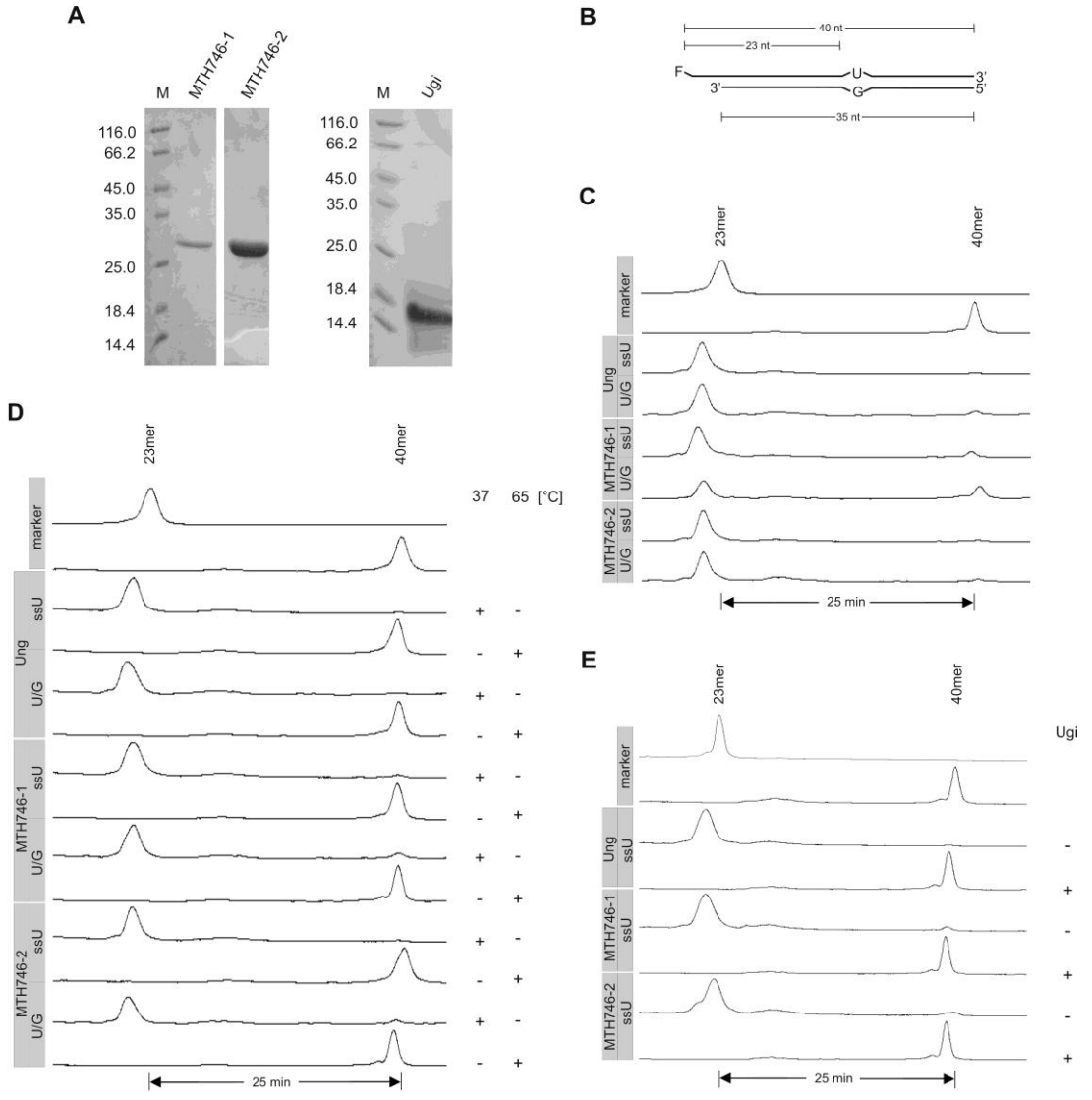
Helix-hairpin-helix protein MJ1434 from *Methanocaldococcus jannaschii* and EndoIV homologue TTC0482 from *Thermus thermophilus* HB27 do not process DNA uracil residues

Lars Schomacher, Sabine Smolorz , Elena Ciirdaeva, Svetlana Ber, Wilfried Kramer and Hans-Joachim Fritz

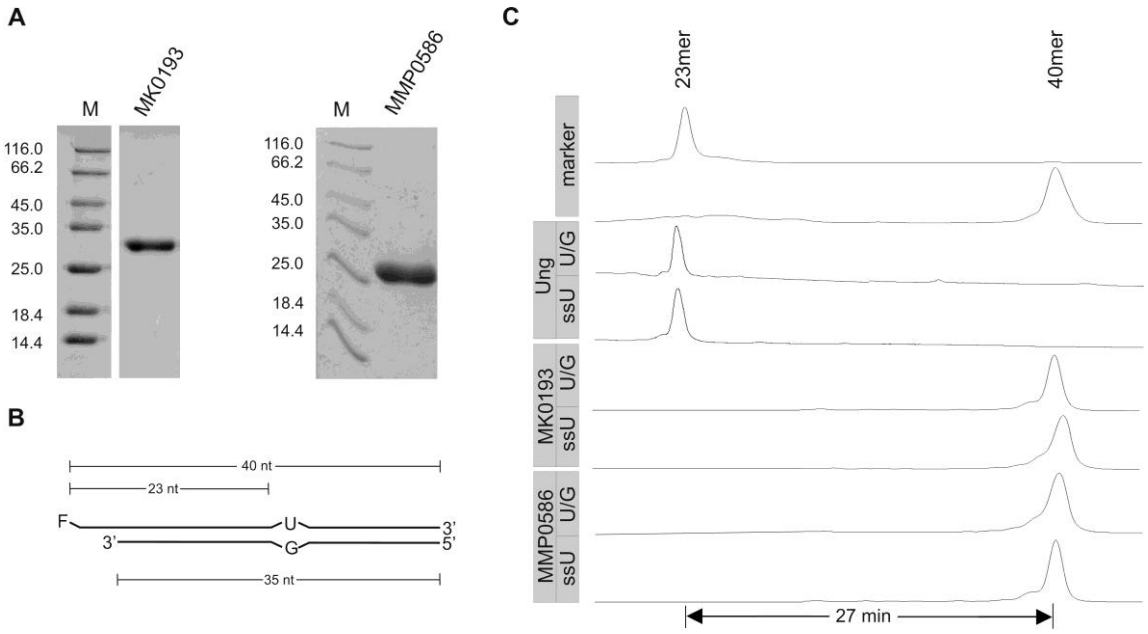
Supplementary Data



Supplementary Figure 1: Characterization of the DNA uracil glycosylase activity found in preparations of MTH746-1 and MTH746-2 from *M. thermautotrophicus* ΔH produced in the Ung-proficient *E. coli* strain Rosetta-gami B(DE3).

(A) SDS-PAGE analysis (Coomassie stain) of purified proteins. MTH746-1 and MTH746-2: N-terminally truncated versions of MTH746 (see main text for details). Ugi: uracil DNA glycosylase inhibitor from *B. subtilis* phage PBS-2. MTH746-1 and MTH746-2 were purified

by IMAC and heparin affinity chromatography, Ugi by IMAC. M: Marker proteins with corresponding relative molecular masses ($x10^{-3}$). Calculated relative molecular masses are 28,020 for MTH746-1, 27,040 for MTH746-2 and 10,510 for Ugi. For production and purification details see 'Materials and Methods'. **(B)** Schematic drawing of the double stranded U/G mismatched DNA substrate. F: fluorescein moiety. The lengths of the substrate oligonucleotides and the expected products are indicated (nt = nucleotides). **(C)** Trackings of fluorescence readout of DNA sequencer. Prior to gel electrophoresis, double stranded (U/G) and single stranded (ssU) uracil-containing oligonucleotide substrates (each 0.12 pmoles) had been incubated at 37 °C for 60 min with 24 pmoles of *E. coli* uracil-N-glycosylase Ung (control) or MTH746-1 and MTH746-2, respectively. For other reaction details refer to 'Materials and Methods'. **(D)** Gel electophoretic analysis of reaction products of glycosylase assay as shown in (C) after 60 min incubation of substrates with proteins at 37 °C and, additionally, at 65 °C as indicated on the right. **(E)** Readout of glycosylase assay essentially as described in (C). When indicated by a "+" sign under "Ugi", Ung, MTH746-1 or MTH746-2 had been incubated in assay buffer with Ugi in equimolar ratio at 37 °C for 30 min before substrate was added.



Supplementary Figure 2: Glycosylase assay of MK0193 from *M. kandleri* AV19 and MMP0586 from *M. maripaludis* S2 produced in Δ*ung*-strain BL21_UX.

(A) Coomassie-stained SDS-PAGE gel of purified proteins after IMAC and heparin affinity chromatography. Marker labelling as in Supplementary Figure 1. Calculated relative molecular masses are 27,620 for MK0193 and 28,160 for MMP0586. For production and purification details see 'Materials and Methods'. **(B)** Schematic drawing of the U/G mismatched substrate (as in Supplementary Figure 1B). **(C)** DNA sequencer readout of glycosylase assays with the indicated proteins (compare to Supplementary Figure 1). Ung was used as a positive control.