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**Re: Ms EMBO/2004/47425**

Dear Valérie:-

We thank you and the referees for the overall positive review of our revised manuscript. We have fully addressed the minor revisions, as outlined below:

**Response to reviewers:**

*1. Control for γ-H2AX foci*
It was clear from the comments of Reviewers 1 & 3 that the conclusions that we draw from the γ-H2AX focus formation assay (now Fig. 8D) would be strengthened by determining whether apoptosis contributes to the formation of DSBs (and hence, γ-H2AX foci) in irradiated XR-1 cell lines. Our new data (Fig. 8E) now clearly indicates that the XR-1 cell lines that we used to measure the rates of γ-H2AX foci following X-irradiation do not show appreciable levels apoptosis as measured by PARP cleavage (detected as a 89 kDa PARP cleavage fragment on immunoblots). PARP cleavage is a hallmark of apopotosis and as a positive control for the assay, we examined the effect of irradiation on the murine pre-B cell line, BaF3. In this cell line, by contrast to the XR-1 cell lines, irradiation triggers a robust apoptotic response.

**These results therefore indicate that apoptosis is not a confounding factor in our γ-H2AX assays, strengthens the validity of the γ-H2AX assay as a mean to measure DSB repair kinetics in XR-1 cells and further supports our conclusion that the Xrcc4 Thr233 residue, and therefore the Xrcc4-PNK interaction, impacts on the DSB repair kinetics in vivo**.

*2- Transfection of linear fragments as mean to examine NHEJ*
As another means of providing further in vivo data supporting a difference in DSB repair, Referee #2 suggested an in vivo plasmid end-joining assay. Although this type of assay has been widely used in mammalian cells to study NHEJ, it does not appear to detect differences in DSB repair in rodent cell lines lacking NHEJ components (the XR-1 cell line is a rodent cell line). Therefore, the experiment suggested by Reviewer #2 is not

amenable to our system. I would like to cite an excerpt from a recent paper by the Jeggo group addressing this issue and concurring with our observations:

> *"A plasmid-based assay has been widely used to characterize the process of V(D)J recombination in mammalian cells. It has been shown to be dependent upon the known NHEJ components and reflects events that occur at the endogenous loci . In contrast, the use of an in vivo plasmid assay to examine DSB rejoining in rodent cell lines lacking NHEJ components failed to demonstrate any significant defect."* Smith et al. Nucleic Acids Research, 2003, Vol. 31, No. 8 **2157-2167**

*3- PNK stimulates end-joining of both 5'-OH and 5'-phosphorylated ends in vitro (Supp Fig. 3)*
In response to the Referee #1 who asked why we did not include the Supplementary Figure 3 in the main manuscript: we felt that despite being a very interesting finding, it does not alter our main conclusion that the Xrcc4-PNK interaction is required for in vitro end-joining of dephosphorylated DNA ends. Therefore, given the current manuscript length restrictions, we felt that this result could be placed in the Supplementary Results section. However, we now discuss the implications of this result in greater length in the Discussion but have elected to leave the figure as Supplementary Figure 3 where it will be available to any reader interested in examining the primary data.

*4- Provision of a loading control for revised Fig. 8A*
We have now included an anti-actin immunoblot as a protein loading control to supplement the revised Fig. 8A as recommended by Referee #3. The immunoblot is now a panel of its own in Figure 8B.

You will also find enclosed hard and electronic copies of the manuscript, prepared as per the EMBO Journal guidelines. I hope the manuscript can now be on course for a rapid publication. On behalf of all the authors, we sincerely thank you for considering our manuscript.

Sincerely,

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