

**Figure S1** The Ras protein level is increased in Lgr5+ stem cells in the murine small intestine. **A-B** Confocal immunofluorescence of **A** Ras (green) and **B** Lgr5-GFP (green) in *Lgr5- EGFP* mouse intestinal sections. Lgr5+ ISCs indicated by arrows. Scale bars represent 20 μm.



**Figure S2** Loss of Wdr76 affects lineage differentiation in the murine small intestine. **A** Quantification of the length of small intestinal crypts. All measurements or counts are based on at least 10 crypts per 5 fields of view. \*\*\* p<0.001. **B-C** Comparative confocal immunofluorescence analysis of lineage differentiation into goblet cells (mucin2, red) and Paneth cell (lysozyme, red) in intestinal sections of **B** *Wdr76+/+* and *Wdr76-/-*mice and **C** *Wdr76+/+*; *ApcMin/+* and *Wdr76-/-*; *ApcMin/+* mice. Boxes indicate the enlarged areas. **B** Crypts and **C** tumors are indicated by dotted lines. Scale bars represent 20 μm.



**Figure S3** RAS protein level is increased in CSC-like cells compared with that in non-CSC-like cells in CRC. **A** Brightfield images of spheroid cultures of CD44lowCD133-CD166- and CD44highCD133+CD166+ cells sorted from D-MT cells by flow cytometry for the indicated antibodies. Scale bars represent 20 μm. **B** Western blots of extracts from CD44lowCD133-CD166- and CD44highCD133+CD166+ cells sorted from D-MT cells using the indicated antibodies.



**Figure S4** Cytosolic WDR76 destabilizes RAS and suppresses CSC activation in CRC. **A** Western blots of extracts from D-MT cells stably expressing GFP-Control, GFP-WDR76FL, or GFP-WDR76ΔNLS using the indicated antibodies. **B-C** Immunoprecipitation **B** and ubiquitination **C** of K-RAS in ALLN-treated (25 μg/mL, 12 h) D-MT cells stably expressing GFP-Control, GFP-WDR76FL, or GFP-WDR76ΔNLS with immunoblotting against the indicated antibodies. **D-G** Five-day spheroid cultures of D-MT cells stably expressing GFP-Control, GFP-WDR76FL, or GFP-WDR76ΔNLS were analyzed. **D** Number and size of spheroids were quantified using Image J. \*\*\* p<0.001. **E** Cell viability assay was performed at the indicated culture day. \*\*\* p<0.001. **F** Immunocytochemistry was performed using the indicated antibodies and counterstaining with DAPI. Scale bars represent 20 μm. **G** Relative mRNA levels of the indicated genes were quantified by RT-qPCR. \* p<0.05, \*\* p<0.01.