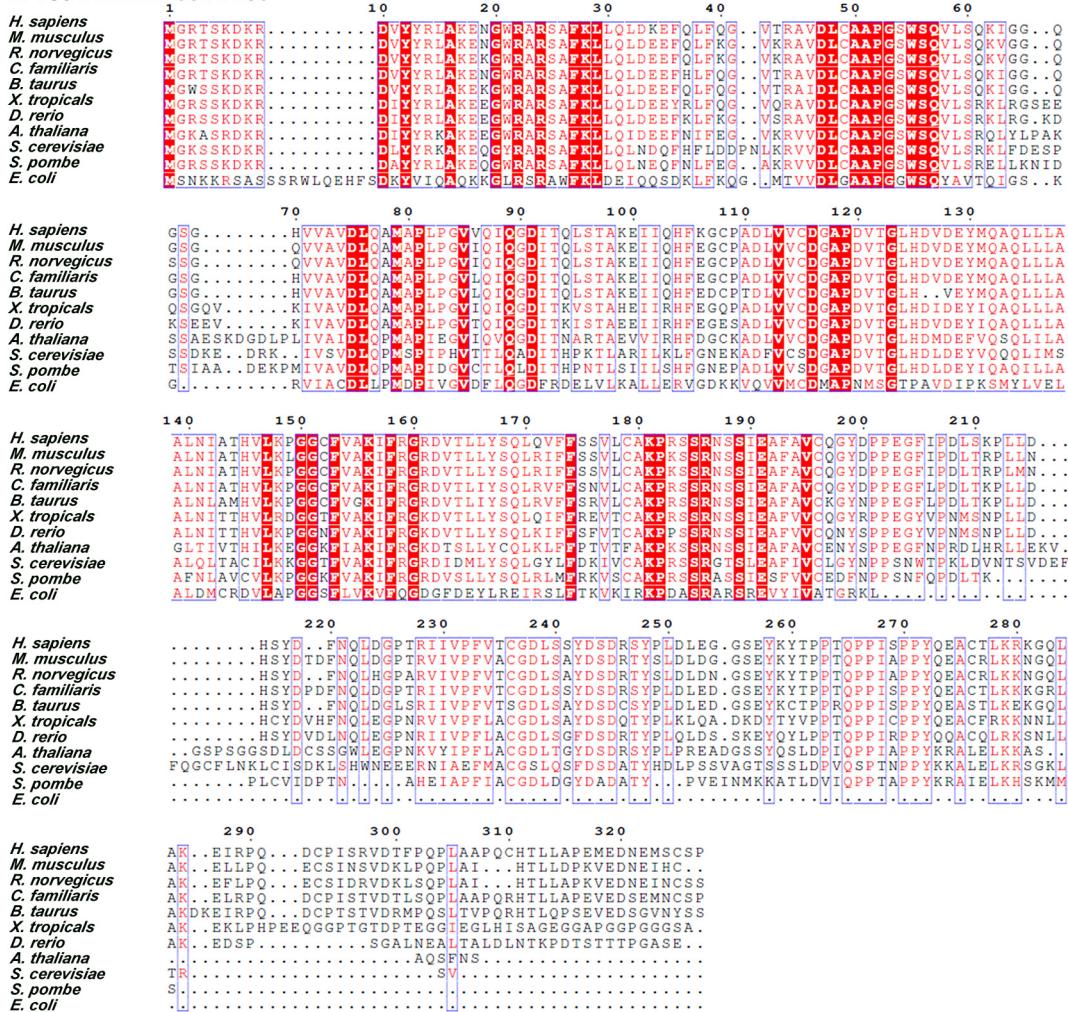


Expanded View Figures

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.

| WDR6/Tmr734 | 1 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
|----------------------|--|-------------------|-----------------------------|-----------------------------|-----------------------------|---------------------------|-------------------|---------------------|
| <i>H. sapiens</i> | M D P L E Q V W Y N R A T S E L I L L Y V T G C E G V E . . . | D R . . . | L L A G G E G P . . . | G V L Y N D . . . | D F G G H I R M . . . | K R V Q N L H Y T H . . . | H G F R . . . | P R V P E N K . . . |
| <i>M. musculus</i> | M D P L C Q V W Y N R A T S E L I L L Y V T G C E G V E . . . | D R . . . | L L A G G E G P . . . | G V L Y N D . . . | D F G G H I R M . . . | K R V Q N L H Y T H . . . | H G F R . . . | P R V P E N K . . . |
| <i>D. rerio</i> | M A N S P E V A S V Y N R A T S E L I L L Y V T G C E G V E . . . | D R . . . | L L A G G E G P . . . | G V L Y N D . . . | D F G G H I R M . . . | K R V Q N L H Y T H . . . | H G F R . . . | P R V P E N K . . . |
| <i>B. taurus</i> | M D H A D G Y W Y N R A T S E L I L L Y V T G C E G V E . . . | D R . . . | L L A G G E G P . . . | G V L Y N D . . . | D F G G H I R M . . . | K R V Q N L H Y T H . . . | H G F R . . . | P R V P E N K . . . |
| <i>R. norvegicus</i> | M D A F G D Y W Y N R A T S E L I L L Y V T G C E G V E . . . | D R . . . | L L A G G E G P . . . | G V L Y N D . . . | D F G G H I R M . . . | K R V Q N L H Y T H . . . | H G F R . . . | P R V P E N K . . . |
| <i>S. cerevisiae</i> | M D P L H S Y G R V L C V Y N R . . . D Y V L A G Y C V E . . . | F R . . . | H Y V D F G G H I R M . . . | K R V Q N L H Y T H . . . | H G F R . . . | P R V P E N K . . . | V Q P E K P . . . | |
| <i>S. pombe</i> | M V B V G D K R V D P W N F V G Y V L C V Y N R . . . | I S L A H I . . . | I N D R V C C Q A C Q . . . | I R V D Y S E S R E D . . . | I S L I R V P H R N R . . . | I C Q P . . . | | |

| | WDR6/Tmr734 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 |
|----------------------|-----------------|----|----|-------|-------|-------|----------|--------|------|--------------|
| <i>H. sapiens</i> | DIDLSCEAMVAVFGC | K | G | FWPEL | GLMMN | W | DWADARWL | P | VVVG | C |
| <i>M. musculus</i> | DIDLSCEAMVAVFGC | K | I | SNGW | PLRSL | W | DWADARWL | P | VVVG | T |
| <i>R. norvegicus</i> | DIDLSCEAMVAVFGC | K | I | SNGW | PLRSL | W | DWADARWL | P | VVVG | T |
| <i>B. taurus</i> | DIDLSCEAMVAVFGC | K | I | SNGW | PLRSL | W | DWADARWL | P | VVVG | T |
| <i>S. cerevisiae</i> | LSSGKEK | I | AY | LRAS | TVT | ELEDV | PLVSL | DFERIN | N | DWATGFATSPDN |
| <i>S. pombe</i> | ...CQKGLFWV | Y | TA | YVAV | MDP | TS | VEYD | RS | DEE | TCV |

| <i>S. pombe</i> | F C A T V Q I S G E N Y I L A S N A F Q Q Y I W K E N Y N N . P P C U S I D T Y V C H E G C A P D L K F S S D O R Y L C S V S B R D T H I M S I E S S P F L . . . |
|---------------------|---|
| WDR17/Tmr734 | 260 270 280 290 300 310 320 |
| <i>H. sapiens</i> | ... I T G H C G B S A N Q V O K L L E N . . X L I S A D E C V C L W S H E G E I D A Q F R Q H G R G I R A A H A H E R . . . Q A W . . . |
| <i>M. musculus</i> | ... I T G H C G B S A N Q V O K L L E N . . X L I S A D E C V C L W S H E G E I D A Q F R Q H G R G I R A A H A H E R . . . Q A W . . . |
| <i>D. rerio</i> | P T C O G C G B S A N Q V O K L L E N . . X L I S A D E C V C L W S H E G E I D A Q F R Q H G R G I R A A H A H E R . . . Q A W . . . |
| <i>C. elegans</i> | I T G H C G B S A N Q V O K L L E N . . X L I S A D E C V C L W S H E G E I D A Q F R Q H G E G R A L A H E R . . . Q A W . . . |

| | 410 | 420 | 430 | 440 | 450 | 460 | 470 | 480 |
|----------------------|------------|--------------|-------------|-------------|----------|----------|----------|----------|
| <i>H. sapiens</i> | PRGGF... | LCALANG... | PTFTAAV... | EEPLL... | EEPLL... | EEPLL... | EEPLL... | EEPLL... |
| <i>M. musculus</i> | PRGGF... | LCALANG... | DQTFP... | GKVVHSLS... | ASAPG... | ASAPG... | ASAPG... | ASAPG... |
| <i>D. discoideum</i> | PRGGF... | LCALANG... | PTFTAAV... | EEPLL... | EEPLL... | EEPLL... | EEPLL... | EEPLL... |
| <i>B. taurus</i> | PEGFG... | LCALANG... | PTFTAAV... | EEPLL... | EEPLL... | EEPLL... | EEPLL... | EEPLL... |
| <i>R. norvegicus</i> | PEGFG... | LCALANG... | PTFTAAV... | EEPLL... | EEPLL... | EEPLL... | EEPLL... | EEPLL... |
| <i>S. cerevisiae</i> | QN... | IAVFSNKSD... | LILLFKSD... | AD1IETT... | PTFL... | PTFL... | PTFL... | PTFL... |
| <i>S. pombe</i> | PEFAAAS... | YIYFQSY... | NLNLRAID... | QKIQQL... | YKQV... | YKQV... | YKQV... | YKQV... |

| WDR6/Tmr734 | 490 | 500 | 510 | 520 | 530 | 540 | 550 | 560 |
|----------------------|--|-----------------------------------|---|---|-----|---------|--|-----|
| <i>H. sapiens</i> | CR...[I] LLEPLPSKQRH WTC I PPGDI | FIVC G BRGSVLLF S P | PGLLKD G VGGKKARAGA G APVGVSSGSSGG S | PTCG T IPV S TL P SLH R | | | | |
| <i>M. musculus</i> | CR...[I] LLEPLPSKQRH WTC I PPGDI | FIVC G BRGSVLLF S P | PGLLKD G VGGKKARAGA G APVGVSSGSSGG S | PTCG T IPV S TL P SLH R | | | | |
| <i>D. rerio</i> | ALLP...[I] LLEPLPSKQRH WTC I PPGDI | FIVC G BRGSVLLF S P | PGLLKD G VGGKKARAGA G APVGVSSGSSGG S | PTCG T IPV S TL P SLH R | | | | |
| <i>B. taurus</i> | ALLP...[I] LLEPLPSKQRH WTC I PPGDI | FIVC G BRGSVLLF S P | PGLLKD G VGGKKARAGA G APVGVSSGSSGG S | PTCG T IPV S TL P SLH R | | | | |
| <i>R. norvegicus</i> | CR...[I] LLEPLPSKQRH WTC I PPGDI | FIVC G BRGSVLLF S P | PGLLKD G VGGKKARAGA G APVGVSSGSSGG S | PTCG T IPV S TL P SLH R | | | | |
| <i>S. cerevisiae</i> | FN...[I] KPEPNFSS TCFSRHN I YLGVS T SLV I NLD I | | | | | ESEEFPI | [I]RR S GD T PT I SIE | |

| <i>S. pombe</i> | STIE | LEP... | ATF | DFT | SLV | DEEYSN | FLIC | CS | TGS | LALY | N1G | | ESNYLS | WWRH | HEDFAI | STH |
|--------------------|---------|--------|------|-----|-----|--------|------|-----|-----|------|------|-------|---------|------|--------|-----|
| WDR6/Tmr7345 | 70 | 580 | 590 | 600 | 610 | 620 | | | | | | | | | | |
| <i>H. sapiens</i> | KQGVISV | TCHG | YVIT | R0G | A | YD | F0R | ... | DG | L0P | VLR0 | ... | R0CRMGN | W1A | GLR1P | ... |
| <i>M. musculus</i> | KQGVISV | TCHG | YVIT | R0G | A | YD | F0R | ... | DG | L0P | VLR0 | ... | R0CRMGN | W1A | GLR1P | ... |
| <i>D. rerio</i> | KQGVISV | TCHG | YVIT | R0G | A | YD | F0R | ... | DG | L0P | VLR0 | ... | R0CRMGN | W1A | GLR1P | ... |
| <i>B. tauris</i> | KQGVISV | TCHG | YVIT | R0G | A | YD | F0R | ... | DG | L0P | VLR0 | ... | R0CRMGN | W1A | GLR1P | ... |

| | | | | |
|----------------------|---|--|---|--|
| <i>R. norvegicus</i> | KGGTIVS ^T CHGGVY ^S STD ^T SYVQF ^H ... | GGRLP ^L LRCKACRGMN ^I MLRRM ^P ... | DGSW... | |
| <i>S. cerevisiae</i> | VE...DRDN ^S AVS ^T RD ^C Y ^V F ^E ... | T...KNSL ^B EGP ^G Y ^R SKV ^L HNSM ^N MKG ^L ... | AFFNS ^G GEV ^I ... | |
| <i>S. pombe</i> | K...SS ^R KDYL ^L IQ ^T TY ^R QY ^N UNL ^E ... | SP ^R VEKS ^A S ^P E ^L CS ^R KC ^D C ^I L ^S KKL ^E KK ^N ... | GKENOW... | |
| WDR6/Trm734 | 630 640 650 660 670 680 690 700 | | | |
| <i>H. sapiens</i> | LGE ^H ANE ^I V ^V VMN ^P RS ^R HE ^K L ^H I ^V ... | CGG ^T W ^R WAF ^S ... | DTEA ^M MAFAY ^L RD ^G IV ^M YRA ^D ... | GCTRPHV ^P REGI ^H ... |

| WDR6/Tpm734 | 710 | 720 | 730 | 740 | 750 | 760 | 770 | 780 | 790 |
|----------------------|--|---|----------------------|---|----------------------|---|----------------------|---|----------------------|
| <i>H. sapiens</i> | V K R G T V T G T I T L G P E Y V S F P M Q D P L D L E P G ... | S G P D P G ... | <i>D. rerio</i> | S G P D P G ... | <i>B. taurus</i> | S G P D P G ... | <i>R. norvegicus</i> | S G P D P G ... | <i>S. cerevisiae</i> |
| <i>M. musculus</i> | V K R G T V T G T I T L G P E Y V S F P M Q D P L D L E P G ... | S G P D P G ... | <i>D. rerio</i> | S G P D P G ... | <i>B. taurus</i> | S G P D P G ... | <i>R. norvegicus</i> | S G P D P G ... | <i>S. cerevisiae</i> |
| <i>D. rerio</i> | V K R G T V T G T I T L G P E Y V S F P M Q D P L D L E P G ... | S G P D P G ... | <i>B. taurus</i> | S G P D P G ... | <i>R. norvegicus</i> | S G P D P G ... | <i>S. cerevisiae</i> | S G P D P G ... | |
| <i>B. taurus</i> | V K R G T V T G T I T L G P E Y V S F P M Q D P L D L E P G ... | S G P D P G ... | <i>R. norvegicus</i> | S G P D P G ... | <i>S. cerevisiae</i> | S G P D P G ... | | | |
| <i>R. norvegicus</i> | V K R G T V T G T I T L G P E Y V S F P M Q D P L D L E P G ... | S G P D P G ... | <i>S. cerevisiae</i> | S G P D P G ... | | | | | |

| | 800 | 810 | 820 | 830 | 840 | 850 | 860 | 870 |
|----------------------|----------------|----------|----------|---------|--------------|----------|---------|----------|
| <i>S. cerevisiae</i> | ISCFVSNITNT | | | | NFRDFLICFTAE | EDS | LYG | GLCOPFIN |
| <i>S. pombe</i> | MDEEN | | | | L | P | FEK | QIESTWS |
| WDR6/TM734 | | | | | | | | |
| <i>H. sapiens</i> | PDPQ...PGLTAAV | WAGGRAEH | HCSFENPT | PPDSTP | RICH | VMILS | HELDY | QPO |
| <i>M. musculus</i> | PQDTR... | WAGGRAEH | HCSFENPT | PPDSTP | RICH | VMILS | HELDY | NDPR |
| <i>D. rerio</i> | RERNNGMETVS | LVLSLVE | WAGGRAQ | QLCYRLL | HELDQO | EVQTCVTO | IAHGRER | WKRNRHKV |

Multiple sequence alignment of Trm734 across four species: *B. taurus*, *R. norvegicus*, *S. cerevisiae*, and *S. pombe*. The alignment highlights the WDR6/Term734 domain (residues 880-950) in red. Conserved residues are shown in blue, and identical residues are shown in black.

| | WDR6/Trm734 | 960 | 970 | 980 | 990 | 1000 | 1010 | 1020 | 1030 | 1040 |
|----------------------|---|-----|-----|-----|-----|---|---|--|------|------|
| <i>H. sapiens</i> | PPVDPGLP M GTP S LTV Q GA C GIN V H T P L P D ... | | | | | RE G H H VL A V G E G D S | SH I H V L A E M L Q E A V G E A L P | Q R VI D E Y S P V | | |
| <i>M. musculus</i> | PA L A P H G L Y C M G T P S L V T O Q A G C I N V H | | | | | RE G H H VL A V G E G D S | SH I H V L A E M L Q E A V G E A L P | Q R VI D E Y S P V | | |
| <i>D. rerio</i> | PA L A P H G L Y C M G T P S L V T O Q A G C I N V H | | | | | RE G H H VL A V G E G D S | SH I H V L A E M L Q E A V G E A L P | Q R VI D E Y S P V | | |
| <i>B. taurus</i> | AA D LG L F G C M G T P S L V T O Q A G C I N V H | | | | | RE G H H VL A V G E G D S | SH I H V L A E M L Q E A V G E A L P | Q R VI D E Y S P V | | |
| <i>R. norvegicus</i> | PPA H E L F G C M G T P S L V T O Q A G C I N V H | | | | | RE G H H VL A V G E G D S | SH I H V L A E M L Q E A V G E A L P | Q R VI D E Y S P V | | |

| <i>D. rerio</i> | AHSPLGGCGLLCSFALLASTS | SPDCKCICWGSYESDGLRP | LVRFRSHIAAAGLWANR | RSSEGQEGGAWAVVCG | GCLGOLFPLTKEIDEAEN |
|----------------------|-----------------------|---------------------|-------------------|------------------|--------------------|
| <i>R. norvegicus</i> | AHSSVQVLLKPLKMLPQV | SDQVIRLDPVQVQVQV | PLVQVQVQVQVQVQV | PLVQVQVQVQVQVQV | PLVQVQVQVQVQVQV |
| <i>S. cerevisiae</i> | ASSTTGGCMLLNGKEVITS | SDQVIRANGITAKGKSLUV | KKRTTVTDTSGLSLEI | ISNDEDADSETLTLC | LSLISWKKI |
| <i>S. pombe</i> | AHASSVQVLLKPLKMLPQV | SDQVIRLDPVQVQVQV | PLVQVQVQVQVQVQV | PLVQVQVQVQVQVQV | PLVQVQVQVQVQVQV |

| | |
|----------------------|---------------------|
| <i>H. sapiens</i> | |
| <i>M. musculus</i> | |
| <i>D. rerio</i> | RAEGERERKVMFSHQCSRT |
| <i>B. taurus</i> | |
| <i>R. norvegicus</i> | |
| <i>S. cerevisiae</i> | |
| <i>S. pombe</i> | |

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.

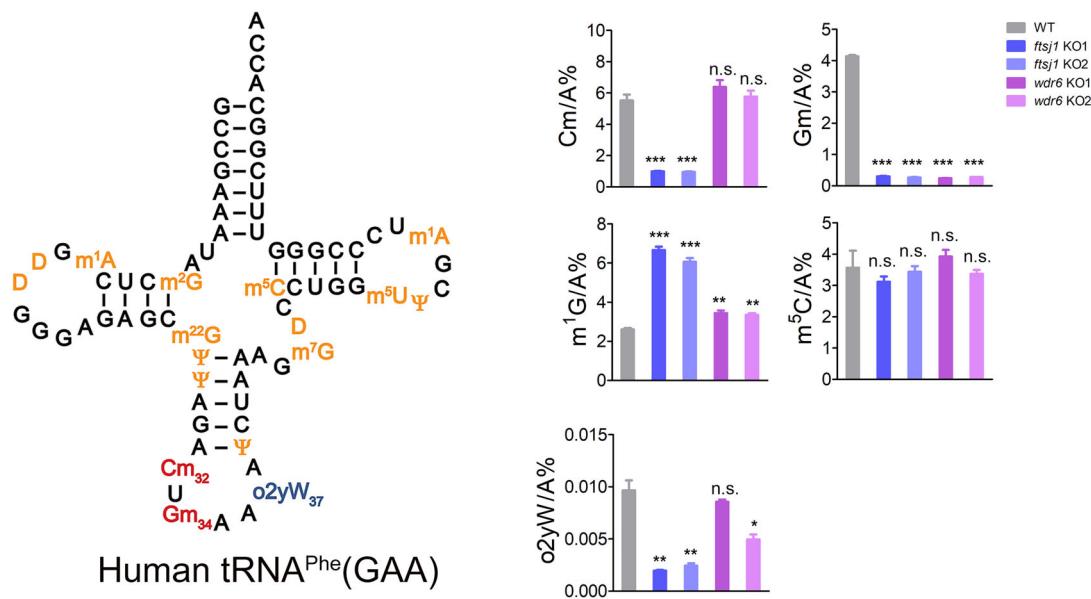


Figure EV3. Knockout of *ftsj1* and/or *wdr6* affects Cm32, Gm34, m¹G37, and o²yW37 levels of tRNA^{Phe}(GAA).

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.

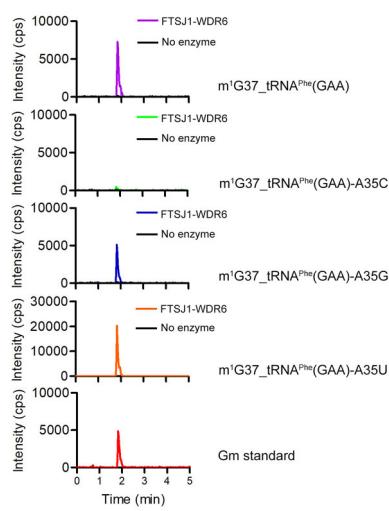


Figure EV4. UPLC-MS/MS analysis of Gm34 of m¹G37_tRNA^{Phe}(GAA), m¹G37_tRNA^{Phe}(GAA)-A35C, m¹G37_tRNA^{Phe}(GAA)-A35G and m¹G37_tRNA^{Phe}(GAA)-A35U after incubation with or without FTSJ1-WDR6.

2 μ l of Gm standard (1 ng/ml) was loaded as control. cps, counts per second.

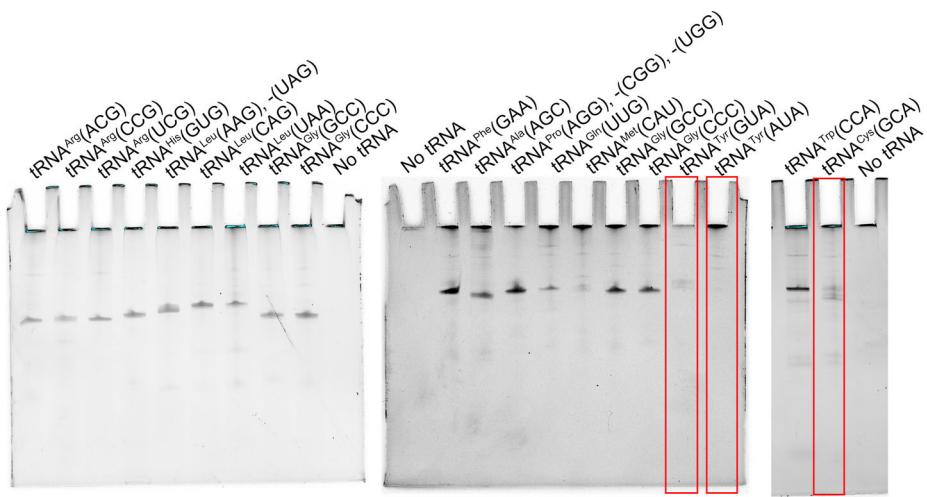


Figure EV5. Analysis of the purity of the fished tRNAs by biotinylated DNA probes using urea PAGE.

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