Cell Metabolism
Short Article

Obesity, Rather Than Diet, Drives Epigenomic Alterations in Colonic Epithelium Resembling Cancer Progression

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SUMMARY

While obesity represents one of several risk factors for colorectal cancer in humans, the mechanistic underpinnings of this association remain unresolved. Environmental stimuli, including diet, can alter the epigenetic landscape of DNA *cis*-regulatory elements affecting gene expression and phenotype. Here, we explored the impact of diet and obesity on gene expression and the enhancer landscape in murine colonic epithelium. Obesity led to the accumulation of histone modifications associated with active enhancers at genomic loci downstream of signaling pathways integral to the initiation and progression of colon cancer. Meanwhile, colon-specific enhancers lost the same histone mark, poising cells for loss of differentiation. These alterations reflect a transcriptional program with many features shared with the program driving colon cancer progression. The interrogation of enhancer alterations by diet in colonic epithelium provides insights into the biology underlying high-fat diet and obesity as risk factors for colon cancer.

INTRODUCTION

Colorectal cancer (CRC) is the third most common human cancer worldwide, with more than 1 million new cases every year (Ferlay et al., 2010). Above normal body weight and obesity are among the principal risk factors for development of colorectal cancer in human populations as well as in animal models (Larsson and Wolk, 2006; Moghaddam et al., 2007; Reddy et al., 1977; Wasan et al., 1997). In humans, the relationship between obesity and colorectal cancer incidence appears to be modified by gender. In men, incidence of colorectal cancer is increased in the obese; in women, this association is weaker (Bardou et al., 2013). While several hypotheses rationalize the link between obesity and colorectal cancer, it seems likely that contributing factors are complex.

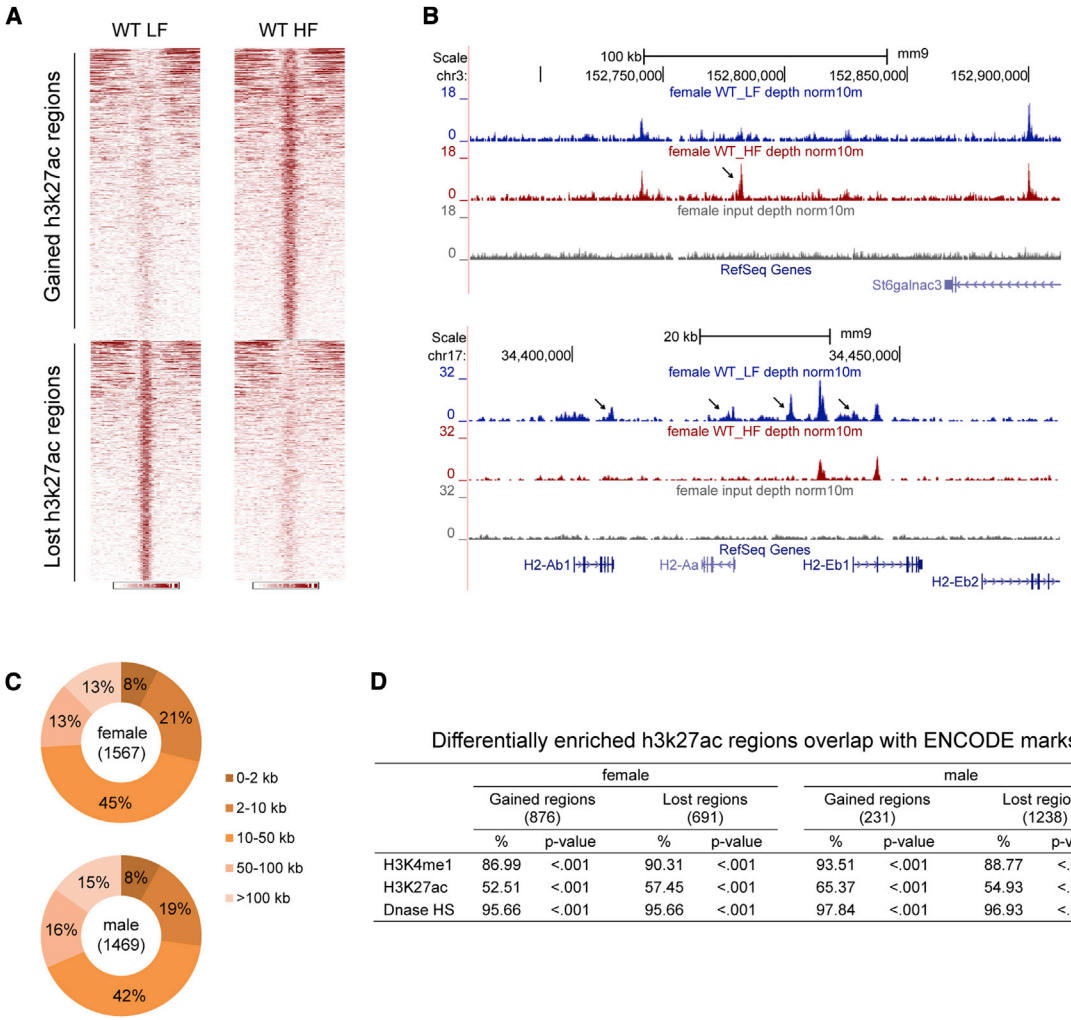
To explore the effects of Western diet, weight gain, and obesity on the epigenomic landscape of colonic epithelial cells, we employed a mouse model of diet-induced obesity. C57BL/6 mice were fed a diet wherein either 10% of calories (LF) or 60% of calories (HF) come from fat. To globally map alterations of the epigenomic landscape induced by high-fat diet and obesity, we performed chromatin immunoprecipitation sequencing (ChIP-seq) analysis of histone H3 lysine 27 acetylation (H3K27ac). Our study revealed striking alterations in enhancer utilization, consistent with a dietary predisposition to cancer.

RESULTS

Acetylation of H3K27 as an Indicator of Chromatin Landscape Changes

To assess the impact of diet and gender on the epigenome in colonic epithelia, male and female C57BL/6 mice were raised on low-fat (10% of calories from fat) and high-fat (60% of calories from fat) diets for periods of 15–20 weeks. Colonic epithelial cells were harvested using a well-established protocol (Roediger and Truelove, 1979) to generate RNA and chromatin. Transcript abundance was determined by conventional microarray; chromatin was evaluated by ChIP for H3K27ac. Analyses were performed on biological replicate animals (two replicates per dietary regimen for females, three replicates per dietary regimen for males).

Local enrichment of H3K27ac was determined using standard peak calling; this analysis revealed ~40,000 significant H3K27ac peaks in each diet group at a false discovery rate of less than 0.001. H3K27ac peaks showed a significant overlap (~75%–91% of peaks) across biological replicates regardless of gender (Table S1A available online). The distribution of H3K27ac relative to annotated genes was similar among groups, regardless of diet or gender, with peaks mapping to intragenic (~43%), intergenic (30%), and promoter regions (±1 kb around the transcription start site [TSS], ~24%) (Table S1B). Chromatin-state models predict a correlation between H3K27ac proximal to the transcription start site and gene expression (Ernst et al., 2011). We determined the relationship between transcript abundance and H3K27ac level in a 2 kb window centered on the transcription



Differentially enriched h3k27ac regions overlap with ENCODE marks

	female				male			
	Gained regions (876)		Lost regions (691)		Gained regions (231)		Lost regions (1238)	
	%	p-value	%	p-value	%	p-value	%	p-value
H3K4me1	86.99	<.001	90.31	<.001	93.51	<.001	88.77	<.001
H3K27ac	52.51	<.001	57.45	<.001	65.37	<.001	54.93	<.001
Dnase HS	95.66	<.001	95.66	<.001	97.84	<.001	96.93	<.001

Figure 1. High-Fat Diet Changes the Enhancer Landscape in Colonic Epithelium

(A) The heatmap depicts H3K27ac levels at differentially enriched H3K27ac regions (female mice) extending 5 kb from the midpoint of the regions. The images were generated using Partek Genomics Suite v.6.11.0321 based on normalized counts of H3K27ac ChIP-seq fragment using 250 bp bins. Read counts were normalized by millions of uniquely mapped, nonduplicate reads, and fragment centers were estimated to be 100 nt downstream of the 5′ mapping location.

(B) UCSC genome browser views illustrating representative examples of gained H3K27ac region (top panel) and lost H3K27ac region (bottom panel) in wild-type mice.

(C) Gained and lost H3K27ac regions were mapped to the closest RefSeq gene TSS, and the distribution of distance relative to the nearest TSS is shown.

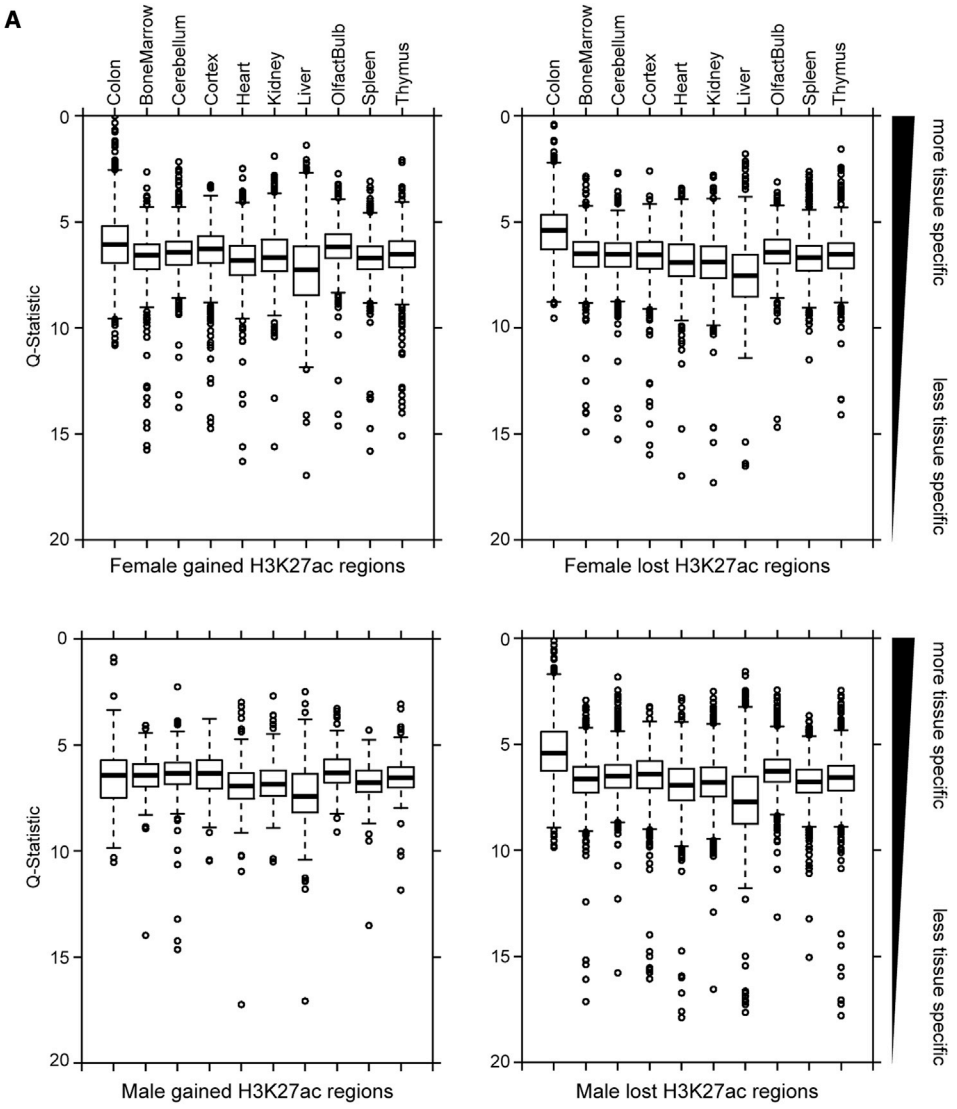
(D) Percent of gained and lost H3K27ac regions overlapping with each ENCODE enhancer mark. See also Figure S1 and Table S1.

start site (Table S1C), observing robust correlation. We concluded that the H3K27ac ChIP-seq data were of sufficient quality to support detailed analysis.

High-Fat Diet Induces Changes in the Enhancer Landscape of Colonic Epithelium

A statistical method (http://www.bioconductor.org/packages/release/bioc/html/DiffBind.html), which detects differential binding sites consistently changed in biological replicates, comparing groups identified 876 regions that gained acetylation at H3K27 and 691 regions that lost this mark in females as a function of diet (Figure 1A); 231 regions gained H3K27ac, and 1,238 regions lost H3K27ac in males (Figure S1A). A subset of these differentially acetylated regions was validated by ChIP-qPCR (Figure S1C). Exemplar loci for each category were depicted in Figure 1B. Differentially enriched H3K27ac regions predominantly localized outside known promoters (>2 kb away from the nearest TSS) (Figure 1C), at regions that are moderately conserved across placental mammals (Figure S1B) and overlap ENCODE marks (H3K4me1, H3K27ac, and DNase HS) (Figure 1D), suggesting that they function as enhancers. We tested this prediction using luciferase reporter assays, finding detectable enhancer activity in 10 of 11 regions tested (Figure S1D).

Direct overlap of differentially acetylated loci was very infrequent across gender, occurring less than 5% of the time. As individual promoters are frequently regulated by multiple enhancers (Thurman et al., 2012), we wondered whether gender



B

Female differential H3K27ac regions

Motif	Best match	p-value	% of target
	ETS	1e-20	31.14
	HNF4a	1e-20	11.23
	Cdx2	1e-18	21.25
	?	1e-15	8.04
	Scl	1e-14	12.95
	AP1	1e-14	13.85
	?	1e-13	19.08
	Klf4	1e-13	12.13
	?	1e-13	13.53
	Foxo1	1e-12	31.65

Male differntial H3K27ac regions

Motif	Best match	p-value	% of target
	Cdx2	1e-24	40.84
	ETS	1e-20	14.57
	Klf4	1e-17	26.48
	Foxo1	1e-16	14.98
	?	1e-16	18.38
	HNF4a	1e-16	29.34
	?	1e-15	2.59
	Foxa1	1e-14	10.48
	AP1	1e-13	16.95

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differences seen here might reflect epigenomic alterations impacting the same promoter by influencing different enhancers. We tested this hypothesis by asking whether differentially acetylated loci were colocalized in genomic space, consistent with the distances between promoter and enhancer defined by current models (Dixon et al., 2012; Sanyal et al., 2012). A statistically significant majority of regions either gaining acetylation in males (126 of 231, $p < 0.0001$, Fisher's exact test) or losing acetylation in females (376 of 691, $p = 0.0015$, Fisher's exact test) was localized within a genomic interval consistent with this hypothesis (Table S1D).

Diet Reprograms the Enhancer Landscape to Resemble Colon Cancer

A recent report describing the enhancer landscape of human colon cancer concluded that *cis*-acting regulatory DNA with decreased levels of histone marks prototypical of enhancers in tumor versus normal tissue were enriched in loci with specific functions in intestinal crypts. Conversely, loci with elevated levels of enhancer marks in the same comparison were not enriched in any tissue-specific pattern (Akhtar-Zaidi et al., 2012), supporting the conclusion that the tumor enhancer landscape reflects a loss of differentiated status. Given the association of obesity with elevated risk of colorectal cancer, we hypothesized that dietary fat and/or obesity might remodel the enhancer landscape of colonic epithelium to resemble the cancer enhancer profile. To test this hypothesis, we assessed the tissue specificity of differentially acetylated (H3K27ac) regions using a function of Shannon entropy (Schug et al., 2005), as employed previously (Akhtar-Zaidi et al., 2012), to rank these regions in order of their specificity in colon and nine other tissue types. Loci losing H3K27ac were enriched in colon-specific acetylated loci, whereas regions gaining H3K27ac were noncolon specific regardless of gender (Figure 2A). These observations suggest that high-fat diet and obesity prompt colonic epithelial cells to remodel their enhancer landscape in a manner analogous to the remodeling of the enhancer signature associated with colorectal cancer.

To decipher the mechanism underlying enhancer reprogramming, we used the HOMER de novo motif algorithm (Heinz et al., 2010) to discover enriched transcription factor motifs at differentially acetylated regions. The most significantly enriched motifs (Figure 2B) exhibited striking concordance across gender, suggesting mechanistic similarities in the biological response to diet. Differential H3K27ac loci were enriched for binding sites of transcription factors downstream of two pathways commonly disrupted in colon cancer (Cancer Genome Atlas Network, 2012)—ETS (RAS signaling pathway) and Foxo1 (phosphatidylinositol 3-kinase [PI3K] signaling pathway)—and AP1 (JNK signaling pathway), which regulates cell proliferation and differentiation in response to a variety of growth factors and cytokines (Hess et al., 2004). To assess the significance of enrichment of the RAS signaling pathway, a constitutively active KRAS (G12V) allele was introduced into a mouse colon epithelial cell line (CMT-93, wild-type for Kras), and a subset of regions that contain putative ETS binding sites were assessed by ChIP-PCR (Figure S2A). The resulting acetylation changes at these loci closely parallel those observed in the ChIP-seq experiment, suggesting that KRAS activation, presumably acting through an ETS family transcription factor(s), could be responsible for a subset of the H3K27ac alterations.

Binding sites of transcription factors essential for colon development and differentiation were also enriched, including Cdx2, Hnf4a, and Klf4 (Cattin et al., 2009; Gao et al., 2009; Garrison et al., 2006; Ghaleb et al., 2011; Hryniuk et al., 2012; Katz et al., 2002; Verzi et al., 2010). To interrogate the significance of this finding, we compared our data to published ChIP-seq data for Cdx2 and Hnf4a in small intestine (Verzi et al., 2011, 2013). Hnf4a peaks colocalized with diet-specific differentially acetylated loci containing putative Hnf4a binding motif at high frequency in females (81% of expected) and a reasonable frequency in males (39% of expected). Cdx2 peaks in small intestine overlapped with diet-specific differentially acetylated loci in colon containing Cdx2 motif with lower frequency in males (Table S2A). These findings are consistent with the hypothesis that diet-specific differential acetylation may reflect alterations in the maintenance of normal colon homeostasis.

We then investigated the biological functions of the differentially enriched H3K27ac regions (Table S2) using the Genomic Regions Enrichment of Annotations Tool (GREAT) (McLean et al., 2010). Gained H3K27ac regions in females were enriched in genes involved in regulation of protein kinase activity (mitogen-activated protein [MAP] kinase and JAK), EGFR- and interleukin-6 (IL-6)-mediated signaling events, presenilin action in Notch and Wnt signaling, and pathways controlling cell adhesion and cell migration ($\alpha6\beta1$ and $\alpha6\beta4$ integrin signaling pathways) (Giancotti and Ruoslahti, 1999). Strikingly, among enriched disease ontology terms, the most enriched terms were colon cancer and diseases known to be correlated with a high-fat diet, such as type 2 diabetes and hyperlipidemia. In addition, gained H3K27ac regions were also significantly enriched with genes upregulated by activated KRAS and with genes upregulated in metastatic, compared with nonmetastatic, tumors (Table S2B). Lost H3K27ac regions in females were associated with genes integral to antigen presentation (Table S2C), suggesting that the colonic epithelial cells from mice on a high-fat diet may be impaired in this function. In addition, lost H3K27ac regions also associated with genes downregulated by activated KRAS (Table S2C).

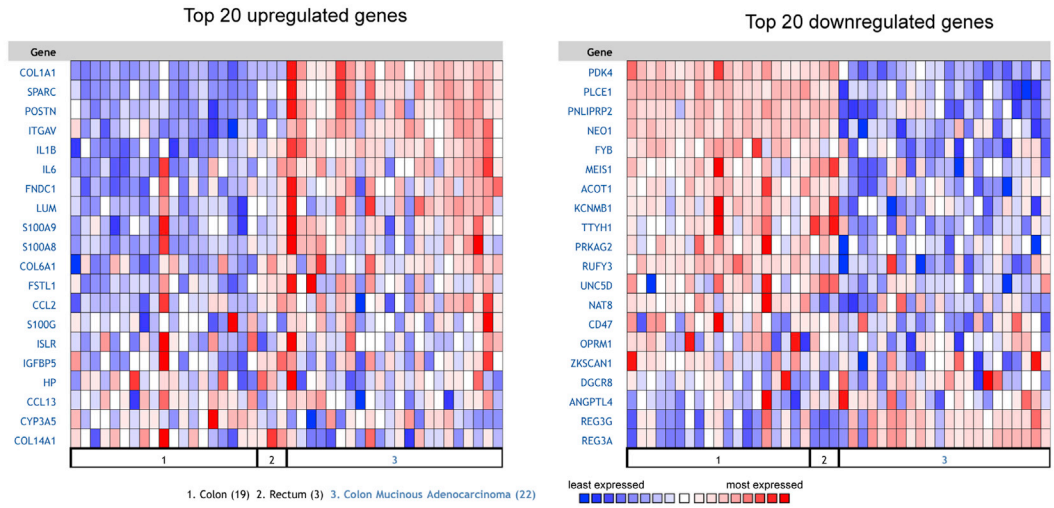
In keeping with the hypothesis that diet impacts similar genes in males and females, the GREAT analysis of differential H3K27ac regions in male mice revealed biological functions similar to those observed in females (Table S2). These included regulation of kinase activity, growth factor signaling (including

Figure 2. Diet-Induced Enhancer Alterations in Colonic Epithelium Resemble the Enhancer Signature of Colon Cancer

(A) Tissue specificity of differentially enriched H3K27ac regions. Tissue specificity scores (Q-statistic, y axis) for H3K27ac signals in ten different tissues are plotted for gained (left panels) and lost (right panels) H3K27ac regions in female mice (upper panels) and male mice (lower panels).

(B) De novo discovery of motifs associated with differential H3K27ac regions. The HOMER package (v.4.1) was used to search for de novo DNA sequence motifs. The motifs were then assigned to transcription factors or transcription factor families based on similarity with known motif matrices. In addition, the fraction of H3K27ac regions containing the motifs and the p value for the overrepresentation of each motif were shown. See also Figure S2 and Table S2.

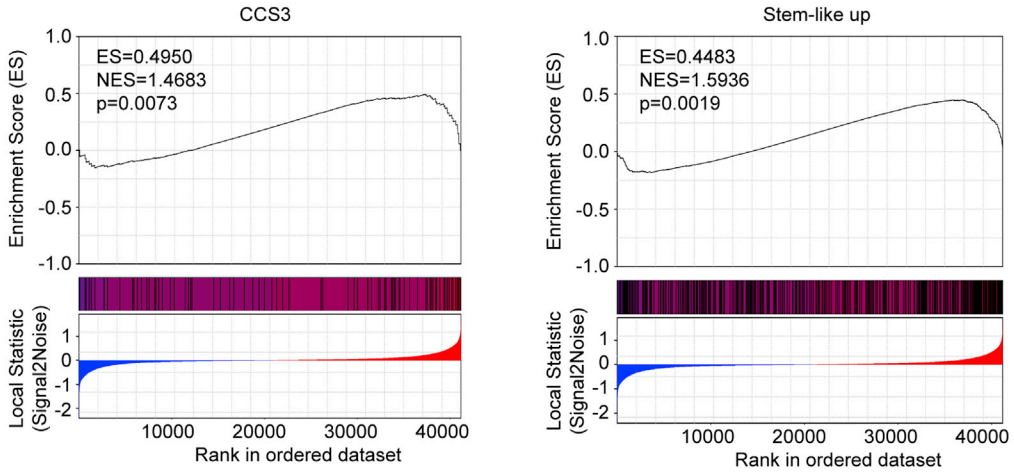
A



B

Concept Name	Overlap	P-Value	Q-Value	Odds Ratio
Mutation Analysis				
Colorectal Carcinoma Metastasis - KRAS Mutation - Top 10% Over-expressed (Khambata-Ford Colon)	39	5.46E-10	2.01E-07	3.7
Multi-cancer Cell Line - PTEN Mutation - Top 1% Over-expressed (Garnett CellLine)	6	0.002	0.084	4.8
Drug Sensitivity Analysis				
BEZ235 Sensitive - Multi-cancer Cell Line - Top 5% Over-expressed (Garnett CellLine)	24	5.00E-08	1.08E-05	4.2
Stage Type Analysis				
Rectosigmoid Adenocarcinoma - Advanced Dukes Stage - Top 5% Over-expressed (Bittner Colon)	37	2.94E-13	2.60E-10	4.9
Colon Adenocarcinoma - Advanced Dukes Stage - Top 10% Over-expressed (Jorissen Colorectal 3)	49	9.80E-11	4.29E-08	3.3
Rectosigmoid Adenocarcinoma - Advanced Stage - Top 5% Over-expressed (Bittner Colon)	33	1.42E-10	5.97E-08	4.3
Rectosigmoid Adenocarcinoma - Advanced N Stage - Top 10% Over-expressed (Bittner Colon)	43	8.86E-08	1.85E-05	2.8
Colon Villous Adenoma - Advanced N Stage - Top 10% Over-expressed (Bittner Colon)	41	6.78E-07	1.08E-04	2.6
Colon Carcinoma - Advanced Dukes Stage - Top 10% Over-expressed (Zou Colon)	11	6.77E-04	0.035	3.9
Colon Carcinoma - Advanced N Stage - Top 10% Over-expressed (Zou Colon)	11	6.77E-04	0.035	3.9
Cancer Histology Analysis				
Colorectal Cancer Type: Colorectal Carcinoma - Top 10% Over-expressed (Bittner Colon)	64	5.83E-20	3.24E-16	4.9
Colon Adenocarcinoma Type: Colon Mucinous Adenocarcinoma - Top 10% Over-expressed (TCGA Colorectal)	50	1.45E-10	6.00E-08	3.2
Colorectal Adenocarcinoma Type: Rectal Adenocarcinoma - Top 5% Over-expressed (Jorissen Colorectal 3)	28	1.38E-07	2.69E-05	3.5

C



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EGFR, PDGF, and FGFR), phosphatidylinositol signaling, and IL-6-mediated signaling events. In the disease ontology category, tumor-related terms were also enriched. These results underscore the similarities in the biological response to diet and obesity in male and female mice.

However, human studies suggest gender-specific differences in the relationship between obesity and cancer. In our genomic regions enrichment analysis, we observed several enriched pathways and terms that were unique to male mice. In males, differential H3K27ac regions were correlated with the RAC1 and RhoA signaling pathways involved in cytoskeletal reorganization, cellular adhesion, and cellular movement (Moorman et al., 1999). Stem cell-related terms were also enriched in males, including stem cell maintenance, stem cell development, and stem cell differentiation (Table S2D). Thus, gender modifies the remodeling of the enhancer landscape by diet.

Diet Induces a Transcriptional Program Enriched in Features of Cancer Progression

To assess whether the observed alterations in active enhancer profile were reflected in the transcriptional program, we performed gene expression microarray analysis. Initial analysis of the data included determination of expression value and ANOVA analysis of differentially expressed genes within gender (Tables S3A and S3B). Gene ontology and pathway analysis indicated similarities in the pattern of genes responsive to diet across gender (Tables S3C and S3D). We focused detailed analysis on the expression data obtained from females.

Consistent with the ChIP-seq, we observed that targets of the RAS, PI3K, and JNK signaling pathways were upregulated (Figure S2B), suggesting that differentially enriched H3K27ac regions are biologically functional and drive a specific transcriptional program in colonic epithelia cells. In line with the GREAT analysis, genes dysregulated by high-fat diet were connected to inflammation, cancer, cellular movement, and tissue development (Figure S4A and Tables S3C and S4A).

Since the epigenomic remodeling by diet resembled the enhancer signature of colon cancer, we asked whether the resulting gene expression profile also recapitulated gene expression profiles in colon cancer. Among the most differentially expressed genes, approximately half were also dysregulated in mouse colon tumor samples when compared with normal colon (Figure S3A). To determine whether the gene expression changes induced by high-fat diet are relevant to human colorectal cancer, we compared differentially expressed genes in our study to colorectal cancer data sets in Oncomine. We evaluated the top 20 genes most highly up- and downregulated in our study across six different tumor/normal data sets (Figure S3B); the majority were changed in the same direction as by diet when comparing cancer with normal colon (Figure 3A).

To ascertain whether the gene signatures induced by high-fat diet associate with particular features of colorectal cancer, we performed Oncomine concepts analysis (http://oncomine.org). Strikingly, the upregulated genes significantly associated with gene signatures characterizing the progression of colorectal cancer to advanced stages (Figure 3B), with enrichment in functions pertinent to tumor invasion and migration (Table S3D). In keeping with the identification of the RAS and PI3K signaling pathways (Figure 2B) and the upregulation of their target genes (Figure S2B), the upregulated gene signature significantly overlapped with gene signatures in KRAS mutant tumors and phosphatase and tensin homolog (PTEN) mutant cell lines (Figure 3B). Accordingly, we also observed significant overlap with gene signatures in cell lines sensitive to inhibitors of these pathways (Figure 3B and Table S3E). However, concepts discriminating colon cancer from normal colon were associated only with downregulated genes at a marginal significance (Table S3E). Finally, we performed gene set enrichment analysis, asking whether genes dysregulated in colonic epithelial cells are enriched with signature genes classifying colorectal cancer (De Sousa E Melo et al., 2013; Sadanandam et al., 2013). We observed significant enrichment with signature genes of the poor-prognosis CCS3 category (De Sousa E Melo et al., 2013) and the stem-like category (Sadanandam et al., 2013) in the high-fat-diet-induced differentially expressed genes (Figure 3C), demonstrating that the gene expression signature induced by diet is complex, with features consistent with a loss of differentiated status and other features associated with colorectal cancer progression.

To validate the specific correlation of gene expression alterations by high-fat diet with colon cancer, we also performed comparison with gene expression in colon of a mouse model of inflammatory bowel diseases (IBD) at both early and late inflammation stages (Russ et al., 2013), showing no correlation with IBD gene expression (Figure S3C).

Obesity, Not Diet, Drives Remodeling of the Enhancer Landscape

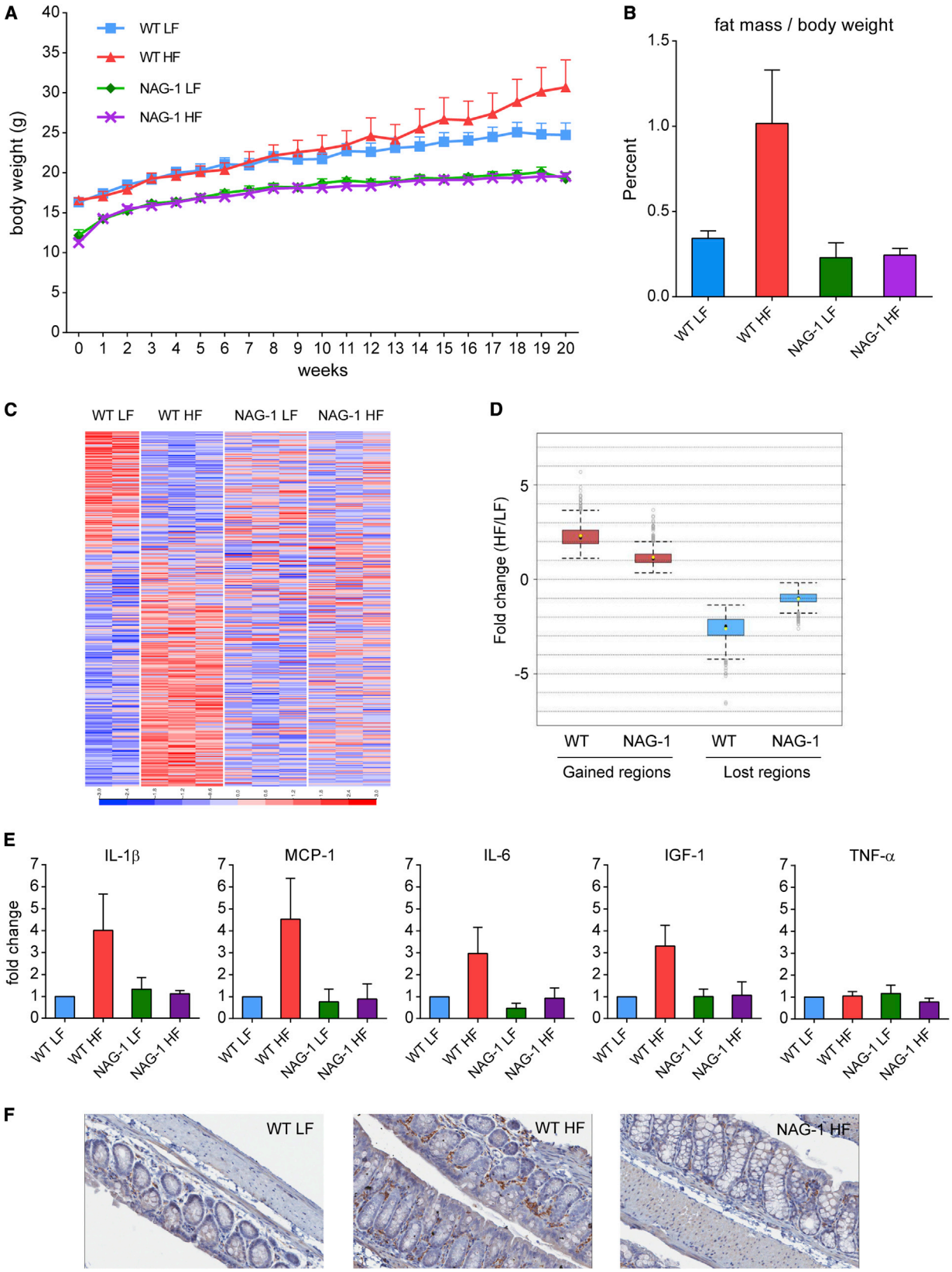
Our results suggest that remodeling of the enhancer landscape by diet and obesity affects critical genes and pathways important for intestinal homeostasis and may render colonic epithelium prone to cancer. It is unclear from any of the above analyses whether the altered landscape results from direct exposure of the colonic epithelia to the contents of the lumen or whether the status of the organism (i.e., obesity) drives the process. To distinguish between these possibilities, we employed transgenic mice (females only) expressing human NAG-1/GDF15 (nonsteroidal anti-inflammatory drug [NSAID]-activated gene-1) (Baek et al., 2006; Eling et al., 2006). NAG-1 transgenic animals, when placed on an identical high-fat diet, failed to gain weight in the same manner as their wild-type counterparts (Figure 4A) and had a lower ratio of fat mass to body weight (Figure 4B). We reasoned that these animals would provide us with a suitable system to ask whether exposure to the colon contents resulting

Figure 3. Diet-Induced Gene Signatures in Colonic Epithelium Are Significantly Enriched with Gene Features in Human Colorectal Cancer

(A) The top 20 most up- (left) and downregulated (right) genes in wild-type mice were identified. Heatmaps display the expression profiles of those genes in human normal colon, rectum, and colon cancer.

(B) Colorectal cancer features enriched in the upregulated genes induced by diet in wild-type female mice using Oncomine concepts analysis.

(C) Gene set enrichment analysis using signature genes of CCS3 subtype and stem-like subtype as gene sets. Genes in our study were ranked by Signal2Noise score comparing WT HF versus WT LF. ES, enrichment score. NES, normalized enrichment score. See also Figure S3 and Table S3.



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from a Western diet predominate over obesity in remodeling the colonic enhancer program.

We first assessed the impact of the transgene on the gene expression alterations induced by diet. A heatmap was generated displaying genes exhibiting diet-induced dysregulation in wild-type animals with the same genes displayed from NAG-1 transgenic animals (Figure 4C). The transgenic mice displayed a pattern of expression of these genes that completely differed from wild-type mice (Figures S4A and S4B and Table S4), suggesting a dominant role of obesity in directing the alterations in transcriptional program. Comparison of H3K27ac ChIP-seq data collected from wild-type and NAG-1 transgenic animals (Figure 4D) demonstrated that loci with significant gains in acetylation in wild-type animals had, on average, no diet-induced change in acetylation level in the NAG-1 transgenic animals. Likewise, loci losing acetylation in wild-type animals were unchanged in the high-fat/low-fat comparison in their transgenic counterparts. These results suggested that obesity, not diet, may be the driving factor in altering the epigenomic landscape in the colons of these mice.

Obesity is closely linked to chronic inflammation, also a hallmark of cancer. Consistent with this notion, expression of inflammatory cytokines in colon tissue was elevated in a diet-specific manner in wild-type, but not transgenic, animals (Figure 4E). Histopathologic examination of colon tissue revealed the presence of substantial macrophage infiltration in wild-type, but not in NAG-1 transgenic animals on high-fat diet (Figure 4F). To exclude the possibility that NAG-1 transgenic mice are defective in fat absorption, we performed the steatocrit test with feces from either WT mice or NAG-1 mice on high-fat diet. NAG-1 transgenic mice did not show malabsorption of fat (Figure S4C). These data demonstrate that NAG-1 transgenic mice were resistant to both the molecular alterations and phenotypes induced by high-fat diet, suggesting that they result from obesity, not diet per se.

DISCUSSION

During intestinal tumorigenesis, the epithelial cells undergo dedifferentiation and acquire stem cell-like properties (Schwitalla et al., 2013); therefore, colon cancer cells lose enhancer marks that typify normal crypt differentiation status while acquiring enhancer marks that are normally found in noncolon cell types (Akhtar-Zaidi et al., 2012). The differentially acetylated regions described here were enriched with binding sites of transcription factors downstream of signaling pathways commonly disturbed in colon cancer and also binding sites of transcription factors essential for colon development. These findings suggest that alterations in the H3K27 acetylation program induced by obesity may reflect both the loss of activities integral to normal colon homeostasis in addition to gain of activity downstream of signaling pathways activated in cancer. Although diet induced alterations in the landscape of H3K27 acetylation, most changes were not readily associated with alterations in expression of a linked gene. This finding implies that the enhancer alterations described here may reflect subtle changes that prime the genome to respond to a subsequent event.

In the absence of oncogenic stimuli, the occurrence of obesity-associated cancer was not increased by high-fat diet in wild-type mice maintained in a specific pathogen-free (SPF) environment (Yoshimoto et al., 2013). However, following treatment with azoxymethane or via introduction of mutant APC, mice on a high-fat diet had an incidence of colon tumors higher than that of mice fed normal chow (Moghaddam et al., 2007; Reddy et al., 1977; Tuominen et al., 2013). In our study, obesity induced widespread remodeling of the acetylation landscape at presumptive *cis*-regulatory regions that likely influence the transcriptional response suggestive of cancer predisposition. The associated gene expression profile was specifically enriched in genes implicated in cancer progression. These data suggest that a premalignant lesion in the colon from obese individuals has an increased probability of evolving into a clinically detectable malignancy, in part due to priming of the enhancer landscape for cancer progression.

EXPERIMENTAL PROCEDURES

Mice

Female h-NAG-1 transgenic mice and wild-type C57BL/6J mice at 5 weeks of age from a colony known to be positive for *Helicobacter* were placed on either 10% fat diet (D12450B) or 60% fat diet (D12492, Research Diets) for 20 weeks. Male C57BL/6J mice on either 10% fat diet (380056 DIO controls) or 60% fat diet (380050 DIO high-fat diet) for 15 weeks were purchased from Jackson Laboratory and acclimated for one additional week. All animal experiments were approved by the NIEHS Institutional Animal Care and Use Committee and were performed according to NIH guidelines for the care and use of laboratory animals.

Isolation of Colonic Epithelial Cells

Colonic epithelial cells were isolated as previously described (Roediger and Truelove, 1979). See also Supplemental Experimental Procedures.

ChIP Sequencing and Data Analysis

Standard crosslinked ChIP DNA was used to construct sequencing libraries, which were processed as single-end 36-mers (detailed methods in

Figure 4. Obesity Drives Alterations in Enhancer Landscape in Colon

(A) Body weight of mice on either low-fat diet or high-fat diet (n = 6 for all groups). Error bars represent SE.

(B) The column graph depicts the ratio of abdominal fat mass to body weight in mice on either low-fat or high-fat diet (n = 3 for all groups). Data are presented as mean ± SEM.

(C) The heatmap depicts expression of genes dysregulated in wild-type mice by diet adjacent to the same genes in NAG-1 transgenic mice.

(D) Diet-induced enhancer alterations were not observed in NAG-1 transgenic mice. The reads per kb per million (RPKM) fold change was calculated for gained and lost H3K27ac regions as identified in the wild-type samples by high-fat diet treatment, with a floor for the ratio denominator set at the estimated noise level (defined as the 2nd percentile in the distribution of RPKM scores for peaks called in nonself samples). The box is the 25^{th}–75^{th} percentile, and the whiskers are the 5^{th} and 95^{th} percentiles; the black dot is the median, and the yellow dot is the mean. Grey circles are points beyond the 5^{th}/95^{th} percentiles.

(E) The column graphs depict quantitative RT-PCR analysis of the indicated inflammatory cytokines in colon tissue (n = 3 for all groups). The expression levels were normalized to β-actin. Data are presented as mean ± SEM.

(F) Immunohistochemistry staining of macrophage marker F4/80 reveals that high-fat-diet-induced inflammation was suppressed in NAG-1 transgenic mice. See also Figure S4 and Table S4.

Supplemental Experimental Procedures). ChIP data were filtered for quality score, aligned, and deduplicated (methodology is detailed in Supplemental Experimental Procedures). Peaks were assigned by SICER (Zang et al., 2009), regions of differentially enriched H3K27ac were identified using the diffBind (v.1.6.2) R package, and motifs were extracted with HOMER (Heinz et al., 2010).

ACCESSION NUMBERS

The NCBI Gene Expression Omnibus (GEO) accession number for the ChIP-seq data reported in this paper is GSE46478, and the GEO accession number for the expression microarray data is GSE46843.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, Supplemental Experimental Procedures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.03.012.

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REFERENCES

Akhtar-Zaidi, B., Cowper-Sal-lari, R., Corradin, O., Saiakhova, A., Bartels, C.F., Balasubramanian, D., Myeroff, L., Lutterbaugh, J., Jarrar, A., Kalady, M.F., et al. (2012). Epigenomic enhancer profiling defines a signature of colon cancer. Science *336*, 736–739.

Baek, S.J., Okazaki, R., Lee, S.H., Martinez, J., Kim, J.S., Yamaguchi, K., Mishina, Y., Martin, D.W., Shoieb, A., McEntee, M.F., and Eling, T.E. (2006). Nonsteroidal anti-inflammatory drug-activated gene-1 over expression in transgenic mice suppresses intestinal neoplasia. Gastroenterology *131*, 1553–1560.

Bardou, M., Barkun, A.N., and Martel, M. (2013). Obesity and colorectal cancer. Gut *62*, 933–947.

Cancer Genome Atlas Network (2012). Comprehensive molecular characterization of human colon and rectal cancer. Nature *487*, 330–337.

Cattin, A.L., Le Beyec, J., Barreau, F., Saint-Just, S., Houllier, A., Gonzalez, F.J., Robine, S., Pinçon-Raymond, M., Cardot, P., Lacasa, M., and Ribeiro, A. (2009). Hepatocyte nuclear factor 4alpha, a key factor for homeostasis, cell architecture, and barrier function of the adult intestinal epithelium. Mol. Cell. Biol. *29*, 6294–6308.

De Sousa E Melo, F., Wang, X., Jansen, M., Fessler, E., Trinh, A., de Rooij, L.P., de Jong, J.H., de Boer, O.J., van Leersum, R., Bijlsma, M.F., et al. (2013). Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nat. Med. *19*, 614–618.

Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., and Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature *485*, 376–380.

Eling, T.E., Baek, S.J., Shim, M., and Lee, C.H. (2006). NSAID activated gene (NAG-1), a modulator of tumorigenesis. J. Biochem. Mol. Biol. *39*, 649–655.

Ernst, J., Kheradpour, P., Mikkelsen, T.S., Shoresh, N., Ward, L.D., Epstein, C.B., Zhang, X., Wang, L., Issner, R., Coyne, M., et al. (2011). Mapping and analysis of chromatin state dynamics in nine human cell types. Nature *473*, 43–49.

Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C., and Parkin, D.M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int. J. Cancer *127*, 2893–2917.

Gao, N., White, P., and Kaestner, K.H. (2009). Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2. Dev. Cell *16*, 588–599.

Garrison, W.D., Battle, M.A., Yang, C., Kaestner, K.H., Sladek, F.M., and Duncan, S.A. (2006). Hepatocyte nuclear factor 4alpha is essential for embryonic development of the mouse colon. Gastroenterology *130*, 1207–1220.

Ghaleb, A.M., McConnell, B.B., Kaestner, K.H., and Yang, V.W. (2011). Altered intestinal epithelial homeostasis in mice with intestine-specific deletion of the Krüppel-like factor 4 gene. Dev. Biol. *349*, 310–320.

Giancotti, F.G., and Ruoslahti, E. (1999). Integrin signaling. Science *285*, 1028–1032.

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell *38*, 576–589.

Hess, J., Angel, P., and Schorpp-Kistner, M. (2004). AP-1 subunits: quarrel and harmony among siblings. J. Cell Sci. *117*, 5965–5973.

Hryniuk, A., Grainger, S., Savory, J.G., and Lohnes, D. (2012). Cdx function is required for maintenance of intestinal identity in the adult. Dev. Biol. *363*, 426–437.

Katz, J.P., Perreault, N., Goldstein, B.G., Lee, C.S., Labosky, P.A., Yang, V.W., and Kaestner, K.H. (2002). The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. Development *129*, 2619–2628.

Larsson, S.C., and Wolk, A. (2006). Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. Int. J. Cancer *119*, 2657–2664.

McLean, C.Y., Bristor, D., Hiller, M., Clarke, S.L., Schaar, B.T., Lowe, C.B., Wenger, A.M., and Bejerano, G. (2010). GREAT improves functional interpretation of cis-regulatory regions. Nat. Biotechnol. *28*, 495–501.

Moghaddam, A.A., Woodward, M., and Huxley, R. (2007). Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events. Cancer Epidemiol. Biomarkers Prev. *16*, 2533–2547.

Moorman, J.P., Luu, D., Wickham, J., Bobak, D.A., and Hahn, C.S. (1999). A balance of signaling by Rho family small GTPases RhoA, Rac1 and Cdc42 coordinates cytoskeletal morphology but not cell survival. Oncogene *18*, 47–57.

Reddy, B.S., Watanabe, K., and Weisburger, J.H. (1977). Effect of high-fat diet on colon carcinogenesis in F344 rats treated with 1,2-dimethylhydrazine, methylazoxymethanol acetate, or methylnitrosourea. Cancer Res. *37*, 4156–4159.

Roediger, W.E., and Truelove, S.C. (1979). Method of preparing isolated colonic epithelial cells (colonocytes) for metabolic studies. Gut *20*, 484–488.

Russ, A.E., Peters, J.S., McNabb, W.C., Barnett, M.P., Anderson, R.C., Park, Z., Zhu, S., Maclean, P., Young, W., Reynolds, G.W., and Roy, N.C. (2013). Gene expression changes in the colon epithelium are similar to those of intact colon during late inflammation in interleukin-10 gene deficient mice. PLoS ONE *8*, e63251.

Sadanandam, A., Lyssiotis, C.A., Homicsko, K., Collisson, E.A., Gibb, W.J., Wullschleger, S., Ostos, L.C., Lannon, W.A., Grotzinger, C., Del Rio, M., et al. (2013). A colorectal cancer classification system that associates cellular phenotype and responses to therapy. Nat. Med. *19*, 619–625.

Sanyal, A., Lajoie, B.R., Jain, G., and Dekker, J. (2012). The long-range interaction landscape of gene promoters. Nature *489*, 109–113.

Schug, J., Schuller, W.P., Kappen, C., Salbaum, J.M., Bucan, M., and Stoeckert, C.J., Jr. (2005). Promoter features related to tissue specificity as measured by Shannon entropy. Genome Biol. *6*, R33.

Schwitalla, S., Fingerle, A.A., Cammareri, P., Nebelsiek, T., Göktuna, S.I., Ziegler, P.K., Canli, O., Heijmans, J., Huels, D.J., Moreaux, G., et al. (2013). Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. Cell *152*, 25–38.

Thurman, R.E., Rynes, E., Humbert, R., Vierstra, J., Maurano, M.T., Haugen, E., Sheffield, N.C., Stergachis, A.B., Wang, H., Vernot, B., et al. (2012). The accessible chromatin landscape of the human genome. Nature *489*, 75–82.

Tuominen, I., Al-Rabadi, L., Stavrakis, D., Karagiannides, I., Pothoulakis, C., and Bugni, J.M. (2013). Diet-induced obesity promotes colon tumor development in azoxymethane-treated mice. PLoS ONE *8*, e60939.

Verzi, M.P., Shin, H., He, H.H., Sulahian, R., Meyer, C.A., Montgomery, R.K., Fleet, J.C., Brown, M., Liu, X.S., and Shivdasani, R.A. (2010). Differentiation-specific histone modifications reveal dynamic chromatin interactions and partners for the intestinal transcription factor CDX2. Dev. Cell *19*, 713–726.

Verzi, M.P., Shin, H., Ho, L.L., Liu, X.S., and Shivdasani, R.A. (2011). Essential and redundant functions of caudal family proteins in activating adult intestinal genes. Mol. Cell. Biol. *31*, 2026–2039.

Verzi, M.P., Shin, H., San Roman, A.K., Liu, X.S., and Shivdasani, R.A. (2013). Intestinal master transcription factor CDX2 controls chromatin access for partner transcription factor binding. Mol. Cell. Biol. *33*, 281–292.

Wasan, H.S., Novelli, M., Bee, J., and Bodmer, W.F. (1997). Dietary fat influences on polyp phenotype in multiple intestinal neoplasia mice. Proc. Natl. Acad. Sci. USA *94*, 3308–3313.

Yoshimoto, S., Loo, T.M., Atarashi, K., Kanda, H., Sato, S., Oyadomari, S., Iwakura, Y., Oshima, K., Morita, H., Hattori, M., et al. (2013). Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature *499*, 97–101.

Zang, C., Schones, D.E., Zeng, C., Cui, K., Zhao, K., and Peng, W. (2009). A clustering approach for identification of enriched domains from histone modification ChIP-Seq data. Bioinformatics *25*, 1952–1958.