

ORIGINAL ARTICLE

KDM6B modulates MAPK pathway mediating multiple myeloma cell growth and survival

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Recent studies have delineated cancer-type-specific roles of histone 3 lysine 27 (H3K27) demethylase KDM6B/JMJD3 depending on its H3K27 demethylase activity. Here we show that KDM6B is expressed in multiple myeloma (MM) cells; and that shRNA-mediated knockdown and CRISPR-mediated knockout of *KDM6B* abrogate MM cell growth and survival. Tumor necrosis factor- α or bone marrow stromal cell culture supernatants induce *KDM6B*, which is blocked by IKK β inhibitor MLN120B, suggesting that *KDM6B* is regulated by NF- κ B signaling in MM cells. RNA-seq and subsequent ChIP-qPCR analyses reveal that KDM6B is recruited to the loci of genes encoding components of MAPK signaling pathway including *ELK1* and *FOS*, and upregulates expression of these genes without affecting H3K27 methylation level. Overexpression of catalytically inactive *KDM6B* activates expression of MAPK pathway-related genes, confirming its function independent of demethylase activity. We further demonstrate that downstream targets of KDM6B, *ELK1* and *FOS*, confer MM cell growth. Our study therefore delineates KDM6B function that links NF- κ B and MAPK signaling pathway mediating MM cell growth and survival, and validates KDM6B as a novel therapeutic target in MM.

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INTRODUCTION

Multiple myeloma (MM) is a plasma cell malignancy that proliferates in the bone marrow.^{1,2} Although novel agents including proteasome inhibitors and immunomodulatory drugs (IMiDs) have remarkably improved MM patient survival, MM remains incurable. Hence, there is a need to decipher the pathogenesis of MM to identify novel therapeutic targets. Recent studies using whole-genome and whole-exome sequencing have identified driver mutations in MM, and revealed relevant pathways in myelomagenesis.³ For example, MAPK pathway genes, including *KRAS*, *NRAS* and *BRAF*, are mutated in about 40% of patients.^{4–6} NF- κ B pathway molecules such as *TRAF3*, *CYLD* and *LTB* are also mutated in about 20% of patients.^{4–6} These results highlight the roles of these two signaling pathways in MM. These pathways are also known to be activated by extrinsic factors in the bone marrow microenvironment.^{1,2} For example, bone marrow stromal cells (BMSCs) produce extrinsic ligands, including interleukin-6, insulin-like growth factor 1 (IGF1), tumor necrosis factor- α (TNF- α), a proliferation inducing ligand (APRIL) and B cell activating factor (BAFF), further activating MAPK and NF- κ B pathways. Thus, MM cells are largely dependent on these two major pathways.

Lysine demethylase 6B (KDM6B, also known as JMJD3) is a Jumonji C-domain-containing histone demethylase, which removes histone 3 lysine 27 (H3K27) di- and tri-methylation (me2/me3) through iron and α -ketoglutarate-dependent oxidative reactions.^{7–9} *KDM6B* is upregulated via NF- κ B binding to its promoter region by inflammatory stimuli in macrophages.⁷ KDM6B contributes not only to inflammatory response, but also to various biological processes, including stress-induced senescence, development and differentiation.^{7–13} Importantly, recent studies have also shown that KDM6B is implicated in the pathogenesis of several cancers in a context-dependent

manner;^{14–19} however, to date the biological function of KDM6B in MM remains to be determined.

Here we investigate the biological impact of KDM6B in MM. We show that KDM6B is highly expressed in MM cells. Depletion of *KDM6B* leads to MM cell death. *KDM6B* is activated by NF- κ B signaling in MM cells, consistent with previous reports in other cell types.^{7,14} Importantly, we demonstrate that KDM6B, in a demethylase-independent manner, upregulates MAPK signaling molecules mediating MM cell growth and survival. Our data therefore delineate the biologic sequelae of KDM6B, and validate KDM6B as a novel therapeutic target in MM.

MATERIALS AND METHODS

Reagents and antibodies

Reagents used in this study are described in Supplementary Materials and Methods. Antibodies used in this study are listed in Supplementary Table 1.

Cell lines

Human MM cell lines MM.1S, RPMI 8226, KMM-1, NCI-H929, KMS-11 and KMS-27 were stocked in our laboratory, and the identity of these cell lines was confirmed before use by STR profiling (GenePrint10 System, Promega, Madison, WI, USA). Human MM cell lines MOLP-8 and KMS-20 were recently obtained from DSMZ (Braunschweig, Germany) and JCRB Cell Bank (Osaka, Japan), respectively. Human embryonic kidney cell line 293T was obtained from ATCC (Manassas, VA, USA). Cell lines were regularly tested to rule out mycoplasma contamination.

Primary MM cell, BMSC and peripheral blood mononuclear cell samples

Bone marrow or peripheral blood samples were obtained from MM patients or healthy volunteers with informed consent and approval of the Institutional Review Board of the Dana-Farber Cancer Institute. After separating mononuclear cells from patients' bone marrow by Ficoll-Paque

PLUS (GE Healthcare, Little Chalfont, UK), MM cells were purified by CD138-positive selection using anti-CD138 magnetic activated cell separation microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Long-term MM patient BMSCs were established and maintained by culturing CD138-negative bone marrow mononuclear cells in DMEM containing 100 U/ml penicillin and 100 µg/ml streptomycin, supplemented with 15% (v/v) fetal bovine serum (FBS). Peripheral blood mononuclear cells were isolated from normal donors' peripheral blood using Ficoll-Paque centrifugation.

RNA-seq analysis

Detailed method is found in Supplementary Materials and Methods. RNA-seq data are deposited at <https://www.ncbi.nlm.nih.gov/geo/> under accession number GSE93395.

Statistical analysis

No statistical method was used to predetermine sample size. Data are presented as mean ± s.d. as indicated in each figure legend. Comparison between two groups assuming normal distribution was made with two-tailed Student's *t*-test, and Welch's correction was applied when variances were unequal, which was determined by *F*-test. A value of *P* < 0.05 was considered significant.

Additional materials and methods are described in Supplementary Materials and Methods, and Supplementary Tables 2–4.

RESULTS

KDM6B confers MM cell growth and survival

We first assessed *KDM6B* expression in MM patient samples using publicly available data sets. Expression of *KDM6B* was significantly elevated in MM patient samples compared with healthy donor plasma cells (Figure 1a), suggesting the role of *KDM6B* in the pathogenesis of MM. *KDM6B* protein expression was also verified by immunoblotting in eight MM cell lines and three primary MM

samples, while *KDM6B* protein was not detected in three healthy-volunteer-derived peripheral blood mononuclear cells (Figure 1b). We therefore explored the biologic role of *KDM6B* in MM cells.

We transduced short hairpin (sh) RNA against *KDM6B* (sh*KDM6B*) or control shRNA against *luciferase* (shLuc) into MM cell lines. Depletion of *KDM6B* protein was confirmed in sh*KDM6B*-transduced cells (Figure 2a). In contrast, transduction of sh*KDM6B* had little effect on closely related *KDM6A* expression, suggesting specific knockdown of *KDM6B* by the hairpin (Figure 2a). Although *KDM6B* is an H3K27 demethylase,^{7–9} we observed that global H3K27me3 level was minimally affected after *KDM6B* knockdown (Figure 2a). Of note, knockdown of *KDM6B* reduced growth of 6 MM cell lines tested (Figure 2b). Cell growth was inhibited even in the presence of MM patient-derived BMSC conditioned medium (BMSC CM) after knockdown of *KDM6B* (Supplementary Figure 1). To confirm the result of shRNA knockdown experiments, we introduced single guide (sg) RNA targeting *KDM6B* into MM.1S and RPMI 8226 cell lines stably expressing *Cas9*. The reduction of *KDM6B* protein level was validated by immunoblot (Figure 2c, left panel). Consistent with shRNA results, CRISPR-mediated *KDM6B* knockout inhibited growth of MM.1S and RPMI 8226 cells (Figure 2c, right panel). Conversely, overexpression of *KDM6B* significantly increased growth of MM.1S cells (Supplementary Figure 2), further confirming the role of *KDM6B* in MM cell growth.

To investigate the underlying mechanism of cell growth inhibition in *KDM6B*-knockdown cells, we quantified apoptotic cells by flow cytometry using Annexin V staining. As shown in Figure 3a, percentages of apoptotic cells were significantly elevated after *KDM6B* knockdown in MM cell lines. Furthermore, cleaved forms of caspase-3, caspase-7 and poly (ADP-ribose) polymerase (PARP) (markers of the execution apoptosis pathway) were increased after *KDM6B* knockdown, except for lack of

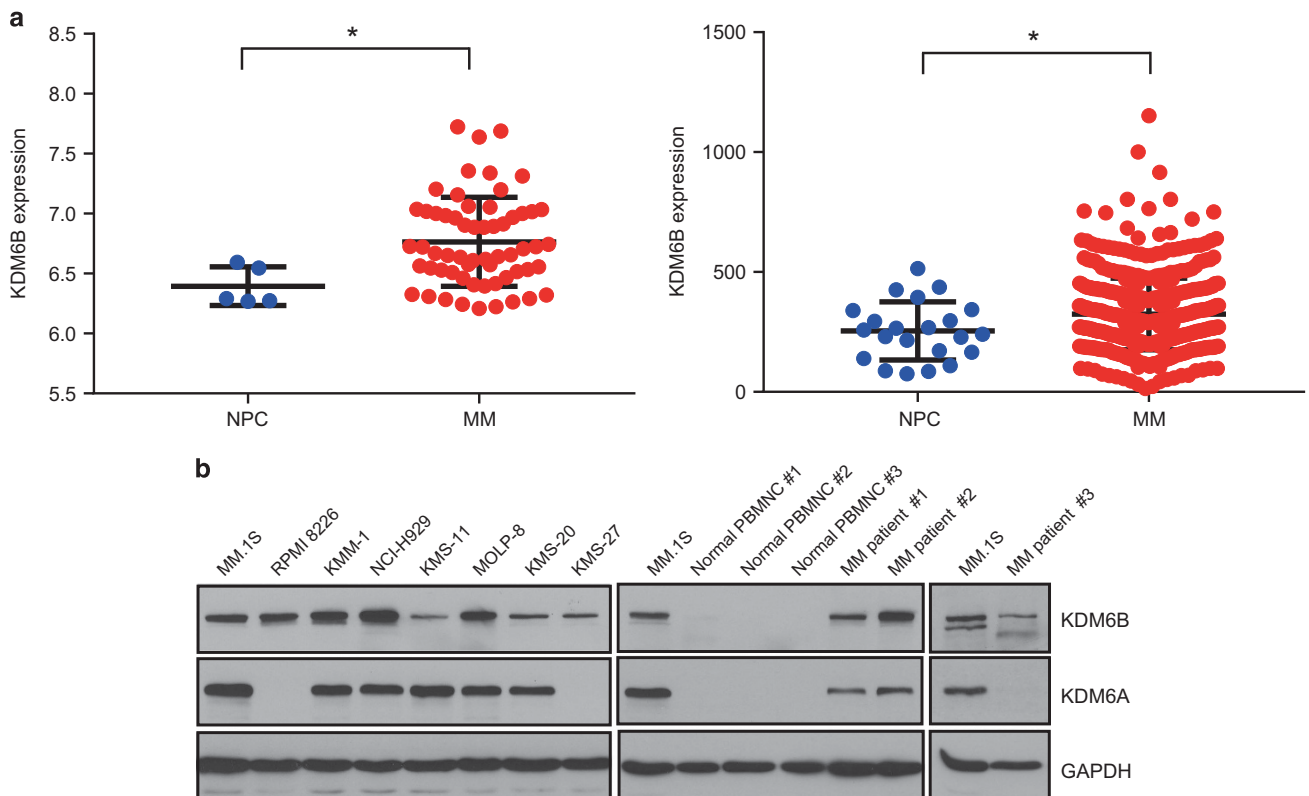


Figure 1. KDM6B expression in MM. (a) *KDM6B* expression was analyzed in normal plasma cells (NPC) and MM patient samples (MM) using publicly available data sets (GSE16558, left panel; combining data from University of Arkansas for Medical Sciences (GSE5900 and GSE2658), right panel). **P* < 0.05. (b) Whole-cell lysates were extracted from MM cell lines, MM patient samples, and normal peripheral blood mononuclear cells (PBMNC), and subjected to immunoblot analysis with the indicated antibodies.

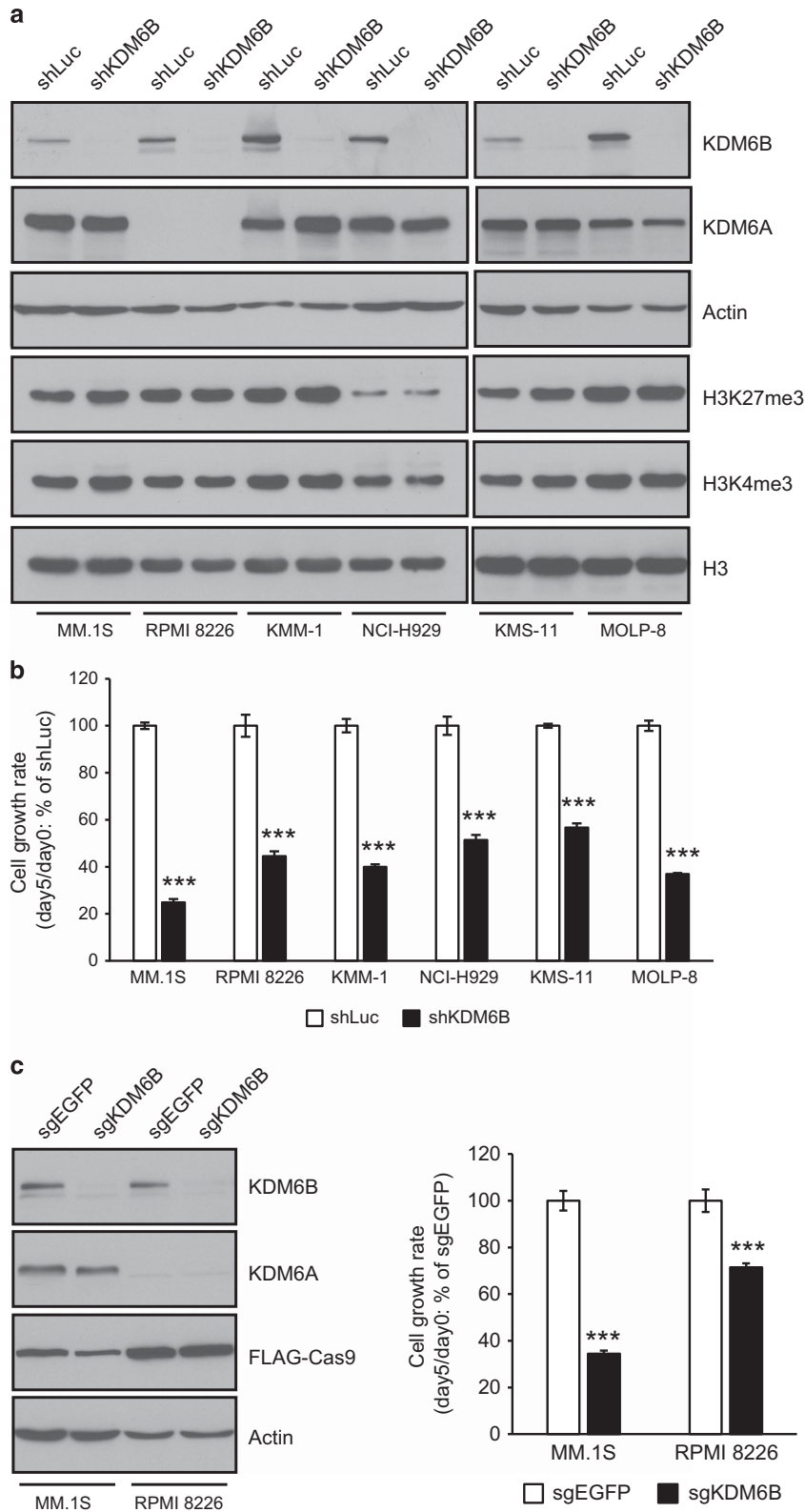


Figure 2. Silencing of *KDM6B* reduces MM cell growth. **(a, b)** MM cell lines were transduced with shRNA targeting *KDM6B* (shKDM6B) or control shRNA targeting *luciferase* (shLuc). Three days post infection (2 days post puromycin selection) is designated as day 0. **(a)** On day 0, whole-cell lysates were extracted and subjected to immunoblot analysis with the indicated antibodies. **(b)** Cell viability was determined by MTT assay on days 0 and 5. The cell growth rate (day 5/day 0) relative to shLuc is shown. **(c)** MM.1S and RPMI 8226 cells expressing *Cas9* were transduced with sgRNA against *KDM6B* (sgKDM6B) or control sgRNA against *EGFP* (sgEGFP). Three days post infection is designated as day 0. On day 0, whole-cell lysates were extracted and subjected to immunoblot analysis with the indicated antibodies (left panel). FLAG-tagged Cas9 was detected by anti-FLAG. Cell growth rate (day 5/day 0) relative to sgEGFP is shown (right panel). **(b, c)** Data represent mean \pm s.d. of quadruplicate cultures. *** $P < 0.001$ compared with control.

cleavage of caspase-3 in RPMI 8226 cells, suggesting that *KDM6B* knockdown-induced apoptosis is dependent on caspase-7 in this cell line (Figure 3b). We further asked whether the intrinsic or extrinsic pathway is involved in the apoptosis induced by *KDM6B* knockdown. Immunoblot analyses showed increased cleavage of caspase-8 in *KDM6B*-knockdown cells with little cleavage of caspase-9, indicating involvement of the extrinsic apoptosis pathway (Figure 3b). Consistent with this finding, caspase-8 inhibitor Z-IETD-FMK partially blocked *KDM6B* knockdown-induced cell growth inhibition in MM.1S cells, whereas caspase-9 inhibitor Z-LEHD-FMK did not; rather it enhanced cell death, similar to the previous observation in B-lineage cells (Supplementary Figure 3).²⁰ We next examined the effect of *KDM6B* knockdown on cell proliferation. As shown in Figure 3c, BrdU incorporation was significantly decreased after silencing of *KDM6B* in MM cell lines, while cell cycle profile was minimally altered (Supplementary Figure 4). Taken together, these results indicate that knockdown of *KDM6B* induces apoptosis and reduces cell proliferation, thereby impairing MM cell growth.

KDM6B is upregulated by NF- κ B pathway in MM

NF- κ B pathway plays an essential role in the survival of MM.^{21,22} Previous studies have shown that NF- κ B regulates *KDM6B* expression in some cell types.^{7,14} We therefore hypothesized that *KDM6B*, as a downstream target of NF- κ B, may mediate MM cell growth. To that end, we examined whether *KDM6B* in MM cells is also regulated by NF- κ B. Activation of NF- κ B pathway by TNF- α , evidenced by increased phospho-I κ B α , resulting I κ B α protein degradation, as well as increased phospho-p65 (Figure 4a, left panel), induced *KDM6B* (Figure 4a, right panel and Supplementary Figure 5a), but not *KDM6A* (Supplementary Figure 5a) expression in MM.1S cells. This *KDM6B* induction was completely blocked by I κ B kinase (IKK) β inhibitor MLN120B (Figure 4a, right panel), indicating that *KDM6B* expression is induced by NF- κ B in MM cells. BMSCs are known to activate NF- κ B pathway in MM cells through cytokines such as TNF- α , APRIL and BAFF.^{1,2} Therefore, we next examined whether BMSCs induce *KDM6B* expression in MM cells. As shown in Figure 4b, MM patient-derived BMSC CM upregulated *KDM6B* expression in MM.1S, RPMI 8226 and KMM-1 cells, but did

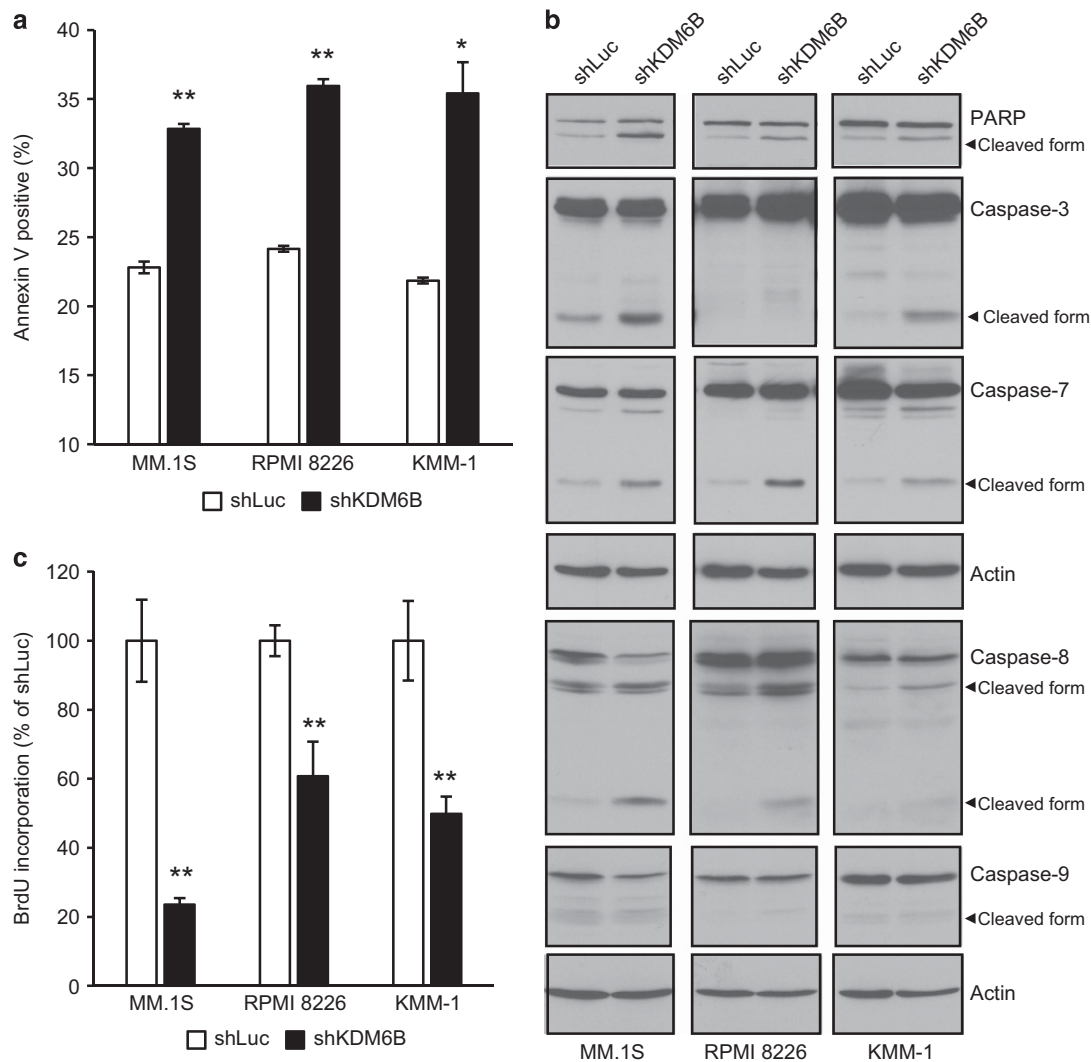


Figure 3. Knockdown of *KDM6B* induces apoptosis and reduces cell proliferation in MM cells. (a–c) MM.1S, RPMI 8226 and KMM-1 cells were transduced with shKDM6B or shLuc. (a) After 3 days (MM.1S) or 5 days (RPMI 8226 and KMM-1) of infection, cells were analyzed for apoptosis by measuring annexin V-positive cells using the flow cytometer. Data represent mean \pm s.d. of duplicate measurements. * P < 0.05, ** P < 0.01 compared with shLuc. (b) After 3 days (MM.1S) or 5 days (RPMI 8226 and KMM-1) of infection, whole-cell lysates were extracted and subjected to immunoblot analysis with the indicated antibodies. Actin served as the loading control for each membrane. (c) After 3 days of infection, the same number of viable cells were seeded and cultured for another 5 days. BrdU incorporation was then determined by BrdU assay. Data represent mean \pm s.d. of triplicate cultures. ** P < 0.01 compared with shLuc.

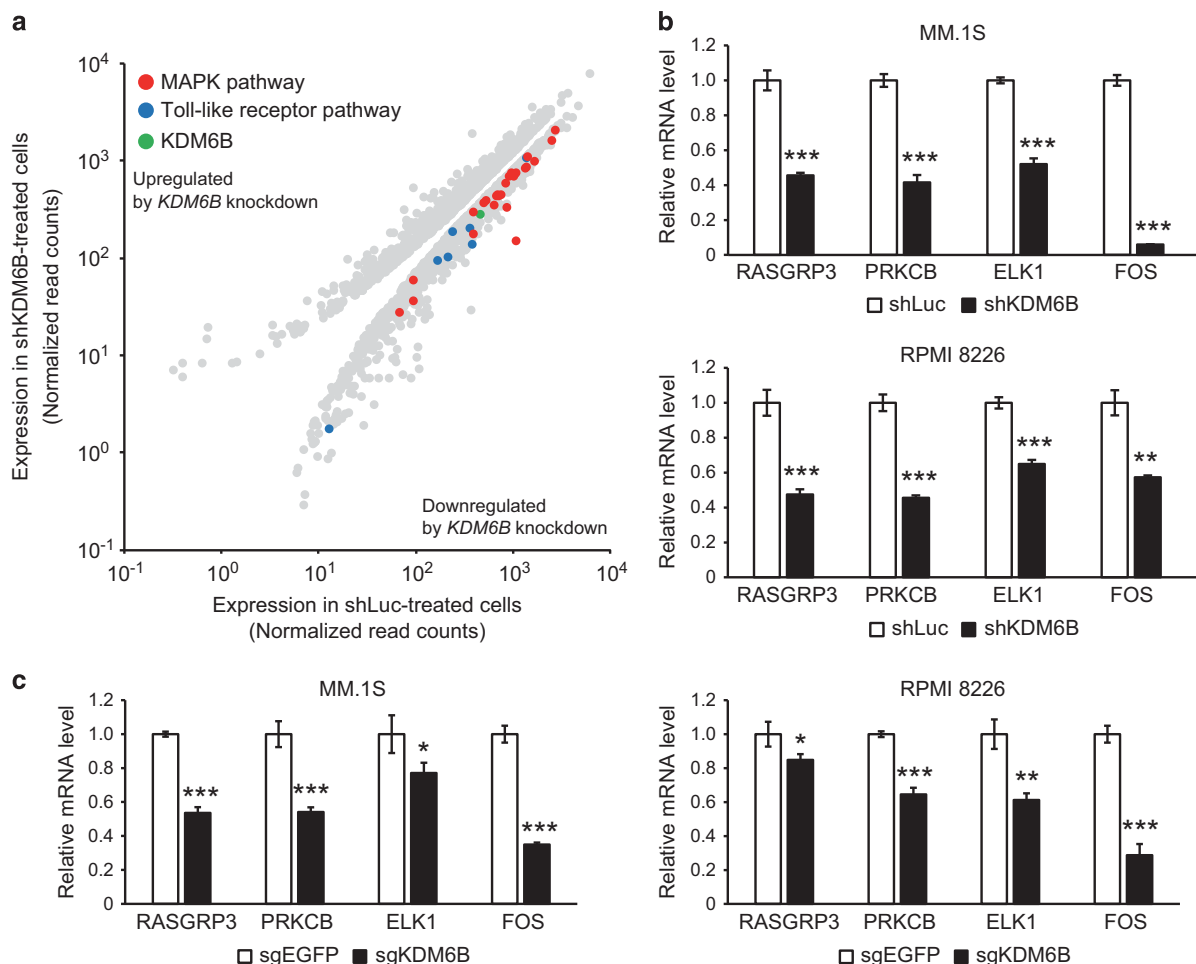


Figure 5. Silencing of *KDM6B* downregulates MAPK pathway-related genes in MM. **(a)** RNA-seq was carried out with MM.1S cells treated with shKDM6B or shLuc. Scatter plot shows differentially expressed transcripts selected based on $>2^{0.3}$ fold-change with adjusted $P < 0.05$. **(b, c)** Total RNA was isolated from MM.1S and RPMI 8226 cells transduced with shKDM6B or shLuc **(b)**, or sgKDM6B or sgEGFP **(c)**. Expression of 4 MAPK pathway-related genes (*RASGRP3*, *PRKCB*, *ELK1* and *FOS*) was then evaluated by quantitative PCR. Expression of each gene relative to control is shown as mean \pm s.d. of triplicate measurements. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control.

Figure 7a). We also observed reduced protein expression of ELK1 and FOS after *KDM6B* knockdown in these MM cell lines (Supplementary Figure 7b). CRISPR-mediated *KDM6B* knockout also demonstrated that depletion of *KDM6B* decreased expression of *RASGRP3*, *PRKCB*, *ELK1* and *FOS* (Figure 5c). Conversely, overexpression of *KDM6B* increased expression of 3 of these MAPK pathway-related genes, but not *ELK1* expression, suggesting that constitutive *ELK1* expression is maintained at the maximum level by endogenous level of *KDM6B* (Supplementary Figure 8).

We next tested whether *KDM6B* is directly recruited to the loci of MAPK pathway-related genes. Since chromatin immunoprecipitation (ChIP)-grade *KDM6B* antibody is unavailable, we generated HA-tagged *KDM6B* (HA-*KDM6B*) stably expressing MM.1S cells for ChIP assays (Figure 6a). Fragments of cross-linked chromatin were immunoprecipitated with anti-HA antibody, followed by quantitative PCR. Empty vector-transduced cells were used as a negative control. As shown in Figure 6b, ChIP signals were enriched at the transcriptional start sites of *RASGRP3*, *PRKCB*, *ELK1* and *FOS* genes in HA-*KDM6B* expressing cells, but not in empty vector-transduced cells, indicating that *KDM6B* binds to the transcriptional start sites of these genes. We next evaluated H3K27me3 level at these regions after knockdown of *KDM6B*, since *KDM6B* is an H3K27 demethylase. Unexpectedly, H3K27me3 level was not increased at these sites in *KDM6B*-knockdown MM.1S cells

compared with control cells (Figure 6c). Previous studies have reported the *KDM6B* functions that are independent of demethylase activity,^{25–27} hence, we hypothesized that *KDM6B* may regulate MAPK pathway genes via an enzymatically independent function in MM cells. To test this possibility, we expressed demethylase-dead *KDM6B* (H1390A) cDNA in MM.1S cells. Expression of mutant *KDM6B* enhanced growth of MM.1S cells (Figure 7a), and induced expression of *RASGRP3*, *PRKCB* and *FOS* (Figure 7b), similar to wild-type *KDM6B*, indicating that *KDM6B* promotes MM cell growth and regulates MAPK pathway genes independent of its catalytic function. Consistent with these findings, GSK-J4, H3K27 demethylases inhibitor which blocks *KDM6B* enzymatic activity but induces *KDM6B* expression (Supplementary Figure 9), upregulates expression of these MAPK pathway molecules (Supplementary Figure 9). Taken together, these results indicate that *KDM6B* regulates MAPK pathway molecules in a demethylase-independent manner in MM cells.

ELK1 and FOS are crucial factors for MM cell growth

We next ascertained whether ELK1 and FOS, the effector transcription factors of MAPK pathway, are required for MM cell growth. shRNA against *ELK1* or *FOS* was transduced into 3 MM cell

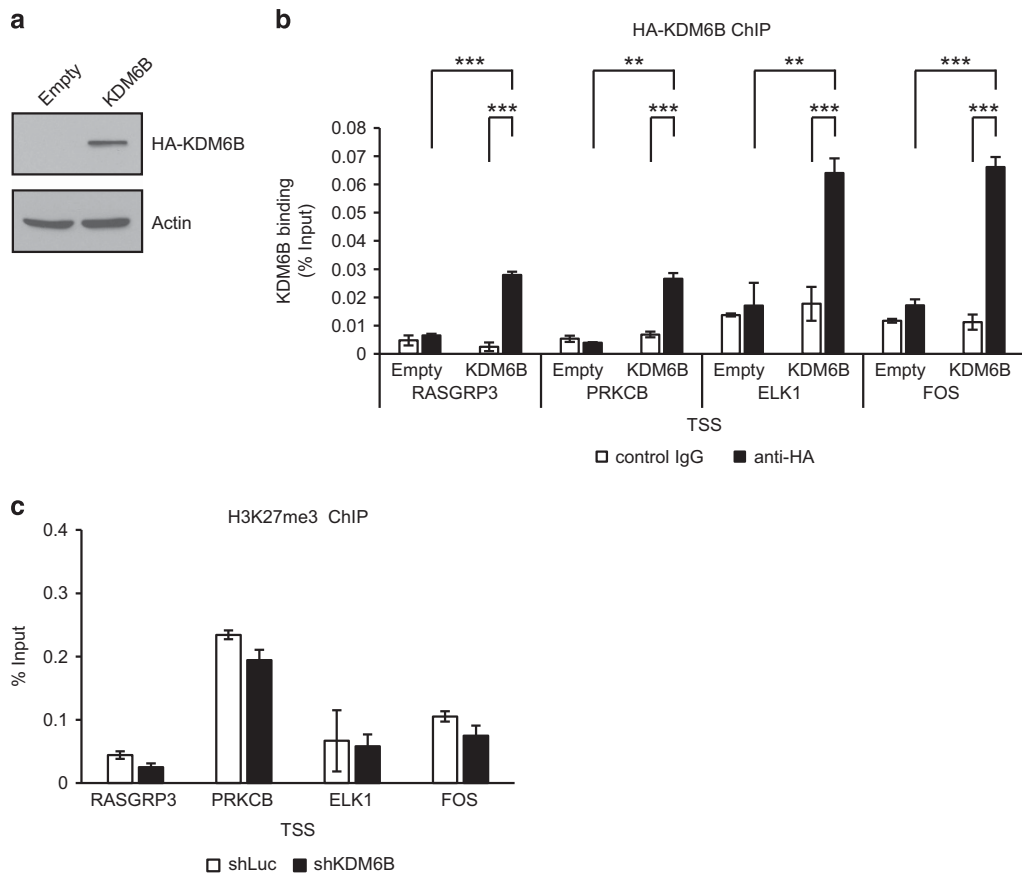


Figure 6. KDM6B is recruited to the transcriptional start sites of MAPK pathway-related genes, but does not affect H3K27me3 level at these sites. **(a)** Whole-cell lysates from MM.1S cells stably expressing HA-tagged *KDM6B* were subjected to immunoblot analysis with anti-HA. **(b)** ChIP assay using anti-HA antibody was performed with MM.1S cells stably expressing HA-tagged *KDM6B*. **(c)** H3K27me3 level was assessed after knockdown of *KDM6B* in MM.1S cells. **(b, c)** Percent recovery over the input was determined by quantitative PCR. TSS; transcriptional start site. Data represent mean \pm s.d. of triplicate measurements. ** $P < 0.01$, *** $P < 0.001$ compared with control.

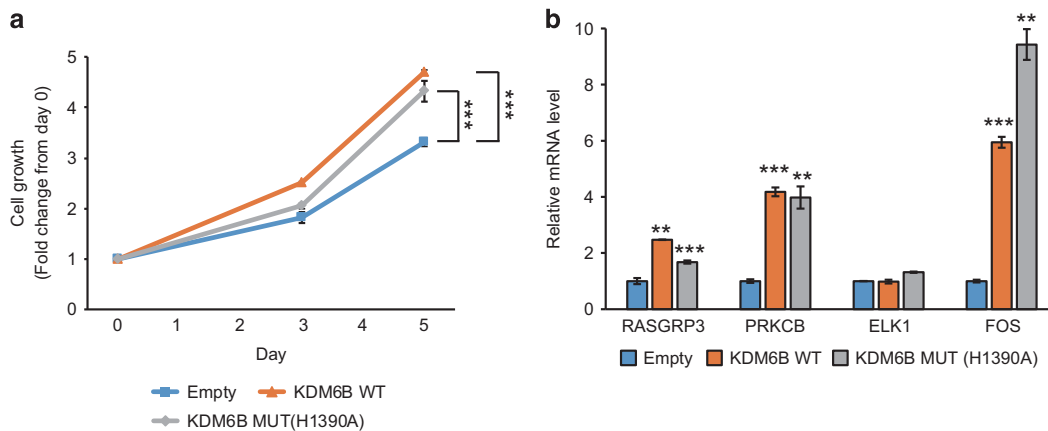


Figure 7. KDM6B regulates MAPK pathway-related genes in a demethylase-independent manner. **(a)** MM.1S cells stably expressing wild-type (WT) or catalytically dead mutant (MUT) *KDM6B* cDNA, or control cells transduced with empty vector were cultured for indicated intervals, and analyzed for growth by MTT assay. Data represent mean \pm s.d. of quadruplicate cultures. **(b)** Total RNA was isolated from MM.1S cells stably expressing WT or MUT *KDM6B*, or control cells. Expression of each gene relative to control is shown as mean \pm s.d. of triplicate measurements. **(a, b)** ** $P < 0.01$, *** $P < 0.001$ compared with control.

lines, and immunoblot analyses confirmed reduction of ELK1 or FOS protein after transduction of each shRNA (Figure 8a). Under these condition, cell growth was markedly reduced in MM cell lines (Figure 8b), indicating that these transcription factors mediate MM cell growth.

Finally, we determined whether BMSCs induce *FOS* expression, as BMSCs activate NF- κ B pathway and induce *KDM6B* expression in MM cells. As shown in Figure 8c, BMSC CM induced *FOS* expression in MM.1S cells. This induction was abrogated by *KDM6B* knockdown (Figure 8c), suggesting that

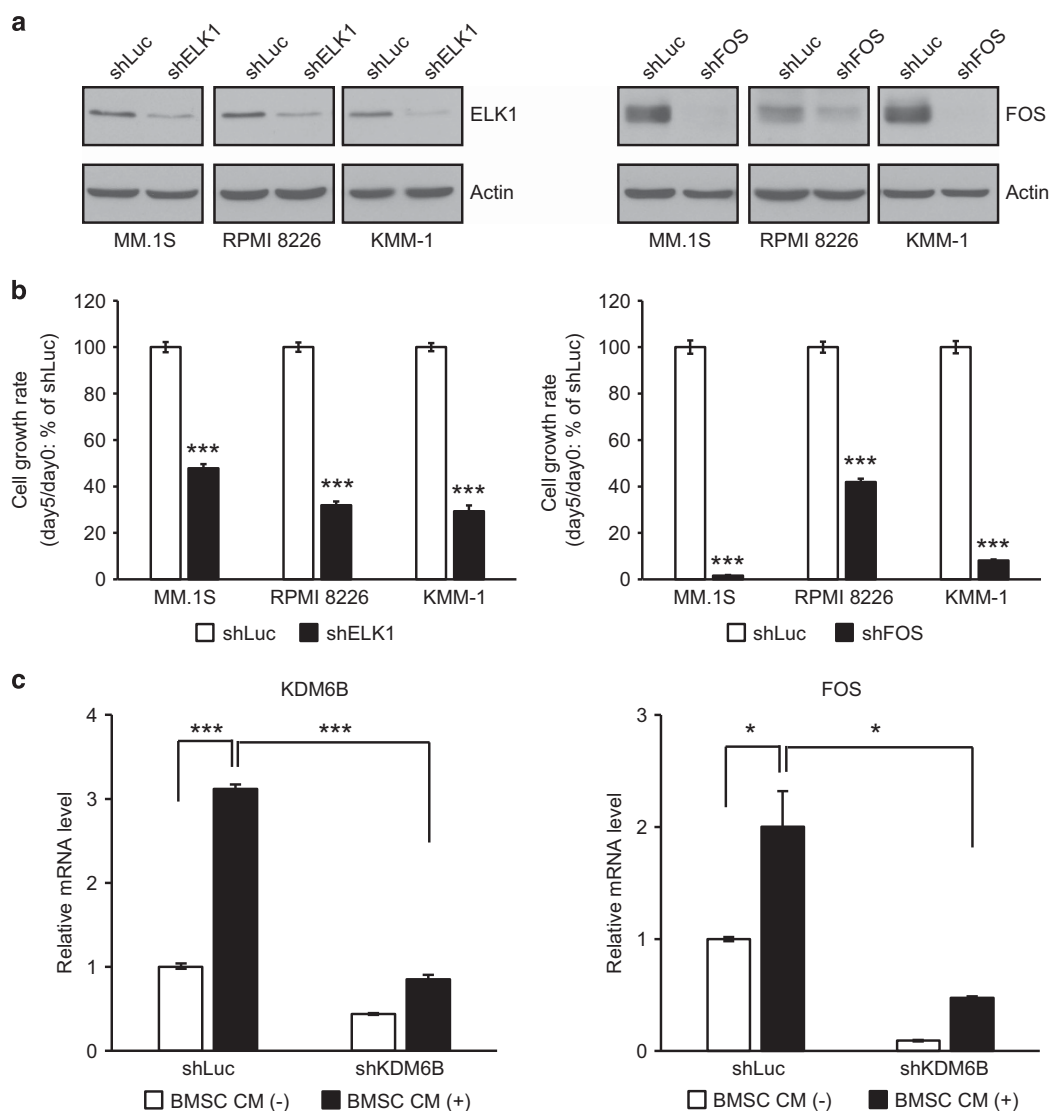


Figure 8. ELK1 and FOS are crucial factors for MM cell growth. **(a, b)** MM.1S, RPMI 8226 and KMM-1 cells were transduced with shELK1, shFOS or shLuc. Three days post infection is designated as day 0. **(a)** On day 0, whole-cell lysates were extracted and subjected to immunoblot analysis with anti-ELK1 or FOS. **(b)** The cell growth rate (day 5/day 0) relative to shLuc is shown. Data represent mean \pm s.d. of quadruplicate cultures. *** $P < 0.001$ compared with shLuc. **(c)** MM.1S cells transduced with shKDM6B or shLuc were treated with BMSC CM or control culture medium for 2 h. Data represent the amount of mRNA relative to shLuc treated with control culture medium. Error bars indicate s.d. of triplicate measurements. * $P < 0.05$, *** $P < 0.001$.

BMSC-induced *FOS* expression is mediated by KDM6B in MM cells.

DISCUSSION

In this study, we show that KDM6B is induced by activation of NF- κ B pathway, and that KDM6B in turn upregulates molecules of MAPK pathway, thereby promoting MM cell growth and survival. Previous reports have shown KDM6B dependence in other types of cancers,^{14–17} but those mechanisms are distinct from our findings in MM. In T-cell acute lymphoblastic leukemia, KDM6B regulates genes related to NOTCH pathway,¹⁴ whereas silencing of *KDM6B* has no effect on NOTCH pathway-related genes in MM cells (Supplementary Table 5). Interestingly, Zhang *et al.*¹⁷ showed subtype-specific KDM6B dependence in diffuse large B-cell lymphoma (DLBCL): KDM6B maintains survival of germinal center B-cell-like DLBCL through activating *BCL2* expression, whereas it promotes activated B-cell-like DLBCL survival via upregulating *IRF4* expression. *IRF4* is also known to be an essential survival factor in

MM,^{28,29} but *IRF4* expression is not affected by knockdown of *KDM6B* in MM cells. A tumor suppressor role of KDM6B has also been reported.^{10,11} Oncogenic stress-induced KDM6B upregulates *INK4A/ARF*, resulting in cellular senescence, but not in MM. These data suggest the cancer-type-specific roles of KDM6B.

We here demonstrate that KDM6B regulates MAPK pathway-related genes in a catalytically independent manner. Several studies have highlighted the demethylase activity-independent functions of KDM6B in different contexts.^{25–27} Using ChIP-sequencing, De Santa *et al.*²⁵ showed that KDM6B is preferentially recruited to transcriptional start sites where H3K27 is not methylated in LPS-activated macrophages, suggesting that KDM6B regulates transcription of target genes independently of H3K27 demethylation. KDM6B has also been shown to be required for chromatin remodeling in a demethylase-independent manner by mediating T-bet interaction with a Brg1-containing SWI/SNF complex in Th1 cells.²⁶ Furthermore, KDM6B facilitates ubiquitination of PHF20 by E3 ubiquitin ligase Trim26, thereby triggering PHF20 degradation.²⁷ These studies support the notion that

KDM6B mediates various biological functions not only *via* its H3K27 demethylase activity, but also its non-enzymatic activity.

Our current data suggest that KDM6B represents a potential therapeutic target in MM, and that targeting KDM6B protein itself, but not enzymatic activity, is necessary, given its demethylase-independent function in MM. Inhibition of NF- κ B pathway may be one of the promising strategies, since *KDM6B* expression is, at least in part, regulated by NF- κ B. For direct targeting of KDM6B, proteolysis targeting chimeras (PROTACs) technology might be suitable. PROTACs are chimera drugs that recruit specific target protein to E3 ubiquitin ligase, thereby facilitating ubiquitination and resulting degradation of target protein.³⁰ Recent reports in this field have shown that target-specific degradation can be achieved at nano molar range within 4 h using two different ubiquitin ligase ligands in cultured cells.^{31–34} Furthermore, two of these reports have demonstrated that their PROTACs reduce the target protein levels even in xenografted tumor cells.^{32,34} Thus, PROTACs might be a promising technology for targeting KDM6B protein, even though there is a requirement to identify a KDM6B-specific small-molecule ligand for the application of PROTACs.

In conclusion, we identify KDM6B as a key molecule promoting growth and survival of MM cells, independent of its demethylase activity. Our work delineates the role of KDM6B in MM pathogenesis, and validates KDM6B as a promising therapeutic target in MM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

HO designed and performed experiments, analyzed the data and wrote the manuscript; T Harada, MS and SK analyzed the data; Y-TT and PGR provided clinical samples. T Hideshima and KCA analyzed the data and edited the manuscript.

REFERENCES

- Hideshima T, Anderson KC. Molecular mechanisms of novel therapeutic approaches for multiple myeloma. *Nat Rev Cancer* 2002; **2**: 927–937.
- Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. *Nat Rev Cancer* 2007; **7**: 585–598.
- Manier S, Salem KZ, Park J, Landau DA, Getz G, Ghorbani IM. Genomic complexity of multiple myeloma and its clinical implications. *Nat Rev Clin Oncol* 2016; **14**: 100–113.
- Lohr JG, Stojanov P, Carter SL, Cruz-Gordillo P, Lawrence MS, Auclair D *et al*. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. *Cancer Cell* 2014; **25**: 91–101.
- Bolli N, Avet-Loiseau H, Wedge DC, Van Loo P, Alexandrov LB, Martincorena I *et al*. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. *Nat Commun* 2014; **5**: 2997.
- Walker BA, Boyle EM, Wardell CP, Murison A, Begum DB, Dahir NM *et al*. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. *J Clin Oncol* 2015; **33**: 3911–3920.
- De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G. The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* 2007; **130**: 1083–1094.
- Agger K, Cloos PA, Christensen J, Pasini D, Rose S, Rappsilber J *et al*. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* 2007; **449**: 731–734.
- Lan F, Bayliss PE, Rinn JL, Whetstone JR, Wang JK, Chen S *et al*. A histone H3 lysine 27 demethylase regulates animal posterior development. *Nature* 2007; **449**: 689–694.
- Agger K, Cloos PA, Rudkjaer L, Williams K, Andersen G, Christensen J *et al*. The H3K27me3 demethylase JMJD3 contributes to the activation of the INK4A-ARF locus in response to oncogene- and stress-induced senescence. *Genes Dev* 2009; **23**: 1171–1176.
- Barradas M, Anderton E, Acosta JC, Li S, Banito A, Rodriguez-Niedenfuhr M *et al*. Histone demethylase JMJD3 contributes to epigenetic control of INK4a/ARF by oncogenic RAS. *Genes Dev* 2009; **23**: 1177–1182.
- Jepsen K, Solum D, Zhou T, McEvilly RJ, Kim HJ, Glass CK *et al*. SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. *Nature* 2007; **450**: 415–419.
- Sen GL, Webster DE, Barragan DI, Chang HY, Khavari PA. Control of differentiation in a self-renewing mammalian tissue by the histone demethylase JMJD3. *Genes Dev* 2008; **22**: 1865–1870.
- Ntziachristos P, Tsigros A, Welstead GG, Trimarchi T, Bakogianni S, Xu L *et al*. Contrasting roles of histone 3 lysine 27 demethylases in acute lymphoblastic leukaemia. *Nature* 2014; **514**: 513–517.
- Hashizume R, Andor N, Ihara Y, Lerner R, Gan H, Chen X *et al*. Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. *Nat Med* 2014; **20**: 1394–1396.
- Park WY, Hong BJ, Lee J, Choi C, Kim MY. H3K27 demethylase JMJD3 employs the NF- κ B and BMP signaling pathways to modulate the tumor microenvironment and promote melanoma progression and metastasis. *Cancer Res* 2016; **76**: 161–170.
- Zhang Y, Shen L, Stupack DG, Bai N, Xun J, Ren G *et al*. JMJD3 promotes survival of diffuse large B-cell lymphoma subtypes via distinct mechanisms. *Oncotarget* 2016; **7**: 29387–29399.
- Svetolis A, Bianco S, Madore J, Huppe G, Nordell-Markovits A, Mes-Masson AM *et al*. H3K27 demethylation by JMJD3 at a poised enhancer of anti-apoptotic gene BCL2 determines ERalpha ligand dependency. *EMBO J* 2011; **30**: 3947–3961.
- Wei Y, Chen R, Dimicoli S, Bueso-Ramos C, Neuberg D, Pierce S *et al*. Global H3K4me3 genome mapping reveals alterations of innate immunity signaling and overexpression of JMJD3 in human myelodysplastic syndrome CD34+ cells. *Leukemia* 2013; **27**: 2177–2186.
- Shah N, Asch RJ, Lysholm AS, Leblin TW. Enhancement of stress-induced apoptosis in B-lineage cells by caspase-9 inhibitor. *Blood* 2004; **104**: 2873–2878.
- Annunziata CM, Davis RE, Demchenko Y, Bellamy W, Gabrea A, Zhan F *et al*. Frequent engagement of the classical and alternative NF- κ B pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* 2007; **12**: 115–130.
- Keats JJ, Fonseca R, Chesi M, Schop R, Baker A, Chng WJ *et al*. Promiscuous mutations activate the noncanonical NF- κ B pathway in multiple myeloma. *Cancer Cell* 2007; **12**: 131–144.
- Huang, da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009; **4**: 44–57.
- Huang, da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 2009; **37**: 1–13.
- De Santa F, Narang V, Yap ZH, Tusi BK, Burgold T, Austenaa L *et al*. Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. *EMBO J* 2009; **28**: 3341–3352.
- Miller SA, Mohn SE, Weinmann AS. Jmjd3 and UTX play a demethylase-independent role in chromatin remodeling to regulate T-box family member-dependent gene expression. *Mol Cell* 2010; **40**: 594–605.
- Zhao W, Li Q, Ayers S, Gu Y, Shi Z, Zhu Q *et al*. Jmjd3 inhibits reprogramming by upregulating expression of INK4a/Arf and targeting PHF20 for ubiquitination. *Cell* 2013; **152**: 1037–1050.
- Shaffer AL, Emre NC, Lamy L, Ngo VN, Wright G, Xiao W *et al*. IRF4 addiction in multiple myeloma. *Nature* 2008; **454**: 226–231.
- Ohguchi H, Hideshima T, Bhasin MK, Gorgun GT, Santo L, Cea M *et al*. The KDM3A-KLF2-IRF4 axis maintains myeloma cell survival. *Nat Commun* 2016; **7**: 10258.
- Deshaijes RJ. Protein degradation: Prime time for PROTACs. *Nature Chem Biol* 2015; **11**: 634–635.
- Lu J, Qian Y, Altieri M, Dong H, Wang J, Raina K *et al*. Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4. *Chem Biol* 2015; **22**: 755–763.
- Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, Dhe-Paganon S *et al*. DRUG DEVELOPMENT. Phthalimide conjugation as a strategy for *in vivo* target protein degradation. *Science* 2015; **348**: 1376–1381.
- Zengerle M, Chan KH, Ciulli A. Selective small molecule induced degradation of the BET bromodomain protein BRD4. *ACS Chem Biol* 2015; **10**: 1770–1777.
- Bondeson DP, Mares A, Smith IE, Ko E, Campos S, Miah AH *et al*. Catalytic *in vivo* protein knockdown by small-molecule PROTACs. *Nat Chem Biol* 2015; **11**: 611–617.

Supplementary Information accompanies this paper on the Leukemia website (<http://www.nature.com/leu>)