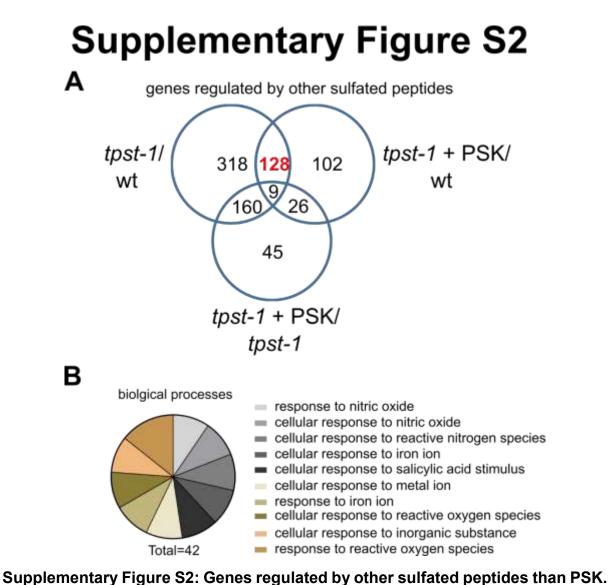


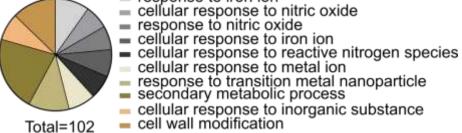
Supplementary Figure S1: Expression analysis of genes involved in triterpene synthesis in response to PSK treatment.  $\log_2$  fold chance of expression of (A) baruol, (B) marneral and (C) thalianol biosynthesis genes. Expression was tested in roots of *tpst-1* seedlings that were grown for five days in hydroponic culture and treated with or without 100 nM PSK for the time indicated. Small or capital letters indicate significant differences between time points of control samples or PSK-treated samples (ANOVA, post-hoc Bonferroni, p<0.05), whereas asterisks indicate significant difference between treatments at a specific time point (Student t-test; p<0.05; n=3 three).



(A) Venn diagram of genes regulated between the different genotypes and treatments indicated. Total number of genes regulated are given. The red number indicates the number of genes regulated by other sulfated peptides than PSK (B) Biological processes that are overrepresented among the genes that are regulated by other sulfated peptides than PSK. The identified genes were analyzed by the PANTHER16.0 programme (Mi *et al.*, 2019; Mi *et al.*, 2021). A total of 42 genes could be assigned to biological processes that are overrepresented.

biological processes response to iron ion

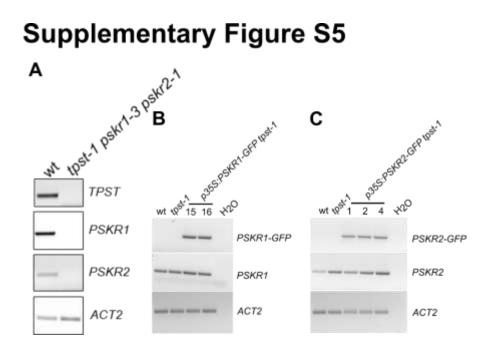
Α



**Supplementary Figure S3: Regulated genes overrepresented in the** *tpst-1* **mutant.** Biological processes that are overrepresented among the genes that are regulated in the *tpst-1* versus wild type. The identified genes were analyzed by the PANTHER16.0 programme (Mi *et al.*, 2019; Mi *et al.*, 2021). A total of 102 genes could be assigned to biological processes that are overrepresented.

Comparison	tpst-1 / wt	tpst-1 + PSK / wt	tpst-1 + PSK / tpst-1	Category
AT1G77640; member of the DREB subfamily A-5 of ERF/AP2 transcription factors	3,27	1.83	0.56	A
AT1G49960; Xanthine/uracil permease family protein	0.33	0.64	1.90	A
At3g22120; CWLP, Cell Wall-plasma Membrane Linker Protein	8.81	3.09	0.35	В
At5g35940; Mannose-binding lectin superfamily protein	0.06	0.29	5.24	В
At3g06019; unknown function	1.73	2.73	1.58	С
At3g21260; GLTP3, Glycolipid Transfer Protein 3	0.53	0.27	0.52	С

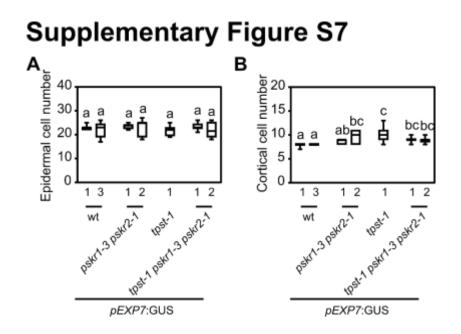
Supplementary Figure S4: Genes that could not be clearly categorized as regulated by PSK or other sulfated peptides and were sorted by different categories. Genes are derived from the microarray experiment. Microarray experiments were performed with three biological replicates.



**Supplementary Figure S5: Semi-quantitative RT-PCR analysis** of **(A)** wt and *tpst-1 pskr1-3 pskr2-1*, **(B)** wt, *tpst-1* and two independent *p35S*:PSKR1-GFP *tpst-1* lines and (C) wt, *tpst-1* and three independent *p35S*:PSKR2-GFP *tpst-1* lines. Transcript levels of *TPST*, *PSKR1*, *PSKR1-GFP* and/ or *PSKR2* and *PSKR2-GFP* were analyzed in 5-day-old seedlings by RT- PCR. *Actin2* was amplified as a control for RNA input.

Comparison Gene	tpst-1 / wt	tpst-1 + PSK / wt	tpst-1 + PSK / tpst-1
At3g59900; ARGOS, Auxin-regulated gene involved in organ size	4.63	1.66	0.36
At5g06080; LBD33, Lob Domain-containing Protein 33	3.05	2.49	0.81
At1g76420; ANAC031, Arabidopsis Nac Domain Containing Protein 3	1 2.77	1.28	0.46
At5g12330; LRP1, Lateral Root Primordium 1	2.36	1.72	0.73
At3g27940; LBD26, Lob Domain-containing Protein 26	0.24	0.20	0.83

Supplementary Figure S6: Differentially regulated genes with functions in lateral root growth and development. Genes are derived from the microarray experiment. Microarray experiments were performed with three biological replicates.



Supplementary Figure S7: Analysis of epidermal and cortical cell numbers (A, B) Quantification of (A) epidermal cell numbers and (B) cortical cell numbers in wild type, *pskr1-3 pskr2-1, tpst-1* and *tpst-1 pskr1-3 pskr2-1* that were determined from cross sections of *pEXP7:GUS*-expressing expressing lines. Numbers indicate independent transgenic lines. Experiments were performed at least three times with similar results. Data are shown for one representative experiment as the mean  $\pm$  SE, (A) n  $\geq$  9, (B) n  $\geq$  9. Different letters indicate significant differences (Kruskal-Wallis, *P*<0.05).

#### Supplementary Table S1

**Table S1: Primers used for analysis by PCR.** For each gene, the upper sequence represents the forward primer, the bottom sequence the reverse primer.

locus	name	primer sequence (5' to 3')	analysis
At3g04120	GAPC1	GATTCTACAATGGCTGACAAGAAGA	qPCR
		ATGAAGGGGTCGTTGACAGC	qPCR
At3g18780	ACT2	ACATTCCAGCAGATGTGGATCTC	qPCR
		GATCCCATTCATAAAACCCCAGC	qPCR
At5g14750	WER	TCGTATTGCCAAAAAGACTGGTTTA	qPCR
		TGATAAGATCCTCTTCTTGCTCGG	qPCR
At5g40330	MYB23	CTCCTCGGCAACAGATGGTC	qPCR
		GGCTTTGACGGCAGTTGAAT	qPCR
At1g11130	SCM	AATCGGGGAAGGGTCGATTG	qPCR
		TTGAGGAATTCGCCGTCACT	qPCR
At1g66800		GCGATGAAGGCATGGTATGG	qPCR
		GCAAAACTGGTCCGATCACG	qPCR
At5g03150	JKD	ATGCGCAAGGTCTATCCGAG	qPCR
		AGGGTTTGTGGAAGTCATTGGA	qPCR
At1g12560	EXP7	TGCATACCGAAGAGTGCCAT	qPCR
		AACGGCCATGCTCTTGATGT	qPCR
At3g18780	ACT2	CAAAGACCAGCTCTTCCATCG	RT-PCR
		AGGTCCAGGAATCGTTCACAG	RT-PCR
At1g08030	TPST	GGCTCTTTTGCGGAACTTGA	RT-PCR
		CTTCAATTTTCGTGCATCTCG	RT-PCR
At2g02220	PSKR1	GTTTCGGAGTTGTGCTTCTCGAG	RT-PCR
		CCAAGAGACTAACTGTTGAGTCGTTG	RT-PCR
At5g53890	PSKR2	GAGGAGACTATCAGCGGGG	RT-PCR
		GGCCTAAGCAACCTCGCTAA	RT-PCR
GFP	GFP	forward primer from PSKR1 or PSKR2	RT-PCR
		CAGATGAACTTCAGGGTCAG	RT-PCR

At1g79840	GL2	CATGGACGTGGGACAATGGA	qPCR
		CATCAGCTGAATAGCCCCGT	qPCR
At1g12560	EXP7	TGCATACCGAAGAGTGCCAT	qPCR
		AACGGCCATGCTCTTGATGT	qPCR
At5g42600	MRN1	TGCATACACTTCCACCGCAT	qPCR
		GCTCCCAAACTGGCATACCT	qPCR
At5g42590	MRO1	CGGGCACACAGGTGATCATT	qPCR
		CGTCCAACAAAATCCCAAGTTGA	qPCR
At5g42580	MRD	TCGCTTTGTCCCAGTCAACA	qPCR
		GTGCATGTGGTCGGAGTAGT	qPCR
At4g15370	BARS1	CTCGACCATGGTGGTGCTAC	qPCR
Al-910070	<i>D,</i> (1001	GAAGGAACCAGAACTCAGGGG	qPCR
At4g15360	CYP705a3	GGTGCAGTGCTTTGACTGGA	qPCR
Al-1910000		GGTTGGGTTCGAAGAACGGA	qPCR
At4g15350	CYP705a2	TGAATGAGGCTGCTGGAACA	qPCR
		AAGCAAGCTGACATCCCCAA	qPCR
At5g48010	THAS	GGTTAGAGTGGCTTAGTCCAGTG	qPCR
		CCCTGGAAACTGTTTGTTAAACTGA	qPCR
At5g48000	ТНАН	GTTACACAATTCCAGCGGGC	qPCR
		GCAACTCTTTCCCCTCCCAT	qPCR
At5g47990	THAD1	GAATGTCTCCTCTCGCCCTC	qPCR
		AGCAGCTTTTGGACCATGAAC	qPCR