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Supplementary Materials for

Differential abundance of CK1α provides selectivity for pharmacological CK1α activators to target WNT-dependent tumors

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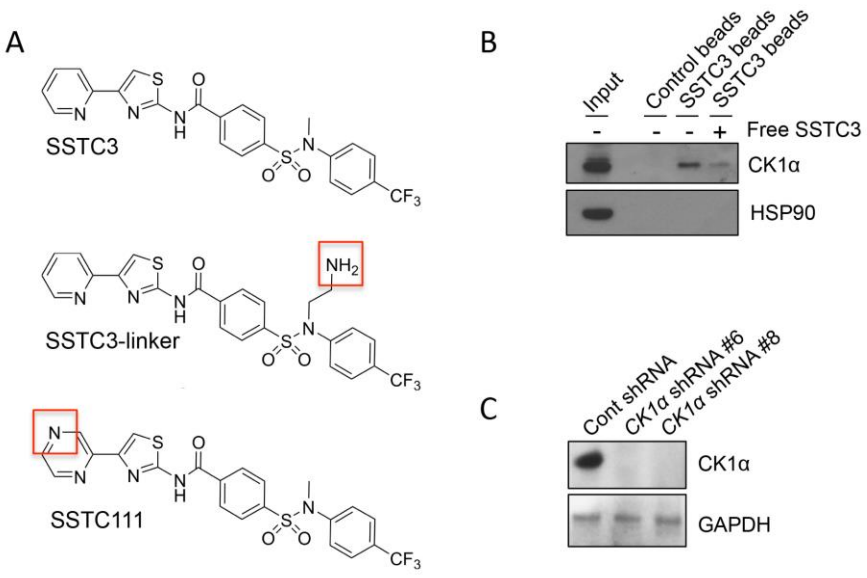

Fig. S1. SSTC3 inhibits WNT signaling via CK1α. (A) Structural formula of SSTC3, its derivative with a free amine (SSTC3 linker) and an inactive analog (SSTC111). **(B)** SSTC3 binds to CK1α in a competitive manner. SSTC3 coupled agarose beads were used to isolate endogenous CK1α from 293T cell lysates in the presence or absence of free SSTC3, followed by analysis of the indicated proteins by immunoblotting. **(C)** CK1α abundance was determined in HCT116 cells expressing control or *CK1α* specific shRNA by immunoblotting. Data are representative of 3 experiments.

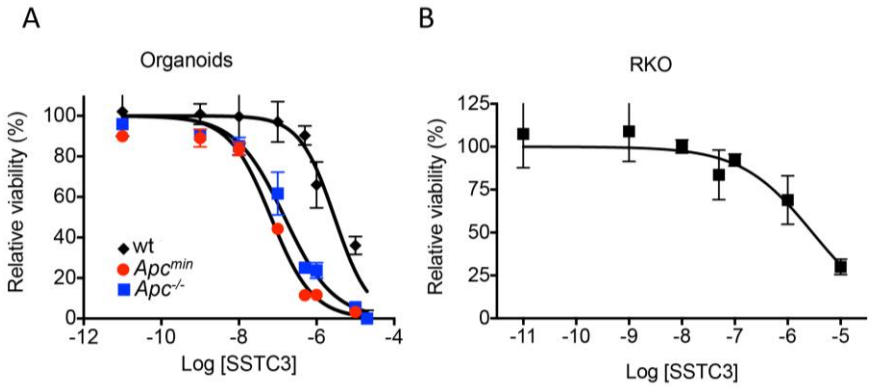

Fig. S2. SSTC3 attenuates the viability of cells dependent on WNT activity. (A) *Apc* mutant organoids are more sensitive to SSTC3 than wild-type (wt) intestinal organoids. The indicated organoids, derived from wt mouse intestines or *Apc* mutant tumors, were treated with the indicated doses of SSTC3 for 4 days and cell viability determined (n= 4 separate cultures). **(B)** The ability of SSTC3 to reduce the viability of non-WNT dependent CRC cells is significantly reduced. RKO cells were treated with the indicated concentrations of SSTC3 for 5 days and viability determined (n= 3 experiments).

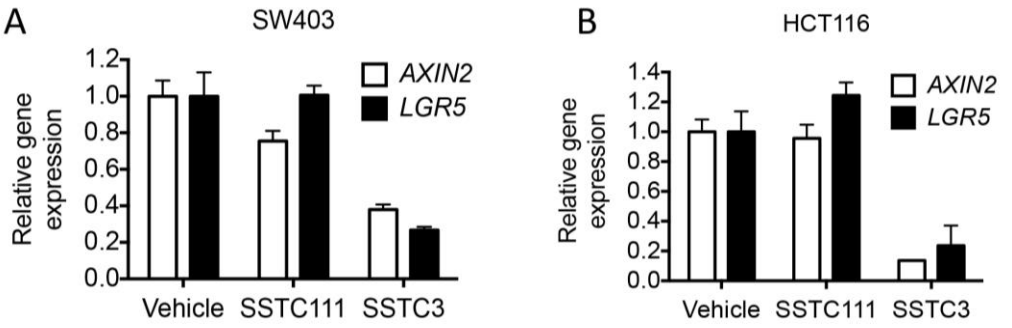

Fig. S3. A structural analog of SSTC3, SSTC111, does not inhibit WNT biomarkers in CRC cells. (A and B) Quantification of *AXIN2* or *LGR5* expression, determined by qRT-PCR, in SW403 (A) or HCT116 (B) cells treated with 2 μM of SSTC3 or SSTC111 for 48 hours. Representative data (mean ± S.D.) from at least two independent experiments are shown.

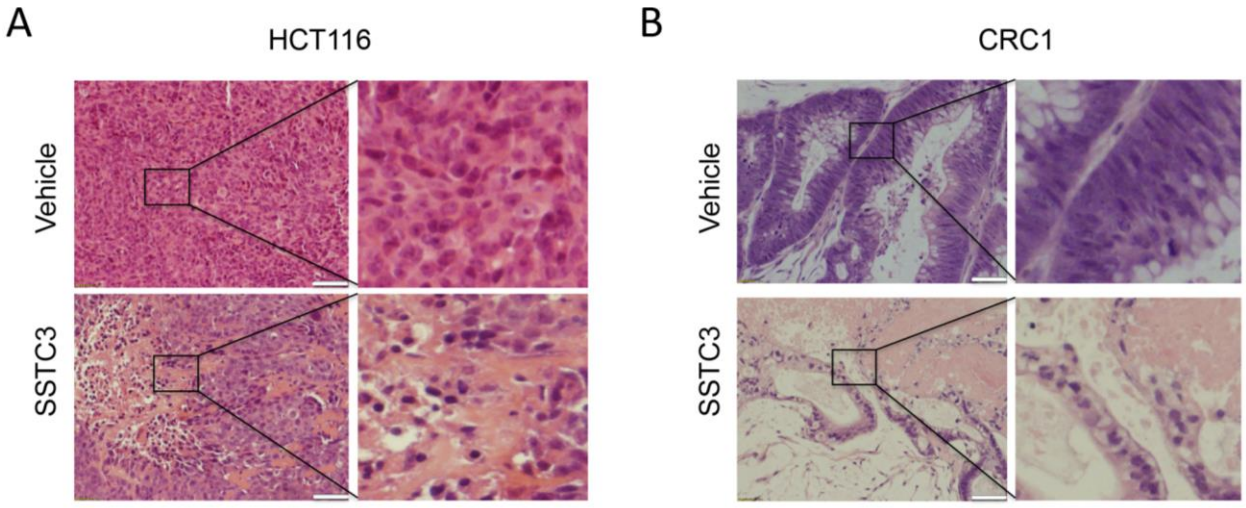

Fig. S4. SSTC3 treatment decreases tumor cell density in CRC xenografts. (A and B) Higher resolution H&E staining images for Fig. 4B and Fig. 4F, respectively.

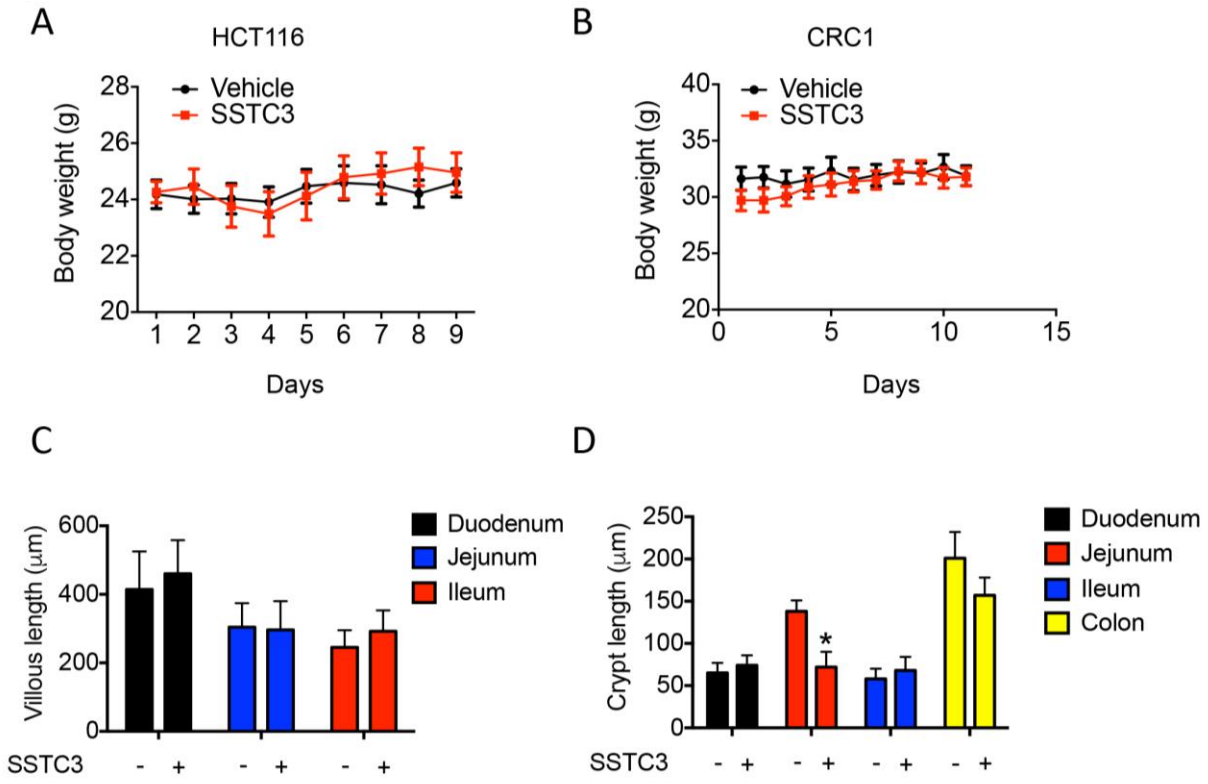

Fig. S5. SSTC3 has limited on-target GI toxicity in mice. (A and B) Body weight data for experiments in Fig. 4B and Fig. 4F respectively. **(C and D)** The intestines of the indicated drug treated mice (n= 4 in each group) were harvested, fixed and subjected to analysis for villous (C) and crypt (D) length. n= 10 random fields; * p≤ 0.05.

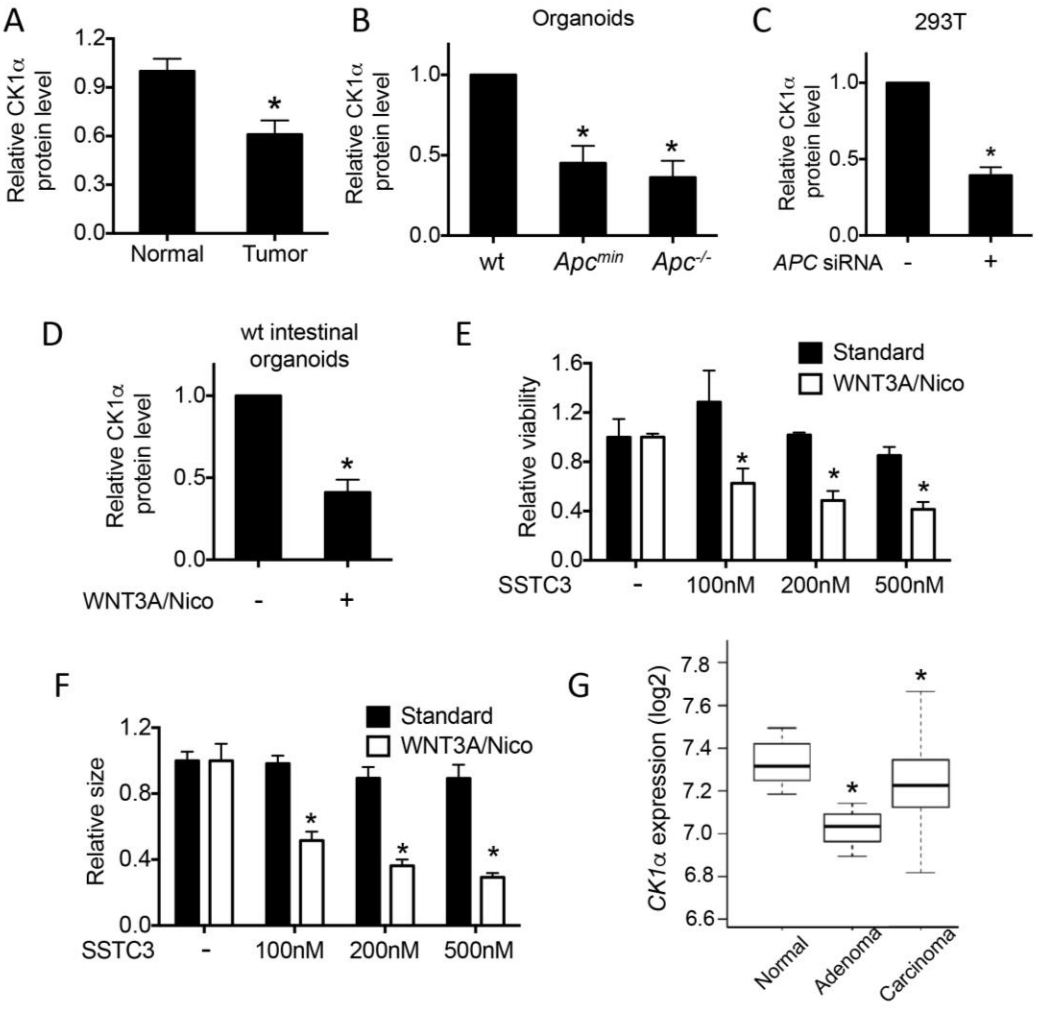

Fig. S6. Decreased abundance of CK1α sensitizes cells to SSTC3. (A and B) *Apc* mutant tumors have decreased amounts of CK1α. Quantitative data for Fig. 6A (n= 3 in normal and 4 in tumor) and Fig 6B (n= 3) are shown. **(C)** Loss of *APC* results in decreased levels of CK1α. Quantification from three independent experiments represented in Fig. 6C is shown. **(D)** Hyperactivated WNT activity decreases CK1α abundance in wild-type (wt) intestinal organoids. Organoids were treated with regular niche factors, or supplemented with WNT3A and 1 mM nicotinamide (Nico) for a week to activate WNT signaling. CK1α abundance was determined by immunoblotting and quantitated (represented in Fig. 6D; n= 3). **(E and F)** WNT hyperactivated intestinal organoids are sensitized to SSTC3. Size of intestinal organoids grown in the indicated medium and treated with increasing amounts of SSTC3 for 4 days (E; n= 4 independent replicates) or viability (F; n= 3 independent replicates). **(G)** *CK1α* expression is decreased in CRC samples. Gene expression data were downloaded from the Gene Expression Omnibus (GSE17538) and normalized using a Robust Multi-array Average (RMA). Shown is a box and whisker plot of log2 transformed *CK1α* expression values for normal colon tissue (n= 10), adenomas (n= 6) or carcinomas (n= 250). * p≤ 0.05, Wilcox rank sum test.