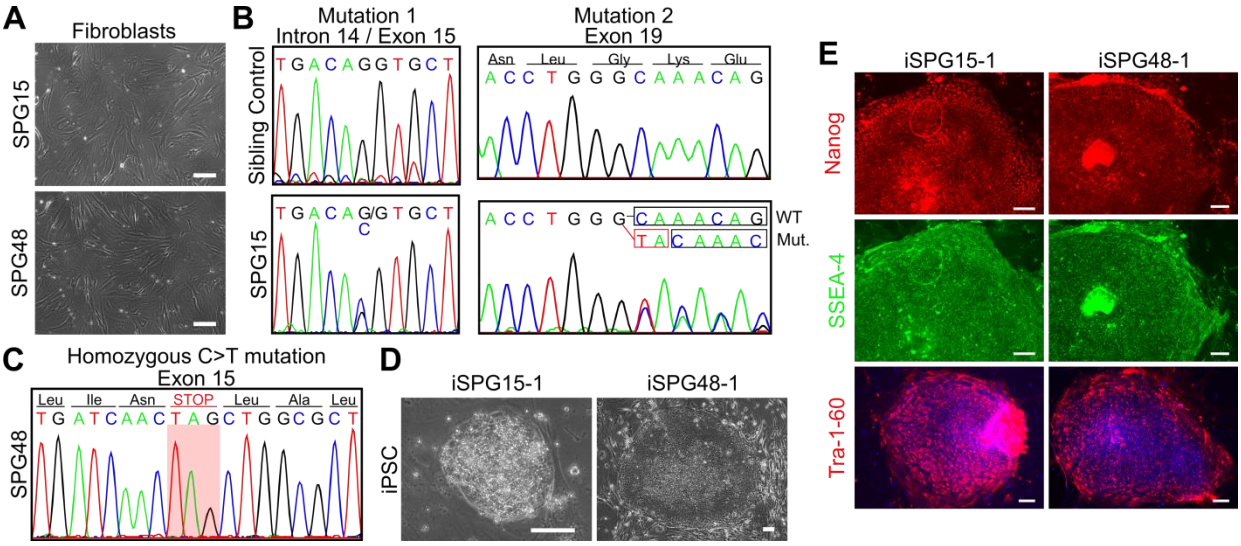
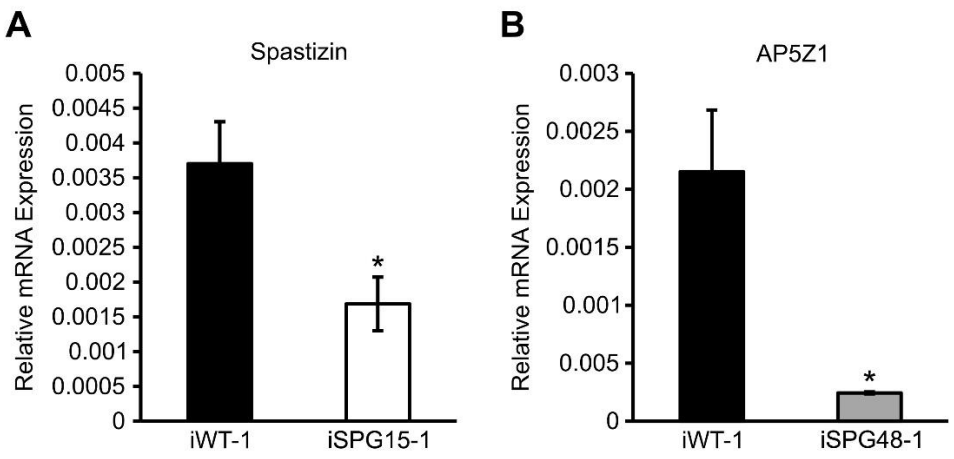
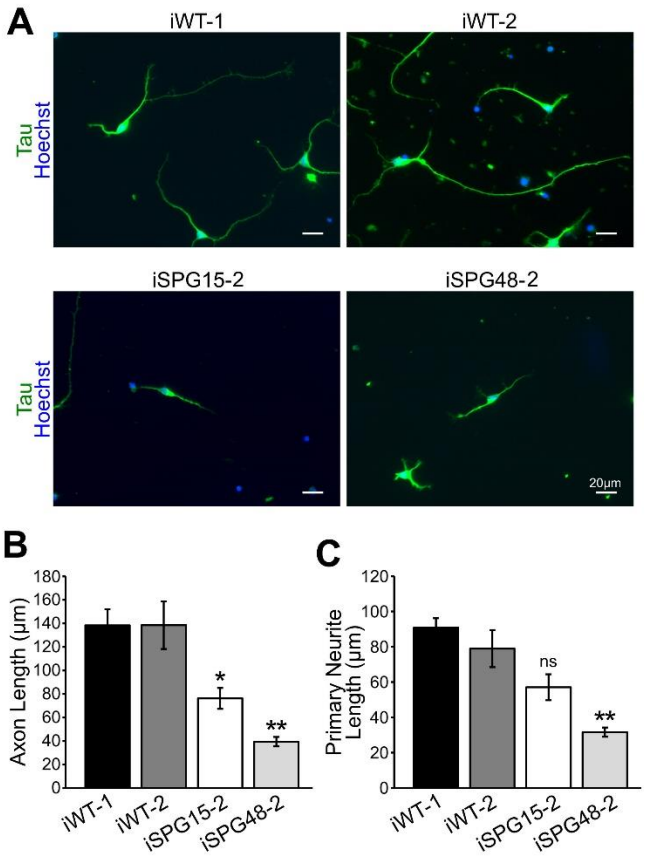
Supplementary Material



Supplementary Figure 1. Generation and characterization of SPG15 and SPG48 iPSC lines. **(A)** Representative phase contrast images of SPG15 and SPG48 fibroblast cells. Scale bars: 200 µm. **(B)** Sanger sequencing identified compound heterozygous mutations in the *ZFYVE26* gene in the SPG15 patient line. One mutation is located at the splice acceptor site of intron 14 (c.2554-1 G>A) resulting in the skipping of exon 15, and the other is a nonsense mutation in exon 19 (c.3417_3418insTA; p. Lys1140X) which results in truncation of the protein. **(C)** Sequencing of DNA from SPG48 iPSCs revealed a homozygous c.1732 C>T nucleotide substitution, resulting in a premature stop codon in exon 15 of the *AP5Z1* gene. **(D)** SPG15 and SPG48-derived iPSC colonies display typical ESC-like morphology. Scale bars: 100 µm. **(E)** All examined lines stained positive for the pluripotency markers Nanog, SSEA-4, and Tra-1-60. Scale bars: 100µm.



Supplementary Figure 2. Reduced spastizin and AP5Z1 mRNA expression in SPG15 and SPG48 forebrain neurons, respectively. **(A)** qRT-PCR analysis of spastizin expression in week 15 telencephalic, glutamatergic iSPG15 neuron cultures. **(B)** AP5Z1 expression in control and iSPG48 week 15 telencephalic, glutamatergic neuron cultures. Data are presented as means ± SD. *$P < 0.05$ versus iWT-1.



Supplementary Figure 3. Reduced neurite outgrowth from additional telencephalic, glutamatergic neuron clones. **(A)** Immunofluorescence images of day 33 forebrain neurons taken 72 h after plating. **(B)** Quantification of the longest neurite. **(C)** Mean length of primary neurites per cell. Data presented as mean ± SEM. *P < 0.05; *P < 0.01 versus iWT; ns, not significant.