Class switch recombination (CSR) is an Ig gene diversification reaction that alters the Ig effector function while retaining antibody specificity. During CSR, one constant region gene is exchanged for another by a deletional recombination reaction between switch regions, which are highly repetitive DNA sequences that precede constant region genes (Chaudhuri et al., 2007; Stavnezer et al., 2008). CSR occurs in the G1 phase of the cell cycle and proceeds through obligate double-strand break (DSB) intermediates (Petersen et al., 2001; Rada et al., 2002). The reaction is initiated by activation-induced cytidine deaminase (AID), an enzyme that deaminates cytidine residues in single-stranded switch region DNA that is exposed during Ig transcription (Petersen-Mahrt et al., 2002; Bransteitter et al., 2003; Chaudhuri et al., 2003; Dickerson et al., 2003; Pham et al., 2003; Ramiro et al., 2003). AID produces multiple lesions in switch DNA, and the resulting U/G mismatches are processed to DSBs via the base excision and mismatch repair pathways (Catalan et al., 2003; Schrader et al., 2005; Stavnezer et al., 2008).

There are several ways to resolve switch region DSBs. For example, a single DSB can be repaired by religation, or two paired DSBs within a single switch region can be ligated together to produce an intra–switch region deletion (ISD; Dudley et al., 2002). Alternatively, synapsis and ligation of paired DSBs in two different switch regions leads to productive CSR (Stavnezer et al., 2008). Finally, in rare instances, an Ig DSB can be joined to a DSB on a heterologous chromosome to produce a translocation (Ramiro et al., 2004, 2006; Franco et al., 2006).

Switch region DSBs occur during G1, and they are ligated by either classical nonhomologous end joining (C-NHEJ; Manis et al., 2002; Lieber, 2008; Stavnezer et al., 2008) or alternative nonhomologous end joining (A-NHEJ). Among the DNA damage response factors, 53BP1 has the most profound effect on CSR. We explore the role of 53BP1 in intrachromosomal DNA repair using I-SceI to introduce paired DSBs in the Igf locus. We find that the absence of 53BP1 results in an ataxia telangiectasia mutated–dependent increase in DNA end resection and that resected DNA is preferentially repaired by microhomology-mediated A-NHEJ. We propose that 53BP1 favors long-range CSR in part by protecting DNA ends against resection, which prevents A-NHEJ–dependent short-range rejoining of intra–switch region DSBs.
Soulas-Sprauel et al., 2007; Yan et al., 2007; Boboila et al., 2010a,b). C-NHEJ ligates DSBs with little or no microhomology and appears to be the dominant pathway involved in CSR, based on the physiological predominance of blunt or small microhomology switch joins (Yan et al., 2007; Boboila et al., 2010a,b). In contrast, little is known about the factors that mediate A-NHEJ (Haber, 2008). However, this is a robust pathway that makes extensive use of junctional microhomologies and can reconstitute up to 20–50% of CSR in the absence of C-NHEJ (Yan et al., 2007; Boboila et al., 2010a,b). A-NHEJ appears to be kinetically slower than C-NHEJ and mediates many of the translocations that are rare byproducts of V(D)J recombination and CSR (Zhu et al., 2002; Han and Yu, 2008; Wang et al., 2008, 2009; Xie et al., 2009; Boboila et al., 2010b).

DSBs incurred during CSR activate the DNA damage response, as indicated by the accumulation of foci of Mre11/Rad50/Nbs1 (MRN), H2AX, and 53BP1 on the IgH locus during CSR (Petersen et al., 2001; Reina-San-Martin et al., 2003). DNA damage response factors are also required for efficient CSR. Deficiency in any of these or ataxia telangiectasia mutated (ATM), a key mediator of the DNA damage response, leads to inefficient switching and concomitant accumulation of DNA damage on chromosome 12 (Reina-San-Martin et al., 2004; Franco et al., 2006; Ramiro et al., 2006; Jankovic et al., 2007). Among DNA damage response factors, the most pronounced defect in CSR occurs upon loss of 53BP1, a chromatin binding protein that is also an ATM and DNA-PKcs substrate (DiTullio et al., 2002; Han and Yu, 2008; Wang et al., 2008, 2009; Xie et al., 2009; Boboila et al., 2010b).

In this paper, we report experiments that investigate the function of 53BP1 using the I-SceI meganuclease to introduce site-specific DSBs in IgH in B lymphocytes undergoing CSR. We show that 53BP1 regulates the choice of end-joining pathways by interfering with resection, thereby favoring C-NHEJ.

**RESULTS**

**IgH locus modification**

To examine DSB repair in the context of IgH during CSR, we used sequential gene targeting in embryonic stem cells to introduce paired loxP and I-SceI sites in mouse chromosome 12. One set of loxP and I-SceI sites was placed 3’ of the intronic enhancer Eq. (Robbiani et al., 2008), and the second set of sites was introduced 3’ of the IgG1 switch region, 95.9 kb downstream of the first. IgH (96k; Fig. S1, A and B). IgH (96k) mice showed normal B cell development and CSR to IgG1 upon stimulation with LPS and IL-4 (Fig. S1, C and D).

In contrast to the DNA lesions introduced by AID, which can involve almost any cytidine residue in the switch region and are processed by a variety of different pathways (Stavnezer et al., 2008), the introduced loxP and I-SceI sites are in specific locations and are targeted by enzymes with well-defined mechanisms of action. Cre is a bacteriophage enzyme that mediates recombination between loxP sites via Holliday junction intermediates (Gopal et al., 1998). Synapsis between two molecules of Cre bound to separate loxP sites precedes and is required for catalysis (Van Duyne, 2001). Because synopsis is limiting in this reaction, the rate of recombination between loxP sites in mammalian cells expressing Cre is inversely proportional to the distance between the sites and can therefore serve as a measure of chromosome topology (Yu and Bradley, 2001; Egli et al., 2004). In contrast, I-SceI, a yeast-derived restriction meganuclease not normally present in the mouse genome, produces a single DSB in an 18-bp consensus sequence (Stoddard, 2005). When this enzyme is expressed in B lymphocytes containing an I-SceI site integrated into the IgH locus, DNA breaks are detectable at a high frequency at IgH, and translocations can form between I-SceI- and AID-generated breaks (Zarrin et al., 2007; Robbiani et al., 2008).

Recombination between loxP or I-SceI sites in IgH (96k) B lymphocytes should result in class switching from IgM to IgG1. However, IgH genes are allelically excluded (Chowdhury and Sen, 2004), with only 50% of the alleles encoding a functional variable region. Therefore, the maximum rate of Cre- or I-SceI-mediated CSR measured by IgG1 surface expression in heterozygous mice is 50% of the true recombination frequency.

**Switching by Cre-mediated recombination**

Although the mechanism that mediates synopsis between switch regions is poorly understood, chromosome conformation capture PCR experiments suggest that AID may contribute to long-range interactions between switch regions (Wuerffel et al., 2007). To determine whether AID expression alters IgH topology sufficiently to change the rate of Cre-mediated recombination between switch regions, we compared
Cre-induced CSR in AID-deficient IgH^{96k} B cells and control IgH^{96k} B cells (Fig. 1).

B cells were stimulated with LPS and IL-4, and infected with a Cre-encoding retrovirus or an inactive retrovirus Cre*. Infected cells were identified by GFP expression and CSR was measured by flow cytometry. Cre expression in AID-sufficient IgH^{96k} B lymphocytes increased CSR to IgG1 from 3.8 to 14.4% and from 16.2 to 32.2% at 72 and 96 h after stimulation, respectively (Fig. 1 B). However, the rate of CSR in IgH^{96k} B cells was similar in the presence or absence of AID 96 h after stimulation (Fig. 1, B and C).

To determine whether the loss of 53BP1 alters the efficiency of Cre-mediated CSR, we compared the frequency of recombination between IgH^{96k}AID^{+/−}53BP1^{+/−} and IgH^{96k}AID^{+/−}53BP1^{+/−} B cells. We found that CSR in Cre-infected IgH^{96k}AID^{+/−}53BP1^{+/−} B cells was indistinguishable from 53BP1-proficient cells (Fig. 1, C and D). We conclude that neither AID nor 53BP1 alters the overall structure of the IgH locus sufficiently to change the rate of synapsis between the IgH^{96k} loxP sites.

I-SceI endonuclease–induced CSR
To further investigate the role of 53BP1 in CSR, we made use of I-SceI, which produces DSBs independently of synapsis. IgH^{96k}AID^{+/−} B cells were stimulated with LPS and IL-4, and infected with retroviruses encoding I-SceI or an inactive form of the enzyme I-SceI* (Fig. S2). Switching to IgG1 by retrovirally infected B cells was initially measured by flow cytometry (Fig. 2 B).

In contrast to Cre, I-SceI mediated CSR was inefficient in IgH^{96k}AID^{+/−} B cells, with a mean recombination frequency of 0.54% 3 d after retroviral infection (five independent experiments; Fig. 2 C and Table S1). The observed rate of I-SceI–induced CSR was somewhat lower than the rate obtained in B cells derived by RAG-blastocyst complementation (Zarrin et al., 2007). However, our experiments differ from previous experiments in several respects: AID was deleted and switch regions were present in our experiments, also, we used only a single I-SceI site in each location as opposed to two I-SceI sites, which doubles the number of breaks.

To determine whether loss of 53BP1 affects the joining efficiency of I-SceI breaks, we assayed IgH^{96k}AID^{+/−}53BP1^{+/−} B cells. In contrast to Cre, where we observed no effects of 53BP1 deficiency, the I-SceI recombination frequency was significantly reduced in IgH^{96k}AID^{+/−}53BP1^{+/−} B cells to 0.31% (five independent experiments; P = 0.0037; Fig. 2 C and Table S1). To obtain an independent measure of recombination frequency, we examined joining between I-SceI sites by sample dilution PCR. In agreement with the

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**Figure 1.** Cre recombinase induces efficient CSR to IgG1 independently of AID and 53BP1. (A) Schematic representation of the IgH^{96k} allele (top) and the Cre-induced recombinant that encodes IgG1 (bottom). LoxP sites are indicated as red triangles, and I-SceI sites are indicated as blue circles. (B) Representative flow cytometry experiments showing CSR to IgG1 of IgH^{96k} B cells infected with retroviruses encoding Cre or catalytically inactive Cre*. IgG1 expression was analyzed at 72 and 96 h after LPS and IL-4 stimulation. (C) Experiment as in B but for IgH^{96k}AID^{+/−} and IgH^{96k}AID^{+/−}53BP1^{+/−} B cells analyzed at 96 h after LPS and IL-4 stimulation. (D) Graph shows the results of three independent flow cytometry experiments measuring CSR to IgG1 after Cre infection of IgH^{96k}AID^{+/−} and IgH^{96k}AID^{+/−}53BP1^{+/−} B cells. The means are shown as horizontal lines. FSC, forward scatter.
flow cytometry analysis, we found that the joining efficiency in stimulated IgH-96kAID−/− B lymphocytes was significantly reduced from 0.76 to 0.48% in the absence of 53BP1 (P = 0.0002; Fig. 2 D and Table S2). We conclude that loss of 53BP1 decreases the efficiency of recombination between I-SceI sites in IgH-96kAID−/− B cells; however, the effect is far less penetrant than it is for CSR.

53BP1 prevents processing of broken ends
53BP1 has been implicated in regulating the choice between HR and NHEJ DNA repair pathways in the S or G2 phase of the cell cycle. CSR does not appear to involve HR and occurs in the G1 phase of the cell cycle (Petersen et al., 2001). Nevertheless, CSR-induced DSBs can be joined by two different pathways: C-NHEJ or A-NHEJ (Yan et al., 2007; Boboila et al., 2010a,b).

To determine whether 53BP1 affects the choice between C-NHEJ and A-NHEJ, we characterized the joins between I-SceI sites from IgH-96kAID−/−53BP1−/− B cells and IgH-96kAID−/− controls. Precise joining between I-SceI sites yields a 336-nt PCR product (Fig. 3 A), which was the predominant species in control IgH-96kAID−/− B cells (Fig. 3 B). End processing results in lower molecular weight species, which can be scored directly by counting the number of products that run ≤300 nt (i.e., more than ~30 nt total end resection). The loss of 53BP1 resulted in an overall increase in the number of PCR products, with lower than expected molecular weight to 51.6% of all products. (P = 0.0001; Fig. 3 C and Table S3).

The precise molecular structure of the joins was determined by sequencing. Although the mean extent of end processing was 34.7 nt in IgH-96kAID−/− B cells, it was 66.8 nt in the absence of 53BP1 (P = 0.012; Fig. 3 D and Table S4). Consistent with the idea that ends are more frequently processed in the absence of 53BP1, the number of precise joins that reconstituted the I-SceI site also decreased from 30.9 to 13.3% in 53BP1−/− B cells (P = 0.0041; Fig. 3 E and

Figure 2. Loss of 53BP1 decreases the joining efficiency of two distal I-SceI–induced DSBs. (A) Schematic representation of the IgH-96k allele (top) and the I-SceI–induced recombinant that encodes IgG1 (bottom). LoxP sites are indicated as red triangles, and I-SceI sites are indicated as blue circles. (B) Representative flow cytometry experiments showing CSR to IgG1 of IgH-96kAID−/− and IgH-96kAID−/−53BP1−/− B cells 72 h after the first infection with an I-SceI–encoding retrovirus. (C) Graph shows the results of five independent flow cytometry experiments, with each dot representing an individual experiment. The p-value was calculated using a two-tailed paired Student’s t test. The means are shown as horizontal lines. (D) Bar graph showing I-SceI to I-SceI recombination frequency in the presence and absence of 53BP1, determined by nine independent PCR experiments. Error bars indicate standard deviation. The p-value was calculated using a two-tailed paired Student’s t test. FSC, forward scatter.
Inhibition of ATM kinase decreases resection

DNA end resection by combined action of MRN, CtIP, Bloom’s helicase, and Exo1 is essential for HR (Mimitou and Symington, 2009). Optimal resection and formation of the single-stranded DNA (ssDNA) substrate for HR requires ATM, because this kinase facilitates the recruitment and activation of the nucleases that attack DNA ends (Jazayeri et al., 2006; You et al., 2009). Although HR does not appear to be involved in CSR, we investigated whether a related mechanism is involved in processing DNA ends for microhomology-based A-NHEJ.

To determine how the loss of ATM activity might affect the processing of I-SceI breaks, we treated IgH I-96kAID−/− B cells (Table S5). Interestingly, all of the minimally processed joins showed 0–4 nt of junctional microhomology, with a mean of 1.5 and 2 nt for AID−/− and AID−/−53BP1−/− B cells, respectively, which is characteristic of C-NHEJ (Fig. 4 and Fig. S3). In contrast, the majority of joining events that involved extensive resection (≥30 nt) used 3 nt or more of junctional microhomology, with a mean of 3.6 and 4 nt for AID−/− and AID−/−53BP1−/− B cells, respectively, indicative of A-NHEJ (P < 0.0001 for AID−/−; P = 0.0004 for AID−/−53BP1−/−; Fig. 4 C and Fig. S3). These data show that loss of 53BP1 leads to increased resection of DNA ends, and that resection is associated with microhomology-mediated end joining irrespective of 53BP1.

Figure 3. Loss of 53BP1 leads to increased end resection. (A) Schematic representation of IgH1.66 allele (top) with the PCR primers used to amplify a 336-nt recombination product, indicated as arrows (bottom). LoxP sites are indicated as red triangles, and I-SceI sites are indicated as blue circles. (B) Representative ethidium bromide–stained agarose gels showing PCR products obtained after I-SceI–induced recombination in IgH1.66AID−/− and IgH1.66AID−/−53BP1−/− B cells. (C) Bar graphs showing frequency of I-SceI–induced recombination products running ≤300 nt for IgH1.66AID−/− and IgH1.66AID−/−53BP1−/− B cells, determined by 12 independent PCR experiments. Error bars indicate standard deviation. The p-value was calculated using a two-tailed Student’s t test. (D) Dot plot showing total resection of I-SceI–infected IgH1.66AID−/− and IgH1.66AID−/−53BP1−/− B cells. Each dot represents one sequence. The p-value was calculated using a two-tailed Student’s t test. The means are shown as horizontal lines. (E) Bar graph shows the frequency of perfect I-SceI joins in I-SceI–infected IgH1.66AID−/− and IgH1.66AID−/−53BP1−/− B cells in five independent experiments. Error bars indicate standard deviation. The p-value was calculated using a two-tailed Student’s t test.
B cells with a small molecule ATM inhibitor (ATMi). In contrast to loss of 53BP1, ATMi did not significantly affect the joining rate of paired I-SceI breaks on the IgH locus, as measured by flow cytometry (Fig. 5, A and B; and Table S6). However, ATMi-treated IgH^{53BP1−/−} B cells showed significantly reduced DNA end resection (17.8% ATMi vs. 34.1% control; P = 0.0024; Fig. 5, C and D; and Tables S3 and S7) and a concomitant increase in the number of precise I-SceI joins (47.6% ATMi treated vs. 30.9% control; P = 0.026; Fig. 5 E; and Tables S5 and S8). Thus, the increase in I-SceI–induced DNA end processing observed in the absence of 53BP1 is dependent on ATM.

Loss of 53BP1 interferes with CSR, but enhances recombination between repeat DNA within switch regions (Manis et al., 2004; Ward et al., 2004; Ramiro et al., 2006; Reina-San-Martín et al., 2007). Because the latter requires DNA end processing, we examined whether inhibition of DNA end processing by ATMi might enhance CSR in 53BP1^{−/−} B cells. In agreement with published studies (Lumsden et al., 2004; Reina-San-Martín et al., 2004; Callén et al., 2007), ATMi reduces CSR to IgG1 in WT B cells (11.6% ATMi vs. 21.5% untreated control; P = 0.0004; Fig. 6, A and B; and Table S9). In striking contrast, ATMi enhanced CSR in 53BP1^{−/−} B cells (3.2% ATMi vs. 1.3% untreated controls; P = 0.0012; Fig. 6, A and B; and Table S9). We conclude that ATMi ameliorates the severe defect in CSR observed in the absence of 53BP1.

**DISCUSSION**

DSBs are dangerous lesions, which, if left unrepaired, can lead to genomic instability and genome rearrangements. These potentially cytotoxic lesions are repaired either by HR, which is conservative and error free, or by NHEJ, which frequently leads to deletions and insertions. NHEJ is mediated by at least two separate pathways: C-NHEJ, which does not require DNA end resection and produces direct joins with little or no microhomology (Lieber, 2008), and A-NHEJ, which is a robust alternative pathway that makes extensive use of microhomology and therefore requires resection of ends (Haber, 2008). Of the two NHEJ pathways, C-NHEJ is the more conservative because there is no obligatory loss of genetic material.

DSBs can be repaired with a high degree of fidelity during the S and G2 phases of the cell cycle by HR, which uses the undamaged sister chromatid as a template for repair. HR requires 5′ to 3′ resection of DNA ends to produce ssDNA, which recruits replication protein A, leading to deposition of Rad51, a factor that is essential in homology search (Sung and Klein, 2006). End resection is believed to occur during two stages: in the first phase, relatively short stretches of ssDNA are produced by the combined action of MRN and CtIP; in the second phase, longer stretches of ssDNA are produced by the combined action of Bloom’s helicase and Exo1 (Sartori et al., 2007; Gravel et al., 2008; Mimitou and Symington, 2008; Nimonkar et al., 2008; Zhu et al., 2008; Rass et al., 2009; Xie et al., 2009). ATM is implicated as a regulator of the resection process because it phosphorylates all of the enzymes known to be involved in resection (Cortez et al., 2001), and is required for optimal ATR activation and for CtIP recruitment to DNA ends (Cuadrado et al., 2006;
level of DNA end resection (Huertas et al., 2008; Huertas and Jackson, 2009; Yun and Hiom, 2009).

In the absence of a template sister chromatid during CSR in G1, DSBs are repaired by error-prone C-NHEJ or
A-NHEJ. However, little is known about the choice between C-NHEJ and A-NHEJ. Under physiological circumstances, the majority of switch joins are blunt or show minimal microhomology, suggesting that C-NHEJ is the dominant pathway (Stavnezer et al., 2008). Nevertheless, A-NHEJ is a robust pathway that can reconstitute up to 50% of normal levels of CSR in the absence of core C-NHEJ factors such as ligase IV, XRCC4, or Ku70/80, or even the combination of Ku70 and ligase IV (Corneo et al., 2007; Yan et al., 2007; Boboila et al., 2010a,b).

A-NHEJ can also mediate plasmid recircularization in transfected cells (Kabotyanski et al., 1998), joining of I-SceI breaks (Guirouilh-Barbat et al., 2007), oncogenic translocations (Zhu et al., 2002; Ramiro et al., 2006; Wang et al., 2008, 2009), and, finally, V(D)J recombination when the end protection function of the RAG recombinase is disabled (Lee et al., 2004). However, other than the preponderance of microhomologies found at the junctions (Haber, 2008; Zha et al., 2009) and the suggestion that MRN is required for A-NHEJ (Deng et al., 2009; Dinkelmann et al., 2009; Rass et al., 2009; Xie et al., 2009), this pathway remains poorly defined.

In addition to NHEJ, the DNA damage response is also essential for physiological CSR (Jankovic et al., 2007). Among the factors that mediate this response, 53BP1 has the most profound effect on CSR and specifically affects long-range joining between different switch regions (Manis et al., 2004; Reina-San-Martin et al., 2007). In contrast, 53BP1-deficient B cells show increased short-range intra-switch joining, which involves ligation of highly repetitive DNA (Reina-San-Martin et al., 2007). These observations and the related findings that loss of 53BP1 results in decreased V(D)J recombination between distal gene segments (DiFilippantonio et al., 2008) and transchromosomal fusions of deprotected telomeres (Dimitrova et al., 2008) support the hypothesis that 53BP1 plays a role in synopsis during CSR. An alternative, nonexclusive explanation of the dominant effect of 53BP1 on long-range joining, which is strongly supported by our data, is that this protein disfavors short-range joining between repetitive sequences in the switch region by blocking the processing of DNA ends. Specifically, end processing is essential for the production of the ssDNA required for microhomology-based A-NHEJ. Therefore, by interfering with end processing, 53BP1 normally impairs A-NHEJ, which leads to enhanced C-NHEJ. Conversely, loss of 53BP1 favors ISDs by enhancing resection-dependent A-NHEJ and disfavors C-NHEJ–dependent long-range recombination between two nonhomologous switch regions. Consistent with this idea, inhibition of end resection by interfering with ATM activity enhances CSR in 53BP1-deficient B cells.

However, a switch from C-NHEJ to A-NHEJ is unlikely to account for the profound defect in CSR in 53BP1 mutant B cells, as there is persistent CSR at up to 50% of normal levels despite increased ISDs in the complete absence of C-NHEJ (Yan et al., 2007; Han and Yu, 2008; Boboila et al., 2010a,b). We therefore propose a model in which 53BP1 has two roles in CSR: it favors synapsis between distal DSBs and also interferes with end resection and A-NHEJ. Alternatively, the rapid formation of a synapse in the presence of 53BP1 could prevent nuclease access and excessive DNA end resection. Consequently, absence of 53BP1 would lead to prolonged access of nucleases, leading to excessive DNA end resection and A-NHEJ, and decreased CSR. Mechanistically, 53BP1 could block DNA end resection either directly by binding to H4K20me2 in the vicinity of the DSB or by acting as an adaptor that mediates the recruitment of additional factors, which in turn would prevent nuclease access. The combined effect of 53BP1 on synopsis and DNA end resection may explain why the absence of 53BP1 leads to a more pronounced defect in CSR than other DNA damage response factors.

Like the choice between HR and NHEJ during the S and G2 phases of the cell cycle (Huertas et al., 2008; Huertas and Jackson, 2009; Yun and Hiom, 2009), DNA end resection...
appears to be a key event in the choice between C-NHEJ and A-NHEJ. Furthermore, as is the case for HR, where ATM is required for the efficient recruitment and activation of the factors mediating DNA end resection (Cudolid et al., 2006; Jazayeri et al., 2006; Myers and Cortez, 2006; Sung and Klein, 2006; You et al., 2009), ATM also regulates the resection that precedes A-NHEJ. However, in contrast to S and G2, where end resection stimulates HR and preservation of genetic material, resection in G1 leads to its oblige loss. Our data suggest a model wherein 53BP1 blocks access of nucleases to DNA ends, which favors C-NHEJ. We speculate that the requirement for obligate deletion of genetic information during A-NHEJ may explain why C-NHEJ, which is more conservative, has evolved as the preferential end-joining pathway in the G1 phase of the cell cycle.

MATERIALS AND METHODS

Mice. IgH*94 mice were produced by sequential gene targeting of C57BL/6 embryonic stem cells (Fig. S1). AID−/− mice (Muramatu et al., 2000) were backcrossed to C57BL/6 for at least 10 generations, and 53BP1−/− mice (Ward et al., 2003) were backcrossed to C57BL/6 for at least 5 generations. All experiments were in agreement with protocols approved by the Rockefeller University and the National Institutes of Health Institutional Animal Care and Use Committee.

B cell cultures. B lymphocytes were isolated from mouse spleens using anti-CD43 MicroBeads (Miltenyi Biotec) and cultured at 0.5 × 10⁶ cells/ml in R10 medium (RPMI 1640 supplemented with l-glutamine, sodium pyruvate, Hepes, 50 µM 2-mercaptoethanol, antibiotic/antimycotic, and 10% fetal calf serum; Hyclone). For B cell stimulation 25 µg/ml LPS (Sigma-Aldrich) and 5 µg/ml of mouse recombinant IL-4 (Sigma-Aldrich) were added to the culture. Where indicated in the figures, 0.5 µg/ml RP105 (BD) or 2.5 µM ATMi Ku55933 was added to the culture.

Retroviral infection. Retroviral supernatants for B cell infections were produced in BOSC23 by transfection with Fugene 6 with pCL-Eco and Retroviral infection or 2.5 µM ATMi Ku55933 was added to the culture.

pyruvate, Hepes, 50 µM 2-mercaptoethanol, antibiotic/antimycotic, and LPS-produced in BOSC23 by transfection with Fugene 6 with pCL-Eco and Retroviral infection.

PCR reactions were performed with the Expand Long Template PCR System (Roche). The first round of PCR was performed with the following primers and conditions: 5'-CCAATACCCGAAGCATTTACAGT-3' and 5'-CCAATACCCGAAGCATTTACAGT-3' for 10 cycles at 92°C for 30 s, 56°C for 30 s, and 68°C for 40 s, with 2 s of additional extension time per cycle. PCR assays.

For the intrachromosomal recombination PCR assay, nested PCR products were isolated from gels and sequenced. Online supplemental material.

Fig. S1 shows the gene targeting strategy and phenotype analysis of IgH*94 mice. Fig. S2 shows that catalytically

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