

Supplementary material

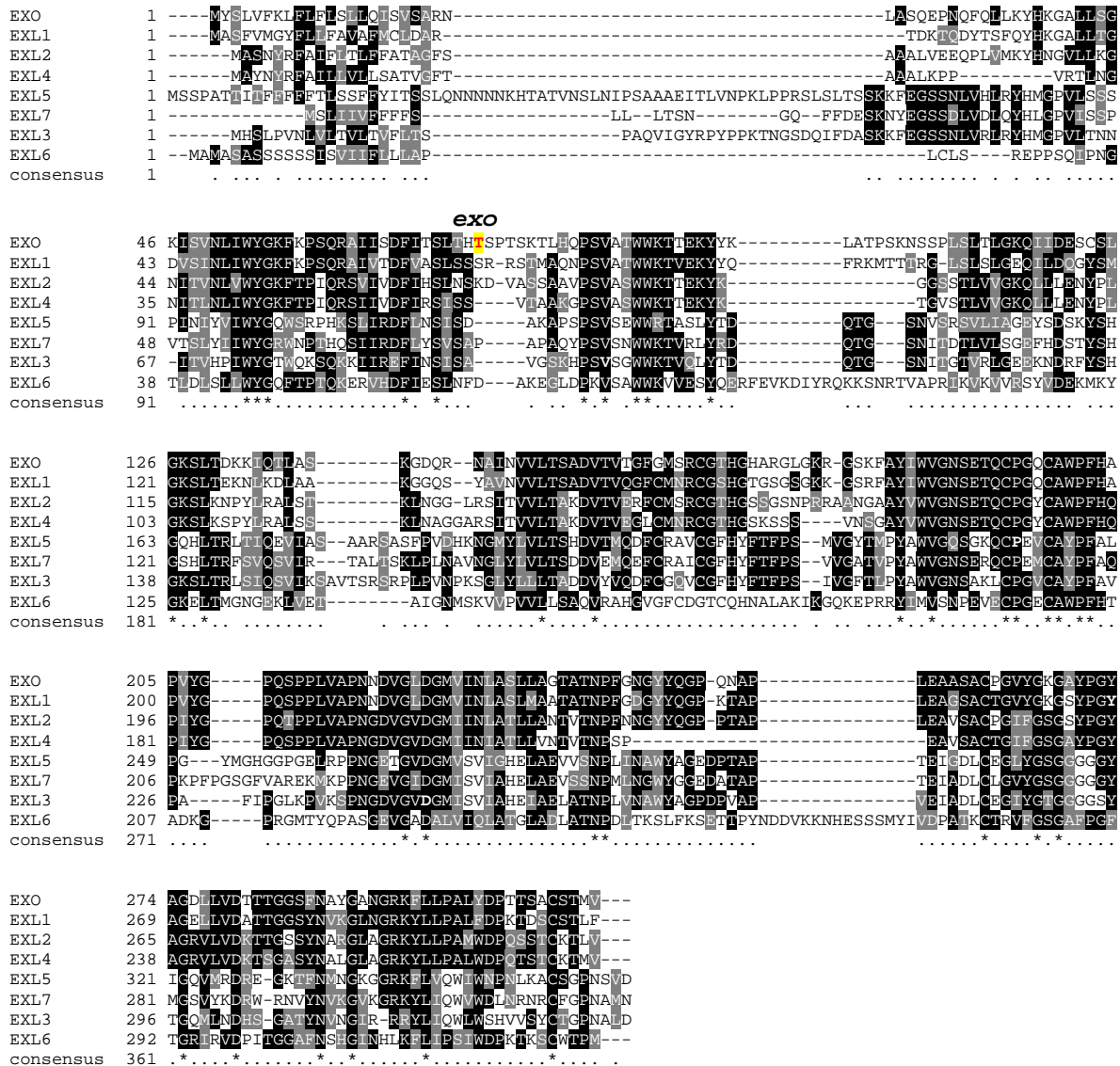


Figure S1. Primary structures of the EXO and EXL proteins.

Coding sequences were derived from full-length cDNAs. The *EXL6* gene has an intron (data not shown), all other genes feature one continuous exon. The coloured amino acid indicates the position of the T-DNA insertion in the *EXO* gene. Sequences were aligned using the BCM Search Launcher (<http://searchlauncher.bcm.tmc.edu/>) and BOXSHADE tools (http://www.ch.embnet.org/software/BOX_form.html).

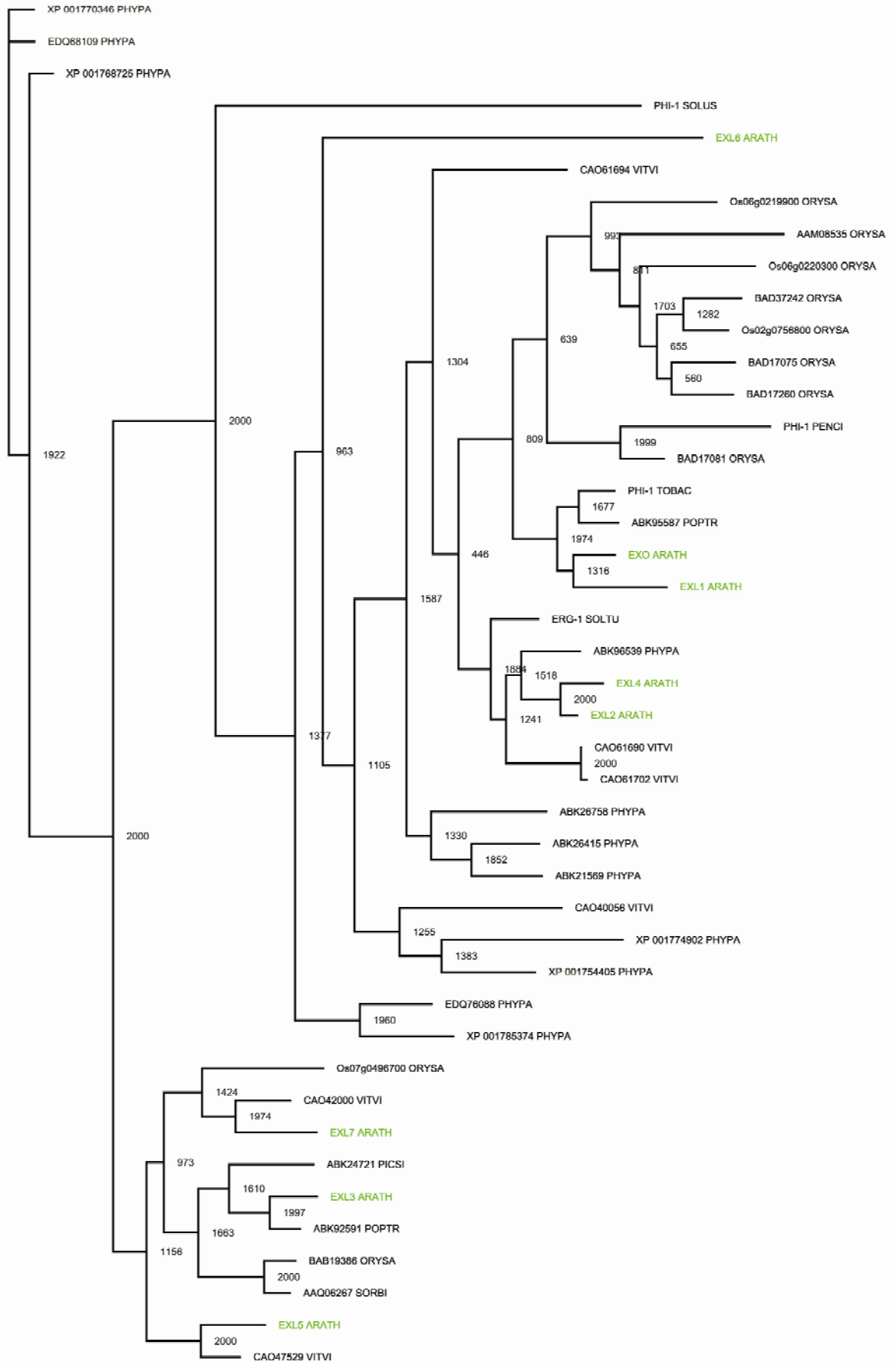


Figure S2. Phylogenetic tree of PHI1/EXO sequence relationships.

EXO homologs were identified using BLAST searches (see Additional file 2). Fragmentary sequences were discarded. Multiple alignments were created by means of the ClustalW2 tool (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>). Sequences were transformed into the PHYLIP format by means of the GeneDoc software (<http://www.nrbsc.org/gfx/genedoc/index.html>). The PhyML software package (*Systematic Biology* 2003, 52: 696-704) with the JTT model for proteins was used to create the phylogenetic tree. Branch support was estimated through 2000 bootstrap samples. TreeView (*Computer Applications in the Biosciences* 1996, 12: 357-358) was used to visualize the tree file.

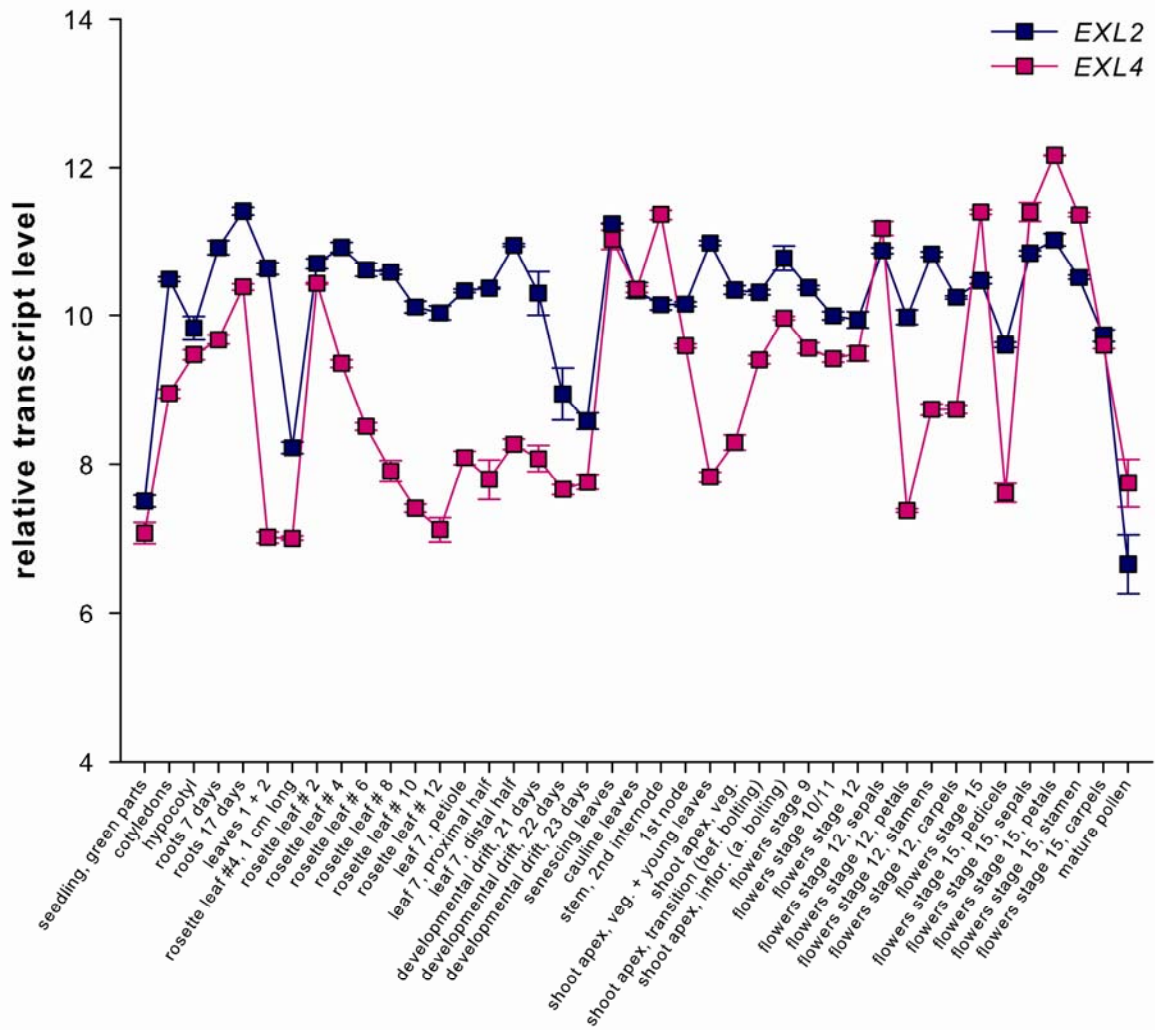


Figure S3. *EXL2* and *EXL4* expression in different organs and developmental stages.

Wild-type expression profiles of the development series (*Nat Genet* 2005, **37**: 501-506) were downloaded from AtGenExpress and normalized using RMA-Express (*Bioinformatics* 2003, **19**: 185-193). The mean and SD of three replicates are shown.

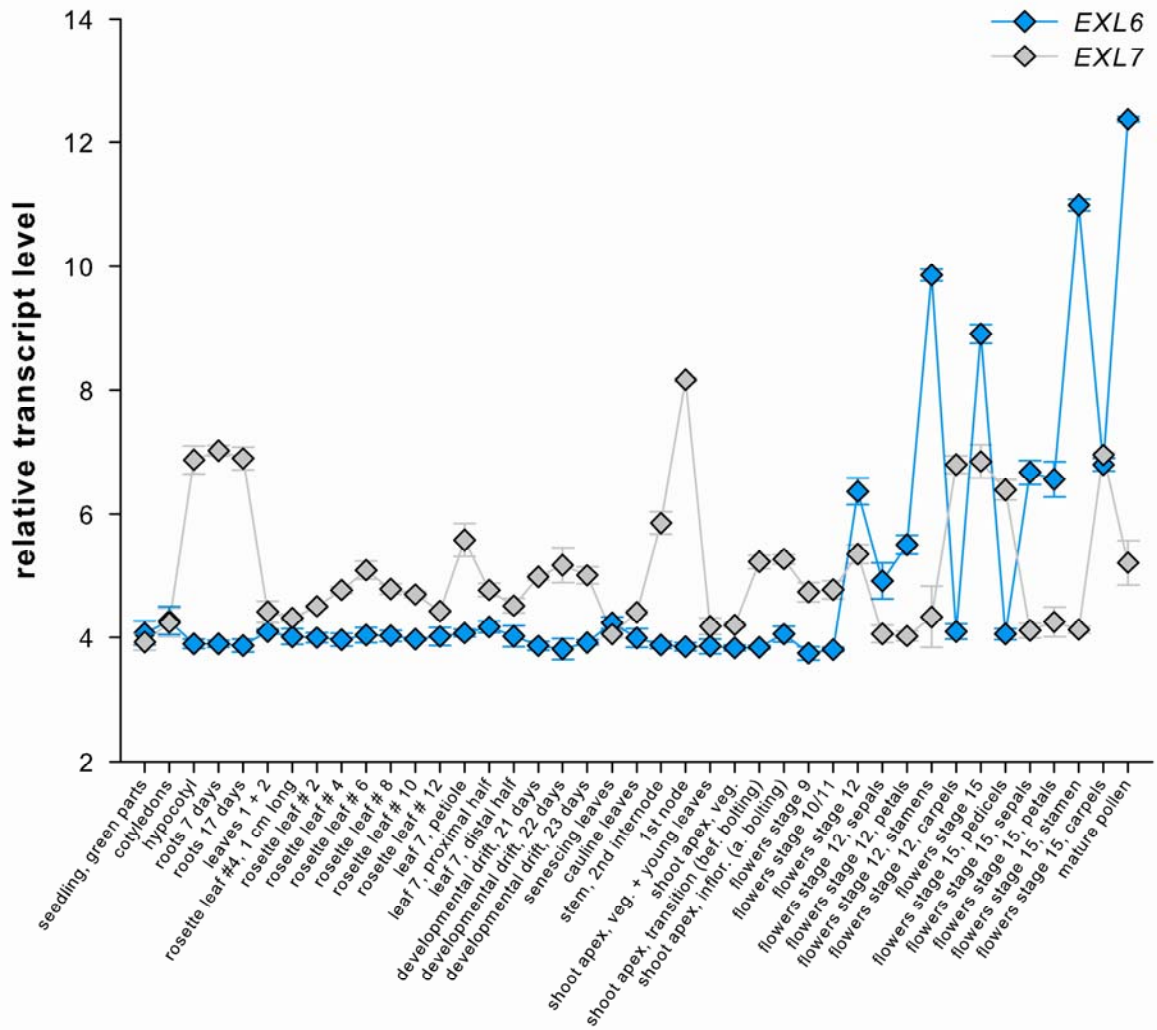


Figure S4. *EXL6* and *EXL7* expression in different organs and developmental stages.

Wild-type expression profiles of the development series (*Nat Genet* 2005, **37**: 501-506) were downloaded from AtGenExpress and normalized using RMA-Express (*Bioinformatics* 2003, **19**: 185-193). The mean and SD of three replicates are shown.

Table S1. *EXO* and *EXL* gene expression in different organs and developmental stages.

All profiles	EXO	EXL1	EXL2	EXL3	EXL4	EXL5	EXL6	EXL7
EXO	-							
EXL1	0,7070	-						
EXL2	0,2601	0,4843	-					
EXL3	0,7014	0,3615	0,0547	-				
EXL4	-0,2984	0,0174	0,5080	-0,2117	-			
EXL5	0,7592	0,5016	-0,0237	0,7780	-0,5258	-		
EXL6	-0,2063	0,0979	0,0841	-0,4850	0,1326	-0,3376	-	
EXL7	0,0789	0,0064	-0,2395	0,2986	0,1322	0,0150	-0,2366	-
Without flower and pollen								
	EXO	EXL1	EXL2	EXL3	EXL4	EXL5	EXL6	EXL7
EXO	-							
EXL1	0,8724	-						
EXL2	0,4487	0,4414	-					
EXL3	0,5818	0,4676	0,2387	-				
EXL4	-0,2094	-0,0031	0,4792	0,1044	-			
EXL5	0,8388	0,7717	0,1685	0,6386	-0,3333	-		
EXL6	0,1868	0,2503	0,1960	-0,0244	-0,1447	0,2534	-	
EXL7	-0,0751	-0,0153	-0,0470	0,2558	0,3718	-0,1239	-0,3748	-

Wild-type expression profiles of the development series (*Nat Genet* 2005, **37**: 501-506) were downloaded from AtGenExpress and normalized using RMA-Express (*Bioinformatics* 2003, **19**: 185-193). Spearman's rank correlation coefficients were calculated for gene pairs based on the transcript levels in all profiles (top) or based on the transcript levels in profiles representing vegetative organs (bottom). Values above 0.5 are in bold type.

Table S2. BR-induced *EXO*, *EXL1*, *EXL3*, and *EXL5* gene expression.

	WT ± EBL 7h		WT ± CS 3h	
	Experiment # 1	Experiment # 2	Experiment # 1	Experiment # 2
	SLR	SLR	SLR	SLR
<i>EXO</i>	1.8		3.2	3.1
<i>EXL1</i>	1.2		4.5	3.3
<i>EXL3</i>	0.8		1.9	1.2
<i>EXL5</i>	0.8		1.1	1.1

	det2 ± BL 1h		det2 ± BL 3h	
	Experiment # 1	Experiment # 2	Experiment # 1	Experiment # 2
	SLR	SLR	SLR	SLR
<i>EXO</i>	2.0	2.0	2.7	2.6
<i>EXL1</i>	2.7	3.2	4.1	5.5
<i>EXL3</i>			1.1	1.3
<i>EXL5</i>	1.0	1.0	1.0	1.0

Affymetrix expression profiles from AtGenExpress (established by Hideki Goda, RIKEN, Japan) and two further profiles (*FEBS Let* 2004, **563**: 82-86) were analyzed using the stringent settings of the statistical algorithms of the GCOS software as described previously (*Nucleic Acids Res* 2005, **33**: 2685-2696). Wild-type plants (top) and the BR-deficient *det2* mutant plants (bottom) were analyzed. Signal log ratios (SLR) of treatment versus control are shown, a SLR of one corresponds to a two-fold change. *EXO*, *EXL1*, *EXL3*, and *EXL5* transcripts were called ‘Present’ at least in profiles of BR-treated plants according to the MAS5.0/GCOS algorithm. Change *P*-values (according to GCOS algorithm) were below 0.001 in all experiments. BL: brassinolide; EBL: 24-epibrassinolide; CS: castasterone. SLRs below 0.8 are not shown.

Table S3. Fresh weight of wild-type and *exo* plants.

	WT	<i>exo</i>	WT	<i>exo</i>	WT	<i>exo</i>
	Soil 32 d	Soil 35 d	Soil 35 d	Soil 35 d	Soil 35 d	Soil 35 d
Fresh weight [mg]	517	202	1268	520	701	263
SD	47	33	303	126	31	50
% WT		39		41		38

	Soil 35 d	Soil 35 d	Soil 35 d	Soil 35 d	Soil 35 d	Soil 35 d
	Fresh weight [mg]	528	427	605	456	619
SD	127	86	104	74	87	109
% WT		81		75		72

Plants were grown in soil in six independent experiments. Student’s t-test *P*-values were below 0.05 in all experiments.

Supplement to figure 5. Relative growth of wild-type, *exo*, and complemented *exo* plants (carrying the 35S::EXOga construct) in response to BL-treatments.

	Cotyledon length			Cotyledon width		
	WT	<i>exo</i>	EXOga in <i>exo</i>	WT	<i>exo</i>	EXOga in <i>exo</i>
0 nM BL						
Mean	100	100	100	100	100	100
SD	11	11	11	17	11	10
1 nM BL						
Mean	114	107	119	102	102	110
SD	14	8	15	11	10	11
10 nM BL						
Mean	122	109	128	110	99	110
SD	16	10	14	17	9	12
100 nM BL						
Mean	119	105	124	100	98	110
SD	14	9	17	14	7	16
	Hypocotyl length					
	WT	<i>exo</i>	EXOga in <i>exo</i>			
0 nM BL						
Mean	100	100	100			
SD	14	9	9			
1 nM BL						
Mean	121	100	117			
SD	16	17	24			
10 nM BL						
Mean	163	115	135			
SD	23	16	24			
100 nM BL						
Mean	197	170	176			
SD	19	28	21			

	WT	Root length exo	EXOga in exo
0 nM BL			
Mean	100	100	100
SD	7	14	8
1 nM BL			
Mean	48	20	43
SD	11	5	12
10 nM BL			
Mean	42	18	34
SD	10	5	9
100 nM BL			
Mean	37	12	36
SD	11	4	9

BL treatments were compared to the respective control treatment (0 nM BL = 100%).

Supplement to figure 5. Statistical analysis (ANOVA).

	Cotyledon length		Cotyledon width		Hypocotyl length		Root length	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Genotype	544,634	<0,001	306,826	<0,001	15,064	<0,001	791,265	<0,001
Treatment	71,127	<0,001	8,366	<0,001	540,574	<0,001	2163,428	<0,001
Genotype x treatment	9,049	<0,001	4,541	<0,001	11,261	<0,001	5,827	<0,001

The ANOVA test was performed using the Holm-Sidak test.