Electronic Supplementary Material (ESI) for Metallomics.
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Supplementary data

Table 1

The milestones for the discoveries of vanadium in chemistry and biological effect.

Time	major events	References
1830	Vanadium was discovered in Sweden by Nils Sefstrom	Sjoberg, Sven Gosta, Journal of Chemical Education, 1951, 28(6):294.
1867	Vanadium metal was first isolated by chemist Henry Enfield Roscoe	Bartel, et al, Chemkon, 2016, 23(3):125-130.
1985	Vanadium (vanadate) first appeared to have an insulin-like action	Heyliger C E, Tahiliani A G, McNeill J H. Science, 1985 , 227:1474 — 1477
1990	Bis(2-ethyl-3-hydroxy-4-pyronato)oxovanadium(IV) (BEOV),was first synthesized	Thompson et al, Metal Ions in Biological Systems, 2004, 41:221-252
2005	Alzheimer's Disease was a Type 3 Diabetes and share common cellular and molecular mechanisms	Eric, et al, Journal of Alzheimer's disease, 2005, 7(1):63-80
2008	BEOV advanced to PhaseII clinical trials for the treatment of diabetes	Thompson et al, Journal of Inorganic Biochemistry, 2009, 103(4):554-558
2019	Vanadium (Vanadyl (IV) acetylacetonate) was first tested on AD in vitro and in vivo models	Dong et al, Science China. Life sciences, 2019, 62(1)
Whether BEOV inhibit AD pathology and possibly be developed for anti-AD drug?		

Table 2

Primary antibody information

Antibody	Host	Application	Source	Identifier
APP	Rabbit	WB (1:10000)	Abcam	Cat#ab32136
BACE1	Rabbit	WB (1:3000)	Abcam	Cat#ab108394
sAPPα	Rabbit	WB (1:500)	Biolegend	Cat#813501
sAPPβ	Rabbit	WB (1:500)	Biolegend	Cat#813401
Aβ1-42	Rabbit	WB (1:500)	Millipore	Cat#AB5078P
Aβ1-16	Mouse	WB (1:500)/IHC (1:50)	Biolegend	Cat#803003
HT5	Mouse	WB (1:1000)	Abcam	Cat#ab80579
HT7	Mouse	WB (1:1000)	Thermo	Cat#MN1000
Tau-pSer404	Rabbit	WB (1:2000)	Abcam	Cat#ab92676
Tau-pThr231	Rabbit	WB (1:5000) /IHC (1:200)	Abcam	Cat#ab151559
Tau-pSer396	Rabbit	WB (1:10000) /IHC (1:200)	Abcam	Cat#ab109390
Tau-pSer422	Rabbit	WB (1:5000)	Abcam	Cat#ab79415
Tau-pSer262	Rabbit	WB (1:2000)	Abcam	Cat#ab64193
PSD95	Rabbit	WB (1:5000) /IHC (1:500)	Abcam	Cat#ab18258

Tau1	Rabbit	WB (1:3000)	Millipore	Cat#MAB3420
Synaptophysin	Rabbit	WB (1:10000)	Abcam	Cat#ab32127
AKT	Rabbit	WB (1:1000)	Abcam	Cat#ab8805
p-AKT	Rabbit	WB (1:1000)	Abcam	Cat#ab81283
GSK3β	Rabbit	WB (1:5000)	Abcam	Cat#ab32391
GSK3β pY216	Rabbit	WB (1:3000)	Abcam	Cat#ab75745
GSK3β pSer9	Rabbit	WB (1:5000)	Abcam	Cat#ab131079
JAK2	Rabbit	WB (1:1000)	Cell Signaling	Cat#3230
p-JAK2	Rabbit	WB (1:1000)	Cell Signaling	Cat#3776
STAT3	Rabbit	WB (1:1000)	Cell Signaling	Cat#12640
p-STAT3	Rabbit	WB (1:1000)	Cell Signaling	Cat#9145
SOCS1	Rabbit	WB (1:1000)	Cell Signaling	Cat#3950
InsR	Rabbit	WB (1:1000)	Cell Signaling	Cat#3025
p-InsR	Rabbit	WB (1:1000)	Cell Signaling	Cat#3024
PTP1B	Rabbit	WB (1:1000)	Cell Signaling	Cat#5311
IDE	Rabbit	WB (1:3000)	Abcam	Cat#ab109538
PPARγ	Rabbit	WB (1:1000)	Cell Signaling	Cat#2443
GAPDH	Rabbit	WB (1:5000)	Proteintech	Cat#10494-1-AP
MAP2	Chicken	IHC (1:10000)	Abcam	Cat#ab5392

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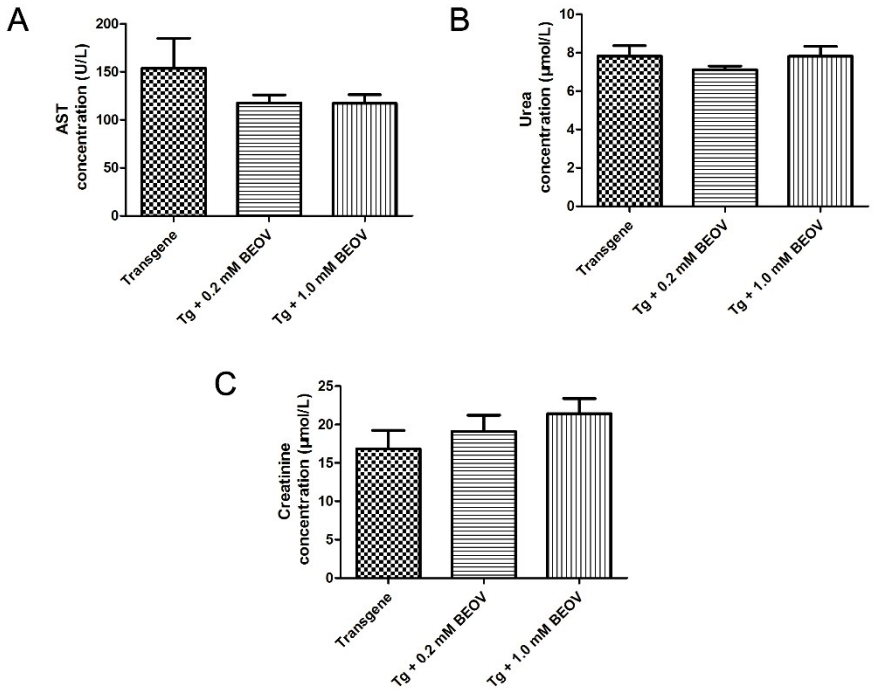
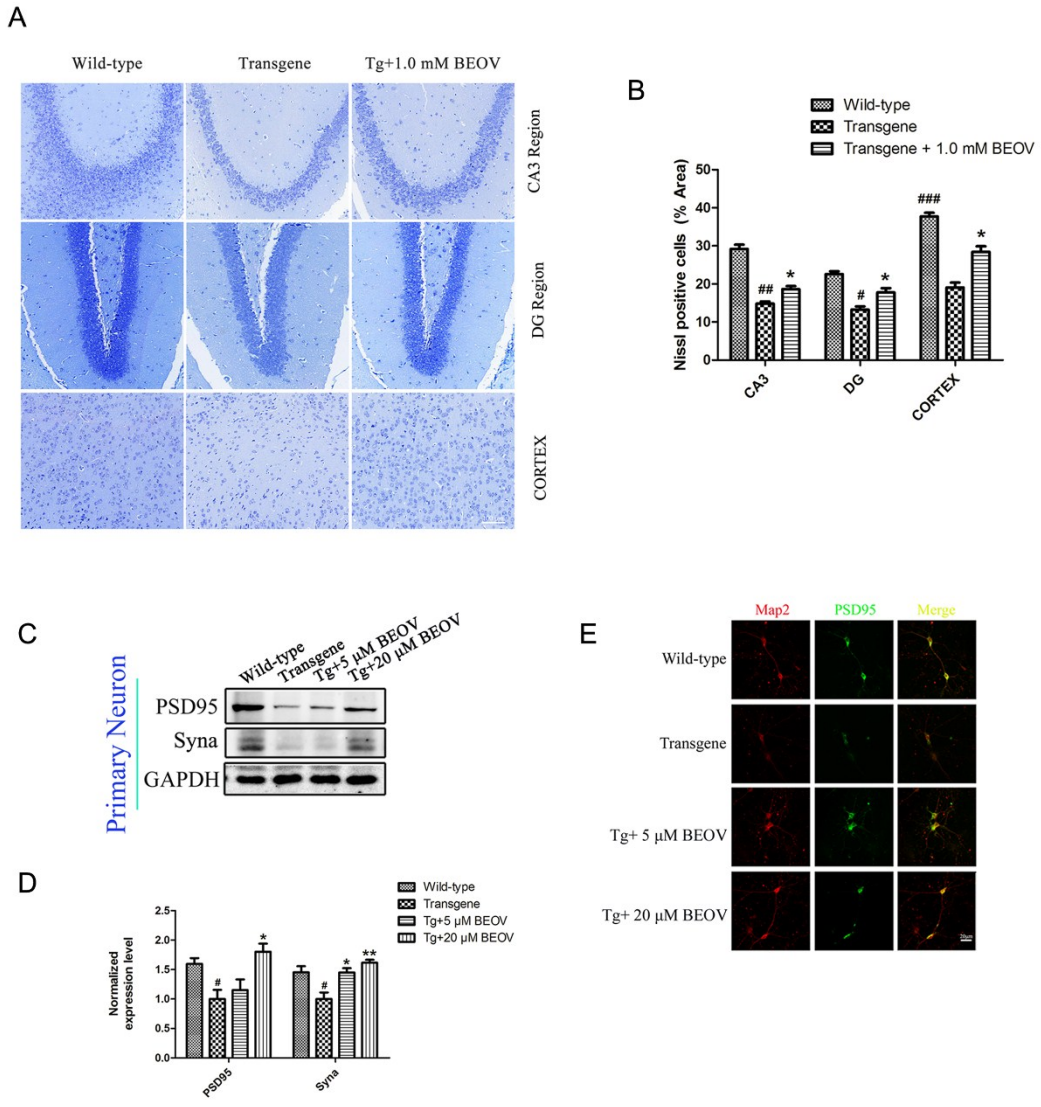
Supplementary Figures

Supplementary Fig. 1



Supplementary Fig. 1 The effect of BEOV on liver and renal function in 3×Tg-AD mice. Serum was collected from triple transgenic AD (3×Tg-AD) and BEOV-treated AD mice. The levels of AST, urea and creatinine were measured (**A-C**), respectively.

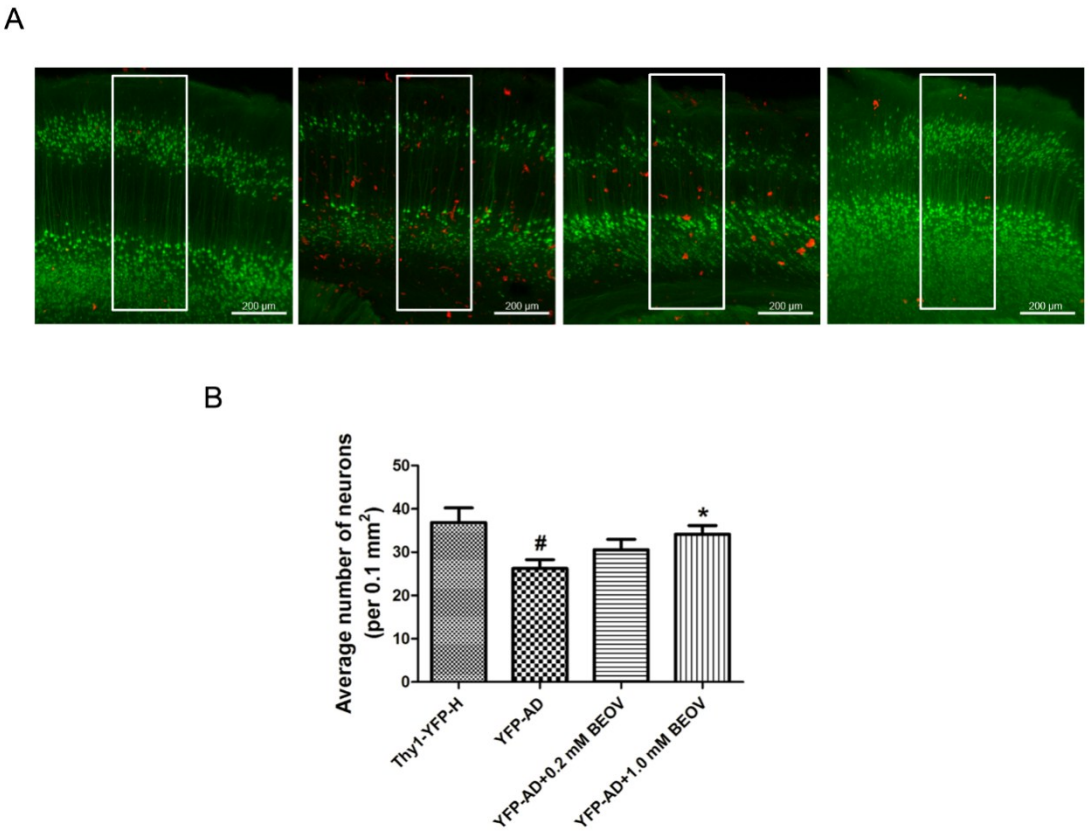
Supplementary Fig. 2



Supplementary Fig. 2 Protective effect of BEOV on hippocampal neurons from 3×Tg-AD mice. Images of Nissl staining in the hippocampal CA3 and DG regions and cerebral cortex of wild-type, AD and BEOV-treated AD mice **(A** and **B)**. Scale bar: 100 μm. Western blots and results of the semiquantitative analysis of synaptophysin and PSD95 levels in primary cultures of hippocampal neurons from the wild-type, AD and BEOV-treated AD mice **(C** and **D)**. Images of immunofluorescence staining for MAP2 and PSD95 expression in primary cultures of hippocampal neurons from the wild-type,

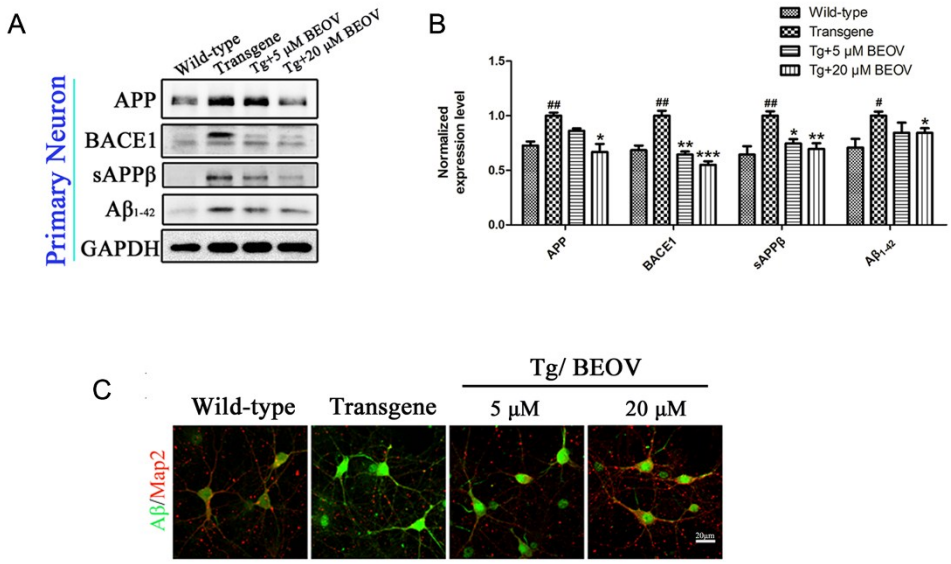
AD and BEOV-treated AD mice (red: MAP2, green: PSD95. n = 3 samples per group) **(E)** (#: WT vs AD; *: AD + BEOV vs AD). (n=3; #P<0.05, ##P<0.01, ###P<0.001, *P<0.05, **P<0.01). Scale bar: 20 μm.

Supplementary Fig. 3



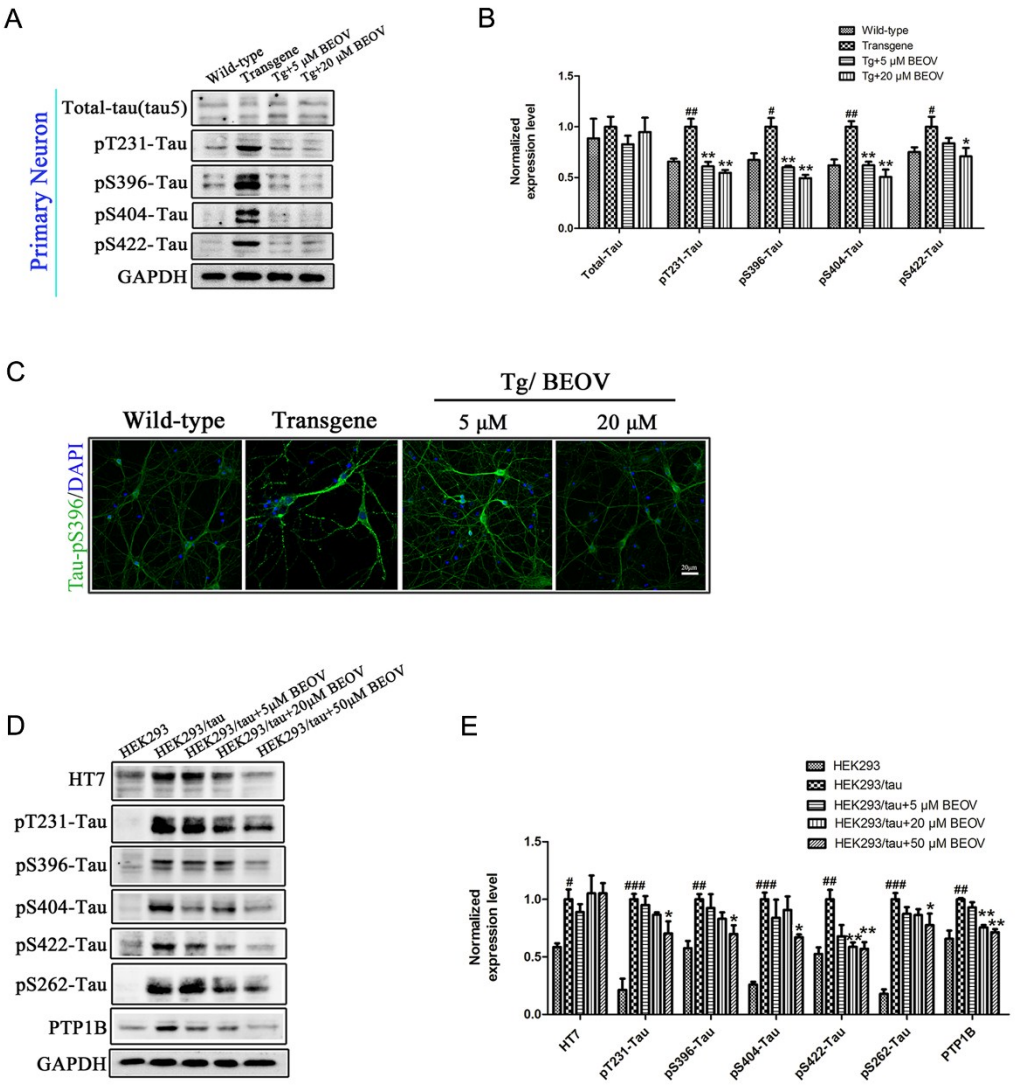
Supplementary Fig. 3. **BEOV attenuated neuronal reduction in the cortex of fluorescent AD mice.** The number of cortical neurons was counted in Thy1-YFP-H, YFP-AD and BEOV-treated YFP-AD mice **(A** and **B)** (#: YFP vs YFP-AD; *: YFP-AD + BEOV vs YFP-AD). (n=3; #P<0.05, *P<0.05). Scale bar: 200 μm

Supplementary Fig. 4



Supplementary Fig. 4 Inhibition of BEOV on the amyloidogenic cascade in primary neuron cultures. Western blots and results of the semiquantitative analysis of several key proteins involved in the amyloidogenic cascade in primary cultures of hippocampal neurons from wild-type, AD and BEOV-treated AD mice **(A** and **B**). Images of immunofluorescence staining for MAP2 and Aβ in those groups of primary cultures of hippocampal neurons (red: MAP2, green: Aβ. n = 3 samples per group) **(C)** (#: WT vs AD; *: AD + BEOV vs AD). (n=3; #P<0.05, ##P<0.01, *P<0.05, **P<0.01 and ***P<0.001). Scale bar: 20 μm.

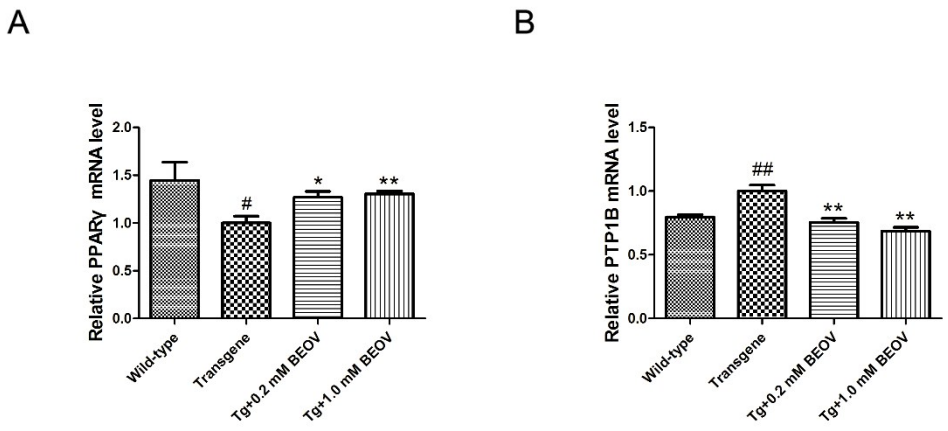
Supplementary Fig. 5



Supplementary Fig. 5 BEOV attenuated tau hyperphosphorylation in primary neuron cultures and HEK293/tau cells. Western blots and results of the semiquantitative analysis of tau phosphorylation at different amino acid positions in primary neuron cultures from the brains of wild-type mice **(A** and **B)**. Images of immunofluorescence staining for phosphorylated tau (pS396-tau, green) and the nucleus (DAPI, blue) in primary cultures of mouse hippocampal neurons (n = 3 samples per group) **(C)**. Western blots and results of the semiquantitative analysis of tau

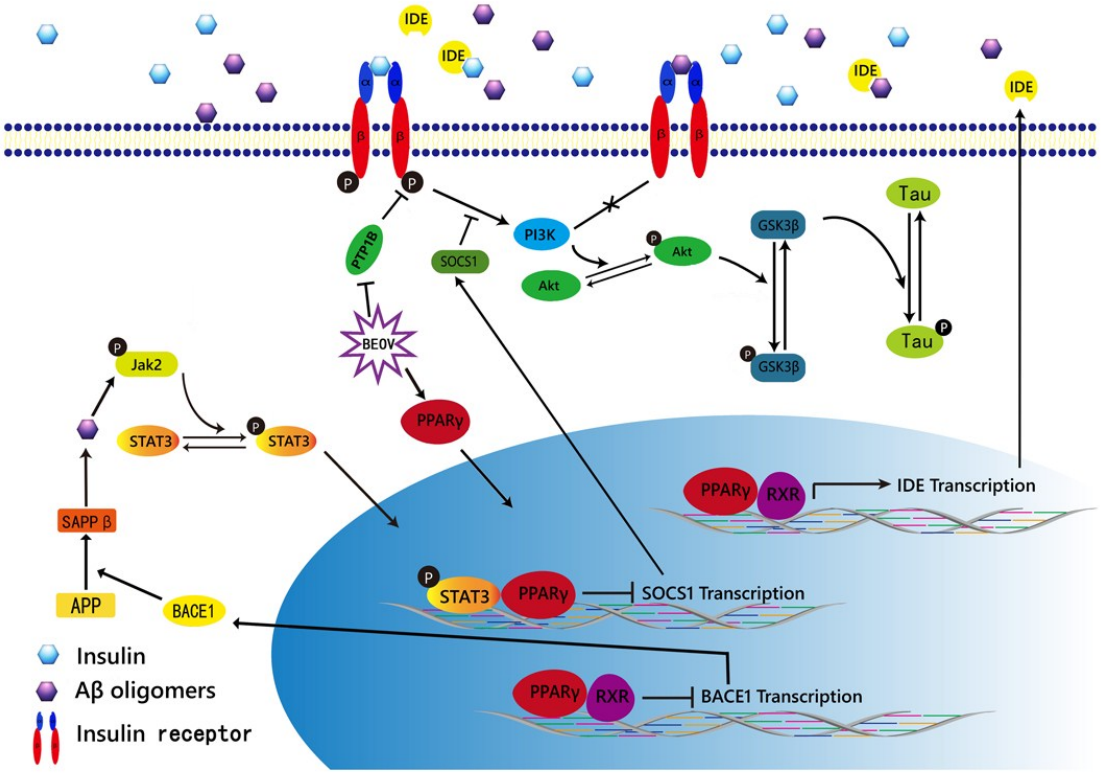
phosphorylation at different amino acid positions and PTP1B levels in HEK293, HEK293/tau and BEOV-treated HEK293/tau cells **(D** and **E**). (n=3; #P<0.05, ##P<0.01, ###P<0.001, *P<0.05, **P<0.01). Scale bar: 20 μm

Supplementary Fig. 6



Supplementary Fig. 6. **BEOV altered the expression of the PPARγ and PTP1B mRNAs in the cortex of 3×Tg-AD mice.** Cortical expression of the PPARγ and PTP1B mRNAs was assessed in WT, 3×Tg-AD and BEOV-treated AD mice at 9 months of age using qRT-PCR **(A** and **B)** (#: WT vs AD; *: AD + BEOV vs AD). (n=5; #P<0.05, ##P<0.01, *P<0.05, **P<0.01).

Supplementary Fig. 7



Supplementary Fig. 7. Schematic depicting the pathways by which BEOV attenuates AD pathological hallmarks in neurons. This image illustrates how BEOV affects the JAK2/STAT3/SOCS1 signaling pathway, IDE expression, Aβ generation, PI3K/Akt/GSK3β signaling pathway and tau hyperphosphorylation through the activation of PPARγ and inactivation of PTP1B. BEOV increases PPARγ activation to induce the expression of IDE and decrease the expression of BACE1 and SOCS1, subsequently attenuating Aβ-induced insulin resistance and stimulating insulin signal transduction. BEOV also inhibits PTP1B activity to promote insulin sensitivity and to inhibit Aβ and tau pathology.

Supplementary videos 1-5