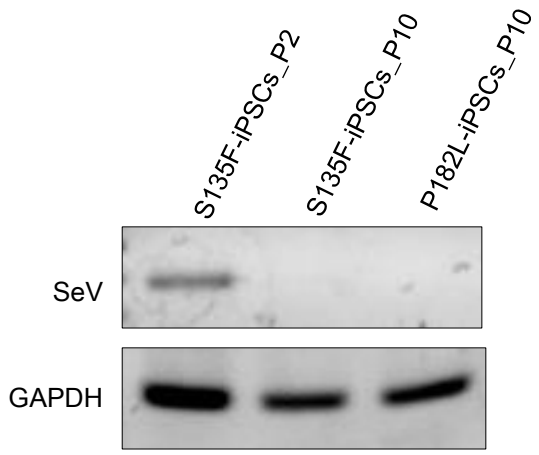
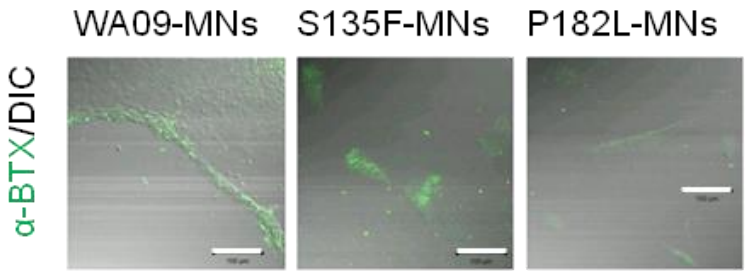
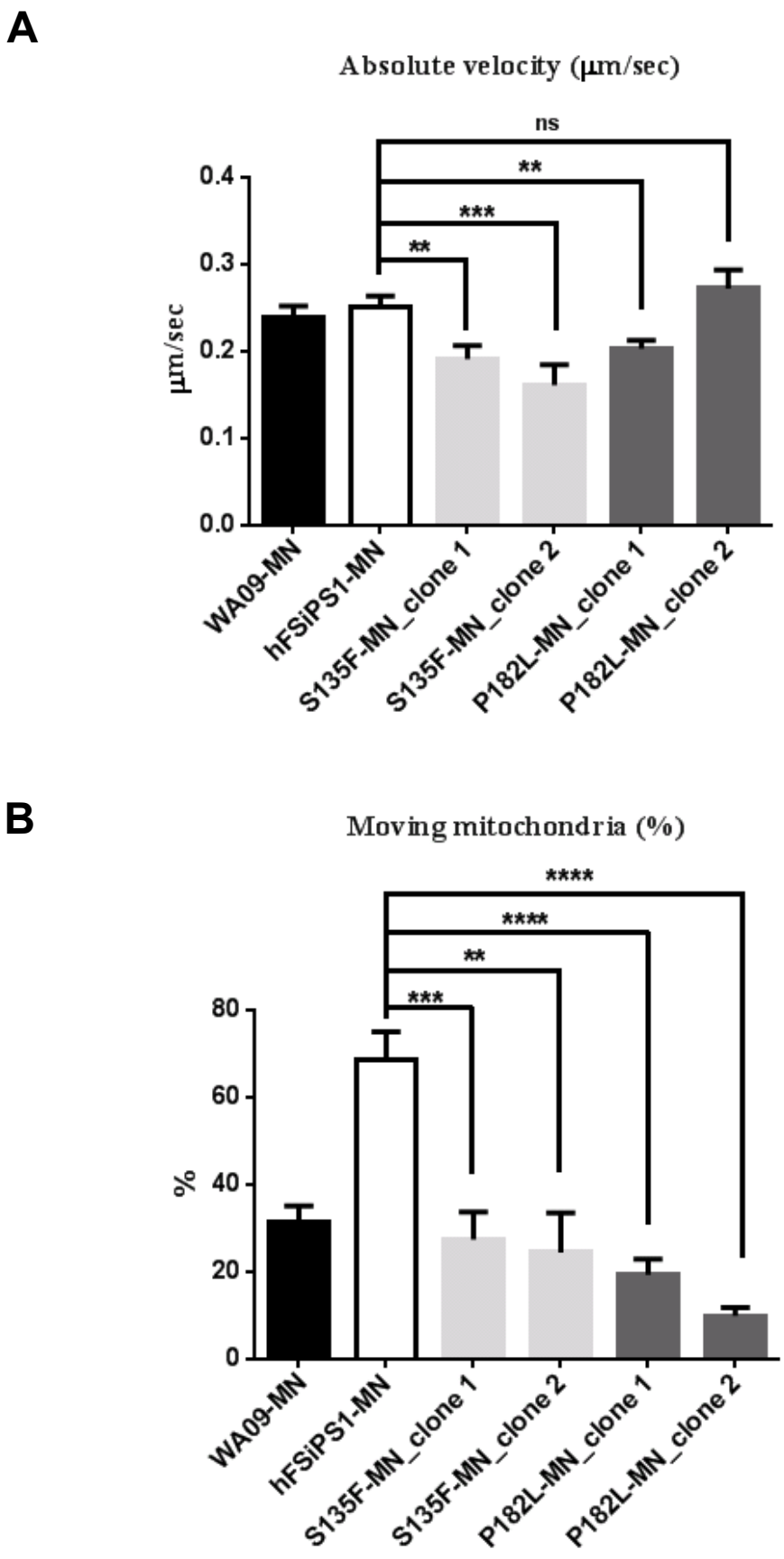


Supplement Figure S1. Assessment of Sendai viral genome contents in iPSCs. Sendai viral genome sequence (SeV) was expressed in S135F-iPSCs at cellular passages of 2 (P2) but SeV was not detected in S135F-iPSCs and P182L-iPSCs at cellular passages of 10 (P10). Primer sequences were 5'-GGA TCA CTA GGT GAT ATC GAG C-3' (forward) and 5'-ACC AGA CAA GAG TTT AAG AGA TAT GTA TC-3' (reverse) for SeV.

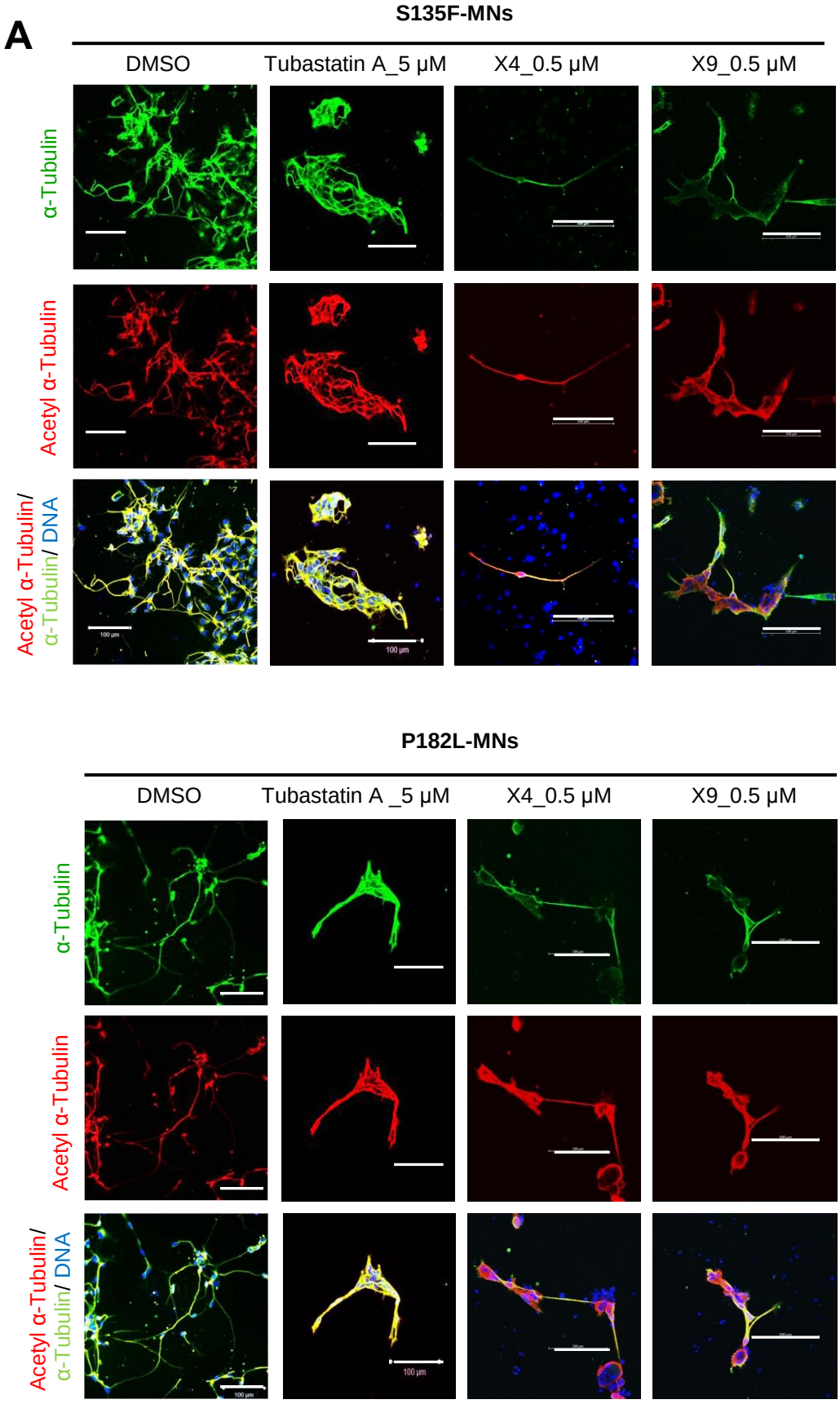


Supplement Figure S2. WA09-MNs, S135F-MNs and P182L-MNs formed neuromuscular junctions by co-culturing with myotube cells differentiated from C2C12 mice myoblast cells. Neuromuscular junctions were visualized by expression of acetylcholine receptors on myotube cells and staining with Alexa 488-conjugated α-BTX (DIC, differential interference contrast image; original magnification, 400x).

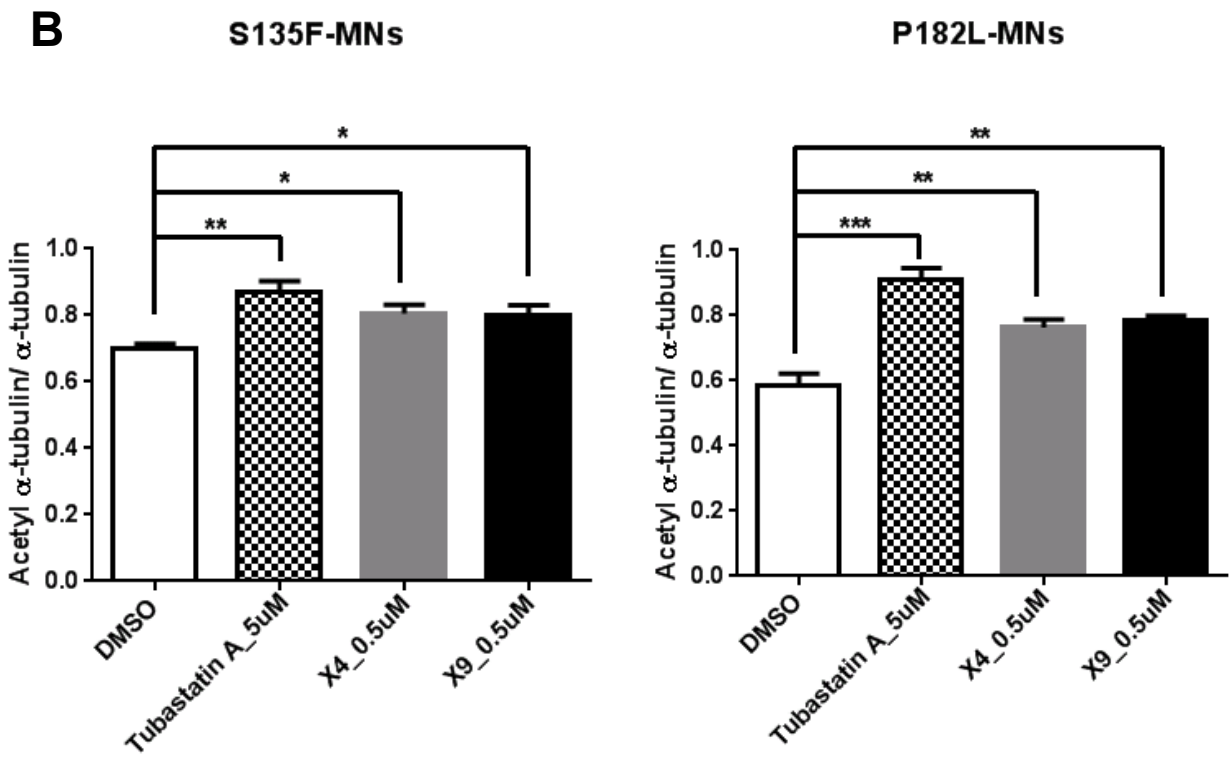


Supplement Figure S3 (Continued)

Supplement Figure S3. Axonal transport in S135F-MNs and P182L-MNs from two different iPSC clones. (**A**) Mitochondrial velocity in hFSiPS1-MNs (N=513), S135F-MNs_clone 1 (N=383), S135F-MNs_clone 2 (N=45), P182L-MNs_clone 1 (N=344) and P182L-MNs_clone 2 (N=152). (**B**) Moving mitochondria (%) in hFSiPS1-MNs (N=6), S135F-MNs_clone 1 (N=13), S135F-MNs_clone 2 (N=7), P182L-MNs_clone 1 (N=21), and P182L-MNs_clone 2 (N=21). Unpaired t-tests, *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001. ns, not significant.



Supplement Figure S4 (Continued)



Supplement Figure S4. Immunostaining showed that treatment of HDAC6 inhibitors increased acetylated α-tubulin levels in S135F-MNs and P182L-MNs. **(A)** Acetylation of α-tubulin was visualized by immunostaining with anti-acetylated α-tubulin Abs (red). Total α-tubulin was also detected by staining with anti-α-tubulin Abs (green). (Original magnification, 400 ×). Scale bars: 100 μm. **(B)** The extent of acetylated α-tubulin was normalized with corresponding total α-tubulin using ImageJ software. Number of fields for image analysis were: S135F-MNs + DMSO (N=4), S135F-MNs + Tubastatin A (N=5), S135F-MNs + X4 (N=3), S135F-MNs + X9 (N=10), P182L-MNs + DMSO (N=5), P182L-MNs + Tubastatin A (N=4), P182L + X4 (N=4) and P182L-MNs + X9 (N=4). Unpaired t-tests, *P<0.05, **P<0.01 and ***P<0.001.

Supplement Table S1. Absolute velocity (μm/sec) of moving mitochondria

	DMSO	Tubastain A	X4		X9	
	0.01 %	5 μM	0.5 μM	5 μM	0.5 μM	5 μM
WA09-MNs	0.24 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.33 ± 0.01	0.30 ± 0.01	0.30 ± 0.02
hFSiPS1-MNs	0.25 ± 0.01	-	-	-	-	-
S135F-MNs	0.19 ± 0.01	0.25 ± 0.02	0.48 ± 0.06	0.38 ± 0.03	0.25 ± 0.02	0.39 ± 0.02
P182L-MNs	0.22 ± 0.01	0.27 ± 0.02	0.28 ± 0.04	0.33 ± 0.02	0.23 ± 0.01	0.29 ± 0.01

Supplement Table S2. Percentage of moving mitochondria (%)

	DMSO	Tubastain A	X4		X9	
	0.01 %	5 μM	0.5 μM	5 μM	0.5 μM	5 μM
WA09-MNs	31.39 ± 3.74	49.71 ± 6.18	35.63 ± 6.28	49.74 ± 4.72	38.31 ± 6.02	34.07 ± 8.86
hFSiPS1-MNs	68.64 ± 6.42	-	-	-	-	-
S135F-MNs	26.37 ± 5.01	21.73 ± 6.53	58.40±10.42	48.31 ± 5.25	55.33 ± 5.86	62.88 ± 5.73
P182L-MNs	14.19 ± 2.14	30.91 ± 5.78	70.83 ± 4.17	37.59 ± 3.86	45.07 ± 4.48	33.14 ± 2.91

Supplement Materials and Methods

Neuromuscular junction formation

C2C12 mouse myoblast cells (CRL-1772, American Type Culture Collection (ATCC), Manassas, VA, USA) were cultured in DMEM supplemented with 10% FBS and 1 mM glutamine and penicillin/streptomycin. At a cell confluence of 70%, 1% insulin-transferrin-selenium supplement (Sigma) was added to the medium for myotube differentiation. After 2 days, 10 μM cytosine arabinoside (Sigma) was added to DMEM containing 0.5% FBS to remove dividing cells. After 2-4 days, differentiated myotubes were trypsinized and plated at a low density of 1×10^4 cells/well onto Matrigel-coated 8-well slide chambers (Nalgene/NUNC). After 1-2 days, CMT2F-MNs and ESC-MNs were co-cultured with myotubes at a ratio of 10:1 and supplemented with MN differentiation medium. After 1 week, functional neuromuscular junctions were visualized by staining MNs with Alexa 488-conjugated α-bungarotoxin (Invitrogen).