**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**PolyA-seq Data Processing**

Six cell lines HCT116, DKO, PrEC, LNCap, DU145, and DICER were prepared for a polyA-sequencing library. Each cell lines were split into 4 replicates and sequenced in more than one flowcells. The 100-bp single-end reads in the FASTQ files are demultiplexed, allowing a maximum of 2 mismatches in the sample barcodes. Total of 24 FASTQ files was generated. Both multiplexing barcode and read tags were stripped off. The former was used for sample identification and the latter was appended to a header of each read in the FASTQ file for a PCR duplicate read removal. Reads with highly enriched A’s from the 3' end (the number of 'A's is >=9 in a 10 bp-long window) are considered for determining polyA sites.

After removing barcodes and trimming the enriched A’s at 3'-end , we aligned the reads to hg19 with Bowtie2 (version 2.3.3.1) in a single-end read mode. No additional parameters were applied.

Reads aligned with higher confidence (i.e., MAPQ>=20) were only retained. Any read mapped to either PhiX control or decoy of chromosome contigs are excluded. A read in which the sample barcode recorded in header and the 5'-end alignment position are identical to the other is considered as PCR duplicated. All duplicated reads were collapsed. Some reads can be potentially originated from a highly-enriched A’s region in the reference genome sequence. Therefore, we examined the mapping coordinate of each read 3' end. If the number of 'A' in the reference genome sequence is >=7 out of 8 bp window, we exclude the reads from further analysis.

**Recruiting Putative PolyA Sites**

Based on the 24 polyA-Seq BAM files after the quality control, we determined poly(A) site positions, or equivalently named ‘processing region’ (PR). All 3' end mapping positions are counted. If two positions are within 10 bp apart from each other), we group them together and both are contributed from the same isoform. A unique poly(A) ID is assigned to a group of 3' ends when it is supported by more than 10 reads.

Which gene does each poly(A) belong to was based on the Ensembl build 87 gene models on GRCh37 and genes annotated in assembled chromosomes (chr1-22, chrX, chrY, and chrM) are only considered. We retained only the genes matched by Ensembl annotation curated by UCSC genome database [<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4937336/> and Table S2]. Biotypes of genes annotated by Ensembl include "protein\_coding", "processed\_transcript", "lincRNA", "antisense", "polymorphic\_pseudogene", "sense\_overlapping", "sense\_intronic", and "3prime\_overlapping\_ncrna". Not only known poly(A) sites but also we kept a *de novo* poly(A) site appeared in either intronic regions or beyond (up to 5,000 bp) the known 3’ UTR end position based on the existing gene model (Table S2).

**Differential PolyA Site Usage Testing**

Differential poly(A)-site usages between two cell lines HCT116 and DKO are tested. The read counts at each PR are normalized across all replicates. A significance testing is conducted with DEXSeq 1.24.4 [<https://genome.cshlp.org/content/22/10/2008>, https://www.pnas.org/content/110/38/15377] with the default parameter. P-value is adjusted by Benjamini-Hochberg

false discovery rate at 0.05. In order to obtain high confidence differential poly(A) sites, multiple quality controls are applied: 1) PR sites were only considered when the difference between two PR usage fractions is ≥ 0.1, 2) the FDR adjusted P-value for differential usage testing is < 0.0001, 3)the log2 fold change of PR usage fraction in absolute value is > 1.5, and 4) at least one group sample’s mean PR usage fraction is ≥ 0.05. As a result, aA total of 718 significant PRs are observed across 546 genes (Table1 and Table S3).

**Genomic function enrichment in regions between putative polyA sites**

**Transcription factor binding site profiles in between putative polyA sites**

Among 546 genes discovered in the differential testing, we focused on 412 proximal APA genes.. We interrogated the genomic regions of shifted PR from HCT116 to DKO at each proximal APA gene to see if it is often associated with transcription factors in common. We randomly sampled 10,000 pairs of PRs in any gene having more than one PR without replacement per each APA gene and compared those with the 412 APA regions. ENCODE [https://science.sciencemag.org/content/306/5696/636.long] regulatory of transcription factor binding sites (including ChIP-Seq binding sites for colon cancer cell lines in a previous study [<https://www.ncbi.nlm.nih.gov/pubmed/26257180>]) are annotated each testing region. Both Z scores and a frequency of binding proteins are calculated (Figure 1C and Table S1F), showing that the APA regions have a higher binding affinity of a certain protein than the others.

**RNA-Seq read Data Processing**

HCT116 and DKO RNA-seq FASTQ files contain 101 bp paired-end reads. The FASTQ files are converted to hg19 BAM using the two-pass mode of STAR (v2.5.4b) with default parameter for paired-end reads. Then, for the gene expression quantification, the BAM files are converted to a read count matrix by RSubread::featureCounts() [<https://academic.oup.com/nar/article/47/8/e47/5345150>] to quantify based on the Ensembl build 87 gene model with the options: ountMultiMappingReads=FALSE, allowMultiOverlap=FALSE, isPairedEnd=T, strandSpecific=2, minMQS=5, ignoreDup=FALSE**,** and fraction=FALSE. Log2-RPKM (read per kilobase per million mapped reads) values adjusted by gene length are computed. In total, 17 known cleavage factors (*CPSF1, CPSF2, CPSF3, CPSF3L, CPSF4, CPSF6, CPSF7, NUDT21, CSTF1, CSTF2, CSTF2T, CSTF3, FIP1L1, CLP1, PCF11, WDR33*, and *SYMPK*) are chosen to compare the gene expression levels between HCT116 and DKO (Figure S1D).

**ChIP-Seq Data Processing**

Twelve ChIP-seq data (two cell lines, HCT116 and DKO, by six proteins, CTCF, SMC1, RAD21, H3K27Ac, Pol2Ser2, and Pol2Ser5) are generated, along with two input controls one for each cell line. The FASTQ files contains 75 bp single-end reads. The primary analysis including alignments and peak callings is done by ENCODE Transcription Factor and Histone ChIP-Seq processing pipeline (https://github.com/kundajelab/chipseq\_pipeline,https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3431496/). The following parameters were used for the software with the default parameters, suggested for unreplicated experiments:

"--se --species hg19 --peak-caller macs2 --blacklist ${blacklist\_file} --fastq1 ${chipseq\_fastq\_file} --ctl\_fastq1 ${input\_control\_fastq\_file} --type $type".

We specified "TF" to "--type" for all transcript factor samples whereas "histone" for the samples tagged by H3K27Ac, Pol2Ser2, and Pol2Ser5. The ENCODE pipeline performs a quality control by collapsing duplicated reads and relying on uniquely mapped reads (e.g., MAPQ score 37). Reproducibility tests of peak detection statistics (e.g., overlapping peaks and irreproducible discovery rate) are all passed. No further quality control is performed.

For MBD-seq data, the FASTQ files are converted to BAM files via Bowtie2 (v.2.3.4.1) with default parameters for a single-end read. All mapped reads are utilized for further analysis.

**Differential ChIP-seq binding site analysis**

Peaks of binding sites for the twelve ChIP-seq data are available from the ENCODE pipeline output. The peak calls are done by MACS2 [https://genomebiology.biomedcentral.com/articles/10.1186/gb-2008-9-9-r137]. Between two cell lines, we conduct a differential binding analysis. For each protein, peaks (\*.narrowPeak) between two binding peaks are compared directly via MANorm v1.1.4 (<https://github.com/shao-lab/MAnorm>, <https://genomebiology.biomedcentral.com/track/pdf/10.1186/gb-2012-13-3-r16>) under the program option: manorm --p2 HCT116.narrowPeak --p1 DKO.narrowPeak --r2 HCT116.tagAlign.bed --r1 DKO.tagAlign.bed -w $width -m 1 -p 0.01 --name2 HCT116 --name1 DKO. In the option (-w) for a window length to calculate the read density, 500 bp is set for CTCF, SMC1, and RAD21 but 1,000 bp is set for H3K27Ac, Pol2Ser5, and Pol2Ser2.

MANorm takes narrow peaks and reads coordinates supporting the peaks (e.g., summit region surrounding by 500 bp extension for a transcription factor ChIP-seq) in BED file format. Then, MAnorm collects peaks commonly bound between two samples. Both fold change (M-Value) and normalized binding depth are reported. All the regions reported are kept for further analysis.

Similarly, MBD-seq peaks are called by MACS2 with the run option: “-f BAM -g hs -B --broad”. Peaks from each sample group (i.e., HCT116 and DKO) are simply concatenated. The mean depth along with the genomic region of the peaks are used for a differential binding analysis using DESeq2 (v1.18.1) under default parameters. Differential binding sites in which log2 fold change is greater than or equal to 1.0 and mean depth in either sample group is greater than 3 before normalization are only kept for further analysis.

**Integrative clustering analysis on multiple differential binding sites**

We compiled the total of 8 genomic tracks with binding sites called by either 6 differential ChIP-seq MANorm or 2 MBD-seq DESeq2 analysis. But, we paid attention to the genomic region where at least one binding site is overlapped by the 412 proximal APA shifted PR regions which we define as a genomic region between two PR locations flanked by 500 bp and the polyA usage is significantly different and the pA site in DKO is shifted toward to 5’ upstream. Then, we generated a feature by count matrix for a clustering analysis. At first, we split the genomic regions of binding sites into smaller segments such that the mean depth is same within each ChIP-seq and thus the differential binding sites can be summarized with either log2 fold change or mean read depthin 8 dimensional feature vector. .

Secondly, we filtered out spurious short segment region of 10 bp or shorter (equivalently, 95% of the regions are retained). A total of 8,013 distinct genomic regions become available for clustering analysis. A matrix of a distinct genomic segment by ChIP-seq tags are filled with a normalized log2 fold change (M-Value from MANorm output) for CTCF, SMC1, RAD21, H3K27Ac, Pol2Ser2, and Pol2Ser5 and raw mean depths from two cell lines MBD-seq, respectively. A feature count matrix represents 8 features for ChIP-seq/MBD-seq binding sites by 8,013 sample objects for genomic segments.

Thirdly, A standard consensus clustering method [https://link.springer.com/article/10.1023/A:1023949509487] was applied based on a non-negative matrix factorization (NMF) method [https://www.sciencedirect.com/science/article/pii/S0167947306004191]. NMF runs on the feature count matrix in 500 times and 80 % of the clustering results are randomly sampled for consensus membership analysis. The job was repeated with the number of clusters K=2 to K=15. The K=9 was found optimal, based on the delta value curves and the consistency of cluster membership. A unique cluster ID is assigned to each genomic segnment in the feature count matrix.he distance between two clusters are measured as the average distance between each point in one cluster to every point in the other cluster.

Fourthly, from ChIP-seq BAM files, we retrieved mean read depths (e.g., read depth normalized by MANorm and raw mean depth for MBD-seq) corresponding to a coordinate of each genomic segment in the feature count matrix for visualization. Finally, we can split the mean depths into 63 groups from 9 clusters by 7 proteins (i.e., CTCF, SMC1, RAD21, H3K27Ac, Pol2Ser2, Pol2Ser5, and MBD). Subsequently, each group is split into two subgroups; one for HCT116 and the other for DKO. An R function ggplot2::geom\_density() is used to visualize two density distributions of the mean depth in log2 value for each group. The difference between two distributions are quantified by Mann–Whitney-Wilcoxon nonparametric test [ref:Myles Hollander and Douglas A. Wolfe (1973). Nonparametric Statistical Methods. New York: John Wiley & Sons. Pages 27--33 (one-sample), 68--75 (two-sample). Or second edition (1999)] since the distribution is not necessary to follow normal distribution (Table S5).

**The Cancer Genome Atlas (TCGA) Dataset**

As of July 2018, we downloaded matched mRNA-seq BAM files and Infinium HumanMethylation 450k BeadChip array data for 5,284 patients across 11 cancer types, Bladder Urothelial Carcinoma (BLCA), Breast invasive carcinoma (BRCA), Colon adenocarcinoma (COAD), Esophageal carcinoma (ESCA), Kidney renal clear cell carcinoma (KIRC), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Pancreatic adenocarcinoma (PRAD), Skin Cutaneous Melanoma (SKCM), Stomach adenocarcinoma (STAD), and Uterine Corpus Endometrial Carcinoma (UCEC) in TCGA database. An average of 480 patient samples per cancer type was analyzed.

**Predicting polyA usage in TCGA RNA-seq data based on our colon cancer cell line model**

A 3’ polyA-seq is not available in the TCGA database. Thus, the polyA isoform usage at each 546 APA gene was inferred from the RNA-Seq BAM files. Under the assumption that when two pA sites exist, the read depth in a region shared by two isoforms is higher than a region uniquely transcribed by either of one, we manually define at least one pair of genomic regions for each of the 546 APA gene observed from the polyA-seq data from our colon cancer cell line model (Table S3). Incorporating with a gene model (e.g., Ensembl GRCh37.87) and the polyA site positions appeared in the 546 APA genes, we prepared a total of 651 pairs of genomic regions in a proximity of the pA sites in a way that, from matched RNA-seq BAM files to our polyA-seq data, the mean depth ratio between the pair of genomic regions for testing is proportional to the polyA usage rate. Each pair has two genomic regions: 1) 5' upstream regions where both proximal and distal isoform commonly share and 2) 3’ downstream region solely used by only one polyA isoform, respectively.

For RNA-seq quality control, we sorted genes by the FPKM-UQ (Fragments Per Kilobase of transcript per Million mapped reads upper quartile) [https://academic.oup.com/bib/advance-article/doi/10.1093/bib/bbx153/4590144] value already available from NCI (National Cancer Institute) Genomic Data Commons (GDC) and considered the genes confidently expressed. We retained the genes whose FPKM-UQ value is within 8,000 from the top for further analysis. Relative polyA usage rate was directly estimated from the ratio of the read depth between the pair of the genomic region in TCGA mRNA-seq BAM files. Cases in which the mean depth in the downstream (unique) region is greater than the one in common were excluded from further analysis.

**Normalization of DNA methylation beta-values**

Among average 485,000 CpG sites reported in the methylation array, approximately, 400,000 probes have valid beta (i.e., methylation fraction) values. In order to correct the technical issues caused by the Illumina type 2 probe, we used the beta mixture quantile dilation (BMIQ) method [<https://academic.oup.com/bioinformatics/article/29/2/189/204142>] to obtain a normalized methylation value at each CpG site.

**Correlation between predicted polyA usage ratio and CG methylation rate**

The samples where both beta values and polyA usage rate are available are only considered. A normalized methylation rate is defined at each CG site whereas a predicted polyA is defined at a gene (or pair of genomic regions defined in the Section, “**Predicting polyA usage in TCGA RNA-seq data based on our colon cancer cell line model”**) which the CG site is included in a region between the transcript start and end position with 5,000 bp extension. Over the multiple TCGA samples, we generated a scatter plot between methylation rate on the x-axis and a polyA usage rate on the y-axis. Pearson correlation (adjusted by Benjamini-Hochberg FDR 𝛼=0.05) is computed to quantify dependence or relationship between two variables.

[Reference]