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SUPPLEMENTAL DATA

Overexpression of MYC and EZH2 Cooperates to Epigenetically Silence MST1 Expression

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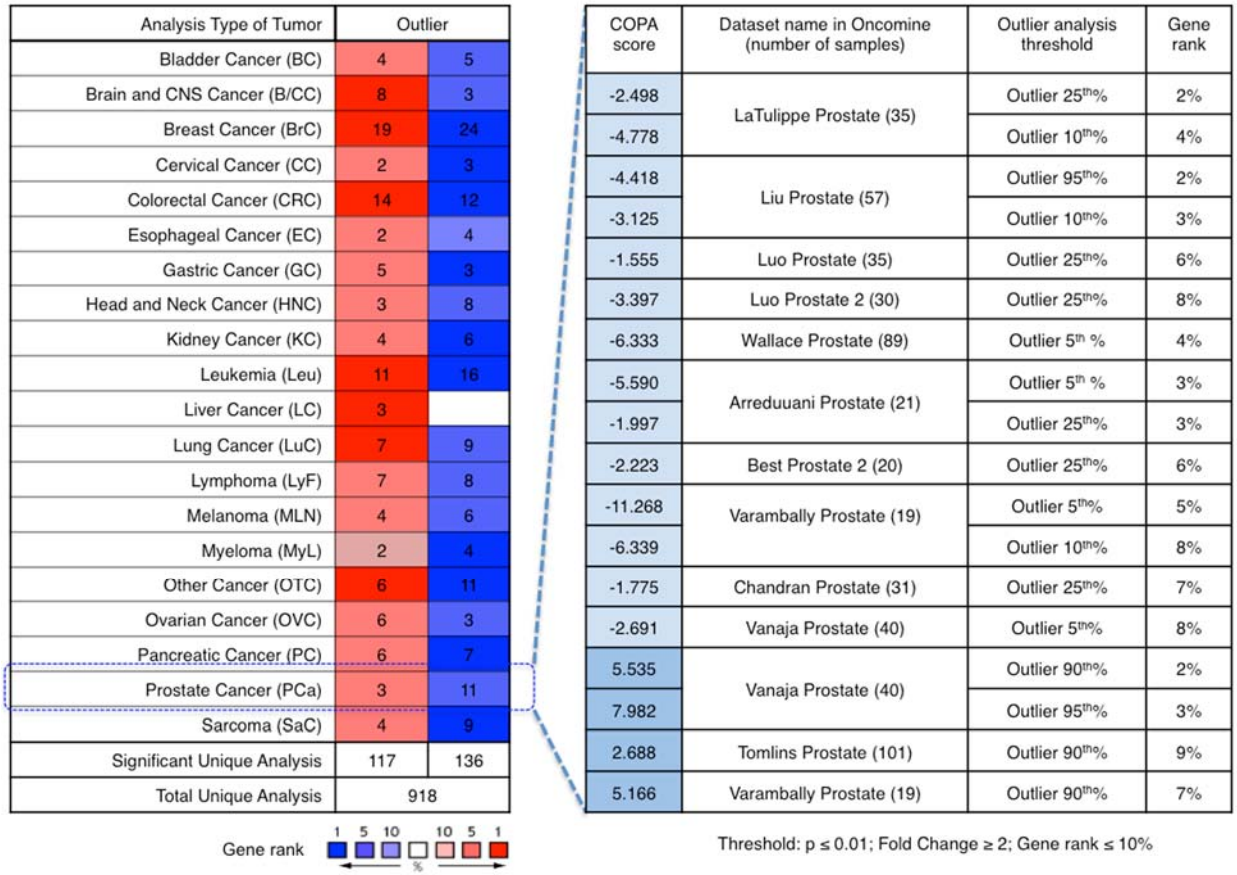
Primer Name	Primer Sequence (5'-3')	Application
MST1	Fw: ACAAATCCTCCTCCCACATTCCG Rv: CACTCCTGACAAATGGGTGCTG	qRT-PCR
EZH2	Fw: TGCACATCCTGACTTCTGTGAGC Rv: AGTCACTGGTCACCGAACACTC	qRT-PCR
MYC	Fw: TCCCTCCACTCGGAAGGAC Rv: CTGGTGCATTTTCGGTTGTTG	qRT-PCR
GAPDH	Fw: CTGGGCTACACTGAGCACC Rv: AAGTGGTCGTTGAGGGCAATG	qRT-PCR
pMST1-Luc2	Fw: AGAGGTACCTGCCATTTTCCTAATACTTACTGATG Rv: AGAAAGCTTCTCACTGCTCCATCCTCCC	Cloning
MST1-ChIP	Fw:TTTGTGGGGTGGGTTTAGGAGGTTTGT Rv: ACCAATAACCCCTCACCAACACAACAA	ChIP-PCR
miR-26a-RT	ACCACACGTCATGTGACTGCCTATCCT	Reverse Transcription
miR-26a	Fw: AGCGTTGTTTCAAGTAATCCAGG Rv: ATCAACCACACGTCATGTGACT	qRT-PCR
miR-26b-RT	CTTAGCGTTGCGATCTTCTCAACCTATCCT	Reverse Transcription
miR-26b	Fw: GGCCGCTTCAAGTAATTCAGG Rv: TGCTTAGCGTTGCGATCTTCTC	qRT-PCR
U6-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAA ATATG	Reverse Transcription
U6	Fw: GCGCGTGAAGCGTTC Rv: GTGCAGGGTCCGAGGT	qRT-PCR

Table S1. Primers used in this study were listed.

Target	Cat. No.	Company	Dilution- Application
MST1	3682	Cell Signaling (Danvers, MA)	1:1000 (WB) 1:50 (IHC)
EZH2	612666	BD Biosciences (San Jose, CA)	1:5000 (WB) 1:100 (IHC)
MYC	sc-40	Santa Cruz Biotechnology (Santa Cruz, CA)	1:1000 (WB)
H3K27me3	07-449	Millipore (Billerica, MA)	1:1000 (WB)
Histone H3	06-755	Millipore (Billerica, MA)	1:1000 (WB)
β-Actin	A2228	Sigma (St Louis, MO)	1:5000 (WB)
Anti-mouse IgG-HRP	7076	Cell Signaling (Danvers, MA)	1:2000 (WB)
Anti-rabbit IgG-HRP	7074	Cell Signaling (Danvers, MA)	1:2000 (WB)
Alexa Fluor 488 conjugated goat anti-rabbit IgG	A-11008	Life Technologies (Grand Island, NY)	1:1000 (IHC)
Alexa Fluor 568 conjugated goat anti-mouse IgG	A-21124	Life Technologies (Grand Island, NY)	1:1000 (IHC)

WB: Western Blot; IHC: Immunohistochemistry

Table S2. Antibodies used in this study were listed.



Analysis Type of Tumor	Outlier	
Bladder Cancer (BC)	4	5
Brain and CNS Cancer (B/CC)	8	3
Breast Cancer (BrC)	19	24
Cervical Cancer (CC)	2	3
Colorectal Cancer (CRC)	14	12
Esophageal Cancer (EC)	2	4
Gastric Cancer (GC)	5	3
Head and Neck Cancer (HNC)	3	8
Kidney Cancer (KC)	4	6
Leukemia (Leu)	11	16
Liver Cancer (LC)	3	
Lung Cancer (LuC)	7	9
Lymphoma (LyF)	7	8
Melanoma (MLN)	4	6
Myeloma (MyL)	2	4
Other Cancer (OTC)	6	11
Ovarian Cancer (OVC)	6	3
Pancreatic Cancer (PC)	6	7
Prostate Cancer (PCa)	3	11
Sarcoma (SaC)	4	9
Significant Unique Analysis	117	136
Total Unique Analysis	918	

COPA score	Dataset name in Oncomine (number of samples)	Outlier analysis threshold	Gene rank
-2.498	LaTulippe Prostate (35)	Outlier 25th%	2%
-4.778		Outlier 10th%	4%
-4.418	Liu Prostate (57)	Outlier 95th%	2%
-3.125		Outlier 10th%	3%
-1.555	Luo Prostate (35)	Outlier 25th%	6%
-3.397	Luo Prostate 2 (30)	Outlier 25th%	8%
-6.333	Wallace Prostate (89)	Outlier 5th %	4%
-5.590	Arreduuani Prostate (21)	Outlier 5th %	3%
-1.997		Outlier 25th%	3%
-2.223	Best Prostate 2 (20)	Outlier 25th%	6%
-11.268	Varambally Prostate (19)	Outlier 5th%	5%
-6.339		Outlier 10th%	8%
-1.775	Chandran Prostate (31)	Outlier 25th%	7%
-2.691	Vanaja Prostate (40)	Outlier 5th%	8%
5.535	Vanaja Prostate (40)	Outlier 90th%	2%
7.982		Outlier 95th%	3%
2.688	Tomlins Prostate (101)	Outlier 90th%	9%
5.166	Varambally Prostate (19)	Outlier 90th%	7%

Threshold: $p \leq 0.01$; Fold Change ≥ 2; Gene rank $\leq 10\%$

Figure S1. Cancer Outlier Profile Analysis (COPA) of publicly available microarray data sets in *Oncomine* for MST1 expression in 20 different tumor types including prostate cancer.

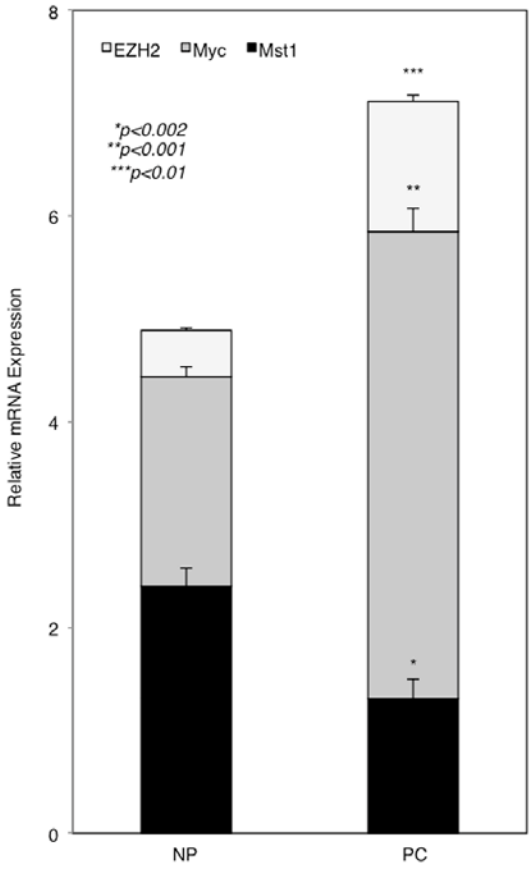


Figure S2. RT-qPCR analysis of MST1, MYC and EZH2 mRNA expression in frozen normal and cancerous prostate tissue samples. GAPDH mRNA was used as an internal control in PCR reactions. NP: Normal prostate (n=4), PC: Prostate cancer (n=8).

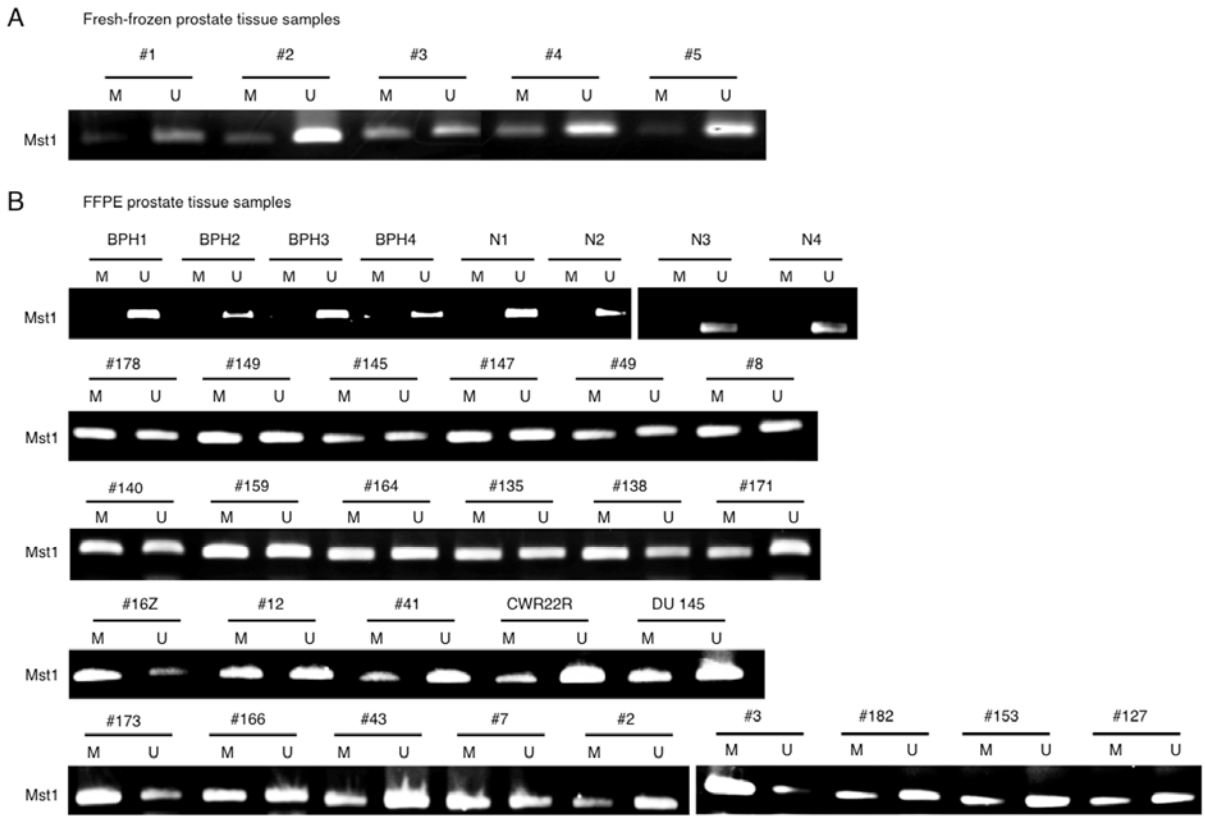


Figure S3. A) Methylation sensitive PCR (MSP) analysis of bisulfite-treated genomic DNA from fresh-frozen cancerous prostate tissue samples for MST1 promoter methylation. **B)** MSP analysis of bisulfite-treated genomic DNA from formalin-fixed and paraffin-embedded (FFPE) cancerous and non-cancerous prostate tissue samples. The procedures for MSP analysis were described in Materials and Methods. BPH: Benign prostate hyperplasia, N: normal (noncancerous) prostate, #: Blindly renamed patient ID number, M: Methylation, U: Unmethylation.

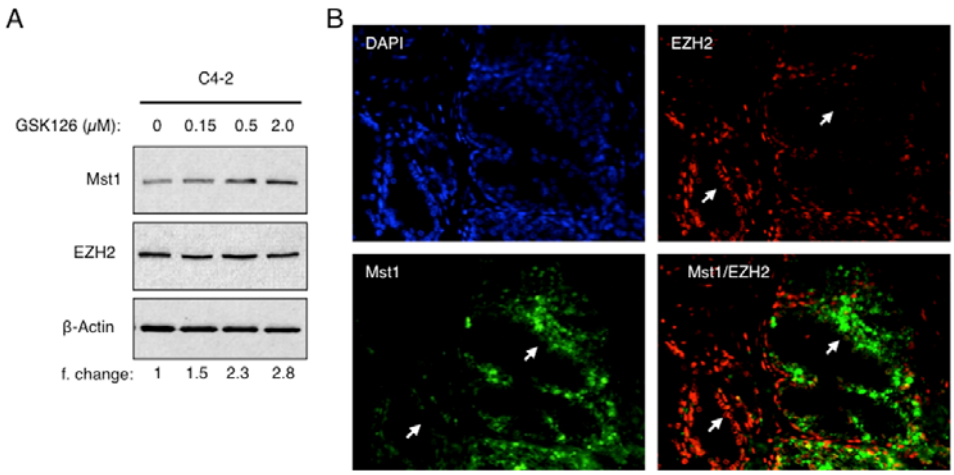


Figure S4. A) Analysis of MST1, EZH2, and β-Actin protein by western blot in C4-2 cells treated with increasing concentrations (0, 0.15, 0.5, and 2 μM) of GSK126 for 72h in serum-fed conditions. MST1 protein levels were normalized to β-Actin (loading control) and the data presented as fold (f) change relative to DMSO control. **B)** Co-IF staining of EZH2 and MST1 protein levels in prostate tumor tissue sections. Alexa Fluor 488 stained MST1 (green), Alexa Fluor 568 stained EZH2 (red), and DAPI stained cell nuclei (blue). Magnification is 20x. Micrographs are representative of multiple images from two independent experiments.

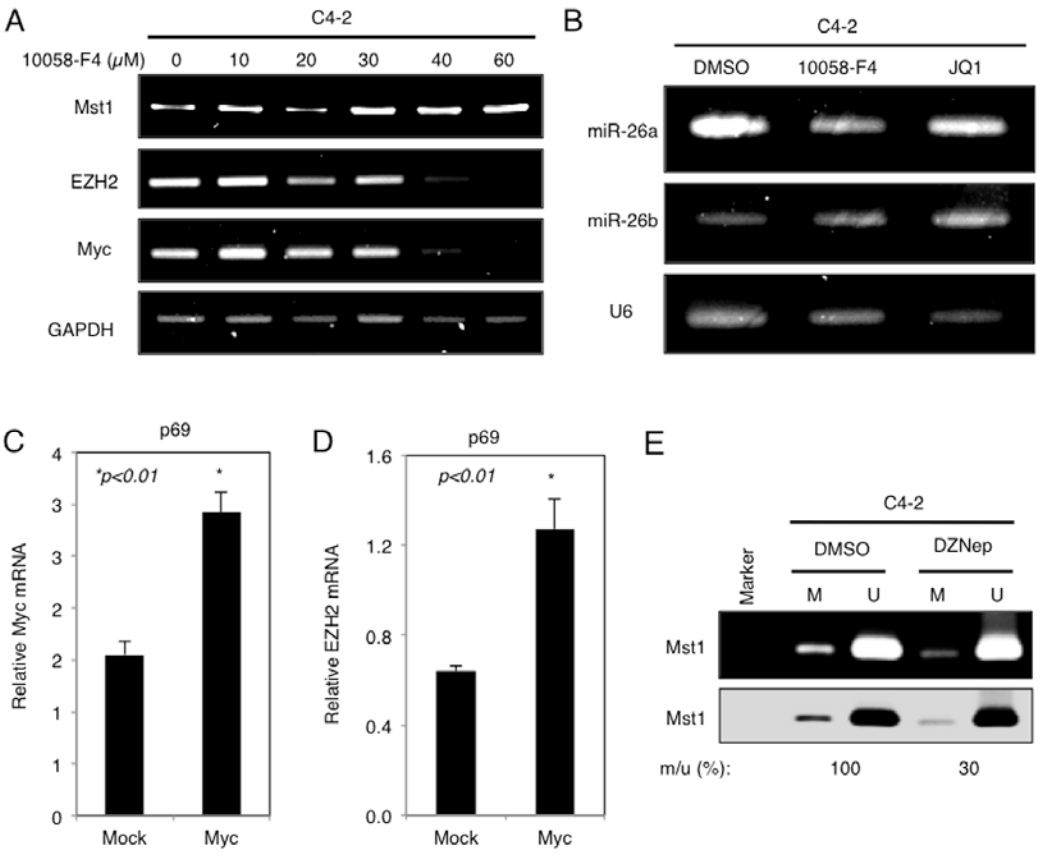


Figure S5. A) Analysis of Mst1, EZH2 and MYC mRNA levels by semi-quantitative RT-PCR in C4-2 cells that were treated with increasing concentrations (0, 10, 20, 30, 40, or 60 μM) of 10058-F4 for 3 days in serum-fed conditions. GAPDH mRNA expression was used as an internal control. **E)** Semi-quantitative RT-PCR analysis of miR-26a and miR-26b expression levels in C4-2 cells treated either with DMSO, 60 μM 10058-F4 or 500 nM JQ1 for 48h. U6 expression was used as an internal control. **C-D)** QPCR analysis of MYC and EZH2 mRNA levels in p69 cells transiently transfected with mock (vector) or MYC expression construct. **E)** MSP analysis of MST1 promoter in C4-2 cells after treatment with DMSO (vehicle) or 5 μM DZNep for 72h. The number below the image represents the percent ratio of methylated (m) to unmethylated (u) DNA intensity quantified by *ImageJ* software. Data are (±S.E.) from multiple experiments