Supplementary Information

Supplementary Figures 1 – 5

Supplementary Tables 1, 6 & 7

The role of histone demethylase PHF2 as a tumour suppressor in hepatocellular carcinoma by regulating SRXN1

Dexter Kai Hao Thng[1,2], Lissa Hooi[1,2], Wai Khang Yong[2], Dennis Kappei[2,3,4], Tan Boon Toh[1,4,5,*], Edward Kai-Hua Chow[1,2,4,5,*]

[1]The N.1 Institute for Health, National University of Singapore, Singapore, Singapore

[2]Cancer Science Institute of Singapore, National University of Singapore, Singapore, Singapore

[3]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

[4]NUS Centre for Cancer Research (N2CR), Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

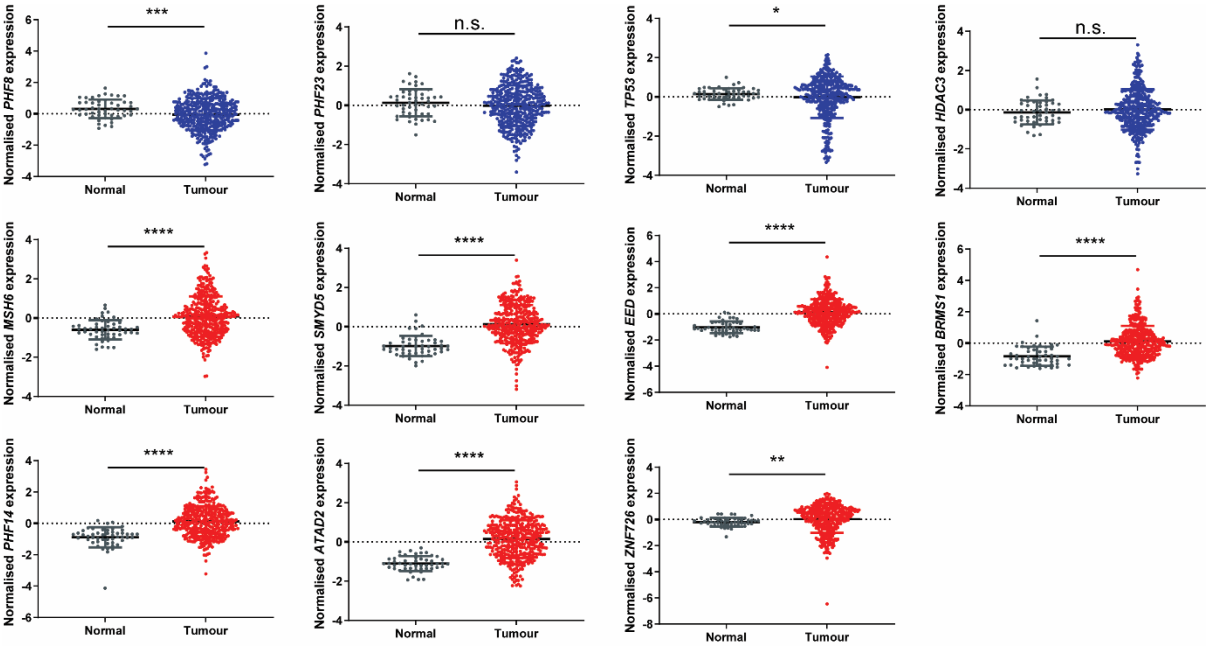
[5]The Institute for Digital Medicine (WisDM), Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

***Co-corresponding authors:**

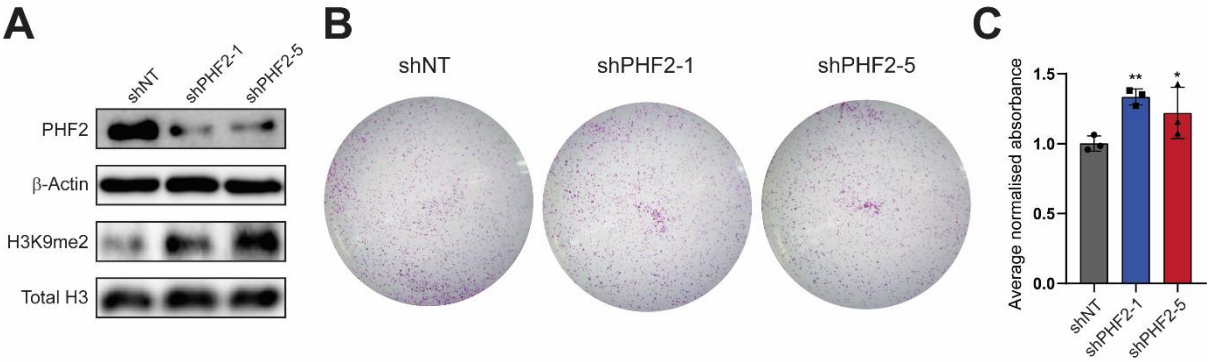
Tan Boon Toh (lsittb@nus.edu.sg), Edward Kai-Hua Chow (csikce@nus.edu.sg)

Running title: PHF2 as a tumour suppressor in hepatocellular carcinoma

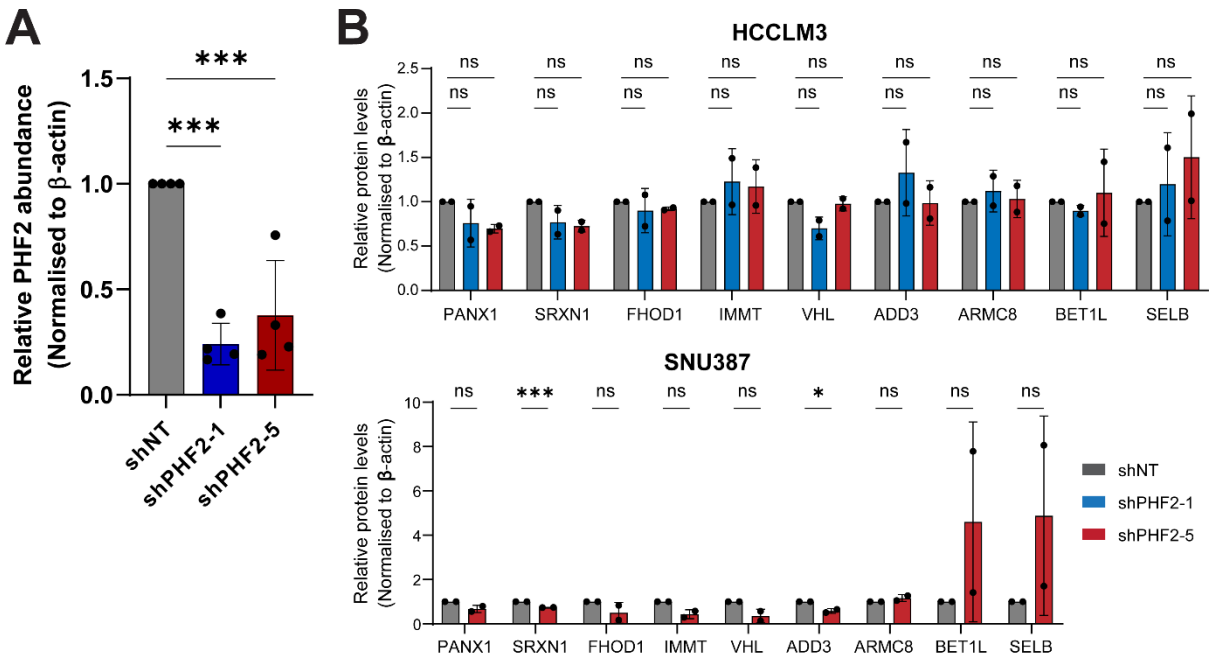
Supplementary Figures



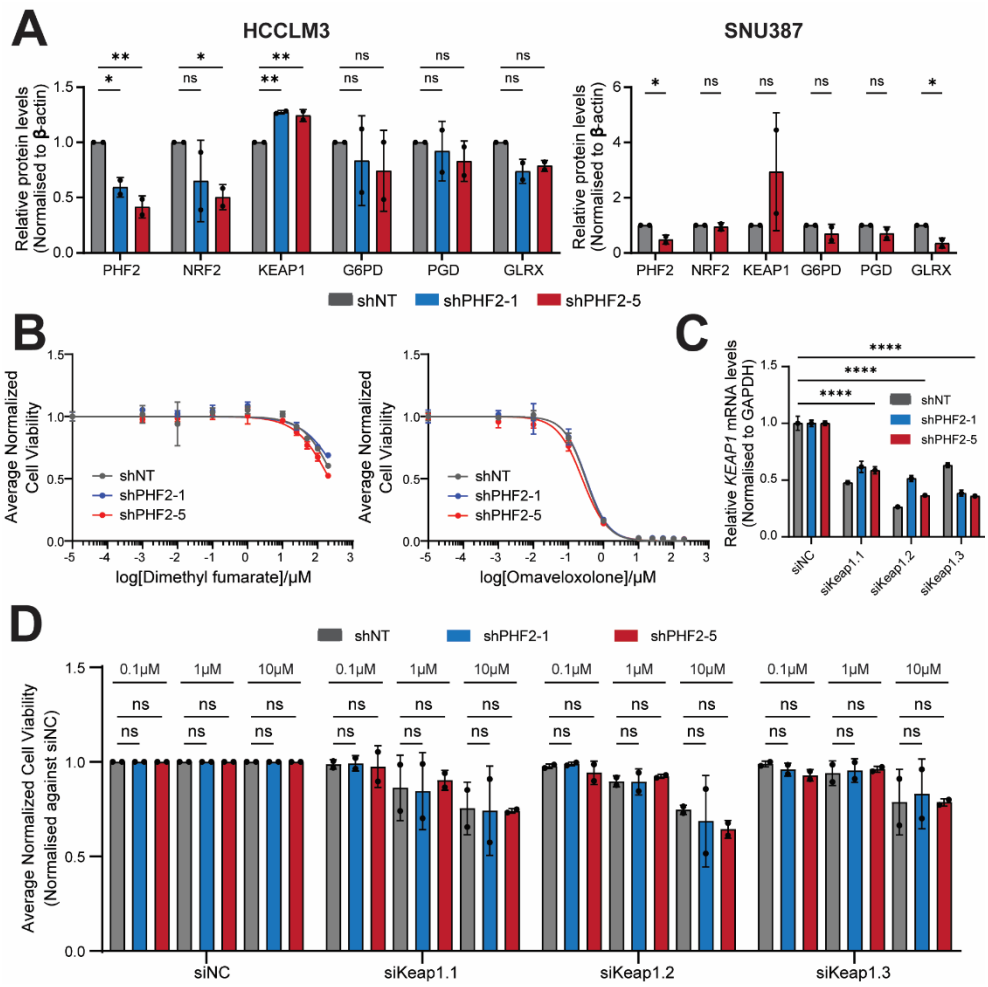
Supplementary Figure 1. Clinical expression of hits from epigenetic esiRNA library screen in HCC patients. Relative expression of hits from epigenetic esiRNA library screen in normal tissues (n = 50) and HCC tumours (n = 374) from the GDC TCGA-LIHC patient cohort. Unpaired t-tests with and without Welch's correction, where appropriate, were performed (n.s.: not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). Transcript expression values (log2(FPKM-UQ+1)) from TCGA were normalized by z-score transformation using the cohort mean and standard deviation prior to downstream analyses



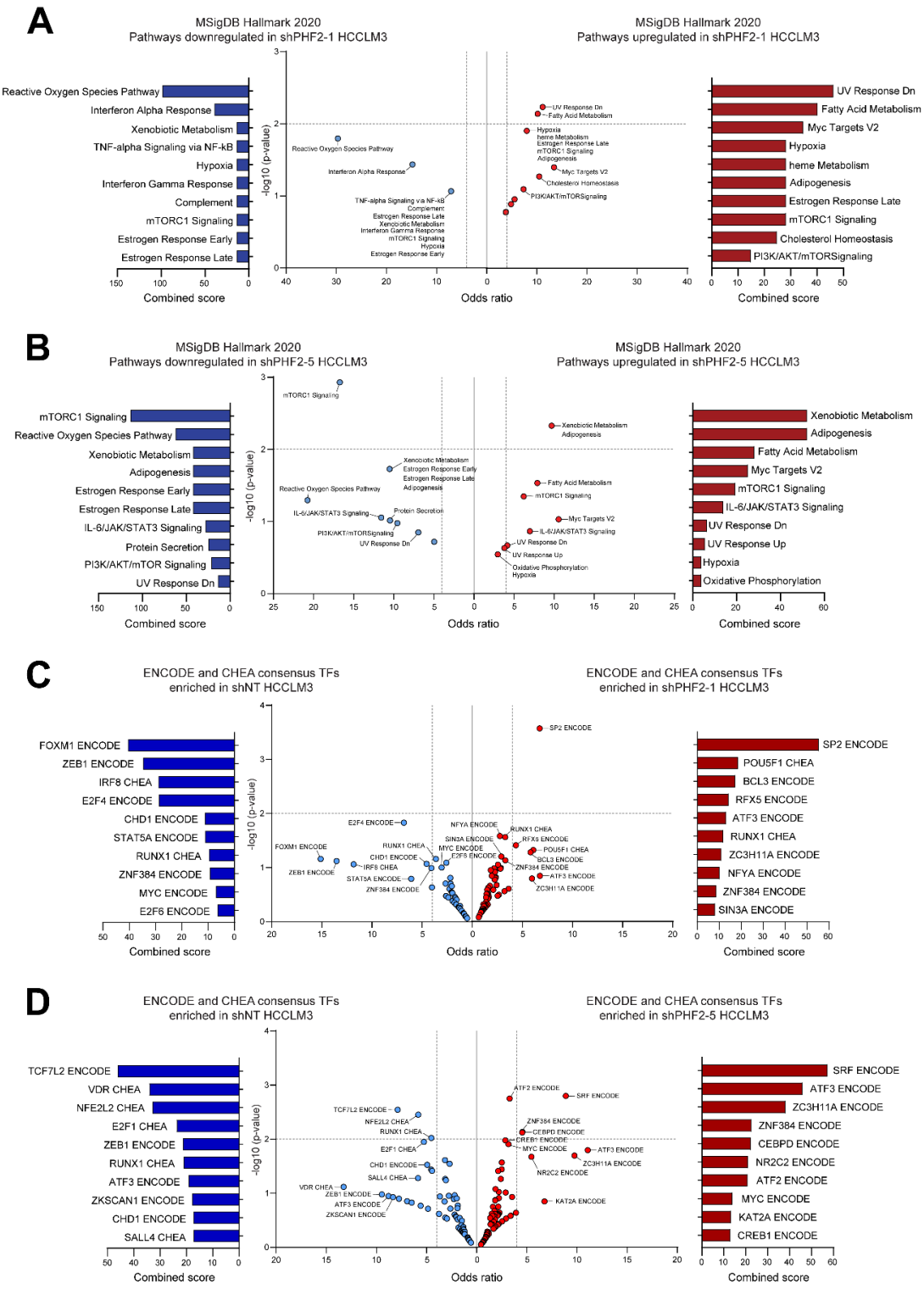
Supplementary Figure 2. Knockdown of PHF2 inhibits cell growth in HCCLM3. (A) Immunoblot of PHF2 and H3K9me2 in shPHF2 HCCLM3 cells. β-Actin and total H3 were used as the loading controls. **(B)** Brightfield images and **(C)** average absorbance of crystal violet-stained HCCLM3 cells following stable knockdown of PHF2 (shPHF2-1 and shPHF2-5) and transduction of non-targeting control (shNT). Ordinary one-way ANOVA and Dunnett's pairwise comparisons were performed as recommended (*$p < 0.05$; **$p < 0.01$). Data represented as means ± SD (n = 3).



Supplementary Figure 3. Proteomic analysis in PHF2-knockdown HCC cells. (A) Densitometry analysis of PHF2 immunoblots of protein lysates isolated from shPHF2 cells used for label-free mass spectrometry. ANOVA and Dunnett's pairwise comparisons were performed as recommended. (***$p <$ 0.001). Data represented as means ± SD (n = 4). **(B)** Densitometry analyses for immunoblot in Figure 4E. ANOVA and Dunnett's pairwise comparisons were performed as recommended for HCCLM3. Unpaired t-tests were performed for SNU387. (ns: not significant; *$p < 0.05$; ***$p < 0.001$). Data represented as means ± SD (n = 2).



Supplementary Figure 4. Stable knockdown of PHF2 downregulates SRXN1 independent of Nrf2 pathway activity. (A) Densitometry analyses for immunoblot in Figure 5B. ANOVA and Dunnett's pairwise comparisons were performed as recommended for HCCLM3. Unpaired t-tests were performed for SNU387. (ns: not significant; *$p < 0.05$; **$p < 0.01$). Data represented as means ± SD (n = 2). **(B)** Representative dose-response curves of Keap1 inhibitors, dimethyl fumarate and omaveloxolone, in shPHF2 HCCLM3. **(C)** Relative gene expression of *KEAP1* in shPHF2 HCCLM3 cells following siRNA-mediated knockdown of Keap1. GAPDH was used as the housekeeping internal control. Two-way ANOVA and Dunnett's pairwise comparisons were performed as recommended. (****p < 0.0001). Data represented as means ± SD (n = 3 replicates). **(D)** Average normalised viability of shPHF2 HCCLM3 following siRNA-mediated knockdown of Keap1 at different siRNA concentrations. ANOVA and Dunnett's pairwise comparisons were performed as recommended. (ns: not significant). Data represented as means ± SD (n = 2).



Supplementary Figure 5. Pathway analysis in stable PHF2-knockdown HCCLM3 cells. **(A)** Pathway enrichment analysis of significantly altered proteins in shPHF2-1 and **(B)** shPHF2-5 HCCLM3 compared to shNT control**,** using the MSigDB Hallmark 2020 database on Enrichr (https://maayanlab.cloud/Enrichr/). **(C)** Upstream transcription factor enrichment analysis of significantly differentially regulated proteins from the PHF2-knockdown proteomics dataset in shPHF2-1 and **(D)** shPHF2-5 HCCLM3 compared to shNT control, performed using the ENCODE and ChEA Consensus TFs from ChIP-X database from Enrichr (https://maayanlab.cloud/Enrichr/).

Supplementary Tables

Supplementary Table 1. List of targets in the pre-arrayed epigenetic esiRNA library.

KIF11	*DPF2*	*MTA1*	*SCMH1*	*UBE2B*	*HLTF*	*MPHOSPH8*	*SMYD4*	*BANF2*	*PDS5B*
RLUC	*DPF3*	*MTA3*	*SCML2*	*UHRF1*	*HMGB1*	*MSL3*	*SP140L*	*BBX*	*PES1*
AIRE	*EED*	*NAP1L1*	*SCML4*	*UTY*	*HMGN1*	*MST1*	*SUDS3*	*BDP1*	*JADE1*
AKAP1	*ELP3*	*NCOA1*	*SETDB1*	*WDR5*	*HMGN2*	*MTA2*	*SUPT7L*	*BOD1*	*PRMT9*
ASH1L	*EP300*	*NCOA2*	*SETDB2*	*WHSC1*	*HMGN3*	*MTF2*	*SUZ12*	*BTAF1*	*PRR12*
ASXL1	*EP400*	*NCOA3*	*SETMAR*	*WHSC1L1*	*HMGN4*	*MUM1*	*TADA2A*	*MRGBP*	*PWWP2A*
ATF2	*EPC1*	*NCOR1*	*SHPRH*	*ACTL6A*	*HMGN5*	*MYSM1*	*TADA2B*	*CDC5L*	*RCOR2*
ATRX	*EZH1*	*NCOR2*	*SIN3A*	*ACTL6B*	*HP1BP3*	*NAT10*	*TAF8*	*CHMP1A*	*RERE*
AURKB	*EZH2*	*NO66*	*SIN3B*	*ACTR5*	*ING2*	*NBN*	*TDRD12*	*CHMP2A*	*REV1*
BAZ1A	*FMR1*	*NSD1*	*SIRT2*	*ACTR6*	*INO80*	*PARP1*	*TDRD6*	*CHMP4B*	*RSAD1*
BAZ1B	*GTF3C4*	*PADI4*	*SIRT3*	*ACTR8*	*INO80B IN080B-WBP1*	*PARP2*	*TDRD7*	*CHMP4C*	*SCML1*
BAZ2A	*HAT1*	*PHC2*	*SIRT4*	*ALKBH1*	*INO80C*	*PARP3*	*TDRD9*	*CHMP5*	*SETBP1*
BAZ2B	*HDAC1*	*PHC3*	*SIRT5*	*ARID1A*	*INO80D*	*PAXIP1*	*TET1*	*CIC*	*SMC1A*
BMI1 COMMD3-BMI1	*HDAC11*	*PHF1*	*SIRT6*	*ARID1B*	*INO80E*	*PBRM1*	*TET2*	*DBF4*	*SMC1B*
BRD1	*HDAC2*	*PHF2*	*SIRT7*	*ARID2*	*INTS12*	*PHF19*	*TET3*	*DBF4B*	*SMC2*
BRD2	*HDAC3*	*PHF10*	*SMARCA1*	*ARID4A*	*JARID2*	*PHF20*	*TP53*	*DEAF1*	*SMC3*
BRD3	*HDAC4*	*PHF11*	*SMARCA2*	*ARID4B*	*JMJD1C*	*PHF20L1*	*TRIM24*	*DMTF1*	*SMC4*
BRD4	*HDAC5*	*PHF12*	*SMARCA4*	*ASF1A*	*JMJD6*	*PHF21A*	*TRIM66*	*DNTT*	*SMN2*
BRD7	*HDAC6*	*PHF13*	*SMARCA5*	*ASF1B*	*KAT2A*	*PHF23*	*TYW5*	*ERCC6L*	*SNAPC4*
BRD8	*HDAC8*	*PHF14*	*SMARCB1*	*ASXL2*	*KAT2B*	*PHF8*	*UBR7*	*FBXL19*	*SRRM2*
BRDT	*HDAC9*	*PHF3*	*SMARCC1*	*ASXL3*	*KAT5*	*PHRF1*	*UHRF2*	*FBXO10 RP11-613M10.8*	*TERF1*
BRPF3	*HDGF*	*PHF5A*	*SMARCC2*	*ATAD2*	*KAT6A*	*PRMT1*	*WDR82*	*FBXO11*	*TERF2*
CARM1	*HELLS*	*PHF6*	*SMARCD1*	*ATAD2B*	*KAT6B*	*PRMT2*	*YEATS2*	*FXR1*	*TOPBP1*
CBX1	*HIF1AN*	*PHF7*	*SMARCD3*	*ATF7IP*	*KAT7*	*PRMT5*	*YEATS4*	*GMEB1*	*TOX*

CBX3	*HIRA C22orf39*	*PHIP*	*SMARCE1*	*BAHD1*	*KAT8*	*PRMT7*	*ZCWPW1*	*GMEB2*	*TOX2*
CBX5	*HR*	*PRDM1*	*SMNDC1*	*BARD1*	*KDM1A*	*PRMT8*	*ZCWPW2 RP11-9J18.1*	*HBP1*	*TOX3*
CBX7	*ING1*	*PRDM2*	*SMYD1*	*BPTF*	*KDM1B*	*PSIP1*	*ZGPAT RP4-583P15.15*	*HMGA1*	*TRAF7*
CBX8	*ING3*	*PRDM10*	*SMYD2*	*BRD9*	*KDM2A*	*PWWP2B*	*ZHX1*	*HMGB2*	*TRDMT1*
CDY1	*ING5*	*PRDM11*	*SMYD3*	*BRWD1*	*KDM2B*	*RAD54B FSBP*	*ZMYND11*	*HN1*	*TRERF1*
CDY2A	*KDM4E*	*PRDM13*	*SMYD5*	*BRWD3*	*KDM3A*	*RAD54L2*	*ZMYND8*	*HN1L*	*TSPY8 TSPY4 TSPY10 TSPY7P TSPY9P TSPY1 TSPY6P*
CDYL	*KDM7A*	*PRDM15*	*SND1*	*CDY1B*	*KDM3B*	*RBX1*	*ZRANB3*	*ITIH4 RP5-966M1.6*	*TSPYL1*
CDYL2	*KMT2A*	*PRDM4*	*SP100*	*CDY2B*	*KDM4A*	*RCOR1*	*HSPBAP1*	*JMJD4*	*TSPYL2*
CHAF1A	*KMT2B*	*PRDM6*	*SP110 AC009950.1*	*CECR2*	*KDM4B*	*RCOR3*	*MGMT*	*JMJD7-PLA2G4B JMJD7*	*TSPYL4*
CHAF1B	*KMT2C*	*PRDM8*	*SP140*	*CHD7*	*KDM4C AL513412.1 RP11-146814.1*	*RPS6KA3*	*NAP1L3*	*JMJD8*	*TSPYL5*
CHD1	*KMT2D*	*PRDM9*	*SRCAP RP11-2C24.9*	*CHD8*	*KDM4D*	*RSF1*	*JADE2*	*KTI12*	*TSPYL6*
CHD1L	*KMT2E*	*PRMT3*	*SSRP1*	*CHD9*	*KDM5A*	*RUVBL1*	*JADE3*	*MDM2*	*TTF2*
CHD2	*KMT5A*	*PRMT6*	*STK31*	*CHRAC1*	*KDM5B*	*SATB2*	*WDR11*	*MDM4*	*TXNDC9*
CHD3	*KMT5B*	*PYGO1*	*SUV39H1*	*CXXC1*	*KDM5C*	*SETD1A*	*ACTR1A*	*MIER1*	*UBTF*
CHD4	*KMT5C*	*PYGO2*	*SUV39H2*	*DAPK3*	*KDM5D*	*SETD1B*	*ACTR2*	*MIER2*	*VEZF1*
CHD5	*L3MBTL2*	*RAI1*	*TAF1*	*DIDO1*	*KDM6A*	*SETD2*	*ACTR3*	*MIER3*	*ZFAT*

CHD6	*LBR*	*RBBP4*	*TCF19*	*DNAJC1*	*KDM6B*	*SETD3*	*AD000671.3*	*MIS18BP1*	*ZHX2*
CLOCK	*MBD3*	*RBBP5*	*TCF20*	*DNAJC2*	*KDM8*	*SETD4*	*AHCTF1 AC009487.6*	*MLLT3*	*ZHX3*
CREBBP	*MBD4*	*RBBP7*	*TDRD1*	*EHMT1*	*L3MBTL1*	*SETD5*	*ALG13*	*MOCS1*	*ZNF726*
CTCF	*MBD5*	*RNF17*	*TDRD3*	*EHMT2*	*L3MBTL3*	*SETD6*	*ARID3A*	*MORF4*	*BRMS1*
DMAP1	*MBTD1*	*RNF2*	*TDRD5*	*EPC2*	*L3MBTL4*	*SETD7*	*ARID3B*	*MUM1L1*	*IDH1*
DNMT1	*MECP2*	*RNF20*	*TDRKH*	*ERCC6*	*MBD6*	*SFMBT1*	*ARID3C*	*NAP1L5*	*IDH2*
DNMT3A	*MLLT10*	*RNF40*	*TP53BP1*	*G2E3*	*MECOM*	*SMARCAD1*	*ARID5A*	*NPTXR*	*ING4*
DNMT3B	*MLLT6*	*RPS6KA4*	*TRIM28*	*GLYR1*	*MGEA5*	*SMARCAL1*	*ARID5B*	*ORC1*	*MEN1*
DOT1L	*MORF4L1*	*RPS6KA5*	*TRIM33*	*HDAC7*	*MINA*	*SMARCD2*	*ATF7IP2*	*ORC2*	*SIRT1*
DPF1	*MSH6*	*SATB1*	*UBE2A*	*HDGFRP2*	*MORF4L2*	*SMN1*	*BANF1*	*PDS5A*	

Supplementary Table 6. List of primers.

Gene name	Direction	Primer sequence
GAPDH	Forward	AAG GTG AAG GTC GGA GTC AA
	Reverse	AAT GAA GGG GTC ATT GAT GG
PHF2	Forward	CTC CCC TAC GAC GTT ACC C
	Reverse	CAG TGG TAT ATG TCG ATG TCG G
MBD4	Forward	TGG TGG TGC ATG CCT GTA AT
	Reverse	TGA GAC AGG GTC TCT CTC TGT CAT
SUPT7L	Forward	GAA TCC CAA TGC ACC ATT CCA
	Reverse	CTG AGT TCA GTC ACA GGT TCC
PRMT5	Forward	CCT GTG GAG GTG AAC ACA GT
	Reverse	AGA GGA TGG GAA ACC ATG AG
NSD1	Forward	AGG TGT AGA ACA CGA TCC CG
	Reverse	AGC CGA CCT TTA GAT GCA GA
SMARCC1	Forward	AGC TGT TTA TCG ACG GAA GGA
	Reverse	GCA TCC GCA TGA ACA TAC TTC TT
TRAF7	Forward	TCT GCG CTC CAC ATT CTC AC
	Reverse	ACG GGA CAC TTC TCT GAC TT
HMGN1	Forward	GCG AAG CCG AAA AAG GCA G
	Reverse	TCC GCA GGT AAG TCT TCT TTA GT
A1CF	Forward	TGT GGA CAA CTG CCG ATT ATT T
	Reverse	TGA CAT CGA CAA CAC CTT CAG TA
ADD3	Forward	TGG CCA ATT CTT TCT CGG GT
	Reverse	GGT GTG CCC ATC CAA ACA AG
ARMC8	Forward	ATC CGC ATG AGC GTC CTT TC
	Reverse	CTT TCT GGG GAT CAG GGT CA
BET1L	Forward	CTC GGA TTT CAC AAG CAT GAC C
	Reverse	GAA GGC CAC AAT TAG ACC CAC
EEFSEC	Forward	ACT TCA GCC ATG CAA GGA GAC
	Reverse	TGG CCC ACT GTA ATG TGG AAC
FAM65A / RIPOR1	Forward	GGC GAG TTT CAT CTC CGA AT
	Reverse	AGA CAC TGC CCA CAA CCA CA
FHOD1	Forward	GGG TCA ACG CTA TCT TGG AAA
	Reverse	CAG CCC CTC TGA ATG CAC AA
IMMT	Forward	CGA TTC AGT CGG GTC CAC TAA
	Reverse	AGC TGG AGT ATC TCC CTT TTG T
NOC4L	Forward	GTG GAT GAG ACA CCG CTA TCA
	Reverse	GTT GCC TTC CCA CTT GGA CT
PANX1	Forward	CCA CGG AGT ACG TGT TCT CG
	Reverse	CCG CCC AGC AAT ATG AAT CC
POLG2	Forward	GCT TAG CCG GGA TTC TCT TCT
	Reverse	CCG AGG TCC ACC ATT CTG C
SRXN1	Forward	AAG GGT GTA CCT GGG AGC AT
	Reverse	CGC CAG GTG CAA AGA GAA TG
VHL	Forward	GCA GGC GTC GAA GAG TAC G
	Reverse	CGG ACT GCG ATT GCA GAA GA

FABP4	Forward	ACT GGG CCA GGA ATT TGA CG
	Reverse	CTC GTG GAA GTG ACG CCT T
CEBPA	Forward	CTC GCC ATG CCG GGA GAA CTC TA
	Reverse	GAG GTG ACT GCT CAT CGG GGG C
KEAP1	Forward	CTG GAG GAT CAT ACC AAG CAG G
	Reverse	GAA CAT GGC CTT GAA GAC AGG
NFE2L2	Forward	TTC CCG GTC ACA TCG AGA G
	Reverse	GCA TAC CGT CTA AAT CAA CAG GG
GSR	Forward	TTC CAG AAT ACC AAC GTC AAA GG
	Reverse	GTT TTC GGC CAG CAG CTA TTG
GLRX	Forward	CCC ATC AAA CAA GGG CTT CTG
	Reverse	CTG CAT CCG CCT ATA CAA TCT T
G6PD	Forward	CGA GGC CGT CAC CAA GAA C
	Reverse	GTA GTG GTC GAT GCG GTA GA
PGD	Forward	GAC ATC ATC ATT GAC GGA GGA AA
	Reverse	GGG CCA CGC TTC TTT GTT C
NQO1	Forward	GGA TTG GAC CGA GCT GGA A
	Reverse	AAT TGC AGT GAA GAT GAA GGC AAC
GSTM3	Forward	GGA GGC AAG GGA CGG AGA
	Reverse	TTC CGA GCC TTC GAG GAC TAG
GSTA3	Forward	CAA AGA AAC CAA GAG ACT GT
	Reverse	CCA AAT CTT CTG CAG ATC C

Supplementary Table 7. List of antibodies.

Protein target	**Brand**	**Catalogue number**
PHF2	Cell Signaling Technology	# 3497
H3K9me2	Cell Signaling Technology	# 4658
Total H3	Cell Signaling Technology	# 9715
Ki-67	Abcam	ab279653
ADD3	Santa Cruz Biotechnology	sc-365178
ARMC8	Santa Cruz Biotechnology	sc-365307
BET1L	Santa Cruz Biotechnology	sc-135846
EEFSEC	Santa Cruz Biotechnology	sc-166521
FHOD1	Santa Cruz Biotechnology	sc-365437
G6PD	Santa Cruz Biotechnology	sc-373886
GLRX	Santa Cruz Biotechnology	sc-293250
IMMT	Santa Cruz Biotechnology	sc-390707
KEAP1	Santa Cruz Biotechnology	sc-365626
NRF2	Cell Signaling Technology	# 12721
PANX1	Santa Cruz Biotechnology	sc-515941
PGD	Santa Cruz Biotechnology	sc-398977
SRXN1	Santa Cruz Biotechnology	sc-514940
VHL	Santa Cruz Biotechnology	sc-135657
β-Actin	Sigma	A5441