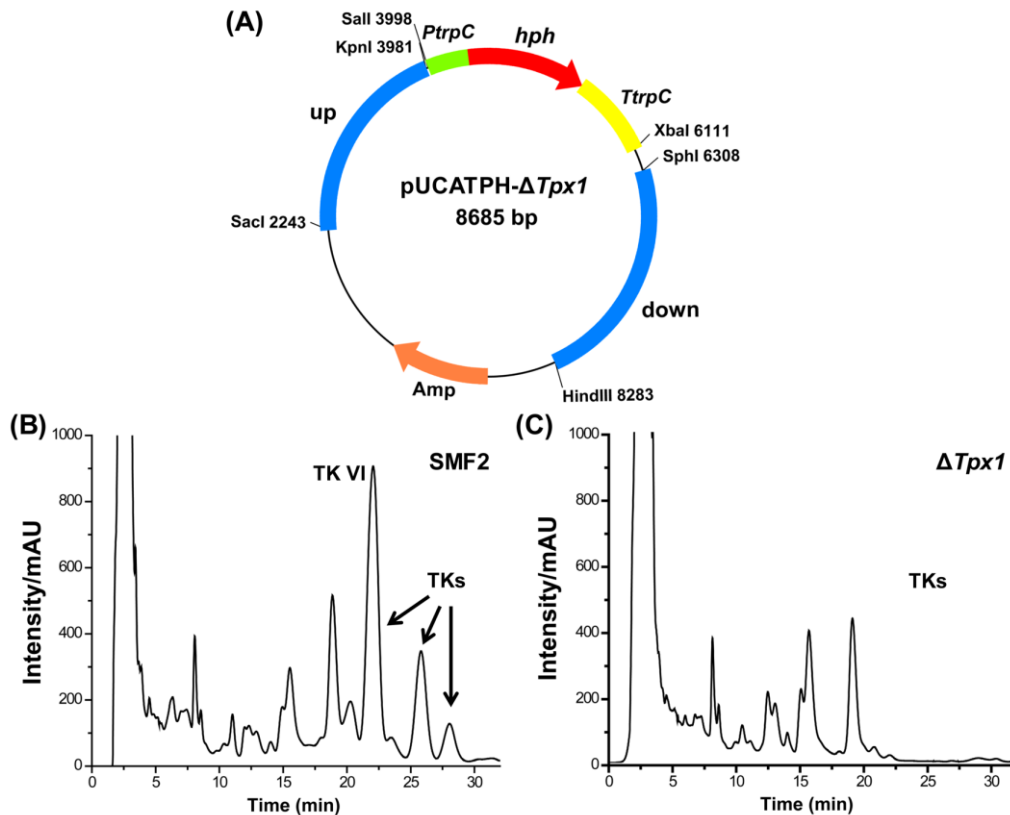


## Supplementary data

### **Cellular and Molecular Insight into the Inhibition of Primary Root Growth of *Arabidopsis* Induced by Peptaibols, a Class of Linear Peptide Antibiotics Mainly Produced by *Trichoderma* spp.**

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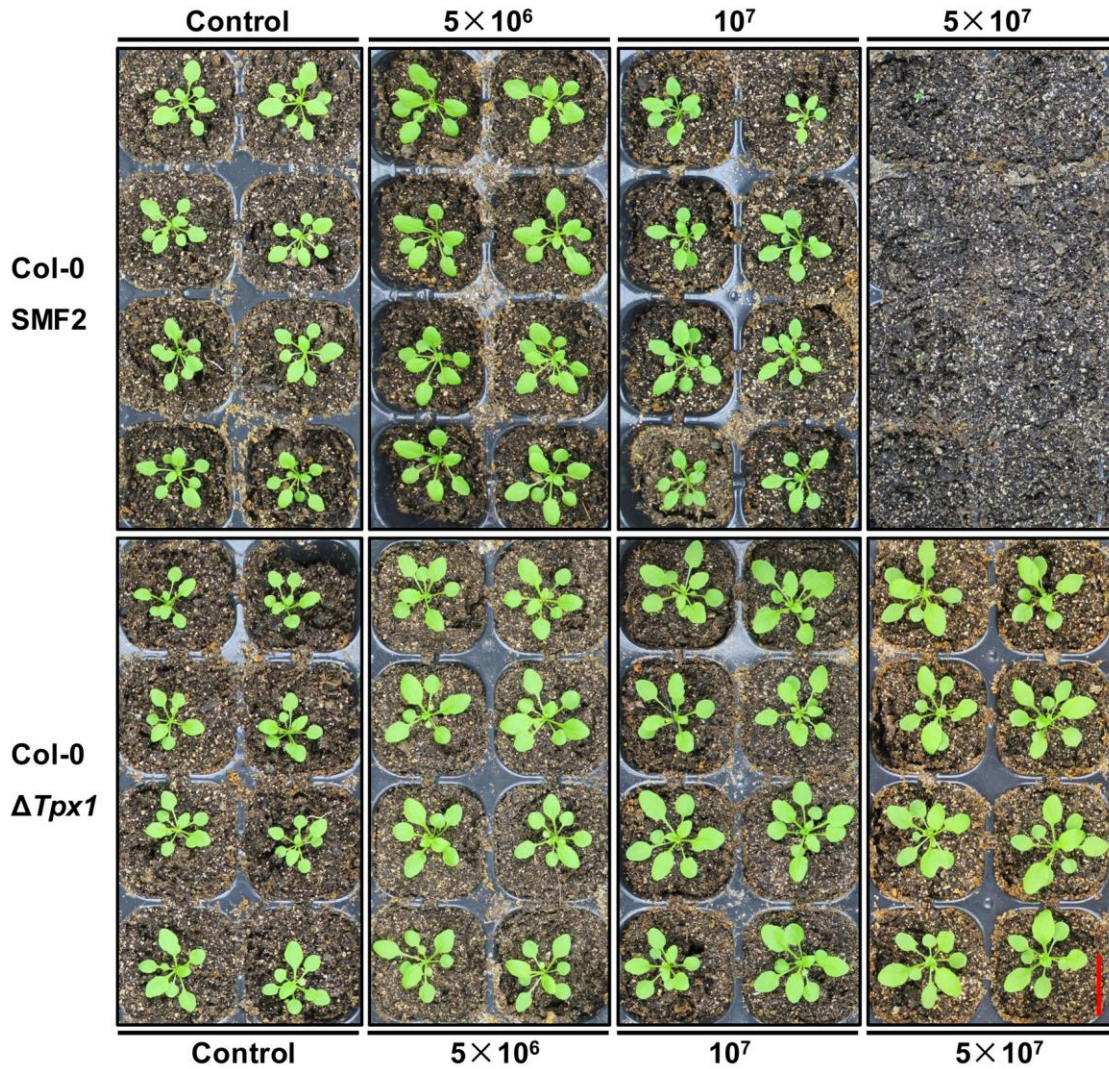


**Fig. S1.** Construction of the  $\Delta Tpx1$  mutant by knocking out the *Tpx1* gene that encodes the NRPS responsible for TKs biosynthesis in SMF2.

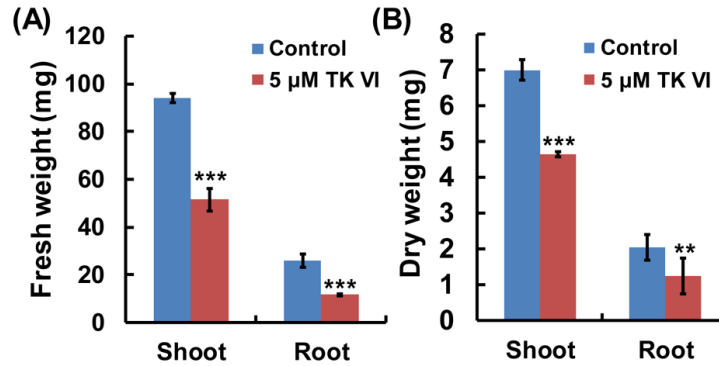
(A) Vector constructed for *Tpx1* knockout. To obtain the mutant with inactivated *Tpx1*, a 1021 bp region of *Tpx1* was replaced by the hygromycin B phosphotransferase (*hph*) gene. To this end, 1739 bp and 1980 bp of the upstream and downstream regions of the *Tpx1* deletion region were amplified and inserted into the pUCATPH vector, respectively. The constructed vector pUCATPH- $\Delta Tpx1$  was transformed into the SMF2 protoplasts to generate the  $\Delta Tpx1$  strain.

(B) TKs production in wild-type SMF2 analyzed by HPLC.

(C) TKs production in  $\Delta Tpx1$  analyzed by HPLC. The TKs are not detectable in  $\Delta Tpx1$  by HPLC.



**Fig. S2.** *Arabidopsis* (Col-0) seedlings grown in soil supplied with different concentrations of spores from the wild-type SMF2 or the  $\Delta Tpx1$  mutant. Eight-day-old Col-0 seedlings were transplanted to soil without (Control) or with the indicated concentrations of spores from wild-type SMF2 and  $\Delta Tpx1$ , respectively, and grown for an additional 2 weeks. Bar = 2 cm.

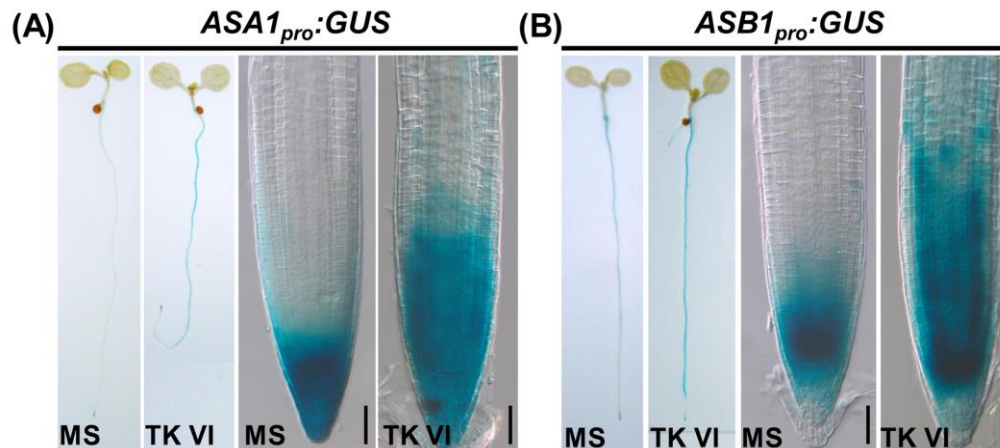


**Fig. S3.** Biomass loss in TK VI-treated *Arabidopsis* (Col-0) shoots and roots.

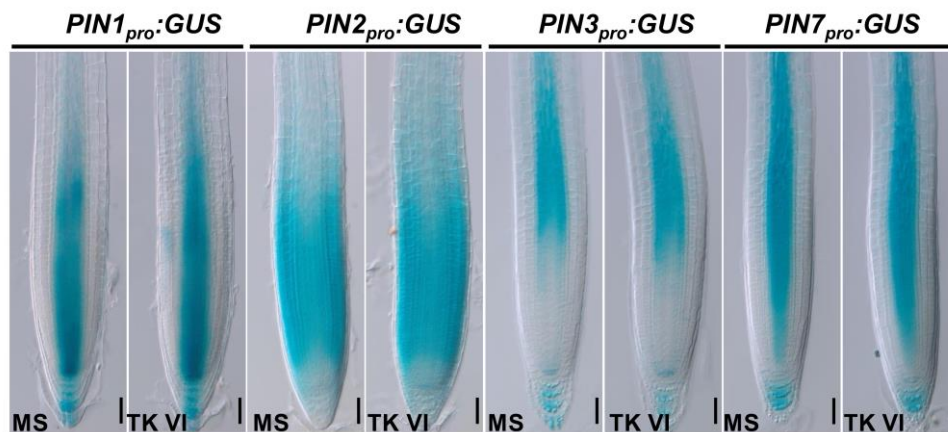
(A) Fresh weight of the shoots and roots of 5-DAG Col-0 seedlings grown on medium without (Control) or with 5 μM TK VI.

(B) Dry weight of the shoots and roots of 5-DAG Col-0 seedlings grown on medium without (Control) or with 5 μM TK VI.

For (A, B), Col-0 seeds were grown on medium without or with 5 μM TK VI for 5 DAG before their shoots and roots were cut apart and weighed immediately (A) or weighed after drying at 65°C for 3 d (B). 30 seedlings were weighted at a time and the error bars represent the SD of triplicate measurements. The asterisks denote Student's *t* test significance compared to untreated plants: \*\**P* < 0.01 and \*\*\**P* < 0.001.

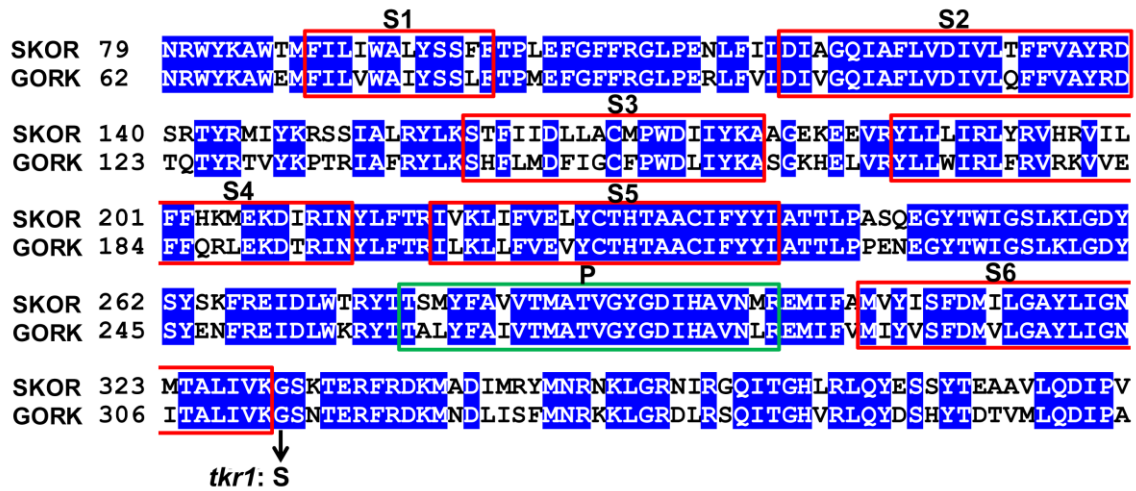


**Fig. S4.** TK VI-induced expression of *ASA1* and *ASB1* analyzed by promoter-GUS reporters. Six-day-old *ASA1<sub>pro</sub>:GUS* (A) and *ASB1<sub>pro</sub>:GUS* (B) seedlings were transferred to medium without (MS) or with 5  $\mu$ M TK VI for 12 h (*ASA1<sub>pro</sub>:GUS*) or 3 h (*ASB1<sub>pro</sub>:GUS*) before the GUS staining assays. Bars = 50  $\mu$ m (A, B).

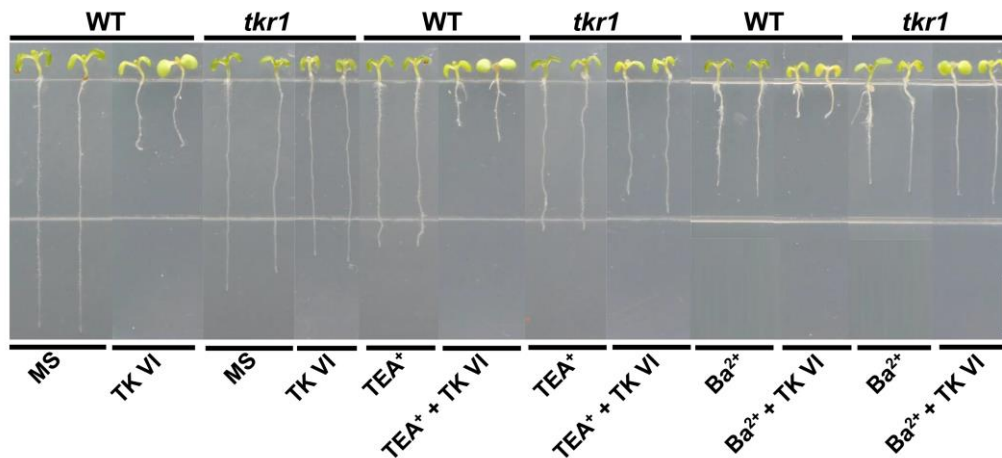


**Fig. S5.** TK VI effect on the expression of the auxin efflux transporter genes. Six-day-old *PIN1<sub>pro</sub>:GUS*, *PIN2<sub>pro</sub>:GUS*, *PIN3<sub>pro</sub>:GUS* and *PIN7<sub>pro</sub>:GUS* seedlings were transferred to medium without (MS) or with 5  $\mu$ M TK VI for 3 h before the GUS staining assays. Bars = 50  $\mu$ m.

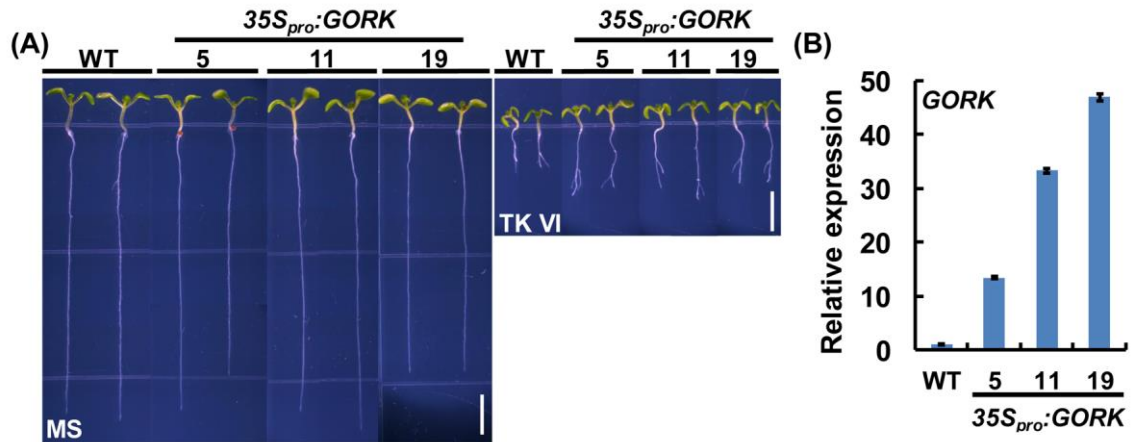




**Fig. S6.** Location of GORK Gly<sup>313</sup> by amino acid sequence alignment with SKOR. Sequence alignment was performed with Clustal X program. Amino acid residues surrounded by blue are identical between SKOR and GORK. S1-S6 are indicated in red boxes. The green box represents the P domain.



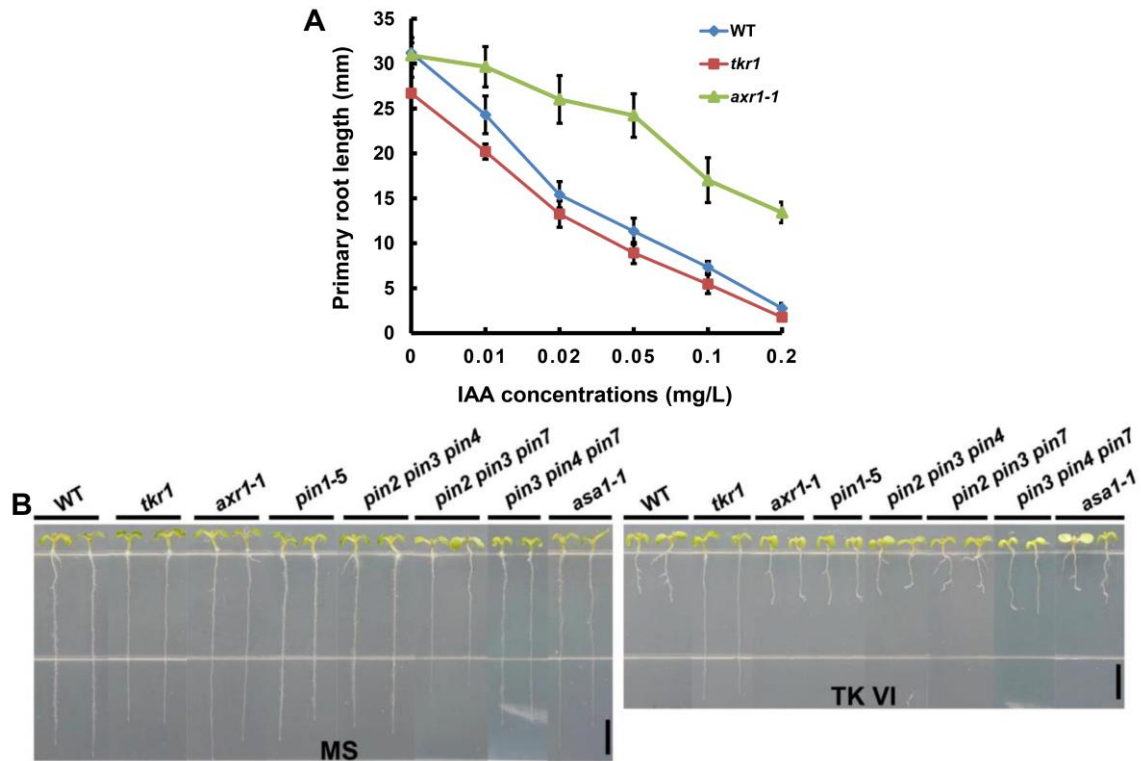
**Fig. S7.** Effect of K<sup>+</sup> channel blockers on TK VI-induced inhibition of primary root growth in the wild type *Arabidopsis* (Col-0) and *tkr1* seedlings. The wild-type (WT) and *tkr1* seeds were germinated on medium without or with 3 μM TK VI in the presence of 4 mM TEA<sup>+</sup> or 4 mM Ba<sup>2+</sup> for 6 DAG. Bar = 5 mm.



**Fig. S8.** Phenotyping of independent  $35S_{pro}:GORK$  transgenic lines upon TK VI treatment.

(A) Wild type (WT) and several independent  $35S_{pro}:GORK$  transgenic lines grown on medium without (MS) or with 3  $\mu$ M TK VI at 7 DAG. Bars = 5 mm.

(B) qRT-PCR analysis of *GORK* expression in the indicated seedlings. Ten-day-old seedlings were harvested for RNA extraction and qRT-PCR analysis. The transcript level of *GORK* in the wild type (WT) was arbitrarily set to 1. The error bars represent the SD of triplicate reactions.

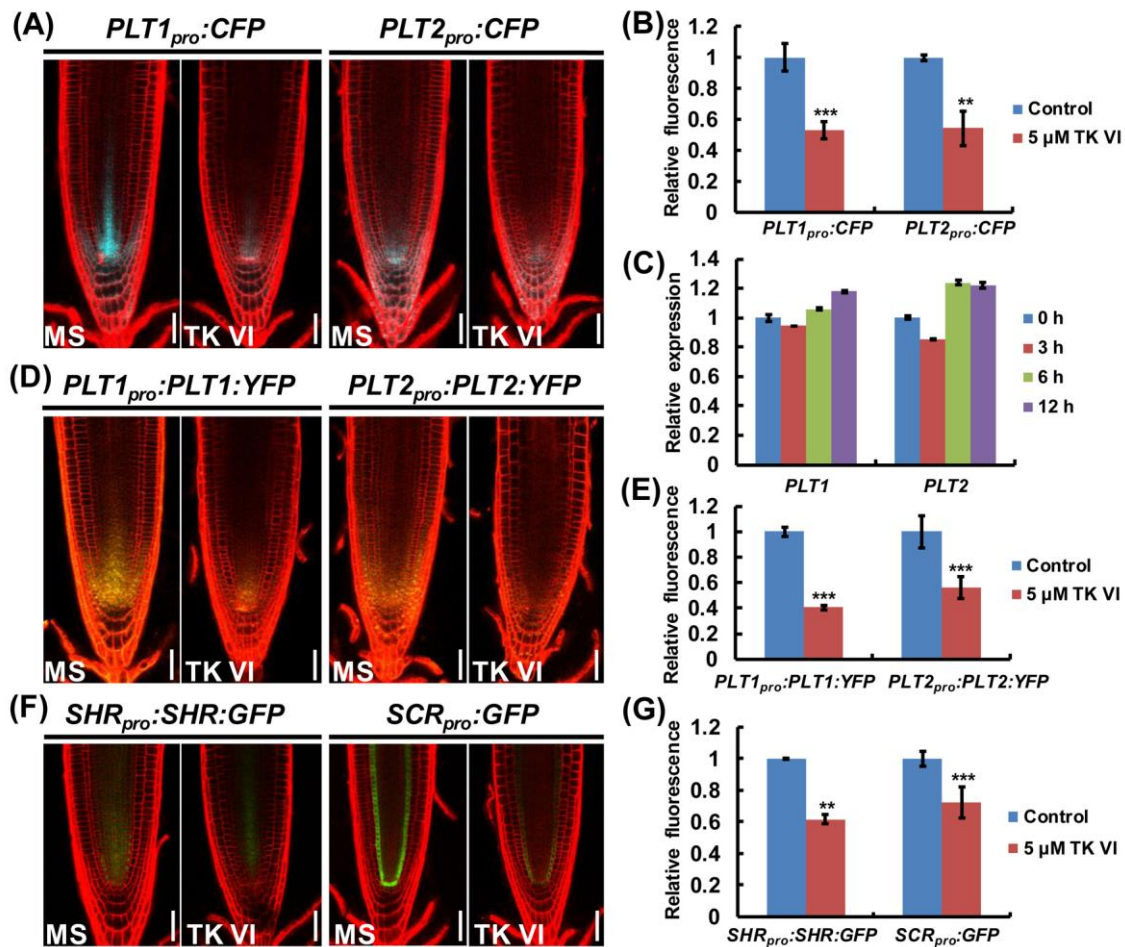


**Fig. S9.** The auxin resistant ability of *tkr1* and phenotyping of auxin-related *Arabidopsis* mutants upon TK VI treatment.

(A) Primary root length of 5-DAG wild type (WT), *tkr1* and *axr1-1* seedlings grown on medium with 0-0.2 mg/L IAA (Sigma, purity  $\geq$  98%). Data shown are averages with SD ( $n > 20$ ).

(B) Phenotyping of auxin-related *Arabidopsis* mutants upon TK VI treatment. The indicated seedlings were grown on medium without (MS) or with 3  $\mu$ M TK VI for 6 DAG. Bars = 5 mm.





**Fig. S10.** TK VI-induced repression of the expression of *PLT1/PLT2* and *SHR/SCR*.

(A) TK VI reduces the expression of *PLT1<sub>pro</sub>:CFP* and *PLT2<sub>pro</sub>:CFP* in Col-0.

(B) Quantification of the CFP fluorescence shown in (A).

(C) Time-course expression of *PLT1* and *PLT2* in response to TK VI treatment. Six-day-old Col-0 seedlings were transplanted to medium without or with 5  $\mu$ M TK VI for the indicated time periods, and the 2-mm root tips were harvested for RNA extraction and qRT-PCR analysis. Transcript levels of *PLT1* and *PLT2* were normalized to *ACTIN2* expression. The transcript levels of *PLT1* and *PLT2* without TK VI treatment were arbitrarily set to 1. Results of one of three independent experiments are shown. Error bars represent the SD of triplicate reactions.

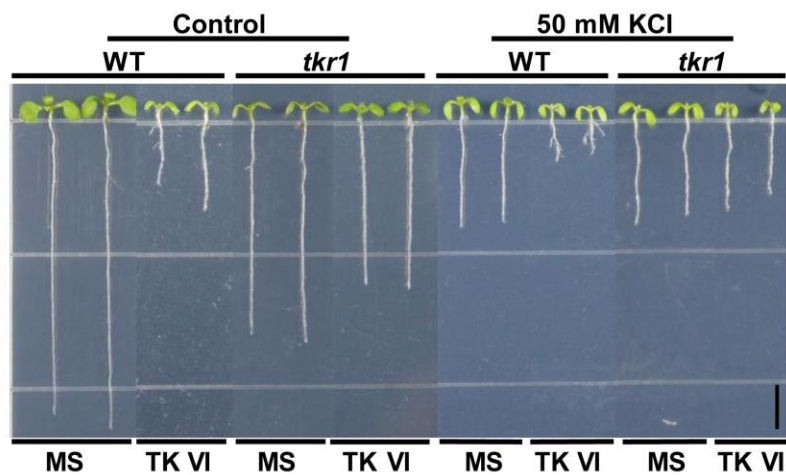
(D) TK VI-induced repression of the expression of *PLT1<sub>pro</sub>:PLT1:YFP* and *PLT2<sub>pro</sub>:PLT2:YFP* in Col-0.

(E) Quantification of the YFP fluorescence shown in (D).

(F) TK VI-induced repression of the expression of *SHR<sub>pro</sub>:SHR:GFP* and *SCR<sub>pro</sub>:GFP* in Col-0.

(G) Quantification of the GFP fluorescence shown in (F).

For (A, B, D, E, F, G), six-day-old seedlings were transplanted to medium without (MS/Control) or with 5  $\mu$ M TK for 24 h before the CFP/YFP/GFP fluorescence was monitored. Data shown are averages with SD (n = 15). The asterisks denote Student's *t* test significance compared to untreated plants: \*\*P < 0.01 and \*\*\*P < 0.001. Bars = 50 $\mu$ m (A, D, F).



**Fig. S11.** The effect of 50 mM external K<sup>+</sup> on TK VI-induced inhibition of root growth. Wild type (WT) and *tkr1* seedlings were grown on the indicated medium for 7 DAG.

**Table S1.** DNA primers used for qRT-PCR assays.

<b>Primer name</b>	<b>Sequence</b>
<i>ACT2</i> -Forward:	5'-TTGACTACGAGCAGGAGATGG-3'
<i>ACT2</i> -Reverse:	5'-ACAAACGAGGGCTGGAACAAG-3'
<i>CYCB1;1</i> -Forward:	5'-CCGGAACCTGAATCTGCTTAGG-3'
<i>CYCB1;1</i> -Reverse:	5'-GCGACTCATTAGACTTGTTCA-3'
<i>CYCB1;4</i> -Forward:	5'-ACGTGGAATCGCAGGTGAAATC-3'
<i>CYCB1;4</i> -Reverse:	5'-AGCCTTGCTTCGAGCTCTTAAG-3'
<i>CYCA1;1</i> -Forward:	5'-TCACTAGTAGCTGCTTCCGCCATT-3'
<i>CYCA1;1</i> -Reverse:	5'-ACACATCCTCTCAACTCCATCGCT-3'
<i>CYCD3;1</i> -Forward:	5'-CATCGTTGAACAGTCCAAGCTGC-3'
<i>CYCD3;1</i> -Reverse:	5'-TACGATTGCCCATGGCAGATGC-3'
<i>E2FA</i> -Forward:	5'-AATGGGCGAAATAGCACCAACAGC-3'
<i>E2FA</i> -Reverse:	5'-TTGTATGGAACGCACCTGCCATTG-3'
<i>MCM3</i> -Forward:	5'-AACACCAAGTGGACGTAGAGGCAA-3'
<i>MCM3</i> -Reverse:	5'-TTCAATCCTTGCTGCAGAGACCGT-3'
<i>PCNA1</i> -Forward:	5'-TGGGTTACATTCGTTACTAC-3'
<i>PCNA1</i> -Reverse:	5'-ATACAAAGGAATCTCACCA-3'
<i>CDKB1;1</i> -Forward:	5'-AGATGGTTCGGAGGCAAGCTCTTT-3'
<i>CDKB1;1</i> -Reverse:	5'-TAGGGTAAACATGCCAGTCACGCA-3'
<i>KRP1</i> -Forward:	5'-GAGAAGGCGAAATTGATGACG-3'
<i>KRP1</i> -Reverse:	5'-TCTAATGGCTTCTCCTTCTCG-3'
<i>KRP2</i> -Forward:	5'-AGAGATCTGGAAGGTGACGTCGTA-3'
<i>KRP2</i> -Reverse:	5'-AATTTCTCGCCACAATTCCACCGC-3'
<i>E2FC</i> -Forward:	5'-TCCCACGGTTTCAGAACCAGACAT-3'
<i>E2FC</i> -Reverse:	5'-CAACTTGTCGCTTGTTTCCGCACT-3'
<i>IAA2</i> -Forward:	5'-AGTCAACGAGCTTAACCTTAAG-3'
<i>IAA2</i> -Reverse:	5'-TCACGAGTTTCCTCAAATAGAC-3'
<i>ASA1</i> -Forward:	5'-GTAGAGAAGCTTATGAACATCGA-3'
<i>ASA1</i> -Reverse:	5'-GGTGCACCACTAACTGTTCCCAC-3'
<i>ASB1</i> -Forward:	5'-GGGGAAGAGTCGTAGAGATGTCT-3'
<i>ASB1</i> -Reverse:	5'-CTGGCAGAGATTGTATGTGAAGC-3'
<i>PAT1</i> -Forward:	5'-ATGGTTATTGCGGTGGCGACGA-3'
<i>PAT1</i> -Reverse:	5'-ATCGTCGCCGACTCAATGTCGG-3'
<i>PAI1</i> -Forward:	5'-CATCAGCCAGAGATGCAGCTA-3'
<i>PAI1</i> -Reverse:	5'-CAGAGGAATCAGCTGCTCTCA-3'
<i>TSA1</i> -Forward:	5'-CAGGCTGCGGCAACAAGGTCGT-3'
<i>TSA1</i> -Reverse:	5'-CAACAGCTCTGATGCTGGACAT-3'
<i>TSB1</i> -Forward:	5'-CTCACACGCACTAGCTTACCTC-3'

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<i>TSB1</i> -Reverse:	5'-CATCAAGATATTTAGCCACTGT-3'
<i>SUR2</i> -Forward:	5'-CTCCCTTACCTAAAGGCAGTCA-3'
<i>SUR2</i> -Reverse:	5'-GCGTTCACCTGAATGATGGTCT-3'
<i>SUR1</i> -Forward:	5'-CGAGACCACCAAGGTGTTACAA-3'
<i>SUR1</i> -Reverse:	5'-TCAACATTATGTTTGAGTATCT-3'
<i>NIT3</i> -Forward:	5'-AGCGAAGTTGGTGTGTTTCCC-3'
<i>NIT3</i> -Reverse:	5'-CCAACCTCAGCCAATCTTTCCAC-3'
<i>CYP79B2</i> -Forward:	5'-CACGATGATGCTCGCGAGACT-3'
<i>CYP79B2</i> -Reverse:	5'-TCACTTCACCGTCCGGTAGAGA-3'
<i>CYP79B3</i> -Forward:	5'-CGTGATCCCTGTGACATGTCCT-3'
<i>CYP79B3</i> -Reverse:	5'-CACGCAGGTTTTGTAGCCGTTA-3'
<i>PLT1</i> -Forward:	5'-GAAGATGGCAAGCAAGGATCGG-3'
<i>PLT1</i> -Reverse:	5'-TCACGTCGTACCGGTTGATCTC-3'
<i>PLT2</i> -Forward:	5'-CAGACGCAGCTTCATCTTCACC-3'
<i>PLT2</i> -Reverse:	5'-ATCCAACGGTAGAGCTTGACCC-3'

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**Table S2.** Genetic analysis of the *tkr1* mutant.

Cross (♀×♂)	Generation	Total seedlings tested	WT	TK VI-resistant <sup>a</sup>	$\chi^2$
WT× <i>tkr1</i>	F1	20	0	20	/
WT× <i>tkr1</i>	F2	568	136	432	0.3380 <sup>b</sup>

<sup>a</sup>TK VI-resistant phenotype was determined by primary root length after treated with 3  $\mu$ M TK VI for 6 DAG.

<sup>b</sup>The calculated value was based on the expected ratio of TK VI-resistant seedlings over wild-type (WT) seedlings equals 3:1,  $P > 0.05$ .