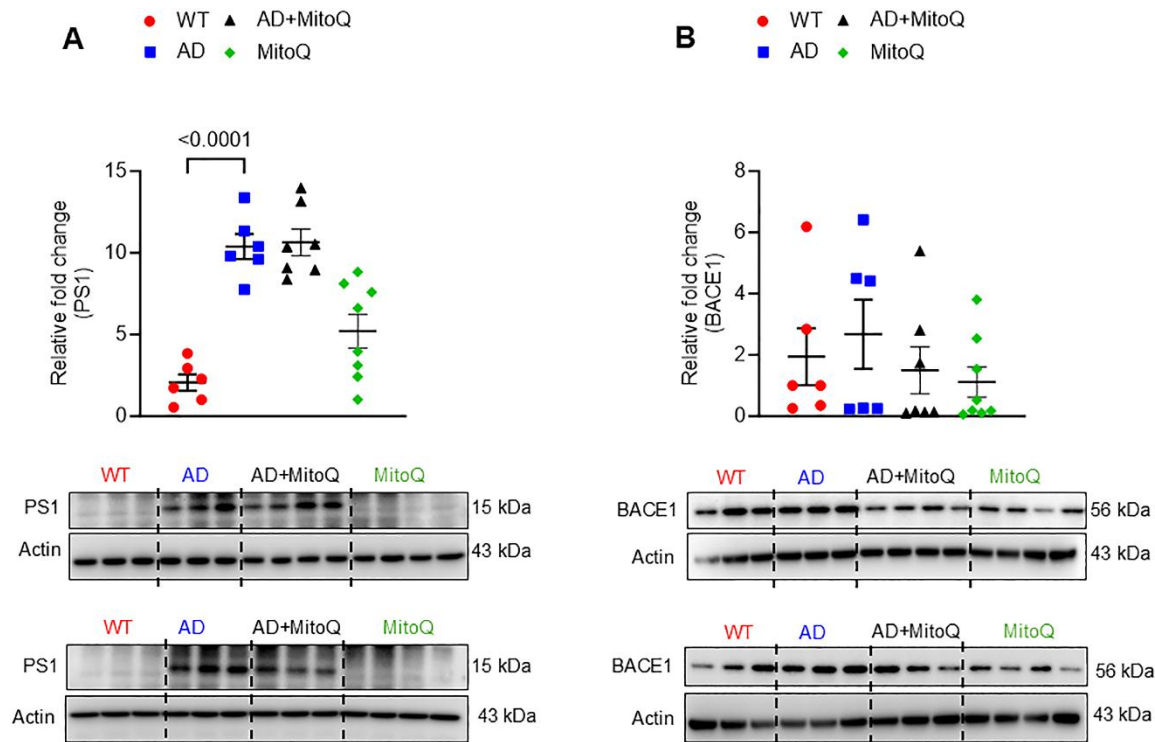


SUPPLEMENTARY DATA

Repurposing MitoQ10 as a Therapeutic for Established Alzheimer's Disease Pathology

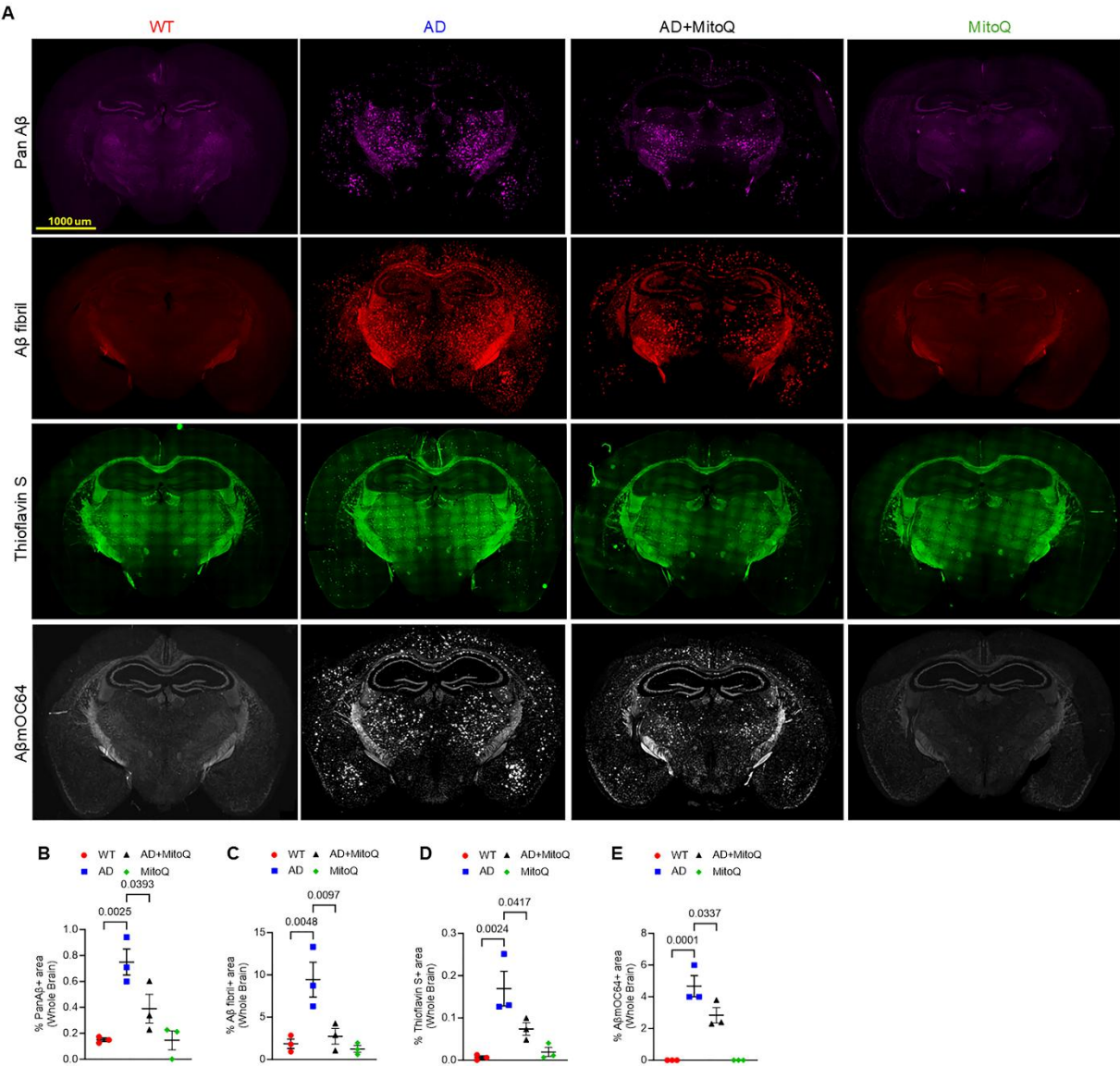
Mohit Kumar, Sudipta Ray, Susmita Sil

SUPPLEMENTARY DATA



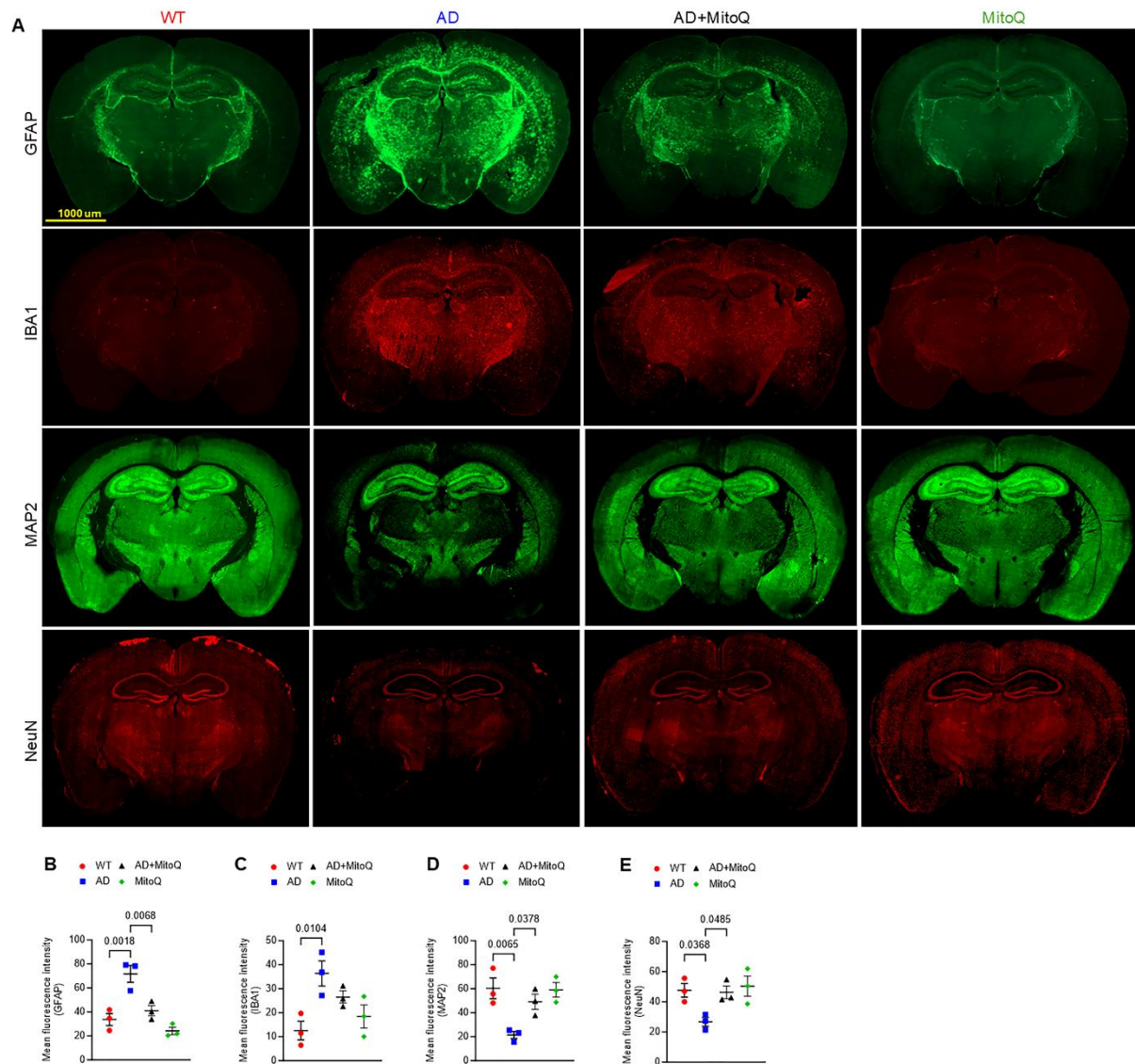
Supplementary Figure 1. Regulation of AD markers in MitoQ-administered mice: (A) Representative western blot images and quantitative analysis of (A) PS1, and (B) BACE1 in the hippocampus. Actin (43 kDa) was used as the internal control for all experiments. Data are presented as mean $\pm$ SEM. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test with the AD group as the reference; $p \leq 0.05$ was considered significant. WT, AD (n= 6 mice/ group); MitoQ, AD+MitoQ (n= 8 mice/ group). PS1- presenilin-1; BACE1- beta-site APP cleaving enzyme 1.

SUPPLEMENTARY DATA



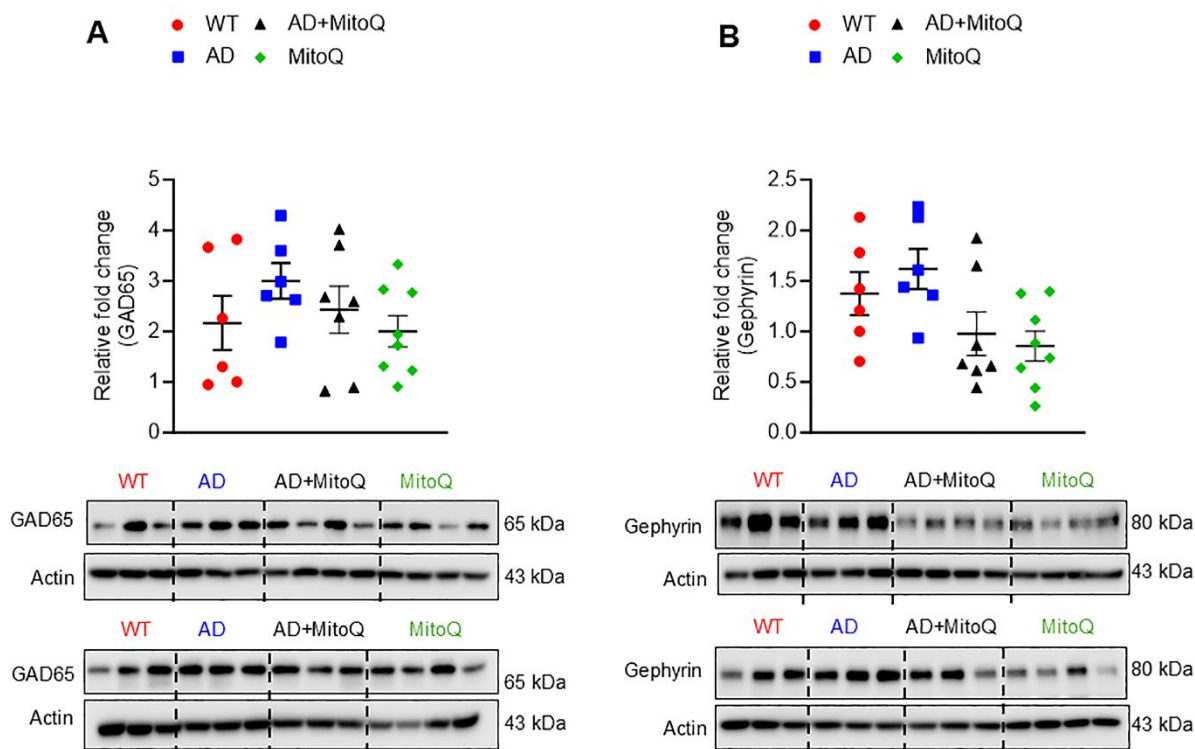
Supplementary Figure 2. MitoQ improves amyloid burden in the whole brain of AD mice. Representative immunofluorescence images and quantitative analysis of (A-B) Pan A β staining (Purple), (A, C) A β fibril (red), (A, D) Thioflavin S (Green), (A, E) A β mOC64 (white) in the whole brain. Data are presented as mean $\pm$ SEM. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test with the AD group as the reference; $p \leq 0.05$ was considered significant. Scale bar: 1000 μ m. $n=3$ mice/group. Pan A β - pan-amyloid beta; A β mOC64- beta-amyloid 1-42.

SUPPLEMENTARY DATA



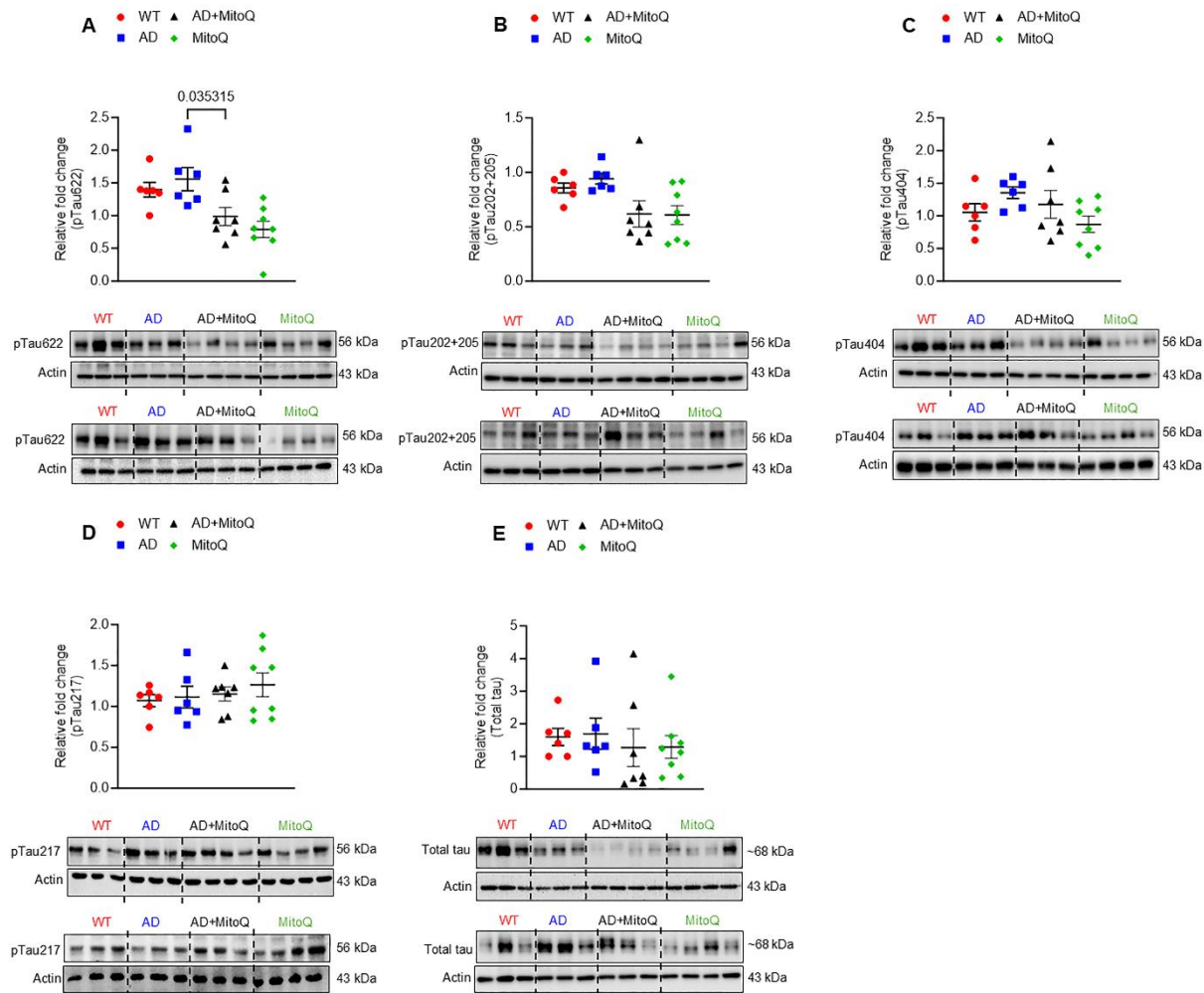
Supplementary Figure 3. MitoQ attenuates glial activation and preserves neuronal integrity in the whole brain of AD mice. Representative immunofluorescence images and quantitative analysis of (A–B) GFAP (Green), (A, C) IBA1 (red), (A, D) MAP2 (Green), (A, E) NeuN (red) in the whole brain. Data are presented as mean ± SEM. Data were analyzed using one-way ANOVA followed by Dunnett’s post hoc test with the AD group as the reference; $p \leq 0.05$ was considered significant. Scale bar: 1000 μ m. $n=3$ mice/group. Pan A β - pan-amyloid beta; A β mOC64- beta-amyloid 1-42. GFAP- Glial fibrillary acidic protein; Iba1- Ionized calcium-binding adaptor molecule 1; MAP2- Microtubule-associated protein 2; NeuN- neuronal nuclear antigen.

SUPPLEMENTARY DATA



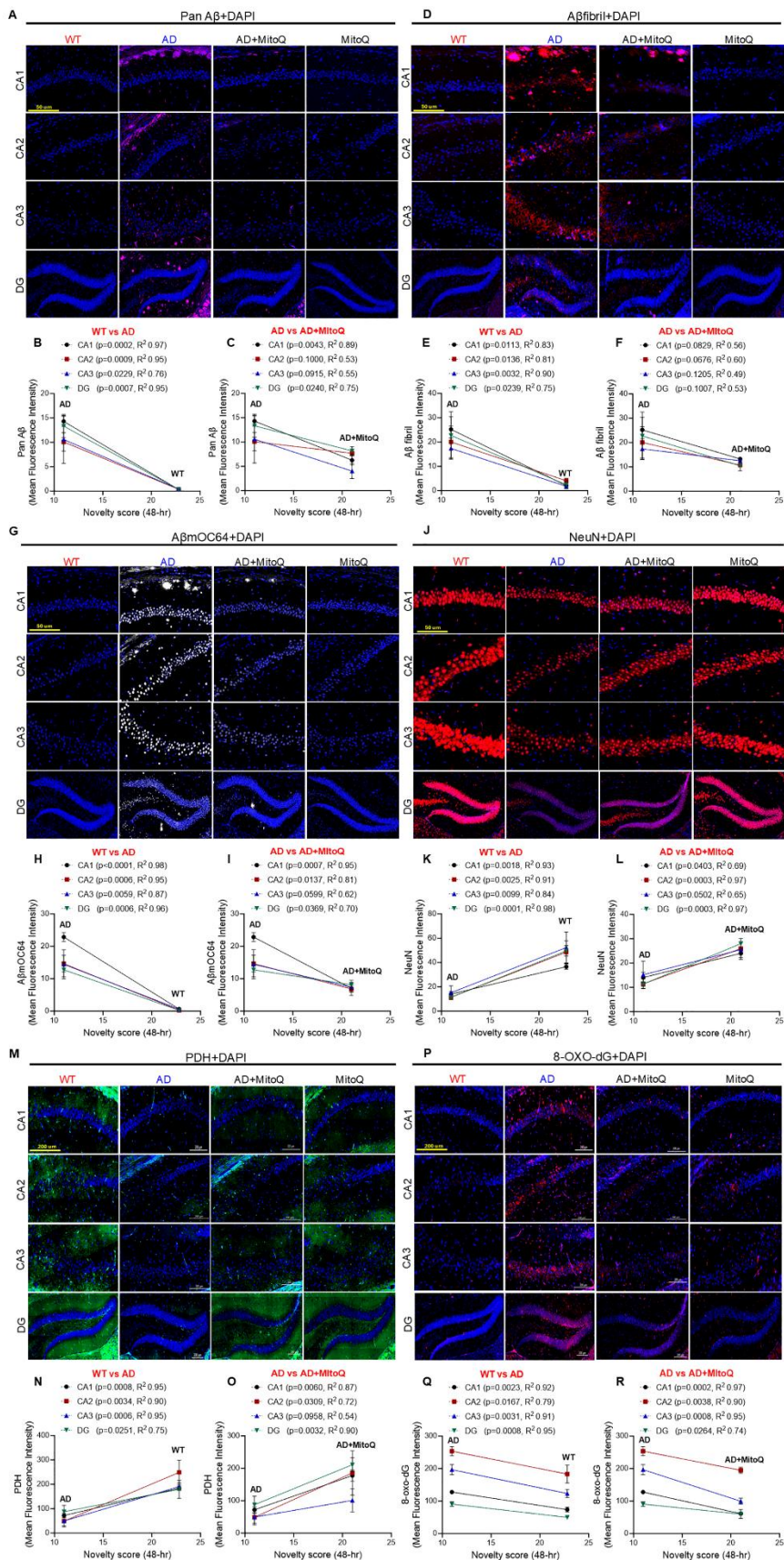
Supplementary Figure 4. MitoQ modulates GAD65 and gephyrin expression in the AD mice brain. Representative western blot images and quantitative analysis of (A) GAD65 and (B) gephyrin in the hippocampus. Actin (43 kDa) was used as an internal control. Data are presented as mean $\pm$ SEM. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test with the AD group as the reference; $p \leq 0.05$ was considered significant. WT, AD (n= 6 mice/ group); MitoQ, AD+MitoQ (n= 8 mice/ group). GAD65- glutamate decarboxylase 65.

SUPPLEMENTARY DATA



Supplementary Figure 5. Exploratory assessment of hippocampal phospho-tau and total-tau abundance following MitoQ administration in 5XFAD mice. Representative western blot images and quantitative analysis of (A) pTau622, (B) pTau202+205, (C) pTau404, (D) pTau217, and (E) total tau in the hippocampus. Actin (43 kDa) was used as the internal control. Data are presented as mean $\pm$ SEM. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test with the AD group as the reference; $p \leq 0.05$ was considered significant. WT, AD (n= 6 mice/ group); MitoQ, AD+MitoQ (n= 8 mice/ group). pTau- phosphorylated tau.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 6. Exploratory descriptive regression analysis between novelty score and hippocampal neuropathological markers. Representative immunofluorescence images and linear regression analysis showing (A-C) Pan A β , (D-F) A β fibril, (G-I) A β mOC64, (J-L) NeuN, (M-O) PDH, and (P-R) 8-oxo-dG intensity versus novelty score in WT vs AD and AD vs AD+MitoQ mice across CA1, CA2, CA3, and DG. Separate regressions were conducted for WT vs AD and AD vs AD+MitoQ comparisons. For each comparison, the novelty-score value on the X-axis represents the pooled group mean at 48h, whereas the Y-axis displays individual pathological-marker values from three animals per group (n = 3 mice/ group). Solid lines show best-fit simple linear regression lines. Slope estimates, 95% confidence intervals, R² values, and two-sided p-values are reported in Supplementary Table 1. Here, Data are presented as R² and p-values. p $\leq$ 0.05 was considered significant. Pan A β , pan-amyloid beta; A β mOC64, beta-amyloid 1-42; NeuN, neuronal nuclear antigen; PDH, pyruvate dehydrogenase; 8-Oxo-dG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; DAPI, 4',6-diamidino-2-phenylindole; CA1, 2, 3, cornu ammonis1, 2, 3; DG, dentate gyrus.

Supplementary Table 1. Exploratory regression analysis between novelty score and hippocampal neuropathological markers

Comparison	Marker	Region	Slope	95% CI	R ²	p value	Nominal interpretation
WT vs AD	A β mOC64	CA1	-1.880	-2.213 to -1.547	0.9840	<0.0001	Significant
WT vs AD	A β mOC64	CA2	-1.215	-1.562 to -0.8682	0.9594	0.0006	Significant
WT vs AD	A β mOC64	CA3	-1.171	-1.779 to -0.5641	0.8776	0.0059	Significant
WT vs AD	A β mOC64	DG	-1.057	-1.355 to -0.7587	0.9604	0.0006	Significant
AD vs AD+MitoQ	A β mOC64	CA1	-1.579	-2.048 to -1.110	0.9562	0.0007	Significant
AD vs AD+MitoQ	A β mOC64	CA2	-0.7790	-1.294 to -0.2644	0.8154	0.0137	Significant
AD vs AD+MitoQ	A β mOC64	CA3	-0.7083	-1.464 to 0.04719	0.6288	0.0599	Not significant
AD vs AD+MitoQ	A β mOC64	DG	-0.4605	-0.8756 to -0.04550	0.7035	0.0369	Significant
WT vs AD	Fibrillar A β	CA1	-1.957	-3.180 to -0.7332	0.8314	0.0113	Significant
WT vs AD	A β fibril	CA2	-1.351	-2.243 to -0.4592	0.8156	0.0136	Significant
WT vs AD	A β fibril	CA3	-1.319	-1.901 to -0.7381	0.9085	0.0032	Significant
WT vs AD	A β fibril	DG	-1.718	-3.063 to -0.3719	0.7584	0.0239	Significant
AD vs AD+MitoQ	A β fibril	CA1	-1.192	-2.631 to 0.2464	0.5696	0.0829	Not significant
AD vs AD+MitoQ	A β fibril	CA2	-0.9303	-1.968 to 0.1078	0.6075	0.0676	Not significant
AD vs AD+MitoQ	A β fibril	CA3	-0.4845	-1.168 to 0.1992	0.4918	0.1205	Not significant

SUPPLEMENTARY DATA

AD vs AD+ MitoQ	A β fibril	DG	-1.225	-2.826 to 0.3753	0.5304	0.1007	Not significant
WT vs AD	Pan-A β	CA1	-1.184	-1.427 to -0.9408	0.9786	0.0002	Significant
WT vs AD	Pan-A β	CA2	-0.8218	-1.079 to -0.5645	0.9516	0.0009	Significant
WT vs AD	Pan-A β	CA3	-0.8618	-1.528 to -0.1959	0.7635	0.0229	Significant
WT vs AD	Pan-A β	DG	-1.103	-1.425 to -0.7818	0.9578	0.0007	Significant
AD vs AD+ MitoQ	Pan-A β	CA1	-0.8019	-1.183 to -0.4205	0.8949	0.0043	Significant
AD vs AD+ MitoQ	Pan-A β	CA2	-0.2377	-0.5472 to 0.07191	0.5318	0.1000	Not significant
AD vs AD+ MitoQ	Pan-A β	CA3	-0.6544	-1.476 to 0.1674	0.5500	0.0915	Not significant
AD vs AD+ MitoQ	Pan-A β	DG	-0.5145	-0.9180 to -0.1110	0.7581	0.0240	Significant
WT vs AD	NeuN	CA1	1.908	1.188 to 2.628	0.9311	0.0018	Significant
WT vs AD	NeuN	CA2	3.142	1.850 to 4.433	0.9194	0.0025	Significant
WT vs AD	NeuN	CA3	3.146	1.255 to 5.036	0.8422	0.0099	Significant
WT vs AD	NeuN	DG	3.237	2.644 to 3.830	0.9829	0.0001	Significant
AD vs AD+ MitoQ	NeuN	CA1	0.9897	0.07169 to 1.908	0.6914	0.0402	Significant
AD vs AD+ MitoQ	NeuN	CA2	1.445	1.107 to 1.784	0.9724	0.0003	Significant
AD vs AD+ MitoQ	NeuN	CA3	1.032	-0.001633 to 2.065	0.6577	0.0502	Not significant
AD vs AD+ MitoQ	NeuN	DG	1.655	1.270 to 2.040	0.9727	0.0003	Significant
WT vs AD	PDH	CA1	9.468	6.549 to 12.39	0.9530	0.0008	Significant
WT vs AD	PDH	CA2	16.92	9.374 to 24.47	0.9064	0.0034	Significant
WT vs AD	PDH	CA3	11.94	8.546 to 15.34	0.9597	0.0006	Significant
WT vs AD	PDH	DG	7.770	1.587 to 13.95	0.7527	0.0251	Significant
AD vs AD+ MitoQ	PDH	CA1	10.51	5.037 to 15.99	0.8766	0.0060	Significant
AD vs AD+ MitoQ	PDH	CA2	13.64	2.039 to 25.24	0.7271	0.0309	Significant
AD vs AD+ MitoQ	PDH	CA3	5.131	-1.434 to 11.70	0.5407	0.0958	Not significant
AD vs AD+ MitoQ	PDH	DG	12.29	6.909 to 17.67	0.9095	0.0032	Significant
WT vs AD	8-oxo-dG	CA1	-4.575	-6.418 to -2.733	0.9224	0.0023	Significant
WT vs AD	8-oxo-dG	CA2	-5.969	-10.16 to -1.782	0.7966	0.0167	Significant

SUPPLEMENTARY DATA

WT vs AD	8-oxo-dG	CA3	-6.212	-8.923 to -3.502	0.9101	0.0031	Significant
WT vs AD	8-oxo-dG	DG	-3.456	-4.502 to -2.410	0.9546	0.0008	Significant
AD vs AD+ MitoQ	8-oxo-dG	CA1	-6.638	-8.094 to -5.181	0.9756	0.0002	Significant
AD vs AD+ MitoQ	8-oxo-dG	CA2	-5.860	-8.561 to -3.159	0.9007	0.0038	Significant
AD vs AD+ MitoQ	8-oxo-dG	CA3	-9.692	-12.61 to -6.778	0.9552	0.0008	Significant
AD vs AD+ MitoQ	8-oxo-dG	DG	-3.068	-5.548 to -0.5873	0.7467	0.0264	Significant

n=3/group. $p \leq 0.05$ was considered significant. Statistical analysis was performed using simple linear regression analysis by the ordinary least squares method.