Supplementary Information

Transfer RNA synthesis-coupled translation and DNA replication in a reconstituted transcription/translation system

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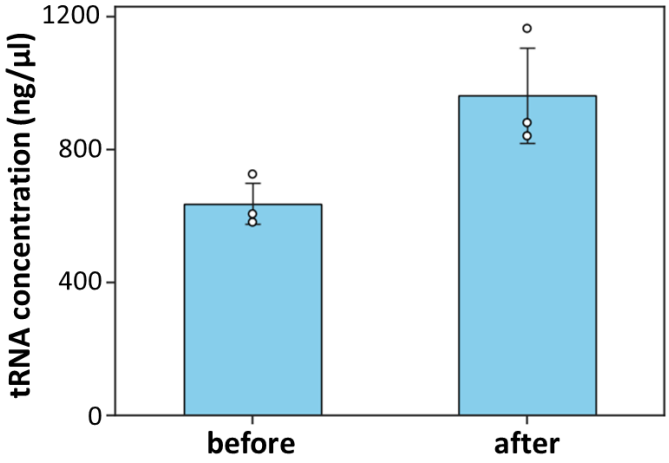

Figure S1. Improvement of *in vitro* transcription

Comparison of *in vitro* tRNA synthesis before and after the modification of the reaction mixture. The reaction conditions are described in Materials and Methods. After incubation, the reaction mixture was subjected to 1% agarose gel electrophoresis, and the tRNA concentration was quantified based on band intensities after 20 min of staining with a fluorescent dye (SAFELOCK Green). Each dot represents three independent results. Error bars represent standard deviation.

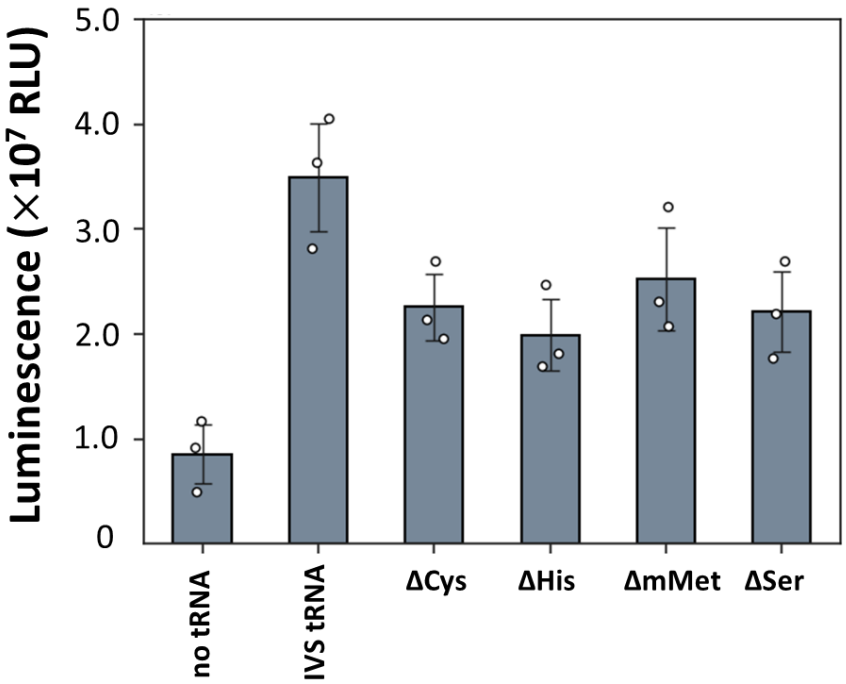

Figure S2. Translation activity in each tRNA-omitted PURE system before repurification

The laboratory-made PURE systems containing different IVS tRNA compositions were incubated with a DNA fragment encoding luciferase (1 nM) and T7 RNA polymerase (0.42 $U \cdot \mu L^{-1}$) at 30°C for 16 h, and luciferase activity was measured. Each dot represents three independent experiments. Error bars represent standard deviation.

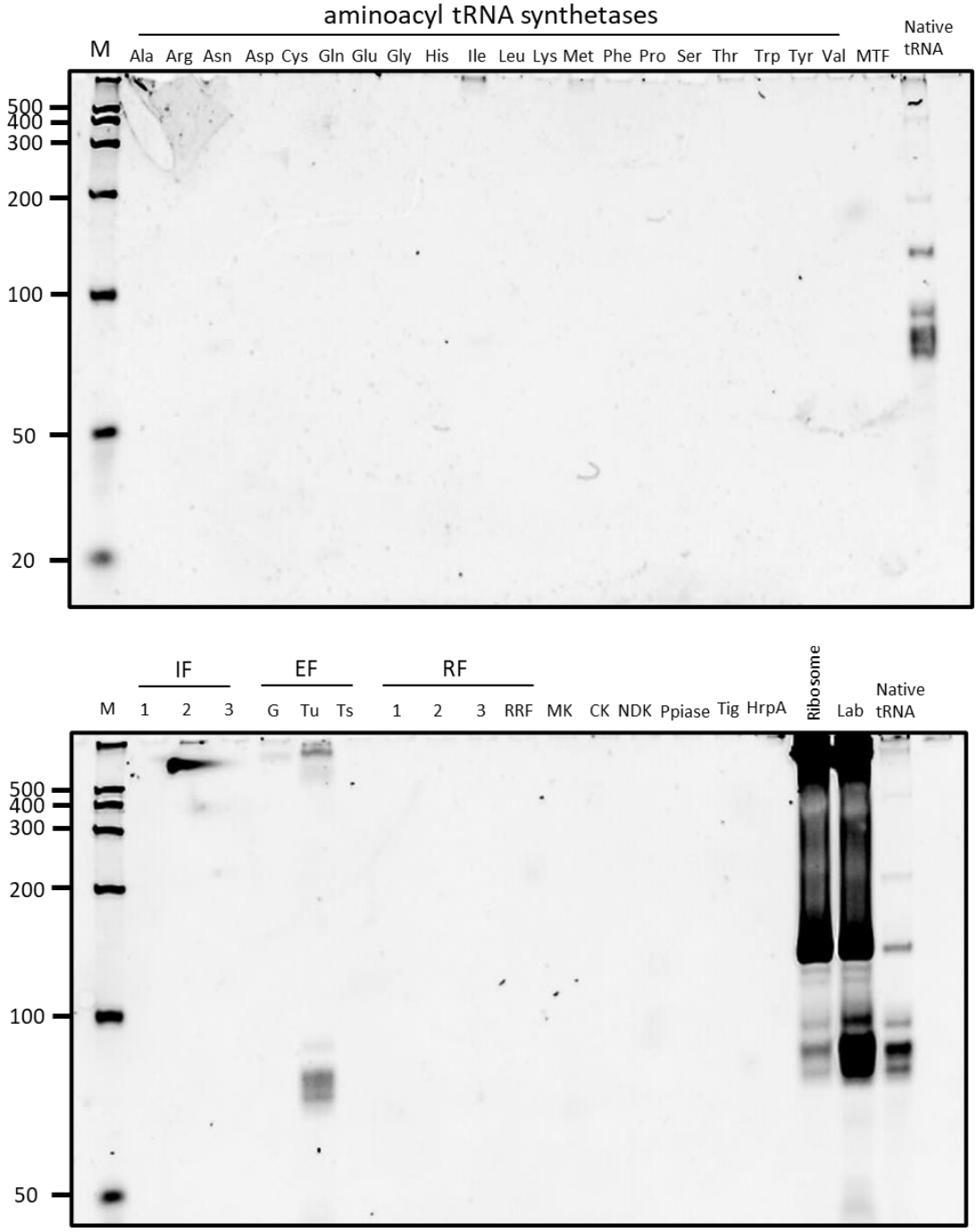

Figure S3. Urea–PAGE analysis of each purified translation factor

Each purified translation factor, corresponding to 5 μL of the PURE system, was subjected to urea–PAGE, and RNA was stained with SYBR Green II. The laboratory-made PURE system (Lab) and native *Escherichia coli* tRNA (100 ng, native tRNA) were used for comparison.

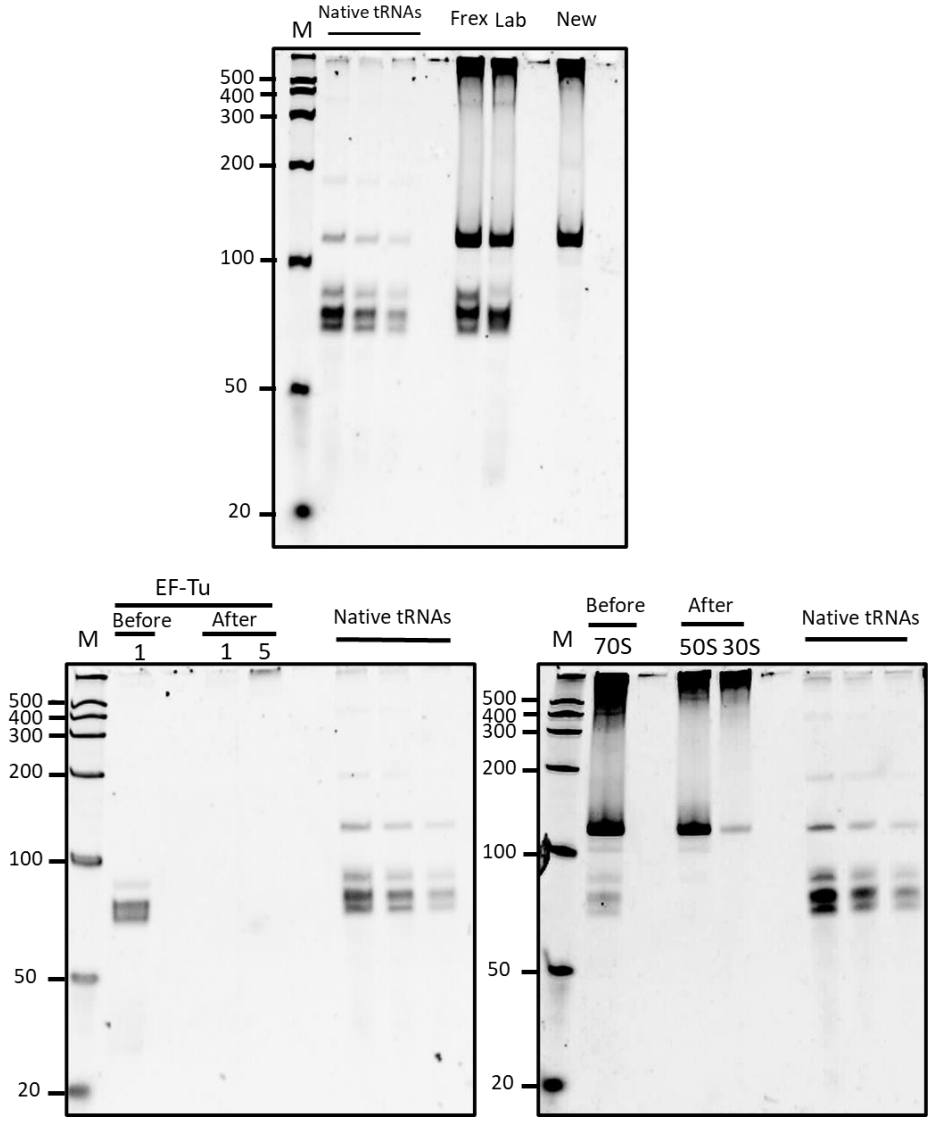

Figure S4. The full images of Figs. 2A and 2B

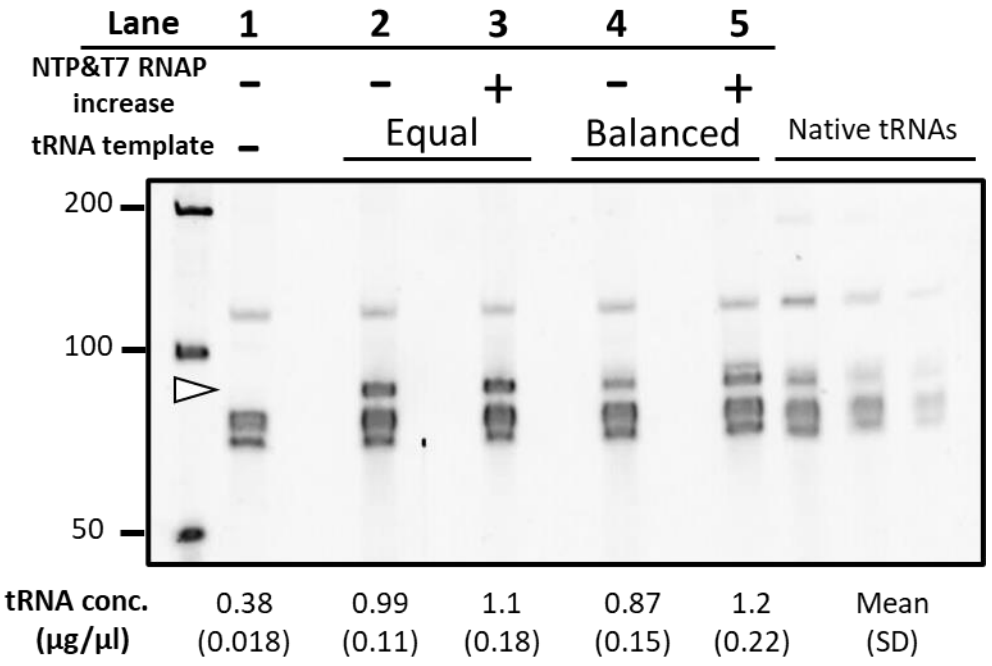

Figure S5. Urea–PAGE analysis of reaction mixtures after the simultaneous expression of 15 tRNAs

The reaction mixtures (0.1 μL) after the reaction performed in Fig. 4B were subjected to urea–PAGE with the native *E. coli* tRNA as a control. The mean values (standard deviation) of tRNA concentration quantified from band intensities of three independent experiments are shown at the bottom. The white arrowhead indicates the high-molecular-weight tRNA bands, corresponding to $tRNA^{Leu}$, $tRNA^{Tyr}$, and $tRNA^{Ser}$.