Supplemental Experimental Procedures

RESOURCE TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
H3K4me2/3	Abcam	ab-6000
H3K27me3	EMD Millipore	07-449
EZH2	Active Motif	ac22
H3K9me3	EMD Millipore	07-442
IgG control	EMD Millipore	12-370
Critical Commercial Assays		
EZ DNA methylation-Gold Kit	Zymo Research	D5005
IQ SYBR Green Supermix	Biorad	1708880
7-Deaza-2'-deoxy-guanosine-5'-triphosphate	Roche	10988537001
SYBRselect	ThermoFisher	4472919
pGEM®-T Easy Vector	Promega	A1360
Superscript III	ThermoFisher	18080-044
Experimental Models: Cell Lines		
SNU-475	ATCC	CRL-2236
SNU-423	ATCC	CRL-2238
Human fibroblasts	ATCC	PCS-201-012
WM793	Wistar Institute	WM793
HepG2	ATCC	HB-8065
U87MG, SK-N-SH, SCaBER, T24 and related lines	University of Colorado, Anschutz Cancer Center Protein Production Shared Resource	U87MG, SCaBER , T24, T24T, FL3, SLT4
See Table S1 for CCLE cell lines.		
Oligonucleotides		
Bisulfite *TERT* CGI Forward 5’- TTTGAGAATTTGTAAAGAGAAATGA-3’	IDT	
Bisulfite *TERT* CGI Reverse 5’-AATATAAAAACCCTAAAAACAAATAC-3’	IDT	
Bisulfite CpG 1-8 sequencing 5’-AAACTAAAAAATAAAAAAACAAAAC-3’	IDT	
Bisulfite CpG 9-18 sequencing 5’- ATATAAAAACCCTAAAAACAAATAC-3’		
EMSA top strand 5’-AATGCGTCCTCGGGTTCGTCCCCAGCCGCGTCTACGCGCCTCCGTCCT - 3’	IDT	
EMSA bottom 5’-AGGACGGAGGCGCGTAGACGCGGCTGGGGACGAACCCGAGGACGCATT - 3’	IDT	
TERT Exon 14 Foward 5’-CATTTCATCAGCAAGTTTGGAAG-3’	IDT	
TERT Exon 14 Reverse 5’-TTTCAGGATGGAGTAGCAGAGG-3’	IDT	
Random Hexamer	ThermoFisher	N8080127
qPCR primer spanning cg11625005 Forward 5’-CTGTGTCAAGGAGCCCAAGT -3’	IDT	
qPCR primer spanningcg11625005 Reverse 5’-CTGGCCTGATCCGGAGAC -3′	IDT	
qPCR primer between cg11625005 and -124 mutation Forward 5’-CGTCCTCCCCTTCACGTC -3’	IDT	

qPCR primer between cg11625005 and -124 mutation Reverse 5'-GCCTAGGCTGTGGGGTAAC -3′	IDT	
qPCR primer spanning -124 mutation Forward 5'-GTCCTGCCCCTTCACCTT-3'	IDT	
qPCR primer spanning -124 mutation Reverse 5'-AGCGCTGCCTGAAACTCG-3′	IDT	
Software and Algorithms		
MethPrimer	(Li and Dahiya, 2002)	http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi
Snapgene viewer		http://www.snapgene.com/products/snapgene_viewer/
cBioPortal for TCGA	(Cerami et al., 2012; Gao et al., 2013)	http://www.cbioportal.org/data_sets.jsp
R	(Therneau, 1999)	https://www.r-project.org/
Other		
Protein G/Protein A agarose beads	EMD Millipore	IP05-1.5 mL
Protease inhibitor Cocktail	ThermoFisher-Pierce	88266
MinElute gel extraction kit	Qiagen	28606
RNase A	ThermoFischer	am2272
HiTrap Heparin column	GE	17-0407-03
RQ1 DNase	Promega	M6101
Amicon Ultra-15 Centrifugal Filter Unit, 30 kDa molecular weight cut-off	Millipore	UFC903024

Chromatin Immunoprecipitation, Bisulfite Conversion and PCR

ChIP was performed as previously described (Stern et al., 2015). For immunoprecipitation, 5-25 µg of solubilized chromatin was used with 2-4 µg of α-H3K4me2/3 (Abcam, ab-6000), α-H3K27me3 (EMD Millipore, 07-449), α-EZH2 (ac22) (Active Motif), α -H3K9me3 (07-442, EMD Millipore) or a non-specific IgG control (12-370, EMD Millipore) of equal mass, and nutated overnight at 4°C. Protein G/Protein A agarose beads (IP05-1.5 mL, EMD Millipore Corporation) were added for three hours and then treated as previously described (Stern et al., 2015). Bisulfite conversion of ChIP-purified material was performed using the EZ DNA Methylation-Gold Kit (D5005, Zymo).

CCLE Methylation Analysis

Cell lines from the CCLE underwent reduced RRBS as previously described (Landau et al., 2014). RNAseq data and *TERT* promoter mutations were annotated as previously described (Huang et al., 2015). A total of 278 lines were analyzed for which the *TERT* promoter mutation status, RNAseq data, and methylation data at the *TERT* locus were determined.

EMSA and PRC2-5mC-DNA Binding

5mC substituted DNA was synthesized by Integrated DNA technologies. All DNA substrates were radiolabeled using T4 PNK (NEB M0201L) by standard protocol. Stock PRC2 was diluted in binding buffer and added to radiolabeled DNA. The binding reaction was carried out for 30 min at 30°C, followed by loading samples onto non-denaturing 1.0% native agarose gel (Fisher BP160-100) buffered with TRIS/borate/EDTA (TBE) at 4°C. Dried gels were exposed to phosphorimaging plates, which were scanned using a Typhoon Trio phosphorimager (GE Healthcare) for signal acquisition. Gel analysis was carried out with ImageQuant software (GE Healthcare) and data fitted to a sigmoidal binding curve using MATLAB (MathWorks).

Cell Culture

SNU-423, SNU-475, HepG2 were obtained from American Type Culture Collection. U87MG, SK-N-SH, SCaBER, T24, T24T, FL3, SLT4, obtained from the University of Colorado, Anschutz, Tissue Culture Shared Resource. WM793 cells were obtained from S. Spencer at the University of Colorado, Boulder. All cells were cultured in DMEM (VWR Scientific) with

2mM GlutaminePlus (Atlanta Biologicals), 10% FBS (Thermo Fisher), 2 mM GlutaMAX-I (GIBCO), 100 units/ml penicillin and 100 mg/ml streptomycin (GIBCO) and 1mM sodium pyruvate (GIBCO), except U87MG and HepG2 cells were cultured in EMEM (American Type Culture Collection) plus serum and glutamxax, but without sodium pyruvate.

RNA Extraction and cDNA Preparation

Following RNA extraction with Trizol (Life Technologies), reverse transcription was performed by treating of 10 µg of RNA with 5 U of RQ1 DNase (Promega) according to the manufacturer's protocol, followed by phenol extraction (pH 7), chloroform:isoamyl alcohol extraction, and then ethanol precipitation. The cDNA was generated from 2 µg of RNA synthesized using random hexamers, oligo(dT) 20-mer, and SuperScript III (Life Technologies). Following treatment with RNase H (New England Biolabs) quantitative PCR was performed with either SybrSelect (Thermo Fisher) or iQ SYBR Green (Bio-Rad) PCR mix using a Roche LightCycler 480 with the program 10 min at 98°C, 30 sec at 95°C, 30 sec at 60°C, 30 sec at 72°C, and 5 min at 72°C, followed by quantification using the Roche LightCycler 480 software. Melt curve analyses were examined to ensure the uniformity of relevant PCR amplicons and all PCR amplicons were sequenced at least once to confirm the product identity. Primers for TERT mRNA exon 2 were forward 5'-CGTGGTTTCTGTGTGGTGTC-3, reverse 5'-CCTTGTCGCCTGAGGAGTAG-3'; and exon 14 were those previously described (Borah et al., 2015).

TERT CpG island coordinates were assessed using the UCSC Genome Browser bona fide CpG island data (Bock et al., 2007). This CGI island is commonly analyzed using the Infinium 450K BeadChip methylation arrays from Illumina which contains a probe (cg11625005) for this region that is designed to hybridized to the 50 bases preceding the chr5:1295737 (HG19). This probe on the Infinium arrays reports on the methylation status of this CpG dinucleotide.

Bisulfite ChIP Analysis

PCR with bisulfite converted samples was performed using BioRad iQ SYBR Green PCR mix using the program: 10 min at 95°C, followed by 17 step-down cycles of 1 min at 95°C, 1 min at 70°C–54° C, and 2 min at 72°C; 30 cycles of 1 min at 95°C, 1 min at 56°C, and 2 min at 72°C; and 10 min at 72°C. Temperature ramp rates for primer annealing were 1°C/min and extension at 2°C/min. Bisulfite primer sequences for amplifying the region of the *TERT* CGI were designed using MethPrimer (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi) and were forward 5'-TTTGAGAATTTGTAAAGAGAAATGA-3'; revervse 5'AATATAAAAACCCTAAAAACAAATAC-3'; and sequenced using 5'-AAACTAAAAAATAAAAAAACAAAAC-3' for CpGs 1-8 and 5'ATATAAAAACCCTAAAAACAAATAC-3' for CpGs 9-18. Three PCR reactions were pooled for each sample, and gel purified (Qiagen miniElute) followed by sequencing (Genewiz). Sequencing analysis was performed using Snapgene and Sequence Scanner 2.0 (Applied Biosystems). To account for non-normal distribution of peak heights, data were log transformed prior to taking averages, and then back-transformed to calculate ratios for ChIP/input values.

cg11625005 Survival Analysis

The methylation data for patients with samples run on the Illumina Human Methylation 450 arrays are reported in the associated TCGA studies. Beta values for cg11625005 were extracted from the associated patient files, and the average was calculated for any patient that had data generated for multiple vials according to the TCGA barcode. Patients were stratified based on a methylation beta value threshold of 0.75 and differences between the survival curves of the stratified patient groups were tested using the log-rank test. The results in this study are in whole or part based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/. Processed files with computed methylation beta values were downloaded from the Genomic Data Commons (https://gdc.cancer.gov/). Clinical information, including overall survival (OS_STATUS and OS_MONTHS) was downloaded from cBioPortal (Cerami et al., 2012) under the bulk data download (http://www.cbioportal.org/data_sets.jsp). All datasets (BLCA, BRCA, CESC, GBM, HNSC, LGG, LIHC, LUAD, LUSC, PRAD, SKCM, THCA) were processed through cBioPortal on June 22, 2016.

EZH2 Survival Analysis for Primary Tumors

Gene expression profiles for EZH2 and overall survival information were selected for patients in the TCGA (SKCM, LIHC, STAD, KIRP) using the CGDS-R R package (v1.2.5; https://github.com/cBioPortal/cgdsr). Each cancer type was filtered to only include primary tumor samples. To find the gene expression threshold that defined the best overall patient survival stratification, EZH2 expression values were ranked, then patients were iteratively stratified into low and high EZH2

expression groups. Overall survival comparisons between these two groups were made using the log rank test in the R survival package (v 2.40-1; https://github.com/therneau/survival). The EZH2 expression value that resulted in the most significant patient stratification defined the best separation threshold and was used for plotting overall patient survival.

Protein Expression and Purification

Human PRC2 5-mer complexes were expressed in insect cells as previously described (Davidovich et al., 2013; Wang et al., 2017). In brief, sequences encoding human EZH2, SUZ12, EED, RBBP4, and AEBP2 were cloned into the pfast-bac1 expression vector (Invitrogen) with PreScission-cleavable N-terminal hexahistidine-MBP tags. Standard Bac-to-Bac baculovirus expression system (Invitrogen) was used to generate baculovirus stocks according to manufacturer's protocol. Following infection, the cells were incubated for 72 h (27°C, 130 rpm) before they were harvested. The harvested cells were snap-frozen for later purification. PRC2 was purified as previously described (Wang et al., 2017). In brief, cell extract was incubated with the amylose resin and washed thoroughly, followed by elution with 10 mM maltose. The eluate was concentrated, then followed by digestion with PreScission protease at a mass ratio of 1:50 protease:protein. After completion of cleavage, protein complex was injected into a 5 mL Hi-Trap Heparin column (GE, 17-0407-03), followed by fractionation over a HiPrep 16/60 Sephacryl S-400 HR sizing column. PRC2 peak fractions were identified using SDS-PAGE, pooled, and concentrated as above. Final protein concentration was measured by absorbance at 280 nm, and the ratio of absorbance at 260 nm/280 nm was <0.7, an indication of no nucleic acid contamination.

EMSA and PRC2-5mC-DNA Binding

5mC substituted DNA was synthesized by Integrated DNA technologies. All DNA substrates were radiolabeled using T4 PNK (NEB M0201L) by standard protocol. After labeling, excess [γ-32P]-ATP in the reaction was removed by running the samples over a G50 Sephadex column (Roche 11 273 949 001). Radiolabeled DNA substrates were purified by native polyacrylamide gel electrophoresis (PAGE). DNA was extracted and pellets were dissolved in TRIS-EDTA buffer, pH 7.5. The radiolabeling efficiency of the purified DNA was determined by liquid scintillation counting. Radiolabeled DNA, with specific activity no less than 100,000 cpm/pmol, was adjusted with binding buffer (50 mM Tris-HCl pH 7.5 at 25°C, 100 mM KCl, 2.5 mM MgCl2, 0.1 mM ZnCl2, 2 mM 2-mercaptoethanol, 0.05% v/v NP-40, 0.1 mg/ml bovine serum albumin, 5% v/v glycerol). Next, stock PRC2 was diluted in binding buffer and added to radiolabeled DNA. The binding reaction was carried out for 30 min at 30°C, followed by loading samples onto non-denaturing 1.0% native agarose gel (Fisher BP160-100) buffered with TRIS/borate/EDTA (TBE) at 4°C. Gel electrophoresis was carried out for 90 min at 66 V packed in an ice box within a 4°C cold room. A Hybond N+membrane (Amersham, Fisher Scientific 45-000-927) and two sheets of Whatman 3 mm chromatography paper were put underneath the gel, which then was vacuum dried for 60 min at 80°C. Dried gels were exposed to phosphorimaging plates, which were scanned using a Typhoon Trio phosphorimager (GE Healthcare) for signal acquisition. Gel analysis was carried out with ImageQuant software (GE Healthcare) and data fitted to a sigmoidal binding curve using MATLAB (MathWorks).

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