Supplementary Figure 1

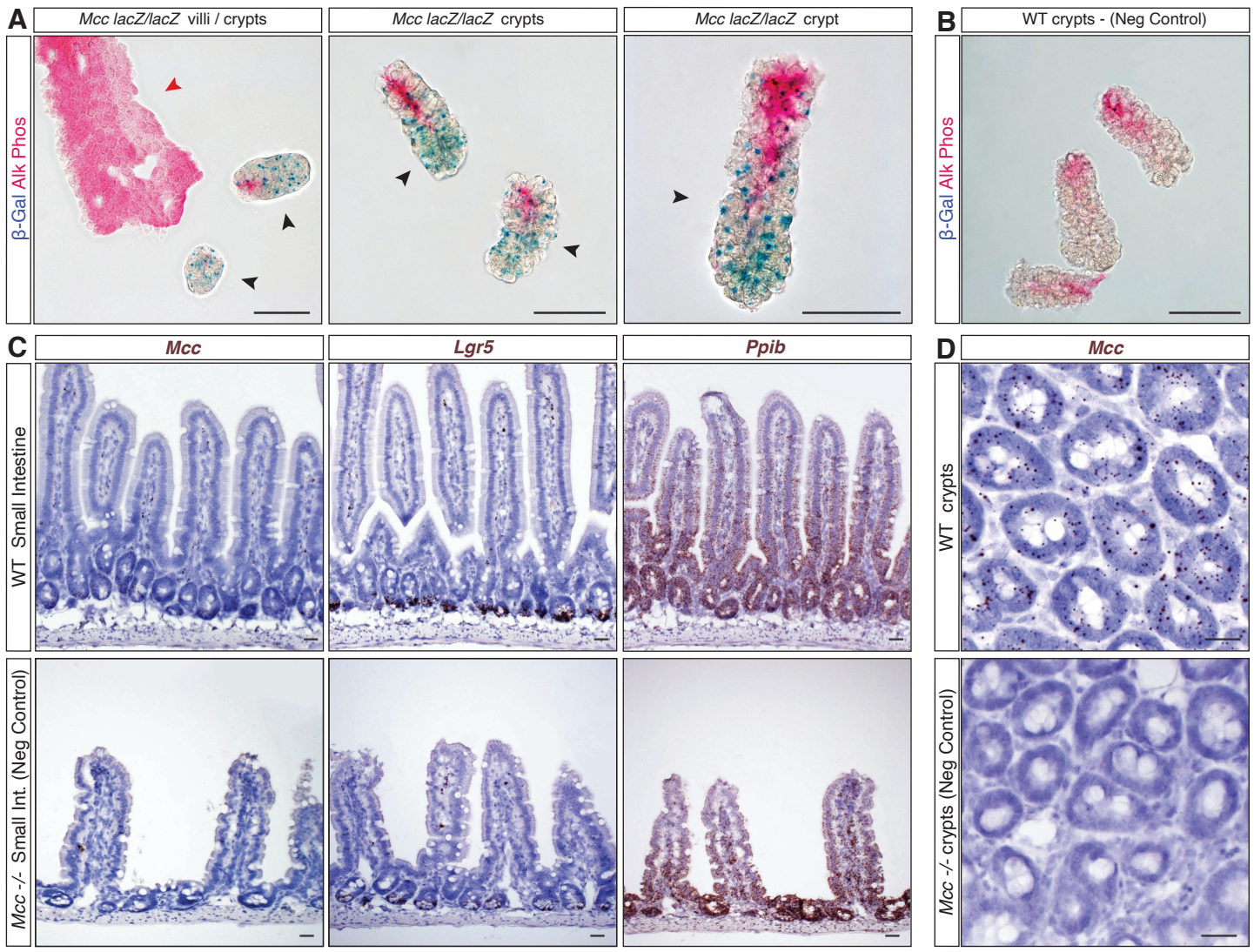


Fig. S1. β-Galactosidase (β-Gal) staining activity and in situ hybridization (ISH) controls. (**A**): Co-staining for β-Gal (blue) activity and intestinal alkaline phosphatase (red) in whole-mount villus (red arrowhead) and crypt (black arrowheads) fractions isolated from *Mcc*$^{lacZ/lacZ}$ adult small intestine (SI). (**B**): Wild type (WT) β-Gal-negative crypt controls. Scale bars, 50 µm. (**C**): ISH for the expression of *Mcc, Lgr5* and *Pbib* (positive control) on WT and *Mcc*$^{lacZ/lacZ}$ (= *Mcc-/-* negative control) mouse SI sections. Endogenous *Mcc* expression is not detected on *Mcc-/-* tissues. (**D**): ISH for Mcc on transverse sections of WT and *Mcc-/-* SI crypts. Mcc expression is not detected on *Mcc-/-* crypts. Scale bars, 20 µm.

Supplementary Figure 2

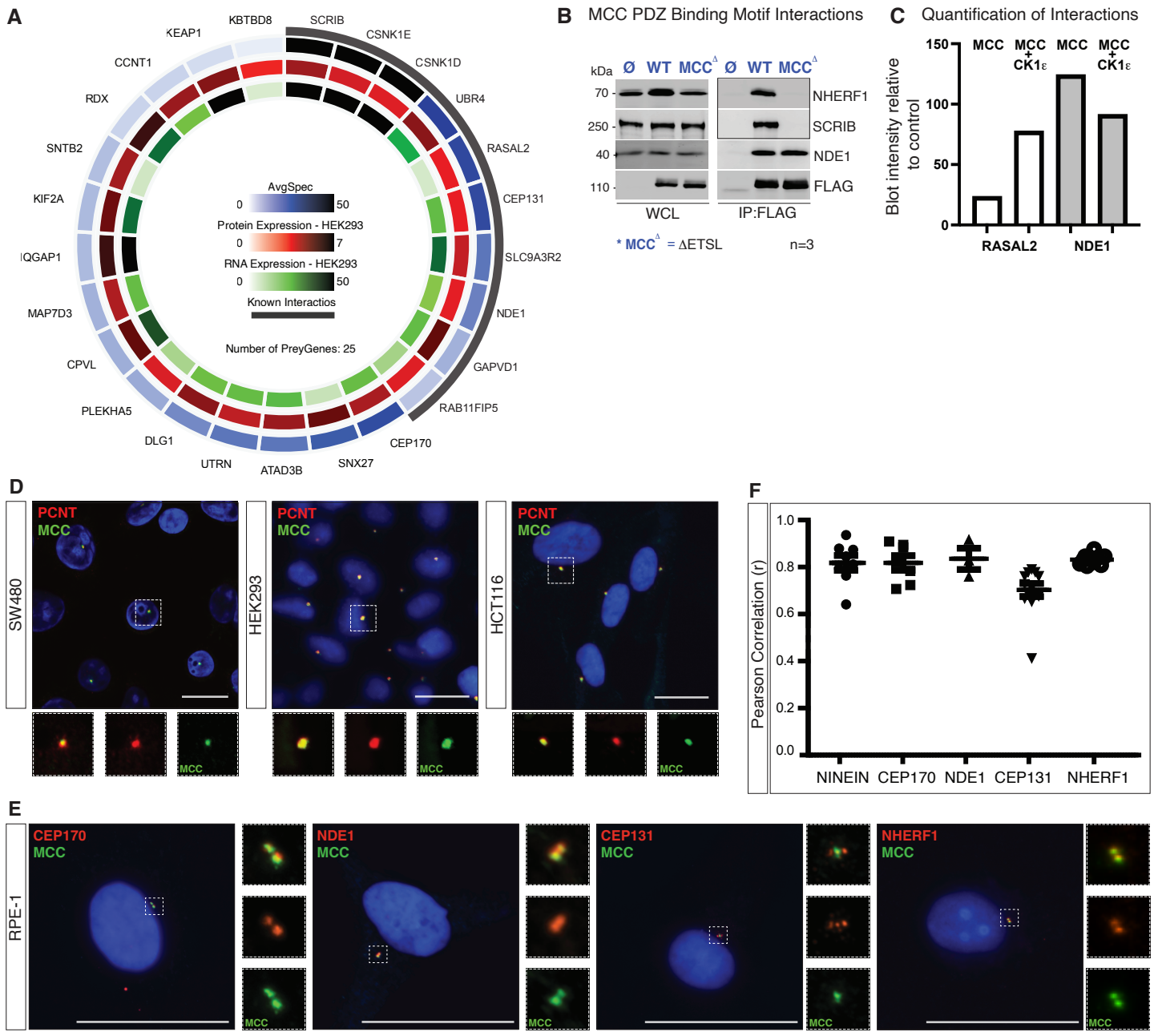


Fig. S2. MCC interactions and subcellular localization across human cell lines. (A): Visualization of high-confidence MCC interactions (showing 25 preys) based on Significance Analysis of INTeractome (SAINTexpress), indicating previously reported interactions from the BioGRID interaction database (thebiogrid.org), protein expression levels from Proteomics DB (proteomicsdb.org), and RNA expression levels from Protein Atlas (proteinatlas.org). **(B):** Truncation of the PDZ binding motif (PBM) (-ETSL) of MCC (MCCΔ) disrupts interaction to NHERF1 and SCRIB, but not to NDE1 in HEK293 cells. WCL, whole cell lysate. IP: Immunoprecipitation. IB: Immunoblotting. **(C):** Western blot quantification analysis for Figure 4D. RASAL2 and NDE1 affinity to MYC-MCC changes upon co-expression of FLAG-CK1ε in HEK293 cells. Western blot images are representative of n = 3. **(D):** Immunofluorescence (IF) shows colocalization of MCC and PERICENTRIN (PCNT) at the centrosome in SW480, HEK293, and HCT116 cells. Scale bars, 50 μm. **(E):** IF in RPE-1 cells shows colocalization of MCC and CEP170, NDE1, CEP131, and NHERF1. All images shown are representative of at least 5 experiments. **(F):** Pearson correlation coefficient (r) reveals strong positive linear correlation (>0.5) between the signal positions of MCC and several of its interacting partners at the centrosome in RPE-1 cells.

Supplementary Figure 3

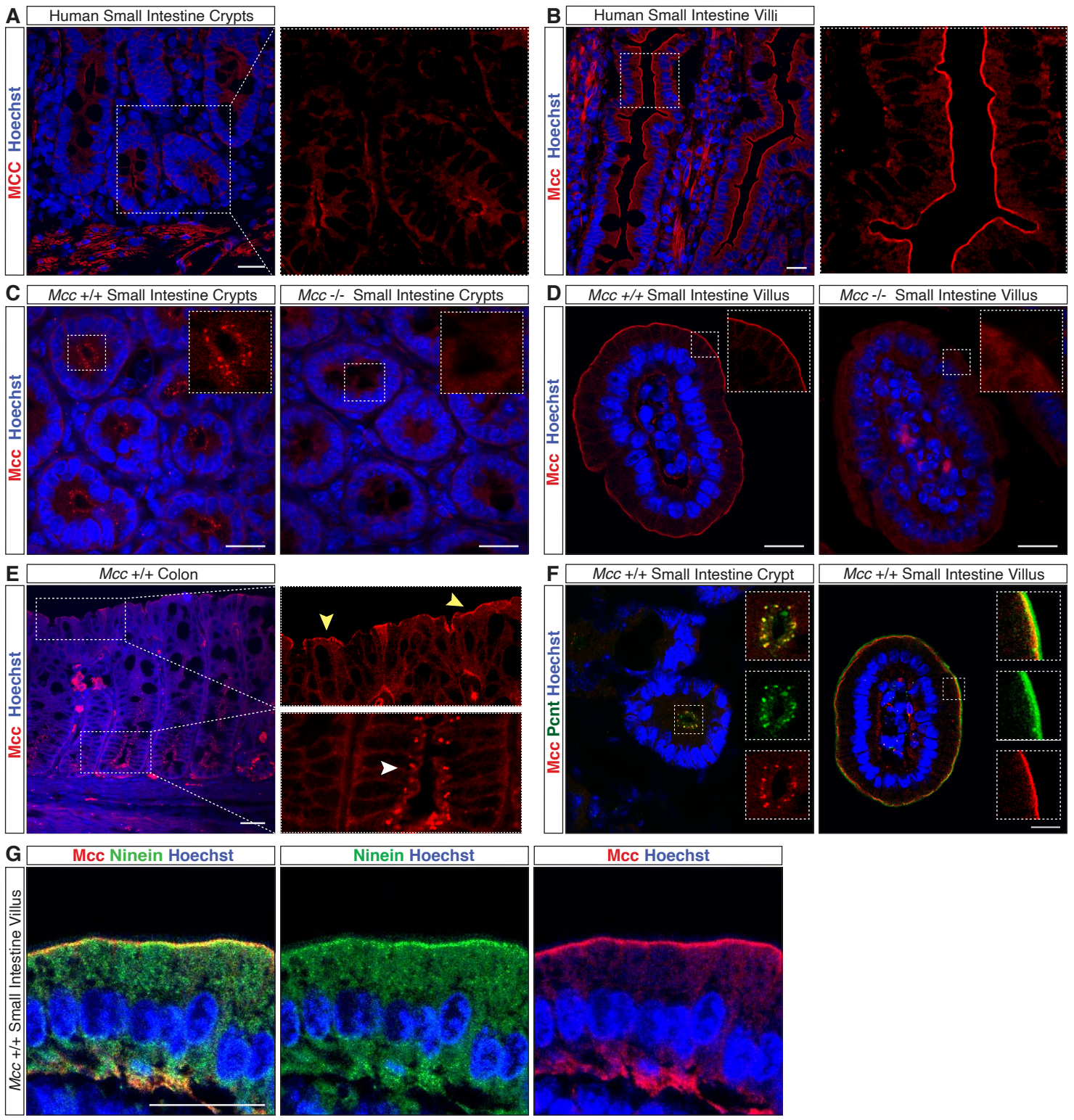


Fig. S3. MCC dynamic localization in the intestine and antibody specificity control for immunofluorescence (IF) in the intestine. (A): IF for MCC on sections of human SI shows MCC localizing at the centrosome in crypt cells. **(B):** IF for MCC on sections of human SI shows MCC localizing at the apical membrane of differentiated cells in villi. **(C-D):** IF for Mcc localization on wild-type (WT) and *Mcc*$^{lacZ/lacZ}$ (= *Mcc-/-* negative control) transverse sections of the mouse small intestine (SI). **(C):** Mcc localizes to the centrosome (punctate staining) in crypts and **(D)** at the apical membrane of differentiated cells in the villus units. *Mcc-/-* sections show absence of Mcc signal in crypts **(C)** and villi **(D). (E):** IF for Mcc localization on WT mouse colonic epithelium reveals Mcc at the centrosome in crypt cells (white arrowhead) and at the apical membrane of differentiated cells (yellow arrowheads). **(F):** IF for Mcc and Pcnt shows colocalization at the centrosome in crypt cells and at the apical membrane of villus cells (transverse sections). **(G): I**F for Mcc and Ninein on a WT transverse section of the villus shows colocalization at the apical membrane. Scale bars, 20 µm.

Supplementary Figure 4

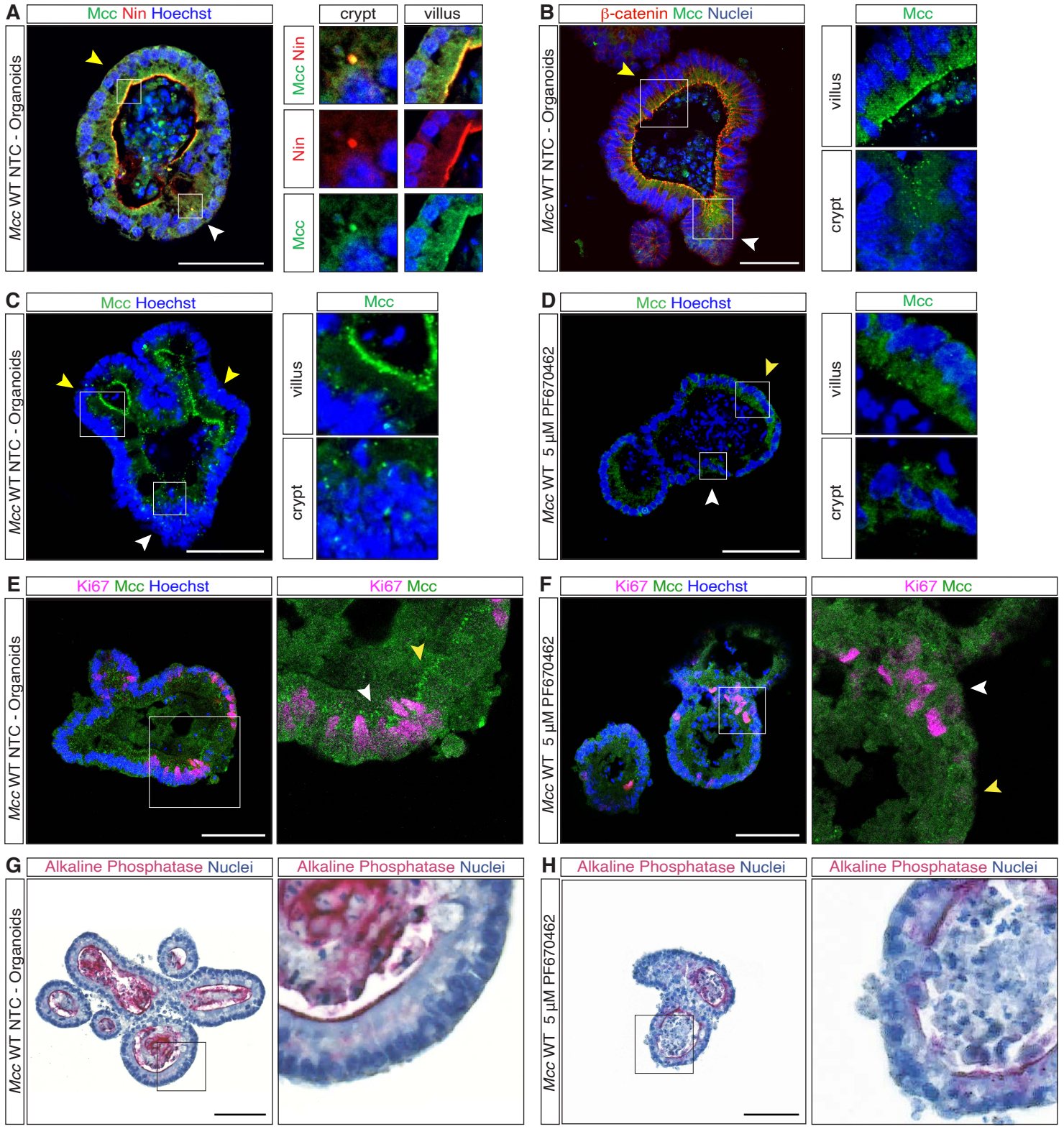


Fig. S4. Phosphorylation by CK1δ/ε triggers MCC redeployment to the ncMTOC at the apical membrane of villus cells. Immunofluorescence (IF) in sections of small intestinal organoids (white arrowheads indicate crypt domains; yellow arrowheads indicate villus domains). **(A):** IF showing Mcc and Ninein localization in the centrosome in crypt cells and along the apical membrane of differentiated cells in non-treated control (NTC) WT organoids. **(B):** IF showing Mcc localization in the centrosome in crypt cells and along the apical membrane of differentiated cells of NTC WT organoids, membrane immunostaining with β-catenin. **(C and D):** IF for MCC in intestinal organoids. Mcc apical membrane localization is disrupted upon treatment with 5 μM of PF670462. **(E and F):** IF for MCC and Ki67 in NTC and PF670462-treated organoids. (B): Histochemical (HC) staining for Alkaline Phosphatase in sections in NTC and PF670462-treated organoids. Nuclei stained with Hema-toxylin. White/Black-dashed squares highlight regions selected for higher magnification. Scale bars, 20 μm.

Table S1. Antibodies and Primers

Primary Antibodies.

Antibody	Species	Catalog #	Brand	Application – Dilution (μL)
β-actin	Rabbit	AB8227	Abcam	WB/IP 1:10000, IF 1:250
β-catenin	Rabbit	AB32572	Abcam	WB/IP 1:10000, IF 1:200
β-tubulin	Mouse	T8328	Sigma	WB/IP 1:10000, IF 1:200
Csnk1e	Mouse	(A6) SC-374069	Santa Cruz	WB/IP 1:15000
Cep131	Rabbit	PA5-54953	Invitrogen	WB/IP 1:10000, IF 1:200
Cep170	Rabbit	AB72505	Abcam	WB/IP 1:10000, IF 1:500
Ep-CAM	Rabbit	AB71916	Abcam	IF 1:250
E-cadherin	Rabbit	AB15148	Abcam	IF 1:250
Iqgap1	Mouse	(C9) SC-376021	Santa Cruz	IP 1:10000
Mcc	Mouse	SC-135982	Santa Cruz	WB/IP 1:5000, IF 1:50
Nde1	Rabbit	PA5-87297	Invitrogen	WB/IP 1:15000, IF 1:500
Ninein	Mouse	637327	Merck	IF 1:250
Ninein	Rabbit	AB4447	Abcam	IF 1:250
Nherf1	Rabbit	SC-271552	Santa Cruz	WB/IP 1:10000, IF 1:500
Pericentrin	Rabbit	AB4448	Abcam	IF 1:250
Rasal2	Rabbit	A302-109A	Bethyl Lab	WB/IP 1:10000, IF 1:500
Scribble	Rabbit	SC-28737	Santa Cruz	WB/IP 1:10000, IF 1:200

WB: Western Blot
IP: Immunoprecipitation
IF: Immunofluorescence

Secondary Antibodies

Antibody	Species	Catalog #	Brand	Application – Dilution (μL)
Alexa Fluor 488 Anti-Mouse	Donkey	A21202	Invitrogen	IF 1:1000
Alexa Fluor 488 Anti-Rabbit	Donkey	A21206	Invitrogen	IF 1:1000
Alexa Fluor 594 Anti-Mouse	Donkey	A21203	Invitrogen	IF 1:1000
Alexa Fluor 594 Anti-Rabbit	Donkey	A21207	Invitrogen	IF 1:1000
Alexa Fluor 488 Anti-Mouse	Goat	A28175	Invitrogen	IF 1:1000

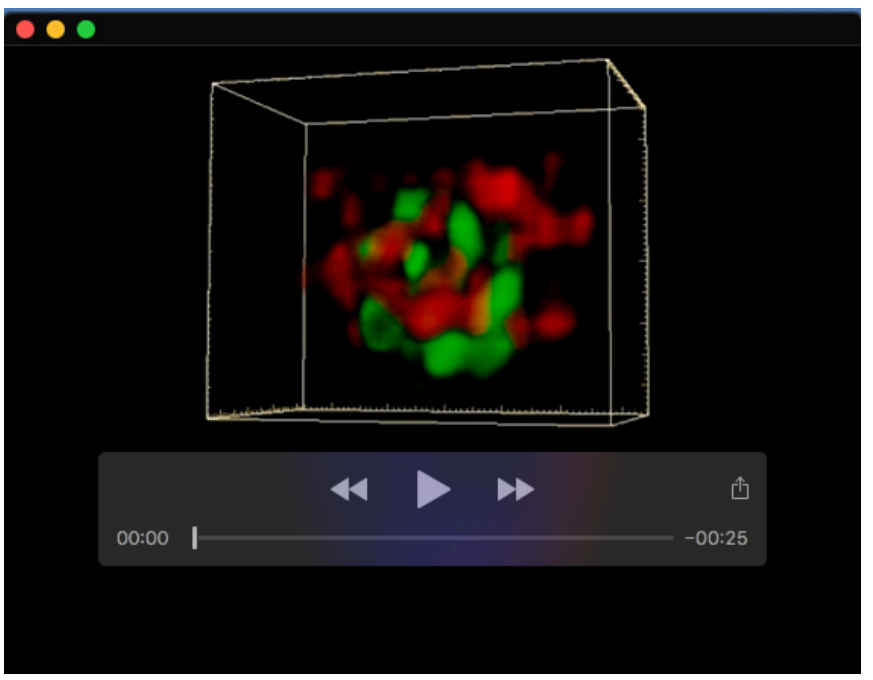
IF: Immunofluorescence

qPCR Primers.

Gene - Target		5’ > 3’ Sequence	TM
Mcc Exon 17	FWD	ACTTGCCGAACTAAGGACGA	52.2
Mcc Exon 18	REV	CTGGTGTCTGATTTCACTGC	53.9
Lgr5	REV	CCACAGCAACAACATCAGGT	55.9
Lgr5	FWD	AACAAATTGGATGGGGTTGT	55.8
β-Actin	FWD	CTTCTTTGCAGCTCCTTCGTTG	56.3
β-Actin	REV	CGCAGCGATATCGTCATCCA	58.4

Table S2. MassIVE SAINTexpress/ProHits output. FLAG AP-MS dataset acquired on Orbitrap classic and Orbitrap Velos instruments (SAINTexpress task 5987). Column A is the “Bait”. Prey Accession is the NCBI protein accession number; Prey Gene is as per NCBI Entrez Gene. Spectra (spectral counts) for the prey (column D, separated by "|" delimiter; column E, summed, column F, averaged), number of replicates performed (column G), spectral counts for the prey across all negative controls (column H), Averaged probability across replicates (column I), maximal probability (column J), log Odds score (column K), Fold Change (counts in the purification divided by counts in the controls plus small factor to prevent division by 0; column L) and Bayesian FDR (column M) are listed for each bait-prey relationship and are directly from the SAINTexpress output. Columns N-P are the unique prey peptides as calculated through ProHits. Column Q is the prey protein length and column R is the UniProt accession number. Significant interactors are those with BFDR ≤0.01, and are bolded. Significant Prey Genes highlighted in yellow contain PDZ domains, those in blue regulate GTPases, and those in orange are centrosomal components.

Click here to download Table S2



Movie 1. Super-resolution 3D Structured Illumination Microscopy (SIM) Video: 3D-SIM video showing co-localization of MCC (green) and PCNT (red) in SW480 cells at high magnification and different angles. 3D-video acquired using Imaris software (https://imaris.oxinst.com).