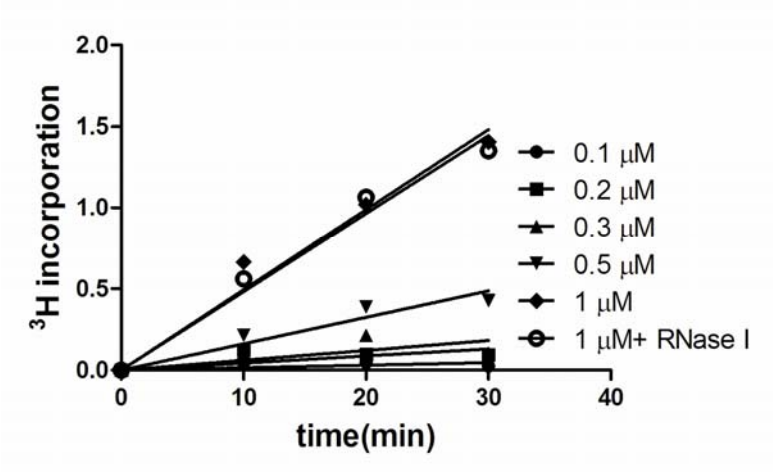
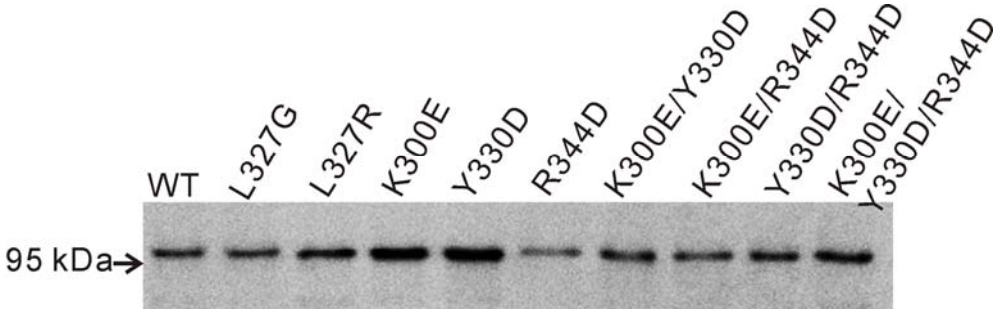


Supplementary Figure 1

Supplementary Figure 1: Self-aminoacylation of *Ec*LeuRS.

Self-aminoacylation of *Ec*LeuRS was performed in the absence of tRNA. The self-aminoacylation reaction was carried out in the reaction solution containing 100 mM Tris-HCl (pH 7.8), 30 mM KCl, 12 mM $MgCl_2$, 0.5 mM dithiothreitol, 4 mM ATP, 20 μM [^{3}H]leucine (15 Ci/mmol) and different concentrations of *Ec*LeuRS at 37 °C. RNase I (final 0.25 U/μl) was added to the 1 μM *Ec*LeuRS reaction mixture. At specific times, aliquots of the reaction solution were removed and quenched on Whatman filter pads, and equilibrated with cold 5% TCA. The pads were sequentially washed with 5% TCA 3 times and with 100% ethanol 3 times. The filter pads were dried, and the radioactivities of the precipitates were quantified using a scintillation counter.



Supplementary Figure 2

Supplementary Figure 2: Western blot analysis of the expression of *Ec*LeuRS and its mutants in KL231.

KL231 cells containing the genes encoding the wild-type (WT) and mutated *Ec*LeuRSs were grown at 42°C overnight. The cells were harvested by centrifugation, lysed by sonication, and cell debris removed by centrifugation. Equal amounts of the protein-containing supernatants (30 μg) were electrophoretically separated on 10% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (NEN Research Products, Boston, MA). The membrane was blocked with 5% non-fat dry milk in PBST (1×PBS plus 0.5‰ Tween-20) and incubated with a 1:2000 dilution of anti-His_6 antibody at room temperature for 2 hours. Subsequently, a 1:5000 dilution of the secondary goat-anti-mouse IgG was added and incubated at room temperature for 2 hours. The bound antibody was visualized by adding the Western Lightning horseradish peroxidase chemiluminescence solution (Perkin-Elmer) and exposing to Fujifilm LAS-4000 (FUJIFILM Life Science).