

Expanded View Figures

hFTSJ1/Trm7/*E. coli* FTSJ

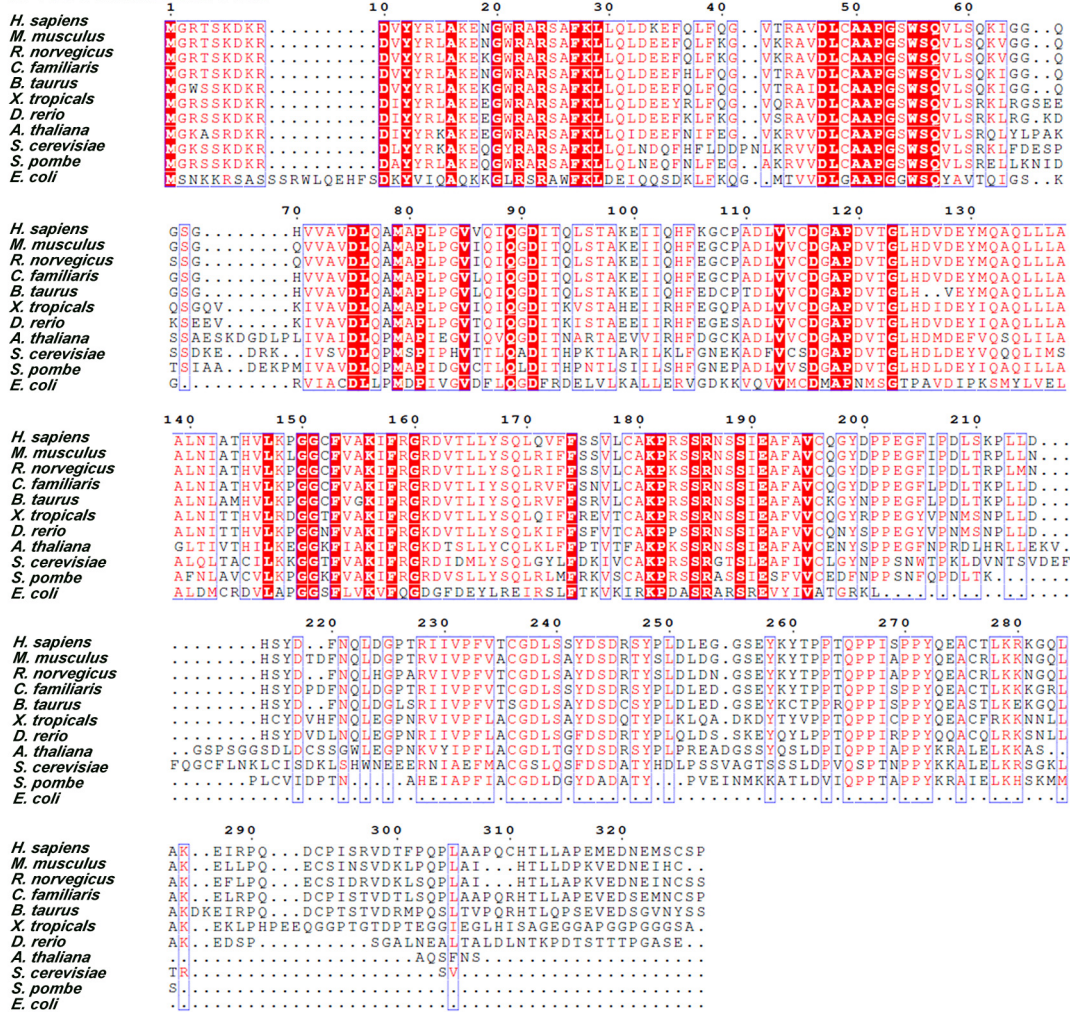


Figure EV1. Sequence alignments of human FTSJ1, eukaryotic Trm7, and *E. coli* FTSJ/RrmJ.

H. sapiens, Homo sapiens; *M. musculus*, *Mus musculus*; *R. norvegicus*, *Rattus norvegicus*; *C. familiaris*, *Canis familiaris*; *B. taurus*, *Bos taurus*; *X. tropicalis*, *Xenopus tropicalis*; *D. rerio*, *Danio rerio*; *A. thaliana*, *Arabidopsis thaliana*; *S. cerevisiae*, *Saccharomyces cerevisiae*; *S. pombe*, *Schizosaccharomyces pombe*; *E. coli*, *Escherichia coli*.

Source data are available online for this figure.

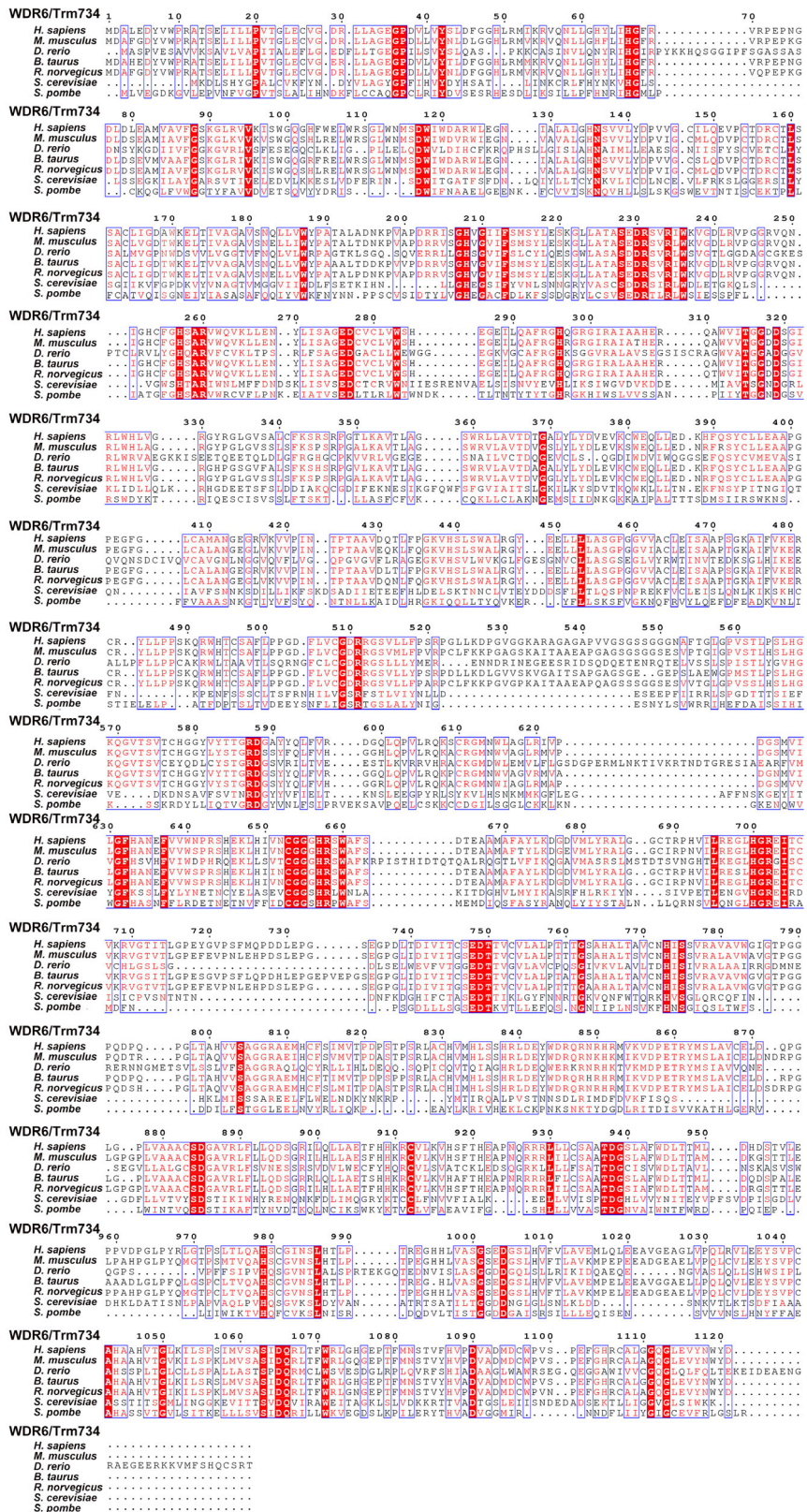


Figure EV2. Sequence alignments of WDR6 or Trm734 from different species.

S. cerevisiae and *S. pombe* contain Trm734, the auxiliary protein for Trm7. Some higher eukaryotes, such as *H. sapiens*, *M. musculus*, *D. rerio*, *B. taurus*, and *R. norvegicus*, contain the Trm734 homolog, WDR6.

Source data are available online for this figure.

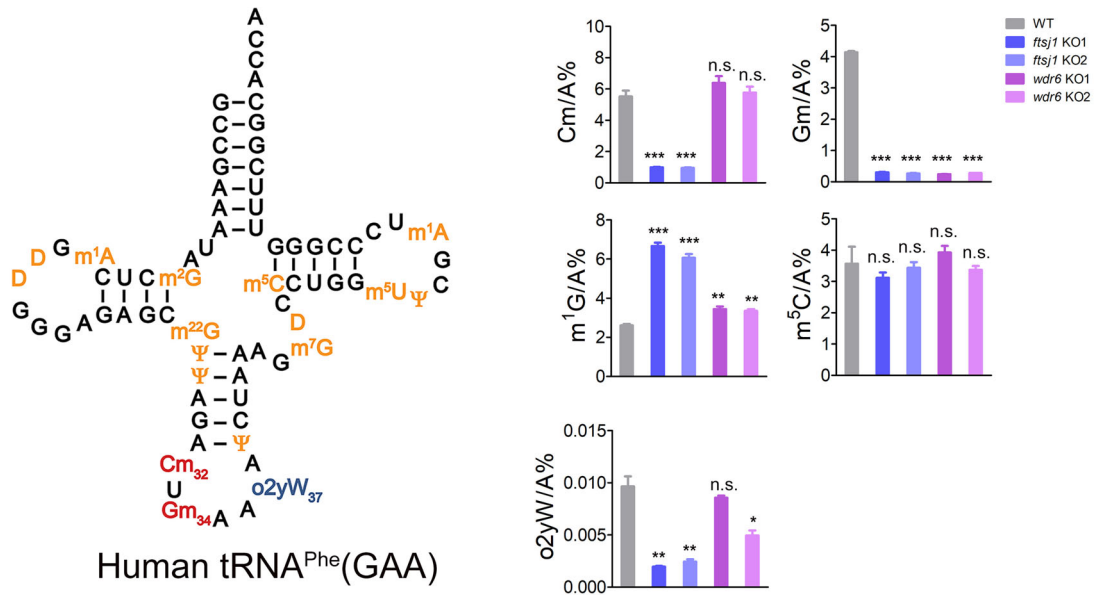


Figure EV3. Knockout of *ftsj1* and/or *wdr6* affects Cm₃₂, Gm₃₄, m¹G₃₇, and o²yW₃₇ levels of tRNA^{Phe}(GAA).

Quantification of the Cm/A, Gm/A, m¹G/A, m⁵C/A, and o²yW/A ratios in endogenous tRNA^{Phe}(GAA) isolated from WT, *ftsj1* KO, and *wdr6* KO cell lines by UPLC-MS/MS analysis. Error bars represent the standard deviation of three independent experiments. *P* values were determined using two-tailed Student's *t*-test for paired samples. **P* < 0.05, ***p* < 0.01, ****p* < 0.001. n.s., no significance.

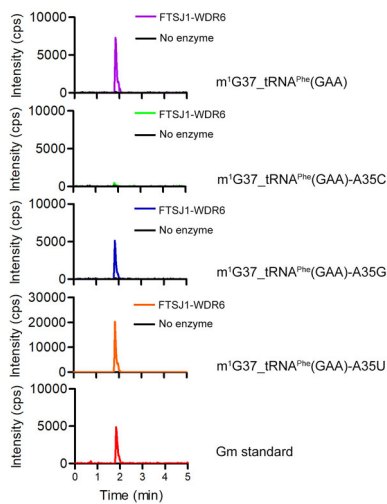


Figure EV4. UPLC-MS/MS analysis of Gm₃₄ of m¹G₃₇-tRNA^{Phe}(GAA), m¹G₃₇-tRNA^{Phe}(GAA)-A35C, m¹G₃₇-tRNA^{Phe}(GAA)-A35G and m¹G₃₇-tRNA^{Phe}(GAA)-A35U after incubation with or without FTSJ1-WDR6. 2 μl of Gm standard (1 ng/ml) was loaded as control. cps, counts per second.

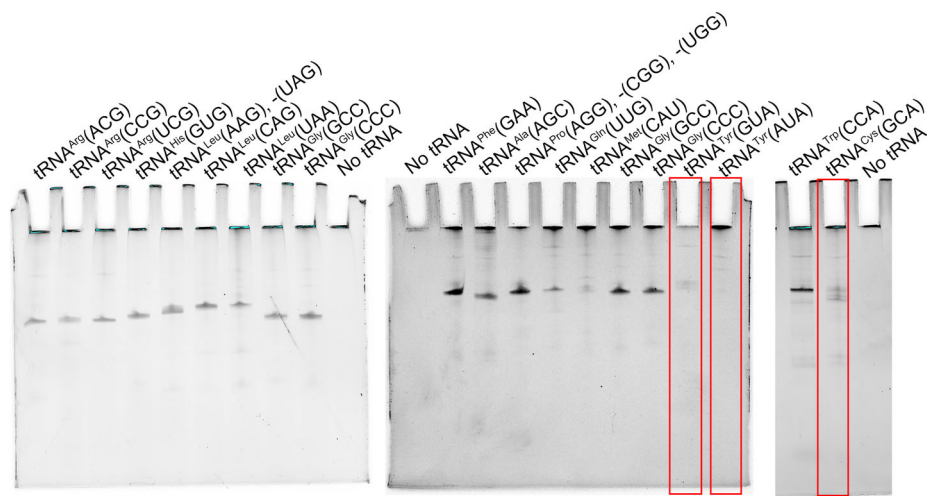


Figure EV5. Analysis of the purity of the fished tRNAs by biotinylated DNA probes using urea PAGE.

Among them, tRNA^{Cys}(GCA), tRNA^{Tyr}(GUA), or tRNA^{Tyr}(AUA) could not be purified (in red box).