# Supplementary Material

## Mutagenesis of the L-Amino Acid Ligase RizA Increased the Production of Bioactive Dipeptides

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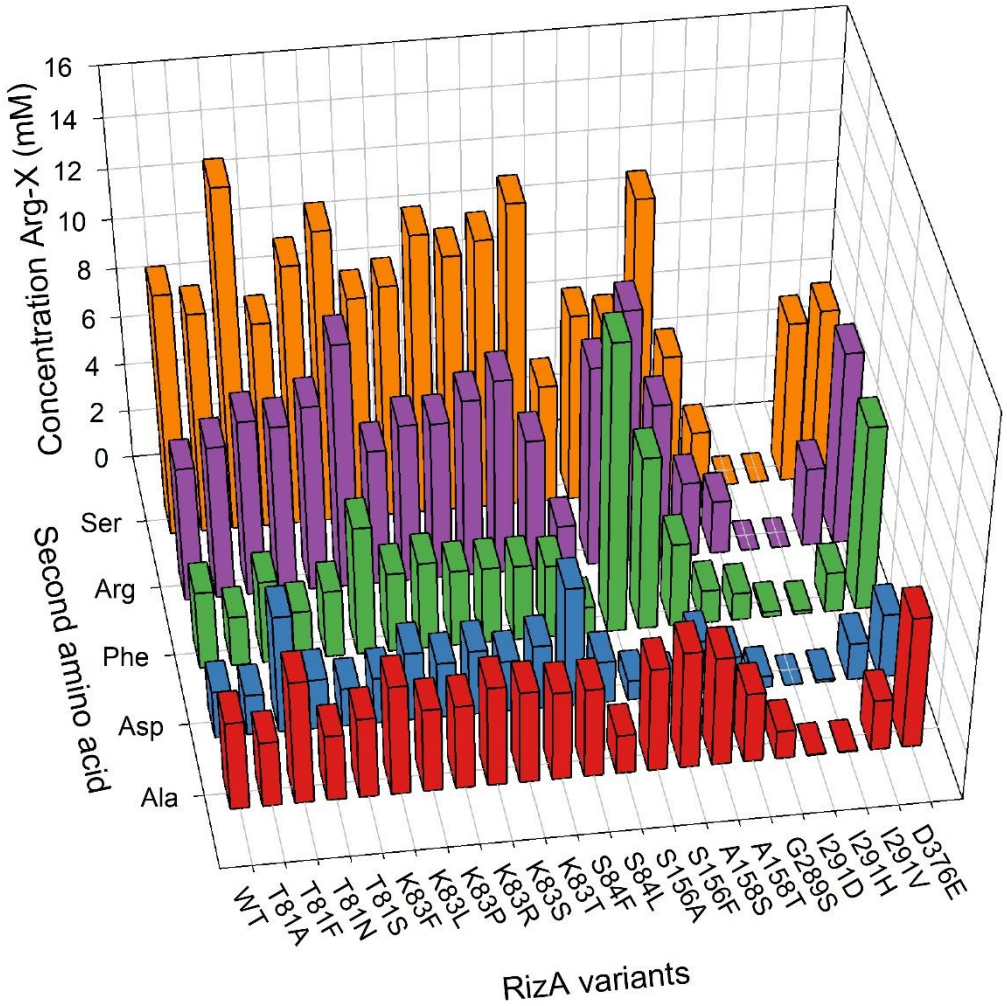


**Figure S1.** 3D bar chart of dipeptide formation. Created with data from Figures 4 to 8.

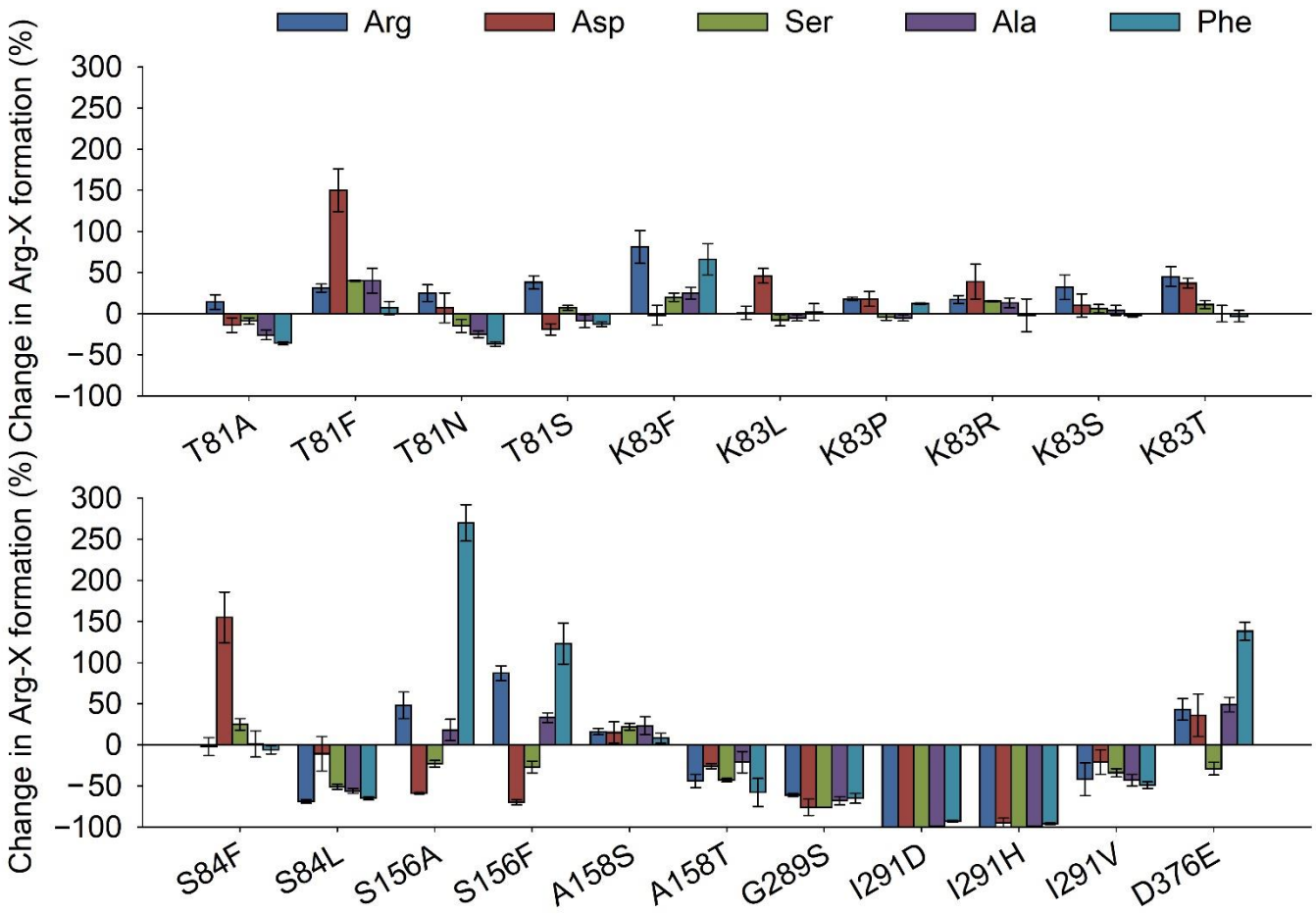


**Figure S2.** Change in product formation of RizA. Reactions were set up in triplicate and data is presented as mean ± standard deviation.

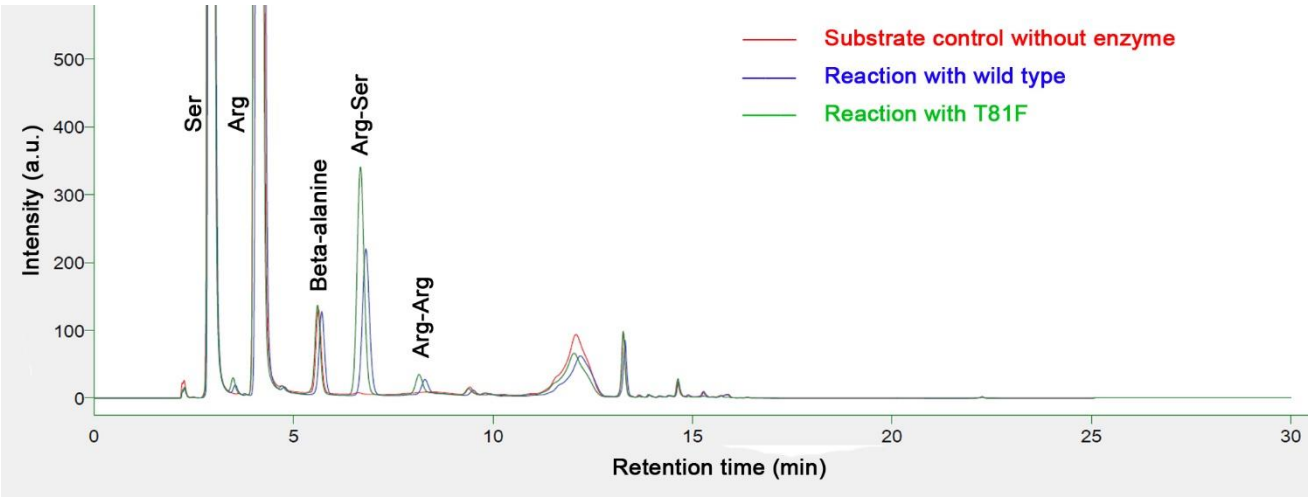


**Figure S3.** Exemplary chromatogram with gradient program 1 for measurement of Arg-Ser. For each reaction, only one of the triplicates is shown. The amino acids could not be quantitated due to the significantly higher intensity of their derivates overloading the fluorescence detector. Substrate controls without enzyme were used to account for signals resulting from impurities.

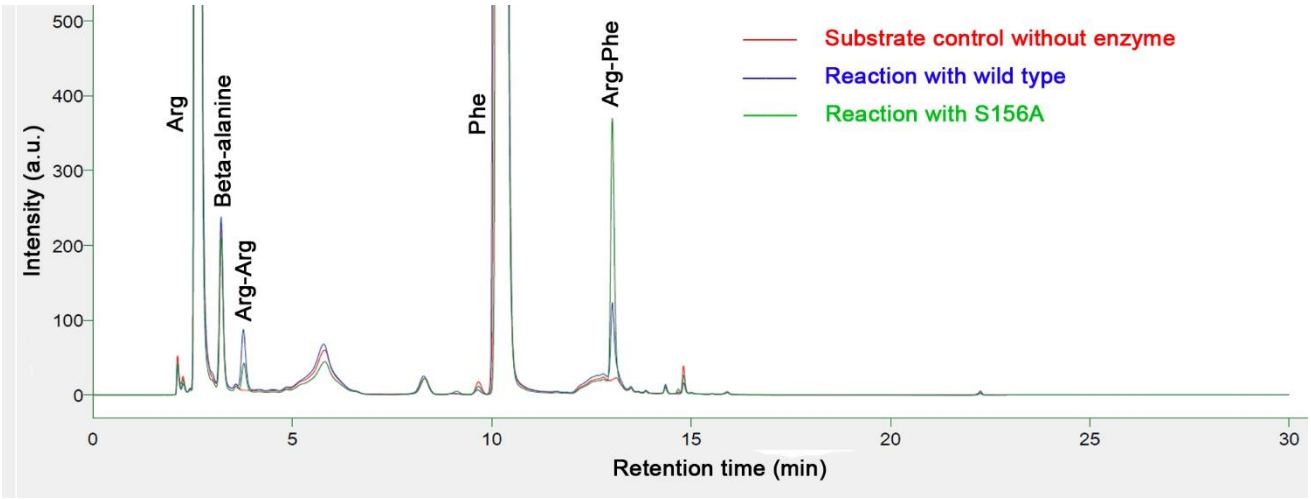


**Figure S4.** Exemplary chromatogram with gradient program 2 for measurement of Arg-Phe. For each reaction, only one of the triplicates is shown. The amino acids could not be quantitated due to the significantly higher intensity of their derivates overloading the fluorescence detector. Substrate controls without enzyme were used to account for signals resulting from impurities.

**Table S1.** Primer pairs for mutagenesis. The given annealing temperatures are for the three-step protocol, in the two-step protocol annealing and elongation were both performed at 72°C (see Materials & Methods).

| Name | Sequence (5'->3') | Annealing temperature (°C) |
|---|---|---|
| T81F_fw | TTAGCACCTTTGAAAAAAGCATTCTGACC | 66 |
| T81F_rv | TTTTTCAAAGGTGCTAACAATATGATCAAACG | 66 |
| T81S_fw | AGCACCAGCGAAAAAAGCATTCTGACCG | 69 |
| T81S_rv | TTTTTCGCTGGTGCTAACAATATGATCAAACG | 69 |
| K83F_fw | ACCGAATTTAGCATTCTGACCGGTGGTTTTC | 67 |
| K83F_rv | AATGCTAAATTCGGTGGTGCTAACAATATG | 67 |
| K83R_fw | ACCGAACGTAGCATTCTGACCGGTG | 68 |
| K83R_rv | AATGCTACGTTCGGTGGTGCTAAC | 68 |
| S156A_fw | TTAGGTGCGGGTGCACTGAATACCTTTATTATC | Two-step protocol |
| S156A_rv | TGCACCCGCACCTAATGCCGGTTTAAC | Two-step protocol |
| S156F_fw | TTAGGTTTTGGTGCACTGAATACCTTTATTATC | 67 |
| S156F_rv | TGCACCAAAACCTAATGCCGGTTTAACAATAATC | 67 |
| A158S_fw | AGCGGTTCTCTGAATACCTTTATTATCCATAGC | 69 |
| A158S_rv | ATTCAGAGAACCGCTACCTAATGCCGGTTTAAC | 69 |
| G289S_fw | TATTGGTGGTAGCGGCATTAGCCGTATG | Two-step protocol |
| G289S_rv | AATGCCGCTACCACCAATACGCATTG | Two-step protocol |
| I291V_fw | GGTGGCGTGAGCCGTATGATTGAGAAAAAATTC | Two-step protocol |
| I291V_rv | AATCATACGGCTCACGCCACCACCACCAATAC | Two-step protocol |
| D376E_fw | TCATTTGAACTGGCACGTCTGTATTTTTG | 67 |
| D376E_rv | TGCCAGTTCAAATGAGCTGCTCTGTTTTTC | 67 |
| T81A fw | AGCACCGCGGAAAAAAGCATTCTGAC | Two-step protocol |
| T81A rv | TTTTTCCGCGGTGCTAACAATATGATCAAACG | Two-step protocol |
| T81N fw | AGCACCAACGAAAAAAGCATTCTGACCG | Two-step protocol |
| T81N rv | TTTTTCGTTGGTGCTAACAATATGATCAAACGGAC | Two-step protocol |
| T81R fw | AGCACCCGCGAAAAAAGCATTCTGAC | Two-step protocol |
| T81R rv | TTTTTCGCGGGTGCTAACAATATGATCAAACG | Two-step protocol |
| K83S fw | ACCGAAAGCAGCATTCTGACCGGTG | Two-step protocol |

| | | |
|---|---|---|
| K83S rv | AATGCTGCTTTCGGTGGTGCTAACAATATG | Two-step protocol |
| K83T fw | ACCGAAACCAGCATTCTGACCGGTG | Two-step protocol |
| K83T rv | AATGCTGGTTTCGGTGGTGCTAACAATATG | Two-step protocol |
| K83L fw | ACCGAACTGAGCATTCTGACCGGTG | Two-step protocol |
| K83L rv | AATGCTCAGTTCGGTGGTGCTAACAATATG | Two-step protocol |
| K83P fw | ACCGAACCGAGCATTCTGACCGGTG | Two-step protocol |
| K83P rv | AATGCTCGGTTCGGTGGTGCTAACAATATG | Two-step protocol |
| S84F fw | GAAAAATTTATTCTGACCGGTGGTTTTCTGCG | 68 |
| S84F rv | CAGAATAAATTTTTCGGTGGTGCTAACAATATGATC | 68 |
| S84L fw | GAAAAACTGATTCTGACCGGTGGTTTTC | 68 |
| S84L rv | CAGAATCAGTTTTTCGGTGGTGCTAACAATATG | 68 |
| A158T fw | AGCGGTACCCTGAATACCTTTATTATCCATAG | 68 |
| A158T rv | ATTCAGGGTACCGCTACCTAATGCCG | 68 |
| I291H fw | GGTGGCCATAGCCGTATGATTGAGAAAAAATTC | Two-step protocol |
| I291H rv | ACGGCTATGGCCACCACCACCAATACG | Two-step protocol |
| I291D fw | GGTGGCGATAGCCGTATGATTGAGAAAAAATTC | Two-step protocol |
| I291D rv | ACGGCTATCGCCACCACCACCAATACG | Two-step protocol |