Supporting Information

Glutamate dehydrogenase from *Thermus thermophilus* is activated by AMP as a complex with catalytically inactive adenine phosphoribosyltransferase homolog

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Table of Contents

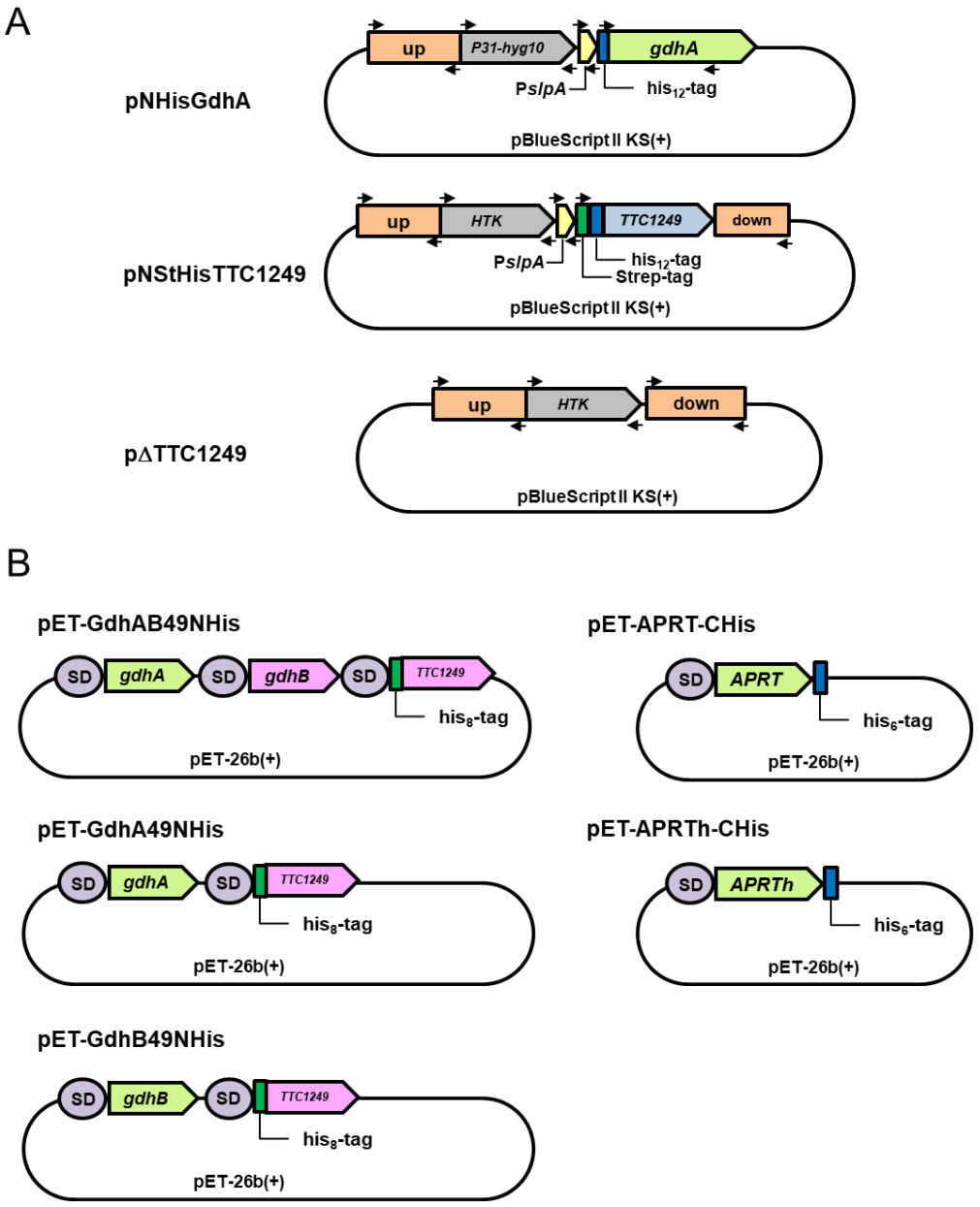

FIG S1. The construct of plasmids used for the homologous recombination of *T. thermophilus* HB27 and for the expression of proteins in *E. coli*

(A) The plasmids used for the homologous recombination of *T. thermophilus* HB27. (B) The plasmids used for the expression of proteins in *E. coli*

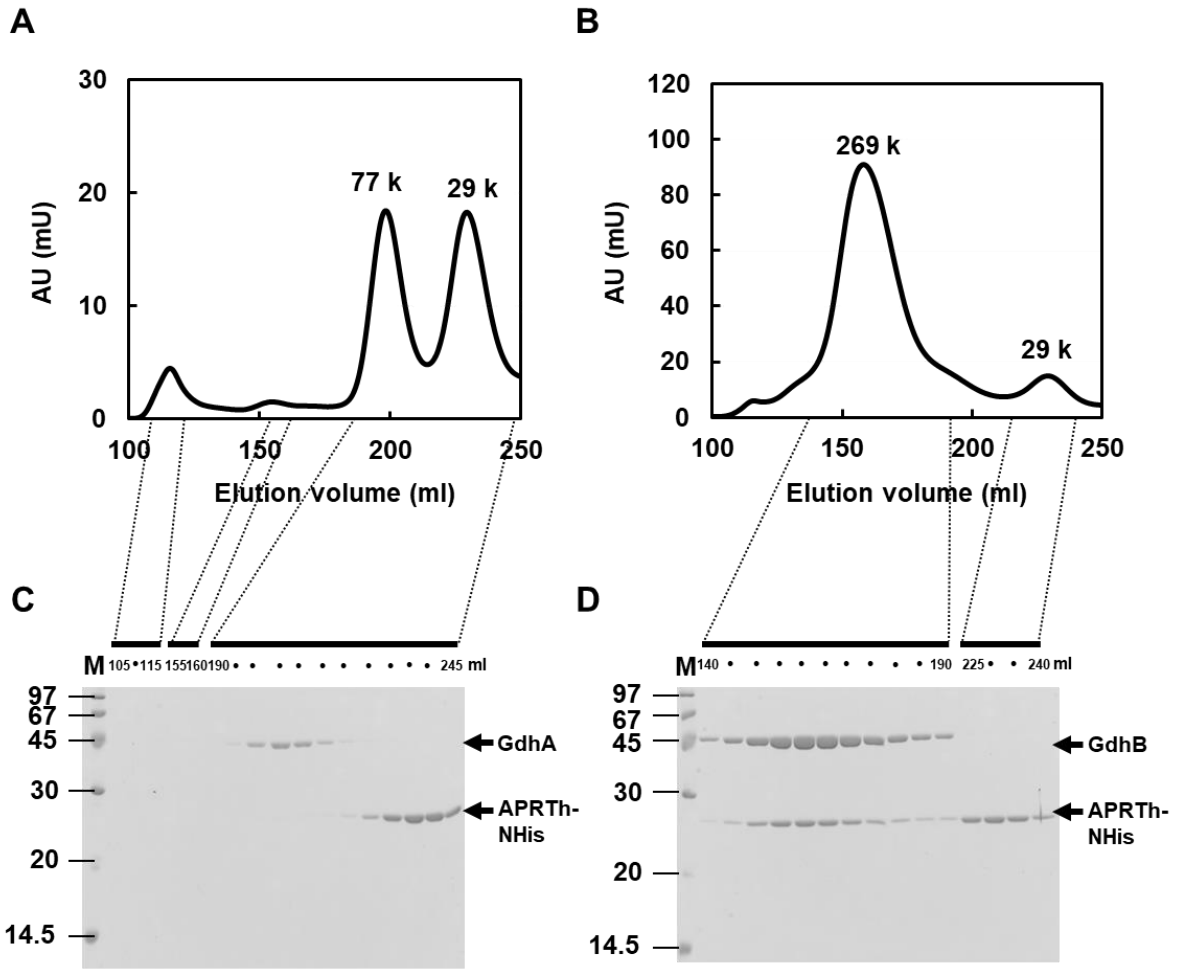

FIG S2. Gel filtration column chromatography of proteins co-purified with His-tagged APRTh

(A) ARPTh with his-tag at the N-terminus and GdhA. Chromatogram of absorption at 280 nm. The estimated molecular weights of the peaks are shown. (B) ARPTh with his-tag at the N-terminus and GdhB. Chromatogram of absorption at 280 nm. The estimated molecular weights of the peaks are shown. (C) SDS-PAGE of the collected fractions from chromatography of (A). The number of corresponding elution volumes are indicated for several fractions. (D) SDS-PAGE of the collected fractions from chromatography of (B). The number of corresponding elution volumes are indicated for several fractions.

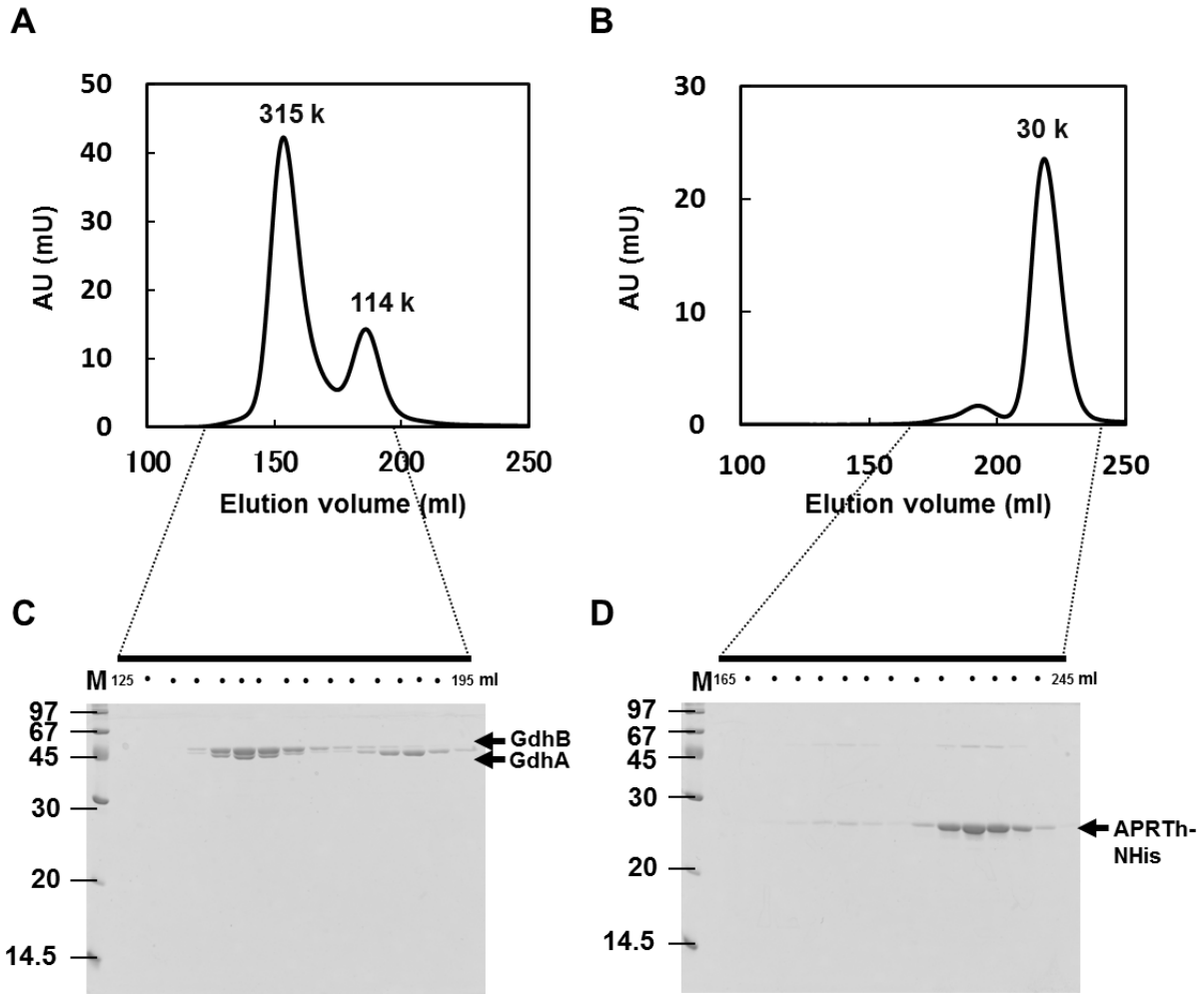

FIG S3. Gel filtration column chromatography of TtGDH (GdhA/GdhB) alone and His-tagged APRTh

(A) TtGDH (GdhA/GdhB) hetero-complex. Chromatogram of absorption at 280 nm. The estimated molecular weights of the peaks are shown. (B) ARPTh with his-tag at the N-terminus. Chromatogram of absorption at 280 nm. The estimated molecular weights of the peaks are shown. (C) SDS-PAGE of the collected fractions from chromatography of (A). The number of corresponding elution volumes are indicated for several fractions. (D) SDS-PAGE of the collected fractions from chromatography of (B). The number of corresponding elution volumes are indicated for several fractions.

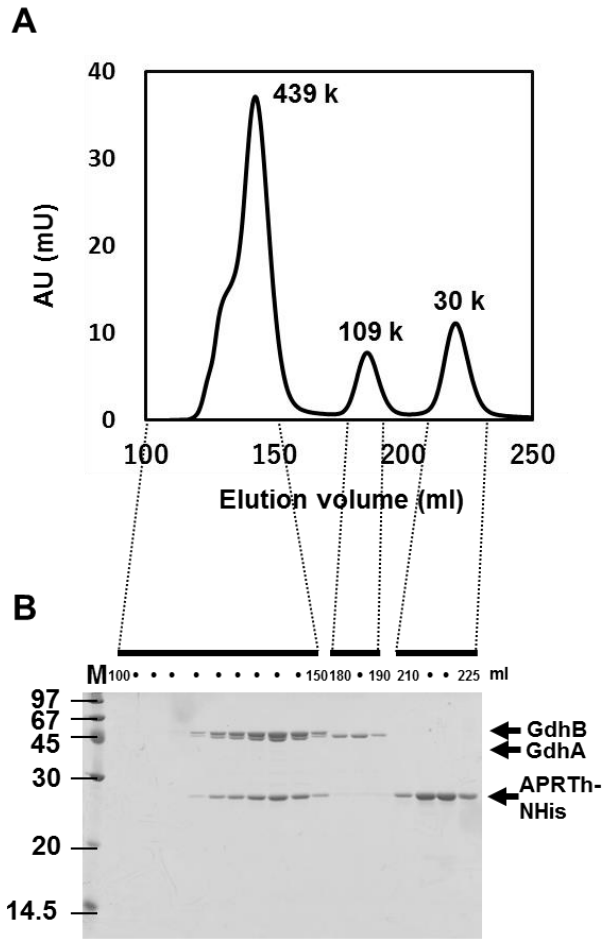

FIG S4. Gel filtration column chromatography of hetero-complex of separately purified TtGDH (GdhA/GdhB) without tag and His-tagged APRTh

(A) TtGDH/APRTh hetero-complex which was formed by incubation of mixture of separately purified TtGDH (GdhA/GdhB) without tag and His-tagged APRTh. Chromatogram of absorption at 280 nm. The estimated molecular weights of the peaks are shown. (B) SDS-PAGE of the collected fractions from chromatography of (A). The number of corresponding elution volumes are indicated for several fractions.

4 mg of purified TtGDH without tag and 4 mg of purified His-tagged APRTh were mixed and incubated at 60 ºC for 30 min. Then, the mixture was equilibrated to room temperature, and the mixture was applied onto gel filtration chromatography using HiLoad 26/60 Superdex 200 pg. The column chromatography was performed in the same condition with that of the analysis of subunit assembly of co-expressed and co-purified TtGDH/APRTh.

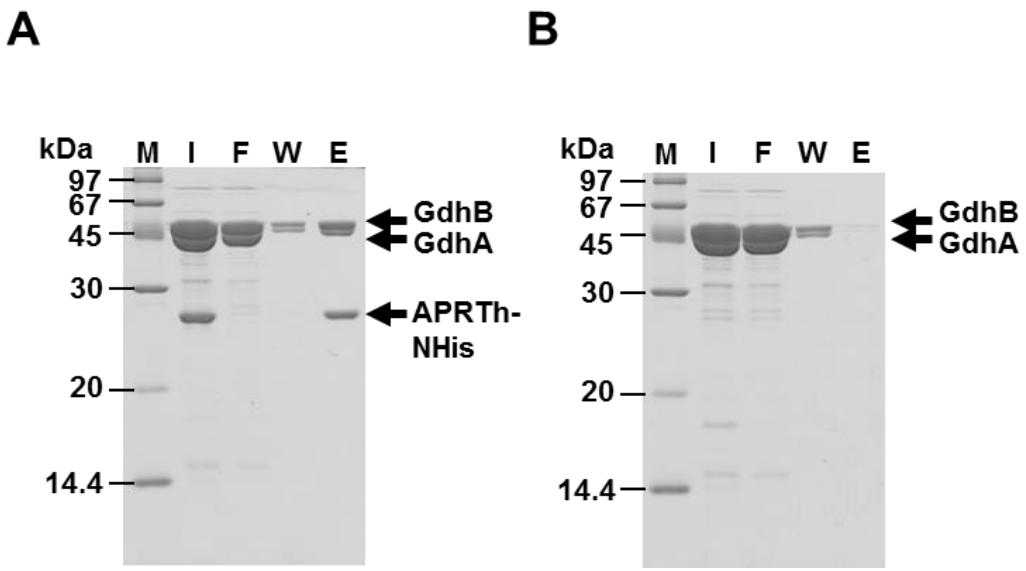

FIG S5. Interaction between separately purified TtGDH (GdhA/GdhB) without tag and His-tagged APRTh.

(A) Pull down assay using TtGDH (GdhA/GdhB) without tag and His-tagged APRTh. (B) Pull down assay using TtGDH (GdhA/GdhB) only. Lane M; Molecular size markers, lane I; the input sample applied on Ni^{2+}-NTA resin, lane F; flow through fraction from the resin, lane W; wash fraction from the resin, lane E; elution fraction from the resin.

0.3 mg of purified His-tagged APRTh and the excess amount (3 mg) of purified TtGDH without tag were mixed and incubated at 60 ºC for 10 min. After the mixture was equilibrated to room temperature, the mixture was applied onto Ni^{2+}-NTA resin. After the sample was passed, the resin was washed with 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 20 mM Imidazole. The absorbed proteins were eluted with 20 mM Tris-HCl (pH8.0), 150 mM NaCl, 500 mM Imidazole.

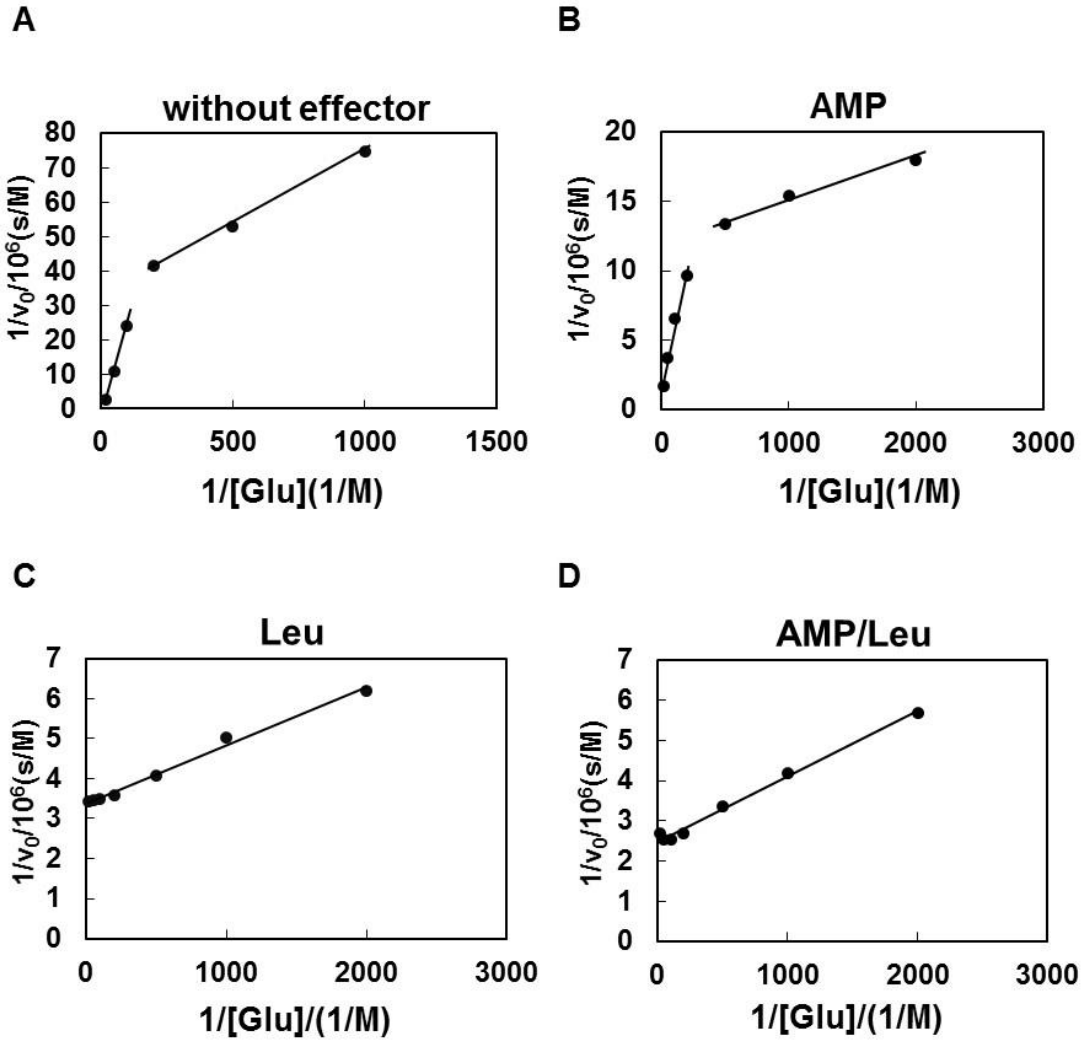

FIG S6. Negative cooperativity in the oxidative deamination reaction of TtGDH/APRTh.

Lineweaver-Burke plots of the oxidative deamination reaction at varied Glu concentration. The negative cooperativity of the reaction was observed in the absence of leucine. Marked breaks at 5~10 mM and 2~5 mM were observed in the plots of without effector and with AMP, respectively. (A) The plot without effector. (B) The plot in the presence of AMP. (C) The plot in the presence of leucine. (D) The plot in the presence of AMP and leucine.

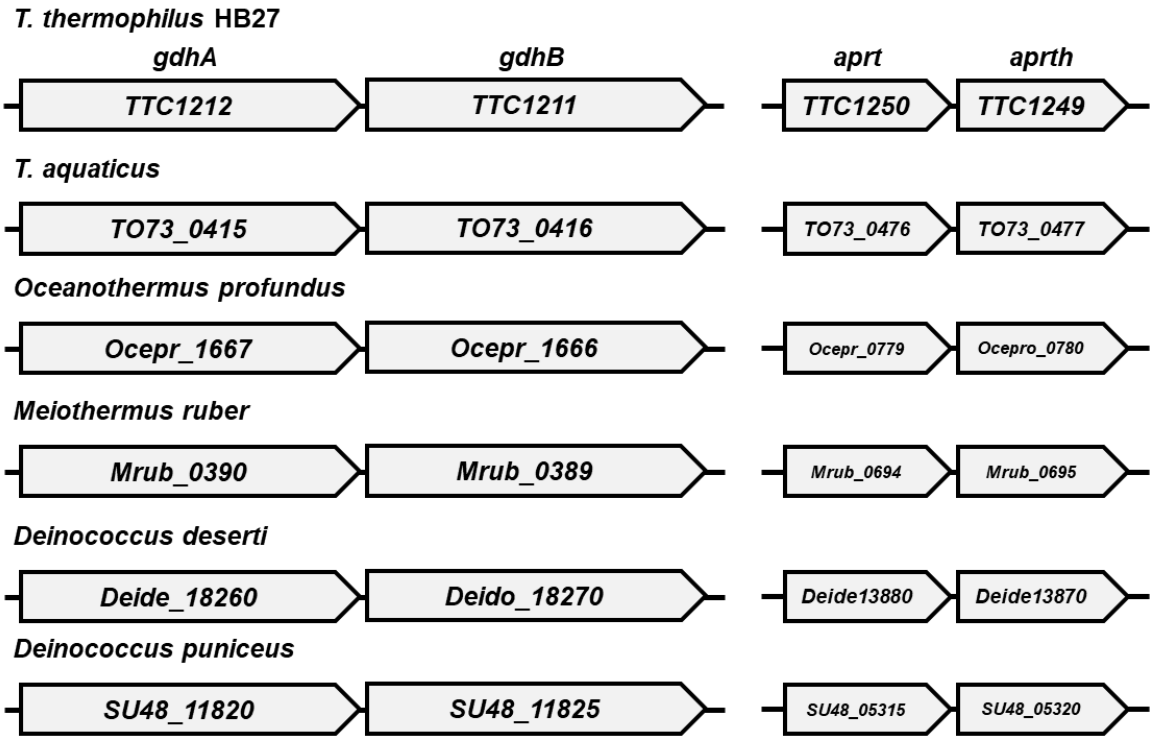

FIG S7. The tandem gene coordination of *gdh* and *aprt* in the genome of several organisms

Table S1 Oligonucleotides used for construction of the plasmids for homologous recombination of *T. thermophilus*.

For construction of pNHisGdhA	
gdhA-up-fw-NotI	AAGGAAAAAAGCGGCCGCGACGCCATCATCGTGGACGAG
gdhA-up-rv-BamHI	CGCGGATCCAGCATCACCGCCGAGGATTAT
hyg10-fw-BamHI	CGCGGATCCATTCGGCCCAAGGTTTACAAA
hyg10-rv-PstI	AACTGCAGCCCGGGGGGAGTATAACAGAA
pslpA-fw-PstI	AACTGCAGCCCGGGGGGAGTATAACAGAA
pslpA-rv-ClaI/NdeI	CCATCGATACATATGCCTCACACCCCCTTAAGGGTC
gdhANHis12-fw-ClaI/NdeI	CCATCGATACATATGCACCATCACCATCACCATCACCATCACCATCACCATGGTGGTCCGCTAAAAGCCTACCGGCCC
gdhANHis12-rv-KpnI	CGGGGTACCTTACGGGTACACGCCCCGAAG
NHisGdhAcheck-fw	CGCGGATCCAGGAGACCCCGCTGGATCTCT
NHisGdhAcheck-rv	CCCCAAGCTTCCAGGTAGGGGACGACCCGG
For construction of pNStHisAPRTh	
APRThup_Fw_BamHI	TCTAGAACTAGTGGATCCCACCACGATGTGGTGGG
APRThup_Rv_EcoRI	TCCGCCGTCAACGGAATTCAGGGTCCTCCTACTCC
APRThhtkStHis_Fw_EcoRI	AGTAGGAGGACCCTGAATTCCGTTGACGGCGGATA
APRThhtkStHis_Rv_HindIII	GGGTAGGTCTCCATAAGCTTGTGGTGGTGGTGGTG
APRTh_Fw_HindIII	CACACCACCACAAGCTTATGGAGACCTACCCCATC
APRTh_Rv_KpnI	AACAAAAGCTGGGTACCTGGGGGTGGCCAAGCCCG
NStHisAPRThcheck-fw	AAGGAAAAAAGCGGCCGCTCCGGAGGAACTGGC
NStHisAPRThcheck-rv	GGGCCTCGCCCCTGAGGAGAC
For construction of pΔAPRTh	
dAPRThup_Fw_BamHI	TCTAGAACTAGTGGATTCCACCACGATGTGGTGGG
dAPRThup_Rv_EcoRI	TCCGCCGTCAACGGAATTCAGGGTCCTCCTACTCC
dAPRThhtk_Fw_EcoRI	AGTAGGAGGACCCTGAATTCCGTTGACGGCGGATA

dAPRThhtk_Rv_HindIII	GGCGCCGGGCTTAGAAGCTTCGTAACCAACATGAT
dAPRThdn_Fw_HindIII	TCATGTTGGTTACGAAGCTTCTAAGCCCGGCGCCC
dAPRThdn_Rv_KpnI	GGGAACAAAAGCTGGGTACCGACGTGCCCGTGGAC
dAPRThcheck-fw	ATTGAGAGCGTGGGGGGTGAG
dAPRThcheck-rv	GGGCCTCGCCCCTGAGGAGAC

Table S2 Oligonucleotides used for construction of the plasmids for expression of recombinant proteins in *E. coli*.

For construction of pET-GdhAB/APRThNH, a plasmid for co-expression of recombinant GdhA, GdhB, and APRTh fused with his tag at the N-terminus.	
gdhA-fw-HindIII/NdeI	CCCAAGCTTCATATGCCGCTAAAAGCCTAC
gdhA-rv-EcoRI	GGGGAATTCTTACGGGTACACGCCCCG
gdhB-fw-EcoRI	GGGGAATTCTAAGAAGGAGATATACATATGCCGCTAAAAGCCTAC
gdhB-rv-SalI	AAACGCGTCGACTTAAGGGTATAGGCCCCG
NHisAPRTh-fw-SalI	AAAGTCGACTAAGAAGGAGATATACAAATGAAACACCACCACCAC
NHisAPRTh-rv-HindIII	CCCAAGCTTTTAGAGCACGGGAAGCTCCGC
For the deletion of *Hin*dIII restriction site in *gdhB* gene in pET-gdhA/gdhB plasmid.	
gdhB-dHindIII-fw	GACCCGAGGAAGCTGTCCCCCGGGGAGCTG
gdhB-dHindIII-rv	CAGCTCCCCGGGGGACAGCTTCCTCGGGTC
For construction of pET-gdhA/APRThNH, a plasmid for co-expression of recombinant GdhA, and APRTh fused with his tag at the N-terminus.	
gdhA-fw-EcoRI/NdeI	GGGGAATTCCATATGCCGCTAAAAGCCTAC
gdhA-rv-SalI	AAACGCGTCGACTTACGGGTACACGCCCCG
For construction of pET-gdhB/APRThNH, a plasmid for co-expression of recombinant GdhB, and APRTh fused with his tag at the N-terminus.	
gdhB-fw-EcoRI/NdeI	GGGGAATTCCATATGAAGAGCGAACCCCTT
gdhB-rv-SalI	AAACGCGTCGACTTAAGGGTATAGGCCCCG
For construction of pET-APRThCH, a plasmid for expression of recombinant APRTh fused with his tag at the C-terminus.	
APRThCHis-fw-EcoRI/NdeI	GGAATTCCATATGGAGACCTACCCCATC
APRTCHis-rv-BamHI	CGCGGATCCTTAGTGATGGTGATGGTGATGGAGCACGGGAAGCTCCGC
For construction of pET-APRTCH, a plasmid for expression of recombinant APRT fused with his tag at the C-terminus.	
APRTCHis-fw-BamHI/NdeI	GGGATCCTAAGAAGGAGATATACATATGAGGACCTACCCCGTT
APRTCHis-rv-EcoRI	GGAATTCTTAGTGATGGTGATGGTGATGCTCCGGCTTGAAGAGGGG