Supplementary data

for " Organoid modelling identifies that DACH1 functions as a tumour promoter in colorectal cancer by modulating BMP signalling "

Supplementary Figures:

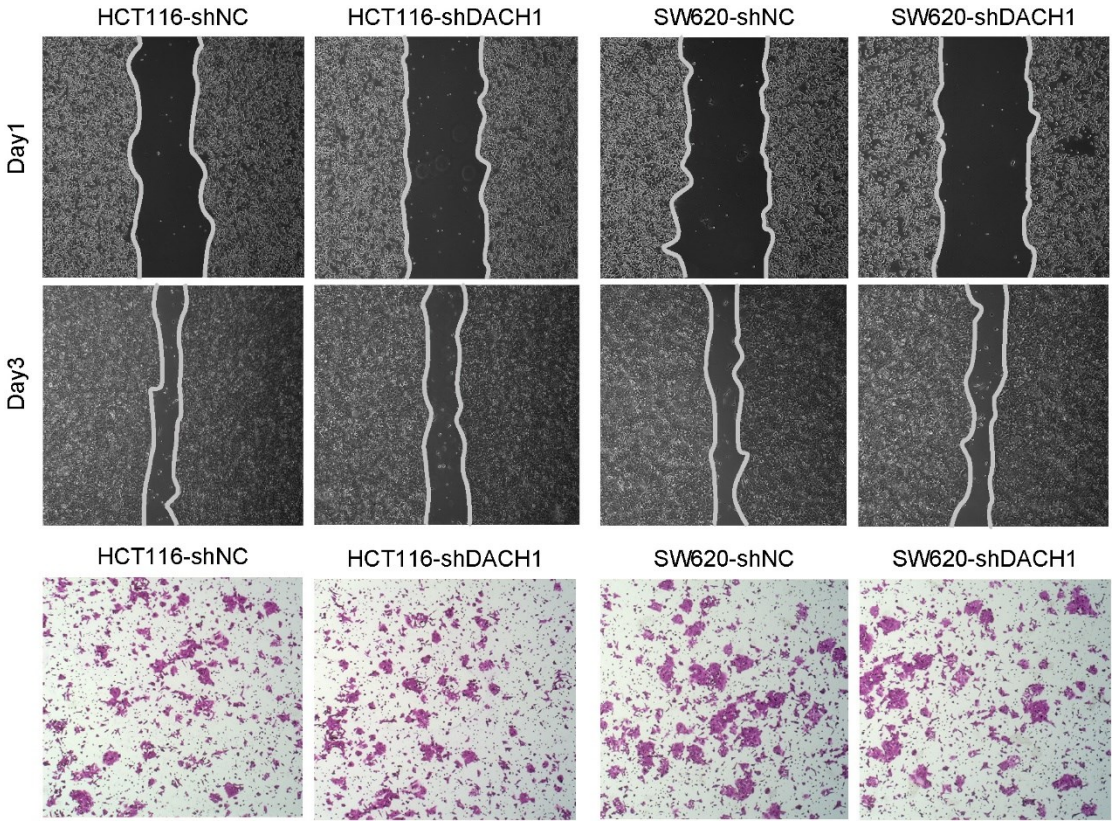

Figure S1. DACH1 showed no significant effects on cell migration in vitro Knocking down DACH1 in HCT116 cells and SW620 cells did not affect the migration of cells in vitro, as detected by scratch assays and transwell assays.

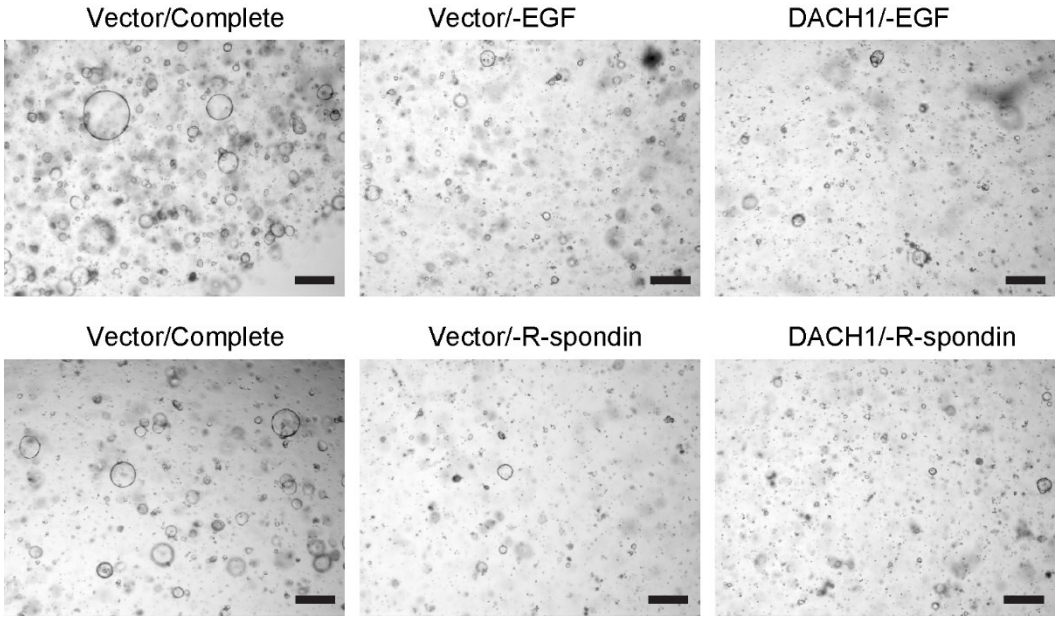

Figure S2. DACH1 overexpression did not compensate for the depletion of EGF or R-spondin

Organoids transfected with empty vector showed normal organoid formation and growth in complete organoid medium. Depletion of EGF or R-spondin resulted in gross inhibition of organoid formation and growth in both control organoids and DACH1-overexpressing organoids. Scale bars=200 μm.

Supplementary methods:

Wound healing assays and transwell assays

To assess colon cancer cell migratory capacity, wound healing assays and transwell assays were conducted. For wound healing assays, 4×10^5 cells were seeded in a well of a 6-well plate. After cells grew to form a monolayer, the well was scratched using a 200 μL pipette tip. Wound healing assay images were taken on days 1 and 3. For transwell assays, 1×10^4 cells were plated in the upper chamber of 8 μm porous transwell inserts (Corning). The cells were

incubated for 3 days in 24-well plates. Cells were fixed with 4% paraformaldehyde/PBS (Yeasen) for 30 min and stained with 2% crystal violet for 30 min at room temperature. After removing the non-migrated cells in the upper chamber with a cotton swab, the stained cells were quantified under a light microscope, and representative images were captured.

Main primers used in the study：

Primers used for qRT-PCR	Sequence
NBL1-qRT-F-primer	AGTCCACAGAGTCCCTGGTT
NBL1-qRT-R-primer	GCAGCTACAGTGCAGGATCT
BMP7-qRT-F-primer	TAGCCATTTCCTCACCGACG
BMP7-qRT-R-primer	ACGTCTCATTGTCGAAGCGT
SMAD4-qRT-F-primer	CTCATGTGATCTATGCCCGTC
SMAD4-qRT-R-primer	AGGTGATACAACTCGTTCGTAGT
LGR5-qRT-F-primer	CTCCCAGGTCTGGTGTGTTG
LGR5-qRT-R-primer	GAGGTCTAGGTAGGAGGTGAAG
Notch1-qRT-F-primer	CGCTGACGGAGTACAAGTG
Notch1-qRT-R-primer	GTAGGAGCCGACCTCGTTG
DACH1-qRT-F-primer	GGGGCTTGCATACGGTCTAC
DACH1-qRT-R-primer	CGAACTTGTTCCACATTGCACA
HES1-qRT-F-primer	TCAACACGACACCGGATAAAC
HES1-qRT-R-primer	GCCGCGAGCTATCTTTCTTCA
ATOH8-qRT-F-primer	CAAGAAGCGCAAGGAGTGAC
ATOH8-qRT-R-primer	GCATCTTGGAGAAGACCACGA
TGFB3-qRT-F-primer	GGAAAACACCGAGTCGGAATAC
TGFB3-qRT-R-primer	GCGGAAAACCTTGGAGGTAAT
MSX1-qRT-F-primer	CGAAGTCTGATCCCTGCCAA
MSX1-qRT-R-primer	ACACCGATTTCTCTGCGCTT
FKBP4-qRT-F-primer	GAAGGCGTGCTGAAGGTCAT
FKBP4-qRT-R-primer	TGCCATCTAATAGCCAGCCAG
FKBP8-qRT-F-primer	AACCTTCCAACAAGACGATCC
FKBP8-qRT-R-primer	CCCAGCATTTTCCGGTACAAG
ID1-qRT-F-primer	CTGCTCTACGACATGAACGG
ID1-qRT-R-primer	GAAGGTCCCTGATGTAGTCGAT
MAPK3-qRT-F-primer	CTACACGCAGTTGCAGTACAT
MAPK3-qRT-R-primer	CAGCAGGATCTGGATCTCCC
GAPDH-qRT-F-primer	GGAGCGAGATCCCTCCAAAAT
GAPDH-qRT-R-primer	GGCTGTTGTCATACTTCTCATGG