**Supplemental Table 1.** Table of *E. coli* tRNAs. The table contains information about the transcript ID, tRNA name, anticodon and tRNA sequences. tRNAs that contained 100% sequence identity were grouped together and named by the first tRNA in the group.

**Supplemental Table 2.** Master table of *E. coli* tRNA misincorporation and termination scores for each position from all tRNA of cultures grown in Luria-Bertani (LB) medium and chemically defined medium (CDM). Listed are the transcript ID, tRNA name, annotated position and corresponding region (a zero refers to locations in which regional information is not available), the reference nucleotide at each position, all annotated modifications, average coverage depth, and misincorporation (mis) and termination (ts) scores according to our sequencing data. Scores were calculated only for tRNAs with read depth greater than 100 reads.

**Supplemental Table 3.** DESeq2 analysis of MTSEA-biotin captured RNA versus input RNA from *E. coli* grown in LB (3A) or CDM (3B). The table contains information about the transcript ID, tRNA name, base mean, log2fold change (l2fc), lfc standard error value, the Wals statistic (stat), p-value and adjusted p-value (padj).

**Supplemental Table 4.** Table of *E. coli* tRNA misincorporation and termination scores for tRNA positions 32, 34 and 37 for cultures grown in both LB and CDM. The table contains information about the transcript ID, tRNA name, annotated position and corresponding region, the reference nucleotide at each position, all annotated modifications, average coverage depths, and misincorporation (mis) and termination (ts) scores. Scores were calculated only for tRNAs with read depth greater than 100 reads.

**Supplemental Table 5.** Table of *E. coli* tRNA misincorporation and termination scores for positions 46 and 47 for cultures grown in LB and CDM. Listed are the transcript ID, tRNA name, annotated position and corresponding region, the reference nucleotide at each position, all annotated modifications (a zero refers to no annotated nucleotide at that position), average coverage depths, and misincorporation (mis) and termination (ts) scores according to our sequencing data. Scores were calculated only for tRNAs with read depth greater than 100 reads.

**Supplemental Table 6.** *P. aeruginosa* tRNA gene information obtained from the Ensembl database (6A). tRNAs transcript ID, *P. aeruginosa* (PA) gene name, and assigned tRNA nomenclature for each tRNA (6B). *E. coli* and *P. aeruginosa* tRNAs were matched on the basis of anticodon identity and sequence similarity (6C).

**Supplemental Table 7.** Master table of matched *E. coli* and *P. aeruginosa* tRNAs. The table contains information about the transcript ID, tRNA name, annotated position and corresponding region. (a zero refers to locations in which regional information is not available), the reference nucleotide at each position, all annotated modifications, average coverage depths, and misincorporation (mis) and termination (ts) scores according to our sequencing data. Scores were calculated only for tRNAs with read depth greater than 100 reads.

**Supplemental Table 8.** DESeq2 analysis of MTSEA-biotin captured RNA versus input RNA of from *P. aeruginosa.* The table contains information about the transcript ID, tRNA name, base mean, log2fold change (l2fc), lfc standard error value, the Wals statistic (stat), p-value, and adjusted p-value (padj).

**Supplemental Table 9.** Data from Supplemental Figure 8 indicating a positive correlation between misincorporation score and log 2-fold enrichment by MTSEA-biotin captured RNAs. The table contains information about the transcript ID, tRNA name, misincorporation scores (mis) and log2fold values (l2f) for each tRNA that had a read depth of at least 100 reads at position 8 and padj value less than 0.05 (padj < 0.05).

**Supplemental Table 10.** Data from Figure 2C comparing log2fold enrichment values between each matched tRNA of *E. coli* (Ec) and *P. aeruginosa* (PA) MTSEA-biotin capture experiments. The table contains information about each anticodon and the subsequent log2fold change values.

**Supplemental Table 11.** This table contains a list of modifications found in tRNA and their corresponding abbreviations or short names. This table was compiled with reference to the literature and the Modomics RNA modification database [8,36].

**Supplemental Table 12.** List of tRNA modifying enzymes from *E. coli* and *P. aeruginosa* and their protein Basic Local Alignment Search Tool (BLASTp) scores.

**Supplemental Table 13.** Master table of *P. aeruginosa* tRNAs from wild-type (Wt) and YfiP mutant (Mut) sequencing data. Listed are the transcript ID, tRNA name, reference base, annotated position and corresponding region (a zero refers to locations in which regional information is not available), the anticodon (AC), and the average misincorporation (mis), depths, and termination scores according to the sequencing data. Scores were calculated only for depths greater than 100, otherwise listed as NA.

**Supplemental Table 14.** List of oligonucleotide sequences used to generate sequencing libraries, perform targeted RT-PCR, and sanger sequencing.