Inactivation of Chk2 and Mus81 Leads to Impaired Lymphocytes Development, Reduced Genomic Instability, and Suppression of Cancer

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Abstract

Chk2 is an effector kinase important for the activation of cell cycle checkpoints, p53, and apoptosis in response to DNA damage. Mus81 is required for the restart of stalled replication forks and for genomic integrity. Mus81ex3-4/ex3-4 mice have increased cancer susceptibility that is exacerbated by p53 inactivation. In this study, we demonstrate that Chk2 inactivation impairs the development of Mus81ex3-4/ex3-4 lymphoid cells in a cell-autonomous manner. Importantly, in contrast to its predicted tumor suppressor function, loss of Chk2 promotes mitotic catastrophe and cell death, and it results in suppressed oncogenic transformation and tumor development in Mus81ex3-4/ex3-4 background. Thus, our data indicate that an important role for Chk2 is maintaining lymphocyte development and that dual inactivation of Chk2 and Mus81 remarkably inhibits cancer.

Introduction

DNA damage response is a result of the coordinated actions of DNA damage signaling and repair pathways, cell cycle checkpoints, and apoptosis [1]. Highlighting the importance of the damage signaling and repair mechanisms, mutations of genes such as ATM, BRCA1, and NBS1, involved in these mechanisms, are associated with increased DNA damage sensitivity, genomic instability, cancer predisposition, immunodeficiency, and developmental defects [2].

Mammalian Mus81 with its partners Emc1 or Emc2 form a heterodimeric structure-specific endonuclease that preferentially cleaves 3’ Flaps and replication fork intermediates [3]. This endonuclease has been shown to facilitate restart of stalled DNA replication forks by generating DNA double-strand breaks (DSBs) [4]. Mus81 also interacts with other DNA damage repair proteins including Rad54, Blm, as well as SLX4 [5–7]. Interestingly, Cds1, the yeast homolog of the mammalian Serine/Threonine kinase Chk2, was reported to phosphorylate and release mus81 from chromatin, presumably to prevent it from cleaving stalled replication forks (RFs) [8].

Three strains of Mus81 mutant mice have been reported. In addition to Mus81 inactivation, Mus81ex3-4 mice have been reported to also display decreased expression of Fibulin-4 gene. Several of these homozygous mutant mice developed cardiovascular complications and died before reaching weaning age [9]. The phenotypes of these mice have been attributed to the decreased expression of Fibulin-4 [9]. Mus81ex9-12 mice have also been reported [10]. These mice displayed increased sensitivity to interstrand crosslinking (ICL) agents including MMC. Genomic instability was reported to be increased in homozygous Mus81ex9-12 MEFs expressing the human papillomavirus type 16 E6 that promotes degradation of p53. While these mutant mice were viable, they showed no increased of tumorigenesis when monitored for a period of 15 months [10]. The Mus81ex3-4 mutant mice and cells that we have generated were also highly sensitive to MMC [11]. Mice homozygous for the Mus81ex3-4 mutation showed no expression of Mus81 protein, and displayed elevated levels of spontaneous genomic instability and cancer predisposition [11]. While the cause for the lack of tumorigenesis in Mus81ex3-4 ‘mutant mice is still not clear, inactivation of p53 in Mus81ex3-4 mice rescued their MMC hypersensitivity and exacerbated their genomic instability and tumorigenesis [12].

Inactivation of Mus81 in human cells also resulted in hypersensitivity to ICL agents and elevated levels of genomic instability[13]. Importantly, Mus81 expression was found...
Author Summary

Failure to repair DNA damage has been associated with a number of human syndromes, neurodegenerative diseases, immunodeficiency, and cancer. In addition, radiotherapy and many cancer chemotherapeutic drugs induce DNA damage, thus allowing the killing of tumors. Recent data indicated Mus81’s role in maintaining genomic integrity and suppressing cancer. Furthermore, inactivation of p53, the most frequently inactivated tumor suppressor in cancer, leads to synergistic tumorigenesis in Mus81 mutant mice. As Chk2 is important for p53 activation, we have examined the effect of its inactivation on the phenotypes associated with Mus81 loss of function. We report that Chk2 is essential for the development of lymphoid cells deficient for Mus81. Chk2 inactivation increased spontaneous cell death of Mus81 deficient cells and impaired the development of T- and B-cell lineages. Chk2 inactivation also reduced the frequency of Mus81-deficient cells that carry elevated levels of spontaneous genomic instability. Importantly, inactivation of Chk2 protected Mus81 mutant mice from developing spontaneous tumorigenesis. These data indicate potential therapeutic benefits for the inactivation of Chk2 and Mus81.

significantly decreased in human hepatocellular carcinomas, and this reduced expression correlates with a poor prognosis for patients with this cancer [14]. Moreover, a variant MUS81 allele (rs545500) was recently associated with increased risk for breast cancer [15].

Results/Discussion

Chk2 Deficiency Does Not Affect Embryonic Development of Mus81<sup>Aox3-4/Aox3-4</sup> Mice

In contrast to the female specific embryonic lethality of Mus81<sup>Aox3-4/Aox3-4</sup> mice in p53 deficient background [12], Mus81<sup>Aox3-4/Aox3-4;Chk2<sup>−/−</sup></sup> mice were viable and born at the expected Mendelian ratio (Table S1). We next examined the fertility of Mus81<sup>Aox3-4/Aox3-4;Chk2<sup>−/−</sup></sup> mice and have also further assessed the viability of double mutant females. Interbreeding of Mus81<sup>Aox3-4/Aox3-4;Chk2<sup>−/−</sup></sup> mice as well as their breeding (males and females) to mice from different genotypes resulted in normal size litters compared to control mice (P=0.05; Table S1). In addition, interbreeding of double mutant mice or heterozygous compound mice resulted in the expected ratio of males and females (P<0.05; Table S2). Double mutant males and females were indistinguishable from their wildtype (WT) and single mutant littermates. Examination of the weight of 6 to 8 week old and 4 to 8 month old double mutant males and females indicated no significant differences compared to WT mice and single mutant controls (Figure S1A). Collectively, these data indicate that Mus81<sup>Aox3-4/Aox3-4;Chk2<sup>−/−</sup></sup> males and females were viable and fertile, and their gross morphology was indistinguishable from their WT and single mutant littermates.

Requirement of Chk2 and Mus81 for Homeostasis of Peripheral T and B-Cells

DNA damage signaling and repair are essential for the development and function of the immune system and their failure impairs hematopoietic cell differentiation [20]. We therefore examined the effect of inactivation of Chk2 on the development and homeostasis of the lymphocytes of Mus81<sup>Aox3-4/Aox3-4</sup> mice. FACs analysis of splenocytes from 6 to 8 week old Mus81<sup>Aox3-4/Aox3-4;Chk2<sup>−/−</sup></sup> mice showed significant impairment of the ratio of T-cells (Thy1.2<sup>+</sup>) to B-cells (B220<sup>+</sup>) (Figure 1A and Figure S2A). The absolute number of splenocytes was significantly decreased in double mutant mice (40.9x10<sup>6</sup>±2.1) compared to Mus81<sup>Aox3-4/Aox3-4</sup> (68.4x10<sup>6</sup>±3.6; P=0.0002), Chk2<sup>−/−</sup> (3.9x10<sup>6</sup>±3.7; P<0.0001) and WT (74.9x10<sup>6</sup>±2.7; P<0.0001) littermates (Figure 1C). The absolute number of B-cells was also significantly reduced in spleen of double mutant mice (12.3x10<sup>6</sup>±0.6) compared to Mus81<sup>Aox3-4/Aox3-4</sup> (32.8x10<sup>6</sup>±1.7; P<0.0001), Chk2<sup>−/−</sup> (46x10<sup>6</sup>±2.3; P<0.0001) and WT (44.5±1.6; P<0.0001) mice (Figure 1C). The absolute number of T-cells in the spleen of double mutant mice (15.9x10<sup>6</sup>±0.8) was also significantly decreased compared to Mus81<sup>Aox3-4/Aox3-4</sup> (21x10<sup>6</sup>±1.1; P=0.007), Chk2<sup>−/−</sup> (19.4x10<sup>6</sup>±0.3; P=0.02) and WT (19.7±10<sup>6</sup>±0.7; P=0.01) mice (Figure 1C). Impaired T-cell lineage homeostasis in the absence of Chk2 and Mus81 was also reflected by the significantly reduced ratio of helper (CD<sup>4+</sup>) to cytotoxic (CD8<sup>+</sup>) T-cells in the spleen of double mutant mice (1.1±0.1) compared to Mus81<sup>Aox3-4/Aox3-4</sup> (1.6±0.1; P=0.02), Chk2<sup>−/−</sup> (1.9±0.3; P=0.03) and WT (2±0.2; P=0.006) mice (Figure 1A and 1D).

To examine the impaired homeostasis of peripheral lymphocytes and the imbalance of the ratio CD4<sup>+</sup> to CD8<sup>+</sup> peripheral T-cells in double mutant mice, we examined the level of spontaneous and activation induced death of these cells. Spontaneous cell death was found significantly increased in both CD4<sup>+</sup> and CD8<sup>+</sup> naive splenocytes from double mutant mice compared to controls (Figure S3A and S3B). In addition, LPS activation of double mutant B-cells also resulted in elevated levels of cell death compared to B-cells from single mutants or WT mice (Figure S7C). While the number of T-cells and B-cells were reduced in spleen of double mutant mice, the number of macrophages in these spleens remained similar to single mutants and WT controls.

Taken together these data demonstrate a specific role for Chk2 in maintaining homeostasis of peripheral T- and B-cells deficient for Mus81.

Chk2 Loss Affects the Development of Mus81<sup>Aox3-4/Aox3-4</sup> Thymocytes

We next assessed the effect of double inactivation of Chk2 and Mus81 on thymocyte development. Similar to peripheral T-cells, FACs analysis indicated a significantly decreased ratio of CD4<sup>+</sup> to CD8<sup>+</sup> thymocytes in Mus81<sup>Aox3-4/Aox3-4;Chk2<sup>−/−</sup></sup> mice compared to controls (Figure 1E and 1F). Moreover, total number of thymocytes was significantly reduced in double mutant mice (86.3x10<sup>5</sup>±5.5) compared to Mus81<sup>Aox3-4/Aox3-4</sup> (121.1x10<sup>5</sup>±4.1; P=0.002), Chk2<sup>−/−</sup> (109.3x10<sup>5</sup>±3.9; P=0.015) and WT (139.9±10<sup>5</sup>±8.7; P=0.005) mice (Figure 1G).
Inactivation of Chk2 and Mus81 Suppresses Cancer

A

B220

WT

Mus81−/−

Chk2−/−

Mus81−/−Chk2−/−

Thy1.2

CD4

CD8

Splenocytes

B

WT

Chk2−/−

Mus81−/−

Mus81−/−Chk2−/−

Cell Number

(x10^6)

**

**

***

***

B-cells

T-cells

CD4+/CD8+ Cells

Splenocytes

E

CD4

CD8

Thymocytes

F

Ratio CD4+/CD8+ cells

Thymocytes

G

Cell Number (x10^6)

Thymocytes
Examination of double negative (CD4+CD8+) thymocytes and their subpopulations DNI (CD44+/CD25-), DNI (CD44+/CD25+), DNNII (CD44+CD25-), DNNIII (CD44+CD25+) and DNNIV (CD44+CD25+) from double mutant mice, single mutants and controls indicated no significant differences in cell numbers (P>0.05; Figure S2C). However, the numbers of CD4+CD8+ and CD4+ thymocytes from Mus81 ex3-4/Chk2−/− mice were significantly reduced compared to control littermates (Figure S2D and S2E).

We next examined the level of expression of TCRβ in thymocytes from the four genotypes using FACS analysis and anti-pan TCR chain constant region, anti-TCRVβ4, anti-TCRVβ5 and anti-TCRVβ17a. Consistent with the normal number of CD4+CD8+ thymocytes, a representative FACS analysis of splenocytes from Mus81 ex3-4/Chk2−/−, Mus81−/−, Chk2−/−, and Mus81−/−; Chk2−/− mice. (A) Representative FACS analysis of total (top panel) and Thy1.2+ (lower panel) splenocytes from WT, Mus81−/−, Chk2−/−, Mus81−/−; Chk2−/−, and Mus81 ex3-4/Chk2−/− mice. (B and C) Number of splenocytes (B), T-cells (C, left panel) and T-cells (C, right panel) in spleen of Mus81 ex3-4/Chk2−/− mice and controls. (D) Ratio of CD4+ to CD8+ T-cells in spleen of Mus81 ex3-4/Chk2−/− mice and controls. (E) Representative FACS analysis of thymocytes from Mus81 ex3-4/Chk2−/− mice and controls. (F) Percentage of T-cells in the CD4+ and CD8+ thymocyte populations indicated their significant decrease in double mutant mice compared to single mutants (P<0.05; Figure S4C). However, the number of spontaneous cell death (CD4+CD8+ T-cells) from these mice also displayed reduced number of total thymocytes (P<0.01) compared to control littermates (Figure S6C). These data indicate that in contrast to the inactivation of either Mus81 or Chk2, their dual inactivation significantly reduces the pool of B-cell precursors. Our data also identify increased cell death as likely to contribute to the defective differentiation of B-cell lineage in Mus81 ex3-4/Chk2−/− mice. The earlier differentiation defect of the B-cell lineage compared to T-cell lineage of Mus81 ex3-4/Chk2−/− mice is likely to contribute to the more pronounced imbalance of the ratio B- to T-cells in the periphery of these mice.

Defects in the T and B-Cell Lineages of Mus81 ex3-4/Chk2−/− Mice Are Cell Autonomous

In order to address whether the phenotypes observed in Mus81 ex3-4/Chk2−/− mice were cell autonomous or nonautonomous, we have transplanted BM cells from the 4 genotypes into Rag1−/− mice, and have examined the reconstituted mice for the number and differentiation status of BM cells, thymocytes and splenocytes (Figure S6). Rag1−/− mice arrest their B-cell development at the pre-B stage [21]. Examination of the number of BM cells in the Rag1−/− mice reconstituted with Mus81 ex3-4/Chk2−/− BM cells indicated no significant differences at the Pre-B-stage (Figure S6A). However, similar to Mus81 ex3-4/Chk2−/− mice, reconstituted Rag1−/− mice with Mus81−/−; Chk2−/− BM cells also displayed significantly reduced number of recirculating B-cells compared to reconstituted controls (P<0.006; Figure S6B). However, Mus81 ex3-4/Chk2−/− mice reconstituted with Mus81 ex3-4/Chk2−/− BM cells, similar to double mutant mice, displayed reduced number of thymocytes (P<0.03; Figure S6C). In addition, spleen from these mice also displayed reduced number of total splenocytes, B-cells, T-cells, CD4+ T-cells and CD8+ T-cells (P<0.05; Figure S6D).

These data indicate that the phenotypes observed with BM, thymus and spleen of Mus81 ex3-4/Chk2−/− mice were largely cell autonomous.

Chk2 Inactivation Does Not Rescue MMC Hypersensitivity of Mus81 ex3-4/Chk2−/− T-Cells

Hypersensitivity to ICL agents including MMC was reported for Mus81 ex3-4/Chk2−/− [4], Mus81 ex3-4/Chk2−/− [10] as well as with Mus81−/−; Chk2−/− mutations [11]. The hypersensitivity of Mus81 ex3-4/Chk2−/− cells was rescued in p53 null background [12]. Examination of the proliferation of activated T-cells (anti-CD3 + IL2) using the Carboxyl fluoroscence succinimidyl ester (CFSE) assay indicated similar level of cell divisions in T-cells from Mus81 ex3-4/Chk2−/− mice and control mice (Figure 3A). However, 72 hours (hr) post-MMC treatment (0.1 μg/ml), double mutant cells

Figure 1. Chk2 Inactivation Impairs Differentiation and Homeostasis of Mus81−/− T and B-Cell Lineages. (A) Representative FACS analysis of total (top panel) and Thy1.2+ (lower panel) splenocytes from WT, Mus81−/−, Chk2−/−, and Mus81−/−; Chk2−/− mice. (B and C) Number of splenocytes (B), T-cells (C, left panel) and T-cells (C, right panel) in spleen of Mus81 ex3-4/Chk2−/− mice and controls. (D) Ratio of CD4+ to CD8+ T-cells in spleen of Mus81 ex3-4/Chk2−/− mice and controls. (E) Representative FACS analysis of thymocytes from Mus81 ex3-4/Chk2−/− mice and controls. (F) Percentage of T-cells in the CD4+ and CD8+ thymocyte populations indicated their significant decrease in double mutant mice compared to single mutants (P<0.05; Figure S4C).
exhibited a pronounced proliferative defect that was similar to the level observed for Mus81Dex3-4/Dex3-4 cells (Figure 3A). Moreover, loss of Chk2 also failed to rescue MMC induced G2 arrest and apoptosis of Mus81Dex3-4/Dex3-4 T-cells (Figure 3B and Figure S7A).

We also examined the effect of Mus81 inactivation on the radioresistance of Chk22/2 activated T-cells. A nearly equivalent resistance was observed in both Mus81Dex3-4/Dex3-4Chk22/2 and Chk22/2 cells 12 hr post ionizing radiation (IR; 4Gy) (Figure S7B), supporting that Mus81 inactivation has no effect on the radioresistant phenotype of Chk22/2 cells.

Therefore, in contrast to p53 inactivation [12], inactivation of Chk2 failed to rescue MMC hypersensitivity of Mus81Dex3-4/Dex3-4 T-cells.

**Effect of Chk2 Inactivation on MMC Hypersensitivity of Mus81Dex3-4/Dex3-4 BM Cells and Mice**

To examine whether Chk2 plays a role in the MMC hypersensitivity of Mus81Dex3-4/Dex3-4 BM cells [12], we performed colony forming assay in the absence or presence of MMC (40 ng/ml), using double mutant, Mus81Dex3-4/Dex3-4, Chk22/2 and WT BM cells. Twelve days post plating, MMC treated BM cells from Chk22/2 and WT mice formed a similar number of colonies (Figure 3C and 3D). However, similarly to Mus81Dex3-4/Dex3-4 BM cells, double mutant BM cells displayed reduced colony-forming capacity post-MMC treatment (Figure 3C and 3D). Therefore, inactivation of Chk2 in Mus81Dex3-4/Dex3-4 BM cells does not rescue their MMC hypersensitivity.

Given the high in vivo MMC sensitivity of Mus81Dex3-4/Dex3-4 mice [11] and Mus81Dex9-12 mice [10], we assessed the effect of Chk2 inactivation on the MMC hypersensitivity of Mus81 mutant mice. Cohorts of mice from the four genotypes received intraperitoneal (i.p) injection of MMC (12.5 mg/kg) and were monitored for survival (Figure 3E). In contrast to the results of the in vitro BM colony forming assay suggestive of equivalent MMC sensitivity of double mutant and Mus81Dex3-4/Dex3-4 BM cells, double mutant mice displayed significantly higher sensitivity to MMC (mean survival = 6 days) than Mus81Dex3-4/Dex3-4 mice (mean survival = 9 days).

As BM failure might contribute to the elevated MMC sensitivity of Mus81Dex3-4/Dex3-4Chk22/2 mice, we examined the in vivo effects
Figure 3. Effect of Chk2 Inactivation on MMC Sensitivity of Mus81−/− Cells and Mice. (A) Representative CFSE staining of WT, Mus81−/−, Chk2−/−, and Mus81−/−Chk2−/− activated T-cells (anti-CD3 + IL2) 120hr post CFSE labelling. Cells were either untreated (UT) or treated with MMC (0.1 μg/ml) for the last 72 hr (MMC). Percentage of cells achieving two cell divisions is shown. (B) Representative FACS analysis showing the level of cell death (7AAD+) of activated T-cells either untreated (UT) or 48 hr post MMC (0.5 μg/ml) treatment. Percent of dead cells is shown. (C and D) BM colony forming assay. BM cells were plated on media containing 0 or 40 ng/ml of MMC and colonies were counted 10 days post-treatment. Representative picture of colonies at day 10 of MMC treatment is shown (C). Data normalized to untreated cells is presented as the mean ± SD of three independent experiments (D). No statistical significance: ns. (E) Kaplan-Meier survival curve of WT, Mus81−/−, Chk2−/−, and Mus81−/−Chk2−/− cohort of mice (n = 10 each) in response to i.p injection of MMC (12.5 mg/kg). (F) Representative FACS analysis of cell death using PI staining and B220 gated BM cells from Mus81−/−Chk2−/− mice and controls. Mice were either untreated (UT) or received MMC (12.5 mg/kg) injection three days prior to analysis (MMC). Data shown is representative of at least three independent experiments. Mus81−/−: doi:10.1371/journal.pgen.1001385.g003
of MMC on BM cell populations from Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> mice, single mutants and WT littermates. Mice were either left untreated or subjected to ip injection of MMC (12.5 mg/kg) and the level of death of B220<sup>+</sup> BM cells was examined three days later using PI staining. Increased cell death was observed in B220<sup>+</sup> BM cells from both untreated and MMC treated double mutant mice compared to control littermates (Figure 3F).

These data indicate that in contrast to p53 inactivation [12], loss of Chk2 synergizes MMC hypersensitivity of Mus81<sup>ex3-4/Aex3-4</sup> mice and BM cells.

### Increased Mitotic Catastrophe of Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> Cells

Mitotic catastrophe is an abnormal mitosis that triggers the death of cells with damaged DNA as they enter mitosis [22]. Mitotic catastrophe is also triggered by agents that affect the stability of microtubules as well as by mitotic failure that results from impaired cell cycle checkpoints. Chk2, which is important for G2/M checkpoint, has been reported to negatively regulate mitotic catastrophe of DNA damaged cells [23]. As Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> cells displayed increased levels of spontaneous cell death and these mutant cells and mice also displayed elevated MMC-induced cell death, we have examined whether cell death of Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> cells is triggered by mitotic catastrophe. Cells undergoing mitotic catastrophe typically display microanuclei, increased frequency of giant cells, multinucleated nuclei, and nuclear bridging [22].

Untreated Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> primary MEFs stained with DAPI displayed increased frequency of abnormal giant cells with multinucleated nuclei compared to controls MEFs (P<0.0055; Figure 4A and 4E). Llagging chromosomes were also observed in untreated Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> MEFs (Figure 4B). The frequency of untreated double mutant MEFs showing micronuclei was also elevated compared to controls (P<0.018; Figure 4F). In response to MMC, Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> MEFs displayed increased frequency of giant cells with multinucleated nuclei (P<0.01; Figure 4G) as well as cells with nuclear bridging (P<0.001; Figure 4D and 4H). These data indicate increased mitotic catastrophe of Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> cells.

Loss or reduction of the expression of survivin [24], an inhibitor of apoptosis protein [IAP], has been shown to lead to cell death by mitotic catastrophe [25]. We have therefore performed Western blot analyses to examine the levels of survivin in untreated or 18 hr and 24 hr post MMC treatment of LPS activated B-cells from Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> mice, single mutants and WT controls. We observed a lower level of survivin in MMC treated Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> B-cells compared to single mutants and WT controls (Figure S8). We propose that decreased survivin level in Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> cells could contribute to their mitotic catastrophe.

Increased mitotic catastrophe would trigger cell death of Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> cells and is therefore likely to contribute to the impaired homeostasis of BM cells, thymocytes and splenocytes in double mutant mice.

### Impaired of Activation of p53 and Defective G2/M Checkpoint in Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> Cells

p53 plays a central role in DNA damage responses and its phosphorylation by Chk2 on Serine 20 is important for its stability and activation [26]. Therefore, we examined the level of p53 expression and activation in double mutants and control B-cells from the four genotypes. While LPS activated double mutant B-cells displayed increased cell death levels (Figure 5A), the basal level of p53 in these activated B-cells remained similarly low compared to single mutants and WT B-cells (Figure 5B). MMC treatment of WT and Mus81<sup>ex3-4/Aex3-4</sup> activated B-cells increased p53 expression levels (Figure 5B). However, double mutant and Chk2<sup>−/−</sup> cells failed to increase their level of p53 in response to MMC treatment (Figure 5B). In accordance with the low level of p53 in untreated and MMC treated double mutant cells, the levels of Serine 15-p53 (a substrate for ATM), and the levels of Bax and p21 (p53 downstream targets) were low to undetectable in double mutant cells under untreated conditions and were not induced in response to MMC (Figure 5C). In contrast, the expression levels of Serine 15-p53, Bax and p21 in Mus81<sup>ex3-4/Aex3-4</sup> and WT cells were significantly increased in response to MMC (Figure 5C).

These data indicate that despite the lack of p53 activation in double mutant B-cells, these cells displayed elevated level of spontaneous and MMC-induced death, supporting that this death is p53-independent. Consistent with these data, mitotic catastrophe has been shown to take place in a p53-independent manner [27].

Chk2 is required for the enforcement of the G2/M cell cycle checkpoint following DNA damage [16,18]. It has been shown that loss of Chk2 expression or inhibition of its kinase activity sensitizes cells to death during mitosis [23]. Therefore, we have examined G2/M cell cycle checkpoint in untreated and MMC-treated double mutant and control activated B-cells (Figure 5D). While the fraction of mitotic cells (positive for phospho-Histone H3) was reduced 18 hr and 24 hr post MMC treatment in WT and Mus81<sup>ex3-4/Aex3-4</sup> B-cells, both Chk2<sup>−/−</sup> and Mus81<sup>ex3-4/Aex3-4</sup> Chk2<sup>−/−</sup> B-cells failed to activate G2/M checkpoint in response to MMC treatment as indicated by their increased fraction of phospho-Histone H3 positive cells compared to untreated controls (Figure 5D).

These data indicate that despite MMC-induced DNA damage, double mutant cells are allowed to enter mitosis, due to their impaired G2/M checkpoint, therefore resulting in mitotic catastrophe and cell death.

### Effect of Chk2 Inactivation on Genomic Instability of Mus81<sup>ex3-4/Aex3-4</sup> Mice

Mus81<sup>ex3-4/Aex3-4</sup> mice display elevated levels of genomic instability [11], likely due to their defective repair of stalled RFs [28]. Genomic instability was also increased in Mus81<sup>ex9-12/Aex9-12</sup> MEFs expressing the oncoprotein E6 [10] and in Mus81<sup>ex1-10/Aex1-10</sup> ES cells treated with Hydroxyurea [28]. In addition, inactivation of MUS81 by gene targeting in the human colon cancer cell line HCT116 also increased the level of genomic instability of these cells [13].

While Chk2 inactivation resulted in increased genomic instability of Brcal<sup>−/−</sup> cells, it did not for Nbs1<sup>−/−</sup> or Mre11<sup>−/−</sup> cells [29,30]. To determine the effect of Chk2 inactivation on the increased spontaneous genomic instability associated with Mus81<sup>ex3-4/Aex3-4</sup> mutation [11], we examined metaphase spreads of activated B-cells from the different genotypes. These analyses indicated elevated level of spontaneous genomic instability of Mus81<sup>ex3-4/Aex3-4</sup> B-cells while Chk2<sup>−/−</sup> B-cells exhibited only a slightly increased level of spontaneous genomic instability (Figure 6A and 6B; Table S3). Remarkably, while inactivation of p53 exacerbated spontaneous genomic instability of Mus81<sup>ex3-4/Aex3-4</sup> cells [12], inactivation of Chk2 in these cells significantly suppressed their spontaneous genomic instability. Multicolor fluorescence in situ hybridization (mFISH) also failed to detect any interchromosomal aberrations like translocations in Mus81<sup>ex3-4/Aex3-4</sup>Chk2<sup>−/−</sup> cells and their controls (Figure 6C). These data suggest that either Chk2 inactivation decreases the...
survival of Mus81−/− cells that carry genomic instability, or alternatively that it prevents genomic instability from occurring in Mus81 mutants. However, the latter is inconsistent with the drastic increased genomic instability of MMC-treated double mutant B-cells compared to Mus81+/+ MEFs controls (Table S3).

To examine whether the reduced level of spontaneous genomic instability of Mus81+/– MEFs in Chk2 null background was restricted to these cells or could be observed in other cell types, we have examined metaphase spreads from activated T-cells and primary MEFs from the four genotypes. Similar to B-cells, Mus81+/– MEFs showed a significant decrease in numbers of abnormal mitoses compared to Mus81+/+ MEFs controls. In addition, in response to MMC treatment, similar to double mutant B-cells, Mus81+/– MEFs exhibited elevated levels of genomic instability compared to Mus81+/+ MEFs controls. In addition, in response to MMC treatment, similar to double mutant B-cells, Mus81+/– MEFs showed a significant decrease in numbers of abnormal mitoses compared to Mus81+/+ MEFs controls. In addition, in response to MMC treatment, similar to double mutant B-cells, Mus81+/– MEFs exhibited increased levels of genomic instability compared to Mus81+/+ MEFs controls (Table S4). The increased genomic instability of MMC treated Mus81+/– MEFs could be observed in other cell types, such as T-cells and primary MEFs from the four genotypes. Similar to B-cells, Mus81+/– MEFs showed a significant decrease in numbers of abnormal mitoses compared to Mus81+/+ MEFs controls. In addition, in response to MMC treatment, similar to double mutant B-cells, Mus81+/– MEFs exhibited increased levels of genomic instability compared to Mus81+/+ MEFs controls. In addition, in response to MMC treatment, similar to double mutant B-cells, Mus81+/– MEFs showed a significant decrease in numbers of abnormal mitoses compared to Mus81+/+ MEFs controls. In addition, in response to MMC treatment, similar to double mutant B-cells, Mus81+/– MEFs exhibited increased levels of genomic instability compared to Mus81+/+ MEFs controls (Table S4).

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Inactivation of Chk2 Suppresses Tumorigenesis of Mus81+/– Mutants

CHk2 is mutated in certain human familial cancers and in a number of other tumors [18]. Tumorigenesis was also observed in a mouse model for CHK2 deficient mice, a mutation associated with increased cancer risk in humans [31]. In addition, inactivation of Chk2 increased cancer risk of BcrAl, Nbs1 and Mus81 mutant mice [30]. Stracker et al. demonstrated that inactivation of Chk2 in DNA-PKcs deficient background did not predispose these mice for cancer, and proposed that Chk2 suppresses the oncogenic potential of DNA damage arising in the S and G2 phases of the cell cycle [30]. Mus81+/– MEFs overexpressed Chk2 null cell cycle and were not G1 phases of the cell cycle [30].

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Inactivation of Chk2 Suppresses Tumorigenesis of Mus81+/–/– Mutants

The remarkable mitigation of tumorigenesis of Mus81+/–/– mice by Chk2 inactivation parallels their reduced level of spontaneous genomic instability, failure of G2/M checkpoint and increased mitotic catastrophe. Our finding is consistent with a beneficial effect of CHK2 inactivation on tumor responses to genotoxic chemotherapeutic drugs [18].

Chk2 Inactivation Suppresses Oncogenic Transformation of Mus81+/–/– Mutants

Dysregulation of oncoproteins, similar to tumor suppressors, plays critical roles in human cancer. Chk2 is activated by oncoproteins such as Ras, and is important for mediating oncogene-induced senescence, a safeguard against cancer development [32]. In addition, Chk2 inactivation has been shown to promote oncogenic transformation [19].

We therefore investigated the effect of Chk2 inactivation on transformation of Mus81+/–/– cells and examined oncogenic transformation of double mutant primary MEFs, single mutants and WT controls. Cells were infected with a retrovirus expressing EIA and Ras and transformed colonies were counted three weeks later (Figure 7B and 7C). Consistent with previous studies [19], the number of EIA-Ras transformed colonies formed by Chk2+/– MEFs was elevated compared to WT controls (P<0.05). Strikingly, while Mus81+/–/– MEFs were able to form EIA-Ras transformed colonies at a similar frequency compared to WT MEFs (P=0.36), no EIA-Ras transformed colonies were observed from Mus81+/–/– MEFs in three independent experiments.

To confirm that the lack of transformation of Mus81+/–/– Chk2−/− MEFs is due to the loss of Mus81 and Chk2 and not due to the inability of these MEFs to be infected with pBabe EIA-HRas V12 retrovirus, MEFs from the four genotypes were infected with this retrovirus and genomic DNA of these cells was prepared 7 days post puromycin selection and PCR analysis was performed to assess the presence of EIA-HRasV12 in the extracted genomic DNA. These analyses demonstrated that despite integration of
Inactivation of Chk2 and Mus81 Suppresses Cancer

ELA-HRaV12 in the genome of Mus81\textsuperscript{lox3-4/Aex3-4}Chk2\textsuperscript{-/-} MEFs (Figure 7D), these cells failed to be transformed.

These data demonstrate that similar to the suppression of spontaneous tumorigenesis of Mus81\textsuperscript{lox3-4/Aex3-4} mice, Chk2 inactivation also suppresses oncogenic transformation of Mus81\textsuperscript{lox3-4/Aex3-4} cells.

Collectively our data demonstrate an important role for Chk2 in maintaining homeostasis of BM cells, thymocytes and splenocytes deficient for Mus81. Remarkably, inactivation of Chk2 also reduced spontaneous genomic instability associated with Mus81 mutation, and significantly protected Mus81 mutants from tumorigenesis and oncogenic transformation. We also report increased mitotic catastrophe in double mutant cells, likely due to the inactivation of G2/M checkpoint associated with loss of Chk2 and increased spontaneous DNA damage associated with loss of Mus81. The phenotypes observed in Mus81\textsuperscript{lox3-4/Aex3-4}Chk2\textsuperscript{-/-} mice are p53-independent. In contrast to Mus81\textsuperscript{lox3-4/Aex3-4}Chk2\textsuperscript{-/-} cells, Mus81\textsuperscript{lox3-4/Aex3-4}p53\textsuperscript{-/-} cells retain proficient G2/M checkpoint that prevents their entry to mitosis in the presence of damaged DNA. Therefore, we propose that G2/M checkpoint failure and
Inactivation of Chk2 and Mus81 Suppresses Cancer

A

Mus81<sup>−/−</sup> Mus81<sup>−/−</sup>-Chk2<sup>−/−</sup> Mus81<sup>−/−</sup>-Chk2<sup>−/−</sup>

UT UT MMC

dm br

B

Total Aberrations (%)

UT MMC

Total Aberrations (%)

WT Mus81<sup>−/−</sup> Chk2<sup>−/−</sup> Mus81<sup>−/−</sup>-Chk2<sup>−/−</sup>

C

Mus81<sup>−/−</sup> Chk2<sup>−/−</sup> Mus81<sup>−/−</sup>-Chk2<sup>−/−</sup>
increased mitotic catastrophe are the mechanisms that result in reduced spontaneous genomic instability, tumorigenesis and oncogenic transformation.

Our data provide in vitro evidence that inhibition of Chk2 can have remarkable inhibitory effects on tumorigenesis. While pharmacological inhibitors of CHK2 are being considered for cancer therapy [18], our data suggest that the therapeutic effect of such inhibitors is likely to depend on the genetic background of the tumors. Namely, while Chk2 inactivation promotes tumorigenesis of B6.129/J mice, we do not observe tumorigenesis in 129/SvJ mice. This result is consistent with our data suggesting a workable therapeutic window for CHK2 inhibitors. While pharmacological inhibition of CHK2 is promising, the work presented here suggests that inhibitors may be limited to specific tumors of the right genetic background.

Materials and Methods

Mice

Mus81<sup>dex3-4/dex3-4</sup>Chk2<sup>−/−</sup> mice were obtained by crossing Mus81<sup>dex3-4/dex3-4</sup> and Chk2<sup>−/−</sup> mice on a mixed 129/SvJ background. Our preclinical data highly support the potential therapeutic value for CHK2 or MUS81 inhibitors for cancer patients with Mus81 or Chk2 mutations, respectively.

Flow Cytometry

BM, thymus and spleen cells from 6–8 week-old mice were stained with antibodies (Pharmani) against B220, CD43, IgM, IgD, Thy1.2, TCR, CD4, CD8, CD44, CD25 and TCRVβ (4, 5.1–5.2, and 17a). Fluorescence-activated cell sorting (FACS) analyses were performed using a FACS Calibur (Becton Dickinson).

Cell Cycle Analysis

Peripheral T-cells stimulated with anti-CD3 and IL-2 (50 U/mL) for 48 h, were treated with 0.5 µg/mL MMC (Sigma) for 18 h. Cells were washed three times with PBS and cultured for an additional 18 h. Cells were fixed in 70% ethanol, and DNA was stained with 5 µg/mL of PI (Sigma). Cells at the G1, S, and G2/M phases of the cell cycle were determined using FLOWJO analysis software.

Phospho-Histone H3 Staining

Peripheral B-cells activated with LPS (10 µg/mL) for 48 h, were treated with 0.1 µg/mL of MMC (Calbiochem) for 18 h. Cells were washed three times with PBS and cultured for an additional 18 h. Cells were fixed in 70% ethanol, and DNA was stained with 5 µg/mL of PI (Sigma). Cells at the G1, S, and G2/M phases of the cell cycle were determined using FLOWJO analysis software.

CFSE Staining

Peripheral T-cells were stained with 5 µM CFSE and then activated for two days with plate bound anti-CD3 in the presence of IL-2. Untreated and 0.1 µg/mL MMC treated cells were grown for an additional 72 h and analyzed by flow cytometry.

MEFs and Retrovirus Infection

Primary MEFs (3x10<sup>5</sup>) were cultured in DMEM plus 10% FCS in the presence of pBabe Ec1A-HrasV12 retrovirus and 8 µg/ml of polybrene. At day three post-infection, Puromycin selection (2 µg/ml) was carried out for three weeks. Colonies on the plates were fixed with ice cold methanol, stained with 0.5% crystal violet and counted. To confirm that MEFs have integrated Ec1A-HrasV12, genomic DNA was prepared from infected cells post 7 days of puromycin selection. PCR was then performed on MEFs genomic DNA using specific primers for HrasV12 (F: CCGTATAAAGCTTGTGGTG and R: CGGTATCCAGGATTGCCACG). Emc2 primers (F: ACGGCTTCTCCCTACGACG and R: AGTGGCTGCTACTCCGTCTCA) were used as controls for the PCR.

Chromosomal Aberrations Analysis

Peripheral T-cells were activated with anti-CD3 (10 µg/mL) and cultured for 12 hr. BM cells (1x10<sup>6</sup>) seeded on 35 mm dishes in Methocult GF M3434 media (Stemcell Technologies Inc.) were either left untreated or treated with MMC (40 ng/mL) and cultured for 12 days prior to counting.

Cell Death Assays

Cell death was assessed using 7AAD, PI or Annexin V-PI staining and FACS analysis as described [12].

Immunoblotting

Activated B-cells treated with 0.1 µg MMC (Sigma) were harvested and lysed 18 hr post-treatment. Proteins were detected using the following anti-rabbit antibodies: anti-p53 (FL393, Santa Cruz), anti-Phospho-p53 Ser-15 (cell signaling), anti-p21 (Santa cruz), anti-Bax (Santa Cruz), anti-Stat3 (Novus) and anti-β-actin (Sigma).

Immunostaining

Passage 1 MEFs derived from WT and Mus81<sup>dex3-4/dex3-4</sup>, Chk2<sup>−/−</sup>, and Mus81<sup>dex3-4/dex3-4</sup>Chk2<sup>−/−</sup> embryos were seeded onto coverslips and either left untreated or treated with MMC (1 µg/mL). 24 hr post treatment, MEFs were fixed with 2% paraformaldehyde, blocked with antibody dilution buffer and

Inactivation of Chk2 and Mus81 Suppresses Cancer

Figure 6. Effects of Chk2 Inactivation on Genomic Instability of Mus81<sup>−/−</sup> Cells. (A) Representative metaphases of LPS activated Mus81<sup>dex3-4/dex3-4</sup> and Mus81<sup>dex3-4/dex3-4</sup>Chk2<sup>−/−</sup> B-cells either untreated (UT) or post-MMC treatment (MMC). f: chromosomal fusion, dm: double minute and br: break. (B) Incidence of total spontaneous (UT, left panel) and MMC-induced chromosomal aberrations (MMC, right panel). Data is presented as the mean ± SD of three independent experiments. A minimum of 50 metaphase spreads were analyzed for each genotype, treatment and experiment. (C) mFISH performed on metaphase spreads from Mus81<sup>dex3-4/dex3-4</sup>, Chk2<sup>−/−</sup> and Mus81<sup>dex3-4/dex3-4</sup>Chk2<sup>−/−</sup> cells. Mus81<sup>−/−</sup>; Mus81<sup>dex3-4/dex3-4</sup>Chk2<sup>−/−</sup> doi:10.1371/journal.pgen.1001385.g006
Bone Marrow Transplantation

Bone marrow cells were harvested from the femur of 8 week old mice. 1 x 10^6 cells were transplanted to 6 week old *Rag1^−/−* mice by tail vein injection. The transplanted mice were scarified and analysed 8 weeks post-transplantation.

In Vivo Sensitivity to MMC–Induced Damage

Mice (n = 10) for each genotype were injected (i.p) with 12.5 mg MMC/Kg of body mass and observed for two weeks post...
treatment. Mice were sacrificed when they became moribund and the day of sacrifice was counted as day of death.

**Statistical Analysis**

The two tailed unpaired student’s t test was used for statistical analysis except for survival curves where Log Rank test was employed (Prism 5, GraphPad Software).

**Ethics Statement**

All experiments were performed in compliance with Ontario Cancer Institute animal care committee guidelines.

**Supporting Information**

**Figure S1** Weight of Mus81+/Chk2-/- Females and Controls. Weight of 6 to 8 week old or 4 to 8 month old WT, Mus81+/ex3-4/ex3-4, Chk2-/-, and Mus81+/ex3-4/ex3-4-Chk2-/- females. No difference was observed. Mus81+/-: Mus81+/ex3-4/ex3-4.

Found at: doi:10.1371/journal.pgen.1001385.s001 (0.55 MB TIF)

**Figure S2** Effects of Chk2 Inactivation on Homeostasis of Lymphocytes from Mus81+/+ Mice. (A) Ratio of Thy1.2+ and B220+ splenocytes from the 6 to 8 week old indicated mice. At least four independent experiments using one mouse per group were performed. (B) Total number of macrophages in spleen of the indicated mice. (C) Total number of CD4+CD8- thymocytes in the indicated mice. (D) Total number of CD4+CD8+ thymocytes in the indicated mice. (E) Total number of CD4+ thymocytes in the indicated mice. (F) Total number of CD8+ thymocytes in the indicated mice. **: P = 0.0002. *: P = 0.0001. At least four independent experiments using one mouse per group were performed. Bar graphs show means ± SEM. Mus81+/-: Mus81+/ex3-4/ex3-4.

Found at: doi:10.1371/journal.pgen.1001385.s002 (1.08 MB TIF)

**Figure S3** Effects of Chk2 Inactivation on Death of Lymphocytes from Mus81+/+ Mice. (A) Percentage of cell death of Naıve CD4+ T-cells from Spleen of Mus81+/ex3-4/ex3-4-Chk2-/- Mice. (B) Percentage of cell death of Naıve CD8+ T-cells from Spleen of Mus81+/ex3-4/ex3-4-Chk2-/- Mice. (C) Representative FACs analysis of cell death performed on untreated (UT) or LPS activated B-cells (48hr) from the indicated mice using PI assay. Numbers indicate the percent of dead cells. At least four independent experiments using one mouse per group were performed. Bar graphs show means ± SEM. *: P<0.04. Mus81+/+; Mus81+/ex3-4/ex3-4.

Found at: doi:10.1371/journal.pgen.1001385.s003 (4.35 MB TIF)

**Figure S4** Mus81+/+Chk2-/- Mice Display Normal TCR Expression. (A) Representative FACs histograms of pan TCRBV expression in thymocytes from WT, Mus81+/ex3-4/ex3-4, Chk2-/-, and Mus81+/ex3-4/ex3-4-Chk2-/- mice. Bars indicate the TCRBV-High positive cells. (B) Percentage of the TCRBV (4, 5,1-5.2 and 17a) positive thymocytes. Numbers indicates the percent of TCRBV positive cells. At least five independent experiments using one mouse per group were performed. Bar graphs show means ± SEM. (C) Representative FACs histograms of pan TCRBV expression in splenocytes from WT, Mus81+/ex3-4/ex3-4, Chk2-/-, and Mus81+/ex3-4/ex3-4-Chk2-/- mice. Bars indicate the TCRBV positive cells. Mus81+/-: Mus81+/ex3-4/ex3-4.

Found at: doi:10.1371/journal.pgen.1001385.s004 (1.90 MB TIF)

**Figure S5** Defective Homeostasis of the B-Cell Lineage in the Mus81+/+Chk2-/- Mice. Representative FACs analysis of anti-B220 and anti-IgM staining of the B220+CD43+ gated BM cells from WT, Mus81+/ex3-4/ex3-4, Chk2-/-, and Mus81+/ex3-4/ex3-4-Chk2-/- mice (top). Representative FACs analysis of anti-IgD and anti-IgM staining of the B220+ gated BM cells from WT, Mus81+/ex3-4/ex3-4, Chk2-/-, and Mus81+/ex3-4/ex3-4-Chk2-/- mice (bottom). At least four independent experiments using one mouse per group were performed. Mus81+/-: Mus81+/ex3-4/ex3-4.

Found at: doi:10.1371/journal.pgen.1001385.s005 (2.77 MB TIF)

**Figure S6** The Developmental Defect of Mus81+/+ Chk2-/- Lymphocytes Is Cell Autonomous. (A) Representative FACs analysis of anti-B220 and anti-CD43 staining of the BM cells from Rag1+/- mice reconstituted with BM cells from WT, Mus81+/ex3-4/ex3-4, Chk2-/-, or Mus81+/ex3-4/ex3-4-Chk2-/- mice. (B) Absolute cell number of recirculating (CD43+ B220+igB2) BM cells from Rag1+/- reconstituted mice as in (A). (C) Absolute number of total, CD4+ and CD8+ thymocytes of Rag1+/- reconstituted mice as in (A). (D) Absolute number of total splenocytes, B-cells, T-cells CD4+ T-cells and CD8+ T-cells from Rag1+/- reconstituted mice as in (A). Numbers of cells are indicated. At least four independent experiments using one mouse per group were performed. Bar graphs show means ± SEM. *: P<0.05. Mus81+/-: Mus81+/ex3-4/ex3-4.

Found at: doi:10.1371/journal.pgen.1001385.s006 (5.17 MB TIF)

**Figure S7** Effect of Chk2 Inactivation on the Proliferation and DNA Damage Response of Mus81+/+ Lymphocytes. (A) Representative cell cycle analysis of WT, Mus81+/ex3-4/ex3-4, Chk2-/-, and Mus81+/ex3-4/ex3-4-Chk2-/- activated T-cells (anti-CD3 + IL2) 120h post stimulation. Cells were either untreated (UT) or treated with 0.5 µg/ml MMC for 18hr (MMC). Percentages of cells in G0/G1, S, and G2/M are shown. (B) Activated (anti-CD3 + IL2) T-cells from WT, Mus81+/ex3-4/ex3-4, Chk2-/-, and Mus81+/ex3-4/ex3-4-Chk2-/- mice were exposed to IR (4 Gy) and 12hr later cell death was examined using FACs analysis 7-AAD. Data presented is normalized to UT cells and bar graphs show means ± SEM. At least three independent experiments using one mouse per group were performed. ns: not statistically significant. Mus81+/-: Mus81+/ex3-4/ex3-4.

Found at: doi:10.1371/journal.pgen.1001385.s007 (1.03 MB TIF)

**Figure S8** Reduced Expression Level of Survivin in MMC Treated Mus81+/+Chk2-/- B-Cells. Western blot analysis of LPS activated WT, Mus81+/ex3-4/ex3-4, Chk2-/- and Mus81+/ex3-4/ex3-4-Chk2-/- B-cells either untreated or MMC treated for 18hr and 24hr. The level of expression of survivin and β-actin proteins is shown. Mus81+/-: Mus81+/ex3-4/ex3-4.

Found at: doi:10.1371/journal.pgen.1001385.s008 (0.16 MB TIF)

**Table S1** Litter sizes and frequency of double mutants from interbreeding of Mus81+/+Chk2-/- mice or compound heterozygotes.

Found at: doi:10.1371/journal.pgen.1001385.s009 (0.04 MB DOC)

**Table S2** Mus81+/ex3-4/ex3-4-Chk2-/- females were born at the expected Mendelian ratio.

Found at: doi:10.1371/journal.pgen.1001385.s010 (0.03 MB DOC)

**Table S3** Spontaneous and MMC-induced chromosomal aberrations of activated Mus81+/ex3-4/ex3-4-Chk2-/- B-cells.

Found at: doi:10.1371/journal.pgen.1001385.s011 (0.07 MB DOC)

**Table S4** Spontaneous and MMC-induced chromosomal aberrations of activated Mus81+/ex3-4/ex3-4-Chk2-/- T-cells.

Found at: doi:10.1371/journal.pgen.1001385.s012 (0.07 MB DOC)
Table S5: Spontaneous chromosomal aberrations of Mus81−/−;ex5−/ex5-Chk2+/− primary MEFs.

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Conceived and designed the experiments: R Hakem, S El Ghamerani, A Pamidi, MJ Halaby, A Hakem. Performed the experiments: S El Ghamerani, A Pamidi, MJ Halaby, M Bohgaki, R Cardoso, L Li, S Venkatesan, S Sethu, A Hakem. Analyzed the data: R Hakem, S El Ghamerani, A Pamidi, MP Hande, A Hakem. Contributed reagents/materials/analysis tools: A Hirao, TW Mak, MP Hande. Wrote the paper: R Hakem, A Hakem.