Stem Cell Reports, Volume *18*

Supplemental Information

A novel protocol to derive cervical motor neurons from induced pluripotent stem cells for amyotrophic lateral sclerosis

Meimei Yang, Min Liu, Yajaira Feller Sánchez, Sahar Avazzadeh, Leo R. Quinlan, Gang Liu, Yin Lu, Guangming Yang, Timothy O'Brien, David C. Henshall, Orla Hardiman, and Sanbing Shen

Supplementary information

Figure S1

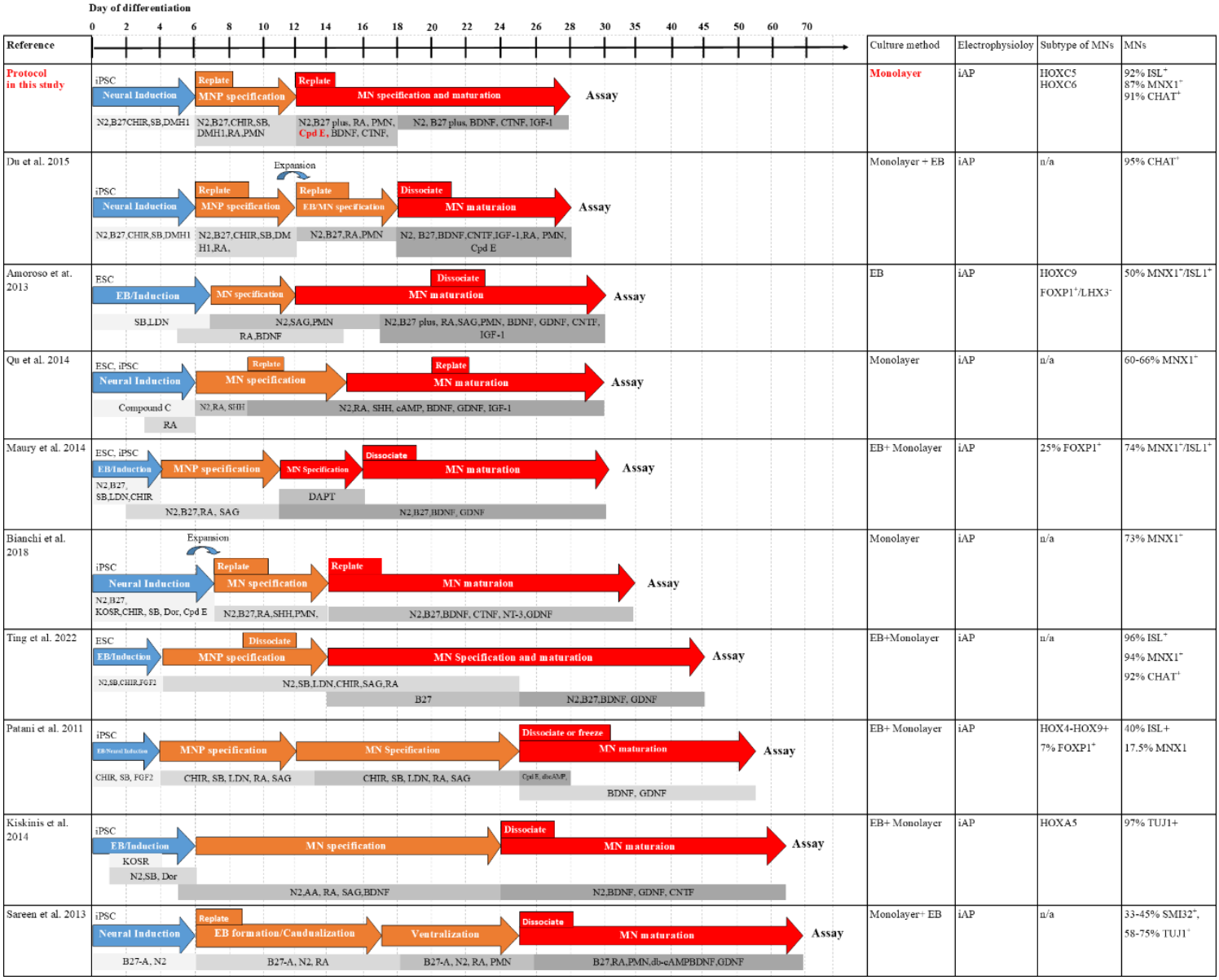

Figure S1. Comparison of Published sMN Differentiation Protocols. Ten protocols of iPSC-sMN differentiation were compared with respect to time (days in vitro). The end of the experiment (Assay) was based on the latest data presented in respective publications. KOSR, N2 and B27-A are the culture mediums. The abbreviations and full names are: BDNF – brain-derived neurotrophic factor, CHIR – CHIR99021, Compound C – a chemical inhibitor of Activin and BMP signalling, Compound E – a γ-secretase and notch pathway inhibitor, CNTF –Ciliary neurotrophic factor, DAPT – a notch response inhibitor, DMH1 – specific BMP-antagonist, Dorsomorphin (Dor, also called compound C) – a BMP inhibitor, FGF2 – Fibroblast growth factor 2, GDNF – glial cell line-derived neurotrophic factor, IGF-1 – Insulin-like Growth Factor 1, LDN193189 (LDN) – selective antagonist of BMP receptor, NT-3 – Neurotrophin-3,

Purmorphamine (PMN) – an SHH agonist, retinoic acid (RA), retinoic acid receptor (RAR)/retinoid X receptors (RXR), SAG – Sonic hedgehog signaling agonist, SB431542 (SB) - a TGF-β/SMAD inhibitor, SHH – Sonic Hedgehog. The right four columns listed a summary of results, including culture method (EB-embryoid body, indicating suspension culture and monolayer indicates adherent culture), induced action potentials (iAP), unique MN subtype markers observed which were expressed in the caudal spinal cord, including Hox family member C (HOXC9), Forkhead Box P1 (FOXP1), LIM Homeobox 3 (LHX3), and approximate yield of MNs which were based on the reported percentage of cells expressing HB9, ISL1 or CHAT.

Figure S2

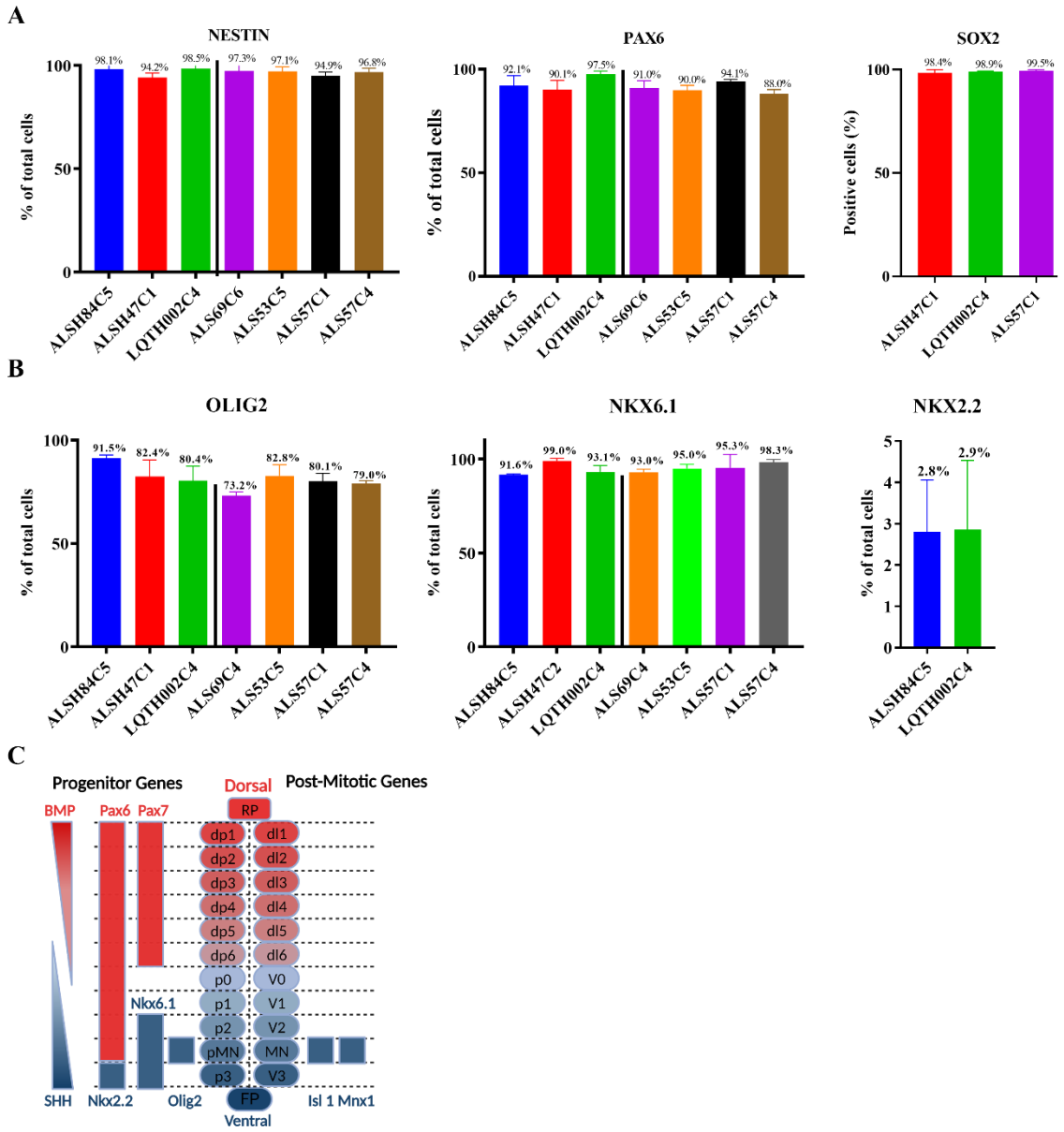

Figure S2. The efficiency of NSC and MNP differentiation from multiple hPSC lines on day 6 and day 12. A) The proportion of NESTIN$^+$, PAX6$^+$, and SOX2$^+$ cells on day 6 of differentiation. More than 600 cells from random fields were automatically counted by the Operetta High Content Imaging System in each cell line. **B) The efficiency of OLIG2$^+$, NKX6.1$^+$ and NKX2.2$^-$ MNP differentiation from multiple hPSC lines on day 12. C)** Schematic showing the expression pattern of pMN domain markers in the developing spinal cord. Adapted from a reference (Sagner and Briscoe, 2019). Created with BioRender.com.

Figure S3.

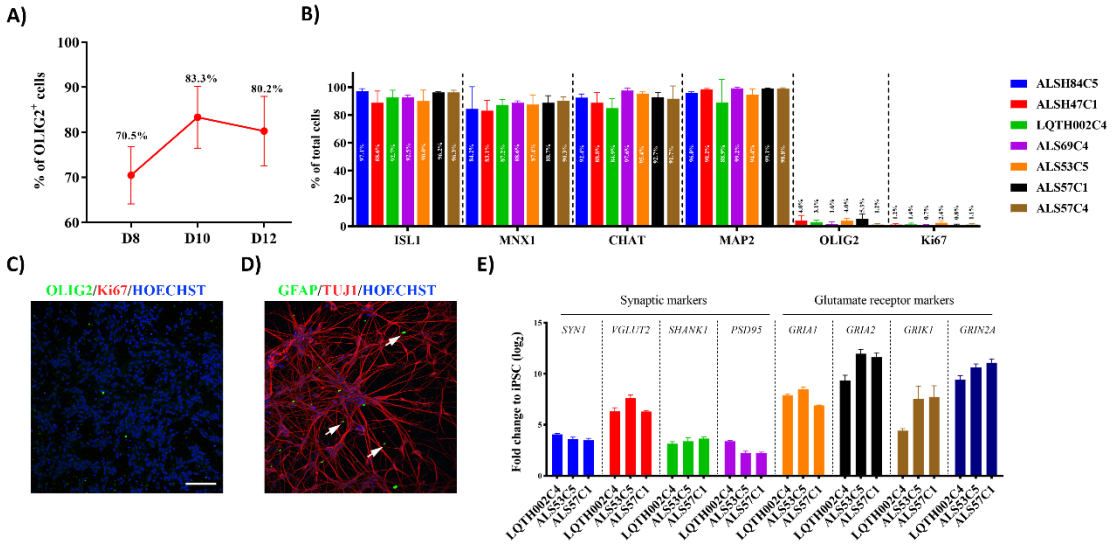

Figure S3. Compound E can effectively drive the MNs to exit the cell cycle and convert OLIG2$^+$ MNP to pure mature sMNs. A) Statistic analysis for the cells positive to OLIG2 from day 8 to day 12. N=3, n=3. **B)** The efficiency of ISL$^+$, MNX1$^+$,CHAT$^+$, MAP2$^+$, OLIG2$^+$, Ki67$^+$ sMN differentiation from multiple hPSC lines on day 18. **C)** Representative images of double staining of OLIG2/Ki67 in iPSC-derived sMNs on day 18. **D)** Representative images of single staining of GFAP and TUJ1 in iPSC-derived sMNs on day 18. Arrows indicate the unspecific staining of GFAP. **E)** RT-qPCR analysis of synaptic excitatory markers of *SYN1*, *SHANK1* and *PSD-95,* vesicular glutamate transporter *VGLUT2*, and glutamatergic neuronal markers, including ionotropic AMPA receptors of *GRIA1* and *GRIA2*, NMDA receptors of *GRIN2A,* kainate receptors of *GRIK1* in iPSC-derived sMNs on day 18 compared to iPSC on day 0. N=3. Scale bar, 100μm.

Table S1. Small molecules for spinal MN induction.

Involved stage	Involved athway	Name	Function	Reference
Neural induction	Wnt activator	CHIR99021	Inhibit GSK3, Promote ectoderm generation and caudalization	(Du et al., 2015; Nordström et al., 2002)
	Activin/TGFβ inhibitor	SB431542	Dual SMAD inhibition for neural induction	(Chambers et al. 2009)
	BMP inhibitor	Noggin		(Chambers et al. 2009)
		DMH1		(Du et al., 2015)
		LDN		(Maury et al., 2015)
		Compound C (Dorsomorphin)	Block both Activin/TGFβ and BMP pathway	(Zhou et al., 2010)
MN caudalization	Retinoid acid	All trans-Retinoid acid	Caudalizing NSCs	(Chambers et al. 2009)
MN ventralization	Sonic Hedgehog activator	SHH protein	Ventralizing NSCs	(Chambers et al. 2009)
		SAG (SHH agonist)		(Gouti et al., 2014; Mak et al., 2012)
		Purmorphamine (PMN, SHH agonist)		(Hu and Zhang, 2009; Mak et al., 2012)
MN maturation	Notch pathway inhibitor	Compound E	Promote neurogenesis by inhibiting Notch signaling and repressing cell proliferation.	(Du et al., 2015)
		DAPT		(Ben-Shushan et al., 2015; Maury et al., 2015)

Table S2. Information of cell lines used in the study.

Status	Cell line	Sex	Age	Ethnicity
Healthy	NUIGi048-C (ALSH84C5)	29	Male	Caucasian
Healthy	NUIGi038-A (LQTH002C4)	47	Female	Caucasian
Healthy	NUIGi049-A (ALSH47C1)	66	Female	Caucasian
Sporadic ALS	NUIGi044-B (ALS69C4)	44	Male	Caucasian
Sporadic ALS	NUIGi050-A (ALS53C5)	60	Male	Caucasian
Sporadic ALS	NUIGi051-A (ALS57C1)	56	Female	Caucasian
Sporadic ALS	NUIGi051-C (ALS57C4)	56	Female	Caucasian