DDX5 promotes oncogene C3 and FABP1 expressions and drives intestinal inflammation and tumorigenesis

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Tumorigenesis in different segments of the intestinal tract involves tissue-specific oncogenic drivers. In the colon, complement component 3 (C3) activation is a major contributor to inflammation and malignancies. By contrast, tumorigenesis in the small intestine involves fatty acid-binding protein 1 (FABP1). However, little is known of the upstream mechanisms driving their expressions in different segments of the intestinal tract. Here, we report that the RNA-binding protein DDX5 binds to the mRNA transcripts of C3 and Fabp1 to augment their expressions posttranscriptionally. Knocking out DDX5 in epithelial cells protected mice from intestinal tumorigenesis and dextran sulfate (DSS)-induced colitis. Identification of DDX5 as a common upstream regulator of tissue-specific oncogenic molecules provides an excellent therapeutic target for intestinal diseases.

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Introduction

Tissue-specific oncogenic molecules drive tumorigenesis in different segments of the intestinal tract. In the colon, complement component 3 protein induces the expression of pro-inflammatory cytokines, such as IL-1β and IL-17 (1, 2, 3). Ablation of C3 genetically protects against colitis and tumorigenesis in mouse models (4, 5, 6, 7). In the small intestine, fatty acid–binding protein 1 (FABP1) is critical for intestinal absorption of dietary long-chain fatty acids (8,9). Ablation of FABP1 genetically protects against tumorigenesis in the small intestine (10).

Regulations of C3 and FABP1 expression at the transcriptional level are described in previous reports. C3 transcription is controlled by the twist basic helix–loop–helix transcription factor 1 (TWIST1), CCAAT/enhancer-binding protein β (C/EBPβ), nuclear receptors farnesoid X receptor, and peroxisome proliferator-activated receptor α in response to stimulation from pro-inflammatory cytokines, such as TNFα, IFNγ, and IL1β (11, 12, 13, 14, 15, 16, 17). Fabp1 transcription is controlled by GATA-binding protein 4 (GATA4), C/EBP, peroxisome proliferator-activated receptor α, pancreatic and duodenal homeobox 1 (PDX1), and hypoxia-inducible factor (HIF1α) (18, 19, 20, 21, 22). However, little is known about how C3 and FABP1 expressions are regulated posttranscriptionally in intestinal epithelial cells (IECs).

Posttranscriptional regulation of gene products can be orchestrated, in part, by RNA-binding proteins (23). One member of the DEAD-box containing RNA-binding protein family, DDX5, is abundantly expressed in the intestinal epithelium (24). Mutation and overexpression of DDX5 are found in human cancers, and its overexpression predicts advanced clinical stage and poor survival in colorectal cancer (CRC) patients (25, 26, 27). Knockdown of DDX5 inhibited the proliferation of cancer cells in vitro and the growth of xenografts in immunodeficient hosts (28, 29).

Mechanistically, DDX proteins have two major modes of action. First, they can directly bind to specific RNA substrates, use ATP hydrolysis energy to unwind RNA duplexes, facilitate RNA annealing, and/or organize RNA–protein complex assembly (30, 31, 32, 33). Second, DDXs can partner with transcription factors to modulate gene transcription (24, 30, 34, 35, 36, 37, 38, 39, 40). In human cancer cell lines, DDX5 interacts with β-catenin protein and the long non-coding RNA NEAT1 to promote oncogene expression (41, 42). However, we know little about how the RNA-binding properties of DDX5 contribute to shaping the epithelial RNA regulome during homeostasis and tumorigenesis in vivo.

Here, we revealed that DDX5 binds to C3 and Fabp1 mRNA and promotes their expressions in primary IECs from the colon and small intestine, respectively. Loss of DDX5 expression in IECs protects against colonic and small intestine tumorigenesis in vivo. Identification of DDX5 as a common upstream regulator of tissue-specific oncogenic molecules provides an excellent therapeutic target for treating intestinal cancers.

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Results

DDX5 regulates the epithelial immune response program and contributes to inflammation in the colon

In the IECs isolated from the colon and small intestine of adult wild-type (WT) mice, mRNAs encoding 35 RNA-binding DEAD-box containing proteins (DDXs) were found at various levels (Fig 5A and B and Table S1). Among these, Ddx5 was the most abundant transcript (Fig 1A). Western blot analyses confirmed that DDX5 proteins were present throughout the intestinal tract (Fig 1B). In the colon, immunohistochemistry (IHC) and nuclear-cytoplasmic fraction revealed that DDX5 proteins predominantly localized to the nucleus of IECs (Figs 1C and S1C). Therefore, we hypothesize that DDX5 may bind to target colonic IEC RNAs in the nucleus and regulate their expression posttranscriptionally.

Hence, we generated an epithelial DDX5 knockout mouse (DDX5ΔIEC) using the Villin1 (Vil1)–Cre recombination system (Fig S2A). WTIEC and DDX5ΔIEC littermates were born in Mendelian ratios and had similar growth curves (Fig S2B). IECs isolated from different segments of the intestinal tract confirmed efficient knockdown of DDX5 at the RNA and protein levels throughout the small intestine and colon (Fig S2C and D). Comparison of the RNA profile of colonic IECs isolated from steady-state WTIEC and DDX5ΔIEC mice revealed that knocking out DDX5 resulted in a down-regulation of 306 and up-regulation of 174 colonic IEC transcripts (Fig 1D and Table S2). DDX5-dependent RNA programs of the colonic IECs were enriched with genes involved in immune response activation (Fig 1E and Table S3). The DDX5 and DDX5ΔIEC knockout mice had lower abundance of transcripts of immune response activation marker, Ki67 (Fig S5C). However, no significant difference of tumor sizes was found on day 120 (Fig 2E).

We hypothesized that DDX5 contributes to CRC by regulating specific RNA programs in colonic IECs. Consistent with this possibility, Kaplan–Meier analysis of survival of CRC patients and relapse-free survival of a third patient cohort indeed reveal strong associations between the CRC patient cohort indeed reveal strong associations between the DDX5-associated 20 down-regulated gene signature we identified in our colonic IEC RNAseq study and worse CRC outcome (Fig 2F).

Epithelial DDX5 directly binds C3 mRNA and enhances its expression posttranscriptionally

To define the direct target of DDX5 in colonic IECs, we performed the enhanced cross-linked immunoprecipitation (eCLIPseq) assay using the anti-DDX5 antibodies (Table S4). Successful pull-down of DDX5 proteins were confirmed by western assays (Fig S6A). Sequencing results were processed by the ENCODE eCLIPseq analysis pipeline, as described in reference 50 and outlined in Fig S6B. Using a cutoff of three in both log10 P-values and log fold changes of immunoprecipitation (IP) signal over input, we identified 201 colonic IEC RNA sites, corresponding to 138 transcripts, that were significantly enriched by the anti-DDX5 antibodies (Table S5). More than 44% of the DDX5-bound sites localized to coding regions on colonic IEC RNAs (Fig S6C). Of the 138 DDX5-bound transcripts, RNA levels of C3, Ahcy1, and Shroom3 were significantly altered in DDX5-deficient colonic IECs (Fig 3A). Notably, the phenotype of the Apcmut C3-deficient mice (4, 5, 6, 7) mirrored those we observed here in the ApcΔIEC DDX5ΔIEC mice. Two independent studies in human CRC patients revealed that higher expression of C3 predicts poor overall and relapse-free survival (47, 48).

C3 mRNA is the highest expressed member of the complement family in wild-type mouse colonic IECs (Fig 5A). Reduced C3 transcripts and proteins were found in DDX5-deficient colonic IECs (Fig 3B–D). If DDX5 promotes C3 mRNA expression at the transcription level, we expect to observe altered RNA polymerase II

Epithelial DDX5 promotes colonic tumorigenesis

Excess inflammation, such as those found in inflammatory bowel diseases, predisposes patients to epithelial dysplasia and cancer (44, 45). Elevated expression of DDX5 predicts worse relapse-free survival in CRC patients (25, 26, 27). To assess the contribution of DDX5 to colonic tumorigenesis in vivo, we crossed the DDX5fl ΔIEC mice with 2% DSS in drinking water. By day 9, DDX5ΔIEC ΔIEC littermates were born in Mendelian ratios and had similar growth curves (Fig S2B). IECs isolated from different segments of the intestinal tract confirmed efficient knockdown of DDX5 at the RNA and protein levels throughout the small intestine and colon (Fig S2C and D). Comparison of the RNA profile of colonic IECs isolated from steady-state WTIEC and DDX5ΔIEC mice revealed that knocking out DDX5 resulted in a down-regulation of 306 and up-regulation of 174 colonic IEC transcripts (Fig 1D and Table S2). DDX5-dependent RNA programs of the colonic IECs were enriched with genes involved in immune response activation (Fig 1E and Table S3). Therefore, we hypothesized that DDX5ΔIEC mice with reduced immune activation in the colon may be protected against intestinal inflammation during colitis. To test this possibility, we challenged WT and DDX5ΔIEC mice with 2% DSS in drinking water. By day 9, DDX5ΔIEC animals experienced less weight loss and recovered more quickly than their WT cohoused littermates (Fig 1F). Colonos from DSS-challenged DDX5ΔIEC animals were longer (Fig 1G) and showed milder histological pathology, particularly in matrices scoring for immune infiltration, submucosal inflammation, and abnormal crypt density (Fig S3A–C). Furthermore, lamina propria cells from DDX5ΔIEC mice expressed less transcripts of inflammatory cytokines, including Il1b and Tnf (Fig S3D).

In humans, colonic tissues from ulcerative colitis (UC) patients have higher DDX5 expression than healthy controls (Fig S4A). Differences in DDX5 expression correlated with Apc, Il1b, and Cdx2 expression posttranscriptionally. Expression of the cell proliferation marker, Ki67 (Fig S5C). However, no significant difference of tumor sizes was found on day 120 (Fig 2E).

We hypothesized that DDX5 contributes to CRC by regulating specific RNA programs in colonic IECs. Consistent with this possibility, Kaplan–Meier analysis of survival of CRC patients and relapse-free survival of a third patient cohort indeed reveal strong associations between the DDX5-associated 20 down-regulated gene signature we identified in our colonic IEC RNAseq study and worse CRC outcome (Fig 2F).

Together, these results demonstrate that DDX5 is a critical contributor to colonic tumorigenesis in vivo.
Figure 1. DDX5 regulates colonic epithelial immune response program and contributes to colitis.

(A) Heat map of average normalized RNAseq read counts of the 10 highest expressed members of the DDX family in the ileum and colon of steady-state WT mice (n = 2).

(B) Representative Western blots showing DDX5 and β-tubulin protein expression in intestinal epithelial cells (IECs) from different sections of the intestine in WT mice.

Experiments were repeated three times using independent biological samples with similar results.

(C) Representative images from immunohistochemistry analysis of DDX5 in the colon of WT mice. Enlarged image is shown on the right. Scale bar represents 50 μm.

(D) Scatterplot of log2 (fold changes: DDX5ΔIEC over WTIEC) and −log10(P-values) of colonic IEC transcripts. RNAseq was performed on two independent pairs of cohoused DDX5ΔIEC over WTIEC littermates. Black dot: DDX5-dependent transcripts.

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recruitment and deposition of H3 lysine 4 trimethylation (H3K4me3) on the C3 gene promoter in colonic IECs from DDX5ΔIEC mice. However, chromatin immunoprecipitation (ChIP) qRT-PCR assay showed that a similar enrichment of RNA polymerase II and H3K4me3 were found on the C3 promoter in colonic IECs from WTIEC and DDX5ΔIEC mice (Fig S7B). In addition, fractionation studies revealed that C3 level is similar in the nuclear compartment but significantly reduced in the cytoplasm of DDX5-deficient IECs (Fig S7C). Next, we asked whether DDX5 regulation of C3 is intrinsic to colonic epithelial cells and independent of inputs from gut microbiota and immune cells using an organoid culture system. Briefly, crypts from DDX5-deficient and WT mice were harvested from WTIEC and DDX5ΔIEC littermates and maintained ex vivo for 5–7 passages. RNaseq of these colonic organoids revealed a similar reduction of C3 RNA in DDX5-deficient cultured cells (Fig S7D), suggesting the regulation of C3 by DDX5 is epithelial cell intrinsic.

Results from the eCLIPseq assay revealed that DDX5 was enriched on a region of the C3 transcript encoded by exon 30 (Fig 3E). Therefore, we hypothesize that DDX5 may bind to and regulate C3 transcripts at the posttranscriptional level. Insertion of the short stretch of DDX5-bound region of mouse C3 into the 3’UTR of the psiCheck2 reporter was sufficient to potentiate DDX5-dependent Renilla luciferase activity in a human epithelial cell line (Fig 3F). In flaviiridol-treated human epithelial cells, C3 mRNAs experienced a greater turnover when DDX5 was knocked down (Fig 3G). Together, these results suggest that DDX5 binds to and promotes C3 mRNA stability in colonic IECs.

**Epithelial DDX5 promotes small intestine tumorigenesis**

In the wild-type mouse small intestine, DDX5 is also abundantly expressed under steady state (Figs 1A and 4A). To ask whether DDX5 may also be involved in tumorigenesis of the small intestine, epithelial DDX5 conditional mice (Ddx5ΔIEC) were crossed to the ApcΔIEC/ΔIEC Cre reporter was sufficient to potentiate DDX5-dependent on the gene promoter in colonic IECs from DDX5ΔIEC mice. Therefore, we hypothesize that DDX5 may bind to and regulate C3 transcripts at the posttranscriptional level. Insertion of the short stretch of DDX5-bound region of mouse C3 into the 3’UTR of the psiCheck2 reporter was sufficient to potentiate DDX5-dependent Renilla luciferase activity in a human epithelial cell line (Fig 3F). In flaviiridol-treated human epithelial cells, C3 mRNAs experienced a greater turnover when DDX5 was knocked down (Fig 3G). Together, these results suggest that DDX5 binds to and promotes C3 mRNA stability in colonic IECs.

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**DDX5 binds a distinct set of RNAs to drive tumorigenesis in the small intestine**

Next, we asked whether DDX5 regulates overlapping and/or distinct RNA programs in the small intestine and colon. As most DDX5-dependent tumorigenesis of the small intestine occurred in the distal end (Fig 4E), we focused on characterizing the DDX5-dependent RNA program in the ileal section of the small intestine. Global transcriptome analyses revealed that DDX5 controls overlapping and distinct programs in the ileum and colon (Fig 5A and Table S6).

To determine the direct targets of DDX5 in the small intestine, eCLIPseq was performed using UV cross-linked cells from WT mouse (Fig S8A). Overall, we found DDX5 binding to 1,276 small intestine IEC RNA sites, corresponding to 466 transcripts (Fig 5B and Table S7). Similar to colonic IECs (Fig S6C), DDX5 was also enriched on coding regions of small intestine IECs (Fig S8B). Of all the DDX5-bound small intestine transcripts, seven experienced significantly altered RNA expression in DDX5-deficient ileal IECs (Fig S9A). Increased expression of Fabp1, but not the others, significantly correlates with worse relapse-free survival in CRC patients (Fig S9B).

Fabp1 encodes FABP1 and is uniquely found in the small intestine IECs (Fig S10A), consistent with previous reports (51, 52). Knocking out FABP1 in mice protects against small intestine tumorigenesis (10), which phenocopied our observations in the DDX5ΔIEC mice. On the Fabp1 RNA, DDX5 localized to a region encoded by exon 2 (Fig 5C). Fabp1 mRNA and its protein were significantly reduced in DDX5-deficient small intestine IECs (Figs 5D and E and S10B). Transcripts coding for other members of the FABP family were DDX5 independent, suggesting a unique regulation of Fabp1 by DDX5. Similar abundance of mature Fabp1 mRNAs was found in the nucleus of WTIEC and DDX5ΔIEC IECs, but cytoplasmic mature Fabp1 mRNAs were significantly lowered in the DDX5ΔIEC IECs (Fig 5F). These results suggest that DDX5 binds to and promotes Fabp1 mRNA stability in small intestine IECs. Last, we asked whether binding of DDX5 to Fabp1 mRNAs in the small intestine IECs may also affect ribosome recruitment for protein translation. We found that ribosomal engagement of Fabp1 mRNA in the small intestine was significantly decreased in cells from DDX5ΔIEC mice (Fig 5G). Together, these results reveal that DDX5 regulates unique IEC targets through overlapping and distinct posttranscriptional mechanisms (modeled in Fig 5H).

**Discussion**

CRC is the fourth most deadly cancer worldwide (53), where DDX5 is often mutated and/or overexpressed (54). The higher expression of DDX5 predicts poor patient survival (25, 26, 27). Here, we...
Figure 2. DDX5 promotes colonic tumorigenesis in Apc-mutant mice.  
(A) Representative bright-field images of tumor-bearing colons from APC^ΔcIEC DDX5^WT and APC^ΔcIEC DDX5^ΔIEC animals. Scale bar equals 1 cm.  
(B) Anal prolapse incidents recorded in mice described in (A).  
(C) Percent weight change of each mice in (A) on day 110 and 120 compared to day 100. Each dot represents one mouse. Weight change from DDX5-sufficient samples are shown in black (n = 15). Weight change from DDX5 knockouts are shown in red (n = 9). Data shown are means ± SD. *P < 0.05 (t test).  
(D) Colonic tumor counts from APC^ΔcIEC DDX5^WT (n = 17) and APC^ΔcIEC DDX5^ΔIEC (n = 12) tumor-bearing animals. Each dot represents one mouse. Data shown are means ± SD. ****P-value < 0.0001 (t test).  
(E) Average colonic tumor diameter (mm) from APC^ΔcIEC DDX5^WT (n = 17) and APC^ΔcIEC DDX5^ΔIEC (n = 9) tumor-bearing animals. Each dot represents one mouse. Data shown are means ± SD. n.s., not significant (t test).  
(F) Expression of the DDX5-dependent colonic gene signature predicts clinical outcome in colorectal cancer patients. Top 20 genes were selected based on criteria listed in the Materials and Methods section. Kaplan–Meier analysis of disease-free survival in cohort 1 (GSE13067, GSE14333, GSE17538, GSE31595, and GSE37892), cohort 2 (GSE87211), and progression-free survival in cohort 3 (GSE58891).  
Source data are available for this figure.
Figure 3. Epithelial DDX5 binds C3 RNA to enhance its expression posttranscriptionally.

(A) Workflow to identify DDX5 direct targets in colonic intestinal epithelial cells (IECs) involved in tumorigenesis. (B) Integrative Genomics Viewer browser displaying RNA expression at the C3 and Ddx5 locus in WT IECs and DDX5−/− (ΔIEC) littermates. (C) qRT-PCR validation of colonic C3 expression in additional independent pairs of WT and DDX5−/− animals. Data shown are means ± SD. *P < 0.05 (t-test). (D) Representative Western analysis of C3 proteins in the colonic IECs from two independent pairs of WT and DDX5−/− mice. Signal quantification was calculated as signal of C3 over signal of total protein.

(D) Integrative Genomics Viewer browser displaying the DDX5 binding to C3 RNAs in WT colonic IECs as defined by eCLIPseq. eCLIPseq was performed on colonic IECs from DDX5 targets tissue-specific RNAs in intestinal diseases Abbasi et al. https://doi.org/10.26508/lsa.202000772 vol 3 | no 10 | e202000772 6 of 15
demonstrated that knocking out DDX5 in IECs in two models resulted in lower tumor counts. Interestingly, tumors that escaped DDX5 regulation had comparable size as those found in WT animals on day 120, indicating that DDX5 plays a more critical role during tumor initiation and that other regulators can compensate for its loss at the later phase of tumor growth in vivo. We observed that mRNAs encoding other DDX members with structural similarities to DDX5, such as DDX17, are also highly expressed in the intestinal epithelium (Fig 1A). Future studies will be needed to examine whether other DDXs have similar or unique roles in the context of intestinal physiology and pathology.

The characterization of the in vivo DDX5 RNA interactome and regulome uncovered several mechanistic surprises of DDX5 biology. First, we demonstrated that DDX5 preferentially localized to coding regions of mRNAs, contributing to RNA stability and/or protein translation of its associated transcripts in mouse IECs. In contrast, previous study in cultured myelogenous leukemia cell line (K562) suggests that DDX5 binding on mRNAs is preferentially localized to introns and 5’ UTRs (30, 31, 32, 55, 56). These results suggest that DDX5 binding to mRNAs is likely tissue- and cell type specific. We speculate that such specificities may be achieved by DDX5 forming tissue-specific protein complexes with other partners yet to be identified. Future proteomics studies will be needed to uncover the DDX5 protein interactomes in different tissues to address this possibility.

In this study, we focused on our mechanistic experiments on two novel targets of DDX5, C3 and Fabp1. Here, we demonstrated that DDX5 binds to and promotes C3 mRNA stability in colonic IECs. In the small intestine, DDX5 binds to Fabp1 transcripts, enhancing cytoplasmic RNA levels, and facilitating ribosome engagement to augment the synthesis of FABP1 protein. It remains to be investigated whether the helicase activity of DDX5 is involved in these regulations of epithelial RNA stability and protein translation. C3 is a potent inducer of the Wnt/β-catenin cascade. DDX5 regulation of C3 uncovered a previously unappreciated role of DDX5 as an upstream regulator of Wnt/β-catenin signaling pathway. Whereas C3 is uniquely expressed in colonic IECs, FABP1 is expressed in the small intestine only. Future studies are needed to investigate the molecular mechanism underlying the region-specific expression of C3 and Fabp1 mRNAs observed here (Fig S10A). Regulation of FABP1 by DDX5 revealed a surprising role of DDX5 in intestinal lipid homeostasis. Highly proliferative cells, such as those found in tumor lesions, require large amounts of fatty acid building blocks from exogenous sources and/or de novo synthesis to sustain the building of cell membranes and organelles. For example, previous reports suggest that the up-regulation of acyl-CoA synthetase long chain family member 4 (ACSL4) promotes tumor cell survival in human colon adenocarcinomas (57), and that fatty acid–binding proteins can channel lipids from surrounding tissues to fuel further tumor growth (58). Future epistasis experiments will be needed to definitively test the contributions of C3, FABP1, and/or other targets acting downstream of DDX5 to promote intestinal inflammation and tumorigenesis. In summary, DDX5 posttranscriptionally orchestrates intestinal RNA programs and drive colitis and intestinal cancers.

Materials and Methods

Mice

C57BL/6 wild-type (Stock No: 000664) and Villin1Cre (Stock No: 021504) mice were obtained from The Jackson Laboratory. Ddx5lox/lox mice were obtained from Dr. Frances Fuller-Pace’s Laboratory and have been previously described in references 59 and 60. Heterozygous mice were bred to yield 6–8-wk-old Ddx5lox/lox Villin1Cre (subsequently referred to as wild-type, WTIEC) and Ddx5lox/lox Villin1Cre (referred to as DDX5ΔIEC) littermates for experiments related to understanding the role of DDX5 in IECs in both the small intestine and colon. Apclox/lox mice were obtained from Dr. Eric Fearon’s Laboratory and previously described in reference 44. For our colonic tumor model, Apcfl/fl Ddx5+/− Cdx2Cre− and Apcfl/fl Ddx5+/− Cdx2Cre+ (referred as APCfl/− DDX5WT and APCfl/− DDX5lox/lox Cdx2Cre+), as well as Apcfl/fl Ddx5fl/fl Cdx2Cre+ (APCfl/− DDX5ΔIEC) cohoused littermates were used. For our small intestine tumor model, Apcfl/fl Ddx5ΔIEC Villin1Cre− and Apcfl/fl Ddx5ΔIEC Villin1Cre+ (APCfl/− DDX5ΔIEC ΔIEC) cohoused littermates were used. All animal studies were approved and followed the Institutional Animal Care and Use Guidelines of the University of California San Diego.

Epithelial cell harvest

Steady-state intestinal epithelial and lamina propria cells were harvested as previously described (61). Briefly, after removing mesenteric fat and Peyer’s patches, the proximal 1/3, middle 1/3, and distal 1/3 of the small intestine were designated as the duodenum, jejunum, and ileum, respectively. To isolate IECs, intestine tissues were first incubated in 5 mM EDTA in HBSS containing 1 mM DTT for 20 min at 37°C with shaking, and then incubated in a second wash of 5 mM EDTA in HBSS without DTT for
Figure 4. DDX5 also promotes tumorigenesis in the small intestine.
(A) Representative images from immunohistochemistry analysis of DDX5 in the ileum of WT mice. Enlarged image is shown on the right. Scale bar represents 50 μm. (B) Genotypes of tumor-bearing APCΔIECDDX5WT and APCΔIECDDX5ΔcIEC littermates used in (C, D, E, F, G). (C) Percent weight change of each mouse in (B) on days 100, 110, and 120. Each dot represents one mouse. Weight change from DDX5-sufficient samples are shown in black (n = 10). Weight change from DDX5 knockouts are shown in red (n = 13). Data shown are means ± SD. *P < 0.05 (multiple t-test). (D) Macroscopic tumor counts in the colon. Each dot represents one mouse. Counts from DDX5-sufficient samples are shown in black (n = 12) and counts from DDX5 knockouts are shown in red (n = 13). Data shown are means ± SD. **P < 0.0001 (multiple t-test). (E) Macroscopic tumor counts in the small intestine. Each dot represents one mouse. Counts from DDX5-sufficient samples are shown in black (n = 12) and counts from DDX5 knockouts are shown in red (n = 13). Data shown are means ± SD. **P < 0.0001 (multiple t-test). (F) Macroscopic tumor diameter in the colon. Each dot represents one mouse. Diameter of tumors from DDX5-sufficient samples are shown in black (n = 12) and diameter of tumors from DDX5 knockouts are shown in red (n = 13). Data shown are means ± SD. n.s. (not significant). (G) Macroscopic tumor diameter in the small intestine. Each dot represents one mouse. Diameter of tumors from DDX5-sufficient samples are shown in black (n = 12) and diameter of tumors from DDX5 knockouts are shown in red (n = 13). Data shown are means ± SD. n.s. (not significant).
20 min at 37°C with agitation. Suspended cells from the EDTA washes were pooled as "IECs." Colon samples were processed similarly.

**Histology and IHC**

Ileal and colonic tissues were fixed overnight in 10% formalin at room temperature. Paraffin-embedded tissues were sectioned into 5-μm slices, stained with H&E, periodic acid–Schiff, or IHC (see Table S4 for antibody information). Briefly, paraffin sections were deparaffinized and rehydrated with TBST washes between each step (Tris-buffered saline, pH 7.8, with 0.1% Tween-20). Sections were blocked first against endogenous peroxidases (immersed for 30 min in 0.3% H2O2) and then blocked against endogenous biotin using unlabeled streptavidin and excess free biotin. Antigen retrieval was induced by heating the slide for 5 min twice in 10 mM sodium citrate buffer, pH 6.0, followed by 20 min of cooling. Finally, the sections were blocked against nonspecific hydrophobic interactions with 1% BSA/TBST. Staining was then performed with either the negative control IgG antibody or anti-DDX5 and anti-Ki67 (1:100) antibodies overnight in a humid chamber at 4°C. The next day, the sections were washed with TBST and then sequentially overlaid with biotinylated goat anti-rabbit (111-065-045; Jackson ImmunoResearch) at 1:500, followed by HRP-labeled streptavidin (16-030-084; Jackson ImmunoResearch) at 1:1000. Substrate was then overlaid (AEC from Vector labs following directions) for 30 min followed by nuclear counterstain with Mayer's hematoxylin. Images were acquired using the AT2 Aperio Scan Scope (UCSD Moores Cancer Center Histology Core).

**Western blot**

For whole cell lysates, cells were lysed in 25 mM Tris, pH 8.0, 100 mM NaCl, and 0.5% NP40 with protease inhibitors for 30 min on ice. Samples were spun down at 14,000g for 15 min, and soluble protein lysates were harvested. The NE-PER kit (Thermo Fisher Scientific) was used for cytoplasmic and nuclear fractionation studies. 30–50 μg protein was loaded on each lane. Blots were blocked in Odyssey Blocking Buffer (LI-COR) and probed for the desired proteins. After incubation with respective IRDye secondary antibody (LI-COR), infrared signals on each blot were measured on the LI-COR Odyssey CLX. Blocking Buffer (LI-COR) and probed for the desired proteins. After incubation with respective IRDye secondary antibody (LI-COR), infrared signals on each blot were measured on the LI-COR Odyssey CLX. The primary antibodies used in this study are listed in Table S4.

**cDNA synthesis and qRT-PCR**

Total RNA was extracted with the RNeasy Plus Kit (QIAGEN) and reverse-transcribed using iScript Select cDNA Synthesis Kit (Bio-Rad). Real-time RT-PCR was performed using iQaq Universal SYBR Green Supermix (Bio-Rad). For IECs and tumor RNA expression, data were normalized to Gapdh. Primers were designed using Primer-BLAST to span across splice junctions, resulting in PCR amplicons that span at least one intron. Primer sequences are listed in Table S8.

**RNaseq**

Ribosome-depleted RNAs were used to prepare sequencing libraries. 100-bp paired-end sequencing was performed on an Illumina HiSeq4000 by the Institute of Genomic Medicine (IGM) at the University of California San Diego. Each sample yielded ~30–40 million reads. Paired-end reads were aligned to the mouse mm10 genome with the STAR aligner version 2.6.1a (62) using the parameters: "--outFilterMultimapNmax 20 --alignSJoverhangMin 8 --alignSJoverhangMin 1 --outFilterMismatchesMin 999 --outFilterMismatchNoverReadLenMax 0.04 --alignIntronMin 20 --alignIntronMax 10000000 --alignMatesGapMax 1000000." Uniquely mapped reads overlapping with exons were counted using featureCounts (63) for each gene in the GENCODE.vM19 annotation. Differential expression analysis was performed using DESeq2 (v1.18.1 package) (64), including a covariate in the design matrix to account for differences in harvest batch/time points. Regularized logarithm (rlog) transformation of the read counts of each gene was carried out using DESeq2. Pathway analysis was performed on differentially expressed protein coding genes with minimal counts of 10, log2 fold change cutoffs of ≥0.5 or ≤−0.5, and P-values < 0.05 using Gene Ontology (http://www.ebi. ac.uk/geneontology) where all expressed genes in the specific cell type were set as background.

Gene set enrichment analysis was carried out using the pre-ranked mode of the gene set enrichment analysis software with default settings (55, 65). The gene list from DESeq2 was ranked by calculating a rank score of each gene as $-log_{10}(P-value) \times sign(log_2\ \text{FoldChange})$, in which FoldChange is the fold change of expression in DDX5-suff over those found in WTIEC.

**Enhanced cross-linked immunoprecipitation (eCLIPseq)**

eCLIPseq analysis was performed as previously described (50). For IEC eCLIPseq, the cells were isolated from two 8–10-wk-old wild-type (C57BL/6) female mice, as described above, and 50 million cells from each mouse were used in the two biological replicates. The cells were subjected to UV-mediated cross-linking, lysis, and treatment with limiting amounts of RNases, followed by IP of the DDX5-containing RNA complexes. RNA fragments protected from RNase digestion were subjected to UV-mediated cross-linking, reverse-transcription, and DNA linker ligation to generate eCLIPseq libraries for high-throughput Illumina sequencing.
Figure 5. DDX5 regulates overlapping and distinct RNA programs in the small intestine and colon.

(A) Venn diagram of the overlapping and distinct DDX5-dependent transcripts from the ileum and colon defined as log2 fold change of ≥0.5 or ≤−0.5 and P-value < 0.05. RNAseq was performed on two independent pairs of cohoused DDX5ΔIEC over WTIEC littermates. (B) Venn diagram showing the overlapping and distinct DDX5-bound transcripts in the small intestine and colonic intestinal epithelial cells (IECs). eCLIPseq was performed on small intestine IECs from two independent WT mice. Peaks were called by a cutoff of three for both log10 P-values and log2 (fold changes: immunoprecipitation over input). (C) Integrative Genomics Viewer browser displaying DDX5 binding on the fatty acid-binding protein 1 (Fabp1) locus as defined by eCLIPseq. (D) Normalized RNAseq read counts of transcripts encoding members of the FABP family in ileal IECs from WT and DDX5-deicient mice. *P < 0.05 (DEseq). (E) Representative Western blots for FABP1, DDX5, β-tubulin, and fibrillarin in cytoplasmic (C) and nuclear (N) extracts of small intestine IECs from WT and DDX5ΔIEC mice. Experiments were repeated three times using independent biological samples with similar results. (F) RNAs from the nuclear and cytoplasmic fractions of small intestine IECs harvested from WT and DDX5ΔIEC mice were evaluated by qRT-PCR for Fabp1. Each dot represents one mouse. This experiment was repeated on two pairs of independent samples. *P < 0.05 (t test). (G) Engagement of Fabp1 mRNA with ribosome RPL10A in small intestine IECs. Results are means of two independent experiments ± SD. *P < 0.05 (t test). (H) Working model: DDX5 posttranscriptionally regulates the expression of tissue-specific oncogenic RNAs in IECs.

Source data are available for this figure.
Peak regions were defined using CLIPper first on the IP sample (https://github.com/YeoLab/clipper/wiki/CLIPper-Home). Enrichment was calculated for both the IP and input samples. Log₂ fold change was calculated as eCLIPSeq reads normalized for read depth over normalized reads found at each peak region in the size-matched input sample. ENCODE Irreproducible Discovery Rate analysis was performed on two independent biological replicates of IECs. Peaks were ranked using the entropy formula, $P_i = \log(P_i/Q_i)/\log_n$, where $P_i$ is the probability of an eCLIPSeq read at that position and $Q_i$ is the probability of input reads at that position. Results were filtered using cutoffs of three for both log₁₀ P-values and log₂ fold changes, respectively, to define a set of true peaks normalized above their respective size-matched input background signal.

**ChiP**

ChiP was carried out as described previously (56). Briefly, 20 million intestinal IECs were fixed with 1% formaldehyde at room temperature for 10 min and quenched with 125 mM glycine for 5 min at room temperature. All buffer compositions were described in reference 56. Nuclear lysates were sonicated with a Bioruptor (Branson Sonifier Cell Disruptor 185) at 4°C using the output setting at 4 for 10 cycles of 30 s on and 30 s off. 30 µg of chromatin was used per IP. Chromatin was diluted 10× in ChiP dilution buffer supplemented with proteinase inhibitor. 5% of the total chromatin used per IP reaction was saved as input samples. 5 µg of antibody was added per 30 µg chromatin per IP reaction and incubated overnight at 4°C. The immune complexes were then incubated with 30 µl of Dynabeads Protein G (10004D; Thermo Fisher Scientific) for 4 h at 4°C on rotation. After washes, protein–DNA complexes were eluted from the beads by adding 200 µl of elution buffer and incubating the beads at 65°C for 15 min with constant shaking at 1,000 rpm. DNA and protein cross-links were reversed by adding 8 µl of 1 NaCl and 2 µl of proteinase K solution (20 mg/ml, AM2546; Thermo Fisher Scientific) by overnight incubation at 65°C under constant shaking. Chromatin was isolated using QIAQuick PCR Purification Kit (28104) and eluted in 40 µl elution buffer. Input samples were diluted five times to make a 1% input control. The ChiP signals were calculated as follows: Adjusted input = Ct (Input) – 6.644. ChiP signal = 100 × Power (2; average of adjusted Input-Ct value ChiP sample). All ChiP qPCR primers are listed in Table S8.

**Ribosome pull-down assay**

Small intestine or colonic IECs were lysed in polysome extraction buffer (10 mM Hepes, pH 7.4, 150 mM KCl, 5 mM MgCl₂, 1% NP40, 2 mM dithiothreitol, 80 U/ml RNaseOUT, 100 µg/ml cycloheximide, and protease inhibitors). Cell extracts were subjected to anti-ribosome IP overnight with 2 µg anti-RPL10A antibodies (Abcam) and harvested in Protein G magnetic Dynabeads (Invitrogen) as described previously (66). cDNAs were synthesized from purified RNA using Superscript III (Invitrogen). Level of RPL10A associated transcripts in pull-down was calculated as fraction of input for each sample.

**DSS-induced colitis**

Mice were provided 2% (wt/vol) DSS (160110; MP Biomedicals) in their drinking water for 7 d, followed by 7 d of access to regular drinking water. Mice were monitored daily for their weight and harvested on day 15 post-DSS treatment. Pathology scoring of distal colon from DSS-challenged mice was performed blind by JE Hernandez following previously published guidelines (67), including parameters for inflammatory infiltrate, crypt density, crypt hyperplasia, muscle thickening, and submucosal inflammation.

**Intestine organoid cultures**

Isolated colonic crypts were embedded in Corning Matrigel Matrix Corning Matrigel GFR Membrane Matrix (CB40230C; Thermo Fisher Scientific) and seeded onto pre-warmed 24-well plates (CytosOne) and overlaid with conditioned media as described in reference 68. The organoid images were acquired using fluorescence microscopy (11350119; Thermo Fisher Scientific).

**RNAi in human IECs**

Caco-2 cell line was cultured in 1× DMEM/F12 media (Gibco, Life Technologies). The media were supplemented with 1× 10% FBS (Gibco, Life Technologies), 1 mM sodium pyruvate (Gibco, Life Technologies) and 1% penicillin streptomycin (Gibco, Life Technologies). Cells were plated on a 24-well plate at 500 liters/well with 2 × 10⁵ cells/ml 1 d before transfection. 50 µM human DDX5 siRNA pool (Cat. no. D-003774-02, D-003774-03, D-003774-04, D-003774-17; GE Healthcare Dharmacon, see Table S9) or scramble siRNA pool (Cat. no. D-001206-14-05; GE Healthcare Dharmacon, see Table S9) were mixed with Opti-MEM medium and Lipofectamine 3000 (L3000001; Invitrogen) reagent according to the manufacturer’s protocol. Solutions were vortexed and incubated for 5 min at room temperature to allow the formation of siRNA–lipid complex. 50 µl of transfection/siRNA (final concentration of 100 nM) mixture was added to the well and incubated at 37°C. Transcription inhibitor, flavopiridol, was purchased from Sigma-Aldrich (F3055). After incubation for 48 h, the cells were treated with DMSO or flavopiridol (2 µM) and were collected 16 h posttreatment. RNA extraction and qRT-PCR were carried out as described above.

**Luciferase assay**

The psiCheck2 construct containing dual Renilla and Firefly luciferase reporters was purchased from Promega (Promega). The DDX5-bound sequence on Fabp1 and Firefly luciferase reporters was purchased from Promega (Promega). The DDX5-bound sequence on Fabp1 was cloned into a multiple cloning site located downstream of the Renilla translational stop codon. SW480 cells were cultured in 1× DMEM/F12 media (Gibco, Life Technologies). 1× 10% FBS (Gibco, Life Technologies), 1 mM sodium pyruvate (Gibco, Life Technologies) and 1% penicillin streptomycin (Gibco, Life Technologies) were added. The cells were plated on a 96-well plate at 0.5 × 10⁵ cells/ml 1 d before transfection. 100 nM of siCTRL or the human DDX5 siRNA pool were introduced to SW480 cells using Lipofectamine 3000 (L3000001; Invitrogen) in Opti-MEM medium according to the manufacturer’s protocol. The transfection mixture was incubated at room temperature for 5 min. 10 µl of the
transfection mixture was added to each well and incubated at 37°C for 24 h. 1 μg of psiCheck2 luciferase reporter plasmids were transfected with Lipofectamine 3000 and 1 μl P3000 enhancer reagent in the Opti-MEM medium. After 24 h, the cell lysates were used to measure both Renilla and firefly activities using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s instructions.

Kaplan–Meier analysis

Top 20 DDX5-dependent genes were selected based on two criteria: log2 FoldChange < -1 (down-regulated in DDX5-deficient colonic IECs) and adjusted P-value < 0.05. Gene expression values were normalized according to a modified Z-score approach centered around StepMiner threshold (formula = (expr – SThr)/3*stddev). A composite gene expression score is computed based on linear combination of normalized and z-scored scaled expression values. StepMiner algorithm (69) is used to classify the final score into high and low values. Kaplan–Meier analysis of disease-free survival in two independent cohorts reveal strong (Pooled gene expression omibus [GEO]: GSE13067, GSE14333, GSE17538, GSE31595, GSE37892, GSE33113, n = 555, P = 0.0014; GSE87211, n = 351, P = 0.044) association between 20 down-regulated gene signatures and worse outcome. Progression-free survival analysis were performed on the mCRC GSE5851 dataset (P = 0.014).

Accession numbers

The accession numbers for the small intestine and colon eCLIPseq results reported in this article are available on GEO (GSE124023). The IEC RNAseq datasets are available on GEO (GSE123881).

Statistical analysis

All values are presented as mean ± SD. Significant differences were evaluated using GraphPad Prism 8 software. The t test was used to determine significant differences between two groups. A two-tailed P-value of <0.05 was considered statistically significant in all experiments.

Supplementary Information

Supplementary Information is available at https://doi.org/10.26508/lsa.202000772.

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Author Contributions

N Abbasi: formal analysis, validation, visualization, and writing—original draft, review, and editing. T Long: data curation, formal analysis, validation, and writing—review and editing. Y Li: data curation, software, formal analysis, and methodology. BA Yee: data curation, software, and formal analysis. BS Cho: data curation, formal analysis, and writing—review and editing. JE Hernandez: data curation, formal analysis, and writing—review and editing. E Ma: formal analysis. PR Patel: formal analysis and writing—review and editing. D Sahoo: formal analysis and visualization. IM Sayed: conceptualization and writing—review and editing. N Varki: data curation and formal analysis. S Das: conceptualization, supervision, and writing—review and editing. P Ghosh: conceptualization, supervision, funding acquisition, visualization, and writing—review and editing. GW Yeo: conceptualization, supervision, funding acquisition, and writing—original draft, review, and editing. WJM Huang: conceptualization, data curation, formal analysis, supervision, funding acquisition, visualization, and writing—original draft, review, and editing.

Conflict of Interest Statement

GW Yeo is co-founder, member of the Board of Directors, on the Science Advisory Board, equity holder, and paid consultant for Locanabio and Eclipse BioInnovations. GW Yeo is a visiting professor at the National University of Singapore. GW Yeo’s interests have been reviewed and approved by the University of California San Diego in accordance with its conflict of interest policies. All other authors declare that they have no conflict of interest.

References

1. Samstad EO, Niyonzima N, Nymo S, Aune MH, Ryan L, Bakke SS, Lappegard KT, Brekke OL, Lambris JD, Damas JK, et al (2014) Cholesterol crystals induce complement-dependent inflammasome activation and cytokine release. J Immunol 192: 2837–2845. doi:10.4049/jimmunol.1302484
2. Mizutani N, Goshima H, Nabe T, Yoshino S (2012) Complement C3-induced IL-17 plays a critical role in an IgE-mediated late-phase asthmatic response and airway hyperresponsiveness via neutrophilic inflammation in mice. *J Immunol* 188: 5694–5705. doi:10.4049/jimmunol.1103176

3. Liu Y, Wang K, Liang X, Li Y, Zhang Y, Zhang C, Wei H, Luo R, Ge S, Xu G (2018) Complement C3 produced by macrophages promotes renal fibrosis via IL-17A secretion. Front Immunol 9: 2385. doi:10.3389/fimmu.2018.02383

4. Leon F, Contractor N, Fuss I, Marth T, Lahey E, Iwaki S, la Sala A, Hoffmann V, Strober W, Kelsall BL (2006) Antibodies to complement receptor 3 treat established inflammation in murine models of colitis and a novel model of psoriasisform dermatitis. *J Immunol* 177: 6974–6982. doi:10.4049/jimmunol.177.10.6974

5. Lu F, Fernandes SM, Davis AE 3rd (2010) The role of the complement and contact systems in the dextran sulfate sodium-induced colitis model: The effect of C1 inhibitor in inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 298: G878–G883. doi:10.1152/ajpgi.00400.2009

6. Ning C, Li YY, Wang Y, Han GC, Wang RX, Xiao H, Li XY, Hou CM, Ma YF, Sheng DS, et al (2015) Complement activation promotes colitis-associated carcinogenesis through activating intestinal IL-1beta/IL-17A axis. *Mucosal Immunol* 8: 1275–1284. doi:10.1038/mi.2015.18

7. Pio R, Corrales L, Lambris JD (2014) The role of complement in tumor inflammation in mice. *Cancer Prev Res (Phila)* 7: 1026–1037. doi:10.1158/1940-6207.capr-13-0120

8. Gajda AM, Storch J (2015) Enterocyte fatty acid-binding proteins (FABPs): Different functions of liver and intestinal FABPs in the intestine. *Procstaglandins Leukot Essent Fatty Acids* 93: 9–16. doi:10.1016/j.plefa.2014.10.001

9. Thumser AE, Moore JB, Plant NJ (2014) Fatty acid binding proteins: Tissue-specific functions in health and disease. *Curr Opin Clin Nutr Metab Care* 17: 124–129. doi:10.1097/MCO.0000000000000031

10. Dharmarajan S, Newberry EP, Montenegro G, Nalbantoglu I, Davis VR, Sheerin NS, Zhou W, Adler S, Sacks SH (1997) TNF-alpha regulation of C3 gene expression in mammalian hepatic-derived cells. *J Biol Chem* 272: 28052–28057. doi:10.1074/jbc.272.45.288.1726

11. Mogilenko DA, Kudriavtsev IV, Shavva VS, Dizhe EB, Vilenskaya EG, Efremov AM, Pervozvshikov AP, Orlov SV (2013) Peroxisome proliferator-activated receptor alpha positively regulates complement C3 expression but inhibits tumor necrosis factor alpha-mediated activation of C3 gene in mammalian hepatic-derived cells. *J Biol Chem* 288: 1726–1738. doi:10.1074/jbc.M112.437525

12. Staloch LJ, Divine JK, Witten JT, Simon TC (2005) C/EBP and Cdx family factors regulate liver fatty acid binding protein transgene expression in the small intestinal epithelium. *Biochim Biophys Acta* 1731: 168–178. doi:10.1016/j.bjbaexp.2005.08.014

13. Bosse T, Piascecky MJ, Burghard E, Fialkovich JJ, Rajagopal S, Pu WT, Krasinski SD (2006) Gat4 is essential for the maintenance of jejunal-ileal identities in the adult mouse small intestine. *Mol Cell Biol* 26: 9060–9070. doi:10.1128/MCB.00124-06

14. Jadoon A, Cunningham P, McDermott LC (2015) Regulation of fatty acid binding proteins by hypoxia inducible factors 1alpha and 2alpha in the placenta: Relevance to pre-eclampsia. *Prostaglandins Leukot Essent Fatty Acids* 93: 25–29. doi:10.1016/j.plefa.2014.09.004

15. Chen C, Fang R, Zhou LC, Lowe AW, Sibley E (2012) POX1 regulation of FABP1 and novel target genes in human intestinal epithelial Caco-2 cells. *Biochim Biochem Biophys Acta* 423: 183–187. doi:10.1016/j.bbabio.2012.05.113

16. Landriat JF, Thomas C, Grober J, Duez H, Percevault F, Souidi M, Linard C, Staels B, Besnard P (2004) Statin induction of liver fatty acid-binding protein (L-FABP) gene expression is peroxisome proliferator-activated receptor-alpha-dependent. *J Biol Chem* 279: 45512–45518. doi:10.1074/jbc.m407461000

17. Pereira B, Billaud M, Almeida R (2017) RNA-binding proteins in cancer: Old players and new actors. *Trends Cancer* 3: 506–528. doi:10.1016/j.trecan.2017.05.003

18. Fuller-Pace FV (2013) The DEAD box proteins DDX5 (p68) and DDX17 (p72): Multi-tasking transcriptional regulators. *Biochem Biophys Acta* 1829: 756–763. doi:10.1016/j.bbagrm.2013.03.004

19. Causevic M, Hilsog RG, Kernohan NM, Carey FA, Kay RA, Steele RJ, Fuller-Pace FV (2001) Overexpression and poly-ubiquitylation of the DEAD-box RNA helicase p68 in colorectal tumours. *Oncogene* 20: 7734–7743. doi:10.1038/sj.onc.1204976

20. Lee H, Flaherty P, Ji HP (2013) Systemic genomic identification of colorectal cancer genes delineating advanced from early clinical stage and metastasis. *BMJ Med Genomics* 6: 54. doi:10.1136/bmjmedgenomics-2012-100428

21. Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, et al (2015) Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med* 21: 449–456. doi:10.1038/nm.3850

22. Du C, Li DQ, Li N, Chen L, Li SS, Yang Y, Hou MX, Xie MI, Zheng ZD (2017) DDX5 promotes gastric cancer cell proliferation in vitro and in vivo through mTOR signaling pathway. *Sci Rep* 7: 42876. doi:10.1038/srep42876

23. Ma Z, Feng J, Guo Y, Kong R, Ma Y, Sun L, Yang X, Zhou B, Li S, Zhang W, et al (2017) Knockdown of DDX5 inhibits the proliferation and tumorigenesis in esophageal cancer. *Onco Res* 25: 887–895. doi:10.3727/096504016x14817158982636

24. Linder P, Jankowsky E (2011) From unwinding to clamping: The DEAD box RNA helicase family. *Nat Rev Mol Cell Biol* 12: 505–516. doi:10.1038/nrm3154

25. Lee YL, Wang Q, Rio DC (2018) Coordinate regulation of alternative pre-mRNA splicing events by the human RNA chaperone proteins hnRNP A1 and DDX5. *Genes* Dev 32: 1060–1074. doi:10.1101/gad.316034.118

26. Das M, Renganathan A, Dighe SN, Bhaduri U, Shettar A, Mukherjee G, Pasyatka P, Satyanarayana Rao MR (2018) DDX5/p68 associated IncRNA LOC284456 is differentially expressed in human cancers and modulates gene expression. *RNA Biol* 15: 214–230. doi:10.1080/15476286.2017.1397261

27. Van Nostrand EL, Freese P, Pratt GA, Wang X, Wei X, Xiao R, Blue SM, Chen JY, Cody NAL, Dominguez O, et al (2020) A large-scale binding and functional map of human RNA-binding proteins. *Nature* 583: 711–719. doi:10.1038/s41586-020-2077-3

28. Giraud G, Terrone S, Bourgeois CF (2018) Functions of DEAD box RNA helicases DDX5 and DDX17 in chromatin organization and transcriptional regulation. *BMB Rep* 51: 613–622. doi:10.5483/BMBRep.2018.51.12.234
35. Endoh H, Maruyama K, Masuhiro Y, Kobayashi Y, Goto M, Tai H, Yanagisawa J, Metzger D, Hashimoto S, Kato S (1999) Purification and identification of p68 RNA helicase acting as a transcriptional coactivator specific for the activation function 1 of human estrogen receptor alpha. Mol Cell Biol 19: 5363–5372. doi:10.1128/mcb.19.8.5363

36. Wang Z, Luo Z, Zhou L, Li X, Jiang T, Fu E (2015) DDX5 promotes proliferation and tumorigenesis of non-small-cell lung cancer cells by activating beta-catenin signaling pathway. Cancer Sci 106: 1303–1312. doi:10.1111/cas.12755

37. Mazurek A, Park Y, Miething C, Wilkinson JE, Gillies J, Lowe SW, Vakoc CR, Stillman B (2014) Acquired dependence of acute myeloid leukemia on the DEAD-box RNA helicase DDX5. Cell Rep 7: 1887–1899. doi:10.1016/j.celrep.2014.05.019

38. Jensen ED, Niu L, Caretti G, Nicol SM, Teplyuk N, Stein GS, Sartorelli V, van Wijnen AJ, Fuller-Pace FV, Westendorf JJ (2008) p68 (Ddx5) interacts with Runx2 and regulates osteoblast differentiation. J Cell Biochem 103: 1430–1451. doi:10.1002/jcb.21526

39. Wortham NC, Ahamed E, Nicol SM, Thomas RS, Periyasamy M, Jiang J, Ochocka AM, Shousha S, Huson L, Bray SE, et al (2009) The DEAD-box protein p72 regulates ERalpha-oestrogen-dependent transcription and cell growth, and is associated with improved survival in ERalpha-positive breast cancer. Oncogene 28: 4035–4046. doi:10.1038/ onc.2009.261

40. Clark EL, Coulson A, Dalgliesh C, Rajan P, Nicol SM, Fleming S, Heer R, Fodde R, Edelmann W, Yang K, van Leeuwen C, Carlson C, Renault B, Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Wang Z, Luo Z, Zhou L, Li X, Jiang T, Fu E (2015) DDX5 promotes proliferation and tumorigenesis of non-small-cell lung cancer cells by activating beta-catenin signaling pathway. Cancer Sci 106: 1303–1312. doi:10.1111/cas.12755

41. Shin S, Ros sow KL, Grande JP, Janknecht R (2007) Involvement of RNA helicase p68 and p72 in colon cancer. Cancer Res 67: 7572–7578. doi:10.1158/0008-5472-can-06-4652

42. Zhang M, Weng W, Zhang Q, Wu Y, Ni S, Tan C, Xu M, Sun H, Liu C, Wei P, et al (2018) The IncRNA NEAT1 activates Wnt/beta-catenin signaling and promotes colorectal cancer progression via interacting with DDX5. J Hematol Oncol 11: 113. doi:10.1186/s13045-018-0656-7

43. Arijs I, De Hertogh G, Lemmens B, Van Lommel L, de Bruyn M, Vanhove W, Cleynen I, Machiels K, Ferrante M, Schuit F, et al (2018) Effect of vedolizumab (anti-alpha4beta7-integrin) on histological healing and mucosal gene expression in patients with UC. Gut 67: 43–52. doi:10.1136/gutjnl-2016-312293

44. Grivennikov SI, Wang K, Micuda D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, et al (2012) Adiponectin regulates the expression of the inflammatory cytokine TNF-alpha via a PI3K-Akt/NF-kappaB signaling pathway in human adipocytes. J Immunol 188: 9713–9721. doi:10.4049/jimmunol.1201346

45. Eaden JA, Abrams KR, Mayberry JF (2007) The risk of colorectal cancer in patients with inflammatory bowel disease. Gut 56: 1052–1063. doi:10.1136/gut.2006-132193

46. Hinot I, Akyol A, Theisen BK, Ferguson DO, GreenSzek J, Williams BO, Cho KR, Fearon ER (2007) Mouse model of colonic adenoma-carcinoma progression based on somatic Apc inactivation. Cancer Res 67: 9721–9730. doi:10.1158/0008-5472-can-07-2735

47. Sano T, Huang W, Hall JA, Yang C, Chen A, Gavzy SJ, Lee JY, Ziel JW, Miraldi ER, Domingos AI, et al (2015) An IL-23R/IL-22 circuit regulates epithelial senescence and cell-cycle arrest after DNA damage. Oncogene 34: 3641–3649. doi:10.1038/onc.2015.77

48. Bates GJ, Nicol SM, Wilson BJ, Jacobs AM, Bourdon J, Wardrop J, Gregory DJ, Lane DP, Perkins ND, Fuller-Pace FV (2005) The DEAD box protein p68: A novel transcriptional coactivator of the p53 tumour suppressor. EMBO J 24: 543–553. doi:10.1038/sj.emboj.7600550

49. Sano T, Huang W, Hall JA, Yang C, Chen A, Gavzy SJ, Lee JY, Ziel JW, Miraldi ER, Domingos AI, et al (2015) An IL-23R/IL-22 circuit regulates epithelial senescence and cell-cycle arrest after DNA damage. Oncogene 34: 3641–3649. doi:10.1038/onc.2015.77

50. Cho KR, Fearon ER, Domingos AI, et al (2015) An IL-23R/IL-22 circuit regulates epithelial senescence and cell-cycle arrest after DNA damage. Oncogene 34: 3641–3649. doi:10.1038/onc.2015.77

51. Liao Y, Smyth GK, Shi W (2014) featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30: 923–930. doi:10.1093/bioinformatics/btu812

52. Liao Y, Smyth GK, Shi W (2014) featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30: 923–930. doi:10.1093/bioinformatics/btu812

53. Liao Y, Smyth GK, Shi W (2014) featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30: 923–930. doi:10.1093/bioinformatics/btu812

54. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15: 550. doi:10.1186/s13059-014-0550-8

55. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Landier W, et al (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102: 15545–15550. doi:10.1073/pnas.0506580102

56. Heiman M, Kulicke R, Fenster RJ, Greengard P, Heintz N (2016) Cell type-specific mRNA purification by translating ribosome affinity purification (TRAP). Nat Protoc 9: 1282–1291. doi:10.1038/nprot.2014.085
67. Koelink PJ, Wildenberg ME, Stitt LW, Feagan BG, Koldijk M, van’t Wout AB, Atreya R, Vieth M, Brandse JF, Duijst S, et al (2018) Development of reliable, valid and responsive scoring systems for endoscopy and histology in animal models for inflammatory bowel disease. J Crohns Colitis 12: 794–803. doi: 10.1093/ecco-jcc/jjy035

68. Miyoshi H, Stappenbeck T (2013) In vitro expansion and genetic modification of gastrointestinal stem cells in spheroid culture. Nat Protoc 8: 2471–2482. doi:10.1038/nprot.2013.153

69. Sahoo D, Dill DL, Tibshirani R, Plevritis SK (2007) Extracting binary signals from microarray time-course data. Nucleic Acids Res 35: 3705–3712. doi:10.1093/nar/gkm284

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