

SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

MTT assay

Cytotoxicity was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously (Guan et al., 2016; Jiang et al., 2017). Briefly, C2C12 myotubes were grown in 6-well plates. After treatment with different concentration of icariin for 24 h, the medium was replaced by 1 mL of fresh DMEM (without phenol red). MTT solution (0.5 mg/mL, pH7.5) was added to cells in each well for 4 h at 37°C. The MTT-containing medium was then aspirated and 1 mL DMSO was added to each well to dissolve the resulting formazan. The plate was covered with tinfoil and agitated on an orbital shaker for 20 minutes. Absorbance at 570 nm was read in an ELISA plate reader (Biotek, Winooski, VT, USA).

REFERENCES

- Guan, F., Ding, Y., Zhang, Y., Zhou, Y., Li, M., and Wang, C. (2016). Curcumin Suppresses Proliferation and Migration of MDA-MB-231 Breast Cancer Cells through Autophagy-Dependent Akt Degradation. *PLoS One* 11, e0146553. doi:10.1371/journal.pone.0146553.
- Jiang, P., Zhang, D., Qiu, H., Yi, X., Zhang, Y., Cao, Y., et al. (2017). Tiron ameliorates high glucose-induced cardiac myocyte apoptosis by PKC δ -dependent inhibition of osteopontin. *Clin. Exp. Pharmacol. Physiol.* 44, 760-770. doi: 10.1111/1440-1681.12762.

FIGURE LEGENDS

FIGURE S1. Impacts of icariin on cell viabilities of C2C12 myotubes. C2C12 myotubes were starved serum for 4 h and then treated with 25 μ mol/L, 50 μ mol/L, 100 μ mol/L, or 200 μ mol/L icariin (ICA) for another 24 h. Cell viability was evaluated by the MTT assay method. N = 4. \$ $p > 0.05$ vs. control group.

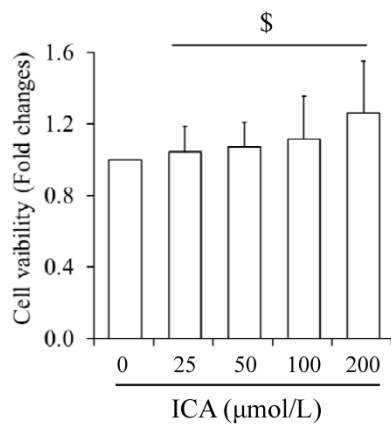


Figure S1