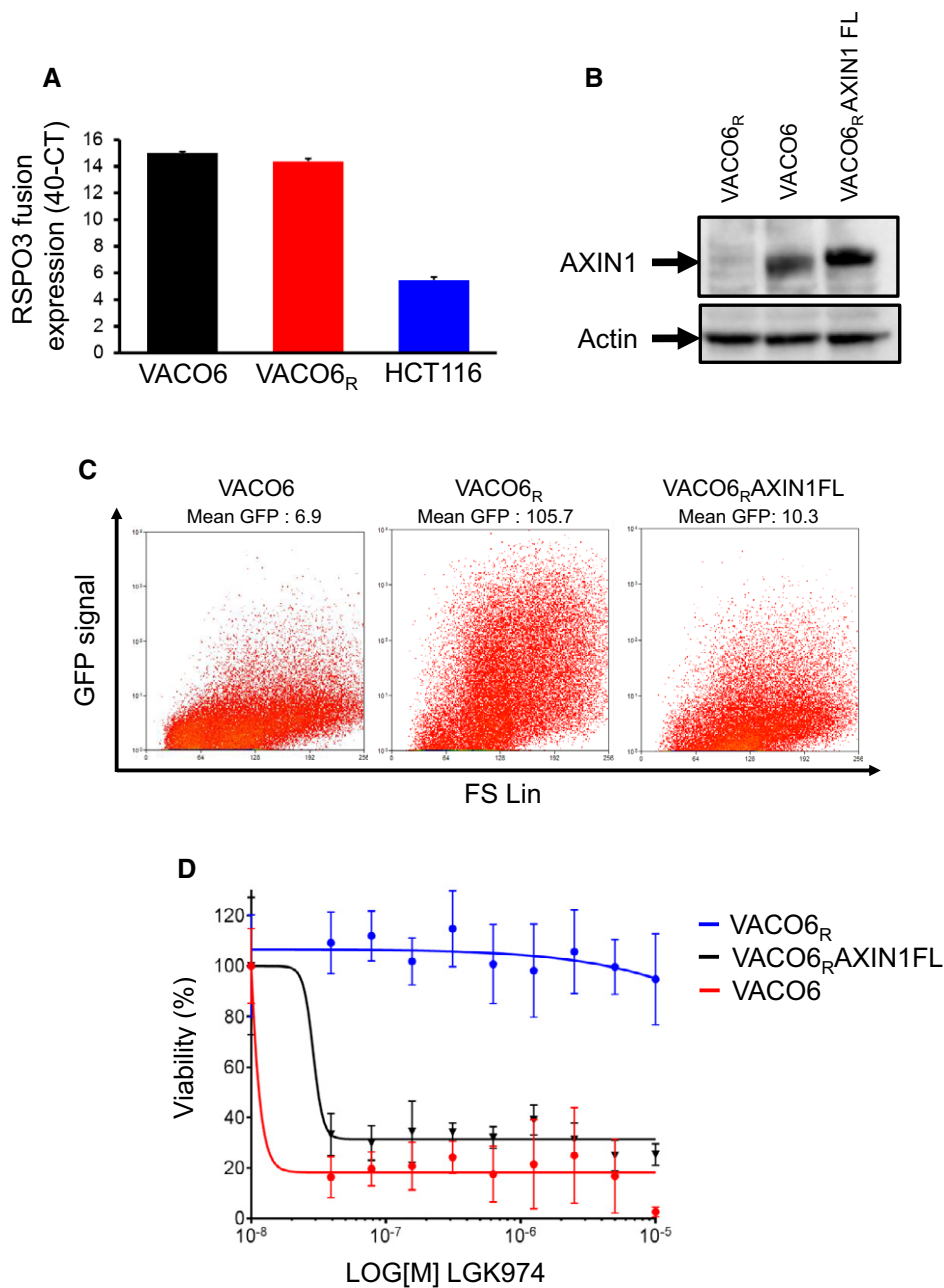


## Expanded View Figures



**Figure EV1. Re-expression of the AXIN1 coding sequence re-sensitizes VACO6<sub>R</sub> cells to PORCN inhibition.**

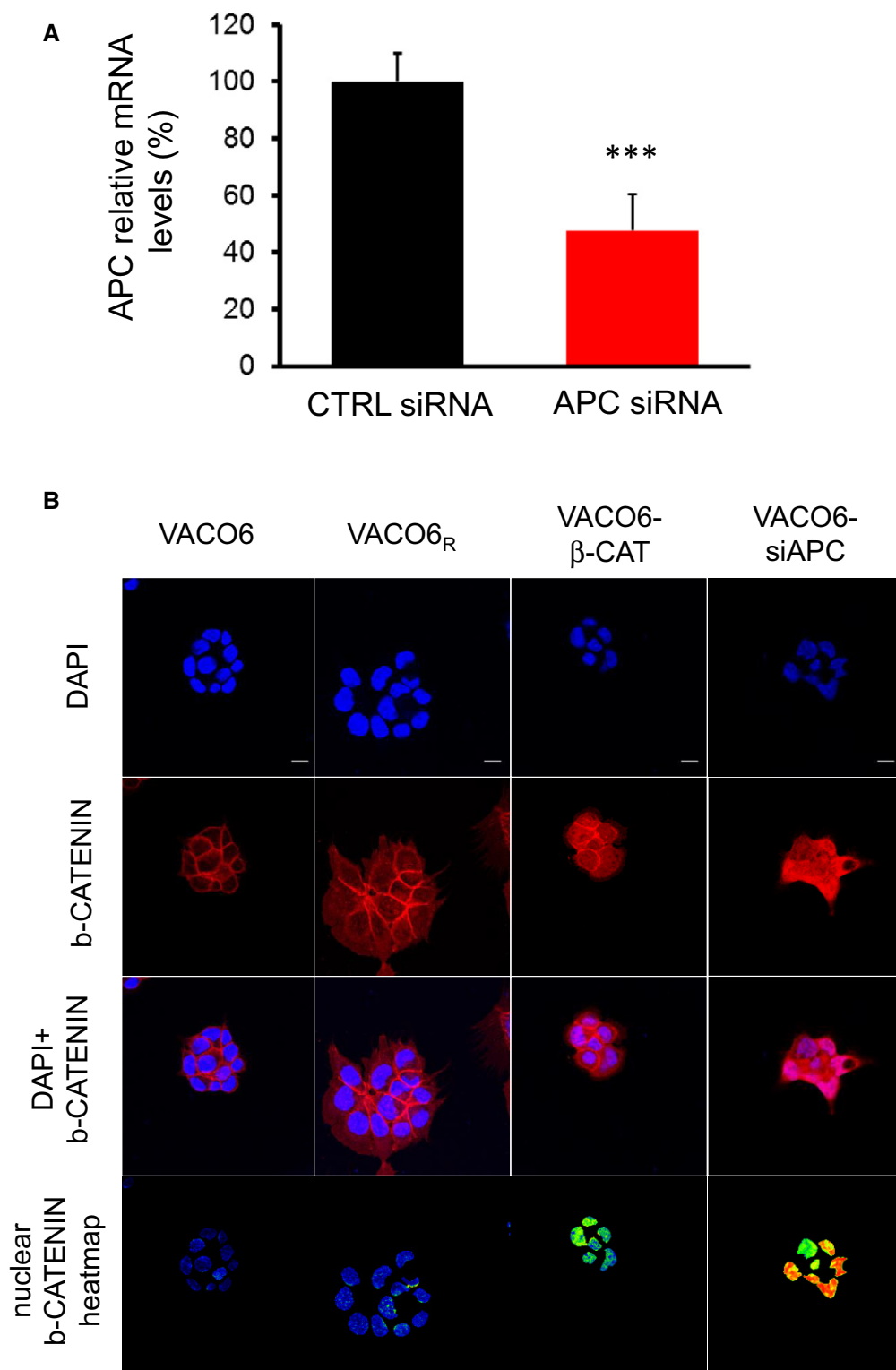
A qRT-PCR evaluation of PTPRK-RSPO3 fusion transcript expression in VACO6, VACO6<sub>R</sub>, and HCT116 cells, as indicated. Data are expressed as means  $\pm$  SD of three technical replicates from one representative experiment.

B Western blot showing AXIN1 protein expression in VACO6<sub>R</sub>, VACO6, and VACO6<sub>R</sub>AXIN1FL cells, as indicated.

C Flow cytometry analysis of the GFP signal in VACO6, VACO6<sub>R</sub>, and VACO6<sub>R</sub>AXIN1FL cells, all transduced with the 7xTcf-eGFP WNT reporter.

D ATP-based assay measuring viability, after 6 days of LGK974 treatment, of parental VACO6, VACO6<sub>R</sub>, and VACO6<sub>R</sub>AXIN1FL cells, as indicated. Data are expressed as average  $\pm$  SD of six technical replicates.

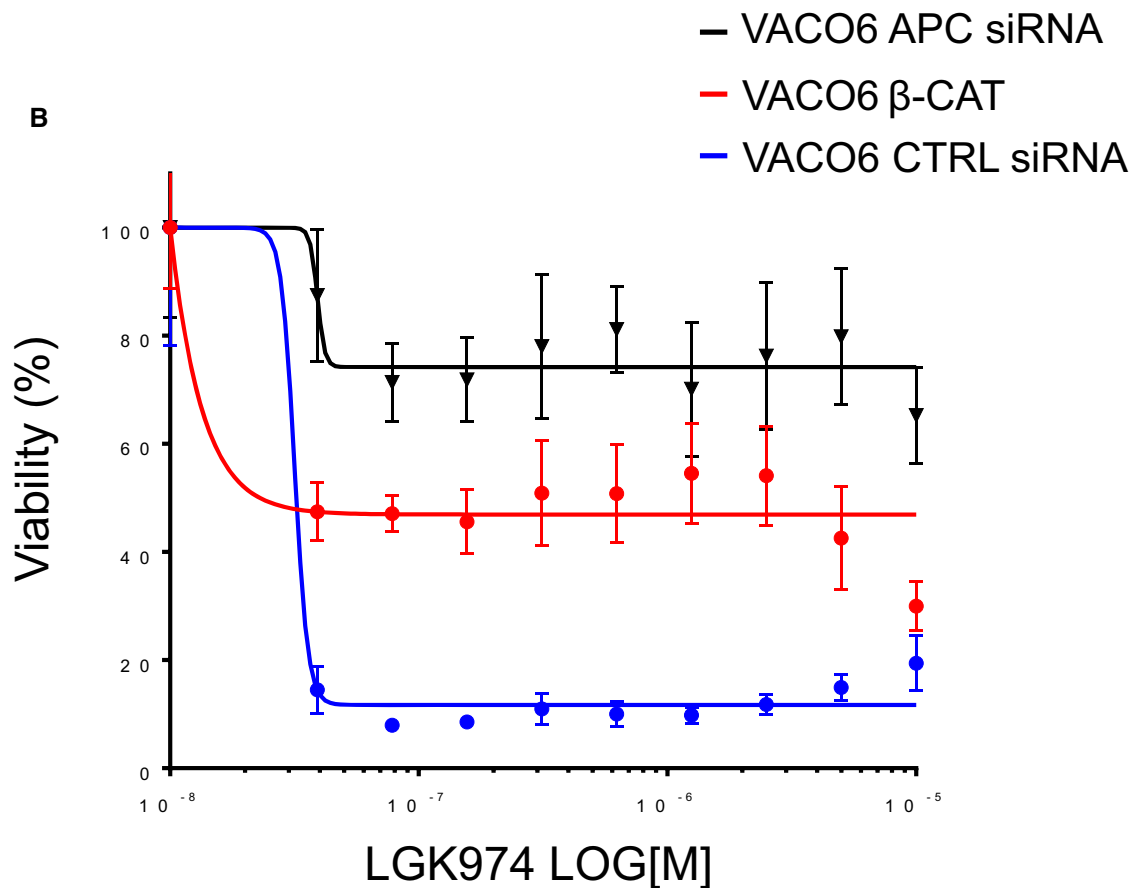
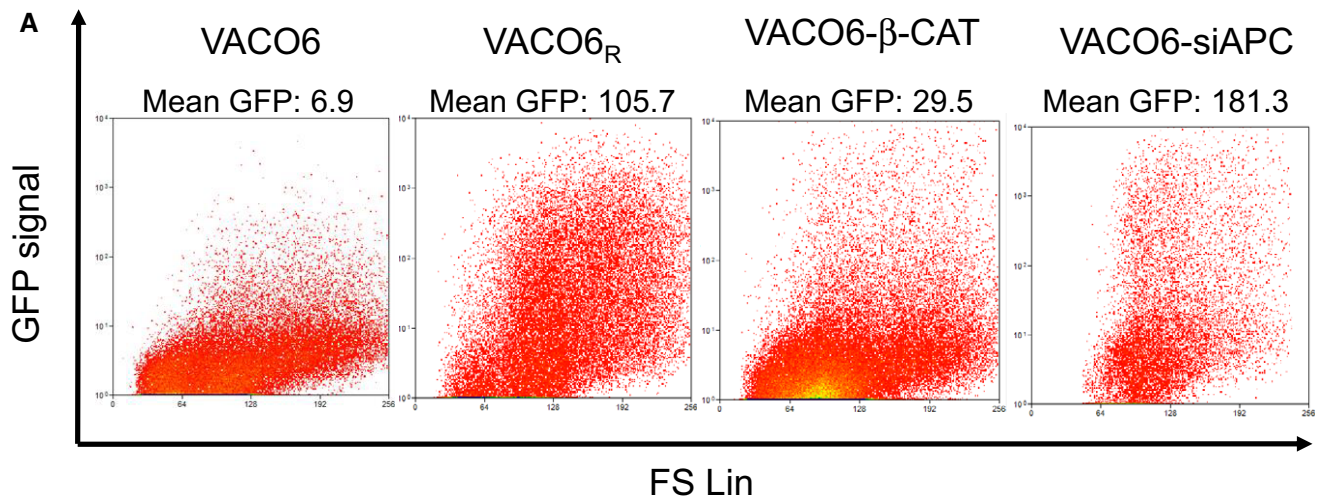
Source data are available online for this figure.



**Figure EV2. Activated  $\beta$ -catenin transduction or transient APC downregulation in VACO6 cells induce increased nuclear  $\beta$ -catenin levels.**

A qRT-PCR evaluation of APC mRNA expression in VACO6 cells transfected with control siRNA or APC siRNA, as indicated. Two-sided Student's *t*-test, \*\*\**P*-value < 0.001, *P* = 8.24E-04. Data are expressed as means  $\pm$  RMSE of three technical replicates from one representative experiment.

B Representative confocal microscopy images of VACO6 cells (as indicated) showing  $\beta$ -catenin distribution and GFP expression. Nuclei are stained with DAPI (blue). Scale bars, 10  $\mu$ m.



**Figure EV3. Activated  $\beta$ -catenin transduction or transient APC downregulation increase WNT pathway activity and induce resistance to WNT pathway inhibitors in VACO6 cells.**

A Flow cytometry analysis of GFP fluorescence in VACO6, VACO6<sub>R</sub>, VACO6- $\beta$ -CAT, and VACO6-siAPC, as indicated, all transduced with the 7xTcf-eGFP WNT reporter.

B ATP-based assay measuring viability, after 7 days of LGK974 treatment, of VACO6 cells transfected with control siRNA or with APC siRNA, or transfected with activated  $\beta$ -catenin. Data are expressed as average  $\pm$  SD of six technical replicates from one representative experiment.