

Proteomic analyses reveal molecular changes underlying the preferential vulnerability of human female CA1 hippocampal subfield across Braak staging in Alzheimer disease

Carmen Soriano-Herrador MS^{1,2}, Isabel Ubeda-Banon PhD^{1,2}, Patricia Villanueva-Anguita¹, Jose Luis Montesinos-Vinader^{1,2}, Daniel Saiz-Sanchez PhD^{1,2}, Veronica Astillero-Lopez PhD^{1,2}, Pilar Alberdi-Velez³, Joaquin Fernandez-Irigoyen⁴, Mercedes Lachen-Montes⁴, Enrique Santamaria⁴, Alino Martinez-Marcos PhD^{1,2*}, Alicia Flores-Cuadrado PhD^{1,2*}

¹Neuroplasticity and Neurodegeneration group, IB-UCLM, Ciudad Real Medical School, University of Castilla-La Mancha (UCLM), Spain.

²Grupo de Neuroplasticidad y Neurodegeneración, Instituto de Investigación Sanitaria de Castilla-La Mancha (IDISCAM), Spain.

³Ciudad Real Medical School, University of Castilla-La Mancha, University of Castilla-La Mancha (UCLM), Spain.

⁴Clinical Neuroproteomics Unit, Proteomics Platform, Navarrabiomed, Hospital Universitario de Navarra (HUN), IdiSNA, Navarra Institute for Health Research, Universidad Pública de Navarra (UPNA), Irunlarrea 3, 13008 Pamplona, Spain.

Supplementary table 1. Comparison between the ages of the experimental groups. A p-value < 0.05 was considered significant

Group	Mean $\pm$ SD	n	One-way ANOVA	
			F (DFn, DFd)	p value
Initial-AD (Braak tau I-II)	81.14 $\pm$ 8.03	7	F (2, 25) = 0.6166	0.5478
Intermediate-AD (Braak tau III-IV)	85.10 $\pm$ 7.39	10		
Advanced-AD (Braak tau V-VI)	81.82 $\pm$ 8.94	11		

Supplementary table 2. Statistical analysis of MALDI-TOF MSI results. Results of the statistical analysis of data obtained from mass spectrometry imaging of hippocampal sections. Peptide intensities obtained in the three experimental groups (Initial-AD, Intermediate-AD, and Advanced-AD) were compared in the CA1, CA2, CA3, and DG subregions. Mr(expt): experimental m/z; HPC1: Initial-AD (Braak tau I-II); HPC2: Intermediate-AD (Braak tau III-IV); HPC3: Advanced-AD (Braak tau V-VI). A p-value < 0.05 was considered significant

Mr(expt)	F value	P value	FDR	HSD Tukey <i>post hoc</i> test
726.588	3.351	3.18E-04	1.08E-02	CA3_HPC3-CA1_HPC2; CA3_HPC3-CA2_HPC2
727.585	3.280	4.08E-04	1.26E-02	CA3_HPC2-CA1_HPC3; CA3_HPC2-CA2_HPC2
730.552	2.958	1.26E-03	2.51E-02	CA1_HPC3-CA1_HPC2; CA2_HPC1-CA1_HPC3; CA2_HPC2-CA1_HPC3; CA2_HPC3-CA1_HPC3; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3
757.578	2.704	3.02E-03	3.94E-02	CA2_HPC3-CA1_HPC3; CA3_HPC1-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3
758.575	2.799	2.18E-03	3.15E-02	CA2_HPC3-CA1_HPC3; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3; DG_HPC3-CA1_HPC3
778.546	3.545	1.60E-04	7.73E-03	CA2_HPC2-CA1_HPC3; CA2_HPC3-CA1_HPC3; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3
779.543	3.648	1.11E-04	6.26E-03	CA1_HPC3-CA1_HPC2; CA2_HPC2-CA1_HPC3; CA2_HPC3-CA1_HPC3; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3; DG_HPC3-CA1_HPC3
785.601	3.249	4.55E-04	1.34E-02	CA1_HPC3-CA1_HPC2; CA2_HPC3-CA1_HPC3; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3; DG_HPC3-CA1_HPC3
836.431	3.526	1.71E-04	7.73E-03	CA1_HPC3-CA1_HPC2; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3; DG_HPC2-CA2_HPC1; DG_HPC2-CA2_HPC3
852.447	3.760	7.45E-05	4.59E-03	CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3; CA3_HPC2-CA2_HPC3; DG_HPC2-CA2_HPC3
853.615	3.601	1.31E-04	6.85E-03	CA1_HPC3-CA1_HPC2; CA2_HPC3-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3; DG_HPC3-CA1_HPC3
868.463	2.855	1.80E-03	3.01E-02	DG_HPC2-CA1_HPC3; DG_HPC2-CA2_HPC1
887.621	3.368	2.99E-04	1.07E-02	CA2_HPC3-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3; DG_HPC3-CA1_HPC3
908.614	2.831	1.95E-03	3.01E-02	CA3_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC1; CA3_HPC3-CA1_HPC3
909.636	3.979	3.41E-05	2.57E-03	CA2_HPC3-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC1; CA2_HPC3-CA1_HPC3; CA3_HPC3-CA1_HPC3
910.633	3.400	2.67E-04	1.07E-02	CA2_HPC3-CA1_HPC1; CA3_HPC3-CA1_HPC1; CA2_HPC3-CA1_HPC3; CA3_HPC3-CA1_HPC3

Mr(expt)	F value	P value	FDR	HSD Tukey <i>post hoc</i> test
1000.686	2.798	2.19E-03	3.15E-02	CA3_HPC1-CA1_HPC1; CA3_HPC1-CA1_HPC3
1001.683	2.840	1.90E-03	3.01E-02	CA2_HPC3-CA1_HPC1; CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC3-CA1_HPC1
1046.71	2.916	1.46E-03	2.67E-02	CA1_HPC3-CA1_HPC2; CA2_HPC2-CA1_HPC3; CA3_HPC3-CA2_HPC2; DG_HPC3-CA2_HPC2
1087.747	2.813	2.08E-03	3.12E-02	CA3_HPC1-CA1_HPC1; CA3_HPC1-CA1_HPC3
1107.692	2.969	1.21E-03	2.49E-02	CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3
1117.74	3.224	4.97E-04	1.40E-02	CA3_HPC2-CA1_HPC1; CA3_HPC3-CA1_HPC1; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3
1148.755	4.708	2.54E-06	3.43E-04	CA2_HPC1-CA1_HPC3; CA3_HPC1-CA2_HPC1; CA3_HPC2-CA2_HPC1; CA3_HPC3-CA2_HPC1; DG_HPC2-CA2_HPC1; DG_HPC3-CA2_HPC1; DG_HPC3-CA2_HPC2
1149.777	3.393	2.74E-04	1.07E-02	CA2_HPC1-CA1_HPC3; CA3_HPC2-CA2_HPC1; CA3_HPC3-CA2_HPC1; DG_HPC2-CA2_HPC1; DG_HPC3-CA2_HPC1
1152.769	3.330	3.42E-04	1.10E-02	DG_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC3; DG_HPC3-CA2_HPC1
1159.749	2.635	3.82E-03	4.62E-02	CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1
1163.788	3.080	8.22E-04	1.90E-02	CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3
1164.761	3.092	7.91E-04	1.90E-02	CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3
1214.774	3.110	7.41E-04	1.90E-02	CA2_HPC3-CA1_HPC3; CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3
1262.817	2.735	2.72E-03	3.61E-02	CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3
1269.822	2.625	3.96E-03	4.70E-02	CA3_HPC2-CA1_HPC1; CA3_HPC2-CA1_HPC3
1272.814	3.064	8.69E-04	1.90E-02	CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC2-CA1_HPC3
1273.787	2.831	1.96E-03	3.01E-02	CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC2-CA1_HPC3
1274.834	2.846	1.85E-03	3.01E-02	CA3_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3
1285.804	2.831	1.96E-03	3.01E-02	CA3_HPC2-CA1_HPC1; CA3_HPC3-CA1_HPC1; CA3_HPC2-CA1_HPC3
1339.905	3.814	6.16E-05	4.17E-03	DG_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC2; CA3_HPC3-CA2_HPC1; DG_HPC3-CA2_HPC1; DG_HPC3-CA2_HPC2
1340.877	2.919	1.44E-03	2.67E-02	DG_HPC3-CA1_HPC2; DG_HPC3-CA2_HPC1; DG_HPC3-CA2_HPC2
1357.881	2.936	1.36E-03	2.63E-02	CA3_HPC1-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC1-CA1_HPC3
1435.942	2.664	3.46E-03	4.42E-02	CA2_HPC2-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3
1483.96	4.321	1.01E-05	8.53E-04	CA3_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC2; DG_HPC3-CA1_HPC3; DG_HPC3-CA2_HPC1; DG_HPC3-CA2_HPC2
1546.987	3.071	8.50E-04	1.90E-02	CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3
1587.95	4.472	5.87E-06	5.68E-04	CA3_HPC2-CA1_HPC1; DG_HPC2-CA1_HPC1; CA1_HPC3-CA1_HPC2; CA2_HPC2-CA1_HPC3; CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3
1644.993	2.761	2.49E-03	3.39E-02	CA3_HPC2-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC2-CA1_HPC3
1709.092	2.655	3.57E-03	4.47E-02	CA3_HPC2-CA1_HPC3

Mr(expt)	F value	P value	FDR	HSD Tukey <i>post hoc</i> test
1746.944	3.204	5.34E-04	1.45E-02	CA2_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC1; CA2_HPC3-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC3-CA1_HPC3
1828.95	5.311	2.98E-07	5.04E-05	CA2_HPC3-CA1_HPC1; CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC3-CA1_HPC1; DG_HPC2-CA1_HPC1; DG_HPC3-CA1_HPC1; CA3_HPC3-CA1_HPC2; DG_HPC3-CA1_HPC2; CA3_HPC3-CA1_HPC3; DG_HPC3-CA1_HPC3; DG_HPC3-CA2_HPC2
1829.947	4.649	3.13E-06	3.53E-04	CA2_HPC3-CA1_HPC1; CA3_HPC1-CA1_HPC1; CA3_HPC3-CA1_HPC1; DG_HPC2-CA1_HPC1; DG_HPC3-CA1_HPC1; CA3_HPC3-CA1_HPC2; DG_HPC3-CA1_HPC2; CA3_HPC3-CA1_HPC3; DG_HPC3-CA1_HPC3
1830.943	3.095	7.81E-04	1.90E-02	CA2_HPC3-CA1_HPC1; CA3_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC1
1839.11	3.383	2.84E-04	1.07E-02	CA2_HPC1-CA1_HPC3; CA3_HPC2-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC2-CA1_HPC3
1906.126	2.759	2.51E-03	3.39E-02	DG_HPC3-CA3_HPC2
1907.099	3.042	9.42E-04	1.99E-02	CA3_HPC2-CA2_HPC3; DG_HPC3-CA3_HPC2
2141.132	5.643	9.21E-08	3.12E-05	CA2_HPC3-CA1_HPC1; CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC3-CA1_HPC1; DG_HPC1-CA1_HPC1; DG_HPC2-CA1_HPC1; DG_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC2; CA3_HPC3-CA1_HPC3; DG_HPC3-CA1_HPC3; DG_HPC3-CA2_HPC1
2142.153	6.590	3.39E-09	2.30E-06	CA2_HPC3-CA1_HPC1; CA3_HPC1-CA1_HPC1; CA3_HPC2-CA1_HPC1; CA3_HPC3-CA1_HPC1; DG_HPC1-CA1_HPC1; DG_HPC2-CA1_HPC1; DG_HPC3-CA1_HPC1; CA3_HPC3-CA1_HPC2; DG_HPC3-CA1_HPC2; CA2_HPC3-CA1_HPC3; CA3_HPC1-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC1-CA1_HPC3; DG_HPC3-CA1_HPC3; DG_HPC3-CA2_HPC1
2143.15	5.462	1.74E-07	3.93E-05	CA2_HPC3-CA1_HPC1; CA3_HPC1-CA1_HPC1; CA3_HPC3-CA1_HPC1; DG_HPC1-CA1_HPC1; DG_HPC2-CA1_HPC1; DG_HPC3-CA1_HPC1; DG_HPC3-CA1_HPC2; CA2_HPC3-CA1_HPC3; CA3_HPC3-CA1_HPC3; DG_HPC3-CA1_HPC3
2274.291	2.837	1.91E-03	3.01E-02	DG_HPC3-CA3_HPC2

Supplementary table 3. Proteins identified by MASCOT from differential peptides between regions of interest. List of proteins that showed significant differences in relative intensity between regions CA1, CA2, CA3, and DG. Mr(exp): experimental m/z; Score: database match score; Rank: minimum score for the peptide sequence to be considered homologous/identical to the databases; Peptide: peptide sequence identified by MS/MS. A p-value < 0.05 was considered significant.

Mr(expt)	Score	P value	Rank	Peptide	Protein	Protein description
726.4781	24	0,026	Score > 20 shows identity	K,PVSPQAK,G	CYCB3	G2/mitotic-specific cyclin-B3
727.4342	32	0,011	Score > 25 shows identity	R,LSVAIPK,A	TRNAU1AP	tRNA selenocysteine 1-associated protein 1
730.4877	25	0,043	Score > 24 shows identity	K,GTALRGR,S	OAS1	2'-5'-oligoadenylate synthase 3
757.5198	29	0,028	Score > 26 shows identity	R,AGDLLLR,H	USP24	Ubiquitin carboxyl-terminal hydrolase 24
836.5084	36	0,015	Score > 30 shows identity	R,GAPGPQGPR,G	COL3A1	Collagen alpha-1(III) chain
852.5291	38	0,0077	Score > 30 shows identity	R,LGALALPR,G	SAMD1	Sterile alpha motif domain-containing protein 1
887.5977	36	0,014	Score > 30 shows identity	K,IDSPTKVK,K	STIL	SCL-interrupting locus protein
1000.6705	31	0,044	Score > 30 shows identity	R,ERAVLSGLR,H	INSRR	Insulin receptor-related protein
1046.6383	38	0,021	Score > 34 shows identity	R,DTGILDSIGR,F	MBP	Myelin basic protein
1087.7184	46	0,0013	Score > 30 shows identity	R,KVSPASGVSKK,V	TTF2	Transcription termination factor 2
1148.6803	42	0,0078	Score > 34 shows identity	R,EVNTSGFAPAR,P	IGHD	Immunoglobulin delta heavy chain
1149.721	34	0,031	Score > 31 shows identity	R,TKPNGHIANR,L + Acetyl (N-term)	NRG1	Pro-neuregulin-1, membrane-bound isoform
1152.6995	42	0,006	Score > 33 shows identity	K,NKILANGGAPR,K + Acetyl (N-term)	FNDC1	Fibronectin type III domain-containing protein 1
1164.734	38	0,011	Score > 31 shows identity	R,TGQVCLFLKR,G	ACAP3	Arf-GAP with coiled-coil, ANK repeat and pH domain-containing protein 3
1214.7364	35	0,038	Score > 34 shows identity	K,LAEAQGTNAALR,T	H2BW2	Histone H2B type F-M
1269.8255	48	8,8E-04	Score > 30 shows identity	R,VGGTGGAAASKLVPR,S	PCDHA8	Protocadherin alpha-8

Mr(expt)	Score	P value	Rank	Peptide	Protein	Protein description
1272.8364	51	3.4E-04	Score > 28 shows identity	K,KTQEEVAAALKR,E	ERC1	ELKS/Rab6-interacting/CAST family member 1
1273.8294	50	4.8E-04	Score > 29 shows identity	R,SLVNSSDKIIR,K + Acetyl (N-term)	PST	CMP-N-acetylneuraminase-poly-alpha-2,8-sialyltransferase
1274.8057	42	0.0042	Score > 31 shows identity	K,LVSETVKKNEK,T	RFC1	Replication factor C subunit 1
1285.8274	66	1.7E-05	Score > 31 shows identity	R,AGGLLGTGSGNLNR,M	DERPC	Decreased expression in renal and prostate cancer protein
1339.8409	32	0.049	Score > 31 shows identity	R,AAGAAGAAAAARAIR,S	C2orf72	Uncharacterized protein C2orf72
1340.8422	60	7.8E-05	Score > 31 shows identity	K,MSYIASTKIIR,I + Acetyl (N-term) Oxidation (M)	CTNNA3	Catenin alpha-3
1357.8339	34	0.022	Score > 33 shows identity Score > 30 shows homology	R,PISSCSQRRGPK,C + Acetyl (N-term)	KPRP	Keratinocyte proline-rich protein
1435.8802	54	3.6E-04	Score > 32 shows identity	K,GTNVSECVLPSTR,P	SYNJ1	Synaptotagmin-1
1546.9574	58	1.1E-04	Score > 31 shows identity	-,MTTRQATKDPLLR,G + Oxidation (M)	TROAP	Tastin
1644.9912	63	5.1E-05	Score > 33 shows identity	K,EKGVVFAHYGPVWR,Q	CYP2U1	Cytochrome P450 2U1
1746.971	42	0.013	Score > 35 shows identity	R,GASLLARPGHSLVAAIR,S + Acetyl (N-term) Oxidation (HW)	NEDL1	E3 ubiquitin-protein ligase HECW1
1828.9596	63	0.0001	Score > 35 shows identity	R,PRSRSYSTSIIEAMK,R	REPS2	RaiBP1-associated Eps domain-containing protein 2
2141.2032	30	0.048	Score > 34 shows identity Score > 29 shows homology	K,DAAGEIMGRGGDNVQVAVVP K,D + Acetyl (N-term) Oxidation (M)	TRIM45	E3 ubiquitin-protein ligase TRIM45
2142.1811	35	0.026	Score > 35 shows identity Score > 32 shows homology	R,GVAAQVLRPGAGVRLPIQPS R,G	NDUFS2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial

Supplementary table 4. Statistical analysis of LC-MS/MS quantification of factors linked to neuroinflammatory processes. Results of the statistical analysis of the quantification of factors related to neuroinflammation and reactive astroglia and microglia in the CA1 subregion comparing the Initial-AD vs. Intermediate-AD (Early Phase) and Intermediate-AD vs. Advanced-AD (Advanced Phase) groups. A p-value < 0.05 was considered significant

Marker	Group	Mean ± SD	n	One-way ANOVA	
				F (DFn, DFd)	valor p
GFAP	Initial-AD (Braak tau I-II)	2.251 ± 0.613	7	F (2, 19) = 6.405	0.0075
	Intermediate-AD (Braak tau III-IV)	1.798 ± 1.053	7		
	Advanced-AD (Braak tau V-VI)	4.085 ± 1.860	8		
MAO-B	Initial-AD (Braak tau I-II)	3.021 ± 0.593	7	F (2, 23) = 8.488	0.0017
	Intermediate-AD (Braak tau III-IV)	3.096 ± 0.358	9		
	Advanced-AD (Braak tau V-VI)	4.099 ± 0.806	10		
VIM	Initial-AD (Braak tau I-II)	6.890 ± 0.410	7	F (2, 21) = 6.659	0.0058
	Intermediate-AD (Braak tau III-IV)	7.254 ± 0.712	9		
	Advanced-AD (Braak tau V-VI)	8.030 ± 0.667	8		
NES	Initial-AD (Braak tau I-II)	1.606 ± 0.438	7	F (2, 21) = 8.656	0.0018
	Intermediate-AD (Braak tau III-IV)	1.702 ± 0.415	9		
	Advanced-AD (Braak tau V-VI)	2.807 ± 0.928	8		
S100β	Initial-AD (Braak tau I-II)	3.561 ± 0.556	6	F (2, 21) = 4.577	0.0224
	Intermediate-AD (Braak tau III-IV)	3.240 ± 0.599	8		
	Advanced-AD (Braak tau V-VI)	4.293 ± 0.941	10		
CD44	Initial-AD (Braak tau I-II)	1.876 ± 0.361	6	F (2, 20) = 14.96	0.0001
	Intermediate-AD (Braak tau III-IV)	1.566 ± 0.535	8		
	Advanced-AD (Braak tau V-VI)	3.344 ± 0.967	9		
CD11C	Initial-AD (Braak tau I-II)	-4.011 ± 0.670	7	F (2, 23) = 15.85	<0.0001
	Intermediate-AD (Braak tau III-IV)	-3.047 ± 0.479	9		
	Advanced-AD (Braak tau V-VI)	-2.134 ± 0.806	10		
CD63	Initial-AD (Braak tau I-II)	-0.076 ± 0.336	6	F (2, 21) = 5.972	0.0088
	Intermediate-AD (Braak tau III-IV)	0.105 ± 0.259	9		
	Advanced-AD (Braak tau V-VI)	0.531 ± 0.443	9		
CD9	Initial-AD (Braak tau I-II)	3.840 ± 0.434	7	F (2, 20) = 12.95	0.0002
	Intermediate-AD (Braak tau III-IV)	4.454 ± 0.530	9		
	Advanced-AD (Braak tau V-VI)	5.146 ± 0.454	7		

Supplementary table 5. Statistical analysis of COX6C quantification by LC-MS/MS. Results of the statistical analysis of COX6C quantification in the CA1, CA2, CA3, and DG subregions comparing the Initial-AD vs. Intermediate-AD (Early Phase) and Intermediate-AD (Advanced Phase) groups. A p-value < 0.05 was considered significant

Subregion	Group	Mean ± SD	n	Student's t-test			
				Initial Phase (Initial-AD vs Intermediate-AD)		Advanced Phase (Intermediate-AD vs Advanced-AD)	
				t, df	p value	t, df	p value
CA1	Initial-AD (Braak tau I-II)	3.978 ± 0.318	7				
	Intermediate-AD (Braak tau III-IV)	4.448 ± 0.473	9	t=2.254, df=14	0.041	t=2.685, df=17	0.016
	Advanced-AD (Braak tau V-VI)	3.659 ± 0.757	10				
CA2	Initial-AD (Braak tau I-II)	3.752 ± 0.540	7				
	Intermediate-AD (Braak tau III-IV)	4.358 ± 0.586	9	t=2.122, df=14	0.052	t=1.861, df=17	0.080
	Advanced-AD (Braak tau V-VI)	3.765 ± 0.776	10				
CA3	Initial-AD (Braak tau I-II)	3.949 ± 0.550	7				
	Intermediate-AD (Braak tau III-IV)	4.054 ± 0.549	9	t=0.379, df=14	0.711	t=1.325, df=17	0.203
	Advanced-AD (Braak tau V-VI)	3.748 ± 0.458	10				
DG	Initial-AD (Braak tau I-II)	3.600 ± 0.700	7				
	Intermediate-AD (Braak tau III-IV)	4.245 ± 0.612	9	t=1.965, df=14	0.067	t=0.697, df=15	0.497
	Advanced-AD (Braak tau V-VI)	3.796 ± 0.470	10				

Supplementary table 6. Statistical analysis of COX6C validation by histoblot. Results of the statistical analysis of COX6C quantification by histoblot, expressed in arbitrary units (a.u.), in the hippocampal regions CA1, CA2, CA3, and DG, comparing the Initial-AD, Intermediate-AD, and Advanced-AD groups. A p-value < 0.05 was considered significant

Subregion	Group	Mean ± SD	n	One-way ANOVA	
				F (DFn, DFd)	p value
CA1	Initial-AD (Braak tau I-II)	40.57 ± 11.88	5	F (2, 11) = 5.177	0.0260
	Intermediate-AD (Braak tau III-IV)	58.55 ± 6.96	5		
	Advanced-AD (Braak tau V-VI)	39.42 ± 11.72	4		
CA2	Initial-AD (Braak tau I-II)	42.48 ± 4.31	5	F (2, 11) = 4.696	0.0335
	Intermediate-AD (Braak tau III-IV)	57.53 ± 3.89	5		
	Advanced-AD (Braak tau V-VI)	42.63 ± 15.39	4		
CA3	Initial-AD (Braak tau I-II)	42.37 ± 9.42	5	F (2, 11) = 5.420	0.0230
	Intermediate-AD (Braak tau III-IV)	57.37 ± 6.94	5		
	Advanced-AD (Braak tau V-VI)	41.11 ± 9.05	4		
DG	Initial-AD (Braak tau I-II)	40.60 ± 10.77	5	F (2, 11) = 6.348	0.0147
	Intermediate-AD (Braak tau III-IV)	59.60 ± 8.19	5		
	Advanced-AD (Braak tau V-VI)	41.24 ± 9.03	4		

Supplementary table 7. Statistical analysis of COX6C validation by histoblot. Results of the statistical analysis of COX6C quantification by histoblot, expressed in arbitrary units (a.u.), comparing the hippocampal regions CA1, CA2, CA3, and DG in the Initial-AD, Intermediate-AD, and Advanced-AD groups. A p-value < 0.05 was considered significant

Group	Subregion	Mean ± SD	n	One-way ANOVA	
				F (DFn, DFd)	p value
Initial-AD (Braak tau I-II)	CA1	40.57 ± 11.88	5	F (3, 16) = 0.062	0.9789
	CA2	42.48 ± 4.31	5		
	CA3	42.37 ± 9.46	5		
	DG	40.60 ± 10.77	5		
InterMeante-AD (Braak tau III-IV)	CA1	58.55 ± 6.96	5	F (3, 16) = 0.120	0.9471
	CA2	57.53 ± 3.89	5		
	CA3	57.37 ± 6.94	5		
	DG	59.60 ± 8.19	5		
Advanced-AD (Braak tau V-VI)	CA1	39.42 ± 11.72	4	F (3, 12) = 0.054	0.9825
	CA2	42.63 ± 15.39	4		
	CA3	41.11 ± 9.05	4		
	DG	41.24 ± 9.03	4		

Supplementary table 8. Statistical analysis of ATP5MK validation by LC-MS/MS. Results of statistical analysis of ATP5MK quantification in the CA1, CA2, CA3, and DG subregions comparing the Initial-AD vs. Intermediate-AD (Early Phase) and Intermediate-AD (Advanced Phase) groups. A p-value < 0.05 was considered significant

Subregion	Group	Mean ± SD	n	Student's t-test			
				Fase Inicial (Initial-AD vs Intermediate-AD)		Fase Avanzada (Intermediate-AD vs Advanced-AD)	
				t, df	valor p	t, df	valor p
CA1	Initial-AD (Braak tau I-II)	3.435 ± 0.248	7				
	Intermediate-AD (Braak tau III-IV)	3.855 ± 0.221	9	t=3.580, df=14	0.003	t=3.422, df=17	0.003
	Advanced-AD (Braak tau V-VI)	3.300 ± 0.438	10				
CA2	Initial-AD (Braak tau I-II)	3.274 ± 0.456	7				
	Intermediate-AD (Braak tau III-IV)	3.647 ± 0.410	9	t=1.721, df=14	0.107	t=1.887, df=17	0.076
	Advanced-AD (Braak tau V-VI)	3.217 ± 0.560	10				
CA3	Initial-AD (Braak tau I-II)	3.644 ± 0.145	7				
	Intermediate-AD (Braak tau III-IV)	3.658 ± 0.437	9	t=0.379, df=14	0.711	t=0.929, df=17	0.661
	Advanced-AD (Braak tau V-VI)	3.481 ± 0.398	10				
DG	Initial-AD (Braak tau I-II)	3.393 ± 0.407	7				
	Intermediate-AD (Braak tau III-IV)	3.736 ± 0.283	9	t=1.987, df=14	0.067	t=0.876, df=17	0.394
	Advanced-AD (Braak tau V-VI)	3.583 ± 0.450	10				

Supplementary table 9. Statistical analysis of ATP5MK validation by histoblot. Results of the statistical analysis of ATP5MK quantification by histoblot, expressed in arbitrary units (a.u.), in the hippocampal regions CA1, CA2, CA3, and DG, comparing the Initial-AD, Intermediate-AD, and Advanced-AD groups. A p-value < 0.05 was considered significant

Subregion	Group	Mean ± SD	n	One-way ANOVA	
				F (DFn, DFd)	p value
CA1	Initial-AD (Braak tau I-II)	39.61 ± 12.82	4	F (2, 11) = 0.8501	0.4536
	Intermediate-AD (Braak tau III-IV)	44.87 ± 11.57	5		
	Advanced-AD (Braak tau V-VI)	36.71 ± 4.29	5		
CA2	Initial-AD (Braak tau I-II)	48.72 ± 16.36	4	F (2, 11) = 0.3512	0.7115
	Intermediate-AD (Braak tau III-IV)	53.83 ± 10.47	5		
	Advanced-AD (Braak tau V-VI)	48.59 ± 5.24	5		
CA3	Initial-AD (Braak tau I-II)	54.81 ± 16.61	4	F (2, 11) = 2.351	0.1412
	Intermediate-AD (Braak tau III-IV)	63.18 ± 7.23	5		
	Advanced-AD (Braak tau V-VI)	49.46 ± 4.47	5		
DG	Initial-AD (Braak tau I-II)	52.44 ± 14.90	4	F (2, 11) = 3.326	0.0742
	Intermediate-AD (Braak tau III-IV)	64.24 ± 5.73	5		
	Advanced-AD (Braak tau V-VI)	49.66 ± 6.66	5		

Supplementary table 10. Statistical analysis of ATP5MK validation by histoblot. Results of the statistical analysis of ATP5MK quantification by histoblot, expressed in arbitrary units (a.u.), comparing the hippocampal regions CA1, CA2, CA3, and DG in the Initial-AD, Intermediate-AD, and Advanced-AD groups. A p-value < 0.05 was considered significant

Group	Subregion	Mean ± SD	n	One-way ANOVA	
				F (DFn, DFd)	valor p
Initial-AD (Braak tau I-II)	CA1	39.61 ± 12.82	4	F (3, 12) = 0.7670	0.5342
	CA2	48.72 ± 16.36	4		
	CA3	54.81 ± 16.61	4		
	DG	52.44 ± 14.90	4		
Intermediate-AD (Braak tau III-IV)	CA1	44.87 ± 11.57	5	F (3, 16) = 5.009	0.0123
	CA2	53.83 ± 10.47	5		
	CA3	63.18 ± 7.23	5		
	DG	64.24 ± 5.73	5		
Advanced-AD (Braak tau V-VI)	CA1	36.71 ± 4.29	5	F (3, 16) = 7.169	0.0029
	CA2	48.59 ± 5.24	5		
	CA3	49.46 ± 4.47	5		
	DG	49.66 ± 6.66	5		

Supplementary table 11. Statistical analysis of H6PD validation by LC-MS/MS. Results of statistical analysis of H6PD quantification in the CA1, CA2, CA3, and DG subregions comparing the Initial-AD vs. Intermediate-AD (Early Phase) and Intermediate-AD vs. Advanced-AD (Advanced Phase) groups. A p-value < 0.05 was considered significant

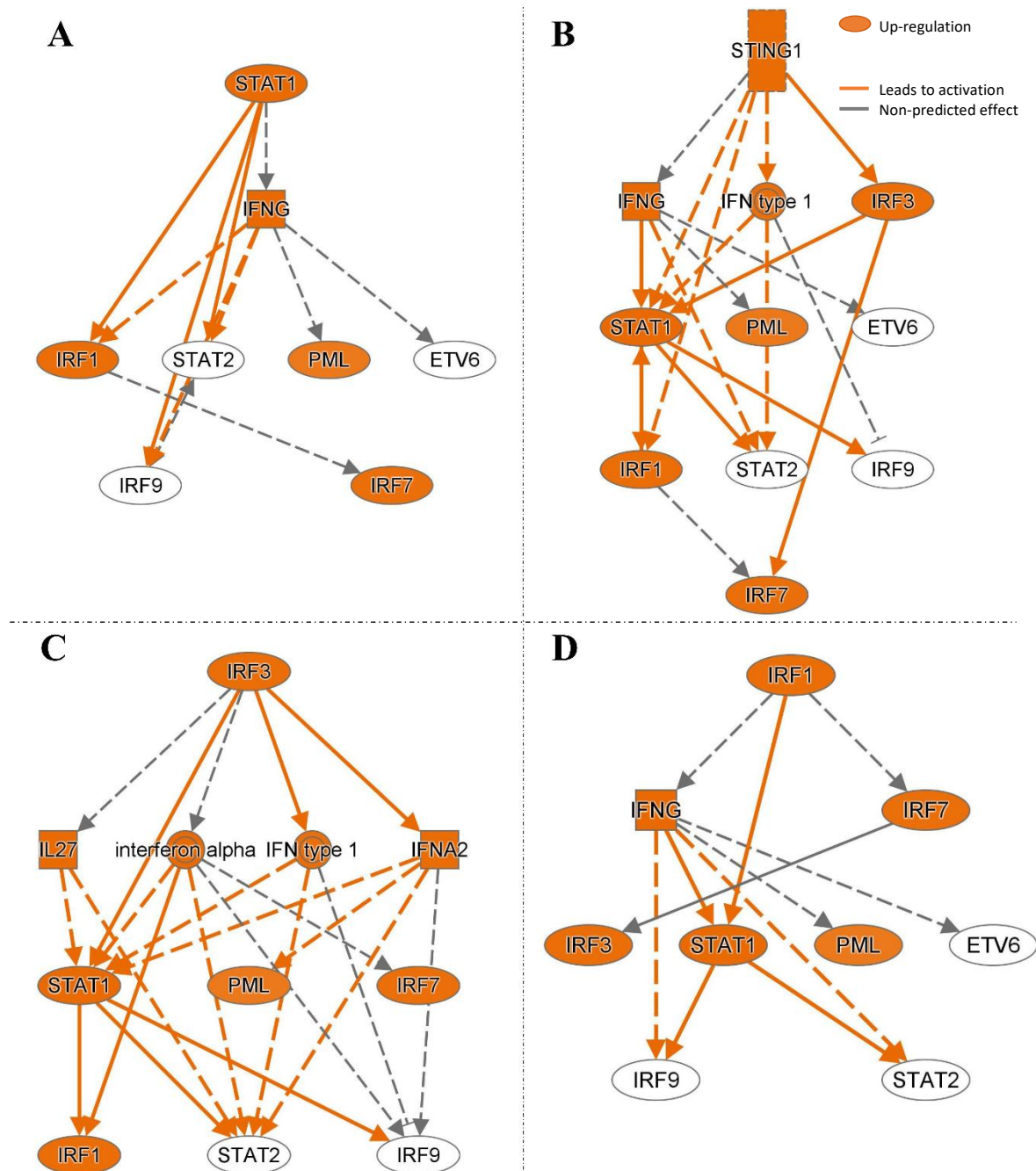
Subregion	Group	Mean ± SD	n	Student's t-test			
				Initial Phase (Initial-AD vs Intermediate-AD)		Advanced Phase (Intermediate-AD vs Advanced-AD)	
				t, df	valor p	t, df	valor p
CA1	Initial-AD (Braak tau I-II)	-2.306 ± 0.953	7				
	Intermediate-AD (Braak tau III-IV)	-1.547 ± 0.345	9	t=2.229, df=14	0.0427	t=2.380, df=17	0.0293
	Advanced-AD (Braak tau V-VI)	-0.722 ± 0.985	10				
CA2	Initial-AD (Braak tau I-II)	-2.067 ± 1.090	7				
	Intermediate-AD (Braak tau III-IV)	-1.582 ± 0.550	9	t=1.164, df=14	0.2640	t=1.236, df=17	0.2333
	Advanced-AD (Braak tau V-VI)	-1.123 ± 0.983	10				
CA3	Initial-AD (Braak tau I-II)	-1.789 ± 1.127	7				
	Intermediate-AD (Braak tau III-IV)	-1.801 ± 1.040	9	t=0.021, df=14	0.9832	t=1.779, df=17	0.0931
	Advanced-AD (Braak tau V-VI)	-0.963 ± 0.320	10				
DG	Initial-AD (Braak tau I-II)	-1.430 ± 0.224	7				
	Intermediate-AD (Braak tau III-IV)	-1.459 ± 0.857	9	t=0.086, df=14	0.9330	t=1.989, df=17	0.0631
	Advanced-AD (Braak tau V-VI)	-0.799 ± 0.576	10				

Supplementary table 12. Statistical analysis of H6PD validation by histoblot. Results of the statistical analysis of H6PD quantification by histoblot, expressed in arbitrary units (a.u.), in the hippocampal regions CA1, CA2, CA3, and DG, comparing the Initial-AD, Intermediate-AD, and Advanced-AD groups. A p-value < 0.05 was considered significant

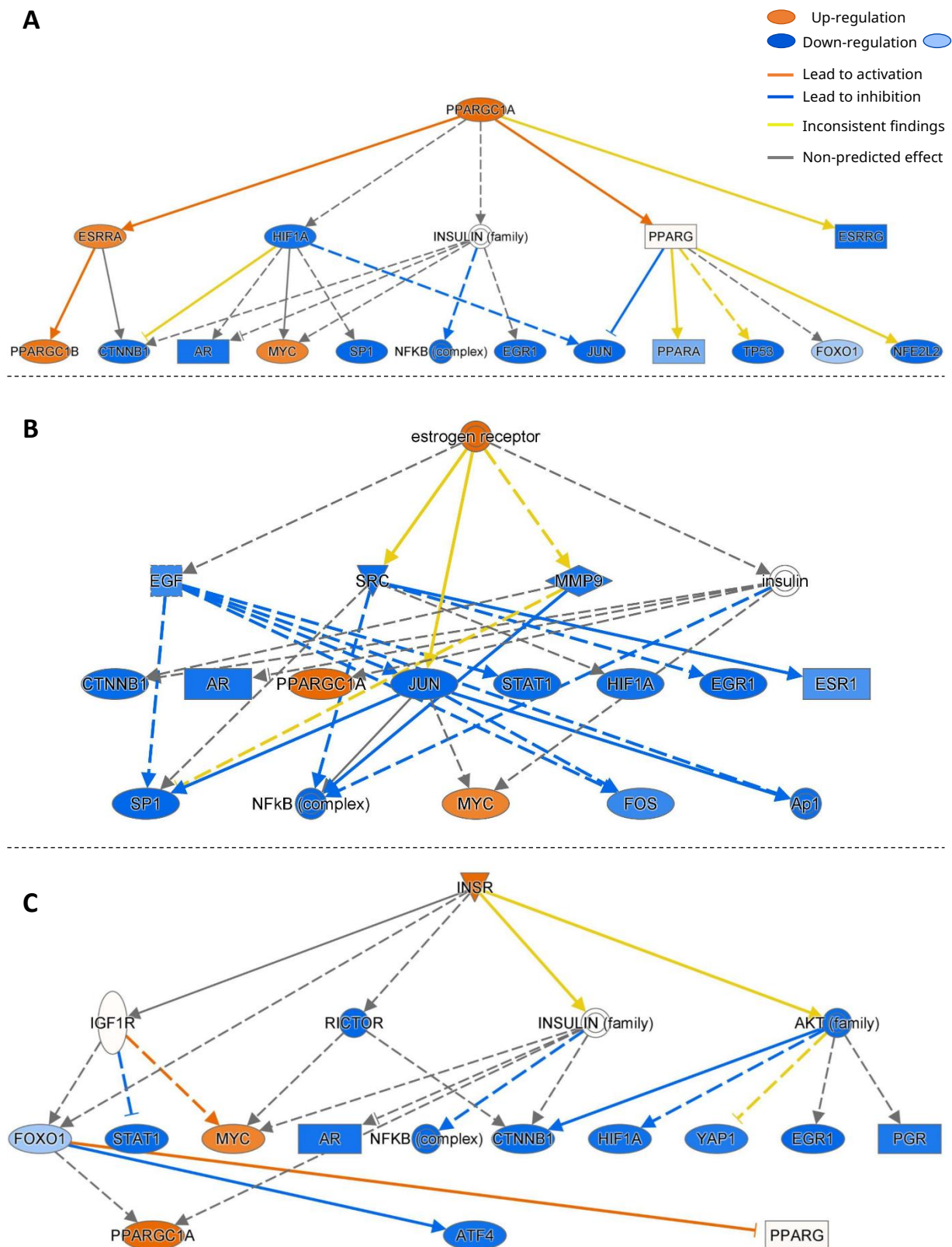
Subregion	Group	Mean ± SD	n	One-way ANOVA	
				F (DFn, DFd)	p value
CA1	Initial-AD (Braak tau I-II)	25.66 ± 5.20	4	F (2, 10) = 24.39	0.0001
	Intermediate-AD (Braak tau III-IV)	60.24 ± 4.41	5		
	Advanced-AD (Braak tau V-VI)	41.53 ± 9.39	5		
CA2	Initial-AD (Braak tau I-II)	52.19 ± 10.28	4	F (2, 10) = 6.814	0.0136
	Intermediate-AD (Braak tau III-IV)	77.31 ± 5.16	5		
	Advanced-AD (Braak tau V-VI)	57.52 ± 12.72	5		
CA3	Initial-AD (Braak tau I-II)	35.50 ± 12.11	4	F (2, 10) = 5.126	0.0249
	Intermediate-AD (Braak tau III-IV)	56.81 ± 8.03	5		
	Advanced-AD (Braak tau V-VI)	43.08 ± 8.38	5		
DG	Initial-AD (Braak tau I-II)	38.50 ± 3.75	4	F (2, 10) = 3.374	0.0759
	Intermediate-AD (Braak tau III-IV)	43.15 ± 10.00	5		
	Advanced-AD (Braak tau V-VI)	29.12 ± 9.30	5		

Supplementary table 13. Statistical analysis of H6PD validation by histoblot. Results of the statistical analysis of H6PD quantification by histoblot, expressed in arbitrary units (a.u.), comparing the hippocampal regions CA1, CA2, CA3, and DG in the Initial-AD, Intermediate-AD, and Advanced-AD groups. A p-value < 0.05 was considered significant

Group	Subregion	Mean ± SD	n	One-way ANOVA	
				F (DFn, DFd)	p value
Initial-AD (Braak tau I-II)	CA1	25.66 ± 5.20	4	F (3, 12) = 6.547	0.0072
	CA2	52.19 ± 10.28	4		
	CA3	35.50 ± 12.11	4		
	DG	38.50 ± 3.75	4		
Intermediate-AD (Braak tau III-IV)	CA1	60.24 ± 4.41	4	F (3, 12) = 14.990	0.0002
	CA2	77.31 ± 5.16	4		
	CA3	56.81 ± 8.03	4		
	DG	43.15 ± 10.01	4		
Advanced-AD (Braak tau V-VI)	CA1	41.53 ± 9.39	5	F (3, 16) = 6.951	0.0033
	CA2	57.52 ± 12.72	5		
	CA3	43.08 ± 8.38	5		
	DG	29.12 ± 9.30	5		



Supplementary figure 1. Upstream regulators associated with specific CA1 DEPs in the Early Phase of AD in CA1. Prediction of altered signalling pathways due to variation in the levels of transcriptional regulators STAT1 (A), STING1 (B), IRF3 (C) and IRF4 (D) in the early stage of AD in CA1. Proteins that would be upregulated (positive z-score) are shown in orange. The coloured lines show the type of predicted connection between proteins.



Supplementary figure 2. Analysis of transcriptional regulators associated with altered proteins in the advanced stage of AD in CA1. Prediction of altered signalling pathways due to variation in the levels of the transcriptional regulators PGC-1 α (A), ER (B), INSR (C) in the advanced stage of AD in CA1. Proteins that would be upregulated (positive z-score) are shown in orange, and those that would be downregulated (negative z-score) are shown in blue. The coloured lines show the type of predicted connection between the proteins.