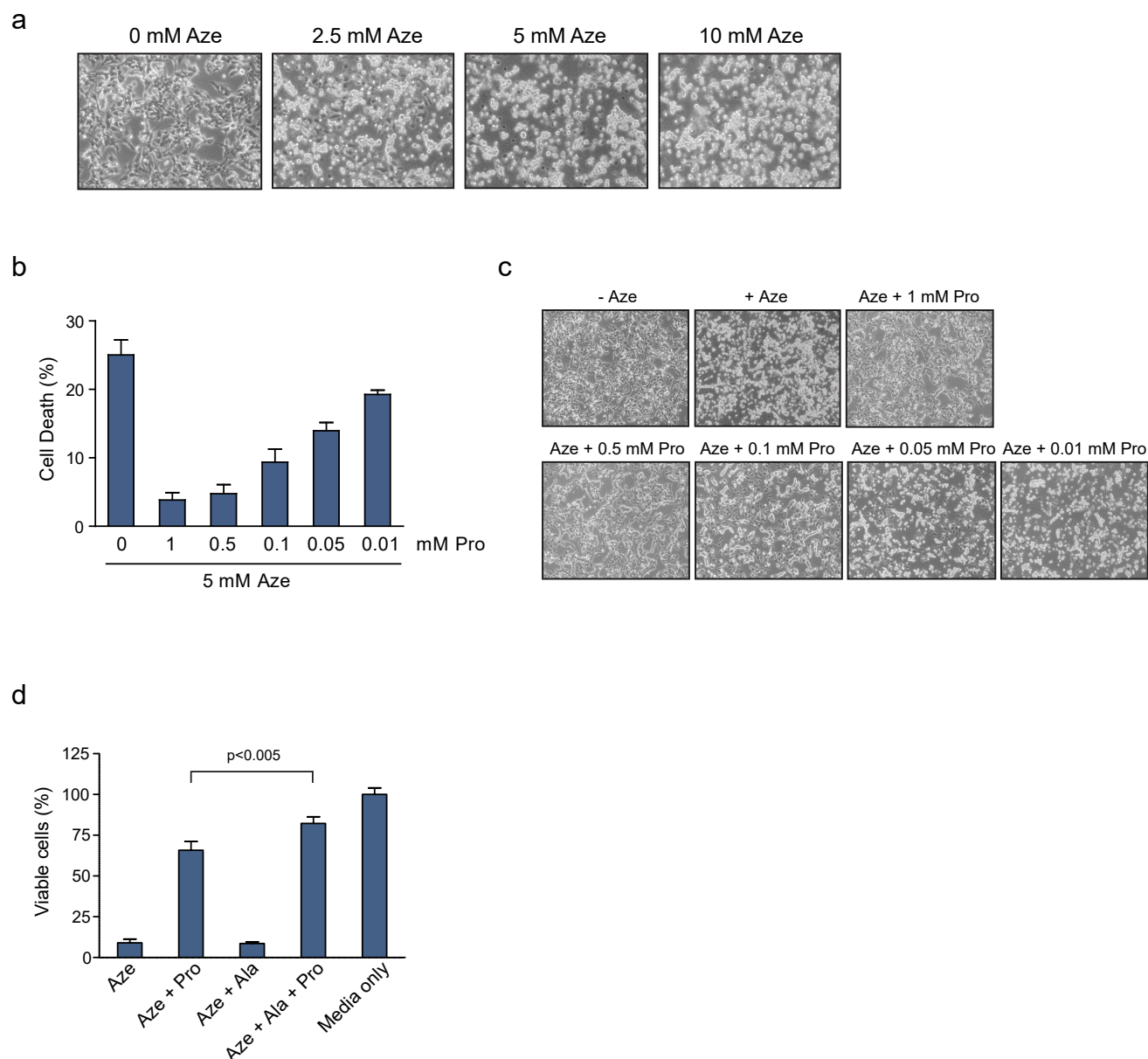


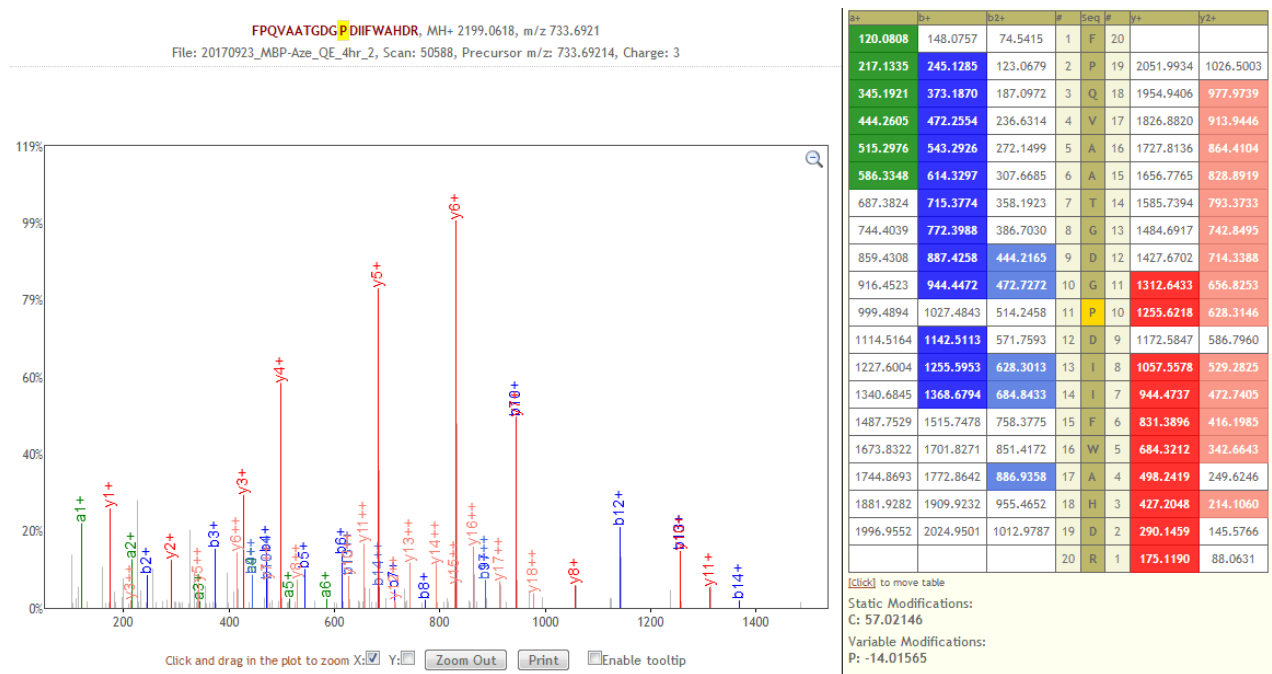
Supplementary Information

Supplementary Figures

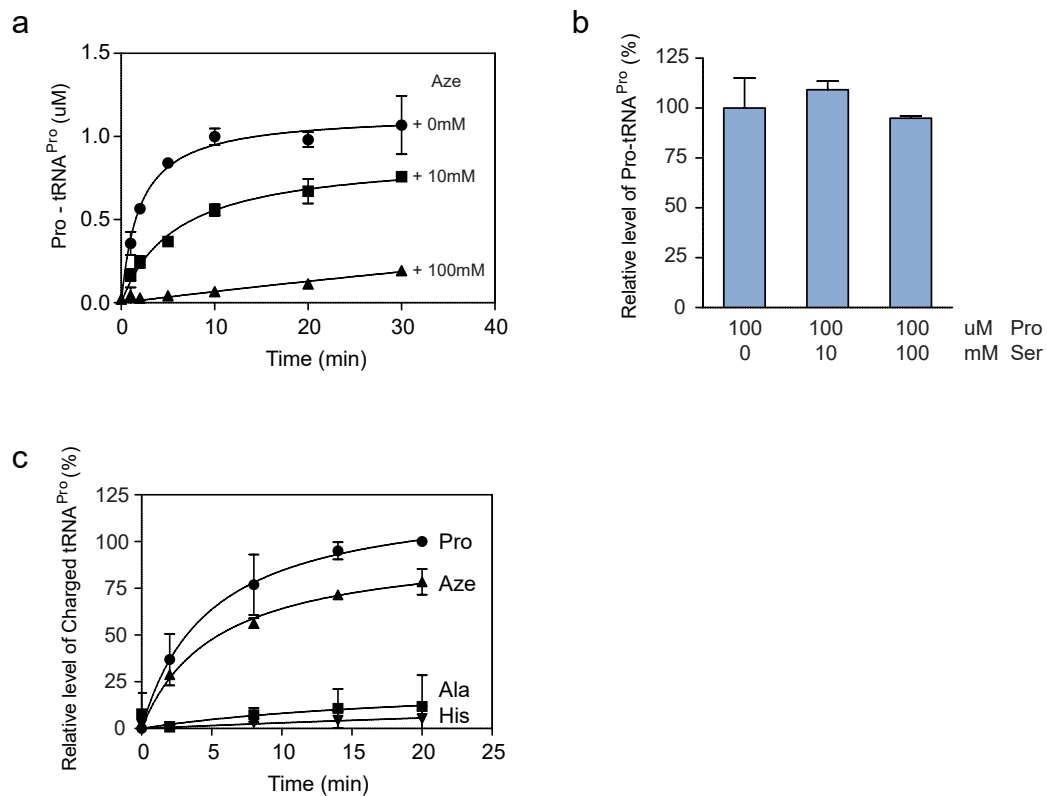


Supplementary Figure 1. Pro rescues Aze toxicity

a. Images of HeLa cells treated with 0-10 mM Aze for 24 hrs. b. HeLa cells were treated with 5 mM Aze and different concentrations (0-1000 μ M) of Pro for 24 hrs. Cell death was quantified and plotted. c. Cell images of b. d. At high concentrations of Aze (40 mM), Ala added to Pro can partially rescue HeLa cell toxicity (Pro and Ala concentrations were 1 mM). All error bars represent s.d..



Supplementary Figure 2. Example of MS/MS spectra showing Aze incorporation at Pro 58 position



Supplementary Figure 3. *Hs* ProRS charging onto tRNA^{Pro}

a. Time course of Aze competition with Pro charging onto tRNA^{Pro} by *Hs* ProRS. b. Pro-tRNA^{Pro} formation by *Hs* ProRS in the presence of different concentrations of Ser at 20 min (negative control). c. Time course of *Hs* ProRS charging tRNA^{Pro}.

Supplementary Tables

	ProRS-AzeSA	AlaRS-AzeSA
Data collection		
Resolution (Å)	50.0-2.59 (2.63-2.59) ^a	50.0-2.03 (2.14-2.03)
Wavelength (Å)	1.5418	1.5418
Space group	<i>p</i> 3 ₂ 21	<i>c</i> 2
Unit cell parameters <i>a</i> , <i>b</i> , <i>c</i> (Å)	<i>a</i> = <i>b</i> =83.76, <i>c</i> =108.77	<i>a</i> =108.33, <i>b</i> =67.27, <i>c</i> =86.35
Unit cell parameters α , β , γ (°)	α = β =90, γ =120	α = γ =90, β =136.23
Unique reflections	14793 (652)	27565 (3800)
Redundancy	10.2 (8.1)	3.9 (3.7)
Completeness (%)	96.9 (86.5)	99.2 (94.8)
Average <i>I</i> / σ (<i>I</i>)	37.2 (4.8)	15.7 (4.0)
<i>R</i> _{merge} ^b (%)	5.7 (33.7)	6.0 (32.0)
Refinement		
Resolution (Å)	35.54-2.59 (2.66-2.59)	59.7-2.03 (2.09-2.03)
Reflections for refinement/test	13977/749 (931/53)	26163/1390 (1803/93)
<i>R</i> _{work} ^c / <i>R</i> _{free} ^d (%)	22.7/27.2 (29.1/33.5)	18.0/21.5 (25.1/27.5)
RMSD bond (Å)	0.004	0.007
RMSD angle (°)	1.05	1.23
Mean B factor (Å ²)	59.4	27.0
Non-hydrogen protein atoms	3749	3046
Water oxygen atoms	26	245
AzeSA atoms	29	29
Zn ²⁺	1	0
MolProbity Ramachandran plot(%)		
Favored regions	98.3	98.2
Allowed regions	100.0	100.0

^aValues in parentheses are for the highest resolution shell.

^b $R_{\text{merge}} = \sum_h \sum_l |I(h)_l - \langle I(h) \rangle| / \sum_h \sum_l I(h)_l$, where $I(h)_l$ is the *l*th observation of the reflection *h* and $\langle I(h) \rangle$ is the weighted average intensity for all observations *I* of reflection *h*.

^c $R_{\text{work}} = \sum_h |F_{\text{obs}}(h) - F_{\text{cal}}(h)| / \sum_h |F_{\text{obs}}(h)|$, where $F_{\text{obs}}(h)$ and $F_{\text{cal}}(h)$ are the observed and calculated structure factors for reflection *h* respectively.

^d R_{free} was calculated as R_{work} using the 5% of reflections which were selected randomly and omitted from refinement.

Supplementary Table 1. Statistics of X-ray diffraction data collection and structure refinement

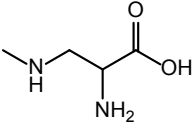
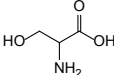
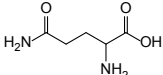
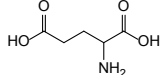
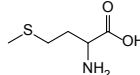
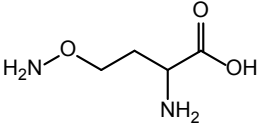
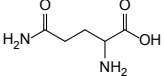
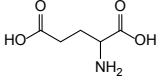
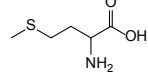
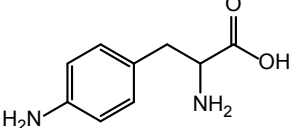
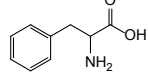
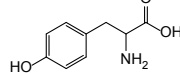
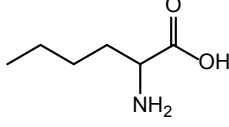
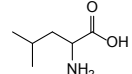
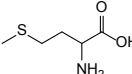
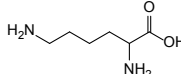
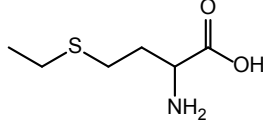
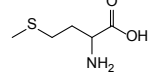
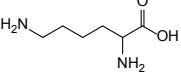
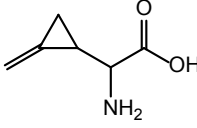
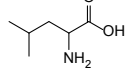
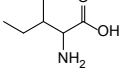
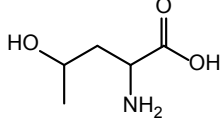
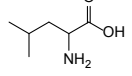
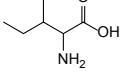
	Amino acid	k_{cat} (s ⁻¹)	K_m (mM)	$k_{cat} K_m^{-1}$ (s ⁻¹ mM ⁻¹)	$k_{cat} K_m^{-1}$ (Relative)
<i>Hs</i> ProRS	Pro	33.0 ± 1.2	0.09 ± 0.01	388.6	1
	Aze	15.8 ± 0.7	2.9 ± 0.4	5.5	1 / 71
<i>Hs</i> AlaRS	Ala	9.6 ± 0.3	0.07 ± 0.01	131.4	1
	Aze	4.1 ± 0.2	38.3 ± 5.5	0.11	1 / 1229
	Ser	9.8 ± 0.4	70.8 ± 7.6	0.14	1 / 950

Supplementary Table 2. Kinetic parameters for activation of Pro and Aze by *Hs* ProRS, and Ala and Aze by *Hs* AlaRS

The ATP-PPi exchange assay was carried out at 37 °C with either 0.05 µM *Hs* ProRS or 0.1 µM *Hs* AlaRS (pH 8.0). For the assays with ProRS, Pro concentrations were 0.03 - 2 mM and Aze concentrations were 1 - 30 mM. For the assays with AlaRS, Ala concentrations were 0.03 - 2 mM, Aze concentrations were 2 - 300 mM and Ser concentrations were 10 - 300 mM.

Non-proteinogenic amino acids

Mimicking amino acids

 BMAA	 Serine  Glutamine  Glutamic acid  Methionine
 Canaline	 Glutamine  Glutamic acid  Methionine
 p-aminophenylalanine	 Phenylalanine  Tyrosine
 Norleucine	 Leucine  Methionine  Lysine
 Ethionine	 Methionine  Lysine
 A-(methylenecyclopropyl)glycine	 Leucine  Isoleucine
 G-hydroxynorvaline	 Leucine  Isoleucine

Supplementary Table 3. Non-proteinogenic amino acids that mimic multiple amino acids