DNA

MDPI

# Exploring the Roles of Different DNA Repair Proteins in Short Inverted Repeat Mediated Genomic Instability: A Pilot Study

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## Supplementary Materials

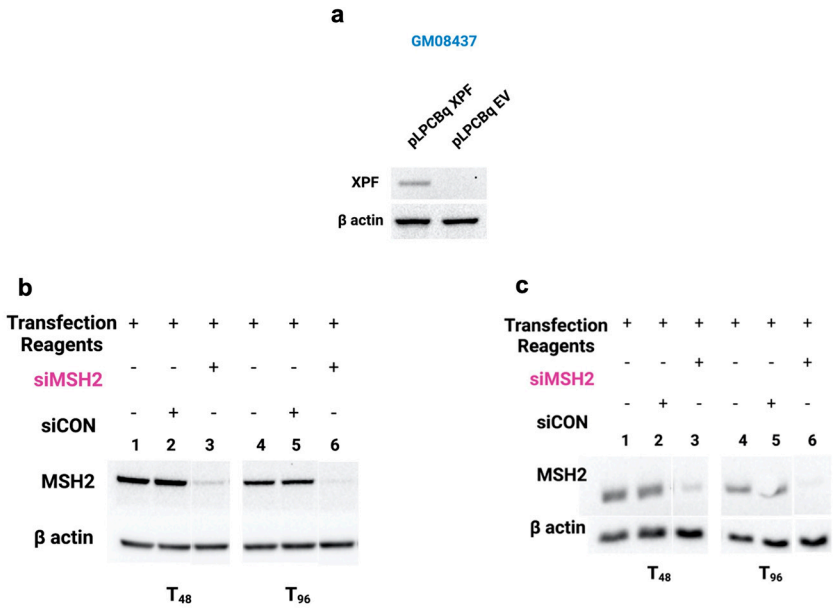
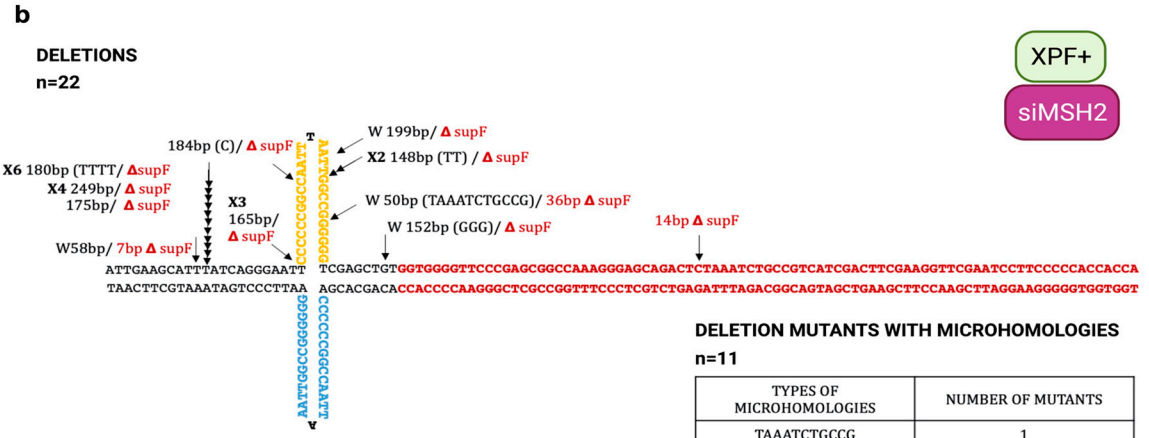



**Figure S1.** Expression of XPF in XPF proficient and deficient cell lines and knockdown of MSH2 in XPF-proficient cells**.** a) Representative image of a Western blot to verify the expression of XPF in the XPF-deficient (GM08347-pLPCBq EV) and XPF-proficient (GM08347-pLPCBq XPF) human cell lines using an anti-XPF antibody and an anti-β-actin antibody as a loading control. b) Representative image of a Western blot to confirm the depletion of MSH2 by siRNA knockdown in XPF-proficient cells using an antibody against MSH2 and an antibody against β-actin as a loading control. Samples were collected at the time of plasmid transfection at 48 hours ($T_{48}$) and at the conclusion of the assay at 96 hours ($T_{96}$). Western blots were quantified by normalizing the MSH2 expression to the β-actin expression and then normalizing to the mock treatment. Experiments were done in triplicate, and error bars represent the mean + SD. c) Representative image of a Western blot to confirm the depletion of MSH2 by siRNA knockdown in XPF-deficient cells using an antibody against MSH2 and an antibody against β-actin as a loading control. Samples were collected at the time of plasmid transfection at 48 hours ($T_{48}$) and at the conclusion of the assay at 96 hours ($T_{96}$). Western blots were quantified by normalizing the MSH2 expression to the β-actin expression and then normalizing to the mock treatment. (Image Created with Biorender.com) (Adapted from [1], corresponding to reference 45 in the main text).


**DELETION MUTANTS WITH MICROHOMOLOGIES**

**n=15**

| TYPES OF MICROHOMOLOGIES | NUMBER OF MUTANTS |
|---|---|
| AAGGATTT | 1 |
| CCCCGGCC | 1 |
| GGTCGAGG | 1 |
| AAATTGG | 1 |
| CGGCCAA | 1 |
| AGGGA | 1 |
| ATT | 1 |
| GA | 2 |
| TT | 3 |
| T | 1 |
| T | 1 |
| A | 1 |




**DELETION MUTANTS WITH MICROHOMOLOGIES**

**n=11**

| TYPES OF MICROHOMOLOGIES | NUMBER OF MUTANTS |
|---|---|
| TAAATCTGCCG | 1 |
| TTTT | 6 |
| GGG | 1 |
| TT | 2 |
| C | 1 |

**Figure S2.** Schematic distribution of short IR-induced deletions from human XPF-proficient cells treated with: a) siCON; b) siMSH2. Distribution of breakpoint junctions depicted along a ~200-bp region of the IR plasmid. Regions in yellow and blue are the 29-bp cruciform-forming short IR sequences, and the region in red is the *supF*-reporter gene. Black arrowheads represent the location and frequency of breakpoint junctions associated with the short IR-induced deletions. The values in black represent the size of the deletion of the mutation-reporter vector as measured from the black arrowhead and including the region right of the arrowhead mark. The values in red represent the status of the *supF* reporter deletion. The types of microhomologies observed and the corresponding

number of mutants are tabulated (Image Created with Biorender.com) (Adapted from [1], corresponding to reference 45 in the main text).

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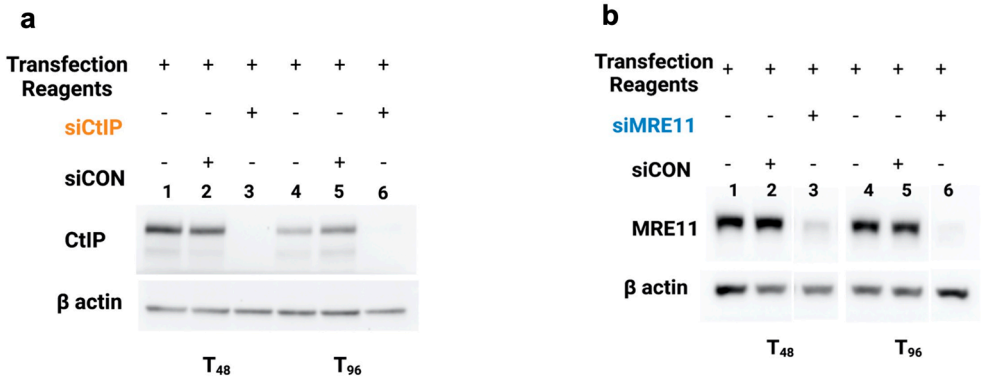


**Figure S3.** Knockdown of CtIP and MRE11 in XPF-proficient cells**.** a) Representative image of a Western blot to confirm the depletion of CtIP by siRNA knockdown in XPF-proficient cells using an anti-CtIP antibody and an anti-β-actin antibody as a loading control. Samples were collected at the time of plasmid transfection at 48 hours ($T_{48}$) and at the conclusion of the assay at 96 hours ($T_{96}$). Quantitation of Western blots occurred by normalizing the CtIP expression to the β-actin expression and then normalizing to the mock treatment. Experiments were done in triplicate, and error bars represent mean ± SD. b) Representative image of a Western blot to confirm the depletion of MRE11 by siRNA knockdown in XPF-proficient cells using an antibody against MRE11 and an antibody against β-actin as a loading control. Samples were collected at the time of plasmid transfection at 48 hours ($T_{48}$) and at the conclusion of the assay at 96 hours ($T_{96}$). Quantitation of Western blots occurred by normalizing the MRE11 expression to β-actin expression and then normalizing to the mock treatment. Experiments were done in triplicate, and error bars represent mean ± SD. (Image Created with Biorender.com) (Adapted from [1], corresponding to reference 45 in the main text).

## References

1. Mandke, P. Elucidation of factors affecting short inverted repeat-induced genomic instability in eukaryotic systems. Dissertation, University of Texas at Austin, Austin, TX, **2022**.