Supportive/Supplementary Material

Clinical utility of neuronal cells directly converted from fibroblasts of patients for neuropsychiatric disorders: studies of lysosomal storage diseases and channelopathy

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Supplementary Material consists of Supplementary Text, Tables S1-S3, Figures S1-S5, and Additional References.

Supplementary Text

Characterization of human induced neuronal (iN) cells directly generated from fibroblasts

Using a method similar to that used in previous studies [1-3], we generated human iN cells by lentiviral transduction of human ASCL1 (also known as MASH1), POU3F2 (also known as BRN2), and MYT1L cDNA into early-passage fibroblasts. These cells have a morphology characteristic of neuronal cells and are stained with the neuronal markers βIII-tubulin and MAP2 (**Supplementary Material, Fig. S1A**). Consistent with previous findings [4], a more efficient conversion to MAP2-positive cells with neuronal morphology was observed in fibroblasts from subjects at younger ages (**Supplementary Material, Fig. S1B and C**). In these cultures, 4-16% of cells are neuronal. We also generated neuronal cells expressing GFP driven by the αCaMKII promoter, and observed a near-complete overlap of MAP2 and GFP (**Supplementary Material, Fig. S1D**). We also observed that almost all the iN cells (MAP2-positive cells with neuronal morphology) are stained with another neuronal marker, NeuN (98.8±1.2%) (**Supplementary Material, Fig. S1E)**. Our culture conditions are favorable for obtaining glutamatergic neuronal cells, as vesicular glutamate transporter 1 (VGLUT1)-positive cells were present, but no detectable glutamate decarboxylase 67-positive or tyrosine hydroxylase-positive cells were observed at the protein level (**Supplementary Material, Fig. S1F**).

We also conducted whole-cell current clamp recordings to characterize the electrophysiological properties of iN cells at 7-37 days after introduction of the three factors (**Supplementary Material, Fig. S1G**). The resting membrane potential of neuronal cells (n=10) was -30.00 ± 12.8 mV, and input resistance was 1169 ± 805 MΩ (mean ± S.D.). Action potentials were observed from cells with a distinct neuronal morphology, i.e. round soma shape and more than one process, in response to depolarizing current pulses after cells were hyperpolarized to approximately -70 mV with intra-somatic current injection. These action potentials were reversibly blocked by the addition of the sodium channel blocker tetrodotoxin (TTX, 1 μM) (**Supplementary Material, Fig. S1H**). The average duration of the action potentials, measured at half-width, was 16.21 ± 5.9 ms. The depolarized resting membrane potential, high input resistance, need to hyperpolarize the membrane to elicit action potentials, and the slow nature of the action potentials indicated that these neuronal cells have a low number of potassium channels. Thus, our protocol results in the

generation of MAP2-positive, glutamatergic iN cells displaying action potentials. We used these iN cells at day 10-37 after lentiviral infection for the subsequent experiments.

Table S1 | List of fibroblasts used for the generation of iN cells

Diseases	OMIM #	Molecular defects	Age	Sex
Tay-Sachs disease (TSD) (GM00502)	272800	Deficiency of Hexosaminidase A	11 M	Male
Tay-Sachs disease (TSD) (GM01110)	272800	Deficiency of Hexosaminidase A	10 M	Male
Tay-Sachs disease (TSD) (TC86-1530)	272800	Deficiency of Hexosaminidase A	11 M	Female
Sandhoff disease (SD)	268800	Deficiency of Hexosaminidase A and B	10 M	Female
GM1 gangliosidosis (GM1)	230500	Deficiency of b-galactosidase	3 M	Male
Gaucher disease (GD) type II	230900	Deficiency of Glucocerebrosidase	1 Y	Male
Metachromatic leukodystrophy (MLD)	250100	Deficiency of Arylsulfatase A	2 Y	Male
Dravet syndrome (D1)	607208	c.2584C>T (p.R862X), *SCN1A* gene	15 Y	Male
Dravet syndrome (D2)	607208	c.3306C>A (p.Y1102X), *SCN1A* gene	2 Y	Female
Dravet syndrome (D3)	607208	c.126delA (K42fxX91) male, samples collected at 24 yo	24 Y	Male
Machado-Joseph disease (MJD) (GM06153)	109150	71 CAG repeats, *Ataxin-3* gene	44 Y	Male
Dentatorubral-pallidoluysian atrophy (DRPLA) (GM06917)	125370	68 CAG repeats, *Atrophin* gene	15 Y	Male
Huntington's disease (HD) (GM09197)	143100	180 CAG repeats, *Huntingtin* gene	6 Y	Male
Control (C1)	---	---	51 Y	Female
Control (C2)	---	---	42 Y	Male
Control (C3)	---	---	57 Y	Female
Control (C4)	---	---	27 Y	Female
Control (GM05399)	---	---	1 Y	Male
Control (TC11-3782)	---	---	10 M	Male
Control (GM05659)	---	---	1 Y	Male

*OMIM: Online Mendelian Inheritance in Man

**Y, year; M, month.

Table S2 | Summary of electrophysiological properties of iN cells

	Control	**Dravet**
Days after infection	21.80 ± 9.43	21.25 ± 5.63
Vm (mV)	-31.00 ± 12.75	-28.40 ± 22.70
Threshold (mV)	-25.72 ± 6.51	-30.25 ± 4.43
AP Amplitude (mV)	44.80 ± 10.42	42.81 ± 9.48
AHP (mV)	-9.57 ± 9.80	-6.81 ± 11.42
Duration (ms) *	**16.21 ± 5.91**	**8.39 ± 3.56**
Rise time (ms) *	**10.46 ± 5.07**	**3.95 ± 1.60**
Decay time (ms)	27.61 ± 11.89	14.46 ± 10.95

*Statistically significant difference ($p<0.05$) between control and Dravet subjects

Table S3 | Variability in the Dravet's electrophysiology data (action potential duration) within versus between groups.

	C1	C2	D1	D2
Number of values	5	5	4	4
Minimum (ms)	6.200	8.54	2.470	7.780
25% Percentile	8.090	14.35	3.143	7.883
Median (ms)	15.74	20.42	6.500	10.37
75% Percentile	17.73	22.50	9.460	13.01
Maximum (ms)	18.02	24.58	10.00	13.16
Mean (ms)	13.47	18.82	6.368	10.42
Std. Deviation (ms)	5.162	6.04	3.267	2.825
Std. Error (ms)	2.308	2.7	1.633	1.413
Lower 95% CI	7.065	11.33	1.170	5.922
Upper 95% CI	19.88	26.32	11.57	14.91

*The two control subjects (C1 and C2) have action potentials with durations larger on average than the Dravet patients (D1 and D2). This is confirmed with a statistical test, where a one-way ANOVA is significant, and driven by C2 vs D1 when correcting for multiple comparisons by using a Tukey's post-hoc test.

Legends for Supplementary Figures

Figure S1 | Generation of induced neuronal (iN) cells directly from human fibroblasts by using human ASCL1, POU3F2, and MYT1L. (**A**) βIII-tubulin and MAP2 staining in iN cells derived from adult human skin fibroblasts with human astrocyte-conditioned medium. Representative images of iN cells from multiple independent conversion experiments are shown. Cells were stained at 2-3 weeks after lentiviral infection. (**B**) iN cell induction from fetal fibroblasts MRC-5. (**C**) Quantification of MAP2-positive iN cells obtained from adult and fetal skin fibroblasts. Data are presented as mean ± S.E.M. HAM: human astrocyte-conditioned medium. (**D**) Considerable overlap of human CaMKII promoter-driven GFP (CaMKII::GFP) and MAP2 staining in iN cells. Quantification is presented as mean ± S.D. of three independent neuronal cultures for control (C1) and Dravet (D2) subjects. (**E**) Representative picture of NeuN staining in iN cells. (**F**) Neuronal subtype analysis at 4 weeks after neuronal induction. Note that no tyrosine hydroxylase- or GAD67-positive cells are observed. (**G**) Whole-cell recordings of iN cells from adult fibroblasts with a typical neuronal morphology. (**H**) Representative traces of changes in membrane potential induced by current injection before and after TTX (1 μM) treatment. Scale bars; 50 μm (**A, B**), 20 μm (**D, E**). Arrowheads indicate MAP2-positive iN cells (**A, B**) and NeuN-positive iN cells (**E**), and VGLUT1/MAP2-double positive iN cells (**F**).

Figure S2 | GM2 ganglioside accumulation in iN cells from Tay-Sachs disease and Sandhoff disease. (**A**) Representative pictures of MAP2-positive iN cells from other two patients and controls. Tay-Sachs MAP2-positive iN cells show robust accumulation of GM2 ganglioside (arrowheads). (**B**) No toxic effects of *N*B-DNJ tratment on Tay-Sachs iN cells. The total number of iN cells in *N*B-DNJ-treated culture is not significantly different from that of iN cells in mock-treated cultures. Scale bars, 20 μm (**A**).

Figure S3 | Representative action potential traces in iN cells from control (C1 and C2) and Dravet syndrome subjects (D1 and D2).

Figure S4 | Electrophysiological characterization of iN cells from Dravet patients and controls. Electrophysiological recordings were performed on iN cells at days 10-37 after lentiviral infection into

fibroblasts. Blue circles, control subjects (n=2); red rectangles, Dravet patients (n=2). Dravet patient iN cells have significantly shorter action potential duration (CONTROL: 16.21 ± 5.91 ms vs DRAVET 9.04 ± 3.29 ms, $t = 3.448$, $p = 0.029$). Even if the two data points recorded at 37 days post-infection are removed from the data set, duration is still significantly different (CONTROL: 14.64 ± 5.09 ms vs DRAVET 9.04 ± 3.29 ms, $t = 2.804$, $p = 0.013$).

In addition, a Pearson correlation analysis did not show a significant correlation between the duration or rise time and culture age (time in culture after conversion). For Duration, Control: Pearson r= 0.53; r2=0.28; P=0.11, Dravet: Pearson r= 0.24; r2=0.06; P=0.51. For Rise time, Control: Pearson r= 0.65; r2=0.43; P=0.08, Dravet: Pearson r= 0.25; r2=0.06; P=0.69.

Figure S5 | iN cell generation from various brain disorders. (**A**) Generation of MAP2-positive iN cells from various lysosomal storage diseases (Gaucher disease, GM1 gangliosidosis, and Metachromatic leukodystrophy). (**B)** MAP2-positive iN cells from patients with three polyglutamine diseases [Machado-Joseph disease, Dentatorubral-pallidoluysian atrophy (DRPLA), and Huntington's disease]. Scale bars, 100 μm.

Additional references

[1] Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. Nature. 2010;463(7284):1035-41.

[2] Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, et al. Induction of human neuronal cells by defined transcription factors. Nature. 2011;476(7359):220-3.

[3] Caiazzo M, Dell'Anno MT, Dvoretskova E, Lazarevic D, Taverna S, Leo D, et al. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature. 2011;476(7359):224-7.

[4] Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, et al. MicroRNA-mediated conversion of human fibroblasts to neurons. Nature. 2011;476(7359):228-31.

Figure S1

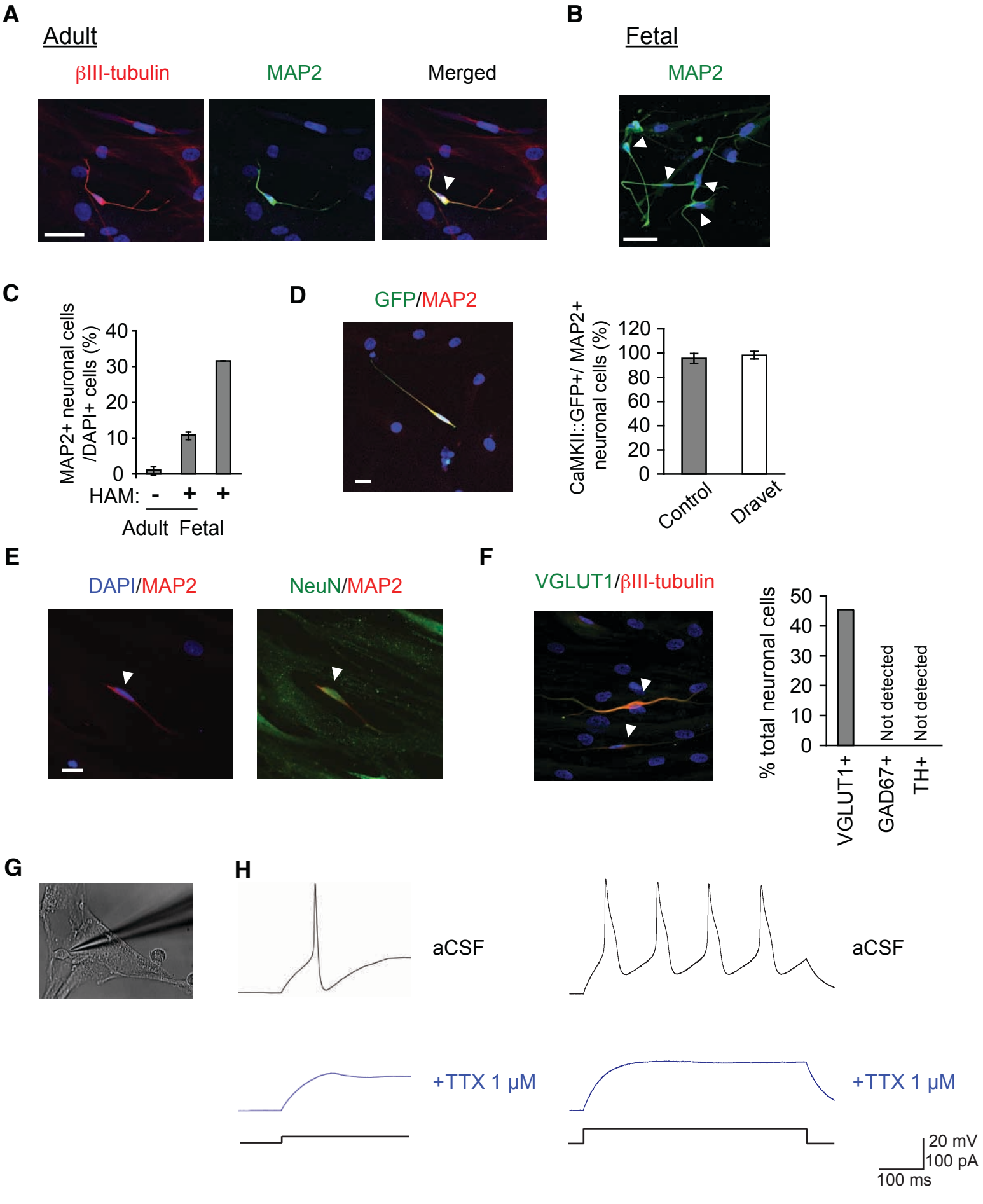
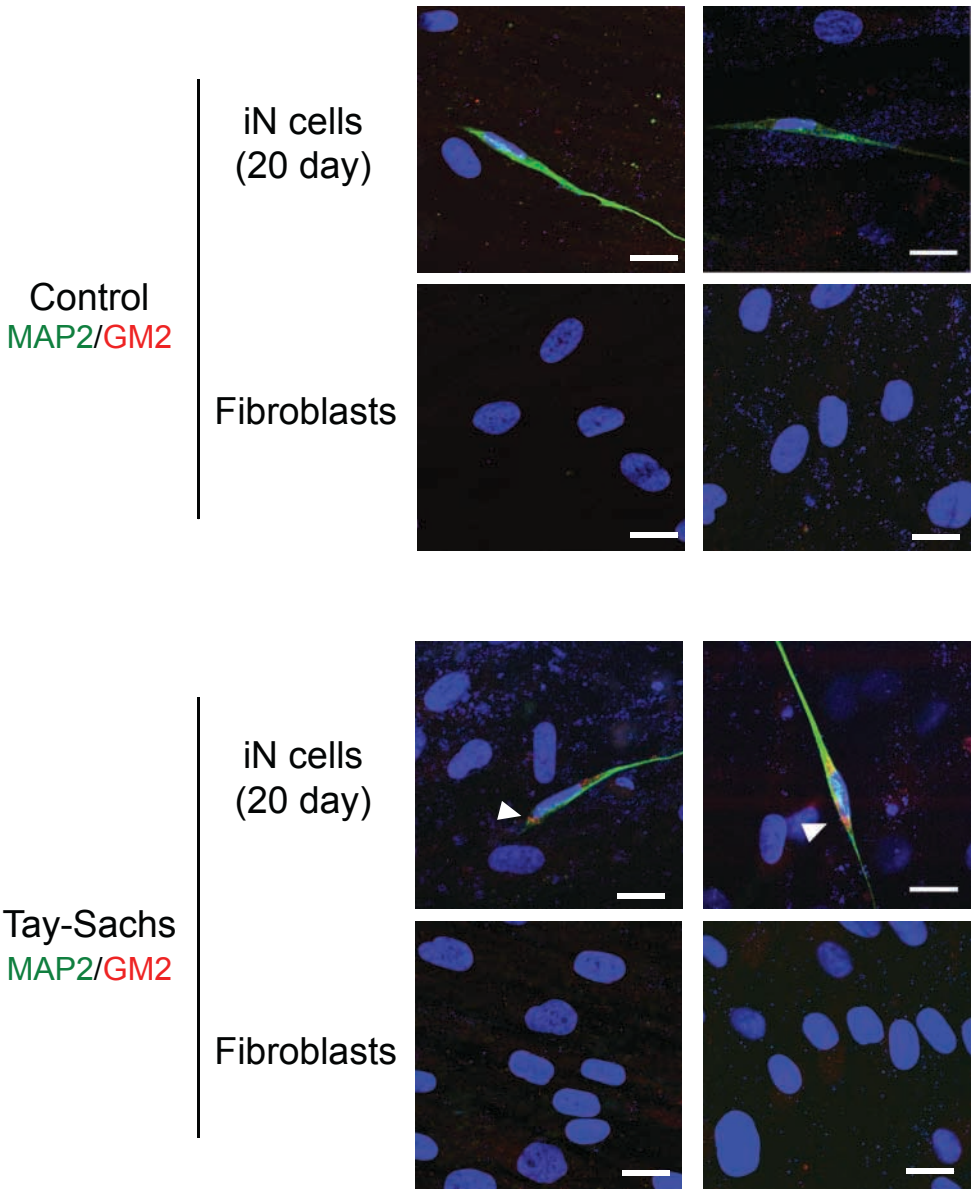

A
Adult
βIII-tubulin
MAP2
Merged
B
Fetal
MAP2
C
MAP2+ neuronal cells /DAPI+ cells (%)
40
30
20
10
0
HAM: - + +
Adult Fetal
D
GFP/MAP2
CaMKII::GFP+/ MAP2+ neuronal cells (%)
120
100
80
60
40
20
0
Control
Dravet
E
DAPI/MAP2
NeuN/MAP2
F
VGLUT1/βIII-tubulin
% total neuronal cells
50
40
30
20
10
0
Not detected
Not detected
VGLUT1+
GAD67+
TH+
G
H
aCSF
+TTX 1 μM
aCSF
+TTX 1 μM
20 mV
100 pA
100 ms

Figure S2

A


Control
MAP2/GM2
iN cells
(20 day)
Fibroblasts
Tay-Sachs
MAP2/GM2
iN cells
(20 day)
Fibroblasts

B

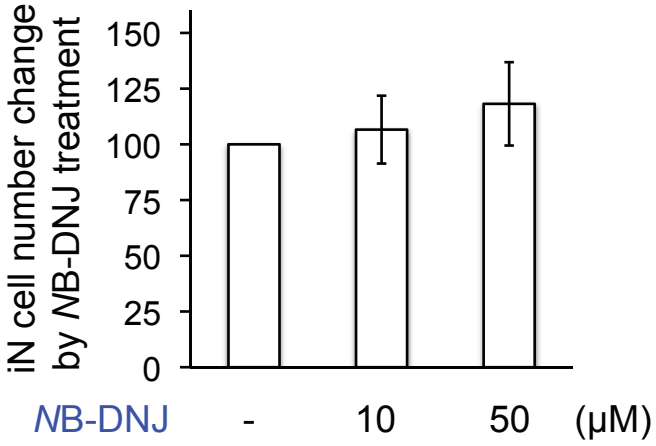

iN cell number change
by NB-DNJ treatment
150
125
100
75
50
25
0
NB-DNJ
-
10
50
(μM)

Figure S3

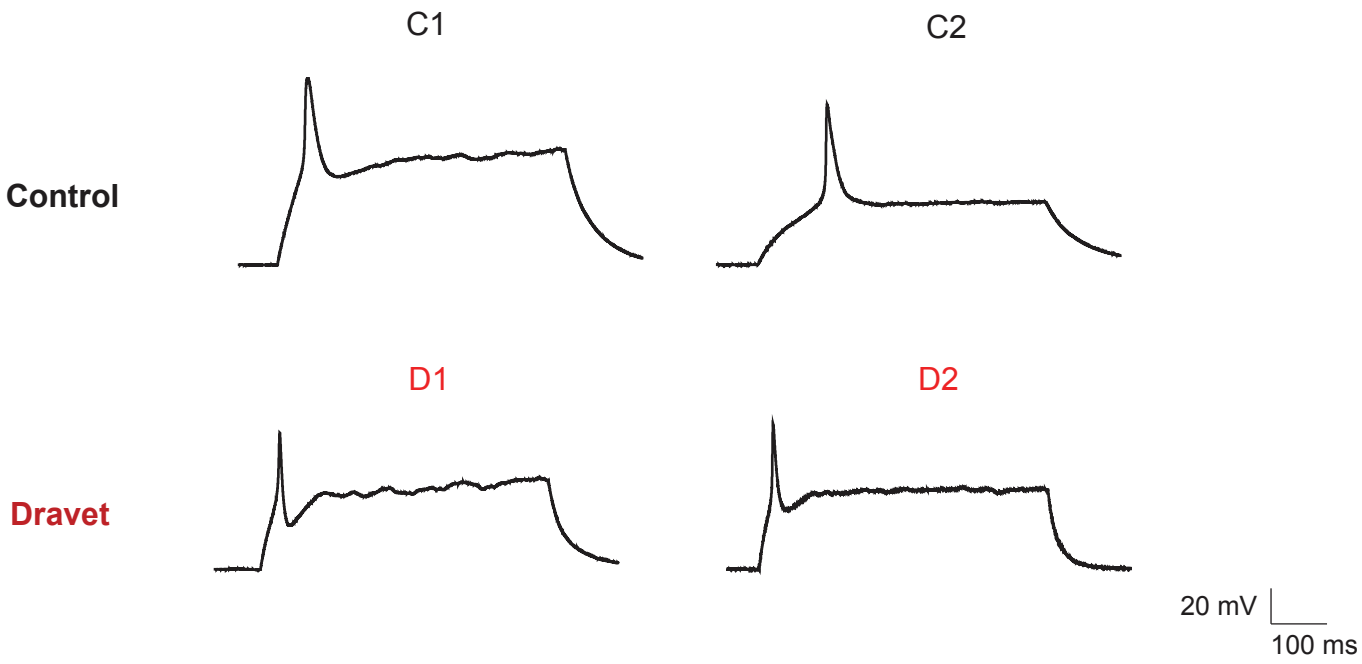


Figure S4

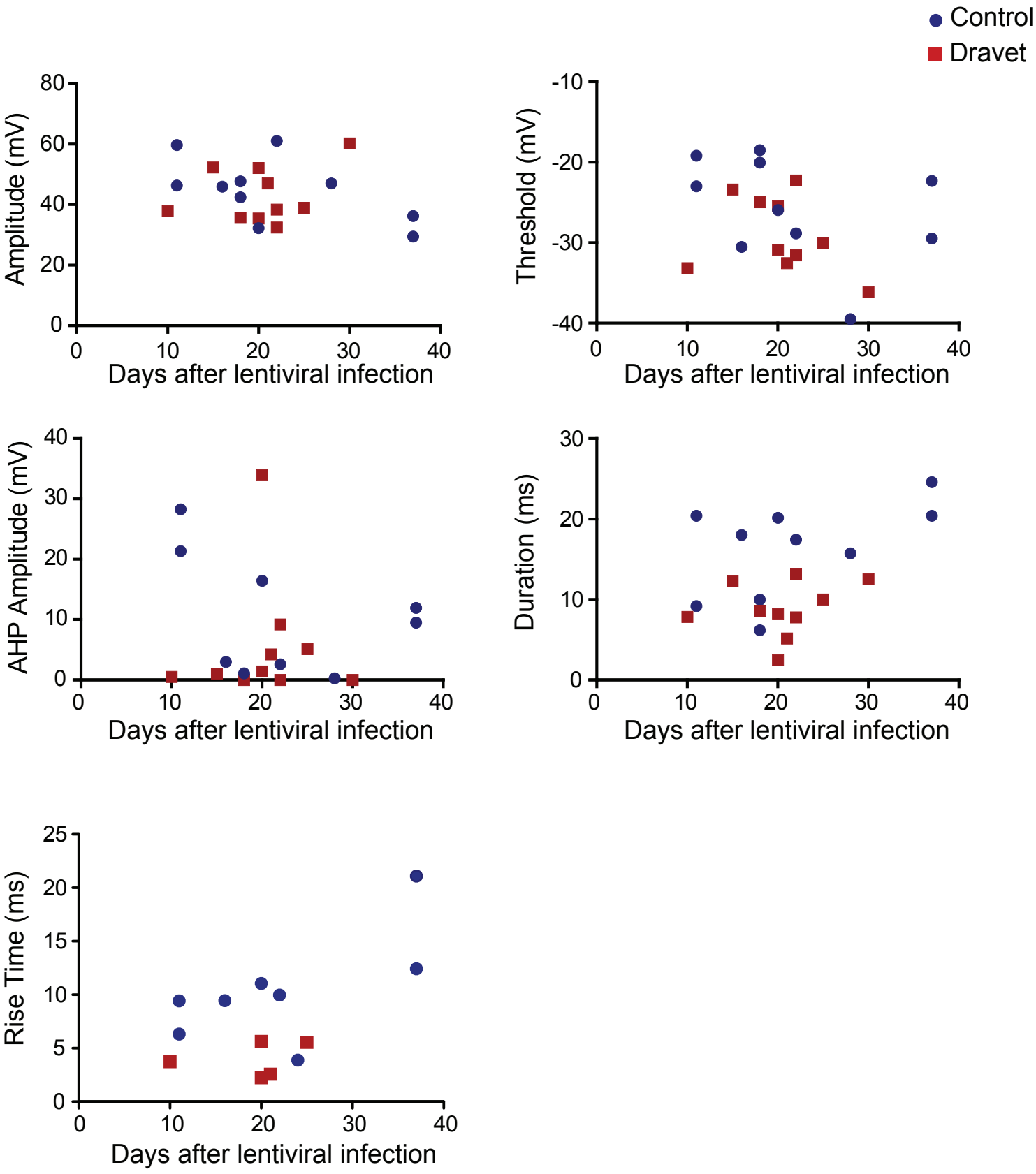
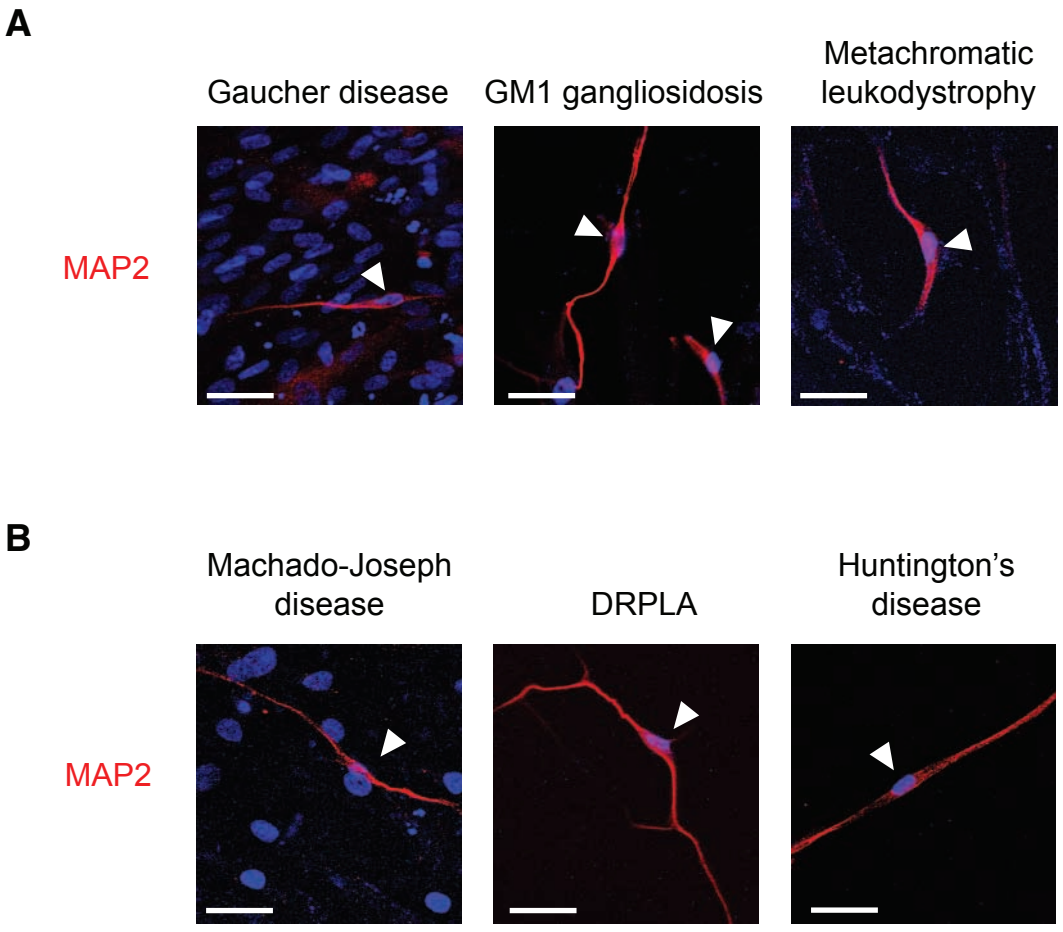

Control
Dravet
Amplitude (mV)
Threshold (mV)
AHP Amplitude (mV)
Duration (ms)
Rise Time (ms)
Days after lentiviral infection

Figure S5


A
Gaucher disease
GM1 gangliosidosis
Metachromatic leukodystrophy
MAP2
B
Machado-Joseph disease
DRPLA
Huntington's disease
MAP2