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Supplemental Information

Targeting the HDAC2/HNF-4A/miR-101b/AMPK Pathway Rescues Tauopathy and Dendritic Abnormalities in Alzheimer's Disease

Dan Liu, Hui Tang, Xin-Yan Li, Man-Fei Deng, Na Wei, Xiong Wang, Ya-Fan Zhou, Ding-Qi Wang, Peng Fu, Jian-Zhi Wang, Sébastien S. Hébert, Jian-Guo Chen, Youming Lu, and Ling-Qiang Zhu

Supplementary Figures

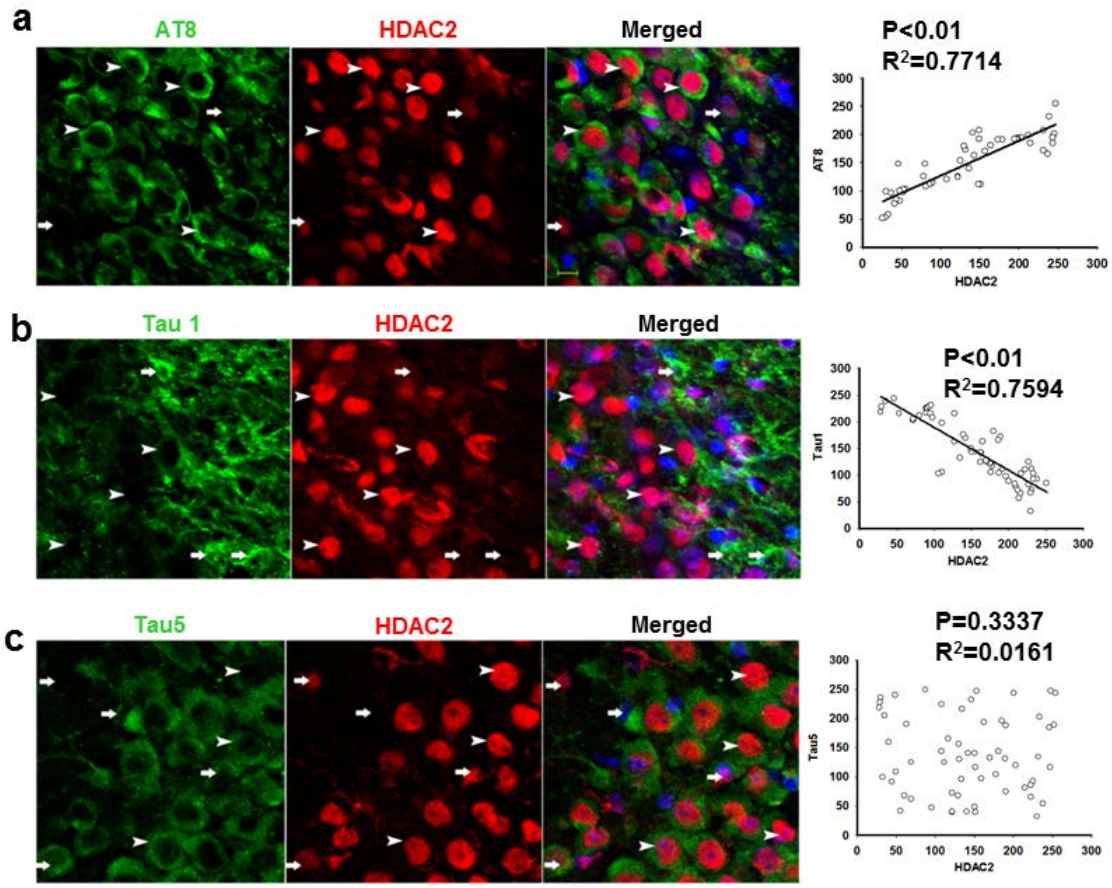

Fig. S1. HDAC2 expression is correlated with tau phosphorylation in P301L Tau mice

(a) The double immunofluorescence were performed in the 9-month P301L Tau mice hippocampal slices by using the AT8 (Green) and HDAC2 (Red) antibodies and the correlation analysis was performed by Sigmaplot after the fluorescent intensity measurement by Image J. Arrowhead, neurons with high level of HDAC2 intensity; Arrow, neurons with low level of HDAC2 intensity. N=52.

(b) The double immunofluorescence were performed in the 9-month P301L Tau mice hippocampal slices by using the Tau1 (Green) and HDAC2 (Red) antibodies and the correlation analysis was performed by Sigmaplot after the fluorescent intensity

measurement by Image J. Arrowhead, neurons with high level of HDAC2 intensity; Arrow, neurons with low level of HDAC2 intensity. N=58.

(c) The double immunofluorescence were performed in the 9-month P301L Tau mice brain slices by using the Tau5 (Green) and HDAC2 (Red) antibodies and the correlation analysis was performed by Sigmaplot after the fluorescent intensity measurement by Image J. Arrowhead, neurons with high level of HDAC2 intensity; Arrow, neurons with low level of HDAC2 intensity. N=55.

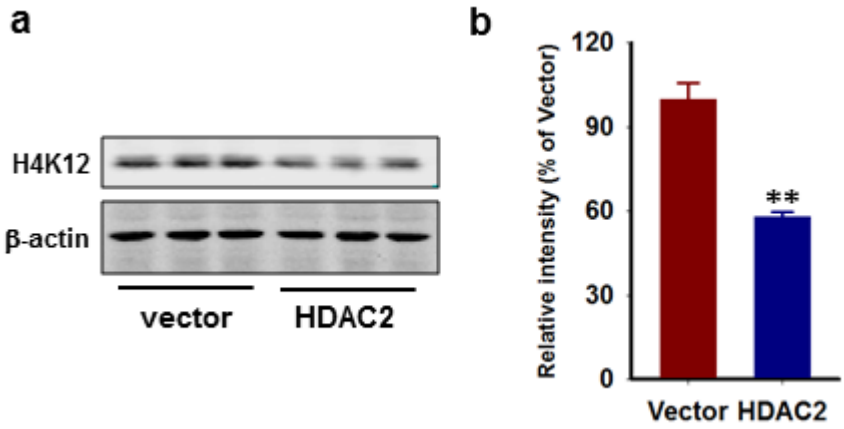

Fig. S2. Overexpression of HDAC2 reduces the acetylation of histone 4 at lysine 12 site.

(a, b) The hippocampal neurons were cultured to 14 DIV and then infected with lenti-HDAC2 (HDAC2) or vector control (vector) viruses. 48 hours later, the neurons were harvest and the cell lyses were used for western blot by the indicated antibodies. The representative images were shown in (a) and the quantitative analysis were performed (b). ** *P<0.01*, *vs.* Vector. N=4.

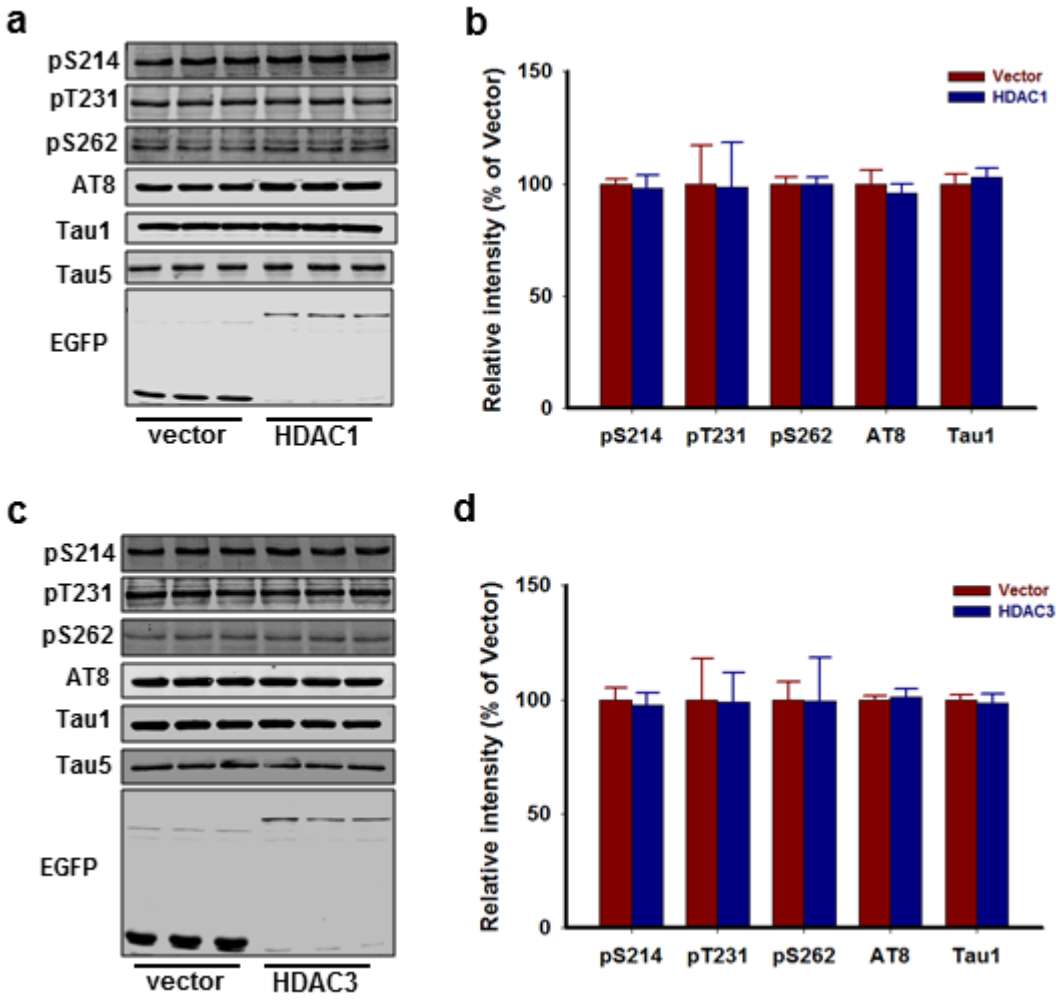

Fig. S3. Overexpression HDAC1 or HDAC3 does not induce the tauopathy *in vitro*

(a, b) The hippocampal neurons were cultured to 14 DIV and then infected with lenti-HDAC1 (HDAC1) or vector control (vector) viruses. 48 hours later, the neurons were harvest and the cell lyses were used for western blot by the indicated antibodies pS214, pT231, pS262, AT8, Tau1 and Tau5. The representative blots were shown in (a) and the quantitative analysis were performed in (b).

(c, d) The hippocampal neurons were cultured to 14 DIV and then infected with lenti-HDAC3 (HDAC3) or vector control (vector) viruses. 48 hours later, the neurons

were harvest and the cell lyses were used for western blot by the indicated antibodies pS214, pT231, pS262, AT8, Tau1 and Tau5. The representative blots were shown in (c) and the quantitative analysis were performed in (d).

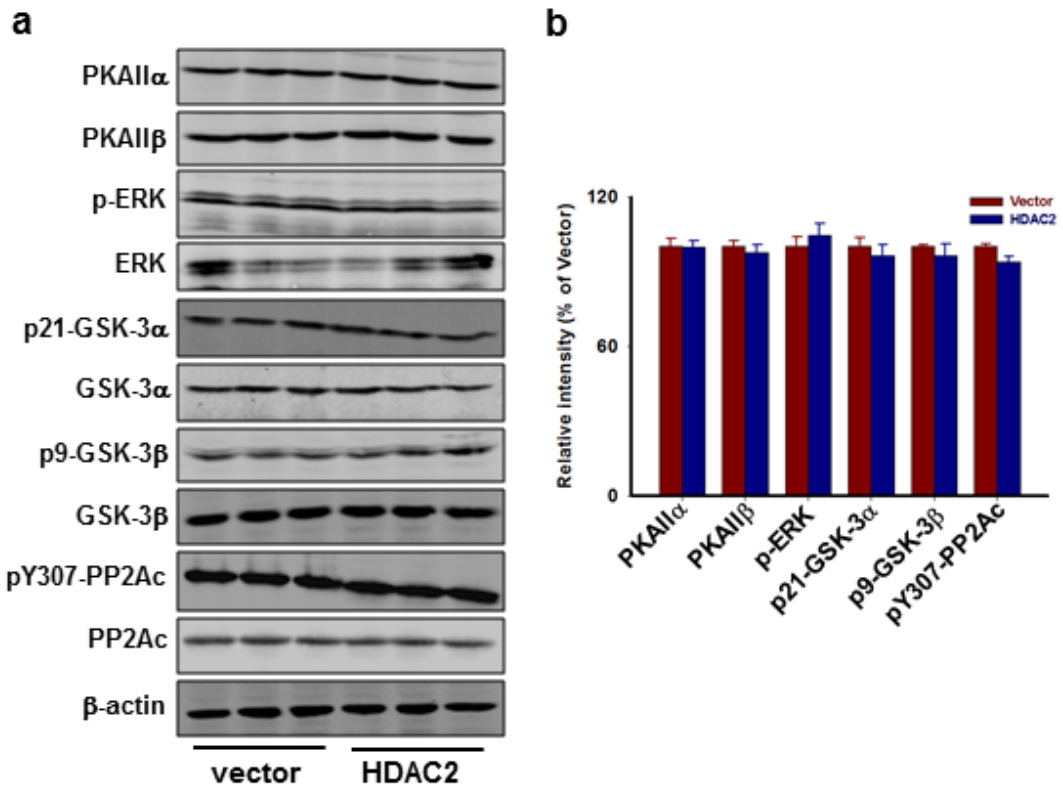

Fig. S4. Overexpression HDAC2 does not alter the activities of PKA, ERK, GSK-3α/β and PP2Ac *in vitro*

(a, b) The hippocampal neurons were cultured to 14 DIV and then infected with lenti-HDAC2 (HDAC2) or vector control (vector) viruses. 48 hours later, the neurons were harvest and the cell lyses were used for western blot by the indicated antibodies above. The representative blots were shown in (a) and the quantitative analysis were performed in (b).

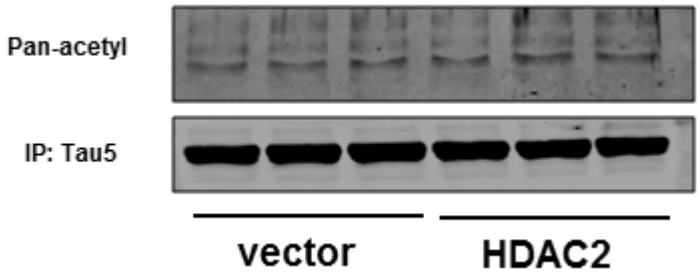

Fig. S5. Overexpression HDAC2 does not alter the acetylation of tau *in vitro*

The hippocampal neurons were cultured to 14 DIV and then infected with lenti-HDAC2 (HDAC2) or vector control (vector) viruses. 48 hours later, the neurons were harvest and the cell lyses were immunoprecipated with Tau 5 and then the pellets were immunoblotted by pan-acetylation antibody.

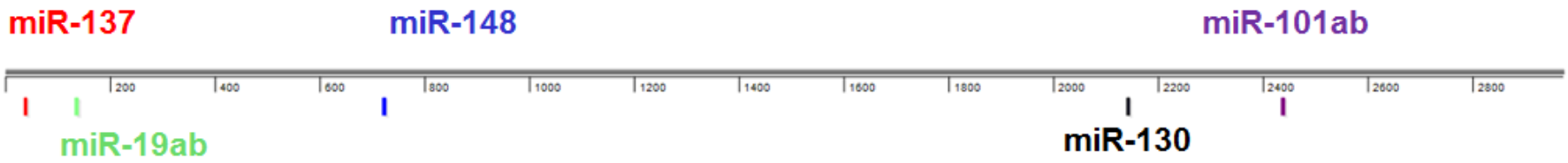

Fig. S6. The predicted binding sites of miRNAs in the 3'UTR of *PRKAA1*.

The 3'UTR sequence of *PRKAA1* was input to the Seqbuilder (Lasergene 7.0) and the different binding sites of predicted miRNAs were indicated.

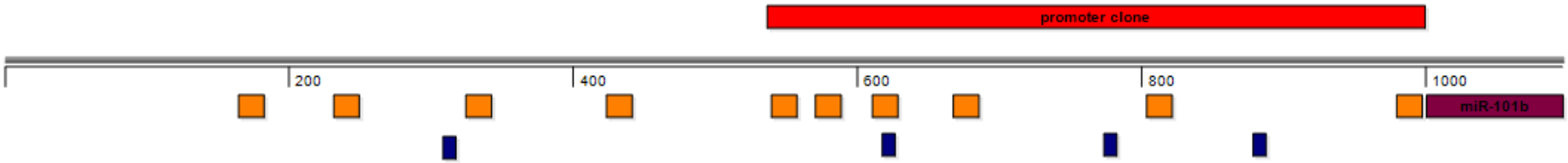

Fig. S7. The predicted binding sites of HNF-4A and Sox5 transcptional factor in the upstream of pri-miR-101b.

The upstream 1000 bp sequence of pri-miR-101b was input to the Seqbuider (Lasergene 7.0). The pri-miR-101b was labeled as purple color; the

cloned promoter sequence in luciferase experiment was labeled as red color. The predicted HNF-4A and Sox5 binding motifs were labeled as yellow color and blue color separatedly.

Supplementary Table 1: Potential binding sites of miR-101 promoter region that predicted by Motifmap

Location	+/-	BBLS	BLS	NLOD	Z-Score	FDR	Motif ID	TF Name	Gene	Distance	Region
chr19:29209757..29209763	+	6.183	7.815	1	3.683	0.176	M01033	HNF4	Mir101b	-11	Upstream
chr19:29209667..29209687	-	0	0.161	0.789	4.302	0.444	M01655	P53	Mir101b	-81	Upstream
chr19:29209634..29209640	-	1.049	2.613	1	4.476	0.471	MA0004	Arnt	Mir101b	-128	Upstream
chr19:29209634..29209640	+	1.049	2.613	1	4.476	0.471	MA0004	Arnt	Mir101b	-134	Upstream
chr19:29209495..29209503	+	2.113	3.181	1	4.232	0.012	M00712	myogenin	Mir101b	-273	Upstream
chr19:29209295..29209305	+	1.764	2.828	1	4.02	0.082	M01734	NFAT3	Mir101b	-473	Upstream
chr19:29209146..29209152	-	1.236	2.453	1	4.476	0.477	MA0004	Arnt	Mir101b	-616	Upstream
chr19:29209146..29209152	+	1.236	2.453	1	4.476	0.477	MA0004	Arnt	Mir101b	-622	Upstream
chr19:29209105..29209125	+	0	0.512	0.817	4.512	0.213	M01200	CTCF	Mir101b	-663	Upstream
chr19:29209006..29209013	+	3.06	5.394	1	3.688	0.495	M01131	SOX10	Mir101b	-762	Upstream
chr19:29208765..29208777	-	0.007	0.161	0.91	4.421	0.363	MA0141	Esrrb	Mir101b	-991	Upstream
chr19:29208762..29208774	-	0.012	0.161	0.909	4.537	0.081	M01589	ERR2 (ESF	Mir101b	-994	Upstream
chr19:29208765..29208771	-	4.625	5.435	1	3.591	0.058	M01032	HNF4	Mir101b	-997	Upstream
chr19:29208617..29208623	-	1.666	2.566	1	3.683	0.171	M01033	HNF4	Mir101b	-1145	Upstream
chr19:29208539..29208546	-	0.927	1.314	1	4.148	0.087	M00468	AP-2rep	Mir101b	-1222	Upstream
chr19:29208536..29208542	-	0.978	1.314	1	3.683	0.171	M01033	HNF4	Mir101b	-1226	Upstream
chr19:29208253..29208261	-	1.402	1.675	1	3.771	0.379	M00494	STAT6	Mir101b	-1507	Upstream
chr19:29208197..29208204	-	1.365	1.513	1	3.958	0	MA0087	SOX5	Mir101b	-1564	Upstream
chr19:29208096..29208111	+	0.012	0.541	0.843	4.377	0.413	M00495	Bach1	Mir101b	-1672	Upstream
chr19:29208091..29208100	+	0.008	0.161	0.941	4.398	0.154	M01139	LMAF	Mir101b	-1677	Upstream
chr19:29208083..29208095	+	0.396	0.996	0.963	4.68	0.125	M01142	LRH1	Mir101b	-1685	Upstream
chr19:29207921..29207935	+	0.003	0.161	0.881	4.42	0.368	M01185	BCL6	Mir101b	-1847	Upstream

Supplementary Table 2. Potential binding sites of miR-101 promoter region that predicted by TFbind

Supplementary Table 3. Primary antibodies used in current study

Antibodies	Type	WB dilution	IHC dilution	References and sources
Tau1	mAb	-	1:200	Millipore (Billerica, MA, USA)
AT8	mAb	-	1:100	Thermo
HDAC2	mAb	1:1000	1:100	Abcam (Cambridge, UK)
pT231	pAb	1:1000	-	SAB (Pearland, TX, USA)
pS214	pAb	1:500	-	SAB (Pearland, TX, USA)
pT262	pAb	1:500	-	SAB (Pearland, TX, USA)
Tau5	mAb	1:1000	-	Thermo
EGFP	pAb	1:1000	-	Abcam (Cambridge, UK)
DM1A	mAb	1:1000	-	Sigma (St. Louis, MO, USA)
GSK-3β	pAb	1:1000	-	SAB (Pearland, TX, USA)
pS9-GSK-3β	pAb	1:1000	-	Cell Signaling (Danvers, MA, USA)
GSK-3α	pAb	1:1000	-	SAB (Pearland, TX, USA)
pS21-GSK-3α	pAb	1:1000	-	Cell Signaling (Danvers, MA, USA)
PP2ac	mAb	1:1000	-	Millipore (Billerica, MA, USA)
pY307-PP2ac	pAb	1:1000	-	Abcam (Cambridge, UK)
Ace-H4K12	pAb	1:1000		Abcam (Cambridge, UK)
CDK5	mAb	1:1000	-	Santa Cruz, CA, USA
P35/25	pAb	1:1000	-	Santa Cruz, CA, USA
PKAα	pAb	1:1000	-	Santa Cruz, CA, USA
PKAβ	pAb	1:1000	-	Santa Cruz, CA, USA
p-ERK	pAb	1:1000	-	Cell Signaling (Danvers, MA, USA)
ERK	pAb	1:1000	-	Cell Signaling (Danvers, MA, USA)
Pan-acetylation	pAb	1:1000		Cell Signaling (Danvers, MA, USA)
HNF-4A	pAb	1:1000		Sigma (St. Louis, MO, USA)
pS304-HNF-A	pAb	1:500		SAB (Pearland, TX, USA)
AMPK	pAb	1:1000		Abcam (Cambridge, UK)
Goat-anti- mouse -peroxidase	-	1:5,000	-	Pierce Chemical Company
Goat-anti- rabbit -peroxidase	-	1:5,000	-	Pierce Chemical Company