Supporting Information

Expression of TWISTED DWARF1 lacking its in-plane membrane anchor leads to increased cell elongation and hypermorphic growth

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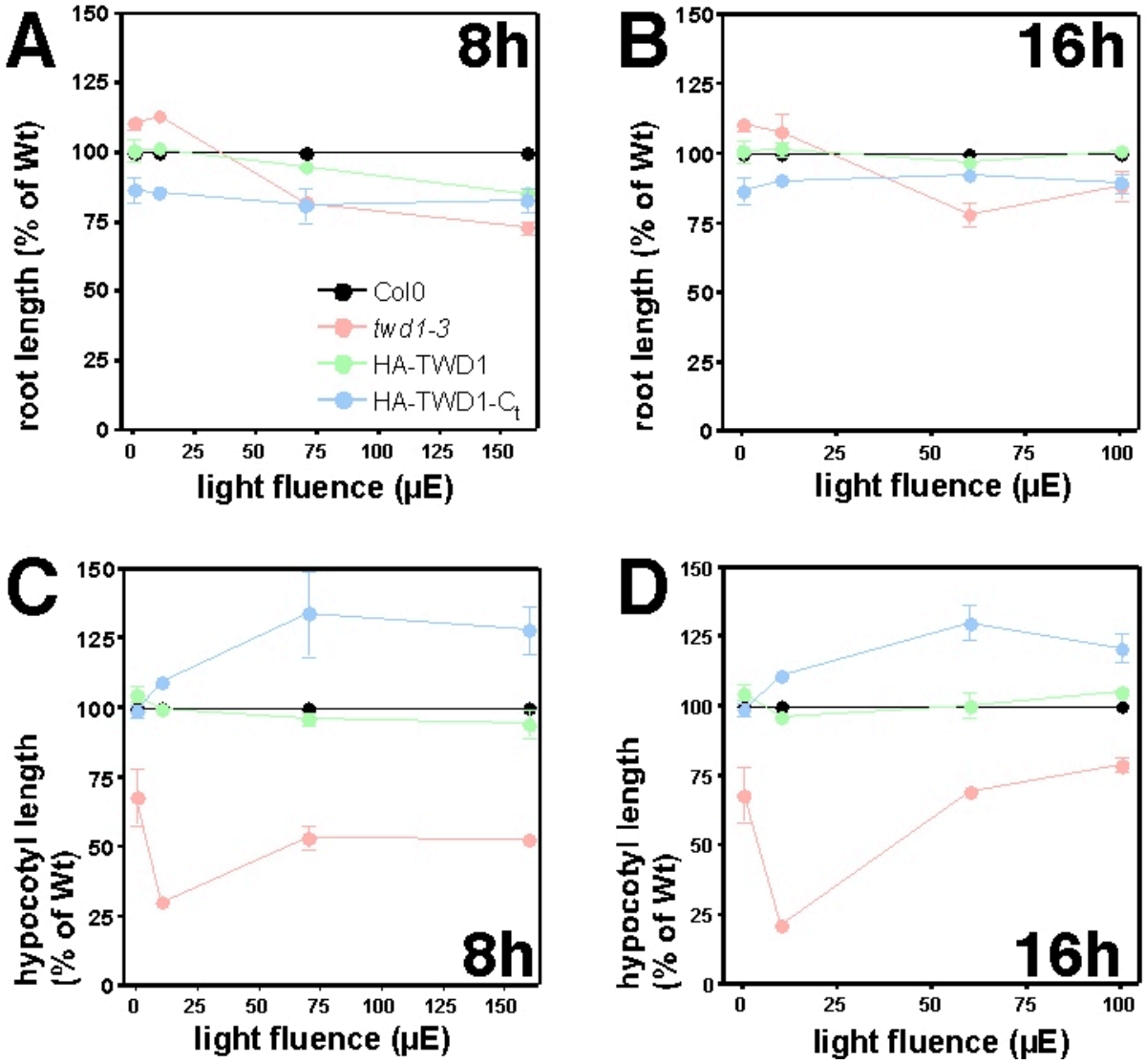
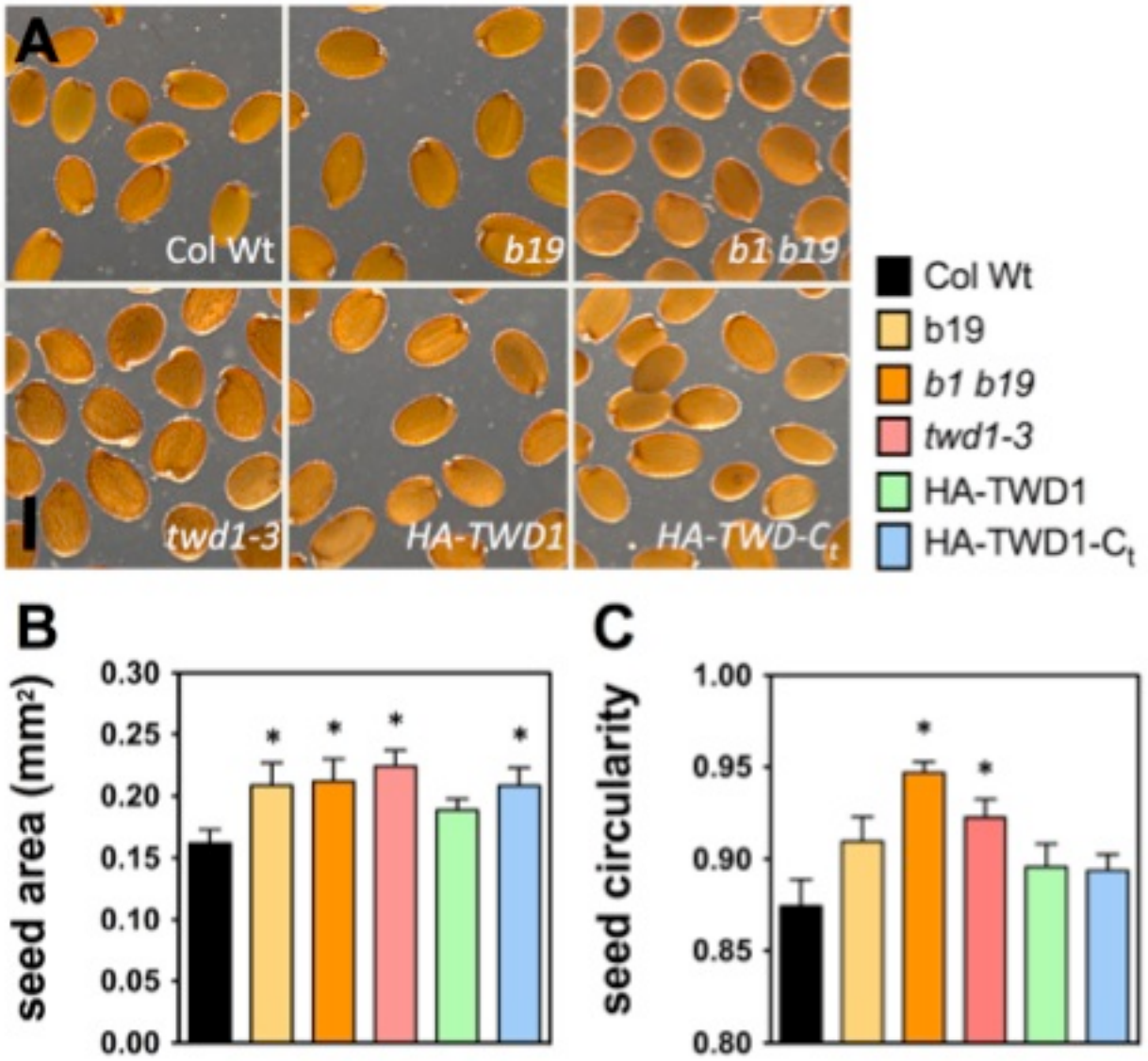
Running title: TWISTED DWARF1 controls cell elongation

Supporting Figures

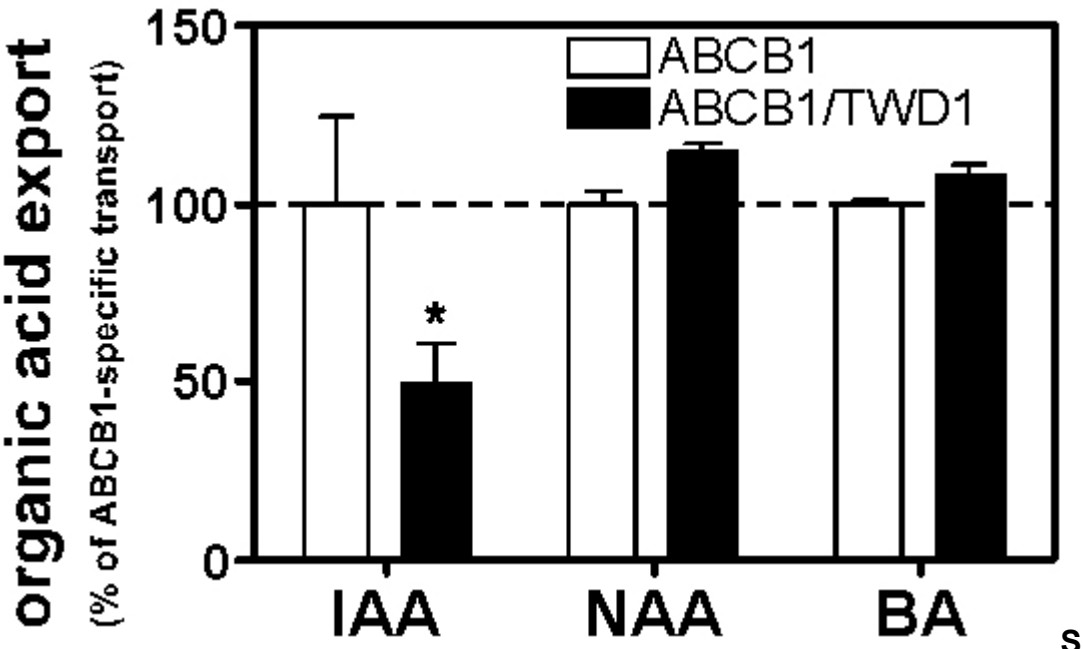
Supporting Figure S1: HA-TWD1-C_t rosettes are bushier than Wt (Col Wt), *twd1* (*twd1-3*) or HA-TWD1 grown for 40 dag under 8h light caused by an elevated number of rosette leaves. See Supporting Table S2 online for quantification.



Supporting Figure S2: Light fluence-dependency of root (A-B) and hypocotyl length (C-D) of wild-type (Col0), *twd1* (*twd1-3*), HA-TWD1 or HA-TWD1-C_t lines under short-day (8h light) and long-day (16h light) conditions. Note inverse behavior of *twd1* roots hypocotyls under dark conditions, respectively, and conversion of long primary roots into short roots by light. Mean ± SE; n = 4.

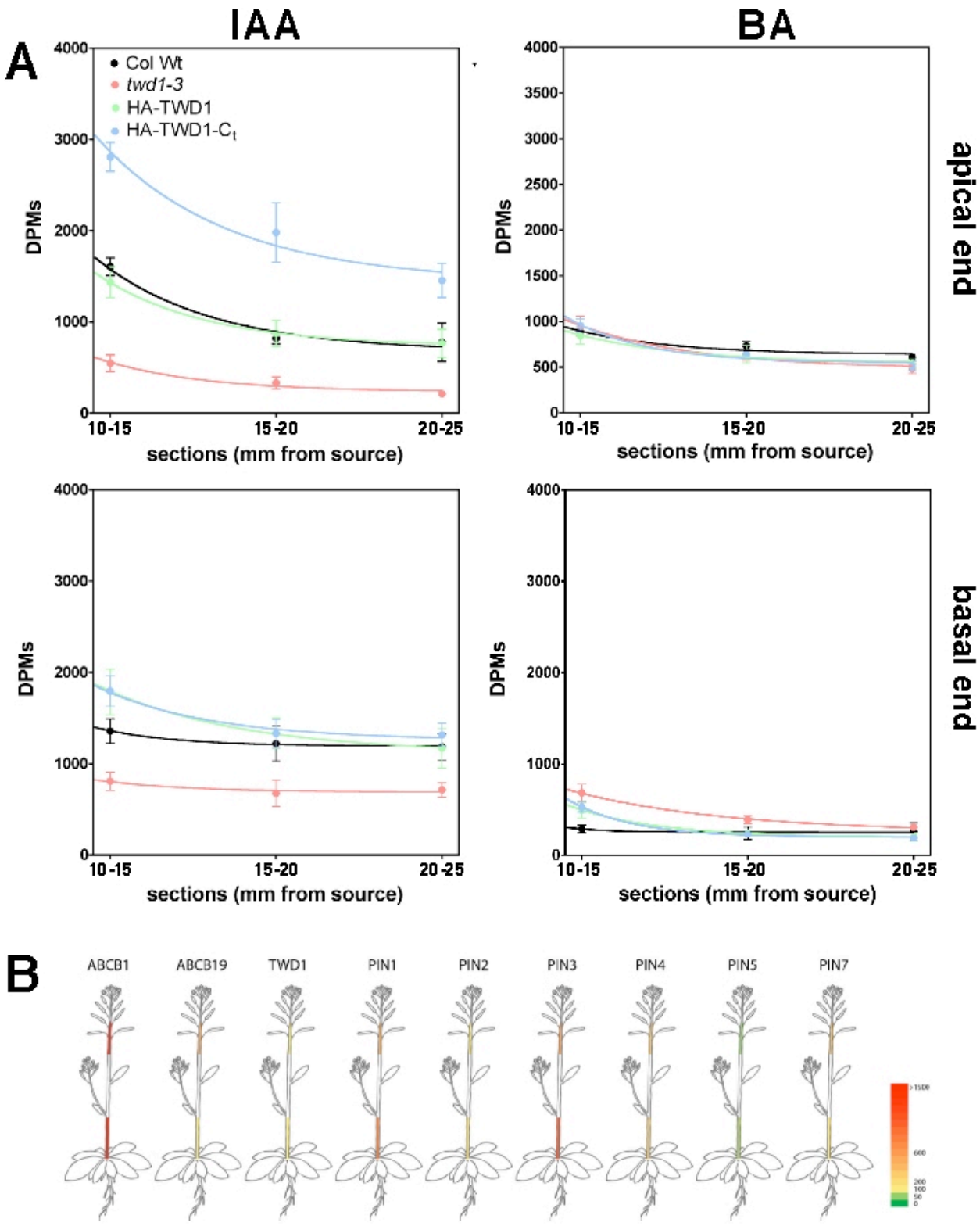


Supporting Figure S3: Seed size and circularity of *TWD1* and *ABCB* mutant alleles. Seed size and circularity was determined after imbibing water for 3 days; mean ± SE; n = 3 Significant differences (ANOVA using the Tukey's test for multiple comparisons: $p < 0.05$) to Wt are indicated by an asterisk.



Supporting

Figure S4: TWD1 inhibits ABCB1-mediated IAA efflux but not of synthetic auxin, NAA, or of specificity control, benzoic acid (BA) from the yeast, *S. cerevisiae*. Mean ± SE; n = 4-10. Significant differences (ANOVA using the Tukey's test for multiple comparisons: $p < 0.05$) -/+ TWD1 is indicated by an asterisk.

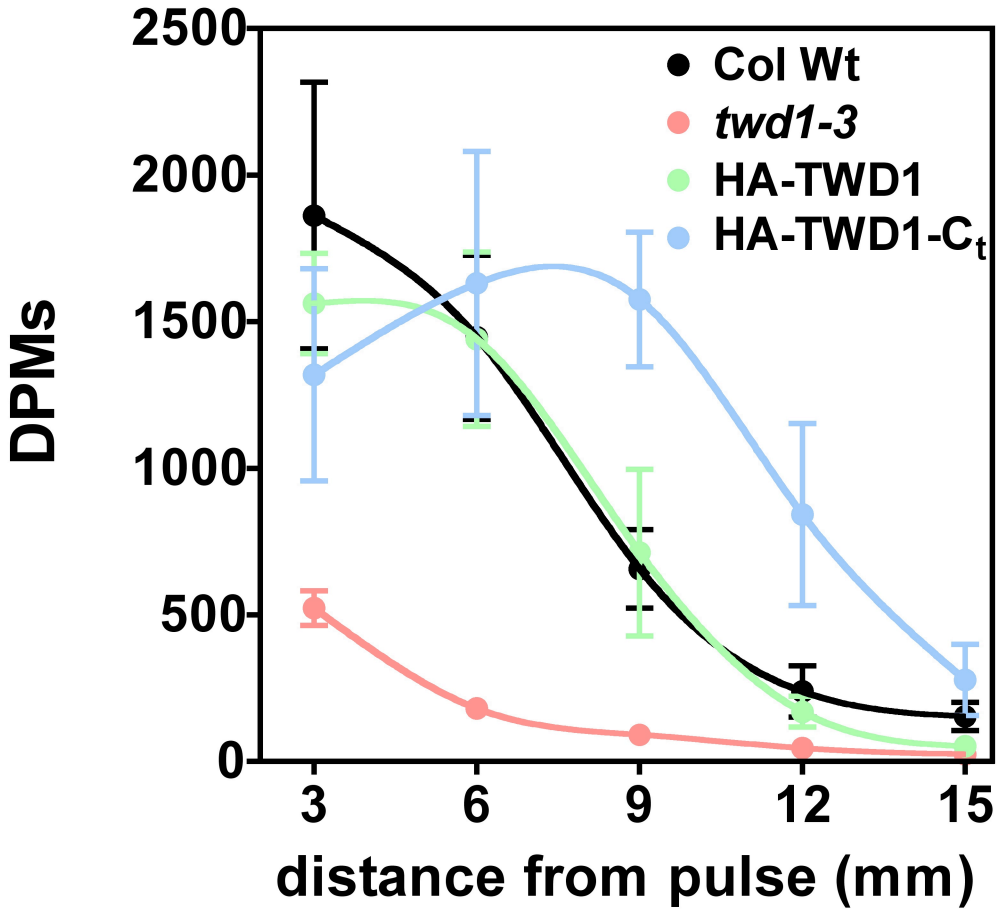


Supporting Figure S5: Basipetal transport of IAA in TWD1 loss- and gain-of-function stems.

(A) Basipetal IAA transport of wild type (Col Wt), *twd1-3*, HA-TWD1 and HA-TWD1-C_t

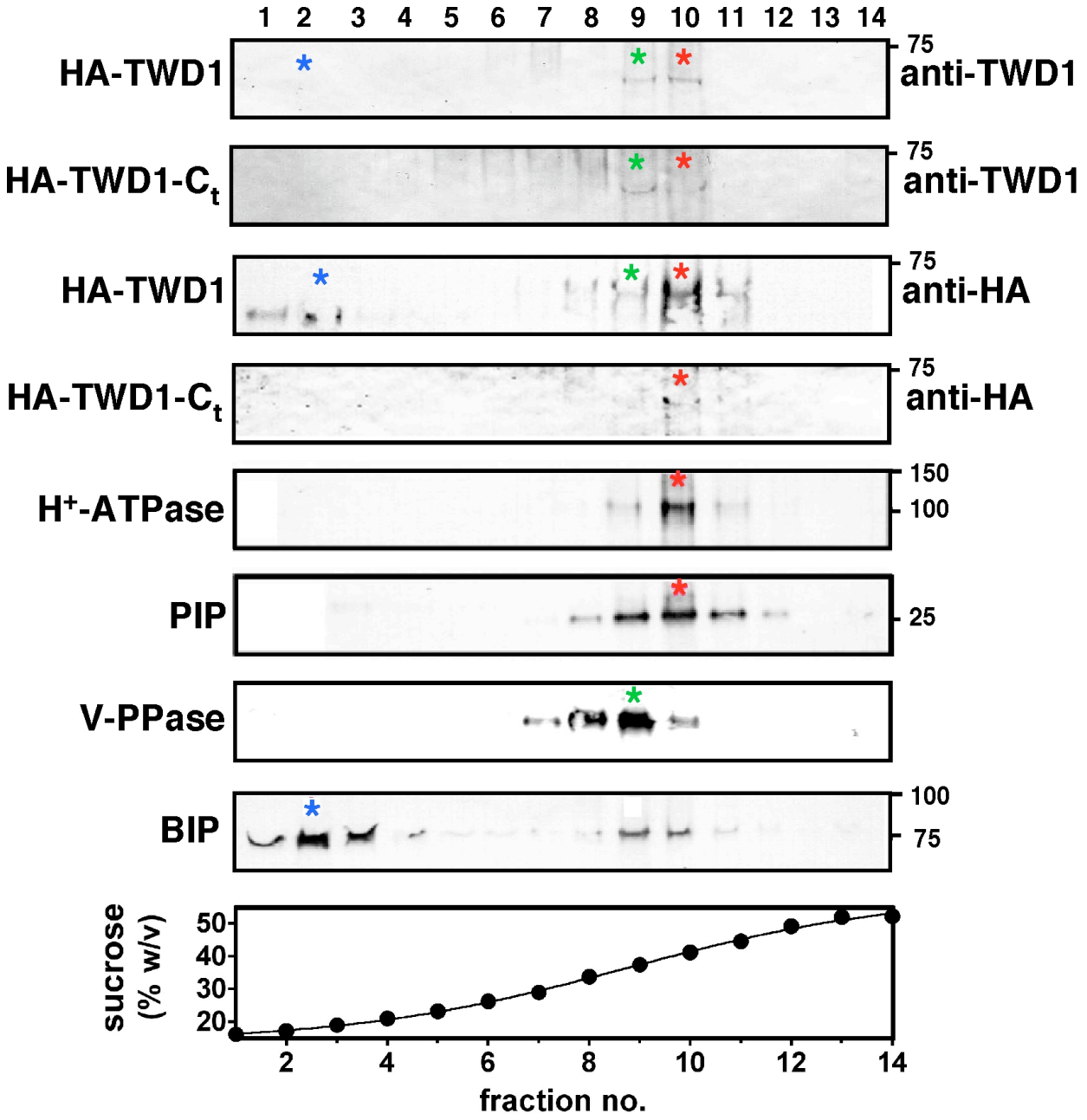
stems. Mean ± SE; n >12. Note different behavior between apical (shoot-ward) and basal (root-ward) stem ends; sections 15-20 mm are presented in Fig. 5A.

(B) Heat-map presentation of ABCB1, B19, TWD1 and PIN1-7 expression in 40 day *Arabidopsis* plants are taken from eFP (www.bar.utoronto.ca/efp/development).



Supporting Figure S6: Pulse-chase transport of IAA in *TWD1* loss- and gain-of-function stems.

Basipetal IAA transport of wild type (Col Wt), *twd1-3*, HA-TWD1 and HA-TWD1-C_t stems after a pulse of cold IAA (1000 x excess). Mean ± SE; n = 12.



Supporting Figure S7: Separation of TWD1 gain-of-function microsomes using discontinuous sucrose gradient centrifugation.

HA-TWD1 and HA-TWD1-C_t detected by using anti-TWD1 and anti-HA co-migrate with PM markers, H^+-ATPase and PIP, in linear sucrose gradients (red asterisks). Note partial overlap between HA-TWD1 and vacuolar marker, V-PPase (green asterisks) and ER marker, BIP (blue asterisks).

Supplemental Tables

Stage	Description	Long Day Col Wt	Long Day *twd1-3*	Long Day HA-TWD1	Long Day HA-TWD1-Ct	Short Day Col Wt	Short Day *twd1-3*	Short Day HA-TWD1	Short Day HA-TWD1-Ct
		(Days mean ± S.D.)				(Days mean ± S.D.)			
Principal growth stage 0	*Seed germination*								
0.1	*Seed imbibition*	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
0.5	*Radicle emergence*	4.75 ± 0.52	5.18 ± 0.78	4.89 ± 0.67	4.8 ± 0.79	4.25 ± 0.47	4.22 ± 0.43	4.31 ± 0.64	4.29 ± 0.46
0.7	*Hypocotyl and cotyledon emergence*	5.33 ± 0.64	5.79 ± 0.78	5.47 ± 0.62	5.43 ± 0.78	5.15 ± 0.50	5.11 ± 0.30	5.17 ± 0.63	5.15 ± 0.39
Principal growth stage 1	*Leaf development*								
1	*Cotyledons fully opened*	6.08 ± 0.93	6.82 ± 1.08	6.11 ± 0.84	6.21 ± 1.03	6.16 ± 0.88	5.89 ± 0.53	5.94 ± 0.71	6.02 ± 0.76
1.02	*2 rosette leaves >1 mm*	11.41 ± 1.58	12.89 ± 1.72	11.44 ± 1.63	11.72 ± 1.47	15.02 ± 2.20	16.10 ± 2.28	14.33 ± 1.91	15.67 ± 2.53
1.04	*4 rosette leaves >1 mm*	13.95 ± 0.94	14.47 ± 1.40	13.89 ± 1.11	14.15 ± 0.79	18.52 ± 1.95	19.69 ± 1.44	17.70 ± 1.93	18.83 ± 1.68

Measurement	Unit	Growth Stage	Long Day Col Wt	Long Day *twd1-3*	Long Day HA-TWD1	Long Day HA-TWD1-Ct	Short Day Col Wt	Short Day *twd1-3*	Short Day HA-TWD1	Short Day HA-TWD1-Ct
			(Mean ± S.D.)				(Mean ± S.D.)			
Number of rosette leaves	*Count*	1	4.69 ± 1.05	3.48 ± 1.24	4.98 ± 1.20	4.93 ± 1.22	4.58 ± 1.59	3.04 ± 1.31	4.99 ± 1.75	4.32 ± 1.49
Length of primary root	*mm*	1	48.63 ± 11.93	38.28 ± 10.6	53.80 ± 16.33	46.55 ± 10.73	37.87 ± 12.41	28.53 ± 8.96	38.70 ± 12.56	32.45 ± 11.33
Number of secondary roots	*Count*	R6*	12.52 ± 5.46	7.98 ± 5.28	14.71 ± 6.00	16.02 ± 4.77	6.10 ± 3.28	2.32 ± 2.73	6.94 ± 3.58	7.94 ± 3.41
Rosette, total exposed leaf area	*mm* ^{2}	R6*	33.33 ± 14.91	13.19 ± 4.54	41.42 ± 16.17	43.16 ± 14.97	22.17 ± 14.98	10.17 ± 2.95	26.26 ± 12.84	26.52 ± 11.43
Rosette, perimeter	*mm*	R6*	52.96 ± 15.31	16.00 ± 4.11	55.71 ± 17.35	61.62 ± 17.80	35.61 ± 16.02	13.7 ± 3.09	42.55 ± 16.76	41.17 ± 14.56
Rosette, major axis	*mm*	R6*	8.19 ± 1.96	4.79 ± 0.91	9.15 ± 2.05	9.73 ± 2.24	6.29 ± 1.94	4.34 ± 1.03	6.66 ± 1.75	6.65 ± 1.60
Rosette, minor axis	*mm*	R6*	5.05 ± 1.24	3.44 ± 0.62	5.61 ± 1.36	5.55 ± 1.08	5.14 ± 1.84	3.78 ± 0.75	5.08 ± 1.38	5.22 ± 1.18
Rosette, eccentricity	*None*	R6*	0.64 ± 0.31	0.68 ± 0.30	0.70 ± 0.30	0.67 ± 0.30	0.51 ± 0.34	0.45 ± 0.31	0.63 ± 0.30	0.62 ± 0.30

Supporting Table 1: Phenotypic analysis of wild-type (Col Wt), *twd1-3*, HA-TWD1 and HA-TWD1-C_t plants grown under long-day (16h light) and short-day (8h light) conditions until growth stage 1 (Boyes et al., 2001). nd; not determined.

		Long Day				Short Day			
		Col Wt	*twd1-3*	HA-TWD1	HA-TWD1-Ct	Col Wt	*twd1-3*	HA-TWD1	HA-TWD1-Ct
Stage	**Description**	(Days mean ± S.D.)				(Days mean ± S.D.)			
Principal growth stage 1	**Leaf development**								
1.02	*2 rosette leaves >1 mm in length*	11.43 ± 0.50	11.37 ± 0.48	11.72 ± 0.55	11.67 ± 0.47	17.00 ± 0.85	17.63 ± 0.94	16.57 ± 0.49	16.48 ± 0.71
1.03	*3 rosette leaves >1 mm in length*	14.42 ± 0.49	14.36 ± 0.49	14.50 ± 0.50	14.37 ± 0.48	19.85 ± 0.99	21.58 ± 1.20	19.29 ± 0.80	19.05 ± 1.06
1.04	*4 rosette leaves >1 mm in length*	15.43 ± 0.50	15.40 ± 0.49	15.50 ± 0.50	15.42 ± 0.49	21.48 ± 1.26	23.80 ± 1.31	20.25 ± 0.43	20.41 ± 0.96
1.05	*5 rosette leaves >1 mm in length*	16.35 ± 0.48	16.67 ± 0.57	16.48 ± 0.50	16.62 ± 0.49	24.85 ± 0.89	28.09 ± 1.74	24.00 ± 0.89	24.25 ± 1.35
1.06	*6 rosette leaves >1 mm in length*	17.60 ± 0.49	18.77 ± 0.72	17.55 ± 0.50	17.82 ± 0.39	26.93 ± 1.12	30.29 ± 1.83	25.82 ± 0.47	26.07 ± 1.27
1.07	*7 rosette leaves >1 mm in length*	18.93 ± 0.63	20.63 ± 0.68	18.90 ± 0.81	19.28 ± 0.52	28.85 ± 0.86	35.09 ± 2.31	27.64 ± 0.97	28.51 ± 1.29
1.08	*8 rosette leaves >1 mm in length*	20.02 ± 0.34	22.23 ± 0.56	20.22 ± 0.80	20.68 ± 0.50	30.15 ± 0.76	37.38 ± 2.10	29.59 ± 0.81	29.89 ± 1.05
1.09	*9 rosette leaves >1 mm in length*	21.35 ± 0.65	23.40 ± 0.71	21.37 ± 0.66	21.82 ± 0.47	31.70 ± 1.08	44.43 ± 3.94	30.70 ± 0.71	31.11 ± 1.14
1.10	*10 rosette leaves >1 mm in length*	22.35 ± 0.51	24.80 ± 0.77	22.53 ± 0.58	22.98 ± 0.29	33.36 ± 1.20	50.91 ± 4.10	32.21 ± 0.56	32.72 ± 0.95
1.11	*11 rosette leaves >1 mm in length*	23.27 ± 0.54	26.17 ± 0.92	23.33 ± 0.62	23.92 ± 0.42	35.46 ± 1.57	57.91 ± 4.17	34.04 ± 1.10	35.13 ± 1.35
1.12	*12 rosette leaves >1 mm in length*	24.32 ± 0.65	27.64 ± 0.92	24.45 ± 0.72	25.03 ± 0.48	37.04 ± 1.61	68.96 ± 4.80	35.50 ± 0.50	35.91 ± 0.87
1.13	*13 rosette leaves >1 mm in length*	25.59 ± 0.85	28.90 ± 0.84	25.53 ± 0.62	26.55 ± 0.69	38.39 ± 1.63	78.91 ± 4.76	36.50 ± 0.82	37.21 ± 1.19
1.14	*14 rosette leaves >1 mm in length*	26.66 ± 0.89	30.25 ± 0.89	26.58 ± 0.74	27.65 ± 0.60	40.14 ± 1.75	87.68 ± 5.64	37.93 ± 1.03	38.71 ± 1.40
Principal growth stage 5	**Inflorescence emergence**								
5.1	*First flower buds visible*	25.68 ± 1.48	31.24 ± 1.37	27.37 ± 1.66	32.98 ± 1.12	96.77 ± 7.96	ND	91.77 ± 7.38	70.88 ± 4.24
Principal growth stage 6	**Flower production**								
6	*First flower open*	33.45 ± 1.16	37.85 ± 1.74	34.67 ± 1.33	41.97 ± 1.74	104.94 ± 5.33	ND	101.47 ± 5.39	81.33 ± 4.42
6.9	*Flowering complete*	53.62 ± 1.44	70.63 ± 3.19	54.02 ± 1.72	73.61 ± 1.64	ND	ND	ND	100.91 ± 3.41
Principal growth stage 8	**Silique ripening**								
8	*First silique shattered*	57.95 ± 2.16	69.03 ± 3.18	57.41 ± 2.02	72.07 ± 2.53	126.19 ± 4.49	ND	128.74 ± 5.88	110.09 ± 3.65

			Long Day				Short Day			
			Col Wt	*twd1-3*	HA-TWD1	HA-TWD1-Ct	Col Wt	*twd1-3*	HA-TWD1	HA-TWD1-Ct
Measurement	**Unit**	**Growth Stage**	(Mean ± S.D.)				(Mean ± S.D.)			
Number of cotyledons	*Count*	**1.04**	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.02 ± 0.13	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00

			Long Day				Short Day			
			Col Wt	*twd1-3*	HA-TWD1	HA-TWD1-Ct	Col Wt	*twd1-3*	HA-TWD1	HA-TWD1-Ct
Measurement	**Unit**	**Growth Stage**	(Mean ± S.D.)				(Mean ± S.D.)			
Number of cotyledons	*Count*	**1.04**	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.02 ± 0.13	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.0
Rosette, total exposed leaf area	*mm2*	**1.12***	484.62 ± 70.67	109.71 ± 18.66	515.73 ± 69.50	674.36 ± 121.86	199.97 ± 26.40	66.09 ± 21.57	208.33 ± 21.94	306.47 ± 26.22
Rosette, perimeter	*mm*	**1.12***	226.88 ± 26.06	41.49 ± 4.29	224.39 ± 43.22	245.92 ± 40.58	163.61 ± 16.88	32.76 ± 6.29	162.81 ± 17.31	196.99 ± 17.25
Rosette, major axis	*mm*	**1.12***	27.08 ± 2.49	12.74 ± 1.26	28.22 ± 2.46	32.06 ± 3.20	17.10 ± 1.14	9.77 ± 1.65	17.94 ± 1.22	21.93 ± 1.34
Rosette, minor axis	*mm*	**1.12***	22.72 ± 1.82	10.91 ± 0.89	23.57 ± 2.28	26.62 ± 2.62	14.84 ± 1.18	8.41 ± 1.44	14.77 ± 1.01	17.8 ± 1.10
Rosette, eccentricity	*None*	**1.12***	0.62 ± 0.27	0.68 ± 0.31	0.52 ± 0.31	0.69 ± 0.30	0.54 ± 0.32	0.64 ± 0.25	0.80 ± 0.22	0.74 ± 0.27
Number of rosette leaves	*Count*	**6.00**	13.03 ± 1.50	14.05 ± 1.22	14.96 ± 1.70	18.60 ± 1.42	ND	ND	ND	ND
Number of stem branches on main bolt	*Count*	**6.50**	3.23 ± 0.67	2.19 ± 0.51	4.07 ± 0.70	5.02 ± 0.79	ND	ND	ND	ND
Number of side bolts >1 cm	*Count*	**6.50**	3.05 ± 1.02	2.53 ± 0.82	2.58 ± 0.98	0.02 ± 0.00	ND	ND	ND	ND
Silique, area	*mm2*	**6.50**	10.33 ± 1.74	4.99 ± 1.10	9.14 ± 1.57	10.22 ± 2.15	12.68 ± 2.86	ND	13.83 ± 3.59	11.23 ± 2.39
Silique, perimeter	*mm*	**6.50**	29.15 ± 3.17	14.78 ± 1.53	25.71 ± 1.81	27.46 ± 1.84	27.42 ± 3.01	ND	28.61 ± 2.60	26.52 ± 2.56
Silique, major axis	*mm*	**6.50**	14.36 ± 1.50	6.53 ± 0.88	12.81 ± 1.37	13.27 ± 1.26	13.67 ± 1.50	ND	14.29 ± 1.31	12.67 ± 1.76
Silique, minor axis	*mm*	**6.50**	0.91 ± 0.10	0.97 ± 0.15	0.91 ± 0.13	0.98 ± 0.18	1.17 ± 0.18	ND	1.22 ± 0.24	1.12 ± 0.15
Stem length	*cm*	**6.90**	32.31 ± 2.70	12.67 ± 2.41	31.88 ± 1.84	60.74 ± 3.58	11.33 ± 11.16	ND	14.04 ± 10.58	57.35 ± 7.33
Seed, area	*mm2*	**9.70**	2.03 ± 0.49	1.88 ± 0.24	1.84 ± 0.46	1.79 ± 0.41	0.20 ± 0.05	ND	0.18 ± 0.04	0.17 ± 0.02
Seed, perimeter	*mm*	**9.70**	18.32 ± 2.42	17.35 ± 1.59	17.09 ± 2.34	16.69 ± 2.04	1.73 ± 0.19	ND	1.61 ± 0.17	1.57 ± 0.10
Seed, major axis	*mm*	**9.70**	6.56 ± 0.76	6.10 ± 0.4	7.07 ± 0.65	5.94 ± 1.44	0.66 ± 0.08	ND	0.61 ± 0.07	0.59 ± 0.04
Seed, minor axis	*mm*	**9.70**	4.93 ± 1.51	4.60 ± 0.45	3.67 ± 1.47	4.60 ± 1.36	0.39 ± 0.05	ND	0.37 ± 0.05	0.36 ± 0.04
Seed , eccentricity	*None*	**9.70**	7.22 ± 1.59	7.49 ± 3.04	7.56 ± 2.12	6.65 ± 1.52	0.62 ± 0.32	ND	0.66 ± 0.32	0.66 ± 0.30

Supporting Table 2: Phenotypic analysis of wild type (Col Wt), *twd1-3*, HA-TWD1 and HA-TWD1-C$_t$ plants grown under long-day (16h light) and short-day (8h light) conditions starting with growth stage 1 (Boyes et al., 2001). nd; not determined.

Supporting Table 3: Summary of morphological and physiological parameters measured in this study.

line	hypocotyl		IAA transport				IAA content			
	length[1]	elongation	cellular	PAT			DR5	free IAA		
			leaf	root	hypocotyl	stem	hyp.	root	hypocotyl	stem
twd1-3	– –	–	–	–	nd	– [2]	–	+	nd	– [2]
HA-TWD1	± [3]	+ + [3]	±	±	±	±	±	±	±	±
HA-TWD1-Ct	+ +	+ +	+	–	–	+	+	+	+	–

[1] Short day conditions.
[2] Discrepancy between stem PAT and free IAA quantification might be caused by technical limitations during PAT measurements of *twd1-3* stems that show a high degree of epidermal twisting in comparison to wild type.
[3] Discrepancy between hypocotyl lengths and elongation rates might be caused by technical limitations of the hypocotyl elongation assays performed on excised hypocotyl segments allowing lateral auxin penetration into segments.