

Supporting Information

Expression of Lanthipeptides in Human Cells

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Codon optimized primers and genes used in this study

SME_CyILL-S15T.F1	CTGCGTCAACGGCTGCATGCGGCTGGGTGGGCGGCG
SME_CyILL-S15T.R1	GCATGCAGCCGTTGACGCAGCAGCAGTAGCAGCCACG
SME_pCMV-NLS.R2	cactttgcgtttctctTGGGCCCGGATTTTCTTCG
SME_pCMV-NLS.F2	aagaagaaacgcaaagtGACTACAAGGACGACGACGAC
SME-NLS_pCMV-FLAG	CGAAGAAAATCCGGGCCCAaagaagaaacgcaaagtGACTACAAG
SME_CyILL_NDT.R3	cctgactccagtgaagattccAHNgccacccaAHNgcacgagccgttgacgcagcAHNagtagcagcAHNggcgcaAHNtggagtcgtc
<i>H. sapiens</i> codon-optimized CylM	tcagaggacaacctcatcaactgtttcaatcaacgagagatgtttcctttgaagcaatctggaagagagaagtagcacataaagaacttgcagacttgaagagagagaaagagtgcttaagcaagcagacttggactacctatcaagtagcaagtagcagcttggacaacttgcgacttggatcacacccatcgagaacttccctgacaaggaagtcgcaatccaatcaccaaggaccaatcctggtacataattctcgagtcactcgcactcatacaacgactctgaggagaagctcctcgaggtggacgcttacccttccgctacttctgcaatagcctgattgttctgctgacactcagactcaacatctgtactaagagttcatcataaacctcttgagactctcactcaagagttgatacacttaccatccaagactctgtccttgactgacacgttcaagaagaacgaccactcaaggggaacgacagttccaagagattcatctactcctcaagaagcagattcaactcaagaagacatcgctttctcacagctgttacccccgagctcatcggatcacagtggtgctgagatacttctgacaatacgaagcagatgctaccgggtcactgaggactgcttccatccaaaactgttcaacatccaatccaggagactcaacagatcagtgagtcacaggggactcacactcaggggaagacagtgctcagcctcactttctcagacggcaagaagatgctcacaagcctaaagataaaactcgagaacaagctcagagacttctcaggttctgaacaaggagctcgaggcagacatctacatcgtaagaaggttacacgtaaaccttactctcagaggagtagacaatcagattacgattacaataatagaagaggtcaagaagtagtagcagcgggaaggtgatcggtatcgcgttctcctcaacgctcactgatctccactacgagaatattatcgcgcacggggagtagccctgtcatcgcgacaacgagacttctccaaacaacataccatcgagttcggcaactctgactgacgctaaagtataagtagtggacagtagatgtaactgggctcgtgcccactctgccaatgaaggacaagtcggacagtaaggacgaggggtgtgaacctctgctcttaactcaaggagcaaaagtgtcccctcaagatattgaagatcaagaacacttctcactgacgagatgaggttcgagtagcaaacacacatcatggacacagtaagaacacacctataatgaacaagagaagattcattcattcacaagagtagatcgttacaggtatgaagtcctcctcaagggtaaggactcaaaaaaagatcctcgcatacatcaacaacaatctccaaaacttgatcgaagaacgtcatccgcccacacaacggtagcggagatgctcaggtcagttaccacccaactgtttctcaacgcaatcgagcgggagaaggtcctcacaacatgtgggcttaccctcaagaacaagaaggtgtccactcagagttctctgacctatagacggagacatccccatcttacaacaacatctcaagacatcccctcatcgctccgacggatgcttggctgagggacttccaagagagcgcattgaaccgatgttgaacaagataaacgaccttgcgacgagacatcagatccaacaagatttgctcgagatagctctcaacatcacaatccatataagtagacataaacgacctaaagaatcaaaactccaataagtagatctactggtcagactcaacggaaagatcacaagctgtcaaaagatcgagaagaagatttcaagcgggcaatttcaataagaagacgaacactgtaaaactggatcgacataaaagctgaccaagactggaactgtggaatcctcaacaacaacatgtacgacgggtcccaggcatattcatctctcagctcgcactcaagtagtacacaagaatcacaagtagcactcagctcatgaggtgatcaagaactcaatatacactatccctccgaggaacatcctcctcgttctcgggaaggggtcctgatataccattgttgggactaccgactcaacaacgacataaacagctcaacgttgcagctcagatcgtgacatgctcatagagaagaagcctataacaacgggagcttaagaacgactggatccacggacacaactccatcatcaaggtcctgctctttgtccgagatcactgaggacgagaagtaggaagttctcactcgagatctcgagaagctcggaggacactcaactcaggg

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<i>H. sapiens</i> codon- optimized CyILL-S15T	tcacaagaccctaactctgagaacctcagtgtagtgcctcttctgaggagcttctgttgaggagatggaagccattcaagggaagtggggat gtgcaagctgagacgactccagtgctgcggcgtgctgactgctgctgctcaacggctgcatgctggctgggtggcgccggaatcttcac tggagtcacggtagtggtgtccctcaagcactgttga
<i>H. sapiens</i> codon- optimized HalM	aaaacccactgacgtcagatccttcaactcagactctgcctcacactaatgacaccgattggctcgaacaactcatgacatactc tccatccccgtgactgaggaatccagaataatcttcacgcagagaacgatctctcattctctatacccttccctgcaattcacatcaga gtatgagcacttattgacctttaaaccgatatgcccctgattgaacgacaatctctcctcaatctactttaactcgtgtacatcatcggt gttccaccttacacatgcacgctgatctcagagatgcacattgataaactacagtggttctaaacggtagcaccctcacgaaaggtat ggattttaatacaaaatcaaaaaacatcaaatcaagaatctctcaacatatacccgatactgggcaagcgtgtagtcaacgagactct ccgaacgataaattttgtaagaaaattatacaactatatagaaggactatcttctcagtcagtcacttcaaggagaagacctgagactg aacccctcaactgggagtcggcgacacctgtaaatggccaatgctgacaattctgacatttgcgagcggacaagaagtagtgataaa gcccggaagtctgtctatcagataagcaattggcgaattatagatgggtaaatcaagggttcaaccttctctcgcattccaatagccatt gacaggcaaacctacgagtggtacgatttatacccatcaagaagctacaagcagggacgaaattgagcgtattactcccgcataggt ggatacctcgcaattgcttctctcggggctacggatctgcaacctggataacctatcgcgtgtggagaacaccccctgctgattgatctcga aaccccttcaactaagcctggactgctatgattccgcttccattccagccttggctcgcgagctcagcaatcagatttccgacccctcat gttaccatcactatcgcactgtgtaagttatggacattgaccttccagcctcggcgggaaagggcgtcgaagcgagaagattaaaa ctgggtcatcgtaaatcaaaagacggatgagatgaaattggtgaaacacctatgtgactgagagcaccgagaataaacccacggcaca cggcaaggaagcaaataggaattacatccacatgtgaccgacgggttccgaaatgtaccgattatttctaatgaaatcgatgaact gatggtcataatggcctatttctgactcagagcgtgcaaacccggcagctgtttagggtactcagatgataatgcaaaattctggaagctct actcactcgtgattactacaagaaccaactaggcgaataaactcttgaagcttctggaatataacatccctcatggcctttaaagaaaat tgtccacacgagatcgagaactcgagaatcagatattccatattctgtttgacatgtggcggcaccatcgtaagatggatagccgca gacatagcagatctgtccaatctagtgtatagaaaggtgactcatagactcagcaactgggactccgaagacgagggcccgaacaatca gataattaagtcagcttggcaacctgaccaacggggactggaccccatcacgaaaagaccccgatgagtcagctagctagcggcga ccgcaagacgggtatttctcggcaagcccaagcgattggagatgacattctgctcaactatttgggaagatgacagggcacgctgcat acctgatcgggtctcagtcggcatgaatgaggcggtagccgtatccctctgactccaggaatctatgattgactcaggggatcgtgctctt ctttgatcaactcgcgcaacaacgggtgagacgcatatagacatgctgcagacgcaatttggaaaggatgtcaacaatgaaagcca gagttgatgccctcaagcgcatacttggactgggagcttcttctacggctctatggtgctcggctcgaacgatccgacagccacattata gaaggcctatgaatacttaagcacttggagaatgtgtgagcatgaagagacgcccgatttctgctcaggccttagtggcgtgctctatag cttaccaaaatatacagttgactaatgagccacgctatttgggtagccaaaaccacagctcccagcttagtcttattagatagcaaac aacccgacaccgcttgacaggttgatcagcggcggcgggttggcctcgcgctcctcactatggtactgcccgtaacgacgaacaa ctcctcaacaaggcactcctatctctgatatagcgaatagattaataagcaagagaataatgggtgacctcggaaaggaaatgc ctatcaaacattctggtgcatggtgcccctgtattggaattagcaggctgtactggtcctcaattttatgatgacgagttgttgacgaggaact aacgccgtctgaataagactataagcagatggtttgggcacaaccattctctgtgccaggaatttggaaacctgatctgtgtgctc cgccaatatacaataatccagaacaaaaaactggcaggaagctggccatctctatagatcaggtcctacatctatggtggaa actcggcctgaatcacagtaccatcaaggaatgatgctcggcgtgacaggaataggataccggttacttagacatataaacctcag gttccctctatattggcctggaactcccagtagtacactgaccgaaaggaaactcagaatccacgaccggca
<i>H. sapiens</i> codon- optimized FactorXa- HalA2	gttaattctaaagatcctcgaatctgaaattcggaaagctcaggcctgcaattcgtcagcagggtaaacgagaagaattgtcaagcctg gccgggtctggagatagaaagcagacatggccctgtgcaacggctcggagtcagcgtcgcgttatgcccaaccactaagtgac ctctcagtgctag

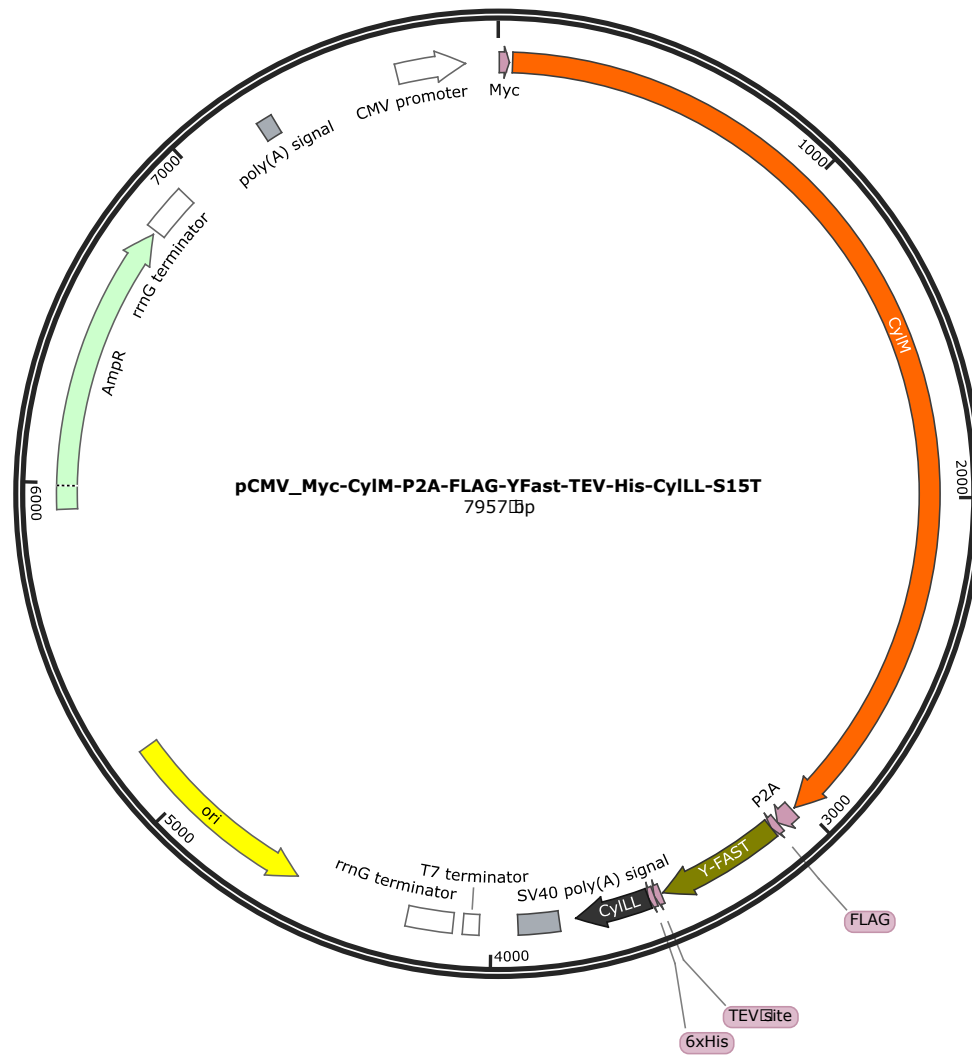


Figure S1: Mammalian expression vector for lanthipeptide production.

Dhb Dhb P V C A V A A Dhb A A A Dha Dha A A C G W V G G G I F Dhb G V Dhb V V V Dha L K H C
 y8 y7 y6 y5

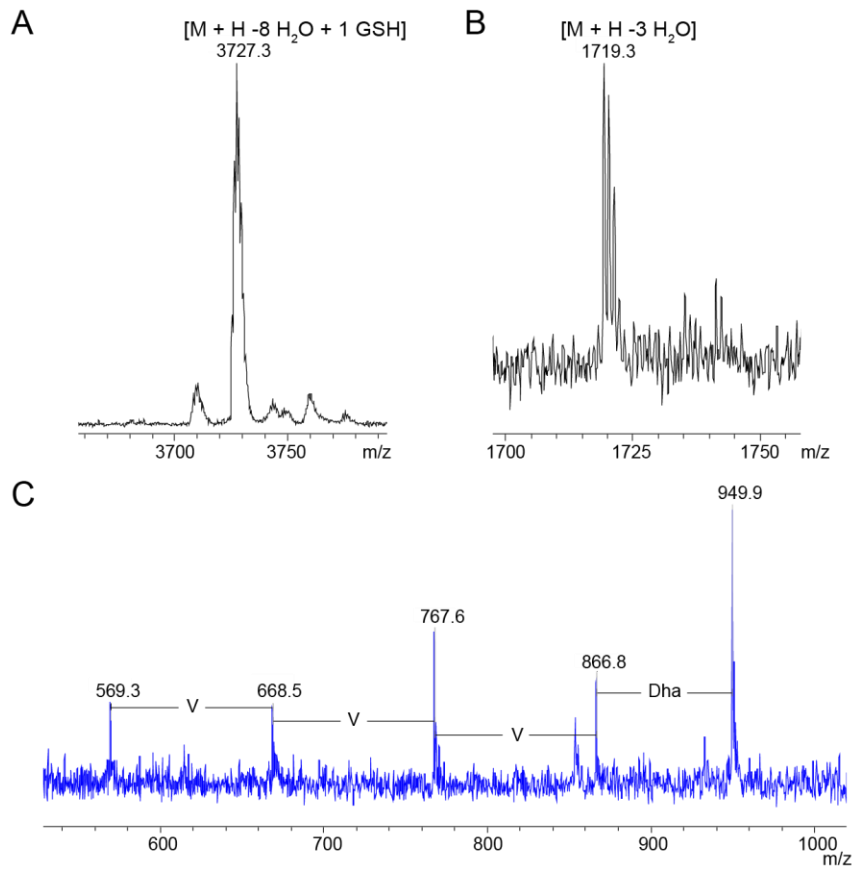


Figure S2: Presence of glutathione adduct in CyLL co-expressed with CyIM in HEK293 cells. A) MALDI-TOF mass spectrum of 8-fold dehydrated CyLL” with a GSH adduct. The peptide was purified via Ni-NTA affinity chromatography and digested with CylA. B) MALDI-TOF mass spectrum of the yellow highlighted CyLL fragment post-chymotrypsin digest. C) LIFT analysis of the 1719 Da fragment.

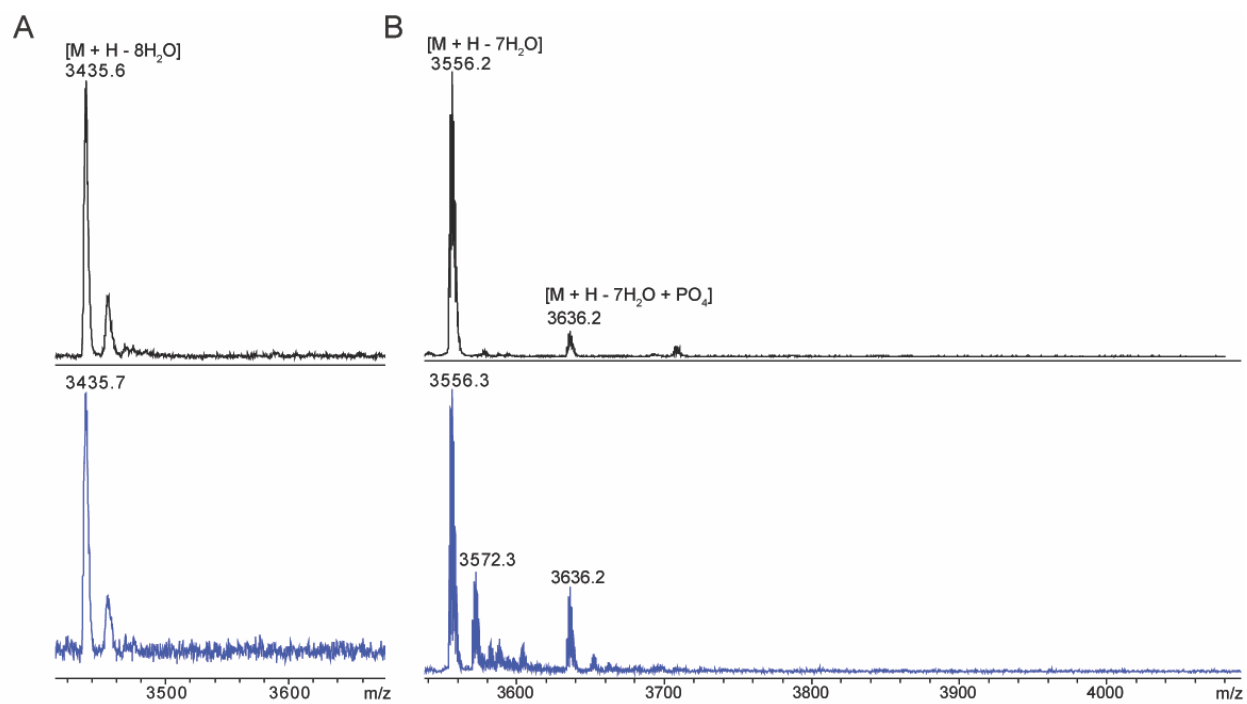


Figure S3: MALDI-TOF mass spectra of (A) CylL-S15T and (B) HalA2 co-expressed with CylM and HalM2, respectively, before (black) and after (blue) IAA (A) or NEM (B) reaction. Peptides were expressed in Expi293 cells and purified via Ni-NTA chromatography and analytical HPLC. CylL-S15T and HalA2 were digested with CylA and GluC, respectively. Reaction products were desalted via C4 ziptip.

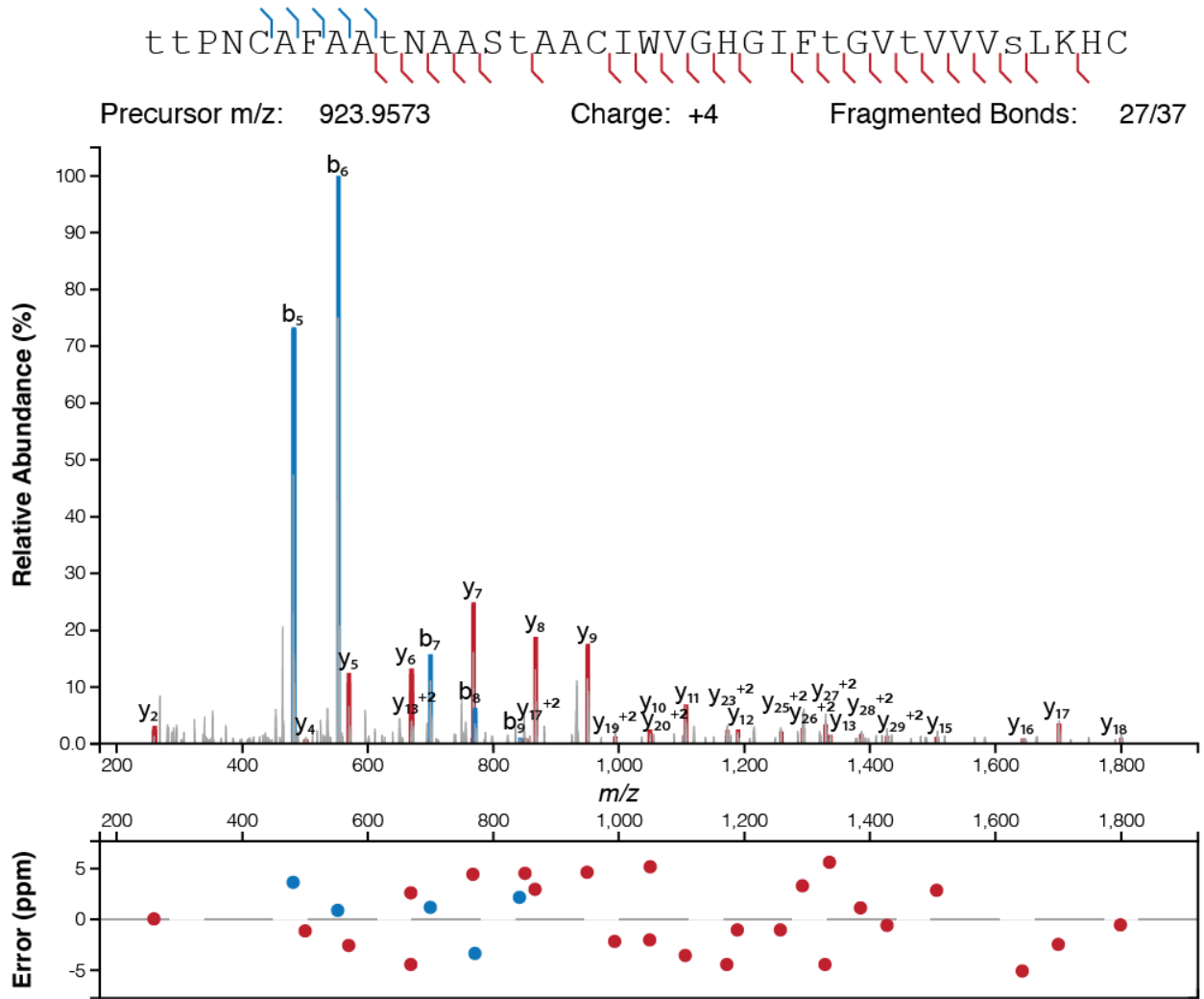


Figure S4: High-resolution MS/MS spectrum of CylL_L-S15T variant NDT1 co-expressed with CylM in Expi293 cells. Peptide was purified via Ni-NTA affinity chromatography and digested with CylA. A graph of the ppm errors for each identified ion is shown.⁷⁴

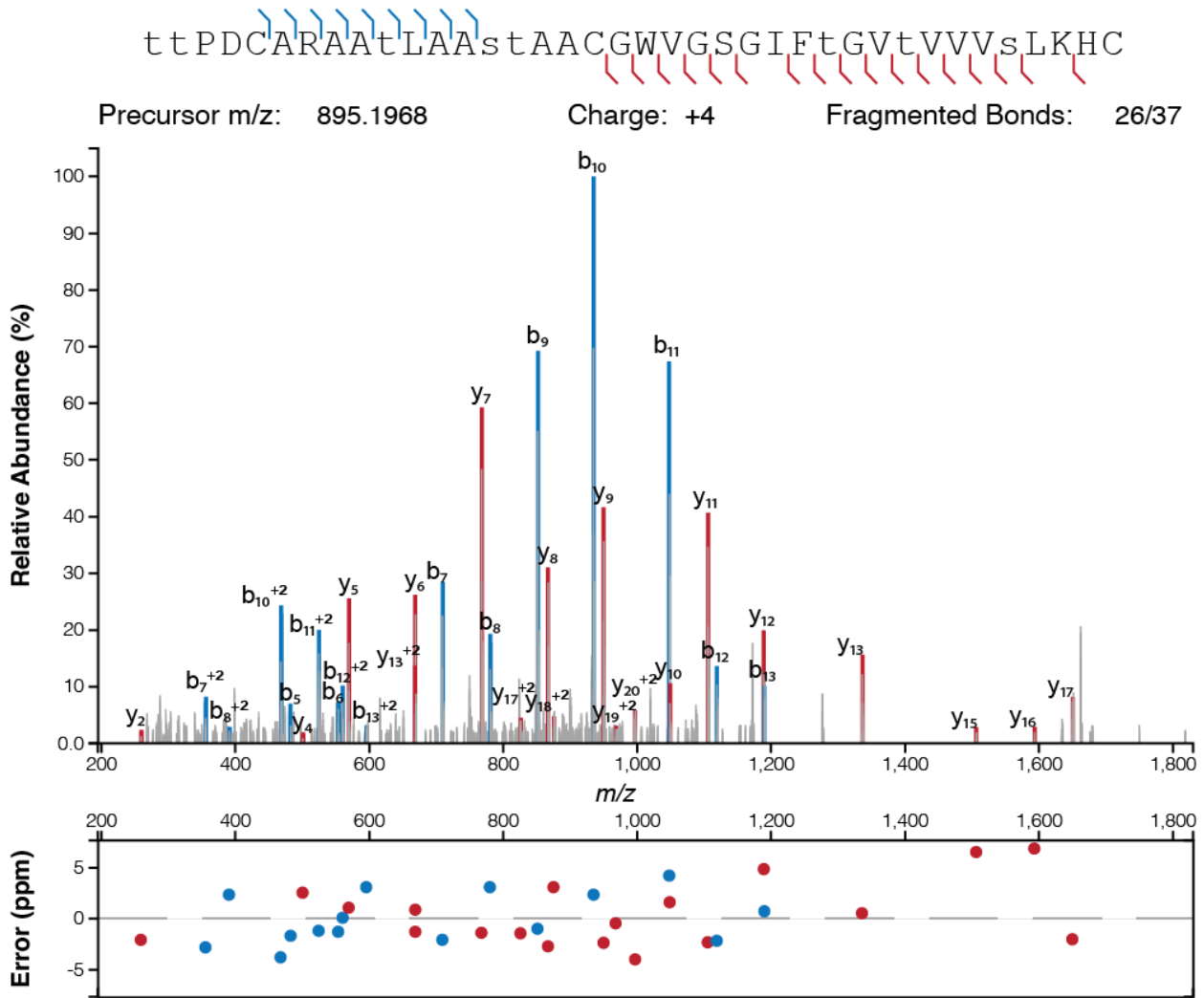


Figure S5: High-resolution MS/MS spectrum of CylL-S15T variant NDT2 co-expressed with CylM in Expi293 cells. Peptide was purified via Ni-NTA affinity chromatography and digested with CylA. A graph of the ppm errors for each identified ion is shown.⁷⁴

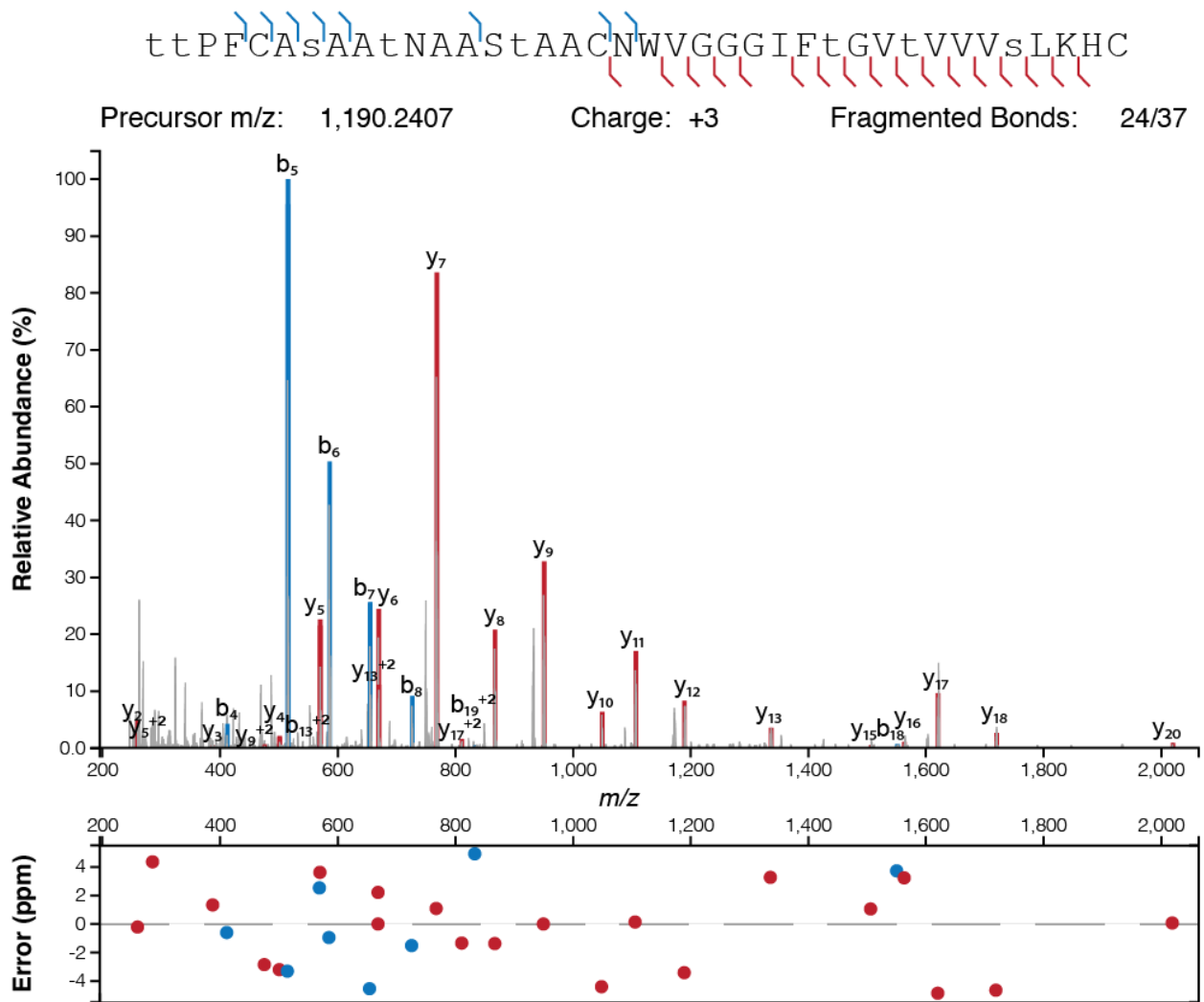


Figure S6: High-resolution MS/MS spectrum of CyLLⁿ-S15T mutant NDT3 expressed in Expi293. Peptide was purified via Ni-NTA affinity chromatography and digested with CylA. A graph of the ppm errors for each identified ion is shown.⁷⁴

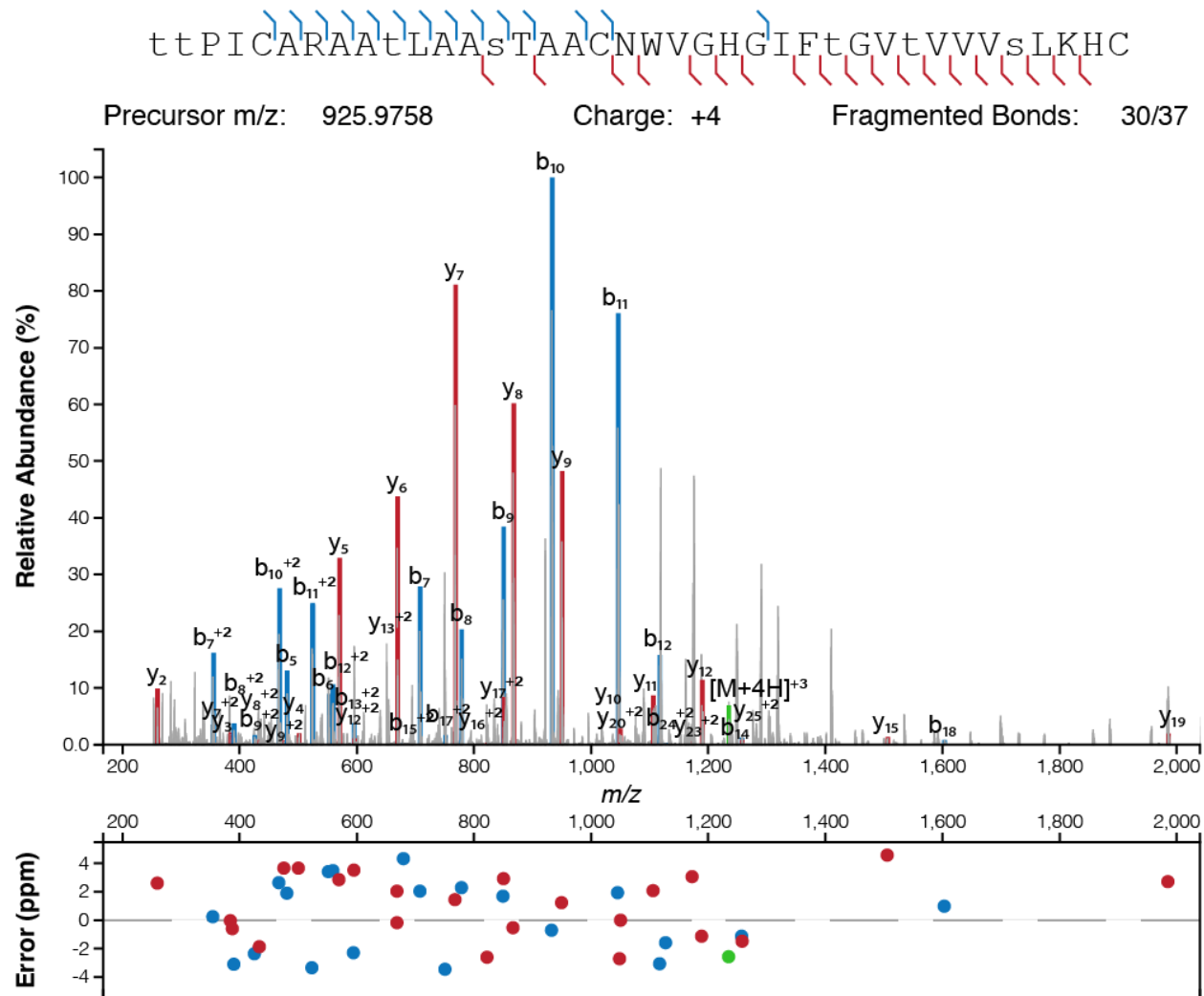


Figure S7: High-resolution MS/MS spectrum of CylL^{S15T} mutant NDT4 expressed in Expi293. Peptide was purified via Ni-NTA affinity chromatography and digested with CylA. A graph of the ppm errors for each identified ion is shown.⁷⁴

CyIL_L"-S15T

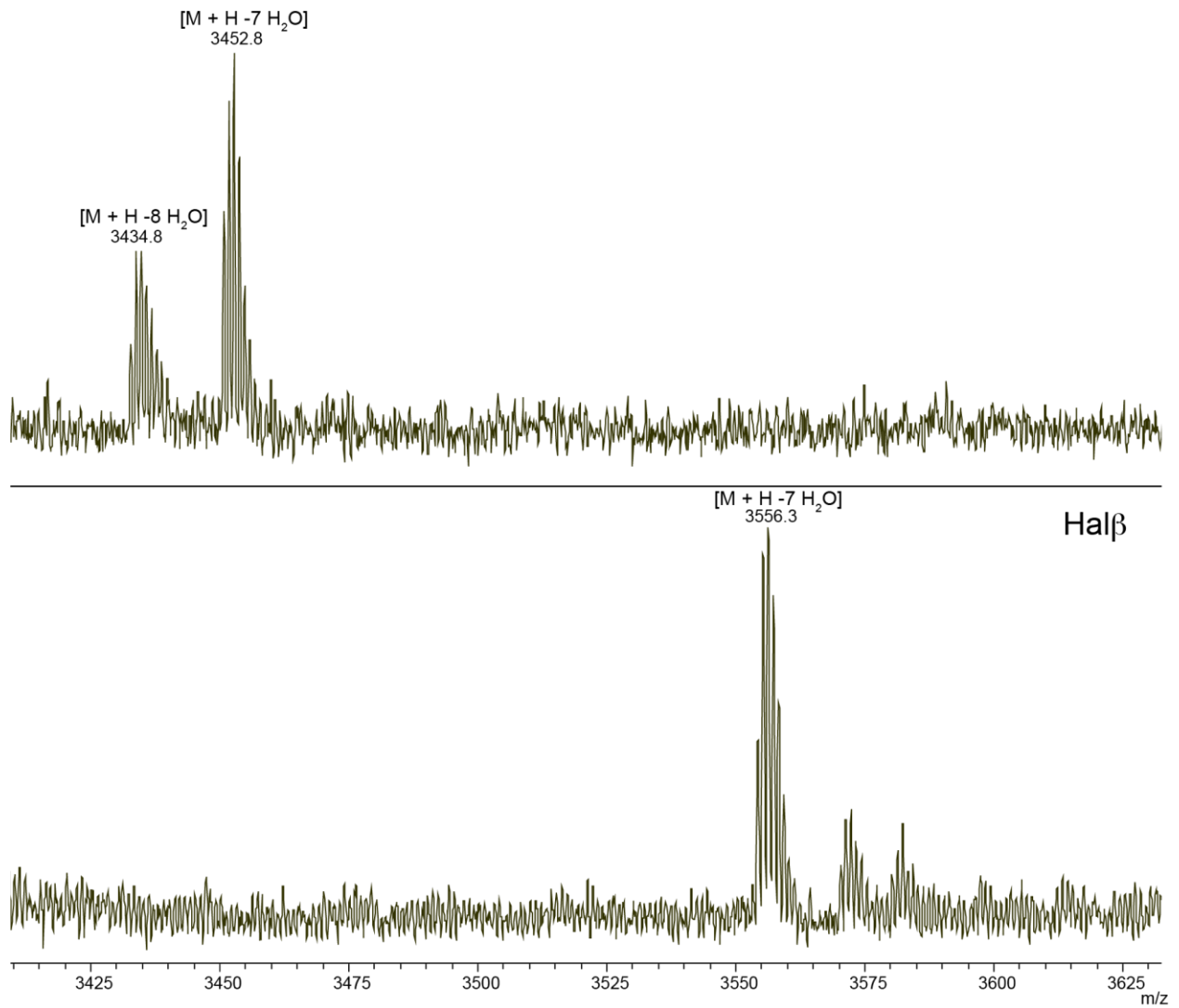


Figure S8: MALDI-TOF mass spectra of nuclear targeted CyIL_L-S15T and nuclear targeted HalA2 co-expressed with CyIM and HalM2, respectively, in Expi293 cells. CyIL_L-S15T and HalA2 were digested with CylA and GluC respectively.