Supplementary information for:

A phosphorylation-based switch controls TAA1-mediated auxin biosynthesis in plants

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Supplementary Figure 1. Immuno-precipitation of *in vivo* TAA1-GFP proteins for mass spectrometric analysis.

(a) Procedure for collection of TAA1-GFP proteins.

(**b**) Image of SDS-PAGE gel stained by Coomassie brilliant blue (CBB). Immunoprecipitation result showed a clear band of TAA1-GFP proteins as the arrow indicated.



Supplementary Figure 2. T101 mutation influences TAA1 binding with PLP.

(a) Transaminase activity detected by measuring IPA production with Salkowski reagent (see methods). Transaminase catalytic reactions were performed in the reaction reagent without PLP using about 2.5 μ g recombinant TAA1-His proteins. The IPA products were measured by reading the absorbance at 530 nm at different time points. Only *E.coli*-purified TAA1^{WT} exhibited normal enzymatic activity without exogenous PLP supply. Values denoted the mean \pm s.d. (n=three biological independent repeats).

(**b**) Transaminase activity detected by measuring IPA production with Salkowski reagent. Transaminase catalytic reactions were performed in the reaction reagent with different concentrations of PLP. *E.coli*-purified TAA1^{T101A} showed increased enzymatic activity when adding sufficient PLP. Values denoted the mean \pm s.d. (n=three biological independent repeats).

(c) PLP detection through measuring the absorbance at 388 nm (see method). The *E.coli*purified TAA1^{WT/T101A/T101D/K217A} proteins were denatured at 95°C to release PLP from proteins. The supernatant containing PLP was obtained by centrifuging and used for PLP detection by reading absorbance at 388 nm (see methods). The data showed that only *E.coli*-purified TAA1^{WT} carried a certain amount of PLP from *E.coli*. Three repeats showed similar results.



Supplementary Figure 3. Auxin regulates root development.

(a) Representative pictures of root apical meristem from 5-day-old seedlings. White arrows show meristem zone, scale bar, 50 μ m. Around 3.19% (3/94: 3 among 94 seedlings showed strong phenotype) of *wei8-3;tar2-1* displayed collapsed root meristem.

(b) Quantification of root meristem size in (a). n denotes number of independent seedlings.

(c) Representative pictures of root hair from 5-day-old seedlings and quantification data. Col-0: n=20; Col-0+NAA: n=8; Col-0+L-Kyn: n=14; *wei8-3;tar2-1*: n=13; *wei8-3;tar2-1*+NAA: n=3. n denotes number of independent seedlings and x represents number of root hairs used for quantification. Scale bar, 1 mm. 20 nM NAA (sigma N0640) or 1.5 μ M L-Kyn (sigma K3750) was added into medium for treatment. One-way ANOVA with Tukey multiple comparisons test was used in (b) and (c). Different letters represent significant difference between each other, P < 0.05(b), P < 0.0001(c).



Supplementary Figure 4. T101 of TAA1 is essential for root hair development. Representative pictures of root hair from 5-day-old seedlings and quantification data. Col-0: n=20; *wei8-3*: n=21; *TAA1^{WT}*; *wei8-3*: n=19; *TAA1^{T101D}*; *wei8-3*: n=20; *TAA1^{T101A}*; *wei8-3*: n=35. n denotes number of independent seedlings and x represents number of root hairs used for quantification. Two independent lines of each transgenic plant showed similar results. Scale bar, 0.5 mm. One-way ANOVA with Tukey multiple comparisons test was used. Different letters represent significant difference between each other, *P*<0.0001.



Supplementary Figure 5. T101A is not fully functional *in vivo*.

(a) Quantification of root meristem size of pTAA1- $TAA1^{T101A}$; wei8-3 transgenic plants. n denotes number of independent seedlings. *P*-values were shown as indicated. One-way ANOVA with Dunnett multiple comparisons test was used comparing with Col-0. *P*<0.05 indicates a significant difference.

(**b**) Quantification of root meristem size of pTAA1- $TAA1^{T101A}$; wei8-3; tar2-1 transgenic plants. n denotes number of independent seedlings. *P*-values were shown as indicated. One-way ANOVA with Dunnett multiple comparisons test was used comparing with Col-0. *P*<0.05 indicates a significant difference.

(**c**, **d**) Western blot showed TAA1-GFP protein levels of different transgenic plants used in (a) and (b). 5-day-old seedlings were used for phenotypic analyses and proteins extraction. TAA1-GFP proteins were detected by anti-GFP antibodies (HT801, TransGen Biotech). (e) Quantification of root meristem size of *pTAA1-TAA1^{T101A};wei8-3* treated with 5 μ M PLP. 5-day-old seedlings grown on 1/2 MS plates with or without 5 μ M PLP were used for phenotypic analysis. n denotes number of independent seedlings. *P*-values were shown as indicated. Two-sided *t*-test. Three independent repeats.



Supplementary Figure 6. TAA1 and TAR1 form heterodimers in vivo.

Co-IP assay showed interactions between TAA1 and TAR1 in Col-0 protoplast. 35S-TAA1-Flag and 35S-TAR1-GFP were transiently co-expressed in *Arabidopsis* protoplasts, single transformation of 35S-TAR1-GFP was used as a negative control. Three independent repeats with similar results.

					T101				
Marpo.0032s0124/188-253	- Q F	FFVQTELDQVIRSLI	IDVIGN.	AVT - EGRHIV	IGVGSTQL	FQAALYAI	S P P D	• • • • R A T	P T K V V S V A P F Y S
Phypa.17 6500/206-272 Phypa.18 15140/174-239	N S Y	LF VD SF LEQTIRQLI VEMENELELOIRAL	IGMIGN.	A V T - E G R F L V A V T - F G R H I V	LGVGSTQL	YQAAL YAI	T S P D	S P T	P T S V V S A I P H Y S P T N I V S A A P F Y S
Phypa.21 15370/173-238	- H V	WFMENELEVQIRELI	RVVGN	AVT - EGRHIV	VGTGSTQL	FQATLYAI	T S S D	Q SK	PTNIVSAAPFYS
Phypa.26 12520/84-149	GCP	WF VSALLDDAIRELI	ISFVGN	AVT - GDRAIV	VGNGSTQL	FQAALYAI	ATRD	G T :	S T P V V S E A P F Y S
Sphfa.0021s0188/293-368	NAF	LFVDPYLEQAIRELI	SLVGN	AVT - KNRHIV	VGVGSTQL	FQAAIYAI	VSLQQPQAAA	AANSSTT	PTDIVSAVPYYS
Sphfa.0026s0096/295-361	VAV	VFMEHELDVQIRALI	AVVGN	AIT-EGRHIV	VGTGSTQL	YQAALYAI	SSPE	Q S K	P T S V I S A A P Y Y S
Sphfa.00/6s0008/210-2// Selmo.171289/31-97	HFL	WFMEHELEVQIKALI WFVEOELENEIROLI	HAIVGN.	A V T - D G R F I V	VGTGSTQL	FOAALYA	VSPRD	EQIK	P A S V V S A A P F Y S P A S V V S A V P Y Y S
Pinab.2120g0020/20-86	NVC	WF LE PELAKEIRS LI	SLVGN	AVT-KGRHLV	VGTGSTQL	FQAALYAI	S P P G	R S K	P T N V I S A A P Y Y S
Pinta.000019549/212-278 Pinta.000027613/206-272	NVC	WFLEPELAKEIRSLI WFLEPELAKEIRSLI	ISLTGN.	AVT - KGRHIV	VGTGSTQL	FQAALYAJ	SPPG	R S R	P T N V I S A A P Y Y S P T N I I S A A P Y Y S
Pinta.000027966/208-274	NIC	WFLEPELAKEIRSLI	ISLVGN	AVT - KGRHIV	VGTGSTQL	FQAALYAI	SPPG	R S T	PTNVISAVPYYS
Anaco.027902/125-194	ALC	WFLEPEFAAEVRRLI	IRLVGN	A V S G D G R H V V	VGTGSTQL	FQAALYAI	SEGGDA	· - RD P	P A S V V S A A P Y Y S
Musac.10G20770 001/148-214	NLC	WFLEPEFASEVRRLI	INLVGN	AV-ADGHFII	VGTGSTQL	FQAALYAI	SPPDA	P E	PMSVVSAVPYYS
Musac.3G20730 001/149-216	NLC	WFLEPEFAHQVRRL	ISLVGN	A V V D D G R F I I	VGTGSTQL	FQAALYAI	SPPDA	A E J	PMN VVSA I PYYS
Musac.6G08900_001/65-131 Spipo.19G0018400/129-195	GLC	WF LQP EFAHEAKKL	INL VGN	A V - A D D K F L V A E - T E G R H I V	VGTGSTOL	FOAALYAI	SPSDA		P M S V V S A V P Y Y S P V S V V S A A P F Y S
Zosma.1g02950/59-125	NMC	W F L E Q K F A E K V R R L I	HKLVGN	ASV-DGKHVI	VGTGSTQL	FQAALFAI	S D P N S	DH	PTNVVTSIPYYP
Zosma.209g00410/77-143 Bradi 2004290/167-234	NVC	WFLERGFEEEVRRL WFLEPFFGROVRRL		A V V - D G K H I I A A A G D F Y H V V	VGTGSTQL	FQAALFAI	SDPNS	DQ	P A N V V S S V P Y Y S P I S I V S T A P Y V S
Bradi.2g34400/104-173	GLC	WFLEPGLEREVRRLI	IRLVGN	AAT-EGYHVL	VGTGSMQL	FQAALYAI	CSPAPV	G S - D G	PVSVVSPAPYYS
Orysa.01g07500.1/175-245	NVC	WFLEPELDRQVRRLI	IRVVGN	A - A VDG YH VL	VGTGSTQL	FMAALYAI	APDAAA	AAAGE	PISVVSTAPYYS PMNVVSDAPYYS
Seita.3G100400/104-170	GFC	WF VEPGFEREVRRL	IRL VGN	AVV - EGYHLL	VGTGSTQL	FQAALYAI	S P A	ED - GK	PMSVVSPAPYYS
Seita.3G100500/180-247	ALCI	WFLEPEFEREVRRL	HRVVGN	AVV-DGYHLV	VGTGATQL	YQAAMYAI	SSPARG	D K	P V P V V S P A P Y Y S
Setta.5G119600/215-284 Seita.6G075800/112-178	GEC	WFLEPGFDHEVKKLI WFVEPGFEROVRRLI	IRLVGN	A I V - DG YH VL A I V - DG YH L L	VGTGSTQL	FOAALFAI	SPPAAA	GAG - A	PMSVVSTAPYYS PMSVVSPAPYYS
Sobic.003G052700/214-284	NVC	WF LEPGLDHEVRRLI	IRLVGN	AAV-DGYHVL	VGTGSTQL	FMAALYAI	SPPAGS	A A G A A	P <mark>M S</mark> V V S T A P Y Y S
Sobic.009G060100/112-178	GIC	WFVEPGFEREVRRLI WELEPELEPEVPPLI	RLVGN	AMV - DGYHVL	VGTGSTQL	FQAVLYAI	SPA	SD - GT	PMN VVSPAPYYS
Maize.2G066345 /209-279	NVCI	WF LEPGLDQEVRRV	IRLVGN	AAV-DGYHVV	VGTGSTQL	FMAALYAI	SPPAGA	- GAGAA	PMSVVSTAPYYS
Maize.2G127160/110-176	GIC	WFLEPGFEREVRRL	IRLVGN	AVV - DGYHVL	VGTGSTQL	FQAVLYAT	SP A	SD - GT	PMN VVSPAPYYS
Maize.2G12/308_/210-2/6 Maize.2G141810_/110-177	VLC	WFLEPGLDHEVKKLI WFLEPELEREVRRLI	IRLVGN	A V V - D G Y H V L	VGTGATOL	YOAAMYAI	SPPA	D O	P V P V V S P A P Y Y S
Aquco.3G274600/71-138	NLC	WFLEPEFAEEIQRLI	INVVGN	AVT - EDRYII	VGTGSTQL	FQAALYAI	S S P E V	S S E	P L N V V S A V P Y Y S
Aqueo.3G276300/31-98 Aqueo.3G276800/145-211	N L C	WFLEPEFAEEIQRLI	INVVGN.	AVT - EDRYII	VGTGSTQL	FQAALYAI	S S P E V	· S S E	P L N V V S A V P Y Y S P I S V V S A A P V V S
Solyc.03g112460/119-185	TVC	WFLEPGFANAVTRL	KLVGN	AET-GNRHIV	VGTGSTQL	FQAVLYAI	C - P YD	A P E	PMSIVSAAPFYS
Solyc.05g031600/69-135	SLC	WFME SKLEEQIKRL	INIVGN	AIV-DDYYIV	VGTGSSQL	MQAALYAI	S - P T D	QLE	P V S V V S A T P F Y S
Soltu.400007680/31-97	SLC	WFLESKLEEQIKRLI	INVVEN	AIV-DDYYIV	VGTGSSQL	MQAALYAI	S - P T D	QLE	PISVVSATPFYS
Soltu.400007681/69-135	SLC	WFLESKLEEQIKRLI	INVVGN	AIV-DDHYTI	VGTGSSQL	MQAALYAI	S - P I D	QLE	PINVVSATPFYS
Soltu.400018110/118-184 Soltu.400025405/69-135	SLC	WFLEPGFANAVIRLI WFLESKLEEOIKRLI	INVVGS	A I V - DDHYIV	VGTVSSOL	MOAALYAI	S - P T Y	OLE	PISVVSATPFYS
Soltu.400027079/111-177	NHC	WF L E <mark>P G F A N A V T R</mark> L I	INLVGN	AQT-RNYHIV	VGTGSTQL	FQAALYAI	<u>S</u> - P S N	A P E J	PMSIVSASPFYS
Vitvi.G01004095001/81-147 Vitvi.G01006656001/81-147	K V C	VFLEKELEEAIRQLI VFLEKELEEAIROLI	IRTVGN	AATD-DRYIN AVTD-DRHIL	VGTGSTQL VGTGSTQL	FQATLYAI YHAALYAI	T S P G G	PE	S VN V V SA V P Y Y S P VN V V SA V P Y Y S
Vitvi.G01007679001/140-206	NLC	WFLEPEFAKQIIRLI	KIVGN	AVT - DNYYIV	VGTGSTQL	FQAALYAI	S - P P N	A S E	PMSVVSAAPFYS
Potri.008G187800/41-107 Potri.010G044500/40-106	NIC	WFLQPQLGDAIKGLI	HRVVGN	AVTD-GRHIV	VGTGSTQL	LMAALYAI	SSPSA	PH	P V S L VAAAP Y Y S D V S L VAAAP F V S
Potri.012G083300 /124-190	SLC	WFLEPEFAKEIIRLI	KTVGN	AVT - EDRYIV	VGTGSTQL	YQAVLYAI	S - P Q D	A V E	PLSVVSAAPYYS
Potri.015G081900/126-192	SLC	WFLEPEFAKEIFRLI	KVVGN	AVT - EDRYIV	VGTGSTQL	YQAVLYAI	S - P L D	A A E	PLSVVSAAPYYS
Citcl.10020085/138-204 Citcl.10033774/68-134	NVC	WFLEPEFAKEVVKLI WFLVPELAEAINNLI	HGVDN	A V S D G - R H I V	IGTGSTQL	YQAAL YAI	SSPGG	A S E	P I S V V SAAPYYS P I S V V SAAPYYS
Citsi.1g012785/136-202	NLC	WF L E P E F A K E V V R L I	KVVGN	AIT-ENHHIV	VGTGSTQL	FQAALYAI	s - sq D	A S E	PISVVSAAPYYS
Citsi.1g018172/32-98 Carpa 35 70/120-186	NVC	WFLVPELAEAINNL WFLFPEFGREIVRL	HVVDN	AVSDG - RHIV	IGTGSTQL	YQAALYAI	SSPGG	P E	PISVVSAAPYYS PISVVSAVPYVS
Carpa.69.95/72-138	NIC	WFLESGLGDAIRRLI	MVVGN	AVAE - DRYIV	VGTGSTQL	YQAALYAI	<u>TFA</u> GG	P D	P VN V V S A A P C Y S
Thecc.1EG010471/71-138	NLC	WFLMPELDHAVRRLI	IRVVGN	A VADDDRFIV	VGTGSTQL	FQALLYAI	SSPDE	P E	PISVVAAAPFYS
Gosra.005G180100/71-140	HFC	WF LMPELDRAIRRLI	ICVVGN	AVVDDDRYIV	VGNGSTQL	FOAVLYAI	SSSYDP	D T E	PLSVVAAAPFYS
Gosra.006G189500/119-185	RLC	WF L E P E F A K Q I V R L I	INVVGN	AVT-ENRHIV	VGTGSTQL	FQAALYAI	S - P C A	E A E	PISVVSAAPYYS
Arath.TAA1/64-13 Arath.TAR1/65-131	NLC	WFLEPELEDAIKDLI WFLEPELEKAIKAL	IGAIGN	AATE - DRYIV AATE - ERYIV	VGTGSTQL	CQAAVHAI	SSLA	R SQ E VK	P V S V VAAA P F Y S P V S I VAA V P Y Y S
Arath.TAR2/118-184	NLC	WFLEPELAKEIVRV	KVVGN	AVT - QDRFIV	VGTGSTQL	YQAALYAI	S - PHD	D S G	PINVVSATPYYS
Araly.1G37110/64-130	NVC	WF ELEKAIKALI	GAIGN.	AATE - ERYIV	VGTGSSQL	CQAALFAI	S S L S	K VK	P V S I V V A V P Y Y S
Araly.7G28530/118-184	NLC	WFLEPELGKEIVRV	KVVGN	AAT - QDRFIV	VGTGSTQL	YQAALYAI	S - PHD	D S G	PINVVSTAPYYS
Brara.A01473 /132-198	ELC	WFLEPELAKEIVRV	KVVGN	AVT-QDRYIV	VGTGSTQL	YQAALYAI	S - PHD	D S G	P I N V V S A A P Y Y C
Brara.G02980/56-122	NMC	WFLEPELAEAIKELI	IDAVGN	AATE - DRYIV	VGTGSTOL	COAAVHAI	SLLAG	G T E	P V S V VAAAPYYS
Brara.103242/60-126	NVC	WF L RQDLAEAIKALI	RAIGN	AATE-ERYIV	VGNGSTQL	CQAALFAI	S S L S	E D K	PLSIVAAVPYYS
Brara.103244/54-120 Prune 1G248200/73-139	NUC	WFLRQDLAEAIKALI WFMFPFFFFAVRRI	RAIGN.	AATE-ERYIV AVVD-DRVIV	VGNGSSQL	CQAAL FAI	TCPGG	· - E DK	P L S I VAA V P Y Y S P V S V V S A A P Y V S
Prupe.1G248300/23-89	TIC	WFMEPEFQEAVRRL	RVVGN	AVVD - DRYIV	VGTGSTQL	YQAALYAI	T S P G G	P E I	P V S V V S A S P Y Y S
Prupe.5G168300/114-180	NPC	WFLEPEFAKQVVRLI	INLVGN	AVT - EGRQIV	VGTGSSQL	FLAALYAT	A - P K D	A S E	PISVISAAPFYS
Maldo.0000267098/125-191	NPC	WFLEPEFAKQVVRLI		AVT - NGRQIV	VGTGSSQL	FLAALYAI	A - P K D	G S E	PMSVV SAAPYYS
Maldo.0000310220/76-143	S V CI	WFLEPALEAAVRRF	H R T V G N .	A V V D G D R H I V	VGTGSTQL	YQAALYAI	T S P G R	S E I	P <mark>V S V V S A A P Y</mark> Y S
Maldo.0000616079/73-140 Medtr 30077250/119-185	NIC	NFLEPALEAA VRRLI NFLEAFFAKE VVRL	IRTVGN.	A V V D G D R H I V A V T - F G R H I V	VGTGSTQL	YQAAL YAI	TSPGG	PE	P V S V V SAAPYYS P I N V V CASPYYS
Medtr.4g105220/115-181	SIC	WF L E P E F S K E V V R L I	INVVGN	AVT - QGRHVV	VGIGSSQL	ILAALYAI	S - S P H	A T E	PISVVSAVPYYS
Medtr.5g033510/76-143 Medtr.5g032520/69.126	NMC	WFML PELRDEIERLI WYML POMPEALL PL	HLVGN	AVTK-DKYIV	VGNGSSQL	FQAALFAI	SPL-DV	P DH	PINVISPT PYYS PINVVA A A PYYS
Frave.03586/74-140	NIC	FFLEPEFEDAVRRL	RTVGN	AVVDG-HYIV	VGTGSTQL	YQAALYAI	TSPGG	A E	PISVVSAAPYYS
Frave.14327/128-194	SLC	WFLEPELAKQIIRLI	KAVGN	AVT-KGRHIV	VGTGSSQL	IMAALFAI	S - P K D	G S E	PMSVVATAPYYS
Frave.31790/109-175 Frave.31791/107-173	NLC	WFLEPEFAKQVIRL WFLEPQLAMOIIKLI	IKAVGN.	AVT - EGRHIV	IGTGSSOL	ILAALFAI	S - P A D		PMSVVSTAPFYS PMSVVSTAPFYS
Glyma.01G027400/65-131	NVC	WYML PETKEAIKRLI	HVVGN	AVTE-DRYIV	VGNGATQL	LQGAVFAI	T P S - E A	S K	PINVVVAAPYYS
Glyma.02G037600/65-132 Glyma.04G186700/124-190	NVC	WYMLPEMKEAIKRLI VELEAEFGPEVVPL	H V V G N A	AVTE - DRYIV	VGNGATQL	FLAALVA	S P S - E A	- N SQ	PINVVAA VPYYS PISVVCA TPYYS
Glyma.05G040400/129-195	NIC	WFLETEFAREVVRLI	IKVVGN	AVT - EGRHIV	VGIGSSQL	ILAALYAI	S - S P D	A A E	PISVVSAAPYYS
Glyma.06G179000 /118-184 Glyma.17G086500 /120.106	NIC	WFLEAEFAREVVRLI NFLETEFAREVVRLI	IN VVGN	AVT - EGRHIV	VGTGSSQL	FLAALYAI	S - P ID	SPE	PISVVCASPYYS PISVVSAAPVVS
oryma.170000300_1130-190						- 1. A A L - A		AAE	
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	129	+ 9 8 5 5 3 8 4 1 4 9 4 3 9	369++	+ 31 - 006599	9 * 7 + 8 8 * *	67 + 787 -	7 - 0 0	02	76799765*9*+

Supplementary Figure 7. Sequence alignment of TAA1 orthologs among 32 species. For detailed information about proteins and species, see Supplementary Table 1. T101 residue of AtTAA1 was highlighted in orange.



Supplementary Figure 8. Phosphorylation modification of Thr224 on MpTAA protein *in vivo*.

(a) PCR results showed successful transformation of constructs. 10-day-old thalli were used for genomic DNA extraction. Plasmid (35S-MpTAA-Flag) was used as a positive control. Arrows show the primers used for PCR. Primer sequences were list in Supplementary Table 2.

(**b**) Western blot results showed MpTAA-Flag protein levels of different transgenic plants. 20-day-old thalli were used for protein extraction. MpTAA-Flag proteins were detected by anti-Flag antibodies (M20008L, Abmart).

(c) Mass spectrometric analysis showed phosphorylation modification at Thr224 residue on MpTAA proteins. The mass increased 80 Da (weight of phosphate group) starting at b_{11} ion that defined the phosphorylation modification at Thr224. 3-week-old *35S-MpTAA-Flag* transgenic *Marchantia* was used to immuno-precipitate MpTAA proteins.



Supplementary Figure 9. Subcellular co-localization of TMK4 and TAA1 in roots. The pictures were taken by TCS SP8 microscopy using 5-day-old transgenic plants (pTMK4-TMK4-GFP crossed with pTAA1-TAA1-RFP). About 7 individual seedlings were observed. The upper pictures showed whole root meristem zone, Scale bar, 50 µm. Boxes indicates two regions magnified to show root epidermal cells and root tip respectively. Scale bar, 8 µm.



Supplementary Figure 10. MS analyses showing TMK4 phosphorylates T101 site on TAA1 protein.

(a) Phosphor-peptide containing T101 site was detected by mass spectrometric analysis. *E.coli*-purified TAA1 proteins were used to perform the *in vitro* kinase reaction together

with TMK4 kinase domain then sent for MS analysis to identify phosphorylation sites. The mass increased 80 Da (weight of phosphate group) starting at y_{16} ion defined the phosphorylation modification at Thr101.

(**b**) Representative mass spectrometric analysis of two non-phosphorylation peptides indicated protein amount of TAA1-GFP in Col-0 and *tmk4-1*. This was shown as the internal reference of Fig.3e.

(c) Three independent biological repeats of mass spectrometric analysis showed T101 phosphorylation level of TAA1 decreased in *tmk4-1*.



Supplementary Figure 11. TMK4 negatively regulates TAA1 enzymatic activity but not the transcription and subcellular localization of TAA1 *in vivo*.

(a) *In vivo* transaminase catalytic activity in Col-0, *tmk4-1*, *wei8-3* and *tmk4-1;wei8-3*. IPA concentration was determined with Salkowski reagent (see methods). Transaminase catalytic reactions were performed with reaction reagents containing protein extracts from Col-0, *tmk4-1*, *wei8-3* and *tmk4-1;wei8-3* roots (n=five biological repeats). *P*-values were shown as indicated, two-sided *t*-test. *P*<0.05 indicates a significant difference. Centre line in box represents mean and whiskers show minimum to maximum.

(**b**) qRT-PCR assay showed *TAA1* families gene expression level in Col-0 and *tmk4-1*. Roots from 5-day-old seedlings were used for RNA extraction. Data represented mean with s.d. from three biological repeats. *P*-values were shown as indicated, two-sided *t*-test.

(c) Subcellular localization of TAA1-GFP in Col-0 and *tmk4-1*. Images were taken from pavement cells and root epidermal cells. Scale bar, 25 μm.



Supplementary Figure 12. TMK4 negatively regulates auxin content and root development-related processes.

(a) Description of T-DNA insertion mutants of *tmk4*. Lines represent introns; black and grey boxes represent exons and untranslated regions. Arrows show primers used for *TMK4* gene expression detection. Primer sequences were listed in Supplementary Table 2.

(**b**) qRT-PCR assay showed *TMK4* expression level in different *tmk4* mutants. Data represented mean with s.d., *P*-values were shown as indicated, two-sided *t*-test.

(c) Western blot results showed TMK4 protein level in *tmk4* mutants detected by TMK4 antibody. The red asterisk denotes a non-specific band.

(d) Representative images of root meristem from 5-day-old seedlings in *tmk4* mutants and pTMK4-gTMK4-GFP;tmk4-l complementation transgenic lines. White arrows show the meristem zone; scale bar, 50 µm.

(e) Quantification of root meristem size in (d). n denotes number of independent seedlings. One-way ANOVA with Tukey multiple comparisons test was used. Different letters represent significant difference between each other, P < 0.0001.

(f) Representative pictures of root hair from 5-day-old *tmk4* mutants and complementation transgenic lines (n=31) and quantification data. n denotes number of individual seedlings and x represents number of root hairs used for quantification. Scale bar, 0.5 mm. 3 independent complementation transgenic lines showed similar results. One-way ANOVA with Tukey multiple comparisons test was used. Different letters represent significant difference between each other, P<0.0001.

(g) Free IAA quantification of 8-day-old seedlings in *tmk4-1* and complementation transgenic line. Values indicated mean \pm s.d. from three individual biological repeats. *P*-values were shown as indicated, two-sided *t*-test.



Supplementary Figure 13. TAA1 contributes to overproduced auxin in *tmk4-1*.

(a) Quantification data and statistical analysis of DR5-GUS activity in different mutants. Six independent biological repeats (n) were performed. Centre line in box represents mean and whiskers show minimum to maximum.

(**b**) Quantification of DII-Venus fluorescence signal in Fig. 4b. 1.5 μ M L-Kyn was used for treatment. n denotes number of independent seedlings. Centre line in box represents mean and whiskers show minimum to maximum.

(c) Representative pictures of root hair from 5-day-old seedlings and quantification data. Col-0: n=20; *tmk4-1*: n=30; *wei8-3*: n=20; *tmk4-1;wei8-3*: n=34; *tmk4-1* +L-Kyn: n=19. n denotes number of independent seedlings and x represents number of root hairs used for quantification. Scale bar, 0.5 mm. One-way ANOVA with Tukey multiple comparisons test was used in (a-c). Different letters represent significant difference between each other. P < 0.05(a), P < 0.01(b), P < 0.0001(c).



b

Replicates		Peptide	Area Ratio (Auxin / mock)	Phosphorylation up regulated ratio
Col-0 1	0-10	Non-phosphopeptides	0.902	0.050
	C0I-0	T101-phosphopeptides	2.031	2.252
	tmk4-1	Non-phosphopeptides	1.110	4 225
		T101-phosphopeptides	1.482	1.335
	0-1-0	Non-phosphopeptides	0.779	0.000
2 —	C0I-U	T101-phosphopeptides	1.744	2.239
	tooled d	Non-phosphopeptides	1.180	4 000
	tmk4-1	T101-phosphopeptides	1.191	1.009

Supplementary Figure 14. TMK4 is involved in self-regulation of auxin biosynthesis. (a) Gel shift assay showed TMK4 phosphorylation level increased in *yuc1-D* but decreased in *wei8-3;tar2-1*. 5-day-old seedlings were used for protein extraction. TMK4 proteins were detected by anti-TMK4 antibody. Two biological repeats with similar results.

(**b**) Auxin enhanced T101 phosphorylation of TAA1 partially through TMK4. TAA1-GFP proteins used for quantitative mass spectrometric analysis were immuno-precipitated from the protoplasts treated with EtOH (as mock) or 500 nM IAA (sigma I2886) for 10 mins. 35S-TAA1-GFP plasmids were transformed into Col-0 and *tmk4-1* protoplasts incubated for 8 hrs.

No.	Name of proteins	Gene ID in Phytozome	Organism	protein
		database		size (aa)
1	Marpo.0032s0124	Mapoly0032s0124	Marchantia polymorpha	531
2	Phypa.17_6500	Pp3c17_6500	Physcomitrella patens	525
3	Phypa.18_15140	Pp3c18_15140	Physcomitrella patens	519
4	Phypa.21_15370	Pp3c21_15370	Physcomitrella patens	511
5	Phypa.26_12520	Pp3c26_12520	Physcomitrella patens	442
6	Sphfa.0003s0188	Sphfalx0003s0188	Sphagnum fallax	551
7	Sphfa.0021s0188	Sphfalx0021s0188	Sphagnum fallax	659
8	Sphfa.0026s0096	Sphfalx0026s0096	Sphagnum fallax	669
9	Sphfa.0076s0008	Sphfalx0076s0008	Sphagnum fallax	559
10	Selmo.171289	171289	Selaginella moellendorffii	359
11	Pinab.2120g0020	MA_2120g0020	Pinus abies	352
12	Pinta.000019549	PITA_000019549-RA	Pinus taeda	565
13	Pinta.000027613	PITA_000027613-RA	Pinus taeda	538
14	Pinta.000027966	PITA_000027966-RA	Pinus taeda	541
15	Anaco.027902	Aco027902	Ananas comosus	433
16	Ambtr.00011.7	evm_27.TU.AmTr_v1.0_	Amborella trichopoda	460
		scaffold00011.7		
17	Musac.3G20730_001	GSMUA_Achr3G20730_	Musa acuminata	468
		001		
18	Musac.6G08900_001	GSMUA_Achr6G08900_	Musa acuminata	387
		001		
19	Musac.10G20770_00	GSMUA_Achr10G20770	Musa acuminata	448
	1	_001		
20	Spipo.19G0018400	Spipo19G0018400	Spirodela polyrhiza	450
21	Zosma.1g02950	Zosma1g02950	Zostera marina	383
22	Zosma.209g00410	Zosma209g00410	Zostera marina	403
23	Bradi.2g04290	Bradi2g04290	Arabidopsis lyrata	498
24	Bradi.2g34400	Bradi2g34400	Brachypodium distachyon	431

Supplementary Table 1: Information on TAA proteins of different species.

25	Orysa.01g07500.1	LOC_Os01g07500.1	Oryza sativa	507
26	Orysa.05g07720.1	LOC_Os05g07720.1	Oryza sativa	441
27	Seita.3G100400	Seita.3G100400	Setaria italica	424
28	Seita.3G100500	Seita.3G100500	Setaria italica	506
29	Seita.5G119600	Seita.5G119600	Setaria italica	540
30	Seita.6G075800	Seita.6G075800	Setaria italica	433
31	Sobic.003G052700	Sobic.003G052700	Sorghum bicolor	538
32	Sobic.009G060100	Sobic.009G060100	Sorghum bicolor	432
33	Sobic.009G060300	Sobic.009G060300	Sorghum bicolor	515
34	Maize.2G066345	GRMZM2G066345	Zea mays	536
35	Maize.2G127160	GRMZM2G127160	Zea mays	431
36	Maize.2G127308	GRMZM2G127308	Zea mays	530
37	Maize.2G141810	GRMZM2G141810	Zea mays	435
38	Aquco.3G274600	Aqcoe3G274600	Aquilegia coerulea	391
39	Aquco.3G276300	Aqcoe3G276300	Aquilegia coerulea	351
40	Aquco.3G276800	Aqcoe3G276800	Aquilegia coerulea	467
41	Solyc.03g112460	Solyc03g112460.2	Solanum lycopersicum	444
42	Solyc.05g031600	Solyc05g031600.1	Solanum lycopersicum	391
43	Solyc.06g071640	Solyc06g071640.2	Solanum lycopersicum	437
44	Soltu.400007680	PGSC0003DMG400007	Solanum tuberosum	355
		680		
45	Soltu.400007681	PGSC0003DMG400007	Solanum tuberosum	393
		681		
46	Soltu.400018110	PGSC0003DMG400018	Solanum tuberosum	443
		110		
47	Soltu.400025405	PGSC0003DMG400025	Solanum tuberosum	385
		405		
48	Soltu.400027079	PGSC0003DMG400027	Solanum tuberosum	439
		079		
49	Vitvi.G01004095001	GSVIVG01004095001	Vitis vinifera	410
50	Vitvi.G01006656001	GSVIVG01006656001	Vitis vinifera	419
51	Vitvi.G01007679001	GSVIVG01007679001	Vitis vinifera	473

52	Potri.008G187800	Potri.008G187800	Populus trichocarpa	377
53	Potri.010G044500	Potri.010G044500	Populus trichocarpa	365
54	Potri.012G083300	Potri.012G083300	Populus trichocarpa	457
55	Potri.015G081900	Potri.015G081900	Populus trichocarpa	457
56	Citsi.1g012785	orange1.1g012785m.g	Citrus sinensis	456
57	Citsi.1g018172	orange1.1g018172m.g	Citrus sinensis	360
58	Citcl.10020085	Ciclev10020085m.g	Citrus clementina	458
59	Citcl.10033774	Ciclev10033774m.g	Citrus clementina	434
60	Carpa.35.70	evm.TU.supercontig_35. 70	Carica papaya	448
61	Carpa.69.95	evm.TU.supercontig_69.	Carica papaya	364
		95		
62	Thecc.1EG010471	Thecc1EG010471	Theobroma cacao	399
63	Thecc.1EG012202	Thecc1EG012202	Theobroma cacao	452
64	Gosra.005G180100	Gorai.005G180100	Gossypium raimondii	401
65	Gosra.006G189500	Gorai.006G189500	Gossypium raimondii	438
66	Arath.TAA1	Arabidopsis TAA1	Arabidopsis thaliana	391
67	Arath.TAR1	Arabidopsis TAR1	Arabidopsis thaliana	388
68	Arath.TAR2	Arabidopsis TAR2	Arabidopsis thaliana	440
69	Araly.1G37110	AL1G37110	Arabidopsis lyrata	387
70	Araly.2G29950	AL2G29950	Arabidopsis lyrata	392
71	Araly.7G28530	AL7G28530	Arabidopsis lyrata	440
72	Brara.A01473	Brara.A01473	Brassica rapa	454
73	Brara.B01919	Brara.B01919	Brassica rapa	381
74	Brara.G02980	Brara.G02980	Brassica rapa	381
75	Brara.I03242	Brara.103242	Brassica rapa	381
76	Brara.I03244	Brara.103244	Brassica rapa	382
77	Prupe.1G248200	Prupe.1G248200	Prunus persica	408
78	Prupe.1G248300	Prupe.1G248300	Prunus persica	354
79	Prupe.5G168300	Prupe.5G168300	Prunus persica	437
80	Maldo.0000203835	MDP0000203835	Malus domestica	431
81	Maldo.0000267098	MDP0000267098	Malus domestica	448

82	Maldo.0000310220	MDP0000310220	Malus domestica	404
83	Maldo.0000616079	MDP0000616079	Malus domestica	407
84	Medtr.3g077250	Medtr3g077250	Medicago truncatula	441
85	Medtr.4g105220	Medtr4g105220	Medicago truncatula	437
86	Medtr.5g033510	Medtr5g033510	Medicago truncatula	401
87	Medtr.5g033520	Medtr5g033520	Medicago truncatula	394
88	Frave.03586	gene03586.1-v1.0-	Fragaria vesca	434
		hybrid		
89	Frave.31790	gene31790.1-v1.0-	Fragaria vesca	432
		hybrid		
90	Frave.31791	gene31791.1-v1.0-	Fragaria vesca	530
		hybrid		
91	Glyma.01G027400	Glyma.01G027400	Glycine max	391
92	Glyma.02G037600	Glyma.02G037600	Glycine max	392
93	Glyma.04G186700	Glyma.04G186700	Glycine max	445
94	Glyma.05G040400	Glyma.05G040400	Glycine max	452
95	Glyma.06G179000	Glyma.06G179000	Glycine max	439
96	Glyma.17G086500	Glyma.17G086500	Glycine max	453

Supplementary Table 2: Primers list.

Primers name	Primers sequence (5'-3')	Notes
gTMK4 GW-F	GGGGACAAGTTTGTACAAAAAGCAGGCTTATGTGGTTTTAGTCATTTATT	PDONR-zeo
gTMK4 GW-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAACCATCAGCTGAATCGAAAGTA	construction
gTAA1 GW-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACTCCATATCGAGGGTATGAA	
gTAA1 GW-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAAAGGTCAATGCTTTTAA	
TAA1 GW-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGTGAAACTGGAGAACTC	
TAA1 GW-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAAAGGTCAATGCTTTTAA	
MpTAA GW-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCGTCTCGAATGGACTCA	
MpTAA GW-R	GGGGACCACTTTGTACAAGAAAGCTGGGTACGACATGTCGACTGCAGACTG	
tmk4-1 LP	TGCGATTGCTCAAAGAGGTCAGA	Genotyping
tmk4-1 RP	GGCTGCATTGGTTGCACTGGAT	primers
tmk4-2 LP	AAATGTCTATGCCTGACC	
tmk4-2 RP	TCCAAGAGCGAGAATCTC	
wei8-3 LP	CATGATGTCATGGTCTTGACG	
wei8-3 RP	TTTACTCCGTAAGTCCCCACC	
tar2-1 LP	CACAATCTCTTTGGCAAGCTC	_
tar2-1 RP	TAGTGGAATTTTTGATTGCTTG	
tar1-1 LP	GATTCGTTATGACATTTGGCG	_
tar1-1 RP	CGATTGAAGATACGATGCCTC	
P35S	GACGCACAATCCCACTATCC	_
PTAA-R	CTACGACATGTCGACTGC	
TMK4 qRT-F	AGGCACTCGACCAAACCTTA	qRT-PCR
TMK4 qRT-R	ACGACGGTTTCCACTTCTCT	primers
TAA1 qRT-F	GATGAAGAATCGGTGGGAGA	_
TAA1 qRT-R	TGACTCGGACATGCTTCTTG	_
TAR1 qRT-F	TCCATTGGTGTGTCGAAGGA	_
TAR1 qRT-R	AAACGCAGGAGAAGTGGAGA	_
TAR2 qRT-F	GCTCTTCACTGCTTCAAAGAGCAC	_
TAR2 qRT-R	TCTGTCTTTCACCAAAGCCCATCC	_
UBQ10 qRT-F	GATCTTTGCCGGAAAACA	_
UBQ10 qRT-R	CGAAGATGAGACGCTGCT	_
PINOID Ndel F	GGCATTCCATATGATGTTACGAGAATCAGAC	pBKT7-
PINOID EcoRI R	CCGGAATTCAAAGTAATCGAACGCCGCTGG	vector
D6PK NdeI F	GGCATTCCATATGATGATGGCTTCAAAAACTCC	construction
D6PK PstI R	CCGCTGCAGGGAAGAAATCAAACTCAAGA	-
FLS2 C NdeI F	GGCATTCCATATGAATTCATCAGAGTCCTCATT	-

FLS2 C BamHI R	AGGTCGACGGATCCCAACTTCTCGATCCTCGTTACG	
BAK1 C NdeI F	GGCATTCCATATGTTCTTTGATGTACCAGCTGA	
BAK1 C BamHI R	CGCGGATCCTTATCTTGGACCCGAGGGGT	
BIN2 Ndel F	GGCATTCCATATGATGGCTGATGATAAGGAGAT	
BIN2 EcoRI R	CCGGAATTCAGTTCCAGATTGATTCAAGAA	
BRI1 C Ndel F	GGCATTCCATATGTTCCATAATGATAGTCTGAT	
BRI1 C EcoRI R	CCGGAATTCTCATAATTTTCCTTCAGGAA	
TAA1 XmaI R	TCGACCCGGGCTAAAGGTCAATGCTTTT	pGEX4T-2
TAA1 BamHI F	CGCGGATCCATGGTGAAACTGGAGAACTC	vector
		construction
TAA1 StuI R	AAAAGGCCTAAGGTCAATGCTTTTAATGA	HBT vector
		construction
TMK4 KD EcoRI F	ATCCGAATTCTTCAGTGAGGATAACATA	His-sumo
TMK4 KD NotI R	ATTATGCGGCCGCTCACCGACCATCAGCTG	vector/
		PGEX4T-2
		vector
		construction
TMK4 KD BmaHI	CCGCGTGGATCCGGGGGTTTCGGTGTCGTG	PET14
TMK4 BclI F	GCGCCTGATCAATGGAGGCTCCTACGCCTC	vector and
		HBT vector
		construction
TMK4 StuI R	AAAAGGCCTCCGACCATCAGCTGAATCGA	Site-directed
TAA1 T101A F	AGTGGTTGGGACCGGTTCGGCGCAGCTTTGTCAAGC	mutagenesis
TAA1 T101A R	GCTTGACAAAGCTGCGCCGAACCGGTCCCAACCACT	
TAA1 T101D F	GGTTGGGACCGGTTCGGACCAGCTTTGTCAAGCCG	
TAA1 T101D R	CGGCTTGACAAAGCTGGTCCGAACCGGTCCCAACC	
MpTAA T224D F	TCCGACCAACTCTTCCAGGCTGCATTG	
MpTAA T224D R	CAATGCAGCCTGGAAGAGTTGGTCGGA	