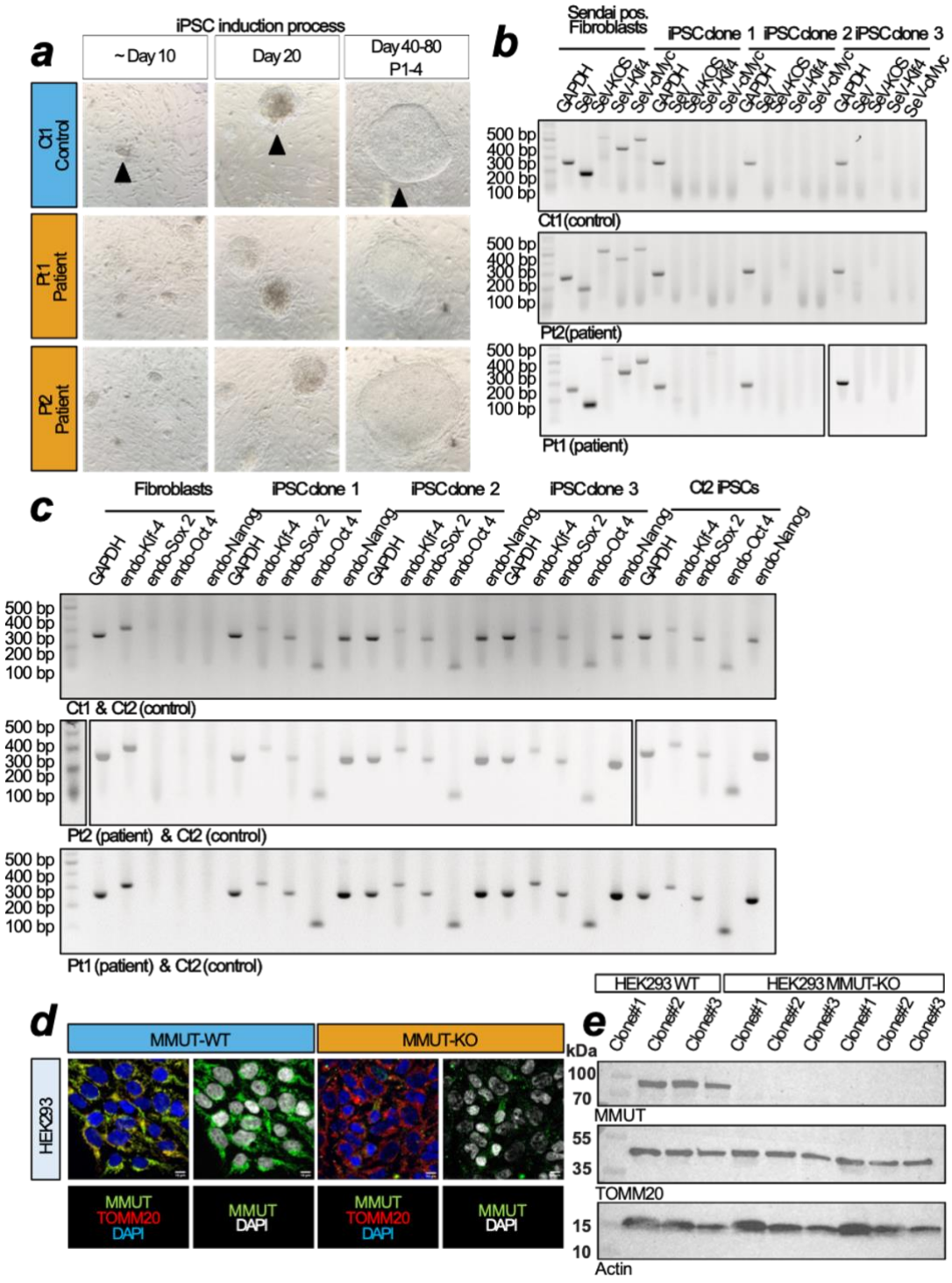
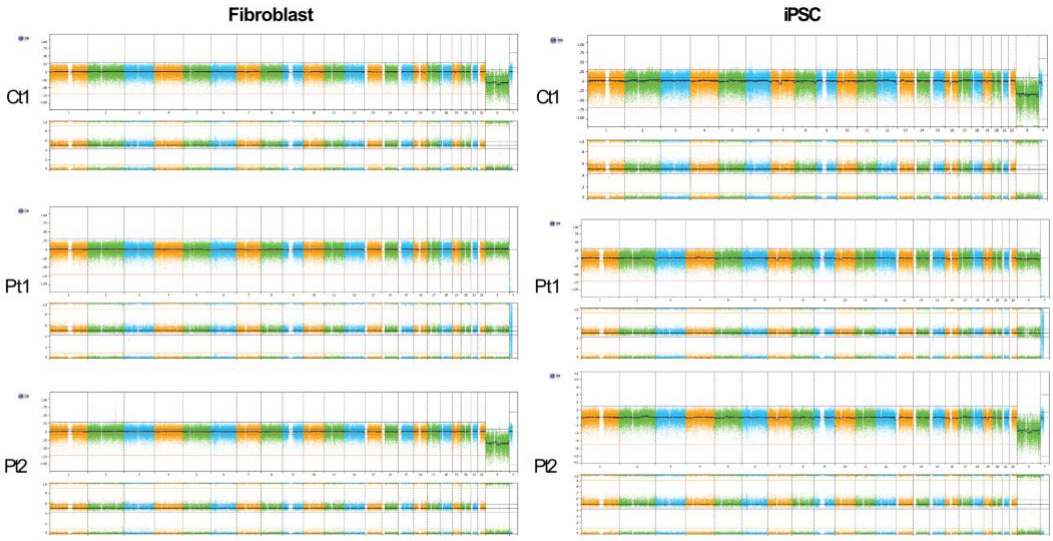
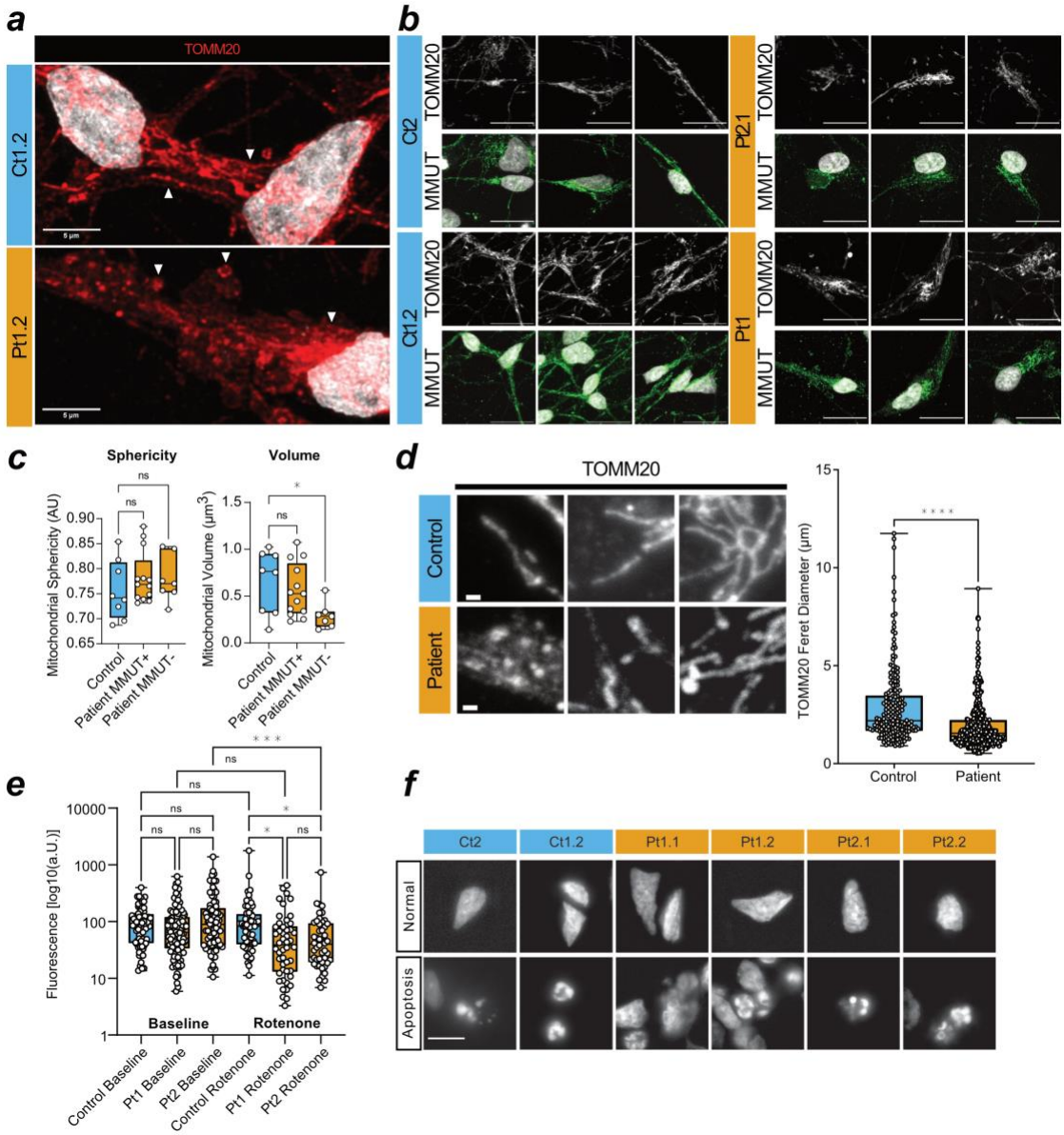


Supplementary Figure 1. **Fibroblasts generate iPSCs without incorporating viral factors. a.** Brightfield microscope images of iPSC generation process. **b.** Sendai virus clearance from fibroblasts to iPSCs in control (Ct1), and patient (Pt1, Pt2) cell lines. **c.** Endogenous mRNA expression of pluripotency markers in generated control, and patient cell lines, with purchased Ct2 control line. **d.** Immunochemistry of selected antibody against MMUT in WT and KO HEK293 cells. Scale bar = 10 µm. **e.** Western blot analysis of MMUT in WT and KO HEK293 cells. Uncropped membranes are available in Supplementary Data Fig. 2c.

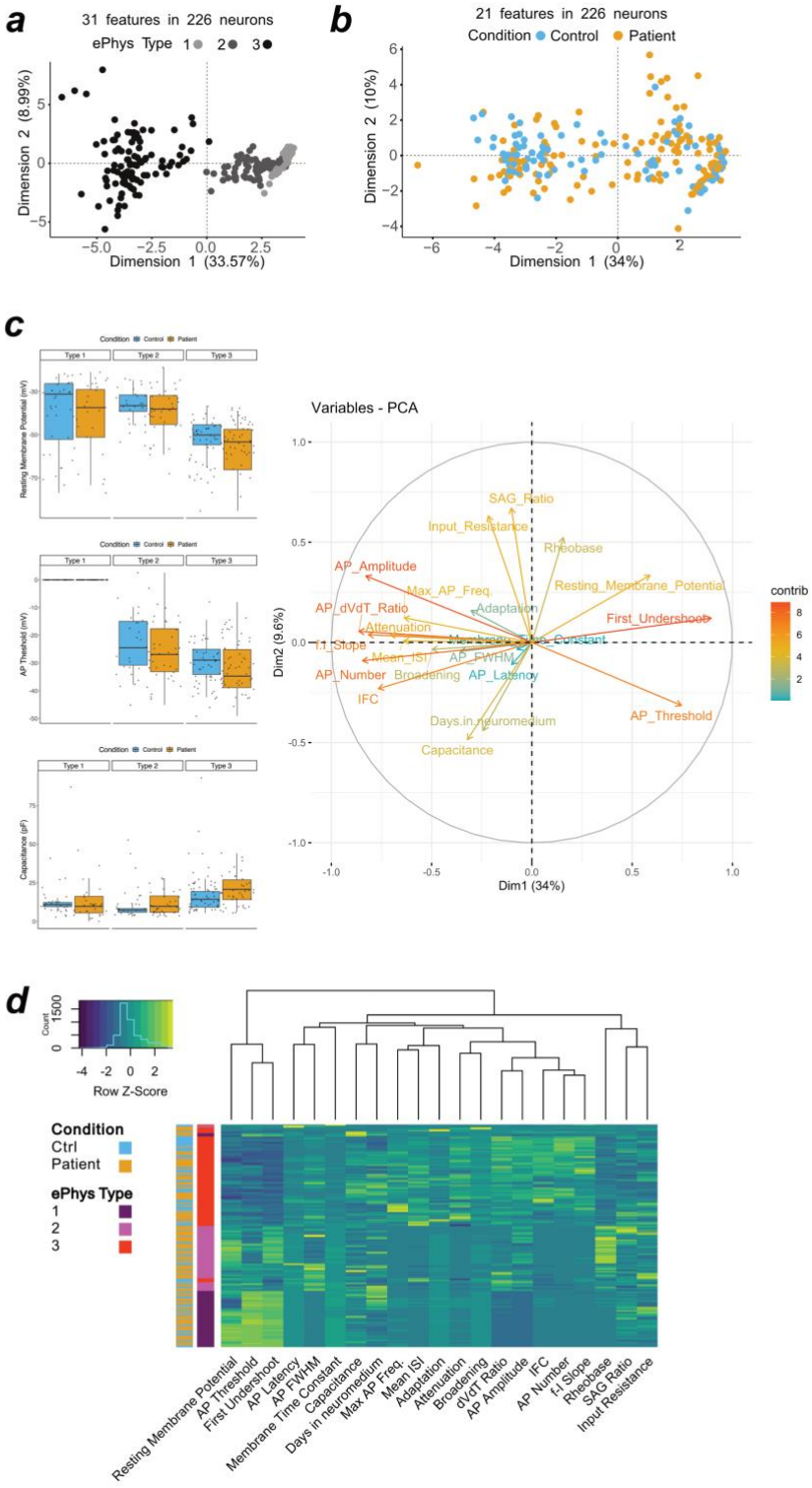


Supplementary Figure 2. Array comparative genomic hybridization (aCGH) analysis of fibroblasts and generated iPSCs. **a**, Visualization of aCGH to identify genomic rearrangements in one control (Ct1) and two patient (Pt1, Pt2) cell lines. Left are fibroblasts before pluripotency induction and right are after induction of pluripotency.

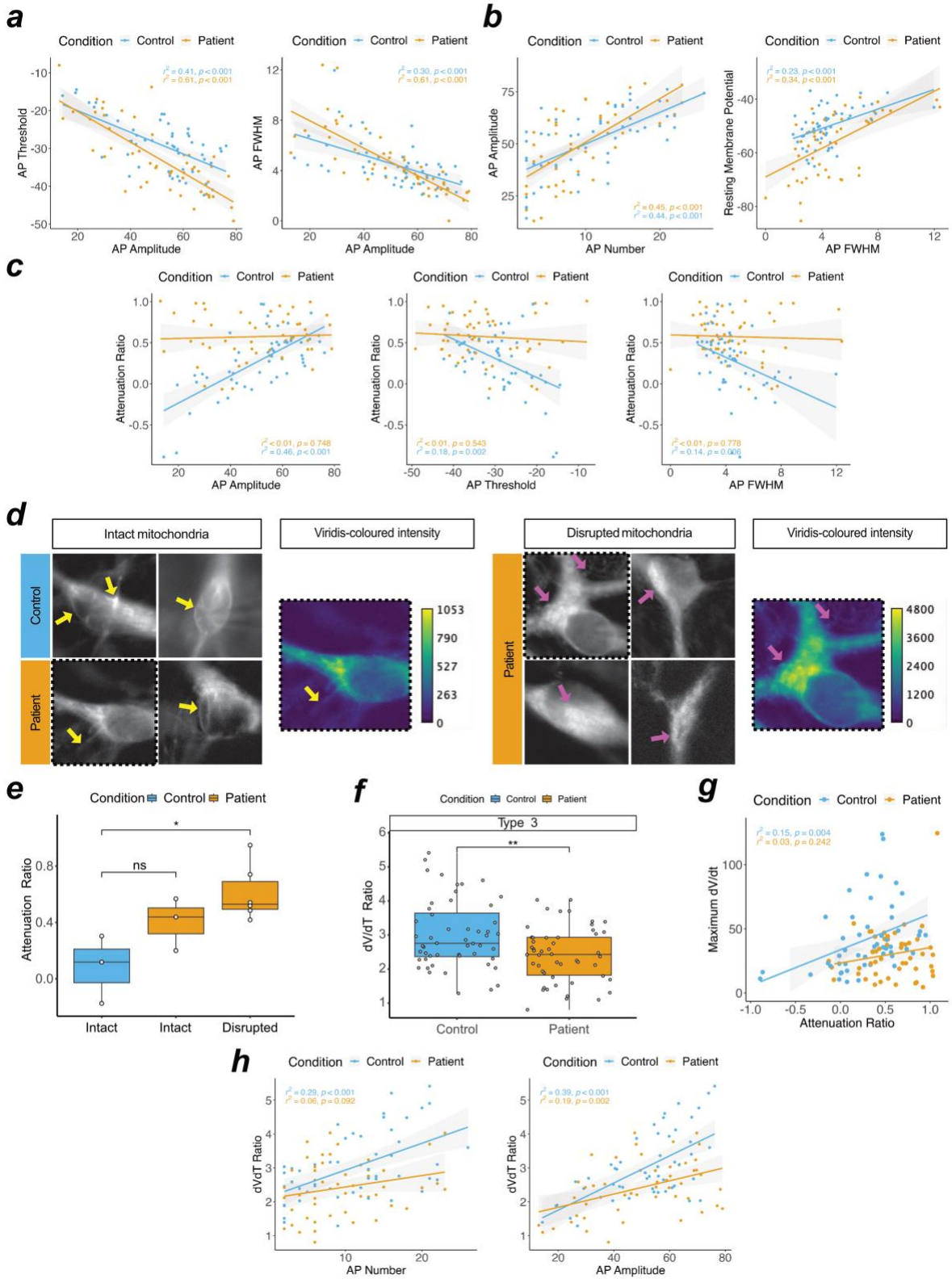


Supplementary Figure 3. **Patient-derived neurons have disrupted mitochondria. a.** Confocal microscope images from control and patient demonstrating TOMM20 staining pattern. Images are representative. Scale bar = 5 µm. **b.** Confocal images of localisation of MMUT (green) to TOMM20 (grey) in control (Ct1, Ct2) and patient (Pt1, Pt2) cell lines. Scale = 20 µm. **c.** Volume and sphericity measurements for Z-stack confocal images of TOMM20 in control and patient cells. Patient images were separated into MMUT localised (MMUT+) or MMUT mislocalised (MMUT-). Datapoints are representative of ROIs from one non-overlapping image. Datapoints represent Ct1 and Ct2 for control, and Pt1.1, Pt1.2, and Pt2.1 for patient. **d.** Maximum projection of confocal TOMM20 immunofluorescent images in control and patient (left), and ferret diameter measurements of those mitochondrial networks identified through TOMM20 staining. Scale =1 µm. Datapoints are representative of one ROI. Statistical test = Mann-Whitney, **** = <0.0001 (Right). Representative images and associated datapoints were selected from Ct2, Pt1.2, and Pt2.1. **e.** MitoSOX fluorescence measurements from confocal microscopy images in control and patient, without (left, baseline) and with (right, rotenone) 1 µM rotenone treatment. Datapoints are representative of one ROI. Statistical test = Kruskal-Wallis, ns = not significant, * =

<0.05, ** = <0.01,*** = <0.001. Datapoints represent Ct2 for control, and Pt1.2 and Pt2.1 for patient. **f.** Representative epifluorescence images of DAPI+ nuclei in control and patient during interphase and apoptosis. Scale = 10 µm.

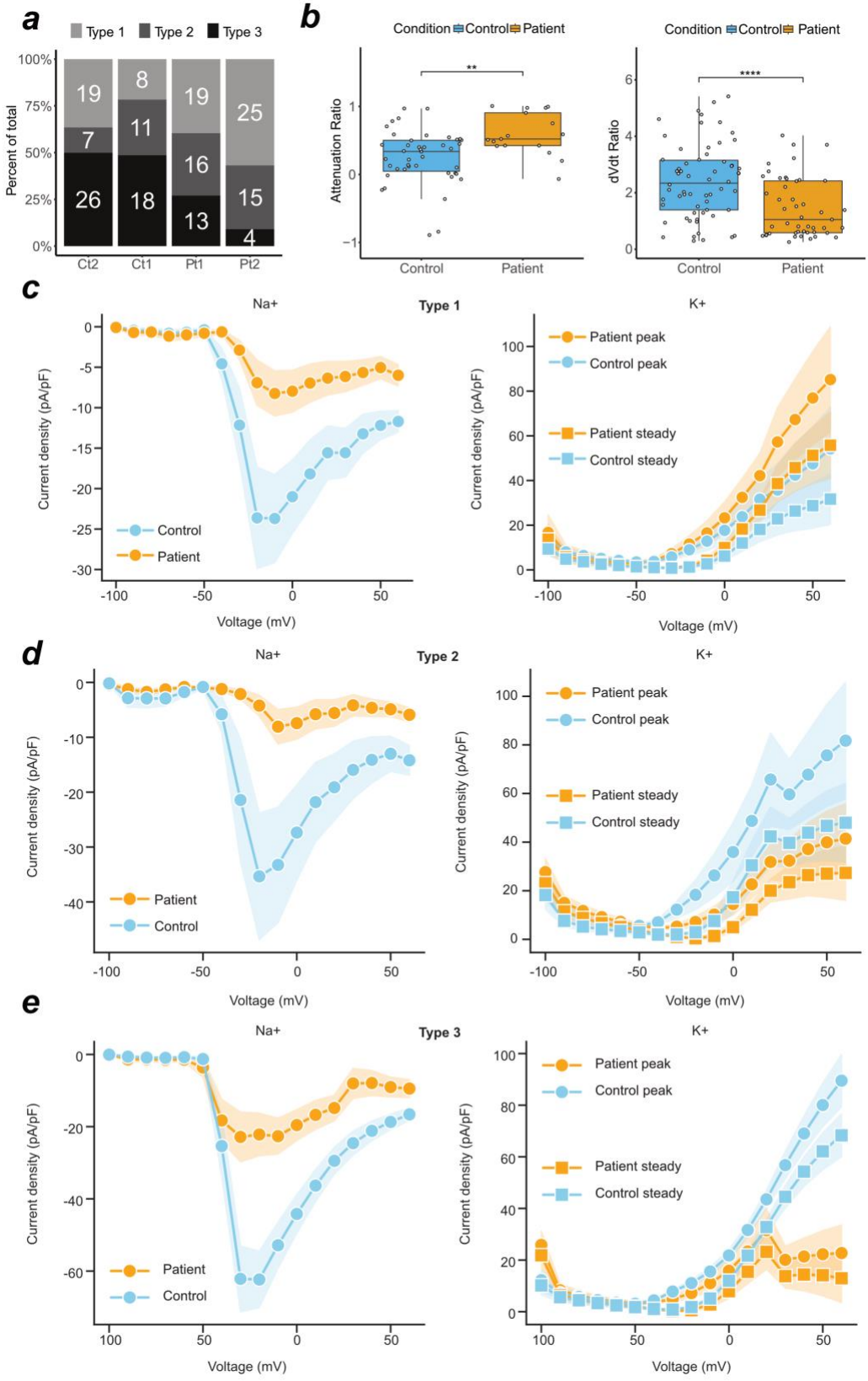


Supplementary Figure 4. **Electrophysiological characterisation of 226 neurons. a.** PCA of 31 features in 226 independent cells highlighting the 3 neuron types, measured during current-clamp recordings. List of features in Supplementary Data Table 1. **b.** Refined PCA of 21 features in 226 independent control (Ct1 n=59, Ct2 n=35) and patient (Pt1 n=77, Pt2 n=55) cells. **c.** Box plots indicate median and IQR 1.5 from Type 1-3 neurons for 3 selected features, each datapoint represents one independent cell (left). PCA loadings for the 21 features on 226 neurons in Fig. 4a, colour = percentage contribution to explained variance (right). **d.** Unguided hierarchical heatmap clustering on measured feature in 226 neurons. One row indicates one cell, one column represent one feature.



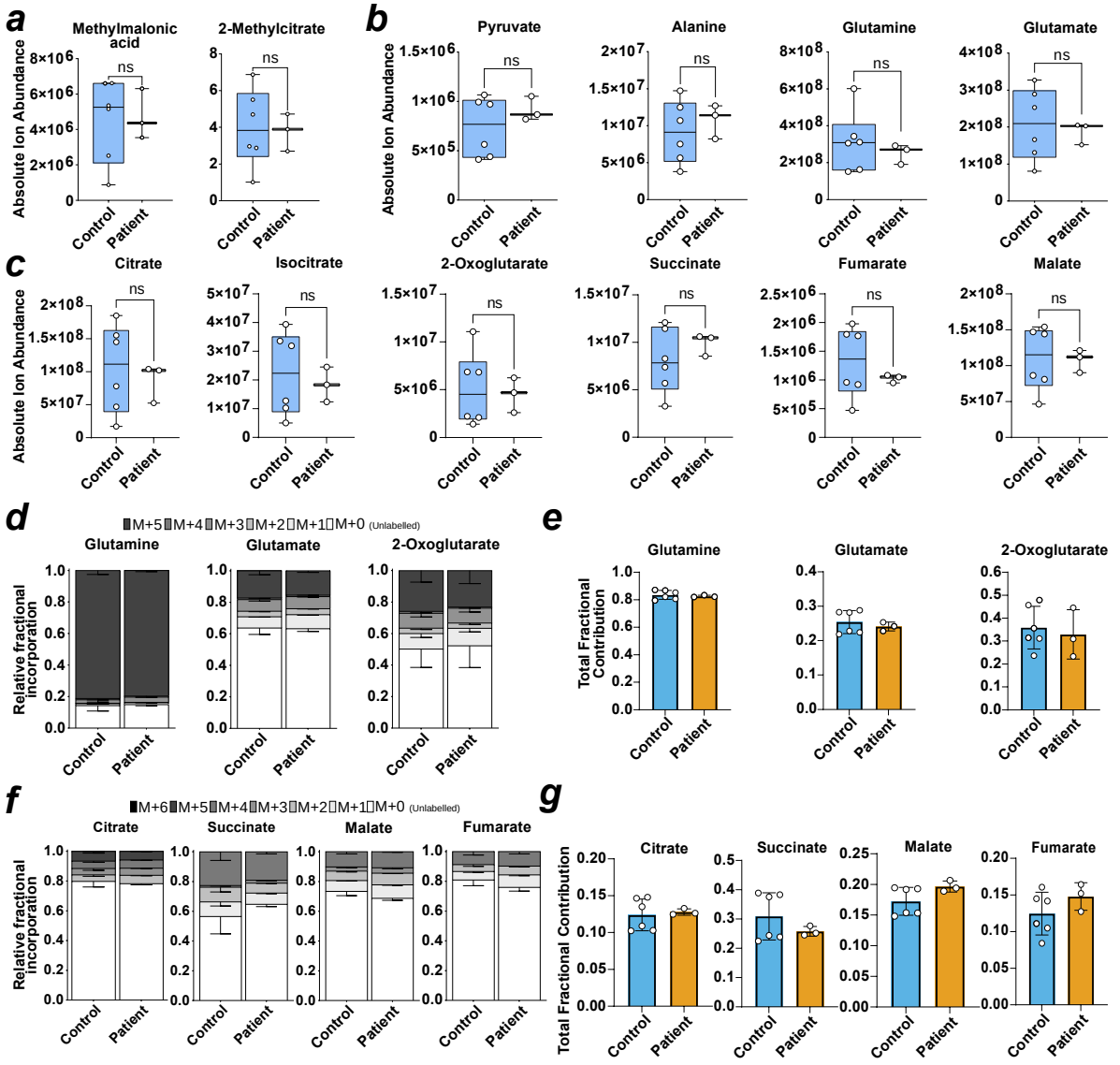
Supplementary Figure 5. **Loss of excitability in patient-derived neurons is explained by mitochondrial disruption. a.** Negative correlation between AP amplitude and threshold or full-width half-max in control and patient type 3 neurons. **b.** Positive correlation between AP amplitude and number or resting membrane potential and AP full-width half-max in control and patient type 3 neurons. **c.** Loss of correlation in patient-derived type 3 neurons between Attenuation ratio and AP amplitude, threshold or full-width half-max. **d.** Immunofluorescent images of MitoTracker in control and patient-

derived neurons, showing intact (yellow arrowhead) or disrupted (magenta arrowhead) mitochondria, images are representative. Inset, pseudo coloured viridis-coloured images from representative cells demonstrate the relative fluorescent differences between intact and disrupted mitochondria. Units are arbitraty and histogram scale is match between images. Representative images were selected from Ct1, Pt1, and Pt2 **e.** Box plot indicates median and IQR 1.5 of attenuation ratio from 12 neurons measured in tandem with MitoTracker visualisation, each datapoint represents one independent cell (right). Datapoints represent data from Ct1, Pt1, and Pt2. **f.** Box plot indicates median and IQR 1.5 of dVdt in type 3 control and patient neurons, each datapoint represents one independent cell. **g.** Correlation between attenuation ratio and dVdt measurements in type 3 neurons, each datapoint represents one independent cell. **h.** Loss of correlation in patient-derived type 3 neurons between dVdt ratio and AP number or amplitude. Panel a-c, f-h feature 102 type 3 cells including control (Ct1 n=29, Ct2 n=23) and patient (Pt1 n=33, Pt2 n=17) cells.

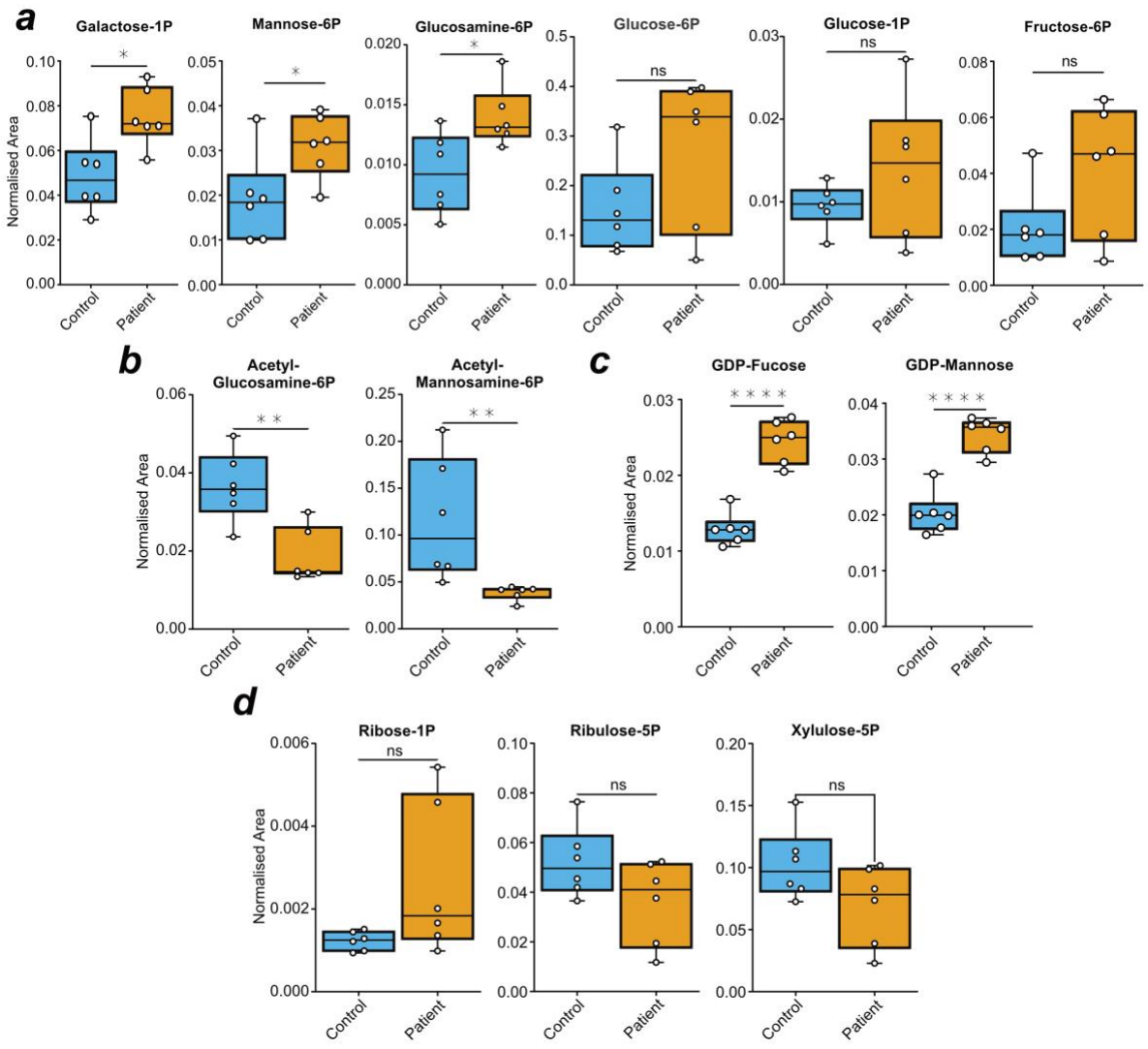


Supplementary Figure 6. **Attenuation phenotype in patient-derived neurons coherent with reduced sodium and potassium current densities a,** Stacked bar graph of proportional generation of type 1, 2, or 3 control (Ct1, Ct2) and patient (Pt1, Pt2) neurons in each cell line of cells measured in both current clamp and voltage clamp (n = 181). **b,** Box plot indicates median and IQR 1.5 of attenuation (left) and dVdt (right) ratios in control and patient neurons. **c,** Sodium and Potassium current density

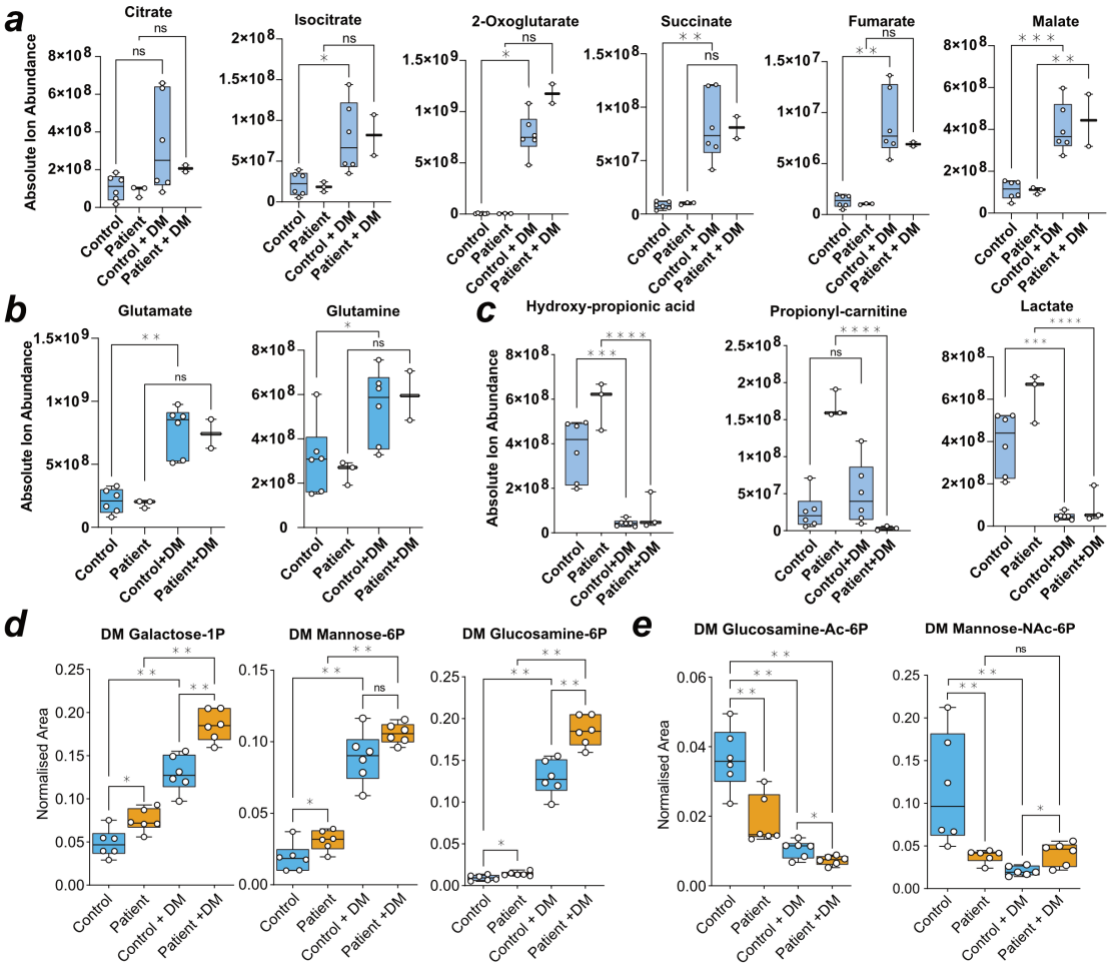
measurements from voltage-clamp recordings in 39 type 1 neurons. **d,** Sodium and Potassium current density measurements from voltage-clamp recordings in 42 type 2 neurons. **e,** Sodium and Potassium current density measurements from voltage-clamp recordings in 53 type 3 neurons.



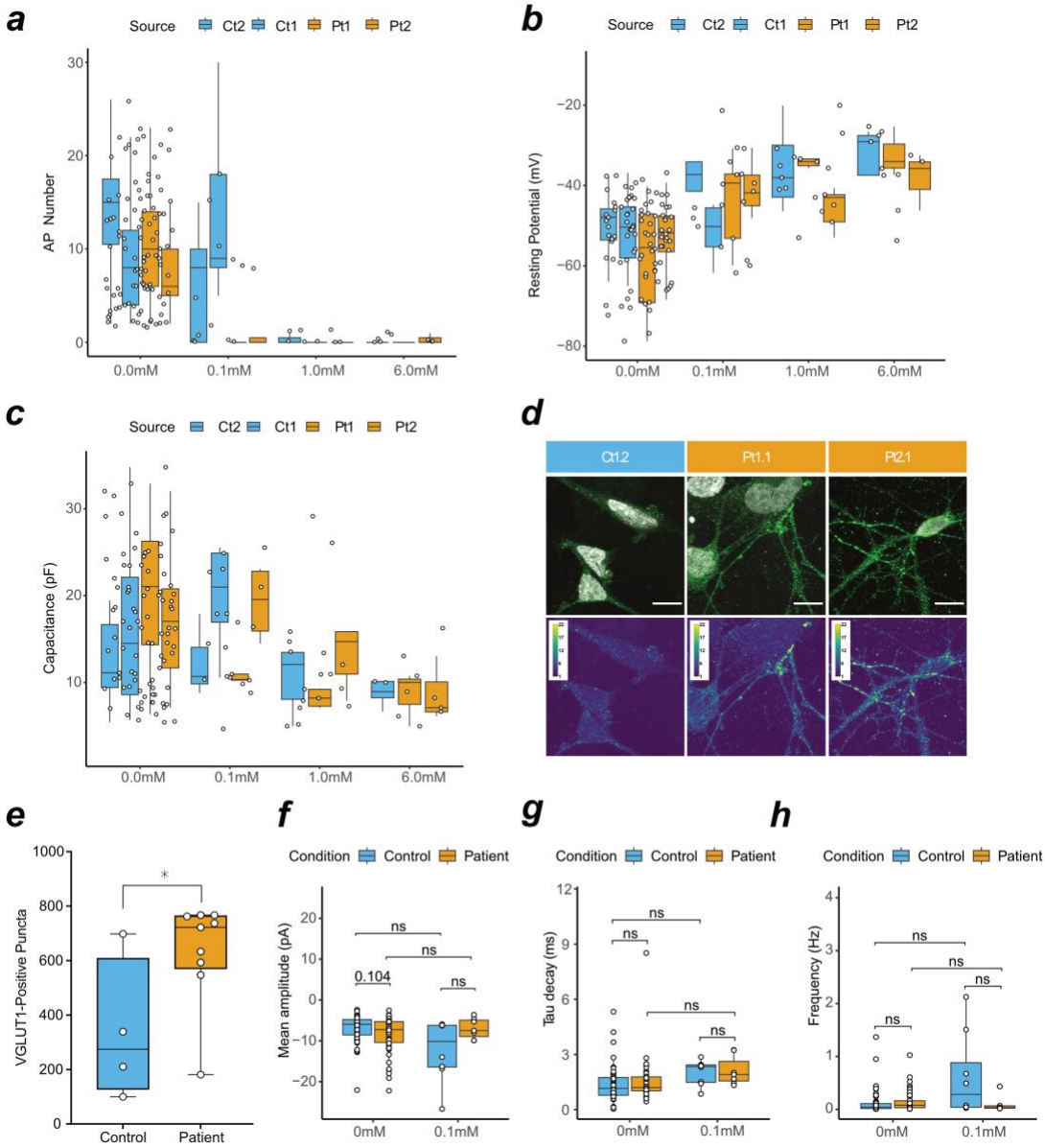
Supplementary Figure 7. **Absolute abundance of TCA cycle metabolites is unchanged in patient-derived neurons. a.** Box plot of ion abundance of disease-related metabolites. **b.** Box plot of ion abundance of anaplerotic metabolites. **c.** Box plot of ion abundance of TCA cycle metabolites. **d.** Relative fractional incorporation of fully labelled glutamine into glutamine, glutamate and 2-oxoglutarate. **e.** Bar plots of fractional contribution of labelled carbons to glutamine, glutamate and 2-oxoglutarate. **f.** Relative fractional incorporation of fully labelled glutamine into TCA cycle metabolites. **g.** Bar plots of fractional contribution of labelled carbons to TCA cycle metabolites. In **a, b, c, e, g,** each datapoint is an averaged result from technical triplicates of an independent sample. In **d, f** data is presented as a fraction of complete labelled and unlabelled measured metabolite. In **a-c**, significance = unpaired t-test. In all panels, datapoints represent Ct1.2 and Ct2 for control, and Pt1.2 and Pt2.2 for patient.



Supplementary Figure 8. **Changes in metabolite pools of selected sugar phosphates. a.** Box plot of normalised area of the peak of targeted hexose phosphates. **b.** Box plot of normalised area of the peak of amine-hexoses. **c.** Box plot of normalised area of the peak of targeted nucleotide sugars. **d.** Box plot of normalised area of the peak of targeted pentose phosphates. In **a-d,** significance testing = paired t-test. In all panels, datapoints represent Ct1.2 and Ct2 for control, and Pt1.2 and Pt2.2 for patient.

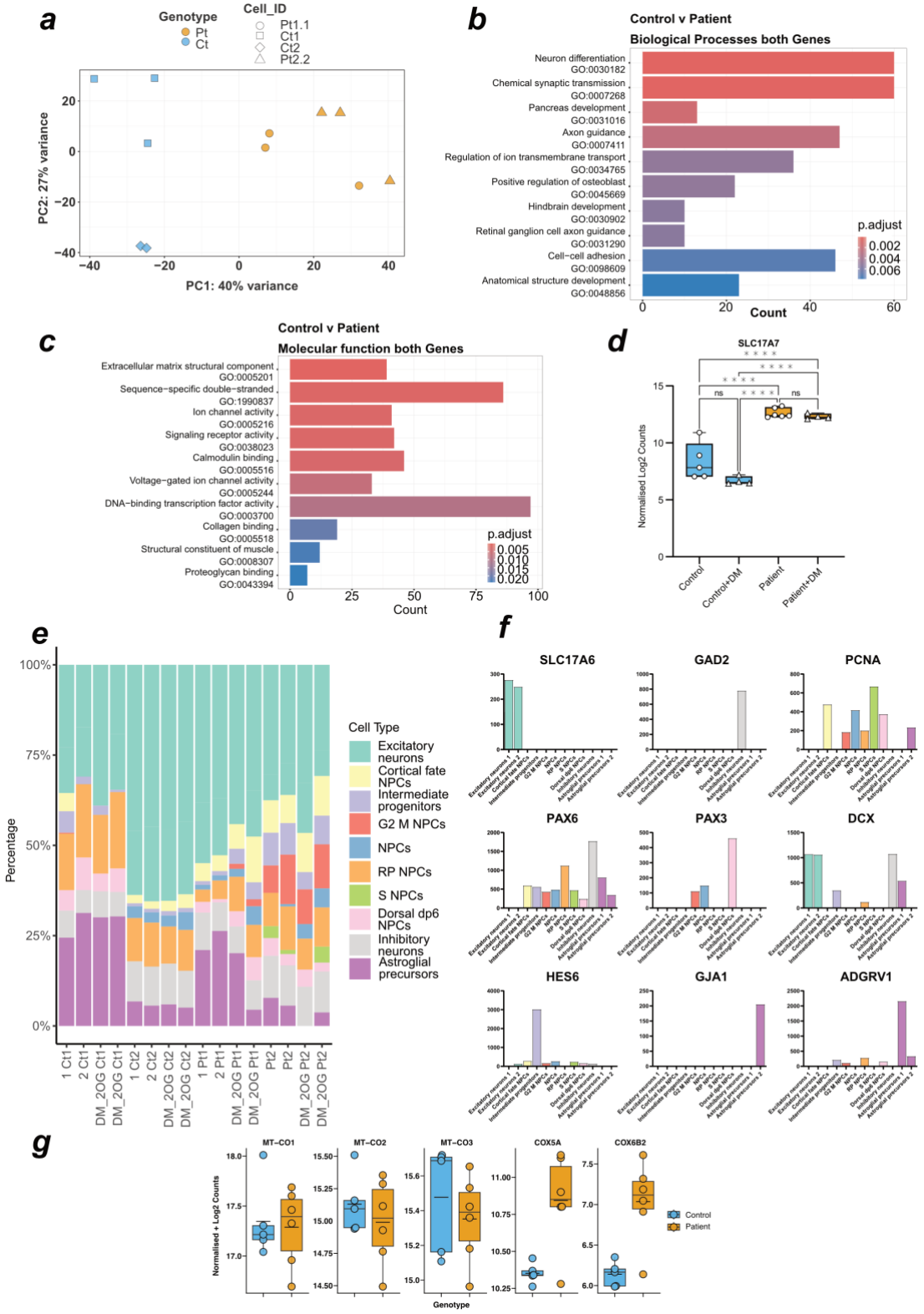


Supplementary Figure 9. **DM-2OG treatment rescues metabolic phenotype in patient neurons. a.** Box plot of response of absolute ion abundance of TCA cycle metabolites to treatment with dimethyl-2-oxoglutarate (DM). **b.** Box plot of glutamate and glutamine response to DM-2OG treatment. **c.** Box plot of response of absolute ion abundance of disease-related metabolites to treatment with dimethyl-2-oxoglutarate (DM). **d.** Box plot of normalised area of the peak of targeted hexoses and response to DM-2OG treatment. **e.** Box plot of normalised area of the peak of *N*-acetylhexosamine phosphates and response to DM-2OG treatment. In **a-e,** datapoints are representative of independent samples measured in triplicate and averaged. In **a-e** multiple testing = Mann-Whitney. In all panels, datapoints represent Ct1.2 and Ct2 for control, and Pt1.2 and Pt2.2 for patient.



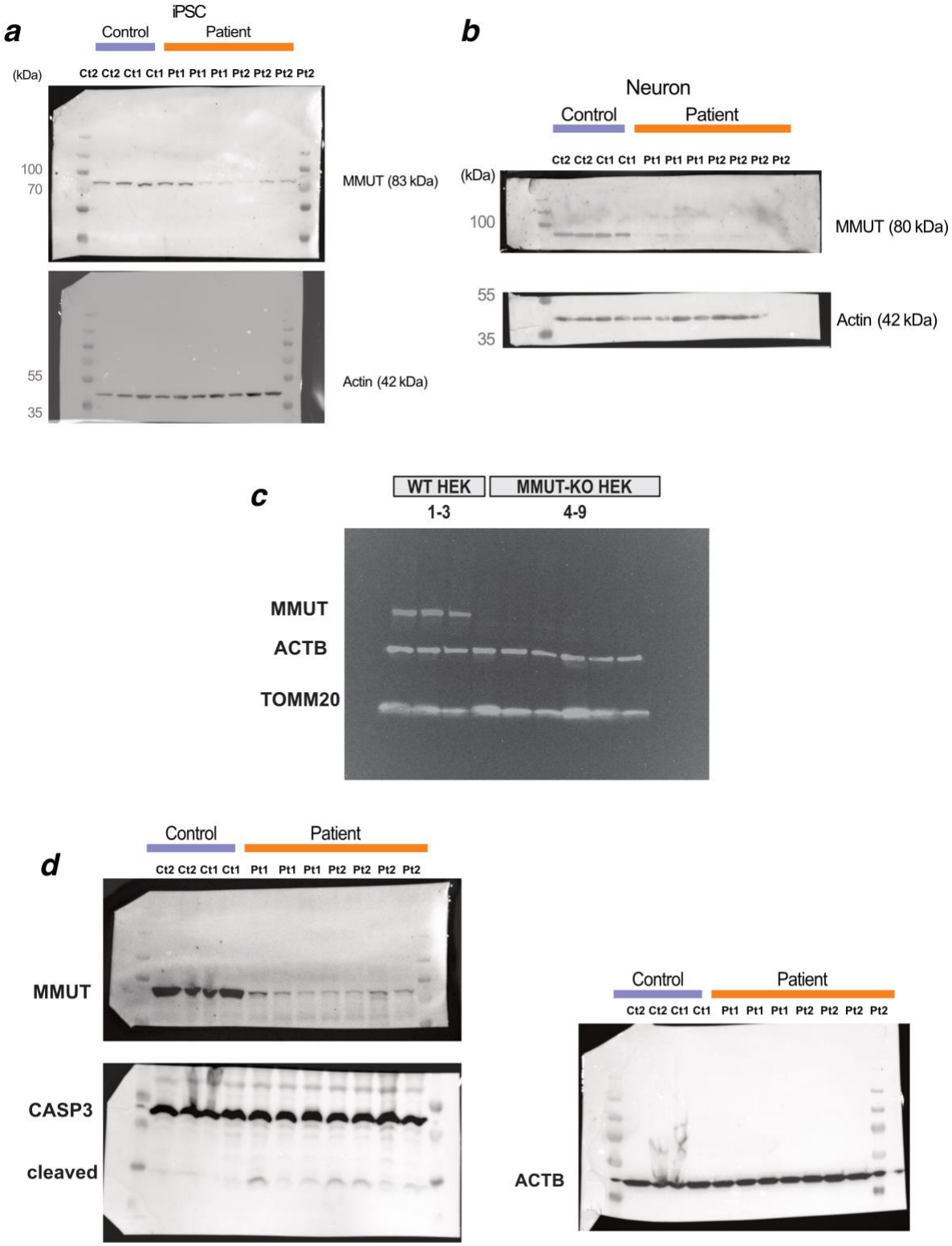
Supplementary Figure 10. **Patient excitatory perturbation can be mimicked in controls using DM-2OG. a.** Box plot of action potential count in current-clamp at various DM-2OG concentrations. **b.** Box plot indicates median and IQR at 1.5 from the measurement of resting membrane potential at different treatments of DM-2OG. **c.** Box plot indicates median and IQR at 1.5 from the measurement of capacitance at different treatments of DM-2OG. In **a, b, c,** datapoints are representative of an independently measured sample. **d.** Confocal immunofluorescence of an antibody against SLC17A7 (VGLUT1), images are representative. Scale = 10 µm. Representative images were selected from Ct1.2, Pt1.1, and Pt2.1. **e.** Box plot indicates median and min/max from quantification of synaptic VGLUT1 events in control (n=4) and patient (n = 9) neurons at DIV 50. Datapoints are representative of averages from non-overlapping images selected from Ct1.2, Pt1.1, and Pt2.1. **f.** Box plot of spontaneous event amplitude in response to DM-2OG treatment at 0.1mM. **g.** Box plot of spontaneous event decay in response to DM-2OG treatment at 0.1mM. **h.** Box plot of spontaneous event frequency in response to DM-2OG treatment at 0.1mM. In **f,g,h,** box plot indicates median and

min/max. Where state numerically, *p* value is Bonferroni significance adjusted. Datapoints are represent recordings from Ct1.2, Ct2, Pt1.1, Pt1.2, and Pt2.2.



Supplementary Figure 11. **DM-2OG influences glutamate-sensitive synaptic apparatus. a.** PCA of control (n = 5) and patient (n = 6) neuronal cultures' transcriptome. **b.** Differentially regulated genes corresponding to GO terms associated to biological processes by overrepresentation analysis. **c.** Differentially regulated genes corresponding to GO terms associated to molecular functions by overrepresentation analysis. **d.** Box plot indicates median and min/max of count of the neuronal glutamate solute carrier extracted from transcriptomic data. Box plot indicates median and min/max of count of the selected genes extracted from transcriptomic data. **e.** Percentage of total transcripts mapped representing cell identity. Each column represents on independent cell sample. **f.** extracted

transcripts for excitatory neurons (SLC17A6), inhibitory neurons (GAD2), proliferating marker (PCNA), dorsal fate markers (PAX6, PAX3), immature neuronal marker (DCX), intermediate progenitor markers (HES6), and astrocytic markers (GJA1, ADGRV1). **g.** Box plot indicates median and min/max, mean is overlaid bar of count of cytochrome c oxidase transcripts extracted from transcriptomic data. In **a, d,** datapoints are representative of an independently measured sample. In **b,c,** the top 10 (in either direction) differentially expressed terms were selected. In all panels, control is represented by Ct1.2 and Ct2, and patient is represented by Pt1.1, and Pt2.2.



Supplementary Figure 12. Uncropped and unprocessed versions of western blots presented in the main and extended figures. **a,** MMUT protein in iPSCs. **b,** MMUT protein in differentiated neurons. **c,** MMUT protein in HEK cells. **d,** MMUT and CASP3 protein in differentiated neurons.

Supplementary Table 1. Electrophysiological variable collections and designations.

Feature	Measurement type	Meaningful in ePhys type(s)...	Contrib. Ct2	Contrib. Ct1	Contrib. Pt1	Contrib. Pt2
Rheobase	Current_Clamp	T1-T3	35	59	77	55
First_Undershoot	Current_Clamp	T2-T3	35	59	77	55
AP_Threshold	Current_Clamp	T2-T3	35	59	77	55
Resting_Membrane_Potential	Current_Clamp	T1-T3	35	59	77	55
AP_Latency	Current_Clamp	T2-T3	35	59	77	55
Adaptation	Current_Clamp	T3	35	59	77	55
Input_Resistance	Current_Clamp	T1-T3	35	59	77	55
Mean_ISI	Current_Clamp	T3	35	59	77	55
Membrane_Time_Constant	Current_Clamp	T1-T3	35	59	77	55
SAG_Ratio	Current_Clamp	T1-T3	35	59	77	55
AP_FWHM	Current_Clamp	T2-T3	35	59	77	55
Max_AP_Freq.	Current_Clamp	T2-T3	35	59	77	55
f.I_Slope	Current_Clamp	T1-T3	35	59	77	55
AP_dVdT_Ratio	Current_Clamp	T2-T3	35	59	77	55
dVdT_Maximum	Current_Clamp	T2-T3	35	59	77	55
dVdT_Minimum	Current_Clamp	T2-T3	35	59	77	55
IFC	Current_Clamp	T3	35	59	77	55
Attenuation	Current_Clamp	T3	35	59	77	55
Broadening	Current_Clamp	T3	35	59	77	55
AP_Amplitude	Current_Clamp	T2-T3	35	59	77	55
AP_Number	Current_Clamp	T1-T3	35	59	77	55
Days.in.neuromedium	Current_Clamp	T1-T3	35	59	77	55
Capacitance	Current_Clamp	T1-T3	35	59	77	55
Batch	Current_Clamp	T1-T3	35	59	77	55
Avg_AP_Freq.	Current_Clamp	T2-T3	35	59	77	55
ISI_CV	Current_Clamp	T3	35	59	77	55
Pause	Current_Clamp	T3	35	59	77	55
Delay	Current_Clamp	T3	35	59	77	55
Min_ISI	Current_Clamp	T3	35	59	77	55

Max_ISI	Current_Clamp	T3	35	59	77	55
Median_ISI	Current_Clamp	T3	35	59	77	55
Last_Undershoot	Current_Clamp	T2-T3	35	59	77	55
Rectification	Current_Clamp	T1-T3	35	59	77	55
Sodium Steady	Voltage_Clamp	T1-T3	34	11	14	9
Sodium Peak	Voltage_Clamp	T1-T3	34	11	14	9
Potassium Steady	Voltage_Clamp	T1-T3	34	11	14	9
Potassium Peak	Voltage_Clamp	T1-T3	34	11	14	9
Synaptic Event Frequency	Voltage_Clamp	T1-T3	27	22	26	18
Synaptic Event Charge	Voltage_Clamp	T1-T3	27	22	26	18
Synaptic Event Amplitude	Voltage_Clamp	T1-T3	27	22	26	18
Mitotracker	C_c & V_c	T1-T3	0	4	14	4
Dimethyl-2-Oxoglutarate	C_c & V_c	T1-T3	17	5	13	12

Supplementary Table 2. Electrophysiological variable qualification into core groupings.

	Variable	Core	Whole
Primary	**Rheobase**	**1**	**1**
	First_Undershoot	**1**	**1**
	AP_Threshold	**1**	**1**
	Resting_Membrane_Potential	**1**	**1**
	AP_Latency	**1**	**1**
	Adaptation	**1**	**1**
	Input_Resistance	**1**	**1**
	Mean_ISI	**1**	**1**
	Membrane_Time_Constant	**1**	**1**
	SAG_Ratio	**1**	**1**
	AP_FWHM	**1**	**1**
	Max_AP_Freq.	**1**	**1**
	f.I_Slope	**1**	**1**
	AP_dVdT_Ratio	**1**	**1**
	IFC	**1**	**1**
	Attenuation	**1**	**1**
	Broadening	**1**	**1**
	AP_Amplitude	**1**	**1**
	AP_Number	**1**	**1**
	Days.in.neuromedium	**1**	**1**
	Capacitance	**1**	**1**
Ancilliary	Batch	0	**1**
	Avg_AP_Freq.	0	**1**
	ISI_CV	0	**1**
	Pause	0	**1**
	Delay	0	**1**
	Min_ISI	0	**1**
	Max_ISI	0	**1**
	Median_ISI	0	**1**
	Last_Undershoot	0	**1**
	Rectification	0	**1**

Supplementary Table 3. Metabolite transition list from acquisition method for hexose and pentose sugars.

Compound Name	Precursor Ion	Product Ion	RT (min)	Delta RT	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Glucosamine 6-phosphate	258	79	1	3	380	45	7	Negative
Glucosamine 6-phosphate	258	97	1	3	380	15	7	Negative
Glucosamine 6-phosphate	258	227	1	3	380	7	7	Negative
Glucosamine 6-phosphate	258	240	1	3	380	9	7	Negative
N-acetyl-D-glucosamine 6-phosphate / N-acetyl-D-mannosamine 6-phosphate	300	79	12.8	3	380	48	7	Negative
N-acetyl-D-glucosamine 6-phosphate / N-acetyl-D-mannosamine 6-phosphate	300	97	12.8	3	380	15.5	7	Negative
N-acetyl-D-glucosamine 6-phosphate / N-acetyl-D-mannosamine 6-phosphate	300	282	12.8	3	380	6	7	Negative
Fructose 6-phosphate - Galactose 1-phosphate - Glucose 1-phosphate - Glucose-6-phosphate - Mannose 6-phosphate	259	79	10	7	380	50.5	7	Negative
Fructose 6-phosphate - Galactose 1-phosphate - Glucose 1-phosphate - Glucose-6-phosphate - Mannose 6-phosphate	259	97	10	7	380	13.3	7	Negative
Fructose 6-phosphate - Galactose 1-phosphate - Glucose 1-phosphate - Glucose-6-phosphate - Mannose 6-phosphate	259	139	10	7	380	10.8	7	Negative
Fructose 6-phosphate - Glucose-6-phosphate - Mannose 6-phosphate	259	169	10	7	380	5.8	7	Negative
Galactose 6-phosphate - Glucose-6-phosphate - Mannose 6-phosphate	259	199	10	7	380	7	7	Negative
Ribose 1-phosphate / Ribulose 5-phosphate / Xylulose 5-phosphate	229	79	12	6.5	380	40	7	Negative
Ribose 1-phosphate / Ribulose 5-phosphate / Xylulose 5-phosphate	229	97	12	6.5	380	9.2	7	Negative
Ribose 1-phosphate / Ribulose 5-phosphate / Xylulose 5-phosphate	229	139	12	6.5	380	10.25	7	Negative

Supplementary Table 4. Antibody list for immunochemistry.

Target	Cat. No.	Manufacturer	Dilution [ICC] [WB]	Incubation [ICC] (Temp.) [WB] (Temp.)	Species
MMUT	Ab67869	Abcam	1:50 1:500	Overnight 4°C Overnight 4°C	Mouse
TOMM20	Ab186735	Abcam	1:200 1:200	2 hours RT Overnight 4°C	Rabbit
TOMM20	sc11415	Santa Cruz	1:250 1:200	2 hours RT Overnight 4°C	Rabbit
SOX2	Ab5603	Millipore	1:400 -	Overnight 4°C -	Mouse
SSEA4	53-8843-42	Invitrogen	1:200 -	2 hours RT -	Rabbit
NANOG	4903S	Cell Signalling	1:25 -	2 hours RT -	Rabbit
NANOG	8750S	Cell Signalling	1:50 -	2 hours RT -	Rabbit
Ki67	Ab15580	Abcam	1:100 -	2 hours RT -	Rabbit
PAX6	901301	Biolegend	1:250 -	2 hours RT -	Rabbit
Nestin	Ab22035	Abcam	1:100 -	2 hours RT -	Mouse
TBR1	Ab31940	Abcam	1:200 -	Overnight 4°C -	Rabbit
Synaptophysin	Ab32127	Abcam	1:100 -	2 hours RT -	Rabbit
PSD-95	Ab2723	Abcam	1:100 -	2 hours RT -	Mouse
NeuN	AB78	Millipore	1:500 -	Overnight 4°C -	Rabbit
MAP2	M1406-2ML	Sigma-Aldrich	1:500 -	2 hours RT -	Mouse
EOMES	Ab23345	Abcam	1:100 -	2 hours RT -	Rabbit
TUBB3	MAB1637	Millipore	1:200 -	2 hours RT -	Mouse
AFP	A25530	Invitrogen	1:100 -	Overnight 4°C -	Mouse
FOXA2/hHNF	AF2400	R&D	1:100 -	Overnight 4°C -	Goat
SMA	A25531	Invitrogen	1:50 -	Overnight 4°C -	Rabbit
Brachyury	AF2085	R&D	1:100 -	Overnight 4°C -	Goat
TUJ1	A25532	Invitrogen	1:500 -	Overnight 4°C -	Rabbit
TUBB3	MAB1637	MilliporeSigma	1:250 -	2 hours RT -	Mouse
20S ß5	BML-PW8895-0025	Enzo	1:100 1:1000	Overnight 4°C Overnight 4°C	Rabbit
LC3	2775S	Cell Signaling Technologies	1:100 1:1000	Overnight 4°C Overnight 4°C	Rabbit
Parkin	66674	ProteinTech	1:100 1:1000	Overnight 4°C Overnight 4°C	Mouse
LAMP1	H4A3-DSHB	Developmental Studies Hybridoma Bank	1:100 1:1000	Overnight 4°C Overnight 4°C	Mouse
ß-Actin	A1978	Sigma-Aldrich	- 1:1000	- Overnight 4°C	Mouse
SQSTM1	5114T	Cell Signalling Technologies	- 1:1000	- Overnight 4°C	Rabbit
Caspase-3	9662S	Cell Signalling Technologies	- 1:1000	- Overnight 4°C	Rabbit
VGLUT1	135303	Synaptic Systems	1:500 1:1000	Overnight 4°C Overnight 4°C	Rabbit

anti-mouse horseradish peroxidase	sc516102-cm	Santa Cruz	- 1:5000	- 2 hours RT	Goat
anti-rabbit horseradish peroxidase	sc2357	Santa Cruz	- 1:5000	- 2 hours RT	Mouse
Alexa Fluor 594 F(ab') (H+L)	A11072	Invitrogen	1:1000 -	1 hour RT -	Goat
Alexa Fluor 488 Cross-Adsorbed	A21121	Invitrogen	1:1000 -	1 hour RT -	Goat

Supplementary Table 5. Primer sequence list used in DNA PCR.

Gene	FWD 5'-3'	REV 5'-3'	Product (bp)
SOX2	GAG CTT TGC AGG AAG TTT GC	TTC AAG GAG AGG CTT CTT GC	190
PAX6	AGC TCG GTG GTG TCT TTG TC	TGC ATC TGC ATG GGT CTG C	128
EOMES	GAG CCC TCA AAG ACC CAG AC	TCT GAA GCG GTG TAC ATG GAA	155
ACTB	AGG CAC CAG GGC GTG AT	GGC GTA CAG GGA TAG CAC AG	318
GAPDH	TGG ACC TGA CCT GCC GTC TA	ATG TGG GCC ATG AGG TCC ACC AC	256
endo-Klf4	ACC CAC ACA GGT GAG AAA CCT T	GTT GGG AAC TTG ACC ATG ATT G	313
endo-Sox2	TTC ATC GAC GAG GCT AAG CG	CAT CAT GCT GTA GCT GCC GT	255
endo-Oct4	GAG GAG TCC CAG GAC ATC AA	CAT CGG CCT GTG TAT ATC CC	100
endo-Nanog	CCA AAT TCT CCT GCC AGT GA	CAG GTG GTT TCC AAA CAA GAA	260
SeV	GGA TCA CTA GGT GAT ATC GAG C	ACC AGA CAA GAG TTT AAG AGA TAT GTA TC	181
SeV-KOS	ATG CAC CGC TAC GAC GTG AGC GC	ACC TTG ACA ATC CTG TAG TGG	528
SeV-Klf4	TTC CTG CAT GCC AGA GGA GCC	AAT GTA TCG AAG GTG CTC AA	410
SeV-cMyc	TAA CTG ACT AGC AGG CTT GTC G	TCC ACA TAC AGT CCT GGA TGA TGA TG	532