

Supplementary Materials

Supplementary figure 1: Enzymatic activity of wild-type *G/LeuRS* and *G/LeuRS* CP2 domain deletion mutant.

(A) The ATP-PPi exchange reaction was carried out using 1 mM Leu with 20 nM *G/LeuRS* (●) and *G/LeuRS*- Δ CP2 (○). (B) Post-transfer editing was performed using 2 μ M [3 H]-Ile-tRNA^{Leu} from *G/tRNA*^{Leu} with 20 nM *G/LeuRS* (●) and *G/LeuRS*- Δ CP2 (○). Control (spontaneous hydrolysis) (▼) was performed in the absence of enzyme.

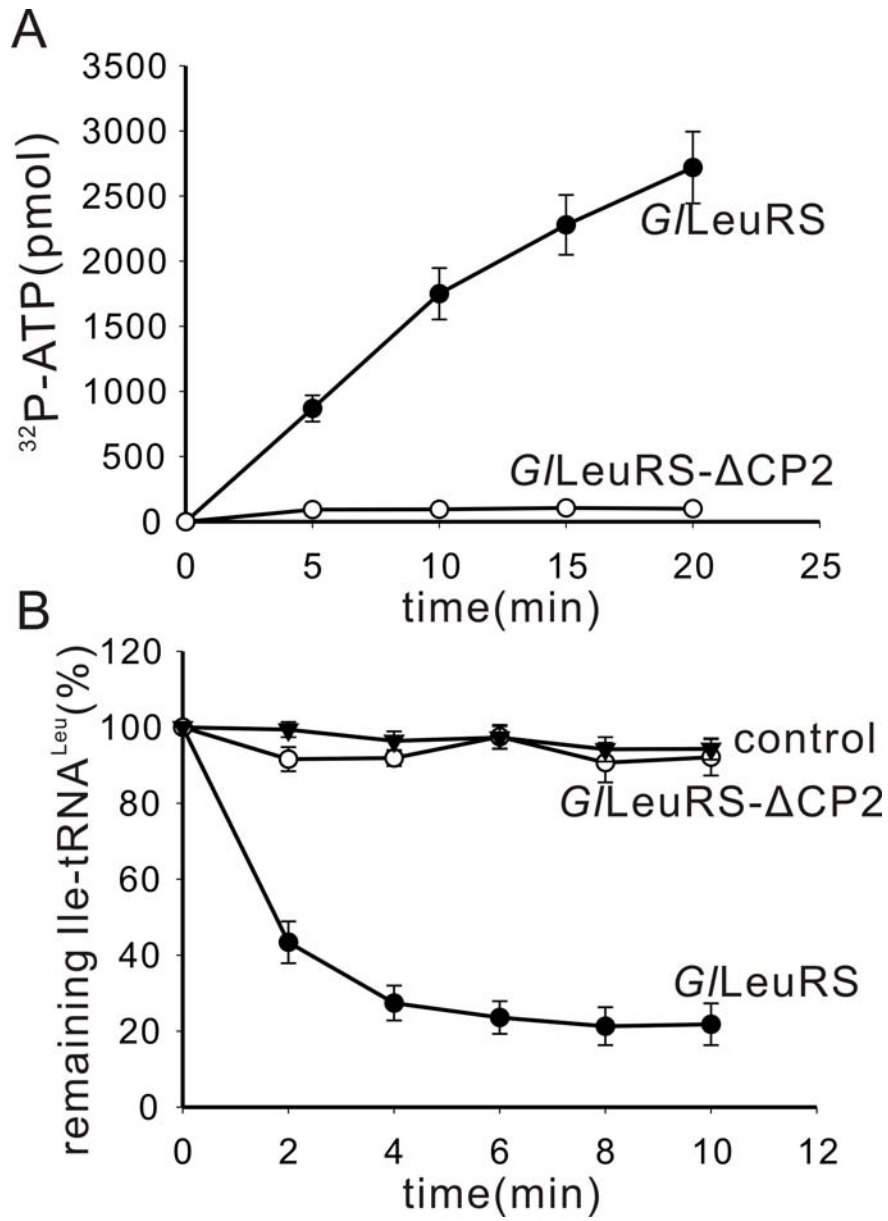
Supplementary figure 2: The conservation of the CP2 domain function in LeuRSs from prokaryotic and archaeal groups.

(A) The ATP-PPi exchange reaction of *EcLeuRS* (●) and its CP2 domain deletion mutant (○) was carried out using 1 mM Leu and 20 nM enzyme. (B) Post-transfer editing was performed using 2 μ M [3 H]-Ile-tRNA^{Leu} from *Ec*tRNA^{Leu}, and 20 nM *EcLeuRS* (■) and its CP2 domain deletion mutant (○). Controls (spontaneous hydrolysis) (▼) were performed in the absence of enzyme. (C) The ATP-PPi exchange reaction of *PhLeuRS* (●) and its CP2 domain deletion mutant (○) was carried out using 2 mM Leu, and 50 nM *PhLeuRS* and its CP2 domain deletion mutant at 65°C. (D) Post-transfer editing was performed using 2 μ M [3 H]-Ile-tRNA^{Leu} from *Ph*tRNA^{Leu}, and 100 nM *PhLeuRS* (○) and its CP2 domain deletion mutant (●) at 37°C. Controls (spontaneous hydrolysis) (▼) were performed in the absence of enzyme.

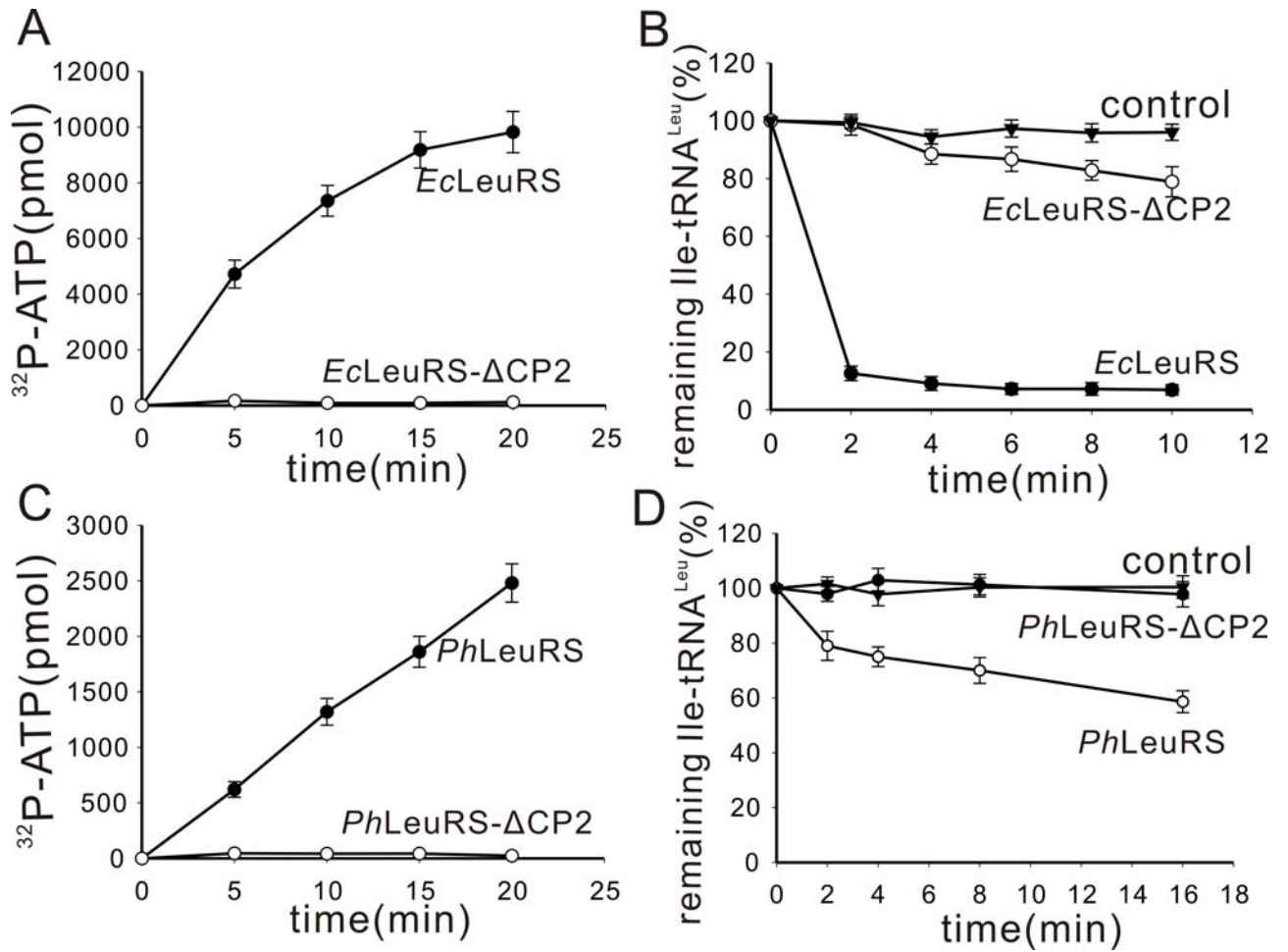
Supplementary table 1. Kinetics of *G/LeuRS* and its mutants in ATP-PPi exchange reaction.

Enzyme	ATP			Leu		
	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1}\text{mM}^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1}\text{mM}^{-1}$)
WT	750.4 ± 61.2	34.5 ± 2.3	46.0	14.2 ± 1.5	30.5 ± 3.5	2147.8
K587A	779.8 ± 80.6	15.6 ± 2.6	20.0	14.6 ± 3.2	13.1 ± 2.1	897.3
D588A	701.8 ± 77.3	18.0 ± 2.9	25.6	17.8 ± 2.9	14.0 ± 2.4	786.5
D603A	775.3 ± 83.5	32.3 ± 3.2	41.7	13.8 ± 1.9	27.9 ± 3.4	2021.7
K606R	735.7 ± 68.9	33.1 ± 3.9	45.0	13.6 ± 2.5	29.0 ± 3.1	2132.4
K606E	789.2 ± 76.2	32.7 ± 3.2	41.3	15.0 ± 3.4	32.0 ± 4.1	2133.3
K606L	803.4 ± 96.2	31.5 ± 2.5	39.2	14.9 ± 2.6	31.3 ± 3.6	2100.7
K606D	813.3 ± 92.1	32.1 ± 3.6	39.5	16.0 ± 3.3	28.5 ± 3.5	1781.3

All values were the average of 3 experiments.



Supplementary figure 1



Supplementary figure 2