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Supplementary Information for

Oxidation of phenylalanyl tRNA synthetase positively regulates translational quality control

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This PDF file includes:

Figure S1: Confirmation of editing deficient βG318W PheRS

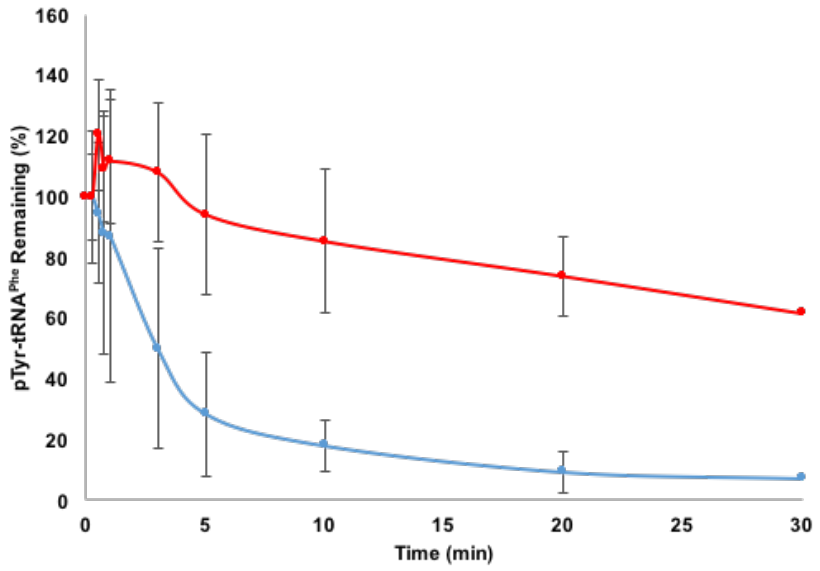

Figure S1: Hydrolysis of H^3-*p*-Tyr-$tRNA^{Phe}$ over time. WT PheRS (blue) clears misacylated H^3-*p*-Tyr-$tRNA^{Phe}$ to 6% remaining. βG318W PheRS only clears H^3-*p*-Tyr-$tRNA^{Phe}$ to 62%.

Figure S2: Deacylation (*p*-Tyr-tRNA^{Phe}) Exponential Fit Curves

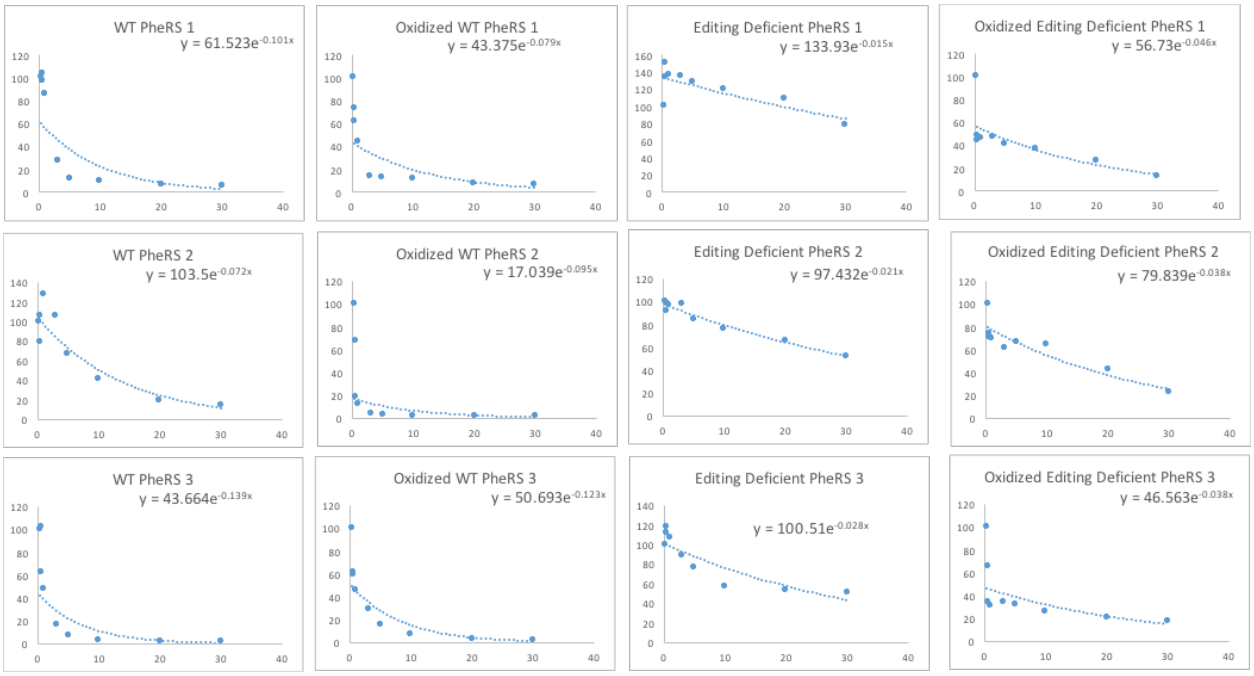

Figure S2: Exponential Curve fits for the deacylation half-life of *p*-Tyr-tRNA^{Phe}. The average half-life determined from these curves is reported in Table S2.

Figure S3: ATP consumption in the absence of tRNA

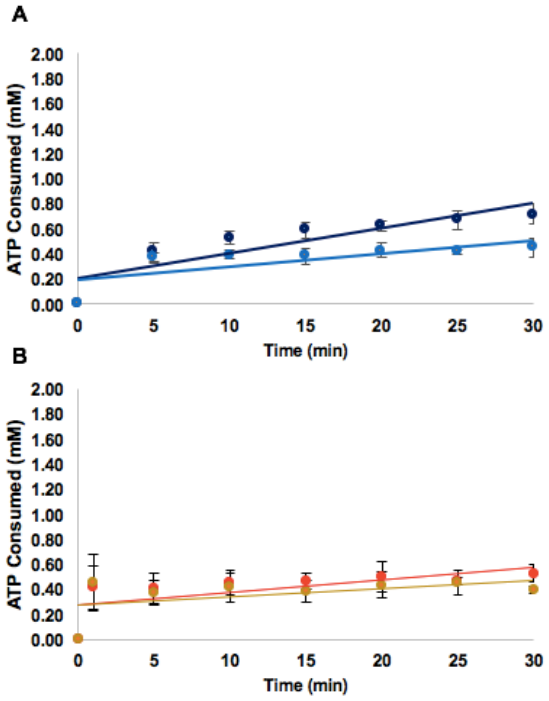

Figure S3: ATP Consumption in the presence of *m*-Tyr but in the absence of $tRNA^{Phe}$. Overall there are lower levels of ATP consumed in the absence of tRNA. A. Non-oxidized WT PheRS (Light Blue) and Oxidized WT PheRS (Dark Blue) consumption of ATP over time. B. Non-oxidized editing deficient PheRS (Yellow) and Oxidized editing deficient PheRS (Orange) consumption of ATP over time.

Figure S4: Aminoacylation non-oxidized vs oxidized PheRS

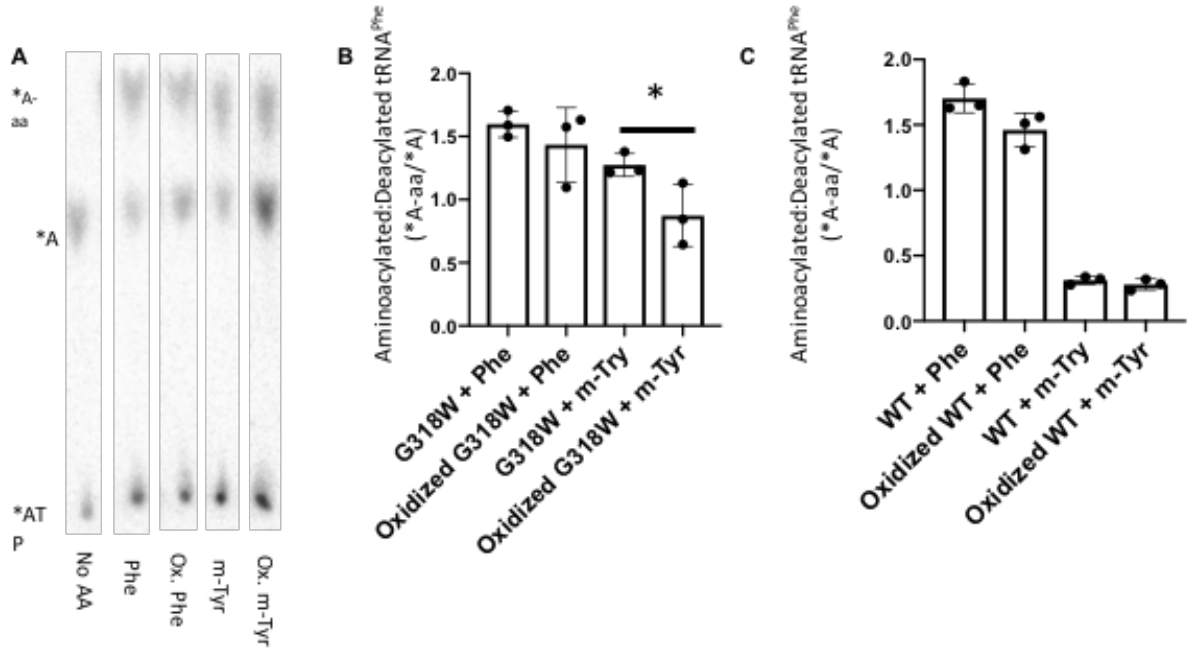

Figure S4: Aminoacylation of $tRNA^{Phe}$ with cognate and non-cognate amino acids using WT and editing deficient proteins. A) Representative TLC plate showing the change of spot intensity. Ration of *A-aa to *A was used to quantify aminoacylated tRNA. B) Quantitative analysis of 3 separate TLC using the editing deficient PheRS to aminoacylate $tRNA^{Phe}$ with Phe or *m*-Tyr ($p \leq 0.05$). C) Quantitative analysis of at least 3 separate TLC using the WT PheRS to aminoacylate $tRNA^{Phe}$ with Phe or *m*-Tyr ($p \leq 0.05$).

Figure S5: Cell Lysate Deacylation (*p*-Tyr-tRNA^{Phe}) Exponential Fit Curves

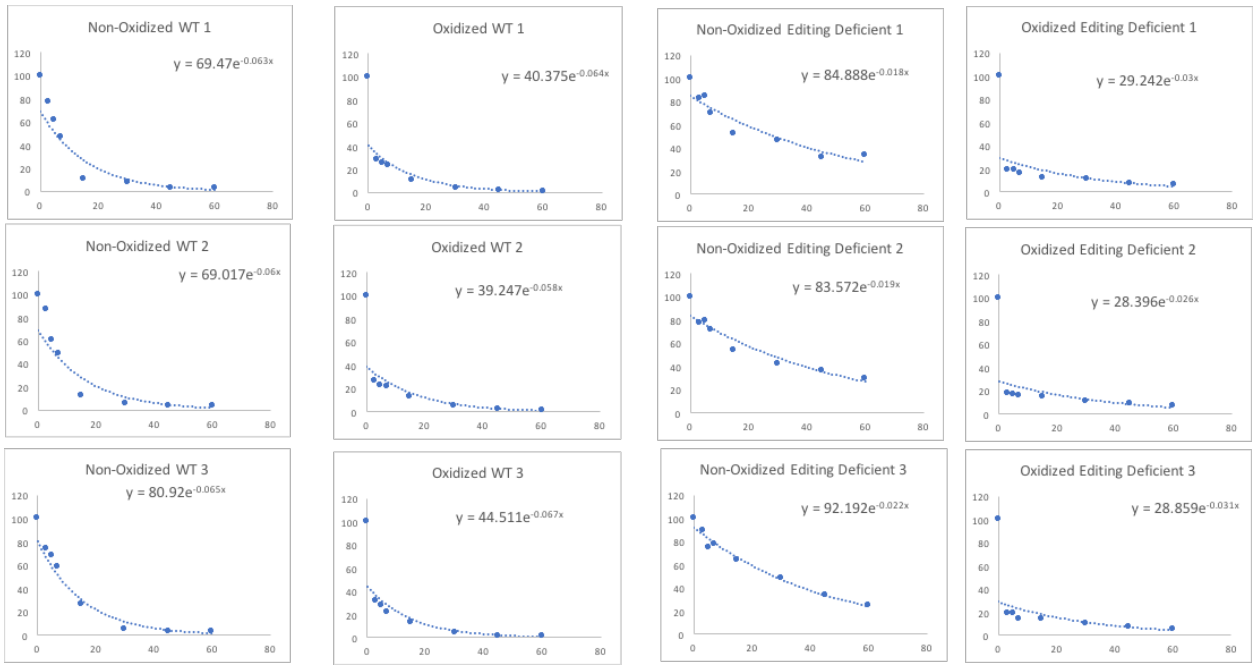

Figure S5: Exponential Curve fits for the deacylation half-life of *p*-Tyr-tRNA^{Phe}. The average half-life determined from these curves is reported in Table S6.

Figure S6: Cell Lysate Deacylation (Phe-tRNA^{Phe}) Exponential Fit Curves

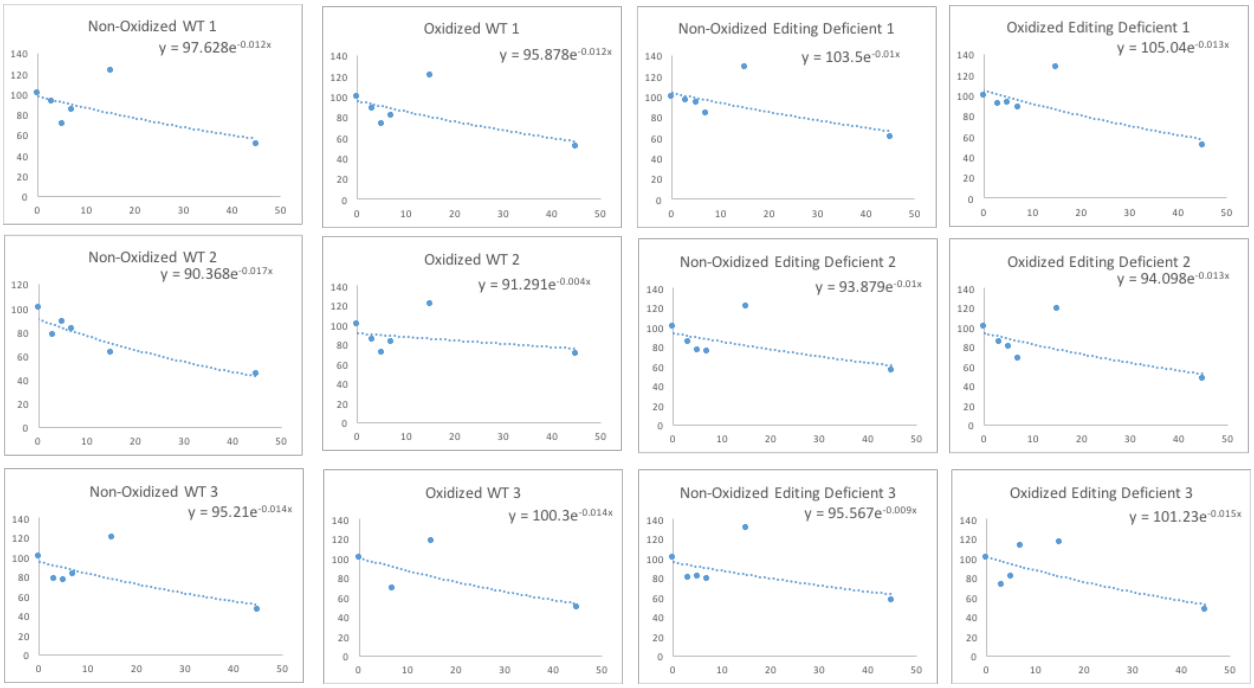

Figure S6: Exponential Curve fits for the deacylation half-life of Phe-tRNA^{Phe}. The average half-life determined from these curves is reported in Table S7.

Figure S7: HPX$^-$ vs. WT *E. coli* Deacylation (*p*-Tyr-tRNA^{Phe}) Exponential Fit Curves

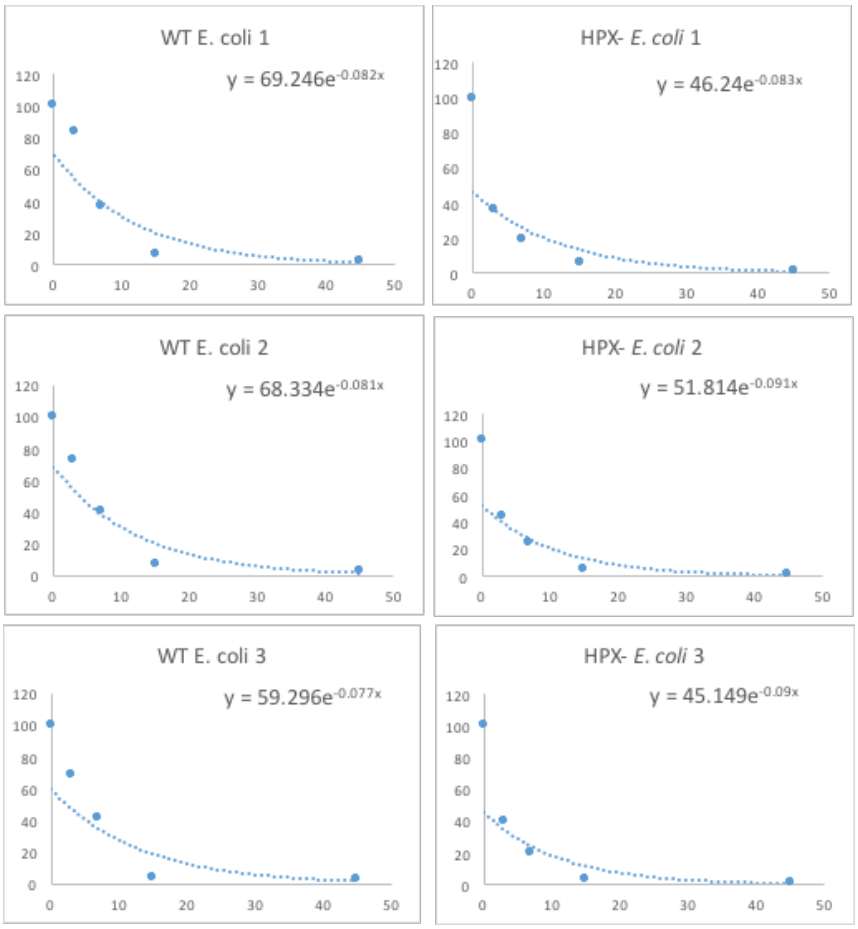

Figure S7: Exponential Curve fits for the deacylation half-life of Phe-tRNA^{Phe}. The average half-life determined from these curves is reported in Table S9.

Figure S8: HPX$^-$ vs. WT *E. coli* Deacylation (Phe-tRNA^{Phe}) Exponential Fit Curves

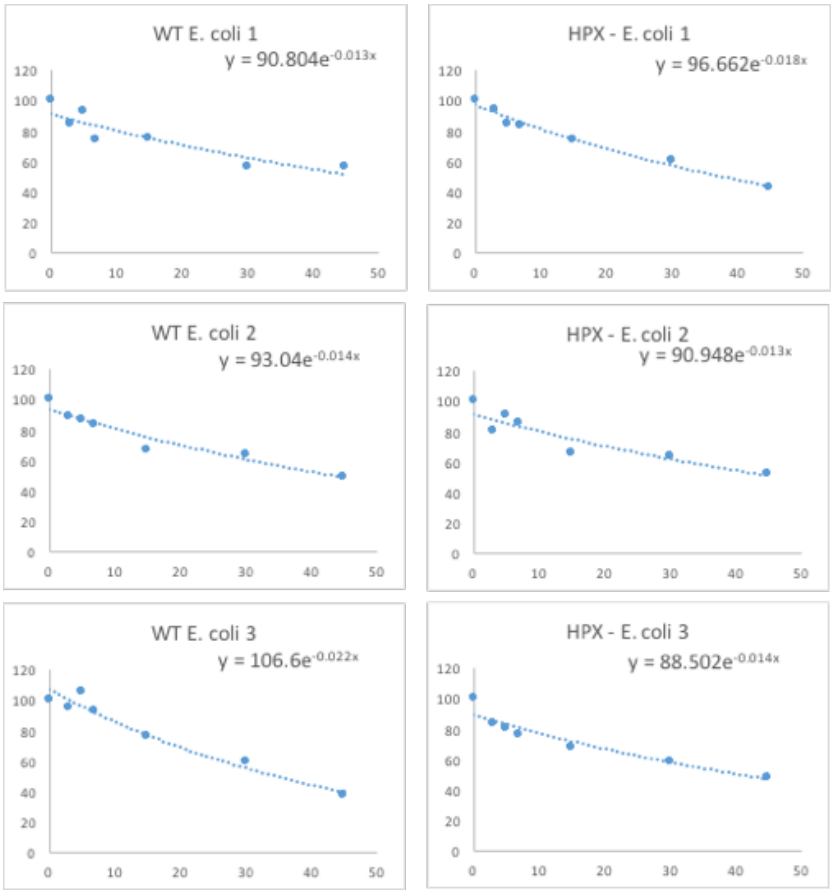

Figure S8: Exponential Curve fits for the deacylation half-life of Phe-tRNA^{Phe}. The average half-life determined from these curves is reported in Table S10.

Table S1 Mass Spectrometry

WT PheRS Only

βG318W PheRS Only

Both PheRS

pheT		pheS	
Position	Residue	Position	Residue
100	P	53	R
102	D	111	R
103	F	139	N
104	K	140	F
111	R	141	D
140	D	179	K
184	K	222	N
280	M	224	K
282	K	307	Y
321	H	316	F
325	N		
423	D		
428	D		
469	Y		
587	R		
601	Y		
616	D		
620	D		
682	F		
717	N		
742	D		

Table S1: Residues that were denoted as oxidized with H_2O_2 treatment when compared to a non-oxidized control. The residues in the WT PheRS are shown in blue, the residues in the editing-deficient PheRS are shown in red, and the residue was oxidized in both cases is shown highlighted in white. The position indicates the amino acid number that is in each specific subunit for pheT and pheS, respectively.

Table S2 Hydrolysis of *p*-Tyr-tRNA^{Phe} Purified Proteins

Protein	Half-Life (min)	$k_{deacylation}$ (min^{-1})
WT PheRS	7.2 ± 2	0.1 ± 0.03
Oxidized WT PheRS	7.2 ± 2	0.1 ± 0.02
βG318W PheRS	36 ± 9	0.02 ± 0.006
Oxidized βG318W PheRS	16 ± 3	0.05 ± 0.01

Table S2**:** Hydrolysis of *p*-Tyr-tRNA^{Phe} using purified proteins treated with H_2O_2 shows decreased half-life in the oxidized editing deficient PheRS. This indicates that the proofreading activity of oxidized editing deficient PheRS is higher than non-oxidized.

Table S3 Average rate of ATP consumed in presence of *m*-Tyr

Protein	Average Rate of ATP Consumed (mM/min)
WT PheRS	0.05 ± 0.006
Ox. WT PheRS	0.06 ± 0.01
βG318W PheRS	0.02 ± 0.003
Ox. βG318W PheRS	0.03 ± 0.003

Table S3: The average rate of ATP consumed was calculated between 1 and 10 minutes of each reaction. WT PheRS and Ox. WT PheRS do not show significant changes in the rate of ATP consumed; however, the results trend towards oxidized WT PheRS having a faster rate of ATP consumption. On the other hand, βG318W PheRS and oxidized βG318W PheRS have a significant difference in which the oxidized βG318W PheRS is consuming ATP at a faster rate than WT. The rate was taken between 1 and 10 minutes because this was the time in which the polynomial equation best fit the data according to the r-squared value.

Table S4 Average rate of ATP consumed in the presence of Phe

Protein	Average Rate of ATP Consumed (mM/min)
WT PheRS	0.04 ± 0.012
Ox. WT PheRS	0.08 ± 0.07
βG318W PheRS	0.02 ± 0.009
Ox. βG318W PheRS	0.02 ± 0.011

Table S4: The average rate of ATP consumed was calculated between 1 and 10 minutes of each reaction. WT PheRS and Ox. WT PheRS do not show significant changes in the rate of ATP consumed. Non-oxidized βG318W PheRS and oxidized βG318W PheRS do not show a significant change in the rate of ATP consumed. The rate was taken between 1 and 10 minutes because this was the time in which the polynomial equation best fit the data according to the r-squared value.

Table S5 Specific activity of cell lysates

	Specific Activity	Normalization Value
WT 0mM H_2O_2	**54**	**1.00**
WT 5mM H_2O_2	**18**	**0.3**
βG318W 0mM H_2O_2	**51**	**0.9**
βG318W5mM H_2O_2	**12**	**0.2**

Table S5: Specific activity of cell lysates from WT and *E. coli* MG1655 with a chromosomal mutation in PheRS to generate an editing defective protein. Specific activity was measured by aminoacylation activity and represented as the ratio of charged ^{14}C-Phe-tRNA^{Phe} (pmol)/minutes) / total protein (mg). Normalization values compare the specific activity value of each protein relative to the non-oxidized WT *E. coli* cell lysates.

Table S6 : Hydrolysis of *p*-Tyr-tRNA^{Phe} Using Cell Lysates Grown in the Presence of H_2O_2

Organism	Half-Life (min)	$k_{deacylation}$ (min^{-1})
WT *E. coli*	11 ± 1	0.06 ± 0.003
WT *E. coli* + 5mM H_2O_2	11 ± 1	0.06 ± 0.005
βG318W *E. coli*	36 ± 4	0.02 ± 0.002
βG318W *E. coli* + 5mM H_2O_2	24 ± 2	0.03 ± 0.003

Table S6**:** Hydrolysis of *p*-Tyr-tRNA^{Phe} using cell lysates grown in the presence of H_2O_2 shows decreased half-life in the oxidized editing deficient PheRS. This indicates that the proofreading activity of oxidized editing deficient PheRS is higher than non-oxidized.

Table S7 : Hydrolysis of Phe-tRNA^{Phe} Using Cell Lysates Grown in the Presence of H_2O_2

Organism	Half-Life (min)	$k_{deacylation}$ (min^{-1})
WT *E. coli*	49 ± 9	0.01± 0.003
WT *E. coli* + 5mM H_2O_2	94 ± 70	0.01± 0.005
βG318W *E. coli*	72 ± 4	0.01± 0.0005
βG318W *E. coli* + 5mM H_2O_2	50 ± 4	0.01± 0.001

Table S7**:** Hydrolysis of Phe-tRNA^{Phe} using cell lysates grown in the presence of H_2O_2 shows similar half-life between non-oxidized and oxidized WT and editing deficient PheRS. This indicates that Phe-tRNA^{Phe} is not being hydrolyzed to a greater extent in the presence of oxidative stress.

Table S8 Specific activity of WT and HPX$^-$ cell lysates

Strain	Specific Activity	Normalization Value
WT *E. coli*	**11 +/- 0.7**	**1**
HPX$^-$ *E. coli*	**6 +/- 0.5**	**0.5**

Table S8: Specific activity of cell lysates from WT *E. coli* MG1655 and HPX$^-$ *E. coli* grown anaerobically. Specific activity was measured by aminoacylation activity and represented as the ratio of charged ^{14}C-Phe-tRNA^{Phe} (pmol)/minutes) / total protein (mg). Normalization values compare the specific activity value of each protein relative to the non-oxidized WT *E. coli* cell lysates.

Table S9: Hydrolysis of *p*-Tyr-tRNA^{Phe} Using WT and HPX$^-$ Cell Lysates

Organism	Half-Life (Min)	$k_{deacylation}$ (min^{-1})
WT *E. coli*	8.7 ± 0.3	0.08 ± 0.003
HPX- *E. coli*	7.9 ± 0.4	0.09 ± 0.01

Table S9**:** Hydrolysis of *p*-Tyr-tRNA^{Phe} using cell lysates from HPX$^-$ and WT *E. coli* shows decreased half-life for HPX$^-$ due to the low levels of oxidative stress that accumulate in this mutant cell. This indicates that in even low levels of oxidative stress, proofreading activity of PheRS is higher than in non-oxidizing conditions.

Table S10 : Hydrolysis of Phe-tRNA^{Phe} Using WT and HPX- Cell Lysates

Organism	Half-Life (min)	$k_{deacylation}$ (min^{-1})
WT *E. coli*	45 ± 12	0.02 ± 0.005
HPX- *E. coli*	47 ± 8	0.02 ± 0.003

Table S10**:** Hydrolysis of Phe-tRNA^{Phe} using WT and HPX- Cell Lysates shows similar half-life. This indicates that Phe-tRNA^{Phe} is not being hydrolyzed to a greater extent in the presence of oxidative stress.

Table S11 Primers List

Number	Primer Name/Use	Sequence
1	**S. Typhimurium pheS/T Fwd**	ACCGACACAATGAGGAAAACC
2	**S. Typhimurium pheS/T Rev**	GCTTTTGTAAGCGCCATAGGT
3	**S. Typhimurium pheS/T Fwd + pET28a overhang**	ATCTCAGTGGTGGTGGTGGTGGTGCTCGAGTCAATCCCTCAATGATGCCTGGAATCG
4	**S. Typhimurium pheS/T Rev + pET28a overhang**	GCGGCCTGGTGCCGCGCGGCAGCCATATGTCACATCTCGCAGAGCT
5	**G318W PheRS Mutagenesis Fwd**	GCTGGGGATAGCCGGTATTTTTTGGGGCGAACATTCCGGCGTGAACG
6	**G318W PheRS Mutagenesis Rev**	CGTTCACGCCGGAATGTTCGCCCCAAAAAATACCGGCTATCCCCAGC

Table S11: List of primers and the 5’ to 3’ sequences used throughout the research.

Supplementary Information Methods

Mass Spectrometry

Purified proteins were treated with 5 mM H_2O_2 for 5 minutes at 37ºC. Excess H_2O_2 was removed by extensive dialysis. Samples were then run on a 10% SDS-PAGE gel and gel extracted and trypsin digested for LC MS/MS analysis at The Ohio State University.

Thin Layer Chromatography: Misaminoacylation

Misacylation of $tRNA^{Phe}$ by *m*-Tyr was analyzed by thin layer chromatography. The 3' end of $tRNA^{Phe}$ was radiolabeled using ^{32}P- αATP (Perkin Elmer) as previously described (1). Aminoacylation reactions were completed using 30 mM KCl, 10 mM $MgCl_2$, 10mM ATP, 300 µM *m*-Tyr, 1 uM PheRS 4 mM H_2O_2 (for oxidized reactions) and radiolabeled $tRNA^{Phe}$. Reactions were incubated at 37ºC for 5 minutes before addition of *m*-Tyr. Reactions were completed at 37ºC for 30 minutes and time points were taken by combining equal volumes of reaction and S1 Quench Buffer (2x S1 Buffer, 0.2M NaOAc, S1 Nuclease, H_2O). Quench left at 25ºC for 30 minutes and then 0.5 µL were spotted on a TLC, pre-ran with H_2O. TLC was run in 100 mM ammonium acetate + 5% acetic acid, dried, exposed to a phosphor screen and imaged on a phosphor imager (Typhoon).

Anaerobic growth

Anaerobic growth of *E. coli* WT MG1655 and HPX^- was completed by plating a lawn LB plates and grown in an anaerobic growth chamber at 37ºC for two days. Cells were harvested, lysed by sonication and cell lysates were used in deacylation experiments.

Western Blot

Cell lysate was run on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane. Membrane was blocked with milk powder and blotted with 1º antibody for *E. coli* PheRS (1:8,000 dilution) and 2º antibody donkey anti-rabbit (1:10,000 dilution). Blots were imaged using BioRad clarity chemiluminescent solutions.

Steady-State Kinetic of PheRS

The K_M of $tRNA^{Phe}$ for oxidized and non-oxidized PheRS was determined using aminoacylation in 30 mM KCl, 10 mM $MgCl_2$, 2 mM ATP, 0-20 µM $tRNA^{Phe}$, 25 nM PheRS, 60 µM ^{14}C or ^{3}H-Phe. Reaction time points between 0-4 minutes were spotted on 3 mm filter papers, pre-soaked with 5% Trichloroacetic acid (TCA), then washed 3x 5% TCA and 1x 95% EtOH. Phe-$tRNA^{Phe}$ was quantified by liquid scintillation counting.

Pyrophosphate Exchange

To determine the K_M for Phe and *m*-Tyr, pyrophosphate exchange was completed in 100 mM HEPES pH 7.5, 30 mM KCl, 10 mM $MgCl_2$ 1 mM NaF, 25 nM PheRS, 2-200 µM Phe or 50 µM-2mM *m*-Tyr, 2 mM ATP, 2 mM ^{32}P-PPi. The reaction was incubated at 37ºC for 5 minutes before enzyme addition. Aliquots were taken at 1-4 minutes by combining with quench solution (1% activated charcoal, 5.6% $HClO_4$, 1.25 M PPi). On a vacuum filter with 3 mm filter discs, pre-rinsed with H_2O, charcoal reaction added, washed 3x H_2O and 1x 95% EtOH. Radiation was quantified using liquid scintillation counting. Michaelis-Menton equation was used to determine kinetic parameters.

Editing of *p*-Tyr-tRNA^{Phe}

Misacylated *p*-Tyr-tRNA^{Phe} was generated using 500 µM ^{3}H-Tyr, 25 µM tRNA^{Phe} and 1 µM *Saccharomyces cerevisiae* mitochondrial PheRS A333G (2). *p*-Tyr-tRNA^{Phe} was then precipitated using acid:phenol chloroform. The aqueous layer was combined with 1/10th volume 3 M NaOAc pH,4.2 and 2.7x volume EtOH and precipitated at -80ºC. RNA was pelleted at 18,600g for 30 minutes at 4ºC and re-suspended in 100 mM NaOAc, pH 4.2. Editing reactions were completed using misacylated tRNA^{Phe} in 30 mM KCl, 10 mM $MgCl_2$, 2.5 nM PheRS, 4 mM H_2O_2 (for oxidized only) at 37ºC for 30 minutes. Before addition of *p*-Tyr-tRNA^{Phe}, reactions were incubated at 37ºC for 5 minutes.

ATP Consumption Assay

Editing of *m*-Tyr-tRNA^{Phe} was analyzed by ATP consumption (3). Reaction contained 2 µM tRNA^{Phe}, 2 mM Phe or *m*-Tyr, 2 mM ^{32}P γ-ATP, 30 mM KCl, 10 mM $MgCl_2$, 2 U/mL pyrophosphatase, 2 µM PheRS, 4 mM H_2O_2 (for oxidized reaction). Without ^{32}P γ-ATP, reactions were incubated at 37ºC for 5 minutes. Reactions were completed at 37ºC for 30 minutes and equal volumes of the reaction and glacial acetic acid were combined at various time points. TLC was completed as above, but was run in 0.7 M KPO_4 (pH 3.5).

***In vitro* Transcription**

tRNA was transcribed using T7 Polymerase in 40 mM Tris pH 8.0, 2 mM Spermidine, 22 mM $MgCl_2$, 5 mM DTT, 50 µg/mL BSA, 5'GMP, pyrophosphatase, 4 mM ATP, 4 mM GTP, 4 mM CTP, 4 mM TTP, RNase inhibitor and 50 µg template DNA at 42ºC for 12-16 hours. tRNA was purified using Diethylaminoethyl cellulose (DEAE-C) resin, washed

with 20 mM Tris-HCl pH 7.0 and 20 mM NaCl and then eluted in 20 mM Tris-HCl pH 7.0 and 1 M NaCl. Fractions containing RNA were precipitated in 1/10th volume 3 M NaOAc, pH 5.2 and 2.7x EtOH at -80ºC. RNA was pelleted at 5000xg for 30 minutes and clear-dried pellet was re-suspended in H_2O. Active tRNA was determined by aminoacylation.

References

1. Ledoux S & Uhlenbeck OC (2008) [3'-32P]-labeling tRNA with nucleotidyltransferase for assaying aminoacylation and peptide bond formation. *Methods* 44(2):74-80.
2. Reynolds NM*, et al.* (2010) Cell-specific differences in the requirements for translation quality control. *Proc Natl Acad Sci U S A* 107(9):4063-4068.
3. Ling J & Soll D (2010) Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. *Proc Natl Acad Sci U S A* 107(9):4028-4033.