PUMILIO proteins promote colorectal cancer growth via suppressing p21

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PUMILIO (PUM) proteins belong to the highly conserved PUF family post-transcriptional regulators involved in diverse biological processes. However, their function in carcinogenesis remains under-explored. Here, we report that \textit{Pum1} and \textit{Pum2} display increased expression in human colorectal cancer (CRC). Intestine-specific knockout of \textit{Pum1} and \textit{Pum2} in mice significantly inhibits the progression of colitis-associated cancer in the AOM/DSS model. Knockout or knockdown of \textit{Pum1} and/or \textit{Pum2} in human CRC cells result in a significant decrease in the tumorigenicity and delayed G1/S transition. We identify p21/Cdkn1a as a direct target of PUM1. Abrogation of the PUM1 binding site in the p21 mRNA also results in decreased cancer cell growth and delayed G1/S transition. Furthermore, intravenous injection of nanoparticle-encapsulated anti-\textit{Pum1} and \textit{Pum2} siRNAs reduces colorectal tumor growth in murine orthotopic colon cancer models. These findings reveal the requirement of PUM proteins for CRC progression and their potential as therapeutic targets.

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ACCORDING TO A GLOBAL EPIDEMIOLOGICAL ANALYSIS IN 2018, colorectal cancer (CRC) accounts for 10% of all cancers, with a mortality rate that has steadily increased in the years since. Although the roles of genetic alterations such as APC, TP53, KRAS, and key signaling pathways such as Wnt, RAS-MAPK, and PI3K in driving CRC have been extensively studied, the incidence and mortality of CRC are still at the forefront of all types of cancer and have been on the rise. There is a pressing need to investigate new molecular mechanisms underlying the pathogenesis of CRC for early detection, diagnosis, and targeted therapy. Recent studies have shown that RNA binding proteins appear to be important for the initiation and development of CRC. However, the exact roles of these proteins in CRC and how they regulate RNA targets in CRC remain largely unknown.

PUMILIO (PUM proteins belong to the highly conserved PUF (PUmilio-Fem3-binding Factor) family of RNA-binding proteins. There are two mammalian PUM homologs, PUM1 and PUM2. They bind to target transcripts through the PUMILIO Response Element (PRE) that contains an eight-nucleotide sequence motif (UGUANAU). In most cases, PUM proteins bind to the 3'UTR of target mRNAs and recruit partner proteins to reduce the stability of the mRNAs and/or inhibit their translation. Many PUF target genes have been identified for their important roles in embryonic development, body axis formation, body size control, neurogenesis, spermatogenesis, and self-renewal of stem cells. In addition, PUM proteins have recently emerged as promising regulators in cancer. Several studies have shown that PUM proteins are required for cancer cell growth. However, an investigation suggested that PUM represses oncogenes in multiple cancer types including CRC.

In this study, we investigated the biological function of PUM proteins in CRC and the underlying molecular mechanism. Moreover, it provides a new approach for the treatment of CRC.

Results

PUM1 and PUM2 display increased expression in human CRC. To investigate the functions of PUM proteins in cancer, we analyzed two online databases containing patients’ clinical features and gene expression data. We first examined the expression of PUM proteins in different tumors in the Human Protein Atlas (www.proteinatlas.org), and found that PUM1 had the highest protein level in CRC compared to other cancer types and PUM2 was also highly expressed in CRC (Supplementary Fig. 1a–b). We then examined Cancer Genome Atlas (TCGA) Datasets (http://software.broadinstitute.org/software/igv/tcga), which revealed that transcripts of Pum1 but not Pum2 were expressed at higher levels in Hong colorectal and Skrzypczak colorectal as compared to normal colon tissues (Supplementary Fig. 1c–d).

To further correlate Pum1 and Pum2 to CRC, we performed a pairwise comparison of mRNA expression in human CRC samples from 22 patients with their adjacent tissues. We found that Pum1 and Pum2 were highly expressed in CRC clinical specimens (Fig. 1a, c). Particularly, the mRNA level of Pum1 in 15 patients and Pum2 in 13 patients were at least 10-fold higher than their paired adjacent normal tissues. Furthermore, TCGA datasets showed that Pum1 but not Pum2 expression was positively correlated with CRC stage (Fig. 1b, d). We then plotted receiver operating characteristic (ROC) curves to further analyze the diagnostic values of Pum1 and Pum2 with Hong and Skrzypczak colorectal datasets. Our analysis showed that Pum1 has a larger area under the curve (AUC) values than Pum2 in CRC (Supplementary Fig. 1e–f). In addition, there may be a negative correlation between Pum1 expression and the survival rate of CRC patients (Supplementary Fig. 1g–h). Together, all these data implicate that PUM1 and PUM2 were involved in the progression of human CRC.

Intestine-specific deletion of Pum1 and Pum2 inhibit AOM/DSS-induced colon carcinogenesis in vivo. To examine the involvement of PUM proteins in CRC, we employed a well-established azoxymethane/dextran sodium sulfate (AOM/DSS) mouse model to evaluate the role of PUM proteins by conditional knockout of Pum1 and Pum2 genes in the intestinal epithelium (Fig. 1e). Lgr5-cre::Pum1/fllox/flox::Pum2/fllox/flox mice were generated by crossing Lgr5-cre with Pum1/fllox/flox::Pum2/fllox/flox mice. We treated Lgr5-cre::Pum1/fllox/flox::Pum2/fllox/flox mice with tamoxifen to obtain mice with Pum1 and Pum2 double knockout in colon epithelial cells (Pum1/2CKO). Tamoxifen-treated Pum1/2CKO mice showed significantly higher levels than the normal cell line, with HCT116 cells expressing Pum2 in adenocarcinoma cell lines.

To further characterize the function of Pum1/2 deficiency significantly inhibits the initiation and development of CRC.

PUM proteins contribute to CRC cell growth in vitro and their tumorigenicity in vivo. To further characterize the function of PUM1 and PUM2 in CRC, we first examined the expression of Pum1 and Pum2 in six different CRC cell lines, with a normal human colon mucosal epithelial cell line, NCM460, as a control (Fig. 2a, b). Pum1 was expressed in all six CRC cell lines at significantly higher levels than the normal cell line, with HCT116
cells showed the highest levels of protein expression. 
Pum2 also showed abnormally high levels of expression in five out of six cancer cell lines, even though the extent of over-expression was generally not as large as Pum1.

In order to study the function of Pum1 and Pum2 in CRC, we chose HCT116 as our cell model since it had the highest levels of Pum1 and Pum2 expression among detected CRC cell lines. We knocked down Pum1 and/or Pum2 in HCT116 cells using siRNAs (Supplementary Fig. 3a). These knockdowns reduced the colony formation ability and increased cell-doubling time (Supplementary Fig. 3b, e), indicating that Pum1 and/or Pum2 knockdown inhibited the growth of CRC cells in vitro. In addition, we found
that Pum1 and/or Pum2 knockout reduced the number of cells in S phase (Supplementary Fig. 3f, g). Moreover, knockout of Pum1/2 did not have a significant impact on the survival and migration of HCT116 cells, as indicated by the transwell assay and flow-cytometry using apoptotic markers (Supplementary Fig. 4). This indicates that the growth deficiency of CRC cells caused by loss of Pum1 and Pum2 was contributed by delayed G1/S transition. Notably, Pum1 and Pum2 knockout showed similar defects in colorectal growth, and double knockout of Pum1 and Pum2 showed additive defects, indicating that they play similar roles.

To assess the entire spectrum of Pum function in CRC, we knocked out Pum1 or Pum2 in both HCT116 and RKO cells, using the CRISPR/Cas9 nickase method. Our designed sgRNAs were able to target all isoforms of Pum1 and Pum2, respectively (Supplementary Fig. 5). Immunofluorescence staining indicated that PUM1 and PUM2 are diffusely distributed in the cytoplasm of HCT116 and RKO cells and that both proteins were no longer detectable in the knockout cells, validating the effectiveness of the deletions (Supplementary Fig. 6a, b). The cytoplasmic localization and depletion of PUM1 and PUM2 were further confirmed by cytoplasm-nuclear fractionation (Supplementary Fig. 6c, d) and western blotting (Fig. 2c), respectively. In addition, we tried to generate Pum1/2 double knockout HCT116 cells by screening more than 400 single cell clones but failed to recover any double knockout cell. This result indicates that Pum1/2 double knockout may seriously impair HCT116 cells survival.

The deletion of Pum1 and Pum2 significantly inhibited the colony formation and proliferation of HCT116 and RKO cells, as indicated by two independent Pum1 (Pum1−/−) and Pum2 (Pum2−/−) knockout clones (Fig. 2d-i and Supplementary Fig. 7). Consistently, Pum1-deficiency caused a delay in G1/S transition in both HCT116 and RKO cells (Fig. 2j, k). In the xenograft assay, knocking out Pum1 significantly inhibited the tumorigenicity of HCT116 and RKO cells (Fig. 2l-q). Pum2−/− clones displayed similar phenotype, though less severe in HCT116 cells (Fig. 2d-q).

Together, these results indicate that both Pum1 and Pum2 are essential to CRC tumorigenesis in vivo and cell growth in vitro. Because long G1 (i.e., slow cycling) and quiescence are hallmarks of cancer stem cells (CSCs), we wondered if Pum1-deficiency affected CSC-related properties of HCT116 and RKO cells. For this reason, we conducted RT-qPCR analysis to detect the expression of putative CSC markers, such as CD13346-49, CD16646-49, CD2430, CD4438,49,40,42, EpCAM48, GLI-143, Msi144,45, ALDH1A146,47 and Lgr538,48 in WT, Pum1−/−, Pum2−/−, Pum2−/−, and Pum2−/−/2 HCT116 cells (Supplementary Fig. 6e). Among them, ALDH1A1 and Lgr5 expression was too low to detect. CD133, CD166, CD44, GLI-1, and Msi1 showed various degrees of reduction in the Pum1/2-deficient cells. The expression of CD26 was significantly downregulated in both Pum1 and Pum2 knockout cells, while EpCAM expression was decreased in Pum2 but not Pum1 knockout cells. CD133 is most drastically downregulated in Pum1−/−/−, Pum1−/−, and Pum2−/−, except for one Pum2−/− replicate (Pum2−/−/−). Thus, Pum1/2 knockout may have an effect on the CSC population.

To further characterize the effect of Pum1/2 on CSC properties, we conducted flow cytometry using CD44 and CD133, two common colorectal CSC markers, and Lgr5+, an intestinal stem cell marker. In Pum1−/− and Pum2−/− cells, the number of CD133+ cells was remarkably reduced, except for one sample (Pum2−/−/− but the number of CD44+ cells was not reduced (Supplementary Fig. 6f, h). Unexpectedly, the number of Lgr5+ cells was increased (Supplementary Fig. 6g, i). These changes reflect that PUM1 and PUM2 have a function in regulating stem cell fate, with their deficiency leads to increased CD133+ Lgr5+ cells.

PUM1 functions in CRC cells mainly by directly regulating cancer-related and cell cycle-related genes. To explore the molecular mechanism underlying the PUM function in CRC, we analyzed the transcriptome of Pum1−/− and Pum2−/− HCT116 cells by deep sequencing (Fig. 3a, b). We identified 1132 and 1226 differentially expressed genes in Pum1 and Pum2 knockout cells, respectively (FDR < 0.1, fold change ≥ 1.5; Supplementary Data 1 and 2). To assess the reproducibility of the replicates, principal component analysis (PCA) was used to visualize the variation among different genotypes. Based on the random clustering of replicates in the PCA plot, we observed a high degree of uniformity among the replicates (Supplementary Fig. 8a). In Pum1−/− cells, 740 genes showed increased mRNA levels and 392 genes showed reduced mRNA levels. Among them, several cell cycle inhibitor genes were upregulated, including Cdkn1a/p21, Cdkn2d, and Cdkn2c (Fig. 3a). The tumor suppressor function of these genes has been well-established. Notably, a key oncogene, Myc, was downregulated. Similarly, In Pum2−/− HCT116 cells, 749 and 477 genes were significantly upregulated and downregulated, respectively. The upregulated genes contain a slightly different set of cell cycle genes. For instance, Ccnd3, Cdk6, and Cdk15 were upregulated in Pum2−/− cells but not Pum1−/− cells (Fig. 3b).

As PUM proteins regulate their targets largely at the post-transcriptional level, we expected a change of their targets at the protein level as well. Therefore, proteomics assays using TMT labeling were performed to identify protein changes between wild type, Pum1−/−, and Pum2−/− HCT116 cells (using fold change ≥ 1.2, a standard cut-off for TMT mass spectrometry because its data are nonlinearly compressed; Fig. 3c, d and Supplementary
The PCA of mass spectrometry based on normalized peptide abundance showed that biological replicates of each genotype could be clustered together (Supplementary Fig. 8b). Consistently, the mRNA and protein level of cell cycle inhibitor gene Cdkn1a/p21 was upregulated in Pum1<sup>+/−</sup> HCT116 cells. This suggested that PUM1 may repress p21 translation in CRC. The protein levels of Wnt signaling inhibitor Dkk1 and tumor suppressor genes Susb2 and Pdlim5 were also upregulated in Pum2<sup>−/−</sup> HCT116 cells, indicating the involvement of the Wnt pathway.

To analyze how much of the changes at the protein level is due to the changes at the mRNA level, because the nonlinear nature of
Fig. 2 PUM1 and PUM2 drive colorectal cancer cell growth in vitro and tumorigenicity in vivo. a The relative levels of Pum1 and Pum2 mRNA in six human colorectal cancer cell lines normalized to their expression levels in a human colon epithelial cell line, NCM460. Actin was used as an internal control to normalize the level of Pum1 or Pum2 among the duplicate samples (n = 3) of the same cell line. Error bars represent SEM. **P < 0.01, ***P < 0.001 by Student’s t-test. b Levels of PUM1 or PUM2 proteins in a normal colon epithelial cell line NCM460 and six human CRC cell lines. p-actin was used as a loading control. c Knockout efficiency of Pum1 and Pum2 in HCT116 and RKO cells, in which HCT116 Pum1−/−/− represents #12, HCT116 Pum1−/−/2 represents #23, HCT116 Pum2−/−/− represents #10-4, HCT116 Pum1−/−/2 and Pum2−/−/2 represents #8-4, RKO Pum1−/−/−/− represents #3-4, RKO Pum1−/−/−/2 represents #3-18, RKO Pum2−/−/−/− represents #2-19 and RKO Pum2−/−/−/2 represents #2-27 according to numbers in Supplementary Fig. 5d, f. Tubulin was used as a loading control. d Representative images of colony formation assay in WT, Pum1−/− and Pum2−/−/− HCT116 (d, n = 2) and RKO (e, n = 3) cells. Bar graphs show the colony numbers per dish. Error bars represent SD. f Growth curves of WT, Pum1−/− or Pum2−/−/− HCT116 (f, n = 4) and RKO (g, n = 3) cells. Error bars represent SD. h I Cell doubling time of WT, Pum1−/− or Pum2−/−/− HCT116 (h, n = 2) and RKO (i, n = 3) cells. Error bars represent SD. j k Cell cycle analysis of WT, Pum1−/− or Pum2−/−/− HCT116 (j, n = 2) and RKO (k, n = 3) cells. Error bars represent SD. l-m Tumor growth (l), tumor volume (m), tumor weight (n) in mice injected with WT, Pum1−/− or Pum2−/−/− HCT116 cells (n = 8). Error bars represent SD. n,o A tumor growth (n), tumor volume (o), tumor weight (p) in mice injected with WT, Pum1−/− or Pum2−/−/− RKO cells (n = 8). Error bars represent SD. Source data are provided as a Source Data file.

the mass spectrometry data precludes direct comparison, we plotted the mRNA and protein log2FoldChange of Pum1−/− and Pum2−/− cells, respectively. The correlation of gene expression changes at the mRNA and protein levels for Pum1 knockout is 0.695 (Fig. 3e) and for Pum2 knockout 0.764 (Fig. 3f). This indicates that changes at the protein level to a large extent resulted from changes at the mRNA levels. In addition, Pum1−/− and Pum2−/− cells showed quite similar changes in gene expression, with a 0.782 correlation on the mRNA level (Fig. 3g) and 0.708 on the protein level (Fig. 3h). In general, Pum1 knockout resulted in slightly greater changes in mRNA abundance than Pum2 knockout. On average, the fold change in mRNA abundance for the upregulated genes is 3.3 in Pum1−/− cells and 2.3 in Pum2−/− cells, and for the downregulated genes is 4.1 in Pum1−/− cells and 2.3 in Pum2−/− cells. With respect to individual genes, Pum1−/− cells also displayed a significantly greater extent of fold change than Pum2−/− cells at both the mRNA and protein levels (Supplementary Fig. 9; Wilcoxon rank sum test \( P = 1.943e-06 \) and 1.658e−11 for mRNAs and proteins, respectively). KEGG pathway analysis of the changed mRNAs and proteins in Pum1−/− and Pum2−/− HCT116 cells revealed that cancer-related genes were enriched (Fig. 3i−l and Supplementary Data 5). Consistent with tumorigenic defects in Pum1−/− and Pum2−/− HCT116 cells, PUM proteins appear to regulate the expression of cancer-related genes to promote CRC growth.

To identify the direct targets of PUM1, we performed PUM1 PAR-CLIP experiments in WT HCT116 cells with Pum1−/− HCT116 cells as negative controls, which would map binding sites of PUM1 on its target mRNAs at the single-nucleotide resolution (Supplementary Fig. 10a, b). Two biological replicates were carried out for each condition, 2228 and 1961 clusters were grouped as PUM1 binding sites in two WT replicates by PARalyzer, respectively. The clusters showed large overlaps for PUM1 binding transcripts (Fig. 4a). We calculated the Spearman correlation of transcripts for two biological replicates of PUM1 PAR-CLIP, which showed high reproducibility (Supplementary Fig. 10c). We found that the binding sites showed the conserved TGTANATA binding motif (N represents A/C/U) (Fig. 4b). This motif was mostly present in the 3’UTRs of the mRNAs, with the second abundant region being the CDS (Fig. 4c). In addition to mRNAs, we identified the long noncoding RNA–NORAD (Supplementary Fig. 10d and Supplementary Data 6), a known target of PUM1 containing 17 binding sites. Targets identified by PAR-CLIP were validated by qPCR (Fig. 4d, e). Given the observed G1/S transition delay in the Pum1-deficient cells, we mainly focused on the genes that are responsible for G1 progression and found that an upregulated gene in Pum1−/− HCT116 cells, p21/Cdkn1a, was enriched in PUM1 PAR-CLIP and contained a canonical PRE site (TGTAnATA) presented in 3’UTR (Fig. 4f). Moreover, cell cycle and cancer pathways such as ErbB signaling pathway, p53 signaling pathway and CRC pathway are also enriched among the PUM1-direct targets (Fig. 4g and Supplementary Data 7).

To explore how PUM1 regulates its targets, we further investigated 1132 mRNAs whose expression were changed in Pum1−/− HCT116 cells and contained PUM1 binding sites as indicated by the PAR-CLIP analysis. We also analyzed 1968 proteins whose expression were changed in Pum1−/− HCT116 cells. We found that 15 changed mRNAs and 48 changed proteins were identified as targets of PUM1 (Fig. 4h−k). KEGG analysis on these 15 mRNAs and 48 proteins as well as on the target genes of PUM1 PAR-CLIP revealed that they are enriched in cell cycle and cancer pathways, etc. (Fig. 4l, m and Supplementary Data 7). The changes of 17 mRNAs in Pum1−/− HCT116 cells were all validated by RT-qPCR (Fig. 4n). These results indicate that PUM1 is required for the growth of CRC cells mainly by directly regulating cancer-related and cell cycle-related genes.

PUM1 directly represses p21 to regulate CRC growth. Among the PUM1 direct targets as revealed by PAR-CLIP-seq experiments, p21 plays an important role in G1/S phase transition. In addition, p21 expression is negatively correlated with the overall survival rate of CRC and, to a less extent, disease-free survival (Supplementary Fig. 11a). We therefore focused on the regulation of PUM proteins towards p21. RNA deep sequencing and protein mass spectrometry revealed that p21 was repressed by PUM1 at both RNA and protein levels. We further confirmed these results by RT-qPCR and western blotting, which indicated that both RNA and protein levels of p21 were increased in Pum1−/− cells (Fig. 5a, b). However, the increase of p21 RNA and protein level in Pum2−/− was not as significant as that in Pum1−/− cells (a similar phenomenon was observed in HCT116 cells transfected with siPum1 and/or siPum2, data not shown), consistent with this, our RNA co-immunoprecipitation (RIP) experiments indicated that p21 mRNA was bound strongly (7.4-fold enrichment) by PUM1, while weaker (1.9-fold enrichment) by PUM2 (Fig. 5c, d). Therefore, we focus our effort on PUM1 regulation of p21.

To test whether PUM1 negatively regulates the stability of p21 mRNA, we used actinomycin to inhibit transcription and then examined the mRNA level in HCT116 cells (Fig. 5e). The stability of p21 mRNA was increased in Pum1−/− HCT116 cells, but not significantly in Pum2−/− HCT116 cells. These results demonstrated that PUM1 repressed p21 by negatively regulating its mRNA stability, but PUM2 had little effect on p21 mRNA stability. We next performed dual luciferase reporter assay to evaluate the PRE contribution in PUM1-mediated p21 regulation. 3’UTR with mutated PRE showed a significant increase of
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Fig. 3 Cancer-related genes are enriched among genes affected in Pum1−/− HCT116 cells. a–d Volcano plot showing the significantly upregulated (red dots) and downregulated (blue dots) mRNA (a, b) or protein (c, d) in Pum1−/− and Pum2−/− cells, including those in cell cycle, Pum1, and Pum2, as labeled. For mRNA, cutoff criteria are false discovery rate (FDR) ≤ 0.1, fold change (FC) ≥ 1.5 or FC ≤ −1.5. For protein, cutoff criteria are FC ≥ 1.2 or FC ≤ −1.2. e, f Scatterplot of log2FoldChange at mRNA and protein levels upon Pum1 (e) or Pum2 (f) knockout. g, h Expression changes in Pum1−/− cells correlate well with that in Pum2−/− cells at both mRNA (g) and protein (h) levels. i, j KEGG analysis of differentially expressed mRNAs in Pum1−/− (i) and Pum2−/− (j) HCT116 cells. k, l KEGG analysis of differentially expressed proteins in Pum1−/− (k) and Pum2−/− (l) HCT116 cells. Source data are provided as a Source Data file.

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luciferase signal over wild-type 3′UTR in the presence of Pum1 expression construct, to a level similar to the control construct without p21 3′UTR (Supplementary Fig. 11b). These results indicate that PUM1 inhibits p21 expression via the PRE in the 3′ UTR.

Given the repression of PUM1 towards p21 and the possible involvement of p21 in CRC, we investigated whether the deletion of PUM1 would reduce cell proliferation and delay G1-S transition by directly upregulating p21 expression. We mutated the PRE in the p21 gene by knocking in a mutated PRE donor sequence in the genome of HCT116 cells (Fig. 5i). DNA sequencing showed that we generated a mutant p21 PRE (p21 PREmut/mut) by converting TGTA to ACAT (Fig. 5g). As expected, mutating the p21 PRE reduced the binding of PUM1 to p21 mRNA (Fig. 5h), and led to increased expression of p21 mRNA and protein (Fig. 5i, j). These results indicated that p21 PREmut/mut relieved p21 mRNA from repression by PUM1.

We next analyzed cell growth and cell cycle of p21 PREmut/mut cells. Again, as expected, p21 PREmut/mut cells showed decreased colony formation, reduced cell proliferation (Fig. 5k–n), and increased proportion of cells in G0/G1 phase (Fig. 5o). Together, these results demonstrated that p21 mRNA is a direct and main target of PUM1 in facilitating CRC cell growth.

Nanoparticle-encapsulated PUM siRNA partially inhibits the development of CRC. To explore the possibility of using PUM1 and PUM2 as targets for anti-cancer treatment, we further investigated the in vivo antitumor activity of siRNA-loaded nanoparticles using the orthotopic colon cancer models (Fig. 6a)37. Two colorectal tumor models, HCT116-luc and
**Fig. 4 PAR-CLIP-seq identification of PUM1 targets.**

**a** Reproducibility of two biological replicates for PUM1 photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP).

**b** De novo discovery of the binding motif for PUM1 by MEME analysis.

**c** Distribution of the PUM1-binding sites in different gene regions.

**d** PUM1 PAR-CLIP peaks in the Arg and Rps9 transcripts in WT and Pum1−/− HCT116 cells. Arg is a target of PUM1 but Rps9 is not a target.

**e** PUM1 RNA immunoprecipitation (RIP)-qPCR validation of the target Arg and non-target Rps9 (n = 3). Error bars represent SEM.

**f** PUM1 PAR-CLIP peaks in the p21 transcript in WT and Pum1−/− HCT116 cells. TGTACATA is the binding site of PUM1.

**g** KEGG analysis of PUM1 PAR-CLIP target mRNAs (i) or proteins (m) that are changed in Pum1−/− HCT116 cells, demonstrating enrichment of genes involved in cell cycle. RT-qPCR validation of overlap genes of PUM1 PAR-CLIP targets with changed mRNAs in Pum1−/− HCT116 cells (n = 3). Error bars represent SEM. Source data are provided as a Source Data file.
Fig. 5 PUM1 regulates CRC growth by directly repressing p21. a The mRNA levels of p21 in WT, Pum1+/− and Pum2−/− HCT116 cells (n = 3) as assayed by RT-qPCR. Actin was used as an internal control. Error bars represent SEM. b Western blot showing the p21 protein levels in WT, Pum1+/− and Pum2−/− HCT116 cells. Tubulin was used as a loading control. The bar graph shows the quantification of the western blot (n = 3). Error bars represent SEM. c Western blot showing the p21 protein levels in WT, Pum1+/− or Pum2−/− HCT116 cells treated with actinomycin D (20 mg/ml) for the indicated period (n = 3). Error bars represent SEM. The bar graph shows the calculated p21 mRNA half-life (n = 3). Error bars represent SEM. d The p21 mRNA levels in WT, Pum1+/− or Pum2−/− HCT116 cells treated with actinomycin D (10 mg/ml) for the indicated period (n = 3). Error bars represent SEM. e Enrichment of p21 mRNA by PUM1 RIP (e) or PUM2 RIP (f) in WT, Pum1+/− or Pum2−/− HCT116 cells (n = 3). Error bars represent SEM. f Schematic diagram of generating p21 PRE mutations in HCT116 cells. DNA sequencing results of PRE in WT and p21 PREmut/+/− cells (g). Enrichment of p21 mRNA by PUM1 RIP in WT, Pum1+/−, and p21 PREmut/+ HCT116 cells (n = 3). Error bars represent SEM. g The mRNA of p21 in WT, Pum1+/− and p21 PREmut/+ HCT116 cells (n = 3). Actin mRNA was used as an internal control. Error bars represent SEM. h Western blot showing the p21 protein levels in WT, Pum1+/− and p21 PREmut/+ HCT116 cells. GAPDH was used as a loading control. The bar graph shows the quantification of the western blot (n = 3). Error bars represent SD. i Images of colony formation assay in WT, Pum1+/− and p21 PREmut/+ HCT116 cells. j Bar graph showing the colony numbers in each view in i (n = 3). Error bars represent SD. k Growth curve of WT, Pum1+/−, and p21 PREmut/+ HCT116 cells (n = 3). Error bars represent SD. l Cell doubling time of WT, Pum1+/−, and p21 PREmut/+ HCT116 cells (n = 3). Error bars represent SD. o Cell cycle analysis of WT, Pum1+/−, and p21 PREmut/+ HCT116 cells (n = 3). Error bars represent SD. Source data are provided as a Data File.
COLO205-luc, were used in this study. The established poly-
ethylenimine (PEI)-coated, glutathione (GSH)-responsive,
mesoporous silica nanoparticles (MSN) were adopted for anti-
Pum1 and Pum2 siRNA delivery. Empty MSN without PEI
coating were spherical with a diameter of ~90 nm as shown in
transmission electron microscopy (TEM; Supplementary
Fig. 12a). The hydrodynamic diameter of empty MSN without
PEI determined by dynamic light scattering (DLS) were 100 nm
(Supplementary Fig. 12b), a little larger than the dimension
acquired by TEM. PEI coating and siRNA loading slightly
increased the nanoparticle size. The zeta potential reversed from
negative (−33 mV) for empty MSN to highly positive (34 mV) for

Fig. 6 In vivo therapy using siRNA-loaded MSN in mice bearing orthotopic HCT116 or COLO205 colorectal tumor. a Schematic diagram of orthotopic implantation and nanoparticle therapy. The mouse icon was from https://smart.servier.com/. b Tumor targeting of the siRNA-loaded nanoparticles was observed using IVIS Spectrum CT multimodal imaging system. Luciferase labeled tumor cells and the Cy5 labeled siRNA@MSN after 24 h i.v. injection. The green signals indicated the orthotopic colorectal tumor and the red fluorescence signals denoted the Cy5-labeled nanoparticles. The nanoparticle distribution in the tumors observed from the orthogonal, coronal, sagittal, and transaxial directions were separately shown. c Treatment regimen. d, h In vivo bioluminescence imaging of HCT116-luc (d) and COLO205-luc (h) tumor at the indicated time. e, i Tumor growth profiles were obtained through quantifying the bioluminescence in panel d and h (n = 5). Panel e: a siPum2@MSN versus saline (P = 4.22E−06) or siNC@MSN (P = 0.0001). b siPum1@MSN versus siPum2@MSN (P = 0.0030). c siPum1/2@MSN versus all other groups: saline (P = 4.52E−07), siNC@MSN (P = 0.0002), siPum1@MSN (P = 0.0006), siPum2@MSN (P = 0.0006). Panel i: a siPum2@MSN versus saline (P = 0.0002) or siNC@MSN (P = 0.0006). b siPum1@MSN versus siPum2@MSN (P = 0.0013). c siPum1@MSN versus siPum2@MSN (P = 5.2E−05), siPum2@MSN (P = 1.22E−06). Error bars represent SD. f, j Tumor weight on the last day (Day 30) of the test (n = 5). Panel f: a siPum1/2@MSN versus saline (P = 2.21E−06) or siNC@MSN (P = 1.25E−06). b siPum1/2@MSN versus siPum1@MSN (P = 0.0403) or siPum2@MSN (P = 0.0122). Panel j: a siPum2@MSN versus saline (P = 3.8E−06) or siNC@MSN (P = 7.24E−05). b siPum1/2@MSN versus siPum1@MSN (P = 0.0011) or siPum2@MSN (P = 8.25E−06). Error bars represent SD. g, k Mice body weight (n = 5). Error bars represent SD. Source data are provided as a Source Data file.

PEI-coated siRNA-loaded MSN (Supplementary Fig. 12c). siRNA molecules were efficiently released in the presence of 5 mM GSH-contained PBS, which well mimicked the environment of cancer cell cytoplasm (Supplementary Fig. 12d,e). Tumor targeting of the siRNA-loaded nanoparticles via the enhanced permeability and retention (EPR) effect was observed using IVIS Spectrum CT multimodal imaging system (Fig. 6b). The Cy5 labeled siRNA@MSN specifically targeted luciferase-labeled tumor cells 24 h after tail vein injection, demonstrating the feasibility of our approach.

We implanted HCT116 and COLO205 tumor cells subcutaneously into 4-week-old male BALB/c nude mice. Twenty-eight days after the implantation, mice showed comparable bioluminescence intensity of the tumors and were divided into five groups (five mice/group). These mice were intravenously injected with nanoparticle formulations every three days for four times (day 0, 3, 6, 9; Fig. 6c). Bioluminescence imaging was used to monitor tumor growth every week (day 7, 14, 21, 28; Fig. 6d, h). For the HCT116 tumor model, non-specific siRNA control (siNC@MSN) did not delay tumor growth. In contrast, injection of nanoparticles loaded with anti-Pum1 siRNA (siPum1@MSN) or anti-Pum2 siRNA (siPum2@MSN) dramatically inhibited tumor growth. Particularly, mice treated with both anti-Pum1 and anti-Pum2 siRNAs (siPum1/2@MSN) showed the strongest antitumor effect (Fig. 6e). At the end of the experiment (day 30), the mice were sacrificed and tumors were excised and weighed. The tumors of mice treated with siPum1/2@MSN were 3.7-4.0-fold smaller than those treated with controls (Fig. 6f). This reduction in tumor weight was not due to overt toxicity, because the body weights of all groups had a similar increase during the treatment (Fig. 6g).

Histological examination and immunohistochemistry staining in siNC@MSN and siPum1/2@MSN groups were performed at day 11. At this time point, the expression of PUM1 and PUM2 was decreased in siPum1/2@MSN samples (Supplementary Fig. 13), validating the effect of the nanoparticle-mediated knock down of PUM1 and PUM2. The PUM1 and PUM2 knockdown did not cause any detectable tissue damage, as examined by H&E staining (Supplementary Fig. 14), indicating the siPum1/2@MSN treatment did not cause non-specific tissue toxicity. Expectedly, the expression of the p21 protein was upregulated in tumors treated with siPum1/2 (Supplementary Fig. 13). H&E staining further revealed a significant decrease of adenomas when Pum1 and Pum2 were knocked down (Supplementary Fig. 15). In addition, Ki-67 staining indicated that the proliferation of the tumor cells in the siPum1/2@MSN group was inhibited compared with siNC@MSN group (Supplementary Fig. 13). These data indicate the important function of PUM1 and PUM2 in tumor progression.

Because metastasis is the main cause of death in patients with CRC61, with nearly 60% of CRC patients having metastasis in the liver62, we examined the anti-metastatic effect of the treatment. On day 30, major organs (heart, liver, spleen, lung, and kidney) from all the mice were excised for anti-metastasis evaluation using bioluminescence imaging (Supplementary Fig. 16). In the saline control group, all mice had metastases in the liver. Two of the five mice also had spleen and lung metastases. No metastases were observed in kidney. The mice in the siNC@MSN control group had similar metastasis pattern to that of the saline group. In contrast, the treatment of siPum1@MSN or siPum2@MSN significantly inhibited metastases in liver, spleen, and lung. Specifically, we observed no metastases in spleen for the siPum1@MSN group and in lung for the siPum2@MSN group. Furthermore, the siPum1,2@MSN group showed no metastases in any of the examined organs, representing the best anti-metastasis effect. Similar antitumor effect was also observed in the COLO205 tumor-bearing mice model (Fig. 6i–k and Supplementary Fig. 17). Taken together, these data indicate that Pum1 or/and Pum2 siRNA delivery by nanoparticles prevented further growth of CRC in vivo, and that Pum1/2 are potential targets for the treatment of CRC.

Discussion

In this paper, we reported the requirement of PUM1 and PUM2, two members of the human PUF protein family, for the initiation and progression of CRC. Furthermore, we discovered that such a cancer cell growth-promoting function was in part achieved by inhibiting the expression of p21, a negative regulator of cell cycle. Finally, we showed that treating CRC models by intravenous injection of nanoparticle-packaged anti-PUM siRNAs effectively reduced tumor growth. These findings provide insights into a new molecular mechanism important for CRC tumorigenesis as well as a potentially new approach for treating CRC.

In recent years, several studies have reported that human PUM proteins play an important oncogenic role. However, the function of PUM proteins in different tumors is different, and there is a debate on whether PUM proteins are oncopgenes or tumor suppressors. Kedde et al. reported that PUM1 and classical oncogenic miRNAs miR-221 and miR-222 co-repress the expression of the cell cycle inhibitory protein p27 in human breast cancer cells and HEK293 cells63. Because the knockdown of PUM1 reduced the number of cells in S phase and inhibit cell proliferation, the authors suggested that PUM1 may promote tumorigenesis in breast cancer. In non-small cell lung cancer, tumor suppressor miR-340 directly binds to the 3′UTR of Pum1 and Pum2 mRNA to inhibit their expression, thus reducing PUM1 and PUM2 that are required for the miR-221/222 interaction with the p27 3′
In addition, recent studies in leukemia cells have shown that PUM proteins can directly bind to the mRNA of the transcription factor FOXP1 to increase the expression level of FOXP1 protein, thereby increasing the proliferation of hematopoietic stem cells and myeloid leukemia cells. Moreover, PUM1 and PUM2 inhibit kinase activator GC-32 by dephosphorylation to promote the growth of EB virus immortalized B cells. Finally, PUM1 can promote the development of ovarian cancer. All of these studies are similar to our findings that knockout or knockdown of PUM1 and PUM2 block cell G1/S phase transition by upregulating p21 expression (Figs. 2 and 5), thus significantly inhibits the proliferation of CRC cells. All of the above studies have revealed the requirement of PUM proteins for oncogenesis. However, Miles et al. found that PUM1 and PUM2 co-inhibit the expression of oncogene E2F3 with miRNA in bladder cancer. In this type of cancer, even though the level of PUM1 or PUM2 protein itself did not change significantly, the level of the miRNAs (miR-503, miR125b) synergizing with PUM proteins was decreased significantly, which allowed oncogenesis. In triple-negative breast cancer cells, PUEs in the 3'UTR of oncogene c-jun were deleted due to alternative polyadenylation, which promotes cancer. These studies indicate an onco-suppressor role of PUM proteins. Thus, PUM proteins have opposite functions in different cancers. This difference may be due to their targeting of different genes or partnering with different proteins in different types of cancer.

An important finding of our study is that p21 mRNA is a direct and main target of PUM1. In order to obtain more credible PUM1 target mRNAs, we used relatively stringent criteria in our CLIP data analysis, including parameter adjustments for PABAlzyer, binding sites intersection from two biological replicates, and presumptive background signal removal. This results in 160 PUM1-bound target mRNAs. When we reduced stringency, more direct target genes were recovered from the CLIP, including an increased number of targets that overlapped with genes showing differentially expressed mRNAs or proteins in the Pum1−/− cells. We limit ourselves to the 160 targets to avoid false positive targets. It was surprising to us as well that only 15 and 48 of the >1000 genes whose expression was changed by Pum1−/− cells were bound by PUM1. This indicates that most of the differentially expressed genes are indirect targets of PUM1. Meanwhile, PUM1 may bind to some mRNAs without any significant regulatory function. In addition, it is possible that both PUM1 and PUM2 binding are needed to generate an easily detectable regulatory effect on some other of the 160 PUM1-target mRNAs, since PUM1 and PUM2 bind to overlapping set of targets. Finally, it is possible that PUM1 might bind to some mRNAs without any significant regulatory function.

Among the 160 targets, p21 is the only one associated with the cell cycle among the direct PUM1-target genes that are regulated by PUM1 at both mRNA and protein levels (Fig. 5). A previously study reported that PUM1 and PUM2 positively regulated FOXP1, and FOXP1 mediates the growth-promoting activity of the PUM proteins by repressing the expression of p21 in human hematopoietic stem/progenitor cells and leukemic cells. Their results suggested that PUM1 negatively regulates p21, which was consistent with our finding. However, we provide strong evidence indicating that PUM1 directly binds to and represses p21 via the 3'UTR PRE of p21 mRNA instead of through FOXP1 or other factors. Our results showed that PUM1 significantly down-regulates both mRNA and protein levels of p21 by promoting p21 mRNA turnover, possibly through the well-known pathway in which PUM proteins interact with the CCR4-POP2-NOT deadenylase complex to inhibit mRNA stability. This eventually results in CRC proliferation and shortened G1/S transition.

Based on our results, we propose the following model to illustrate the function of PUM proteins in human CRC. PUM1 is highly expressed in CRC and bind to p21 mRNA to reduce its expression. This relieves p21 suppression of cell cycle and cell growth mechanisms, allowing CRC progression. Either Pum1 deletion or p21 RPE mutation abolishes PUM repression of p21, which results in CRC growth defects and delayed G1/S transition. PUM proteins are widely expressed in many tissues, so in order to evaluate whether a PUM protein can be used as a target for cancer treatment, one needs to determine whether such treatment will cause broad side effects. Remarkably, Pum1;Pum2 double knockout in small intestinal epithelial cells did not cause any detectable effect on intestinal homeostasis and function in our mouse CRC model (Supplementary Fig. 2). Instead, we found that conditional knockout of Pum1 and Pum2 effectively blocked the occurrence and development of colorectal tumors (Fig. 1e-i). Perhaps most importantly, when we treated colon orthotopic implant tumors by tail vein injection of nanoparticles encapsulated with anti-Pum1/2 siRNAs, these siRNAs prevented the further growth of colorectal tumors without obvious effects on other organs (Figs. 6g and S14). This might be because PUM proteins are highly expressed in CRC at higher levels than in normal tissues, and are therefore more sensitive to Pum1 knockdown and/or because nanoparticles are highly accumulated in these tumor cells. Our results point to the feasibility of using PUM proteins as targets for CRC treatment. A systematic examination of the biodistribution of Pum1/2 siRNA loaded nanoparticles, and the levels of Pum1 and Pum2 in other tissues in the future will further test this feasibility.

**Methods**

**Mice.** Male or female BALB/c nude mice were purchased from Shanghai Ling-chang Biotechnology Co., Ltd (Shanghai, China). All animals were housed and maintained in pathogen-free conditions and allowed free access to food and autoclaved water ad libitum in a 12 h light/dark cycle, with room temperature at 21 ± 2 °C and humidity between 45 and 65%. All animal experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Biomedical Research Ethics Committee of the ShanghaiTech University or Shanghai Jiao Tong University School of Medicine (SJTU-SM).

**Cell culture.** HCT116, RKO, COLO205, LOVO, SW480, SW620, HT29 cells were purchased from the American Type Culture Collection (ATCC) and cultured according to the culture methods of ATCC. Colon normal immortalized epithelial cell line NCM460 was obtained from In Cell (San Antonio, TX) and cultured according to the method of manufacturer.

**Cell cycle assay.** For each sample, 2–5 × 10^6^ cells were collected by centrifugation at 400 × g for 5 min at 4 °C and washed twice with 1 ml ice cold PBS. Supernatant was discarded and cell pellet was resuspended with 250 μl ice cold PBS. Seven hundred and fifty microliter absolute ethyl alcohol (pre-cooled at −20 °C) was slowly added into the suspension drop by drop to 75% final concentration on low speed vortex. Cell suspension was fixed at 4 °C for overnight or for 4 h at −20 °C. Cells were washed once with 1 ml ice cold PBS, collected by centrifugation at 1000 × g for 5 min at 4 °C, stained by 400 μl PI Staining Solution/RNase solution for 30–60 min in the dark at 4 °C, and then analyzed by flow cytometry. The maximum excitation wavelength is 488 nm. Data analyses were performed using Modfit LT 5.0.

**Apoptosis assay.** HCT116 or RKO cells were stained with the FITC Annexin V apoptosis detection kit (556547, BD Biosciences) or PE Annexin V apoptosis detection Kit (559763, BD Biosciences) according to the manufacturer protocol and analyzed using BD FACSAria III (BD Biosciences). Data analyses were performed using CytoExpert (version 2.0).

**Cell growth curve and colony formation.** For growth curve assay, a total of 1 × 10^4^ HCT116 or RKO cells per well were seeded in 12-well plate, triplicate wells were seeded. Cells were counted every 24 or 48 h for a total of 5–8 days. For colony formation assay, a total of 1000 HCT116 or RKO cells per well were seeded in 6-well plate and triplicate wells were seeded. After 10 days, cells were stained with Crystal Violet Staining Solution (C0121, Beyotime).

**RNA extraction and real-time PCR.** Total RNA from cultured cells of desired genotypes was extracted with TRIzol reagent (Thermo, 15596026) and genomic
DNA was removed using DNA-free™ Kit. D Nasate Treatment and Removal Reagents (Thermo, AM1906) according to the manufacturer’s protocol. Total RNA was then purified using the High Capacity DNA Reverse Transcription Kit (Thermo, 4368814). Quantitative real-time PCR reactions were performed according to the protocol of the iQ™ Sybr Green Supermix (Bio-Rad, 1725125). The same PCR program was used for all quantifications: 95°C for 3 min, 95°C for 10 s, 55°C for 10 s, and 72°C for 30 s. This cycle was repeated 40 times, followed by a dissociation curve measurement. The efficiency of primers used for mRNA are listed in Supplementary Table 1. Primers of the actin gene were designed as a real-time PCR control. ΔCt method was used for normalized expression. Data analysis was performed in accordance with MIQE guidelines.

Western blot. Western blot analysis was performed according to our previous method. The following antibodies were used: Recombinant Anti-Pumilio 1 antibody (EPR3795) (1:1000, Abcam, Cat#: ab292390), Recombinant Anti-Pumilio 2 antibody [EPR3813] (1:1000, Abcam, Cat#: ab108395), p21 Waf1/Cip1 (1:200, Rabbit mAb (1:1000, cell signaling technology, Cat#: 29973), α-Tubulin (1:100) Rabbit mAb (1:1000, cell signaling technology, Cat#: sc-51973), GAPDH (1:1000, cell signaling technology, Cat#: 21185), β-Actin (1:1000) Rabbit mAb (1:1000, cell signaling technology, Cat#: 49705) and HRP-conjugated AffiniPure Goat Anti-Rabbit IgG (H + L) (1:5000, ProteinTech, Cat#: SA00001-2).

RNA immunoprecipitation (RIP) and statistical analysis. For each condition in each biological repeat, cells in a 10 cm dish reaching about 90% density were homogenized in RIP buffer (20 mM Tris-HCl, 150 mM NaCl, 1% IGEPA, 0.5 mM CaCl2 (SIGMA, A8996), 1 mM EDTA, 0.5 M DTT, CompleteTM EDTA-free Protease Inhibitor Cocktail Tablets (MERCK, 4693132001), PhosStop Tablets (MERCK, 496837001) and SUPER In RNase Inhibitor (Thermo, AM2694), spun at 13,000 × g for 15 min to remove the debris. Ten microliter of empty Dynabeads Protein A Theromodified™ (Thermo, 3480201008) were added to the lysate and incubated for 30 min for pre-clearing. Recombinant Anti-Pumilio 1 antibody [EPR3795] (0.25 mg/ml, Abcam, ab92545) or Recombinant Anti-Pumilio 2 antibody [EPR3813] (0.25 mg/ml, Abcam, ab92390) was incubated with the pre-cleared lysate overnight at 4°C, and 50 μl of Dynabeads were added. The incubation of lysates-antibody-Dynabeads was done with rotation at 4°C for 4 h. Five percent of the lysate-antibody-Dynabeads was saved as input sample. Next, the lysates-antibody-Dynabeads were washed three times with the RIP wash buffer (20 mM Tris-HCl, 200 mM NaCl, 0.05% IGEPA, 630 and 0.5 mM DTT), and the RNA was eluted using 1 μl of TRIzol (Thermo, 15598026) following the manufacturer’s manual. For quantification and statistical analysis, Percent Input Method was used for enrichment determination. In brief, signals obtained from the RIP were divided by signals obtained from an input sample. Before that, Input Ct value was corrected following the equation: C_{input} − C_{input} − log2 (1 + C_{input}). Since three triplicates were analyzed for each sample, the average C_{input} was calculated for target and non-target genes (primers of GAPDH were designed as a non-target control) in the input and the ΔCt in the RIP sample. Subsequently, the average ΔCt was calculated for each target RNA and samples (negative control: RIP in knockout cells, RIP −/− cells and measure them by mass spectrometry separately). Different from TMT, the label-free quantification approach aims to correlate the mass spectrometric signal of intact peptides, or the number of peptide sequencing events with the relative protein quantity directly.

RNA deep sequencing. Wild type, Pum1+/− and Pum2−/− HCT116 cells were cultured in (100 cm2) 5 Teflon-bottom tissue culture flasks (Corning, 1239003) with 5 Teflon-bottom tissue culture flasks (Corning, 1239003) with 0.05% Trypsin-EDTA for 14 h. Cells were then scraped from culture plates, transferred into a 15 ml Falcon tube, washed once with PBS, pelleted by centrifugation at 5000 × g for 5 min and flash-frozen in liquid nitrogen for storage at −80°C.

On the day of the experiment, the cell pellets were thawed on ice, suspended in three volumes of lysis buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA) and digested with 0.5 U/μl IGEPA, 630 and 0.5 mM DTT. The RNA was purified using QIA kit from RNase T1 (Thermo Scientific, EN0541) at 22°C for 15 min. Meanwhile, the Protein A bead-antibody complexes were prepared. For each sample, 0.25 mg/ml Pum1 antibody (Abcam, Cat#: ab92545) was conjugated to 100 μl protein A beads at 4°C for 2 h, the unbound antibody was removed and the pre-cleared lysate was added to antibody-coupled beads and incubated overnight at 4°C.

In the following day, the beads were washed five times with wash buffer [20 mM Tris-HCl pH 7.4, 200 mM NaCl, 0.05% (v/v) IGEPA, 630, 0.5 mM DTT, CompleteTM EDTA-free Protease Inhibitor Cocktail Tablets, and PhosStop Tablets] and incubated with 1 U/μl RNase T1 (Thermo Scientific, EN0541) at 22°C for 15 min. Meanwhile, the Protein A bead-antibody complexes were prepared. For each sample, 0.25 mg/ml Pum1 antibody (Abcam, Cat#: ab92545) was conjugated to 100 μl protein A beads at 4°C for 2 h, the unbound antibody was removed and the pre-cleared lysate was added to antibody-coupled beads and incubated overnight at 4°C.
Subsequently, NuPAGE LDS Sample Buffer (Thermo Fisher Scientific, Cat#: NP0007) was added to each sample and incubated at 95 °C for 5 min. Protein-RNA complexes were observed using NuPAGE 1-12% Bis-Tris-HCl Gels (Thermo Fisher Scientific, Cat#: NP03353BO) and desired complexes were excised from gel using a clean scalpel. The gel pieces were transferred to a D-tube Dialyzer Mide tube (Millipore, Cat#: 71506) and 800 μl of 1×SDS buffer (Thermo Fisher Scientific, Cat#: NP0001) was added. After electroelution the cross-linked RNA-RBP complex, the solution was transferred to tubes, added 800 μl of proteinase K buffer (100 mM Tris-HCl pH = 7.4, 150 mM NaCl, 12.5 mM EDTA, 2% (v/v) SDS (Bio-Rad, Cat#: 1610418)), and with 1.2 mg/ml of proteinase K (New England Biolabs, Cat#:P8910S), and incubated at 55 °C for 30 min. Then the RNA was recovered using peptides reagents according to the manufacturer's protocol.

The following steps of library preparation were performed as described according to the NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1).

Analysis of PAR-CLIP data. The forward read1 was taken from the paired-end sequenced PAR-CLIP data of each sample. This is because the RBP (RNA-binding protein)-bound fragments were short (<70 bp), the 150 bp long paired-read2 read and read2 will share the same fragment, thus one read (either read1 or read2) is sufficient. TrimGalore (version 0.4.4_dev, http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to trim off the adapter sequences. Only reads in the size range of 17–50 nucleotides were retained for downstream analyses. The size-selected reads were then aligned to the human hg19 genome using 0, 1, and 2 mismatches sequentially using bowtie (version 1.2.1.1) and the following procedure: 1) map reads to the genome using 0 mismatch (+ 0), retain the mapped reads, and align the unmapped genome to the genome using 1 mismatch (+ 1); 2) retain the unmapped reads and map the unmapped reads to the genome using 2 mismatches (+ 2).

A maximum of two mismatches were used because the RBP-binding sites contained T-to-C conversions during the PAR-CLIP treatment. To increase the signal-to-noise ratio, only reads resulting from up to 2 T-to-C conversions were selected, yet reads containing more than 2 mismatches were discarded. Customized scripts and command tools (upon request) were developed for processing aligned SAM files to identify reads that perfectly aligned to the genome, and those aligned to the genome with 1 or 2 mismatches resulting from T-to-C conversion(s) only. Subsequently, the three SAM files (0 mismatch, 1 T-to-C converted SAM, 2 T-to-C converted SAM) were merged and sorted using samtools (version 1.4.1) and acted as the input for PARalyzer (v1.58).

The parameters in the ".ini" file used as the input for PARalyzer are as follows:
- BANDWIDTH = 3
- CONVERSION = T-C
- MINIMUM_READ_COUNT_PER_GROUP = 5
- MINIMUM_READ_COUNT_PER_CLUSTER = 2
- MINIMUM_READ_COUNT_FOR_KDE = 3
- MINIMUM_CLUSTER_SIZE = 11
- MINIMUM_CONVERSION_LOCATIONS_FOR_CLUSTER = 2
- MINIMUM_CONVERSION_COUNT_FOR_CLUSTER = 2
- MINIMUM_READ_COUNT_FOR_CLUSTER_INCLUSION = 1
- MINIMUM_READ_LENGTH = 20
- MAXIMUM_NUMBER_OF_NON_CONVERSION_MISMATCHES = 1
- GENOME_2BIT_FILE = Homo_sapiens.GRCh19.dna.primary_assembly.2bit
- LIGHT_READS = False
- USE_NUCLEOTIDE_INDEX = False
- MAXIMUM_READ_LENGTH = 200
- FASTA_ALIGNMENT = True
- MINIMUM_ALIGNMENT_SCORE = 20
- MAXIMUM_ALIGNMENT_SCORE = 200

The bound regions identified by PARalyzer from the "resulting.cluster" file were processed with the hg19 gene annotations using bedtools (version 2.22.0) for detailed annotation (for instance, 3'UTR, 5'UTR, or intron of particular genes of the hg19 genome). Two replicates for each genotype (wild type and knockout) were analyzed separately. To identify PUM1-bound genes, the bound genes from each replicate of the wild type were combined by taking the union not the intersect, and those genes identified to be bound by PUM1 in the knockout were excluded, as they presumably contain non-specific binding sites not resulting from PUM1 but other non-specific activities of the antibody. The bound regions were analyzed for enriched motif analysis and visualized in IGV to infer the potential binding affinity expressed as the percentage of T-to-C conversions among all mapped reads covering the region of interest.

Immunofluorescence microscopy. Immunofluorescence microscopy assay was performed according to a previously described standard method55. The following antibodies were used: Recombinant Anti-Pumilio 1 antibody [EPR3795] (1:200, Abcam, Cat#: ab92545), Recombinant Anti-Pumilio 2 antibody [EPR8313] (1:200, Abcam, Cat#: ab92390) and Goat anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555 (1:10,000, invitrogen, Cat#: A32732). Immunofluorescence signal was transferred to 800 μl of proteinase K buffer [100 mM Tris-HCl pH = 7.4, 150 mM NaCl, 12.5 mM EDTA, 2% (v/v) SDS (Bio-Rad, Cat#: 1610418), Santa Cruz Biotechnology Inc, Cat#: sc-7846] and HRP-conjugated AffiniPure Goat Anti-Rabbit IgG (H + L) (1:1000, Proteintech, Cat#: SA00001-2). TUNEL staining was performed using an in situ Cell Death Detection Kit (Roche) based on the manufacturer's instructions.

Luciferase reporter assay. The 3’UTR of p21 cDNA was amplified and cloned into psiCHECK2 vector (C8021, Promega) using primers specified in Supplementary Table 1. In the 3’UTR reporter assay, HCT116 cells in 24-well plates were transfected with 100 ng of the psiCHECK2-p21-3’UTR-WT or 3’UTR mutant plasmid, with cotransfections in psiCHECK2 without mutations in p21 promoter and with mutations in p21 promoter along with 2 μl Lipofectamine 2000 (Invitrogen). Lysates were harvested after 48 h of transfection. The reporter activity was measured with the Dual Luciferase Assay (E1910, Promega).

Tumorigenesis assay. Four-week-old BALB/c nude mice (male) were purchased from Shanghai Lingzhang Biotechnology Co., Ltd (Shanghai, China). All experiments were performed under a protocol approved by the laboratory animals. BALB/c nude mice were randomly divided into two groups (n = 8 per group). Wild type, Pum1-/- and Pum2-/- HCT116 (2 × 10⁵) or RKO (3 × 10⁵) cells in 0.1 ml of medium mixture (medium: Matrigel = 1: 1) were subcutaneously injected into the nude mice. The tumor size was measured at time intervals as indicated in Fig. 2m, p. The mice were euthanized by cervical dislocation and the tumors were dissected. The tumor weight was measured, and the tumor volume was calculated using the following formula: D × d²/2, with “D” representing the longest diameter and “d” representing the shortest diameter. The maximum diameter of the tumor permitted by the animal ethics committee is 1.5 cm, and the size of all tumors in our experiment does not exceed 1.5 cm.

AOM/DSS model. There are two types of mice used in the AOM/DSS model: Lgr5^LGFP or Pum1^FLPflox or Pum2^FLPflox and Pum1^FLPflox or Pum2^FLPflox. Six to eight-week-old C57BL/6J (male) mice were first induced by intraperitoneal injection with tamoxifen (120 mg/kg) every other day for a total of 7 days. Sixteen days later, mice were injected intraperitoneally with 12.5 mg/kg anoxymethane (Sigma, St.Louis, MO). Dextran sodium sulfate (DSS) (2.5% wt/vol) (molecular mass, 36–50 kilodaltons; Medium biotechnology) was given for 5 days. Mice were then given regular drinking water for 14 days, followed by one additional cycle with DSS. In the third cycle, the DSS was adjusted from 2.5 to 2%. On day 65, mice were sacrificed for testing.

Preparation and characterization of siRNA-loaded MSN. We previously developed a novel polyethyleneimine (PEI) (1.8 kDa)-coated, glutathione (GSH)-responsive mesoporous silica nanoparticles (MSN) (for the delivery of miRNA-145) delivery57. The nanoparticles can quickly escape from the lysosome into the cytosol for miRNA-145 delivery, mediated by the proton sponge effect of PEI57. The reducing environment (0.5 mM GSH-contained PBS in the centrifuge tubes. The tubes were placed in a gas bath at 37 °C shaking at 100 rpm. At pre-determined time point (1, 2, 4, 8, 12, and 24 h), empty Dipalmitoylphosphatidylcholine (DPPC) liposomes (50 kDa) and centrifuged under 2350 × g at 4 °C for 30 min. FAM-labeled siRNA (Ex 488 nm, Em 520 nm) in the filtrate was collected for the quantification of nucleic acid release. The in vivo tumor targeting of the siRNA-loaded nanoparticles 24 h after i.v. injection was examined using the IVIS Spectrum/CT imaging system (PerkinElmer, USA), as previously described57.

siRNA molecules specifically targeting the mRNA of Pum1 and Pum2 were purchased from RIBOBIO (China). siPum1: GGTCAGAGTTTCCATGTGA, siPum2: CTCAGAAGTTTCCATGTGA, siPum2: CTCAGAAGTTTCCATGTGA, siPum2: CTCAGAAGTTTCCATGTGA. siRNA (FAM labeled) release test was performed in the presence of 5 mM GSH-contained PBS in the centrifuge tubes. The tubes were placed in a gas bath at 37 °C shaking at 100 rpm. At pre-determined time point (1, 2, 4, 8, 12, and 24 h), empty Dipalmitoylphosphatidylcholine (DPPC) liposomes (~30 mM, 120 nm, 25 mg/ml) were added to bind free PEI and PEI-siRNA complex. Then, the solution was transferred into Ultra Centrifugal Filters (molecular weight cut-off 50 kDa) and centrifuged under 2350 × g at 4 °C for 30 min. FAM-labeled siRNA (Ex 488 nm, Em 520 nm) in the filtrate was collected for the quantification of nucleic acid release.

Antitumor therapy in orthotopic colorectal tumor model. The orthotopic CRC model was developed in female BALB/c nude mice as described previously57. Twenty-eight days after orthotopic tumor cell (HCT116-luc, COLO205-luc) inoculation, the tumor-bearing mice were randomly allocated into five groups (n = 6 per group). mice were inoculated via the tail vein of C57BL/6J mice (male) intravenously treated with saline (control), MSN loaded with negative control siRNA with a scrambled sequence (siNC@MSN), and MSN loaded with siPum1 (siPum1@MSN), siPum2
were collected for H&E histological assay for toxicity evaluation. The major organs included heart, liver, spleen, lung, and kidney were harvested for ex vivo bioluminescence imaging (IVIS spectrum CT). The body weight was recorded throughout the study. At the end of the study (Day 30), the mice were sacrificed, the tumors were removed and weighted. Other major organs included heart, liver, spleen, lung, and kidney were harvested for ex vivo bioluminescent imaging to examine the metastasis. In a separate study, on Day 11 (2 days after the last injection of the nanoparticle), three mice from each group were sacrificed. The major organs, including heart, liver, spleen, lung, and kidney were collected for H&E histological assay for toxicity evaluation.

Statistics and reproducibility. For comparisons of decay curves, significance between fitted curves is indicated. For comparisons between groups, two-tailed Student’s t-tests were used. Exact P values are shown in the figures. Data were presented as the mean ± SD or mean ± SEM and P < 0.05 was considered significant. Statistical analysis was performed using GraphPad Prism 8 software. Pearson’s correlation analyses were used to calculate the regression and correlation between two groups. As indicated in the figure legends, all assays were performed in three biological replicates unless stated otherwise. Representative micrographs and western blot shown in figures were repeated three times independently with similar results.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The accession number for the RNA-seq and PAR-CLIP data reported in this paper is GEO: PRJNA648706. The accession number for the Mass spectrum data reported in this paper is PXD: 027513. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository83 with the dataset identifier PXD027513. Source data are provided with this paper.

Code availability
We used the following publicly available software for analysis of the PAR-CLIP data: TrimGalore version 0.4.4.dev, available at http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/, bowtie version 1.2.1, available at http://bowtie. bio.sourceforge.net/index.shtml, samtools version 1.4.1, available at https://github.com/ samtools/samtools/releases/, PARalyzer version 1.5, available at https://ohkuchi-lab. deis.de/ software/PARalyzer_85/bedtools version 2.22.0, available at https://bedtools.readthedocs.io/en/latest/index.html. A custom script used to analyze the PAR-CLIP data is available in the Zenodo repository under https://doi.org/10.5281/ zenodo.5981256.

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References
1. Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J. Clin. 68, 394–424 (2018).
2. Fearon, E. R. Molecular genetics of colorectal cancer. Annu. Rev. Pathol. 6, 479–507 (2011).
3. Cancer Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. Nature 487, 330–337 (2012).
4. Pereira, B., Billaud, M. & Almeida, R. RNA-binding proteins in cancer: old players and new actors. Trends Cancer 3, 506–528 (2017).
5. Chatterji, P. & Rustgi, A. K. RNA binding proteins in intestinal epithelial biology and colorectal cancer. Trends Mol. Med. 24, 490–506 (2018).
6. Gor, R., Sampath, S. S., Lazer, L. M. & Ramalingam, S. RNA binding protein PUM1 promotes colon cancer cell proliferation and migration. Int. J. Biol. Macromol. 174, 549–561 (2021).
7. Spassov, D. S. & Jurecic, R. The PUF family of RNA-binding proteins: does evolutionarily conserved structure equal conserved function? JURMB Life 55, 359–366 (2003).
8. Van Etten, J. et al. Human Pumilio proteins recruit multiple deadenylases to efficiently repress messenger RNAs. J. Biol. Chem. 287, 36370–36383 (2012).
9. Zamore, P. D., Williamson, J. R. & Lehmann, R. The Pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. Nucleic Acids Res. 23, 1421–1433 (1997).
10. Miller, M. A. & Olivas, W. M. Roles of Puf proteins in mRNA degradation and translation. Wiley Interdiscip. Rev. RNA 2, 471–492 (2011).
11. Lin, H. & Spalding, A. C. A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the Drosophila ovary. Development 124, 2463–2476 (1997).
12. Doniach, T. & Hodgkin, J. A sex-determining gene, fem-1, required for both male and hermaphrodite development in Caenorhabditis elegans. Dev. Biol. 109, 223–235 (1984).
13. Barton, M. K., Schedl, T. B. & Kimble, J. A sex-determining gene in Caenorhabditis elegans. Genetics 115, 107–119 (1987).
14. Chen, D. et al. Pumilio 1 suppresses multiple activators of p53 to safeguard mammalian oocyte maturation and maternal phase of embryogenesis. Cell 146, 855–863 (2011).
15. Mak, W., Fang, C., Holden, T., Dravet, M. B. & Lin, H. An important role of pumilio 1 in regulating the development of the mammalian female germline. Biol. Reprod. 94, 134 (2016).
16. Mak, W., Xia, J., Cheng, E. C., Lowther, K. & Lin, H. A role of Pumilio 1 in mammalian oocyte maturation and maternal phase of embryogenesis. Cell 146, 855–863 (2011).
17. Zhang, M. et al. Post-transcriptional regulation of mouse neurogenesis by Pumilio proteins. Genes Dev. 31, 1354–1369 (2017).
18. Galgano, A. et al. Comparative analysis of miRNA targets for human PUF-family proteins suggests extensive interaction with the miRNA regulatory system. PLoS ONE 3, e0030164 (2008).
19. Hafner, M. et al. Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 141, 129–141 (2010).
20. Morris, A. R., Mukherjee, N. & Keene, J. D. Ribonomic analysis of human Pumilio reveals cis-trans conservation across species despite evolution of diverse miRNA target sets. Mol. Cell Biol. 28, 4093–4103 (2008).
21. Breitkreutz, B. et al. Mammalian Pum1 and Pum2 control body size via translational regulation of the cell cycle inhibitor Cdkn1b. Cell Rep. 26, 2434–2450.e2436 (2019).
22. Dai, H. et al. PUM1 knockdown prevents tumor progression by activating the PERK/eIF2α/ATF4 signaling pathway in pancreatic adenocarcinoma cells. Cell Death Dis. 10, 595 (2019).
23. Guan, X. et al. PUMI1 promotes ovarian cancer proliferation, migration and invasion. Biochim. Biophys. Acta. 204, 313–318 (2018).
24. Naudin, C. et al. PUMI1/FoxP1 signaling drives expansion of hematopoietic stem/progenitor and leukemia cells. Blood 129, 2493–2506 (2017).
25. Xia, L. H., Yan, Q. H., Sun, Q. D. & Gao, Y. P. MiR-411-5p acts as a tumor suppressor in non-small cell lung cancer through targeting PUM1. Eur. Rev. Med. Pharmacol. Sci. 22, 5546–5553 (2018).
26. Spassov, D. S. & Jurecic, R. Cloning and comparative sequence analysis of PUM1 and PUM2 genes, human members of the Pumilio family of RNA-binding proteins. Gene 299, 195–204 (2002).
27. Lee, S. et al. Noncoding RNA NORAD regulates genomic stability by sequestering PUMI1O proteins. Cell 164, 69–80 (2016).
28. Miles, W. O., Tschop, K., Herr, A., Ji, J. Y. & Dyson, N. J. Pumilio facilitates miRNA regulation of the E2F3 oncogene. Genes Dev. 26, 356–368 (2012).
29. Dong, H., Downey, T., Eu, K. W., Koh, P. K. & Cheah, P. Y. A. ‘metastasis-prone’ signature for early-stage mismatch-repair proficient sporadic colorectal cancer patients and its implications for possible therapeutics. Clin. Exp. Metastasis-27, 83–90 (2010).
30. Skrzypczak, M. et al. Modeling oncogenic signaling in colon tumors by multidirectional analyses of microarray data directed for maximization of analytical reliability. PLoS One 5, e13091 (2010).
31. Sohn, O. S., Fiala, E. S., Requejo, S. P., Weisburger, J. H. & Gonzalez, F. J. Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogen azoxymethane and methylazoxymethanol. Cancer Res. 61, 8435–8440 (2001).
32. O'Brien, C. A., Pollett, A., Gallinger, S. & Dick, J. E. A human colon cancer cell line capable of initiating tumour growth in immunodeficient mice. Nature 445, 106–110 (2007).
33. Ricci-Vitiani, L. et al. Identification and expansion of human cancer-initiating cells. Nature 445, 111–115 (2007).
34. Vermeulen, L. et al. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. Proc. Natl Acad. Sci. USA 105, 13427–13432 (2008).
35. Zhu, L. et al. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. Nature 457, 603–607 (2009).
36. Dalerba, P. et al. Phenotypic characterization of human colorectal cancer stem cells. Proc. Natl Acad. Sci. USA 104, 10158–10163 (2007).
Bai, F., Chan, H. L., Smith, M. D., Kiyokawa, H. & Pei, X. H. p19(INK4d) is a metastasis and stem cell survival and expansion. EMBO Mol. Med. 1, 338–351 (2009).

Weichert, W., Knösel, T., Bellach, J., Dietel, M. & Kristiansen, G. ALCAM/CD166 is overexpressed in colorectal carcinoma and correlates with shortened patient survival. J. Clin. Pathol. 57, 1160–1164 (2004).

Todaro, M., Perez Alea, M., Scopelliti, A., Medema, J. P. & Stass, G. IL-4-mediated drug resistance in colon cancer stem cells. Cell Cycle 7, 309–313 (2008).

Huang, E. H. et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colon stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Res. 69, 3382–3389 (2009).

Carpentino, J. E. et al. Aldehyde dehydrogenase-expressing colon stem cells contribute to tumorigenesis in the transition from colitis to cancer. Cancer Res. 69, 8208–8215 (2009).

Barker, N. et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449, 1003–1007 (2007).

Sato, T. et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459, 262–265 (2009).

Barker, N. et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 457, 608–611 (2009).

Scheper, A. G. et al. Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. Science 337, 730–735 (2012).

Wiedemeyer, R. et al. Feedback circuit among INK4 tumor suppressors constrains human glioblastoma development. Cancer Cell 13, 355–364 (2008).

Eastham, J. A. et al. In vivo gene therapy with p53 or p21 adenovirus for prostate cancer. Cancer Res. 55, 5151–5155 (1995).

Bai, F., Chan, H. L., Smith, M. D., Kiyokawa, H. & Pei, X. H. p19(INK4d) is a tumor suppressor and controls putative anterior lobe cell proliferation. Mol. Cell Biol. 34, 2121–2134 (2014).

Krimpenfort, P. et al. p16(INK4b) is a critical tumour suppressor in the absence of p16(INK4a). Nature 448, 943–947 (2011).

Corcoran, D. L. et al. PARalyzer: definition of RNA binding sites from PAR-CLIP short-read sequence data. Genome Biol. 12, R79 (2011).

Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26, 841–842 (2010).

Robinson, J. T. et al. Integrated genomics viewers. Nat. Biotechnol. 29, 24–26 (2011).

Ma, J. et al. iProX: an integrated proteome resource. Nucleic Acids Res. 47, D1211–D1217 (2019).

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Author contributions

H.L and S.L conceived the project. Y.G., Z.L., Y.Y., C.F., S.L., and H.L designed the experiments and interpreted the results. In details, Y.G. performed the AOM-DSS mouse experiments and S.L. did data analysis. Y.G. and Z.L. performed the experiments on Pum1/2 biological function in vitro and data analysis. Y.G. performed the experiments of PAR-CLIP, Z.Y. and Y.G. performed analyses of PAR-CLIP data. Z.L. performed RNAseq and PAR-CLIP experiments. Y.G. and Z.L. performed Pum1 target gene validation experiments and data analysis. Y.G. generated p21 PRE mutations and studied their biological function. Y.Y. and Q.L. performed RNAi therapy experiments and data analysis. S.L. analyzed public cancer database. Y.G., S.L., C.F., and H.L. wrote the manuscript. Z.Y. and Z.L. commented on the manuscript. H.L. and S.L. supervised the overall work and C.F. was responsible for RNAi therapy.

Competing interests

The authors declare no competing interests.

Additional information

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