CTCF orchestrates long-range cohesin-driven V(D)J recombinational scanning

Zhaoqing Ba1*, Jiangman Lou1*, Edward W. Dring1, Adam Yongxin Ye1, Sherry G. Lin1, Suvi Jain1, Kyong-Rim Kieffer-Kwon2, Rafael Casellas2#, Frederick W. Alt1#

1Howard Hughes Medical Institute, Program in Cellular and Molecular Medicine, Boston Children’s Hospital, and Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
2Lymphocyte Nuclear Biology, NIAMS, NIH, and Center of Cancer Research, NCI, NIH, Bethesda, MD 20892, USA.

*These authors contributed equally

#Correspondence
alt@enders.tch.harvard.edu
rafael.casellas@nih.gov
Abstract

RAG endonuclease initiates V(D)J recombination in progenitor (pro)-B cells\(^1\). Upon binding a recombination center (RC)-based J\(_H\), RAG scans upstream chromatin via loop extrusion, potentially mediated by cohesin\(^2\)-\(^{10}\), to locate Ds and assemble a DJ\(_H\)-based RC\(^11\). CTCF looping factor-bound elements (CBEs) within IGCR1 upstream of Ds impede RAG-scanning\(^{12,15}\); but their inactivation allows scanning to proximal V\(_H\)s where additional CBEs activate rearrangement and impede scanning any further upstream\(^ {15,16}\). Distal V\(_H\) utilization is thought to involve diffusional RC access following large-scale Igh locus contraction\(^ {17-23}\). Here, we test the potential of linear RAG-scanning to mediate distal V\(_H\) usage in G1-arrested, v-Abl-pro-B cell lines\(^ {24,25}\), which undergo robust D-to-J\(_H\) but little V\(_H\)-to-DJ\(_H\) rearrangements, presumably due to lack of locus contraction\(^ {11,15}\). Through an auxin-inducible approach\(^ {26,27}\), we degrade the cohesin-component Rad21\(^ {4,7,27}\) or CTCF\(^ {7,9}\) in these G1-arrested lines, which maintain substantial viability throughout four-day experiments. Rad21 degradation eliminated all V(D)J recombination and RAG-scanning-associated interactions, except RC-located DQ52-to-J\(_H\) joining in which synopsis occurs by diffusion\(^ {11}\). Remarkably, while CTCF degradation suppressed most CBE-based chromatin interactions, it promoted robust RC interactions with, and robust V\(_H\)-to-DJ\(_H\) joining of, distal V\(_H\)s, with patterns similar to those of “ locus-contracted” primary pro-B cells. Thus, down-modulation of CTCF-bound scanning-impediment activity promotes cohesin-driven RAG-scanning across the 2.7Mb Igh locus.

Cohesin is a highly conserved chromosome-associated multi-subunit ring-shaped ATPase complex built upon subunits belonging to the structural maintenance of chromosomes (Smc) family and is important for diverse chromosome-based processes\(^ {28-33}\). The cohesin complex is proposed to form contact loop domains by extrusion of chromatin until reaching convergent CTCF-bound CBE anchors\(^ {2-10}\). In this regard, cohesin extrudes both naked and nucleosome-containing DNA in an NIPBL-MAU2 and ATP-hydrolysis-dependent manner in vitro\(^ {34,35}\). Our studies implicated the cohesin complex in chromatin loop extrusion-mediated mechanisms of lymphocyte V(D)J and IgH class switch recombination\(^ {11,14-16,36,37}\). To further elucidate potential cohesin role(s) in chromatin scanning during long-range V(D)J recombination, we employed a mini auxin-inducible degron (mAID) approach\(^ {4,7,26,27}\) to conditionally degrade Rad21, a core component of the cohesin complex, in v-Abl-kinase-transformed, E\(_\mu\)-Bcl2-expressing mouse pro-B cells (“v-Abl pro-B cells”).
These cells can be viably arrested in the G1 cell-cycle stage by treatment with Abl kinase inhibitor (STI-571), activating RAG1/2 endonuclease and V(D)J recombination\textsuperscript{24,25}. We targeted the last codon of both mouse $\text{Rad21}$ alleles to introduce in-frame sequences that encode mAID and a green fluorescent protein (GFP) marker in a RAG2-deficient $\nu$-$\text{Abl}$ pro-B line (Extended Data Fig. 1a,c). We then targeted a transgene into the $\text{Rosa26}$ locus that over-expresses the OsTir1-V5 protein that binds mAID in the presence of auxin (Indole-3-acetic acid, IAA) to trigger proteasome-dependent degradation of the mAID-fused $\text{Rad21}$ protein (Extended Data Fig. 1a-d). The resulting RAG2-deficient line is referred to as the "Rad21-degron $\nu$-$\text{Abl}$ pro-B line".

To test effects of cohesin depletion on $\text{Igh}$ loop domain formation, we split $\text{Rad21}$-degron $\nu$-$\text{Abl}$ cells into two sub-populations, one non-treated ("NT") and the other treated with IAA for 6 hours and then with STI-571 for 4 days to induce G1 arrest (Extended Data Fig. 1b,f). Treatment of $\text{Rad21}$-degron $\nu$-$\text{Abl}$ cells with IAA led to depletion of mAID-GFP-tagged $\text{Rad21}$ to background levels by 6 hours, and ChiP-seq confirmed nearly complete depletion of chromatin-bound $\text{Rad21}$ at known cohesin-occupancy sites across $\text{Igh}$\textsuperscript{11,15,17,38,39} (Fig. 1a,b, Extended Data Fig. 2a). While there was cell loss in the initial day while cells were cycling, cohesin-depleted $\text{Rad21}$-degron $\nu$-$\text{Abl}$ pro-B cells underwent normal STI-571-induced G1 arrest (Extended Data Fig. 1e,g), and showed no decrease in viability throughout the 4-day experimental period (Extended Data Fig. 1e). We applied the sensitive chromatin interaction 3C-HTGTS assay\textsuperscript{15} to G1-arrested cells at day 4, and found that cohesin loss essentially abrogated $\text{Igh}$ chromatin loop domains mediated by the proximal $\text{V}_{\text{H}}$81X-CBE\textsuperscript{15} or by $\text{Igh}$ intronic enhancer (iE\textsubscript{H})/RC\textsuperscript{11,15}, with the exception of interaction of RC/iE\textsubscript{H} with the closely associated, diffusion-accessible locale that harbors the infrequently rearranged (due to its poor RSS) DST4.1/D\textsubscript{H}3-2\textsuperscript{11} (Figure 1c, Extended Data Fig. 2b,c). Similar to mutational inactivation of the $\text{V}_{\text{H}}$81X-CBE\textsuperscript{15}, $\text{Rad21}$ degradation completely eliminated proximal $\text{V}_{\text{H}}$81X interaction with downstream $\text{Igh}$ sequences, including the non-CBE-based RC.

To test effects on $\text{Igh}$ V(D)J recombination, we introduced RAG2 into $\text{Rad21}$-degron $\nu$-$\text{Abl}$ pro-B cells, with or without IAA treatment to induce mAID-GFP-tagged $\text{Rad21}$ depletion, and treated both with STI-571 to induce G1 arrest and activation of V(D)J recombination (Extended Data Fig.1f). After four days, we applied HTGTS-V(D)J-seq\textsuperscript{11,40,41} to NT and treated cells using a J\textsubscript{H}4 primer as bait\textsuperscript{15} to analyze both D-to-J\textsubscript{H} and $\text{V}_{\text{H}}$-to-DJ\textsubscript{H} junctions (Fig. 1a,d). Similar to V(D)J joining patterns in other $\nu$-$\text{Abl}$ lines, the NT $\text{Rad21}$-
degron v-Abl pro-B cells displayed robust D-to-JH rearrangements, with the JH-distal DFL16.1 having highest rearrangement frequency and JH-proximal DQ52 an intermediate frequency\(^\text{11}\) (Fig. 1d, right panel, Supplementary Table 1). We also observed low-level rearrangement of the most proximal VHs and little or no rearrangement of distal VHs\(^\text{11,14,15}\) (Fig.1d, left panel, Supplementary Table 1). Strikingly, Rad21 depletion abolished all VH rearrangements and largely eliminated all D rearrangements, except that of the JH-proximal DQ52, which retained substantial rearrangement levels (Fig. 1d, Supplementary Table 1). In additional studies, we found Rad21 degradation abrogated robust proximal VH utilization on IGCR1-deleted alleles along with nearly complete abrogation of all D rearrangements except, again, that of proximal DQ52, which occurred at nearly 70% of control levels (Fig. 1e, Supplementary Table 1). Consistent with impeded loop extrusion, Rad21 degradation also abrogated the greatly robust IγH RC interaction with proximal VHs in the absence of IGCR1, but had little effect on RC interactions with the diffusion-accessible D2;3-2 locale (Extended Data Fig. 2d).

The substantial level of residual DQ52 rearrangement in the various RAG-sufficient, Rad21-depleted v-Abl pro-B lines provides an internal control for maintenance of RAG activity and integrity of a JH-based RC following cohesin depletion, as DQ52 is known to substantially access RC-bound RAG via diffusion due to its RC-based location\(^\text{11}\). To further confirm integrity of the IγH RC, we performed GRO-seq on G1-arrested Rad21-degron cells, which confirmed robust sense and anti-sense transcription through the RC before and after cohesin degradation (Extended Data Fig. 3a). Furthermore, while Rad21-depletion led to expected subtle genome-wide transcriptional changes\(^\text{4}\) (Extended Data Fig. 3b), we detected no substantial differences in transcription levels of several genes involved in V(D)J recombination per se or in chromosomal regulation of V(D)J recombination (Examples highlighted in Extended Data Fig. 3b). Taken together, our findings strongly implicate cohesin-mediated loop extrusion in moving chromatin past RC-bound RAG for scanning during IγH V(D)J recombination.

How the 100-plus VHs embedded across the 2.4Mb VH domain gain access to the DJH RC has been a major question. In this regard, there are over 100 CBEs across this domain in convergent orientation with the upstream IGCR1 CBE and 10 CBEs ("3’CBEs") downstream of IγH\(^\text{12,13,17,21,22,42}\). While most of these CBEs are not physically adjacent to VHs, several dozen D-proximal VHs have CBEs directly downstream that promote associated VH utilization through interactions with the DJH RC during RAG scanning\(^\text{15,16}\). In addition to
IGCR1 CBEs, these proximal V_H CBEs are barriers to direct RAG scanning to more distal V_Hs\cite{15}. Distal V_H utilization might circumvent CBE linear scanning barriers through a physical locus contraction process that brings all V_Hs into proximity with the DJ_H RC for diffusional access\cite{17-23}. On the other hand, extrusion-mediated RAG scanning could, in theory, also contribute to distal V_H utilization if CBE impediments were neutralized or circumvented\cite{15,16}. To test the latter hypothesis, we asked whether we could activate RAG scanning to distal V_Hs in G1-arrested \(\nu\)-Abl pro-B cells by down-regulating CTCF scanning barriers across the locus. For this purpose, we generated a CTCF degron line from the parental RAG2-deficient \(\nu\)-Abl line using a similar approach to that used for establishing the Rad21-degron line (Extended Data Fig. 4a-e). We then followed the same experimental protocol used to study the Rad21-degron line.

Treatment of CTCF-degron \(\nu\)-Abl cells with IAA rapidly led to depletion of mAID-GFP-tagged CTCF to background levels by 6 hours, which were maintained throughout the course of the subsequent 4-day experiment (Extended data Figure 4b). Treatment with STI-571 induced normal G1 arrest of both non-treated and treated CTCF-degron \(\nu\)-Abl cells (Extended Data Fig. 4f). However, CTCF degradation moderately impacted viability of treated G1-arrested, CTCF-degron \(\nu\)-Abl cells, which decreased to approximately 55% of NT or parental control cells over the 4-day experimental course (Extended Data Fig. 4g). We also found that the non-treated CTCF-degron \(\nu\)-Abl cells had a modestly leaky phenotype for CTCF degradation\cite{7,9}, which provided additional insights (Extended Data Fig. 5 and legend). However, to simplify presentation of our findings, we focus here on description of the striking results obtained from comparison of the parental \(\nu\)-Abl cells and IAA-treated CTCF-degron \(\nu\)-Abl lines derived from them.

To characterize effects of CTCF degradation, we performed ChIP-seq for CTCF- and Rad21-binding across the \(lgh\) of G1-arrested parental and treated CTCF-degron \(\nu\)-Abl cells (Fig. 2a,b, Extended Data Fig. 6a). In parental \(\nu\)-Abl cells, there was a high density of co-localized CTCF and Rad21 peaks throughout the V_H locus, with more robust binding in the distal region\cite{17,38}. In treated CTCF-degron \(\nu\)-Abl cells, CTCF occupancy across \(lgh\) was greatly diminished; but residual binding\cite{9,17} was retained at certain CBEs in the distal region (e.g. V_H1-67, V_H1-11), as well as CBEs associated with proximal V_H5-6, V_H2-3, and CBE1 of IGCR1 (Fig. 2b, Extended Data Fig. 6a). Notably, CTCF binding increased at the RC upon CTCF depletion (Fig. 2b, Extended Data Fig. 6a). While increases in apparent CTCF-binding to the non-CBE-containing RC upon CTCF depletion could be considered surprising, it may
likely occur indirectly due to cohesin-mediated loop extrusion-driven dynamic associations of the RC sub-loop anchor with residual-CTCF-bound CBEs across Igh. The, apparently, uneven depletion of CTCF at various CBEs may reflect differential intrinsic CTCF-binding affinity, local chromatin context and/or other factors. Upon CTCF depletion, Rad21 occupancy throughout Igh was also greatly diminished, with residual binding sites and levels largely reflecting those of CTCF (Fig. 2b, Extended Data Fig. 6a). Residual CTCF binding upon CTCF depletion likely reflects average states of depletion of different cells in the population of G1-arrested CTCF-degron v-Abl cells, with the latter also being influenced by loss of particular cells in which CTCF depletion reaches a critical level for viability. Therefore, occurrence of continuous sub-populations of G1-arrested, CTCF-degron v-Abl cells in which residual CTCF maintains viability implies that such cells may also maintain RAG-scanning in the face of variable CTCF depletion across Igh. If so, this would further predict increased RC scanning anchor interactions with the distal V_{H} locus and increased utilization of distal V_{H}S.

GRO-seq confirmed that transcription of the RC and distal V_{H}S occurred similarly in parental and treated CTCF-degron v-Abl lines (Fig. 2c, Extended Data Fig.6b), predicting an intact RC and accessibility of distal V_{H}S if distal scanning reached them. Correspondingly, IAA-treated, G1-arrested CTCF-degron v-Abl lines had a dramatic increase in utilization of many V_{H}S upstream of proximal V_{H}81X and V_{H}2-2, including transcribed distal V_{H}S that lie several Mb upstream (Fig. 3a-c, Supplementary Table 2). Remarkably, the patterns of V_{H} rearrangements in CTCF-degraded v-Abl cells were reminiscent of those in "locus-contracted" bone marrow (BM) pro-B cells in terms of similar highly rearranging V_{H} clusters and comparable levels of some distal V_{H} rearrangements (Fig. 3c,d, Supplementary Table 2). Moreover, compared to parental v-Abl cells, the V_{H}DJ_{H} to DJ_{H} ratio in CTCF-degraded v-Abl cells reached that of BM pro-B cells, confirming that CTCF deletion greatly increased the overall rate of V_{H} to DJ_{H} joining by permitting relatively unimpeded far upstream scanning for diverse V_{H} rearrangements to DJ_{H} RCs (Fig. 3). Utilization of the most proximal functional V_{H}S (V_{H}81X and V_{H}2-2) decreased relative to that of IGCR1-inactivated normal G1-arrested v-Abl cells or even that of normal pro-B cells. This latter finding may reflect CTCF-depletion dampening at V_{H}81X- and V_{H}2-2-associated CBEs, as well as at IGCR1 CBEs (Fig. 2b, Extended Data Fig. 6a). Taken together, these results confirm the hypothesis that accessibility of distal V_{H}S to RC-based RAG is greatly enhanced by broad diminution of CTCF-based scanning impediments across Igh.

3C-HTGTS from an RC/iE_{H} bait on G1-arrested parental v-Abl cells confirmed lack of
interaction with the more distal $V_H$s (Fig. 4a, b, top panel). Remarkably, in treated CTCF-degron $\nu$-$Abl$ cells, the RC/iE4 was gained robust interactions with sequences across the entire $V_H$ locus, including the most distal $V_H$s, with patterns reminiscent of those in normal locus-contracted BM pro-B cells (Fig. 4b, middle and bottom panels). We confirmed these chromatin interaction analyses with other RC-based baits (Extended Data Fig. 7a-c).

Notably, many $V_H$s near prominent RC/iE4-interacting peaks corresponded to those with greatly enhanced utilization (Fig. 3c, 4b). In addition, we observed that, compared to parental $\nu$-$Abl$ cells, the RC retained interactions with IGCR1, had diminished interactions with $V_H$81X- and $V_H$2-2-associated CBEs, and had increased interactions with proximal $V_H$2-3 and $V_H$5-6 in CTCF-degraded $\nu$-$Abl$ cells (Fig. 4b, Extended Data Fig. 7b, c, top two panels). These chromatin interaction changes corresponded well to proximal $V_H$ utilization changes (Fig. 3d, c). Together with our finding of CTCF degradation-dependent enhancement of distal $V_H$ rearrangements, our chromatin interaction findings indicate that diminution of CTCF-based scanning impediments allows linear interaction by the RC and, correspondingly, RC-bound RAG, to proceed across the entire 2.7Mb $IgH$ $V_H$ locus, likely to different distances in individual cells in the population.

Our cohesin-depletion studies in G1-arrested $\nu$-$Abl$ pro-B cells strongly support cohesin-mediated loop extrusion as a major driver in moving chromatin past RC-bound RAG for $IgH$ V(D)J recombinational scanning. Moreover, our studies show that RAG scanning can progress linearly across the entire $V_H$ locus upon down-modulation of CBE impediment activity allowing other local scanning impediments (e.g. transcription, Fig. 2c, Extended Data Fig. 6b)\textsuperscript{11,37,46} to mediate increased access to particular sets of $V_H$s (Fig. 4, Extended Data Fig. 7, 8 and legend). Indeed, CTCF-degradation actually increased the overall level of $V_H$-to-$DJ_H$ rearrangements, including those of distal $V_H$s, in $\nu$-$Abl$ pro-B lines to levels similar to those found in primary pro-B cells (Fig. 3). While CTCF depletion provides proof of principle that linear RAG scanning can access to distal $V_H$s, physiological modulation of scanning activity may involve mechanisms that down-regulate CBE impediments and/or mechanisms that circumvent impediments by modulating cohesin activity. One such possibility would be modulating activity of the WAPL cohesin unloader, given that WAPL depletion extends length of CBE-based loops\textsuperscript{6,7}. Thus, mechanisms that, directly or indirectly, impact CTCF-bound CBE scanning impediments during early B cell development could contribute to IgH variable region repertoire diversification via linear scanning. Cohesin-mediated loop extrusion may be similarly regulated in the context of V(D)J recombination in other antigen receptor loci.
including Igκ\textsuperscript{47,48} and T cell receptor loci\textsuperscript{36,49,50}, consistent with cohesin being required for TCRα rearrangement in developing T cells\textsuperscript{49}. The system we describe for analyzing cohesin-complex protein functions in G1-arrested v-Abl pro-B cells should be useful for further testing functions and mechanisms of these or other factors in physiological chromatin loop extrusion \textit{in vivo}.

REFERENCES

1. Alt, F. W., Zhang, Y., Meng, F.-L., Guo, C. & Schwer, B. Mechanisms of programmed DNA lesions and genomic instability in the immune system. \textit{Cell} \textbf{152}, 417–429 (2013).
2. Fudenberg, G. \textit{et al.} Formation of Chromosomal Domains by Loop Extrusion. \textit{Cell Rep} \textbf{15}, 2038–2049 (2016).
3. Sanborn, A. L. \textit{et al.} Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. \textit{Proc. Natl. Acad. Sci. U.S.A.} \textbf{112}, E6456–65 (2015).
4. Rao, S. S. P. \textit{et al.} Cohesin Loss Eliminates All Loop Domains. \textit{Cell} \textbf{171}, 305–320.e24 (2017).
5. Haarhuis, J. H. I. \textit{et al.} The Cohesin Release Factor WAPL Restricts Chromatin Loop Extension. \textit{Cell} \textbf{169}, 693–707.e14 (2017).
6. Schwarzer, W. \textit{et al.} Two independent modes of chromatin organization revealed by cohesin removal. \textit{Nature} \textbf{551}, 51–56 (2017).
7. Wutz, G. \textit{et al.} Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins. \textit{EMBO J.} \textbf{36}, 3573–3599 (2017).
8. Vian, L. \textit{et al.} The Energetics and Physiological Impact of Cohesin Extrusion. \textit{Cell} \textbf{173}, 1165–1178.e20 (2018).
9. Nora, E. P. \textit{et al.} Targeted Degradation of CTCF Decouples Local Insulation of Chromosome Domains from Genomic Compartmentalization. \textit{Cell} \textbf{169}, 930–944.e22 (2017).
10. Rowley, M. J. & Corces, V. G. Organizational principles of 3D genome architecture. \textit{Nat. Rev. Genet.} \textbf{19}, 1–800 (2018).
11. Zhang, Y. \textit{et al.} The fundamental role of chromatin loop extrusion in physiological V(D)J recombination. \textit{Nature} \textbf{573}, 600–604 (2019).
12. Guo, C. \textit{et al.} CTCF-binding elements mediate control of V(D)J recombination. \textit{Nature} \textbf{477}, 424–430 (2011).
13. Lin, S. G., Guo, C., Su, A., Zhang, Y. & Alt, F. W. CTCF-binding elements 1 and 2 in the Igκ intergenic control region cooperatively regulate V(D)J recombination. \textit{Proc. Natl. Acad. Sci. U.S.A.} \textbf{112}, 1815–1820 (2015).
14. Hu, J. \textit{et al.} Chromosomal Loop Domains Direct the Recombination of Antigen Receptor Genes. \textit{Cell} \textbf{163}, 947–959 (2015).
15. Jain, S., Ba, Z., Zhang, Y., Dai, H.-Q. & Alt, F. W. CTCF-Binding Elements Mediate Accessibility of RAG Substrates During Chromatin Scanning. \textit{Cell} \textbf{174}, 102–116.e14 (2018).
16. Lin, S. G., Ba, Z., Alt, F. W. & Zhang, Y. RAG Chromatin Scanning During V(D)J Recombination and Chromatin Loop Extrusion are Related Processes. *Adv. Immunol.* 139, 93–135 (2018).

17. Degner, S. C. *et al.* CCCTC-binding factor (CTCF) and cohesin influence the genomic architecture of the Igh locus and antisense transcription in pro-B cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9566–9571 (2011).

18. Lucas, J. S., Bossen, C. & Murre, C. Transcription and recombination factories: common features? *Curr. Opin. Cell Biol.* 23, 318–324 (2011).

19. Lucas, J. S., Zhang, Y., Dudko, O. K. & Murre, C. 3D trajectories adopted by coding and regulatory DNA elements: first-passage elements for genomic interactions. *Cell* 158, 339–352 (2014).

20. Khanna, N., Zhang, Y., Lucas, J. S., Dudko, O. K. & Murre, C. Chromosome dynamics near the sol-gel phase transition dictate the timing of remote genomic interactions. *Nat Commun* 10, 2771 (2019).

21. Bossen, C., Mansson, R. & Murre, C. Chromatin topology and the regulation of antigen receptor assembly. *Annu. Rev. Immunol.* 30, 337–356 (2012).

22. Proudhon, C., Hao, B., Raviram, R., Chaumeil, J. & Skok, J. A. Long-Range Regulation of V(D)J Recombination. *Adv. Immunol.* 128, 123–182 (2015).

23. Ebert, A., Hill, L. & Busslinger, M. Spatial Regulation of V-(D)J Recombination at Antigen Receptor Loci. *Adv. Immunol.* 128, 93–121 (2015).

24. Muljo, S. A. & Schlissel, M. S. A small molecule Abl kinase inhibitor induces differentiation of Abelson virus-transformed pre-B cell lines. *Nat. Immunol.* 4, 31–37 (2003).

25. Bredemeyer, A. L. *et al.* ATM stabilizes DNA double-strand-break complexes during V(D)J recombination. *Nature* 442, 466–470 (2006).

26. Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T. & Kanemaki, M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat. Methods* 6, 917–922 (2009).

27. Natsume, T., Kiyomitsu, T., Saga, Y. & Kanemaki, M. T. Rapid Protein Depletion in Human Cells by Auxin-Inducible Degron Tagging with Short Homology Donors. *Cell Rep* 15, 210–218 (2016).

28. Yatskevich, S., Rhodes, J. & Nasmyth, K. Organization of Chromosomal DNA by SMC Complexes. *Annu. Rev. Genet.* (2019). doi:10.1146/annurev-genet-112618-043633

29. Hassler, M., Shaltiel, I. A. & Haering, C. H. Towards a Unified Model of SMC Complex Function. *Curr. Biol.* 28, R1266–R1281 (2018).

30. Uhlmann, F. SMC complexes: from DNA to chromosomes. *Nat. Rev. Mol. Cell Biol.* 17, 399–412 (2016).

31. Haarhuis, J. H. I., Elbatsh, A. M. O. & Rowland, B. D. Cohesin and its regulation: on the logic of X-shaped chromosomes. *Dev. Cell* 31, 7–18 (2014).

32. Peters, J.-M., Tedeschi, A. & Schmitz, J. The cohesin complex and its roles in chromosome biology. *Genes Dev.* 22, 3089–3114 (2008).

33. Wu, N. & Yu, H. The Smc complexes in DNA damage response. *Cell Biosci.* 2, 5 (2012).
34. Davidson, I. F. et al. DNA loop extrusion by human cohesin. *Science* (2019). doi:10.1126/science.aaz3418

35. Kim, Y., Shi, Z., Zhang, H., Finkelstein, I. J. & Yu, H. Human cohesin compacts DNA by loop extrusion. *Science* **366**, 1345–1349 (2019).

36. Zhao, L. et al. Orientation-specific RAG activity in chromosomal loop domains contributes to Tcrd V(D)J recombination during T cell development. *J. Exp. Med.* **213**, 1921–1936 (2016).

37. Zhang, X. et al. Fundamental roles of chromatin loop extrusion in antibody class switching. *Nature* **575**, 385–389 (2019).

38. Choi, N. M. et al. Deep sequencing of the murine IgH repertoire reveals complex regulation of nonrandom V gene rearrangement frequencies. *J. Immunol.* **191**, 2393–2402 (2013).

39. Bolland, D. J. et al. Two Mutually Exclusive Local Chromatin States Drive Efficient V(D)J Recombination. *Cell Rep* **15**, 2475–2487 (2016).

40. Lin, S. G. et al. Highly sensitive and unbiased approach for elucidating antibody repertoire. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 7846–7851 (2016).

41. Hu, J. et al. Detecting DNA double-stranded breaks in mammalian genomes by linear amplification-mediated high-throughput genome-wide translocation sequencing. *Nat Protoc* **11**, 853–871 (2016).

42. Aiden, E. L. & Casellas, R. Somatic Rearrangement in B Cells: It's (Mostly) Nuclear Physics. *Cell* **162**, 708–711 (2015).

43. Nakahashi, H. et al. A genome-wide map of CTCF multivalency redefines the CTCF code. *Cell Rep* **3**, 1678–1689 (2013).

44. Canzio, D. et al. Antisense lncRNA Transcription Mediates DNA Demethylation to Drive Stochastic Protocadherin α Promoter Choice. *Cell* (2019). doi:10.1016/j.cell.2019.03.008

45. Ghirlando, R. & Felsenfeld, G. CTCF: making the right connections. *Genes Dev.* **30**, 881–891 (2016).

46. Hsieh, T.-H. S. et al. Resolving the 3D landscape of transcription-linked mammalian chromatin folding. *bioRxiv* **176**, 638775 (2019).

47. Xiang, Y., Park, S.-K. & Garrard, W. T. A major deletion in the Vκ-Jκ intervening region results in hypertranscribed transcription of proximal Vκ genes and a severely restricted repertoire. *J. Immunol.* **193**, 3746–3754 (2014).

48. Ribeiro de Almeida, C. et al. The DNA-binding protein CTCF limits proximal Vκ recombination and restricts κ enhancer interactions to the immunoglobulin κ light chain locus. *Immunity* **35**, 501–513 (2011).

49. Seitan, V. C. et al. A role for cohesin in T-cell-receptor rearrangement and thymocyte differentiation. *Nature* **476**, 467–471 (2011).

50. Chen, S. & Krangel, M. S. Diversification of the TCR β Locus Vβ Repertoire by CTCF. *Immunohorizons* **2**, 377–383 (2018).

**ACKNOWLEDGEMENTS**

We thank lab members for stimulating discussions. This work was supported by NIH R01 AI020047 (to F.W.A.). R.C. is partially funded by the NIH Regulome Project. F.W.A. is
an investigator of the Howard Hughes Medical Institute. Z.B. was a Cancer Research Institute Irvington fellow.

AUTHOR CONTRIBUTIONS

Z.B., J.L., R.C., and F.W.A. designed the study; Z.B. and J.L. performed most of the experiments with assistance from E.D., S.G.L., and K.-R.K.-K. on certain experiments; A.Y.Y. designed some of the bioinformatics pipelines for data analysis; S.J. provided helpful discussions early in the study; Z.B., J.L., and F.W.A. analyzed and interpreted data, designed figures, and wrote the paper. R.C., S.G.L., and S.J. helped polish the paper. F.W.A. supervised the study.

AUTHOR INFORMATION

The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to F.W.A. (alt@enders.tch.harvard.edu). F.W.A. is a co-founder of Otoro Biopharmaceuticals.

FIGURE LEGENDS

Fig. 1 | Cohesin depletion abrogates Igh loop domains and V(D)J rearrangements except RC-based DQ52-to-JH joining in G1-arrested Rad21-degron v-Abl pro-B cells.

a, Schematic of the murine Igh locus showing proximal VHs, DHs, CHs, and regulatory elements as indicated, with Igh recombination center (RC) that comprises JH-proximal DQ52 segment, four JH segments, and the intronic enhancer (iEμ) highlighted. Purple arrowheads indicate organization of CBES. Cen. centromere. Blue arrow denotes the JH4 coding end (CE) bait primer used for generating HTGTS-V(D)J-seq libraries. b, c, Representative profiles of Rad21 ChIP-seq (b) and 3C-HTGTS chromatin interactions using VH81X-CBE (blue asterisk) and RC/iEμ (red asterisk) as bait (c) at indicated Igh locus in G1-arrested Rad21-degron v-Abl pro-B cells without (NT) or with (+IAA) IAA treatment. See related Extended Data Fig. 2 for additional biological repeats. d, e, Average utilization frequencies ± s.d. of indicated proximal VH (left) and D (right) segments on IGCR1-intact (d, n=3) and IGCR1-deleted (e, n=4) alleles in RAG2-complemented, G1-arrested Rad21-degron cells without (NT) or with (+IAA) IAA treatment. A JH4-CE bait primer was used for generating HTGTS-V(D)J-seq libraries. P values were calculated using unpaired two-tailed Student's t-test, NS: P > 0.05, *: P ≤ 0.05, **: P ≤ 0.01, and ***: P ≤ 0.001. NA: not applicable. See Methods for more details.

Fig. 2 | Effects of CTCF degradation on chromatin CTCF/Rad21-binding and
transcription across \textit{Igh} locus in G1-arrested CTCF-degron v-Abl pro-B cells.

\textit{a}, Schematic of the entire murine \textit{Igh} locus with details as shown in Fig. 1. \textit{b}, \textit{c}, Representative profiles of ChIP-seq (\textit{b}) using CTCF (top panels) and Rad21 (bottom panels) antibodies and GRO-seq (\textit{c}) across the entire \textit{Igh} locus as indicated in G1-arrested parental and CTCF-degraded v-Abl pro-B cells. CBES with residual CTCF-binding are indicated in \textit{b}. The entire \textit{VH} locus is diagrammed at the top. Additional biological repeats of each experiment are shown in related Extended Data Fig. 6. See Methods for more details.

\textbf{Fig. 3 | CTCF degradation activates distal \textit{VH} rearrangements in G1-arrested CTCF-degron v-Abl pro-B cells.}

\textit{a}, Schematic of the murine \textit{Igh} locus. Blue arrow denotes the J4-CE bait primer used for generating HTGTS-V(D)J-seq libraries. Other details are as in Fig. 2. \textit{b}-\textit{d}, Average utilization frequencies ± s.d. of all \textit{VH} segments in RAG2-complemented, G1-arrested parental (\textit{b}, \textit{n}=4), CTCF-degraded (\textit{c}, \textit{n}=6) v-Abl pro-B cells and mouse BM pro-B cells (\textit{d}, \textit{n}=3). Average percentage ± s.d. of VDJH and DJH rearrangements among total V(D)J junctions for each sample are indicated. See Methods for more details. For comparison of \textit{VH} utilization patterns among samples, the location of several \textit{VH}S are indicated.

\textbf{Fig. 4 | CTCF degradation restores long-distance \textit{Igh} RC interactions with distal \textit{VH} sequences in G1-arrested CTCF-degron v-Abl pro-B cells.}

\textit{a}, Schematic of the entire murine \textit{Igh} locus with details as shown in Fig. 2. \textit{b}, Representative 3C-HTGTS chromatin interaction profiles of RC/\textit{E}H bait (red asterisk) across the entire \textit{Igh} locus in G1-arrested parental (top), CTCF-degraded (middle) v-Abl pro-B cells and RAG2-deficient mouse BM pro-B cells (bottom). See Methods for more details. For comparison, several distal interaction peaks located proximal to \textit{VH}S highlighted in Fig. 3 are indicated. The entire \textit{VH} locus is diagrammed at the top. See related Extended Data Fig. 7 for additional 3C-HTGTS profiles with other RC-based baits.

\textbf{EXTENDED DATA FIGURE LEGENDS}

\textbf{Extended Data Fig. 1 | Generation and characterization of Rad21-degron v-Abl pro-B lines.}

\textit{a}, Diagram of the Rad21-degron mouse v-Abl pro-B line. \textit{b}, Representative flow-cytometry plots showing the percentage of Clover-positive Rad21-degron v-Abl cells that are non-treated (NT) and treated by IAA (+IAA) for 6h (left) followed by treatment with STI-571 (without or with IAA) for 4 days to induce G1 arrest (right). \textit{c}, \textit{d}, Top: Schematic of the
targeting strategy for introducing in-frame mAID-Clover sequences into the last codon of mouse both endogenous Rad21 alleles (c), and OsTir1-V5 expression cassette into both endogenous Rosa26 alleles (d) in a rag2-/-; Eμ-Bcl2+ v-Abl pro-B line. Positions of Cas9/sgRNAs and Southern blot probes are indicated. H: HindIII; B: BglII; S: SspI. Bottom: Southern blot confirmation of correctly targeted alleles as indicated. e, Time-course cell viability assay for Rad21-degron v-Abl pro-B cells without (-IAA, NT) and with (+IAA) IAA treatment following STI-571 treatment for G1-arrest (+STI). Top: the assay timeline. Bottom: time-course cell viability curves. Average percentage ± s.d. of viable cells for each timepoint and under each condition was shown (n=4 experiments with biologically independent clones). NS: P > 0.05. f, Diagram of the experimental strategy with Rad21-degron v-Abl cells for various assays presented in this study. g, Fucci cell cycle assay of Rad21-degron v-Abl cells without (NT) and with (+IAA) IAA treatment following 4-day STI-571 treatment for G1-arrest. Representative flow-cytometry plots and average percentage ± s.d. of cells arrested in G1 stage at indicated condition (n=3) are shown. See Methods for details.

Extended Data Fig. 2 | Cohesin depletion abrogates Igh loop domains in the presence or absence of IGCR1 in G1-arrested Rad21-degron v-Abl pro-B cells.

a, An additional biologically independent repeat of Rad21 ChIP-seq profiles at indicated Igh locus in G1-arrested Rad21-degron v-Abl pro-B cells without (NT) or with (+IAA) IAA treatment as shown in Fig. 1b. b, c, Two additional biologically independent repeats for 3C-HTGTS chromatin interaction profiles of Vκ81X-CBE bait (b, blue asterisk) and iΕμ bait (c, red asterisk) at indicated Igh locus in G1-arrested Rad21-degron v-Abl pro-B cells without (NT) or with (+IAA) IAA treatment as shown in Fig. 1c. d, Two biologically independent repeats for 3C-HTGTS chromatin interaction profiles of iΕμ bait (red asterisk) at indicated Igh locus in G1-arrested IGCR1-deleted Rad21-degron v-Abl pro-B cells without (NT) or with (+IAA) IAA treatment. See Methods for more details.

Extended Data Fig. 3 | Effects of cohesin loss on Igh and genome-wide gene transcription in G1-arrested Rad21-degron v-Abl pro-B cells.

a, Three biologically independent GRO-seq repeats of G1-arrested Rad21-degron v-Abl cells without (NT) or with (+IAA) IAA treatment across the entire Igh locus as indicated. b, Scatter plots of transcriptome-wide GRO-seq (three biologically independent repeats) counts in G1-arrested Rad21-degron v-Abl cells without (NT, x axis) or with (+IAA, y axis) IAA treatment. Representative known requisite genes for V(D)J recombination and chromatin interaction are highlighted by red circles and blue arrows in each of the three scatter plots. See Methods for more details.
Extended Data Fig. 4 | Generation and characterization of CTCF-degron v-Abl pro-B lines.

a, Diagram of the CTCF-degron mouse v-Abl pro-B line. b, Representative flow-cytometry plots showing the percentage of GFP-positive CTCF-degron v-Abl cells that are non-treated (NT) and treated by IAA (+IAA) for 6h (left) followed by treatment with STI-571 (without or with IAA) for 4 days to induce G1 arrest (right). c, Schematic of the targeting strategy for introducing in-frame mAID-GFP sequences into the last codon of mouse both Ctcf alleles in a rag2Δ; Eµ-Bcl2+ v-Abl pro-B line. Positions of Cas9/sgRNAs and Southern blot probes are indicated. B: BglII. d, e, Southern blot confirmation of correctly targeted Ctcf (d) and Rosa26 (e) alleles as indicated. Targeting OsTir1-V5 cassette into Rosa26 alleles follows the same targeting strategy as shown in Extended Data Fig. 1d. f, Fucci cell cycle assay of CTCF-degron v-Abl cells without (NT) and with (+IAA) IAA treatment following 4-day STI-571 treatment for G1-arrest. Representative flow-cytometry plots and average percentage ± s.d. of cells arrested in G1 stage at indicated condition (n=3) are shown. g, Time-course cell viability assay for CTCF-degron v-Abl pro-B cells without (-IAA, NT) and with (+IAA) IAA treatment following STI-571 treatment for G1-arrest (+STI). Top: the assay timeline. Bottom: time-course cell viability curves. Average percentage ± s.d. of viable cells for each timepoint and under each condition was shown (n=3 experiments with biologically independent clones). NS: P > 0.05, *: P ≤ 0.05, **: P ≤ 0.01. See Methods for details.

Extended Data Fig. 5 | CTCF-degron v-Abl pro-B lines show modest leakiness.

a, Schematic of the murine Igh locus as in Fig. 2a. b, Average utilization frequencies ± s.d. of all VhS in RAG2-complemented, G1-arrested parental v-Abl pro-B cells (top, n=4) and non-treated CTCF-degron v-Abl pro-B cells (bottom, n=6). For comparison, locations of several VhS are indicated. c, Representative 3C-HTGTS chromatin interaction profiles of RC/iEµ (red asterisk), RC/DQ52-Jµ1 (blue asterisk), and RC/Jµ4 (purple asterisk) baits across the Igh locus in G1-arrested parental v-Abl pro-B cells (top) and non-treated CTCF-degron v-Abl pro-B cells (bottom). See Methods for details. For comparison, several distal interaction peaks near VhS highlighted in Fig.3 are indicated. The Vh locus is diagrammed at the top. See Extended Data Fig. 7a for diagrams of 3C-HTGTS RC baits employed. To facilitate direct comparisons, the HTGTS-V(D)J-seq and 3C-HTGTS data for the Parental v-Abl cells are the same as those presented in Figs. 3, 4 and Extended Data Fig. 7.

Discussion: The apparent greater leakiness with respect to RC interactions versus RAG scanning activity across the upstream locus might result from the latter being done in RAG2-
sufficient cells. As proposed to explain a related phenomenon involving dCas9-binding, extrusion of chromatin impediments past the RC may be more efficient when not RAG-bound\textsuperscript{11}.

**Extended Data Fig. 6** | Effects of CTCF degradation on chromatin CTCF/Rad21-binding and transcription across \textit{Igh} locus in G1-arrested CTCF-degron \textit{v-Abl} pro-B cells.

\textit{a, b,} Two additional biologically independent repeats of ChIP-seq (\textit{a}) using CTCF (top panels) and Rad21 (bottom panels) antibodies and GRO-seq (\textit{b}) across the entire \textit{Igh} locus as indicated in G1-arrested parental and CTCF-degraded \textit{v-Abl} pro-B cells. CBEs with residual CTCF-binding are indicated in \textit{a}. The entire \textit{VH} locus is diagrammed at the top. See Methods for more details.

**Extended Data Fig. 7** | CTCF degradation restores long-distance \textit{Igh} RC interactions with distal \textit{VH} sequences in G1-arrested CTCF-degron \textit{v-Abl} pro-B cells.

\textit{a,} Top: Schematic of the murine \textit{Igh} locus as in Fig. 2\textit{a}. Bottom: Zoom-in schematic of \textit{Igh} RC showing 3C-HTGTS assay baiting strategies used for iE\textsubscript{\textmu} (red asterisk), DQ52-J\textsubscript{H}1 (blue asterisk), and J\textsubscript{H}4 (purple asterisk) locales. NlaIII restriction fragments and the relative positions of biotinylated (cayenne arrow) and nested (blue arrow) PCR primers employed are illustrated. Brown and teal blue triangles represent DQ52-12RSSs and J\textsubscript{H}-23RSSs, respectively. \textit{b,} Representative 3C-HTGTS chromatin interaction profiles of RC/DQ52-J\textsubscript{H}1 (blue asterisk) across the entire \textit{Igh} locus in G1-arrested parental (top), CTCF-degraded (middle) \textit{v-Abl} pro-B cells and RAG2-deficient mouse BM pro-B cells (bottom). \textit{c,} Representative 3C-HTGTS chromatin interaction profiles of RC/J\textsubscript{H}4 (purple asterisk) across the entire \textit{Igh} locus in G1-arrested parental (top) and CTCF-degraded (bottom) \textit{v-Abl} pro-B cells. See Methods for details. For comparison, several distal interaction peaks located proximal to \textit{VH}s highlighted in Fig. 3 are indicated. The entire \textit{VH} locus is diagrammed at the top.

**Extended Data Fig. 8** | A working model for CTCF orchestrating long-range cohesin-driven V(D)J recombinational scanning.

\textit{a,} Diagram shows mouse \textit{VH} locus with 100-plus \textit{VH}s in four domains\textsuperscript{38,39}, illustrated in different colors from J\textsubscript{H}-proximal to J\textsubscript{H}-distal. Besides harboring different \textit{VH} families, proximal domains are transcriptionally down-regulated and have CBEs immediately adjacent to \textit{VH}-RSSs, whereas distal domains are transcriptionally active and have CBEs between but
rarely adjacent to V_Hs. All V_H CBEs are oriented convergently with the IGCR1 upstream CBE1 and their RSSs convergently oriented with the DJ_H recombination center (RC)-RSS and must join by deletion. Details of V_H locus organization have been discussed.\textsuperscript{13,15,21-23,38,39}

\textbf{b}, With normal CBE-impediment activity (red arrowheads), RAG scanning is strongly impeded by CBE-based IGCR1 and non-CBE-based DJ_H RC impediments\textsuperscript{11,15}, allowing scanning-based D-to-J_H rearrangement to proceed V_H-to-DJ_H rearrangement\textsuperscript{11}. Low-level scanning beyond IGCR1 allows proximal V_H-to-DJ_H rearrangement in a minor subset of pro-B cells. Proximal V_H-CBEs impedes further upstream scanning\textsuperscript{15,16}.

\textbf{c, d}, Down-modulation of CTCF reduces CTCF-occupancy of CBEs (grey arrowheads) across \textit{Igh}, but residual binding is retained at certain CBEs (pink arrowheads). Down-modulation of CTCF/CBE activity allows RC-bound RAG to scan various linear distances across the \textit{Igh} in some cases the full-length. Upon reaching remaining scanning impediments, which could reflect transcription (dark arrows)\textsuperscript{11,37,46}, transcription-factor (TF)-binding sites\textsuperscript{15,21-23,38,39} (orange diamonds), and/or CBEs with residual CTCF binding, impeded scanning focuses RC-based RAG activity on sequences in the impeded region\textsuperscript{11} for V_H-to-DJ_H joining\textsuperscript{1} (\textbf{e}). Down-modulation of CTCF binding activity is one manner for extending extrusion but this could also be achieved by circumventing CBE impediments, for example, through modulation of cohesin activity (See Text).

\textbf{METHODS}

\textbf{Experimental procedures}

No statistical methods were used to predetermine sample size. Experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

\textbf{Mice}

Wild-type (WT) 129SV mice were purchased from Charles River Laboratories International. RAG2-deficient mice in 129SV background were generated\textsuperscript{51} and maintained in the Alt laboratory. The \textit{RAG2}\textsuperscript{-/-}; \textit{E\_\textmu-Bcl2}\textsuperscript{+} mice were generated by crossing RAG2-deficient mice with \textit{E\_\textmu-Bcl2} transgenic mice\textsuperscript{52} that had been backcrossed to the 129SV background. All animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of Boston Children’s Hospital.

\textbf{Bone marrow pro-B cell purification}
Bone marrow (BM)-derived pro-B cells (B220⁺CD43⁻IgM⁻) were purified via FACS sorting as previously described\textsuperscript{15,40} from 4-week-old WT 129SV mice. B220-positive BM pro-B cells were purified via anti-B220 biotin/streptavidin beads (Miltenyi, #130-049-501) from 4-week-old RAG2-deficient mice and were cultured in opti-MEM medium containing 10% (v/v) FBS plus IL-7/SCF for 4 days as previously described\textsuperscript{14}.

Cell lines

The parental v-Abl-kinase-transformed pro-B lines were derived by retroviral infection of BM pro-B cells derived from RAG2\textsuperscript{-/-}; E\textsubscript{µ}-Bcl2\textsuperscript{+} mice with the pMSCV-v-Abl retrovirus, as described previously\textsuperscript{25}. Infected cells were cultured in RPMI medium containing 15% (v/v) FBS for three months to obtain stably transformed v-Abl lines. The RAG2\textsuperscript{-/-}; E\textsubscript{µ}-Bcl2\textsuperscript{+} v-Abl pro-B lines were further targeted by Cas9/sgRNA approach\textsuperscript{53} to randomly introduce 1~4 bp indels into the site ~90 bp downstream of J\textsubscript{H}4-RSS-heptamer and ~10 bp upstream of J\textsubscript{H}4-coding end (CE) bait primer on both Igh alleles. The Igh allele-specific barcodes permit the separation of the sequenced reads amplified from each allele with the identical bait primer from the same HTGT-V(D)J-seq libraries, thus, permitting in parallel examination of V(D)J recombination events that occur on each Igh allele under the same cellular context. HTGT-S-V(D)J-seq analyses on RAG2-complemented above-mentioned Igh-barcoded v-Abl cells confirmed very comparable V(D)J rearrangement patterns and frequencies between Igh alleles, which were also very similar with those on germline Igh alleles in the parental RAG2\textsuperscript{-/-}; E\textsubscript{µ}-Bcl2\textsuperscript{+} v-Abl cells. The human colorectal carcinoma line HCT116 was purchased from American Type Culture Collection (ATCC, #CCL-247) and were cultured in McCoy’s 5A medium (ThermoFisher Scientific, #16600082) containing 10% (v/v) FBS.

Generation of Rad21-degron and CTCF-degron v-Abl pro-B lines and their derivatives

The targeting constructs used for introducing the in-frame mAID sequences into mouse endogenous CTCF locus (pEN84, Addgene #86230) and for introducing the OsTir1-V5 expression cassette into endogenous Rosa26 locus (pEN114, Addgene #92143) were described in a previously published study\textsuperscript{9}. Using a similar strategy\textsuperscript{9}, we generated the targeting construct for introducing the in-frame mAID sequences into mouse endogenous Rad21 locus. Briefly, the 5’ and 3’ homology arms (1.5 kb each) flanking Rad21 gene stop codon were PCR amplified from RAG2\textsuperscript{-/-}; E\textsubscript{µ}-Bcl2\textsuperscript{+} v-Abl pro-B cell genomic DNA (gDNA). The mAID-Clover cassette was PCR amplified from the plasmid pMK290 (Addgene,
These fragments together with a puro selection cassette amplified from pEN84 were assembled in an order diagramed in Extended Data Fig. 1c using Gibson assembly kit (NEB, #E2611S). To promote the targeting efficiency, Cas9/sgRNAs were cloned by annealing pairs of oligos into pX330 (Addgene, #42230) following the protocol previously described\(^5\). The parental \textit{RAG2}^{-}; \textit{E}_{H-Bcl2}^{+} \textit{v-Abl} pro-B cells were nucleofected with Rad21- or CTCF-targeting donors and their corresponding Cas9/sgRNAs via Lonza 4D Nucleofector as shown before\(^{14,15}\), selected with 1 \(\mu\)g/ml Puromycin (Gibco, #A1113802) for 4 days, and subcloned by dilution. Candidate clones with desired gene modifications were screened by PCR and confirmed by Southern blot as outlined in Extended Data Fig. 1c and 4c. The confirmed clones were further processed to remove the puromycin selection cassette as previously described\(^9\). Similarly, the resultant clones were further targeted to introduce into both \textit{Rosa26} alleles the OsTir1-V5 expression cassette followed by removal of the puromycin selection cassette as outlined in Extended Data Fig. 1d.

The resulting lines were referred to as the Rad21-degron or CTCF-degron \textit{v-Abl} pro-B line and used in this study. To delete IGCR1, the Rad21-degron \textit{v-Abl} pro-B line was targeted with Cas9/sgRNAs as previously described\(^{14,15}\). To determine which \textit{Igh} allele harbors IGCR1 deletion in the IGCR1^{+/-} Rad21-degron \textit{v-Abl} clones, we developed and applied an efficient allele-specific assay based on the examination of Cas9/sgRNA-induced translocations via LAM-HTGTS\(^{14,54}\). Briefly, we introduced the bait and prey Cas9/sgRNAs that, respectively, target the site located between \textit{J}_{H4}-RSS-heptamer and \textit{Igh}-allele-specific barcodes and IGCR1 locale into the IGCR1^{+/-} Rad21-degron \textit{v-Abl} clones, extracted the gDNA from the cells 3 days post the nucleofection, and performed LAM-HTGTS using \textit{J}_{H4}-CE bait primer. The bait-prey site translocation junctions derived from each of the two \textit{Igh} alleles were separated through the barcodes, and the combined junction numbers from both strands that fall into bait and prey regions on each allele were plotted under a 1.5 Mb bin size. The relative numbers of prey junctions within IGCR1 locale indicate the presence or absence of IGCR1 on each allele, which can be further confirmed by HTGTS-V(D)J-seq analyses, as the rearrangements of proximal \textit{V}_{H}S are enhanced by IGCR1 deletion\(^{14,54}\). Sequences of all sgRNAs and oligos used are listed in Supplementary Table 3.

**Treatment of Rad21-degron and CTCF-degron \textit{v-Abl} pro-B lines with IAA**

To deplete mAID-tagged Rad21 or CTCF protein in Rad21-degron or CTCF-degron
v-Abl pro-B cells, the auxin analog, Indole-3-acetic acid (IAA, Sigma-Aldrich, #I3750-25G-A), was added in the medium at 500 µM from a 1000X stock that were prepared by dissolving IAA with DMSO. DMSO solvent was applied as the mock to non-treated (NT) cells. After 6 hours, the depletion efficiency of mAID-tagged proteins in IAA-treated cells compared to non-treated cells was examined by FACS. Both non-treated and IAA-treated cells were then treated by 3 µM STI-571 without or with IAA for 4 days to induce G1 arrest as previously described11,14,54. The cells were then collected and examined by FACS for protein depletion confirmation prior to various assays as described below.

Cell viability assay
For time-course cell viability assay, Rad21-degron and CTCF-degron v-Abl pro-B cells were non-treated or treated with IAA for 6 hours and then subject to STI-571 treatment (set as 0 h). The viability of NT and +IAA cells at the following 4 days was determined by the percentage of viable lymphocyte population gated by FACS side (SSC) and forward (FSC) scatters out of the total cells. Average percentage ± s.d. of viable cells for each timepoint and for each condition was calculated from >3 biologically independent experiments and was normalized to the number of NT cells at 0 hours, which was set to 100%.

G1 cell cycle stage analysis
For cell cycle analysis, the fluorescent, ubiquitination-based cell cycle indicator (Fucci) 55 cassette (pEN435, Addgene #92139) was stably introduced into the Rad21-degron and CTCF-degron v-Abl pro-B lines following the strategy as described previously9. The parental v-Abl cells without Fucci cassette, Rad21-degron and CTCF-degron v-Abl pro-B cells with Fucci cassette were non-treated or treated with IAA for 6 hours followed by STI-571 treatment (set as 0 h). The distribution of cells at G1 cell cycle stage (mCherry-hCdt1+; TagBFP-hGeminin population) at the following 4 days was determined by FACS using the parental v-Abl cells without Fucci cassette as the gating control. Average percentage ± s.d. of cells arrested in G1 stage for each timepoint and for each condition was calculated from >3 biologically independent experiments. The data collected at day 4 post STI-571 treatment are shown in Extended Data Fig. 1g, 4f.

HTGTS-V(D)J-seq
For Igh V(D)J recombination analyses, we purified BM pro-B cells from WT 129SV mice as described above and introduced RAG2 into RAG2-deficient Rad21-degron and
CTCF-degron ν-Abl pro-B cells as well as their derivatives via the approach described previously\textsuperscript{11}. HTGTS-V(D)J-seq libraries were prepared as previously described\textsuperscript{11,15,40,41}. Briefly, 2 µg of gDNA from sorted mouse BM pro-B cells or 30 µg of gDNA from G1-arrested RAG2-complemented parental ν-Abl cells, Rad21-degron and CTCF-degron ν-Abl cells without or with IAA treatment was sonicated and subjected to LAM-PCR using biotinylated J\textsubscript{H}4-CE bait primer\textsuperscript{15}. Single-stranded LAM-PCR products were purified using Dynabeads MyONE C1 streptavidin beads (Life Technologies, #65002) and ligated to bridge adaptors. Adaptor-ligated products were amplified by nested PCR with indexed J\textsubscript{H}4 primers and the primer annealed to the adaptor. The PCR products were further tagged with Illumina sequencing adaptor sequences, size-selected via gel extraction and loaded onto an Illumina MiSeq machine for paired-end 250-bp or 300-bp sequencing. Primer sequences are listed in Supplementary Table 3.

**HTGTS-V(D)J-seq data processing and analyses**

HTGTS-V(D)J-seq libraries were processed via the pipeline described previously\textsuperscript{41}. I\textgreek{h} allele-specific sequencing reads were separated via barcodes and aligned to AJ851868/mm9 hybrid genome that was generated by combining all of the annotated I\textgreek{h} sequences of 129SV background (AJ851868) and the distal V\textgreek{h} sequences from the C57BL/6 background (mm9) starting from V\textgreek{h}8-2 as previously described\textsuperscript{15,40,56}. For statistical analyses, utilization data of V\textgreek{h} and D segments in G1-arrested CTCF-degron ν-Abl pro-B cells was the sum up of the numbers obtained from both I\textgreek{h} alleles, each of which was normalized to 10,000 total recovered junctions as previously described\textsuperscript{15} and showed very similar utilization patterns. For comparisons, utilization data of V\textgreek{h} and D segments in BM pro-B cells was normalized to 20,000 total recovered junctions. We also extracted and re-analyzed the V(D)J recombination data obtained from 129SV BM pro-B cells in our prior publication\textsuperscript{40} and compared them with the newly generated data following the same normalization approach, which showed essentially same relative V\textgreek{h} and D utilization patterns. As the number of junctions used for normalization of Rad21 depletion experiments was greatly decreased comparing non-treated experiments due to the abrogation of nearly all V(D)J rearrangements by cohesin loss, we normalized the utilization data for Rad21 depletion experiments to the total aligned reads which include all bait primer-containing sequencing reads including V(D)J junctions for statistical analyses following the previously
described strategy\textsuperscript{14,15}. In this regard, utilization data from G1-arrested Rad21-degron v-Abl pro-B cells with or without IAA treatment was the sum up of the numbers obtained from both \textit{Igh} alleles, each of which was normalized to 110,000 total aligned reads. Utilization data from WT or IGCR1-deleted allele in G1-arrested IGCR1\textsuperscript{+/-} Rad21-degron v-Abl cells without or with IAA treatment was normalized to 110,000 total aligned reads. For each HTGTS-V(D)J-seq data plotted in figures and shown in Supplementary Table 1 and 2, average utilization frequencies \pm s.d. were presented.

3C-HTGTS and data analyses

3C-HTGTS on short-term cultured RAG2-deficient BM pro-B cells, G1-arrested parental v-Abl cells, Rad21-degron and CTCF-degron v-Abl cells without or with IAA treatment, was performed as previously described\textsuperscript{15}. Briefly, 10 million cells were crosslinked with 2\% formaldehyde (Sigma-Aldrich, \#F8775) for 10 min at room temperature and quenched with glycine at a final concentration of 125 mM. Cells were lysed on ice for 10 min followed by centrifugation to get nuclei. Nuclei were resuspended in NEB Cutsmart buffer for NlalII (NEB, \#R0125) digestion at 37 °C overnight, followed by T4 DNA ligase (Promega, \#M1801) mediated ligation under dilute conditions at 16 °C overnight. Ligated products were treated with Proteinase K (Roche, \#03115852001) and RNase A (Invitrogen, \#8003089) followed by DNA purification to get the 3C templates. 3C-HTGTS library preparation follows the standard LAM-HTGTS library preparation procedures as previously described\textsuperscript{15}. 3C-HTGTS libraries were sequenced via Illumina NextSeq550 using paired-end 150-bp sequencing kit or Miseq using paired-end 300-bp sequencing kit. Sequencing reads were processed as previously described\textsuperscript{15} and were aligned to AJ851868/mm9 hybrid genome as described above. Data were plotted for comparison after normalizing junction from each 3C-HTGTS library by random selection to the total number of chr12-wide junctions recovered from the smallest library in the set of comparing libraries. For G1-arrested Rad21-degron v-Abl pro-B cells with and without IAA treatment, 3C-HTGTS libraries using \textit{V}_{H}81X-CBE (Fig.1c, Extended Data Fig.2b) and \textit{iE}_{\mu} (Fig.1c, Extended Data Fig.2c) as bait were normalized to 9,844 and 74,339 total junctions from chromosome 12, respectively. For G1-arrested IGCR1\textsuperscript{-/-} Rad21-degron v-Abl pro-B cells with and without IAA treatment, 3C-HTGTS libraries using \textit{iE}_{\mu} as bait (Extended Data Fig.2d) were normalized to 74,339 total junctions from chromosome 12. For G1-arrested parental, non-treated and IAA-treated
CTCF-degron v-Abl pro-B cells and RAG2-deficient BM pro-B cells, 3C-HTGTS libraries using RC/iEμ, RC/DQ52-JH1, and RC/JH4 as bait were normalized to 333,127 total junctions from chromosome 12 (Fig. 4b, Extended Data Fig.5c, 7b, c). Chromosomal interaction patterns were very comparable before and after normalization. The sequences of primers used for generating 3C-HTGTS libraries are listed in Supplementary Table 3.

**ChIP-seq**

ChIP-seq was performed as previously described\(^{11,37}\) on G1-arrested parental v-Abl cells, Rad21-degron and CTCF-degron v-Abl cells without or with IAA treatment. In brief, 20 million cells in fresh medium were crosslinked with 1% formaldehyde for 10 min at 37 °C, and quenched with glycine at final concentration of 125 mM. Crosslinked samples were collected and lysed in cell lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP-40) followed by centrifugation to get the nuclei. Nuclei were lysed in nuclei lysis buffer (50 mM Tris-HCl pH 8.1, 10 mM EDTA, 1% SDS) and fragmented using Bioruptor at average size of 200-300 bp. Following the same procedure, fragmented chromatin from HCT116 cells were prepared and used as spike-in controls. Fragmented chromatin from G1-arrested cells with or without spike-in was precleared with 40 µl Dynabeads Protein A (ThermoFisher Scientific, #10002D) and then incubated with Rad21 (Abcam, #ab992) or CTCF (Millipore, #07-729) antibody in cold room overnight, followed by incubating with 40 µl Protein A beads for at least 2 hours. The immunoprecipitated DNA fragments were washed, eluted, de-crosslinked and purified for library preparation. ChIP-Seq libraries were prepared using NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB, #E7645) and sequenced by paired-end 75-bp sequencing kit on Illumina NextSeq550.

**ChIP-seq analysis**

We used bowtie2 to align reads to reference genome, used MACS2 to obtain the IP peak signal tracks after normalized to 1 million IP reads. Results were displayed in IGV. Rad21 ChIP-seq on Rad21-degron v-Abl pro-B cells with and without IAA treatment (Fig.1b, Extended Data Fig.2a) were performed with human HTC116 chromatin as spike-in\(^{57}\). For Rad21 ChIP-seq on parental and IAA-treated CTCF-degron v-Abl pro-B cells, one repeat was performed without human HTC116 chromatin as spike-in (Fig.2b, bottom panels), and two repeats were performed with human HTC116 chromatin as spike-in (Extended Data Fig.6a, bottom panels). Three repeats show very reproducible results. For CTCF ChIP-seq
on parental and IAA-treated CTCF-degron v-Abl pro-B cells, all three repeats (Fig.2b, Extended Data Fig.6a, top panels) were performed without human HCT116 chromatin as spike-in. For ChIP-seq without human spike-in chromatin, reads were aligned to mouse AJ851868/mm9 hybrid genome and libraries were normalized to 1 million reads for display. For ChIP-seq with human spike-in chromatin, reads were aligned to the mixture genome of mouse (AJ851868/mm9) and human (hg19), and the mouse IP signal track was normalized to 1 million human IP reads, after adjusting the input spike-in chromatin ratio to be 1:1. To estimate and adjust for the actual spike-in chromatin ratio, we counted the number of reads aligned to human or mouse genome in the IP-corresponding input library, and calculated their ratio. We finally scaled the mouse IP signal track by multiplying its normalization factor = (1 million)/(human IP read number) /([((mouse input read number)/(human input read number))] for display. For the samples that were processed with or without spike-in controls, we compared the results obtained from both ways and found little differences between them.

**GRO-seq and data analyses**

GRO-seq libraries were prepared as described previously on G1-arrested parental v-Abl cells, Rad21-degron and CTCF-degron v-Abl cells without or with IAA treatment. Briefly, 10 million cells were collected and permeabilized with the DEPC treated buffer (10 mM Tris-HCl pH 7.4, 300 mM sucrose, 10 mM KCl, 5 mM MgCl2, 1 mM EGTA, 0.05% Tween-20, 0.1% NP40 substitute, 0.5 mM DTT, protease inhibitors and RNase inhibitor). The permeabilized cells were resuspended in 100 µl of DEPC treated storage buffer (10 mM Tris-HCl pH 8.0, 25% (v/v) glycerol, 5 mM MgCl2, 0.1 mM EDTA and 5 mM DTT) followed by nuclear run-on with 100 µl 2X run-on mix (5 mM Tris-HCl pH 8.0, 2.5 mM MgCl2, 0.5 mM DTT, 150mM KCl, 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 0.5 mM Br-UTP, RNase inhibitor, 1% Sarkosyl) at 37 °C for 5 min. Total RNA was extracted by Trizol and followed by hydrolyzation with NaOH at a final concentration of 0.2 N on ice for 18 min. After quenching with ice-cold Tris-HCl pH 6.8 at a final concentration of 0.55 M and exchanging buffer via Bio-Rad P30 columns, the total RNA was incubated with Br-dU antibody-conjugated beads (Santa Cruz, #sc-32323-ac) for 1 h. The enriched Br-dU labeled RNAs were incubated with RppH (NEB, #M0356S) and with T4 PNK (NEB, #M0201S) for hydroxyl repair, followed by ligating the 5’ and 3’ RNA adaptors. RT-PCR was performed after adaptor ligation to obtain cDNAs. Half of the cDNAs was subjected to library
preparation by two rounds of PCR with barcoded primers. The second round of PCR products were purified by AMPure beads (Beckman Coulter, #A63880). GRO-seq libraries were sequenced via paired-end 75 bp sequencing on Illumina NextSeq550. For GRO-seq analysis, we used bowtie2 to align GRO-seq reads to AJ851868/mm9 hybrid genome, and run MACS2 to generate bigwig graph. To visualize the genome-wide RNA expression level, we run htseq-count to count read number on each gene, and made scatter plot after down-sampling each sample to 10 million reads mappable to any genes. We used DESeq2 to call differentially expressed genes. Each experiment was repeated three times with multiple biologically independent clones.

Quantification and statistical analysis

An unpaired, two-tailed Student’s t test was used to determine the statistical significance of differences between samples. At least three repeats were done for each statistical analysis. P values are calculated and shown in the figures as the follows: non-significant (NS): P > 0.05, *: P ≤ 0.05, **: P ≤ 0.01, and ***: P ≤ 0.001.

Data availability

HTGTS-V(D)J-seq, 3C-HTGTS, ChIP-seq, and GRO-seq sequencing data reported in this study have been deposited in the GEO database under the accession number GSE142781. Specifically, HTGTS-V(D)J-seq data related to Fig. 1d, e, 3b-d, Extended Data Fig. 5b, and Supplementary Tables 1, 2 are deposited in the GEO database under the accession number GSE142777. 3C-HTGTS data related to Fig. 1c, 4b, and Extended Data Fig. 2b-d, 5c, 7b, c are deposited in the GEO database under the accession number GSE142778. ChIP-seq data related to Fig. 1b, 2b, and Extended Data Fig. 2a, 6a are deposited in the GEO database under the accession number GSE142779. GRO-seq data related to Fig. 2c, and Extended Data Fig. 3, 6b are deposited in the GEO database under the accession number GSE142780.

Code availability

HTGTS-V(D)J-seq, 3C-HTGTS, ChIP-seq, and GRO-seq data were processed through the published pipelines as previously described. Specifically, these pipelines are available at [http://robinmeyers.github.io/transloc_pipeline/](http://robinmeyers.github.io/transloc_pipeline/) (HTGTS pipeline), [http://bowtie-bio.sourceforge.net/bowtie2/index.shtml](http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) (Bowtie2 v.2.2.8), [https://sourceforge.net/projects/samtools/files/samtools/1.8/](https://sourceforge.net/projects/samtools/files/samtools/1.8/) (SAMtools v.1.8) and
http://rseqc.sourceforge.net/# bam2wig-py (RSeQC tool v.2.6).

51. Shinkai, Y. et al. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* **68**, 855–867 (1992).

52. Strasser, A. et al. Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8661–8665 (1991).

53. Cong, L. et al. Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 (2013).

54. Frock, R. L. et al. Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. *Nat. Biotechnol.* **33**, 179–186 (2015).

55. Sakaue-Sawano, A. et al. Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* **132**, 487–498 (2008).

56. Medvedovic, J. et al. Flexible long-range loops in the VH gene region of the Igh locus facilitate the generation of a diverse antibody repertoire. *Immunity* **39**, 229–244 (2013).

57. Orlando, D. A. et al. Quantitative ChIP-Seq normalization reveals global modulation of the epigenome. *Cell Rep* **9**, 1163–1170 (2014).

58. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
**Fig. 1**

(a) Proximal V\(_H\)s

(b) Rad21 ChIP-seq profiles

(c) 3C-HTGTS using the V\(_H\)81X-CBE\(^+\) or RC/iE\(_\mu\)\(^+\) locale as bait

(d) HTGTS-V(D)J-seq using J\(_H\)4-CE as bait (in the presence of IGCR1)

(e) HTGTS-V(D)J-seq using J\(_H\)4-CE as bait (in the absence of IGCR1)
Fig. 2

a) CTCF/Rad21 ChIP-seq profiles at Igh

b) GRO-seq profiles at Igh

c) CTCF binding

CTCF-degraded

Parental

VH81X

VH1-67

VH1-11

VH5-6

VH2-3

CBE1

Rad21 binding

Parental

VH81X

VH1-67

VH1-11

VH5-6

VH2-3

CBE1

GRO-seq profiles at Igh

RC

Parental

0

1000

800

Antisense

Sense

0

1000

800

Antisense

chr12:114,400,000–117,638,400
Fig. 3

a

Distal $V_{\mu s}$ Proximal

IGCR1 $Ds$ $J_{\mu s}$ $iE_{\mu}$ $C_{\mu s}$ $3'RR$ $3'CBEs$ Cen.

Recombination Center (RC)

b

Parental $v$-Abl cells

Normalized $V_H$ usage

VDJ$_H$ DJ$_H$

% 100 80 60 40 20 0

$V_{\mu 81X}$ $V_{\mu 2-2}$ $V_{\mu 5-4}$ $V_{\mu 2-3}$ $V_{\mu 5-6}$ $V_{\mu 5-1}$ $V_{\mu 5-12}$

c

CTCF-degraded $v$-Abl cells

Normalized $V_H$ usage

VDJ$_H$ DJ$_H$

% 100 80 60 40 20 0

$V_{\mu 1-11}$ $V_{\mu 1-67}$ $V_{\mu 14-4}$ $V_{\mu 9-2-1}$ $V_{\mu 5-4}$ $V_{\mu 5-1}$ $V_{\mu 81X}$ $V_{\mu 2-2}$

d

Mouse BM pro-B cells

Normalized $V_H$ usage

VDJ$_H$ DJ$_H$

% 100 80 60 40 20 0

$V_{\mu 1-11}$ $V_{\mu 1-67}$ $V_{\mu 14-4}$ $V_{\mu 9-2-1}$ $V_{\mu 5-4}$ $V_{\mu 5-1}$ $V_{\mu 81X}$ $V_{\mu 2-2}$

Distal $V_{\mu s}$ Proximal
3C-HTGTS using RC/iEμ* locale as bait

Fig. 4

chr12:114,400,000–117,638,400
a) Proteasome ubiquitination by SCF

b) % of cells

Mouse Rad21 locus

Mouse Rosa26 locus

c) Mouse Rad21 locus

d) Mouse Rosa26 locus

e) Timeline for cell viability assay

f) Rad21-degron v-Abl cells

g) Fucci cell cycle reporter:
Rad21 ChIP-seq profiles

Repeat #2
+IAA

3C-HTGTS using the V_{i}81X-CBE\(^{+}\) locale as bait

Repeat #2
+IAA

Repeat #3
+IAA

3C-HTGTS using the RC/iE\(_{i\mu}^{+}\) locale as bait (in the presence of IGCR1)

Repeat #2
+IAA

Repeat #3
+IAA

3C-HTGTS using the RC/iE\(_{i\mu}^{+}\) locale as bait (in the absence of IGCR1)

Repeat #1
+IAA

Repeat #2
+IAA

chr12:114,440,000–114,915,000
GRO-seq profiles at IgH

Repeat #1
NT
+IAA

Repeat #2
NT
+IAA

Repeat #3
NT
+IAA

Genome-wide GRO-seq analyses highlighting V(D)J recombination and chromatin interaction related genes

Repeat #1
+IAA
NT

Repeat #2
+IAA
NT

Repeat #3
+IAA
NT
**Extended Data Fig. 4**

**a** CTCF protein turnover. CTCF interacts with zinc fingers to recruit CBP/P300 to the CTCF-binding site. SCF-OsTir1 ubiquitinates CTCF to target it for degradation by the proteasome. CTCF degradation results in the loss of the CTCF-dependent CTCF-mAID-GFP reporter.

**b** Flow cytometry analysis of GFP expression in target cells. +IAA/6h treatment results in a significant increase in the number of GFP-positive cells compared to NT and +IAA/STI treatments.

**c** Mouse Ctfc genomic locus. The targeted allele includes a loxpP flanked mAID-GFP cassette. After Cre deletion, the mAID-GFP cassette is removed, allowing for CTCF degradation.

**d** Genomic analysis of Cre deletion efficiency. Digestion with BglII and SspI shows a decrease in the 8.0kb band, indicating Cre-mediated deletion.

**e** OsTir1 targeting into Rosa26 locus. Digestion with BglII probes A and B shows specific bands indicating Cre-mediated targeting.

**f** Fucci cell cycle reporter. TagBFP-hGeminin and mCherry-hCdt1 reporters are used to monitor cell cycle progression. +IAA and +IAA/STI treatments show a decrease in viable cells over time, whereas parental and +STI treatments show minimal changes.

**g** Timeline for cell viability assay. The assay is performed over 4 days, with +IAA and +IAA/STI treatments showing a significant decrease in viable cells compared to parental and +STI treatments.
a

Extended Data Fig. 5

b

Parental v-Abi cells

NT CTCF-degron v-Abi cells

Normalized V\textsubscript{H} usage

800

600

400

200

0

Distal

V\textsubscript{H}s

Proximal

V\textsubscript{H}1-11

V\textsubscript{H}s

V\textsubscript{H}1-67

V\textsubscript{H}1-11

V\textsubscript{H}14-4

V\textsubscript{H}9-2-1

V\textsubscript{H}2-3

V\textsubscript{H}s

V\textsubscript{H}1-67

V\textsubscript{H}1-11

V\textsubscript{H}14-4

V\textsubscript{H}9-2-1

V\textsubscript{H}2-3

V\textsubscript{H}s

V\textsubscript{H}1-67

V\textsubscript{H}1-11

V\textsubscript{H}14-4

V\textsubscript{H}9-2-1

V\textsubscript{H}2-3

V\textsubscript{H}s

V\textsubscript{H}1-67

V\textsubscript{H}1-11

V\textsubscript{H}14-4

V\textsubscript{H}9-2-1

V\textsubscript{H}2-3

NlaIII

chr12:114,400,000–117,638,400

Cen.

IGCR1

Recombination Center (RC)

DQ52

J\textsubscript{H}1

RC

Distal

Proximal

V\textsubscript{H}s

Parental v-Abi cells

NT CTCF-degron v-Abi cells

Normalized V\textsubscript{H} usage

800

600

400

200

0

Distal

V\textsubscript{H}s

Proximal

V\textsubscript{H}1-11

3'CBEs

Parental v-Abi cells

NT

v-Abi cells

3C-HTGTS using RC/iE\textsubscript{\mu}\textsuperscript{*} locale as bait

3C-HTGTS using RC/DQ52-J\textsubscript{H}1\textsuperscript{*} locale as bait

3C-HTGTS using RC/J\textsubscript{\mu}4\textsuperscript{*} locale as bait

chr12:114,400,000–117,638,400
Extended Data Fig. 6

CTCF/Rad21 ChIP-seq profiles at Igh

CTCF binding

Parental
CTCF-degraded

Parental
CTCF-degraded

Rad21 binding

Parental
CTCF-degraded

Parental
CTCF-degraded

GRO-seq profiles at Igh

b

GRO-seq profiles at Igh

b

 chr12:114,400,000–117,638,400
Extended Data Fig. 7

(a) Schematic representation of the genomic region around the Recombination Center (RC) on chromosome 12. The RC is indicated by the red box. The genomic region is shown with restriction enzyme sites (NlaIII) indicated. The V\textsubscript{H} genes and C\textsubscript{\textmu} genes are shown. The distances between the sites are indicated.

(b) Diagram showing the 3C-HTGTS experiments using RC/DQ52-J\textsubscript{H}1* locale as bait. The V\textsubscript{H} genes and the C\textsubscript{\textmu} genes are shown with peaks indicating the distances between them.

(c) Diagram showing the 3C-HTGTS experiments using RC/J\textsubscript{H}4* locale as bait. The V\textsubscript{H} genes and the C\textsubscript{\textmu} genes are shown with peaks indicating the distances between them.
Extended Data Fig. 8

A working model

Mouse V<sub>H</sub>s (~2.4 Mb)

J558/3609

J558

Middle

7183/Q52

Distal

Proximal

(a) Normal CTCF activity

RAG1/2

DJ<sub>H</sub>RC

IGCR1

Cohesin

V<sub>H</sub>S<sub>1</sub>

(b) Downregulated CTCF activity

Extrusion/scanning impeded by transcription

Extrude

(c) Downregulated CTCF activity

Extrusion/scanning impeded by CBE with residual CTCF-binding

VDJ<sub>H</sub>

(d) Downregulated CTCF activity

VDJ<sub>H</sub>

(e) Extrude

CBEs (normal CTCF binding)

CBEs (depleted CTCF binding)

CBEs (residual CTCF binding)

Cohesin

Transcription

TFs

23RSS

12RSS