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Supplemental information

miR-23b-3p rescues cognition in Alzheimer's disease by reducing tau phosphorylation and apoptosis via GSK-3β signaling pathways

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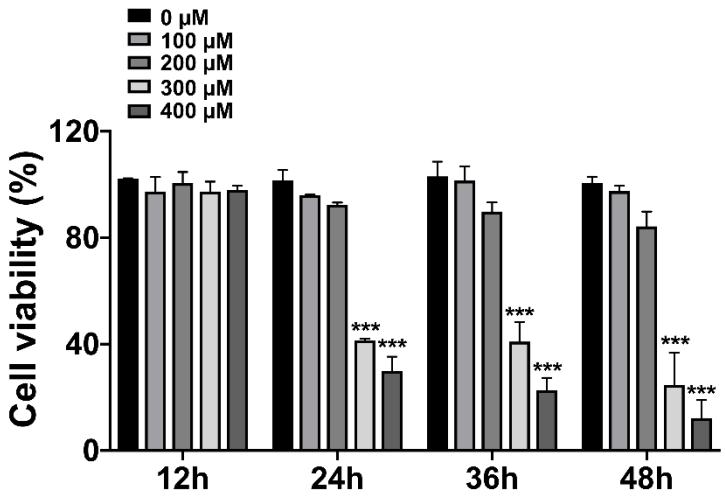
Supplementary Tables and Figures

Supplementary Table 1. List of miRNAs with low expression and unchanged expression in APP/PS1 mice at different disease stages compared with age-matched WT controls using high-throughput sequencing analysis.

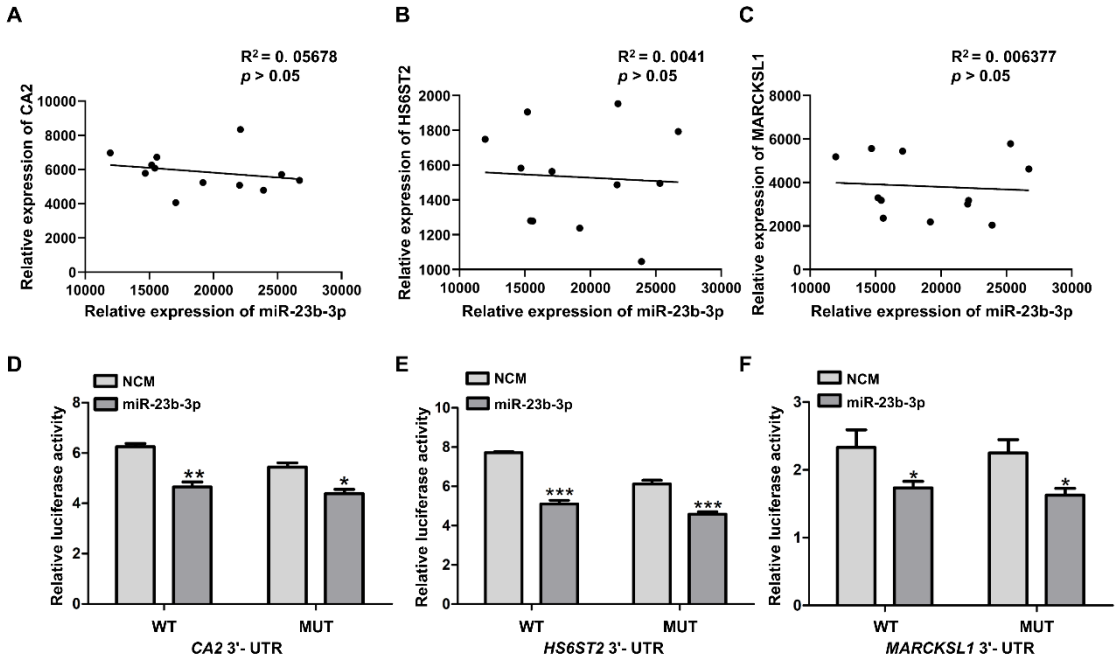
	1-month-old APP/PS1 mice versus WT control			3-month-old APP/PS1 mice versus WT control			6-month-old APP/PS1 mice versus WT control			9-month-old APP/PS1 mice versus WT control		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
miR-23b-3p	1.00	0.58	0.68	0.95	1.06	0.88	0.88	0.47	0.62	0.61	0.76	0.60
miR-195a-5p	1.30	0.50	0.57	1.23	0.82	0.89	1.04	0.47	0.82	0.76	0.87	0.54
miR-486a-3p	1.61	0.92	1.96	0.35	0.46	0.58	1.15	0.58	0.58	0.46	1.15	0.58
miR-339-5p	1.09	0.79	1.24	1.00	1.12	1.15	0.88	1.21	0.72	1.13	1.00	1.24
miR-19a-3p	2.14	1.39	1.57	1.07	1.52	1.27	1.39	0.86	0.54	0.75	1.16	0.96
miR-29a-3p	0.90	0.58	0.67	0.87	1.10	0.94	0.95	0.75	0.65	0.78	0.89	0.77
miR-130a-3p	1.50	1.00	1.50	2.00	1.00	2.00	2.00	1.50	1.00	1.00	1.00	1.00
miR-21a-5p	1.15	0.23	0.23	0.23	1.15	0.92	1.38	0.23	0.92	1.15	0.92	1.15
miR-1298-5p	0.69	0.98	1.03	0.52	1.08	0.69	0.82	0.63	0.30	0.96	0.54	1.04
miR-26b-3p	1.23	0.59	0.77	0.95	1.19	0.80	0.94	0.56	0.64	0.73	1.17	0.69
miR-192-5p	1.33	0.70	0.90	0.94	1.16	0.97	1.04	0.77	0.54	1.46	0.76	0.67
miR-10b-5p	2.14	1.00	0.88	1.17	0.70	0.65	1.06	0.70	0.40	1.20	0.88	0.63
miR-193a-5p	1.14	1.00	0.71	0.57	0.43	0.71	0.71	0.14	0.29	1.14	0.29	0.86
miR-1298-3p	0.91	1.02	2.12	0.61	1.90	1.00	1.37	1.17	0.29	0.87	0.59	1.36
miR-133a-3p	1.21	0.63	0.75	1.48	1.35	1.04	1.25	0.73	0.96	0.79	0.97	0.70
miR-138-5p	1.24	0.94	1.40	1.01	1.70	1.20	1.22	0.60	0.57	0.78	0.71	0.99
miR-218-3p	1.30	0.81	1.16	1.25	1.42	1.22	1.08	0.61	0.67	0.86	0.93	0.76
miR-28a-3p	1.46	0.84	1.10	1.21	1.58	0.98	1.20	1.10	0.66	1.18	1.15	1.04
miR-3072-3p	1.89	1.25	1.39	1.54	1.67	1.09	1.15	0.44	0.38	0.93	1.19	1.11
miR-449a-5p	0.88	0.74	1.38	0.85	1.54	1.07	1.32	0.50	0.47	0.41	0.41	1.57

Supplementary Table 2. Predicted miR-23b-3p targets obtained from TargetScan, miRDB and Tarbase with SVR and PhastCons scores.

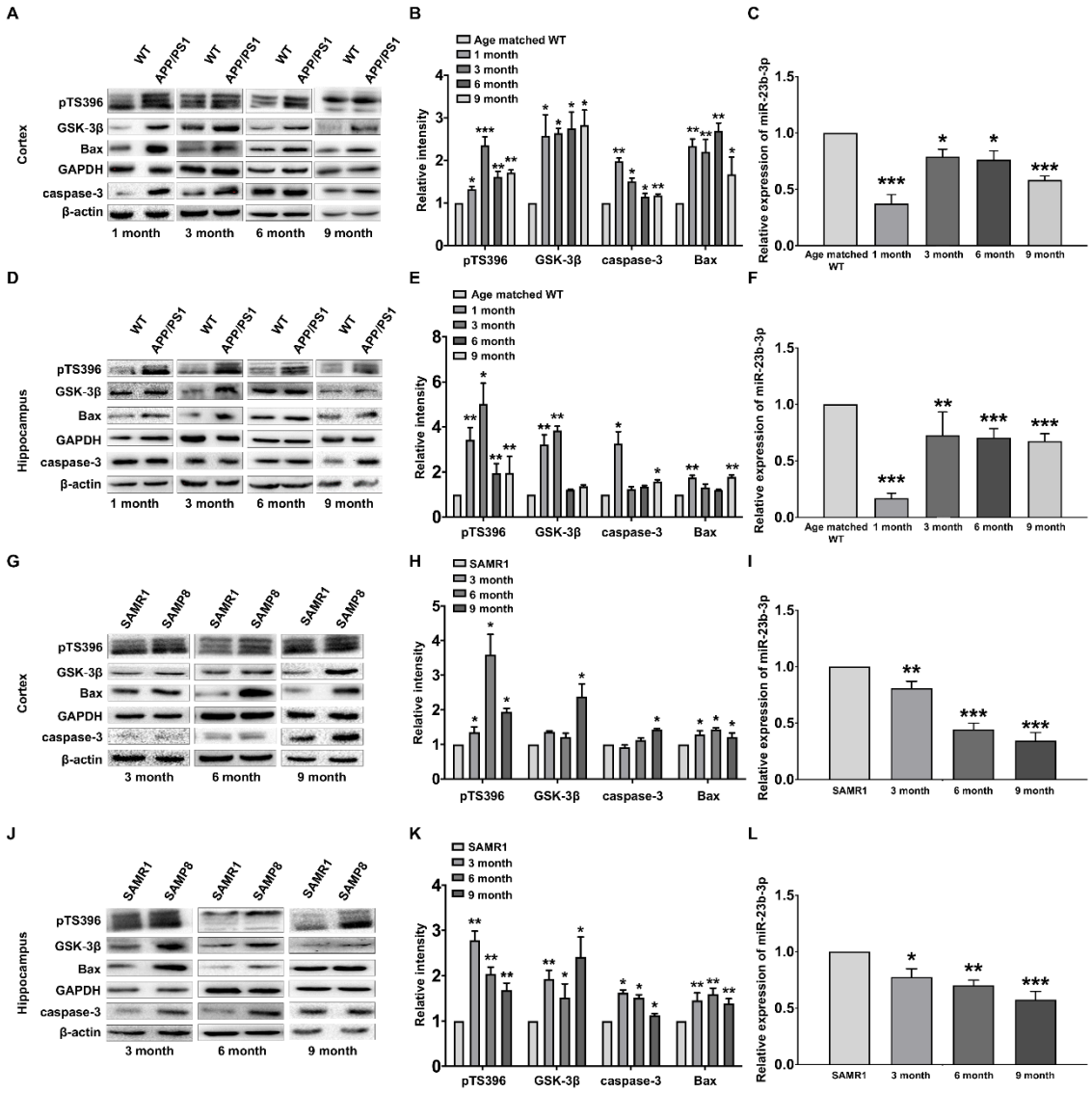
Target gene	SVR score	PhastCons score
ASAP1	-0.3285, -0.6249	0.5245, 0.7704
CA2	-1.2158	0.5660
CEP350	-0.1122, -1.1661	0.6767, 0.6834
GSK3B	-0.5377	0.6265
HS6ST2	-0.8510, -1.1982	0.6642, 0.6872
KIAA1109	-1.0751	0.5632
MARCKSL1	-1.1870	0.7188
MCFD2	-0.3414, -0.6952	0.7049, 0.7040
NEK6	-0.8075, -1.3071	0.6007, 0.7370
NLGN4X	-0.2045, -0.1165, -0.8240	0.5776, 0.5717, 0.6554
PTEN	-0.8937, -0.4545	0.6396, 0.7878
TAB3	-0.3918, -0.2632	0.5845, 0.6660
TARDBP	-0.2616, -0.3191	0.6722, 0.5824
TMEM38B	-0.2125	0.6526
TTC33	-0.1832	0.5430
ZNF423	-1.2174	0.8157
CACUL1	/	/
SIPA1L1	/	/



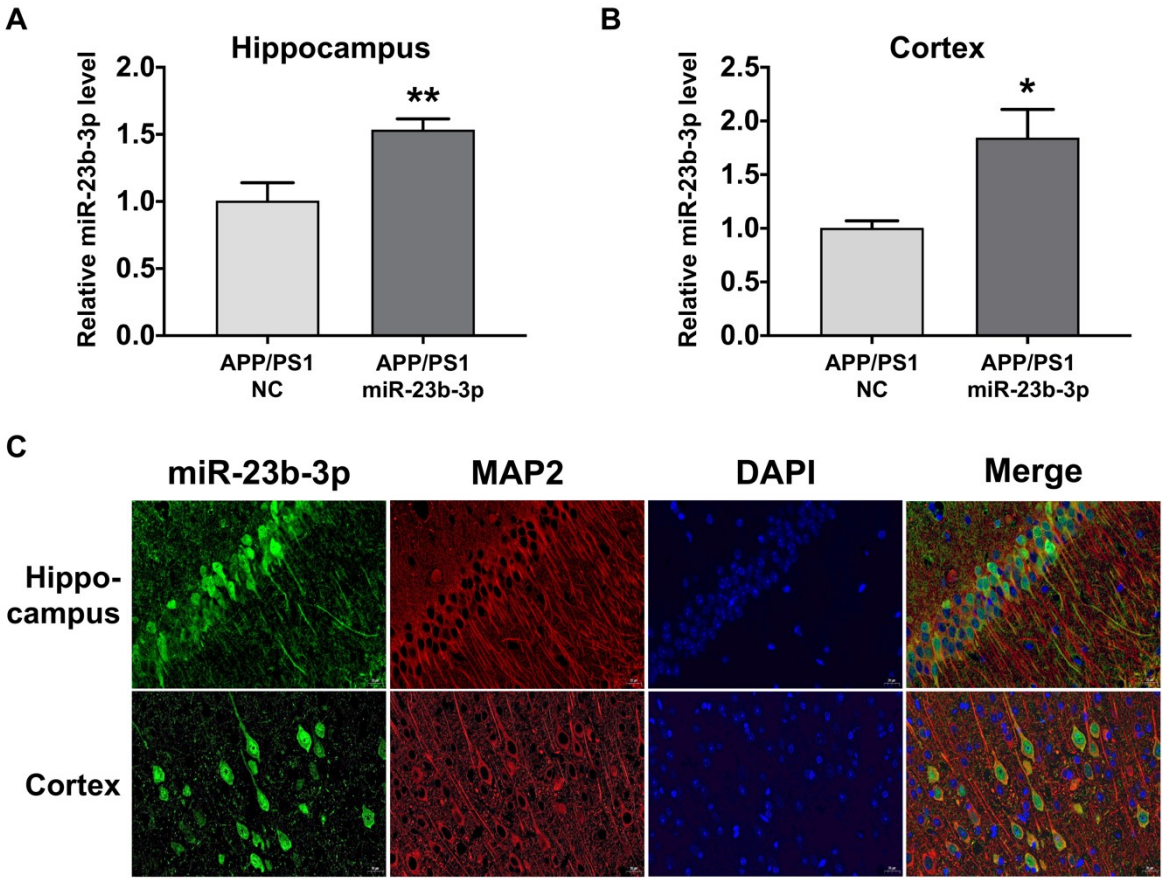
Supplementary Figure 1. Cytotoxicity of copper in APPswe cells. Results represent means ± SEM. $n = 3$. ***$p < 0.001$ *vs.* control. Comparisons among multiple groups were analyzed by one-way ANOVA, followed by Turkey's *post-hoc* testing to analyze differences between groups.



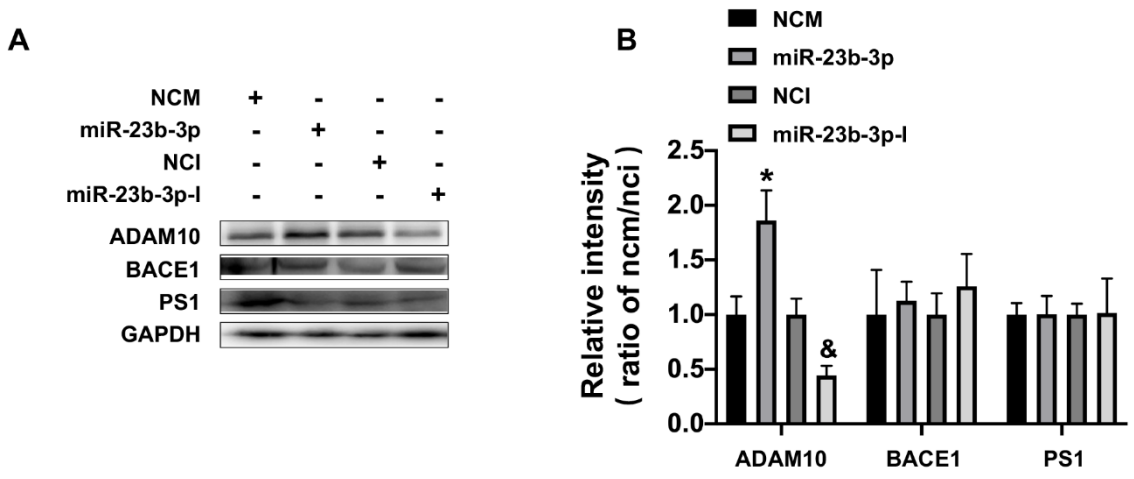
Supplementary Figure 2. Validation of the relationship between miR-23b-3p and three additional predicted targets. (A-C) The correlation analysis between miR-23b-3p and CA2 (A), HS6ST2 (B), and MARCKSL1 (C). (D-F) Dual-luciferase reporter assay of HEK293 cells transfected with WT 3'-UTR or MUT 3'-UTR reporter of CA2 (D), HS6ST2 (E), and MARCKSL1 (F) together with miR-23b-3p mimics or NCM. Comparisons between two groups were analyzed by unpaired *t*-test. Results represent means ± SEM. $n = 4$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* NCM.



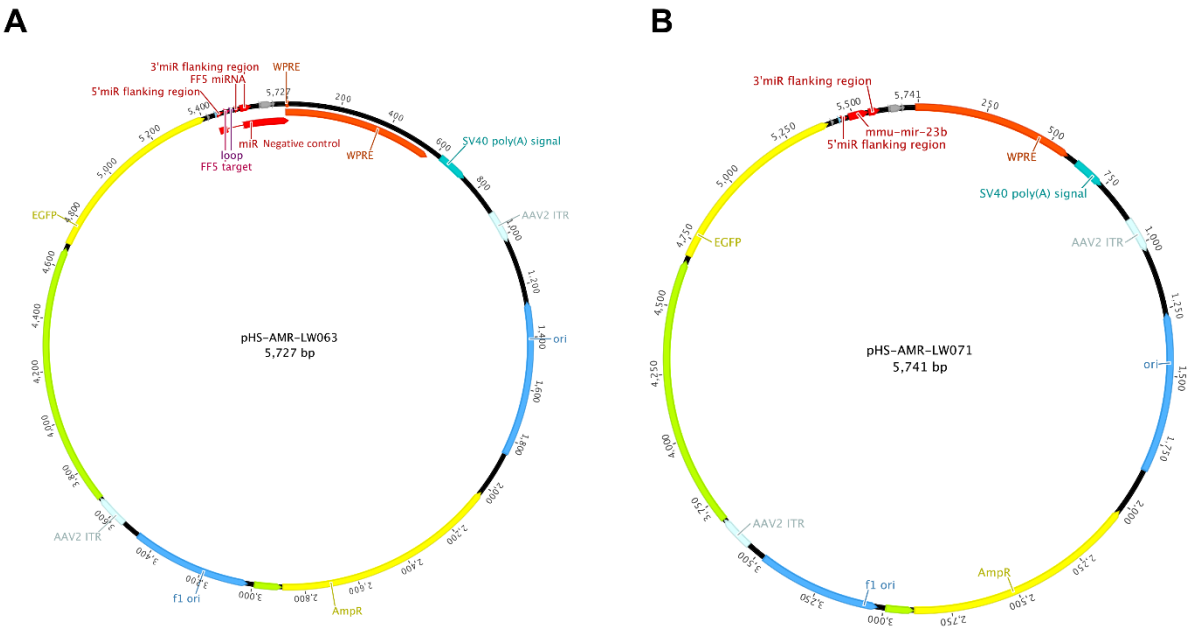
Supplementary Figure 3. Expression of miR-23b-3p-related GSK-3β signaling pathways in the brain of APP/PS1 and SAMP8 mice. (A-F) Expression of p-Tau-Ser396, GSK-3β, caspase-3, miR-23b-3p, and Bax protein in the cortex (A-C) and hippocampus (D-F) of APP/PS1 mice. (G-L) Expression of p-Tau-Ser396, GSK-3β, caspase-3, miR-23b-3p, and Bax protein in the cortex (G-I) and hippocampus (J-L) of SAMP8 mice. β-actin and GAPDH were used as loading controls. Comparisons among multiple groups were analyzed by one-way ANOVA, followed by Turkey's *post-hoc* testing to analyze differences between groups. Results represent means ± SEM. $n = 4$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* WT or SAMR1.



Supplementary Figure 4. miR-23b-3p upregulation and distribution in the brain. (A-B) miR-23b-3p upregulation in the hippocampus (A) and cortex (B) of AAV-miR-23b-3p-treated APP/PS1 mice. Comparisons between two groups were analyzed by unpaired *t*-test. (C) Double-labeling for AAV-CAG-EGFP-miR-23b-3p and microtubule associated protein 2 (MAP2, neuron-specific cytoskeletal protein; Proteintech Group, Inc., Rosemont, IL, USA) in the hippocampus and cortex of AAV-miR-23b-3p-treated APP/PS1 mice. Results represent means ± SEM. $n = 4$. Bar, 20 μm. *$p < 0.05$, **$p < 0.01$ *vs.* APP/PS1 mice treated with NC.



Supplementary Figure 5. Effects of miR-23b-3p on the amyloidogenic and non-amyloidogenic pathways in APPswe cells. (A) Western blot images of ADAM10, BACE1, and PS1 in copper-treated APPswe cells after transfection of negative control mimics (NCM), miR-23b-3p mimics (miR-23b-3p), and inhibitor (miR-23b-3p-I); (B) Quantitative analysis of these proteins measure in western blot. Comparisons between two groups were analyzed by unpaired *t*-test. Results represent means ± SEM. $n = 3$. $^{*}p < 0.05$ *vs.* NCM, $^{\&}p < 0.05$ *vs.* NCI.



Supplementary Figure 6. Structural diagram of the AAV-NC (A) and AAV-miR-23b-3p (B).