

Figure S1 related to Figure 1: Expression of RIP140 in mouse models. A) RT-qPCR analysis of RIP140 expression in mouse tissues. The results are expressed in arbitrary units (AU) after normalization to RS9 mRNA levels. Values are the means \pm S.D.; $n=6$ mice for each genotype. **B)** RIP140 expression in paraffin-embedded sections of small intestine from RIPKO, WT and RIPTg mice. Images show merged RIP140 detection (green) and nuclear staining (blue). Original magnification $\times 20$. **C)** RT-qPCR analysis of mouse *Rip140* gene expression together with the human *RIP140* and *LacZ* transgene expression in small intestine from RIPKO, WT and RIPTg mice. The results are expressed in arbitrary units (AU) after normalization to RS9 mRNA levels. Values are the means \pm S.D.; $n=6$ mice for each genotype. **D)** Small intestines of RIPKO, WT and RIPTg mice were collected. The small intestine length was measured and their total area calculated. Quantification data were represented as means \pm S.D.; $n=6$ mice for each genotype. A Mann-Whitney test was used for statistical analysis (* $p < 0.05$ and *** $p < 0.001$).

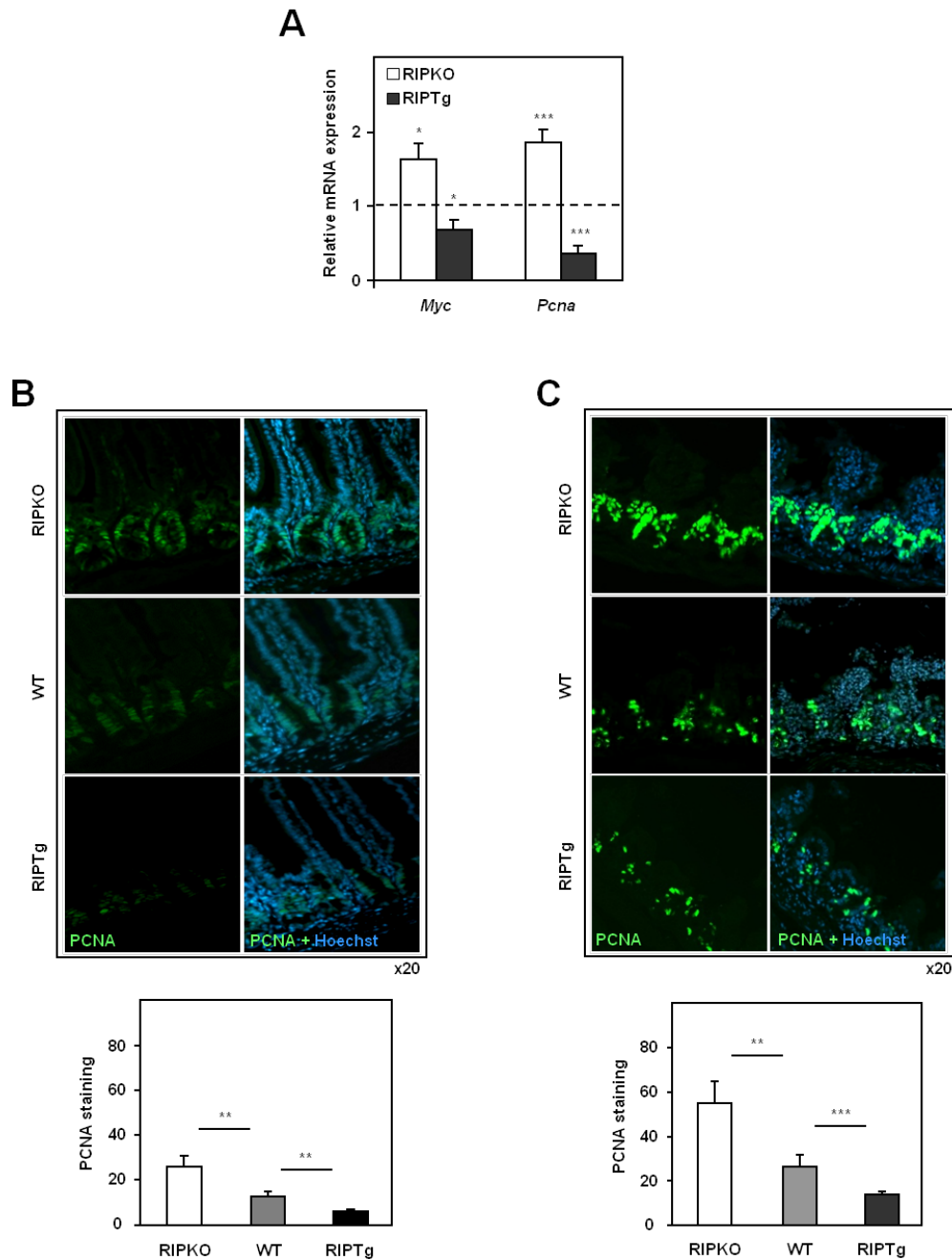


Figure S2 related to Figure 2: RIP140 inhibits the expression of proliferation markers. A) RT-qPCR analysis of *Myc* and *Pcna* expression in small intestine of RIPKO, WT and RIPTg mice. Values represent fold change \pm S.D., after normalization to RS9 mRNA and wild-type mice; n=6 mice for each genotype. **B)** PCNA staining in paraffin-embedded sections of small intestine from non-irradiated RIPKO, WT and RIPTg mice. Images show merged PCNA detection (green) and nuclear staining (blue) in small intestine. PCNA staining was quantified in sections from RIPKO, WT and RIPTg mice. Values are the means \pm S.D. and are expressed in arbitrary units; n=6 mice for each genotype. **C)** Same as in panel B, 2.5 days after a 12 Gy irradiation. A Mann-Whitney test was used for statistical analysis (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

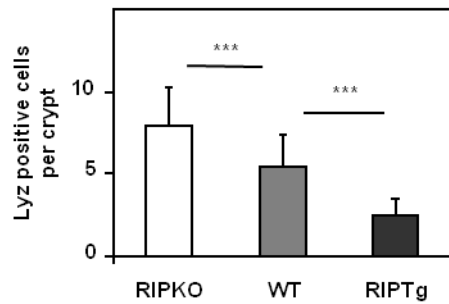
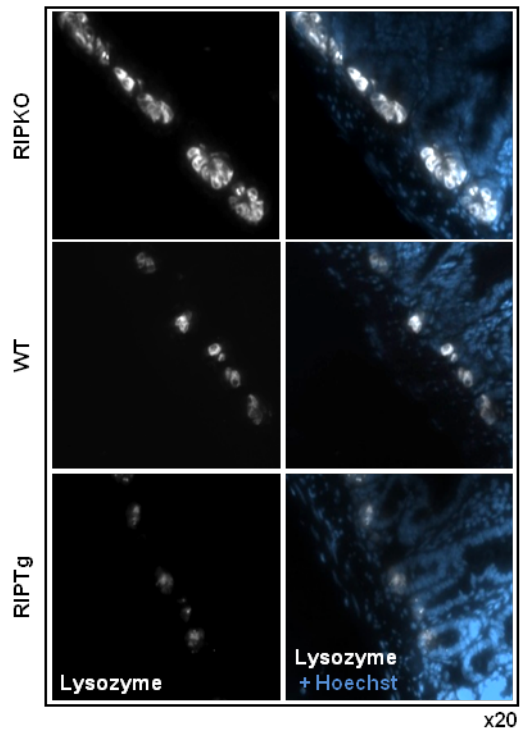


Figure S3 related to Figure 3: RIP140 inhibits Paneth cell differentiation in mouse intestinal epithelium. Lysozyme (Lyz) staining was performed in paraffin-embedded sections of small intestine from RIPKO, WT and RIPTg mice in order to detect Paneth cells. Images show merged Lysozyme detection (white) and nuclear staining (blue). Quantifications correspond to the number of Lyz positive cells per crypt (mean \pm S.D.), n=6 mice for each genotype. A Mann-Whitney test was used for statistical analysis (***) p<0.001).

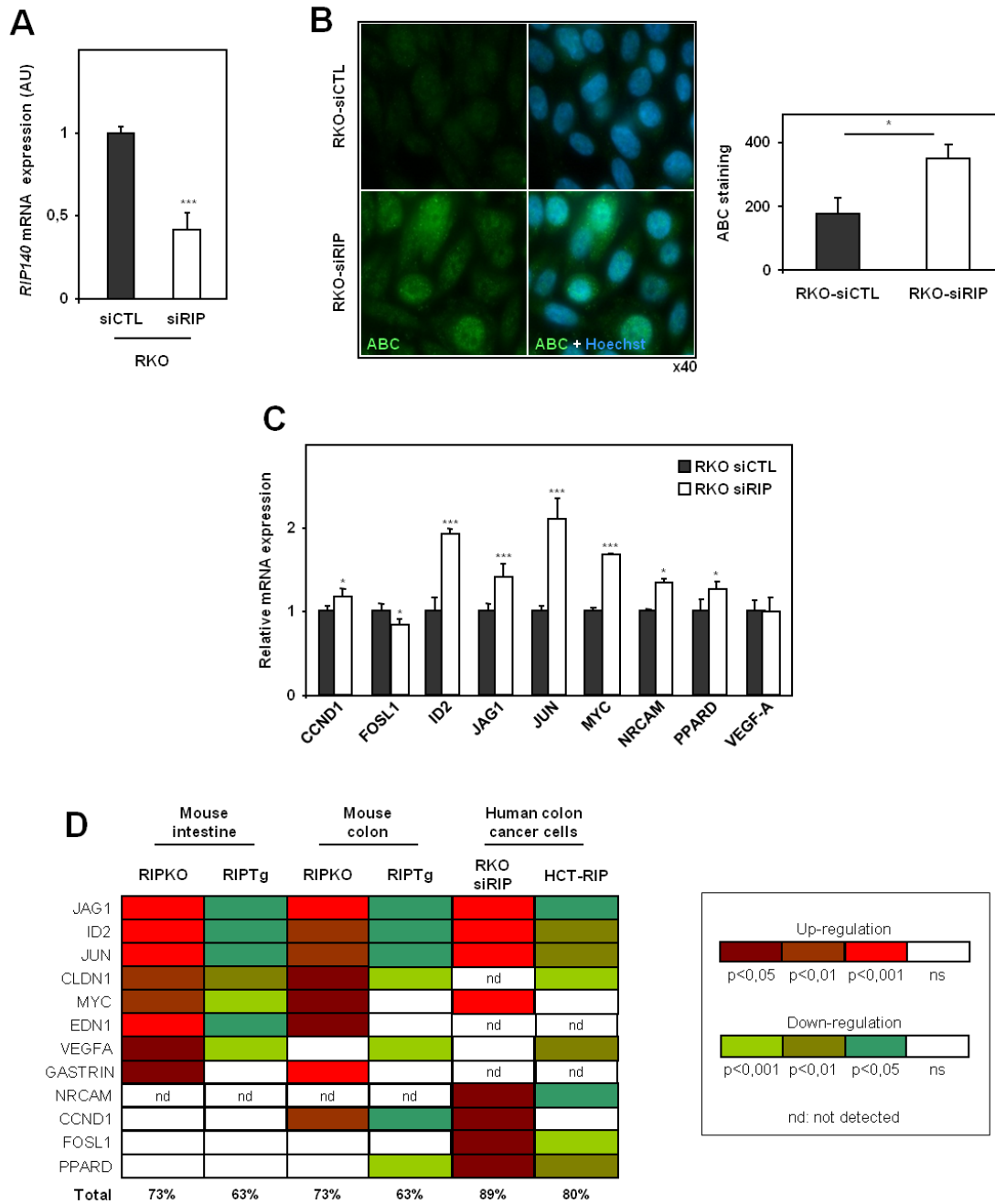


Figure S4 related to Figure 5: RIP140 controls β -catenin activation in human colon cancer cells. **A)** RIP140 mRNA quantification in RKO colon cancer cells with (RKO-siRIP) or without (RKO-siCTL) siRNA-mediated knock-down of RIP140 expression. **B)** Active β -catenin labeling in RKO-siCTL and RKO-siRIP cells. Images represented merged active β -catenin detection (green) and nuclear staining (blue). Quantification represent means \pm S.D.; n=3 independent experiments. **C)** Expression of β -catenin target genes in RKO-siCTL and RKO-siRIP cells. Values represent fold change \pm S.D. as compared to RKO-siCTL cells, after normalization to 28S mRNA; n=3 independent experiments. **D)** Regulation of Wnt target gene expression by RIP140 in mouse tissues and human cell lines. The expression of the different Wnt target genes was quantified in mouse small intestine and colon tissues of RIPKO, wild-type and RIPtg mice as well in human colorectal cancer cells (HCT116 overexpressing or not RIP140 and RKO cells with or without RIP140 knock-down). The color code indicates whether the genes were up-regulated (red) or down-

regulated (green) in the transgenic mice as compared to wild-type animals or in transfected cells as compared to the respective controls. A Mann-Whitney test was used for statistical analysis: $p < 0.05$, $p < 0.01$ and $p < 0.001$ corresponding to a given color intensity. In each column, the percentage of genes (among the 12 genes tested) which appeared significantly regulated by RIP140 is indicated.

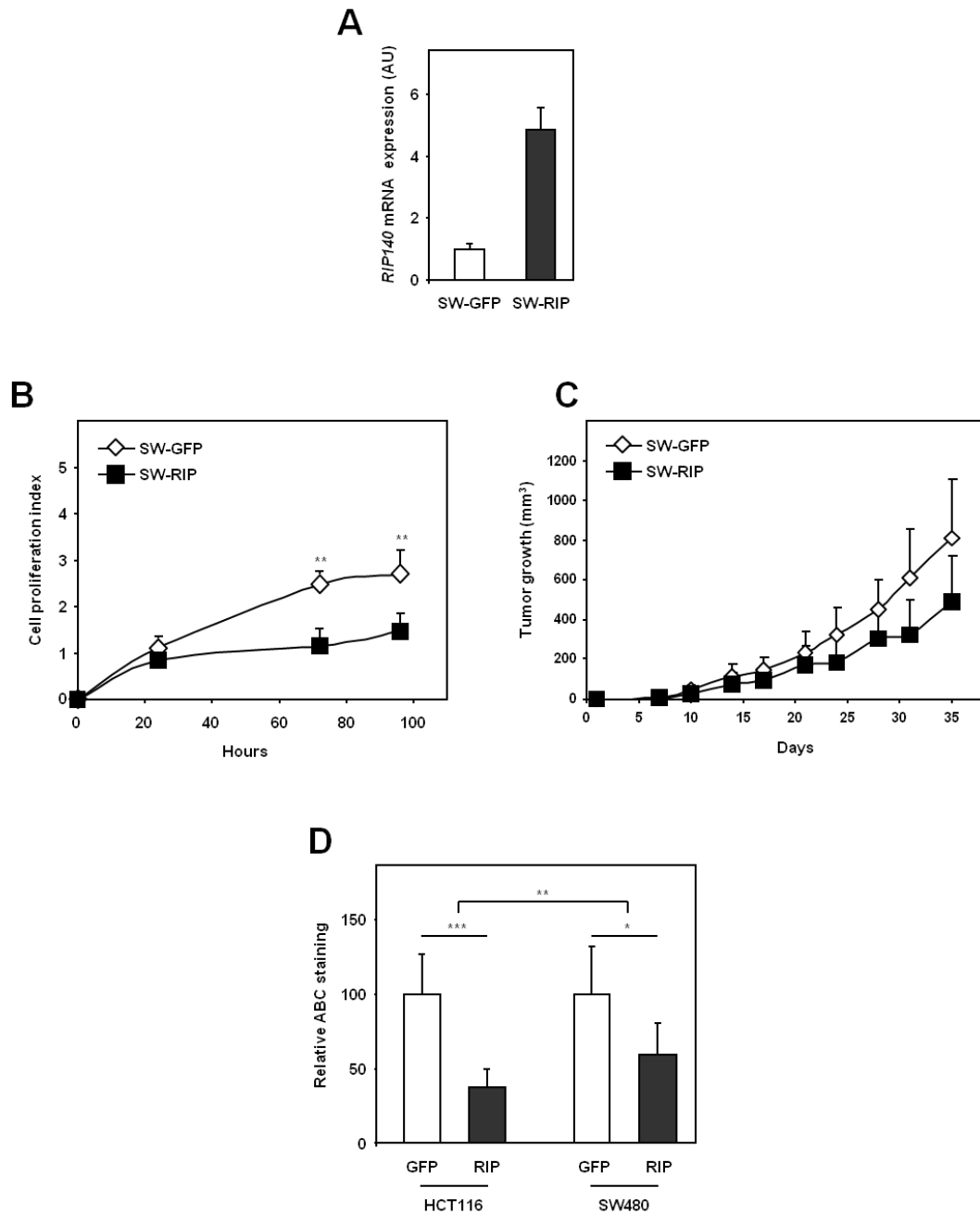
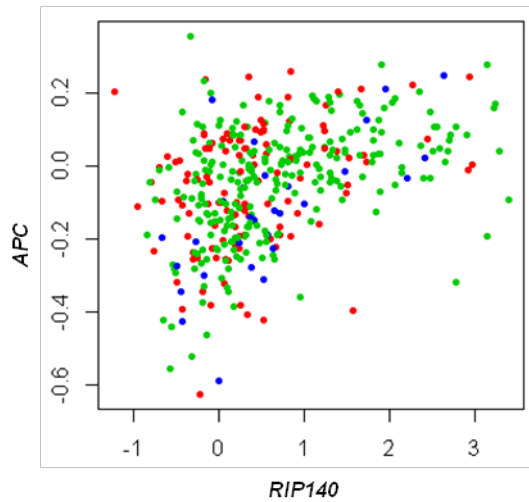


Figure S5 related to Figure 6: Effect of RIP140 overexpression in SW480 human colon cancer cells. A) RT-qPCR analysis of SW480 cells overexpressing (SW-RIP) or not (SW-GFP) RIP140. Data are expressed in arbitrary units (AU) as means \pm S.D. after normalization by RS9 mRNA levels; n=5 independent experiments. **B)** Cell index corresponding to the number of SW-GFP and SW-RIP viable cells were monitored using the XCELLigence system during 72 hours. Values are means \pm S.D., n=3 independent experiments. **C)** Volumes of SW-GFP and SW-RIP cell xenografts were measured at different times after grafting and expressed as means \pm S.D.; n=12 xenografts per cell line. **D)** Effects of transient overexpression of GFP or GFP-RIP140 on active β -catenin staining in HCT116 and SW480 cells. A Mann-Whitney test was used for statistical analysis (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).



Red : unknown Microsatellite status
 Green : Microsatellite Stable
 Blue : Microsatellite Instable

	rho	p-value
All samples (n=396)	0,412	<2,2E-16
MSS samples (n=247)	0,4387	4,85E-13
MSI samples (n=29)	0,6589	1,02E-04

Figure S6 related to Figure 7: Correlation between *RIP140* and *APC* gene expression in colorectal adenocarcinomas. *RIP140* and *APC* mRNA were quantified in 396 human colon cancer biopsies (Salazar *et al*, JCO, 2011, 29:17-24) containing 247 microsatellite stable (MSS - green dots) and 29 microsatellite instable (MSI - blue dots) samples. Tumors with unknown status are represented by red dots. Statistical significance was assessed using a Spearman correlation analysis. Values of the corresponding rho coefficients and p-values are indicated in the table below for the whole cohort and for the MSS and MSI samples.

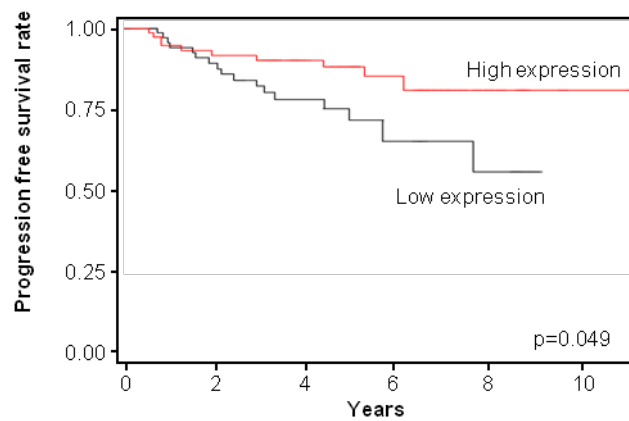
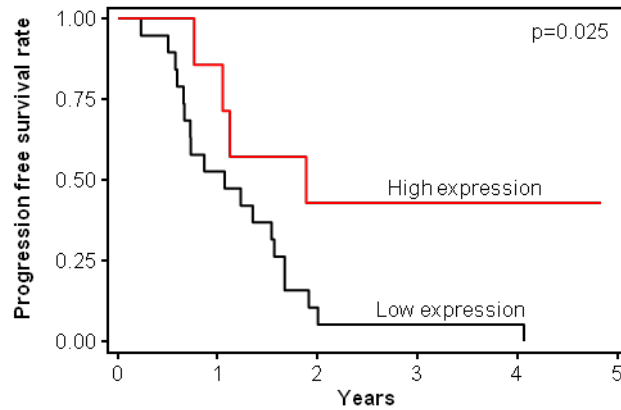
A**B**

Figure S7 related to Figure 8: RIP140 expression is a prognosis marker in colon cancer. RIP140 mRNA was quantified in **A)** 396 tumors human colon cancer biopsies (Salazar *et al*, JCO, 2011, 29:17-24) and in **B)** 22 colorectal adenocarcinomas (Del Rio *et al*, JCO, 2007, 25:773-780). Patients were ranked according to *RIP140* gene expression in their tumors and divided into two groups exhibiting low and high *RIP140* expression, respectively. A Kaplan-Meier plot of the cumulative progression-free survival of patients with low or high *RIP140* gene expression was performed. A log-rank test was used for statistical analysis.

Table S1: primer sequences

Gene Symbol	Forward Sequence	Reverse Sequence
mApc	TGAGTGCCTTATGGAACCTGT	CTCCGGTAAGTGAGGGTGC
mAxin2	CCAGGAAAGTCCGGAAGAGGTATG	GAGTAGCGCCGTGTTAGTGACT
mCldn1	AGCACCGGGCAGATACAGT	ATGCCAATTACCATCAAGGC
mCsnk1E	GAGCTGCGTGTGGGAAATAAG	ACATTCGAGCTTGATGGCTACT
mGsk3β	TCCATTCTTTGGAATCTGC	CAATTCAGCCAACACACACAGC
mLacZ	CCGGTCGCTACCATTACCAG	CCGATTGTCTGTTGTGCC
mLyz	CATGGCGAGCACACTGTCA	GCGGTCAGACTCCGCAGTT
mMuc2	CGACACCAGGGATTTGCTTAAT	CACTTCCACCCTCCCGGCAAAC
mMyc	GCACAAGCTCACCTCTGAAAAGGAC	CTCACGAGAGATTCCAGCTCCTCC
mPcna	GGTCTCGGCATATACGTGCAA	AGCAACTTGGAATCCCAGAACA
mRip140	AGAACGCACATCAGGTGGCA	GATGGCCAGACACCCCTTTG
mhEDN1	TCCCGTGATCTTCTCTCTGC	ACTCCATTCTCAGCTCCGGT
mhJAG1	GCTTCGGCTCAGGGTCTAC	GGCGAAACTGAAAGGCAGTA
mhJUN	GAAAAGTAGCCCCAACCTC	GGGACACAGCTTTCACCCTA
mhPP2A	CCACAGCAAGTCACACATTGG	CAGAGCACTTGATCGCCTACAA
hAPC	CCTCATCCAGCTTTTACATGGC	CGCCTGCCTCTCTTGTCAT
hAXIN2	GTGATGGAGGAAAATGCCTACC	GTCCCCCATTACTCATGTAAGC
hCLDN1	TTGACTCCTTGCTGAATCTGA	TTCTGCACCTCATCGTCTTC
hCSNK1E	GCTGAGGGCGACTACAACG	GCACCGTCTTGAGGCTGAAT
hGSK3β	GGTCTATCTTAATCTGGTGCTGG	TGGATATAGGCTAACTTCGGAAC
hRIP140	AATGTGCACTTGAGCCATGATG	TCGGACACTGGTAAGGCAGG
pAPC	TAGGGCTAGGCAGGCTGTG	CTGCACCAATACAGCCACAT

Supplemental Experimental Procedures.

Real-time quantitative PCR. RT-qPCR were performed with a LightCycler® 480 SYBR Green I Master (Roche Applied Science) and were carried out in a final volume of 10µl using 0.25µl of each primer (25µM), 5µl of the supplied enzyme mix, 2.5µl of H₂O and 2µl of the template diluted at 1:10. After pre-incubation at 95°C, runs corresponded to 35 cycles of 15s each at 95°C, 5s at 60°C and 15s at 72°C. Melting curves of the PCR products were analysed using the LightCycler® software system to exclude amplification of unspecific products. Results were normalized to RS9 or 28S housekeeping gene transcripts.

Cell culture and transfections. RKO cells were transiently transfected with small interfering RNA against RIP140 (Dharmacon). SW480 human colon adenocarcinoma cells were stably transfected with the empty pEGFP vector (Clontech) or with the same vector containing the full-length human RIP140 cDNA (25). Pools of G418 resistant cells (respectively named SW-GFP and SW-RIP) were selected. Both RKO and SW480 cells were grown in RPMI medium supplemented with 10% FCS, 100U/ml penicillin, 100mg/ml streptomycin and 100mg/ml sodium pyruvate. All transfections were carried out using Lipofectamine2000 (Invitrogen) as recommended by the manufacturer.

Immunofluorescence (IF) analysis. Mouse tissues were fixed with 4% paraformaldehyde, embedded in paraffin and sectioned (5µm). For IF analyses, following incubation in citrate buffer solution, paraffin-embedded tissue sections were incubated with blocking serum for 3h to reduce non-specific binding. Sections were then incubated with antibodies specific for RIP140 (Ab42126, Abcam), PCNA (sc-56, Santa Cruz) and Lysozyme (A0099, DAKO Cytomation). RKO cells were fixed with 4% paraformaldehyde and incubated with active β-catenin antibody (05-665, clone 8E7, Millipore). IF revelation was performed using an Alexa-conjugated secondary

antibody (Invitrogen). After washing, sections were counterstained with Hoechst (Sigma Aldrich) and mounted for fluorescence microscopy. Negative controls using rabbit or mouse IgGs were performed and no staining was observed in these conditions. Quantification of staining was realized by the Axiovision software.

Cell proliferation assay. SW480 cells, overexpressing or not RIP140, were seeded at a density of 3000 cells/well into three E-Plate 16 (ACEA Biosciences, Inc., San Diego, CA) containing 150 μ l medium per well, supplemented with 10% FCS. Cells were monitored every 24 hours at 37 °C in a 5% CO₂ atmosphere, during 4 days. Dynamic monitoring of the growth inhibition pattern was determined using the impedance-based xCELLigence system (Roche Applied Science, Germany). The cell index was derived from measured cell-electrode impedance that correlates with the number of viable cells.

Tumorigenicity assay. 6 week-old nu/nu female mice from Harlan Laboratories, housed in a pathogen-free facility, were used to assess the effect of RIP140 on tumor growth. SW-GFP and SW-RIP cells (10⁶ cells) were injected subcutaneously and tumor formation was monitored every 3-4 days using a caliper. Tumor volume was calculated as width x length x thickness. Mice were sacrificed 36 days after injection.

DNA microarray analysis. Published DNA microarray data (Salazar *et al*, JCO, 2011, 29:17-24) (Del Rio *et al*, JCO, 2007, 25:773-780) were reanalyzed for *RIP140* and *APC* expression.

Statistical analysis. Statistical comparisons were performed with Mann-Whitney or Spearman tests. Differences were considered statistically significant at p<0.05.