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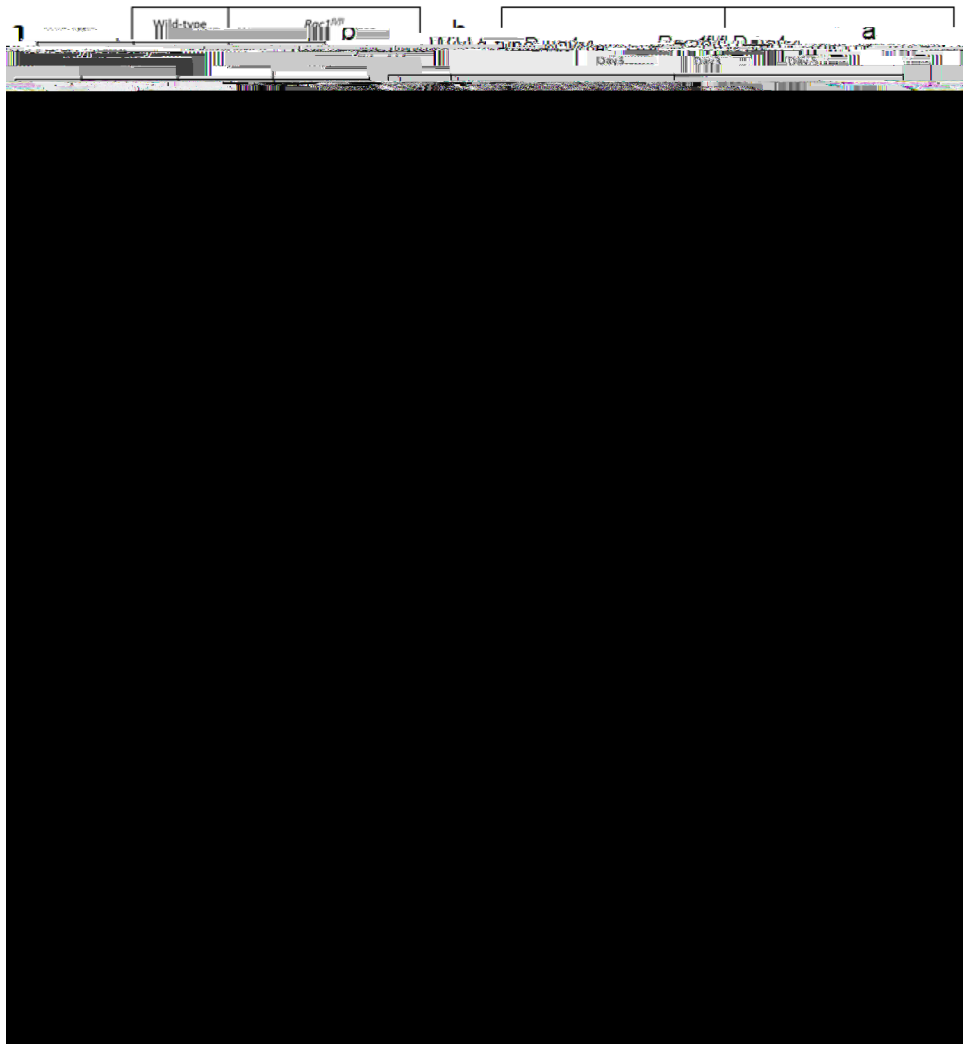


Fig. 1 Loss of Rac1 perturbs villus homeostasis. **a** Images showing H&E, BrdU incorporation and Cleaved caspase-3 IHC in *CreERT2 Rac1^{fl/fl}* (wild-type), *CreERT2 Rac1^{fl/fl}* (*CreERT2 Rac1^{fl/fl}*) mice day 3 post induction and *CreERT2 Rac1^{fl/fl}* (*CreERT2 Rac1^{fl/fl}*) mice 3 and 5 days post induction. Red arrow indicates disintegrating villus. Scale bar represents 100 μ m in each case (See amplified images in Supplementary Fig. 1d-f). **b** Scanning EM on *CreERT2 Rac1^{fl/fl}* (Wild-type) or *CreERT2 Rac1^{fl/fl}* (*CreERT2 Rac1^{fl/fl}*) intestines 4 days post induction. Red arrows indicate rounded, blebbing cells. Scale bar represents 100 μ m in the upper panels and 50 μ m in the lower panels. **c** *CreERT2 Rac1^{fl/fl}* driving expression of

Results and discussion

Rac1 is a key regulator of the actin cytoskeleton and is essential for the formation and maintenance of the intestinal villus. We first examined the effect of Rac1 loss on villus homeostasis in *CreERT2 Rac1^{fl/fl}* mice. Mice were induced with tamoxifen at 3 days of age and examined at 3, 4, and 5 days post-induction. H&E staining of the small intestine revealed that villi in *CreERT2 Rac1^{fl/fl}* mice were shorter and more irregular in shape compared to wild-type controls (Fig. 1a). BrdU incorporation analysis showed that the proliferative zone in the crypts of *CreERT2 Rac1^{fl/fl}* mice was expanded, suggesting that the loss of Rac1 leads to increased proliferation in the crypts. Cleaved caspase-3 staining revealed that there was an increase in apoptosis in the villi of *CreERT2 Rac1^{fl/fl}* mice, particularly at the tips of the villi (Fig. 1a). Scanning electron microscopy (SEM) of the intestinal surface 4 days post-induction showed that villi in *CreERT2 Rac1^{fl/fl}* mice were shorter and more rounded compared to wild-type controls (Fig. 1b). Red arrows in the SEM images indicate rounded, blebbing cells. These findings indicate that the loss of Rac1 leads to a perturbation of villus homeostasis, characterized by increased proliferation in the crypts, increased apoptosis in the villi, and a change in villus morphology.

To determine whether the loss of Rac1 leads to a change in the expression of other genes, we performed a microarray analysis of the small intestine in *CreERT2 Rac1^{fl/fl}* mice 4 days post-induction. We identified several genes that were upregulated in the *CreERT2 Rac1^{fl/fl}* mice, including *Vil*, *Clca4*, *Clca3*, *Clca1*, *Clca2*, *Clca5*, *Clca6*, *Clca7*, *Clca8*, *Clca9*, *Clca10*, *Clca11*, *Clca12*, *Clca13*, *Clca14*, *Clca15*, *Clca16*, *Clca17*, *Clca18*, *Clca19*, *Clca20*, *Clca21*, *Clca22*, *Clca23*, *Clca24*, *Clca25*, *Clca26*, *Clca27*, *Clca28*, *Clca29*, *Clca30*, *Clca31*, *Clca32*, *Clca33*, *Clca34*, *Clca35*, *Clca36*, *Clca37*, *Clca38*, *Clca39*, *Clca40*, *Clca41*, *Clca42*, *Clca43*, *Clca44*, *Clca45*, *Clca46*, *Clca47*, *Clca48*, *Clca49*, *Clca50*, *Clca51*, *Clca52*, *Clca53*, *Clca54*, *Clca55*, *Clca56*, *Clca57*, *Clca58*, *Clca59*, *Clca60*, *Clca61*, *Clca62*, *Clca63*, *Clca64*, *Clca65*, *Clca66*, *Clca67*, *Clca68*, *Clca69*, *Clca70*, *Clca71*, *Clca72*, *Clca73*, *Clca74*, *Clca75*, *Clca76*, *Clca77*, *Clca78*, *Clca79*, *Clca80*, *Clca81*, *Clca82*, *Clca83*, *Clca84*, *Clca85*, *Clca86*, *Clca87*, *Clca88*, *Clca89*, *Clca90*, *Clca91*, *Clca92*, *Clca93*, *Clca94*, *Clca95*, *Clca96*, *Clca97*, *Clca98*, *Clca99*, and *Clca100*. We also identified several genes that were downregulated, including *Rac1*, *Rac2*, *Rac3*, *Rac4*, *Rac5*, *Rac6*, *Rac7*, *Rac8*, *Rac9*, *Rac10*, *Rac11*, *Rac12*, *Rac13*, *Rac14*, *Rac15*, *Rac16*, *Rac17*, *Rac18*, *Rac19*, *Rac20*, *Rac21*, *Rac22*, *Rac23*, *Rac24*, *Rac25*, *Rac26*, *Rac27*, *Rac28*, *Rac29*, *Rac30*, *Rac31*, *Rac32*, *Rac33*, *Rac34*, *Rac35*, *Rac36*, *Rac37*, *Rac38*, *Rac39*, *Rac40*, *Rac41*, *Rac42*, *Rac43*, *Rac44*, *Rac45*, *Rac46*, *Rac47*, *Rac48*, *Rac49*, *Rac50*, *Rac51*, *Rac52*, *Rac53*, *Rac54*, *Rac55*, *Rac56*, *Rac57*, *Rac58*, *Rac59*, *Rac60*, *Rac61*, *Rac62*, *Rac63*, *Rac64*, *Rac65*, *Rac66*, *Rac67*, *Rac68*, *Rac69*, *Rac70*, *Rac71*, *Rac72*, *Rac73*, *Rac74*, *Rac75*, *Rac76*, *Rac77*, *Rac78*, *Rac79*, *Rac80*, *Rac81*, *Rac82*, *Rac83*, *Rac84*, *Rac85*, *Rac86*, *Rac87*, *Rac88*, *Rac89*, *Rac90*, *Rac91*, *Rac92*, *Rac93*, *Rac94*, *Rac95*, *Rac96*, *Rac97*, *Rac98*, *Rac99*, and *Rac100*.

CreER^{T2} *Rac1* *Vil-*



Fig. 2 VAV3 and TIAM1 are upregulated following APC loss. a Heatmap derived from RNA-seq analysis comparing whole tissue from wild-type ($n = 3$) and APC intestines ($n = 3$) = 3 biologically independent animals for both APC and WT intestinal tissue. Log₂

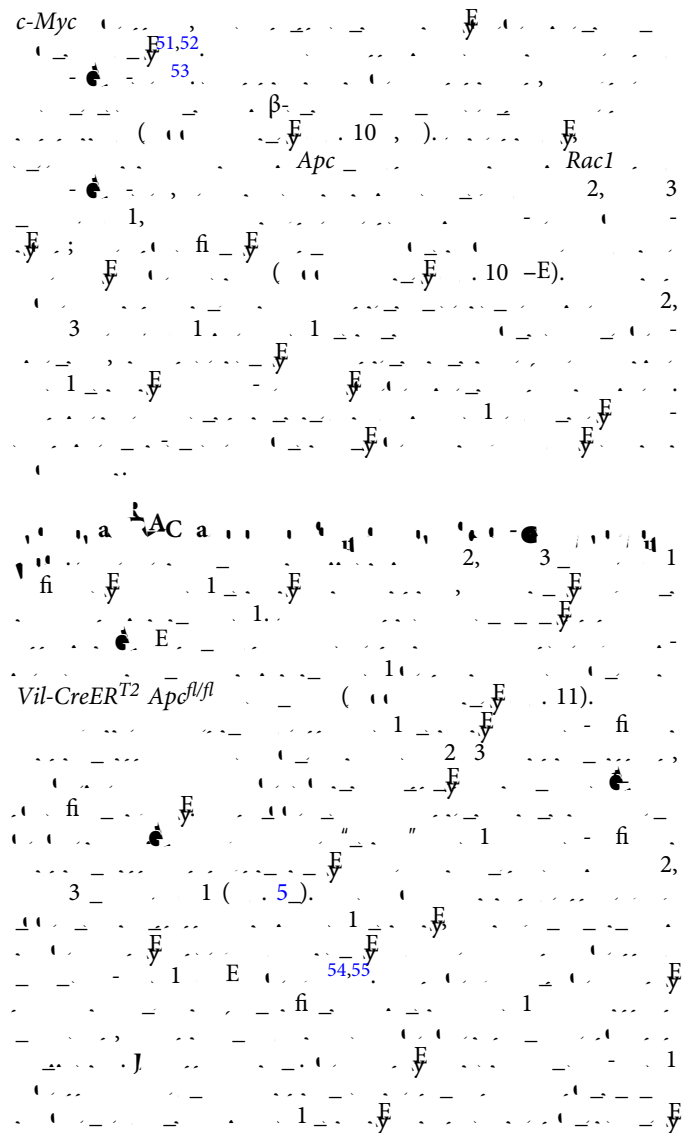
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Fig. 4 Loss of free GEF is able to suppress the loss of Apc phenotype. a RNAscope staining for *Rac1* in intestine from *Vil-CreER^{T2} Apc^{fl/fl}*.

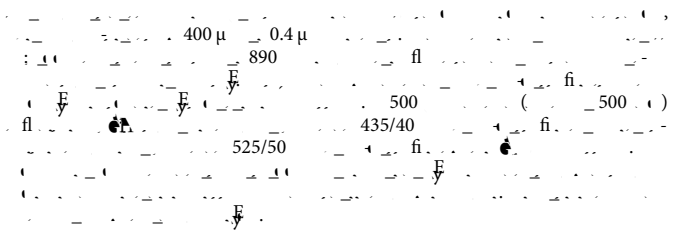


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Acknowledgements