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LETTER TO THE EDITOR Open Access

Integrated transcriptomic and lipidomic analyses reveal altered lipid homeostasis in mutant *FUS*^{P525L} astrocytes from HiPSCs

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Letter to the editor

We recently reported a pathological lipid signature—characterized by alterations in glycerophospholipid metabolism at both metabolite and gene expression levels—in oligodendroglial progenitors (OPCs) and astrocytes derived from human induced pluripotent stem cells (hiPSCs) of Amyotrophic Lateral Sclerosis (ALS) patients harboring a *FUS*^{R521H} mutation [1, 2], underscoring the complex and active role of lipid abnormalities in ALS pathogenesis [3]. The *FUS*^{P525L} mutation carriers display a specific ALS phenotype characterized by a severe course, early disease onset (most are juvenile onset), rapid disease progression, and (in some patients) multiple system degeneration and presence of basophilic inclusions. Whether *FUS*^{P525L} mutant astrocytes exhibit a similar lipid signature remains unknown.

To generate the *FUS*^{P525L} iSOX9-iPSC line, the *P525L* mutation was introduced into iSOX9 SIGi001-A iPSCs via base editing (Supplementary Fig. 1a). Afterward, iPSC colonies were isolated and screened using Sanger sequencing to identify clones wherein the *FUS*^{P525L} mutation had been correctly introduced (Supplementary Fig. 1b). Sanger sequencing of in silico predicted off-target regions demonstrated no off-target events (Supplementary Fig. 1c). The hiPSCs carrying the *FUS*^{P525L} and an inducible *SOX9* transcription factor cassette, differentiated towards astrocytes similarly to the isogenic controls (Fig. 1a). Transcript levels of the pluripotency gene *OCT4* decreased in all lines, while doxycycline treatment between day 12 and 18 induced *SOX9* expression (Fig. 1b). Immunostaining at day 48 showed comparable percentages of S100B, and EAAT1-positive cells in *FUS*^{P525L} mutant and control astrocyte populations (Fig. 1c–e), indicating that the *FUS*^{P525L} mutation does not impair astrocyte differentiation.

To investigate the phenotypic differences between *FUS*^{P525L} mutant and isogenic control astrocytes, we performed RNA sequencing. Principal component analysis (PCA) demonstrated clear separation between the two groups, indicating distinct transcriptomic profiles (Fig. 1f). Gene set enrichment analysis (GSEA) using Gene Ontology (GO) terms, based on genes ranked from most significantly upregulated to most significantly downregulated, revealed significant enrichment of upregulated genes in the cellular component categories "integral component of plasma membrane" and "intrinsic component of plasma membrane" (Fig. 1g). Interestingly, these same categories were also significantly enriched among downregulated genes (Fig. 1h), suggesting a complex and broader dysregulation of membrane-associated processes in *FUS*^{SP525L} mutant astrocytes. We next performed mass spectrometry to assess whether the

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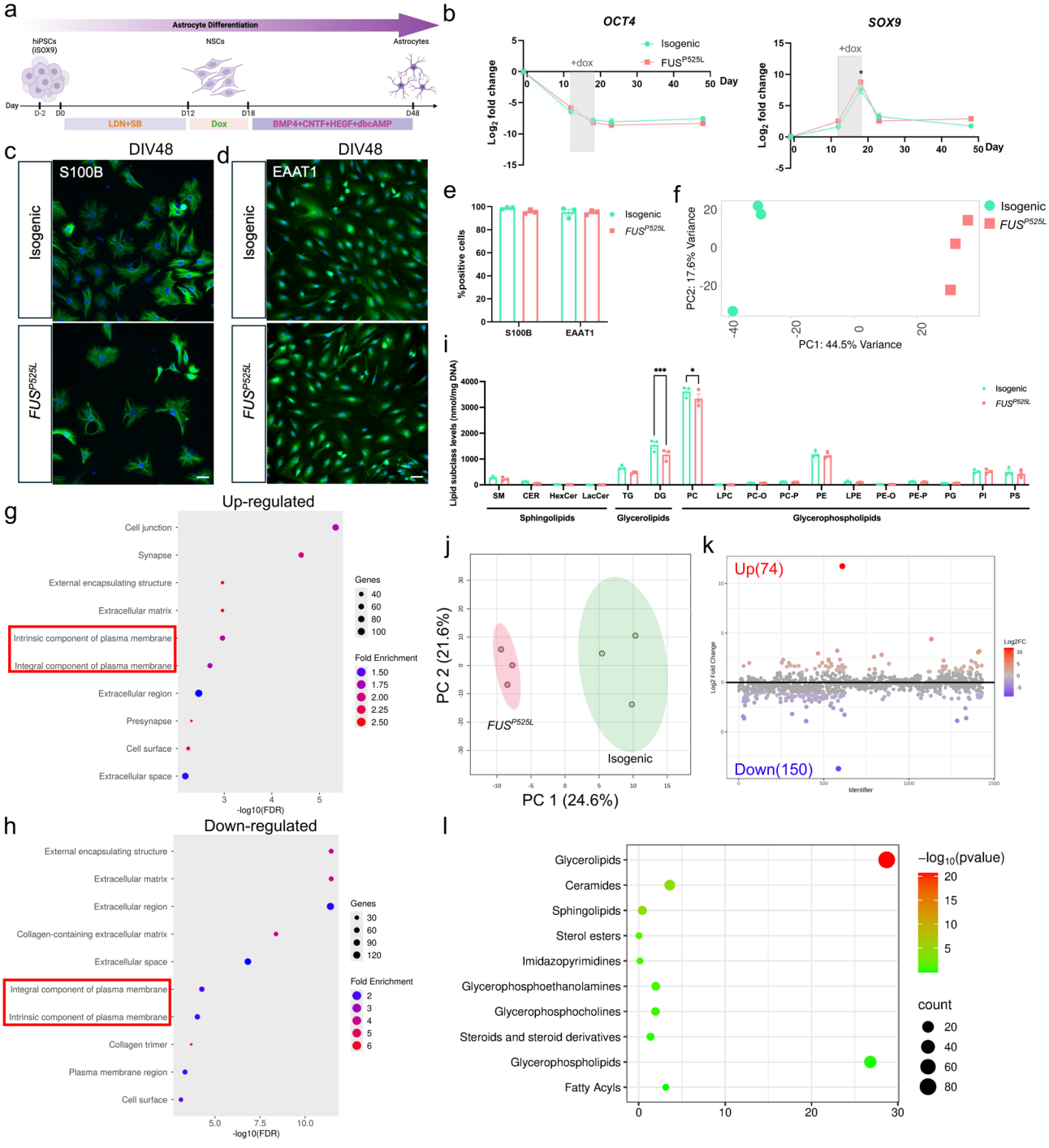

Fig. 1 (See legend on next page.)

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Fig. 1 Altered lipid homeostasis in mutant *FUS*^{P525L} astrocytes from hiPSCs. **a** Schematic overview of the astrocyte differentiation protocol. **b** RT-qPCR analysis of *OCT4* and *SOX9* transcript levels during astrocyte differentiation of *FUS*^{P525L} mutant and its isogenic control. **c**, **d** Immunofluorescent staining for S100B, and EAAT1 in Day 48 (DIV48) astrocytes derived from isogenic and *FUS*^{P525L} mutant iPSCs (scale bar: 50 µm). **e** Quantification of the percentage of S100B, and EAAT1-positive cells ($N = 3$ independent differentiations). **f** Principal component analysis (PCA) of *FUS*^{P525L} mutant and isogenic control astrocytes ($N = 3$ independent differentiations). **g**, **h** Ranked gene set enrichment analysis (GSEA) of *FUS*^{R521H} mutant astrocytes and isogenic controls. The top ten significantly enriched gene ontology (GO) terms of upregulated (**g**) and downregulated (**h**) genes in cellular component categories are presented. **i** Quantification of sphingolipid concentrations (SM, CER, HexCer, and LacCer), glycerolipid concentrations (TG and DG), glycerophospholipid subclasses (PC, LPC, PC-O, PC-P, PE, LPE, PE-O, PE-P, PG, PI, and PS) in *FUS*^{P525L} mutant astrocytes and isogenic controls. **j** PCA plot of lipidomic profiles distinguishes *FUS*^{P525L} mutant from isogenic astrocytes. **k** Differentially abundant lipid species identified by fold change analysis; upregulated lipids ($\log_2 FC > 1.0$) are shown in red, and downregulated lipids ($\log_2 FC < -1.0$) in blue. **l** Dot plot of enriched metabolites sets (top 10) of differentially abundant lipid species. (Abbreviations: CER, ceramide; DG, diglyceride; HexCer, hexosyl-ceramides; LacCer, lactosyl-ceramides; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; PC, phosphatidylcholine; PC-O, alkylphosphatidylcholine; PC-P, alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE-O, alkylphosphatidylethanolamine; PE-P, alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; TG, triglyceride). Statistical analyses were performed using two-way ANOVA (**b**, **i**) with Bonferroni's multiple comparisons test. Data are presented as mean ± SEM. *$p < 0.05$, ***$p < 0.001$

FUS^{P525L} mutation affects lipid composition. Phosphatidylcholine (PC) levels were reduced in *FUS*^{P525L} mutant astrocytes compared to their isogenic controls (Fig. 1i). Moreover, the most abundant glycerophospholipid species—such as PC(34:1), PC(36:1), PC(36:2), PC(38:4), PC-O(34:1), PC-O(36:1), PC-P(34:1), PC-P(36:1), LPC(18:0), LPC(18:1), PE(36:1), PE-O(36:1), PE-P(34:1), PE-P(36:1), PE-P(38:3), LPE(18:1), LPE(20:3), and LPE(20:3)—were decreased in *FUS*^{P525L} mutant astrocytes relative to their isogenic controls (Supplementary Figs. 2–3). Regarding glycerolipid, the overall diglycerides (DG) levels, TG(52:2), TG(54:2), TG(54:3), TG(56:4), TG(54:7), TG(56:8), and DG(36:2), were reduced in *FUS*^{P525L} mutant astrocytes compared to their isogenic controls (Fig. 1i and Supplementary Fig. 4). To visualize this robust phenotypes, we conducted a multivariate analysis using Metaboanalyst 5.0 based on normalized data. PCA of lipidomic data showed the segregation of 2 groups among the 6 samples analyzed. Samples of the same genotype clustered together (Fig. 1j). A total of 74 lipid species were significantly increased and 150 were decreased in *FUS*^{P525L} mutant astrocytes compared to isogenic controls (Fig. 1k). The enriched metabolites sets of differentially abundant lipid species suggest glycerophospholipid and glycerolipid classes (Fig. 1l).

To investigate a potential link between the *FUS*^{P525L} mutation and aberrant lipid metabolism, we performed comparative transcriptomic profiling across *FUS*^{P525L} mutant astrocytes, and MNs [4] derived from hiPSCs, alongside spinal cord tissue from sporadic ALS patients [5]. As shown in Fig. 2a, some differentially expressed genes (DEGs) were shared among these cell types. Importantly, *FUS*^{P525L} mutant astrocytes, heterozygous MNs, and homozygous MNs exhibited 11, 19, and 46 lipid metabolism-associated DEGs, respectively, while sporadic ALS spinal cords showed 48 such DEGs (Fig. 2b). Compared to isogenic control, *PLPP3* was downregulated, whereas *ST6GALNAC5*, *PLAAT3*, *PAFAH1B3*, *ENPP2*, *A4GALT*, *GCNT2*, *ST6GALNAC3*, *PPP2R2B*, *PRKCZ*, and *RAC3* were upregulated in *FUS*^{P525L} mutant astrocytes (Fig. 2c). Notably, transcriptomic data from sporadic ALS patients revealed more pronounced lipid dysregulation signatures, particularly in pathways related to glycerophospholipid metabolism (Fig. 2d), glycerolipid and sphingolipid metabolism (Fig. 2e), other lipid-related pathways (Fig. 2f), and sphingolipid signaling (Fig. 2g). Among MN subtypes, *PAFAH1B2*, *GNAI3*, *PPP2R5C*, and *LYPLA1* were consistently upregulated, while *ACHE*, *PAFAH1B3*, and *TNFRSF1A* were commonly downregulated in both heterozygous and homozygous MNs (Fig. 2h–i). As previously reported, FUS binds and/or regulates the splicing of a substantial number of lipid metabolism-related genes, including those involved in nearly all steps of the glycerophospholipid metabolism pathway [1]. Furthermore, several lipid metabolism-associated DEGs were found to participate in the glycerophospholipid metabolism pathway, as indicated by the arrows in Fig. 5j. Together, these results reveal a shared lipid dysregulation signature across *FUS*^{P525L} mutant astrocytes, and MNs, as well as in sporadic ALS spinal cords, suggesting a conserved pathogenic mechanism involving lipid metabolism.

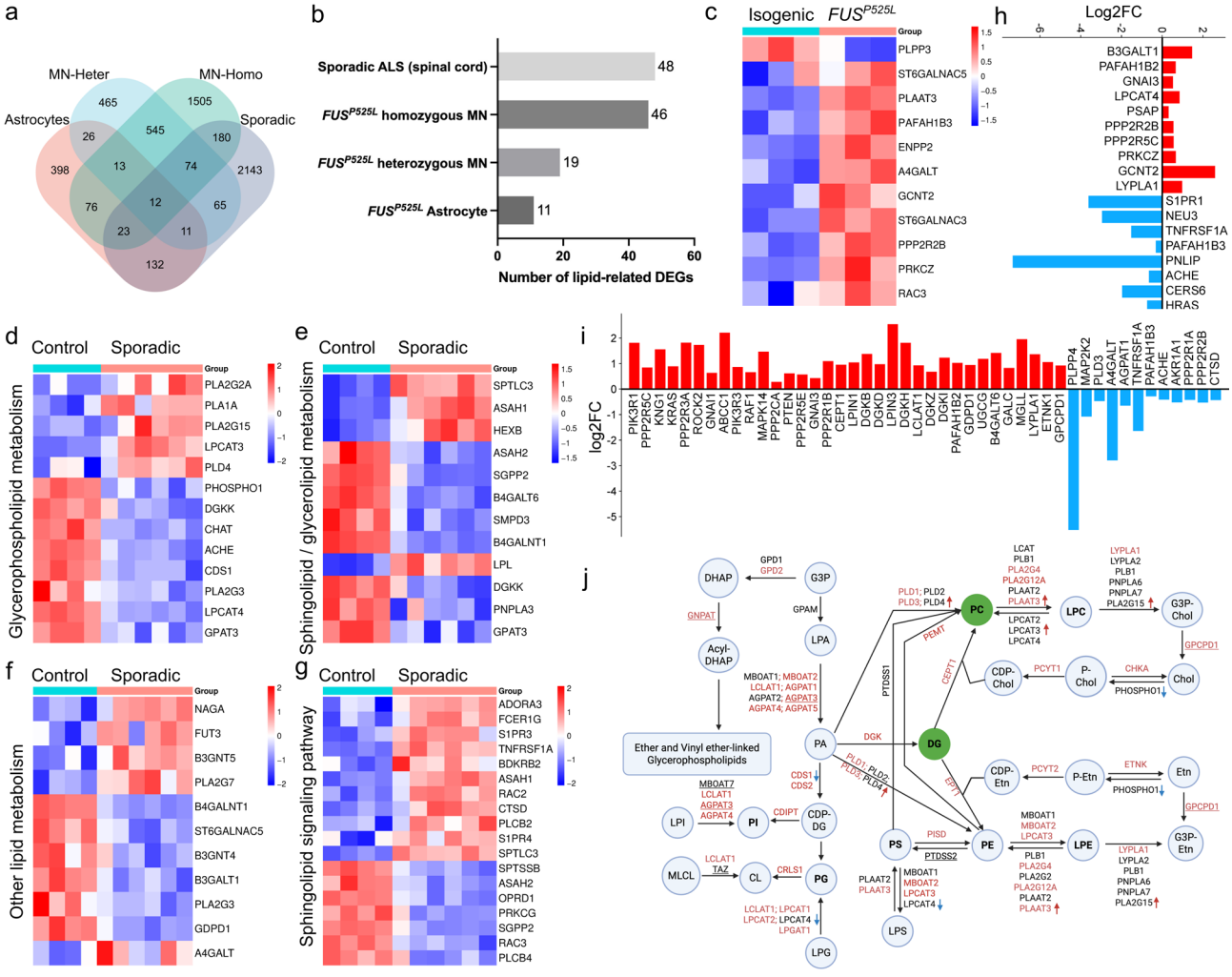

Fig. 2 Shared Lipid Dysregulation in *FUS*^{P525L} mutant Astrocytes, Motor Neurons, and Sporadic ALS Spinal Cord. **a** Venn diagram showing the overlap of DEGs across *FUS*^{P525L} mutant astrocytes, and motor neurons (MNs) derived from hiPSCs, as well as spinal cord tissues from sporadic ALS patients. **b** Bar graph summarizing the number of DEGs associated with major lipid metabolism pathways. **c** Heatmap illustrating the expression patterns of differentially expressed genes (DEGs) related to lipid metabolism in *FUS*^{P525L} mutant astrocytes compared to isogenic controls. **d–g** Heatmaps displaying the expression patterns of lipid metabolism-related DEGs in spinal cord tissues from sporadic ALS patients, categorized by pathway: glycerophospholipid metabolism (**d**), glycerolipid/sphingolipid metabolism (**e**), other lipid metabolism (**f**), and sphingolipid signaling (**g**). **h**, **i** Bar graph showing lipid metabolism-related DEGs in *FUS*^{P525L} heterozygous (**h**) and homozygous (**i**) MNs compared to isogenic controls. Upregulated genes are shown in red, and downregulated genes in blue. **j** Scheme of glycerophospholipid metabolism, in which circles indicate metabolites and arrows indicate the enzymatic reaction with the gene name encoding the enzyme. Altered lipid classes in *FUS*^{P525L} astrocytes are highlighted in green, and arrow colors indicate gene expression changes from RNA-seq data: red for upregulated and blue for downregulated genes. Genes targeted by FUS are highlighted in red, while genes spliced by FUS are underlined. This figure was adapted and modified from Fig. 5n in Zhu et al. (2024), with permission. The datasets include bulk RNA-sequencing data from spinal cord tissues of sporadic ALS patients [5] and single-cell RNA-sequencing data from *FUS*^{P525L} heterozygous (MN-heter) and homozygous (MN-homo) MNs [4]. Differentially expressed genes (DEGs) were identified using DESeq2. In bulk RNA-seq, genes with $|\log_2 FC| > 1.0$ and $-\log_{10}(FDR) > 1.0$ were considered differentially expressed. For single-cell RNA-seq datasets, statistical significance was determined using an FDR-adjusted p-value threshold of < 0.05

Abbreviations

ALS	Amyotrophic lateral sclerosis
CNS	Central nervous system
DEGs	Differentially expressed genes
DG	Diglycerides
GSEA	Gene set enrichment analysis
GO	Gene ontology
hiPSCs	Human induced pluripotent stem cells
MNs	Motor neurons
OPCs	Oligodendrocyte progenitor cells
PC	Phosphatidylcholine
PCA	Principal component analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-07120-y.

Supplementary Material 1

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Author contributions
YZ: study design and coordination, astrocytes differentiation, immunofluorescence, RT-qPCR, lipidomics, statistical analysis, and manuscript drafting and preparation. JH: lipidomics, RNAseq and critical review of the manuscript. KN: genome engineering. ML, TY, KA, and YCC: critical review of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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