

Quantitative analysis of TMT phosphorylation modification to investigate the effect of verbascoside on the expression of phosphorylated protein in AD cell model

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SUBJECT AREAS

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KEYWORDS

verbascoside, Alzheimer's disease, phosphorylation modification, vesicle protein

Abstract

Ethnopharmacological relevance— The active monomer Verbascum glycosides in Cistanche tubulosa has good development prospects in terms of neuroprotection and delaying neurodegenerative diseases, and it has become one of the research hot spots. **Aim of the study—** To investigate the effect of verbascoside (OC1) on the expression of phosphorylated protein in the protective effect of AD cell model by TMT labeling and phosphorylation enrichment technique and high-resolution liquid chromatography-mass spectrometry quantitative proteomics research strategy.

Materials and Methods— The normal control group, the model group (A β 1-42 10 μ mol/L) group and the OC1 administration group (10 μ g/ml) group were set. (1)protein extraction quality control. (2)TMT mark.(3)HPLC classification and modification enrichment. (4)Analysis of mass spectrometry by liquid chromatography-mass spectrometry. (5)Analysis of bioinformatics results.(6)Western Blotting was used to detect the expression levels of p-CaMKII(Thr286), p-Synapsin (Ser9) / Synapsin, Synaptophysin and Synaptotagmin-1 protein.

Results— The study finally identified 9020 phosphorylation sites on 3227 proteins, of which 8635 sites of 3134 proteins contained quantitative information. Screening of differential sites follows the following criteria: 1.2 times the change threshold and CV value < 0.1. Based on the above data and standards, we performed a systematic bioinformatics analysis of proteins containing quantitative information sites. Western Blotting results showed that Verbascoside could promote the expression of p-CaMKII (Thr286), p-Synapsin (Ser9) / Synapsin, Synaptophysin and Synaptotagmin-1 protein.

Conclusions— Verbascoside (OC1) can increase the expression of phosphorylated protein in AD cell model, which provides a basis for further study on the molecular mechanism of verbascoside promoting neurotransmitter release.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Tables

Due to technical limitations, tables are only available as a download in the supplemental files section.

Figures

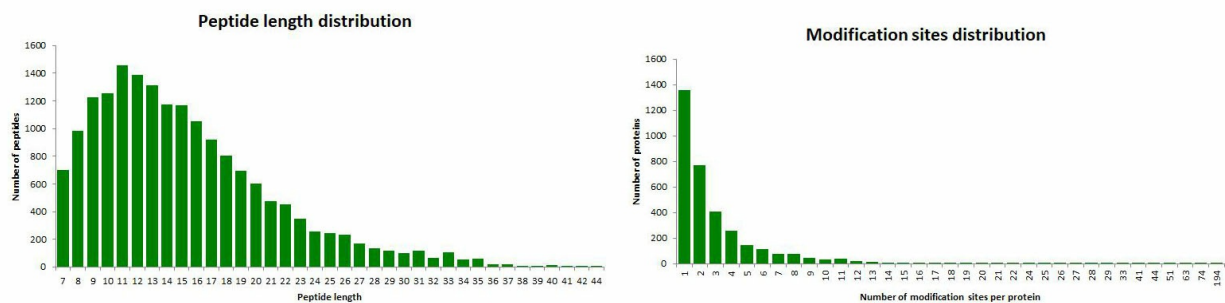


Figure 1

Mass spectrometry identified the length distribution of peptides and identification of protein corresponding modification site distribution

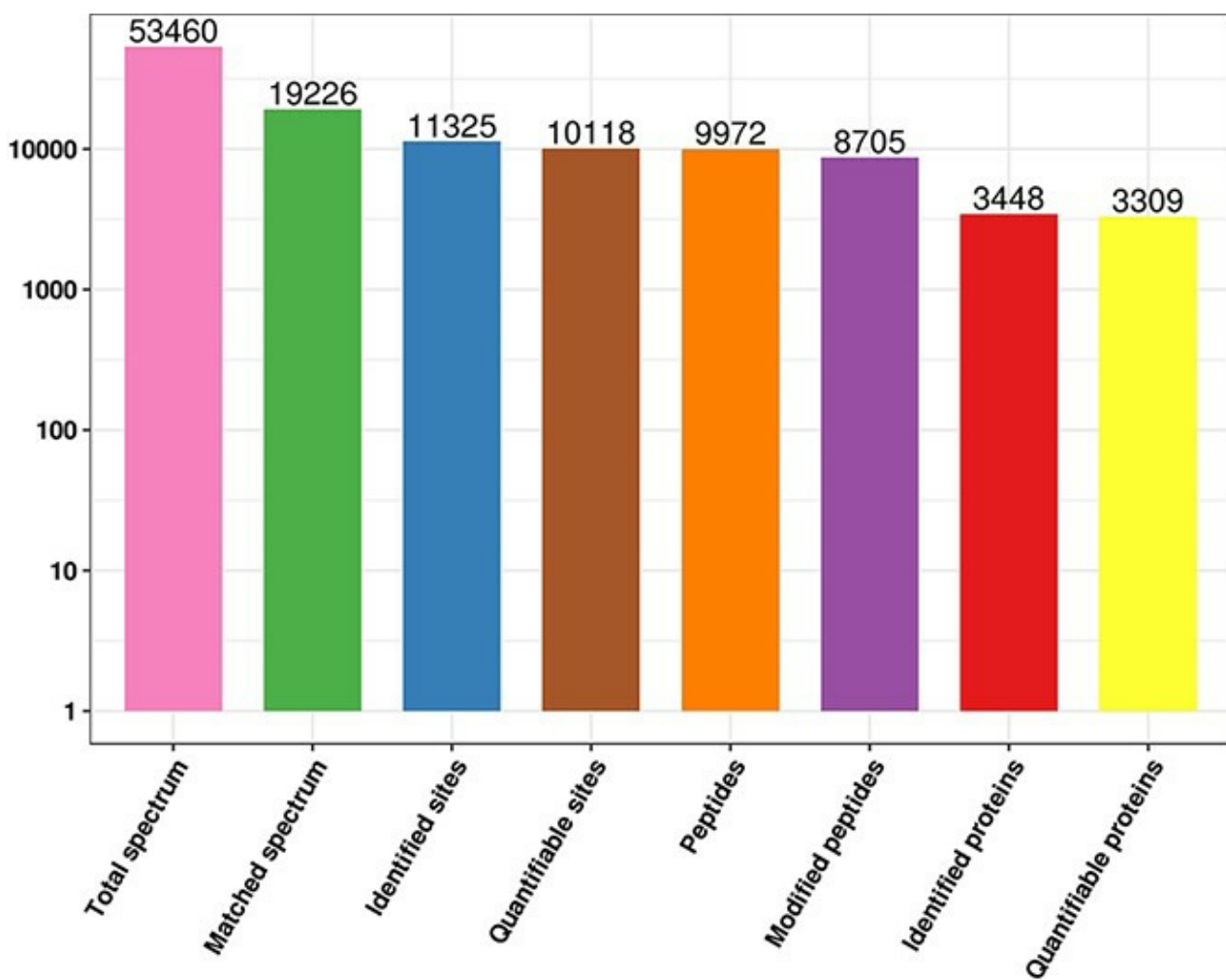


Figure 2

Basic statistics of mass spectrometry data results

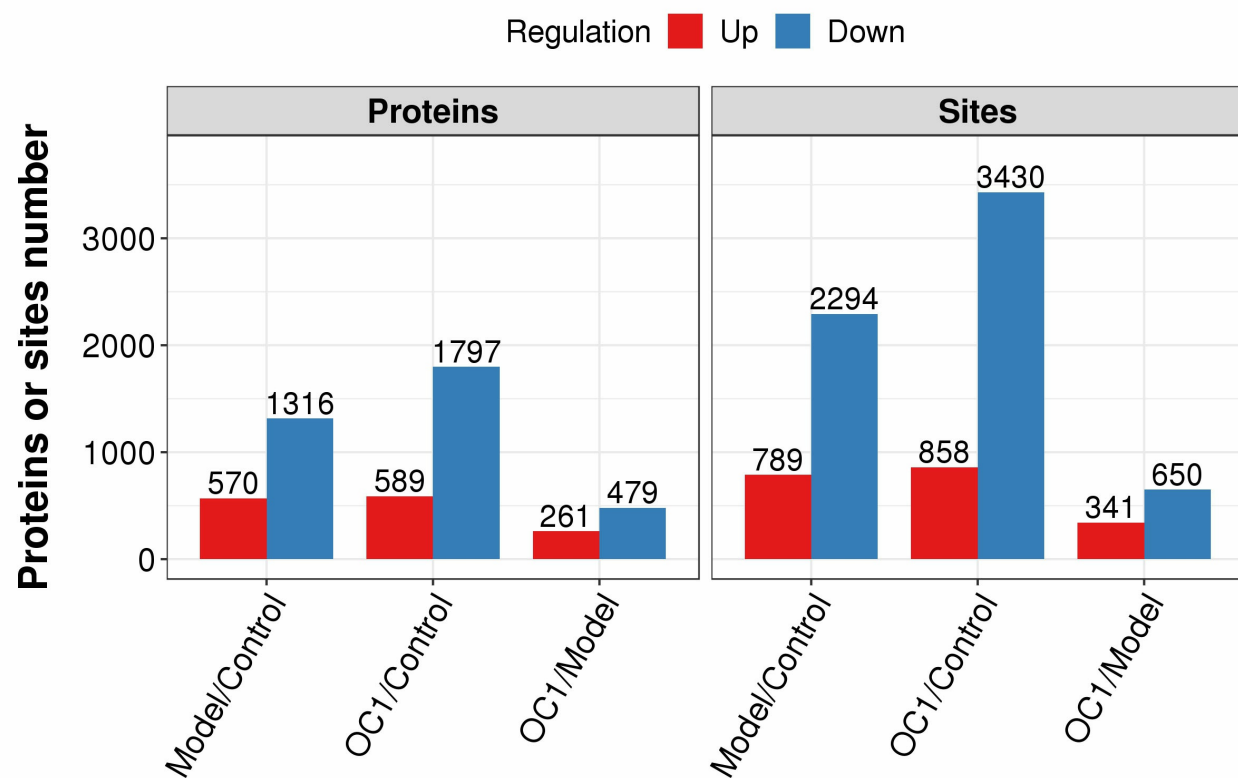


Figure 3

Bar graph of the number distribution of differentially modified proteins and modified sites in different comparison groups.

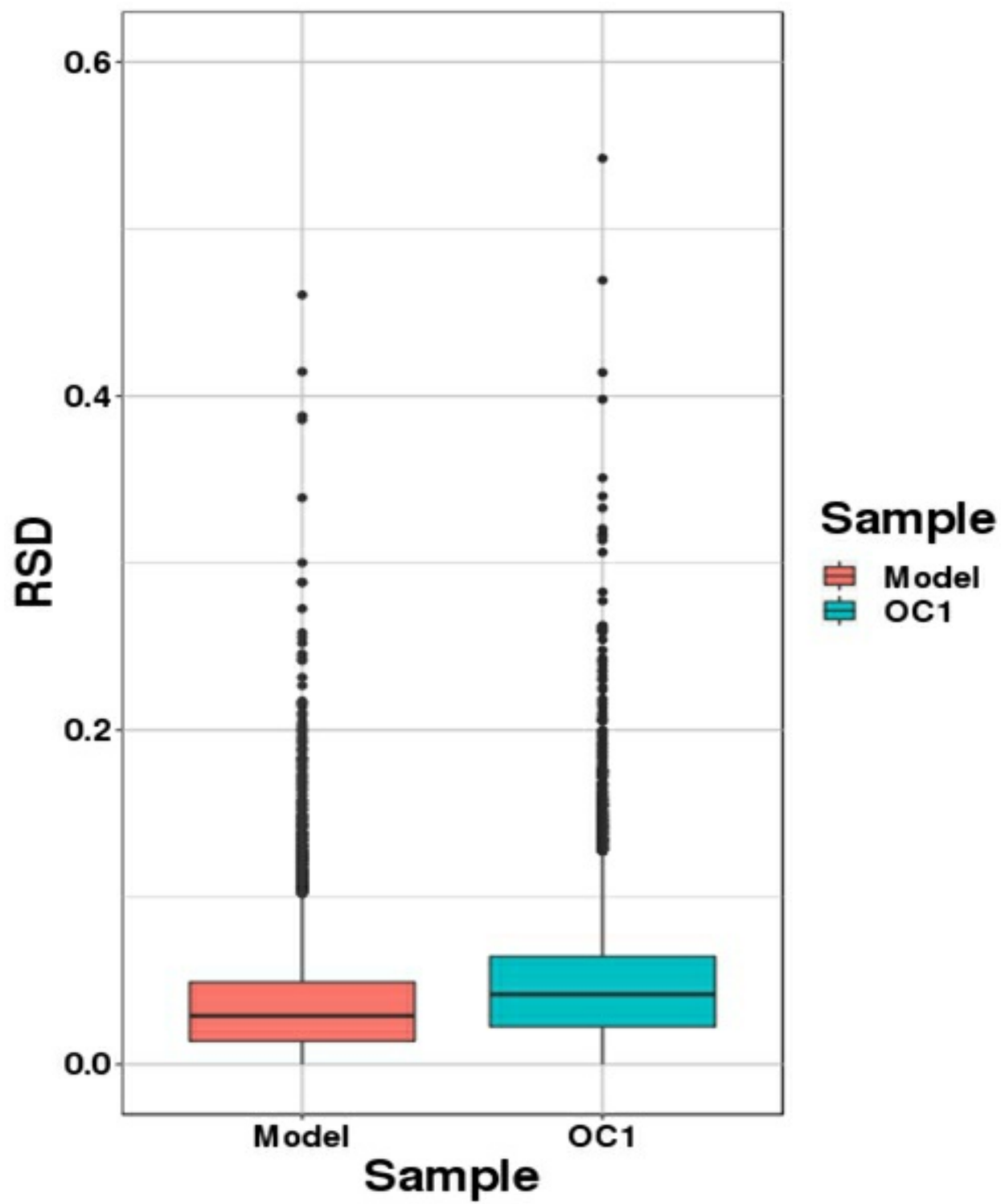


Figure 4

Repeated sample-to-sample modification quantitative RSD distribution box line diagram

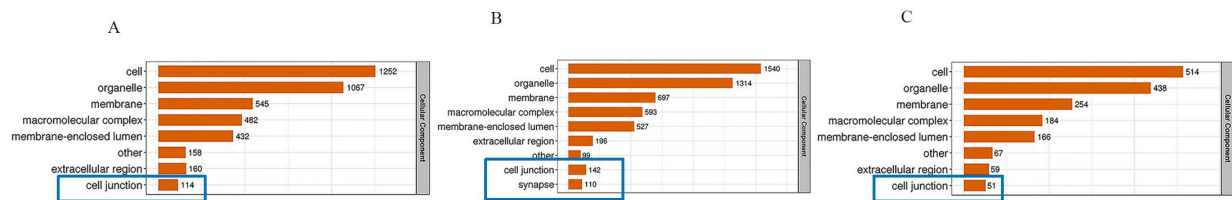


Figure 5

Statistical distribution map of the corresponding phosphorylated modification sites
corresponding to Camk and Syn1 in GO secondary classification

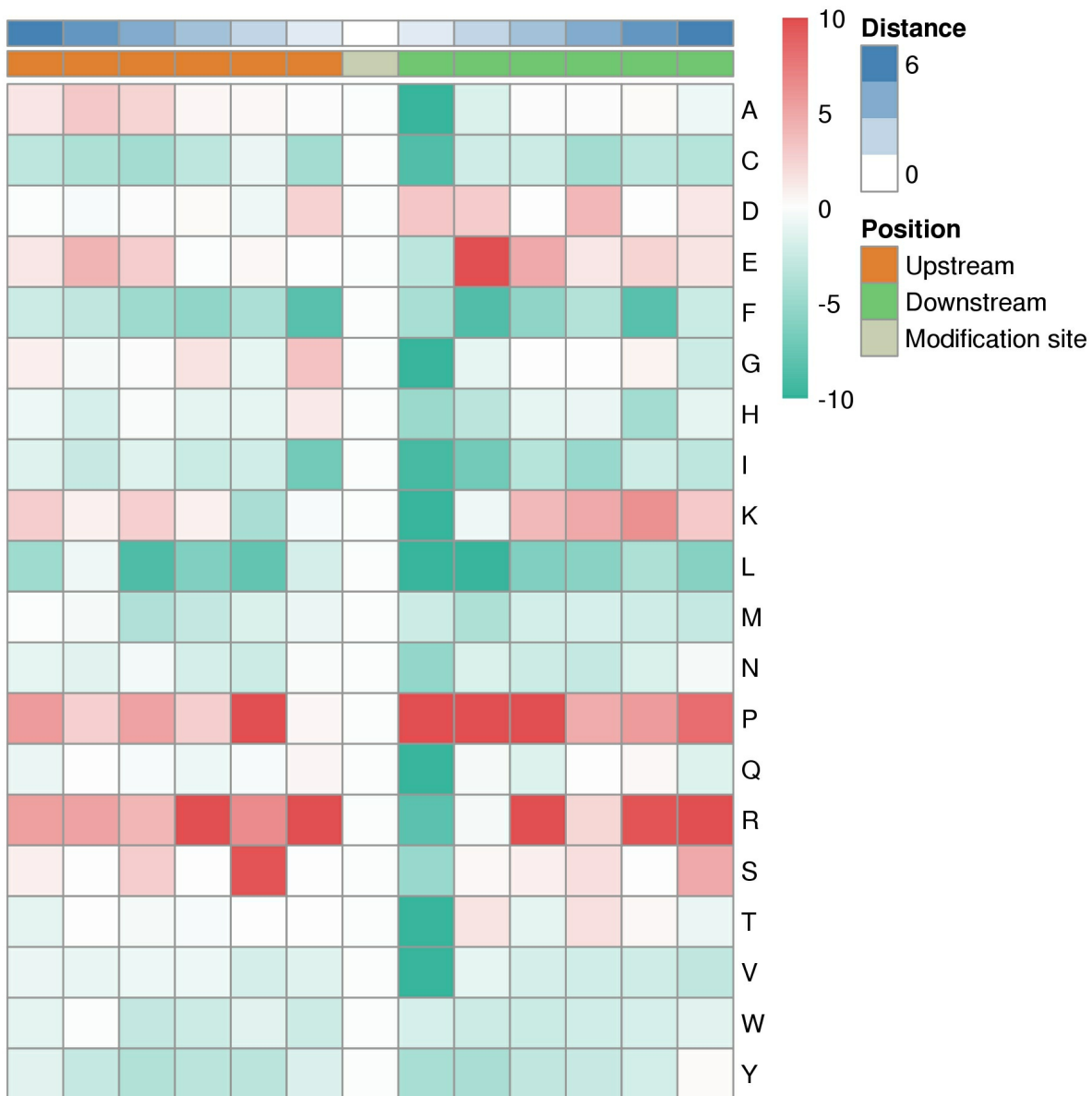


Figure 6

shows the enrichment heat map of the upstream and downstream amino acids of the
identified phosphorylation modification sites.

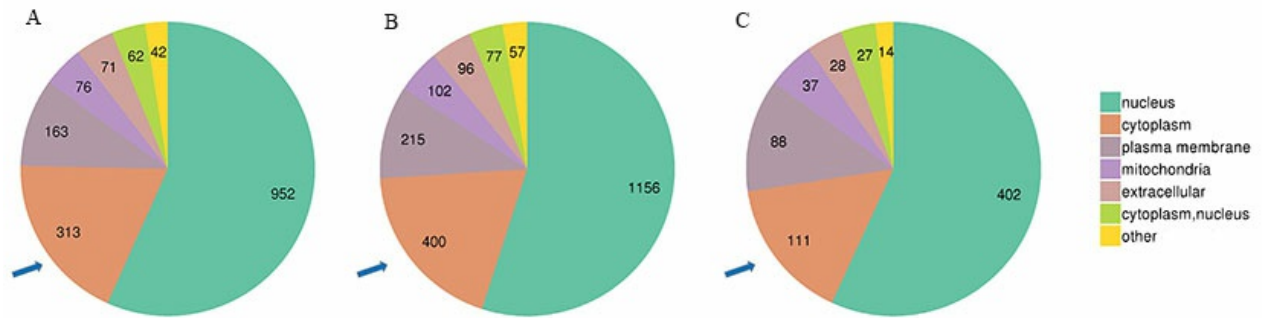


Figure 7

Subcellular structure localization map of the corresponding phosphorylated modification sites corresponding to CaMK and Syn1

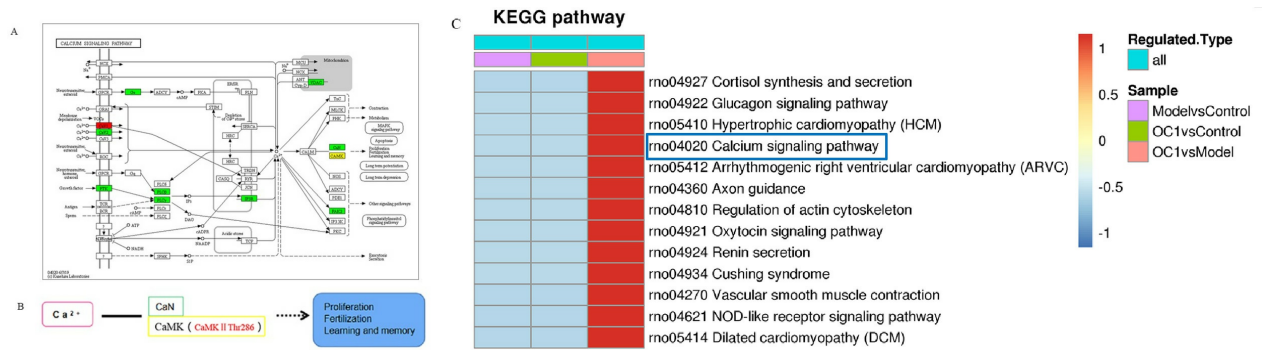


Figure 8

8A Schematic diagram of the KEGG pathway with significant enrichment of the corresponding phosphorylated modification site; Up-downstream relationship of p-CaMKII (Thr286) protein Note: Red in the figure indicates differentially upregulated protein; green indicates differentially downregulated protein; yellow indicates the presence of multiple proteins in this node, and contains differentially up-regulated and differentially down-regulated proteins. 8C Cluster analysis heat map based on KEGG pathway.

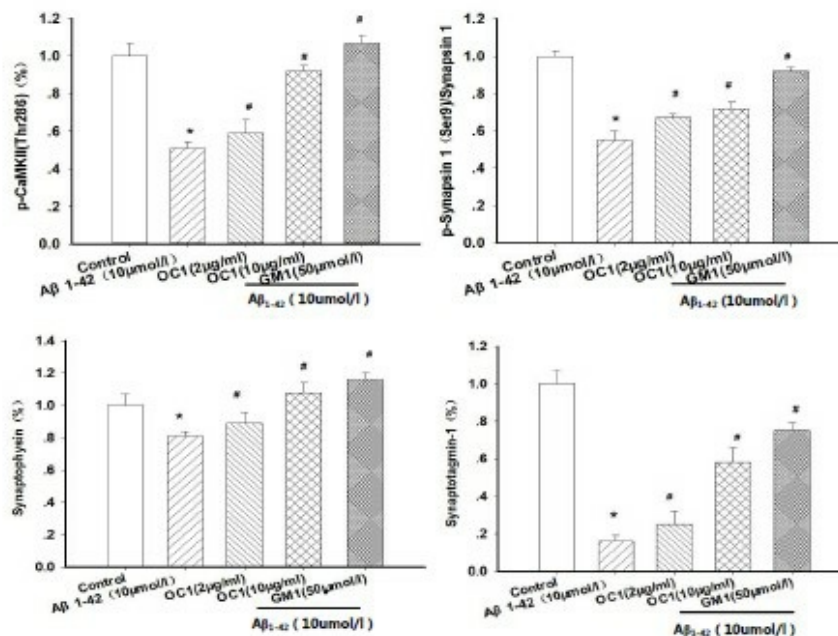
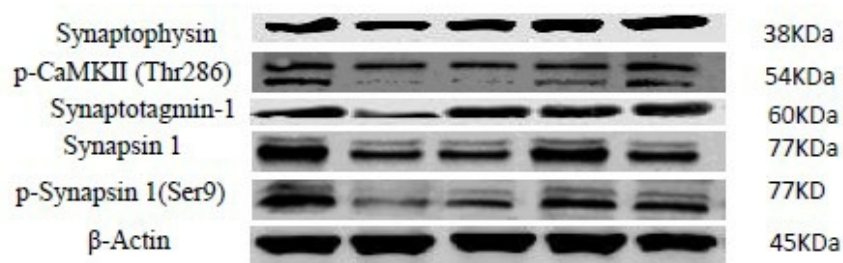


Figure 9

Effect of verbascoide on the expression of p-CaMKII (Thr286) □ p-Synapsin1(Ser9)/Synapsin1 □ Synaptophysin and Synaptotagmin-1 protein in AD cell model(n=3) Note: *P<0.05 compared with the blank group; #P<0.05 compared with the model group

Supplementary Files

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