Supplemental Materials

Fig. S1. Identification of the EF-Tu-binding sites on bovine mt tRNA$^{Ser}_{GCU}$ by the ENU modification interference assay. (Left) Autoradiographs of the ENU-modification interference assay using *T. thermophilus* EF-Tu. The lanes are the same as described in Figure 1C. (Right) Sites of bovine mt tRNA$^{Ser}_{GCU}$ that are bound by canonical EF-Tu. The tRNA is portrayed as simplified backbone with the aminoacyl moiety depicted by a filled circle. The phosphate positions recognized by each EF-Tu are indicated by arrowheads in the left and right panels.

Fig. S2. Autoradiographs of the ENU-modification interference assay using bovine mt EF-Tu (*A*) and *C. elegans* mt EF-Tu2 (*B*). These are the results for the region that spans from the D-arm replacement loop of bovine mt tRNA$^{Ser}_{GCU}$ to the anticodon arm. The lanes are the same as described in Figure 1C. The numbers at the left and right of the autoradiographs are the positions of G. The ladder of the 5'-labeled ENU-modified fragments is shifted upwards a bit compared to the alkaline ladder because of its 3'-phosphate ethylation.

Fig. S3. Sequences of tRNA^{Ser}s that can bind to EF-Tu2. We have confirmed EF-Tu2 binding to the following tRNA transcripts charged with serine; *C. elegans* mt tRNAs^{Ser} (T. Ohtsuki, unpublished observation), *A. suum* mt tRNAs^{Ser} (1), mutated *A. suum* mt tRNAs$^{Ser}_{UCU}$ (1, this study), bovine mt tRNA$^{Ser}_{GCU}$ (1), and the G15-deletion mutant of *E. coli* tRNA$^{Ser}_{GGA}$ (this study). Modified bases and the CCA end sequence are not included.

1. T. Ohtsuki, A. Sato, Y. Watanabe, K. Watanabe (2002) *Nat. Struct. Biol.*, **9**, 669-673.

Table S1. Half-lives (min) of the aminoacyl-bond of *E. coli* Ser-tRNA$^{Ser}_{GGA}$ and its mutants in the presence of EF-Tu2

	wild-type	transcript	G15-deletion	C48-deletion	G15A
without EF-Tu	27	35	28	29	27
EF-Tu2	35	65	165	151	120
E.coli EF-Tu	1155	385	315	495	2310

These values were estimated from the deacylation curves shown in Figure 2.

Table S2. Half-lives (min) of the aminoacyl-bond of nematode mt Ser-tRNA$^{Ser}_{UCU}$ and bovine mt Ser-tRNA$^{Ser}_{GCU}$ in the presence of EF-Tu2 mutants

	A. suum mt tRNA$^{Ser}_{UCU}$	bovine mt tRNA$^{Ser}_{UCU}$
without EF-Tu	35	44
EF-Tu2	161	248
(-) 3aa	79	81
(-) 7aa	36	41
K429A	128	133
K433A	65	78
K434A	133	131
K438A	80	68

These values were estimated from the deacylation curves shown in Figure 4.