Contrasting roles of histone 3 lysine 27 demethylases in acute lymphoblastic leukaemia

Panagiotis Ntziachristos1,2*, Aristotelis Tsirigos1,3*, G. Grant Welstead4,5,6, Thomas Trimarchi1,2, Sofia Bakogianni1,2, Luyao Xu4, Evangelia Loizou1,2, Linda Holmfeldt7, Alexandros Strikoudis1,2, Bryan King2, Jasper Mullenders1,2, Jared Beckoff4, Jelena Nedjic1,2, Elisabeth Paitetta8, Martin S. Tallman9, Jacob M. Rowe11,12, Giovanni Tononi10, Takashi Sato14,15, Laurens Kruidenier16, Rab Prinjha16, Shizuo Akira14,15, Pieter Van Vlierberghe6,17, Adolfo A. Ferrando6,18,19, Rudolf Jaenisch4,5, Charles G. Mullighan7 & Iannis Aifantis1,2

T-cell acute lymphoblastic leukaemia (T-ALL) is a haematological malignancy with a dismal overall prognosis, including a relapse rate of up to 25%, mainly because of the lack of non-cytotoxic targeted therapy options. Drugs that target the function of key epigenetic factors have been approved in the context of haematopoietic disorders8, and mutations that affect chromatin modifiers in a variety of leukaemias have recently been identified12; however, ‘epigenetic’ drugs are not currently used for T-ALL treatment. Recently, we described that the polycomb repressive complex 2 (PRC2) has a tumour-suppressor role in T-ALL4. Here we delineated the role of the histone 3 lysine 27 (H3K27) demethylases JMJD3 and UTX in T-ALL. We show that JMJD3 is essential for the initiation and maintenance of T-ALL, as it controls important oncogenic gene targets by modulating H3K27 methylation. By contrast, we found that UTX functions as a tumour suppressor and is frequently genetically inactivated in T-ALL. Moreover, we demonstrated that the small molecule inhibitor GSKJ4 (ref. 5) affects T-ALL growth, by targeting JMJD3 activity. These findings show that two proteins with a similar enzymatic function can have opposing roles in the context of the same disease, paving the way for treating haematopoietic malignancies with a new category of epigenetic inhibitors.

In recent studies, we and other researchers have revealed that PRC2 has a key tumour-suppressor function, catalysing the methylation of H3K27 (refs 2, 4, 6). Since net H3K27me3 levels are dictated by the balance between histone methylation and active histone demethylation, we hypothesized that the removal of methyl groups from H3K27 is also an important process in T-ALL progression. We therefore investigated the possible roles of H3K27 demethylases in T-ALL (see Supplementary Notes for an extended introduction). Ubiquitously transcribed tetratricopeptide repeat X-linked protein (UTX)7,8 (also known as KDM6A) is a ubiquitously expressed protein that controls the basal levels of H3 K27me3 and the induction of ectoderm and mesoderm differentiation9,10 and is essential for somatic cell reprogramming11. Junoonj D3 (JMJD3)7,8 (also known as KDM6B) is induced upon inflammation12 or exposure to viral and oncogenic stimuli13,14, and it controls neuronal and epidermal differentiation15,16 and inhibits reprogramming12. UTX is a tumour suppressor in several solid tumours16,17. However, the roles of these two demethylases as direct modulators of the oncogenic state are largely uncharacterized11,14.

We have generated and studied NOTCH1-induced T-ALL animal models (Fig. 1a), because activating mutations of NOTCH1 are a defining feature of T-ALL2. Jmjd3 messenger RNA and protein expression levels were significantly higher in leukaemic cells than in untransformed CD4+ CD8+ (double positive) control T cells, which exhibit low levels of active NOTCH1, whereas Utx (and Ezh2) expression did not change significantly (Fig. 1b, c and Supplementary Table 1) upon transformation. It has previously been shown that the transcription factor nuclear factor-κB (NF-κB) controls JMJD3 expression during inflammation12 and that NOTCH1 induces the NF-κB pathway in T-ALL2. Here we showed increased expression of the p65 subunit (also known as RELA) of NF-κB and its—but not NOTCH1—binding to Jmjd3 control elements in mouse T-ALL cells (Extended Data Fig. 1a, b). Modulation of the levels of intracellular NOTCH1 or the activity of the NF-κB pathway significantly decreased the amount of NF-κB bound to the Jmjd3 elements, as well as Jmjd3 mRNA expression (Extended Data Fig. 1b–f). We then probed for JMJD3 binding to specific oncogenic loci, which has previously been shown to be important in T-ALL2. We found that JMJD3 binding was highly enriched on the Hes1 promoter (Fig. 1d, left), and this binding depended on the activation of the NOTCH1 pathway and negatively correlated with the H3K27me3 levels (Extended Data Fig. 1g, h).

Analyses of human leukaemia cases2,24–26 have shown that JMJD3 is more highly expressed in T-ALL cells than in normal T-cell progenitors24 or in other types of leukaemia25,26, which is similar to the expression of the classic NOTCH1 target HES1 (Fig. 1e). Genes that are co-expressed with JMJD3 in human primary samples were found to exhibit loss of H3K27me3 during leukaemia progression (Extended Data Fig. 11), suggesting a connection between the expression of JMJD3 and the H3K27me3 levels on specific targets.

Chromatin immunoprecipitation followed by sequencing (ChIP-seq) studies in human T-ALL cells (the cell line CUTTL1) showed that JMJD3 was bound to important NOTCH1 targets with oncogenic functions (such as HEY1, NRARP and HES1) (Fig. 1f). There was a significant co-occupancy of JMJD3 with NOTCH1 (ref. 2) (33% of the top JMJD3 peaks were occupied by NOTCH1, a 6.9-fold enrichment over control, P < 10−7), the NOTCH1 partner RBP-Jk and the activating mark H3K4me3 (ref. 27 (Extended Data Fig. 1j)). The majority of JMJD3 binding sites were localized around the transcription start sites (TSSs) of...
Figure 1 | JMJD3 is highly expressed in T-ALL and controls the expression of important oncogenic targets. a, Size comparison of the spleens (left) and haematoxylin and eosin staining of the liver (centre) of healthy (WT, top) and leukaemic (T-ALL, bottom) mice. The arrows denote leukaemic infiltration of the liver of the T-ALL mouse. Scale bar, 50 μm. Representative samples from n = 3 mice are shown. b, c, Protein (b) and transcript (c) levels of the demethylases JMJD3 and UTX in control T cells (CD4+CD8+ (double positive) thymocytes) and mouse T-ALL cells. Representative samples (b) or the mean ± s.d. (c) of three mice is shown; values were normalized according to the sample with the highest expression value. d, ChIP for JMJD3 on the Hes1 promoter in control T cells and mouse T-ALL cells (left) and upon γ-secretase inhibitor (γSI) treatment of T-ALL cells (right) (n = 3); data are shown as mean ± s.d. DMSO, dimethylsulphoxide. e, Expression analysis of JMJD3 and HESI among samples of acute T-cell leukaemia (T-ALL; 83 samples), acute B-cell leukaemia (B-ALL; 23) and acute myeloid leukaemia (AML; 537), as well as physiological T-cell subsets (24) (quantile normalization across samples, see Methods). The data are shown as mean ± s.d. The P values (Wilcoxon test) are as follows: for JMJD3, T-ALL versus physiological T cells, 4.0 × 10⁻³; T-ALL versus AML, 1.1 × 10⁻³; T-ALL versus B-ALL, 2.2 × 10⁻⁵; and for HESI, T-ALL versus physiological T cells, 3.7 × 10⁻⁴; T-ALL versus AML, 3.5 × 10⁻⁴; T-ALL versus B-ALL, 1.3 × 10⁻⁶, ***, significant. f, Snapshots of JMJD3 binding in human T-ALL. Three NOTCH1 targets and the interferon-β (IFNB) gene (negative control) are shown. Chr, chromosome.

genes (Extended Data Fig. 1k) in a fashion similar to NOTCH1 binding sites. These results suggest a key role for JMJD3 in oncogenic programs in T-ALL, through interaction with NOTCH1. Protein immunoprecipitation studies in 293T cells (human embryonic kidney cells), as well as in mouse T-ALL cell lines, showed that JMJD3 is part of the NOTCH1 transcriptional complex, as it interacts directly with NOTCH1 and MAML1 (Extended Data Fig. 2a–c). By contrast, there was no NOTCH1 interaction with EZH2 or UTX. As JMJD3 has been shown to be a member of MLL complexes, we tested whether JMJD3 interacted with WDR5, a key subunit of the MLL complex. We found that JMJD3 interacted with WDR5 (Extended Data Fig. 2b), suggesting a potential NOTCH1–JMJD3–MLL complex on target promoters.

To clarify the role of JMJD3 and UTX in the maintenance of leukaemia, we performed genomic knockdown of JMJD3 in human T-ALL cells using two short hairpin RNAs (shRNAs) (Fig. 2a, b and Extended Data Fig. 2d). Treatment with shJMJD3 but not shUTX affected the viability of leukaemic cells, as shown in loss of representation studies and apoptosis assays, and this finding is in contrast to the viability of myeloid leukaemia lines used as controls (Fig. 2c and Extended Data Fig. 2e, f). The expression of NOTCH1 targets was negatively affected by shJMJD3.
Figure 3 | The demethylase UTX acts as a tumour suppressor in T-ALL. a–c, Monitoring the initiation and progression of T-cell leukaemia in a NOTCH1-overexpressing model of T-ALL. Leukaemic blasts (expressed as a percentage of GFP–NOTCH1-positive cells) were scored in the peripheral blood (a, mean ± s.d.) and in a blood smear (b) and leukaemic cell infiltration of the liver (c) of male wild-type (UTx+/Y, n = 10) and knockout (UTx−/Y, n = 6) mice are shown. NOTCH1-IC, intracellular part of NOTCH1. d, Survival studies of mice transplanted with haematopoietic progenitors from the wild-type (UTx+/Y, n = 10) and knockout (UTx−/Y, n = 6) backgrounds expressing NOTCH1-IC. e, Scatter plot summarizing the major genome-wide expression differences between T-ALL tumours of the wild-type (UTx+/Y) and knockout (UTx−/Y) backgrounds. RNA sequencing was performed using three pairs of wild-type and UTx knockout (KO) NOTCH1-IC tumours (spleen and bone marrow). f, g, Analysis of genetic status of the UTX (KDM6A) locus in paediatric T-ALL leukaemia (n = 107). Affymetrix SNP6.0 microarrays (f) for assessing genomic deletions. Illustration of the human UTX protein (g) depicting three frameshift (fs) mutations in paediatric T-ALL (grey circles), as well as one in-frame deletion (p.Ala14_Ala17del), one splice acceptor site mutation (exon (e) 4 splice) and one missense mutation (p.Ile598Val) in adult T-ALL (white circles), as identified by targeted Sanger sequencing. The jumonji domain (JMJC) and the tetratricopeptide repeats are shown. SJTALL, St. Jude’s Children’s Research Hospital sample depository of T-ALL samples.
of primary human samples of paediatric T-ALL using single nucleotide polymorphism (SNP) arrays identified two patients with focal deletions of the UTX locus (Fig. 3f). Further targeted sequencing in paediatric and adult T-ALL led to the identification of six more patient cases with UTX mutations (Fig. 3g, Extended Data Fig. 5j, k and Supplementary Table 2), including in-frame deletions, missense (Ile598Val) mutations and frameshift alterations. Analysis of bone marrow remission genomic DNA confirmed the somatic origin of the UTX splice site mutation (Extended Data Fig. 5k). Seven out of the eight alterations belonged to male patients, further underlining that the roles of UTX and UTY do not seem to be interchangeable. These genetic alterations are predicted to have an inactivating role20,21 and provide further evidence that UTX is a tumour suppressor in T-ALL. Indeed, overexpression of UTX using a doxycycline-inducible lentiviral system in T-ALL cell lines (Extended Data Fig. 5l) led to suppression of tumour growth and a significant increase in apoptosis (Extended Data Fig. 5m, n).

Jmjd3−/− mice22 lack the catalytic domain of the JMJD3 protein (Extended Data Fig. 6a, b) and die perinatally23. Haematopoiesis and T-cell development were largely unaffected by the absence of JMJD3 (Extended Data Fig. 6c–h). Genetic ablation of Jmjd3 in T-ALL led to fewer leukemic blasts in the peripheral blood, significantly reduced leukemic cell infiltration of the spleen and liver and improved survival rates in the recipients (Extended Data Fig. 7a–f), consistent with Jmjd3 having an oncogenic role. These striking phenotypes supported our previous in vitro and in vivo findings and led us to further explore the therapeutic potential of targeting JMJD3 activity in T-ALL.

We next tested whether the small molecule GSKJ4 (ref. 5), which is directed against JMJD3 and UTX (half-maximum inhibitory concentration (IC50) as determined by matrix-assisted laser desorption mass spectrometry, JMJD3, 18 μM; UTX 56 μM; ref. 5), affects maintenance of the disease. We used GSKJ4 at the IC50 determined for T-ALL cells (2 μM) (Fig. 4a) to treat a panel of T-ALL cell lines. GSKJ4 significantly affected the growth of human T-ALL cell lines and primary human T-ALL cells (T-ALL-1-3), leading to cell cycle arrest and increased apoptosis compared with control-inhibitor-treated cells (Fig. 4b and Extended Data Fig. 8a–h). The first detectable changes started at 24 h, and we observed significantly altered phenotypes at 48 h and 72 h (Extended Data Fig. 8i). These GSKJ4 effects appear to be connected to the demethylase activity of JMJD3, as overexpression of catalytically inactive JMJD3 did not rescue the phenotype (Extended Data Fig. 8j, k). The growth of myeloid leukaemia cells, stromal cells and haematopoietic progenitor cells (Extended Data Fig. 8l, m) was unaffected by GSKJ4, demonstrating specificity of function. Mechanistically, we detected gene expression changes starting at 24 h post-GSKJ4 treatment, and significant changes were noted at 48 h and 72 h (Extended Data Fig. 8n) and were coupled to an increase in the H3K27me3 levels at repressed genes (Extended Data Fig. 9a–c). The NOTCH1 and JMJD3 occupancy at specific NOTCH1 target genes that were tested, as well as the total cellular levels of NOTCH1 and JMJD3 and the chromatin H3K27me3 levels, did not significantly change over the treatment duration (Extended Data Fig. 9a–e).

Genome-wide studies identified 486 downregulated genes after 72 h of treatment of human T-ALL cells (CUTLL1) with GSKJ4 (Fig. 4c). There was a significant overlap between the shJMJD3 and GSKJ4 signatures for both downregulated genes (P = 4.88 × 10−44; Fig. 4d and Supplementary Table 3) and upregulated genes (P = 2.57 × 10−20). By contrast, the shUTX-upregulated gene signature significantly overlapped with the GSKJ4-downregulated gene signature. Furthermore, there was a significant overlap between genes upregulated in Utx knockout blasts and downregulated by GSKJ4 treatment (P = 2.49 × 10−5; Figs 3e and 4d and Supplementary Table 3), suggesting again that UTX and JMJD3 play opposing roles in T-ALL. Genome-wide study of H3K27me3 localization demonstrated that the GSKJ4-downregulated genes experienced gain of H3K27me3 upon GSKJ4 treatment and were marked by the presence of H3K4me3, NOTCH1 and JMJD3 at their promoters (Fig. 4c and Extended Data Fig. 9j). Well-characterized NOTCH1 and JMJD3 targets are highlighted as representative examples of the GSKJ4-downregulated/shJMJD3-downregulated signature and show a significant gain in H3 K27me3 upon GSKJ4 treatment (Fig. 4e and Extended Data Fig. 9f). UTX was not involved in the regulation of the oncogenic NOTCH1 targets, as revealed by ChIP studies (Extended Data Fig. 9g).

We propose targeting JMJD3 as a novel therapeutic option for paediatric and adult T-ALL. This proposal is based on recent studies2,4,6 that demonstrate that H3K27me3 catalysed by the PRC2 complex plays...
a key role in T-ALL, through antagonism with oncogenic NOTCH1. We demonstrate here that NOTCH1-mediated recruitment of JMJD3 to promoters can explain this antagonism (Extended Data Fig. 10 and see also Supplementary Discussion for extended discussion). We propose that NOTCH1 recruitment leads to PRC2 eviction as a result of the active demethylation of H3K27 through the catalytic activity of JMJD3 and the recruitment of JMJD3 to target promoters. By contrast, the reported increases in the levels of the activating H3K4me3 mark on to these sections appear only in the online paper.

GSKJ4 affects other important epigenetic modulators or signalling pathways. Nevertheless, we consider that the main action of this inhibitor in T-ALL is channelled through the inhibition of JMJD3 activity and propose that such compounds should be tested either as single drugs or in combination with standard chemotherapy.

Online Content Methods, along with any additional Extended Data display items, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions I.A. and P.N. designed the experiments and wrote the manuscript. P.N. performed most of the experiments. A.T. designed and performed the analysis of genome-wide data and wrote the manuscript. T.T., E.L., A.S., J.M., B.K., S.B. and J.N. performed experiments and contributed ideas. G.T. provided materials and tips related to the study. A.A.F. and L.X. designed and performed xenograft luciferase experiments and helped with ideas and concepts. P.V.V., E.P., M.S.T., J.M.R. and A.A.F. performed and analysed the mutational studies in adult T-ALL. G.S.W., R.J.T.S. and S.A. provided mouse tissues and helped with ideas and concepts. L.K. and R.P. helped with guidance on the biology and use of GSK1 inhibitors and with manuscript preparation. L.H., J.B. and C.G.M. performed and analysed the mutational studies in paediatric T-ALL.

Author Information The high-throughput sequencing data have been deposited in the Gene Expression Omnibus with accession number GSE56696. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to I.A. (Ianis.Afantis@nyumc.org), A.T. (Aristotelis.Tsirigos@nyumc.org) or C.G. (charles.mullighan@sludge.org).
METHODS

Mice, cell culture and primary cell samples. The Jmjd3 (ref. 29) and Utx knockout mouse models, as well as the corresponding genotyping strategy, have been described previously4. All animals used in this study were treated according to IACUC protocols for the laboratories of I.A., A.A.F. and R.J. The human T-ALL cell lines CEM, LA-1, Jurkat and the myeloid leukaemia cell lines (THP-1 and HL-60), as well as the mouse T-ALL line (720)31, were cultured in RPMI 1640 medium supplemented with 20% FBS and penicillin and streptomycin. All cell lines were tested for the presence of mycoplasma, and only mycoplasma-free lines were used for these studies. Primary human samples were collected by collaborating institutions with informed consent and were analysed under the supervision of the Columbia University Medical Center and St Jude Children’s Research Hospital Institutional Review Boards. The primary cells treated with GSJK4 inhibitor (for more information on these cells, see ref. 32) were cultured in MEMx medium plus 10% FBS (StemCell Technologies, #06400), human AB” serum (Invitrogen), 1% penicillin/streptomycin, 1% GlutaMAX, human interleukin-7 (IL-7) (R&D Systems; 10 ng ml−1), human Flk3 ligand (Peprotech; 20 ng ml−1), human SCF (Peprotech; 50 ng ml−1) and insulin (Sigma; 20 ng ml−1). Irradiated M5S stromal cells expressing delta-like-1 (DLL1) were used as a feeder layer, as previously described32. In vitro drug treatment and shRNA treatment and cell growth, apoptosis and cell cycle analysis. T-ALL cells were infected twice with shRNA-expressing retroviruses and selected using puromycin. Expression studies took place up to different time points during the selection period, and we present the results from day 4 during selection. To calculate the IC50 of GSJK4 (GlaxoSmithKline)3 normalized to the control inhibitor GSJK5 (GlaxoSmithKline), T-ALL lines were treated with different concentrations of the drug for 5 days. For cell growth, cell lines and primary cultures were treated with 2 μM GSJK4 and GSJK5 for various times (24 h to 72 h) and stained with annexin V and subjected to cell cycle analysis. γ-Secretase inhibitor (γSI, specifically Compound E (Alexis Biochemicals)) was used at 500 nM for various periods. For the cell cycle analysis, 5-bromo-2’-deoxyuridine (BrdU;10 μM) was added for a 1 h pulse, and incorporation into DNA was determined by using the BrdU Flow Kit (BD Biosciences). Apoptosis was studied by quantification of annexin V staining using the BD Biosciences kit and flow cytometry according to standard protocols provided by the manufacturer. Doxycycline was used at 1 μg ml−1 final concentration.

Intravenous and subcutaneous xenograft studies. Studies were conducted as previously published4. In both cases, CULTLL1, P12 or CEM T-ALL cells expressing luciferase (FUW-LUC) and the corresponding shRNA (shJMJD3, shUTX or shRenilla) were used. For the intravenous studies, 1 × 106 cells were injected retroorbitally into sublethally irradiated female NRG (NOD Rag1−/−129g-2Rj) mice. For subcutaneous studies, 1 × 106 cells were mixed with an equal volume of BD Matrigel and injected into the flanks of female NOD-SCID mice. In both cases, cell growth was monitored every 2 days using IVIS (Caliper, PerkinElmer). Transplantation for reconstitution of the haematopoietic system and for disease progression analysis. Fetal livers from Jmjd3−/−, Utx−/− and Jmjd3−/−/Utx−/− embryos (E13.5, Ly45.2 background) were provided by S.A.’s laboratory, and 1 × 106 total (unfractionated) fetal liver cells were used for the reconstitution of the haematopoietic system of lethally irradiated recipients on a Ly45.1 background. Bone marrow was isolated from the recipients, followed by isolation of cells of the Ly45.2 background using flow cytometry. Total Ly45.2 bone marrow mononuclear cells (2.5 × 106 cells) were mixed with equal numbers of Ly45.1 (wild-type) bone marrow cells and transplanted into lethally irradiated recipients to study haematopoietic reconstitution in a competitive setting.

For the Utx−/−, Utx−/− and Utx−/− (Ly45.2) background, 2.5 × 106 cells of total Ly45.2 bone marrow mononuclear cells were mixed with equal numbers of Ly45.1 (wild-type) bone marrow cells and transplanted into lethally irradiated recipients to study haematopoietic reconstitution in a competitive setting similar to the Jmjd3 study.

In both cases, reconstitution of the haematopoietic system was monitored by analysis of the peripheral blood for the main haematopoietic lineages. The thymus and spleen of some recipients were isolated and analysed at 3 months post transplantation.

For analysis of leukaemia progression, c-Kit+ haematopoietic progenitors from the bone marrow of both Jmjd3 and Utx knockout models were magnetically selected (STEMCELL Technologies) using an antibody against CD117 (c-Kit) and were cultured overnight in the presence of 50 ng ml−1 SCF, 50 ng ml−1 Flt3 ligand, 10 ng ml−1 IL-3 and 10 ng ml−1 IL-6. Overexpression of oncogenic Notch1 mutants (the intracellular part of NOTCH1 (NOTCH1-IC) and DeltaE (NOTCH1-AE)) in bone marrow haematopoietic progenitors following transplantation into mouse recipients led to the development of T-ALL, characterized by the presence of leukaemic blasts in the peripheral blood that infiltrated the peripheral lymphoid organs, progressively leading to the death of the animals (Extended Data Fig. 5c). The cells were infected with NOTCH1-IC or NOTCH1-AE (and green fluorescent protein (GFP)) expressing retroviruses twice (24 h and 48 h post c-Kit selection). Viral transduction efficiency was determined by measuring reporter fluorescence over a total period of 4 days, and total populations were transferred via retro-orbital injection into lethally irradiated congenic recipients along with 2.5 × 106 total (wild-type) bone marrow mononuclear cells for haemogenic support. GFP+ cells (4 × 105) were transplanted in both NOTCH1-IC and NOTCH1-AE mice. The Mantel–Cox test was used for the analysis of the survival data. No randomization or blinding method was used during these animal studies.

Antibodies, reagents, kits and virus production. Protein-G–coated magnetic beads were purchased from Invitrogen. Antibodies against the following proteins were used: monoclonal mouse H3K27me3 (histone H3 migrates at around 17 kDa) (Abcam, ab6002), monoclonal mouse H3K27m1 (Active Motif, 60151), polyclonal rabbit H3K4me3 (Active Motif, 39159), polyclonal rabbit NOTCH1 (the intracellular part of the protein migrates at around 110 kDa), polyclonal rabbit JMJ3 (protein migrates at around 170 kDa) (Abgent, AP1022A (human) and AP1022B (mouse)), as well as polyclonal rabbit JMJD3 (Cell Signaling Technology, 3457), polyclonal rabbit UTX (protein migrates at around 160 kDa) (Abcam, ab30938, and Bethyl Laboratories, A302-374A), polyclonal rabbit NF-kB (p65, protein migrates at around 65 kDa) (Santa Cruz Biotechnology, sc-109 and sc-372) and control IgG (Santa Cruz Biotechnology, sc-m225 (mouse) and sc-m2027 (rabbit)). All antibodies for flow cytometry were from eBioscience. All antibodies used had been tested and shown to be specific for the purposes we used them for. The antibodies used for the specific attachment or labelling of the corresponding shRNA.

Histopathology. Organs were obtained from the animals and specimens fixed for 4 days. Samples were washed with PBS three times for 1 h at room temperature and dehydrated in 70% ethanol. Samples were embedded in paraffin blocks. Sections (6-μm thick) were stained with haematoxylin and eosin following standard procedures. Peripheral blood smears were brieﬂy stained in methanol and stained with Wright–Giemsa solution (Fisher). Slides were rinsed with water, dried, mounted with Cytoseal 60 and covered with Cytoseal 60.

Protein immunoprecipitation for interaction studies. For the interaction studies between the NOTCH1 complex (NOTCH1 and MAML1) and the epigenetic modifiers UTX, JMJD3 and EZH2, we used standard non-commercial antibodies. In brief, cells were resuspended in TEB buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 150 mM NaCl and 0.05% (v/v) Tween-20) supplemented with the inhibitors at a concentration of 20 × 106 cells ml−1 buffer. Cell lysates were passed through a 25G syringe ﬁve times and incubated on ice for 30 min, followed by centrifugation to remove cell debris (5 min, 13,000g). The cleared lysate was precleared with beads for 1 h at 4 °C to decrease non-speciﬁc binding and incubated overnight with the corresponding antibody-bound bead complexes. Five micrograms of antibody was used for 3 mg of extracts.

RNA-seq library preparation and analysis. Whole RNA was extracted from 1–5 × 106 T-ALL cells or primary cells using the RNeasy kit (QIAGEN) according to the manufacturer’s protocol. Poly(A)+ RNA was enriched using magnetic oligo(dT)-converting beads (Invitrogen). cDNA was prepared and strand-specific libraries were constructed using the dUTP method as described previously5. Libraries were sequenced on the Illumina HiSeq 2000 using the 50-base pair single-read method.

ChIP and ChIP-seq library preparation. ChIP experiments were performed as described previously4. In brief, for the analysis of histone marks, we ﬁxed the cells with 1% formaldehyde for 10 min at 25 °C and lysed them by the addition of nucleic acid incu-
volumes of IP dilution buffer (0.01% SDS, 1% Triton X-100, 1.2 mM EDTA, pH 8.0, 16.7 mM Tris-HCl, pH 8.0, and 167 mM NaCl) and magnetic Dynabeads. One per cent of the chromatin was kept as input. We coupled 2.5 μg antibody with 25 μl of beads for 4 h in reaction buffer, and the complex was added to precleared chromatin (the equivalent of 10^−10^ cells, depending on the antibody) followed by overnight incubation at 4 °C with rotation. We washed the complexes bound to the beads using buffers with increasing salt concentration: once with wash A (20 mM Tris-HCl, pH 8, 150 mM NaCl, 2 mM EDTA, 1% (w/v) Triton X-100 and 0.1% (w/v) SDS), twice with wash B (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 2 mM EDTA, 1% (w/v) Triton X-100 and 0.1% (w/v) SDS), once with wash C (10 mM Tris-HCl, pH 8.0, 250 mM LiCl, 1 mM EDTA, 1% (w/v) NP-40 and 1% (w/v) deoxycholic acid) and twice with TE, followed by treatment with RNase and protease K. The cross-links were then reversed, and the DNA was precipitated using ethanol and glycogen.

For [MJ]D3 ChIP, the cells were fixed with 1% formaldehyde for 10 min at 25 °C and lysed on ice using 1 ml cell lysis buffer (50 mM HEPES-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40 and 0.25% Triton X-100) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells. We further resuspended the nuclei in buffer III (10 mM Tris-HCl, pH 8, 200 mM NaCl, 1 mM EDTA, pH 8, and 0.5 mM EGTA) per 1 × 10^7 cells.
TSSs between cells treated with the inhibitor GSKJ4 and the control GSKJ5 were determined using GenomicTools ("genomic_apps peakdiff" tool) as described in a previously published study. Epigenetic changes between the treatment (shJMJD3 or GSKJ4) and control samples were determined by evaluating sliding windows across the genome using the following protocol. First, enriched ChIP-seq windows were identified separately for each of the two samples under comparison using a window-based approach and the binomial probability distribution to compare signal reads with control reads in each window. Subsequently, for each genomic window enriched in at least one of the two samples, the total number of reads was determined, and the window read counts were normalized using quantile normalization across biological replicates and samples before comparison. Finally, for each window, the fold change between the two samples was calculated (GSKJ4 versus control, and vice versa). To estimate the false discovery rate, the distribution of the observed H3K27me3 fold changes was compared with the distribution of fold changes between replicates of the same treatment. This comparison was performed independently at different H3K27me3 read density levels to control for artificially high fold changes due to low read counts in the denominator. Significant epigenetic changes are reported at 5% false discovery.

JMJD3, NOTCH1, H3K4me3 and H3K27me3 heatmaps were generated using GenomicTools ("genomic_apps heatmap" utility) over log-transformed read counts in 200-nucleotide non-overlapping bins of 4-kb-flanked TSSs. Box plots of H3K27me3 log-fold changes (GSKJ4 versus control) show the distribution of values in (a) JMJD3 targets, (b) commonly downregulated genes upon shJMJD3 and GSKJ4 treatment, and (c) the intersection of GSKJ4-upregulated and shJMJD3-upregulated genes as a negative control. P values were computed using a one-sided Wilcoxon unpaired test for (a) and (b) versus the control (c).

RNA-seq and ChIP-seq replicate reproducibility. For RNA-seq experiments, we focused on the reproducibility of gene expression levels as measured by FPKM values. For each pair of replicates, we computed the Spearman and Pearson correlations, as well as the Pearson correlation on log-transformed FPKM values. In general, Pearson correlations were much higher because values are dominantly expressed, and highly expressed genes tend to be more reproducible. Using a Pearson correlation on log-transformed values attempts to balance the expression distribution and allow contributions from genes that are expressed at a lower level, thereby providing a more realistic genome-wide reproducibility metric. Spearman correlations focus on the ranking of gene expression, and in our experiments, in general, were a more conservative (that is, lower) and consistent (lower variability across various settings, and when comparing different cell lines that is, CUTLL1 and CEM) estimate of reproducibility; therefore, for simplicity, we have reported only the Spearman correlations.

For ChIP-seq ‘broad peak’ experiments (H3K27me3), we also used Pearson, log-transformed Pearson and Spearman correlations on (a) TSSs and (b) all genome-wide peaks. As before, Spearman correlation was the most conservative and consistent estimate of reproducibility.

For ChIP-seq ‘narrow peak’ experiments (JMJD3), in addition to TSS-based and genome-based correlations, we used the IDR method guidelines and pipeline available for narrow peaks at the URL https://sites.google.com/site/anshulkundaje/projects/idr. Apart from determining the reproducibility, we also used the IDR method to determine high-confidence peaks supported by both JMJD3 replicates.

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Extended Data Figure 1 | JMJD3 is induced through activation of the NF-κB pathway in a NOTCH1-dependent mode in T-ALL and binds to NOTCH1 target genes. a, Levels of p65 (RELA) protein in control T cells and T-ALL tumour cells. A representative sample from three mice is shown. b, Schematic representation of the Jmjd3 locus showing the p65 binding site (upper) and ChIP analysis for p65 binding to the Jmjd3 locus in mouse control T cells and T-ALL tumour cells, as well as T-ALL cells upon treatment with γ-secretase inhibitor (γSI), which affects NOTCH1 levels (centre). NOTCH1 binding to this region upon γSI treatment in T-ALL cells is also shown (right). c, Analysis of JMJD3 and HES1 messenger RNA levels upon γSI treatment of CUTLL1 cells. The average of three independent studies is shown. d, e, Expression levels of the JMJD3 transcript (d) and protein (e) upon treatment of human T-ALL lines (DND41 and CEM) with a NEMO binding domain (NBD) inhibitor of the NF-κB pathway. f, JMJD3 levels in T-ALL cells upon inhibition of the NF-κB pathway using a dominant negative form of IκBα (DN-IκBα). g, h, ChIP for NOTCH1 (g) and H3K27me3 (h) on the Hes1 promoter upon γSI treatment of mouse T-ALL cells. In d and f–h, the average of three studies is shown. In e, a representative example from three studies is shown. i, Genes correlated with selected human genes (including JMJD3 and NFKB1) were tested for enrichment in loss-of-H3K27me3 genes during the transition to T-ALL in the mouse model. j, Overlap of JMJD3 peaks with peaks of important activating (H3K4me3 and H3K4me1) and repressive (H3K27me3) epigenetic marks, as well as members of the NOTCH1 complex. The percentage of TSSs containing JMJD3 peaks was used as a conservative control and is an alternative to the much lower genome-wide JMJD3 occupancy. k, Genome-wide distribution of JMJD3 peaks in human T-ALL.
Extended Data Figure 2 | JMJD3 is vital for T-ALL growth through participation in NOTCH1 transcriptional programs. 

**a**. NOTCH1 interaction analyses for JMJD3, MAML1 and WDR5 proteins in 293T cells. Interaction with JMJD3 was confirmed in a reciprocal way (right-most lane, immunoprecipitation (IP) using an anti-haemagglutinin (HA) antibody). 

**b**. Expression of JMJD3 and WDR5 in 293T cells, followed by immunoprecipitation using the anti-HA antibody against HA–JMJD3. An anti-Flag antibody was used for the detection of both proteins. 

**c**. NOTCH1 interaction studies for JMJD3 and MAML1 proteins in mouse T-ALL cells expressing a Flag/Strep form of intracellular NOTCH1. StrepTactin beads were used for NOTCH1 precipitation in the absence of detectable intracellular NOTCH1, and different antibodies were used for the detection of JMJD3, MAML1, EZH2 and UTX. Extracts from green fluorescent protein (GFP)-expressing cells were used as negative control. All experiments were repeated three times (biological replicates), and a representative example is shown. 

**d**. mRNA expression of JMJD3 and UTX upon treatment with shRNA against JMJD3 or UTX. The expression after treatment of CEM cells with two shRNAs against JMJD3 and one shRNA against UTX and one control (Renilla) is shown. 

**e**. The effects on cell proliferation as measured by the loss of GFP-expressing shRNA. HL-60 is an acute promyelocytic leukaemia cell line (APL), which is a subtype of acute myeloid leukaemia (AML) and is used as control in this study. For both cell lines, the average results from three representative studies are shown. 

**f**. Annexin V staining upon shJMJD3 and shRenilla treatment of CUTLL1 cells (top) and HPB-ALL cells (bottom).
Extended Data Figure 3 | JMJD3 binds to genes with important oncogenic functions and is vital for T-ALL growth. 

**a**, JMJD3 but not UTX genetic inactivation impairs the expression of important oncogenic genes. NOTCH1, MYC and MAZ, as well as JMJD3, expression levels are shown. shUTX treatment results in significant upregulation of JMJD3 compared with shRenilla (control)-treated cells. The average results from three studies are shown.

**b**, Significant expression changes in NRARP transcript levels upon JMJD3 knockdown.

**c**, ChIP for H3K27me3 on the NRARP locus.

**d, e**, Binding of JMJD3 to the NOTCH1 (**d**) and MAZ (**e**) promoters upon shJMJD3 and shRenilla (control) treatment. The average results from three studies are shown.

**f**, Numbers of upregulated and downregulated genes are shown for shJMJD3- and shUTX-treated cells compared with shRenilla-treated cells.

**g**, Scatter plot showing the expression levels of important genes in shJMJD3- and shUTX-treated CUTLL1 T-ALL cells. Emphasis is given to the NOTCH1 pathway and apoptosis-related genes. This is a scatter plot representation of an expression analysis comparing three independent studies for shJMJD3 and two for shUTX.

**h, i**, Scatter plots showing the expression levels of important genes in shJMJD3- and shRenilla-treated CCRF-CEM T-ALL cells (**h**) and in shUTX-treated CCRF-CEM T-ALL cells (**i**). CCRF-CEM cells exhibit increased NOTCH1 levels through mutations in the heterodimerization (HD) domain of NOTCH1 and in the NOTCH1-associated ligase FBXW7. Emphasis is given to the NOTCH1 pathway and apoptosis-related genes. This is a scatter plot representation of an expression analysis comparing two studies for shJMJD3, two for shUTX and two for shRenilla.
Extended Data Figure 4 | In vivo studies of the role of JMJD3 in T-ALL using luciferase analysis of CEM-, P12- and CUTLL1-based xenograft models in immunocompromised (NRG) mouse recipients. 

**a, b. In vivo** growth of CEM T-ALL cells in subcutaneous xenograft studies upon genomic ablation of JMJD3 and UTX (red and green circles denote shJMJD3-expressing cells; blue denotes shUTX-expressing cells; and black circles denote shRenilla-expressing cells). One million CEM cells were injected into the animals, and representative graphs from five mouse recipients and an image of a representative mouse on days 0 and 6 are shown (a). Representative graphs from five mouse recipients and the average luciferase intensity on days 0 and 6 are shown (b). 

**c.** Results for growth of CEM cells at different time points post transplantation in subcutaneous xenograft studies (n = 5). 

**d.** Comparison of in vivo cell growth in the subcutaneous model of shJMJD3-, shUTX- and shRenilla-expressing P12 cells (n = 5). 

**e, f, g.** Intravenous xenograft studies using CUTLL1 cells injected into sublethally irradiated NRG (immunocompromised) recipients (n = 4). 

One million P12 cells were injected into sublethally irradiated NRG (immunocompromised) recipients, and the mice were monitored every day for luciferase activity. Day 0 was the first day that a substantially detectable luciferase intensity was measured. The last day of the experiment was the day that either luciferase intensity reached saturation or the mice were euthanized for humanitarian reasons. Red and green circles denote shJMJD3-expressing cells (two different shRNAs, shJMJD3A and shJMJD3B); blue denotes shUTX-expressing cells; and black circles denote shRenilla-expressing cells. 

Monitoring the change in luciferase intensity over a period of seven days in the subcutaneous xenograft model using CUTLL1 T-ALL cells (n = 4). 

In e-g, 0.5 × 10^6 CUTLL1 cells were transplanted, and the mice were monitored every day for luciferase activity.
Extended Data Figure 5 | UTX is a tumour suppressor and is genetically inactivated in T-ALL but is dispensable for physiological T-cell development. a, b, Study of lymphoid development in Utx<sup>−/−</sup> compared with Utx<sup>+/+</sup> (or Utx<sup>+/y</sup>, data not shown) background mice. Flow cytometric analyses of CD4<sup>+</sup> and CD8<sup>+</sup> expression (a), and the relative proportions of CD4<sup>+</sup>CD8<sup>−</sup> (double-positive) thymocytes across different genotypes (b) are shown. A representative example from three independent samples (biological replicates) is shown. c, Illustration of the transplantation scheme for the in vivo leukaemia studies. d, e, T-ALL progresses faster in the male Utx knockout background (Utx<sup>2y</sup>) than in the female wild-type background (Utx<sup>+/+</sup>) in recipients transplanted with NOTCH1-IC-GFP-expressing haematopoietic progenitors, as is demonstrated by the white blood cell counts in the peripheral blood (d), as well as the percentage of GFP<sup>+</sup> leukaemic cells in the peripheral blood upon transplantation of wild-type progenitors (e) from female mice (Utx<sup>+/y</sup>) compared with the corresponding knockout cells (Utx<sup>−/−</sup>). f, Survival study of the recipients of cells from male wild-type (Utx<sup>+/y</sup>, n = 7) and knockout (Utx<sup>−/y</sup>, n = 5) mice expressing NOTCH1-deltaE(DE)-GFP (an allele with weaker oncogenic action than NOTCH1-IC). g, h, Survival analysis of recipients upon transplantation of wild-type progenitors from female mice (Utx<sup>+/+</sup>) compared with the corresponding knockout cells (Utx<sup>−/−</sup>) carrying NOTCH1-IC (g) or NOTCH1-DE (h). i, Quantitative PCR (qPCR) validation of the expression levels of one downregulated gene (Suz12) and one upregulated gene (Il7r) in Utx<sup>−/−</sup> compared with Utx<sup>+/+</sup> mice. The average results from three independent samples (studies) are presented. j, Targeted Sanger sequencing in paediatric T-ALL led to the identification of three cases with frameshift mutations. The positions of the mutations are indicated by dashed lines in the electropherograms. k, Identification of one in-frame deletion (p.Ala14_Ala17del, #1, top panel), one splice acceptor site (#2, second panel) and one missense mutation (#3, third panel) in adult T-ALL. Case 4 is an adult T-ALL case with wild-type UTX (control, bottom panel). Mutations are indicated by red characters. l, The levels of UTX in CUTLL1 T-ALL cells in the absence (−dox) or presence (+dox) of doxycycline. m, n, Apoptosis analysis through measuring annexin V staining using control LacZ-expressing and UTX-expressing CUTLL1 cells in the absence or presence of doxycycline. Representative plots (l, n), as well as the average results (l, m), from three independent experiments are shown.
Extended Data Figure 6 | Physiological development of the haematopoietic system in the absence of JMJD3. a, b, Targeting scheme for the generation of the Jmjd3<sup>−/−</sup> mouse (a) and PCR-based quantification of the wild-type and mutant transcripts (b) using a specific primer set for the 3′ end of Jmjd3 cDNA. c, d, Analysis of the fetal liver for lineage markers (c), as well as the bone marrow (d) of recipients for haematopoietic progenitors (the Lin<sup>−</sup> c-Kit<sup>−</sup> Sca1<sup>−</sup> (LSK) population), for the Jmjd3<sup>+/+</sup> and Jmjd3<sup>−/−</sup> genotypes. Representative plots from three independent experiments are shown.

e–g, Analysis of major thymic subsets in Jmjd3<sup>+/+</sup> (n = 7) and Jmjd3<sup>−/−</sup> (n = 7) mice. Schematic representation of the flow cytometric analysis performed (e). Relative proportions of the major cell populations in the thymi of Jmjd3<sup>+/+</sup> and Jmjd3<sup>−/−</sup> mice (f). The mRNA expression of the Jmjd3 gene at different stages of thymic development (g). h, The expression of NOTCH1 target genes (such as Hes1, n = 7) in CD4<sup>+</sup>CD8<sup>+</sup> (double positive) and CD4<sup>+</sup>CD8<sup>+</sup>CD25<sup>+</sup> lymphocyte progenitor cells. Representative plots (e), as well as average results (g, h), from seven independent thymi are shown.
Extended Data Figure 7 | JMJD3 is necessary for disease initiation in an animal model of T-ALL. Initiation of the disease was studied by transplanting c-Kit⁺ haematopoietic progenitors. a, b, Blood analysis of the recipients for NOTCH1-IC–GFP leukaemic blasts (Jmjd3⁻⁺, n = 8; Jmjd3⁻⁻, n = 8; a) and white blood cells (WBCs, Jmjd3⁻⁺, n = 4; Jmjd3⁻⁻, n = 6; b). c–e, Comparison of the organ size (c), histochemistry (haematoxylin and eosin staining) (d) and flow-cytometry-based analysis (e) of the leukaemic cell infiltration of the spleen. f, Survival studies of recipients. Eight recipients for the Jmjd3⁻⁺ and eight for the Jmjd3⁻⁻ background were used in c–f.
Extended Data Figure 8 | GSKJ4 inhibitor induces apoptosis and cell cycle arrest of T-ALL but not myeloid leukaemia or physiological LSK cells. 
a, Effect of GSKJ4 (at 2 μM concentration) on a panel of T-ALL and myeloid lines. The average results from three representative studies are shown. 
b–d, Effects on cell growth (b), apoptosis (c) and the cell cycle (d) in three primary T-ALL lines. The average results from three representative studies are shown. 
e, f, Measurement of apoptosis (e, n = 3) and cell cycle effects (f, representative study from three experiments) on CUTLL1 cells 72 h post treatment with the inhibitor. 
g, h, Apoptosis assays using annexin V staining of CEM cells (g) after a period of 72 h of treatment and measuring caspase 7/9 activity upon treatment of CUTLL1 T-ALL cells with GSKJ5 or GSKJ4 over a period of 24 h (h). 
i, Time course studies of annexin V (top) and cell cycle analysis (bottom) of CUTLL1 cells over a period of 72 h during GSKJ4 treatment according to the scheme on top of the figure. 
j, Expression of the wild-type and catalytic mutant of JMJD3 in T-ALL (CEM) cells. 
k, Cell growth analysis of T-ALL cells overexpressing wild-type JMJD3 or a catalytic mutant of JMJD3 upon GSKJ4 treatment over a period of 72 h. 
Average results from three independent experiments are shown. 
l, Cell growth of LSK cells upon treatment with the control (2 μM) and different concentrations of the inhibitor GSKJ4. 
m, Annexin V staining of THP-1 (AML) cells after a period of 72 h of GSKJ4 or GSKJ5 (control) treatment at 2 μM concentration. The average results from three independent experiments are shown. 
n, The mRNA levels are shown for three classical NOTCH1 targets (HEY1, NRARP and NOTCH1) over a period of 72 h during GSKJ4 treatment. The average results from three independent experiments are shown.
Extended Data Figure 9 | GSKJ4 treatment leads to increased H3K27me3 levels on NOTCH1 target genes through specific inhibition of JMJD3 activity. a–c, Analysis of the promoter area of HEY1 (a), NOTCH1 (b) and NRARP (c) for H3K27me3, H3K27me1, NOTCH1 and JMJD3 enrichment over a period of 24 h during GSKJ4 treatment. The average results from three independent experiments are shown. d, Analysis of the total protein extracts from CUTLL1 cells for JMJD3 and NOTCH1. e, Analysis of the chromatin fraction from CUTLL1 cells for the repressive mark H3K27me3, the activating marks H3K27me1 and H3K4me3, as well as total histone H3 levels. Representative plots from three independent experiments are shown. f, Snapshots of GSKJ4-associated H3K27me3 changes in major NOTCH1 and JMJD3 targets. g, ChIP-qPCR analyses for UTX binding to the NOTCH1 target genes HEY1, NRARP and NOTCH1. (RBBP6 was used as positive control). The average results from three independent experiments are shown.
Extended Data Figure 10 | JMJD3 as a pivotal factor in NOTCH1-mediated oncogenic activation in T-cell leukaemia. a, Schematic representation of the H3K27me3 writer (the polycomb complex, left) and eraser (JMJD3, right). EZH2 contains the catalytic subunit of the complex through its SET domain, whereas the EED subunit recognizes the H3K27me3 mark and aids in polycomb binding. JmjC domain activity is inhibited by the small molecule inhibitor GSKJ4. b, The main idea about the key role of JMJD3 in the NOTCH1 transcriptional complex. Before activation of the NOTCH1 signalling pathway, the promoters of classical NOTCH1 target genes are bound by RBP-Jκ, together with components of the co-repressor complexes and PRC2, leading to low gene expression. After the binding of NOTCH1 and its co-activator MAML1, the genes are activated through the recruitment of JMJD3 and the MLL complex, with simultaneous eviction of PRC2, which leads to the demethylation of H3K27me3 and the methylation of H3K4me3.