An iPSC-Derived Neuron Model of CLN3 Disease Facilitates Small Molecule Phenotypic Screening

Nihar Kinarivala[¶#], Ahmed Morsy[‡#], Ronak Patel[¶#], Angelica V. Carmona[‡], Md. Sanaullah Sajib[¶], Snehal Raut[¶], Constantinos M. Mikelis[¶], Abraham Al-Ahmad[¶*] and Paul C. Trippier[‡,¶,§,*]

[‡]Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE 68198, USA.

[¶]Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, School of Pharmacy, Amarillo, TX 79106, USA.

[§]UNMC Center for Drug Discovery, University of Nebraska Medical Center, Omaha, NE 68198, USA.

[#]Contributed equally to this work.

[*]Corresponding Author: paul.trippier@unmc.edu

[*]Corresponding Author: abraham.al-ahmad@ttuhsc.edu

Supporting Information

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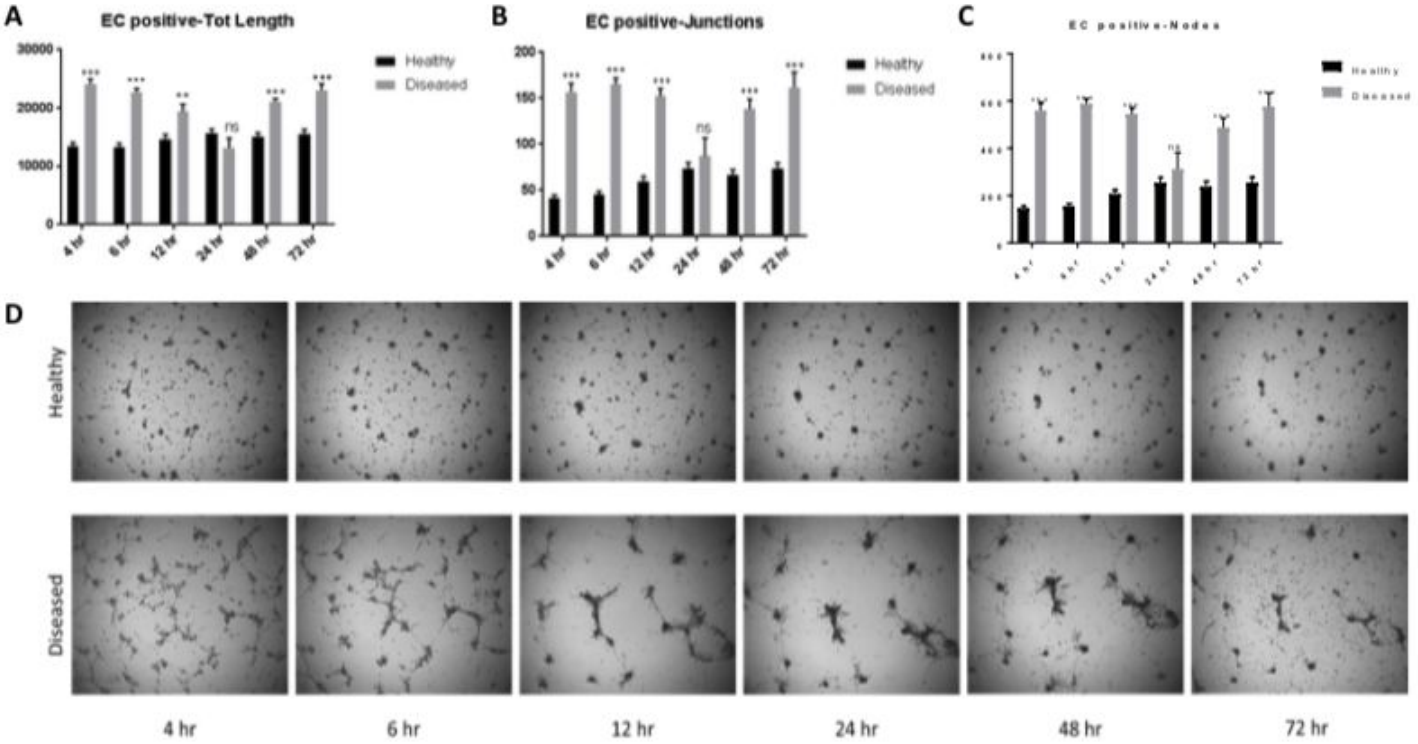
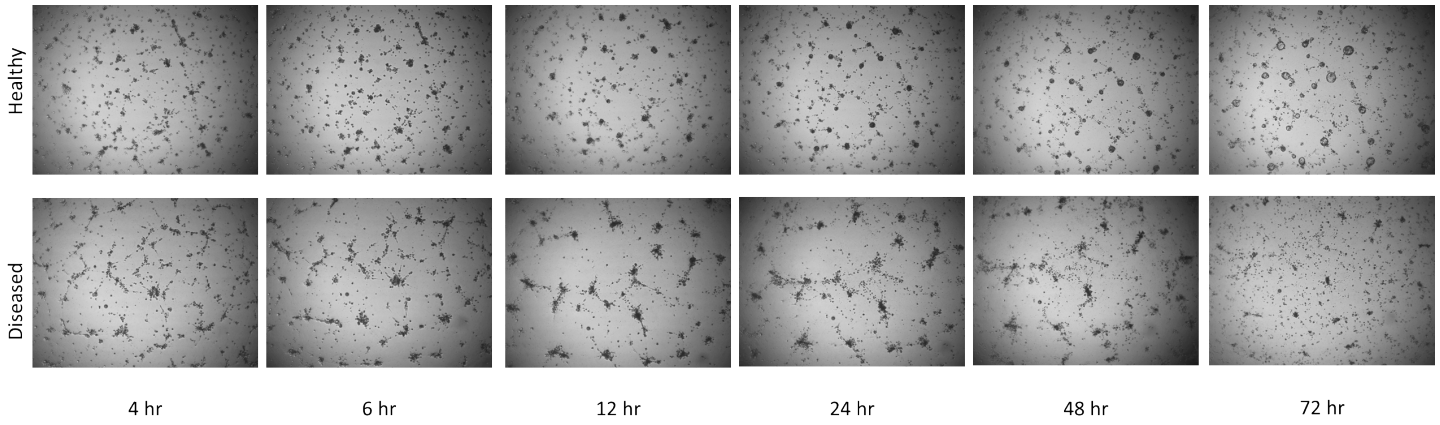
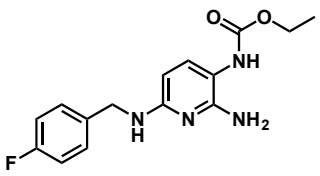
Supplementary Figure S-1a. At regular intervals, tube length (A), number of nodes (B) and number of junctions (C) were quantified in EC++ medium. Representative images of tube formation assay in EC++ medium at each time point are shown (D).



Supplementary Figure S-1b. Representative images of tube formation assay in EC- medium at each time point.



Supplementary Figure S-2. Structures of aromatic carbamates used herein.



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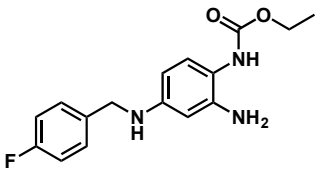

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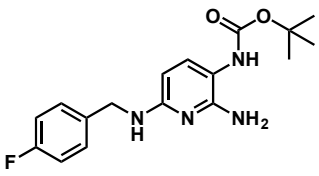

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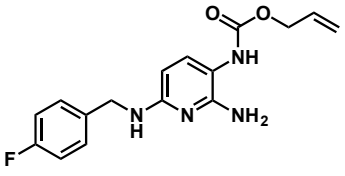

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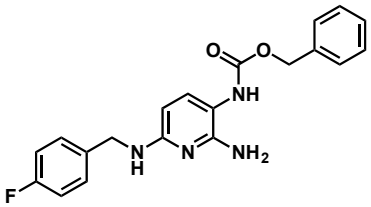
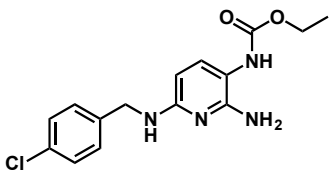
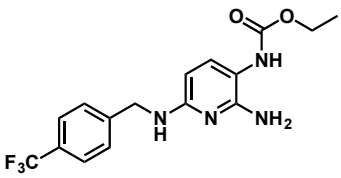


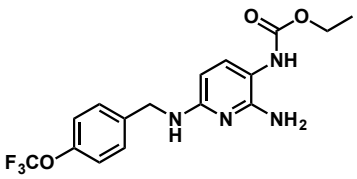
(8l)



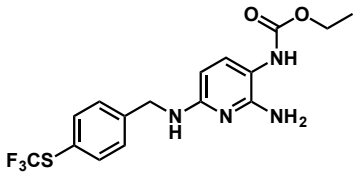
(9a)



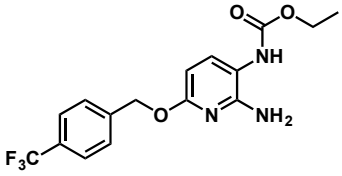
(9e)



9g)

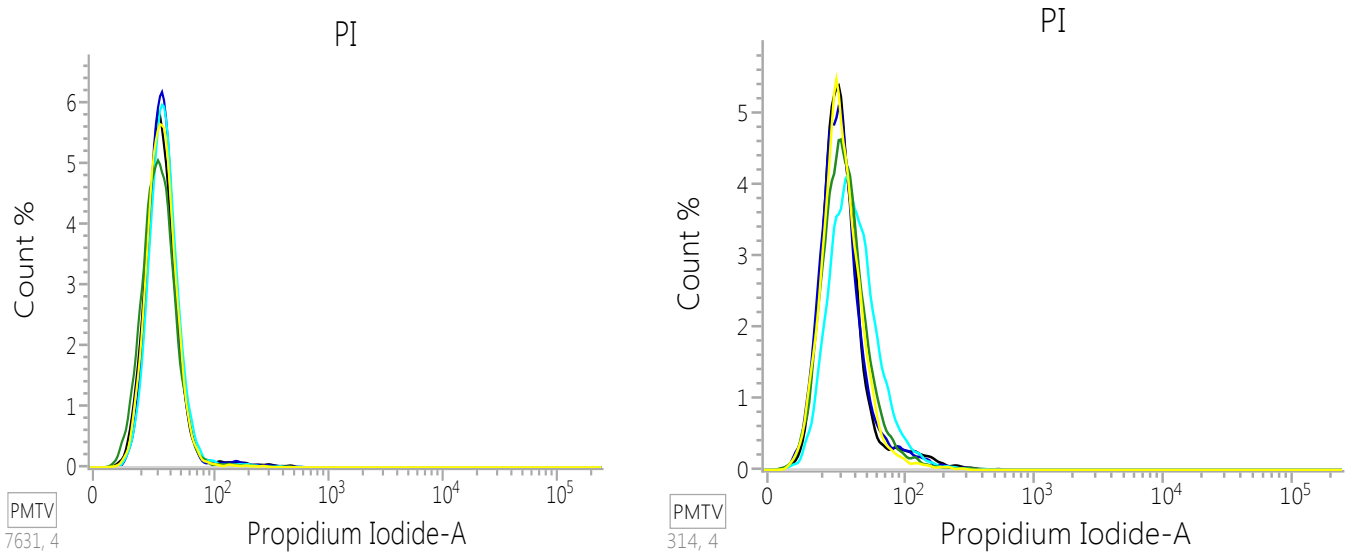


(9h)

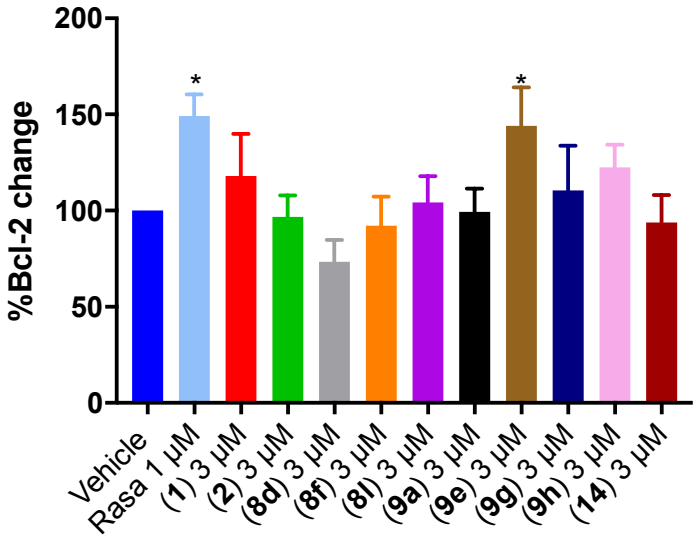


(14)

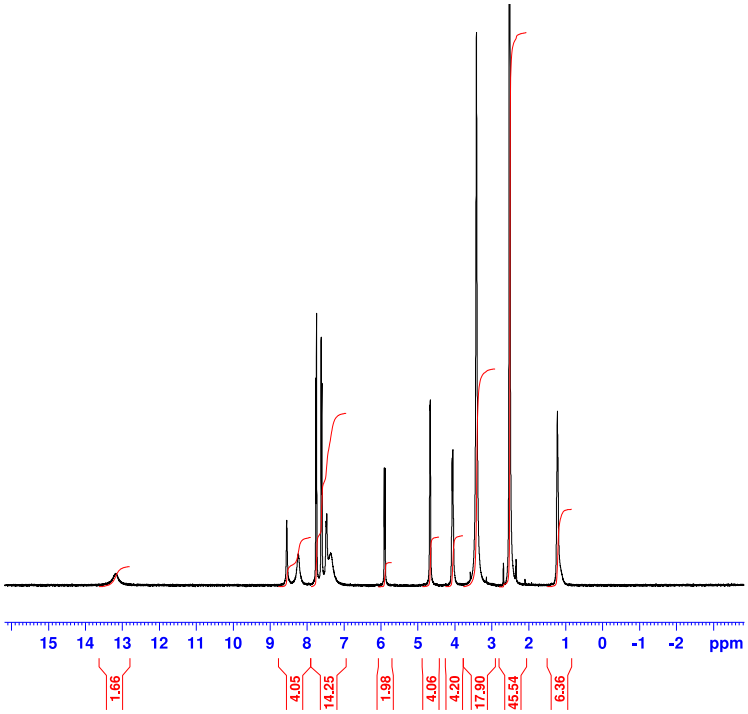
Supplementary Figure S-3. Propidium Iodide staining of CLN3 iPSC-derived neurons with selected aromatic carbamate small molecules. No toxicity is observed at 3 μM concentration for 48 hours in CLN3 iPSC-derived neurons.



Supplementary Figure S-4. Bcl-2 induction of all compounds tested expressed as percentage of Bcl-2 change upon small molecule treatment of CLN3-derived neurons at 3 μM. n = 3, expressed as mean ± SD. One-way ANOVA; 95% Confidence Interval; *, p= <0.012.



Supplementary Figure S-5a. ^{1}H NMR spectra of **9e** at day zero.

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DS                     2
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AQ             4.0894465 sec
RG                   161
DW                62.400 usec
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TE                291.9 K
D1             1.00000000 sec
TD0                    1

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NUC1                  1H
P1                15.00 usec
PLW1           9.85000038 W

F2 - Processing parameters
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WDW                   EM
SSB       0
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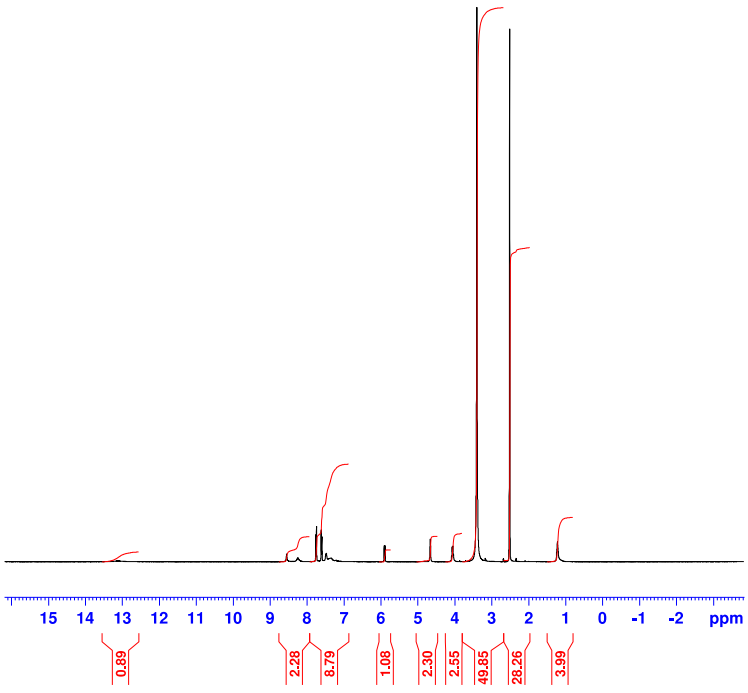
Supplementary Figure S-5b. ^{1}H NMR spectra of **9e** after 10 days under assay conditions at 37 ^{o}C.

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F2 - Processing parameters
SI                 65536
SF            400.1300000 MHz
WDW                   EM
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LB                0.30 Hz
GB        0
PC                1.00
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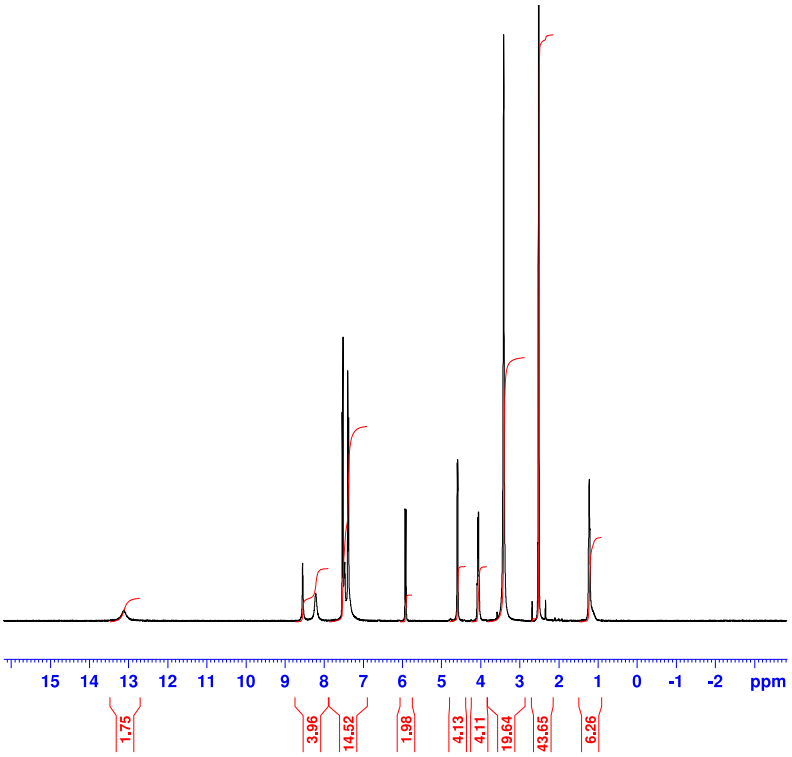
Supplementary Figure S-6a. ^{1}H NMR spectra of **9g** at day zero.

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FIDRES        0.122266 Hz
AQ            4.0894465 sec
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DW               62.400 usec
DE                6.50 usec
TE               292.4 K
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TD0                  1

======== CHANNEL f1 ========
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F2 - Processing parameters
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SF           400.1300000 MHz
WDW                  EM
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PC                1.00
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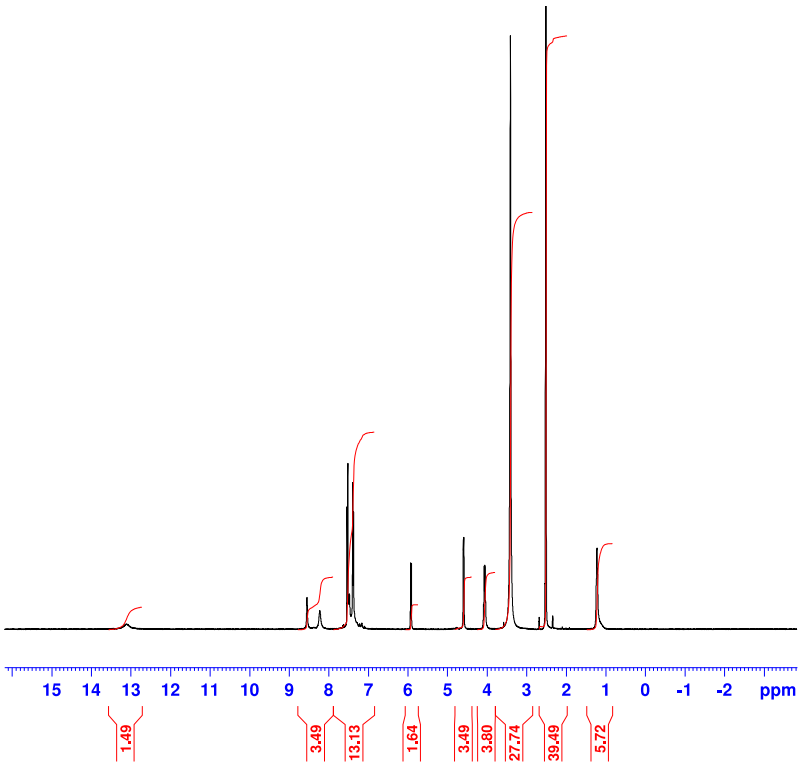
Supplementary Figure S-6b. ^{1}H NMR spectra of **9g** after 10 days under assay conditions at 37 ºC.

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AQ            4.0894465 sec
RG                 144
DW               62.400 usec
DE                6.50 usec
TE               293.3 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
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NUC1                1H
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PLW1         9.85000038 W

F2 - Processing parameters
SI                65536
SF           400.1300000 MHz
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GB        0
PC                1.00
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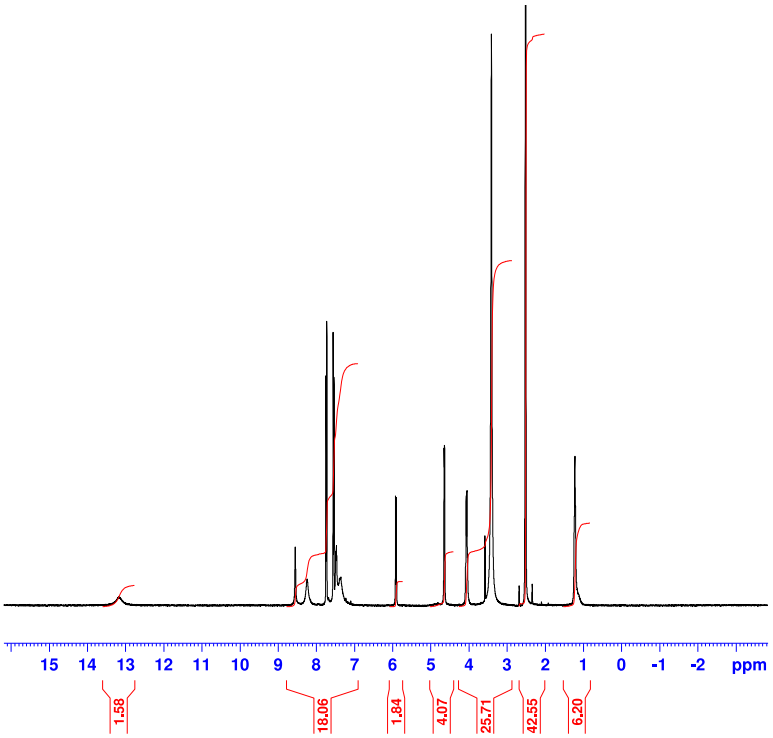
Supplementary Figure S-7a. ^{1}H NMR spectra of **9h** at day zero.

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TD                65536
SOLVENT            DMSO
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DS                    2
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FIDRES        0.122266 Hz
AQ            4.0894465 sec
RG                  144
DW               62.400 usec
DE                6.50 usec
TE               292.6 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        400.1324710 MHz
NUC1                1H
P1                15.00 usec
PLW1         9.85000038 W

F2 - Processing parameters
SI                65536
SF          400.1300000 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
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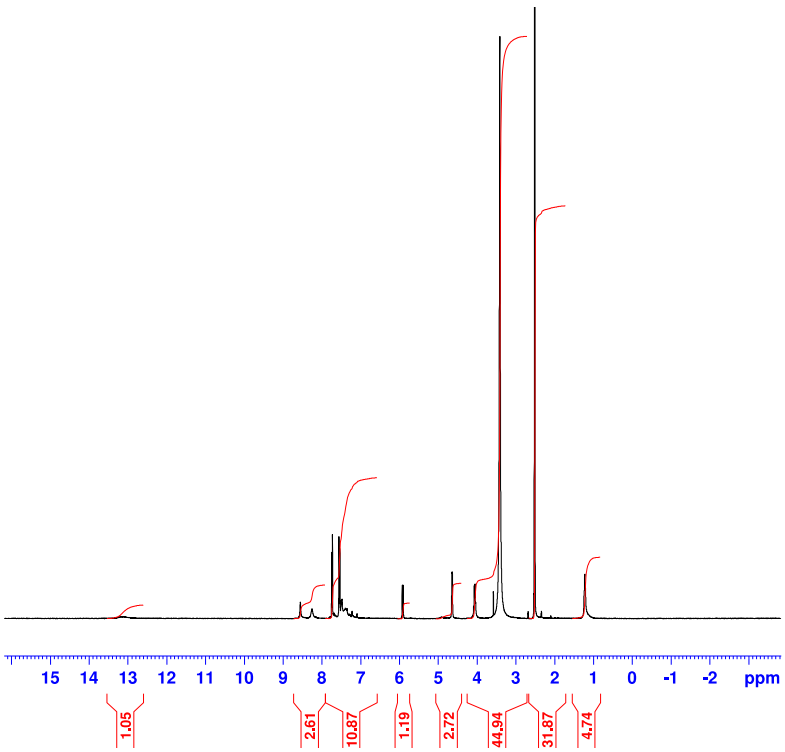
Supplementary Figure S-7b. ^{1}H NMR spectra of **9h** after 10 days under assay conditions at 37 ºC.

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PROCNO                1

F2 - Acquisition Parameters
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TD                65536
SOLVENT            DMSO
NS                   16
DS                    2
SWH            8012.820 Hz
FIDRES        0.122266 Hz
AQ            4.0894465 sec
RG                  144
DW               62.400 usec
DE                6.50 usec
TE               293.4 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
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NUC1                1H
P1                15.00 usec
PLW1         9.85000038 W

F2 - Processing parameters
SI                65536
SF          400.1300000 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
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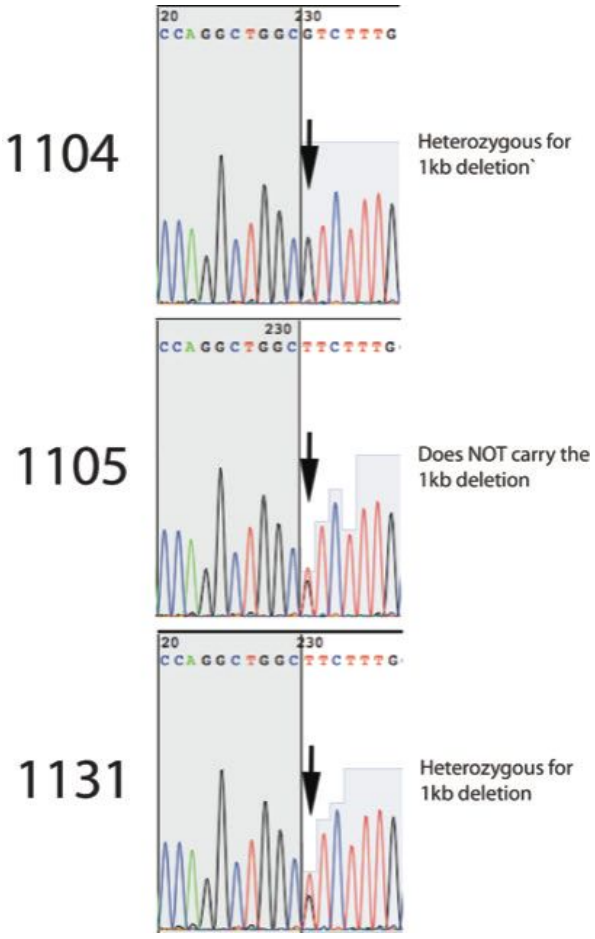
Figure S-8. Confirmation of genotypes by sequencing of CLN3 iPSC line employed in this study. Obtained from the New York Stem Cell Foundation as family group 5003. 1104) Maternal iPSCs heterozygous for common (1 kb) deletion. 1105) Paternal iPSCs wild-type; carrier of E13 c.988>T, p.Val330Phe mutation. 1131) Compound mutation: Heterozygous for both common (1 kb) deletion and E13 c.988>T, p.Val330Phe mutation.



Supplementary Table S-1: Compound **9e** inhibits the Kv7.1 channel in a dose-dependent manner.

Compound ID	Client Compound ID	Concentration (μM)	% inhibition		
			n1	n2	mean
US034-0009898-1	AM-9e	0.1	9.47	6.08	7.77
US034-0009898-1	AM-9e	1	19.20	17.50	18.35
US034-0009898-1	AM-9e	10	39.24	40.06	39.65
Time-Matched Vehicle Control	DMSO	0.003	2.35	6.82	4.58
Time-Matched Vehicle Control	DMSO	0.003	8.12	8.87	8.49
Time-Matched Vehicle Control	DMSO	0.003	15.00	24.51	19.76
Positive Reference Control	Chromanol 293B	0.3	7.61	13.97	10.79
Positive Reference Control	Chromanol 293B	1	19.09	19.69	19.39
Positive Reference Control	Chromanol 293B	3	34.73	36.72	35.72
Positive Reference Control	Chromanol 293B	10	43.16	43.27	43.22
Positive Reference Control	Chromanol 293B	30	74.30	76.58	75.44
Positive Reference Control	Chromanol 293B	100	93.28	95.70	94.49

Supplementary Table S-2: Antibody source and use.

Antibody (clone)	Vendor	IF	Flow Cytometry	WB
CD31/PECAM1 (polyclonal)	Labvision	1:20 (MeOH)	N.D.	N.D.
GLUT 1 (SPM498)	Labvision	1:100 (MeOH)	N.D.	N.D.
BCRP (5D3)	Millipore	1:50 (MeOH)	1:20 (MeOH)	N.D.
P-glycoprotein (F4)	ThermoFisher	1:50 (MeOH)	1:20 (MeOH)	N.D.
MRP 1 (QCRL 1)	Millipore	1:50 (MeOH)	1:20 (MeOH)	N.D.
Claudin-5 (4C3C2)	ThermoFisher	1:100	N.D.	N.D.
Occludin (OC3F10)	ThermoFisher	1:100	N.D.	N.D.
VE-Cadherin (F8)	Santa Cruz Biotechnology	1:50	N.D.	N.D.
Nestin (25)	BD Biosciences	1:100	N.D.	N.D.
PAX6 (13B10)	BD Biosciences	1:100	N.D.	N.D.
Beta tubulin, Class III (TUJ1)	BD Biosciences	1:100	N.D.	N.D.
Caveolin-1	Cell Signaling Technology	N.D.	N.D.	1:1000
Clathrin	Cell Signaling Technology	N.D.	N.D.	1:1000
Actin	Cell Signaling Technology	N.D.	N.D.	1:1000
Bcl-2	ThermoFisher	N.D.	N.D.	1:1000
Beclin 1	Cell Signaling Technology	N.D.	N.D.	1:1000
P70 S6 Kinase	Cell Signaling Technology	N.D.	N.D.	1:1000
Subunit C	Abcam	N.D.	N.D.	1:1000

N.D. = Not determined