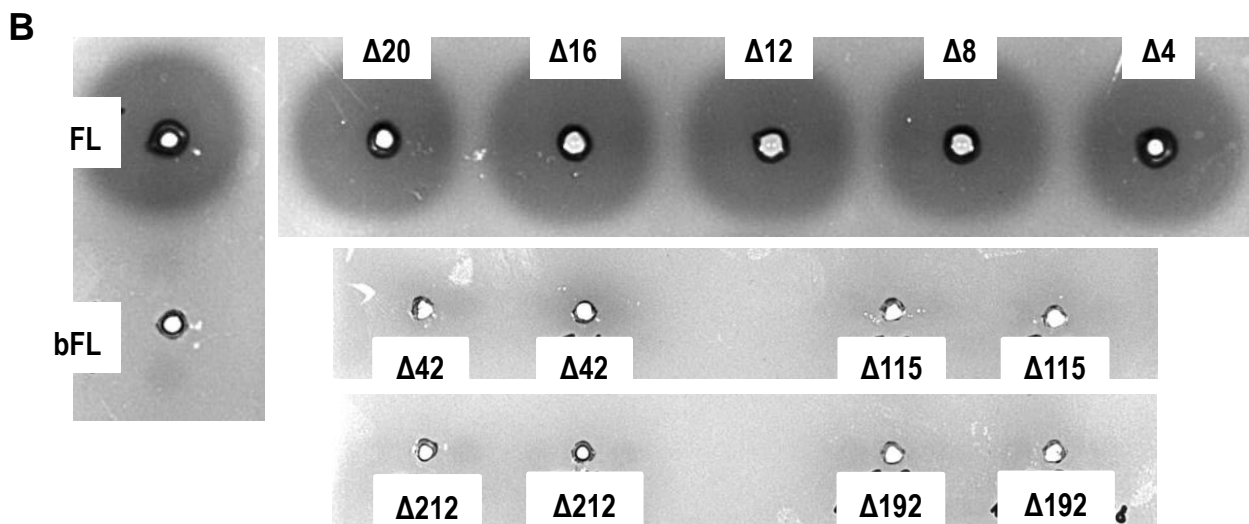


A

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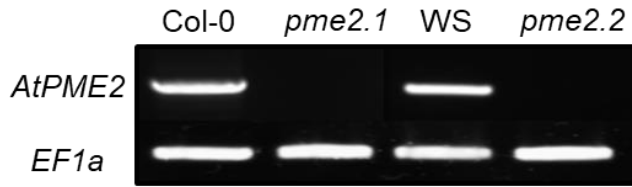
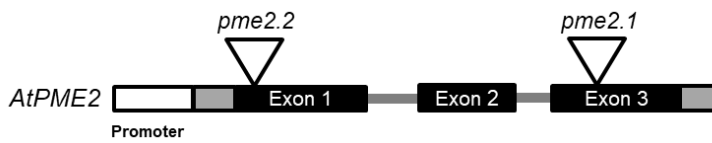
1  MAPIKEFISK  FSDFKNNKKL  ILSSAAIALL  LLASIVGIAA  TTTNQNKNQK  ITTLSSTSHA
                                FL  Δ4  Δ8
61  ILKSVCSSTL  YPELCFSAVA  ATGGKELTSQ  KEVIEASLNL  TTKAVKHNYF  AVKKLIAKRK
    Δ12  Δ16  Δ20                                Δ42
121  GLTPREVTAL  HDCLETIDET  LDELHVAVED  LHQYPKQKSL  RKHADDLCTL  ISSAITNQGT
                                Δ115
181  CLDGFSYDDA  DRKVRKALLK  GQVHVEHMCS  NALAMIKNMT  ETDIANFELR  DKSSTFTNNN
                                Δ192
241  NRKLKEVTGD  IDSDGWPKWL  SVGDRLLQG  STIKADATVA  DDGSGDFTTV  AA AVAAAPEK
                                Δ212                                MAT *****
301  SNKRFVIHIK  AGVYRENVEV  TKKKTNIMFL  GDGRGKTIIT  GSRNVVDGST  TFHSATVAAV
                                *****  ***
361  GERFLARDIT  FQNTAGPSKH  QAVALRVGSD  FSAFYQCDMF  AYQDTLYVHS  NRQFFVKCHI
421  TGTVDFIFGN  AAVLQDCDI  NARRPNSGQK  NMVTAQGRSD  PNQNTGIVIQ  NCRIGGTSDL
                                ** *****
481  LAVKGTFPTY  LGRPWKEYSR  TVIMQSDISD  VIRPEGWHEW  SGSFALDTLT  YREYLNRGGG
                                *****  *****
541  AGTANRVKWK  GYKVITSDE  AQPFTAGQFI  GGGWLASTG  FPFSLSL*

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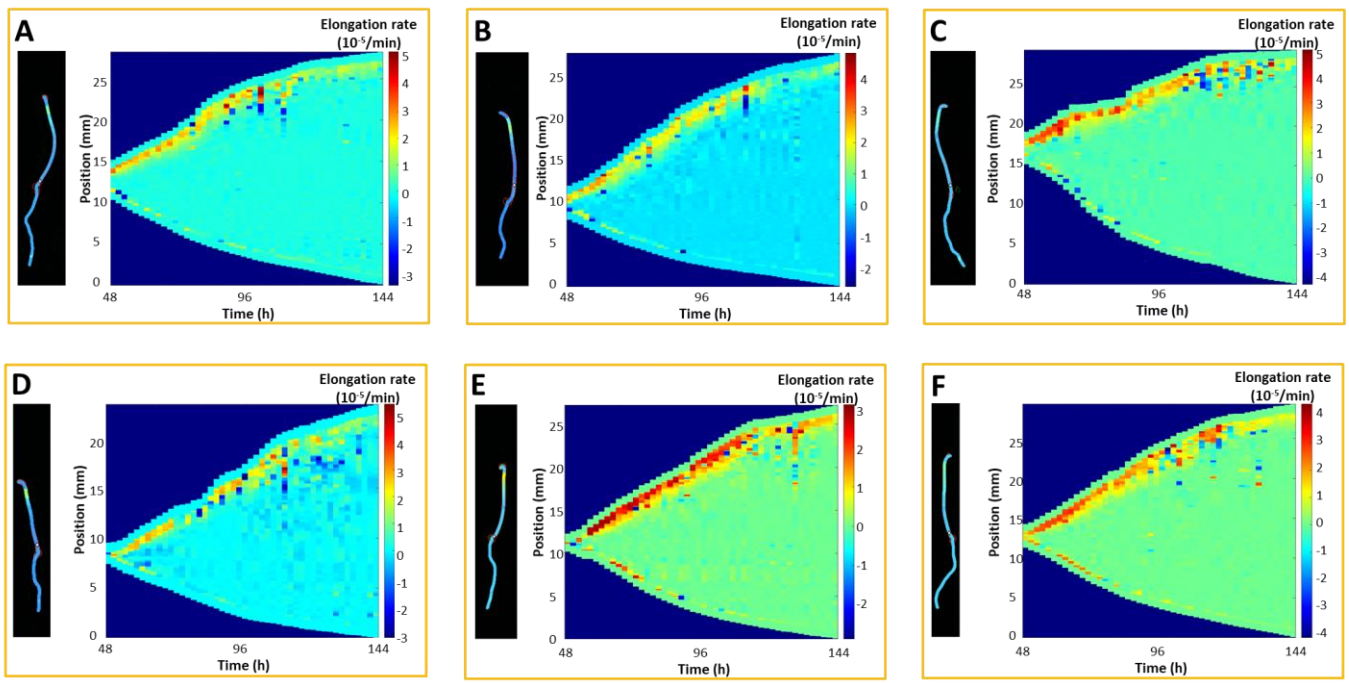


Deletion	FL	Δ4	Δ8	Δ12	Δ16	Δ20	Δ42	Δ115	Δ192	Δ212
Activity	+	+	+	+	+	+	-	-	-	-

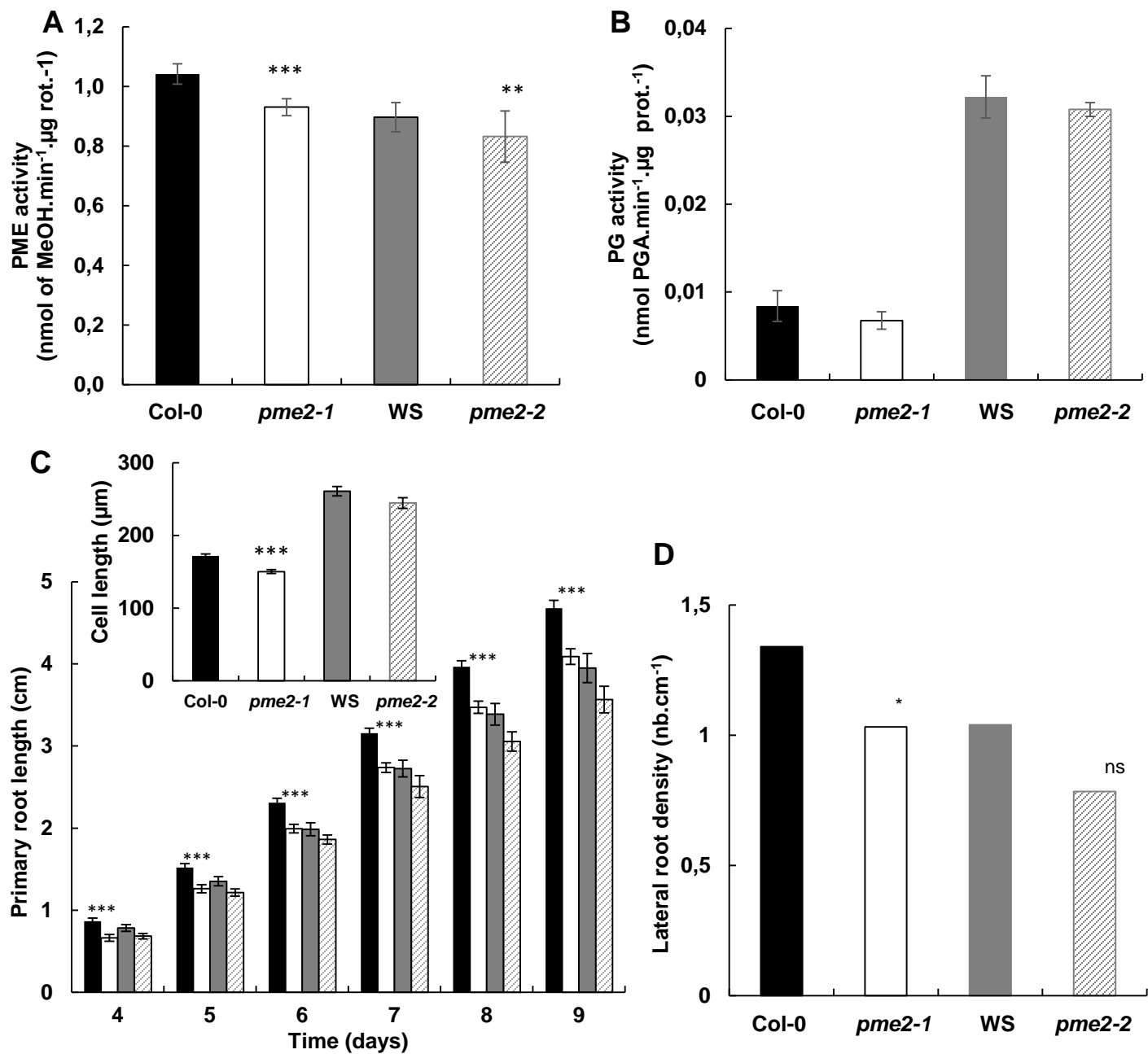
Supplemental Figure 1: (A) AtPME2 amino-acid sequence highlighting the sequences used for expression in *Pichia pastoris* (signal peptide in bold, PRO part in green, mature part in cyan and putative processing motifs indicated in red in the intermediate region in yellow, red arrows indicate the different truncated versions). Peptides mapping AtPME2 in cell wall-enriched extracts of Col-0 dark-grown hypocotyls using nano LC-MS/MS are underlined with asterisks. **(B)** Effects of deletion of the N-terminus sequence of AtPME2 on the production of an active enzyme in *Pichia*. FL: Full length AtPME2, FLb: heat-denaturated AtPME2, Δ4FL to Δ212FL: Deletion of 4 to 212 amino acids of the PRO part. PME activity was assessed on concentrated supernatants using gel diffusion assay.



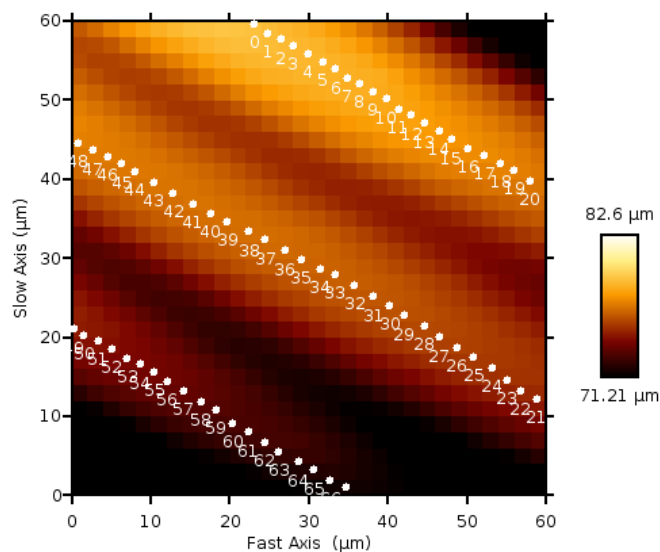
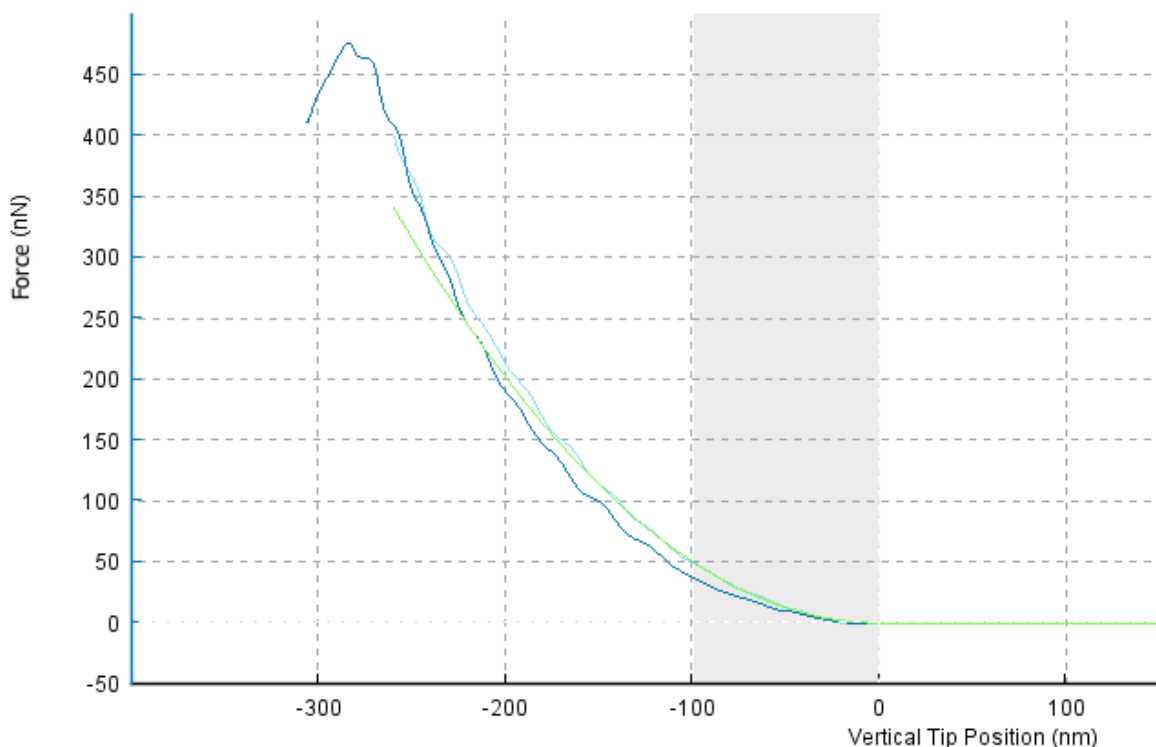
Supplemental Figure 2: Schematic representation of *AtPME2* gene structure and localization of the T-DNA insertions for *pme2-1* (GK-835A09, in the third exon) and *pme2-2* (FLAG_445B05, in the first exon). PCR analysis of *pme2-1*, *pme2-2* and WT (Col-0 and WS) hypocotyl cDNAs using specific primers flanking the T-DNA insertion sites. *EF1α* was used as reference gene.



Supplementary figure 3: Elemental relative rates of elongation for 3 wild-type Col-0 (A-C) and 3 *pme2-1* seedlings (D-E). In each panel, the colour scale on the left indicates relative increase in length of hypocotyl and root ($10^{-5}/\text{min}$ corresponds to 0.001% increase per minute or to 0.06% per hour). Elongation rate is shown along the seedling at 96h on the right. The kymograph in the middle shows elongation rate along the seedling at one time point, with all time points assembled horizontally.



Supplemental Figure 4 : (A) PME activity of cell wall-enriched protein extracts from 7-day-old roots of wild type Col-0/WS and *pme2-1/pme2-2* mutants. Data represent the means of PME activity in nmol of methanol.min⁻¹/μg of protein-1 ± SE of three independent protein extractions and three technical replicates (n=9). (B) PG activity of cell wall-enriched protein extracts from 7-day-old roots of wild type Col-0/WS and *pme2-1/pme2-2* mutants. Data represent the means of PG activity in nmol of GalA.min⁻¹/μg of protein-1 ± SE of three independent protein extractions and three technical replicates (n=9). (A-B) Significant differences with p<0.05 (*), p < 0.01 (**), p < 0.001 (***), were determined according to Mann-Whitney. (C) Time course analysis of root elongation of wild type Col-0 (black bar), WS (grey bar), *pme2-1* (white bar) and *pme2-2* (hatched bar). Data represent the means of length, in cm ± SE (n > 30), for each condition. Inset: Length of fully elongated cells of 7-day-old roots measured between two root hairs. 300 cells were measured for each condition. Significant differences were determined according to a Student t-test with p < 0.001 (***). (D) Lateral root density of wild type Col-0 (black bar), WS (grey bar), *pme2-1* (white bar) and *pme2-2* (hatched bar). Data represent the means ± SE (n > 30), for each condition. Significant differences were determined according to a Student t-test with p<0.05 (*).

A**B****C**

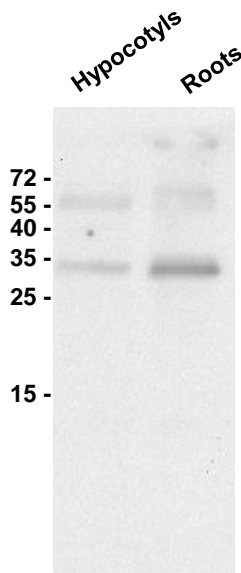
Supplemental Figure 5: AFM analysis of 3-day-old hypocotyls. **(A)** Top view of a wild-type hypocotyl under the atomic force microscope; the cantilever (rectangular with a triangular end) is located over the hypocotyl at about 1mm from the hook. Scale bar 100 μm . **(B)** Topographic image of a 60 μm x 60 μm region of the hypocotyl (height scale on right), with three cells partially visible. The white dots show points where force-depth curves were obtained in order to characterise cell wall stiffness. **(C)** Typical force-depth curve with approach in light blue, retract in dark blue. The green curve is a fit of the approach to the Sneddon model, which yields the apparent Young's modulus.

A

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At3g62170 IPDKLEADK---LTIKSYLGRPWKFKFATTVIIIGTEIGDLIKPEGWTEWQG-EQN--HK
At2g47030 VPDRLKTPER---LTVATYLGRPWKFFSTTVIMSTEMGDLIRPEGWKIWDG-ESF--HK
At2g47040 VPDKLLAAER---LIVESYLGRPWKFFSTTVIINSEIGDVIIRPEGWKIWDG-ESF--HK
At2g26450 AANEDLKPVK---EYKSYLGRPWKNYSRTIIMESKIENVIDPVGWLRWQETDFA--ID
At4g33230 APNEDLKPVK---AQFKSYLGRPWKPHSRTVVMESTIEDVIDPVGWLRWQETDFA--ID
At3g10710 SPLGDLTD-----VMTFLGRPWKNFSTTVIMDSYLHGFIIDRKGWLPWTG-DSA--PD
At5g04960 KPLDNLTD-----IQTFLLGRPWKDFSTTVIMKSFMDKFINPEGWLPWTG-DTA--PD
At3g10720 KAAPDLAAEP---KSAMTFLGRPWKPYSTRVFMQSYISDVIQVPGWLEWNG-TIG--LD
At5g04970 GAAPDLAADP---KSTMTFLGRPWKPYSTRVYIQSYISDVIQVPGWLEWNG-TTG--LD
At4g03930 TASSDLDTAT---VKTYLGRPWRI FSTVAVLQSFIGDLVDPAGWTFWEG-ETG--LS
At1g11590 TASSDLDTT---VKTYLGRPWRI FSTVAVMQSFIGDLVDPAGWTFWEG-ETG--LS
At3g27980 TTSSDLDTAT---VKTYLGRPWRRFSTVAVLQSFIGDLVDPAGWTFWEG-ETG--LS
At5g20860 RTDSDLSPVK---HKYSSYLGRPWRRKYSRAIVMESYIDDAIAGGGWAGWLD-SGDEVLK
At5g51490 LEAPDLKPVV---GTVKTYMGRPWKFRSRTVVLQTYLDNVSPVPGWSPWIE-GSVFGLD
At5g51500 IPAPDLKPVV---RSVKTYMGRPMMYSRTVVLKTYIDSVVSPVPGWSPWTK-GSTYGLD
At4g15980 TGDASYLQV---AKNRAFLGRPWKEFSRTIIMNTEIDVIDPEGWLRWNE-TFA--LN
At3g47400 IAASDLKQVI---RAYKTYLGRPWQAYSRTVIMKTYIDNSISPLGWSFWLR-GSNFALN
At3g14300 SPNGNVT-----ATTYLGRPWKLFSTTVIMQSVIGSFVNPAGWIAWNS-TYDPPPR
At1g23200 ATA-----SETYLGRPWRSHSRTVFMKCNLQALVSPAGWLPWSG-SFA--LS
At5g64640 NGTEEYMKEFQANPEGHKNFLGRPWKEFSRTVFNVCNLSLISPDGWMFPWNG-DFA--LK
At5g53270 LATPDLQASK---GSYPTYLGRPWKLYSRVVYMSMDMGDHDIPRGWLEWNG-PFA--LD
At5g49180 TGEPAYIPVK---SINKAYLGRPWKEFSRTIIMGTTIDVDIDPAGWLPWNG-DFA--LN
At5g09760 NGTEEYMKLFKANPKVHKNFLGRPWKDYSTRVFIGCNLEALITPDGWLFPWSG-DFA--LK
At4g02320 LAAPDLIPVQ---ANFKAYLGRPWQLYSRTVIMKSFIDDLVDPAGWLRWNG-DFA--LE
At4g02300 LAAPDLIPVK---ENFKAYLGRPWKYSRTVIMKSFIDDLIHPAGWLEGGK-DFA--LE
At4g00190 KGAPGVQ--L---GGVKTYLGRPWRYSARTVVIIGTYLDLIEPNGWLRWNG-VTA--LS
At3g49220 LAASDLQATN---GSTQTYLGRPWKLFSTTVYMSYIGGHVHTRGWLEWNT-TFA--LD
At3g06830 TGDPAYIPMK---SVNKAYLGRPWKEFSRTIIMKTTIDVDIDPAGWLPWSG-DFA--LK
At2g26440 LASEDLFNS---NKVKSYLGRPWREFSRTVVMESYIDEIFDGSWGSKWNG-GEA--LD
At1g53840 SANGNVI-----APTLYLGRPWKEFSSTVIMETVIGAVVRPSGWSWNS-GVDP-PA
At4g33220 SADADLVPLYL---NTRTYLGRPWKLYSRTVFI RNNMSDVVRPEGWLRWNG-DFA--LD
At3g60730 RAAPEFEAVK---GRFKSYLGRPWKYSRTVFLKTDIDELIDPRGWREWSG-SYA--LS
At3g43270 AADTDLNLLN---NTTATYLGRPWKLYSRTVFMQNYMSDAINPVGWLEWNG-NFA--LD
At3g05620 LAT-----QPTYLGRPWKLYSRTVYMNNTYMSQLVQPRGWLEWFG-NFA--LD
At3g05610 AGEPTYLAVK---ETSKAYLGRPWKEYSRTIIMNTFIPDFVQPGWQPLWG-DFG--LK
At2g45220 TAASDLRPVL---GSTKTYLGRPWQYSRTVFMKTSLSLIDPRGWLEWNG-NFA--LK
At1g11580 TASSDLAPVK---GSVKTFLGRPWKLYSRTVIMQSFIDNHDIPAGWTFWEG-EFA--LS
At3g14310 GATSDLQSVK---GSFPTYLGRPWKEYSQTVIMQSAISDVIIRPEGWSEWTG-TFA--LN
At5g27870 VGEPDYLAVK---EQSKTYLGRPWKEYSRTIIMNTFIPDFVQPGWQPLWG-EFG--LN
At3g59010 TGS-----TKTYLGRPWQYSRTVVMQSFIDGSIHPSGWSWNS-NFA--LK
At2g43050 TAE-----SMTYLGRPWKEYSRTVVMQSFIDGSIHPSGWSWNS-GFG--LK
At1g53830 GGTSDLLAVK---GTFTTYLGRPWKEYSRTVIMQSDISDVIIRPEGWHEWSG-SFA--LD
At4g02330 KPADDLVSSN---YTVKTYLGRPWKEYSRTVFMQSYIDEVVEPVGWREWNG-DFA--LS
At2g47550 RPADDLATS---YTVKTYLGRPWKEYSRTVVMQTYIDGFLEPSGWNWSG-DFA--LS
At1g02810 KPADDLVSSN---YTVKTYLGRPWKEYSRTVIMQSYIDGFVEPVGWREWNG-DFA--LS
Antibody CKTYLGRPWKEYSRTV
::**** :.

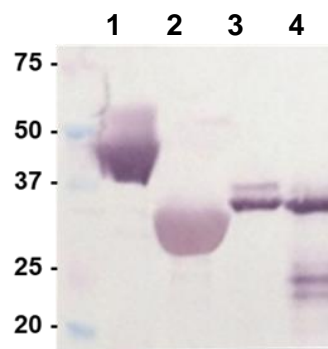
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B**C**

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CsPME2      GSFEIYLGRPWKRYSTRVVMQ
CsPME4      GSFPPTYLGRPWKEYSRTVIMQ
CsTTPME     TEFKTYLGRPWKEYSRTVFM
SIPME1      KEFPTYLGRPWKEYSRTVME
            . * * * * * * * * * * * * *
            CKTYLGRPWKEYSRTV

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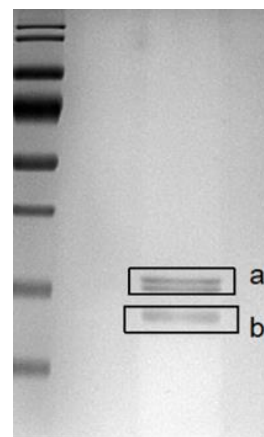
Supplemental Figure 6: (A) Sequence alignment of 45 Arabidopsis PME isoforms showing the conservation of the epitope used for production of the generic anti-PME antibody. (B) Western blot analysis of cell-wall-enriched protein extracts from 7 day-old roots and 4 day-old dark grown hypocotyls using the designed anti-PME peptide antibody. Both processed (~35kDa) and non-processed (~55kDa) forms of PME can be detected. (C) Sequence alignment of previously characterized PMEs from Citrus and Solanum showing the conservation of the epitope. Western blot analysis of purified PMEs, 1: CsTT-PME (Citrus sinensis thermally-tolerant isozyme; (Savary et al., 2013)), 2: CsPME2 (C. sinensis fruit-specific salt-independent isozyme, (Savary et al., 2010)), 3: CsPME4 (C. sinensis salt-dependent isozyme, (Savary et al., 2010)), and SIPME1 (Solanum lycopersicum isozyme (Savary, 2001)).

PRO-region sequence (~30 kDa)

```
1  MAPIKEFISK  FSDFKNNKLL  ILSSAAIALL  LLASIVGIAA  TTTNQNKNQK
51  ITTLSSTSHA  ILKSVCSSSTL  YPELCFSAVA  ATGGKELTSQ  KEVIEASLNL
101 TTKAVKHNYF  AVKKLIAKRK  GLTPREVTAL  HDCLETIDET  LDELHVAVED
151 LHQYPKQKSL  RKHADDLKTLL  ISSAITNQGT  CLDGFSYDDA  DRKVRKALLK
201 GQVHVEHMCS  NALAMIKNMT  ETDIANFELR  DKSSTFTNNN  NRKLKEVTGD
251 LDSDGWPKWL  SVGDRRL
```

Catalytic region sequence (~35 kDa)

```
                QG  STIKADATVA  DDGSGDFTTV  AA AVAA APEK
301 SNKRFVIHIK  AGVYRENVEV  TTKKTNIMFL  GDGRGKTIIT  GSRNVVDGST
351 TFHSATVAAV  GERFLARDIT  FQNTAGPSKH  QAVALRVGSD  FSAFYQCDMF
401 AYQDTLYVHS  NRQFFVKCHI  TGTVDFFIGN  AAAVLQDCDI  NARRPNSGQK
451 NMVTAQGRSD  PNQNTGIVIQ  NCRIGGTSDL  LAVKGTFTPT  LGRPWKYSR
501 TVIMQSDISD  VIRPEGWHEW  SGSFALDTLT  YREYLNRRGG  AGTANRVKWK
551 GYKVITSDTE  AQPFTAGQFI  GGGWLASTG  PPFSLSL
```



Catalytic region tryptic peptides – 12 observed (2 truncated) (“Band a”)

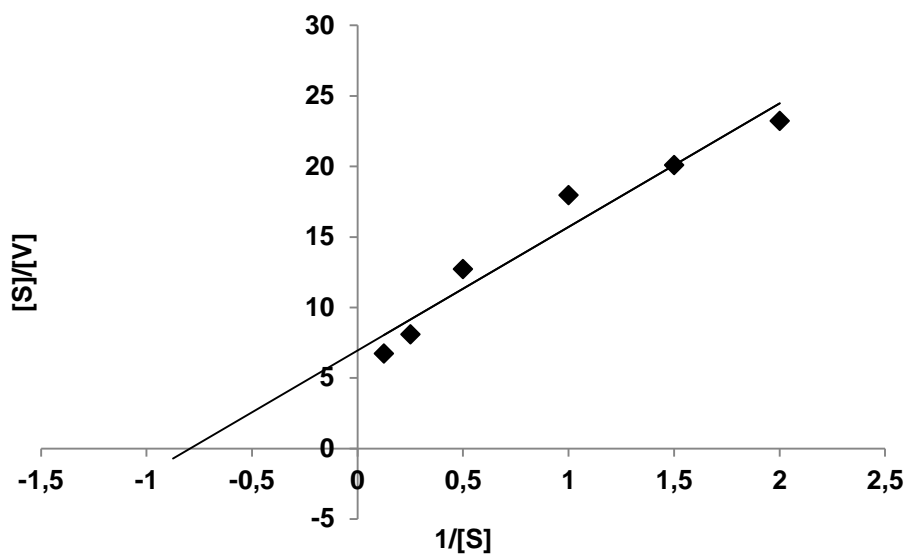
Position	Peptide sequences
75-300	ADATVADDGSGDFTTVAA AVAA APEK*
316-322	ENVEVTK*
325-334	TNIMFLGDGR
344-363	NVVDGSTTFHSATVA AVGER
368-379	DITFQNTAGPSK
387-412	VGSDFSAFYQCDMFAYQDTL YVHSNR
451-458	NMVTAQGR
459-473	SDPNQNTGIVIQNCR*
474-484	IGGTSDLLAVK*
485-496	GTFPTYLGRPWK*
501-532	TVIMQSDISDVIRPEGWH (+EWSGSFALDTLYR) – fragment observed
554-587	VITSDTEAQPFTAGQFIGGG (+GWLASTGFPPFSLSL) – fragment observed

PRO-region tryptic peptides – 7 observed (“Band b”)

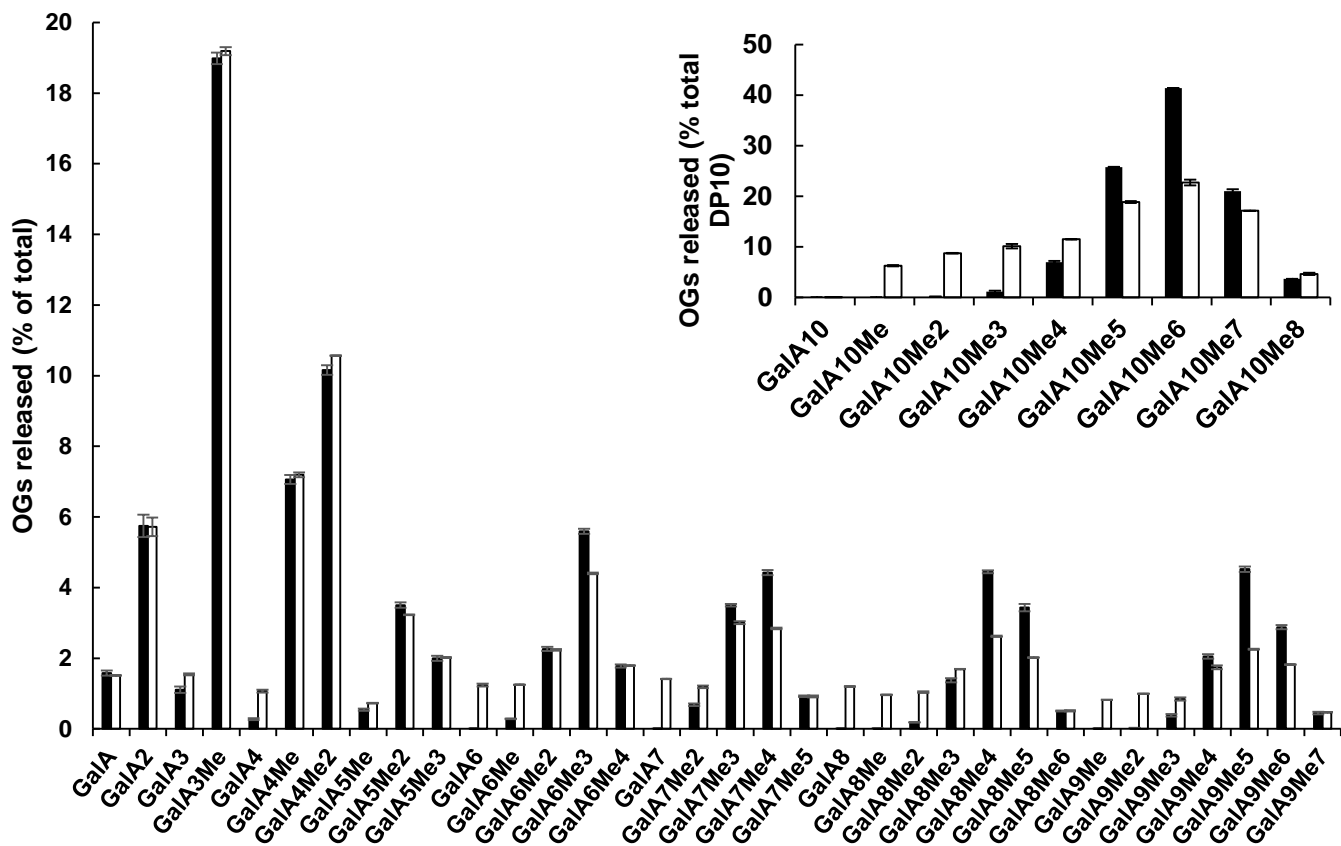
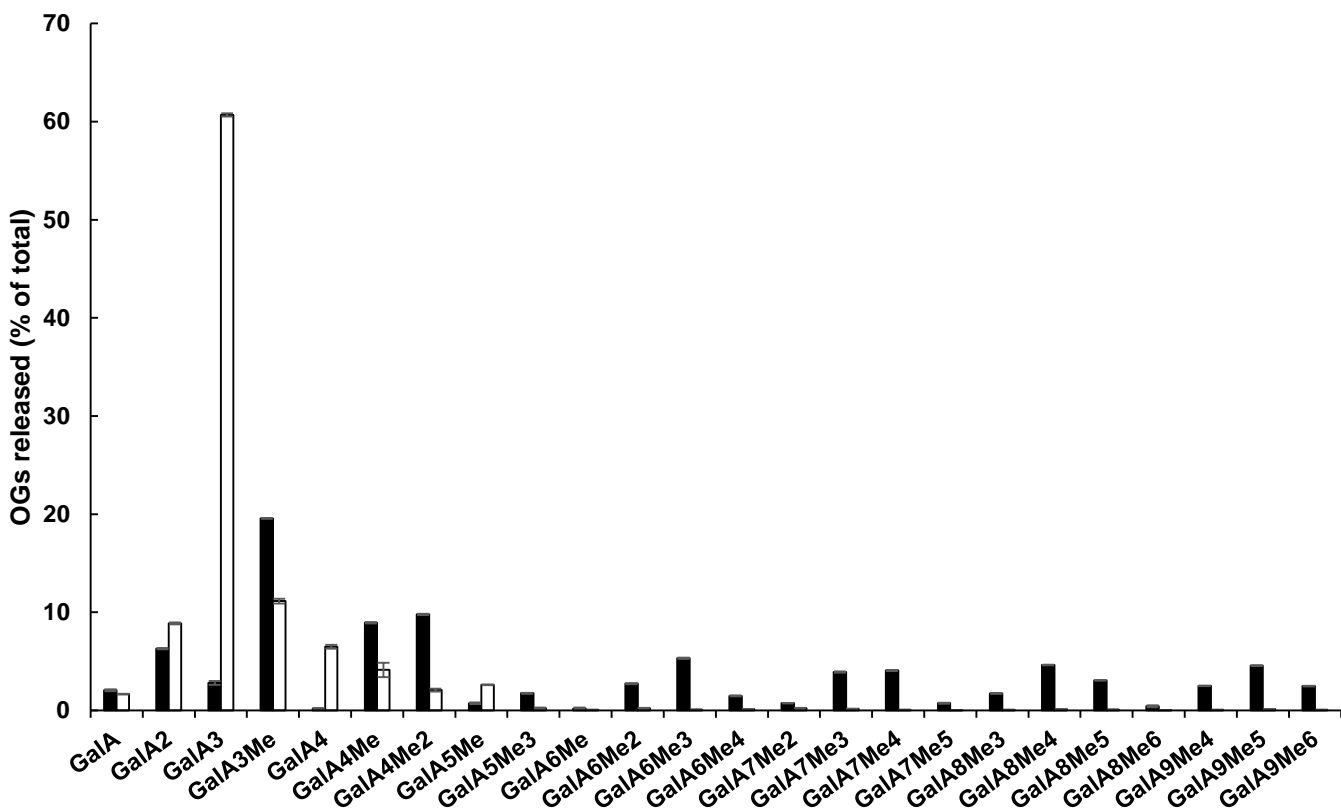
Position	Peptide sequences
51-63	ITTLSSTSHAILK
64-85	SVCSSSTLYPELCFSAVAATGGK
107-113	HNYFAVK
169-192	TLISSAITNQGTCLDGFSYDDADR
201-217	GQVHVEHMCSNALAMIK
218-230	NMTETDIANFELR
233-242	SSTFTNNNNR

*Peptides observed in cell wall extract and indicated by sequence alignment in Supp Fig 1

Supplemental Figure 7: Identification of peptides mapping the PRO and mature (catalytic) regions of AtPME2 following cation exchange purification. Band **a** corresponds to the two isoforms at ~35 kDa while band **b** corresponds to the band below ~35kDa (see gel).



Supplemental Figure 8: Determination of K_m and V_{max} for AtPME2. Activity was assessed using various concentrations of pectins DM 55-70% at 37°C and pH 7.5. $[S]$ is expressed in mg.mL^{-1} and $[V]$ in $\text{nmol.MeOH.min}^{-1}.\mu\text{g.prot}^{-1}$.

A**B**

Supplemental Figure 9: Determination of the processivity of CsPME at (A) pH 5 and (B) pH 8 using LC-MS/MS. Non-digested samples (black bars); CsPME digestion (white bars). Data represent the mean \pm SE of three replicates.

		Col-0		WS	
		% coverage	Nb peptides	% coverage	Nb peptides
AT1G30600.1	Subtilase family protein			8,65	4
AT1G32940.1	Subtilase family protein	20,8	14		
AT1G53830.1	Pectin methylesterase 2	18,91	5	16,87	4
AT2G04160.1	Subtilisin-like serine endopeptidase	15,16	9	18,39	11
AT2G05920.1	Subtilase family protein	62,2	29	62,2	30
AT2G45220.1	Plant invertase/pectin methylesterase inhibitor	30,53	13	31,12	13
AT3G14067.1	Subtilase family protein	38,87	17	38,35	17
AT3G14310.1	Pectin methylesterase 3	23,99	8	19,93	7
AT3G16850.1	Pectin lyase-like superfamily protein	18,46	5	17,58	5
AT3G43270.1	Plant invertase/pectin methylesterase inhibitor			11,2	3
AT3G57790.1	Pectin lyase-like superfamily protein			14,69	7
AT4G19410.1	Pectinacetylerase family protein	63,94	14	47,39	14
AT4G20430.2	Subtilase family protein	4,21	2	4,21	2
AT4G21650.1	Subtilase family protein			11,1	6
AT4G23500.1	Pectin lyase-like superfamily protein	4,65	2	12,53	4
AT4G25260.1	Plant invertase/pectin methylesterase inhibitor			11,94	2
AT4G33220.1	pectin methylesterase 44			13,9	3
AT4G34980.1	Subtilase family protein	18,85	11	23,04	13
AT5G09760.1	Plant invertase/pectin methylesterase inhibitor	13,43	5	11,8	5
AT5G44530.1	Subtilase family protein	13,81	7	9,05	4
AT5G45280.2	Pectinacetylerase family protein	47,83	10	43,22	10
AT5G59090.2	Subtilase 4.12	30,23	16	35,15	21
AT5G62350.1	Plant invertase/pectin methylesterase inhibitor			36,63	4
AT5G67360.1	Subtilase family protein	32,76	17	42,8	21

Supplemental Table I: Identification of homogalacturonan remodeling enzymes and regulators in cell wall-enriched protein extracts of Col-0/WS roots.

Genotyping	
<i>pme2-1</i> F	5'-GACGGAAGCGGTGACTTTAC-3'
<i>pme2-1</i> R	5'-AGTGTC AACGCAAACTCC-3'
<i>pme2-2</i> F	5'-CTCAACACTATACCCGGA-3'
<i>pme2-2</i> R	5'-ACTTTCCTATCGGCGTCG-3'
GK F	5'-ATATTGACCATCATACTCATTGC-3'
FLAG F	5'-CTACAAATTGCCTTTTCTTATCGAC-3'
RT-qPCR	
AtPME2 F	5'-TACGACGACGCCGATAGGAAAG-3'
AtPME2 R	5'-ATGTGCTCCACGTGTACCTGAC-3'
APT1 F	5'-GAGACATTTTGCGTGGGATT-3'
APT1 R	5'-CGGGGATTTTAAGTGGAACA-3'
CLA F	5'-GTTTGGGAGAAGAGCGGTTA-3'
CLA R	5'-CTGATGTC ACTGAACCTGAACTG-3'
TIP41 F	5'-GCTCATCGGTACGCTCTTTT-3'
TIP41 R	5'-TCCATCAGTCAGAGGCTTCC-3'
Promoter cloning	
primer F	5'-AAGCTTGTACAATGATGGTTCTATTGT-3'
primer R	5'-TCTAGAGGTGGAATAGGGTTTATATTG-3'
CDS cloning for confocal imaging	
primer F	5'-CACCATGGCACCAATCAAAG-3'
primer R	5'-AAGACTTAACGAGAAAGGAA-3'
CDS cloning for Pichia expression	
primer F	5'-CTGCAGGAGCCACAACAACAAC-3'
primer R	5'-GCGGCCGCAAGACTTAACGAGAAAGGA-3'

Supplemental Table II: Primers used in the study.

Parameter	Value used	Default value
Image channel	Green	No
Spatial resolution	20.55 μ m	No
Time interval	120 min	No
Smoothing method	Box filter	Yes
Radius	2	Yes
Threshold	Automatic	Yes
Threshold method	Max entropy	Yes
Smoothing factor for contours	20	Yes
First point of skeleton	Bottom	Yes
Curvature smoothing	10	Yes
Number of points for resampling	500	Yes
Channel for computing displacement	Green	No
Step between two measurements of displacements	1	No
Size of the correlating window #1	5	Yes
Spatial smoothing	0.1	Yes
Value smoothing	0.05	No
Resampling distance	0.05	No
Size of the correlating window #2	20	Yes

Supplemental Table III: Parameters used for spatial growth pattern determination using KymoRod (Bastien et al. Plant J 2016).