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Supplemental information

iPSC motor neurons, but not other derived cell types, capture gene expression changes in postmortem sporadic ALS motor neurons

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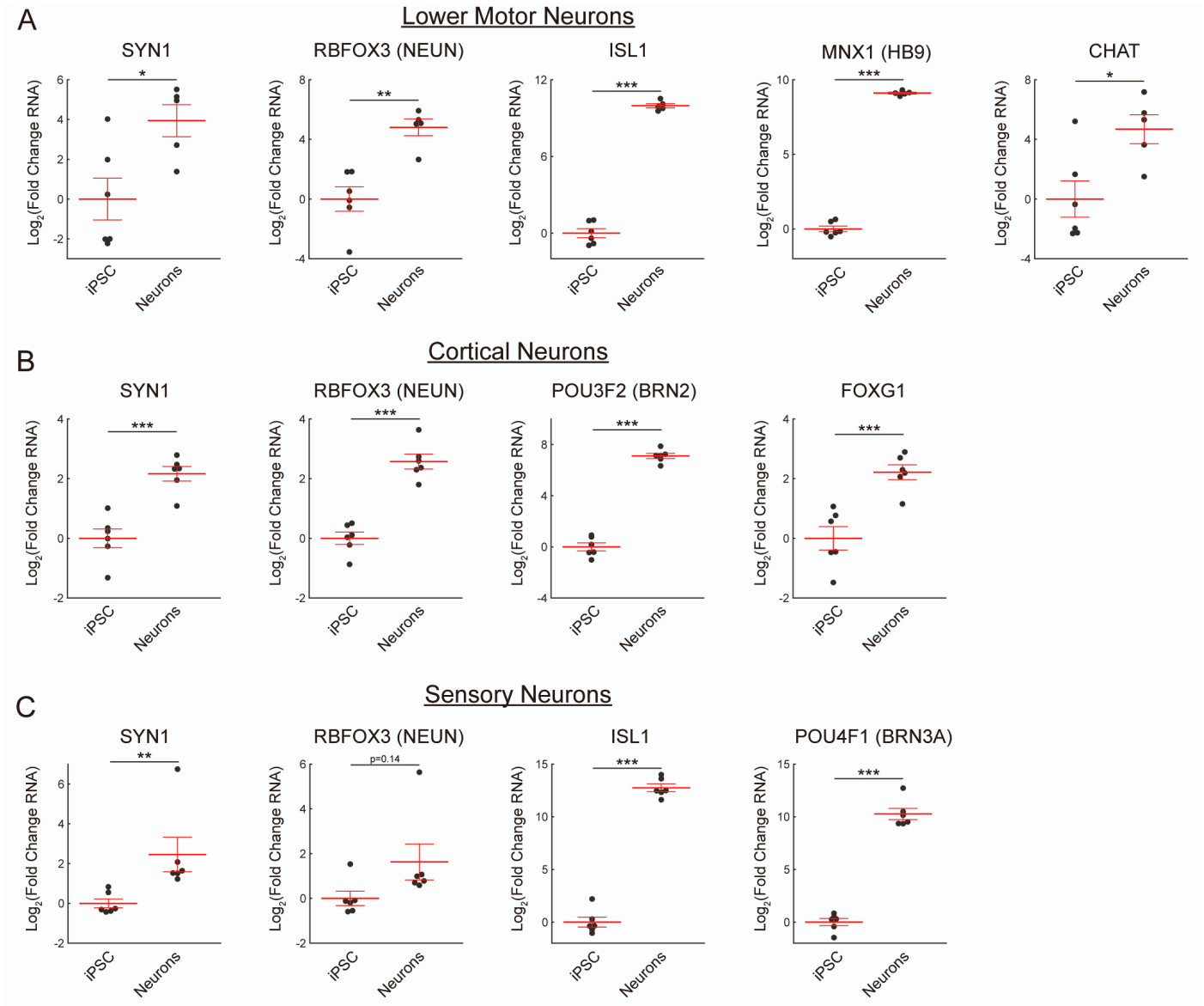

Figure S1. qRT-PCR analysis for LMN, cortical neuron, and sensory neuron markers. (A) LMN markers in LMNs, (B) cortical neuron markers in NGN2 neurons, and (C) sensory neuron markers in sensory neurons; normalized to *GAPDH* and undifferentiated iPSCs containing the differentiation construct; n=6 wells for all groups. Two-sided t-tests were used to compare between groups. ***, **, and * represent $p<0.001$, $p<0.01$, and $p<0.05$, respectively.

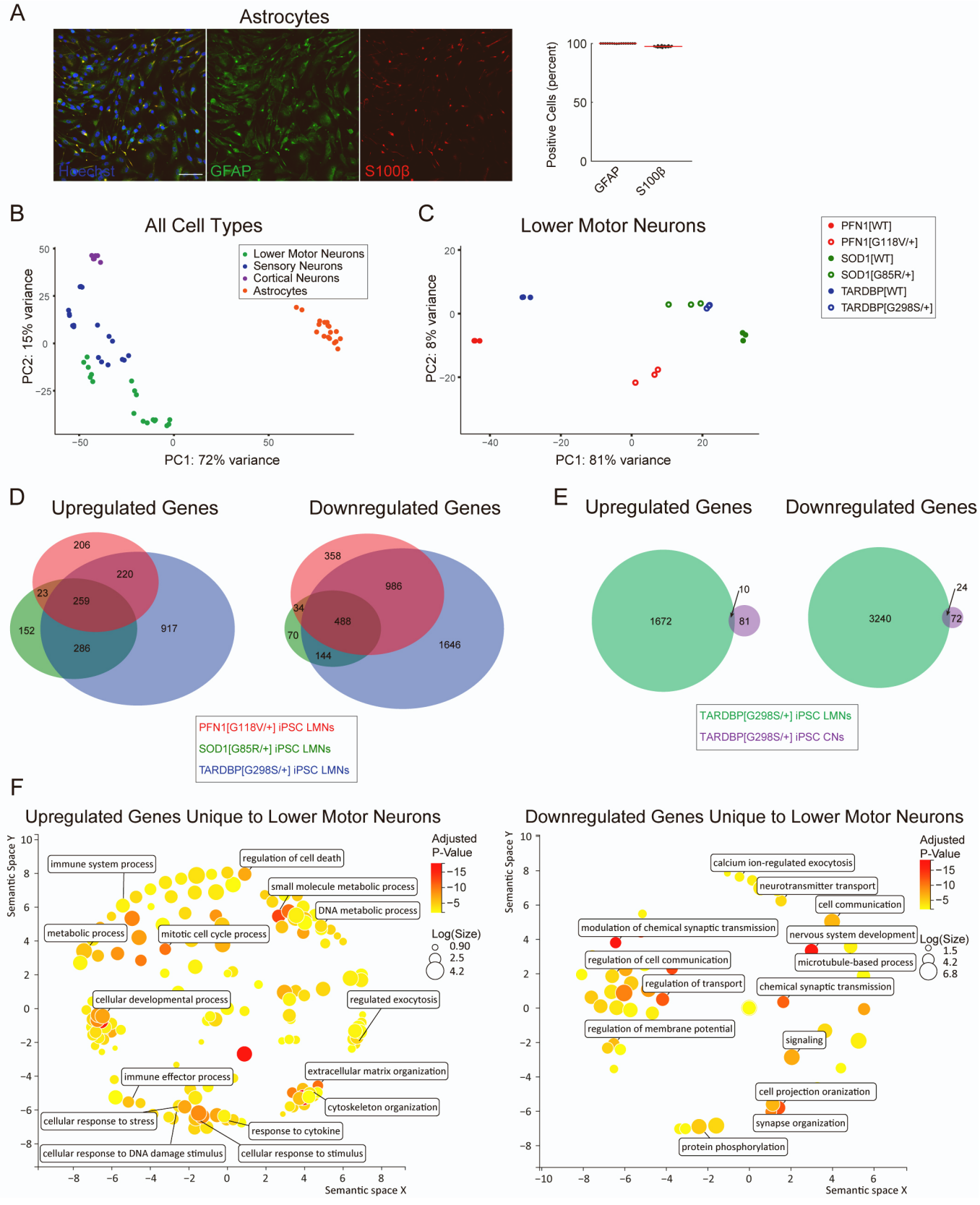

Figure S2. Additional RNA-seq information. (A) Differentiation of astrocytes and staining for astrocyte markers in the parental line used for iPSC editing (scale bar = 100um; n=16 wells per group). (B) Principal component analysis of all RNA-seq libraries. (C) Principal component analysis of LMN RNA-seq libraries. (D) Venn diagram comparing differentially expressed genes in fALS iPSC LMNs vs all controls. (E) Venn diagram comparing differentially expressed genes in *TARDBP*$^{G298S/+}$ LMNs and cortical neurons. (F) Gene ontology analysis of upregulated and downregulated genes specific to LMNs.

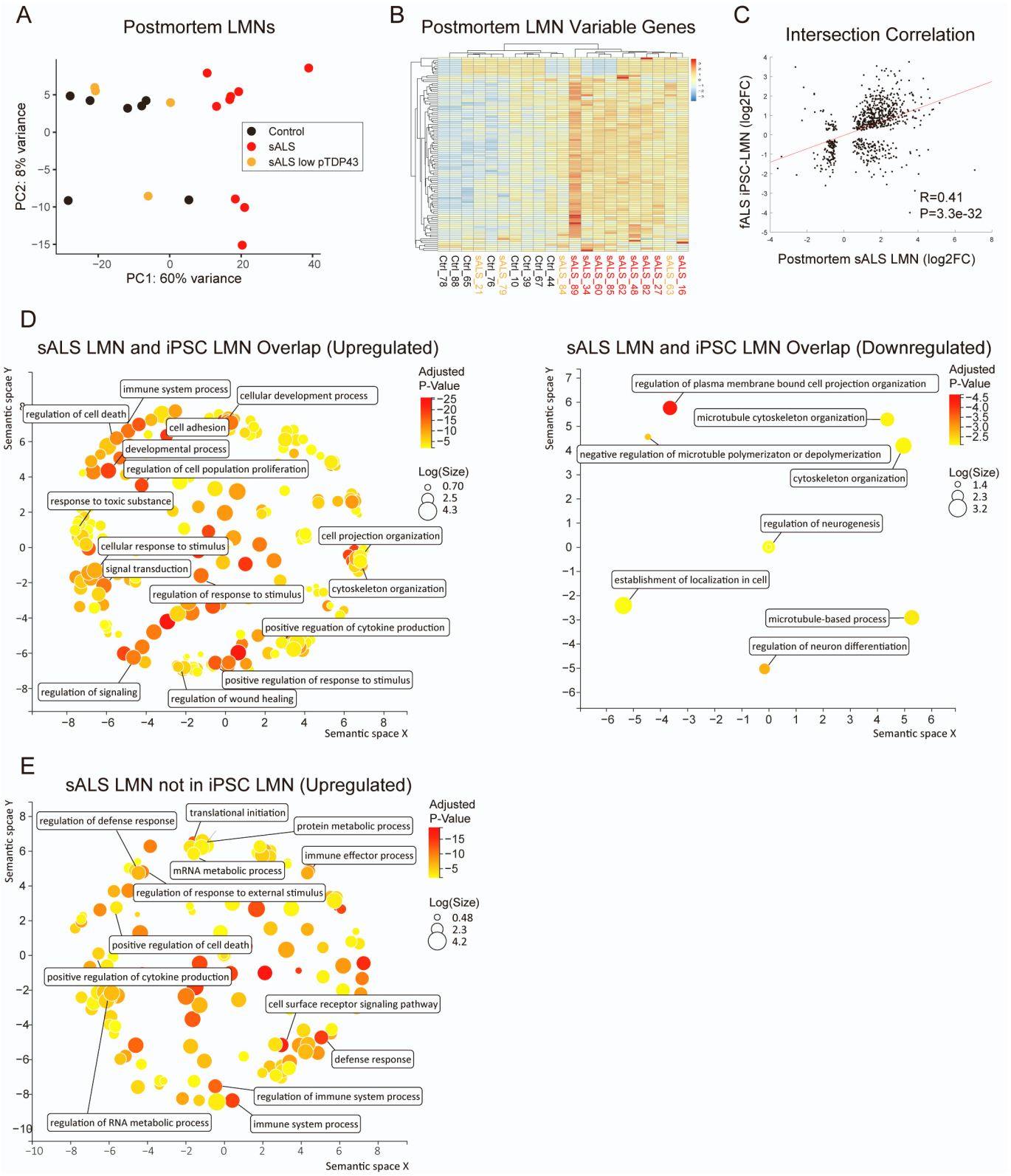

Figure S3. Comparison of differential gene expression in fALS iPSC LMNs (this study) to postmortem sALS LMNs[36]. (A) Principal component analysis of laser captured postmortem sALS LMNs. (B) Heatmap clustering of genes with the highest variance. (C) Correlation of differentially regulated genes in iPSC LMNs with postmortem sALS LMNs. (D) GO terms for postmortem sALS LMN gene expression changes that are also differentially regulated in iPSC LMNs. (E) GO terms for gene expression changes in postmortem sALS LMNs that are not differentially regulated in iPSC LMNs.

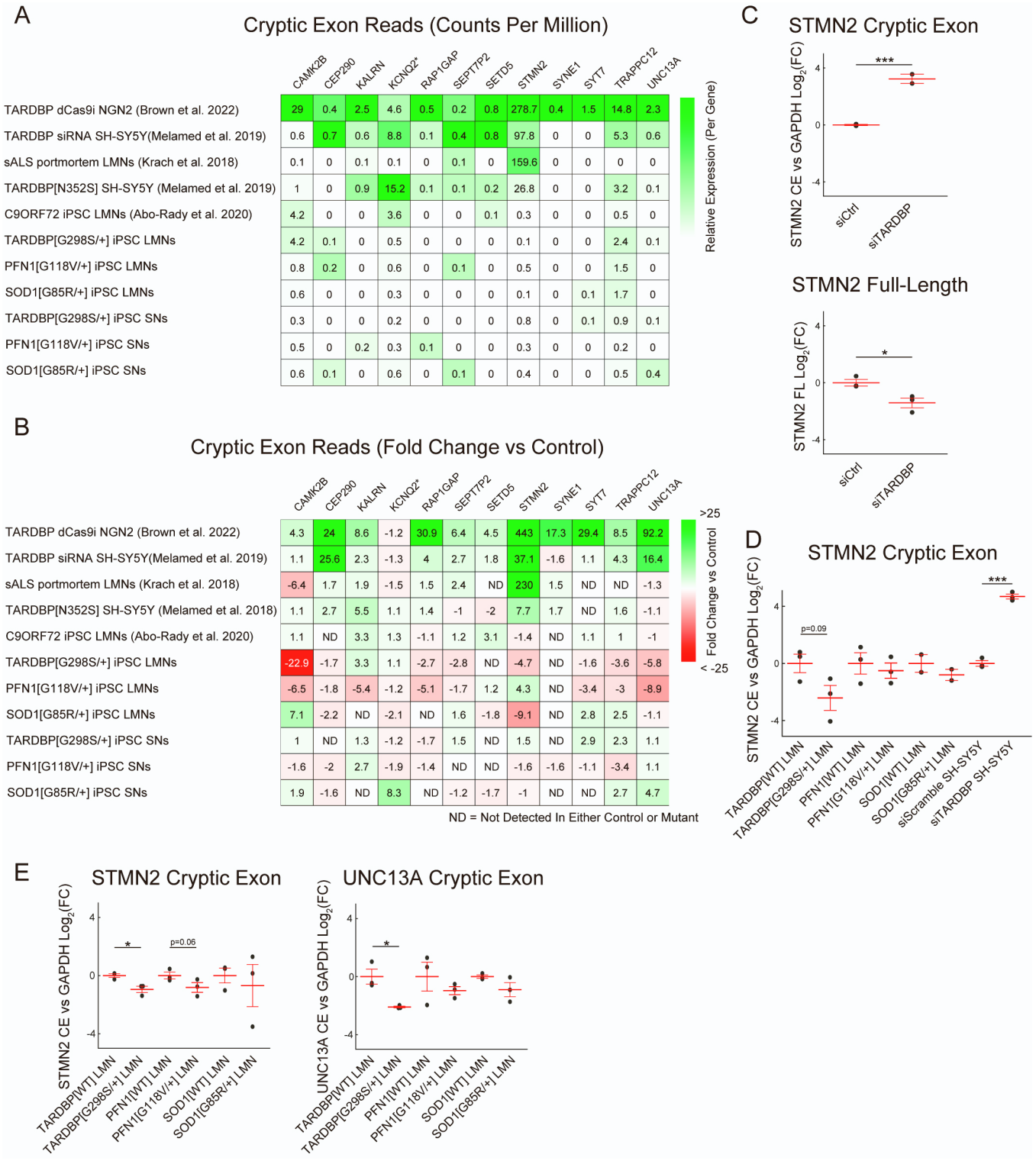

Figure S4. Legend on next page.

Figure S4. Cryptic exons have very low abundance in iPSC LMNs. (A) RNA-seq counts aligning to cryptic exons in genes with previously reported cryptic splicing. Previously published TDP-43 knockdown experiments have reads aligning to cryptic exons[10-13], but neither our iPSC-derived neurons nor another study of *C9ORF72* iPSC LMNs[41] have comparable numbers of reads aligning to cryptic exons despite similar sequencing depths. *KCNQ2* is marked with an asterisk (*) to denote that it is an exon skipping event instead of a cryptic exon. (B) The same analysis as in (A) but comparing fold change vs control. A pseudocount of 1 was used if reads were detected in only one group. (C) qRT-PCR of *STMN2* cryptic exon and full length when *TARDBP* is knocked down in SH-SY5Y cells, demonstrating fidelity of the primers; n=3 wells for all groups except the *siTARDBP* cryptic exon group where n=2. (D) qRT-PCR using hydrolysis probes for the *STMN2* cryptic exon in iPSC LMN samples and in SH-SY5Y cells with *TARDBP* knockdown; n=3 for all groups except the *SOD1* isogenic pair where n=2. (E) qRT-PCR for the *STMN2* and *UNC13A* cryptic exons normalized to *GAPDH*. Two-sided t-tests were used to compare between groups. *** and * represent $p<0.001$ and $p<0.05$, respectively.

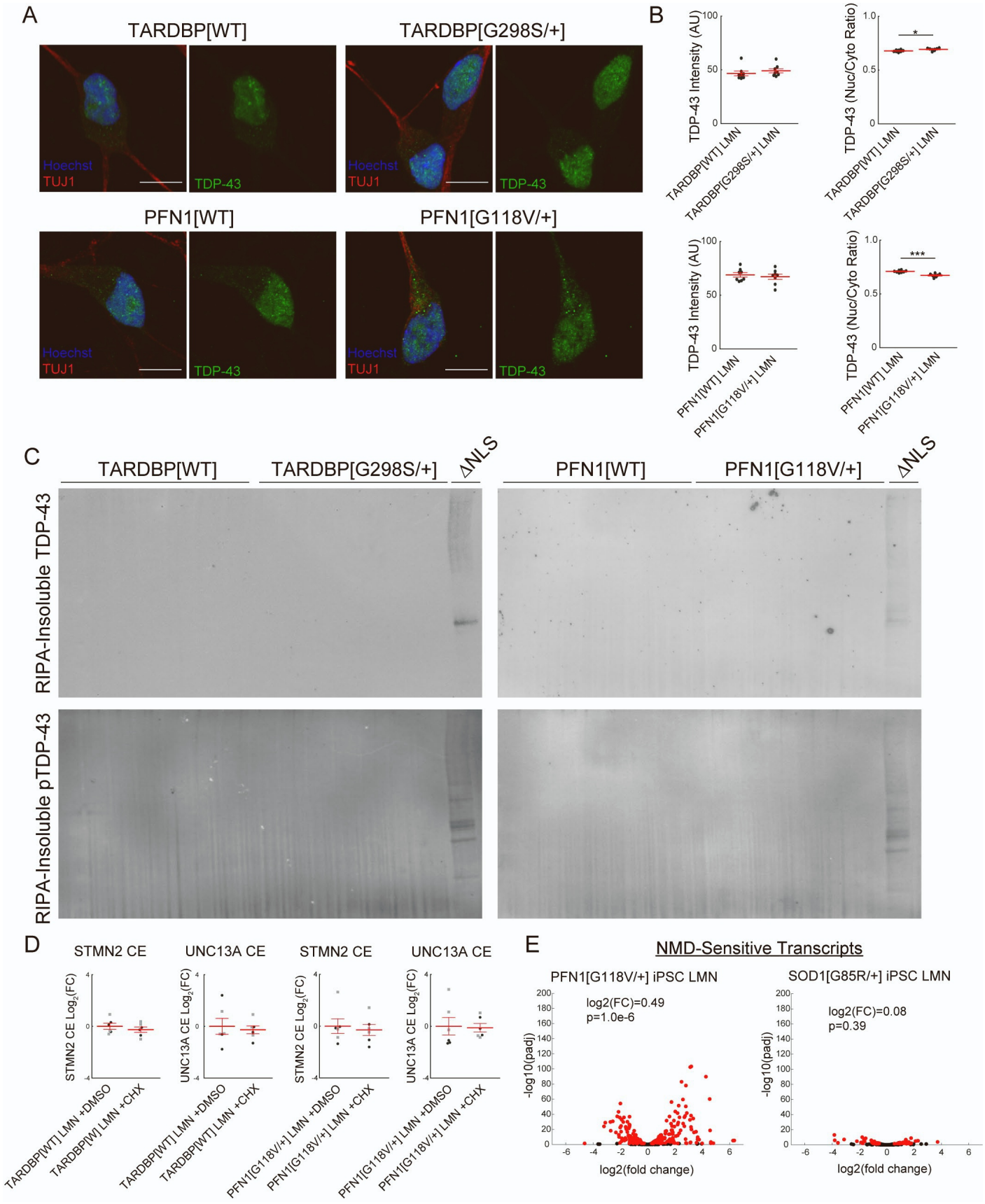

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Figure S5. Insoluble or phosphorylated TDP-43 are not detected in *TARDBP*$^{G298S/+}$ or *PFN1*$^{G118V/+}$ iPSC LMNs. (A) Immunofluorescence images of TDP-43 in *TARDBP*$^{G298S/+}$, *PFN1*$^{G118V/+}$, and isogenic control iPSC LMNs (scale bar = 10um); quantified in (B); n=8 wells per genotype. (C) Western blots of RIPA-insoluble fractions from *TARDBP*$^{G298S/+}$ LMNs, *PFN1*$^{G118V/+}$ LMNs, isogenic control iPSC LMNs, and hTDP-43ΔNLS mice[42]. (D) qRT-PCR for *STMN2* and *UNC13A* cryptic exons in *TARDBP*^{WT} and *PFN1*$^{G118V/+}$ LMNs treated with either CHX or DMSO. (E) Expression of NMD-sensitive transcripts in *PFN1*$^{G118V/+}$ and *SOD1*$^{G85R/+}$ iPSC LMNs compared to their isogenic controls. Two-sided t-tests or GSEA were used to compare between groups. *** and * represent $p<0.001$ and $p<0.05$, respectively.