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and then washed twice with PBS. The cells were scraped in 1 ml of RIPA buffer containing protease and phosphatase inhibitors.

The proteins were collected by centrifugation at 15,000 g for 30 min at 4°C. The concentration of the extracted protein was detected using a BCA kit. Next, the protein was separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-

PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 1 h in a blocking buffer containing 5% skim milk and 0.1% Tween-20 in Tris-buffered saline (TBS), incubated with primary antibodies overnight at 4°C, and then incubated with secondary antibodies for 1 h at room temperature. The protein bands were visualized using an enhanced chemiluminescence (ECL) assay kit. Statistical analysis ----- Statistical analysis was performed using the

SPSS 13.0 software (SPSS, Chicago, IL, USA). All data were presented as the mean \pm standard deviation. The analysis of variance (ANOVA) was used to compare the differences among the different treatment groups, and Dunnett's test was used for pairwise comparison. P \ 520fdb1ae7

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