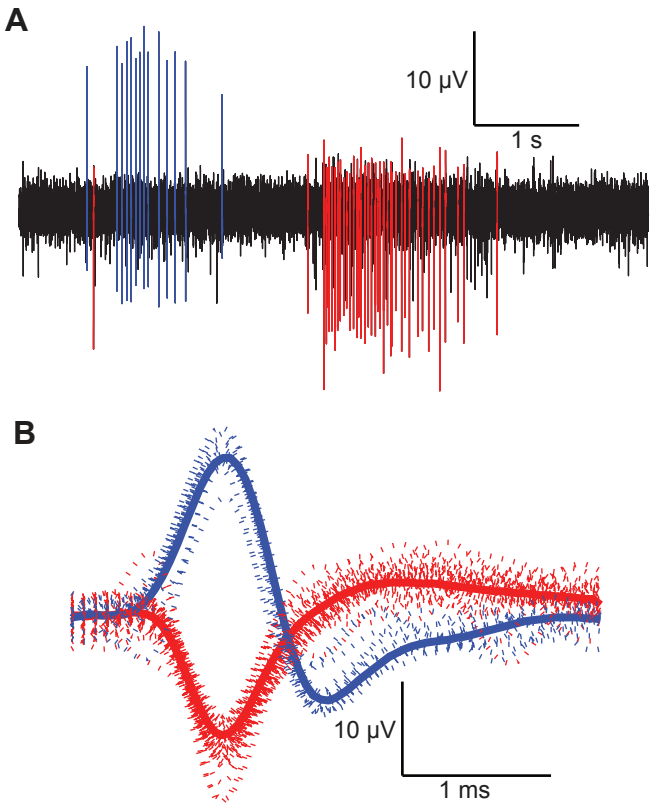


Figure S1, related to Figure 1, Clustering of Spikes to Identify Individual Neurons.
(A) Example of MEA data from a single electrode with spikes from two clusters in blue and red.
(B) Traces of clusters (solid lines) and individual spikes classified within each cluster (broken lines).

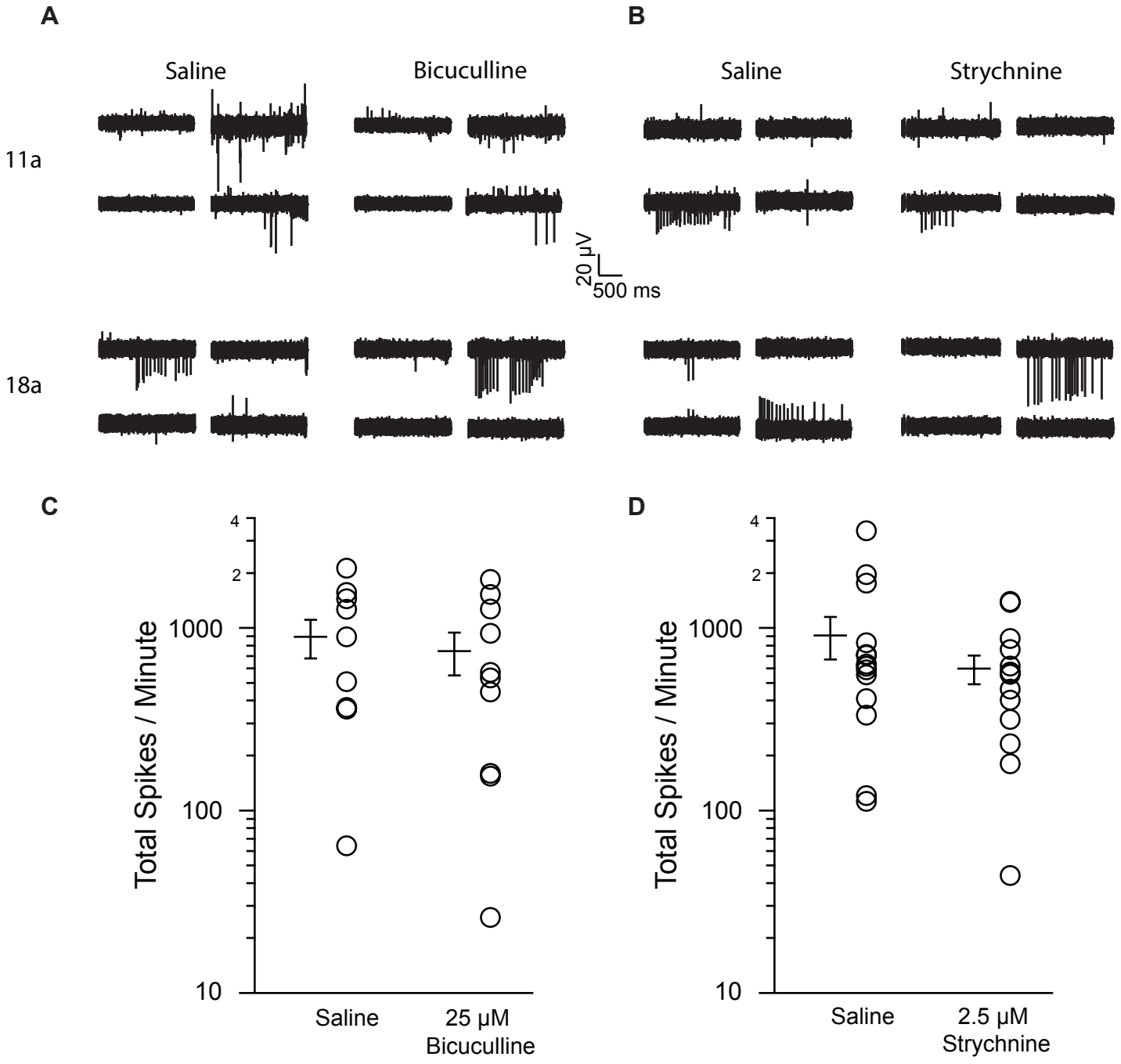


Figure S2, related to Figure 1, Effect of Bicuculline and Strychnine on Spike Firing.
(A) Representative recordings from four/64 MEA electrodes before (left) and after (right) the application of 25 µM bicuculline in neurons derived from 11a and 18a.
(B) Representative recordings from four/64 MEA electrodes before (left) and after (right) the application of 2.5 µM strychnine in neurons derived from 11a and 18a.
(C) Total spikes/minute before and after treatment with 25 µM bicuculline (n=10).
(D) Total spikes/minute before and after treatment with 2.5 µM strychnine (n=14).

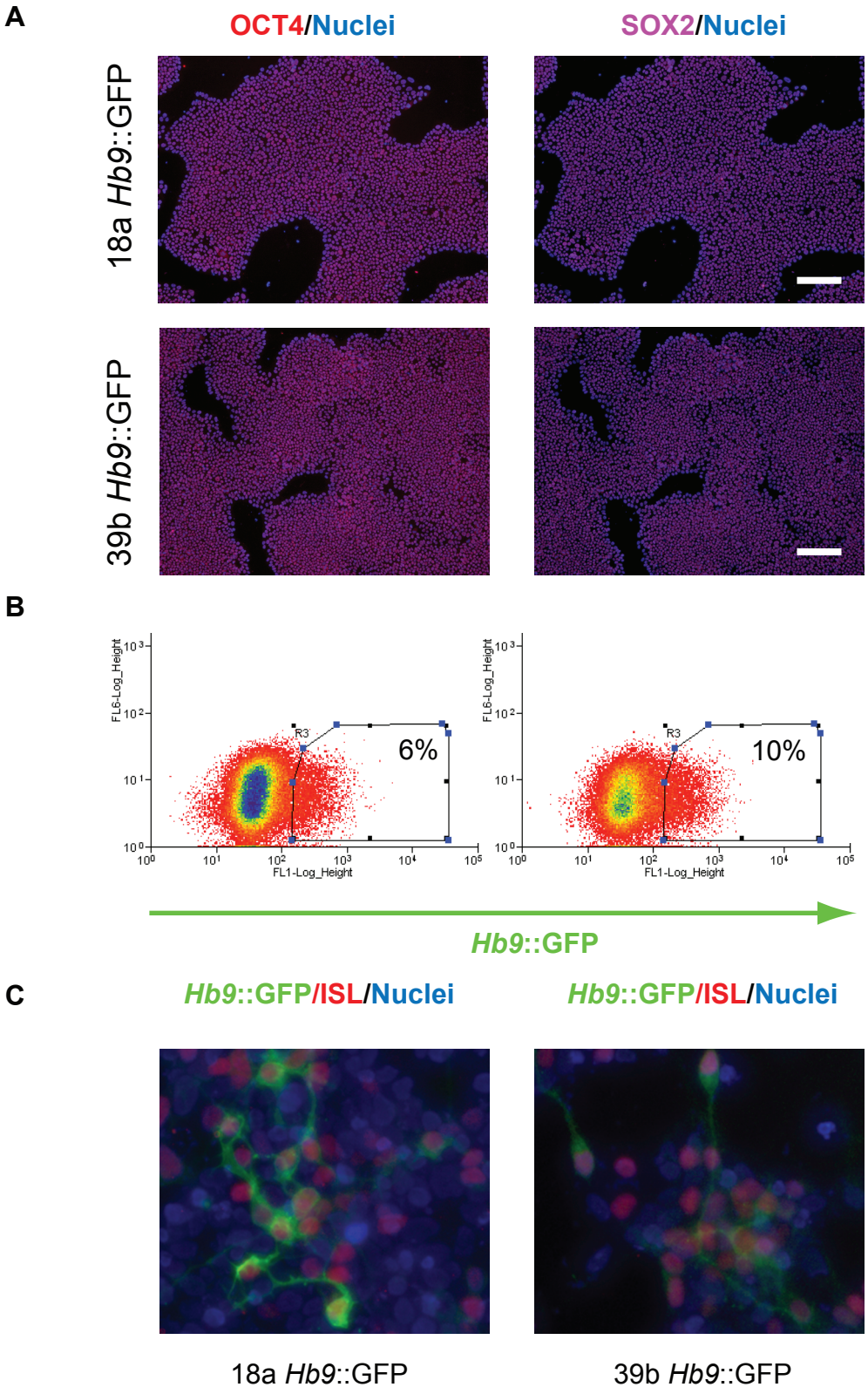


Figure S3, related to Figure 1. Characterization and Sorting of 18a *Hb9*::GFP and 39b *Hb9*::GFP reporter iPS Cell Lines.

(A) Representative immunofluorescence staining of 18 and 39 iPS *Hb9*::GFP reporter cell lines demonstrates expression of the pluripotency marker OCT4(red) and SOX2 (magenta). Scale bar is 100 μm.

(B) Fluorescence cell sort analysis of the reporter cell lines after motor neuron differentiation.

(C) ISL expression indicates motor neuron identity of *Hb9*::GFP positive cells derived from differentiating 18a and 39b reporter iPS cell lines.

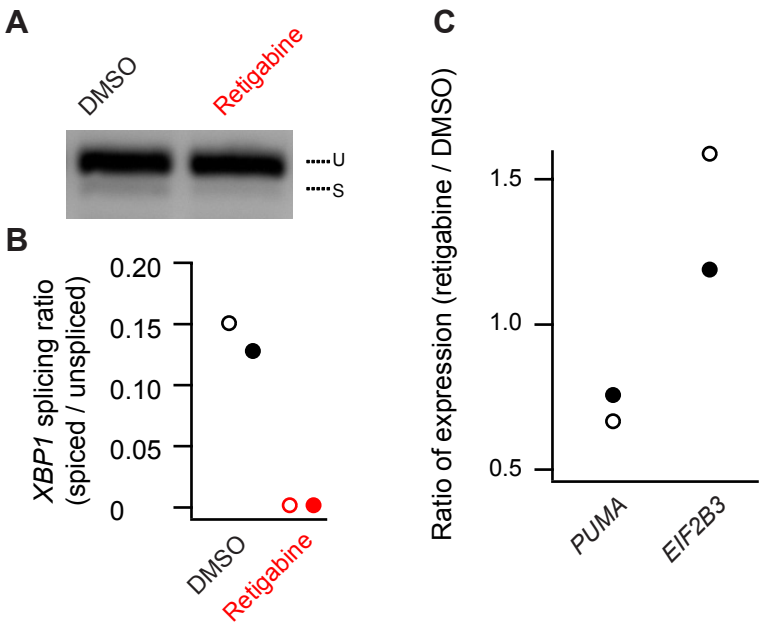


Figure S4, related to Figure 5, Retigabine Decreases Activation of ER Stress Pathways.

(A) Blots of unspliced (u) and spliced (s) *XBP1* in sorted 39b-*SOD1*$^{+/A4V}$ Hb9:RFP-sorted motor neurons treated with DMSO vehicle or 1 μM retigabine from days 15-30.

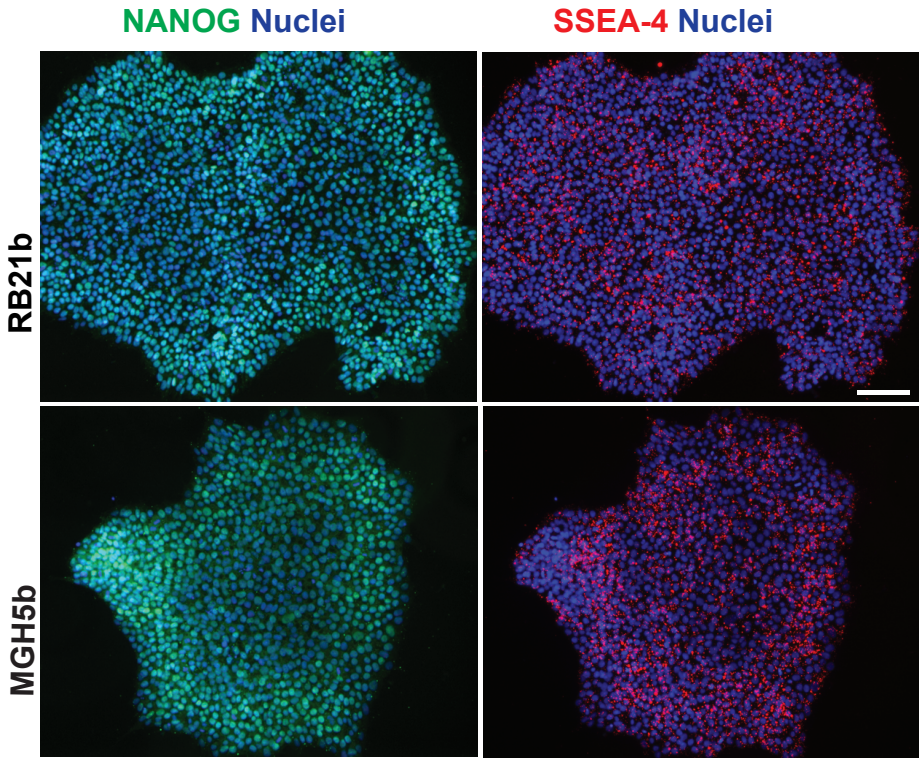
(B) Quantification of effect on *XBP1* splicing in motor neurons from two independent replicates (open and filled circles).

(C) Ratio of qRT-PCR product for genes associated with ER stress following retigabine compared to DMSO treatment in the same two independent replicates.

A

Patient ID	Gender	Mutation	Age of Onset	Age at Skin Biopsy
RB21	Female	FUS H517Q	43	49
MGH5	Female	FUS M511Nfs*6	19	19

B



C

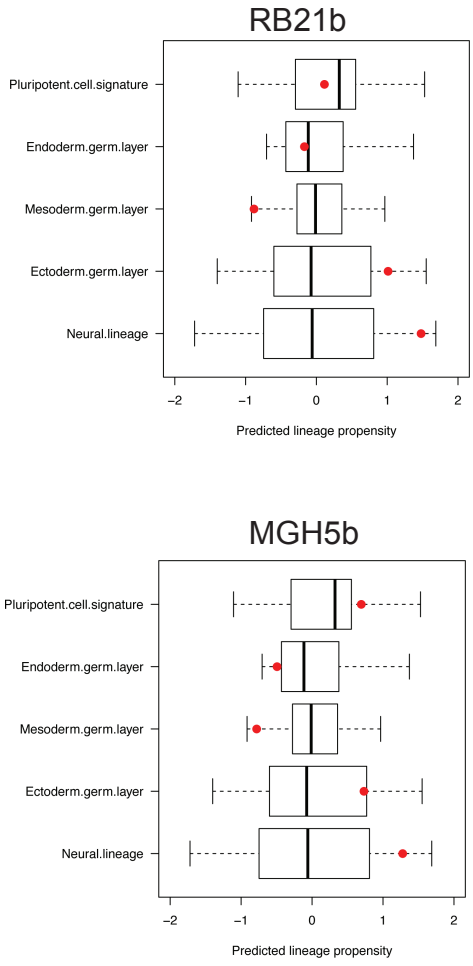


Figure S5, related to Figure 5, Characterization of FUS iPS Cell Lines.
(A) FUS-ALS patient line characteristics
(B) Representative immunofluroescence staining of iPS cell colonies demonstrating the expression of pluripotency markers NANOG (green) and SSEA-4 (red). Cell nuclei are fluorescently labeled with Hoescht. Scale bar is 100 μm.
(C) Scorecard analysis of pluripotency and lineage specific genes at day 16 of undirected iPS cell differentiation

Table S1, related to Figures 1-3, 5, 6, Electrophysiological Comparisons of Motor Neurons.
Summary of lines, numbers of MEAs/cells and differentiations for individual experiments.

Property and Technique	Figure and Time	Lines	Total MEAs/Cells	Differentiations
Total spikes/minute (MEA)	Figure 1C 28 days	11a, 18a vs 39b, RB9d	9 MEAs	4
Neuronal mean firing rate (MEA)	Figure 1E 28 days	11a, 18a vs 39b, RB9d	1754 cells	4
Total spikes/minute (MEA)	Figure 1F 4-28 days	18a vs 39b (both HB9::GFP)	23 MEAs	3
Total spikes/minute (MEA)	Figure 1G 14 days	39b-Cor vs 39b	8 MEAs	3
Number of spikes on ramp (Patch)	Figure 2C 28 days	11a, 18a vs 39b, RB9d	48 cells	4
Number of spikes on ramp (Patch)	Figure 2D 28 days	39b-Cor vs 39b	36 cells	3
Relative K(DR)/ Total Na Current (Patch)	Figure 3B 28 days	11a, 18a vs 39b, RB9d	48 cells	4
Relative K(DR)/ Total Na Current (Patch)	Figure 3C 28 days	39b-Cor vs 39b	37 cells	3
Absolute K(DR) and Na Current (Patch)	Figure 3D-E 28 days	39b-Cor vs 39b	37 cells	2
Total spikes/minute (MEA)	Figure 5B 14 days	11a, 18a vs 19f, RB8b	10 MEAs	2
Neuronal mean firing rate (MEA)	Figure 5C 14 days	11a, 18a vs 19f, RB8b	1621 cells	2
Total spikes/minute (MEA)	Figure 6A 14 days	Control: 11a, 15b, 17a, 18a, 18b, 20b SOD1: 25b, 27b, 39b, RB9d C9orf72: 19f, RB8b FUS: MGH5b, RB21	Control: 38 MEAs SOD1: 20 MEAs C9orf72: 15 MEAs FUS: 14 MEAs	4

Table S2, related to Figures 2-3, Electrophysiological Properties of Motor Neurons. No comparisons between control and ALS motor neurons reached statistical significance, in either the initial 4 Lines (11a, 18a, 39b, and RB9d) or later gene correction (39b-Cor and 39b) experiments.

	4 Lines		**Gene Correction**	
	Control: 11a, 18a n=23	ALS: 39b, RB9d n=25	Control: 39b-Cor n=18	ALS: 39b n=19
Capacitance (pF)	38.8 ± 4.1	39.3 ± 2.6	34.7 ± 3.8	36.3 ± 4.7
Resting Vm (mV)	-50.7 ± 2.5	-46.0 ± 2.3	-45.1 ± 2.1	-48.0 ± 2.3
Input Resistance (MΩ)	1148 ± 130	856 ± 94	1295 ± 183	1047 ± 97
Rheobase (pA)	9.0 ± 2.0	6.8 ± 0.9	4.1 ± 1.5	3.0 ± 0.7
Spike Voltage Threshold (mV)	-42.3 ± 1.8	-42.5 ± 1.2	-43.5 ± 0.6	-44.7 ± 1.0
Spike Width (ms)	3.4 ± 0.3	3.1 ± 0.2	1.8 ± 0.2	1.6 ± 0.1
Spike Peak (mV)	16.3 ± 2.6	23.6 ± 2.7	25.8 ± 3.0	31.4 ± 1.7
Maximum Upstroke Velocity (mV/ms)	76.2 ± 8.8	109.9 ± 13.0	117.9 ±18.6	146 ± 9.9
Maximum Downstroke Velocity (mV/ms)	24.4 ± 2.3	28.3 ± 2.1	37.1 ± 4.2	40.8 ± 3.1
After-hyperpolarization (mV)	-60.9 ± 2.4	-60.5 ± 1.9	-63.8 ± 0.4	-65.4 ± 1.2

Table S3, related to Figure 4, RNA-Seq Expression of Voltage-Gated Potassium Channels. Mean normalized transcript counts per million of voltage-gated potassium channels present in 39b-Cor and 39b-derived motor neurons.

Gene	39b-Cor	39b
KCNA1	56.85	22.98
KCNA2	25.16	24.87
KCNA3	16.34	35.63
KCNA4	10.43	8.26
KCNA5	7.807	7.05
KCNA6	646.65	422.77
KCNB1	142.77	161.55
KCNB2	41.96	36.99
KCNC1	311.56	293.43
KCNC2	14.49	28.52
KCNC3	132.75	74.13
KCNC4	88.24	108.16
KCND1	17.33	47.55
KCND2	307.23	243.56
KCND3	706.92	1425.60
KCNF1	93.49	28.68
KCNG1	81.24	134.45
KCNG2	15.10	7.42
KCNH1	23.82	14.79
KCNH2	372.19	300.71
KCNH3	13.406	14.32
KCNH5	16.68	58.85
KCNH7	12.85	22.20
KCNH8	135.75	245.41
KCNQ2	476.93	427.27
KCNQ3	133.21	155.91
KCNQ4	6.06	17.35
KCNQ5	37.39	76.20
KCNV1	210.74	81.48