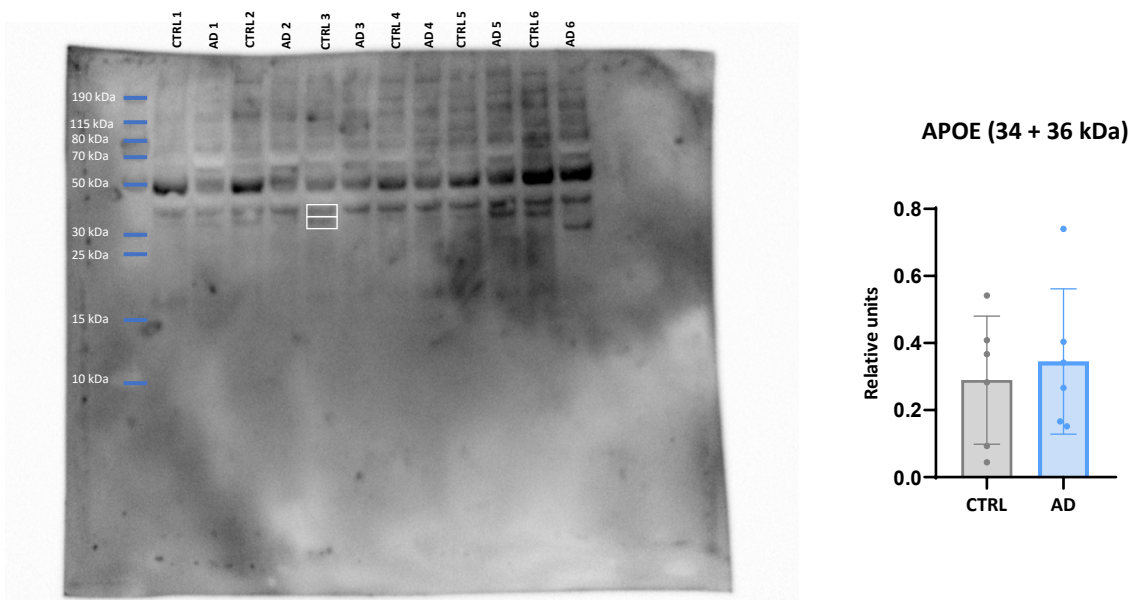


Supplementary Figures

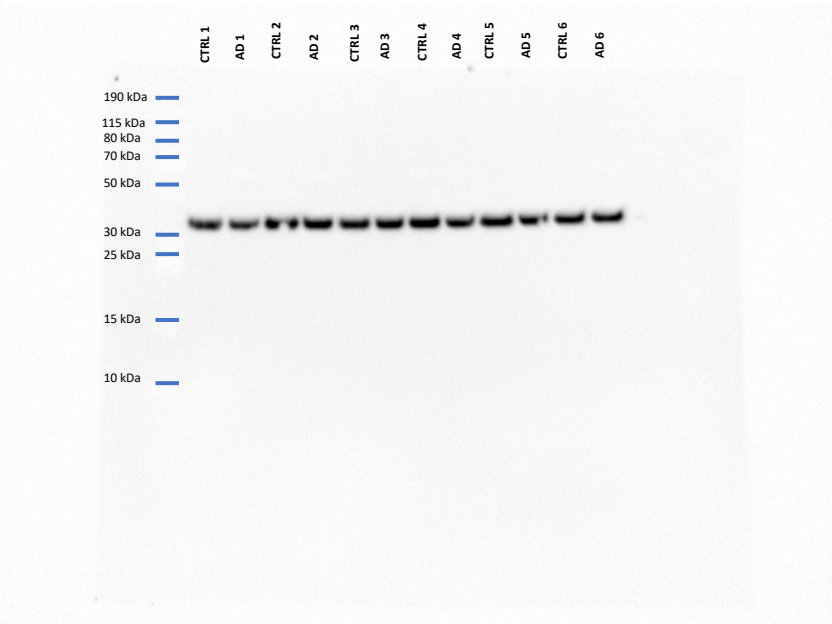
HUMAN iPSC-DERIVED APOE4/4 ALZHEIMER'S DISEASE ASTROCYTES EXHIBIT A SENESCENT AND PRO-INFLAMMATORY STATE THAT COMPROMISES NEURONAL SUPPORT

Laura Caceres-Palomo, Elisabeth Sanchez-Mejias, Laura Trujillo-Estrada, Juan José Pérez-Moreno, Elba Lopez-Oliva, Tau En Lim, Leah DeFlicht, Serena H. Chang, Lucas Kampman, M. Ryan Corces, Mathew Blurton-Jones, Ines Moreno-Gonzalez, Alberto Pascual, Javier Vitorica, Juan Antonio Garcia-Leon[‡] and Antonia Gutierrez[‡]

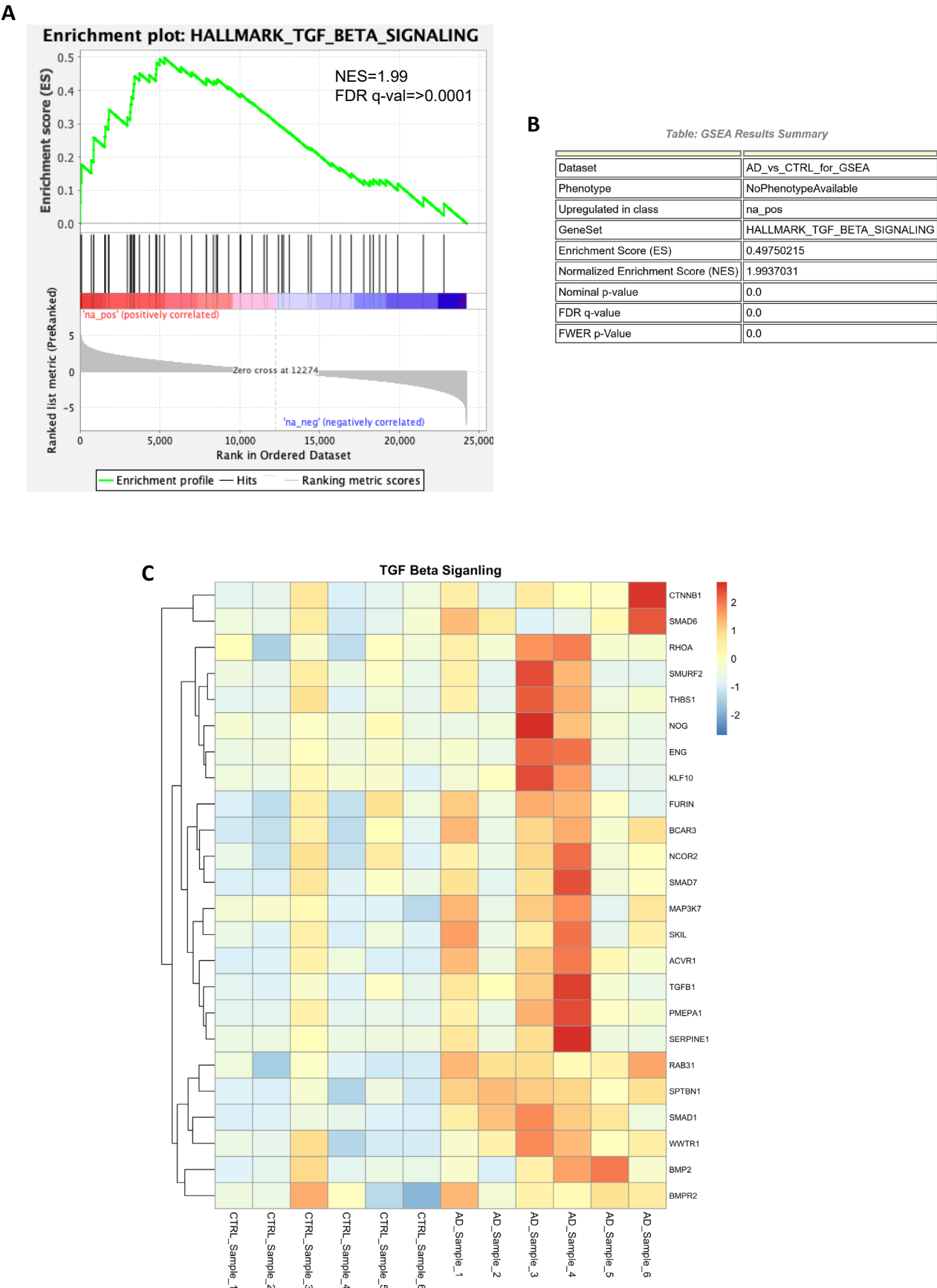
APOE



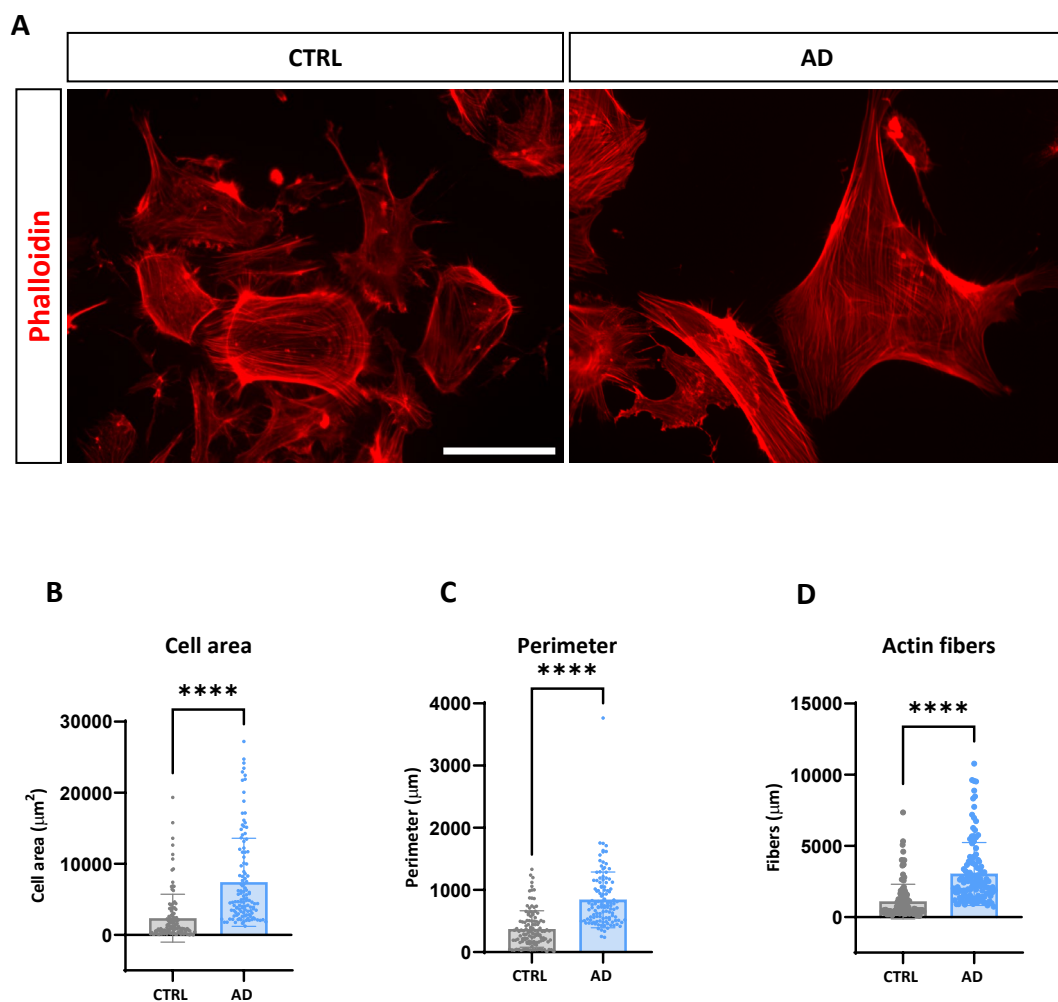
GAPDH



Supplementary Figure 1. APOE quantification in cell extracts by wester blot (WB).

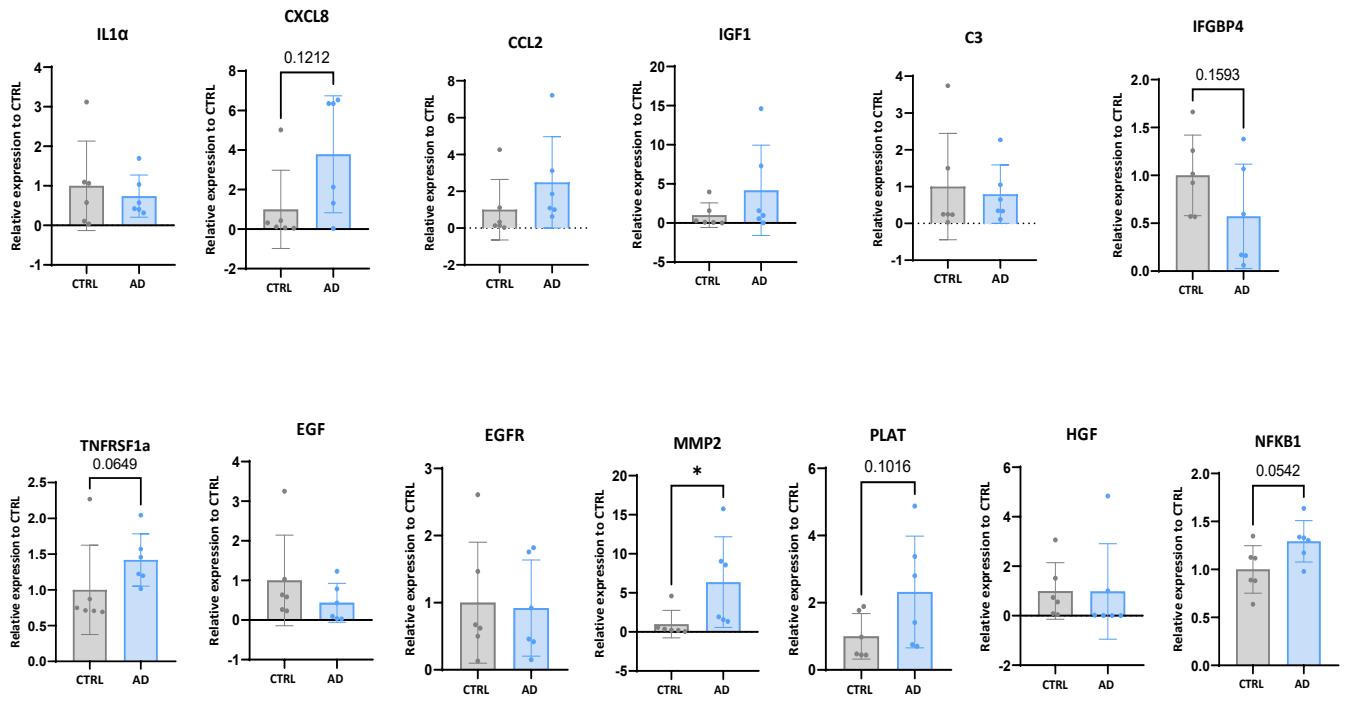


Supplementary Figure 2. Gene set enrichment analysis (GSEA) of the transforming growth factor beta (TGFb) pathway. A) Enrichment plot, B) GSEA results summary and C) heatmaps of the top 24 ranking leading edge genes.

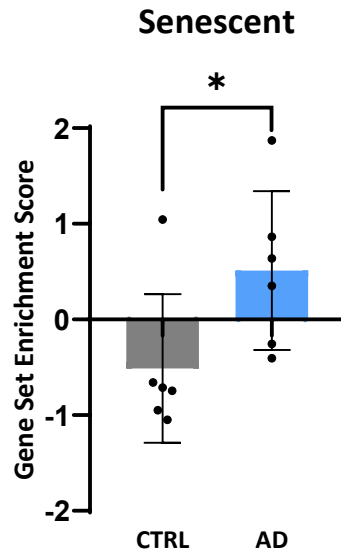


Supplementary Figure 3. Morphological characterization by Phalloidin staining of astrocytes. A) Representative fluorescence images of Phalloidin staining of cytoskeletal actin filaments in CTRL and AD astrocytes. Scale bar: 100 μm . Quantification of astroglial cell area (B), perimeter (C), and total actin fiber length (D) of astrocytes. Individual cell values are represented, along with the mean and standard deviation.

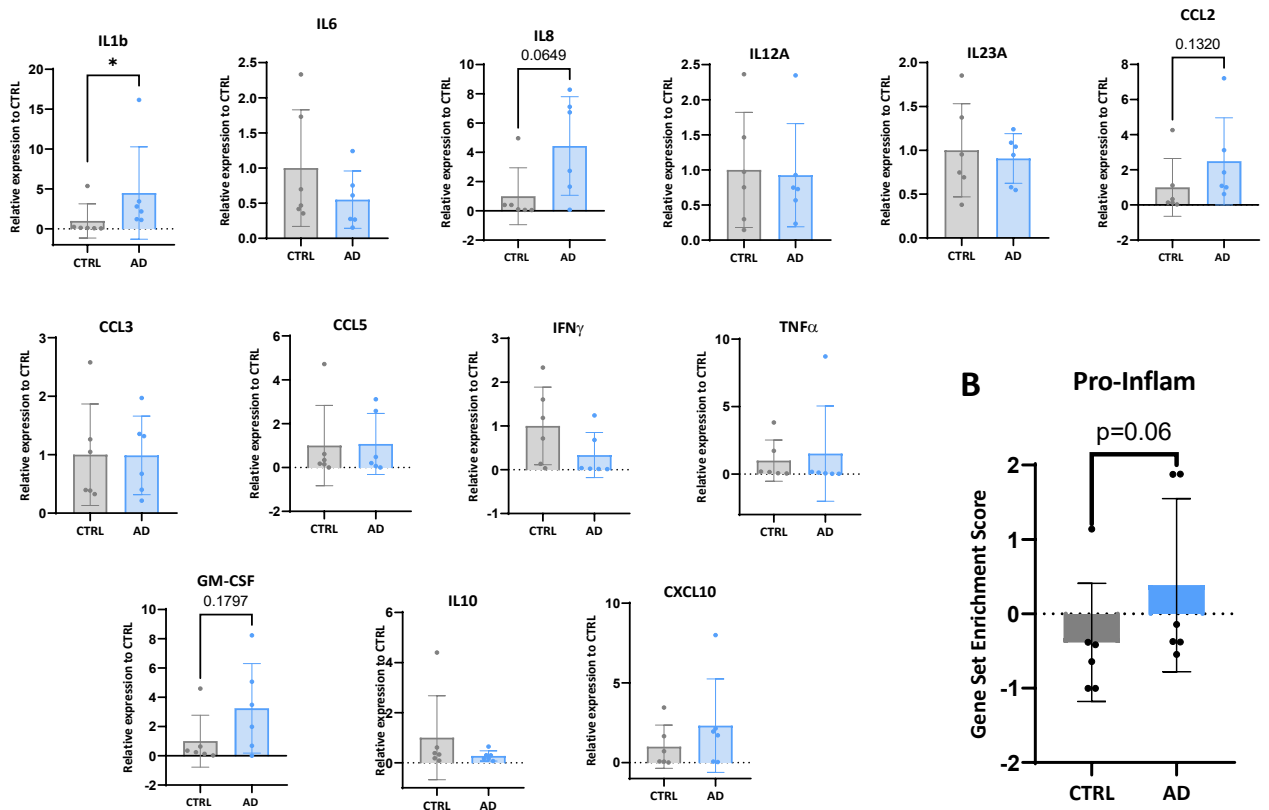
A



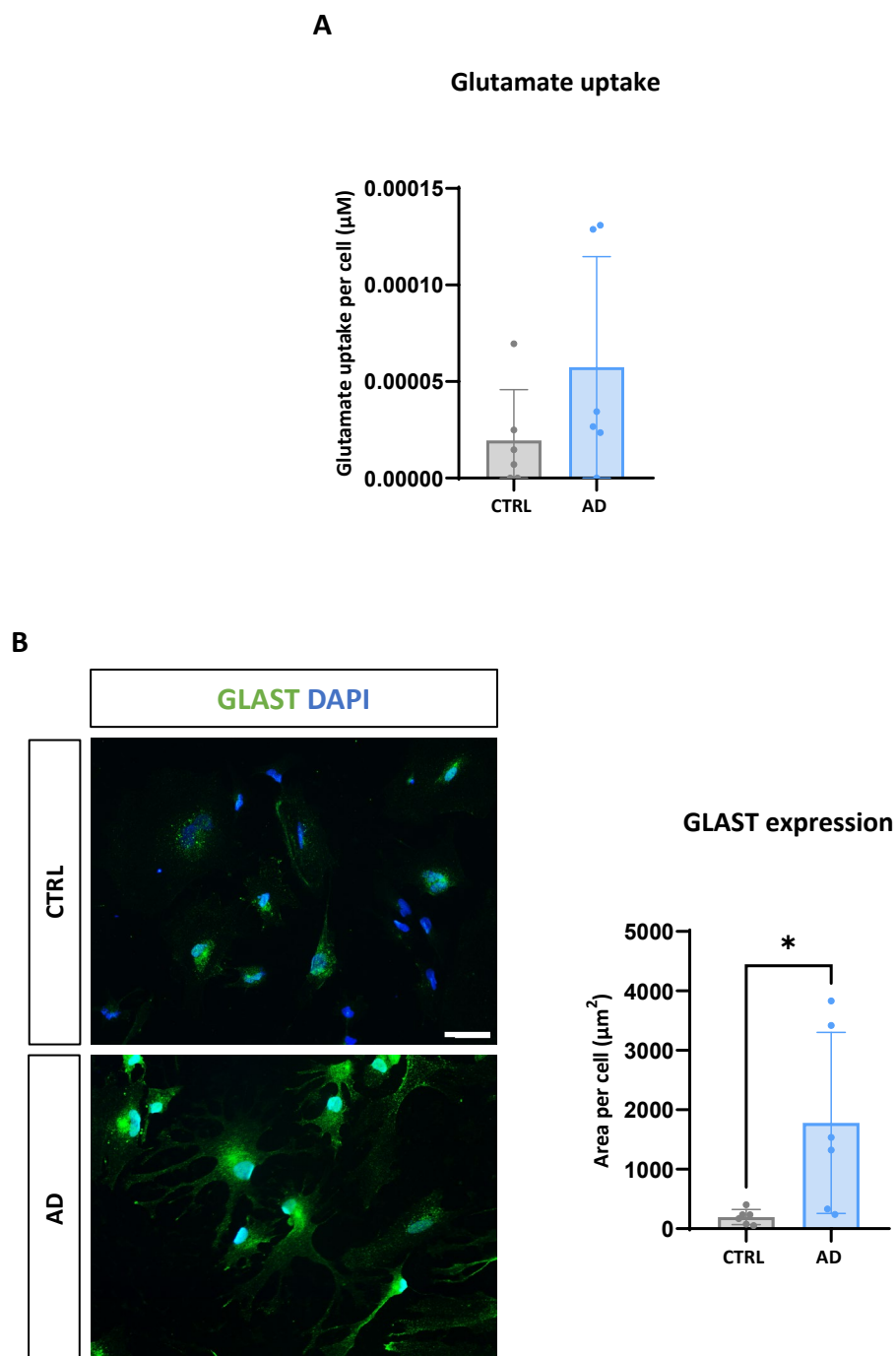
B



Supplementary Figure 4. Expression of senescent markers by human iPSC-derived AD APOE4/4 astrocytes. A) Analysis of the expression by qPCR of the set of genes most influencing the acquisition of a senescent phenotype (Saul D. et al. 2022). B) Gene set score analysis of the above senescence genes. Individual values for each cell line are represented, along with the mean and standard deviation. * $p < 0.05$.



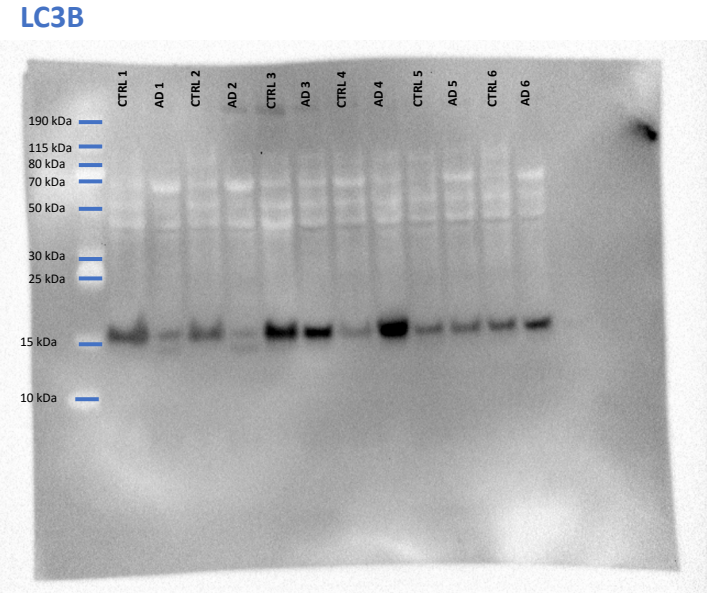
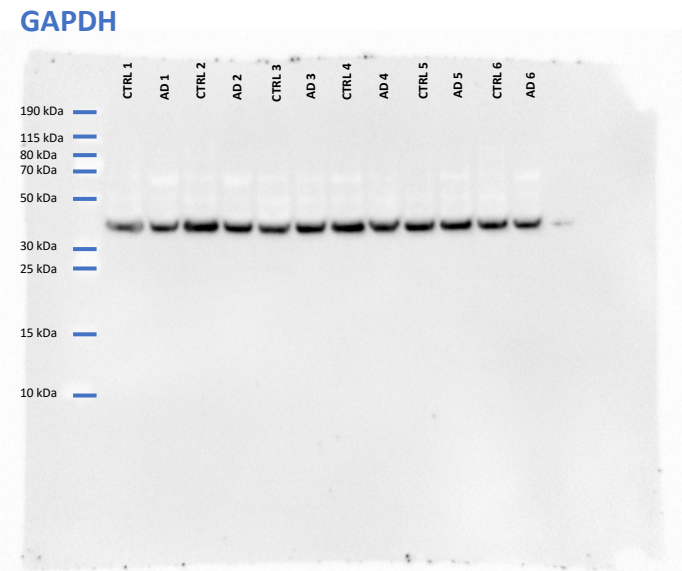
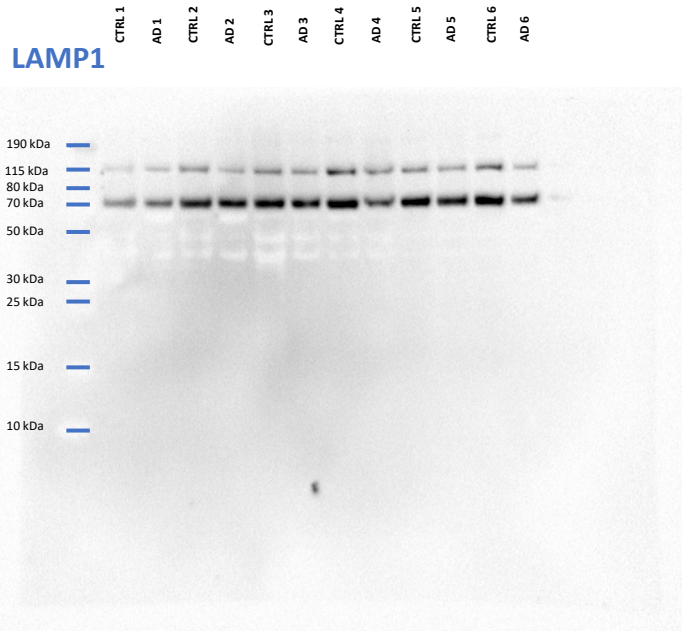
Supplementary Figure 5. Expression of inflammatory cytokines and chemokines by human iPSC-derived AD APOE4/4 astrocytes. A) Evaluation by qPCR of the gene expression of several pro- and anti-inflammatory cytokines and chemokines under basal conditions in CTRL and AD astrocytes (IL1 β , IL6, IL8, IL12A, IL23A, CCL2, CCL3, CCL5, IFN γ , TNF α , GM-CSF, IL10 and CXCL10). B) Gene score analysis of the main proinflammatory genes (IL1 β , IL6, IL8, IL12A, IL23A, CCL2, CCL3, CCL5, IFN γ , TNF α). B) Gene set score analysis of the above senescence genes. Individual values for each cell line are represented, together with the mean and standard deviation.



Supplementary Figure 6. Glutamate uptake by astrocytes. A) Quantification of glutamate consumption per cell by astrocytes from the two experimental groups. B) Images and quantification of the expression of the glutamate transporter GLAST in the two study groups. Nuclei were stained blue with DAPI. Scale bar: 50 μm. Individual values for each cell line are represented, along with the mean and standard deviation.

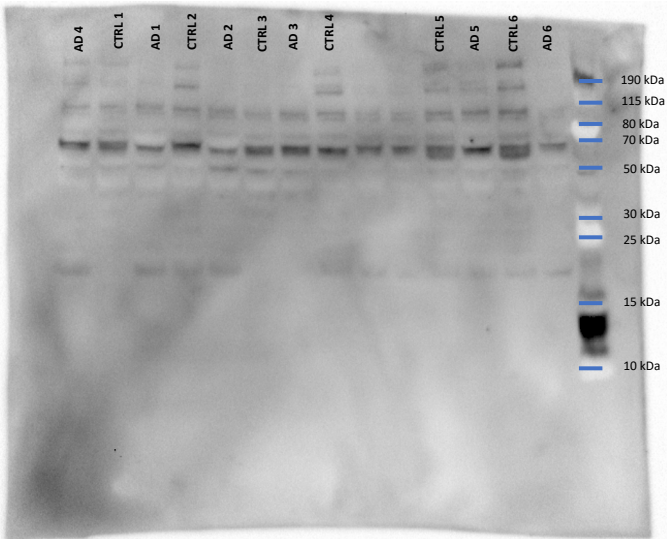
WESTERN BLOTS

WB1

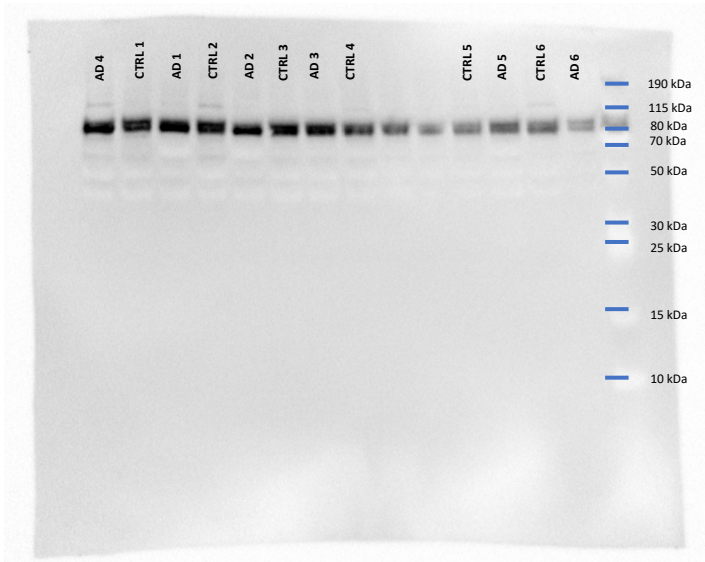


WB2

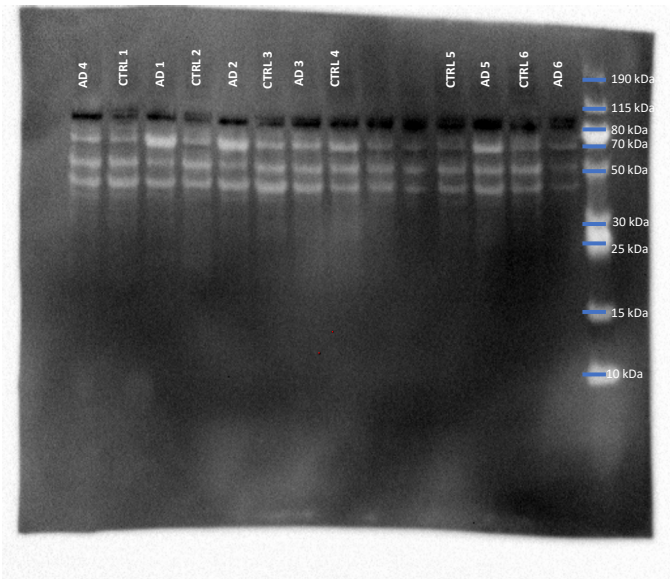
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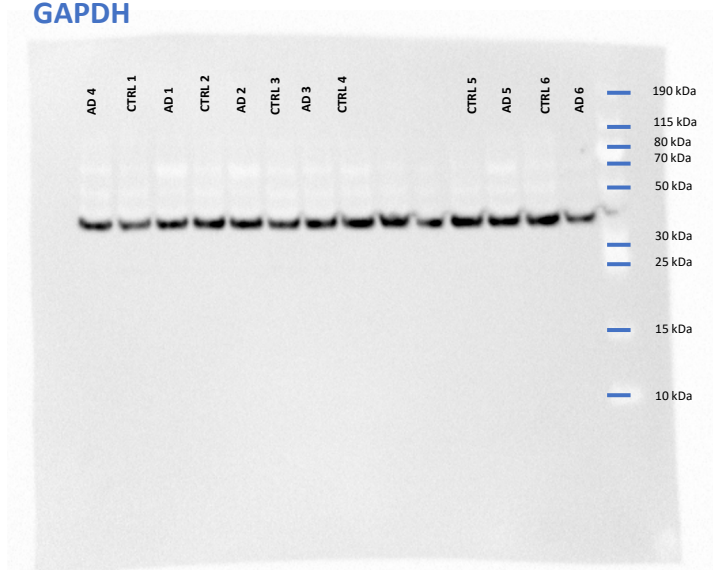
OPA1



Mfn2

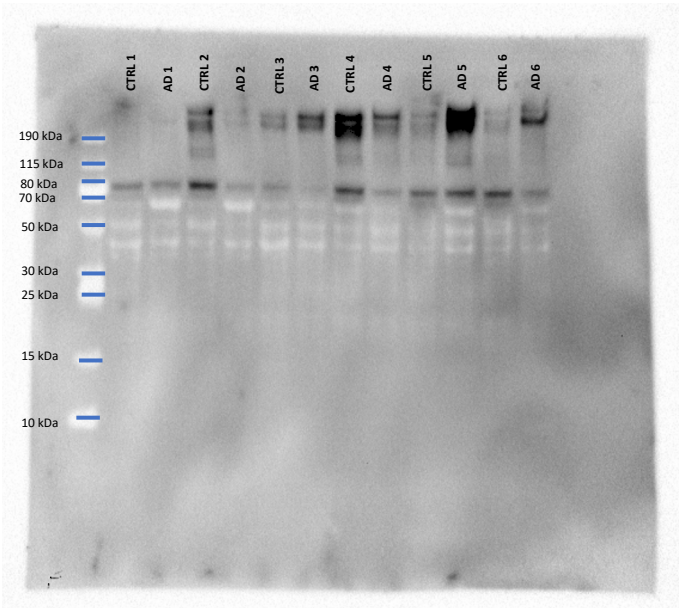


GAPDH

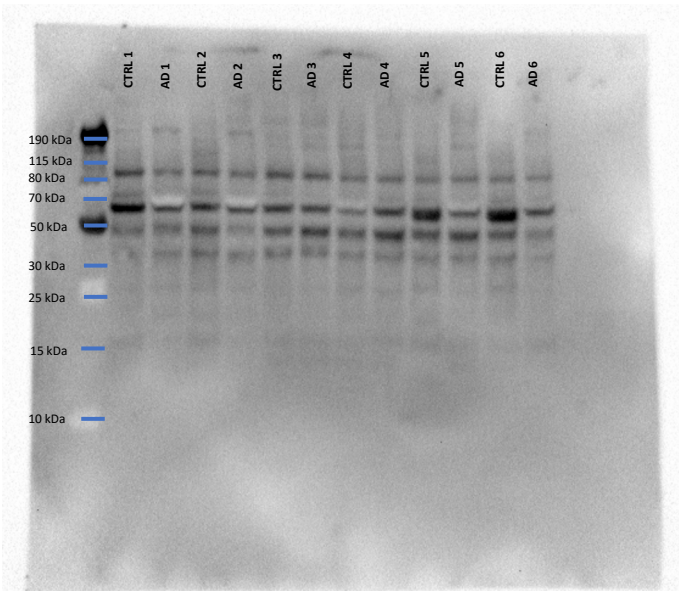


WB3

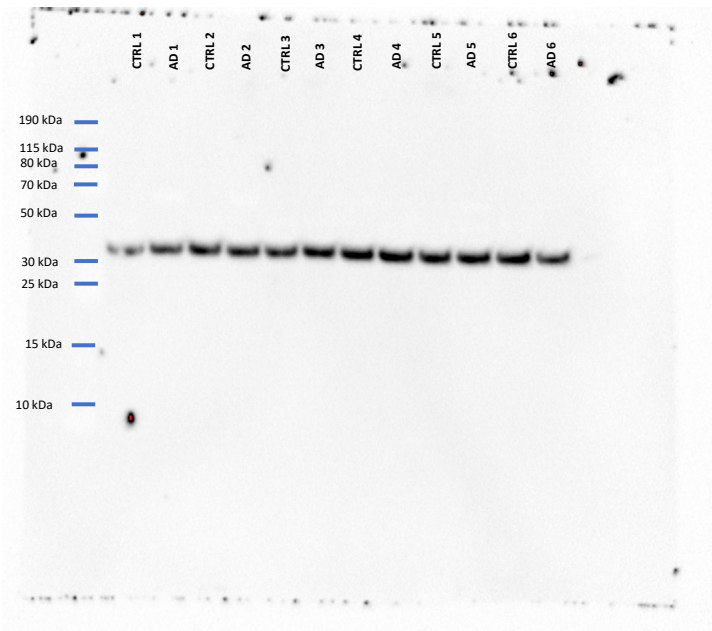
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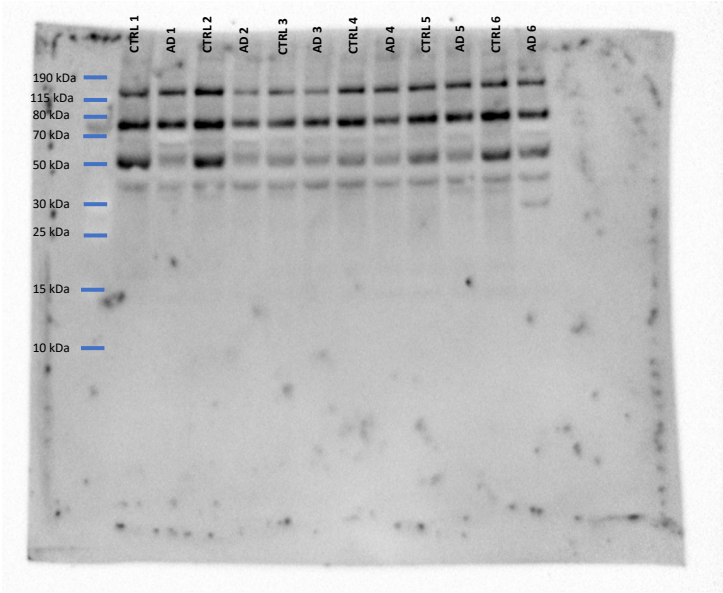
PARK2



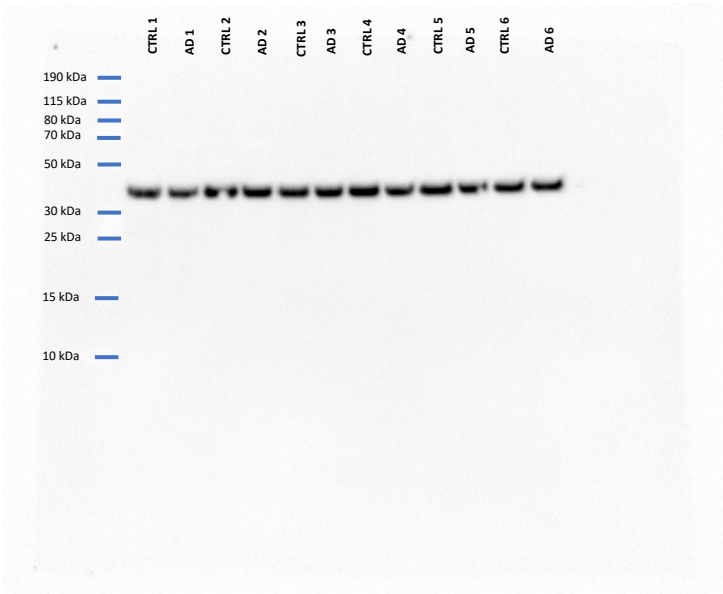
GAPDH



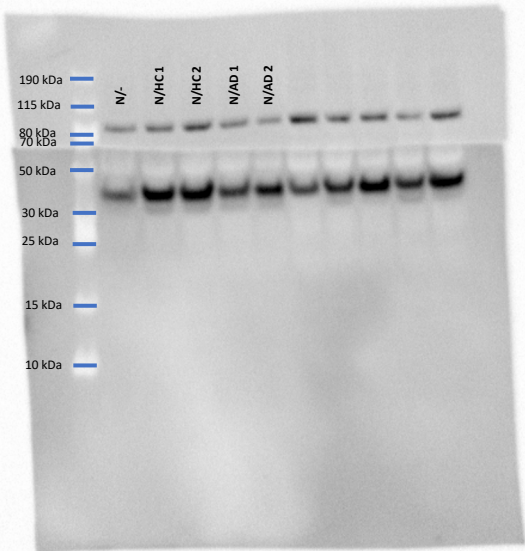
Lamp2



GAPDH



PSD-95 & SYN



GAPDH

