Expanded View Figures

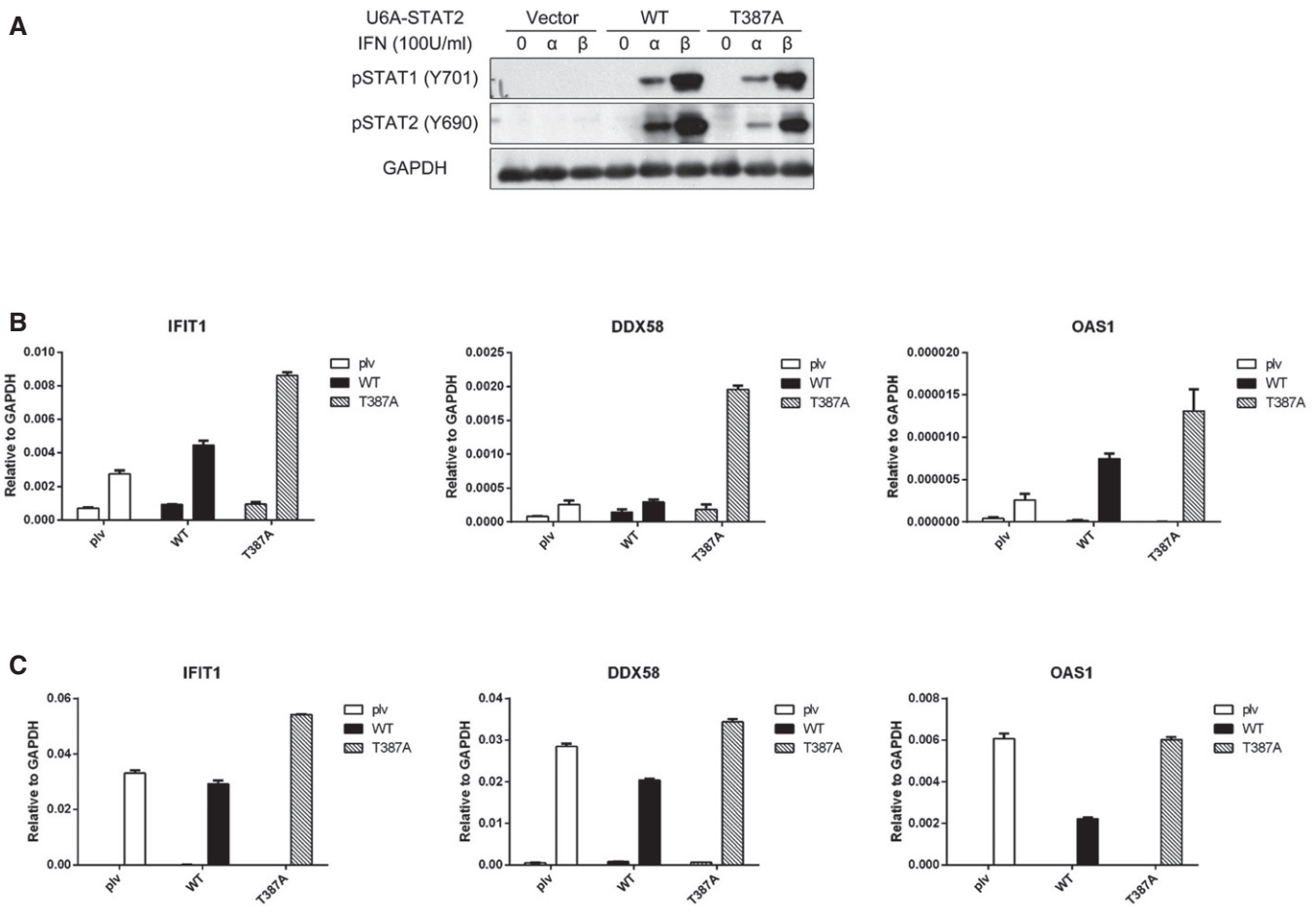

Figure EV1. Tyrosine phosphorylation of STAT1 and STAT2 in U6A cells expressing wild-type or T387A STAT2, and high expression of STAT2 does not change gene expression in response to IFN-β.

A The cells were treated with IFN-α or IFN-β (100 IU/ml) for 30 min or were untreated. Whole-cell lysates were analyzed by Western blot.

B, C 293T (B) or HME (C) cells expressing wild-type or T387A STAT2 were treated with IFN-β (100 IU/ml). Cells were harvested after 4 h, and RNAs were analyzed by real-time PCR. Values are the means ± SD from three independent experiments.

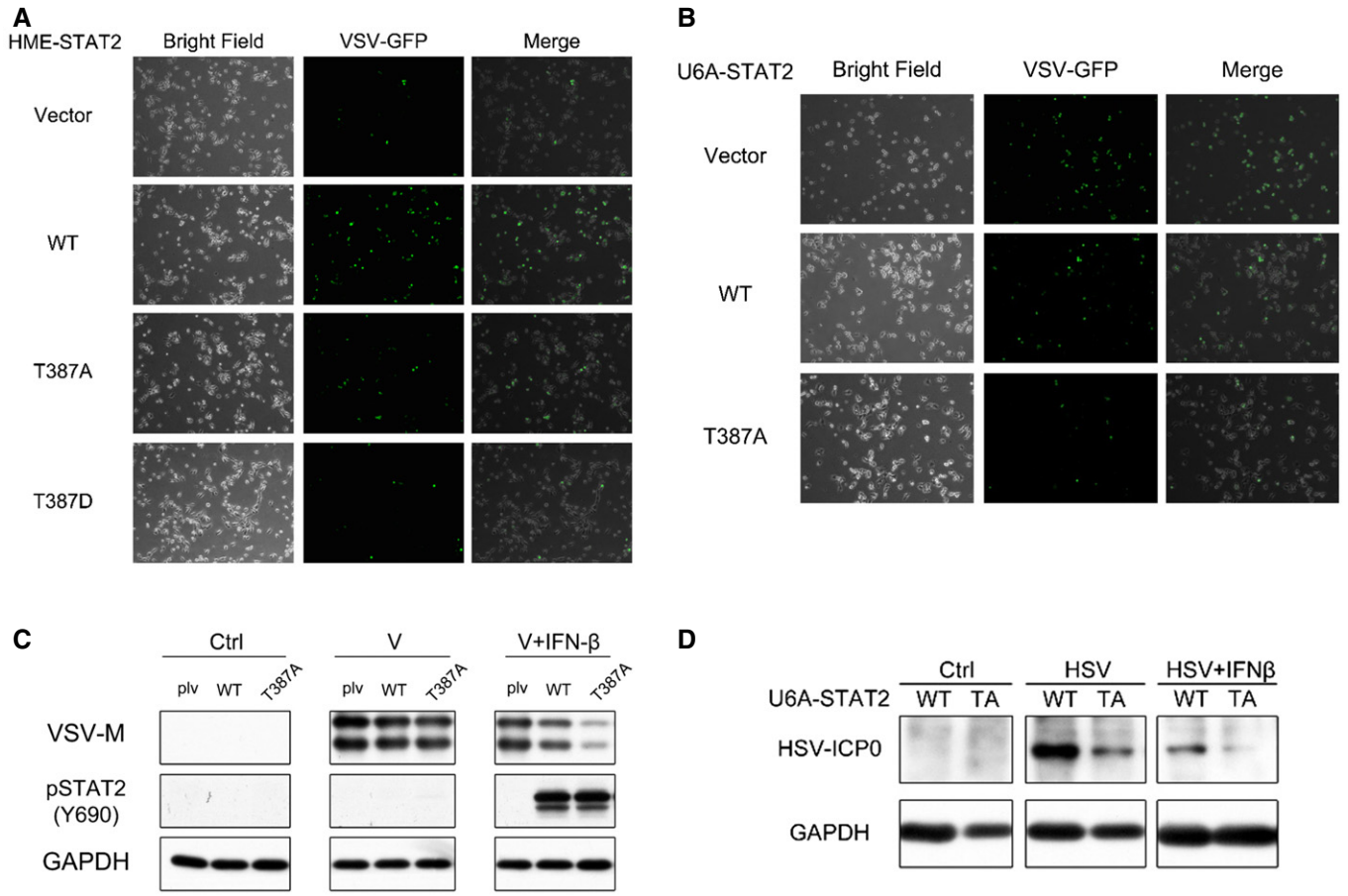

Figure EV2. Phosphorylation of T387 STAT2 inhibits the antiviral effect of IFN-β in HME and U6A cells.

A HME cells expressing wild-type, T387A, or T387D STAT2 were seeded at 8,000 cells/well. The cells were exposed to VSV for 2 h, with or without pre-treatment with IFN-β (100 IU/ml). After 20 h, the expression of GFP from modified VSV was analyzed by microscopy.

B, C U6A cells expressing wild-type or T387A STAT2 were seeded at 8,000 cells/well. The cells were exposed to VSV for 2 h, with or without pre-treatment with IFN-β (100 IU/ml). After 20 h, the expression of GFP from modified VSV was analyzed by microscopy (B) and the VSV-M protein was analyzed by Western blot (C).

D U6A cells expressing wild-type or T387A STAT2 were seeded at 8,000 cells/well. The cells were exposed to HSV for 2 h, with or without pre-treatment with IFN-β (100 IU/ml). After 20 h, the HSV ICP0 protein was analyzed by Western blot.

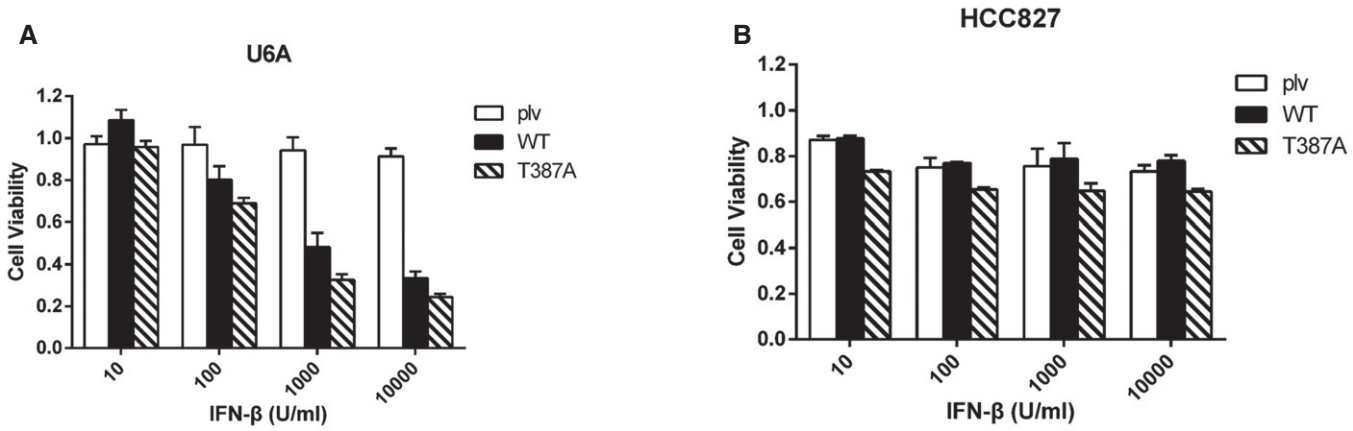

Figure EV3. Phosphorylation of T387 of STAT2 compromises the ability of IFN-β to inhibit cancer cell growth.

A, B U6A (A) or HCC827 (B) cells expressing wild-type or T387A STAT2 were placed into 96-well plates (2,000 cells/well). The cells were incubated with IFN-β (0, 10, 100, 1,000 or 10,000 IU/ml) for 96 h. Cell survival was analyzed by the MTT assay. Values are the means ± SD from three independent experiments.

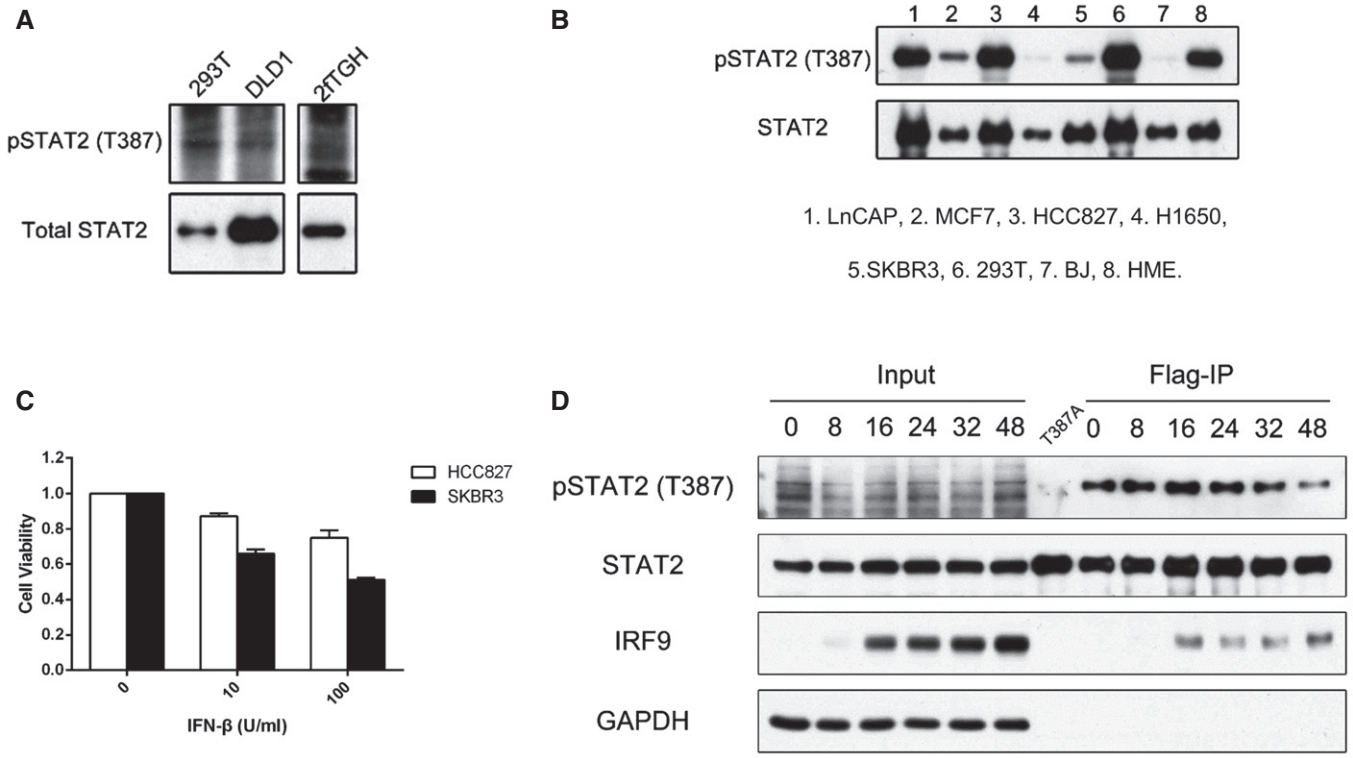

Figure EV4. Phosphorylation of STAT2 T387 in various cells.

A Immunoprecipitations of STAT2 were performed with whole-cell lysates of 293T, DLD1, and 2fTGH cells, and samples were analyzed by Western blot.

B STAT2, immunoprecipitated by means of a Flag tag from cells expressing wild-type STAT2, was analyzed by Western blot. 1: LnCAP; 2: MCF7; 3: HCC827; 4: H1650; 5: SKBR3; 6: 293T; 7: BJ; 8: HME.

C HCC827 and SKBR3 cells were placed into 96-well plates (2,000 cells/well), and the cells were treated with IFN-β (1,000 IU/ml) for 96 h. Cell survival was analyzed by the MTT assay. Each experiment was carried out two independent times, with results similar to the representative examples that are shown. Values are the means ± SD from three independent experiments.

D STAT2 was immunoprecipitated by means of a Flag tag from U6A cells expressing wild-type or T387A STAT2, treated with IFN-β (100 IU/ml) at the times indicated. Cell extracts were analyzed by Western blot.

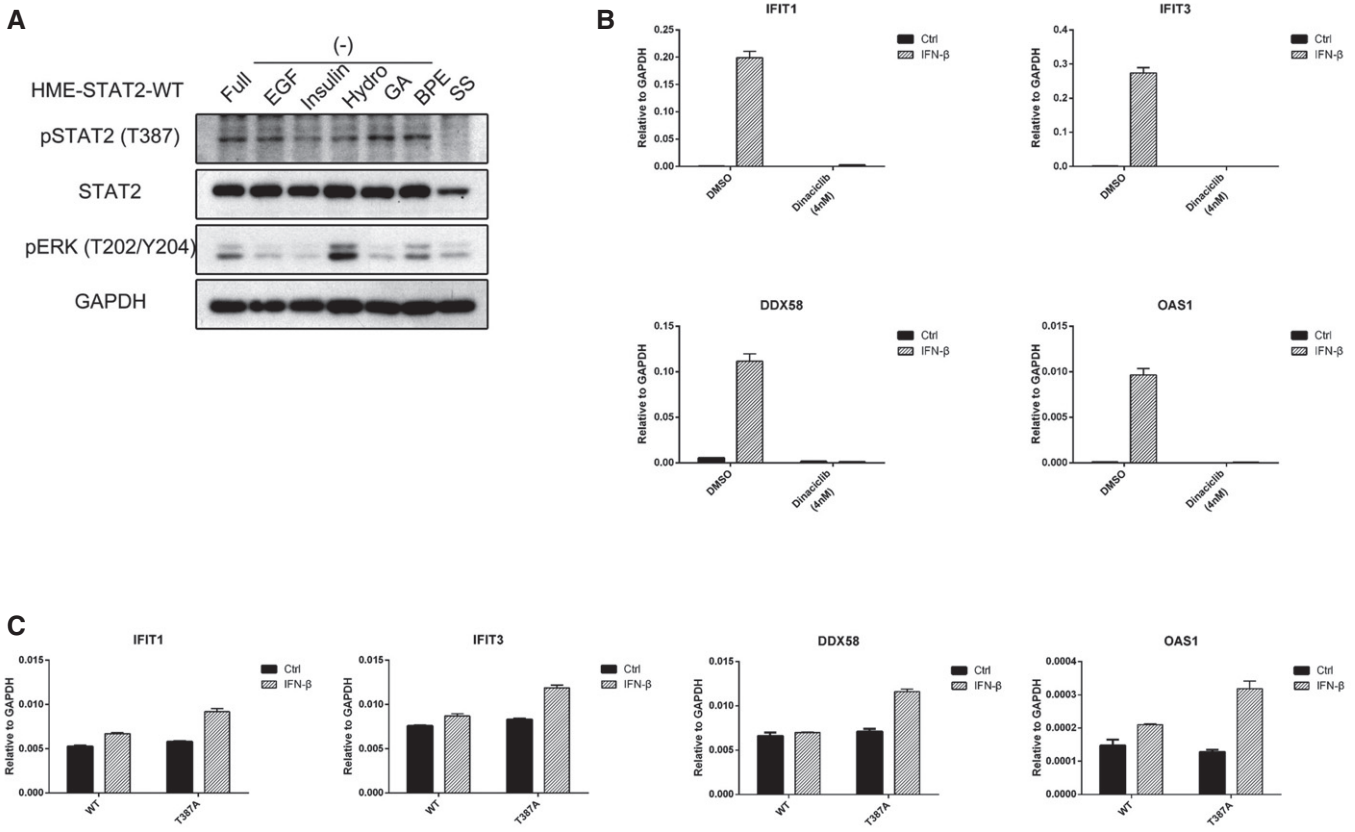

Figure EV5. ISG induction by IFN-β in response to dinaciclib treatment and in the absence of STAT1.

A HME cells expressing wild-type STAT2 were seeded in complete medium. On the second day, the medium was replaced with fresh medium lacking each component. On the third day, the cells were harvested and total lysates were analyzed by Western blot.

B HME cells were pre-treated with dinaciclib (4 nM) for 3 h and then treated with IFN-β (100 IU/ml). Cells were harvested after 4 h, and total RNAs were analyzed by real-time PCR. Values are the means ± SD from three independent experiments.

C STAT1-null U3A cells expressing wild-type (WT) or T387A STAT2 were treated with IFN-β (100 IU/ml). Cells were harvested after 4 h, and total RNAs were analyzed by real-time PCR. Values are the means ± SD from three independent experiments.