-VPR\ and}$ dCas9^{-KRAB} NPC lines, shown as graphs (top) and histogram plots (bottom). **B-C.** Normalized relative *SNAP91*:*dCas9* mRNA levels (compared to scrambled gRNA control (grey)) following transduction of dCas9^{VPR} (**B**) and dCas9^{KRAB} (**C**) 8-day NGN2-neurons with lentivirus expressing gRNAs targeted to three different locations (green, blue, yellow) upstream of the TSS for *SNAP91*. Data are presented as mean ± s.e.m (bar graph) from at least 3 independent biological replicates. Each biological replicate is depicted by a circle. *=$p < 0.05$, **=$p < 0.01$, ***=$p < 0.001$, ****=$p < 0.0001$.



SI Figure 3. Evaluation of impact of antibiotic selection for dCas9-VP64 on gRNA efficacy. Normalized relative mRNA levels (compared to scrambled gRNA control (grey)) following transduction of dCas9^{VP64} NPCs with lentivirus expressing gRNAs targeted to three different locations (green, blue, yellow) upstream of the TSS for *NRXN1*. Data are presented as mean ± s.e.m (bar graph) from at least 3 independent biological replicates. Each biological replicate is depicted by a circle.



SI Figure 4. Evaluation of changes in SNAP91 protein levels by dCas9^{VP64} in NPCs. A. Representative western blots following transduction of dCas9^{VPR} NPCs with lentivirus expressing *SNAP91* gRNA#2, day 8 *NGN2*-neurons. **B.** Representative western blots following transduction of dCas9^{KRAB} NPCs with lentivirus expressing *SNAP91* gRNA#2, day 21 *NGN2*-neurons.



SI Figure 5. Evaluation of impact of gRNA efficacy in HEK293Ts. A-D. Normalized relative mRNA levels (compared to scrambled gRNA control (grey)) following transduction of antibiotic-selected and/or non-selected dCas9^{VP64} (**A-C**) and dCas9^{VPR} (**D-F**) HEK293Ts with lentivirus expressing gRNAs targeted to three to six different locations (green, blue, yellow, orange, purple and pink) upstream of the TSS for *KCTD13* (**A**), *TOAK2* (**B**), *NRXN1*

(**C-D**), *SNAP91* (**E**) and *CLCN3* (**F**). Data are presented as mean ± s.e.m (bar graph) from at least 3 independent biological replicates. Each biological replicate is depicted by a circle. *=p < 0.05, **=p < 0.01, ***=p < 0.001, ****=p < 0.0001.

SI Table 1. Control hiPSC NPC lines.

ID	Source	Patient ID	Source Cell	Reprogramming method	NPC line	Sex	Dx	Ethnicity	Age at Biopsy	IQ
C1	NIMH	NSB553	Fibroblast	SV-KOSM	NSB553 hiPSC#S1 NPC#1	M	Control	caucasian, non-Hispanic	31	127
C2	NIMH	NSB2607	Fibroblast	SV-KOSM	NSB2607 hiPSC#1 NPC#4	M	Control	caucasian, non-Hispanic	15	126
C3	NIMH	NSB690	Fibroblast	SV-KOSM	NSB690 hiPSC#2 NPC#1	M	Control	caucasian, non-Hispanic	25	115

SI Table 2. gRNA sequences.

gRNA Target	Oligo Sequence	
hKCTD13 gRNA #1	caccgGGAGCGCACGTCGACCCGCC	aaacGGCGGGTCGACGTGCGCTCCc
hKCTD13 gRNA #2	caccgGGTCGGCCGCATCCTCGATC	aaacGATCGAGGATGCGGCCGACCc
hKCTD13 gRNA #3	caccgAGCGCACGTCGACCCGCCCG	aaacCGGGCGGGTCGACGTGCGCTc
hTAOK2 gRNA #1	caccgGCGCAAAGATTCCTCGCACT	aaacAGTGCGAGGAATCTTTGCGCc
hTAOK2 gRNA #2	caccgGCGATCTGCGACTGCGCGCA	aaacTGCGCGCAGTCGCAGATCGCc
hTAOK2 gRNA #3	caccgGGCGATCTGCGACTGCGCGC	aaacGCGCGCAGTCGCAGATCGCCc
hNRXN1 gRNA #1	caccgCGTAGCCTACTGAGCATGCC	aaacGGCATGCTCAGTAGGCTACGc
hNRXN1 gRNA #2	caccgAGGAGTCGATAATTATGATG	aaacCATCATAATTATCGACTCCTc
hNRXN1 gRNA #3	caccgGCTCGGAACCCTTGAAAAGA	aaacTCTTTTCAAGGGTTCCGAGCCc
hNRXN1 gRNA #4	caccgCCGGGGCCGACAGGGTCAAAATG	aaacCATTTTGACCCTGTCGGCCCCGGc
hNRXN1 gRNA #5	caccgCAGTGGTACAGGGTAGCCACAGA	aaacTCTGTGGCTACCCTGTACCACTGc
hNRXN1 gRNA #6	caccgCCAGAGCCTGAAGCATGCATCGG	aaacCCGATGCATGCTTCAGGCTCTGGc
hSNAP91 gRNA #1	caccgGCGCGACGACGCCCTTGCCT	aaacAGGCAAGGGCGTCGTCGCGCc
hSNAP91 gRNA #2	caccgGACGGTCGCGGATGGCCGGC	aaacGCCGGCCATCCGCGACCGTCc
hSNAP91 gRNA #3	caccgGTTGGCCAAGACGGGCGAGT	aaacACTCGCCCGTCTTGGCCAACc
hCLCN3 gRNA #1	caccgGAGTAGCGTCGGCGCCTATT	aaacAATAGGCGCCGACGCTACTCc
hCLCN3 gRNA #2	caccgCGCCGACGCTACTCAGCGAG	aaacCTCGCTGAGTAGCGTCGGCGc
hCLCN3 gRNA #3	caccgGTGAGCTAATCGCTAATGAC	aaacGTCATTAGCGATTAGCTCACc
IVT *SNAP91* gRNA#3	TAATACGACTCACTATAGTTGGCCAAGACGGGCG	TTCTAGCTCTAAAACACTCGCCCGTCTTGGCCAA
IVT *FUT9* gRNA #1	TAATACGACTCACTATAGTACACGCGCGAGATCCAG	TTCTAGCTCTAAAACCTGGATCTCGCGCGTGTA
IVT *FUT9* gRNA #2	TAATACGACTCACTATAGACACGCGCGAGATCCAGA	TTCTAGCTCTAAAACTCTGGATCTCGCGCGTGT
IVT *FUT9* gRNA #3	TAATACGACTCACTATAGATGTTATGCATTACACCA	TTCTAGCTCTAAAACTGGTGTAATGCATAACAT
IVT *FUT9* gRNA #4	TAATACGACTCACTATAGCCAGATATTGGTTAGCAA	TTCTAGCTCTAAAACTTGCTAACCAATATCTGG
IVT *FUT9* gRNA #5	TAATACGACTCACTATAGATCCATTGTTGTAACCAG	TTCTAGCTCTAAAACCTGGTTACAACAATGGAT
IVT *FUT9* gRNA #6	TAATACGACTCACTATAGATTACTTGATGGGCTGGG	TTCTAGCTCTAAAACCCCAGCCCATCAAGTAAT
IVT *FUT9* gRNA #7	TAATACGACTCACTATAGGGGTATAGCTGCAAAAGG	TTCTAGCTCTAAAACCCTTTTGCAGCTATACCC
IVT *FUT9* gRNA #8	TAATACGACTCACTATAGGCTGGGGATGTAAGCTGG	TTCTAGCTCTAAAACCCAGCTTACATCCCCAGC
IVT *FUT9* gRNA #9	TAATACGACTCACTATAGGGTAATTGGGGATAGACA	TTCTAGCTCTAAAACTGTCTATCCCCAATTACC
IVT *FUT9* gRNA #10	TAATACGACTCACTATAGACAGTGGGTGAATTTGCA	TTCTAGCTCTAAAACTGCAAATTCACCCACTGT

IVT *CEP162* gRNA #1	TAATACGACTCACTATAGCAGATCATTTCAACCACT	TTCTAGCTCTAAAACAGTGGTTGAAATGATCTG
IVT *CEP162* gRNA #2	TAATACGACTCACTATAGGTGAAAACCCTTGAGGGG	TTCTAGCTCTAAAACCCCCTCAAGGGTTTTCAC
IVT *CEP162* gRNA #3	TAATACGACTCACTATAGAGATTGGCAAGTAAGGTG	TTCTAGCTCTAAAACCACCTTACTTGCCAATCT
IVT *CEP162* gRNA #4	TAATACGACTCACTATAGAAAGGTCTGACAAAAGGG	TTCTAGCTCTAAAACCCCTTTTGTCAGACCTTT
IVT *CEP162* gRNA #5	TAATACGACTCACTATAGAGACAGTGAAAACCCTTG	TTCTAGCTCTAAAACCAAGGGTTTTCACTGTCT
IVT *CEP162* gRNA #6	TAATACGACTCACTATAGACAGTGAAAACCCTTGAG	TTCTAGCTCTAAAACCTCAAGGGTTTTCACTGT
Negative Control (NC) gRNAs		
IVT *FUT9* NC gRNA #1	TAATACGACTCACTATAGTCTCCCAAATAGTATCTG	TTCTAGCTCTAAAACCAGATACTATTTGGGAGA
IVT *FUT9* NC gRNA #2	TAATACGACTCACTATAGATGCCACTCATCCAAACC	TTCTAGCTCTAAAACGGTTTGGATGAGTGGCAT
IVT *FUT9* NC gRNA #3	TAATACGACTCACTATAGGAGTGCAACATGACCAAG	TTCTAGCTCTAAAACCTTGGTCATGTTGCACTC
IVT *FUT9* NC gRNA #4	TAATACGACTCACTATAGGAGTGGCATCAGTATCAG	TTCTAGCTCTAAAACCTGATACTGATGCCACTC
IVT *FUT9* NC gRNA #5	TAATACGACTCACTATAGAACAAAGACATTGACCCA	TTCTAGCTCTAAAACTGGGTCAATGTCTTTGTT
IVT *FUT9* NC gRNA #6	TAATACGACTCACTATAGTACTGCTCTGTACCATGA	TTCTAGCTCTAAAACTCATGGTACAGAGCAGTA
IVT *FUT9* NC gRNA #7	TAATACGACTCACTATAGCCAACCACCACTTCAGCA	TTCTAGCTCTAAAACTGCTGAAGTGGTGGTTGG
IVT *FUT9* NC gRNA #8	TAATACGACTCACTATAGatATCTATGCCAAGATGG	TTCTAGCTCTAAAACCCATCTTGGCATAGATAT
IVT *FUT9* NC gRNA #9	TAATACGACTCACTATAGAACCACTTCAGCAGGGAGT	TTCTAGCTCTAAAACACTCCCTGCTGAAGTGGT
IVT *FUT9* NC gRNA #10	TAATACGACTCACTATAGTGGAAATATTTACCAAGG	TTCTAGCTCTAAAACCCTTGGTAAATATTTCCA
IVT *CEP162* NC gRNA #1	TAATACGACTCACTATAGGGGAAGAAGCGATCAGGG	TTCTAGCTCTAAAACCCCTGATCGCTTCTTCCC
IVT *CEP162* NC gRNA #2	TAATACGACTCACTATAGCCACCACCACTTACCCAA	TTCTAGCTCTAAAACTTGGGTAAGTGGTGGTGG
IVT *CEP162* NC gRNA #3	TAATACGACTCACTATAGAAGATAGAACCCAGCTGA	TTCTAGCTCTAAAACTCAGCTGGGTTCTATCTT
IVT *CEP162* NC gRNA #4	TAATACGACTCACTATAGAAAGGGATTAGCCTTAGG	TTCTAGCTCTAAAACCCTAAGGCTAATCCCTTT
IVT *CEP162* NC gRNA #5	TAATACGACTCACTATAGCAAGATAGAACCCAGCTG	TTCTAGCTCTAAAACCAGCTGGGTTCTATCTTG
IVT *CEP162* NC gRNA #6	TAATACGACTCACTATAGACAATAAAGGACGACCAA	TTCTAGCTCTAAAACTTGGTCGTCCTTTATTGT
IVT *CEP162* NC gRNA #7	TAATACGACTCACTATAGCATGGAAGTCCCTCAGCT	TTCTAGCTCTAAAACAGCTGAGGGACTTCCATG
IVT *CEP162* NC gRNA #8	TAATACGACTCACTATAGGGAAGAAGCGATCAGGGA	TTCTAGCTCTAAAACTCCCTGATCGCTTCTTCC
IVT *CEP162* NC gRNA #9	TAATACGACTCACTATAGTAGACTTTGCACACTGTG	TTCTAGCTCTAAAACCACAGTGTGCAAAGTCTA
IVT CEP162 NC gRNA #10	TAATACGACTCACTATAGACAGAAGTCCTACATACC	TTCTAGCTCTAAAACGGTATGTAGGACTTCTGT

SI Table 3. qPCR primer sequences.

mRNA Target	Oligo Sequence	
	Forward	**Reverse**
GAPDH	AGGGCTGCTTTTAACTCTGGT	CCCCACTTGATTTTGGAGGGA
B-ACTIN	TGTCCCCCAACTTGAGATGT	TGTGCACTTTTATTCAACTGGTC
KCTD13	TAACAGGACACCTGCAAACG	CTGGATGATGCCATGTCTTG
TAOK2	AGTCTAGCTCTTCTCCCCGC	CCGCCAAGCCCGAGTG
NRXN1	AGAAAGATGCCAAGCACCCA	CCCATGTCCAGGAGGAGGTA
SNAP91	AGGACCCATTAGCGGATCTTAACA	GCTCCCTTTGAAACTCAGCATCAA
CLCN3	AATCATAGGTCAAGCAGAGGGTCC	CCACAGGCATATGGAGCAAATACC
CEP162	TGCCTTGGTGGATAACTGAA	GAGAGAGGTTCCACTGCTCTT
FUT-9	TGTCTACGTGCTTCCCATGA	AACAGCCCAGGATAATGCAG
IVT SNAP91 #3	TTGGCCAAGACGGGCGAGT	CGACTCGGTGCCACTTTTTC

SI Table 4. Antibodies.

Antibody	Species	Supplier	Product Number	Dilution
GFAP	Ck	Aves Lab	GFAP	1:1000
EAAT1/GLAST	Rb	Boster Bio	PA2185	1:100
S100β	Ms	Sigma	S2532	1:1000
VIMENTIN	Rb	Cell Signaling	#3932	1:500
Cas9:AF-488	Ms	CST	34963S	5uL/1x10^6 cells

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

hiPSC and NPC culture

All hiPSC and NPC lines were derived and validated as previously described; full details of the donors of the fibroblasts and validation of the hiPSC and NPC lines available (Topol et al., 2016) (**SI Table 1**). NPCs were maintained at high density, grown on growth factor reduced Matrigel (BD Biosciences) coated plates in NPC media (Dulbecco's Modified Eagle Medium/Ham's F12 Nutrient Mixture (ThermoFisher Scientific), 1x N2, 1x B27-RA (ThermoFisher Scientific) and 20 ng/ml FGF2 and split 1:3 every week with Accutase (Millipore, Billerica, MA, USA). Routine (every 4 weeks) mycoplasma testing was conducted using the MycoAlert Mycoplasma detection kit (Lonza); all cells used in this study were consistently negative.

Astrocyte differentiation

Antibiotic-selected dCas9-effector NPCs were differentiated to astrocytes and maintained as previously described (TCW et al., 2017). Astrocytes were cultured in astrocyte medium (ScienCell: 1801, astrocyte medium (1801-b), 2% fetal bovine serum (0010), astrocyte growth supplement (1852) and 10U/ml penicillin/streptomycin solution (0503) on Matrigel. Immunocytochemistry and FACS were used to validate NPC-astrocytes. Vendors, catalog numbers and dilutions of all antibodies used are listed in **SI Table 4**.

NGN2-induced neuronal differentiation

NGN2-induced neurons were derived from dCas9-effector NPCs as previously described (Ho et al., 2015) using the human *NGN2*-Neo lentiviral expressing vector (Addgene #79049). gRNA lentiviruses generated as above were co-transduced with induction viruses and cultures were spinfected (1 hour, 1000xg, 25°C). Cells were harvested at 9 days or 21 days later.

SUPPLEMENTAL REFERENCES

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Ho, S.M., Hartley, B.J., Tcw, J., Beaumont, M., Stafford, K., Slesinger, P.A., and Brennand, K.J. (2015). Rapid Ngn2-induction of excitatory neurons from hiPSC-derived neural progenitor cells. Methods.

TCW, J., Wang, M., Pimenova, A.A., Bowles, K.R., Hartley, B.J., Lacin, E., Machlovi, S., Abdelaal, R., Karch, C.M., Phetnani, H.*, et al.* (2017). An efficient platform for astrocyte differentiation from human induced pluripotent stem cells. bioRxiv.

Topol, A., Zhu, S., Hartley, B.J., English, J., Hauberg, M.E., Tran, N., Rittenhouse, C.A., Simone, A., Ruderfer, D.M., Johnson, J.*, et al.* (2016). Dysregulation of miRNA-9 in a Subset of Schizophrenia Patient-Derived Neural Progenitor Cells. Cell reports *15*, 1024-1036.