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Supplemental information

Warburg-like metabolic transformation underlies neuronal degeneration in sporadic Alzheimer's disease

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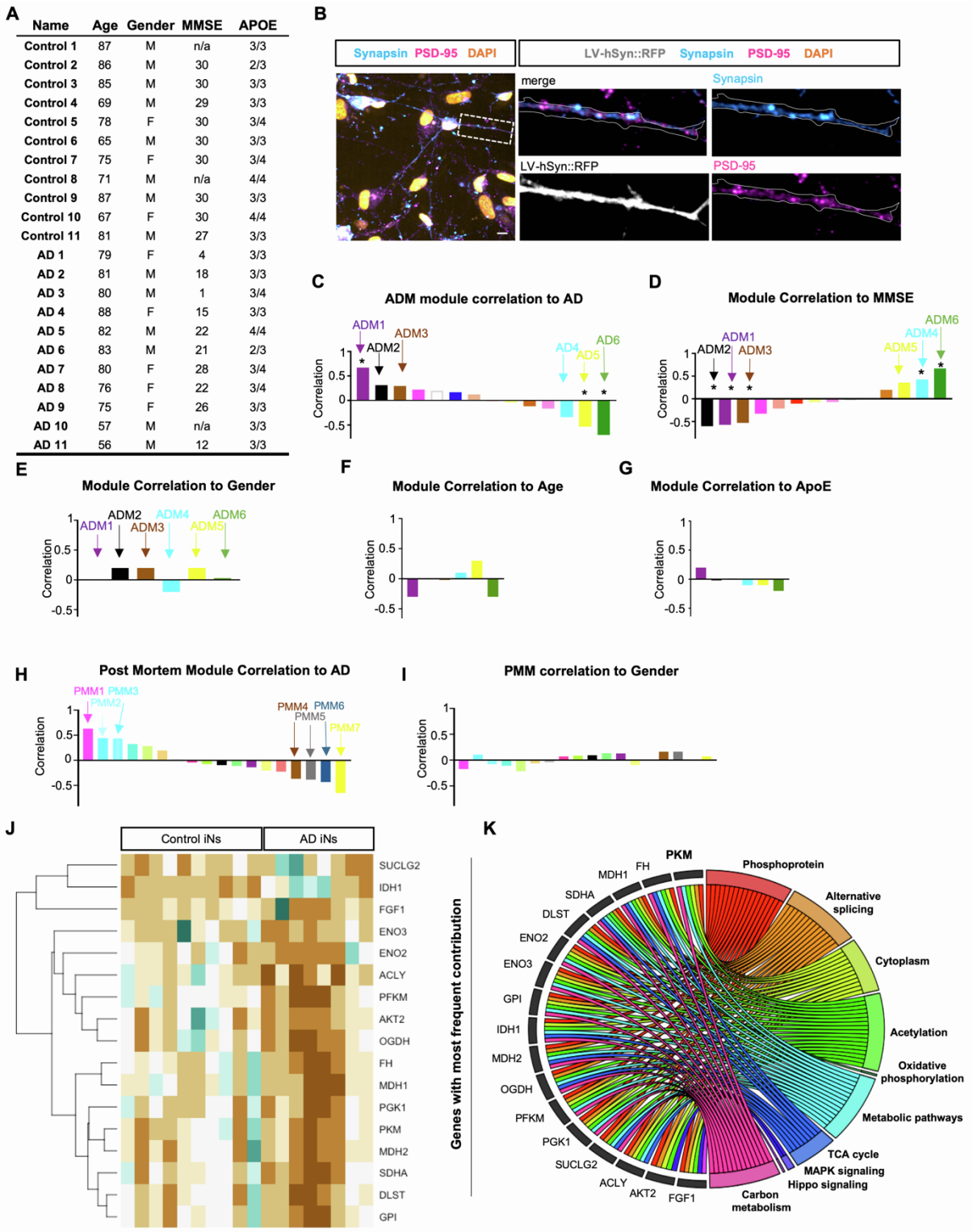

Figure S1. Gene network analysis of a cohort of control and AD iNs

Related to Figure 2. (**A**) Clinical data of 11 control and 11 patients with AD in this study. **(B)** iNs labeled with lentivirus expressing RFP under synapsin promoter, stained for synapsin and PSD95. Scale bar= 10 µm **(C-D)** Gene modules of iNs identified by WGCNA and their correlation to AD and MMSE. **(E-G)** Correlation of ADM1-ADM6 to gender, age and ApoE. **(H-I)** Gene modules of post-mortem brain and their correlation to AD and the only known variable, gender. **(J)** Chordplot of the 17 most abundant genes in the top 20 KEGG pathways and UniProt keywords and their pathway contribution. **(K)** Heatmap of gene expression of the 17 most abundant genes across the iN cohort.

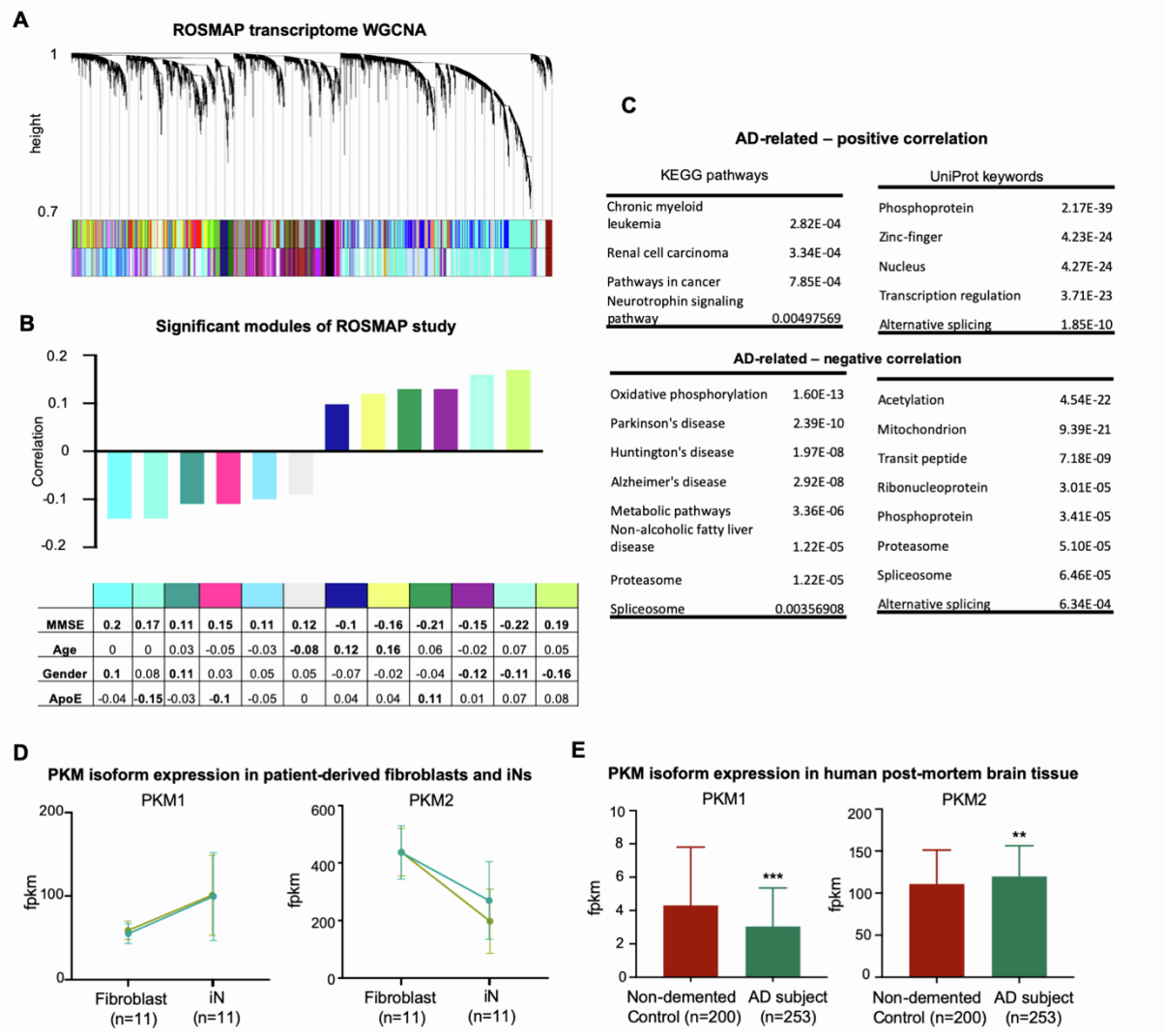

Figure S2. Gene network analysis of post-mortem brains of ROSMAP study

Related to Figures 2 and 3. **(A)** Cluster dendrogram of WGCNA analysis of vst-normalized ROSMAP transcriptomic data. **(B)** Gene modules significantly correlated to AD as a bar plot and their correlation values presented in the table. **(C)** KEGG pathways and UniProt keywords of AD-related gene modules. **(D)** PKM1 and PKM2 isoform counts comparing starting fibroblasts and iNs from the same donors. **(E)** FPKM-normalized PKM1 and PKM2 isoform counts in ROSMAP based on post-mortem diagnosis (n=633). Significance: Mann-Whitney test, **p=0.005, ***p=0.0005.

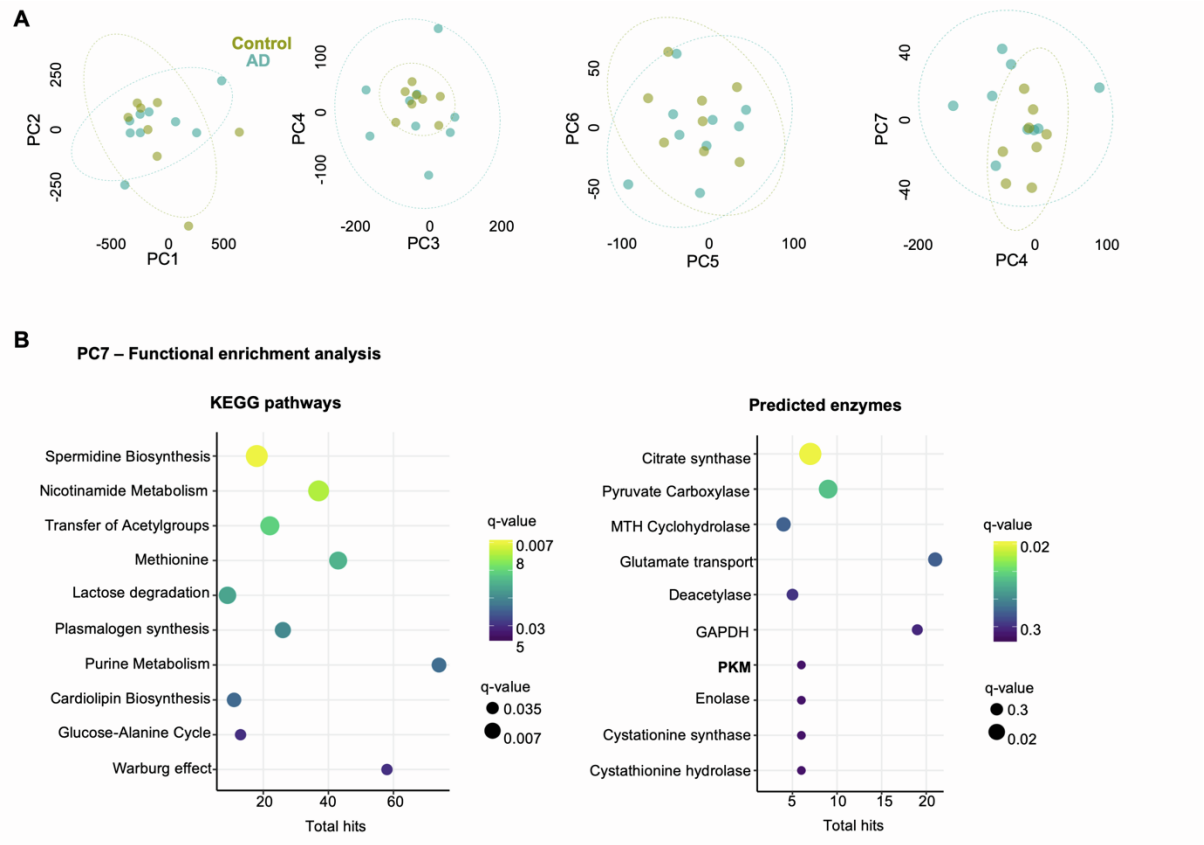

Figure S3. PCA of UHPLC-MS metabolomics in iNs

Related to Figure 4. **(A)** Dotplots of PC1-PC7 comparing control and AD iNs based on their metabolic profile (n=10 per group). **(B)** Enrichment analysis using Metaboanalyst of top 20 metabolites of PC7.

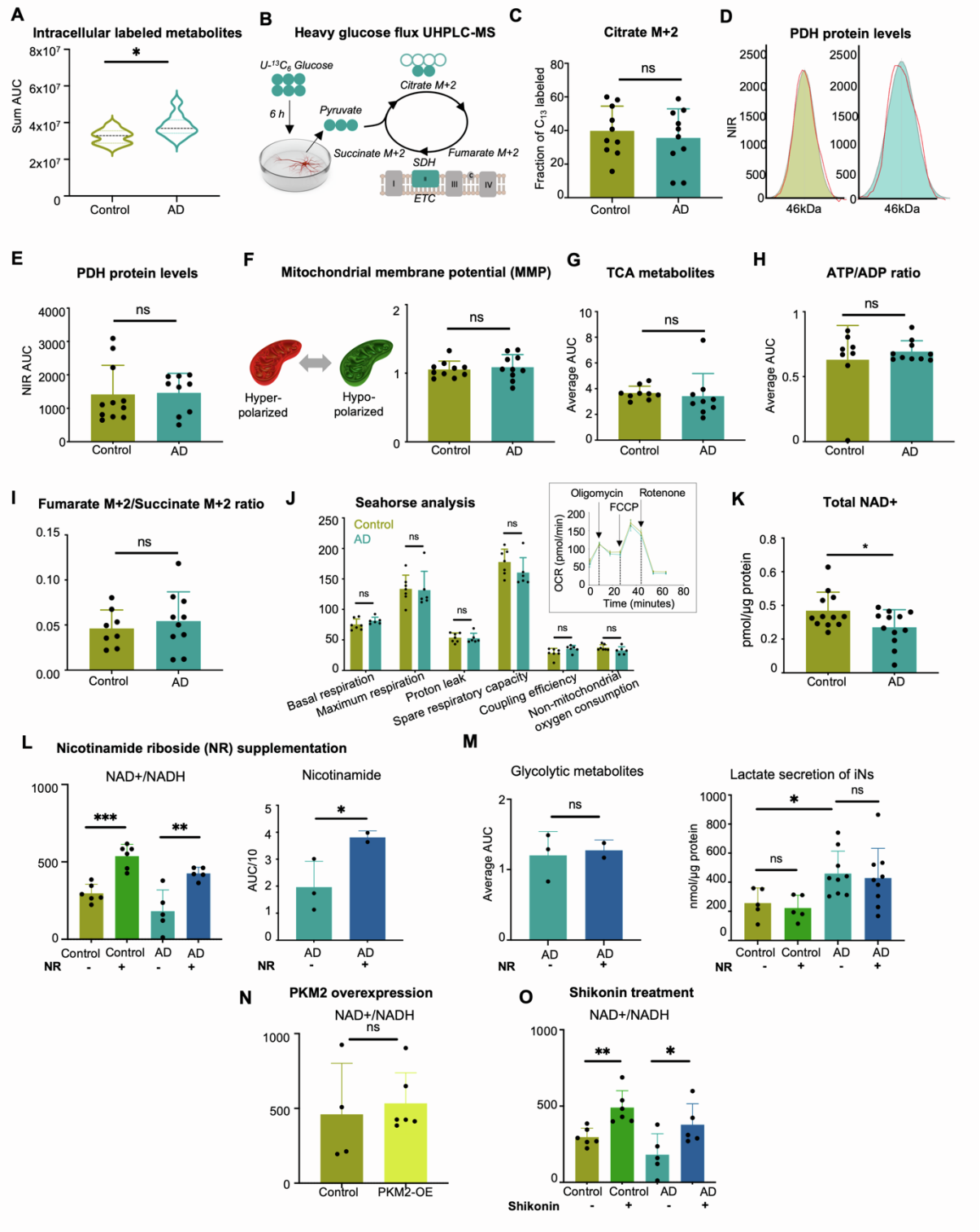

Figure S4. Mitochondrial function remains unaffected in AD iNs

Related to Figure 4. **(A)** Glucose consumption as sum of labeled metabolic intermediates after six hours of treatment with U-$^{13}C_6$-Glucose. **(B)** Scheme of glucose flux to TCA cycle and involvement in electron transport chain (ETC). **(C)** Fraction of heavy-labeled Citrate. **(D-E)** Protein level of PDH measured by ProteinSimple (n=11 control, n=9 AD). **(F)** Mitochondrial membrane potential (JC-1 dye) analyzed by FACS (control n=9, AD n=10). **(G)** Average of TCA metabolites in AD iNs (n=9) compared to controls (n=9). **(H)** Ratio of ATP and ADP levels as measured by UHPLC-MS. **(I)** Heavy-labeled fumarate/succinate level (succinate dehydrogenase (SDH) activity). **(J)** Mitochondrial respiration measured by seahorse mitochondrial stress test (n=7 control, n=6 AD). **(K)** Colorimetric assay measuring total free NAD$^+$ levels. **(L)** Restoration of NAD$^+$/NADH ratio in control and AD iNs measured by a luminescence assay (n=6 control, 5 AD; one-way ANOVA, DF: 21; F-value 18.31, p-value <0.0001), and the precursor nicotinamide measured by UHPLC-MS (n=3 control, 2 AD) after NR supplementation (300 µM, 72 hours). **(M)** Effects of NR supplementation on lactate secretion (colorimetric assay, n=5 control, n=9 AD, average for each donor of four independent experiments,

one-way ANOVA, DF: 27, F-value 3.677, p-value 0.02) and total glycolytic metabolite levels (UHPLC-MS, n=3 AD, n=2 AD + NR). **(N-O)** Effects of PKM2 overexpression (n=4 control, n=6 overexpression, unpaired t-test) and shikonin treatment (n=6 control, n=5 AD, one-way ANOVA, p-value 0.0021, F-value 7.335, DF 21) on total NAD^+ levels and NAD^+/NADH ratios measured by UHPLC-MS after 10 days of treatment and FACS-purification. Significance: unpaired t-test, *p<0.05, **p<0.001, ***p<0.005.

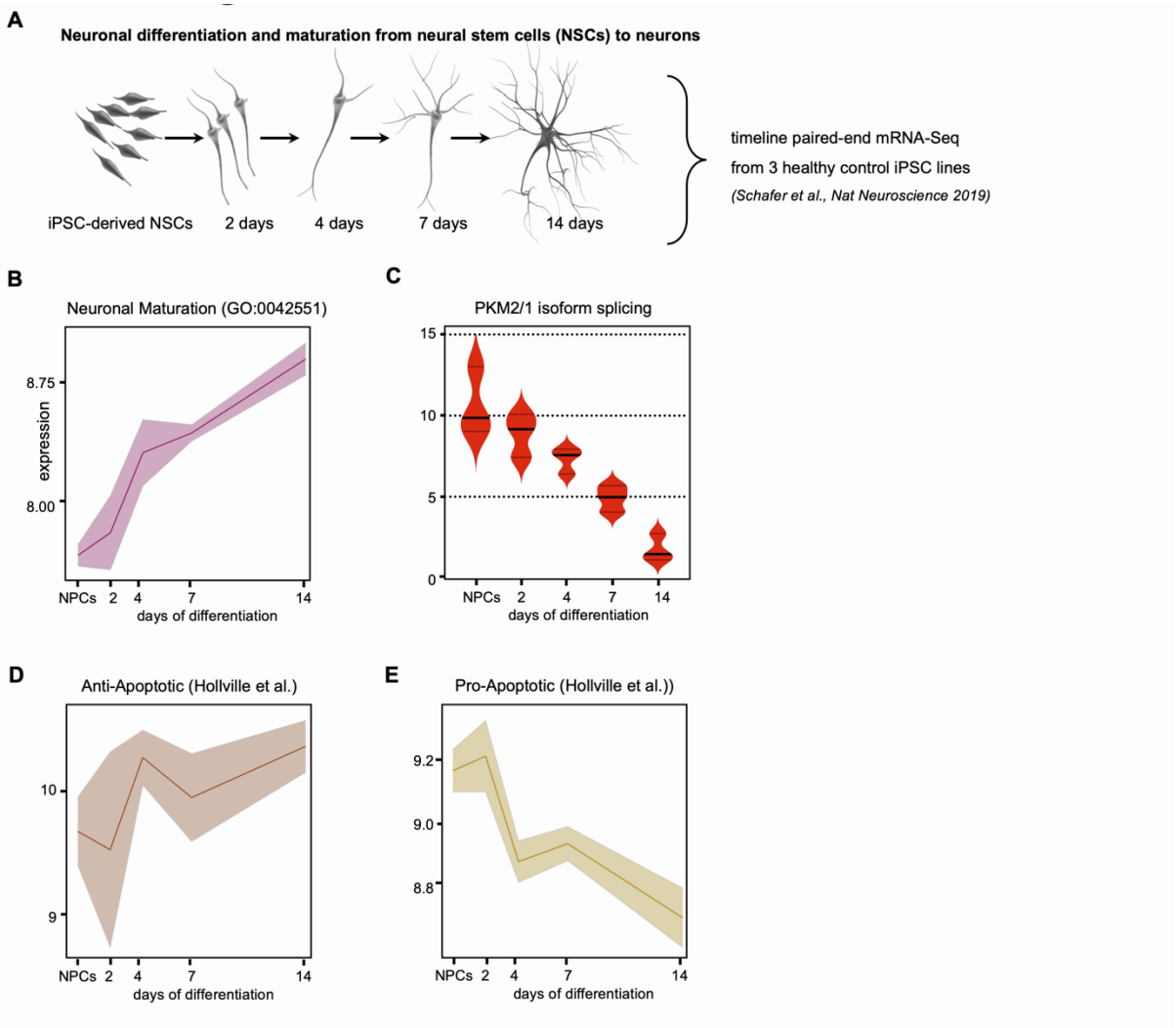

Figure S5. Apoptotic and PKM gene expression during neuronal differentiation

Related to Figure 6**. (A)** Schematic: timeline RNA-Seq differentiating iPSC-derived NSCs (Schafer et al., 2019). **(B)** Genes associated with the GO term "neuronal maturation" (GO:0042551) increase their expression along differentiation. **(C)** PKM2/PKM1 ratio decreases as neurons differentiate. **(D-E)** Differentiating neurons increase gene expression related to the neuronal apoptotic brake and decrease neuronal pro-apoptotic genes, as identified according to Holville et al. (2019).

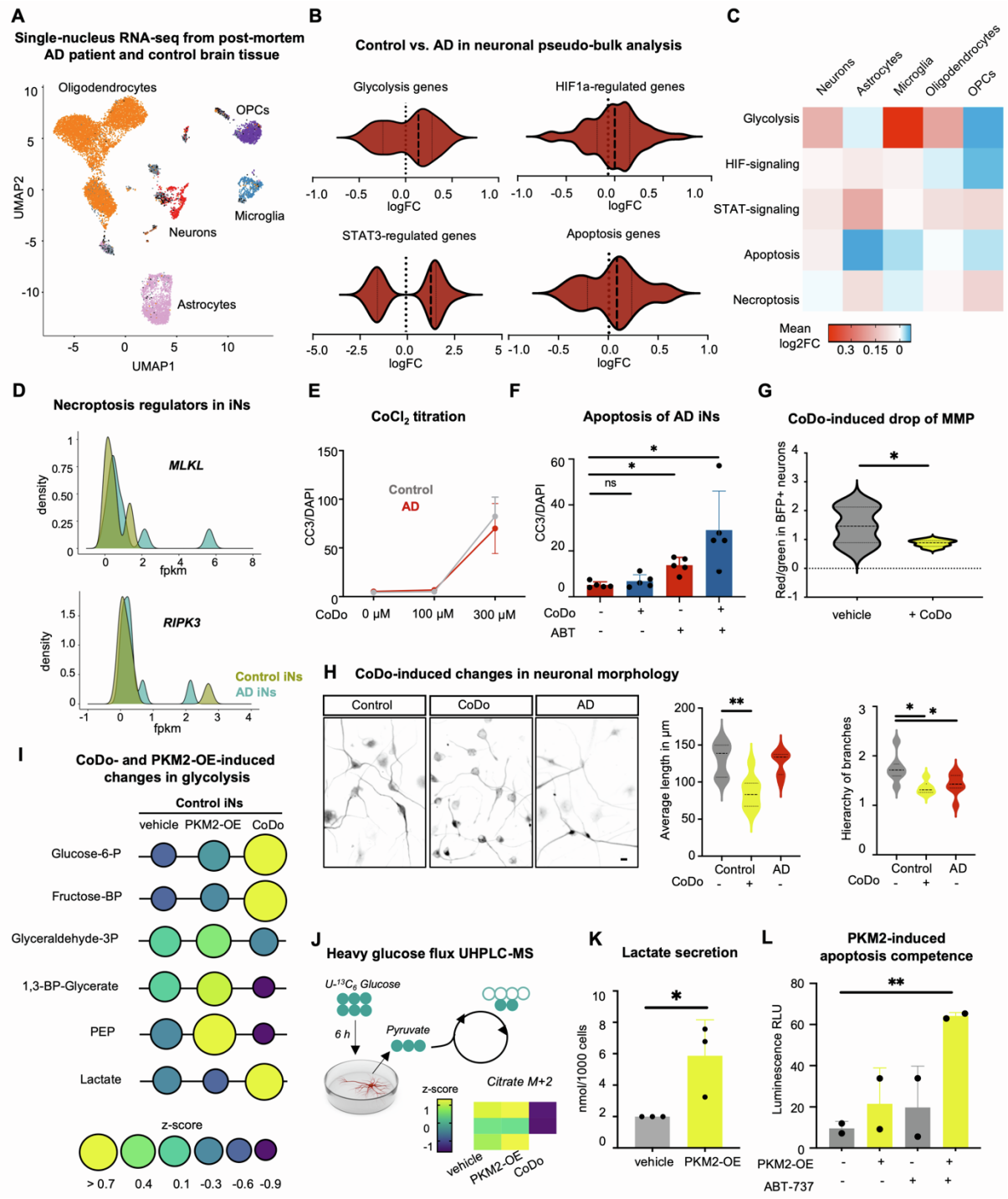

Figure S6. Induction of aerobic glycolysis in AD iNs using CoDo and PKM2 overexpression

Related to Figure 6. **(A)** UMAP of single-nuclei transcriptomic data of post-mortem brain tissue, modified from Grubman et al. (2019). **(B)** Violin plots representing log2FC of PKM-related gene expression in the neuronal subpopulation, and **(C)** heatmap showing median of log2FC of genes related to the indicated pathways comparing different cell populations in the human brain. **(D)** Distribution of FPKM-normalized counts of main necroptosis-related genes in control and AD iNs (n=10 per group). **(E)** Cell death of control and AD iNs with vehicle treatment, 100 µM and 300 µM $CoCl_2$. **(F)** Cell death assessed by cleaved caspase3/β-tubulin-positive cells of AD iNs. **(G)** Mitochondrial membrane potential in control and AD iNs with and without CoDo treatment (n=3 per group). **(H)** Neuronal morphology assessed by β-tubulin staining in control, CoDo-treated control, and AD iNs (n=9, scale bar 10 µm). Average length measured from cell body to end of longest dendrites; hierarchy representing complexity of branching (one-way ANOVA, length: p-value <0.0001, F-value 14.27, DF 26; hierarchy: p-value 0.0089, F-value 6.12, DF 21). **(I)** UHPLC-MS metabolomics comparing control iNs overexpressing GFP::PKM2 for 48 hours (n=3), and CoDo

treated iNs (n=2) compared to GFP-vehicle treated control iNs (n=3). Size and color of circles represent z-scores of respective metabolites. **(J)** UHPLC-MS measurement of labeled citrate after six hours of $^{13}C_6$ Glucose treatment. **(K)** Lactate secretion measured by colorimetric assay in control iNs overexpressing GFP-vehicle or GFP::PKM2 (n=3 per group). **(L)** Apoptosis competence measured by the Luminescence Caspase 3/7-Glo assay (n=2 per group, one-way ANOVA, DF: 7, F-value 6.496, p-value 0.04). Significance: unpaired t-test, *<0.05, **<0.01.

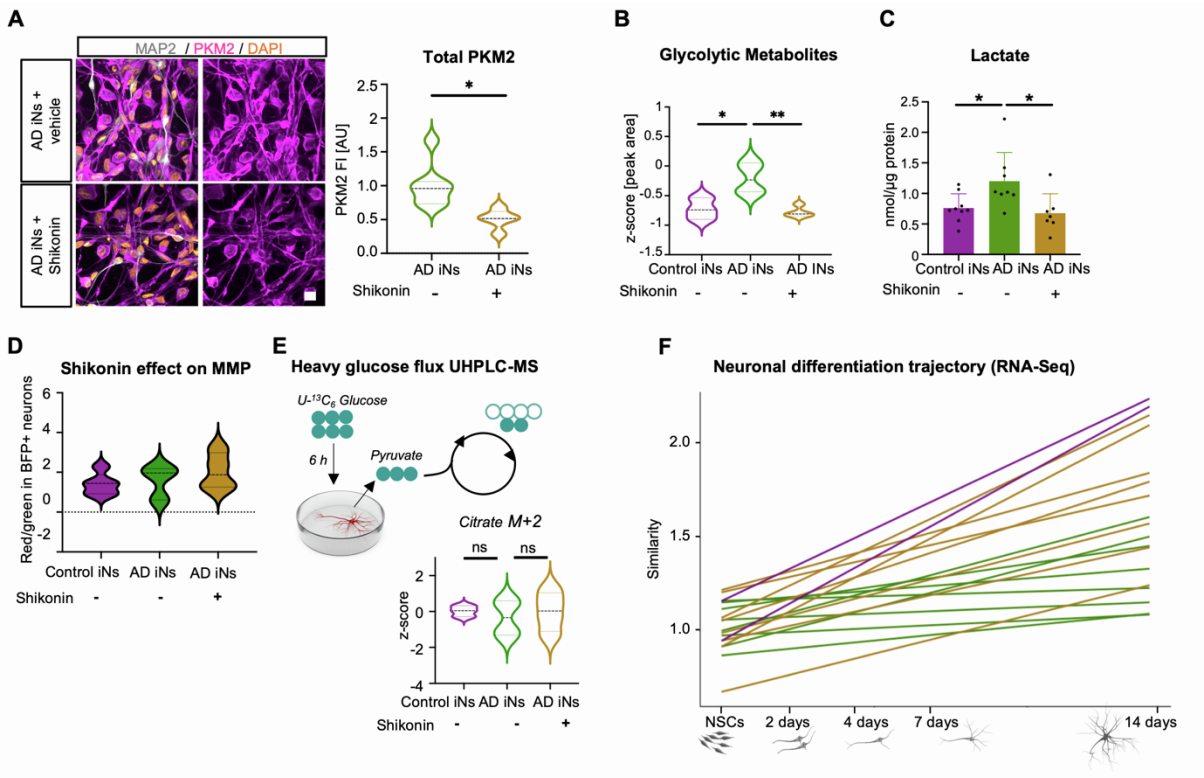

Figure S7. Shikonin restores mature neuronal phenotype
Related to Figure 7. **(A)** Immunostaining and quantification of total PKM2 FI in MAP2-ROIs in AD iNs with and without shikonin (vehicle n=6, shikonin n=5, unpaired t-test). Scale bar = 10 µm. **(B)** Average of all detected glycolytic metabolites measured by UHPLC-MS-based metabolomics (DF:10, F-value 10.12, p-value 0.0064). **(C)** Colorimetric assay measuring lactate levels in supernatant of control (n=9), AD (n=8) and shikonin-treated AD (n=7) iNs normalized to total protein (DF:23, F-value 5.087, p-value 0.01). **(D)** Mitochondrial membrane potential of iNs treated with and without shikonin measured by JC-1 in PSA-NCAM$^+$ neurons (n=3 per group, DF:11, F-value 0.78, p-value 0.48). **(E)** Tracing of isotope-labeled glucose after six hours of incubation with $^{13}C_6$-Glucose. Fraction of labeled glucose detected in citrate (n=10 per group, DF:10, F-value 0.18, p-value 0.8). **(F)** Correlation of trajectory genes in control (n=3), AD (n=8) and AD + shikonin (n=8) iNs to neuronal differentiation from NSCs to neurons (Schafer et al. 2019) for each patient. **(B-E)** Significance: one-way ANOVA *<0.05, **<0.01.