Supplemental figure legends

Supplemental Figure 1. (**a**) **Stable expression of V3Nter, SFRP-1 and V2Nter in HCT116 cells.** Cells were transfected with empty vector ("–", *top*) or the indicated expression vectors ("+", *top*) and selected with G418. Stable expression was screened by western blot with antibodies directed against the indicated epitopes (*bottom*). V3Nter shows a major ~80 kD polypeptide, SFRP-1, a ~36 kD polypeptide, and V2Nter, two ~50 kD polypeptides. (**b**) Gene assays using the CRT reporter Super8•TOP Flash or the negative control Super8•FOP Flash in HCT116 cells. (**c**) Reporter gene assays using cyclin D1 promoter driving luciferase expression. Values are expressed relative to cells stably transfected with empty vector. In (**b**) and (**c**), mean±SD were obtained from three independent experiments performed in triplicate, normalized to *Renilla luciferase* activity and pooled (*p,* Student's *t* test). (**d**) V3Nter-HCT116 cell batches #1 and #2 show reduced baseline CRT. Reporter gene assay using a β-catenin-TCF responsive reporter driven by TCF binding sites in Vector-HCT116 cells and two V3Nter-HCT116 cell batches. Values are expressed relative to cells stably transfected with empty vector (mean±SD). NS, non significant; *, $p<0.05$ (Student's *t* test).

Supplemental Figure 2. SFRP-1 and V3Nter inhibit Wnt3a-induced signaling in cancer cells carrying either wild-type or mutant β-catenin. (**a**) Relative CRT in SW480 cells after transient transfection with different amounts of V3Nter or APC cDNAs. V3Nter does not affect CRT in these cells. *Below,* immunoblots with anti-V5 epitope tag antibody detecting V3Nter and GAPDH as a loading standard. (**b**) Reporter gene assays in Huh-7 human liver cancer cells and HeLa human cervical carcinoma cells. After transient transfection with the indicated vectors and with the CRT reporters, cells were incubated with either control-CM or 50% Wnt3a-CM for 16h. Values are expressed relative to cells transfected with empty vector

and incubated with control-CM. (**c**) Gene assay using the CRT reporter in HCT116 cell clones carrying either wild-type ($CTNNB1^{WT/-}$) or mutant β-catenin ($CTNNB1^{-/\Delta S45}$) alleles. Cells were transiently transfected with the indicated vectors and incubated with either control-CM or Wnt3a-CM. (**d**) Relative CRT of HCT116 cell clones carrying either wild-type ($CTNNB1^{WT/-}$) or mutant β-catenin ($CTNNB1^{-/\Delta S45}$) alleles, transiently co-transfected with the indicated vectors and with the negative control CRT reporter Super8•FOP Flash and incubated with either control-CM or Wnt3a-CM (mean±SD from three replications). (**e**) Relative CRT of Huh-7 cells transiently transfected with the Super8•FOP Flash reporter and the indicated vectors. CRT reporter activities are expressed relative to activity in assays containing empty vector.

Supplemental Figure 3. V3Nter and SFRP-1 reduce proliferation of HCT116 cells without increased cell death. (**a**) Analysis of [^{3}H] thymidine incorporation in HCT116 cells stably expressing the indicated vectors. Cell cycle synchronized cells were stimulated with 10% FCS in a 48h time course. At the indicated time points, cells were pulsed with [^{3}H] thymidine and lyzed. Incorporated [^{3}H] thymidine is shown as mean±SD from three replications. (**b**) Proliferation of HCT116 cells stably expressing the indicated vectors was assessed by the MTT colorimetric assay measuring mitochondrial activity in living cells on a 96h time course and is shown as mean±SD from three replications. Note that no significant differences in cell survival are seen 24h after seeding. (**c**) Apoptotic rate in a 72h time course of HCT116 cells stably expressing the indicated vectors measured by quantification of the subG1 cell population by flow cytometry. Results show mean ± SD percent of apoptotic cells from triplicates of 1×10^4 cells at each time point.

Supplemental Figure 4. Colony formation assays in HCT116 cells stably expressing empty vector, V3Nter, SFRP-1 or V2Nter. Colony formation assays were performed on cells stably expressing the indicated vectors that contain the neomycin resistance gene. Cells were seeded at low density and incubated in G418 for 14 days. After fixing in paraformaldehyde and staining in hematoxylin, images from whole petri dishes were acquired and colonies counted blindly with Scion Image (NIH). (**a**) Percentages of colony formation efficiencies relative to Vector-HCT116 cells (mean±SD from three replications). The Student's *t* test was used to calculate *p*-values. (**b**) *p*-values (Student's *t* test) comparing the proportions of colonies in each category of colony size, as shown in Figure 4d.

Supplemental Figure 5. V3Nter overexpression inhibits the growth of HCT116 tumor xenografts *in vivo*. Athymic nude mice received subcutaneous injections of HCT116 cells and tumor volume was estimated in a 22-day time course. (**a**) *p*-values (Mann-Whitney U-test) comparing mean tumor volumes from mice receiving injections of Vector-, V3Nter-, SFRP-1- or V2Nter-HCT116 cells at each time point ("D", Day). Six mice per group received cells in both hind legs (n=12 tumors per group). (**b**) Analysis of tumor volume of mice injected with Vector-HCT116 cells or two different HCT116-V3Nter cell batches. For vector and batch #1, data were from 12 tumors from 6 mice; for batch #2, data were from 10 tumors from 5 mice (mean±SD). The Mann-Whitney U-test was used to compare mean tumor volumes between batch #2-V3Nter-HCT116 and Vector-HCT116 (*, $p<0.05$ and **, $p<0.005$).

Supplemental Figure 6. Immunostaining in tumor cryosections assessing V3Nter expression and cell proliferation *in vivo*. (**a**) Cryosections derived from Vector- or V3Nter-HCT116 tumors, as indicated, were stained with monoclonal mouse anti-V5 tag, followed by

peroxydase-conjugated goat anti-mouse antibodies to detect V3Nter (*brown*). Slides were counterstained with hematoxylin and images acquired with a Micro Imager M1 microscope (Zeiss) and Zeiss AxioVision. (**b** and **c**) Cell proliferation was assessed in cryosections derived from Vector- or V3Nter-HCT116 tumors by immunohistochemical staining with anti-Ki67. This marker is expressed by all proliferating cells during late G1, S, M and G2 phase of the cell cycle. Ki67 (+) cells (*green*) are shown relative to nuclear staining with the fluorescent dye DAPI (*blue*). We acquired images from whole mount tissue sections using a Micro Imager M1 microscope (Zeiss) and Zeiss AxioVision Software, and Ki67 signal was quantified with Simple PCI 6.1.2. Each result is the means±SD of 3 analyzed tumors.