Supplemental Information

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Supplementary Figure Legends

Figure S1: TAPa-tagged TWD1 functionally complements *twd1.*

(A) Phenotype of homozygous *twd1-3* transformed with 35S:TAPa-TWD1 (no. 3, line 3 and no. 5, line 5) in comparison to Columbia wild-type (Col Wt) 45 dag.

(B) Western blot verification of TAP1-TWD1 expression using anti-MYC.

(C) Auxin efflux from protoplasts of *twd1-3* complemented with 35S:TAPa-TWD1 (TAPa-TWD1) is restored to wild-type (Col Wt) levels (mean ± SE; n = 4). Significant differences (unpaired *t* test with Welch's correction, $p<0.05$) to wild type are indicated by an asterisk.

(D) Protein sequences of TWD1 and PID peptides with protein coverages identified by MS/MS after TAPa-TWD1 co-immunoprecipitation.

(E) Phenotype of homozygous *twd1-3* complemented with C- (TWD1-Rluc) and N-terminally tagged *Renilla* luciferase (Rluc-TWD1) in comparison to Columbia wild-type (Col Wt) 40 dag.

Figure S2: Co-expression and mutagenesis does not significantly alter location and expression of ABCB1 in yeast

(A) Co-expression with PID, MPID or BIN2 does not significantly alter plasma membrane location of ABCB1-YFP as revealed by Western analysis using anti-GFP and anti-PMA1 directed against plasma membrane marker, H^+-ATPase, after linear sucrose gradient centrifugation. Peak fractions are marked by red asterisks.

(B) S634A and S634E mutagenesis does not significantly alter plasma membrane location of ABCB1-YFP as revealed by Western analysis using anti-GFP and anti-PMA1 directed against plasma membrane marker, H^+-ATPase, after linear sucrose gradient centrifugation. Peak fractions are marked by red asterisks.

(C) S634A and S634E mutagenesis does not significantly alter expression of ABCB1-YFP. Each 20 μg of protein was subjected to PAGE and Western analysis using anti-GFP and anti-PMA1 directed against plasma membrane marker, H^+-ATPase.

(D) S634A and S634E mutagenesis does not significantly alter expression and location of ABCB1-YFP that localized primarily to raft-like structures surrounding the plasma membrane (Bailly et al, 2008). Bars, 2 μm.

Figure S3: Co-expression and mutagenesis does not significantly alter location and expression of ABCB1 in yeast and tobacco

(A) Co-expression of ABCB1-MYC with PID-FLAG (PID), TWD1-YFP (TWD1), WAG1-YFP (WAG1) and PID-FLAG/TWD1-YFP (PID + TWD1) does not significantly alter expression of ABCB1-YFP. Each 10 μg of protein was subjected to PAGE and Western analysis using anti-MYC and anti-AHA2, directed against plasma membrane marker, H^+-ATPase.

(B-C) Time-course of auxin efflux from *PID* gain- and loss-of-function protoplasts. IAA (**B**) and NAA efflux (**C**) from Arabidopsis *pid* protoplasts is strongly enhanced while *PID* gain-of function has in agreement with described phentotypes only a non-significant effect (means ± SE; n = 3). 7.5 min. values were taken for bar presentation in Fig. 3C.

(D-E) 35S:WAG1-YFP (**D**, WAG1-YFP) and 35S:TWD1-GFP (**E**, TWD1-GFP) localize to the plasma membrane of *N. benthamiana* protoplasts isolated 4 days after leaf infiltration. Bars, 5 μm.

(F-G) 35S:ABCB1-YFP co-localizes with 35S:PID-GFP (**F**, PID-GFP) and 35S:TWD1-GFP (**G**, TWD1-GFP) on the plasma membrane of *N. benthamiana* protoplasts isolated 4 days after leaf infiltration. Bars, 5 μm.

(H) Co-transfection of PID (PID-GFP) and TWD1 (TWD1-YFP) in *N. benthamiana* protoplasts does not significantly alter ABCB1 (ABCB1-CFP) location in comparison to vector control co-expression. Note co-localization of ABCB1, TWD1 and PID on the plasma membrane of tobacco protoplasts (lower row; insets in upper row show indicated details at higher magnification). Bar, 10 μm.

Figure S4: Inhibition of *in vitro* auto-phosphorylation of PID-GST by quercetin correlates with inhibition of MBP trans-phosphorylation

(A-B) Coomassie stains (left panels) of non-phosphorylated PID (PID) was used as loading control (**A**). Autoradiographies (right panels) of autophosphorylated PID (PID-P), presumably represented by the upper band in the Coomassie stain (Christensen et al, 2000), were quantified (**B**) and signal intensities were plottet against solvent controls (lower panel; means ± SE; n = 3). Significant differences (unpaired *t* test with Welch's correction, $p<0.05$) to solvent controls are indicated by asterisks. Note that in contrast to PID autophosporylation (Fig. 5), chelerythrine has only a slight effect on MBP trans-phosphorylation.

Figure S5: The ABCB1 linker domain is a target of protein phosphorylation

Overview of ABCB1 linker (amino acid 607-680, grey background) phosphorylation sites either predicted *in silico* using NetPhos (red letters) or identified by phosphoproteomics (black P, (Benschop et al, 2007; Nuhse et al, 2004); red P, this study). *In vitro* verification of putative linker phosphorylation sites using synthetic linker peptides (blue background) and mammalian PKC; numbers of PKC phosphorylation sites (X x P) identified by MS/MS are indicated.

Figure S6: Staurosporine and phorbole ester do not rescue the *pin2* agravitropic phenotype.

Root bending analysis after 12h kinase inhibitor treatment (100 nM quercetin, 400 nM chelerythrine, 50 nM staurosporine, 10 μM phorbole ester). The length of each bar represents the mean percentages ± SD of seedlings showing the same direction of root growth of at least three independent experiments; numbers correspond to the mean percent occurrence of 60 and 90° bending (sum of 60 and 90° sectors); nd, experiment not done.

Figure S7: Protein kinase inhibitors do not significantly alter ABCB1-, ABCB19, PIN1 and PIN2 root locations.

Arrows mark ectopic PIN1 expression at epidermal and cortical PIN2 locations caused by quercetin and chelerythrine, and enhanced ABCB1 expression in Columella cells upon chelerythrine treatments, as well as slightly elevated ABCB19 expression in the stele induced by quercetin and chelerythrine.

Figure S8: Model of PID-ABCB-TWD1 interaction and ABCB1 linker regulation

(A) Simplified model of the dual, counter-active, regulatory impact of PID on ABCB activity. In the absence of TWD1, PID has a positive (+) regulatory effect on ABCB activity, most probably by S634 linker phosphorylation. Moreover, TWD1 functions in recruiting PID for ABCB1 linker phosphorylation resulting in block of auxin efflux (-). This negative regulatory role on auxin transport *in planta* is supported by Arabidopsis protoplast efflux data (Fig. 3C), however, target residues phosphorylated by PID have not yet been identified. Quercetin (Quer) binds to PID resulting in PID inhibition. Functional domains of TWD1 are in blue (FKBD), green (TPR), yellow (calmodulin-binding domain) and grey (in-plane membrane anchor).

(B) Inward-facing structural model of the *Arabidopsis* ABCB1 modeled on the crystal structure of murine ABCB1/PGP1 (PDBID 3G5U) indicating a location of the linker region (orange) with S634 (depicted as calotte) between N- and C-terminal NBDs (Bailly et al, 2012).

(C) Impact of the linker location on electrostatic surface potentials. Bottom view into the transmembrane region of the translocation chamber (dark grey in B.) displaying electrostatic surface potential of AtABCB1 with (+ linker) and without linker domain (- linker). Note negative shift of surface potentials in the absence of the ABCB1 linker. Scale of legend, +/- 10 kT.

Table S1: TWD1 interactors identified by co-imunoprecipitation using TAP-TWD1 as bait followed by shotgun MS/MS analysis

MASCOT-identified vector control proteins and TAP-TWD1 proteins with their AGI codes (prot_acc), protein scores (prot_score) and protein cover (prot_cover). Proteins with a score above 30 that are not found in vector controls are colored in yellow and summarized in detail in Fig. 1A.

Supplemental references

Bailly A, Sovero V, Vincenzetti V, Santelia D, Bartnik D, Koenig BW, Mancuso S, Martinoia E, Geisler M (2008) Modulation of P-glycoproteins by auxin transport inhibitors is mediated by interaction with immunophilins. *J Biol Chem* **283:** 21817-21826

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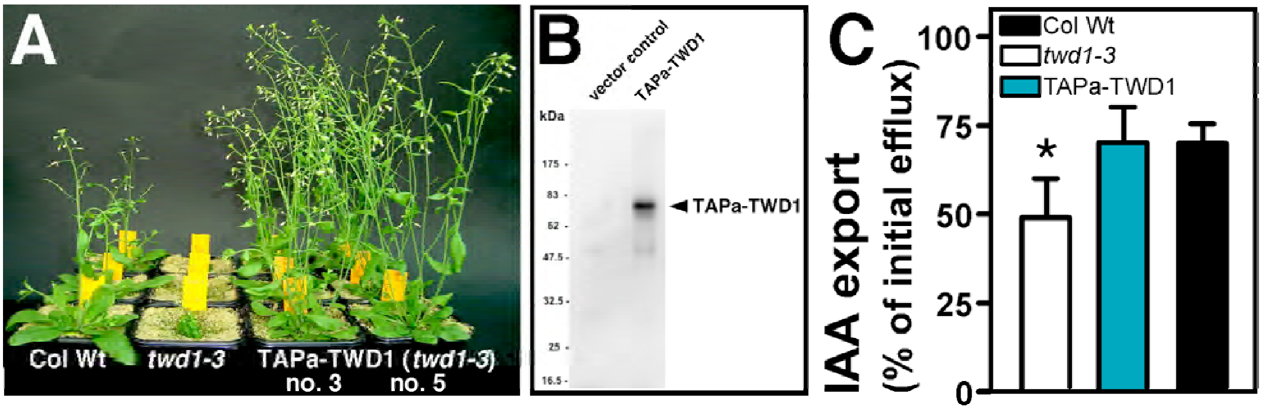
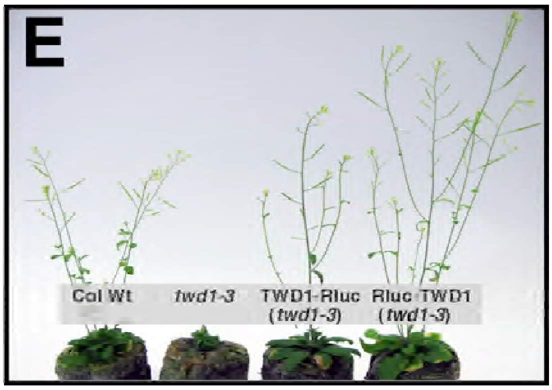
Benschop JJ, Mohammed S, O'Flaherty M, Heck AJ, Slijper M, Menke FL (2007) Quantitative phosphoproteomics of early elicitor signaling in Arabidopsis. *Mol Cell Proteomics* **6:** 1198-1214

Christensen SK, Dagenais N, Chory J, Weigel D (2000) Regulation of auxin response by the protein kinase PINOID. *Cell* **100:** 469-478

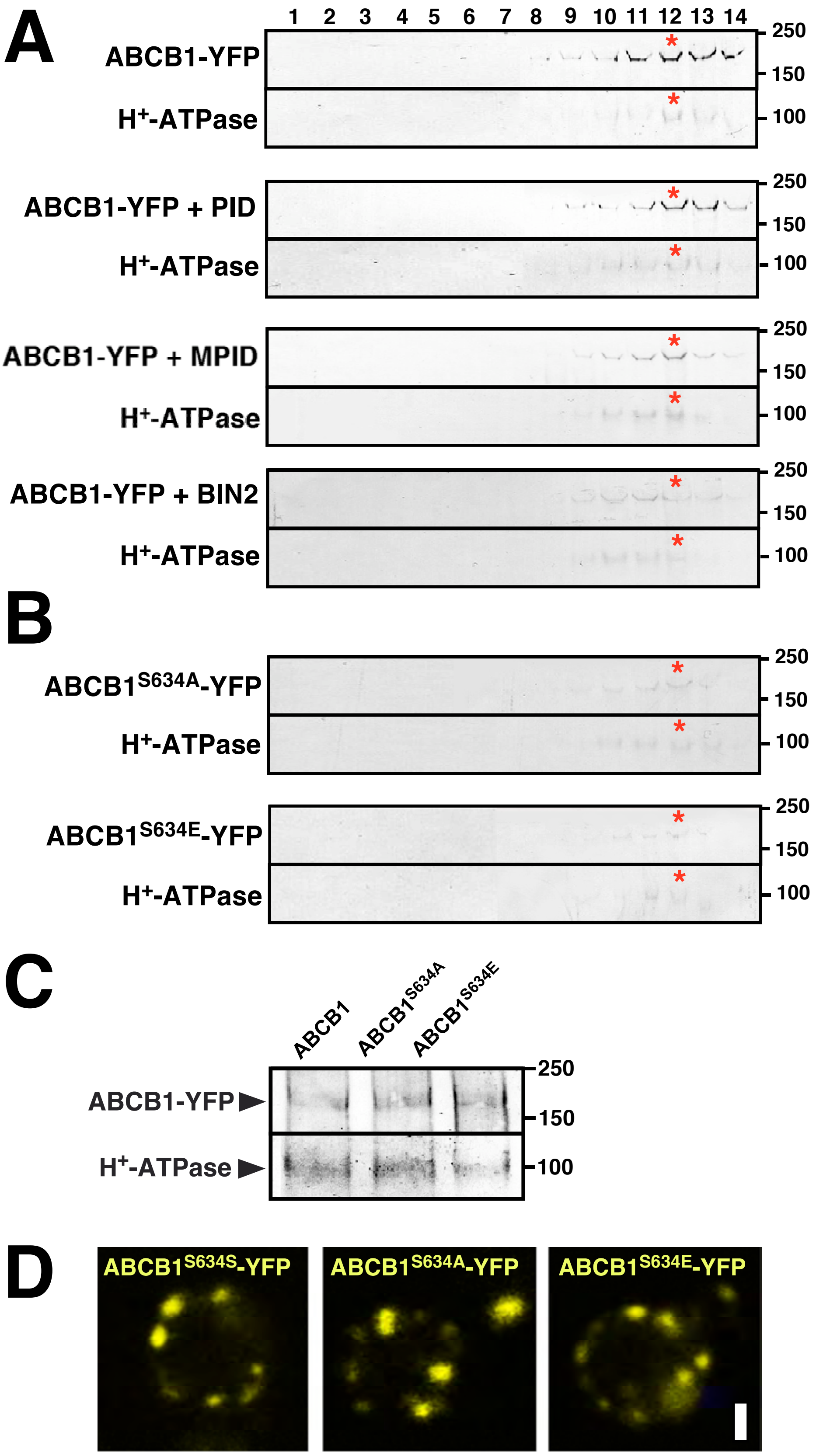
Nuhse TS, Stensballe A, Jensen ON, Peck SC (2004) Phosphoproteomics of the Arabidopsis plasma membrane and a new phosphorylation site database. *Plant Cell* **16:** 2394-2405



D	TWD1	PID
Peptide	ALYQK	TPFVAPTNDVILR
	SDMTVEER	
	AELGQMDSAR	
	ARSDMTVEER	
	AKAELGQMDSAR	
	NSQHKFEDTWHEQQPIELVLGK	
Cover (%)	13.4	3



Henrichs et al. Fig. S1



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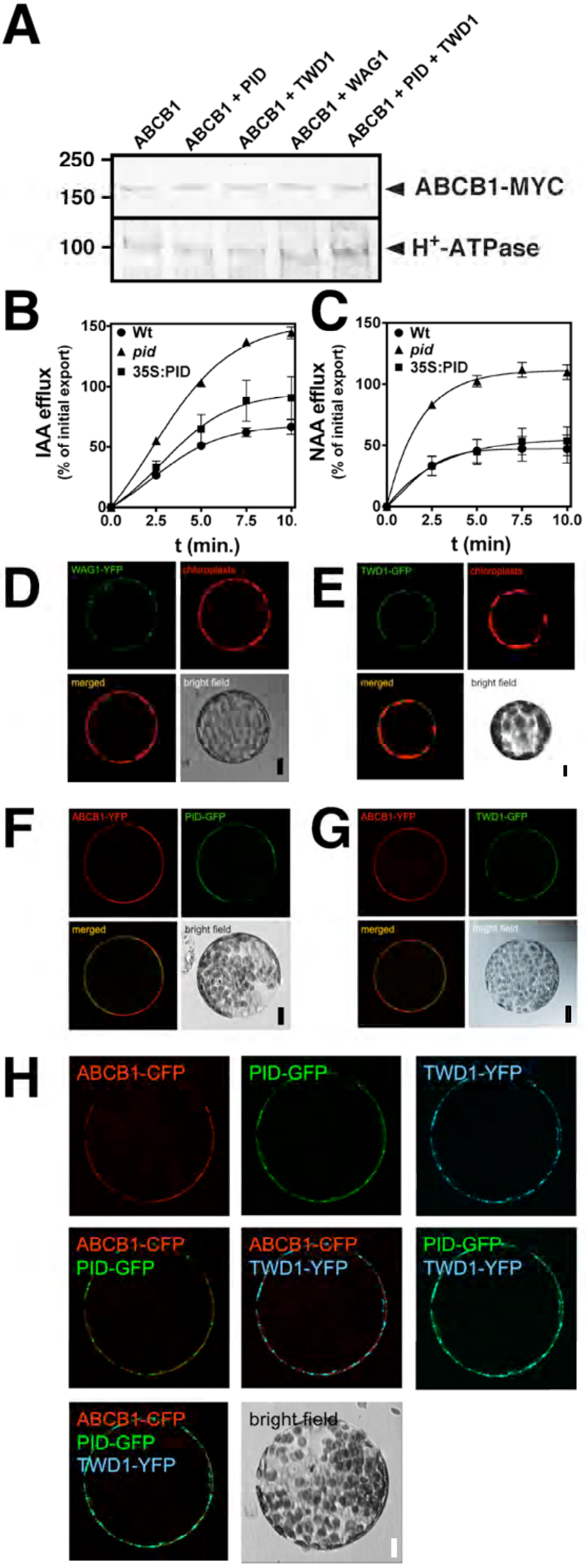

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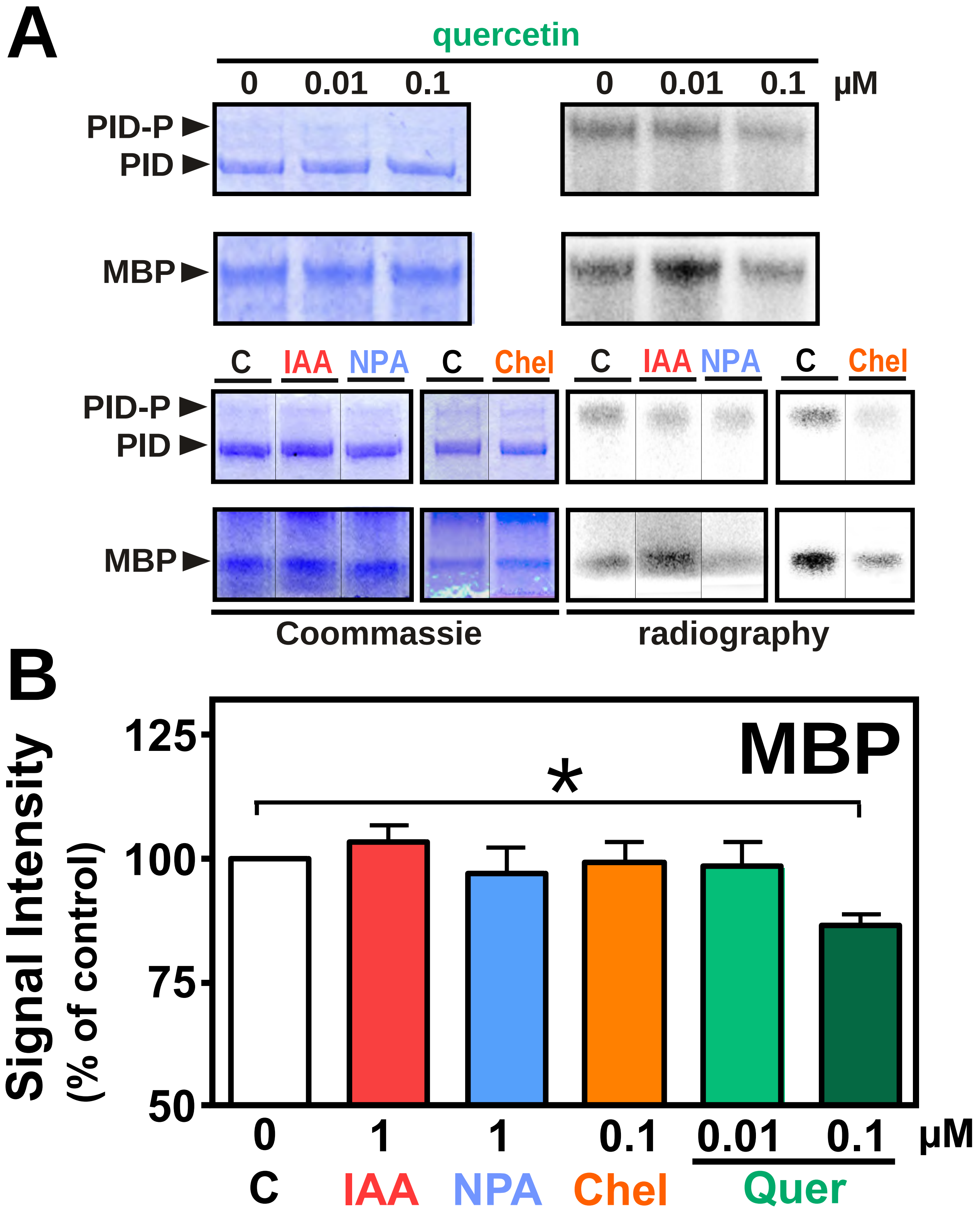

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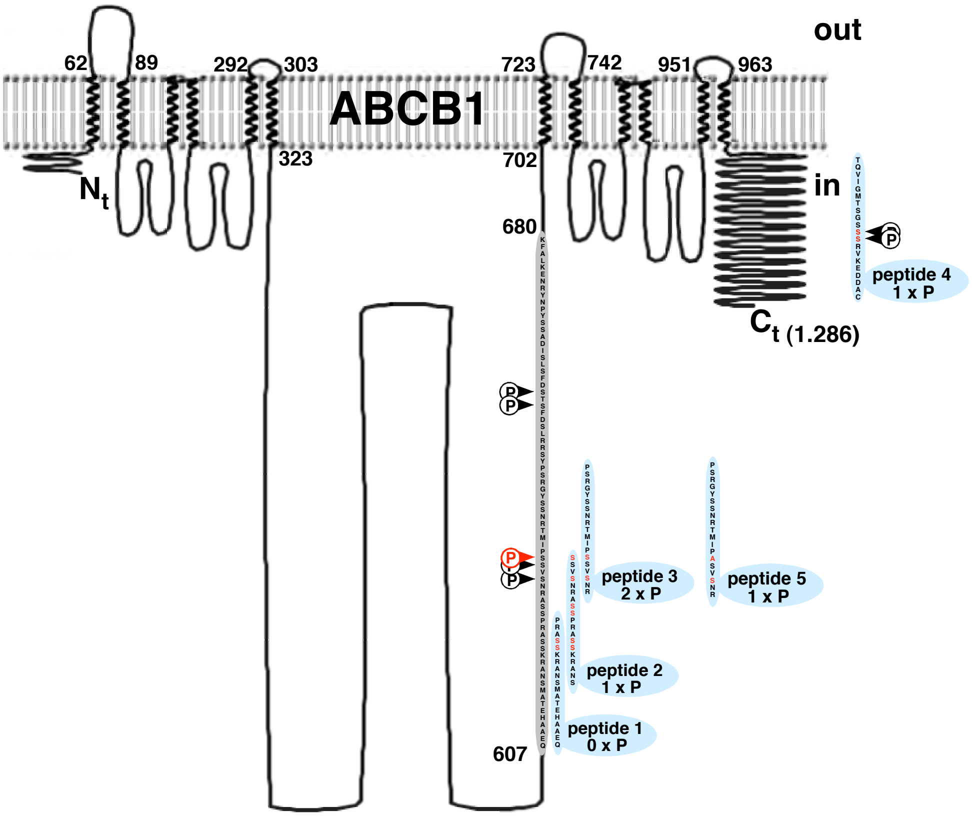

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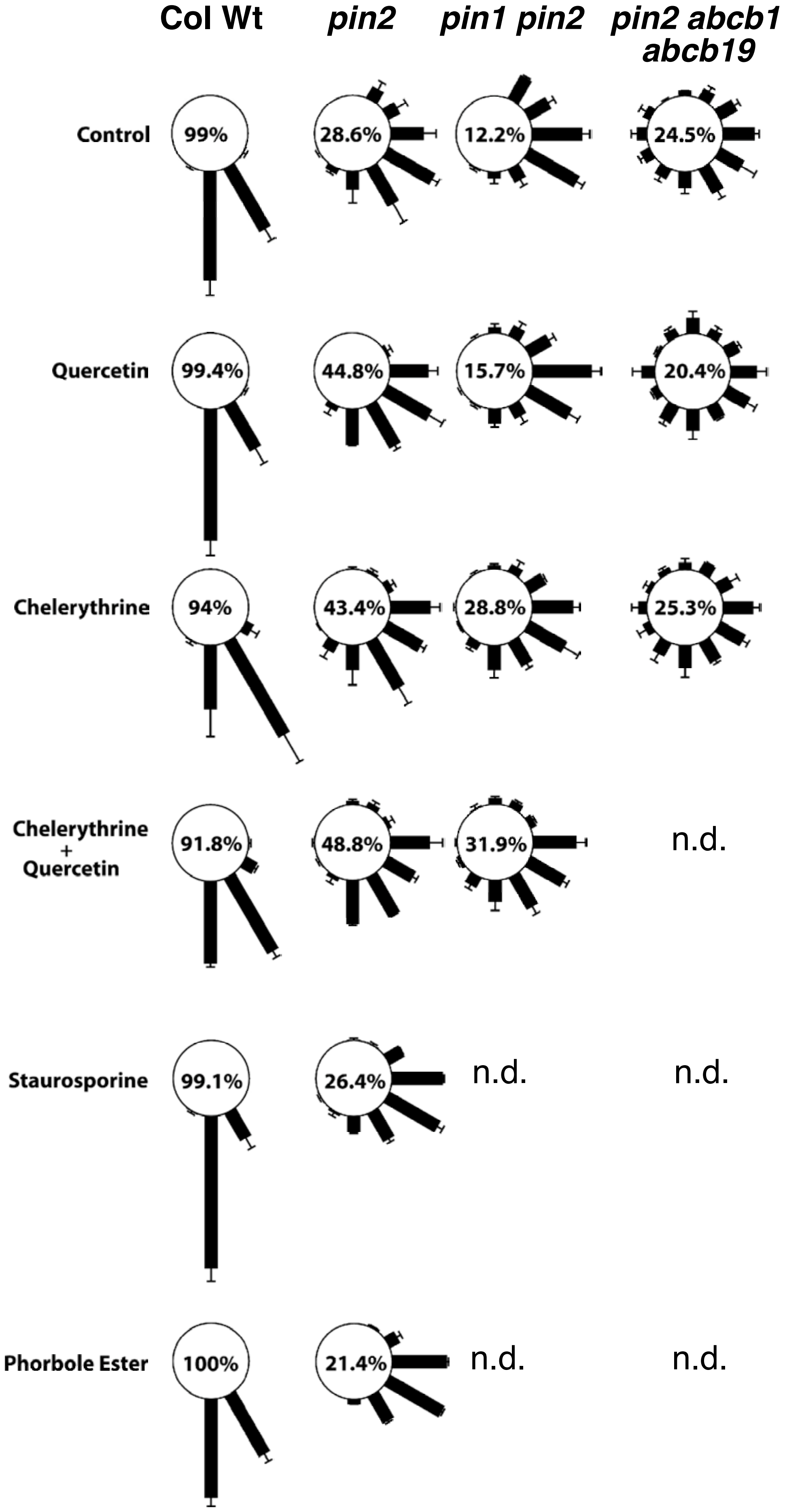

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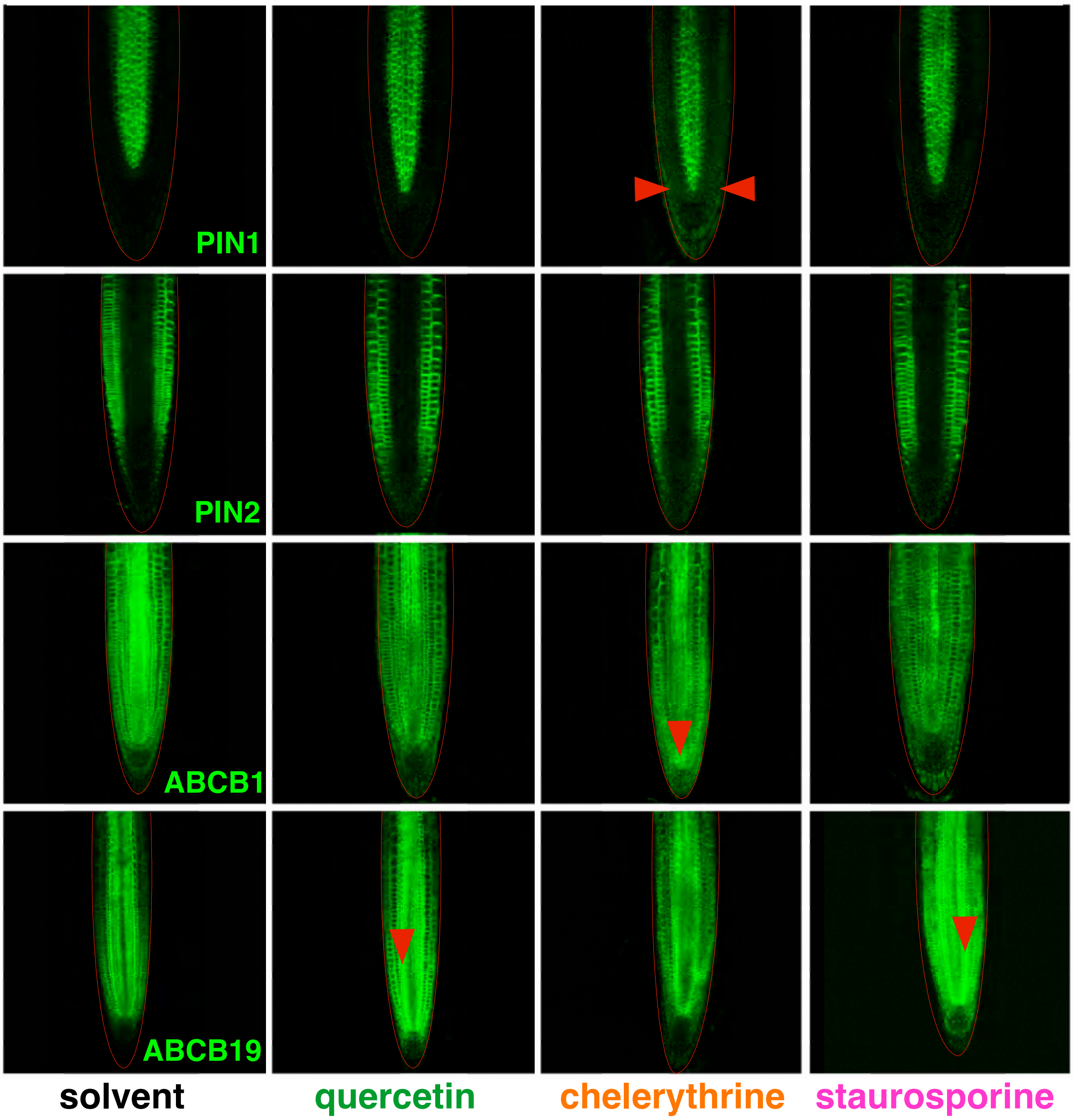

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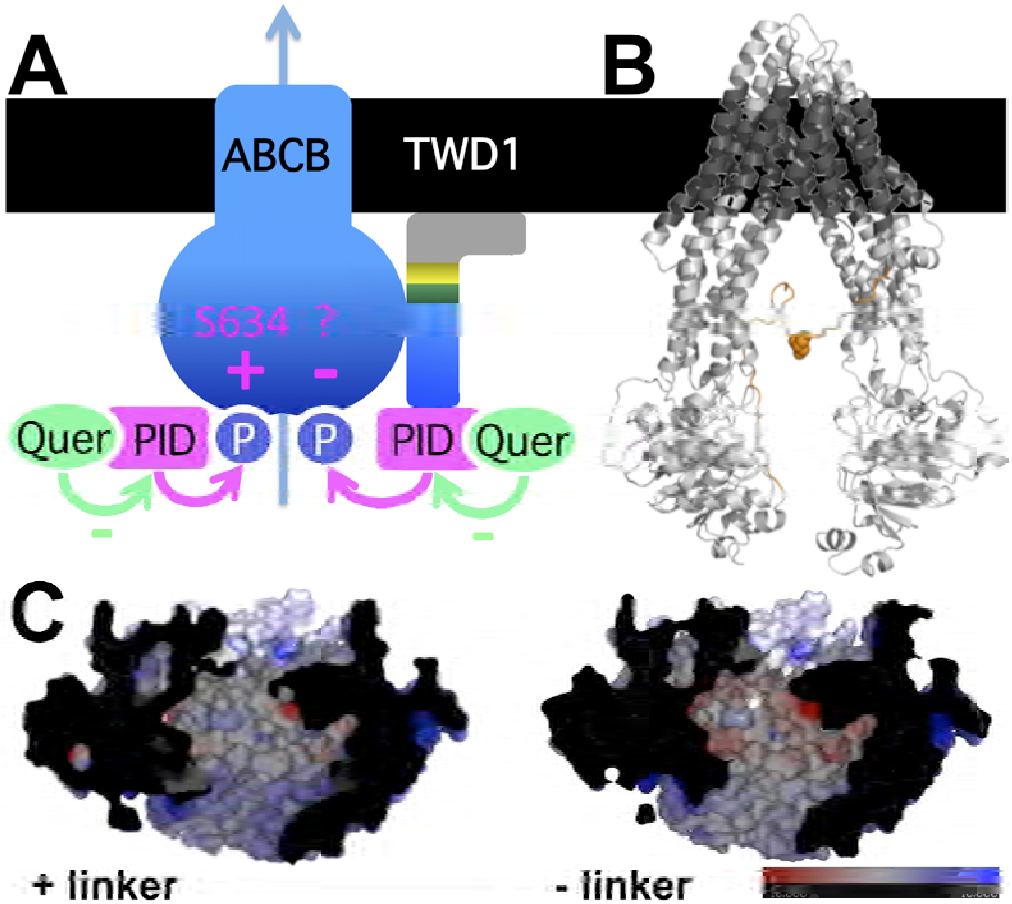

Henrichs et al. Fig. S8

prot_acc	Identified Proteins in TWD1-TAP	prot_score	prot_cover
AT3G09260	PYK10 (phosphate starvation-response 3.1); hydrola	1813	50
AT3G16420	PBP1 (PYK10-BINDING PROTEIN 1)	840	60.1
AT3G16460	jacalin lectin family protein	614	32.6
AT3G15730	PLDALPHA1 (PHOSPHOLIPASE D ALPHA 1); phos	330	26.9
AT1G66280	glycosyl hydrolase family 1 protein	308	12.6
ATCG00490	large subunit of RUBISCO.	283	21.3
AT1G54010	myrosinase-associated protein, putative	223	28.5
AT3G21640	TWD1 (TWISTED DWARF 1); FK506 binding / peptid	129	6
AT3G14210	ESM1 (EPITHIOSPECIFIER MODIFIER 1); carboxyle	122	24.5
AT3G16450	jacalin lectin family protein	120	41
AT2G39730	RCA (RUBISCO ACTIVASE)	93	3.2
AT4G33630	EX1 (EXECUTER1)	86	0.7
AT3G16470	JR1 (Jacalin lectin family protein)	72	10.9
AT3G48830	polynucleotide adenylyltransferase family protein /	61	1.1
AT4G24750	similar to rhodanese-like domain-containing protein	44	2.1
AT1G54000	myrosinase-associated protein, putative	43	7.9
AT5G26280	meprin and TRAF homology domain-containing prot	41	16.9
AT1G58270	ZW9	40	3.8
AT1G80450	VQ motif-containing protein	39	2.8
AT2G27120	POL2B/TIL2 (TILTED2); DNA-directed DNA polyme	39	0.2
AT2G47300	ribonuclease P	39	0.6
AT3G20370	meprin and TRAF homology domain-containing prot	38	7.1
AT4G25920	similar to unknown protein [Arabidopsis thaliana] (T	38	4.1
AT1G52570	PLDALPHA2 (PHOSPHLIPASE D ALPHA 2); phosp	35	4.6
AT5G10720	AHK5 (CYTOKININ INDEPENDENT 2)	34	1.2
AT2G25980	jacalin lectin family protein	33	3.3
AT2G34650	PID (PINOID); kinase	32	3
AT2G30020	protein phosphatase 2C, putative / PP2C, putative	32	2.5
AT3G14270	phosphatidylinositol-4-phosphate 5-kinase family pr	30	0.4
AT1G01360	similar to unknown protein [Arabidopsis thaliana] (T	30	2.7
AT3G62110	glycoside hydrolase family 28 protein / polygalactur	30	1.1
AT5G28910	similar to unknown protein [Arabidopsis thaliana] (T	30	2.2
AT1G33240	AT-GTL1 (Arabidopsis thaliana GT2-like 1); transcr	30	1.2
AT4G37460	binding	29	0.8
AT1G47765	F-box family protein	28	1.6
AT3G54230	nucleic acid binding	28	0.8
AT2G18850	similar to SET domain-containing protein [Arabidops	28	4.2
AT2G39190	ATATH8 (ABC2 homolog 8)	28	2.2
AT4G01650	similar to unknown protein [Arabidopsis thaliana] (T	27	5.9
AT1G70840	MLP31 (MLP-LIKE PROTEIN 31)	27	11.1

AT1G27190	leucine-rich repeat transmembrane protein kinase,	26	3.8
AT2G29480	ATGSTU2 (GLUTATHIONE S-TRANSFERASE 20);	26	5.8
AT5G61280	remorin family protein	26	1.9
AT2G27170	TTN7 (TITAN7); ATP binding	25	1.1
AT1G61010	CPSF73-I; protein binding	25	1.3
AT1G06470	phosphate translocator-related	24	2.4
AT4G00750	dehydration-responsive family protein	24	1.7
AT4G38825	similar to auxin-responsive protein, putative [Arabid	24	12.4
AT1G49240	ACT8 (ACTIN 8); structural constituent of cytoskel	24	3.2
AT5G43020	leucine-rich repeat transmembrane protein kinase,	24	2.7
AT2G33040	ATP synthase gamma chain, mitochondrial (ATPC)	23	2.8
AT3G45610	Dof-type zinc finger domain-containing protein	23	3.7
AT1G71390	disease resistance family protein / LRR family prot	23	1.7
AT5G53670	similar to hypothetical protein [Vitis vinifera] (GB:C	23	2.9
AT5G67100	ICU2 (INCURVATA2); DNA-directed DNA polymera	23	1.4
AT2G23390	similar to hypothetical protein OsI_015489 [Oryza s	23	1.1
AT3G01680	similar to unknown protein [Arabidopsis thaliana] (T	22	1.5
AT2G36740	ATSWC2/SWC2; DNA binding	22	6.3
AT4G14310	similar to hypothetical protein OsI_009189 [Oryza s	21	2.1
AT4G14385	similar to unnamed protein product [Vitis vinifera]	21	12.9

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prot_acc	Identifeid Proteins in vector control	prot_score	prot_cover
AT3G09260	PYK10 (phosphate starvation-response 3.1); hydrolase, hy	2040	55.5
AT3G15730	PLDALPHA1 (PHOSPHOLIPASE D ALPHA 1); phospholipa	895	43.3
AT3G16420	PBP1 (PYK10-BINDING PROTEIN 1)	519	55.4
AT1G66280	glycosyl hydrolase family 1 protein	355	12.6
AT3G16460	jacalin lectin family protein	323	23.3
AT3G16470	JR1 (Jacalin lectin family protein)	178	25.1
AT3G01670	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	171	13.7
AT1G52400	BGL1 (BETA-GLUCOSIDASE HOMOLOG 1); hydrolase, hy	169	13.6
AT3G14210	ESM1 (EPITHIOSPECIFIER MODIFIER 1); carboxylesterase	159	15.8
ATCG00490	large subunit of RUBISCO.	158	16.3
AT1G52570	PLDALPHA2 (PHOSPHLIPASE D ALPHA 2); phospholipas	153	6.9
AT1G54010	myrosinase-associated protein, putative	129	20.5
AT5G26280	meprin and TRAF homology domain-containing protein / M	81	8.9
AT4G33630	EX1 (EXECUTER1)	81	0.7
AT3G20370	meprin and TRAF homology domain-containing protein / M	71	7.7
AT3G16450	jacalin lectin family protein	53	13.7
AT3G01680	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	51	6.4
AT4G24750	similar to rhodanese-like domain-containing protein [Arabi	47	2.1
AT1G54000	myrosinase-associated protein, putative	37	9
AT1G58270	ZW9	37	3.8
AT2G27120	POL2B/TIL2 (TILTED2); DNA-directed DNA polymerase	35	0.2
AT2G47300	ribonuclease P	35	0.6
AT1G29120	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	32	1.1
AT1G50030	TOR (TARGET OF RAPAMYCIN)	32	0.2
AT1G80450	VQ motif-containing protein	32	2.8
AT2G20240	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	32	0.8
AT1G06170	basic helix-loop-helix (bHLH) family protein	32	2.6
AT4G01650	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	31	5.9
AT1G52000	jacalin lectin family protein	30	1.8
AT2G33040	ATP synthase gamma chain, mitochondrial (ATPC)	29	2.8
AT1G32375	F-box family protein	28	2.6
AT3G50820	PSBO-2/PSBO2 (PHOTOSYSTEM II SUBUNIT O-2); oxyg	28	10.6
AT1G48370	YSL8 (YELLOW STRIPE LIKE 8); oligopeptide transporter	27	1.5
AT3G16940	calmodulin-binding protein	26	1.2
AT4G25920	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	26	4.1
AT1G01360	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	25	2.7
AT3G62110	glycoside hydrolase family 28 protein / polygalacturonase (	25	1.1
AT5G38750	asparaginyl-tRNA synthetase family	25	4.4
AT5G28910	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	25	2.2
AT5G19950	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT	24	4.1

AT2G36240	binding	24	5
AT1G64530	RWP-RK domain-containing protein	23	2
AT1G53880	GTP binding / translation initiation factor	23	1.7
AT2G25980	jacalin lectin family protein	22	3.1
AT2G23390	similar to hypothetical protein OsI_015489 [Oryza sativa (ir	22	1.1
AT2G35110	GRL/NAP1/NAPP (NCK-ASSOCIATED PROTEIN); transc	22	1.4
AT1G50140	ATPase	22	0.5
AT3G05050	protein kinase family protein	22	0.8
AT1G09440	protein kinase family protein	21	2.1
AT5G53670	similar to hypothetical protein [Vitis vinifera] (GB:CAN812	21	2.9