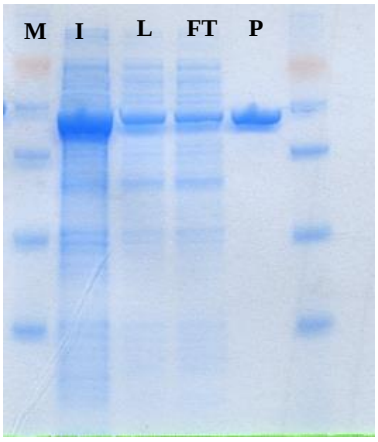
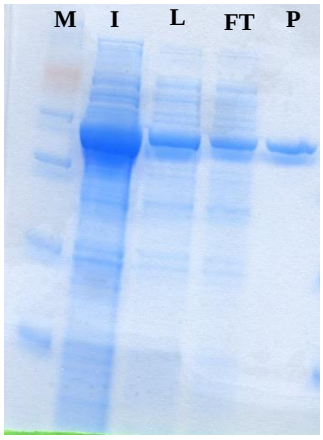
Supplementary material

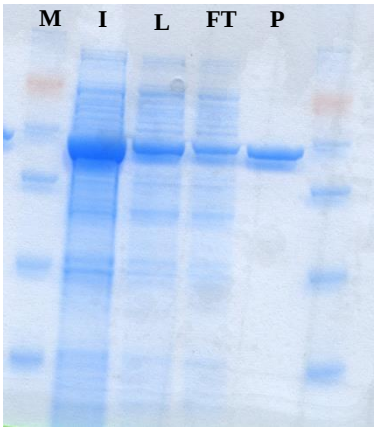
A



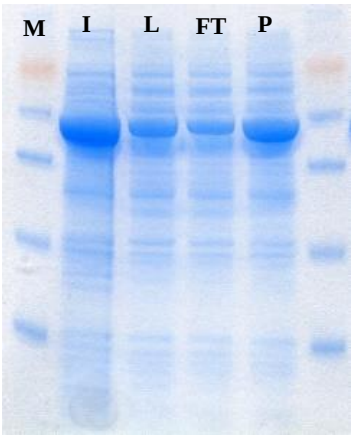
W259G



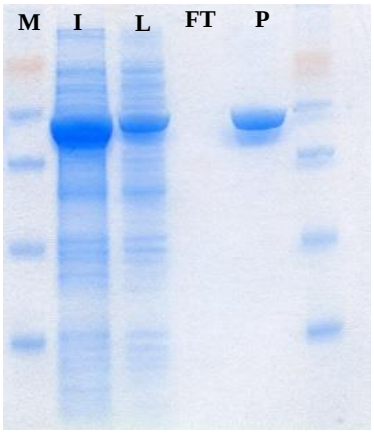
V238T



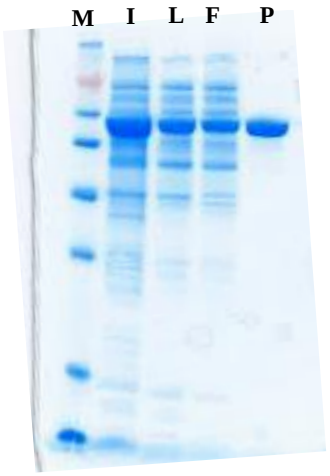
F350Y



W259G/V238T

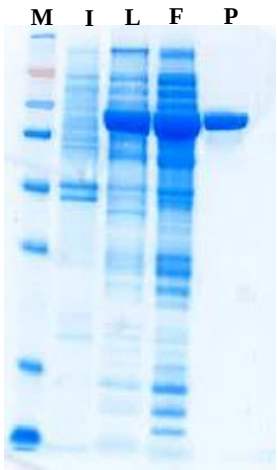


W259G/F350Y

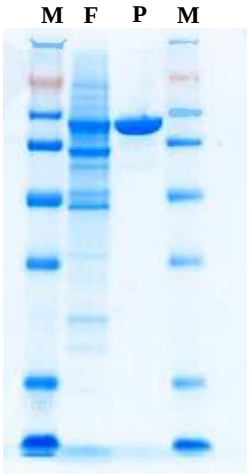


F350A

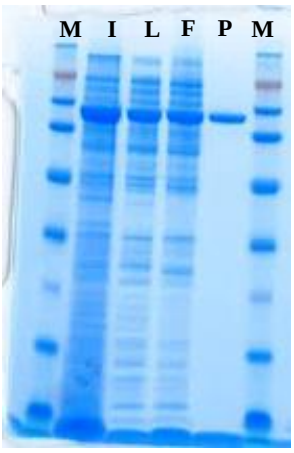
F350T



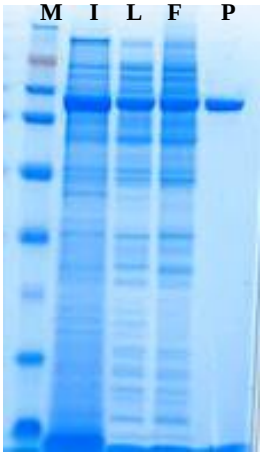
F350V



W259G/V328T/F350A

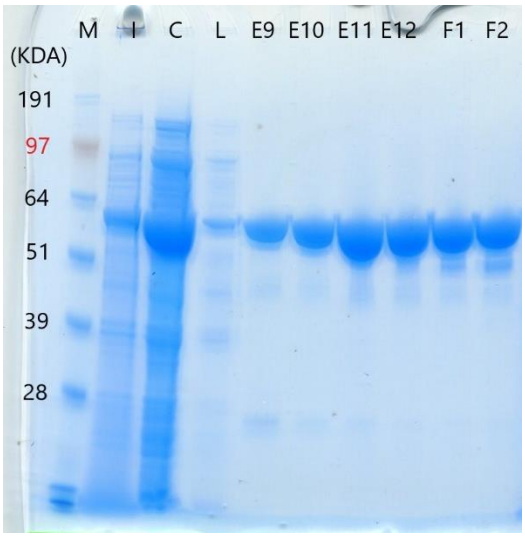


W259G/V238T/F350T

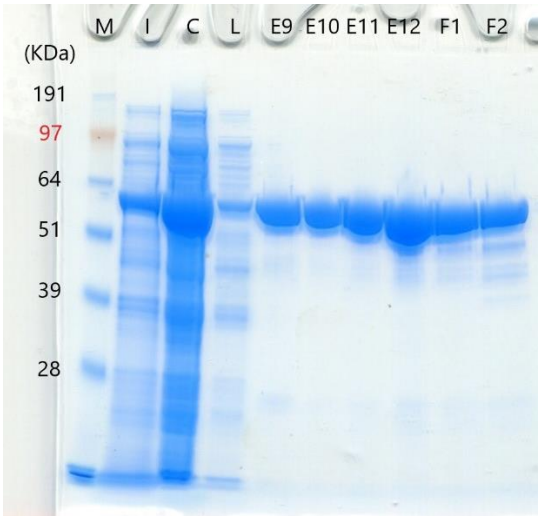


W259G/V238T/F350V

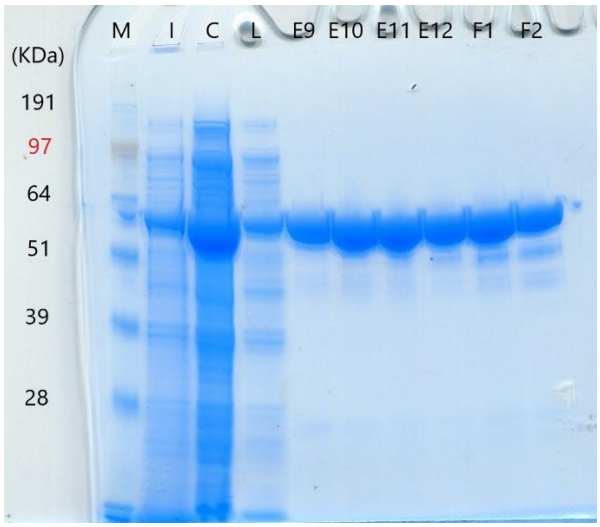
B



MSACL WT



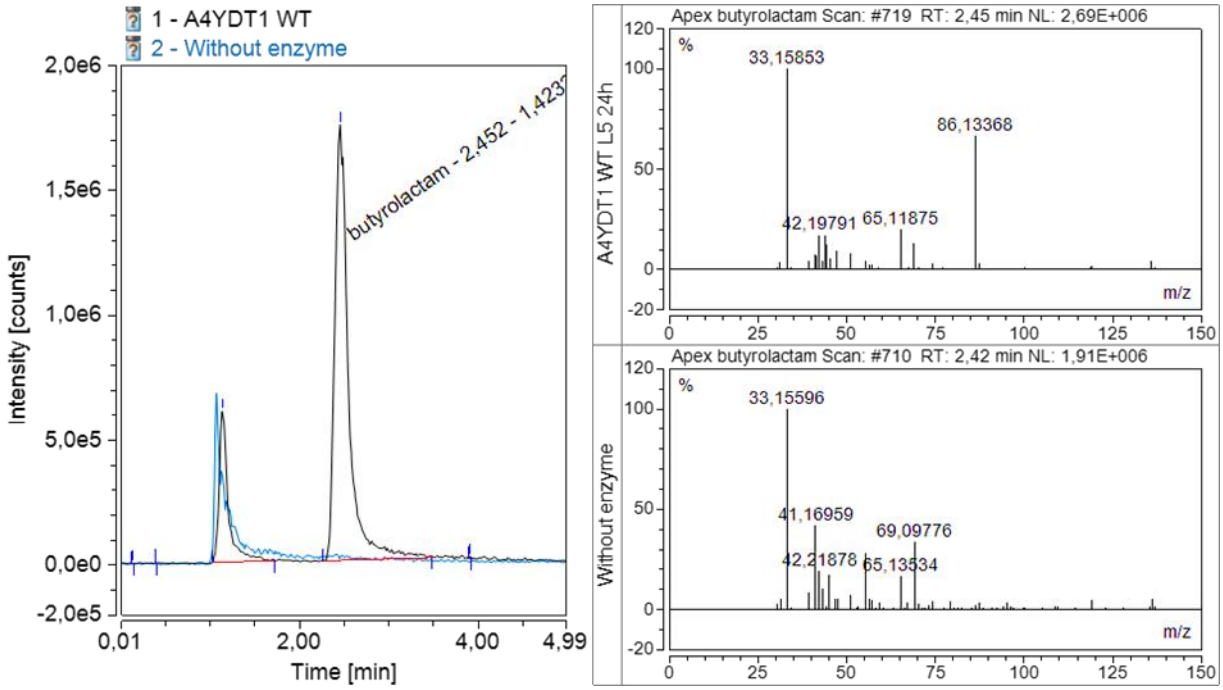
W259G



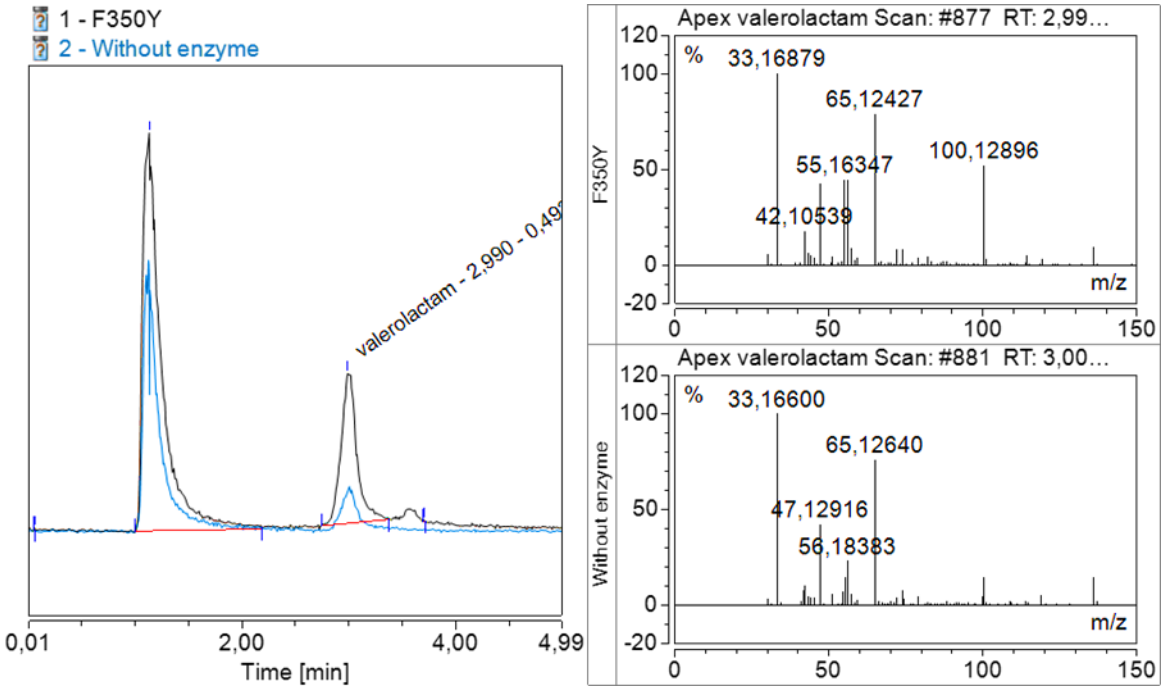
W259G/V238T

Supplementary Figure 1. SDS gel using the Nu-PAGE system (Invitrogen) of the MSACL WT and mutants. M: Molecular marker SeeBlue® Plus2 pre-strained standard (6 µl deposited), I: Induction (15 µg deposited), L: lysate (surpernatant, 15 µg deposited), FT: Flow Through NiNTA (15 µg deposited) and P: purified enzyme (5 µg deposited). **A**. Small-scale purification. **B**. Large-scale purification.

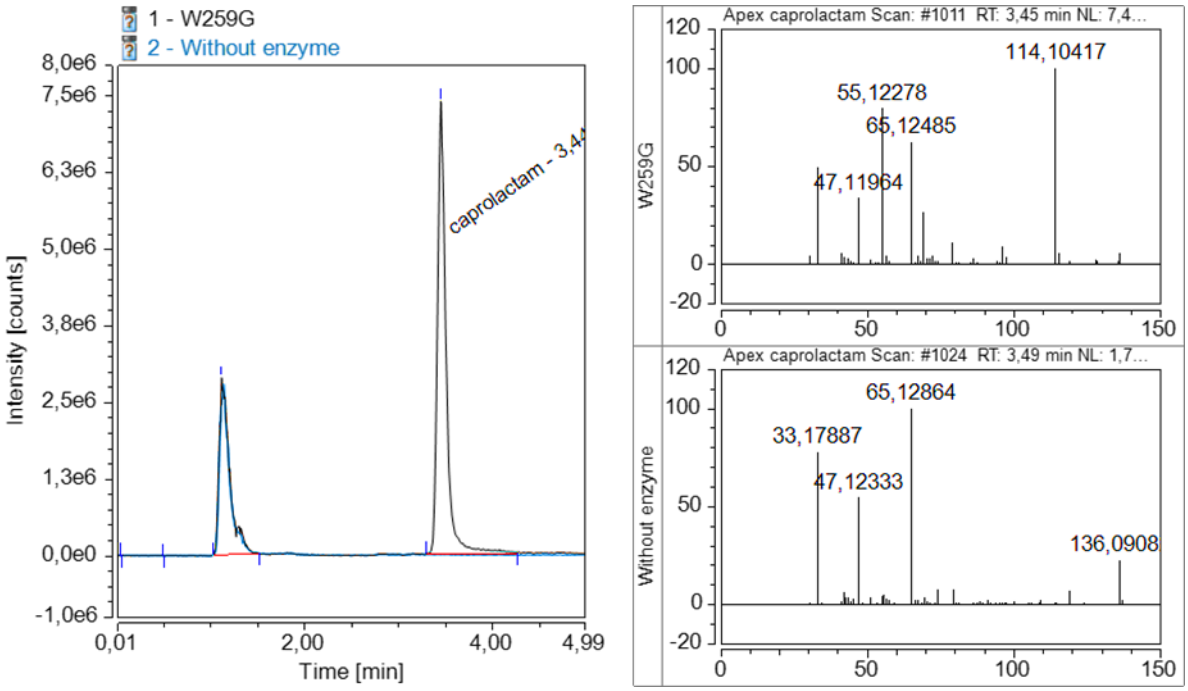
A


1 - A4YDT1 WT
2 - Without enzyme
Intensity [counts]
Time [min]
butyrolactam - 2,452 - 1,423
Apex butyrolactam Scan: #719 RT: 2,45 min NL: 2,69E+006
A4YDT1 WT L5 24h
33,15853
86,13368
42,19791
65,11875
m/z
Apex butyrolactam Scan: #710 RT: 2,42 min NL: 1,91E+006
Without enzyme
33,15596
41,16959
69,09776
42,21878
65,13534
m/z

B

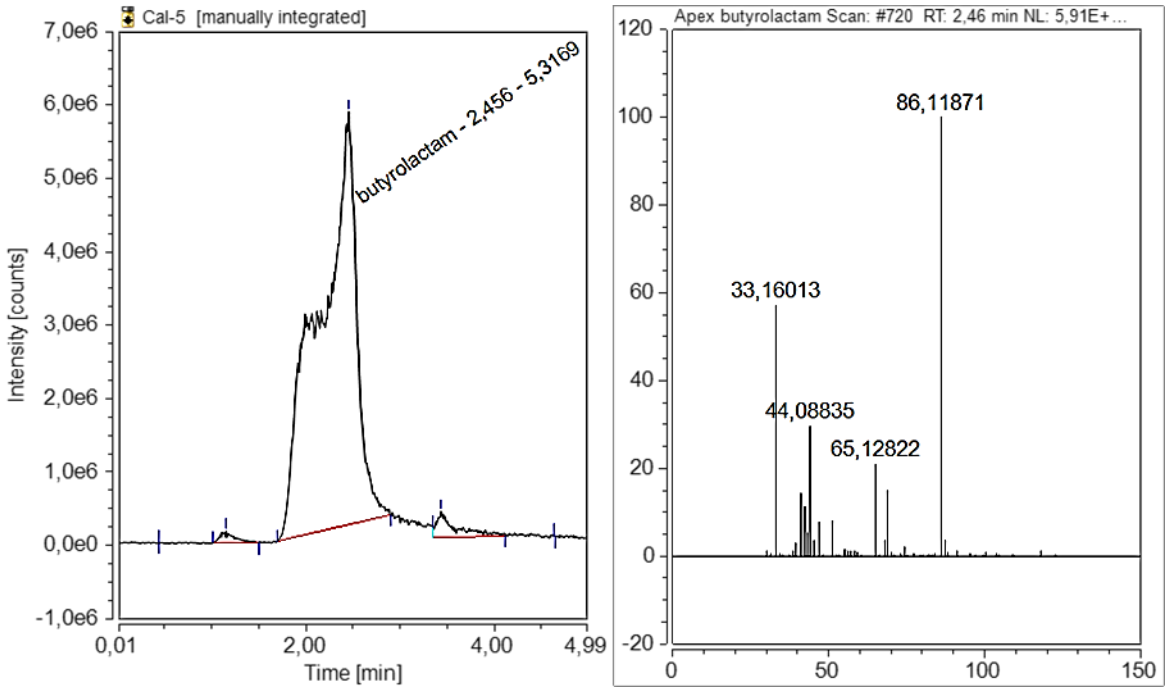

1 - F350Y
2 - Without enzyme
Time [min]
valerolactam - 2,990 - 0,49
Apex valerolactam Scan: #877 RT: 2,99...
F350Y
33,16879
65,12427
55,16347
100,12896
42,10539
m/z
Apex valerolactam Scan: #881 RT: 3,00...
Without enzyme
33,16600
65,12640
47,12916
56,18383
m/z

C

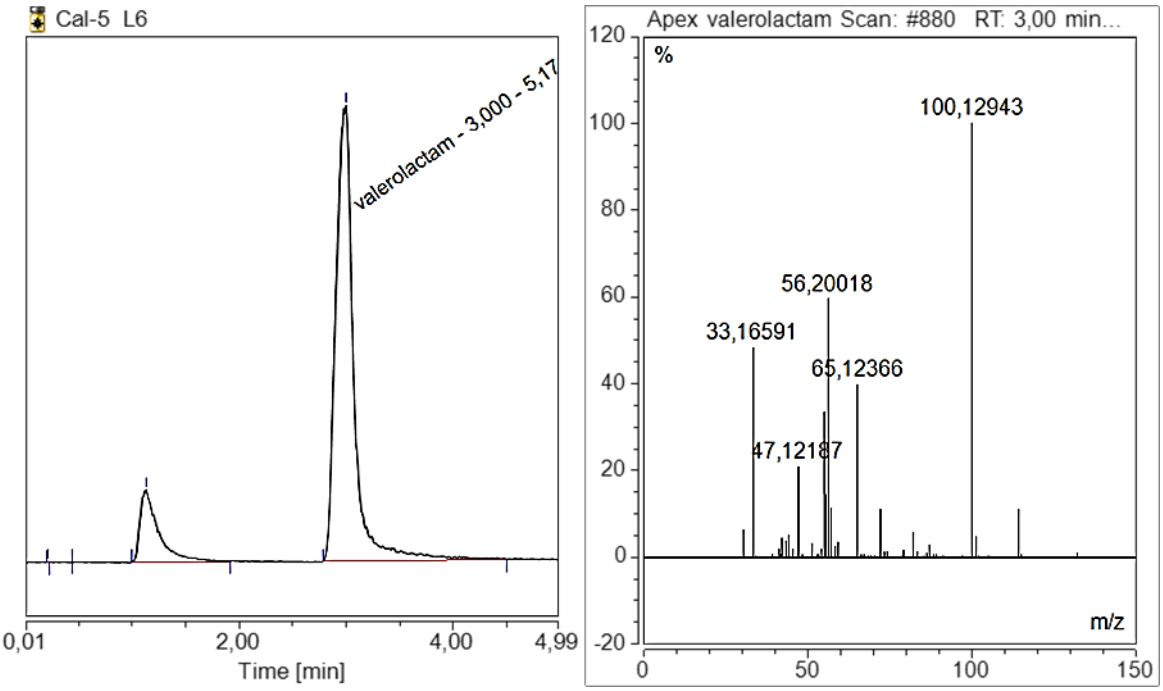


Supplementary Figure 2. UHPLC-MS (APCI) chromatograms and product mass spectra of blank reaction mixture without enzyme (blue) and reaction mixtures (black) of **A**. 4-aminobutyric acid and wild-type *Ms*ACL; MS: extraction of the mass m/z = 86 from the Total Ion Current (TIC) signal, tr = 2.3 min. **B**. 5-aminovaleric acid and *Ms*ACL F350Y; MS: extraction of the mass m/z = 100 from the TIC signal, tr = 3.0 min. **C**. 6-aminohexanoic acid and *Ms*ACL W259G; MS: extraction of the mass m/z = 114 from the TIC signal, t_r = 3.4 min.

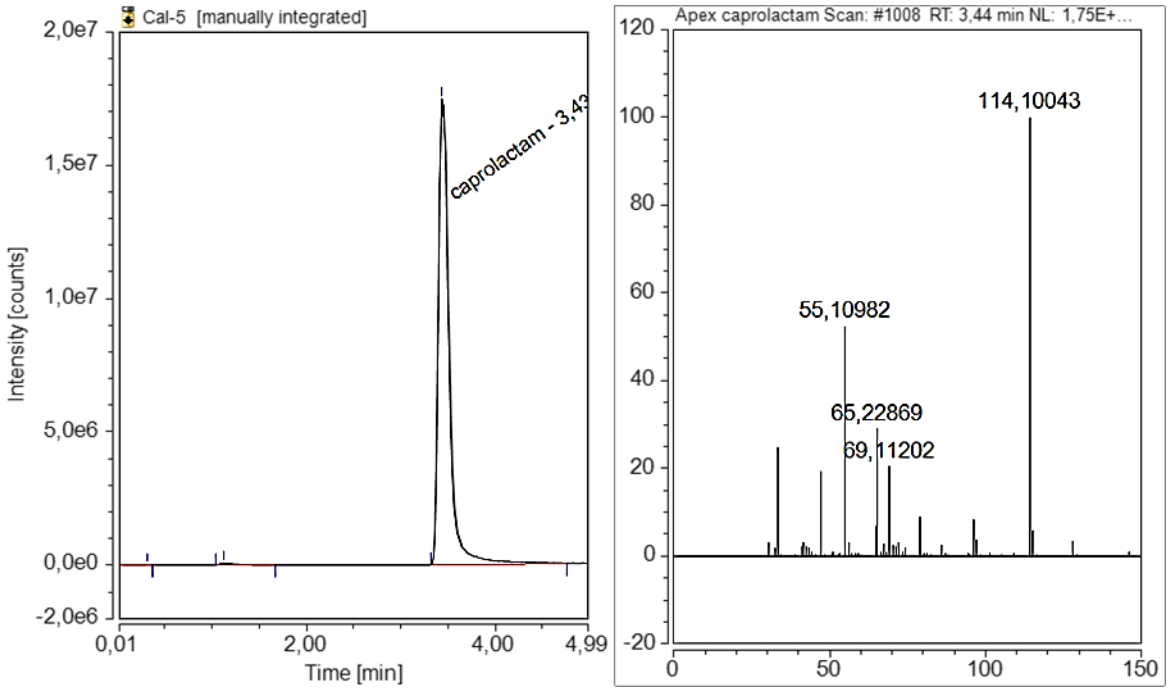
A


Cal-5 [manually integrated]
butyrolactam - 2,456 - 5,3169
Intensity [counts]
Time [min]
Apex butyrolactam Scan: #720 RT: 2,46 min NL: 5,91E+...
86,11871
33,16013
44,08835
65,12822

B

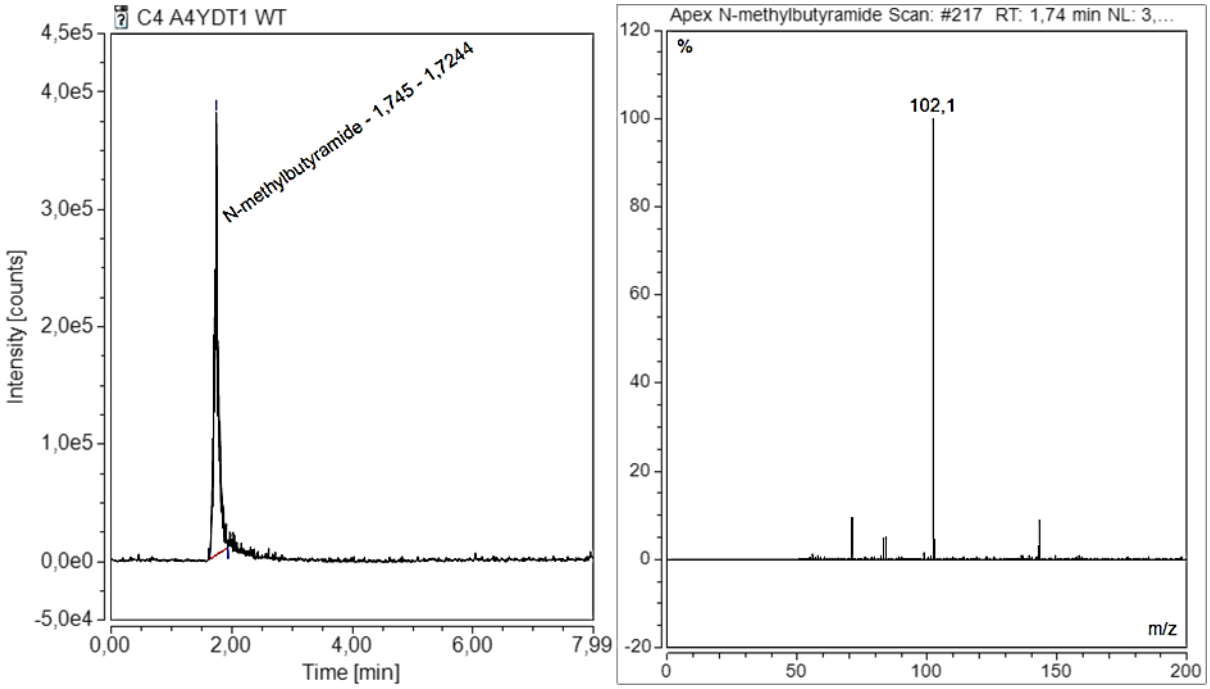

Cal-5 L6
valerolactam - 3,000 - 5,17
Time [min]
Apex valerolactam Scan: #880 RT: 3,00 min...
%
100,12943
56,20018
33,16591
65,12366
47,12187
m/z

C

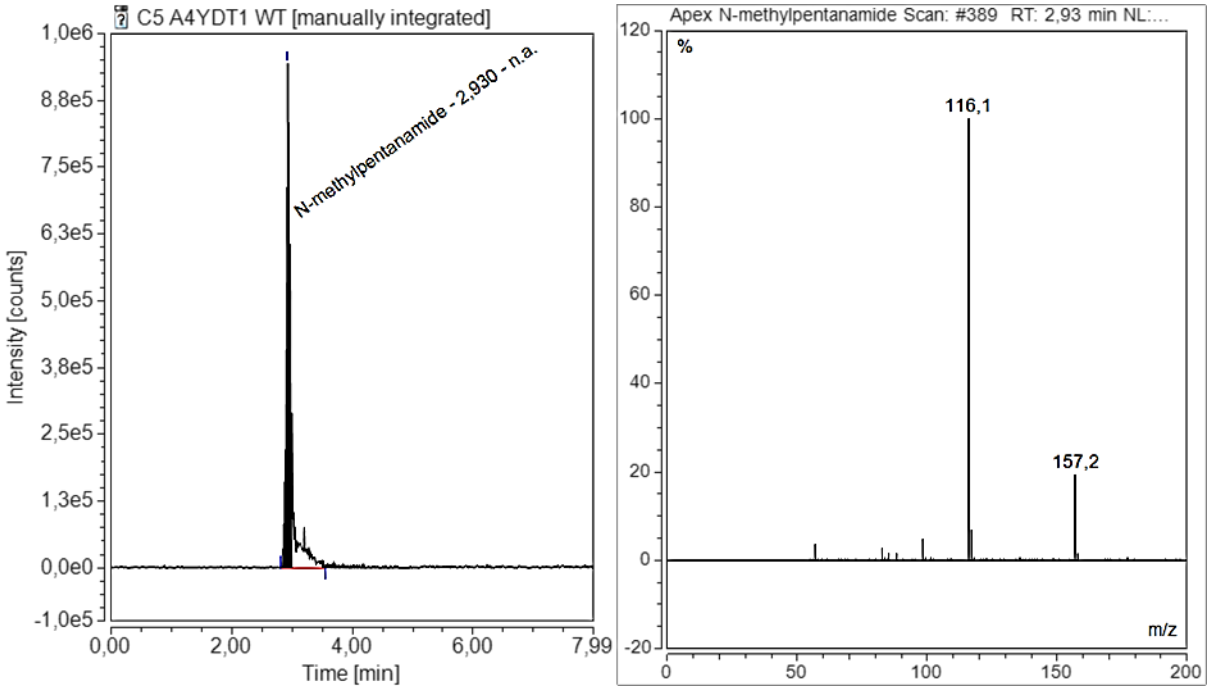


Supplementary Figure 3. UHPLC-MS (APCI) chromatograms and product mass spectra of standard product of **A**. 4-aminobutyric acid; MS: extraction of the mass m/z = 86 from the TIC signal, tr = 2.3 min. **B**. 5-aminovaleric acid; MS: extraction of the mass m/z = 100 from TIC signal, tr = 3.0 min. **C**. 6-aminohexanoic acid; MS: extraction of the mass m/z = 114 from the TIC signal, t_r = 3.4 min.

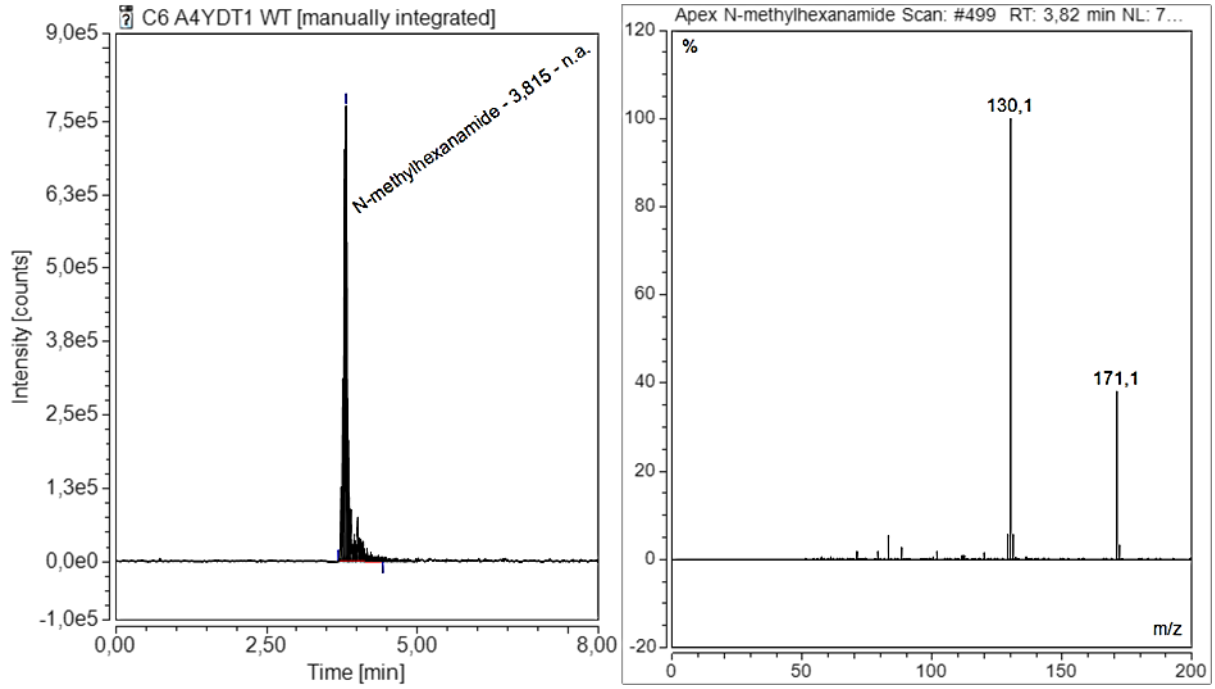
A


C4 A4YDT1 WT
N-methylbutyramide - 1,745 - 1,7244
Intensity [counts]
Time [min]
Apex N-methylbutyramide Scan: #217 RT: 1,74 min NL: 3,...
%
102,1
m/z

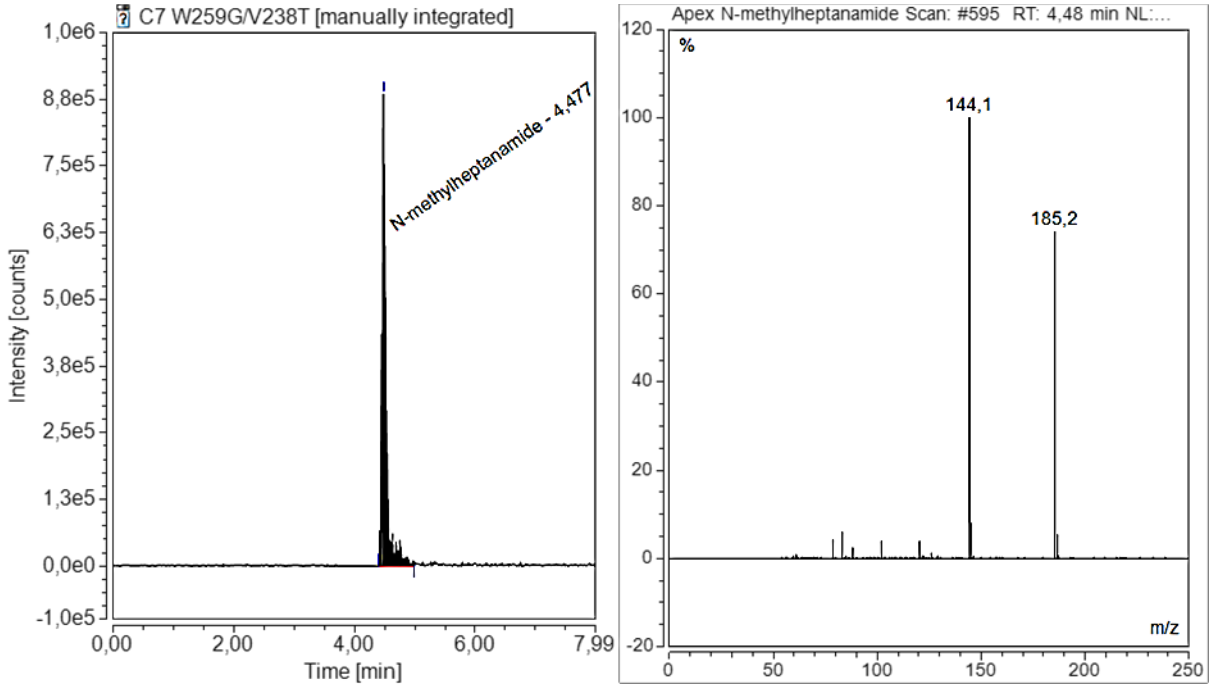
B


C5 A4YDT1 WT [manually integrated]
N-methylpentanamide - 2,930 - n.a.
Intensity [counts]
Time [min]
Apex N-methylpentanamide Scan: #389 RT: 2,93 min NL:...
%
116,1
157,2
m/z

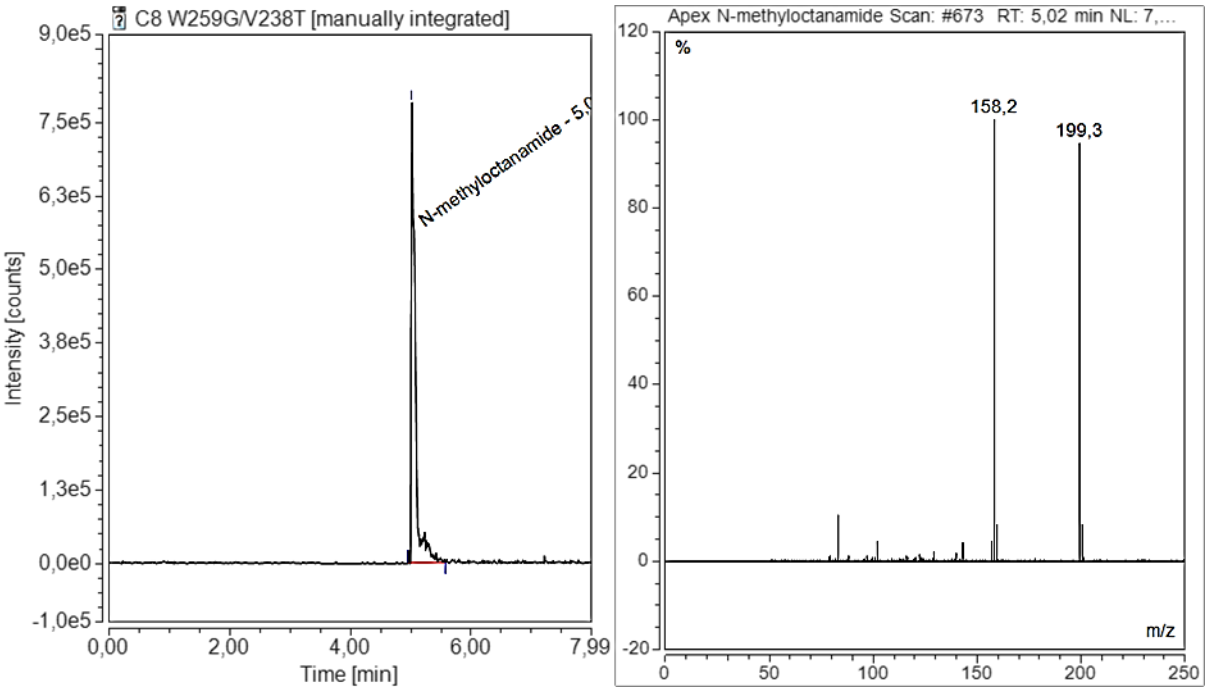
C


C6 A4YDT1 WT [manually integrated]
N-methylhexanamide - 3,815 - n.a.
Intensity [counts]
Time [min]
Apex N-methylhexanamide Scan: #499 RT: 3,82 min NL: 7...
%
130,1
171,1
m/z

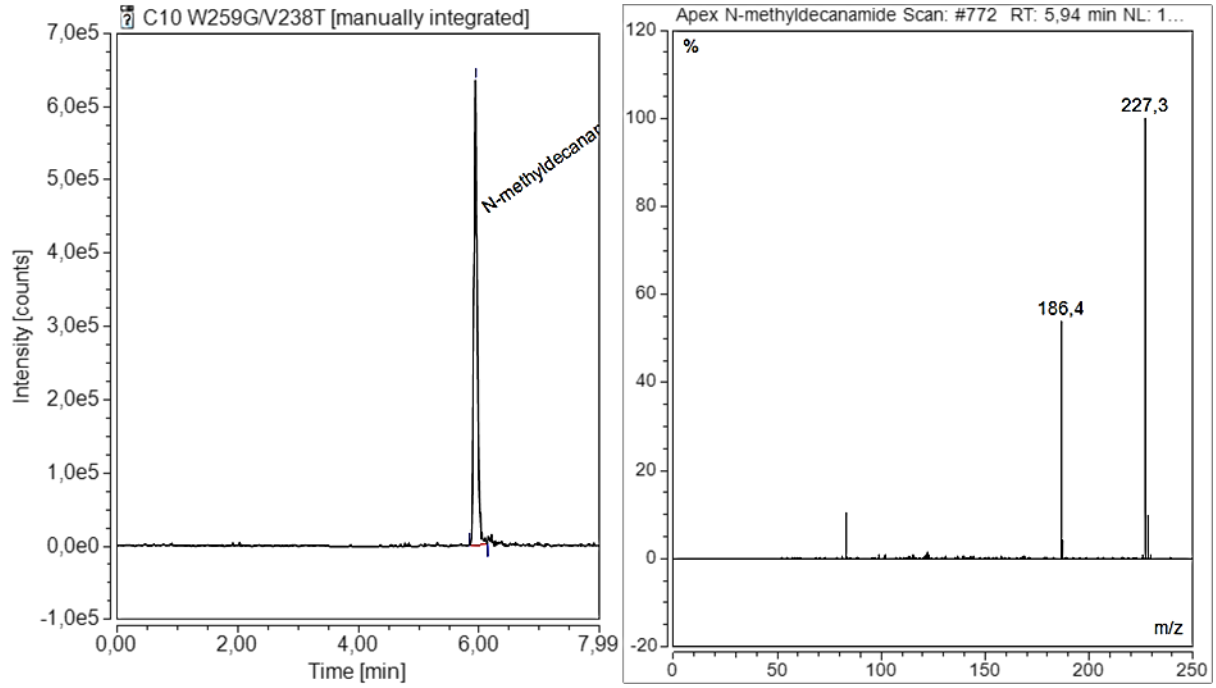
D


C7 W259G/V238T [manually integrated]
N-methylheptanamide - 4,477
Intensity [counts]
Time [min]
Apex N-methylheptanamide Scan: #595 RT: 4,48 min NL:...
%
144,1
185,2
m/z

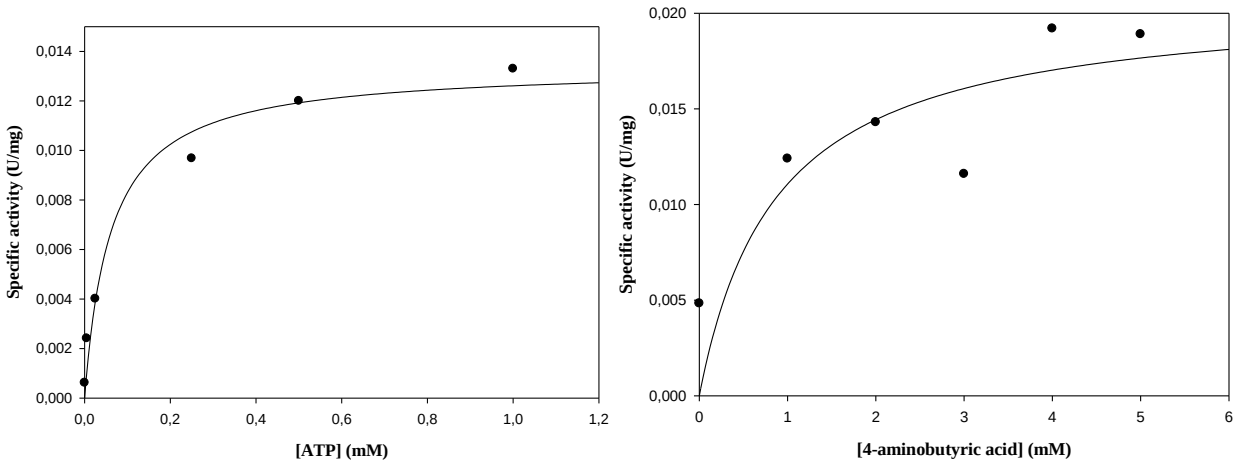
E



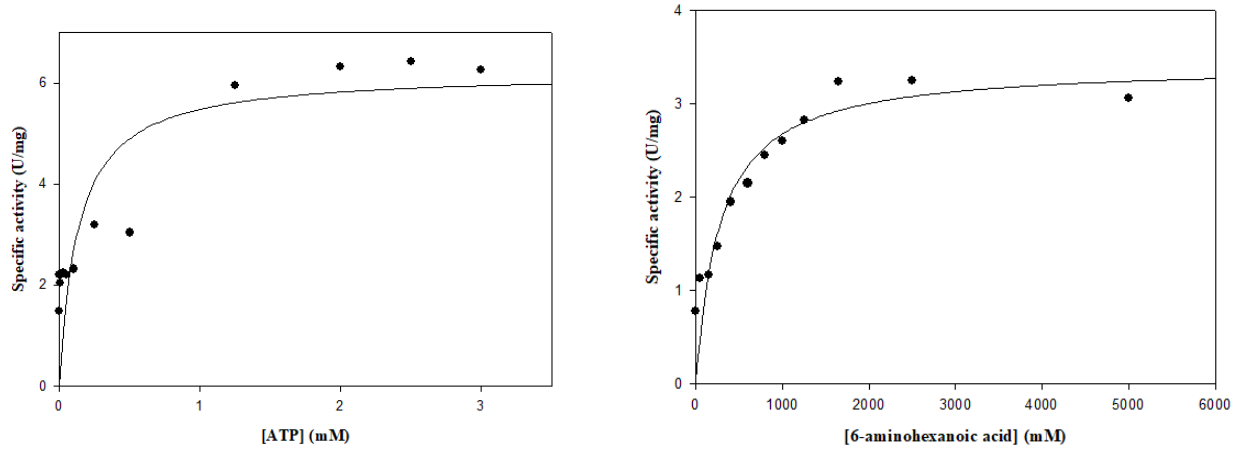
F



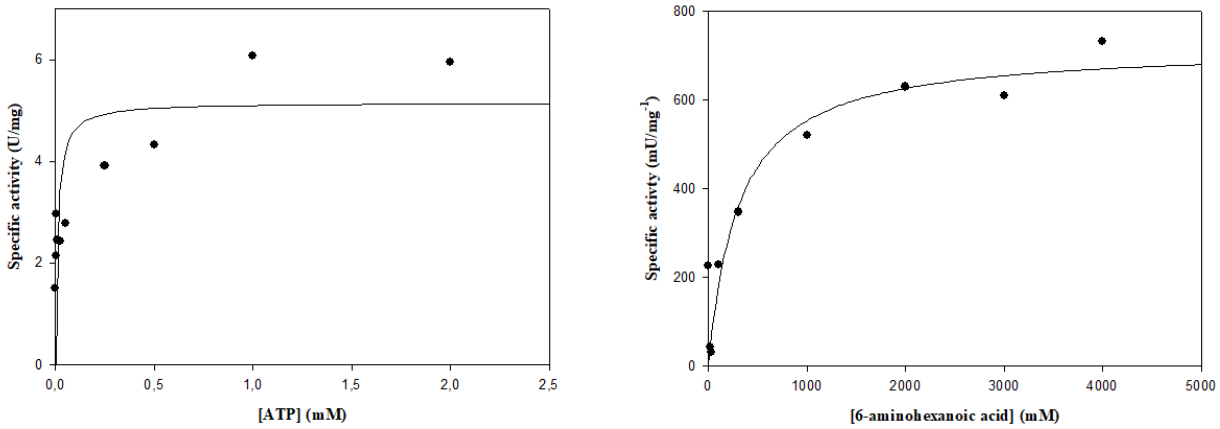
Supplementary Figure 4. UHPLC-MS (ESI) chromatograms of reaction mixture of various carboxylic acids with *N*-methyl amine and *Ms*ACL WT and mutants. **A**. *N*-methylbutyramide. **B**. *N*-methylpentanamide. **C**. *N*-methylhexanamide. **D**. *N*-methylheptanamide. **E**. *N*-methyloctanamide. **F**. *N*-methyldecanamide.



Supplementary Figure 5. Michaelis-Menten plot for the determination of the kinetic parameters of *Ms*ACL wild-type.
Reactions conditions: TRIS pH 7.5; ATP 0-10 mM; 4-aminobutyric acid 10 mM; *Ms*ACL wild-type 0.5 mg/mL.

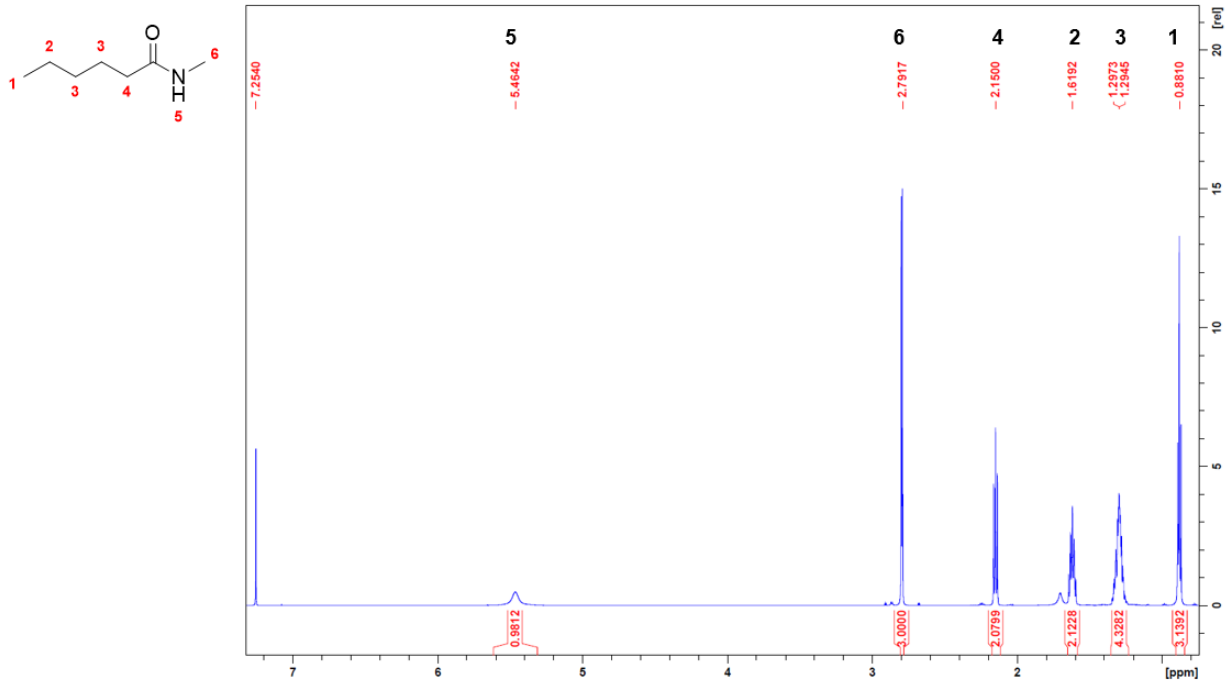


Supplementary Figure 6. Michaelis-Menten plot for the determination of the kinetic parameters of *Ms*ACL W259G. Error represent standard deviations from two independent experiments. The uncertainties of the kinetic parameters values are those generated by the fitting.
TRIS pH 7.5; ATP 10 mM; 6-aminohexanoic acid 6 M; *Ms*ACL W259G 0.05 mg/mL.

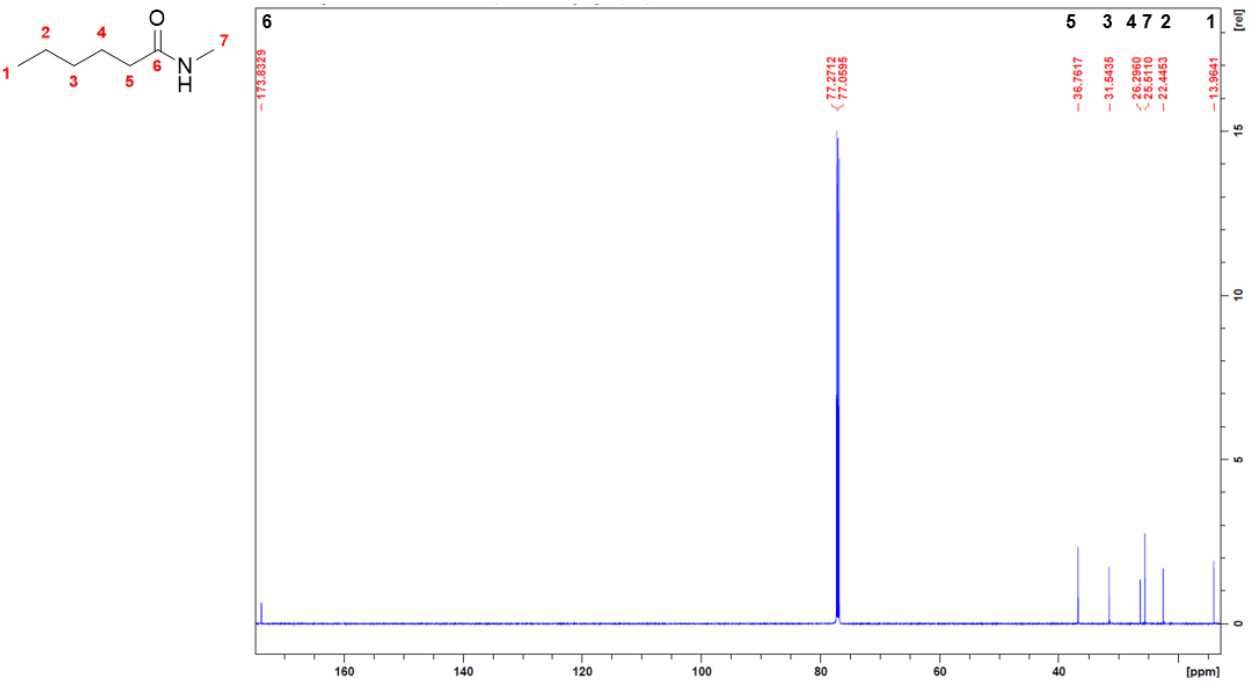


Supplementary Figure 7. Michaelis-Menten plot for the determination of the kinetic parameters of MSACL W259G/V238T. Error represent standard deviations from two independent experiments. The
Uncertainties of the kinetic parameters values are those generated by the fitting.
TRIS pH 7.5; ATP 10 mM; 6-aminohexanoic acid 6 M; *Ms*ACL mutant W259G/V238T 0.05 mg/mL.

A

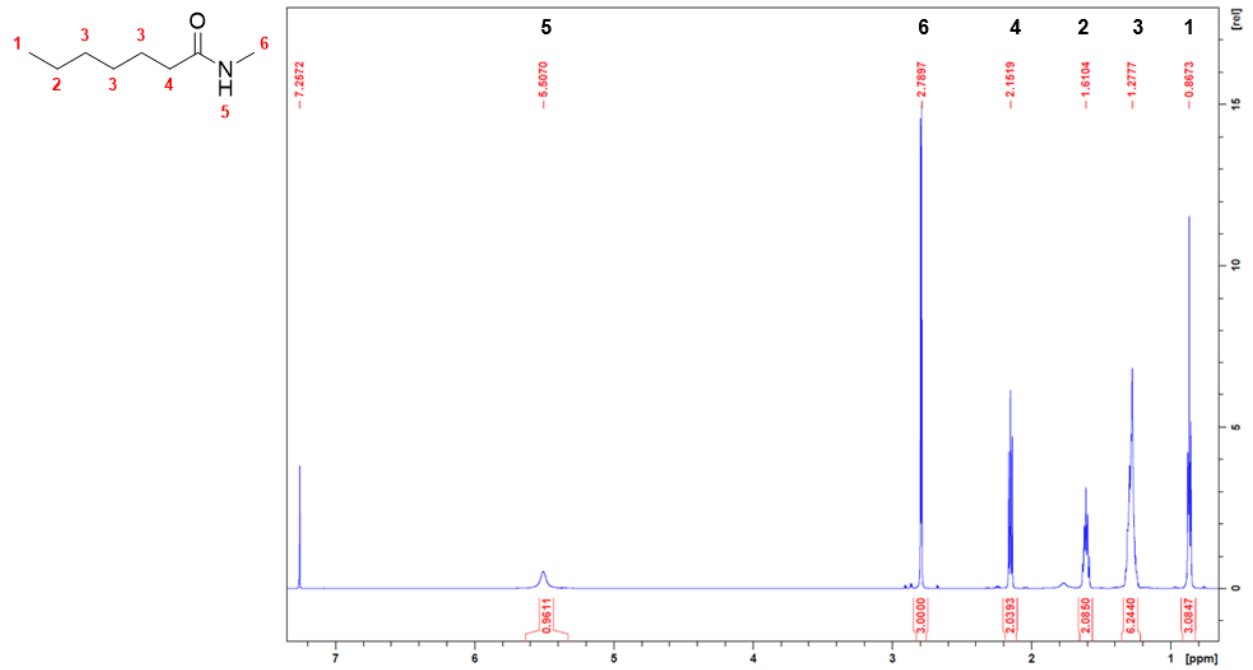


B

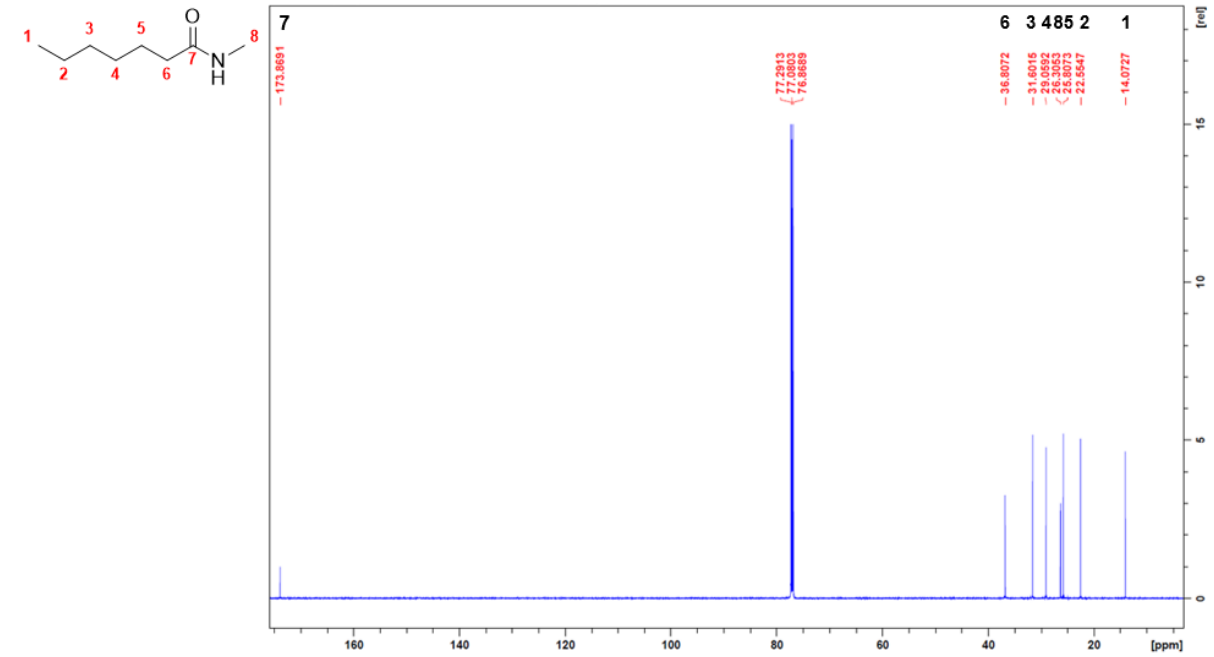


Supplementary Figure 8. NMR spectra of standard *N*-methylhexanamide in $CDCl_3$. **A**. ^{1}H 600 MHz. **B**. ^{13}C 125 MHz.

A

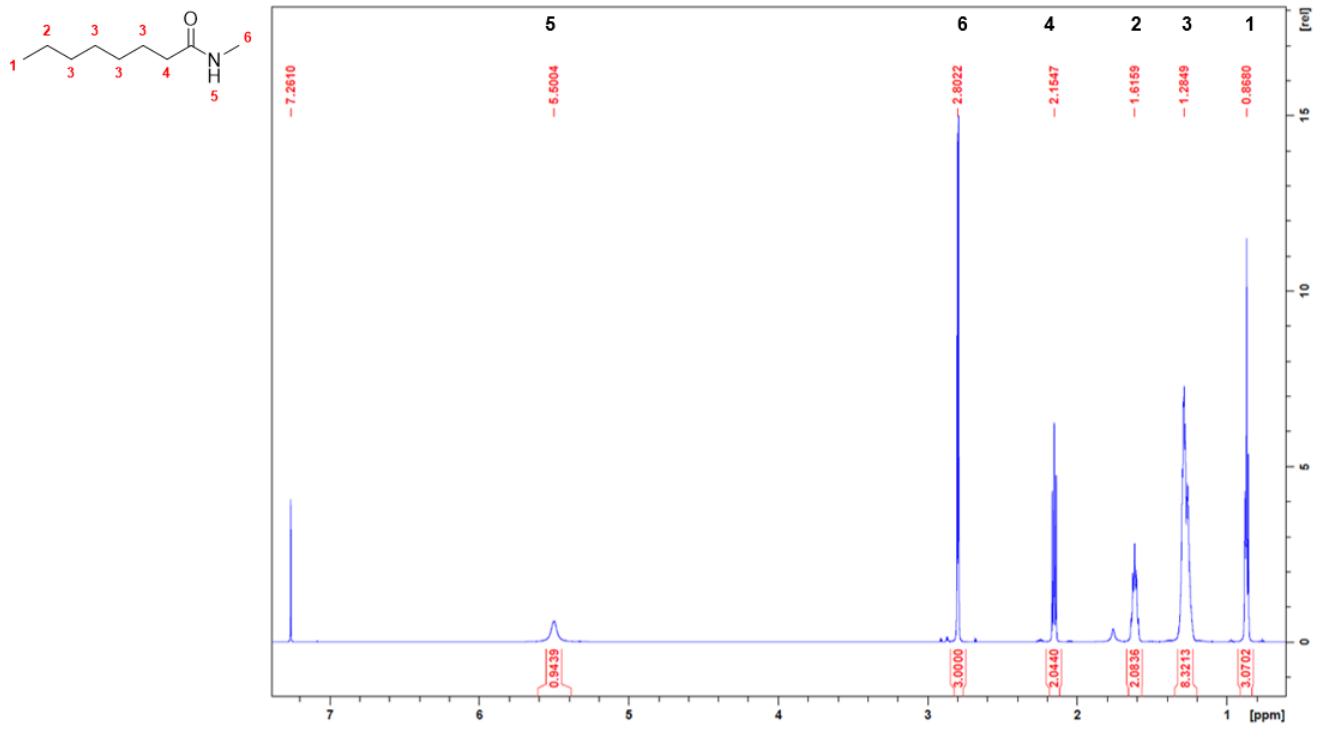


B

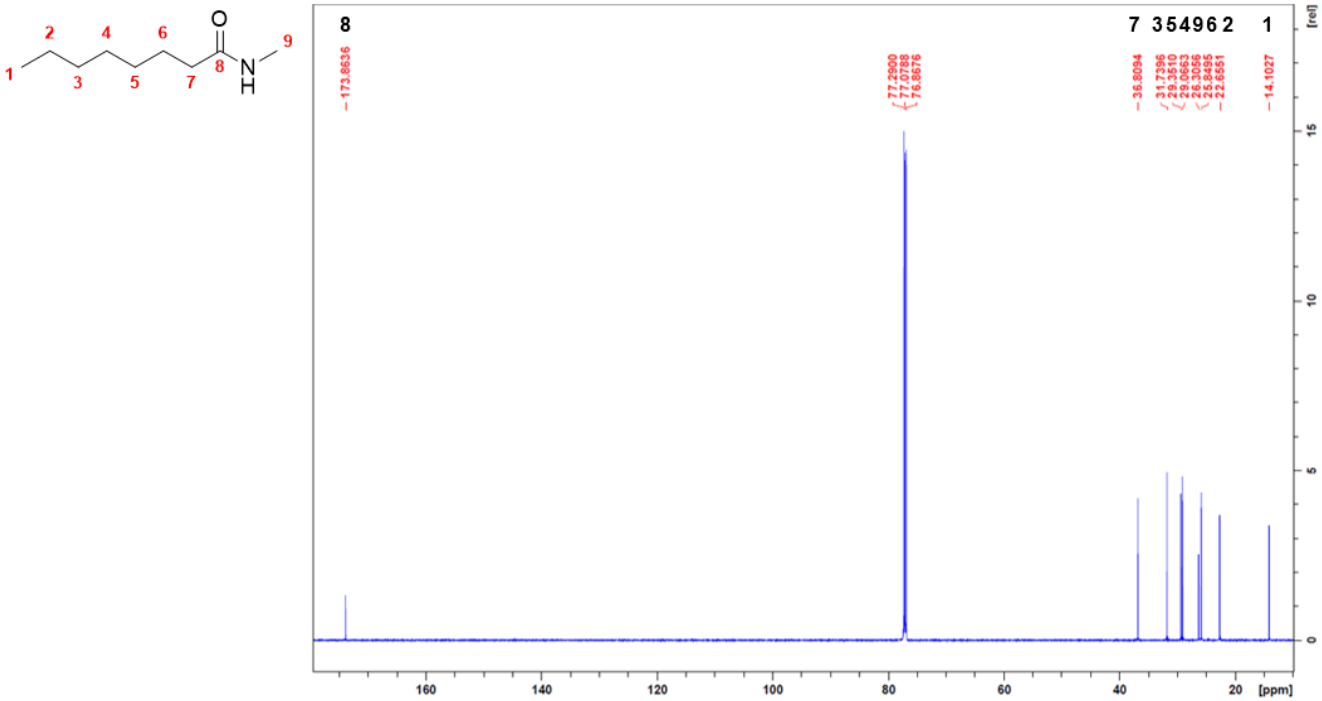


Supplementary Figure 9. NMR spectra of standard *N*-methylheptnamide in $CDCl_3$. **A**. ^{1}H 600 MHz. **B**. ^{13}C 125 MHz.

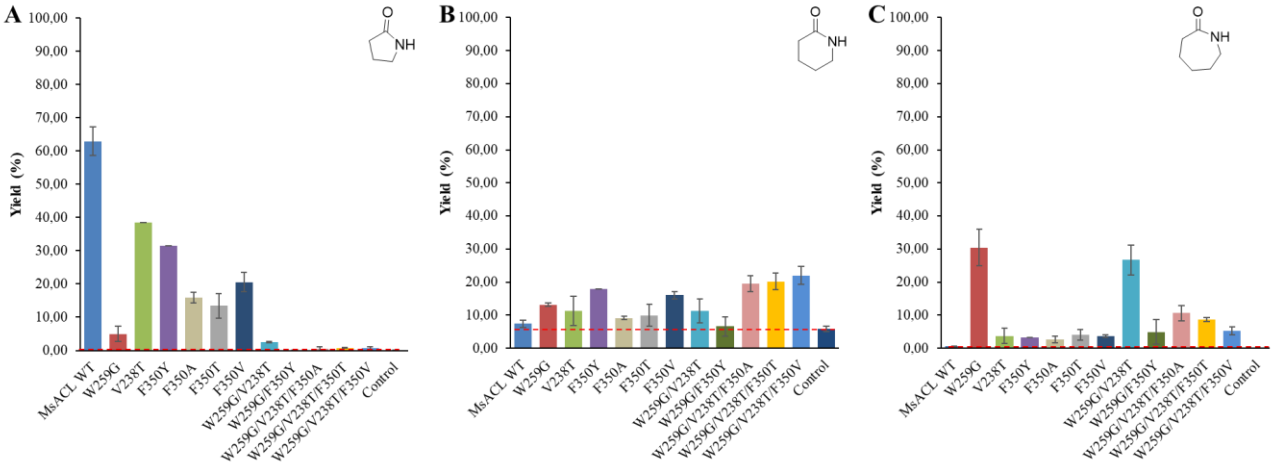
A



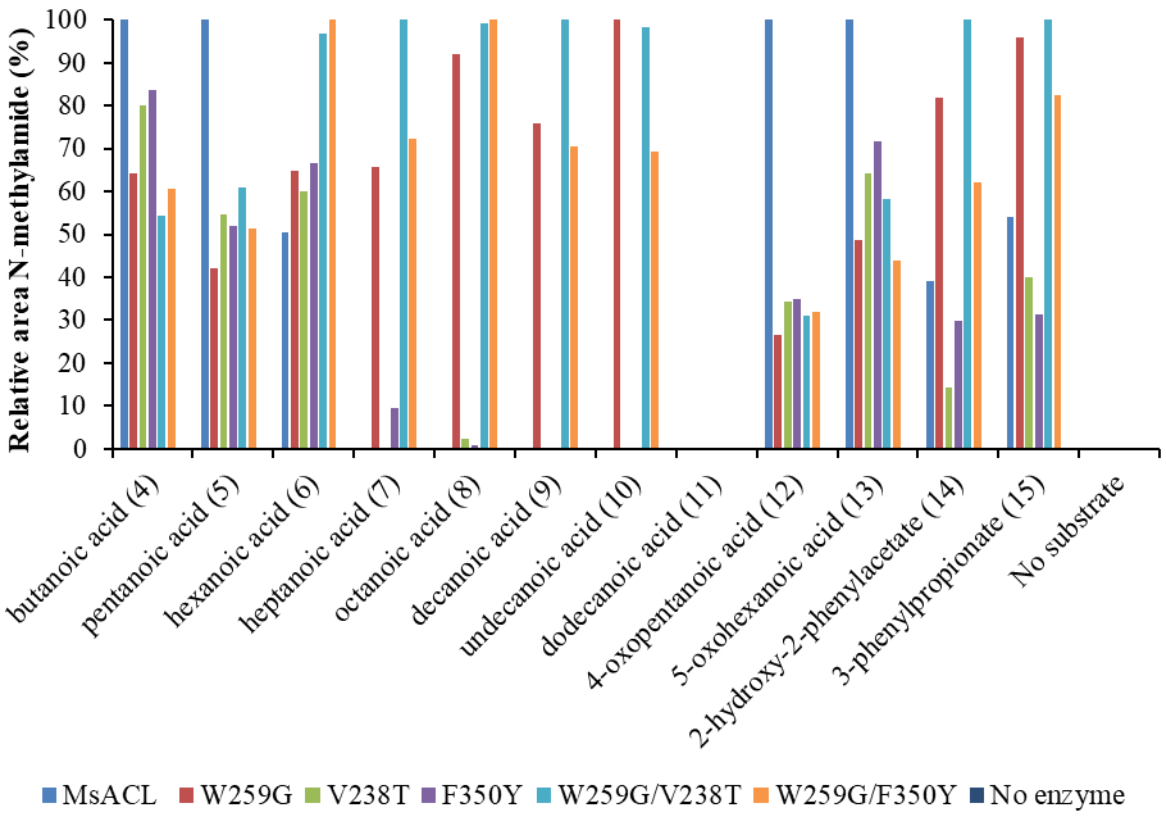
B



Supplementary Figure 10. NMR spectrum of standard *N*-methyloctanamide in $CDCl_3$. **A**. ^{1}H 600 MHz. **B**. ^{13}C 125 MHz.



Supplementary Figure 11. Analytical yields of lactam formation from ω-amino acids catalyzed by WT and mutant enzymes. **A**. γ-butyrolactam. **B**. δ-valerolactam. **C**. ε-caprolactam. The dotted red line indicates the yield of spontaneous cyclisation. Reaction conditions: 5 mM ω-amino acid (**1**-**3**), 5 mM ATP, 5 mM $MnCl_2$ in 50 mM MOPS buffer (pH 8.5), 2 mg/mL of purified enzyme, 400 rpm at 60 °C for 24 h. Control reactions correspond to samples without enzyme. Analytical yields were deduced from calibration curves by UHPLC-MS (APCI).



Supplementary Figure 12. Comparison of the relative areas of various substrates compared to the best variant enzyme for each substrate by monitoring the formation of the corresponding N-methyl amide compounds by UHPLC-MS. Reaction conditions: 5 mM substrate (**4**-**6**; **8**-**9**; **10**-**14**), 5 mM ATP, 5 mM MgCl2, 50 mM MeNH2 in 50 mM phosphate buffer (pH 8) was added 0.2 mg/mL of purified enzyme, 400 rpm at 60 °C for 24 h.

Supplementary Table 1. Primer sequence for variant. Mutated codons are in bold and underlined.

A4YDT1 variant	Primer	Sequence
W259G	Forward (F)	TGGGCCAAGTTCGCG**GGC**AGCTCATTCTTCTCTCCCCTT
	Reverse (R)	AAGGGGAGAGAAGAATGAGCT**GCC**CGCGAACTTGGCCCA
V238T	Forward (F)	ACCACAGCCTCCATA**ACC**GGCGTCAGGGAGAGCGATCTCCACTTA
	Reverse (R)	TAAGTGGAGATCGCTCTCCCTGACGCC**GGT**TATGGAGGCTGTGGT
F350Y	Forward (F)	CTTACCATAAGGGAC**TAT**TACGGCCAGACTGAGACC
	Reverse (R)	GGTCTCAGTCTGGCCGTA**ATA**GTCCCTTATGGTAAG
F350A	Forward (F)	CTTACCATAAGGGAC**GCC**TACGGCCAGACTGAGACC
	Reverse (R)	GGTCTCAGTCTGGCCGTA**GGC**GTCCCTTATGGTAAG
F350T	Forward (F)	CTTACCATAAGGGAC**ACC**TACGGCCAGACTGAGACC
	Reverse (R)	GGTCTCAGTCTGGCCGTA**GGT**GTCCCTTATGGTAAG
F350V	Forward (F)	CTTACCATAAGGGAC**GTG**TACGGCCAGACTGAGACC
	Reverse (R)	GGTCTCAGTCTGGCCGTA**CAC**GTCCCTTATGGTAAG