

Supplementary Information

Inlets of side channels	Outlets of side channels	Center channel	Interconnecting grooves
Width / Height (μm)			Length / Width / Height (μm)
200 / 150	1000 / 150	1500 / 150	250 / 100 / 150

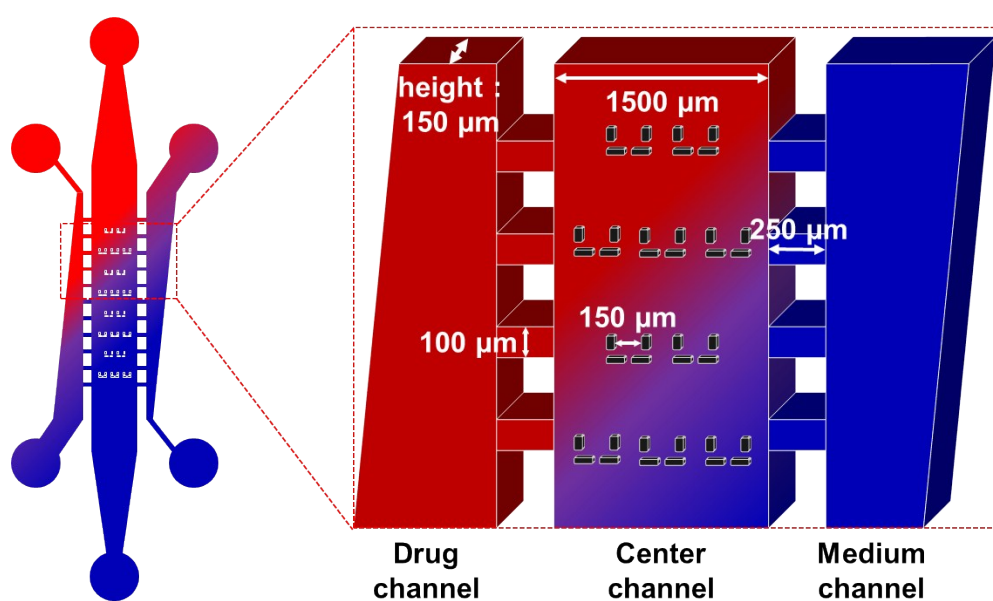


Fig. S1. Schematic diagram of microfluidic gradient device with detail channel dimensions.

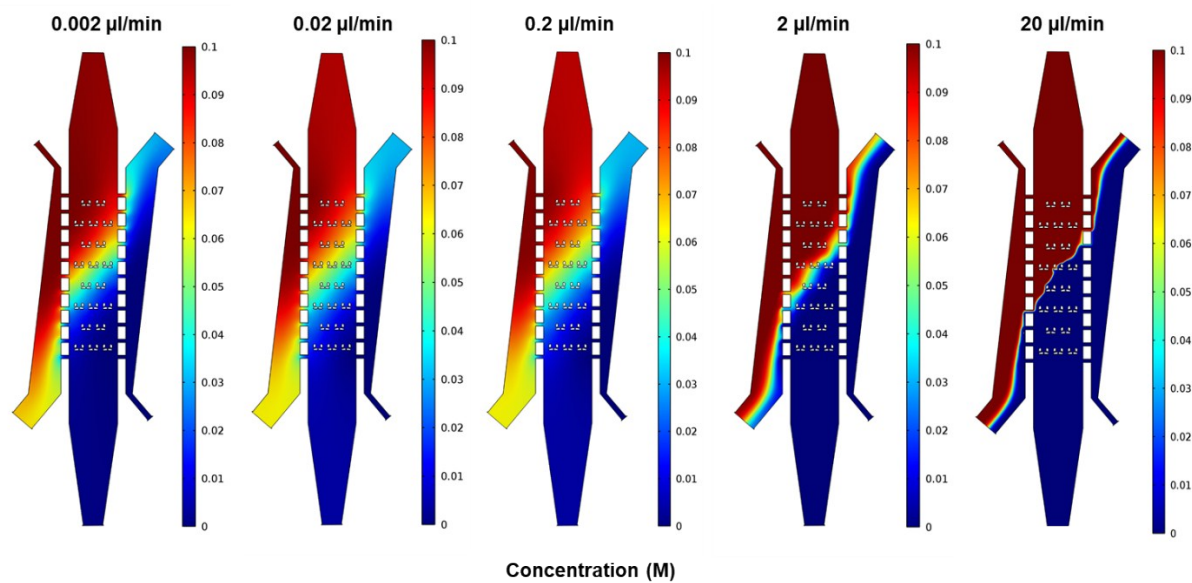


Fig. S2. Computational modeling of gradient with various flow rates (from 0.002 μL/min to 20 μL/min). At a flow rate of 0.2 μl/min, average flow speed $V = 1.48 \times 10^{-5}$ driven by a syringe pump in the center channel (height $h = 150 \mu\text{m}$, width $w = 1500 \mu\text{m}$, length = 1 cm).

Table. S1. Primer sequences used for real-time PCR

Gene	Primer (Forward)	Primer (Reverse)
OCT4	CTGGTTCGCTTTCTCTTTTCG	CTTTGAGGCTCTGCAGCTTA
SOX1	ACTTTTATTTCTCGGCCCGT	GGAATGGGAGGACAGGATTT
TUJ1	GGCCAAGGGTCACTACACG	GCAGTCGCAGTTTTCACACTC
NeuN	TCGTAGAGGGACGGAAAATTGA	GCCGTTGGTGTAGGGGTTTC
ISLET1	TACGGGATCAAATGCGCCAA	CACACAGCGGAAACACTCGAT
ChAT	CAGCCCTGCCGTGATCTTT	TGTAGCTGAGTACACCAGAGATG
GAPDH	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG

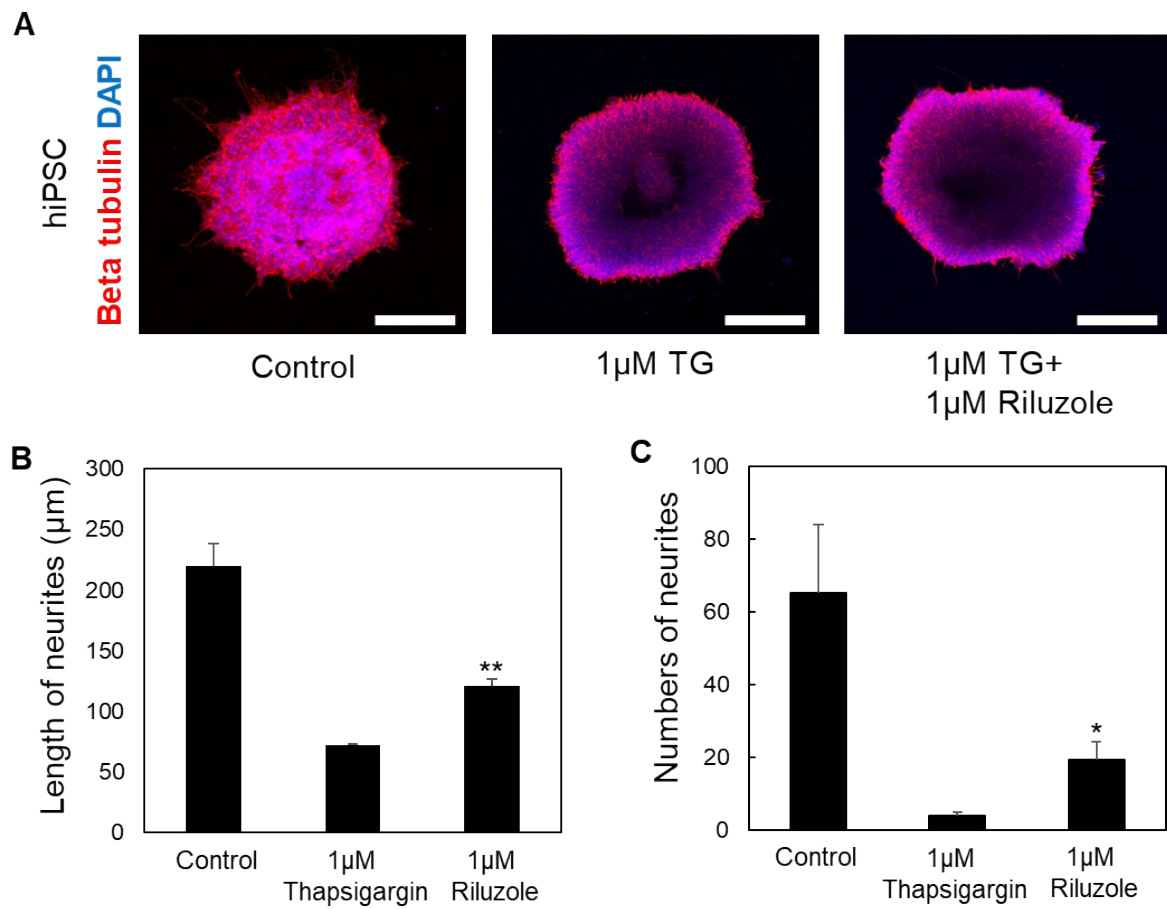


Fig. S3. Confocal microscope images of iPSC-derived motoneuron spheroids in a control and drug treatment on cell culture plate. (A) Analysis of length (B) and number (C) of neurites after drug treatment drug treatment (Student's *t*-test, * $p < 0.05$, ** $p < 0.01$; $N=3$).