DNA-PK Promotes DNA End Resection at DNA Double Strand Breaks in G0 cells

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Abstract

DNA double-strand break (DSB) repair by homologous recombination is confined to the S and G2 phases of the cell cycle partly due to 53BP1 antagonizing DNA end resection in G1 phase and non-cycling quiescent (G0) cells where DSBs are predominately repaired by non-homologous end joining (NHEJ). Unexpectedly, we uncovered extensive MRE11- and CtIP-dependent DNA end resection at DSBs in G0 mammalian cells. A whole genome CRISPR/Cas9 screen revealed the DNA-dependent kinase (DNA-PK) complex as a key factor in promoting DNA end resection in G0 cells. In agreement, depletion of FBXL12, which promotes ubiquitylation and removal of the KU70/KU80 subunits of DNA-PK from DSBs, promotes even more extensive resection in G0 cells. In contrast, a requirement for DNA-PK in promoting DNA end resection in proliferating cells at the G1 or G2 phase of the cell cycle was not observed. Our findings establish that DNA-PK uniquely promotes DNA end resection in G0, but not in G1 or G2 phase cells, and has important implications for DNA DSB repair in quiescent cells.

Introduction

DNA double-strand breaks (DSBs) are particularly deleterious lesions which, if left unrepaired, can lead to cell death, or if repaired aberrantly, can lead to oncogenic chromosomal translocations and deletions (Jackson and Bartek 2009). Eukaryotic cells utilize two main mechanisms of DSB repair: non-homologous end joining (NHEJ), where the broken DNA ends are ligated together with minimal processing of the DNA termini; and homologous recombination (HR), which uses a homologous sequence, usually on a sister chromatid, as a template for accurate DNA repair. Because HR relies on a homologous template for accurate repair, HR is mostly restricted to S and G2 phases of
the cell cycle when sister chromatids exist. On the other hand, cells can employ NHEJ in any phase of the cell cycle and it is the only option in quiescent (G₀) cells and G₁ phase cells (Scully et al. 2019).

Extensive DNA end resection of the broken DNA ends, which generates long tracts of 3’ ssDNA overhangs at DSBs, is a critical step in committing the cell to use HR to repair DSBs. DNA end resection is initiated by nucleases MRE11 and CtIP, and subsequently extended by nucleases including EXO1 and DNA2/BLM (Paull and Gellert 1998; Trujillo et al. 1998; Sartori et al. 2007; Gravel et al. 2008; Mimitou and Symington 2008; Zhu et al. 2008; Bunting et al. 2010). The 3’ ssDNA overhangs are quickly bound by the single-stranded binding protein trimer replication protein A (RPA) to stabilize and protect the ssDNA, and later in repair RPA is replaced by the RAD51 recombinase protein that leads to the homology search to find a homologous template to achieve accurate HR repair (Sugiyama and Kowalczykowski 2002; San Filippo et al. 2008; Wright et al. 2018). NHEJ is initiated by the KU70/KU80 heterodimer binding to broken DNA ends (Zahid et al. 2021). KU70/KU80 recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) which together form a complex called DNA-PK (Gottlieb and Jackson 1993; Hammarsten and Chu 1998). Once the DNA-PK complex is formed, the KU heterodimer translocates inwards along the DNA and DNA-PKcs remains at the DNA ends, undergoing activation via conformational changes mediated by autophosphorylation of the ABCDE cluster (Yaneva et al. 1997; Chen et al. 2021b). Recent cryo-EM structures of DNA-PK also implicate dimerization of DNA-PK as important in recruiting downstream NHEJ factors by bringing broken DNA ends together (Chaplin et al. 2021; Zha et al. 2021). In addition to autophosphorylation, DNA-PKcs phosphorylates members of the NHEJ
machinery, including the KU heterodimer, XRCC4, XLF, and Artemis (Bartlett and Lees-Miller 2018).

The critical bifurcation point in the choice to use HR or NHEJ to repair DSBs is the processing of broken DNA ends to form single-stranded 3’ DNA overhangs, which blocks NHEJ and commits the cell to HR (Symington and Gautier 2011). Therefore, DNA end resection is tightly regulated to prevent aberrant DNA end resection in G₀ and G₁ phase cells, where NHEJ is the major DSB repair pathway. Several factors have been identified as critical DNA end protection factors that limit resection of DNA DSBs including 53BP1, RIF1, and the Shieldin complex. The proposed mechanism of action of 53BP1 and its downstream effectors include acting as a physical barrier to protect DNA ends from nucleases and promoting DNA polymerase α activity to quickly fill in any resected ends (Dev et al. 2018; Mirman et al. 2018; Noordermeer et al. 2018; Setiaputra and Durocher 2019; Paiano et al. 2021). Additionally, KU70/KU80 has also been shown in budding yeast Saccharomyces cerevisiae to inhibit DNA end resection in G₁ and G₂ phases of the cell cycle, and in S phase in mammalian cells (Lee et al. 1998; Clerici et al. 2008; Shao et al. 2012).

While nuclease activity is largely limited in G₀/G₁ phase cells to prevent aberrant DNA end resection, evidence exists suggesting that nuclease-mediated DNA end processing occurs at some DSBs in G₀/G₁. For example, Artemis is required to open hairpin-sealed DNA ends generated during V(D)J recombination in lymphocytes (Menon and Povirk 2016). Additionally, DNA end resection has been observed in G₁ phase after DNA damage at complex DNA lesions (Averbeck et al. 2014; Biehs et al. 2017), suggesting that DNA end resection is not completely inhibited in the absence of sister
chromatids. To investigate what additional factors may regulate DNA end resection in cells lacking sister chromatids, we performed a genome-wide CRISPR/Cas9 screen for genes whose inactivation either increases or decreases RPA bound to chromatin after irradiation (IR) in G0-arrested murine cells. We discovered, unexpectedly, that KU70, KU80, and DNA-PKcs promote extensive DNA end resection in G0 cells, but not in G1 or G2 phases of the cell cycle.
Results

RPA associates with IR-induced DNA DSBs in G₀ cells:

Murine pre-B cells transformed with Abelson murine leukemia virus (termed abl pre-B cells hereafter) continuously proliferate in vitro and can be efficiently arrested in G₀, also referred to as the quiescent state, upon treatment with the abl kinase inhibitor imatinib (Figure S1A) (Bredemeyer et al. 2006; Chen et al. 2021a). To investigate how DNA end resection is regulated in G₀ cells, we used a flow cytometric approach to assay RPA bound to chromatin after detergent extraction of soluble RPA, as a proxy for ssDNA generated at DSBs after exposing cells to irradiation (IR) (Forment et al. 2012; Chen et al. 2021a). This assay was performed in abl pre-B cell lines deficient in DNA Ligase IV (Lig4⁻/⁻), to maximize our ability to detect chromatin-bound RPA at DSBs, given that completion of NHEJ is prevented in the absence of DNA Ligase IV. We also performed the analysis in Lig4⁻/⁻:53bp1⁻/⁻ abl pre-B cells which lack the DNA end protection protein 53BP1 and accumulate high levels of RPA on chromatin after IR (Chen et al. 2021a). In agreement with our previous work, we detected a high level of chromatin-bound RPA in G₀-arrested Lig4⁻/⁻:53bp1⁻/⁻ abl pre-B cells after IR, consistent with the role of 53BP1 in DNA end protection (Figure 1A). Surprisingly, we also observed RPA associated with chromatin after IR of G₀-arrested Lig4⁻/⁻ abl pre-B cells, although at lower levels than in Lig4⁻/⁻:53bp1⁻/⁻ abl pre-B cells (Figure 1A). Moreover, the increase in IR-induced chromatin-bound RPA does not require DNA Ligase IV deficiency as we were able to observe similar results using the RPA flow cytometric assay in wild-type (WT) abl pre-B cells arrested in G₀ (Figure S1B). These data indicate that extensive DNA end resection...
occurs at DSBs in G0 cells, despite the presence of the DNA end protection proteins 53BP1 and KU70/KU80.

To determine whether higher levels of chromatin-bound RPA in irradiated G0-arrested Lig4−/ abl pre-B cells is a result of DNA end resection, we depleted the nucleases that are required for the initiation of DNA end resection during HR in cycling cells. We found that the depletion of CtIP or MRE11 reduced the levels of RPA on chromatin in irradiated G0-arrested Lig4−/ abl pre-B cells (Figure 1B and S1C), indicating that the RPA we observe with our flow cytometric assay after IR is indeed a result of DNA end resection.

To determine whether the DNA end resection that we observed was unique to abl pre-B cells or not, we performed the RPA flow cytometric chromatin association assay in the human breast epithelial cell line MCF10A. We arrested the MCF10A cells in G0 by EGF deprivation (Chen et al. 2021a). Similar to Lig4−/ and WT abl pre-B cells in G0, we observed IR-induced chromatin-bound RPA in G0 MCF10A cells (Figure S1D), consistent with DNA end resection occurring in these cells at DSBs. RPA binding to ssDNA surrounding DSBs often form distinct nuclear foci that can be easily detected by immunofluorescence staining and microscopy analysis (Golub et al. 1998). Therefore, we performed immunofluorescence staining for RPA in EGF-deprived MCF10A cells. We observed discrete IR-induced RPA foci, consistent with the RPA associated with ssDNA accumulating at DNA damage sites (Figure 1C). Together, these results suggest that broken DNA ends are resected in a CtIP and MRE11-dependent manner, leading to RPA accumulation on ssDNA in G0 mammalian cells.
DNA end resection and RPA loading occurs at site-specific DSBs in G₀ cells:

As irradiation induces DNA base lesions and single-stranded DNA breaks in addition to DSBs, it could potentially complicate our analysis of DNA end processing at regions surrounding DSBs. Therefore, we investigated DSBs at specific locations in the mouse genome upon induction of the AsiSI endonuclease. We performed RPA chromatin immunoprecipitation sequencing (RPA ChIP-seq) after induction of AsiSI DSBs in G₀-arrested Lig4⁻/⁻ abl pre-B cells. We detected RPA binding adjacent to AsiSI DSBs, consistent with ssDNA generated by resection around DNA DSBs (Paiano et al. 2021) (Figure 1D and S1E). Moreover, the association of RPA with chromatin was strand specific around the DSBs, consistent with the 5'-3' nature of DNA end resection which generates 3' ssDNA overhangs (Paiano et al. 2021) (Figure 1D). To determine the extent of DNA end processing in G₀ cells, we performed END-seq (Canela et al. 2016; Wong et al. 2021) to directly measure DNA end resection at nucleotide resolution at AsiSI-induced DSBs. Using END-seq, we detected extensive DNA end resection in G₀-arrested Lig4⁻/⁻ abl pre-B cells at 4 and 8 hours after AsiSI DSB induction (Figure 1E). Together, these data indicate that in G₀-arrested cells, DNA ends are resected at DSBs induced by IR or site-specific endonucleases, generating ssDNA that is bound by RPA.

A CRISPR/Cas9 screen identifies the DNA-PK complex as promoting DNA end resection in G₀ cells:

To identify factors that influence DNA end resection in G₀ cells, we performed a genome-wide CRISPR/Cas9 screen in G₀-arrested Lig4⁻/⁻ abl pre-B cells 2 hours after irradiation to identify factors that either promote or impair DNA end resection (Figure 2A).
We isolated the 10% of cells with the lowest RPA (low RPA) and the 10% cells with the highest RPA (high RPA) staining intensity using our RPA flow cytometric assay followed by flow assisted cell sorting. We then amplified the guide RNAs (gRNAs) in these populations of cells and determined their frequencies using high throughput sequencing. gRNAs enriched in the low RPA staining population correspond to genes encoding proteins that normally promote DNA end resection, while gRNAs enriched in the high RPA population correspond to genes encoding proteins that normally impair resection. In this screen we identified several gRNAs enriched in the low RPA staining population to \textit{Rbbp8} which encodes the nuclease CtIP, and \textit{Nbn}, which encodes the NBN subunit of the MRE11-RAD50-NBN (MRN) complex, consistent with their established roles in promoting DNA end resection (Figure 2B). Unexpectedly, we also found gRNAs of \textit{Ku70}, \textit{Ku80}, and \textit{Prkdc} (the gene encoding DNA-PKcs) highly enriched in our low RPA population (Figure 2B). This suggested that DNA-PK may promote DNA end resection in G\textsubscript{0} cells, contrary to the established role of these factors in preventing DNA end resection in other phases of the cell cycle.

To validate the screen and determine if DNA-PK is required for DNA end resection, we generated \textit{Lig4}\textsuperscript{−/−}:\textit{Prkdc}\textsuperscript{−/−} abl pre-B cells that do not express DNA-PKcs by CRISPR/Cas9-mediated gene inactivation (Figure S2A). G\textsubscript{0}-arrested \textit{Lig4}\textsuperscript{−/−}:\textit{Prkdc}\textsuperscript{−/−} abl pre-B cells had lower levels of chromatin-bound RPA after IR compared to \textit{Lig4}\textsuperscript{−/−} abl pre-B cells (Figure 2C). DNA-PKcs and Ataxia-telangiectasia mutated (ATM) are two major serine/threonine kinases that are activated in response to DNA DSBs and share some overlapping functions due to similar substrate specificity (Blackford and Jackson 2017). Because DNA-PKcs but not ATM was identified in our screen, we wanted to determine if
the pro-resection activity in G₀-arrested cells is unique to DNA-PKcs or also shared by ATM. We treated G₀-arrested Lig4⁻/⁻ abl pre-B cells with the ATM inhibitor KU55933 or the DNA-PKcs inhibitor NU7441 before IR and performed flow cytometric analysis of IR-induced chromatin-bound RPA. In contrast to the consistent reduction in the levels of chromatin-bound RPA observed in G₀-arrested Lig4⁻/⁻ abl pre-B cells treated with DNA-PKcs inhibitor, ATM inhibition did not have a detectable effect on the levels of IR-induced binding of RPA in G₀-arrested Lig4⁻/⁻ abl pre-B cells (Figure 2D and S2B). The role of DNA-PK in promoting DNA end resection in G₀ is not limited to murine abl pre-B cells as we also observed a reduced number of IR-induced RPA foci in G₀-arrested human MCF10A cells upon inhibition of DNA-PKcs (Figure S2C). These results indicate that DNA-PKcs activity, but not ATM, uniquely promotes resection and RPA binding to damaged chromatin after IR in G₀ cells.

To directly observe if DNA-PKcs influenced DNA end resection at DSBs, we performed nucleotide resolution END-seq on G₀-arrested Lig4⁻/⁻ abl pre-B cells with and without DNA-PKcs inhibitor treatment before the induction of AsiS I DSBs. Consistent with our RPA flow cytometric assay results, DNA-PKcs inhibitor-treated G₀-arrested Lig4⁻/⁻ abl pre-B cells showed greatly reduced END-Seq signals distal to DSBs, consistent with limited DNA end processing when DNA-PK is inactivated (Figure 2E and S2D). These results demonstrate that DNA-PK activity promotes DNA end resection of DSBs in G₀ mammalian cells.

FBXL12 inhibits KU70/KU80-dependent DNA end resection in G₀ cells:
Given that DNA-PKcs promotes DNA end resection in G0 cells (Figure 2C, 2D, 2E), and that Ku70 and Ku80 were enriched in the low RPA loading population of cells in the CRISPR/Cas9 screen (Figure 2B), we determined whether Ku70/Ku80 may also promote resection in G0 cells. We generated Lig4−/−:Ku70−/− abl pre-B cells and measured DNA end resection using our RPA flow cytometric approach. Consistent with our observations in DNA-PK inhibitor-treated G0-arrested Lig4−/− abl pre-B cells and Lig4−/−:Prkdc−/− abl pre-B cells, the level of chromatin-bound RPA after IR was greatly reduced in G0-arrested Lig4−/−:Ku70−/− abl pre-B cells compared to Lig4−/− abl pre-B cells (Figure 3A and S3A). As such, the entire DNA-PK complex is required for DNA end resection in G0 cells.

KU70/KU80 is removed from DSBs via ubiquitylation, which has been shown to be mediated by E3 ligases including RNF138, RNF8, RNF126, and the SCF^{Fbxl12} complex (Postow et al. 2008; Feng and Chen 2012; Postow and Funabiki 2013; Ismail et al. 2015; Ishida et al. 2017). In agreement, gRNAs of Fbxl12, which encodes the substrate recognition subunit FBXL12 of the SCF^{Fbxl12} E3 ubiquitin ligase complex, were highly enriched in our screen in the high RPA staining cell population (Figure 2B), consistent with the idea that the persistent presence of KU70/KU80 at DSBs in cells lacking FBXL12 would lead to persistent DNA end resection. Indeed, we observed that in G0-arrested Lig4−/−:Fbxl12−/− abl pre-B cells, the level of IR-induced chromatin-bound RPA increased compared to Lig4−/− abl pre-B cells (Figure 3B and Fig S3B). Given the role of FBXL12 on limiting the levels of KU70/KU80 at broken DNA ends, we tested whether the increased DNA end resection phenotype in Lig4−/−:Fbxl12−/− abl pre-B cells depended on DNA-PK activity or the presence of the KU70/KU80 complex. Indeed, inhibition of DNA-PK with...
NU7441 (Figure 3C) and depletion of KU70 (Figure 3D and Fig S3C) in G0-arrested Lig4-/-:Fbxl12-/- abl pre-B cells prevented excessive accumulation of RPA on chromatin after IR. Our results suggest that the ability of DNA-PK to promote DNA end resection in G0 cells is regulated through maintaining proper levels of KU70/KU80 at DNA DSBs by the SCF\textsuperscript{Fbxl12} E3 ubiquitylation complex.

**DNA-PK uniquely promotes DNA end resection exclusively in G0 cells:**

KU70/KU80 have been shown to prevent DNA end resection in G1 and G2 phases in budding yeast and in S phase in mammalian cells but has not been examined in G0 cells (Lee et al. 1998; Clerici et al. 2008; Shao et al. 2012). Thus, we set out to determine whether DNA-PK-dependent DNA end resection is limited to G0 or can occur in other phases of the cell cycle. To this end, we compared the levels of IR-induced chromatin bound RPA in Lig4-/-, Lig4-/-:Prkdc-/- and Lig4-/-:Ku70-/- abl pre-B cells arrested in G0 by imatinib, arrested in G2 by the CDK1 inhibitor RO3306, and in G1 phase (cells with 2N DNA) in a proliferating population. In contrast to G0 cells, loss of DNA-PKcs (Lig4-/-:Prkdc-/-) did not reduce the levels of IR-induced chromatin-bound RPA in G2-arrested or cycling G1 phase cells (Figure 4A and S4A). Similar results were obtained when analyzing Lig4-/-:Ku70-/- abl pre-B cells (Figure 4B). The unique function of DNA-PK activity in promoting DNA end resection in G0-arrested cells was confirmed with END-seq analysis of AsiSI-induced DSBs in Lig4-/- abl pre-B cells arrested in G0 or G2 and treated with or without DNA-PKcs inhibitor. Whereas G0-arrested Lig4-/- abl pre-B cells treated with DNA-PKcs inhibitor exhibited significantly reduced END-seq signals in regions distal to the DSBs, the same treatment had little effect in cells arrested in G2 phase of the cell cycle (Figure
4C and S4B). These results suggest that DNA-PK distinctly promotes DNA end resection at DSBs in G₀ but not in other cell cycle phases.
Discussion

DNA end resection is one of the key events that determines whether cells utilize NHEJ, HR, or other repair pathways utilizing homologous sequences. During G₀ and G₁ phase of the cell cycle, NHEJ is the predominant DSB repair pathway and DNA end resection is largely limited compared to other phases of the cell cycle. However, in this study we revealed that DNA end resection dependent on CtIP and MRE11, which are required for resection in S and G₂ phases of the cell cycle, occurs at DSBs in G₀ mammalian cells (Figures 1B). In addition to CtIP and MRE11, we identified additional factors that promote resection in G₀ cells as components of the DNA-PK complex, including KU70, KU80 and DNA-PKcs, in a genome-wide CRISPR/Cas9 screen and showed that the kinase activity of DNA-PK is critical as resection of DSBs diminishes upon DNA-PKcs inhibitor treatment (Figures 2 and 3). Interestingly, we also found in our genome-wide CRISPR/Cas9 screen that inactivating FBXL12, the substrate recognition subunit of the SCF<sub>FBXL12</sub> E3 ubiquitin ligase complex, promotes extensive resection of DNA ends in G₀ cells (Figure 3B). As the SCF<sub>FBXL12</sub> E3 ubiquitin is thought to limit the abundance of the KU70/KU80 heterodimer (Postow and Funabiki 2013), our data are in line with the notion that loss of FBXL12 results in aberrant accumulation of KU70/KU80 at DSBs, and consequently elevated or prolonged activation of DNA-PK at DSBs which promotes resection in G₀ cells (Figure 5).

Why would resection occur in G₀ cells? Chemical modifications or secondary structures at DSBs have been identified as requiring DNA end processing to create a more accessible repair environment, which could presumably be the case at DSBs in G₀ cells (Weinfeld and Soderlind 1991). For example, Artemis is an endo and exonuclease
which is activated by DNA-PKcs and uses its nuclease activity to open DNA hairpins at coding ends, which is required for V(D)J recombination, and cleaves 3’ ssDNA overhangs during NHEJ (Ma et al. 2002; Ma et al. 2005). Though Artemis was not identified in our screen as having a role in G₀ DSB repair, it serves as an example of nuclease activity being critical for DSB repair outside of HR. Interestingly, DNA end resection has a role in recruiting anti-resection factors to limit extensive DNA end resection. The SHLD2 component of Shieldin binds ssDNA, suppresses RAD51 loading, and ultimately recruits 53BP1 to DSBs (Noordermeer et al. 2018). HELB, a 5’-3’ DNA helicase, binds to RPA and limits EXO1 and BLM-DNA2-mediated DNA end resection (Tkáč et al. 2016). Limited DNA end resection in G₀ cells could be important in preventing extensive DNA end resection. Altogether, we propose that DNA end resection in G₀ cells is likely not resulting in aberrant HR but may be required to create more accessible DNA ends and/or to recruit anti-resection factors.

Studies investigating the role of KU70/KU80 during DSB repair have found that KU70/KU80 protects DSBs from nuclease activity. For example, at HO endonuclease breaks in budding yeast, deletion of KU70/KU80 leads to ssDNA accumulation in G₁ cells and increased MRE11 recruitment to DSBs compared to wild-type cells (Lee et al. 1998; Clerici et al. 2008). Also in budding yeast, at inducible I-SceI DSBs, deletion of KU70 results in increased RFA1 foci formation in G₁, but deletion of NHEJ factor DNA Ligase IV leads to no defect in RFA1 foci formation compared to wild-type cells, indicating that KU70 itself, not NHEJ, is a barrier to DNA end resection (Barlow et al. 2008). In mammalian cells, complementation of KU70/KU80 knockout cells with a M. tuberculosis KU homolog persistently bound to DSBs in S phase results in reduced RPA and RAD51. 

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foci formation after IR (Shao et al. 2012). Contrary to these roles for KU70/KU80 in protecting DNA ends from nucleolytic attack, we found that in G₀ cells, KU70/KU80 promotes DNA end resection (Figure 3A and 4B). We hypothesize that KU70/KU80 promotes resection through recruitment and activation of DNA-PKcs at DSBs (Gottlieb and Jackson 1993), as we also found that DNA-PKcs inhibition and genetic deletion of Prkdc leads to more RPA on chromatin after IR and more DNA end resection in G₀ cells (Figure 2C-E, S2C, S2D, 4A, 4C). It is important to note that most studies establishing the role of KU70/KU80 in protecting DNA ends were performed in S. cerevisiae which do not have a homolog to DNA-PKcs. Therefore, we hypothesize that the function of DNA-PK promoting DNA end resection in G₀ cells may not be evolutionarily conserved. Moreover, previous studies in S. cerevisiae and mammalian cells establishing DNA-PK as a pro-NHEJ complex did not analyze G₀ cells. We found that DNA-PK does not promote DNA end resection in G₁ or G₂ phase cells, only in G₀-arrested cells, indicating that DNA-PK-dependent DNA end resection is unique to G₀, but is not contradictory to its anti-resection function in G₁ or G₂ phase cells (Figure 4). In G₀ cells, KU70/KU80 could protect some DNA ends, but after recruitment and activation of DNA-PKcs, the net effect is DNA end resection. Additional studies may elucidate how the balance between DNA end protection and DNA end resection is regulated in G₀.

ATM and DNA-PK have been shown to have some overlapping functions in DNA damage response and repair, including phosphorylation of H2A.X in response to IR and signal join formation during V(D)J recombination (Stiff et al. 2004; Zha et al. 2011). However, we find that this is not the case during DNA end resection in G₀ cells as DNA-PK promotes resection in G₀ cells, but ATM does not have a detectable impact (Figure
S2B). ATM has been implicated in promoting HR repair by phosphorylating CtIP and promoting KU removal from DSBs, as well as phosphorylating DNA-PKcs at single-ended DSBs to remove it from these breaks that require DNA end resection (Wang et al. 2013; Britton et al. 2020). DNA-PKcs autophosphorylation promotes HR by removing it from DSBs to allow nuclease access, but is typically associated with promoting NHEJ by phosphorylating Artemis, XRCC4, and XLF (Zhou and Paull 2013; Bartlett and Lees-Miller 2018). So while ATM often promotes DNA end resection and HR, it appears that DNA-PKcs could be acting in place of ATM to promote DNA end resection in G₀ cells. It is additionally possible that DNA-PKcs phosphorylates a unique substrate(s) in G₀ cells that promotes DNA end resection.

In summary, we provide here evidence that DNA-PK promotes DNA end resection uniquely in G₀ cells, and that this DNA end resection is counteracted by FBXL12. We speculate that some aspects of DSB repair in G₀ function differently than DSB repair in cycling cells, and future studies may reveal the mechanism and utility of these key differences.
Figure 1

A  

Lig4^{-/-}  Lig4^{-/-}:53bp1^{-/-}  

RPA  RPA  

-IR  +IR  -IR  +IR  

Lig4^{-/-} + gMre11 Lig4^{-/-} + ... IR IR  

0 10 20 30  

# of RPA foci/cell  

****  

B  

Lig4^{-/-} - gRNA  Lig4^{-/-} + gMre11  Lig4^{-/-} + gCtIP  

RPA  RPA  

-IR  +IR  -IR  +IR  

C  

DAPI  RPA  Merge  

- IR  + IR  

D  

chr 2  

5 kb  

+ strand  - strand  

+ strand  - strand  

+ strand  - strand  

AsiSI  RPA  RPA  

E  

chr 2  

5 kb  

+ strand  - strand  

+ strand  - strand  

+ strand  - strand  

AsiSI  RPA  RPA  

344 345 346 347
Figure 1. RPA is loaded onto ssDNA after DSBs in G\(_0\) mammalian cells

(A) Flow cytometric analysis of chromatin-bound RPA in G\(_0\)-arrested Lig4\(^{-/-}\) and Lig4\(^{-/-}\):53bp\(^{1/-}\) abl pre-B cells before and 3 hours after 20 Gray IR. Representative of three independent experiments. (B) Flow cytometric analysis of chromatin-bound RPA before and 2 hours after 15 Gy IR in G\(_0\)-arrested abl pre-B Lig4\(^{-/-}\) cells (left), Lig4\(^{-/-}\) cells depleted of Mre11 (middle), and Lig4\(^{-/-}\) cells depleted of CtIP (right). Representative of three independent experiments. (C) Representative images and quantification of IR-induced RPA foci from 3 independent experiments in G\(_0\)-arrested MCF10A cells before and 3 hours after 10 Gray IR. n=365 cells in No IR and n=433 cells in IR. Red bars indicate average number of RPA foci in No IR=0.96 and average number of RPA foci in IR=9.4 (**p<0.0001, unpaired t test). (D) RPA ChIP-seq tracks at AsiSI DSBs on chromosome 2, 5, and 4 at 4 hours (top) and 8 hours (bottom) after AsiSI endonuclease induction in G\(_0\)-arrested Lig4\(^{-/-}\) abl pre-B cells. (E) Representative END-Seq tracks showing resection at AsiSI DSBs at chromosome 2, 5, and 4 at 4 hours (top) and 8 hours (bottom) after AsiSI induction in G\(_0\)-arrested Lig4\(^{-/-}\) abl pre-B cells. END-seq data is representative from two independent experiments.
Figure S1

A) Cycling pre-B abl cells

B) STI treated pre-B abl cells

C) Lig4−/−
gCtip - + -
gMre11 - - +
CtIP
MRE11
GAPDH

D) WT MCF10A

E) Lig4−/− 4 hr  Lig4−/− 8 hr

Low High
Figure S1. RPA is loaded onto ssDNA after DSBs in G0 mammalian cells

(A) Flow cytometric analysis of cycling and STI treated Lig4−/− abl pre-B cells for BrdU content (y-axis) and DNA content (7AAD, x-axis). (B) Flow cytometric analysis of chromatin-bound RPA in wild-type G0-arrested abl pre-B cells before and 3 hours after 20 Gray IR. (C) Western blot of bulk CtIP and MRE11 knockout in Lig4−/− abl pre-B cells. (D) Flow cytometric analysis of chromatin-bound RPA loading in wild-type G0-arrested MCF10A cells before and 3 hours after 20 Gray IR. (E) Heat maps of RPA ChIP-seq results at top 200 AsiSI sites in G0-arrested Lig4−/− abl pre-B cells 4 hours (left) and 8 hours (right) after AsiSI-endonuclease induction.
Figure 2

A. **Lig4**−/− Abelson Cells

- Dox for 7 days
- Gene inactivation
- Gleevec for 48 hrs
- G0 Arrest
- 20 Gray IR
- Collect cells 2 hours post-IR
- Stain cells with anti-RPA and sort

B. **Table**

<table>
<thead>
<tr>
<th>Gene</th>
<th>RPA Low Fold Enrichment</th>
<th>Gene</th>
<th>RPA High Fold Enrichment</th>
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<td>Rbbp8</td>
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C. **Graph**

- **Lig4**−/−-IR
- **Lig4**−/−:Prkdc−/−-IR
- **Lig4**+/−IR
- **Lig4**−/−:Prkdc−/−+IR

D. **Graph**

- **Lig4**−/−-IR
- **Lig4**−/−+DNA-PK inhibitor
- **Lig4**+/−IR
- **Lig4**−/−+DNA-PK inhibitor

E. **Graphs**

chr 3, 4 hr
- + strand
- - strand
- DNA-PK inhibitor

chr 13, 4 hr
- + strand
- - strand
- DNA-PK inhibitor

chr 3, 8 hr
- + strand
- - strand
- DNA-PK inhibitor

chr 13, 8 hr
- + strand
- - strand
- DNA-PK inhibitor
Figure 2. A genome-wide gRNA screen identifies DNA-PK as a factor that promotes DNA end resection in G₀

(A) Schematic of genome-wide guide RNA screen for factors promoting (low RPA) or inhibiting (high RPA) chromatin-bound RPA loading 2 hours after 20 Gray IR in G₀-arrested Lig4⁻/⁻ abl pre-B cells. (B) Fold enrichment of selected guide RNAs in low RPA high RPA populations. (C) Flow cytometric analysis of chromatin-bound RPA in G₀-arrested Lig4⁻/⁻ and Lig4⁻/⁻:Prkdc⁻/⁻ abl pre-B cells before and 3 hours after 15 Gray IR. Data is representative of three independent experiments in two different cell lines. (D) Flow cytometric analysis of chromatin-bound RPA in G₀-arrested Lig4⁻/⁻ abl pre-B cells with and without 10 µm NU7441 (DNA-PKcs inhibitor) pre-treatment 1 hour before 20 Gray IR. Data is representative of three independent experiments in two different cell lines. (E) Representative END-seq tracks at chromosome 3 (left) and chromosome 13 (right) in G₀-arrested Lig4⁻/⁻ abl pre-B cells 4 hours (top) and 8 hours (bottom) after AsiSI DSB induction, with and without 10 µm NU7441 treatment.
**Figure S2**

**A**

DNA-PKcs

KAP1

**B**

Lig4<sup>-/-</sup>

Lig4<sup>-/-</sup>:Prkdc<sup>-/-</sup>

**C**

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**D**

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Figure S2. A genome-wide gRNA screen identifies DNA-PK as a factor that promotes DNA end resection in G₀.

(A) Western blot analysis of DNA-PKcs protein in Lig4⁻/⁻ and Lig4⁻/⁻:Prkdc⁻/⁻ abl pre-B cells. (B) Flow cytometric analysis of chromatin-bound RPA in G₀-arrested abl pre-B cells with and without 1 hour pre-treatment with 15 μm KU-55933 (ATM inhibitor), before and 3 hours after 20 Gray IR. (C) Representative images and quantitation from 3 independent experiments of IR-induced RPA foci in G₀-arrested MCF10A cells with and without 10 μm NU7441, before and 3 hours after 10 Gray IR. For No Inhibitor -IR condition, n=426, average number of RPA foci=0.34. For No Inhibitor +IR condition, n=389, average number of RPA foci=5.2. For DNA-PK inhibitor treated -IR condition, n=266, average number of RPA foci=0.66. For DNA-PK inhibitor treated +IR condition, n=441, average number of RPA foci=1.13. Red bar indicates mean number of RPA foci (**p=0.003). (D) Heat maps of END-seq at top 200 AsiSI DSBs with and without 10 μm NU7441 treatment in G₀-arrested Lig4⁻/⁻ abl pre-B cells 4 hours (left) and 8 hours (right) after AsiSI DSB induction.
Figure 3

A

- Lig4<sup>-/-</sup> -IR
- Lig4<sup>-/-</sup>:Ku70<sup>-/-</sup> -IR
- Lig4<sup>-/-</sup> +IR
- Lig4<sup>-/-</sup>:Ku70<sup>-/-</sup> +IR

B

- Lig4<sup>-/-</sup> -IR
- Lig4<sup>-/-</sup>:Fbxl12<sup>-/-</sup> -IR
- Lig4<sup>-/-</sup> +IR
- Lig4<sup>-/-</sup>:Fbxl12<sup>-/-</sup> +IR

C

- IR
- IR +DNA-PK inhibitor
- +IR
- +IR +DNA-PK inhibitor

D

- IR
- IR + gKu70
- +IR
- +IR + gKu70
Figure 3. FBXL12 inhibits KU70/KU80-promoted DNA end resection

(A) Flow cytometric analysis of chromatin-bound RPA in G0-arrested Lig4- abl pre-B cells vs Lig4-:Ku70- abl pre-B cells before and 3 hours after 20 Gray IR. Data is representative of three independent experiments in two different cell lines. (B) As in A, in G0-arrested Lig4- and Lig4-:Fbxl12- abl pre-B cells. Data is representative of three independent experiments in at least two different cell lines. (C). Flow cytometric analysis of chromatin-bound RPA in G0-arrested Lig4-:Fbxl12- abl pre-B cells with and without 10 μm NU7441 treatment, before and 3 hours after 20 Gray IR. Data is representative of three independent experiments in at least two different cell lines (D) Flow cytometric analysis of chromatin-bound RPA in G0-arrested Lig4-:Fbxl12- abl pre-B cells before and after KU70 knockout, before and 3 hours after 15 Gray IR. Data is representative of three independent experiments.
**Figure S3**

A

KU70

KAP1

B

FBXL12 reference sequence (gRNA sequence in red):
395th to 492nd nucleotide of exon 4
TGCCGGCCTTTCCGATGAGCATTGCAGGCGCCACCCGATTCGAGCGCTGCTCTGCTTT
GCTGGCCTGACCTACCGGTCCTGAGACGGGG

Point Mutation

Insertion

Allele 1:
TGCCGGCCTTTCCGGG (69 nt deletion) TCAGGAGACGGGG

Allele 2:
TGCCGGCCTTTCCGAGAG (41 nt deletion)
CTGCTGCTTGGGCGACCTACGGGTCACTGAGACGGGG

C

Lig4+/Fbxl12+/gKu70

KU70

GAPDH

KAP1
Figure S3. FBXL12 inhibits KU70/KU80-promoted DNA end resection

(A) Western blot analysis of KU70 protein in Lig4-/- and Lig4-/-:Ku70-/- abl pre-B cells.

(B) Gene sequence of Lig4-/-:Fbxl12-/- abl pre-B cell clones indicating deletions. (C)

Western blot analysis of KU70 protein in Lig4-/-:Fbxl12-/- abl pre-B cells.
Figure 4

A

G0 arrested  G2 arrested  G1 Cycling Cells

- Lig4<sup>−/−</sup> - IR
- Lig4<sup>−/−</sup>:Prkdc<sup>−/−</sup> - IR
- Lig4<sup>−/−</sup> + IR
- Lig4<sup>−/−</sup>:Prkdc<sup>−/−</sup> + IR

B

G0 arrested  G2 arrested  G1 Cycling Cells

- Lig4<sup>−/−</sup> - IR
- Lig4<sup>−/−</sup>:Ku70<sup>−/−</sup> - IR
- Lig4<sup>−/−</sup> + IR
- Lig4<sup>−/−</sup>:Ku70<sup>−/−</sup> + IR

C

G3 arrested

chr 1, 4 hr

+ strand  No inhibitor  Resection
- strand  DNA-PK inhibitor  Resection

chr 1, 8 hr

+ strand  No inhibitor  Resection
- strand  DNA-PK inhibitor  Resection

G2 arrested

chr 1, 4 hr

+ strand  No inhibitor  Resection
- strand  DNA-PK inhibitor  Resection

chr 1, 8 hr

+ strand  No inhibitor  Resection
- strand  DNA-PK inhibitor  Resection
Figure 4. DNA-PK mediates DNA end resection in G₀ but not G₁ or G₂

(A) Flow cytometric analysis of chromatin-bound RPA in Lig4⁻/⁻ and Lig4⁻/⁻:Prkdc⁻/⁻ abl pre-B cells arrested in G₀ (left), arrested in G₂ by 10 μm RO-3306 treatment for 16 hours and gated on 4N (middle), and G₁ cells gated on 2N DNA content in cycling cells (right), before and 3 hours after 20 Gray IR. Data is representative of three independent experiments in at least two different cell lines. (B) As in A in Lig4⁻/⁻ and Lig4⁻/⁻:Ku70⁻/⁻ abl pre-B cells. (C) Representative END-seq tracks in G₀ (left) and G₂-arrested (right, by 10 μm RO-3306 treatment for 16 hours) Lig4⁻/⁻ abl pre-B cells, with and without 10 μm NU7441 treatment on chromosome 1, 4 hours (top) and 8 hours (bottom) after AsiSI endonuclease induction.
Figure S4

A

Cycling Cells

G₂ Arreísted Cells

DNA Content

2N 4N

B

G₀ arrested

No Inhibitor DNA-PK inhibitor

4 hr

-3 (kb) ▲ +3 (kb) ▲ +3

AsiSI AsiSI AsiSI

8 hr

-3 (kb) ▲ +3 (kb) ▲ +3

AsiSI AsiSI AsiSI

G₂ arrested

No Inhibitor DNA-PK inhibitor

4 hr

-3 (kb) ▲ +3 (kb) ▲ +3

AsiSI AsiSI AsiSI

8 hr

-3 (kb) ▲ +3 (kb) ▲ +3

AsiSI AsiSI AsiSI

Low

High
Figure S4. DNA-PK mediates DNA end resection in G0 but not G1 or G2

(A) Flow cytometric analysis of DNA content (7AAD) in RO-3306 treated (right) and cycling cells (left) showing gating used for RPA flow cytometry analysis. (B) Heat maps of END-seq at top 200 AsiS I DSBs in G0-arrested Lig4−/− abl pre-B (left) and G2-arrested Lig4−/− abl pre-B (right) 4 hours (top) and 8 hours (bottom) after AsiS I DSB induction, with and without 10 μm NU7441 treatment.
Figure 5

G₀ Cells

- SCF complex
- FBXL12
- DNA-PKcs
- KU

- Normal end resection
- No DNA-PK
- No end resection
- No FBXL12
- Excess end resection
Figure 5. Model of DNA-PK-mediated DNA end resection in G₀ cells

Normally in G₀ at DSBs, the DNA-PK complex promotes DNA end resection. This resection is counteracted by FBXL12. Without DNA-PK, there is no DNA end resection in G₀. Without FBXL12, DNA-PK persists at DSBs which leads to more extensive DNA end resection.
## Materials and Methods

### Key Resources Table

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### Cell Lines and Maintenance

Abelson virus-transformed pre-B cell lines were maintained in DMEM (Thermo Fisher #11960-077) supplemented with 10% fetal bovine serum, 1% Penicillin-Streptomycin, 2 mM glutamine, 1 mM sodium pyruvate, 1X nonessential amino acids, and 0.4% betamercaptoethanol at 37°C with 5% CO₂. MCF10A cells were maintained in DMEM/F12 (Gibco, #11330032), 5% horse serum, 20 ng/mL EGF, 0.5 μg/mL hydrocortisone, 100 ng/mL cholera toxin, 10 μg/mL insulin, and 1% Penicillin-Streptomycin at 37°C with 5%
CO₂. 293T cells were maintained in DMEM (Corning, #10-013-CM) supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin at 37°C with 5% CO₂.

Lig4⁻/⁻ abl pre-B cells contain pCW-Cas9 (addgene, #50661) which expresses cas9 under a doxycycline-induced promoter. To generate single cell clones of Lig4⁻/⁻:53bp1⁻/⁻, Lig4⁻/⁻:Ku70⁻/⁻, Lig4⁻/⁻:Prkdc⁻/⁻, and Lig4⁻/⁻:Fbxl12⁻/⁻, guide RNAs (gRNAs) against each gene were cloned into pKLV-U6gRNA-EF(BbsI)-PGKpuro2ABFP (addgene, #62348) modified to express human CD2 as a cell surface marker. Lig4⁻/⁻ abl pre-B cells were grown in 3 μg/mL of doxycycline for 2 days and then nucleofected with the pKLV-gRNA plasmid using a Lonza Amaxa Nucleofector. The next day, cells were magnetically selected for human CD2 cell surface expression, and selected cells were grown in 3 μg/mL doxycycline overnight. Serial dilution in 96 well plates was used to isolate single cells. After cell growth, potential clones were confirmed to have the gene of interest knocked out by Sanger sequencing or western blotting.

**Bulk gene inactivation**

gRNAs against Mre11, CtlP, and Ku70 were cloned into pKLV-U6gRNA-EF(BbsI)-PGKpuro2ABFP (addgene, #62348). 293T cells were transfected with the pKLV-gRNA plasmid along with lentiviral packaging and lentiviral envelope plasmids. 3 days post-transfection, supernatant containing pKLV-gRNA lentivirus was filtered with a 0.45 micron filter. Lig4⁻/⁻ cells were resuspended in the filtered viral supernatant supplemented with 5 μg/mL polybrene (Sigma-Aldrich, #S2667) in 6-well plates and centrifuged at 1,800 RPM for 1.5 hrs at room temperature. After spin infection, virally transduced cells were supplemented with DMEM containing 3 μg/mL doxycycline for 3
days before flow cytometry-assisted cell sorting or magnetic-assisted cell sorting based on hCD2 cell surface expression.

**Flow Cytometry**

Abl pre-B cells were arrested in G₀ using 3 μM imatinib (Selleck Chemicals, #S2475) for 48 hours. MCF10A cells were arrested in G₀ by withdrawing EGF for 48 hours. To arrest cells in G₂, abl pre-B cells were treated with 10 μM RO-3306 (Selleck Chemicals, #S7747) overnight. For experiments analyzing DNA-PKcs and ATM inhibition, 10 μM NU7441 (Selleck Chemicals, #S2638) or 15 μM KU-55933 (Selleck Chemicals, #S1092) was added 1 hour prior to irradiation. After irradiation with 20 Gray, cells were allowed to recover for 3 hours. Cells were then pre-extracted with 0.05% Triton-X (STI-treated abl pre-B cells), 0.2% Triton-X (proliferating abl pre-B cells) or 0.5% Triton-X (MCF10A cells) in PBS and fixed with BD Cytofix/Cytoperm solution (BD Biosciences, #554722) containing 4.2% formaldehyde. Fixed cells were stained with anti-RPA32 (Cell Signaling Technology, #2208S) for 2 hours at room temperature, and then treated with a fluorescent conjugated secondary antibody (BioLegend, #405416 or BioLegend, #405418) for 1 hour at room temperature. 7-AAD was added to each sample to stain for DNA content. Cells were analyzed using a BD LSRII Flow Cytometer or a BD FACSCelesta and flow cytometry results were further analyzed using FlowJo.

**Nuclear RPA Immunofluorescence Staining**

60,000 G₀-arrested MCF10A cells grown on cover slips were irradiated with 10 Gray and then allowed to recover for 3 hours at 37°C with 5% CO₂. Cells were then washed with PBS containing 0.1% Tween-20 (PBST), pre-extracted using cold 0.5% Triton-X in PBS for 5 minutes, fixed with 4% formaldehyde for 15 minutes, and blocked in 3% BSA-
PBST for 1 hour at room temperature. Cells were incubated overnight at 4°C in primary antibody (anti-RPA32, Cell Signaling Technology, #2208). diluted in 3% BSA-PBST

Samples were then washed 3x with PBST, incubated with secondary antibody diluted in 3% BSA (Alexa Fluor 594 Goat anti-Rat IgG, BioLegend, #405422) in the dark for 1 hr at room temperature, washed 3x with PBST, and mounted in Prolong Gold Antifade Mountant with DAPI (Life Technologies, #P-36931). Images were taken using a Biotek Lionheart Automatic Microscope and foci quantification was performed using Biotek Gen5 software.

**END-Seq and RPA ChiP-Seq**

Sequencing assays were performed in *Lig4⁻/⁻* abl pre-B cells after arrest in G₀ with imatinib for 24 hours or arrest in G₂ with RO-3306 for 12 hours, then treated with doxycycline for 24 hours followed by tamoxifen treatment for 4 or 8 hours to induce AsiSI breaks in the nucleus. End-seq was performed as previously described (Canela et al. 2016; Chen et al. 2021; Wong et al. 2021). Cells were embedded in agarose plugs, lysed, and treated with proteinase K and RNase A. The DNA was then blunted with ExoVII (NEB) and ExoT (NEB), A-tailed, and ligated with a biotinylated hairpin adaptor. DNA was then recovered and sonicated to a length between 150 and 200 bp and biotinylated DNA fragments were purified using streptavidin beads (MyOne C1, Invitrogen). The DNA was then end-repaired and ligated to hairpin adaptor BU2 and amplified by PCR. RPA single-strand DNA sequencing was performed as previously described (Paiano et al. 2021). Cells were fixed in 1% formaldehyde (Sigma, F1635) for 10 min at 37°C, quenched with 125 mM glycine (Sigma), washed twice with cold 1× PBS. After centrifugation, pellets were frozen on dry ice, and stored at −80°C. Sonication, immunoprecipitation, and library preparation
were performed as previously detailed (Tubbs et al. 2018). Before immunoprecipitation, sheared chromatin was precleared with 40 µL of Dynabeads Protein A (Thermo Fisher) for 30 min at 4°C. Sheared chromatin was enriched with 10 µg of anti-RPA32/RPA2 antibody (Abcam, ab10359) on Dynabeads Protein A overnight at 4°C. During library preparation, kinetic enrichment of single-strand DNA was performed by heating sheared DNA for 3 min at 95°C and allowing DNA to return to room temperature (Tubbs et al. 2018). All END-seq and RPA ChIP-seq libraries were collected by gel purification and quantified using qPCR. Sequencing was performed on the Illumina NextSeq500 (75 cycles) as previously described (Chen et al. 2021a).

**Genome Alignment and Visualization**

END-seq and RPA ChIP-seq single-end reads were aligned to the mouse genome (mm10) using Bowtie v1.1.2 (Langmead et al. 2009) with parameters (-n 3 -k 1 -l 50) for END-seq and (-n 2 -m 1 -l 50) for RPA ChIP-seq. All plots or analysis were done for the top 200 AsiSI sites determined by END-seq. Alignment files were generated and sorted using SAMtools (Li et al. 2009b) and converted to bedgraph files using bedtools genomecov (Quinlan and Hall 2010) following by bedGraphToBigWig to make a bigwig file (Kent et al. 2010). Visualization of genomic profiles was done by the UCSC genome browser (Kent et al. 2002) and normalized to present RPM. Heat maps were produced using the R package pheatmap.

**Guide RNA Library Screen**

144 million *Lig4*−/− abl pre-B cells were transduced with a viral tet-inducible guide RNA library (Pooled Library #67988, Addgene) containing 90,000 gRNAs targeting over 18,000 mouse genes. 3 days post-infection, cells were sorted for gRNA vector
expression using a BD FACSARia flow assisted cell sorter. The next day, sorted cells were treated with 3 µg/ml doxycycline to induce gRNA expression. 7 days later, cells were treated with Gleevec to arrest cells in G0. 48 hours later, cells were irradiated with 20 Gray and allowed to recover for 2 hours. After collection, cells were permeabilized, fixed, and stained with anti-RPA32 in the same manner as described in the Flow Cytometry section. After staining, the top 10% and bottom 10% of RPA stained cells were collected using flow assisted cell sorting and genomic DNA was extracted. An Illumina sequencing library was generated using two rounds of PCR to amplify the gRNA and add a barcode, then purified PCR products containing the barcoded enriched gRNAs were sequenced on an Illumina HiSeq2500. Sequencing data were processed as previously described (Chen et al. 2021a).

Western Blotting

The following antibodies were used for western blot analysis: CtIP (gift from Dr. Richard Baer, [Columbia University, New York], 1:1000), MRE11 (Novus Biologicals, NB100-142, 1:2000), GAPDH (Sigma, G8795, 1:10,000), DNA-PK (Invitrogen, MA5-32192, 1:1000), KAP1 (Genetex, GTX102226, 1:2000), KU70 (Cell Signaling Technology, #4588, 1:1000).

Plasmid Constructs

pCW-Cas9 was a gift from Eric Lander and David Sabatini (Addgene plasmid #50661) (Wang et al. 2014). pKLV-U6gRNA(BbsI)-PGKpuro2ABFP was a gift from Kosuke Yusa (Addgene plasmid #50946) (Koike-Yusa et al. 2014). Mouse Improved Genome-wide Knockout CRISPR Library v2 was a gift from Kosuke Yusa (Addgene #67988) (Tzelepis et al. 2016).
Acknowledgements

The authors thank Chitra Mohan for designing the model graphic and Yinan Wang for performing the bioinformatics for the high throughput screen. We thank the Weill Cornell Flow Cytometry Core for flow cytometry and thank the Weill Cornell Epigenomics Core for providing advice and performing the sequencing for the high throughput screen. JKT is supported by NIH R35 GM139816 and R01 CA95641. BPS is supported by NIH R01 AI047829 and R01 AI074953. FCF is supported by NIH F31 CA239442. JKT and BPS were also supported by the Starr Cancer Consortium and Emerson Collective Cancer Research Fund.
References


