Supporting Information

Effect of Akt-activating peptide obtained from walnut protein degradation on the prevention of memory impairment in mice

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1.Synthesis EPEVLR and EPEVLR-FITC

1.1 Solid Phase Synthesis

The walnut-derived Akt-activating peptide EPEVLR was synthesized using the classical solid phase synthesis method with Fmoc-Arg(pbf)-Wang resin (0.400 mmol/g). Firstly, the mass of resin required is calculated and weighed based on the molecular weight of EPEVLR. After the resin was soaked in DMF, the Fomc groups were removed using 20 % Piperidine/DMF. Then, the color of the resin was analyzed using Kaise-test, and the blue color of the resin indicated that Fmoc groups were successfully removed. Next, amino acids, HBTU and HOBt were weighed according to the three times molar amount of resin, dissolved in DMF and reacted with stirring for 1 h. Similarly, the color of the resin is detected using Kaise-test. If the resin is transparent, the reaction is complete. According to the amino acid sequence of EPEVLR, the operation is repeated until the last amino acid. Finally, the Fomc groups were removed, and the resin was washed three times using DCM and methanol, and vacuum dried to obtain the EPEVLR-containing resin.

EPEVLR-FITC was obtained by attaching a FITC moiety after accessing an aminocaproic acid at the end of EPEVLR-containing resin. In detail, Fmoc-6-aminohexanoic acid is bonded to the N end of EPEVLR-containing resin as an alkyl spacer. The synthetic steps are consistent with the bonding of EPEVLR amino acids. Then, the Fomc groups were removed using 20 % Piperidine/DMF. Finally, FITC-EPEVLR was obtained by synthesis using an addition reaction between the amino group and the SCN group of FITC in an alkaline environment.

The Kaise-test procedure is described as follows: firstly, reagent A, reagent B and reagent C were prepared separately. Reagent A: Dissolve 16.5 mg of KCN in 25 mL of distilled water. Dilute 1.0 mL of above solution with 49 mL of pyridine (freshly distilled from ninhydrin). Reagent B: Dissolve 1.0 g of ninhydrin in 20 mL of n-butanol. Reagent C: Dissolve 40 g of phenol in 20 mL of n-butanol. 10-15 resin beads were taken into a test tube and washed twice with anhydrous ethanol. Next, 2 to 3 drops of reagent A, reagent B and reagent C were added to tube. Finally, Heating the tubes at 110°C for 5 minutes and observing the change in resin colour.

1.2 Peptide purification

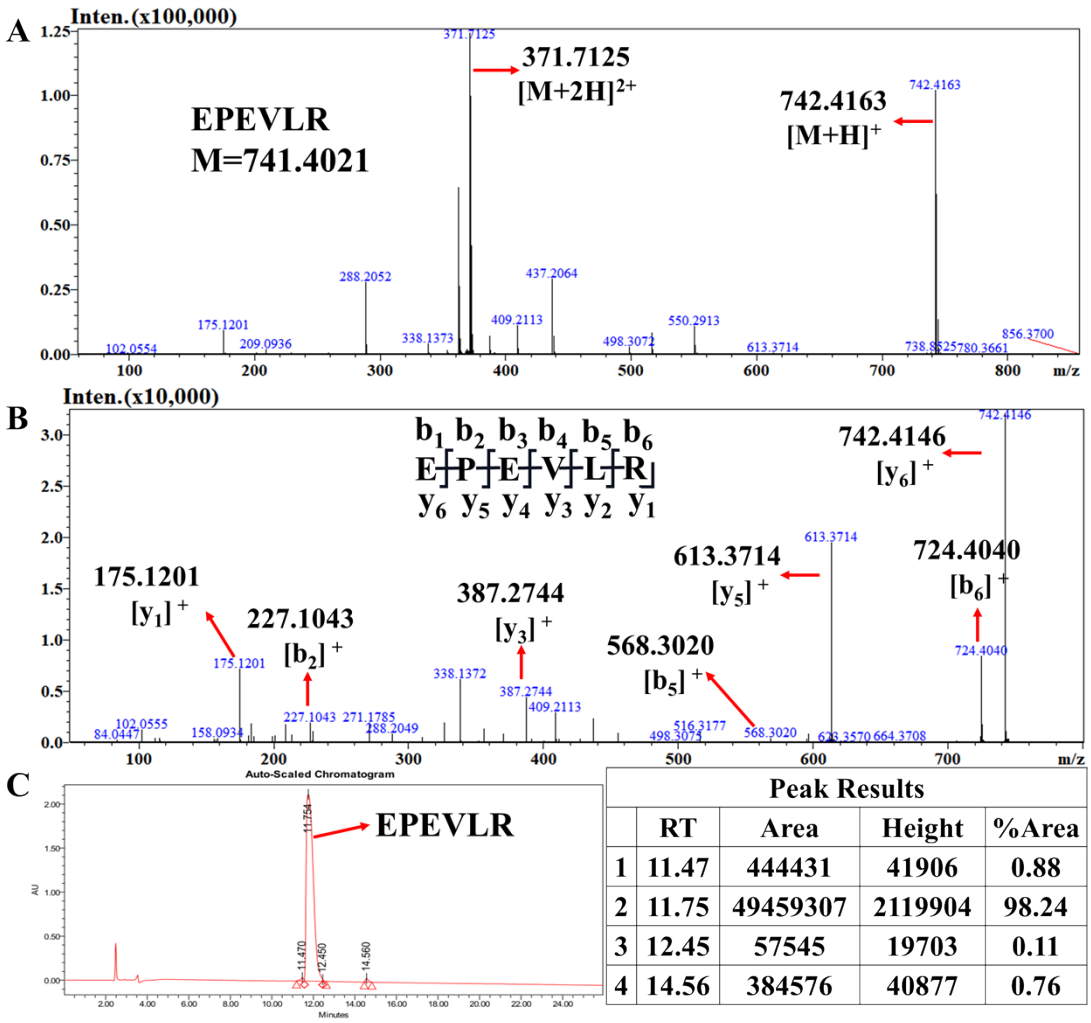
The peptides were separated from the resin using a lysis solution (95 % TFA, 2.5 % H_2O, 2.5 % TIS) for 2 h. The crude peptide was obtained by precipitating with pre-cooled ether and centrifugation (8000 r/min, 10 min). Then, the peptide sequence was identified using LC-MS/MS and the peptide was purified by Semi-Preparative Chromatograph. The LC-MS/MS parameters were as follows: LC-2030 Plus-QTOF-MS/MS (Shimadzu, Japan), positive ion mode, Material C18 column (3 μm, 4.6 μm×150 mm), water and acetonitrile containing 0.1 % TFA as mobile phases, and peptides was eluted using a linear gradient of 5~90 % acetonitrile (5 %~90 %/30 min), flow rate was 0.2 mL/min. The LC parameters for purification were as follows: LC-P1201 (Elitehplc, Dalina, China), SinoChrom ODS-BP column (5 μm, 10 ×250 mm), water and acetonitrile containing 0.1 % TFA as mobile phases, and peptides was eluted using a linear gradient of 5~75 % acetonitrile (5 %~75 %/40 min), flow rate was 1.0 mL/min. The distillate containing EPEVLR was collected, freeze-dried, and stored at -

20℃.

2. Results and discussion

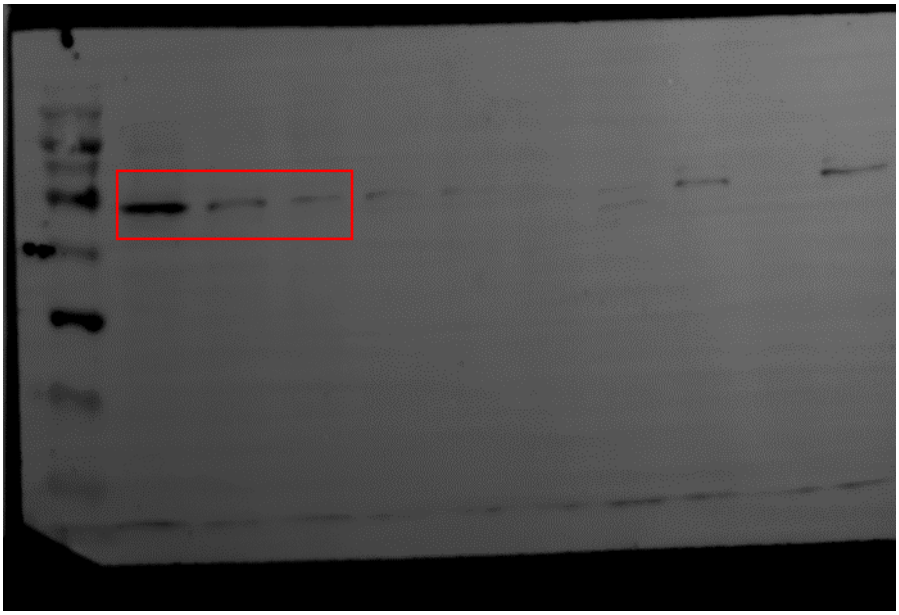
2.1 Structure and purity characterization of EPEVLR

EPEVLR with high purity was obtained using solid-phase synthesis and liquid chromatography purification. Firstly, the sequence of EPEVLR after solid-phase synthesis was identified by LC-MS/MS. As shown in Fig. S1A, there are two major molecular ion peaks ($[M+H]^+$=742.4163 and $[M+2H]^{2+}$=371.7125) for EPEVLR (M=741.4021) in the positive ion mode. Then, the fragment ion information was obtained after $[M+H]^+$ was destroyed, which was further compared using Fragment Ion Calculator Tool (http://db.systemsbiology.net/proteomicsToolkit/FragIonServlet.html). As shown in Fig. S1B, the ion information in MS2 spectra matches perfectly with the standard fragment ion information, indicating that correct EPEVLR sequences were synthesized. The purity analysis of purified EPEVLR showed that the purity of EPEVLR was 98.24 % (Fig. S1C). Therefore, EPEVLR was further used for subsequent experiments.

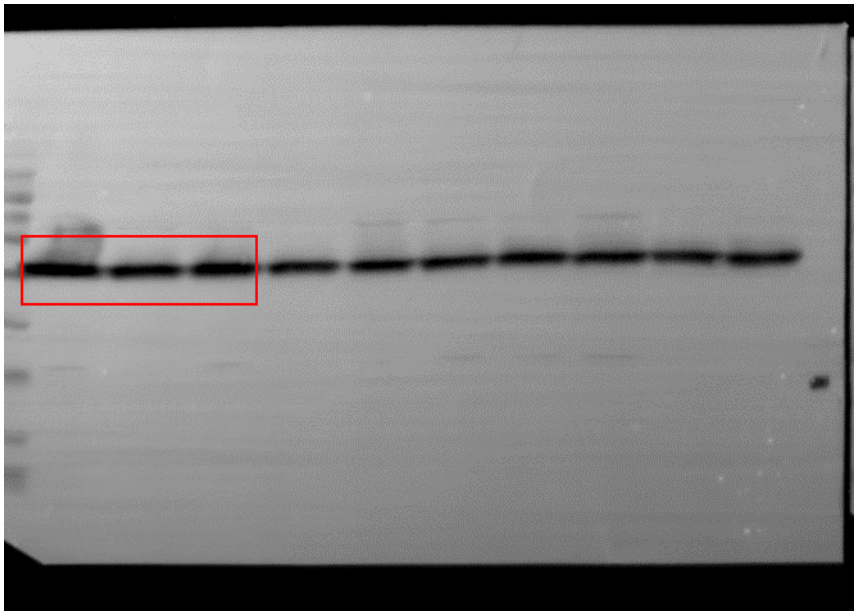


Peak Results				
	RT	Area	Height	%Area
1	11.47	444431	41906	0.88
2	11.75	49459307	2119904	98.24
3	12.45	57545	19703	0.11
4	14.56	384576	40877	0.76

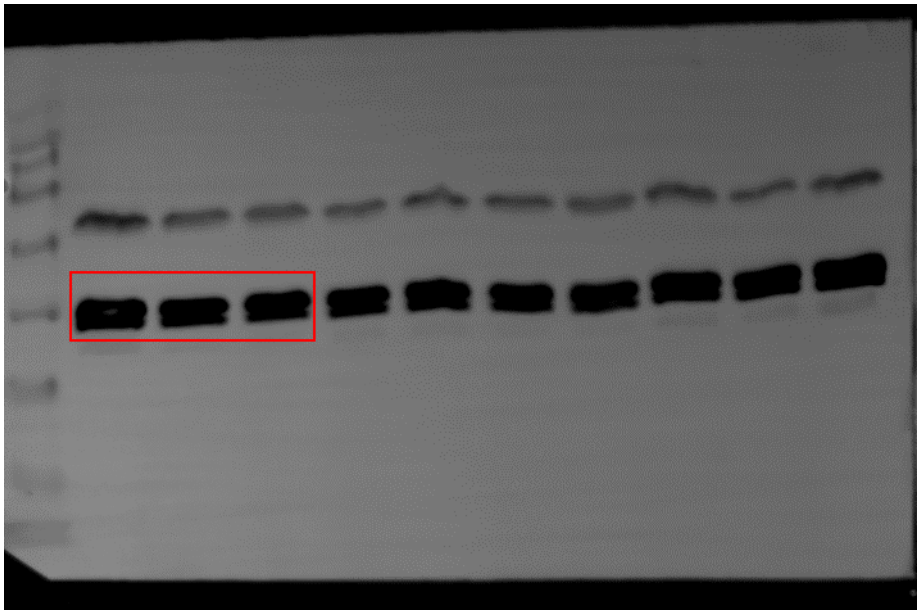
Fig S1. Sequence characterization and purity identification of solid-phase synthesized EPEVLR. MS (A) and MS^2 (B) spectra of EPEVLR. (C) The purity of EPEVLR was characterized using the peak area in the LC.



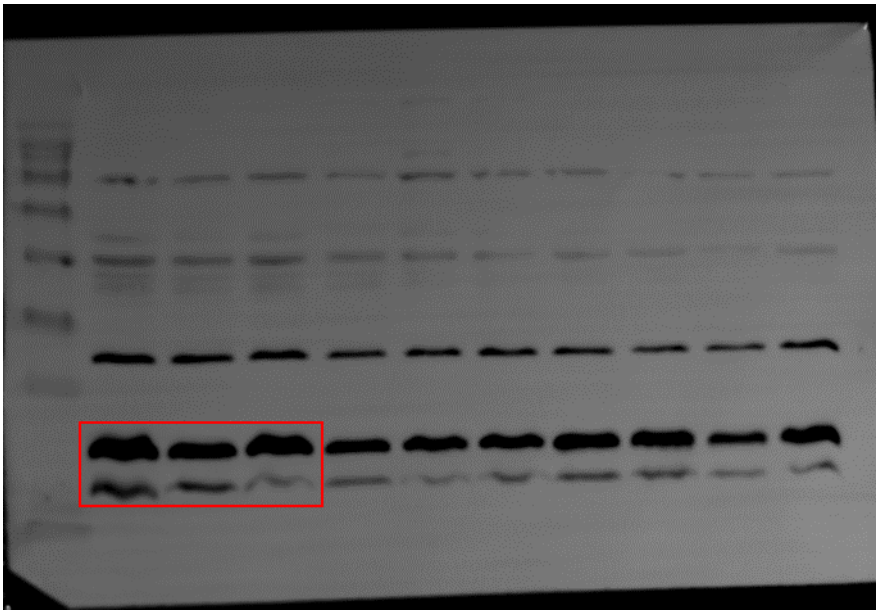
The raw images of western blots of p-Akt



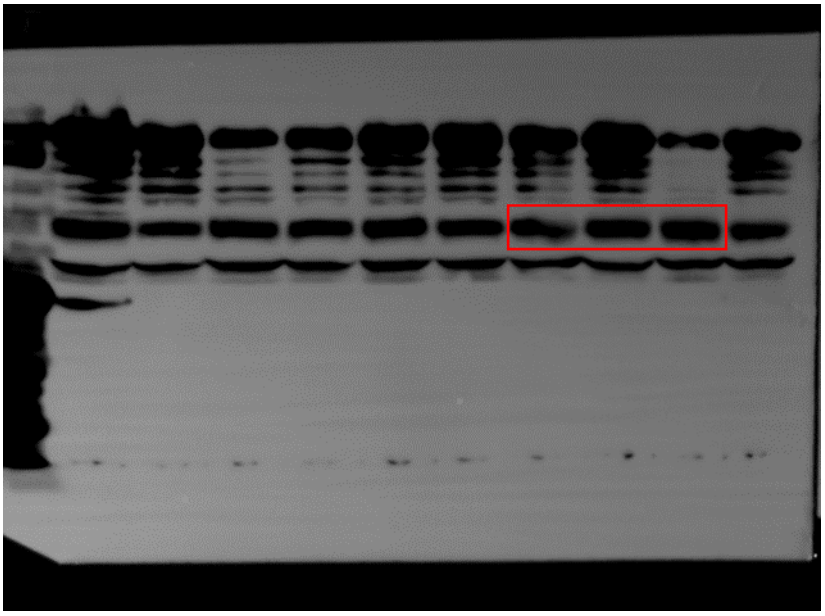
The raw images of western blots of Akt



The raw images of western blots of Beclin-1



The raw images of western blots of LC3-II



The raw images of western blots of p62