

SUPPLEMENTAL DATA

Mutations in *DARS2* result in global dysregulation of mRNA metabolism and splicing

Guang S^{1,2}, O'Brien BM¹, Fine AS³, Ying, M^{2,4}, Fatemi A^{1,2}, Nemeth CL^{1,2*}

¹Moser Center for Leukodystrophies at Kennedy Krieger, Kennedy Krieger Institute, Baltimore, MD, USA;

²Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA;

³Department of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, MD, USA

⁴Department of Neurology, Kennedy Krieger Institute, Baltimore, MD, USA

*Corresponding Author:

Christina Nemeth Mertz, PhD
Kennedy Krieger Institute
707 N Broadway
Baltimore, MD 21205
667-205-4288 (p)
443-923-2695 (f)
Mertz@kennedykrieger.org

SUPPLEMENTAL DATA

Supplemental Table 1. Primers used for DARS2 exon 3 inclusion/exclusion

Experiments	Primers	Primer sequence (5'-3')	Primer length (bp)
PCR	E2A-fwd	GTGGAGAGTTGCGTTCGTCT	20
	E4-rev	AGGACGGGAAATGACTGTACC	21
RT-qPCR (Transcripts with exon 3)	E2A-fwd	GTGGAGAGTTGCGTTCGTCT	20
	E3_4-rev	TGAACAAGCCCATCGAAATCTC	22
RT-qPCR (Transcripts without exon 3)	E2B-fwd	CCAACACATGTGGAGAGTTGC	21
	E2_4-rev	TTCAGTACCGAAGTCGGCAG	20
	PPIA-fwd	GTCAACCCACCGTGTCT	19
RT-qPCR (Housekeeping genes)	PPIA-rev	CTGCAAACAGCTCAAAGGAGAC	22
	PSMB4-fwd	CGTCCACTCCCGATTCTTC	20
	PSMB4-rev	AATGCGAGAGATGTTGCGGA	20

Supplemental Table 2. Top 20 significant DTU genes in two groups of LBSL cells

Gene	# Transcripts Detected	# Sig Transcripts	Gene Overall FDR	Min Transcript OFDR	Max DTU Difference	Chr	% Cells Expressing
MIS LBSL cells VS. Control cells							
<i>SLIRP</i>	9	2	1.85E-78	1.58E-86	0.173	14	0.973
<i>TPD52L1</i>	12	3	1.16E-67	1.67E-57	0.085	6	0.905
<i>MORF4L1</i>	11	3	3.51E-57	1.16E-20	-0.158	15	0.991
<i>TRIM22</i>	8	3	6.66E-50	2.10E-38	-0.464	11	0.606
<i>STMN1</i>	5	3	8.92E-47	2.61E-50	0.241	1	0.943
<i>SEPTIN7</i>	10	3	3.73E-45	2.63E-18	0.084	7	0.900
<i>GPI</i>	10	4	9.21E-42	5.47E-11	-0.066	19	0.896
<i>RAB1A</i>	4	3	1.07E-40	1.26E-39	0.166	2	0.953
<i>RBIS</i>	12	3	7.53E-39	1.36E-32	0.196	8	0.764
<i>WSB1</i>	12	1	3.31E-38	3.02E-23	-0.159	17	0.975
<i>ZFYVE16</i>	13	3	3.39E-37	9.17E-19	-0.126	5	0.912
<i>CALCOCO2</i>	19	1	1.44E-35	2.78E-20	0.094	17	0.712
<i>AKAP8L</i>	20	3	1.54E-34	8.89E-06	0.092	19	0.667
<i>IFITM2</i>	10	3	2.34E-34	5.73E-17	-0.198	11	0.873
<i>SNRPN</i>	9	2	1.71E-32	1.73E-22	-0.101	15	0.971
<i>GLUL</i>	7	2	7.12E-32	1.22E-27	0.127	1	0.912
<i>SNX3</i>	4	2	1.97E-31	3.94E-23	-0.150	6	0.948
<i>MYO10</i>	12	4	2.01E-30	9.14E-09	0.100	5	0.943
<i>CLU</i>	9	1	1.41E-29	2.33E-17	-0.050	8	0.973
<i>JTB</i>	5	4	1.69E-29	4.67E-23	-0.197	1	0.950
SPLICE LBSL cells VS. Control cells							
<i>MAGED2</i>	8	2	1.07E-116	1.35E-121	0.288	X	0.894
<i>IFITM2</i>	10	6	4.47E-91	3.55E-43	-0.3296	11	0.826
<i>SULF1</i>	16	4	1.82E-68	6.92E-22	0.187	8	0.807
<i>SCOC</i>	4	2	3.90E-62	7.21E-64	0.196	4	0.853
<i>GAPDH</i>	2	2	5.25E-60	0	-0.114	12	0.995
<i>GNAS</i>	21	2	1.41E-56	3.17E-08	0.239	20	0.909

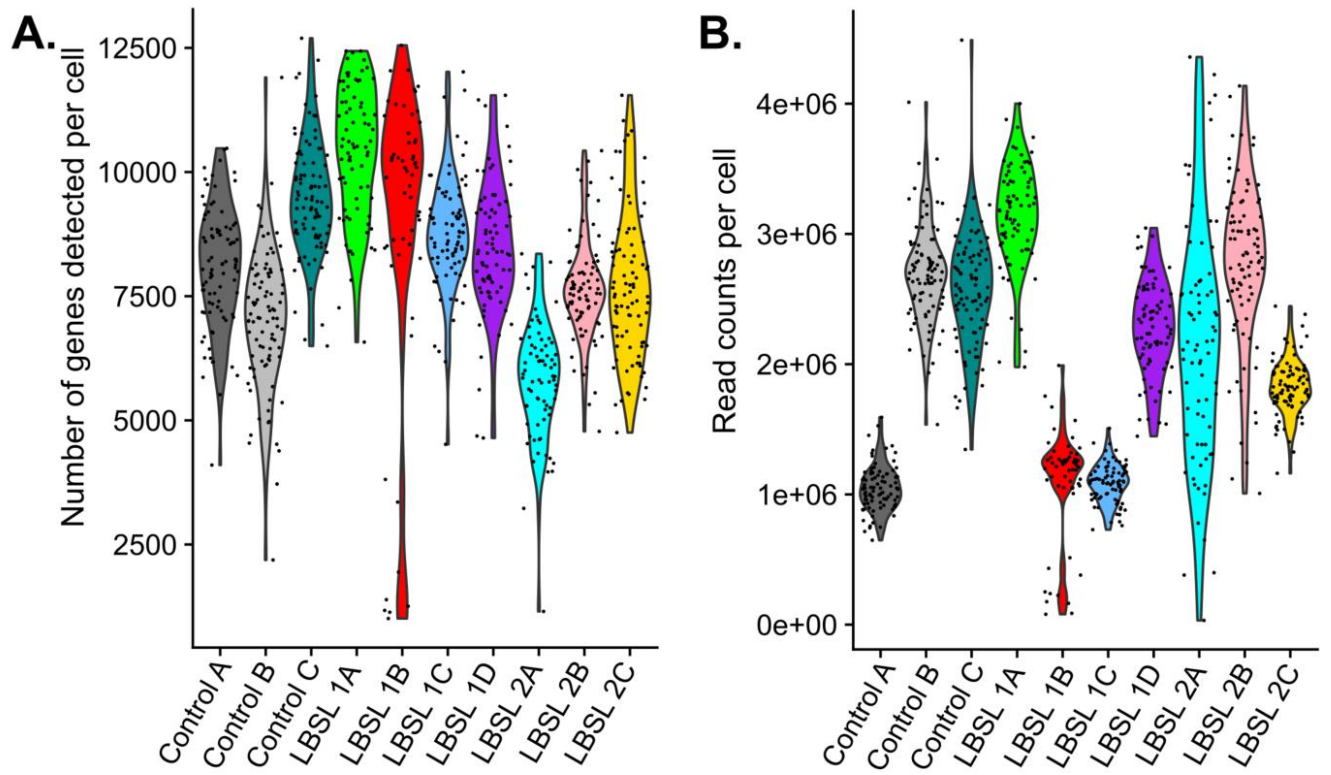
SUPPLEMENTAL DATA

<i>RPL29</i>	4	2	1.04E-54	1.24E-53	0.243	3	0.977
<i>ITM2B</i>	5	2	3.56E-54	2.05E-37	-0.129	13	0.985
<i>RPL9</i>	6	2	3.36E-53	6.84E-34	0.048	4	0.983
<i>GOLGA8B</i>	10	3	1.14E-49	5.84E-48	-0.202	15	0.683
<i>SF3B2</i>	110	4	1.88E-46	1.64E-28	0.317	11	0.844
<i>YWHAZ</i>	15	2	3.18E-44	2.47E-23	0.179	8	0.878
<i>CALM2</i>	15	2	9.97E-42	8.27E-22	0.150	2	0.990
<i>HNRNPC</i>	13	3	7.35E-41	7.59E-15	-0.093	14	0.948
<i>NDUFB5</i>	11	1	7.02E-39	1.42E-37	0.076	3	0.929
<i>RPL17- C18orf32</i>	3	3	2.54E-38	8.16E-39	0.194	18	0.932
<i>EIF4G2</i>	12	2	7.91E-37	1.91E-24	0.165	11	0.954
<i>CKB</i>	7	3	5.21E-36	3.81E-25	-0.266	14	0.905
<i>RPL10</i>	7	2	9.59E-36	1.13E-41	0.188	X	0.958
<i>CYCS</i>	4	2	2.15E-33	3.90E-36	-0.153	7	0.960

SUPPLEMENTAL DATA

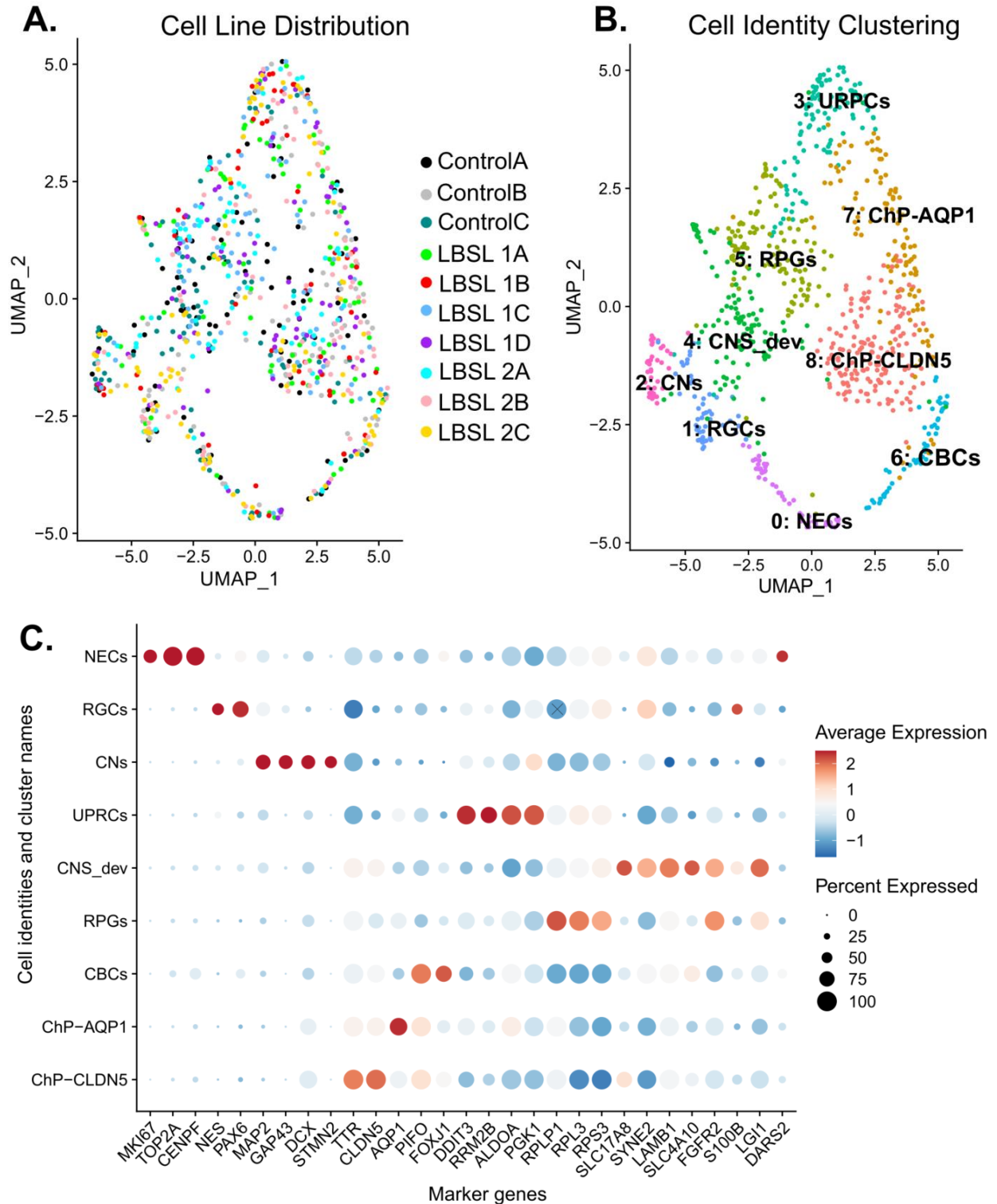
Supplemental Table 3. LBSL Associated Differentially Spliced Exons

DSE genes	Chr	5' adjacent exon	DSE exon	3' adjacent exon	cell_coeff	FDR
<i>LGMN.AS2</i>	14	92703807-92704361	92704640-92704707	92709672-92709872	8.724	3.34E-43
<i>PDCD4</i>	10	110871795-110872018	110875966-110876070	110881233-110881535	-3.554	4.97E-15
<i>TARDBP.AS</i>	1	11013716-11013965	11016844-11017007	11018733-11018873	4.871	1.79E-12
<i>UBE2VI</i>	20	50081124-50082914	50084129-50084254	50096672-50096820	-3.844	6.88E-12
<i>KARSI</i>	16	75635912-75636098	75636454-75636547	75641564-75641723	3.688	1.29E-10
<i>NDUFS7</i>	19	1383900-1383942	1386518-1386639	1387811-1387847	-5.339	8.41E-09
<i>NASP.AS2</i>	1	45591223-45591270	45606482-45606591	45613169-45613248	2.526	1.41E-07
<i>MIA2</i>	14	39319209-39319291	39320928-39321056	39326864-39327022	-3.392	2.32E-07
<i>HAGH</i>	16	1817168-1817271	1819115-1819223	1819897-1820014	-2.416	5.29E-07
<i>RP11-</i>	15	68197440-68197628	68199541-68199681	68205121-68205151	-3.22	5.31E-07
<i>MIF4GD</i>	17	75267538-75267630	75268083-75268192	75270114-75270245	-3.45	7.73E-07
<i>FNTA</i>	8	43056308-43056546	43064101-43064215	43069555-43069659	-3.053	2.52E-06
<i>SRP72</i>	4	56478592-56478649	56483139-56483270	56484736-56484864	2.571	3.40E-06
<i>GALNT10</i>	5	154329572-154329738	154376277-154376462	154380448-154380631	6.409	4.22E-06
<i>COX7B</i>	X	77899440-77899593	77901744-77901867	77902643-77902767	1.636	6.91E-06
<i>PIFO</i>	1	111346910-111347049	111347674-111347772	111348516-111348646	-2.052	7.15E-06
<i>DARS2.AS2</i>	1	173826687-173826786	173828333-173828399	173830660-173830761	-6.781	7.99E-06
<i>NUCB2.AS2</i>	11	17312028-17312120	17315386-17315475	17330127-17330297	-2.757	2.63E-05
<i>EXOC5</i>	14	57239595-57239659	57244165-57244359	57246711-57246858	-4.015	6.46E-05
<i>CLYBL</i>	13	99858861-99859049	99862991-99863092	99864818-99864911	-4.974	9.54E-05



Supplemental Figure 1. Violin plots after quality control. (A) Number of genes detected per cell (> 1000) for each cell line. (B) Read counts per cell indicated by cell line.

SUPPLEMENTAL DATA

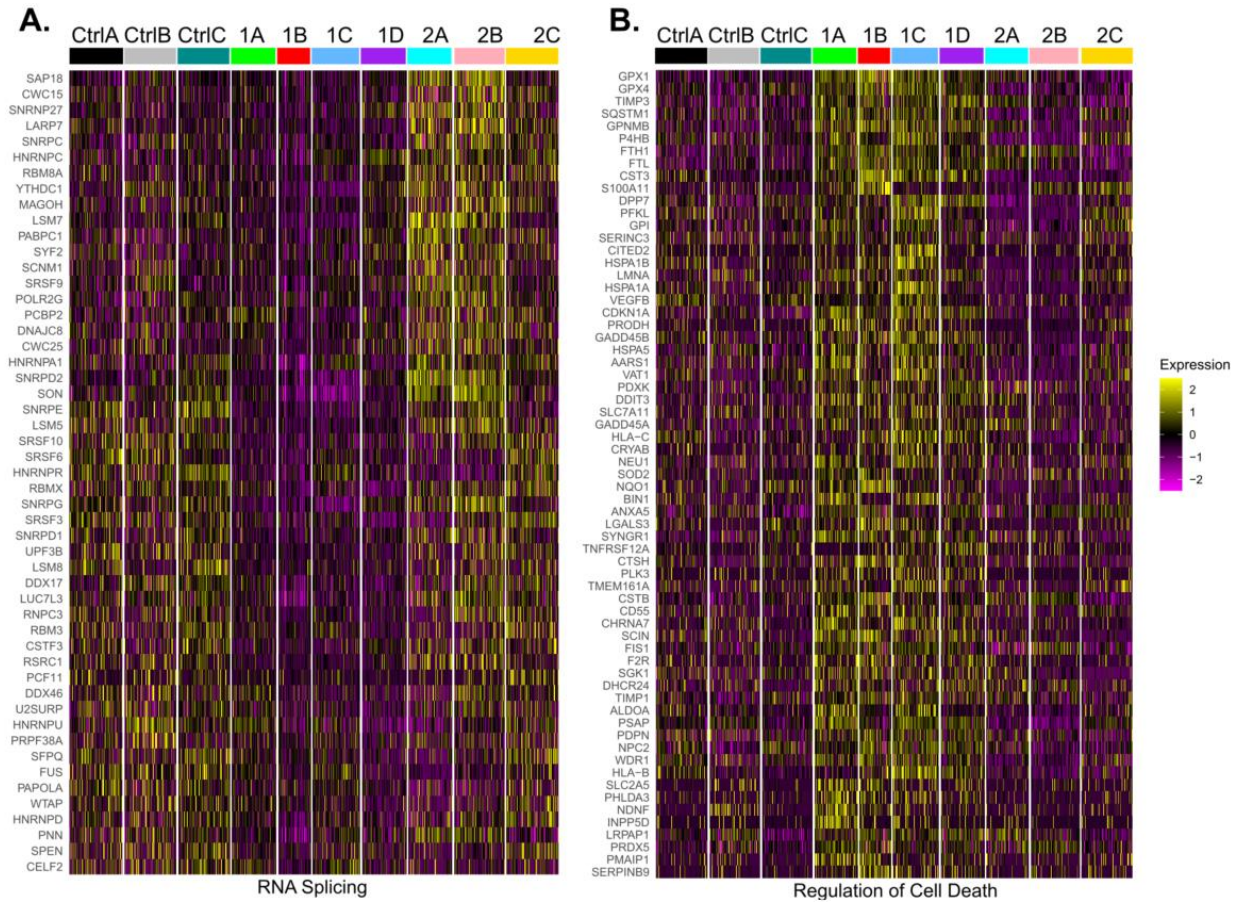


Supplemental Figure 2. Unsupervised clustering and dimension reduction by Uniform Manifold Approximation and Projection (UMAP). (A) Cells from different cell lines were intermixed without an obvious batch effect. (B) Nine transcriptionally distinct clusters were detected. (C)

SUPPLEMENTAL DATA

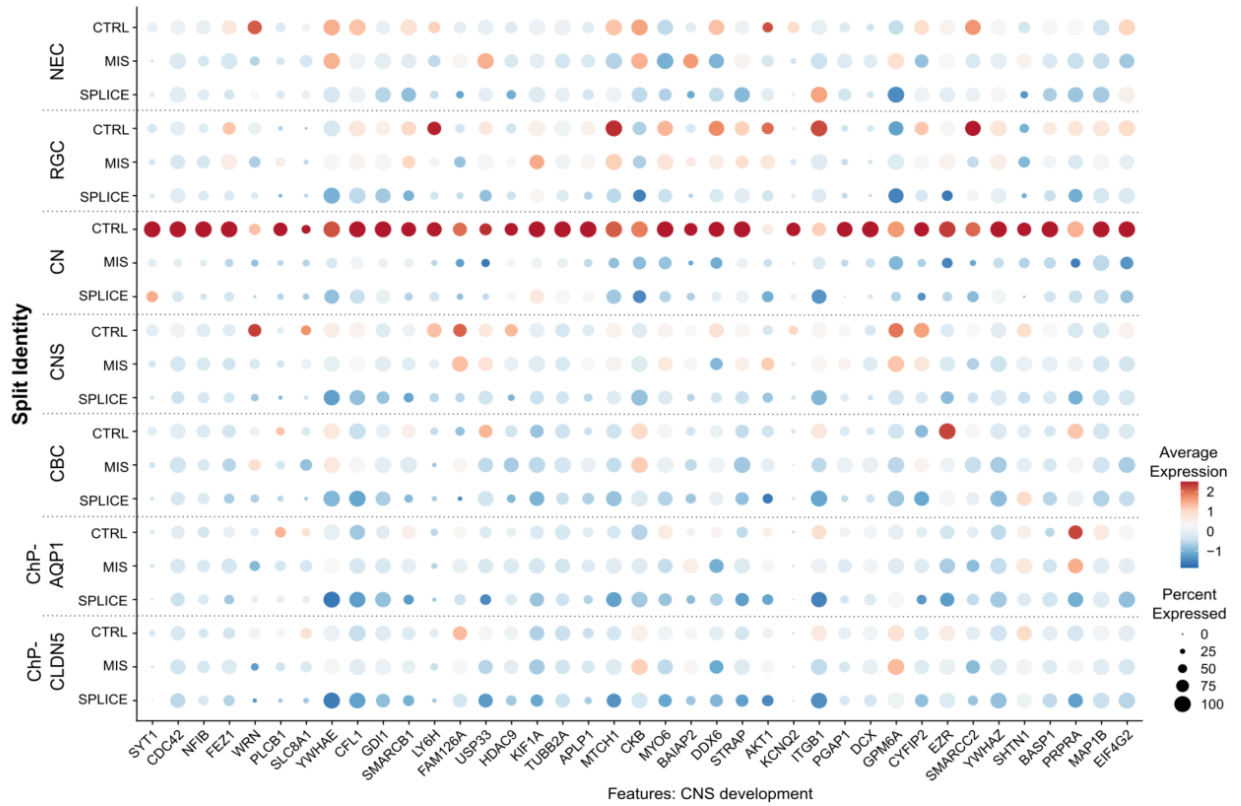
Dot plot of marker genes to identify each cell cluster. NECs: neuroepithelial cells; RGCs: radial glial cells; CNs: cortical neurons; UPRCs: unfolded protein response-related cells; CNS_dev: cells related to the development of CNS; RPGs: cells highly expressing ribosomal protein genes; CBCs: cilium bearing cells; ChP: choroid plexus.

SUPPLEMENTAL DATA



Supplemental Figure 3. Heatmaps of DEGs under the GO terms “RNA splicing” (A) and “Regulation of cell death” (B).

SUPPLEMENTAL DATA



Supplemental Figure 4. Dot plot of DEGs related to CNS development and neuronal differentiation.



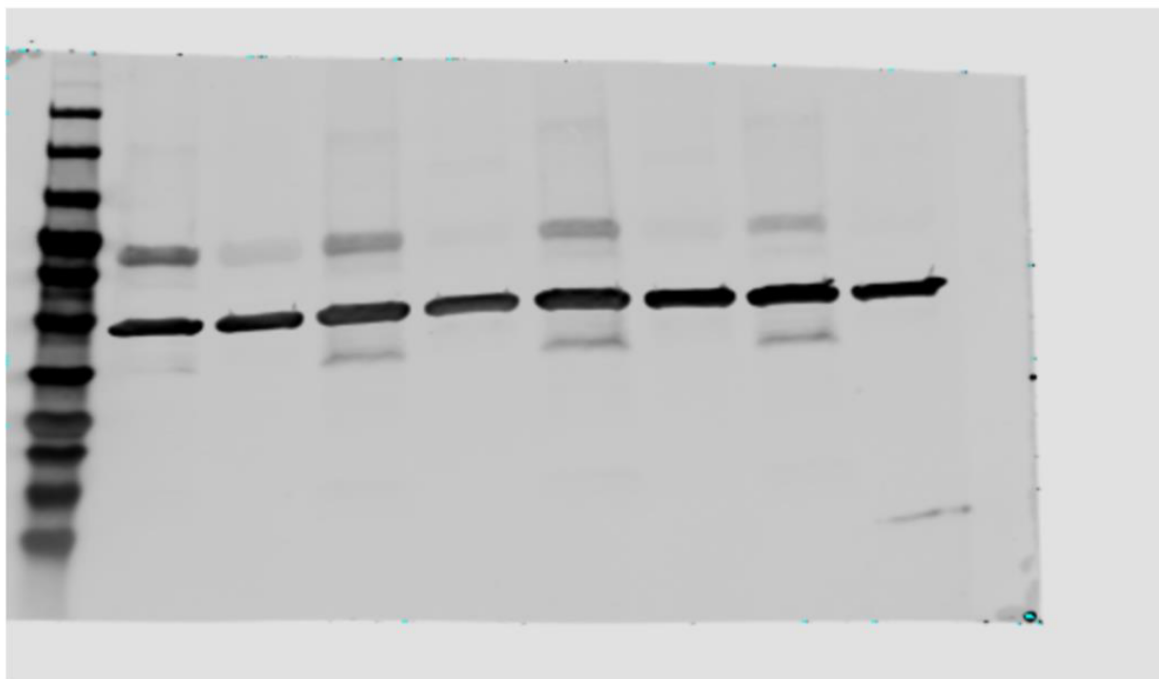
Image ID: 0004804_02
 Acquire Time: Mar 5, 2020 3:25:19 PM

Acquisition Information

#	Image ID	Acquire Time	Channels	Resolution	Intensities	Quality	Analysis	Image Name	Comment
1	0004804_02	Mar 5, 2020 3:25:19 PM	700 800	169um	3.0 3.0	medium	Manual	0004804_02	

Image Display Values

Channel	Color	Minimum	Maximum	K
700	Gray Scale (Black on White)	5.26	6480	0
800	Gray Scale (Black on White)	0.437	6700	0



Supplemental Figure 5. LI-COR report of raw western blot data. All samples were run and analyzed from a single gel.