

SUPPLEMENTARY INFORMATION

Clustered PHD domains in KMT2/MLL proteins are attracted by H3K4me3 and H3 acetylation-rich active promoters and enhancers

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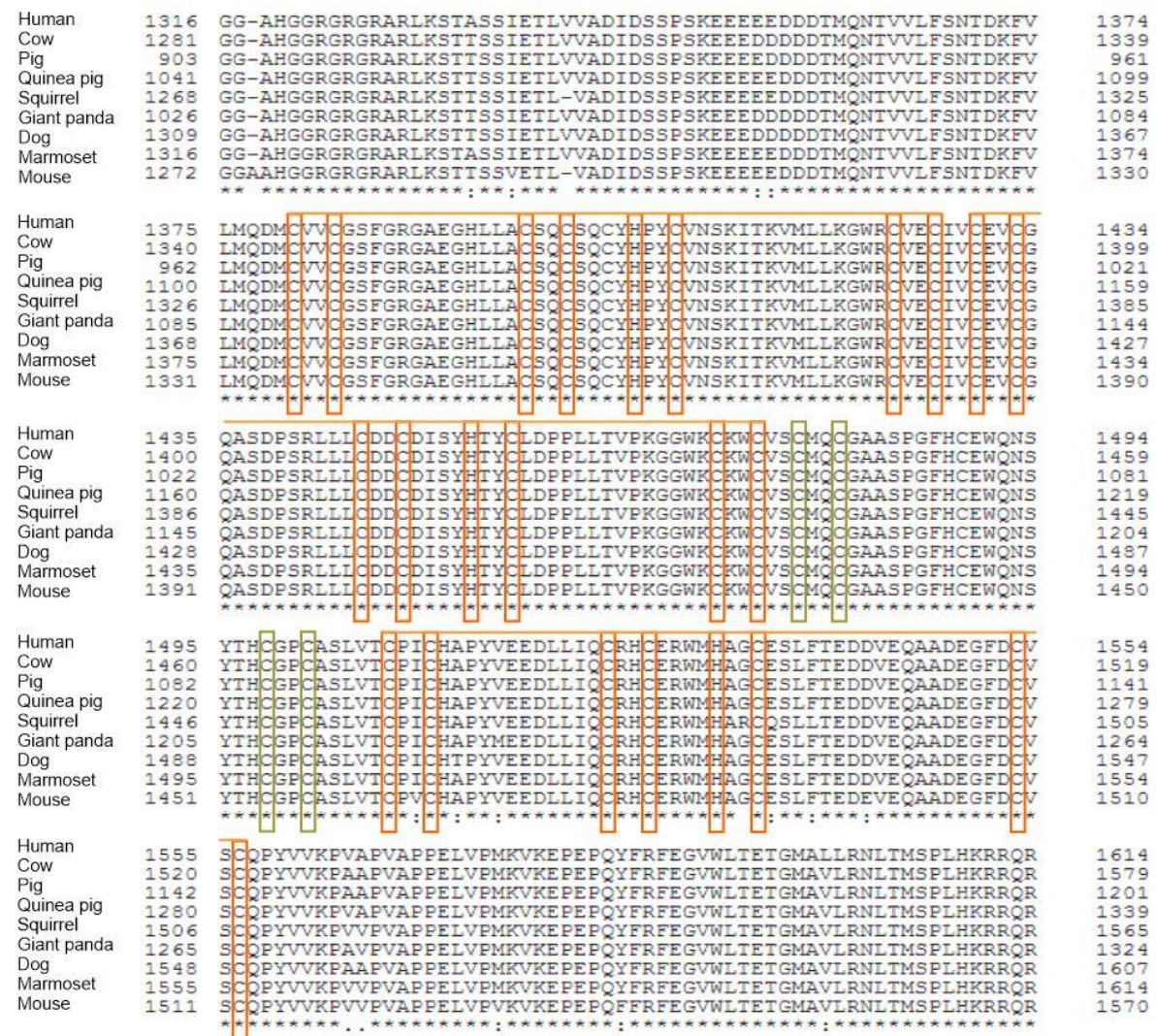
Supplementary methods

Immunofluorescence

Cells for immunofluorescence were seeded onto cover slides at the bottom of 24 well plates (50 000 cells per well). After 24 hours, the cells were washed twice with PBS, fixed for 10 minutes at room temperature (4% paraformaldehyde in PBS), and washed twice with PBS again. The cover slides were either used immediately or stored sealed with parafilm for up to two weeks at 4 °C. For immunostaining, cells were incubated with permeabilisation buffer (0.25% TritonX100 in PBS) for 20 minutes at room temperature, washed once with PBS, placed in blocking buffer (3% bovine serum albumin, BSA in PBS) for 30 minutes, incubated with primary antibody diluted in Ab buffer (1% BSA in PBS) in a humid box for 1 hour at room temperature or overnight at 4 °C, washed two times with PBS, incubated for 1 hour with secondary antibody and DAPI in Ab buffer, and washed two times with PBS. The cover slides were then briefly washed with MilliQ water and put on ethanol-cleaned glass slides containing Mowiol. The specimens were kept protected from light and analysed by confocal microscopy (LSM 800, Zeiss). All antibodies used are listed in Table S3.

VPA treatment

24 hours after seeding the HeLa cell line on a 15 cm plate, the cells were treated with 1mM VPA for 24 hours. As a control non-treated HeLa cell line was used. Mononucleosome isolation was carried out as described in the main text.



b

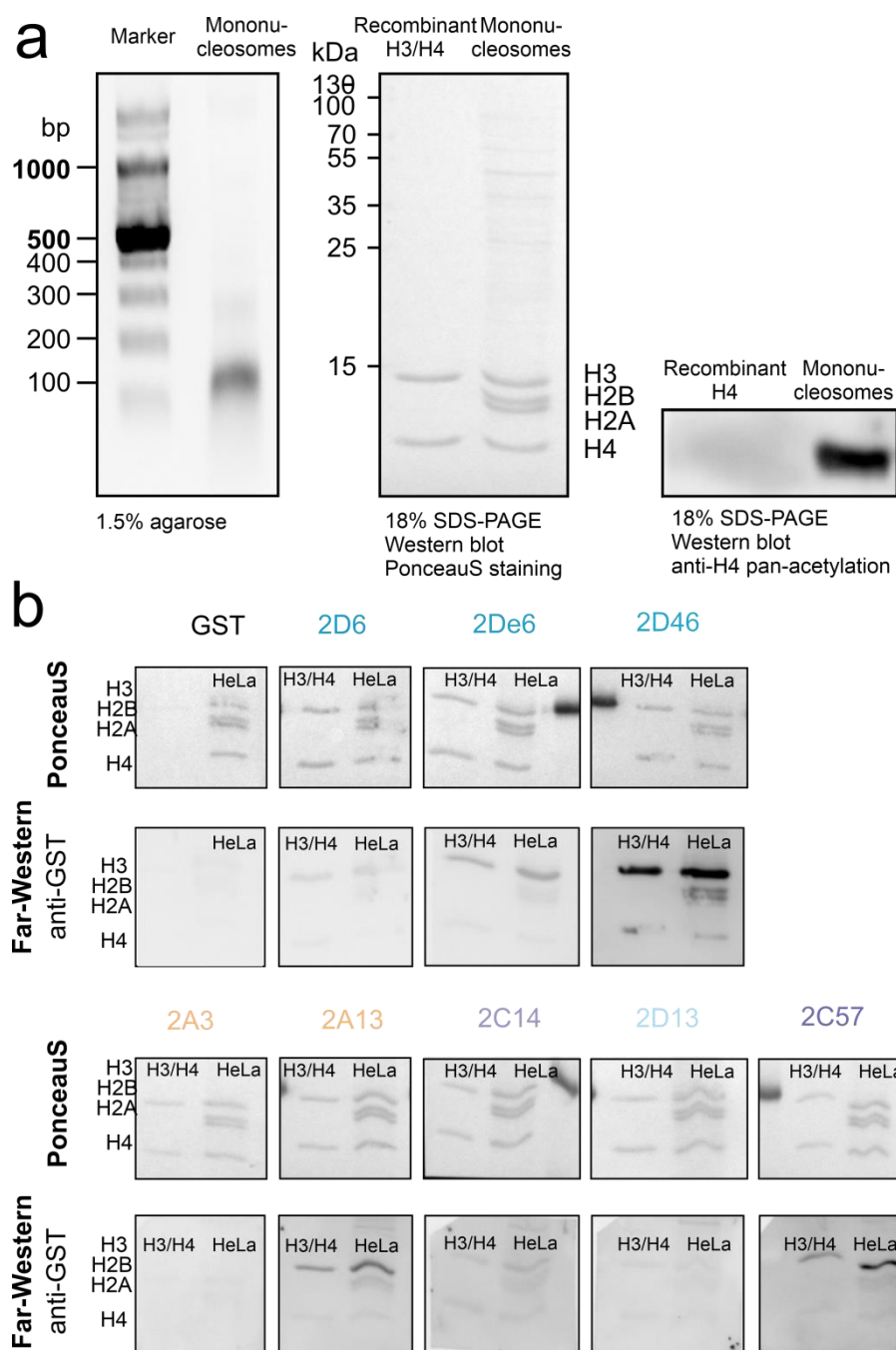
IDENTITY

	KMT2A PHD1-3	KMT2B PHD1-3	KMT2C PHD1-4	KMT2C PHD5-7	KMT2D PHD1-3
KMT2B PHD1-3	65%				
KMT2C PHD1-4	32%	32%			
KMT2C PHD5-7	31%	34%	36%		
KMT2D PHD1-3	28%	35%	59%	41%	
KMT2D PHD4-6	33%	34%	35%	75%	39%

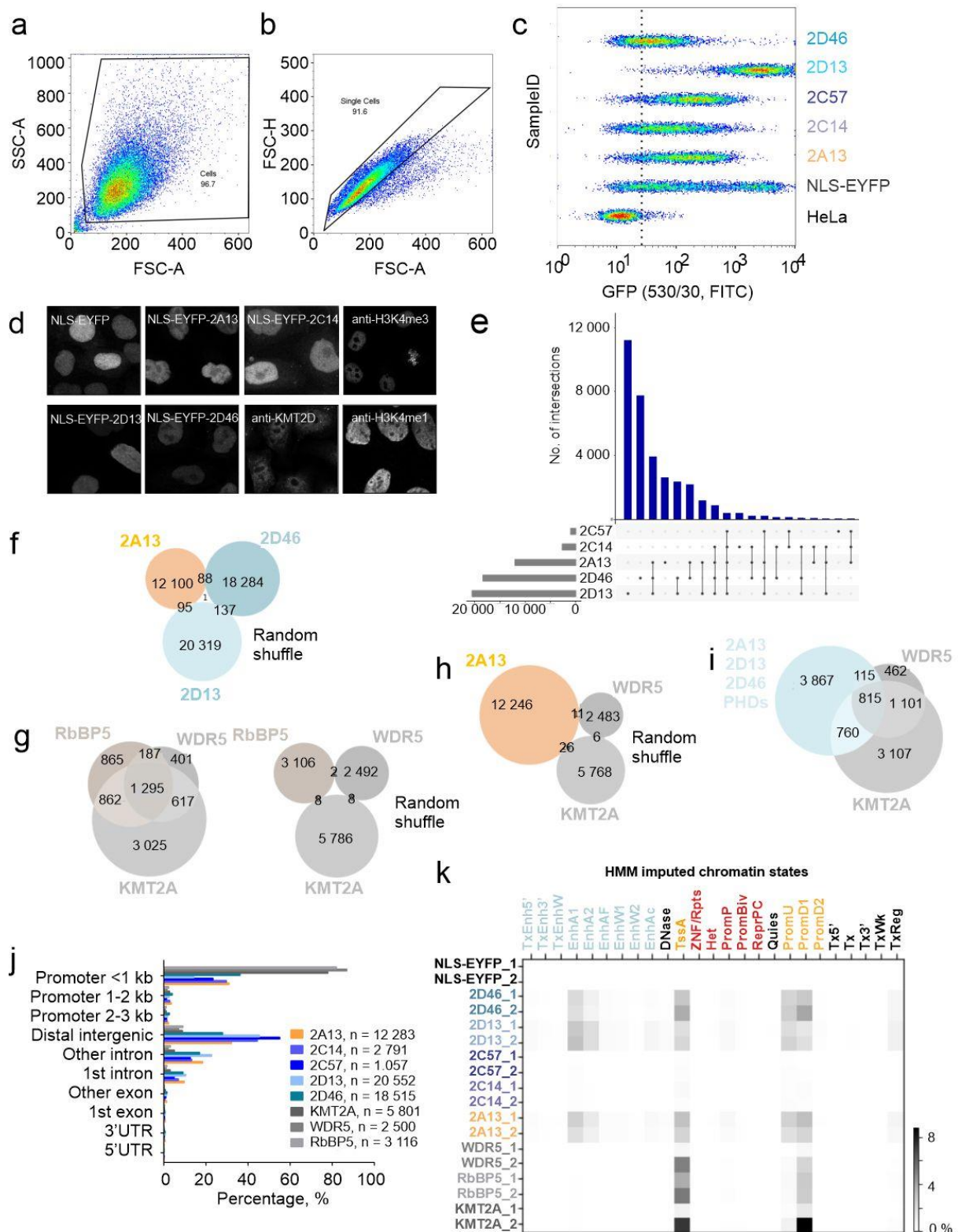
SIMILARITY

	KMT2A PHD1-3	KMT2B PHD1-3	KMT2C PHD1-4	KMT2C PHD5-7	KMT2D PHD1-3
KMT2B PHD13	80%				
KMT2C PHD1-4	48%	44%			
KMT2C PHD5-7	50%	46%	56%		
KMT2D PHD1-3	37%	41%	73%	56%	
KMT2D PHD4-6	49%	46%	53%	84%	55%

Supplementary Figure 1. Alignment of PHDs. **a** MUSCLE alignment for 2D46 from selected mammals (human, cow, pig, guinea pig, squirrel, giant panda, dog, marmoset, and mouse). Aminoacids of PHD folding motif are labelled in an orange frame and Zn knuckle in green. **b** Pairwise identity and similarity between clustered PHDs in human KMT2A-D proteins, based on blastp alignment.

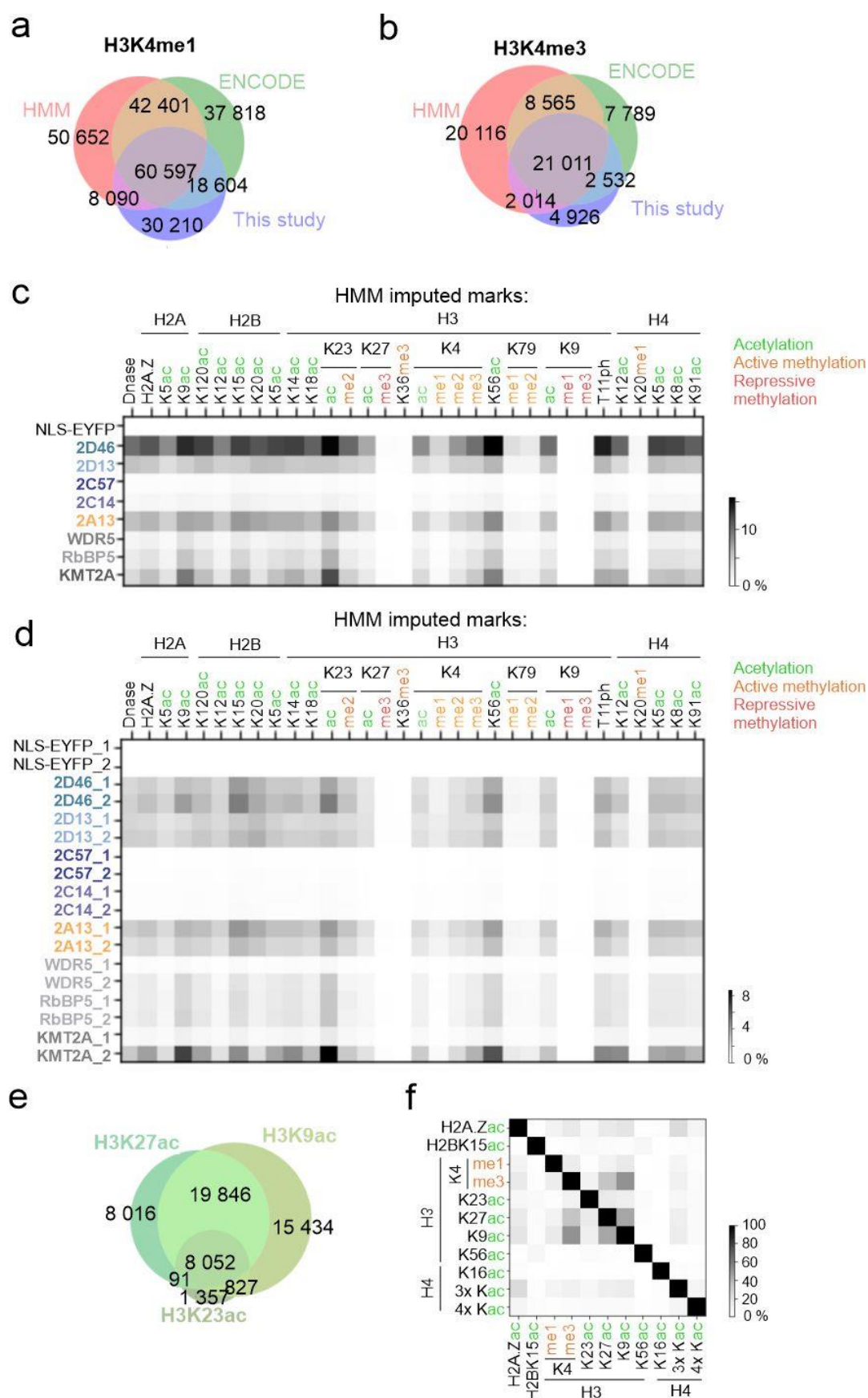


Supplementary Figure 2. Biochemical characterisation of clustered PHDs binding histones.
a Characterization of isolated mononucleosomes from HeLa S3 cell line. Left panel – length of isolated DNA. Middle panel – PonceauS staining of mononucleosome extract. Right panel – Western blot using an antibody against exemplary histone mark, anti H4 pan-acetylation. **b** Far-Western of 100 nM singlets and clustered PHDs and recombinant H3/H4 or endogenous HeLa histones.



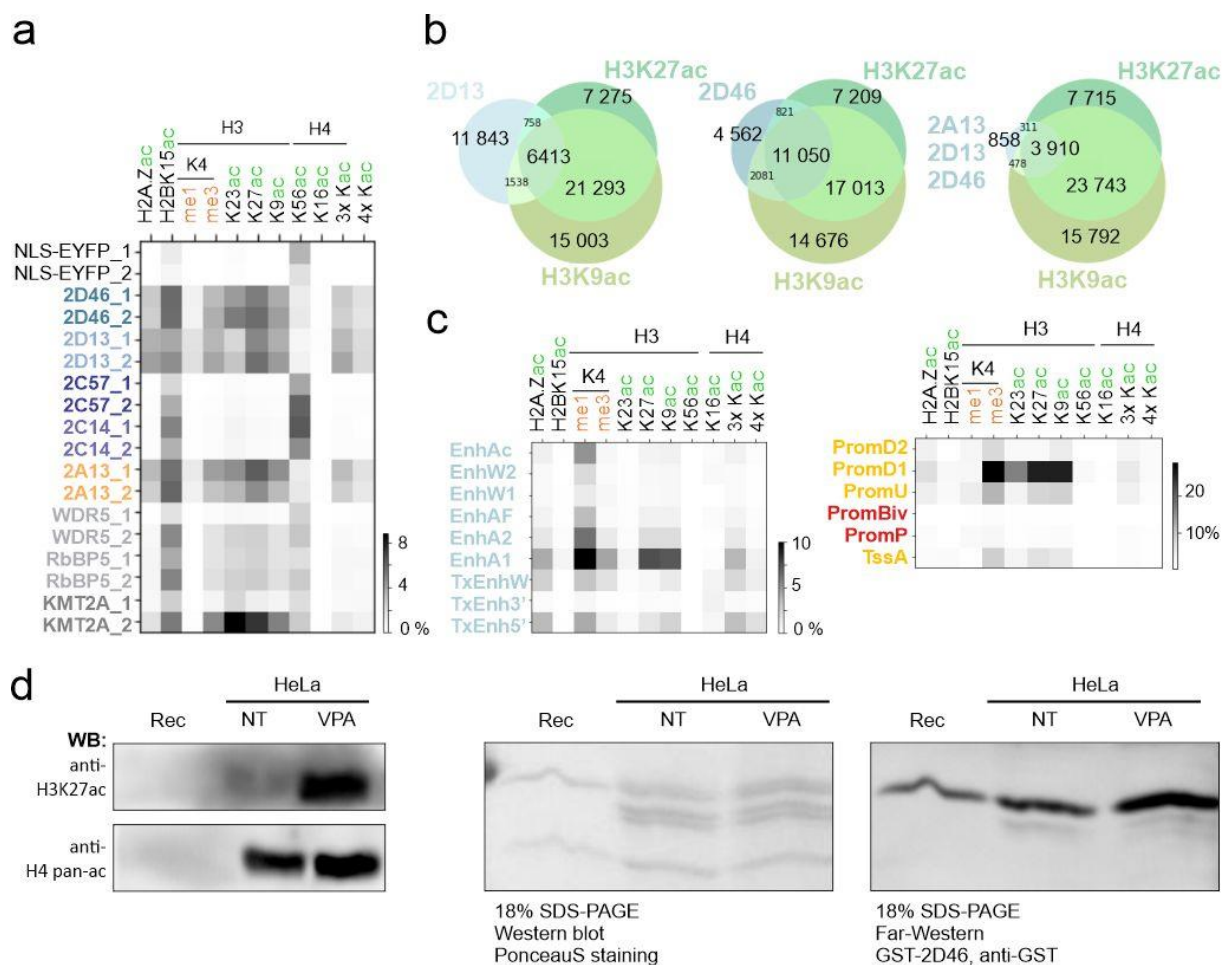
Supplementary Figure 3. Characterisation of clustered PHDs in HeLa cells by greenCUT&RUN experiments. **a-c** Flow cytometry analysis of HeLa S3 cell lines. **a** Cells were gated based on Forward (FSC-Area) and Side Scatter (SSC-Area) parameters. **b** Cell doublets were discriminated from "Cells" based on Forward Scatter FSC-Height vs. Forward Scatter FSC-Area parameters. **c** EYFP fluorescence signal was detected using FITC (530/30) emission filter. For each cell line, 5 000 cells were plotted. **d** Confocal microscopy of EYFP fluorescence in stable cell lines as well as immunofluorescence of KMT2D and selected histone modifications (H3K4me1 and H3K4me3). **e** UpSet plot comparing peaks of all clustered PHDs. **f** Venn diagram

comparing randomly shuffled peaks of the PHDs (2A13, 2D13, 2D46), **g** full-length hCOMPASS-like subunits (KMT2A, RbBP5, WDR5), **h** random shuffle of 2A13 PHDs and full-length WDR5 and KMT2A, **i** common peaks of PHDs (2A13, 2D13, 2D46) with full-length WDR5 and KMT2A. **j** Genomic distribution of binding sites of PHDs and hCOMPASS-like subunits as determined using MACS2. **k** Jaccard analysis comparing MACS2 bed files from single gC&R and C&R experiments with bed files for HMM (Hidden Markov Model) imputed chromatin states.

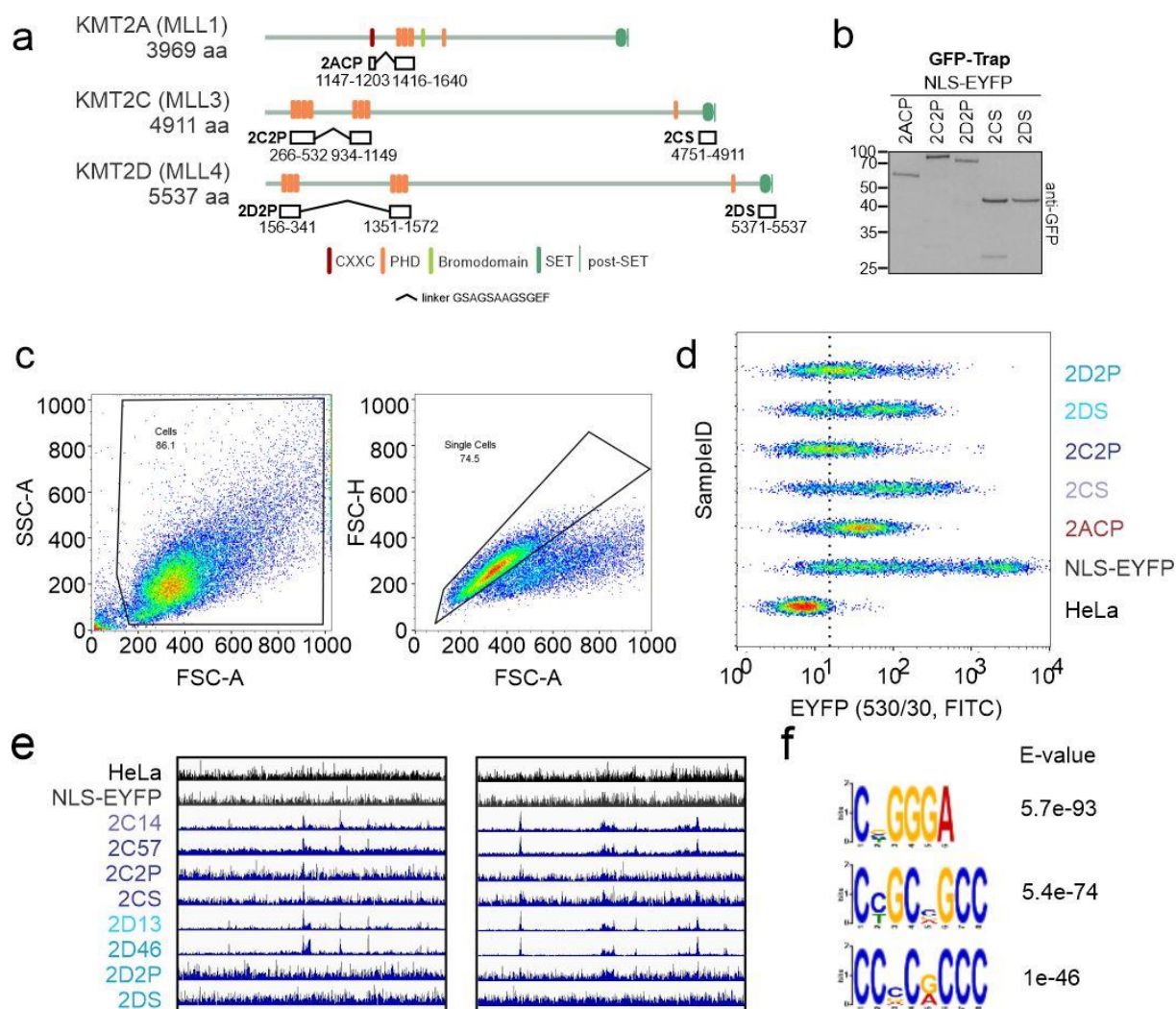


Supplementary Figure 4. Characterization of the investigated chromatin marks in HeLa S3 cell lines. a-b Venn diagrams comparing **a** H3K4me1 or **b** H3K4me3 from our CUT&RUN experiments with ENCODE ChIP-seq and HMM imputed data. **c** Jaccard analysis comparing MACS2 peaks of PHDs and full-length hCOMPASS-

like subunits (KMT2A, Wdr5, RbBP5) with HMM imputed chromatin marks with merged replicas and **d** with single replicas. **e** Venn diagrams comparing the overlap of chromatin marks most associated with PHD domain binding (H3K4me1/me3, H3K9ac, H3K27ac, H3K23ac and H2BK15ac). **f** Jaccard analysis showing the overlap between chromatin marks analysed by CUT&RUN.



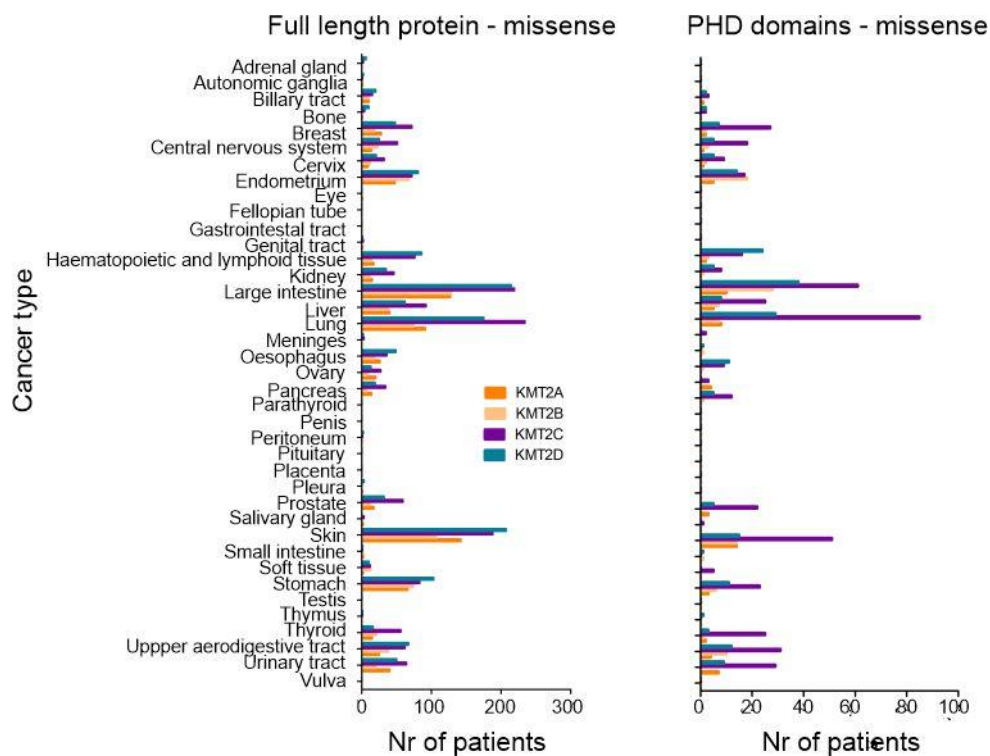
Supplementary Figure 5. Chromatin marks at binding sites of PHDs. **a** Jaccard analysis of binding sites of PHDs and histone modifications, without replica merging. **b** Venn diagrams comparing the MACS2 peaks of selected PHDs (2D13, 2D46) with the strongest histone marks (H3K9ac, H3K27ac). **c** Histone marks at enhancer (left) and promoter (right) imputed chromatin states. **d** Left Panel - Western blot of recombinant H3/H4 histones (Rec) and 10 ug mononucleosomes isolated from HeLa cells non-treated (NT) and treated for 24 hours with 1mM valproic acid (VPA), histone deacetylase inhibitor. Antibodies against selected acetylation marks were used: H3K27ac and H4 pan-acetylation histone marks. Middle panel - PonceauS staining of recombinant histones (Rec) and NT or VPA mononucleosomes. Right panel - Far-Western of 100 nM 2D46 and recombinant H3/H4, endogenous NT or VPA HeLa histones.



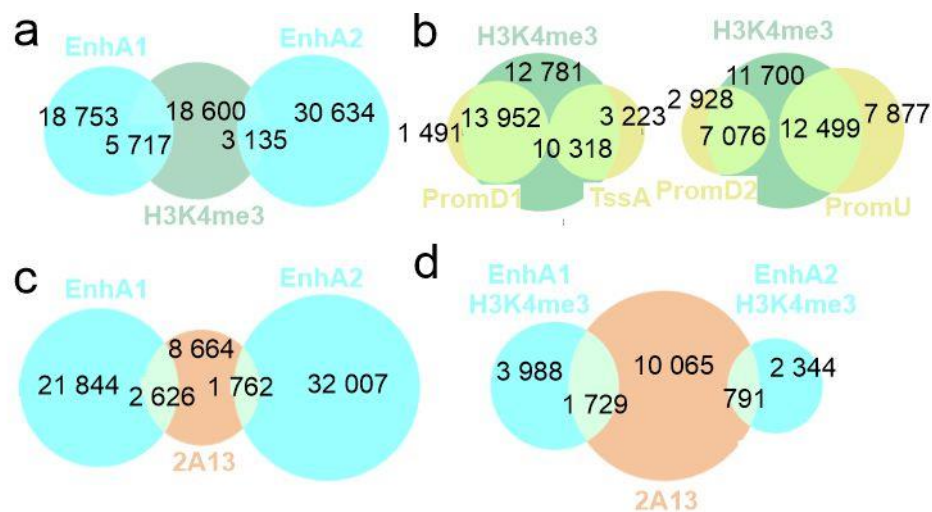
Supplementary Fig. 6 **a** Tandem reader domain constructs. 2ACP – the fusion of CXXC and PHDs from KMT2A. 2C2P/2D2P – tandem of two clustered PHDs from KMT2C or KMT2D proteins, respectively. 2CS/2DS – SET domain of KMT2C or KMT2D proteins, respectively. **b** Western blot analysis with anti-GFP antibody after GFP-Trap enrichment of stable HeLa cell line lysates. **c-d** Flow cytometry analysis of HeLa S3 stable cell lines. **c** Left panel - cells were gated based on Forward (FSC-Area) and Side Scatter (SSC-Area) parameters. Right panel - Cell doublets were discriminated from "Cells" based on Forward Scatter FSC-Height vs. Forward Scatter FSC-Area parameters. **d** EYFP fluorescence signal was detected using FITC (530/30) emission filter. For each cell line, 5 000 cells were plotted. **e** IGV browser snapshot showing the reads of greenCUT&RUN for tandem 2C2P/2D2P and 2CS/2DS stable cell lines. **f** Top 3 hits of the MEME-ChIP DNA motif analysis for 2ACP binding sites as determined by MACS2.



Supplementary Figure 7. Characterisation of the clustered PHDs and full-length KMT2A binding sites.
a Top twelve KEGG pathways (with the lowest FDR) of KMT2A and selected clustered PHDs (2A13, 2D13, 2D46). **b** Top twelve GO biological processes (with the lowest FDR) of KMT2A and selected clustered PHDs (2A13, 2D13, 2D46).



Supplementary Figure 8. Distribution of missense mutations in the cancer patients analyzed for full-length proteins or PHD domains only.



Supplementary Figure 9. H3K4me3 and 2A13 at active promoters and enhancers. Venn diagrams comparing peaks of H3K4me3 with imputed active **a** enhancers EnhA1, EnhA2, **b** promoters TssA and PromD1 (left panel), PromD2 and PromU (right panel), **c** 2A13 PHDs with imputed enhancers EnhA1/2 and **d** fraction of these enhancers decorated with H3K4me3.

Table S1. Synthetic genes used in this study.

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Synthetic genes for recombinant proteins		
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TATAAAAATAGGCGTATCACGAGGCATATGGGATCCGAA GAACCGCTGCTGGTTAATGTTGATAAAGCAGTTGTTAGC GGTAGCACCGAACGTTGTGCATTTTGTAAACATCTGGGT GCCACCATTAAATGCTGTGAAGAAAAATGTACGCAGATG TATCATTATCCGTGTGCAGCCGGTGCAGGCACCTTTCAGG ATTTTAGCCATATTTTCTGCTGTGTCCGGAACATATTGA TCAGGCACCGGAACGTAGCAAAGAAGATGCAAAATTGCG CAGTTTGTGATAGTCCGGGTGATCTGCTGGATCAGTTTTT TTGTACCACCTGTGGTCAGCATTATCATGGTATGTGTCTG GATATTGCAGTTACACCGCTGAAACGTGCAGGTTGGCAG TGTCTGAATGTAAAGTTTGTGAGAATTGTAAACAGAGC GGTGAGGATAGCAAAATGCTGGTTTTCGATACTGTGAT AAAGGCTATCACACCTTTTGTCTGCAGCCTGTTATGAAAA GCGTTCCGACCAATGGTTGGAAATGTAAAAATTGCCGCA TTTGCAATTGAATGCGGCACCCGTAGCAGCAGCCAGTGGC ATCATAATTGTCTGATTTGCGATAATTGCTATCAGCAGCA GGATAATCTGTGTCCGTTTGTGGTAAATGTTATCATCCG GAACTGCAGAAAGACATGCTGCATTGTAATATGTGTA CGTTGGGTTTCATCTGGAATGTGATAAACCGACCGATCAT GAACTGGATACCCAGCTGAAAGAAGAATATATCTGCATG TATTGCAAACACCTGGGAGCAGAAATGGATCGTCTGCAA	ThermoScientific	2C14

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GGAAGATCAGCTGGAAAACTGGTGCTGCCGGCGGTGCAA GTTCTGTCTGTGTGGCAGACAGCACCAGGCCACCAA ACAGCTGCTGGAATGCAACAAGTGCCGGAACAGCTATCA CCCCGAGTGTCTGGGCCCTAACTACCCACCAAGCCTAC CAAGAAAAAGAAAGTGTGGATCTGCACCAAATGCGTCCG GTGCAAGAGCTGCGGCTCTACCACACCTGGCAAAGGCTG GGATGCTCAGTGGTCCCACGATTTACGCCTGTGCCACGA TTGCGCCAAGCTGTTCCGCAAGGGCAACTTCTGCCCTCTG TGCGACAAGTGCTACGACGACGATGACTACGAGAGCAA GATGATGCAGTGCGGCAAGTGCACAGATGGGTGCACTC CAAGTGCAGAGAATCTGAGCGGCACCGAGGACGAGATGT ACGAGATCCTGAGCAACCTGCCTGAGAGCGTGGCCTACA CCTGTGTGAACTGCACCGAAAGACACCCTGCCGAATGGC GGCTGGCCCTGGAAAAAGAACTTTAAGGATCCCCTCGAGG CGGCCGCCGCTGAGCAATAACTAGCA		
TACCTCACCGGTAGGCATATGTGTACAAGAGGAATGTGCG ACGAGGAAGAAGAAGAGGACGACGATACCATGCAGAAC ACCGTGGTGCTGTTTCAGCAACACCGACAAGTTCGTGCTG ATGCAGGATATGTGCGTCGTGTGCGGCAGCTTTGGAAGA GGCGCTGAAGGACATCTGCTGGCCTGTAGCCAGTGCTCC CAGTGCTATTACCCCTACTGCGTGAACAGCAAGATCACC AAAGTGATGCTGCTGAAAGGCTGGCGCTGCGTGGAATGC ATCGTGTGTGAAGTGTGCGGCCAGGCCAGCGATCCTTCT AGACTGCTGCTGTGCGACGACTGCGACATCAGCTACCAC ACCTACTGTCTGGACCCTCCACTGCTGACCGTGCCTAAAG GCGGATGGAAGTGCAAGTGGTGCCTGTATGCAGT GCGGAGCCGCCTCTCCTGGATTTCACTGCGAGTGGCAGA ACAGCTACACCCACTGTGGCCCTTGTGCCAGCCTGGTCA CCTGTCCTATTTGTACGCCCCCTTACGTGGAAGAGGACCT GCTGATCCAGTGACGACACTGCGAGAGATGGATGCACGC CGGCTGCGAGAGCCTGTTACAGAGGATGATGTGGAACA GGCCGCCGACGAGGGCTTCGATTGTGTGTCTTGTACGCC CTACGTGGTCAAGCCCGTGGCTCCTGTTGCTCCTCCTGAA CTTTAAGGATCCCTCGAGGCGGCCGCCGCTGAGCAATA ACTAGCA	ThermoScientific	2D46* H1405Y
TACCTCACCGGTAGGCATATGTGTACAAGAGGAATGTGCG ACAAGAAAGGCCGAGATCTCGGAGATGCGGCCAGTGTC CTGGATGCCAGGTTCCAGAAGATTGCGGCGTCTGCACCA ACTGCCTGGACAAGCCTAAGTTGCGCGGCAGAAACATCA AGAAACAGTGCTGCAAGATGCGGAAGTGCCAGAACCTG CAGTGATGCTTCTAAGGGCTCTGCCGATCTGCTGCC GGAAGCGGAGAGTTTGAAATGGGCGGACTGGGCATCCTG ACCAGCGTGCCAATCACTCCTAGAGTCGTGTGCTTCCTGT GTGCCAGCTCTGGCCACGTGGAATTCGTGTACTGCCAAG TGTGCTGCGAGCCCTTCCACAAGTTCTGCCTGGAAGAGA ACGAGCGGCCCTGGAAGATCAGCTGGAAGAACTGGTGCT GCCGCGGTGCAAGTTCTGTCTGTGTGCGCAGACAGC ACCAGGCCACCAACAGCTGCTGGAATGCAACAAGTGCC GGAACAGCTATACCCCGAGTGTCTGGGCCCTAACTACC CCACCAAGCCTACCAAGAAAAAGAAAGTGTGGATCTGCA CTAAGTGCGTCCGGTGCAAGAGCTGCGGCTCTACCACAC CTGGCAAAGGCTGGGATGCTCAGTGGTCCCACGATTTCA GCCTGTGCCACGATTGCGCCAAGCTGTTCCGCAAGGGCA ACTTCTGCCCTCTGTGCGACAAGTGCTACGACGACGATG ACTACGAGAGCAAGATGATGCAGTGCGGCAAGTGCGAC AGATGGGTGCACTCCAAGTGCGAGAATCTGAGCGACGAG ATGTACGAGATCCTGAGCAACCTGCCTGAGAGCGTGGCC TACACCTGTGTGAACTGCACCGAAAGACACCCTGCCGAA TGGCGGCTGGCCCTGGAAAAAGAACTTTAAGGATCCTCT AGATAACTGAATTCTCGACCTCCGCCCGCTGAGCAATAA	ThermoScientific	2ACP

CTAGCAG		
TACCTCACCGGTAGGCATATGTGTACAAGAGGAATGTCTG ACGAGGAACCCCTGCTGGTCAACGTGGACAAAGCCGTGG TGTCTGGCTCTACCGAGAGATGCGCCTTCTGCAAACACCT GGGCGCCACCATCAAGTGCTGCGAAGAGAAGTGCACCCA GATGTATCACTACCCCTGTGCCGCTGGCGCCGGAACCTTT CAGGATTTACGCCACATCTTTCTGCTGTGCCCCGAGCACA TCGATCAGGCCCCTGAGAGAAGCAAAGAGGACGCCAATT GCGCCGTGTGCGATAGCCCTGGCGATCTGCTGGATCAGT TCTTCTGCACCACCTGTGGCCAGCACTACCACGGCATGTG CCTGGATATTGCCGTGACACCCCTGAAGCGAGCCGGATG GCAATGCCCTGAGTGCAAAGTGTGCCAGAACTGCAAGCA GAGCGGCGAGGACAGCAAGATGCTCGTGTGCGACACCTG TGACAAGGGCTACCACACCTTCTGCCTGCAACCTGTGAT GAAGTCCGTGCTTACCAACGGCTGGAAGTGCAAGAACTG CCGGATCTGCATCGAGTGCGGCACCAGATCTAGCAGCCA GTGGCACCAACAATTGCCTGATCTGCGACAACCTGCTACCA GCAGCAGGACAATCTGTGCCCTTCTGCGGCAAGTGCTA TCACCCCGAGCTGCAGAAAGACATGCTGCACTGCAACAT GTGCAAGAGATGGGTGCACCTGGAATGCGACAAGCCAC CGATCACGAGCTGGACACCCAGCTGAAAGAAGAGTACAT CTGCATGTATTGCAAGCACCTCGGAGCCGAGATGGACAG ACTGCAGCCTGGCGAAGAAGGATCTGCAGGCTCTGCTGC AGGCAGCGGCGAGTTCGACGATGAAGAGAACAGCATGC ACAACACCGTGGTGCTGTTTCAGCAGCTCCGACAAGTTCA CCCTGAACCAGGATATGTGCGTCTGTGCGGCTCTTTTGG ACAGGGCGCTGAAGGCAGACTGCTGGCCTGTTCTCAGTG CGGACAGTGCTACCATCCTTACTGCGTGTCATCAAGATC ACCAAGGTGGTGCTGAGCAAAGGCTGGCGGTGCCTGGAA TGTACCGTGTGTGAAGCCTGTGGCAAGGCCACAGATCCT GGAAGGTGCTGCTGTGCGACGACTGCGATATCAGCTAT CACACCTACTGTCTGGACCCTCCACTGCAGACCGTGCCTA AAGGCGGATGGAAATGCAAGTGGTGGTGTGGTGTAGAC ACTGCGGCGCTACATCTGCCGACTGAGATGCGAGTGCG AGAACAATAACCCAGTGCGCCCTTGTGCCAGCCTGA GTTCTTGCCCTGTGTGCTACCGGAACTACAGAGAAGAGG ACCTGATCCTGCAGTGCCGCGAGTGCGATAGATGGATGC ATGCCGTGTGTGTCAGAACCTGAACACCGAGGAAGAGGTGG AAAACGTCGCCGACATCGGCTTCGACTGCTCCATGTGCA GACCCTACATGCCCGCCAGCAATGTGCCTAGCTCTGACT AAGGATCCTCTAGATAACTGAATTCTCGACCTCCGCCCCG CTGAGCAATAACTAGCAG	ThermoScientific	2C2P
TACCTCACCGGTAGGCATATGTGTACAAGAGGAATGTCTG ACAGCAAGCAGTTCGTGCACAGCAAGAGCAGCCAGTACC GGAAGATGAAGACCGAGTGGAAGTCCAACGTGTACCTG GCCAGAAGCAGAATCCAAGGCCTGGGCTGTATGCCGCC AGAGACATCGAGAAGCACACCATGGTCATCGAGTACATC GGCACCATCATCCGGAACGAGGTGGCCAACCGGAAAGA GAAGCTGTACGAGAGCCAGAACCGGGGCGTGTACATGTT CAGAATGGACAACGACCACGTGATCGACGCCACACTGAC AGGTGGCCCTGCCAGATATATCGCCCACAGCTGCGCCCC TAATTGCGTGGCCGAGGTGGTCAATTTGAGCGGGGCCA CAAGATCATCATCAGCAGCTCCAGAAGAATCCAGAAAGG CGAGGAACTGTGCTACGACTACAAGTTCGACTTCGAGGA CGACCAGCACAAGATCCCTTGTCACTGTGGCGCCGTGAA CTGCCGGAAGTGGATGAACTAAGGATCCTCTAGATAACT GAATTCTCGACCTCCGCCCCGCTGAGCAATAACTAGCAG	ThermoScientific	2CS
TACCTCACCGGTAGGCATATGTGTACAAGAGGAATGTCTG ACGAGCTGTGCGGCGTGGACAAGGCCATCTTTAGCGGCA TCAGCCAGAGATGCAGCCACTGCACAAGACTGGGCGCCA	ThermoScientific	2D2P

GCATTCCTTGTAGAAGCCCTGGCTGCCCCAGACTGTATCA CTTCCCTTGTGCTACAGCCAGCGGCAGCTTCCTGAGCATG AAGACACTGCAGCTGCTGTGCCCTGAGCACTCTGAAGGC GCCGCTTATCTGGAAGAGGCCAGATGCGCTGTGTGTGAA GGACCTGGCGAGCTGTGTGACCTGTTCTTCTGTACCAGCT GCGGCCACCACTATCACGGCGCCTGTCTGGATACAGCCC TGACCGCCAGAAAACGCGCTGGATGGCAATGCCCCGAGT GCAAAGTTTGGCAGGCCTGCAGAAAGCCCGGCAACGACT CTAAGATGCTCGTGTGCGAGACATGCGACAAGGGCTACC ACACCTTCTGCCTGAAGCCTCCAATGGAAGAACTGCCCC CTCACAGCTGGAAGTGCAAGGCCTGTAGAGTGTGCAGAG CCTGTGGCGCCGATCTGCCGAGCTGAATCCTAACTCTG AGTGGTTCGAGGGCAGCGCCGGTTCTGCTGCTGGATCTG GCGAGTTCGAGGAAGAAGAAGAGGACGACGATACCATG CAGAACACCGTGCTGCTGTTTCAGCAACACCGACAAGTTC GTGCTGATGCAGGATATGTGCGTCGTGTGCGGCTCCTTTG GCAGAGGCGCTGAAGGACATCTGCTGGCCTGTAGCCAGT GCTCCCAGTGCTATCACCCCTACTGCGTGAACAGCAAGA TCACCAAAGTGATGCTGCTGAAAGGCTGGCGCTGCGTGG AATGCATTGTGTGCGAAGTGTGCGGCCAGGCCAGCGATC CTAGTAGACTGCTGCTGTGTGATGACTGCGACATCTCCTA CCACACATACTGTCTGGACCCTCCACTGCTGACCGTGCCT AAAGGCGGATGGAAATGCAAGTGGTGCGTGTCTGTATG CAGTGCGGAGCCGCTCTCCTGGATTTCCTGCGAGTGG CAGAACAGCTACACCCACTGTGGCCCTTGTGCCAGCCTG GTCACCTGTCTATTGTACGCCCCCTTACGTGGAAGAGG ACCTGCTGATCCAGTGCAGACACTGCGAGAGATGGATGC ACGCCGGCTGCGAGAGCCTGTTACAGAGGATGATGTGG AACAGGCCCGCCGACGAGGGCTTCGATTGTGTGTCTTGTG AGCCCTACGTGGTCAAGCCCGTGGCTCCTGTTGCTCCTCC TGAACCTTAAAGGATCCTCTAGATAACTGAATTCTCGACCT CCGCCCCGCTGAGCAATAACTAGCAG		
TACCTCACCGGTAGGCATATGTGTACAAGAGGAATGTGCG ACGAGACAAACACCCCTTACAGCAAGCAGTTCTGTGCACA GCAAGAGCAGCCAGTACAGACGGCTGAGAACCGAGTGG AAGAACAACGTGTACCTGGCCAGAAGCAGAATCCAAGG CCTGGGTCATCGAGCCGCCAAGGACCTGGAAAAGCACAC CATGGTTCATCGAGTACATCGGCACCATCATCCGGAACGA GGTGGCCAACAGAAGAGAGAAGATCTACGAGGAACAGA ACCGGGGCATCTACATGTTCCGGATCAACAACGAGCAGG TGATCGACGCCACACTGACAGGTGGCCCTGCCAGATATA TCGCCCACAGCTGCGCCCCCTAATTGCGTGGCCGAGGTGG TCACCTTCGACAAAGAGGACAAGATCATCATCTCCA GCCGGCGCATCCCCAAGGGCGAAGAAGTACCTACGACT ACCAAGTTCGACTTCGAGGACGACCAGCACAAGATCCCTT GTCACTGCGGCGCCTGGAAGTCCCGGAAATGGATGAAGT AAGGATCCTCTAGATAACTGAATTCTCGACCTCCGCCCCG CTGAGCAATAACTAGCAG	ThermoScientific	2DS

Table S2. Oligonucleotides used in this study.

OLIGONUCLEOTIDE SEQUENCE	SOURCE	IDENTIFIER
Cloning using SLIC to pEYFP		
CGGTAGGCATATGTGTACAAGAG	Genomed	Synthetic_F
TAGTTATTGCTCAGCGGGCG	Genomed	Synthetic_R
GCGGCTGGCCCTGGAAAAAGAACTT TGAGGATCCACCGGATCTAGAT	Genomed	PEY_A13_F
TCAGGATGCCCAGGCCGCCCATCTC	Genomed	PEY_A13_R

GTCGACTGCAGAATTCGAAGCT		
GGACAGACTGCAGCCTGGCGAAGAA TGAGGATCCACCGGATCTAGAT	Genomed	PEYFP_C14_F
TGTCCACGTTGACCAGCAGGGGCTC GTCGACTGCAGAATTCGAAGCT	Genomed	PEYFP_C14_R
TGCTGTTCTCTTCGTCGTCGTCGACTGCAGAATTCGAAG	Genomed	PEY_C57-CELL-OPTR
ATGTGCCTAGCTCTGACTAAGGATCCACCGGATCTAGA TAACTG	Genomed	PEY_C57-CELL-OPTF
TGGCCTTGTCACGCCGCACAGCTCGTCGACTGCAGAA TTCGAAG	Genomed	PEY_D13-CELL-OPTR
GAATCCTAACAGCGAGTGGTTCGAATGAGGATCCACCG GATCTAGAT	Genomed	PEY_D13-CELL-OPTF
TGCTCCTCCTGAACTTTAAGGATCCACCGGATCTAG	Genomed	PEY_D46-CELL-OPTF
GGTATCGTCGTCCTCTTCTTCTCCTCGTCGACTGCAGA ATTCTGAAG	Genomed	PEY_D46-CELL-OPTR
Cloning using SLIC from pEYFP to pLJM1		
CCATTTGTCTCGAGGTCGAGAATTCAGTTATCTAGATCC GGTGGATCC	Sigma-Aldrich	revSLICpljm1
CGTCAGATCCGCTAGCGCTACC	Sigma-Aldrich	5UTRpCMV

Table S3. Biotinylated peptides used in this study.

PEPTIDE SEQUENCE	SOURCE	IDENTIFIER
ARTKQTARKSTGGKAPRKQLATKAARKSAP	GenScript	H3
ARTKQTARK me3 STGGKAPRKQLATKAARSAP	GenScript	H3K4me3
ARTKQTARK Kac STGGKAPRKQLATKAARK Kac SAP	GenScript	H3K9acK27ac

Table S4. The key reagent used in this study.

REAGENT	SOURCE	Catalogue number
Antibodies		
anti-GFP-Mnase	Nizamuddin et al, 2021; a kind gift from Marc Timmers	N/A
Anti-GFP	R&D Systems	Cat#AF4240
anti-GST-HRP	Abcam	Cat#ab3416
anti-H3	Cell Signaling Technology	Cat#4499
normal rabbit IgG	Sigma-Aldrich	Cat#I8140
anti-KMT2A	Diagenode	Cat#C15310264
anti-KMT2D	Invitrogen	Cat#701869
anti-WDR5	Diagenode	Cat#C15410027
anti-RbBP5	ActiveMotif	Cat#61406
anti-H3K4me1	Cell Signaling Technology	Cat#5326
anti-H3K4me3	Cell Signaling Technology	Cat#9751
anti-H3K9ac	Diagenode	Cat# C15410004
anti-H3K27ac	Diagenode	Cat# C15410196
anti-H3K23ac	Diagenode	Cat# C15410344
anti-H3K56ac	Diagenode	Cat# C15410213
anti-H2A.Zac	Diagenode	Cat#C15410173
anti-H2BK15ac	Diagenode	Cat# C15410220
anti-H4K16ac	ActiveMotif	Cat#39168

anti-H4K5acK8acK12ac	Diagenode	Cat#C15410021
anti-H4K5acK8acK12acK16ac	Diagenode	Cat#C15410024
Chemicals and recombinant proteins		
MNase	New England Biolabs	Cat#M0247S
CUT&RUN pAG-MNase	Cell Signaling Technologies	Cat#40366
Digitonin	Sigma-Aldrich	Cat#D141
Spermidine	Sigma-Aldrich	Cat#S2626
BioMag®Plus Concanavalin A	Polysciences	Cat# 86057-3
GFP-Trap magnetic Agarose	Chromotek/Proteintech	Cat# gtma
Dynabeads MyOne Streptavidin C1	ThermoScientific	Cat#65001
cOmplete mini EDTA-free protease inhibitors cocktail	Sigma-Aldrich	Cat#4693159001
Valproic acid sodium salt	Sigma-Aldrich	Cat# P4543
Recombinant protein H4	New England Biolabs	Cat#M2504S
Recombinant protein H3.1	New England Biolabs	Cat#M2503S
ChIP DNA Clean & Concentrator™	Zymo Research	Cat#D5201
NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®	New England Biolabs	Cat#E7645
NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 1)	New England Biolabs	Cat#E7335
NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 2)	New England Biolabs	Cat#E7500
NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 3)	New England Biolabs	Cat#E7710
NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 4)	New England Biolabs	Cat#E7730
NovaSeq 6000 S1 Reagent Kit (200 cycles)	Illumina	Cat#20028318