Supplemental materials and methods

Cell culture, transfection and reporter assays

HCT116 human CRC cells were obtained from the European Collection of Cell Cultures (EACC) and cultured as described (Quelard *et al.*, 2008). Cells were transfected with the indicated cDNAs in pcDNA3.1 vectors using Lipofectamine Plus (Invitrogen), plated in 96-well plates and selected with 0.6 mg/ml G418 (Invitrogen). Antibiotic resistant cells were characterized by immunoblot. Allele-targeted HCT116 cells carrying only wild-type ($CTNNB1^{WT/-}$) or mutant β-catenin ($CTNNB1^{-/\Delta S45}$) were kindly provided by B. Vogelstein and cultured as described (Chan *et al.*, 2002). Mouse L and L-Wnt3a cells were purchased from the American Type Culture Collection (ATCC). Culture and collection of L and L-Wnt3a conditioned medium were performed as recommended by the vendor and by R. Nusse's lab at http://www.stanford.edu/~rnusse/wntwindow.html. β-catenin-T-Cell factor Regulated Transcription (CRT) was assessed as described (Quelard *et al.*, 2008). Briefly, the β-catenin-TCF responsive reporter driven by wild-type (Super8•TOP Flash) or the negative control with mutated TCF binding sites (Super8•FOP Flash), kindly provided by R. Moon, were transiently cotransfected with pGL4.70[*hRluc*] (Promega, France) expressing Renilla luciferase as a normalization standard. Luciferase activity was measured by the Dual Luciferase Reporter Assay (Promega). Where indicated, V3Nter, SFRP-1 (250 ng each), wild-type, S33Y or S37A β-catenins (10 ng each) were transiently transfected with Lipofectamine Plus (Invitrogen) and the amounts of cDNA were kept constant using empty vector. For co-culture assays, 24h after transfection with the luciferase reporter, HCT116 cells were co-cultured with HEK293 cells stably expressing FZC18 or empty vector in the presence of 50% Wnt3a-CM.

Cell Growth Assays

For MTT assay, cells were incubated with 0.5 mg/mL MTT (Sigma) in culture medium at 37°C for 2h and absorbance was read at 540 nm. For [^{3}H] thymidine incorporation, cells were synchronized in the G1 phase of the cell cycle by serum starvation for 72h, and pulsed with 1 µCi/ml of [^{3}H] thymidine (Amersham) at 37°C for 1h30. Incorporated [^{3}H] was quantified by liquid scintillation counting and normalized to total protein content using the DC protein assay kit (Bio-Rad). Colony formation assay and flow cytometry were done as described (Quelard *et al.*, 2008).

Immunological Methods

Anti-SFRP-1 (clone H-90), GAPDH (FL-335), β-catenin (E-5) and Hsc-70 (B-6) were from Santa Cruz (Tebu, France). Anti-mouse Wnt3a (MAB1324) was from R&D. Anti-DUF-959 (Saarela *et al.*, 1998) and FZC18 (Elamaa *et al.*, 2003) were kindly provided by Prof. T. Pihlajaniemi's team and used as previously described (Quelard *et al.*, 2008). Anti-V5 and anti-myc (Invitrogen) were used as previously described (Quelard *et al.*, 2008). Immunostainings were performed on tumor cryosections of 5 Vector-HCT116 and 5 V3Nter-HCT116 tumors. BrdU was detected using monoclonal anti-BrdU (clone BU-1; GE Healthcare). Detection of microvessels was done with monoclonal rat anti-CD31 antibody (Becton & Dickinson), followed by biotinylated goat anti-rat (Becton & Dickinson) and a Streptavidine-Texas Red conjugate (Amersham). The TUNEL (dUTP nick end labeling) assay was performed with the DeadEnd Fluorometric TUNEL System (Promega) according to the manufacturer. For Ki67 staining, tumor sections were incubated with rabbit monoclonal anti-Ki67 (Clone SP6, Interchim) and FITC-conjugated anti-rabbit IgG (Jackson).

Image Acquisition and Analysis

We used a fluorescence microscope (Axio Imager M1 and Colibri LED system, Zeiss) and AxioVision software (Zeiss). Automatic image acquisition from whole mount tumor sections was done by tiling of adjacent fields of view, after excluding necrotic regions. Quantification of microvessel density (% of labeled area using anti-CD31) and of apoptotic cells (numbers of positive cells per mm^2, using the TUNEL assay) was performed by automated image analysis (Simple PCI 6.1.2 software, Compix Inc.). Quantification of proliferating cells (*in vivo* BrdU uptake) was done in well-perfused areas (positive for carbocyanine *in vivo* staining) by counting BrdU-positive cells using Image J (NIH).

In vivo Magnetic Resonance Imaging (MRI)

MRI was carried out on a 4.7-Tesla horizontal Biospec 47/40 imaging system (Bruker Biospin, Wissembourg, France). Mice were placed in a supine position and held in a contention system equipped with a face mask for anesthesia with 1-3% isoflurane in air. Breathing was monitored with a probe. T2-weighted images were obtained using a rapid acquisition with relaxation enhancement method (Hennig *et al.*, 1986), where repetition time = 3500 ms; effective echo time = 36 ms; relaxation enhancement factor = 8; field of view = 3.2 x 3.2 cm; matrix = 128 x 128; 29 sagittal contiguous slices of 500 µm, 4 averages. T1-weighted images were acquired using a 2D Fast Low Angle SHot sequence (Frahm *et al.*, 1986), where repetition time = 500 ms; echo time = 3.4 ms; flip angle = 40; field of view = 3.2 x 3.2 cm; matrix 160 x 160; 29 sagittal contiguous slices of 500 µm, 8 averages.

Statistical analysis

The Student's t or the Mann-Whitney's "U" tests were used, as indicated (Statistica 8, StatSoft 2008).

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