

Supplementary Material

Material and Methods

WT and ANP Peptide Sequencing by Mass Spectrometry

Peptide sequencing by LC/MS/MS was carried out at The Proteomics Core Facility, Michigan State University (East Lansing, MI, USA). Samples were suspended in 2% acetonitrile/0.1% TFA to 25 μ L and from this 10 μ L were automatically injected (4 μ L/min in 2% acetonitrile/0.1% formic acid) by a Waters nanoAcquity Sample Manager onto a Waters Symmetry C18 peptide trap column (5 μ m, 180 μ m \times 20 mm). Bound peptides were eluted from the C18 column onto a Michrom MAGIC C18AQ column (3 μ m, 200A, 100U \times 150 mm) and the peptides were further eluted over 15 minutes with a gradient of 5% B to 35% B in 8 min (Buffer A = 99.9% Water/0.1% formic acid; Buffer B = 99.9% acetonitrile/0.1% formic acid) on the C18AQ column. The C18AQ column elution gradient was then quickly ramped to 90% B at 9 min, held for 0.5 min and returned to 5% B for the remainder of the run at a constant flow rate of 0.8 μ L/min.

Peptides eluted from the C18AQ column were sprayed into a ThermoFisher LTQ-FT Ultra mass spectrometer using a Michrom ADVANCE nanospray source. Survey scans were taken in the FT (25,000 resolution determined at m/z 400) and the top five ions in each survey scan were subjected to automatic low energy collision induced dissociation (CID) in the LTQ. The resulting MS/MS spectra were converted to peak lists using BioWorks Browser v3.3.1 by default LTQ-FT parameters and searched against a custom protein database consisting of Arabidopsis sequences (TAIR v10, downloaded from www.Arabidopsis.org) and common processing contaminants such as trypsin and human keratins (downloaded from NCBI, www.ncbi.nlm.nih.gov), using the Mascot searching algorithm, v 2.3 (www.matrixscience.com). The Mascot output was then analyzed using Scaffold, v3.5.1 (www.proteomesoftware.com) to probabilistically validate protein identifications using the ProteinProphet computer algorithm. Assignments validated above the Scaffold 95% confidence filter were considered true.

Sequencing of Peptides from MT

The MT HFI trypsin digest was re-suspended in 2% acetonitrile/0.1% TFA (designated Blank Ssolution) to 25 μ L and purified by solid phase extraction using OMIX c18 tips (www.agilent.com) according to the manufacturer's instructions. Eluted peptides were dried by vacuum centrifugation and re-suspended in 20 μ L Blank Solution. From this, 5 μ L were automatically injected by a Thermo (www.thermo.com) EASYnLC onto a Thermo Acclaim PepMap RSLC 0.075 mm \times 150 mm C18 column and eluted over 60 min with a gradient of 5% B to 30% B in 49 min, ramping to 100% B at 50 min and held at 100% B for the duration of the run (Buffer A = 99.9% water/0.1% formic acid; Buffer B = 99.9% acetonitrile/0.1% formic acid).

Eluted peptides were sprayed into a ThermoFisher Q-Exactive mass spectrometer (www.thermo.com) using a FlexSpray spray ion source. Survey scans were taken in the Orbi trap (35,000 resolution, determined at m/z 200) and the top twelve ions in each survey scan are then subjected to automatic higher energy collision induced dissociation (HCD) with fragment spectra acquired at 17,500 resolution. The resulting MS/MS spectra are converted to peak lists using Mascot Distiller, v2.4.3.3 (www.matrixscience.com) and searched against all entries in the TAIR, v10, protein sequence database

(downloaded from The Arabidopsis Information Resource, www.arabidopsis.org) appended with common laboratory contaminants (downloaded from www.thegpm.org, cRAP project) and also against all protein entries available from SwissProt (v2013_10, downloaded from www.uniprot.org), using the Mascot searching algorithm, v2.4. The Mascot output was then analyzed using Scaffold, v4.2.1 (www.proteomesoftware.com) to probabilistically validate protein identifications. Assignments validated above the Scaffold 95% confidence filter were considered true.

Table S1. Amino acid composition (mole %) of major reverse-phase peptide peaks from the trypsin digested cell wall HF insoluble pellets.

Amino Acid	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	F1	F2	F3	F4	A1	A2	A3	A4	A5	A6
Asp	0	0	0	0	0	3	5	6	8	0	0	0	5	8	4	11	5	6	6	10
Glu	0	0	0	3	0	3	4	2	0	0	0	0	0	6	2	6	7	4	5	7
Hyp	23	8	36	18	33	33	23	16	28	33	32	41	28	14	16	3	0	17	18	17
Ser	10	1	15	9	15	10	12	13	13	20	18	25	13	10	8	22	6	14	15	11
Gly	0	2	5	12	0	7	10	7	0	8	0	8	8	13	7	21	22	13	17	14
His	0	0	20	34	0	0	12	6	0	10	22	0	0	9	0	0	0	15	10	0
Arg	0	0	0	0	0	0	5	4	0	0	0	0	0	6	0	0	0	4	4	4
Thr	2	2	0	0	0	12	3	1	0	0	0	0	10	0	7	7	0	3	0	7
Ala	7	81	11	14	6	6	8	7	51	18	12	0	8	0	39	0	23	0	0	0
Pro	0	1	2	0	0	2	3	4	0	0	0	0	0	2	2	8	9	3	4	5
Tyr	0	0	0	0	0	0	0	14	0	12	0	0	0	2	0	0	0	3	0	0
Val	2	0	0	0	3	0	4	2	0	0	0	0	0	3	2	6	3	3	5	4
Ile	6	0	0	0	5	0	0	5	0	0	0	0	0	5	0	0	6	0	0	0
Leu	34	0	0	0	25	0	0	7	0	0	0	0	0	7	0	0	11	0	0	0
Phe	0	0	0	0	0	5	0	0	0	0	0	0	6	0	0	0	0	0	0	0
Lys	17	5	12	10	15	19	10	5	0	0	15	25	22	16	14	16	9	14	15	20

1. Zero value indicated a low signal during analysis that could not be integrated by the analyzer. 2. The amino acid composition of F5 and A7 were not determined due to the lack of material.

EXT1 (AT1G76930):

*MGAPMASFLV LAFSLAFVSQ TT*ANYFYSSP PFPVKHYSPP PVYKSPPPPV **KHYSPPPVYK**
 SPPPPVKHYS PPPVYKSPPP PVK**YYSPPPV** **YKSPPPPVYK** SPPPPVKHYS PPPVYKSPPP
 PVKHYSPPPV YKSPPPPVKH YSPPPVYKSP PFPVKHYSPP PSYTTLHHHR FTTHLLQSYT
 TLHHHRFTTH LLQLYTTPHH HPRSTTNTNL LLLRSITLPL RYTILLLLQY TTTHLHTNPT
 FTNHLLLLTT RLLNRL

EXT3 (AT1G21310):

*MGSPMASLVA TLLVLTISLT FVSQST*ANYF YSSPPPPVKH **YTPPVKHYS**P PPVYHSPPPP
 KKHYEYKSP PPVKHYSPPP VYHSPPPPKK HYVYKSPPPP VKHYSPPPVY HSPPPPKKH
 VYKSPPPPVK HYSPPPVYHS PPPPKKHVY **KSPPPVKH**Y SPPPVYHSPP PPKKHVYK
 PPPPVKHYS PPVYHSPPPP KKHVYKSP PVPKHYSPPP **VYHSPPPK**K HYVYKSPPPP
 VKHYSPPPVY HSPPPPKKH VYKSPPPPVK HYSPPPVYHS PPPPKKHVY KSPPPPVKH
 SPPPVYHSPP PPKKHVYKSP PPPPVKHYS PPVYHSPPPP KKHVYKSP PPVKHYSPPP
 VYHSPPPPK HYVYKSPPPP VKHYSPPPVY HSPPPKKEY VYKSPPPPV HHYSPPHPY
 LYKSPPPPYH Y

EXT17 (AT3G54580):

MGPSTHLICA LGVVIMATMV AAYDPYTDSS PPPLYSSPLP KIEYKTPPLP YIDSSPPPTY
 SPAPEVEYKS PPPPYVYSSP PPPTYSPSPK VEYKSPPPPY VYSSPPPTY SPSKVEYKS
 PPPPYVYSSP PPPTYSPSPK VEYKSPPPPY VYSSPPPTY SPSKVEYKS PPPPYVYSSP
 PPPTYSPSPK VEYKSPPPPY VYSSPPPPYY SPSKVEYKS PPPPYVYSSP PPPTYSPSPK
 VDYKSPPPPY VYSSPPPPYY SPSKVEYKS PPPPYVYSSP PPPTYSPSPK VDYKSPPPPY
 VYSSPPPPYY SPSKVYKSP PPPPYVYSSP PPPTYSPSPK VEYKSPPPPY VYSSPPPTY
 SPSKVEYKS PPPPYVYSSP PPPTYSPSPK VEYKSPPPPY VYSSPPPTY SPSKVEYKS
 PPPPYVYSSP PPPYSPSPK VEYKSPPPPY VYSSPPPTY SPSKVYKSP PPPPYVYSSP
 PPPYSPSPK VYKSPPPPY VYSSPPPPYY **SPSKVYKSP** PPPPYVYSSP PPPYSPSPK
 VYKSPPPPY YSPSPKVYK SPPPPYVYSS PPPPYSPSP KVYKSPPPP VYSSPPPPY
 HSPSPKVQYK SPPPPYVYSS PPPPYSPSP KVYKSPPPP VYSSPPPPY YSPSPKVYK
 SPPPPYVYSS PPPPYSPSP KVYKSPPH HVCVCPPPP CYSKVPVY KSPPPPYVY
 SPPPPHYSPP PKVYKSPPP PYVYSSPPPP YSPSPKVHY KSPPPPYAP TPKVHYKSP
 PPVYSSPPP PYSPSPKVH YKSPPPPYVY SPPPPYSP SPKVEYKSP PPVYSSPPP
 PYSKVPVY YKSPPPPYVY SPPPPYSP SPKVYKSP PPVYSSPPP PYSPSPVVD
 YKSPPPPYVY SPPPPYSP SPKVEYKSP PPVYKSPPP PSYSPKTE Y

EXT21 (AT2G43150):

MATPAWSHAK AQWVAMLAL LVGSAMATEP YYSSPPPPY EYKSPPPPVK SPPPPYEYKS
 PVPKSPPP PYHSPPPP VKSPPPPYVY **SSPPPVKSP** PYPYHSPPP PVPKSPPPY
 YHSPPPPVK SPPPPYYHS PVPKSPPP **PYPYHSPPP** **VKSPPPYYY** HSPPPPVKSP
 PYPYSSPP PVPKSPPPV YIYASPPPT HY

Figure S1. Cont.

LRX1 (AT1G12040):

*MLFPPLRSLF LFTLLLSSVC FLQIKAD*HDD ESDLGSDIKV DKRLKFENPK LRQAYIALQS
 WKKAI FSDPF NFTANWNGSD VCSYNGIYCA PSPSYPKTRV VAGIDLNHAD MAGYLASELG
 LLSDLALFHI NSNRFCGEVP LTFNRMKLLY *ELDLSNNRFV* GKFPK*VVLSL* *PSLK*FLDLRY
 NEFEGKIPSK LFDRELD AIF LNHNRF RFGI PKNMGNSPVS ALVLADNNLG GCIPGSIGQM
 GKTLNELILS NDNLTGCLPP QIGNLKK*VTV* *FDITSNRLQG* *PLPSSVGNMK* SLEELHVANN
 AFTGVIPPSI CQLSNLENFT YSSNYFSGRP PICAASLLAD IVVNGTMNCI TGLARQRSDK
 QCSSLLARPV DCSKFGCYNI FSPPPTFKM SPEVRTLPPP IYVYSSPPPP PSSKMSPTVR
 AYSPPPPSS KMSPSVRAYS PPPPYSKMS PSVRAYPPP PPSPPPPPY VYSSPPPPYV
 YSSPPPPYV YSSPPPPYV YSSPPPPYVY SSPPPPVYSS SPPPPPSPP PPCPESSPPP
 PVVYAPVTQ SPPPPSPVY PVVTQSPPPP SPVYYPVVTN SPPPPSPVY PVVTYSPPPP
 SPVYYPQVTP SPPPPSPLY PVVTPSPPPP SPVYYPVTP SPPPPSPVY PVVTPSPPPP
 SPVYYPSETQ SPPPPTEYYY SPSQSPPTK ACKEGHPPQA TPSYEPPEY SYSSSPPPPS
 PTSYFPPMPS VSYDASPPP PSYY

LRX2 (AT1G62440):

MESSDDCFIQ LEVSVRLGVN DLLSFENYRT SFPWDSYSGL DGGFHLGAVD DEGNLTEIGQ
 LMNLEIRGDD DDDDISDDNI KVDPSLKFEN PSLRQAYIAL QSWKQAI FSD PFNFTANWNG
 SDVCSYNGIF CAPSPSSPKT RVVAGIDLNH ADMAGYLPRE *LGLLTDLALF* *HLNSNRFCGE*
 VPLTFKHMKL LFELDLSNNR FVGK*FPNVVL* *SLPSLK*FLDL RYNEFEGSIP SKLFDKELDA
 IFLNHNRFMF GIPENMGNSP VSALVLADND LGGCIPGSIG LMGKTLNEII LSNNDLGTCL
 PPQIGNLKNV TVFDISFNRL *SGPLPSSIGN* *MKSLEQLNVA* *NNR*FTGVIPS SICQLSNLEN
 FTYSSNFFTG DAPRCVALLG DNVVVNGSMN CIDGKEDQRS SKECSSPASR SVDCSKFGCN
 NFFSPPPPSF KMSPTVRVLP PPPPSSKMSF TFRATPPPPS SKMSPSFRAT PPPPSSKMSF
 SFRATPPPPS SKMSPSVKAY PPPPPPEYE PSPPPSSEM SPSVRAYPPP PPLSPPPPSP
 PPPYIYSSPP PPSPPPPPY IYSSPPPVN CPPTTQSPPP PKYEQTSPR EYYPSPSPPY
 YQYTSPPPPP TYATQSPPP PPPPTYAVQ SPPPPPVY PVVTASPPP PVYTPVIQS
 PPPPVYVYSP VTQSPPPPPP VYYPVVTQSP PPSPVYYPV TQSPPPPVY YLPVTQSPPP
 PSPVYYPVA KSPPPSPVY YPPVTQSPPP PSTPVEYHPP ASPNQSPPE YQSPPPKGCN
 DSPSNDHHYQ TPTPPSLPPP YYEDTLPPI RGVSYASPPP PSIPYY

LRX3 (AT4G13340):

MKKTIQILLF FFFLINLTNA LSISSDGGVL SDNEVRHIQR RQLLEFAERS VKITVDPSLN
 FENRRLNAY IALQAWKQAI LSDPNNFTSN WIGSNVCNYT GVFCSPALDN RKIRTVAGID
 LNHADIAGYL PEELGLLSDL ALFHVNSNRF CGTVPHRFNR *LKLLFELDLS* *NNRFAGKFPT*
*VVLQLPSLK*F LDLR*FNEFEG* *TVPKELFSK*D *LDAIFINHR* *FRFELPENFG* *DSPVSVIVLA*
*NNR*FHGCVPS SLVEMKNLNE IIFMNNGLNS CLPSDIGRLK NVTVFDVSN ELVGPLPESV
 GEMVSVEQLN VAHNMLSGKI PASICQLPKL ENFTYSYNFF TGEAPVCLRL PEFDDRRNCL
 PGRPAQRSPG QCKAFLSRPP VNCGSFSCGR SVSPRPVVT PLPPPSLSP PPPAPIFSTP
 PTLTSPPPPS PPPPVYSPPP PPPPPPVYS PPPPPPPPP PPVYSPPPP PPPPPPPVY
 SPPPPSPPP PPPVYSPPP PPPPPPPVY SPPPPPVYSS PPPPPSPAPT PVYCTRPPPP
 PPHSPPPPQF SPPPEPEYYY SPPPPHSSP PPHSPPPPHS PPPPIYPYLS PPPPTPVSS
 PPPTPVYSPP PPPPCIEPPP PPPPCIEYSPP PPPPVHYSS PPPPPVYSS PPPPPVYSS
 PPPPPVHYSS SPPPEVHYH SPPPSPVHYS SPPPPSAPC EESPPAPV HHSPPPMVH
 HSPPPVHIQ SPPPPSPEYE GPLPPVIGVS YASPPPPPFY

Figure S1. Cont.

AGP30 (AT2G33790):

MGIIGKSVSL TLFALLCFTS SVFTLGVNQP GSSDPFHSLP QHLPLPPIKL PTLPPAKAPI
KLPAYPPAKA PIKLPTLPPA KAPIKLPTLP PIKPPVLPPV YPPKYNKTLV *AVRGVVYCKA*
 CKYAGVNNVQ GAKPVKDAVV RLVCKNKKNS ISETKTDKNG YFMLLAPKTV TNYDIKGCRA
 FLVKSPDTC SKVSSLHDGG *KGSVLKPVLK* PGFSSTIMRW FKYSVYNVGP FAFEPTCPK

AGP 31 (AT1G28290):

MGFIGKSVLV SLVALWCFTS SVFTTEEVNHNK TQTPSLAPAP APYHHGHHHP HPPHHHHHPH
 HPHPHPPAKS *PVKPPVKAPV* SPPAKPPVKP *PVYPPTKAPV* KPPTKPPVKP PVSPPAKPPV
 KPPVYPPTKA PVKPPTKPPV KPPVYPPTKP *PVYPPTKAPV* KPPVSPPTKP PVTTPVYPPK
 FNRSLVAVRG TVYCKSCKYA AFNTLLGAKP IEGATVKLVC KSKKNITAET *TTDKNGYFLL*
LAPKTVTNGF FRGCRVYLK SKDYKCSKVS *KLFGGDVGAE* *LKPEK*KLKGS TVVVNKLVIY
 LFNVGPFAPN PSCPK

FLA7 (AT2G04780):

MAKMQLSIFI AVVALIVCSA SAKTASPPAP VLPPTPAPAP APENVNLTEL LSVAGPFHTF
 LDYLLSTGVI ETFQNQANNT EEGITIFVPK DDAFKAQKNP PLSNLTKDQL KQLVLFHALP
 HYYSLSEFKN LSQSGPVSTF AGGQYSLKFT *DVSGTVRIDS* LWTRTKVSSS VFSTDPVAVY
 QVNRVLLPEA IFGTDVPPMP APAPAPIVSA PSDSPSVADS EGASSPKSSH KNSGQKLLLA
 PISMVISGLV ALFL

FLA15 (AT3G52370):

MDDLKLLFF LLLTISITTA LPDKPGSGQI NSNSVLVALL DSHYTELAEL VEKALLLQTL
 EEAVGQHNIT IFAPRNDAL KNLDPFKSF LLQPKNLKSL QSLLMFHILP KRITSPQFSS
 AVVSHRTLSN DHLHFTNGKV NSAEITKPPD LTRPDGIIHG IERLLIPRSV QEDFNRRSL
 RSIAAVLPEG APEVDPRTHR LKKKPAPIPA GAPPVLPVYD AMSPGPSLAP APAPGGGPR
HHFNGEAQVK DFIHTLLHYG GYNEMADILV NLTSLATEMG RLVSEGYVLT VLAPNDEAMA
 KLTTDQLSEP GAPEQIMYYH IPEYQTEES MYNSVRRFGK IRYDSLRFPH KVEAQEADGS
 VKFGHGDGSA YLFDPIYTD GRISVQGIDG VLFPEEKTPV EKKTGVPVVK KAPKPRRGKL
 MEVACTMLGS QFPTCQ

FLA16 (AT2G35860):

MDSSYGATKF LLLLFLTTSI ATALPDNKPV PGQINSNSVL VALLDSHYTE LAELVEKALL
 LQTL EEAVGK HNITIFAPRN DALERNLDPL FKSFLLEPRN LKSLQSLLMF HILPKRITSP
 QWPSLSHHHR TLSNDHLHLT VDVNTLKVDS AEIIRPDDVI RPDGIIHGIE RLLIPRSVQE
 DFNRRRSLRS ISAVIPEGAP EVDPRTHRLK KPSPAVPAGA PPVLPIDAM SPGPSLAPAP
 APGGGPRGH FNGDAQVKDF IHTLLHYGGY NEMADILVNL TSLATEMGRL VSEGYVLTVL
 APNDEAMAKL TTDQLSEPGA PEQIMYYHII PEYQTEESMY NAVRRFGKVK YDSLRFPHKV
 LAQEADGSVK FGHGDGSAYL FDPDIYTDGR ISVQGIDGVL *FPKEETPATE* *IKPAAPVVKK*
 VSKSRRGKLM EVACRMMGSR FIPCQ

Figure S1. Cont.

PAG2 (AT2G25060):

*MFLSASM*ASS *SLHV*AIFSLI *FLFSL*AAANE VTVGGKSGDW KIPPSSSYSF TEWAQKARFK
 VGDFIVFRYE SGKDSVLEVT KEAYNSCNTT NPLANYTDGE TKVKLDRSGP FYFISGANGH
 CEKGQKLSLV *VISPR*HVIS PAPSVEFED GPALAPAPIS GSVRLGGCYV VLGLVLGLCA
 WF

PRP3 (AT3G62680):

*MAITR*SSLAI *CLIL*SLVTIT *TADYY*SPSSP PVYKSPEHKP TLPSPVYTPP VYKPTLSPPV
 YTKPTIPPPV YTPPVYKHTP SPPVYTKPTI PPPVYTPPVY KPTLSPPVYT KPTIPPPVYT
 PPVYKPTPVY TKPTIPPPVY TPPVYKPTPS PPVYKKSPPY SSPPPPVYVK PTYTPTTKPY
 VPEILKAVDG IILCKNGYET YPILGAKIQI VCSDPASYGK *SNTE*VVIYSN *PTDS*KGYFHL
 SLTSIKDLAY CRVKLYLSPV ETCKNPTNVN *KGLT*GVPLAL *YGYR*FYPDKN LELFSVGPFY
 YTGPKAAPAT PKY

PRP4 (AT4G38770):

*MRIL*PEPRGS *VPCL*LLLLVSV *LLSAT*LSLAR VVEVVGYAES KIKTPHAFSG LRVTIDCKVN
 KGHFVTKGSG NIDDKGKFGI NIPHDIVSDN GALKEECYAO LHSAGTPCP AHDGLESTKI
 VFLSKSGDKH ILGLKQNLKF SPEICVSKFF WMPKLPFPK GFDHPFPLPP PLELPPFLKK
 PCPPKYSPPV EVPPPVPVYE PPPKKEIPPP VPVYDPPPCK *EVPPP*VPVYK *PPP*KVELPPP
 IPKKPCPPK PKIEHPPVP VYKPPKIEH PPPVPVYKPP PKIEHPPVP VHKPPKPCP
 PKKVDPPPVP VHKPPTKKPC PPKVDPPPVP PVHKPPPCKV IPPPKIEHPP *VVP*VYKPPP
 IEHPPPIYIP IVKKPCPPP PIYKPPVIP KKPCPPPVPV YKPPVVVIP KPCPPLQLP
 PLPKFPPLPP KYIHHPKFGK WPPLPPH

PRP10 (At5G09530):

MALMKKLSA ALLSSPLLI CLIALADPF SVGARRLED PKPEIPKLPE LPKFVVPKLP
*EF*PKPELPLK PEFPKPELPEL IPEIPKPELP *KV*PEIPKPEE *TK*LDPDKLE LPKFPEIPK
 ELPKMPEIPK *PE*LKVPKPEIQ KPELPMPEI PKPELKFPE IPKDLKFP ENSKPEVPKL
 METEKPEAPK VPEIPKPELP KLPEVPKLEA PKVPEIQKPE LPKMPELPM PEIQKPELPEL
 LPEVPKLEAP KVPEIQKPEL PKMPELPM EIQKPELPM *PEIQ*KPELPEL VPEVPKPEL
 TVPEVPKSEA PKFPEIPKPE LPKIPEVPKPE ELPKVPEITK PAVPEIPKPE LPTMPQLPKL
 PEFKVPVPGTP

Figure S1. Protein sequences of HRGPs identified in wild type and AtEXT3 mutant lines. Color-codes of peptides indicate the Arabidopsis lines from which were they identified: WT only—green, ANP only—blue, MT only—red, WT+ANP—underlined, WT+MT—purple, ANP+MT—orange, All—BOLD, signal peptides—*italic dark red*.

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