

Figure S1: TRAIL-R2 mediates TRAIL-induced cell death and activation of TAK-AMPK1 pathway.

(A) The expression levels of TRAIL receptors on the surface of MCF10A cells were determined by a standard flow cytometry protocol using monoclonal antibodies to TRAIL-R1 (HS101), TRAIL-R2 (HS201), TRAIL-R3 (HS301) and TRAIL-R4 (HS401) from Alexis Biochemicals Corp. (San Diego, CA, USA). Representative flow cytometry profiles are shown.

(B) MCF10A cells were treated with 0.5 $\mu\text{g/ml}$ cyclohexymide (CHX) alone or in combination with 5 $\mu\text{g/ml}$ antagonist TRAIL-R2 antibody for 1 h before adding 500 ng/ml TRAIL for 24 h. Cells were analyzed for the DNA content by flow cytometry. The percentage of apoptotic cells with sub-G1 DNA content is shown and the values represent mean \pm SD for two independent experiments.

(C) MCF10A cells were left untreated (-) or treated with 500 ng/ml TRAIL for 2 h. When indicated, cells were pre-incubated with 5 $\mu\text{g/ml}$ antagonist TRAIL-R2 antibody for 1 h. Immunoblot analyses of P-ACC, P-TAK1, TAK1, P-p70S6K, p70S6K and GAPDH (loading control) are shown. The values indicate the P-TAK1/GAPDH, P-ACC/GAPDH and P-p70S6K/p70S6K ratios as percentages of the ratios in control cells. Similar results were obtained in three independent experiments.

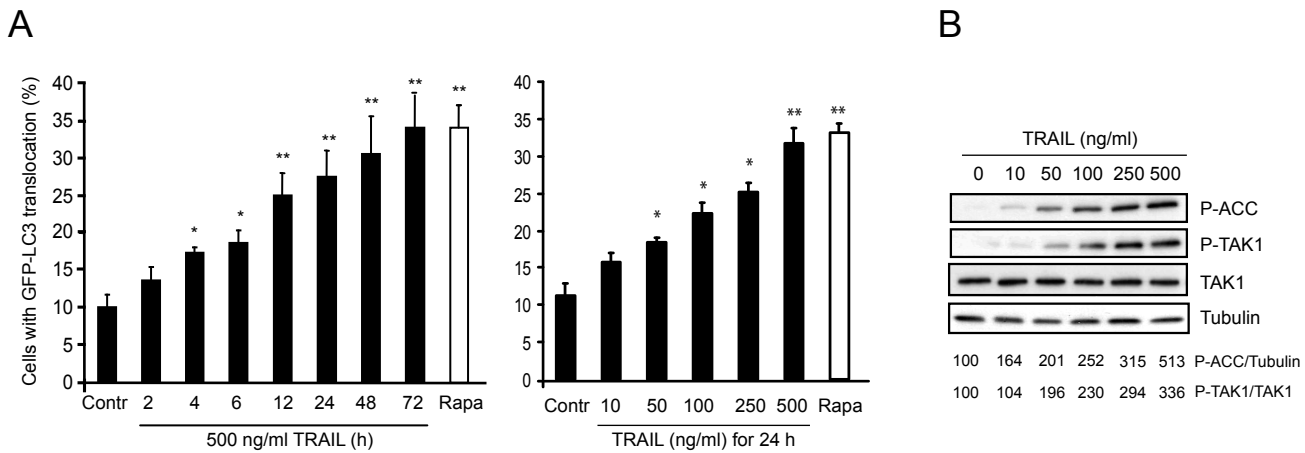


Figure S2: Kinetics and/or dose dependence of TRAIL-induced LC3 translocation and TAK-1 and AMPK activation.

(A) MCF10A cells treated with TRAIL at times and concentrations indicated were analyzed for LC3 translocation. A 24 h treatment with 5 μM rapamycin (Rapa) served as a positive control. The values represent mean ± SD for three independent experiments. *p value < 0.05, **p value < 0.01 as compared to untreated samples.

(B) Lysates of MCF10A cells treated with indicated concentrations of TRAIL for 24 h were analyzed by immunoblot for P-ACC, P-TAK1, TAK1 and tubulin (loading control). The values show the P-ACC/Tubulin and P-TAK1/TAK1 ratios as percentages of the ratios in control cells.

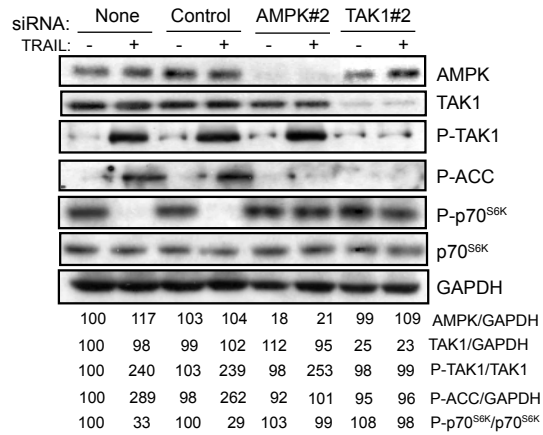


Figure S3: TAK1 is upstream of AMPK in TRAIL-induced pathway leading to the inhibition of mTORC1.

Lysates of MCF10A cells transfected with indicated siRNAs for 48h and treated with 500 ng/ml TRAIL for 2 h were analyzed by immunoblotting for AMPK, TAK1, P-TAK1, P-ACC, P-p70S6K, p70S6K and GAPDH (loading control). The values show the AMPK/GAPDH, TAK1/GAPDH, P-ACC/GAPDH, P-TAK1/TAK1, and P-p70S6K/p70S6K ratios as percentages of the ratios in cells with no siRNA, and are representative of two independent experiments.