Supplemental Information

Targeting histone demethylases in MYC-driven neuroblastomas with ciclopirox

	Non-MNA cell lines			MNA cell lines						Hits (viability)		Correlation with MYCN	
Target	SK-N-SH	SK-N-AS	CHLA20	NB-1643	BE(2)-C	NB7	NB15	Nagai	NB-1691	Reduce	Increase	R	p value
C14orf169	-0.23	-0.15	-0.03	-0.20	-0.13	-0.13	-0.24	-0.21	-0.38	4	0	0.286	0.0069
HIF1AN	-0.14	-0.23	0.03	-0.11	0.06	-0.30	-0.30	-0.17	-0.18	3	0	-0.113	0.3
HR	-0.27	0.03	-0.01	-0.33	-0.12	-0.26	0.03	-0.12	-0.26	4	0	0.102	0.35
HSPBAP1	-0.32	-0.49	0.18	-0.26	-0.11	-0.07	0.15	-0.31	-0.21	5	0	0.132	0.22
JARID2	-0.28	-0.12	0.03	-0.07	-0.13	-0.14	-0.02	-0.29	-0.26	3	0	-0.045	0.68
JHDM1D	-0.22	0.04	0.09	-0.07	-0.09	0.02	0.11	-0.16	-0.04	1	0	-0.141	0.19
JMJD1C	-0.06	0.28	0.12	-0.02	0.36	0.15	0.10	-0.11	-0.10	2	0	0.195	0.07
JMJD4	-0.19	-0.18	0.08	0.01	-0.16	0.16	0.19	-0.22	-0.04	1	0	0.224	0.02
JMJD6	-0.15	-0.29	0.29	0.01	-0.47	-0.52	-0.57	-0.27	-0.20	6	1	0.274	0.0099
JMJD7	-0.18	-0.03	0.01	-0.07	-0.03	-0.11	-0.18	-0.20	-0.07	0	0	-0.198	0.06
JMJD8	-0.15	0.27	0.29	-0.14	0.11	0.02	0.01	-0.02	-0.01	1	1	0.281	0.0081
KDM1A	-0.10	-0.22	0.12	-0.21	0.01	-0.50	-0.04	0.05	-0.12	3	0	-0.034	0.75
KDM1B	-0.18	0.26	0.14	-0.10	-0.02	-0.01	-0.23	-0.04	-0.07	1	1	-0.028	0.79
KDM2A	0.06	0.07	0.27	0.03	0.12	-0.25	-0.01	-0.24	-0.12	2	1	-0.021	0.85
KDM2B	-0.43	-0.37	-0.21	-0.14	-0.17	-0.09	-0.02	-0.28	-0.30	5	0	0.216	0.04
KDM3A	-0.11	-0.23	0.06	-0.10	0.29	0.06	0.36	-0.08	-0.05	1	2	0.315	0.0028
KDM3B	0.11	-0.37	0.34	0.06	-0.49	-0.62	-0.06	-0.12	-0.16	3	1	-0.01	0.92
KDM4A	-0.10	-0.16	0.08	-0.17	-0.01	-0.56	-0.27	-0.15	-0.18	2	0	-0.217	0.04
KDM4B	-0.27	0.02	0.06	-0.21	-0.24	-0.32	-0.30	-0.28	-0.32	7	0	0.385	0.00021
KDM4C	-0.01	-0.16	0.09	-0.17	0.12	0.11	0.20	-0.13	-0.16	0	1	-0.312	0.0031
KDM4D	-0.30	-0.44	0.11	-0.07	-0.26	-0.35	-0.16	-0.12	-0.33	5	0	0.249	0.02
KDM4E	-0.45	-0.41	-0.03	-0.28	-0.10	-0.36	-0.41	-0.22	-0.36	7	0	N/A	N/A
KDM5A	-0.13	-0.19	-0.03	-0.17	-0.33	-0.19	0.06	-0.19	-0.18	1	0	0.171	0.11
KDM5B	-0.31	-0.14	0.06	-0.22	0.00	-0.14	-0.02	-0.10	-0.21	3	0	0.357	0.00064
KDM5C	-0.15	-0.51	-0.06	-0.18	0.00	-0.43	-0.13	-0.23	-0.41	4	0	0.299	0.0047
KDM5D	-0.12	-0.19	0.10	-0.14	-0.11	-0.17	0.06	-0.28	-0.01	1	0	0.011	0.92
KDM6A	0.14	0.25	0.14	-0.04	-0.32	0.06	-0.12	-0.11	-0.17	1	1	0.07	0.52
KDM6B	-0.52	-0.34	-0.06	-0.18	-0.18	-0.30	-0.31	-0.23	-0.15	5	0	0.231	0.03
KDM8	-0.35	0.06	0.11	0.01	-0.09	-0.24	-0.17	-0.19	-0.19	2	0	0.146	0.17
MINA	-0.04	-0.18	0.20	-0.23	0.16	-0.05	0.04	-0.03	-0.11	1	1	0.193	0.07
PHF2	-0.23	-0.35	0.09	-0.13	0.02	-0.30	-0.24	-0.24	-0.05	5	0	0.129	0.23
PHF8	0.09	0.09	0.21	-0.08	-0.12	0.02	-0.28	-0.23	-0.08	2	1	0.173	0.11
TYW5	-0.13	0.08	0.10	0.01	-0.08	-0.09	-0.02	-0.25	-0.22	2	0	0.061	0.57
UTY	-0.13	0.22	0.21	-0.21	-0.03	-0.21	-0.05	-0.26	-0.17	3	2	0.043	0.69

Figure S1. siRNA screening in neuroblastoma cells.

Cell viability assessment after siRNA knockdown of histone demethylases and genes encoding JmjC domain containing non-histone demethylase proteins. The first nine columns represent the value of each gene subtracted by 1 after being normalized to the control. The viability threshold is set to (+0.2, -0.2). A value of less than -0.2 defined the genes that reduced cell viability. A value of greater than 0.2 defined the genes that increased cell viability. MNA = MYCN amplification. The green rows indicate siRNA knockdown reduced proliferation in at least 4 cell lines. HR and HSPBAP1, two genes without histone demethylase activity were heighted in the grey rows.

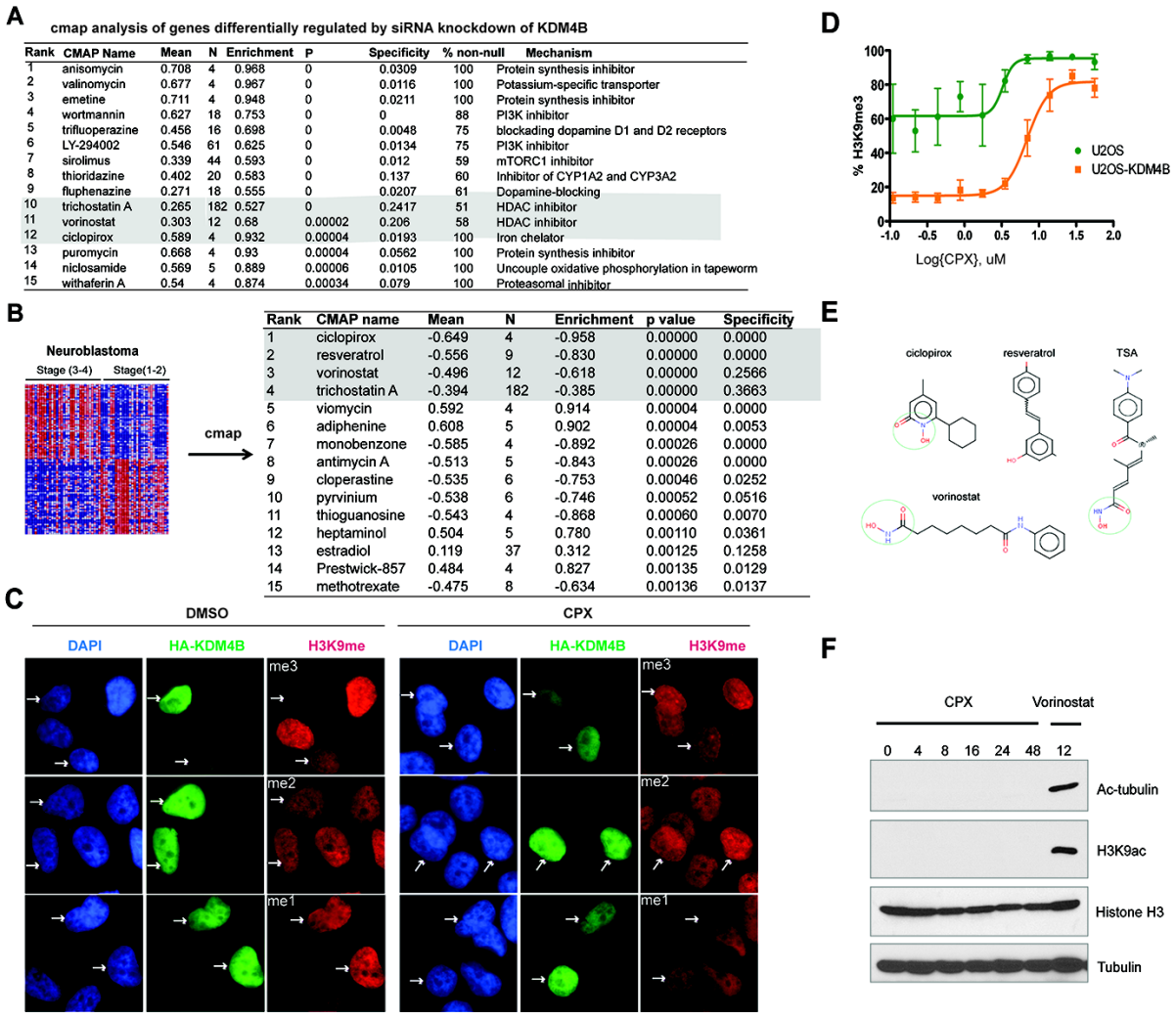

Figure S2. Identification of ciclopirox that targets KDM4B

(**A**) CMAP analysis to identify small molecules that generate a transcriptome inversely matching the KDM4B knockdown. The rank, name, mean connectivity score, the number of instances (n), enrichment of those instances in the order list of all instances, a permutation p-value for that enrichment score (P), the specificity of that enrichment (specificity), and the non-null percentage are shown in the table. (**B**) A similar strategy was used to identify small molecules that generate a transcriptome inversely matching the advanced neuroblastoma signature. The neuroblastoma gene expression profile (GSE12460, Janoueix-Lerosey et al) was reanalyzed according to INSS stage.

The top differential genes were used to perform CMAP. (**C**) Immunofluorescence shows a global change of H3K9me3/me2/me1 marks after overexpression of HA-tagged KDM4B in SK-N-AS cells (left). 5.0 μM of CPX treatment for 48 hours blocked erasure of H3K9me3 by HA-KDM4B (right). Arrows indicate the cells overexpressing HA-KDM4B. (**D**) U2OS parental and U2OS-KDM4B were treated with different concentrations of CPX. Positive H3K9me3 cells were quantified by immunofluorescence. (**E**) Structures of CPX and HDAC inhibitors. (**F**) NB-1691 cells were treated with 5μM of CPX and 2.5μM of vorinostat for the indicated duration. Western blotting was used to assess acetylated tubulin and histone H3.

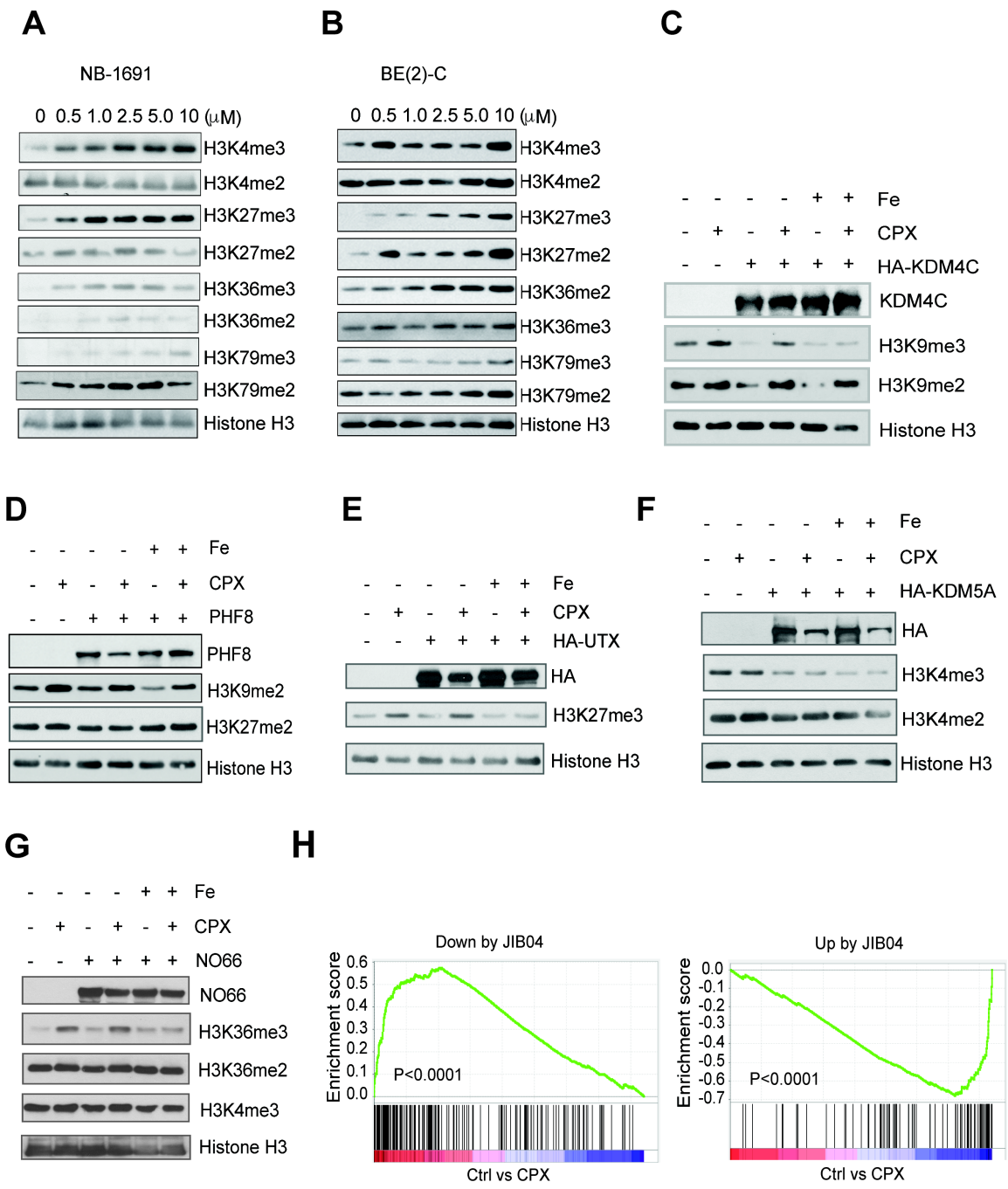

Figure S3. CPX effect on other histone methyl marks

(A-B) Different concentrations of CPX were used to treat NB-1691 and BE(2)-C cells for 48 hours. Whole cell lysates from samples as shown in Figure 2B were subject to western blotting with indicated antibodies. (C-G) 293T cells were transfected with expression constructs for the indicated

histone demethylases. 8 hours after transfection, cells were treated with 5.0 μM of CPX and/or 500μg/ml of holo-transferrin that carries iron into cells, for 24 hours. Western blotting was used to assess the indicated markers. (H) CPX induces a similar expression profile as JIB-04. GSEA analysis of the JIB-04 signatures (downregulated and upregulated) with the CPX expression prolife.

A

GSEA for enrichment of CPX targets (Genetic and chemical perturbations)

NAME	NES	NOM p-val	FDR q-val
SCHUHMACHER_MYC_TARGETS_UP	2.1	0	0.001
JYANG_MYCN	2.0602424	0	0.001740632
MORI_EMU_MYC_LYMPHOMA_BY_ONSET_TIME_UP	1.9022601	0	0.009949825
MENSSEN_MYC_TARGETS	1.8271015	0	0.020422729
SANSOM_APC_TARGETS_REQUIRE_MYC	1.826927	0	0.019973619
TARTE_PLASMA_CELL_VS_PLASMABLAST_DN	1.7787449	0	0.028089175
SCHLOSSER_MYC_TARGETS_REPRESSED_BY_SERUM	1.7770976	0	0.028022494
KIM_MYC_AMPLIFICATION_TARGETS_UP	1.6246115	0	0.077343486
DANG_MYC_TARGETS_UP	1.6043347	0	0.0859786
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_DN	1.707464	0.0019724	0.04636353
DANG_REGULATED_BY_MYC_UP	1.5659686	0.0019763	0.10390965
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_UP	1.709521	0.0041929	0.045992367
ACOSTA_PROLIFERATION_INDEPENDENT_MYC_TARGETS_UP	1.6639152	0.0056711	0.063507766
SCHLOSSER_MYC_AND_SERUM_RESPONSE_SYNERGY	1.7851715	0.0084211	0.026890123
BILD_MYC_ONCOGENIC_SIGNATURE	1.3102815	0.0293542	0.3030681
KIM_MYCL1_AMPLIFICATION_TARGETS_DN	1.4097298	0.0658436	0.2076127

B

GSEA for enrichment of CPX targets (Oncogenic signatures)

NAME	NES	NOM p-val	FDR q-val
MYC_UP.V1_UP	2.0591497	0	0
EIF4E_UP	1.6493754	0.004056795	0.02482508
CSR_LATE_UP.V1_UP	1.6395335	0	0.018402291
RPS14_DN.V1_DN	1.6152741	0	0.017571498
GCNP_SHH_UP_LATE.V1_UP	1.4219851	0.004210526	0.10633502
STK33_NOMO_DN	1.3973626	0	0.11529338
STK33_DN	1.3874437	0.001976285	0.10959221
BMI1_DN.V1_DN	1.3646958	0.030181086	0.122080706
RB_P107_DN.V1_UP	1.3415922	0.029350106	0.13575825

C

GSEA for enrichment of CPX targets (Transcription factor binding motif)

NAME	NES	NOM p-val	FDR q-val
V$MYCMAX_01	1.74	0	0.056
TMTCGCGANR_UNKNOWN	1.57	0	0.253
GKCGCNNNNNNNTGAYG_UNKNOWN	1.46	0	0.478
V$USF_C	1.45	0	0.429
V$USF_Q6_01	1.41	0	0.477
V$NFMUE1_Q6	1.39	0.022	0.513
V$ZF5_B	1.39	0	0.441
V$USF_Q6	1.3	0.019	0.841
V$MYCMAX_B	1.3	0.042	0.81

D

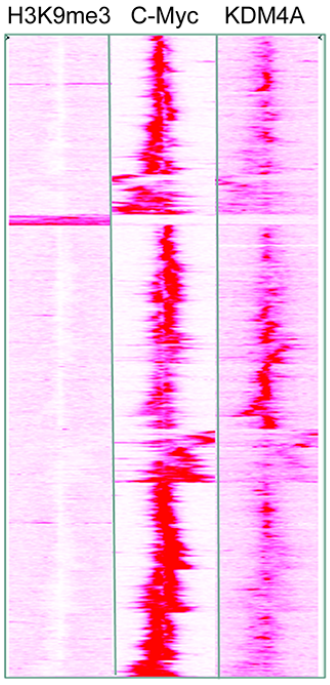

Figure S4. CPX targets the Myc pathway

(**A**) Gene set (from genetic and chemical perturbations) representing Myc pathways or signatures that are enriched with CPX targets, as analyzed by GSEA. NES = Normalized enrichment score. NOM = normalized. FDR = false discovery rate. (**B**) Gene set (oncogenic signatures) enrichment analysis for CPX targets. (**C**) Gene set (transcription factor binding motif signatures) enrichment analysis for CPX targets. (**D**) Heat map analysis of occupancy peaks of H3K9me3, c-Myc and KDM4A in human embryonic stem cells (ENCODE data).

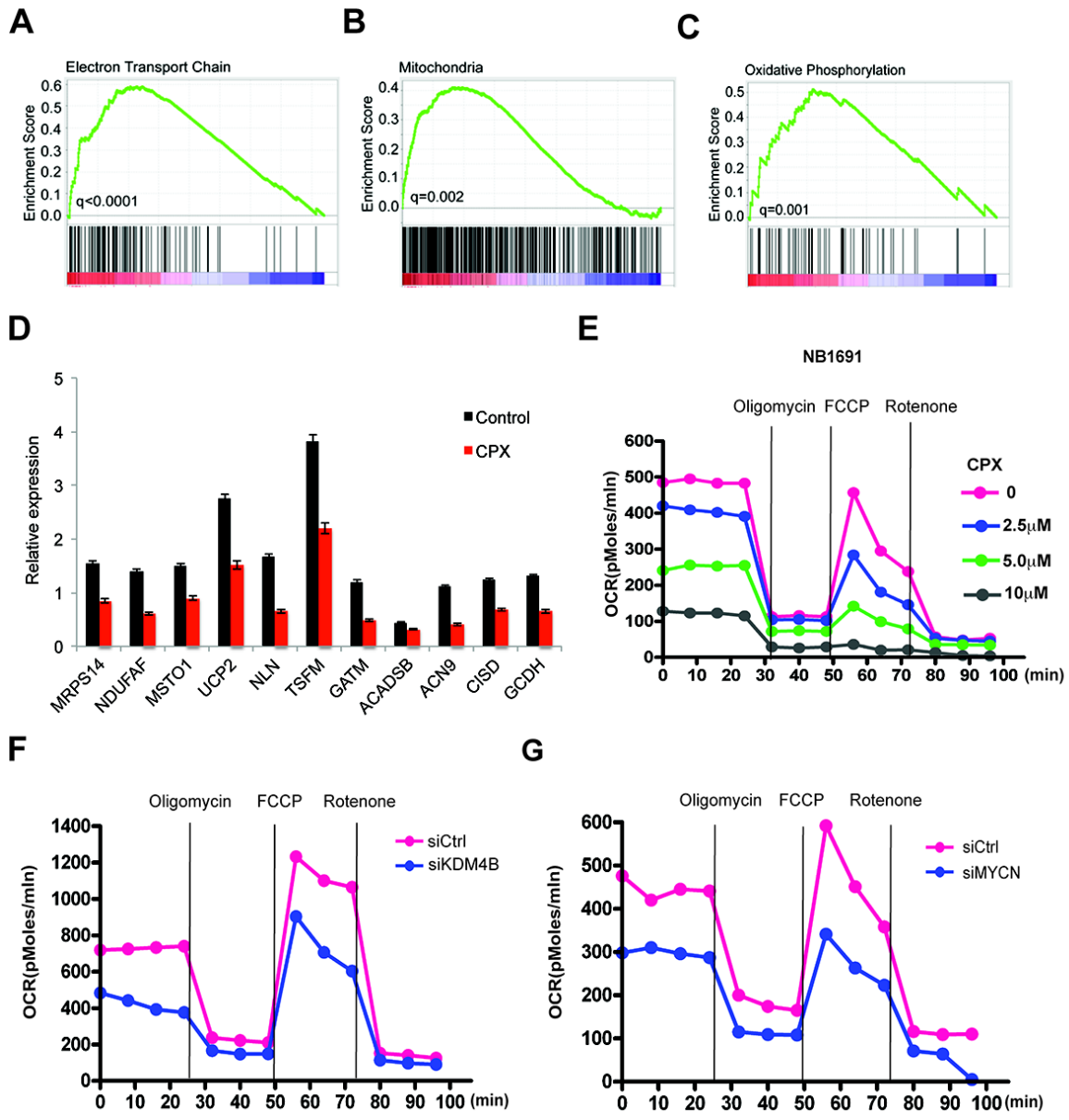

Figure S5. CPX inhibits oxidative phosphorylation

(**A-C**) GSEA analysis of our microarray data reveals that CPX suppresses genes involved in oxidative phosphorylation or electron transport chain reaction. (**D**) After treating NB-1691 cells for 48h with 2.5 μM CPX, RT-PCR was used to validate the genes involved in mitochondria respiration. (E) Oxygen consumption analysis showing consumption was compromised by CPX treatment of NB-1691 cells for 48 hours. (**F**) Oxygen consumption was compromised by depletion

of KDM4B in NB-1691 cells. (**G**) Oxygen consumption was also compromised by depletion of MYCN in NB-1691 cells.

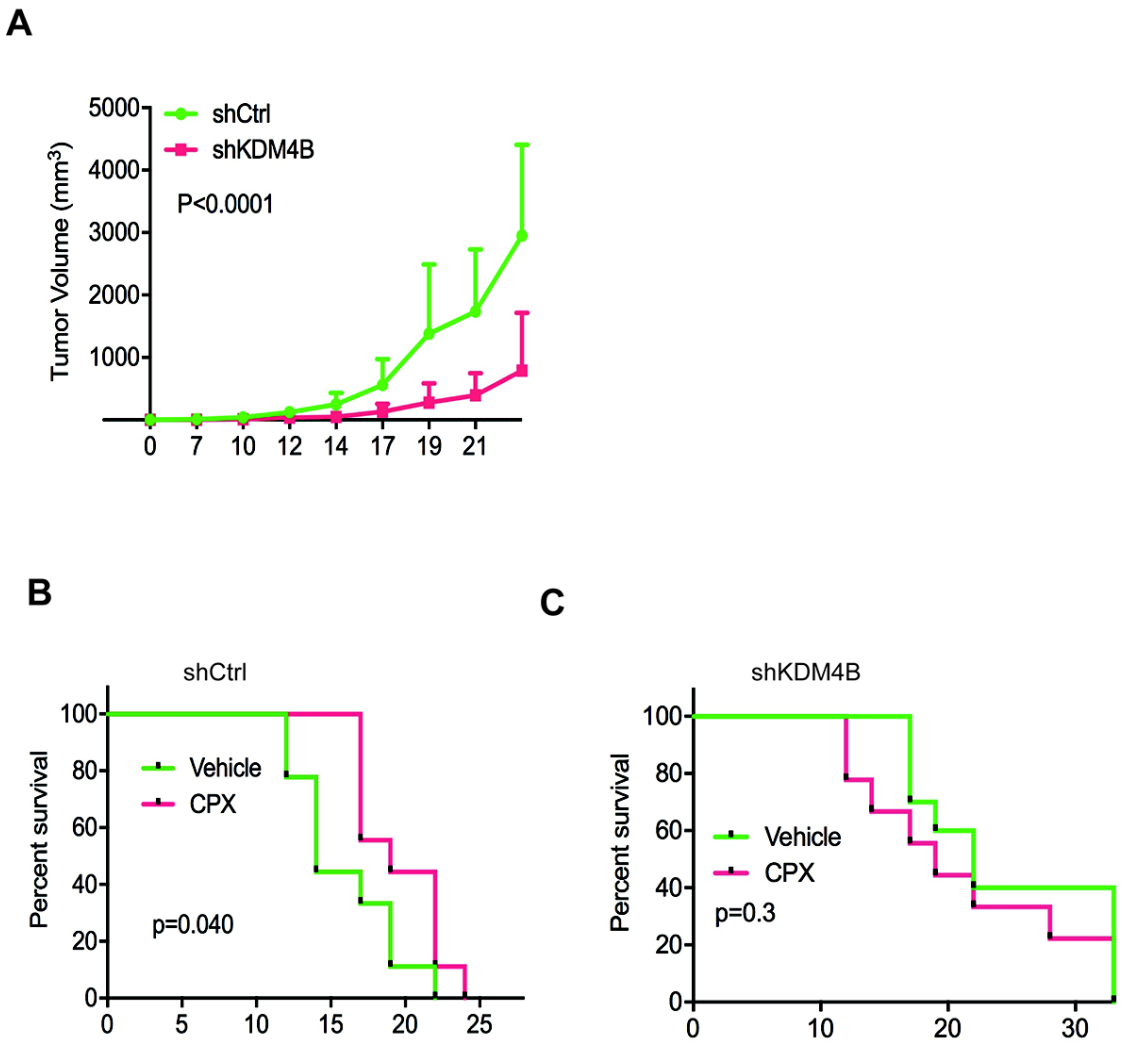

Figure S6. CPX effect on BE2C xenografts with KDM4B knockdown

(A) Growth of BE2(C) subcutaneous xenografts, shRNA control (n=5/group) and KDM4B knockdown (n=5/group). P value was computed by Student's t test.

(B-C) shRNA-ctrl and shKDM4B tumor bearing mice were treated with vehicle or 20 mg/kg of CPX twice daily via oral gavage. Kaplan-Meier analysis of survival curve with the Wilcoxon test

being used to determine the P value for shRNA control (n=5/group) (**B**) or shKDM4B-814 group (n=5/group) (**C**).

Table S1. The sequences of the focused KDM siRNA library

G-CUSTOM-68386								
Plate	Well	Pool Catalog Number	Duplex Catalog Number	Gene Symbol	GENE ID	Gene Accession	GI Number	Sequence
Plate 1	A02	M-014930-01	D-014930-01	KDM2B	84678	NM_001005366	54112379	GCAAUAAGGUCACUGAUCA
Plate 1	A02	M-014930-01	D-014930-02	KDM2B	84678	NM_001005366	54112379	GACCUCAGCUGGACCAAUA
Plate 1	A02	M-014930-01	D-014930-03	KDM2B	84678	NM_001005366	54112379	GGGAGUCGAUGCUUAUUGA
Plate 1	A02	M-014930-01	D-014930-04	KDM2B	84678	NM_001005366	54112379	CAGCAUAGACGGCUUCUCU
Plate 1	A03	M-012458-00	D-012458-01	KDM2A	22992	NM_012308	16306579	CAACAGCGAUCCCAAGUUA
Plate 1	A03	M-012458-00	D-012458-02	KDM2A	22992	NM_012308	16306579	GGACACGGGUUCCAAAUAA
Plate 1	A03	M-012458-00	D-012458-03	KDM2A	22992	NM_012308	16306579	CAACAUCCCUAUGCAGUUA
Plate 1	A03	M-012458-00	D-012458-04	KDM2A	22992	NM_012308	16306579	GCAAGCAGAUCACUCGAAA
Plate 1	A04	M-004073-02	D-004073-01	HIF1AN	55662	NM_017902	148596935	GAAAUUUCAUGAGUUCGUU
Plate 1	A04	M-004073-02	D-004073-02	HIF1AN	55662	NM_017902	148596935	CACAUAGAGUCAUUACUAA
Plate 1	A04	M-004073-02	D-004073-04	HIF1AN	55662	NM_017902	148596935	UCAUGGACUUCUUAGGUUU
Plate 1	A04	M-004073-02	D-004073-18	HIF1AN	55662	NM_017902	148596935	AGUUAUAGCUUCCCGACUA
Plate 1	A05	M-011872-01	D-011872-01	HR	55806	NM_018411	70906481	GGACUGGGCUGUGUUCCAA
Plate 1	A05	M-011872-01	D-011872-02	HR	55806	NM_018411	70906481	CCAGAGAACGGCAUCGUGA
Plate 1	A05	M-011872-01	D-011872-03	HR	55806	NM_018411	70906481	CAUUACAGGAGGCCAAAUA
Plate 1	A05	M-011872-01	D-011872-04	HR	55806	NM_018411	70906481	CGAUAGGGAACAAGGAUGU
Plate 1	A06	M-004287-01	D-004287-01	HSPBAP1	79663	NM_024610	142383186	GAAAGUACAUUGUGGAUUG
Plate 1	A06	M-004287-01	D-004287-02	HSPBAP1	79663	NM_024610	142383186	GAACAGAUUGCCUCAAAUA
Plate 1	A06	M-004287-01	D-004287-03	HSPBAP1	79663	NM_024610	142383186	CAACUAGAAUCCCUUAUGA
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Plate 1	A10	M-010820-01	D-010820-04	KDM5D	8284	NM_004653	56243542	CAGUAGAAGUUGACAAUUU
Plate 1	A11	M-009244-01	D-009244-02	JARID2	3720	NM_004973	11863151	UGAAUGGGCUCCUUGGUAA
Plate 1	A11	M-009244-01	D-009244-03	JARID2	3720	NM_004973	11863151	GAAAGACCCAAGAGGAAUA
Plate 1	A11	M-009244-01	D-009244-04	JARID2	3720	NM_004973	11863151	CCAACAGCAUGGUGUAUUU
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Plate 1	B02	M-017301-01	D-017301-01	KDM3A	55818	NM_018433	156602657	GAUCAAAGCUGGUAUUUAG
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Plate 1	B03	M-020378-01	D-020378-01	KDM3B	51780	NM_016604	54873608	CUACCUAGCUACUUUGUAA
Plate 1	B03	M-020378-01	D-020378-02	KDM3B	51780	NM_016604	54873608	GAUCAUAUGCAAACGACUA
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Plate 1	B04	M-012686-01	D-012686-03	JMJD1C	221037	NM_004241	68342035	GGAUAGCACUGUAAUCUGU
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Plate 1	B05	M-004292-01	D-004292-03	KDM4A	9682	NM_014663	98986458	UCAAGAGGCUGGAGAGUUU
Plate 1	B05	M-004292-01	D-004292-04	KDM4A	9682	NM_014663	98986458	GGUCUUACCUUCCCAGAUU
Plate 1	B06	M-004290-01	D-004290-01	KDM4B	23030	NM_015015	45504379	GGAAGAACCUCACCUUUGU
Plate 1	B06	M-004290-01	D-004290-05	KDM4B	23030	NM_015015	45504379	GGGAAUUCAUGAUCACAUU
Plate 1	B06	M-004290-01	D-004290-06	KDM4B	23030	NM_015015	45504379	CAAAUACGUGGCCUACAUA
Plate 1	B06	M-004290-01	D-004290-07	KDM4B	23030	NM_015015	45504379	GCAGGCACCGUCCACAUUU
Plate 1	B07	M-004293-02	D-004293-18	KDM4C	23081	NM_001146696	226442896	ACGAAGAUUUGGAGCGCAA
Plate 1	B07	M-004293-02	D-004293-19	KDM4C	23081	NM_001146696	226442896	GCAUAUAUGAUGAGGGUGU
Plate 1	B07	M-004293-02	D-004293-20	KDM4C	23081	NM_001146696	226442896	UUGCAUACAUGGAGUCUAA
Plate 1	B07	M-004293-02	D-004293-21	KDM4C	23081	NM_001146696	226442896	GGCAAAUAUUGUACUCCAA
Plate 1	B08	M-020709-00	D-020709-01	KDM4D	55693	NM_018039	39653316	GGAAGAACCGCAUCUAUAA
Plate 1	B08	M-020709-00	D-020709-02	KDM4D	55693	NM_018039	39653316	AGAGAGACCUAUGAUAAUA
Plate 1	B08	M-020709-00	D-020709-03	KDM4D	55693	NM_018039	39653316	CCCAGAAUCCAAAUUGUAA
Plate 1	B08	M-020709-00	D-020709-04	KDM4D	55693	NM_018039	39653316	UGUCAUAGAAGGCGUCAAU
Plate 1	B09	M-029951-01	D-029951-01	KDM4E	390245	XM_372429	113422553	CUGGAGAGAUGAUAUAGUA
Plate 1	B09	M-029951-01	D-029951-03	KDM4E	390245	XM_372429	113422553	CUAGUAAUCUCCCUGAUGU
Plate 1	B09	M-029951-01	D-029951-17	KDM4E	390245	XM_372429	113422553	GGAAGAGCCACCCCGGUAA
Plate 1	B09	M-029951-01	D-029951-18	KDM4E	390245	XM_372429	113422553	UGUAUGAUGAUAUCGAAGA
Plate 1	B10	M-023013-01	D-023013-01	KDM6B	23135	NM_001080424	122937250	GGAAUGAGGUGAAGAACGU
Plate 1	B10	M-023013-01	D-023013-02	KDM6B	23135	NM_001080424	122937250	GGAGACCUCGUGUGGAUUA
Plate 1	B10	M-023013-01	D-023013-03	KDM6B	23135	NM_001080424	122937250	CCACAGCGCCCUUCGAUAC
Plate 1	B10	M-023013-01	D-023013-04	KDM6B	23135	NM_001080424	122937250	GCACAAACGGAACUAUGGA
Plate 1	B11	M-014238-00	D-014238-01	JMJD4	65094	NM_023007	12711669	CUACAUCACCUACUGGAAA
Plate 1	B11	M-014238-00	D-014238-02	JMJD4	65094	NM_023007	12711669	CAACAUGUGGCGCUUCUUG
Plate 1	B11	M-014238-00	D-014238-03	JMJD4	65094	NM_023007	12711669	CCGACUGGCUGAAUGAGUU
Plate 1	B11	M-014238-00	D-014238-04	JMJD4	65094	NM_023007	12711669	GGUCAUCGCUGAGAAGAGG
Plate 1	C02	M-003983-02	D-003983-01	KDM8	79831	NM_024773	13376122	CCACUGAGCUCUUCUACGA
Plate 1	C02	M-003983-02	D-003983-02	KDM8	79831	NM_024773	13376122	GAAGUUGGUUCGAGGUACA
Plate 1	C02	M-003983-02	D-003983-03	KDM8	79831	NM_024773	13376122	UCAACGAGUUCAUCAGCAA
Plate 1	C02	M-003983-02	D-003983-17	KDM8	79831	NM_024773	13376122	AGCCAAGGGACGUCGGGUA
Plate 1	C03	M-010363-03	D-010363-01	JMJD6	23210	NM_015167	125988388	GAACUGGGAUUCACAUCGA
Plate 1	C03	M-010363-03	D-010363-02	JMJD6	23210	NM_015167	125988388	GGAUAACGAUGGCUACUCA
Plate 1	C03	M-010363-03	D-010363-04	JMJD6	23210	NM_015167	125988388	GACCAAAGUUAUCAAGGAA
Plate 1	C03	M-010363-03	D-010363-05	JMJD6	23210	NM_015167	125988388	GGACCCGGCACAACUACUA
Plate 1	C04	M-187551-00	D-187551-13	JMJD7	100137047	NM_001114632	167860113	CGGAGCGAGUUACGAGAAU
Plate 1	C04	M-187551-00	D-187551-14	JMJD7	100137047	NM_001114632	167860113	UCUGGUAUGACAUGGAAUA
Plate 1	C04	M-187551-00	D-187551-15	JMJD7	100137047	NM_001114632	167860113	CGCCAGACCUAGCACGGUA
Plate 1	C04	M-187551-00	D-187551-16	JMJD7	100137047	NM_001114632	167860113	GAAAGAUGCCCGAUGCUGU

Plate 1	C05	M-022873-01	D-022873-01	JMJD8	339123	NM_001005920	56090145	CGUAAGCGCUGGUUCCUUU
Plate 1	C05	M-022873-01	D-022873-02	JMJD8	339123	NM_001005920	56090145	CGAGUGGGCCUCUCUCUUU
Plate 1	C05	M-022873-01	D-022873-04	JMJD8	339123	NM_001005920	56090145	GGUACUCAGAAGUGAUCUA
Plate 1	C05	M-022873-01	D-022873-17	JMJD8	339123	NM_001005920	56090145	CCAGUAAAGCCUUCGAUAA
Plate 1	C06	M-025357-01	D-025357-02	JHDM1D	80853	NM_030647	90093354	GCACAGACAUGACUACACA
Plate 1	C06	M-025357-01	D-025357-03	JHDM1D	80853	NM_030647	90093354	GAUACCAUGUCAAGACUGA
Plate 1	C06	M-025357-01	D-025357-04	JHDM1D	80853	NM_030647	90093354	GGAAACUUCGAGAUCAUAA
Plate 1	C06	M-025357-01	D-025357-17	JHDM1D	80853	NM_030647	90093354	CUAUAAACAACCCGUGUAA
Plate 1	C07	M-009223-01	D-009223-01	KDM1A	23028	NM_015013	58761545	UGAAUUAGCUGAAACACAA
Plate 1	C07	M-009223-01	D-009223-02	KDM1A	23028	NM_015013	58761545	GACAAGCUGUUCCUAAAGA
Plate 1	C07	M-009223-01	D-009223-03	KDM1A	23028	NM_015013	58761545	GUAAAGCCACCCAGAGAUA
Plate 1	C07	M-009223-01	D-009223-04	KDM1A	23028	NM_015013	58761545	CUAUAAAGCUCCAAUACUG
Plate 1	C08	M-008121-01	D-008121-01	KDM1B	221656	NM_153042	116256450	CUGCAUAACUUUGGAAUUA
Plate 1	C08	M-008121-01	D-008121-02	KDM1B	221656	NM_153042	116256450	GGACCACAAUGAAUUCUUU
Plate 1	C08	M-008121-01	D-008121-04	KDM1B	221656	NM_153042	116256450	UAACAACCCAGUAGCAUUA
Plate 1	C08	M-008121-01	D-008121-17	KDM1B	221656	NM_153042	116256450	GGUCAGUUUAUAACGAGUA
Plate 1	C09	M-016031-01	D-016031-01	MINA	84864	NM_153182	110227620	GUACAUAACUCCCGCAGGA
Plate 1	C09	M-016031-01	D-016031-02	MINA	84864	NM_153182	110227620	GUAAGCAGAUGAAGUUAGA
Plate 1	C09	M-016031-01	D-016031-03	MINA	84864	NM_153182	110227620	GGGCAACGAUUCAGUUUCA
Plate 1	C09	M-016031-01	D-016031-17	MINA	84864	NM_153182	110227620	UCACAGUACUGCCGGAUCA
Plate 1	C10	M-014397-01	D-014397-01	C14orf169	79697	NM_024644	106879205	GCGAAGAACCGCUUUCAUG
Plate 1	C10	M-014397-01	D-014397-02	C14orf169	79697	NM_024644	106879205	CCCGAGACUUCAUGGAUUA
Plate 1	C10	M-014397-01	D-014397-03	C14orf169	79697	NM_024644	106879205	GUGCACCGCGCAACACUUA
Plate 1	C10	M-014397-01	D-014397-04	C14orf169	79697	NM_024644	106879205	GACCAGCUGUCCUUGGCAA
Plate 1	C11	M-012912-01	D-012912-01	PHF2	5253	NM_005392	117190341	GCAAGCGCCUGACGUCAAG
Plate 1	C11	M-012912-01	D-012912-02	PHF2	5253	NM_005392	117190341	AGGAGUUUGUGGACUAUUA
Plate 1	C11	M-012912-01	D-012912-03	PHF2	5253	NM_005392	117190341	ACGGGAAACUACUCCUUUA
Plate 1	C11	M-012912-01	D-012912-04	PHF2	5253	NM_005392	117190341	CGCCCGACAUCGACAUAUA
Plate 1	D02	M-004291-00	D-004291-01	PHF8	23133	NM_015107	32698699	GAACCAAGAUAGCAAAGAA
Plate 1	D02	M-004291-00	D-004291-02	PHF8	23133	NM_015107	32698699	CAGCAGACCUCUUCAGAUU
Plate 1	D02	M-004291-00	D-004291-03	PHF8	23133	NM_015107	32698699	GGAGGGAACUUCUUACACA
Plate 1	D02	M-004291-00	D-004291-04	PHF8	23133	NM_015107	32698699	GGACAUCUUUCGCGGUUUG
Plate 1	D03	M-016468-01	D-016468-01	TYW5	129450	NM_001039693	89242133	GGUGAUGUAUUAUUCAUUC
Plate 1	D03	M-016468-01	D-016468-02	TYW5	129450	NM_001039693	89242133	GGACUUCUAUGCACGACGA
Plate 1	D03	M-016468-01	D-016468-03	TYW5	129450	NM_001039693	89242133	CGAGAUGCCCAGUAUUUAU
Plate 1	D03	M-016468-01	D-016468-04	TYW5	129450	NM_001039693	89242133	CCGAGUUACCAGAGGAAUA
Plate 1	D04	M-014140-01	D-014140-01	KDM6A	7403	NM_021140	10863942	GAACAGCUCCGCGCAAAUA
Plate 1	D04	M-014140-01	D-014140-02	KDM6A	7403	NM_021140	10863942	GAGAGUAAUUCACGAAAGA
Plate 1	D04	M-014140-01	D-014140-04	KDM6A	7403	NM_021140	10863942	CAGCACGAAUUAAGUAUUU
Plate 1	D04	M-014140-01	D-014140-05	KDM6A	7403	NM_021140	10863942	GCUAUUGGCUGGUGCAACA
Plate 1	D05	M-017344-00	D-017344-01	UTY	7404	NM_007125	33188430	GCAAAUGAGACACAAAGAA
Plate 1	D05	M-017344-00	D-017344-02	UTY	7404	NM_007125	33188430	GCUAGGCAGUAAUUGUAUA
Plate 1	D05	M-017344-00	D-017344-03	UTY	7404	NM_007125	33188430	CCAUGGAGAUUCACCAAAU
Plate 1	D05	M-017344-00	D-017344-04	UTY	7404	NM_007125	33188430	CAACUAGCACUGGUAUUAA

Table S2. Primers for Real time PCR

Primer Sequence	Gene Symbol	Forward or reverse
AACCCGTTGAACCCCATT	18S	F
CCATCCAATCGGTAGTAGCG	18S	R
TGGCAAAAGACTATTTGATTTTCA	ACADSB	F
GCAGCATTGTATGTTAGTAATCTTGC	ACADSB	R
AGGCACAGCGTTTCTTGC	ACN9	F
TTTGTTCATCACGAAAGTCATTAAG	ACN9	R
CCAACCGCGAGAAGATGA	ACTIN	F
CCAGAGGCGTACAGGGATAG	ACTIN	R
GCCCACTATGCCCACTCTC	ANGPTL2	F
CTGCAGGCAGTCTCTCCAT	ANGPTL2	R
GAAGAAGAGGTGCCTGAGGA	ARID4A	F
CAGGTGGTTTGTTGATTGGA	ARID4A	R
TCTGAGCCCGAACTGGAG	BOP1	F
TGCTGTGGCTGAGAGGAGA	BOP1	R
GCGAGCAGAGGCTTAAGGT	BTG2	F
GGGAAACCAGTGGTGTTTGTA	BTG2	R
GAAGACCCCCATCAAGGTG	CISD3	F
AAGAAGTGGGAGCCGTCAC	CISD3	R
TCAAGAGGTGCCACGTCTCC	CMYC	F
TCTTGGCAGCAGGATAGTCCTT	CMYC	R
TGCTCTTTGCCAACCAGA	CYB5R3	F
TGCAGAATGTTTGTTCCTGAGT	CYB5R3	R
GCCATGGTCAAGGGCTAC	DHCR7	F
TTGTAAAAGAAATTGCCTGTGAA	DHCR7	R
CGGAGCCTTCAGGGAAAG	DHODH	F
CACTCTCCGCAAGCCATC	DHODH	R
CACACTTCTCTCCGCGTACAT	ELOVL2	F
GTTGTAGCCTCCTTCCCAAGT	ELOVL2	R
ACTTTGTCAGGGACTATCCTGTG	ENO2	F
TCCCTACATTGGCTGTGAACT	ENO2	R
TGGCTCAACCTCATCTTCAA	G6PC3F	F
AGAAGAGGGGAACTGGTGAAC	G6PC3R	R
TTTGCAATATGACTTTGGAGGA	GADD45A	F
CATCCCCCACCTTATCCAT	GADD45A	R
GAGGATGCTGCTGCCAAG	GAP43	F
GGCACTTTCCTTAGGTTTGGT	GAP43	R
TTTTCAAGAAAGCAGGATGGA	GATM	F
TGGAAAGCCATTTGGATGA	GATM	R

GATGGGGGAGTTGGGTGT	GCDH	F
CATAGGCCACAGACGAAACC	GCDH	R
CCGGATACTCACGCCAGA	GDF15	F
AGAGATACGCAGGTGCAGGT	GDF15	R
TGGACCTTCGTCAGTTTGG	GPM6A	F
TCAAGAAATTCTCAGAGACAGTACAAA	GPM6A	R
CCATGGATGTGGTCTACGC	HIST1H4J	F
TAGAAAGGGACGCTCAACCA	HIST1H4J	R
TCCCCTGCCACCAGACTA	HK2	F
TGGACTTGAATCCCTTGGTC	HK2	R
CGATTCAATGACCCTTCTGG	HMMR	F
AGGATACTGGTCCTTTCAATACTTCT	HMMR	R
CGTGGACCACCTGACCTT	HPDL	F
GGAACCAGCGCAAAAGTG	HPDL	R
GAATCCTGAATCGAGCTGAGA	hRARA	F
GGGCCATGTCCTGTGATG	hRARA	R
TCACCAGCCACATCTACCAG	JMJD2B	F
GATGTCCCCACGCTTCAC	JMJD2B	R
GAGGAATCCTGGAGCACAGA	KIAA1712	F
GCAAGATAGGTTCACAAATCAGG	KIAA1712	R
TTCATGTGCAAGTTCGTCAAC	KISS1R	F
CACACTCATGGCGGTCAG	KISS1R	R
GACGGAGTACACGGAGCAG	LASS2	F
CGTTCCCACCAGAAGTAATCA	LASS2	R
CTCCCGAAGAACCCTCTTTC	LRP8	F
GCTTAATGCCACTCGCTTG	LRP8	R
CTACGGCACCTGGTTTGG	LSS	F
CCTCTGCACAGGCAGTCC	LSS	R
CCTGTGTTAAGCGGAAAACC	MAP2	F
AGAGACTTTGTCCTTTGCCTGT	MAP2	R
GCACATTCCTTTTCACCTCAG	MAT2A	F
CTGCTGAAGGTGGGCATC	MAT2A	R
GCGTTACTTAGGCAAAGGTGT	mENO2	F
ATCCTGCTGTTGATGTGGTCC	mENO2	R
AGATGGTGTCAAGCCGGAAG	mGAP43	F
TTTTGGTCGCAGCCTTATGAG	mGAP43	R
CTCGCTCTATCTCTGCTCGTG	mNPY	F
GGCTGGATCTCTTGCCATATCT	mNPY	R
AGACACGCAGACGGGTTG	mRARA	F
GAGGATGCCACTCCCAGA	mRARA	R
TTGTGCTTTTAAACCCAAAGC	MRPL35	F
TATTCAGGGGCCGTAGGATT	MRPL35	R
CCTCTAAAGTTCTTGGATAAAGCAA	MRPL40	F

GCTCTCCTCTCAGTCTCCTCAA	MRPL40	R
TCACTCAGGAAGAATACCATTTTG	MRPS14	F
CCGATTTCTGATTCTAACAGGAC	MRPS14	R
GGCACTCGGAGCTTCTACA	MRPS28	F
GATGCAAAGGATTCCACATTTT	MRPS28	R
GTCCACCGGGTATGGTTCT	MSTO1	F
AACACTGGGATGCTCTCCAC	MSTO1	R
CCAAGGTTCATTGGACGGC	mTH	F
GGGCATCCTCGATGAGACTC	mTH	R
CCACAAGGCCCTCAGTACC	MYCN	F
CCTCTTCATCATCTTCATCATCTG	MYCN	R
AGGACATCACCGAAGACAGC	NDUFAF3	F
CCCACCACCACGATCTCTAT	NDUFAF3	R
TCTCAGCGCTCCCATGAT	NLN	F
AATCCTGGAACCACCAACTCT	NLN	R
TCCTAGCTGAATGCTATAACCTCTG	NTRK2	F
GGCATCCTTCAGGGTCTTC	NTRK2	R
CACTTCGGCTGCAGAGTTTT	PLOR3G	F
AGTGGGCAAATTCTGAAAGG	PLOR3G	R
CCGATCACTTCTTTTCTTGGAT	QTRTD1	F
CCACCTTCAATCACTCCAATG	QTRTD1	R
AATTGTGCGGAAATATCTAGTATCAG	RAB3IP	F
CCAGCTGTGACTCCAGAGGT	RAB3IP	R
GAATGCAGGAAAGAGATCGAA	RANBP1	F
TCTCCTCAGCATCCTCCTTG	RANBP1	R
AGTAGGTGCTTGGCGGTTC	RFC3	F
CACAGTAGATAACACGTGGCAAA	RFC3	R
TTTTTCCTTGTGGTGTGAGTGT	RPL27A	F
CCCAATGCACACAGGATGTA	RPL27A	R
CTGTCTCAAGGGGTGATTGC	SKP2	F
TTCGATAGGTCCATGTGCTG	SKP2	R
CATGAGTCTCCGGAAAGCAG	SQLE	F
ACAACACCTTCAATAAACTTTGCAT	SQLE	R
ACTATAGCAAAACTCACGGGAAGA	ST18	F
GAGAGCTCACTGGGTCTTTCTCT	ST18	R
AAGCCTCAGCAGTTCTTTGG	TAC1	F
TCTGGCCATGTCCATAAAGAG	TAC1	R
TGCACAGGAGCCAAGAGTGAA	TBP	F
TGCAGACCCTGGACCAACTGTA	TBP	R
ACTACTACGCCAAGGAGGTCAC	TGFB1	F
TGCTTGAACTTGTCATAGATTTCG	TGFB1	R
GCCAAGGACAAGCTCAGG	TH	F
AGCGTGTACGGGTCGAACT	TH	R

CCGGACTCAGCTGCAAAC	TRIL4	F
TTGCATTTCTGTATAAAACTGTTTCTC	TRIL4	R
GCTCACTCAAGGATCAGTTGG	TSFM	F
CCATGCAGCTCGTTTAAGAA	TSFM	R
GACGTGGATGAAGAATAGCTCTG	TSHZ2	F
CGAAGAAGGGTTCAGTGTGAG	TSHZ2	R
CCAGATCCCCGTGTATGACT	UCK2	F
ACGTCTGCGGGATAGACAGT	UCK2	R
TGAAAGCCAACCTCATGACA	UCP2	F
CGATGACAGTGGTGCAGAA	UCP2	R

ChIP PCR Primer Sequence	**Gene symbol**	**F/R**
AGCCACGAGGTCTTGATTG	MIR17HG	F
AGCAATCACCGACTCCACT	MIR17HG	R

Materials and Methods

The individual siRNA target sequences from the pool for validation

KDM2B-01 (5' G.C.A.A.U.A.A.G.G.U.C.A.C.U.G.A.U.C.A.U.U 3'),

KDM5C-01 (5' G.A.G.C.G.G.A.G.G.U.U.U.C.C.U.A.A.U.A.U.U 3')

KDM2B-02 (5' G.A.C.C.U.C.A.G.C.U.G.G.A.C.C.A.A.U.A.U.U 3')

KDM5C-02 (5' G.U.G.G.A.C.A.A.C.U.U.C.A.G.G.U.U.U.A.U.U 3')

KDM4B-01 (5' G.G.A.A.G.A.A.C.C.U.C.A.C.C.U.U.U.G.U.U.U 3')

KDM4B-05 (5' G.G.G.A.A.U.U.C.A.U.G.A.U.C.A.C.A.U.U.U.U 3')

KDM4D-01 (5' G.G.A.A.G.A.A.C.C.G.C.A.U.C.U.A.U.A.A.U.U 3')

KDM4D-02 (5' A.G.A.G.A.G.A.C.C.U.A.U.G.A.U.A.A.U.A.U.U 3')

KDM4E-01 (5' C.U.G.G.A.G.A.G.A.U.G.A.U.A.U.A.G.U.A.U.U 3')

KDM4E-03 (5' C.U.A.G.U.A.A.U.C.U.C.C.C.U.G.A.U.G.U.U.U 3')

KDM6B-01 (5' G.G.A.A.U.G.A.G.G.U.G.A.A.G.A.A.C.G.U.U.U 3')

KDM6B-02 (5' G.G.A.G.A.C.C.U.C.G.U.G.U.G.G.A.U.U.A.U.U 3')

JMJD6-01 (5' G.A.A.C.U.G.G.G.A.U.U.C.A.C.A.U.C.G.A.U.U 3')

JMJD6-02 (5' G.G.A.U.A.A.C.G.A.U.G.G.C.U.A.C.U.C.A.U.U 3')

C14ORF169-01 (5' G.C.G.A.A.G.A.A.C.C.G.C.U.U.U.C.A.U.G.U.U 3')

C14ORF169-02 (5' C.C.C.G.A.G.A.C.U.U.C.A.U.G.G.A.U.U.A.U.U 3')

PHF2-01 (5' G.C.A.A.G.C.G.C.C.U.G.A.C.G.U.C.A.A.G.U.U 3')

PHF2-02 (5' A.G.G.A.G.U.U.U.G.U.G.G.A.C.U.A.U.U.A.U.U 3')

MYCN knockdown target sequences (purchased from Origene) were as follows: siMYCN#1, ACGCUGAUACAUAACUAAAUUUGAA; siMYCN#2, 5-AGUUCAUACCUAAGUACUGUAAUAA-3.

High Content Method for H3K9me3 quantification

1000 U2OS-KDM4B expressing cells in 25 µl of media were plated into each well of a poly-D-lysine coated Perkin Elmer 384-well View plates (Perkin Elmer 6007710) with a Thermo Scientific Wellmate. The cells were then grown for 18 hours overnight before they were drugged using a VP scientific pintool with S100 pins. The cells were then treated with compound for a twenty-four hours. Following treatment, the cells were fixed with 4% formaldehyde for 20 minutes at 37°C and permeabilized with 0.1% Triton-X 100 for 15 minutes at 25°C. Fixative was removed and each well washed with PBS. Cells were blocked using 1% BSA in PBS for 1 hour at 25°C. The primary antibody against trimethyl-histone H3 at Lys9 (Millipore 07-442) was used at 1/400 dilution in 1% BSA in

PBS. This mixture was added to each well before incubation overnight at 4°C. Each well was then washed 3 times with PBS using a Biotek plate washer, and incubated for 1 hour at 25°C with a solution containing 1/400 goat α-rabbit-Alexa-488 (Cell Signaling 4412S) and 1 μM Hoechst 34580 to detect nuclear material (H21486 Molecular Probes.) Two images were captured of each well at 10X using a GE Healthcare InCell 6000 at 405 to detect nuclear staining and 488 nM to detect H3K9me3. The number of nuclear objects in each well, as detected through Hoechst staining, was compared to the number of cells in each well expressing a minimum amount of H3K9me3 as determined by Alexa-488 fluorescence (1.5 million counts total intensity), to identify the percentage of cells in each considered "H3K9me3 Positive." Averages shown are the result of eight replicate measurements per data point.

Western Blotting

Antibodies H3K9me2 (4658), H3K9me1 (9538), H3K4me3 (9751), H3K4me2 (9725), H3K27me3 (9733), H3K27me2 (9728), H3K36me3 (9763), H3K36me2 (2901), H3K79me3 (4260), H3K79me2 (5427), Histone H3 (9715) were purchased from Cell Signaling. H3K9me3 (07-523), H3K9me2 (07-441), H3K9me1 (107-450), Phosphorylated H2AX (05-636), Acetyl H3 (06-599) were purchased from Millipore. KDM4B (A301-478A), KDM4C (A300-885A), KDM6A (A302-374A), KDM5A (A300-897A) and PHF8 (A301-772A) were purchased from Bethyl Laboratories. β-actin (A1978) antibody was purchased from Sigma. Anti-HA (3F10) antibody was purchased from Roche.

ENCODE data analysis

BigWig files for ChIP-seq data of H3K9me3, Myc and KDM4A from human embryonic stem cells were downloaded from UCSC/ENCODE. Coverage at TSS +/- 2500bp of RefSeq genes was generated in 100 bins for each mark and the combined data were clustered using R.