Phosphorylation of xeroderma pigmentosum group C regulates ultraviolet-induced DNA damage repair

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SUPPLEMENTARY METHODS

Plasmids and site-directed mutagenesis

The following primers were used to generate the mutations: S61A sense 5'-TGAACCCCCAGGATGAGCGCAGCCTCTTTTCCTC-3' and S61A antisense 5'-GAGGAAAAGAGGCTGCGCTCATCCTGGGGGTTCA-3', S94A sense 5'-AAAGGATGAAGCCCTCGCCGATGGGGATGACCTC-3' and S94A antisense 5'-GAGGTCATCCCCATCGGCGAGGGCTTCATCCTTT-3', T169A sense 5'-CTGCTCTGGCGCTTCAATCTCTATCTCCACTGG-3' and T169A antisense 5'-CCAGTGGAGATAGAGATTGAAGCGCCAGAGCAG-3', S399A sense 5'-GCAAGCCCTCCTCCGCCGAGGAAGATGAGG-3' and S399A antisense 5'-CCTCATCTTCCTCGGCGGAGGAGGGCTTGC-3', S397A sense 5'-CGGAGCAAGCCCGCCTCCAGCGAGG-3' and S397A antisense 5'-CCTCGCTGGAGGCGGGCTTGCTCCG-3', S884A sense 5'-CCTCCTCTTCATCAGCAGAGAGTCCACCTCC-3' and S884A antisense 5'-GGAGGTGGACTCTCTGCTGATGAAGAGGAGG-3', S883A sense 5'-CCTCTTCATCAGAAGCGAGTCCACCTCCTGC-3' and S883A antisense 5'-GCAGGAGGTGGACTCGCTTCTGATGAAGAGG-3', S892A sense 5'-GCTTCTGCTTGAGCGCTGGTCCCCTCC-3' and S892A antisense 5'-GGAGGGGACCAGCGCTCAAGCAGAAGC-3', S892D sense 5'-GCCGCTTCTGCTTGATCGCTGGTCCCCTCCTC-3' and S892D antisense 5'-GAGGAGGGGACCAGCGATCAAGCAGAAGCGGC-3'.

SUPPLEMENTARY FIGURES LEGENDS

Supplementary Figure S1. Role of XPC phosphorylation at S61, T169, S397, S399, S883, and S884 in CPD repair. **(A)** Immunoblot analysis of XPC and GAPDH in XPC^{Null} cells expressing pLenti vector or pLenti-XPC (WT or Ser/Thr→ Ala mutant) constructs. **(B)** Slot blot analysis of the levels of CPD at the indicated times post-UVB (20 mJ/cm^2) in XPC^{Null} cells expressing pLenti vector or pLenti-WT XPC. Methylene blue staining was used for loading control. **(C)** Quantification of percentage (%) of CPD repair from (B). *, $P < 0.05$,

compared with vector, Student's *t*-test. **(D)** Slot blot analysis of the levels of CPD in sham control and UVB (20 mJ/cm^2) irradiated XPC^{Null} cells expressing pLenti vector or pLenti-WT XPC. Methylene blue staining was used for loading control. **(E-J)** Slot blot analysis of the levels of CPD at the indicated times post-UVB (20 mJ/cm^2) in XPC^{Null} cells expressing pLenti-XPC WT and mutant constructs S397A (E), S399A (F), T169A (G), S883A (H), S884A (I), S61A (J). Methylene blue staining was used for loading control. The results were obtained from three independent experiments.

Supplementary Figure S2. Role of XPC phosphorylation at S61, T169, S397, S399, S883, and S884 in 6-4PP repair. **(A)** Slot blot analysis of the levels of 6-4PP at the indicated times post-UVB (20 mJ/cm^2) in XPC^{Null} cells expressing pLenti vector or pLenti-WT XPC. Methylene blue staining was used for loading control. **(B)** Quantification of percentage (%) of 6-4PP-repair from (A). ***, $P \leq 0.001$, compared with vector, Student's *t*-test. **(C)** Slot blot analysis of the levels of 6-4PP in sham control and UVB (20 mJ/cm^2) irradiated XPC^{Null} cells expressing pLenti vector or pLenti-WT XPC. Methylene blue staining was used for loading control. **(D-H)** Slot blot analysis of the levels of 6-4PP at the indicated times post-UVB (20 mJ/cm^2) in XPC^{Null} cells expressing pLenti-XPC WT and mutant constructs T169A (D), S397A (E), S883A or S399A (F), S884A (G), S61A (H). Methylene blue staining was used for loading control. The results were obtained from three independent experiments.

Supplementary Figure S3. Effect of pharmacological CK2 inhibition on CPD repair and UVB regulation of CK2 levels. **(A)** HaCaT cells were pretreated with vehicle or CK2 inhibitor CX-4945 (5μM) for 1 h, exposed to UVB (20 mJ/cm^2), and incubated for 30 min. The levels of XPC and GAPDH were analyzed by immunoblot assay. **(B)** Slot blot analysis of the levels of CPD at the indicated times post-UVB (20 mJ/cm^2) in HaCaT cells pretreated with vehicle or CX-4945 (5 μM) for 1 h. Methylene blue staining was used for loading control. **(C)** Quantification of percentage (%) of CPD repair from (B). *, $P < 0.05$; ***, $P \leq 0.001$; compared with the vehicle group, Student's *t*-test. **(D)** Immunoblot analysis of CK2A1, CK2A2, CK2B and GAPDH in HaCaT cells 30 min after UVB exposure (20 mJ/cm^2). The results were obtained from three independent experiments.

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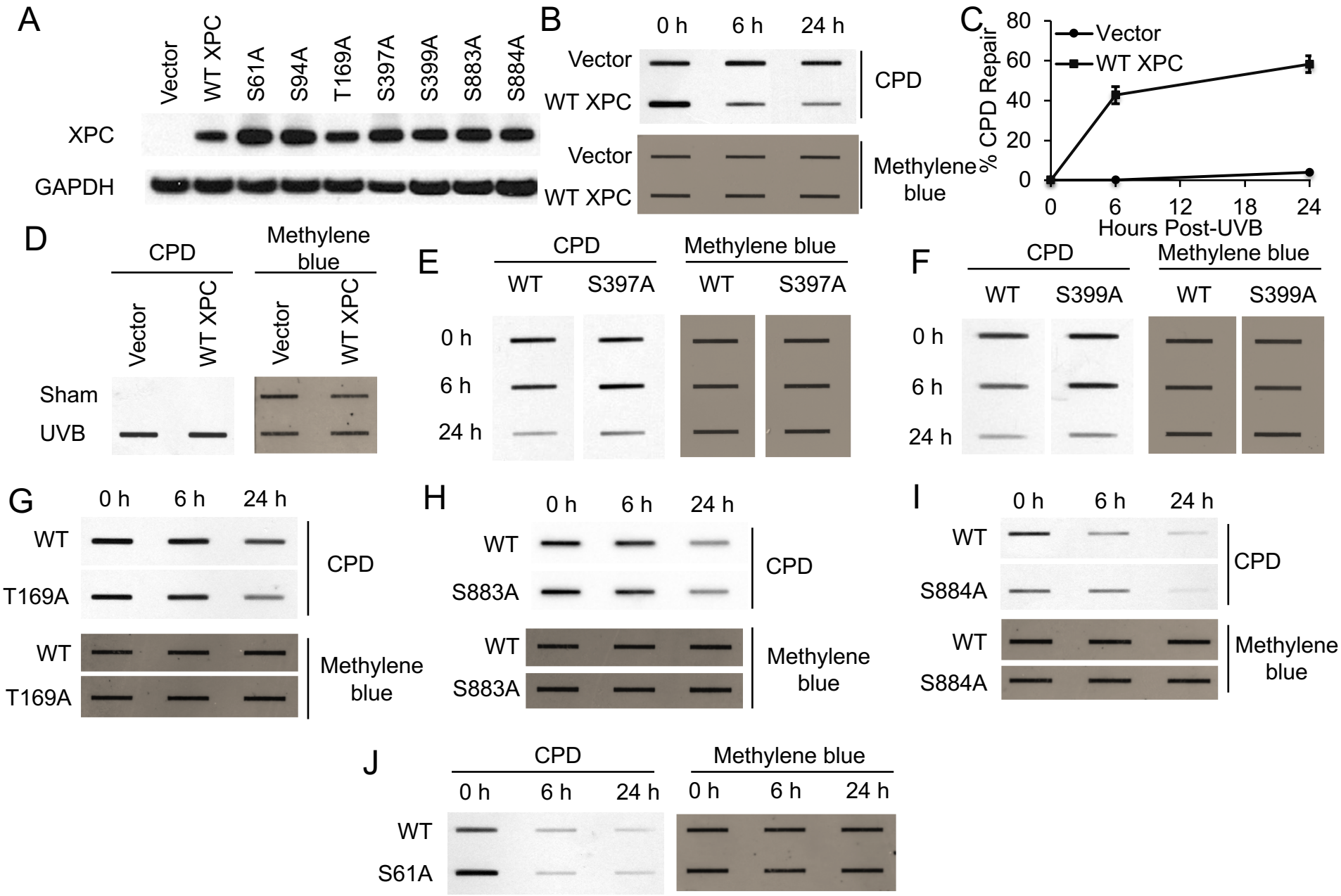
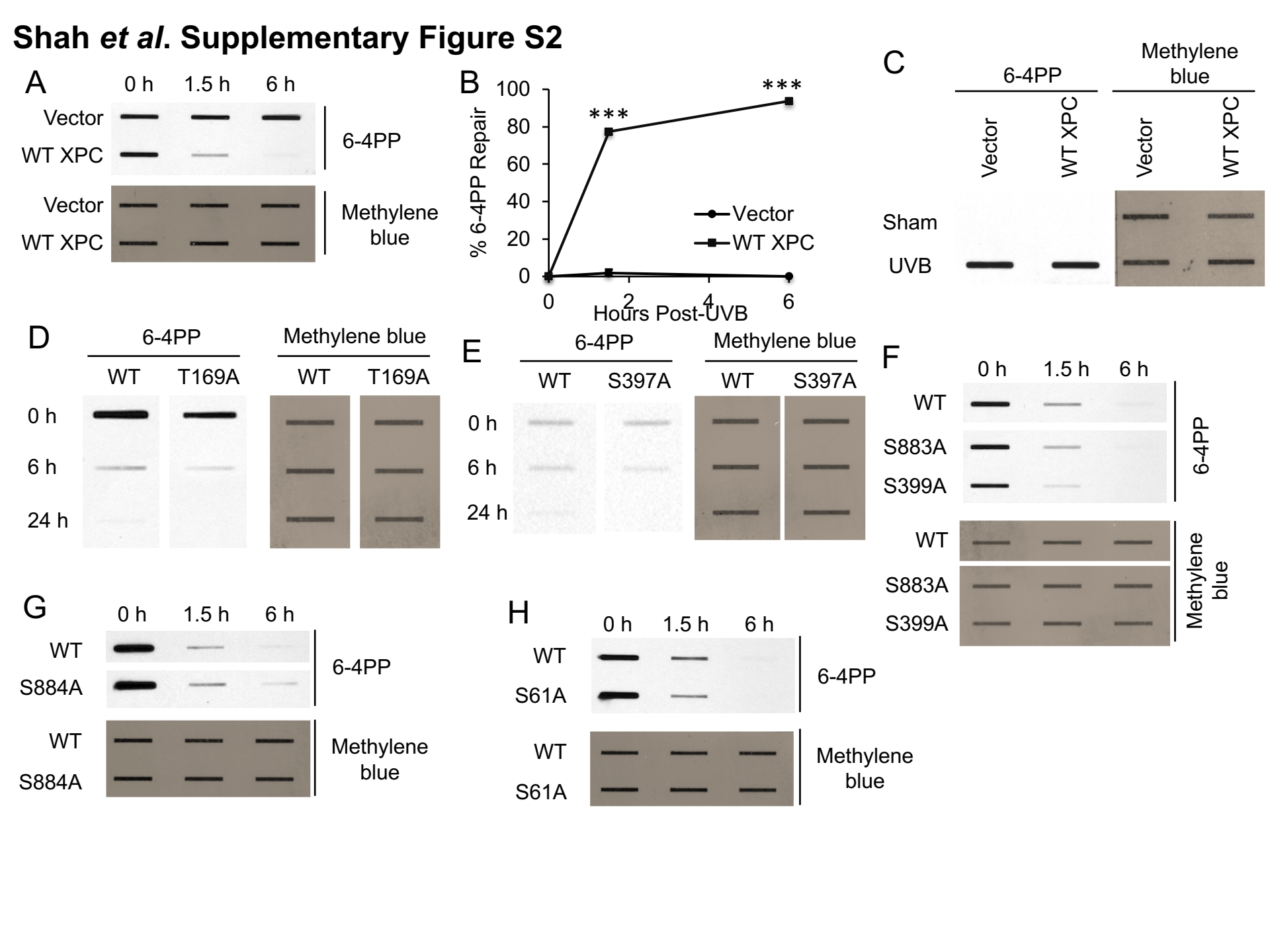


Shah *et al*. Supplementary Figure S2



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