Histone H1 loss drives lymphoma by disrupting 3D chromatin architecture

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Linker histone H1 proteins bind to nucleosomes and facilitate chromatin compaction¹, although their biological functions are poorly understood. Mutations in the genes that encode H1 isoforms B-E (H1B, H1C, H1D and H1E; also known as H1-5, H1-2, H1-3 and H1-4, respectively) are highly recurrent in B cell lymphomas, but the pathogenic relevance of these mutations to cancer and the mechanisms that are involved are unknown. Here we show that lymphoma-associated H1 alleles are genetic driver mutations in lymphomas. Disruption of H1 function results in a profound architectural remodelling of the genome, which is characterized by large-scale yet focal shifts of chromatin from a compacted to a relaxed state. This decompaction drives distinct changes in epigenetic states, primarily owing to a gain of histone H3 dimethylation at lysine 36 (H3K36me2) and/or loss of repressive H3 trimethylation at lysine 27 (H3K27me3). These changes unlock the expression of stem cell genes that are normally silenced during early development. In mice, loss of H1c and H1e (also known as H1f2 and H1f4, respectively) conferred germinal centre B cells with enhanced fitness and self-renewal properties, ultimately leading to aggressive lymphomas with an increased repopulating potential. Collectively, our data indicate that H1 proteins are normally required to sequester early developmental genes into architecturally inaccessible genomic compartments. We also establish H1 as a bona fide tumour suppressor and show that mutations in H1 drive malignant transformation primarily through three-dimensional genome reorganization, which leads to epigenetic reprogramming and derepression of developmentally silenced genes.

Linker histones are encoded in humans by ten different genes, five of which (H1A (also known as H1-1), H1B, H1C, H1D and H1E) are expressed in a replication-dependent manner. Linker histones act as transcriptional repressors by limiting chromatin accessibility², and are depleted from actively transcribed domains³. Their functions are mediated either directly, through condensation of chromatin fibre; or indirectly, through recruitment of transcriptional repressors or by impairing the access of transcriptional activators to core nucleosomes¹. A triple knockout of three H1 isoforms (H1C, H1D and H1E) in mouse embryonic stem cells impaired differentiation⁴, suggesting that H1 is involved in the epigenetic programming of cellular phenotypes. Although mutations in core nucleosomal histones are well-documented as having a role in cancer, little is known about H1 mutations^{5,6}. Recurrent H1 mutations occur in around 30-40% of diffuse large B cell lymphomas (DLBCLs), around 30% of follicular lymphomas and around 50% of Hodgkin lymphomas⁷⁻⁹. These diseases originate from germinal centre (GC) B cells, which arise transiently from resting B cells during the

T-cell-dependent humoral immune response¹⁰. During the GC reaction, the immunoglobulin loci undergo extensive mutagenesis through the activity of activation-induced cytosine deaminase (AICDA), and mutant H1 alleles in lymphoma exhibit AICDA mutation signatures¹¹. H1 mutations in lymphoma are often highly clonal and their mutational landscape suggests loss of function; however, their effect at the chromatin or functional level has not been defined, although one study of a single H1 mutant reported impaired binding to mononucleosomes⁷. In mice, homozygous knockout of one or both of *H1c* and *H1e*, the most commonly mutated isoforms of H1 in human lymphoma, did not induce a developmental phenotype¹². Whether or how the dosage or functionality of H1 isoforms contribute to lymphomagenesis is unknown.

Lymphoma H1 alleles are driver mutations

We examined data from the Pan-Cancer Atlas of The Cancer Genome Atlas (TCGA) project and observed that B cell lymphomas have the

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highest frequency of mutant H1 alleles as compared to all other cancers. The majority (97%) of mutant H1 alleles encode missense mutations that affect the globular and C-terminal domains, with H1C and H1E being the most commonly affected isoforms (Extended Data Fig. 1a, b). Although H1 mutations occurred across DLBCL subtypes, there was significant enrichment for H1 single-nucleotide variants (SNVs) and focal deletions in the newly defined MCD subtype of DLBCLs¹³ (Extended Data Fig. 1c-f). We analysed germline-controlled whole-genome sequencing profiles from 101 patients with DLBCL and observed H1 mutation rates of 8.9% (*H1B*), 24.7% (*H1C*), 11.0% (*H1D*) and 42.6% (*H1E*) (Extended Data Fig. 1g). A rigorous analysis controlling for genomic and epigenomic covariates (Supplementary Methods) found that H1C and H1E were among the top ten driver mutations (Extended Data Fig. 1h). The variant-allele frequency ranged from 0.2 to 0.4, consistent with clonal heterozygous mutation. There was significant co-occurrence between H1C and H1E mutations, as well as those affecting other H1 alleles (Extended Data Fig. 1i, j). Furthermore, 85% of mutations in H1B-H1E that altered the globular domain of the protein scored as deleterious and affected amino acids within conserved interaction interfaces, including an ASGS motif that directly binds to DNA¹⁴ (Extended Data Fig. 2a).

Expression of wild-type or C-terminal-mutant H1C tagged with monomeric eGFP in mouse 3T3 cells showed patterns consistent with localization to chromatin, whereas globular domain mutants affecting the ASGS loop formed extensive nuclear aggregates. In fluorescence recovery after photobleaching (FRAP) assays, wild-type H1C and C-terminal H1C mutants showed similar in vivo dynamics, with recovery rates in the order of minutes (Extended Data Fig. 2b, c). By contrast, globular domain mutants recovered rapidly, consistent with failure to incorporate into chromatin. In line with this, using biolayer interferometry we observed that globular domain mutants had higher mononucleosome dissociation constants than did wild-type or C-terminal mutants. Furthermore, Mg2⁺ precipitation and atomic force microscopy of 12-mer nucleosome arrays revealed impaired chromatin compaction upon loading of C-terminal-mutant H1C relative to wild-type H1C (Extended Data Fig. 2d-f). Together, these results show that H1 mutations may result in loss of function through several biochemical mechanisms.

H1 deficiency increases GC B cell fitness

Quantitative PCR with reverse transcription (RT-PCR) analysis showed that the expression of H1B-H1E was twofold to fourfold higher in GC B cells than in naive B cells (Extended Data Fig. 3a, b). Given the common co-occurrence of H1C and H1E mutations, we assessed GC formation in $H1c^{-/-}H1e^{-/-}$ mice, which were previously reported to have no developmental phenotype^{2,12}. Immunized $H1c^{-/-}H1e^{-/-}$ mice had no splenomegaly (Extended Data Fig. 3c) or disruption of splenic architecture (Fig. 1a). However, they exhibited enlarged and more abundant GCs based on PNA (peanut agglutinin) and Ki67⁺ staining (Fig. 1b-d, Extended Data Fig. 3d). There was no observable effect on apoptosis (active caspase-3) or DNA damage (γ -H2AX) (Extended Data Fig. 3e, f). The increase in GC B cells was confirmed by flow cytometry (Extended Data Fig. 3g-i, Supplementary Fig. 2). The proportions of other mature and immature B cells were similar to wild-type mice, with minor differences in the number of Ki67⁺ cells (Extended Data Fig. 3j-m). After sequential immunization with NP-KLH and NP-CGG to induce formation of NP-specific plasma cells, we found no difference between H1c^{-/-}H1e^{-/-} and wild-type mice in the ratio of high- to low-affinity NP antibody titres or in the number of plasma cells secreting anti-NP immunoglobulins (Extended Data Fig. 3n-q). GCs are composed of a dark zone that contains proliferative B cells (centroblasts) and a light zone that contains mostly non-dividing B cells (centrocytes). $H1c^{-/-}H1e^{-/-}$ mice showed a selective increase in the number of centrocytes (Extended Data Fig. 3r). To determine whether a lack of H1C and H1E endows GC B cells with a fitness advantage, we performed mixed bone marrow chimaera experiments and observed a robust competitive advantage for *H1c^{-/-}H1e^{-/-}* GC B cells at both time points (day 10 and day 16) as shown by flow cytometry (Fig. 1e–h) and immunofluorescence analysis (Extended Data Fig. 3s, t). The competitive advantage was specific to centrocytes (Fig. 1i, j). Administering 5-ethynyl-2'-deoxyuridine (EdU) revealed a significant increase in the replication of GC B cells that was specific to centrocytes (Fig. 1k, Extended Data Fig. 3u). Therefore, H1 deficiency leads to increased fitness of GC B cells, which manifests as an increase in the self-renewal of centrocytes.

H1 deficiency induces stem cell genes

RNA sequencing (RNA-seq) in sorted $H1c^{-/-}H1e^{-/-}$ and wild-type GC B cells revealed distinct transcriptional profiles, with 782 differentially expressed genes that were markedly skewed towards transcriptional activation (Fig. 2a, Extended Data Fig. 4a). Many upregulated genes were linked to stem cell functionality, including Klf4, Klf5, Meis1, Prdm5, Mycn, Spry2 and Hoxa9. Pathway analysis revealed enrichment for (i) signatures associated with partially reprogrammed induced pluripotent stem (iPS) cells, adult tissue stem cells and haematopoietic stem and progenitor cells; (ii) direct targets of stem cell transcription factors such as SOX2, NANOG and SUZ12 (a subunit of polycomb repressive complex 2; PRC2); and (iii) genes marked by H3K27me3 in stem and mature haematopoietic cells, including monovalent H3K27me3 in GC B cells (Fig. 2b). H3K27me3 is formed by the PRC2 complex and is opposed by H3K36me2, which is mediated by NSD2 and related histone methyltransferases¹⁵. Notably, NSD2 is induced in wild-type human and mouse GC B cells, and $H1c^{-/-}H1e^{-/-}$ GC B cells showed a marked upregulation of genes activated by NSD2 gain of function in B and T cells¹⁶ (Fig. 2b. Extended Data Fig. 4b. c).

Among normal immune and haematopoietic cells, only long-term repopulating haematopoietic stem cells were enriched for the *H1c^{-/-}H1e^{-/-}* signature, and there was significant enrichment for mesenchymal-like state transitions, and cancer stromal cells (Extended Data Fig. 4d, e). Of note, genes repressed by EZH2 in GC B cells through the formation of bivalent chromatin were not de-repressed by loss of H1c and H1e¹⁷. Therefore, H1C and H1E deficiency primarily reversed the silencing of developmental PRC2 targets (Fig. 2b, Extended Data Fig. 4f). When we examined RNA-seq profiles from patients with double-mutant DLCBL (that is, with mutations in both H1C and H1E; hereafter, H1C/ H1E-mutant DLCBL) and compared these to RNA-seq profiles from patients with DLCBL with wild-type H1 alleles¹⁸, we identified 453 significantly differentially expressed genes in the patients with H1 mutations. These genes were again skewed towards transcriptional upregulation and enrichment for iPS cell signatures, H3K27me3-marked genes in haematopoietic cells and cistromes for NANOG and PRC2 (Extended Data Fig. 4g-i).

To determine whether the effect of H1 deficiency on gene expression was derived from an aberrant subpopulation of GC B cells, we performed single-cell RNA-seq in GC B cells from $H1c^{-/-}H1e^{-/-}$ and wild-type mice. When we plotted centroblasts and centrocytes across a pseudotime axis (Fig. 2c, Extended Data Fig. 4j, k), we again observed an increased abundance of centrocytes among $H1c^{-/-}H1e^{-/-}$ GC B cells (Fig. 2d, e). Yet genes upregulated in $H1c^{-/-}H1e^{-/-}$ GC B cells or H1C/H1E-mutant DLBCLs were uniformly upregulated across centroblasts and centrocytes (Fig. 2f, g) with no new subpopulations among H1-deficient GC B cells (data not shown). Notably, $H1c^{-/-}H1e^{-/-}$ centrocytes (but not centroblasts) showed a significant upregulation of proliferation-related genes (Extended Data Fig. 4l), consistent with aberrant self-renewal being most evident among centrocytes.

H1 deficiency decompacts stem cell genes

We next performed chromatin conformation capture (Hi-C) analyses in sorted $H1c^{-/-}H1e^{-/-}$ and wild-type GC B cells. These showed distinct contact profiles, and analyses of chromatin compartment



Fig. 1 | **Characterization of** *HIc^{-/-}HIe^{-/-}* **GC B cells. a**, **b**, Spleens of wild-type (WT) and *HIc^{-/-}HIe^{-/-}* mice at day 9 after immunization with sheep red blood cells (SRBC). Images were stained for haematoxylin and eosin (H&E) or B220 antibody (**a**), or PNA or Ki-67 antibody (**b**). Scale bars, 500 µm (low-magnification images); 100 µm (high-magnification images). Images are representative of three independent experiments. **c**, **d**, Quantification of GC area (**c**; n = 11 per genotype, ***P = 0.0004) and number of GCs (**d**; n = 10 per genotype, *P = 0.044) in the spleens of wild-type and *HIc^{-/-}HIe^{-/-}* mice. Mean ± s.d., unpaired *t*-test. **e**, Schematic of competitive mixed bone marrow chimaera experiments. **f**, Representative flow cytometry plots at day 10 after immunization for relative fractions of CD45.1⁺ and CD45.2⁺ cells within naive B220⁺ cells and GC B cells. **g**, **h**, Relative ratio of GC B cells to B220⁺ cells in chimaeras injected with wild-type CD45.1⁺ cells or *HIc^{-/-}* CD45.2⁺ cells, measured at day 10 (**g**; n = 4 chimaeras, ****P < 0.0001) or day 16 (**h**; n = 7

states (compartment scores; c-scores) revealed a strong separation between $H1c^{-/-}H1e^{-/-}$ and wild-type GC B cells (Extended Data Fig. 5a, b). Compartment-B chromatin is highly compacted and transcriptionally silent, whereas compartment A is associated with transcriptionally poised or active chromatin¹⁹. We observed extensive, yet focal, decompaction that affected 5,320 discrete 100-kb chromatin domains, and increased compaction of only 386 regions (Fig. 3a, b, Extended Data Fig. 5c). Among these, 637 domains shifted entirely from compartment B to compartment A (B to A). The remaining shifts consisted of decompaction within respective compartments (Fig. 3a). Focal compartment shifting occurred across all chromosomes, only excluding regions with the most-extreme compartment-B c-scores, which contain gene deserts and pericentric heterochromatin²⁰ (Extended Data Fig. 5d, e). Accordingly, regions that underwent B-to-A decompaction had a significantly higher content of genes and CpG islands than did non-shifting B regions (P = 0.007 and P = 0.0005, respectively). Boundaries of topologically associating domains (TADs) were not significantly affected, but 26 TADs exhibited a significant gain of intra-TAD interactivity (Extended Data Fig. 5f) and were enriched for regions with compartment decompaction (Fig. 3b). Assay for transposase-accessible chromatin using sequencing (ATAC-seq) profiling revealed 488 differentially accessible peaks, 90% of which manifested gain of accessibility (Extended Data Fig. 5g), and significant enrichment within regions that experienced decompaction

chimaeras, *P=0.0138) after immunization. Paired *t*-test. GC B/B220⁺ ratios in chimaeras injected with wild-type CD45.1⁺ cells and wild-type CD45.2⁺ cells were unchanged at day 10 (*n* = 5) and day 16 (*n* = 7) after immunization. **i**, Relative fraction of centroblasts (CXCR4⁺CD86⁻) among GC B cells for wild-type (CD45.1⁺) and *H1c^{-/-}H1e^{-/-}* (CD45.2⁺) fractions in mixed chimaeras at day 10 (*n* = 4, ***P = 0.0004) or day 16 (*n* = 7, ****P < 0.0001) after immunization. Paired *t*-test. **j**, Relative fraction of centrocytes (CXCR4⁻CD86⁺) among GC B cells for wild-type (CD45.1⁺) and *H1c^{-/-}H1e^{-/-}* (CD45.2⁺) fractions at day 10 (*n* = 4, ***P = 0.0005) or day 16 (*n* = 7, ****P < 0.0001) after immunization. Paired *t*-test. **k**, Relative fraction of EdU⁺ cells among centroblasts (*n* = 4) or centrocytes (*n* = 3, **P = 0.0040, two-sided paired *t*-test) for wild-type (CD45.1⁺) and *H1c^{-/-}H1e^{-/-}* (CD45.2⁺) fractions at day 7 after immunization.

(Fig. 3c), whereby more than 99% of differentially accessible sites gained accessibility (Extended Data Fig. 5h).

Chromatin shifts from compartment B to compartment A affected genes that are upregulated in H1-deficient GC B cells, such as *Klf5*, *Meis1*, *Tusc1* and *Spry2* (Fig. 3d, Extended Data Fig. 5i). In line with this, we observed a significant upregulation of genes shifting from B to A (n = 224, normalized enrichment score (NES) = 1.73, false discovery rate (FDR) < 0.001), as well as genes decompacting within cognate compartments (Extended Data Fig. 5j, k). Decompacted genes were enriched for iPS cell reprogramming, mesenchymal-transition states, stem cell transcription factor cistromes, H2K27me3-marked genes in haematopoietic cells, and genes that are induced by NSD2 gain of function. GC-B-cell-specific EZH2 targets were unaffected, consistent with a previous report on the absence of H1 from poised gene promoters²¹ (Extended Data Fig. 5]).

Notably, we observed a significant association between genomic domains that decompact during early iPS cell reprogramming and those that decompact in H1-deficient GC B cells (Extended Data Fig. 6a–d). Further analysis of the stem-cell-associated *Klf5* locus using virtual 4C (v4C) revealed a gain of promoter interaction with distal elements that shift from B compartments to A compartments in *H1c^{-/-}H1e^{-/-}* GC B cells, similar to the gains in *Klf5* promoter–enhancer interactions that occur in iPS cells (Extended Data Fig. 6e). Notably, these newly



Fig. 2 | Loss of H1c and H1e induces stem-cell-like transcriptional profiles in GCB cells. a, Heat map of differentially expressed genes as log₂-transformed ratio of every sample to the mean wild-type gene expression (FDR < 0.05, fold change > 1.5) in sorted GC B cells from independent $H1c^{-/-}H1e^{-/-}$ (n = 3) and wild-type (n = 4) mice. Mll3 is also known as Kmt2c. b, Gene pathway enrichment analysis of upregulated and downregulated genes in H1c^{-/-}H1e^{-/-} versus wild-type GC B cells (hypergeometric mean test). ES cell, embryonic stem cell; KO, knockout; NSD2 GoF, NSD2 gain of function (in three cell lines: SEM, RCH-ACV and RPMI); shEZH2, short hairpin RNA against EZH2. c, Single-cell RNA-seq density plot showing the frequency of centroblasts and centrocytes across the Slingshot pseudotime axis. d, Density plot of the frequency of $H1c^{-/-}H1e^{-/-}$ (n = 9,807 cells) and wild-type (n = 6,774 cells) GC B cells across the pseudotime axis. Data are pooled from two independent biological replicates. e, Differential density plot between $H1c^{-/-}H1e^{-/-}$ and wild-type GC B cells, with analysis by two-sided Wilcoxon test. f, g, Top, expression of the upregulated H1c^{-/-}H1e^{-/-} GC B cell gene signature (f) and human H1C/H1E-mutant DLCBL gene signature (g) was plotted for each cell on the yaxis, with spline curves representing the average for H1c^{-/-}H1e^{-/-} and wild-type cells. Bottom, differential expression shown as a delta spline plot across pseudotime, tested by two-sided Wilcoxon test within ten bins of equal cell number (dashed lines). BCCA, British Columbia Cancer Agency.

interactive *Klf5* sites were significantly enriched for two canonical consensus motifs for transcription factors that are associated with GC B cells–OCT2 (*P* = 0.00295) and IRF8 (*P* = 0.00699)–suggesting that decompacted genes could become targets for GC-B-cell associated transcription factors. We wondered whether these iPS-cell-like architectural states would facilitate stem cell reprogramming from differentiated H1C-and-H1E-deficient cells. Indeed, expression of the pluripotency-related transcription factors OCT4, KLF4, SOX2 and MYC (OKSM) in $H1c^{-/-}H1e^{-/-}$ and wild-type mouse embryonic fibroblasts resulted in a three- to fourfold-increased efficiency in forming H1C-and-H1E-deficient colonies of iPS cells as compared to wild-type

cells (Extended Data Fig. 6f–h). These data suggest that the H1C and H1E isoforms maintain the inactivation of primitive stem cell genes that are silenced during lineage specification and differentiation.

H1 deficiency reprograms epigenetic states

Mass-spectrometry-based quantification of histone post-translational modifications in wild-type and $H1c^{-/-}H1e^{-/-}$ GC B cells revealed a significant gain of H3K36me1 and H3K36me2–but not H3K36me3–in $H1c^{-/-}H1e^{-/-}$ cells. As expected given that H3K36me2 can license chromatin for transcription and antagonize PRC2¹⁶, there was a significant reduction in H3K27me2 and H3K27me3, but little change in other histone modifications (Extended Data Fig. 7a, b). Gain of H3K36me2 and loss of H3K27me3 was confirmed by western blotting, whereas the levels of EZH2 and NSD2 were unchanged (Extended Data Fig. 7c). We found little difference in the relative abundance of H3.3 versus H3.1 or H3.2 in $H1c^{-/-}H1e^{-/-}$ GC B cells, and a similar gain of K36me2 and loss of K27me3 across H3 variants (Extended Data Fig. 7d, e).

Using chromatin immunoprecipitation with reference exogenous genomic DNA (ChIP-RX) analysis for H3K36me2 and H3K27me3, we found that there was a clear difference in the distribution of these marks in *H1c^{-/-}H1e^{-/-}* relative to wild-type GC B cells, yielding 7,901 gained versus 33 lost H3K36me2 peaks, and 792 gained versus 4,736 lost H3K27me3 peaks, with a general inverse correlation of these marks (Fig. 4a, Extended Data Fig. 7f, g). Notably, H3K36me2 gain more closely followed B-to-A compartment shifts as compared to H3K27me3 loss (Fig. 4b, Extended Data Fig. 7h). Further analysis of H3K36me2 and H3K27me3 marks in normal GC B cells showed that both were generally absent from compartment-B chromatin, except for H3K27me3 at the least compacted B regions, whereas H3K36me2 increased progressively across compartment-A chromatin, and H3K27me3 was depleted from the most decompacted regions (Extended Data Fig. 7i). Thus, the degree of chromatin interactivity is reflected in alternatively demarcated H3K27me3 or H3K36me2 epigenetic states. In line with this, hierarchical clustering of these histone marks in regions with compartment decompaction revealed five distinct epigenetic states (Fig. 4c, d, Extended Data Fig. 7j): (1) regions that remain in compartment B, with both marks stably absent; (2) regions that start in compartment B, with low H3K36me2 and H3K27me3, and exhibit modest gains of both, with H3K27me3 gains preferentially remaining in compartment B (Extended Data Fig. 7k); (3) regions that start in compartment B or A and undergo moderate gain of H3K26me2 and loss of H3K27me3; (4) regions that start in compartment A and show substantial gain of H3K36me2 and modest loss of H3K27me3; and (5) regions in compartment A that become highly decompacted and mostly show a reduction of H3K27me3.

Genes within the epigenetic state groups 3, 4 and 5 exhibited significant transcriptional activation, which was consistent across centroblasts and centrocytes (Extended Data Fig. 8a, b). Accordingly, upregulated promoters gained H3K4me3 and H3K27ac marks, primarily in compartment A (Extended Data Fig. 8c, d). By contrast, genes in groups 1 and 2 remained mostly silenced. Profiling of the distribution of H3K9me2 and H3K9me3 in wild-type GC B cells revealed that these histone marks were confined to compartment B (Extended Data Fig. 8e). Profiling these marks in H1c^{-/-}H1e^{-/-} GC B cells showed a reduction in H3K9me3 but not in H3K9me2 peaks (Extended Data Fig. 8f). However, regions undergoing decompaction in H1c^{-/-}H1e^{-/-}GCB cells exhibited a significant reduction in both H3K9me2 and H3K9me3 (Extended Data Fig. 8g-i). Moreover, H3K9me2 and H3K9me3 were reduced but not completely lost in group 1 and 2 regions, thus marking compacted territories that are more resistant to full decompaction (Extended Data Fig. 8j).

To gain further insight into how the abundance of H1 influences chromatin compaction, we performed in silico modelling of 50-mer nucleosome arrays²². At higher concentrations, H1 formed a rigid



Fig. 3 | **Loss of** *Hic* **and** *Hie* **induces three-dimensional decompaction in GC B cells. a**, Schematic of directionality of shifting within defined compartments (left) and number of shifted compartment regions (100-kb scale) (right), showing decompaction in red bars and compaction in blue bars (BwA, A decompacting shift in compartment B; AwA, A decompacting shift in compartment A). **b**, Bar graph showing the proportion of stable or decompacting 100-kb compartment bins within TADs gaining (n = 26 TADs) or showing no change in (n = 1,444 TADs) intra-TAD interactivity in $H1c^{-/-}H1e^{-/-}$ GC B cells. Two sided Fisher's exact test, $P < 2.2 \times 10^{-16}$. **c**, Gene set enrichment

analysis (GSEA) of ATAC-seq peaks from decompacting compartments against ranked log₂-transformed fold changes in ATAC-seq peaks in $H1c^{-l-}H1e^{-l-}$ versus wild-type GC B cells. **d**, Top, contact heat map of chromosome 14, showing wild-type GC B cells on the top right and $H1c^{-l-}H1e^{-l-}$ GC B cells on the bottom left. Bottom, contact heat map in the region containing the *KlfS* locus. Integrated genome viewer (IGV) tracks below represent the eigenvector for compartments A (red) and B (blue) positioned on chr14:98,000,000– 101,000,000. ic, iterative correction (normalization method).

stem and straightened chromatin fibres (Extended Data Fig. 9a). These rigid conformations occupied smaller volumes as compared to nucleosome arrays with lower H1 concentrations and demonstrated negligible long-range contacts between nucleosomes. With decreasing H1:nucleosome ratio, fewer DNA linkers were shielded by H1, allowing greater bending and loop formation²² and resulting in enhanced long-range nucleosome–nucleosome contacts (Extended Data Fig. 9b-f). These results mimic the chromatin state changes that were observed by Hi-C and provide a rationale for the transition states that we captured experimentally.

Collectively, these data suggest that H1 dosage affects gene expression by: (i) sequestering genes within compartment B through dense compaction; (ii) making less-compact compartment-B regions accessible enough to enable PRC2 to form H3K27me3 domains²³; and (iii) contributing to the regulation of compartment-A genes by establishing chromatin compaction states that are optimal for specific epigenetic complexes (for example, PRC2 and NSD2), with regions that lack H3K27me3 at baseline primarily gaining H3K36me2, and regions that contain H3K27me3 primarily experiencing loss of this mark without substantial gain of H3K36me2. These data suggest that there is a critical functional link between H1 and NSD2, as NSD2 gain of function mimics the H1-deficient transcriptional signature in B cells¹⁶, disrupts genomic compartmentalization²⁴ and leads to the upregulation of genes associated with epithelial–mesenchymal transition²⁵.

H1 deficiency induces lymphomagenesis

We crossed *H1c^{-/-}H1e^{-/-}* mice with *VavP-Bcl2* mice to model DLBCLs of the MCD subtype (Fig. 5a), as MCD DLBCLs have the highest expression of BCL2 among DLBCLs¹³. At early time points, *VavP-Bcl2* mice had intact lymph node architecture, albeit with hyperplastic follicles containing foci of small lymphocytes with condensed chromatin. By contrast, additional loss of *H1c* and *H1e* resulted in disrupted lymph nodes that were diffusely infiltrated by immunoblastic cells with large nuclei, open chromatin and increased H3K36me2 (Fig. 5b, Extended

Data Fig. 10a, b). *VavP-Bcl2;H1c^{-/-}H1e^{-/-}* mice also exhibited a more extensive invasion of the liver and the lungs by neoplastic Ki67⁺ B cells, with T cell infiltrates as often observed in activated B cell-like (ABC) DLBCLs²⁶, and more evident expansion of monoclonal B cell populations (Fig. 5c, Extended Data Fig. 10c-f). Long-term observation revealed that VavP-Bcl2;H1c+/-H1e+/- and VavP-Bcl2;H1c-/-H1e-/mice had significantly shorter survival times than did VavP-Bcl2-only mice (Fig. 5d). $H1c^{-/-}H1e^{-/-}$ and $H1c^{+/-}H1e^{+/-}$ mice without Bcl2 also had shorter survival times compared to controls, and at early time points manifested lymphoproliferative disease invading extranodal tissues (Extended Data Fig. 10g). We noted a trend for greater lethality of the heterozygous condition, with two *VavP-Bcl2;H1c^{+/-}H1e^{+/-}* mice already suffering from immunoblastic DLBCL at the early time point, which did not occur in VavP-Bcl2 or VavP-Bcl2;H1c^{-/-}H1e^{-/-} mice (Extended Data Fig. 10h). These findings are consistent with H1 mutations being heterozygous in humans, perhaps to avoid loss of isoform-specific functions (for example, mouse H1D uniquely interacts with DNA methyltransferases^{8,27}), which might place homozygous mutants at a relative disadvantage.

RNA-seq performed in mouse lymphomas showed that there was significant similarity between the *VavP-Bcl2;H1c^{+/-}H1e^{+/-}* and *VavP-Bcl2;H1c^{-/-}H1e^{-/-}* transcriptional profiles, as well as *H1c^{-/-}H1e^{-/-}* GC B cells (Extended Data Fig. 10i, j). H1-deficient lymphomas in mice exhibited upregulation of many genes-including stem cell factors such as Klf5 (Extended Data Fig. 10k), which was also induced at the protein level (Fig. 5f). We observed significant enrichment for stem cell and mesenchymal-transition genes, target genes of PRC2 and H3K27me3, and NSD2 gain-of-function signatures (Fig. 5e). The genetic signature of human H1C/H1E-mutant DLBCL was significantly enriched in *VavP-Bcl2;H1c^{+/-}H1e^{+/-}* lymphomas, with a trend also seen in *VavP-Bcl2;H1c^{-/-}H1e^{-/-}* (Extended Data Fig. 10I). The common theme of stem cell signatures among mouse and human H1-deficient lymphomas prompted us to perform secondary transplantation of lymphoma cells from moribund *VavP-Bcl2;H1c^{+/-}H1e^{+/-}* or *VavP-Bcl2*-only mice into NOD-SCID recipients (Fig. 5g). All mice were euthanized



Fig. 4 | Loss of H1c and H1e reprograms H3K36me2 and H3K27me3 epigenome trajectories. a, Differential chromatin immunoprecipitation followed by sequencing (ChIP-seq) peaks for H3K36me2 and H3K27me3 between $H1c^{-/-}H1e^{-/-}$ and wild-type GC B cells (fold change > 1.5). **b**, Heat map of c-score and H3K36me2 and H3K27me3 fold enrichment centred within shifting B-to-A compartment 'islands' (100 kb) and surrounding 300 kb in H1c^{-/-}H1e⁻ and wild-type GC B cells. c, Heat map of changing fraction peak coverage for H3K27me3 and H3K36me2 in H1c^{-/-}H1e^{-/-} compared to wild-type GC B cells within decompacting compartments (n = 5,320), subdivided into the five groups captured by unsupervised hierarchical clustering. d, Density plots showing the distribution of c-scores for shifting compartment groups 1-5 defined in (c) for $H1c^{-/-}H1e^{-/-}$ versus wild-type GC B cells.

within six weeks, at which point 100% of the VavP-Bcl2;H1c^{+/-}H1e^{+/-} mice, but none of the VavP-Bcl2-only mice, had developed lymphomas (Fig. 5h, i) Tertiary transfer into recipient NOD-SCID mice again yielded 100% engraftment of *VavP-Bcl2;H1c*^{+/-}H1e^{+/-} cells (Fig. 5h). Hence, loss of H1 endows lymphoma cells with enhanced self-renewal properties, consistent with the highly aggressive nature of H1-mutant DLBCL.

In conclusion, our studies point to decompaction of threedimensional chromatin as the dominant effect of H1 loss of function in GC B cells. By contrast, however, compartment shifting was not observed in mouse embryonic stem cells with triple knockout of H1c, H1d and H1e²⁸. Chromatin compartmentalization by H1 might therefore occur specifically during differentiation, consistent with recent findings in conditional H1 triple-knockout haematopoietic cells²⁹. H1 isoforms could thus function as key maintenance factors for compaction and epigenetic memory, particularly for regions that are initially marked for repression by PRC2 during lineage specification. We propose a stoichiometric gradient model to suggest that GC B cells are especially sensitive to H1 dosage (Extended Data Fig. 10m) owing to their extremely rapid proliferative rate¹⁰. Reduced H1 dosage in this context could impair the maintenance of proper chromatin compartmentalization (and hence 'epigenetic memory') in daughter cells (that is, centrocytes). The greater inefficiency in



Fig. 5 | Loss of H1c and H1e leads to aggressive VavP-Bcl2 lymphomas. a, Experimental scheme for lymphomagenesis. Disease burden was examined by immunohistochemistry (IHC). b, IHC images of H&E-stained lymph nodes from mice of indicated genotypes. Scale bars, 25 µm (top); 10 µm (bottom). Images are representative of two independent experiments. c, Representative IHC images of liver tissue stained for H&E, B220 and Ki67 from mice of indicated genotypes. Scale bars, 100 µm. d, Kaplan-Meier curves depicting overall survival of *VavP-Bcl2;H1c^{-/-}H1e^{-/-}* (n = 10), *VavP-Bcl2;H1c^{-/+}H1e^{-/+}* (n = 11), *VavP-Bcl2*-only (that is, with wild-type H1 alleles) (n = 11), $H1c^{-/+}H1e^{-/+}$ (n = 7), $H1c^{-/-}H1e^{-/-}$ (n = 5) and wild-type mice (n = 1), assessed by time of death or euthanasia after bone marrow transplantation. Pvalues are shown compared to VavP-Bcl2-only control (log-rank test). e. Normalized enrichment score of GSEA on indicated gene sets, using ranked log₂-transformed fold change in expression between indicated genotypes versus VavP-Bcl2-only control. EMT, epithelial-mesenchymal transition; NS, not significant. f, Immunoblot for KLF5 protein from VavP-Bcl2;H1c^{-/+}H1e^{-/+} and control VavP-Bcl2-only B220-enriched lymphoma cells with GAPDH loading control. Sample pairs collected over two independent experiments are shown. g, Schematic of tumour engraftment assay. h, Diameter of engrafted tumours after six weeks for secondary (2nd) engraftments (n = 7 recipients per genotype) and tertiary (3rd) engraftments of VavP-Bcl2; H1c^{-/+}H1e^{-/+} cells into seven recipients. i, H&E staining of secondary engrafted *VavP-Bcl2; H1c^{-/+}H1e^{-/+}* lymphomas. Scale bar, 25 μ m. Image is representative of n = 7 mice.

node tumours

generating iPS cells from terminally differentiated cells has been attributed to their restrictive epigenetic states³⁰, an effect that our data suggest is reversed by H1 deficiency. Given that stem-cell-like transcriptional programs are linked to cancer³¹, it is possible that silencing of these through H1-mediated compartmentalization represents a major barrier to transformation of mature B cells. The highly recurrent nature of H1 loss-of-function alleles in lymphoma may reflect the fitness advantage conferred to these mature cells by primitive stem cell programs that are normally sequestered within compartment B.

Online content

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Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All sequencing data that support the findings of this study have been deposited in the NCBU Gene Expression Omnibus (GEO) with accession number GSE143293. All other data that support the findings of this study are available from the corresponding authors upon request. Source data are provided with this paper.

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Author contributions N.Y., A.A.S., E.C. and A.M.M conceived and designed the study and wrote the manuscript. N.Y. performed in vivo experiments. A.A.S., J.M.C. and A.O. performed biochemical experiments. A.K., M.T., C.R.C., C.E.M., A.S.D. and M.I. performed bioinformatics analysis on sequencing-based data. N.Y., A.A.S., A.O., O.E., J.M.P., E.A., Y.D., N.L.K., A.T. and L.M.S. analysed data. J.C. performed mouse genotyping and breeding. B.J.V. and M.-C.K. provided CUT&RUN data. D.W.S. and C.D.A. provided resources. W.B. and E.A. were involved in designing experiments. J.D.L. analysed data and helped write the manuscript. A.I.S. provided the H1 knockout mouse model. T.S. and S.P.-L. performed the in silico modelling. All authors contributed to the written manuscript.

Competing interests A.M.M. has research funding from Janssen Pharmaceuticals, Sanofi and Daiichi Sankyo, has consulted for Epizyme and Constellation and is on the advisory board for KDAC Pharma. A.M.M. has also been on advisory boards for BMS and ExoTherapeutics. N.L.K. is a consultant for Thermo Fisher Scientific. C.D.A is a co-founder of Chroma Therapeutics and Constellation Pharmaceuticals, and a Scientific Advisory Board member of EpiCypher. EpiCypher is a commercial developer of the CUTANA CUT&RUN platform. M.I. has received consultancy fees from Novartis Venture Fund outside of the scope of the work. The remaining authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to A.A.S., E.C. or A.M.M. Peer review information *Nature* thanks lannis Aifantis, Ralph Stadhouders and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Reprints and permissions information is available at http://www.nature.com/reprints.



Extended Data Fig. 1 | Characterization of H1 allele mutations and deletions in DLBCL. a, A PanCancer Atlas cancer mutation survey was performed using the cBioPortal to search the cumulative mutation frequency of *H1A–H1E* across a total of 10,953 non-redundant patient samples across all cancer types. b, *H1B– H1E* mutation landscape across non-redundant TCGA and BCCA (British Columbia Cancer Agency) DLBCL samples (number of samples indicated for each H1 isoform); location of mutations is plotted onto protein structure, with bar height corresponding to mutation counts, total frequency of mutations in each H1 isoform is plotted to the right (red bars). c, Prevalence of mutant H1 versus wild-type H1 cases in ABC and GC B DLBCLs (Fisher's exact test for enrichment in ABC or GC B-DLBCL, *P* > 0.05) d, Prevalence of *H1A–H1E* missense mutations and heterozygous loss in MCD-subtype DLBCL and their enrichment (–log₁₀(*P* value)) over non-MCD DLBCLs. e, Summary table of frequency of mutations and heterozygous loss of *H1A–H1E* in DLBCL subtypes (MCD, ST2, BN2, EZB, N1 and AP53). **f**, Co-occurrence as odds ratio and $-\log_{10}(P$ value) among H1 alleles in 101 germline-matched WGS DLBCLs. **g**, Frequency and location of missense mutations for *H1A–H1E* in 101 germline-controlled DLBCLs. **h**, Quantile-quantile plot showing the *P* values for SNVs across 101 germline-matched WGS DLBCLs. Driver analysis derived as probability of mutation count greater than or equal to the observed mutation count under *P* values expected from a Gamma-Poisson distribution for SNVs. Shaded grey zone contains mutant genes with FDR < 0.01, depicted as blue or red dots, several of which are listed in the figure. **i**, Oncoprint for *H1A–H1E* in 101 germline-matched WGS DLBCLs.**j**, Co-occurrence as odds ratio (OR) and – log₁₀(*P* value) among H1 alleles in 101 germline-matched WGS DLBCLs.



Extended Data Fig. 2 | **H1 mutations are genetic drivers in lymphoma and confer loss of function. a**, Crystal structure of the linker histone globular domain (grey) bound to nucleosome (Protein Data Bank (PDB), 4QLC), with magnified view of ASGS amino acid residues highlighted in red. **b**, Representative images of FRAP of ectopically expressed, monomeric eGFP-tagged wild-type H1C and H1C mutants S102F and P118S in 3T3 cells before, immediately after, and at 10, 30 and 90 s after bleaching the area (yellow dashed square). Scale bars, 5 μm. **c**, Quantification of normalized intensity as representation of turnover kinetics from **b** for wild-type H1C (*n* = 18) and mutants A101V (*n* = 15), S102F (*n* = 9), S104F (*n* = 10), G103A (*n* = 10) and P118S (*n* = 10); shaded area indicates 95% confidence intervals. Data are pooled from

two independent biological experiments. **d**, Dissociation constant (K_d) of the binding to mononucleosomes of recombinant H1C mutants S102F and P118S compared to wild-type H1C, as determined by biolayer interferometry. Data are mean ± s.e.m. (two-sided unpaired *t*-test, ****P < 0.0001). Data are global fits from five concentration measurements. **e**, Chromatin fibre oligomerization upon serial precipitation by Mg2⁺ as per cent soluble 12-mer arrays was determined for no H1, wild-type H1C and the C-terminal P118S mutant. Data are mean ± s.d. Data are pooled from three independent biological experiments. **f**, Atomic force microscopy imaging of chromatin arrays in the presence of wild-type H1 and the C-terminal P118S mutant. Scale bars, 200 nm. Images are representative of two independent biological experiments.



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | H1c^{-/-} H1e^{-/-} mature B cells show normal development in spleen and bone marrow. a, mRNA of human H1B-H1E normalized to *RPL13A* in GC B cells relative to naive B cells (H1B, **P=0.004; H1E, *P=0.027), isolated from three independent specimens of human tonsils. Data are mean ± s.d., two-sided unpaired *t*-tests. **b**, Mouse *H1b–H1e* mRNA levels normalized to Gapdh in sorted GC B cells (n = 3) relative to naive B cells(n = 3)(*H1b*, ****P < 0.0001). Data are mean ± s.d., two-sided unpaired *t*-tests. **c**, Quantification of spleen/body weight ratios of two-month-old H1c^{-/-}H1e^{-/-} (n=13) and wild-type littermate control (n=14) mice. Data are pooled from two independent experiments. P < 0.05; NS, not significant; two-sided unpaired t-tests. Data are mean ± s.d. d, Quantification of GC area (Ki67 staining) in the spleens of $H1c^{-/-}H1e^{-/-}$ (n = 10) and WT (n = 10) mice. ***P = 0.0005. Data are mean ± s.d., two-sided unpaired t-tests. e, f, Immunohistochemistry images of spleen sections of cleaved caspase-3 (e) and y-H2AX (f) staining (left) and quantification (right) of positively stained follicular cells from H1c^{-/-}H1e^{-/-} (n=3) and wild-type littermate control (n=3) mice immunized with SRBCs and euthanized 10 days after immunization. Scale bars, 100 µm. P < 0.05 (not significant: NS), two-sided unpaired *t*-tests. Data are mean ± s.d. g. Flow cytometry analysis and quantification of (Fas⁺CD38⁻) GC B cells within total B cells from $H1c^{-/-}H1e^{-/-}$ and wild-type mice (n = 10 per genotype). **P = 0.0018, two-sided unpaired t-tests. Data are mean \pm s.d. h, Quantification of the percentage of B220⁺ splenocytes in $H1c^{-/-}H1e^{-/-}$ (n = 10) and wild-type (n = 10) mice 9 days after SRBC immunization. P < 0.05, two-sided unpaired t-tests. Data are mean ± s.d. i, Flow cytometry analysis and quantification of GC B cells (Fas⁺GL7⁺) from $H1c^{-/-}H1e^{-/-}$ (n = 10) and wild-type (n = 10) mice. Two-sided unpaired t-tests, *P = 0.041. Data are mean \pm s.d.j, Flow cytometry analysis and quantification of mature B cells (B220 $^{+}\text{Ig}\text{M}^{+}\text{)}$ and transitional B cells $(B220^{+}IgD^{int}IgM^{+})$ in spleens from $H1c^{-/-}H1e^{-/-}$ (n = 10) and wild-type (n = 10) mice. P < 0.05, two-sided unpaired t-tests. Data are mean \pm s.d. k, Flow cytometry quantification of follicular B cells (B220⁺D23⁺CD21⁺) and marginal zone B cells (B220⁺D23¹⁰CD21⁺) in spleens from $H1c^{-/-}H1e^{-/-}$ (n = 10) and

wild-type (n = 10) mice. P < 0.05, two-sided unpaired t-tests. Data are mean \pm s.d. l, Flow cytometry analysis gated on B220 $^{\scriptscriptstyle +}\text{CD24}^{\scriptscriptstyle +}$ and quantification of $ProBPreB\,(IgM^-IgD^-), immature\,(IgM^-IgD^{\rm lo}), transitional\,(IgD^+IgM^-) \, and \, early$ mature (IgD⁺IgM⁺) B cells in bone marrow of $H1c^{-/-}H1e^{-/-}$ (n = 4) and wild-type (n = 5) mice. P < 0.05, two-sided unpaired t-tests. Data are mean \pm s.d. m, Percentage of Ki67⁺early B cells (B220⁺CD24⁺) in bone-marrow of H1c^{-/-}H1e^{-/-} (n=4) and wild-type (n=5) mice, as well as naive B cells (***P=0.0004) and marginal zone B cells (***P = 0.001) in the spleens of $H1c^{-/-}H1e^{-/-}$ (n = 5) and wildtype (n=5) mice. **n**, Schematic diagram of primary immunization with NP-KLH and secondary immunization 21 days after with NP-CGG. o, Ratio between high (NP₈) and low (NP₃₀) affinity NP-specific IgG1 antibody titres in sera of $H1c^{-/-}H1e^{-/-}$ (n = 5) and wild-type (n = 5) mice by enzyme-linked immunosorbent assav (ELISA). P < 0.05. two-sided unpaired *t*-tests. Data are mean \pm s.d. p, Enzyme-linked immunosorbent spot (ELISPOT) quantification of NPspecific (anti-NP $_8$ and anti-NP $_{30}$) IgG1-secreting cells from the bone marrow of $H1c^{-/-}H1e^{-/-}$ (n = 5) and wild-type (n = 5) mice. P < 0.05, two-sided unpaired t-test. Data are mean \pm s.d. Data are representative of two independent experiments. q, Representative images of anti-NP₈ and anti-NP₃₀ 96-well ELISPOT. r, Flow cytometry analysis and quantification of centroblasts within the dark zone (DZ) (CXCR4⁺CD86⁻) (***P = 0.0002) and centrocytes within the light zone (LZ) $(CXCR4^{-}CD86^{+})$ (****P* = 0.0002) within GC B cells from $H1c^{-/-}H1e^{-/-}$ (*n* = 10) and wild-type (n = 10) mice. Two-sided unpaired t-test. Data are representative of three independent experiments. s, Immunofluorescence confocal microscopy images of GCs at day 7 after immunization in mixed chimaeras. Scale bar, $50\,\mu m$. Images are representative of two independent experiments. t, Quantification from s of the fraction of PNA⁺CD45.1 or CD45.2 cells (17 GCs, n = 3 mice). Two-sided paired t-test, ***P = 0.0004. **u**, Relative EdU⁺GC B cell/GC B cell fraction for wild-type CD45.1⁺ and *H1c^{-/-}H1e^{-/-}*CD45.2⁺ cells at day 7 after immunization (n = 4 chimaeras). Two-sided paired t-test, **P = 0.0065. Data are representative of two independent experiments.



Extended Data Fig. 4 | Loss of H1c and H1e induces stem-cell-like transcriptional profiles in GC B cells and DLBCLs. a, Unsupervised hierarchical clustering analysis of RNA-seq data from sorted H1c^{-/-}H1e^{-/-} and wild-type H1 GC B cells, based on genes in the top 90th percentile of variability. b, Expression of NSD2 (measured as fragments per kilobase of exon model per million reads mapped; FPKM) from RNA-seq profiles of human and mouse naive B and GC B cells. c, GSEA analysis of genes linked to NSD2 gain-of-function mutation in three cell lines (RCHACV, SEM, RPMI) against ranked gene expression changes in mouse $H1c^{-/-}H1e^{-/-}$ GC B cells. **d**, Box plot of log₂-transformed relative gene expression normalized to the average expression value of all genes from the top 200 differentially upregulated genes in the H1c^{-/-}H1e^{-/-} signature against the ImmGen database. Box plot centre represents median, bounds of box are the first and third quartiles and whiskers extend to 1.5 × the interguartile range. e, GSEA analysis with indicated gene sets, using ranked log₂-transformed fold change in expression between $H1c^{-/-}H1e^{-/-}$ and wild-type mouse GC B cells. **f**, GSEA analysis of gene sets linked to EZH2 against ranked gene expression changes in mouse H1c^{-/-}H1e^{-/-}GC B

cells. g, Volcano plot showing differentially expressed genes between patients with H1C/E-mutant DLCBL (n = 18) versus patients with H1-wild-type DLBCL (n = 237) (FDR < 0.05, fold change > 1.5). Red field denotes upregulated and blue field downregulated genes. h, GSEA analysis of upregulated genes in patients with H1C/E -mutant versus H1-wild-type DLBCL, using ranked changes in log₂-transformed fold change in mouse H1c^{-/-}H1e^{-/-} GC B cells. i, Gene pathway enrichment analysis of upregulated and downregulated genes from g, hypergeometric mean test. j, k, Sorted H1c^{-/-}H1e^{-/-} or wild-type GC B cells (n = 2 per genotype) were subjected to droplet based (10X) single-cell RNA-seq. Centroblasts (\mathbf{j}) and centrocytes (\mathbf{k}) were defined on the basis of enrichment for centroblast and centrocyte signature profiles, respectively projected onto the uniform manifold approximation and projection (UMAP) distribution of cells. I, Top, expression of G2M cell cycle proliferation gene signature was plotted for each cell on the yaxis with spline curves representing the average for H1c^{-/-}H1e^{-/-} and wild-type GC B cells. Bottom, differential expression is shown as a delta spline plot (blue) across pseudotime and tested by two-sided Wilcoxon test within ten bins of equal cell number (dashed lines).



Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5|HiC compartment analysis and integration with ATAC-seq in H1c^{-/-}H1e^{-/-} and wild-type GCB cells. a, Genome-wide correlation score (Stratum adjusted correlation score, SCC) of HiC matrices within same genotype (***P = 0.0002171, two-sided unpaired *t*-test), and across genotypes (P<0.05, two-sided Wilcoxon test). b, Principal component analysis (PCA) of compartment bins processed with Hi-C bench at a resolution of 10 0kb from Hi-C replicates for $H1c^{-/-}H1e^{-/-}$ and wild-type GC B cells. **c**, Volcano plot showing significant c-score shifts in $H1c^{-/-}H1e^{-/-}$ mouse GC B cells based on Δc -score and -log10(permutation FDR P value): decompacted (red) compartment bins (n = 5,320) and compacted (blue) compartment bins (n=386). **d**, Δ c-score $(H1c^{-/-}H1e^{-/-}$ versus wild type) across mouse chromosomes (positive y axis is in red for decompacting loci and negative y axis is blue for compacting loci) plotted as ideograms. **e**, Box plots of Δ c-score $(H1c^{-/-}H1e^{-/-}$ versus wild type) across c-score range (-1.0 to 1.0) separated into 0.1 bins. No statistical evaluation was derived for this graph. ${f f}$, Volcano plot showing TADs with significant gain of intra-TAD interactivity (n = 26, red) and reduction of intra-TAD interactivity (n = 2, blue; two-sided unpaired t-test, FDRadjusted *P* value < 0.05, fold change > 1.5). **g**, Volcano plot showing significant ATAC-seq peaks gaining accessibility (n = 438) and losing accessibility (n = 53); two-sided unpaired *t*-test FDR-adjusted *P* value < 0.05, fold-change > 1.5).

h, Scatter plot showing log₂-transformed fold change in ATAC-seq peaks between $H1c^{-/-}H1e^{-/-}$ and wild-type GC B cells in decompacting compartments versus wild-type c-score: B to BwA (left), B to A (middle), and A to AwA (right). Peaks gaining accessibility (FC > 1.5, P_{adj} < 0.05) are marked in red. Estimated odds ratios and P values were calculated using Fisher's exact test. Although ATAC-seq peaks are more prevalent in the A-to-AwA compartment (right), increased accessibility is more enriched in B-to-BwA and B-to-A compartments. i, HiC contact maps of regions surrounding Spry (left), Tusc1 (middle) and Meis1(right) genes. The top of each square shows H1 wild-type contacts and the bottom of each square shows those in H1c^{-/-}H1e^{-/-} GC B cells. Heat maps represent the Pearson correlation of interactions in wild-type and $H1c^{-/-}H1e^{-/-}$ GCB cells. Bottom tracks represent the eigenvector (PC1) for compartments A and B in red and blue, respectively, and show the position of genes within these loci.j, k, GSEA analysis of genes shifting to A compartments (B to A, B to BwA, A to AwA) or genes contained in stable compartments using ranked log₂transformed gene expression in H1c^{-/-}H1e^{-/-}GCB cells. (NES and FDR values as implemented by GSEA). I, Gene pathway enrichment analysis of genes in decompacting and stable compartments in H1c^{-/-}H1e^{-/-} versus wild-type GC B cells (hypergeometric mean test).



Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | Three-dimensional changes in chromatin architecture owing to a deficiency of H1C and H1E in GCB cells recapitulate decompaction during iPS cell differentiation. a, Schematic of a previous study of B cell differentiation to iPS cells³² with time points for HiC analysis. b, Correlation plots comparing shifting to A c-scores in iPS cells (day 2, day 4, day 6, day 8) compared to control B cells versus c-score changes in H1c^{-/-}H1e^{-/-} GC B cells. Estimated odds ratios and P values were calculated using Fisher's exact test. c, GSEA analysis of shifting to A compartments in iPS cells (day 2, day 4, day 6, day 8) against ranked Δ c-scores derived from $H1c^{-/-}H1e^{-/-}$ minus wildtype mouse GC B cells (NES and FDR values as implemented by GSEA). d, Violin plots comparing stable and shifting B to A compartments during iPS cell differentiation (day 2, day 4, day 6, day 8 and fully undifferentiated) to the estimated Δ c-score due to deficiency of H1C and H1E in those same regions in GC B cells. $(day 2, P < 2.2 \times 10^{-16}; day 4, P < 2.2 \times 10^{-16}; day 6, P = 0.002; day 8, P < 0.00$ 0.0001; estimated with two-sided Wilcoxon test). Box plot centre represents median, bounds of box are the first and third quartiles and whiskers extend to $1.5 \times$ the interquartile range. **e**, v4c analyses on the *Klf5* locus (chr14:

99,000,000-100,200,000) anchored on the Klf5 promoter for (top) B cell reprogramming (blue) to iPS cells (red) with four intermediate time states (grey) from a previous study³², as well as GC B cells (bottom) ($H1c^{-/-}H1e^{-/-}$ and wild-type H1). IgV tracks below comprise Δ c-score and ATAC-seq signal in $H1c^{-/-}H1e^{-/-}$ versus wild-type GC B cells. Gained HiC interactions in $H1c^{-/-}H1e^{-/-}$ compared to wild-type GC B cells (shaded in grey 1–3: pval = 0.04; pval = 0.059; pval = 0.02, respectively, two-sided unpaired t-test) have OCT2 motif sequences as shown. **f**, Schematic of experimental set-up with $H1c^{-/-}H1e^{-/-}$ or wild-type littermate mouse embryonic fibroblasts. g, Representative images of alkaline phosphatase (AP)-stained H1c^{-/-}H1e^{-/-} and wild-type iPS cell colonies at day 21. **h**, Per cent iPS cell reprogramming efficiency of $H1c^{-/-}H1e^{-/-}$ (n = 5 transfections on two biological replicates) and wild-type (n = 4 transfections on two biological replicates) mouse embryonic fibroblasts determined as the ratio of AP⁺ colonies to the number of seeded mCherry⁺ cells. P = 0.01, twosided unpaired t-tests. Data are mean \pm s.d. Data are representative of three independent experiments.





Extended Data Fig. 7 | Altered H3K36me2 and H3K27me3 distribution in H1c^{-/-}H1e^{-/-}GCB cells. a, b, Mass spectrometry of H3K36 (a) and H3K27 (b) post-translational modifications, \log_2 -transformed and normalized to average wild-type peak area from $H1c^{-/-}H1e^{-/-}$ (n = 5) and wild-type (n = 7) acid-extracted samples from GC B cells; two-sided unpaired t-test: K36 unmodified (unmod.), ****P*=0.0005; K36me1, ****P*=0.0003; K36me2****P*=0.0002; K36me3, *P*=0.93; K36ac, P=0.56; K27 unmod., *P=0.0157; K27me1, **P=0.0072; K27me2, *P=0.0175; K27me3, ***P=0.0007; K27ac, P=0.9337. Box plots show median and 25th to 75th percentiles, whiskers indicate data range. Data are representative of two independent experiments. c, Immunoblots for H1 (D4J5Q and AE-4 antibodies), H3K36me2, H3K27me3, EZH2 and NSD2 from sorted wild-type and H1c^{-/-}H1e^{-/-}GCB cells. Direct blue stain is included as loading control. A representative image of at least three experiments is shown. Uncropped gels are shown in Supplementary Fig. 1. d, Mass-spectometrybased relative abundance of H3.1 or H3.2 (replication-dependent) and H3.3 (replication-independent) isoforms, shown as the average percentage of the total peak area of H3K27-K36 peptide containing H3.3-specific Ser31 in acidextracted histones from wild-type (H3.3, 15.76%, n = 7) and $H1c^{-/-}H1e^{-/-}$ (H3.3, 15.07%, n = 5) GC B cells; two-sided unpaired *t*-test, P = 0.0004, Data are mean \pm s.d. e, Mass spectrometry of H3 K36 (top) and K27 (bottom) posttranslational modifications across H3.1/2 (left) and H3.3 (right) isoforms, log₂transformed and normalized to average wild-type peak area from samples acidextracted from wild type (n = 7) and $H1c^{-/-}H1e^{-/-}$ (n = 5) GC B cells; two-sided unpaired *t*-test: H3.1/2 K36 unmod., ***P=0.0005; H3.1/2 K36me1, ***P=0.0003; H3.1/2K36me2, ****P<0.0001; H3.1/2K36me2, P=0.88; H3.1/2 K27 unmod., *P=0.0100; H3.1/2 K27me1, *P=0.0162; H3.1/2 K27me2, **P*=0.0129; H3.1/2 K27me3, ****P*=0.0002; H3.3 K36 unmod., *****P*<0.0001; H3.3K36me1,*P=0.036;H3.3K36me2,P=0.15;H3.3K36me3,P=0.5974;H3.3

K27 unmod., P=0.1187; H3.3 K27me1, P=0.4743; H3.3 K27me2, P=0.1199; H3.3 K27me3, P=0.0628. Box plots show median and 25th to 75th percentiles, whiskers indicate data range. f, Unsupervised hierarchical clustering analysis of ChIP-seq data for H3K27me3 and H36me2 in biological triplicates from sorted H1c^{-/-}H1e^{-/-} and wild-type GC B cells. g, Genome-wide correlation plot of \log_2 -transformed fold change ($H1c^{-/-}H1e^{-/-}$ versus wild type) of normalized reads within ChIP-seq peak union for H3K36me2 and H3K27me3 (Pearson correlation coefficient R = -0.453, $P < 1 \times 10^{-16}$). **h**, Heat map of HiC c-score, H3K36me2 and H3K27me3 centred within shifting B-to-A compartments (100 kb) and surrounding 300 kb for H1c^{-/-}H1e^{-/-} and wild-type GC B cells for compartment 'extensions' (top). i, Fraction of ChIP-seq peak coverage (H3K36me2 in red and H3K27me3 in blue) within 100-kb compartments across HiC c-score (x axis, -1 to 1) for wild-type GC B cells. Cubic smoothing splines of data are presented with shaded regions indicating 99% confidence intervals. j, Fraction peak (H3K27me3 and H3K36me2) coverage of regions within shifting compartment groups 1-5 in H1c^{-/-}H1e^{-/-} and wild-type GC B cells. Paired Wilcoxon test, group 1: H3K27me3, *P* < 1×10⁻¹⁶; H3K36me2, *P* = 0.597; group 2: H3K27me3, P<1×10⁻¹⁶; H3K36me2, P<1×10⁻¹⁶; group 3: H3K27me3, $P = 3.38 \times 10^{-13}$; H3K36me2, $P < 1 \times 10^{-16}$; group 4: H3K27me3, $P = 8.71 \times 10^{-15}$; H3K36me2, *P*<1×10⁻¹⁶; group 5: H3K27me3, *P*<1×10⁻¹⁶; H3K36me2, *P*<1×10⁻¹⁶. Box plot centre represents median, bounds of box are the first and third quartiles and whiskers extend to $1.5 \times$ the interquartile range. **k**, Scatter plot of H3K27me3 peak \log_2 -transformed fold change ($H1c^{-/-}H1e^{-/-}$ versus wild-type GC B cells) versus wild-type c-score score for decompacting group 2. Gain of H3K27me3 (red dots) largely occurred within regions shifting from compartment B whereas loss of H3K72me3 (blue dots) was more prevalent within regions shifting compartments from compartment A.



Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Changes in activation marks H3K4me3 and H3K27ac and repressive marks H3K9me2 and H3K9me3 in H1-deficient GCB cells are associated with compartment-B decompaction. a, Genes defined as groups 3 and 4 (n = 108 genes) and group 5 (n = 152 genes) show transcriptional activation and significant upregulation in $H1c^{-/-}H1e^{-/-}$ GCB cells compared to wild-type GCB cells. Paired Wilcoxon test. Box plot centre represents median, bounds of box are the first and third quartiles and whiskers extend to 1.5 × the interquartile range. b, Top, expression of the genes defined as groups 3-5 was plotted for each cell on the yaxis. Average expression is represented by the different coloured spline curves for each genotype as indicated. Bottom, differential expression between H1c^{-/-}H1e^{-/-} and wild-type cells is represented as the delta spline plot across pseudotime. Cells are divided by pseudotime into bins of equal cell number (grey vertical dashed lines) and tested for signature enrichment compared to wild-type GC B cells by two-sided Wilcoxon test ($P < 6.81 \times 10^{-50}$) **c**, GSEA with genes marked with gain in H3K4me3 (top) or H3K27Ac (bottom) on their promoters (transcription start site (TSS) ± 500 bp) using the ranked \log_2 -transformed fold change in mouse $H1c^{-/-}H1e^{-/-}$ GC B cells. d, Fraction of histone peak coverage for H3K4me3 and H3K27ac within 100-kb compartments across HiC c-score (x axis) for wild-type GC B cells. Cubic smoothing splines of data are presented with shaded regions indicating 99%confidence intervals. e, Fraction of histone peak coverage (for H3K9me2 and

H3K9me3) within 100-kb compartments across HiC c-score (x axis) for wildtype GC B cells. Cubic smoothing splines of data are presented with shaded regions indicating 99% confidence intervals. f, CUT&RUN peaks for H3K9me2 and H3K9me3 show altered abundance between H1c^{-/-}H1e^{-/-} and wild-type GC B cells (FC > 1.5, n = 5,277 gained and n = 4,717 lost H3K9me2 and n = 2,511 gained and n = 7,529 lost H3K9me3 peaks). g, Genome-wide correlation plot of \log_2 transformed fold change ($H1c^{-/-}H1e^{-/-}$ versus wild-type H1GC B cells) in H3K9me2 (left) and H3K9me3 (right) peaks versus Δ c-score (*H1c^{-/-}H1e^{-/-}* minus wild-type H1). Both H3K9me2 and H3K9me3 changes were largely loss and were anti-correlated with compartment decompaction (Pearson correlation coefficient R = -0.438, $P < 1 \times 10^{-16}$ and R = -0.543, $P < 1 \times 10^{-16}$, respectively). h, i, Heat maps of H39me2 and H3k9me3 centred within shifting B-to-A compartments (100 kb) and surrounding 300 kb for H1c^{-/-}H1e^{-/-} and wild-type GCB cells for compartment 'extensions' (h) and 'islands' (i). j, Fraction peak $(H3K9me2\,and\,H3K9me3)\,coverage\,of\,regions\,within\,shifting\,compartment$ groups and 2 in H1c^{-/-}H1e^{-/-} and wild-type GC B cells. Paired Wilcoxon test, group 1: H3K9me2, P<1×10⁻¹⁶, H3K9me3, P<1×10⁻¹⁶; group 2: H3K9me2, $P < 1 \times 10^{-16}$; H3K9me3, $P < 1 \times 10^{-16}$. Box plot centre represents median, bounds of box are the first and third quartiles and whiskers extend to $1.5 \times$ the interquartile range.



Extended Data Fig. 9 | Linker histone incorporation reduces interactivity of chromatin fibre. a, Representative equilibrated configurations of 50-nucleosome chromatin fibres obtained in silico in the absence of H1 and in the presence of 0.25, 0.5, 0.75 and 1H1 molecule per nucleosome (H1C (left) and H1E (right)). Fibre contour (in red) on which the H1 mean positions are shown is shown on the top right of each model. Colour key shows DNA, linker histone, H2A, H2B, H3 and H4 tails. **b**, **c**, Contact maps for the 1,000-configuration ensembles obtained from left to right, in the absence of H1 and in the presence of 0.25, 0.50, 0.75 and 1H1 per nucleosome(**b**, H1C; **c**, H1E). **d**, The nucleosome– nucleosome interaction patterns, or a one-dimensional decomposition of each contact map shown in **b** and **c**, indicate the dominant zigzag pattern of the fibre (*i*±2) and increase of long-range interactions as the density of H1C (left) or H1E

(right) decreases. **e**, Packing ratio calculated as the number of nucleosomes contained in 11 nm of fibre for systems without H1 and in the presence of 0.25, 0.50, 0.75 and 1 H1 molecule per nucleosome (left, H1C; right, H1E); n = 1,000 chromatin ensemble configurations for each H1 per nucleosome ratio. Ordinary one-way analysis of variance for multiple comparisons, ****P<0.0001. **f**, Volume of chromatin fibres calculated assuming a cylindrical shape for systems with no H1 and in the presence of 0.25, 0.50, 0.75 and 1 H1 molecule per nucleosome (left, H1C; right, H1E); n = 1,000 chromatin ensemble configurations for each H1 per nucleosome ratio. Systems with no H1 and in the presence of 0.25, 0.5, 0.75 and 1 H1 molecule per nucleosome (left, H1C; right, H1E); n = 1,000 chromatin ensemble configurations for each H1 per nucleosome ratio. Ordinary one-way analysis of variance for multiple comparisons, ****P<0.0001. Box plot centre represents median, bounds of box are the first and third quartiles and whiskers extend to 1.5× the interquartile range (**e**, **f**).



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | Loss of H1c and H1e leads to aggressive Vav-PBcl2 lymphomas with DLBCL-like morphology. a, Immunohistochemistry images of lymph node stained for H&E and B220 from VavP-Bcl2;H1c^{-/-}H1e^{-/-}, VavP-Bcl2;H1c^{-/+}H1e^{-/+} and VavP-Bcl2-only mice at day 164. Scale bar, 1 mm. Images are representative of n = 11 mice per genotype examined over two independent experiments. b, Representative immunohistochemistry images of lymphomatous VavP-Bcl2;H1c^{-/-}H1e^{-/-}, VavP-Bcl2;H1c^{-/+}H1e^{-/+}and VavP-Bcl2only lymph nodes stained for H3K36me2, and quantification of intensity (binned as high, mid, low and negative). Scale bar, 50 µm. Tissue derived from three mice per genotype with four tumour lymph nodes each. Data are mean ± s.d., two-sided unpaired t-test. c, Immunohistochemistry images of lung tissue stained for H&E, B220 and Ki67 from VavP-Bcl2;H1c^{-/-}H1e^{-/-}, VavP-*Bcl2;H1c^{-/+}H1e^{-/+}* and *VavP-Bcl2*-only mice at day 164. Scale bar, 100 µm. Images are representative of n = 11 mice per genotype examined over two independent experiments. d, Quantification of B220⁺lesion areas in liver tissue (Fig. 5c) from VavP-Bcl2;H1c^{-/-}H1e^{-/-} (****P<0.0001) and VavP-Bcl2;H1c^{-/+}H1e^{-/+} (*P = 0.0308) compared to VavP-Bcl2-only (n = 7 mice per genotype, mean \pm s.d.; two-sided unpaired *t*-tests). **e**. Immunohistochemistry stains for CD3 from *VavP-Bcl2;H1c^{-/-}H1e^{-/-}* and *VavP-Bcl2;H1c^{-/+}H1e^{-/+}* lymphomas. Scale bar, 50 µm. Images are representative of n = 4 mice per genotype examined over two independent experiments. f, PCR for $Ig\lambda$ clonal rearrangement to report on tumour clonality of B220⁺ cells from VavP-Bcl2;H1c^{-/-}H1e^{-/-}, VavP-Bcl2;H1c^{-/+} H1e^{-/+} and VavP-Bcl2-only mice at day 164. g, Immunohistochemistry images of liver and lung tissues stained for H&E and B220 in from H1c^{-/-}H1e^{-/-}, H1c^{-/+}H1e^{-/+}

and wild-type mice at day 164. Scale bar, 500 µm. Images are representative of n=6 mice per genotype examined over two independent experiments. h, Immunohistochemistry images of lymph node tissue stained for H&E and B220 from VavP-Bcl2;H1c^{-/+}H1e^{-/+} and VavP-Bcl2-only mice. Images are representative of n = 4 mice per genotype examined over two independent experiments. Scale bars, 100 μm. i, GSEA with the *VavP-Bcl2;H1c^{-/-}H1e^{-/-}* versus VavP-Bcl2 lymphoma upregulated gene set ranked against log₂-transformed changes in fold change from mouse *VavP-Bcl2;H1c^{-/+}H1e^{-/+}* versus *VavP-Bcl2*. j, Top, GSEA for genes upregulated in VavP-Bcl2;H1c^{-/+}H1e^{-/+} versus VavP-Bcl2 lymphomas using the ranked log₂-transformed fold change in mouse H1c^{-/-}H1e^{-/-}GCB cells. Bottom, GSEA for genes upregulated in VavP-Bcl2;H1c^{-/-} $H1e^{-/-}$ versus VavP-Bcl2 mice using the ranked \log_2 -transformed fold change in mouse *H1c^{-/-}H1e^{-/-}* GC B cells. **k**, Heat map showing differential expression of leading-edge genes (n = 898) from VavP-Bcl2;H1c^{-/-}H1e^{-/-} and VavP-Bcl2;H1c^{-/+} *H1e*^{-/+} lymphomas. I, GSEA for genes upregulated in human *H1C/E*-mutant DLBCL, against the ranked log₂-transformed fold change gene expression profiles of mouse *VavP-Bcl2;H1c^{-/+}H1e^{-/+}* (left) and *VavP-Bcl2;H1c^{-/-}H1e^{-/-}* (right) lymphomas, m. Summary model depicting chromatin as contiguous B-to-A space, with H3K27 and H3K36 methylations occupying distinct compartments within. Loss of H1 results in a global shift of compartment interactivity towards A, with both H3K27 and H3K36 methylations shifting into ectopic regions. Most compacted regions devoid of either K27 or K36 methylation appear largely protected from H1 loss. The biological effects of H1 loss in GC B cells are summarized below.

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Reporting Summary

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Statistics

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	firmed
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\square	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
		The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
	\square	A description of all covariates tested
	\square	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
		A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
		For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable</i> .
	\square	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
	\square	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	\square	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	FACS BD Aria II, Influx, FACS Canto II were used to collect flow cytometry sorting and analysis data. Halo software (v3.0.311.201) was used to quantify data from immunohistochemistry. Skyline software (v4.1) was used for raw mass spectometry files. Asylum Research sotware packager version IX was used for height profiles on atomic force microscopy. ELISPOT plates were evaluated using an automated Zeiss ELISPOT reader system (ZellNet Consulting, Inc.). Biolayer interferometry measurements were taken on an Octen Red96e system (v11).
Data analysis	FlowJo software v10.5.3, R version 3.4.4 (2017-11-30), R package Slingshot version 1.2.0, R package Seurat 3.0.2, R package EdgeR v3.16.5 and v3.20.9, R package EDAseq 2.22.0, R package DeSeq2 1.27.1, Sarek pipeline version 2.5.1 ChIP-seq reads were aligned using bwa-mem function of the BWA suite. Peaks were called using the SICER algorithm. RNA-seq reads were aligned to mm10 using STAR and annotated to RefSeq using the R subread package. Whole-genome sequencing data aligned to human genome hg-19 was retrieved from the European Genome Archive and used to generate unmapped bam files using picard tools RevertSam according to GATK best practice. Bam files were aligned to human genome hg38 using BWA mem. Somatic alterations were called from matched tumor-normal pairs using Manta followed by Strelka2, and annotated by VEP (https://github.com/nf-core/sarek). FishHook (https://github.com/mskilab/fishHook) was used to model background mutational processes. All study and public Hi-C data used have been pre-processed with the hic-bench pipeline. FRAP data were analyzed using ImageJ (v1.51j) and FRAP Profiler plugin (http://worms.zoology.wisc.edu/research/4d/4d.html)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information. Policy information about availability of data

- All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
 - Accession codes, unique identifiers, or web links for publicly available datasets
 - A list of figures that have associated raw data
 - A description of any restrictions on data availability

The sequencing datasets generated in this study are available at GEO (GSE143293). No restriction of data availability.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For high throughput NG, mass spec experiments, a minimum of three independent biological replicates were used where possible (ChIP-seq, ATAC-seq, RNA-seq, Cut and Run). Two biological replicates were used for HiC and Single cell RNA-seq. Sample size for in vivo experiments were chosen based on our prior experience for detecting statistically significant differences compared to control animals (Béguelin et al Cancer Cell, Vol37, Issue 5, Pages 655-673). Sample size for in vitro experiments were determined with preliminary experiments with a minumum of 2 samples per group, and full cohort experiments repeated at least twice.
Data exclusions	No samples were excluded.
Replication	All findings were reproducible. Confirmation of reproducibility was assessed with at least three independent experiments for in vivo and at least two independent experiments for in vitro assays, and in the case of high throughput NGS analysis, through the use of sample sized that provided comprehensive genome-wide coverage of chromatin changes.
Randomization	Sex-matched littermates were randomly assigned to experimental arms. Samples were allocated to groups according to genotype.
Blinding	Studies were not blinded and group allocation was done before data collection as only one investigator ran the experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems	Methods
n/a Involved in the study	n/a Involved in the study
Antibodies	ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology	MRI-based neuroimaging
Animals and other organisms	

Antibodies

Human research participants

Clinical data

 \mathbf{X}

 \boxtimes

Antibodies used	For immunoblotting, the following primary antibodies were used: D4J5Q, anti-H1E, Cell-Signaling 41328; AE-4, anti-H1, Millipore 05-457; C36B11 anti-H3 K27me3, Cell Signaling 9733; C75H12 anti-H3 K36me2, Cell Signaling 2901; AC22 anti-Ezh2, Cell Signaling 3147; 29D1 anti-NSD2, Millipore Mabe191; rabbit polyclonal anti-KLF5, Proteintech 21017-1-AP; 6C5 anti-GAPDH, Ab8245) For ChIP, the following primary antibodies were used: anti-H3 K27me3, C36B11 - Cell Signaling 9733 (20 micrograms), anti-H3 K36me2, C75H12 - Cell Signaling 2901 (20 micrograms). For CutandRun, the following antibodies were used: anti-H3K4me3 (Epicypher 13-0041) (0.5ug each).
Validation	anti-H3 K27me3 and anti-H3 K36me2 antibodies have been extensively validated in literature, including Weinberg et al., 2019

Nature; Papillon-Cavanagh et al., Nat Genet 2017; Lu et al., Science 2016. Cut and Run antibodies are validated to SNAP-ChIP nucleosome standards (S1hah et al., Mol Cell 2018).D4J5Q and AE-4 anti-H1 antibodies have been validated in mouse knock-out and human shRNA-treated knock-down cells. C36B11, C75H12, Ac22 and 29D1 have been validated in human and mouse knock-out and transgenic cell lines (using ChIP-qPCR in wild type and loss-of-function (K27M, K36M, knock-ou, Ezh2 knock-out) backgrounds.

Eukaryotic cell lines

Policy information about <u>cell lines</u>	
Cell line source(s)	NIH-3T3 (CRL-1658) purchased from ATCC; Kc167 (RRID:CVCL_Z834) cells were purchased from Drosophila Genomics Resource Center
Authentication	Cell lines were not specifically authenticated for these studies
Mycoplasma contamination	Cell line was tested negative for mycoplasma contamination
Commonly misidentified lines (See <u>ICLAC</u> register)	No commonly misidentified lines were used in this study

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	The study involved H1c-/-/e-/- mice and littermate controls (C57BL/GJ) that were age- and sex-matched (tested within 8-10-weeks old). Bone-marrow recipient mice were female, 8-weeks old and C57BL/GJ strain with donor bone marrow (CD45.1 and CD45.2) pooled from sex-matched, 8-10-week old mice. VavP-Bcl2 (C57BL/GJ), crossed with H1c-/-/e-/-, were used sex-matched 8-week old mice as bone-marrow donors for lymphomagenesis and survival studies.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Animal care was in strict compliance with institutional guidelines established by Weill Cornell Medicine, the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences 1996) (Silverman et al., 2006), and the Association for Assessment and Accreditation of Laboratory Animal Care International. All mice were followed until any one of several criteria for euthanizing were met, including severe lethargy, more than 10% body weight loss, and palpable splenomegaly that extended across the midline, in accordance with our Weill Cornell Medicine Institutional Animal Care and Use Committee (IACUC)– approved animal protocols.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE143293 Publicly available
May remain private before publication. Files in database submission	Publicly available GSM4256478 Sample_1_GCB_WT_repl1_RNAseq GSM4256479 Sample_2_GCB_WT_repl3_RNAseq GSM4256480 Sample_3_GCB_WT_repl3_RNAseq GSM4256481 Sample_4_GCB_WT_repl4_RNAseq GSM4256483 Sample_6_GCB_H1_DKO_repl1_RNAseq GSM4256483 Sample_6_GCB_H1_DKO_repl3_RNAseq GSM4256484 Sample_7_GCB_H1_DKO_repl3_RNAseq GSM4256485 Sample_8_GCB_WT_H3K27me3_repl1_ChIPseq GSM4256486 Sample_9_GCB_WT_H3K27me3_repl3_ChIPseq GSM4256488 Sample_11_GCB_H1_DKO_H3K27me3_repl2_ChIPseq GSM4256498 Sample_13_GCB_H1_DKO_H3K27me3_repl3_ChIPseq GSM4256491 Sample_14_GCB_WT_H3K36me2_repl1_ChIPseq GSM4256491 Sample_15_GCB_WT_H3K36me2_repl3_ChIPseq GSM4256493 Sample_15_GCB_WT_H3K36me2_repl3_ChIPseq GSM4256494 Sample_17_GCB_H1_DKO_H3K36me2_repl3_ChIPseq GSM4256494 Sample_17_GCB_H1_DKO_H3K36me2_repl3_ChIPseq GSM4256494 Sample_17_GCB_H1_DKO_H3K36me2_repl3_ChIPseq GSM4256494 Sample_17_GCB_H1_DKO_H3K36me2_repl3_ChIPseq
	GSM4256495 Sample_18_GCB_H1 DKO_H3K36me2_repl2_ChlPseq GSM4256496 Sample_19_GCB_H1 DKO_H3K36me2_repl3_ChlPseq GSM4256497 Sample_20_GCB_WT_Input_repl1_ChlPseq

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GSM4256498 Sample_21_GCB_WT_Input_repl2_ChIPseq
GSM4256499 Sample_22_GCB_WT_Input_repl3_ChIPseq
GSM4256500 Sample 23 GCB H1 DKO Input repl1 ChIPseq
GSM4256501 Sample 24 GCB H1 DKO Input repl2 ChIPseq
CSM/256502 Sample 25 GCB H1 DKO Input rend ChiPsed
CSM4250264 Sample 25_CCD_MT_repl3_cfm/scq
GSIVI4365365 Sample_27_GCB_WI_repl2_Hic
GSM4363366 Sample_28_GCB_H1_DKO_repl1_HiC
GSM4363367 Sample_29_GCB_H1_DKO_repl2_HiC
GSM4523349 Sample 30 GCB WT repl1 ATACseq
GSM4523350 Sample 31 GCB WT repl2 ATACseq
GSM/523351 Sample 32 GCB WT repl3 ATACseq
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GSM4523353 Sample_34_GCB_H1_DKO_repl1_ATACseq
GSM4523354 Sample_35_GCB_H1_DKO_repl2_ATACseq
GSM4523355 Sample_36_GCB_H1_DKO_repl3_ATACseq
GSM4523356 Sample 37 GCB H1 DKO repl4 ATACseq
GSM4523357 Sample 38 GCB WT H3K4me3 CutAndRun
GSM4523358 Sample 39 GCB WT H3K9me2 CutAndRun
CSM45235260 Sample 40 CCP WT H2K0mc2 CutAndRun
GSM4523360 Sample_41_GCB_H1_DKO_H3K4me3_CutAndKun
GSM4523361 Sample_42_GCB_H1_DKO_H3K9me2_CutAndRun
GSM4523362 Sample_43_GCB_H1_DKO_H3K9me3_CutAndRun
GSM4523363 Sample 44 GCB WT H3K27Ac rep1 ChIPseq
GSM4523364 Sample 45 GCB WT H3K27Ac rep2 ChIPseq
GSM4523365 Sample 46 GCB WT H3K274c rep3 ChiPseq
CSM4523366 Sample 47 CCB H1 DKC H2/27Ac rep1 CbDcg
GSN4523506 Sample_4/_GCB_H1_DKO_H3K2/AC_rep1_Chirsed
GSM4523367 Sample_48_GCB_H1_DKO_H3K27Ac_rep2_ChIPseq
GSM4523368 Sample_49_GCB_H1_DKO_H3K27Ac_rep3_ChIPseq
GSM4752544 Sample_DKO2-GCB-3_10X_SingleCell
GSM4752545 Sample_WT4-GCB-1_10X_SingleCell
GSM4752546 Sample_WT5-GCB-2_10X_SingleCell
CSM4752547 Sample DKOG-GCB-4 10X SingleCell
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Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates per group were tested. For ATAC-seq with primar mouse GCB-cells, at least three biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end 1-WT-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WT-H3K27me3, total number reads: mm10, 15,809,793; dm6, 21,020,327 1-H1DKO-H3K27me3, total number reads: mm10, 12,020,4274; dm6, 26,505,971 2-H1DKO-H3K27me3, total number reads: mm10, 17,020,424; dm6, 25,308,467
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Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates per group were tested. For ATAC-seq with primar mouse GCB-cells, at least three biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end 1-WT-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WT-H3K27me3, total number reads: mm10, 15,809,793; dm6, 21,688,452 3-WT-H3K27me3, total number reads: mm10, 18,663,073; dm6, 21,202,327 1-H1DKO-H3K27me3, total number reads: mm10, 17,020,424; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 17,020,424; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 16,444,400; dm6, 23,322,436 1-WT-H3K36me2, total number reads: mm10, 19,010,885; dm6, 2,397,743
Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates per group were tested. For ATAC-seq with primar mouse GCB-cells, at least three biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end 1-WT-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WT-H3K27me3, total number reads: mm10, 15,809,793; dm6, 21,688,452 3-WT-H3K27me3, total number reads: mm10, 120,042,774; dm6, 26,505,971 2-H1DKO-H3K27me3, total number reads: mm10, 17,020,424; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 16,444,400; dm6, 23,322,436 1-WT-H3K36me2, total number reads: mm10, 18,804,732; dm6, 2,397,743
Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates per group were tested. For ATAC-seq with primar mouse GCB-cells, at least three biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end 1-WT-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WT-H3K27me3, total number reads: mm10, 15,809,793; dm6, 21,202,327 1-H1DKO-H3K27me3, total number reads: mm10, 12,0042,774; dm6, 26,505,971 2-H1DKO-H3K27me3, total number reads: mm10, 17,020,424; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 16,444,400; dm6, 23,322,436 1-WT-H3K36me2, total number reads: mm10, 18,804,732; dm6, 2,275,684 3-WT-H3K36me2, total number reads: mm10, 18,804,732; dm6, 2,275,684
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Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates per group were tested. For ATAC-seq with primary mouse GCB-cells, at least three biological replicates (n=3) per group were tested. For ATAC-seq with primary mouse GC B-cells, two biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end 1-WT-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WT-H3K27me3, total number reads: mm10, 18,663,073; dm6, 21,202,327 1-H1DKO-H3K27me3, total number reads: mm10, 18,663,073; dm6, 21,202,327 1-H1DKO-H3K27me3, total number reads: mm10, 16,402,774; dm6, 26,505,971 2-H1DKO-H3K27me3, total number reads: mm10, 16,444,400; dm6, 23,322,436 1-WT-H3K36me2, total number reads: mm10, 18,804,732; dm6, 2,397,743 2-WT-H3K36me2, total number reads: mm10, 18,804,732; dm6, 2,275,684 3-WT-H3K36me2, total number reads: mm10, 16,474,978; dm6, 1,760,707 1-H1DKO-H3K26me2, total number reads: mm10, 22,605,400; dm6, 2,585,188 2-H1DKO-H3K26me2, total number reads: mm10, 22,605,400; dm6, 2,585,188 2-H1DKO-H3K26me2, total number reads: mm10, 22,605,400; dm6, 2,585,188
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Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates per group were tested. For ATAC-seq with primary mouse GCB-cells, at least three biological replicates (n=3) per group were tested. For ATAC-seq with primary mouse GC B-cells, two biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end 1-VTI-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WTI-H3K27me3, total number reads: mm10, 18,663,073; dm6, 21,202,327 1-H1DKO-H3K27me3, total number reads: mm10, 20,042,774; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 10,0042,774; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 16,444,400; dm6, 23,322,436 1-WTI-H3K36me2, total number reads: mm10, 18,804,732; dm6, 2,725,684 3-WTI-H3K36me2, total number reads: mm10, 25,400; dm6, 2,310,400 3-W1DKO-H3K26me2, total number reads: mm10, 22,605,400; dm6, 2,310,400 3-W1DKO-H3K27Me2, total number reads: mm10, 22,811,922; dm6, 2,900,788 1-H1DKO-H3K26me2, total number reads: mm10, 22,811,922; dm6, 8,933,764
Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates per group were tested. For ATAC-seq with primary mouse GC B-cells, at least three biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end -WT-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WT-H3K27me3, total number reads: mm10, 18,663,073; dm6, 21,202,327 1-H1DKO-H3K27me3, total number reads: mm10, 12,0042,774; dm6, 26,505,971 2-H1DKO-H3K27me3, total number reads: mm10, 17,020,424; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 16,444,400; dm6, 23,322,436 -WT-H3K36me2, total number reads: mm10, 18,644,732; dm6, 2,397,743 2-WT-H3K36me2, total number reads: mm10, 16,474,978; dm6, 1,760,707 1-H1DKO-H3K27me3, total number reads: mm10, 22,605,400; dm6, 2,385,188 2-H1DKO-H3K26me2, total number reads: mm10, 22,605,400; dm6, 2,300,788 -WT-H3K27Ac, total number reads: mm10, 22,811,922; dm6, 8,253,764 2-WT-H3K27Ac, total number reads: mm10, 23,571,881; dm6, 8,717,785
Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For HiC experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For ATAC-seq with primary mouse GCB-cells, at least three biological replicates per group were tested. For ATAC-seq with primary mouse GC B-cells, two biological replicates (n=3) per group were tested. For ATAC-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end 1-WT-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WT-H3K27me3, total number reads: mm10, 15,809,793; dm6, 21,688,452 3-WT-H3K27me3, total number reads: mm10, 16,643,073; dm6, 21,202,327 1-H1DKO-H3K27me3, total number reads: mm10, 17,020,422, dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 16,444,400; dm6, 23,322,436 1-WT-H3K36me2, total number reads: mm10, 16,474,978; dm6, 2,397,743 2-WT-H3K36me2, total number reads: mm10, 18,804,732; dm6, 2,275,684 3-WT-H3K36me2, total number reads: mm10, 27,599,022; dm6, 2,585,188 2-H1DKO-H3K26me2, total number reads: mm10, 22,605,400; dm6, 2,310,040 3-H1DKO-H3K26me2, total number reads: mm10, 22,812,922; dm6, 8,933,764 2-WT-H3K27Ac, total number reads: mm10, 22,811,922; dm6, 8,933,764 2-WT-H3K27Ac, total number reads: mm10, 22,811,922; dm6, 8,933,764 </th
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Not applicable - visualized data using IGV For ChIP-seq experiments with primary mouse GC B-cells, three biological replicates (n=3)per group were tested. For CutandRun experiments with primary mouse GC B-cells, two biological replicates (n=2) per group were tested. For ATAC-seq with primary mouse GC B-cells, two biological replicates per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, at least three biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates (n=3) per group were tested. For Single Cell RNA-seq with primary mouse GC B-cells, two biological replicates per group were tested. ChIP-seq experiments: 75 bp single-end -WT-H3K27me3, total number reads: mm10, 18,627,064; dm6, 23,345,945 2-WT-H3K27me3, total number reads: mm10, 18,63,073; dm6, 21,202,327 1-H1DKO-H3K27me3, total number reads: mm10, 20,042,774; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 17,020,424; dm6, 25,308,467 3-H1DKO-H3K27me3, total number reads: mm10, 19,010,885; dm6, 2,397,743 2-WT-H3K36me2, total number reads: mm10, 19,010,885; dm6, 2,796,84 -WT-H3K36me2, total number reads: mm10, 22,605,400; dm6, 2,810,040 3-H1DKO-H3K26me2, total number reads: mm10, 22,811,922; dm6, 8,933,764 2-H1DKO-H3K26me2, total number reads: mm10, 28,325,523; dm6, 2,900,788 -WT-H3K27Ac, total number reads: mm10, 22,811,922; dm6, 8,933,764 2-WT-H3K27Ac, total number reads: mm10, 28,92351; dm6, 6,909,044 2-H1DKO-H3K27Ac, total number reads: mm10, 16,7
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CutAndRun experiments:

Genome browser session

Sequencing depth

(e.g. <u>UCSC</u>) Methodology Replicates

> 1-WT-H3K4me3, total number reads: mm10, 3,815,224 2-WT-H3K4me3, total number reads: mm10, 5,541,738 1-H1DKO-H3K4me3, total number reads: mm10, 4,812,484 2-H1DKO-H3K4me3, total number reads: mm10, 5,854,955 1-WT-H3K9me2, total number reads: mm10, 6,981,537 2-WT-H3K9me2, total number reads: mm10, 6,448,278 1-H1DKO-H3K9me2, total number reads: mm10, 7,147,881 2-H1DKO-H3K9me2, total number reads: mm10, 6,496,731

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	2-WT-H3K9me3, total number reads: mm10, 9,044,632 1-H1DKO-H3K9me3, total number reads: mm10, 7,585,753 2-H1DKO-H3K9me3, total number reads: mm10, 8,276,380
	ATAC-seq experiment: DK01-GCB: 75,394,476 DK02-GCB: 68,819,276 DK03-GCB: 63,692,712 WT1-GCB: 79,495,180 WT2-GCB: 68,697,976 WT3-GCB: 73,202,966
	Hi-C number of intra-chromosomal reads used for downstream reads ('ds.accepted intra'): WT_H1_1, total number of reads: 216,296,915 WT_H1_2, total number of reads: 222,714,033 H1DKO_1, total number of reads: 246,312,138 H1DKO_2, total number of reads: 237,201,022
	Single cell-RNA seq: WT4-GCB: 283,715,494 WT5-GCB: 237,596,846 DKO2-GCB: 210,177,503 DKO6-GCB: 257,475,313
Antibodies	For ChIP, the following primary antibodies were used: anti-H3 K27me3, C36B11 - Cell Signaling 9733 (20micrograms), anti-H3 K36me2, C75H12 - Cell Signaling 2901 (20micrograms)
Peak calling parameters	Peaks were called using SICER algorithm: GCB_WT_K27me3_pooledSicer: 24,131 GCB_H1DKO_K27me3_pooledSicer: 13,603 GCB_WT_K36me2_pooledSicer: 29,466 GCB_H1DKO_K36me2_pooledSicer: 35,553 GCB_WT_K27ac_pooledSicer: 10,522 GCB_H1DKO_K27ac_pooledSicer: 10,522 GCB_H1DKO_K27ac_pooledSicer: 13,393 GCB_H1DKO_K4me3_pooledSicer: 14,761 GCB_WT_K9me2_pooledSicer: 77,330 GCB_H1DKO_K9me2_pooledSicer: 90,670 GCB_WT_K9me3_pooledSicer: 103,436 GCB_H1DKO_K9me3_pooledSicer: 73,051 ATAC-seq peaks were called: Sample_30_GCB_WT_repl1_ATACseq: 141,700 Sample_31_GCB_WT_repl3_ATACseq: 147,478 Sample_32_GCB_MT_repl3_ATACseq: 157,608 Sample_35_GCB_H1_DKO_repl3_ATACseq: 13,4151
Data quality	Resulting FASTQ files were aligned to mouse mm10 and Drosophila dm6 genomes using bwa-mem function of the BWA suite. Individual sequencing experiments were assessed for their percent of mapped reads (to mm10 and dm6) to ensure proper coverage. ChIPseq data was normalized to dm6 spike-in reads using CompChIPseq algorithm. H3K27me3 and H3K36me2 ChIPseq peaks were called using the SICER algorithm on pooled BAM files from all respective replicates. Loci showing differences in ChIPseq abundance were determined by calculating the Comp-ChIPseq normalized read count within the union of peaks from both genotypes using the multiBigwigSummary function of the deepTools package. For Hi-C, mapped reads were filtered by the GenomicTools tools-hic filter command integrated in HiC-bench for known artifacts of the Hi-C protocol. The filtered reads include multi-mapped reads ('multihit') read-pairs with only one mappable read, duplicated read-pairs, low mapping quality reads, read-pairs resulting from self-ligated fragments, and short-range interactions, resulting from read-pairs aligning within 25kb. For the downstream analyses, all the accepted intra-chromosomal read-pairs ('ds.accepted intra') were used.
Software	ChIP-seq reads were aligned using bwa-mem function of the BWA suite. Peaks were called using the SICER algorithm. Read density tracks were visualized using the Integrative Genomics Viewer (IGV).

1-WT-H3K9me3, total number reads: mm10, 7,617,674

Flow Cytometry

Plots

Confirm that:

The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Single cell suspensions from mouse spleens were separated by Ficoll gradient centrifucation and stained using fluorescent labeled anti mouse antibodies, incubated on ice in the dark for 30min, then washed 2x with BS with .5% BSA and 5mM EDTA and resuspended in 200ul washing buffer for acquisition
Instrument	BD FACS Canto II
Software	FlowJo v10
Cell population abundance	Sorted human and murine GC B cells were confimed to be >90% following each sorting.
Gating strategy	For selection of single cells, cells were first gated based on FSC-H/FSC-A, then DAPI/FSC-A followed by FSC-A/SSC-A gating. For Sorting: lymphocytes were further gated on APC-B220 positive population on a SSC-A/APC-A dotplot. GC B cells were gated on GL7+FAS+ populations on a BV421-A/PE-Cy7 boxplot (Log axes). For regular flow: live B cells were selected as B220-APC+ cells on APC-A/SSC-A dotplot followed by gating GC B cells either as GL7-FITC+/FAS-PE+ or CD38-APC negative /FAS-PE positive on dotplot with logarithmic axes.

X Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.