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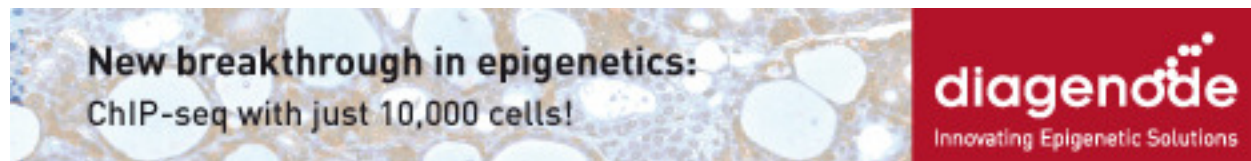
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RESEARCH COMMUNICATION

Flexible ordering of antibody class switch and V(D)J joining during B-cell ontogeny

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V(D)J joining is mediated by RAG recombinase during early B-lymphocyte development in the bone marrow (BM). Activation-induced deaminase initiates isotype switching in mature B cells of secondary lymphoid structures. Previous studies questioned the strict ontological partitioning of these processes. We show that pro-B cells undergo robust switching to a subset of immunoglobulin H (IgH) isotypes. Chromatin studies reveal that in pro-B cells, the spatial organization of the *Igh* locus may restrict switching to this subset of isotypes. We demonstrate that in the BM, V(D)J joining and switching are interchangeably inducible, providing an explanation for the hyper-IgE phenotype of Omenn syndrome.

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Humoral immunity is dependent on antigen receptors that are assembled from immunoglobulin (Ig) heavy chain V_H, D, and J_H segments during early B-cell development. RAG recombinase mediates V(D)J gene segment assembly in the bone marrow (BM) (Zhang et al. 2010; Schatz and Ji 2011). In mature B cells located in secondary lymphoid structures, activation-induced deaminase (AID) initiates a switch from IgM expression to secondary Ig classes (IgG3, IgG1, IgG2b, IgG2a, IgE, and IgA) that have specialized effector functions (Stavnezer 1996, 2000). Class switch recombination (CSR) occurs via an intrachromosomal deletion process while maintaining the original V(D)J assembly (Kenter 2012). The failure to assemble V(D)J exons or signal through the B-cell receptor (BCR) creates a block in the developmental progression of pro-B cells (Jankovic et al. 2004; von Boehmer and Melchers 2010). However, the strict ontological separation of V(D)J

joining and CSR may break down under some circumstances (Milili et al. 1991; Rolink et al. 1996; Weller et al. 2001; Dudley et al. 2002; Mao et al. 2004; Han et al. 2007; Ueda et al. 2007; Scheeren et al. 2008; Kuraoka et al. 2009; Wesemann et al. 2011). For example, in μ MT mice, membrane Ig μ is not expressed, and B-cell development is halted at the pro-B-cell stage; nevertheless, IgA is selectively expressed (Melamed et al. 2000; Macpherson et al. 2001), implying an alternative pathway for CSR that can circumvent the pro-B-cell block. However, it is unclear where and under what circumstances the switch to IgA occurs.

Omenn syndrome is a severe combined immunodeficiency disease associated with autoimmunity and atopy, most commonly arising from hypomorphic mutations in the Rag recombinase genes. The syndrome is characterized by the severe paucity of B and T lymphocytes, hypogammaglobulinemia, and, paradoxically, elevated IgE levels (Wong and Roth 2007; Ozcan et al. 2008). To account for hyper-IgE in Omenn syndrome, we hypothesized that (1) CSR occurs in BM pro-B cells prior to V(D)J joining, (2) CSR is predisposed to IgE, and (3) V(D)J joining events can follow CSR. Here, we show that Rag-deficient or *Mb1*-deficient pro-B cells can be induced to undergo robust CSR, albeit to a restricted subset of Ig constant (C_H) region genes, including IgG2b and IgE, prior to or following V(D)J joining. Immunization of *Rag1*^{-/-} mice with LPS promotes IgG2b switching in BM pro-B cells. We also demonstrate that V(D)J joining can follow CSR in Abelson transformed pro-B-cell and pre-B-cell lines. Studies of *Igh* locus chromatin structure revealed a unique stage-specific organization in pro-B cells that is correlated with preferential IgG2b and IgE switching. The flexible expression of the V(D)J recombination and CSR programs has implications for the genesis of Omenn syndrome hyper-IgE, autoimmune repertoire development, and leukemagenesis via the coexpression of the RAG and AID recombinases (Tsai et al. 2008).

Results and Discussion

To test the proposition that robust CSR can occur in BM pro-B cells prior to V(D)J recombination, we evaluated Rag2, AID, and isotype-specific germline transcript (GLT) expression in an *E2A*^{-/-} pre-pro-B cell line, in pro-B cells isolated from *Rag1*- or *Mb1*-deficient mice, and in a panel of Abelson transformed pro-B-cell lines (Rag-deficient R2K2 and 445.3) and pre-B-cell lines (PA112.1, PA112.2, PA48.1, A70.2, and ATM2A). *E2A* and *Rag* deficiencies preclude V(D)J joining, whereas *Mb1*-deficient pro-B cells assemble V(D)J exons but cannot signal through the pre-BCR and are blocked at the pro-B-cell stage (Pelanda et al. 2002). Elevated Rag2 but not AID transcripts are detected in all strains of unstimulated pre-pro-B and pro-B cells but not in the Abelson transformed cell lines, as expected (Fig. 1A, top; Supplemental Fig. S1; Muljo and Schlissel 2003). Upon activation with the CSR inducers LPS+CD40L

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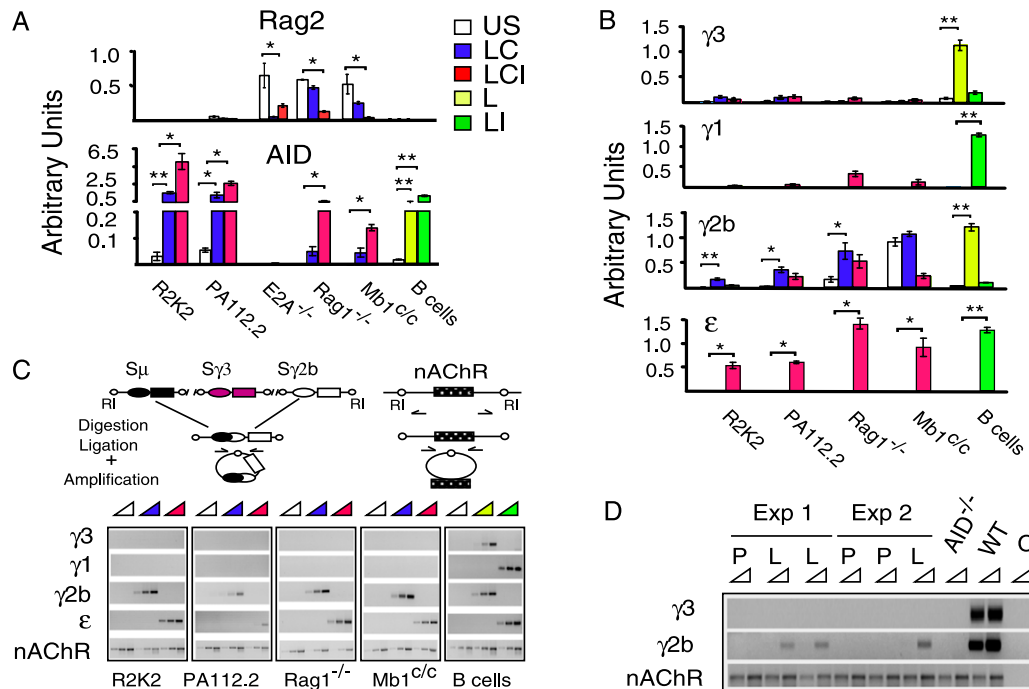


Figure 1. Pro-B cells undergo inducible CSR. *E2A*-deficient pre-pro-B cells, *Rag1*-deficient or *Mb1*-deficient pro-B cells, Abelson transformed pro-B-cell lines (R2K2), and pre-B-cell lines (PA112.2) were unstimulated (US) or activated with LPS+CD40L (LC) or LPS+CD40L+IL4 (LCI), and wild-type (WT) resting splenic B cells were unstimulated or activated with LPS (L) or LPS+IL4 (LI). (*) $P \leq 0.05$; (**) $P \leq 0.001$ – 0.0001 . (A,B) Quantitative RT-PCR assays for Rag2 and AID (A) and GLTs $\gamma 3$, $\gamma 1$, $\gamma 2b$, and ϵ (B) from five samples from three to five independent experiments were normalized to the rRNA 18S transcript. (C, top) DC-PCR assay schematic, EcoRI (RI) sites, and *nAChR* gene loading control. (Bottom) DC-PCR analyses of $\mu \rightarrow \gamma 3$, $\mu \rightarrow \gamma 1$, $\mu \rightarrow \gamma 2b$, and $\mu \rightarrow \epsilon$ CSR are representative of three independent experiments. (D) DC-PCR analysis of pro-B cells from *Rag1*^{-/-} mice injected with LPS or PBS. Wild-type or *AID*^{-/-} splenic B cells activated with LPS or no template control (C) are representative of three independent experiments.

or LPS+CD40L+IL4, *Rag2* expression is reduced in *E2A*^{-/-} pre-pro-B cells and *Rag1*-deficient and *Mb1*-deficient pro-B cells (Fig. 1A). In contrast, AID expression is markedly induced in *Rag1*-deficient and *Mb1*-deficient pro-B cells but not *E2A*^{-/-} pre-pro-B cells (Fig. 1A, bottom). Similarly, in R2K2 and PA112.2 as well as other Abelson transformed cell lines, AID expression substantially increases in response to CSR stimuli (Fig. 1A, bottom; Supplemental Fig. S1A,B). *E2A*^{-/-} pre-pro-B cells were not further considered because AID is not expressed. Previous studies of BM pro-B cells concluded that AID transcription was undetectable (Crouch et al. 2007) but did not evaluate expression in response to CSR activators.

We next examined the pattern of GLT expression in pro-B cells. C_H genes are organized in transcription units that include a noncoding intronic (I) exon, the switch (S) region, and the C_H exons. Downstream S regions are targeted for recombination with S_μ by selective activation of GLT promoters in response to specific stimuli. GLTs initiate from a promoter located upstream of each I exon and terminate 3' of the C_H region (Stavnezer 2000). Typically, GLTs $\gamma 3$ and $\gamma 2b$ are induced by LPS or LPS+CD40L, whereas GLTs $\gamma 1$ and ϵ also require IL4 in mature B cells (Fig. 1B). However, in response to the appropriate CSR stimuli, *Rag1*-deficient and *Mb1*-deficient pro-B cells and the Abelson transformed cell lines expressed GLT $\gamma 2b$ and ϵ but not $\gamma 1$ and $\gamma 3$ (Fig. 1B; Supplemental Fig. S1A). Accordingly, expression of GLT ϵ and not $\gamma 1$ was noted for immature B cells (Wesemann et al. 2011).

Activation of AID expression and germline transcription prompted us to investigate whether CSR occurs in pro-B or pre-B cells. We used semiquantitative digestion circularization PCR (DC-PCR) to detect CSR events, since pro-B cells do not express surface Ig (Fig. 1C, top). The nonrearranging *Acetylcholine receptor* (*nAChR*) gene was used as a loading control (Fig. 1C). DC-PCR products representing $\mu \rightarrow \gamma 2b$ and $\mu \rightarrow \epsilon$ CSR events are detected in the *Rag1*-deficient and *Mb1*-deficient pro-B cells and Abelson transformed cell lines, whereas $\mu \rightarrow \gamma 3$ or $\mu \rightarrow \gamma 1$ events are not detected in either case (Fig. 1C, bottom; Supplemental Fig. S2). In contrast, in mature splenic B cells activated with LPS or LPS+IL4, a full spectrum of $\mu \rightarrow \gamma 3/\gamma 2b$ and $\mu \rightarrow \gamma 1/\epsilon$ CSR events is detected, respectively (Fig. 1C). This restricted pattern of CSR in the pro-B versus mature B cells parallels their GLT expression profiles. Strikingly, the levels of DC-PCR products in primary pro-B cells and activated splenic B cells were similar, indicating robust CSR in early B cells (Fig. 1C; Supplemental Fig. S2).

High-frequency $\mu \rightarrow \gamma 2b/\epsilon$ CSR in pro-B cells was confirmed by several independent methods, including analysis of post-switch transcripts (PSTs), circle transcript PCR (CT-PCR) assays, and S/S junction analyses. Following CSR, the I_μ exon is in close proximity with a new downstream C_H region, thereby permitting PST expression that was detected using a forward primer in I_μ together with a reverse primer in the relevant C_H exon. PST $\gamma 2b$ and ϵ are induced in *Rag1*-deficient and *Mb1*-deficient pro-B cells and Abelson transformed cell lines

upon activation with CSR stimuli (Supplemental Fig. S3). CT-PCR assays indicate that $\mu \rightarrow \gamma 2b$ or $\mu \rightarrow \epsilon$ CSR occurs dynamically in *Rag1*^{-/-} pro-B cells and Abelson transformed cell lines (Supplemental Fig. S4). We sequenced S/S recombination junctions from appropriately activated *Rag1*^{-/-} pro-B cells or R2K2 cells and found that μ joined to a variety of sites in $\gamma 2b$ or ϵ (Supplemental Figs. S5–S8). Switch junctions were blunt or contained short junctional microhomologies or occasional insertions in normal proportions (Stavnezer et al. 2010) and confirmed that the mechanism of CSR in pro-B cells is operationally indistinguishable from that observed in mature B cells.

To determine whether CSR can be induced in vivo, *Rag1*^{-/-} mice were injected with LPS or PBS, and pro-B cells were isolated directly from the BM and tested for CSR by DC-PCR. Wild-type or *AID*-deficient splenic B cells that were activated with LPS in ex vivo culture served as positive and negative controls for CSR, respectively. *nAChR* amplification is shown as a loading control, and the absence of PCR products in reactions devoid of template demonstrate PCR specificity. Under these activation conditions, $\mu \rightarrow \epsilon$ CSR is negligible. Switching $\mu \rightarrow \gamma 2b$ but not $\mu \rightarrow \gamma 3$ was observed in 67% (six out of nine) of LPS immunized mice ($P = 0.007$; χ^2) but not (zero out of six) for PBS-injected controls from three independent experiments (Fig. 1D). Targeting of CSR to the $\gamma 2b$ but not the $\gamma 3$ locus reflects the established GLT expression pattern that we observed for pro-B cells activated in ex vivo culture (Fig. 1B,C). We conclude that CSR can occur in BM *Rag1*^{-/-} pro-B cells prior to V(D)J recombination.

To address the question of whether a B cell that experienced CSR can subsequently undergo V(D)J joining, switched clones from the R2K2 pro-B-cell line were isolated by limiting dilution. CSR was confirmed in clones B5 ($\mu \rightarrow \gamma 2b$) and A2 ($\mu \rightarrow \epsilon$) by DC-PCR, and clonality was demonstrated by virtue of unique intra-S μ region rearrangements (Fig. 2A, Supplemental Fig. S9). Treatment of these cell lines with STI571, a pharmacologic inhibitor of Abl kinase activity, resulted in activation of V(D)J joining and differentiation to the late pre-B-cell state (Muljo and Schlissel 2003). STI571 stimulation of B5 and A2 clones led to induction of the V(D)J joining gene program (Fig. 2B); Muljo and Schlissel 2003). The *Rag2*⁺ but not the empty expression construct induced diverse D_{DFL16.1}–J_{H1} coding joins (CJs), while PST expression persisted (Fig. 2C; Supplemental Figs. S10, S11). Thus, pro-B-cell clones that experienced CSR retain the ability to undergo V(D)J recombination. Importantly, evidence indicates that BM pro-B cells expressing IgG1 ($\gamma 2b$) chains (Storb et al. 1994) are capable of mature B-cell development that is indistinguishable from wild type.

To determine whether class-switched pre-B cells could complete the developmental program of recombination, we assayed V–J joining at the light chain *Igk* locus.

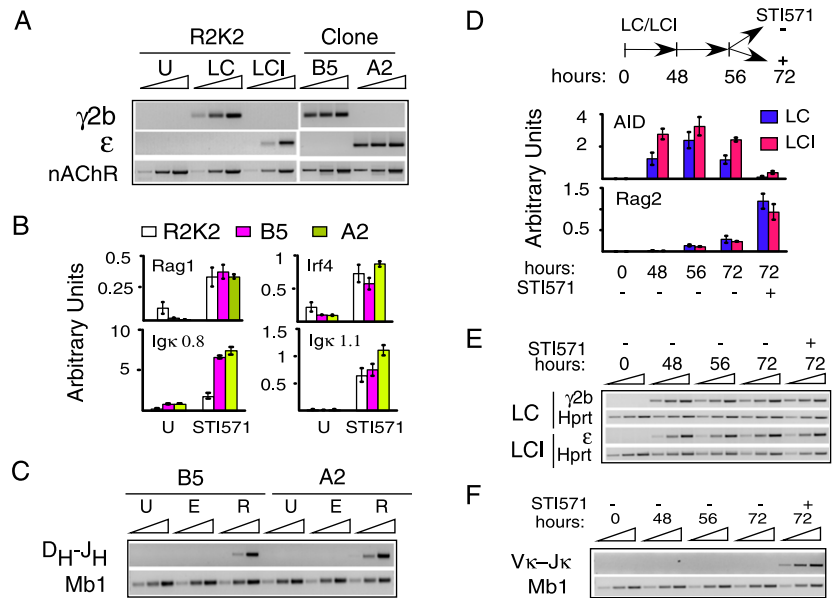


Figure 2. V(D)J joining can follow CSR in early B-cell lines. Cells were untreated (U) or activated with LPS+CD40L (LC) or LPS+CD40L+IL4 (LCI). Results are representative of three to nine samples from three independent experiments. (A) DC-PCR analysis of CSR in R2K2 and in clones A2 and B5. (B) STI571 treatment induces *Rag1*, *Igk* GLT, and *Irf4* expression as assessed by quantitative RT-PCR in B5 and A2 clones. (C) D_{DFL16.1}–J_{H1} CJs assessed in A2 and B5 clones transfected with empty (E) or *Rag2* (R) expression constructs and the Mb1 loading control. (D, top) Treatment timeline in PA112.2 cells. (Bottom) Quantitative RT-PCR for AID and *Rag2* expression. (E) Semiquantitative RT-PCR for PST $\gamma 2b$ and ϵ or Hprt. (F) V $\kappa 6-23$ –J $\kappa 1$ CJs and Mb1 assessed by semiquantitative PCR.

PA112.2 cells (strain 129; L κ , germline) were exposed to CSR inducers for 48 h, and switching was activated as indicated by increased expression of AID and PSTs $\gamma 2b$ or ϵ , which persisted for 8 h following withdrawal of the CSR inducers (Fig. 2D,E). In cells exposed to STI571, AID diminished, and *Rag2* concomitantly increased, accompanied by active V $\kappa 6-23$ –J $\kappa 1$ CJ formation (Fig. 2D,F) with appropriate junctional diversity (Supplemental Fig. S12). CSR induction had no effect on subsequent generation of V $\kappa 6-23$ –J $\kappa 1$ CJs (Supplemental Fig. S13). Active PST expression following STI571 treatment indicates that populations of switched cells are fully capable of V(D)J joining (Fig. 2D,E). We conclude that B cells expressing BCR with secondary isotypes may emerge directly from the BM. Intriguingly, the CSR or V(D)J gene programs can be alternately expressed in response to specific inducers during early B-cell ontogeny.

The pattern of CSR in pro-B cells is skewed toward IgG2b and IgE and is atypical of mature B cells. Germline transcription in activated mature B cells is regulated by the spatial organization of the *Igh* locus through long-range chromatin contacts between the E μ and 3'E α enhancers and GLT promoters (Supplemental Fig. S14A; Wuerffel et al. 2007; Sellars et al. 2009). Could $\gamma 3$: $\gamma 1$ contacts in a developmental stage-specific chromatin structure restrict CSR in pro-B cells (Supplemental Fig. S14B)? Using the chromosome conformation capture (3C) carbon copy (5C) method for unbiased detection of chromatin contacts (Dostie et al. 2006), we simultaneously examined a matrix of 12,656 possible looping interactions in parallel across the entire *Igh* locus and focused here on the 220-kb C_H domain spanning E μ and

3'E α (Supplemental Material, Supplemental Fig. S15; Supplemental Tables S2, S3). We detected abundant $\gamma 3:\gamma 1$ chromatin interactions (Fig. 3, orange circle), indicating a compact conformation in *Rag2*^{-/-} pro-B cells that is absent in resting splenic B cells (Fig. 3). We examined the log₂ ratio pro-B/resting B 5C interactions (represented by red [pro-B] and blue [resting B] in Fig. 3) and confirmed that $\gamma 3:\gamma 1$ interactions are elevated in pro-B cells (Fig. 3). We found that E μ : $\gamma 2b$ - ϵ and E μ : $\gamma 3$ - $\gamma 1$ contacts are associated with pro-B and resting B cells, respectively, in accord with the pattern of potential GLT expression and CSR. In contrast, few long-range interactions were detected for a gene desert region on chromosome 5 (Supplemental Fig. S16).

3C studies confirmed abundant $\gamma 3:\gamma 1a$ (1.7-fold, $P = 0.053$) and $\gamma 3:\gamma 1b$ (twofold, $P = 0.01$) chromatin contacts in *Rag2*^{-/-} pro-B cells as compared with resting B cells or ConA-activated splenic T cells, where $\gamma 1a$ and $\gamma 1b$ are opposite ends of Hind III fragment C (Fig. 4A,B). *Rag2*^{-/-} pro-B cells and the 445.3 pro-B-cell line display a similar profile of $\gamma 3:\gamma 1$ interactions, indicating that in early B cells, a compacted higher-order chromatin structure involving $\gamma 3:\gamma 1$ may limit that accessibility to the CSR machinery (Supplemental Fig. S17). In contrast, $\gamma 3:\gamma 2b$ contacts observed in both *Rag2*^{-/-} pro-B cells and wild-type resting B cells are similar to each other and may be related to a spatial organization that is absent in T cells (Fig. 4B).

Because interaction of distal Igh enhancers with the targeted *C_H* locus is essential to bring participating S regions into close proximity (Wuerffel et al. 2007), we examined *Rag2*^{-/-} pro-B and 445.3 cells for these chromatin contacts in 3C assays. 3'E α :E μ (H-A) interactions in *Rag2*^{-/-} pro-B cells and resting splenic B cells are similarly abundant and significantly higher than in ConA-stimulated splenic T cells (Fig. 4C, left panel). When CSR is induced in LPS+IL4-activated B cells,

3'E α :E μ (H-A) interactions become elevated relative to resting B cells (Fig. 4C, left panel). Notably, when the 445.3 pro-B-cell line is activated with LPS+CD40L to initiate CSR, 3'E α :E μ (H-A) ($P < 0.006$) and hs3b,4: $\gamma 2b$ ($P < 0.002$) interactions significantly increase, whereas $\gamma 3:3'E\alpha$ interactions remain unchanged (Fig. 4C, right panel). Thus, our results demonstrate a unique three-dimensional chromatin structure in pro-B cells that supports isotype-specific CSR and appears to preclude $\gamma 3$ and $\gamma 1$ GLT expression and CSR in a developmental stage-specific fashion (Supplemental Fig. S14B).

Our findings establish that pro-B cells in the BM can undergo robust CSR, biased toward IgG2b and IgE, in response to specific stimuli followed by V(D)J recombination. Thus, pro-B cells that are delayed in the BM by genetic predisposition (as in Omenn syndrome) or environmental cues have the capacity to undergo CSR in response to bacterial infections and to eventually populate the periphery following V(D)J joining. Two additional conclusions flow from our findings. The origin of DNA damage reminiscent of both RAG and AID activity in some human leukemias (Tsai et al. 2008) has been difficult to explain. This footprint of DNA damage in leukemia may be related to coexpression of AID and RAG1/2 that we observed in pro-B cells induced to switch. Most notably, alternating expression of CSR versus V(D)J joining in early B cells reveals an unanticipated flexibility of these gene programs, with implications for our understanding of developmental and lineage commitment.

Materials and methods

In brief, mice were handled according to institutional and National Institutes of Health (NIH) guidelines. Standard protocols were used for cell culture, quantitative PCR, RT-PCR, DC-PCR, CT-PCR, retroviral transductions, statistical analyses, and switch, V κ -J κ , and D H -J H junction

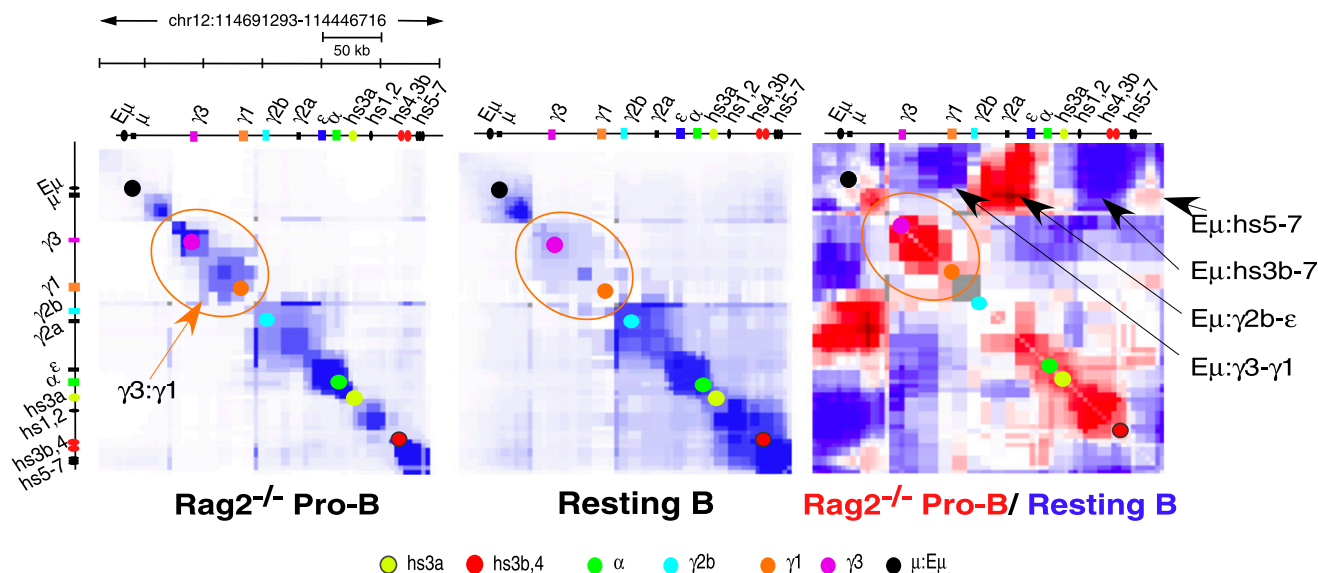


Figure 3. A developmentally stage-specific chromatin configuration of the *Igh* locus. 5C analyses of the *Igh* locus in *Rag2*^{-/-} pro-B cells and resting splenic B cells are represented in heat maps of 5C data (bin size 30 kb; step size 3.0 kb). Interaction frequencies are plotted against genomic distance. Blue intensity represents the contact frequency between genomic coordinates. The diagonal represents frequent proximal intrachromosomal interactions along the linear *Igh* locus. 5C signals off the diagonal indicate looping interactions. (Right panel) Log₂ ratio of pro-B/resting B 5C interactions where pro-B (red) and resting B (blue) contact.

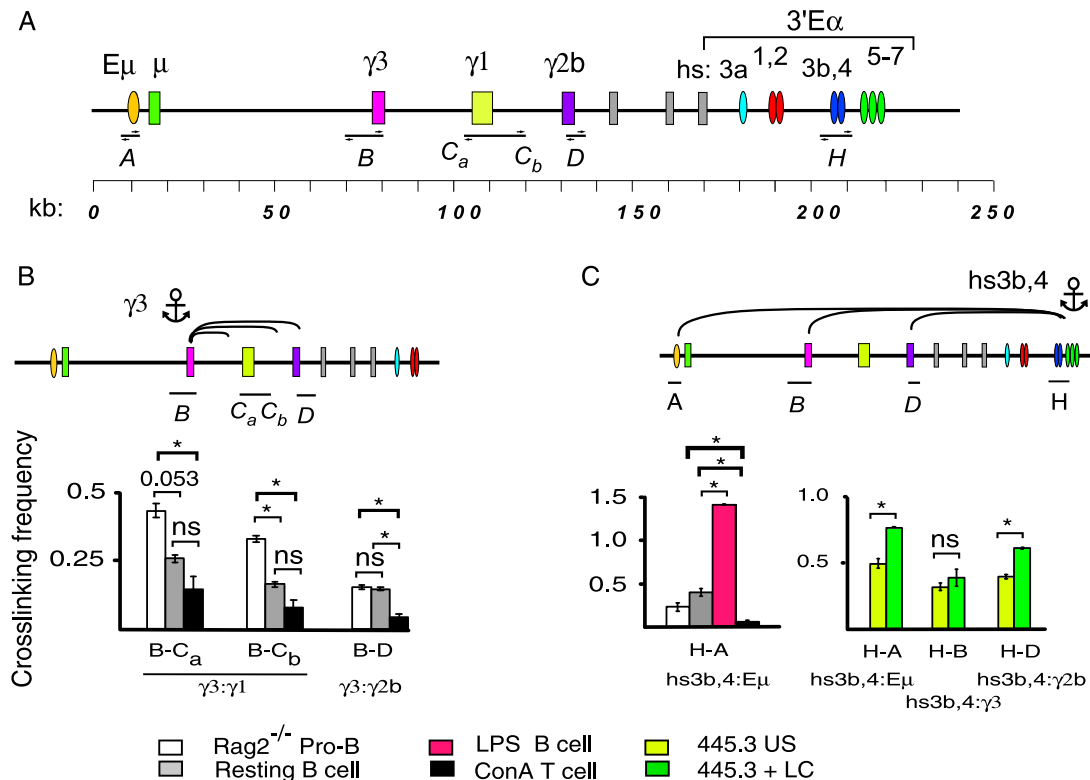


Figure 4. Absence of IgG3 and IgG1 CSR in pro-B cells is related to looping interactions between the $\gamma 3$ and $\gamma 1$ loci. (A) A segment of the *Igh* locus spanning 220 kb (shown at the top) in which 3C HindIII restriction fragments (A, B, C, D, and H) are shown. (B,C) 3C assays using chromatin from *Rag2*^{-/-} pro-B cells, unstimulated (US) or LPS+CD40L (LC)-stimulated 445.3 cells, wild-type (WT) resting splenic B cells, or ConA-stimulated T cells. (*) $P \leq 0.05$; (ns) Not significant. (B) 3C assays anchored at $\gamma 3$ were analyzed for interactions with $\gamma 1$ or $\gamma 2b$. (C) Hs3b,4 (3'Eα) anchor was analyzed for interactions with Eμ, $\gamma 3$, or $\gamma 2b$.

cloning. Abelson-MuLV transformed pro-B-cell or pre-B-cell lines were kindly provided by Dr. B. Sleckman (Washington University, St. Louis). 3C assays were carried out (Wuerffel et al. 2007) in combination with 5'FAM/ 3'BHQ1-modified probes. 5C primers were designed using online tools (<http://my5C.umassmed.edu>). 5C library construction was performed as described (Dostie et al. 2006). Additional methods are available in the Supplemental Material.

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Flexible ordering of antibody class switch and V(D)J joining during B cell ontogeny

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INVENTORY OF SUPPLEMENTAL INFORMATION

SUPPLEMENTAL MATERIALS AND METHODS

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Suppl. Figure S14. Model of CSR in pro-B cells. (Related to Figure 3)

Suppl. Figure S15. 5C analysis of a 244kb segment of the Igh locus in pro-B and resting B cells. (Related to Figure 3)

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Suppl. Table S1. PCR Primers (Related to Figure 1,2)

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SUPPLEMENTAL MATERIALS AND METHODS

Mice and Primary Cell Culture

Mice, C57BL/6 (WT), Rag deficient, or homozygous Mb1-Cre (Hobeika et al. 2006) on the C57BL/6 background were purchased from Jackson Laboratories or maintained in colonies at the University of Illinois College of Medicine, The Scripps Research Institute (TSRI) or the NIA. All procedures involving mice were approved by the Institutional Animal Care Committee of the University of Illinois College of Medicine, TSRI, or the NIA animal facilities, in accordance with protocols approved by the TSRI or NIA Institutional Animal Care and Use Committees. 3C chromatin was prepared from Rag2^{-/-} pro-B cells that were purified from the bone marrow by positive selection using anti-CD19-coupled magnetic beads (Stem Cell Technologies, BC, Canada) and then expanded on OP9 cells as described (Pongubala et al. 2008). For all other pro-B cell analyses, Rag1^{-/-} and homozygous Mb1-Cre pro-B cells were cultured as previously described (Sayegh et al. 2005) with minor modifications. CD19⁺ cells were isolated using anti-CD19 coupled magnetic beads (Miltenyi) and cultured in the presence of IL7 (1% vol/vol supernatant of a J558L cell line stably expressing IL7) for 4 days. After 4 days in IL7 the pro-B cells were induced with LPS (*Salmonella typhimurium*; 50 µg/ml) (Sigma-Aldrich) and CD40L (10% vol/vol) or LPS+CD40L+IL4 (10 ng/ml) (R & D Systems) for 42 hours and DNA or RNA was isolated. CD40L is the rCD40L-CD8 fusion protein secreted by J558 mouse myeloma cell line (Lane et al. 1993) and was prepared as described (Ma et al. 2002). E2A^{-/-} pre-pro-B cells were a kind gift from C. Murre (University of California, San Diego) and were cultured on S17 stromal cells in the presence of IL7, SCF, and FLT3 as previously described (Ikawa et al. 2004) and then LPS+CD40L or LPS+CD40L+IL4 were

added in the presence of IL7, SCF, and FLT3, for 42 hours. Splenocytes were sorted for CD43⁻ resting B cells using anti-CD43 magnetic microbeads (MACS, Miltenyi) and cultured with LPS or LPS+IL4 as previously described (Wuerffel et al. 2007) or were enriched for T cells with the mouse T cell enrichment column kit (MTCC-5, R&D) according to the manufacturer's instructions. For in vivo studies, Rag1^{-/-} mice were immunized with LPS (10 µg) in PBS (200 µl) or PBS alone and animals were sacrificed on day 3 post-injection and pro-B cells were isolated and analyzed directly.

Abelson-MuLV transformed cell lines and induction of CSR and VDJ joining

Seven Abelson-MuLV transformed pro- or pre-B cell lines were kindly provided by Dr. B. Sleckman, (Washington University, St. Louis). Bone marrow cells from mice were cultured with the pMSCV-v-abl retrovirus to generate stable v-abl transformed pro- or pre-B cell lines, as previously described (Mainville et al. 2001). The pre-B cell lines, PA112.1 and PA112.2, are independent lines from the same mouse on the 129 background and PA48.1 from a different mouse on the C57Bl/6 background. The pro-B cell lines are R2K2 (Rag2^{-/-}), on the C57Bl/6x129 background, and 445.3 (Rag1^{-/-}) on the C57Bl/6 background. The C57Bl/6x129 Abelson-MuLV transformed pre-B cell lines A70.2 and ATM2A were previously described (Bredemeyer et al. 2006). Abelson transformed cell lines were cultured in RPMI 1640 (Cellgro), 10% (v/v) FBS, 4µM glutamine (Gibco), 1mM sodium pyruvate (Gibco), 1X non essential amino acid (Gibco), 5000 units/ml Penicillin and 5000 µg/ml Streptomycin (Gibco), 50 µM β-mercaptoethanol (Sigma) and maintained at approximately 5x10⁵ cells/ml. To activate the V(D)J joining program cells were treated with 10 µM STI571 (Novartis) for 16 hours. To

stimulate CSR cells were induced with LPS (*Salmonella typhimurium*; 50 µg/ml) (Sigma-Aldrich) and CD40L (10% vol/vol) or LPS+CD40L and IL4 (10 ng/ml) (R & D Systems) for 42 hours then DNA was isolated by standard methods. RNA was isolated using TRIzol (Ambion), as prescribed by the manufacturer.

Isolation of isotype switched clones from an Abelson transformed cell line and PST assays

To derive switched isolates, the Abelson transformed pro-B cell line, R2K2, was stimulated with LPS+CD40L in the presence or absence of IL4 for 48 hours, then cloned by limiting dilution. Following clonal expansion, RNA was isolated and cDNA prepared using SuperScript II reverse transcriptase (Invitrogen) with random primers (Promega). Post-switch transcripts (PST) $\gamma 2b$ or ϵ were screened by RT-PCR using primers $I\mu F$ and $C\gamma 2bR$ or $C\epsilon R$ (Table S1) and Phire Hot Start II DNA polymerase (Thermo Scientific) in 25 µl, as advised by the manufacturer, then PCR amplified at 98°C/5 min, 35 cycles of 98°C for 5 sec, 58°C for 5 sec and 72°C for 15 sec. The CSR status of PST positive clones was confirmed by DC-PCR for $\mu \rightarrow \gamma 2b$ or $\mu \rightarrow \epsilon$ switching. Clonality of the isotype switched clones was verified by Southern analyses using the 5'S μ probe (chr 12:114,663,849-114,665,084; mm9) and standard procedures. In semiquantitative PST assays products were harvested at 26, 29 and 32 cycles as shown in Figures 2B, S3, S11.

Real time quantitative RT-PCR, DC-PCR, CT-PCR

Real-time quantitative (q) RT-PCR for AID or germline transcripts (GLTs) expression was performed using a viiA7 system and SYBR Green PCR Mix (Applied Biosystems) as described (Wuerffel et al. 2007) except that primers for 18S rRNA were used (Rhinn et al. 2008) to normalize samples. Digestion circularization (DC)-PCR was performed as described (Bhattacharya et al. 2010). For ex vivo studies DC-PCR products of $\mu \rightarrow \gamma 3$, $\mu \rightarrow \gamma 1$, $\mu \rightarrow \gamma 2b$ and $\mu \rightarrow \epsilon$ CSR harvested at 26, 29, and 32 cycles as shown in Figure 1C. DC-PCR and nAChR PCR products for in vivo studies were harvested at 36 and 39 cycles or 29 and 32 cycles, respectively as shown in Figure 1D. Circle transcript (CT) RT-PCR assays were carried out as previously described (Kinoshita et al. 2001) with modifications. The CT-PCR amplification was done using Phire Hot Start II polymerase (Thermo Scientific) for $I\gamma 2b-C\mu$ with initial denaturation for 5 min at 98°C followed by 32 cycles of 98°C for 5 sec, 60°C for 5 sec, 72°C for 15 sec; $I\epsilon-C\mu$ with initial denaturation for 5 min at 98°C followed by 34 cycles of, 98°C for 5 sec, 60°C for 5 sec, 72°C for 8 sec, both in a 25 μ l reaction on 5 fold serial diluted cDNA. QRT-PCR, DC-PCR, CT and PST assay primers are listed in Table S1.

Statistical analyses

Statistical analyses were done using a two-tailed Student's t-test except where indicated.

PCR amplification of endogenous $S\mu/S\gamma 2b$ and $S\mu/S\epsilon$ junctions

For $S\mu/S\gamma 2b$ and $S\mu/S\epsilon$ junction analyses genomic DNA (1 μ g) from Rag1^{-/-} pro-B cells or the R2K2 cell line was PCR amplified in a total volume of 30 μ l with Expand Long Template System (Roche) in Buffer 1, dNTPs (200 μ M) and primers (400nM) for 38 cycles of 94° for 15

sec / 60° for 30 sec / 68° for 3 min (plus an additional 10 sec added every succeeding cycle) with S_μ (C3F-5'GGTATCAAAGGACAGTGCTTAG 3') and S_{γ2bR} (5' TTCAACATCCCAAGCCCTCCATAG 3') or S_{εR} (5' GAAAGTTCAACTAAACCAAAAGACCTC 3') primers, respectively. S/S junction PCR products were cloned into pCR4-TOPO (Invitrogen) and then identified by colony lift hybridization using an S_μ probe (chr12:114,664,506-114,664,076; mm9), analyzed for DNA sequence (ABI 3730 XL) and aligned with genomic sequences. The 431 bp S_μ probe template was PCR amplified from genomic DNA (50ng) in a 25μl reaction with Platinum Taq (1.25units) (Invitrogen) using the provided buffer, dNTPs (100μM) with primers 5'S_μ (5'GCAGAAAATTTAGATAAAATGGATACCTCAGTG-3') (Schrader et al. 2005) and 3' FG (5'-GGCTTTCTCTGTCTGGGTCA-3') (400nM) and amplified at 95°, 5 min, then 32 cycles of 94°/15 sec, 62°/15 sec, 72°/30 sec. The S_μ probe was radiolabeled in a 25 μl reaction using Klenow fragment (12.5 U) (MBI Fermentas) polymerization, random hexamers (2 μg) (MBI Fermentas), 50μCi of α-dCTP 32P (3000Ci/mmol, Perkin Elmer), and dATP/dTTP/dGTP (100 μM each), at room temperature for 1 h and then was passed over a fine Sephadex G-25 column (Roche 11 273 922 001) to remove free nucleotides.

PCR amplification of endogenous V_K-J_K and D_H-J_H rearrangements

PA112.2 cells were treated with ST571 for 16 hours and V_K6-23-J_K1 coding joins (CJs) were PCR amplified in 25μl reactions using genomic DNA (0.1μg) and Phire Hot Start II DNA polymerase (0.5μl) (Thermo Scientific) according to the manufacturers instructions with primers p_KJa and p_K6c.SK (400nM) (Table S1) using 98°C/5 min followed by 38 cycles 98°C/5

sec, 64°C/5 sec, 72°C/15 sec. DFL16.1-J_H1 CJs were isolated from the R2K2 clones A2 and B5 that were transfected with pMX2-RAG2-EGFP (Subrahmanyam et al. 2012) or empty vector using Nucleofection II Kit L and a Nucleofactor machine (model AAD-1001N) with program Y001 (Lonza) according to manufacturers instructions. Cells were monitored 16 hr post transfection for GFP expression and genomic DNA was isolated. CJs were PCR amplified in 25µl reactions using genomic DNA (0.1µg) and Phire Hot Start II DNA polymerase (0.5µl) (Thermo Scientific) per the manufacturers instructions with DFL16.1 F1 and JH1 R1 primers (400nM) (Table S1). DFL16.1-J_H1 CJs were PCR amplified at 98°C/5min followed by 38 cycles of 98°C/5sec, 58°C/5 sec, and 72°C/15 sec. All CJs were visualized by gel electrophoresis and verified by cloning into pCR4-TOPO (Invitrogen) and DNA sequence analysis. For both V_κ6-23-J_κ1 and DFL16.1-J_H1 CJs in semiquantitative PCR products were harvested at 32, 35 and 38 cycles as shown in Figures 2C,F.

5C primer design

5C primers were designed at HindIII restriction sites using 5C primer design tools previously developed (Dostie et al. 2006) and made available online at <http://my5C.umassmed.edu> (Lajoie et al. 2009). A total of 112 forward and 113 reverse primers were designed for all HindIII restriction fragments in the Igh locus between coordinates chr12:114390223-117301828 (mm9) a 2.91 Mb region producing a total of 12,656 *cis* interactions that were studied (Table S2). Although a gene desert region on chromosome 5 between coordinates chr5:133050833-133608757 (mm9) spanning 0.56 Mb was interrogated using 24 forward and 25 reverse primers (Table S2) for a total of 525 interactions this information will be presented

elsewhere. 5C primers located at highly repetitive sequences were excluded. Primers settings were: U-BLAST: 3; S-BLAST: 80; 15-MER: 800; MIN_FSIZE: 1000; MAX_FSIZE: 15,000; OPT_TM: 65; OPT_PSIZE: 30. Several primers with slightly relaxed parameters were included to provide a more complete coverage of the *Igh* locus. DNA sequence of the universal tails of forward and reverse primers were TAATACGACTCACTATAGCC and TCCCTTTAGTGAGGGTTAATA, respectively.

5C libraries construction

5C library construction was performed as described (Dostie and Dekker 2007). The 5C library composition of each experiment is summarized in Table S3. 5C analysis of the *Igh* locus was carried out on two biological replicates of 3C libraries from Rag2^{-/-} pro-B cells, or CD43⁺ resting splenic B cells. For Rag2^{-/-} pro-B cells we obtained 22,049,248 and 22,300,921 mapped reads for two biological replicates of which 21,927,201 and 21,689,397 could be specifically mapped back to interactions within the *Igh* locus using Novoalign (<http://www.novocraft.com>), respectively. For resting B cells we obtained 20,682,018 and 20,580,457 mapped reads for two biological replicates of which 20,059,382 and 19,878,261 could be specifically mapped back to interactions within the *Igh*. In all instances the mappable reads were proportional to the degree of multiplexing, indicating equivalent library quality despite different read numbers. The heat maps are scaled as follows: for Figure 3A, Rag1^{-/-} pro B cells: 72-29673, resting B cells: 204-25913. Raw data for 5C and a complete 3C protocol are available by request.

5C analyses of the Igh locus and chromosome 5 gene desert

We generated comprehensive interaction maps of the Igh constant region locus by performing 5C analyses in Rag2^{-/-} pro-B cells and resting splenic B cells. 3C libraries were constructed and used as a template for 5C analyses. As described above, we designed 5C primers at Hind III sites using computational algorithms posted online in the My5C software package (Lajoie et al. 2009). A total of 112 forward and 113 reverse primers were designed for alternating HindIII restriction fragments in the Igh locus producing a total of 12,656 *cis* interactions that were studied. We focus the analysis here on the 3'-end of the Igh locus which spans the 244 kb C_H subregion and extends to the end of 3'Ea (chr12:114,691,293-114,446,716, mm9). As a specificity control, we examined a gene desert region spanning 270 kb on chromosome 5 (133,194,370-133,464,774) using 21 forward and 25 reverse primers for a total of 525 intrachromosomal interactions. Paired-end Solexa sequencing was used to determine the number of 5C ligation products corresponding to pairs of interacting fragments. To visualize particular long range interactions along the Igh locus, interaction frequencies between forward and reverse 5C primers are presented as two dimensional heatmaps where color intensity indicates contact frequency (Suppl. fig. S15A). The diagonal reflects interaction frequencies along the chromosome between fragments that are closely spaced in the genome whereas squares off the diagonal represent long range looping interactions between designated fragments. The frequency of interacting restriction fragments is inversely related to the genomic distance separating them (Suppl. Fig. S15B) consistent with previous reports (Dostie et al. 2006). The 5C analysis on chromosome 5 revealed few long range chromatin interactions at the gene desert locus indicating that the looping interactions

observed at the *Igh* locus are specific events (Suppl. Fig. S16). Our 5C data was next submitted to binning analysis, using a bin size of 30kb and a step size of 3 kb, to transform the fragment based interaction maps to distance specified and symmetrical contact matrices (fig. 3, left and middle panels). This approach also permits estimation of interaction frequency between non-interrogated restriction fragments. Thus, the heat maps shown in Supplemental Figure S15-S16 and Figure 3, represent the 5C data prior to and following binning analysis, respectively.

Difference heatmaps, shown in Figure 3 (right panel) allow us to visualize the log₂ pro-B cell/resting B cell data. This function transforms the data so that both higher and lower pro-B cell/resting B cell ratios are on the same scale and the values will range between -1 and 1. Values between 0 and 1 (pro-B cell; red) will indicate an interaction frequency that is higher than resting B cells and values between -1 and 0 will represent a (resting B cell; blue) frequency that is higher than pro-B cells. Note that all pro-B cell (red) interactions will be compressed between 0 and 1. Thus, interactions which are low frequency but still uniquely occurring in pro-B cell will be evident. Reciprocally, all resting B cell interactions will be compressed between 0 and -1 and marked as blue. This approach confirms that in the binning analysis of Figure 3, $\gamma 1:\gamma 3$ interactions, encircled by the orange oval, are more frequent than expected in pro-B cells than in resting splenic B cells. It is useful to note that low frequency interactions which are not very evident in binning analysis (Fig. 3, left panel) but which nevertheless occur uniquely in pro-B cells are also marked in red in the difference plot (Fig. 3, right panel). For example, in pro-B cells (red) $E\mu:hs5-7$ looping interactions are

detected in accord with previous studies (Degner et al. 2011; Guo et al. 2011), as are $E_{\mu:\gamma 2b-\epsilon}$. In contrast, some $E_{\mu:hs3b,4}$ (and $E_{\mu:\gamma 3-\gamma 1}$) looping interactions are detected in resting B cells but are lower in pro-B cells, an observation that is confirmed in 3C assays (fig. 4C, left panel).

Chromosome conformation capture (3C)

The chromosome conformation capture (3C) assay (Dekker et al. 2002) was adapted for use on the mouse *Igh* locus using *Hind* III to digest crosslinked chromatin (Wuerffel et al. 2007) with modifications. Cells were crosslinked with 1% formaldehyde for 8 minutes at room temperature and the reaction was quenched with glycine. All primers and probes for 3C product analyses were designed to avoid strain polymorphisms (Table S4). Controls to confirm the efficacy of the 3C procedure were performed. Template concentration using the mb1 primers (Table S4) was determined as previously described (Wuerffel et al. 2007). The efficiency of *Hind* III restriction site digestion which was monitored by real-time PCR analysis using primers spanning the restriction sites under study (Table S5). To control for differences in amplification efficiency between primer sets we constructed a control template in which all possible 3C ligation products are present in equimolar concentration (Table S6). DNA fragments that span restriction sites studied were PCR amplified, and mixed in equimolar amounts, then digested with *Hind* III, ligated and added to genomic DNA that had been digested and ligated, to serve as the 3C control template and is used in a standard curve in quantitative 3C PCR analyses. To permit sample to sample comparisons the data were normalized using the interaction frequency between two fragments within the non-expressed

chr5 gene desert (GD) (chr5:133050833-133608757), which are separated by 3.5 kb (Table S4). The equation used to calculate the relative crosslinking frequency between two *Igh* restriction fragments: $X_{Igh} = [S_{Igh}/S_{GD}] \text{ Cell Type} / [S_{Igh}/S_{GD}] \text{ Control mix}$. S_{Igh} is the signal obtained using primer pairs for two different *Igh* restriction fragments and S_{GD} is the signal obtained with primer pairs for the GD locus fragments (Table S4). The crosslinking frequency for the two GD fragments was arbitrarily set to a value of 1 to permit sample comparisons.

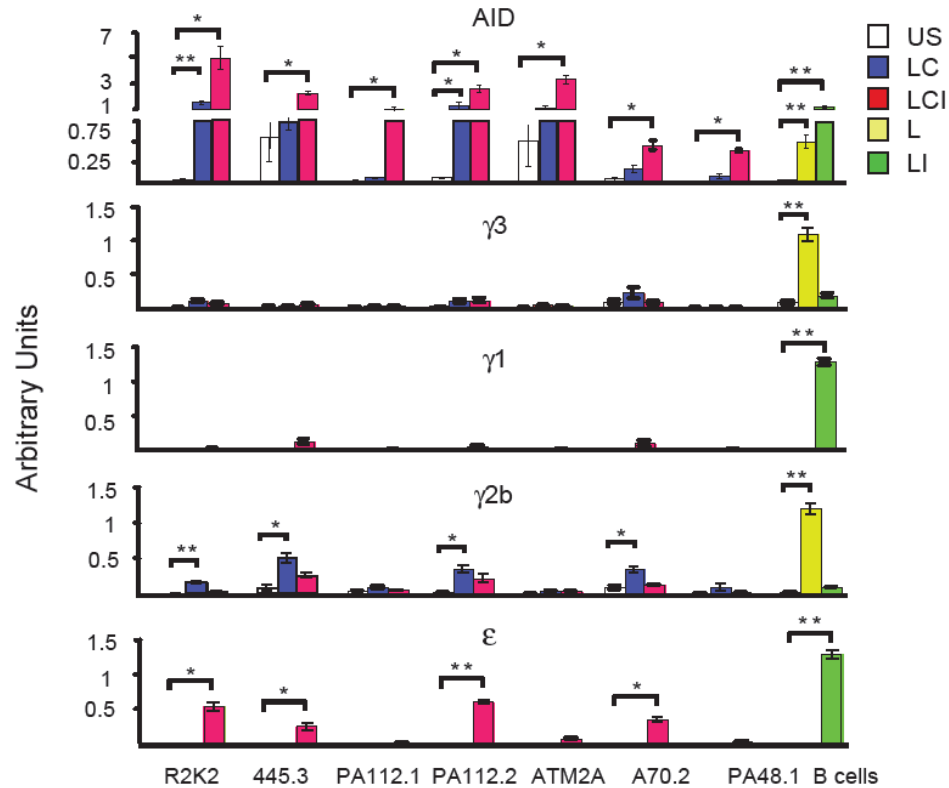
Quantitative PCR for 3C Ligation Products

Quantitative PCR (qPCR) for 3C was used in combination with 5'FAM and 3'BHQ1 modified probes (IDT) for detection of 3C products and primers were designed using Primer Express software (ABI) (Table S4). PCR control fragments for determination of primer efficiency of each primer combination (Table S6) were generated using genomic DNA and PCR primers for the regions of interest. The qPCR protocol was run at 59C for 90 sec. Primer and probe optimization were carried out according to the manufacturer's recommendations, (http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042996.pdf). Dilutions of control mix (64 to 0.03 pg of ligated mix per 50 ng of genomic DNA per μ l) were used to establish standard curves and all 3C reactions used 100 ng of 3C chromatin. A complete laboratory protocol for 3C is available upon request. For all qPCR 3C reactions, 100 ng of chromatin was used.

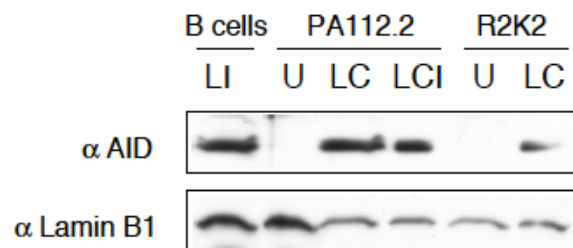
SUPPLEMENTAL FIGURES

Supplemental Figure S1

A



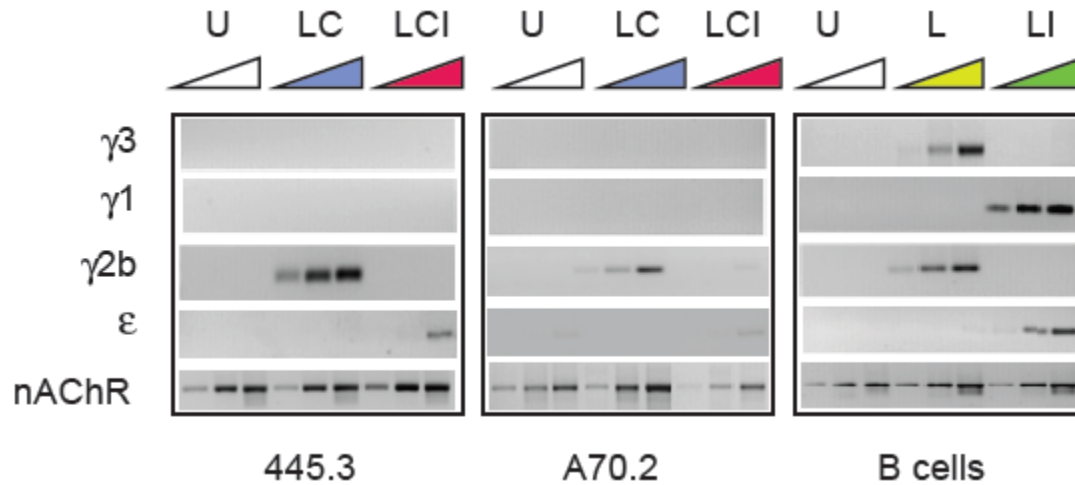
B



Supplemental Figure S1. AID and GLT expression is induced in pro-B and pre-B cell lines. **A)** Abelson transformed pro-B (R2K2 and 445.3) and pre-B (PA112.1, PA112.2, ATM2A, A70.2, PA48.1) cell lines were unstimulated (U) or activated with LPS+CD40L (LC) or LPS+CD40L+IL4 (LCI). Controls were WT resting splenic B cells were unstimulated (U) or activated with LPS (L) or LPS+IL4 (LI). AID and GLTs γ3, γ1,

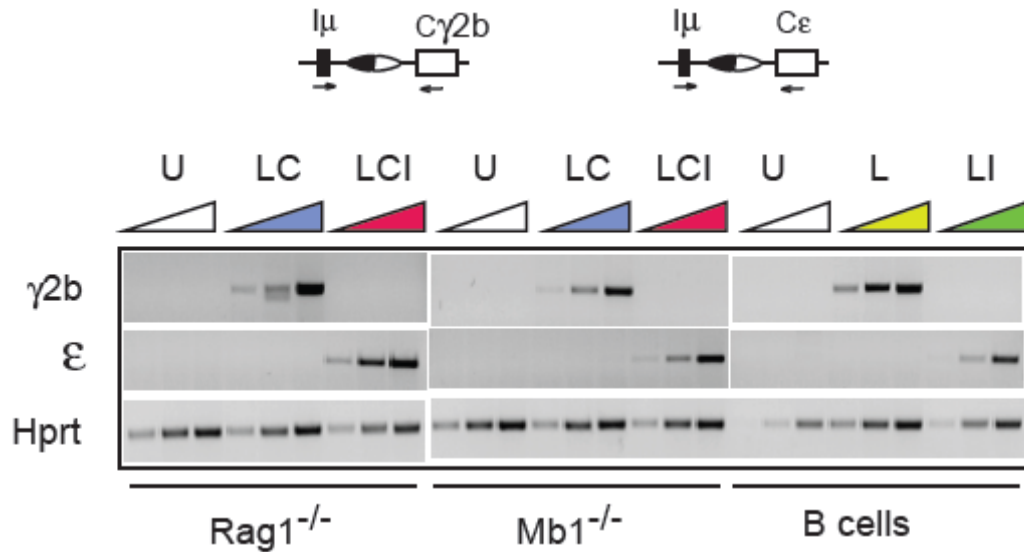
$\gamma 2b$ and ε from five samples from 3-5 independent experiments were normalized to the rRNA 18S transcript. p values; $p \leq 0.05$ (*) or 0.001-0.0001 (**). **B)** Resting splenic B cells were activated with LPS+IL4, R2K2 and PA112.2 were unstimulated (U) or activated with LPS+CD40L (LC) or LPS+CD40L+IL4 (LCI) for 48 hours then nuclear extracts analyzed for expression of AID and the LAMIN B1 loading control in Western assays using anti-AID (Chaudhuri et al. 2003) or anti-LAMIN B1 (Abcam, ab16048) and standard techniques. A Western blot representative of 3 independent experiments is shown.

Supplemental Figure S2



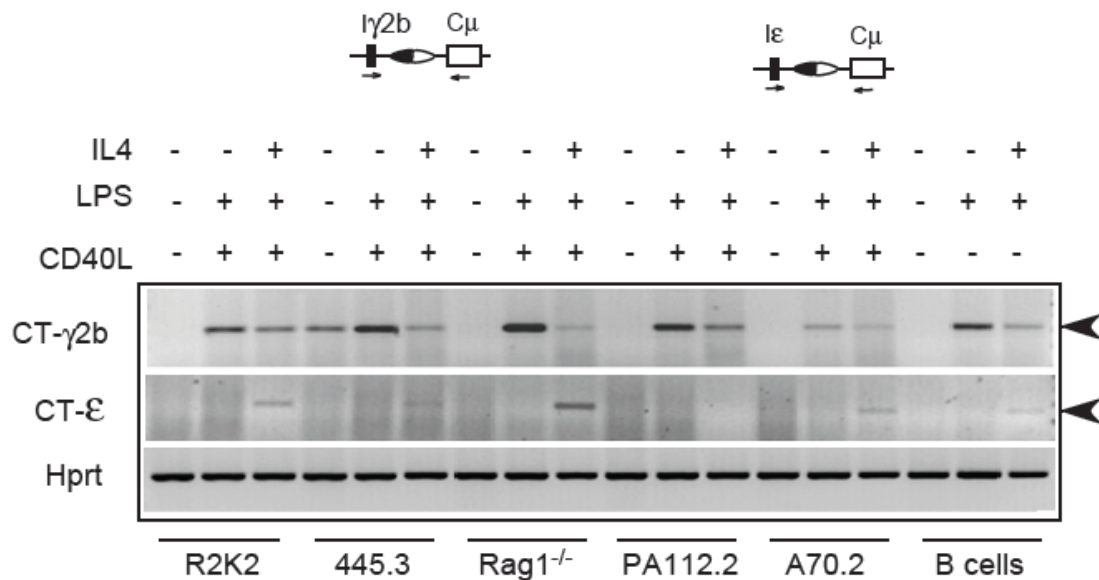
Supplemental Figure S2. Abelson transformed pro-B and pre-B cell lines undergo robust CSR. Abelson transformed pro-B (445.3) and pre-B (A70.2) cell lines were unstimulated (U) or activated with LPS+CD40L (LC) or LPS+CD40L+IL4 (LCI). Controls were WT resting splenic B cells were unstimulated (U) or activated with LPS (L) or LPS+IL4 (LI). DC-PCR analyses of $\mu \rightarrow \gamma 3$, $\mu \rightarrow \gamma 1$, $\mu \rightarrow \gamma 2b$ and $\mu \rightarrow \epsilon$ CSR. PCR products harvested at 26, 29, and 32 cycles (bottom) are representative of three independent experiments.

Supplemental Figure S3



Supplemental Figure S3. Bone marrow pro-B cells induced for CSR express post-switch transcripts (PSTs). Rag1^{-/-} and Mb1^{-/-} pro-B cells were unstimulated (U) or activated with LPS+CD40L (LC) or LPS+CD40L+IL4 (LCI). Controls were WT resting splenic B cells that were unstimulated (U) or activated with LPS (L) or LPS+IL4 (LI). PSTs were analyzed for $\mu \rightarrow \gamma 2b$ (top) and $\mu \rightarrow \epsilon$ (middle) CSR. Hprt was used as a loading control (bottom). Results are representative of three independent experiments. Samples were harvested at 29, 32 and 35 cycles.

Supplemental Figure S4



Supplemental Figure S4. Circle transcript (CT) assays indicate that CSR in early B cells is cytokine inducible and dynamic. CT RT-PCR assays demonstrate whether induced CSR occurs dynamically. Hybrid $I\gamma 2b$ - $C\mu$ or $I\epsilon$ - $C\mu$ transcripts arise from excised circular DNA generated by direct $\mu \rightarrow \gamma 2b$ or $\mu \rightarrow \epsilon$ CSR and are detected by inverse PCR (top). CT-PCR generates a single band since the S_x - S_μ junction is spliced out in the processed post-switch mRNA. Rag1 deficient pro-B cells, Abelson transformed pro-B cell lines (R2K2 and 445.3) and pre-B cell lines (PA112.2 and A70.2) were activated with as indicated for 42 hours or unstimulated (U). Controls were WT resting splenic B cells that were unstimulated (U) or activated with LPS (L) or LPS+IL4 (LI) for 48 hours. CTs were analyzed for $\mu \rightarrow \gamma 2b$ (top) and $\mu \rightarrow \epsilon$ (middle) CSR. Hprt was used as a loading control (bottom). Results are representative of three independent experiments. Samples were harvested at 32 and 35 cycles for Hprt and CT respectively.

Supplemental Figure S5

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Sμ      TAC TC TGA G CTA AGC AAA GCT GGG CTTU AGC CAA AAT GAA GTA GAC TTTA
      |||
P1       TAC TC TGA AGC TAA GCA AAG C TU G TAC G TU GAG ATT GTA GGA A TTTG A GG
      |||
Sγ2b     G ACTACTTCCTAGC AGC CGT G GAGAG C TU GAG ATT GTA GGA ATTU TUG AGG

Sμ      GG TTAG C TU AGC TTAGCTTGG C TU AGCTAGG GTU AGC TUG GCTU AGC TUG
      |||
P5       GG TTAG C TU AGC TTAGCTTGG C TU A AG GGA GTTGGGG CAG GTTGGAG GTU
      |||
Sγ2b     A TTAGGGG ACC AGTTC TTAGCA GCTAGGAGGG AGC TUG GGCAGGTGGG AGTTC

Sμ      C TTAGCTT GGG TTAG C TTAG C TU AGC TGGG GTU AGCTGAG C TU AG C TUG G
      |||
P10      C TTAGCTTGG GTU AGC TTAGCTTAG G GGG ATT TUG AATAAG TAG TAG AGT
      |||
Sγ2b     TU GGGAGCC AGTTCCTAGGAGG CTA TUG GGGAGTCTGGAA TAG G TAG TAG AGT

Sμ      AG C TTAGGG TTAG C TU GGC TTAGCTT GGG TTAGCTGAG C TU AGCTGGG GTA
      |||
P15      AGC TAG GGTGAG C TU GGC TAAAC TU C TU GGG CAG GTTG GAG TTTGAG GGA
      |||
Sγ2b     ACCAGTTC TTAGCA GCTAGGAGGG AGC TUG GGC AGG TUG GAG TTTGAG GGA

Sμ      CTACACTU GAC TTTCTU AGC TTAGATGAGC TUG GGTGAG CTT AGCTATU
      |||
P40      CTAC ACTGAGCTU TTT TTAGACTT AG C TU GGA TAGG TUG GAG TATTAG GGA
      |||
Sγ2b     AC CAG TTT TTAGAGC TATGCG GGA GCTTGGG ATA GGTGAG AGTATTAGGG A

Sμ      TGGG GTTAGCTGAG C TU AGCTGGAGC TU AGC TTAG C TU GGC TTAGCTT GGG
      |||
P31      TU GGGTGA C TU AGC TTAGCTTGA GAG TTTGAGG GAC CAG ACC TAG CAG C
      |||
Sγ2b     CTAGGAGGGAGCTTGG GCAAG GTU GGAAG TTTGAGG GAC CAG ACCTAGC AGC

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Supplemental Figure S5. S μ /S γ 2b junctions derived from Rag1^{-/-} pro B cells. BM derived Rag1^{-/-} pro B cells were activated with LPS+CD40L to undergo CSR in *ex vivo* cultures (see Suppl. Methods). S μ /S γ 2b junctions were amplified by PCR, cloned and analyzed by DNA sequence analysis. The S μ donor and S γ 2b acceptor germline sequences are aligned above or below the recombined switch junction sequences, respectively. Microhomology between the S μ and the downstream S γ 2b regions is a common feature in switch junctions (Stavnezer et al. 2010). Microhomology, highlighted in red, was determined by identifying the longest region at the S μ /S γ 2b junction of perfect, uninterrupted donor/acceptor sequences. Mutations or insertions are highlighted in blue.

Supplemental Figure S6



Supplemental Figure S6. S μ /S ϵ junctions derived from Rag1^{-/-} pro B cells. BM derived Rag1^{-/-} pro B cells were activated with LPS+CD40L+IL4 to undergo CSR in ex vivo cultures (see Suppl. Materials and Methods). S μ /S ϵ junctions were amplified by PCR, cloned and analyzed by DNA sequence analysis. The S μ donor and S ϵ acceptor germline sequences are aligned above or below the recombined switch junctional

sequences. Microhomology between the S_{μ} and the downstream S_{ε} is a common feature in switch junctions (Stavnezer et al. 2010). Direct joints are indicated by the vertical line and no microhomology or insertions are observed at the junction. Microhomology, highlighted in red, was determined by identifying the longest region at the S junction of perfect, uninterrupted S_{μ} donor/ S_{ε} acceptor sequences. Mutations or insertions are highlighted in blue.

Supplemental Figure S7

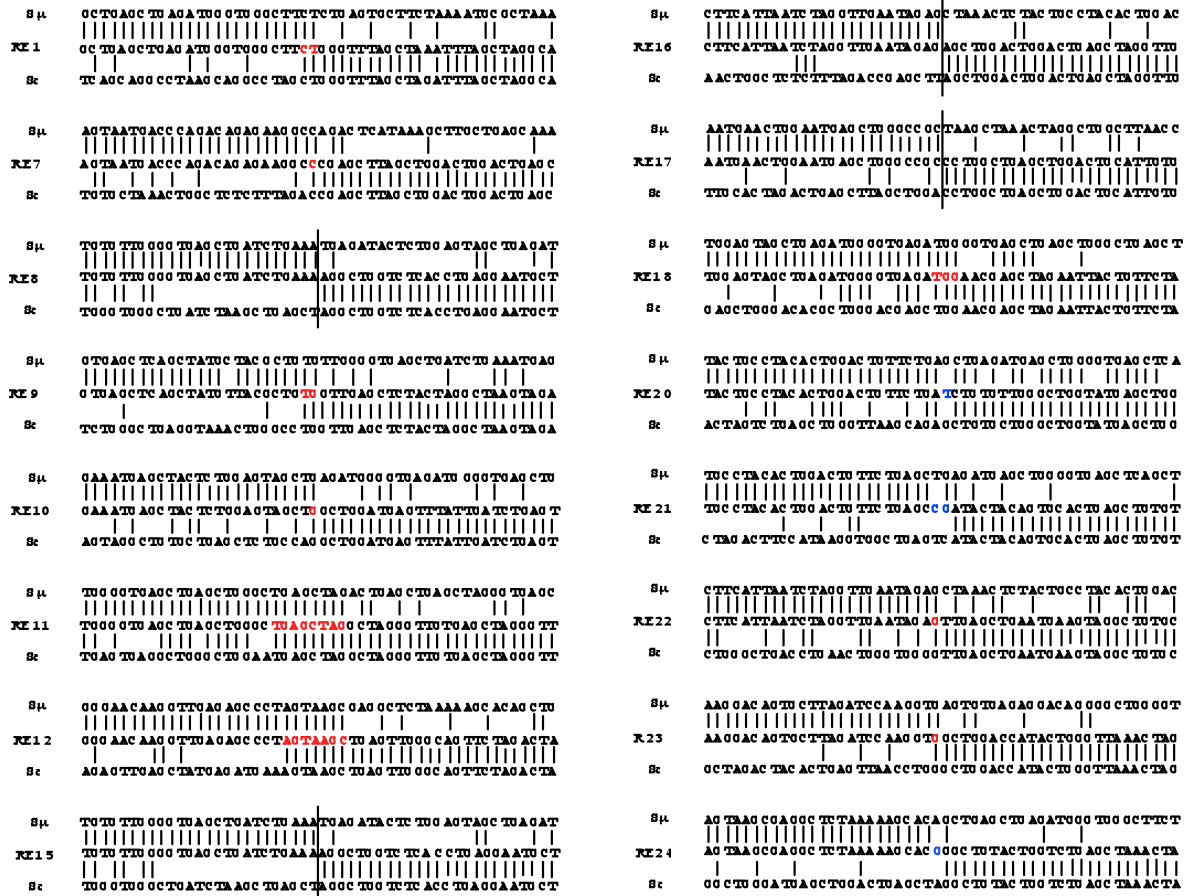


Supplemental Figure S7. S μ /S γ 2b junctions derived from R2K2 pro-B cell line.

Abelson transformed R2K2 pro-B cells were activated with LPS+CD40L to undergo CSR (see Suppl. Methods). S μ /S γ 2b junctions were amplified by PCR, cloned and analyzed by DNA sequence analysis. The S μ donor and S γ 2b acceptor germline sequences are aligned above or below the recombined switch junction sequences, respectively. Microhomology between the S μ and the downstream S γ 2b regions is a common feature

in switch junctions (Stavnezer et al. 2010). Microhomology, highlighted in red, was determined by identifying the longest region at the $S_{\mu}/S_{\gamma}2b$ junction of perfect, uninterrupted donor/acceptor sequences. Mutations or insertions are highlighted in blue.

Supplemental Figure S8

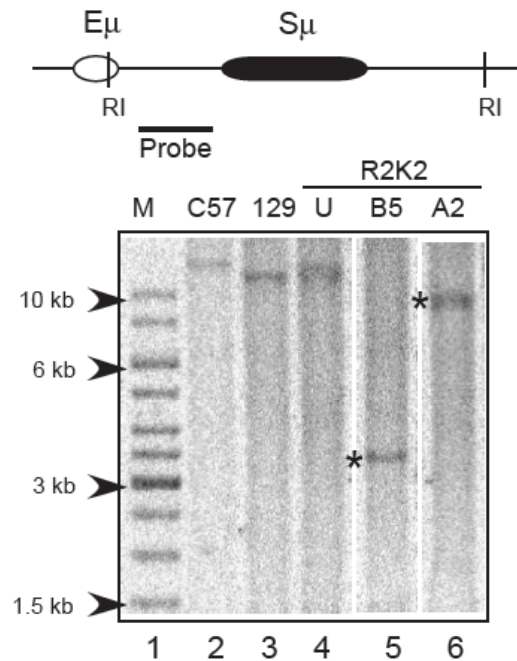


Supplemental Figure S8. S μ /S ϵ junctions derived from R2K2 pro-B cell line.

Abelson transformed R2K2 pro B cells were activated with LPS+CD40L+IL4 to undergo CSR (see Suppl. Methods). S μ /S ϵ junctions were amplified by PCR, cloned and analyzed by DNA sequence analysis. The S μ donor and S ϵ acceptor germline sequences are aligned above or below the recombined switch junctional sequences. Microhomology between the S μ and the downstream S ϵ is a common feature in switch junctions (Stavnezer et al. 2010). Direct joints are indicated by the vertical line and no

microhomology or insertions are observed at the junction. Microhomology, highlighted in red, was determined by identifying the longest region at the S junction of perfect, uninterrupted S_{μ} donor/ S_{ε} acceptor sequences. Mutations or insertions are highlighted in blue.

Supplemental Figure S9.



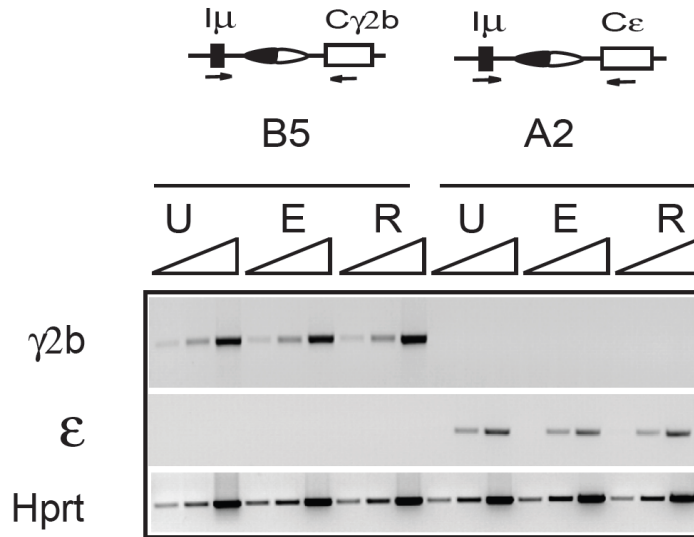
Supplemental Figure S9. Clonality of $S\mu$ rearrangements in R2K2 clones B5 and A2. $Rag2^{-/-}$ R2K2 cells were induced with LPS+CD40L (LC) or LPS+CD40L+IL4 (LCI) for 48 hours then switched clones, B5 and A2 were isolated by limiting dilution (see Suppl. Materials and Methods). A2 and B5 cells were assessed by Southern for rearrangement of the $S\mu$ region. Schematic representation of the $S\mu$ region shows the intronic μ enhancer ($E\mu$), $S\mu$ region, location of the 5' $S\mu$ probe and $EcoR1$ (RI) restriction sites (top). Genomic DNA was digested with $EcoR1$, and Southern blots were analyzed using 5' $S\mu$ probes. Molecular weight ladder (lane 1), $S\mu$ region in germline configuration in C57Bl/6 (C57) (12 Kb) (lane 2) and 129 (11kb) splenic B cells (lanes 2,3). Unstimulated (U) R2K2 cells are in the C57Bl/6x129 germline configuration (lane 4) and clones B5 and A2 are rearranged, as indicated (*) (lanes 5,6).

Supplemental Figure S10.

TAATGATGCCATCATCGATG) ATTACTACGGTAGTAGCTAC)		(GATGACCATGAAGCTACAGT CTACTGGTACTTCGATGTCT	
A2 (μ→ε)			
DJ25	ATTACTACGGTAGTAG		CTACTGGTACTTCGATGTCT
DJ26	ATTACTACGGTA		CTGGTACTTCGATGTCT
DJ27	ATTACTACGGTAGTAGCTA		CTACTAGTACTTCGATGTCT
DJ28	ATTACTACGGTA	CTG	GTACTTCGATGTCT
DJ32	ATTACTACGGTAGTAGC		TGGTACTTCGATGTCT
DJ35	ATTACTACGGTAGTAG		CTGGTACTTCGATGTCT
DJ41	ATTACTACGGTAGTAGCTAC	CA	ACTGGTACTTCGATGTCT
DJ42	ATTACTACGGTAGTAGCT	TC	ACTGGTACTTCGATGTCT
DJ44	ATTACT		GGTACTTCGATGTCT
DJ29	ATTACTACGGTAGTAGCTAC	GTGG	ACTGGTACTTCGATGTCT
B5 (μ→γ2b)			
DJ45	ATTACTACGGTAGTAGC	CC	CTACTGGTACTTCGATGTCT
DJ49	ATTACTACGGTAGTAGCT	T	CTGGTACTTCGATGTCT
DJ54	ATTACTACGGTAGTAGC	CTT	TACTGGTACTTCGATGTCT
DJ15'	ATTACTACGGTAGTAGC		TACTGGTACTTCGATGTCT

Supplemental Figure S10. $D_{DFL16} \rightarrow J_{H1}$ CJs induced by RAG2 expression in switched R2K2 clones A2 and B5. Clones A2 ($\mu \rightarrow \epsilon$) and B5 ($\mu \rightarrow \gamma 2b$) were transfected with Rag2 (R) whereupon $D_{DFL16} \rightarrow J_{H1}$ CJs were amplified by PCR, cloned, then examined by DNA sequence analysis. Coding regions of D and J are shown at the top and DNA sequences were aligned to the bottom strand.

Supplemental Figure S11.



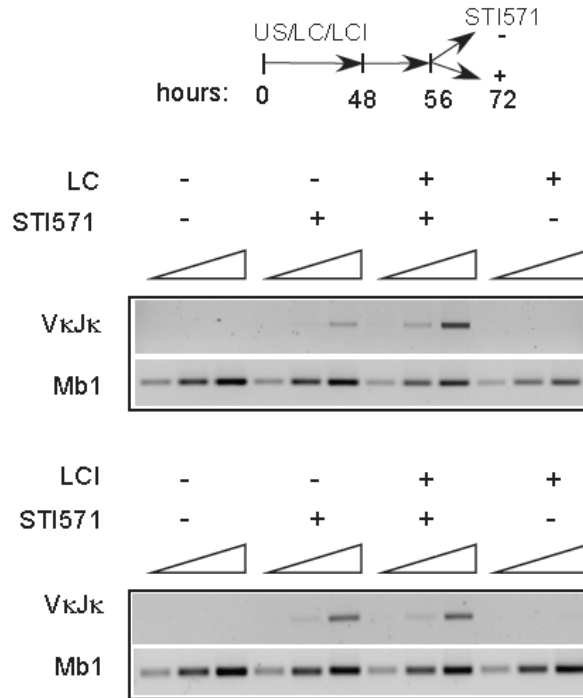
Supplemental Figure S11. Rag2 expression has no effect on the CSR status of R2K2 clones. R2K2 switched clones B5 ($\mu \rightarrow \gamma 2b$) and A2 ($\mu \rightarrow \epsilon$) were untreated (U) or transfected with either Rag2 (R) or empty (E) vector then grown in culture for 16 hours. PST $\mu \rightarrow \gamma 2b$ (top) and $\mu \rightarrow \epsilon$ (middle) were analyzed by semiquantitative RT-PCR before and after transfection. Hprt was used as loading control (bottom). Results are representative of three independent experiments and PCR products were harvested at 29, 32 and 35 cycles.

Supplemental Figure S12.

	AGTCGTTATATCGTCGATAG) TCAGCAATATAGCAGCTATC		(GAGACACCTGCAAGCCACCT CTCTGTGGACGTTTCGGTGGA
VJ1	TCAGCAATATAGCAGCTATC	C	GTGGACGTTTCGGTGGA
VJ4	TCAGCAATATAGCAGCTA	CC	GTGGACGTTTCGGTGGA
VJ5	TCAGCAATATAGCAGCTATC	CCCC	GGACGTTTCGGTGGA
VJ6	TCAGCAATATAGCAGCTATC	CAC	GTGGACGTTTCGGTGGA
VJ7	TCAGCAATATAGCAGCTA		TGGACGTTTCGGTGGA
VJ8	TCAGCAATATAGCAGCTATC	C	GGACGTTTCGGTGGA
VJ9	TCAGCAATATAGCAGCTATC	CTC	TGGACGTTTCGGTGGA
VJ10	TCAGCAATATAACAGCTATC	CCC	GGACGTTTCGGTGGA
VJ12	TCAGCAATATAGCAGCTATC	CC	GTGGACGTTTCGGTGGA
VJ14	TCAGCAATATAGCAGCTATC	TCCAC	GTGGACGTTTCGGTGGA
VJ19	TCAGCAATATAGCAGCTATC	CCTC	GTGGACGTTTCGGTGGA
VJ22	TCAGCAATATAGCAGCTATC	CTA	GACGTTTCGGTGGA

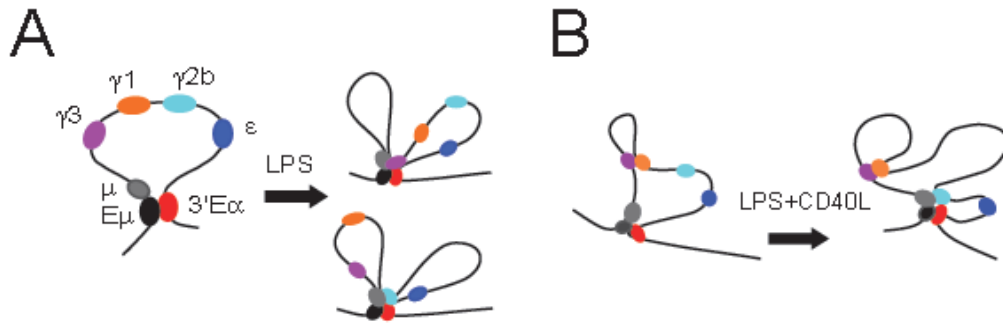
Supplemental Figure S12. $V_{K6-23} \rightarrow J_K$ joining following CSR in an Abelson transformed pre-B cell line. PA112.2 was treated with LPS+CD40L for 48 hours, cells were washed and cultured for 8 hours whereupon STI571 was added for 16 hours. $V_{K6-23} \rightarrow J_K$ CJs were PCR amplified, cloned then submitted to DNA sequence analysis. Coding regions of V and J are shown on the top and DNA sequences were aligned to the bottom strand.

Supplemental Figure S13



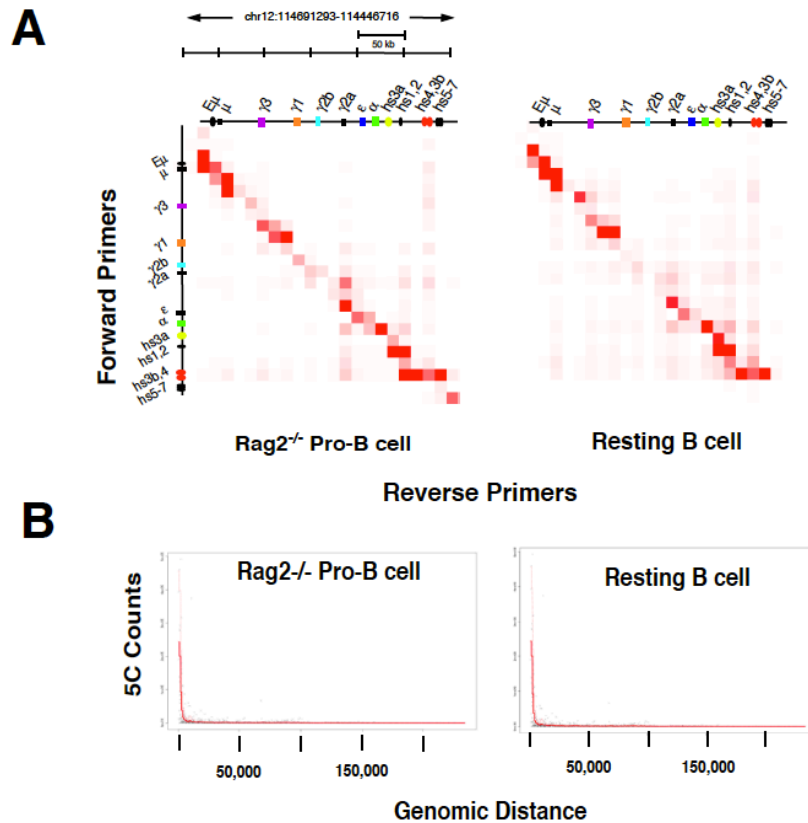
Supplemental Figure S13. The frequency of STI571 induced V_{K6-23}→J_{K1} CJs in an Abelson transformed cell line is not diminished following activation of CSR. PA112.2 cells were unstimulated (US) or exposed to CSR inducers, LPS+CD40L (LC), LPS+CD40L+IL4 (LCI), for 48 hours, then washed and allowed to recover for 8 hours, then cultured in the presence or absence of STI571 for 16 hours (top). At the end of the culture period DNA was isolated and tested for V_{K6-23}→J_{K1} CJs and Mb1 by semi-quantitative PCR. Primers were V_K.1 (5'-CTTACGGAGTTACTCTGTATCTTTGC-3') and pk6c.SK (Table S1) for CJs as described (Supplementary Methods) except that annealing was 60°C/5 sec. Triangles indicate that PCR products were harvested at increasing cycles (32, 35 and 38) of amplification. Mb1 is shown as a loading control. Results are representative of 3 independent experiments.

Supplemental Figure S14.



Supplemental Figure S14. Model of CSR in pro-B cells. A) A schematic of the looped IgH locus in mature resting and LPS activated B cells. **B)** In pro-B cells, $E\mu:3'E\alpha$ and $\gamma 3:\gamma 1$ interactions are evident before CSR activation precluding IgG3 and IgG1 CSR. Following CSR activation in an Abelson transformed pro-B cell line, 445.3, $E\mu:3'E\alpha$ and $\gamma 2b:3'E\alpha$ looping interactions increase (Fig. 4C). Factors previously shown to inhibit the I ϵ GLT promoter region include Bcl6 and ID2. Bcl6 inhibits I ϵ GLT by antagonizing Stat6-binding activity (Harris et al. 1999; Harris et al. 2005) and ID2 down-regulates I ϵ GLT by inhibiting E2A proteins from binding to the I ϵ promoter (Sugai et al. 2003). Both Bcl6-deficient (Harris et al. 2005) and ID2-deficient (Sugai et al. 2003) mature splenic B cells display an increased propensity for IgE switching. Steady-state Bcl6 and ID2 transcript levels are significantly lower in early B cells as compared to mature B cells (Wesemann et al. 2011). However, over-expression of Bcl6 alone does not alter the IgE switching pattern in early B cells (Wesemann et al. 2011). Further studies are required to determine whether Bcl6 or Id2 are involved in the long range interactions identified by our work shown in Figures 3 and 4.

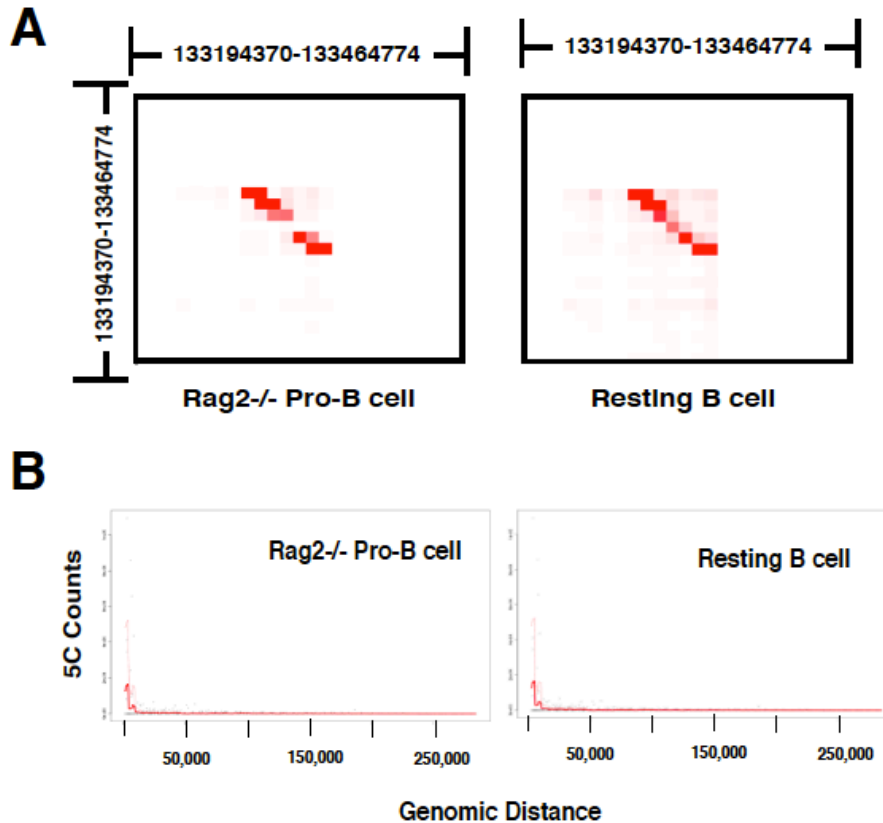
Supplemental Figure S15



Supplemental Figure S15. 5C analysis of a 244kb segment of the IgH locus in pro-B and resting B cells. **A)** 5C experimental data for Rag2^{-/-} pro-B cells and resting splenic B cells. Plots show heat maps colored red to white to indicate high to low counts. The axis labels are replaced by a linear representation of the genetic elements associated with the Hind III fragments in the IgH locus. The heat maps shown here and in Figure 3 represent the 5C data prior to and following binning analysis, respectively. **B)** 5C counts for all detected interactions were plotted against the genomic distance between the corresponding restriction fragments. The graph shows an inverse relationship between interaction frequency and genomic distance (30kb or larger, excluding self ligations and adjacent ligations) separating Hind III restriction fragments. The average expected level of interaction was determined using LOESS smoothing ($\alpha = 0.05$) (red line). The gray circles (visualized best when figure is expanded) show the observed 5C counts for each queried fragment. The average profile provides an

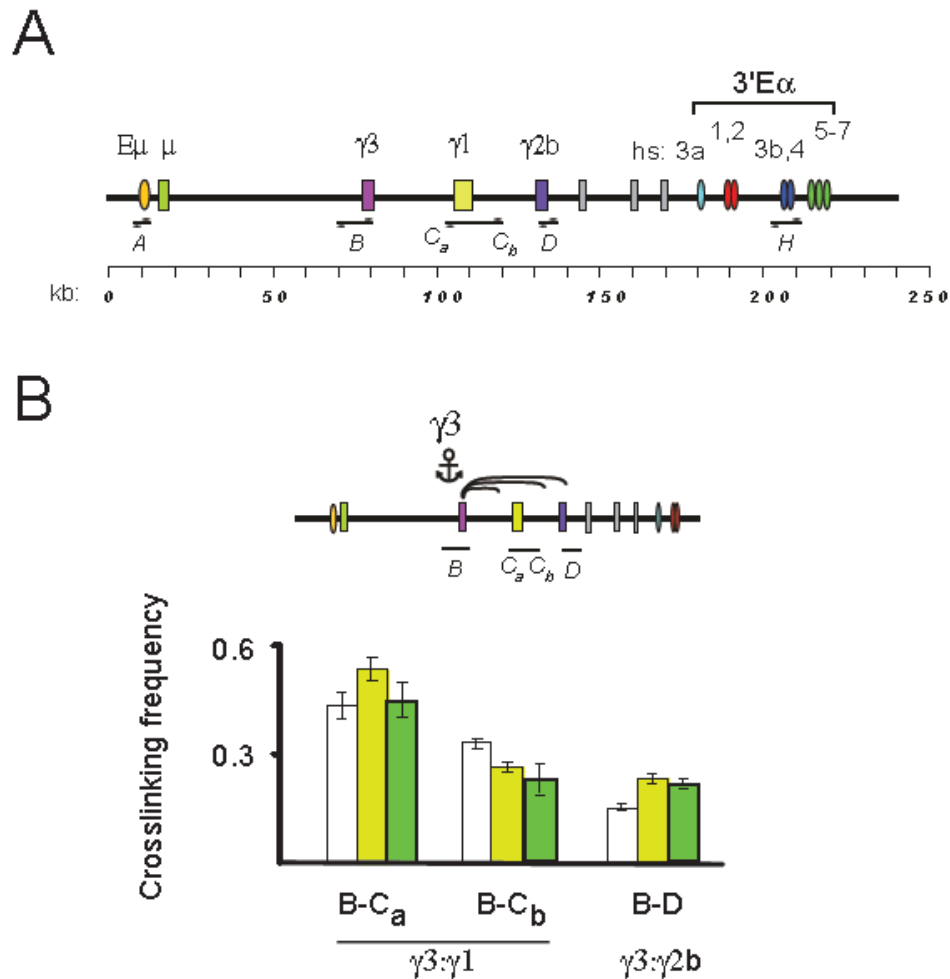
estimate for the level of interaction expected when no specific chromatin looping interactions occur.

Supplemental Figure S16



Supplemental Figure S16. 5C analysis of a gene desert on chromosome 5 in pro-B and resting B cells. A) 5C experimental data for Rag2^{-/-} pro-B cells and resting splenic B cells. Plots show heat maps colored red to white to indicate high to low counts. The axis labels are replaced by a linear representation of the genetic elements associated with the Hind III fragments in the Igh locus. **B)** 5C counts for all detected interactions were plotted against the genomic distance between the corresponding restriction fragments. The graph shows an inverse relationship between interaction frequency and genomic distance (30kb or larger, excluding self ligations and adjacent ligations) separating Hind III restriction fragments. The average expected level of interaction was determined using LOESS smoothing ($\alpha = 0.05$) (red line). The gray circles (visualized best when figure is magnified) show the observed 5C counts for each queried fragment. The average profile provides an estimate for the level of interaction expected when no specific chromatin looping interactions occur.

Supplemental Figure S17



Supplemental Figure S17. 3C analyses indicate that $\gamma 3$ and $\gamma 1$ loci preferentially interact in pro-B cells. **A)** A diagram showing a segment of the *Igh* locus spanning 220 kb (top) in which *HindIII* restriction fragments (A,B,C,D,H) analyzed in 3C assays are indicated. **B)** Chromatin from Rag2^{-/-} BM pro-B cells, 445.3 cell lines either unstimulated (US) or stimulated with LPS+CD40L (LC) were used in 3C assays, as indicated. 3C assays anchored at $\gamma 3$ (fragment B) were analyzed for interactions with $\gamma 1$ (fragment C, at both C_a and C_b ends) and at $\gamma 2b$ (fragment D). P values of ≤ 0.05 or 0.001-0.0001 are indicated by (*) and (**) respectively.

Supplemental Table S1. PCR Primers

Assay	Primer ¹	Sequence	References
RT-PCR	18S F	5' TTGACGGAAGGGCACCACCAG 3'	(Rhinn et al. 2008)
"	18S R	5' GCACCACCACCCACGGAATCG 3'	"
"	I μ F	5' CTCTGGCCCTGCTTATTGTTG 3'	(Muramatsu et al. 2000)
"	γ 1 F	5' AGGAATGTGTTTGGCATGGAC 3'	(Snapper et al. 1996)
"	γ 1 R	5' CACTGTCACTGGCTCAGGGAA 3'	"
"	γ 3 F	5' TGTCTGGAAGCTGGCAGGA 3'	(Wuerffel et al. 2001)
"	γ 3 R	5' GGCTCAGGGAAGTAGCCTTTG 3'	This paper
"	γ 2b F	5' TAAGCTGCGCACACCTACAGACAA 3'	"
"	γ 2b R	5' AGGGATCCAGAGTTCCAAGTCACA 3'	"
"	ϵ F	5' CAGAAGATGGCTTCGAATAAGAACA 3'	(Warren and Berton 1995)
"	ϵ R	5' CGTTGAATGATGGAGGATGTGT 3'	"
"	AID F	5' CCATTTCAAAAATGTCCGCT 3'	(Wuerffel et al. 2007)
"	AID R	5' CAGGTGACGCGGTAACACC 3'	"
"	Rag1 F1	5' ATGGCTGCCTCCTTGCCGTCTACC 3'	(Hassaballa et al. 2011)
"	Rag1 R1	5' CTGAGGAATCCTTCTCCTTCTGTG 3'	"
"	Rag2 F1	5' CTCTGGCCTTCAGTGCCAAAAT 3'	This paper
"	Rag2 R1	5' AGTGAGAAGCCTGGTTGAATTAAGG 3'	"
"	IgK 0.8 F1	5' CAGTGAGGAGGGTTTTTGTACAGCC 3'	(Grawunder et al. 1995)
"	IgK 1.1 F1	5' CATGGAAGTGAAATGGCTGTAGCC 3'	"
"	IgK R1	5' CTCATTCTCTGTTGAAGCTCTTGACAAT 3'	"
"	IRF4 F1	5' AGCGCACAAAGCCCAGGAG 3'	This paper
"	IRF4 R1	5' AGCGTCCTCCTCACGATTGTAGT 3'	"
DC-PCR	DC- μ R1	5' TGAAGCCGTTTTGACCAGAAT 3'	"
"	DC- μ R2	5' GGAGACCAATAATCAGAGGGAAGAA 3'	"
"	DC- γ 3 F1	5' CTATGTCCAATGTTCTGAGGAATCA 3'	"
"	DC- γ 3 F2	5' TCACCAGCATCTGATGCCGTCT 3'	"
"	DC- γ 1 F1	5' GCTACCAAGGATCAGGGATAGACA 3'	"
"	DC- γ 1 F2	5' TCTCCTGGGTAGGTTACAGGTCAAG 3'	"
"	DC- γ 2b F1	5' GGGTACTCAGGAAGTGACAAGGA 3'	"
"	DC- γ 2b F2	5' GGAAAGCACTCCGGGCTC 3'	"
"	DC- ϵ F1	5' CCGATTTGACCTACCAGATGCT 3'	"
"	DC- ϵ F2	5' TCTATGGGCATCAGGACCACTCC 3'	"
CT-PCR	I γ 2bF	5' ACTGGGGCAAGGACTGTGAATC 3'	"
"	I ϵ F	5' GCGGCCCTAGGTACTACCA 3'	"
"	C μ R	5' AATGGTGCTGGGCAGGAAGT 3'	(Kinoshita et al. 2001)
V _K →J _K	pK6c(SK)	5' AGATGGAGACACATTCTCAGGTCTTT 3'	This paper
"	pKJa	5' GGAGAGTGCCAGAATCTGGTTTCAG 3'	(Bredemeyer et al. 2006)
D _H →J _H	DFL16.1 F1	5' ACACCTGCAAAACCAGAGACCATA 3'	(Subrahmanyam et al. 2012)
"	JH1 R1	5' GGCCTGACATGGGGAGATCTGAGAATATC 3'	"

¹ F and R indicate forward and reverse primers, respectively

Supplemetal Table S2. 5C library statistics					
<u>MatrixFile</u>	<u>LibraryName</u>	<u>totalRead</u>	<u>totalInteractions</u>	<u>totalCis</u>	<u>totalTrans</u>
2d97cea72765fa51aac4a5a1184d92e1.5C.interactions	AKIgHExGD-B-ProB-R1	22,049,248	15,357	21,927,201	122,047
5e4f7a42d4c93aa4fe2df836462dd7e7.5C.interactions	AKIgHExGD-B-ProB-R2	22,300,921	14,508	21,689,397	611,524
02699d065c6a868301c56e6294e7cc9c.5C.interactions	AKIgHExGD-B-BCell-Unstim-index4-R	20,682,018	15,548	20,059,382	622,636
9b3a8312f94d11d1c5dc62b1c75a7fad.5C.interactions	AKIgHExGD-B-BCell-Unstim-index2-R	20,580,457	15,608	19,878,261	702,196

Supplemental Table S3. 5C Primers

PRIMER_NAME	REGION	TYPE	ASSEMBLY	CHROMOSOME	FRAGMENT_ID	PRIMER_ID	P_STARTPOS	P_ENDPOS	P_SPECIFIC	P_SPECIFIC_SIZE	P_FILLER	_FILLER_SIZ	P_TM	P_GC	F_STARTPOS
5C_2822_GeneDesertCh5_FOR_3	GeneDesertCh5	FOR	mm9_dna	chr5	3	1	133194370	133194399	GAGGGAAGTAACTATATAGTCAAAAG	30		0	54.03	30	133181428
5C_2822_GeneDesertCh5_FOR_5	GeneDesertCh5	FOR	mm9_dna	chr5	5	1	133203809	133203829	TAGTGAATTTCTCAGCCCTCATCTGATAAG	30		0	59.8	40	133196012
5C_2822_GeneDesertCh5_FOR_8	GeneDesertCh5	FOR	mm9_dna	chr5	8	1	133212412	133212412	GGAGAGCGGCACCACTCAGCTGTAGAAG	28	GT	2	64.4	53	133209647
5C_2822_GeneDesertCh5_FOR_11	GeneDesertCh5	FOR	mm9_dna	chr5	11	1	133229213	133229242	ATAAACCTAGCTAGCCTACTAGATGCGAAG	30		0	60.7	40	133222596
5C_2822_GeneDesertCh5_FOR_16	GeneDesertCh5	FOR	mm9_dna	chr5	16	1	133241044	133241073	GGGTGTGTGGCGAAGGCACTTGAAG	25	CTGCA	5	64.34	56	133239397
5C_2822_GeneDesertCh5_FOR_19	GeneDesertCh5	FOR	mm9_dna	chr5	19	1	133248971	133249000	CAAAACCTTCAAAACCCCTTTCAGGAAG	30		0	62.41	43.33	133246157
5C_2822_GeneDesertCh5_FOR_21	GeneDesertCh5	FOR	mm9_dna	chr5	21	1	133266728	133266757	CAAGTGTCACTGTTCTCACTATACCCAAG	30		0	60.81	43.33	133261954
5C_2822_GeneDesertCh5_FOR_23	GeneDesertCh5	FOR	mm9_dna	chr5	23	1	133272450	133272479	GTGTGAAACAACTGTTGTGACGAGAAGAAG	30		0	60.62	40	133268300
5C_2822_GeneDesertCh5_FOR_27	GeneDesertCh5	FOR	mm9_dna	chr5	27	1	133304535	133304564	CAGAGAGGACAGCGCAGGAAGAAGACAAG	30		0	63.93	50	133295558
5C_2822_GeneDesertCh5_FOR_30	GeneDesertCh5	FOR	mm9_dna	chr5	30	1	133313224	133313224	TTCTGTAGCTCTCTGATCGTATGGAGAAG	30		0	62.64	46.67	133308827
5C_2822_GeneDesertCh5_FOR_32	GeneDesertCh5	FOR	mm9_dna	chr5	32	1	133319102	133319131	GAGGGAAGGAAGTGGAAATCAAGCACTAAG	30		0	59.33	40	133314344
5C_2822_GeneDesertCh5_FOR_34	GeneDesertCh5	FOR	mm9_dna	chr5	34	1	133330866	133330895	TCTTGCTAGAGTGTAGTGTGGAAGTAAG	30		0	60.29	40	133329497
5C_2822_GeneDesertCh5_FOR_36	GeneDesertCh5	FOR	mm9_dna	chr5	36	1	133336680	133336709	GCTACTAGAAGTCTCAGAGAACCCTCTGAAG	30		0	60.84	46.67	133334157
5C_2822_GeneDesertCh5_FOR_39	GeneDesertCh5	FOR	mm9_dna	chr5	39	1	133349631	133349660	TAGGGAATCTCTTTGTCTATGTTGTCAAG	30		0	59.93	40	133346648
5C_2822_GeneDesertCh5_FOR_47	GeneDesertCh5	FOR	mm9_dna	chr5	47	1	133371482	133371511	AGGGCCGTGTCTTCCAGGCTTTCAAAAAG	28	GC	2	64.96	50	133368466
5C_2822_GeneDesertCh5_FOR_50	GeneDesertCh5	FOR	mm9_dna	chr5	50	1	133382725	133382754	CTTGTGACTTGATCAGAAATATTTCTCAAG	30		0	55.4	33.33	133381614
5C_2822_GeneDesertCh5_FOR_52	GeneDesertCh5	FOR	mm9_dna	chr5	52	1	133392356	133392385	CTCTGAACACTGCTCACTTGGAAACCAAG	30		0	62.88	46.67	133384257
5C_2822_GeneDesertCh5_FOR_55	GeneDesertCh5	FOR	mm9_dna	chr5	55	1	133396293	133396322	AGGATTAAACATCGATTACGATAATAAG	30		0	54.08	26.67	133393576
5C_2822_GeneDesertCh5_FOR_57	GeneDesertCh5	FOR	mm9_dna	chr5	57	1	133406887	133406916	GGATCAGTTACACAGTGTCCAGAGCTACTAAG	30		0	61.6	46.67	133400513
5C_2822_GeneDesertCh5_FOR_59	GeneDesertCh5	FOR	mm9_dna	chr5	59	1	133422985	133423014	GGGGAGGAGGAGTCCCGTGAAG	22	ACCAACAC	8	64.46	68.18	133413032
5C_2822_GeneDesertCh5_FOR_61	GeneDesertCh5	FOR	mm9_dna	chr5	61	1	133437014	133437043	TAAACGGTTTAAAGATATGTTCCACAAAAG	30		0	56.66	30	133429793
5C_2822_GeneDesertCh5_FOR_65	GeneDesertCh5	FOR	mm9_dna	chr5	65	1	133444758	133444787	GGTGCTCTATTGCTGAACAAAGCTCTTAAAG	30		0	61.51	43.33	133440484
5C_2822_GeneDesertCh5_FOR_67	GeneDesertCh5	FOR	mm9_dna	chr5	67	1	133451023	133451052	CCGCTCGCTAGCAGGGAAGTGAAG	24	GCCTAG	6	64.76	62.5	133449849
5C_2822_GeneDesertCh5_FOR_70	GeneDesertCh5	FOR	mm9_dna	chr5	70	1	133463472	133463501	TACATAGTTAGTGGCTCAGTGTCCGACTAAG	30		0	60.94	43.33	133459116
5C_2822_GeneDesertCh5_REV_1	GeneDesertCh5	REV	mm9_dna	chr5	1	2	133179972	133180001	CTTGATAGCAACTGTACAGAGTAAATTTTA	30		0	55.69	30	133174214
5C_2822_GeneDesertCh5_REV_4	GeneDesertCh5	REV	mm9_dna	chr5	4	2	133195982	133196011	CTTCCCTCGGCTGCTTCTAGAAGAACTG	28	TC	2	64.68	53.57	133194400
5C_2822_GeneDesertCh5_REV_6	GeneDesertCh5	REV	mm9_dna	chr5	6	2	133205057	133205086	CTTCAAGTGAATTCATAGAGTCTCTAAGTGG	30		0	59.06	40	133203830
5C_2822_GeneDesertCh5_REV_10	GeneDesertCh5	REV	mm9_dna	chr5	10	2	133222566	133222595	CTTGGGGTTCATCCCAAAAAGATCTTAAG	30		0	61.49	43.33	133212656
5C_2822_GeneDesertCh5_REV_14	GeneDesertCh5	REV	mm9_dna	chr5	14	2	133238879	133238908	CTTGGCTACATGACTCTTCAACAGCAATTC	30		0	60.25	40	133234372
5C_2822_GeneDesertCh5_REV_17	GeneDesertCh5	REV	mm9_dna	chr5	17	2	133243169	133243198	CTTAAACAGTCAGGACGGCACAACAG	26	TACA	4	64.17	53.85	133241074
5C_2822_GeneDesertCh5_REV_20	GeneDesertCh5	REV	mm9_dna	chr5	20	2	133261924	133261953	CTTAAAGATCTAATGAAGAACTATGTGTTT	30		0	53.69	26.67	133249001
5C_2822_GeneDesertCh5_REV_22	GeneDesertCh5	REV	mm9_dna	chr5	22	2	133268270	133268299	CTTTTTTTCTTTCTGCTCCCAAGCCG	28	GT	2	64.69	50	133266758
5C_2822_GeneDesertCh5_REV_26	GeneDesertCh5	REV	mm9_dna	chr5	26	2	133295528	133295557	CTTGCCCATAAAATGAGCTACCCACCAAAAT	30		0	63.21	43.33	133279882
5C_2822_GeneDesertCh5_REV_29	GeneDesertCh5	REV	mm9_dna	chr5	29	2	133308797	133308826	CTTGTAGATCTGTGAACATTCCTGTATCAA	30		0	58.55	36.67	133305259
5C_2822_GeneDesertCh5_REV_31	GeneDesertCh5	REV	mm9_dna	chr5	31	2	133314314	133314343	CTTTCAGCAGCAGCTGCTGCTTTTG	24	AGTC	4	63.94	53.85	133313225
5C_2822_GeneDesertCh5_REV_33	GeneDesertCh5	REV	mm9_dna	chr5	33	2	133329467	133329496	CTTGAGTAGATTGTAAAGCTGAAGAACACAC	30		0	59.33	40	133319132
5C_2822_GeneDesertCh5_REV_35	GeneDesertCh5	REV	mm9_dna	chr5	35	2	133334127	133334156	CTTCTATTCTCTAAATGCTTCTGTGAGAG	30		0	57.27	36.67	133330896
5C_2822_GeneDesertCh5_REV_38	GeneDesertCh5	REV	mm9_dna	chr5	38	2	133346618	133346647	CTTCACTCTCTGTGTTCCATTTAGTGTTCT	30		0	60.01	40	133337167
5C_2822_GeneDesertCh5_REV_41	GeneDesertCh5	REV	mm9_dna	chr5	41	2	133352391	133352420	CTTGGGAAGTACAGGAAGGACGCAACTG	29	C	1	64.84	51.72	133350057
5C_2822_GeneDesertCh5_REV_49	GeneDesertCh5	REV	mm9_dna	chr5	49	2	133381584	133381613	CTTCAGATAAAATGGCATATATTGGA	30		0	54.9	26.67	133375816
5C_2822_GeneDesertCh5_REV_51	GeneDesertCh5	REV	mm9_dna	chr5	51	2	133384227	133384256	CTTTCAGAGCTGTACAGGGGAC	23	GCACGGG	7	60.87	60.87	133382755
5C_2822_GeneDesertCh5_REV_53	GeneDesertCh5	REV	mm9_dna	chr5	53	2	133393114	133393143	CTTAATGCTGCTCCGCCCTCC	22	CGTTTCTA	8	63.71	63.64	133392386
5C_2822_GeneDesertCh5_REV_56	GeneDesertCh5	REV	mm9_dna	chr5	56	2	133400483	133400512	CTTAAACCAAGAACTCTAGGTGGGCACAA	30		0	63.9	46.67	133396323
5C_2822_GeneDesertCh5_REV_58	GeneDesertCh5	REV	mm9_dna	chr5	58	2	133413002	133413031	CTTTTTTTTCTATGATCAACTAAATTTT	30		0	51.14	16.67	133406917
5C_2822_GeneDesertCh5_REV_60	GeneDesertCh5	REV	mm9_dna	chr5	60	2	133429763	133429792	CTTCAGGTCACGCCAGAGACCTG	25	GGGGT	5	64.33	60	133423015
5C_2822_GeneDesertCh5_REV_63	GeneDesertCh5	REV	mm9_dna	chr5	63	2	133439789	133439818	CTTTGACAGCAAAAGTGTGTATGGGATCA	30		0	62.41	43.33	133437662
5C_2822_GeneDesertCh5_REV_66	GeneDesertCh5	REV	mm9_dna	chr5	66	2	133449848	133449877	CTTGCGACTCTGTGGGAGAGCTTGA	29	GT	2	64.87	51.72	133444788
5C_2822_GeneDesertCh5_REV_69	GeneDesertCh5	REV	mm9_dna	chr5	69	2	133459086	133459115	CTTGTGGTGTGTGCTGTGATGCGACT	28		0	64.99	50	133451389
5C_2822_GeneDesertCh5_REV_72	GeneDesertCh5	REV	mm9_dna	chr5	72	2	133464745	133464774	CTTTCGCGACCTAGTGTCTGTTTCTTCTC	30		0	61.92	43.33	133463922
5C_2821_IgHBiggier_FOR_3	IgHBiggier	FOR	mm9_dna	chr12	3	1	114353686	114353715	CTTCTCGACGCTGTGACTAGCTGCTCTTAAG	30		0	63.37	50	114341252
5C_2821_IgHBiggier_FOR_6	IgHBiggier	FOR	mm9_dna	chr12	6	1	114367360	114367389	AAACTGAGTCTTCTGCGACTCTGAGAGAAG	30		0	64.18	50	114364563
5C_2821_IgHBiggier_FOR_9	IgHBiggier	FOR	mm9_dna	chr12	9	1	114375748	114375777	CTACCTGGGTCAGCTTGGCACAAGAG	26	AAAA	4	64.58	57.69	114372427
5C_2821_IgHBiggier_FOR_11	IgHBiggier	FOR	mm9_dna	chr12	11	1	114389458	114389487	TCTCAACCTCAGTCTGTATGATCTCTCAAG	30		0	64.63	50	114387141
5C_2821_IgHBiggier_FOR_14	IgHBiggier	FOR	mm9_dna	chr12	14	1	114395257	114395286	CTCTTATGATGCACAGCTTAGCGATCTTAAAG	30		0	63.32	50	114394005
5C_2821_IgHBiggier_FOR_16	IgHBiggier	FOR	mm9_dna	chr12	16	1	114400194	114400223	GGTGAGGGCTGGATGACCATTTGACAAAG	29	C	1	64.98	51.72	114399193
5C_2821_IgHBiggier_FOR_18	IgHBiggier	FOR	mm9_dna	chr12	18	1	114408116	114408145	GAGAGGATGCTGGGCTACACTTTCAAG	28	TA	2	63.92	53.57	114404465
5C_2821_IgHBiggier_FOR_20	IgHBiggier	FOR	mm9_dna	chr12	20	1	114420723	114420752	CTGCTGTCTCTCCGCTTGTGCTAAG	25	AAGTC	5	64.92	60	114409003
5C_2821_IgHBiggier_FOR_23	IgHBiggier	FOR	mm9_dna	chr12	23	1	114447031	114447060	AAACTATTAAATTAACAGGGAATAAAAG	30		0	53.62	23.33	114441222
5C_2821_IgHBiggier_FOR_25	IgHBiggier	FOR	mm9_dna	chr12	25	1	114450755	114450784	AACATAGTTTAAACGGTTAAATCTACTAAG	30		0	54.32	26.67	114449888
5C_2821_IgHBiggier_FOR_28	IgHBiggier	FOR	mm9_dna	chr12	28	1	114474023	114474052	ATACCTTTTCATCTGTTTTCGTGTGGAAAG	30		0	60.12	36.67	114465909
5C_2821_IgHBiggier_FOR_31	IgHBiggier	FOR	mm9_dna	chr12	31	1	114480844	114480873	GAGAGACAGCAAGGAGGCGCAGAAAG	28	TC	2	63.59	53.57	114477117
5C_2821_IgHBiggier_FOR_34	IgHBiggier	FOR	mm9_dna	chr12	34	1	114493822	114493851	AATGTTTAAAGATCAGTCCAGATCTCAAG	30		0	58.84	36.67	114491123
5C_2821_IgHBiggier_FOR_36	IgHBiggier	FOR	mm9_dna	chr12	36	1	114501501	114501530	CCACGCTCAAGTCTGACATCTCAGCAAAG	30		0	62.72	46.67	114496829
5C_2821_IgHBiggier_FOR_38	IgHBiggier	FOR	mm9_dna	chr12	38	1	114510135	114510164	CCAGGTGCCACACACAGACGGTAAAG	25	CGAAA	5	64.1	60	114502800
5C_2821_IgHBiggier_FOR_40	IgHBiggier	FOR	mm9_dna	chr12	40	1	114528676	114528705	CTCCCATGCTCCAGCTCTGGAAG	24	GGACAA	6	64.59	62.5	114522182
5C_2821_IgHBiggier_FOR_43	IgHBiggier	FOR	mm9_dna	chr12	43	1	114539099	114539128	CTCCCTTCCACCAAGTATAGACCTCAAG	29	A	1	64.52	55.17	114537058
5C_2821_IgHBiggier_FOR_46	IgHBiggier	FOR	mm9_dna	chr12	46	1	114557930	114557959	GAAAAAATAATCTGAGGCGCTGCAGCAAG	30		0	61.77	40	114551907
5C_2821_IgHBiggier_FOR_49	IgHBiggier	FOR	mm9_dna	chr12	49	1	114579769	114579798	AATGAACCTGAATCCACATGACAATAAAG	30		0	57.93	33.33	114559616
5C_2821_IgHBiggier_FOR_54	IgHBiggier	FOR	mm9_dna	chr12	54	1	114601367	114601396	CCATGATAGATCTCAGCTGCACTTCAAG	30		0	62.04	46.67	114594537
5C_2821_IgHBiggier_FOR_58	IgHBiggier	FOR	mm9_dna	chr12	58	1	114616981	114617010	TCTGGTGTTCCTGTTTCTGTTGAATCAAG	30		0	59.93	36.67	114614961
5C_2821_IgHBiggier_FOR_68	IgHBiggier	FOR	mm9_dna	chr12	68	1	114643256	114643285	AAAGCTTGGCTTTATGACTTTACACGAGAAG	30		0	61	40	114638362
5C_2821_IgHBiggier_FOR_71	IgHBiggier	FOR	mm9_dna	chr12	71	1	1146496								

SC_2821_IgHBigger_FOR_191	IgHBigger	FOR	mm9_dna	chr12	191	1	115093785	115093814	GATGTAACCTCACTGATGATGAACAAAAAAG	30		0	57.03	33.33	115091955
SC_2821_IgHBigger_FOR_204	IgHBigger	FOR	mm9_dna	chr12	204	1	115145634	115145663	AAACTGAGGAGGACAGCTGAGATGTGGAAG	30		0	64.53	50	115139357
SC_2821_IgHBigger_FOR_216	IgHBigger	FOR	mm9_dna	chr12	216	1	115166547	115166576	CTTTCTCATGAGGGCTGACAGATGATCTAAG	30		0	60.94	43.33	115163800
SC_2821_IgHBigger_FOR_233	IgHBigger	FOR	mm9_dna	chr12	233	1	115215965	115215995	TATCAAGAGAGGAACCAAGTATAGGAAG	30		0	59.31	33.33	115211655
SC_2821_IgHBigger_FOR_241	IgHBigger	FOR	mm9_dna	chr12	241	1	115243663	115243693	ATAAAGGGCTCTAAATAACCAAGCAAAAG	30		0	59.73	36.67	115242106
SC_2821_IgHBigger_FOR_266	IgHBigger	FOR	mm9_dna	chr12	266	1	115344570	115344599	TGAATTCCTTCAGAGATGATGTGAGTAAG	30		0	56.83	33.33	115341931
SC_2821_IgHBigger_FOR_272	IgHBigger	FOR	mm9_dna	chr12	272	1	115363960	115363989	ATCCAGACATGAAGGAAGTAAACAGTACAAAG	30		0	60.36	40	115362252
SC_2821_IgHBigger_FOR_279	IgHBigger	FOR	mm9_dna	chr12	279	1	115385290	115385319	ATAAAATCAACATTTGTTGAGAGCTTTAAG	30		0	56.38	30	115382611
SC_2821_IgHBigger_FOR_283	IgHBigger	FOR	mm9_dna	chr12	283	1	115396622	115396651	GATGATCTAGCCAGCAGTGCATTTGAGAAAG	30		0	62.54	46.67	115390339
SC_2821_IgHBigger_FOR_287	IgHBigger	FOR	mm9_dna	chr12	287	1	115404368	115404397	TCAAGGGCTTCTATCAGTTGGGTTAAGAAAG	30		0	60.49	40	115402100
SC_2821_IgHBigger_FOR_295	IgHBigger	FOR	mm9_dna	chr12	295	1	115444952	115444981	CACATATTCTACACATTAATCTCCCAATAG	30		0	55.48	33.33	115443133
SC_2821_IgHBigger_FOR_306	IgHBigger	FOR	mm9_dna	chr12	306	1	115495531	115495560	TGTTGGACAAATAATGTAATGAAGGGAAAG	30		0	58.11	33.33	115498003
SC_2821_IgHBigger_FOR_308	IgHBigger	FOR	mm9_dna	chr12	308	1	115510111	115510140	TAGAAGATCAACTCCCTCACCACAAAAG	30		0	62	43.33	115504302
SC_2821_IgHBigger_FOR_317	IgHBigger	FOR	mm9_dna	chr12	317	1	115549463	115549492	AGGAATCTGGGTGAAGTAATTAAGACAAG	30		0	58.43	36.67	115544761
SC_2821_IgHBigger_FOR_324	IgHBigger	FOR	mm9_dna	chr12	324	1	115577264	115577293	CATTCTGTAAATAGATTGAGAGTGGAAAG	30		0	58.25	36.67	115564709
SC_2821_IgHBigger_FOR_329	IgHBigger	FOR	mm9_dna	chr12	329	1	115589657	115589686	GTAACGACCAACATGACGGGTGTTTGGAAAG	30		0	62.25	43.33	115586578
SC_2821_IgHBigger_FOR_334	IgHBigger	FOR	mm9_dna	chr12	334	1	115605144	115605173	ACACAAATCCGACATCTCTCATCTTCTAAG	30		0	61.99	43.33	115594002
SC_2821_IgHBigger_FOR_338	IgHBigger	FOR	mm9_dna	chr12	338	1	115616133	115616162	ATTTTATTTAGTTGTTGTGAACAAGCAAG	30		0	57.45	30	115609122
SC_2821_IgHBigger_FOR_340	IgHBigger	FOR	mm9_dna	chr12	340	1	115620201	115620230	AAATCAGAGTGAAGTAAGTCTCAGCTACAAG	30		0	59.74	40	115618625
SC_2821_IgHBigger_FOR_344	IgHBigger	FOR	mm9_dna	chr12	344	1	115623576	115623605	GTCTTGACAAAGCATTAAGATATAAATAAG	30		0	54.91	30	115621851
SC_2821_IgHBigger_FOR_346	IgHBigger	FOR	mm9_dna	chr12	346	1	115630183	115630212	CCAAGCCTCTCCAGACTCCCTCAAG	25	AACGT	5	63.92	60	115628291
SC_2821_IgHBigger_FOR_350	IgHBigger	FOR	mm9_dna	chr12	350	1	115639529	115639558	CTTATTTTTTCTGAATTTCTGTTTGAAG	30		0	53.76	23.33	115637370
SC_2821_IgHBigger_FOR_364	IgHBigger	FOR	mm9_dna	chr12	364	1	115688895	115688924	TGATATCTGAAGATGGAGACAACCTTAAG	30		0	58.56	36.67	115684492
SC_2821_IgHBigger_FOR_367	IgHBigger	FOR	mm9_dna	chr12	367	1	115694856	115694885	AATGTATTAATCTAGGAGAGGTTTAAAG	30		0	54.06	26.67	115693734
SC_2821_IgHBigger_FOR_369	IgHBigger	FOR	mm9_dna	chr12	369	1	115699902	115699931	GATCTATGAGCTAGCAGGAAGATCCAAAG	30		0	60.39	43.33	115696906
SC_2821_IgHBigger_FOR_371	IgHBigger	FOR	mm9_dna	chr12	371	1	115705555	115705584	GAACCAGGCAAGTTTACAGTAAAGTAAG	30		0	58.52	36.67	115701558
SC_2821_IgHBigger_FOR_377	IgHBigger	FOR	mm9_dna	chr12	377	1	115724753	115724782	CTGAGGAAAGATGTTTATGTGAATCAAG	30		0	55.29	30	115723732
SC_2821_IgHBigger_FOR_399	IgHBigger	FOR	mm9_dna	chr12	399	1	115778785	115778814	TGACAAAGGGGACCATATAAAAGACAAG	30		0	59.42	36.67	115776652
SC_2821_IgHBigger_FOR_402	IgHBigger	FOR	mm9_dna	chr12	402	1	115788374	115788403	TGTGTATTCTGATACCGATGGGATGAAAG	30		0	62.54	43.33	115786561
SC_2821_IgHBigger_FOR_404	IgHBigger	FOR	mm9_dna	chr12	404	1	115793964	115793993	CATTCACATCCAGGCCCTGTCCAAAG	25	TCTAC	5	63.44	56	115792871
SC_2821_IgHBigger_FOR_412	IgHBigger	FOR	mm9_dna	chr12	412	1	115809205	115809234	TTAATGTCTAAGTCTCAGTTGCTGTCTAAG	30		0	60.17	40	115807614
SC_2821_IgHBigger_FOR_415	IgHBigger	FOR	mm9_dna	chr12	415	1	115821987	115822016	GAATGTGAGCCAGTACGCTCTCAGGAAAG	30		0	64.56	50	115816276
SC_2821_IgHBigger_FOR_419	IgHBigger	FOR	mm9_dna	chr12	419	1	115826270	115826299	GACACTTGGATATAGGAGTCAATGTGAAG	30		0	58.96	40	115824505
SC_2821_IgHBigger_FOR_423	IgHBigger	FOR	mm9_dna	chr12	423	1	115840194	115840223	TATTGAGGAGGACAGAGATTTAAAGCAGAAG	30		0	61.6	43.33	115830886
SC_2821_IgHBigger_FOR_429	IgHBigger	FOR	mm9_dna	chr12	429	1	115858034	115858063	CTTATTTTGATACATGCTCAGTAGTGAAG	30		0	54.05	30	115855285
SC_2821_IgHBigger_FOR_436	IgHBigger	FOR	mm9_dna	chr12	436	1	115885561	115885590	ATATGTAGCATGTGTGATTACAGATAAAG	30		0	54.38	26.67	115880011
SC_2821_IgHBigger_FOR_451	IgHBigger	FOR	mm9_dna	chr12	451	1	115951275	115951304	CAGACTGCCCTCCAGAACTCGGTGTACAAG	29	G	1	64.13	51.72	115941646
SC_2821_IgHBigger_FOR_458	IgHBigger	FOR	mm9_dna	chr12	458	1	115975855	115975884	AAAGAGTAAAAAACCTGTTGTGTGCGAAG	30		0	58.68	33.33	115974288
SC_2821_IgHBigger_FOR_494	IgHBigger	FOR	mm9_dna	chr12	494	1	116152204	116152233	GGCCTTGGCTCTGATGAGATGCTTGAAAG	29	A	1	64.79	51.72	116143528
SC_2821_IgHBigger_FOR_501	IgHBigger	FOR	mm9_dna	chr12	501	1	116169944	116169973	AAAATCAGCAGTGTGAAAAGGAAGTAAG	30		0	58.4	33.33	116168801
SC_2821_IgHBigger_FOR_510	IgHBigger	FOR	mm9_dna	chr12	510	1	116203871	116203900	TTATTTGTGGAGGGGACGCTGACTACTAAG	30		0	64.01	46.67	116198884
SC_2821_IgHBigger_FOR_513	IgHBigger	FOR	mm9_dna	chr12	513	1	116216098	116216127	TATAGTGTCTGAGGGTGTAGCTCAAG	30		0	61.68	43.33	116215116
SC_2821_IgHBigger_FOR_517	IgHBigger	FOR	mm9_dna	chr12	517	1	116231650	116231679	GGTCCAAAGGATTAAGCTTGTTATTAAGAAG	30		0	58.88	40	116222484
SC_2821_IgHBigger_FOR_526	IgHBigger	FOR	mm9_dna	chr12	526	1	116284475	116284504	TTGTGTTTCTCCACAGTGCTCGAGAAG	30		0	64.89	46.67	116282495
SC_2821_IgHBigger_FOR_542	IgHBigger	FOR	mm9_dna	chr12	542	1	116337101	116337130	CTGCATAATCTTCACTCAACTCTCTCAAG	30		0	58.44	36.67	116335333
SC_2821_IgHBigger_FOR_548	IgHBigger	FOR	mm9_dna	chr12	548	1	116357139	116357168	TTTATCAGATGTTTGATGCTCTCGCAAAAG	30		0	60.15	36.67	116356077
SC_2821_IgHBigger_FOR_556	IgHBigger	FOR	mm9_dna	chr12	556	1	116386629	116386658	ATAAATTACTGAAAGGCTTAAGCAATTAAG	30		0	56.3	30	116385510
SC_2821_IgHBigger_FOR_573	IgHBigger	FOR	mm9_dna	chr12	573	1	116439857	116439886	AATGCACATATAGTACATATGAGTGAAG	30		0	58.47	36.67	116436677
SC_2821_IgHBigger_FOR_609	IgHBigger	FOR	mm9_dna	chr12	609	1	116556493	116556522	GGGTACAGAAATTAACATGTTATAGCTAAG	30		0	55.79	33.33	116553198
SC_2821_IgHBigger_FOR_611	IgHBigger	FOR	mm9_dna	chr12	611	1	116561297	116561326	GAAACAGAGAGGACGAATGGCCCTGAAG	29	T	1	64.13	51.72	116559256
SC_2821_IgHBigger_FOR_624	IgHBigger	FOR	mm9_dna	chr12	624	1	116610547	116610576	CAGATCTCAAGCTGCCACACCTTCAAG	27	CCG	3	64.67	55.56	116605743
SC_2821_IgHBigger_FOR_634	IgHBigger	FOR	mm9_dna	chr12	634	1	116643809	116643838	CAGAGGCTTCTTGAAGTCCAGCATATAAG	30		0	60.51	43.33	116640887
SC_2821_IgHBigger_FOR_661	IgHBigger	FOR	mm9_dna	chr12	661	1	116742114	116742143	GGAAATCTGAGGACACGAGGAAGCAACAAAG	30		0	64.15	50	116735642
SC_2821_IgHBigger_FOR_671	IgHBigger	FOR	mm9_dna	chr12	671	1	116772149	116772178	AATGTGTGGGAATATGCTGACTTTAGAAG	30		0	62.16	43.33	116768031
SC_2821_IgHBigger_FOR_685	IgHBigger	FOR	mm9_dna	chr12	685	1	116811044	116811073	GCTTGGAAACAGCATACTGCTTCCACAAAG	30		0	63.39	46.67	116809961
SC_2821_IgHBigger_FOR_694	IgHBigger	FOR	mm9_dna	chr12	694	1	116850980	116851009	AATCTAAAGGATTAATACTGATATTACATAG	30		0	53.45	26.67	116846437
SC_2821_IgHBigger_FOR_702	IgHBigger	FOR	mm9_dna	chr12	702	1	116868345	116868374	CTCTATGCTAATATTAGTGAAGGAAG	30		0	53.23	30	116864979
SC_2821_IgHBigger_FOR_712	IgHBigger	FOR	mm9_dna	chr12	712	1	116894405	116894434	TTTTCTGAAGTGAGCAGCTATGAAGAAG	30		0	62.34	43.33	116891916
SC_2821_IgHBigger_FOR_729	IgHBigger	FOR	mm9_dna	chr12	729	1	116952104	116952133	TTTGAATAGGAGACACATAAGAGCTCAAAG	30		0	59.11	36.67	116948848
SC_2821_IgHBigger_FOR_764	IgHBigger	FOR	mm9_dna	chr12	764	1	117081646	117081675	TTTCATGAACACCAAGTAGAAGTGTTTAAG	30		0	57.88	33.33	117080054
SC_2821_IgHBigger_FOR_769	IgHBigger	FOR	mm9_dna	chr12	769	1	117108865	117108894	CTTCTAAGTGAATTAATGATTAATGCTAAG	30		0	54.47	30	117102069
SC_2821_IgHBigger_FOR_792	IgHBigger	FOR	mm9_dna	chr12	792	1	117190665	117190694	CTTCTGTCATCGTCAAGCCCTGGGAAG	27	TTA	3	64.21	55.56	117189453
SC_2821_IgHBigger_FOR_817	IgHBigger	FOR	mm9_dna	chr12	817	1	117288575	117288604	GTGTGTCCTTCTCTCACTAAACCTTGAAG	30		0	62.61	46.67	117278387
SC_2821_IgHBigger_FOR_820	IgHBigger	FOR	mm9_dna	chr12	820	1	117295244	117295273	TCAGTGTGTATTAACAGGTTTATTAAGAAG	30		0	53.85	26.67	117292549
SC_2821_IgHBigger_FOR_825	IgHBigger	FOR	mm9_dna	chr12	825	1	117300154	117300183	CTTACAGGCCAGCCTCTAATTGTGACAAAG	30		0	62.8	46.67	117298879
SC_2821_IgHBigger_FOR_828	IgHBigger	FOR	mm9_dna	chr12	828	1	117304751	117304780	CACCTTGAATGGCAGGTTATTAACAACAAG	30		0	59.14	36.67	117303409
SC_2821_IgHBigger_FOR_832	IgHBigger	FOR	mm9_dna	chr12	832	1	117314220	117314249	CTCATACAGGCTCTAATGCTCTGATAAG	30		0	58.23	40	117307900
SC_2821_IgHBigger_FOR_837	IgHBigger	FOR	mm9_dna	chr12	837	1	117319513	117319542	AGCACTCAACCTTTTGGCATCTTTTCAAG	30		0	63.17	43.33	117317889
SC_2821_IgHBigger_FOR_842	IgHBigger	FOR	mm9_dna	chr12	842	1	117328487	117328516	CTGCACCTCTTGGATCATGACATGAGGAAG	30		0	63.55	50	117325410
SC_2821_IgHBigger_FOR_850	IgHBigger	FOR	mm9_dna	chr12	850	1	117346590	117346619	CTGACCTCTCACTGTGCAATCCCAAG	28	AG	2	64.59	53.57	117345114
SC_2821_IgHBigger_REV_1	IgHBigger	REV	mm9_dna	chr12	1	2	114341024	114341053	CTTCGTGTTCTGGGATTTAAACACAGAGAGC	30		0	64.32	50	114339456
SC_2821_IgHBigger_REV_4	IgHBigger	REV	mm9_dna	chr12	4	2	114363514	114363543	CTTAATTTGAAGACAGATACATCGGGGAAG	30		0	59.29	40	114353716
SC_2821_IgHBigger_REV_7	IgHBigger	REV	mm9_dna	chr12	7	2	114368951	114368980	CTTAAT						

SC_2821_IgHBigger_REV_70	IgHBigger	REV	mm9_dna	chr12	70	2	114649322	114649351	CTTGGTCCTGTTGCTATGCCTCGGGATCT	29									
SC_2821_IgHBigger_REV_73	IgHBigger	REV	mm9_dna	chr12	73	2	114650809	114650838	CTTACCAAAAGCACTAACATCTGAGTAGT	30		A	1	64.95	51.72	114647312			
SC_2821_IgHBigger_REV_75	IgHBigger	REV	mm9_dna	chr12	75	2	114656243	114656272	CTTTGGTGATGTGGGTGTGCTTTTGG	27		CCT	3	63.9	51.85	114652514			
SC_2821_IgHBigger_REV_79	IgHBigger	REV	mm9_dna	chr12	77	2	114659589	114659588	CTTTTTCACACTTCACAAAGATGATGTC	30			0	56.65	33.33	114658751			
SC_2821_IgHBigger_REV_81	IgHBigger	REV	mm9_dna	chr12	79	2	114664034	114664063	CTTGTCTGAGCAAAATTAAGCGACAAGGTT	30			0	62.03	40	114662159			
SC_2821_IgHBigger_REV_83	IgHBigger	REV	mm9_dna	chr12	81	2	114667066	114667095	CTTGAAGCTCGAGGCAAGATCTTGTCAGG	30			0	63.98	50	114664824			
SC_2821_IgHBigger_REV_85	IgHBigger	REV	mm9_dna	chr12	83	2	114670090	114670119	CTTCTCCTCGAGGAATGAAGCAGAGATCATC	27		GTG	3	64.91	59.26	114667887			
SC_2821_IgHBigger_REV_90	IgHBigger	REV	mm9_dna	chr12	85	2	114675686	114675715	CTTAAGCGGGGAAATGAAGCAGAGATCATC	30			0	62.4	46.67	114672759			
SC_2821_IgHBigger_REV_94	IgHBigger	REV	mm9_dna	chr12	90	2	114686538	114686567	CTTGCCGAGAACCACTCAGTGTGCT	24		GTGTGT	6	64.92	58.33	114679904			
SC_2821_IgHBigger_REV_106	IgHBigger	REV	mm9_dna	chr12	94	2	114722984	114723013	CTTGGGAGAAATGAGAATAGAGAAAGTGTG	30			0	56.39	33.33	114722112			
SC_2821_IgHBigger_REV_125	IgHBigger	REV	mm9_dna	chr12	106	2	114755013	114755042	CTTTGACCTCTGGTAGCCTCTCTTCCACCT	30			0	64.64	50	114751970			
SC_2821_IgHBigger_REV_129	IgHBigger	REV	mm9_dna	chr12	125	2	114823580	114823609	CTTTGTCAGAAATAAAGCCAAGACTTACA	30			0	60.89	40	114809751			
SC_2821_IgHBigger_REV_131	IgHBigger	REV	mm9_dna	chr12	129	2	114838790	114838819	CTTATGGATAGGCAAGATTAACATTAATGAA	30			0	55.52	30	114836439			
SC_2821_IgHBigger_REV_137	IgHBigger	REV	mm9_dna	chr12	131	2	114842465	114842494	CTTATATCTAGTAACCAATATGTGTGTG	30			0	55.03	30	114840678			
SC_2821_IgHBigger_REV_153	IgHBigger	REV	mm9_dna	chr12	137	2	114868474	114868474	CTTGCAATGTGGCATATTACGATGTGCTA	30			0	61.57	40	114864513			
SC_2821_IgHBigger_REV_161	IgHBigger	REV	mm9_dna	chr12	153	2	114920995	114921024	CTTGTAACTTACAATATATGTGATTCATG	30			0	54.24	30	114919053			
SC_2821_IgHBigger_REV_171	IgHBigger	REV	mm9_dna	chr12	161	2	114952492	114952521	CTTTAAGCGAGCCTCTACTAATGTATTCT	30			0	59.73	40	114950757			
SC_2821_IgHBigger_REV_181	IgHBigger	REV	mm9_dna	chr12	171	2	115006213	115006242	CTTGAAGAATCTAGGGAGGCTTATGTCGAG	30			0	64.78	53.33	114996747			
SC_2821_IgHBigger_REV_187	IgHBigger	REV	mm9_dna	chr12	181	2	115052542	115052571	CTTAAGGTCTTTTGGTCTGGTGTG	30			0	62.63	43.33	115048372			
SC_2821_IgHBigger_REV_192	IgHBigger	REV	mm9_dna	chr12	187	2	115074036	115074065	CTTCCCAGCCTAACTAGTATTGAATG	30			0	59.08	40	115061883			
SC_2821_IgHBigger_REV_208	IgHBigger	REV	mm9_dna	chr12	192	2	115096345	115096374	CTTCAATTTGATGCAACACAGCACTAGCCC	30			0	64.06	46.67	115093815			
SC_2821_IgHBigger_REV_235	IgHBigger	REV	mm9_dna	chr12	208	2	115152711	115152740	CTTAAAGATCATCTCAAGTTGGGCTGTGCTA	30			0	62.87	43.33	115148612			
SC_2821_IgHBigger_REV_249	IgHBigger	REV	mm9_dna	chr12	231	2	115211262	115211291	CTTAAGACTCAAGGTAGGGAACTTAGATA	30			0	57.42	36.67	115209244			
SC_2821_IgHBigger_REV_260	IgHBigger	REV	mm9_dna	chr12	235	2	115233347	115233376	CTTTGATATTGTATCTAAGTGTGCTGCACA	30			0	58.77	36.67	115225314			
SC_2821_IgHBigger_REV_271	IgHBigger	REV	mm9_dna	chr12	249	2	115262195	115262224	CTTCATCTTTAACCAGACCAAGTATCTCTA	30			0	59.93	40	115259493			
SC_2821_IgHBigger_REV_274	IgHBigger	REV	mm9_dna	chr12	260	2	115330456	115330485	CTTATAGCAAACTACCTCTTTTACTCACTA	30			0	55.05	30	115327864			
SC_2821_IgHBigger_REV_277	IgHBigger	REV	mm9_dna	chr12	271	2	115362222	115362251	CTTCTCAGTATTAGTGAGAACTAATTCAC	30			0	53.8	30	115359372			
SC_2821_IgHBigger_REV_280	IgHBigger	REV	mm9_dna	chr12	274	2	115370769	115370798	CTTTTACAATTAAGCATGTCTTCTTCCCA	30			0	57.54	33.33	115364817			
SC_2821_IgHBigger_REV_286	IgHBigger	REV	mm9_dna	chr12	280	2	115386773	115386802	CTTGATCATTAATCTCTGAGAGAGCCCTC	30			0	63.8	50	115385320			
SC_2821_IgHBigger_REV_292	IgHBigger	REV	mm9_dna	chr12	286	2	115402070	115402099	CTTGTGTCTATGTGGTCTGTAAGAGCTTC	30			0	59.8	40	115400266			
SC_2821_IgHBigger_REV_300	IgHBigger	REV	mm9_dna	chr12	292	2	115431993	115432022	CTTACAGTAGGAGAGGTTGTATTTATG	30			0	58.72	40	115430826			
SC_2821_IgHBigger_REV_307	IgHBigger	REV	mm9_dna	chr12	300	2	115466905	115466934	CTTGCAATCCCCAATGAGGAGTGTGTTCT	27		GAG	3	63.79	51.85	114655028			
SC_2821_IgHBigger_REV_312	IgHBigger	REV	mm9_dna	chr12	307	2	115504272	115504301	CTTTGTGGTGGTATTAGTGACAAAACCAA	30			0	60.02	36.67	115499561			
SC_2821_IgHBigger_REV_322	IgHBigger	REV	mm9_dna	chr12	312	2	115520981	115521010	CTTATTGACTAAACCTTTAAGGCCATATA	30			0	56.87	33.33	115517402			
SC_2821_IgHBigger_REV_326	IgHBigger	REV	mm9_dna	chr12	322	2	115561328	115561357	CTTGTGTAATCTTGAAGCTTTAAATATCAC	30			0	59.82	40	115558984			
SC_2821_IgHBigger_REV_333	IgHBigger	REV	mm9_dna	chr12	326	2	115579412	115579441	CTTATGCTTATTGCTCAGACTCCCATCTC	30			0	62.37	46.67	115578210			
SC_2821_IgHBigger_REV_335	IgHBigger	REV	mm9_dna	chr12	333	2	115593972	115594001	CTTGGGCTCCCCTAGACAGTCAAGTAGACA	30			0	64.09	50	115592852			
SC_2821_IgHBigger_REV_339	IgHBigger	REV	mm9_dna	chr12	335	2	115607560	115607589	CTTGGTCAATCATCATATAAATCTCCA	30			0	54.55	30	115605174			
SC_2821_IgHBigger_REV_342	IgHBigger	REV	mm9_dna	chr12	339	2	115618595	115618624	CTTGTGACAGATTGCCCTATGCTCTGAACCA	30			0	64.02	46.67	115616163			
SC_2821_IgHBigger_REV_345	IgHBigger	REV	mm9_dna	chr12	342	2	115621662	115621691	CTTCTCCTGAAGGTTGTCAGGACAATTTTG	30			0	61.95	43.33	115620492			
SC_2821_IgHBigger_REV_348	IgHBigger	REV	mm9_dna	chr12	345	2	115628261	115628290	CTTTAACAATAGTAGAGATGATTCTATA	30			0	51.41	23.33	115623606			
SC_2821_IgHBigger_REV_357	IgHBigger	REV	mm9_dna	chr12	348	2	115636408	115636437	CTTCCAAGACATAGTCTCAACAGCACTAA	30			0	59.11	36.67	115632508			
SC_2821_IgHBigger_REV_365	IgHBigger	REV	mm9_dna	chr12	357	2	115671952	115671981	CTTCTAGGAGGCTTCAGCAAGTTTACTCTG	30			0	64.44	50	115668148			
SC_2821_IgHBigger_REV_368	IgHBigger	REV	mm9_dna	chr12	365	2	115693192	115693221	CTTCCCTTAAACAGATCTTACCAACCACTA	30			0	59.99	40	115688925			
SC_2821_IgHBigger_REV_370	IgHBigger	REV	mm9_dna	chr12	368	2	115696876	115696905	CTTTTAACTTCAATGCTCTCAAGCTAAGA	30			0	57.13	33.33	115694886			
SC_2821_IgHBigger_REV_374	IgHBigger	REV	mm9_dna	chr12	370	2	115701528	115701557	CTTATTITCCCCTGTGATTTTAAAGTGTCT	30			0	57.12	33.33	115699932			
SC_2821_IgHBigger_REV_398	IgHBigger	REV	mm9_dna	chr12	374	2	115709820	115709849	CTTTATGAGAACTTCAGCAGGCCCAAGG	30			0	58.75	36.67	115706727			
SC_2821_IgHBigger_REV_401	IgHBigger	REV	mm9_dna	chr12	398	2	115776651	115776680	CTTCTGCTGAGTGAAGTTCACAAAGCAAC	30			0	62.35	46.67	115774724			
SC_2821_IgHBigger_REV_403	IgHBigger	REV	mm9_dna	chr12	401	2	115786531	115786560	CTTCTTTTGAATAGCATCTGATAACCTTCT	30			0	56.95	33.33	115780319			
SC_2821_IgHBigger_REV_410	IgHBigger	REV	mm9_dna	chr12	403	2	115792841	115792870	CTTCAGGCGTCTGGGAAAATTAACACCTGT	30			0	64.18	46.67	115788404			
SC_2821_IgHBigger_REV_413	IgHBigger	REV	mm9_dna	chr12	410	2	115807315	115807344	CTTGCTCTGGGAAACAAATCAGCACTGAGA	30			0	64.35	46.67	115806128			
SC_2821_IgHBigger_REV_418	IgHBigger	REV	mm9_dna	chr12	413	2	115810714	115810743	CTTAGTAGTAGAGAGACTAATAGAGATTGAT	30			0	52.35	30	115809235			
SC_2821_IgHBigger_REV_420	IgHBigger	REV	mm9_dna	chr12	418	2	115824477	115824506	CTTTTATATGTGCTCTCTACTCAGTTTTC	30			0	57.43	36.67	115823136			
SC_2821_IgHBigger_REV_428	IgHBigger	REV	mm9_dna	chr12	420	2	115827627	115827656	CTTGAAGAGGCACTACATATATCATATAA	30			0	56.54	26.67	115826305			
SC_2821_IgHBigger_REV_435	IgHBigger	REV	mm9_dna	chr12	428	2	115855255	115855284	CTTAGCAATATCAAAAATATCTTGGAAAT	30			0	53.51	23.33	115848051			
SC_2821_IgHBigger_REV_450	IgHBigger	REV	mm9_dna	chr12	435	2	115879981	115880010	CTTGAGACCGCAATGSCCTCCAC	23		ACGTCTG	7	64.37	60.87	115877939			
SC_2821_IgHBigger_REV_457	IgHBigger	REV	mm9_dna	chr12	450	2	115941616	115941645	CTTTTAAAGATTAACAGGTTATTAATACAG	30			0	50.42	23.33	115938466			
SC_2821_IgHBigger_REV_464	IgHBigger	REV	mm9_dna	chr12	457	2	115974258	115974287	CTTTTCAAGGGCACTCCATGAATTACATG	30			0	60.62	40	115972764			
SC_2821_IgHBigger_REV_499	IgHBigger	REV	mm9_dna	chr12	464	2	116000799	116000828	CTTTGGTTACAATCTCTGTTTATACAAG	30			0	55.17	30	115993659			
SC_2821_IgHBigger_REV_509	IgHBigger	REV	mm9_dna	chr12	499	2	116168719	116168748	CTTTGAAGAACTCAGCAGGCCCAAGG	27		GGT	3	63.35	51.85	116166285			
SC_2821_IgHBigger_REV_512	IgHBigger	REV	mm9_dna	chr12	509	2	116198854	116198883	CTTCAATAGTTTCATCGCAGGTGAGGTTTC	30			0	62.96	46.67	116193782			
SC_2821_IgHBigger_REV_514	IgHBigger	REV	mm9_dna	chr12	512	2	116215086	116215115	CTTTGTTCTGTAGAGTATTGTTAACATGT	30			0	56.03	30	116206582			
SC_2821_IgHBigger_REV_522	IgHBigger	REV	mm9_dna	chr12	514	2	116218035	116218064	CTTAGGAATATCAGGTTAAATCTCATGAAC	30			0	55.68	33.33	116216128			
SC_2821_IgHBigger_REV_528	IgHBigger	REV	mm9_dna	chr12	522	2	116273247	116273276	CTTAGCCCTATAACCACTGATGCGCAAGTTT	30			0	62.33	43.33	116268138			
SC_2821_IgHBigger_REV_545	IgHBigger	REV	mm9_dna	chr12	528	2	116293317	116293346	CTTACAATATTACAGAAATGTTATTGTA	30			0	51.27	20	116286772			
SC_2821_IgHBigger_REV_552	IgHBigger	REV	mm9_dna	chr12	545	2	116353384	116353413	CTTGGGAAATTTGATACATGATCTATGATC	30			0	57.34	36.67	116342486			
SC_2821_IgHBigger_REV_571	IgHBigger	REV	mm9_dna	chr12	552	2	116366835	11636686											

F_ENDPOS	F_SIZE	ELEMENTID	TERSECTION	E_NAME	P_MER	P_UBLAST	P_SBLAST	TAIL	PRIMER_SEQUENCE
133194399	12971	'0'	'0'		169	1	8	TAATACGACTCACTATAGCCGAAGGGATTAACTATTATTAGGTCAAAG	TAATACGACTCACTATAGCC
133203829	7817	'0'	'0'		261	1	13	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTAGTAATTCTCAGCCCTCATCTGATAAG
133212412	2140	'0'	'0'		62	1	10	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGTGGAAAGAGGCGACCACTCAGCTATAGAG
133229242	6646	'0'	'0'		100	5	5	TAATACGACTCACTATAGCCATAAACTAGCCTAGCCACTAGATTGCAAG	TAATACGACTCACTATAGCCCTTAGTCTGTATGACCTATGAGGAAAG
133241073	1676	'0'	'0'		25	1	7	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCGAGGGTTGTTGCCAAGGAGCTTGAAG
133249000	2843	'0'	'0'		185	1	27	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAAACCTTTCAACCCCTTTCCAGGAAG
133266757	4803	'0'	'0'		121	1	7	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAAGTGTGATGTTCTCAACTATACCCAAAG
133272479	4179	'0'	'0'		186	1	23	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGTGTGAAACACTGTTGTGACCAAGAAAG
133304564	9006	'0'	'0'		781	1	67	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCGAAGGAGGACAGGCGAGGAAGAACAAG
133313224	4397	'0'	'0'		61	1	2	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCTGTAGCTCTGTACCTATGAGGAAAG
133319131	4787	'0'	'0'		93	1	19	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAGGGAAGGAATCGAAATCAAGACTAAG
133330895	1398	'0'	'0'		185	1	8	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCTGTAGAGTGAGTTGTTTGAAGTAAG
133336709	2552	'0'	'0'		365	1	40	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGCTACTAGAAGTCTCAGAGAAGCTCTGAAG
133349660	3012	'0'	'0'		233	1	12	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTAGGGAATCTCTTTGTCTATGTTGTCAAG
133371511	3045	'0'	'0'		311	1	37	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCGAGGSCCTGTTCTCAGGCTTCAAAAAG
133382754	1140	'0'	'0'		240	1	46	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTGTAAGTCTGATCAAGATATCTCAAG
133392385	8128	'0'	'0'		169	1	10	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCTGAACACTGCTACACTTGAACCAAG
133396322	2746	'0'	'0'		251	1	3	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAGATTATAAACATGCATTACGATAAAG
133406916	6403	'0'	'0'		175	1	5	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGGATCAGTTCAAGTGTGAGCTACTAAG
133423014	9982	'0'	'0'		102	1	9	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAACACCGGGAGGAGGGTCCAGCTGAAG
133437043	7250	'0'	'0'		132	1	22	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTAAACGGTTTAAAGATATGTTCCACAAAG
133444787	4303	'0'	'0'		235	1	21	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTGTAAGTCTGATCAAGATATCTCAAG
133451052	1203	'0'	'0'		173	1	24	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTAGCCCTGCTGACAGGAGGTTGAAAG
133463501	4385	'0'	'0'		107	1	1	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTATAGTTTATGGCTCAGTGTCCGACTAAG
133180001	5787	'0'	'0'		165	1	8	TCCTCTTAGTGAGGGTTAATA	CTTGATAGCAACTTGACAGAGTAAATTTTATCCCTTTAGTGAGGGTTAATA
133196011	1611	'0'	'0'		157	1	7	TCCTCTTAGTGAGGGTTAATA	TCCTCCCTGGCTGCTTCATGAAGAACTGCTCCCTTTAGTGAGGGTTAATA
133205086	1256	'0'	'0'		122	1	7	TCCTCTTAGTGAGGGTTAATA	CTTCAAGTGATTCAATGAGTCTCTAAGTGGTCCCTTTAGTGAGGGTTAATA
133222595	9939	'0'	'0'		237	1	41	TCCTCTTAGTGAGGGTTAATA	CTTGCGAGTCTGTAACATTCCTGTATCAATCCCTTTAGTGAGGGTTAATA
133238908	4536	'0'	'0'		269	1	16	TCCTCTTAGTGAGGGTTAATA	CTTGCTCATAGTCTTCAACAGGATTTCTCCCTTTAGTGAGGGTTAATA
133243198	2124	'0'	'0'		66	1	7	TCCTCTTAGTGAGGGTTAATA	CTTTAAAGTCTGAGGAGGCGCACAGCTACATCCCTTTAGTGAGGGTTAATA
133261953	12952	'0'	'0'		192	1	12	TCCTCTTAGTGAGGGTTAATA	CTTAAAGTCTTAATAGGAAACATATGTTTTTCCCTTTAGTGAGGGTTAATA
133268299	1541	'0'	'0'		848	1	1	TCCTCTTAGTGAGGGTTAATA	CTTTTTTTCTTTGCTGCTCCAGCGGCTTCCTTTAGTGAGGGTTAATA
133295557	7575	'0'	'0'		113	1	16	TCCTCTTAGTGAGGGTTAATA	CTTGCCCAATAAATGAGCTACCCCAAAATCCCTTTAGTGAGGGTTAATA
133308826	3567	'0'	'0'		199	1	13	TCCTCTTAGTGAGGGTTAATA	CTTGATAGTCTGTAACATTCCTGTATCAATCCCTTTAGTGAGGGTTAATA
133314343	1118	'0'	'0'		273	1	21	TCCTCTTAGTGAGGGTTAATA	CTTTCAGGACCAAGCTCTCTTTGAGTCTCCCTTTAGTGAGGGTTAATA
133329496	10364	'0'	'0'		174	1	6	TCCTCTTAGTGAGGGTTAATA	CTTGAGTAGATTGAGTCTGAGAAACACACTCCCTTTAGTGAGGGTTAATA
133334156	3260	'0'	'0'		317	1	28	TCCTCTTAGTGAGGGTTAATA	CTTCTATTCTATAAATGCTTCTGTGAGAGTCCCTTTAGTGAGGGTTAATA
133346647	9480	'0'	'0'		410	1	49	TCCTCTTAGTGAGGGTTAATA	CTTCACCTCTCTGTGTTTCCATTATGAGTCTCCCTTTAGTGAGGGTTAATA
133352420	2363	'0'	'0'		294	1	37	TCCTCTTAGTGAGGGTTAATA	CTTGGGAAGTAAACAGAAAGGCGCACTGCTCCCTTTAGTGAGGGTTAATA
133381613	5797	'0'	'0'		396	2	32	TCCTCTTAGTGAGGGTTAATA	CTTCAGATATAAATGGCATAATATTTGCATCCCTTTAGTGAGGGTTAATA
133384256	1501	'0'	'0'		70	1	17	TCCTCTTAGTGAGGGTTAATA	CTTGCAGAGTCTGAGGAGGCGACGCGAGGGTCTTTAGTGAGGGTTAATA
133393143	757	'0'	'0'		298	1	17	TCCTCTTAGTGAGGGTTAATA	CTTAAGTCTGCTGCCCTCCCGGTTCTATCCCTTTAGTGAGGGTTAATA
133400512	4189	'0'	'0'		139	1	22	TCCTCTTAGTGAGGGTTAATA	CTTAAACAGAAACCTCTAGGGTGGCAATCCCTTTAGTGAGGGTTAATA
133413031	6114	'0'	'0'		265	1	1	TCCTCTTAGTGAGGGTTAATA	CTTTTTTTTCTTATGCTAACTAAATTTTCCCTTTAGTGAGGGTTAATA
133429792	6777	'0'	'0'		168	1	11	TCCTCTTAGTGAGGGTTAATA	CTTCAGGTCGAGGCAAGAGACCTTGGGGGTTCCCTTTAGTGAGGGTTAATA
133439818	2156	'0'	'0'		195	1	23	TCCTCTTAGTGAGGGTTAATA	CTTTGACAGAAAGTGTGGTATGGGATCATCCCTTTAGTGAGGGTTAATA
133449848	5069	'0'	'0'		227	1	22	TCCTCTTAGTGAGGGTTAATA	CTTCCCTTAGTGAGGGTTAATA
133459115	7726	'0'	'0'		202	1	19	TCCTCTTAGTGAGGGTTAATA	CTTGCTGGTGTGCTGTGATGACAGACTTCCCTTTAGTGAGGGTTAATA
133472010	8088	'0'	'0'		382	1	9	TCCTCTTAGTGAGGGTTAATA	CTTTGCCAGACTAGTGTCTGTTTTTCTCCCTTTAGTGAGGGTTAATA
134353715	12463	'0'	'0'		101	1	28	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTCTGACCGGTGACTAGTCTCTTAAG
134367389	2826	'0'	'0'		72	1	13	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAAAGTGAAGTCTCGGACTCTGGAGAGAAG
134375777	3350	'0'	'0'		141	1	20	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAAACCTACCTGGGTGAGTCTGGCAGAGAAG
134389458	2355	'0'	'0'		166	1	17	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCCAACTGAGTCTGTAGTCTCTCAAG
134395286	1281	'0'	'0'		44	1	13	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCTTAGTCCAGGCTTACGAGGTTCTAAG
134400223	1030	'0'	'0'		114	1	14	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCGGTGAGGCGTGGATGACCATTTGACAAG
134408145	3680	'0'	'0'		43	1	6	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTAGAGAGGATCGTGGGCTACACTTTCAAG
134420752	11749	'0'	'0'		80	1	4	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAAGTCCCTGGTGTCTTCCGCTTGCTAAG
134447060	5838	'0'	'0'		454	2	37	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAAACCTATTAAATCAAGGGGAATAAAG
134450784	896	'0'	'0'		117	1	3	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAACATAGTTTAAACGGTTAAACTTACTAAG
134474052	8143	'0'	'0'		397	3	34	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCATACCTTCTCATCTGTTTGTGTGAAAG
134480873	3756	'0'	'0'		861	1	50	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCGAAGAGACAGAAAGGAGGCGCAGAAAG
134493851	2728	'0'	'0'		185	1	10	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAATGTTTGAAGATCAGGTCCAGATCTCAAG
134501530	4701	'0'	'0'		182	1	17	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCCAGCTCAATTATGATTCACTCAGCCAAAG
134510164	7364	'0'	'0'		120	1	13	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCGAAGCAGGTCCACACAGCAGAGGTAAG
134528705	6523	'0'	'0'		273	2	48	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGGAACCTCCCATGCTCCAGCTCTGGAAG
134539128	2070	'0'	'0'		26	1	1	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCTCCACGAGTAAAGACCTTAAG
134557959	6052	'0'	'0'		2098	1	10	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAAAGAAAAAATCTGAGGCTTGCAGCAAG
134579798	20182	'0'	'0'		238	1	26	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAATGAAGTGAATCCACATGTACAATAAAG
134601396	6859	'0'	'0'		97	1	11	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCATGTAGATACTCAGCTGAGCACTTCAAG
134617010	2049	'0'	'0'		1643	1	33	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGGTGTCTTCTGTTTCTGTTGAATCAAG
134643285	4923	'0'	'0'		199	1	32	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAAGACTTGCTTTATGACTTTCACAGGAAG
134649657	305	'0'	'0'		131	1	13	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAGGCTGTCTGACAGCTTCACTCTCAAG
134652513	1674	'0'	'0'		179	1	36	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCACTAATTGCTAGGCTCTTCTGGCATTAAG
134658750	2477	'0'	'0'		131	1	7	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGACTTACCTGCTGCACAGGTTAGGCAAG
134662158	2199	'0'	'0'		169	1	5	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGACTCAGCAGCTCAGCCCGCTCAITCAAG
134664823	759	'0'	'0'		187	1	24	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAGTCCCTTTAAGCAGAAACAGTTTACAAG
134667886	790	'0'	'0'		130	1	20	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAGGATGAAGACCTTACTCTTACAGAAAG
134672758	1857	'0'	'0'		365	8	6	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGTGCTCAGAGAGGAGTAAATATGCAAG
134676753	1037	'0'	'0'		757	1	60	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTATGATGATGAGATGACATGACAAG
134691293	4725	'0'	'0'		163	1	12	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAGTTCATGCTCTGTAGCTCTATAGAAG
134750051	2614	'0'	'0'		270	1	15	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTAGCAGCTGGTCTTTAGTCCATGACCAAG
134787052	8909	'0'	'0'		98	1	6	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGACCAAGTCCAAAGTTGAACCCACCAAG
134794187	2619	'0'	'0'		395	1	66	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCACTCTAATCAGTAAAAAATGTTGAAGAAAG
134829182	5572	'0'	'0'		460	3	49	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGCTTAGTAGTCACTACATACAGAGTCCCAAG
134840677	1857	'0'	'0'		296	1	64	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCACTTAGGTGCGCTAAGGAAGGAACAAG
134847397	4902	'0'	'0'		458	1	86	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCACTTAGTGGGCTGATGAAAGGAACAAG
134900764	3617	'0'	'0'		611	1	29	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGATTCCCTCTGTGATGTGACAGAACCAAG
134939720	1393	'0'	'0'		64	1	11	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCAAGCCATCCCTGGGCGCTCAAGTCTAAG
134977500	3789	'0'	'0'		144	1	16	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCATAGGAGAAGGATCATAGATAGGACAAG
135018809	7361	'0'	'0'		151	1	17	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGACTCTATTTAGGTTTAAAACTCAAG
135061882	1183	'0'	'0'		595	1	1	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTTATACAGTGGTTTTTTTTTTCACAAAG

115093814	1859	0'	0'	258	1	36	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGATGTAACCTCATGATGATGAACAAAAAG
115145663	6306	0'	0'	235	1	32	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGCAAGGAGGACAGTGGAGTATGGAG
115166576	2776	0'	0'	149	1	9	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTCTCATGAGGCTGCAGACTGTTCAG
115215965	4310	0'	0'	734	1	25	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCTTAAACAAAGGAAGAAAAACAGTAAGGAAG
115243663	1557	0'	0'	217	1	46	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCAATAGAGGGCTCTAAATAATACCAAGCAAG
115344599	1287	0'	0'	287	1	31	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGATCTTTCAGGATGATGAGGAGGAGG
115363989	1737	0'	0'	490	1	41	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATCCAGACAGCTGGAAGGTAACTACATCAAG
115385319	2708	0'	0'	157	2	26	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATAAATAACCAATTGTGTGAGAGCTTAAAG
115396651	6312	0'	0'	62	1	10	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGATGATCTAGGACAGCTGTTGTGAGAG
115404397	1229	0'	0'	132	1	11	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCTCAAGGCTCTCATACGTTAGTGTGGAAG
115444953	2827	0'	0'	124	1	9	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCATATTTCTACGACTTAATGCTTCCAG
115499560	1127	0'	0'	132	1	16	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGCTGAGGAGGAGGAGGAGGAGGAGG
115510140	5838	0'	0'	168	1	20	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCTTAGAAGATCCAACTCCCTCACCACAAAAG
115549492	4731	0'	0'	154	1	28	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCAGGAATCTGGGTGTAAAGTAATAGACAAG
115577293	12584	0'	0'	256	1	34	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCTGTGTAATGTTCAGGAAGTGAAG
115589686	3108	0'	0'	165	1	28	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGTACAGCAACAGTAGAGGGTGTTTGGAAAG
115605123	1881	0'	0'	181	1	31	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCACACATCTCAGAGTCACTGAGGAGGAGG
115616162	7040	0'	0'	630	2	16	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATTTTATTATGTTGTGTGGAACAGCAAG
115620230	1605	0'	0'	132	1	8	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCAAATCAGAGTAGGAATAGCTCAGCTCAAG
115623605	1754	0'	0'	628	1	50	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGTTCTGCACAAAGCATATGAGATAAATAG
115630212	1921	0'	0'	233	2	38	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCAACTGCAAGGCTCCCTCCAGCTCTCCAA
115639558	2188	0'	0'	482	1	5	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTATTTTTCATTGGAATTCGTTTGAAG
11568892	4432	0'	0'	204	1	16	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGATGATGAGGAAGGAGGAGGAGGAGG
115694885	1151	0'	0'	242	1	32	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCAAATCTATATACCTATGAGGTTTATAAG
115699931	3025	0'	0'	185	1	24	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGATCTGAGGACTGAGGAGGAAGTCAAAG
115705584	4026	0'	0'	757	1	21	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAACCGCAAGTATTTACAGTTAAAGTAG
115724782	1410	0'	0'	734	2	35	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGAGGAATGATTTTATGTGTAATCAAG
115778814	2162	0'	0'	322	2	50	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCTGACAGAGGGGAGCATTAAGAGAGCAAG
115788403	1841	0'	0'	346	1	32	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGTTTGTGCGGCTGATGAGGATGAAG
115793993	1122	0'	0'	191	1	16	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTACCAATCATTCACAGGCCCTCTCAAG
115809234	1620	0'	0'	101	1	34	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTAATGCTCAAGTGCAGTTGCTCTCAAG
115822016	5740	0'	0'	86	1	9	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGGAAGTGTAGGCGAGTGAAGTCTCGAGAA
115826299	1792	0'	0'	97	1	9	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGACATCGGATATGAGGAGTCAATGTGAAG
115840228	932	0'	0'	219	2	27	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAGGAGGAGGAGGAGGAGGAGGAGGAGG
115858063	2778	0'	0'	179	1	4	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTATTTTGTATAACATCTCTAGTAGGAAG
115885590	5579	0'	0'	246	1	31	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGTATGACATGCTGATTACAGATAAAG
115951304	9658	0'	0'	642	2	6	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGCGAGACTGCCTTCAGAACTGGGTGCAAG
115975884	1596	0'	0'	214	2	4	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGCAAGAGTAAAAAACTGTTGTTGTGCAAG
116152233	8705	0'	0'	258	1	19	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGAGGCTGGTGTGTGATGAGATGCTTGAAG
116169971	1841	0'	0'	285	1	32	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGCAAGAAATGAGGAGTGAAGGAGGAGG
116203900	5016	0'	0'	87	1	4	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTATTGTGCGAGGCGACGCTGCTAAAG
116216127	1011	0'	0'	39	1	6	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCTATAGTTGTGCTAAGGCTGTGCGACAGAAG
116231679	9195	0'	0'	109	1	18	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGGTTCGACAGGATTAAGCTGTATTACTAAG
116284504	2009	0'	0'	377	1	15	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGTGTTTCTTCCACAGCTGTCTGCAGAAAG
116337130	1794	0'	0'	226	1	14	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGATGATGATGATGATGATGATGATGATG
116357168	1091	0'	0'	236	1	14	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTATTACAGATTTGTATGCTCTGCAAAAG
116386658	1148	0'	0'	110	1	15	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATAAATCTCAAGGGGCTTAAAGCAATTAG
116439886	3209	0'	0'	148	1	18	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGAACGATTAAGTAGTACTCTTACGCTGAAG
116565522	3324	0'	0'	252	1	12	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGGGTGACAGAATTAACACTGTTATAGTACAG
116561326	2070	0'	0'	357	2	20	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGTGAACAGGAGGACGAAGTGGCCCTGAAG
116610526	4045	0'	0'	197	1	12	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGATGATGATGATGATGATGATGATGATGATG
116643838	2951	0'	0'	197	1	17	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGACAGGCTCTTGAAGTCAAGTACATAAG
116742143	6901	0'	0'	300	2	24	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGGAATCTTGAGGACGAGGAGGAAGCAAAAG
116772149	4118	0'	0'	43	1	9	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATAATGCTGAGGATATGCTGATTAGAAG
116811073	1212	0'	0'	202	1	19	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGCTGGGACACAGATATGCTATTCCAAAG
116851009	4572	0'	0'	215	1	36	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATTAAGGATTAAGCTGATATTATTAAG
116868132	3395	0'	0'	177	1	29	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGCTGATGAGGAGGAGGAGGAGGAGGAGG
116894434	2518	0'	0'	222	3	29	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTTCTGCAAGGTGACGACTTGAAGAAG
116952133	3285	0'	0'	167	1	23	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTTGAATGAGGACAGATCAAGAGCTCAAAG
117081675	1621	0'	0'	153	2	22	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTTGATCAACAGCAGTAGAAGGTTTGAAG
117108895	6826	0'	0'	129	1	13	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGTTTCAAGTGAATTTAGTATGATTTGAAG
117190698	124	0'	0'	179	1	27	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTGATGATGATGATGATGATGATGATGATGATG
117288604	10217	0'	0'	249	1	17	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGTGTGCTGCTCTCTCTCTCAATTAACCTGAAG
117295273	2724	0'	0'	139	1	16	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTAGTGTGATTTACAGGGTTTATAATGAAG
117300183	1304	0'	0'	142	1	9	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTTACAGTGCAGCGCTCTAATGTGACAAAG
117304780	1371	0'	0'	164	1	14	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCATTTGAAGTAGGCTAGTATACACAAAG
117314249	6349	0'	0'	200	1	17	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCCTCATACAGGCTGCTTAATGCTCTGATAG
117319548	1261	0'	0'	179	1	14	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCATGAGGCTCAAGCTCAAGCTTTTGAAG
117328516	3106	0'	0'	128	1	14	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCGCTCACTTCTTGATCATGACCTAGGAG
117346619	1505	0'	0'	157	1	10	TAATACGACTCACTATAGCC	TAATACGACTCACTATAGCCAGCTGCAGCTCTGATGCTGCAAAATCCCCAAG
114341053	1597	0'	0'	93	1	10	TCCTTTTGTGAGGGGTTAATA	TCCTTGCTTGTGGGATTAATACAGGAGGAGCTCCCTTTGTGAGGGGTTAATA
114363543	9827	0'	0'	286	1	28	TCCTTTTGTGAGGGGTTAATA	CTTTAATTGAAGACAGATCAATCGGGGAAAGCTTCCTTTAGTGAGGGGTTAATA
114368951	1561	0'	0'	124	1	37	TCCTTTTGTGAGGGGTTAATA	CTTTGATGATGCT

114649351	2039	'0'	'0'	81	1	23	TC0CCTTTAGTGAGGGTTAATA	CTTGGTCTGTTGTCTATGCCCTCGGATCTATCCCTTTAGTGAGGGTTAATA
114650838	1169	'0'	'0'	144	1	12	TC0CCTTTAGTGAGGGTTAATA	CTTACCAAGCCCAATCAATCTAGTGTAGTTC0CCTTTAGTGAGGGTTAATA
114656272	3758	'0'	'0'	266	1	46	TC0CCTTTAGTGAGGGTTAATA	CTTTTGGTATGTGGGTGTGCCCTTGCCCTTC0CCTTTAGTGAGGGTTAATA
114659958	1181	'0'	'0'	213	27	18	TC0CCTTTAGTGAGGGTTAATA	CTTCCCTTTAGTGAGGGTTAATACTTCAACATAGATATGCTCCCTTTAGTGAGGGTTAATA
114664063	1904	'0'	'0'	216	1	16	TC0CCTTTAGTGAGGGTTAATA	CTTGCTGAGCAAAATTAAGGGAACAGGGTTCC0CCTTTAGTGAGGGTTAATA
114667095	2271	'0'	'0'	353	1	46	TC0CCTTTAGTGAGGGTTAATA	CTTGAAGTCTGAGGCAGAATCTTGTCAGGTC0CCTTTAGTGAGGGTTAATA
114670119	2232	'0'	'0'	150	1	30	TC0CCTTTAGTGAGGGTTAATA	CTTCCTCTGGGAGGTGAGATCTGTCGGTGTC0CCTTTAGTGAGGGTTAATA
114675715	2956	'0'	'0'	629	1	41	TC0CCTTTAGTGAGGGTTAATA	CTTAAGGGGGGAAATGAAGCAGAGATCATCC0CCTTTAGTGAGGGTTAATA
114686567	6663	'0'	'0'	262	1	18	TC0CCTTTAGTGAGGGTTAATA	CTTGCCAGGGAACCACTCAGTGCTGTGTGTC0CCTTTAGTGAGGGTTAATA
114723013	901	'0'	'0'	627	1	38	TC0CCTTTAGTGAGGGTTAATA	CTTGGAGAAAGTAGAAATAGAAAGGTGATCC0CCTTTAGTGAGGGTTAATA
114755042	3072	'0'	'0'	174	1	16	TC0CCTTTAGTGAGGGTTAATA	CTTTGACCTCTGGTATGCTCTTCCACCTTCC0CCTTTAGTGAGGGTTAATA
114823609	13858	'0'	'0'	228	1	16	TC0CCTTTAGTGAGGGTTAATA	CTTTGTGAGAAATGAAGCCAAAGCTTACATCC0CCTTTAGTGAGGGTTAATA
114838819	2380	'0'	'0'	221	1	31	TC0CCTTTAGTGAGGGTTAATA	CTTATGATAGGCGAGAATTAACCTAATGAATCC0CCTTTAGTGAGGGTTAATA
114842494	1816	'0'	'0'	130	1	11	TC0CCTTTAGTGAGGGTTAATA	CTTATATCTAGTAACACAATTTATGTGGTTC0CCTTTAGTGAGGGTTAATA
114868474	3961	'0'	'0'	55	2	7	TC0CCTTTAGTGAGGGTTAATA	CTTGCAATGTTGGCTATTACGATGTTGCTATCC0CCTTTAGTGAGGGTTAATA
114921024	1971	'0'	'0'	220	1	22	TC0CCTTTAGTGAGGGTTAATA	CTTGTAACTACAAATATATGTGATTCATGTC0CCTTTAGTGAGGGTTAATA
114952521	1764	'0'	'0'	123	1	20	TC0CCTTTAGTGAGGGTTAATA	CTTTAAGCAGCCTCTAGATTAATCTGATATCC0CCTTTAGTGAGGGTTAATA
115006242	9495	'0'	'0'	123	1	16	TC0CCTTTAGTGAGGGTTAATA	CTTGAGAAAGTCTAGGGGAGGCTTAGTGCACTCC0CCTTTAGTGAGGGTTAATA
115052571	4199	'0'	'0'	224	1	10	TC0CCTTTAGTGAGGGTTAATA	CTTAAGGTCACTCTTTTGTGCTTGCTGTGTC0CCTTTAGTGAGGGTTAATA
115074065	12182	'0'	'0'	75	1	8	TC0CCTTTAGTGAGGGTTAATA	CTTCCCAGCCATAACTAGATTTAGTGAATGTC0CCTTTAGTGAGGGTTAATA
115096374	2559	'0'	'0'	207	1	31	TC0CCTTTAGTGAGGGTTAATA	CTTCAATTTGATGCAACACGACCTAGCCCTCC0CCTTTAGTGAGGGTTAATA
115152740	4128	'0'	'0'	574	1	25	TC0CCTTTAGTGAGGGTTAATA	CTTAAATGCATCCTAAGTTGGGCTGGTCACTCC0CCTTTAGTGAGGGTTAATA
115211291	1047	'0'	'0'	180	1	19	TC0CCTTTAGTGAGGGTTAATA	CTTAAAGCTCAAAAGTGAGGGAACCTTAGATATCC0CCTTTAGTGAGGGTTAATA
115233376	8062	'0'	'0'	104	1	17	TC0CCTTTAGTGAGGGTTAATA	CTTTGATATGTATCTAAGTGGTCTGCACATCC0CCTTTAGTGAGGGTTAATA
115262224	2731	'0'	'0'	212	1	29	TC0CCTTTAGTGAGGGTTAATA	CTTCATCTTAAACCAGACAGTGATCCTATCC0CCTTTAGTGAGGGTTAATA
115330485	2621	'0'	'0'	276	2	14	TC0CCTTTAGTGAGGGTTAATA	CTTATAGCAAAATACATCTTTATCACTCATCC0CCTTTAGTGAGGGTTAATA
115362251	2879	'0'	'0'	156	1	15	TC0CCTTTAGTGAGGGTTAATA	CTTTCTAGTATTTAGTGAGAACTAATTCACCTCC0CCTTTAGTGAGGGTTAATA
115370798	5981	'0'	'0'	274	1	27	TC0CCTTTAGTGAGGGTTAATA	CTTTTACAATATAAGCATGTCTTCCCATCC0CCTTTAGTGAGGGTTAATA
115386802	1482	'0'	'0'	237	2	23	TC0CCTTTAGTGAGGGTTAATA	CTTTGCACTTAATCTCCCTGAGAAGCTCTCC0CCTTTAGTGAGGGTTAATA
115402099	1833	'0'	'0'	167	1	19	TC0CCTTTAGTGAGGGTTAATA	CTTGTGTCATGTGTGCTCTGTAAGAGCTCTCC0CCTTTAGTGAGGGTTAATA
115432022	1196	'0'	'0'	230	1	26	TC0CCTTTAGTGAGGGTTAATA	CTTACAGTGAGAGGAGGTGTGTTATTTATGTC0CCTTTAGTGAGGGTTAATA
115466934	1906	'0'	'0'	520	1	26	TC0CCTTTAGTGAGGGTTAATA	CTTGCAATCCCCAATGGAGGTGTGTCGAGTCC0CCTTTAGTGAGGGTTAATA
115504301	4740	'0'	'0'	176	1	18	TC0CCTTTAGTGAGGGTTAATA	CTTTTGGTGGTTATAGTGACAAAACCAATCC0CCTTTAGTGAGGGTTAATA
115521010	3608	'0'	'0'	120	1	39	TC0CCTTTAGTGAGGGTTAATA	CTTATTGACTAAACCTTTAAGGCCCATATATCC0CCTTTAGTGAGGGTTAATA
115561357	2373	'0'	'0'	211	1	8	TC0CCTTTAGTGAGGGTTAATA	CTTGGTACTCACTGAGCCCTTTAAATCACTCC0CCTTTAGTGAGGGTTAATA
115579441	1231	'0'	'0'	84	1	9	TC0CCTTTAGTGAGGGTTAATA	CTTTATCTTTGCCAGAGTCCCATCACTCC0CCTTTAGTGAGGGTTAATA
115594001	1149	'0'	'0'	86	1	11	TC0CCTTTAGTGAGGGTTAATA	CTTGGGGTCC0CCTAGAGATCAAGTAGACATCC0CCTTTAGTGAGGGTTAATA
115607589	2415	'0'	'0'	139	1	7	TC0CCTTTAGTGAGGGTTAATA	CTTGGTCATAACTATCATAATAATCATCCATCC0CCTTTAGTGAGGGTTAATA
115618624	2461	'0'	'0'	100	1	10	TC0CCTTTAGTGAGGGTTAATA	CTTGTGACAGATTCGCTTATGCTCTGAACCATCC0CCTTTAGTGAGGGTTAATA
115621691	1199	'0'	'0'	176	1	29	TC0CCTTTAGTGAGGGTTAATA	CTTTCCTGAAGGGTGTTCAGGACAATTTTGTCC0CCTTTAGTGAGGGTTAATA
115628290	4684	'0'	'0'	162	1	21	TC0CCTTTAGTGAGGGTTAATA	CTTTTACAATAGTAGAGATGATTTCTATATCC0CCTTTAGTGAGGGTTAATA
115636437	3929	'0'	'0'	175	1	18	TC0CCTTTAGTGAGGGTTAATA	CTTCCAGAAACATAGATGCTCAACAGCATATCC0CCTTTAGTGAGGGTTAATA
115671981	3833	'0'	'0'	164	1	20	TC0CCTTTAGTGAGGGTTAATA	CTTCAGGGGGTCTGACCAGTGTACTGTC0CCTTTAGTGAGGGTTAATA
115693221	4296	'0'	'0'	122	1	17	TC0CCTTTAGTGAGGGTTAATA	CTTCCCCTAAACCAGATCTTACCAAACCTATCC0CCTTTAGTGAGGGTTAATA
115696905	2019	'0'	'0'	169	1	21	TC0CCTTTAGTGAGGGTTAATA	CTTTTAACTTACACATGTCTCGAAGCTAAGATCC0CCTTTAGTGAGGGTTAATA
115701557	1625	'0'	'0'	280	1	31	TC0CCTTTAGTGAGGGTTAATA	CTTATTTTCCCCTGTTGATTTTAAAGTGTCTCC0CCTTTAGTGAGGGTTAATA
115709849	3122	'0'	'0'	188	1	23	TC0CCTTTAGTGAGGGTTAATA	CTTTTGGAGAACCTTCACTCATTTGCTAGATCC0CCTTTAGTGAGGGTTAATA
115776651	1927	'0'	'0'	359	2	48	TC0CCTTTAGTGAGGGTTAATA	CTTCCGAGGTGAGGAATTCAGAAAGACCTCC0CCTTTAGTGAGGGTTAATA
115786560	6241	'0'	'0'	252	1	37	TC0CCTTTAGTGAGGGTTAATA	CTTCTTTGTGAATGTCATCTGATAACCTCTCC0CCTTTAGTGAGGGTTAATA
115792870	4466	'0'	'0'	252	1	38	TC0CCTTTAGTGAGGGTTAATA	CTTCAGGCTCTGGGAAATTAACACCTGTTC0CCTTTAGTGAGGGTTAATA
115807344	1216	'0'	'0'	284	1	28	TC0CCTTTAGTGAGGGTTAATA	CTTGCTCTGGGAAACAAATCAGCACTGAGATCC0CCTTTAGTGAGGGTTAATA
115810743	1508	'0'	'0'	144	1	6	TC0CCTTTAGTGAGGGTTAATA	CTTAGTATAGGAGACTAAATAGAAAGTTATCTCC0CCTTTAGTGAGGGTTAATA
115824506	1370	'0'	'0'	186	1	32	TC0CCTTTAGTGAGGGTTAATA	CTTTTATATGTGCTCTTACTCAGTTTCCCTCC0CCTTTAGTGAGGGTTAATA
115827627	1327	'0'	'0'	226	2	26	TC0CCTTTAGTGAGGGTTAATA	CTTTGAAAGGGTAGCATATATATCACTCAATCC0CCTTTAGTGAGGGTTAATA
115855284	7233	'0'	'0'	488	1	25	TC0CCTTTAGTGAGGGTTAATA	CTTAGCAATATCAAAAATATCTTGGAAATTC0CCTTTAGTGAGGGTTAATA
115880010	2071	'0'	'0'	55	1	1	TC0CCTTTAGTGAGGGTTAATA	CTTGAGCAGCGAATGGCCTCCACAGCTGTGTC0CCTTTAGTGAGGGTTAATA
115941645	3179	'0'	'0'	313	2	30	TC0CCTTTAGTGAGGGTTAATA	CTTTTAGAATAAAGGTTAATAATACAGTC0CCTTTAGTGAGGGTTAATA
115974287	1523	'0'	'0'	158	1	15	TC0CCTTTAGTGAGGGTTAATA	CTTTTCAAGGGGCACTCATGAATTACATGTC0CCTTTAGTGAGGGTTAATA
116000828	7169	'0'	'0'	297	2	21	TC0CCTTTAGTGAGGGTTAATA	CTTTGGTTACATAATCTCTGTTTACAAAGTCC0CCTTTAGTGAGGGTTAATA
116168748	2463	'0'	'0'	147	1	22	TC0CCTTTAGTGAGGGTTAATA	CTTTGAAGAACTCTCAGCAGGCCAAGGGTTC0CCTTTAGTGAGGGTTAATA
116198983	5101	'0'	'0'	146	1	22	TC0CCTTTAGTGAGGGTTAATA	CTTCCATGTTTATGCCAGGTGAGGTTTCC0CCTTTAGTGAGGGTTAATA
116215115	8533	'0'	'0'	198	1	30	TC0CCTTTAGTGAGGGTTAATA	CTTTGTCTGTAGAGTATTTGTTAAACATGTC0CCTTTAGTGAGGGTTAATA
116218064	1936	'0'	'0'	126	1	15	TC0CCTTTAGTGAGGGTTAATA	CTTAGGAATATCAGGTAATCTCATGAAATCC0CCTTTAGTGAGGGTTAATA
116273276	5138	'0'	'0'	118	3	14	TC0CCTTTAGTGAGGGTTAATA	CTTAGCCCTATACAACTGATGGCAAGTTTTCC0CCTTTAGTGAGGGTTAATA
116293346	6574	'0'	'0'	414	1	28	TC0CCTTTAGTGAGGGTTAATA	CTTACAATATTTCAGAAATAGTATTTTGAATCC0CCTTTAGTGAGGGTTAATA
116353413	10927	'0'	'0'	228	1	35	TC0CCTTTAGTGAGGGTTAATA	CTTGGGAAATGTATACATGACTGATAGTCTCC0CCTTTAGTGAGGGTTAATA
116366864	2504	'0'	'0'	413	3	39	TC0CCTTTAGTGAGGGTTAATA	CTTGATGAAATTAAGAAAGCTGTTTAAATATCC0CCTTTAGTGAGGGTTAATA
116436207	2454	'0'	'0'	169	1	6	TC0CCTTTAGTGAGGGTTAATA	CTTCAATAGTCCATCAGGTCCTGAGTTGTCC0CCTTTAGTGAGGGTTAATA
116542358	2928	'0'	'0'	257	2	36	TC0CCTTTAGTGAGGGTTAATA	CTTTGAAAAATTTCTTGGTTCAGTGTGATCC0CCTTTAGTGAGGGTTAATA
116559255	2732	'0'	'0'	361	2	18	TC0CCTTTAGTGAGGGTTAATA	CTTAAATGGATGATGAAGTGAAGAAACCACTTCC0CCTTTAGTGAGGGTTAATA
116580013	2015	'0'	'0'	341	1	24	TC0CCTTTAGTGAGGGTTAATA	CTTATATGAATTAAGAAAGTGTATTTTAAATCC0CCTTTAGTGAGGGTTAATA
116627666	5370	'0'	'0'	525	1	35	TC0CCTTTAGTGAGGGTTAATA	CTTTCACAAATTAATACATGAACACAATATCC0CCTTTAGTGAGGGTTAATA
116715742	5307	'0'	'0'	56	1	3	TC0CCTTTAGTGAGGGTTAATA	CTTACTGAGTATGAGCACCAGTTTCAACAGAGAGATCC0CCTTTAGTGAGGGTTAATA
116762407	2180	'0'	'0'	242	2	32	TC0CCTTTAGTGAGGGTTAATA	CTTCCCTTTCCATATACCACACAACCTCAGTTC0CCTTTAGTGAGGGTTAATA
116781489	2641	'0'	'0'	228	1	10	TC0CCTTTAGTGAGGGTTAATA	CTTGTTTCAGCACTAAGTTCAAAATGTTTTC0CCTTTAGTGAGGGTTAATA
116816454	5380	'0'	'0'	475	1	1	TC0CCTTTAGTGAGGGTTAATA	CTTTGAAAAAATCTTGGTCCAGTGTGATCC0CCTTTAGTGAGGGTTAATA
116852283	1273	'0'	'0'	182	1	24	TC0CCTTTAGTGAGGGTTAATA	CTTGTCTCTCATGATTAACTGTGCTTAATATCC0CCTTTAGTGAGGGTTAATA
116877871	2850	'0'	'0'	381	1	22	TC0CCTTTAGTGAGGGTTAATA	CTTATTTCAAGCATTCGTGAATATTTTACTCC0CCTTTAGTGAGGGTTAATA
116897428	2828	'0'	'0'	202	1	26	TC0CCTTTAGTGAGGGTTAATA	CTTGTGTTTCTTGGCAAACTTTTAACTCTCC0CCTTTAGTGAGGGTTAATA
116981159	8554	'0'	'0'	302	1	25	TC0CCTTTAGTGAGGGTTAATA	CTTATTTCCCTGAAGTTACATCCACAACATCTCC0CCTTTAGTGAGGGTTAATA
117102068	6398	'0'	'0'	133	2	16	TC0CCTTTAGTGAGGGTTAATA	CTTAGCCACATCAGTTAAATGCGATGAATTTCC0CCTTTAGTGAGGGTTAATA
117185079	2034	'0'	'0'	181	1	5	TC0CCTTTAGTGAGGGTTAATA	CTTAAAGGGTGGTTATATAGAAACACTTTATCC0CCTTTAGTGAGGGTTAATA
117268163	2905	'0'	'0'	72	1	4	TC0CCTTTAGTGAGGGTTAATA	CTTAGGTGGTATGGTAGAGTAATCAAACTCC0CCTTTAGTGAGGGTTAATA
117292548	2954	'0'	'0'	181	1	20	TC0CCTTTAGTGAGGGTTAATA	CTTCAAGCAGTTTCCCATGTTGTGGGAATCC0CCTTTAGTGAGGGTTAATA
117298106	2832	'0'	'0'	614	1	30	TC0CCTTTAGTGAGGGTTAATA	CTTCTTTGGAAGATTACATTAGTGGTGTGTTCC0CCTTTAGTGAGGGTTAATA
117303400	2826	'0'	'0'	245	1	48	TC0CCTTTAGTGAGGGTTAATA	CTTCTCTCTTCTAGCTGTTTAAAGTATGATCTCC0CCTTTAGTGAGGGTTAATA
117307899	2066	'0'	'0'	124	1	17	TC0CCTTTAGTGAGGGTTAATA	CTTCTTTAGGATGACCTGCTGCACAGCTGTCC0CCTTTAGTGAGGGTTAATA
117317137	1980	'0'	'0'	53	1	8	TC0CCTTTAGTGAGGGTTAATA	CTTAGTTGGCTCCCTCAGCCGGAGCGGATCC0CCTTTAGTGAGGGTTAATA
117324621	4297	'0'	'0'	197	1	33	TC0CCTTTAGTGAGGGTTAATA	CTTTTCTCCAGGTTCTACCATGGCTCAGGCTCC0CCTTTAGTGAGGGTTAATA
117337990	3536	'0'	'0'	311	1	10	TC0CCTTTAGTGAGGGTTAATA	CTTTGGCCCACAATATGCCTTAAACACTATGTCC0CCTTTAGTGAGGGTTAATA
117350186	3566	'0'	'0'	174	1	25	TC0CCTTTAGTGAGGGTTAATA	CTTTGAGAAATCACAACCTCCCATGTGGCTCC0CCTTTAGTGAGGGTTAATA

Supplemental Table S4. Primer and Probe Sequences Used in the Quantitative 3C assay.

Probe ¹	Primer ^{2,3,4}	Sequence	References
GD	mb1F	5' CCACGCACTAGAGAGAGACTCAA 3'	This paper
	mb1R	5' CCGCCTCACTTCCTGTTTCAGCCG 3'	"
		5' ACCTTGCTACTCTTCCCTGGTGTGTTGTTGG 3'	"
A (Eμ)	T.GD.F	5' AGGCTTCTGACCTGCATCTTGA 3'	"
	T.GD.R	5' TTCCAGAGCATTGTCAGCAAA 3'	"
B (γ3)		5' TGGCTTACCATTGCGGTGCCTGGTTT 3'	"
	T.A-A*	5' TCCACACAAAGACTCTGGACCTCT 3'	"
H.1 (hs3b,4)		5' CTGACCCAGGAGCTG 3'	"
	T.B-B*	5' CAGATCACAGGGTCCCAGGTT 3'	"
	T.H-H.1*	5' CTCATCCACATCACCTTGCCTGTGTATTGTC 3'	"
		5' ATAGGCCCTCCTCCCACCA 3'	"
	T.A	5' GAAACCAGGCACCGCAAATG 3'	"
	T.B	5' AGTAGATAGGACAGATGGAGCAGTTACA 3'	"
	T.Ca	5' GTGATAATGAACTGAATCCCACATGTAC 3'	"
	T.Cb	5' GATTTAGAGGACTGGACATCCTTCC 3'	"
	T.D	5' AGTACCCAGCATGTTTCACATC 3'	"
	T.H	5' GCCCCTAAGACCCTACTCTGCTA 3'	"

1 3C probes specific for unique HindIII fragments are denoted by a capital letter referring to the 3C fragments shown in Figure 4 and Supplemental Figure S14. GD indicates a probe located in the *Gene desert1* gene. The probes with their associated anchor primers were designed to be used in combination with any other 3C primers.

2 F and R indicate forward and reverse primers, respectively

3 Primers marked with the asterisk (*) are used in combination with anchor probes.

4 3C assay primers specific for each HindIII fragment are denoted by a capital letter referring to the 3C fragments shown in Supplemental Figure S14.

Supplemental Table S5. Analysis of HindIII digestion of crosslinked genomic chromatin templates ^{1,2}

Genotype	Stimulation²	<i>Amy 2</i>	<i>Amy6</i>	<i>Igh C</i>	<i>Igh H</i>
WT	Un	77		80	75
WT	Un	79		82	76
WT	LPS	76		78.4	81.2
WT	LPS	72		74	80
WT	L+I	68		78	81
WT	L+I	77		74	89
Rag2 ^{-/-} Pro-B	Un			99.9	99.9
Rag2 ^{-/-} Pro-B	Un			99.9	99.9
445.3	Un			66.0	69.9
445.3	Un			70.2	68.3
445.3	Un		83.6	71.0	
445.3	Un		69.0	57.9	
445.3	Un		73.3	75.4	
445.3	LC		73.4	64.6	
445.3	LC		76.4	70.0	
445.3	LC		85.7	74.8	
445.3	LCI		78.1	79.9	
445.3	LCI		83.9	76.5	
445.3	LCI		82.6	73.2	
T cells	ConA		92.6	80.3	63.4
T cells	ConA		93.6	83.2	74.8
T cells	ConA		92.6	79.0	73.9
T cells	ConA		92.0	76.9	70.3

1 Individual 3C sites (A,B,C,H) analyzed for Hind III digestion are shown in Supplemental Figure S14.

2 Formaldehyde treated nuclei of splenic B cells and T cells from WT mice were unstimulated or stimulated using LPS (L) or LPS+IL4 (LI) and Con A respectively. Pro-B cells from Rag1^{-/-} mice and Abelson transformed cell line 445.3 that were unstimulated (Un), or activated with LC (LPS+CD40L), or LCI for 48 hours were digested with HindIII overnight at 37°C. An aliquot of each sample was assessed for digestion efficiency of relevant HindIII sites prior to ligation. DNA was analyzed in real-time PCR with primer pairs spanning each of the HindIII sites used to generate the restriction fragments examined in 3C assays (Supplemental Table S5). To correct for varying input DNA concentrations from sample to sample, primers amplifying the mb1 gene were used. The degree of cutting of each site was determined by comparison to undigested and fully digested naked control DNA. 3C chromatin analyzed here are used in Supplemental Figure S14.

Supplemental Table S6. Primers used for 3C control fragment amplification.

Primer ^{1,2}	Sequence	Reference
C.Amy2F	5' CCCTGCATCCTATCTGACAACCAGC 3'	This paper
C.Amy2R	5' GAGAACATAATGGGAACAAATTCAC 3'	"
C.Amy6F	5' GGGTCTAGGAATAGTAGAGATGAAG 3'	"
C.Amy6R	5' GTGCATGAAAAAATCCTTAACAAAAC 3'	"
C.xF	5' CATTAGCAGCCGTGAGGTG 3'	"
C.xR	5' TCAACGTGTGCATGTGTGTGTG 3'	"
C.AF	5' CTGGGAATGTATGGTTGTGG 3'	(Wuerffel et al, 2007)
C.AR	5' GAGCTCTATGATTATTGGTTAACAGGC 3'	"
C.BF	5' GGGAGCTGGGGCTATCAGA 3'	"
C.BR	5' AGTTCCTGTGCTTGACCTGGTA 3'	"
C.CF	5' CTTATTATCCTCCAAGAAGGCTG 3'	"
C.CR	5' GCAAACATCATTGACAGCAGTATC 3'	"
C.DF	5' TGCTGGGCAGGTTAGAGTGT 3'	"
C.DR	5' ATTCACTTTTGGCACAGCTGT 3'	"
C.EF	5' GAGTAAGTAGACTGGCCAA 3'	"
C.ER	5' TCAGCACAACCCACCCA 3'	"
C.FF	5' CTCACAGACTTTCCTGAGCA 3'	"
C.FR	5' CTA GGGACCAGTTATTAGAATG 3'	"
C.G'F	5' CTGGGAGAGACAAAGATACTGA 3'	"
C.G'R	5' GAGGCTCCTGCTGTTCCACC 3'	"
C.GF	5' TGACATCTAATCTGACATGGAGG 3'	"
C.GR	5' AGGTGGAGATGTGTAGAGAC 3'	"
C.HF	5' CAGACATGAGTGATCTCAGC 3'	This paper
C.HR	5' CATGCGTTTGTGTCTACATGC 3'	"
C.IF	5' CTACCTGTCCTGAGCTCTGA 3'	"
C.IR	5' CCAAACTAAGTATCATGTACCAG 3'	"
C.JF	5' GCTCGTTGTAAATGGTGCCA 3'	"
C.JR	5' CCAAATCTATCACCTTGGTCAG 3'	"
C.KF	5' TGCACTCTCCCTGCTGATAGAC 3'	"
C.KR	5' AACAAGGTAGCCCACTAGGGAAG 3'	"
C.QF	5' ACAGGGCCAACAGCAGTTCC 3'	"
C.QR	5' GCTCCTCTCGCTGTTG 3'	"
C.GD53.F	5' ACCACTGCCTGTCTCATTGCT 3'	"
C.GD53.R	5' GCCTGAGATTGGTTCTTAGCGG 3'	"
C.GD55.F	5' GTTGAATTGAGCGTTTCCAG 3'	"
C.GD55.R	5' AAGCTGGAATCCTAAAGAATTAGGTT 3'	"
C.GD56.F	5' TGCAAATCAGTCCTCTGCTTGAG 3'	"
C.GD56.R	5'CAAGATCAACAAAGCATCAATTAACA 3'	"

1 F and R indicate forward and reverse primers, respectively

2 3C control primers spanning the HindIII site for each 3C fragment tested are denoted by a capital letter referring to the 3C fragments associated with them.

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