

Supplementary Materials for

Epigenetic Activation of AP-1 Promotes Squamous Cell Carcinoma Metastasis

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Fig. S1. KDM4A is required for the abundance of JUN and FOSL1 induced by HGF.

Fig. S2. KDM4A, but not KDM4C, is required for the abundance of JUN and FOSL1 induced by HGF.

Fig. S3. KDM4A knockdown does not cause global changes of H3K9me3 and H3K36me3

Fig. S4. JUN and KDM4A co-occupy on the promoters of *FOSL1* and *JUN* in SCC23/MET cells.

Fig. S5. Over-expression of JUN cannot restore the abundance of FOSL1 in KDM4A-depleted cells.

Fig. S6. Inefficient KDM4A knockdown might be responsible for lymph node metastasis from KDM4A-depleted SCC23/MET cells.

Table S1. A list of genes that were repressed by KDM4A knockdown

Table S2. A list of genes that were more abundantly expressed by KDM4A knockdown

Table S3. Real-time RT-PCR primers for human histone demethylases

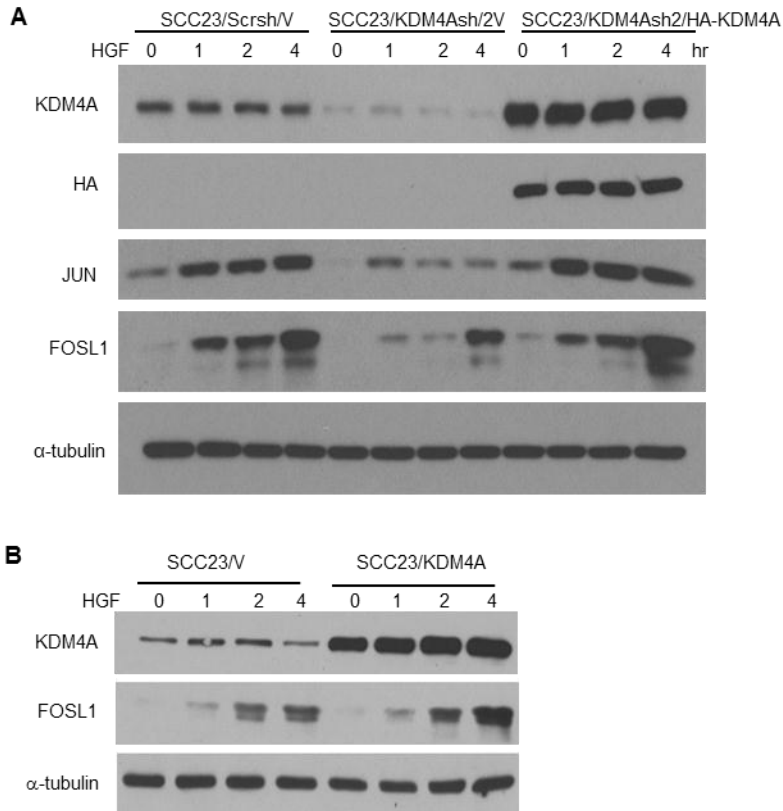


Fig. S1. KDM4A is required for the abundance of JUN an FOSL1 induced by HGF. (A)

Western blot analysis of the abundance of JUN an FOSL1 induced by HGF in SCC23 control (SCC23/Scrsh/V), KDM4A-depleted (SCC23/KDM4Ash/V) and KDM4A-restored (SCC23/KDM4Ash/HA-KDM4A) cells. (B) Western blot analysis of the abundance of FOSL1

in cells overexpressing KDM4A (SCC23/KDM4A) compared with control cells (SCC23/V).

Data are representative of 2 experiments.

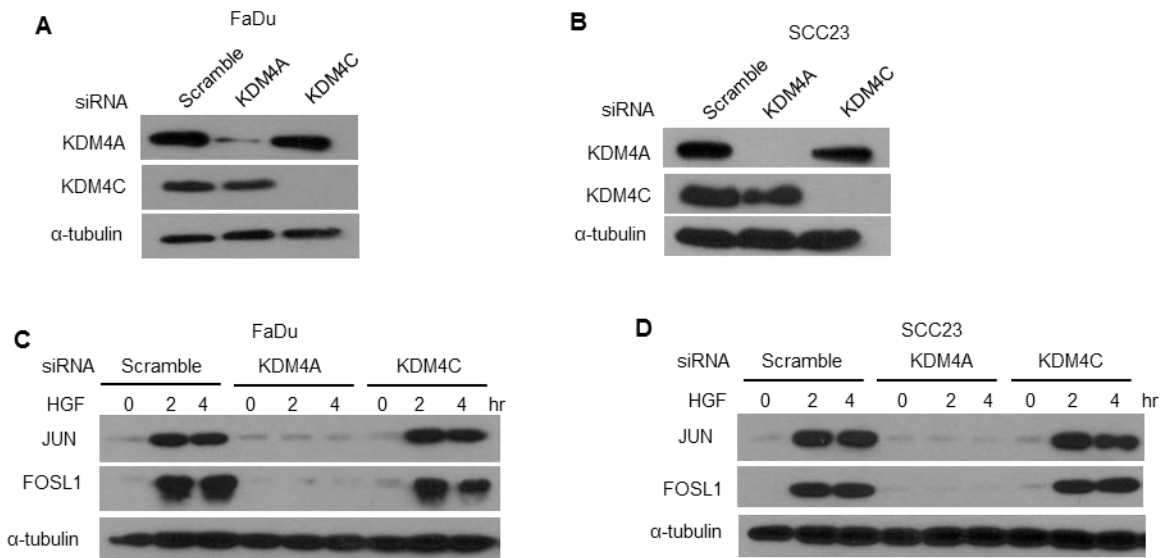


Fig. S2. KDM4A, but not KDM4C, is required for the abundance of JUN and FOSL1 induced by HGF. (A and B) Western blot analysis showing KDM4A and KDM4C knockdown by siRNA in (A) FaDu cells or (B) SCC23 cells. (C and D) Western blot analysis of the abundance of JUN and FOSL1 induced by 20 ng/ml of HGF in KDM4A- or KDM4C-depleted FaDu (C) or SCC23 cells (D). Data are representative of 2 experiments.

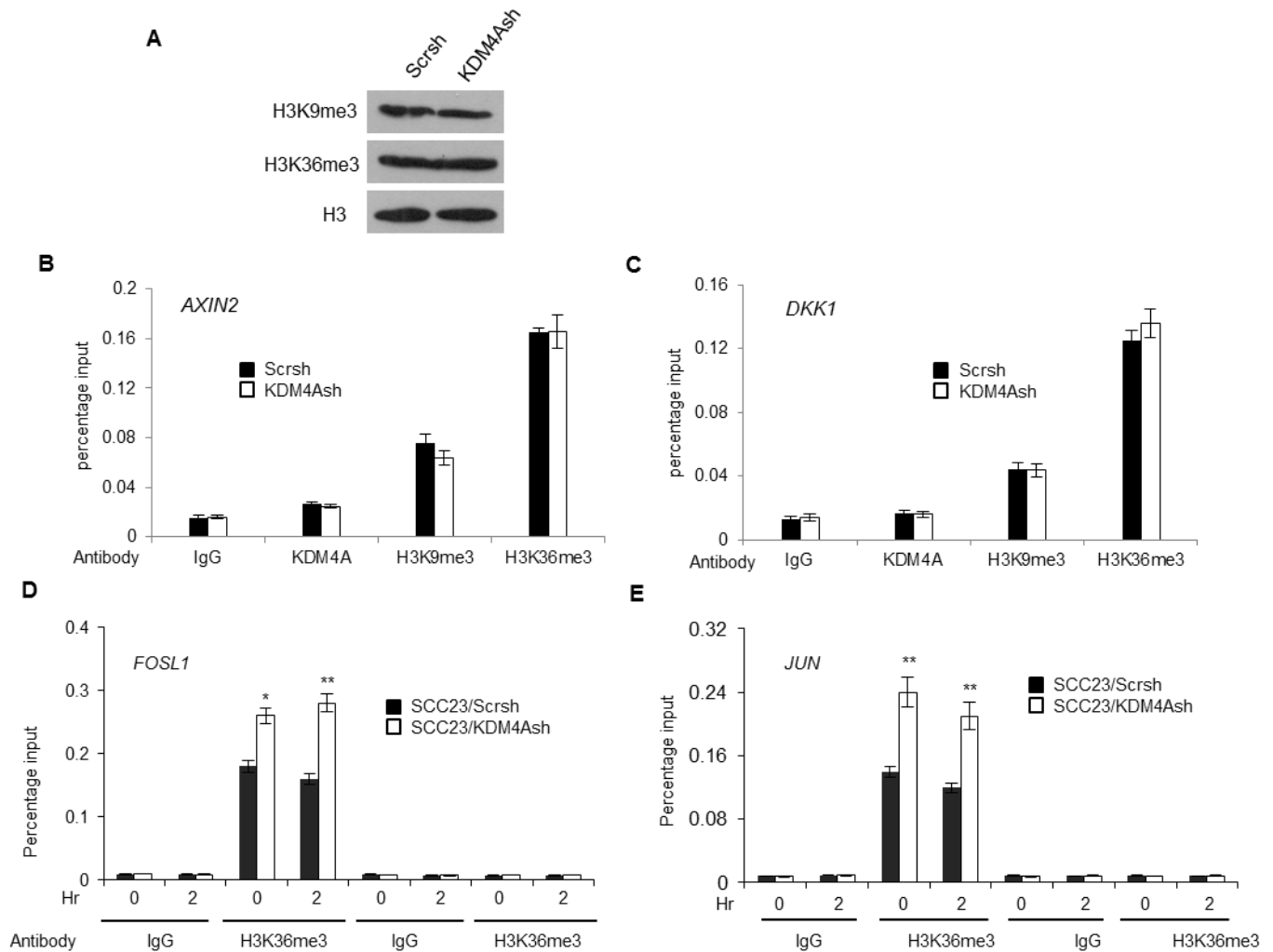


Fig. S3. KDM4A knockdown does not cause global changes of H3K9me3 and H3K36me3.

(A) Western blot analysis of H3K9me3 and H3K36me3 marks in KDM4A-depleted cells. Scrsh, SCC23/MET/Scrsh cells; KDM4Ash2, SCC23/MET/KDM4Ash2 cells. Blot is representative of 2 experiments. (B and C) ChIP assays of KDM4A, H3K9me3 and H3K36me3 on the (B) *AXIN2*

or (C) *DKK1* promoter in KDM4A-depleted SCC23/MET cells. (D and E) ChIP assays of H3K36me3 changes on the (D) *FOSL1* or (E) *JUN* promoter in KDM4A-depleted cells, either at the AP-1 site or 8kb upstream. Data in (B to E) are means \pm s.d. of 3 experiments. * $P < 0.05$; ** $P < 0.01$, unpaired two-tailed Student's *t*-test.

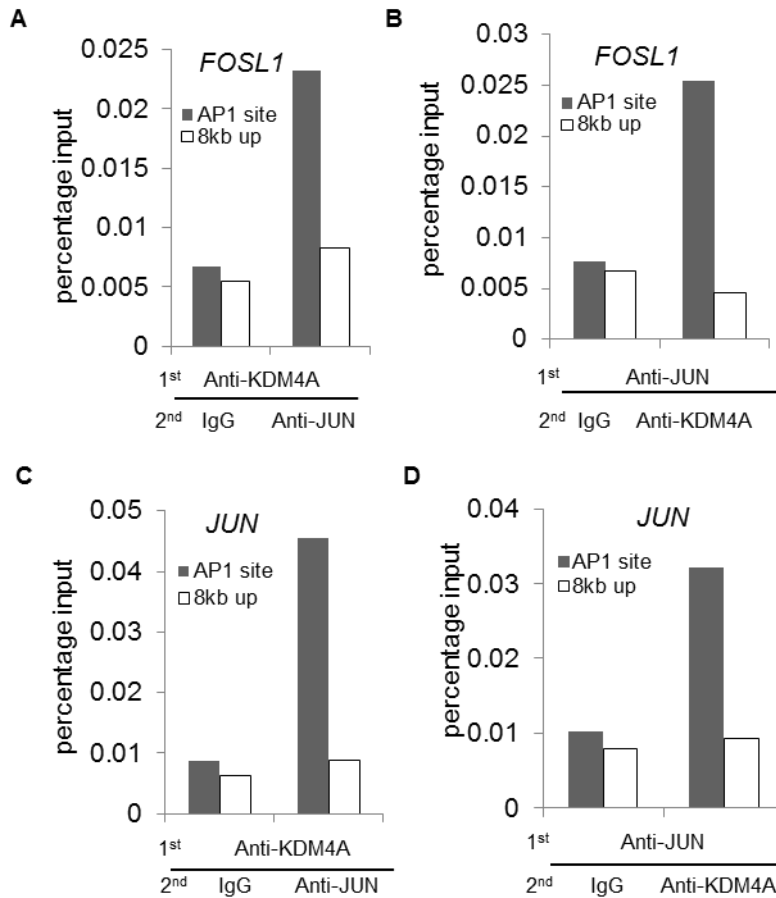


Fig. S4. JUN and KDM4A co-occupy on the promoters of *FOSL1* and *JUN* in SCC23/MET cells. (A and B) Reciprocal re-ChIP assays of the co-occupancy of JUN and KDM4A on the *FOSL1* promoter in SCC23/MET cells. The result represents one of three independent experiments. (C and D) The reciprocal re-ChIP assays of the co-occupancy of JUN and KDM4A on the *JUN* promoter in SCC23/MET cells. The data represents one of three independent experiments.

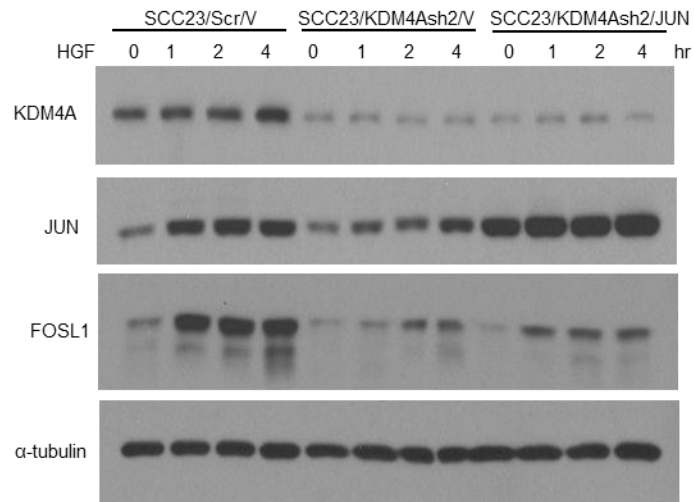


Fig. S5. Over-expression of JUN cannot restore the abundance of FOSL1 in KDM4A-depleted cells. Western blot analysis of the abundance of JUN and FOSL1 in SCC23 cells transduced with control shRNA and vector (SCC23/Scr/V), KDM4A-depleted SCC23 cells (SCC23/KDM4Ash2/V), and KDM4A-depleted SCC23 cells overexpressing JUN (SCC23/KDM4Ash2/JUN) treated with 20 ng of HGF per ml for up to 4 hours.

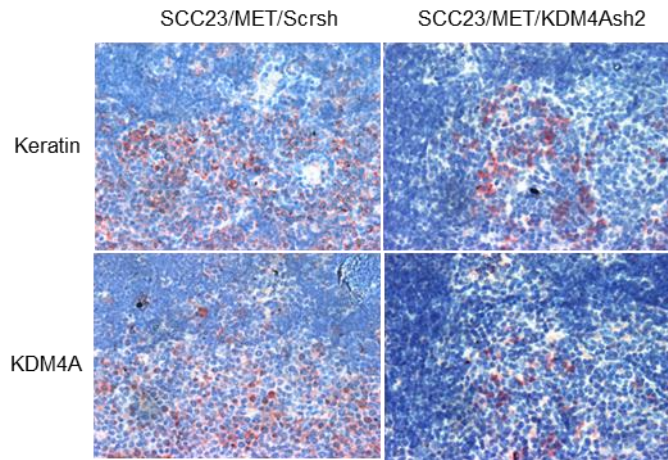


Fig. S6. Inefficient KDM4A knockdown might be responsible for lymph node metastasis from KDM4A-depleted SCC23/MET cells. Histological analysis of the abundance of KDM4A in lymph node metastasis from mice with tumor xenografts of either SCC23/MET/Scrsh or SCC23/MET/KDM4Ash2 cells. Cells were stained with antibodies against KDM4A and keratin (red) and counterstained with DAPI (blue).

Table S1. A list of genes that were repressed by KDM4A knockdown

Gene expression profiling of SCC23/MET/Scrsh and SCC23/MET/KDM4Ash2 cells was compared.

Genes which had decreased expression by at least two-fold after KDM4A knockdown were listed.

Probe id	Gene Symbol	Fold Change
201041_s_at	DUSP1	0.09732242
226682_at	RORA	0.1078467
242517_at	KISS1R	0.11411252
209183_s_at	C10orf10	0.11673515
222847_s_at	EGLN3	0.12201877
223333_s_at	ANGPTL4	0.12437738
219232_s_at	EGLN3	0.13060744
210479_s_at	RORA	0.132921
221009_s_at	ANGPTL4	0.13939667
212143_s_at	IGFBP3	0.14458242
210095_s_at	IGFBP3	0.14884875
235857_at	KCTD11	0.1560953
210426_x_at	RORA	0.16137589
227868_at	LOC154761	0.16940014
219410_at	TMEM45A	0.18543005
227337_at	ANKRD37	0.1893493
202672_s_at	ATF3	0.19179207
201170_s_at	BHLHE40	0.21027482
236266_at	RORA	0.21060667
201044_x_at	DUSP1	0.2147104
225557_at	CSRNPI	0.22307891
224797_at	ARRDC3	0.22714761
202887_s_at	PPP2R1B	0.22764963
202219_at	SLC6A8	0.23013575
212099_at	RHOB	0.23181904
209034_at	PNRC1	0.23414482
203438_at	STC2	0.23857768
224657_at	ERRFI1	0.24359527
215813_s_at	PTGS1	0.24913817
205969_at	AADAC	0.25156079
201694_s_at	EGR1	0.25280204
212188_at	KCTD12	0.25419702
207574_s_at	GADD45B	0.25696397
211559_s_at	CCNG2	0.26673629
203439_s_at	STC2	0.2707417
221479_s_at	BNIP3L	0.27305434
227404_s_at	EGR1	0.27428849
226452_at	PDK1	0.27762666
210854_x_at	SLC6A8	0.27807308
204614_at	SERPINB2	0.28218046
1564027_a_at	FAM115C	0.28636737
202770_s_at	CCNG2	0.29501455
213843_x_at	SLC6A8	0.29682675
227697_at	SOCS3	0.29714273
209305_s_at	GADD45B	0.30295323
207517_at	LAMC2	0.30511986
201465_s_at	JUN	0.30732861

203504_s_at	ABCA1	0.30854119
203505_at	ABCA1	0.31018353
209822_s_at	VLDLR	0.31421697
212110_at	SLC39A14	0.3164953
230630_at	AK3L1	0.31721436
225342_at	AK3L1	0.31779889
211708_s_at	SCD	0.31784405
205476_at	CCL20	0.31880651
227038_at	SGMS2	0.31958088
200831_s_at	SCD	0.32225821
1554008_at	OSMR	0.32327369
202769_at	CCNG2	0.32433531
208893_s_at	DUSP6	0.3253729
202364_at	MXI1	0.32719033
205199_at	CA9	0.3276951
223394_at	SERTAD1	0.32926544
221478_at	BNIP3L	0.33253106
216867_s_at	PDGFA	0.3339037
215177_s_at	ITGA6	0.3347514
202619_s_at	PLOD2	0.33617492
202628_s_at	SERPINE1	0.33697349
228846_at	MXD1	0.33880146
205128_x_at	PTGS1	0.33962063
201169_s_at	BHLHE40	0.34033395
211162_x_at	SCD	0.34049441
204194_at	BACH1	0.34156499
201531_at	ZFP36	0.34171228
226436_at	RASSF4	0.3449942
214446_at	ELL2	0.34862671
219181_at	LIPG	0.34906679
212192_at	KCTD12	0.35279451
209457_at	DUSP5	0.35603624
201466_s_at	JUN	0.3584662
1554980_a_at	ATF3	0.3599494
202627_s_at	SERPINE1	0.36237366
238063_at	TMEM154	0.3624852
221011_s_at	LBH	0.36540544
227180_at	ELOVL7	0.36612241
1569582_at	LOC201651	0.37136429
209304_x_at	GADD45B	0.37280575
229396_at	OVOL1	0.37374055
201294_s_at	WSB1	0.3748889
205281_s_at	PIGA	0.37516314
208892_s_at	DUSP6	0.3752201
215812_s_at	LOC653562 /// SLC6A10P /// SLC6A8	0.37583231
202081_at	IER2	0.37605276
219888_at	SPAG4	0.37660133
203108_at	GPRC5A	0.37750954
226021_at	RDH10	0.37941431
234932_s_at	CDCP1	0.38056295
211527_x_at	VEGFA	0.38416953
208891_at	DUSP6	0.38467127
204036_at	LPAR1	0.38523054
222108_at	AMIGO2	0.38546907
228149_at	C7orf60	0.38776755
212368_at	ZNF292	0.38781137

201848_s_at	BNIP3	0.38784524
209566_at	INSIG2	0.38805606
201849_at	BNIP3	0.3905754
210512_s_at	VEGFA	0.39145164
202441_at	ERLIN1	0.39196697
203574_at	NFIL3	0.39218058
227719_at	SMAD9	0.39232631
202768_at	FOSB	0.39613663
203705_s_at	FZD7	0.39740943
202241_at	TRIB1	0.39756151
220239_at	KLHL7	0.40022911
219239_s_at	ZNF654	0.40213477
201626_at	INSIG1	0.40302242
200920_s_at	BTG1	0.40307522
226431_at	FAM117B	0.40527947
225366_at	PGM2	0.4062757
201464_x_at	JUN	0.40944739
205227_at	IL1RAP	0.40978185
204420_at	FOSL1	0.40994919
204668_at	RNF24	0.41027752
206686_at	PDK1	0.41044591
213281_at	LOC100288387	0.41206276
204669_s_at	RNF24	0.41573468
227458_at	CD274	0.41801337
200921_s_at	BTG1	0.41894594
205266_at	LIF	0.42233538
211719_x_at	FN1	0.42253478
224580_at	SLC38A1	0.42574781
239572_at	GJA3	0.42576964
213865_at	DCBLD2	0.42577289
216442_x_at	FN1	0.426884
225144_at	BMP2	0.42740598
209596_at	MXRA5	0.42762942
215446_s_at	LOX	0.42824936
201631_s_at	IER3	0.43071247
203987_at	FZD6	0.4322821
224606_at	KLF6	0.43286078
212464_s_at	FN1	0.43316242
224973_at	FAM46A	0.43390376
201325_s_at	EMP1	0.43499418
223423_at	GPR160	0.4352783
202620_s_at	PLOD2	0.43920306
202444_s_at	ERLIN1	0.43959504
209185_s_at	IRS2	0.44004989
229010_at	CBL	0.44019419
225524_at	ANTXR2	0.44220983
219496_at	ANKRD57	0.44223221
202696_at	OXSRI	0.44374722
203499_at	EPHA2	0.44420052
212079_s_at	MLL	0.44430892
202014_at	PPP1R15A	0.44623296
210495_x_at	FN1	0.44811161
235216_at	ESCO1	0.44843848
210139_s_at	PMP22	0.44856066
207528_s_at	SLC7A11	0.44885084
1555832_s_at	KLF6	0.44956543
201565_s_at	ID2	0.45040258

201983_s_at	EGFR	0.4504847
225946_at	RASSF8	0.45126538
209212_s_at	KLF5	0.45147844
212113_at	ATXN7L3B	0.45292152
203706_s_at	FZD7	0.45293722
227345_at	TNFRSF10D	0.45327453
208936_x_at	LGALS8	0.45402952
228328_at	KLHL28	0.45409152
201627_s_at	INSIG1	0.45581522
205910_s_at	CEL	0.45656716
228188_at	FOSL2	0.45712639
211506_s_at	IL8	0.45734222
203476_at	TPBG	0.45762888
203083_at	THBS2	0.457725
221753_at	SSH1	0.45848708
202843_at	NDUFB7	0.45861581
225492_at	TMEM33	0.45915049
203204_s_at	KDM4A	0.45942046
223839_s_at	SCD	0.4601726
226464_at	C3orf58	0.46022556
200832_s_at	SCD	0.46024597
219911_s_at	SLCO4A1	0.4628652
221766_s_at	FAM46A	0.46318903
201473_at	JUNB	0.46337207
226863_at	FAM110C	0.46431314
228498_at	B4GALT1	0.46491665
226275_at	MXD1	0.46493019
202131_s_at	RIOK3	0.4650604
202284_s_at	CDKN1A	0.4651368
224314_s_at	EGLN1	0.46518484
232291_at	MIR17HG	0.46529158
235907_at	TMEM33	0.46635969
212902_at	SEC24A	0.46674355
207543_s_at	P4HA1	0.4672333
201324_at	EMP1	0.4686413
208961_s_at	KLF6	0.46866469
205729_at	OSMR	0.46881187
208078_s_at	SIK1	0.46907971
1563900_at	FAM83B	0.46917173
226199_at	UPRT	0.46925662
228051_at	KIAA1244	0.46954033
218451_at	CDCP1	0.47012327
1554462_a_at	DNAJB9	0.47153704
209946_at	VEGFC	0.47164131
209373_at	MALL	0.47214798
219326_s_at	B3GNT2	0.47264274
223092_at	ANKH	0.472998
219549_s_at	RTN3	0.47373626
202912_at	ADM	0.47373954
1555950_a_at	CD55	0.47376253
224888_at	EPT1	0.47402038
233819_s_at	RNF160	0.47420211
1557257_at	BCL10	0.47480005
226982_at	ELL2	0.47535031
218880_at	FOSL2	0.4767104
205463_s_at	PDGFA	0.47766133
201299_s_at	MOBK1B	0.47798889

205220_at	GPR109B	0.47844633
219747_at	C4orf31	0.48005347
226621_at	OSMR	0.4800904
223059_s_at	FAM107B	0.48050722
231873_at	BMPR2	0.48084873
227810_at	ZNF558	0.48175181
222662_at	PPP1R3B	0.48188473
227268_at	RNFT1	0.48226834
201926_s_at	CD55	0.48246661
213446_s_at	IQGAP1	0.48257531
201996_s_at	SPEN	0.48282758
209920_at	BMPR2	0.48291829
225262_at	FOSL2	0.4845784
209101_at	CTGF	0.48473629
205198_s_at	ATP7A	0.48499541
227467_at	RDH10	0.48597028
221752_at	SSH1	0.48601037
217678_at	SLC7A11	0.48705851
222664_at	KCTD15	0.4879134
212665_at	TIPARP	0.48809741
210845_s_at	PLAUR	0.48834106
224675_at	MESDC2	0.48928467
202067_s_at	LDLR	0.49002559
36711_at	MAFF	0.4903324
202668_at	EFNB2	0.49216228
227101_at	ZNF800	0.49275144
242918_at	NASP	0.49279824
226899_at	UNC5B	0.49329241
201883_s_at	B4GALT1	0.4943381
223028_s_at	SNX9	0.49464418
230440_at	ZNF469	0.49514295
208960_s_at	KLF6	0.49529227
209921_at	SLC7A11	0.49539527
205865_at	ARID3A	0.49544403
211165_x_at	EPHB2	0.49678276
206662_at	GLRX	0.49710689
204029_at	CELSR2	0.49779581
202842_s_at	FUCA1	0.49847394
201925_s_at	CD55	0.49922947
229115_at	DYNC1H1	0.49956178

Table S2. A list of genes that were more abundantly expressed by KDM4A knockdown

Gene expression profiling of SCC23/MET/Scrsh and SCC23/MET/KDM4Ash2 cells was compared.

Genes which had increased expression by at least two-fold after KDM4A knockdown were listed.

Probe id	Gene Symbol	Fold Change
207339_s_at	LTB	3.830801
222717_at	SDPR	3.800539
218330_s_at	NAV2	3.581865
221232_s_at	ANKRD2	3.291787
204279_at	PSMB9	2.987768
225355_at	NEURL1B	2.949297
203153_at	IFIT1	2.84178
218032_at	SNN	2.750336
204159_at	CDKN2C	2.75005
239710_at	FIGN	2.653337
241994_at	XDH	2.611372
1555812_a_at	ARHGDIB	2.589005
1555310_a_at	PAK6	2.579088
227420_at	TNFAIP8L1	2.555685
205083_at	AOX1	2.514485
206504_at	CYP24A1	2.499747
225817_at	CGNL1	2.431342
1555758_a_at	CDKN3	2.411858
229579_s_at	DISP2	2.40829
230720_at	RNF182	2.407355
206632_s_at	APOBEC3B	2.398149
238964_at	FIGN	2.388475
224480_s_at	AGPAT9	2.381937
229450_at	IFIT3	2.367701
205547_s_at	TAGLN	2.356659
218755_at	KIF20A	2.354288
242828_at	FIGN	2.352474
201288_at	ARHGDIB	2.348331
202270_at	GBP1	2.343887
213201_s_at	TNNT1	2.328715
219461_at	PAK6	2.324283
238989_at	C1GALT1C1	2.307249
1555724_s_at	TAGLN	2.289264
238756_at	GAS2L3	2.279381
219493_at	SHCBP1	2.257145
209714_s_at	CDKN3	2.246927
221436_s_at	CDCA3	2.223754
227609_at	EPSTI1	2.223709
202954_at	UBE2C	2.218814
214858_at	PP14571	2.217766
202609_at	EPS8	2.2041
223707_at	RPL27A	2.200567
242470_at	EID2B	2.199034
228885_at	MAMDC2	2.182855
227687_at	HYLS1	2.174374
222016_s_at	ZNF323	2.168375
203562_at	FEZ1	2.166705
201896_s_at	PSRC1	2.12653
200783_s_at	STMN1	2.124477

202283_at	SERPINF1	2.108871
204962_s_at	CENPA	2.107512
205225_at	ESR1	2.09808
228933_at	NHS	2.091451
219716_at	APOL6	2.090762
203390_s_at	KIF3C	2.078011
213906_at	MYBL1	2.076351
210538_s_at	BIRC3	2.073879
203638_s_at	FGFR2	2.070224
203018_s_at	SSX2IP	2.064685
223307_at	CDCA3	2.036918
218033_s_at	SNN	2.032911
213929_at	EXPH5	2.030645
228729_at	CCNB1	2.017431
209172_s_at	CENPF	2.012435
201058_s_at	MYL9	2.010891
229068_at	CCT5	2.010091
232197_x_at	ARSB	2.002876

Table S3. Real-time RT-PCR primers for human histone demethylases

Gene Name	Forward Primers (5' – 3')	Reversed Primers (5' – 3')
KDM4A	CCTCACTGCGCTGTCTGTAT	CCAGTCGAAGTGAAGCACAT
KDM4B	CGGGTTCTATCTTTGTTTCTCTCACCCG	AAGGAAGCCTCTGGAACACCTG
KDM4C	GGCATAGGTGACAGGGTGTGTC	CGGGGACCAAACCTCTGGAAACCCG
KDM4D	CGGGATCTGCACAGATTATCCACCCG	AGTTTCTGAGGAGGGCGACCA
KDM5A	GTTTCTTAAGGTGGCAAGTC	TCTTTTGTACTGTTCCCTAC
KDM5B	AGCTTTCTCAGAATGTTGGC	GCAGAGTCTGGGAATTCACA
KDM5C	GGGTTTCTAAAGTGTAGATCT	CCACACATCTGAGCTTTAGT
KDM3A	ACCTGCAGTTATTCTTCAGC	TAATGCCAGTCCTATGCCAT
KDM3B	TGTTCCCTGGGGACTCCTCT	GGGCACTACAGTACAGCTGG
JMJD1C	TTTGTTGAAGCTATTGACTG	CACTTTAACAAAAGCAAGCC
KDM2A	CGGATAGTTGAGAAAGCCAAGATCCG	CTCTTTGGTGGGCCTCTGTAGC
KDM2B	GTTAGTGGTAGTGGTGTGTTTGG	AGCAGATGTGGTGTGTGGTC
KDM6A	CGTCCGAGTGTCAACCAACTGGACG	TGAGAGTCCTGGAGTAGGAGCAG
KDM6B	CTCAACTTGGGCCTCTTCTC	GCCTGTCAGATCCCAGTTCT
PHF2	TCGGCACTTCTCTGTTCTCCC	AAATCCAGCCCCTCCGTGTC
PTH8	CTTTCTCTACCTTGGGGACC	TAGGGACTCACGTGCTAGGG
JMJD4	GGCCCTTCCAGAAATAAAGACC	CAGGCAGTGGCCATGAACAG
JMJD5	GTGGGGAGAGCCCAGAAGGACATT	GACCCACCCGTTTCCCAAAA
HR	AGACCTCTGCCCTCTCTGCT	GCTTGGAACACAGCCCAGTC
FIH1AN	TTACTTAACCTCTCTGAGCC	CCAACAACCCTGAGGTAGAT
HSPBAP1	ACGTAGAAGCTACACTCGAAG	TAAGCCCAGAACTTGAATG
JARID2	AAGTGCTGCTTACATCACTG	AGTGGATCATAGGACGTTCC
PTDSR	AGGTGGATCACTTGAGGTCA	CACCACACCTGGCTAATTTT
JMJD7	GCCCAGGCCACACATAATTT	AGTGGTAGCTTTCCATGTGG
JHDM1D	ATCTGTGAACCTTTGGAGAGG	TAGGGACTCACGTGCTAGGG