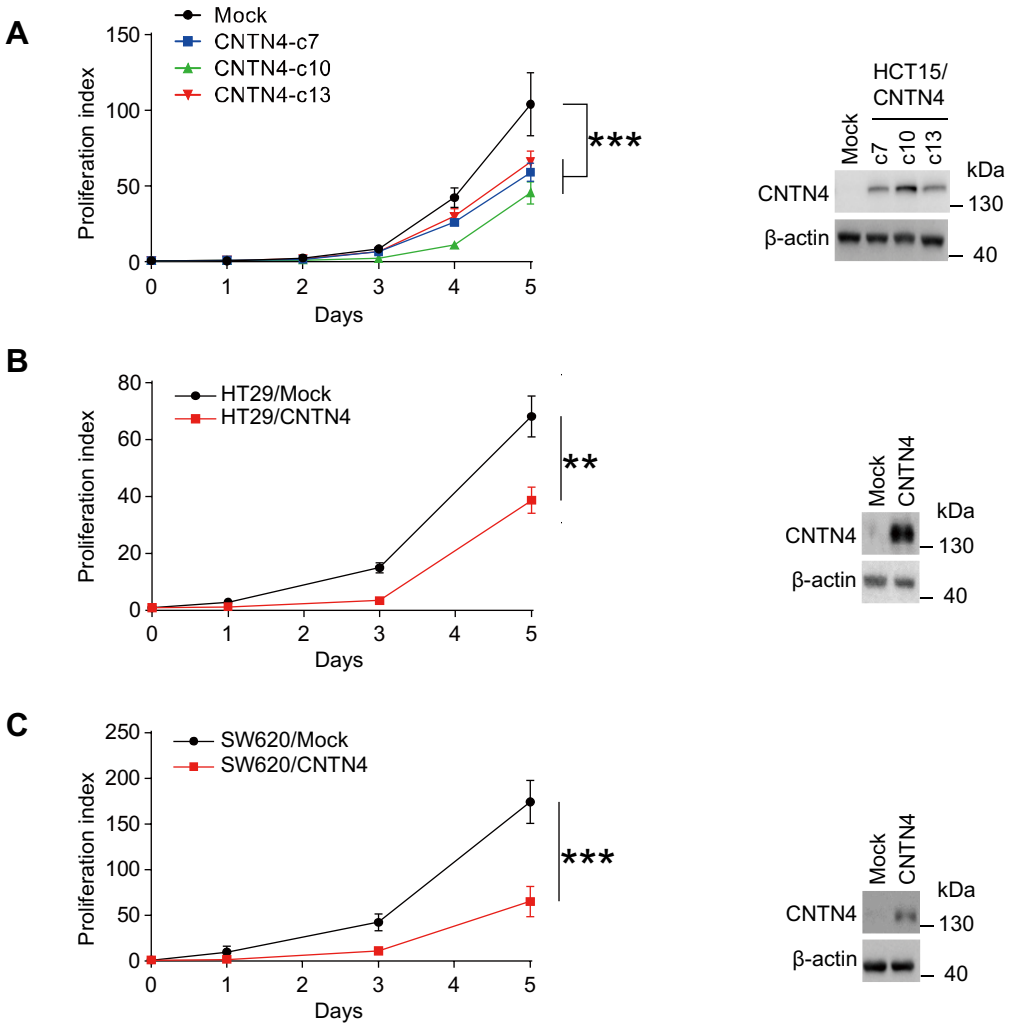
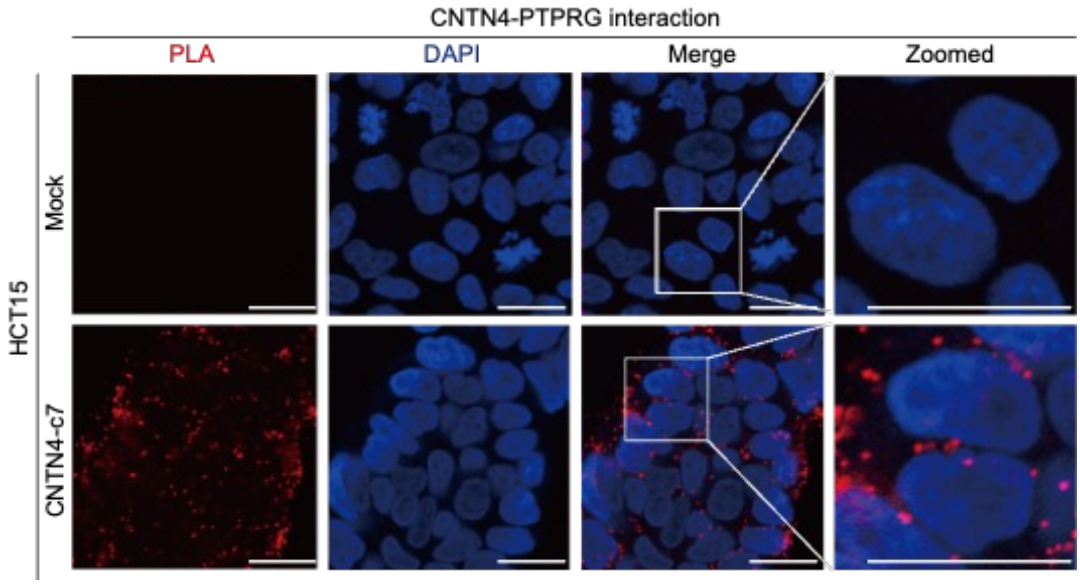
Supplementary information

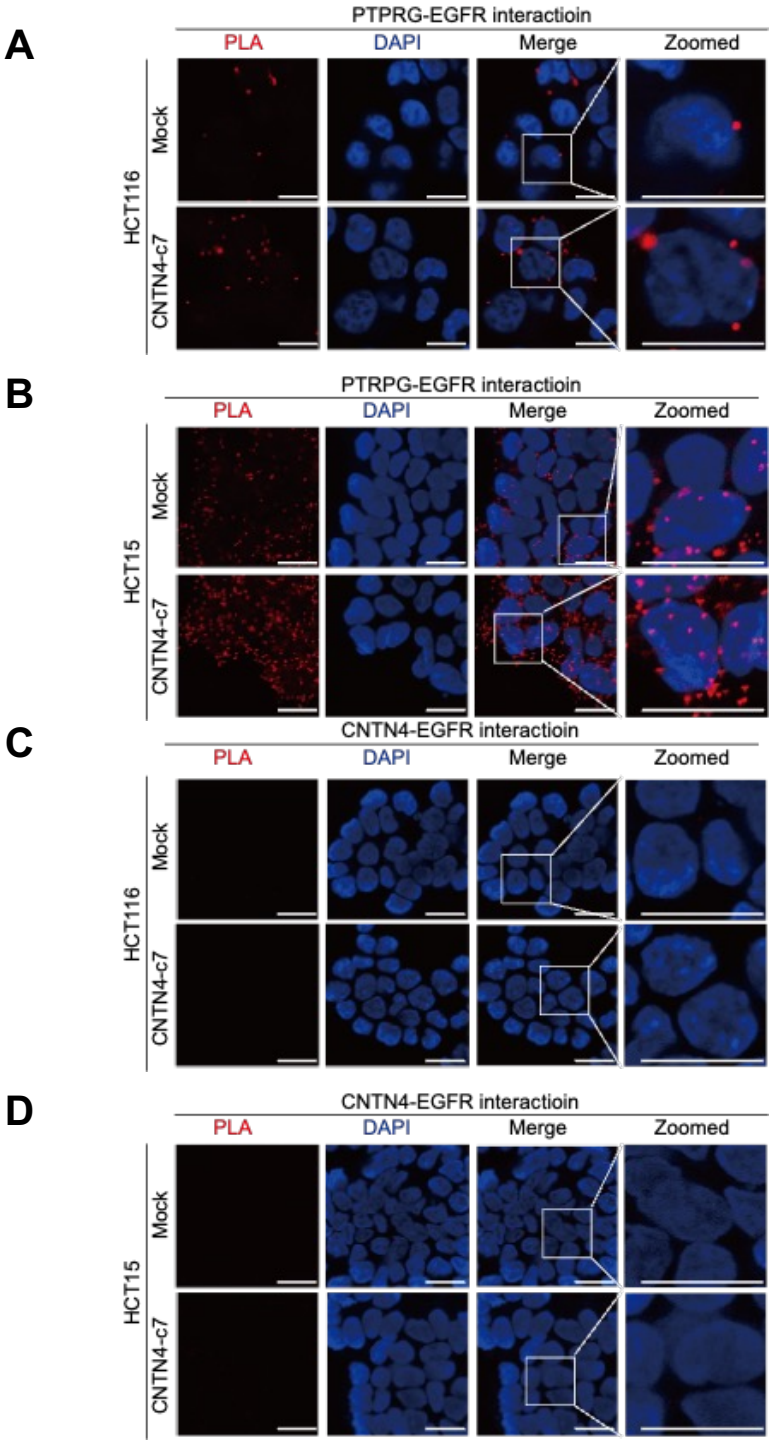
Supplementary figures



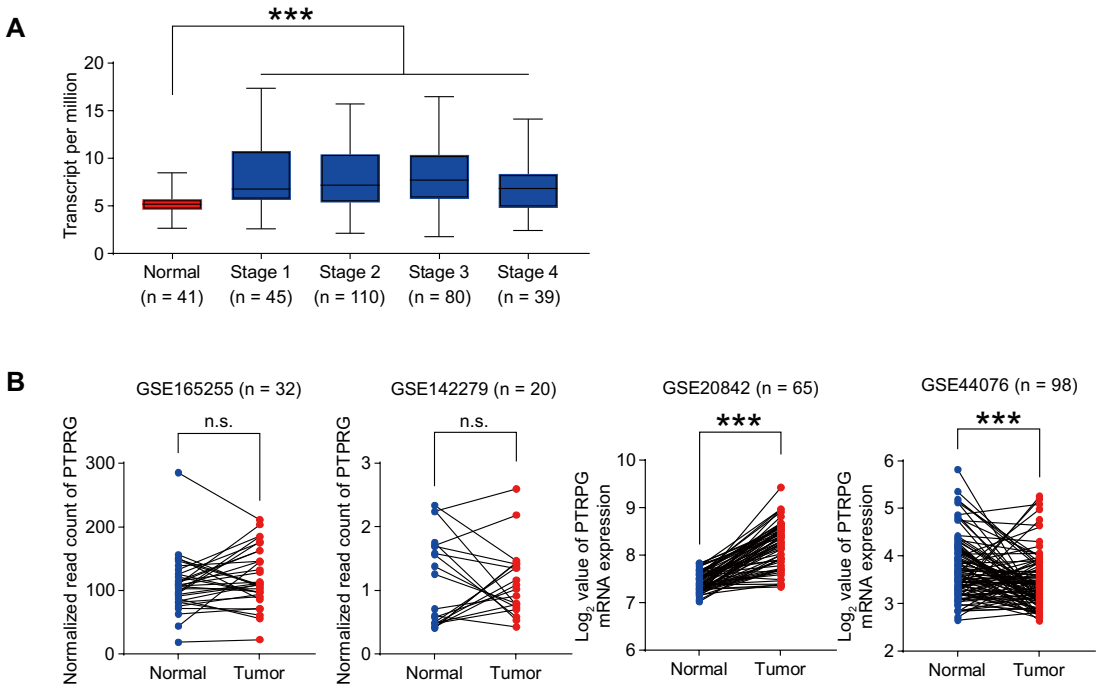
Supplementary Fig. 1 CNTN4 expression inhibits proliferation of CRC cells. Ectopic expression of CNTN4 suppresses proliferation of HCT15 (**A**), HT29 (**B**) and SW620 (**C**) cells. CNTN4 protein expression is confirmed by Western blot analysis. Data are representative of three independent experiments. Values are presented as mean±SD. ***P* < 0.01; ****P* < 0.001, determined by One-way ANOVA.



Supplementary Fig. 2 CNTN4-PTPRG interaction in HCT15 cells.
Representative micrograph shows proximity ligation assay (PLA) results. Red fluorescent signals indicate the close proximity of CNTN4 and PTPRG proteins, suggesting a physical interaction between them in HCT15 cells. Scale bars, 20 μm.



Supplementary Fig. 3 PTPRG-EGFR, but not CNTN4-EGFR, interaction is observed in HCT116 and HCT15 cells. A, **B** Representative micrographs show proximity ligation assay (PLA) results. Red fluorescent signals indicate the close proximity of PTPRG and EGFR proteins, suggesting a physical interaction in CRC cells. **C**, **D** No red fluorescent signals are detected between CNTN4 and EGFR, indicating no direct interaction as assessed by PLA assay. Scale bars, 20 μm.



Supplementary Fig. 4 *PTPRG* mRNA expression is analyzed in multiple independent CRC cohorts from public databases. A *PTPRG* mRNA expression is upregulated in tumor tissues compared to normal tissues, based on data from The Cancer Genome Atlas (TCGA) database. **B** Divergent patterns of *PTPRG* expression between normal and tumor tissues are observed across datasets from the Gene Expression Omnibus (GEO) database. ****P* < 0.001; ns, not significant. Statistical analysis is performed using One-way ANOVA in panel **A** and two-tailed Student's *t*-test in panel **B**.

Supplementary methods

RNA extraction

Total RNA of cells and tissues were extracted by using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The concentration and purity of RNA were determined by a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity was confirmed by agarose gel electrophoresis.

Reverse transcription-polymerase chain reaction (RT-PCR)

Complementary DNA (cDNA) was reverse-transcribed from total RNA by using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription was conducted under the following conditions: 25°C for 10 minutes, 37°C for 2 hours, and 85°C for 5 minutes. The resultant cDNA was diluted 5-fold with diethylpyrocarbonate (DEPC)-treated H_2O. PCR amplification was conducted in a final volume of 25 μL by using 2.5 μL of diluted cDNA, 1 μM of each of respective primers, 250 μM of each dNTP, and 1 unit of Super-Therm Gold DNA Polymerase (Bertec Enterprise, Taipei, Taiwan). PCR was performed under the following cycling conditions: a pre-PCR incubation step at 95°C for 10 minutes; 35 or 28 (for internal control) cycles of 95°C for 15 seconds, annealing temp for 45 seconds, and 72°C for 45 seconds; followed by a final extension of 72°C for 3 minutes. The amplified fragments were analyzed by agarose gel electrophoresis. The primer sequences are listed in the Supplementary Table 4.

Real-time RT-PCR using SYBR Green system

To quantify *PTPRG*, *uPA*, *EGFR* mRNA expression, the SYBR Select Master Mix (Applied Biosystems) was used. The *ACTB* served as an internal reference. Quantitative PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System, and data were analyzed with ABI PRISM 7000 SDS Software (Applied Biosystems). Each 20 μL reaction mixture contained 10 or 50 ng of diluted cDNA, 10 μL of 2X SYBR Select Master Mix, 1.6 μL (400 nM) of forward and reverse primer, and nuclease-free ddH_2O to

reach the final volume. Reactions were carried out in triplicate under the following thermal cycling conditions: an incubation step at 50°C for 20 sec, an enzyme activation step at 95°C for 10 min, and 40 cycles of 95°C for 15 sec and 60°C for 1 min. The *PTPRG*, *uPA*, *EGFR* expression levels were normalized to *ACTB* expression. The relative expression ratio of tumor tissue to matched normal mucosa was calculated using the comparative Ct method, $2^{-\Delta\Delta Ct}$.

Cell proliferation assay

The cells were seeded in 96-well plates ($2x10^4$ cells/mL) with 100 μL cell suspension in each well and incubated for 24, 48, 72, 96, and 120 hours. At each time point, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed by adding 100 μL of MTT (0.5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) for 1.5 hours at 37°C. The reaction was stopped by adding 100 μL of dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA). The absorbance value of each well was measured with a microplate reader set at 570 nm.

Anchorage-dependent growth assay

The HCT116 stable transfectants ($1.5x10^2$ cells/well) were seeded in six-well culture plates, and were incubated at 37°C for 10 days. The colonies were stained with 0.05% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) and photographed. Colonies equal to and larger than 0.2 mm in diameter were counted. All experiments were performed three times in triplicate.

Anchorage-independent growth assay

Anchorage-independent growth of HCT116 stable transfectants was assayed by colony formation on soft agar. The cells ($8x10^2$ cells/well) suspended in complete medium containing 0.35% low melting agarose were overlaid on a base layer containing 0.7% agarose in six-well culture plates, and were incubated at 37°C for 21 days. The colonies were stained with 0.05% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) and photographed. Colonies equal to and larger than 0.2 mm in diameter were counted. All experiments were performed three times in triplicate.

Apoptosis study

Cells were detached by Accutase (Millipore, Billerica, MA, USA) treatment. After washing twice with cold PBS, cells were resuspended in Annexin V binding buffer at a concentration of 1×106 cells/mL. After incubation of 100 µL cell suspension with 5 µL Annexin V-FITC and 5 µL PI at room temperature for 15 minutes in dark, 400 µL of binding buffer was added to each tube. Samples were then analyzed on a flow cytometer (Beckman Coulter, Pasadena, CA, USA). The percentage of apoptotic cells was determined including early apoptotic cells (Annexin V+/PI-) and late apoptotic/necrotic cells (Annexin. V+/PI+).

The human angiogenesis array

Cell culture supernatants of CNTN4 transfectants were harvested for human angiogenesis array analysis following the manufacturer's instructions (R&D Systems, MN, USA). The immunoblot images were captured using LAS-4000 Luminescent Image Analyzer (FujiFilm, Tokyo, Japan) and the intensity of each spot in membranes was analyzed using ImageJ software (National Institutes of Health, Maryland, USA).