Contribution of the IGCR1 regulatory element and the 3' Igh CBEs to Regulation of Igh V(D)J Recombination

Zhuoyi Liang\textsuperscript{a,b,c,1,2}, Lijuan Zhao\textsuperscript{a,b,c,1}, Adam Yongxin Ye\textsuperscript{a,b,c,1} Sherry G. Lin\textsuperscript{a,b,c,1}, Yiwen Zhang\textsuperscript{a,b,c}, Chunguang Guo\textsuperscript{a,b,c}, Hai-Qiang Dai\textsuperscript{a,b,c,d}, Zhaoqing Ba\textsuperscript{a,b,c,2}, Frederick W. Alt\textsuperscript{a,b,c,2,3}

\textsuperscript{a}HHMI, Boston Children’s Hospital, Boston, MA 02115; \textsuperscript{b}Program in Cellular and Molecular Medicine, Boston Children’s Hospital, Harvard Medical School, Boston, MA 02115; \textsuperscript{c}Department of Genetics, Harvard Medical School, Boston, MA 02115; \textsuperscript{d}Present address: Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, Shanghai, China; \textsuperscript{e}Present address: National Institute of Biological Sciences, #7 Science Park Rd, Beijing, 102206, China

\textsuperscript{1}Equal Contribution

\textsuperscript{2}Z.L., Z.B., F.W.A. share senior authorship.

\textsuperscript{3}To whom correspondence may be addressed. Email: alt@enders.tch.harvard.edu;

**Author Contributions:** Z.L., L.Z., S.G.L. Z.B., and F.W.A. designed the study. Z.L., L.Z., S.G.L., Y. Z., H-Q.D., and Z.B. performed experiments. S.G.L., C.G., and Z.B. generated IGCR1 CBEs deletion and inversion mutant mouse lines and their \textit{v-Abl} transformed pro-B cell line derivates. Z.L., A.Y.Y., S.G.L., Z.B. and F.W.A. analyzed data. Z.L., A.Y.Y., Z.B. and F.W.A. prepared the figures; Z.L., L.Z., Z.B. and F.W.A. drafted the manuscript. All authors helped polish the manuscript.

**Competing Interest Statement:** None.

**Classification:** Biological Sciences; Immunology.

**Keywords (3-5):** V(D)J Recombination, CTCF-binding elements (CBEs), CTCF, Antibody Repertoires, Chromatin 3D structure

This PDF file includes:
- Main Text
- Figures 1 to 5
ABSTRACT (249 words)

Immunoglobulin heavy chain variable region exons are assembled in progenitor-B cells, from \( V_H \), \( D \), and \( J_H \) gene segments located in separate clusters across the \( Igh \) locus. RAG endonuclease initiates V(D)J recombination from a \( J_H \)-based recombination center (RC). Cohesin-mediated extrusion of upstream chromatin past RC-bound RAG presents Ds for joining to \( J_H \)s to form a DJH-RC. \( Igh \) has a provocative number and organization of CTCF-binding-elements (CBEs) that can impede loop extrusion. Thus, \( Igh \) has two divergently oriented CBEs (CBE1 and CBE2) in the IGCR1 element between the \( V_H \) and D/JH domains, over 100 CBEs across the \( V_H \) domain convergent to CBE1, and 10 clustered 3'\( Igh \)-CBEs convergent to CBE2 and \( V_H \) CBEs. IGCR1 CBEs segregate D/JH and \( V_H \) domains by impeding loop extrusion-mediated RAG-scanning. Down-regulation of WAPL, a cohesin unloader, in progenitor-B cells neutralizes CBEs, allowing DJH-RC-bound RAG to scan the \( V_H \) domain and perform \( V_H \)-to-DJH rearrangements. To elucidate potential roles of IGCR1-based CBEs and 3'\( Igh \)-CBEs in regulating RAG-scanning and elucidate the mechanism of the "ordered" transition from D-to-\( J_H \) to \( V_H \)-to-DJH recombination, we tested effects of deleting or inverting IGCR1 or 3'\( Igh \)-CBEs in mice and/or progenitor-B cell lines. These studies revealed that normal IGCR1 CBE orientation augments RAG-scanning impediment activity and suggest that 3'\( Igh \)-CBEs reinforce ability of the RC to function as a dynamic loop extrusion impediment to promote optimal RAG scanning activity. Finally, our findings indicate that ordered V(D)J recombination can be explained by a gradual WAPL down-regulation mechanism in progenitor B cells as opposed to a strict developmental switch.
SIGNIFICANCE STATEMENT (119 words)

To counteract diverse pathogens, vertebrates evolved adaptive immunity to generate diverse antibody repertoires through a B lymphocyte-specific somatic gene rearrangement process termed V(D)J recombination. Tight regulation of the V(D)J recombination process is vital to generating antibody diversity and preventing off-target activities that can predispose the oncogenic translocations. Recent studies have demonstrated V(D)J rearrangement is driven by cohesin-mediated chromatin loop extrusion, a process that establishes genomic loop domains by extruding chromatin, predominantly, between convergently-oriented CTCF looping factor-binding elements (CBEs). By deleting and inverting CBEs within a critical antibody heavy chain gene locus developmental control region and a loop extrusion chromatin-anchor at the downstream end of this locus, we reveal how these elements developmentally contribute to generation of diverse antibody repertoires.
INTRODUCTION

Variable region exons that encode antigen-binding sites of antibodies are assembled in progenitor ("pro")-B cells from germline V_H, D, and J_H gene segments (1). V(D)J recombination is initiated by RAG1/2 endonuclease (RAG) (2). RAG introduces DNA double-stranded breaks (DSBs) between V_H, D, and J_H coding segments and flanking recombination signal sequences (RSSs) (2). RSSs comprise a conserved heptamer, a spacer of 12 or 23 base pairs, and an AT-rich nonamer. To robustly initiate V(D)J recombination, RAG must bind and cleave a pairs of gene segments flanked by RSSs with complementary 12 and 23 bp spacers (termed 12-RSSs and 23-RSSs respectively) (2). After RAG cleavage, 12/23-RSS matched gene segment ends and, separately, their RSS ends are fused by the classical nonhomologous end-joining (3). The mouse IgH locus (Igh) spans 2.7 megabases (Mbs) on chromosome 12 with over 100 V_Hs interspersed within a several Mb distal portion (1). This V_H domain lies 100 kb upstream of a 50 kb region containing up to 13 Ds, with 4 J_Hs embedded within a 2 kb region just downstream of the most proximal D (DQ52) (1). RAG initiates Igh V(D)J recombination from a recombination center (RC) formed within highly transcribed chromatin that spans DQ52, the four J_Hs, and the intronic enhancer (iE_Mu) (4, 5). The V_Hs and J_Hs have 23RSSs and cannot be directly joined. Ds are flanked on either side by 12RSSs, allowing them to join to a downstream J_H and an upstream V_H to form a V(D)J exon (3). V(D)J recombination is developmentally ordered with Ds joined to a J_H to form a DJ_H RC, after which V_Hs are joined to the upstream D12RSS of the DJ_H RC (3).

The CTCF chromatin looping factor binds target DNA sequences, termed CTCF binding elements ("CBEs or "CTCF sites"), in an orientation-specific manner (6, 7). In this regard, the numerous genomic CBEs, when in adjacent regions, can occur in the same, divergent, or in convergent orientations (8). The cohesin complex mediates extrusion of chromatin loops genome-wide, forming contact loops when extrusion in each direction reaches CTCF-bound CBEs that impede extrusion (9, 10). Such CBE-anchored chromatin loops occur most dominantly between CBEs in convergent orientation (8, 11), an orientation that forms the most stable anchor (9, 10). Convergent CBE orientation has been implicated in mediating physiological functions (12–17). However, CTCF-bound CBEs can impede loop extrusion regardless of orientation (18), and non-CBE-based impediments, for example highly transcribed chromatin, can impede extrusion and contribute to developmental or tissue-specific regulation of loop extrusion (18–24). The Igh contains numerous CBEs with remarkable relative orientation. The IGCR1 element in the V_H to D domain just upstream of the distal DFL16.1 has two
divergently-oriented CBEs, namely upstream CBE1 and downstream CBE2 (25). The $V_H$ domain has over 100 CBEs with most lying in convergent orientation to IGCR1 CBE1 (26). Ten consecutive CBEs, termed 3'$Ig\!h$ CBEs (27, 28) lie at the just downstream of $Ig\!h$ in convergent orientation to IGCR1 CBE2 and $V_H$ domain CBEs (25, 26). This organization has been proposed to have various functions in regulating V(D)J recombination (25, 29–31).

Cohesin-mediated loop extrusion provides the mechanistic underpinnings for RC-bound RAG to scan chromatin across the $Ig\!h$ locus for substrates (18, 19, 22, 24, 32). In pro-B cells, RC-bound RAG initiates scanning upon binding a JH-23RSS into one of its two active sites (1, 18, 24). For this scanning process, the active RC, which lacks CBEs, serves as a transcription-based dynamic downstream loop extrusion anchor, while IGCR1 serves as a CBE-based upstream anchor that terminates RAG scanning, preventing scanning from entering the $V_H$ domain (18, 24, 33). The upstream orientation of the RAG-bound JH programs RAG scanning of upstream D-containing chromatin extruded past the RC (24). During RAG scanning of the D locus, only downstream D-12RSSs, convergently oriented to the JH23RSSs, are used, resulting in D-to-JH joining that deletes all sequences between the participating D and JH (24). Predominant utilization of RSSs, or cryptic RSSs, in convergent orientation to the initiating RC RSS is a mechanistic property of the linear RAG scanning process (22, 32).

While $V_{H5}$-23RSSs are compatible for joining to D12-RSSs, the IGCR1 impedes access of $V_{H5}$ to the D locus during the D-to-JH rearrangement and, thereby, enforces ordered D-to-JH before $V_H$-to-$DJ_H$ V(D)J recombination in pro-B cells (18, 25, 32). Mutational inactivation of IGCR1 CBEs allows RC-bound RAG to scan directly into the proximal $V_H$ locus, causing the most proximal functional $V_{H5}$-2 to robustly rearrange and dominate the $V_H$ repertoire, with little rearrangement of more distal $V_{H5}$ (18, 25, 32). The mechanism by which $V_{H5}$-2 dominates rearrangement when IGCR1 CBEs are inactivated is based on its RSS-associated CBE that impedes extrusion past the RC, making it accessible for rearrangement (18). Indeed, dozens of the proximal $V_{H5}$ have RSS-associated CBEs. Thus, while deletion of the $V_{H5}$-2 CBE results in a 50-fold reduction of $V_{H5}$-2 rearrangement along with greatly reduced RC interaction, the next upstream $V_H$ becomes dominantly rearranged based on its RSS-associated CBE (18). Because dozens of the D-proximal $V_{H5}$ have RSS-associated CBEs, this portion of the $V_H$ locus is a major barrier to RAG scanning to further upstream $V_{H5}$ when IGCR1 CBEs are inactivated (18).

Fluorescent in situ hybridization and chromosome conformation capture (3C)-based studies revealed the $V_H$ domain to undergo large-scale contraction in pro-B cells (34–42). $V_H$
locus contraction was proposed to bring distal VHs into proximity with the DJH RC for recombinational access (43). Recent studies revealed that locus contraction is mediated by loop extrusion, which extends across the several Mb V_H locus due to approximately 4-fold down-regulation of the WAPL cohesin unloading factor in pro-B cells (44). In this context, depletion of WAPL in non-lymphoid cells extends genome-wide loop extrusion by increasing cohesin density, allowing it to bypass CBEs and potentially other impediments (45–47). WAPL down-regulation in pro-B cells down-regulates impediment activity of IGCR1 CBEs, proximal V_H-associated CBEs, and other impediments, allowing RAG scanning from a DJH RC to extend linearly across the V_H domain (22). Abelson murine leukemia virus-transformed pro-B cell lines ("v-Abl lines") can be viably arrested in the G1-cell cycle phase in which V(D)J recombination occurs (48). Introduction of RAG into G1-arrested RAG-deficient v-Abl lines activates robust RAG scanning across the D domain (19, 22, 24). However, scanning is impeded at IGCR1 and there is little V_H-to-DJH joining (19, 22, 24). Inactivation of IGCR1 CBEs leads to dominant rearrangement of V_H5-2 in v-Abl lines due to its RSS-associated CBE (18). In this regard, v-Abl lines have high WAPL levels and do not neutralize V_H locus CBEs (19, 22). Neutralization of CBE impediments by depletion of CTCF or WAPL extends cohesin loop extrusion and RAG-scanning past IGCR1 and proximal V_HS to the most distal V_HS (19, 22). Thus, v-Abl pro-B cell studies have provided substantial mechanistic insights into locus contraction and RAG (1).

Despite recent advances in elucidating functions of Igh CBEs during V(D)J recombination, many questions remain. While an early study indicated that IGCR1 CBEs act synergistically to segregate the V_H domain from the D/JH domain (49), the question of whether orientation of these CBEs is critical to their function remained. Likewise, 3'Igh CBEs confine Igh class switch recombination (CSR) activity to the Igh (50). However, their roles in V(D)J recombination have not been resolved (1). Finally, a long-standing question is how the developmental transition from D-to-JH joining to V_H-to-DJH joining is regulated. While a role for the DJH intermediate in signaling the transition (51, 52) has been considered, such a transition could, in theory, be mediated by gradual WAPL-down-regulation during pro-B cell development (1). We now describe studies that address these questions.

RESULTS
Role of IGCR1 CBEs in D-to-JH and Proximal V_H-to-DJH rearrangements.

To gain insight into factors that determine primary BM pro-B cell V_H repertoires, we assessed effects of IGCR1 CBE1 and CBE2 inactivation via high resolution HTGTS-V(D)J-Seq
For these assays, we purified B220⁺CD43ʰɪᵍʰIgM⁻ pro-B cells from bone marrow (BM) of wild-type (WT) 129SV controls and previously generated IGCR1/CBE1&2⁻/⁻ mice. We performed HTGTS-V(D)J-Seq on DNA from these samples using a JH4 bait primer to compare their levels of D-to-JH and VH-to-DJH rearrangements (Fig.1A and SI Appendix, Table S1). A JH4 bait primer was used for HTGTS-V(D)J-Seq analyses in this study to eliminate potential confounding effects of rearrangements within extra-chromosomal deletion products. For these analyses, individual peaks found in HTGTS libraries can be normalized as a fraction of total HTGTS reads for a given experiment, which reveals absolute V(D)J levels of each rearranging gene segment. Alternatively, peaks can be normalized as a fraction of total recovered junctions across a given locus or section of a locus to reveal the relative utilization of a given gene segments to each other as a percentage of all junctions in the analyzed region (SI Appendix, Fig. S1). Such analyses are useful for examining effects of potential regulatory element mutations. For example, finding a decrease in total reads between two samples in the absence of differences in the junction profile, reflects decreased RAG or RC activity without changes in long range scanning patterns.

WAPL down-regulation in mouse pro-B cells allows RAG to scan the entire VH locus, which results in highly reproducible utilization of the different VHs (Fig. 1B,C). However, in WT pro-B cells, VH5-2 (“VH81X”) and three immediately upstream VHs with RSS-associated CBEs are much more highly utilized than any VH further upstream (Fig. 1B, C). In such steady-state BM pro-B cell populations, DJH rearranged alleles are more prevalent than VH(D)JH rearranged alleles (Fig. 1B, inset), likely reflecting steady-state distributions within pro-B cells entering the compartment and successively generating DJH and VH-to-DJH rearrangements before leaving the compartment. Inactivation of IGCR1 by mutational inactivation of CBE1 and CBE2, allows VH5-2 to dominate the VHDJH repertoire (Fig.1D,E). Moreover, the great majority of VH5-2 rearrangements are non-productive (SI Appendix, Fig. S2 and Table S2), consistent with selection against productive VH5-2 rearrangements. This finding also confirms that dominant VH5-2 rearrangements in pro-B cells do not result from cellular selection (25). Rearrangement frequencies of more distal VHs are dramatically decreased upon IGCR1 inactivation (Compare Fig. 1 panels B and C with panels D and E; also see SI Appendix, Table S1). Strikingly, however, in CBE1&2⁻/⁻ pro-B cell populations as compared to WT pro-B cell populations, the absolute level of VHDJH rearrangements is greatly increased with the vast
majority utilizing $V_\text{H}5-2$, while the absolute level of $DJ_\text{H}$ rearrangements and upstream $V_\text{H}$ rearrangements is, correspondingly, decreased (Fig. 1 E-G and SI Appendix, Table S1).

The above findings support the notion that, in the absence of IGCR1 CBE activity in normal pro-B cells, RAG scanning continues between the $DJ_\text{H}$ RC and $V_\text{H}5-2$, which allows $V_\text{H}5-2$ to dominate $V_\text{H}$-to-$DJ_\text{H}$ rearrangements due to its robust CBE-mediated interaction with the $DJ_\text{H}$ RC (18). Moreover, the finding that the increased frequency of $V_\text{H}$-to-$DJ_\text{H}$ rearrangements in the steady state CBE1&2$^{/-}$ pro-B results almost totally from increased $V_\text{H}5-2$ rearrangements indicates that these dominant rearrangements occur at the normal $DJ_\text{H}$ scanning stage before sufficient WAPL down-regulation neutralizes proximal $V_\text{H}$-RSS associated CBE impediments to allow upstream scanning. These high resolution HTGTS-V(D)J-seq studies also revealed another notable finding. Thus, despite the dramatically reduced levels of upstream $V_\text{H}$ rearrangements in CBE1&2$^{/-}$ pro-B cells, the upstream $V_\text{H}$s beyond the several most proximal $V_\text{H}$s, relative to each other, have rearrangement junction patterns (i.e. relative levels compared to each other), that were nearly identical to those of WT pro-B cells (Fig.1, Compare panels H and I). Thus, $V_\text{H}81X$, and to a lesser extent immediately upstream $V_\text{H}$s, dominate initial rearrangements in the absence of IGCR1 CBE activity, and, in doing so, terminate most RAG upstream scanning. However, RAG scanning that does proceed beyond $V_\text{H}5-2$ continues through the remainder of the $V_\text{H}$ locus, with similar $V_\text{H}$ usage patterns as those of WT cells. Overall, these findings indicate that, while most $V_\text{H}5-2$ rearrangements in CBE1&2$^{/-}$ pro-B cells occur before WAPL down-regulation, a small fraction of CBE1&2$^{/-}$ pro-B that do not form $V_\text{H}5-2$ rearrangements on one or both $Igh$ alleles undergo upstream $V_\text{H}$-to-$DJ_\text{H}$ recombination events at normal frequencies when WAPL-down-regulation reaches appropriate levels.

**Role of IGCR1/CBE1 and CBE2 in regulating RAG scanning into the $V_\text{H}$ locus.**

To further assess potential mechanisms by which IGCR1 CBE impediment activity is modulated to promote RAG scanning of the $V_\text{H}$ domain, we applied the highly sensitive 3C-HTGTS chromatin interaction assay to explore interactions of the RC-based $iE_\mu$ enhancer element with upstream and downstream $Igh$ locus chromatin domains in WT $rag2^{/-}$ and IGCR1/CBE1&2$^{/-}rag2^{/-}$ cultured pro-B cells derived from the corresponding mouse lines. RAG-deficient cells must be used for such assays to eliminate confounding effects of V(D)J recombination events on such interactions (18, 19, 22, 24). These studies revealed that the $iE_\mu$ /RC interacts robustly with 15 highly focused regions across the 2.4 Mb $V_\text{H}$ locus in WT $rag2^{/-}$ pro-B cells (Fig. 2A; SI Appendix, Fig. S3) (19, 22). Among the most robust of these $iE_\mu$ /RC
interacting peaks are peaks associated with robustly transcribed PAX5-activated intergenic repeat (PAIR) elements (38, 54, 55) in the J558, and J558/3609 V_H-containing regions in the distal portion of the V_H locus (Fig. 2A and SI Appendix, Fig. S3; Peaks 1,4,6,8-10). RC interactions with transcribed PAIR element-associated sequences are considered a hallmark of loop extrusion-mediated V_H locus contraction in pro-B cells (38). In this regard, locus contraction results from an approximately 4-fold developmental down-regulation of WAPL in pro-B cells(44), which at least partially neutralizes IGCR1-CBEs, proximal V_H CBEs, and likely other V_H locus CBEs, and potentially transcription-associated impediments to RC-based RAG linear scanning (22, 44). Although V_H5-2 and proximal V_Hs are the most dominantly utilized V_Hs in normal pro-B cells (Fig 1B), they show only low-level interactions with the RC in RAG2-deficient WT pro-B cells at steady-state (Fig. 2A, B), consistent with most CBE-based interactions being diminished by WAPL-downregulation in a large fraction of the pro-B cells (22). In this regard, some WT rag2^−/− pro-B cells retain interactions between the RC and IGCR1(Fig. 2B, upper), indicating that some cells in the population have not fully down-regulated WAPL and/or that IGCR1 impediment activity is not completely neutralized by physiological levels of WAPL down-regulation (Fig. 2B; upper).

Notably, in CBE1&2^−/− rag2^−/− cultured pro-B cells, the RC gained greatly increased interaction with proximal V_H5-2 and the 3 proximal V_Hs immediately upstream, which, based on prior studies (18), is dependent on their RSS-associated CBEs (Fig. 2A,B, and SI Appendix, Fig. S3; Peaks 16-18). Strikingly, nearly all major further upstream interaction peaks were also present in chromatin from CBE1&2^−/− rag2^−/− pro-B cells, mostly at similar relative levels to those in WT rag2^−/− pro-B cells (Fig. 2A. upper and lower). Given that the chromatin interactions are investigated in RAG-deficient cells and upstream interactions are not impacted by proximal V_H-to DJH recombination events, it is not unexpected that large number of IGCR1/CBEs mutated pro-B cells would have robust interactions between the RC to the upstream V_Hs after developmental WAPL down-regulation. As previously described (18), the RC also robustly interacts downstream with the transcribed enhancer-like element between Cγ1 and Cγ2b (38) and with the 3′Igh CBEs in pro-B cells (Fig. 2B, upper); these interactions were not altered by IGCR1 CBE1 and CBE2 deletion (Fig. 2B, lower).

**Influence of IGCR1 CBEs and their orientation on V_H-utilization in pro-B cells.**

We have previously generated CBE1^−/− and CBE2^−/− mice (49). To assess whether orientation of IGCR1/CBEs is critical for Igh V(D)J recombination control, we replaced CBE1
or CBE2 with their inverted sequences to generate an CBE1\textsuperscript{inv} or CBE2\textsuperscript{inv} alleles in mouse 129SV ES cells (SI Appendix, Fig. S4 A-B) (25, 49) (see Methods). We employed HTGTS-V(D)J-Seq to assay Jh4-based utilization of the various V\textsubscript{H}S in pro-B populations harboring IGCR1 CBE deletion or inversion mutations (Fig. 3). CBE1\textsuperscript{-/-} pro-B cell populations have markedly increased V\textsubscript{H}5-2 rearrangements and markedly decreased rearrangements of more distal V\textsubscript{H}S, with the degree of increases and decreases modestly, but significantly, less than those observed for CBE1&2\textsuperscript{-/-} pro-B cells (Fig. 3A, E and SI Appendix, Table S1). In contrast, CBE2\textsuperscript{-/-} pro-B cells had very modestly increased V\textsubscript{H}5-2 rearrangements (2.5-fold; Fig. 3 B, E) and little impact on more distal V\textsubscript{H} rearrangements (Fig. 3B, E and SI Appendix, Table S1). These findings indicate that CBE1 and CBE2 cooperatively provide the full impact of IGCR1 scanning impediment activities and unequivocally demonstrate that CBE1 plays a much more dominant role. CBE1\textsuperscript{inv/inv} BM pro-B cells also have significantly increased levels of proximal V\textsubscript{H}5-2 utilization relative to WT; but to a much lower extent than CBE1\textsuperscript{-/-} pro-B cells, indicating that ability of CBE1 to impede RAG scanning is dampened, but not abrogated, when inverted (Fig. 3C, E and SI Appendix, Table S1). In contrast, CBE2\textsuperscript{inv/inv} pro-B cells were very similar to WT pro-B cells with respect to utilization of V\textsubscript{H}5-2 and upstream V\textsubscript{H}S (Fig. 3D, E and SI Appendix, Table S1). Consistent with our findings for IGCR1/CBE1&2\textsuperscript{-/-} pro-B cells (Fig.1), the rearrangement pattern of upstream V\textsubscript{H}S, relative to each other, was not markedly impacted by CBE1 or CBE2 deletions or inversions (SI Appendix, Fig. S5 A-F and Table S1).

We performed 3C-HTGTS on RAG-deficient IGCR1/WT, IGCR1/CBE1&2\textsuperscript{-/-}, IGCR1/CBE1\textsuperscript{inv/inv} and IGCR1/CBE2\textsuperscript{inv/inv} \textsuperscript{\textmu}Abl lines with bait primers to the iE\textmu in the RC (SI Appendix, Fig. S6 A) and V\textsubscript{H}5-2 (SI Appendix, Fig. S6 B) locales. In CBE1\textsuperscript{-/-} pro-B cell populations, we observed robust, albeit somewhat diminished, interactions between the iE\textmu/RC bait and the proximal V\textsubscript{H}-CBEs compared to interactions in CBE1&2\textsuperscript{-/-} pro-B cells (SI Appendix, Fig. S6 A, CBE1\textsuperscript{-/-} vs. CBE1&2\textsuperscript{-/-}). In CBE2\textsuperscript{-/-} pro-B cells we observed a modest increase in the interactions between the iE\textmu/RC bait and the proximal V\textsubscript{H}-CBEs (SI Appendix, Fig. S6 A, CBE2\textsuperscript{-/-} vs. CBE1&2\textsuperscript{-/-}). These findings indicate that IGCR1 CBEs play a cooperative role in impeding loop extrusion-mediated proximal V\textsubscript{H}-CBEs and RC interactions and, again, that CBE1 has a more dominant role. In contrast, when CBE1 or CBE2 was inverted, interactions between proximal V\textsubscript{H}-CBEs and iE\textmu/RC showed only very modest changes compared to those when IGCR1 CBEs are in normal orientation (SI Appendix, Fig. S6 A-B). These studies confirm unequivocally that CBE1 and CBE2 function synergistically to provide the full RAG scanning.
impediment activity of IGCR1 and that CBE1 provides the major portion of this activity. Moreover, reminiscent of the effects of V_{H5-2} RSS-associated CBE inversion as compared to complete inactivation on proximal V_{H5-2} rearrangements (18), IGCR1 CBEs in their normal orientation provide physiological levels of RAG-scanning impediment activity, but retain significant activity when inverted.

**Influence of 3’Igh CBEs on D-to-JH and V_{H}-to-DJH rearrangement patterns and levels.**

To investigate potential contributions of 3’Igh CBEs on RAG scanning, we deleted all 3’CBEs in RAG1-deficient C57BL/6 WAPL-degron v-Abl cells that contain a single copy of the Igh locus (22). For analyses, WT or 3’CBEs-deleted (3’CBE-) WAPL-degron v-Abl cells were arrested in G1 and then left untreated or treated with Dox plus IAA to degrade WAPL (22). Subsequent introduction of RAG into untreated WT WAPL-degron v-Abl cells-activated V(D)J recombination leading to robust D-to-JH recombination, very low-level V_{H5-2} to DJH recombination, and extremely low-level upstream V_{H}-to-DJH recombination (Fig. 4A and SI Appendix, Table S3), as v-Abl lines have high WAPL levels and RAG scanning is impeded at IGCR1(22). Dox plus IAA treatment completely depletes WAPL in these G1-arrested v-Abl lines, but they remain substantially viable (22). Introduction of RAG into WAPL-depleted WT v-Abl cells activated D-to-JH rearrangement, but the level of DJH rearrangements was reduced 6.5-fold compared to that of untreated cells (Fig. 4I and SI Appendix, Table S3). This reduction was associated with dramatically decreased distal DFL16.1-JH and, to a lesser degree, most other DJH absolute rearrangement levels (Fig. 4M, N). Notably, however, DQ52 rearrangement levels showed little change (Fig. 4M). WAPL-depletion also led to RAG-scanning and utilization of V_{HS} across the 2.4 Mb V_{H} locus (Fig. 4 A,B and SI Appendix, Table S3) as expected (22). The absolute level of V_{H} DJH rearrangements in WAPL-depleted WT v-Abl cells was similar to that of the low level of proximal V_{H} rearrangements in untreated cells; but represented a 5.3-fold increase with respect to their fraction of DJH rearrangements (Fig. 4 I, J; see Discussion). In both untreated and WAPL-depleted 3’CBE- lines, we observed a further 25% decrease in DJH rearrangement levels from their baseline levels. We observed a similar 25% decrease in V_{H} DJH rearrangements in untreated 3’CBE- lines and a nearly 50% decrease in WAPL-depleted 3’CBE- lines (compare Fig. 4 panels B and D, Fig.4 I-L). Despite the further reduction of V_{H} DJH rearrangement levels in WAPL-depleted 3’CBE- v-Abl cells, their relative V_{H} usage pattern was very similar to that of WAPL-depleted WT lines (compare Fig. 4, panel F and H).
We performed 3C-HTGTS in RAG-deficient WT and 3’CBE- v-Abl cells baiting from iEμ/RC (Fig. 5 A, B and SI Appendix Fig. S7 B, C, S8). These studies revealed, as previously described (22), that complete depletion of WAPL substantially increased a number of interaction peaks in the J558/3609, J558 and middle VH regions (Fig. 5 A and SI Appendix, Fig. S8 Dox+IAA vs untreated; Peaks 1-13). Many of the sequences that contribute to these peaks were highly transcribed including the well-characterized PAIR elements (Fig. 5A and SI Appendix, Fig. S8; Peaks, 1-8). In contrast, peaks in the proximal 7183/Q52 region that are dominant in untreated v-Abl cells are mainly associated with proximal V_H RSS-CBEs and were significantly diminished by WAPL depletion (Fig. 5 A, and SI Appendix, Fig. S8, Dox+IAA vs untreated; Peaks 14-16). Notably, in 3’CBE- cells, these same major interaction peaks, including those associated with proximal V_HRSS-CBEs in untreated and those associated with the upstream VH regions in treated and untreated cells, remain robust and largely correspond to those in the same locations as in the WT line (Fig. 5 A and SI Appendix, Fig. S8, 3’CBE- vs WT). While the intensity of some peaks in the untreated or WAPL-depleted 3’CBE- v-Abl cells were somewhat diminished compared those of the untreated or WAPL-depleted WT v-Abl cells, when viewed at high resolution they are clearly still associated with same transcriptional or CBE impediments (Fig. 5 A, and SI Appendix, Fig. S8). Upon the deletion of 3’Igh CBEs, multiple CBEs downstream of the 3’Igh CBEs appear to gain robust interactions with the iEμ/RC (Fig. 5B). Finally, WAPL depletion also substantially diminishes interactions of IGCR1 CBE1 with both CBE-based (proximal VHs and 3’Igh CBEs and transcription-based (RC and γ1-γ2b enhancer) loop extrusion impediments (Fig. 5C).

**DISCUSSION**

HTGTS-V(D)J-Seq analyses provided a deep analysis of V_H repertoires in primary WT and IGCR1/CBE1&2−/− pro-B cells (Fig. 1). In addition, 3C-HTGTS-Seq analyses of iEμ/RC interactions across the Igh locus in RAG2-deficient primary WT and IGCR1/CBE1&2−/− pro-B cells complemented HTGTS-V(D)J-Seq studies to reveal a likely mechanism by which ordered transition from D-to-J_H versus VH-to-DJ_H rearrangement is regulated (Fig. 2). Our overall findings suggest a model in which robust D-to-J_H rearrangements occur in WT pro-B cells before WAPL is sufficiently down-regulated to allow scanning to pass IGCR1 or proximal V_H-associated CBEs. In CBE1&2−/− pro-B cells, robust iEμ/RC interactions with proximal V_HRSS-associated CBEs promote their robust rearrangements and suppress scanning to upstream V_HS. Our finding that low-level rearrangements of upstream V_HS in CBE1&2−/− pro-B cells have
normal RAG-scanning patterns is consistent with these rearrangements occurring after WAPL-down-regulation in the cells that have not formed proximal V_HDJ_H rearrangements on both alleles. In CBE1&2^+/rag2^/- pro-B cells, lack of V(D)J recombination upon WAPL down-regulation allows extrusion of the iE\(\mu\)/RC to continue into the upstream V_H domain where it reaches normal impediments in a substantial fraction of the cells. In this context, the relatively robust contribution of V_H5-2 and immediately upstream V_Hs to the WT pro-B repertoire indicates that these V_Hs are dominantly utilized in normal pro-B cells until WAPL levels are sufficiently down-regulated. Finally, in support of this model, proximal V_Hs are poorly utilized in v-Abl cells in which RAG is introduced after complete WAPL-depletion (22) (Fig 4).

Our findings on the impact of WAPL-depletion on chromatin interactions and RAG scanning activity support a model in which 3'I\(\gamma\)gh CBEs reinforce RC activity during I\(\gamma\)gh V(D)J recombination (SI Appendix, Fig. S9) (1). In WT v-Abl cells with high WAPL expression, the RC robustly interacts with the downstream \(\gamma_1-\gamma_2b\) enhancer and the 3'I\(\gamma\)gh CBEs, which, as proposed (1), could reinforce its loop-extrusion impediment activity (Fig. 5B). Complete WAPL depletion in v-Abl cells substantially diminishes downstream RC interactions (Fig. 5B). In addition, interactions of the IGCR1/CBE1 with the RC, as well as with the \(\gamma_1-\gamma_2b\) enhancer and 3'I\(\gamma\)gh CBEs, are also greatly diminished in WAPL-depleted v-Abl cells (Fig. 5C), consistent with WAPL-depletion diminishing transcription-based RC impediment activity. In contrast, in WT pro-B cells, in which WAPL levels are modestly reduced (44), these interactions are relatively robust (Fig. 2B). We propose that the 6.5-fold decrease in DJ_H rearrangements in WAPL-depleted v-Abl cells results from decreased RC impediment activity (Fig. 4I and 5B), as previously proposed for a similar reduction in V_e,J_k rearrangements upon WAPL depletion in this v-Abl line (22). Notably, WAPL-depletion reduced rearrangement levels of all Ds, other than DQ52, leading to DQ52 contributing more substantially to residual DJ_H rearrangements (Fig. 4N). A similar overall trend was obtained when previously reported data that employed J_H 1-4 baits (22) was analyzed for absolute levels, as well as relative percentages (SI Appendix, Fig. S7D, E; Table S4). We propose that DQ52 recombination, after WAPL depletion, may be less affected, because it accesses RAG by diffusion (versus scanning) from its RC location (24).

Finally, diverse V_HDJ_H rearrangements in WAPL-depleted cells occurred at a similarly low, absolute level to those of proximal V_Hs in untreated cells. However, V_H rearrangements in WAPL-depleted cells contributed to a 5.3-fold increase in the proportion of V_HDJ_H/DJ_H rearrangements (Fig. 4I, J). This latter finding may reflect increased levels of V_HDJ_H...
recombination in WAPL-depleted v-Abl cells compensating for reduced RC activity (Fig 4). However, the net effect is that overall V(D)J recombination levels are much lower in WAPL-depleted v-Abl lines than in BM pro-B cells as reported (22).

Deletion of 3’Igh CBEs decreased DJH and VhDJH rearrangement levels in both untreated and WAPL-depleted v-Abl cells. These decreases support the model that 3’ Igh CBEs contribute to reinforcing RC impediment activity (SI Appendix, Fig. S9). In this regard, the similarity in Vh usage patterns across the Vh locus, despite the nearly 50% decrease in V(D)J junctions, in WAPL-depleted 3’CBE- versus untreated 3’CBE- v-Abl cells indicates that the impact on V(D)J recombination in these WAPL-depleted lines affects V(D)J recombination activity per se, but apparently not scanning patterns. We note that the extent to which the 3’ Igh CBEs reinforce RC activity may be compensated, in its absence, by interactions of the RC with downstream CBEs; it is also notable that these downstream CBE interactions are diminished by complete WAPL down-regulation (Fig. 5B). Such compensatory activity of downstream CBEs, in the absence of 3’ Igh CBEs, was also implicated in the context of Igh class switch recombination (50). Finally, normal pro-B cells do not completely down-regulate WAPL levels (44), which may contribute to preserving 3’Igh CBEs/RC interactions and RC activity in these cells. In this regard, WT and 3’CBE- pro-B cells have indistinguishable Igh V(D)J recombination patterns (22).

When the RC-interacting peaks found in the 129SV pro-B cells (Fig. 2A), and C57BL/6 v-Abl cells (Fig. 5A), a number of the peaks C57BL/6 Peaks 6-9, 12-13 (Fig. 5A) are shared; but others are unique due to the significant differences in the Vh loci in these two cell types. Major peaks in J558/3609, distal Vhs region, are often associated with PAIR elements (Peaks 1, 4, 6, 8-10 in Fig. 2A and peaks 1-8 in Fig. 5A), while other peaks in J558/3609, J558, and middle Vh regions (Peaks 2, 3, 5, 7, 11-15 in Fig. 2A and peaks 9-13 in Fig. 5A) are associated with transcription or CBE binding motifs. These observations support the notion that when WAPL is down regulated, upon the neutralization of IGCR1, various transcription sites and CBEs still form sufficiently active loop extrusion impediments to promote interactions with the RC during RAG scanning of upstream Vhs locus sequences.

METHODS

Mice
Wildtype 129SV mice were purchased from Charles River Laboratories International. RAG2-deficient mice in 129SV background were purchased from Taconic. All animal experiments were
performed under protocols approved by the Institutional Animal Care and Use Committee of Boston Children’s Hospital.

**Generation of IGCR1 CBE-inversion mice.**

A previously described pLNTK targeting vector (49) containing inversion mutations of the 20-bp CBE1 and corresponding upstream activating sequence (WT sequence: 5’- TGCTTCCCCCTTGTGGCCATGAGCATTACTGCA-3’; inverted: 5’- TGCAGTAATGCTCATGGCCACAAGGGGAAGCA-3’); or the 19-bp CBE2 (WT sequence: 5’-TCTCCACAAGAGGGAGAA-3’; inverted sequence: 5’-TTCTGCCCCTCTTGTGGAGA-3’) sites within IGCR1 were electroporated into TC1 ES cells. Successfully targeted clones with CBE1 or CBE2 inversion integration were assessed by Southern blot analyses using StuI-digested (13.9 kb untargeted; 10 kb targeted) or SpeI-digested (16.3 kb untargeted; 12.7 kb targeted) genomic DNA with appropriate probes. Two independently targeted clones containing each inversion mutation were subjected to adenovirus mediated Cre deletion to remove the NeoR gene, karyotyped, and injected for germline transmission. Homozygous mice were generated through breeding and genotype was confirmed by PCR genotyping (Primer sequences are listed in Table S5).

**Generation of v-Abl cell lines**

The WT v-Abl-kinase-transformed pro-B cell line (v-Abl pro-B cells) was derived by retroviral infection of BM pro-B cells derived from rag2−/− mice as described (48). IGCR1 mutated RAG2-deficient v-Abl lines were established by breeding each IGCR1 mutant mice with rag2−/− germline mice to generate RAG2-deficient homozygous IGCR1 mutated mice (i.e. IGCR1/CBE1−/−rag2−/−), and deriving v-Abl lines as described (48). All these mutations were confirmed by PCR genotyping. 3’Igh CBEs deletion in single Igh WAPL-degron v-Abl (22) were generated by designed sgRNAs and screened by PCR. The sequence of all sgRNAs and oligos used are listed in Table S5.

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

Pro-B cells used in HTGTS-V(D)J-seq experiments were purified from WT or IGCR1 mutated mice as described (53). 2ug pro-B cell genomic DNA were used to generate each library. The sequence of the JH4 coding end primer (129SV background) used to generate HTGTS-V(D)J-seq libraries is listed in Table S5. HTGTS-V(D)J-seq libraries were prepared as described (24). HTGTS-V(D)J-seq libraries were sequenced using paired-end 300-bp sequencing on a Mi-Seq (Illumina) machine. The WT 129SV pro-B cells data shown in Fig.1 and Fig. S1-2
was extracted from a prior publication (GSM2183881- GSM2183883) (53). All libraries were normalized to total reads (junctions+germine reads) or junctions across a given locus, and the V_{H}DJ_{H} and DJ_{H} junctions were described in Table S1, S2. When normalized to total reads, all libraries were normalized to the smallest libraries from the same batch of experiments. The number of normalized reads or junctions is indicated in the figure and figure legends. D usage from the V_{H}DJ_{H} joins was analysed via the VDJ_annotation pipeline. Productive and nonproductive V_{H}DJ_{H} joins were analyzed via VDJ_productivity_annotation pipeline (see ‘code availability’).

RAG recombination and treatment of WAPL-degron v-Abl cells was performed as described (22), and the J_{H}4 coding end primer (C57BL/6 background) used to generate HTGTS-V(D)J-seq libraries. All libraries were normalized to total reads or junctions, and the V_{H}DJ_{H} and DJ_{H} junctions were described in Table S3. D usage from the V_{H}DJ_{H} joins was analyzed by the VDJ_annotation pipeline.

### 3C-HTGTS and data analyses

RAG2-deficient pro-B cells for 3C-HTGTS were purified and cultured as described (19). Cycling or G1-arrested RAG2-deficient v-Abl pro-B cells for 3C-HTGTS were prepared as described (18). Treatment of WAPL-degron v-Abl cells was performed as described (22). 3C-HTGTS was performed as described (18). Briefly, 10 million cells were crosslinked with 2% (v/v) formaldehyde for 10 min at RT. Cells were lysed in 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 1% Triton X-100 and protease inhibitors (Roche, #11836153001). Nuclei were digested with 700 units of NlaIII (NEB, #R0125) restriction enzyme at 37°C overnight, followed by ligation (T4 DNA ligase NEB M0202L) at 16°Covernight. Crosslinks were reversed and samples were treated with Proteinase K (Roche, #03115852001) and RNase A (Invitrogen, #8003089) prior to DNA precipitation. 3C-HTGTS libraries were generated using LAM-HTGTS (56), and primers are listed in Table S5.

3C-HTGTS libraries were sequenced using paired-end 150-bp sequencing on a Next-seq550 (Illumina) or paired-end 300-bp sequencing on a Mi-Seq (Illumina) machine. Data were processed as described previously (18). In addition, the PCR artificial junctions at Chr12: 114,692,680 in Fig. S6 A were removed from the total junctions. The junctions from Chr12 were extracted and counted for normalization. All 3C-libraries were normalized to the smallest libraries from the same batch of experiments. The number of normalized junctions is indicated in the figure legends. For peak analysis, 3C-HTGTS profiles were analyzed by MACS2 pipeline to
call robust interaction peaks (macs2 bgdpeakcall -c20 -l400 -g1000 was used for pro-B 3C-HTGTS in Fig. 2 and macs2 bgdpeakcall -c30 -l400 -g1000 was used for v-Abl 3C-HTGTS in Fig. 5). The peaks that showed >2-fold intensity change were annotated as unique peaks of indicated condition.

**Data availability**

HTGTS-V(D)J-seq, 3C-HTGTS and GRO-seq sequencing data reported in this study are available through GEO (GSExxxx). All study data are included in the article and/or supporting information. Previously published data were used for this work [GSE151910 (22) and GSE821126 (53)].

**Code availability**

3C-HTGTS and HTGTS-V(D)J-seq data were processed through published pipelines (http://robinmeyers.github.io/transloc_pipeline/). D usage in VhDJJi joins was processed via a custom pipeline (https://github.com/Yyx2626/VDJ_annotation/). Productive and non-productive junctions were processed via another pipeline (https://github.com/Yyx2626/VDJ_annotation/).

**ACKNOWLEDGMENTS**

We thank members of the Alt laboratory for stimulating discussions. This work was supported by the National Institutes of Health Grant R01AI020047 to F.W.A. and Grant F31-AI117920 to S.G.L.; Z.B. and H-Q.D. were supported in part by Cancer Research Institute Irvington Fellowships. F.W.A. is an investigator of the Howard Hughes Medical Institute.

**REFERENCES**


**FIGURE LEGENDS**

**Figure 1. Role of IGCR1/CBE1 and CBE2 in D-to-JH and proximal V\textsubscript{H}-to-DJH rearrangements.** (A) Schematic of the murine *Igh* locus showing V\textsubscript{HS}, D\textsubscript{s}, J\textsubscript{HS}, C\textsubscript{HS} and IGCR1. The red arrow indicates the J\textsubscript{H4} coding end (CE) bait primer. (B-E) Utilization of V\textsubscript{HS} across the entire *Igh* locus in WT (B-C) and CBE1\&2\textsuperscript{-/-} (D-E) pro-B cells. The V\textsubscript{H}DJH and DJH junctions are shown in the insets. (n=3 mice, mean±SEM; all HTGTS libraries are normalized to 78,091 total reads; see SI Appendix, Table S1). (F-G) Proximal V\textsubscript{H} usage in V\textsubscript{s}DJH junctions and D usage DJH junctions of WT (F) and CBE1\&2\textsuperscript{-/-} (G) pro-B cells (n=3 mice, mean±SEM). (H-I) Each panel shows the relative percentage of upstream V\textsubscript{HS} beyond the five most proximal V\textsubscript{HS} normalized to the indicated V\textsubscript{H}DJH junction number. Upstream V\textsubscript{HS} junctions are extracted from the data of Fig. 1B, D (n=3 mice, mean±SEM).

**Figure 2. Role of IGCR1/CBE1 and CBE2 in regulating RAG scanning into the V\textsubscript{H} locus.** (A) 3C-HTGTS signal counts of all V\textsubscript{HS} in WT (red) and CBE1\&2\textsuperscript{-/-} (blue) RAG2-deficient pro-B cells baiting from RC/iE\textsubscript{μ} (*). Each library was normalized to 160,314 total junctions (n=3 mice, mean±SEM). 18 peaks across V\textsubscript{HS} region are called by MACS2 pipeline and highlighted in gray (peaks 1-9, 12-15 are called in both conditions), orange (peaks 10-11 are called only in WT), green (peaks 16-18 are called only in CBE1\&2\textsuperscript{-/-}) (see SI Appendix, Fig. S3). (B) Zoom-in 3C-HTGTS profiles of *Igh* locus from proximal V\textsubscript{HS} to 3’*Igh* CBEs.
Figure 3. The mutation of IGCR1/CBEs alters VH utilization in pro-B cells. (A-D) Each panel shows the utilization of VHs across the entire Igh locus in indicated IGCR1/CBEs mutated pro-B cells. The V\textsubscript{H}DJ\textsubscript{H} and DJ\textsubscript{H} junctions are shown in insets. (\textit{n}=3 mice, mean±SEM; All HTGTS libraries are normalized to 78,091 total reads; see SI Appendix, Table S1). (E) VHs usage in WT and indicated IGCR1/CBEs mutated pro-B cells (\textit{n}=3 mice, mean±SEM).

Figure 4. Role of 3’Igh CBEs in RC activity during loop extrusion. (A-D) Utilization of VHs across the entire Igh locus in (A, B) and 3’CBE\textsuperscript{-} (C, D) v-Abl cells with or without Dox/IAA treatments. The V\textsubscript{H}DJ\textsubscript{H} and DJ\textsubscript{H} junctions are shown in insets. (\textit{n}=3 repeats from 3 independent clones, mean±SEM; all HTGTS libraries are normalized to 1,964,102 total reads; see SI Appendix, Table S3). (E-H) Relative percentage of VHs utilization normalized to the indicated V\textsubscript{H}DJ\textsubscript{H} junction number (\textit{n}=3 repeats, mean±SEM; percentages are plotted from the data of Fig. 4 A-D). (I-J) Absolute level of V\textsubscript{H}DJ\textsubscript{H} and DJ\textsubscript{H} rearrangements in WT and 3’CBE\textsuperscript{-} lines (\textit{n}=3 repeats, mean±SEM; \textit{t}-test, \textit{P}<0.01, **). (K-L) Relative percentages of VH\textsubscript{H}DJ\textsubscript{H} and DJ\textsubscript{H} normalized to untreated or treated WT conditions. (M-N) Absolute (M) and relative (N) D usage in DJ\textsubscript{H} rearrangements in untreated and WAPL-depleted WT v-Abl cells (relative percentage was normalized to 106,936 DJ\textsubscript{H} junctions in untreated or 16,565 DJ\textsubscript{H} junctions in WAPL-depleted cells; see SI Appendix, Table S3).

Figure 5. 3C-HTGTS profiles at Igh locus baiting from RC in WT and 3’CBE\textsuperscript{-} WAPL-degron v-Abl cells. (A) 3C-HTGTS signal counts at VHs domains of WT and 3’CBE\textsuperscript{-} RAG1-deficient v-Abl lines baiting from nRC/iE\textsubscript{\mu} (*) with (red) or without (blue) Dox/IAA treatment. Each library was normalized to 160,314 total junctions (\textit{n}=3 repeats from 3 independent clones, mean±SEM). 16 peaks across VHs region are called by MACS2 pipeline and highlighted in gray (peak 1, 6-9, 12-13 are called in WAPL-depleted WT v-Abl cells and WT pro-B cells), red (peaks 2-5, 10-11 are called in WAPL-depleted v-Abl cells), orange (peak 1 is called only in WAPL-depleted WT v-Abl cells), green (peaks are called in 3’CBE\textsuperscript{-} v-Abl cells); Peaks 14-16 are present in untreated WT and 3’CBE\textsuperscript{-} v-Abl lines (See SI Appendix, Fig. S8). (B) Zoom-in 3C-HTGTS profiles of Igh locus from proximal VHs to 3’Igh CBEs. (C) 3C-HTGTS signal counts of Igh locus from proximal VHs to 3’Igh CBEs in RAG-deficient WT v-Abl cells (red), WAPL-depleted v-Abl cells (blue) and pro-B cells (red) baiting from IGCR1/CBE1 (*). Each library was normalized to 112,525 total junctions (\textit{n}=3 repeats from 3 independent clones or 3 mice, mean±SEM).
Figure 1

**A**

*Tel.*

**B**

**C**

**D**

**E**

**F**

**G**

**H**

**I**

**J**

**K**

**L**

**M**

**N**

**O**

**P**

**Q**

**R**

**S**

**T**

**U**

**V**

**W**

**X**

**Y**

**Z**

**Figure 1**

**A**

*Tel.*

**B**

**C**

**D**

**E**

**F**

**G**

**H**

**I**

**J**

**K**

**L**

**M**

**N**

**O**

**P**

**Q**

**R**

**S**

**T**

**U**

**V**

**W**

**X**

**Y**

**Z**

**Figure 1**

**A**

*Tel.*

**B**

**C**

**D**

**E**

**F**

**G**

**H**

**I**

**J**

**K**

**L**

**M**

**N**

**O**

**P**

**Q**

**R**

**S**

**T**

**U**

**V**

**W**

**X**

**Y**

**Z**

**Figure 1**

**A**

*Tel.*

**B**

**C**

**D**

**E**

**F**

**G**

**H**

**I**

**J**

**K**

**L**

**M**

**N**

**O**

**P**

**Q**

**R**

**S**

**T**

**U**

**V**

**W**

**X**

**Y**

**Z**
Figure 2
Figure 3

Absolute $V_H$ usage in CBE-mutated pro-B cells

A

B

C

D

E

[Graphs and figures showing $V_H$ usage for different genotypes and conditions]
Figure 4

Absolute $V_H$ usage in WAPL-depleted $v$-Abl cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$V_H$ usage</th>
<th>$V_D$$V_J$ rearrangement</th>
<th>$V_H$$V_D$$V_J$ rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT untreated</td>
<td>100000</td>
<td><strong>0</strong></td>
<td><strong>2500</strong></td>
</tr>
<tr>
<td>WT Dox+IAA</td>
<td>100000</td>
<td><strong>0</strong></td>
<td><strong>2000</strong></td>
</tr>
<tr>
<td>3'CBE- untreated</td>
<td>100000</td>
<td><strong>0</strong></td>
<td><strong>1000</strong></td>
</tr>
<tr>
<td>3'CBE- Dox+IAA</td>
<td>100000</td>
<td><strong>0</strong></td>
<td><strong>800</strong></td>
</tr>
</tbody>
</table>

Relative $V_H$ usage in WAPL-depleted $v$-Abl cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of $V_H$$V_D$$V_J$ rearrangement</th>
<th>% of $V_D$$V_J$ rearrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT untreated</td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>WT Dox+IAA</td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>3'CBE- untreated</td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>3'CBE- Dox+IAA</td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
</tr>
</tbody>
</table>

Absolute $D$ usage in DJH rearrangements

<table>
<thead>
<tr>
<th>DJH usage</th>
<th>$D$ usage</th>
<th>Relative $D$ usage in DJH rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT untreated</td>
<td>100000</td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>WT Dox+IAA</td>
<td>100000</td>
<td><strong>0</strong></td>
</tr>
</tbody>
</table>

**Legend:**
- **WT**
- **Dox+IAA**

**Note:**
- The data represents the absolute and relative usage of $V_H$ and $D$ segments in $v$-Abl cells following treatment with or without WAPL depletion.
Figure 5

A 3C-HTGTS using the RC/iE\* locale as bait

B 3C-HTGTS using the CBE1\* locale as bait

C 3C-HTGTS using the CBE1\* locale as bait