Mediator facilitates transcriptional activation and dynamic long-range contacts at the IgH locus during class switch recombination

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Immunoglobulin (Ig) class switch recombination (CSR) is initiated by the transcription–coupled recruitment of activation–induced cytidine deaminase (AID) to Ig switch regions (S regions). During CSR, the IgH locus undergoes dynamic three–dimensional structural changes in which promoters, enhancers, and S regions are brought to close proximity. Nevertheless, little is known about the underlying mechanisms. In this study, we show that Med1 and Med12, two subunits of the mediator complex implicated in transcription initiation and long–range enhancer/promoter loop formation, are dynamically recruited to the IgH locus enhancers and the acceptor regions during CSR and that their knockdown in CH12 cells results in impaired CSR. Furthermore, we show that conditional inactivation of Med1 in B cells results in defective CSR and reduced acceptor S region transcription. Finally, we show that in B cells undergoing CSR, the dynamic long–range contacts between the IgH enhancers and the acceptor regions correlate with Med1 and Med12 binding and that they happen at a reduced frequency in Med1–deficient B cells. Our results implicate the mediator complex in the mechanism of CSR and are consistent with a model in which mediator facilitates the long–range contacts between S regions and the IgH locus enhancers during CSR and their transcriptional activation.
Mediator is an evolutionarily conserved multiprotein complex composed of 31 subunits organized in four modules that is required for gene transcription by RNA polymerase II (Pol II; Malik and Roeder, 2010; Conaway and Conaway, 2011). The head, middle, and tail modules form a stable core complex that associates reversibly with the CDK8 module (consisting of cyclin-dependent kinase 8, cyclin C, Med12, and Med13) to control interactions of mediator with the Pol II machinery (Malik and Roeder, 2010; Conaway and Conaway, 2011). Mediator behaves as an interface between Pol II and transcription factors and is capable of promoting Pol II preinitiation complex assembly, transcription initiation by Pol II, regulation of Pol II pausing and elongation, recruitment of transcription elongation factors, and control of the phosphorylation state of the C-terminal domain of Pol II (Malik and Roeder, 2010; Conaway and Conaway, 2011; Allen and Taatjes, 2015). The Med1 subunit of mediator, part of the middle module, interacts with distinct transcriptional activators (Borggreve and Yue, 2011) and has been shown to play a key role in embryonic development (Ito et al., 2000; Zhu et al., 2000), erythropoiesis (Stumpf et al., 2010), and iNKT cell development (Yue et al., 2011). In addition, Med1 recruitment to chromatin is one of the features that characterizes super enhancers (Whyte et al., 2013). Interestingly, mediator has also been implicated, together with cohesin, in the formation of long-range DNA loops (Malik and Roeder, 2010; Conaway and Conaway, 2011; Allen and Taatjes, 2015), and chromatin immunoprecipitation sequencing (ChIP-Seq) analysis for Smc1, Smc3, Med1, and Med12 revealed that cohesin–mediator binding predicts genomic sites of long-range promoter–enhancer interactions (Kagey et al., 2010; Phillips-Cremins et al., 2013). As we have recently implicated the cohesin complex in the mechanism of CSR (Thomas-Claudepierre et al., 2013), we have examined the role of mediator in CSR by performing shRNA-mediated knockdowns of the Med1 and Med12 subunits of mediator (belonging to different modules) in CH12 cells and by conditionally inactivating the Med1 subunit in developing B cells.

RESULTS AND DISCUSSION

The mediator complex is dynamically recruited to the IgH locus

Mediator has been suggested to generate DNA loops by binding to promoters and enhancers in embryonic stem cells to induce gene expression (Kagey et al., 2010; Phillips-Cremins et al., 2013). Recently, a ChIP-Seq experiment for the Med12 subunit of mediator performed on ex vivo–activated WT B cells has been reported (Wang et al., 2014). Analysis of the available data (SRR975483) showed that in B cells induced to switch to IgG1 (LPS + IL-4, Med12 is significantly recruited at Eμ, the 3′ RR (hs1,2 and hs4), the γ1 promoter (γ1p), the Sγ1 S region, and a region downstream of Cγ1 containing a putative enhancer (γ1E; Medvedovic et al., 2013) that is also bound by Med1 in developing B cells (Whyte et al., 2013; Predeus et al., 2014).

To confirm these results and investigate the dynamics of mediator binding during CSR, we performed ChIP–quantitative PCR (qPCR) on resting B cells and B cells stimulated with LPS + IL-4 or LPS from control (Med1+/−) and Med1−/− mice (Med1+/−/MblCre+; see section CSR and transcription S regions are compromised by Med1 deficiency in primary B cells) using Med1- and Med12–specific antibodies. We used primer pairs spanning across the IgH locus: Eμ, the S region promoters (γ3p, γ1p, γ2bp), the γ1E enhancer, two elements of the 3′ RR bound by Med12 (hs1,2 and hs4), and Cy2a as negative control. In control resting B cells, we found that Med1 and Med12 are exclusively recruited at the Eμ enhancer and at the hs1,2 and hs4 elements of the 3′ RR (Fig. 1 A). Binding was specific, as the ChIP signal was significantly enriched relative to Med1−/− deficient B cells. Upon CSR induction to IgG1 with LPS + IL-4, Med1 and Med12 are in addition recruited to γ1p and γ1E but not to γ3p and γ2p (Fig. 1 B and Table S1). Conversely, stimulation with LPS, which causes CSR to IgG3 and IgG2b, leads to the specific recruitment of Med1 and Med12 to γ3p, γ2bp, and γ1E, but not to γ1p (Fig. 1 C and Table S1). Additionally, ChIP-qPCR for Med12 performed on Med1−/− deficient cells did not show reduced binding (Fig. 1, A–C; and Table S1), indicating that recruitment of Med12 is not dependent on Med1.

The Med1 and Med12 subunits, which belong to different modules of mediator, show similar recruitment profiles, suggesting that they are part of the same complex in B cells undergoing CSR. Consistent with this, we could show that Med1 and Med12 reciprocally commounoprecipitate from extracts prepared from both CH12 cells and ex vivo–activated splenic B cells (Fig. 1, D and E). We conclude that the mediator complex is dynamically recruited to the IgH locus at the promoters driving the transcription of acceptor S regions in a stimulation-specific manner and that Med12 can be recruited in the absence of Med1.

Knockdown of Med1 or Med12 impairs CSR and transcription of the acceptor S region in CH12 cells

To investigate the functional significance of Med1 and Med12 recruitment to the IgH locus during CSR, we undertook knockdown experiments in CH12 cells, which can be efficiently stimulated to undergo CSR from IgM to IgA in vitro (Thomas-Claudepierre et al., 2013). CH12 cells were transduced with lentiviruses expressing shRNAs specific for Med1 or Med12. As controls, we used a nontarget shRNA and AID-specific shRNA (Thomas-Claudepierre et al., 2013). Knockdown efficiency was confirmed by Western blot (Fig. 2 A), and the ability of transduced cells to undergo CSR to IgA was determined by flow cytometry (Fig. 2, B and C). As expected, knockdown of AID resulted in a robust reduction in the efficiency of CSR (65%) relative to the nontarget shRNA control (Fig. 2, B and C). Similarly, knockdown of the Med1 or Med12 subunits resulted in a significant reduction in the efficiency of CSR (37%) relative to the nontarget shRNA (Med1 vs. nontarget: P = 3.66 × 10−5; Med12 vs. nontarget: P = 3.66 × 10−5; Med12 vs. nontarget: P = 3.66 × 10−5; Med12 vs. nontarget: P = 3.66 × 10−5; Med12 vs. nontarget: P = 3.66 × 10−5).
Figure 1. Med1 and Med12 are dynamically recruited to the IgH locus during CSR. 
(A–C) Med1 and Med12 ChIP-qPCR experiments performed on chromatin prepared from 10^7 splenic resting B cells (A) or cells stimulated with LPS + IL-4 (B) or LPS alone (C) for 72 h. qPCR was performed at the indicated locations (see Table S3 for primers). Results are expressed as the mean of the percentage input (+SD) from three independent biological replicate experiments. Statistical significance from WT results versus Cγ2a (two-tailed Student’s t test) is indicated. *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001. White bars represent ChIP performed on WT samples; black bars represent ChIP performed on Med1-deficient samples. See Table S1 for full statistical analysis. (D and E) Nuclear extracts (D) from CH12 cells and whole-cell extracts (E) prepared from WT splenic B cells stimulated with LPS + IL-4 for 72 h were immunoprecipitated and blotted with antibodies specific for Med1 and Med12. Input represents 1% of material used. Theoretical molecular masses in kilodaltons are indicated. Data are representative of two independent experiments.
vs. nontarget: P = 4.03 × 10^{-10}; Fig. 2 B and C). The CSR defect observed was independent of abnormalities in AID expression as determined by Western blot (Fig. 2 A). We conclude that knockdown of Med1 and Med12 in CH12 cells results in defective CSR without affecting AID expression. To determine whether Med1 and Med12 knockdown affects S region transcription, we analyzed the level of donor (I\(\mu\)-C\(\mu\)) and acceptor (I\(\alpha\)-C\(\alpha\)) germline transcripts by real time (RT–qPCR) in CH12 cells expressing shRNAs specific for Med1, Med12, or a nontarget control (Fig. 2 D and E). Although the level of donor germline transcript was not affected (Fig. 2 D), we found that the level of the acceptor S region germline transcripts was significantly reduced upon Med1 and Med12 knockdown when compared with the nontarget control (Fig. 2 E). We conclude that Med1 and Med12 are required for robust transcription of the S region.

**CSR and transcription of acceptor S regions are compromised by Med1 deficiency in primary B cells**

To inactivate the Med1 subunit in developing B cells, we bred Med1-floxed mice (Med1\(^{F/F}\); Jia et al., 2004) with Mb1\(^{Cre/+}\) knock-in mice (Hobeika et al., 2006). Despite efficient Cre-mediated deletion (not depicted), normal B cell numbers (not depicted) and frequencies were found in the bone marrow and the spleen (not depicted). The only difference observed was an increase in the proportion of marginal zone relative to follicular B cells in the spleen of Med1-deficient mice (not depicted). Therefore, mature B cell populations in the spleen are efficiently generated in the absence of Med1.

To determine whether conditional inactivation of Med1 results in defective CSR, we cultured in vitro CFSE-labeled splenic B cells isolated from Med1\(^{F/F}\)Mb1\(^{Cre/+}\) mice and control mice (Med1\(^{+/+}\), Mb1\(^{Cre/+}\), Med1\(^{F/F}\), or Med1\(^{F/F}\)Mb1\(^{Cre/+}\)) under conditions that induce CSR to different isotypes (Fig. 3, A and B). Although no differences were observed among the different control genotypes, interestingly, we found that Med1 deficiency resulted in a 30–60% reduction in CSR to all isotypes tested (Fig. 3, A and B). To determine whether Med1 deficiency affects AID expression, we measured the level of AID mRNA and protein in activated Med1\(^{F/F}\)Mb1\(^{Cre/+}\) and control B cells by RT-qPCR and Western blot (Fig. 3 C). We did not find any significant reduction in AID expression level in Med1\(^{F/F}\)Mb1\(^{Cre/+}\) mice compared with control mice (Fig. 3 C). Therefore, reduced CSR in Med1-deficient B cells cannot be explained by defective AID expression. In addition, the effect of Med1 deficiency on CSR was not caused by decreased survival (not depicted), strong proliferation defects (not depicted), or defective cell cycle progression (not depicted), nor by an increased proportion of marginal zone B cells in Med1\(^{F/F}\)Mb1\(^{Cre/+}\) mice (not depicted). We conclude that Med1 deletion results in a B cell–intrinsic CSR defect that is independent of defective AID expression or strong proliferation abnormalities.

To determine whether Med1 deletion has a general impact on S region transcription, we measured the level of donor (I\(\mu\)-C\(\mu\)) and acceptor S region germline transcripts (I\(\gamma1\)-C\(\gamma1\), I\(\gamma3\)-C\(\gamma3\), I\(\gamma2b\)-C\(\gamma2b\), and I\(\gamma2a\)-C\(\gamma2a\)) by RT–qPCR in activated Med1\(^{F/F}\)Mb1\(^{Cre/+}\) and control B cells (Fig. 3 D). We found that the level of I\(\mu\)-C\(\mu\) germline transcripts was increased by Med1 deletion (Fig. 3 D), consistent with the fact that these transcripts accumulate when CSR is not efficient (Thomas–Claudepierre et al., 2013). Interestingly, however, we found that the level of all the acceptor S region germline transcripts was significantly reduced in Med1-deficient B cells when compared with control B cells (Fig. 3 D). This is consistent with reduced levels of S\(\gamma\) transcripts resulting from Med1 or Med12 knockdown in CH12 cells (Fig. 2 E). We conclude that Med1 is required for robust transcription of the S region. This could be explained by two nonmutually exclusive hypotheses. Med1 could be required to initiate transcription at the acceptor S region promoters. Alternatively, Med1 could mediate the long-range interactions between the IgH enhancers and the acceptor S region that are necessary to activate transcription, to bring the acceptor S region to the proximity of S\(\mu\) and to successfully undergo CSR.

**Med1 deficiency results in reduced interactions between E\(\mu\) and the C\(\gamma3\), C\(\gamma1\), C\(\gamma2b\), and C\(\gamma6\) genes**

To evaluate whether the dynamic three-dimensional changes occurring at the IgH locus during CSR are dependent on the Med1 subunit of the mediator complex, we performed high-resolution 4C–Seq (circular chromosome conformation capture) experiments on resting and activated B cells isolated from the spleens of Med1\(^{F/F}\)Mb1\(^{Cre/+}\) and control (Med1\(^{F/F}\)) mice (Fig. 4 and not depicted). Purified B cells from three different mice per genotype were cultured (or not) for 48 h in the presence of LPS and IL-4 to induce CSR to IgG1 or with LPS alone to induce CSR to IgG3 and IgG2b. As a viewpoint, we used a bait located at the E\(\mu\) enhancer.

In control resting B cells, we observed a strong interaction between the two IgH enhancers, E\(\mu\) and 3′ R\(\gamma\), that was substantially increased upon activation (Fig. 4 and not depicted). This is consistent with the long-range interactions described in primary B cells and in CH12 B cells (Wuerffel et al., 2007; Pefanis et al., 2015) and with the promoter–enhancer interactome of the IgH locus revealed by Pol II ChIA-PET in primary B cells (Qian et al., 2014). We also observed close-range contacts between E\(\mu\) and the C\(\gamma1\) and C\(\gamma6\) genes in resting B cells, which were diminished upon stimulation (Fig. 4 and not depicted). Consistent with the fact that culturing B cells with LPS and IL-4 induces CSR primarily to IgG1, we observed a significant increase in the interaction between E\(\mu\) and the γ1 promotorm1 (γ1p), Sγ1, and the γ1E enhancer (Fig. 4 and not depicted). Conversely, LPS stimulation led to a significant increase in the interaction between E\(\mu\) and the γ2b promoter and Sγ2b, as well as the γ1E enhancer. Importantly, no interaction was detected between E\(\mu\) and Sγ1 (Fig. 4 and not depicted), confirming that the formation of loops involving recombining acceptor S regions is stimulation dependent (Wuerffel et al., 2007). The γ3 promoter and the
Sy3 S region appear to be involved in a constitutive interaction with Eμ in resting B cells (Fig. 4 and not depicted). Although this interaction is significantly lost when B cells are stimulated to undergo CSR to IgG1 or IgE but not IgG3 (LPS + IL-4; Fig. 4 and not depicted), it is maintained when B cells are stimulated to undergo CSR to IgG3 but not IgG1 or IgE (LPS alone; Fig. 4 and not depicted).

Interestingly, the three-dimensional contacts revealed by 4C-Seq in control activated B cells (Fig. 4 and not depicted) perfectly correlate with Med1 and Med12 binding (Fig. 1).
Figure 3. **CSR and acceptor S region transcription are compromised by Med1 deficiency in primary B cells.** (A, left) Percentage (+SD) of CSR relative to control cells from three to six independent experiments. The genotypes tested and number of mice were as follows: Med1<sup>F/F</sup>Mb<sup>Cre/+</sup> (n = 37), Med1<sup>+/+</sup> (n = 6), Mb<sup>Cre/+</sup> (n = 4), Med1<sup>F/F</sup> (n = 28), Med1<sup>F/+</sup> (n = 11), or Med1<sup>F/+Mb<sup>Cre/+</sup> (n = 16). No difference between control genotypes (Med1<sup>+/+</sup>, Mb<sup>Cre/+, Med1<sup>F/F</sup>, Med1<sup>F/+</sup>, and Med1<sup>F/+Mb<sup>Cre/+</sup>) was observed. CSR in control cells was set to 100%. Statistical analysis was performed using Student’s t test. **, P ≤ 0.01; ***, P ≤ 0.0001. (A, right) Right: CSR to IgE was evaluated by the levels of Igμ-Cε post-switch transcripts by RT-qPCR in control and Med1<sup>F/FMb<sup>Cre/+</sup> B cells cultured for 72 h with LPS + IL-4. Expression is normalized to Igβ and is presented relative to expression in control B cells (set as 1). Mean and SD of triplicate samples are shown. Statistical analysis was performed using two-tailed Student’s t test. **, P ≤ 0.01. Data are representative of three experiments with two mice per genotype. (B) Representative example of surface expression of IgG1, IgG3, and CFSE dilution as determined by flow cytometry in Med1<sup>F/F</sup> and Med1<sup>F/FMb<sup>Cre/+</sup> B cells stimulated for 72 h with LPS + IL-4. Percentage of switched cells is indicated. (C, top) RT-qPCR analysis for AID mRNA in control and Med1<sup>F/FMb<sup>Cre/+</sup> B cells cultured for 72 h with LPS + IL-4. Expression is normalized to Igβ and is presented relative to expression in control B cells (set as 1). Mean and SD of triplicate samples are shown. Statistical analysis was performed using two-tailed Student’s t test. Data are representative of three experiments with two mice per genotype. (C, bottom) Western blot for β-actin and AID on whole-cell extracts from splenic B cells from Med1<sup>F/F</sup> and Med1<sup>F/FMb<sup>Cre/+</sup> mice cultured for 72 h with LPS + IL-4. Theoretical molecular masses in kilodaltons are indicated. Data are representative of three independent experiments. (D) RT-qPCR analysis for germline transcripts (GLT) at donor and acceptor S regions in Med1<sup>F/FMb<sup>Cre/+</sup> and control (Med1<sup>F/F</sup>) B cells cultured for 72 h with LPS alone or with LPS + IL-4 or LPS + IFN-γ. Expression is normalized to Igβ and is presented relative to expression in control B cells, set as 1. Mean and SD of triplicate samples are shown. Statistical analysis was performed using two-tailed Student’s t test. *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001. Data are representative of three independent experiments with two mice per genotype.
Figure 4. *Med1* deficiency affects IgH chromatin dynamics during CSR. (A and B) High-resolution 4C-Seq was performed using a bait on the Eμ enhancer (red dot). A schematic map of the IgH locus indicates the I exons (black dots), S regions (gray boxes), the constant region exons (white boxes), the Eμ enhancer, the γ1 enhancer (γ1E), and the DNaseI hypersensitive sites (hs) located in the 3' RR. Curved lines indicate long-range interactions. 4C-Seq signal was calculated using 10-kb windows centered on DpnII sites located in the constant region of IgH (chr12: 113175000–113475000, mm10). Full lines represent the mean of three replicates and dashed lines the signal for each replicate. The 4C-Seq analysis was conducted with three biological replicates.
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In Med1F/FMb1Cre/+ B cells, architectural changes within the IgH locus exhibit a similar profile upon activation (Fig. 4 and not depicted). Nevertheless, the long-range interactions between Eμ and the γ1p-Sγ1 region and γ1E enhancer were no longer significant (Fig. 4 and not depicted). This suggests a role for Med1 in facilitating these chromosomal interactions that occur after activation.

Across the entire IgH locus, no significant differences could be detected when comparing activated Med1F/FMb1Cre/+ with control B cells (Fig. 4). However, when we focused our analysis on the regions whose interactions with Eμ change upon activation and whose transcription is induced in a stimulation-dependent manner (Fig. 4), we detected significant differences between Med1F/FMb1Cre/+ and control B cells (Fig. 4). Notably, the interaction between Eμ and the γ1p-Sγ1 region and γ1E enhancer was significantly reduced (P = 0.01 and P = 0.05, respectively) in activated (LPS + IL-4 Med1F/FMb1Cre/+ B versus control B cells (Fig. 4 A). This shows that the Cγ1 gene is not efficiently brought into close proximity with Sμ in Med1-deficient B cells. Similarly, Eμ-γ2b-Sγ2b interactions induced by LPS tend to be less frequent in Med1-deficient B cells (Fig. 4 B). The lower robustness of CSR induction by LPS alone (when compared with LPS + IL-4), in which only a small percentage of cells underwent CSR (Fig. 3 B), could explain why these interactions are not as frequent in control cells and why the difference between Med1-deficient and control B cells is not more pronounced.

Altogether, the decrease in interactions observed in Med1-deficient B cells relative to control B cells (Fig. 4) is consistent with the reduction in acceptor S region transcription (Fig. 3 D) and the defect in CSR observed (Fig. 3, A and B). We conclude that the long-range contacts occurring in resting B cells between both of the IgH enhancers are robust and remain intact upon Med1 deletion. In contrast, the dynamic three-dimensional changes occurring at the IgH locus upon activation between Eμ and the Cγ3, Cγ1, Cγ2b, and Cε genes are facilitated by the Med1 subunit of the mediator complex. It is impossible, however, to determine whether the reduced frequency of interaction between Eμ and the C genes observed in Med1-deficient B cells is the cause or the consequence of reduced transcription at the acceptor S regions.

Med1 deficiency results in a 50% reduction in the efficiency of CSR, and it is possible that those B cells, which successfully underwent CSR in the absence of Med1, managed to properly synapse and transcribe the donor and acceptor S regions by a mediator-independent mechanism. Alternatively, as the Med12 subunit can be recruited to the IgH locus in the absence of Med1 (Fig. 1, A and B) and mediator complexes lacking the Med1 subunit can exist (Borggrefe and Yue, 2011; Allen and Taatjes, 2015), it is possible that the Med1-deficient B cells that managed to undergo CSR were able to assemble and recruit a mediator complex independently of the Med1 subunit. Our results implicate the mediator complex in the mechanism of CSR and are consistent with a model in which mediator, possibly together with cohesin, facilitates the transcriptional activation of S regions and their long-range contacts with the IgH locus enhancers during CSR.

MATERIALS AND METHODS

Mice. Mb1Cre/+ mice (Hobeika et al., 2006) were obtained from M. Reth (Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany). Med1F/F (Jia et al., 2004) and Mb1Cre/+ mice were on a B6/129 mixed background and bred and maintained under specific pathogen–free conditions. 8–12-wk-old mice were used in all experiments. All animal work was performed under protocols approved by the Direction des Services Vétérinaires du Bas-Rhin (authorization number 67–343).

Lentiviral transduction. The lentiviral vectors (pLKO.1 and pLKO.1–puro–CMV-TurboGF) expressing shRNAs specific for AID (TRCN0000112031), Med1 (TRCN00000099576), Med12 (TRCN00000096466), or a nontarget control (SHC002) were obtained from Sigma-Aldrich. CH12 cells were infected with viral particles produced in Lenti-X 293T cells as described previously (Thomas-Claudepierre et al., 2013).

Cell culture and flow cytometry. Resting splenic B cells were isolated using CD43 microbeads (Miltenyi Biotech), stained with 5 μM CFSE (Invitrogen), and cultured for 72 h with 50 μg/ml LPS (Sigma-Aldrich) to switch to IgG3 and IgG2b, LPS and IL-4 (5 ng/ml; PeproTech) to switch to IgG1, and LPS and IFN-γ (100 ng/ml; PeproTech) to switch to IgG2a. CSR was assayed by flow cytometry as described previously (Jeevan-Raj et al., 2011). CH12 cells were transduced with shRNA-expressing lentiviral vectors and were cultured with IL-4, TGF-β, and anti-CD40 antibody to switch to IgA as described previously (Thomas-Claudepierre et al., 2013). After 72 h, enhanced GFP expression and surface IgA was analyzed by flow cytometry.

Western blot analysis. Proteins were fractionated by SDS-PAGE on 4–12% gradient gels (Invitrogen), transferred to Immobilon polyvinylidene difluoride membranes (EMD...
Millipore), and analyzed by Western blot. See Table S2 for antibodies used in this study.

ChIP-qPCR. Analytical scale ChIP was performed on chromatin prepared from 10^7 (resting or activated) splenic B cells isolated from a pool of two to three mice as described previously (Yamane et al., 2011). qPCR was performed at several locations across the IgH locus using primers listed in Table S3. Results are expressed as percent input and represent the mean of three biological replicates. Error bars represent the corresponding SD.

ChIP-Seq analysis. Sequence reads (SRR975483 [Wang et al., 2014] and SRR620145 [Whyte et al., 2013; Predeus et al., 2014]) were mapped to reference genome mm10/MMR38 using Bowtie v1.12.8. Peak calling was performed using MACS (Zhang et al., 2008) with default parameters.

RT-qPCR. RNA and cDNA were prepared using standard techniques. qPCR was performed in triplicates using the Universal Probes Library system (Roche) or SyberGreen (QIA GEN) and a LightCycler 480 (Roche). Transcript quantities were calculated relative to standard curves and normalized to β-actin, Igβ, or hypoxanthine-guanine phosphoribosyltransferase (HPRT) mRNA. See Table S3 for primers and probes used in this study.

High-resolution 4C-Seq. 4C-Seq was performed as described previously (Rocha et al., 2012). The primary restriction enzyme used was DpnII and secondary restriction enzyme Csp6I. Primers for the E-AGA-3′, chr12:113428514; C-δ, chr12:113423031–113428514; Cμ-Cδ, chr12:113384000–113420500; γ3p-γ3, chr12:113361553–113371500; γ1p-Sγ1, chr12:11332600–113349000; γ1E, chr12:113319346–113325934; γ2bp-Sγ2b, chr12:113310111–113315500; εp-Sc, chr12:113273549–113280029; hs1.2-hs3A, chr12:113242500–113255414; and hs3B/hs7, chr12:113220000–113233375.

Accession number. 4C-Seq data on resting and activated, control, and Med1−-deficient B cells were submitted to the Gene Expression Omnibus (accession number GSE62969).

Online supplemental material. Table S1 includes statistics for ChIP-qPCR. (Fig. 1). Table S2 lists antibodies used in this study. Table S3 lists primers and probes used in this study. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20141967/DC1.

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