Functional and structural studies of the nucleotide excision repair helicase XPD suggest a polarity for DNA

Jochen Kuper\textsuperscript{1}\textsuperscript{*}, Stefanie C. Wolski\textsuperscript{1}\textsuperscript{§}, Gudrun Michels\textsuperscript{1}, and Caroline Kisker\textsuperscript{1}\textsuperscript{*}

\textsuperscript{1}Rudolf Virchow Center for Experimental Biomedicine, Institute for Structural Biology, University of Würzburg, Josef-Schneider-Str. 2, 97080 Würzburg, Germany

\textsuperscript{§}These two authors contributed equally to this work

\textsuperscript{*}Authors to whom correspondence should be addressed

Phone (+49 931 31 80381)
E-Mail: caroline.kisker@virchow.uni-wuerzburg.de

Phone (+49 931 31 80391)
E-Mail: jochen.kuper@virchow.uni-wuerzburg.de
Fig. S4
Supplementary Figure legends

Figure S1: Sequence alignment of taXPD and eukaryotic XPD homologs (organisms are indicated in the figure). Investigated variants are marked by a green dot. If variants are not strictly conserved blue boxes are indicating residues in close proximity that could be the relevant homologs but are not captured by the sequence alignment.

Figure S2: Overall structure of taXPD (A) Topography diagram of taXPD. The colours are chosen as for the domains in the ribbon diagram. Helices are depicted as cylinders and β-strands as arrows. All helices and β-strands are numbered and the first and last residue of each secondary structure element is indicated by a number as well. (B) Ribbon diagram of XPD. The two RecA-like domains HD1 and HD2 are labeled and coloured in yellow and red, respectively. The FeS domain is shown in cyan and the FeS cluster is shown in all-bonds representation. The arch domain is shown in green.
**Figure S3: The DNA and Sulfate Binding Sites.** (A) Initial difference electron density maps after molecular replacement contoured at 2.2σ and final 2Fo-Fc electron density map contoured at 1σ, coloured in green and grey, respectively. (B) and (C) Difference electron density maps contoured at 2.5σ after molecular replacement for the two sulfate molecules which indicate potential phosphate positions. The XPD protein is shown in ribbon presentation and is coloured as in Supplementary Figure 1.

**Figure S4: The ATP Binding site.** Superposition of NS3 Helicase from Hepatitis virus C (3gpl), UvrB from *Bacillus caldotenax* (1d9z) and *T. acidophilum* XPD. For clarity only the bound nucleotides from NS3 helicase and UvrB are shown. XPD is shown in ribbon presentation, the sulfate molecule in all bonds-representation and the two motor domains are color coded as in Supplementary Figure 1.

**Figure S5: Thermal stability of XPD variants.** Unfolding curves of taXPD and the variants analysed in this study. Representative run out of several. None of the variants unfolds earlier than wild type XPD indicating no negative influence on protein fold. WT colored in darkblue, D582N in red, K170A in green, W549S in purple, F326A in black, R88H in brown, R59A in grey, F133A in orange, E107A in lightblue, Y166A in pink and R584E in yellow.