Supplementary Material

Cholestane-3β,5a,6β-triol induces multiple cell death in A549 cells via ER stress and autophagy activation

Jiaxi Chen [1,†], Jieping Zhang [1,†], Lijuan Cai [1], Li Guo [1], Zhenyu Cai [1], Hua Han [1,*] and Wen Zhang [1,2,*]

[1]   School of Medicine, Tongji University, 1239 Si-Ping Road, Shanghai 200092, China

[2]   Ningbo Institute of Marine Medicine, Peking University, 56 Kang-Da Road, Ningbo 315832, China

[*]   Correspondence: hanhua@tongji.edu.cn (H.H.); wenzhang1968@163.com (W.Z.)

[†]   These authors contributed equally to this work.
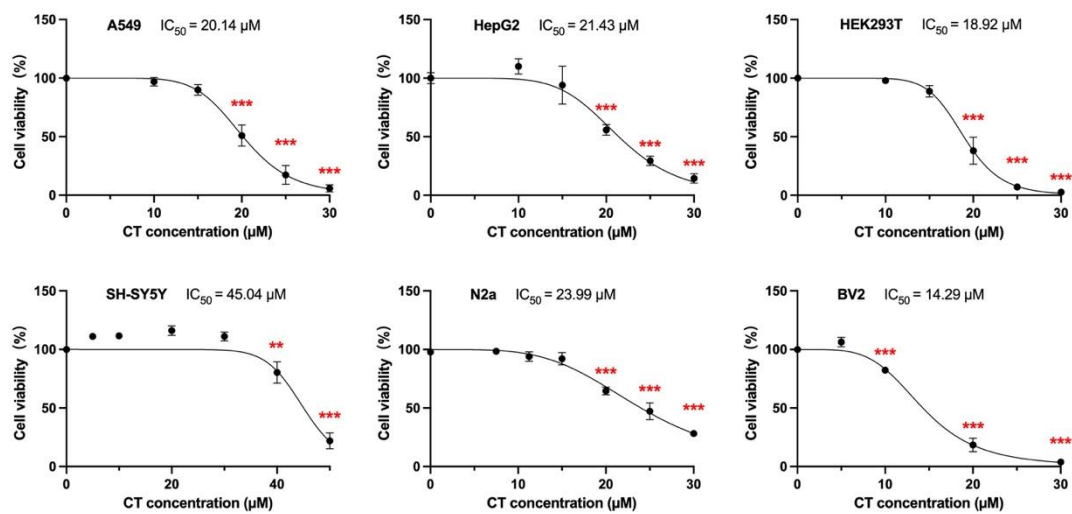
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Figure S1. CT induced the cell death of different cell lines. CT inhibited the cell viability of A549, HepG2, HEK293T, SH-SY5Y, N2a, BV2 cells. Cell viability was assessed by CCK-8 kit after the treatment of CT at different concentrations for 24 h. Statistical significance was determined using one-way ANOVA with Dunnett's post-test, **$p < 0.005$, ***$p < 0.001$ compared with the NC group.
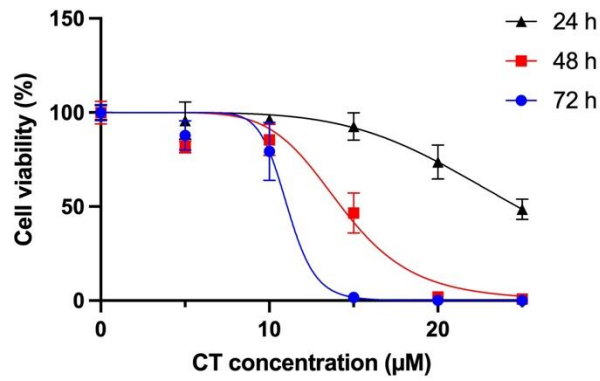
Figure S2. CT induced the cell death of A549 cells at concentration- and time-dependent manner. Cell viability was assessed by CCK-8 kit after the treatment of CT at different concentrations for 24, 48, 72 h.
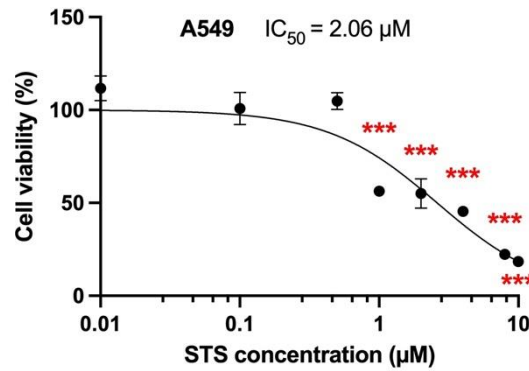

Figure S3. STS induced the cell death of A549 cells. Cell viability was assessed by CCK-8 kit after the treatment of STS at different concentrations for 24 h. Statistical significance was determined using one-way ANOVA with Dunnett's post-test, $n = 2$, ***$p < 0.001$ compared with the NC group.
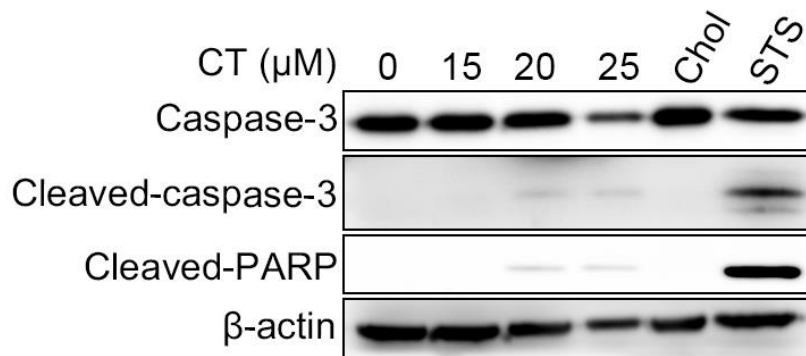
Figure S4. Western blot of cleaved-PARP and cleaved-caspase-3 in A549 cells.

Cells were treated with series doses of CT for 24 h. Cells were treated with 1 μM STS for 24 h as the positive control, and 20 μM cholesterol (Chol) for 24 h as an isotype control.
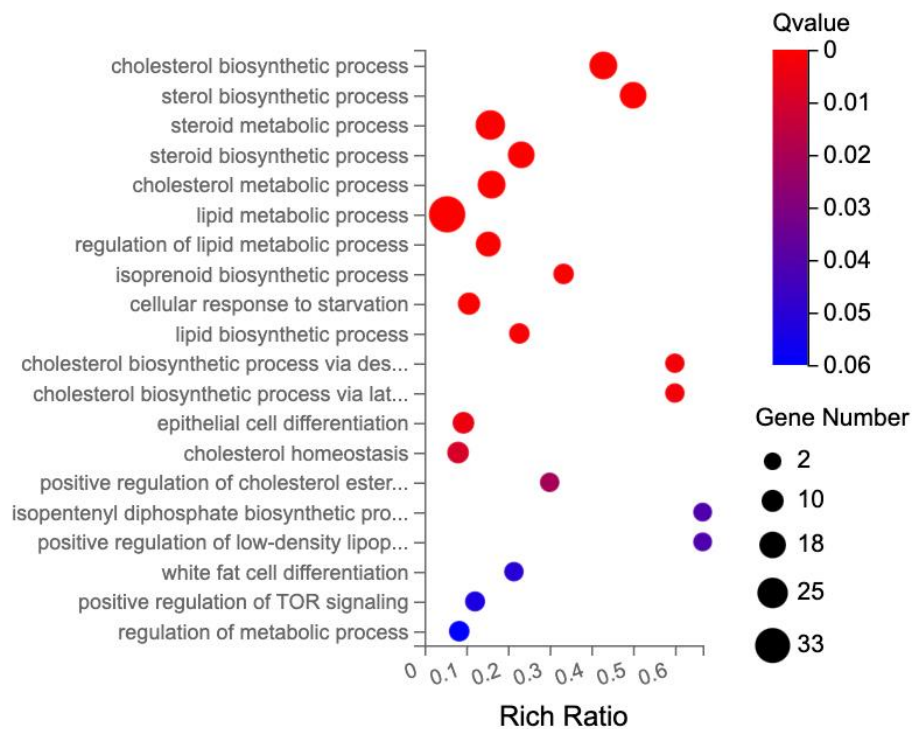

Figure S5. RNA-seq analysis of 10 μM CT-treated A549 cells. GO analysis of

the upregulated gene clusters in CT 10 μM treatment groups. The function of
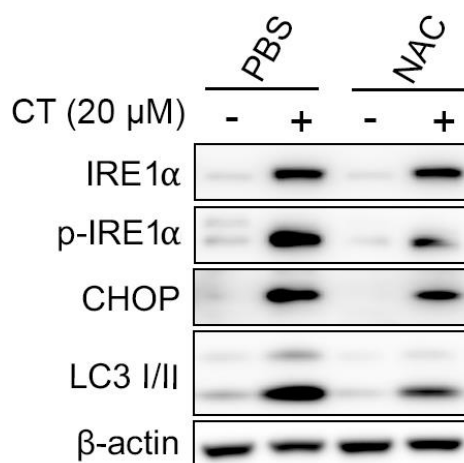
Q value ≤ 0.05 was considered as significant enrichment.

Figure S6. Western blot of p-IRE1α and LC3 II levels in A549 cell lines treated with CT. The cells were pre-treated with 1 mM 4-PBA or 3 mM NAC for 1 h, then incubated with CT at 20 μM for 24 h.