## Appendix. Supplementary materials

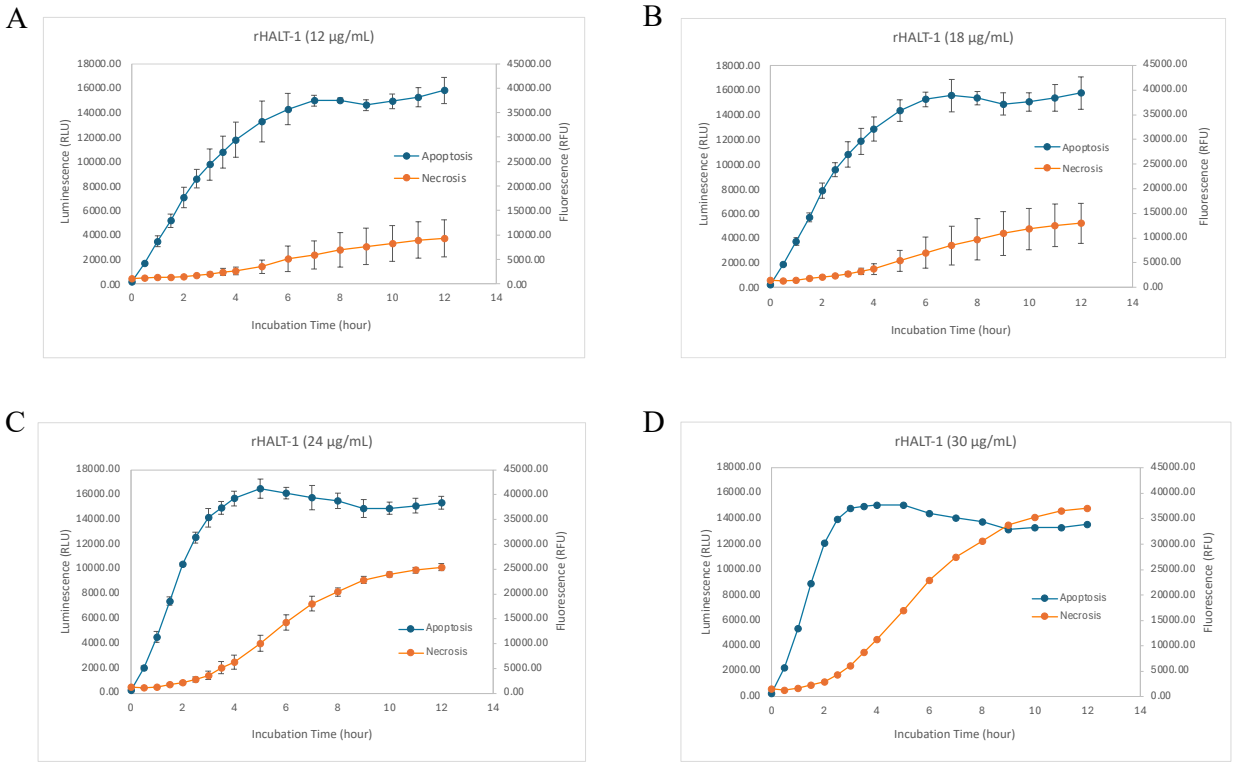



**Figure S1. Mode of apoptosis and necrosis of rHALT-1-treated HeLa cells at four different concentrations.** HeLa cells were cultured in a 96-well plate at a density of 1 x $10^4$ cells/well and incubated overnight for 16 hours. The line graphs represent the trends of apoptosis and necrosis observed over 12 hours, with **(A)** 12 µg/mL, **(B)** 18 µg/mL, **(C)** 24 µg/mL, and **(D)** 30 µg/mL of rHALT-1. The data were collected from three independent experiments with triplicate readings (n = 3) and presented as the mean ± SD of relative luminescence or fluorescence units (RLU or RFU).

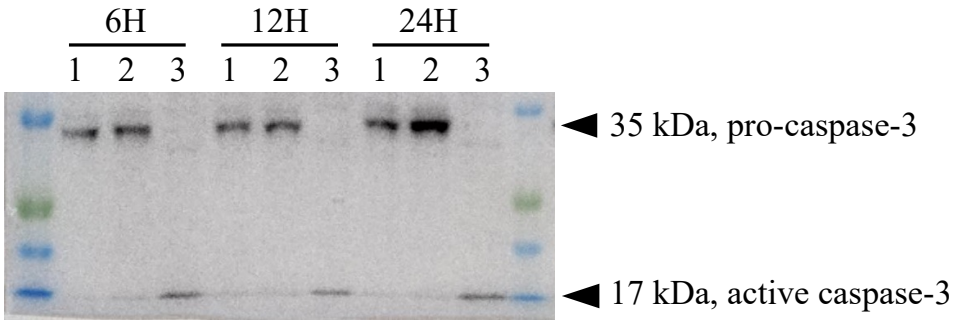
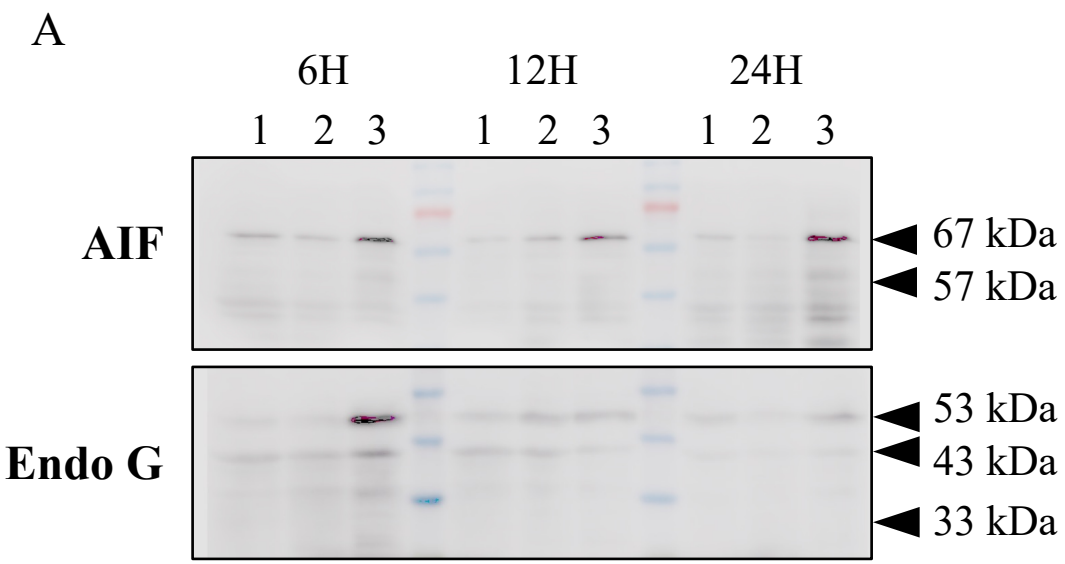



**Figure S2**. **Detection of pro-caspase-3 and active caspase-3 in treated and untreated HeLa cells by Western blot.** HeLa cells were untreated or treated with 12 μg/mL rHALT-1 or 100 ng/mL hTRAIL, and subsequently cell lysates (10 μg) were collected at 6, 12, and 24 hours for SDS-PAGE followed by Western blot analysis. Lanes 1, 2, and 3 represent lysates from untreated, rHALT-1–treated, and hTRAIL-treated cells, respectively, showing the presence of pro-caspase-3 and cleaved (active) caspase-3. Arrowheads indicate the detected bands and their corresponding molecular weights in kilosdaltons (kDa).


A
6H
12H
24H
1 2 3 1 2 3 1 2 3
AIF
67 kDa
57 kDa
Endo G
53 kDa
43 kDa
33 kDa

B

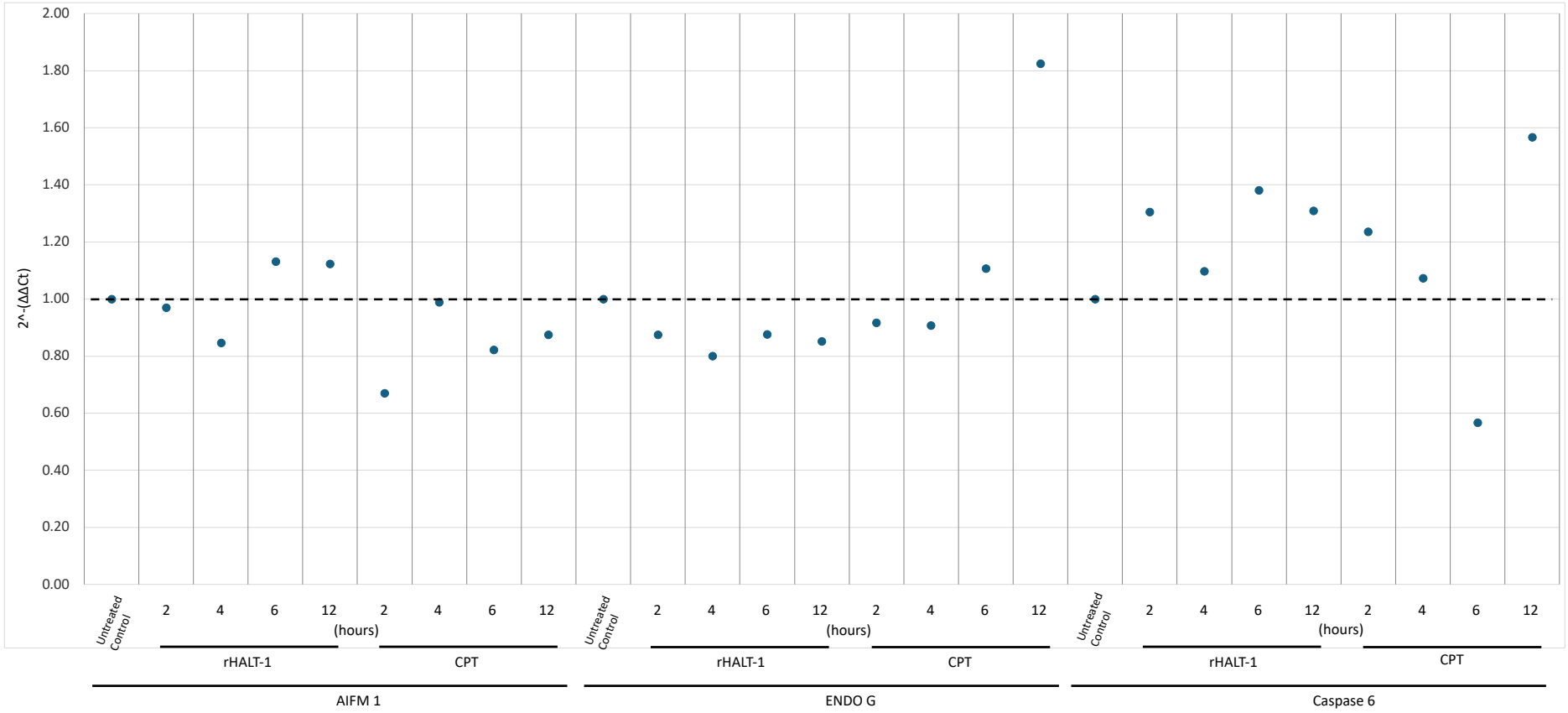



**Figure 3S**. **Time-dependent apoptotic responses to rHALT-1 in HeLa cells involving AIF, Endo G, and Caspase 6. (A)** HeLa cells were left untreated (1), and treated with 12 µg/mL rHALT-1 (2) and 100 ng/mL hTRAIL (3) for 6, 12, and 24 h. Whole-cell lysates (10 µg) were fractionated by 12 % SDS-PAGE and subjected to western blotting analysis. Specific monoclonal antibodies were used to detect the expression of AIF and Endo G. **(B) Relative gene expression changes of *AIFM*1, *Endo G,* and *caspase* 6 at time points 2, 4, 6, and 12 hours following rHALT-1 or CPT treatment.** Gene expression levels were measured using qRT-PCR and normalized to the GAPDH gene. The y-axis represents fold changes in

expression, 2^(-ΔΔCt), relative to untreated controls (dashed line at 1.0). The x-axis represents different genes (*AIFM*1*,* *ENDOG*, and *caspase* 6), with corresponding time points (2, 4, 6, and 12 hours) post-treatment, along with an untreated control for each gene. Each dot (blue) represents the mean of data duplicate data.