INVENTORY OF SUPPLEMENTAL INFORMATION

Modeling motor neuron resilience in ALS using stem cells

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The Supplemental Information includes Supplemental Figure legends, Supplemental Figures S1-3 and Supplemental Tables S1-3.

1. Figure S1 shows the isolation of oculomotor and spinal neurons using FACS and their further characterization, accompanying the data presented in Figure 1.

2. Figure S2 shows the differential expression of axon guidance genes in oculomotor and spinal motor neurons generated from stem cells, accompanying Figure 2.

3. Figure S3 shows the preferential expression of Calcium-regulating genes in oculomotor neurons, accompanying Figure 3.

4. Supplemental Table S1 (Excel) contains the DESeq analysis of the RNA sequencing from *in vitro* generated oculomotor neurons and spinal motor neurons, accompanying data presented in Figure 1.

5. Supplemental Table S2 (Excel) contains the PAGODA analysis of the RNA sequencing data of *in vitro* generated oculomotor neurons and spinal motor neurons, accompanying data presented in Figure 1.

6. Supplemental Table S3 (Word) contains information on the characteristics of non-demented clinical cases used for LCM-seq RNA sequencing of spinal and culomotor neurons from *post mortem* tissues.

SUPPLEMENTAL INFORMATION:

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Supplemental Figure S1. Generation of oculomotor neurons and spinal motor neurons from stem cells and their characterization. (a) FACS reanalysis plots of Hb9-GFP and (c) Islet1-GFP/NesEPhox2A cells sorted based on GFP expression demonstrated an enrichment of motor neurons (MNs) (Hb9-GFP 85%, Islet-GFP/NesEPhox2A 96% in P3 plots). (b) FAC sorted spinal motor neurons (SC MNs) express Islet1 and Hb9, while oculomotor neurons (OMNs) express Islet1, while lacking Hb9 (d) scale bar = 60 μm. (e) Graph showing mean number of detected genes (and RPKM > 1) (mean ± SEM, t test for RPKM > 0.1 P=0.1196, t test for RPKM > 1 P=0.4047) after mRNA-seq analysis performed on EB dissociation day following FACS of Hb9GFP+ (SC MNs) and Islet1GFP/NesEPhox2A+ (OMNs) cells. Both conditions show similar amounts of expressed genes. (f) Heatmap showing the differential Hox gene expression between OMNs and SC MNs, confirming their positional identity. (g) The top 100 DEGs obtained from our RNAseq analysis separate a previously published microarray dataset generated from stem cell derived-brain stem and spinal motor neurons. (h) Graph indicated percentage of BrdU+ cells over Islet1+ cells at D7 assay. BrdU pulses were performed at D0, D2 and D3 survival assay quantifications were performed at D7. (mean ± SEM, 2way ANOVA and Tukey's multiple comparison test, $F(2, 169)=0.3278$, $P=0.7209$, n=3). 10 random areas were quantified per each time point per experiment (in duplicates), with at least 100 cells quantified per condition.

Supplemental Figure S2. Differential expression of axon guidance genes in oculomotor and spinal motor neurons. (a) DESEQ analysis performed on transcripts obtained from generated oculomotor neurons (OMNs) and spinal motor neurons (SC MNs) reveal significant differences in genes controlling axon guidance, Log_{10}-transformed data for *Sema6d*, *Plxa4*, *Cdh6* and *Cdh12*, preferentially expressed in OMNs, and *Sema4a*, *Sema5b*, *Epha3* and *Ephx4* preferentially expressed in SC MNs (adjusted *P < 0.05). (b) Preferential expression of *SEMA6D* and *PLXNA4* was found in human OMNs isolated by LCM-seq when compared with human SC MNs. Graph shows Log_{10}-transformed data for differentially expressed transcripts (adjusted *P < 0.05).

Supplemental Figure S3. Preferential expression of Ca-regulating genes in oculomotor neurons. (a) After DESEQ analysis of generated oculomotor neuron (OMN) and spinal motor neuron (SC MNs) mRNA expression, several transcripts of Ca^{2+} binding proteins could be found differentially expressed. Graph shows four of them (*Cald1*, *Esyt1*, *Camk2a*, *Hpcal1*) with preferential expression in OMNs suggesting increased capability of intracellular Ca^{2+} buffering during excitotoxicity (adjusted *P<0.05, log_{10}-transformed RPKM values). (b-c) ESYT1 immunohistochemistry performed on OMNs and SC MNs at D7 toxicity assay revealed preferential expression in OMNs at protein level. Scale bar = 60 μm. (d) Semi-quantification of ESYT1 staining in control and KA20 conditions (mean ± SEM, 2way ANOVA and Tukey's multiple comparison test, F(1, 239)=31,84, *** P < 0.0001, n=3). Experiments were performed with technical replicates and with at least 100 motor neurons counted per condition.

Supplemental Table S3. **Characteristics of non-demented clinical cases used for LCM-seq**

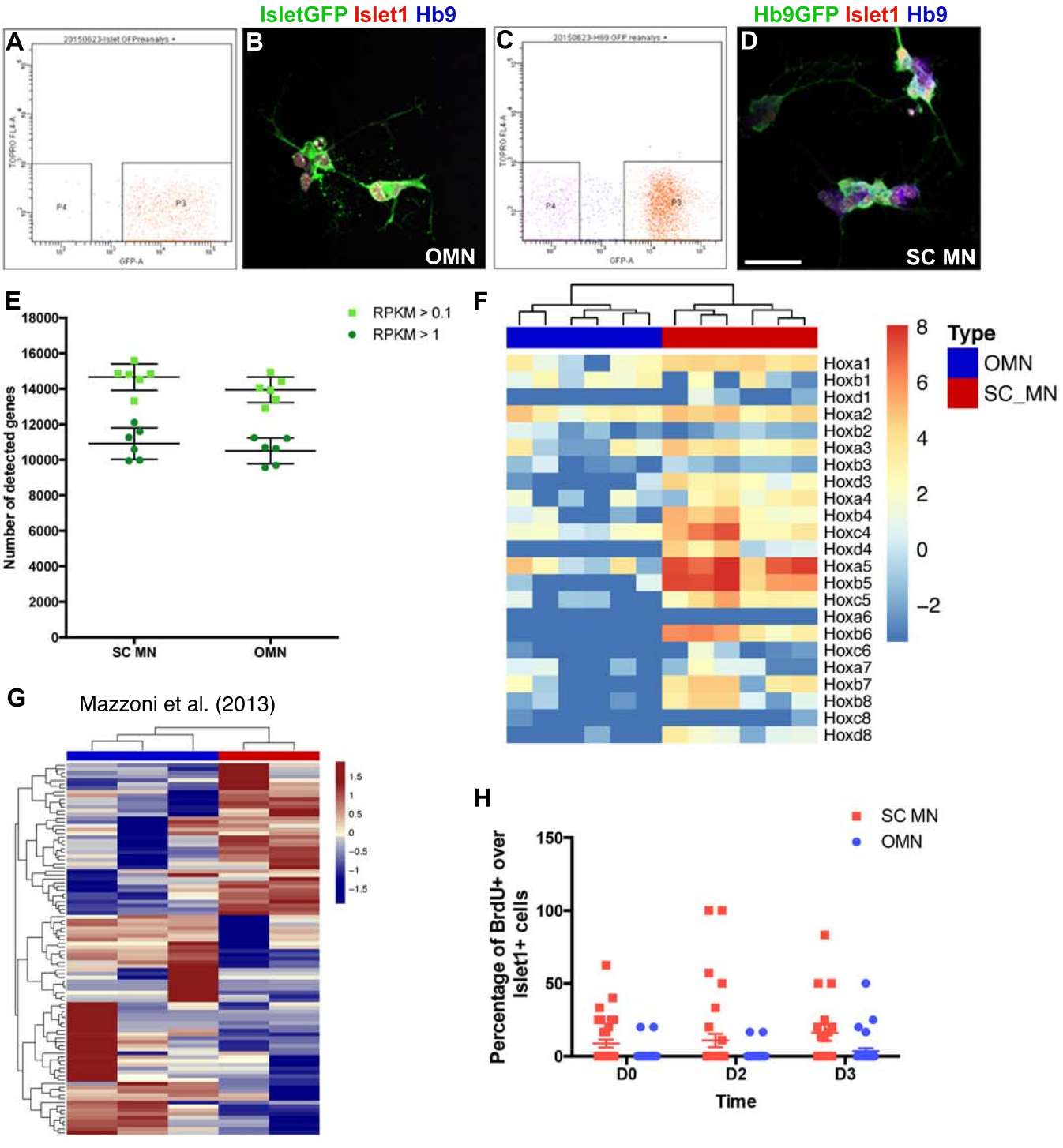
Case number	Sex	Age at death	Cause of death	Postmortem delay time	Source	Published GEO accession*
1	F	47	ND (mammal carcinoma)	4:00	NBB	
2	M	51	ND (suicide)	7:45	NBB	
3	M	63	ND (respiratory arrest)	2:10	NDRI	
4	F	68	ND (obstructive pulmonary disorder)	3:00	NDRI	GSM2027419
5	F	71	ND (renal insufficiency)	7:10	NBB	GSM2027414
6	F	72	ND (metastasized bile duct cancer)	7:30	NDRI	GSM2027415 GSM2027417
7	F	79	ND (respiratory arrest)	13:20	NDRI	
8	M	79	ND (bronchopneumonia and sepsis)	7:40	NBB	
9	M	82	ND (pleuritic carcinomatosis)	10:00	NBB	
10	F	88	ND (metastasized lung cancer)	10:00	NDRI	GSM2027416 GSM2027418
11	M	88	ND (metastasized prostate cancer)	7:25	NBB	
12	M	98	ND (cardiac tamponade)	8:40	NBB	

ND – non-demented

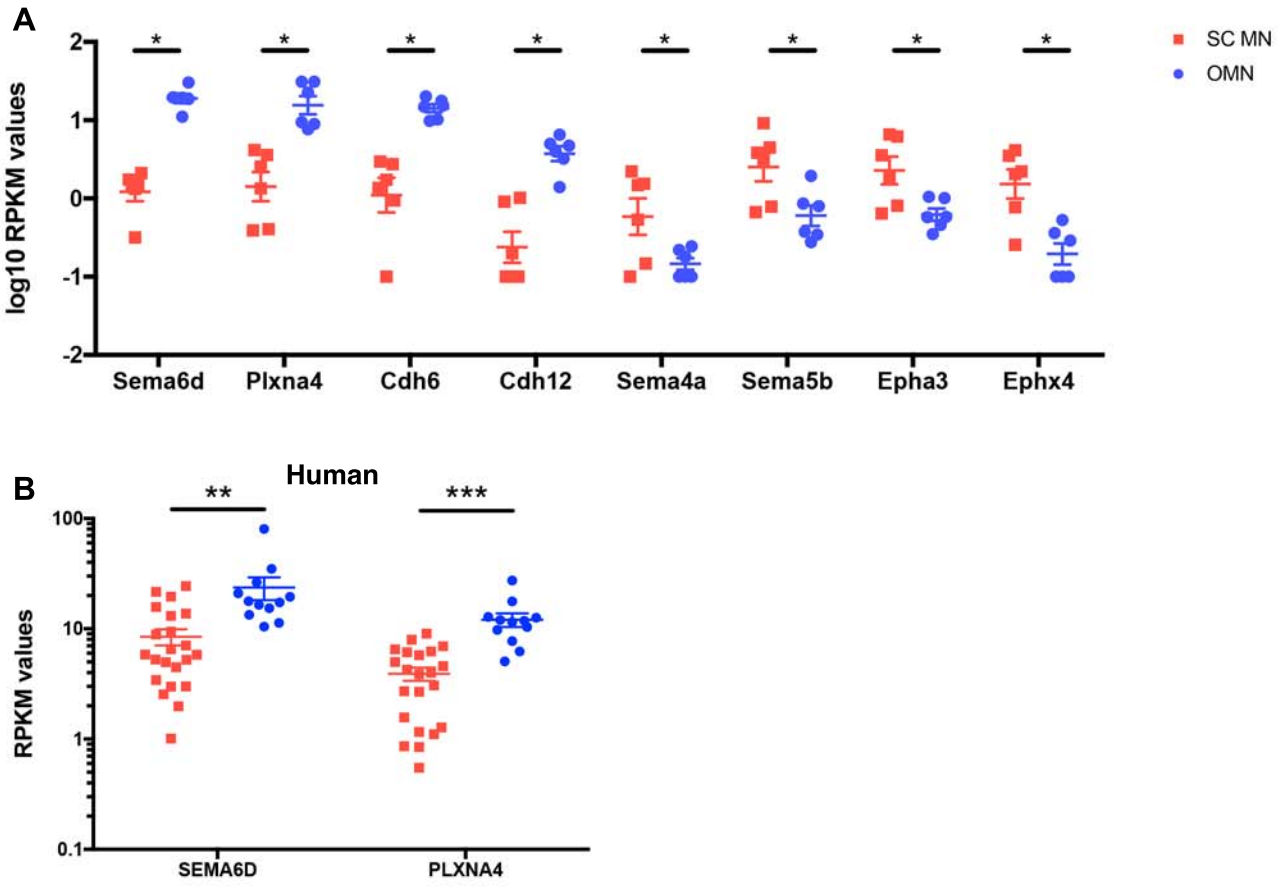
NBB - Netherland's Brain Bank (http://www.brainbank.nl)

NDRI - National Disease Research Interchange (http://www.ndriresource.org/)

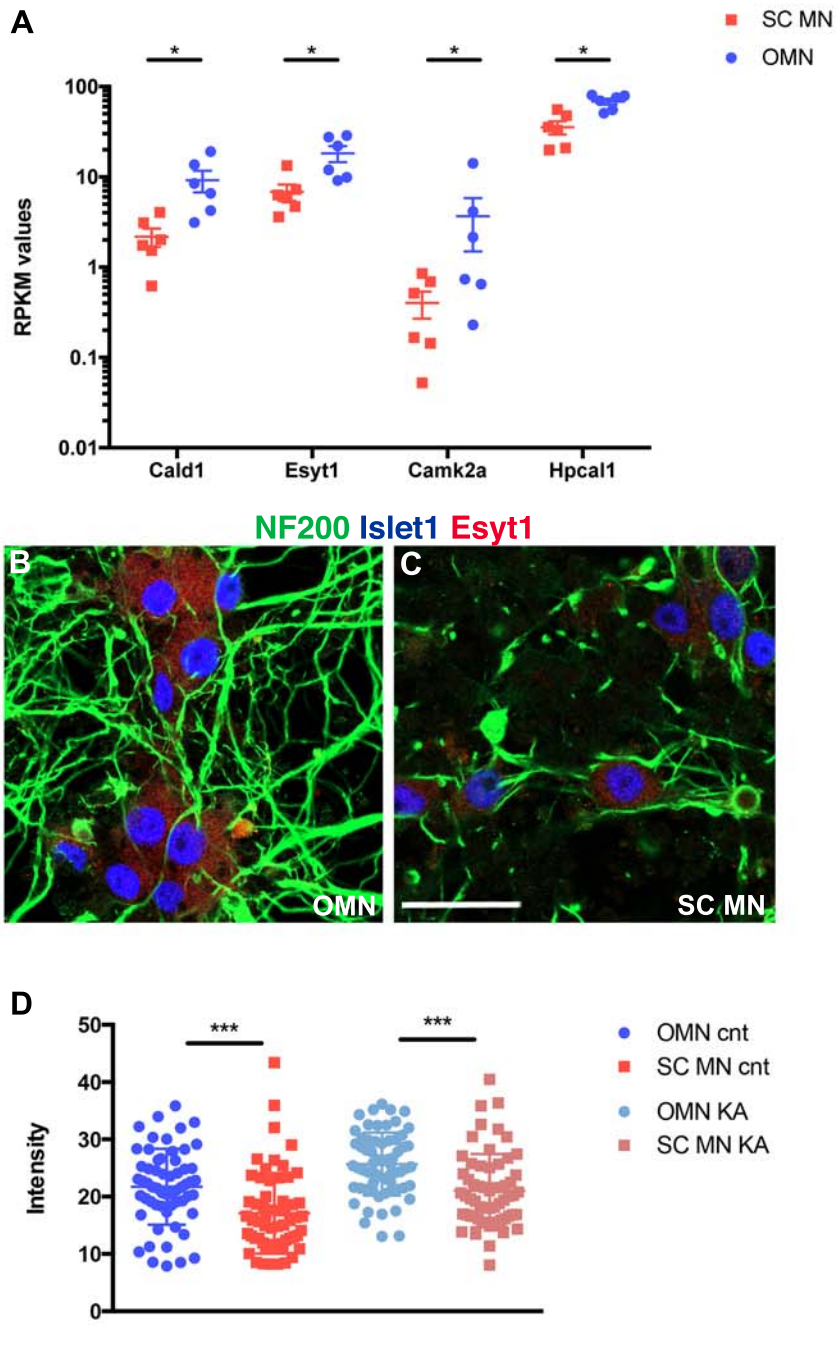
* Additional samples included for the bioinformatics analysis



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3