Supporting Information

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SI Materials and Methods

Cell Culture, Transfection, and Retroviral Infection. Cell lines were obtained from the American Type Culture Collection. DLD-1 and T-47D cells were cultured in RPMI1640. HEK293 cells were cultured in minimum essential medium and HCT116 cells were cultured in McCoy's 5A medium. Media were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen). Transfections were performed with either Lipofectamine (shRNA) or Oligofectamine (siRNA) according to the manufacturer's suggestions. Unless stated otherwise, cells were seeded at $\approx 2 \times 10^5$ per well in a six-well dish and transfected with 2 μg of the indicated construct or 100 nM siRNA for 24 h (DLD-1) or 72 h (T-47D). Plasmids encoding shRNA used the pSUPERretro system, where the shRNA sequences for SIRT11 and SIRT12 correspond to nucleotide positions 589 or 1091 in NM_012238. SMART pool siRNA against SIRT1 and SIRT2 were obtained from Dharmacon. Cambinol and MG132 were obtained from Sigma and lactacystin from Cayman Chemical.

Western Blots and Immunoprecipitations. Antibodies included β-actin (sc-47778; Santa Cruz Biotechnology, Inc.) 1:20,000, SIRT1 (sc-19857; Santa Cruz Biotechnology, Inc. and DB083; Delta Biolabs), Dvl-1 (sc-8025; Santa Cruz Biotechnology, Inc.), Dvl-2 (3216 and 3224; Cell Signaling Technology), Dvl-3 (sc-8027; Santa Cruz Biotechnology, Inc. and 3218), and SIRT2 (2313; Cell Signaling Technology). Membranes were probed with horseradish peroxidase (HRP) -conjugated secondary antibodies for 1 h. Membranes were probed with HRP-conjugated secondary antibodies for 1 h. Membranes were washed with Tris-buffered saline Tween-20 and deionized water before visualization by enhanced chemiluminescence. For immunoprecipitation, cells were harvested in MPER lysis buffer (Pierce) supplemented with 1× Complete protease mixture (Roche). The appropriate antibodies described above were used for immunoprecipitation overnight at 4 °C followed by a 2-h incubation with Protein A/G agarose beads (Santa Cruz Biotechnology). Complexes were then washed four times in MPER lysis buffer and subjected to Western blot analysis.

Endpoint and Real-Time Quantitative PCR. Intron-spanning primers specific for each of the targets were designed and human BMP-4, cMyc, cyclin-D1, and β-actin gene expression was measured by endpoint using JumpStart RedTaq (Sigma) and quantitative real-time (RT) PCR using Fermentas Maxima SYBR Green, and fluorescence was detected on an ABI PRISM 7900 sequence detector (Applied Biosystems). Total RNA was isolated using TRIzol (Invitrogen), and 2 μg RNA was reverse-transcribed with M-MLV Reverse Transcriptase (Promega). For quantitative PCR, an ABI PRISM 7900 sequence detector was used with an initial denaturation at 95 °C for 15 min, followed by 40 cycles, each cycle consisting of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 1 min. The following primers were used: human *BMP-4*: 5′-TGCCCT GGATTGTTGACATGA-3′ (sense) and 5′-TTTGTCAGGCT-GGGGGTAGG-3′ (antisense); cMyc: 5′-TTGAGGAGTGCGA-GGTGCTG-3′ (sense) and 5′-ATCTGGGCCAGCAGAAGTGC-3′ (antisense); cyclin-D1: 5′-AGGCGAGGGCGATCTTGACAG-3′ (sense) and 5′-GATGCGGATGGCCACCTCTTT-3′ (antisense). Dvl-1: ttatctaccacatggacgagga (sense) and caaagatctcctccttcaccac (antisense); Dvl-2: cgtcacagattccacaatgtct (sense) and tcgttgct-catgttctcaaagt (antisense); Dvl-3: catgtcactcaacatcatcacg (sense) and acaacatatctcctggctcgat (antisense). SYBR Green fluorescence emissions were monitored after each cycle. mRNA quantities were normalized against each group's control sample.

Migration and Cell-Viability Assays. HCT116 cells (5×10^5) were suspended in serum-free McCoy's 5A media, and from this suspension 100 μl was placed on the upper chamber already containing 200 μl of serum-free McCoy's 5A media; 260 μg/mL + 130 μM cambinol or vehicle control (dimethyl sulfoxide; DMSO) were placed in the lower chamber. HCT116, HT-29, and T-47D cell migration occurred for 8 h, 24 h, and 24 h, respectively. HT-29 media were supplemented with 2% FBS and allowed to migrate for 24 h, 2×10^5 cells were suspended, and 100 μL seeded from this suspension. T-47D media were supplemented with 2% FBS, 5×10^5 cells were suspended, and 100 μL seeded from this suspension. After the incubation period, cells were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet. Nonmigrant cells were gently swabbed off the top insert and discarded. Each condition was performed in duplicate and representative fields were taken of each duplicate, counted, and imaged on an (10×) Olympus DP 70 (680 × 512) field.

Cell viability was measured using Cell Titer Blue Viability Assay obtained from Promega (G8080) and performed according to the manufacturer's instructions. Concentrations of cambinol, Wnt3a, and vehicle corresponded to those of the migration assay. Fluorescence was measured using a BioTek Synergy HT.

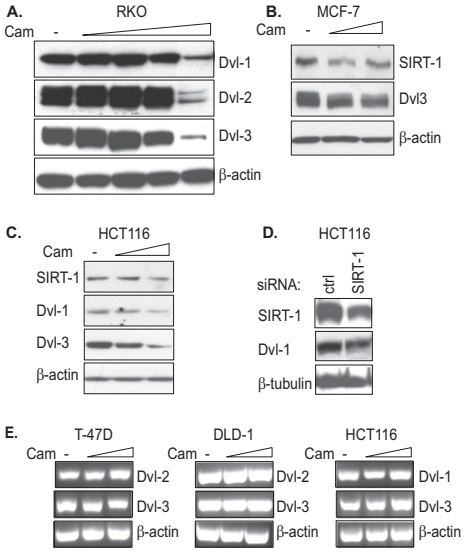

Fig. S1. (*A*) RKO, (*B*) MCF-7, and (*C*) HCT116 cells were treated with vehicle control (DMSO) or 50 μM or 100 μM cambinol (SIRT1 inhibitor) for 24 h and the indicated proteins were analyzed by Western blot. (*C*) HCT116 cells were treated with vehicle control (DMSO) or 50 μM or 100 μM cambinol (SIRT1 inhibitor) for 24 h and the indicated proteins were analyzed by Western blot. (*D*) Control or SIRT1 siRNAs were transfected into HCT116 cells for 42 h and Dvl-1 was analyzed by Western blot. (*E*) T-47D, DLD-1, and HCT116 cells were treated with either vehicle control (DMSO) or 50 μM or 100 μM cambinol (SIRT1 inhibitor) for 18 h (T-47D) or 24 h (DLD-1 and HCT116), and total RNA was isolated and semiquantitative RT-PCR analysis of Dvl levels and β-actin was performed using intron-spanning primers.

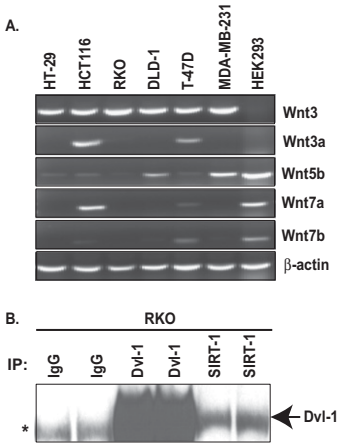

Fig. S2. (*A*) Total RNA was isolated from the indicated cell lines and RT-PCR analysis of the indicated Wnt genes and β-actin was performed using intron-spanning primers. (*B*) Interaction between endogenous SIRT1 and Dvl-1 in RKO cells was performed by immunoprecipitation of Dvl-1 or SIRT1 followed by blots for Dvl-1.

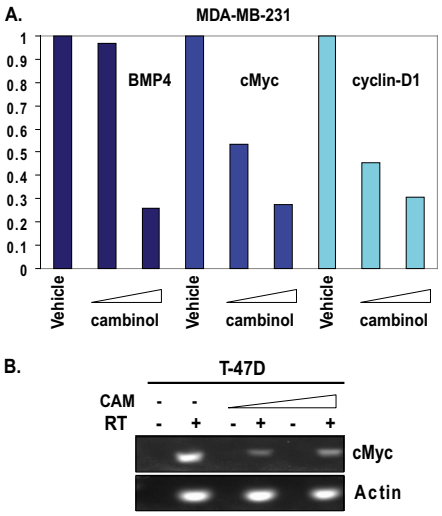

Fig. S3. (*A*) MDA-MB-231 cells were treated with either vehicle control (DMSO) or 50 μM or 100 μM cambinol (SIRT1 inhibitor) for 24 h, and total RNA was isolated and quantitative RT-PCR analysis of Wnt target genes (BMP4, cMyc, and cyclin D1) was performed using intron-spanning primers. (*B*) Total RNA was isolated from T-47D cells and semiquantitative analysis of the c-Myc gene was performed using intron-spanning primers. For each amplification, either the reverse transcriptase was excluded (–) or included (+) in the reverse-transcription reaction, and β-actin was included as a control.

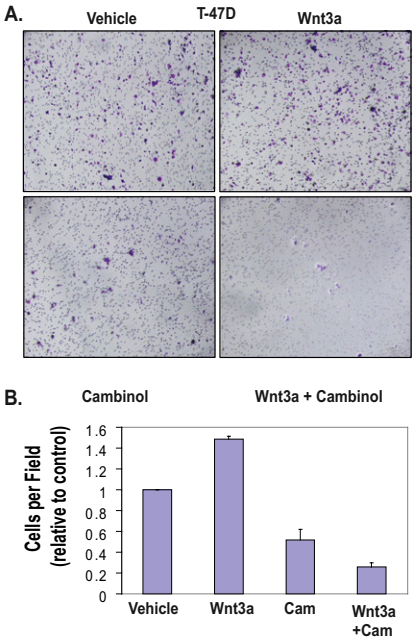

Fig. S4. (*A*) T-47D cells were analyzed in a transwell migration assay in the absence or presence of purified Wnt3a ligand (200 μg/mL) ± cambinol (50 μM). (*B*) T-47D cells were manually counted in a (680 × 512) still image field with an Olympus DP 70 microscope. One assay was counted with duplicates per condition.

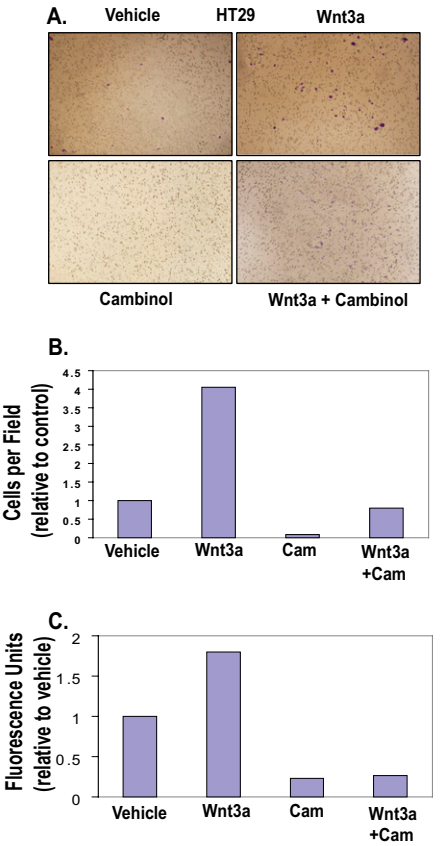

Fig. S5. (*A*) HT-29 cells were analyzed in a transwell migration assay in the absence or presence of purified ligand (260 ng/mL) ± cambinol (130 μM). (*B*) HT-29 cells were manually counted in a (680 × 512) still image field with an Olympus DP 70 microscope. One assay was counted with duplicates per condition. (*C*) A Cell Titer Blue Viability assay was performed to test the viability of cells when treated with Wnt3a, cambinol, or vehicle (DMSO) in HT-29 cells.