- Supplemental material -

A key enzyme of NAD^+ salvage pathway in *Thermus thermophilus:* Characterization of nicotinamidase and the impact of its gene deletion at high temperatures

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Table S1. Composition of chemically defined medium for *T. thermophilus*

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Components	Final concentration
$CaCl_2$	0.04 mM
$MgSO_4$	0.41 mM
KCl	1.02 mM
Na_2SO_4	0.80 mM
NaCl	9.0 mM
K_2HPO_4	2.8 mM
KH_2PO_4	2.2 mM
$NaHCO_3$	5 mM
EDTA	0.04 mM
Tris	40 mM
ferric citrate	3.5 μM
$MnSO_4$	9.2 μM
$ZnSO_4$	1.9 μM
Na_2MoO_4	0.1 μM
$CoCl_2$	0.2 μM
$CuSO_4$	1.0 μM
H_2SO_4	2.1 μM
d-biotin	8 μM
monosodium glutamate	50 mM
NH_4Cl	9.5 mM

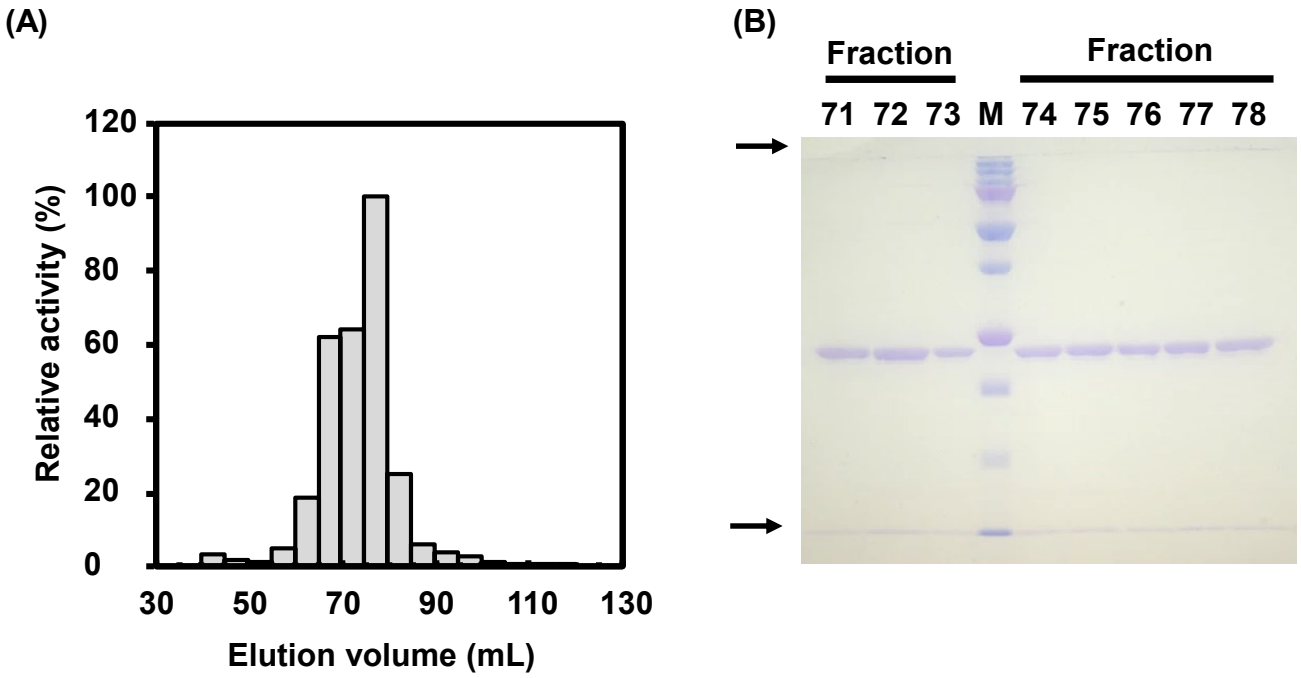

Figure S1. Protein purification by size exclusion chromatography

(A) The activity of each fraction separated by size exclusion chromatography (gray scale bar) is shown. Five continuous fractions were pooled and the activity of pooled samples were measured (Fraction 41-45, 46-50, 51-55, 56-60, 61-65, 66-70, 71-75, 81-85, 86-90, 91-95, 96-100, 101-105, 106-110, 111-115, 116-120). The activity was normalized by the highest activity of fractions 76-80. (B) SDS-PAGE result of fractions from 71 to 78 is shown. Protein concentration of each fraction was determined by the Bradford method and 1 μg of total protein was used for each sample. SDS-PAGE was performed with 12% of polyacrylamide with Tris-glycine SDS buffer. Precision Plus Protein™ Dual Color Standards (Bio-Rad, USA) was used as protein maker (M). The molecular weight of the band was calculated to be 23.6 kDa which is comparable to molecular weight of TTHA0328 product (22.0 KDa). The arrows indicate the start of running gel and the frontline of gel electrophoresis.

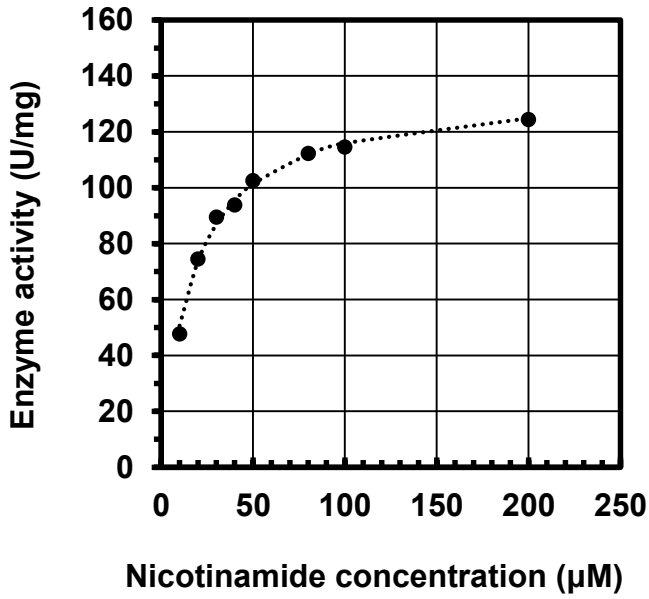

Figure S2. Determination of kinetic parameters of TTHA0328

Enzyme activity at different concentration of nicotinamide (10, 20, 30, 40, 50, 80, 100, 200 μM) was shown. The dotted line indicates the fitted Michaelis-Menten curve. The fitting was performed by the least squares method.

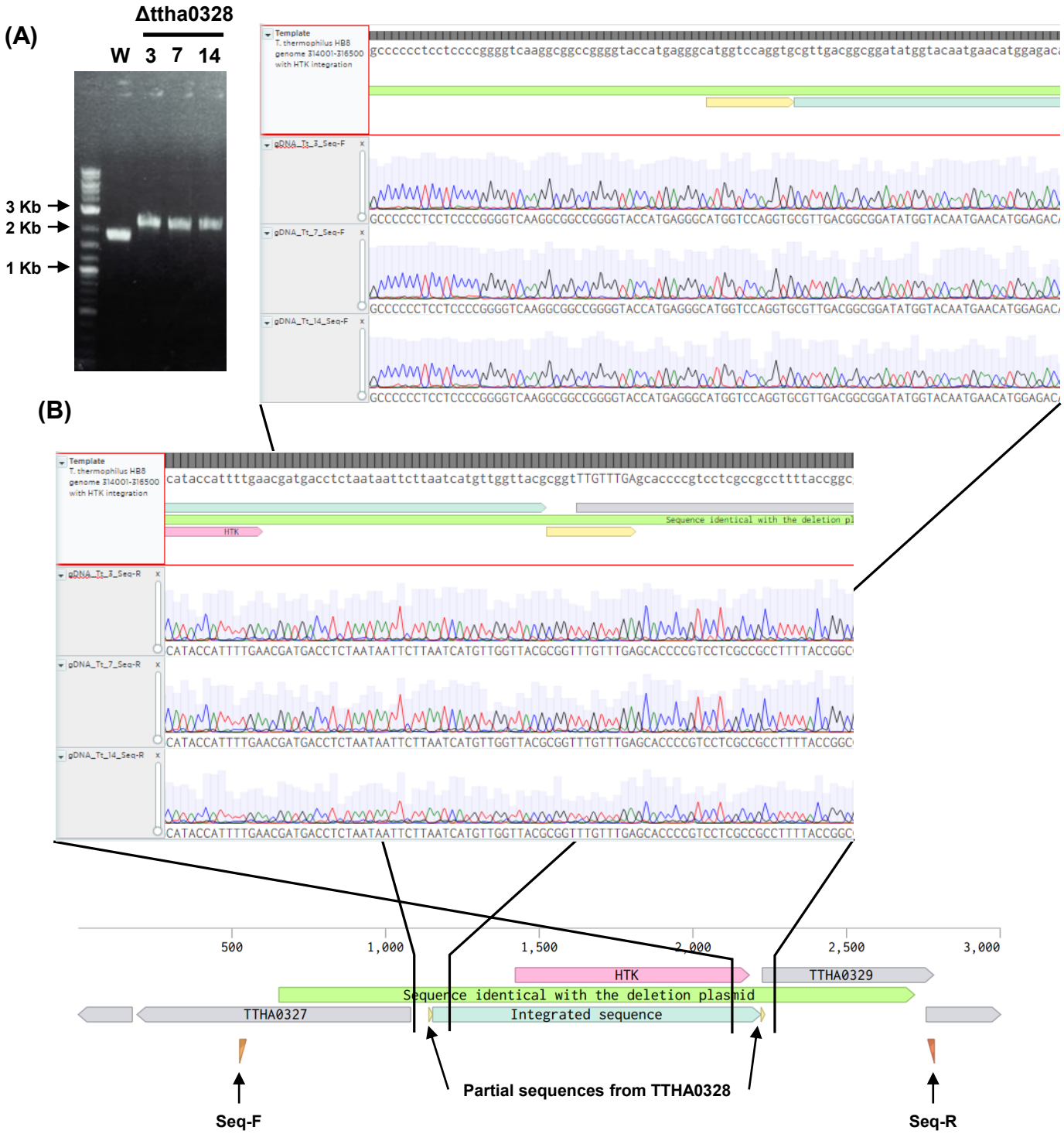

Figure S3. Confirmation of the deletion of ttha0328

(A) The integrated region in the chromosome was amplified by PCR with the oligonucleotide pairs (Seq-F/Seq-R). The genome DNAs extracted from Δttha0328 strains (Colony No. 3, 7, 14) were used as templates. The genomic DNA of *T. thermophilus* wild type strain was used as control (W). The PCR products were analyzed by gel electrophoresis (Expected size, 2251 bp for the integreated fragment,1759 bp for the native fragment). **(B)** The genome organization of Δttha0328 and the part of sequence results of PCR products was visualized by Benchiling (https://benchling.com).

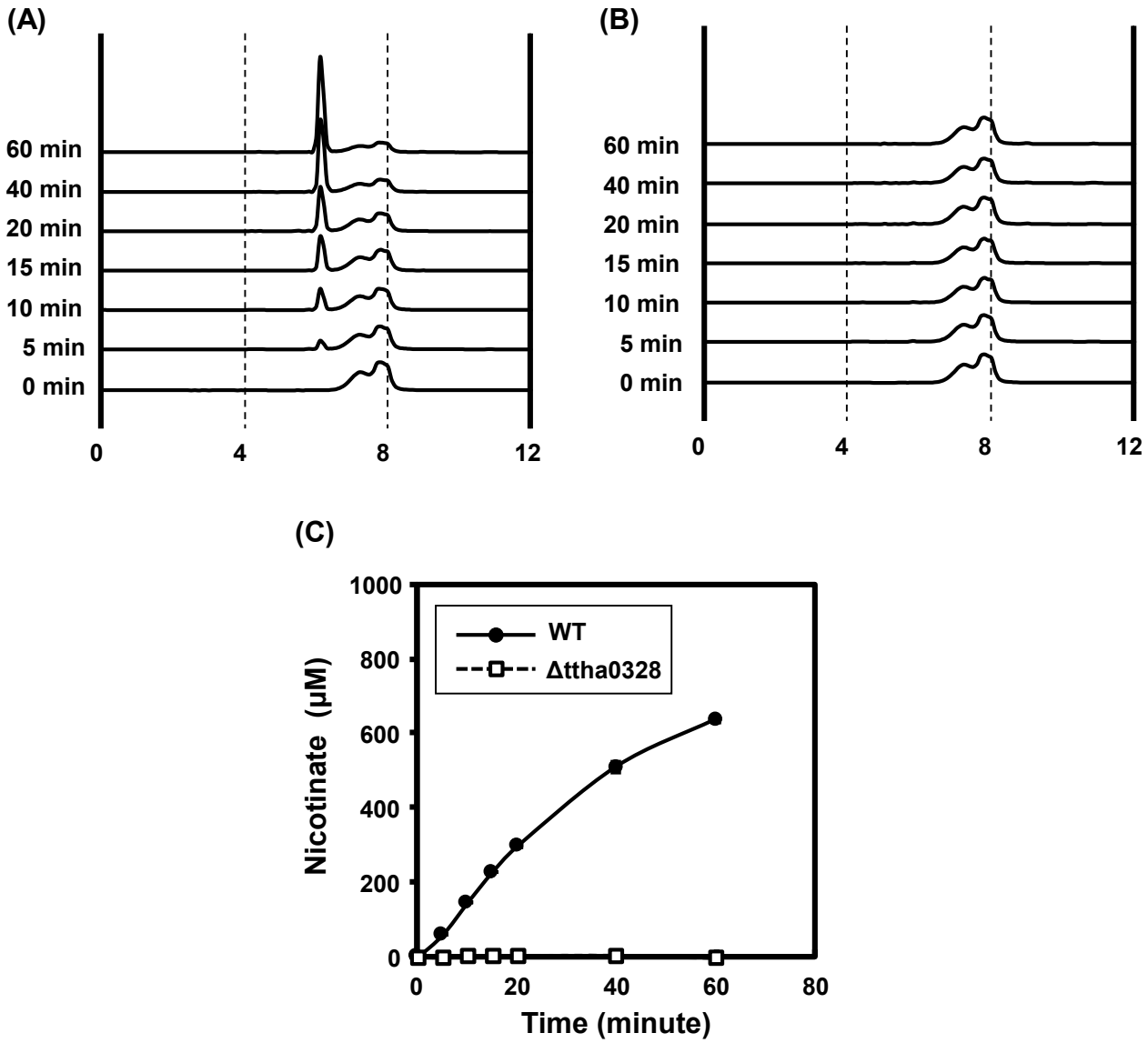

Figure S4. Determination of nicotinamidase activity in the cell lysate

The HPLC chromatograms of reaction mixtures with the lysates from WT **(A)** and Δttha0328 **(B)** at different time points are shown. Two peaks at 7.2 and 7.8 min correspond to nicotinamide and the peak at 6.1 corresponds to nicotinate. **(C)** Nicotinate concentration in the reaction is shown over time. The initial concentration of nicotinamide is 1 mM.