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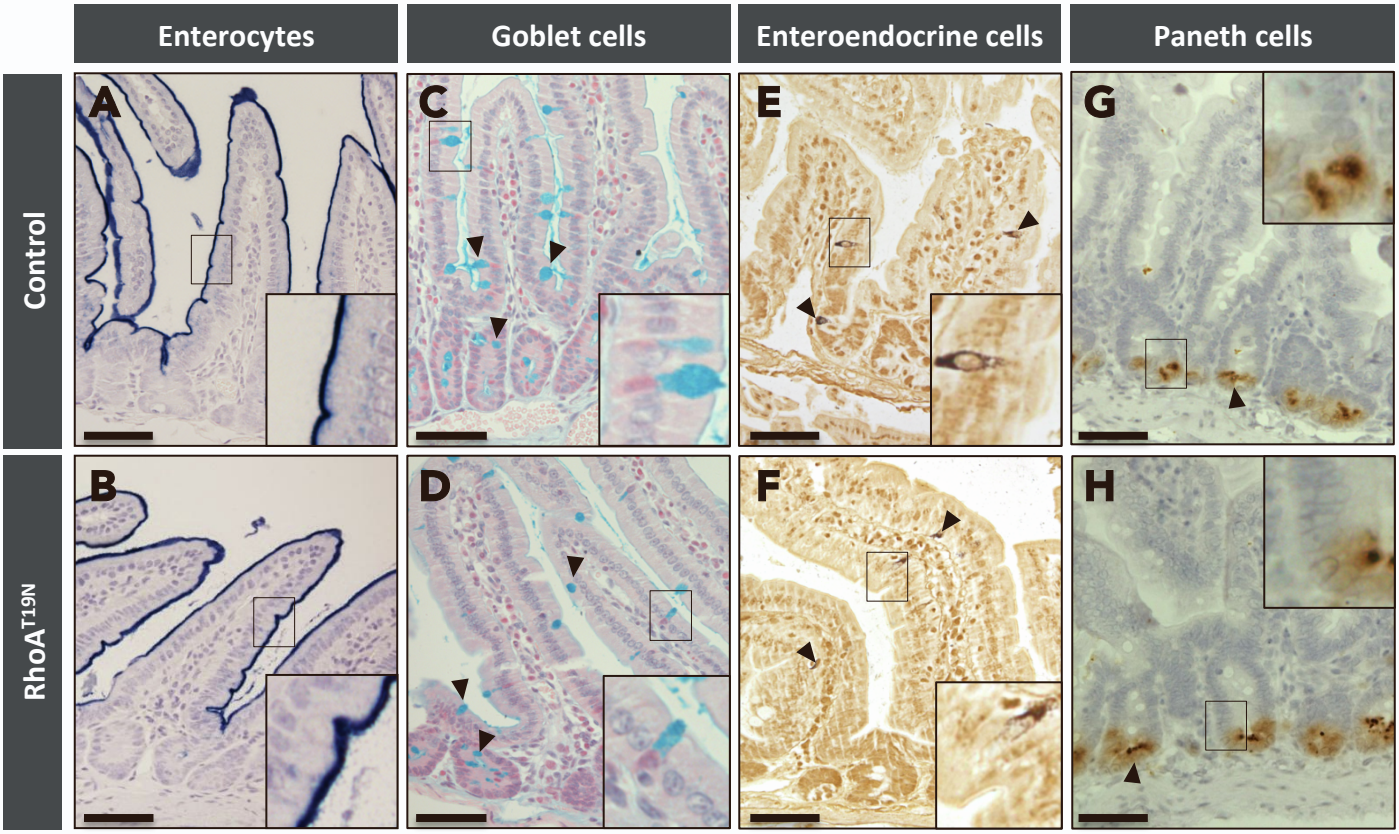
Supplemental information

RhoA downregulation in the murine intestinal epithelium results in chronic Wnt activation and increased tumorigenesis

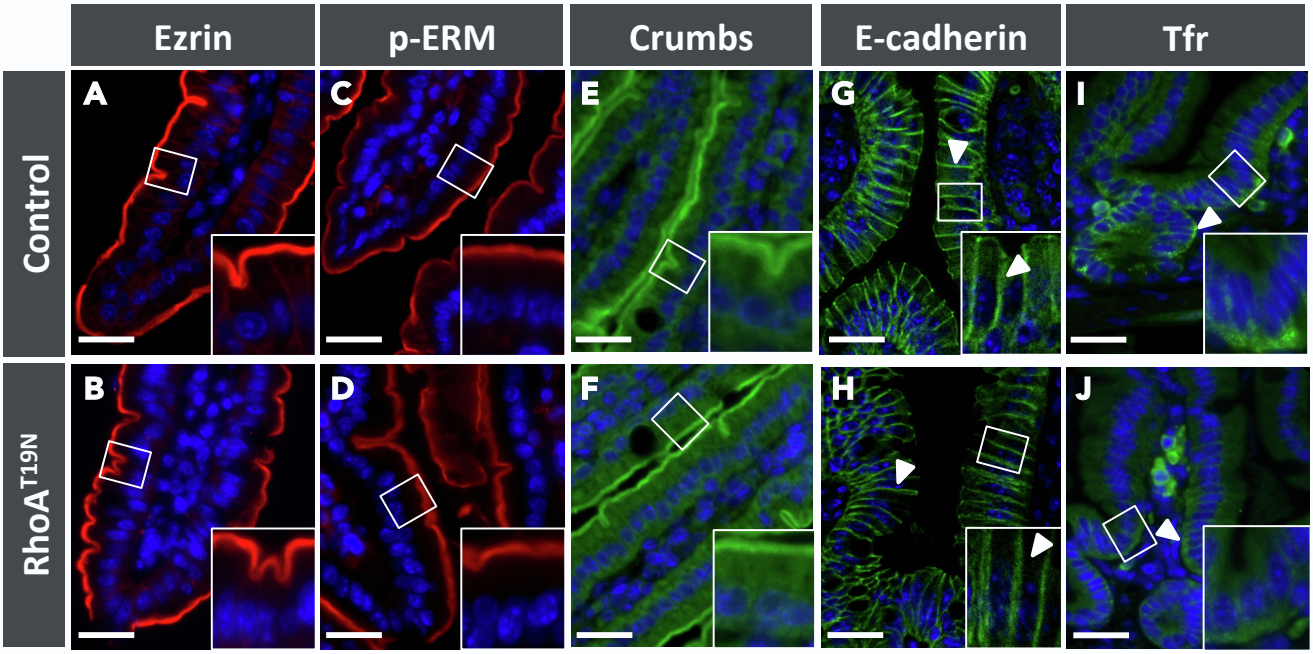
Higinio Dopeso, Paulo Rodrigues, Fernando Cartón-García, Irati Macaya, Josipa Bilic, Estefanía Anguita, Li Jing, Bruno Brotons, Núria Vivancos, Laia Beà, Manuel Sánchez-Martín, Stefania Landolfi, Javier Hernandez-Losa, Santiago Ramon y Cajal, Rocío Nieto, María Vicario, Ricard Farre, Simo Schwartz Jr., Sven C.D. van Ijzendoorn, Kazuto Kobayashi, Águeda Martinez-Barriocanal, and Diego Arango

Supplementary Figure 1



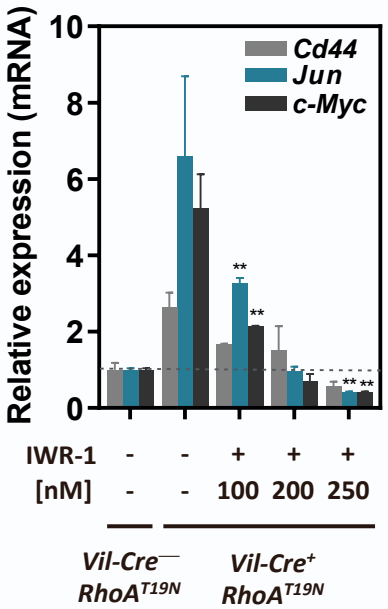
Supplementary Figure 1: Effects of RhoA inhibition on intestinal epithelial cell lineage fate, related to Figure 5. The number of total **(A-B)**, goblet **(C-D)**, enteroendocrine **(E-F)** and Paneth **(G-H)** cells were quantified in both villus and crypt compartments of the small intestine of control (*Vil-Cre*$^{-}$*;RhoA*^{T19N}) and RhoA^{T19N} (*Vil-Cre*$^{+}$*;RhoA*^{T19N}) mice. Total cells were detected by hematoxylin counterstaining in alkaline phosphatase stained preparations (differentiated enterocytes; **A-B)**, goblet cells were detected with alcian blue staining **(C-D)**, enteroendocrine cells were detected with Grimelius staining **(E-F)** and Paneth cells were immunostained with an anti-lysozyme antibody **(G-H)**. Arrows heads indicate examples of the different epithelial cell types. Scale bar: 50 µm.

Supplementary Figure 2



Supplementary Figure 2: Localization of apical and basolateral protein markers in the intestinal cells of control and RhoA^{T19N} mice, related to Figure 5. Immunofluorescence staining of Ezrin **(A-B)** p-ERM **(C-D)**, Crumbs **(E-F)**, E-cadherin **(G-H)**, and transferrin receptor (TfR) **(I-J)** in sections of the small intestine of control (*Vil-Cre^{-};RhoA^{T19N}*) and RhoA^{T19N} (*Vil-Cre^{+};RhoA^{T19N}*) mice. Scale bar: 25 µm.

Supplementary Figure 3



Supplementary Figure 3: Effects of the inhibition of RhoA^{T19N}-dependent Wnt activation, related to Figure 8. Relative levels of expression of the Wnt target genes *Cd44*, *Jun* and *c-Myc* in small intestinal organoids derived from *RhoA^{T19N}* (*Vil-Cre^{+};RhoA^{T19N}*) and control (*Vil-Cre^{-};RhoA^{T19N}*) mice after treatment with increasing concentrations of the Wnt inhibitor IWR-1-endo. The mean ±SEM in three independent experiments each of them carried out in triplicate is shown. Student's t-test *p<0.05; **p<0.01 (IWR1-treated *Vil-Cre^{+};RhoA^{T19N} vs Vil-Cre^{-};RhoA^{T19N}).*