

Supplementary table 1. Table showing publications mentioned in this review that have produced or utilised *in vitro* models suitable for ALS research, and the experimental readout methods utilised in

Publication	Model description	Experimental readouts
2D Models		
Stoklund-Dittlau et al., 2021 ¹	2-chamber microfluidic platform. iLMNs projecting through microchannels to differentiated human myoblasts (figure 3B).	Immunocytochemistry Confocal microscopy Calcium imaging
Southam et al., 2013 ² and Blizzard et al., 2015 ³	2-chamber microfluidic platform. Primary spinal neurons (on a bed of astrocytes) projecting through microchannels onto C2C12 myoblasts (figure 3C).	Scanning electron microscopy Immunocytochemistry Confocal Microscopy
Feiler et al., 2015 ⁴	2-chamber microfluidic platform. Primary cortical neurons grown through microchannels to H4 cells in the second chamber (figure 3D).	Luciferase complementation assay
3D models		
Machado et al., 2019 ⁵	3-chamber, open-top microfluidic platform. Differentiated primary myoblasts in the central chamber, ESC-derived motor neuron/astrocyte spheroids in peripheral chambers (figure 3E).	Immunocytochemistry Optogenetic stimulation Electrophysiology (patch clamp) Quantitative reverse transcription PCR (RT-qPCR)
Osaki et al., 2018 ⁶	ALS patient-derived motor neuron spheroid grown through the device onto iPSC derived skeletal muscle fibre. Micropillars in the skeletal muscle chamber measure contraction force (figure 3F).	Optogenetic stimulation Micropillar biosensor integration Calcium imaging Immunocytochemistry Confocal imaging
Faustino Martins et al., 2020 ⁷	Non-compartmentalised hESC-derived neuromuscular organoids	Calcium imaging Immunohistohcemistry RT-qPCR Live imaging Single cell RNA sequencing Microelectrode array recordings
Andersen et al., 2020 ⁸	Non-compartmentalised iPSC-derived corticomotor assembloid.	Calcium imaging Electrophysiology (patch clamp) Viral tracing Optogenetic stimulation Transmission electron microscopy RT-qPCR

the study.

1. Stoklund Dittlau K, Krasnow EN, Fumagalli L, Vandoorne T, Baatsen P, Kerstens A, et al. Human motor units in microfluidic devices are impaired by FUS mutations and improved by HDAC6 inhibition. *Stem Cell Reports.* 2021;16(9):2213-27.
2. Southam KA, King AE, Blizzard CA, McCormack GH, Dickson TC. Microfluidic primary culture model of the lower motor neuron-neuromuscular junction circuit. *J Neurosci Methods.* 2013;218(2):164-9.
3. Blizzard CA, Southam KA, Dawkins E, Lewis KE, King AE, Clark JA, et al. Identifying the primary site of pathogenesis in amyotrophic lateral sclerosis - vulnerability of lower motor neurons to proximal excitotoxicity. *Dis Model Mech.* 2015;8(3):215-24.
4. Feiler MS, Strobel B, Freischmidt A, Helferich AM, Kappel J, Brewer BM, et al. TDP-43 is intercellularly transmitted across axon terminals. 2015(1540-8140 (Electronic)).
5. Machado CB, Pluchon P, Harley P, Rigby M, Sabater VG, Stevenson DC, et al. In Vitro Modeling of Nerve–Muscle Connectivity in a Compartmentalized Tissue Culture Device. *Advanced Biosystems.* 2019;3(7):1800307.
6. Osaki T, Uzel SGM, Kamm RD. Microphysiological 3D model of amyotrophic lateral sclerosis (ALS) from human iPS-derived muscle cells and optogenetic motor neurons. *Sci Adv.* 2018;4(10):eaat5847.
7. Faustino Martins JM, Fischer C, Urzi A, Vidal R, Kunz S, Ruffault PL, et al. Self-Organizing 3D Human Trunk Neuromuscular Organoids. *Cell Stem Cell.* 2020;27(3):498.
8. Andersen J, Revah O, Miura Y, Thom N, Amin ND, Kelley KW, et al. Generation of Functional Human 3D Cortico-Motor Assembloids. *Cell.* 2020;183(7):1913-29.e26.