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Efficient repair of DNA double-strand breaks (DSBs) requires a coordinated DNA Damage Response (DDR), which includes phosphorylation of histone H2Ax, forming γH2Ax. This histone modification spreads beyond the DSB into neighboring chromatin, generating a DDR platform that protects against end disassociation and degradation, minimizing chromosomal rearrangements. However, mechanisms that determine the breadth and intensity of γH2Ax domains remain unclear. Here, we show that chromosomal contacts of a DSB site are the primary determinants for γH2Ax landscapes. DSBs that disrupt a topological border permit extension of γH2Ax domains into both adjacent compartments. In contrast, DSBs near a border produce highly asymmetric DDR platforms, with γH2Ax nearly absent from one broken end. Collectively, our findings lend insights into a basic DNA repair mechanism and how the precise location of a DSB may influence genome integrity.
A ll cells continuously face DNA damage resulting from environmental insults or from normal physiological processes, including replication and transcription. Perhaps the most dangerous type of damage to DNA is double-strand breaks (DSBs), since their aberrant repair can produce oncogenic rearrangements. When DNA damage occurs in mammalian cells, DSB sensors activate the serine-threonine kinases ATM, ATR, and DNA-PK, which initiate the DNA Damage Response (DDR) via phosphorylation of ~900 protein targets. An important chromatin-based substrate for these kinases is the histone variant H2Ax that, when phosphorylated on serine 139, is referred to as γH2AX. Formation of γH2Ax serves as a checkpoint for the homologous recombination (HR) and non-homologous end joining (NHEJ) repair pathways, through mechanisms that indirectly or directly retain effector proteins.

These effectors include 53BP1, which prevents end degradation and disassociation of DDR factors around a DSB site. Likewise, H2Ax deficiency destabilizes chromosomes harboring DSBs, leading to numerous aberrations, including translocations and deletions. Pursuant to a break, the DDR generates γH2Ax domains that are thought to spread over neighboring chromatin for 1–2 Mb, perhaps by propagation along the chromosome. However, a classic, processive model cannot fully explain the observed profiles of γH2Ax, which can be asymmetric, and may have gaps and varying levels of the modification throughout a domain. Likewise, γH2Ax foci are not contiguous when visualized by high-resolution microscopy, which revealed spatially distinct nano-domains clustered around DSB sites. The mechanisms that sculpt γH2Ax domains have important implications, especially given the critical role of these platforms in damage responses, including: (1) tethering broken chromosomes until they are repaired (2) repression of transcription, and (3) sequestration of DDR factors around a DSB site. Prior studies have shown that perturbation of DDR mechanisms, including mutations in ATM and MDC1 alter γH2Ax densities, but do not affect the extent of its spread. We now show that γH2Ax domains are established via chromosomal contacts with the DSB site. Indeed, the break site interactome precisely defines the densities and spread for this damage-induce histone modification. γH2Ax domains are largely, but not exclusively, confined within self-interacting chromatin regions, called topologically associated domains (TADs), which functionally compartmentalize the genome. Disruption of a TAD border by a targeted DSB extends γH2Ax domains into both adjacent TADs. In contrast, DSBs adjacent to TAD borders generate asymmetric γH2Ax domains, which may influence repair efficiencies and could explain the enrichment of structural variants near topological boundaries.

Results
Physiologic DNA breaks induce locus-restricted γH2Ax domains. To probe genomic features that limit γH2Ax propagation, we characterized DDR platforms in precursor lymphocytes resulting from physiological DSBs, which are mediated by the RAG endonuclease complex during V(DJ) recombination. Initially, we profiled chromatin following RAG-induced DSBs at the Igκ antigen receptor locus in G1-arrested v-abl transformed pre-B cells. We employed a particular line of v-abl cells in which RAG breaks are persistent due to a crippling mutation in the essential NHEJ gene, Lig4. In Lig4−/− cells, but not in control Rag1−/− cells, Lig4Wt, which lack Igκ breaks, γH2Ax covered the entire Igκ locus, as revealed by chromatin immunoprecipitation (ChIP)-seq analysis (Fig. 1a, p < 0.01 Fisher’s Exact Test). The boundaries of the γH2Ax domain, as well as the Igκ locus, coincided with the encompassing TAD, as computed from global interactomes in Rag1−/− cells (Fig. 1a and Supplementary Fig. 1A). Moreover, γH2Ax profiles correlated with the magnitude of chromosomal contacts measured by 4C from the viewpoint of the small Jκ cluster, which always harbor a DSB in the v-abl system (R = 0.60, Pearson’s correlation). Overall patterns in the Jκ interactome did not differ substantially in cells with Lig4+ or without Igκ-DSBs (Rag1−/−), as revealed by 4C analysis (Fig. 1a). Importantly, contours and borders of the RAG-induced γH2Ax domain did not reflect, at a gross level, those of the un-phosphorylated histone substrate, H2Ax (Fig. 1a).

In addition, in the small Jκ cluster, the RAG complex targets DSBs to synapsed Vκ gene segments, which are distributed throughout the 2.5 Mb Vκ cluster. As such, profiles of γH2Ax within Igκ may simply correspond to a broad distribution of DSBs throughout the Vκ cluster in this pre-B cell population.

To circumvent this complication, we examined γH2Ax patterns in thymocytes from Artemis-deficient, Bcl2-Tg mice, which harbor persistent, RAG-mediated DSBs at the Dbf4 recombination center (RC) of Tcrb. In this case, we employed both Cas9 and Runx1/Siphonogly (CR-seq), which, importantly, avoids potential artifacts associated with chromatin crosslinking in conventional ChIP-seq. As shown in Fig. 1b, γH2Ax spread throughout most of the Tcrb locus, despite confinement of the DSBs to its 3’RC portion. In these primary cells with Tcrb damage, γH2Ax values in CR-seq data correlated almost precisely with RC chromosomal contacts in Rag1−/− thymocytes, which we defined quantitatively using deep Hi-C data that were flattened to show the RC viewpoint (herein called virtual 4C).

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H2Ax profiles parallel cell type-specific contacts. To define determinants for DSB-induced γH2Ax domains, we designed a flexible experimental platform, targeting the Cas9 endonuclease with guide RNAs in preformed ribonuclear particles (RNP), which were delivered into cells by nucleofection. We validated the system by targeting Cas9 breaks to the Eβ enhancer in G1-arrested RagL−/− cells. The Eβ-proximal DSB accumulated to near maximum levels (>80%) within 2 h following RNP nucleofection (Fig. 2a and Supplementary Fig. 2A, B). As shown in Fig. 2b, the Cas9 system recapitulated γH2Ax profiles observed for either RAG- or ZFN-induced DSBs in the Tcrb-RC (compare to Fig. 1b). Likewise, in cycling, repair-sufficient cells (Rag2−/−), which undergo continual cycles of cut and repair, targeted Cas9-DSBs at Eβ produced a nearly indistinguishable DDR platform. Hence, γH2Ax profiles arising from DSBs within the Tcrb-RC were identical across distinct cellular sources and experimental systems that had comparable Tcrb interactomes.

If the interactome of a break site is the primary determinant for γH2Ax profiles, one would predict that cell types with different interaction magnitudes would produce DDR platforms with distinct contour densities. Indeed, largely tracking with distinctions in their Eβ interactomes, a targeted DSB at this enhancer generated significantly higher levels of γH2Ax across distal Vβ...
ChIP-seq (representative of two independent replicates) for indicated v-abl cell genotypes. The bottom Juicebox snapshot shows Hi-C data derived from Lig4 windows across Tcrb. The Hi-C data show TADs and their insulation scores as in b Tcrb antigen receptor regions. Each panel includes diagrams indicating antigen receptor loci, genes, and DSB location (lightning bolt) on top. Myc, the established long-range contacts with an H3K27ac-dense region, associated domains (TADs), which are assigned based upon TAD insulation scores, derived from 40 kb bins (blue lines) or 20 kb bins (gray lines). Gene segments in a pro-lymphocyte cell line (63–12) when compared with a pre-lymphocyte line (p5424) (Fig. 2c, d and Supplementary Fig. 2C). These differences are highlighted in subtraction plots for both γH2Ax and V.4C data (bottom tracks). Conversely, the pre-lymphocyte cell line exhibited elevated interactions between the Tcrb-RC and a +500 kb gene cluster, located in an adjacent TAD, which was reflected in significantly higher downstream γH2Ax intensities (Fig. 2d and Supplementary Fig. 2C).

We observed similar results at an independent locus containing the proto-oncogene cMyc, which can adopt cell type-specific topologies. As shown in Fig. 2e, the Myc promoter region established long-range contacts with an H3K27ac-dense region, the Myc super-enhancer (Myc-SE), situated ~1 Mb away. Long-range chromatin contacts between the Myc promoter and enhancers that compose the Myc-SE change during development or differentiation, as distinct regulatory regions become activated or are decommissioned. In keeping with this, pro- and pre-lymphocyte lines preferentially activated and formed promoter contacts with distinct enhancer elements in the large Myc-SE region (Fig. 2e, HiCCompare p < 0.05, M test). Although the γH2Ax borders were similar in both cell types, DSB induction at the Myc promoter (RNP-Myc-P) generated relative changes in γH2Ax densities that mirrored differences in cell type-specific promoter contacts. We conclude that γH2Ax intensities in response to a DSB can differ between cell types in a contact-dependent manner.

We also performed complementary studies to examine the impact of a DSB on chromosomal contacts. For this purpose, we generated Hi-C contact maps for G1-arrested pre-B cells following Imatinib treatment (72 h). UCSC genome browser tracks show RPKM-normalized histograms for γH2Ax ChIP-seq (mean of three independent replicates), Jk interactome 4C-Seq (representative of two independent replicates), and H2Ax ChIP-seq (representative of two independent replicates) for indicated v-abl cell genotypes. The bottom Juicebox snapshot shows Hi-C data derived from G1-arrested Lig4−/− cells (n = 2, merged independent samples). Scale indicates interaction counts. The IGV tracks under the Hi-C data show topologically associated domains (TADs), which are assigned based upon TAD insulation scores, derived from 40 kb bins (blue lines) or 20-100 kb bins (gray lines). Tcrb locus snapshot with data derived from both primary and G1-arrested v-abl cells. The UCSC genome tracks show RPKM-normalized histograms for γH2Ax CR-seq (n = 2, representative of independent replicates), Tcrb-RC viewpoint virtual V.4C-seq (n = 2, merged independent replicates), and H2Ax ChIP-seq (representative of two independent replicates) for indicated Abl cell genotypes. To generate V.4C tracks, 10 kb-binned Hi-C data were extracted for the RC viewpoint and plotted. Bottom: Hi-C Juicebox plot derived from Rag−/− DN thymocytes (n = 2, merged independent samples). The tracks under the Hi-C data show TADs and their insulation scores as in (a). c DN thymocyte Pearson’s correlation of γH2Ax RPKM and interaction counts in 5 kb windows across Tcrb.

Interactomes rather than TADs limit DDR platforms. If γH2Ax propagates when chromatin interacts with a DSB, we reasoned that nearly any RNP targeted to a single self-interacting region (i.e., a TAD) would generate DDR profiles with similar patterns.
Fig. 2 Contact-dependent γH2Ax profiles in a tractable cell model. a Southern blotting analysis for DSBs targeted to the Eβ enhancer in Tcrb. Bands corresponding to uncut (top band) or cut (bottom band) loci are indicated. Genomic DNA was harvested 4 h after Lig4−/− or Rag2−/− v-abl cells were nucleasefected with an RNP targeting Eβ. See Supplementary Fig. 2A for replicates. **b** UCSC genome browser tracks showing γH2Ax CR-seq performed 2 h after Lig4−/− or Rag2−/− v-abl cells were nucleasefected with RNP-Eβ (n = 2, representative of independent replicates). Tcrb gene segments, neighboring genes (red arrows) and the DSB location (lightning bolt) are shown at the top. TAD locations are indicated on bottom. c UCSC genome browser tracks showing the Tcrb loci in pro- (63-12 cell line) or pre-lymphocyte cell lines (p5424). For each panel, the locations of gene segments, regulatory elements, and RNP target (lightning bolt and dashed line) are shown at the top. Tracks represent values for γH2Ax CR-seq (red, RPKM, n = 3, representative of independent replicates). V.4C (gray, interaction count, n = 2 merged independent replicates). In panels with interactome data, V.4C is extracted from the DSB site (dark blue line). c, e include subtraction plots (labeled Δ) for γH2Ax and V.4C data, showing differences in (mean of independent replicates) signal from pro- versus pre-lymphocytes. d Box and whisker plot showing γH2Ax in 25 kb bins after pre- (p5424: gray) or pro- (63-12: gray) lymphocyte cell lines were nucleasefected with an RNP-Eβ. Data points show three biologically independent replicates. Means, quartiles, and outlier limits (1.5 × interquartile range) are indicated by the median line, box and whiskers, respectively. Relative locations are indicated above the graph. *p < 0.05, two-sided Paired Student’s T test. Enrichment and statistics for all bins across the Tcrb locus is shown in Supplementary Fig. 2C. e UCSC genome browser tracks showing the Myc locus, as in (c). Green tracks represent H3K27ac CR-seq (green, RPKM, n = 1). Cell type-specific H3K27ac+ regions near the Myc Super-Enhancer (SE) are indicated by colored dots, showing positions for pre- (black) or pro- (gray) lymphocyte enhancers.

**Discussion**

To test this hypothesis, we computationally defined TADs by measuring insolation scores across a series of 10–200 kb bins (Fig. 3a, b, bottom)35. Next, we targeted DSBs to two distinct locations within the Tcrb-TAD (Vβ30 and Eβ). As shown in Fig. 3a, both lesions produced γH2Ax profiles that paralleled the contours of their interactomes, including large, TAD-TAD gaps, and a sharp γH2Ax boundary at the 5′ TAD border. However, both DSBs also generated a γH2Ax region at a gene cluster in the adjacent 3′ TAD, with which the break sites also formed significant contacts. Thus, in Tcrb, the DSB interactome, rather than strict TAD borders, served as the primary determinant of γH2Ax boundaries. Likewise, introduction of DSBs at either the 5′ (Myc-P) or 3′ (Myc-SE) end of the Myc-TAD generated robust γH2Ax domains that were contact dependent and largely restricted to the Myc-TAD, but also extended into adjacent TADs (Fig. 3b). Strikingly, the two Myc DSBs generated highly asymmetric γH2Ax domains relative to the break sites, which were situated on the extreme ends of the Myc-TAD (i.e., producing one long and one short γH2Ax domain, see “Discussion”).

Importantly, DSBs at sites in topological domains adjacent to either Tcrb (Agk) or Myc (Fam84b and Fam49b), produced γH2Ax domains that were largely restricted to their distinct TADs, but also consistently exhibited inter-TAD deposition of γH2Ax that reflected the DSB interactome (Fig. 3a, b). Persistent DSBs at three randomly chosen genomic locations also generated γH2Ax domains whose termini coincided well with those of their parent interactome, and whose (a)symmetries reflected the DSB location relative to adjacent TAD borders (Supplementary Fig. 4).

We conclude that interactomes are the primary determinants of DDR platforms, and that TAD borders can impede, but may not completely block, the spread of γH2Ax into neighboring domains.

**Deletion of a CTCF motif reduces γH2Ax spread within a TAD.** To directly determine if chromatin interactions, which are often controlled by the architectural protein CTCF, mediate γH2Ax propagation, we used Cas9-RNPs to remove, from the Rag2−/− v-abl line, a CTCF motif positioned only 3 kb from the Myc promoter (5′CTCF KO, Fig. 4a and Supplementary Fig. 5A). We then verified depletion of CTCF binding at the targeted motif by CR-seq (Fig. 4a). Comparison of Hi-C data from WT and 5′ CTCF KO cells revealed reduced contacts throughout the Myc-TAD in the mutant line (Fig. 4b–d, p < 0.05 HiCompare, M test), a conclusion consistent with previous studies using cells lacking this CTCF motif31. Following RNP-induced damage at the Myc promoter, 5′ CTCF KO cells had reduced γH2Ax at distal sites within the Myc-TAD, when compared with WT cells. Indeed, subtraction of γH2Ax or V.4C data revealed comparable shifts between the interactome and γH2Ax profiles (Fig. 4c). To further
compare interactome and chromatin datasets, we quantified sequencing data in 10 kb bins, representing the Hi-C resolution, across the Myc-TAD or neighboring 5′ TAD. We observed consistently less γH2Ax across the KO TAD, correlating well with lower interaction intensities (Fig. 4d). Similar results were observed when we directed a DSB to the super-enhancer (SE) at the 3′ end of the locus (Supplementary Fig. 5B, C). In contrast, neither interactions nor γH2Ax intensities in the KO cells differed significantly for the 5′ TAD, suggesting that removal of this single CTCF site did not remove the topological border. Thus, depletion of the Myc 5′CTCF site perturbs interactions of the promoter and SE within its encompassing TAD, leading to a commensurate reduction of γH2Ax deposition following a DSB.

**DSBs at TAD borders extend γH2Ax domains bidirectionally.**

Our findings with mutants lacking an architectural element in the Myc locus spurred us to test how γH2Ax distribution is impacted when a DSB occurs precisely at a CTCF motif within a topological border. In contrast to CTCF mutant cells, in which a new interactome is formed following deletion of the architectural element (see Fig. 4b), targeting a DSB to the CTCF site itself would examine γH2Ax propagation in cells with wild-type chromosomal contacts. For this purpose, we targeted an RNP to the same CTCF site, which is associated with the Myc 5′ TAD border (Myc-CTCF), using nucleofection of v-abl cells. We then compared γH2Ax between the Myc-CTCF and Myc-P breaks, the latter of which was situated only 3 kb downstream (Fig. 5a).

Strikingly, a DSB occurring within the Myc-CTCF site propagated γH2Ax into both the Myc- and neighboring 5′-TAD (Fig. 5a, b), whereas γH2Ax was largely confined to the Myc-TAD following a break in the promoter. γH2Ax profiles within the Myc-TAD were not significantly different when comparing Myc-P and Myc-CTCF breaks (Fig. 5b, c). Similarly, DSBs at sites located within 40 bp of the CTCF motif (5′ or 3′), potentiated γH2Ax spreading into adjacent TADs, while having little impact on DDR platforms over the Myc-TAD (Supplementary Fig. 6A, B).

We observed similar multi-TAD γH2Ax propagation when additional CTCF sites corresponding to TAD borders were targeted by RNP nucleofection, either at the Tcrb (RNP-SE) or the Rasl10b-TAD (RNP-Rasl10b) (Supplementary Fig. 6C). We conclude that CTCF-containing TAD borders can contribute as γH2Ax insulators for DSBs occurring at sites within a TAD, but lesions at the borders themselves allow for spreading of the DDR platform into two adjacent TADs. This is in sharp contrast to DSBs introduced even a short distance from a TAD border (e.g., at Myc-P or -SE), which generates relatively asymmetric γH2Ax domains, with a short and long DDR platform on each side of the broken chromosome (Model, Supplementary Fig. 6D).

**Discussion**

It has been appreciated for some time that DSBs generate γH2Ax and a DDR platform encompassing a large swath of the neighboring genome, in which repair factors are concentrated and transcription is repressed^{21,36}. We now find that γH2Ax landscapes reflect the basal interactome of a DSB site, whose
CTCF binding motif (representative of three independent results). For subtraction plots of nucleofection. Relative interactome measurements have been normalized using coverage normalization. For all plots, statistical enrichment (two-sided

\textit{Fig. 4 CTCF is necessary for robust γH2Ax propagation in the Myc-TAD. a} CTCF motif deletion within a TAD border upstream of Myc. Top: Sequence of the upstream Myc-CTCF (green) and RNP target sites (gray and red). RNP targets labeled 5' and 3' CTCF were used for deletion. Bottom: Myc locus in 5'CTCF WT or KO pre-lymphocyte cells (63-12). Myc exons and UTRs are shown (top). Tracks represent CTCF CR-seq values (black, RPKM, \(n = 1\)) and the arrow indicates the CTCF motif. \textit{b} Interactome of 5'CTCF KO cells. Top left insert shows CTCF ChIP-seq data (0-60 RPKM, \(n = 1\)), CTCF motif orientation (blue arrows), TAD border, and deletion site (red X). Bottom: JuiceBox Hi-C plots show the wild-type Myc locus (left) or relative Hi-C interactome changes following CTCF deletion (right) \((n = 1,\) each). Blue points are \(\geq 2\) interaction counts higher in WT; red points are \(\geq 2\) counts higher in the 5'CTCF KO. The location of the Myc-TAD is indicated on bottom. \textit{c} UCSC snapshot, as described in Fig. 2, showing the Myc locus in 63-12 cells with and without the 5’ CTCF binding motif (representative of three independent results). For subtraction plots of γH2Ax or V.4C data, red represents WT enrichment, while purple represents KO enrichment. \textit{d} Myc promoter interactome and γH2Ax derived from 5'CTCF WT or KO cell lines, quantified in 10 kb bins across either the Myc-containing TAD or immediate 5' TAD. Left: γH2Ax 2 h after 5'CTCF WT or KO cells were nucleofected with RNP Myc-P. Each γH2Ax dataset was first normalized to RPKM values at the DSB-containing bin. Colors represent biologically independent replicates \((n = 3)\). Right: Comparison of Myc promoter interactomes, derived from Hi-C data, across the Myc-containing TAD or immediate 5' TAD in cells of indicated genotypes, without RNP nucleofection. Relative interactome measurements have been normalized using coverage normalization. For all plots, statistical enrichment (two-sided Paired Student’s t test) is shown. Non-significant (ns) \(p\) values are 0.6, 0.27, and 0.26 for replicates 1, 2, and 3, respectively. Means, quartiles, and outlier limits (1.5 × interquartile range) are indicated by the median line, box and whiskers, respectively.

boundaries often, but do not always, correspond to those of its native TAD. In this regard, TAD boundaries are defined statistically using relative insolation scores; thus, a called border does not absolutely exclude interactions between neighboring compartments, as observed, for example, at Tcrb. Indeed, recent high-resolution microscopy has visualized γH2Ax and 53BP1 foci as single and multi-TAD rings surrounding their DSB. Mechanistically, chromatin contacts might compartmentalize the spread of γH2Ax, spatially concentrating DDR repair factors, perhaps via 53BP1-dependent phase separation. The spatially-defined DMR compartments may simultaneously restrict separation of DSB ends to facilitate their efficient re-ligation. The contact-dependent nature of γH2Ax domains suggests that recruitment of ATM and DNA-PKc may be restricted to a DSB-proximal region, which then phosphorylates H2Ax via physical contacts, rather than by linear propagation along the chromosome. The contact-dependent model (Supplementary Fig. 6D) would also explain contours we observed in DDR platforms; certain regions within a domain would remain free of γH2Ax due to limited interactions with the site that suffered damage. Therefore, although TADs define the same γH2Ax borders in a wide array of cell types, the profiles of this modification within DDR platforms will differ, depending on the actual DSB site and the cell type-specific patterns of chromosomal contacts.

Another important finding of our study is that DSBs can generate γH2Ax domains of widely divergent symmetries. We find that γH2Ax spreads symmetrically from breaks incurred near the center of a TAD, whereas a DSB near a TAD border leads to one short and one long DDR platform. This is in sharp contrast to our finding that DSBs at TAD borders generate long γH2Ax domains that spread throughout the two adjacent TADs, which often alternate between euchromatic and heterochromatic states. Prevailing evidence indicates that DSBs occurring in euchromatic regions favor end resection and HR, while heterochromatin favors NHEJ pathways. It remains unclear how the hybrid γH2Ax domains formed by DSBs at TAD borders would direct repair; however, we would point out that many cancer types are characterized by TAD border mutations.

Aside from damage at CTCF-containing TAD borders, wherein γH2Ax propagation becomes bidirectional, we find that the distribution of this DSB-induced modification largely reflects the pre-DSB interactome. Indeed, interactions observed in intact chromosomes are modestly enhanced throughout γH2Ax domains following a persistent DSB (see Supplementary Fig. 3).
This observation suggests that mechanisms involved in establishing chromosomal contacts are preserved, even in persistently damaged loci. One such mechanism is loop extrusion, during which cohesin drives the formation of progressively larger chromatin loops until it stalls at TAD boundaries or convergent CTCF sites. Indeed, loop extrusion is likely active on severed alleles during immunoglobulin class switch recombination, wherein it is required to align switch regions. Conversely, defects in extrusion, or the TAD architecture, may compromise normal DDR signaling, a hypothesis that remains to be tested. A remaining unknown, however, is how the loop extrusion mechanism following a DSB could generate domains with large γH2A.X voids, which were observed at multiple loci in our study.

Given that γH2A.X is the platform for break stabilization, likely via mechanisms involving 53BP1 retention, we predict that the location of damage relative to a TAD border may also contribute to the stability of a broken chromosome. DSBs close to, but not at a TAD border (i.e., the Myc promoter), may have reduced stability due to highly asymmetric γH2A.X domains. Indeed, DSBs in this border-proximal Myc region are involved in chromosomal translocations associated with lymphocytic malignancies. Moreover, structural variants, especially those resulting from chromothripsis, are enriched at TAD borders in many types of cancer. TAD-proximal DSBs may leave one chromosome end relatively unprotected by 53BP1, especially when the break persists, which could lead to extensive end resection and/or drifting of the two chromosome fragments. Importantly, sites lying close to topological borders are common sources of damage, especially when the relief of ongoing torsional stress in chromosomes is inhibited. Such a scenario occurs when topoisomerase poisons are employed as chemotherapeutics, and can lead to therapy-associated leukemia. Thus, contact-driven mechanisms for generating DDR platforms are likely to be critical determinants of genome integrity in the wake of natural or agent-induced DSBs.

**Methods**

**Mouse models.** Bcl2−/−;Igh−/− Rag−/−, and Artemis−/− mouse strains have been described previously. DN thymocytes and CD19− prec-B cells were isolated from 6-week-old animals, enriched for lymphocytes using an Ammonium–Chloride–Potassium lysing buffer (Thermo Fisher), and selected with microbeads (Miltenyi Biotec), based upon established protocols. All animal studies were reviewed and approved by the Washington University Animal Review Board.

**Cell lines.** V-abl pro B cell lines 63–12, Ligi−/− and Ligi−/−FOK1-ZFN were created previously. For creation of 5′CTCF KO cells, 63–12 cells were nucleofected with RNP-Myc-P or RNP-CTCF, quantified in 10 kb bins across either the Myc-containing TAD or immediate 5′ TAD. Each γH2A.X dataset was first normalized to RPKM values at the DSB-containing bin. Colors represent independent replicates. Statistical enrichment (two-sided Paired Student’s t test) is shown above (ns non-significant). Means, quartiles, and outlier limits (1.5 × interquartile range) are indicated by the median line, box and whiskers, respectively.
Cut and Run-Seq. For Cut and Run-Seq 500 k cells were processed as described28,53. Cells were eluted using a sodium bicarbonate buffer at 55 °C and purified using a PCR cleanup kit (EZbioresearch M1001). All antibody incubations and digestion steps were performed for 5 min at room temperature and included protease, phosphatase and deacetylase inhibitors (Roche).

Hi-C SEQ. Hi-C was performed as follows, based upon in-situ protocols35, Briefly, 5 × 10⁶ formaldehyde-cross-linked cells were lysed on ice for 15 min with 250 µl of ice-cold Hi-C lysis buffer (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 0.2% Igepal CA630) containing protease inhibitors (Roche). Chromatin was digested using DpnII (100 U) at 37 °C for 6 h. The digested DNA ends were then filled and marked with biotin using Klenow, followed by ligation with T4 DNA ligase. After reversing the cross-links, DNA was fragmented using a Covaris E220 Evolution Sonicator followed by size-selection for 300–500 bp using AMPure XP Beads (Beckman Coulter). DNA end repair was performed using NEBNext Ultra II DNA Library Prep Kit according to the manufacturer’s instructions using 1 µg of the Hi-C DNA. Adapter-ligated DNA was then selected for 300–400 bp using AMPure XP beads and the biotinylated DNA fragments were pulled down using MyOne Streptavidin T1 beads (Life Technologies). The final Hi-C library was generated with 5 PCR cycles using the NEBNext Ultra II DNA Library Prep Kit and NEBNext Dual Index primers (NEB) for Illumina sequencing.

Sequencing and analysis. Libraries were constructed as described35. Finished libraries were sequenced using Illumina HiSeq2000 instrument (Hi-C, CR- and 4C-seq 50-bp single-end) or NovaSeq S1 instrument (Hi-C: 101-bp paired-end; 500M-1B reads). Unique reads were aligned to the reference build (GTCm38/ mm9) using TopHat and Bowtie2. RPMK values were obtained using Deeptools56. Hi-C Reads were processed using Juicer pipeline57 and visualized using JuiceBox.HiC files were extracted, and 25 kb binned using straw, then uploaded to R and processed for significance with the HiCompare pipeline base settings. Pearson’s correlations were generated using the UCSC genome browser Table Browser Correlate function, using settings for 25 kb bins and a 5 Mb interval surrounding the DSB site. Fisher’s Exact Tests were performed on TAD and yH2Ax peak files using bedtools Fisher. TADs and TAD insulation scores were called using HiExplorer’s hicFindTADs55 function with 20–200 kb bins, with the following settings:—threshold:Comparisons 0.01—delta 0.2. Peak files were generated using HOMER’s CallPeaks-histone, with a non-targeting guide as the input setting.

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

Data availability
Next-generation sequencing data are available at the Gene Expression Omnibus under accession number GSE150384. All other data are available from the authors upon reasonable request.

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References
1. Tübs, A., Nussenzweig, A. & Endogenous DNA damage as a source of genomic instability in cancer. Cell 168, 644–656 (2017).
2. Jackson, S. P. & Bartek, J. The DNA-damage response in human biology and disease. Nature 461, 1071–1078 (2009).
3. Matsuoka, S. et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science 316, 1160–1166 (2007).
4. Bonner, W. M. et al. H2AX and cancer. Nat. Rev. Cancer 8, 957–967 (2008).
5. Rogakou, E. P., Püch, D. R., Orr, A. H., Ivanova, V. S. & Bonner, W. M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J. Biol. Chem. 273, 5858–5868 (1998).
6. Scully, R. & Xie, A. Double strand break repair functions of histone H2AX. Nat. Rev. Fundam. Mol. Mech. Histogen. 730, 5–14 (2013).
7. Shen, B. R. et al. XLF and H2AX function in series to promote replication fork stability. J. Cell Biol. 218, 2113–2123 (2019).
8. Helmink, B. A. et al. H2AX prevents CIP-mediated DNA end resection and aberrant repair in G1-phase lymphocytes. Nature 469, 245–249 (2011).
9. Xie, A. et al. Control of sister chromatid recombination by histone H2AX. Mol. Cell 66, 1017–1025 (2014).
10. Bassing, C. H. et al. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. Proc. Natl Acad. Sci. USA 99, 8173–8178 (2002).
11. Yin, B. et al. Histone H2AX stabilizes broken DNA strands to suppress chromosome breaks and translocations during V(DJ) recombination. J. Exp. Med. 206, 2625–2639 (2009).
12. Bassing, C. H. et al. Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. Cell 114, 359–370 (2003).
13. Céleste, A. et al. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. Cell 114, 371–383 (2003).
14. Stocki, M. et al. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell 123, 1213–1226 (2005).
15. Iacovoni, J. S. et al. High-resolution profiling of gammaH2AX around DNA double-strand breaks in the mammalian genome. EMBO J. 29, 1446–1457 (2010).
16. Cloarec, T. et al. Comprehensive mapping of histone modifications at DNA double-strand breaks decipher repair pathway chromatin signatures. Mol. Cell 72, 250–262.e6 (2018).
17. Savic, V. et al. Formation of dynamic γH2AX domains along broken DNA strands is distinctly regulated by ATM and MDC1 and dependent upon H2AX densities in chromatin. Mol. Cell 34, 298–310 (2009).
18. Nistala, F. et al. Identification of the elementary structural units of the DNA damage response. Nat. Commun. 8, 15760 (2017).
19. Mojmard, A. et al. Nej1 interacts with Mre11 to regulate tethering and Dna2 binding at DNA double-strand breaks. Cell Rep. 28, 1564–1573.e3 (2019).
20. Shanbhag, N. M., Rafalska-Metcalf, I. U., Balane-Bolivar, C., Janicki, S. M. & Greenberg, R. A. ATM-dependent chromatin changes silence transcription in cis to dna double-strand breaks. Cell 141, 970–981 (2010).
21. Purman, C. E. et al. Regional gene repression by DNA double-strand breaks in G1 phase cells. Mol. Cell Biol. 39, e00181-19 (2019).
22. Ochs, F. et al. Stabilization of chromatin topology safeguards genome integrity. Nature 574, 571–574 (2019).
23. Dixon, J. R. et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485**, 376–380 (2012).
24. Bredemeyer, A. L. et al. ATM stabilizes DNA double-strand-break complexes during V(D)J recombination. *Nature* **442**, 466–470 (2006).
25. Lee, R.-S. et al. Functional intersection of ATM and DNA-dependent protein kinase catalytic subunit in coding end joining during V(D)J recombination. *Mol. Cell. Biol.* **33**, 3568–3579 (2013).
26. Strasser, A., Harris, A. W. & Cory, S. bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. *Cell* **67**, 889–899 (1991).
27. Rooney, S. et al. Leaky Scid phenotype associated with defective V(D)J coding end processing in Artemis-deficient mice. *Mol. Cell* **10**, 1379–1390 (2002).
28. Skene, P. J., Henikoff, J. G. & Henikoff, S. Targeted in situ genome-wide profiling with high efficiency for low cell numbers. *Nat. Protoc.* **13**, 1006–1019 (2018).
29. Majumder, K. et al. Lineage-specific compaction of Tcer requires a chromatin barrier to protect the function of a long-range tethering element. *J. Exp. Med.* **212**, 107–120 (2015).
30. Majumder, K. et al. Domain-specific and stage-intrinsic changes in Tcer conformation during thymocyte development. *J. Immunol.* **195**, 1262–1272 (2015).
31. Schuijers, J. et al. Transcriptional dysregulation of MYC reveals common enhancer-docking mechanism. *Cell Rep.* **23**, 349–360 (2018).
32. Bahr, C. et al. A Myc enhancer cluster regulates normal and leukemic haematopoietic stem cell hierarchies. *Nature* **553**, 515–520 (2018).
33. Sanchez, O. & Herranz, D. The MYC enhancer-ome: long-range transcriptional regulation of MYC in cancer. *Trends Cancer* **4**, 810–822 (2018).
34. Aymard, F. et al. Genome-wide mapping of long-range contacts unveils clustering of DNA double-strand breaks at damaged active genes. *Nat. Struct. Mol. Biol.* **24**, 353–361 (2017).
35. Ramírez, F. et al. High-resolution TADs reveal DNA sequences underlying genome organization in flies. *Nat. Commun.* **9**, 189 (2018).
36. Fernandez-Capetillo, O. et al. H2AX is required for chromatin remodeling and DNA repair compartments. *EMBO J.* **38**, e10379 (2019).
37. Caron, P. et al. Non-redundant functions of ATM and DNA-PKcs in response to DNA double-strand breaks. *Cell Rep.* **13**, 1598–1609 (2015).
38. Baldyroy, C., Soria, G., Roche, D., Cook, A. J. L. & Almouzni, G. HP1alpha recruitment to DNA damage by p53CAF-I promotes homologous recombination repair. *J. Cell Biol.* **193**, 81–95 (2011).
39. Aymard, F. et al. Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. *Nat. Struct. Mol. Biol.* **21**, 366–374 (2014).
40. Rivera-Reyes, A., Haye, K. E. & Bassing, C. H. Genomic alterations of non-coding regions underlie human cancer: lessons from T-ALL. *Trends Mol. Med.* **22**, 1035–1046 (2016).
41. Fudenberg, G. et al. Formation of chromosomal domains by loop extrusion. *Cell Rep.* **15**, 2038–2049 (2016).
42. Zhang, X. et al. Fundamental roles of chromatin loop extrusion in antibody class switching. *Nature* **575**, 385–389 (2019).
43. Hnisz, D. et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. *Science* **351**, 1454–1458 (2016).
44. Akdemir, K. C. et al. Disruption of chromatin folding domains by somatic genomic rearrangements in human cancer. *Nat. Genet.* **52**, 294–305 (2020).
45. Noordermeer, S. M. et al. The shieldin complex mediates 53BP1-dependent DNA repair. *Nature* **560**, 117–121 (2018).
46. Lottersberger, F., Boitnour, A., Robbiani, D. F., Nussenzweig, M. C. & de Lange, T. Role of 53BP1 oligomerization in regulating double-strand break repair. *Proc. Natl Acad. Sci. USA* **110**, 2146–2151 (2013).
47. Gothe, H. J. et al. Spatial chromosome folding and active transcription drive DNA fragility and formation of oncogenic MLL translocations. *Mol. Cell* **75**, 287–283.e12 (2019).
48. Canala, A. et al. Topoisomerase II-induced chromosome breakage and translocation is determined by chromosome architecture and transcriptional activity. *Mol. Cell* **75**, 252–266.e8 (2019).
49. Bednarski, J. J. et al. RAG-induced DNA double-strand breaks signal through Pim2 to promote pre-B cell survival and limit proliferation. *J. Exp. Med.* **209**, 11–17 (2012).
50. Mombaerts, P., Terhorst, C., Jacks, T., Tonegawa, S. & Sancho, J. Characterization of immature thymocyte lines derived from T-cell receptor or recombination activating gene 1 and p53 double mutant mice. *Proc. Natl Acad. Sci. USA* **92**, 7420–7424 (1995).
51. Chichaybam, L. et al. An efficient electroporation protocol for the genetic modification of mammalian cells. *Front. Bioeng. Biotechnol.* **4**, 99 (2016).
52. Cella, M. et al. Subsets of ILC3-ILC1-like cells generate a diversity spectrum of innate lymphoid cells in human mucosal tissues. *Nat. Immunol.* **20**, 980–991 (2019).
53. Rao, S. S. P. et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665–1680 (2014).
54. Koues, O. I. et al. Distinct gene regulatory pathways for human innate versus adaptive lymphoid cells. *Cell* **165**, 1134–1146 (2016).
55. Ramírez, F. et al. deepTool2: a next generation web server for deep-sequence data analysis. *Nucleic Acids Res.* **44**, W160–W165 (2016).
56. Durand, N. C. et al. Juicebox provides a visualization system for Hi-C contact maps with unlimited zoom. *Cell Syst.* **3**, 99–101 (2016).
57. Stansfield, J. C., Cresswell, K. G., Vladimirov, V. I. & Dozmorov, M. G. HiCompare: an R-package for joint normalization and comparison of Hi-C datasets. *BMC Bioinformatics* **19**, 279 (2018).