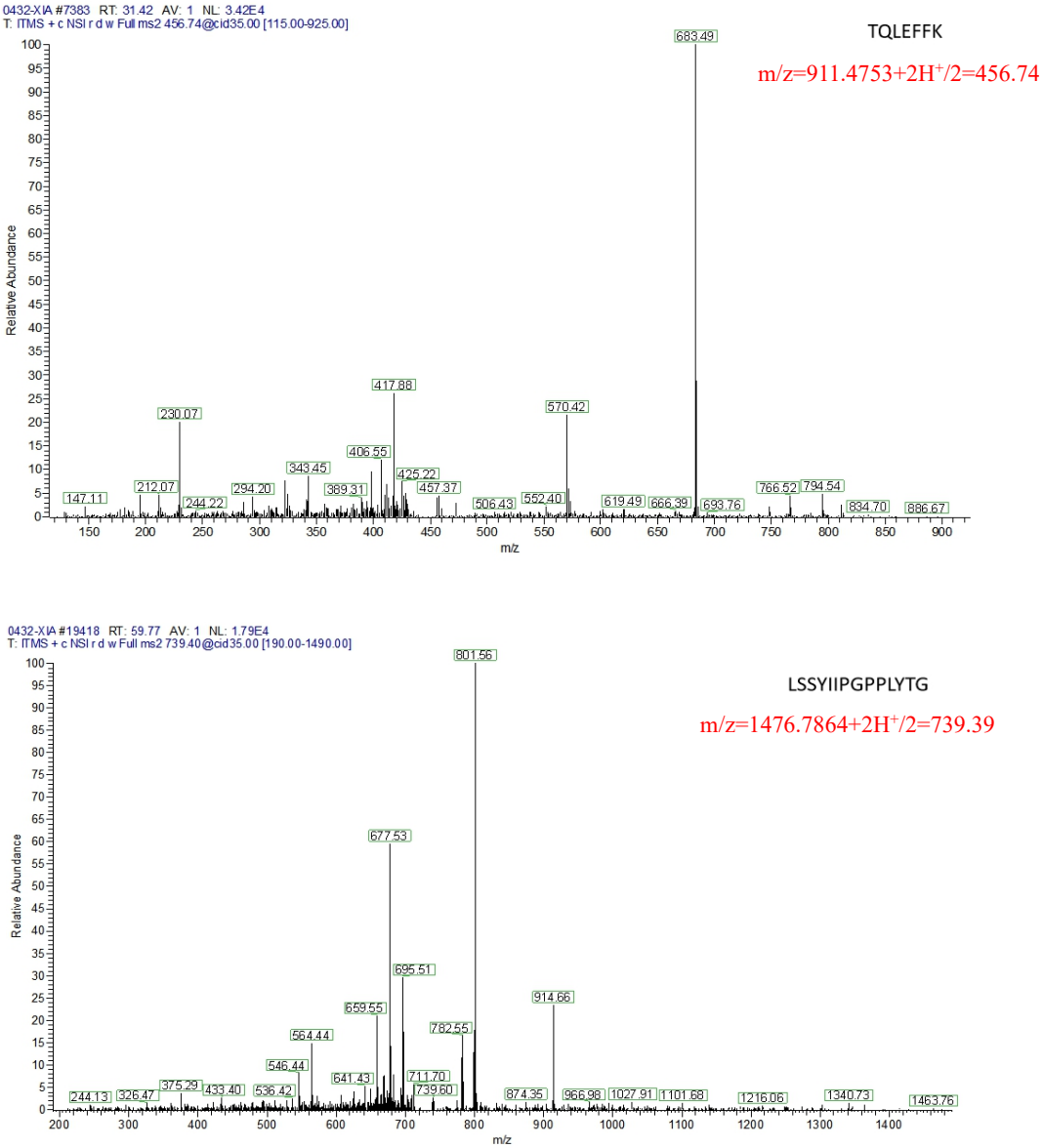
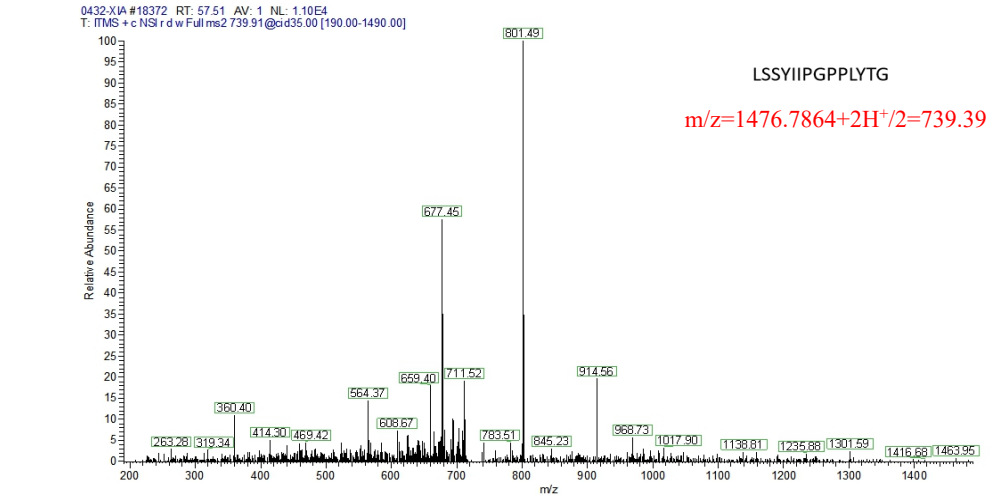
Supplementary Information

Figure S1 Identification of *Ta*AA9A using LC-MS/MS. The *Ta*AA9A band was cut from the SDS-PAGE gel, extracted, and digested with trypsin. The extracted-ion chromatograms (EIC) (a) and Mascot search results (b) of the digested *Ta*AA9A peptides are shown. The molecular ions of m/z 456.74, 739.40, 739.91, 1744.80, 1313.60, 933.46, 1526.25, and 1528.75 were confirmed by MS/MS fragmentation analysis. Fragmentation *m/z* values agree with the molecular weight of the corresponding fragmentations of the digested *Ta*AA9A peptides. The observed and calculated molecular mass of the normal and modified *Ta*AA9A peptides were shown in Mascot search results.

a

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LSSYIIPGPPLYTG
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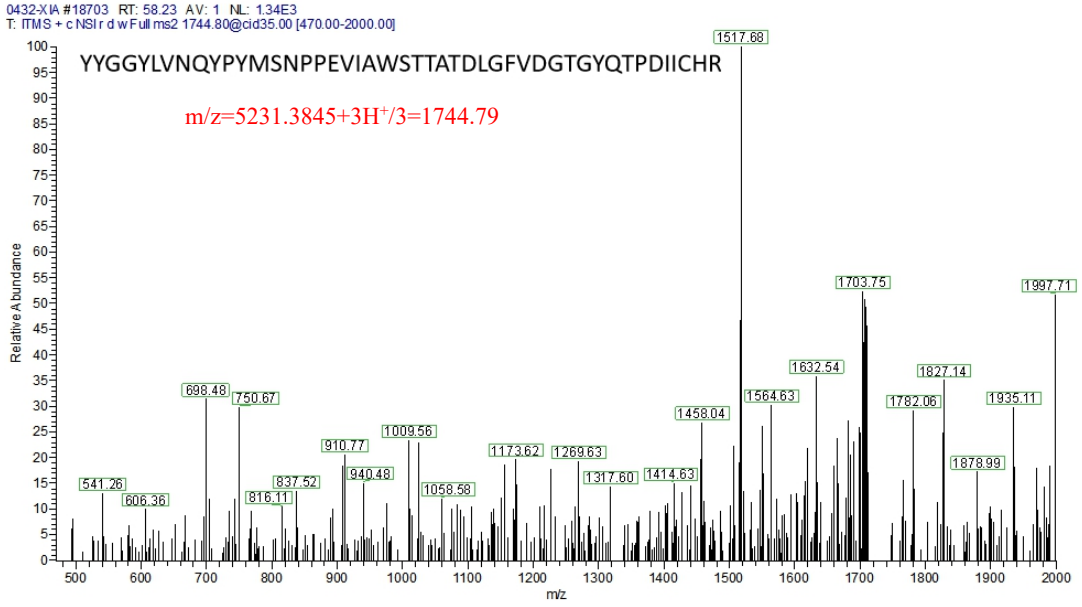
Relative Abundance
m/z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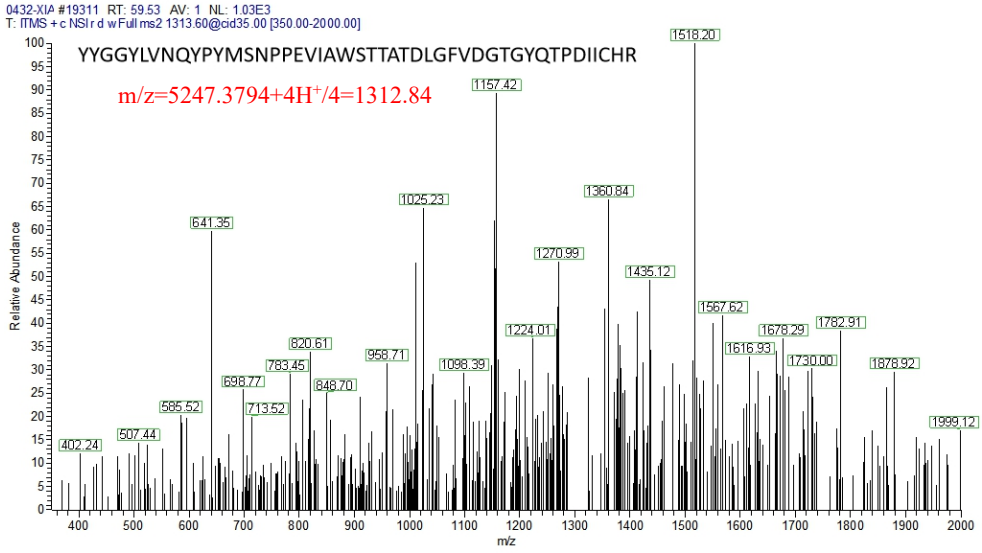
Relative Abundance
m/z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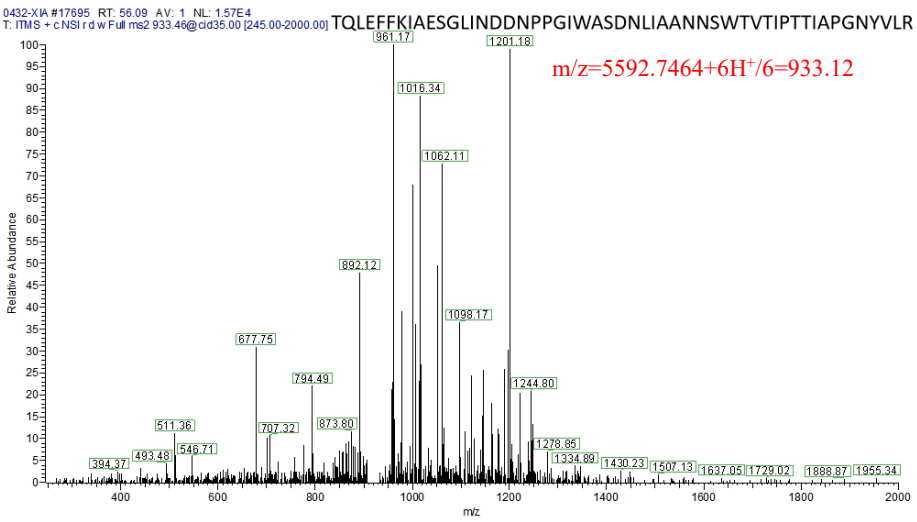
Relative Abundance
m/z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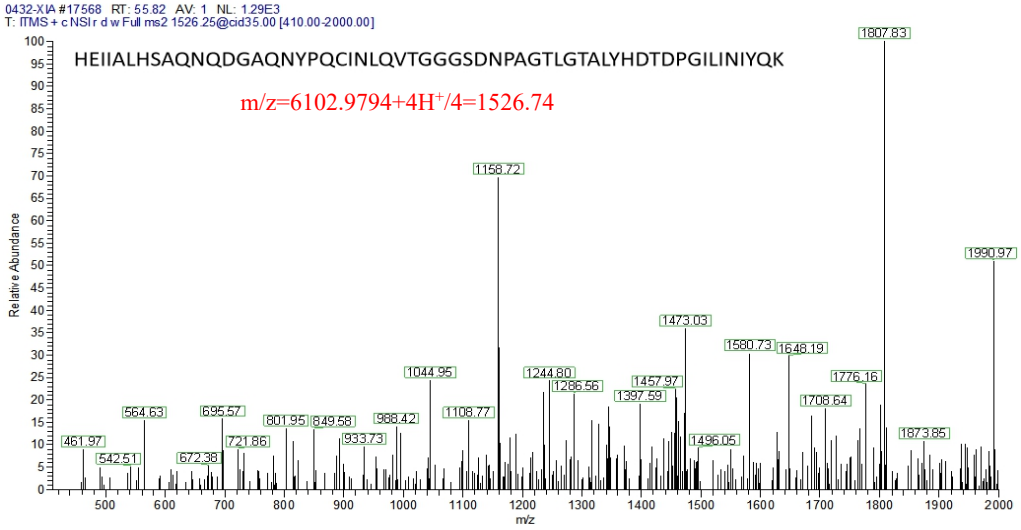
Relative Abundance
m/z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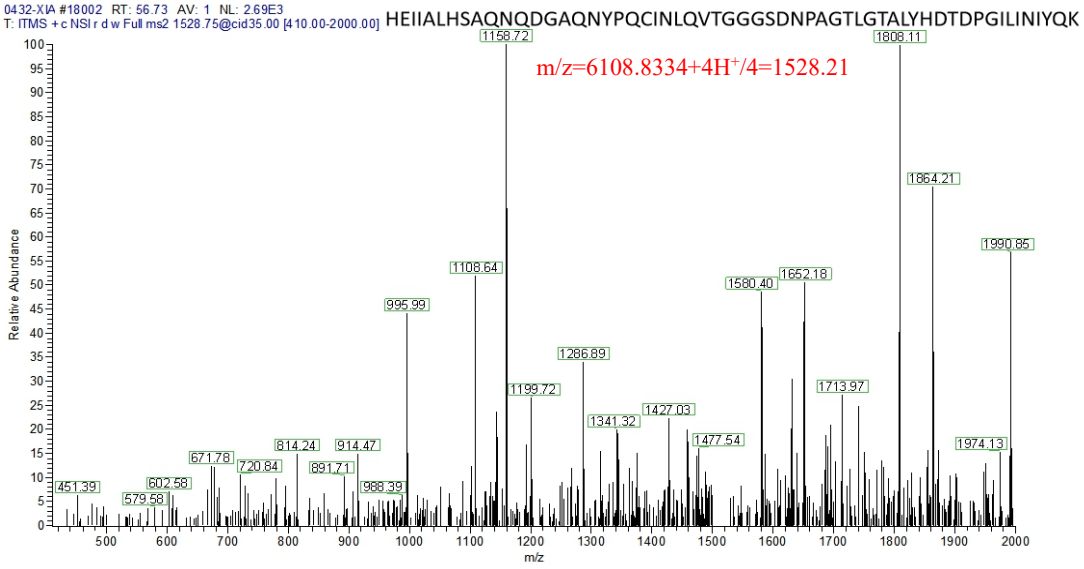
Relative Abundance
m/z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m/z=6108.8334+4H+/4=1528.21
Relative Abundance
m/z

b

Mascot Search Results

Protein View

Match to: **gi|724466400|dbj|DI380449.1|** Score: **354**
ER 1020140001161-A/1: Fusion proteins of GH61 with enhanced secretory capability comparing to its wild-type
Found in search of C:\Users\DELL\Desktop\LCMSMS\7.2\0432-XIA.mgf
Translated in frame 1

NB Matches were also found in other frames indicating a possible frame shift.
Only matches in frame 1 are shown in this report

Show frame 1

Nominal mass (M_r): **26756**; Calculated pI value: **4.83**
NCBI BLAST search of gi|724466400|dbj|DI380449.1| against nr
Unformatted sequence string for pasting into other applications

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Acetyl (Protein N-term),Deamidated (NQ),Dioxidation (W),Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: **67%**

Matched peptides shown in **Bold Red**

```
  1 MSFSKIIAIA GVLASASLVA GHGFVQNIVI DGKVYYGGYL VNQYPYMSNP
 51 PEVIAWSTTA TDLGFVDGTG YQTPDIICHR GARPGALTAP VSPGGTVELQ
101 WTPWPDSHHG PVINYLAPCN GDCSTVDKTQ LEFFKIAESG LINDDNPPGI
151 WASDWLIAAN NSWTVTIPTT IAPGNYVLRH EIIALHSAQN QDGAQNYPQC
201 INLQVTGGGS DNPAGTLGTA LYHDTDPGIL INIYQKLSSY IIPGPPLYTG
251 _
```

Show predicted peptides also

Sort Peptides By: (•) Residue Number ( ) Increasing Mass ( ) Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence	
35 - 80	1744.1329	5229.3770	5229.4165	-8	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	(Ions score 0)
35 - 80	1744.1522	5229.4348	5229.4165	4	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	(Ions score 3)
35 - 80	1744.4689	5230.3848	5230.4005	-3	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	Deamidated (NQ) (Ions score 24)
35 - 80	1744.4749	5230.4027	5230.4005	0	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	Deamidated (NQ) (Ions score 9)
35 - 80	1744.7961	5231.3666	5231.3845	-3	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	2 Deamidated (NQ) (Ions score 3)
35 - 80	1744.7968	5231.3684	5231.3845	-3	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	2 Deamidated (NQ) (Ions score 21)
35 - 80	1745.1301	5232.3686	5232.3685	0	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	3 Deamidated (NQ) (Ions score 35)
35 - 80	1312.3594	5245.4084	5245.4114	-1	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	Oxidation (M) (Ions score 2)
35 - 80	1312.6049	5246.3903	5246.3954	-1	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	Deamidated (NQ); Oxidation (M) (Ions score 11)
35 - 80	1312.6082	5246.4035	5246.3954	2	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	Deamidated (NQ); Oxidation (M) (Ions score 6)
35 - 80	1312.8511	5247.3752	5247.3794	-1	0	K.YYGGYLVNQYPYMSNPPEVIAWSTTATDLGFVDGTGYQTPDIICHR.G	2 Deamidated (NQ); Oxidation (M) (Ions score 4)
129 - 135	456.7441	911.4737	911.4753	-2	0	K.TQLEFFK.I	(Ions score 27)
129 - 135	456.7453	911.4761	911.4753	1	0	K.TQLEFFK.I	(Ions score 25)
129 - 135	456.7457	911.4768	911.4753	2	0	K.TQLEFFK.I	(Ions score 26)
129 - 135	456.7458	911.4770	911.4753	2	0	K.TQLEFFK.I	(Ions score 19)
129 - 135	456.7458	911.4770	911.4753	2	0	K.TQLEFFK.I	(Ions score 22)
129 - 135	456.7458	911.4770	911.4753	2	0	K.TQLEFFK.I	(Ions score 22)
129 - 135	456.7458	911.4770	911.4753	2	0	K.TQLEFFK.I	(Ions score 24)
129 - 135	456.7459	911.4771	911.4753	2	0	K.TQLEFFK.I	(Ions score 28)
129 - 135	456.7459	911.4772	911.4753	2	0	K.TQLEFFK.I	(Ions score 29)
129 - 135	456.7459	911.4772	911.4753	2	0	K.TQLEFFK.I	(Ions score 22)
129 - 135	456.7459	911.4772	911.4753	2	0	K.TQLEFFK.I	(Ions score 19)
129 - 135	456.7459	911.4773	911.4753	2	0	K.TQLEFFK.I	(Ions score 25)
129 - 135	456.7459	911.4773	911.4753	2	0	K.TQLEFFK.I	(Ions score 25)
129 - 135	456.7460	911.4775	911.4753	2	0	K.TQLEFFK.I	(Ions score 19)
129 - 135	456.7460	911.4775	911.4753	2	0	K.TQLEFFK.I	(Ions score 24)
129 - 135	456.7460	911.4775	911.4753	2	0	K.TQLEFFK.I	(Ions score 21)
129 - 135	456.7461	911.4776	911.4753	3	0	K.TQLEFFK.I	(Ions score 21)
129 - 135	456.7461	911.4777	911.4753	3	0	K.TQLEFFK.I	(Ions score 18)
129 - 135	456.7461	911.4777	911.4753	3	0	K.TQLEFFK.I	(Ions score 19)
129 - 135	456.7461	911.4777	911.4753	3	0	K.TQLEFFK.I	(Ions score 22)
129 - 135	456.7462	911.4779	911.4753	3	0	K.TQLEFFK.I	(Ions score 17)
129 - 135	456.7462	911.4779	911.4753	3	0	K.TQLEFFK.I	(Ions score 24)
129 - 135	456.7462	911.4779	911.4753	3	0	K.TQLEFFK.I	(Ions score 23)
129 - 135	456.7462	911.4779	911.4753	3	0	K.TQLEFFK.I	(Ions score 21)
129 - 135	456.7463	911.4780	911.4753	3	0	K.TQLEFFK.I	(Ions score 21)
129 - 135	456.7463	911.4781	911.4753	3	0	K.TQLEFFK.I	(Ions score 20)
129 - 135	456.7463	911.4781	911.4753	3	0	K.TQLEFFK.I	(Ions score 23)
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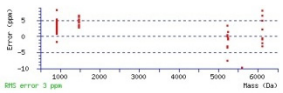
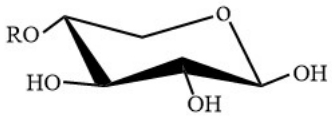
129 - 135	456.7464	911.4782	911.4753	3	0	K.TQLEFFK.I	(Ions score 22)
129 - 135	456.7464	911.4782	911.4753	3	0	K.TQLEFFK.I	(Ions score 19)
129 - 135	456.7464	911.4782	911.4753	3	0	K.TQLEFFK.I	(Ions score 17)
129 - 135	456.7464	911.4782	911.4753	3	0	K.TQLEFFK.I	(Ions score 19)
129 - 135	456.7464	911.4782	911.4753	3	0	K.TQLEFFK.I	(Ions score 28)
129 - 135	456.7464	911.4783	911.4753	3	0	K.TQLEFFK.I	(Ions score 19)
129 - 135	456.7465	911.4784	911.4753	3	0	K.TQLEFFK.I	(Ions score 23)
129 - 135	456.7465	911.4784	911.4753	3	0	K.TQLEFFK.I	(Ions score 21)
129 - 135	456.7465	911.4785	911.4753	4	0	K.TQLEFFK.I	(Ions score 23)
129 - 135	456.7466	911.4786	911.4753	4	0	K.TQLEFFK.I	(Ions score 23)
129 - 135	456.7466	911.4786	911.4753	4	0	K.TQLEFFK.I	(Ions score 21)
129 - 135	456.7466	911.4786	911.4753	4	0	K.TQLEFFK.I	(Ions score 20)
129 - 135	456.7466	911.4787	911.4753	4	0	K.TQLEFFK.I	(Ions score 22)
129 - 135	456.7467	911.4789	911.4753	4	0	K.TQLEFFK.I	(Ions score 24)
129 - 135	456.7467	911.4789	911.4753	4	0	K.TQLEFFK.I	(Ions score 28)
129 - 135	456.7468	911.4790	911.4753	4	0	K.TQLEFFK.I	(Ions score 17)
129 - 135	456.7469	911.4792	911.4753	4	0	K.TQLEFFK.I	(Ions score 22)
129 - 135	456.7470	911.4795	911.4753	5	0	K.TQLEFFK.I	(Ions score 23)
129 - 135	456.7472	911.4798	911.4753	5	0	K.TQLEFFK.I	(Ions score 18)
129 - 135	456.7473	911.4800	911.4753	5	0	K.TQLEFFK.I	(Ions score 19)
129 - 135	456.7474	911.4802	911.4753	5	0	K.TQLEFFK.I	(Ions score 22)
129 - 135	456.7487	911.4828	911.4753	8	0	K.TQLEFFK.I	(Ions score 14)
129 - 135	457.2375	912.4605	912.4593	1	0	K.TQLEFFK.I	Deamidated (NQ) (Ions score 15)
129 - 179	933.1226	5592.6917	5592.7464	-10	1	K.TQLEFFKIAESGLINDDNPPGIWASDNLIAANNSWTVTIPTTIAPGNYVLR.H	4 Deamidated (NQ); Dioxidation (W) (Ions score 2)
180 - 236	1526.2469	6100.9587	6100.9613	-0	0	R.HEIIALHSAQNQDGAQNYPQCINLQVTGGGSDNPAGTLGTALYHDTDPGILINIYQK.L	(Ions score 13)
180 - 236	1221.3927	6101.9271	6101.9454	-3	0	R.HEIIALHSAQNQDGAQNYPQCINLQVTGGGSDNPAGTLGTALYHDTDPGILINIYQK.L	Deamidated (NQ) (Ions score 1)
180 - 236	1526.4905	6101.9328	6101.9454	-2	0	R.HEIIALHSAQNQDGAQNYPQCINLQVTGGGSDNPAGTLGTALYHDTDPGILINIYQK.L	Deamidated (NQ) (Ions score 34)
180 - 236	1526.4971	6101.9592	6101.9454	2	0	R.HEIIALHSAQNQDGAQNYPQCINLQVTGGGSDNPAGTLGTALYHDTDPGILINIYQK.L	Deamidated (NQ) (Ions score 17)
180 - 236	1526.7472	6102.9597	6102.9294	5	0	R.HEIIALHSAQNQDGAQNYPQCINLQVTGGGSDNPAGTLGTALYHDTDPGILINIYQK.L	2 Deamidated (NQ) (Ions score 17)
180 - 236	1221.6031	6102.9794	6102.9294	8	0	R.HEIIALHSAQNQDGAQNYPQCINLQVTGGGSDNPAGTLGTALYHDTDPGILINIYQK.L	2 Deamidated (NQ) (Ions score 7)
180 - 236	1222.5762	6107.8445	6107.8494	-1	0	R.HEIIALHSAQNQDGAQNYPQCINLQVTGGGSDNPAGTLGTALYHDTDPGILINIYQK.L	7 Deamidated (NQ) (Ions score 2)
180 - 236	1528.2256	6108.8732	6108.8334	7	0	R.HEIIALHSAQNQDGAQNYPQCINLQVTGGGSDNPAGTLGTALYHDTDPGILINIYQK.L	8 Deamidated (NQ) (Ions score 23)
237 - 250	739.4025	1476.7905	1476.7864	3	0	K.LSSYIIPGPPLYTG.-	(Ions score 20)
237 - 250	739.4027	1476.7909	1476.7864	3	0	K.LSSYIIPGPPLYTG.-	(Ions score 9)
237 - 250	739.4030	1476.7914	1476.7864	3	0	K.LSSYIIPGPPLYTG.-	(Ions score 17)
237 - 250	739.4032	1476.7918	1476.7864	4	0	K.LSSYIIPGPPLYTG.-	(Ions score 2)
237 - 250	739.4037	1476.7929	1476.7864	4	0	K.LSSYIIPGPPLYTG.-	(Ions score 36)
237 - 250	739.4038	1476.7931	1476.7864	4	0	K.LSSYIIPGPPLYTG.-	(Ions score 10)
237 - 250	739.4044	1476.7942	1476.7864	5	0	K.LSSYIIPGPPLYTG.-	(Ions score 17)
237 - 250	739.4045	1476.7944	1476.7864	5	0	K.LSSYIIPGPPLYTG.-	(Ions score 30)
237 - 250	739.4045	1476.7944	1476.7864	5	0	K.LSSYIIPGPPLYTG.-	(Ions score 9)
237 - 250	739.4050	1476.7955	1476.7864	6	0	K.LSSYIIPGPPLYTG.-	(Ions score 16)
237 - 250	739.4054	1476.7962	1476.7864	7	0	K.LSSYIIPGPPLYTG.-	(Ions score 18)

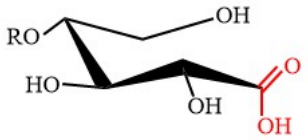


RMS error 3 ppm

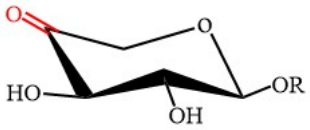
Figure S2. Structure of C1- and C4-oxidized and non-oxidized xylo-oligosaccharides



non-oxidized xylo-oligosaccharides



C1-oxidized xylo-oligosaccharides



C4-oxidized xylo-oligosaccharides

Figure S3. Structure-based sequence alignment of n*Ta*AA9A and *Cv*AA9_A. Structural superposition and figure creation were carried out with Chimera.

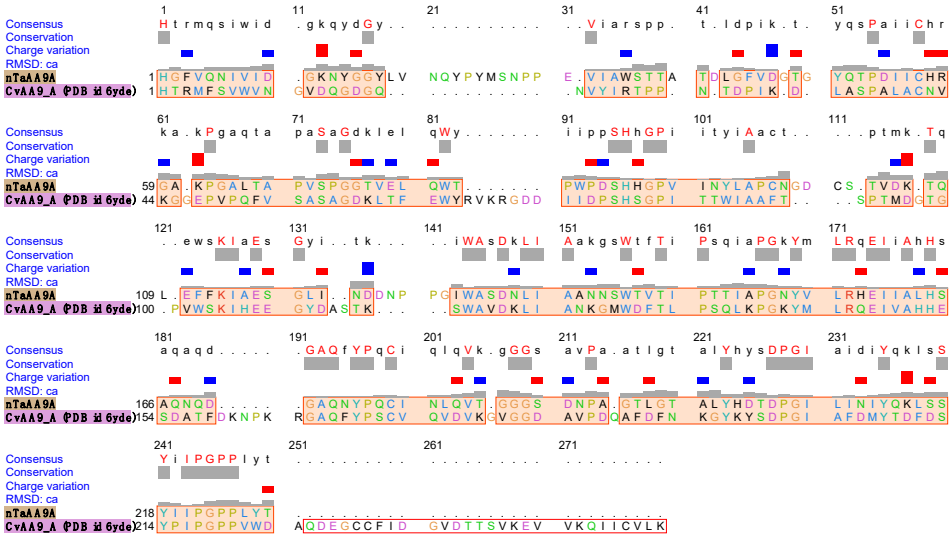


Figure S4. The cluster of the four residues used for the construction of a tetramutant in AfAA9_B with enhanced thermostability. The four residues (Val90, Ser131, Leu134, and Trp141) are shown in sphere representation. The glycosylation site and the Cu-binding site are also depicted (in stick representation).