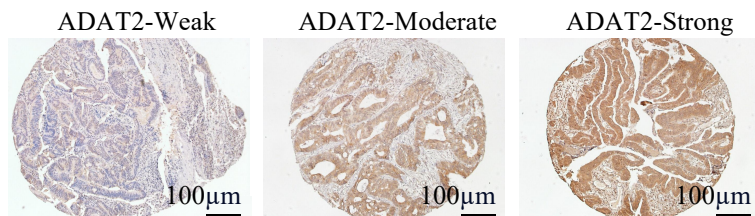
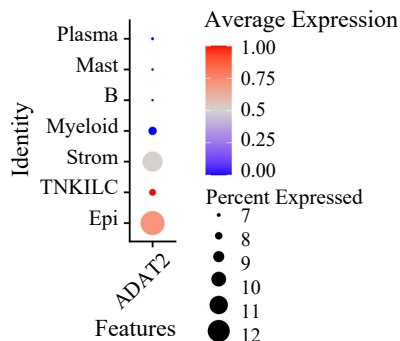
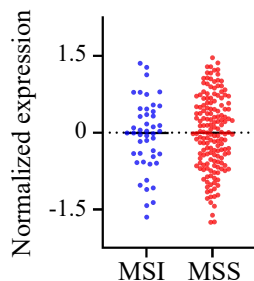


ACohort IV – IHC on tissue microarray**B****C****Figure S1. ADAT2 expression in CRC.**

(A) Representative IHC of ADAT2 protein expression in a subset of paired samples from Cohort IV.

(B) Single-cell analysis of ADAT2 expression in CRC tissues (GSE178341).

(C) Analysis of ADAT2 mRNA expression in the TCGA-COAD microarray cohort (AgilentG4502A_07_3) comparing microsatellite stable (MSS, N=163) and microsatellite instable (MSI, includes MSI-H and MSI-L, N=46) tumors.

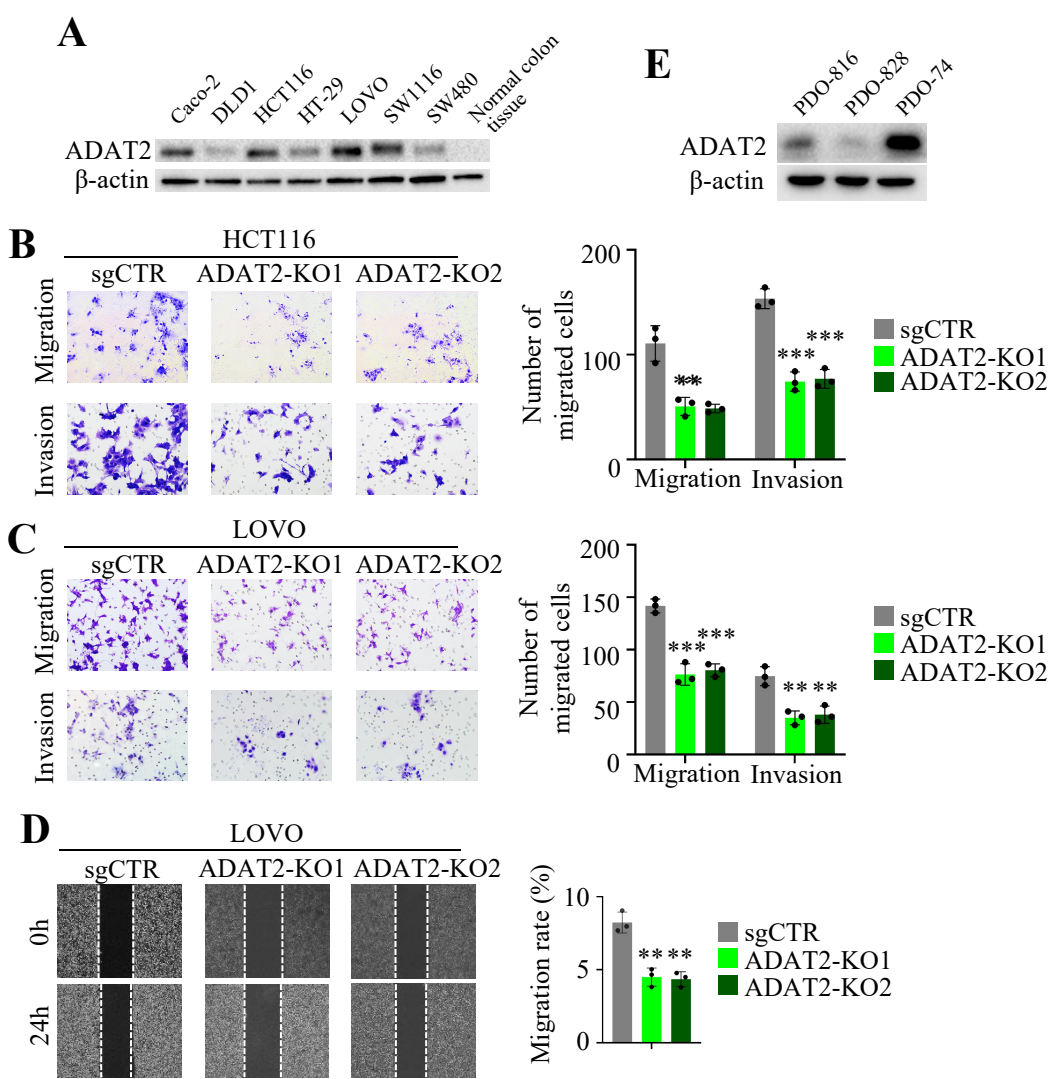


Figure S2. ADAT2 drives pro-metastatic phenotypes in CRC.

(A) ADAT2 protein expression in CRC cell lines by western blot.

(B) Transwell migration and Matrigel invasion assays of control and ADAT2-knockout HCT116 cells (*left*). Statistical analysis (N=3) (*right*).

(C) Transwell migration and Matrigel invasion assays of control and ADAT2-knockout LOVO cells (*left*). Statistical analysis (N=3) (*right*).

(D) Wound healing assay of control and ADAT2-knockout LOVO cells (*left*). Statistical analysis (N=3) (*right*).

(E) Western blot analysis of ADAT2 protein expression in CRC patient-derived organoids.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

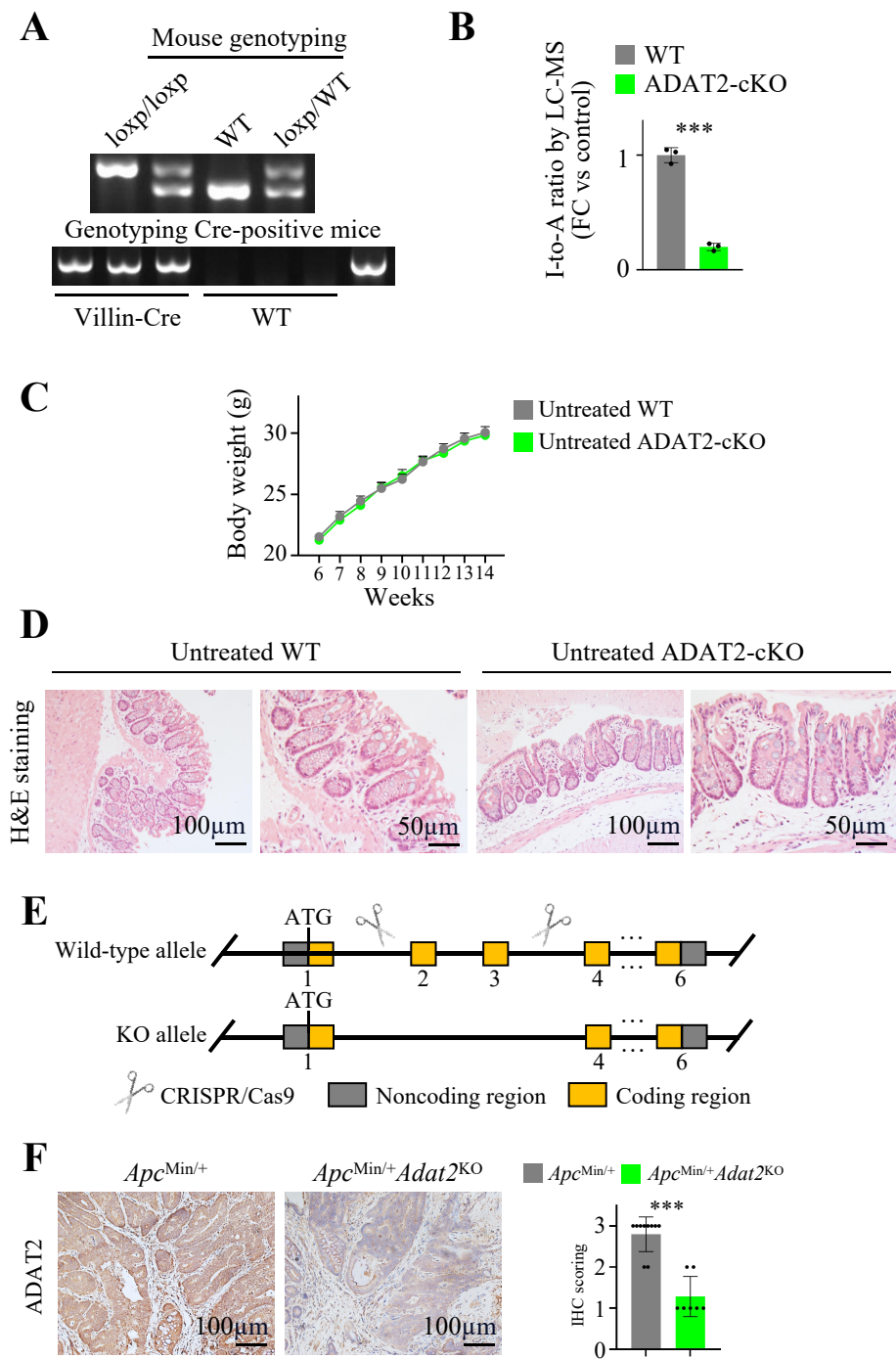


Figure S3. Genetic ablation of ADAT2 suppresses colorectal tumorigenesis *in vivo*.

(A) PCR genotyping confirmed the presence of the floxed *Adat2* allele and *Villin-Cre* transgene.

(B) LC-MS analysis of I-to-A ratio in colon tissues from ADAT2 cKO mice (N=3) and wildtype littermates (N=3).

(C) Body weight analysis of ADAT2 cKO mice and wildtype littermates (N=3).

(D) Histological assessment of normal colon tissues from untreated wildtype and untreated ADAT2 cKO mice.

(E) Schematic for whole body *Adat2*-KO mice.

(F) Immunohistochemistry analysis of ADAT2 expression in colon tumors from *Apc*^{Min/+} *ADAT2*^{KO} (N=7) mice compared with controls (N=10).

P*<0.05; *P*<0.01; ****P*<0.001.

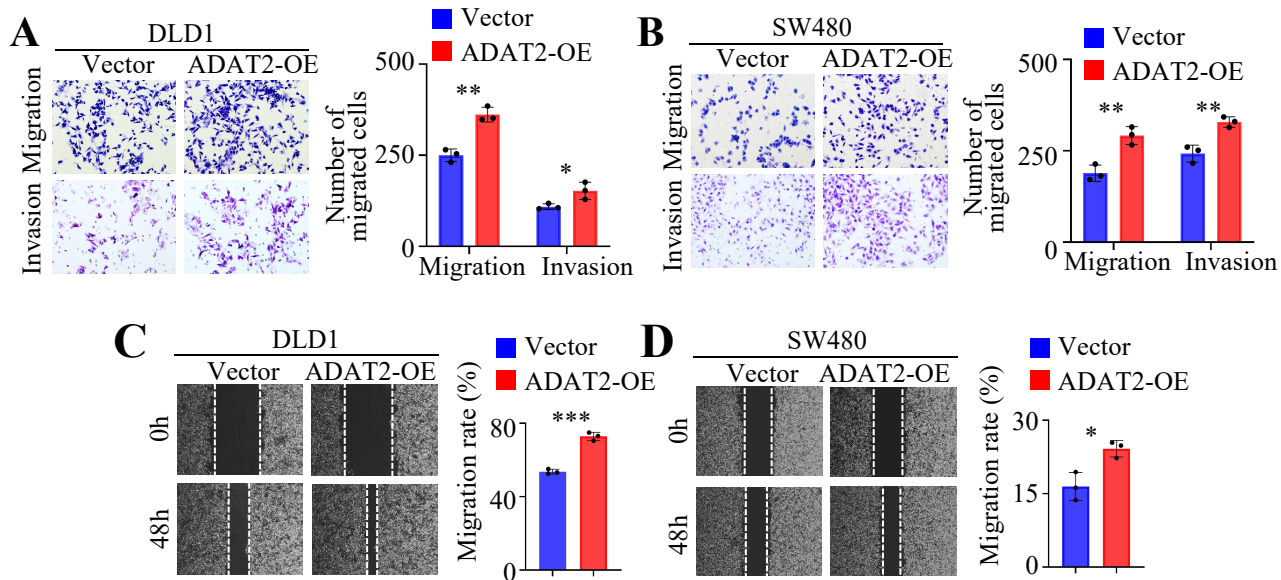


Figure S4. ADAT2 overexpression promotes pro-metastatic phenotypes in CRC cells.

(A) Transwell migration and Matrigel invasion assays of control and ADAT2-overexpressing DLD1 cells (*left*). Statistical analysis (N=3) (*right*).

(B) Transwell migration and Matrigel invasion assays of control and ADAT2-overexpressing SW480 cells (*left*). Statistical analysis (N=3) (*right*).

(C) Wound healing assay of control and ADAT2-overexpressing DLD1 cells (*left*). Statistical analysis (N=3) (*right*).

(D) Wound healing assay of control and ADAT2-overexpressing SW480 cells (*left*). Statistical analysis (N=3) (*right*).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

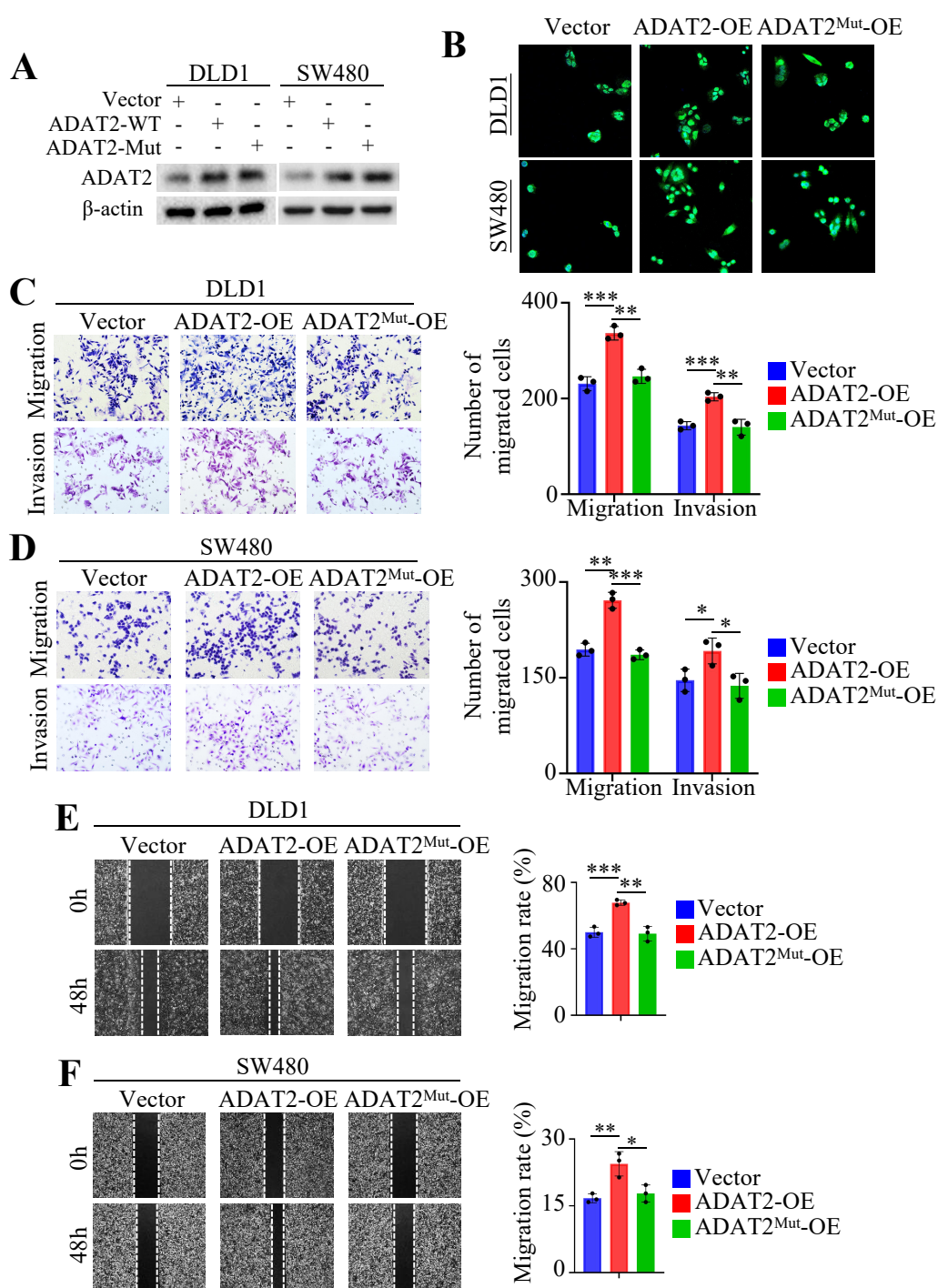


Figure S5. ADAT2 A-to-I catalytic activity is required for its pro-metastatic function.

(A) Western blot validated overexpression of ADAT2-WT and catalytically-dead ADAT2 mutant in DLD1 and SW480 cells.

(B) Subcellular localization of wild-type and catalytically dead ADAT2.

(C) Transwell migration and Matrigel invasion assays of control, ADAT2-WT-overexpressing and ADAT2 mutant-overexpressing DLD1 cells (*left*). Statistical analysis (N=3) (*right*).

(D) Transwell migration and Matrigel invasion assays of control, ADAT2-WT-overexpressing and ADAT2 mutant-overexpressing SW480 cells (*left*). Statistical analysis (N=3) (*right*).

(E) Wound healing assay of control, ADAT2-WT-overexpressing and ADAT2 mutant-overexpressing DLD1 cells (*left*). Statistical analysis (N=3) (*right*).

(F) Wound healing assay of control, ADAT2-WT-overexpressing and ADAT2 mutant-overexpressing SW480 cells (*left*). Statistical analysis (N=3) (*right*).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

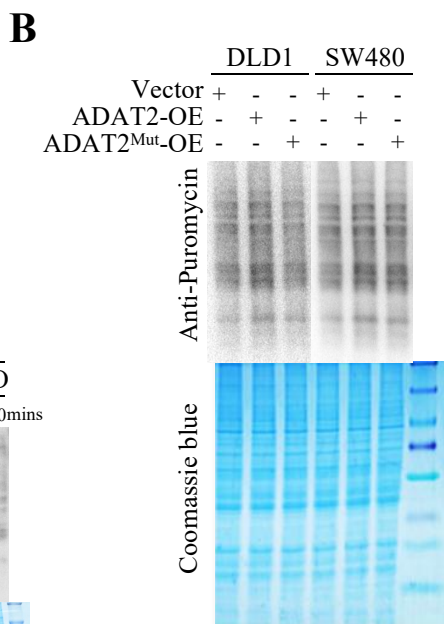
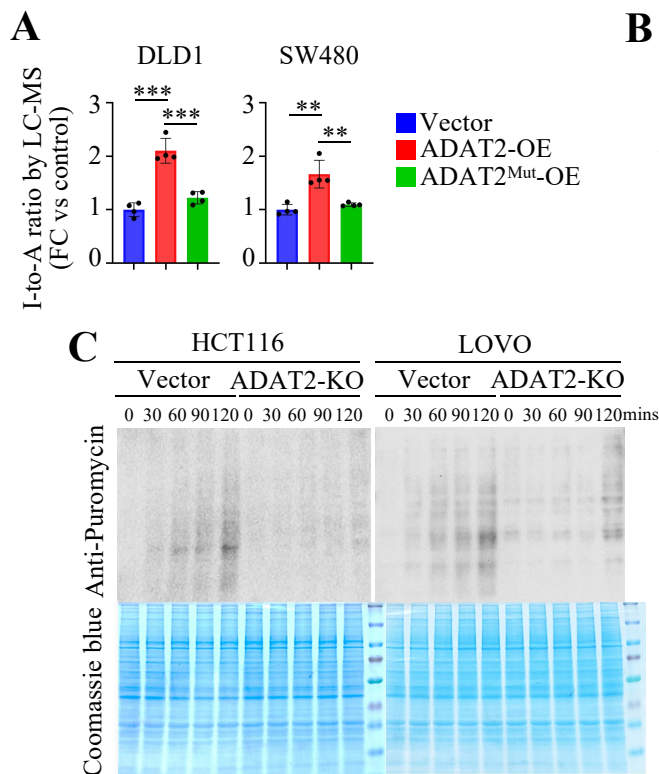


Figure S6. ADAT2 promotes A-to-I tRNA modification-dependent translation in CRC cells.

(A) LC-MS analysis of I-to-A ratio in control, ADAT2-WT-overexpressing, and catalytically-dead ADAT2 mutant-overexpressing DLD1 and SW480 cells (N=3).

(B) Puromycin incorporation assay in cells with overexpression of ADAT2-WT or ADAT2 mutant. Immunoblotting with anti-puromycin antibody showed the levels of newly synthesized proteins.

(C) Puromycin incorporation assay in control and ADAT2-knockout HCT116 and LOVO cells. Immunoblotting with anti-puromycin antibody showed the levels of newly synthesized proteins.

** $P < 0.01$; *** $P < 0.001$.

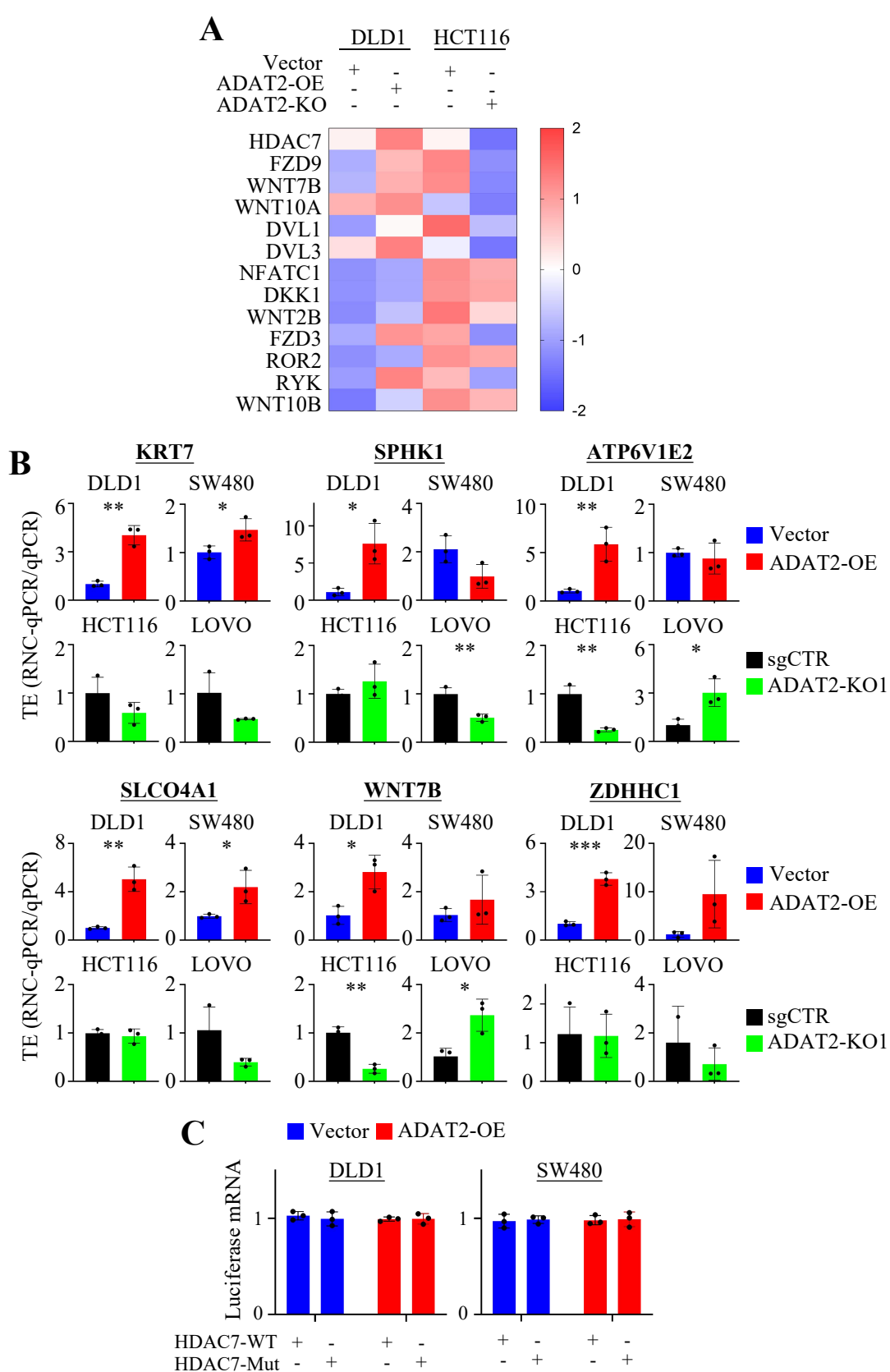


Figure S7. Downstream targets of ADAT2 in CRC.

(A) Heatmap of Ribo-seq data showing the effect of ADAT2 overexpression and knockout on the translation of WNT pathway genes.

(B) Translation efficiency (RNC-qPCR to qPCR ratio) of other candidate genes, ATP6V1E2, KRT7, SLCO4A1, SPHK1, WNT7B, and ZDHHC1. (N=3)

(C) Quantitative RT-PCR analysis of luciferase mRNA derived from wildtype (WT) and codon-mutated (Mut) HDAC7 reporters. (N=3)

* $P < 0.05$; ** $P < 0.01$.

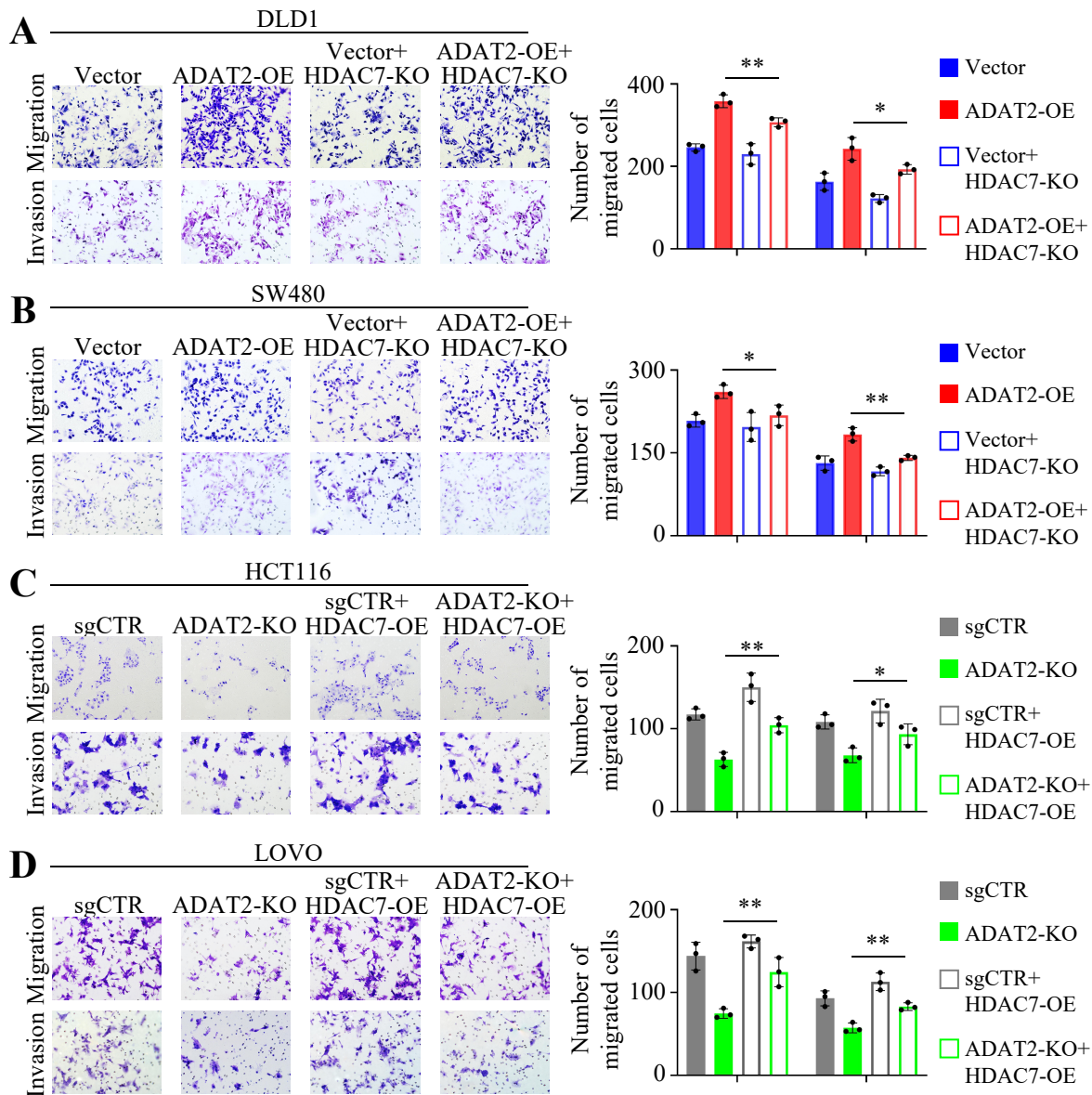


Figure S8. ADAT2 promotes pro-metastatic phenotypes through HDAC7.

(A) Transwell migration and Matrigel invasion assays of control and ADAT2-OE DLD1 cells, with or without HDAC7 knockout (*left*). Statistical analysis (N=3) (*right*).

(B) Transwell migration and Matrigel invasion assays of control and ADAT2-OE SW480 cells, with or without HDAC7 knockout (*left*). Statistical analysis (N=3) (*right*).

(C) Transwell migration and Matrigel invasion assays of control and ADAT2-KO HCT116 cells, with or without HDAC7 overexpression (*left*). Statistical analysis (N=3) (*right*).

(D) Transwell migration and Matrigel invasion assays of control and ADAT2-KO LOVO cells, with or without HDAC7 overexpression (*left*). Statistical analysis (N=3) (*right*).

* $P < 0.05$; ** $P < 0.01$.

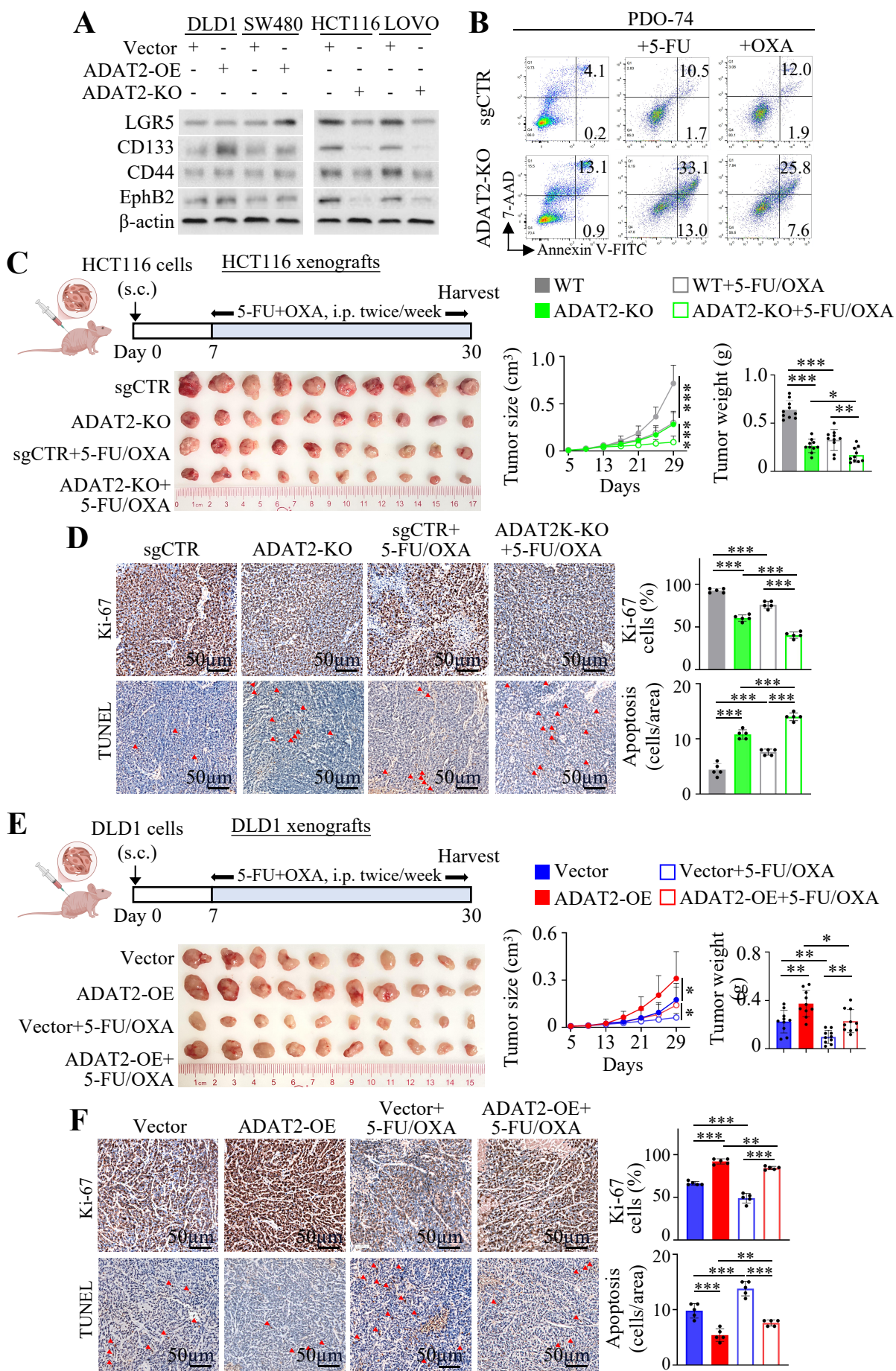


Figure S9. ADAT2 promotes stemness and chemoresistance in CRC.

(A) Western blot of stemness markers in ADAT2-overexpressing and knockout CRC cells.

(B) Flow cytometry analysis of apoptosis induced by ADAT2 knockout plus chemotherapy.

(C) HCT116 cells with or without ADAT2 knockout were subcutaneously injected into mice, followed by 5-FU+OXA treatment (*left*). Tumor growth curve and weight (N=10). (*right*).

(D) Ki-67 and TUNEL staining of HCT116 xenografts.

(E) DLD1 cells with or without ADAT2 overexpression were subcutaneously injected into mice, followed by 5-FU+OXA treatment (*left*). Tumor growth curve and weight (N=10). (*right*).

(F) Ki-67 and TUNEL staining of DLD1 xenografts.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

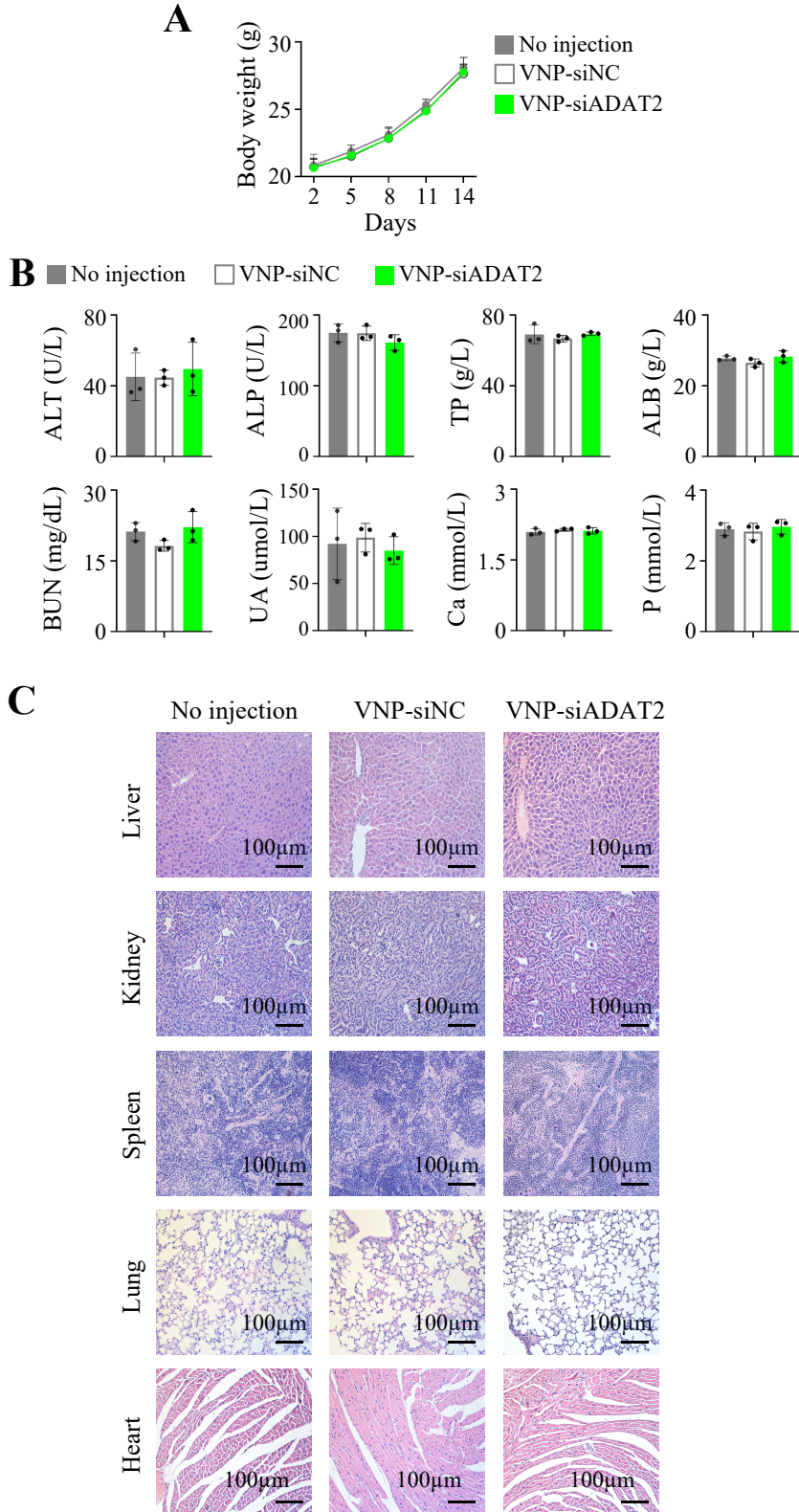


Figure S10. *In vivo* safety and toxicity assessment of VNP-siADAT2 therapy.
 (A) Body weight curves for untreated, VNP-siNC, and VNP-siADAT2 groups (N=3).
 (B) Plasma biochemistry analysis of hepatic and renal function (N=3).
 (C) Representative histopathology of liver, kidney, spleen, lung, and heart tissues.

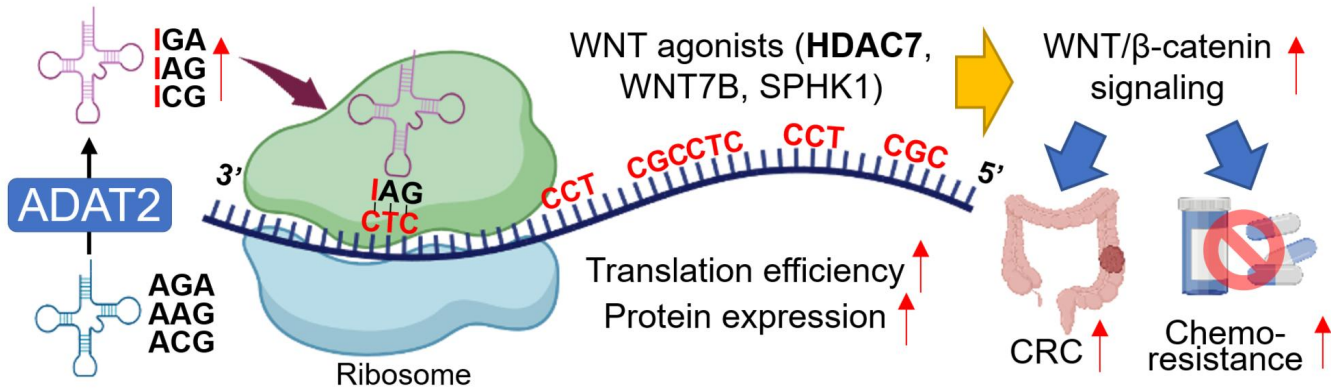


Figure S11. Schematic diagram showing the mechanism of action of ADAT2 in tumorigenesis and chemoresistance of CRC.