

Functional Neuronal Differentiation of Injury-Induced Muscle-Derived Stem Cell-Like Cells with Therapeutic Implications*

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Supplementary Table 1: Primary and secondary antibodies.

Primary antibodies	Host	Dilution	Manufacturer	Secondary antibodies
AChE	Mouse monoclonal IgG2b	1:200	Abcam	Goat anti-mouse AlexaFluor 488
CNPase	Mouse monoclonal IgG1	1:200	Millipore	Goat anti-mouse AlexaFluor 594
Gfap	Rabbit polyclonal IgG	1:150	Santa Cruz	Goat anti-rabbit AlexaFluor 488
Nefm	Mouse monoclonal IgG2a	1:200	Abcam	Goat anti-mouse AlexaFluor 594
Nestin	Goat polyclonal IgG	1:150	Santa Cruz	Donkey anti-goat AlexaFluor 488
Myosin heavy chain	Mouse monoclonal IgG1	1:200	Sigma	Goat anti-mouse AlexaFluor 488
β -Tubulin III	Rabbit polyclonal IgG	1:500	Abcam	Goat anti-rabbit AlexaFluor 594

Supplementary Table 2: PCR primers.

Gene	5'Primer	3'Primer
AChE	AAGGGCTGGGATATAATACGAC	CTTAGCCCAAGACATGCAGA
Agrin	CCTCAACTTGGACACGAAGCT	AGGCCGATGCCACAGA
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Chrna1	GAATCCAGATGACTATGGAG	GACAATGATCTCACAGTAGC
Chrng	ATCCGGCACCGACCGGCTAA	CATTTCTGCCC GCCCGCCTT
Dok7	TCTCCCAGACCCGAGTTCTG	TCTAGCTGCAGGGCTTCCA
Gapdh	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT
Gfap	GCCCGGCTCGAGGTCGAG	GTCTATACGCAGCCAGGTTGTTCT CT
Lrp4	GGACTGCACGTCAGCTATGC	CGCGATCACCAACAAAATCA
Musashi	G TTCATCGGAGGACTCAG	GCTCTCAAACGTGACAAA
Musk	TGAGAACTGCCCTTGGA ACT	GGGTCTATCAGCAGGCAGCTT
Mtap2	ACACCCCGAACCAGGAGGA	GCGTTGGACGTGCCCTTCT
Ncam1	ATGGAAACTCTATTAAAGTGAAC CTGA	TAGACCTCATACTCAGCATTCCAG T
Nefm	AGTGGTTCAAATGCCGCTAC	TTTTCCA ACTGCTGGATGGT
Nefl	CCATGCAGGACACAATCAAC	CGCCTTCCAAGAGTTTTCTG
Nestin	CTGGAAGGTGGGCAGCAACT	ATTAGGCAAGGGGGAAGAGAAGG ATG

Nrg1	CCTGGGAGGCCCTCGCGAAT	CCGTCATGCTGGACACGGGT
Olig1	ACGTCGTAGCGCAGGCTTAT	CGCCCAACTCCGCTTACTT
Olig2	GGGAGGCGCCATTGTACA	GTGCAGGCAGGAAGTTCCA
Otx2	CTGTTACCAGCCATCTCAATC	ATAGCTTCTACAGGTCTTCAC
Pax6	AGGGGGAGAGAACACCACT	CATTTGGCCCTTCGATTAGA
Rapsyn	ACGAGTGC GTGGAGGAGACT	TGTTCTCTCCCCGATGGA
β- TubulinIII	AGACA ACTTCGTTTTTCGGTCAGT	CCTTTAGCCCAGTTGTTGCCT

Supplementary Table 3: Tested medium for neuronal differentiation of iMuSCs.

ND1 medium	DMEM/F12, 1% Glutamax, 1% non-essential amino acids, 0.1mM β -mercaptoethanol, 1% ITS (5 μ g/ml insulin, 5 μ g/ml transferrin, 29nM selenium), 20nM progesterone, 100 μ M putrescine dihydrochloride, 1 μ g/ml laminin, 5 ng/ml human recombinant basic fibroblast growth factor, 0.5% antibiotics
ND2 medium	Neurobasal Medium, 1% Glutamax, 2% B-27, 0.5% antibiotics
ND3 medium	Neurobasal Medium, 1% Glutamax, 1% B-27, 20ng/ml brain-derived neurotrophic factor, 0.5% antibiotics

Supplementary Table 4: Morphological characteristics of the neuromuscular junctions.

	Terminal Nodes	Branching Nodes	Nb of Segments	Total Length
PBS	79±7	13±3	59±6	1135.5±87
MuSCs 1week	83±6	21±2	49±3	1205.3±110
MuSCs 3weeks	85±8	28±3	63±5	1167.3±78
iMuSCs 1week	80±8	31±5	95±9*	1879.4±98*
iMuSCs 3weeks	144±11*	58±4*	163±1*	3099.7±112*
MuSCs extract 1week	86±8	10±2	45±2	995.34±98
MuSCs extract 3weeks	89±5	18±6	53±8	1058.4±82
iMuSCs extract 1weeks	89±8	45±9*	104±97*	2920.3±101*
iMuSCs extract 3weeks	135±13*	63±7*	142±12*	3233.5±125*

All data are presented as mean ±SEM, $p < 0.05$. *, indicates statistical significance from control PBS injected *mdx* mice