Stem cell protein Piwil2 modulates chromatin modifications upon cisplatin DNA damage

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Supplementary Data

Material and methods

Real-time quantitative RT-PCR. Total RNA was purified from MEFs using Trizol (Invitrogen). The cDNA was generated by reverse transcription using Superscriptase III (Invitrogen) and oligo (dT) in a 20 µl reaction containing 1 µg of total RNA. An aliquot of 0.5 µl cDNA was used in each 20 µl PCR reaction, using Applied Biosystem's Power SYBR Green PCR Master Mix and the reactions were run on an ABI 7500 Fast Real-Time PCR system. The following primers were used: XPA, forward, 5'- AAC CAA GAC AGA AGC GAA GC -3'; reverse, 5'- GCC CGC TTT ACC ACT AGA CA -3'; XPC, forward, 5'- ATC ATT CCA ATT CGC TTT ACC AA -3'; reverse, 5'- GTT CCG ATG AAC CAC TTT ACC AG -3'; XPF, forward, 5'- CAG GGC ACA CAG AAT CAT TG -3'; reverse, 5'- ACA AAA AGG TTC CGC ATC AC -3'; XPG, forward, 5'- ATT TAC AAC CGC GAG TCA CC -3'; reverse, 5'- CCC TCC ACC TCA CAT TCA CT -3'; ERCC1, forward, 5'- CCA CAA CCT CCA TCC AGA CT -3'; reverse, 5'- CTG CTG GGG ATC TTT CAC AT -3'; DDB2, forward, 5'- GCC GAT ACC CAG ATC CTA ATC TT -3'; reverse, 5'- ACA CAT CAT CTT CCC TGA GCT TC -3'; and GAPDH, forward, 5'- TTG TGA TGG GTG TGA ACC ACG A -3'; reverse, 5'- AGC CCT TCC ACA ATG CCA AAG T -3'.

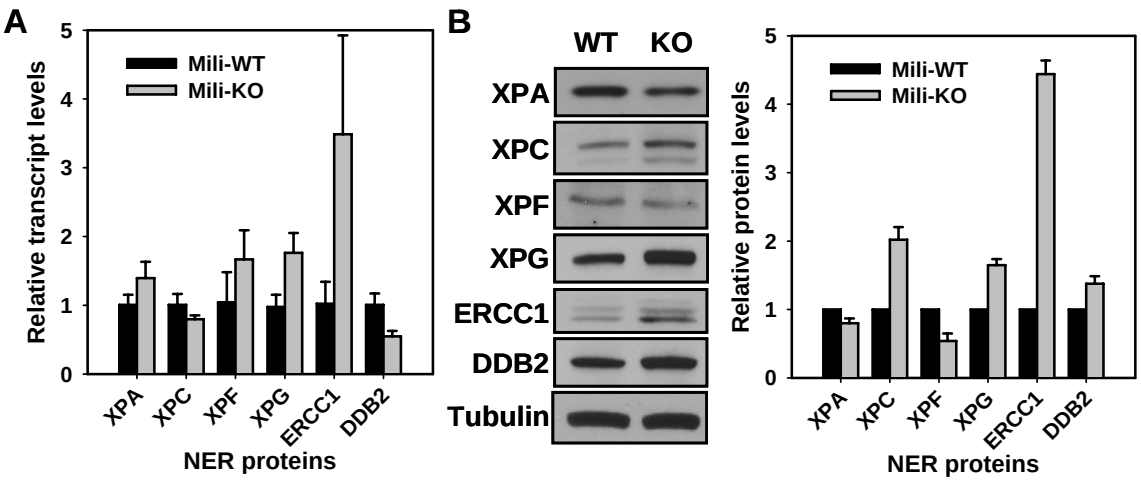

Figure S1. The expression of NER factors in Mili-WT and Mili-KO MEFs. The expression of various NER-related factors in Mili-WT and Mili-KO MEFs were detected at both mRNA level (A) and protein level (B) using Real-time PCR and Western blotting, respectively.

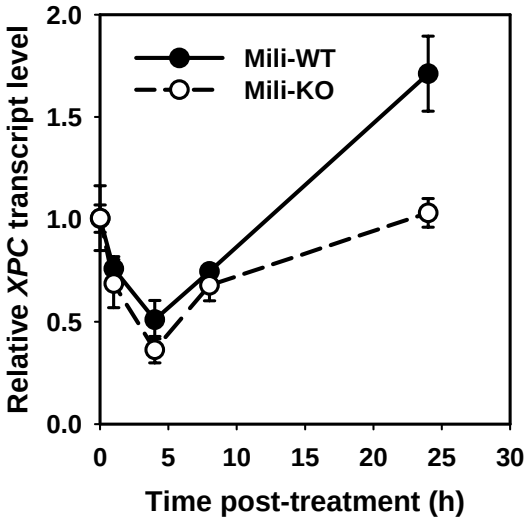

Figure S2. **Mili is required for the transcriptional activation of *XPC* following cisplatin treatment.** Mili-WT and Mili-KO MEFs were treated with 80 μM cisplatin for 1 h, further cultured in drug-free medium for the indicated time periods. Total RNA was isolated and subjected to Real-time RT-PCR to detect the transcript level of XPC.