Figure S6. COUP-TFII_V1 expression is influenced by COUP-TFII_V2. (A) Western blot analysis of MiaPaca2 cells transfected with increasing quantities of COUP-TFII_V2 expression plasmid indicated that COUP-TFII_V1 expression slightly decreased with higher COUP-TFII_V2 expression. (B) Western blot analysis of PANC-1 cells transfected with EGFP-COUP-TFII_V2 or EGFP-COUP-TFII_V1 fusion protein for 48 h. Western blot was performed with a pan-COUP-TFII antibody. (C) Western blot analysis of MiaPaca2 cells transfected with a combination of plasmids expressing COUP-TFII_V1, COUP-TFII_V2 or shRNA for NR2F2, indicating that COUP-TFII_V2 has effects similar to those of the shRNA for COUP-TFII. (D) Efficiency of shNR2F2 in reducing COUP-TFII expression. COUP-TFII_V1 mRNA is reduced in PANC-1 and MiaPaca2 cells transiently transfected for 48 h with the plasmid for shNR2F2 expression, compared to the same cells transfected with shNEG (expression in shNEG-transfected cells is normalized to 1 and represented as a dashed line). Values are expressed as the mean ± standard deviation of the fold change (n=3), normalized to housekeeping genes. *P<0.05 (E) Anti-ubiquitin western blot of total proteins extracted from MiaPaca2 and PANC-1 cells transfected with COUP-TFII_V2. Prior to western blot, proteins were immunoprecipitated with the specific COUP-TFII_V1 antibody. (F) Co-IP western blot of proteins extracted from wild-type PANC-1 and MiaPaca2 cells. Total proteins were incubated overnight with the mouse monoclonal COUP-TFII-V1 antibody. Immunoprecipitated proteins were subjected to standard SDS-PAGE western blot. A polyclonal pan-COUP-TFII antibody was then used. Bands putatively associated with COUP-TFII_V1, COUP-TFII_V2 and COUP-TFII_V3/V4 were observed. (G) Brightfield and fluorescence microphotographs of PANC-1 cells transiently expressing EGFP-COUP-TFII_V1 alone (top row), indicating the expected nuclear localization; co-transfection of EGFP-COUP-TFII_V1 and COUP-TFII_V2 (lower row) instead suggested that an excess of V2 forces COUP-TFII_V1 to localize in the cytosol (original magnification, x200). shRNA, short hairpin RNA; shNR2F2, shRNA targeting NR2F2 (COUP-TFII); shNEG, negative control shRNA; NR2F2, nuclear receptor 2 family 2; TF, transcription factor; EGFP, enhanced green fluorescence protein; IP, immunoprecipitation.

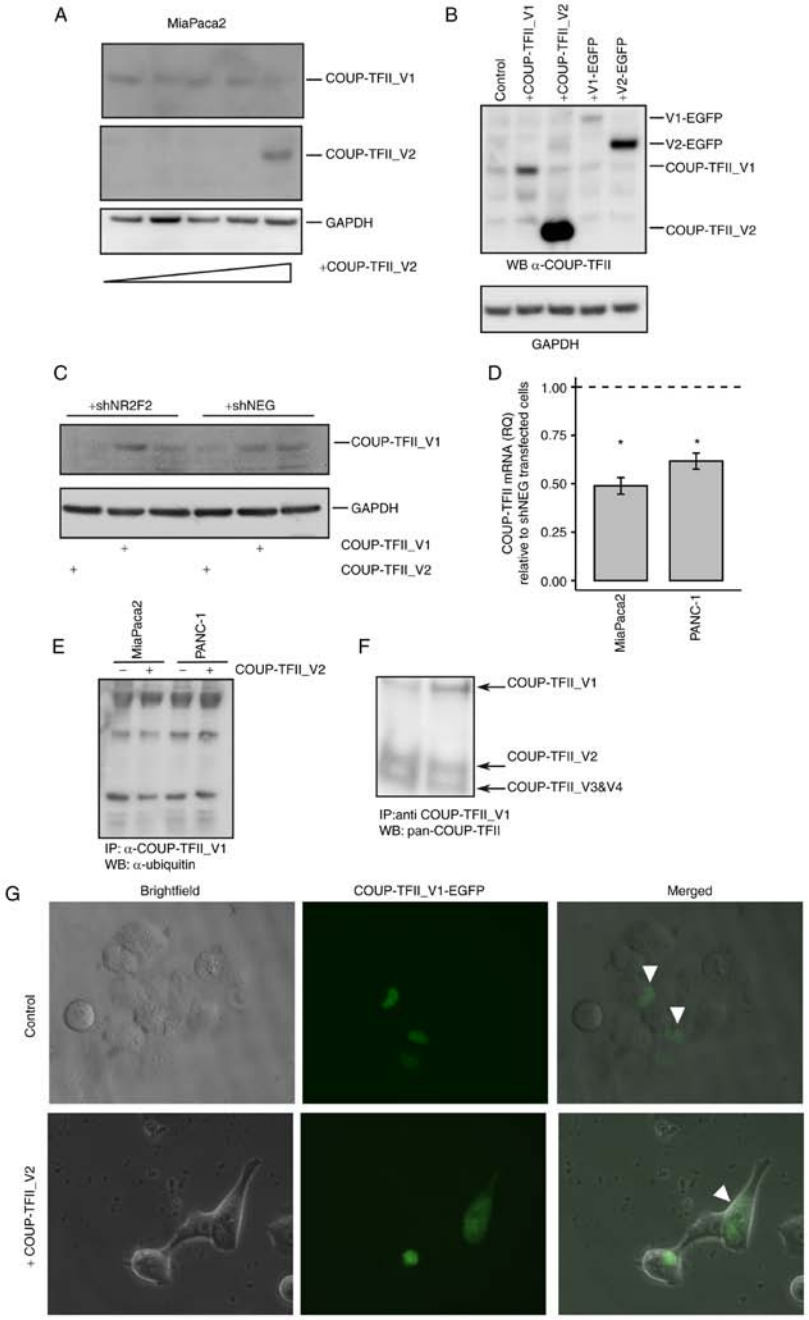

Figure S7. (A) Electrophoretic mobility shift assay. The arrow indicates the specific NHE-COUP-TFII_V1 complex (that is more evident in PANC-1 cells transfected with COUP-TFII_V1); this band is less evident in pcDNA 3.1(+) transfected cells (control) and COUP-TFII_V2-expressing cells and it disappeared when proteins were preincubated with the COUP-TFII_V1 antibody (indicated as '#Ab V1') prior to complex formation. (B) Transcriptional activity of COUP-TFII. PANC-1 clones were transiently transfected with the NHE luciferase reporter for 48 h then tested with the Luciferase reporter assay (Promega Corporation). In agreement with the gel shift results, a reduction of transcriptional activity was observed in the presence of excess V2 (n=4). Reported data are normalized to *Renilla* luciferase and to MOCK expression.; TF, transcription factor; Ab, antibody; NHE, sodium-hydrogen exchanger; PANC-V1, PANC-1 cell line overexpressing COUP-TFII_V1; PANC-V2, PANC-1 cell line overexpressing COUP-TFII_V2; MOCK, PANC-1 cell line resistant to G418; control, PANC-1 cells transfected with pcDNA 3.1(+).

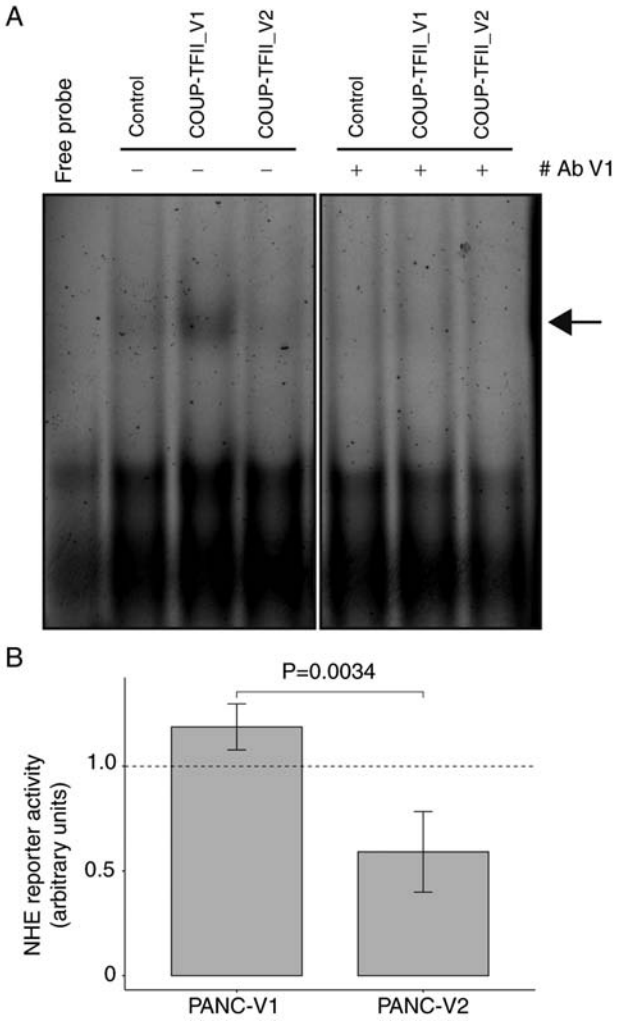

Figure S8. (A) COUP-TFII_V2 increases chemotherapy resistance. PANC-1 cells were transiently transfected with COUP-TFII-V2 or -V1 and 24 h later treated with various concentrations of gemcitabine (10, 50 and 100 μM) for 48 h. Western blot was then performed to determine the expression of COUP-TFII isoforms; as clearly indicated, the expression of COUP-TFII_V2 was not diminished after drug treatment, whereas the expression of COUP-TFII_V1 was markedly reduced compared with the controls. This result suggests that COUP-TFII_V2 may protect cancer cells from chemotherapy. (B) Reverse transcription-quantitative PCR for proliferation marker in PCNA performed on total RNA extracted from 9-day-old MOCK, PANC-V1 and PANC-V2 spheroids. (C) Immunofluorescence of intermediate filaments (vimentin and actin) of spheroids obtained from MOCK, PANC-V1 and PANC-V2 cells cultured in methocel suspension for 9 consecutive days; under the tested conditions, PANC-V2 cells, and, to a lesser extent, PANC-V1 cells, formed bigger spheroids. PANC-V1 spheroids were characterized by the formation of vessel-like cavities (scale bar, 50 μm). (D) Representative wound-healing images of the results reported in Fig. 4. Cellular migration fronts are outlined in yellow and the straight green lines mark the wound edges used for the distance measurement (Bar 400 mm). PANC-V1, PANC-1 cell line overexpressing COUP-TFII_V1; PANC-V2, PANC-1 cell line overexpressing COUP-TFII_V2; MOCK, PANC-1 cell line resistant to G418; TF, transcription factor; PCNA, proliferating cell nuclear antigen.

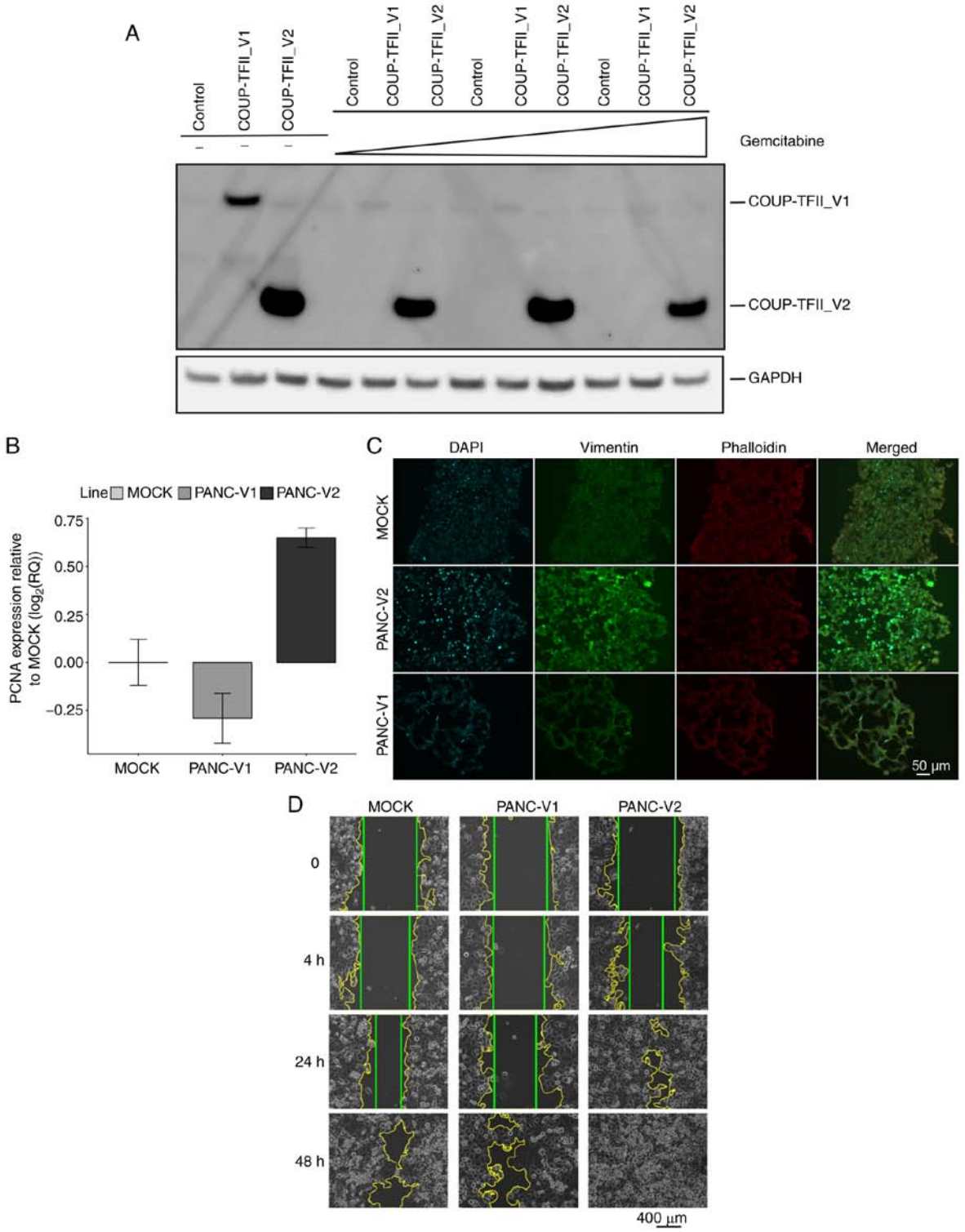

Figure S9. (A) Sirius red staining of collagen fibers (in red) of representative xenograft tumors (original magnification, x100). (B) Quantification of Sirius Red staining indicated no differences among the MOCK, PANC-V1 and PANC-V2 xenografts. (C) Stromal infiltration in xenograft tumor. Representative immunohistochemistry images for α-smooth muscle actin. Positive cells infiltrated the tumor masses (original magnification, x200). PANC-V1, PANC-1 cell line overexpressing COUP-TFII_V1; PANC-V2, PANC-1 cell line overexpressing COUP-TFII_V2; MOCK, PANC-1 cell line resistant to G418; TF, transcription factor.

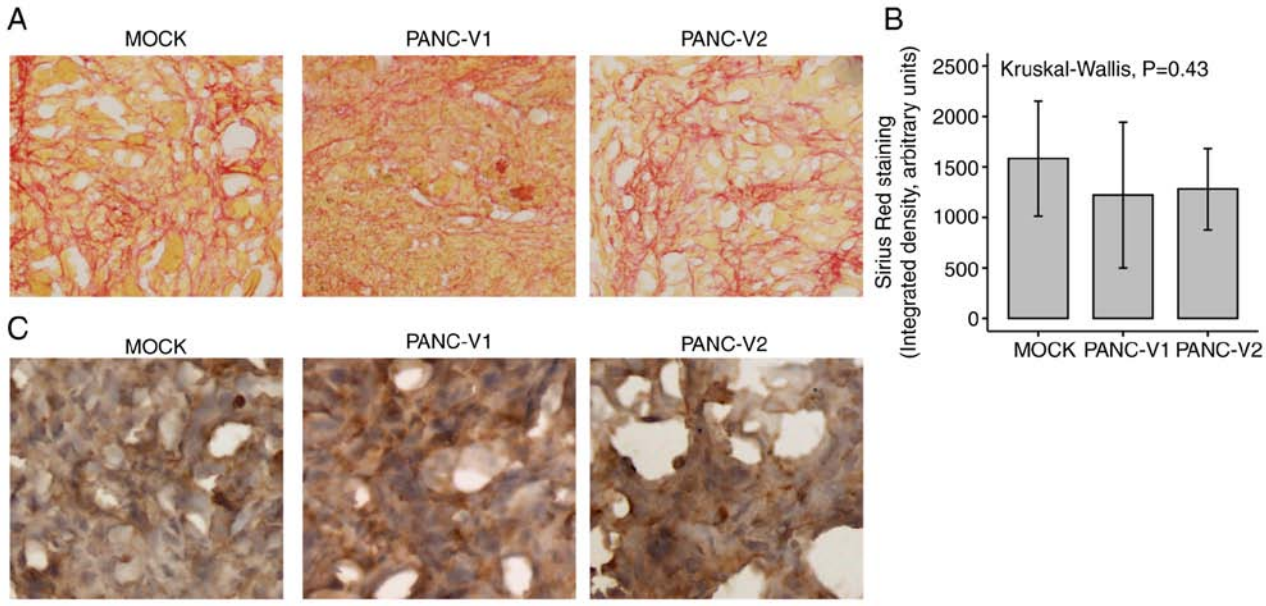

Figure S10. (A) Heatmaps of gene expression in BxPC3, CAPAN-2, PANC-1 and MiaPaca2 cells transiently transfected with COUP-TFII_V1, COUP-TFII_V2 or pcDNA 3.1(+) (control). (B) Reverse transcription-quantitative PCR of selected genes expressed in 9-day-old spheroids obtained from PANC-1 clones. Data are normalized to housekeeping genes and the RQ is calculated with the MOCK as a reference. (C) Representative photomicrographs of senescence staining of PANC-1 clones (native β-gal). Positive cells are stained in blue (original magnification, x100). MOCK, PANC-1 cell line resistant to G418, PANC-V1, PANC-1 cell line overexpressing COUP-TFII_V1PANC-V2, PANC-1 cell line overexpressing COUP-TFII_V2; TF, transcription factor; E-cad, E-cadherin; Vim, vimentin; β-cat, β-catenin; hTERT, human telomerase reverse transcriptase; ABCG2, ATP binding cassette subfamily G member 2; KLF4, Kruppel-like factor 4.