

SUPPLEMENTARY INFORMATION

Kinetics of the secoisolariciresinol glucosyltransferase LuUGT74S1 and its mutants

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Table S1. Forward and Reverse Primers used for site-directed mutagenesis of *LuUGT74S1*.

Primer ID	Direction	Primer sequence (5'-3')
1 Y144F	F	GCGGTGAACGCGATTTTTTATCATCTGCGCGAAGGC
	R	GCCTTCGCGCAGATGATAAAAAATCGCGTTCACCGC
2 S115A	F	TTATTTATGATGCGGCCATGCCGTGGTTTCTGGATG
	R	CATCCAGAAACCACGGCATGGCCGCATCATAAATAA
3 S115F	F	TTATTTATGATGCGTTTATGCCGTGGTTTCTGGATG
	R	CATCCAGAAACCACGGCATAAACGCATCATAAATAA
4 Q136E	F	CGCGGCGTTTCTGACGGAGAGCTGCGCGGTGAACG
	R	CGTTCACCGCGCAGCTCTCCGTCAGAAACGCCGCG
5 H194S	F	AATTTCTGGCGGCGTCTCTGCGTCAGTTTAGCAACG
	R	CGTTGCTAAACTGACGCAGAGACGCCGCCAGAAATT
6 Q136F	F	GCGCGGCGTTTCTGACGTTTAGCTGCGCGGTGAACG
	R	CGTTCACCGCGCAGCTAAACGTCAGAAACGCCGCGC
7 A17S	F	CCTGGTGGTGCCGGCGAGCGCGCAAGGCCATTTTAC
	R	GTAAAATGGCCTTGCGCGCTCGCCGGCACCACCAGG
8 Q136S	F	GCGGCGTTTCTGACGTCAAGCTGCGCGGTGAACGCG
	R	CGCGTTCACCGCGCAGCTTGACGTCAGAAACGCCGCG
9 S82F	F	AAGATCCGGAACATTATTTTCAGACCTTTCGCCGCG
	R	CGCGGCGAAAGGTCTGAAAATAATGTTCCGGATCTT
10 E189L	F	GATGATCTGCATACCTTATTTCTGGCGGCGCATCTG
	R	CAGATGCGCCGCCAGAAATAAGGTATGCAGATCATC

Gene specific primers, 100% complementary, No overhangs

Table S2. The mass to charge ratios m/z of the substrate and the glucoside products used for LC-MS detection. M = molecule; H = proton; HCOO⁻ = formate, Cl⁻ = chloride.

Substrat/ product	M [g/mol]	[M-H] ⁻ [m/z]	[M+CHOO] ⁻ [m/z]	[M+Cl] ⁻ [m/z]
SECO	362	361	407	397
SMG	524	523	569	559
SDG	686	685	731	721

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optimized LuUGT74S1 1 ATGGCGGGCGATGAAAGGCAAAAAGCCGATGCGTGGTGGTCCCGCGCGCGCCCAAGGCCAATTAA
LuUGT74S1_JX011632.1 1 ATGGCGGGCGATGAAAGGCAAAAAGCCGATGCGTGGTGGTCCCGCGCGCGCCCAAGGCCAATTAA
optimized LuUGT74S1 70 CCCCGCTGCTGCAAGTTAGCAATCGCGTGGTGGTCCCGCGCGCGCCCAAGGCCAATTAA
LuUGT74S1_JX011632.1 70 CCCCGCTGCTGCAAGTTAGCAATCGCGTGGTGGTCCCGCGCGCGCCCAAGGCCAATTAA
optimized LuUGT74S1 140 CTTATCAAGCACCATGACCGTACCCTGCGAGCGGATATCAATGAGAACATAGCGAAGGC
LuUGT74S1_JX011632.1 140 CTTATCAAGCACCATGACCGTACCCTGCGAGCGGATATCAATGAGAACATAGCGAAGGC
optimized LuUGT74S1 210 TTGATCAAGCGGCTGATGTCGAAGACCGGAACATATAGCAGACCTTTCCCGTGGGCA
LuUGT74S1_JX011632.1 210 TTGATCAAGCGGCTGATGTCGAAGACCGGAACATATAGCAGACCTTTCCCGTGGGCA
optimized LuUGT74S1 270 CCGAAACCCGACGATCTGATCGAAACAGAGCGAAAGCGCACCCGGTGCATGATATTTA
LuUGT74S1_JX011632.1 270 CCGAAACCCGACGATCTGATCGAAACAGAGCGAAAGCGCACCCGGTGCATGATATTTA
optimized LuUGT74S1 340 LGATGCGAGCATGCCGTGGTTCTGGAGTCCGAAACCGCTTGGCATTGTGGCGCGCTTCTC
LuUGT74S1_JX011632.1 340 LGATGCGAGCATGCCGTGGTTCTGGAGTCCGAAACCGCTTGGCATTGTGGCGCGCTTCTC
optimized LuUGT74S1 410 ACCCAGATGCGCGGTAAACCGCATATATTAACAATCGCGAAGGCAACATAAACGCCCGTGG
LuUGT74S1_JX011632.1 410 ACCCAGATGCGCGGTAAACCGCATATATTAACAATCGCGAAGGCAACATAAACGCCCGTGG
optimized LuUGT74S1 470 TGAGCATCCGGCGCGGACCTGGTGTATGATGGCTGCCCGCCCTGGAAGTGAAGCATGCGC
LuUGT74S1_JX011632.1 470 TGAGCATCCGGCGCGGACCTGGTGTATGATGGCTGCCCGCCCTGGAAGTGAAGCATGCGC
optimized LuUGT74S1 540 GAGCTTATTGGGATGATGTCGACAGCAATTTCTGGCGCGCATGTCGTCAGTTAGCAACAA
LuUGT74S1_JX011632.1 540 GAGCTTATTGGGATGATGTCGACAGCAATTTCTGGCGCGCATGTCGTCAGTTAGCAACAA
optimized LuUGT74S1 610 GGCGCGATTGGGTGTTGCAACACAGTATACAGCTGGAAGTGAAGCATGCGCAGTTCACAA
LuUGT74S1_JX011632.1 610 GGCGCGATTGGGTGTTGCAACACAGTATACAGCTGGAAGTGAAGCATGCGCAGTTCACAA
optimized LuUGT74S1 680 AACAGTGGTAATAACTTCGACATTTGGCCGACATCCGAGCTTATGGAACAAGAT
LuUGT74S1_JX011632.1 680 AACAGTGGTAATAACTTCGACATTTGGCCGACATCCGAGCTTATGGAACAAGAT
optimized LuUGT74S1 740 CCGGATGAAAGATAAGATAGCATTTAAACCCGCAAGATGACCTGCATGAACCTGGCTG
LuUGT74S1_JX011632.1 740 CCGGATGAAAGATAAGATAGCATTTAAACCCGCAAGATGACCTGCATGAACCTGGCTG
optimized LuUGT74S1 810 CCGGATGAAAGATAAGATAGCATTTAAACCCGCAAGATGACCTGCATGAACCTGGCTG
LuUGT74S1_JX011632.1 810 CCGGATGAAAGATAAGATAGCATTTAAACCCGCAAGATGACCTGCATGAACCTGGCTG
optimized LuUGT74S1 880 CAGAGCAACCCGATGGCAGCTGCTGTATGTGAGCTTGGCAGCCTGGCGCGCTGAGCCCGAGC
LuUGT74S1_JX011632.1 880 CAGAGCAACCCGATGGCAGCTGCTGTATGTGAGCTTGGCAGCCTGGCGCGCTGAGCCCGAGC
optimized LuUGT74S1 940 CGAAGTCGCCAAACTCCAAAGAGATACTGAGCGGCGAATAAGGCTGGTGGTGGTGG
LuUGT74S1_JX011632.1 940 CGAAGTCGCCAAACTCCAAAGAGATACTGAGCGGCGAATAAGGCTGGTGGTGGTGG
optimized LuUGT74S1 1,010 AGCAGCTCAAGTCTGGCAGCGGAAAGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGG
LuUGT74S1_JX011632.1 1,010 AGCAGCTCAAGTCTGGCAGCGGAAAGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGG
optimized LuUGT74S1 1,080 CCTGGAAGCGCTGAGCTGGGCTGCGGATGGTGGGATGCCGGAATGCGCGATCACTGCCAA
LuUGT74S1_JX011632.1 1,080 CCTGGAAGCGCTGAGCTGGGCTGCGGATGGTGGGATGCCGGAATGCGCGATCACTGCCAA
optimized LuUGT74S1 1,140 CGCAAAATTAATAAGAGTGTGAAAACCGGCTGCGCGGAAAGCGGATGATGCCAAAGGAT
LuUGT74S1_JX011632.1 1,140 CGCAAAATTAATAAGAGTGTGAAAACCGGCTGCGCGGAAAGCGGATGATGCCAAAGGAT
optimized LuUGT74S1 1,210 ATGTTGGGATGATCAAGCGGAAAGTGTGAAAACCGGCTGCGCGGAAAGCGGATGATGCCAA
LuUGT74S1_JX011632.1 1,210 ATGTTGGGATGATCAAGCGGAAAGTGTGAAAACCGGCTGCGCGGAAAGCGGATGATGCCAA
optimized LuUGT74S1 1,280 CCGCGCAAGCGGAAAGTGGGCAAAATTAATAAGAGTGTGAAAACCGGCTGCGCGGAAAG
LuUGT74S1_JX011632.1 1,280 CCGCGCAAGCGGAAAGTGGGCAAAATTAATAAGAGTGTGAAAACCGGCTGCGCGGAAAG
optimized LuUGT74S1 1,350 TAAAAACACCGAGGATTTTGCAGGAGCTGATTAACCTTCCGGAACCTTCACTTTAGCTGCTA
LuUGT74S1_JX011632.1 1,350 TAAAAACACCGAGGATTTTGCAGGAGCTGATTAACCTTCCGGAACCTTCACTTTAGCTGCTA
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MAGDEREKAHCLVVPAAQGHFTPLLQFSKRLIPKRIRVTLALTRFIHSTMTVTAQSGIHIDTISDGFHDSGLILQDPEHYSQTFRR
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VSDLPSFIWDDLHTEFLAAHLRQFSNDGADWVFCNTVYQLELEAVDWLTKQWLNFRITGPTIPSFYLDKQIPDDKDYDISIFNPQ
NQTCMNWLQSKPDGSSVVYVYFSGSLARLSPQQTEELYFGLKNSNHFLWVWVRESEVAKLPKEEYLSGEKGLVSWCSQLQVLA
SGKVGCFVTHCGWNSTLEALSLGVPMVAMPECGDQLTNAKFIKDVWKTGVRAEADDGKIMWGMKREVIERCIREVMEEGET
RRNADKWKIIEAVVEGGSSDKNTEDFATSLINFAETTFQFSC
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Figure S1. The original and optimized (for *Escherichia coli*) gene sequence of *LuUGT74S1* (from Flax seed) and the protein sequence. (A) Nucleotide sequence of the optimized and original *LuUGT74S1* gene sequence. The deviating nucleotides are highlighted by individual colors. (B) The amino acid sequence of *LuUGT74S1* derived from both sequences.

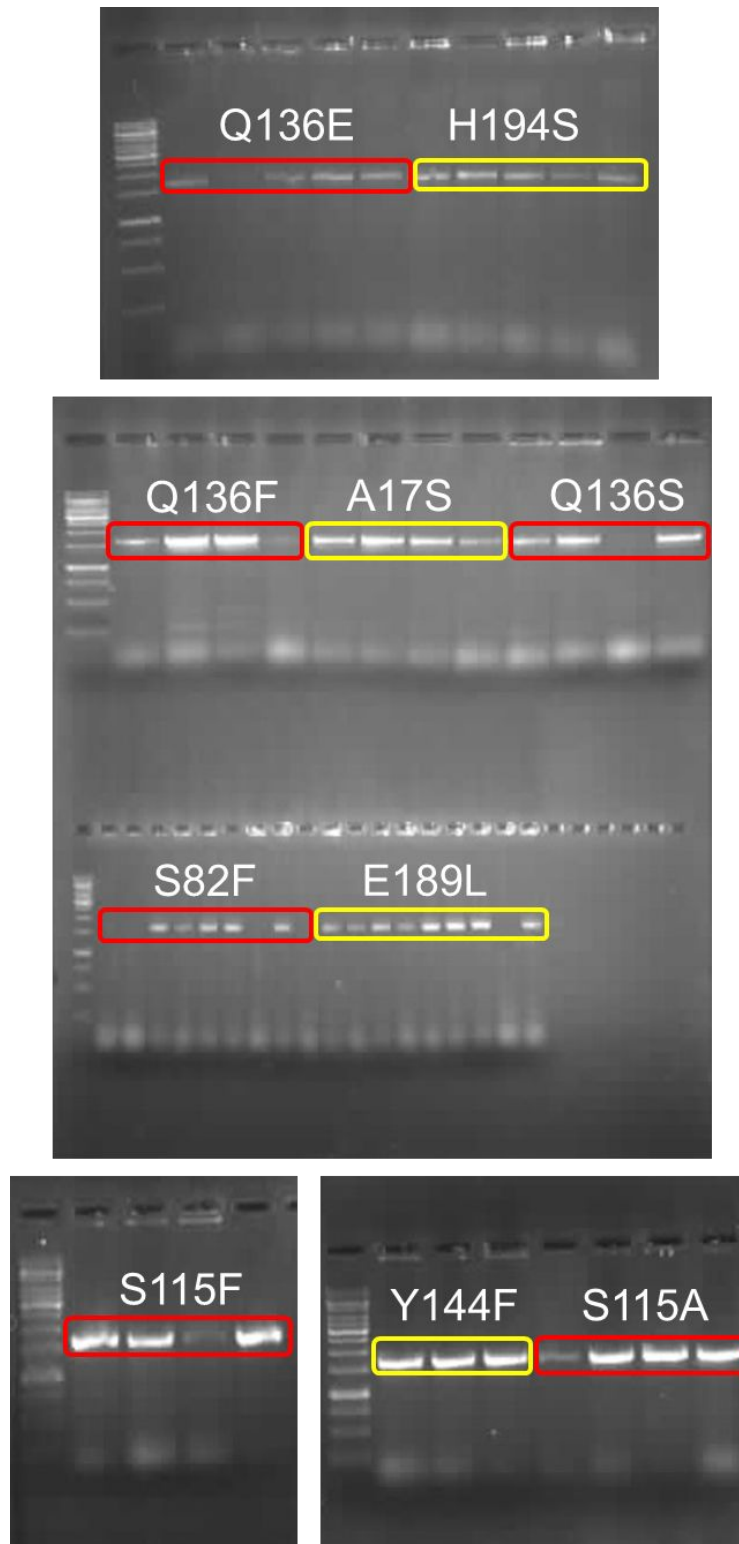


Figure S2. Control of the size of the ligated PCR products of the mutated *LuUGT74S1* genes by agarose gel electrophoresis. Colony PCR of the ten *LuUGT74S1* mutants. The boxes confirm the sizes of the mutant genes isolated from different colonies. For comparison, a 1 kb DNA ladder is shown on the left.

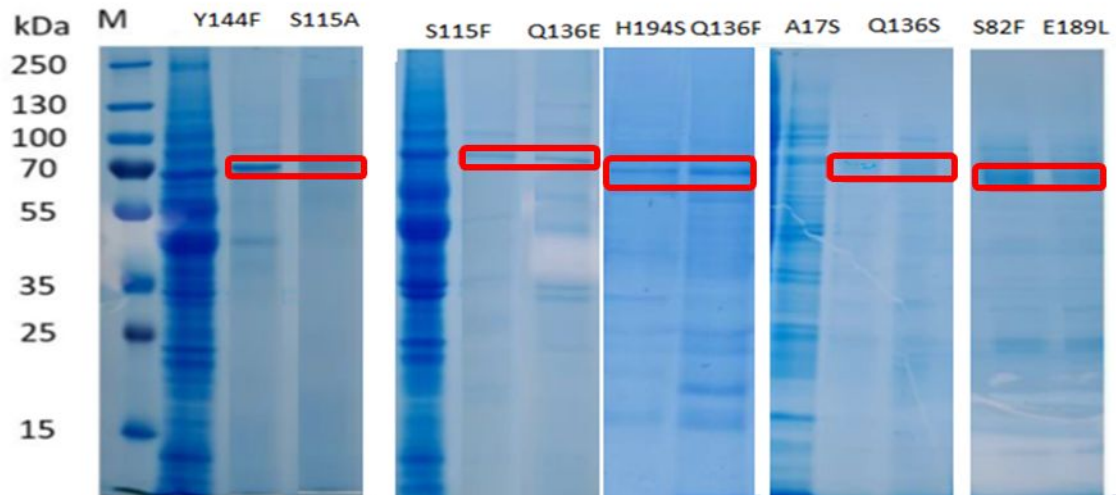


Figure S3. SDS-PAGE analysis to separate and identify protein sizes of LuUGT74S1 wild type and mutations. The bands framed in red represent proteins with a size of about 73 kDa (LuUGT74S1 and its mutants with 47 kDa, bound to GST with 26 kDa), as shown by the protein ladder on the left. All bands with red frame have the same size.

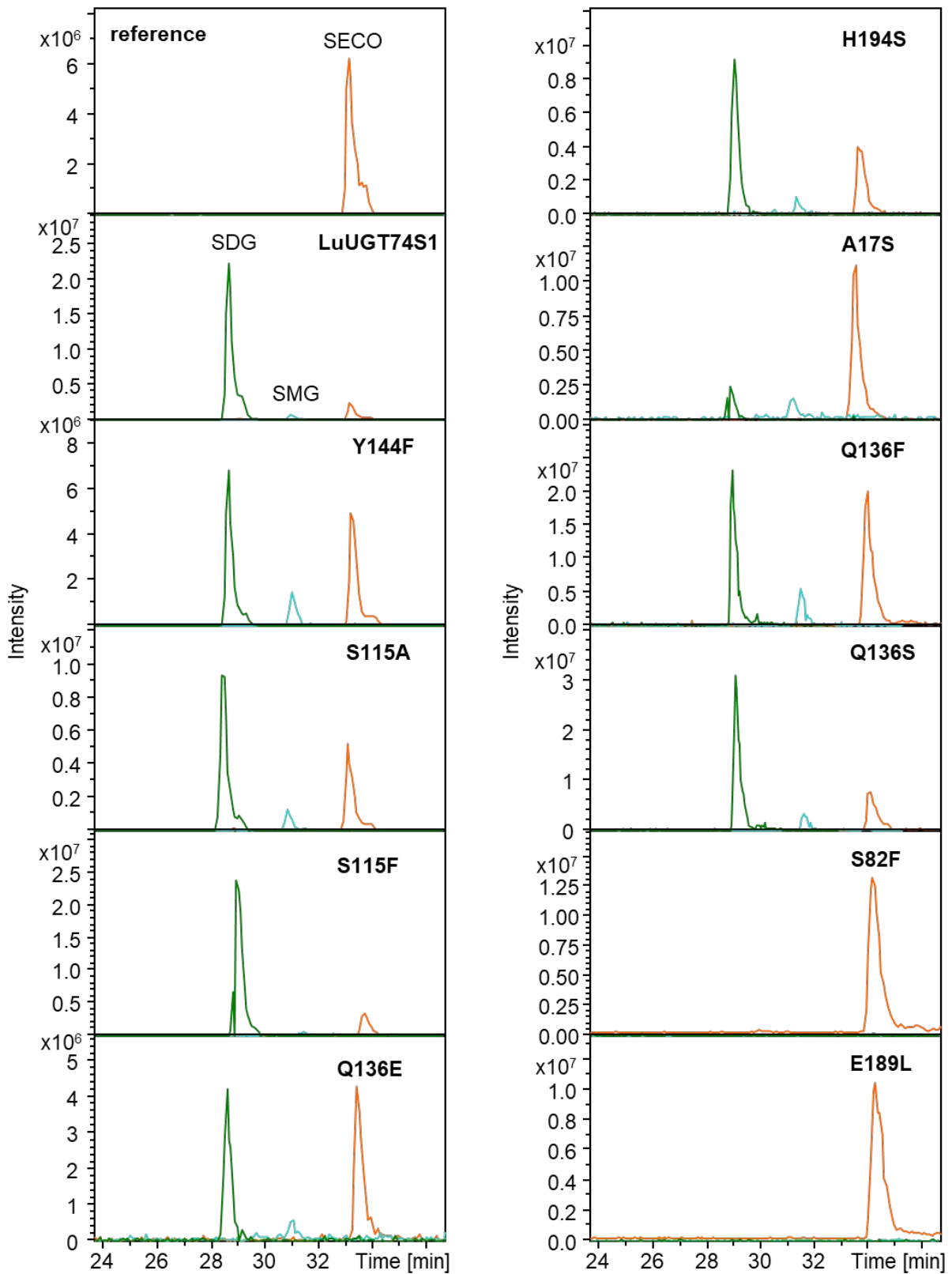


Figure S4. LC/MS analysis of the reaction products of the wild-type enzyme and all mutant proteins. Note that S82F and E189L do not produce SMG or SDG. Ion trace m/z 361 (orange) shows SECO, m/z 523 (light blue) shows SMG, and m/z 731 (green) shows SDG.

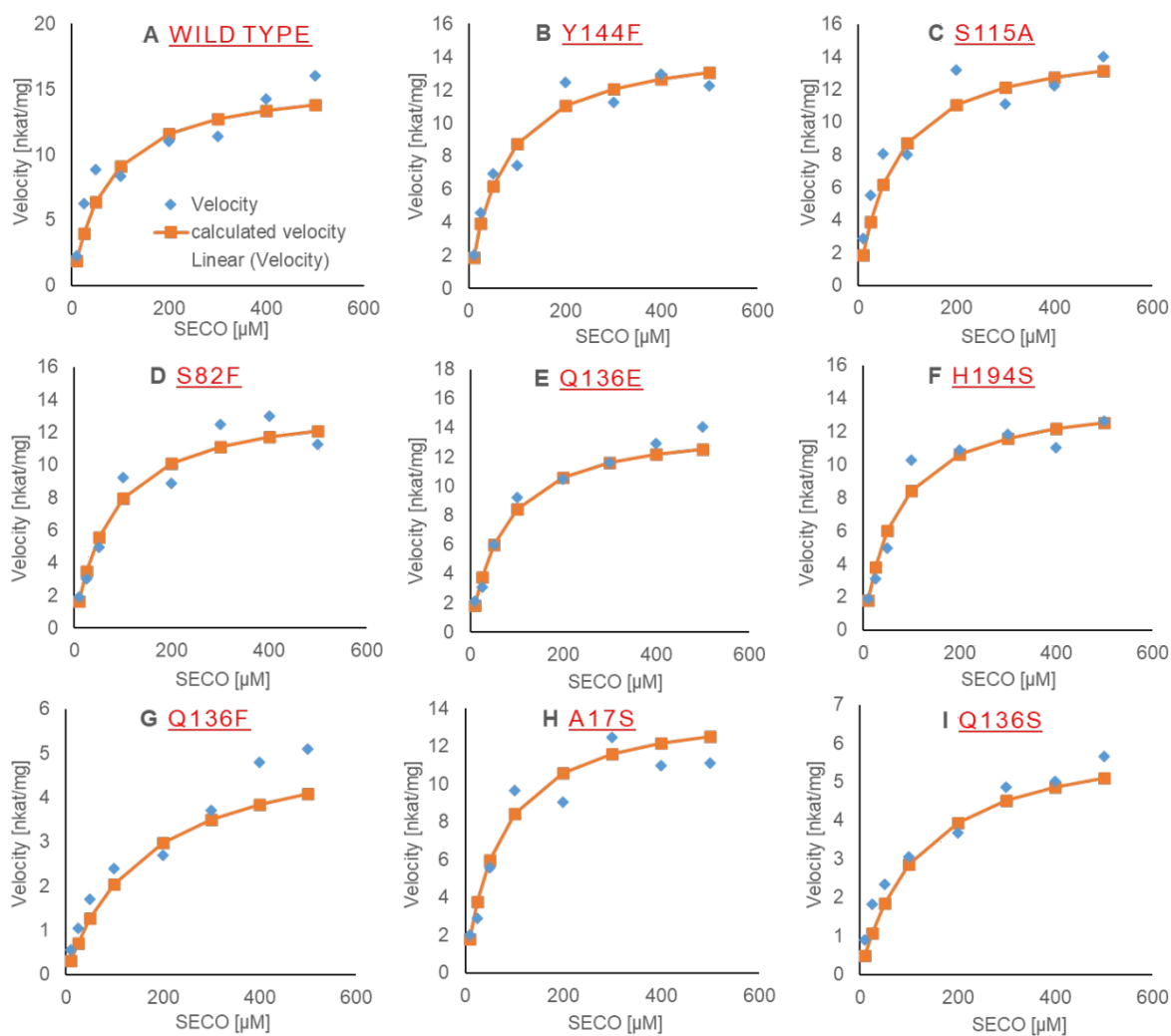


Figure S5. Michaelis-Menten diagrams of mutant proteins. Plots of acceptor substrate concentration versus velocity. **(A)** wild type, **(B)** Y144F, **(C)** S115A, **(D)** S82F, **(E)** Q136E, **(F)** H194S, **(G)** Q136F, **(H)** A17S and **(I)** Q136S. Data represent mean \pm SD of $n = 3$ technical replicates.