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Supplemental information

Implications of miR-148a-3p/p35/PTEN signaling in tau hyperphosphorylation and autoregulatory feedforward of Akt/CREB in Alzheimer's disease

Li Zeng, Hailun Jiang, Ghulam Md Ashraf, Jianghong Liu, Linlin Wang, Kaiyue Zhao, Mimin Liu, Zhuorong Li, and Rui Liu

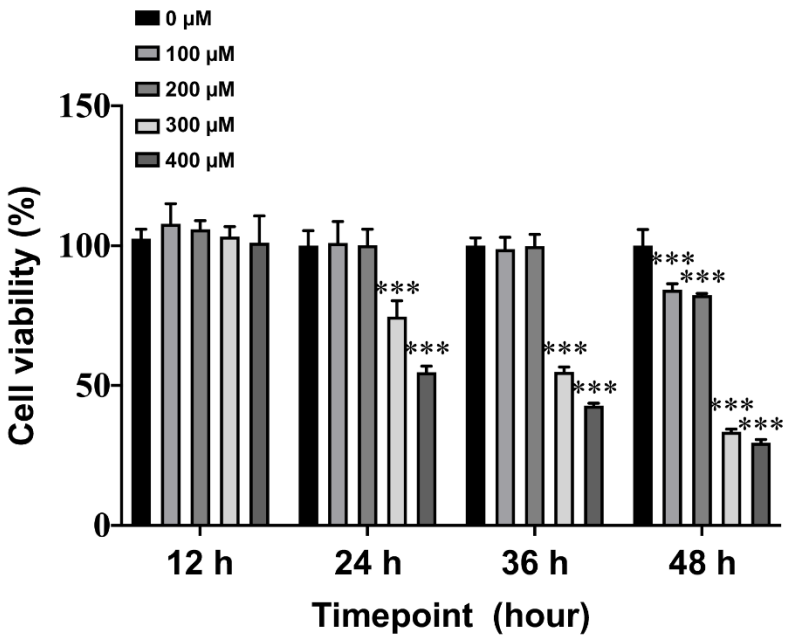
Supplementary Table 1. List of downregulated miRNAs in the cortex of APP/PS1 mice at different disease stages compared with age-matched WT controls obtained by high-throughput sequencing analysis.

	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-148a-3p	20.74	23.92	27.1	16.03	16.62	16.47	18.51	20.77	16.2	15.66	21.1	18.33
miR-10a-5p	36.67	35.53	29.97	42.02	34.17	35.58	70.21	119.84	61.51	171.43	144.53	93.66
miR-144-3p	397.72	274.3	258.69	293.64	186.61	169.01	275.52	137.5	211.6	25.92	65.72	103.83
miR-144-5p	24.98	16.7	13.6	13.48	10.44	6.02	15.35	11.31	12.43	2.08	3.05	3.29
miR-706	0.16	0	0.22	0	0.08	0	0.33	0	0.94	0.55	0	0.43
miR-451a	307.24	218.96	207.15	251.13	176.26	150.75	338.44	256.77	195.71	31.6	51.74	107.98
miR-7651-5p	0.08	0.85	1	0.29	0.17	0.63	0.22	0.57	0.63	0	0.25	0.14
miR-190b-3p	0.08	0.42	0.22	0.2	0	0	0.44	0	0.16	0	0.25	0.14
miR-3093-3p	0.56	0.85	0.77	0.39	0.75	0.63	1.09	1.13	1.42	0.42	1.4	1.29
miR-361-5p	0.24	0.28	0.33	0.1	0.33	0.42	0.54	0.42	0.16	0.42	0.51	0.57
miR-6966-5p	0.08	0	0.11	0	0.25	0	0.54	0.28	0.31	0.55	0	0.29
miR-1960	0.16	0.28	0.11	0	0	0.11	0.65	0.42	0.47	0.14	0	0

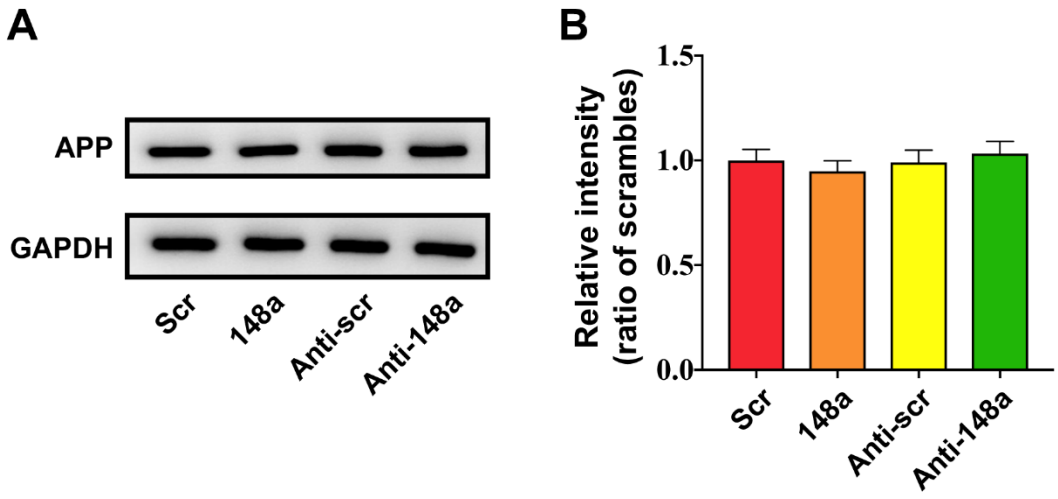
Supplementary Table 2. Predicted miR-148a-3p targets obtained from TargetScan, miRDB, and Tarbase with SVR and PhastCons scores by miRanda database.

Target gene	SVR score	PhastCons score
CDK5R1	-0.1732, -1.1160	0.6726, 0.7310
PTEN	-0.8271, -1.1250	0.7479, 0.8259
RAB14	-0.6152	0.7585
CCT6A	-1.2625	0.5446
KLF6	-0.6231, -0.2811	0.7672, 0.5823
NRP1	-1.3151	0.8190
RASSF8	/	/
DSTYK	/	/
SESTD1	-0.4249	0.7870
STARD13	-1.0885	0.6333
RNF219	-1.1764, -0.1473	0.7210, 0.7876
MET	-0.6736	0.7518
DICER1	-1.0661	0.6992
BMP3	-0.4221	0.5468
DNMT1	-1.0394	0.5812
LDLR	-0.6683, -0.3894	0.5086, 0.5129
MTMR9	-0.1911, -0.3442	0.6080, 0.6620
ARL6IP1	-1.1916	0.6270
TNRC6A	-1.0862	0.6934
TNRC6B	/	/
PRNP	-0.7594	0.6691

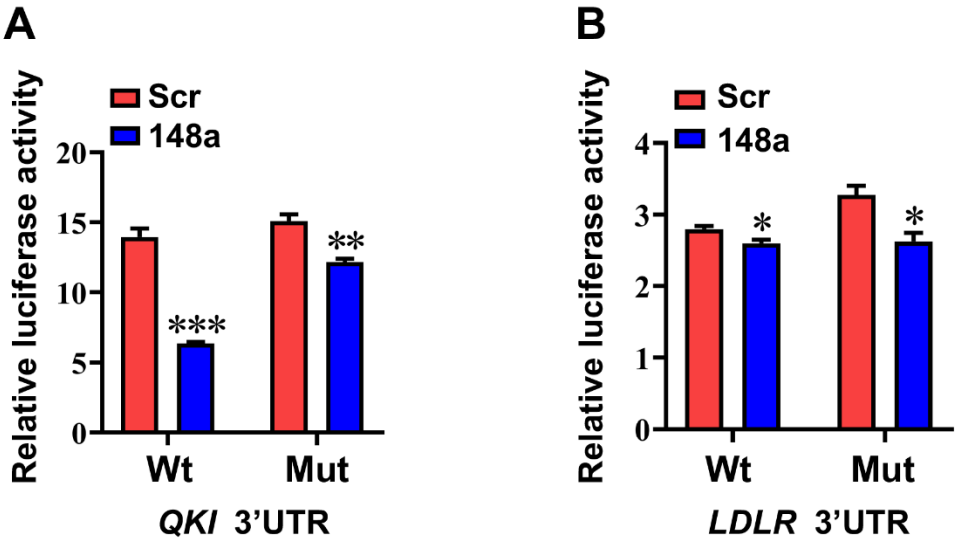
USP38	-0.4790	0.6421
NPTX1	-0.6437	0.6847
MAP3K4	-0.9967	0.6347
QKI	-0.8427	0.7996
INO80	-0.4482, -0.8962	0.6178, 0.6178
PHACTR2	/	/
ALCAM	-0.7482	0.6758
BCL2L11	-1.0531	0.8164
TGIF2	/	/
YWHAB	-1.3163	0.6428
FXR1	-1.3371	0.7250
ZFYVE26	-0.5147,-1.1036	0.5476,0.6386
MAP3K9	-0.8604	0.5968
RPS6KA5	-0.0467,-1.0577,-0.1758,-0.8790,-1.1316	0.6114,0.6956,0.6500,0.6500
TGFB2	-1.1434	0.6894
LBR	-0.6630	0.4498
CDKN1B	-0.2472	0.6546
DYRK1A	-0.0207,-0.2277	0.6207,0.7089
DYNLL2	-0.5181	0.6807
SH3PXD2A	-0.0028,-0.005,-0.002	0.6362,0.5135,0.5511



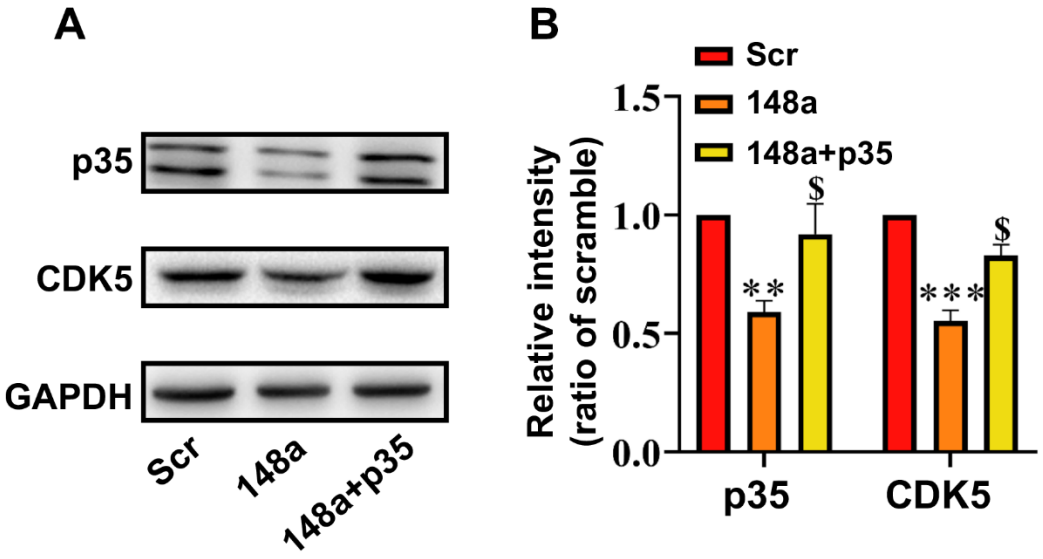
Supplementary Figure 1. Cytotoxicity of copper in APPswe cells. Results represent means ± SEM. $n = 3$. ***$P < 0.001$ *vs.* 0 μM copper at 12 h.



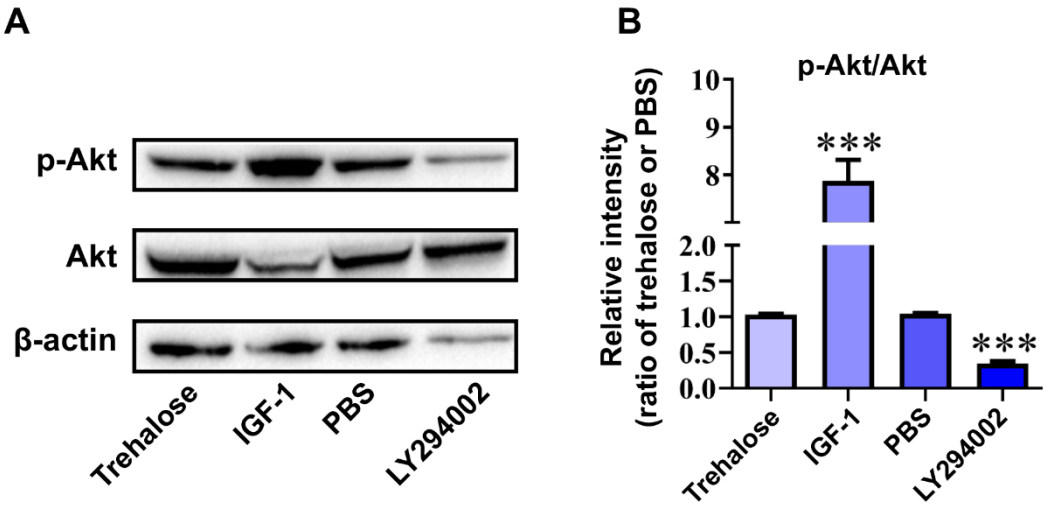
Supplementary Figure 2. Unchanged APP expression in APPswe cells after transfection with miR-148a-3p mimics and anti-miR-148a-3p. Results represent means ± SEM. $n = 4$. Abbr.: 148a, miR-148a-3p mimics; Anti-148a, anti-miR-148a-3p; Scr, scrambled control; Anti-scr, anti-scrambled control.



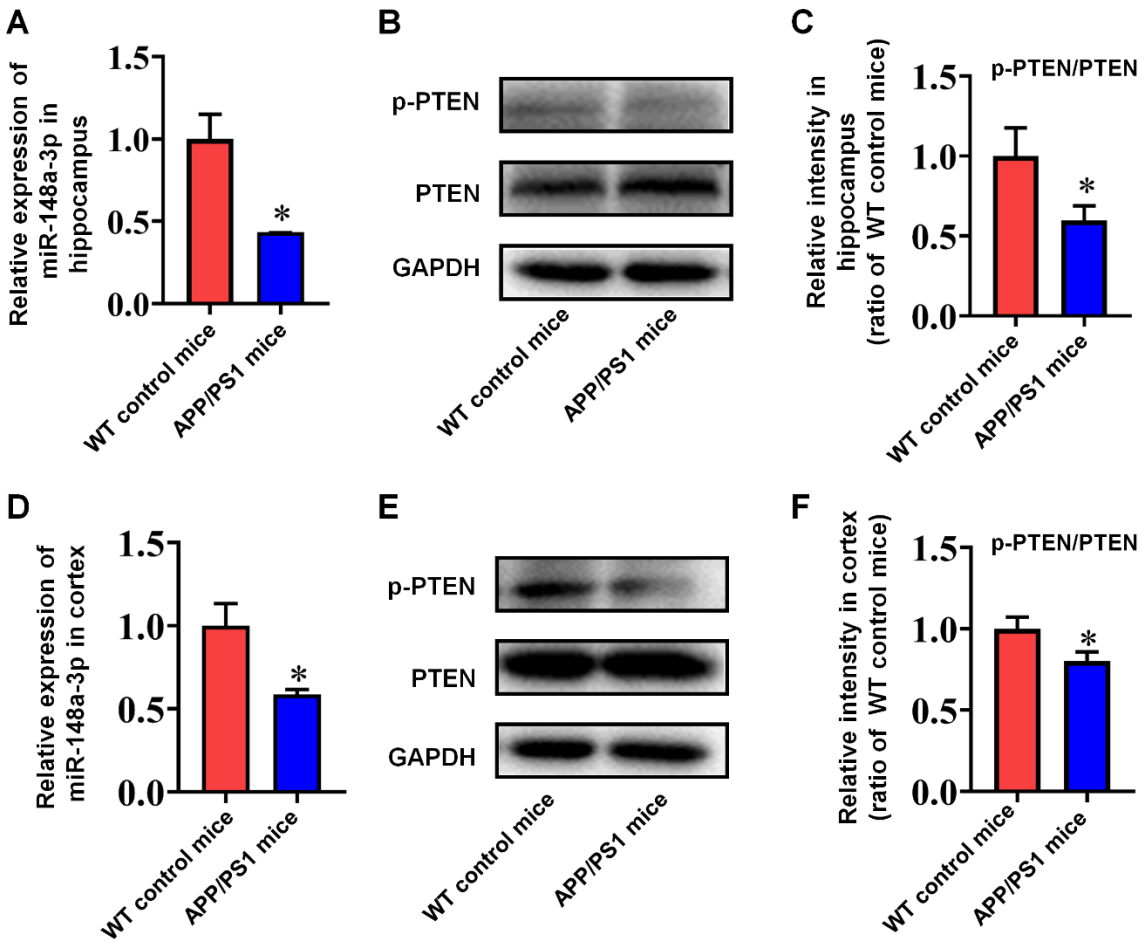
Supplementary Figure 3. Dual-luciferase reporter assay in HEK293 cells transfected with wide-type (WT) 3'-UTR or mutant (MUT) 3'-UTR reporter of *QKI* (A) and *LDLR* (B) together with miR-23b-3p mimics (148a) or scrambled control (Scr). Results indicated that the predicted genes *QKI* and *LDLR* were not specific targets of miR-148a-3p. Results represent means ± SEM, $n = 5$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Scr.



Supplementary Figure 4. Expression of p35 and CDK5 in APPswe cells transfected with scrambled control (Scr), miR-148a-3p mimics (148a) and p35 overexpressing plasmid (p35). Representative Western blot images of p35 and CDK5 (A) and qualification of expression of p35 and CDK5 (B). Results represent mean ± SEM, $n = 4$. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* Scr. $^{\$}P < 0.05$ *vs.* 148a.



Supplementary Figure 5. Expression of p-Akt and Akt in APPswe cells subjected to the stimulus of Akt by IGF and inhibition of PI3K by LY294002. Representative Western blot images of p-Akt and Akt (A) and qualification of ratio of p-Akt/Akt (B). Results represent means ± SEM, $n = 4$. $^{***}P < 0.001$ *vs.* trehalose/PBS.



Supplementary Figure 6. Expression of miR-148a-3p and PTEN in the hippocampus and cortex of APP/PS1 mice. (A) Decreased level of miR-148a-3p in the hippocampus of APP/PS1 mice. (B,C) Representative Western blot images of p-PTEN and PTEN (B) and qualification of decreased ratio of p-PTEN/PTEN (C) in the hippocampus of APP/PS1 mice. (D) Decreased level of miR-148a-3p in the cortex of APP/PS1 mice. (E,F) Representative Western blot images of p-PTEN and PTEN (E) and qualification of decreased ratio of p-PTEN/PTEN (F) in the cortex of APP/PS1 mice. Results represent means ± SEM, $n = 4$. *$P < 0.05$ *vs.* WT control mice.