A local tumor microenvironment acquired super-enhancer induces an oncogenic driver for efficient growth under oxidative conditions in colorectal carcinoma

Royce Zhou

Icahn School of Medicine at Mount Sinai

Jia Xu

Icahn School of Medicine at Mount Sinai

Tiphaine Martin

Icahn School of Medicine at Mount Sinai <https://orcid.org/0000-0001-9990-1455>

Sait Ozturk

Icahn School of Medicine at Mount Sinai

Alexis Zachem

Icahn School of Medicine at Mount Sinai

John He

Icahn School of Medicine at Mount Sinai

Ankita Bansal

Icahn School of Medicine at Mount Sinai

Andrew Trotta

Icahn School of Medicine at Mount Sinai

Bruno Giotti

Icahn School of Medicine at Mount Sinai

Berkley Gryder

Genetics Branch, NCI, NIH <https://orcid.org/0000-0003-0130-2302>

Shen Yao

Icahn School of Medicine at Mount Sinai

Xuewei WU

The University of Hong Kong

Saul Carcamo

Icahn School of Medicine at Mount Sinai

Kaitlyn Bosch

Icahn School of Medicine at Mount Sinai

Benjamin Hopkins

Icahn School of Medicine at Mount Sinai <https://orcid.org/0000-0002-7970-6430>

Alexander Tsankov

Icahn School of Medicine at Mount Sinai

Randolph Steinhagen

Icahn School of Medicine at Mount Sinai

Drew Jones

New York University Langone Medical Center

John Asara

Beth Israel Deaconess Medical Center

Jerry Chipuk

Icahn School of Medicine at Mount Sinai

Rachel Brody

Icahn School of Medicine at Mount Sinai

Steven Itzkowitz

Icahn School of Medicine at Mount Sinai

Christine Chio

Columbia University

Dan Hasson

Icahn School of Medicine at Mount Sinai

Emily Bernstein

Icahn School of Medicine <https://orcid.org/0000-0001-6533-8326>

Ramon Parsons (✉ ramon.parsons@mssm.edu)

Icahn School of Medicine at Mount Sinai <https://orcid.org/0000-0002-6656-3514>

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A local tumor microenvironment acquired super-enhancer induces an oncogenic driver for efficient growth under oxidative conditions in colorectal carcinoma

Royce W. Zhou^{1,2,3}, Jia Xu^{1†}, Tiphaine C. Martin^{1†}, Sait Ozturk^{1†}, Alexis L. Zachem¹, John He^{1,2}, Ankita Bansal¹, Andrew P. Trotta¹, Bruno Giotti⁴, Berkley Gryder⁵, Yao Shen¹, Xuwei Wu¹, Saul Carcamo^{1,2}, Kaitlyn Bosch^{1,2}, Benjamin Hopkins^{1,2,4}, Alexander Tsankov^{2,4}, Randolph Steinhagen⁶, Drew R. Jones⁷, John Asara⁸, Jerry E. Chipuk^{1,2}, Rachel Brody⁹, Steven Itzkowitz¹⁰, Iok In Christine Chio¹¹, Dan Hasson^{1,2}, Emily Bernstein^{1,2}, Ramon E. Parsons^{1,2*}

¹Department of Oncological Sciences, The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai; New York, NY, 10029, USA.

²Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai; New York, NY, 10029, USA.

³Medical Scientist Training Program, Icahn School of Medicine at Mount Sinai; New York, NY, 10029, USA.

⁴Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai; New York, NY, 10029, USA.

⁵Department of Genetics and Genome Sciences, Case Western Reserve University; Cleveland, OH, 44106, USA.

⁶Division of Colon and Rectal Surgery, Department of Surgery, Icahn School of Medicine at Mount Sinai; New York, NY, 10029, USA.

⁷Metabolomics Core Resource Laboratory, NYU Langone Health; New York, NY, 10016, USA.

⁸Mass Spectrometry Core, Beth Israel Deaconess Medical Center; Boston, MA, 02215, USA.

⁹Mount Sinai Biorepository, Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA.

¹⁰Division of Gastroenterology, Department of Medicine, Icahn School of Medicine at Mount Sinai; New York, NY, 10029, USA.

¹¹Institute for Cancer Genetics, Department of Genetics and Development; Columbia University Medical Center, New York, NY, 10032, USA.

*Corresponding author. Email: ramon.parsons@mssm.edu.

†These authors contributed equally to this work.

Abstract

Tumors exhibit widespread enhancer landscape reprogramming compared to normal tissue. The etiology is believed to be largely cell-intrinsic in non-hormonal cancers, attributed to such genomic alterations as focal amplification of non-coding regions, aberrant activation of transcription factors, and non-coding mutations creating de novo transcription factor binding sites. Here, using freshly resected primary CRC tumors and patient-matched adjacent normal colon epithelia, we find divergent epigenetic landscapes between primary CRC tumors and CRC cell lines. We identify a unique super-enhancer signature largely absent in cell culture. Intriguingly, this phenomenon extends to highly recurrent aberrant super-enhancers gained in CRC over patient-matched normal epithelium suggesting novel insight into the etiology of enhancer reprogramming in CRC and its downstream relevance to tumor biology. We find one such super-enhancer activated in epithelial cancer cells due to surrounding inflammation in the tumor microenvironment. We restore this super-enhancer and its expressed gene, *PDZK1IP1*, following treatment with cytokines or xenotransplantation into nude mice, thus demonstrating its etiology via local tumor microenvironment acquisition. Building on its known role in glucose uptake via SGLT receptors, we demonstrate mechanistically that *PDZK1IP1* enhances the reductive capacity CRC cancer cells via the pentose phosphate pathway using polar metabolomic profiling. We show this activation enables efficient growth under oxidative conditions both *in vitro* and *in vivo*, challenging the previous notion that *PDZK1IP1* acts as a tumor suppressor in CRC. Collectively, these observations highlight the biologic significance of epigenomic profiling on patient-matched primary specimens and identify this microenvironment-acquired super-enhancer as an oncogenic driver in the setting of the inflamed tumor.

Introduction

Cells commit exceptional density of histone 3 lysine 27 acetylation (H3K27ac) and transcriptional machinery to large genomic regulatory regions termed super-enhancers (SEs)(Whyte et al., 2013). First described in embryonic stem cells, SEs control expression of genes critical for cell identity required for pluripotency in this context(Hnisz et al., 2013; Whyte et al., 2013). Dysregulation of the SE landscape occurs in cancer(Akhtar-Zaidi et al., 2012; Alonso-Curbelo et al., 2021; Bradner et al., 2017; Hnisz et al., 2015; Loven et al., 2013; Mack et al., 2018; Mansour et al., 2014; Morton et al., 2019; Roe et al., 2017; Sengupta and George, 2017; Zhang et al., 2016). Certain SE loci exhibit increased H3K27ac to potentiate oncogene expression, including *MYC* in several malignancies, *KLF5* in CRC, brachyury (*T*) in chordoma, *F3* in osteosarcoma, and *TALI* in T-cell acute lymphoblastic leukemia(Loven et al., 2013; Mansour et al., 2014; Morrow et al., 2018; Sharifnia et al., 2019; Zhang et al., 2018). Identification of dysregulated SEs between primary tumors and patient-matched normal remains incomplete for many cancers, and downstream validation for functional relevance for tumor growth is scarce.

The etiology of SE reprogramming in cancer is incompletely understood but is widely attributed to cancer cell-intrinsic genomic alterations. In CRC, highly prevalent somatic mutations in the *APC* tumor suppressor result in aberrant β -catenin translocation to the nucleus to activate the TCF family of transcription factors (TFs) that converge on the epigenome(Akhtar-Zaidi et al., 2012; Cohen et al., 2017; Hnisz et al., 2015). Non-coding SE can become focally amplified in multiple malignancies(Zhang et al., 2018; Zhang et al., 2016). Somatic non-coding mutations that create a *de novo* *MYB* TF binding site resulting in formation of an oncogenic SE at *TALI* has been described in T-ALL(Mansour et al., 2014). Similarly, inherited polymorphisms within SEs contribute to their activation and susceptibility to neuroblastoma(Oldridge et al., 2015).

The enhancer and SE landscapes are dynamic with regard to environment. One study observed the SE landscape of hair follicle stem cells to be niche-dependent mediated by pioneer and lineage factors(Adam et al., 2015). Pro-inflammatory cytokines upstream of NF- κ B and STAT family TFs have been reported to alter the SE landscape in endothelial as well as immune cells(Brown et al., 2014; Vahedi et al., 2012). Androgen and estrogen receptors respond to physiologic endocrine signaling to converge on the epigenome in hormonal cancers(Pomerantz et al., 2015; Ross-Innes et al., 2012; Wang et al., 2009). Whether the local tumor microenvironment influences the SE landscape of cancer cells is poorly understood. One previous study observed divergent enhancer landscapes between primary medulloblastoma tumors versus medulloblastoma cell lines, suggesting the possibility of local microenvironment influence(Lin et al., 2016).

First isolated based on its overexpression in epithelial cancers relative to normal tissue, *PDZK1IP1* (also known as DD96 and MAP17) encodes a 17 kDa transmembrane protein that interacts with the protein PDZK1, which contains four PDZ domains(Kocher et al., 1995; Kocher et al., 1996). PDZK1IP1 stimulates the sodium dependent uptake of mannose and glucose through the regulation of sodium-glucose linked transporter (SGLT) family and bypasses TNF- α dependent growth arrest(Blasco et al., 2003; Calado et al., 2018; Coady et al., 2017; Guijarro et al., 2007a; Kocher et al., 1998; Perez et al., 2013). PDZK1IP1 is reported to have contradictory effects on cell growth in culture and tumor growth in nude mice. This may be explained by the different cancer cell line models used and its modest induction of reactive oxygen species measured in one of the model systems, which is reported to inhibit PTEN to activate AKT signaling(Guijarro et al., 2007b; Guijarro et al., 2007d; Kocher et al., 1996). Expression of PDZK1IP1 in cancer is broadly correlated with markers of inflammation; moreover, its expression occurs in many other inflammatory diseases(Garcia-Heredia et al., 2017).

Here, we identify that one of the most highly recurrent SEs in CRC upregulates the
105 expression of *PDZK1IP1* and that this is activated by inflammatory cytokines and the tumor
microenvironment. *PDZK1IP1* overexpression or deletion in multiple model systems altered
growth of CRC in the tumor microenvironment more dramatically than in tissue culture.
PDZK1IP1 overexpression was associated with an increase in glucose uptake, which generated
enhanced levels of reductive capacity as measure by glutathione and NADPH. Our findings argue
110 that the tumor microenvironment activates *PDZK1IP1* in colorectal epithelial cells where it acts
as an oncogenic driver of tumor growth in collaboration with genetic drivers of the disease.

Results

The super-enhancer landscape of primary CRC and patient-matched adjacent normal mucosa

115 CRC is the second leading cause of cancer-related deaths in the United States and one of
the first cancers in which a divergent enhancer landscape between tumor and normal was described
(Akhtar-Zaidi et al., 2012; Siegel et al., 2020). We profiled SE landscapes in 15 CRC patients
using H3K27ac chromatin immunoprecipitation and sequencing (ChIP-seq), with the aim of
leveraging recurrently dysregulated SEs to identify novel dependency genes in CRC. To control
120 for batch effect and patient heterogeneity, we processed each tumor in parallel with matched
adjacent normal colon mucosa, totaling 30 unique tissue samples (Figure 1A). We identified 2026
total SEs in tumor and normal, which accounted for approximately 50% of the H3K27ac signal,
with 95% saturation of unique SE discovery at 10 and 11 patients for adjacent normal and primary
CRC, respectively (Figure S1A-C). H3K27ac deposition at these 2026 SEs exhibits tissue-of-
125 origin specificity, clustering separately from other malignancies under hierarchical unsupervised
analyses when re-processed together with our datasets using the same pipeline (Figure S1D, see
Methods for accessions).

Divergent super-enhancer landscapes between primary CRC and matched normal

To compare CRC and normal mucosa SE landscapes, we performed principal component analysis (PCA) of H3K27ac signal at all 2026 SEs. We observed distinct clustering of CRC and normal mucosa suggestive of divergent SE landscapes (Figure 1B). As further validation, we included previous datasets from colon crypts harboring normal intestinal stem cells and Familial Adenomatous Polyposis adenomas (Cohen et al., 2017). Crypts clustered near normal mucosa and adenomas occupied the boundary between normal mucosa and CRC, corroborating a previously described enhancer transition state in these early lesions (Cohen et al., 2017) (Figure 1B).

To identify dysregulated SEs between CRC and normal colon epithelium, we rank-ordered SEs by mean $\log_2$ fold-change in H3K27ac signal. SE changes corresponded with proximal gene expression enabling prediction of target genes (Figure 1C-D). The most up-regulated SE in CRC (H3K27ac signal $\log_2$ fold change 2.73; $P = 0.0027$) was associated with *ASCL2*, a TF for intestinal stem cell fate (Figure 1E, S2A) (Schuijers et al., 2015). To prioritize SEs for downstream analyses, we overlapped 1) the top 100 SEs up-regulated in CRC with 2) SEs recurrent in 9 or more out of the total of 15 patients (Figure 1F-G). We identified 12 candidate SEs enriched in primary CRC over adjacent normal with high recurrence in patients, including SEs assigned to genes previously implicated in CRC including Wnt target genes *MYC* and *ASCL2* (Figure 1F, S2B) (He et al., 1998; Sansom et al., 2007; Schuijers et al., 2015).

Unique super-enhancers in primary CRC over CRC cell lines

Due to the technical challenges and cellular material demands for high-resolution ChIP-seq, many investigators continue to rely on commercially available cell lines as starting input for epigenomic studies. We investigated the extent commercially available CRC cell lines recapitulated the primary CRC tumor SE landscape, with the aim of identifying SEs that are unique to tumor biopsies. Given the propensity of SE regulation of oncogenes in cancer, we wished to

assess whether such SEs could reveal oncogenic factors in epithelial cells that are not discoverable in tumor cell lines.

To assess whether any of our identified 2026 SEs were unique to primary specimens, we compared them with 13 commercially available CRC cell lines routinely used in laboratory studies, from previously published H3K27ac datasets as well as those generated for this study (Supplementary Table 1)(Cohen et al., 2017; McClelland et al., 2016; Nakamura et al., 2017). PCA analysis revealed distinct clustering of H3K27ac signal between primary CRC and CRC cell lines at all 2026 SEs, including three CRC cell lines assayed in our laboratory as an internal control for potential batch effects (Figure 2A). This observation suggests some SEs in CRC may be dependent upon the physiological environment within the living human body and not recapitulated in common laboratory cell line models of CRC and corroborates a previous finding in medulloblastoma(Lin et al., 2016).

We identified 221 SEs (~11%) as significantly under-represented in CRC cell lines compared to primary CRC ($P < 0.01$ Student's t -test, H3K27ac signal $\log_2$ fold change < -1), hereafter referred to as primary specimen enriched SEs (Figure 2B). 18 of these SEs were highly under-represented with a mean H3K27ac signal $\log_2$ fold change less than -2. In contrast, only 12 SEs were significantly over-represented in CRC cell lines (Supplementary Table 2). It is possible this is an under-estimate as the master list of SEs queried were derived from primary CRC and matched normal colon mucosa and therefore biased towards physiologically relevant loci.

Upstream TF regulation of primary specimen enriched SEs

We next wished to better understand the TFs upstream of this SE discrepancy between primary CRC and CRC cells. We queried publicly available assay for transposase-accessible chromatin using sequencing (ATAC-seq) datasets from 81 patients with primary CRC from TCGA for motif analysis using the TRAP algorithm(Corces et al., 2018; Thomas-Chollier et al., 2011).

We found open chromatin at primary specimen enriched SEs was enriched for motifs of TFs with niche-dependent activity particularly in response to inflammation including *HNF4A*, NF- κ B, *STAT3*, and *KLF4* (Figure S3A)(Babeu and Boudreau, 2014; Ghaleb and Yang, 2017). SEs boundaries are often flanked by CTCF sites for structural regulation, which validated in this analysis as the third most enriched motif(Gong et al., 2018; Shin, 2019).

We next wished to assess whether primary specimen enriched SEs had functional consequences on gene expression. We queried RNA-seq datasets from all 51 CRC cell lines from the Cancer Cell Line Encyclopedia (CCLE) in comparison with data from our primary CRCs (PRJNA523380)(Ghandi et al., 2019). Integrating our target gene predictions with this CCLE analysis, we derived a gene signature downstream of primary specimen enriched SEs up-regulated in primary CRCs over CCLE cell lines (Figure 2C). Pathway analysis indicated an enrichment in genes involved in ion transport and homeostasis which are among the physiologic roles of the colon, as well as cytokine signaling (Figure S3B) (Field, 2003). Furthermore, ARCHS4 algorithm analysis of these genes to predict upstream transcriptional regulation identify large intestine lineage factors *CDX1* and *CDX2* as top hits (Figure S3C)(Guo et al., 2004; Lachmann et al., 2018; Silberg et al., 2000). Interestingly, ARSHS4 analysis also corroborated TRAP motif analysis identification of *HNF4A* as a significantly discovered motif in primary specimen enriched SEs (Figure S3C).

Many of these genes have been implicated in cancer progression (Supplementary Table 3). The phosphatase *PTP4A3* is associated with greater risk for metastasis in uveal melanoma patients(Laurent et al., 2011). The transmembrane protein *TMEM176B* attenuates the inflammasome and hinders anti-tumor activity in response to immune checkpoint blockade therapy(Segovia et al., 2019). *MAPK3* (also known as *ERK1*) is a major unit of the MAP kinase signaling pathway frequently activated in CRC through *BRAF* or *KRAS* mutations(Cancer Genome

Atlas, 2012). *KLF4* has been described as an oncogenic driver in some contexts in transformed cells (Rowland et al., 2005).

A local tumor microenvironment acquired super-enhancer at *PDZK1IP1*

Of the primary specimen enriched SEs, 9 SEs were among the top 100 SEs we found to be enriched in primary CRC over patient-matched normal colon (Figure 2D, Supplementary Table 4). Intriguingly, two of them were previously identified in Figure 1 as among the top recurrently dysregulated SEs in CRC tumors over patient-matched normal epithelium. One SE, associated with the gene *PDZK1IP1*, ranked second out of 2026 SEs for recurrence in CRC, meeting ROSE criteria for SE calling in 12 out of 15 primary CRC tumors, 1 out of 15 patient-matched normal colon epithelia, and 0 out of 13 CRC cell lines (H3K27ac signal $\log_2$ fold change -2.07, $P = 1.7 \times 10^{-6}$) (Figure 3A-B, S4). The other SE, adjacent to and most significantly associated with the expression of interferon induced transmembrane protein *IFITM1*, met ROSE criteria for SE calling in 9 out of 15 primary CRC tumors, 0 out of 15 patient-matched normal colon epithelia, and 0 out of 13 CRC cell lines (H3K27ac signal $\log_2$ fold change -2.89, $P = 1.8 \times 10^{-5}$) (Figure S5A).

The SE at *PDZK1IP1* spans the entire gene body of *PDZK1IP1* as well as the long non-coding RNA LINC00853. RNA-seq analysis of all primary CRCs in our cohort found LINC00853 expression to be significantly below 1 TPM, our absolute minimum cutoff for expression, whereas the mean RNA-seq expression of *PDZK1IP1* was 88.8 TPM. This observation suggests the H3K27ac and open chromatin peak at LINC00853 may serve as a distal regulatory region for *PDZK1IP1*. As validation, we assessed *PDZK1IP1* mRNA expression between CRC cell lines from the CCLE and primary CRCs. We discovered a 21.5-fold higher median *PDZK1IP1* mRNA levels in primary CRCs over CCLE cell lines, suggesting widespread inactivation of *PDZK1IP1* expression in culture (Figure 3C). CCLE cell line expression was lower than normal mucosa, suggesting up-regulation of *PDZK1IP1* in CRC is microenvironment acquired (Figure 3C). As

proof-of-principle, high-expressing CRC surgical specimens significantly reduced *PDZK1IP1* mRNA levels when transferred to culture as 3D organoids in two separate cohorts (Della Chiara et al., 2021) (Dr. Benjamin Hopkins, personal communication) (Figure 3D-E).

The tumor microenvironment contains cancer cells as well as immune, stromal, and other non-cancer cells. We wished to assess the expression distribution of *PDZK1IP1* and *IFITM1* across these cell populations. Analysis of published single cell RNA-seq analyses identified epithelial cancer cells as having the highest expression of *PDZK1IP1* within primary CRCs (Figure 3F-G) (Lee et al., 2020). In contrast, *IFITM1* exhibited significant expression in stromal and immune cells in addition to epithelial cells (Figure S5B-D). This suggests the *PDZK1IP1* expression discrepancy between primary specimens and tissue culture is unlikely due to the exclusion of other cell populations but rather is an epithelial cell re-programming phenomenon. For this reason, we chose to focus on *PDZK1IP1* for downstream functional analyses.

The *IFITM1* expression pattern led us to consider the level contamination of bulk tumor H3K27ac ChIP-seq datasets with immune and stromal cell types, an unavoidable confounder when starting with primary tumor specimens. To this end, we performed de-convolution of cell type populations all primary CRCs for which sample-matched bulk tumor RNA was available using the xCell algorithm (Aran et al., 2017). Our analyses showed a dominant epithelial cell signature for most tumors (Figure S5E). Interestingly, the two cases predicted to have a low epithelial signature score were among the three cases that did not meet ROSE criteria for *PDZK1IP1* SE calling (Figure S4E, see patients 17787 and 19282). Sample-matched RNA-seq data was not available to assess the third case with the absent *PDZK1IP1* SE.

Single cell RNA-seq also revealed *PDZK1IP1* expression in all four consensus molecular subtypes (CMS) of CRC (Figure S5F) (Dienstmann et al., 2017; Guinney et al., 2015; Lee et al., 2020). Expression was mostly enriched in CMS1-3, and especially prevalent in CMS3, previously

described as a predominantly metabolic signature (Figure S5F) (Guinney et al., 2015). Most CRC cases are sporadic, initiated by mutations in the *APC* tumor suppressor gene, which largely
250 comprise CMS2 and CMS3(Rajamaki et al., 2021). *PDZK1IP1* single cell expression is less prevalent among CMS4, described as stromal-like and best recapitulates the transcriptomes of colitis-associated colorectal carcinomas, a small subset of the disease(Rajamaki et al., 2021). This finding supports the observed absence of the *PDZK1IP1* SE in two tumors annotated as having a high stromal composition by xCell (Figure S5E). These data further support the association of
255 *PDZK1IP1* expression with intra-tumoral epithelial cells as a re-programming event.

Unique super-enhancers in primary CRC over 3D CRC organoids

H3K27ac ChIP-seq datasets from human CRC 3D organoids were re-analyzed through our pipeline for comparison to our primary CRCs (Della Chiara et al., 2021). We similarly observed distinct clustering away from primary CRCs (Figure S6A). We identified 180 (~8.9%) SEs
260 differentially expressed between 3D organoids and primary tumors ($P < 0.01$ Student's *t*-test, H3K27ac signal absolute $\log_2$ fold change >1) (Figure S6B). In contrast to 2D commercial cell lines, most of these loci (127 SEs) were enriched in organoids over primary CRC (Figure S6B), notably at TGF- β signaling genes *BMP4* and *RGMB* (Figure S6D). Our data suggests 3D organoids improve recapitulation of the SE landscape of primary CRC at the 221 loci under-represented in
265 commercial 2D CRC cell lines but nevertheless diverge from primary CRC (Figure S6C).

Importantly, 3D CRC organoid culture validated the decreased H3K27ac signal at the *PDZK1IP1* ($\log_2$ fold change -0.48, $P = 0.05$) and *IFITM1* ($\log_2$ fold change -1.87, $P = 0.0002$) SEs observed in 2D culture (Figure S6E-F). The findings corroborate the observed decrease in *PDZK1IP1* mRNA expression between patient-matched primary CRCs and derived 3D CRC
270 organoids (Figure 3D-E).

Xenotransplantation as tumors results in acquisition of the *PDZK1IP1* super-enhancer

We first assessed genomic alterations as a possible etiology of the *PDZK1IP1* SE. The SE resides on chromosome 1p which rarely exhibits amplification in 195 CRC cases from TCGA (Cancer Genome Atlas, 2012). Inspection of H3K27ac reads within the *PDZK1IP1* SE did not reveal any recurrent mutations in CRC over patient-matched normal. Moreover, *PDZK1IP1* SE status failed to correlate with individual somatic protein-coding mutations at other loci, microsatellite instability (MSI), or tumor stage (Supplementary Table 5-7). One normal colon epithelium case in our cohort met ROSE criteria for *PDZK1IP1* SE calling, further suggesting tumor initiating mutations may be dispensable for induction.

As a proof-of-principle that the *PDZK1IP1* SE is under cell-extrinsic control by the tumor environment, we grew the CRC cell line HT29, which expresses low levels of *PDZK1IP1*, in culture or as subcutaneous xenografts in nude mice (Figure 4A). Remarkably, we observed increased H3K27ac deposition ($\log_2$ fold change 1.24) at the *PDZK1IP1* locus that met SE criteria in three independent xenograft tumors, but not parental cells grown *in vitro* (Figure 4B). Although the H3K27ac antibody employed reacts to both human and mouse epitopes, sequenced ChIP-seq reads were mapped only to the human genome to specifically capture signal from human CRC cells within the xenograft. Broadly, HT29 xenotransplantation failed to restore SE expression at other loci under-represented in cell lines, including at *IFITM1* ($\log_2$ -0.34-fold).

We next assessed the effects of SE induction on *PDZK1IP1* expression, using antibodies and quantitative real-time PCR (qRT-PCR) primers specific to human *PDZK1IP1* to exclude any signal contribution from mouse cell xenograft tumor infiltrates. HT29 xenograft tumors exhibited a 34.4-fold increase of *PDZK1IP1* mRNA, which validated by immunoblot (Figure 4C-D). To assess whether SE induction regulates other proximal genes, we performed RNA-seq on HT29 xenograft tumors and parental cells. *PDZK1IP1* was the only differentially expressed protein

coding gene within 500kb of the SE (Figure 4E). Importantly, HT29 xenograft tumors recapitulate *PDZK1IP1* mRNA and protein levels observed in most primary CRCs (Figure S7).

The *PDZK1IP1* super-enhancer is regulated by inflammation

To better understand *PDZK1IP1* epigenetic regulation by the microenvironment, we queried bulk tumor ATAC-seq and RNA-seq datasets from TCGA (Corces et al., 2018). We uncovered several enriched inflammatory TF motifs including NF- κ B, STAT1, STAT3, and CTCF at open chromatin in the *PDZK1IP1* promoter and SE (Figure S8A-D). We validated TF binding in various tissues and cell lines based on analysis of published ChIP-seq data (Figure S8E, see Methods for accessions). In support, gene set enrichment analysis (GSEA) demonstrated a strong correlation between *PDZK1IP1* mRNA expression and inflammatory signatures including TNF α , IFN γ , IL-6, and IFN β signaling from TCGA (Figure 5A, S8F-I). As proof-of-principle, stimulation of HT29 cells *in vitro* with the upstream pro-inflammatory cytokines TNF α , IFN γ , and IL-6, but not IFN β , induced *PDZK1IP1* expression (Figure 5B). Cytokines resulted in additive potentiation of *PDZK1IP1* expression suggesting these TFs act independently and cooperatively, and phenocopies levels observed in xenografts (Figure S8J). In support, stimulation of HT29 in culture with TNF α , IFN γ , and IL-6 likewise phenocopied and induced the *PDZK1IP1* SE (Figure 5C).

Multiple orthogonal approaches link inflammation in the setting of HT29 xenografts with *PDZK1IP1* induction. Consistent with our observations in human samples, we observed increased inflammatory TF signaling in HT29 xenografts relative to cells grown in culture. GSEA demonstrated enriched inflammatory response and NF- κ B signatures in HT29 xenografts over parental cells (Figure S8K). Next, the ChIP-X Enrichment Analysis (ChEA) algorithm identified NF- κ B subunit RELA as the top TF central to up-regulated genes ($\log_2$ fold change >2) in HT29 xenografts over parental cells (Figure S8L) (Keenan et al., 2019). In support of this data, gained

enhancers in HT29 xenografts over parental cells ($\log_2$ fold change H3K27ac signal >1) similarly enriched for NF- κ B and STAT3 motifs via the TRAP algorithm (Figure S8M-N). Cytokine array
320 profiling of xenografts detected elevated human as well as murine pro-inflammatory cytokines and chemokines, including TNF α and IL-6 (Figure S9). Immunohistochemical analysis of HT29 xenograft tumors readily detected CD68+ and Ly6G+ cell staining, suggesting robust infiltration by macrophages and neutrophils or other granulocytes, respectively (Figure S9)(Daley et al., 2008; Holness and Simmons, 1993).

325 **Cell-specific super-enhancer reprogramming in response to microenvironment**

CRC cells grown in culture exhibit decreased STAT3 signaling compared to primary tumors, and growing HT29 cells as a xenograft tumor reactivates this signaling(Corvinus et al., 2005). Intriguingly, we validated this phenotype as unique to HT29 cells compared to COLO205 and DLD1, two other human CRC cell lines with low expression of *PDZK1IP1*. COLO205 and
330 DLD1 failed to phenocopy induction of *PDZK1IP1* protein levels, correlating with poor STAT3 reactivation in these lines (Figure S10). In line with this finding, we did not observe increased H3K27ac signal in COLO205 liver metastasis xenografts in nude mice from orthotopic splenic vein injections (GSE126188) nor in subcutaneous DLD1 xenografts in nude mice (this study), over respective parental lines maintained in culture (Figure S10)(Hung et al., 2019). Multiple other
335 CRC cell lines we screened (including CACO2, SW48, SW480, SW620, RKO, and HCT116) exhibited no detectable expression of *PDZK1IP1* by RNA-seq or immunoblot. These cells failed to express *PDZK1IP1* in response to inflammatory cytokines or xenotransplantation (Figure S10C). The finding that some cell lines lose the ability to respond to inflammation at the *PDZK1IP1* locus is perhaps explained by selection of known immune modulators to avoid growth
340 inhibitory effects of inflammation(Inagaki-Ohara et al., 2013).

Tumor microenvironment recapitulates enhancer reprogramming in cancer

A comparison of log₂ fold change in H3K27ac signal at all enhancers between xenografts and *in vitro* cytokine stimulated cells demonstrate that pro-inflammatory cytokines correlate with xenograft tumor induced enhancer reprogramming ($R = 0.22$, $P < 0.0001$) (Figure S11). In addition, xenograft induced enhancer changes modestly but significantly recapitulated those observed in CRC ($R = 0.11$, $P < 0.0001$) (Figure S11). This trend validated in independent COLO205 peritoneal deposit and liver metastasis xenografts ($R = 0.19$, $P < 0.0001$) (Figure S11)(Hung et al., 2019). These findings more broadly implicate cytokines and the tumor environment as novel contributors to reprogramming throughout the CRC enhancer landscape.

***PDZK1IP1* is an oncogenic driver specific to the tumor microenvironment setting**

PDZK1IP1 (also known as MAP17, DD96) encodes a 114-residue protein overexpressed in multiple carcinomas (Figure S4)(Guijarro et al., 2007c; Kocher et al., 1995; Kocher et al., 1996). Although *PDZK1IP1* has been previously linked to tumorigenesis, there is conflicting data on its role in modulating tumor growth. At least two groups have reported that over-expression of *PDZK1IP1* in cell line models of CRC and prostate adenocarcinoma inhibit cell growth *in vitro* and tumor growth in xenografted mice (Ikeno et al., 2019; Kocher et al., 1996). On the other hand, multiple studies published by other groups have reported *PDZK1IP1* is oncogenic in *in vitro* and in xenograft models of cell lines derived from melanoma and hepatocellular carcinoma (HCC) (Dong et al., 2021; Garcia-Heredia et al., 2017; Guijarro et al., 2007a; Guijarro et al., 2007b; Guijarro et al., 2007c; Guijarro et al., 2007d). High mRNA expression of *PDZK1IP1* negatively prognosticates patient survival in multiple cancers including low-grade gliomas, glioblastomas, pancreatic adenocarcinoma, but not CRC using data from TCGA (Figure S12).

Given the presence of a highly recurrent CRC-enriched and microenvironment acquired super-enhancer at *PDZK1IP1*, we assessed whether it reflects a novel transcriptional dependency for CRC growth. Previous studies report *PDZK1IP1* loss of function via short hairpin RNA

(shRNA) silencing decreases growth of human breast and HCC cell lines in *in vitro* assays (Dong et al., 2021; Guijarro et al., 2007b). We deleted *PDZK1IP1* using two independent and non-overlapping CRISPR-cas9 guide RNAs (hereafter referred to as sgPDZK1IP1) and assessed the ability of human CRC lines to proliferate *in vitro* (Figure S13A-D). We observed a very modest decrease in growth rate in HT29 cells whereas DLD1 cells remained unaffected using a 2D Incucyte proliferation assay over 6 days (Figure S13A-D). This observation conflicts with previous observations in other cancers, suggesting *PDZK1IP1* may exhibit cancer-specific effects on proliferation in culture. As a possible explanation, we notably observed severe off-target toxicity on CRC proliferation in culture in nearly all commercially available shRNAs (Sigma) against *PDZK1IP1*, that were non-reproducible in isogenic CRISPR-cas9 loss of function models.

Given the observed induction of *PDZK1IP1* *in vivo* in HT29 cells, we grew the same lines as subcutaneous xenografts in nude mice. Remarkably, *PDZK1IP1* was critical for CRC tumor growth *in vivo* (Figure 6A-B, S13E-F). The same trend is repeated in DLD1, which expressed *PDZK1IP1* at a much lower level, although the sgPDZK1IP1 growth defect was not as large as observed in HT29. These data suggest that PDZK1IP1 contributes to tumor growth when grafted into mice.

As validation, we observed the same trend using gain of function models of *PDZK1IP1*. Given low levels of *PDZK1IP1* *in vitro*, we re-introduced V5-tagged *PDZK1IP1* cDNA (PDZK1IP1-V5) by titrating to protein levels comparable to that observed in primary CRCs and HT29 xenograft tumors (Figure S13G). PDZK1IP1-V5 and EV cells grew at similar rates in culture in HT29, COLO205, and DLD1 cells (Figure S13H-J). When the same lines were grown as xenograft tumors, additional ectopic overexpression of PDZK1IP1-V5 significantly increased tumor growth in HT29 over empty vector controls (Figure 6C). PDZK1IP1-V5 also significantly increased tumor growth of DLD1 (xenografts tumors for this model fail to endogenously induce

390 *PDZK1IP1*) (Figure 6D). Taken together, our data demonstrate *PDZK1IP1* is more important for tumor cell growth in mice, where it can be induced, than in tissue culture where it is largely silent (Figure 6E).

***PDZK1IP1* regulates cancer metabolism**

395 The mechanism by which *PDZK1IP1* regulates tumor growth remains incompletely understood. Under normal physiology, *PDZK1IP1* is largely expressed in the proximal tubules of the kidney, where it is proposed to facilitate glucose reabsorption via the SGLT family of sodium-dependent glucose transporters (Coady et al., 2017). Indeed, in a cohort of familial renal glucosuria primarily composed of patients with inactivating mutations in *SLC5A2* encoding the renal glucose transporter SGLT2, one patient was found to have a mutually exclusive homozygous truncating
400 mutation in *PDZK1IP1* (Coady et al., 2017; Santer and Calado, 2010).

As previously observed, PDZK1IP1-V5 and EV cells grew at similar rates in culture and thus any metabolic phenotypes identified are independent of proliferation in this context (Figure S13). However, *PDZK1IP1* over-expression has been reported to increase glucose uptake in breast cancer cells in a SGLT1 and SGLT2-dependent manner (Guijarro et al., 2007b). To determine
405 whether *PDZK1IP1* could affect glucose uptake in CRC cells, we measured its uptake in DLD1 overexpressing PDZK1IP1-V5 relative to empty vector and found that it could indeed enhance glucose uptake. Moreover, consistent with the literature, use of sotagliflozin, a SGLT1 and SGLT2 inhibitor approved for the management of diabetes mellitus in Europe but not currently by the FDA, could rescue the increase in glucose uptake caused by PDZK1IP1-V5 (Figure S14A) (Bhatt
410 et al., 2021). Sotagliflozin did not affect baseline glucose levels in DLD1 EV cells, suggesting an inactivation of surplus glucose uptake. As an additional control, we performed a cellular uptake assay using 2-deoxyglucose (2DG), which is a poor substrate for SGLT family glucose transporters but is readily taken up by the GLUT family of passive glucose transporters. We observed no

difference in 2DG uptake between PDZK1IP1-V5 and EV DLD1 cells, further supporting the
415 increase in intracellular glucose is due to SGLT glucose transporters (Figure S14B).

The association of *PDZK1IP1* expression and metabolism gene signatures was independently noted in primary CRC tumors. *PDZK1IP1*-high primary CRCs from TCGA were enriched for glycolysis and oxidative phosphorylation gene sets by GSEA to nearly the same extent detected for inflammation and cytokines (Figure S14C-D). In single cell analyses, we previously
420 observed CMS3 as the CRC subtype with the highest incidence of *PDZK1IP1* expression across 23 primary CRCs, which is defined by a highly metabolic gene signature (Figure S5F) (Guinney et al., 2015).

Thus, we considered whether *PDZK1IP1* affects downstream metabolism in CRC. A recent study demonstrated increased glycolytic rate in Seahorse live-cell assays with *PDZK1IP1*
425 expression in HCC cells (Dong et al., 2021). Surprisingly, in Seahorse live-cell assays, PDZK1IP1-V5 cells reproducibly exhibited decreased extracellular acidification rate (ECAR), a read-out of glycolysis, and oxygen consumption rate (OCR), a read-out of mitochondrial respiration, compared to EV cells (Figure S15A-D). We corroborated this result by performing a rapid glycolysis carbon tracing experiment using uniformly labeled C¹³ glucose. We observed a
430 significant decrease in M+6 fructose 1,6 bisphosphate isotopologues in PDZK1IP1-V5 cells compared to EV, suggesting *PDZK1IP1* may modulate phosphofructokinase, a key rate-limiting regulatory enzyme in control of glycolysis (Figure S15E-F) (Al Hasawi et al., 2014; Yi et al., 2012). However, these decreases in ECAR and OCR did not significantly negatively impact cell proliferation. Furthermore, we did not notice consistent decreases in the ATP/ADP ratio with
435 PDZK1IP1-V5 expression (Figure S15G-H). Similarly, reverse phase protein array screening between PDZK1IP1-V5 and EV cells did not indicate a difference in phosphorylation of AMPK α suggesting energy signaling is not compromised.

We next performed global polar metabolite profiling by liquid chromatography-mass spectrometry to assess the effects of increased glucose uptake and decreased glycolytic rate on in
440 CRC cells. We identified five consensus metabolites up-regulated in PDZK1IP1-V5 (>1.25 -fold, $P < 0.05$) in HT29, DLD1, and COLO205 lines. Intriguingly, four of these—6-phosphogluconate, ribose 5-phosphate, IMP, and AMP—are intermediates or derivatives of the pentose phosphate pathway (PPP) (Figure 7A-B, S16A). To assess increased PPP activity, we performed a carbon tracing experiment utilizing 1,2- C^{13} -glucose. PDZK1IP1-V5 increased relative abundance of C^{13} -
445 labeled 6-phosphogluconolactone, the first and committed intermediate of the PPP in HT29, COLO205, and DLD1 lines (Figure 7C-F).

The ratio of M+1:M+2 lactate is a readout of PPP flux relative to glycolysis(Jang et al., 2018). We did not observe an increase in M+1:M+2 lactate between PDZK1IP1-V5 and EV cells despite increased M+2 6-phosphogluconolactone labeling (Figure S16B-D). This suggests the
450 increase in PPP activity is more likely due to increased glucose uptake rather than shunting of glycolytic intermediates.

The best understood role of the PPP in cancer is protection against reactive oxygen species (ROS)(Patra and Hay, 2014). In this regard, we observed an increase in both NADPH/NADP⁺ ratios and glutathione levels in PDZK1IP1-V5 cells at steady state (Figure 7G-J, S16E). To assess
455 whether this metabolic change in reductive capacity exhibits a cellular phenotype, we challenged cells with oxidative stress. We observed lower intracellular ROS levels in PDZK1IP1-V5 cells in response to the thiol oxidizing agent diamide in HT29 and DLD1 lines (Figure 7K-L). Furthermore, PDZK1IP1-V5 exhibited increased cell viability when cultured under 1% O_2 or treated with diamide in HT29 lines (Figure S16F-H). Taken together, we demonstrate *PDZK1IP1*
460 protects against oxidative stress in CRC cells.

We next assessed whether *PDZK1IP1* regulation of PPP and reductive capacity was relevant towards the observed growth phenotype *in vivo*. Importantly, supplementing nude mice bearing sgPDZK1IP1 xenograft tumors with the antioxidant N-acetylcysteine in drinking water partially rescued tumor growth (Fig. 7M).

Discussion

Super-enhancers are select areas of the genome at which cells commit exceptionally high levels of histone acetylation and active transcriptional machinery. Cancer cells are associated with enhancer reprogramming that extends to the super-enhancer landscape to activate oncogenes and other transcriptional dependencies. Profiling the super-enhancer landscape has emerged as a powerful tool for uncovering novel target genes in cancer, particularly in those with few recurrently mutated genes(Mack et al., 2018).

The mechanisms by which super-enhancer reprogramming occur in cancer are not fully understood. They include somatic mutations resulting in oncogenic signaling converging on the epigenome such as *APC* mutations activating TCF family TFs, focal amplification of TF binding sites within SE regions, endocrine signaling to estrogen and androgen receptors that similarly converge on the epigenome in hormonal cancers, somatic non-coding mutations that create *de novo* TF binding sites, as well as inherited polymorphisms that create *de novo* TF binding sites. The enhancer landscape of cancer cell lines has been observed to diverge from their cancer counterparts, suggesting the possibility of cell-extrinsic microenvironment influences on the epigenetic landscape that could contribute to tumor cell development and growth.

Here, we demonstrate the local tumor microenvironment induces super-enhancer reprogramming in CRC. We find the SE landscape of primary CRC to diverge from *in vitro* CRC models, corroborating a previous observation in medulloblastoma(Lin et al., 2016). Attempts to restore the tumor microenvironment in cell lines to model changes in the human epigenome are

485 inherently flawed due to the requirement of immunocompromised mouse models. We expect the recapitulation of super-enhancer changes in this model to be limited given the notable absence of T cells in the microenvironment of our nude mouse model, especially at loci under regulation by inflammatory signaling such as *PDZK1IP1* which may explain failure of some cell lines to induce *PDZK1IP1* upon xenotransplantation. Nevertheless, its super-enhancer in HT29 cells is restored
490 upon treatment with pro-inflammatory cytokines or xenotransplantation into nude mice, correlating with phosphorylation of STAT3 specifically in this line.

We identify its target, *PDZK1IP1*, as a novel positive regulator of CRC growth. Oncogenic non-coding regions have been described but most occur in a small fraction of cases. We identified the *PDZK1IP1* SE as among the most recurrently up-regulated SEs across 15 patient CRC samples,
495 which is consistent with the common up-regulation of *PDZK1IP1* in epithelial carcinomas(Kocher et al., 1995; Kocher et al., 1996). Pro-tumorigenic roles for inflammation in the tumor environment are well-described, including paracrine signaling between immune and cancer cells(Alonso-Curbelo et al., 2021; Coussens and Werb, 2002; Greten and Grivnickov, 2019; Wyckoff et al., 2004). Activation of NF- κ B and STAT3 signaling and transcriptional programs within cancer cells
500 promote disease progression in multiple malignancies, including CRC(Grivnickov and Karin, 2010; Huynh et al., 2019; Taniguchi and Karin, 2018; Xia et al., 2014; Yu et al., 2014). Although regulation of *PDZK1IP1* expression by hypoxia was recently noted, we found its up-regulation to be negligible compared to levels induced by cytokines and xenotransplantation(Dong et al., 2021). Our findings suggest that inflammation in the microenvironment activates *PDZK1IP1* to stimulate
505 tumor growth in a large proportion of CRC, likely in situations of moderate inflammation where *PDZK1IP1* is part of an adaptive response to signals that inhibit epithelial cell growth and viability.

In cancer cells, we find *PDZK1IP1* facilitates surplus glucose uptake via SGLT glucose transporters. SGLT2 inhibitors are FDA approved and undergoing Phase I/II clinical trials in solid

tumors following promising pre-clinical results as monotherapy or in combination with PI3K or
inhibitors or other therapies (canagliflozin: NCT04073680; dapagliflozin: NCT02695121,
NCT04899349, NCT04542291)(Hopkins et al., 2018; Scafoglio et al., 2015; Scafoglio et al.,
2018). Previous studies suggest *PDZK1IP1* increases ROS to contribute to cell proliferation.
However, this does not agree with growing literature on *KRAS* and *BRAF* mutant tumors
demonstrating the opposite. Rather, it is the mitigation of ROS that benefits tumor growth in this
context (Lim et al., 2019; Sayin et al., 2014; Shaw et al., 2011; Yun et al., 2015). Moreover, *KRAS*
and *BRAF* are among the most frequently mutated oncogenes in CRC(Cancer Genome Atlas,
2012). We notably observed increased PPP activity, reductive capacity, and resistance to oxidative
stress in HT29 (*BRAF* mutant), DLD1 (*KRAS* mutant), and COLO205 (*BRAF* mutant) cells. Our
data show this novel function of *PDZK1IP1* endows tumor cells with a growth advantage in the
tumor microenvironment but not in tissue culture. Thus, we elucidate a novel link between the
context of *PDZK1IP1* super-enhancer activation and *PDZK1IP1* relevance to CRC growth.

It is unclear why our results conflict directly with previous other studies on *PDZK1IP1*,
which report increased ROS levels as well as glycolytic and mitochondrial respiration rate (Dong
et al., 2021; Guijarro et al., 2007b). While all studies over-expressed *PDZK1IP1*, they used
different cancer cell lines with different lineage and mutation backgrounds to measure its effect.
In addition, we chose a level of ectopic expression that mimics endogenous protein levels observed
upon induction in xenografts as well as primary CRC tumors. Thus, a second possible explanation
is that the other studies may be reporting the effect at different gene doses. Finally, ROS levels
were measured by others using different probes.

Collectively, our observations broaden our notion of super-enhancers and highlights the
importance of epigenomic profiling of primary tumor specimens and patient-matched controls.
Our work further supports the need to assess and validate tumor-enriched super-enhancers to

identify novel target genes. Antagonizing distal cell-extrinsic androgen or estrogen signaling to the cancer epigenome is a mainstay of initial therapy for hormonal cancers(Takeda et al., 2018).

535 A greater understanding of local microenvironment factors and upstream intracellular signaling expands the scope for therapeutic strategies towards silencing cell-extrinsic enhancers and super-enhancers in control of transcriptional dependencies *in vivo*. Targeted therapy that reduces inflammation to lower the level of expression of *PDZK1IP1* could be of benefit for patients with colorectal cancer.

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Author contributions:

Conceptualization: R.W.Z., R.E.P.

Methodology: R.W.Z., A.L.Z., S.O., J.X., T.C.M., A.B., J.H., A.P.T., B.G., Y.S., X.W.,
S.C., K.B., J.A.,

Investigation: B.H., A.T.

Funding acquisition: R.E.P., R.W.Z.

Project administration: R.B., R.S.,

Supervision: D.R.J., J.E.C., S.I., I.I.C.C., D.H., E.B., R.E.P.

Writing – original draft: R.W.Z, R.E.P.

Data and material availability: H3K27ac ChIP-seq and RNA-seq datasets generated in this study
are deposited at the NCBI Gene Expression Omnibus under the accession GSE166254. Any
materials are available from the corresponding author upon reasonable request.

Figure 1. Recurrently dysregulated super-enhancers in CRC patients.

(A) Study overview.

(B) PCA of H3K27ac signal at 2026 SEs in CRC ($n = 15$ independent tissue samples), normal mucosa ($n = 15$), crypts ($n = 4$), and FAP adenomas ($n = 2$).

(C-D) GSEA between SE proximal genes and differentially expressed genes between CRC and normal.

(E) H3K27ac ChIP-seq track near *ASCL2*. Two proximal SEs are underlined.

(F) 2026 SEs by log₂ fold change in H3K27ac signal with 12 candidate SE target genes based on overlap of ranking and recurrence annotated.

(G) Heatmap of H3K27ac signal at 583 differentially expressed SEs ($P < 0.01$, Student's t -test).

Figure S1. The super-enhancer landscape in primary CRC and patient-matched adjacent normal colon epithelium.

(A) Percentage of H3K27ac signal deposited solely at SEs. Each red or blue bar is an independent primary CRC ($n = 15$ independent samples) or normal colon epithelium sample ($n = 15$ independent samples), respectively.

(B-C) Power analyses indicating cumulative percentage saturation of unique super-enhancer discovery with the addition of each patient in cohort. CRC in red, normal mucosa in blue.

(D) Unsupervised hierarchical clustering, H3K27ac signal Pearson correlations at 2026 SEs from 30 colorectal samples in our cohort ($n = 15$ independent CRC, $n = 15$ independent normal mucosae) against clear cell renal cell carcinomas (ccRCC, $n = 10$ independent tumors), chordomas ($n = 8$ independent tumors), diffuse large B-cell lymphomas (DLBCL, $n = 4$ independent tumors), gastric carcinomas ($n = 17$ independent tumors), glioblastomas ($n = 20$ independent tumors), luminal

breast cancer ($n = 34$ independent tumors), osteosarcoma ($n = 5$ independent tumors), and prostate carcinomas ($n = 20$ independent tumors). Please see Methods for accessions.

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Figure S2. The super-enhancer landscape in CRC identifies target genes.

(A) Linear correlation of H3K27ac signal deposition at the *ASCL2* SE and *ASCL2* gene expression using sample matched CRC ($n = 11$ patients) and normal epithelium ($n = 10$ patients) Although 15 primary CRCs and 15 normal colons were used for ChIP-seq analyses, sample-matched RNA-seq data was only available for 11 CRCs and 10 normal colons, respectively. All available data was used in this analysis. Please see Methods for details.

(B) Histogram of 12 candidate SEs rank-ordered by gained recurrence (number of patients exhibiting SE gain, out of a total of 15).

Figure 2. A subset of super-enhancers is specific to primary CRC specimens and not recapitulated in CRC cell lines.

(A) PCA of H3K27ac signal at 2026 SEs in CRC ($n = 15$ independent tissue samples) and CRC cell lines ($n = 13$ independent cell lines). Please see Methods for accessions.

(B) Heatmap of H3K27ac signal (Z-score) between CRC tumors and CRC cell lines with unsupervised hierarchical clustering. 221 SEs (out of 2026) down-regulated in CRC cell lines compared to primary tumors (mean H3K27ac signal $\log_2$ fold change < -1 , $P < 0.01$).

(C) GSEA validation of a tumor-enriched SE gene signature imposed on differentially expressed genes between primary CRC and CRC cell lines. See Supplementary Table 3 for gene list.

(D) Venn diagram showing overlap between 221 SEs down-regulated in CRC cell lines from (B) with the top 100 gained SEs in primary CRC over patient-matched normal.

Figure S3. Characterization of super-enhancers is specific to primary CRC under-represented in CRC cell lines.

(A) TRAP motif analysis of open chromatin regions within primary CRC-enriched SEs. Open chromatin regions defined by ATAC-seq data from 81 independent primary CRC tumors from TCGA. Please see Methods for accessions.

(B) ENRICHR pathway analysis of a primary CRC-enriched SE gene signature, using the Reactome gene set database.

(C) ENRICHR prediction of upstream transcription factors of the primary CRC-enriched SE gene signature using the ARCHS4 tool.

Figure 3. A context-dependent, primary CRC enriched super-enhancer at the locus of *PDZK1IP1*.

(A) H3K27ac ChIP-seq tracks at the *PDZK1IP1* SE in primary CRCs ($n = 8$ representative independent tumors) and CRC cell lines ($n = 8$ representative lines).

(B) Frequency of samples meeting ROSE criteria for SE calling at the *PDZK1IP1* locus. $n = 15$ independent CRC tumors, $n = 15$ independent patient-matched normal colon mucosae, $n = 13$ independent CRC cell lines, $n = 9$ independent 3D CRC organoids.

(C) RNA-seq expression of *PDZK1IP1* in primary CRC ($n = 16$ independent tumors), normal mucosa ($n = 15$ independent tissue samples), and CCLE CRC cell lines ($n = 51$ independent cell lines). Data presented as median $\pm$ interquartile range. Significance was determined using Student's *t*-test.

(D-E) RNA-seq expression of *PDZK1IP1* in primary CRCs and sample-matched patient-derived 3D CRC organoids. For the Weill cohort, $n = 3$ for *PDZK1IP1*-high tumors and organoids, $n = 2$ for *PDZK1IP1*-low tumors and organoids. For the Milan cohort, $n = 6$ for *PDZK1IP1*-high tumors

and organoids, $n = 3$ for *PDZK1IP1*-low tumors and organoids. Significance determined by Student's *t*-test. Please see Methods for accessions.

(F-G) Uniform Manifold Approximation and Projection (UMAP) of single cells from 23 independent primary CRC tumors and 10 independent adjacent patient-matched normal colons merged into a single plot, annotated with cell type and *PDZK1IP1* expression. Please see Methods for accessions.

Figure S4. *PDZK1IP1* and its super-enhancer are up-regulated in CRC.

(A) H3K27ac ChIP-seq track at the *PDZK1IP1* SE (underlined) in 6 representative independent primary CRCs, 2 FAP adenomas, 6 representative independent normal colon epithelium samples, and 4 normal colon crypts. See Methods for accessions used for FAP and crypt samples.

(B) Correlation of *PDZK1IP1* SE H3K27ac signal and *PDZK1IP1* mRNA expression using sample-matched sequencing data. For CRC $n = 11$ independent patient samples, for normal mucosa

$n = 10$. Although 15 primary CRCs and 15 normal colons were used for ChIP-seq analyses, sample-matched RNA-seq data was only available for 11 CRCs and 10 normal colons, respectively. All available data was used in this analysis.

(C-D) *PDZK1IP1* mRNA expression by RNA-seq in our cohort of patients. For CRC $n = 16$ independent tumors; for normal mucosa $n = 15$ independent tissue samples. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's *t*-test.

(E) *PDZK1IP1* mRNA expression by RNA-seq between normal colon ($n = 42$ independent cases), normal rectum ($n = 10$ independent cases) rectal adenocarcinoma (READ, $n = 166$ independent cases), and colon adenocarcinoma (COAD, $n = 475$ independent cases) from TCGA. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's *t*-test.

(F-G) *PDZK1IP1* protein levels by immunoblot with ImageJ quantification (fold change, CRC over normal for each pair). $n = 14$ de-identified patients, each with a matched tumor normal pair. T – tumor, N – matched normal. Five-digit de-identified patient number. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's *t*-test.

Figure S5. Characterization of CRC-enriched SEs at *IFITM1* and *PDZK1IP1*.

(A) Frequency of samples meeting ROSE criteria for SE calling at the *IFITM1* locus. $n = 15$ independent CRC tumors, $n = 15$ independent patient-matched normal colon mucosae, $n = 13$ independent CRC cell lines, $n = 9$ independent 3D CRC organoids.

(B-C) UMAP of single cells from 23 independent primary CRC tumors and 10 independent adjacent patient-matched normal colons merged into a single plot, annotated with cell type and *IFITM1* expression. Please see Methods for accessions.

(D) Dot plot of *PDZK1IP1* or *IFITM1* expression in single cells from primary CRC or adjacent normal colon epithelium.

(E) xCell de-convolution of bulk tumor RNA-seq into putative epithelial, immune, and stromal compositions. Out of the 15 primary CRCs for which H3K27ac ChIP-seq data is available, only 11 have sample-matched bulk tumor RNA-seq data. All available data was used in this analysis.

(F) Dot plot of *PDZK1IP1* expression in single cells of epithelial origin, from either primary CRC ($n = 23$ independent cases) or patient-matched adjacent normal epithelium ($n = 10$ independent cases). CMS refers to the consensus molecular subtypes of CRC as previously assigned. Please see Methods for accessions.

Figure S6. Super-enhancers specific to primary CRC specimens and not recapitulated in 3D CRC organoids.

(A) PCA of H3K27ac signal at 2026 SEs in CRC ($n = 15$ independent tissue samples) and CRC 3D organoids (derived from $n = 9$ independent patients). Please see Methods for accessions.

780 **(B)** Heatmap of H3K27ac signal (Z-score) between CRC tumors and CRC 3D organoids with
unsupervised hierarchical clustering at 180 differentially expressed SEs (absolute mean log₂ fold
change >1, $P < 0.01$).

(C) Incorporation of 3D CRC organoids into the heatmap of 221 SEs down-regulated in 2D CRC
cell lines from Figure 2.

785 **(D)** H3K27ac ChIP-seq track of the SE at *BMP4* (underlined) in $n = 8$ representative primary CRCs
and $n = 8$ representative 3D CRC organoid cultures.

(E) H3K27ac ChIP-seq track of the SE at *PDZK1IP1* (underlined) in $n = 8$ representative primary
CRCs and $n = 8$ representative 3D CRC organoid cultures.

(F) H3K27ac ChIP-seq track of the SE at *IFITM1* (underlined) in $n = 8$ representative primary
790 CRCs and $n = 8$ representative 3D CRC organoid cultures.

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Figure 4. Xenotransplantation restores the *PDZK1IP1* super-enhancer and expression.

(A) Experiment overview.

(B) H3K27ac ChIP-seq track of the *PDZK1IP1* SE (underlined) in HT29 xenografts ($n = 3$ independent tumors) or HT29 parental cells maintained in culture.

(C) Immunoblot of *PDZK1IP1* protein levels between HT29 xenografts ($n = 3$ independent tumors) or HT29 parental cells maintained in culture ($n = 3$ biological replicates).

(D) Quantitative real-time PCR (qRT-PCR) of *PDZK1IP1* mRNA expression between HT29 xenografts ($n = 3$ independent tumors) or HT29 parental cells maintained in culture ($n = 3$ biological replicates). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(E) Fold change gene expression by RNA-seq between HT29 xenografts ($n = 3$ independent tumors) or HT29 parental cells maintained in culture ($n = 3$ biological replicates). X-axis represents all protein-coding genes within 500 kb on either side of the *PDZK1IP1* SE. Data presented as mean $\pm$ s.e.m.

Figure S7. *PDZK1IP1* expression levels in HT29 xenografts recapitulate those in primary CRC.

(A) *PDZK1IP1* expression by RNA-seq, HT29 cells ($n = 3$ biological replicates), HT29 xenografts ($n = 3$ independent tumors), normal mucosa ($n = 15$ independent tissue samples), and primary CRC ($n = 16$) independent tumors. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's *t*-test.

(B-C) *PDZK1IP1* expression by immunoblot across four independent patient-matched CRC-normal colon pairs, versus HT29 cells or HT29 xenograft tumors.

Figure S8. *PDZK1IP1* is regulated by inflammation.

(A) ATAC-seq track at the *PDZK1IP1* SE in primary CRCs from TCGA ($n = 81$ independent tumors, merged into one track).

(B-D) TRAP motif analysis at consensus open chromatin regions from (A) at the *PDZK1IP1* SE in primary CRC.

(E) H3K27ac, STAT1, STAT3, RELA, and CTCF ChIP-seq tracks at the *PDZK1IP1* SE. See Methods for accessions.

(F-I) Hallmarks of Cancer GSEA enrichment signatures from *PDZK1IP1*-high (top 50% mRNA expression) versus *PDZK1IP1*-low (bottom 50% mRNA expression) primary CRCs from TCGA
850 ($n = 342$ independent tumors).

(J) *PDZK1IP1* expression levels by immunoblot in cytokine stimulated HT29 cells (10 ng/mL, 16 hours) or HT29 subcutaneous xenografts in nude mice.

(K) Hallmarks of Cancer GSEA performed on differentially expressed genes from RNA-seq between HT29 xenograft tumors ($n = 3$ independent tumors) and HT29 parental cells maintained
855 in culture ($n = 3$ biological replicates).

(L) ChEA TF predictions upstream of up-regulated genes ($\log_2$ fold change > 2) from RNA-seq data in HT29 xenograft tumors over HT29 parental cells in culture.

(M) Metagene of up-regulated H3K27ac enhancer peaks (mean $\log_2$ fold change > 1) in HT29 xenograft tumors ($n = 3$ independent tumors) over HT29 parental cells maintained in culture.

860 (N) TRAP TF motif analysis output for H3K27ac enhancers shown in (M).

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Figure 5. The *PDZK1IP1* super-enhancer is regulated by inflammation.

(A) Hallmarks of Cancer GSEA of RNA-seq expression data from *PDZK1IP1*-high (top 50% mRNA expression) versus *PDZK1IP1*-low (bottom 50% mRNA expression) primary CRCs from TCGA ($n = 342$ independent tumors).

(B) *PDZK1IP1* protein levels by immunoblot in HT29 cells following 16-hour stimulation with the indicated recombinant human cytokine in culture media at 10 ng/mL.

(C) H3K27ac ChIP-seq track of the *PDZK1IP1* SE (underlined) in unstimulated or TNF α , IFN γ , and IL-6 co-stimulated HT29 cells at 10 ng/mL for 16 hours.

Figure S9. Xenograft tumors exhibit increased pro-inflammatory cytokines, chemokines, and growth factors upstream of *PDZK1IP1*.

(A-S) Luminex-based human (prefixed with “h” on the y-axis, with cayenne red bars) cytokine profiling of HT29 xenograft tumor lysates ($n = 11$ biological replicates) and HT29 parental cells

($n = 4$ biological replicates) in culture (performed by EveTechnologies). Data presented as mean
890 $\pm$ s.e.m. Significance was determined using Student's t -test or Mann-Whitney t -test.

(T-GG) Luminex-based mouse (prefixed with “m” on the y-axis, with cantaloupe yellow bars)
cytokine profiling of HT29 xenograft tumor lysates and HT29 parental cells in culture as above
(performed by EveTechnologies). Data presented as mean $\pm$ s.e.m. Significance was determined
using Student's t -test or Mann-Whitney t -test.

895 **(HH-LL)** IHC staining of immune cell markers in HT29 xenograft tumors.

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Figure S10. Cell-specific super-enhancer reprogramming in response to microenvironment.

(A) Experiment overview. A xenograft model of COLO205 liver metastases in nude mice via splenic vein injection. H3K27ac ChIP-seq of COLO205 xenograft tumors in nude mice ($n = 4$ independent tumors) and COLO205 parental cells maintained in culture ($n = 4$ biological replicates).

(B) Experiment overview. A subcutaneous xenograft tumor model of DLD1 cells in nude mice. H3K27ac ChIP-seq of DLD1 xenograft tumors or DLD1 parental cells maintained in culture.

(C) *PDZK1IP1* protein levels by immunoblot with or without $\text{TNF}\alpha$ stimulation in HCT116, SW480, or HT29 cells.

(D) *PDZK1IP1* protein levels correlated with phosphorylation of STAT3 at the Y705 residue in parental cells maintained in culture or subcutaneous xenografts in nude mice.

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Figure S11. The tumor microenvironment recapitulates enhancer reprogramming in cancer.

(A) Linear correlation between log₂ fold change H3K27ac signal in HT29 nude mice subcutaneous xenografts ($n = 3$ independent tumors) over parental cells *in vitro*, and TNFα, IFNγ, IL-6 stimulated cells (10 ng/mL, 16 hours) over unstimulated cells. Each dot represents a unique typical enhancer, called in our primary CRC tumors using ROSE. For HT29 xenografts

(B) Linear correlation between log₂ fold change H3K27ac signal in HT29 xenografts/parental cells and CRC/normal mucosa.

(C) Linear correlation between log₂ fold change H3K27ac signal correlation in COLO205 splenic vein injection xenografts ($n = 7$ independent tumors, 4 in nude mice, 3 in NSG mice) over parental cells against CRC ($n = 15$ independent tumors) over normal mucosa ($n = 15$ normal colon tissue samples).

Figure S12. High *PDZK1IP1* expression negatively prognosticates patient survival in multiple cancers.

(A-D) Kaplan-Meier survival curves of *PDZK1IP1* high (top 20% mRNA, in red) and low (bottom 20% mRNA, in blue) cancer cases. Patient survival and gene mRNA expression data from TCGA, visualized using OncoLnc.

Figure S13. *PDZK1IP1* does not affect CRC proliferation in culture.

(A-B) Incucyte 2D growth curves of sgNeg or sgPDZK1IP1 cells ($n = 6$ biological replicates per group). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(C-D) *PDZK1IP1* protein levels by immunoblot in HT29 and DLD1 cells between sgPDZK1IP1 and sgNeg. Data presented as mean $\pm$ s.e.m.

(E-F) *PDZK1IP1* protein levels by immunoblot in HT29 and DLD1 subcutaneous xenograft tumors in nude mice between sgPDZK1IP1 and sgNeg.

(G) Re-introducing exogenous *PDZK1IP1* expression *in vitro*.

(H-J) Incucyte 2D growth curves of EV or PDZK1IP1-V5 expressing cells in HT29, COLO205, or DLD1 ($n = 4-6$ biological replicates per group). Data presented as mean $\pm$ s.e.m.

(K) *PDZK1IP1* protein levels by immunoblot in HT29 subcutaneous xenograft tumors in nude mice between EV and PDZK1IP1-V5 expressing cells.

Figure 6. *PDZK1IP1* is a novel context-dependent regulator of CRC tumor growth.

(A-B) Growth curves, sgPDZK1IP1.329 and sgNeg subcutaneous xenograft tumors in nude mice. HT29 $n = 9$ mice per group, DLD1 $n = 6$ mice per group. 1 million cells injected per mouse for both HT29 and DLD1. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(C-D) Growth curves, PDZK1IP1-V5 and EV xenograft tumors. HT29 $n = 10$ mice per group, DLD1 $n = 5$ mice for PDZK1IP1-V5, $n = 8$ mice for EV. 3-5 million cells injected for both HT29 and DLD1. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(E) Meta-comparison of 2D Incucyte growth curves *in vitro* with xenograft tumor growth *in vivo*. KO – sgPDZK1IP1.329, WT – sgNeg, EV – empty vector, OE – PDZK1IP1-V5. Δ Growth presented as (KO-WT) as percentage of WT, or (OE-EV) as a percentage of EV. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

Figure S14. *PDZK1IP1* increases glucose uptake and is linked to cancer metabolism.

(A) Cellular glucose levels by fluorometric assay in DLD1 cell lysates in the presence or absence of 25 μ M sotagliflozin. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's *t*-test.

(B) 2DG uptake by luminescent assay in DLD1 cell lysates. Data presented as mean $\pm$ s.e.m.

(C-D) Hallmarks of Cancer GSEA of RNA-seq expression data from *PDZK1IP1*-high (top 50% mRNA expression) versus *PDZK1IP1*-low (bottom 50% mRNA expression) primary CRCs from TCGA ($n = 342$ independent tumors).

Figure S15. *PDZK1IP1* decreases glycolytic and mitochondrial respiration rate.

(A-B) Seahorse glycolytic stress test. ECAR – extracellular acidification rate, a readout of glycolysis rate ($n = 6$ biological replicates per group). Data presented as mean $\pm$ s.e.m.

(C-D) Seahorse mitochondrial stress test. OCR – oxygen consumption rate, a readout of oxidative respiration rate ($n = 6$ biological replicates per group). Oligo – oligomycin, an ATP synthase

inhibitor which decreases OCR and increase ECAR. FCCP – trifluoromethoxy carbonylcyanide phenylhydrazone, an inner mitochondrial membrane gradient decoupler that increases OCR. Rot/AA – Rotenone and antimycin A, complex I and complex III inhibitors, respectively, which
1050 decrease OCR. Data presented as mean $\pm$ s.e.m.

(E-F) Relative peak area of uniformly labeled fructose 1,6-bisphosphate, reported as % of M+6 isotopomer of total labeled and unlabeled metabolite, from U-C¹³ glucose tracing ($n = 4$ biological replicates per group) as a readout of phosphofructokinase enzyme live-cell activity. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's *t*-test.

1055 **(G-H)** ATP/ADP ratio from total polar metabolite peak area in HT29 and DLD1 cells ($n = 4$ biological replicates per group). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's *t*-test.

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Figure 7. *PDZK1IP1* regulates cancer metabolism.

(A-B) Steady state levels (total peak area) of PPP intermediates and downstream metabolites ($n = 3$ per group). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

(C) Schematic of PPP entry from glycolysis.

(D-F) Total peak area of labeled 6-phosphogluconolactone ($n = 4$ per group), 1,2C¹³-glucose tracing. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(G-H) Steady state NADPH/NADP⁺ ratios from total metabolite peak area ($n = 3$ per group). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(I-J) Steady state levels of total glutathione peak area ($n = 4$ per group). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(K-L) Cellular ROS levels by luminescent assay following diamide treatment for 1 hour at the indicated concentrations ($n = 4$ per group). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(M) Growth curves of sgNeg and sgPDZK1IP1.329 xenograft tumors with or without 30 mM N-acetylcysteine in drinking water ($n = 9$ mice per group, 4 million cells injected). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

Figure S16. *PDZK1IP1* maintains redox homeostasis.

(A) Steady state levels (total peak area) of PPP intermediates and downstream metabolites ($n = 3$ per group). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test. * $P < 0.05$, ** $P < 0.01$.

(B-D) Ratio of M+1/M+2 lactate levels by total peak area, from 5 mM 1,2C¹³-glucose labeling for 4 hours. Data presented as mean $\pm$ s.e.m.

(E) Steady state levels of total glutathione peak area ($n = 4$ per group). Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

(F-H) Cell viability by colorimetric MTT assay under normoxia, hypoxia for 48 hours, or with 30 minutes of diamide treatment. Data presented as mean $\pm$ s.e.m. Significance was determined using Student's t -test.

Materials and Methods

Patient samples and power analysis

All human subjects work was approved by the Mount Sinai Hospital Institutional Review Board (IRB) under Protocol IRB-19-01860. Patient surgical specimens were resected by the Department of Colon and Rectal Surgery at Mount Sinai Hospital followed by pathologist separation into matched tumor and adjacent normal pairs. All samples were de-identified by The Mount Sinai Biorepository. For power analysis, total unique SEs for 15 primary CRCs and 15 adjacent normal mucosae were tallied. The percentage of total unique discovered SEs contributed by the acquisition of each patient sample, cumulative in the order in which the patient samples were received, was plotted.

Cell lines

HT29, COLO205, DLD1, and HEK293T cells were purchased from the American Type Culture Collection (ATCC) and grown according to their specifications, supplemented with 10% fetal bovine serum (Thermo Fisher), 1% penicillin/streptomycin (Corning), and 5 µg/mL plasmocin prophylactic against mycoplasma (Invivogen). Furthermore, cells were tested for mycoplasma contamination at regular intervals to ensure mycoplasma-negative status (Lonza). All cell lines were maintained at 37 degrees Celsius and 5% CO₂. Recombinant human TNFα, IFN-β, IFN-γ, and IL-6 were purchased from Peprotech. For cytokine stimulation experiments, cells were stimulated overnight for 16 hours.

Animal experiments

6-8 week old male nude mice (*Foxn1^{nu}*) were purchased from The Jackson Laboratory. Male mice were used to better recapitulate CRC demographics. For HT29 xenograft experiments, 1 million cells suspended in 100uL serum-free RPMI media were injected per nude mouse. During the HT29 N-acetylcysteine rescue experiment, 4 million cells were injected. For DLD1 xenograft

experiments, 2 million cells suspended in 100uL serum-free RPMI media were injected per nude mouse. For animal studies involving N-acetylcysteine (Sigma), drinking water supplemented with 30 mM NAC was used as the only drinking source. NAC solution was pH adjusted to 7.0 and refreshed weekly. Tumors were measured using calipers every 5 days, with volume calculated using the following formula: $\text{volume} = 0.523(\text{length})(\text{width}^2)$. All animal experiments were approved by the Mount Sinai Institutional Animal Care and Use Committee (IACUC) (protocol #IACUC-2019-0048 & LA13-00024). For mouse studies, no statistical method was used to predetermine sample size, mice were randomly distributed among the treatment groups and no blinding was performed during data collection.

Intracellular ROS and cell death

5,000 to 10,000 cells were plated in a 96-well plate overnight. Media containing 100 ng/mL doxycycline was refreshed the next day to induce expression of PDZK1IP1-V5 or empty vector (EV) for 48 hours. Cells were treated with media containing diamide (Sigma) at the indicated concentrations for one hour. Intracellular ROS levels were determined using the ROS-Glo H_2O_2 assay (Promega) according to manufacturer's instructions before being read on a spectrophotometer. Viable cells after diamide treatment were assessed using the CellTiter 96 Non-Radioactive Cell Proliferation MTT Assay (Promega) according to manufacturer's instructions.

Hypoxia chamber

For experiments involving hypoxia, 5,000 to 10,000 cells were plated in a 96-well plate. Media containing 100 ng/mL doxycycline was refreshed the next day and the plate was moved into a HypOxystation H35 hypoxia chamber. The hypoxia chamber was maintained at 37 degrees, humidified, 5% CO_2 , 1% O_2 , and 94% N_2 . Cells were kept in the hypoxia chamber for 96 hours. Media containing 100 ng/mL doxycycline was maintained and refreshed every 48 hours for EV or PDZK1IP1-V5 cells. Cellular assays were performed at this time point.

Quantitative real-time PCR

RNA was isolated using the RNeasy Mini Kit (Qiagen). cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad) according to manufacturer's instructions and analyzed by qPCR using SYBR green master mix (Life Technologies) on an ABI Prism 7500 instrument. 10 ng of cDNA was used per reaction. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method as previously described (Livak and Schmittgen, 2001). For a list of primers used in this study, see Supplementary Figure 2.

Immunoblot

Cell and tissue lysates were collected in RIPA buffer supplemented with PhosSTOP and cOmplete protease inhibitors (Roche). Tissue samples were homogenized using a handheld TissueRuptor (QIAGEN). Samples were sonicated with a probe sonicator for 12 pulses before spinning down cellular debris at 14,000 RPM in a 4-degree centrifuge. Total protein concentrations were determined and normalized using a BCA assay (Thermo Fisher). Lysates were mixed 1:1 with 2x sample buffer (125 mM Tris-HCl at pH 6.8, 10% β -mercaptoethanol, 2% SDS, 20% glycerol, 0.05% Bromophenol Blue, 8 M urea) and boiled for 5 minutes. Protein lysates were resolved by electrophoresis using 4-12% Bis-Tris gels (Thermo Fisher). Samples were blotted on PVDF membrane (Millipore) by wet transfer. Membranes were blocked in 5% milk or 5% BSA in TBST for 1 hour prior to overnight staining with primary antibody at 4 degrees Celsius with agitation. Membranes were rinsed three times with TBST followed by incubation with horseradish peroxidase-conjugated secondary antibodies in 5% milk for 1 hour, and then rinsed again in TBST three times. Membranes were visualized using chemiluminescence (Thermo Fisher) and autoradiography film (Denville). The following primary antibodies were used for immunoblot: anti-PDZK1IP1 (Sigma #HPA014907), anti-GAPDH (Cell Signaling Technologies, CST #2118),

anti-V5 (CST #13202), anti-phosphorylated STAT3 at Y705 (CST #9145). Band exposure was quantified using ImageJ.

CRISPR-cas9 gene silencing

LentiCRISPRv2-puro (Plasmid #52961) and lentiCRISPRv2-blast (Plasmid #98293) were purchased from Addgene. Sense and antisense sgRNA oligos were synthesized using Fisher Scientific Eurofins, annealed, phosphorylated, and ligated into BsmBI (NEB) digested plasmids according to depositor's instructions as previously described (Sanjana et al., 2014). The Quick Ligation Kit (NEB) was used to ligate DNA into the digested vectors. DNA was transformed into Stbl3 competent cells (Thermo Fisher) and ampicillin-resistant colonies were picked and screened for successful sgRNA integration by Sanger Sequencing (Genewiz) using the hU6-F primer (5'-GAGGGCCTATTTCCTCATGATT-3'). For a list of sgRNAs used in this study, please see Supplementary Figure 3.

Ectopic overexpression of V5-tagged PDZK1IP1

The consensus coding sequence of *PDZK1IP1* was acquired from NCBI and PCR amplified from HT29 cell cDNA, derived as above. Inserts were cloned into the doxycycline-inducible pTRI-blast vector, a gift from Dr. Poulikos Poulikakos and Dr. Stuart Aaronson using the EcoR1 and BamH1 restriction enzyme sites (both from NEB). The *PDZK1IP1* carboxy-terminus comprises a PDZ binding domain. To minimize disruption of this domain which may require a free carboxy-terminus, the V5 peptide tag (GKPIPPLLGLDST) was inserted in-frame immediately upstream of the PDZ binding domain. For a list of primers used in this study, see Supplementary Figure 2. The Quick Ligation Kit (NEB) was used to ligate DNA into the digested vectors. DNA was transformed into Stbl3 competent cells (Thermo Fisher) and ampicillin-resistant colonies were picked and screened for successful sgRNA integration by Sanger Sequencing (Genewiz) using the pTRIPZ_seq_F primer (5'-GGCCATGGTGGCCCTCCTATAGTGA-3'). For doxycycline-

induction of PDZK1IP1-V5 expression, cells were incubated with 100 ng/mL doxycycline for 48 hours prior to assay.

Lentivirus production and cell transduction

1215 Packaging plasmids psPAX2 (Plasmid # 12260) and pMD2.G (Plasmid # 12259) were purchased from Addgene. Lentiviral plasmids and packaging plasmids were assembled in Opti-MEM media (Corning) and transfected into HEK293T cells using the TransIT-Lenti Transfection Reagent (Mirus) according to the manufacturer's instructions. The mass ratio of psPAX2 to pMD2.G used for packaging was 3:1. Media was collected 48 hours after transfection and passed through a 0.45
1220 μ m filter and concentrated using Amicon Ultra-15 centrifugal filter (30 kDa, Millipore). Cells were transduced for 16 hours followed by 48-72 hours of puromycin selection or 6 days of blasticidin selection, depending on the vector. For puromycin, 2 μ g/mL was used for HT29 cells and 3 μ g/mL was used for DLD1 cells. For blasticidin, 5 μ g/mL was used for HT29, COLO205, and DLD1 cells, refreshed every 72 hours. We aimed for low MOI infections by empirically determining
1225 lentivirus dilution factors to achieve <50% cell survival during antibiotic selection.

Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq)

Xenograft tumors and patient surgical specimens were digested with 1X collagenase/hyaluronidase (Stem Cell Technologies), minced, and passed through a 100 μ m filter. Cells were double crosslinked with 0.25 M disuccinimidyl glutarate followed by 1%
1230 formaldehyde. ChIP-seq was performed as previously described (Fontanals-Cirera et al., 2017). Briefly, 2X crosslinked chromatin was sonicated on a Diagenode Bioruptor for 15 cycles 30s ON/30s OFF on the low power setting. Cells were precleared using 100 μ L of Magna-ChIP Protein A+G magnetic bead slurry (Millipore) before overnight incubation with 10 μ g ChIP antibody conjugated to 100 μ L beads. Beads were washed once with IP Wash Buffer I, twice with High Salt

1235 Wash Buffer, once with IP Wash Buffer II, and twice with TE buffer. For buffer recipes refer to
Fontanals-Cirera, Hasson et al (Fontanals-Cirera et al., 2017). Beads were eluted with 1% SDS at
65 degrees Celsius with agitation and reverse crosslinked overnight with high salt with RNase
treatment (Roche). The next day, DNA underwent Proteinase K treatment at 42 degrees Celsius
before elution using QIAquick PCR purification kit (QIAGEN). DNA was run on an Agilent High
1240 Sensitivity DNA chip using an Agilent Technologies 2100 Bioanalyzer for quality control.
H3K27ac ChIP-seq antibodies were purchased from abcam (#ab177178).

Incucyte *in vitro* cell proliferation assay

10,000 cells were plated in a 48-well plate (Corning Falcon). Cells were monitored for 5-6 days
using an Incucyte live cell imaging system (Essen Bioscience), which is placed in a cell culture
1245 incubator operated at 37 degrees Celsius and 5% CO₂. For relevant experiments, 100 ng/mL
doxycycline containing media was refreshed every 48 hours.

DNA library construction and sequencing

DNA library construction was performed as previously described (Fontanals-Cirera et al., 2017).
ChIP DNA was prepared as above. Briefly DNA underwent end repair, poly-adenylation, and
1250 barcoding using enzymes from New England Biolabs. ChIP libraries were amplified 12-15 PCR
cycles using the 2X KAPA HiFi DNA HotStart ReadyMix before quality assessment by running
on a High Sensitivity chip using a Bioanalyzer 2100 (Agilent) followed by a Qubit fluorometer
(Thermo Fisher). The Mount Sinai Oncological Sciences Sequencing Core Facility prepared poly-
A-capture RNA-seq libraries using the TruSeq RNA Library Prep Kit v2 (Illumina) according to
1255 manufacturer's instructions. All libraries were sequenced as 75 bp single end reads by the Mount
Sinai Oncological Sciences Sequencing Core Facility on an Illumina NextSeq instrument.

ChIP-seq data analysis and super-enhancer calling

Adapter sequences were removed from reads using Cutadapt. Reads were mapped to the hg19 human genome using bowtie. Duplicate reads were removed using samtools. Matching input control was used to call peaks. Peak calling was performed using MACS2. For H3K27ac, a *P*-value cutoff of 10^{-10} was used for peak calling. Bigwig tracks were generated using deepTools bamCoverage with RPKM normalization. H3K27ac ChIP-seq tracks were promoter normalized prior to direct comparison within and between patients. Blacklisted regions (Duke_Hg19SignalRepeatArtifactRegions.bed, downloaded from the Broad Institute) were excluded from called peaks using bedtools. Super-enhancer and enhancer calling were performed using Rank Ordering of Super-Enhancers (ROSE) on H3K27ac enrichment (Loven et al., 2013) using default parameters. Principal component analysis was performed using deepTools multiBigwigSummary and plotPCA functions. Summary plots were generated using deepTools computeMatrix and plotProfile. Pearson correlation heatmap and unsupervised hierarchical clustering analysis was performed using deepTools plotCorrelation. Student's t-test was used to call significant differentially expressed super-enhancers among the 2026 total identified between 15 primary CRCs and 15 patient-matched adjacent normal colon mucosa, using normalized H3K27ac RPKM as input. These significantly differential super-enhancers were visualized as a heatmap in Figure 1 using the R package pheatmap on R version 4.0.3 and RStudio version 1.1.463. Please see Supplementary Table 8 for quality control metrics for ChIP-seq datasets generated in this study.

RNA-seq data analysis

Sequenced reads were mapped to the hg38 transcriptome using Salmon quasi-mapping under the default parameters and expression output in TPM was extracted (Patro et al., 2017). Output files (expression in TPM) were directly integrated into the R package DESeq2 for differential gene expression analysis, again under default parameters (Love et al., 2014). Genes with expression >2

TPM were used for downstream analyses. Pathway analyses were performed using pre-ranked Gene Set Enrichment Analysis (GSEA), using the pre-ranked (by $\log_2$ fold change gene expression) function, as well as Enrichr (which includes many tools including ChEA)(Kuleshov et al., 2016; Subramanian et al., 2005). $\log_2$ fold change cut-offs for differentially expressed genes used in downstream analyses are stated in the text. For epithelial, immune, and stromal cell population de-convolution from bulk CRC tumor RNA-seq data, xCell was used with Salmon output and gene expression in TPM(Aran et al., 2017). Out of the 15 CRC tumors, 11 had sample-matched RNA-seq data, for which xCell was performed to assess non-epithelial cell contribution to H3K27ac signal from the bulk tumor ChIP-seq. The ratio of epithelial cell, immune cell, and stroma scores for each independent CRC is reported.

Prediction of super-enhancer target genes

Sample-matched RNA-seq and H3K27ac ChIP-seq across multiple patient cohorts were utilized to predict SE target genes. For a list of patient samples with sample matched RNA-seq and H3K27ac ChIP-seq used in this analysis, please see Supplementary Fig. 5. For each of the 2026 SEs identified in this study, a linear regression analysis was performed, comparing natural variation of H3K27ac signal at the SE with sample-matched mRNA expression from RNA-seq for each protein coding gene with a transcriptional start site within 500kb of the center of the SE. The gene with the most significant expression correlation ($P < 0.05$) is assigned as the most confident target gene. Several SEs have multiple target genes with significant correlation.

ATAC-seq data and motif analysis

Previously generated ATAC-seq bigwig files mapped to human genome hg38 from primary colorectal carcinomas were downloaded, with permission, from TCGA(Corces et al., 2018). LiftOver (UCSC) was used to convert coordinates between hg38 and hg19 human genomes. Tracks were visualized using the IGV browser(Robinson et al., 2011). Accessible chromatin

regions of interest were subject to motif analysis using Transcription Factor Affinity Prediction (TRAP) web tools(Thomas-Chollier et al., 2011). <500bp regions were analyzed using Jaspar vertebrates as the matrix file, human promoters as the background model, and Benjamini-Hochberg as the multiple test correction. For TRAP motif analysis on multiple sequences, only the top 3000 sequences were analyzed per program input requirements.

Single cell RNA-seq analysis

Single cell 3' mRNA sequencing data from a cohort of 23 CRC patients (SMC cohort) was obtained from GSE132465(Lee et al., 2020). R package Seurat (v.4.0) was used to analyze the data, using built in functions for normalization, dimensionality reduction, and visualization. To display cell clusters in UMAP plots and dotplots we colored and grouped cells by the annotations provided by the study, where malignant and non-malignant epithelial cells were combined into one category called epithelial cells. A customized version of the Seurat dotplot was used to display *PDZK1IP1* expression across cell types and patients.

Mutation analysis of *PDZK1IP1* super-enhancer

H3K27ac ChIP-seq bam files for 15 patient tumor normal pairs were loaded into the IGV genome browser where the *PDZK1IP1* super-enhancer locus (chr1:47644434-47659486, hg19 coordinates) was visualized. Single nucleotide polymorphisms (SNPs), insertions, and deletions against the hg19 reference human genome within the super-enhancer locus were highlighted and assessed for specificity in CRC tumors over patient-matched normal in all 15 patients.

Seahorse assays

Cellular glycolysis was measured using the XF Glycolysis stress test kit and XP Mitochondrial stress test kit (Agilent) according to the manufacturer's instructions. Briefly, PDZK1IP1-V5 and EV cells were seeded in XFe96 well plates (8000-10,000/well). The next day, media containing 100 ng/mL doxycycline was added to the cells for 48 hours to induce PDZK1IP1-V5 or EV

expression. The next day, growth media was aspirated and replaced with Seahorse media (DMEM supplemented with 2mM glutamine, pH 7.4) and cells were incubated in a were incubated at 37°C in the absence of CO₂ for 1 hr. Extracellular acidification rates (ECAR) and oxygen consumption rate (OCR) were measured using the XFe96 Extracellular Flux Analyzer. Baseline ECAR measurements were determined before administration of glucose (10mM) and oligomycin (1μM). Baseline OCR measurements were determined before administration of any drug. At the end of the assay, cells were stained with methylene blue, de-stained, and the absorbance was measured at 668nm using a plate reader (Synergy H1 Hybrid multi-mode micro-plate reader, Biotek). ECAR and OCR measurements were normalized against the cell densities and all data analysis was conducted with the Seahorse Wave software version 2.6.1.53 (Agilent).

Medical illustrations

Figure 1a contains medical illustrations from SMART Servier Medical Art, reproduced with permission.

Cytokine profiling

Cell line and tumor lysates were collected in RIPA buffer as described above, without the addition of 2X sample buffer. Supernatant lysates were diluted 1:1 with PBS supplemented with protease inhibitors. Cytokine profiling performed by Eve Technologies using the Human Cytokine Array/Chemokine Array 48-plex platform and Mouse Cytokine Array/Chemokine Array 31-plex panels. Cytokine concentrations were normalized to protein levels determined by BCA assay (Thermo Fisher).

Targeted metabolomics and data analysis

For steady state polar metabolite profiling, full RPMI media was refreshed on cells for two hours. Polar metabolites were extracted from cancer cells in vitro using 80% (v/v) aqueous methanol as described before(Yuan et al., 2012). Targeted liquid chromatography-tandem mass spectrometry

(LC-MS/MS) was performed using a 6500 or 5500 QTRAP triple quadrupole mass spectrometer (AB/SCIEX) coupled to a Prominence UFLC HPLC system (Shimadzu) with Amide HILIC chromatography (Waters). Data were acquired in selected reaction monitoring (SRM) mode using positive/negative ion polarity switching for steady-state polar profiling of greater than 260 molecules. Peak areas from the total ion current for each metabolite SRM transition were integrated using MultiQuant v2.0 software (AB/SCIEX). The original data were normalized to protein concentration determined by BCA assay.

1,2C¹³ glucose carbon labeling

For carbon labeling experiments to assess the pentose phosphate pathway, cells were plated and PDZK1IP1-V5 induced in full RPMI. 24 hours prior to extraction, media was changed to RPMI with 5mM glucose, 2mM glutamine, and 10% dialyzed FBS as previously described(Yi et al., 2012). Media was refreshed using RPMI with 5mM glucose, 2mM glutamine, and 10% dialyzed FBS for three hours to normalize metabolism. Media was then changed to RPMI with 5mM 1,2C¹³ glucose (Cambridge Isotope Laboratories), 2mM glutamine, and 10% dialyzed FBS to allow carbon labeling for 4 hours. Polar metabolites were extracted as above.

Uniform C¹³ glucose carbon labeling

For carbon labeling experiments to assess glycolysis, cells were plated and PDZK1IP1-V5 induced in full RPMI. 24 hours prior to extraction, media was changed to RPMI with 5mM glucose, 2mM glutamine, and 10% dialyzed FBS as previously described(Yi et al., 2012). Media was refreshed using RPMI with 5mM glucose, 2mM glutamine, and 10% dialyzed FBS for three hours to normalize metabolism. Media was then changed to RPMI with 5mM U-C¹³ glucose (Cambridge Isotope Laboratories), 2mM glutamine, and 10% dialyzed FBS to allow carbon labeling for 15 minutes. Polar metabolites were extracted as above.

Immunohistochemistry

Xenograft tumors were fixed in 10% formalin (Thermo Fisher). IHC was performed by the Oncological Sciences Core Facility. Paraffin sections were dewaxed as previously described. Antigen retrieval was performed using a Dako pressure cooker in Antigen Unmasking Solution (Vector Laboratories). Briefly, sections were boiled in 0.01 M sodium citrate, pH 6.0, 0.05% Tween-20 twice for 10 min and cooled at room temperature for 20 min. Sections were washed with ddH₂O 3 times for 5 min, followed by a PBS wash for 5 min. HRP conjugated secondary antibody was used to detect signal. Endogenous HRP was blocked with 3% H₂O₂ in PBS or methanol for 10 min. The slide was washed in PBS three times for 5 minutes. The following primary antibodies were used for IHC: anti-CD11c (CST #97585), anti-CD68 (CST #97778), anti-perforin (CST #31647), and anti-Ly-6G (CST #87048).

Glucose 6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate (6PGD) activity assays

In vitro enzyme activity assays were performed as previously described (Stanton et al., 1991). Briefly, for glucose 6-phosphate dehydrogenase activity, the following substrates were re-constituted *in vitro* in PPP activity buffer (50 mM Tris, 1 mM MgCl₂): 200 μM glucose 6-phosphate (Sigma) and 100 μM NADP⁺ (Roche). 10 μg of protein lysate from PDZK1IP1-V5 or EV expressing cells were added to the reaction mixture. Reduction of NADP⁺ to NADPH was read by absorption at 340 nm on a spectrophotometer as a readout of glucose 6-phosphate dehydrogenase activity. For 6-phosphogluconate dehydrogenase activity, the following substrates were re-constituted *in vitro* in PPP activity buffer: 200 μM 6-phosphogluconate (Sigma) and 100 μM NADP⁺ (Roche). 10 μg of protein lysate from PDZK1IP1-V5 or EV expressing cells were added to the reaction mixture. Reduction of NADP⁺ to NADPH was read by absorption at 340 nm on a spectrophotometer as a readout of 6-phosphogluconate dehydrogenase activity.

Statistics and data reproducibility

Statistical analyses were performed using Microsoft Excel for Mac Version 16 and Prism Graphpad Version 7. Data are expressed as mean with standard error of the mean unless otherwise noted. A Student's *t*-test (two-tailed with equal variance) was used when comparing two groups. When unequal variance was noted (for example, in some cytokine profiling measurements detectable in one group but not the other in fig. S4), a Mann-Whitney test was used instead. A *P* value of <0.05 was considered statistically significant. No statistical method was used to predetermine sample size. We were not blinded to allocation during experiments and outcome assessment. Experiments presented in Fig. 2G,I,K,L,N, 3A,M,N, and in fig. S2G-I,M, 4HH-LL, 6G, 7A,D-F,I-K, 8A-F, 9A-G were independently repeated and similar results were obtained.

Data availability

H3K27ac ChIP-seq and RNA-seq datasets performed in this study are deposited at the NCBI Gene Expression Omnibus under the accession GSE166254. H3K27ac ChIP-seq datasets previously performed in FAP adenomas and normal colon crypts presented in Fig. 1b-c, fig. S6A were accessed from the Gene Expression Omnibus under the accession GSE77737(Cohen et al., 2017). Broad Institute Cancer Cell Line Encyclopedia (CCLE) RNA-seq datasets for CRC cell lines presented in Fig. 2g, fig. S4C-F were accessed from the Gene Expression Omnibus under the accession PRJNA523380(Ghandi et al., 2019). COLO205 in vitro and orthotopic tumor H3K27ac ChIP-seq datasets presented in fig. S2C-E, 3A-B were accessed under the accession GSE126188(Hung et al., 2019). The following datasets are presented in fig. S2I: RELA ChIP-seq datasets from adipocytes were accessed under the accession GSM1566735(Schmidt et al., 2015); RELA ChIP-seq datasets from A549 cells were accessed under the accession GSM847876(Raskatov et al., 2012); STAT1 ChIP-seq datasets from HeLa cells were accessed under the accession GSM385505(Robertson et al., 2007); STAT1 ChIP-seq datasets from monocytes were accessed under the accession GSM1057011(Qiao et al., 2013); STAT3 ChIP-seq

datasets from MDA-MB-468 cells were accessed under the accession GSM2278003(McDaniel et al., 2017); CTCF ChIP-seq from normal colon tissue was accessed from ENCODE(Davis et al., 2018). Cancer H3K27ac datasets presented in the heatmap in fig. S1D contains the following: osteosarcoma H3K27ac ChIP-seq datasets accessed under accession GSE74230(Morrow et al., 2018); gastric cancer H3K27ac ChIP-seq datasets accessed under accession GSE76153 & GSE75898(Ooi et al., 2016); luminal breast cancer H3K27ac ChIP-seq datasets accessed at the ENA under accession PRJEB22757(Patten et al., 2018); prostate cancer H3K27ac ChIP-seq datasets accessed under accession GSE96652(Kron et al., 2017); GBM H3K27ac ChIP-seq datasets accessed under accession GSE119834(Mack et al., 2019); chordoma H3K27ac ChIP-seq datasets accessed under accession GSE109794(Sharifnia et al., 2019); ccRCC H3K27ac ChIP-seq datasets accessed under accession GSE86095(Yao et al., 2017); DLBCL H3K27ac ChIP-seq datasets accessed under accession GSE46663(Chapuy et al., 2013). OncoLnc was used to generate Kaplan Meier curves for PDZK1IP1-high and -low mRNA expression cases based off TCGA data. ATAC-seq data presented in Fig. 3a and RNA-seq data presented in fig. S6C accessed from TCGA. RNA-seq datasets from CRC GEMMs with reversible *Apc* silencing using an endogenous doxycycline-inducible shRNA in the presence of absence of *KRAS*^{G12D} mutations accessed under accession GSE67186(Dow et al., 2015). Single-cell RNA-seq data from primary CRC and normal colon epithelium was accessed under the accession EGAS00001003779 and EGAS00001003769 from the European Genome-phenome Archive database(Lee et al., 2020). Raw H3K27ac ChIP-seq sequencing data from primary CRC and matched human colon organoids, also referred to in the manuscript as the Milan cohort, were accessed under E-MTAB-8416, and raw RNA-seq sequencing data was accessed under E-MTAB-8448(Della Chiara et al., 2021). Raw H3K27ac ChIP-seq sequencing data from commercially available human CRC cell lines were accessed under

GSE96069, GSE73319, GSE126188, and GSE77737(Cohen et al., 2017; Hung et al., 2019;
1450 McClelland et al., 2016; Nakamura et al., 2017).

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Supplementary Table 1: List of commercially available CRC cell lines commonly used in laboratory studies.

	HCT15
1830	HCT116
	COLO320
	CACO2
	HT29
	DLD1
1835	LS180
	RKO
	SW480
	SW620
	COLO741
1840	SW480
	COLO205

1845

1850

Supplementary Table 2: Hg19 coordinates of super-enhancers identified in primary CRC and normal colon tissue that are over- or under-represented in 13 commercial CRC cell lines.

'chr'	'start'	'end'	log2 cell lines/primary CRC	P-value
chr2	219921304	219926326	-3.933334181	4.7453E-06
chr19	12827411294598	-3.032709913	7.59682E-05	
chr16	49886677	49893398	-2.949821466	0.000519631
chr19	42210448	42221456	-2.898911644	0.004772279
chr11	312970	318010	-2.894756705	1.76456E-05
chr8	142422272	142429622	-2.644337941	0.001276849
chr19	950367	958117	-2.495988143	0.007449326
chr5	149539500	149568866	-2.335044971	1.37598E-06
chr7	27226673	27235671	-2.171925116	0.002112967
chr7	100607523	100631215	-2.158768857	7.52654E-06
chr11	76773786	76787721	-2.116481306	9.32179E-10
chr3	38025920	38036323	-2.084897042	1.80235E-06
chr1	47644434	47659486	-2.078868512	1.73654E-06
chr7	113722871	113728455	-2.054225966	0.003951842
chr16	31137030	31147852	-2.037706389	1.65113E-08
chr19	11292401134649	-2.035016076	5.13254E-09	
chr17	38564963892930	-2.015277075	9.50179E-07	
chr11	76796563	76803020	-2.005698624	1.14188E-06
chr10	85918778	85965078	-1.956797767	6.89249E-07
chr16	67460809	67504574	-1.945311673	1.21339E-07
chr9	130905976	130914424	-1.91203976	0.000189521
chr14	94848020	94862793	-1.890862615	0.009692258
chr20	22548732	22567099	-1.887185684	0.001244224
chrX	70835396	70848211	-1.868040926	0.000105237
chr17	74485442	74490614	-1.851351981	2.512E-05

1885	chr7	70158103	70166365	-1.815465956	2.41185E-05
	chr7	73180110	73189479	-1.810669041	1.09706E-05
	chr2	14762896	14776676	-1.803198778	0.000146674
	chr8	142315603	142357428	-1.803098563	0.00033056
	chr1	205532653	205568500	-1.800685286	0.000129067
1890	chr8	48646118	48651966	-1.760217332	6.43184E-08
	chr7	156393561	156419142	-1.759424939	0.000451767
	chr7	150477778	150505955	-1.753099976	1.77004E-05
	chr16	85449100	85526770	-1.752547912	0.000214569
	chr16	582919 587823	-1.75068509	5.44416E-06	
1895	chr4	15002818	15007221	-1.704048064	8.80386E-08
	chr19	18611824	18621473	-1.694070408	8.10599E-07
	chr19	40419549	40457265	-1.692289026	0.000447315
	chr9	140186990	140203083	-1.674837677	8.93647E-05
	chr1	33342282	33373754	-1.670767379	3.25312E-06
1900	chr4	149348610	149367220	-1.66504665	4.70202E-07
	chr17	73634586	73644264	-1.663655009	0.000271978
	chr8	76314903	76322046	-1.663653639	0.000200429
	chr4	160023546	160026111	-1.660029638	2.20781E-06
	chr19	50668992	50738128	-1.654229726	1.38886E-05
1905	chr20	60979569	60991115	-1.651679206	0.001274464
	chr16	31487207	31500133	-1.646142188	2.01116E-05
	chr1	10563061073787	-1.625633377	4.36938E-05	
	chr7	27140197	27149295	-1.590910353	0.002221693
	chr15	68479899	68503417	-1.588524898	3.10799E-08
1910	chr22	31666929	31694556	-1.583014828	6.12579E-07
	chr19	33723635	33815979	-1.576082011	4.14475E-06
	chr16	53163214	53168804	-1.562522215	0.001147531

	chr16	68384973	68432029	-1.562204677	1.67925E-07
	chr17	37779956	37790775	-1.552808833	3.35556E-05
	chr21	43718828	43759283	-1.549372024	0.000136642
	chr22	38493842	38517899	-1.539972117	5.67556E-07
1915	chr20	17902067	17921342	-1.522326512	2.29737E-06
	chr16	30123335	30135353	-1.518808525	9.5405E-06
	chr2	241540948	241546332	-1.517004878	0.000736344
	chr8	144938952	144989216	-1.503213086	6.16809E-06
	chr19	35599346	35623141	-1.500696097	0.000173606
1920	chr22	50319146	50364826	-1.495054142	2.28937E-06
	chr9	92263575	92302155	-1.49031086	1.82918E-06
	chr18	28676993	28682939	-1.489553055	0.001023615
	chr11	29301682936397	-1.486304394	1.33944E-05	
	chr9	130864490	130880714	-1.485125129	8.30681E-05
1925	chr4	17481801818534	-1.482977267	0.000172748	
	chr1	16059949	16070046	-1.480374457	0.000183684
	chr9	95929205	96033139	-1.480201283	3.42275E-06
	chr2	219148537	219162345	-1.476482263	0.001992198
	chr19	41914345	41942398	-1.473144331	1.3812E-07
1930	chr18	19741865	19781074	-1.464659281	0.000491763
	chr17	38327873	38351879	-1.464426274	6.48712E-05
	chr2	239169490	239219160	-1.463708595	1.68293E-07
	chr7	29601890	29607677	-1.452014835	0.001734837
	chr17	81005827	81069196	-1.442303168	1.50685E-05
1935	chr2	242704267	242763347	-1.441783842	7.53839E-06
	chr13	27522316	27597035	-1.42852943	0.008113301
	chr15	31616912	31698718	-1.42833867	2.07408E-07
	chr19	55756765	55767392	-1.42726789	0.006850812

1940	chr10	134197375	134245029	-1.427197048	5.09147E-09
	chr1	61508480	61523688	-1.412822061	2.24545E-05
	chr9	140087151	140096306	-1.410234098	0.006737055
	chr16	67423853	67447927	-1.407506127	1.03681E-05
1945	chr17	79763639	79788092	-1.388498587	8.97146E-08
	chr10	134257773	134301784	-1.380589018	7.23145E-06
	chr1	199990289	200023200	-1.374864468	0.002411763
	chr3	53166834	53230973	-1.374491386	8.47178E-08
1950	chr8	142203985	142256046	-1.372888112	6.06341E-05
	chr3	71612437	71634637	-1.372196278	0.000179723
	chr19	54979246	54985496	-1.363581356	0.000548464
	chr14	38052216	38072548	-1.363489967	0.002279186
1955	chr9	134496283	134555283	-1.35721224	0.005416658
	chr22	37406071	37423323	-1.357177296	4.84104E-05
	chr7	30322996	30327105	-1.355003336	6.64456E-06
	chr12	64684736473930	-1.354950301	0.000145933	
1960	chr4	140096935	140110107	-1.354221017	1.09865E-07
	chr4	79579547988605	-1.340452856	1.32515E-05	
	chr17	26120397	26151772	-1.335406068	0.000614658
	chr11	15250341597870	-1.334335	6.89398E-06	
1965	chr5	40676729	40700663	-1.333969521	0.005834136
	chr7	27236867	27251265	-1.333213185	0.008969102
	chr5	149586118	149597726	-1.327771064	0.000134751
	chr1	155050463	155059415	-1.32584885	0.000636383
1965	chr19	40345564150644	-1.318671308	4.72375E-08	
	chr17	73568797	73577073	-1.314979465	3.52102E-05
	chr14	55031455	55035147	-1.314499318	0.00014642
	chr9	117124745	117165720	-1.313797327	0.000198119

	chr17	37827160	37837462	-1.311567369	0.000914851
	chr7	991118	994980	-1.306250732	0.006732574
	chr8	1913890	1926709	-1.295545411	0.000272981
1970	chr12	121650947	121685811	-1.29244219	0.001279391
	chr19	1247317	1256284	-1.290320659	7.93516E-05
	chr7	25979727	26008574	-1.288425537	0.005901451
	chr16	11290981	11360138	-1.28593865	2.36031E-06
	chr8	145536936	145562791	-1.282480618	2.95262E-05
1975	chr5	1073524	1188157	-1.28018389	0.000552488
	chr2	43035897	43038998	-1.279825977	7.19543E-09
	chr9	139578763	139591286	-1.278519633	5.51206E-05
	chr1	51761624	51802404	-1.276336427	7.98952E-06
	chr19	35485150	35505427	-1.273803969	2.20636E-06
1980	chr21	42866102	42955540	-1.271553665	0.003990368
	chr9	140203344	140212896	-1.268492344	0.003718446
	chr7	948975	956672	-1.267631416	0.000589779
	chr7	100482826	100510426	-1.262130127	4.80322E-06
	chr11	64654508	64663543	-1.26061998	0.001093014
1985	chr9	110243788	110268173	-1.251133259	0.000442417
	chr1	226288057	226325426	-1.248226489	8.31645E-07
	chr12	32543708	32556907	-1.244848297	1.22568E-06
	chr18	60381757	60385355	-1.235852828	3.78748E-05
	chr20	57463250	57471189	-1.214365622	0.000611273
1990	chr5	32433949	32447867	-1.213848352	0.000267944
	chr19	923608	929931	-1.211890102	0.004619643
	chr21	45658980	45670752	-1.200354979	0.001115286
	chr17	76988200	76995813	-1.19781147	0.002996369
	chr3	169375755	169387326	-1.197762721	0.00030491

1995	chr15	40634403	40643032	-1.192001967	0.003206032
	chr9	100821574	100909505	-1.19111333	7.61172E-05
	chr11	818110 822789	-1.190429173	1.75958E-05	
	chr1	19965334	19984929	-1.189885971	0.00011409
	chr3	127449108	127543524	-1.181684473	0.000712362
2000	chr17	73519791	73524830	-1.179960513	4.78328E-05
	chr5	172195280	172206972	-1.177488882	6.764E-05
	chr11	117944260	117963999	-1.175196218	0.001450218
	chr20	62164679	62175099	-1.174885529	0.002258229
	chr19	12892449	12906321	-1.172881308	1.56507E-06
2005	chr6	35438934	35474161	-1.172216596	4.51858E-06
	chr1	1092834 1107947	-1.171099652	0.002064843	
	chr17	18864569	18923164	-1.170180749	0.005223225
	chr1	17828633	17961597	-1.162184065	0.000116939
	chr1	32127999	32154793	-1.1590256	0.001938253
2010	chr1	233743752	233761980	-1.158896275	0.001413337
	chrX	68047881	68069392	-1.15695134	0.001230527
	chr16	81299560	81312418	-1.156884193	3.82558E-05
	chr2	206948329	206952121	-1.152646404	2.06789E-07
	chr6	157097603	157103712	-1.152413919	0.000154177
2015	chr1	1076920 1083409	-1.149284102	0.002793414	
	chr15	31725421	31791946	-1.146208447	0.000106421
	chr22	46497766	46507175	-1.144533411	0.005056484
	chr15	31546526	31573312	-1.1437809	7.76493E-06
	chr7	1568954 1574711	-1.142482389	0.000235901	
2020	chr8	134335470	134391007	-1.136072787	0.002367042
	chr6	35691386	35701797	-1.134733443	6.61822E-05
	chr1	207098037	207154650	-1.131390166	0.003585078

2025	chr11	29223972926733	-1.127961438	0.000137631	
	chr9	14309511	14323573	-1.125174648	0.002250256
	chr20	56104107	56204341	-1.122036256	0.00336621
	chr20	56229753	56295325	-1.118689646	0.004286441
	chr19	49178577	49202140	-1.111287415	8.87727E-05
2030	chr17	73529524	73542716	-1.109659761	0.008782904
	chr21	44858977	44873702	-1.109649262	0.001078597
	chr9	20141952023271	-1.108875373	7.58311E-06	
	chr14	105944150	105959031	-1.106254332	0.002882295
	chr19	11353793	11380147	-1.104940631	1.86943E-06
2035	chr8	81397760	81421513	-1.104026822	0.000173483
	chr8	145724875	145736551	-1.102944841	0.000375006
	chr10	135156522	135179866	-1.10253336	0.004821712
	chr19	45220249	45288652	-1.101828316	0.000358198
	chr1	155161643	155165270	-1.101255846	0.006195042
2040	chr11	25319692557416	-1.099006162	0.000600571	
	chr10	112562258	112639452	-1.095353097	0.00213485
	chr16	88443224	88621560	-1.094897966	0.002762185
	chr17	10676053	10719009	-1.094674354	0.004648038
	chr11	606195 622242	-1.094121442	5.98525E-05	
2045	chr5	172327125	172346151	-1.093982888	2.61373E-05
	chr22	50962309	50971267	-1.093835024	0.003064404
	chr6	168413323	168503148	-1.09366734	2.40523E-05
	chr8	145043674	145052590	-1.093621486	0.000623648
	chr17	57442508	57459901	-1.092540175	0.003262118
2050	chr1	228349132	228354871	-1.091068244	0.000204448
	chr6	89788647	89793074	-1.089729814	8.93332E-09
	chr2	28542414	28677214	-1.088175117	9.13028E-05

	chr22	46406761	46411260	-1.088019864	0.000118634
	chr1	65530848	65534992	-1.086156021	0.000454408
	chr13	41237892	41242018	-1.08574238	0.000736638
	chr1	37936562	37964770	-1.084054544	0.000176254
2055	chr1	20796697	20823686	-1.080299094	0.004959933
	chr8	61811816	61840052	-1.080121891	4.14865E-05
	chr12	106620916	106644657	-1.079291391	3.6892E-05
	chr14	103367432	103415466	-1.07815608	2.60995E-05
	chr3	169755228	169768436	-1.072797034	7.40788E-06
2060	chr14	100569955	100660583	-1.069741409	0.000293944
	chr2	208027703	208032208	-1.068574986	0.00013338
	chr5	149941195	150017647	-1.064230609	5.22559E-06
	chr4	124315353	124341272	-1.055196918	0.001277395
	chr9	139739767	139758297	-1.045706755	4.37139E-05
2065	chr4	141157126	141174915	-1.044484983	0.00687941
	chr1	226029826	226110273	-1.037503403	7.20714E-06
	chr9	139683661	139691005	-1.034391734	9.9218E-05
	chr5	32298136	32333194	-1.033402799	0.003793373
	chr16	57118390	57188393	-1.032752273	2.17302E-05
2070	chr2	242280263	242316360	-1.030467192	0.002928121
	chr1	48150864	48195520	-1.024145926	2.274E-05
	chr19	39887706	39902964	-1.023371562	0.000581726
	chr2	238380339	238457019	-1.019570375	0.00548691
	chr12	57519739	57575138	-1.017545226	0.000325957
2075	chr2	241895868	241951777	-1.014868666	0.004083038
	chr20	62150329	62155053	-1.010226977	0.000818134
	chr19	49114785	49123342	-1.009885182	0.000928071
	chr19	17875523	17914613	-1.007297164	9.04413E-05

2080	chr10	98337456	98394081	1.007035335	0.002355826
	chr14	91817703	91881574	1.054473572	0.001319197
	chr2	242784751	242844988	1.055869807	0.000500672
	chr6	30691362	30762214	1.06354511	0.001788619
	chr8	27189458	27266603	1.105089489	0.006499779
2085	chr1	839207 879441	1.23821519	0.008747137	
	chr4	149281615843061	1.253051461	0.003244989	
	chr18	358327436261741	1.292623519	4.46798E-05	
	chr7	130567798	130646840	1.301600783	0.000842286
	chr12	46761555	46797433	1.368447694	0.00332708
2090	chr17	48176137	48195174	1.393480425	0.000480532
	chr17	17795500	17864058	1.41147405	0.007475802

2095

2100

2105

Supplementary Table 3: Gene signature downstream of tumor-enriched SEs. Genes are mRNA up-regulated in primary CRCs over CCLE cell lines.

##37 SE associated tumor specific genes

PTP4A3
IFITM2
SERPINA1
TMEM176B
COL16A1
C10orf99
PDZK1IP1
NOS2
SLC7A10
TMEM82
FXVD3
SHC2
GADD45G
CEACAM6
TMEM220
SOCS1
NR3C2
CYP27A1
ATP2A3
PALM
HSD11B2
FKBP5
PADI2
LRRC36
IFITM1
KIF25
PDE4C
SMPD3
MATK
TST
SMIM5
MAPK3
AMN
LCN12
CCDC183
CAMK2N1
KLF4

Supplementary Table 4: Super-enhancers up-regulated in primary CRC over patient-matched normal tissue, and also up-regulated in primary specimens over CRC cell lines.

'chr'	'start'	'end'
chr11	312970	318010
chr8	142422272	142429622
chr19	950367	958117
chr1	47644434	47659486
chr17	74485442	74490614
chr8	142203985	142256046
chr19	923608	929931
chr20	56229753	56295325
chr2	208027703	208032208

Supplementary Table 5: Juxtaposition of primary CRC cases with mutational status and *PDZK1IP1* SE status. All patients for which data are available are shown.

	Patient	PDZK1IP1 SE?	Gene	Mutation	Variant_Classification	Variant Allele Frequency
2140	18481T	Yes	PRDM2	c.4027G>A	Missense_Mutation	0.150344828
	18481T	Yes	SZT2	c.2138del	Frame_Shift_Del	0.383838384
	18481T	Yes	CACNA1S	c.5012G>A	Missense_Mutation	0.182072829
	18481T	Yes	KISS1	c.225del	Frame_Shift_Del	0.41025641
	18481T	Yes	PIK3C2B	c.2698C>T	Missense_Mutation	0.399463807
2145	18481T	Yes	C2orf71	c.3839C>T	Missense_Mutation	0.2
	18481T	Yes	CASP8	c.1557del	Frame_Shift_Del	0.350308642
	18481T	Yes	CASP8	c.1586T>C	Missense_Mutation	0.396258503
	18481T	Yes	TGFBR2	c.644G>A	Missense_Mutation	0.351687389
	18481T	Yes	TGFBR2	c.1043T>C	Missense_Mutation	0.364628821
2150	18481T	Yes	PBRM1	c.1961A>G	Missense_Mutation	0.189873418
	18481T	Yes	PBRM1	c.518T>C	Missense_Mutation	0.390728477
	18481T	Yes	ATR	c.7375C>T	Missense_Mutation	0.155555556
	18481T	Yes	TET2	c.371A>G	Missense_Mutation	0.179447853
	18481T	Yes	TET2	c.4354C>T	Nonsense_Mutation	0.377919321
2155	18481T	Yes	TET2	c.4871A>G	Missense_Mutation	0.208805031
	18481T	Yes	FAT1	c.12608G>A	Missense_Mutation	0.376744186
	18481T	Yes	FAT1	c.7366G>A	Missense_Mutation	0.352859135
	18481T	Yes	ARID1B	c.1988A>G	Missense_Mutation	0.19895288
	18481T	Yes	EGFR	c.2029C>T	Missense_Mutation	0.193548387
2160	18481T	Yes	EGFR	c.2930G>A	Missense_Mutation	0.393333333
	18481T	Yes	MET	c.40C>T	Missense_Mutation	0.418390805
	18481T	Yes	KMT2C	c.7238C>T	Missense_Mutation	0.18814433
	18481T	Yes	WRN	c.2743T>C	Missense_Mutation	0.181081081
	18481T	Yes	ANK1	c.1231G>A	Missense_Mutation	0.420454545

2165	18481T	Yes	SMARCA2	c.980G>A	Missense_Mutation	0.33333333
	18481T	Yes	GATA3	c.1134del	Frame_Shift_Del	0.40833333
	18481T	Yes	RET	c.169C>T	Missense_Mutation	0.343023256
	18481T	Yes	ADK	c.731C>T	Missense_Mutation	0.152777778
	18481T	Yes	KAT6B	c.4121G>A	Missense_Mutation	0.37628866
2170	18481T	Yes	TCF7L2	c.1485del	Frame_Shift_Del	0.452830189
	18481T	Yes	FGFR2	c.181C>T	Missense_Mutation	0.368512111
	18481T	Yes	DRD4	c.763del	Frame_Shift_Del	0.344827586
	18481T	Yes	FAT3	c.971A>G	Missense_Mutation	0.158798283
	18481T	Yes	KMT2A	c.5338del	Frame_Shift_Del	0.179824561
2175	18481T	Yes	CHD4	c.2402T>C	Missense_Mutation	0.338461538
	18481T	Yes	KMT2D	c.9417del	Frame_Shift_Del	0.401574803
	18481T	Yes	KMT2D	c.7061del	Frame_Shift_Del	0.25
	18481T	Yes	KMT2D	c.1300del	Frame_Shift_Del	0.227272727
	18481T	Yes	ERBB3	c.2033G>A	Missense_Mutation	0.332129964
2180	18481T	Yes	POLE	c.1309G>A	Missense_Mutation	0.370481928
	18481T	Yes	POLE	c.1280C>T	Missense_Mutation	0.341397849
	18481T	Yes	BRCA2	c.2957del	Frame_Shift_Del	0.33611691
	18481T	Yes	RB1	c.971T>A	Missense_Mutation	0.33333333
	18481T	Yes	PCDH8	c.1975C>T	Missense_Mutation	0.36
2185	18481T	Yes	PCDH8	c.1432C>T	Missense_Mutation	0.413043478
	18481T	Yes	PCDH8	c.1397C>T	Missense_Mutation	0.295454545
	18481T	Yes	PCDH8	c.277G>A	Missense_Mutation	0.371273713
	18481T	Yes	PCDH17	c.1898A>G	Missense_Mutation	0.394160584
	18481T	Yes	PCDH17	c.2714A>T	Missense_Mutation	0.364
2190	18481T	Yes	DICER1	c.4458del	Frame_Shift_Del	0.368088468
	18481T	Yes	DICER1	c.1859A>G	Missense_Mutation	0.123214286
	18481T	Yes	AXIN1	c.1565C>T	Missense_Mutation	0.28

	18481T	Yes	PIGQ	c.1679G>A	Missense_Mutation	0.142857143
	18481T	Yes	TSC2	c.1723C>A	Missense_Mutation	0.333333333
2195	18481T	Yes	PLEKHG4	c.1574del	Frame_Shift_Del	0.214285714
	18481T	Yes	PLEKHG4	c.1575C>G	Missense_Mutation	0.214285714
	18481T	Yes	C16orf86	c.461A>G	Missense_Mutation	0.191780822
	18481T	Yes	CD68	c.84del	Frame_Shift_Del	0.368181818
	18481T	Yes	TP53	c.998G>A	Missense_Mutation	0.355102041
2200	18481T	Yes	NF1	c.8158A>G	Missense_Mutation	0.310344828
	18481T	Yes	ERBB2	c.2516T>C	Missense_Mutation	0.169934641
	18481T	Yes	KEAP1	c.1639G>A	Missense_Mutation	0.35978836
	18481T	Yes	DAND5	c.37C>T	Missense_Mutation	0.222222222
	18481T	Yes	FCHO1	c.2354T>C	Missense_Mutation	0.436170213
2205	18481T	Yes	JAK3	c.911A>T	Missense_Mutation	0.48
	18481T	Yes	PIK3R2	c.862C>T	Missense_Mutation	0.150943396
	18481T	Yes	KMT2B	c.1833G>A	Nonsense_Mutation	0.443820225
	18481T	Yes	ASXL1	c.2993C>T	Missense_Mutation	0.34765625
	18481T	Yes	SRC	c.659G>A	Missense_Mutation	0.356321839
2210	18481T	Yes	SRC	c.800G>A	Missense_Mutation	0.473684211
	18481T	Yes	SMC1A	c.2701G>A	Missense_Mutation	0.373608903
	18481T	Yes	SMC1A	c.1732-1G>T	Splice_Site	0.363914373
	18481T	Yes	SMC1A	c.401T>C	Missense_Mutation	0.320486815
	18481T	Yes	AR	c.2086G>A	Missense_Mutation	0.349887133
2215	18481T	Yes	STAG2	c.3584G>A	Missense_Mutation	0.323943662
	17627T	Yes	APC	c.3733A>T	Nonsense_Mutation	0.395480226
	17627T	Yes	ATM	c.6679C>T	Missense_Mutation	0.310559006
	17627T	Yes	SETBP1	c.4511C>T	Missense_Mutation	0.363636364
	17627T	Yes	SMAD2	c.1272_1279dup	Frame_Shift_Ins	0.643410853
2220	17627T	Yes	NXNL1	c.376C>G	Missense_Mutation	0.142857143

	18946T	No	NRAS	c.34G>T	Missense_Mutation	0.726744186
	18946T	No	APC	c.1495C>T	Nonsense_Mutation	0.441201717
	18946T	No	APC	c.4339del	Frame_Shift_Del	0.459482038
	18946T	No	SMC3	c.212C>T	Missense_Mutation	0.41686747
2225	18946T	No	ATM	c.2084T>C	Missense_Mutation	0.45041816
	18946T	No	ATM	c.9022C>T	Missense_Mutation	0.443911793
	18946T	No	CHD4	c.4216C>T	Missense_Mutation	0.419981499
	18946T	No	FLT3	c.106G>T	Missense_Mutation	0.620921802
	18946T	No	ERBB2	c.2033G>A	Missense_Mutation	0.550724638
2230	19395T	Yes	PIK3CA	c.323G>A	Missense_Mutation	0.376528117
	19395T	Yes	PIK3CA	c.1634A>C	Missense_Mutation	0.251655629
	19395T	Yes	APC	c.2336dup	Frame_Shift_Ins	0.39544808
	19395T	Yes	APC	c.4459_4460insT	Frame_Shift_Ins	0.393899204
	19395T	Yes	APC	c.4460C>A	Missense_Mutation	0.394946809
2235	19395T	Yes	BRAF	c.1799T>A	Missense_Mutation	0.269349845
	19395T	Yes	FAT3	c.2762C>A	Missense_Mutation	0.3671875
	19395T	Yes	FAT3	c.4718C>T	Missense_Mutation	0.352380952
	19395T	Yes	KMT2D	c.2021C>T	Missense_Mutation	0.307692308
	19395T	Yes	AGRP	c.262G>A	Missense_Mutation	0.486631016
2240	19395T	Yes	TP53	c.743G>A	Missense_Mutation	0.6125
	19395T	Yes	SOX9	c.736C>T	Nonsense_Mutation	0.363888889
	19395T	Yes	SMAD2	c.1389C>A	Nonsense_Mutation	0.379496403
	19395T	Yes	SMAD2	c.904T>C	Missense_Mutation	0.380514706
	19395T	Yes	KMT2B	c.2732C>T	Missense_Mutation	0.41509434
2245	19442T	Yes	PRDM2	c.2933C>T	Missense_Mutation	0.121718377
	19442T	Yes	PRDM2	c.4184G>A	Missense_Mutation	0.170694864
	19442T	Yes	ARID1A	c.2402dup	Frame_Shift_Ins	0.102857143
	19442T	Yes	IGFN1	c.10258G>A	Missense_Mutation	0.175

	19442T	Yes	FH	c.535G>A	Missense_Mutation	0.176870748
2250	19442T	Yes	ALK	c.474dup	Frame_Shift_Ins	0.190082645
	19442T	Yes	ALS2	c.4136_4138del	In_Frame_Del	0.178907721
	19442T	Yes	ERBB4	c.3817C>T	Missense_Mutation	0.151821862
	19442T	Yes	BAP1	c.1628G>A	Missense_Mutation	0.154185022
	19442T	Yes	PBRM1	c.1601G>A	Missense_Mutation	0.209191759
2255	19442T	Yes	PIK3CA	c.331A>G	Missense_Mutation	0.180987203
	19442T	Yes	FGFR3	c.1075C>T	Nonsense_Mutation	0.144230769
	19442T	Yes	TET2	c.4317dup	Frame_Shift_Ins	0.193014706
	19442T	Yes	TET2	c.5597C>T	Missense_Mutation	0.196048632
	19442T	Yes	FAT1	c.9857C>G	Missense_Mutation	0.173780488
2260	19442T	Yes	FAT1	c.4184G>A	Missense_Mutation	0.121510673
	19442T	Yes	FAT1	c.2833G>A	Missense_Mutation	0.201787995
	19442T	Yes	APC	c.4666dup	Frame_Shift_Ins	0.210526316
	19442T	Yes	KMT2C	c.13940dup	Frame_Shift_Ins	0.166276347
	19442T	Yes	ACTR3B	c.829C>A	Missense_Mutation	0.197740113
2265	19442T	Yes	FGFR1	c.1588G>A	Missense_Mutation	0.177446103
	19442T	Yes	KAT6A	c.76C>T	Missense_Mutation	0.180327869
	19442T	Yes	SMARCA2	c.4354dup	Frame_Shift_Ins	0.243589744
	19442T	Yes	FAM166B	c.470C>T	Missense_Mutation	0.5625
	19442T	Yes	PTCH1	c.3793G>A	Missense_Mutation	0.155555556
2270	19442T	Yes	PTCH1	c.3622G>A	Missense_Mutation	0.185714286
	19442T	Yes	TSC1	c.1257del	Frame_Shift_Del	0.217687075
	19442T	Yes	NOTCH1	c.4828G>A	Missense_Mutation	0.203389831
	19442T	Yes	KAT6B	c.4523del	Frame_Shift_Del	0.195439739
	19442T	Yes	OR2D3	c.234dup	Frame_Shift_Ins	0.19524618
2275	19442T	Yes	NRXN2	c.2011C>T	Missense_Mutation	0.223300971
	19442T	Yes	EHD1	c.490C>T	Missense_Mutation	0.18694362

2280	19442T	Yes	FAT3	c.3998A>G	Missense_Mutation	0.244705882
	19442T	Yes	FAT3	c.6679C>T	Missense_Mutation	0.12424608
	19442T	Yes	KMT2D	c.7109G>A	Missense_Mutation	0.216216216
	19442T	Yes	KMT2D	c.7061del	Frame_Shift_Del	0.351351351
	19442T	Yes	KMT2D	c.4841G>A	Missense_Mutation	0.174563591
	19442T	Yes	KMT2D	c.2993del	Frame_Shift_Del	0.170731707
	19442T	Yes	NCOR2	c.6923C>T	Missense_Mutation	0.141304348
2285	19442T	Yes	RB1	c.1478T>A	Missense_Mutation	0.149797571
	19442T	Yes	PCDH17	c.2579C>T	Missense_Mutation	0.202925046
	19442T	Yes	AKT1	c.745C>T	Missense_Mutation	0.285714286
	19442T	Yes	BLM	c.2268del	Frame_Shift_Del	0.213296399
	19442T	Yes	AXIN1	c.1523del	Frame_Shift_Del	0.407407407
	19442T	Yes	CREBBP	c.2469G>T	Missense_Mutation	0.111111111
	19442T	Yes	FANCA	c.623C>T	Missense_Mutation	0.18018018
2290	19442T	Yes	MAP2K2	c.329C>T	Missense_Mutation	0.209923664
	19442T	Yes	SMARCA4	c.2714G>A	Missense_Mutation	0.166240409
	19442T	Yes	SMARCA4	c.4336C>G	Missense_Mutation	0.156593407
	19442T	Yes	OR7A17	c.113C>T	Missense_Mutation	0.156097561
	19442T	Yes	SLC27A1	c.1259G>A	Missense_Mutation	0.183431953
	19442T	Yes	PIK3R2	c.107C>T	Missense_Mutation	0.2
	19442T	Yes	SRC	c.1259C>T	Missense_Mutation	0.174496644
2300	19442T	Yes	RUNX1	c.1108G>T	Missense_Mutation	0.193548387
	19442T	Yes	EP300	c.5375A>C	Missense_Mutation	0.177570093
	19442T	Yes	SMC1A	c.2695G>A	Missense_Mutation	0.203262233
	19442T	Yes	AMER1	c.3009A>G	Missense_Mutation	0.197101449
	19442T	Yes	ATRX	c.3682C>T	Missense_Mutation	0.169724771
	19442T	Yes	PLXNB3	c.1970G>A	Missense_Mutation	0.11627907
	17787T	No	TP53	c.701A>G	Missense_Mutation	0.618421053

2305	17787T	No	KEAP1	c.1133G>A	Missense_Mutation	0.663793103
	18081T	Yes	APC	c.646C>T	Nonsense_Mutation	0.635416667
	18081T	Yes	PDE6A	c.2302G>A	Missense_Mutation	0.333333333
	18081T	Yes	POLE	c.5573G>A	Missense_Mutation	0.381294964
	19106T	Yes	PRDM16	c.1898C>T	Missense_Mutation	0.951612903
2310	19106T	Yes	PRDM16	c.3369_3371dup	In_Frame_Ins	0.92
	19106T	Yes	IDH1	c.532G>A	Missense_Mutation	0.302449414
	19106T	Yes	MLH1	c.655A>G	Missense_Mutation	0.372986369
	19106T	Yes	TACC3	c.427G>A	Missense_Mutation	0.358422939
	19106T	Yes	UGT2B7	c.801_802delinsTC	Missense_Mutation	0.319034853
2315	19106T	Yes	FAT1	c.8535G>A	Missense_Mutation	0.355665025
	19106T	Yes	PNPLA1	c.1268C>A	Missense_Mutation	0.914285714
	19106T	Yes	TSC1	c.1079C>A	Missense_Mutation	0.337777778
	19106T	Yes	ATE1	c.499C>T	Missense_Mutation	0.378723404
	19106T	Yes	TACC2	c.2488C>T	Missense_Mutation	0.363043478
2320	19106T	Yes	FAT3	c.8983C>A	Missense_Mutation	0.290087464
	19106T	Yes	TECTA	c.1111A>G	Missense_Mutation	0.33492823
	19106T	Yes	TP53BP1	c.1074C>G	Missense_Mutation	0.949874687
	19106T	Yes	MPDU1	c.685G>A	Missense_Mutation	0.969465649
	19106T	Yes	TP53	c.326dup	Frame_Shift_Ins	0.910714286
2325	19282T	No	SMO	c.2081del	Frame_Shift_Del	0.117021277
	18607T	Yes	ADCY3	c.2387G>A	Missense_Mutation	0.167630058
	18607T	Yes	MSH6	c.2079del	Frame_Shift_Del	0.16286645
	18607T	Yes	CASP8	c.1596del	Frame_Shift_Del	0.121794872
	18607T	Yes	PIK3CA	c.3140A>G	Missense_Mutation	0.159292035
2330	18607T	Yes	TET2	c.3157C>T	Nonsense_Mutation	0.166666667
	18607T	Yes	FAT1	c.13717G>A	Missense_Mutation	0.153439153
	18607T	Yes	EGFR	c.1282G>A	Missense_Mutation	0.174208145

	18607T	Yes	PVRIG	c.892del	Frame_Shift_Del	0.115942029
	18607T	Yes	BRAF	c.1799T>A	Missense_Mutation	0.165254237
2335	18607T	Yes	WT1	c.496G>A	Missense_Mutation	0.142857143
	18607T	Yes	CCDC73	c.1341del	Frame_Shift_Del	0.363636364
	18607T	Yes	KMT2D	c.1940del	Frame_Shift_Del	0.24
	18607T	Yes	BRCA2	c.10248del	Frame_Shift_Del	0.136842105
	18607T	Yes	C14orf79	c.83C>A	Missense_Mutation	0.171929825
2340	18607T	Yes	IDH2	c.435del	Frame_Shift_Del	0.184466019
	18607T	Yes	SPNS1	c.1133G>A	Missense_Mutation	0.217391304
	18607T	Yes	TRADD	c.286C>T	Missense_Mutation	0.285714286
	18607T	Yes	ERBB2	c.2446C>T	Missense_Mutation	0.154589372
	18607T	Yes	ERBB2	c.3235G>A	Missense_Mutation	0.159663866
2345	18607T	Yes	SMARCE1	c.903G>T	Missense_Mutation	0.157258065
	18607T	Yes	SOX9	c.984_1036del	Frame_Shift_Del	0.121428571
	18607T	Yes	RGL3	c.925G>A	Missense_Mutation	0.141025641
	18607T	Yes	CHEK2	c.1081C>T	Missense_Mutation	0.158798283
	18810T	Yes	PIK3CA	c.1633G>A	Missense_Mutation	0.406779661
2350	18810T	Yes	FAT1	c.7699C>T	Missense_Mutation	0.437694704
	18810T	Yes	APC	c.1958+2T>G	Splice_Site	0.42019544
	18810T	Yes	APC	c.4464_4467del	Frame_Shift_Del	0.382075472
	18810T	Yes	KRAS	c.38G>A	Missense_Mutation	0.426273458
	18810T	Yes	FZD10	c.1127C>T	Missense_Mutation	0.446327684
2355	18810T	Yes	SMAD2	c.961C>T	Nonsense_Mutation	0.112676056
	18810T	Yes	SMAD4	c.1065C>A	Missense_Mutation	0.535947712
	18739T	Yes	PIK3CA	c.1624G>A	Missense_Mutation	0.290187891
	18739T	Yes	FBXW7	c.941del	Frame_Shift_Del	0.367454068
	18739T	Yes	APC	c.4166_4167ins	Frame_Shift_Ins	0.430107527
2360	18739T	Yes	KRAS	c.35G>A	Missense_Mutation	0.486166008

	18739T	Yes	TP53	c.743G>A	Missense_Mutation	0.696296296
	19441T	Yes	APC	c.2711_2712del	Frame_Shift_Del	0.392703863
	19441T	Yes	NOTCH1	c.4864C>T	Missense_Mutation	0.465116279
	19441T	Yes	TP53	c.880G>T	Nonsense_Mutation	0.361313869
2365	19441T	Yes	FAM129C	c.1967G>A	Missense_Mutation	0.380614657
	19342T	Yes	SMARCA2	c.2363G>A	Nonsense_Mutation	0.339756592
	19342T	Yes	TP53	c.853G>T	Nonsense_Mutation	0.611515553
	19342T	Yes	TP53	c.659A>G	Missense_Mutation	0.231943629
	19342T	Yes	SUZ12	c.1105T>C	Missense_Mutation	0.601747815
2370	19342T	Yes	SMAD4	c.1594_1596del	In_Frame_Del	0.573435504

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Supplementary Table 6: Juxtaposition of primary CRC cases with patient demographics and *PDZK1IP1* SE status.

Samples Has PDZK1IP1 super-enhancer? Sex Age Colon Anatomy

2390	18481T	Yes	no data		
	17753T	Yes	Male	63	Sigmoid colon and rectum
	18607T	Yes	Male	71	Terminal ileum appendix and ascending colon
	18810T	Yes	Male	67	Terminal ileum and cecum
	19441T	Yes	Male	71	Descending colon
2395	19282T	No	Female	82	Terminal ileum and ascending colon
	19106T	Yes	Male	74	Sigmoid colon and rectum
	19342T	Yes	Male	53	Transverse colon
	18739T	Yes	Male	63	Sigmoid colon
	18946T	No	Male	83	Descending colon
2400	18081T	Yes	Male	62	Sigmoid colon and rectum
	19442T	Yes	Female	61	Terminal ileum appendix and ascending colon
	19395T	Yes	Male	61	Terminal ileum, cecum, appendix, and ascending colon
	17627T	Yes	Male	64	Sigmoid colon
	17787T	No	Male	67	Descending and sigmoid colon

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Supplementary Table 7: Juxtaposition of primary CRC cases with pathological features and *PDZK1IP1* SE status.

Samples	Has PDZK1IP1 super-enhancer?	Tumor Staging	Primary Tumor Description
18481T	Yes	No data	
17753T	Yes	pT4aN2a	Invasive, moderately differentiated adenocarcinoma with mucinous features. The tumor invades through the wall to the serosal surface with acute inflammatory reaction. Lymphovascular and perineural invasion identified. Extramural large vessel invasion identified.
18607T	Yes	pT3N1a	Invasive poorly differentiated adenocarcinoma with medullary feature. Invades into subserosal soft tissue. Perineural invasion also present. Extensive lymphatic invasion seen. IHC shows MLH1 and PMS2 loss, possible MSI.
18810T	Yes	pT1N0	Invasive moderately differentiated adenocarcinoma. The tumor invades the submucosa. Foci suspicious for lymphovascular invasion are identified. Perineural invasion is not identified.
19441T	Yes	pT4aN0	Invasive moderately differentiated adenocarcinoma extending through the serosal surface. Perineural and lymphovascular space invasion are present. Peritumoral abscess with foreign body giant cell reaction. Large extramural vein invasion.
19282T	No	no data	Poorly differentiated adenocarcinoma. No adenomatous component is found to confirm primary colonic origin. The tumor is almost entirely intralymphatic and there is focal intramural venous invasion. The tumor is CDX2 negative which is uncommon for primary CRC but not exclusionary. Additional immunostains are negative for primary breast, GYN, or pulmonary origin.
19106T	Yes	pT3N1a	Invasive moderately differentiated adenocarcinoma with invasion to the pericolorectal adipose tissue. Lymphovascular and neural

invasions present.

19342T Yes pT2N0 Moderately differentiated mucinous adenocarcinoma with invasion into the muscularis propria. India ink tattoo noted.

2445 18739T Yes pT4aN0 Moderately differentiated adenocarcinoma with transmural invasion to the visceral peritoneum. Focal high-grade tumor budding and extramural large vein invasion are identified.

18946T No pT4N0 Invasive, moderately differentiated adenocarcinoma extending to the serosal surface. Submucosal ink tattoo. Lymphovascular

2450 or perineural space invasion is not identified. Exuberant fibrohistiocytic reaction of the subserosal soft tissue.

18081T Yes pT3N0 Invasive moderately differentiated adenocarcinoma. The tumor invades the muscularis propria and into subserosal soft tissue. Tumor budding.

2455 19442T Yes pT4aN0 Invasive moderately differentiated mucinous adenocarcinoma. Extended through the serosal surface into the visceral peritoneum with associated fibroinflammatory response. Lymphovascular or perineural space invasion is not identified. No evidence of large extramural vein invasion.

2460 19395T Yes Moderately differentiated mucinous adenocarcinoma. The tumor invades through muscularis propria into pericolic fat. Vascular invasion is not identified. Perineural invasion is not identified.

17627T Yes no data No data

2465 17787T No pT4bN2b Moderate to poorly differentiated carcinoma with mixed morphology. Extensive lymphovascular and perineural invasion are noted. Extramural large vessel invasion is identified. Background colon with mildly active chronic colitis and melanosis coli. Long history with quiescent ulcerative colitis.

2470 **Supplementary Table 8: H3K27ac ChIP-seq metrics.**

17627T: 74,355 called peaks stitched to 17,726 enhancers.

17627N: 75,466 called peaks stitched to 21,636 enhancers.

17753T: 59,688 called peaks stitched to 13,726 enhancers.

17753N: 175,589 called peaks stitched to 5,392 enhancers.

2475 17787T: 76,011 called peaks stitched to 20,341 enhancers.

17787N: 66,728 called peaks stitched to 17,567 enhancers.

18081T: 54,343 called peaks stitched to 15,075 enhancers.

18081N: 52,082 called peaks stitched to 14,110 enhancers.

18481T: 29,640 called peaks stitched to 7,921 enhancers.

2480 18481N: 75,102 called peaks stitched to 24,268 enhancers.

18607T: 47,801 called peaks stitched to 24,704 enhancers.

18607N: 80,274 called peaks stitched to 19,847 enhancers.

18739T: 56,757 called peaks stitched to 15,073 enhancers.

18739N: 34,545 called peaks stitched to 5,692 enhancers.

2485 18946T: 51,652 called peaks stitched to 19,214 enhancers.

18946N: 27,010 called peaks stitched to 15,655 enhancers.

18810T: 72,790 called peaks stitched to 13,804 enhancers.

18810N: 57,932 called peaks stitched to 6,217 enhancers.

19106T: 58,973 called peaks stitched to 16,857 enhancers.

2490 19106N: 61,476 called peaks stitched to 15,331 enhancers.

19282T: 63,909 called peaks stitched to 17,020 enhancers.

19282N: 59,106 called peaks stitched to 15,434 enhancers.

19342T: 53,155 called peaks stitched to 14,616 enhancers.

19342N: 52,206 called peaks stitched to 13,616 enhancers.

2495 19395T: 63,693 called peaks stitched to 18,157 enhancers.

19395N: 47,387 called peaks to 13,483 enhancers.

19441T: 70,089 called peaks stitched to 18,884 enhancers.

19441N: 63,324 called peaks stitched to 16,074 enhancers.

19442T: 62,613 called peaks stitched to 16,760 enhancers.

2500 19442N: 77,621 called peaks stitched to 19,239 enhancers.

HT29 cells: 43,055 called peaks stitched to 10,784 enhancers.

HT29 xenograft tumor rep. 1: 47,940 called peaks stitched to 13,097 enhancers.

HT29 xenograft tumor rep. 2: 61,713 called peaks stitched to 18,869 enhancers.

HT29 xenograft tumors rep. 3: 27,347 called peaks stitched to 7,049 enhancers.

2505 HT29 TNF-alpha, IFN-gamma, and IL-6 treated cells: 60,741 called peaks stitched to 15,139 enhancers.

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