Supplementary Information

Effects of taurine-conjugated bile acids on colorectal cancer cell proliferation and tumor-associated cellular properties

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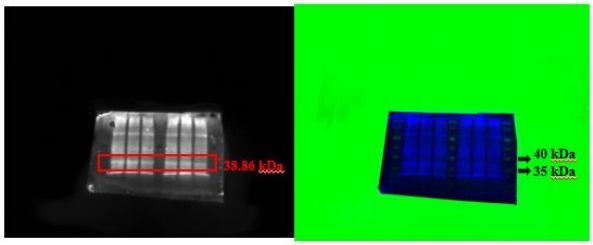
* Shared first authorship

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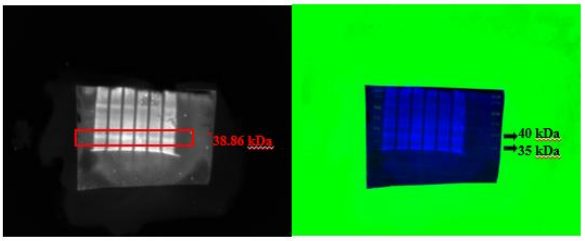
(a)

HCT-116:

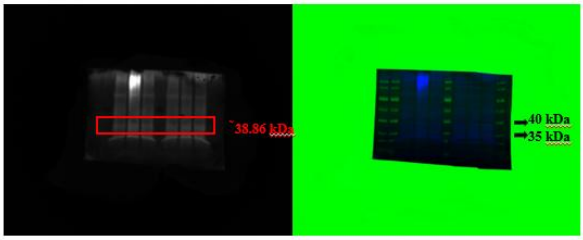
S1PR2: **Gel.1**



Gel.2



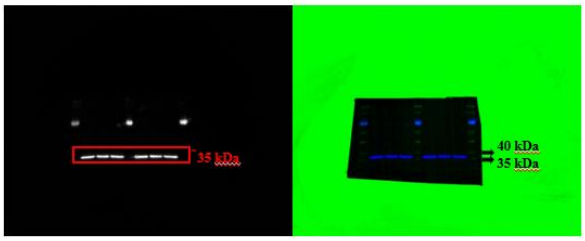
Gel.3



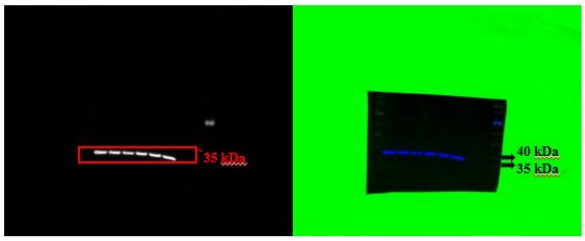
(b)

HCT-116:

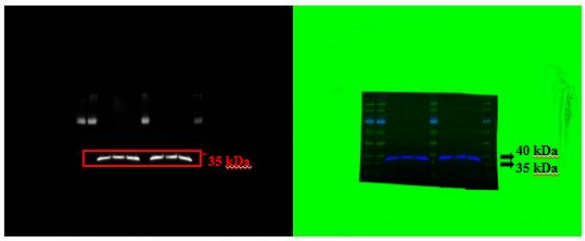
GAPDH: **Gel.1**



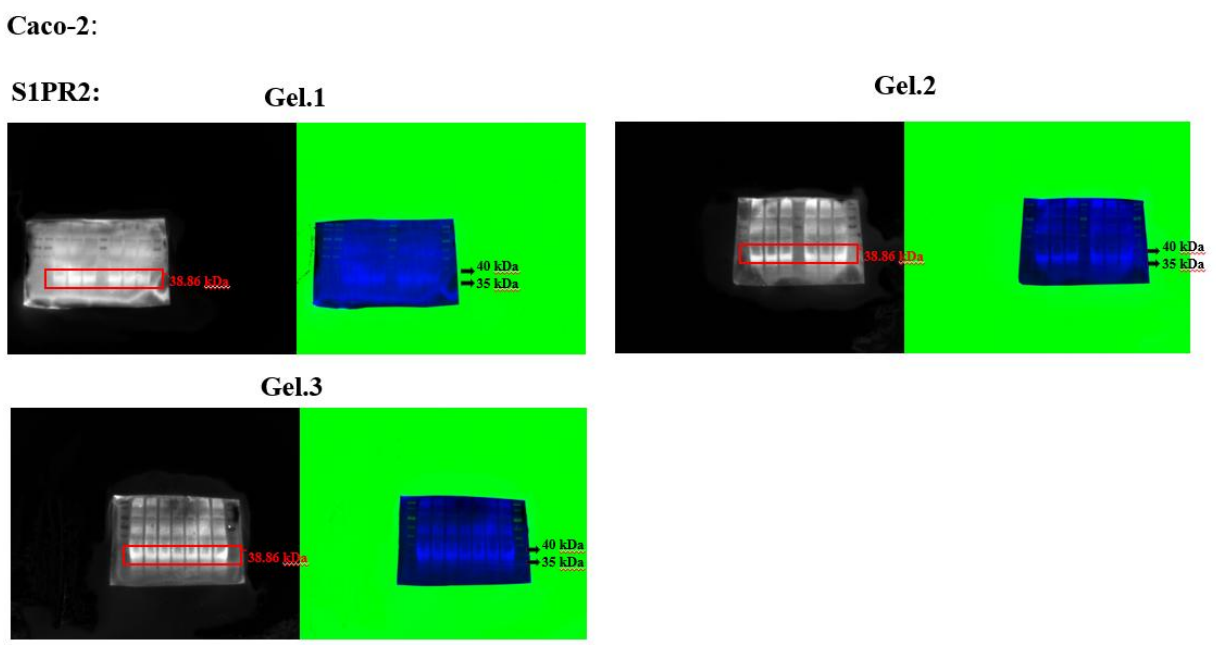
Gel.2



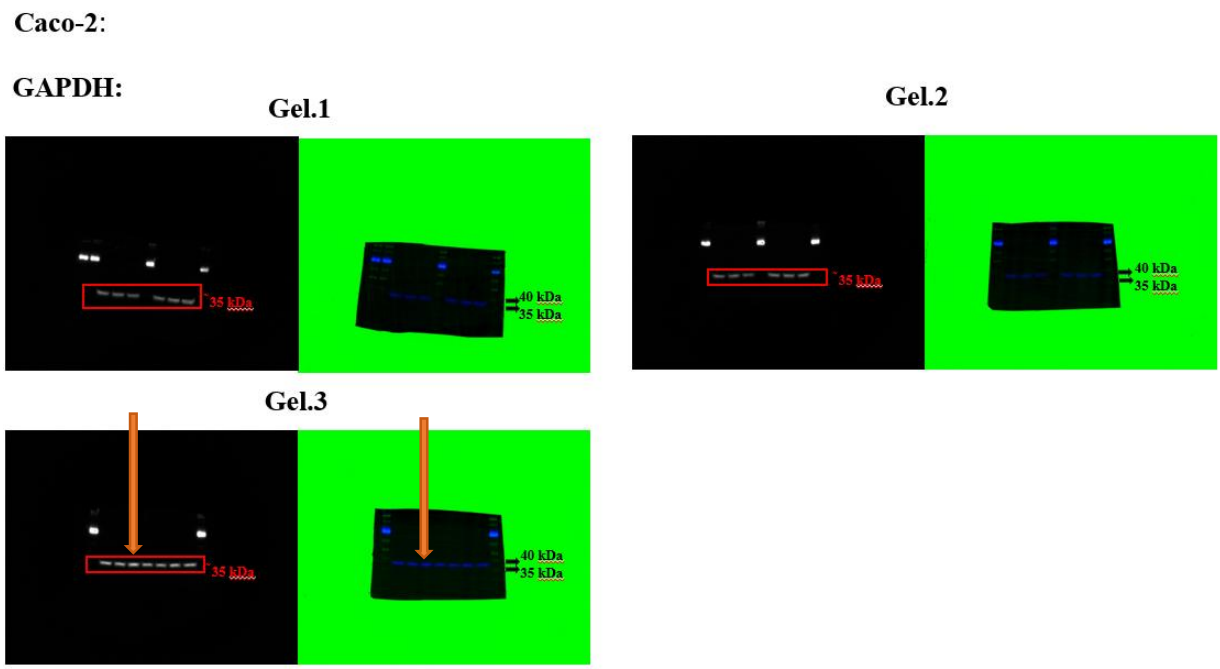
Gel.3



(c)



(d)



Supplementary Figure 6. Taurocholic acid (TCA) increases sphingosine-1-phosphate receptor 2 (S1PR2) reduces proliferation under TCA-treated conditions in HCT-116 and Caco-2 cells.

(**c**) Representative Western blot images showing S1PR2 protein expression, with GAPDH used as the loading control, in HCT-116 and Caco-2 cells following TCA treatment.

HCT-116 and Caco-2 cells were seeded in 75-cm² culture flasks at a density of 250,000 cells per flask and allowed to attach for 24 h. Cells were subsequently treated with 300 µM taurocholic acid (TCA) for 72 h. Control cells received culture medium alone and were maintained under the same experimental conditions. Following treatment, cells were harvested for protein extraction. Total cellular proteins were extracted using RIPA lysis buffer supplemented with protease inhibitors. Protein concentrations were determined using the Bradford assay, and 25 µg of total protein per sample was separated by SDS-PAGE on 10% acrylamide gels.

Western blot analyses were performed in three independent experiments for each cell line using different protein samples in each experiment. In each experiment, three samples per experimental group (control and TCA-treated) were analyzed. Densitometric values for S1PR2 were normalized to GAPDH for each sample. The mean normalized value of the three samples within each experimental group was calculated for each independent experiment. These experiment-level means from the three independent experiments were used for statistical analysis (n = 3 per group).

Panel descriptions: (a) S1PR2 bands in HCT-116 cells, showing the chemiluminescent and composite images. Molecular weight markers (Thermo Fisher Scientific, Cat. No. 26619) are also shown. (b) Corresponding GAPDH loading controls for HCT-116 cells. (c) S1PR2 bands in Caco-2 cells, showing the chemiluminescent and composite images. (d) Corresponding GAPDH loading controls for Caco-2 cells. Within each set of six sample lanes, the bands are arranged from right to left as three control samples followed by three TCA-treated samples. In the Caco-2 blot, the fifth well from the right was excluded from the analysis due to a technical error as indicated by the orange arrow.

Cropped images were extracted from 8-bit images using ImageJ (Fiji), version 1.54p. Image processing was limited to color inversion and horizontal flipping or rotation, where applicable. Brightness and contrast were not adjusted.

Statistical significance was assessed using Student's *t*-test.