

Supplementary Information

An Efficient Pyrrolysyl-tRNA Synthetase for Economical Production of MeHis-containing Enzymes

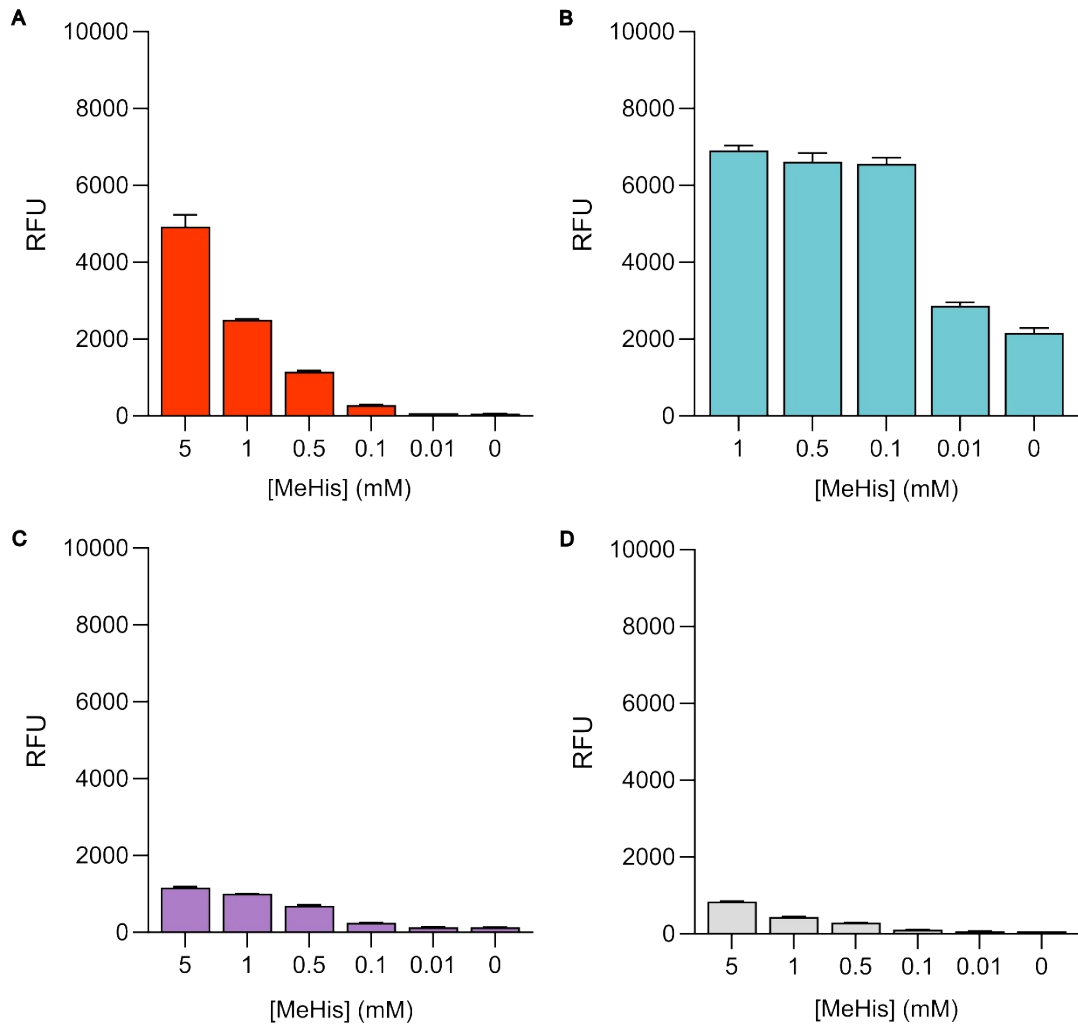
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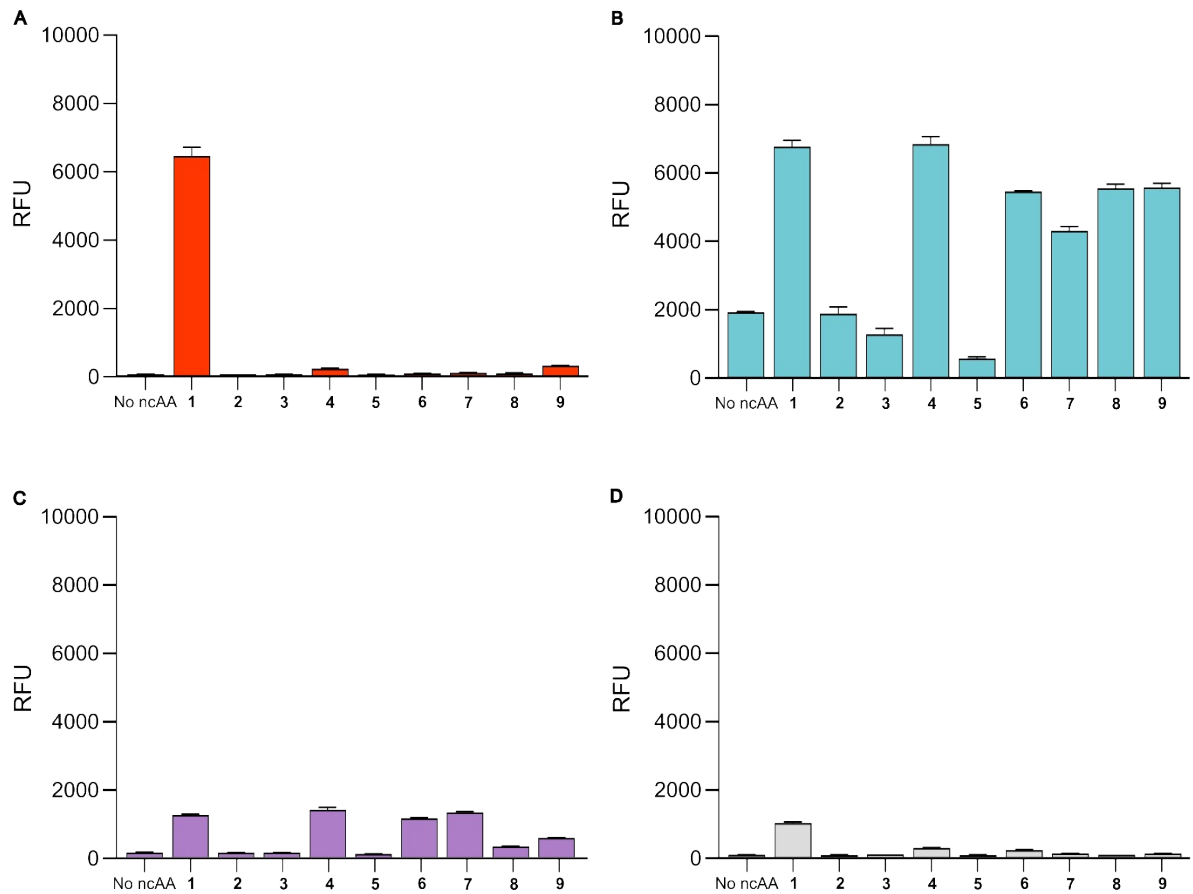
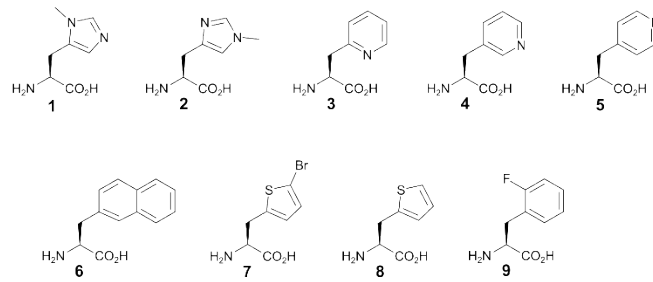
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Supplementary Figure 1: Bar charts showing GFP production containing MeHis at position 150 in cultures with varying MeHis concentrations (0-5 mM), using either **A**) *MaPyIRS*^{MIFAF}; **B**) *G1PyIRS*^{IFGFF}; **C**) *RumEnPyIRS*^{IFGFF}; **D**) *RumEnPyIRS*^{MIFAF}. Error bars represent the standard deviation of measurements made in triplicate.



Supplementary Figure 2: Bar charts showing the activities of **A)** *MaPyIRS*^{MIFAF}; **B)** *G1PyIRS*^{IFGFF}; **C)** *RumEnPyIRS*^{IFGFF}; **D)** *RumEnPyIRS*^{MIFAF}, towards a small panel of ncAAs (10 mM, 1-9) for the production of GFP through the suppression of the UAG codon at position 150. Error bars represent the standard deviation of measurements made in triplicate.

Table S1: Mass spectrometry of GFP variants.

	PyIRS	ncAA used	GFP construct	Predicted	Observed
1	<i>G1PyIRS</i> ^{IFGFF}	MeHis	GFP 150UAG (6His-tagged)	27864	27864
2	<i>G1PyIRS</i> ^{MIFAF}	MeHis	GFP 150UAG (6His-tagged)	27864	27864
3	<i>RumEnPyIRS</i> ^{IFGFF}	MeHis	GFP 150UAG (6His-tagged)	27864	27864
4	<i>RumEnPyIRS</i> ^{MIFAF}	MeHis	GFP 150UAG (6His-tagged)	27864	27864
5	<i>G1PyIRS</i> ^{IFGFF}	No ncAA	GFP 150UAG (6His-tagged)	-	27860 ^a
6	<i>G1PyIRS</i> ^{MIFAF}	MeHis	GFP 40 and 150UAG (6His-tagged)	27901	27901
7	<i>G1PyIRS</i> ^{MIFAF}	4	GFP 150UAG (6His-tagged)	27861	27861
8	<i>G1PyIRS</i> ^{MIFAF}	6	GFP 150UAG (6His-tagged)	27946	27946
9	<i>G1PyIRS</i> ^{MIFAF}	7	GFP 150UAG (6His-tagged)	27945	27945
10	<i>G1PyIRS</i> ^{MIFAF}	8	GFP 150UAG (6His-tagged)	27866	27866

^a observed mass corresponds to incorporation of phenylalanine.