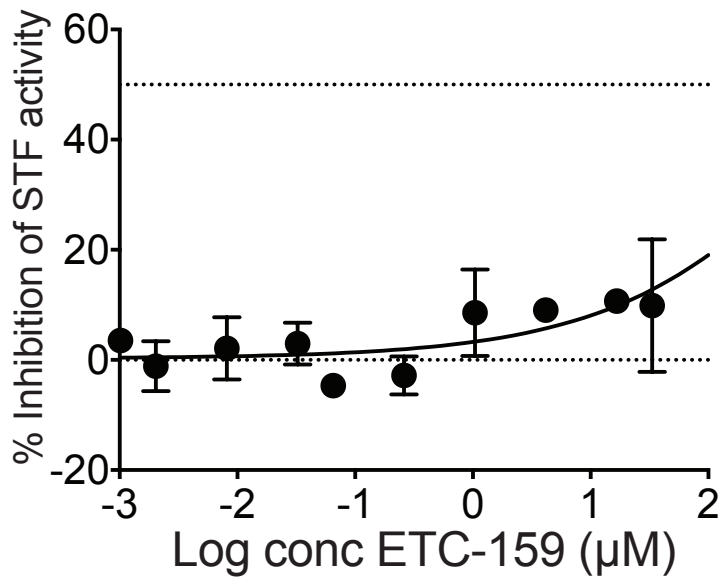
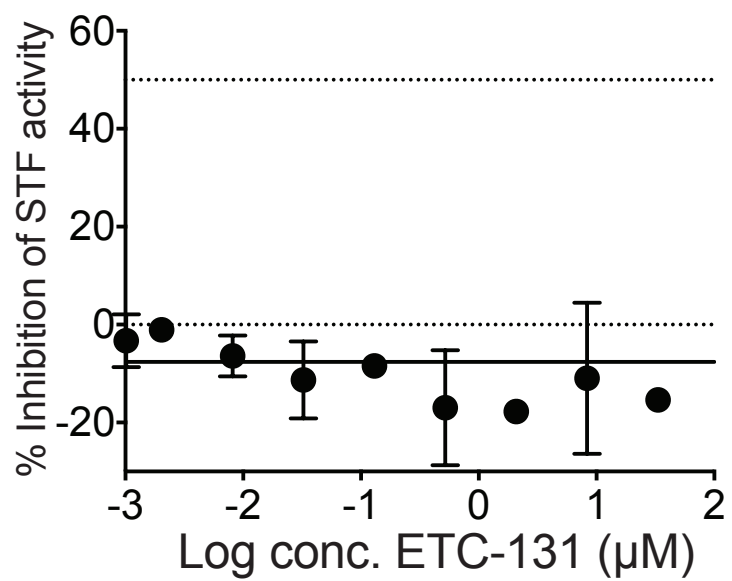


Supplementary Figure 1

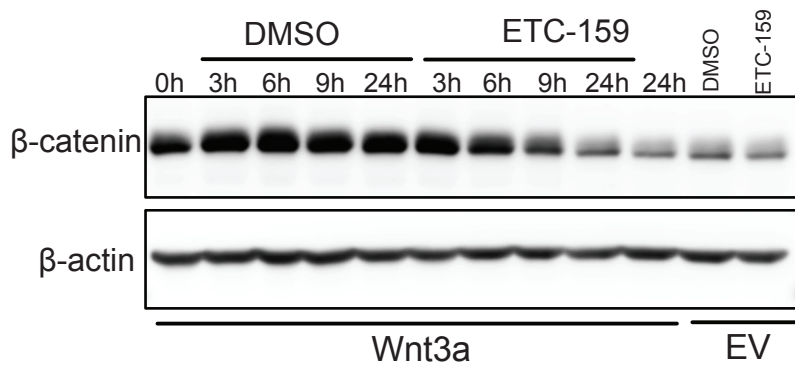
A.



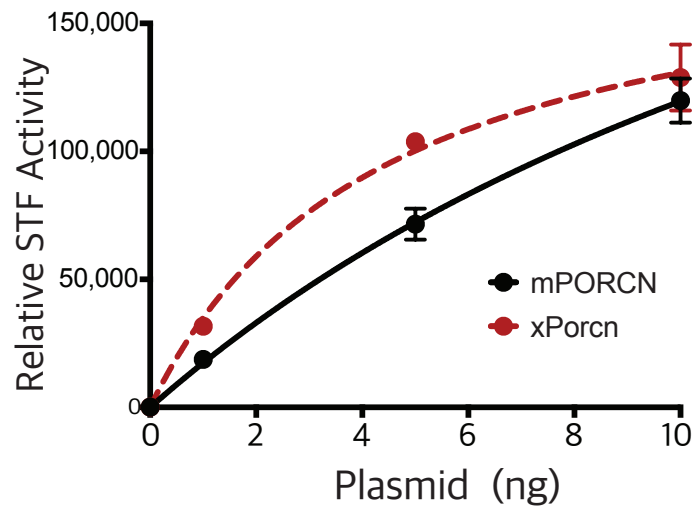
B.



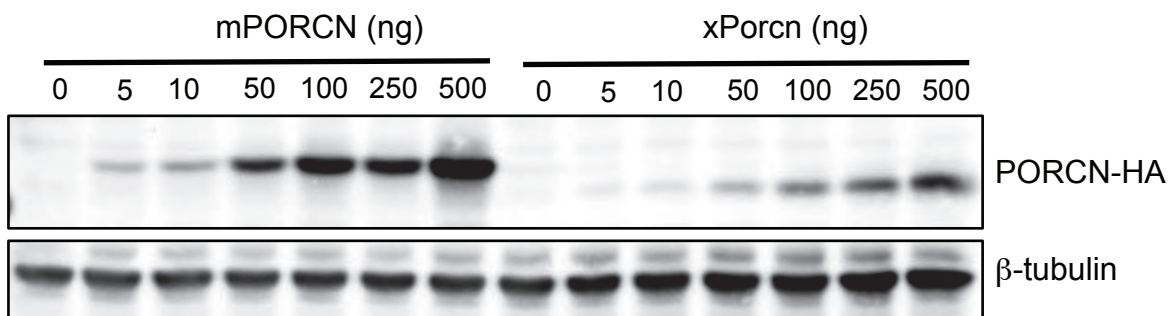
C.



D.



E.



Supplementary Figure Legends:

Figure S1: (A-B) *WNT3A* rescues the inhibition by *ETC-159* and *ETC-131*: HEK293 cells stably transfected with TOPflash reporter were treated with indicated concentrations of *ETC-159* or *ETC-131* in presence of conditioned media from LWNT3A cells (mouse fibroblasts stably expressing *WNT3A*) for 24 hours. The cells were harvested and luciferase activity measured. Data represents mean \pm SD. **(C) *ETC-159* promotes β -catenin degradation:** Following 24 h of transfection with *Wnt3a* expressing plasmid, HEK293 cells were treated with DMSO or 100 nM *ETC-159*. The cells were harvested at indicated time points and cytoplasmic β -catenin levels were assessed by immunoblot. **(D)** HT1080 null cells were transfected with *Wnt3a* and indicated amounts of mouse and *Xenopus Porcn* expression plasmids and Super8xTOPFLASH reporter. After 24 hours the cells were lysed and luciferase activity was measured. Data represents mean \pm SD. **(E)** HT1080 null cells were transfected with indicated amounts of mouse and *Xenopus Porcn* expression plasmids. The cells lysates were resolved on a 10% SDS gel and probed with HA or β -tubulin antibodies. Data shown is representative of 3 independent experiments.

Supplementary Figure 2

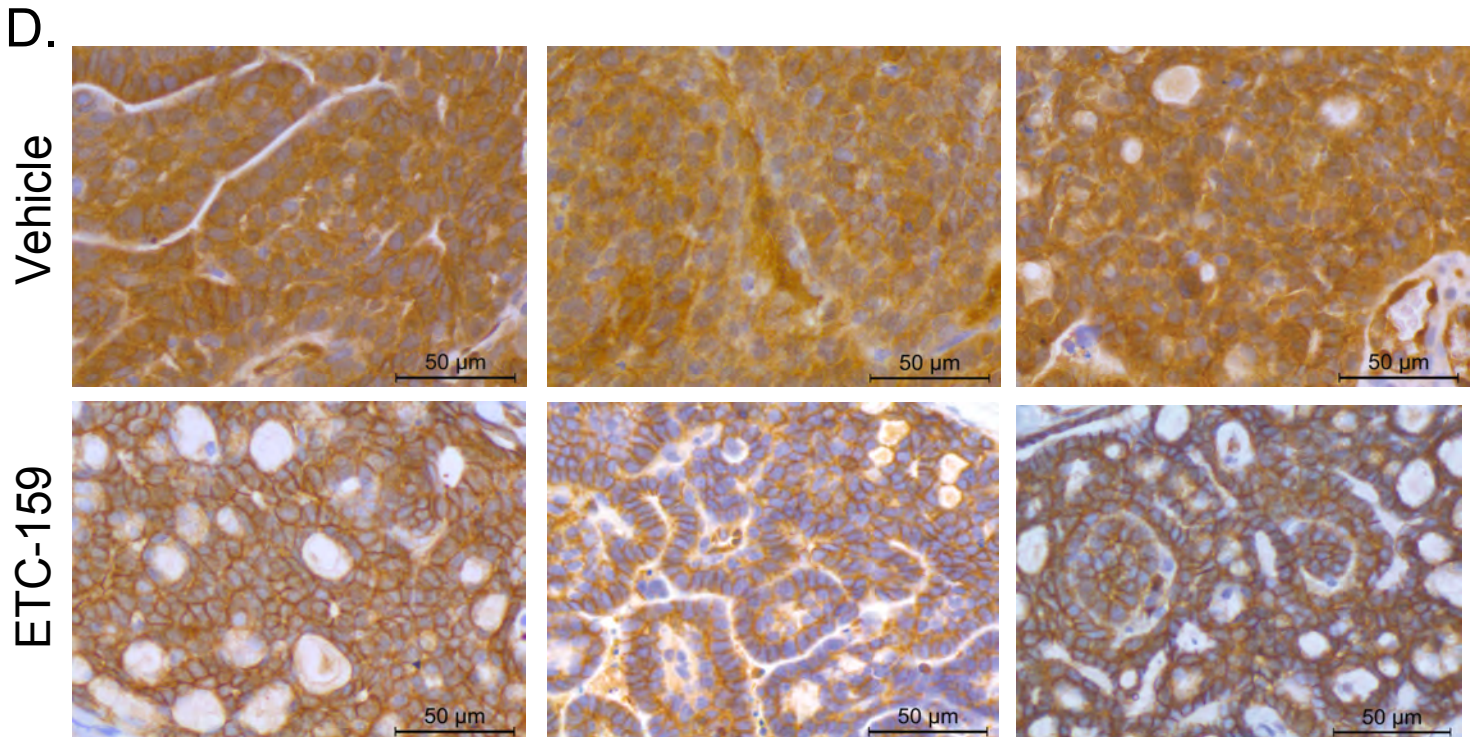
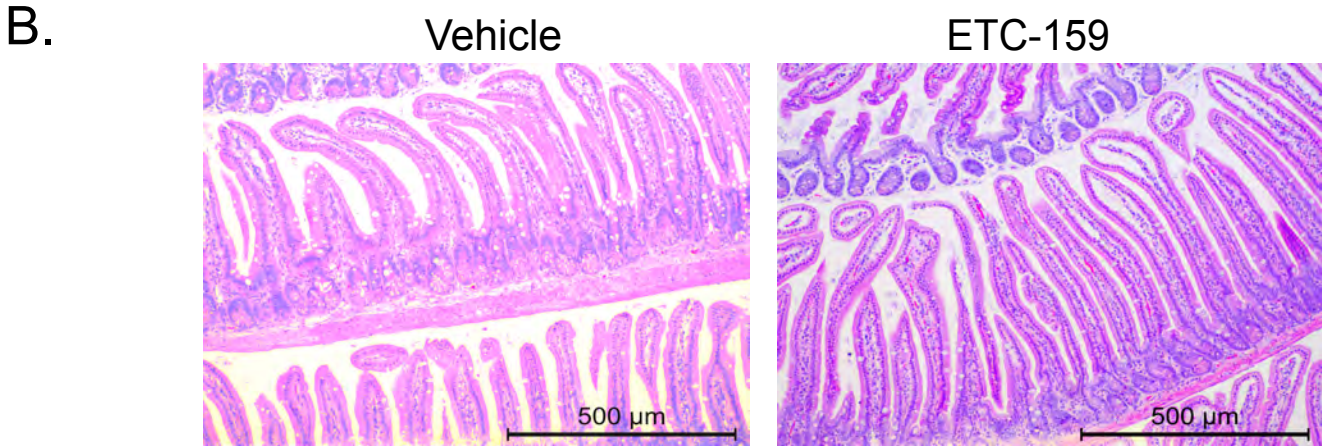
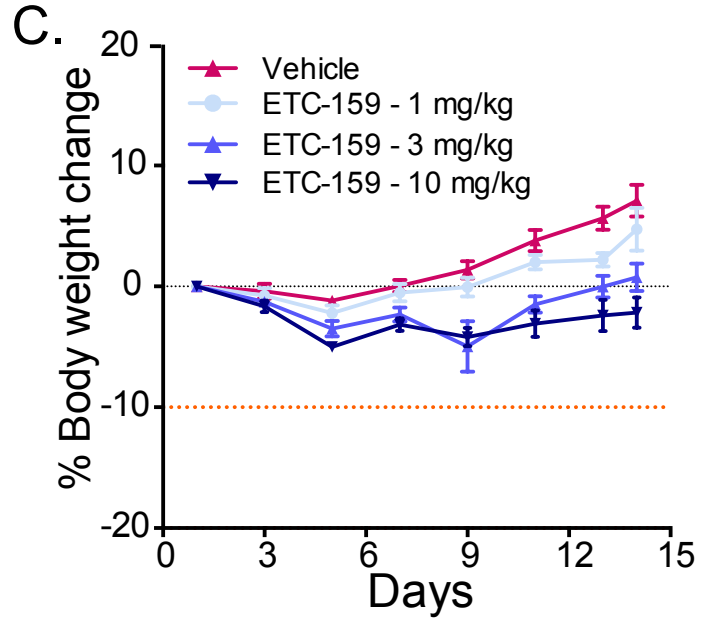
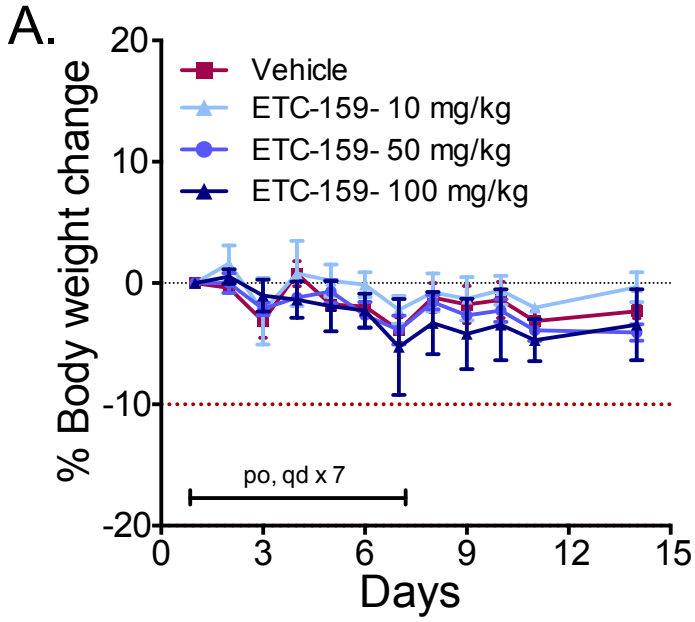
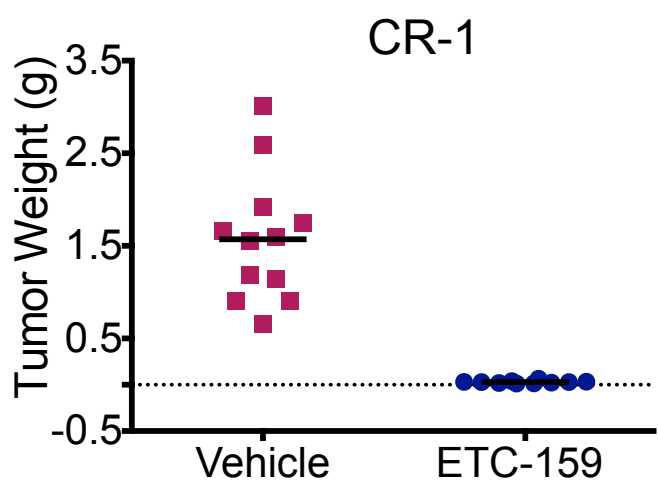


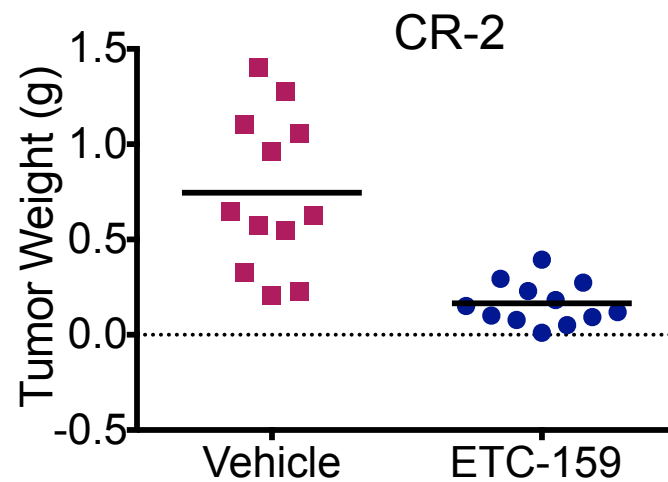
Figure S2: (A) *ETC-159 is non-toxic to the mice:* BALB/C nude mice were treated daily with 10-100 mg/kg ETC-159 as indicated for 7 days. Body weights were measured daily and are presented as percentage of the body weight on day 1. **(B) *Therapeutic doses of ETC-159 are well tolerated in mice:*** Hematoxylin and Eosin staining of intestinal sections from mice treated with 100 mg/kg ETC-159 for 4 weeks. **(C) *ETC-159 treatment is well tolerated in the MMTV-Wnt1 orthotopic mouse model:*** Female BALB/C nude mice implanted orthotopically with MMTV-Wnt1 tumor fragments from a transgenic mouse were treated daily with indicated doses of ETC-159, starting on day 1, at an average tumor volume of 100 mm³. Body weight changes were measured daily and are presented as percentage of the body weight on day 1. **(D) *PORCN inhibition promotes nuclear and cytoplasmic exclusion of β -catenin:*** Tumors harvested at the end of the study were stained for β -catenin. Representative sections from 3 independent tumors of vehicle and ETC-159 treatment group are shown. Scale bar = 50 μ m.

Supplementary Figure 3

A.



B.



C.

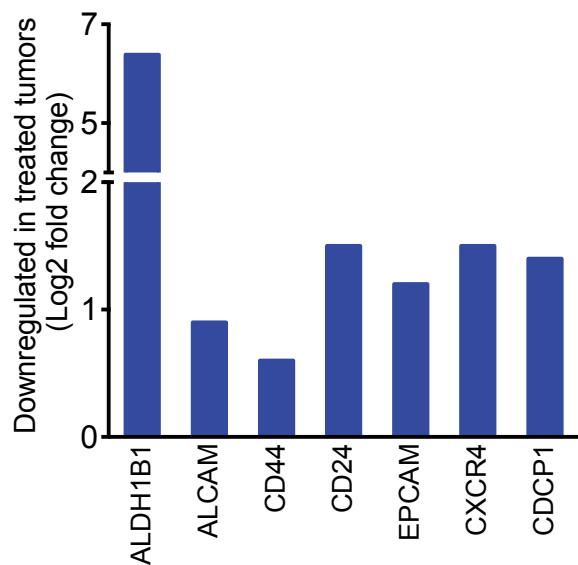


Figure S3: *ETC-159* effectively inhibits the growth of colorectal patient derived xenografts with *RSPO* translocations: Female BALB/C nude mice were implanted subcutaneously with tumor fragments from 2 independent patient derived xenografts CR-1 and CR-2. Oral, daily treatment with vehicle or 75 mg/kg *ETC-159* was started at an average tumor volume of $\sim 120 \text{ mm}^3$. The tumors were excised at the end of the study and weighed individually CR-1 (A) and CR-2 (B). (C) ***Downregulation of cancer stem cell genes in ETC-159 treated colorectal cancers:*** The bar graph shows the fold changes in expression of colorectal cancer stem cell genes in *ETC-159* treated CR-1 tumors.

Supplementary Figure 4

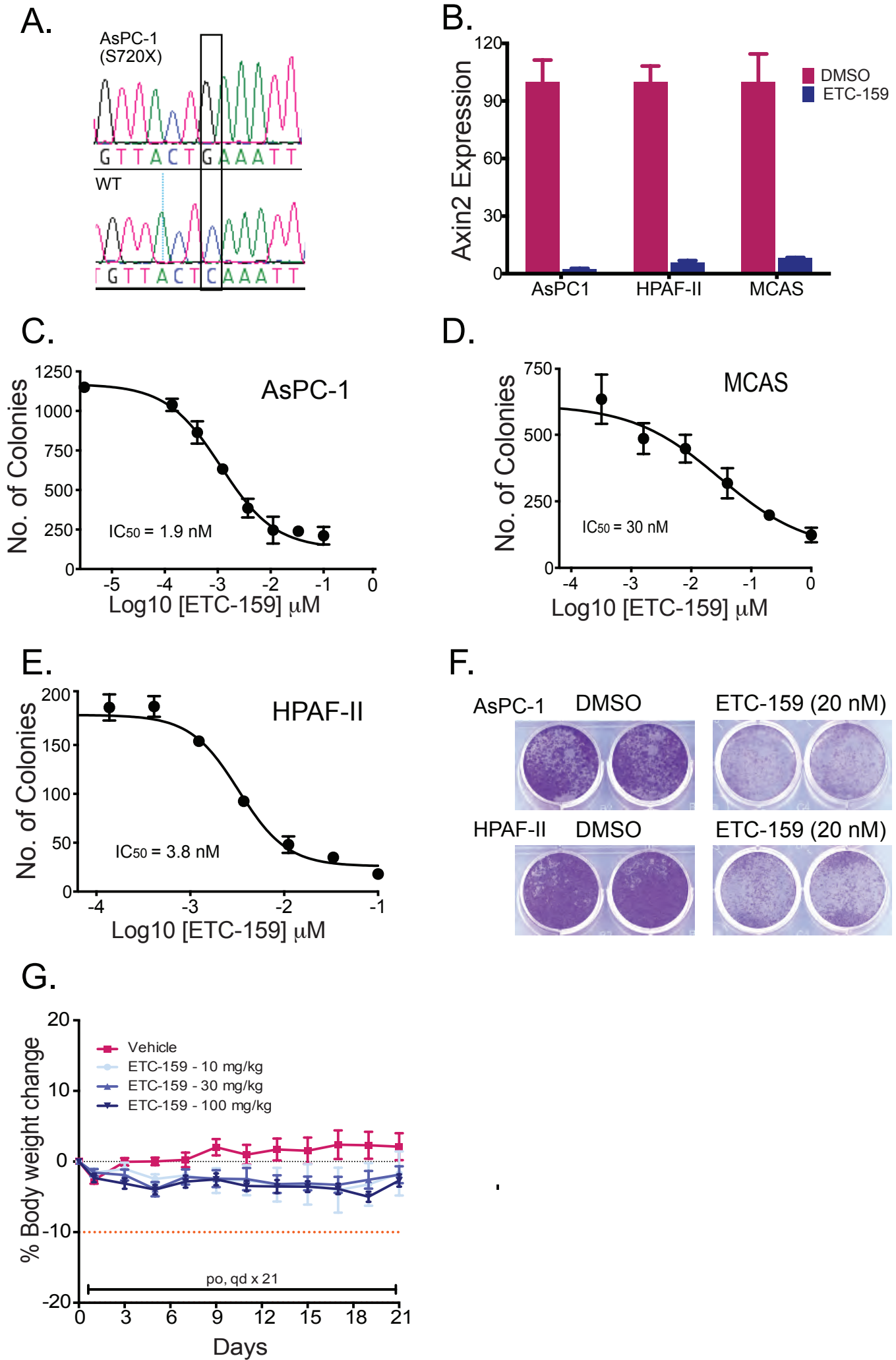


Figure S4: (A) RNF43 mutation in AsPC-1 cells, corresponding sequence of wild type RNF43 is shown below. (B) *Axin2 expression in ETC-159 treated pancreatic cell lines:* Pancreatic and ovarian cell lines were treated with 100 nM ETC-159 for 24h. Total RNA was isolated and AXIN2 expression analyzed by qRT-PCR. Expression of AXIN2 in the ETC-159 treated cells is represented as the percentage of DMSO treated controls. (C-E) **Reduction in anchorage independent growth of pancreatic and ovarian cancer cell lines:** Cells were plated in soft agar in the presence of DMSO or the indicated concentrations of ETC-159. Colonies were counted two weeks later. Each data point represents an average count of two wells. (F) *Effect of ETC-159 on foci formation in pancreatic cell lines:* 3000 cells plated in 24 well plates were treated with indicated concentration of ETC-159. Cells were fixed with methanol prior to staining with crystal violet. (G) *Effect of ETC-159 treatment on body weight in HPAF-II mouse model:* Female NCr nude mice with established HPAF-II tumors were treated daily with indicated doses of ETC-159 for 21 days. Body weight changes were measured daily and are presented as percentage of the body weight at day 1.