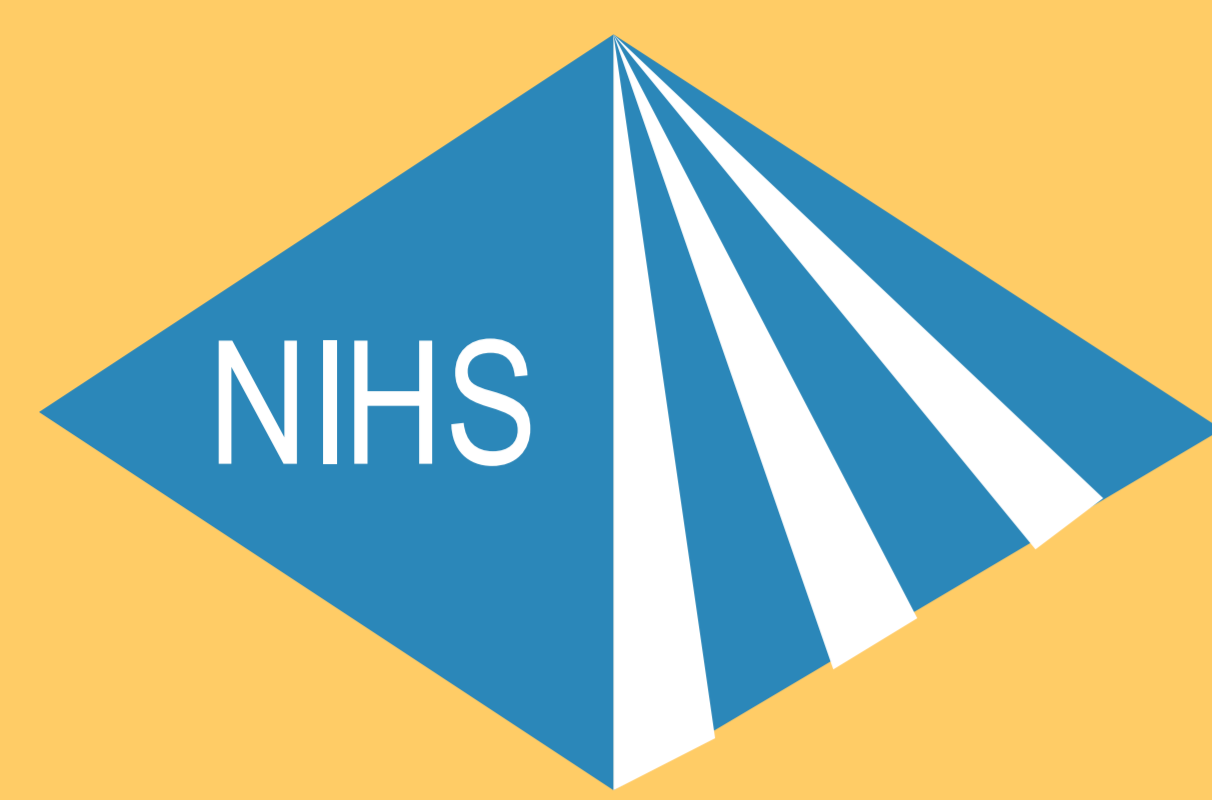


Partial restoration of UV-mutagenesis in a *umuDC*-deletion mutant of *Escherichia coli* with human DNA polymerase η



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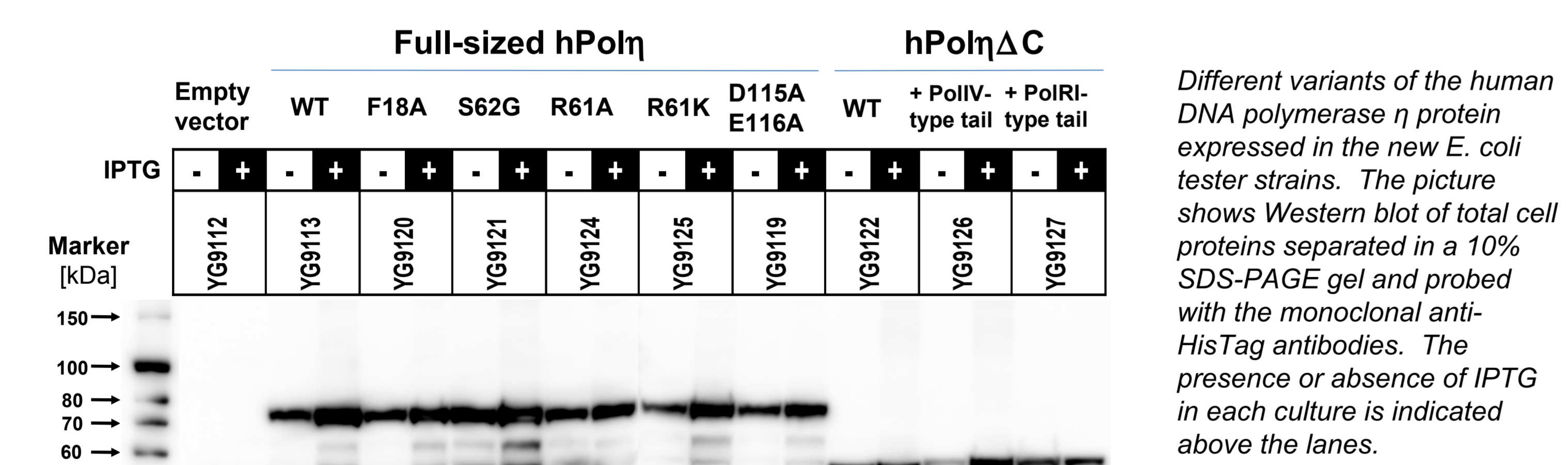
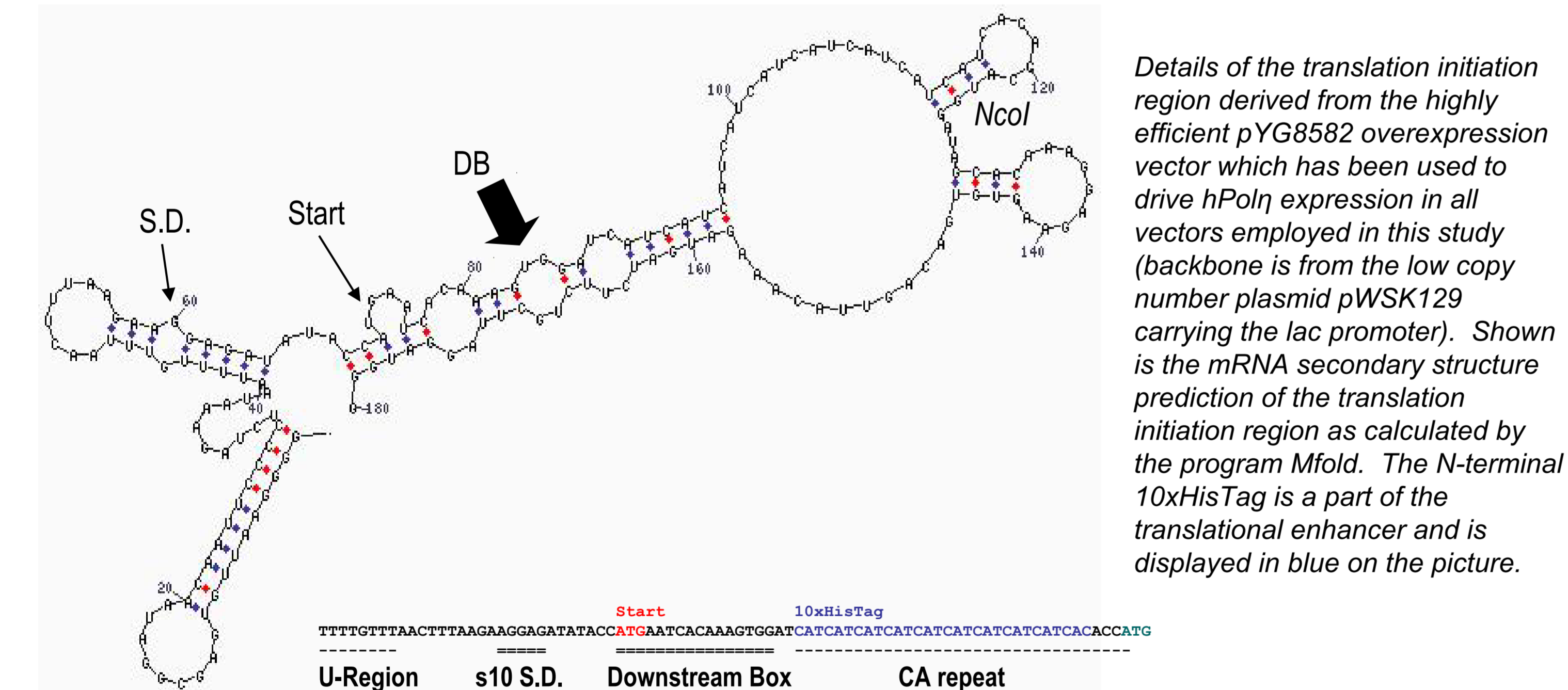
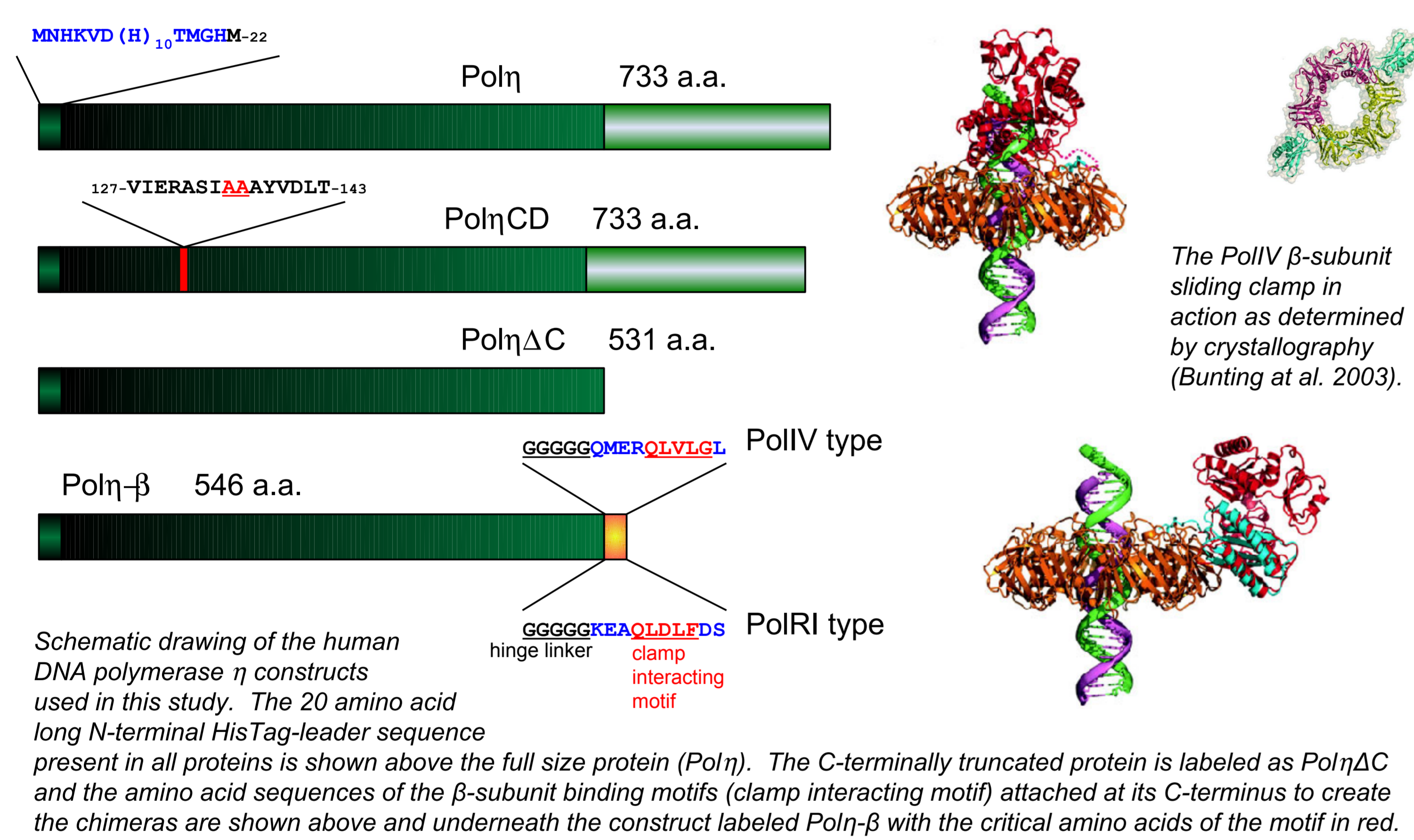
Abstract

DNA polymerase η (hPol η) is a key protein in translesion DNA synthesis (TLS) in human cells. Its primary function is the *error free* replication through UV-induced TT cyclobutane dimers which present a barrier to DNA synthesis by other eukaryotic replicative polymerases. hPol η defects underlie the genetic disease xeroderma pigmentosum variant (XPV) characterized by higher susceptibility to UV-light induced skin cancers due to erroneous replication of the UV adducts. However, hPol η is also a very low fidelity enzyme when copying undamaged DNA or DNA with other adducts and is actively recruited during the somatic hypermutation of the immunoglobulin genes.

We demonstrate that hPol η restores partially the mutability and completely the survival of a UV non-mutable *umuDC*-deletion mutant of *Escherichia coli* after UVB irradiation. The catalytic core lacking the C-terminal part of hPol η was even more biologically active than the full size protein and its activity was further enhanced by attaching the prokaryotic β -subunit binding motif to it. The mutagenicity and survival effects were enhanced upon the induction of hPol η expression and its catalytically inactive variant was unable to promote any mutagenesis. This suggests that hPol η directly participates in the replication of damaged DNA in the prokaryotic bacteria.

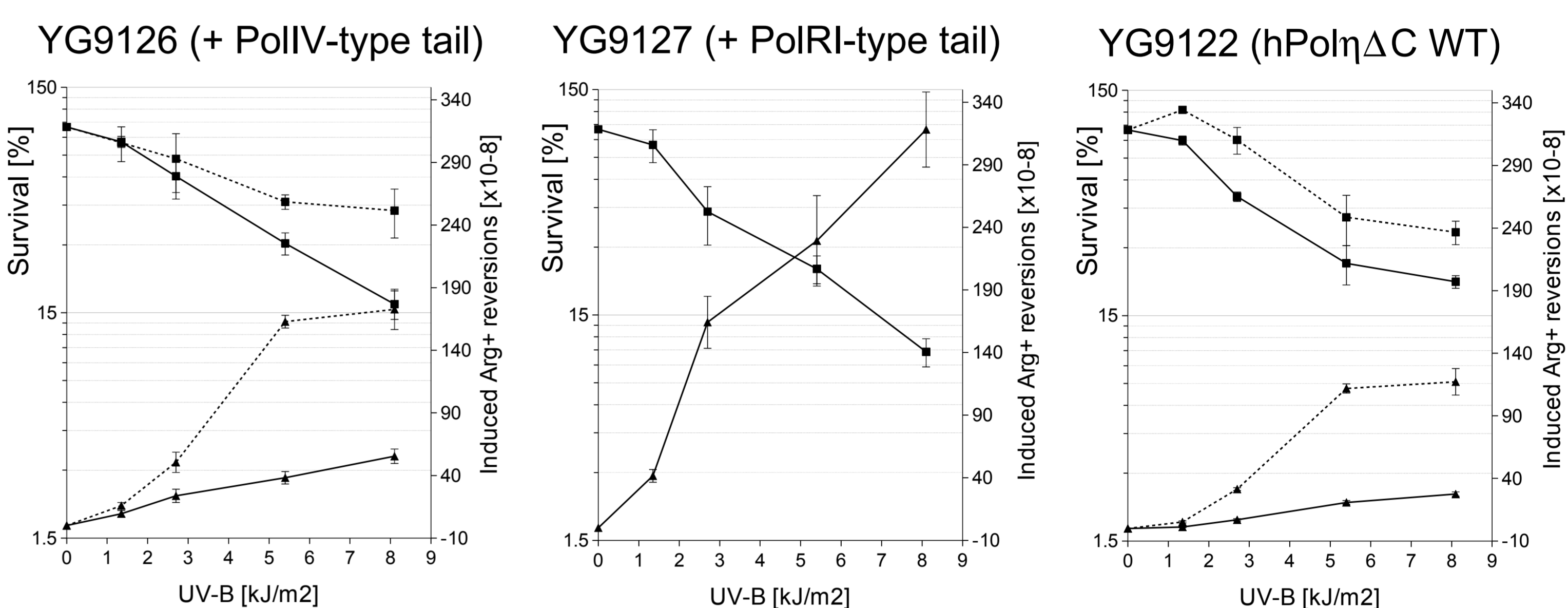
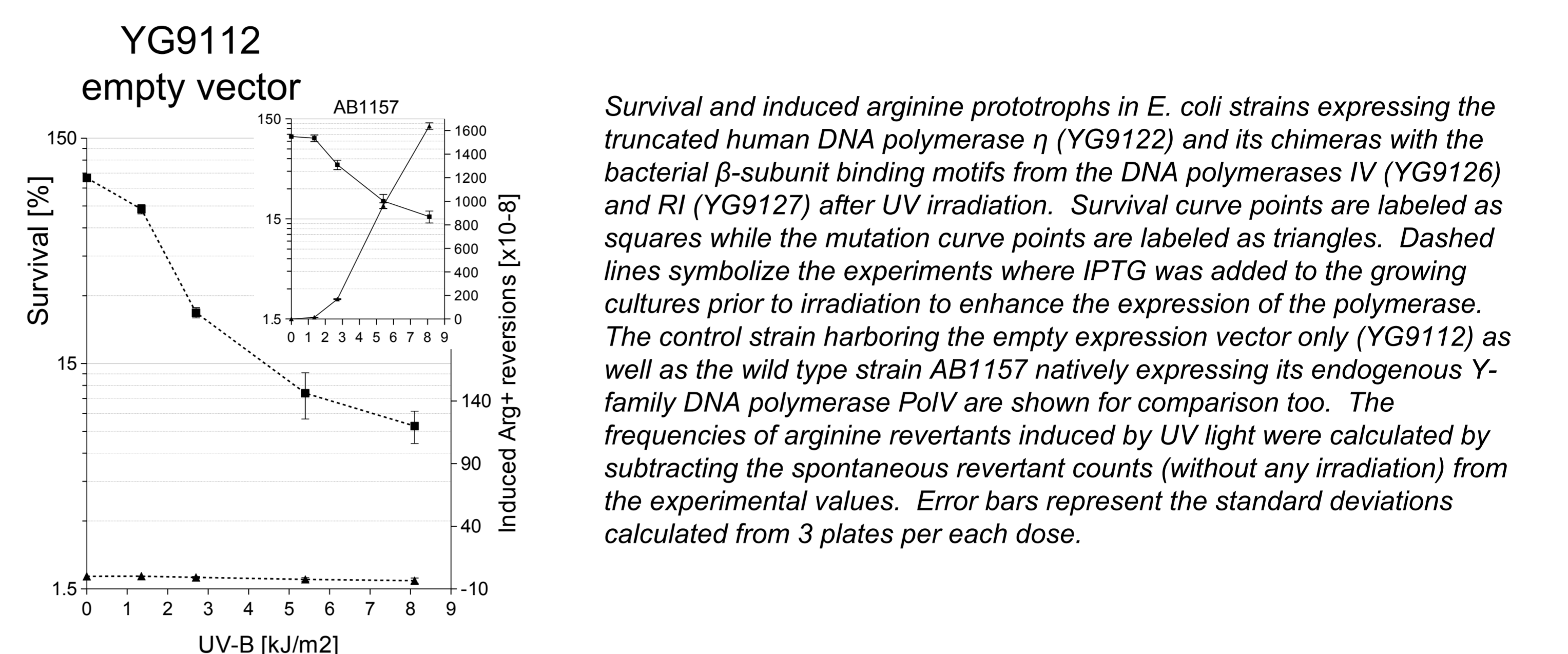
To demonstrate that our system can be useful in studying different variants of hPol η *in vivo* we have constructed 4 amino acid substitution mutants with altered geometry of the catalytic site analyzed previously biochemically and confirmed their altered abilities to promote mutagenesis and survival after UVB irradiation. This study paves a way to generate a variety of useful derivatives of hPol η in prokaryotic systems.

Results & Discussion

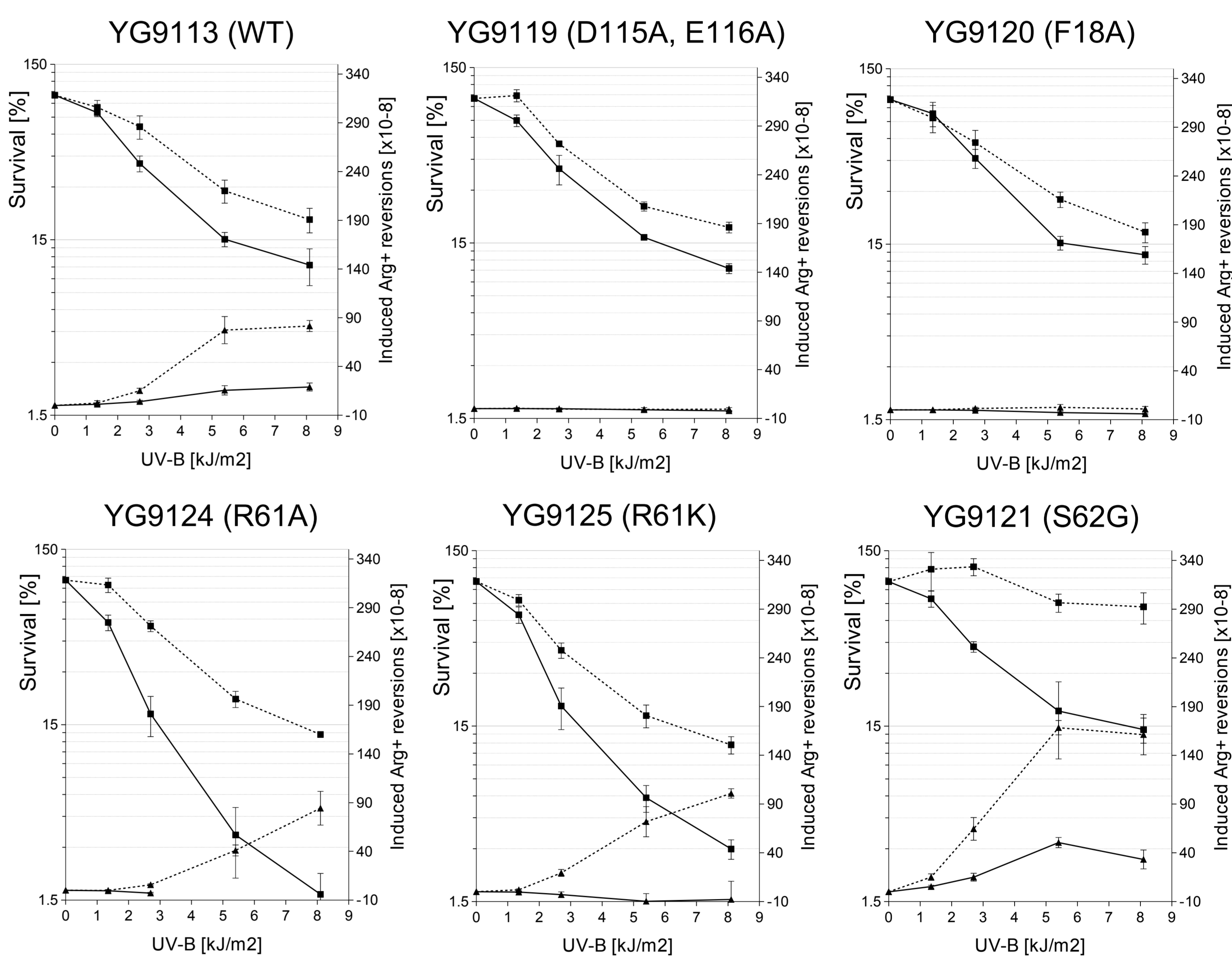


Enterobacterial strains used in the study

<i>E. coli</i> strain	Description	hPol η mutation
AB1157	Wild type K-12 derivative carrying the <i>argE3</i> (ochre) reversion marker	
YG6168	UV non-mutable derivative of AB1157 carrying the $\Delta(umuDC)596::ermGT$ disruption	
YG9112	YG6168 derivative harboring the empty expression vector pWSK129	
YG9113	YG6168 derivative expressing human DNA polymerase η from the plasmid pYG8626	wild type
YG9119	YG6168 derivative expressing catalytically dead human DNA polymerase η from the plasmid pYG8636	D115A+E116A
YG9120	YG6168 derivative expressing the steric gate mutant of human DNA polymerase η from the plasmid pYG8637	F18A
YG9121	YG6168 derivative expressing the superactive mutant of human DNA polymerase η from the plasmid pYG8638	S62G
YG9124	YG6168 derivative expressing the Arg61 \rightarrow Ala61 mutant of human DNA polymerase η from the plasmid pYG8639	R61A
YG9125	YG6168 derivative expressing the Arg61 \rightarrow Lys61 mutant of human DNA polymerase η from the plasmid pYG8640	R61K
YG9122	YG6168 derivative expressing C-terminally truncated DNA polymerase η from the plasmid pYG8633 containing only its essential catalytic domain	ΔC
YG9126	YG6168 derivative expressing the chimerical human DNA polymerase η with the attached DinB (PolIV) β -subunit binding motif from the plasmid pYG8634	ΔC +PolIV-type tail
YG9127	YG6168 derivative expressing the chimerical human DNA polymerase η with the attached MucB (PolRI) β -subunit binding motif from the plasmid pYG8635	ΔC +PolRI-type tail



Survival and induced arginine prototrophs in *E. coli* strains expressing different mutants of the human DNA polymerase η after UV irradiation. Survival curve points are labeled as squares while the mutation curve points are labeled as triangles. Dashed lines symbolize the experiments where IPTG was added to the growing cultures prior to irradiation to enhance the expression of the polymerase. The frequencies of arginine revertants induced by UV light were calculated by subtracting the spontaneous revertant counts (without any irradiation) from the experimental values. Error bars represent the standard deviations calculated from 3 plates per each dose.



Conclusions

Human DNA polymerase η (hPol η) is the most well studied Y-family DNA polymerase responsible for the inherited disease XPV syndrome and is mechanistically involved in the initiation of cancers associated with the BRCA1 mutations.

We have successfully expressed hPol η in the prokaryotic model bacterium *Escherichia coli* as confirmed by Western blotting and response to IPTG induction.

hPol η remained functional in *E. coli* and participated in the translesion DNA synthesis across CPD lesions induced by UVB radiation.

The activity of hPol η in *E. coli* was remarkably increased by removing its C-terminal part carrying motifs specific to eukaryotes and completely abolished by substituting 2 essential residues within its catalytic site.

Chimeric hPol η proteins carrying the prokaryotic DNA targeting motifs showed increased ability to promote UVB mutagenesis.

A single amino acid substitution within the motif II of finger domain (S62G) significantly enhanced hPol η abilities to increase survival and mutagenesis after UVB irradiation *in vivo*.

The hPol η steric gate mutant (F18A), although biochemically active *in vitro*, completely lost its activity *in vivo*.

Substitution of the amino acid residue guiding the 8oxo-dGTP misincorporation opposite template dA *in vitro* (R61) produced mutants of hPol η which lost the ability to promote mutagenesis at low expression level *in vivo* and suppressed the survival after UVB irradiation.

Our new system allows for the generation and analysis of the effects of various hPol η mutations on this enzyme's functionality during TLS in enterobacteria as well as for testing the intrinsic ability of hPol η to modulate TLS across other types of DNA adducts *in vivo*.

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