SUPPLEMENTAL FIGURES

Meta-analysis of human and mouse ALS astrocytes reveals multiomic signatures of inflammatory reactive states

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A

B
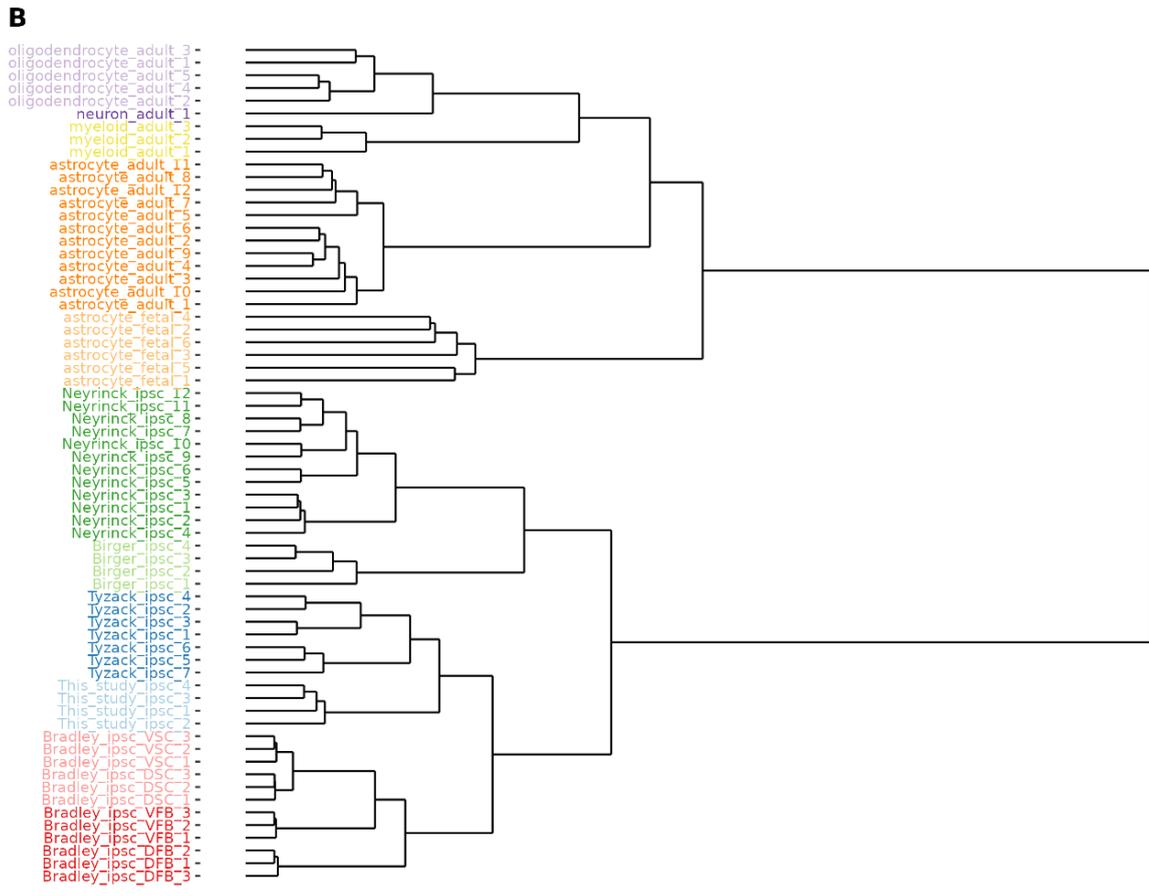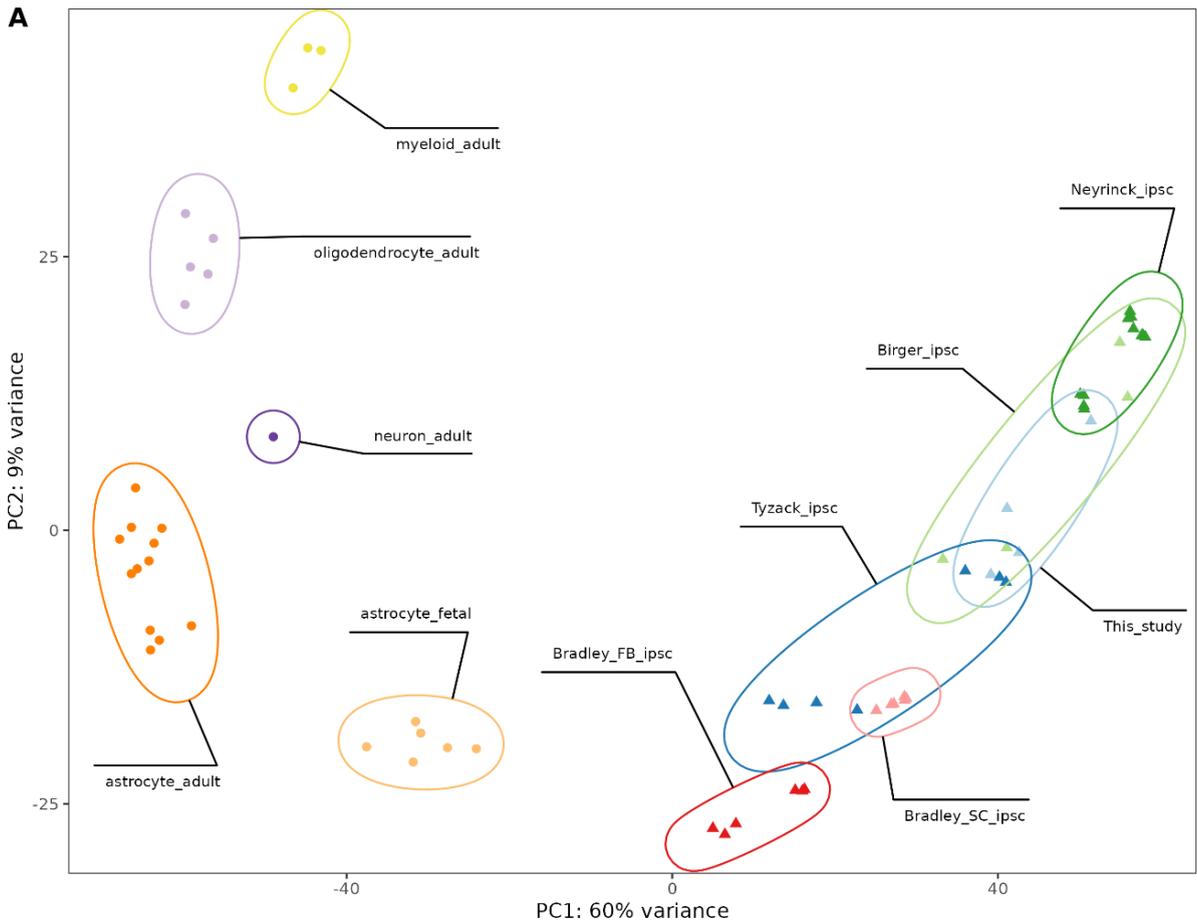
Supplementary Figure 1

A: Principal component analysis (PCA) on variance stabilised gene counts plotted by their coordinates along the first two principal components across all human samples. Samples include human biopsy purified cell type samples from Zhang et al. 2016 (Neuron, Myeloid, Oligodendrocyte, adult and fetal Astrocyte) together with hiPSC-astrocytes (This study: *VCP* mutant and control, Tyzack: *SOD1* mutant and control, Birger: *C9orf72* mutant and control, Neyrinck: *FUS* mutant and control and Bradley: regionally specified dorsal (D) and ventral (V) forebrain (FB) and dorsal and ventral spinal cord (SC). Samples are labeled and coloured by their dataset and shape according to source tissue.

B: Dendrogram showing unsupervised hierarchical clustering of variance stabilised gene counts across the same samples.

A GFAP/DAPI
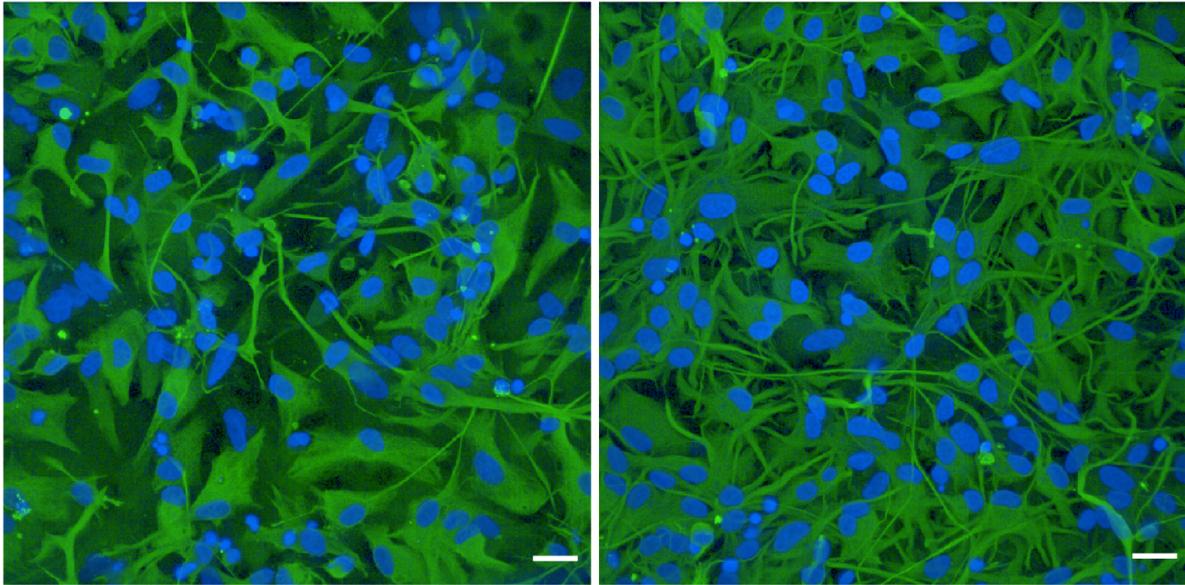
CTRL   VCP mutant
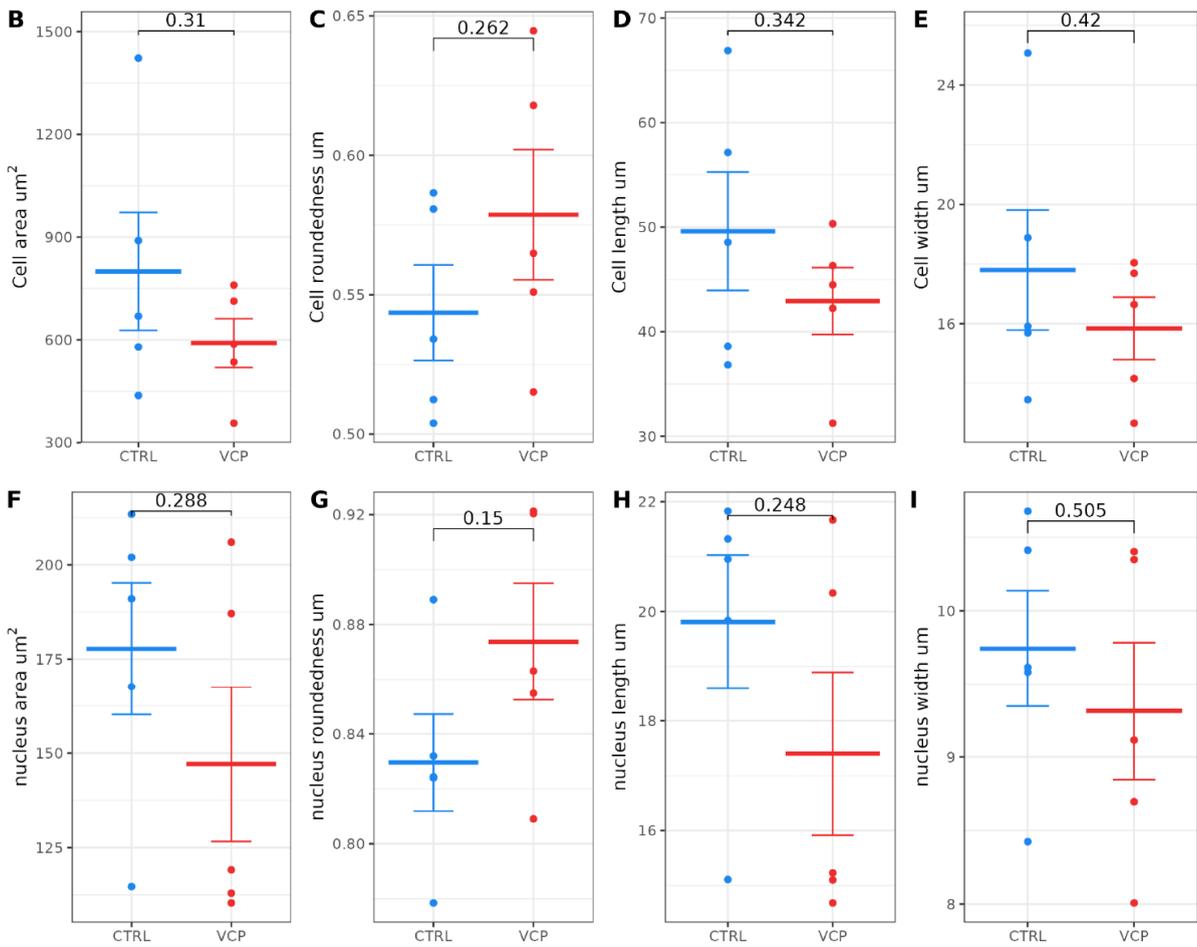
Supplementary Figure 2

A: Representative immunolabeling images of control (left) and *VCP* mutant (right) astrocytes staining for GFAP (green). Nuclei were counterstained by DAPI (blue). Scale bar, 20um.

B-E: Quantification of morphological parameters of whole cell astrocytes in controls (blue, n = 5) and *VCP* mutants (red, n=5).

F-I: Quantification of morphological parameters of astrocyte nucleus in controls (blue, n = 5) and *VCP* mutants (red, n = 5). Results plotted are the mean of 2 technical repeats from 2 experimental repeats. 2-3 biological lines were used in both control and *VCP* mutant for each experimental repeat. P values are reported from the t-test.
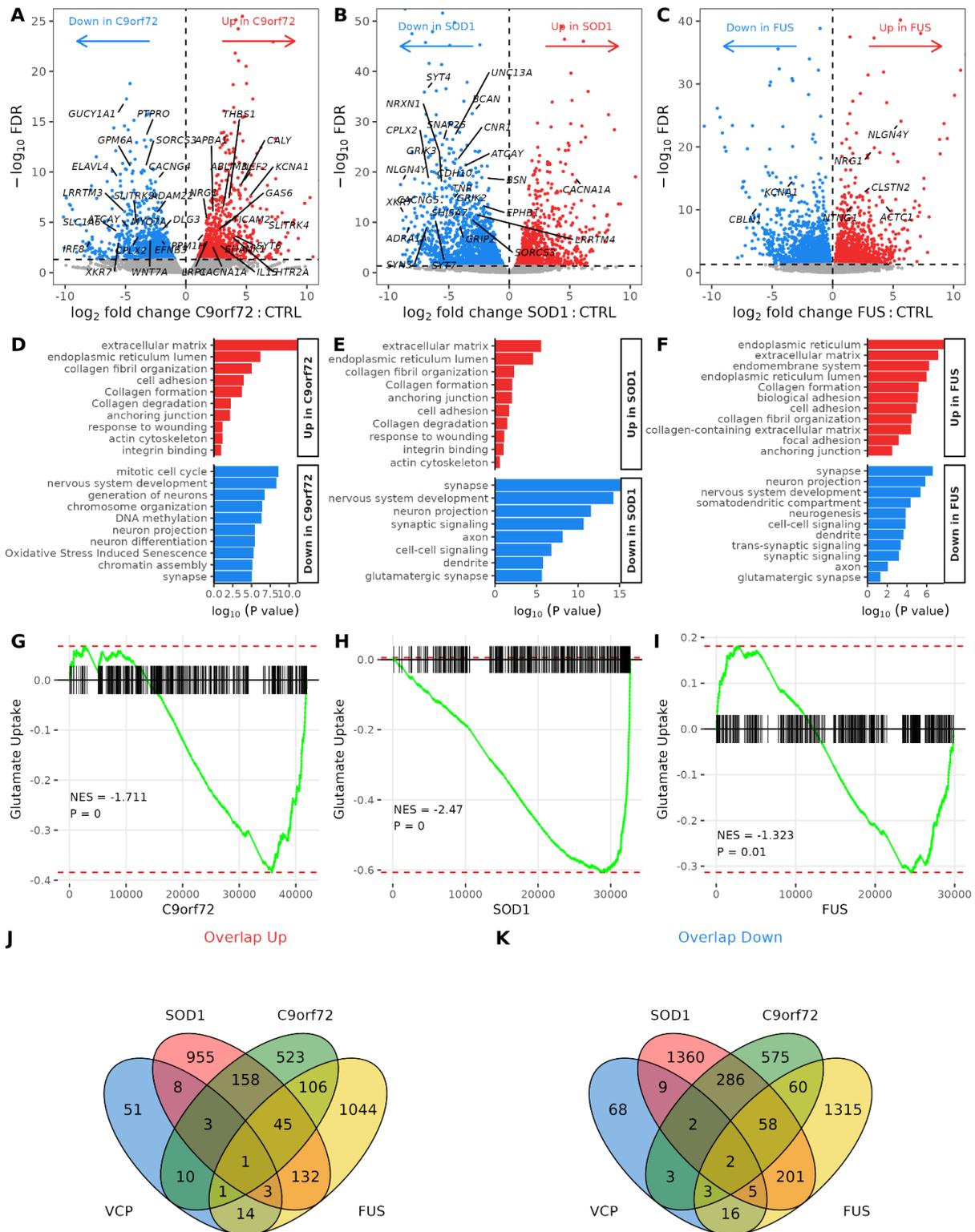
Supplementary Figure 3

A-C: Volcano plots showing log$_2$ fold change in differential gene expression from RNA sequencing for ALS mutant *C9orf72* (A), *SOD1* (B) and *FUS* (C) versus control astrocytes. Genes with significantly

(FDR < 0.05) increased expression are shown in red and those decreased in expression are shown in blue.

D-F: Gene ontology terms enriched in up (red) and downregulated (blue) differentially expressed genes in ALS mutant *C9orf72* (D), *SOD1* (E) and *FUS* (F) vs control astrocytes.

G-I: Gene set enrichment analyses (GSEA) plots for glutamate uptake in *C9orf72* (G), *SOD1* (H), *FUS* (I).

J-K: Venn diagrams showing overlapping differentially expressed genes between *VCP* (blue), *SOD1* (red), *C9orf72* (green) and *FUS* (yellow) for upregulated (J) and downregulated (K) genes. See Table S3 for details of overlapping genes.
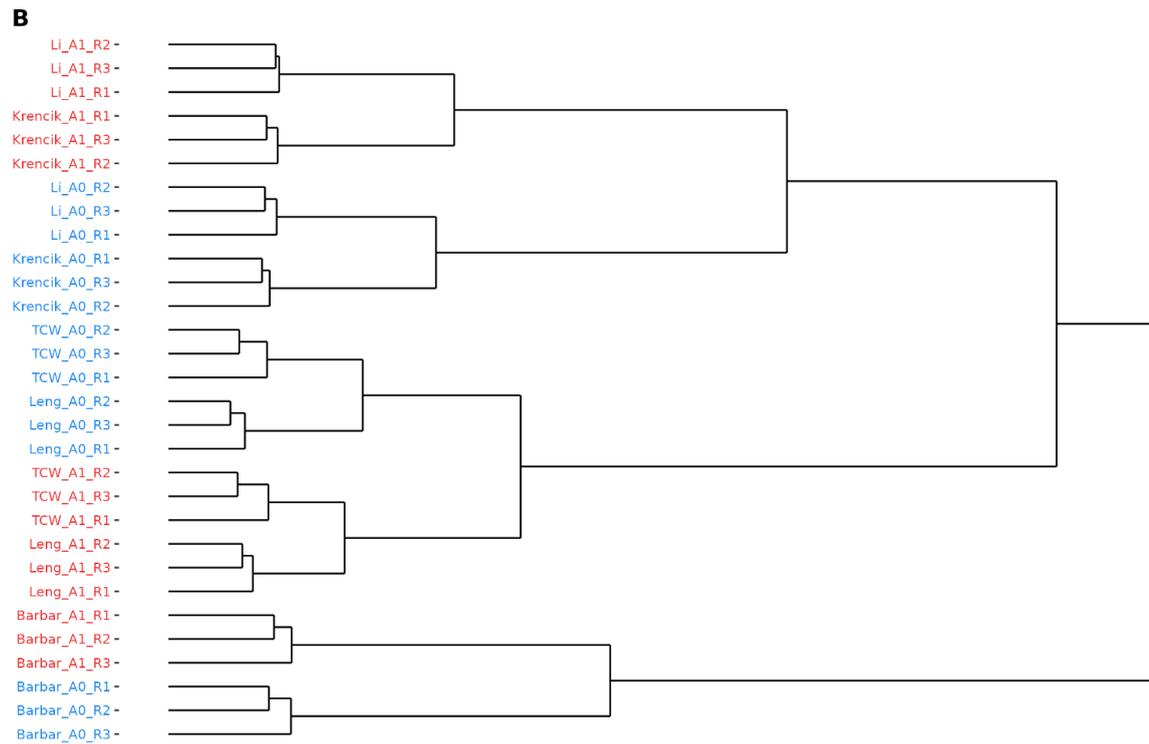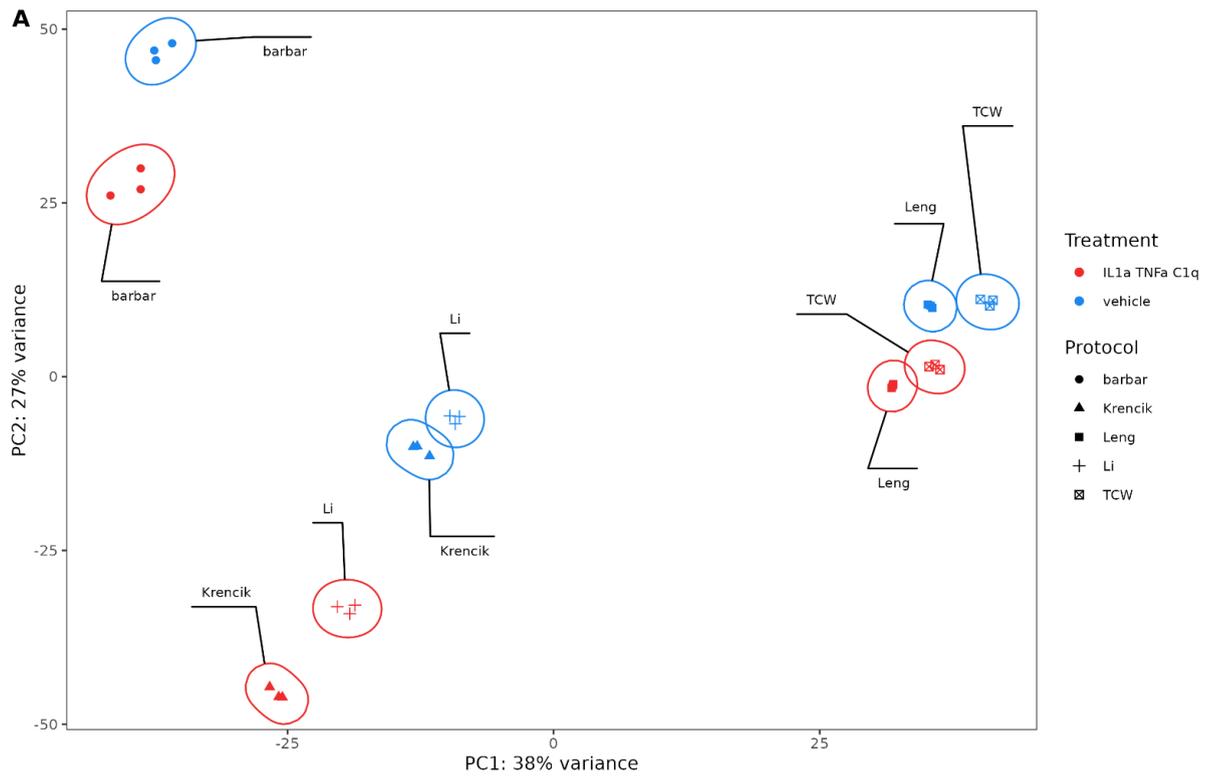
Supplementary Figure 4

A: Principal component analysis (PCA) on variance stabilised gene counts plotted by their coordinates along the first two principal components across TNF, IL1A, C1q and vehicle treated hiPSC derived astrocyte samples.

B: Dendrogram showing unsupervised hierarchical clustering of variance stabilised gene counts across the same samples.
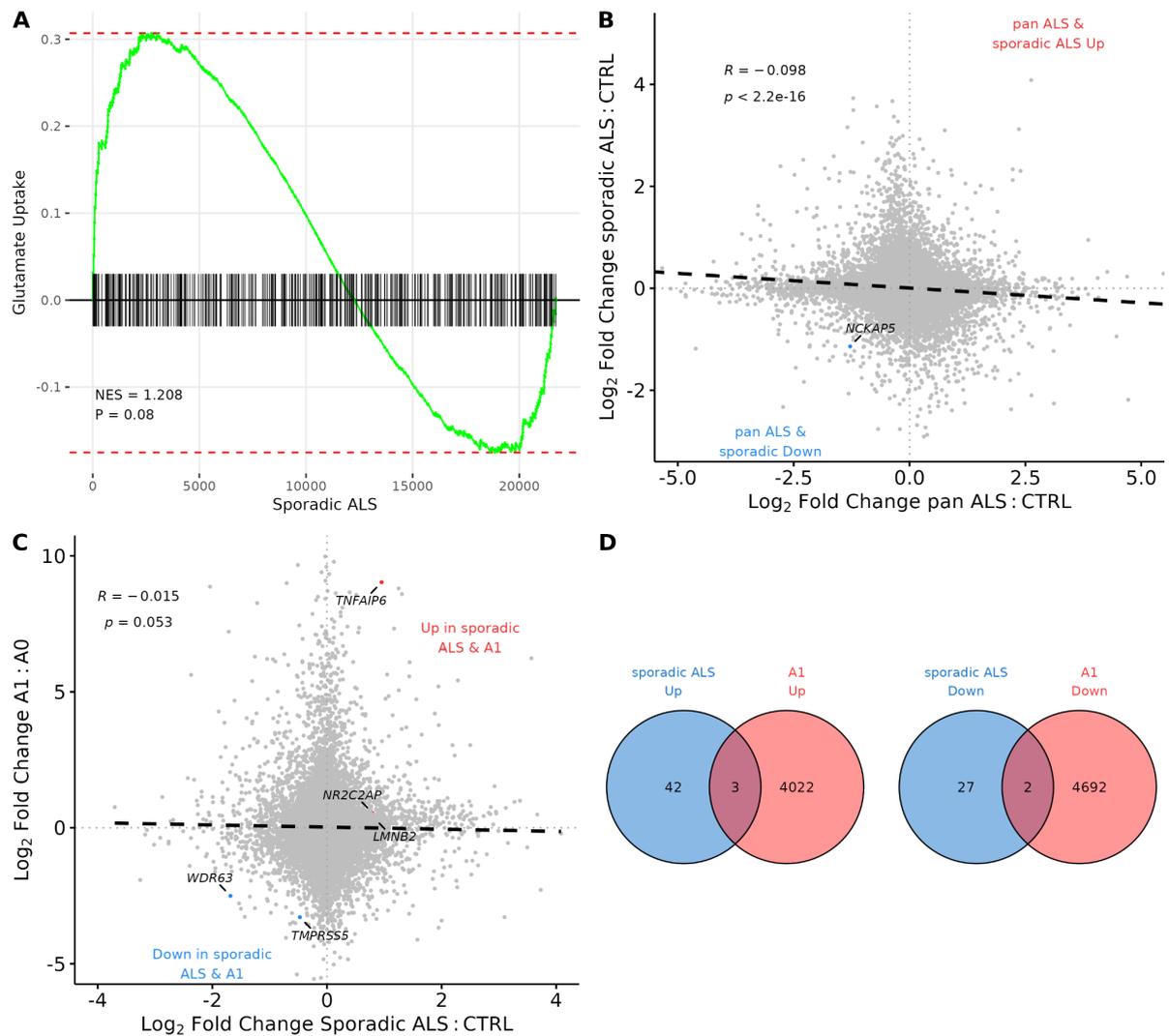
Supplementary Figure 5

A: Gene set enrichment analysis of glutamate uptake in sporadic ALS astrocytes
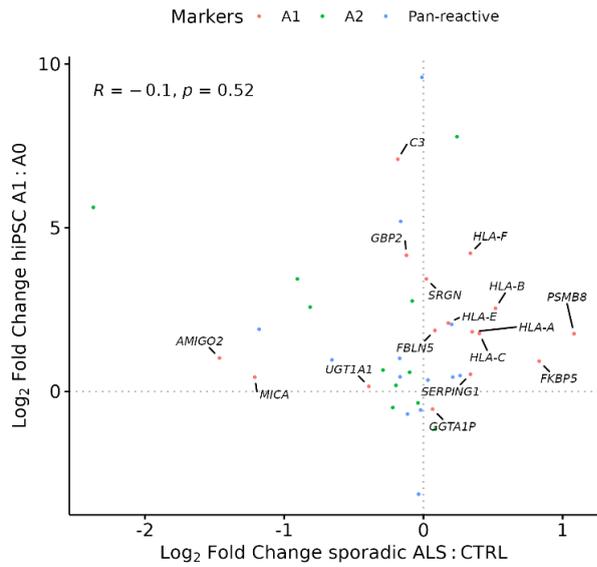
B: Scatterplot of meta-analysis panALS vs control (x-axis) against $\log_2$ fold change in sporadic ALS vs control microarray.

C: Scatterplot of sporadic ALS vs control (x-axis) against $\log_2$ fold change in hiPSC A1 vs A0

D: Venn overlap of differentially expressed genes in sporadic ALS (blue) and A1 (red) hiPSC astrocytes.
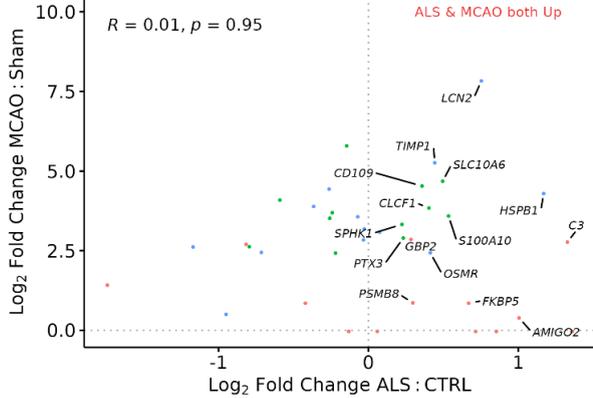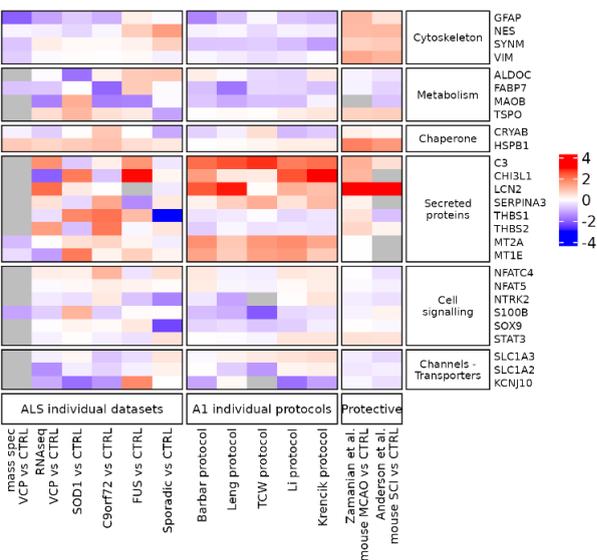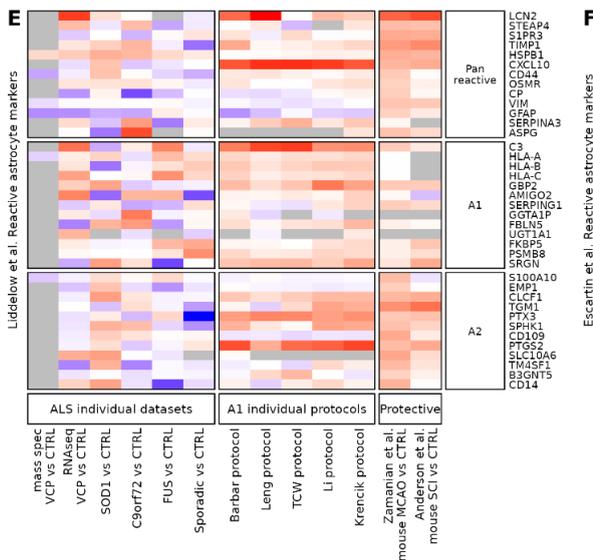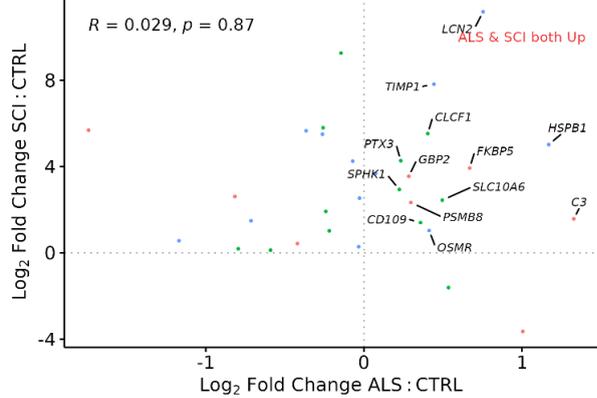
Supplementary Figure 6

A-D: Scatterplots of (Liddelow et al. 2017) astrocyte reactivity markers showing $\log_2$ fold change in hiPSC A1 vs A0 (y-axis) against (A) pan ALS vs control, (B) sporadic ALS vs control (x-axis) and pan ALS vs control (x-axis) against in (C) MCAO vs Sham, (D) SCI vs control (y-axis).

E-F: Heatmaps of (Liddelow et al. 2017) pan-reactive, A1 and A2 specific astrocyte reactivity markers (E) and (Escartin et al. 2021) markers of astrocyte reactivity (F; rows) in the hiPSC ALS astrocyte individual datasets, hiPSC A1 protocols (TNF, IL1A, C1q vs vehicle) and protective astrocyte datasets (columns). Colour intensity represents the scaled differential gene expression $\log_2$ fold changes in each dataset.
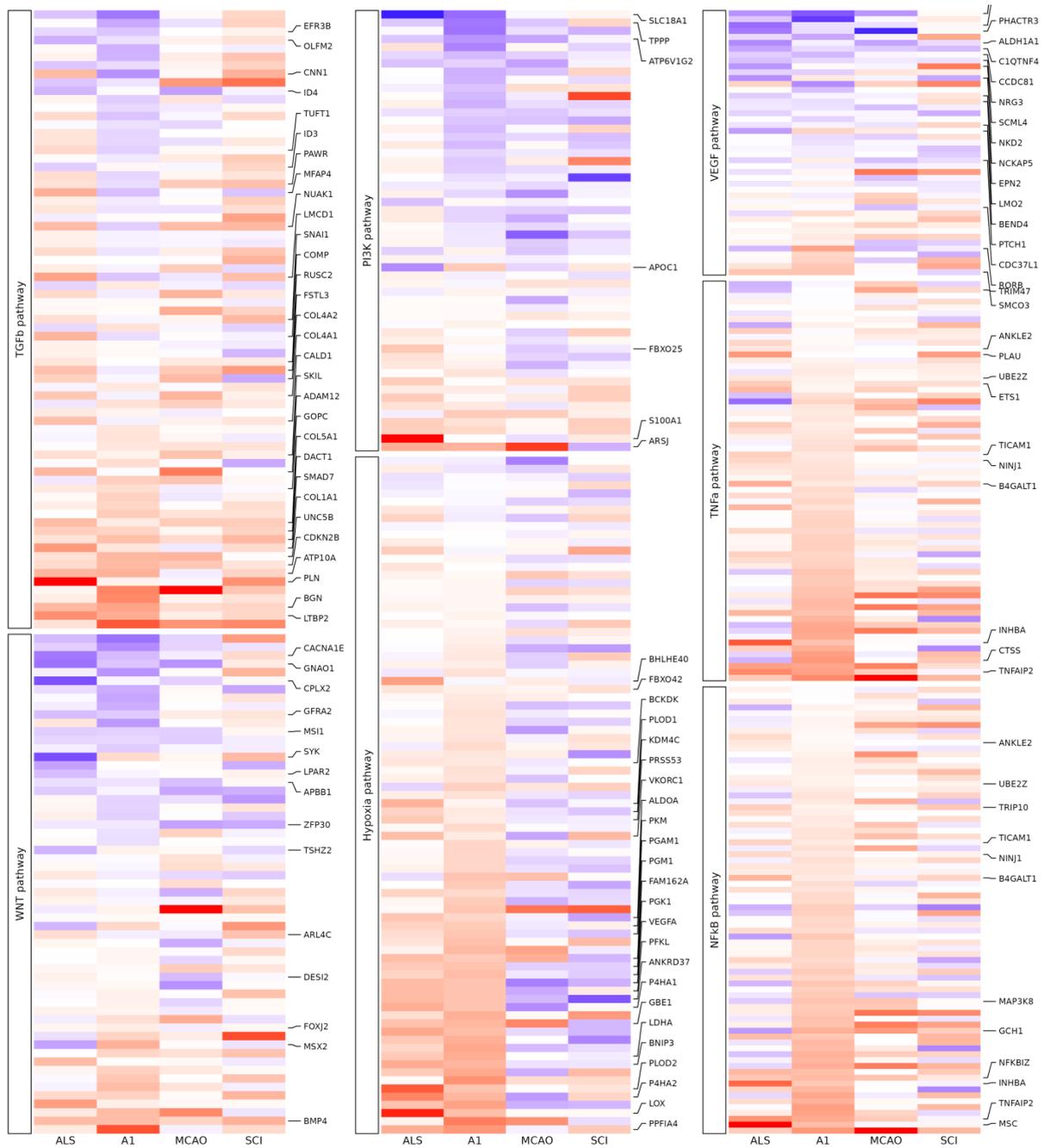
Supplementary Figure 7

PROGENy signaling pathway responsive genes for the top 6 pathways upregulated in pan-ALS vs control. Each heatmap shows the $\log_2$ fold change in (left to right columns) pan-ALS vs control, hiPSC A1 vs A0, MCAO vs sham, SCI vs control. Genes changing significantly in ALS (FDR < 0.05) are labelled.
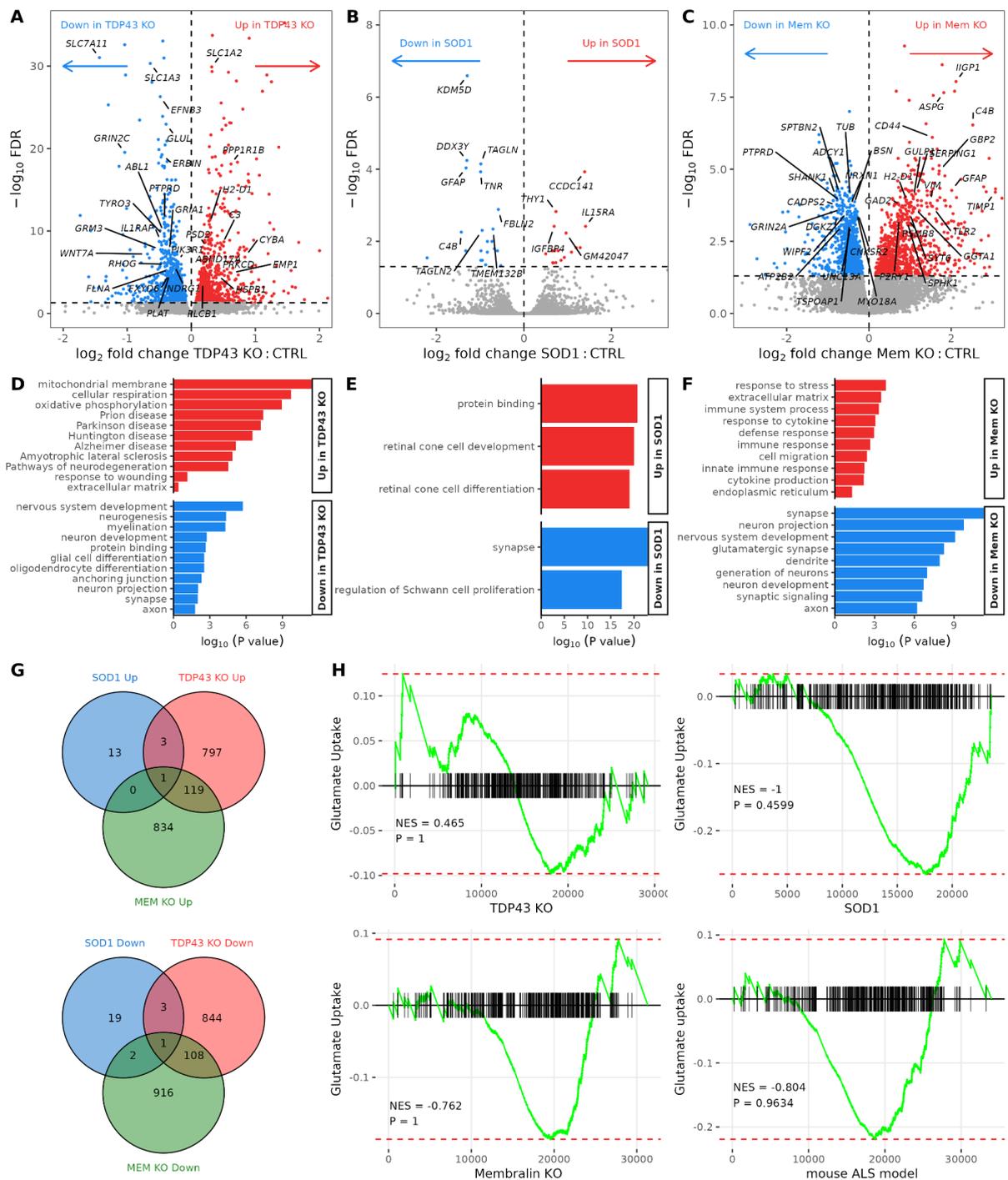
Supplementary Figure 8

A-C: Volcano plots showing $\log_2$ fold change in differential gene expression from RNA sequencing for ALS mutant (A) Tardbp knockout, (B) Sod1^{G93A} and (C) membralin knockout versus control mouse astrocytes. Genes with significantly (FDR < 0.05) increased expression are shown in red and those

decreased in expression are shown in blue.

D-F: Gene ontology terms enriched in up (red) and downregulated (blue) differentially expressed genes in ALS mutant (D) Tardbp knockout , (E) Sod1^{G93A} and (F) membralin knockout vs control astrocytes.

G: Venn diagrams showing overlapping differentially expressed genes between Sod1^{G93A} (blue), Tardbp knockout (red), membralin knockout (green) for upregulated (top) and downregulated (bottom) genes.

H: Gene set enrichment analyses (GSEA) plots for glutamate uptake in Tardbp KO (top left), Sod1^{G93A} (top right), membralin KO (bottom left) and mouse ALS meta-analysis (bottom right).

A mouse ALS models vs human pan-ALS

$R = 0.051$, $p = 1.6e{-}10$

mouse ALS models & human pan-ALS Up

GJB2
COL3A1
MYLK
GBP3
CTSZ
ASS1
CXCL16
BCL3
OSTF1
PREX1
TNR
STMN4
SLC30A10
NRXN1
GRM3
CACNG3
CPLX2

mouse ALS models & human pan-ALS Down

Log$_2$ Fold Change human pan ALS : CTRL

Log$_2$ Fold Change mouse ALS models : CTRL

B
mouse ALS Up | human ALS Up
388 | 41 | 1268

mouse ALS Down | human ALS Down
541 | 83 | 1479

C human & mouse ALS overlap

upregulated:
- cytoplasm
- response to laminar fluid shear stress
- positive regulation of apoptotic process
- endoplasmic reticulum
- positive regulation of programmed cell death
- endoplasmic reticulum lumen
- endomembrane system
- positive regulation of cell death

downregulated:
- neuron projection
- synapse
- axon
- synaptic signaling
- nervous system development
- somatodendritic compartment
- dendrite
- synaptic membrane
- glutamatergic synapse
- postsynapse

log$_{10}$ (P value)

Supplementary Figure 9

A: Scatterplot of human and mouse meta-analyses: log$_2$ fold change in human ALS vs control (y-axis) against log$_2$ fold change in mouse ALS models vs control (y-axis). Genes coloured red are upregulated in both meta-analyses whilst blue genes are downregulated in both.

B: Venn overlap of differentially expressed genes in the human ALS meta-analysis (red) and mouse ALS models meta-analysis (blue) for upregulated (top) and downregulated (bottom) genes.

C: Bar graph showing curated overrepresented functional categories (FDR < 0.05) by gene ontology analysis of genes co-upregulated (red) and co-downregulated (blue) between human pan-ALS and mouse ALS model meta-analyses.
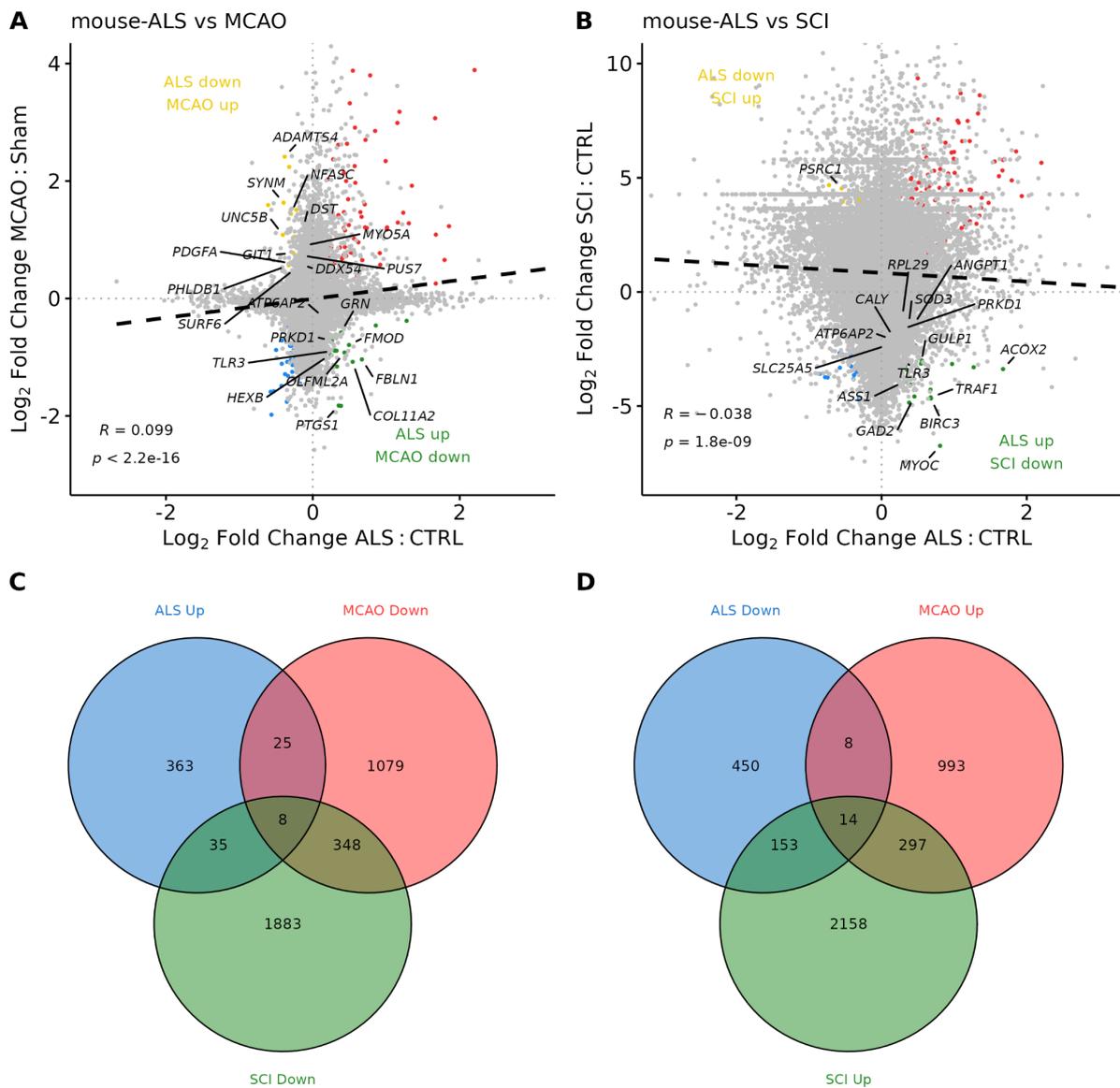
Supplementary Figure 10

A-B: Scatterplots of $\log_2$ fold changes in gene expression in mouse ALS vs control astrocytes (x-axis) against (A) MCAO vs Sham astrocytes and (B) SCI vs control astrocytes (y-axis). Black dashed line indicates linear regression correlation (Pearson correlation MCAO R = +0.1, SCI R = -0.04). Overlapping differentially expressed genes are coloured red (upregulated) and blue (downregulated). Genes increased in ALS but decreased in the protective astrocytes are shown in green, whilst genes decreased in ALS and increased in protective astrocytes are yellow.

C-D: Venn diagram depicting the number of differentially expressed genes (FDR < 0.05) increased

(up) in ALS but decreased (down) in the protective astrocytes (C) and genes decreased in ALS but increased in protective astrocytes (D).
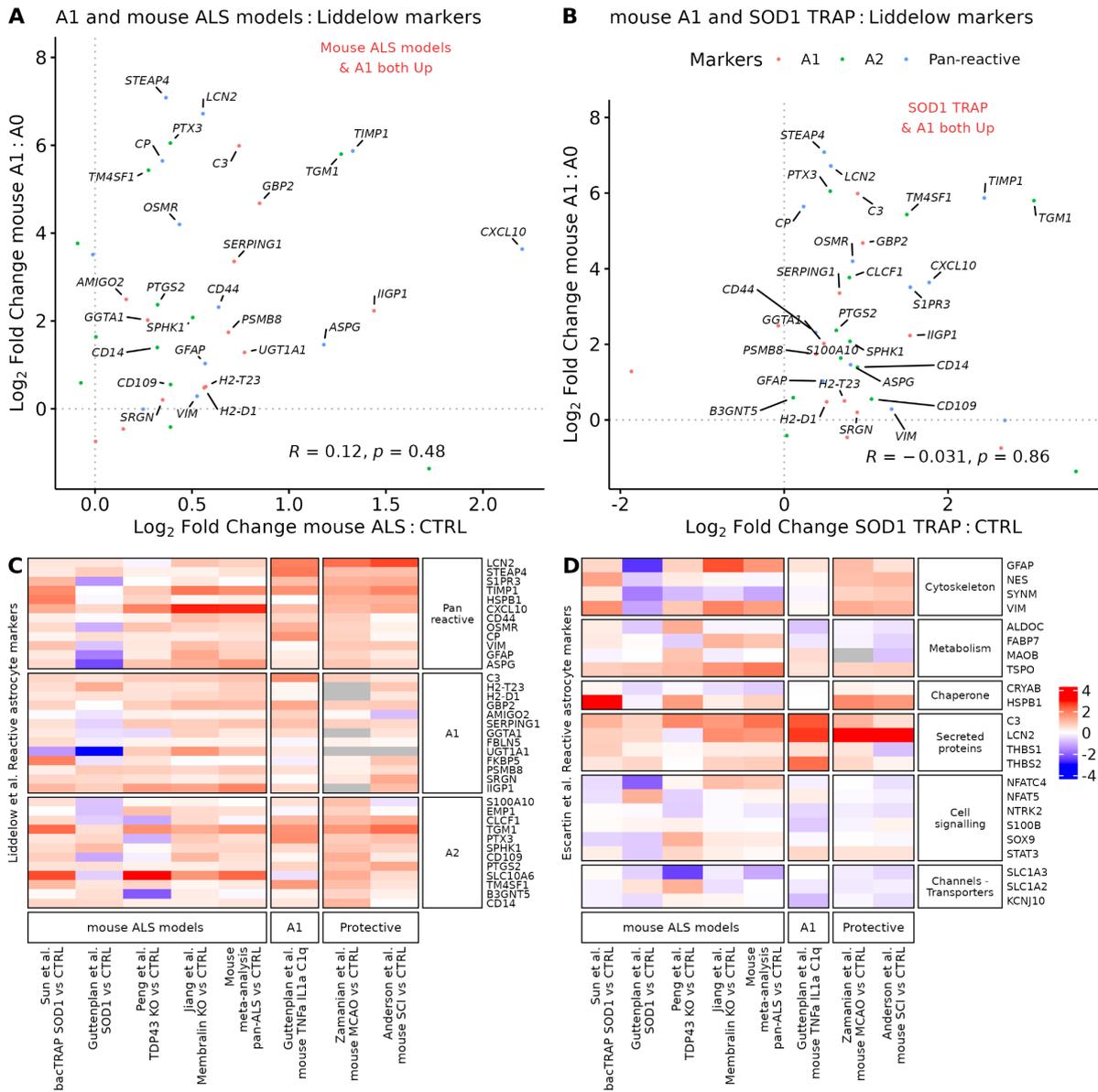
Supplementary Figure 11

A-B: Scatterplots of (Liddelow et al. 2017) astrocyte reactivity markers showing log2 fold change in (A) mouse ALS models vs control and (B) Sod1 TRAP mouse vs control (x-axis) against log2 fold change in mouse A1 vs A0 (y-axis).

C-D: Heatmaps of (Liddelow et al. 2017) pan-reactive, A1 and A2 specific astrocyte reactivity markers (C) and (Escartin et al. 2021) markers of astrocyte reactivity (D; rows) in the mouse ALS models individual datasets (left columns) and meta-analysis and A1 and protective datasets (columns). Colour intensity represents the scaled differential gene expression log2 fold changes in ALS vs control; A1 vs A0 astrocytes.

A SOD1^{G37R} astrocyte TRAP-seq | *Aldh1l1 bacTRAP* *EGFP-Rpl10a* | *Lox* *SOD1^{G37R}* → Spinal cord dissection → RNA library preparation / translating mRNA immuno-precipitation → mRNA purification

B $R = 0.064$ $p = 1.4e\text{-}15$
Sod1 TRAP & A1 Up
GCH1, C3, GBP5, IFI27, HCK, GNA15, BST2, ISG20, CCL3, CCL2, TIMP1, TCIM, LAMC2, TGM1, FKBP5, HSPB1
Sod1 TRAP & A1 Down
y-axis: Log$_2$ Fold Change hiPSC A1 : A0
x-axis: Log$_2$ Fold Change Sod1 TRAP : CTRL

C $R = 0.072$ $p < 2.2e\text{-}16$
Sod1 TRAP & A1 Up
C3, BCL3, CCL2, TM4SF1, GBP2, TIMP1, TGM1, CRISPLD2, NFKBIA, CEBPD, ECM1, LAMC2, FN1, TCIM
FZD10
Sod1 TRAP & A1 Down
y-axis: Log$_2$ Fold Change mouse A1 : A0
x-axis: Log$_2$ Fold Change Sod1 TRAP : CTRL

D Sod1.TRAP down MCAO up
RND3, RND1, PRR5L, ACOX2, MAPK7, THY1, UCP3, PODP1, RAMP2, FMOD, AIFM1, ITPKA, NRROS, MAP2K6
$R = 0.13$ $p < 2.2e\text{-}16$
Sod1.TRAP up MCAO down
y-axis: Log$_2$ Fold Change MCAO : Sham
x-axis: Log$_2$ Fold Change Sod1 TRAP : CTRL

E Sod1.TRAP down SCI up
TNFRSF13C, RAB7B, ZFPM1, RHOBTB2, RFFL, RAP2A, RBM15B, PRR5L, SPATA2, EPRS, MAP6D1, MYO10, BCL2L11, RBMS2, TRAP1, CETN2, S100A10, UCP3, AIFM1, CALY, PEPD, SOD1, DYNC1I1, TRMT61B
$R = 0.064$ $p < 2.2e\text{-}16$
Sod1.TRAP up SCI down
y-axis: Log$_2$ Fold Change SCI : CTRL
x-axis: Log$_2$ Fold Change Sod1 TRAP : CTRL

F Sod1 TRAP Up / hiPSC A1 Up / mouse A1 Up
348, 78, 3240, 99, 65, 642, 1167
Sod1 TRAP Down / hiPSC A1 Down / mouse A1 Down
262, 76, 4045, 74, 40, 533, 1279

G A1 & pan-ALS overlap
response to cytokine
defense response
response to stress
immune system process
immune response
TNF signaling pathway
Interferon alpha/beta signaling
inflammatory response
response to interferon-gamma
Interferon Signaling
extracellular matrix
A1 & Sod1 TRAP Up
x-axis: log$_{10}$ (P value)

Supplementary Figure 12

A: Schematic depicting mouse in vivo (top row) ALS Sod1^{G37R} mutant mouse with astrocyte-specific (Aldh1l1) bacTRAP reporter, which expresses the EGFP-tagged ribosome protein (Rpl10a) within astrocytes. Polyribosome-associated mRNAs undergoing translation are then isolated by EGFP immunoprecipitation before mRNA purification and RNA translatome sequencing. B-E: Scatterplots of log$_2$ fold changes in gene expression in astrocyte-specific bacTRAP mouse Sod1 mutant vs control (x-axis) against log$_2$ fold changes in (B) hiPSC A1 vs A0 astrocytes, (C) mouse A1 vs A0 astrocytes, (D) middle cerebral artery occlusion (MCAO) vs sham, and (E) spinal cord injury (SCI) vs control (y-axis) against. Black dotted line indicates linear regression correlation (Pearson correlation R reported in each plot). Overlapping differentially expressed genes are coloured red (upregulated, top right quadrant) and blue (downregulated, bottom left quadrant).

F: Venn diagrams depicting the overlap of upregulated (left) and downregulated (right) differentially expressed genes between bacTRAP Sod1 (blue), hiPSC A1 (red) and mouse A1 (green) astrocytes.

G: Bar graph showing curated overrepresented functional categories (FDR < 0.05) by gene ontology analysis of 154 genes Sod1 TRAP, hiPSC & mouse A1 co-upregulated (red). There were no relevant terms enriched amongst the 102 co-downregulated (blue) genes.