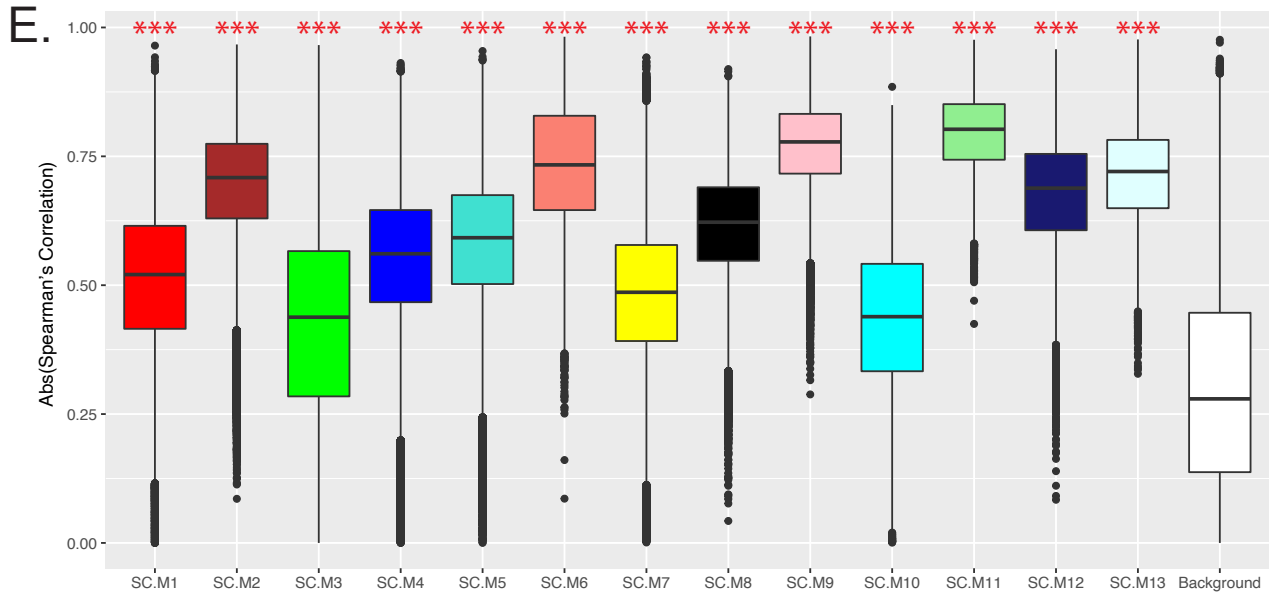
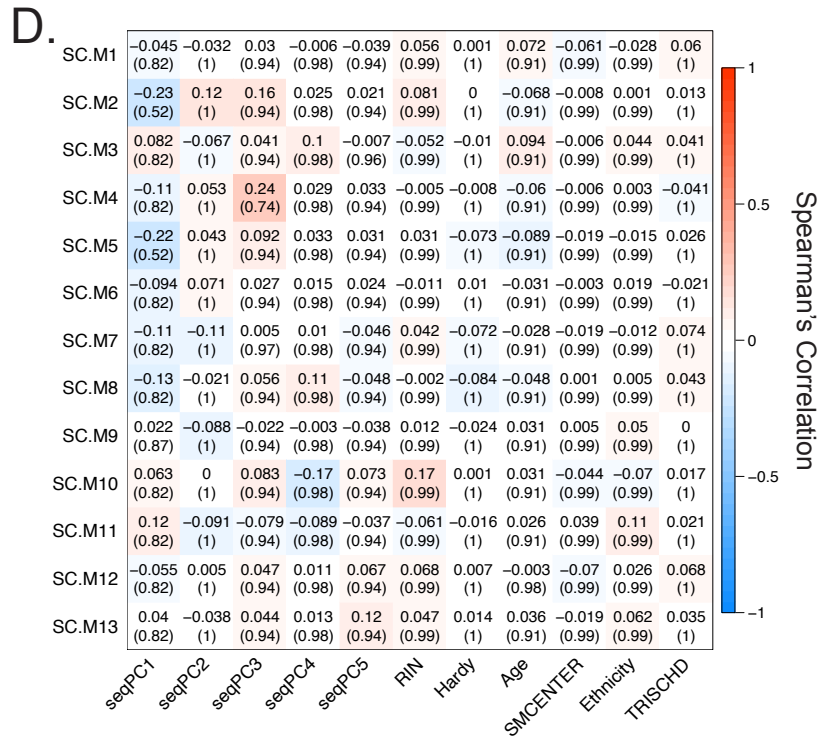
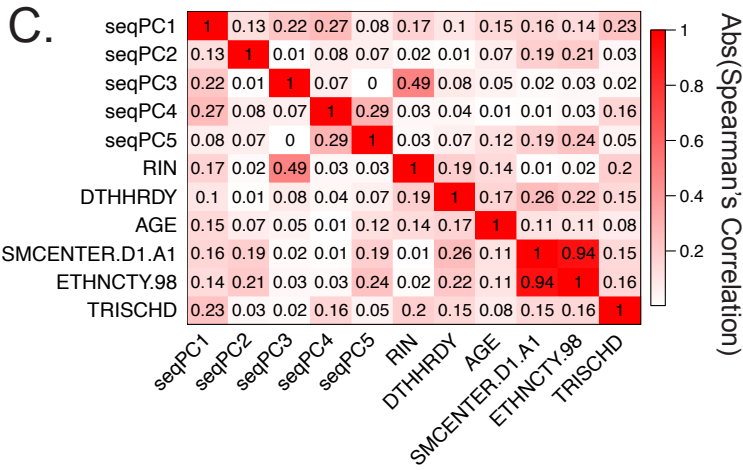
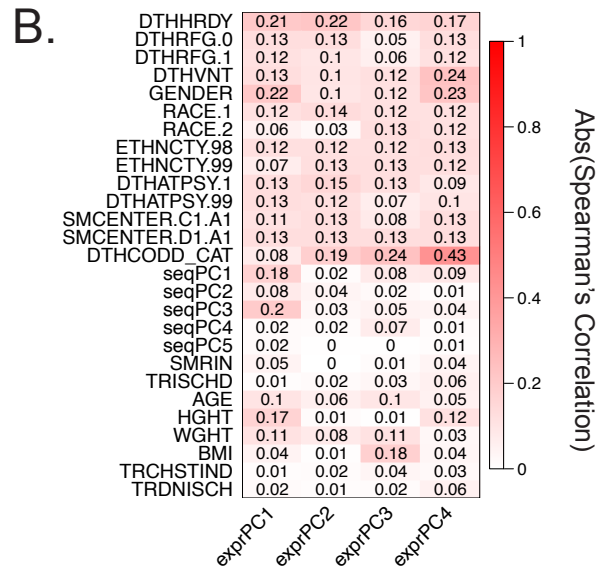
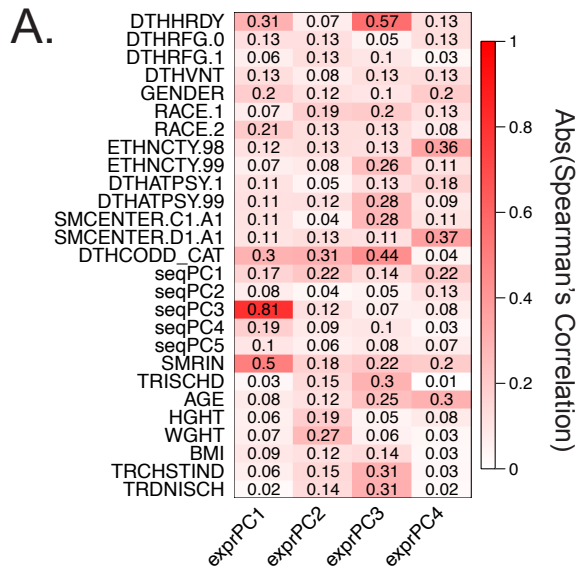


Gene co-expression network analysis in human spinal cord highlights mechanisms underlying amyotrophic lateral sclerosis susceptibility

Jerry C. Wang, Gokul Ramaswami, Daniel H. Geschwind



**Supplementary Figure S1.** Correction for technical and biological drivers of expression variance in the GTEx human spinal cord dataset.

(A) Spearman's correlations between the top four expression principal components (exprPCs1-4) and technical and biological covariates. RIN is RNA Integrity Number. DTHHRDY indicates Hardy's Death Classification which measures time from first onset of symptoms to death.

TRISCHD indicates ischemic time as defined by the time of death to when tissue was fixed or frozen. Complete code annotations can be found in the GTEx Portal v7.

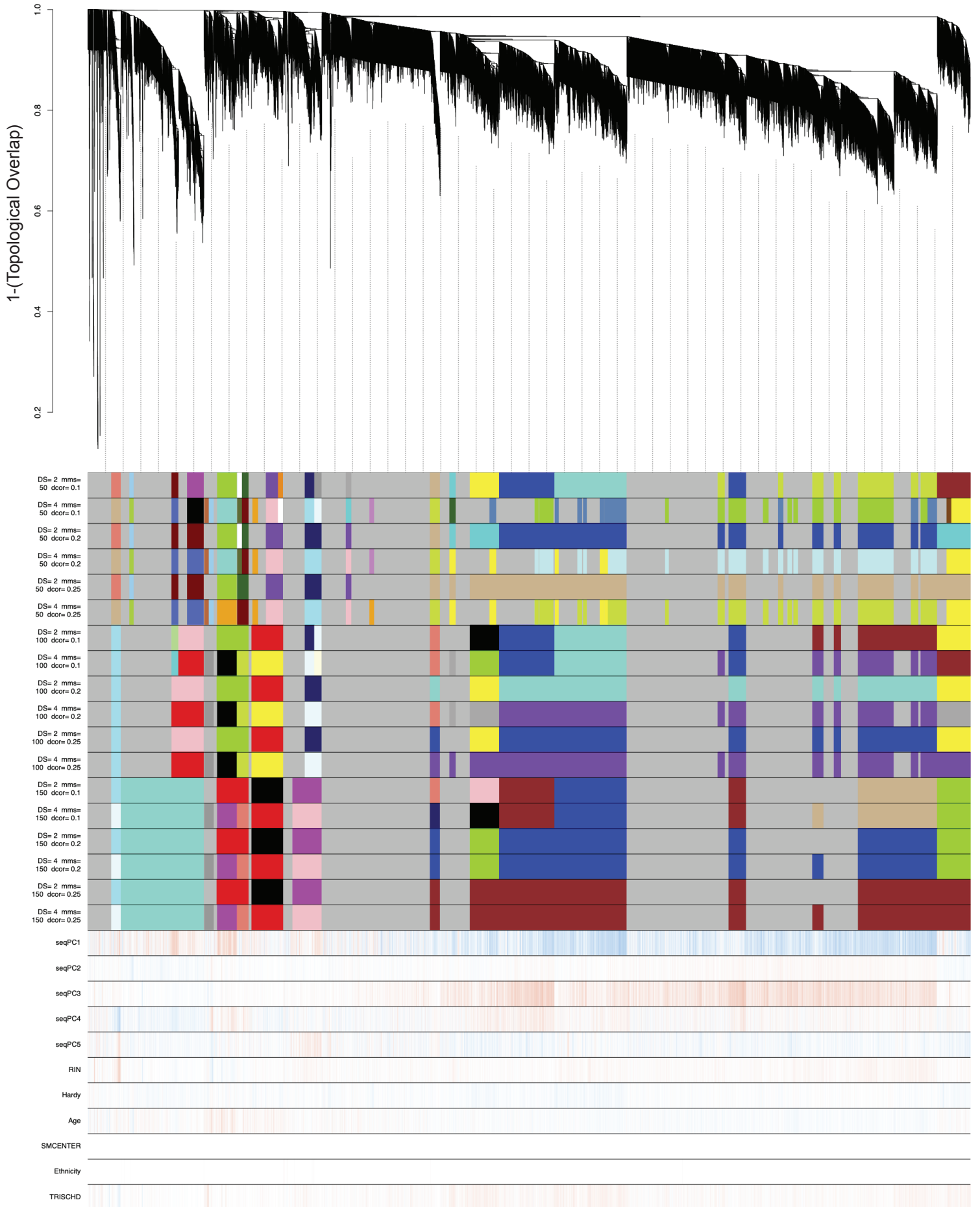
(B) Same as (A), except using the covariate-corrected human spinal cord expression matrix.

(C) Pairwise Spearman's correlations between biological and technical covariates in the human spinal cord dataset to check for covariate collinearity.

(D) Spearman's correlations of module eigengenes to traits in the covariate-corrected human spinal cord dataset. Significance was assessed using Student's t-test.

(E) Pairwise Spearman's correlations of 1,000 randomly sampled genes from each module and a background set. Significance of difference between each module with the background set was assessed using Student's t-test. \*\*\* (FDR corrected p-value < 0.001).

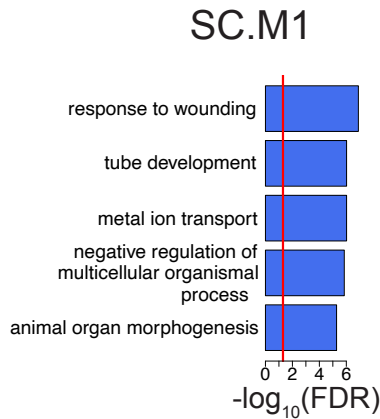
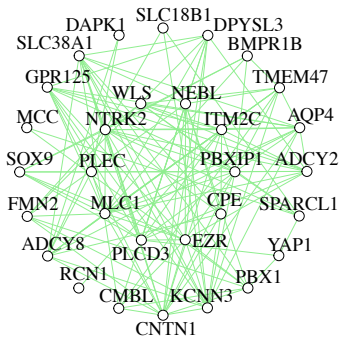
Dendrogram With Different Module Cutting Parameters



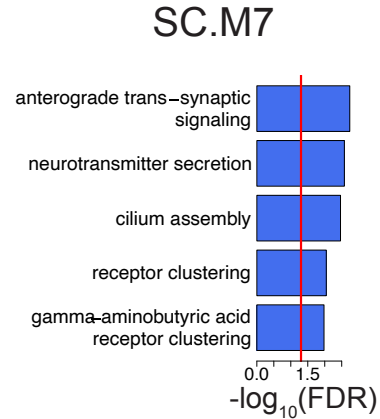
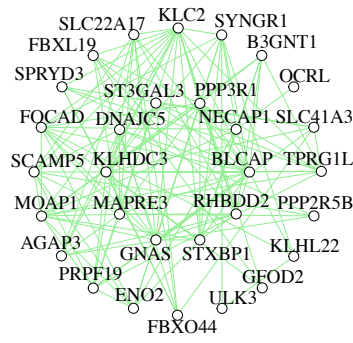
**Supplementary Figure S2.** Optimization of tree cutting parameters to generate co-expression modules.

Dendrogram of the topological overlap matrix for gene co-expression dissimilarity. Co-expression module assignments are shown in each color track. Various permutations of minimum module size (mms), module merging (dcor), and deep split parameters (DS) were tested. Spearman correlations for each gene to biological and technical covariates are shown below the color tracks (Blue: Negative; Red: Positive).

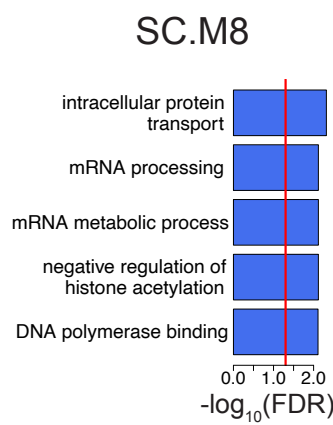
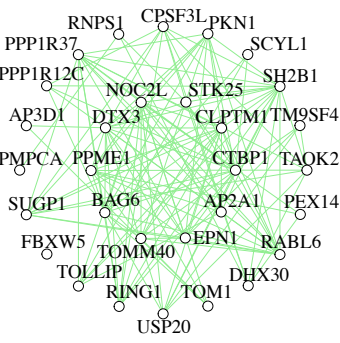
**A.**



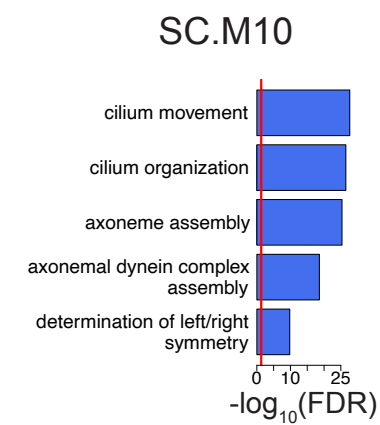
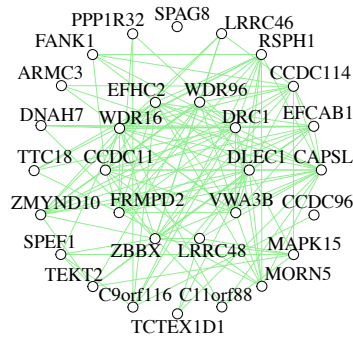
**B.**



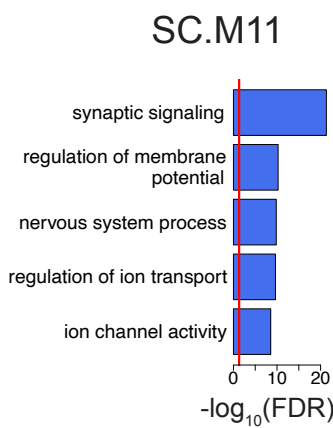
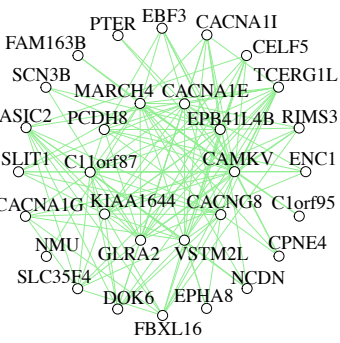
**C.**



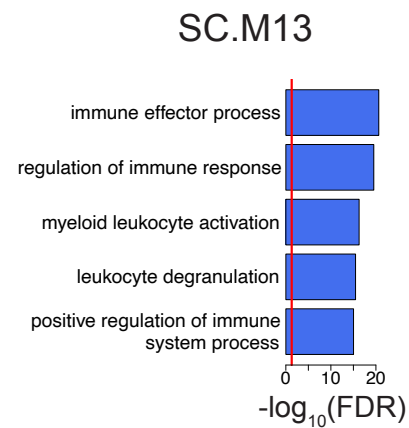
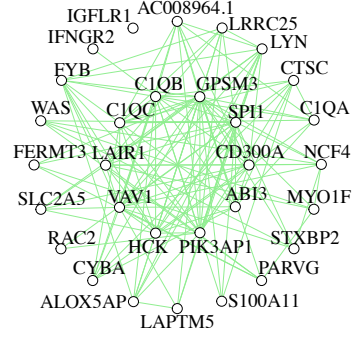
**D.**



**E.**



**F.**



**Supplementary Figure S3.** GO term annotations and top hubs of co-expression modules not displayed in the main figures.

(A, B, C, D, E, F) Top 30 hub genes and 300 connections for SC.M1, SC.M7, SC.M8, SC.M10, SC.M11, and SC.M13 respectively (left panels). Top 5 enriched GO terms for each of the respective modules (right panels). Red lines mark an FDR corrected p-value threshold of 0.05.





**Supplementary Figure S4.** Enrichment of cell-type markers from mouse spinal cord within co-expression modules.

(A) Enrichments of glial cell-type markers from mouse spinal cord within co-expression modules. FDR corrected enrichment p-values < 0.05 are shown within the parentheses.

(B) Same as (A), except using neuronal cell-type markers.



**Supplementary Figure S5.** Covariate correction, module preservation, and module-trait correlations in postmortem human ALS spinal cord.

(A) Spearman's correlations between the top five expression principal components (exprPCs1-5) and technical and biological covariates.

(B) Same as (A), except using the covariate-corrected expression matrix.

(C) Module preservation analysis was used to calculate the  $Z_{\text{summary}}$  statistic for each module in postmortem human ALS spinal cord.

(D) Spearman's correlations of module eigengenes to traits correlated in postmortem human ALS spinal cord. Significance was assessed using Student's t-test. FDR corrected enrichment p-values < 0.05 are shown within the parentheses.



**Supplementary Figure S6.** Covariate correction, module preservation, and module trait correlations in presymptomatic motor neurons from human ALS spinal cord.

(A) Same as Supplementary Fig. S5a except using a dataset of presymptomatic motor neurons in the human ALS spinal cord. RIN is RNA Integrity Number. PMI is postmortem interval.

(B) Same as Supplementary Fig. S5b except using the covariate-corrected expression matrix generated from presymptomatic motor neurons in the human ALS spinal cord.

(C) Same as Supplementary Fig. S5c except module preservation was assessed in presymptomatic motor neurons from human ALS spinal cord dataset.

(D) Same as Supplementary Fig. S5d except using a dataset of presymptomatic motor neurons in the human ALS spinal cord. FDR corrected enrichment p-values < 0.05 are shown within the parentheses.



**Supplementary Figure S7.** Covariate correction, module preservation, and module-trait correlations in iPSC-derived motor neurons from ALS8 patients.

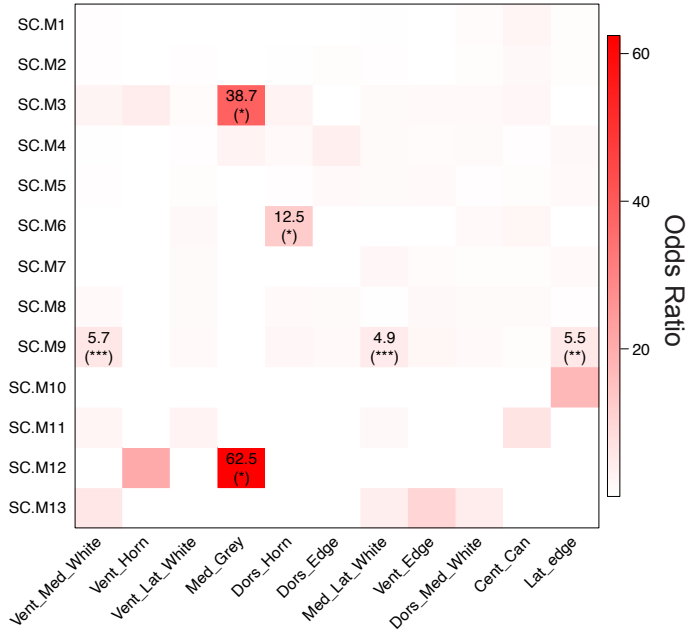
(A) Same as Supplementary Fig. S5a except using a dataset of iPSC-derived motor neurons from ALS8 patients.

(B) Same as Supplementary Fig. S5b except using the covariate-corrected expression matrix generated from iPSC-derived motor neurons from ALS8 patients.

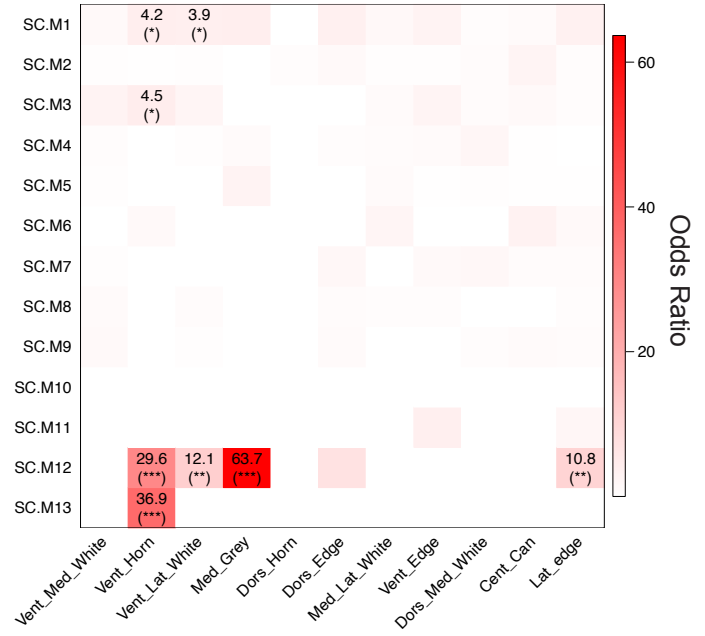
(C) Same as Supplementary Fig. S5c except module preservation was assessed in iPSC-derived motor neurons from ALS8 patients.

(D) Same as Supplementary Fig. S5d except using a dataset of iPSC-derived motor neurons from ALS8 patients. FDR corrected enrichment p-values  $< 0.05$  are shown within the parentheses.

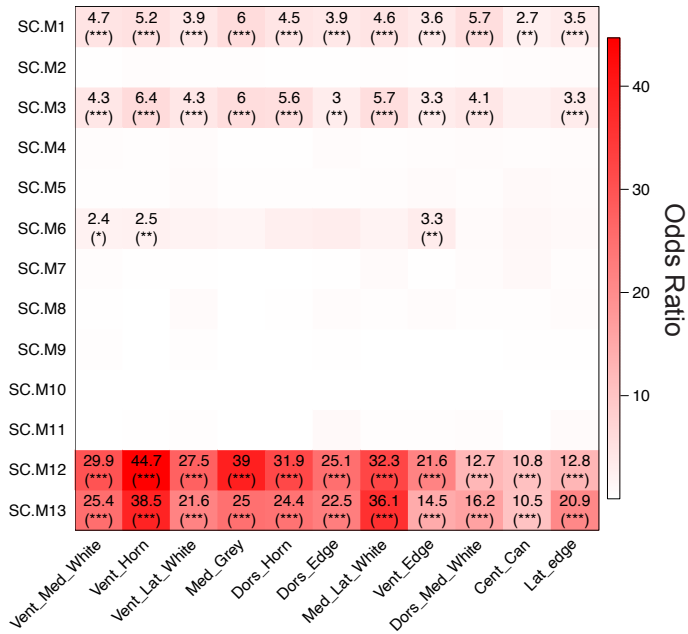
### A. p30 Up-regulated DEGs



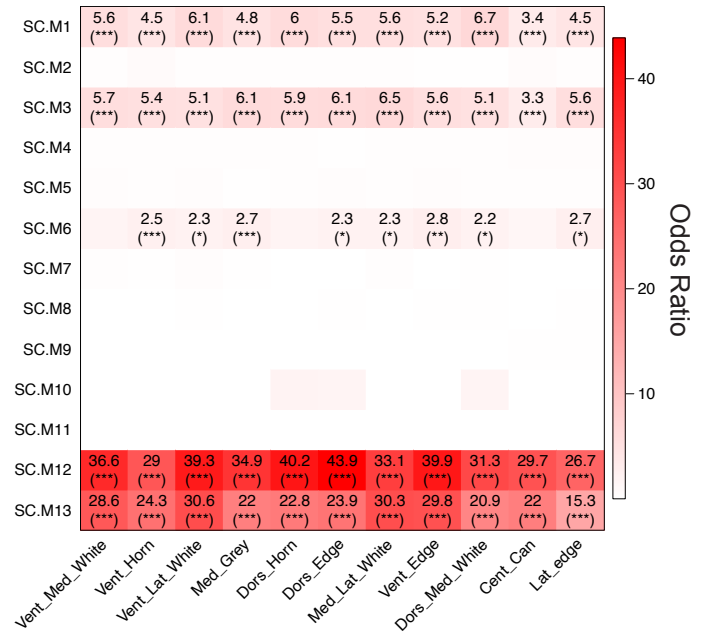
### B. p70 Up-regulated DEGs



### C. p100 Up-regulated DEGs



### D. p120 Up-regulated DEGs



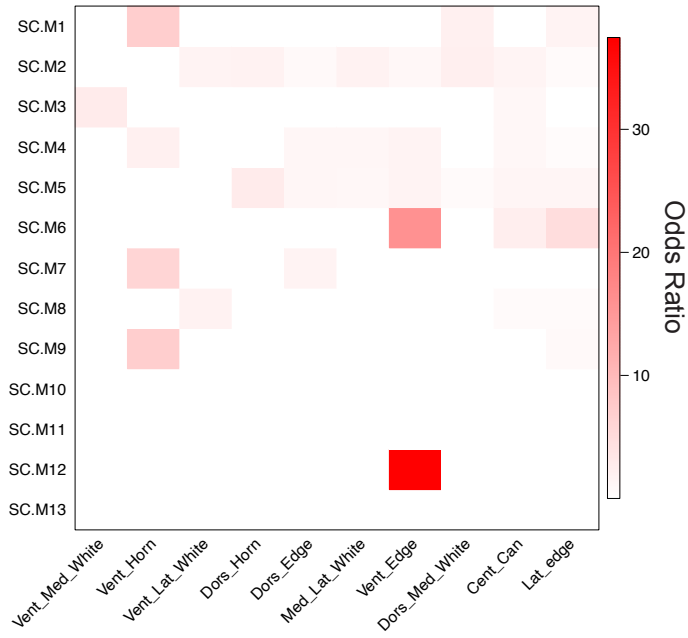


**Supplementary Figure S8.** Enrichment of ALS up-regulated genes for each Anatomical Annotated Region (AAR) from spatial transcriptomics study using SOD1-G93A mice in co-expression modules.

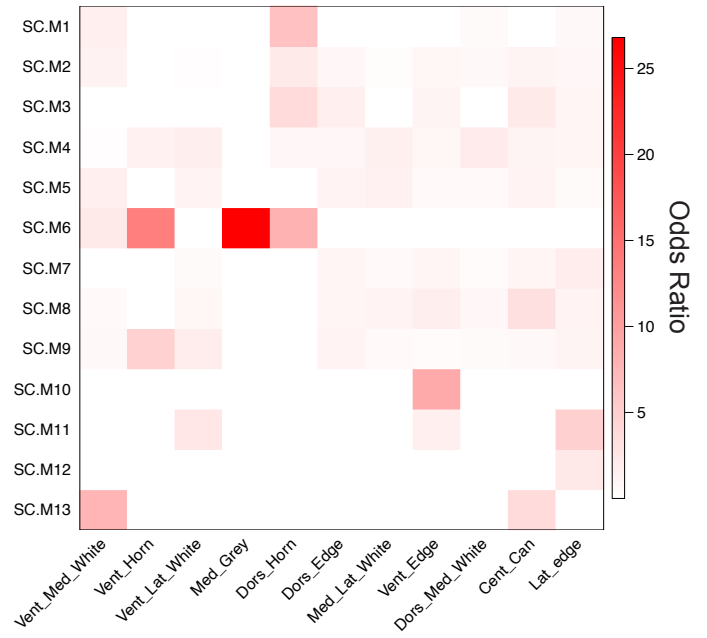
(A) Enrichment of up-regulated DEGs at stage p30 within co-expression modules. Only enrichments with an FDR corrected p-value  $< 0.05$  are displayed. \* (FDR corrected p-value  $< 0.05$ ). \*\* (FDR corrected p-value  $< 0.01$ ). \*\*\* (FDR corrected p-value  $< 0.001$ ).

(B, C, E) Same as (A) except for the p70, p100, and p120 mouse respectively.

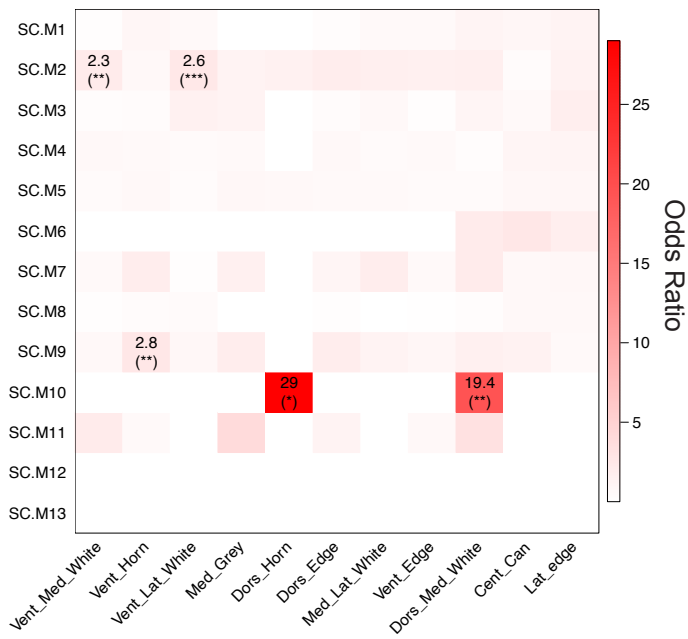
### A. p30 Down-regulated DEGs



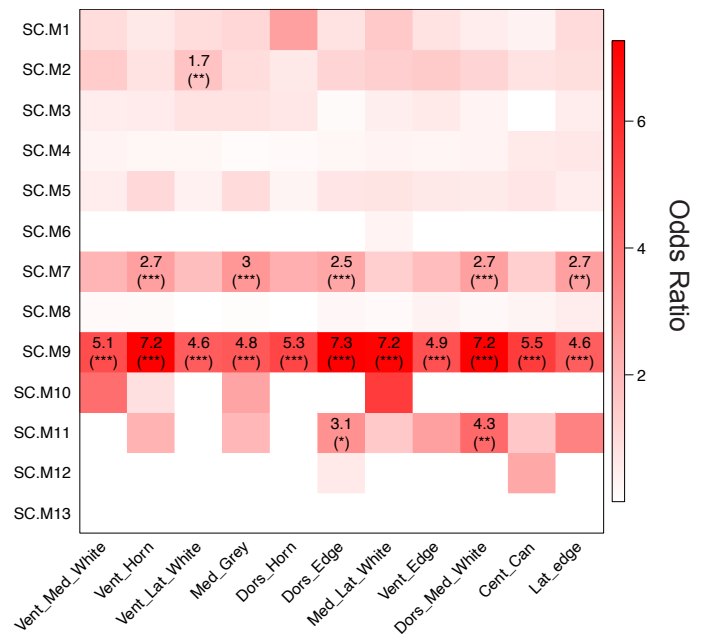
### B. p70 Down-regulated DEGs



### C. p100 Down-regulated DEGs



### D. p120 Down-regulated DEGs



**Supplementary Figure S9.** Enrichment of ALS down-regulated genes for each Anatomical Annotated Region (AAR) from spatial transcriptomics study using SOD1-G93A mice in co-expression modules.

(A) Enrichment of down-regulated DEGs at stage p30 within co-expression modules. Only enrichments with an FDR corrected p-value  $< 0.05$  are displayed. \* (FDR corrected p-value  $< 0.05$ ). \*\* (FDR corrected p-value  $< 0.01$ ). \*\*\* (FDR corrected p-value  $< 0.001$ ).

(B, C, E) Same as (A) except for the p70, p100, and p120 mouse respectively.