Supplementary Information

Supplementary Methods:

Primary cell culture

Primary FL cell-suspensions were defrosted at 37°C and layered onto 3ml of lymphoprep (STEMcell Technologies). Lymphocytes were isolated by centrifugation at 1150g for 12 minutes, washed in RPMI and resuspended in fresh RPMI before treatment. Written consent was obtained for the collection and use of specimens for research purposes with ethical approval obtained from the London Research Ethics Committee of the East London and the City Health authority (10/H0704/65, 06/Q0605/69) and Southampton and South West Hampshire (t228/02/t).

Western blots

To assess histone mark levels, an isotonic lysis buffer (20mM Tris, 100mM NaCl, 5mM $MgCl_2$, 10% glycerol, 0.2% NP40, 0.5mM DTT) and centrifugation was used to isolate nuclei, which where lysed in a high-salt buffer (50mM Tris, 600mM NaCl, 10% glycerol, 0.2% NP40, 0.5mM DTT) followed by sonication to fragment chromatin (Diagenode Bioruptor). Buffers were supplemented with phosphatase and Complete ULTRA protease inhibitor cocktails (Roche) and lysates quantified by Pierce 660nm Protein Assay Reagent (ThermoFisher). 1-2.5µg of nuclear protein was loaded in 4-12% Bis-Tris gels (NuPAGE), resolved by SDS-PAGE and transferred to polyvinylidene fluoride membranes via the iBlot™ transfer device (Invitrogen). After blocking with 5% BSA/Milk in Tris-buffered saline, membranes were probed with primary antibody, stained with horseradish peroxidase-conjugated secondary antibodies (DAKO) and bands detected using ECL Plus (GE Healthcare) or

SuperSignal West Femto (ThermoFisher). To examine multiple histone marks, equal amounts of protein were loaded onto multiple gels from a single loading solution, and quantified relative to H3.

For total protein analysis, western blots were performed as before except that cells were lysed in RIPA buffer (150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium-dodecyl-sulphate, 50mM tris(hydroxymethyl)aminomethane hydrochloride, pH 8.0), supplemented with 1X protease inhibitor and phosphatase inhibitor cocktail 2 and 3 (Sigma-Aldrich), for 30 minutes. Densitometry analysis was performed using ImageJ software and all the antibodies used are listed in Supplementary Table 12.

Proliferation and apoptosis assays

2000 cells were seeded in 100µl growth media in triplicate in 96-well plates 24h before treatment, followed by treatment with DMSO and 6 concentrations of KDM5i diluted 8-fold (0.0003-10µM) in 100µl of growth medium. The plates were incubated for five days, when viable cell numbers were determined using the Guava ViaCount assay (Millipore) or CellTitreGlo (Promega). The percentage of apoptotic cells was quantified by the Guava Nexin assay (Millipore), which measures binding of Annexin V to phosphatidyl serines and the incorporation of 7-AAD, a cell impermeable dye. Apoptotic cells were defined as Annexin V+/7-AAD+, early-apoptotic as Annexin V+/7-AAD- and nucleated debris as Annexin V-/7-AAD+.

For 10-day treatments, 20,000 cells were seeded in 1ml in 12-well plates and incubated overnight. The cells were then treated with DMSO or KDM5-inh1 (0.0024-10µM) and incubated for 5 days. Viable cell numbers were determined, and the cells re-seeded in

triplicate in 96-well plates at 4000 cells/well and treated with the same concentration of KDM5-inh1 or DMSO as before. Viable cell numbers were determined by Guava ViaCount assay after a further 5-day incubation.

Cell-cycle analysis

After treatment with DMSO or KDM5-inh1 for 72h, cells were permeabilized in ice-cold 70% ethanol, stored at -80°C and stained in PBS containing 50µg/ml propidium iodide and 100µg/ml RNase A. DNA content was then quantified using the YG610/20 filter on a Fortessa II flow cytometer.

Surface IgM analysis

Cells were washed, re-suspended in FACS buffer (1% BSA, 4mM ethylenediaminetetraacetic acid (EDTA) and 0.15mM NaN_3 in PBS) and stained for surface IgM expression ($5x10^5$ cells/100µl) in the dark on ice for 30 minutes, using R-Phycoerythrin-conjugated anti-IgM (DAKO). Following incubation, cells were washed, re-suspended in FACS buffer and $1x10^4$ lymphocytes were acquired on a FACS Canto (BD Biosciences). Analysis of mean fluorescence intensities was performed with FlowJo software.

Synergy analysis

Cells were treated with 5 concentrations of KDM5-inh1 for 5 days as per the standard proliferation assay described above, except that cells were also treated with 5 concentrations of S63845 and Venetoclax for 2 days or Ibrutinib for 3 days. Synergy was assessed for each combination using the DrugSynergy portal (https://synergyfinder.fimm.fi/)[59].

ChIP-PCR and –seq

ChIP reactions were prepared using a modified version of the Active Motif ChIP-IT High Sensitivity Kit. For cell lines, 5-15 million cells were treated for 72h with DMSO or 1μM KDM5-inh1, cross-linked in a 1% formaldehyde/PBS solution for 5 minutes, washed in PBS, and nuclei isolated by 5 minute incubation on ice in a cytoplasmic lysis buffer (50mM Tris·Cl, 140mM NaCl, 1.5mM $MgCl_2$, 0.5% (v/v) Nonidet P-40 (1.06g/ml)) and centrifugation. Nuclei were sonicated using a BioRuptor for 10-20 cycles of 30s on (high)/60s off. After confirming correct fragmentation of input DNA, ChIP reactions were performed overnight at 4°C (antibodies listed in Supplementary Table 12), followed by DNA precipitation with agarose beads, reversal of cross-links and purification of DNA by columns. Samples were analyzed by qPCR using the probes listed in Supplementary Table 2. Lymphocytes from primary FL cell-suspensions were examined identically except that 1-5 million cells were used per ChIP and that the cells were treated for 48h. For ChIP-seq, the chromatin was spiked with 15ng of Drosophila chromatin (Active Motif; 53083) and ChIP was performed with an anti-*Drosophila* chromatin antibody (Active Motif; 61686) alongside the H3K4me3/H3K4me1 antibodies.

Libraries were prepared for sequencing using the NEBNext Ultra II and Multiplex Oligios for Illumina kits (New England Biolabs) according to the manufacturers protocol. Briefly, ChIP and input DNA were end-repaired, adaptors ligated and size-selected using SPRIselect beads for 300-400bp DNA fragments and amplified by PCR. Correct library size (400-500bp) was confirmed by Tapestation. Sequencing was performed on the Illumina HiSeq 4000 to generate 75bp paired-end reads or NextSeq

500 to generate 40bp single-ended reads.

CRISPR

To generate *KMT2D* mutant cells, four pooled guide-RNAs (gRNAs) were designed targeting exon 3 of *KMT2D*, while individual gRNAs were used for KDM5A/KDM5C (Supplementary Table 3). gRNAs were combined with tracrRNA at equimolar concentrations, heated at 95ºC before cooling to anneal. 460pmol of the gRNA/tracrRNA pool was then complexed with 401pmol of Cas9 protein (Alt-R Cas9 Nuclease 3NLS; IDT) and transfected by Nucleofection (Supplementary Table 4). After transfection, cells were left to recover in 4ml of complete growth medium and after 48h a cell-sorter was used to isolate single cell clones. Editing was identified by Sanger sequencing and validated by TA-cloning for complex mutations (Supplementary Table 5). To correct the 1bp insertion present in *KMT2D* within SU-DHL-8 cells, CRISPR was performed as above except that a donor-template containing 119bp of WT sequence, with a silent mutation to alter the PAM site, was co-transfected alongside the gRNA (targeting the mutation site) and Cas9 protein.

Xenograft studies

SU-DHL-6 xenograft studies were performed by Crown Bioscience Inc. (Beijing). 24h after irradiation with Co^{60} (150 rads), $5x10^6$ SU-DHL-6 cells (in 0.1ml PBS mixed with matrigel 1:1) were inoculated subcutaneously into the right flank of NOD/SCID mice (weighing 18-20g). Once tumours reached an average size of $100mm^3$, mice were randomized into three groups of 10; vehicle (6% Captisol + 94% ddWater, pH=2), KDM5-inh1 and ibrutinib. Mice were orally dosed daily with 50mg/kg KDM5-inh1

and 10mg/kg ibrutinib up to 21 days, with a scheduled dosing holiday for the KDM5-inh1 group between days 8-14. Six mice were additionally randomized into two groups (n=3) and treated with vehicle or 50mg/kg KDM5-inh1 for 1 week. Tumour volumes were calculated in two dimensions 3x a week. Mice were euthanized when the mean tumour size of the vehicle group exceeded 2000mm^3 or once the study endpoint was reached.

Mutational profiling of cell lines

Targeted resequencing was performed on 28 frequently mutated genes using the Access Array platform (Fluidigm) as previously described[1]. Amplification was performed in multiplex using 50ng genomic DNA while sequencing was performed on pooled libraries using the Illumina MiSeq platform. Data were aligned to hg19 using Bowtie2[60] and VarScan2[61] was used to call variants, including SNPs and short indels, implementing a threshold VAF > 5%, a read depth > 10 and a Fisher's exact test *P* value of <0.05. Identified variants were annotated using SNPnexus. For OCI-LY-18, whole exome capture libraries were prepared using the Agilent SureSelect Human All Exon Kit V5 (Agilent Technologies). Paired-end multiplex sequencing of samples was performed on the Illumina HiSeq 2500. Data were aligned to hg19 using the Burrows-Wheeler Aligner (BWA)[62], and converted into BAM files with the exclusion of PCR duplicates and removal of low quality reads using Picard (version 1.86), while the Genome Analysis Toolkit (GATK) version 2.3.9[63] was used for realignment of discordantly mapped read pairs around indels and recalibration of base quality scores. Somatic single nucleotide variants (SNVs) were identified using the Strelka pipeline and independently verified by Sanger sequencing (Supplementary Table 1).

RNA-seq and analysis

mRNA isolation and strand-specific cDNA libraries were prepared using the Illumina TruSeq stranded mRNA Prep kit according to the manufacturer's instructions. For SU-DHL-6 and OCI-LY-18, two hundred cycles of sequencing on the Illumina HiSeq 2500 instrument were performed to generate 2x100bp paired-end sequencing reads. HT libraries were sequenced by 300 cycles of sequencing on the HiSeq 4000 to generate 2x150bp paired-end sequencing reads while WSU-DLCL2/WSU#22 cells were sequenced using 2x40bp reads generated on the NextSeq 500. Raw data was aligned to hg19 using STAMPY[64] and BWA[62]. The number of uniquely aligned reads (quality score $q > 10$) aligned to the exonic region of each gene were counted using HTSeq[65] based on the Ensembl annotation (version 75). Only genes that achieved at least one read per million reads (CPM) in at least 3 samples were kept. Aligned reads numbers were further normalised using the 'cqn' method[66], accounting for gene length and GC content. Differential expression (DE) analysis was then performed using the edgeR R package[67], employing the generalised linear model (GLM) approach, for the treated versus control pairwise matched comparisons. DE genes were selected based on a false discovery rate (FDR) of <0.05 and a fold change >2 or <0.5.

Raw read counts across 55,765 annotated genes for 97 FL and 75 DLBCL samples were obtained from the ICGC and converted to RPKM expression values. Gene-level transcription estimates of 20,531 genes (log2(transformed RSEM normalized count + 1) (level_3 data)) from were obtained from the TCGA Large B-cell Lymphoma (DLBC) cohort (n=48) using the UCSC Xena Browser (https://xenabrowser.net/datapages/). RPKM expression values from healthy GC B-

cells were downloaded from the BLUEPRINT EPICO data portal (http://blueprint-data.bsc.es/release_2016-08/#!/).

To identify clusters within the KDM5-inhibition/*KMT2D* mutation RNA-seq datasets, log2 fold change values from 897 genes DE following either KDM5-inhibition or *KMT2D* mutation were selected. K-means clustering was performed for 1-20 clusters using the kmeans function in R, with six identified as the optimal cluster number by plotting the cluster number verses the "within-cluster sum of squares".

ChIP-seq analysis

To normalize ChIP-seq data, protocols published by Active Motif and used to investigate other epigenetic drugs were followed[68]. Input FASTQ files were mapped to hg19 using Bowtie 2[60], while H3K4me3/me1 FASTQ files were mapped to hg19 and dm3. The number of reads mapping to the dm3 genome, derived from the spike-in, were used to calculate a scaling factor and sequencing reads were randomly removed from samples requiring normalization using SAMtools[69] (samtools view -b -s <scaling.factor> <filename.bam> > <scaled.bam>) i.e. samples with higher spike-in read counts were downscaled so that the overall read number would be relative to the level of the histone mark in that sample. Peaks were identified using the broad source option in MACS2 (macs2 callpeak -t <filename.bam> -c <input.bam> --broad -n <filename> -g hs --broad-cutoff 0.1 -q 0.05) and regions known to result in poor quality sequencing ([24]) removed using BEDtools[70]. Analysis of differential H3K4 methylation was performed in R using EdgeR within the DiffBind package[71]. Peak annotation was performed using ChIPseeker package[72] and Genomic Regions Enrichment of Annotations Tool (GREAT)[73]. The overlap with publicly available ChIP-seq datasets

was performed using Intervene[74] to generate pairwise intersection matrices, BEDtools intersect[70] to assess individual relationships and the Genomic Association Tester to calculate significance[75].

To examine the relationship between H3K4me3 and H3K4me1 at promoters following KDM5-inhibition, 1kb intervals were generated from H3K4me3 positive promoters, centered (+/- 500bp) on the TSS. Bedtools coverage (bedtools coverage -a <Intervals.bed> -sorted -b <chip.bam>) was then used to count reads in each interval and calculate fold changes following KDM5-inh1 exposure. Profile plots for histone marks were generated using the computeMatrix and plotProfile tools from Deeptools[57].

To assess the enrichment of histone marks and epigenetic regulators at promoters (TSS +/- 500bp), TSS locations for each gene were downloaded from BioMart and any overlapping intervals merged. Intervals overlapping genes in each cluster were then selected and Deeptools MultiBigwig summary (MultiBigwigSummary bins -b <bigwig files> --bed <Cluster.bed> --outRawCounts <filename>)[57] used to calculate a summary score for each promoter using bigwig files downloaded from the ENCODE or BLUEPRINT projects. The control promoters were generated as above, using genes that were not DE or differentially methylated in any RNA- or ChIP-seq experiment, and then overlapped with H3K27me3 deposition[31] to identify non-bivalent (H3K4me3+/H3K27me3-) and bivalent (H3K4me3/H3K27me3+) promoters. Mean values were then scaled between clusters for each dataset using the scale function in R.

All generated ChIP-seq data have been deposited in the GEO database under the accession number GSE153671.

Pathway enrichment

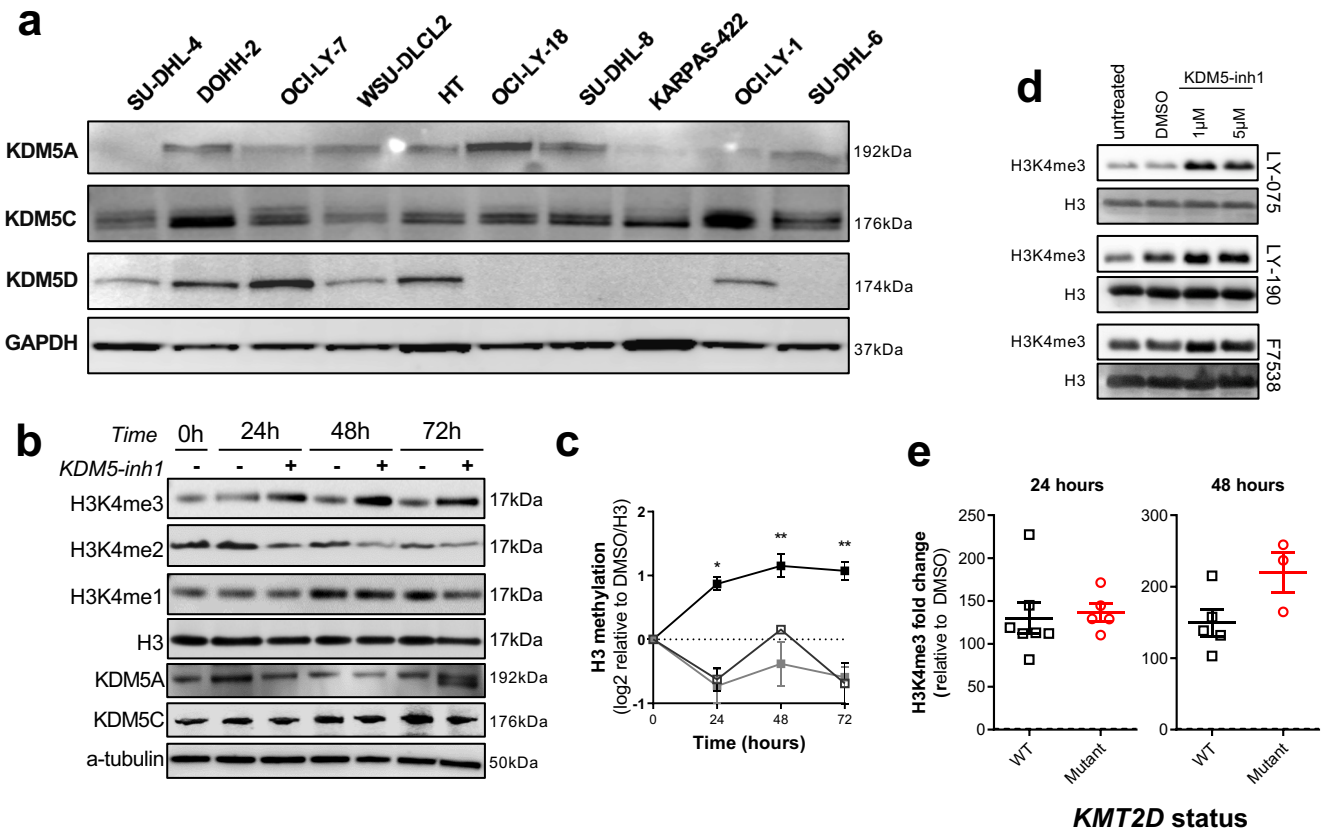
Pathway enrichment on genes proximal to ChIP-seq intervals was performed using GREAT[73]. For RNA-seq data and analysis of promoters with differential H3K4me3 deposition, Gene Set Enrichment Analysis (GSEA) was performed[26] using pre-ranked option and "classic" scoring. To interrogate publicly available datasets from different B-cell/lymphoma studies, we used a manually curated version of the lymphochip database[28] where key studies from the literature were manually added.

Mass spectrometry phosphoproteomics

Cells were lysed with urea lysis buffer (8M urea, 25 mM HEPES) supplemented with phosphatase inhibitors, and extracted proteins digested with trypsin. Resultant peptides were then enriched for phosphopeptides using TiO2 chromatography and analysed in a LC-MS/MS system consisting of a Q-Exactive orbitrap instrument connected to a Ultimate-3000 nanoflow chromatograph (ThermoFisher Scientific). Phosphopeptides were identified with the Mascot search engine and quantified using label-free methods as previously described[76].

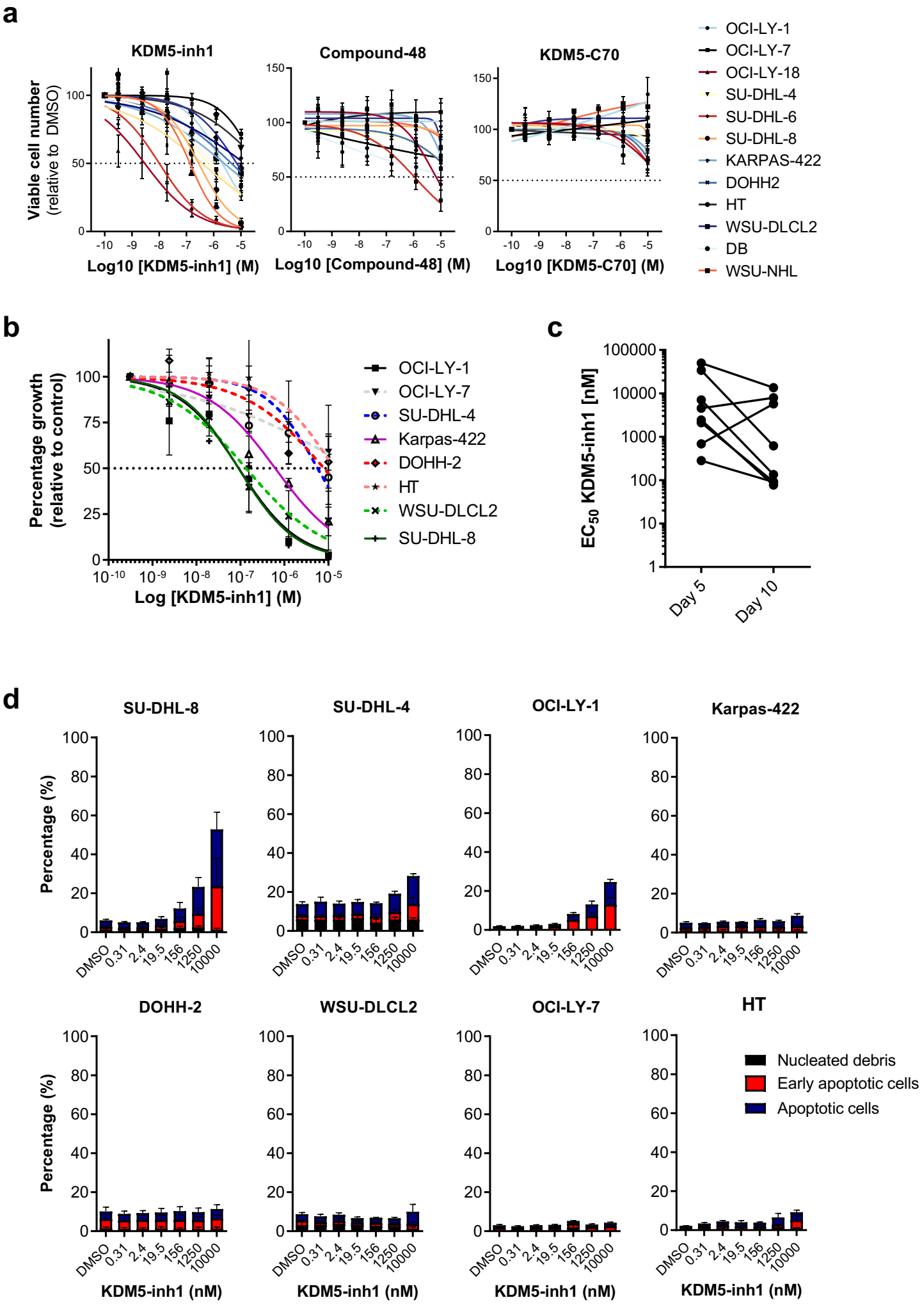
Supplementary Figures:

Supplementary Figure 1



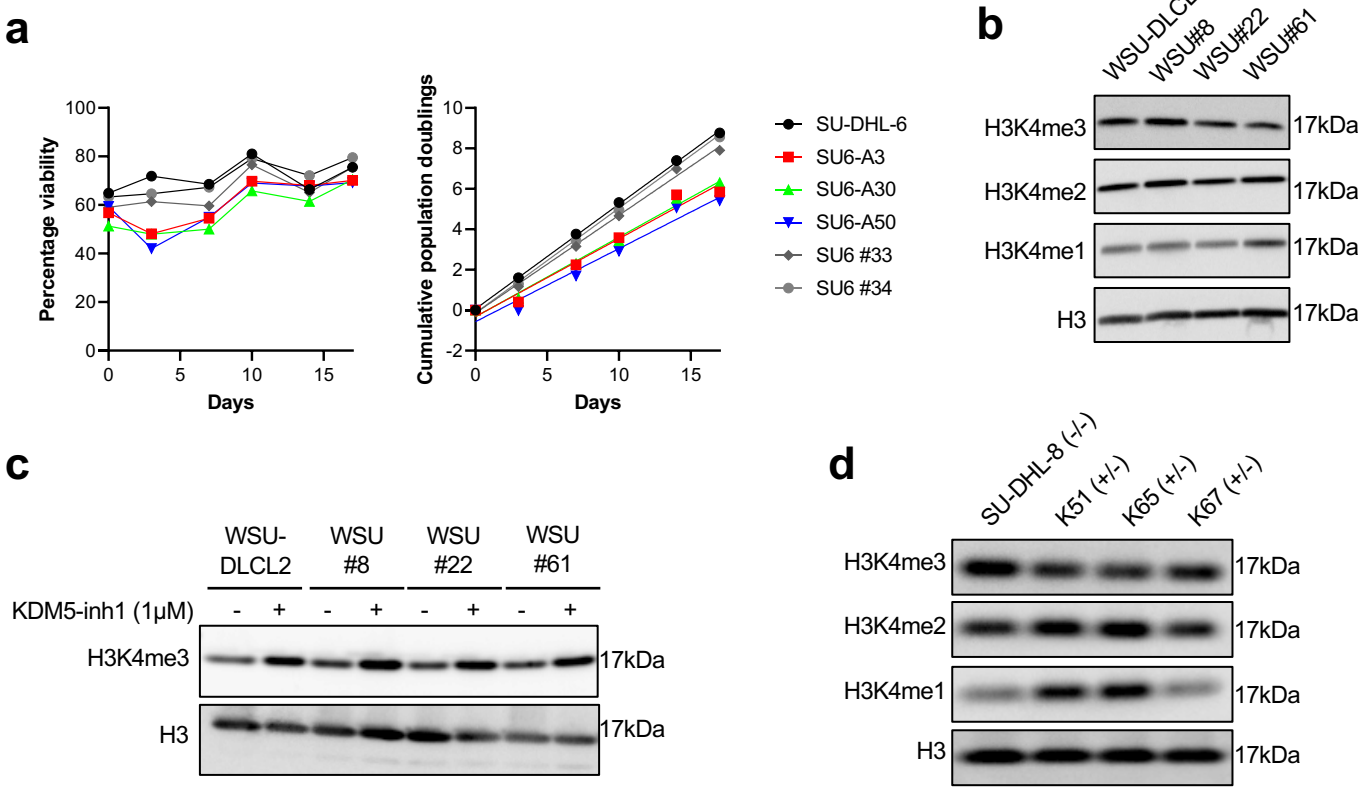
Supplementary Figure 1. Validation of KDM5 expression and effect of KDM5-inhibition on H3K4-methylation in DLBCL cell lines and FL cell suspensions. (a) Expression of KDM5A, C and D was confirmed by western blot in 10 DLBCL cell lines. **(b+c)** SU-DHL-6 cells were treated with DMSO or 1μM KDM5-inh1 for increasing lengths of time, followed by western blots to quantify H3K4me3/me2/me1, KDM5A, KDM5C and H3 levels. Representative westerns blots are show in **(b)** and the quantification and the quantification of H3K4me3/me2/me1 relative to H3 in **(c)**. Data are the mean ± SEM of 3 independent experiments, with significance determined using a one-way ANOVA with a Dunnett's post-test versus untreated control, where ** $P<0.01$ and *** $P<0.001$. H3K4me3 levels and PARP cleavage (no difference – data not shown) were analysed by western blot in primary FL cell suspensions exposed to 1μM and 5μM KDM5-inh1 for 24 and 48h. H3K4me3 levels after 48h are displayed in **(d)** and the quantification of H3K4me3 relative to H3, in *KMT2D* mutant and WT samples, in **(e)**. The primary cell analysis was performed on 5 mutant *KMT2D* and 7 WT cell suspensions at 24h, and 3 mutant and 5 WT at 48h.

Supplementary Figure 2



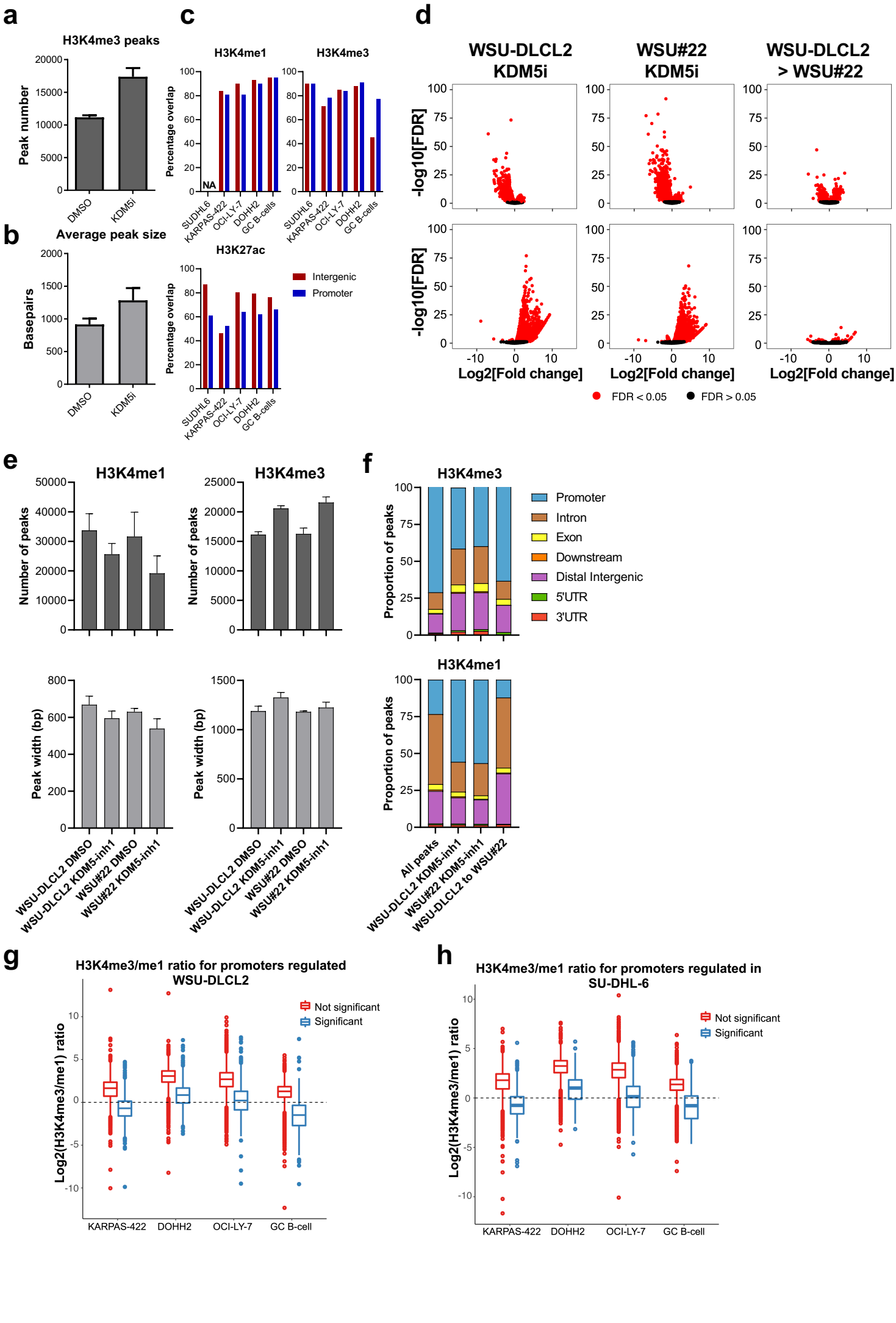
Supplementary Figure 2. KDM5-inhibition is cytostatic and cytotoxic in cell lines. (a) DLBCL, FL, myeloma and Burkitt's lymphoma cell lines were treated with DMSO or increasing concentrations of KDM5-inh1, Compound-48 or KDM5-C70, and viable cells quantified after 5 days. Data are the mean ± SEM of 3-6 independent experiments. **(b)** 8 DLBCL cell lines were treated with DMSO or increasing concentrations of KDM5-inh1 for 10 days, with the cells re-seeded in fresh drug/media after 5 days. Data are the mean ± SEM of 3 independent experiments. **(c)** Mean EC_{50} values after 5 and 10 days. **(d)** Induction of apoptosis was quantified by the Guava Nexin assay in 8 DLBCL cell lines treated with DMSO or increasing concentrations of KDM5-inh1 for 5 days**.** Data are the mean ± SEM of 3 independent experiments.

Supplementary Figure 3



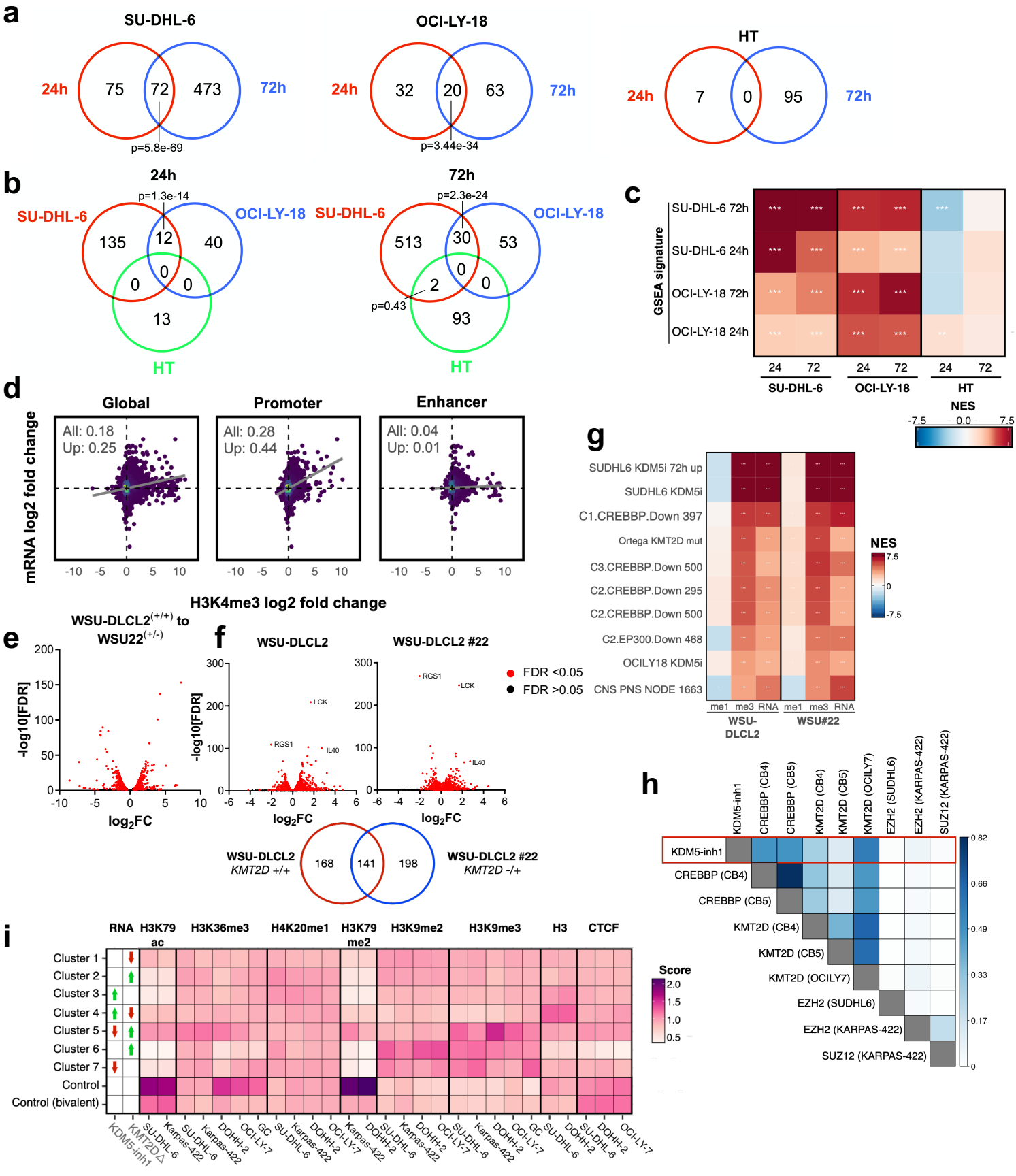
Supplementary Figure 3. Profiling of *KDM5A/C* and *KMT2D* CRISPR models. (a) Parental, control and *KDM5A* knockout SU-DHL-6 cells were seeded at 3x10^5 cells/ml at every passage, allowing a population doubling value to be calculated (left panel), with the percentage viability also monitored using the Guava ViaCount assay (right panel). **(b)** H3K4me3/me2/me1 and H3 levels were quantified by western blot in WSU-DLCL2 parental and *KMT2D* mutant cells. **(c)** H3K4me3 and H3 protein levels were quantified by western blot in WSU-DLCL2 parental and KMT2D mutant cells treated with DMSO or 1μM KDM5-inh1 for 48h. **(d)** H3K4me3/me2/me1 and H3 levels were quantified by western blot in homozygous mutant *KMT2D* SU-DHL-8 parental cells and in three clones with a corrected allele. Results are representative of 3 independent experiments.

Supplementary Figure 4



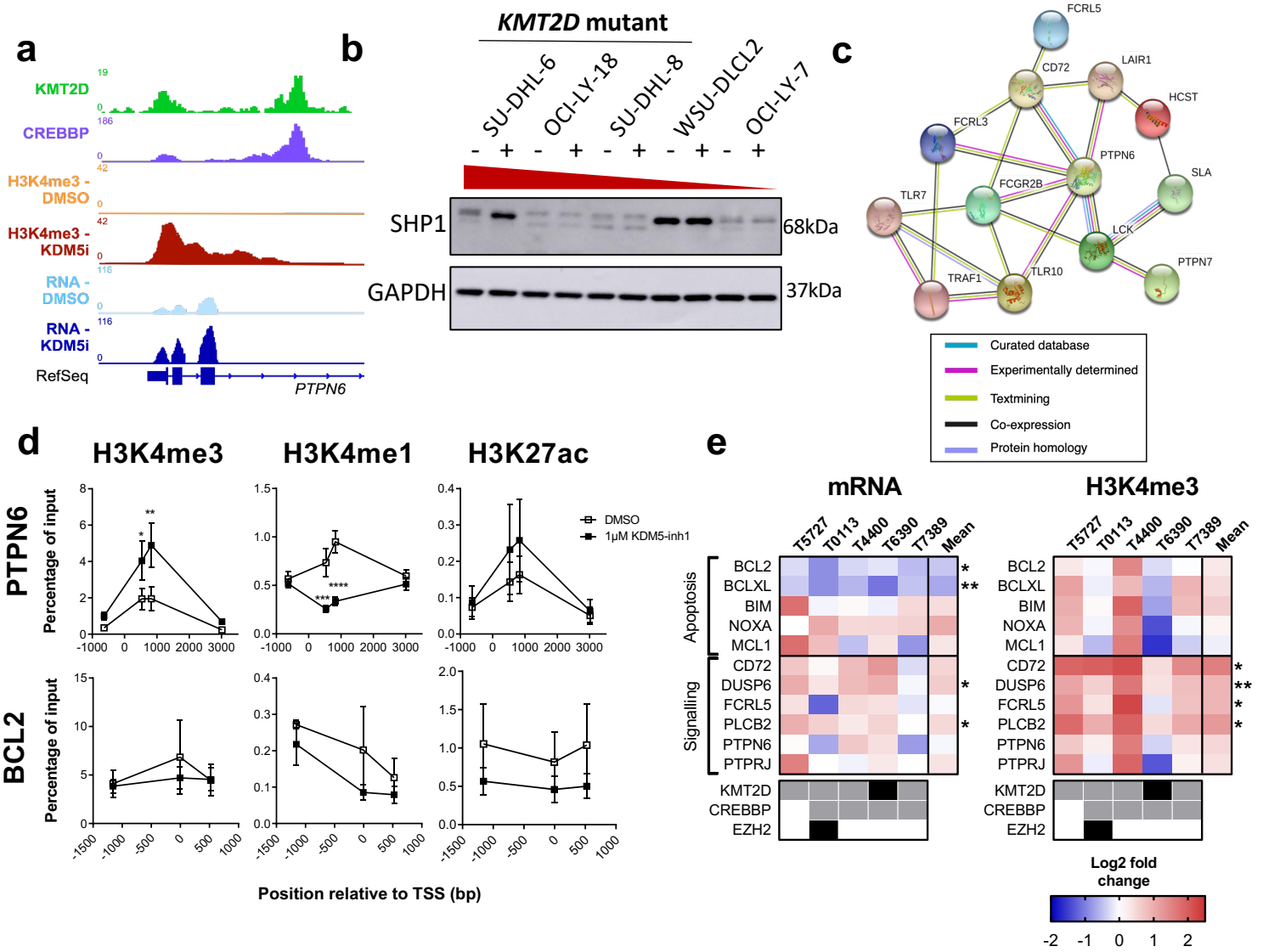
Supplementary Figure 4. KDM5-inhibition targets regions marked by high H3K4me1. (a) Mean number of H3K4me3 peaks and **(b)** mean peak width identified by ChIP-seq in DMSO and 1μM KDM5-inh1 treated SU-DHL-6 cells. **(c)** Summary of percentage overlaps between KDM5-inhibition regulated intergenic regions or promoters, and H3K4me1, H3K4me3 and H3K27ac peaks. **(d)** Volcano plots displaying changes in H3K4me3 or H3K4me1 following ChIP-seq analysis of 1μM KDM5-inh1 treated WSU-DLCL2 and WSU#22$^{-/+}$ cells, or between WSU-DLCL2 and WSU#22$^{-/+}$ cells. **(e)** Number and width of H3K4me1/H3K4me3 peaks in DMSO or 1μM KDM5-inh1 treated WSU-DLCL2 and WSU#22$^{-/+}$ cells. **(f)** Annotation of differential H3K4me1/H3K4me3 peaks following 1μM KDM5-inh1 treatment or between WSU-DLCL2 and WSU#22$^{-/+}$ cells, alongside total peak annotations. **(g+h)** Deeptools[57] was used to calculate summary scores from H3K4me3 and H3K4me1 ChIP-seq datasets, using H3K4me3 peaks called in **(g)** WSU-DLCL2/WSU#22$^{-/+}$ and **(h)** SU-DHL-6 cells. Peaks were subdivided on the basis of being significantly altered by KDM5-inhition in WSU-DLCL2/WSU#22$^{-/+}$ or SU-DHL-6 cells, and log2 ratios of the H3K4me3 score/H3K4me1 score calculated for each examined cell type.

Supplementary Figure 5



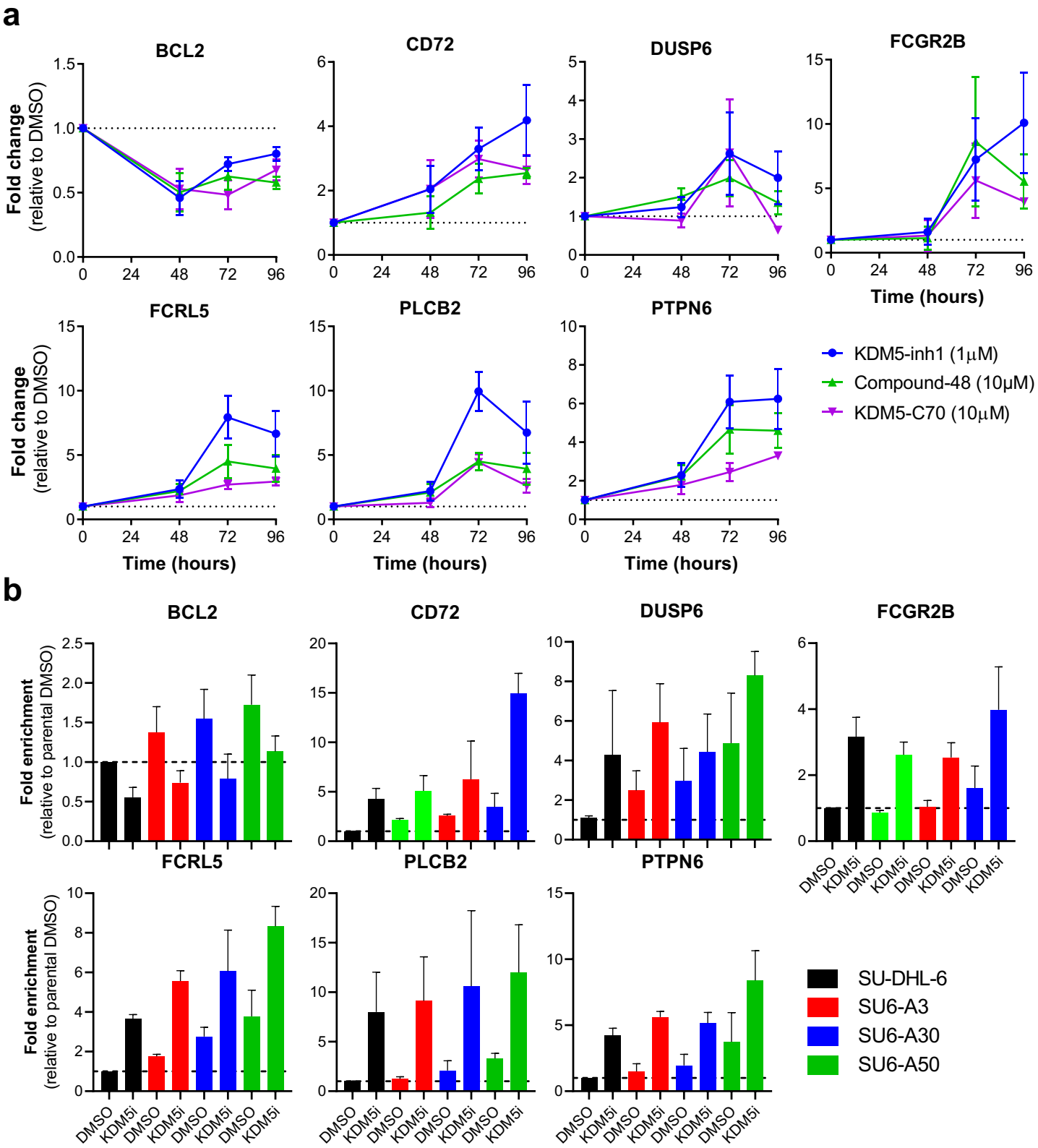
Supplementary Figure 5. Epigenetic and transcriptomic analysis of KDM5-inhibition. Overlap between KDM5-inh1 regulated genes at **(a)** 24h and 72h in SU-DHL-6, OCI-LY-18 and HT cells or **(b)** between cell lines at 24h and 72h, with the hypergeometric p-values of the overlaps displayed. **(c)** GSEA was performed using signatures derived from each cell line (y axis) to examine their enrichment in all cell types and timepoints (x axis). Note that signatures derived from HT cells were not enriched in any condition, likely due to low numbers of protein-coding genes. **(d)** Correlation analysis between mRNA expression and H3K4me3 levels following KDM5-inhibition for all H3K4me3 peaks (Global), promoters or putative enhancers. Peaks were annotated according to the nearest genes by ChIPseeker[72]. Pearson's correlation coefficients are indicated in the top left of each panel for all genes and genes with a log2 fold change > 0 (Up). Volcano plots showing DE genes between **(e)** WSU-DLCL2 and WSU#22$^{-/+}$ cells and **(f)** WSU-DLCL2 and WSU#22$^{-/+}$ cells exposed to 1μM KDM5-inh1 for 72h, with significant genes highlighted in red. **(g)** GSEA of RNA and H3K4me3/H3K4me1 (promoter) datasets following KDM5-inhibition, using a manually curated database of B-cell/lymphoma signatures. **(h)** Intersection matrix[74] showing overlap between KDM5-inhibition regulated regions and KMT2D[9,10], CREBBP[27] and EZH2/SUZ12[58] binding. **(i)** Deeptools[57] was used to calculate summary scores at the promoters (TSS+/-500bp) of genes in each cluster, plus non-bivalent (H3K4me3+/H3K27me3-) and bivalent (H3K4me3/H3K27me3+) control promoters, for ChIP-seq datasets of histone mark deposition and CTCF binding (ENCODE/BLUEPRINT). The overall direction of change in RNA expression, following KDM5i or *KMT2D* loss (Figure 3f), is indicated for each cluster in the first two columns.

Supplementary Figure 6



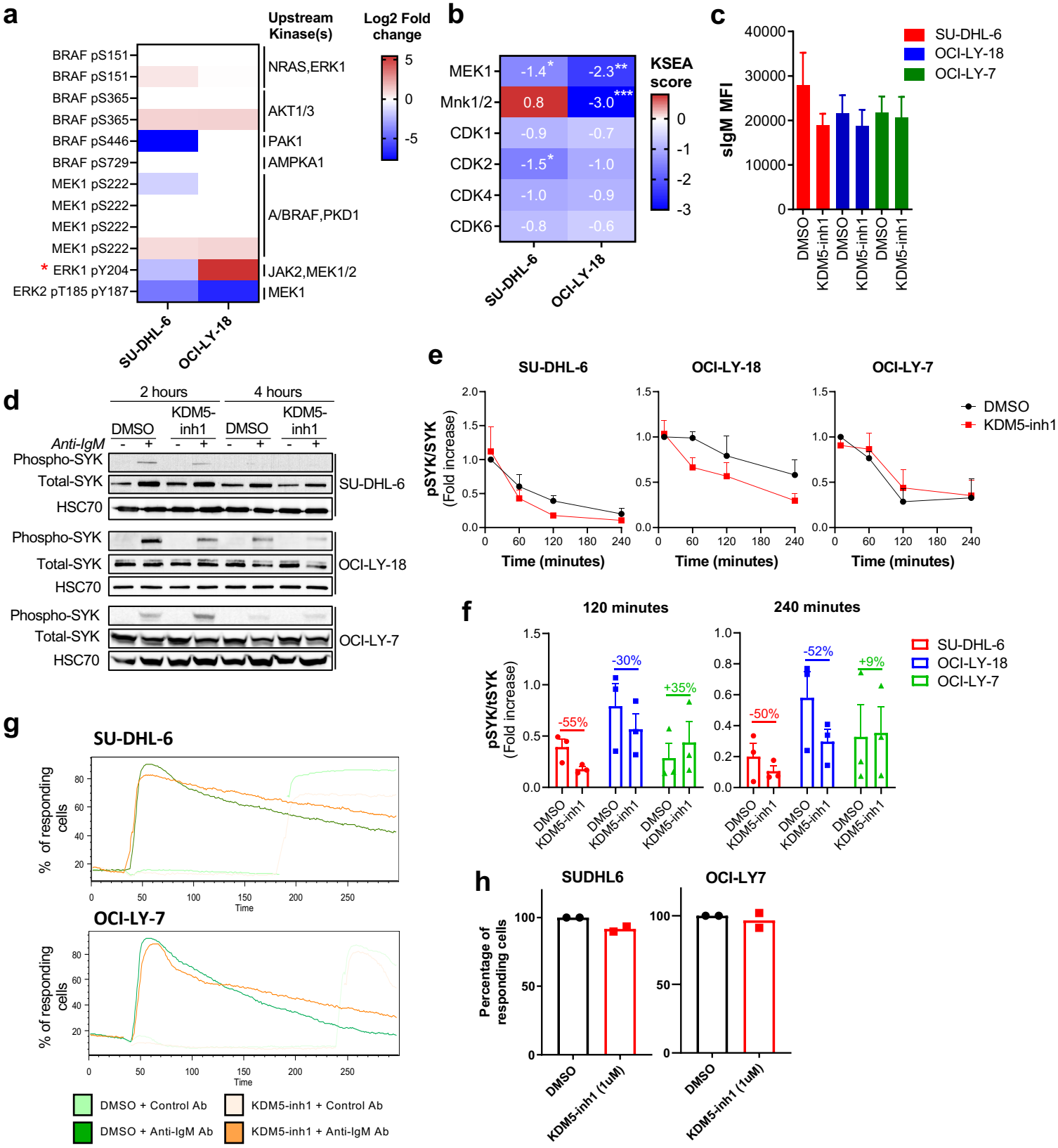
Supplementary Figure 6. KDM5-inhibition induces B-cell signalling regulators (a) ChIP-seq and RNA-seq tracks, centred on the *PTPN6* promoter, from SU-DHL-6 cells treated with DMSO or 1μM KDM5-inh1 for 72h, plus ChIP-seq tracks of KMT2D[9] and CREBBP[27] binding in GC centroblasts. **(b)** Western blots of SHP-1 protein in SU-DHL-6, OCI-LY-18, SU-DHL-8, WSU-DLCL2 and HT cells treated with DMSO or 1μM KDM5-inh1 for 5 days. **(c)** String analysis **(**https://string-db.org/) showing the interaction network of identified B-cell signalling regulators. **(d)** ChIP-PCR analysis of H3K4me3, H3K4me1 and H3K27ac at the *PTPN6* and *BCL2* promoters, following 72h DMSO or 1μM KDM5-inh1 exposure in SU-DHL-6 cells. Data was expressed as the percentage of input and plotted relative to the Transcription Start Site (TSS) of *PTPN6/BCL2.* Data are the mean ± SEM of 3 independent experiments. Statistical significance was calculated using a two-way ANOVA with a Dunnett's post-test, where * P <0.05, ** P<0.01, *** P<0.001 and **** P<0.0001. **(e)** Five primary FL cell suspensions were exposed to DMSO or 1μM KDM5-inh1 for 48h, followed by qRT-PCR and H3K4me3 ChIP-PCR analysis of KMD5i target genes. A summary heatmap is displayed, with the mutational status of patient samples indicated by grey (mono-allelic) and black (bi-allelic) squares in the lower panel. Statistical significance was calculated using a paired students T-test, where * P <0.05 and ** P <0.01.

Supplementary Figure 7



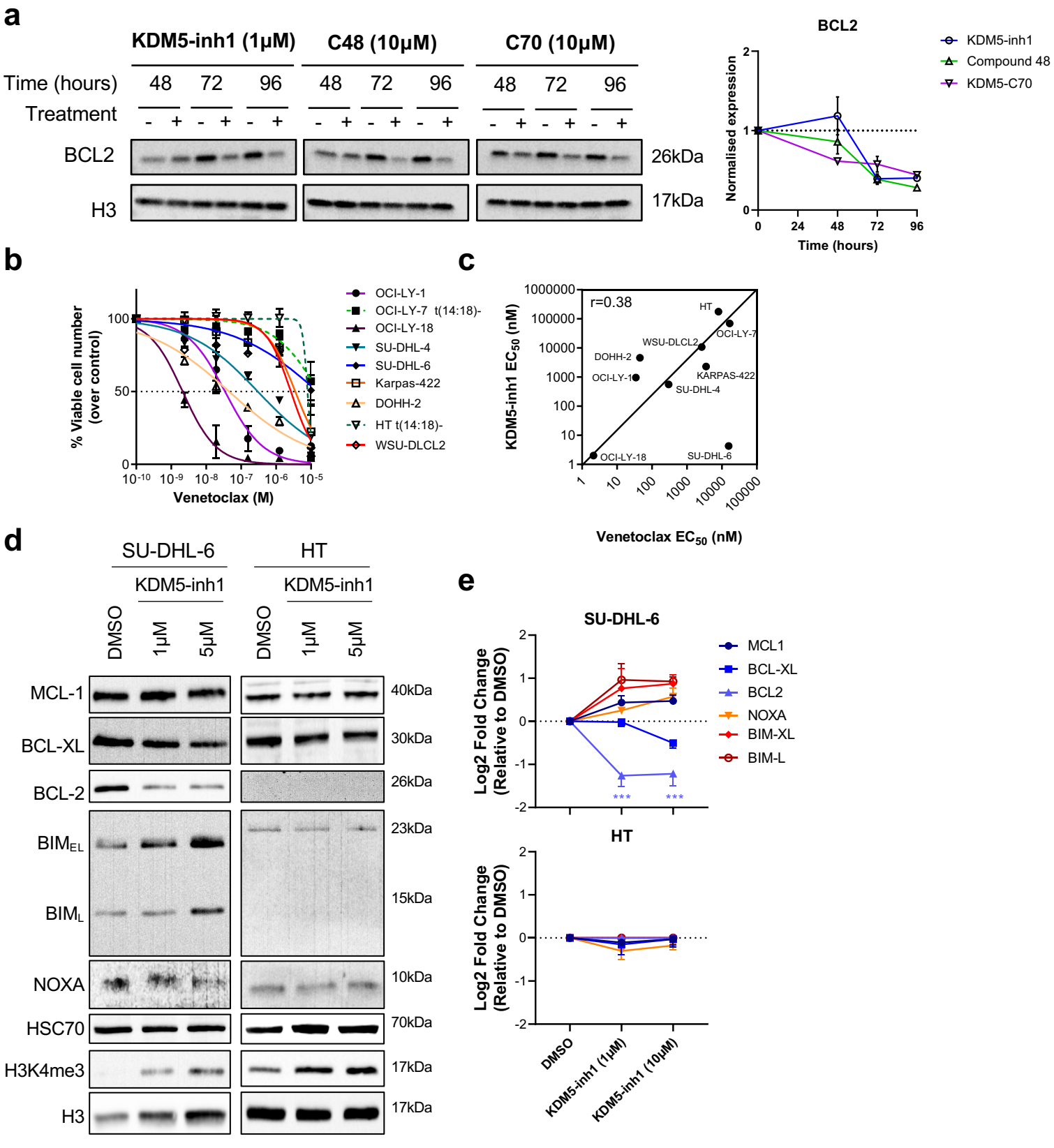
Supplementary Figure 7. Expression of KDM5 target genes following KDM5-inhibition or *KDM5A* knockout (a) qRT-PCR analysis of KDM5-inhibition target genes was performed on SU-DHL-6 cells treated with 1μM KDM5-inh1 or 10μM Compound 48 and Compound 70 for 48h, 72h and 96h. Data are the mean ± SEM of 3 independent experiments. **(b)** qRT-PCR analysis of KDM5-inhibition target genes in SU-DHL-6 and *KDM5A* knockout clones exposed to DMSO or 1μM KDM5-inh1 for 72h. Data are the mean ± SEM of three independent experiments.

Supplementary Figure 8



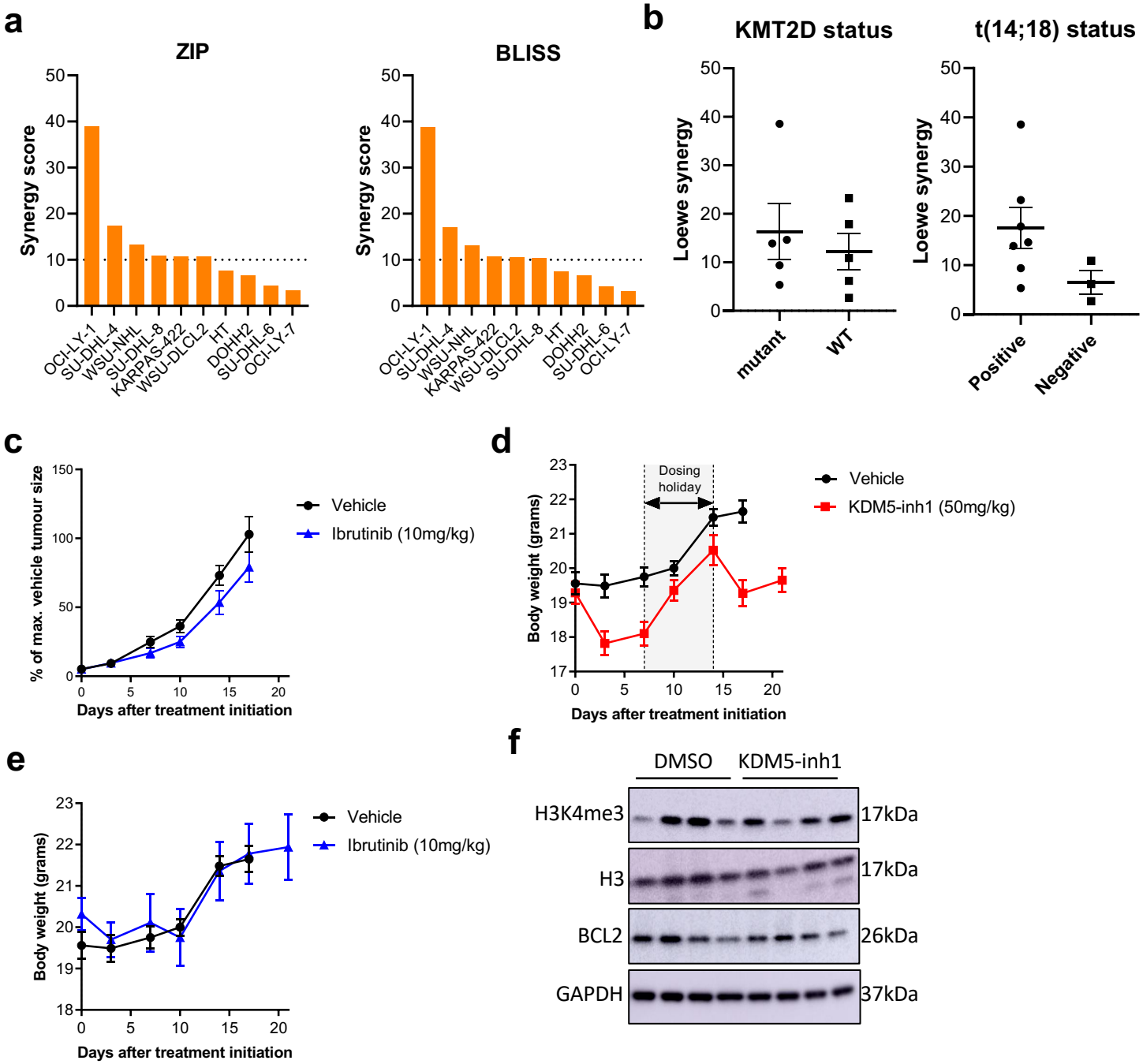
Supplementary Figure 8. KDM5-inhibition results in diminished B-cell signalling (a) Log2 fold change values of key phospho-peptides within the MAPK-ERK pathway, measured by phospho-proteomics, following exposure of OCI-lY-18 and SU-DHL-6 cells to 1μM KDM5-inh1 for 72h. The MAPK3 p204 phospho-peptide, measured by western blot in Figure 4g is highlighted. **(b)** Log2 KSEA values[36] for kinases with altered activity following KDM5-inhibition. **(c)** Surface IgM (sIgM) expression was quantified by flow cytometry in SU-DHL-6, OCI-LY-18 and OCI-LY-7 cells exposed to DMSO or 1μM KDM5-inh1 for 72h. **(d-f)** Activation of the BCR-associated kinase SYK was investigated in SU-DHL-6, OCI-LY-18 and OCI-LY-7 cells pre-treated with DMSO or 1μM KDM5-inh1 for 72h, followed by stimulation with anti-IgM $F(ab')_2$ antibody for 2h and 4h. Representative western blots for phospho-SYK, total SYK, and HSC70 are displayed in **(d)**, with quantification displayed in **(e+f)**. Data are the mean ± SEM of 3 independent experiments. SU-DHL-6 and OCI-LY-7 cells were treated with either DMSO or 1μM KDM5-inh1, and labelled with the calcium sensitive dye Fluo-3-AM and analysed by flow cytometry before and after addition of either $F(ab')_2$ anti-IgM or control antibody. **(g)** Panels showing sIgM-mediated intracellular calcium mobilisation as a percentage of responding cells, with quantification displayed in **(h)**. Data are the median of 2 independent experiments.

Supplementary Figure 9



Supplementary Figure 9. Response to BCL2i and expression analysis of BCL2 family members (a) SU-DHL-6 cells were treated with 1μM KDM5-inh1 or 10μM Compound-48 or KDM5-C70 for 48h, 72h and 96h, followed by western blot analysis of BCL2 expression relative to H3. The quantification from three independent western blots (mean ± SEM) is shown in the right panel. Note that the H3 loading control was from the same experiment in Figure 1c. **(b)** Viable cells counts following exposure to DMSO or increasing concentrations of Ventoclax for 48h. t(14;18)-negative cell lines are indicated with dashed lines. **(c)** Correlation of Venetoclax and KDM5-inh1 EC_{50} values, with the Pearson's correlation coefficient indicated. Results are representative of 3 independent experiments. **(d)** The expression of BCL2 family members was investigated by western blot analysis of SU-DHL-6 cells and HT cells treated with DMSO or 1μM and 5μM KDM5-inh1 for 2 days, with HSC70 used as a loading control. Representative western blots of 3 independent experiments are shown in **(d)**, with the quantification relative to HSC70 for in **(e)**. Statistical significance was determined using a one-way ANOVA with a Dunnett's post-test versus untreated control, where *** $P < 0.001$.

Supplementary Figure 10



Supplementary Figure 10. Synergy and *in vivo* analysis of KDM5-inh1. (a) Overall ZIP and BLISS synergy scores calculated for each cell line using Synergy Finder[59], with a score >10 indicating significant synergy. **(b)** Plots showing differences in Loewe synergy scores between *KMT2D* mutant and WT cells, or t(14;18)-positive or negative cell lines **(c)** Activity of 10mg/kg ibrutinib on the growth of SU-DHL-6 xenografts, in comparison to vehicle treated mice. Effect of **(d)** 50mg/kg KDM5-inh1 and **(e)** 10mg/kg ibrutinib on the body weight of mice, in comparison to vehicle treated mice. Data are the mean ± SEM of 10 individual mice, except in the vehicle group where one mouse was removed due to insufficient tumour growth (<300mm^3). **(f)** Levels of H3K4me3, H3, BCL2 and GAPDH were quantified by western blot in tumours from mice treated with vehicle or 50mg/kg KDM5-inh1 for three weeks (including dosing holiday).

Supplementary Tables:

Supplementary Table 1. Details of cell lines used in study.

Cell line	Source	Disease	Growth medium	Mutation profiling	Cosmic exome available?	KMT2D allele 1 (amino acid)	KMT2D allele 2 (amino acid)	t(14;18)
OCI-LY-1	DSMZ	GCB-DLBCL	20% FBS, IMDM	Targeted	No	P4929Lsf*66	R1903*	Yes
OCI-LY-7	DSMZ	GCB-DLBCL	20% FBS, IMDM	Targeted	Yes	WT	WT	No
OCI-LY-18	DSMZ	GCB-DLBCL	10% FBS, RPMI	Exome	No	P658fs	splice site disrupted (chr12, 49446988; A>C)	Yes
SU-DHL-4	Tissue bank	GCB-DLBCL	10% FBS, RPMI	Targeted	Yes	WT	WT	Yes
SU-DHL-6	Tissue bank	GCB-DLBCL	10% FBS, RPMI	Targeted	Yes	E4712*	Q211*	Yes
SU-DHL-8	DSMZ	GCB-DLBCL	10% FBS, RPMI	NA	Yes	P648Tfs*2	P648fs*2	Yes
KARPAS-422	Tissue bank	GCB-DLBCL	10% FBS, RPMI	Targeted	Yes	Q3278*	WT	Yes
DOHH2	Tissue bank	GCB-DLBCL	10% FBS, RPMI	Targeted	Yes	WT	WT	Yes
HT	DSMZ	GCB-DLBCL	10% FBS, RPMI	Targeted	No	WT	WT	No
WSU-DLCL2	DSMZ	GCB-DLBCL	10% FBS, RPMI	Targeted	Yes	WT	WT	Yes
OMP2	Tissue bank	Multiple myeloma	10% FBS, RPMI	NA	Yes	G1486D	Q3322*	No
RAJI	Tissue bank	Burkitt's lyphoma	10% FBS, RPMI	NA	Yes	WT	WT	No
WSU-NHL	DSMZ	GCB-DLBCL	10% FBS, RPMI	NA	Yes	V2281fs*32	L2425fs*26	Yes
DB	DSMZ	GCB-DLBCL	10% FBS, RPMI	NA	Yes	Q2736*	P480fs*	Yes

Supplementary Table 2. Primers used in study.

qRT-PCR

Target	Forward	Reverse
KMT2D	CCCCTGAGAGCTGCTGTG	GTAACGGGTGATGGGCAAAA
BCL2	GGTGGGGTCATGTGTGTGG	CGGTTCAGGTACTCAGTCATCC
CD72	ATCTGAGGTTTGTGAAGGCTCC	AACATTCTCGTAGGTGATTTCCC
FCGR2B	AGCCAATCCCACTAATCCTGA	GGTGCATGAGAAGTGAATAGGTG
FCRL5	ACCCAGGCCCATTATTTTCCT	AGTAGAAGCGAAATCCCTTGC
PTPN6	GGAGAAGTTTGCGACTCTGAC	GCGGGTACTTGAGGTGGATG
DUSP6	GAAATGGCGATCAGCAAGACG	CGACGACTCGTATAGCTCCTG
PLCB2	ATCCGGGATACTCGCTTTGG	CACCACCGTGAGTGTCTTCAG
PTPRJ	TGTAACATCACAGGCTTACGTC	CCCCAAGTCTCATTGCCTATTC
BCL-XL	TAAGTTCTGAGTGTGACCGAGA	GCTCTGTCTGTAGGGAGGTAGG
BIM	GAGCTGGTGGTTGACTTTCTC	TCCATCTCCGATTCAGTCCCT
NOXA	ACCAAGCCGGATTTGCGATT	ACTTGCACTTGTTCCTCGTGG
MCL1	CTGTGCCAGGAAGGGTTAGG	GCGTCGATGAGAAAAGACCC

ChIP-PCR

Target	Forward	Reverse
PTPN6 (537bp)	TTCTCGCTCTCCGTCAGGTA	GGGGAACCAGGAATGAGTGG
PTPN6 (827bp)	ATGGAGGGGAGAAGTTTGCG	GGAGCCCTCACCTCTCACTA
PTPN6 (-634bp)	GCTCAGGGTCATGTTGTCCA	GGTGCCCTCCTCTAACCAAC
PTPN6 (3016bp)	CATCCGCCTTCCTTGTGACT	AAGAGAAACGCAGACCGAGG
FCRL5 promoter	GCCCATGGTGAGCCCTTTTA	CCTTGGTGGTCCAGGTCTTC
DUSP6 promoter	ATTCGGACTCCGTGCTACTG	AAGACGCCCGGGTAGATTTG
FCGR2B promoter	AGAGAGGAACGGGAACCTCA	ATCTTCACCAGCCTGCCTTC
PLCB2 promoter	AAAGGTTGGGAGCACCGTAG	AAGAGGAGCCGTGTGTTCAG
CD72 promoter	GCTCTCCAGACCTGCTTTGT	GAATGTTCAAGTGCCCGCAG
PTPRJ promoter	ATCGATCCCTTTTCCGGCTG	AGGCTTTCCCCGGGAGG
BCL-XL promoter	GTATTCGGTTCGCTGCGTTC	GCGTTTCTCAGTCCGAGAGT
BIM promoter	TGCCTTCCTCGGAAAGTCAC	AAAAGATCTTCCGGGGGCTG
NOXA promoter	ACTGGACAAAAGCGTGGTCT	ATGCCAACTACACACGGTGA
MCL1 promoter	CTCCCGAAGGTACCGAGAGA	CTGGAGTTGGTCGGGGAATC
BCL2 promoter (-6bp)	AATGAATCAGGAGTCGCGGG	GGGATTCCTGCGGATTGACA
BCL2 promoter (-1558bp)	ATCCACAGGGCGATGTTGTC	GAACTGGGGGAGGATTGTGG

BCL2 promoter (523bp)	CTGCTACGAAGTTCTCCCCC	ACCAGGAGGAGGAGAAAGGG

Supplementary Table 3. guideRNAs used in study.

ID	Sequence
KMT2D gRNA 1	TGATTGGCCCCGGTGTCCAG
KMT2D gRNA 2	AGTTGCCATTTGATTGGCCC
KMT2D gRNA 3	TACACGGGCAGCGGGAGCTA
KMT2D gRNA 4	CGTTGTGCTCTCTGTAACTG
SU-DHL-8 KMT2D gRNA 1	TAGGCGCGATACCTCAGGTG
SU-DHL-8 donor template	TCCCCACCTGAGGACTCGCCTATGTCCCCACCACC TGAAGAATCACCTATGTCCCCACCACCTGAGGTAT CGCGCCTATCCCCCCTGCCTGTGGTGTCACGCCTGT CTCCACCGCC
KDM5A gRNA 1	TCTCTGGTATGAAAGTGCCG
KDM5C gRNA 1	GAAACCGCTGCCAAATTCTT

Supplementary Table 4. Transfection conditions.

Cell Line	Instrument	Buffer	Program	Cell number
WSU-DLCL2	Nucleofector II	Buffer T	G-016	5 million
HT	Nucleofector II	Buffer T	A-023	5 million
SU-DHL-8	Nucleofector II	Buffer T	A-032	5 million
SU-DHL-6	Nucleofector 4D	Buffer SF	CA-137	1 million

Supplementary Table 5. KMT2D, KDM5A and KDM5C clones generated by CRISPR

Gene	Parental cell	Clone ID	Allele 1	Allele 2	TA cloned?
KMT2D	WSU-DCLCL2	#8	c.245_284del (40bp); L82Qfs*35	WT	Yes
KMT2D	WSU-DCLCL2	#22	c.284_284delC; P95Qfs*35	WT	Yes
KMT2D	WSU-DCLCL2	#61	c.245_284del (40bp); L82Qfs*35	WT	Yes
KMT2D	HT	#4	c.245_283del (39bp); L82_C94del	WT	No
KMT2D	HT	#9	c.245_284del (40bp); L82Qfs*35	WT	No
KMT2D	HT	#14	c.244_257del (144bp); L82Vfs*4	c.245_284del (40bp); L82Qfs*35	No
KMT2D	HT	#16	c.243-244insG; L82fs*5	WT	No
KMT2D	HT	#18	c.245_272del (28bp); L82Rfs*39	c.245_284del (40bp); L82Qfs*35	No
KMT2D	HT	#21	c.244_305del (62bp); L82Pfs*5	WT	No
KMT2D	SU-DHL-8	K51	c.1940_1941insC; P648fs*2	WT	Yes
KMT2D	SU-DHL-8	K65	c.1940_1941insC; P648fs*2	WT	Yes
KMT2D	SU-DHL-8	K67	c.1940_1941insC; P648fs*2	WT	Yes
KDM5A	SU-DHL-6	A3	c.1404_1404delG; P469Rfs*49	c.1404_1404delG; P469Rfs*49	No
KDM5A	SU-DHL-6	A30	c.1404_1404delG; P469Rfs*49	c.1404_1404delG; P469Rfs*49	No
KDM5A	SU-DHL-6	A50	c.1404_1404delG; P469Rfs*49	c.1404_1404delG; P469Rfs*49	No
KDM5A	WSU-DLCL2	A3	c.1753_1769del(17bp); S464Vfs*16	c.1753_1769del(17bp); S464Vfs*16	No
KDM5A	WSU-DLCL2	A4	c.1767_1767delG; P469Rfs*49	c.1767_1767delG; P469Rfs*49	No
KDM5A	WSU-DLCL2	A16	c.1768_1772del (5bp); P469Afs*15)	c.1768_1772del (5bp); P469Afs*15)	No
KDM5C	WSU-DLCL2	C11	c.1674_1675del (2bp); E381Ifs*9	c.1674_1675del (2bp); E381Ifs*9	No
KDM5C	WSU-DLCL2	C36	c.1669_1678del (1bp); S379Lfs*14	c.1669_1678del (1bp); S379Lfs*14	No
KDM5C	WSU-DLCL2	C47	c.1141_1200+68del (128bp); E381_E400del	c.1141_1200+68del (128bp); E381_E400del	No

Supplementary Table 6. H3K4me3/H3K4me1 ChIP-seq analysis.

Please see separate excel spreadsheet**.**

Supplementary Table 7. RNA-seq analysis.

Please see separate excel spreadsheet.

Supplementary Table 8. GSEA analysis of KDM5-inh1 treated cell lines.

Please see separate excel spreadsheet.

Supplementary Table 9. H3K4me1, H3K4me3 and RNA pathway analysis in WSU-DLCL2 and WSU#22 cells.

Please see separate excel spreadsheet.

Supplementary Table 10. Pathway analysis of SUDHL6 ChIP-seq data

Please see separate excel spreadsheet.

Supplementary Table 11. Summary of drug combinations

DrugA	DrugB	Cell_line	CSS	ZIP	BLISS	LOEWE	HSA	KMT2D	BCL2
MCL1i	KDM5i	OCILY1	95.9	30.54	30.92	34.21	38.42	M	Y
MCL1i	KDM5i	KARPAS-422	78.5	4.38	4.06	8.27	11.69	M	Y
MCL1i	KDM5i	SUDHL6	71.48	5.1	4.82	7.92	9.04	M	Y
MCL1i	KDM5i	WSU-NHL	69.03	5.6	5.63	8.96	10.87	M	Y
MCL1i	KDM5i	WSU-DLCL2	66.5	6.81	6.92	14.55	16.47	WT	Y
MCL1i	KDM5i	SUDHL4	62.78	9.88	12.84	5.94	17.96	WT	Y
MCL1i	KDM5i	DOHH2	17.15	-9.38	1.41	1.05	2.42	WT	Y
MCL1i	KDM5i	OCILY7	53.34	-1.52	-0.15	0.24	3.87	WT	N
MCL1i	KDM5i	SUDHL8	71.77	5.29	7.2	9.06	11.96	M	N
MCL1i	KDM5i	HT	69.71	1.12	3.2	5.71	8.59	WT	N

Supplementary Table 12. Antibodies used in study.

Target	Supplier	Product no.
phospho-SYK (Tyr525/526)	Cell Signaling Technologies	2710
SYK	Cell Signaling Technologies	2712
pERK (Thr202/Tyr204)	Cell Signaling Technologies	9101
ERK	Cell Signaling Technologies	9102
GAPDH	Cell Signaling Technologies	2118
alpha-Tubulin	Sigma Aldrich	T9026
H3K4me3 (for western blots)	Cell Signaling Technologies	9751
H3K4me2 (for western blots)	Cell Signaling Technologies	9725
H3K4me1 (for western blots)	Cell Signaling Technologies	5326
H3	Cell Signaling Technologies	4499
H3K9me3	Active Motif	39161
H3K36me3	Diagenode	C15200183
H3K27me3	Cell Signaling Technologies	9733
H3K27ac	Active Motif	39133
H3K4me3 (for ChIP)	Diagenode	C15410003
H3K4me2 (for ChIP)	Diagenode	C15200151
H3K4me1 (for ChIP)	Diagenode	C15410194
Spike-in Antibody	Active Motif	61686
KDM5A	Abcam	ab70892
KDM5B	Sigma Aldrich	HPA027179
KDM5C	Abcam	ab34718
KDM5C	Diagenode	C15410338
KDM5D	Sigma Aldrich	HPA049086
PTPN6	Santa Cruz	sc-7289
BCL2	BD sciences	610538
PARP	BD sciences	556494
MCL-1	Santa Cruz	sc-819
BCL-XL	Cell Signaling Technologies	2764
BCL-2	DAKO	M0887
NOXA	Merck millipore	OP180
BIM	Cell Signaling Technologies	2933
HSC70	Santa Cruz	sc-7298
Goat F(ab')2 Anti-Human IgG-UNLB	Cambridge Bioscience	0110-01
Goat F(ab')2 Anti-Human IgM-UNLB	Cambridge Bioscience	2022-01
Anti-mouse	DAKO	P0448
Anti-Rabbit	DAKO	P0447