Molecular Cell, Volume *32*

Supplemental Data

Human DNA2 Is a Mitochondrial Nuclease/Helicase for Efficient Processing of DNA Replication and Repair Intermediates

Li Zheng, Mian Zhou, Zhigang Guo, Huiming Lu, Limin Qian, Huifang Dai, Junzhuan Qiu, Elena Yakubovskaya, Daniel F. Bogenhagen, Bruce Demple, and Binghui Shen

Table S1: Evolutionary trends of changes of nuclear localization signals in DNA2 proteins from different species

Species	Genbank #	Classic NLS		
		Pat4	Pat7	Bipartite
Human	NP_001073918	-	-	-
Mouse	BAC97861	-	-	-
Rat	XM_241671	-	-	-
Cattle	XP_613907	-	-	-
Chicken	NP_001006497	-	-	-
Xenopus	NP_001079231	-	+	-
Puffer fish	CAG05680	-	+	-
Drosophila	XP_001354415	-	+	+
C. elegans	CAB54253	+	+	-
Arabidopsis	NP_172361	+	+	-
A. fumigatus	EDP50582	+	+	+
S. cerevisia	AAB68010	+	+	+
S. pombe	NP_596499	+	+	+

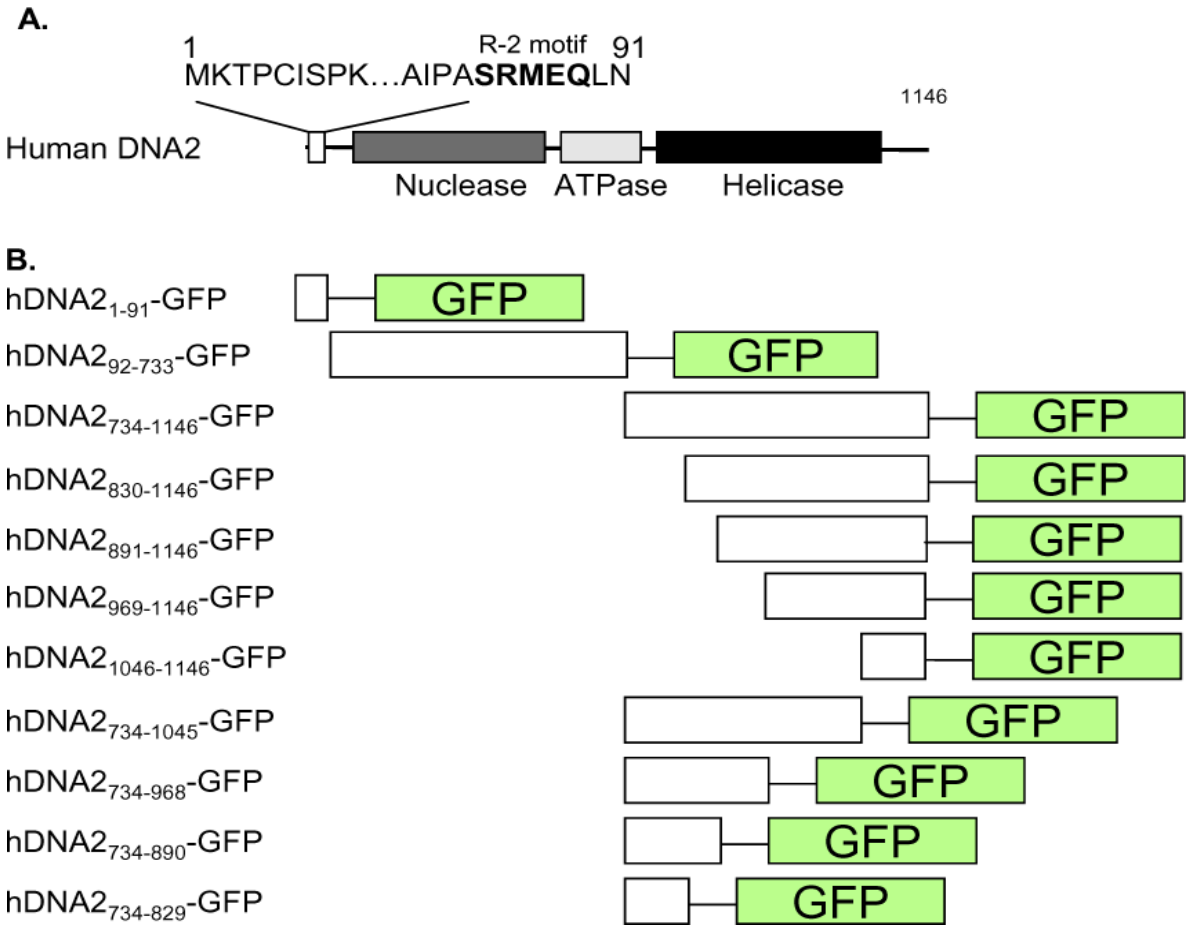

Figure S1. Identification of the mitochondrial targeting motif of hDNA2. A. Domain structure of hDNA2. Nuclease, ATPase, and Helicase domains were defined. An R-2 motif (-SRMEQ-), which is a potential cleavage site of mitochondrial targeting peptides, was predicted by pSORT II. **B.** Constructs for expressing of varying hDNA2 deletion mutant-GFP fusion proteins to map the mitochondrial targeting motif of hDNA2. A DNA fragment encoding a specific peptide fragment of hDNA2 (NP_001073918) was sub-cloned into the pEGFP-N1 plasmid. The plasmid was transfected into HeLa cells to transiently express a specific DNA2 deletion mutant-GFP fusion protein.

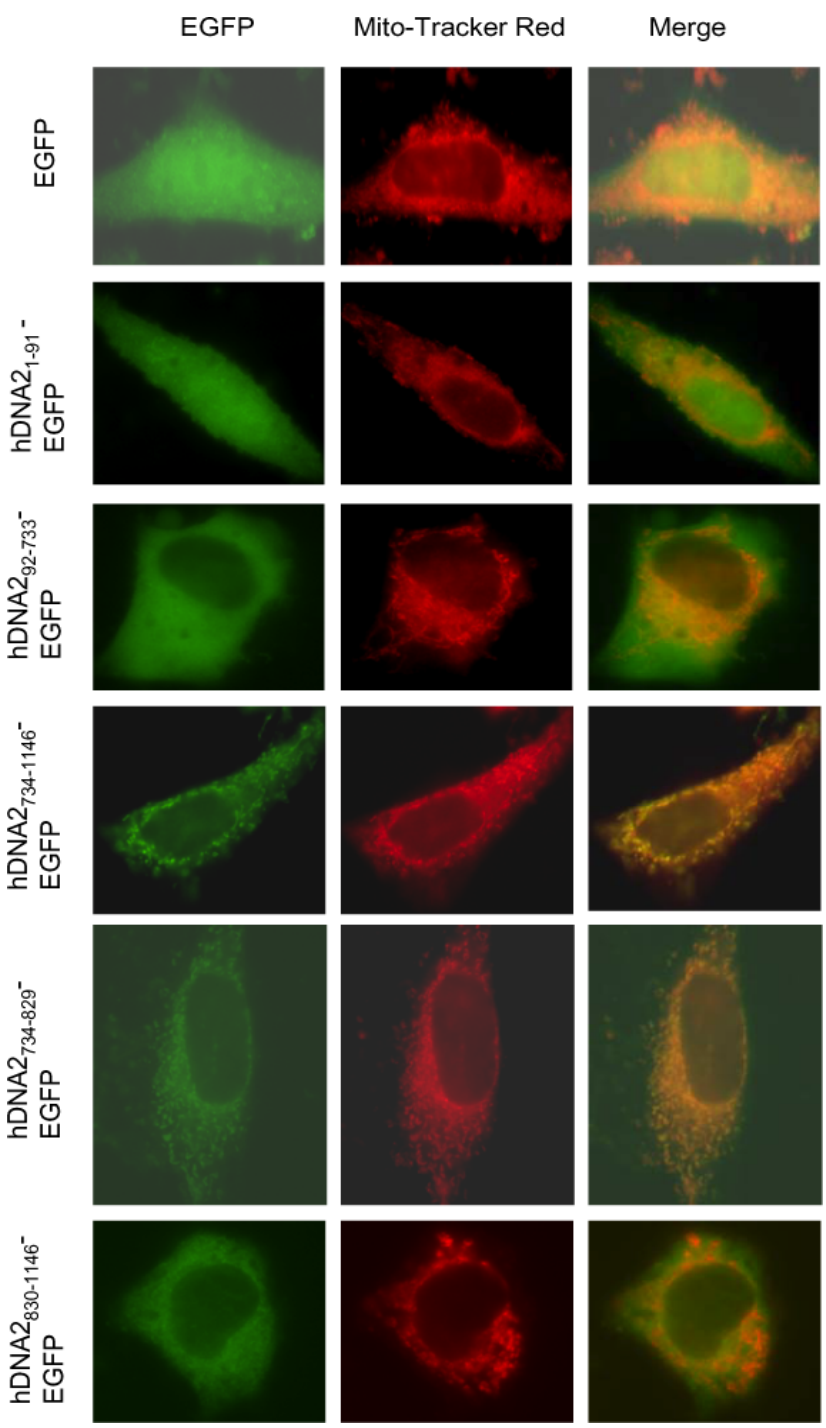

Figure S2. The internal peptides 734-829 of hDNA2 is a mitochondrial targeting signal. HeLa cells were transfected with plasmids expressing varying hDNA2 deletion mutant-EGFP fusion proteins. After 5 hours transfection, the cells were further incubated in fresh DMEM for 24 hours. The cells were fixed with 2% paraformaldehyde. The signal of EGFP or mito-tracker red was observed with a fluorescence microscope. The EGFP fusion proteins containing the internal peptides 734-829 were able to translocate into mitochondria. Conversely, cells expressing EGFP fusion proteins without the 734-829 peptides failed to show discrete co-localization signals of EGFP and mito-tracker red. Representative microscopic images of hDNA2$_{734-1146}$-EGFP and hDNA2$_{734-829}$-EGFP, which contain the 734-829 peptides and hDNA2$_{92-933}$-EGFP and hDNA2$_{830-1146}$-EGFP, which do not have the 734-829 peptides, were displayed.

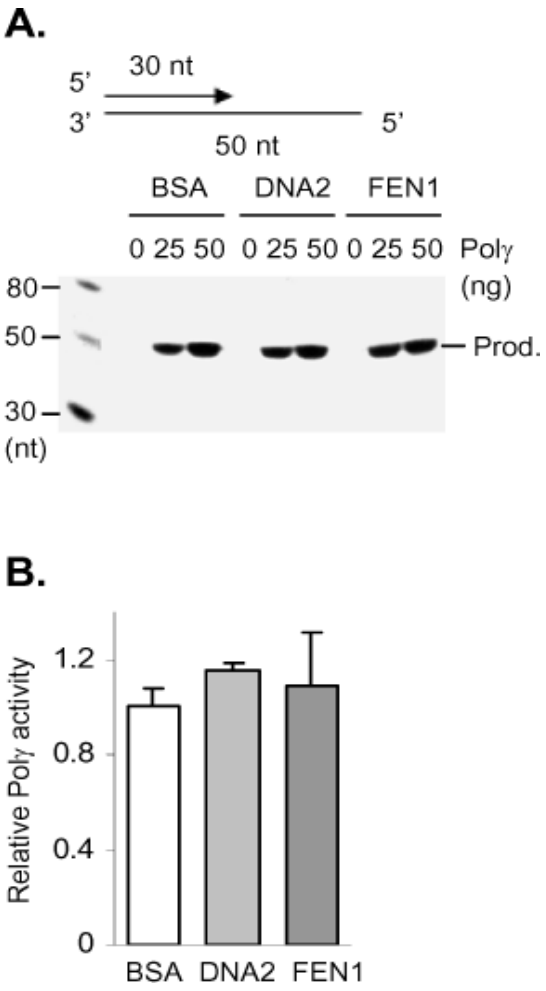

Figure S3. Polγ-driven primer extension on an oligo-based DNA substrate. 25 or 50 ng of purified Polγ was incubated with 1 pmol biotinylated oligo-based DNA substrates, 5 μCi [α-32P] dATP, 1 μM dATP, and 50 μM each of the other three dNTP, in the presence of 100 ng BSA, 100 ng hDNA2 or 40 ng hFEN1. Reactions were carried out at 37 ºC for 30 min. Products were run on 15% denaturing PAGE and visualized by autoradiography (A). The products from reactions by 50 ng Polγ as specified in panel A were purified by streptavidin Dynabeads and quantified by a scintillation counter (B). The relative Polγ activity was calculated by dividing the CPM of each reaction by the CPM in the presence of BSA. The relative Polγ activity in the presence of BSA was arbitrarily set as 100. Values are mean ± SD (n = 3).