Expanded View Figures

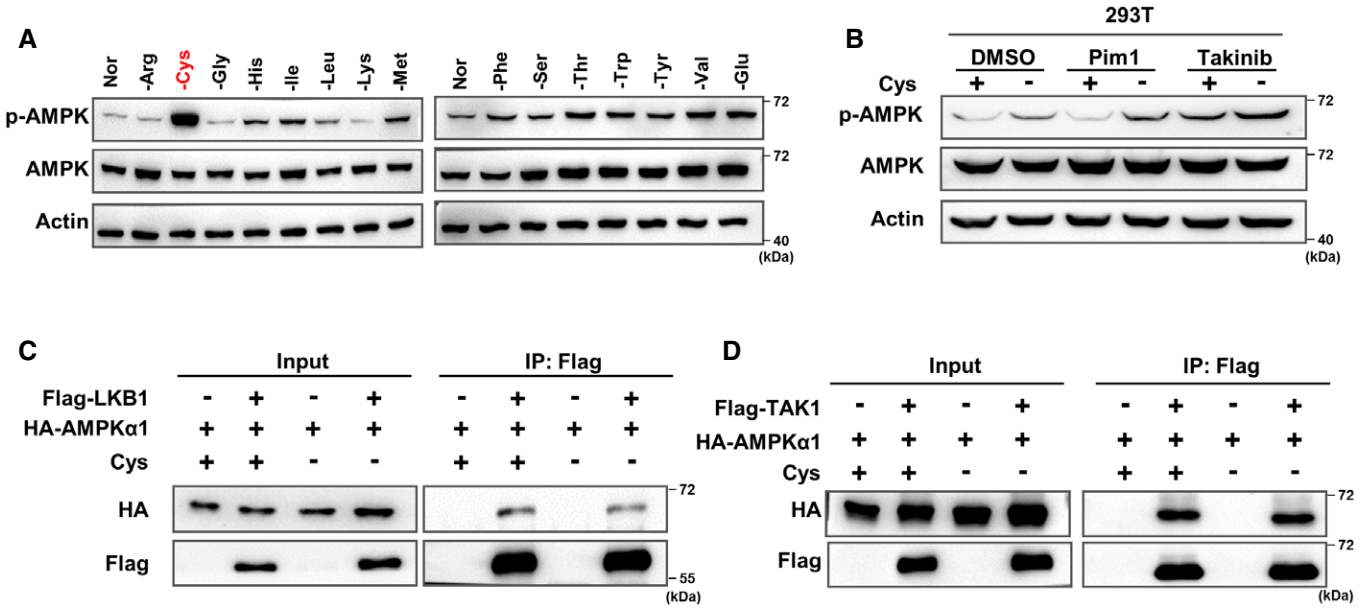

Figure EV1. Cystine starvation activates AMPK through CaMKK2 instead of LKB1 or TAK1.

A RCC4 cells were cultured in complete medium for 24 h and then replaced and cultured with the corresponding amino acid-deficient medium for 24 h, followed by WB detection of p-AMPK and total AMPK protein expression. Actin served as the loading control.

B WB analysis of p-AMPK and total AMPK in 293T cells treated with DMSO, 1 μM Pim1 or 100 nM Takinib for 8 h under cystine deprivation. Actin served as the loading control.

C, D 293T cells were transfected with HA-AMPKα1 alone or with Flag-LKB1 (C) or Flag-TAK1 (D) alone for 48 h and then cultured with cystine-deficient medium for 8 h. Cell lysates were immunoprecipitated with anti-Flag antibody, and a WB analysis was performed.

Source data are available online for this figure.

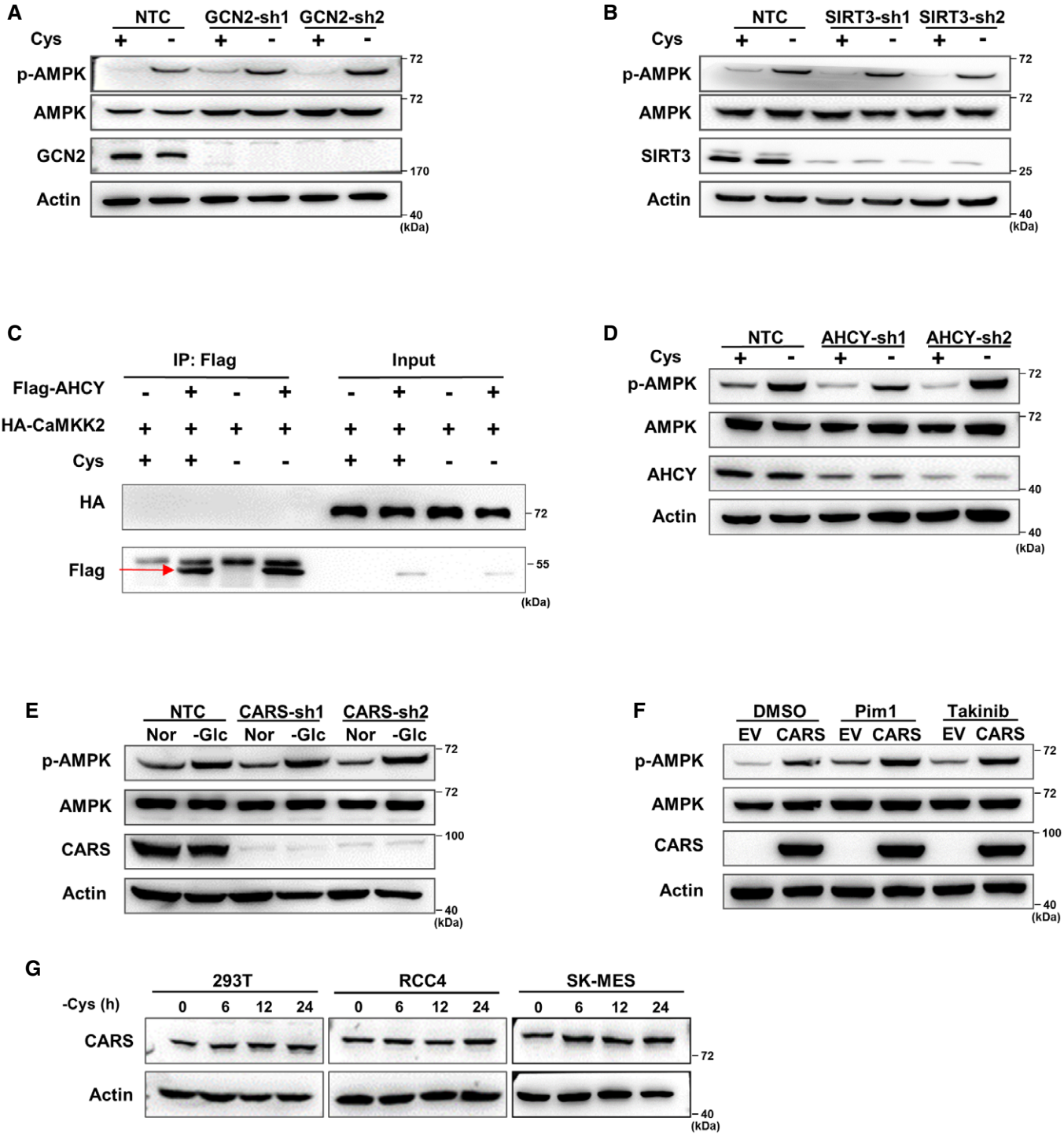

Figure EV2.

Figure EV2. CARS is critical for AMPK activation under cystine starvation condition.

A, B WB analysis of p-AMPK and total AMPK protein expression in 293T cells transfected with shRNAs targeting GCN2 (A) or Sirt3 (B) and further treated with cystine-deficient medium for 8 h. Actin served as the loading control.

C 293T cells were transfected with HA-CaMKK2 alone or with Flag-AHCY for 48 h and then cultured with cystine-deficient medium or complete medium for 8 h. Cell lysates were immunoprecipitated with anti-Flag, and a WB analysis was performed. The red arrow indicates the target band.

D WB analysis of p-AMPK and total AMPK protein expression in 293T cells transfected with shRNAs targeting AHCY and further treated with cystine-deficient medium for 8 h. Actin served as the loading control.

E WB analysis of p-AMPK and total AMPK protein expression in 293T cells transfected with shRNAs targeting CARS and further treated with glucose-deficient medium for 2 h. Actin served as the loading control.

F WB analysis of p-AMPK and total AMPK protein expression in CARS-overexpressing 293T cells treated with DMSO, pim1, or Takinib for 8 h. Actin served as the loading control.

G WB analysis of CARS protein expression in 293T, RCC4 and SK-MES cells treated with cystine-deficient medium for 0, 6, 12 or 24 h. Actin served as the loading control.

Source data are available online for this figure.

Figure EV3. CARS senses cystine starvation to activate AMPK through direct combination with AMPKγ2.

A Coomassie Blue staining of His-CARS protein purified from *E. coli*. "M" = marker. The red arrow indicates the target band.

B Protein sequences of AMPKγ1 and AMPKγ2 were compared using Jellyfish software. The model below shows the segments of AMPKγ1 and AMPKγ2.

C 293T cells were transfected with HA-AMPKγ2 alone or with Flag-CARS, Flag-CARS-N-terminus, Flag-CARS-M domain or Flag-CARS-C-terminus for 48 h. Cell lysates were immunoprecipitated with anti-Flag, and WB analysis was performed. The model above shows the segments of CARS.

D In 293T cells transfected with endogenous knocked down AMPKγ2, wild-type AMPKγ2 or AMPKγ2^{R531G} was treated with cystine-deficient medium for 8 h, and WB was performed to detect of p-AMPK, total AMPK and AMPKγ2 protein expression. Actin served as the loading control.

Source data are available online for this figure.

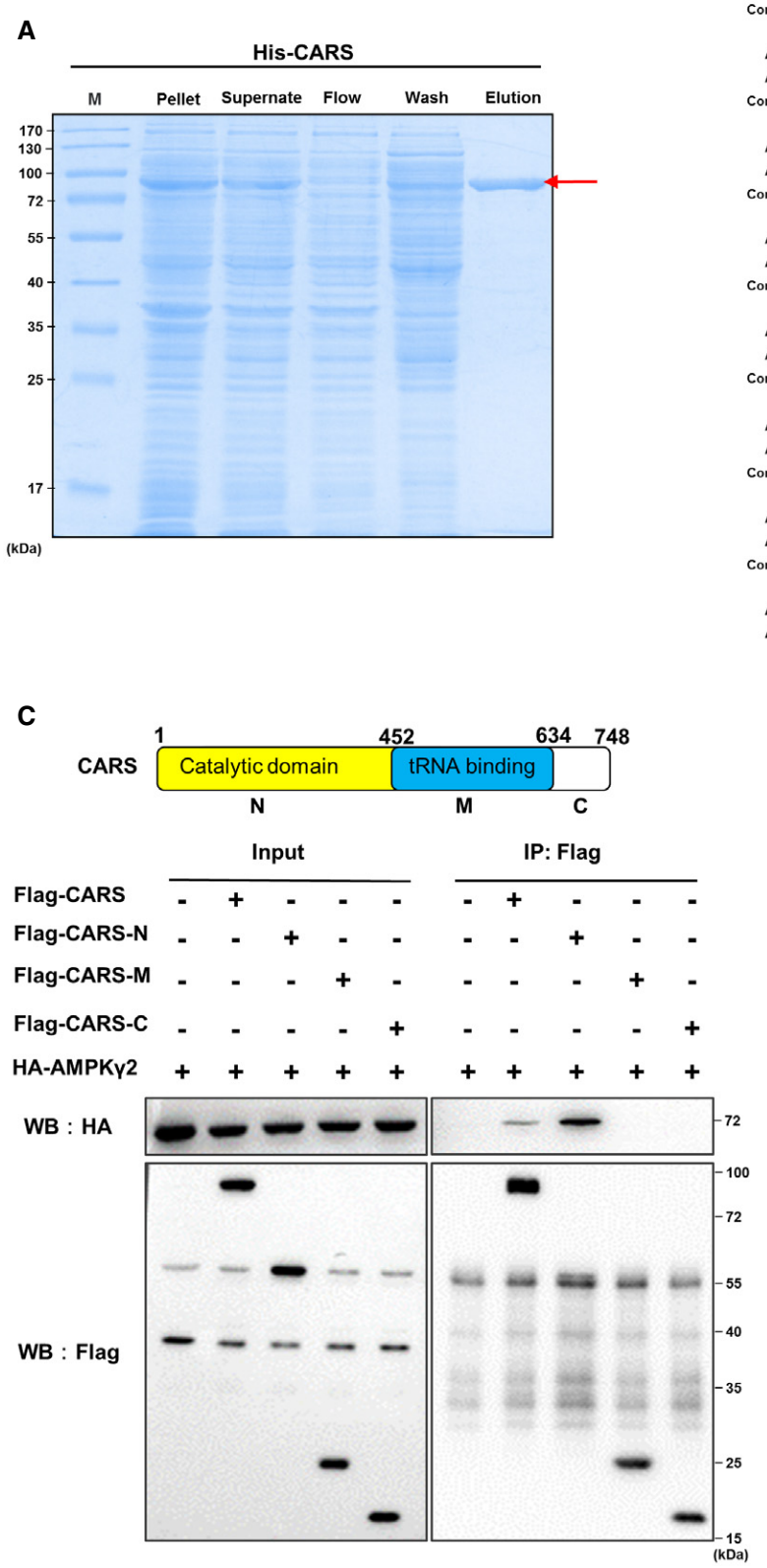

Figure EV3.

Figure EV4. The cysteine–CARS–AMPK pathway promotes cell survival.

A, B Cell death levels were determined in cystine-deficient medium-cultured RCC4 or SK-MES cells with or without 1 μg/ml STO-609 for 24 h. Data are presented as the mean (± SD) of three independent experiments. "Nor" = normal medium; "-Cys" = cystine-deficient medium. *$P < 0.05$ [two-tailed Student's *t*-test], compared with the indicated groups.

C, D The crystal violet assay (C) and apoptosis rate assay (D) were performed in 293T cells transfected with shRNAs targeting NTC, CARS, CaMKK2 or AMPKγ2 and further treated with cystine-deficient medium for 24 h with or without 100 μM PT1 or 30 μM GSK621. Data are presented as the mean (± SD) of three independent experiments. NS, not significant; *$P < 0.05$ [two-tailed Student's *t*-test], compared with the indicated groups.

E 786-O, NCI-1650 and HT1080 cells were treated with cystine-deficient medium for 0, 6, 12 and 24 h, respectively. The protein levels of p-AMPK and total AMPK were measured by WB, and actin served as the loading control.

F The crystal violet assay was performed in cystine-deficient medium-cultured 786-O, NCI-1650 and HT1080 cells with or without 1 mM AICAR.

Source data are available online for this figure.

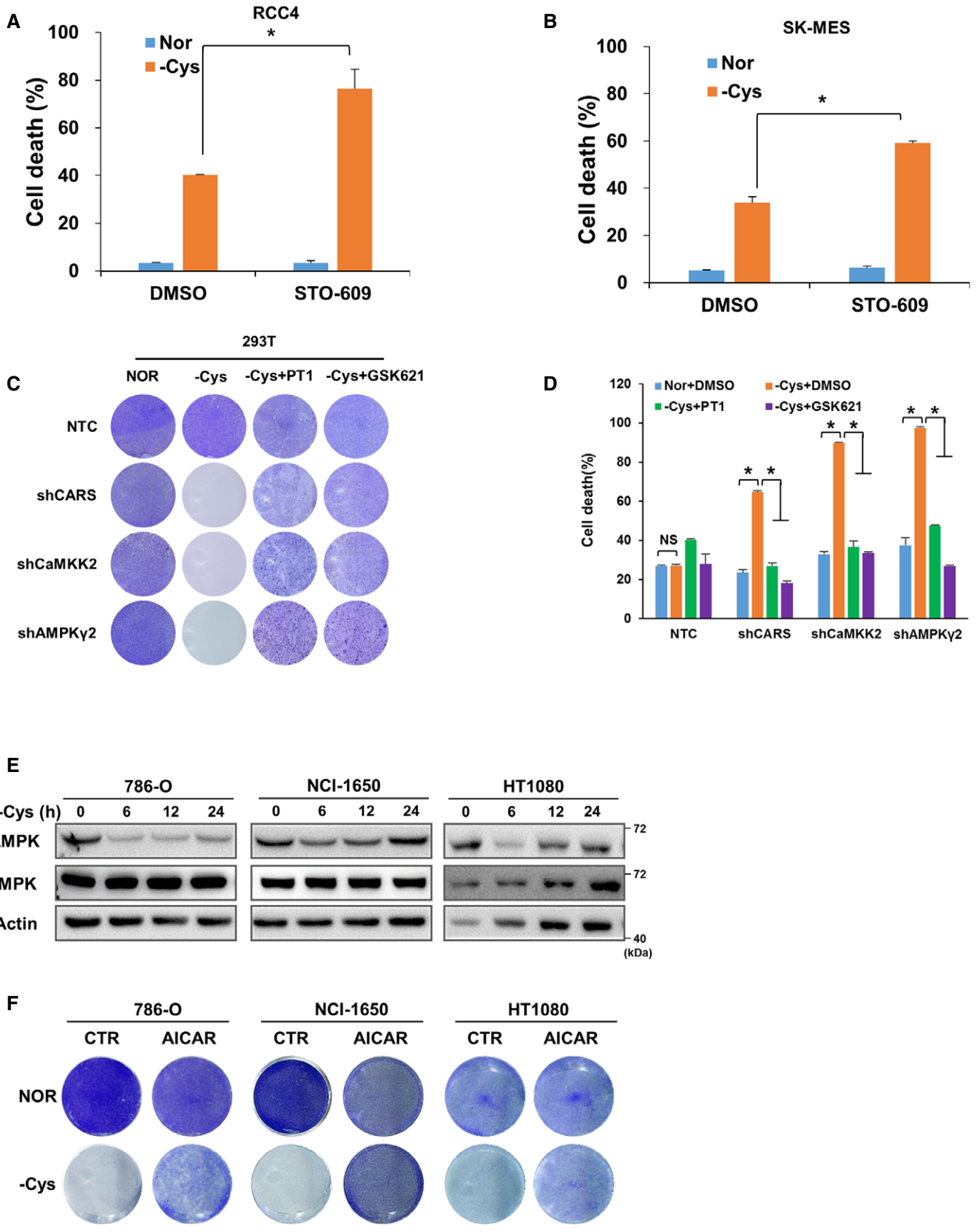

Figure EV4.