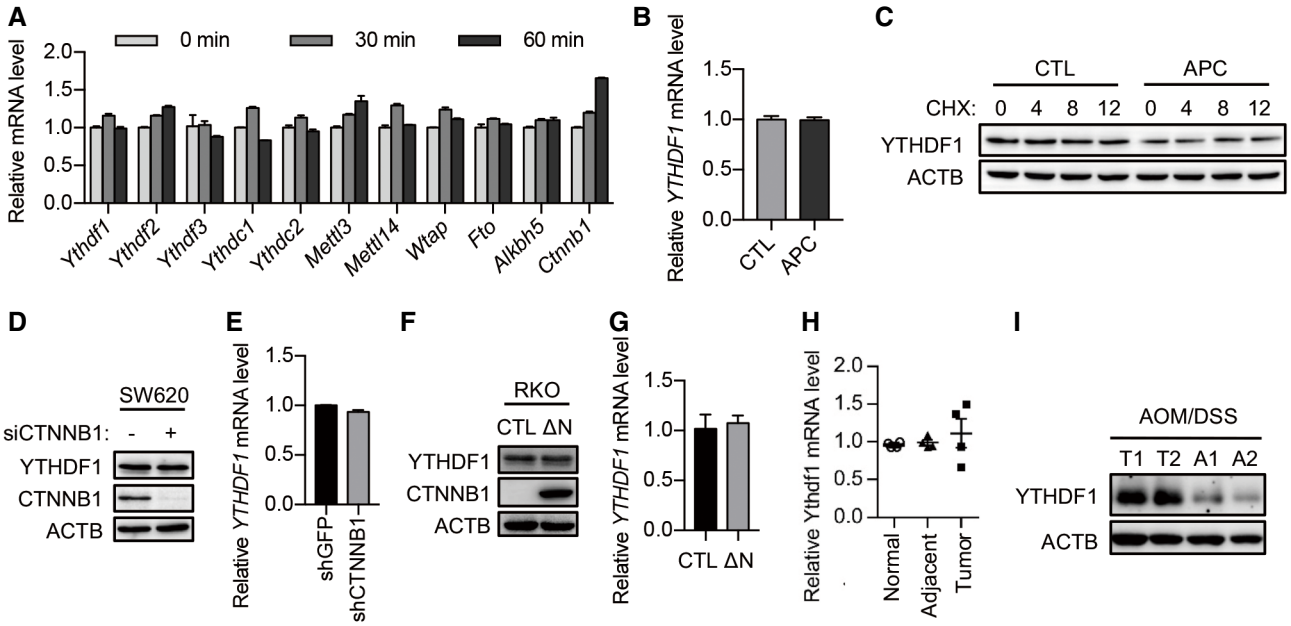
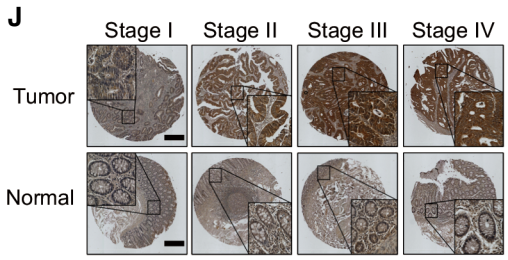
Expanded View Figures





K

AJCC stage	Total(%)	-	+	++	P
I	12(16.0%)	0(0.0%)	6(50.0%)	6(50.0%)	0.046
II	23(30.7%)	1(4.3%)	2(8.7%)	20(87.0%)	
III	31(41.3%)	0(0.0%)	2(6.5%)	29(93.5%)	
IV	9(12.0%)	1(11.1%)	1(11.1%)	7(77.8%)	

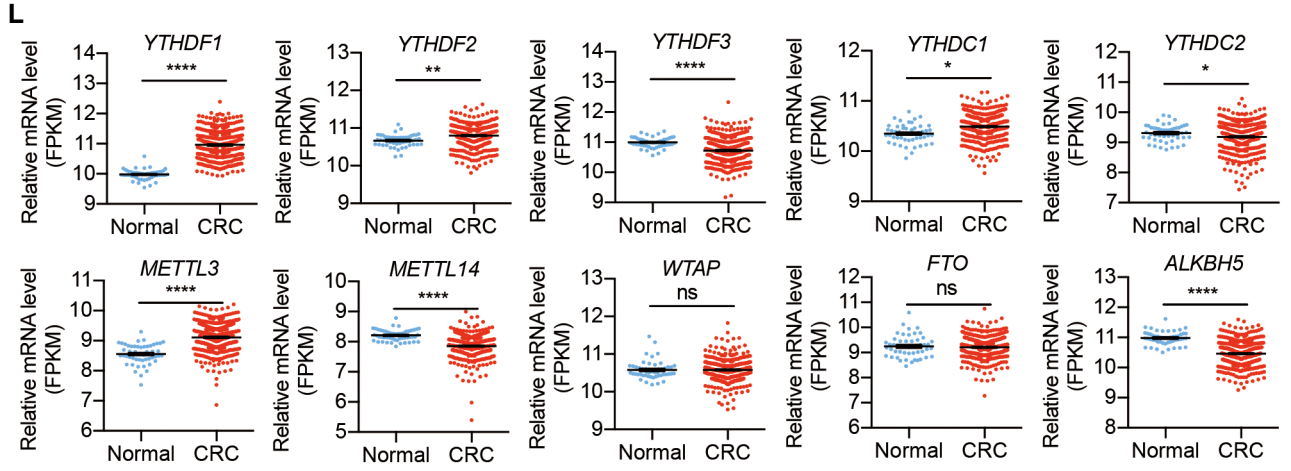


Figure EV1.

Figure EV1. Wnt signaling promotes YTHDF1 expression.

A RT–qPCR analysis of mouse intestinal crypt treated with Wnt3a (60 ng/ml) for the indicated time. Data are represented as mean ± SEM. Three biological replicates.
B RT–qPCR analysis of SW620 cells with or without APC overexpression. Data are represented as mean ± SEM. Three biological replicates.
C Immunoblot analysis of SW620 cells with or without APC overexpression treated with CHX for the indicated time.
D Immunoblot analysis of SW620 cells with or without β-catenin (CTNNB1) knockdown.
E RT–qPCR analysis of SW620 cells with or without β-catenin (CTNNB1) knockdown. Data are represented as mean ± SEM. Three biological replicates.
F Immunoblot analysis of RKO cells expressing a non-degradable β-catenin mutant (N90).
G RT–qPCR analysis of RKO cells expressing a non-degradable β-catenin mutant (N90). Data are represented as mean ± SEM. Three biological replicates.
H *Ythdf1* mRNA expression in intestinal tissue from 4 pairs of $Apc^{+/+}$ (Normal), $Apc^{min/+}$ tumor (Tumor), and $Apc^{min/+}$ tumor-adjacent tissues (Adjacent). Data are represented as mean ± SEM. Three biological replicates.
I YTHDF1 protein expression in intestinal tissue from 2 pairs of AOM/DSS-treated mice. T: tumor tissue; A: adjacent non-tumor tissue.
J YTHDF1 staining in matched samples of human CRC tissue at different stages and adjacent non-tumor tissues. Scale bar, 450 μm.
K IHC scoring and analysis of YTHDF1 expression in (J). Chi-square was used for analysis.
L Analysis of mRNA level of m^6A-related genes from The Cancer Genome Atlas (TCGA) datasets. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ (*t*-test).

Source data are available online for this figure.

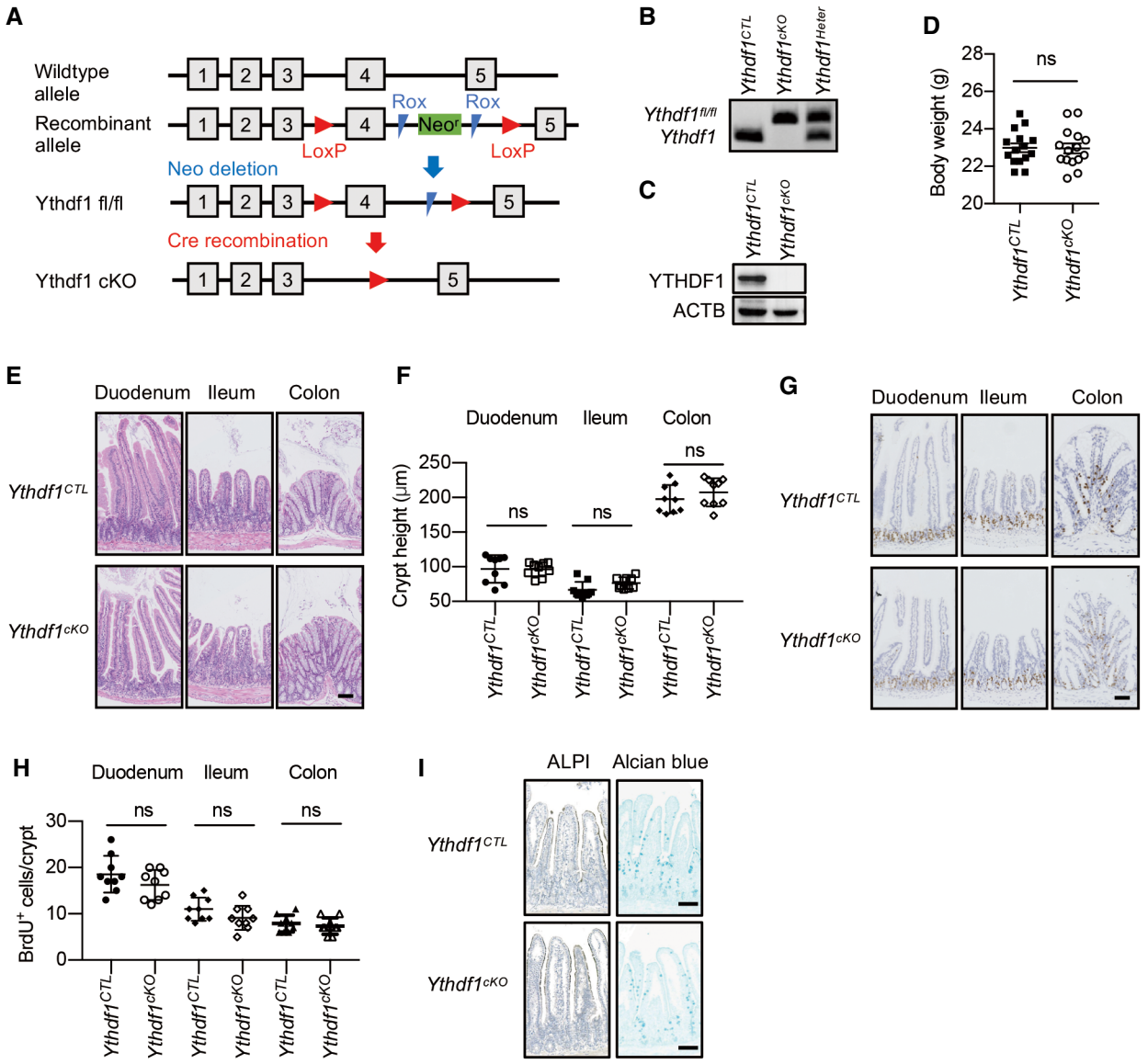


Figure EV2.

Figure EV2. YTHDF1 is dispensable for intestinal development.

A Generation of *Ythdf1* conditional knockout (*Ythdf1*^{cKO}) mouse.
B Genotyping of wild-type (*Ythdf1*^{CTL}), heterogeneous (*Ythdf1*^{Heter}), and *Ythdf1*^{cKO} mice.
C Immunoblot analysis of intestinal epithelia from *Ythdf1*^{CTL} and *Ythdf1*^{cKO} mice.
D Bodyweight of *Ythdf1*^{CTL} and *Ythdf1*^{cKO} mice at the age of 2 months. Data are represented as mean ± SEM. (15 mice for each group, *t*-test)
E H&E staining of duodenum, ileum, and colon from wild-type (*Ythdf1*^{CTL}) and *Ythdf1*^{cKO} mice.
F The quantification of crypt height in (E). Scale bar, 50 μm. Data are represented as mean ± SEM. (9 mice for each group, *t*-test)
G BrdU staining of duodenum, ileum, and colon from wild-type (*Ythdf1*^{CTL}) and *Ythdf1*^{cKO} mice.
H The quantification of BrdU^{+} cells per crypt in (G). Scale bar, 50 μm. Data are represented as mean ± SEM. (9 mice for each group, *t*-test)
I Alkaline phosphatase staining (ALPI) and Alcian blue staining (Alcian blue) of small intestine from wild-type (*Ythdf1*^{CTL}) and *Ythdf1*^{cKO} mice. Scale bar, 50 μm.

Source data are available online for this figure.

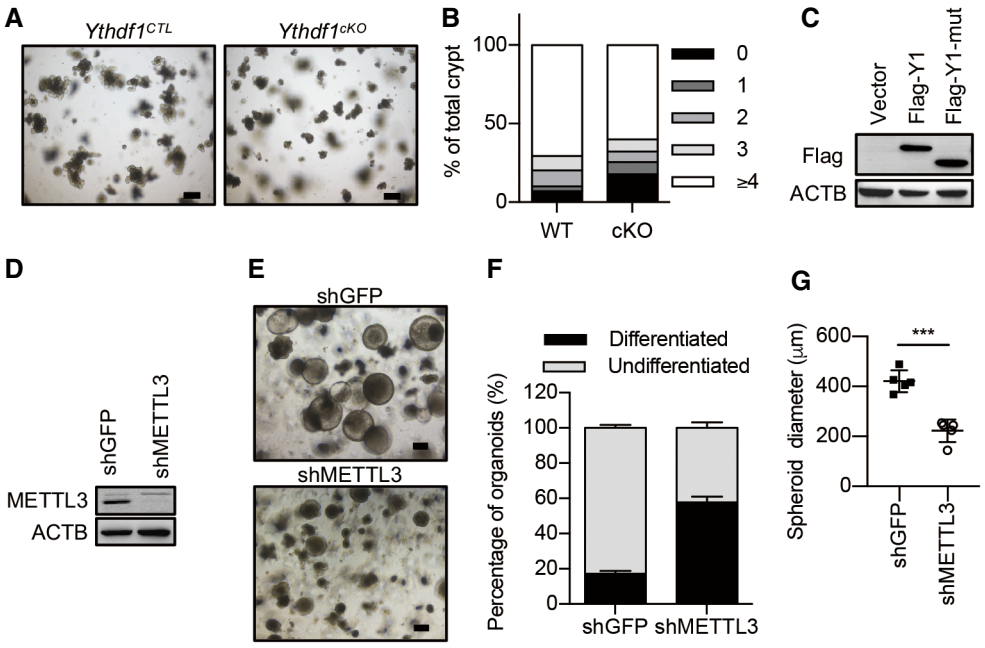


Figure EV3. The role of m^{6}A in the maintenance of intestinal stemness.

A Morphology of organoids from wild-type (*Ythdf1*^{CTL}) and *Ythdf1*^{cKO} mice at day 6. Scale bar, 250 μm.
B Quantification of percentage of organoid budding in (A) (3 independent replicates).
C Immunoblot analysis of organoids infected with lentivirus expression Flag-tagged YTHDF1 (Flag-Y1) and YTH domain-deleted mutant (Flag-Y1-mut).
D Immunoblot analysis of organoids with or without METTL3 knockdown.
E Morphology of organoids in Wnt3a-conditioned medium with or without METTL3 knockdown. Scale bar, 200 μm.
F Quantification of differentiated versus undifferentiated organoids from (E). Data are represented as mean ± SEM. Three biological replicates.
G Spheroid diameter analysis from (E). Data are represented as mean ± SEM. ***$P < 0.001$ (3 biological replicates, *t*-test).

Source data are available online for this figure.

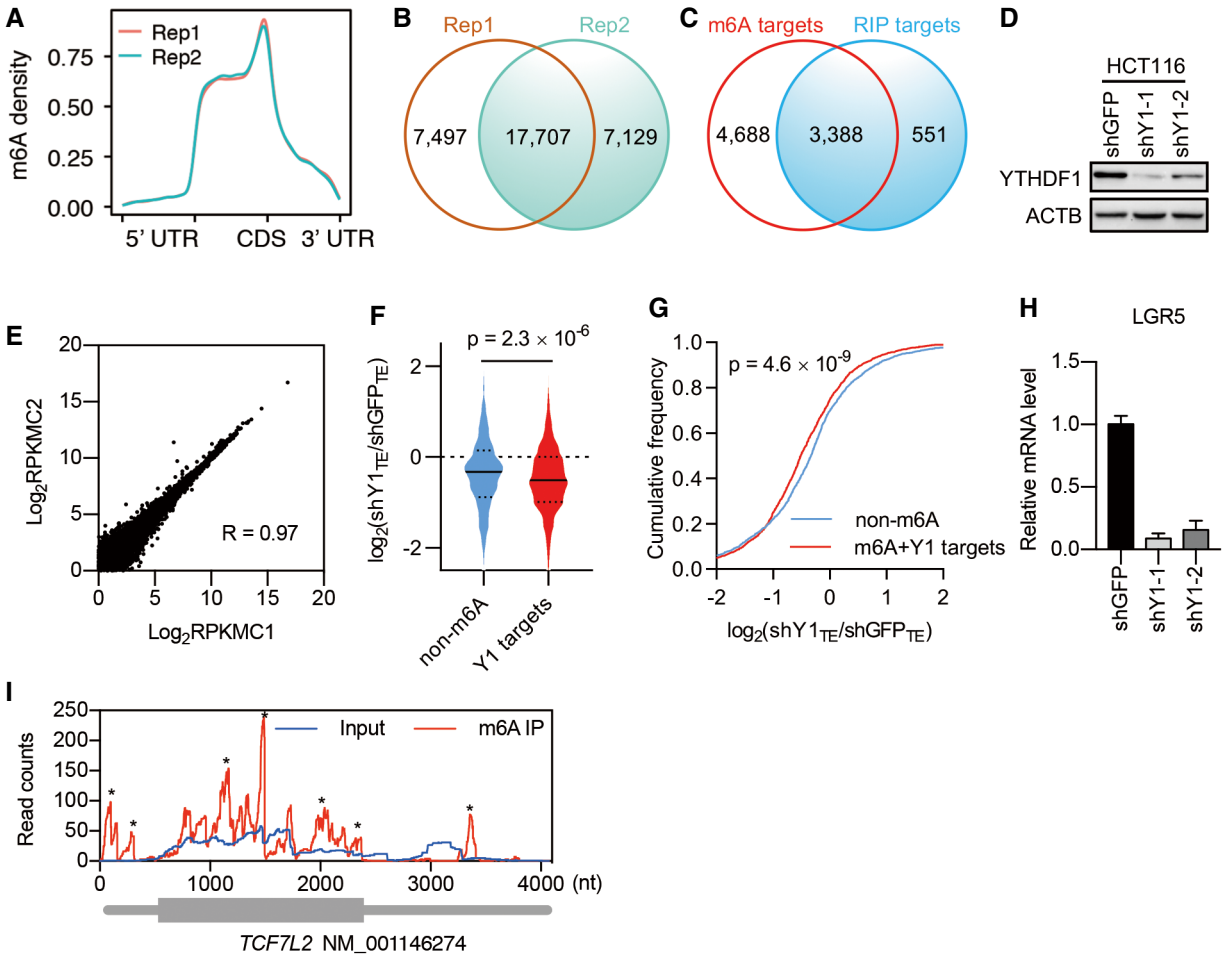


Figure EV4. Analysis of YTHDF1-regulated mRNA translation.

A Metagene plot of m^{6}A peak distribution from two biological replicates.
B m^{6}A peaks identified from two biological replicates.
C Overlap of m^{6}A-containing transcripts (m^{6}A targets) with YTHDF1-binding transcripts (RIP targets).
D YTHDF1 knockdown efficiency in HCT116 cells.
E Correlation of ribosome density between two control biological replicates.
F Violin plots showing translational efficiency (TE) change after YTHDF1 knockdown for non-methylated (non-m^{6}A) transcripts and YTHDF1 targets. The upper and lower quartiles and the median are indicated for each group. Mann–Whitney test.
G Cumulative distributions of TE change after YTHDF1 knockdown for non-m^{6}A transcripts and YTHDF1 targets as in (E). Kolmogorov–Smirnov test.
H RT–qPCR analysis of *LGR5* expression in HCT116 cells after YTHDF1 knockdown. Data are represented as mean ± SEM. 3 biological replicates.
I m^{6}A peak distribution within TCF7L2 transcript. * indicates the predicted m^{6}A peak.

Source data are available online for this figure.

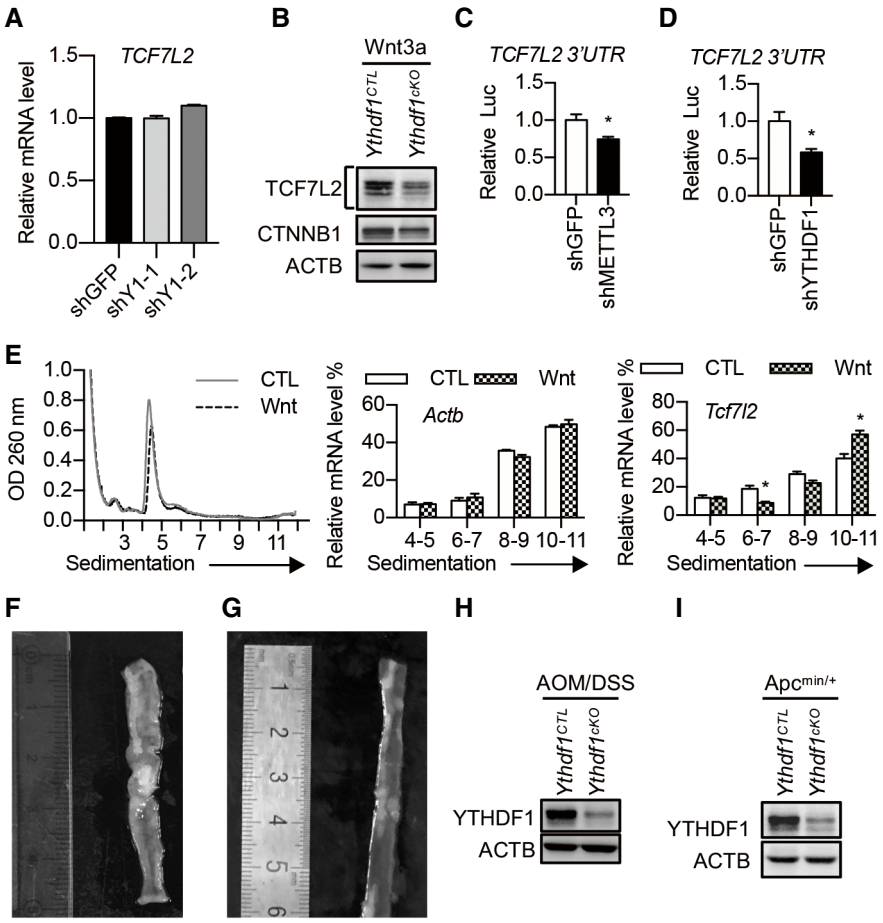


Figure EV5. YTHDF1 regulates the translation of TCF7L2 during Wnt activation.

A TCF7L2 mRNA level in control and YTHDF1 knockdown HCT116 cells. Data are represented as mean ± SEM. 3 biological replicates.

B Immunoblot analysis of intestinal crypts from WT or *Ythdf1* cKO mice treated with Wnt3a (60 ng/ml) for 30 min.

C Dual-Luciferase Assay with a construct bearing the 3′UTR of *TCF7L2* in HCT116 cells with or without METTL3 knockdown. Data are represented as mean ± SEM. *$P < 0.05$ (3 biological replicates, *t*-test).

D Dual-Luciferase Assay with a construct bearing the 3′UTR of *TCF7L2* in HCT116 cells with or without YTHDF1 knockdown. Data are represented as mean ± SEM. *$P < 0.05$ (3 biological replicates, *t*-test).

E Polysome profiles of mouse intestinal crypt treated with or without Wnt3a treatment for 30 min. The left panel is the same experiment as Fig 1B. The right panels show the distributions of *Tcf7l2* and *Actb* in polysome fractions. Data are represented as mean ± SEM. *$P < 0.05$ (3 biological replicates, *t*-test).

F Mouse colon after 6 weeks of AOM/DSS induction.

G Small intestine from a 3-month-old $Apc^{min/+}$ mouse.

H Western blot showing YTHDF1 knockout efficiency in AOM/DSS-induced tumors.

I Western blot showing YTHDF1 knockout efficiency in $Apc^{min/+}$ tumors.

Source data are available online for this figure.