

Figure & Table captions for Supplementary materials:

Table S1. Root traits of *A. thaliana* accessions on NPA and MS media, Related to Figure 1 and 2

ID: Identifier of *A. thaliana* natural accessions as used in GWAPP (Seren et al., 2012); Accession name: common accessions name; Origin: country of collection; 10 μ M NPA treatment: traits acquired from images of plants (6 DAG) grown on media (1 \times MS media, 1% sucrose, 0.8% agar, 16 hours light conditions, 21 $^{\circ}$ C) containing 10 μ M of auxin transport inhibitor NPA; Control: traits acquired from images of plants (5 DAG) grown on media (1 \times MS media, 1% sucrose, 0.8% agar, 16 hours light conditions, 21 $^{\circ}$ C). Control data from (Slovak et al., 2014). Haplogroup: classification based on haplotypes around the *EXOCYST70A3* locus. C: Common-sensitivity to NPA; H: High-sensitivity to NPA.

Table S2. Gene models in proximity to the significantly associated locus for Horizontality under NPA treatment, Related to Figure 1 and 2.

Tair10 gene models in proximity (3 KB distance) to the significant SNP (passing the Bonferroni corrected threshold for approximately 215,000 tests, p -value $< 10^{-7}$).

Table. S3. Seed production under control (sufficient water) and drought conditions, Related to Figure 7

Haplogroup: classification based on haplotypes around the *EXOCYST70A3* locus. C: Common-sensitivity to NPA; H: High-sensitivity to NPA.; Treatment: water conditions during plant growth. Control: 65% water/soil.; Drought: 25% water/soil.; Replicate: experimental replicates; GWAS ID: Identifier of *A. thaliana* natural accessions as used in GWAPP (26).

Figure S1. Trait correlations of untreated and NPA treated roots of 215 *A. thaliana* natural accessions, Related to Figure 1

(A) Histograms of data distribution for traits of NPA treated plants. x-axis: phenotype value; y-axis: Frequency. (B) z-scores of each root trait were compared between control and NPA treatments. x-axis: control; y-axis: 10 μ M NPA treatment. r indicates Pearson's correlation coefficient between control and NPA treatment in relation to the phenotype. p -value shows the significance of these correlations.

Figure S2. Analysis of the locus around the GWA-significant SNP, Related to Figure 1, 2 and 3

(A) Two haplogroups that divide 215 natural accessions according to 40k bp sequences around the SNP that was found to be significantly associated with Horizontality by GWA mapping (Table S2). Haplotype structures were analyzed by fastPHASE (Scheet and Stephens, 2006) and visualized by blocks with different colors using 250K SNPs data from the Regional Mapping Project (Horton et al., 2012) with the dendrogram indicating the haplogroups. Trait (Horizontality, white circles) values and the nucleotides of the accessions at the SNP position (green and orange bars) are indicated. Each peripheral node in the top dendrogram shows a natural accession of *A. thaliana*. Black horizontal lines show the *EXO70A3* gene. Accessions between green dashed lines belong in haplogroup H and other accessions in haplogroup C.

(B) Gene model and polymorphisms among 30 natural accessions for the genomic region surrounding the significant GWA SNP. Red line: location of the significant GWA SNP. Top of the

panel: a magnification of the Manhattan plot for this genomic region. x-axis: chromosomal position; y-axis: significance (negative logarithmic of *p*-value); Black dashed line: the threshold of significance (Bonferroni-corrected *p*-value); Red dot: top SNP; Blue dots: other SNPs in the region. Middle of the panel: a gene model of this locus. arrows: gene; boxes: exon. Bottom of the panel: SNPs and amino acid substitutions in this locus in 30 accessions. Depicted are representative accessions of haplogroups C and H. Data from 1001 genome project (Schmitz et al., 2013, 1001genomes.org). Gray boxes: genomic locus equivalent to Col-0; Gray lines: SNP in non-coding region; Cyan lines: synonymous SNP; Magenta lines: non-synonymous SNP which is exclusively for Haplogroup H; Yellow lines: other non-synonymous SNPs. (C) Gene models of natural accessions that were used in *EXO70A3* allelic complementation analysis. Orange arrow: genomic sequence of Col-0 *EXO70A3* coding region; Light green boxes: exon; Gray bar: intergenic region; Black bars: genomic locus of various natural accessions that are equivalent to Col-0 *EXO70A3*; Gray lines: SNP in non-coding region; Cyan lines: synonymous SNP; Magenta lines: non-synonymous SNP which is exclusively for Haplogroup H; Yellow lines: other non-synonymous SNPs.

Figure S3. Gene expression patterns of *EXO70A3* and neighboring genes, Related to Figure 2, 4 and 5

(A-D) Tissue-, cell-type specific, and auxin related treatment-dependent expression pattern of *EXO70A3* gene from publicly available microarray datasets visualized by the Arabidopsis eFP browser ((Winter et al., 2007), <http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi>). Signals less than or equal to 20 (the background value of AtGEN Express (Goda et al., 2008; Schmid et al., 2005)) were masked with a grey color. Signals above this threshold were color coded in relation to their average levels in the respective datasets (see color legend for each data set). (A) AtGen Express data (organ level). (B) Cell-type specific RootMap (cellular level). (C, D) IAA and NPA treatments (whole seedlings). (E) *EXO70A3* expression profile in various root cells and tissues which was quantified by RNA-Seq (data collected from Li *et al.*, 2017). x-axis: cells/tissues; y-axis: transcript level (Fragments per kilo base per million mapped reads: FPKM). (F-I) qRT-PCR results of expression levels of *EXO70A3* and related genes. Transcript levels of proximal genes of *EXO70A3*, AT5G52340 (F) and AT5G52360 (G), in various organs of Col-0 and *EXO70A3ox*. x-axis: sample; y-axis: relative expression level (normalized to Col-0 Whole); Error bars: standard deviation. (H) Gene expression levels of five *PIN* and *EXO70A3* genes in root tip of *EXO70A3ox*. x-axis: gene; y-axis: transcript level (normalized to Col-0); error bars: standard deviation. Asterisk: significance evaluated by Welch t-test (p -value = 2.2×10^{-3} , $n = 6$). (I) Expression levels of *EXO70A3* in root tips of 4 natural accessions of the haplogroups C and H, Col-0, and *EXO70A3ox* lines. Expression levels normalized to Col-0. x-axis: sample; y-axis: normalized expression level; Error bars: standard deviation; Asterisk: significance evaluated by Student t-test (p -value = 0.014). Haplogroups C and H were not distinctive (ANOVA > 0.05).

Figure S4. PIN:GFP localization in *EXO70A3ox/PIN::PIN:GFP* lines, Related to Figure 4

Confocal images of representative roots grown on MS media. Green: GFP signal; Red: cell wall stained with propidium iodide (PI); Yellow bars: 100 μ m. (A) Median longitudinal optical sections of root tips (5 DAG) of *PIN1::PIN1:GFP*, *PIN2::PIN2:GFP*, *PIN3::PIN3:GFP*, and *PIN7::PIN7:GFP* lines and crosses with the *EXO70A3ox* line are shown. (B) Confocal images of representative lateral roots (15 DAG) of

PIN4::PIN4-GFP and a cross with *EXO70A3ox* line. Upper panels: overview of low resolution images of emerging lateral roots. Boxes indicate the position of high-resolution images depicted below. Lower panel: median longitudinal optical sections of lateral root tips.

Figure S5. Phenotypic analysis of *EXO70A3*-related lines, Related to Figure 4 and 5

(A-E) Various auxin-related traits including Statocytes and statolith, IAA responses in primary roots and hypocotyl, lateral root density, shoot gravitropism, rosette development, phyllotaxy, floral stem height and inflorescence structure in *EXO70A3*-related lines. *CRISPR-exo70A3-1* (A), *CRISPR-exo70A3-2* (B), *EXO70A3ox* (C), *35S::GFP:EXO70A3* (D) and *pin4-3* (E) were compared with Col-0. Statocytes and statolith: representative root tips of plants (5 DAG) grown on MS plates as visualized by Lugol staining. Black bars: 20 μ m. IAA response in primary root: Plants were treated with 0.1, 1, 10, or 100 nM IAA on 1 \times MS plates and pictured at 5 DAG. x-axis: treatments; y-axis: primary root length; Error bars: standard deviation; “a” and “b”: two significantly different groups (evaluated by post-hoc Tukey test after ANOVA, p -value < 0.05). Hypocotyl elongation in response to IAA: Plants were treated with 10 or 100 nM IAA on 1 \times MS plates and pictured at 5 DAG. x-axis: treatments; y-axis: hypocotyl length; Error bars: standard deviation; “a” and “b”: two significantly different groups (evaluated by post-hoc Tukey test after ANOVA, p -value < 0.05). Lateral root density: representative lateral root structures of plants. Plants were grown on 1 \times MS plates until 5 DAG and equal size 5 plants were transferred to fresh 1 \times MS plate for further growth, then pictured at 20 DAG. Yellow bars: 5 cm; x-axis: average lateral root density [number/cm primary root]; y-axis: genotype; Error bars: standard deviation. Shoot gravitropism: inflorescence angle changes during shoot gravitropism response of 40 DAG plants. x-axis: time after rotation [h]; y-axis: average angle change [$^{\circ}$]; Error bars: standard deviation. Rosette development, phyllotaxy, floral stem height and floral structure: Mutant plants were grown with Col-0 plants on soil side by side under long day conditions (16 hours light, 21 $^{\circ}$ C/8 hours dark, 16 $^{\circ}$ C) and pictured at 15 and 30 DAG (rosette development and phyllotaxy), 45 DAG (inflorescence structure) and 50 DAG (floral stem height). Yellow bars: 5 cm; x-axis: genotype; y-axis: phenotypes (indicated in charts); Error bars: standard deviation; WT: Col-0; Mutant: mutant line which is indicated in the title of each figure. NPA response (E): Col-0 and *pin4-3* plants were grown on 1 \times MS plates until 5 DAG and equal size plants were transferred to chemical treatment plates (mock and 0.5 μ M NPA), then pictured (1 day after transfer). Beanplot: x-axis: treatment; y-axis: root tip angle [$^{\circ}$]; Red lines: mean; short lines: individual data points; shape: density of the data distribution. **(F)** Variances of root tip angles during dynamic root re-orientation of *pin4-3* and *pin4-3 EXO70A3ox*. Significance of the difference of variances between Col-0 and mutant was analyzed by Fisher test and color coded. x-axis: time [hours], y-axis: standard deviation of root tip angles. **(F)** Average angles and variances of root tips during dynamic root re-orientation of *EXO70A3*-related lines and *pin4-3*-related lines. x-axis shows genotype. Top panel: y-axis shows average root tip angle (6 hour after plate re-orientation), black point: genotype which showed significant phenotype in comparison with Col-0; Middle panel: y-axis shows root tip angle variation (10 hour after plate re-orientation), black point: genotype which showed significant phenotype in comparison with Col-0; Bottom panel: Rose plots to show root tip angle distribution (10 hour after plate re-orientation). black bar: occupancy (%) of roots in the range of direction in all roots (distance from center of circles shows the occupancy level); *pin4-3 EXO70A3ox* showed *pin4-3*-type phenotypes (no delay of root bending and the larger variation of root tip angles) but not *EXO70A3* mutants-type phenotypes (the delay of root bending and the larger variation of root tip angles), indicating that

EXO70A3 is upstream of *PIN4*.

Figure S6. Exploration of signals of selection in *EXO70A3* locus, Related to Figure 7

(A) Selection signals around the significantly associated SNP as shown in the Selection Browser (Horton et al., 2012). Orange line: the position of the significant GWAS SNP; Red arrow: the closest significant selection signal (p -value < 0.01). (B) Distribution of associations of SNPs with precipitation seasonality that were reported in GWAS for climate adaptation (Hancock et al., 2011). x-axis: significance of association (Spearman's correlation coefficient: ρ). y-axis: frequency. Area between the blue lines shows the range which contains 95% of SNPs. Red line: Correlation of the significant SNP identified by our GWA mapping (chromosome 5: 21256465) which is located outside of the 95% range.

Figure S7. Hypothetical multilevel model of *EXO70A3* function and effects on variations in traits, Related to Figure 6 and 7

Model of *EXO70A3* function based on the presented data. Lower left: Through the vesicle tethering function of *EXO70A3* which is specific to vesicles that contain *PIN4*, *PIN4* is delivered to the plasma membrane. Upper left: This occurs asymmetrically upon gravity stimulus in a subset of columella cells that are localized at the side of the root that points in the direction of gravity, leading to an auxin flux to the root side located towards the gravity direction and therefore establishes an asymmetric auxin distribution. Allelic variation of *EXO70A3* leads to altered *EXO70A3* activity and determines the threshold and dynamics of this auxin flux resulting in distinct root growth direction control properties and lateral root density patterns among *A. thaliana* natural accessions, potentially involved in adaptation to various environments including water or nutrient deprived areas.

movie S1. Dynamic root gravitropism responses of Col-0, *CRISPR-exo70A3-1* and *EXO70A3ox* after 90 degree re-orientation of roots, Related to Figure 5

Plants grown for 5 days after germination on vertical 1 × MS agar plate under long day conditions (16 hour light) at 21 °C were transferred to another 1 × MS agar plate and incubated for 1 hours under the same conditions. Then the plate was turned 90 degrees and time dependent re-orientation of root tips to gravity was captured every 4 minutes using scanners for 20 hours. The movie is a time-lapse of these images and is a mosaic assembled from 3 representative individuals for each genotype. Upper row: Col-0 wildtype plants; Middle row: *EXO70A3ox* plants; lower row: *CRISPR-exo70A3-1* plants.

movie S2. Dynamic root gravitropism responses of different *EXO70A3* allele accessions (haplogroups C and H) after 90 degree re-orientation of roots, Related to Figure 5

Plants grown for 5 days after germination on vertical 1 × MS agar plate under long day conditions (16 hour light) at 21 °C were transferred to another 1 × MS agar plate and incubated for 1 hours under the same conditions. Then the plate was turned 90 degrees and time dependent re-orientation of root tips to gravity was captured every 4 minutes using scanners for 20 hours. The movie is a time-lapse of these images and is a mosaic assembled from 1 representative individual for each accession.

Table S1. Root traits of *A. thaliana* accessions on NPA and MS media^a, Related to Figure 1 and 2

ID: Identifier of *A. thaliana* natural accessions as used in GWAPP (Seren et al., 2012); Accession name: common accessions name; Origin: country of collection; 10 μ M NPA treatment: traits acquired from images of plants (6 DAG) grown on media (1 \times MS media, 1% sucrose, 0.8% agar, 16 hours light conditions, 21 °C) containing 10 μ M of auxin transport inhibitor NPA; Control: traits acquired from images of plants (5 DAG) grown on media (1 \times MS media, 1% sucrose, 0.8% agar, 16 hours light conditions, 21 °C). Control data from (Slovak et al., 2014). Haplogroup: classification based on haplotypes around the *EXOCYST70A3* locus. C: Common-sensitivity to NPA; H: High-sensitivity to NPA.

GWAS ID	Accession Name	Origin	10 μ M NPA treatment				Control				Haplogroup (<i>EXO70A3</i> locus)				
			Length traits		Angle traits		Width traits		Length traits			Angle traits		Width traits	
			Total length	Horizontality	Skewness ^b	Average root width	Total length	Horizontality	Skewness ^b	Average root width					
86 CUR-8	France		240.00	81.82	57.20	7.69	713.69	116.04	-4.49	4.07	C				
96 LAC-5	France		394.50	309.69	57.66	8.02	619.23	70.45	2.37	4.21	H				
149 LDV-58	France		125.00	106.37	58.12	8.26	650.11	160.30	-6.63	4.79	C				
204 MIB-60	France		259.00	418.65	57.98	8.03	ND	ND	ND	ND	H				
224 MIB-86	France		263.00	101.16	58.09	7.32	ND	ND	ND	ND	H				
236 MOG-11	France		141.00	85.50	58.10	8.54	ND	ND	ND	ND	C				
260 PAR-5	France		241.00	159.01	58.17	8.13	ND	ND	ND	ND	H				
266 RAN	France		237.00	196.73	58.63	8.12	ND	ND	ND	ND	C				
394 Vou-5	France		219.50	71.89	57.78	7.08	ND	ND	ND	ND	C				
461 EM-183	UK		123.00	49.82	57.68	7.47	484.78	55.10	-3.98	3.94	C				
936 FOR-5	USA		72.00	90.24	57.25	NA	505.25	45.33	-2.99	3.98	C				
1874 MNF-Pot-80	USA		302.00	179.09	57.65	7.95	677.08	59.58	2.24	3.94	C				
2057 Map-42	USA		261.00	120.12	57.71	7.86	733.46	74.27	-0.75	4.32	C				
2171 Paw-26	USA		303.00	159.59	58.26	7.67	ND	ND	ND	ND	C				
2290 Ste-3	USA		231.50	135.80	57.50	7.27	ND	ND	ND	ND	C				
2320 Wilcox-4	USA		309.50	55.07	57.84	6.75	ND	ND	ND	ND	C				
5719 Bur-0	Ireland		294.00	66.06	56.76	8.08	963.52	307.15	-9.02	4.90	C				
5723 Chr-1	UK		17.50	53.20	57.25	NA	305.51	52.61	3.71	4.04	C				
5729 Coc-1	UK		194.00	368.71	57.77	7.76	497.95	33.21	-1.24	4.32	H				
5731 Crl-1	UK		280.00	68.90	57.71	7.27	650.44	78.34	-3.74	4.10	C				
5736 Ema-1	UK		324.00	237.27	45.74	7.93	736.29	56.40	-1.32	4.44	C				
5742 Frd-1	UK		215.50	130.35	57.45	7.70	643.36	83.76	0.24	3.67	H				
5745 Hil-1	UK		246.00	86.17	48.23	7.10	703.56	164.80	-6.06	4.35	C				
5751 Kyl-1	UK		123.00	91.49	58.20	NA	632.51	53.46	0.28	4.18	C				
5752 Lan-1	UK		258.00	154.01	57.88	7.67	701.96	138.87	-6.76	4.30	C				
5837 Bor-1	Czech		341.00	155.98	56.95	7.68	736.86	95.06	3.63	4.02	C				
6008 Duk	Czech		263.00	114.94	47.58	7.34	691.98	72.97	-4.03	4.02	C				
6074 Ör-1	Sweden		167.00	129.89	57.83	7.58	524.12	81.34	-1.54	3.79	C				
6243 Tottarp-2	Sweden		233.50	124.53	57.47	7.31	ND	ND	ND	ND	C				
6730 CIBC-5	UK		210.00	56.77	57.21	8.12	576.78	150.21	-12.35	4.42	C				
6897 Ag-0	France		249.00	130.12	57.52	7.59	ND	ND	ND	ND	C				
6898 An-1	Belgium		275.50	121.24	51.06	7.83	887.99	151.42	-3.33	4.03	C				
6899 Bay-0	Germany		304.00	104.38	55.95	6.95	777.79	74.45	-4.93	4.12	C				
6903 Bor-4	Czech		296.00	252.80	56.97	7.89	788.25	78.98	-2.61	4.26	C				
6904 Br-0	Czech		191.00	71.94	57.28	8.86	786.50	52.92	-1.45	4.24	C				
6906 C24	Portugal		208.00	109.57	57.09	8.19	614.46	83.09	-3.70	4.14	C				
6907 CIBC-17	UK		257.50	343.27	57.19	8.62	672.07	125.16	-4.63	4.39	C				
6909 Col-0	USA		340.50	160.68	57.63	6.99	741.51	48.11	-1.72	3.76	C				
6910 Ct-1	Italy		230.00	134.62	57.70	6.96	683.83	70.82	-5.15	4.01	C				
6911 Cvi-0	Cape Verde		202.00	193.77	57.90	7.51	860.06	352.44	-6.75	4.74	C				
6915 Ei-2	Germany		311.50	186.85	57.47	NA	721.11	40.90	-3.16	4.09	C				
6916 Est-1	Russia		342.00	342.86	49.21	7.69	805.90	161.72	-4.46	4.23	C				
6919 Ga-0	Germany		359.00	235.26	57.20	7.84	744.24	251.23	-13.66	4.01	C				
6920 Got-22	Germany		265.00	275.36	57.58	8.14	664.33	121.37	-6.01	3.75	H				
6922 Gu-0	Germany		297.00	183.39	57.27	8.01	559.92	56.60	-2.30	4.12	C				
6923 HR-10	UK		13.00	2.00	57.25	NA	614.31	61.44	-1.97	4.35	C				
6926 Kin-0	USA		21.00	20.34	57.25	NA	504.88	50.99	0.72	4.08	H				
6928 Kno-18	USA		230.50	118.28	57.50	7.44	804.14	157.42	-3.22	4.23	C				
6929 Kondara	Tajikistan		57.00	20.54	57.28	8.56	662.68	97.95	-5.15	4.20	C				
6930 Kz-1	Kazakhstan		351.00	107.75	57.44	7.19	862.87	172.36	-6.40	4.43	C				
6931 Kz-9	Kazakhstan		209.00	1059.35	57.43	7.98	757.69	108.73	-3.32	4.44	C				
6932 Ler-1	Germany		165.50	92.13	57.41	8.40	793.83	192.32	-9.62	4.01	C				
6933 LL-0	Spain		275.00	117.04	57.35	7.60	502.61	81.00	-4.30	3.73	C				
6936 Lz-0	France		375.00	106.63	57.23	7.62	ND	ND	ND	ND	C				
6937 Mrk-0	Germany		329.50	190.76	57.67	7.88	855.12	86.75	0.27	4.15	C				
6938 Ms-0	Russia		213.00	121.80	57.24	8.05	ND	ND	ND	ND	C				
6939 Mt-0	Libya		256.50	89.84	57.28	8.66	ND	ND	ND	ND	C				
6940 Mz-0	Germany		253.00	186.07	57.25	8.41	ND	ND	ND	ND	C				
6942 Nd-1	Switzerland		342.00	206.23	57.32	8.63	ND	ND	ND	ND	C				

6943 NFA-10	UK	179.00	95.88	57.27	8.59	ND	ND	ND	ND	C
6944 NFA-8	UK	148.00	136.46	58.09	7.26	ND	ND	ND	ND	C
6945 Nok-3	Netherlands	229.00	209.43	57.79	7.70	449.52	35.37	1.58	3.97	C
6946 Oy-0	Norway	324.00	139.61	57.25	8.31	ND	ND	ND	ND	H
6951 Pu2-23	Czech	212.50	127.99	56.85	7.72	ND	ND	ND	ND	C
6956 Pu2-7	Czech	195.00	124.97	56.88	7.78	ND	ND	ND	ND	C
6958 Ra-0	France	433.50	173.81	56.81	8.07	ND	ND	ND	ND	C
6959 Ren-1	France	281.00	279.23	56.78	8.09	ND	ND	ND	ND	H
6960 Ren-11	France	368.00	506.50	56.78	8.30	ND	ND	ND	ND	H
6961 Se-0	Spain	267.00	114.71	57.47	6.65	ND	ND	ND	ND	C
6962 Sha	Tajikistan	277.00	98.35	57.49	7.54	ND	ND	ND	ND	C
6963 Sorbo	Tajikistan	148.00	119.33	57.45	8.15	ND	ND	ND	ND	C
6966 Sq-1	UK	203.00	121.44	57.42	7.92	ND	ND	ND	ND	C
6967 Sq-8	UK	327.00	221.05	57.50	7.25	ND	ND	ND	ND	C
6968 Tamm-2	Finland	242.00	141.16	57.81	7.53	710.58	104.49	-4.28	4.20	C
6969 Tamm-27	Finland	242.00	133.31	57.81	7.80	804.65	91.11	2.56	4.10	C
6970 Ts-1	Spain	268.00	83.22	57.50	6.77	ND	ND	ND	ND	C
6971 Ts-5	Spain	241.50	116.50	57.86	6.69	613.48	107.25	-4.83	3.88	C
6972 Tsu-1	Japan	453.00	207.46	57.49	8.15	ND	ND	ND	ND	C
6973 Ull2-3	Sweden	270.00	171.43	57.77	8.18	ND	ND	ND	ND	C
6975 Uod-1	Austria	260.50	153.57	57.74	7.32	ND	ND	ND	ND	C
6976 Uod-7	Austria	338.00	141.75	57.80	7.05	745.19	44.78	1.76	3.89	C
6977 Van-0	Canada	202.50	85.93	57.76	7.62	ND	ND	ND	ND	C
6978 Wa-1	Poland	260.00	164.83	57.61	8.46	ND	ND	ND	ND	C
6979 Wei-0	Switzerland	279.50	286.86	57.73	7.40	ND	ND	ND	ND	C
6980 Ws-0	Russia	376.00	235.58	57.75	8.15	ND	ND	ND	ND	C
6981 Ws-2	Russia	197.00	69.04	57.80	7.29	623.98	78.34	-3.89	3.72	C
6982 Wt-5	Germany	338.00	271.95	57.76	7.27	ND	ND	ND	ND	C
6984 Zdr-1	Czech	256.00	93.36	57.40	6.91	ND	ND	ND	ND	C
6985 Zdr-6	Czech	192.00	147.94	57.40	7.40	ND	ND	ND	ND	C
6987 Ak-1	Germany	227.00	91.75	52.46	7.23	524.12	100.35	-5.47	3.97	C
6990 Amel-1	Netherlands	428.00	167.37	56.98	7.85	900.22	137.70	0.82	4.41	C
6992 Ang-0	Belgium	251.00	151.11	51.41	6.93	637.17	103.64	0.80	3.79	C
6994 Ann-1	France	212.00	158.95	57.48	8.06	ND	ND	ND	ND	C
7000 Aa-0	Germany	344.00	214.82	51.10	7.95	700.92	71.99	-1.64	4.32	C
7002 Baa-1	Netherlands	253.00	606.71	50.56	8.18	463.09	38.30	4.65	4.20	C
7004 Bs-2	Switzerland	310.50	193.10	56.08	7.61	862.03	100.65	-4.66	3.87	C
7014 Ba-1	UK	184.00	153.83	57.04	8.70	535.61	63.22	-2.68	4.13	C
7015 Bla-1	Spain	201.00	100.80	57.25	NA	745.21	60.33	-2.28	4.01	C
7026 Boot-1	UK	291.00	221.85	56.41	8.23	677.96	63.44	-1.07	4.16	H
7031 Bsch-0	Germany	218.00	181.73	55.94	7.80	784.06	89.08	-2.97	4.13	C
7062 Ca-0	Germany	343.00	368.35	56.04	8.02	888.75	113.22	-2.99	4.34	C
7071 Chat-1	France	320.00	181.70	55.97	7.54	633.74	63.08	-0.29	3.98	C
7081 Co	Portugal	476.00	382.83	57.66	7.69	1066.93	144.97	-5.62	4.58	C
7092 Com-1	France	260.50	101.00	57.86	7.00	613.26	133.03	-6.70	4.25	C
7094 Da-0	Germany	208.50	146.59	57.60	6.99	655.74	67.80	-5.74	3.81	C
7098 Di-1	France	291.50	128.08	47.43	7.09	742.22	180.49	-7.00	4.24	C
7102 Do-0	Germany	282.00	94.90	47.30	6.90	717.56	53.51	-1.09	4.22	C
7123 Ep-0	Germany	227.00	102.05	50.30	7.29	543.55	62.24	-4.67	3.58	C
7143 Gel-1	Netherlands	360.00	143.63	50.49	7.40	776.15	61.73	2.50	4.24	C
7147 Gie-0	Germany	310.00	277.21	57.28	7.88	840.00	190.18	-4.95	3.85	C
7163 Ha-0	Germany	274.00	166.59	49.15	7.17	718.88	100.83	-6.25	4.46	C
7166 Hey-1	Netherlands	232.00	102.46	54.66	6.86	790.97	77.57	-1.83	4.17	C
7172 Hl-3	Germany	310.00	241.92	48.05	7.78	679.64	85.74	-4.57	4.70	C
7176 Is-1	Germany	249.50	141.84	57.97	7.05	ND	ND	ND	ND	C
7178 Jm-1	Czech	348.00	159.75	58.24	8.05	857.07	69.41	-1.78	4.68	C
7181 Je-0	Germany	215.00	127.80	57.25	8.79	593.43	60.74	0.81	4.52	C
7192 Kil-0	UK	284.50	231.59	58.08	8.19	890.60	193.84	-5.87	4.45	C
7199 Kl-5	Germany	313.50	164.14	58.02	7.35	728.28	230.72	-11.62	4.23	C
7201 Kr-0	Germany	347.00	125.13	58.20	7.12	762.80	101.52	-3.12	4.32	C
7205 Krot-2	Germany	285.00	81.88	58.07	7.21	772.04	81.66	-1.52	4.23	C
7210 La-1	Poland	334.00	141.09	57.42	7.81	877.06	69.03	2.37	4.34	C
7224 Li-3	Germany	528.00	226.80	57.64	8.00	1007.75	88.92	-2.48	4.25	C
7231 Li-7	Germany	436.00	337.58	57.67	7.65	743.78	89.73	-1.82	3.89	C
7242 Lo-2	Germany	275.00	172.12	58.10	7.70	806.71	79.19	-2.33	4.03	C
7244 Mnz-0	Germany	287.00	192.29	58.00	7.72	ND	ND	ND	ND	C
7246 Ma-2	Germany	265.50	413.39	58.01	7.76	ND	ND	ND	ND	C
7255 Mh-0	Poland	287.00	181.61	58.05	7.82	ND	ND	ND	ND	C

7262 Nw-4	Germany	312.00	130.95	58.02	7.84	ND	ND	ND	ND	C
7268 Np-0	Germany	281.00	211.16	58.04	7.45	ND	ND	ND	ND	H
7275 No-0	Germany	254.50	90.96	57.99	7.13	ND	ND	ND	ND	C
7276 Ob-0	Germany	261.00	153.66	58.08	7.11	ND	ND	ND	ND	C
7280 Old-1	Germany	316.00	118.56	58.22	7.80	ND	ND	ND	ND	C
7282 Or-0	Germany	260.50	108.39	58.27	7.54	ND	ND	ND	ND	C
7283 Ors-1	Romania	253.00	111.04	57.82	7.44	621.58	87.11	-3.08	3.87	C
7287 Ove-0	Germany	382.00	193.04	58.28	8.06	ND	ND	ND	ND	C
7291 Pa-2	Italy	218.00	118.39	58.21	7.57	ND	ND	ND	ND	C
7297 Pf-0	Germany	274.50	97.28	58.19	6.95	ND	ND	ND	ND	C
7299 Pi-2	Austria	274.00	94.06	58.79	7.13	ND	ND	ND	ND	C
7300 Pla-0	Spain	354.00	133.19	56.83	7.78	ND	ND	ND	ND	C
7306 Pog-0	Canada	306.50	113.51	58.81	7.93	ND	ND	ND	ND	C
7307 Pn-0	France	206.00	76.51	58.47	8.39	ND	ND	ND	ND	C
7309 Po-1	Germany	284.50	133.28	56.79	7.93	ND	ND	ND	ND	C
7310 Pr-0	Germany	227.00	117.43	58.75	7.50	ND	ND	ND	ND	C
7316 Rhen-1	Netherlands	308.00	150.75	57.81	7.80	790.89	126.45	-3.18	3.98	C
7317 Ri-0	Canada	69.00	89.59	58.16	8.23	ND	ND	ND	ND	H
7320 Rou-0	France	218.00	119.92	58.74	7.70	ND	ND	ND	ND	C
7330 Sapporo-0	Japan	239.00	158.57	57.48	7.28	ND	ND	ND	ND	C
7331 Sh-0	Germany	349.00	137.93	57.56	6.88	ND	ND	ND	ND	C
7337 Si-0	Germany	293.00	175.70	57.50	7.30	ND	ND	ND	ND	C
7344 Sg-1	Germany	273.00	246.39	57.55	7.68	ND	ND	ND	ND	C
7351 Ty-0	UK	233.00	305.17	57.83	8.20	ND	ND	ND	ND	H
7352 Te-0	Finland	165.00	185.87	57.48	7.74	ND	ND	ND	ND	C
7353 Tha-1	Netherlands	341.50	258.24	57.47	7.56	ND	ND	ND	ND	C
7372 Tscha-1	Austria	331.50	240.36	57.49	7.91	ND	ND	ND	ND	C
7378 Uk-1	Germany	117.00	99.45	57.83	8.23	ND	ND	ND	ND	C
7382 Utrecht	Netherlands	378.00	123.48	57.79	6.94	ND	ND	ND	ND	C
7384 Ven-1	Netherlands	303.00	213.22	57.88	7.99	835.70	188.58	-7.71	4.02	C
7404 Wc-1	Germany	304.50	132.72	57.79	7.55	ND	ND	ND	ND	C
7418 Zu-1	Switzerland	292.00	84.97	57.42	7.36	ND	ND	ND	ND	C
7424 Ji-3	Czech	171.50	136.25	47.83	7.90	650.54	86.47	-2.79	4.48	C
7438 N13	Russia	144.00	104.89	57.81	8.06	485.42	81.23	-0.69	4.20	C
7477 WAR	USA	235.50	79.63	57.54	7.49	ND	ND	ND	ND	C
7514 RRS-7	USA	295.00	149.70	57.82	7.12	816.22	97.62	3.95	3.66	C
7519 Omo2-3	Sweden	194.50	128.78	57.80	7.26	625.71	102.13	-5.20	3.81	C
7520 Lp2-2	Czech	291.00	73.45	57.44	7.23	ND	ND	ND	ND	C
7521 Lp2-6	Czech	266.00	340.15	57.69	NA	724.12	76.71	0.46	4.03	C
7522 Mr-0	Italy	33.00	52.16	57.94	9.97	642.79	163.14	-5.75	4.71	C
7523 Pna-17	USA	346.00	182.87	57.84	7.42	742.23	95.92	5.45	4.02	C
7525 Rmx-A180	USA	251.00	210.12	57.55	7.58	ND	ND	ND	ND	C
8214 Gy-0	France	255.50	117.42	50.38	7.25	593.18	89.12	-2.28	4.34	C
8215 Fei-0	Portugal	261.00	88.84	57.13	7.77	564.26	114.18	-6.46	4.06	C
8233 Dem-4	USA	269.00	139.98	57.46	7.74	772.48	45.52	2.36	4.04	C
8236 Hsm	Czech	348.00	98.51	47.98	6.78	855.57	59.02	0.96	4.25	C
8240 Kulturen-1	Sweden	274.00	128.55	57.71	7.68	627.40	53.31	3.18	4.01	C
8241 Liarum	Sweden	205.50	190.61	57.90	8.10	628.58	50.29	-1.61	4.24	C
8245 Seattle-0	USA	177.50	161.26	57.47	7.72	ND	ND	ND	ND	H
8249 Vimmerby	Sweden	229.00	95.79	57.75	7.59	639.78	84.40	-0.60	4.11	C
8256 B41-2	Sweden	386.50	298.62	50.62	7.68	677.80	94.26	-1.56	4.22	C
8258 B44-1	Sweden	299.00	146.86	51.55	7.27	705.96	54.41	-1.12	4.21	C
8259 B45-1	Sweden	308.00	125.62	51.27	7.76	719.20	86.78	1.18	4.15	C
8265 Blh-1	Czech	252.50	152.99	57.54	7.55	ND	ND	ND	ND	C
8270 Bs-1	Switzerland	283.00	227.77	56.32	7.87	724.54	144.87	-4.06	4.45	C
8271 Bu-0	Germany	370.00	92.72	56.01	7.26	1038.26	125.54	-1.07	4.58	C
8284 DralI-1	Czech	329.00	101.77	57.18	8.24	833.79	53.06	0.66	4.13	C
8285 DralII-1	Czech	357.00	313.69	48.06	7.84	821.53	158.18	-6.86	4.10	C
8290 En-1	Germany	294.00	142.48	45.33	7.28	685.56	101.88	-4.81	4.03	C
8296 Gd-1	Germany	262.00	116.39	50.69	7.44	753.09	92.17	-2.70	4.10	C
8300 Gr-1	Austria	279.00	91.50	49.17	6.97	764.09	103.12	4.89	4.11	C
8304 Hi-0	Netherlands	271.00	107.89	48.06	6.86	776.44	80.94	-2.88	4.35	C
8310 Hs-0	Germany	393.00	160.15	48.02	7.47	917.83	79.21	-0.48	3.97	C
8311 In-0	Austria	324.00	383.22	57.29	8.33	827.97	89.10	-4.67	4.29	C
8312 Is-0	Germany	304.50	124.84	47.63	6.97	904.86	216.96	-10.92	4.38	C
8313 Jm-0	Czech	318.00	389.45	47.77	7.42	826.24	314.00	-12.98	4.16	C
8314 Ka-0	Austria	392.00	344.09	58.11	7.89	792.84	116.00	-0.23	4.32	C
8323 Le-0	UK	221.50	147.55	57.69	7.67	408.41	52.06	-1.10	3.90	C

8325 Lip-0	Poland	350.00	201.17	57.63	7.69	620.03	71.78	-0.36	3.88	C
8329 Lm-2	France	356.50	257.40	57.68	7.24	759.93	105.32	-4.73	4.06	C
8334 Lu-1	Sweden	189.50	109.07	57.23	8.04	ND	ND	ND	ND	C
8337 Mir-0	Italy	101.00	42.00	58.26	7.37	ND	ND	ND	ND	C
8343 Na-1	France	203.00	128.17	57.99	6.87	ND	ND	ND	ND	C
8348 Nw-0	Germany	258.00	92.03	58.03	6.60	ND	ND	ND	ND	C
8353 Pa-1	Italy	302.00	173.34	57.76	7.22	526.96	129.49	-6.07	4.03	C
8354 Per-1	Russia	193.00	55.96	58.26	7.83	ND	ND	ND	ND	C
8366 Rd-0	Germany	244.50	173.24	58.73	7.86	ND	ND	ND	ND	C
8374 Rsch-4	Russia	391.50	193.14	57.50	7.47	ND	ND	ND	ND	C
8378 Sap-0	Czech	315.00	120.73	57.64	7.01	ND	ND	ND	ND	C
8387 St-0	Sweden	272.00	83.10	57.54	6.81	ND	ND	ND	ND	C
8388 Stw-0	Russia	226.00	252.34	57.54	6.72	ND	ND	ND	ND	C
8395 Tu-0	Italy	441.50	127.77	57.49	6.89	ND	ND	ND	ND	C
8420 Kelsterbach-4	Germany	370.00	144.82	57.97	7.43	676.23	113.79	-4.57	4.00	C
9104 Lag1-6	Georgia	253.50	117.59	57.73	7.29	688.64	94.81	1.15	3.95	C
9165 Truk-5	Ukraine	167.00	306.85	57.48	7.63	ND	ND	ND	ND	C
9302 Edinburgh-5	UK	188.00	129.58	48.90	7.02	621.72	110.45	-4.47	4.07	C
9308 Ullapool-3	UK	237.00	128.81	57.76	7.55	ND	ND	ND	ND	C
100000 Wil-1-Dean-Lab	Lithuania	285.50	108.26	57.85	6.66	ND	ND	ND	ND	C
Average		267.00	136.25	57.50	7.67	709.73	102.51	-2.77	4.15	–
Standard deviation		80.47	106.41	3.06	0.51	130.08	55.83	3.66	0.25	–

^aND indicates that no data is available.

^bAverage value (an average of five values measured for 2 DAG - 6 DAG, respectively) was used.

Table S2. Gene models in proximity of the significantly associated locus for Horizontality under NPA treatment, Related to Figure 1, 2, 3 and 4.

TAIR10 gene models in proximity (3 KB distance) of the significant SNP (passing the Bonferroni corrected threshold for approximately 215,000 tests, p -value $< 10^{-7}$).

Chromosome	SNP position	GWAS p -value	Effect size [%] ^a	Associated traits	Associated gene				
					Gene start	Gene end	Distance between SNP and gene body	AGI code	Gene annotation
5	21256465	4.65×10^{-8}	13.58	Horizontality	21250802	21253939	2526	AT5G52340	exocyst subunit exo70 family protein A2
					21254911	21257618	0 (on gene)	AT5G52350	exocyst subunit exo70 family protein A3
					21257834	21257915	1369	AT5G52355	pre-tRNA
					21257934	21259342	1469	AT5G52360	actin depolymerizing factor 10

^a Calculated as previously reported (Kerdaffrec *et al.* 2016, eLife)

Table. S3. Seed production under control (sufficient water) and drought conditions, Related to Figure 7

Haplogroup: classification based on haplotypes around the *EXOCYST70A3* locus. C: Common-sensitivity to NPA; H: High-sensitivity to NPA.; Treatment: water conditions during plant growth. Control: 65% water/soil.; Drought: 25% water/soil.; Replicate: experimental replicates; GWAS ID: Identifier of *A. thaliana* natural accessions as used in GWAPP (Seren et al. 2012).

General name	Haplogroup	Treatment	Replicate	Seed number/plant	Flowering time (days after germination) ^{a,b}	GWAS ID
Col-0	-(C)	Control	1	59954	23	6909
Col-0	-(C)	Control	1	44209	22	6909
Col-0	-(C)	Control	1	48739	23	6909
Col-0	-(C)	Control	1	41328	23	6909
Col-0	-(C)	Control	2	41088	23	6909
Col-0	-(C)	Control	2	59202	23	6909
Col-0	-(C)	Control	2	49795	23	6909
Col-0	-(C)	Control	2	44522	23	6909
Col-0	-(C)	Control	3	79380	24	6909
Col-0	-(C)	Control	3	67973	23	6909
Col-0	-(C)	Control	3	95171	24	6909
Col-0	-(C)	Control	3	73260	24	6909
Col-0	-(C)	Control	4	90249	23	6909
Col-0	-(C)	Control	4	72053	23	6909
Col-0	-(C)	Control	4	65993	22	6909
Col-0	-(C)	Control	4	95371	22	6909
Col-0	-(C)	Drought	1	3112	20	6909
Col-0	-(C)	Drought	1	3213	20	6909
Col-0	-(C)	Drought	1	0	20	6909
Col-0	-(C)	Drought	1	1504	20	6909

Col-0	-(C)	Drought	2	1505	20	6909
Col-0	-(C)	Drought	2	1086	20	6909
Col-0	-(C)	Drought	2	1152	20	6909
Col-0	-(C)	Drought	2	1691	20	6909
Col-0	-(C)	Drought	3	1142	25	6909
Col-0	-(C)	Drought	3	1288	25	6909
Col-0	-(C)	Drought	3	1588	25	6909
Col-0	-(C)	Drought	3	2401	25	6909
Col-0	-(C)	Drought	4	1914	25	6909
Col-0	-(C)	Drought	4	1815	25	6909
Col-0	-(C)	Drought	4	1858	25	6909
Col-0	-(C)	Drought	4	836	25	6909
<i>EXO70A3ox</i>	-	Control	1	46763	22	-
<i>EXO70A3ox</i>	-	Control	1	14531	22	-
<i>EXO70A3ox</i>	-	Control	1	37094	22	-
<i>EXO70A3ox</i>	-	Control	1	37296	22	-
<i>EXO70A3ox</i>	-	Control	2	28951	22	-
<i>EXO70A3ox</i>	-	Control	2	38528	22	-
<i>EXO70A3ox</i>	-	Control	2	35370	22	-
<i>EXO70A3ox</i>	-	Control	2	42682	22	-
<i>EXO70A3ox</i>	-	Control	3	59494	22	-
<i>EXO70A3ox</i>	-	Control	3	65354	22	-
<i>EXO70A3ox</i>	-	Control	3	44308	22	-
<i>EXO70A3ox</i>	-	Control	3	78958	22	-
<i>EXO70A3ox</i>	-	Control	4	30367	22	-
<i>EXO70A3ox</i>	-	Control	4	35279	22	-
<i>EXO70A3ox</i>	-	Control	4	50978	22	-
<i>EXO70A3ox</i>	-	Drought	1	1277	20	-
<i>EXO70A3ox</i>	-	Drought	1	1501	20	-
<i>EXO70A3ox</i>	-	Drought	1	1048	20	-
<i>EXO70A3ox</i>	-	Drought	1	495	20	-

<i>EXO70A3ox</i>	-	Drought	2	685	20	-
<i>EXO70A3ox</i>	-	Drought	2	1764	20	-
<i>EXO70A3ox</i>	-	Drought	2	338	25	-
<i>EXO70A3ox</i>	-	Drought	2	1820	20	-
<i>EXO70A3ox</i>	-	Drought	3	1512	25	-
<i>EXO70A3ox</i>	-	Drought	3	739	25	-
<i>EXO70A3ox</i>	-	Drought	3	1104	25	-
<i>EXO70A3ox</i>	-	Drought	3	409	25	-
<i>EXO70A3ox</i>	-	Drought	4	0	NF	-
<i>EXO70A3ox</i>	-	Drought	4	691	25	-
<i>EXO70A3ox</i>	-	Drought	4	1294	25	-
<i>EXO70A3ox</i>	-	Drought	4	272	25	-
Ler-1	C	Control	1	15576	23	6932
Ler-1	C	Control	1	12186	23	6932
Ler-1	C	Control	1	10182	24	6932
Ler-1	C	Control	1	14526	24	6932
Ler-1	C	Control	2	13498	23	6932
Ler-1	C	Control	2	9977	23	6932
Ler-1	C	Control	2	8471	24	6932
Ler-1	C	Control	2	15375	23	6932
Ler-1	C	Control	3	27440	23	6932
Ler-1	C	Control	3	11290	23	6932
Ler-1	C	Control	3	24710	23	6932
Ler-1	C	Control	3	25591	23	6932
Ler-1	C	Control	4	16051	23	6932
Ler-1	C	Control	4	14780	23	6932
Ler-1	C	Control	4	17081	23	6932
Ler-1	C	Control	4	22333	23	6932
Ler-1	C	Drought	1	1428	20	6932
Ler-1	C	Drought	1	995	20	6932
Ler-1	C	Drought	1	1417	20	6932

Ler-1	C	Drought	1	1895	20	6932
Ler-1	C	Drought	2	2288	20	6932
Ler-1	C	Drought	2	1903	20	6932
Ler-1	C	Drought	2	1832	20	6932
Ler-1	C	Drought	2	836	20	6932
Ler-1	C	Drought	3	1357	25	6932
Ler-1	C	Drought	3	1180	25	6932
Ler-1	C	Drought	3	806	25	6932
Ler-1	C	Drought	3	884	25	6932
Ler-1	C	Drought	4	1050	25	6932
Ler-1	C	Drought	4	1215	25	6932
Ler-1	C	Drought	4	1273	25	6932
Ler-1	C	Drought	4	1158	25	6932
Shakdara	C	Control	1	28800	22	6962
Shakdara	C	Control	1	18290	24	6962
Shakdara	C	Control	1	21802	22	6962
Shakdara	C	Control	1	18274	24	6962
Shakdara	C	Control	2	31040	22	6962
Shakdara	C	Control	2	44968	22	6962
Shakdara	C	Control	2	19286	22	6962
Shakdara	C	Control	2	23959	22	6962
Shakdara	C	Control	3	71903	22	6962
Shakdara	C	Control	3	37200	22	6962
Shakdara	C	Control	3	34002	22	6962
Shakdara	C	Control	3	54017	22	6962
Shakdara	C	Control	4	35088	23	6962
Shakdara	C	Control	4	41748	19	6962
Shakdara	C	Control	4	39138	24	6962
Shakdara	C	Control	4	37598	23	6962
Shakdara	C	Drought	1	1648	20	6962
Shakdara	C	Drought	1	1902	20	6962

Shakdara	C	Drought	1	1604	20	6962
Shakdara	C	Drought	1	1710	20	6962
Shakdara	C	Drought	2	2247	20	6962
Shakdara	C	Drought	2	2142	20	6962
Shakdara	C	Drought	2	1797	25	6962
Shakdara	C	Drought	2	1852	20	6962
Shakdara	C	Drought	3	3038	20	6962
Shakdara	C	Drought	3	2453	20	6962
Shakdara	C	Drought	3	2012	20	6962
Shakdara	C	Drought	3	1815	20	6962
Shakdara	C	Drought	4	2041	25	6962
Shakdara	C	Drought	4	1402	25	6962
Shakdara	C	Drought	4	1479	25	6962
Shakdara	C	Drought	4	945	20	6962
Lp2-2	C	Control	1	59564	27	7520
Lp2-2	C	Control	1	57462	27	7520
Lp2-2	C	Control	1	52994	28	7520
Lp2-2	C	Control	1	59428	27	7520
Lp2-2	C	Control	2	58531	26	7520
Lp2-2	C	Control	2	40511	27	7520
Lp2-2	C	Control	2	49896	27	7520
Lp2-2	C	Control	2	73265	27	7520
Lp2-2	C	Control	3	102778	26	7520
Lp2-2	C	Control	3	109754	26	7520
Lp2-2	C	Control	3	82600	26	7520
Lp2-2	C	Control	3	112543	26	7520
Lp2-2	C	Control	4	72530	26	7520
Lp2-2	C	Control	4	72409	26	7520
Lp2-2	C	Control	4	69163	26	7520
Lp2-2	C	Control	4	89082	26	7520
Lp2-2	C	Drought	1	884	25	7520

Lp2-2	C	Drought	1	815	20	7520
Lp2-2	C	Drought	1	1068	20	7520
Lp2-2	C	Drought	1	988	20	7520
Lp2-2	C	Drought	2	561	25	7520
Lp2-2	C	Drought	2	856	25	7520
Lp2-2	C	Drought	2	1241	25	7520
Lp2-2	C	Drought	2	96	20	7520
Lp2-2	C	Drought	3	167	35	7520
Lp2-2	C	Drought	3	0	35	7520
Lp2-2	C	Drought	3	0	35	7520
Lp2-2	C	Drought	3	5418	40	7520
Lp2-2	C	Drought	4	0	30	7520
Lp2-2	C	Drought	4	0	35	7520
Lp2-2	C	Drought	4	0	35	7520
Lp2-2	C	Drought	4	0	30	7520
Per-1	C	Control	1	41658	26	8354
Per-1	C	Control	1	44868	24	8354
Per-1	C	Control	1	27103	25	8354
Per-1	C	Control	1	57143	23	8354
Per-1	C	Control	2	61402	24	8354
Per-1	C	Control	2	48125	24	8354
Per-1	C	Control	2	56719	25	8354
Per-1	C	Control	2	68521	26	8354
Per-1	C	Control	3	61320	23	8354
Per-1	C	Control	3	79596	25	8354
Per-1	C	Control	3	87498	23	8354
Per-1	C	Control	3	90758	24	8354
Per-1	C	Control	4	70278	24	8354
Per-1	C	Control	4	67210	24	8354
Per-1	C	Control	4	61904	23	8354
Per-1	C	Control	4	93404	23	8354

Per-1	C	Drought	1	1024	25	8354
Per-1	C	Drought	1	1217	25	8354
Per-1	C	Drought	1	946	20	8354
Per-1	C	Drought	1	2897	20	8354
Per-1	C	Drought	2	1224	20	8354
Per-1	C	Drought	2	1535	25	8354
Per-1	C	Drought	2	680	20	8354
Per-1	C	Drought	2	1075	20	8354
Per-1	C	Drought	3	98	35	8354
Per-1	C	Drought	3	5683	40	8354
Per-1	C	Drought	3	42	35	8354
Per-1	C	Drought	3	264	35	8354
Per-1	C	Drought	4	209	35	8354
Per-1	C	Drought	4	114	35	8354
Per-1	C	Drought	4	57	35	8354
Per-1	C	Drought	4	6710	35	8354
LAC-5	H	Control	1	60237	31	96
LAC-5	H	Control	1	37245	31	96
LAC-5	H	Control	1	60522	31	96
LAC-5	H	Control	2	53159	31	96
LAC-5	H	Control	2	61534	32	96
LAC-5	H	Control	2	49903	32	96
LAC-5	H	Control	2	44078	31	96
LAC-5	H	Control	3	62516	32	96
LAC-5	H	Control	3	72228	31	96
LAC-5	H	Control	3	56917	31	96
LAC-5	H	Control	3	49544	31	96
LAC-5	H	Control	4	68069	31	96
LAC-5	H	Control	4	53850	31	96
LAC-5	H	Control	4	62850	31	96
LAC-5	H	Control	4	82702	31	96

LAC-5	H	Drought	1	0	30	96
LAC-5	H	Drought	1	0	30	96
LAC-5	H	Drought	1	155	25	96
LAC-5	H	Drought	1	96	30	96
LAC-5	H	Drought	2	550	35	96
LAC-5	H	Drought	2	0	30	96
LAC-5	H	Drought	2	965	30	96
LAC-5	H	Drought	2	370	35	96
LAC-5	H	Drought	3	0	35	96
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LAC-5	H	Drought	3	0	35	96
LAC-5	H	Drought	3	0	35	96
LAC-5	H	Drought	4	0	NF	96
LAC-5	H	Drought	4	0	NF	96
LAC-5	H	Drought	4	0	NF	96
LAC-5	H	Drought	4	0	NF	96
MIB-60	H	Control	1	32158	28	204
MIB-60	H	Control	1	54078	28	204
MIB-60	H	Control	1	31898	27	204
MIB-60	H	Control	1	79740	27	204
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MIB-60	H	Control	2	23998	27	204
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MIB-60	H	Control	2	37364	28	204
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MIB-60	H	Control	3	49329	27	204
MIB-60	H	Control	4	59582	27	204
MIB-60	H	Control	4	68096	28	204
MIB-60	H	Control	4	51968	28	204

MIB-60	H	Control	4	64630	27	204
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MIB-60	H	Drought	1	1443	20	204
MIB-60	H	Drought	1	186	20	204
MIB-60	H	Drought	1	349	20	204
MIB-60	H	Drought	2	1637	20	204
MIB-60	H	Drought	2	2195	20	204
MIB-60	H	Drought	2	1246	20	204
MIB-60	H	Drought	2	2041	20	204
MIB-60	H	Drought	3	1490	30	204
MIB-60	H	Drought	3	680	35	204
MIB-60	H	Drought	3	283	35	204
MIB-60	H	Drought	3	746	35	204
MIB-60	H	Drought	4	1178	35	204
MIB-60	H	Drought	4	612	35	204
MIB-60	H	Drought	4	706	35	204
MIB-60	H	Drought	4	408	35	204
Coc-1	H	Control	1	49172	27	5729
Coc-1	H	Control	1	26831	29	5729
Coc-1	H	Control	1	42148	27	5729
Coc-1	H	Control	1	29068	27	5729
Coc-1	H	Control	2	40326	25	5729
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Coc-1	H	Control	2	38021	26	5729
Coc-1	H	Control	2	52345	28	5729
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Coc-1	H	Control	4	78730	26	5729
Coc-1	H	Control	4	64813	29	5729

Coc-1	H	Control	4	59413	27	5729
Coc-1	H	Control	4	106848	27	5729
Coc-1	H	Drought	1	887	30	5729
Coc-1	H	Drought	1	538	25	5729
Coc-1	H	Drought	1	637	25	5729
Coc-1	H	Drought	1	371	30	5729
Coc-1	H	Drought	2	488	25	5729
Coc-1	H	Drought	2	0	NF	5729
Coc-1	H	Drought	2	606	25	5729
Coc-1	H	Drought	2	412	30	5729
Coc-1	H	Drought	3	1176	25	5729
Coc-1	H	Drought	3	605	25	5729
Coc-1	H	Drought	3	636	20	5729
Coc-1	H	Drought	3	1700	20	5729
Coc-1	H	Drought	4	0	NF	5729
Coc-1	H	Drought	4	0	45	5729
Coc-1	H	Drought	4	0	NF	5729
Coc-1	H	Drought	4	0	45	5729
Ty-0	H	Control	1	18914	36	7351
Ty-0	H	Control	1	17897	36	7351
Ty-0	H	Control	1	21862	36	7351
Ty-0	H	Control	1	15853	36	7351
Ty-0	H	Control	2	14245	36	7351
Ty-0	H	Control	2	18391	36	7351
Ty-0	H	Control	2	23727	36	7351
Ty-0	H	Control	2	21835	36	7351
Ty-0	H	Control	3	30996	37	7351
Ty-0	H	Control	3	17035	37	7351
Ty-0	H	Control	3	16989	37	7351
Ty-0	H	Control	3	25668	36	7351
Ty-0	H	Control	4	43280	37	7351

Ty-0	H	Control	4	34630	37	7351
Ty-0	H	Control	4	25637	37	7351
Ty-0	H	Control	4	47174	37	7351
Ty-0	H	Drought	1	0	NF	7351
Ty-0	H	Drought	1	0	NF	7351
Ty-0	H	Drought	1	0	NF	7351
Ty-0	H	Drought	1	0	NF	7351
Ty-0	H	Drought	2	0	NF	7351
Ty-0	H	Drought	2	0	NF	7351
Ty-0	H	Drought	2	0	NF	7351
Ty-0	H	Drought	2	0	NF	7351
Ty-0	H	Drought	3	78	30	7351
Ty-0	H	Drought	3	130	30	7351
Ty-0	H	Drought	3	0	30	7351
Ty-0	H	Drought	3	26	30	7351
Ty-0	H	Drought	4	0	30	7351
Ty-0	H	Drought	4	0	30	7351
Ty-0	H	Drought	4	0	30	7351
Ty-0	H	Drought	4	0	30	7351

^a NF: no flowering.

^b Flowering was observed every five days in the drought experiment.

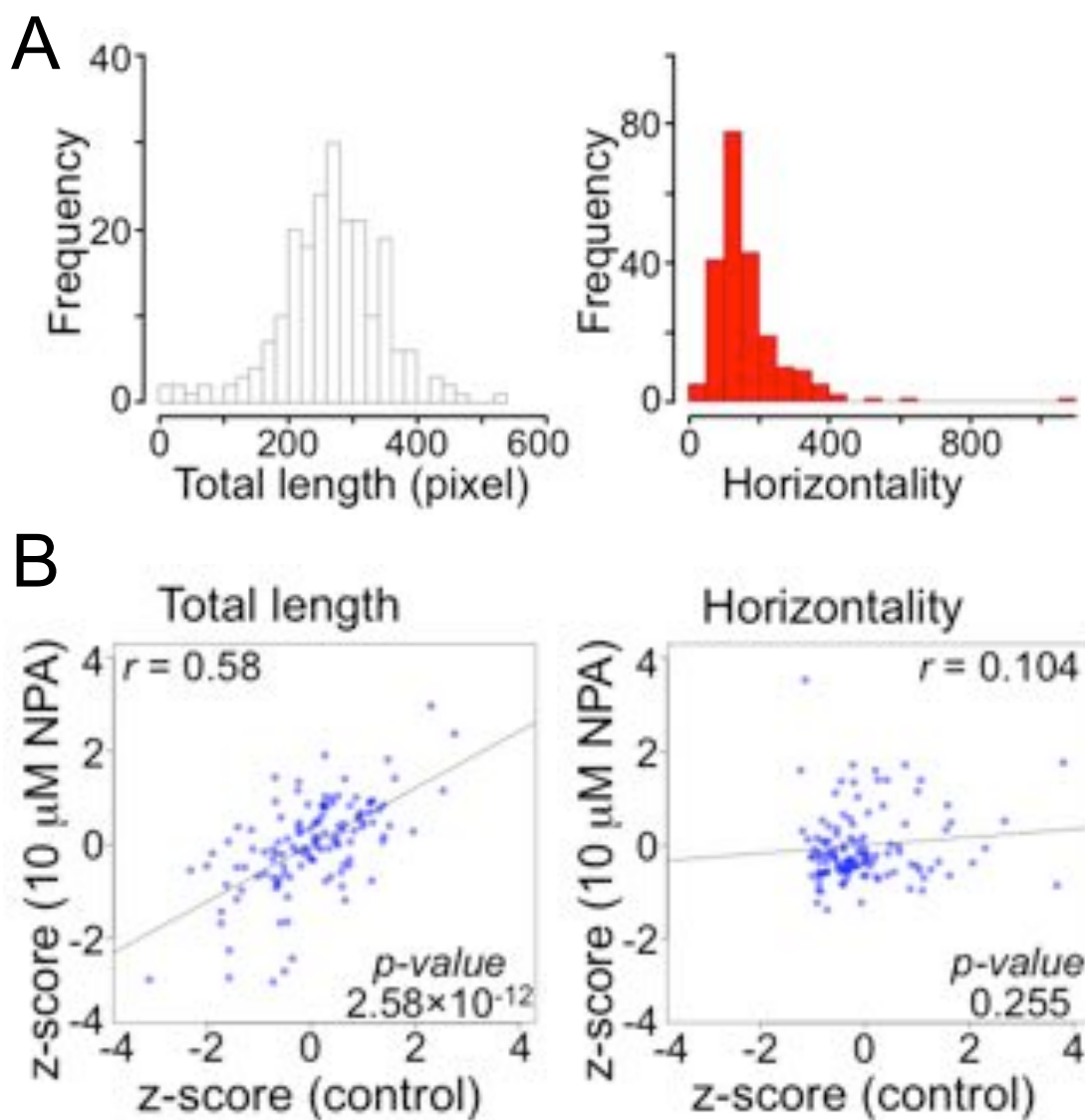


Figure S1 Ogura et al. 2018

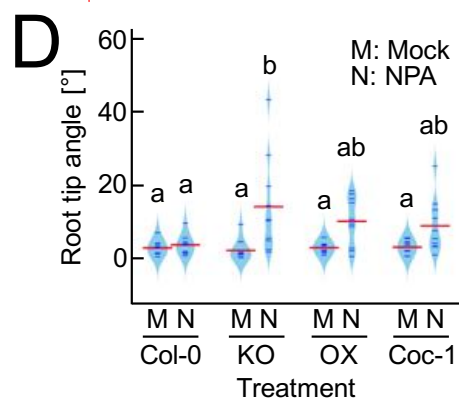
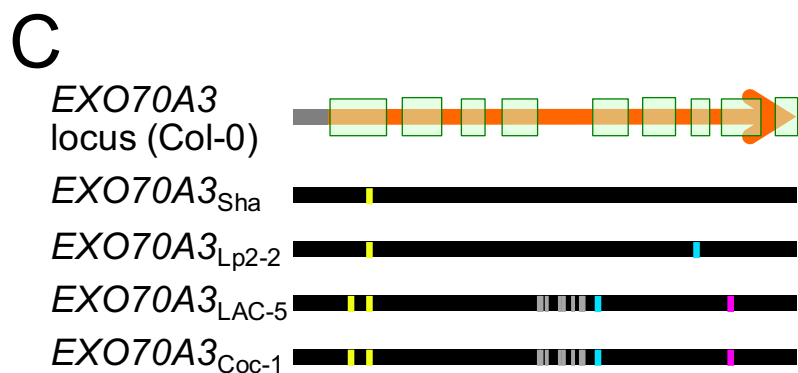
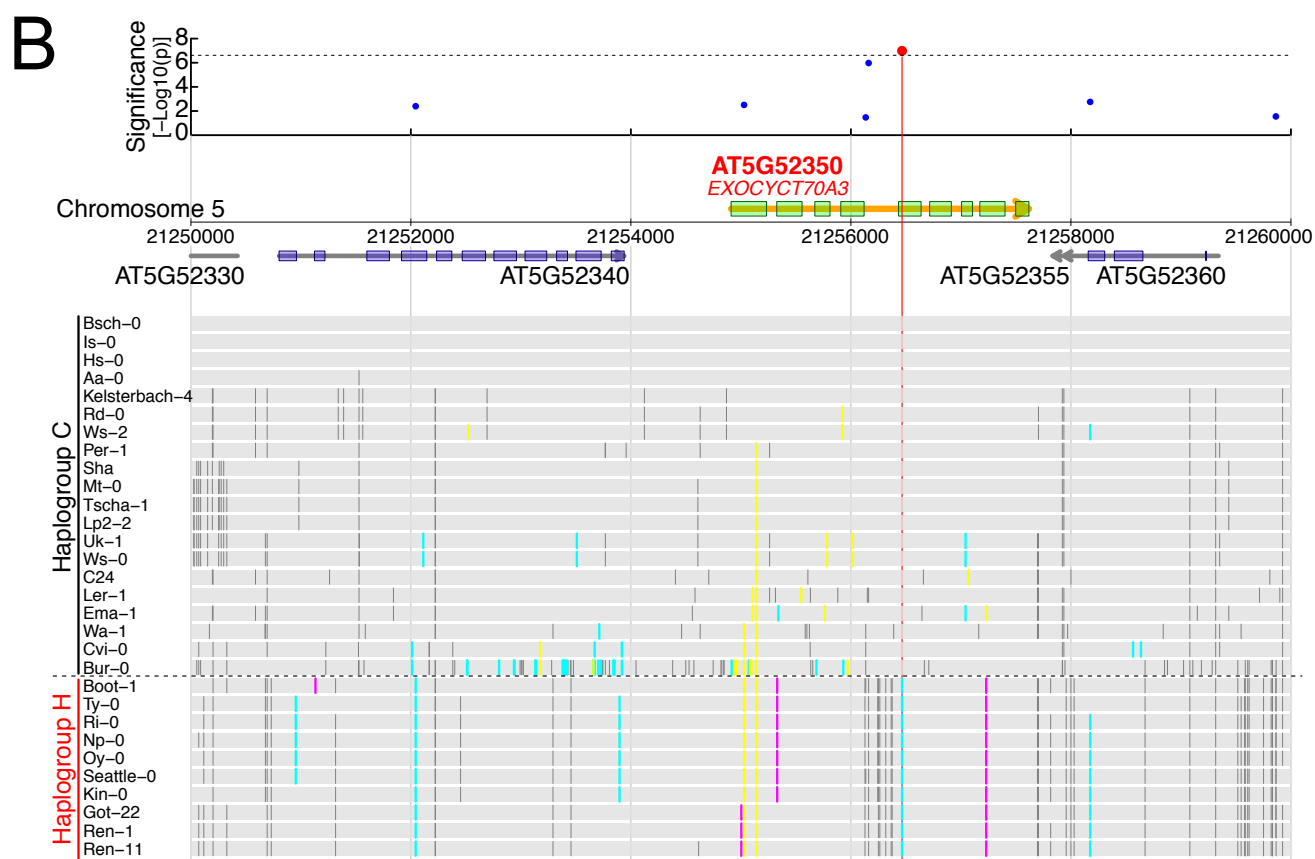
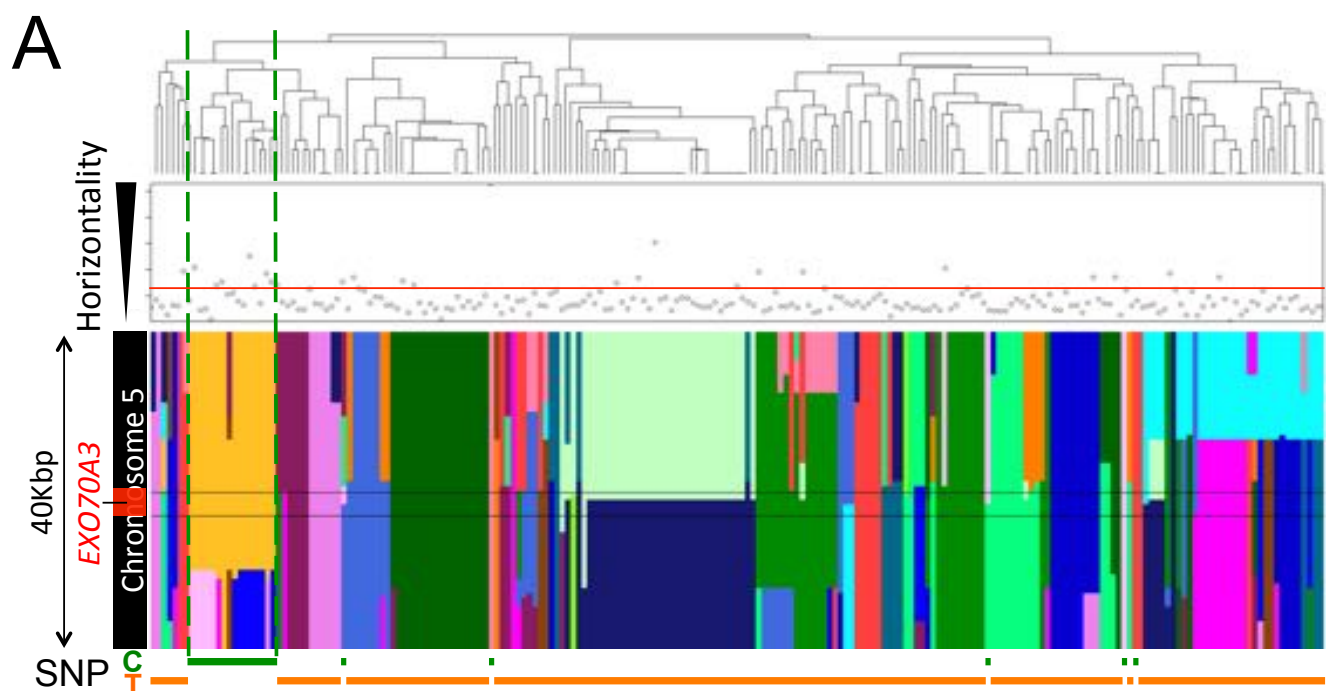


Figure S2 Ogura et al. 2018

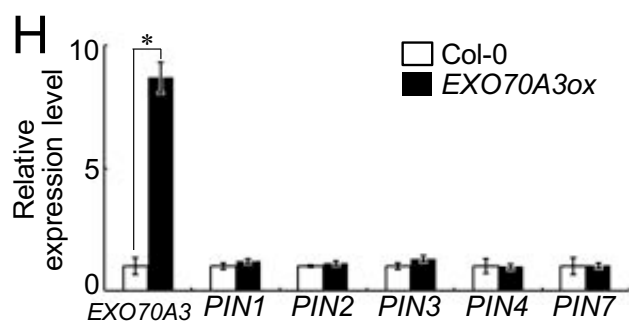
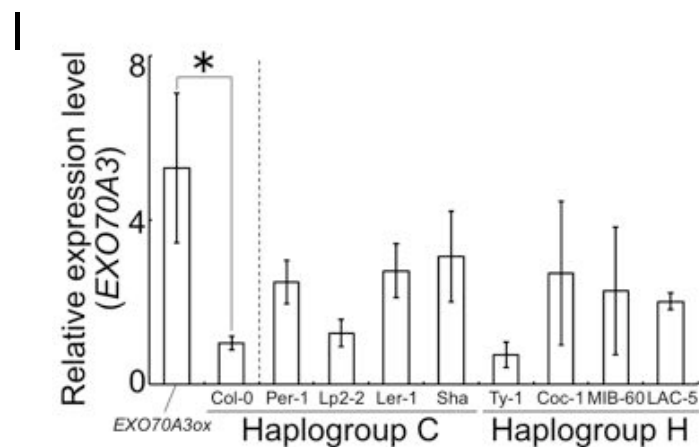
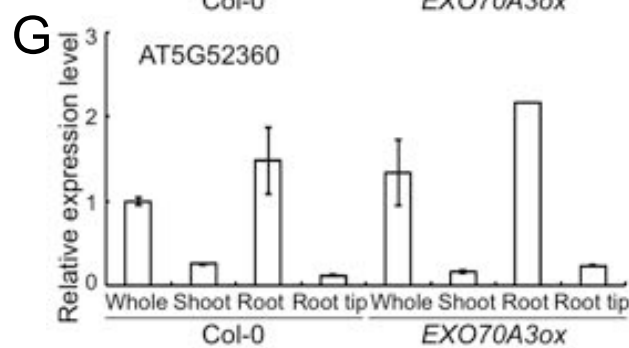
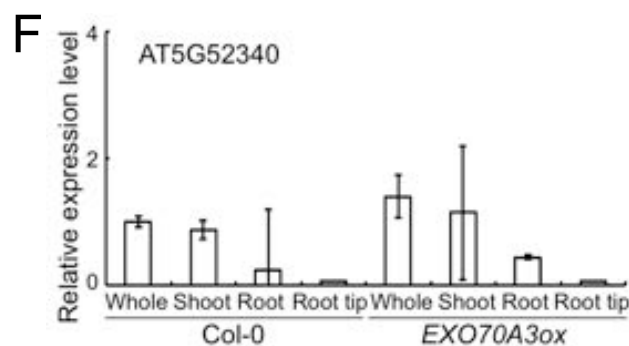
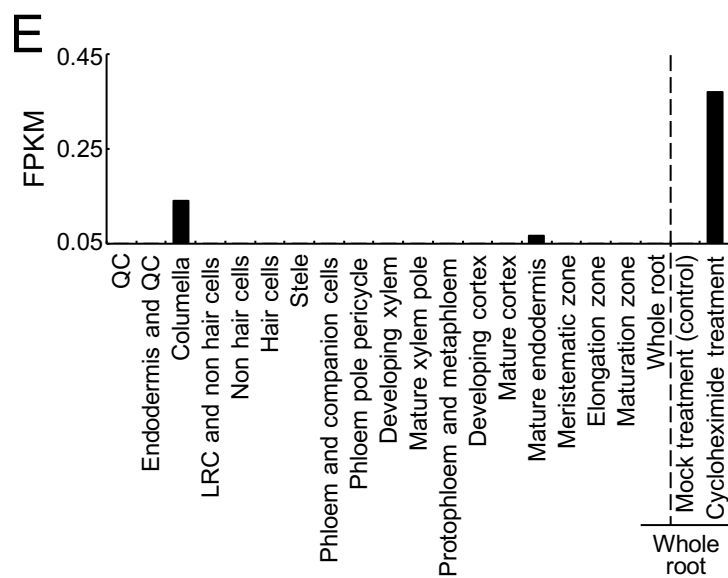
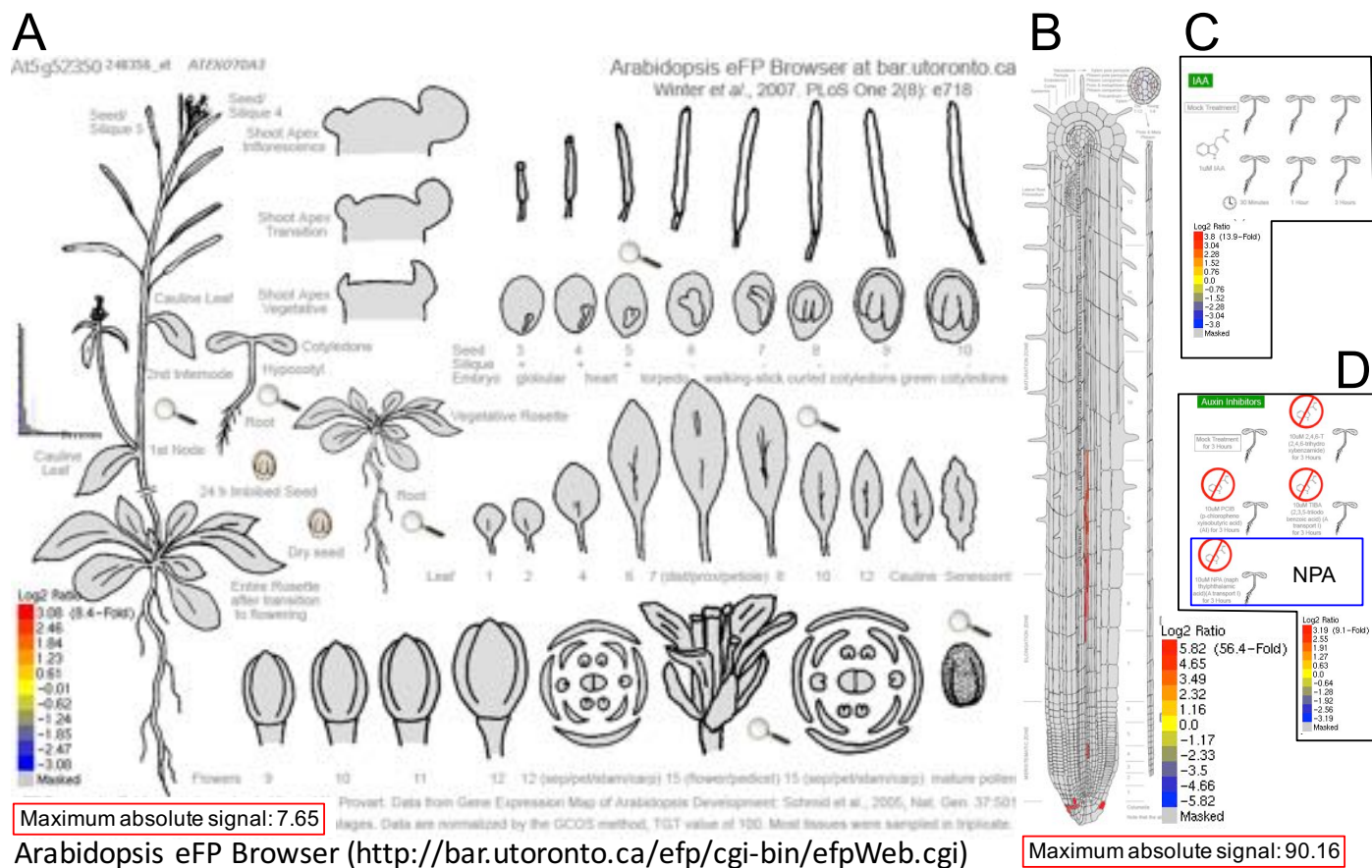
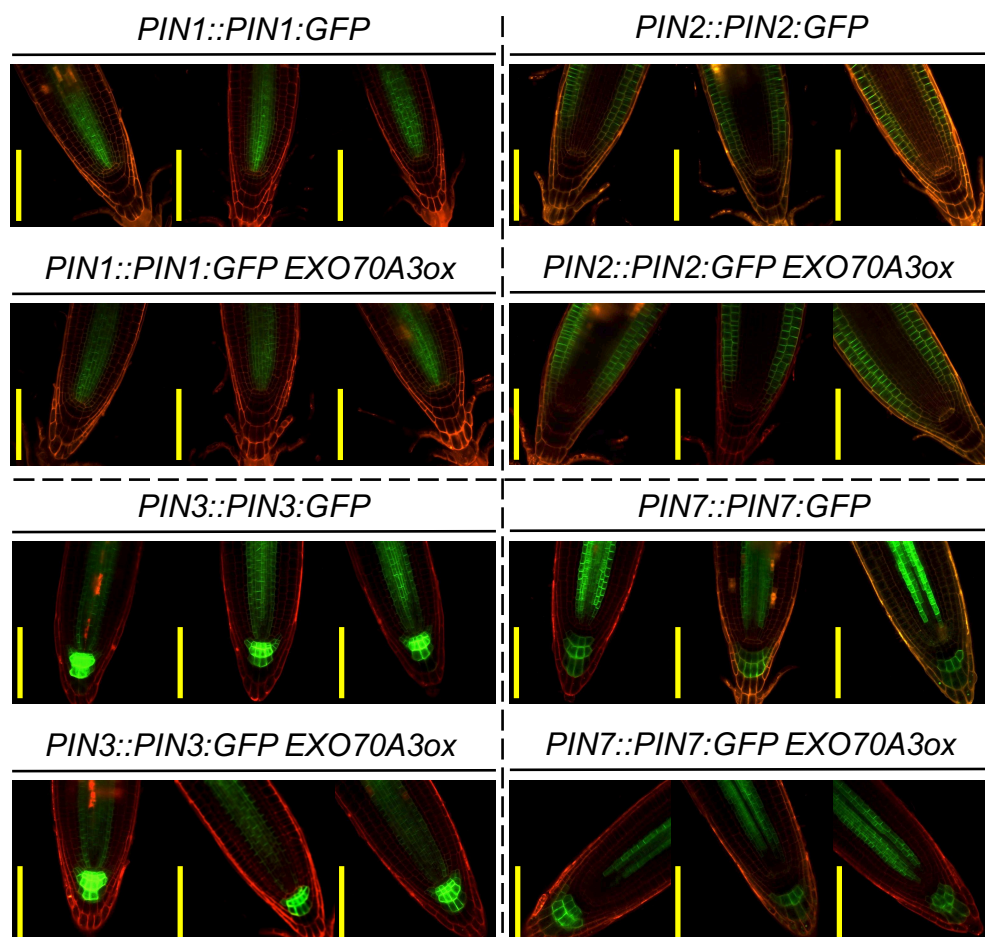


Figure S3 Ogura et al. 2018

A



B

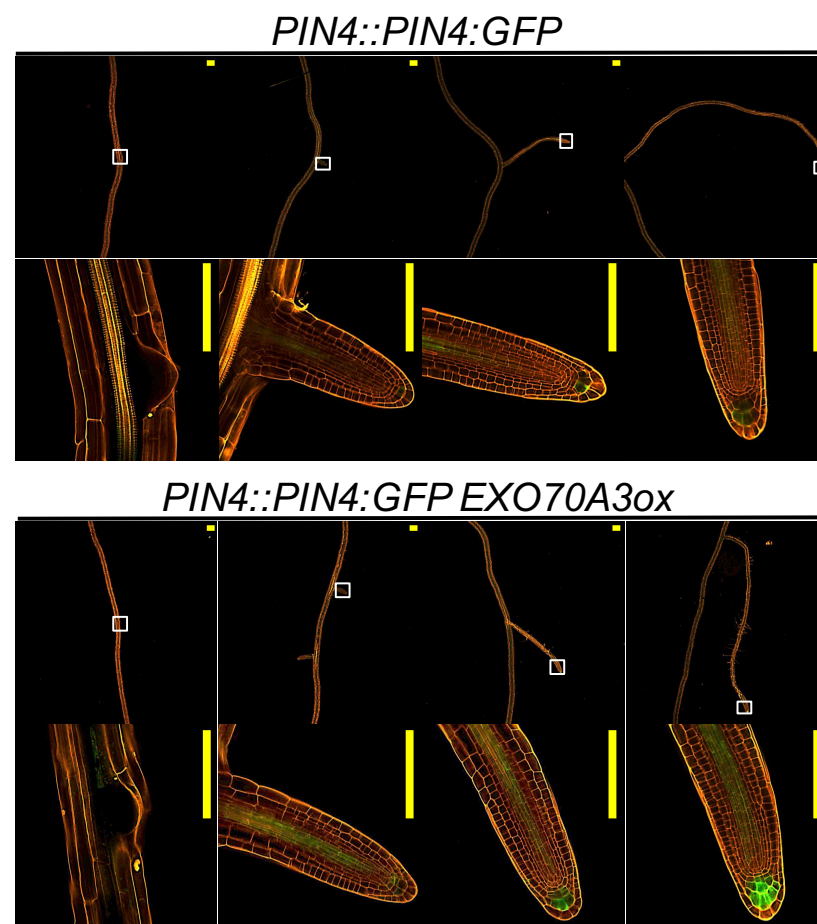
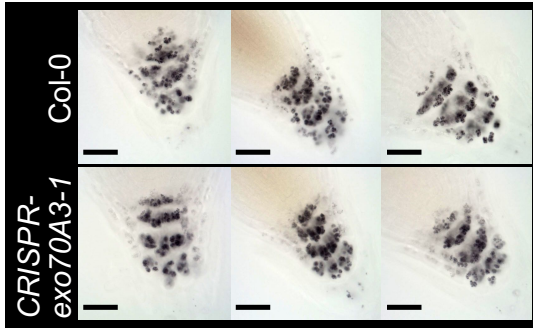


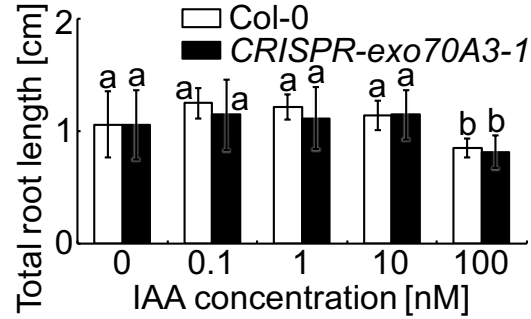
Figure S4 Ogura et al. 2018

A. *CRISPR-exo70A3-1*

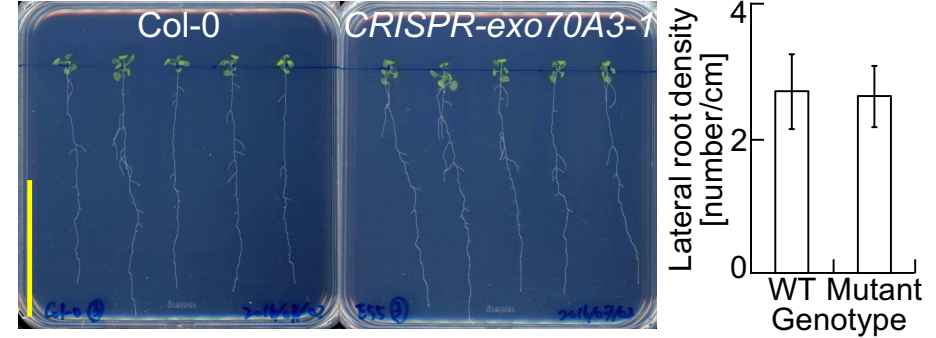
Statocytes & statolith



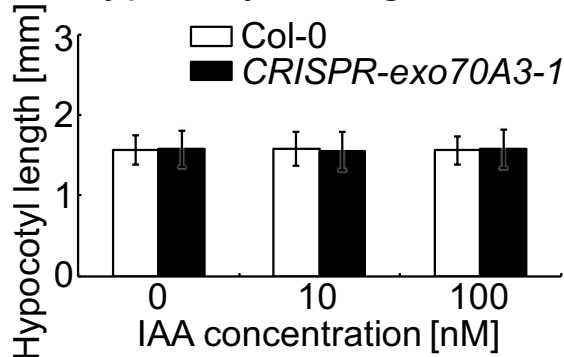
Primary root length



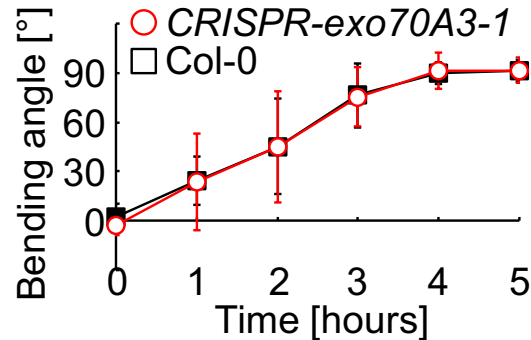
Lateral root density



Hypocotyl elongation



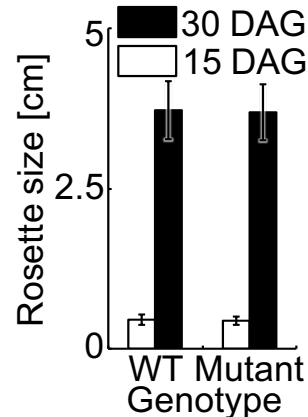
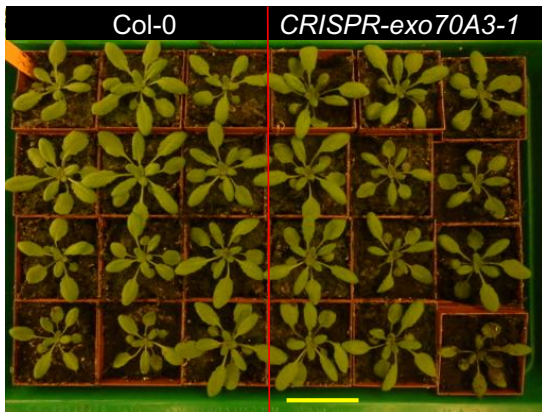
Shoot gravitropism



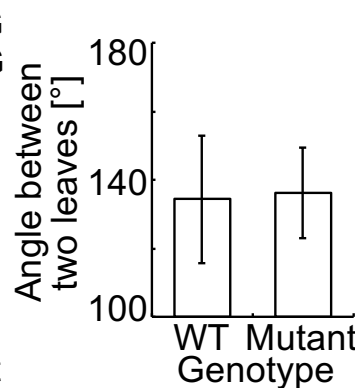
Inflorescence structure



Rosette development



Phyllotaxy



Floral stem height

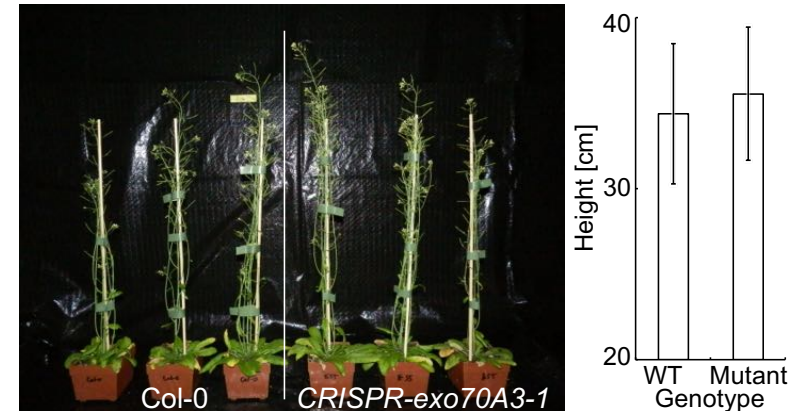
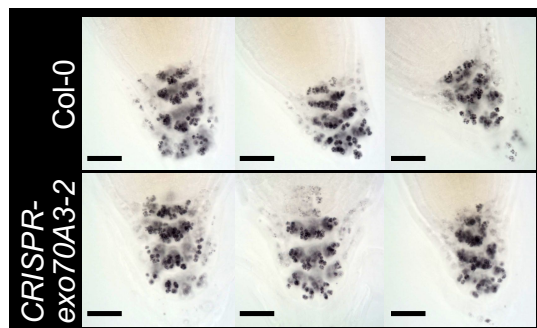


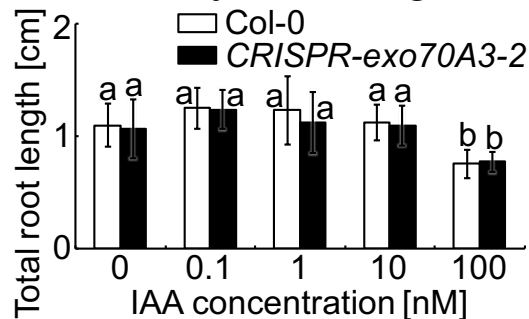
Figure S5 Ogura et al. 2018

B. *CRISPR-exo70A3-2*

Statocytes & statolith



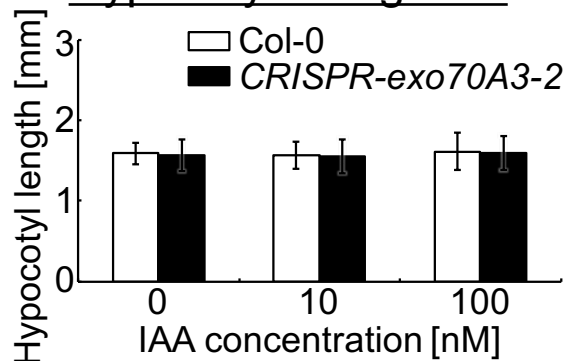
Primary root length



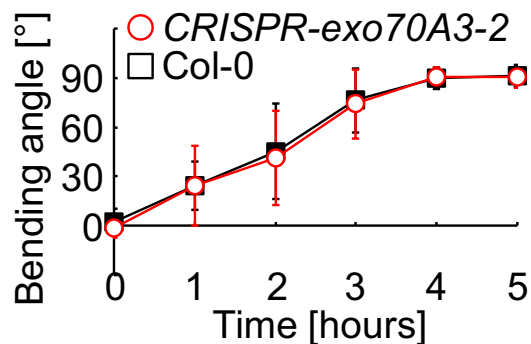
Lateral root density



Hypocotyl elongation



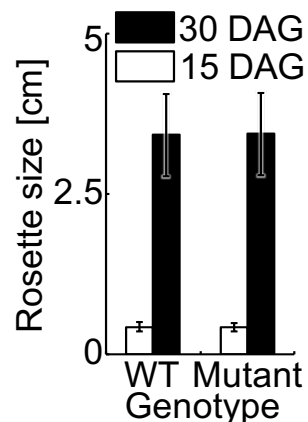
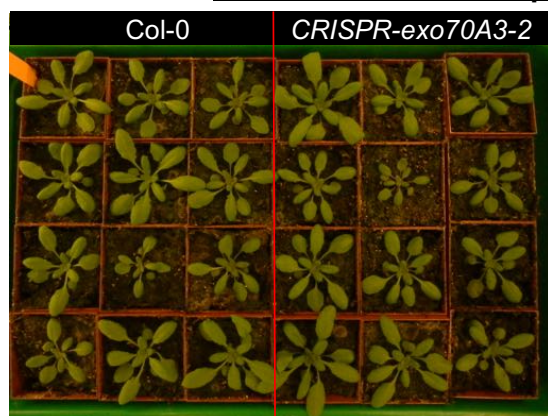
Shoot gravitropism



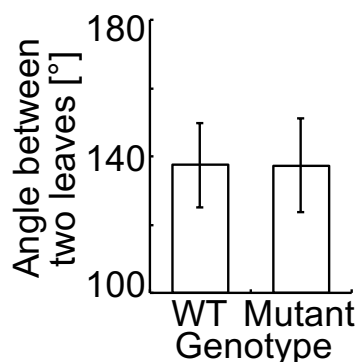
Inflorescence structure



Rosette development



Phyllotaxy



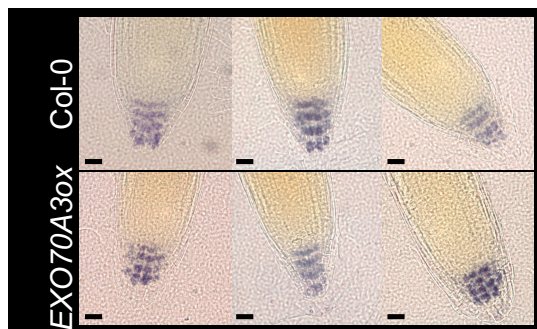
Floral stem height



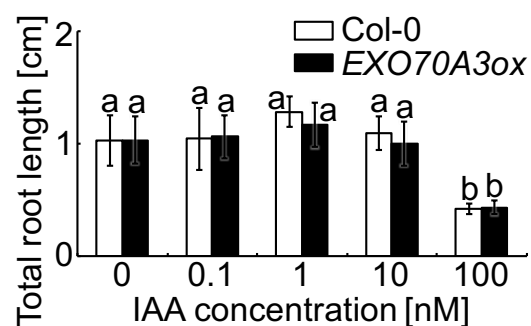
Figure S5 Ogura et al. 2018 (continued)

C. *EXO70A3ox*

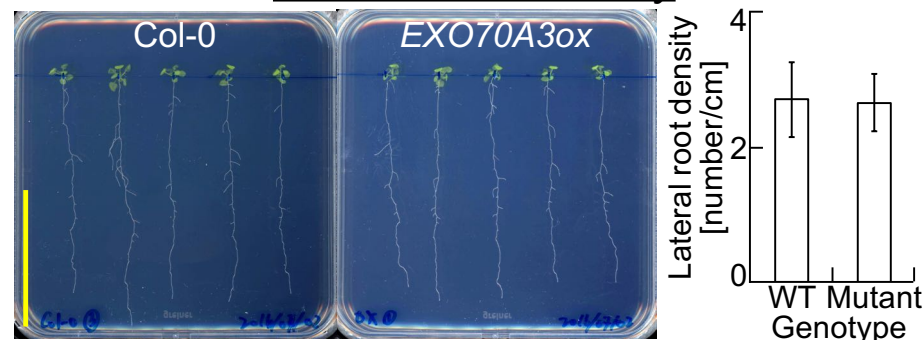
Statocytes & statolith



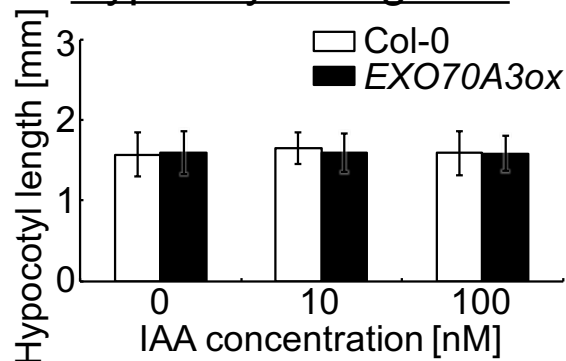
Primary root length



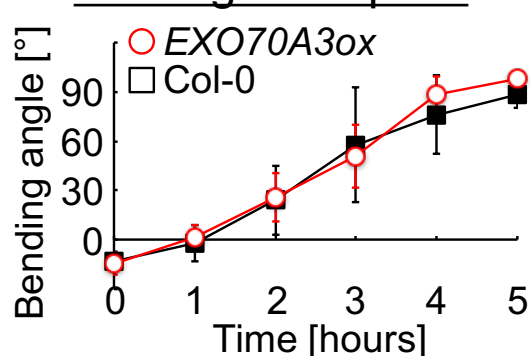
Lateral root density



Hypocotyl elongation



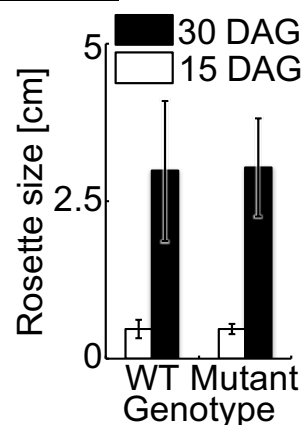
Shoot gravitropism



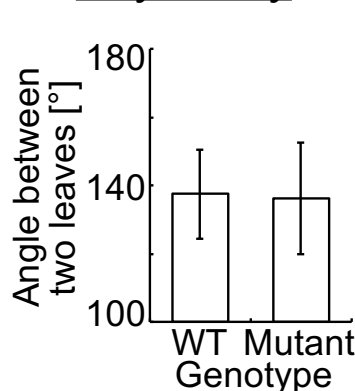
Inflorescence structure



Rosette development



Phyllotaxy



Floral stem height

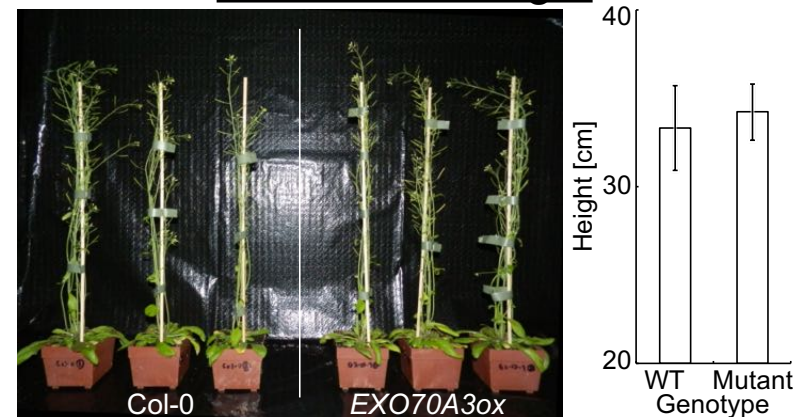
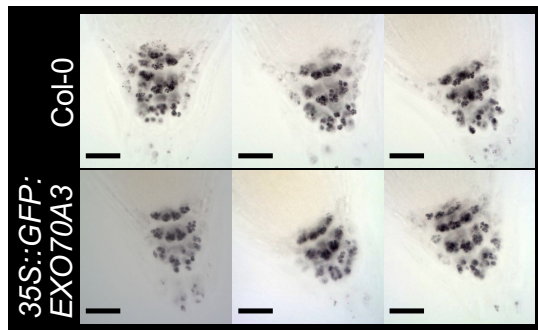


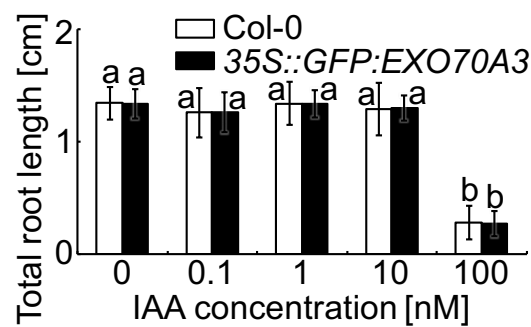
Figure S5 Ogura et al. 2018 (continued)

D. *35S::GFP:EXO70A3*

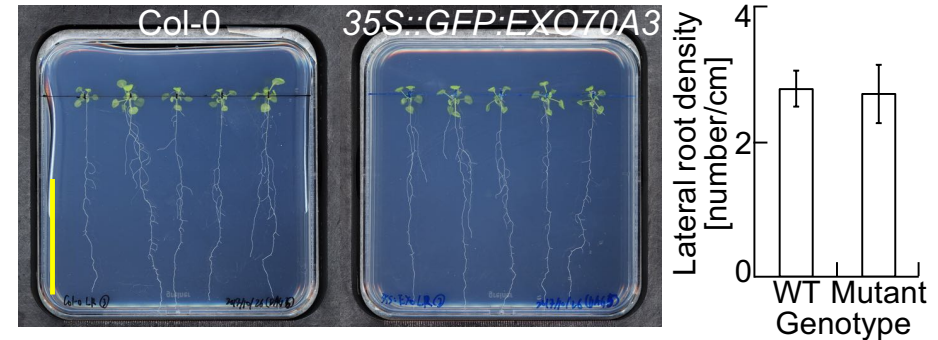
Statocytes & statolith



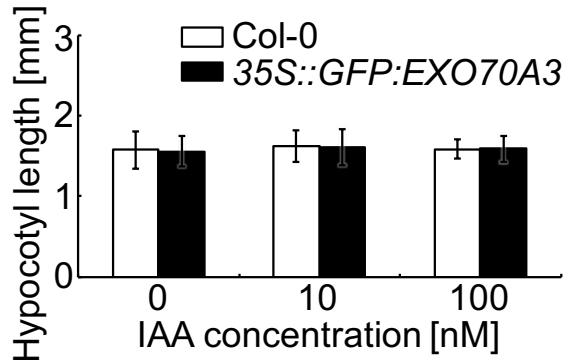
Primary root length



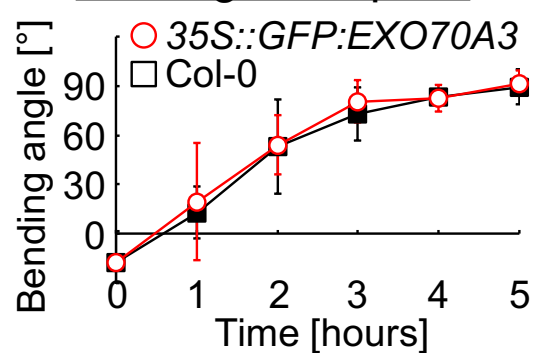
Lateral root density



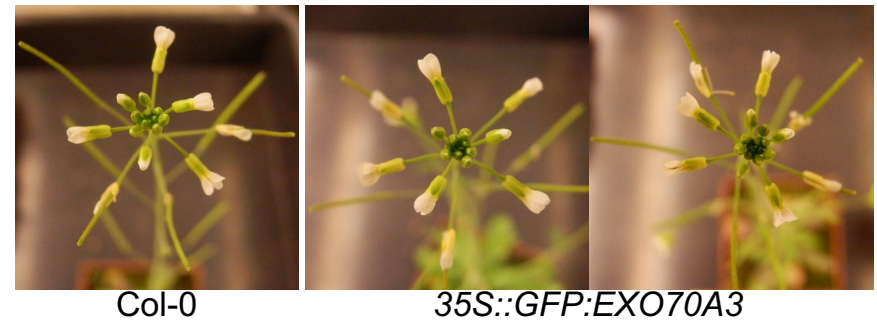
Hypocotyl elongation



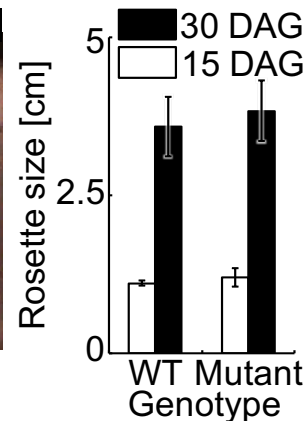
Shoot gravitropism



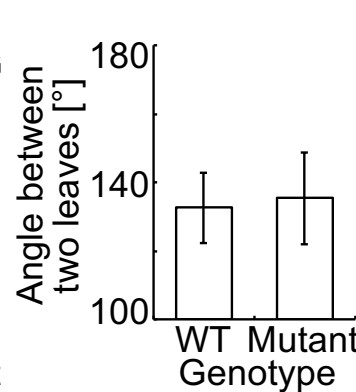
Inflorescence structure



Rosette development



Phyllotaxy



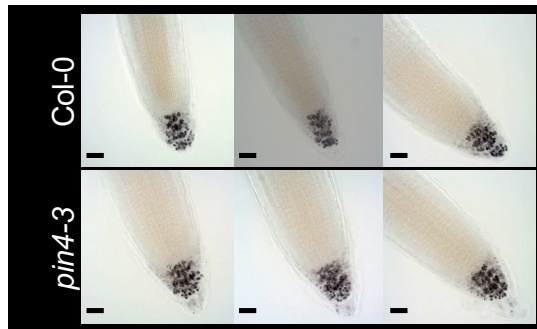
Floral stem height



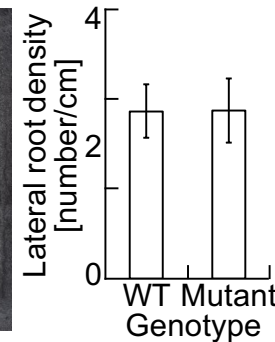
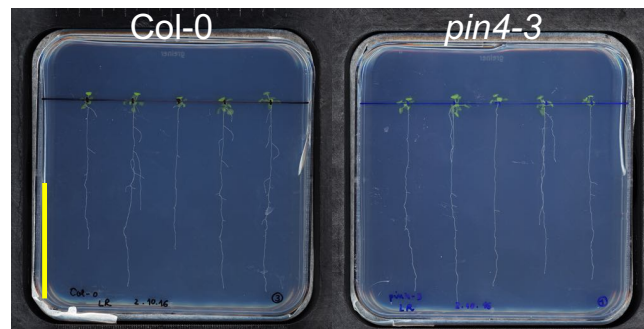
Figure S5 Ogura et al. 2018 (continued)

E. *pin4-3*

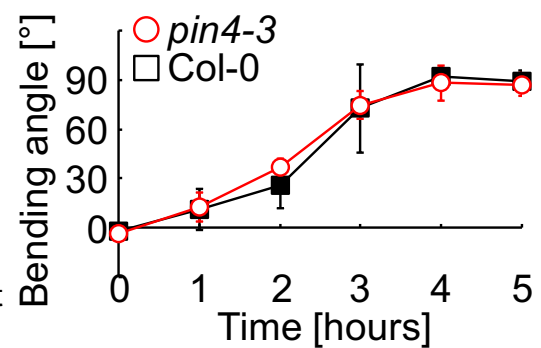
Statocytes & statolith



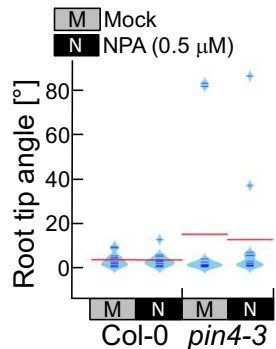
Lateral root density



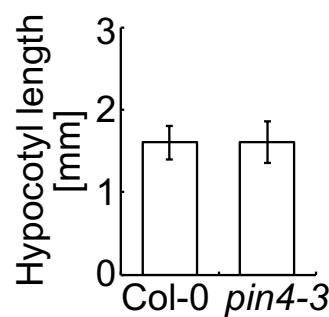
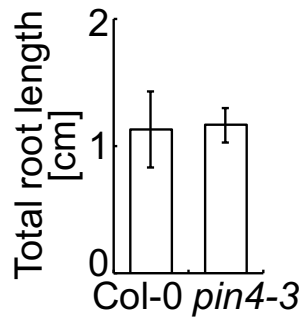
Shoot gravitropism



NPA response



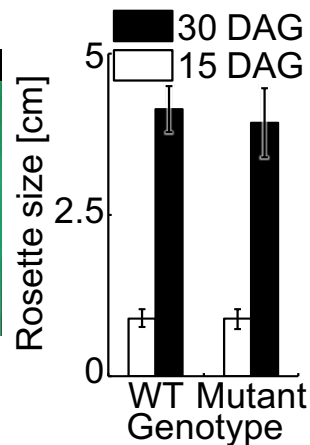
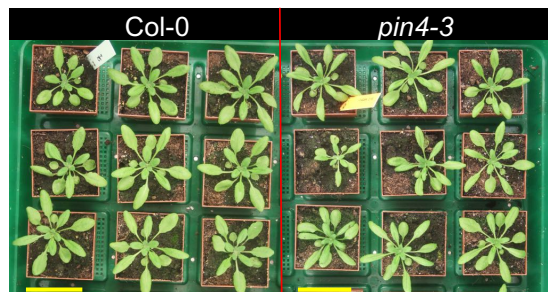
Primary root and Hypocotyl



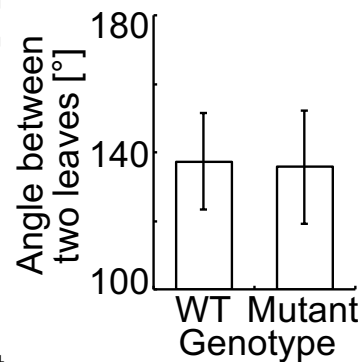
Inflorescence structure



Rosette development



Phyllotaxy



Floral stem height

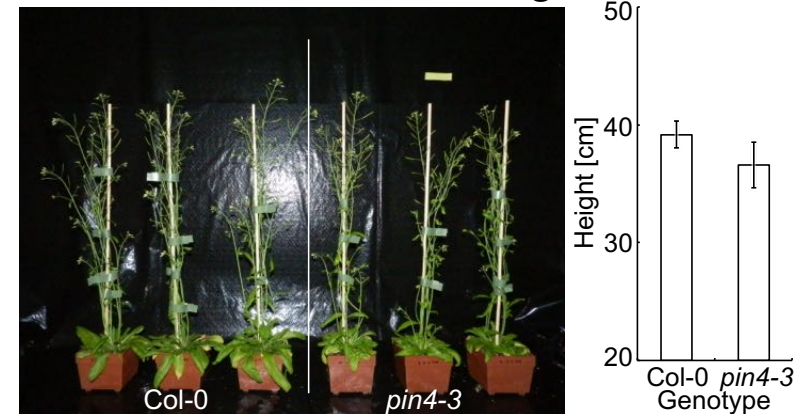


Figure S5 Ogura et al. 2018 (continued)

F. Variances in root tip angles

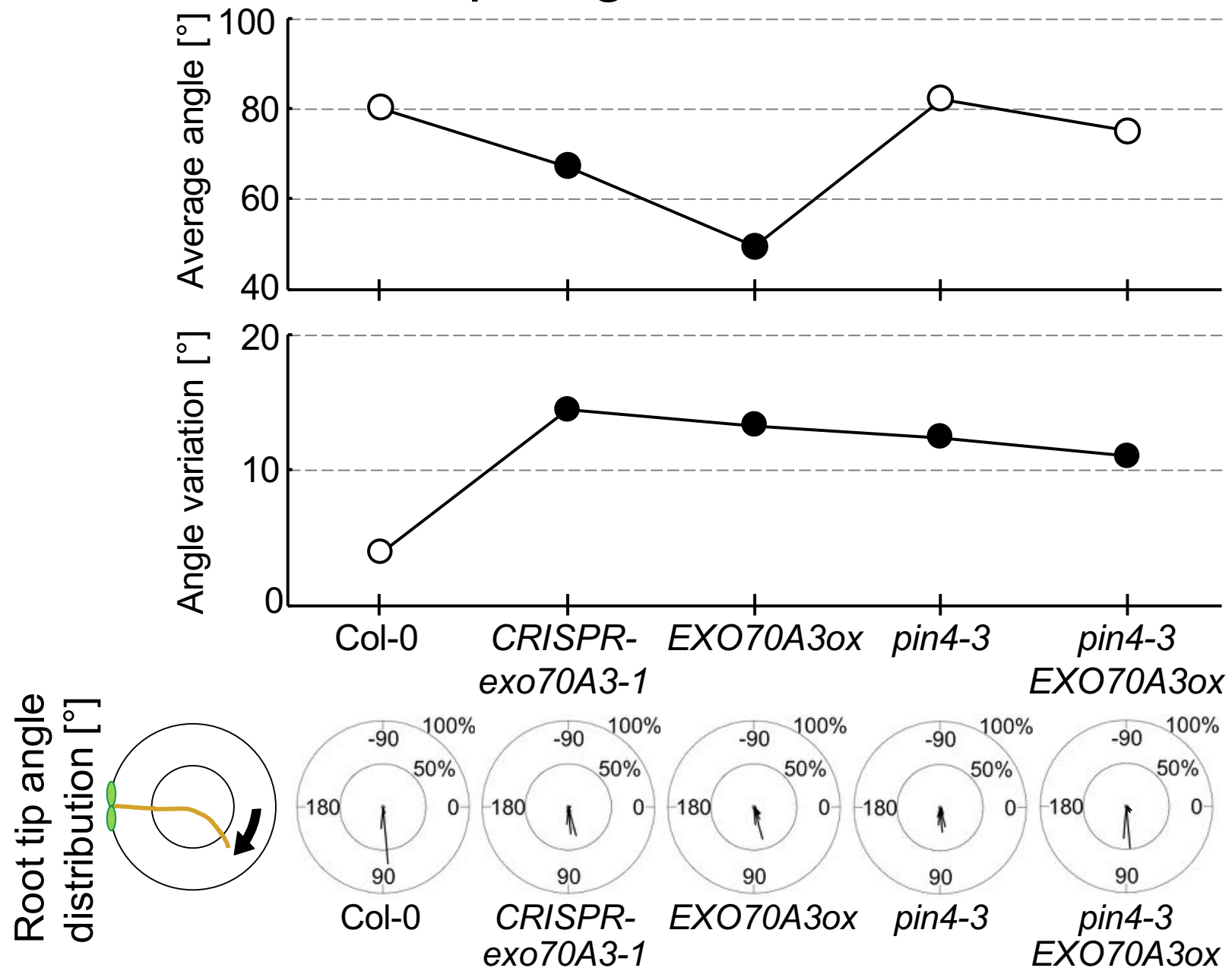
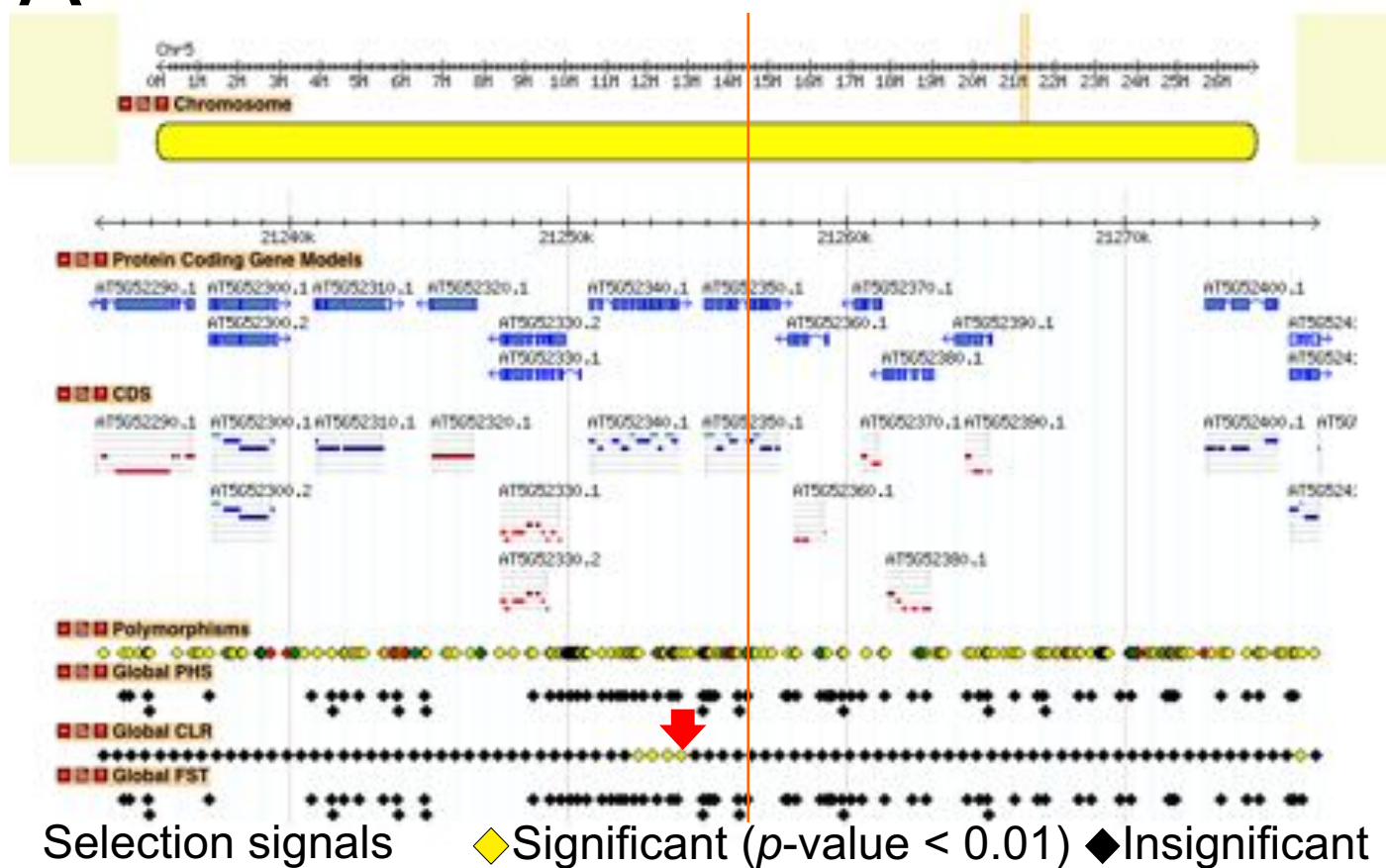


Figure S5 Ogura et al. 2018 (continued)

A



B

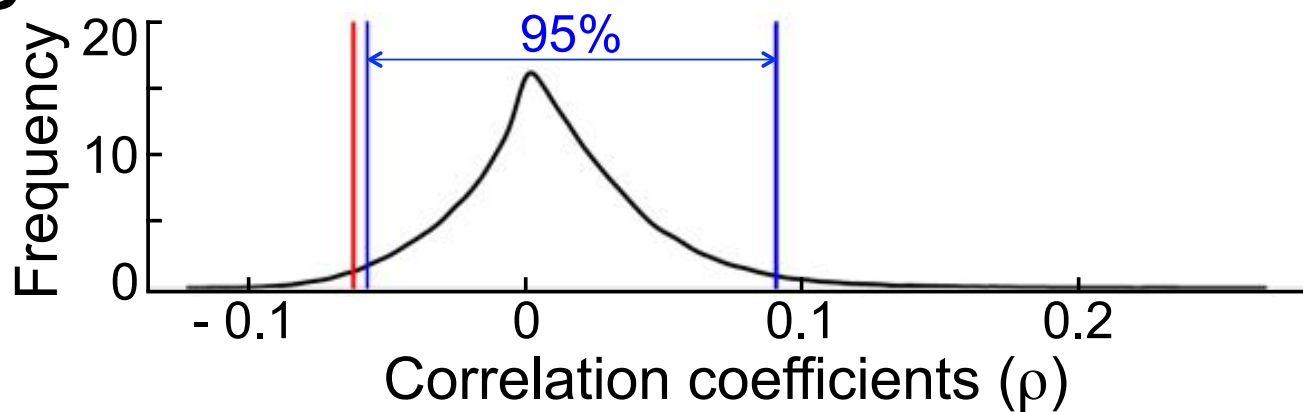


Figure S6 Ogura et al. 2018

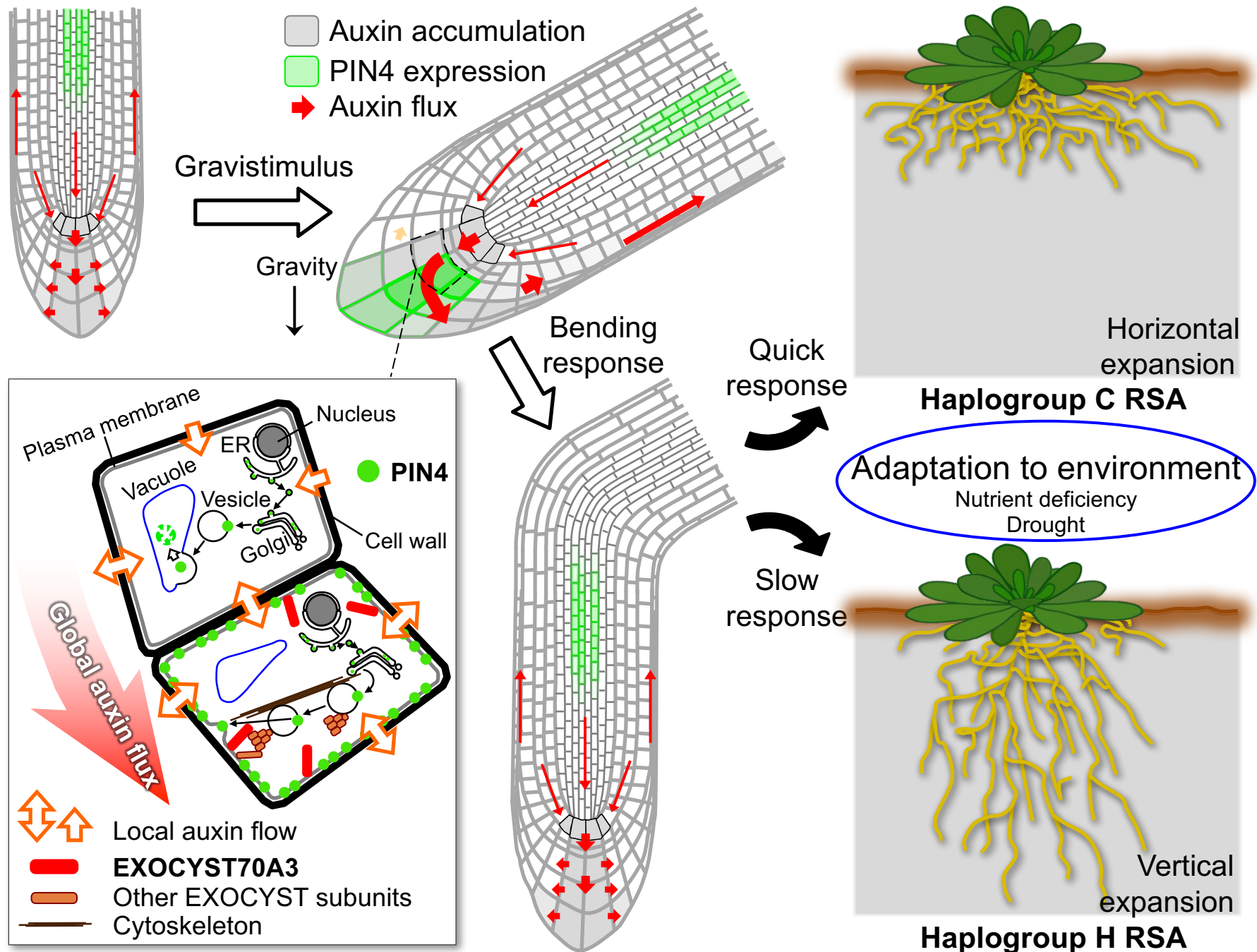


Figure S7 Ogura et al. 2018

Supplemental Information

Supplemental Experimental Procedures

Plant materials and growth conditions

All plants for the image acquisition on plates were grown on vertical 1 × MS agar plates (1 % sucrose, 0.8 (phenotyping for GWA mapping) or 1.0 % (other experiments) agar, pH = 5.7, phenotyping for GWA mapping: 35 ml/plate, other experiments: 53 ml/plate) in 12 cm × 12 cm square plates (Greiner Bio One International AG, Kremsmünster, Austria) that were sealed with Leucopore tape (1.25 cm width, Duchefa Biochemie B.V., Haarlem, The Netherlands), with or without chemicals under long day conditions (16 hours light) at 21°C. OSRAM Fluora L36W/77 (OSRAM Licht AG, Munich, Germany) was used as the light source. Indole-3-acetic acid (IAA), *N*-1-naphthylphthalamic acid (NPA), and propidium iodide were purchased from SIGMA-ALDRICH Co. LLC. (Saint Louis, MO, USA). Sucrose was obtained from AppliChem GmbH (Darmstadt, Germany). Plant Agar and Murashige & Skoog medium incl. MES buffer were purchased from Duchefa Biochemie B.V. 6.5 cm × 6.5 cm × 5.2 cm plastic pots for plant growth on soil (Blumentopf Typ2) were obtained from Alpaco Lück GmbH & Co. KG (Meinerzhagen, Germany).

The seeds of the 215 natural accessions of *Arabidopsis thaliana* (Extended Data Table 1) were a gift from Dr. Magnus Nordborg's laboratory (GMI, Vienna, Austria). *CRISPR-exo70A3-1* and *CRISPR-exo70A3-2* were prepared following previously published method (Mao *et al.*, 2016) and genotypes were confirmed by sequencing. *EXO70A3ox* (SALK_075426) was purchased from Nottingham Arabidopsis Stock Center (Leicestershire, UK). *35S::GFP:EXO70A3* was a gift from Dr. Viktor Žárský (IEB AS CR, Prague, Czech Republic). *PIN1::PIN1:GFP*, *PIN2::PIN2:GFP*, *PIN3::PIN3:GFP*, *PIN4::PIN4:GFP*, *PIN7::PIN7:GFP*, *DR5::GFP* and *pin4-3* were a gift from Dr. Jürgen Kleine-Vehn (University of Natural Resources and Life Sciences, Vienna, Austria). Crosses between *EXO70A3*-related mutants and other mutants were made by manually pollinating emasculated pistils of those marker lines with *EXO70A3ox* pollen. For allelic complementation analysis, *EXO70A3* promoter sequence from Col-0 and *EXO70A3* protein coding genomic sequence from five natural accessions (Col-0, Shakhara (Sha), Lp2-2, LAC-5 and Coc-1) were PCR amplified and cloned into pGreen0029 binary vector (Hellens *et al.* 2000) using In-Fusion Cloning system (Takara Bio Europe, Saint-Germain-en-Laye, France) to obtain 5 constructs that have fused *EXO70A3* promoter sequence from Col-0 with different *EXO70A3* protein coding genomic sequence. The five constructs and pGreen0029 itself as a vector control were used for floral dipping transformation to create transgenic lines. For each construct, at least three individual transformed lines were established, and a line which showed median phenotype in these lines in terms of NPA response was selected for further analysis as a representative line of the allele. Plants for crosses, seed amplification and phenotyping of aerial parts were grown on soil (PROFI SUBSTRAT, Einheitserde Werkverband e.V., Sinntal-Altengronau, Germany) with a bottom layer of Premium Perlite (Gramoflor GmbH & Co. KG, Vechta, Germany) at 21°C, 16 hours light/16°C, 8 hours dark conditions.

Quantitative phenotyping of NPA treated roots for GWAS

Seeds were sterilized by chlorine gas generated from 130 ml of 10% sodium hypochlorite and 3.9 ml of 37% hydrochloric acid and then stratified in sterile water for 3 days at 4°C in the dark. The 215 natural accessions were grown on 1 × MS agar plates containing 10 μM NPA under long day conditions (16 hours light) at 21°C. 24 plants were used for each accession. Starting at 2 days after germination (DAG), root images were acquired by scanners (EPSON

Perfection V600 Photo, Seiko Epson CO., Nagano, Japan) each 24 hours for 5 days (2 DAG – 6 DAG). Root image analyses and phenotype quantification were performed using the BRAT software (Slovak et al., 2014). In particular, from the traits quantified by BRAT, we used a length phenotype (Total length), a width trait (Average root width), and two derivatives of angle phenotypes of roots (Horizontal: Root horizontal index/ Root vertical index \times Total length of the root, Skewness: the averages of angles of all lines, which were drawn between each pixel of the root and the root-hypocotyl junction, to the gravity vector). Horizontality represents both “the trend of root growth to horizontal direction” and “the size of root growth in horizontal direction”. Simply stating, “Horizontality” shows the intensity of root growth in horizontal direction.

Genome wide association studies (GWAS) and bioinformatics analyses

GWAS were conducted using the average trait value for each trait of 215 natural accessions by an accelerated mixed model (EMMAX (Kang et al., 2010)) followed by EMMA (Kang et al., 2008) for the most significant 200 associations as implemented in the web-based GWAS application GWAPP (Seren et al., 2012), conducted on December 15, 2014). For “horizontality” phenotype, box-cox transformation was conducted before GWAS to obtain better data distribution. All other statistical analyses in this study were performed using R language (<http://www.R-project.org/>). For the test of the correlation between phenotypes on control and NPA plates, z-scores for phenotype values of each accession were calculated according to the following formula: $z = (\text{“Individual phenotype value”} - \text{“Median of phenotype values”}) / \text{“Standard deviation of phenotype values”}$, and used for the analysis. For multiple tests, one-way, two-way or three-way factorial ANOVA (analysis of variance) was first conducted to assess the existence of distinct groups using a threshold p -value < 0.05 . Then the post-hoc Tukey test was performed to find significantly different groups (threshold: p -value < 0.05). To test whether population structure does explain a significant portion of phenotypic variation observed between two haplogroups, the significance of phenotypic differences between haplogroups was assessed using the mixed model procedure as implemented in EMMAX (Kang et al., 2010) with a kinship matrix estimated from the Regional Mapping Project data (Horton et al., 2012). The analysis regarding signals of selection was conducted using the Selection Browser of the Regional Mapping Project (Horton et al., 2012). The eFP browser (Winter et al., 2007) was used as a general reference for gene expression profiles of candidate genes as well as a RNA-Seq data from Li et al. 2017 (Li et al., 2017). The SALK genome browser created by the *Arabidopsis thaliana* 1001 genome project (Schmitz et al., 2013), signal.salk.edu/atg1001/3.0/gebrowser.php) was used to analyze polymorphism among natural accessions. Signals of selection was investigated using Selection Scan (Horton et al., 2012). The search for an association between the SNP which we identified by GWAS using Horizontality and the climate condition was conducted by comparing correlations that were calculated between each SNP and 19 climate conditions (WorldClim, <http://www.worldclim.org/>) in Hancock et al., 2011.

Haplogroup analysis

For grouping the 215 natural accessions of *A. thaliana* according to haplotypes around the single nucleotide polymorphisms (SNPs) of interest, SNPs in a 40KB window around the SNPs of interest were extracted from a pre-imputation version of the Regional Mapping Project panel described previously (Horton et al., 2012). These SNPs were used as the input into fastPHASE (Scheet and Stephens, 2006) version 1.4.0, which was run using the default settings, with the exception of invoking the $-Pzp$ option to output raw likelihood values. For each SNP in the analysis, the cluster membership with the highest likelihood was designated as the haplogroup for that SNP. In the resulting

dendrograms, accessions were ordered using the distance matrix calculated from all SNPs in the window of interest.

Plant observations

All manual measurements and analyses of plant images were conducted using Fiji (Schindelin *et al.*, 2012). For root phenotyping of *CRISPR-exo70A3-1*, *CRISPR-exo70A3-2*, *EXO70A3ox*, *35S::GFP::EXO70A3* and *pin4-3* young seedlings, seedlings were grown on 1 × MS agar plates. For root angle changes of these mutants in response to NPA, we conducted transfer experiments instead of germinating the plants on NPA as for our GWAS screen, to directly and more precisely measure the effect of NPA on the direction of root growth. First seeds were sown on normal 1 × MS agar plates and grown until 5 days after germination. Immediately after that, plants grown to a similar size were transferred to control (DMSO) or treatment plates that contained 0.1 μM NPA (allelic complementation) or 0.5 μM NPA (other experiments) and images were acquired one day after the transfer by scanners (EPSON Perfection V600 Photo). The angle of the root tip to the vector of gravity was measured from these images. For each genotype, 10 or more plants were used and the whole experiment was repeated at least twice. For lateral root density, plants grown on 1 × MS agar plates were transferred to fresh plates 5 days after germination (DAG) and images of plants were acquired 20 days after germination. Then numbers of lateral roots and the primary root lengths (cm) of plants were manually measured. The lateral root density (the number of lateral roots/cm) was calculated by dividing the lateral root numbers by the primary root lengths. To compare lateral root densities among natural accessions, we used eight accessions (haplogroup C: Ler-1, Shakdara (Sha), Lp2-2 and Per-1; haplogroup H: LAC-5, MIB-60, Coc-1 and Ty-0). For hypocotyl length and total root length measurements, various concentrations of IAA in DMSO solutions were added to 1 × MS agar plates (final IAA concentrations: 0.1, 1, 10 and 100 nM, DMSO concentration: 0.1% v/v). 0.1% DMSO plates were used as a mock treatment. Plants were grown on these plates for 5 days after germination and images were acquired. 10 or more plants of each genotype were used for each experiment and the experiment was repeated twice.

For the observation of statocytes, plants were grown on 1 × MS agar plates for 5 days after germination. They were stained in Lugol's solution (Appllichem) for 2 minutes and transferred into a chloral hydrate solution (chloral hydrate 8 g, glycerol 1 ml, water 2 ml). Images were observed using an AXIO image.M1 or an AXIO observer Z1 (Carl Zeiss AG, Oberkochen, Germany) combined with a CMOS camera (SPOT Idea 1.3 Mp Color Digital Camera, SPOT imaging, Sterling Heights, MI, USA). More than 5 plants per genotype were observed in an experiment and the experiment was repeated 2 times.

For analysis of above-ground organs of mature plants, images were acquired using a Nikon D80 (Nikon Co., Tokyo, Japan) digital camera. Images were taken after 15 and 30 (phyllotaxis and rosette development), 45 (inflorescence) and 50 (height) days growth on soil for shoot traits comparison between Col-0 and *EXO70A3*-related mutant. At least 9 plants were observed for each line and the experiment was repeated twice.

For analyses of fluorescent protein containing lines, crossbred lines (*DR5::GFP EXO70A3ox*, *DR5::GFP CRISPR-exo70A3-2*, *PIN1::PIN1::GFP EXO70A3ox*, *PIN2::PIN2::GFP EXO70A3ox*, *PIN3::PIN3::GFP EXO70A3ox*, *PIN4::PIN4::GFP EXO70A3ox*, *PIN7::PIN7::GFP EXO70A3ox* and *PIN4::PIN4::GFP CRISPR-exo70A3-1*) and control lines for these crossbreds (*DR5::GFP*, *PIN1::PIN1::GFP*, *PIN2::PIN2::GFP*, *PIN3::PIN3::GFP*, *PIN4::PIN4::GFP* and *PIN7::PIN7::GFP*, respectively) were grown on 1 × MS agar plates for 5 days after germination. GFP signals in plant roots were observed under a laser scanning confocal microscope LSM700 (Carl Zeiss) with a 10× objective (EC plan Neofluar 10×0.3 M27), a 20× objective (Plan Apochromat 20×0.8 M27) or a 40× objective (Plan-Apochromat 40×/0.95 Korr M27, Zeiss). GFP-fusion proteins and PI were excited by a 488-nm diode array and

detected by collecting light with wavelengths below 555 nm and above 560 nm, respectively. Gravity responses of GFP signals were observed using *DR5::GFP EXO70A3ox*, *DR5::GFP CRISPR-exo70A3-1* and control *DR5::GFP* lines following the dynamic root gravitropism assay (see below) and images were acquired 3 hours after re-orientation of plates. Cell outlines were visualized by propidium iodide (red signals). At least 10 plants per genotype were observed for an experiment and the experiments were repeated at least twice. FRAP (Fluorescent recovery after photo bleaching) was conducted using *PIN4::PIN4:GFP* and *PIN4::PIN4:GFP EXO70A3ox* lines by Visitron Spinning Disc microscope, which is based on Nikon Eclipse Ti E inverted microscope (Nikon Instech Co., Ltd., Tokyo, Japan) equipped with CSU-W1 spinning disc unit (Yokogawa Electric Corporation, Tokyo, Japan), with a 60× water objective (Plan Apo VC 60x/1.2 WI water, Nikon) and Immersol WTM (Carl Zeiss). Plants were grown for 5 DAG and whole seedlings were mounted on glass slides with distilled water. FRAP observation was done using EMCCD (Ixon Ultra 888, Andor Technology Ltd., Belfast, UK) as follows: laser 488 nm (20% for observation, 100 % for bleaching), emission filter: ET 525/50, exposure 500 ms, FRAP time 150 ms/pixel, interval 0.5 minutes, pre-count 3 times, total time 20 minutes and EMCCD gain 645 nm. Fluorescent intensity was manually measured using Fiji. A ratio of fluorescent intensity to a neighboring cell, which was not photobleached, is calculated for each intensity data and then normalized by an average ratio of all pre-counts of *PIN4::PIN4:GFP* and used for the analysis. At least 10 plants were used in an experiment and the experiment was repeated twice.

Dynamic root and shoot gravitropism assays

To observe the dynamic shoot gravitropic responses (negative gravitropism), *EXO70A3*-related mutants and control Col-0 plants were grown on soil for 40 days. The whole pots were turned by 90° and images of the time-dependent angle changes of floral stems were acquired every hour for 5 hours using a Nikon D80 camera. Quantification of the angles of floral stems relative to gravity on these images was conducted using Fiji. At least Four plants were used for each line, and the experiment was repeated twice.

For the dynamic root gravitropism assay (positive gravitropism), plants were grown on 1 × MS agar plates for 5 days after germination under normal growth conditions. Plants of the same size were transferred to new vertical 1 × MS agar plates and incubated for a further one hours under the same growth conditions. These plates were then turned 90 degrees (in a manner that plates remained vertical) and fixed on vertically oriented scanners (EPSON Perfection V600 Photo). Images were acquired every four minutes for 20 hours under the same growth conditions. Root re-orientation (root tip angle change to the vector of gravity on images representing 32 minute intervals) was measured using Fiji as Root tip angle. 10 or more roots were measured for each genotype and the experiment was repeated at least three times. To compare the dynamic root gravitropism among accessions, we used eight accessions (haplogroup C: Ler-1, Shakdara (Sha), Lp2-2 and Per-1; haplogroup H: LAC-5, MIB-60, Coc-1 and Ty-0).

Root system architecture (RSA) assay

To assess the RSA in soil, we used three plants from each genotype. The experiment was conducted blind so that the plant genotypes were not known to the researcher conducting and analyzing the experiment, except for *pin4-3* and *CRISPR-exo70A3-1*. Each plant was grown on soil for 30 days in a pot (ϕ 20 cm, 17 cm height, Fiskars Germany GmbH, Herford, Germany) at 21°C, 16 hours light/16°C, 8 hours dark conditions. The whole pot was then sectioned in two halves at the center of the plant using a band saw machine (STARTRITE 20RWS, Startrite, A.L.T. Saws & Spares Ltd., Gillingham, UK) equipped with a specially modified band (10 mm BAND SAW, LENOX Newell Rubbermaid

Inc., East Longmeadow, MA, USA). The soil at the section was gradually removed with a paint brush, and an image was acquired by a Nikon D80 camera. Using Fiji, the roots on the image were marked (overlay) and subsequently the coordinates of all root pixels were determined. The Shallowness (standard deviation of all root pixels in X axis direction in a picture divided by that in Y axis direction) was calculated using these data as an indicator of shallow or deep RSA. Note that, while many recent studies used rhizotron (Devienne-Barret *et al.* 2006, Plant and Soil) and related systems, we could not use these for our analysis. Images acquired by the rhizotron system, GLO-Roots system (Rella'n-A'lvarez *et al.* 2015, eLife) and other related systems are optically appealing and probably useful to understand morphological patterning of roots. However, all these systems do not allow roots grow in 3-dimensions (only in 2-dimensions, sometimes with a rounded shape plane) due to their system requirements for root visualization, particularly in case of Arabidopsis roots. Since roots in nature do not grow in 2-dimensions, these systems have severe limitations in studying natural root system architectures. In fact, no Col-0 root system architectures grow vertically deeper in 3-dimensional growth space while the rhizotron and related systems always showed vertically expanded roots (they do not have the space to grow anywhere else but down in an space that is essentially 2-dimensional).

Seed number assay

To analyze fitness of different genotypes in drought conditions, we conducted an assay similar to that reported previously (Schmalenbach *et al.*, 2014). In particular, we used four plants from each genotype and four sets of the whole drought experiment, resulting in a total number of 16 plants per genotype. Each experiment contained five pots that were arranged in a block in a growth chamber. In each block, positions of five pots were randomized. The experiment was conducted blindly so that the plant genotypes were not known to the researcher conducting and analyzing the experiment. Each four plants of a line were grown on soil for more than 60 days in a pot (ϕ 20 cm, 17 cm height, Fiskars Germany GmbH, Herford, Germany) at 60% humidity, 21°C, 16 hours light/16°C, 8 hours dark conditions until the seed production was completed. Four plants of two different genotypes were grown within one pot. Before starting the experiment, the initial weight of each pot filled with humid soil was measured. Subsequently, the soil in pots without plants was dried up by heating to 95 °C for 8 days and the initial water contents (% g water/g soil) of the soil was determined. Then water content of each pot was calculated with the total soil weight and the initial dry soil weight. During plant growth, water contents of pots were decreased from 60% (DAG5) to 30% (DAG 35). After that, pots were watered every two to three days as necessary by spray from top to keep the water contents 25 %. As the water-sufficient control, same experiment was repeated while water level was kept 65% through the whole experimental period in this control. Mature siliques from each plant were harvested and the number of seeds in five siliques was counted to determine the average number of seeds per silique for each plant. Subsequently, the total silique number of each plant was counted and the total seed number was decided using the average seed number per silique and the total silique number. An average seed number of four plants of each line in a pot was calculated, rounded and used in statistical analyses.

The significance of effects from seed number experiments in terms of responses to drought was determined using Poisson regression models. For the Col-0 versus *EXO70A3ox* experiment, the following model was fit using the glm function in the stats package in R:

$$\text{Seed number} \sim \mu + \text{Treatment} + \text{Genotype} + \text{Treatment*Genotype}$$

1 Where Seed number is the number of seeds per plant, Treatment refers to the watering regime, Genotype is Col-0 or
2 *EXO70A3ox*, and Treatment*Genotype is the interaction between these two variables. In this model, all terms are
3 significant as shown below.

Coefficients ^a					
	Estimate	Standard error	z value	Pr (> z)	
(Intercept)	11.070813	0.001972	5613.143	$< 2.0 \times 10^{-16}$	***
Treatment-Drought	-3.673558	0.012535	-293.067	$< 2.0 \times 10^{-16}$	***
GWAS_ID- <i>EXO70A3ox</i>	-0.406473	0.003119	-130.304	$< 2.0 \times 10^{-16}$	***
Treatment-Drought*GWAS_ID- <i>EXO70A3ox</i>	-0.151038	0.02075	-7.279	3.36×10^{-13}	***

5 ^aSignificance codes: 0, '***', 0.001, '**', 0.01, '*', 0.05, '.', 0.1, ' ', 1

6
7 For the experiment comparing haplogroups C and H of *EXO70A3*, the following mixed-effect Poisson model was fit
8 using the glmer function in the lme4 package for R:

9
10 Seed number $\sim \mu + \text{Treatment} + \text{Haplogroup} + \text{Treatment} * \text{Haplogroup} + 1 | \text{Accession} + \varepsilon$

11
12 Where seed number is the number of seeds per plant, Treatment is a fixed effect referring to the watering regime,
13 Haplogroup is a fixed effect representing the H or C haplogroup, and Accession is a random effect fitted for each
14 individual accession. Treatment*Haplogroup is the interaction between these two variables. In this model, the
15 Treatment and Treatment*Haplogroup interaction were significant, while the marginal Haplogroup effect was not as
16 shown below.

Coefficients ^a					
	Estimate	Standard error	z value	Pr (> z)	
(Intercept)	10.61369	0.23522	45.1	$< 2.0 \times 10^{-16}$	***
Treatment-Drought	-3.53378	0.00686	-515.1	$< 2.0 \times 10^{-16}$	***
Haplogroup-H	0.08876	0.33269	0.3	0.79	***
Treatment-Drought*Haplogroup-H	-1.19095	0.01409	-84.5	$< 2.0 \times 10^{-16}$	***

18 ^aSignificance codes: 0, '***', 0.001, '**', 0.01, '*', 0.05, '.', 0.1, ' ', 1

19
20 In order to apply a population structure correction to the comparison of these haplogroups, a Poisson regression model
21 was fit using the glm function in the stats package in R:

22
23 Seed number $\sim \mu + \text{Treatment} + \text{Accession} + \text{Treatment} * \text{Accession}$

24
25 Where Seed number is the number of seeds per plant, Treatment refers to the watering regime, Accession is the
26 accession tested, and Treatment*Accession is the interaction between these two variables. For each accession, the
27 coefficients for these effects were extracted from the model output and used to calculate the following “modeled”

phenotypes as shown below: seed number (SN) in well-watered conditions, seed number in drought conditions, and response to drought.

GWAS_ID	SN (well-watered)	Response to drought	SN (drought)	Haplogroup
6932	9.692257	-2.48811	7.204149	H
6962	10.45793	-2.91863	7.539293	H
7520	11.19351	-4.56579	6.627711	H
8354	11.06028	-3.7566	7.303675	H
204	10.82579	-3.90585	6.919931	C
5729	10.90723	-4.68564	6.221584	C
7351	10.11186	-7.43771	2.674149	C
96	10.96822	-6.07412	4.894101	C

A population-structure corrected test of differences between the haplogroups H and C was then performed for each of these phenotypes as described in “Genome wide association studies (GWAS) and bioinformatics analyses” part on Supplemental Experimental Procedure page 2. The resulting *p*-values for these tests are shown below.

Control: 0.844

Drought: 0.11051

Response: 0.09257

The significance of the differences in absolute seed numbers produced per plant in drought between Col-0 and *EXOCYST70A3ox* and between haplogroups C and H was analyzed by ANOVA.

Quantitative reverse transcription PCR (qRT-PCR)

For qRT-PCR, primers were purchased from Eurofins MWG Operon Co. (Ebersberg, Germany). Plants were grown on 1 × MS agar plates under normal growth conditions with or without 100 nM IAA for 7 days after germination. Tissues were collection by excision with fine scissors. Whole shoots and roots were simply cut from whole plants. For root tips, 2.5 mm root tips were excised from primary roots. 5 plants for the whole plant, 5 plants for the shoot, 10 plants for the root and more than 100 plants for the root tip samples were used. Samples were immediately frozen in liquid nitrogen. Frozen plant materials were ground using a ball mill (MM301, Retch, Haan, Germany) with 5 mm glass beads and total RNA was extracted using RNeasy Plant Mini (QIAGEN GmbH, Hilden, Germany). RNA concentrations were quantified using Nanodrop (Thermo Fisher Scientific Inc., Waltham, MA USA). cDNA was synthesized using RevertAid Reverse Transcriptase (Thermo Fisher Scientific Inc.) and Oligo (dT)₁₈ primer (Thermo Fisher Scientific Inc.) according to the manufacturer’s protocol. qrt-PCR reaction was prepared using 2x SensiMix™ SYBR & Fluorescein Kit (PEQLAB LLC, Wilmington, DE, USA) according to the manufacturer’s instructions and PCR was conducted by a combination of iCycler and IQ5 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). All transcript levels were normalized to the tubulin β gene (AT5G62690). For AT5G52340 and AT5G52360, gene expression levels were calculated as relative values to that of Col-0 whole plants. For the other genes, expression levels were compared as the relative values to that in Col-0 root tips. *EXO70A3* gene expression in IAA treated plants was calculated as the relative value to the transcript level in mock treated (1% DMSO) Col-0 root tips. All experiments were repeated at least twice. Genes quantified include *EXO70A3* (AT5G52350), AT5G52340, AT5G52360, PIN1 (AT1G73590), PIN2 (AT5G57090),

PIN3 (AT1G70940), PIN4 (AT2G01420) and PIN7 (AT1G23080). For the comparison of *EXO70A3* expression levels among natural accessions, we used eight accessions (haplogroup C: Ler-1, Shakdara (Sha), Lp2-2 and Per-1; haplogroup H: LAC-5, MIB-60, Coc-1 and Ty-0).

Supplemental References:

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