Supplementary data

Article title: DEG10 contributes to mitochondrial proteostasis, root growth and seed yield in Arabidopsis

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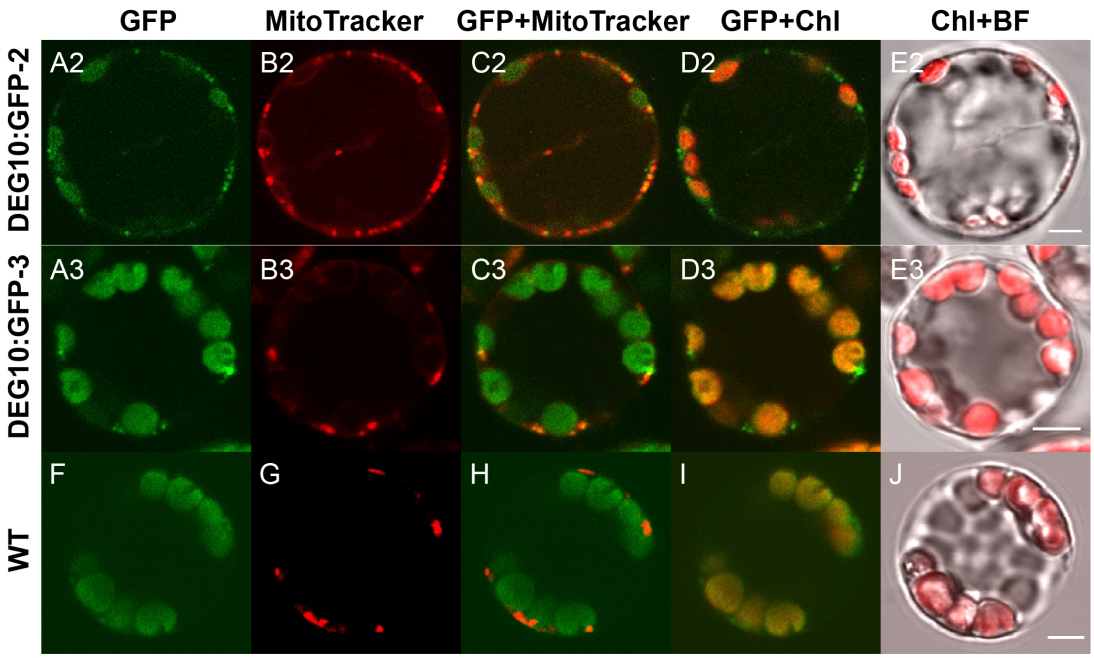
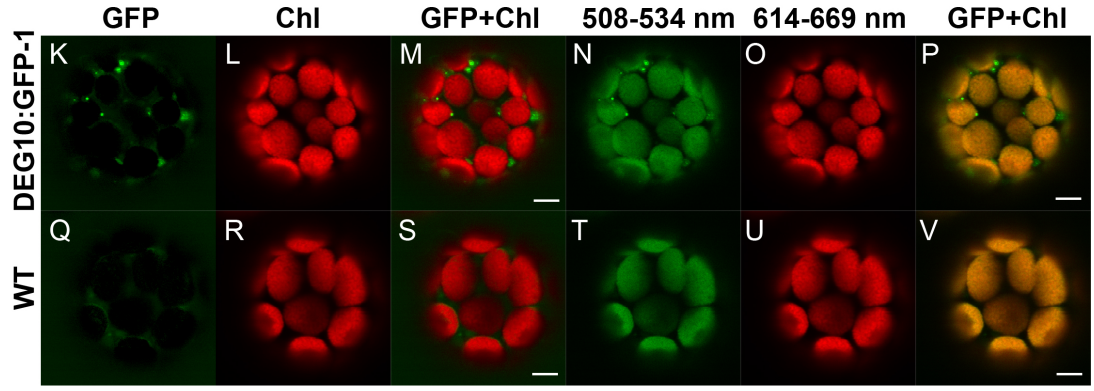

GFP
MitoTracker
GFP+MitoTracker
GFP+Chl
Chl+BF
DEG10:GFP-2
DEG10:GFP-3
WT
A2
B2
C2
D2
E2
A3
B3
C3
D3
E3
F
G
H
I
J


GFP
Chl
GFP+Chl
508-534 nm
614-669 nm
GFP+Chl
DEG10:GFP-1
WT
K
L
M
N
O
P
Q
R
S
T
U
V

W


subcellular location
chloroplast
mitochondrion
secr.pathway
nucleus
AtSubP
BaCelLo
ChloroP_v1.1
iPSort
Mitopred
MitoProt_v2
MultiLoc
NLStradamus
NucPred
PCLR_v0.9
PProwler_v1.1
PredictNLS
PrediSi
Predotar_v1
PredSL
SignalP_v4.1
SLP-Local
TargetP_v1
WoLF-PSort
YLoc
AramLocCon
7.0
21.7
-1.2
0.1
Subcellular location predictions
chloroplast location weakly...strongly predicted
mitochondrion location weakly...strongly predicted
secretory pathways location weakly...strongly predicted
nucleus location weakly...strongly predicted
See also SUBA 3 database

Fig. S1. Mitochondrial localization of DEG10.

(**A2-E3**) Confocal fluorescence pictures of MitoTracker stained protoplasts isolated from two additional, independently transformed *A. thaliana* plants expressing DEG10:GFP (2&3) or (**F-J**) wildtype (WT) plants. The pictures are false-colored, both MitoTracker and chlorophyll (Chl) signals are depicted in red for a better visualization of co-localization in merged pictures. (**A+F**) green fluorescence (497 nm to 550 nm); (**B+G**) MitoTracker Orange (572 nm to 615 nm, depicted in red); (**C+H**) merged image of GFP (green) and MitoTracker (red) fluorescence showing overlap (orange to yellow) of the most intense GFP signals with stained mitochondria except for some cases in which the mitochondria moved slightly between acquisition of the two pictures; (**D+I**) merged image of of GFP (green) and Chl auto-fluorescence (657 nm to 690 nm, depicted in red); (**E+J**) merged image of Chl auto-fluorescence (red) and a bright field image. In each panel, offset, gain and contrast settings are identical for DEG10:GFP expressing and WT protoplasts. (**K-V**) Comparison of spectral deconvolution and channel separation for the protoplasts shown in Fig. 1K-P. (**K-P**) are identical to the pictures in Fig. 1; (**Q+T**) average intensity projection of three channels between 508 and 534 nm to simulate a narrow-pass GFP filter; (**R+U**) average intensity projection of 6 channels between 614 and 669 nm to simulate a long pass Chl filter; (**S+V**) merge of green and red fluorescence. Scale bars = 5 μm. (**W**) Localization prediction as derived from the ARAMEMNON database on Apr. 27, 2019 (http://aramemnon.botanik.uni-koeln.de/tm_sub.ep?GeneID=61781&ModelID=0).

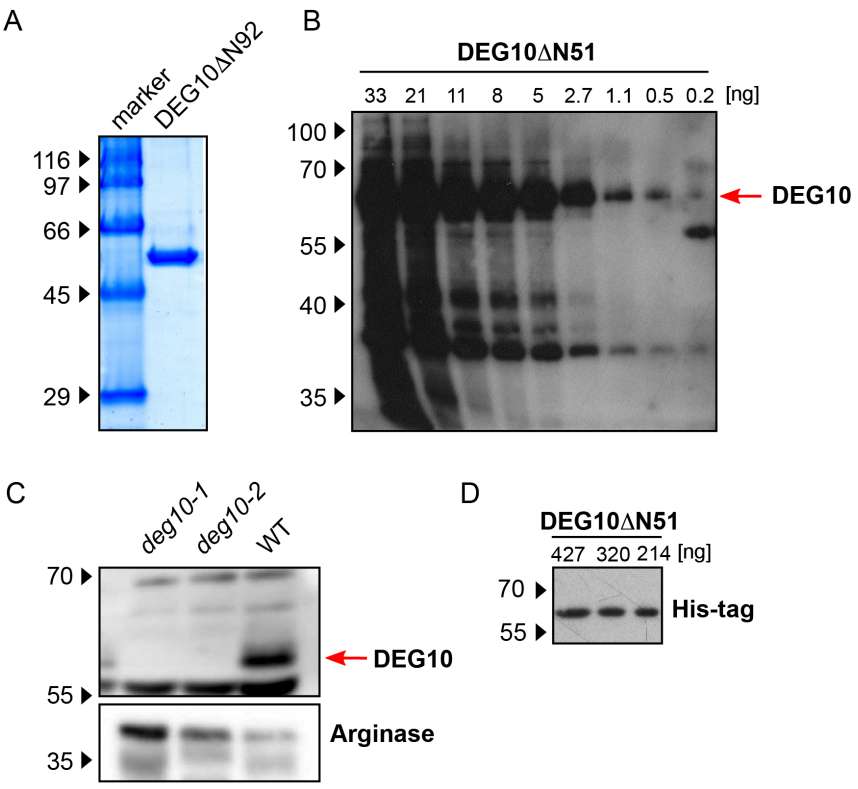


Fig. S2. Characterization of the polyclonal anti-DEG10 immune serum by immunoblot analyses. (**A**) Coomassie gel of the 6xHis:DEG10ΔN92 used for immunization of a rabbit after purification by nickel affinity chromatography and size exclusion chromatography. (**B**) Detection of recombinant 6xHis:DEG10ΔN51 (63 kDa, marked by a red arrow) heterologously expressed in *Escherichia coli*. Aliquots with known amounts of the recombinant protein were separated by SDS-PAGE on a 10% gel and detected by the anti-DEG10 serum (exposure time: 30 s). (**C**) In protein extracts of mitochondria isolated from *A. thaliana* WT plants as well as *deg10-2* and *deg10-1* mutants, DEG10 is exclusively detected in the WT sample. Detection of Arginase demonstrates that the mutant samples contained at least as many mitochondrial proteins as the WT sample. (**D**) Detection of recombinant 6xHis:DEG10ΔN51 by an anti-5xHis antibody. The molecular weights of protein markers are indicated on the left in kDa.

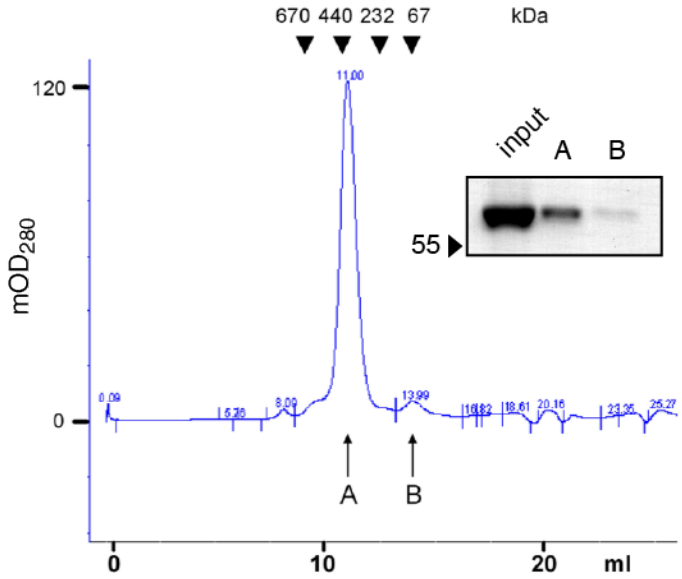
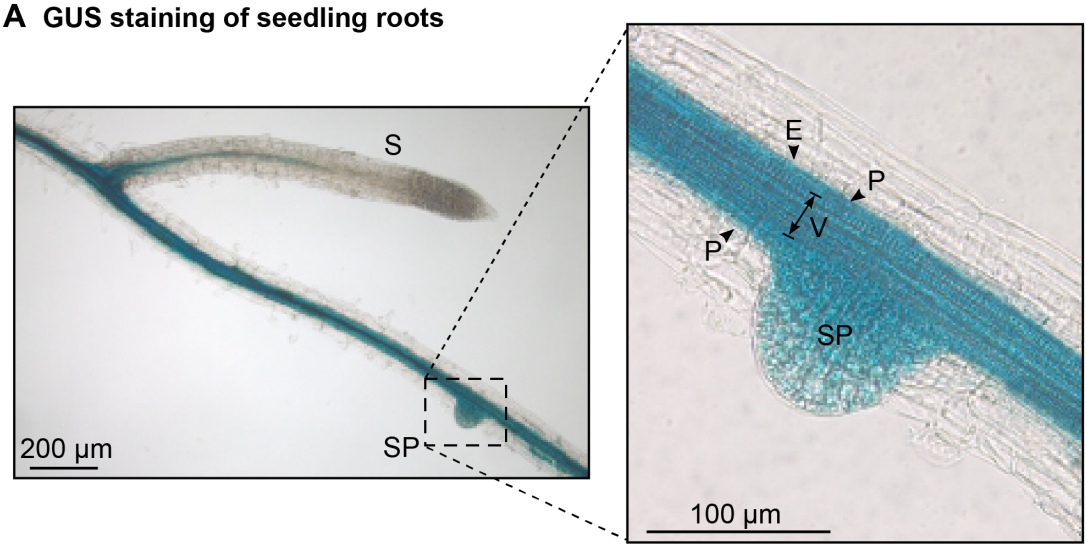


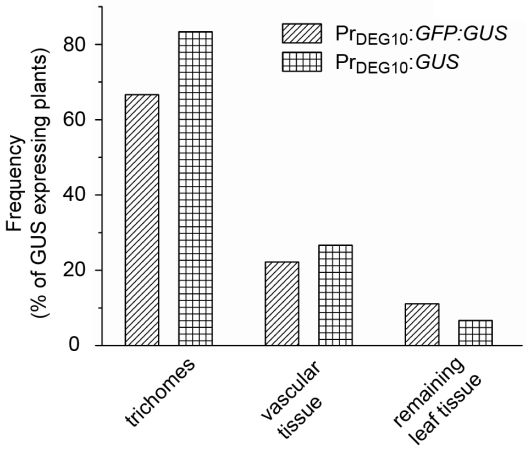
Fig. S3. Apparent molecular mass of recombinant DEG10.

Recombinant, purified DEG10ΔN92 (truncated by 92 amino acids at the N-terminus) was subjected to size exclusion chromatography by FPLC (Äkta Purifier with a Superdex 200 16/300 GL column, GE healthcare). Calibration was performed in a parallel run with commercial marker proteins (GE healthcare) of known sizes, indicated by black triangles above the chromatogram . The inset shows a Coomassie-stained denaturing gel with the input and the peak fractions A and B as indicated below the chromatogram.

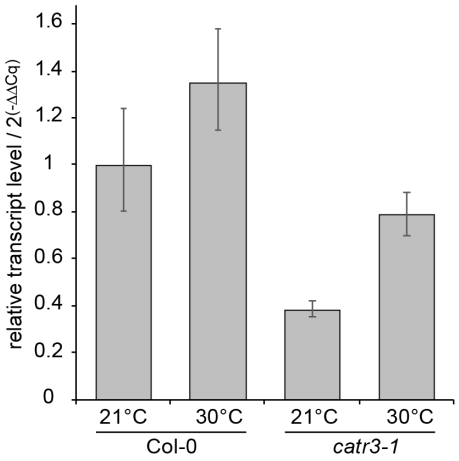
A GUS staining of seedling roots



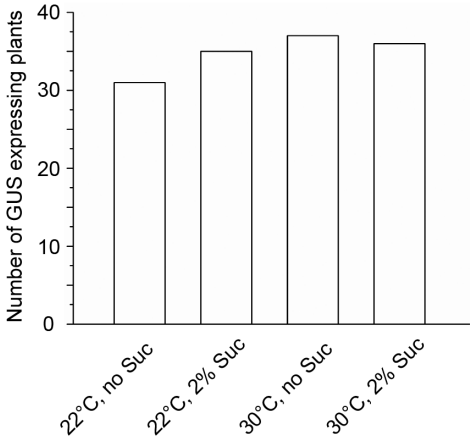
B GUS staining in mature leaves



C *DEG10* transcript level in seedlings



D GUS staining of seedling rosettes



E GUS staining of seedling roots

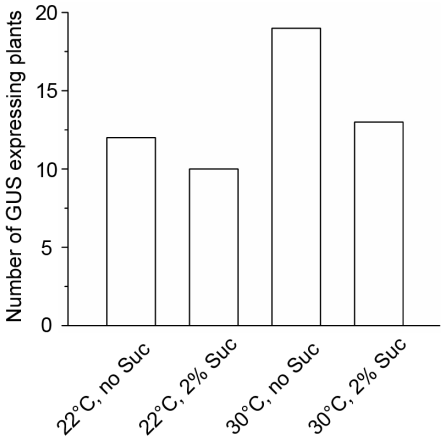


Fig. S4. Regulation of *DEG10* promoter activity and localization in roots. (previous page)
(**A**) The localization of GUS expression in roots of three-week-old *A. thaliana* seedlings transformed with Pr_{DEG10}*:GFP:GUS* grown in axenic culture. The central cylinder of the main root, including a side root primordium (SP) were stained, but not the root tips, also not of the side root (S). Scaled-up section: vascular bundle (V), pericycle (P), endodermis (E). (**B**) Frequencies of GUS expression patterns (expressed in % of all plants with detectable GUS activity) in mature leaves of six-week-old T1 Arabidopsis plants carrying either a Pr_{DEG10}*:GUS* (159 plants) or a Pr_{DEG10}*:GFP:GUS* (49 plants) expression cassette. (**C**) Relative quantification of *DEG10* transcript levels in 13-day-old seedlings (see Fig. S6). Transcript levels were normalized to *TUBULIN*α*1* and *UBIQUITIN5* and to Col-0, 21°C. Columns represent $2^{(-\Delta\Delta Cq \pm SD)}$. Two-way ANOVA analysis of $\Delta\Delta$Cq-values confirmed a significant effect of temperature that was reproduced in an independent experiment. (**D**,**E**) Impact of elevated temperature (30°C) and the presence or absence of 2% (w v^{-1}) sucrose on the *DEG10* promoter activity in T2 seedlings carrying the Pr_{DEG10}*:GFP:GUS* construct and cultivated in axenic culture. After two weeks of growth at 22°C, seedlings were either kept at 22°C or transferred for one week to 30°C. (**D**) Numbers of plants with GUS expression in aboveground tissues among 40 T2 seedlings of four independent lines. (**E**) Numbers of plants with GUS expression in roots among 20 plants of two independent lines. All stainings were performed for 20 h at 37°C.

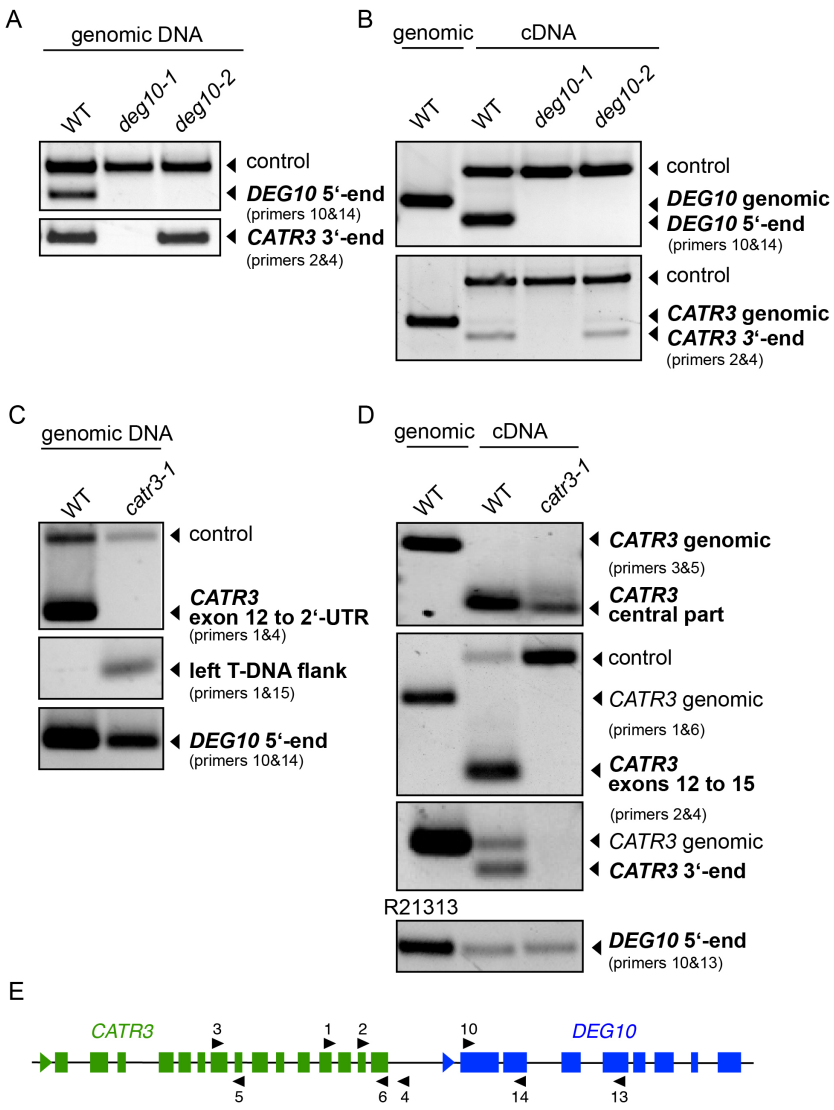


Fig. S5. Molecular characterization of *deg10-1*, *deg10-2* and *catr3-1*

(**A**) Amplification of *DEG10* fragments or T-DNA flanking sites from genomic DNA isolated from WT plants or homozygous *deg10* mutants. (**B**) Detection of *DEG10* and *CATR3* transcripts in cDNA obtained from WT or homozygous *deg10* mutants. (**C**) Amplification of *CATR3* fragments or T-DNA flanking sites from genomic DNA of WT plants or homozygous *catr3-1* mutants. (**D**) Detection of *DEG10* and *CATR3* transcripts in cDNA obtained from WT plants or homozygous *catr3-1* mutants. R21313: fullength *DEG10* cDNA clone RAFL_21313. (**E**) Schematic representation of *CATR3* and *DEG10* with primer binding sites. Primer numbers correspond to the numbers given in Supplementary Table S1. In order to validate negative results as evidence for the absence of specific genomic or cDNA fragments, control primers amplifying an unrelated, larger fragment were added to the PCR reactions. The relevant PCR products are indicated in bold letters.

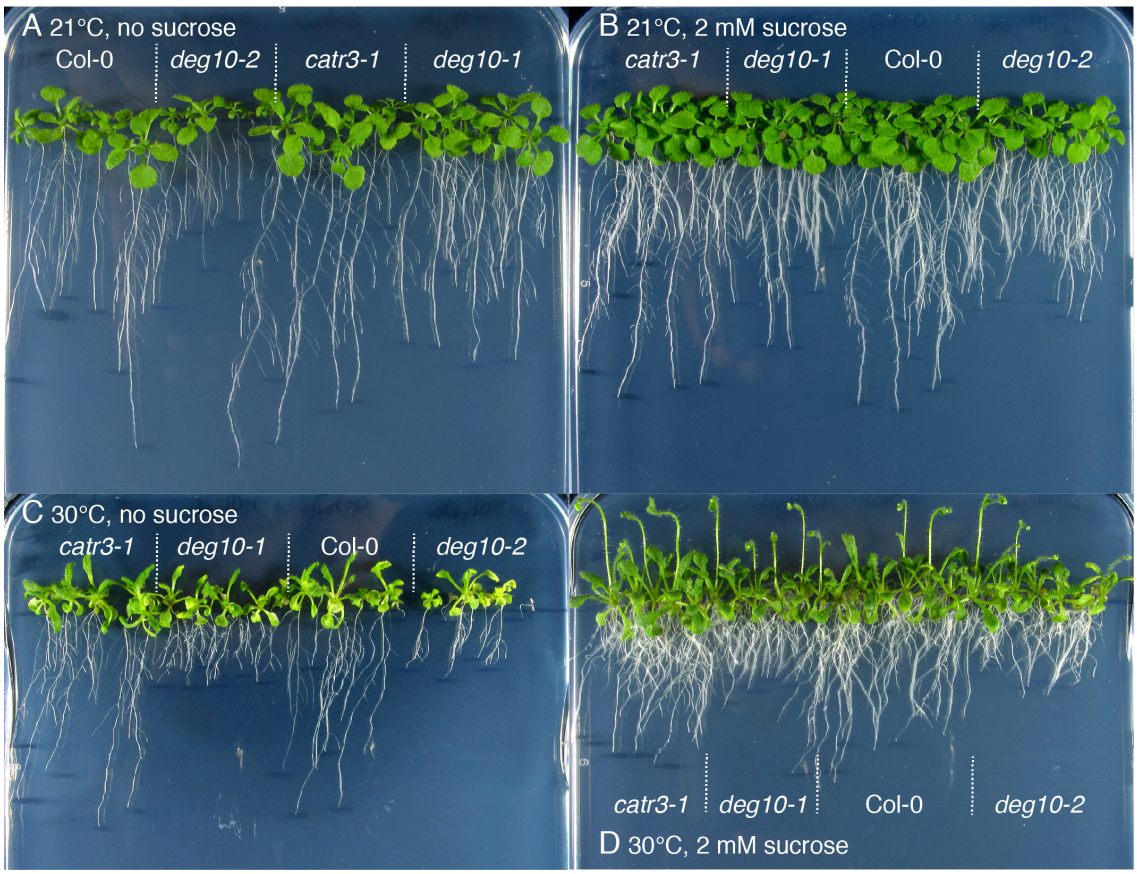


Fig. S6. Effect of temperature and sucrose on root growth of Arabidopsis wildtype (WT), *deg10-1, deg10-2* and *catr3-1* seedlings.

Pictures of 13-day-old plants used for the quantitative assessment (shown in Figure 6) of root growth on medium with or without sucrose and at 21°C or 30°C daytime temperature. Representative plates with a low proportion of seedlings that stopped growing during the first 13 days are shown. Early flowering was not observed on all plates with sucrose incubated at 30°C.

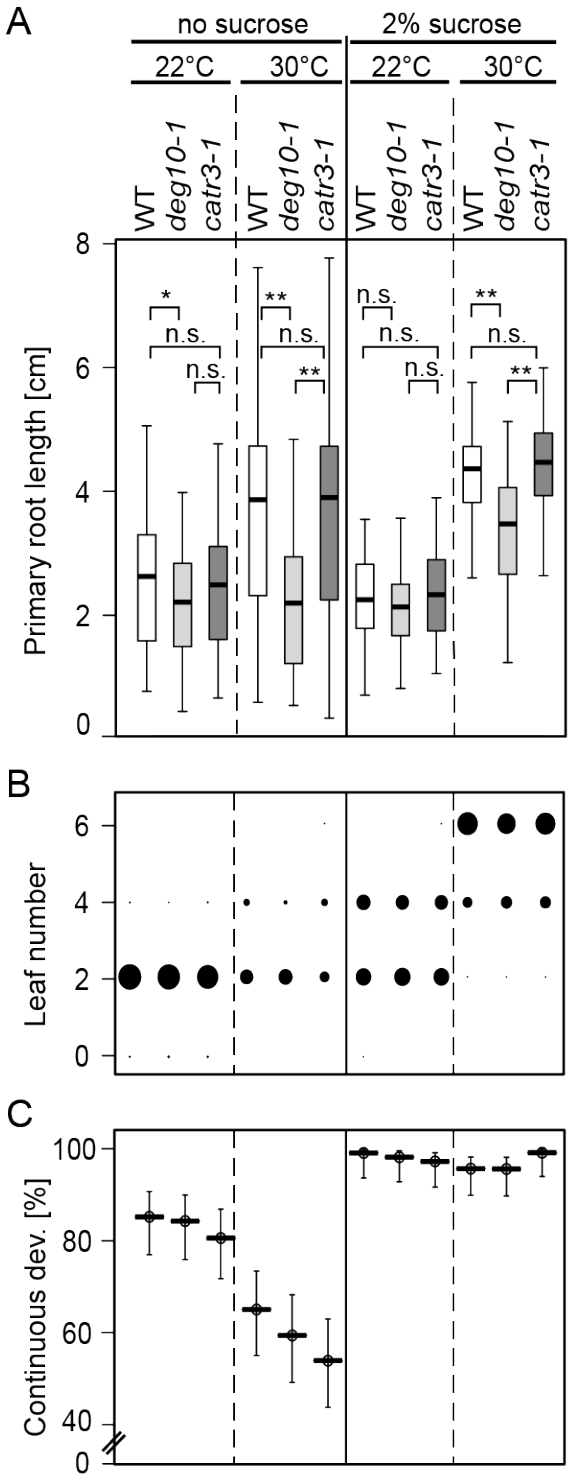


Fig. S7. Effect of temperature and sucrose on development of Arabidopsis wildtype (WT), *deg10-1* and *catr3-1* seedlings.

Per genotype and condition, at least 108 seeds from three parental plants were analyzed in axenic culture at the indicated temperatures and in the presence or absence of 2% (w v^{-1}) sucrose in the medium. (**A**) Primary root length at day 18, boxes show interquartile range with medians. Whiskers extend to the most extreme data point within 1.5 times the interquartile range. Significance of differences was assessed by Tukey's post-hoc comparisons and is indicated as follows: n.s.: not significant; *: $p<0.05$; **: $p<0.001$. (**B**) Leaf number at day 10, dot size is proportional to the number of seedlings with the given number of true leaves. (**C**) Percentage of seedlings which showed continuous development until day 18, horizontal lines indicate means, error bars indicate 95% confidence intervals. In (B) and (C), no significant differences between the genotypes were detected.

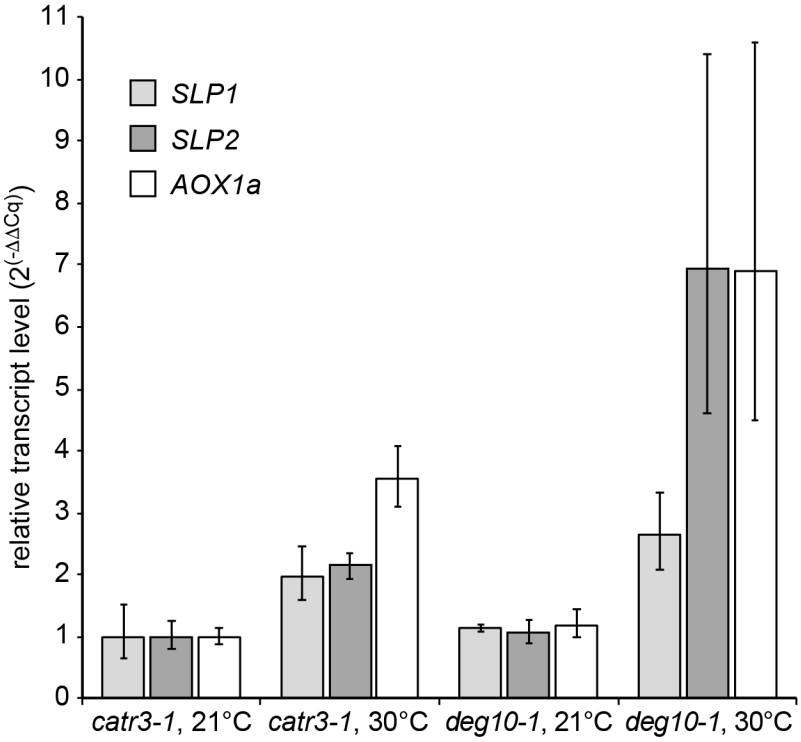


Fig. S8. Transcript levels of *SLP1*, *SLP2* and *AOX1a* in *catr3-1* and *deg10-1* mutants at different temperatures.

RNA was isolated from 13-day-old seedlings grown for 3 days at ambient temperature (21°C day/17°C night) followed by 10 days incubation at ambient temperature or elevated temperature (30°C day/25°C night) on medium with 2% (w v^{-1}) Suc (see Supplementary Fig. 6). Transcript levels were normalized to *TUBULIN*α*1* and *UBIQUITIN5* and to Col-0, 21°C. Columns represent $2^{(-\Delta\Delta Cq \pm SD)}$. Statistical analysis of $\Delta\Delta$Cq values confirmed a significant effect of the *deg10-1* mutation on *SLP2* transcript levels (Supplementary Table S4).

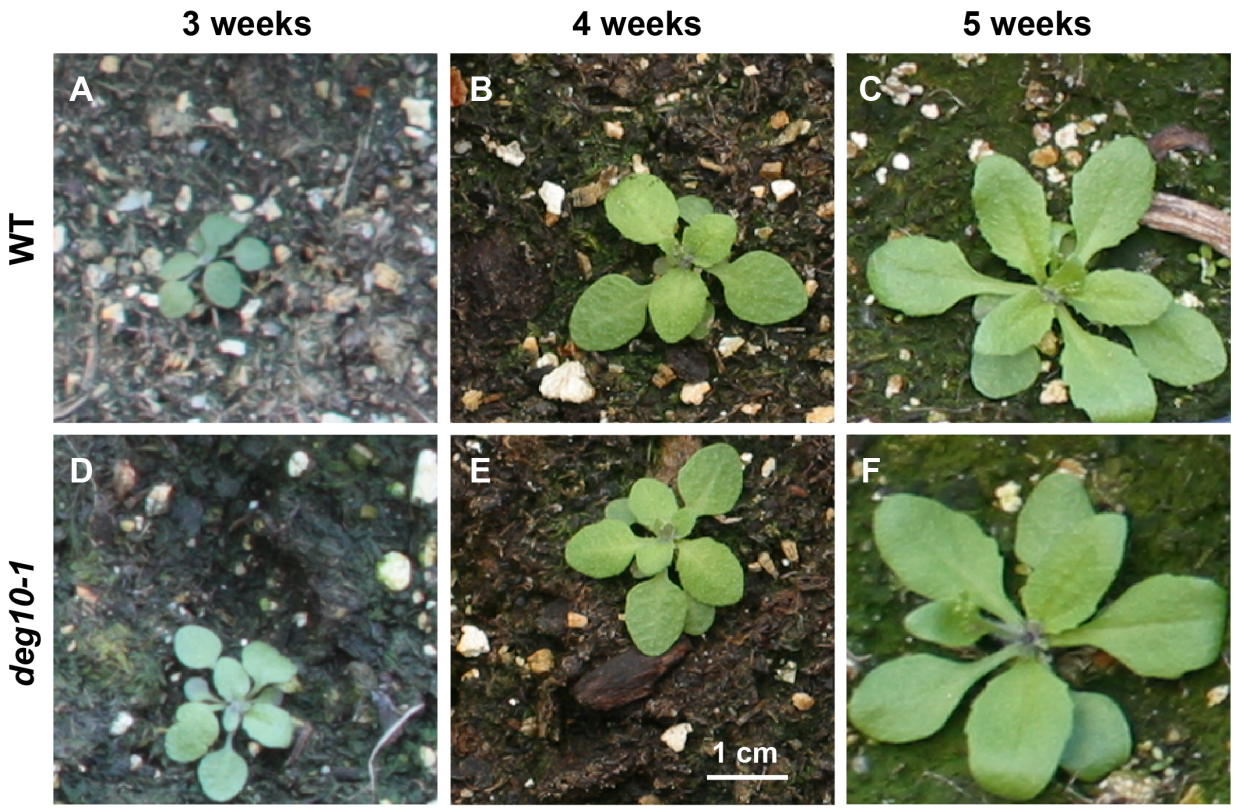


Fig. S9. Growth and development of WT plants and *deg10-1* mutants under field conditions. Photographs of representative WT (**A-C**) and *deg10-1* (**D-F**) plants grown for three, four and five weeks in a garden lot in Umeå, Sweden.

Table S1. Overview of T-DNA insertion lines analyzed for T-DNA insertions affecting *DEG10* expression

Name	NASC code	T-DNA location[a]
SALK_033779	N533779	n.d.
SALK_051527	N551527	n.d.
SALK_051519	N551519	promoter
SALK_007681	N507681	promoter
SALK_011327	N511327	promoter
SALK_051528	N551528	n.d.
SALK_020354	N520354	n.d.
SALK_135850 (*deg10-1*)	N635850	8th exon[c]
SALK_127867 (*deg10-1*)	N627867	8th exon[c]
SAIL_133_C08	N806457	n.d.
GK_088E04[b] (*deg10-2*)	N408404	1st intron[c]
GK_393C09[b]	N437665	promoter

[a] n.d.: not detected
[b] The lines were obtained from GABI KAT (University of Bielefeld)
[c] Exact position of T-DNA location was determined by sequencing

Table S2. Primers used in this study. Primers 1 to 16 were used to characterize the *deg10* and *catr3-1* T-DNA insertion mutants and primers 23 to 34 for qPCR analysis of *DEG10*, *SLP1*, *SLP2* and *AOX1a* transcript levels. Primers 17-22 are control primers for qPCR used pairwise in the order indicated. Primers 35 to 42 were used for molecular cloning.

Number	Name[a]	Sequence 5' – 3'
1	CATR3-LP-1	AGCTATGCAGCGTCAAGCTTC
2	CATR3-LP-2	GCCCGTTTGTTCCGCTTTTGC
3	CATR3-LP-3	TGGGATTTCGCTCCTGCTTTGT
4	CATR3-RP-1	AGTCGCCAGATTCAACATAGTC
5	CATR3-RP-2	TGTAAATGCAACAAGTGTCCCGA
6	CATR3-RP-3	TTAAGCCAAAGAATGTCCAGAAGTGC
7	DEG10-LP-1	GCAATGATGTCTCGCCATTTATG
8	DEG10-LP-2	CAACGCACGCAGGTTCC
9	DEG10-LP-3	GTGCATCAATTTGATCAGCTTCC
10	DEG10-LP-4	ATGCTGCTCCGGTCATTTCGC
11	DEG10-RP-1	CATTGGAACAAGAGATGGTAATG
12	DEG10-RP-2	TCAAACCGCAGAACAAGAAGCC
13	DEG10-RP-3	CCACTACGAAGTTCACCATTCTCC
14	DEG10-RP-4	CTAGCTCCAAAGCATTCATACC
15	T-DNA-LB-1	TTCGGAACCACCATCAAACAG
16	T-DNA-RB-1	CAGACGTGAAACCCAACATAC

17	OAT-F	AGTCTTGGATTAACTTAGGAGAG
18	OAT-R	GTTCATAGGAAGCACCATATC
19	ProDH1-F	TCTCCTCTATCCCAACCTCTG
20	ProDH1-R	CGCAATCCCGGCGATTAATCTC
21	P5CR-F	CACCATAATGGAGATTCTTCCGATTCC
22	P5CR-R	TGTGAGGTGAAACAATAGCAG
23	DEG10-qF	CCTTGGCAGAATAAGTCTCAACG
24	DEG10-qR	TCGACGATCCATGCTTTCTAACTAA
25	SLP1-qF	TCCACCTCCGATTTTCTCCG
26	SLP1-qR	GTCGGCGGCGTCAATTGAAAA
27	SLP2-qF	GCAACTTCAGCTCTACCGGA
28	SLP2_qR	AAGCCTGGAAGTGAAGGATTTTGA
29	AOX1a-qF	GAATGGAAGTGGAACTGTTTCAG
30	AOX1a-qR	CTATCAAGAAACGTCGTTGGAACA
31	TUA1-qF	GGTATCCAACCCGATGGCA
32	TUA1-qR	TGAGCTTGTCTCGCTAAAGAATG
33	UBQ5-qF	ACGCTTCATCTCGTC
34	UBQ5-qR	CCACAGGTTGCGTTA
35	DEG10ΔN51-F	CACCCCTTCACACATCTCTCGATTCT
36	DEG10ΔN92-F	CACCAGGAAGGCGGGAAAATCATTATC
37	DEG10S-R	GAGTCAAACCGCAGAACAAGAAGCC
38	DEG10-F	CACCATGCTGCTCCGGTCATTTCGC
39	DEG10NS-R	AACCGCAGAACAAGAAGCCAAC
40	DEG10Pr-F	ttgtacaagaaagctgggTTATTTTCCGGTACTGCTTTTTTGAG
41	DEG10Pr-R	tgtacaaaaaagcaggctGTCTACATGAAACCATCAATGGAAG
42	DEG10Pr-Xba-F	GATCTAGAGTCTACATGAAACCATCAATGGAAG
43	DEG10Pr-Xba-R	CTTCTAGAGAGCAGCATTATTTTCCGGTACT

[a]OAT = ornithine-δ-amino-transferase; ProDH1 = proline dehydrogenase 1; P5CR = pyrroline-5-carboxylate reductase; LP primers are orientated in the same direction as *DEG10* and *CATR3* transcription, while RP primers bind to the sense strand.

Table S3. Antibodies and conjugates used in this study

Target	Dilution	Source (product Nr.)	
Lhcb2	1:15,000	Agrisera (AS01 003)	
COXII	1: 1,000	Agrisera (AS04 053A)	
GDC-H	1: 5,000	Agrisera (AS05 074)	
DEG10	1: 500	this study	
Hcf101	1: 3,000	Agrisera (AS06 163)	
Arginase	1: 5,000	Todd and Gifford 2002	
5x-His	1: 5,000	Qiagen (34660)	HRP conjugate
Rabbit IgG	1:15,000	Sigma-Aldrich	HRP conjugate

Table S4. Statistical analyses of qRT-PCR data. ΔΔCq values were analyzed with linear models (R base function *lm*) including genotype, temperature and their interaction as fixed effects. p-values for the model effects were obtained using the *Anova* function in the *car* package. Values in bold indicate significant differences.

Col-0 *vs catr3-1*	***DEG10*** (Exp. 1)		***DEG10*** (Exp. 2)	
Factor	$F_{1,8}$ [a]	p-value	$F_{1,8}$ [a]	p-value
Temperature (T)	**32.34**	**< 0.001**	**22.27**	**0.002**
Genotype (G)	**69.65**	**< 0.001**	**8.36**	**0.020**
Interaction (G*T)	**5.37**	**0.049**	3.62	0.093

Col-0 *vs deg10-2*	***SLP1***		***SLP2***		***AOX1a***	
Factor	$F_{1,8}$ [a]	p-value	$F_{1,8}$ [a]	p-value	$F_{1,8}$ [a]	p-value
Temperature (T)	**51.9**	**< 0.001**	**68.57**	**< 0.001**	**15.15**	**0.005**
Genotype (G)	4.49	0.067	**40.95**	**< 0.001**	**16.96**	**0.003**
Interaction (G*T)	0.64	0.446	**8.64**	**0.019**	1.30	0.287
catr3-1 vs deg10-1						
Factor						
Temperature (T)	**25.96**	**< 0.001**	**83.01**	**< 0.001**	**104.96**	**< 0.001**
Genotype (G)	1.84	0.212	**17.93**	**0.003**	**8.07**	**0.022**
Interaction (G*T)	0.32	0.589	**14.86**	**0.005**	2.67	0.141

[a] F-factor with one degree of freedom for the analyzed effect and 8 degrees of freedom for the residuals

Table S5. Statistical analyses of seedling development. Complete results of the analyses of the effects of temperature and addition of sucrose on the development of *A. thaliana* wildtype (WT), *deg10-2*, *deg10-1* and *catr3-1* seedlings presented in Figs. 6 and S6. Likelihood-Ratio Tests ($\chi 2$) were performed to assess the significance of the fixed effects genotype (G), temperature (T), medium (M) and their interactions on continuous seedling development, leaf number and primary root length. Values in bold indicate significant differences depending on the given factor or interaction at the specified levels (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$).

			Continuous development[a] N=360-376[e]	**Leaf number**[b] N=355-371[e]	**Root length Fig. S8**[c] N=350-353[e]		**Root length Fig. 5**[d] N=166-209[e]
Fixed effects		df[f]	χ^2	χ^2	χ^2	df[f]	χ^2
Genotype (G)[g]		2	2.3	0.5	**92.14** ***	3	**46.87** ***
Temperature (T)[g]		1	**13.47** ***	**68.69** ***	**60.47** ***	1	**44.35** ***
Medium (M)[g]		1	**80.57** ***	**78.90** ***	**7.46** **	1	0.088
Interactions	G*T[h]	2	0.39	0.11	**33.31** ***	3	**107.5** ***
	G*M[i]	2	1.1.64	0.7	**6.19** *	3	**134.5** ***
	T*M[j]	1	**9.00** **	**8.35** **	**16.51** ***	1	**14.13** ***
	G*T*M[k]	2	3.17	0.5	2.56	3	**72.77** ***

[a] Binomial error distribution
[b] Poisson error distribution
[c] Gaussian error distribution, square root transformed data
[d] Gaussian error distribution, log transformed data
[e] Number of analyzed seedlings per genotype
[f] df = degrees of freedom
[g] Removal of the main effects genotype (G), temperature (T) or medium (M) compared to: random part + G + T + M
[h] Removal of G*T interaction compared to: random part + G + T+ M + G*M + T*M
[i] Removal of G*M interaction compared to: random part + G + T+ M + G*T + T*M
[j] Removal of T*M interaction compared to: random part + G + T+ M + G*T + G*M
[k] Removal of G*T*M interaction compared to: random part + G + T + M + G*T + G*M + T*M

Table S6 Proteins identified and quantified by comparative label-free proteome analysis of *deg10-1*, *deg10-2*, *catr3-1* and Col-0 roots (separate spreadsheet)

Table S7 Proteins with significant differences in abundance among *deg10-1*, *deg10-2*, *catr3-1* and Col-0 WT roots (separate spreadsheet)

Table S8. Analyses of vegetative growth of *A. thaliana* wildtype (WT) and *deg10-1* plants grown under field conditions in Umeå. Plants were grown in a randomized setup. After three, four and five weeks the rosette diameters were measured. Values in bold indicate significant difference.

Year	Weeks	Genotype	Rosette diameter[a] [mm]	N $_{plants}$[b]	t-value[c]	p-value[c]
2011	3	WT	26.6±1.3	10		
		deg10-1	30.1±2.5	10	1.25	0.23
	4	WT	62.5±3.1	10		
		deg10-1	54.2±5.2	10	-1.38	0.18
	5	WT	64.4±3.3	10		
		deg10-1	55.9±5.2	10	-1.38	0.18
2012	3	WT	9.2±0.7	30		
		deg10-1	**11.5±0.6***	30	2.6	**0.01**
	4	WT	25.9±1.7	29		
		deg10-1	29.5±1.5	30	1.57	0.12
	5	WT	45.4±2.3	29		
		deg10-1	46.2±1.9	30	0.28	0.78

[a] Mean ± SE

[b] Indicates number (N) of analyzed plants

[c] For comparison to WT reference (model intercept)

Table S9. Seeds per silique of *A. thaliana* WT plants and *deg10-1* or *catr3-1* mutants under glasshouse conditions.

Location	Year	Genotype	Seeds per silique[a]	$N_{siliques}$ (N_{plants})[b]	t-value[c]	p-value[c]
Glasshouse	2013	WT	45.5±1.9	150 (15)		
		deg10-1	47.4±1.3	140 (14)	0.84	0.40
		catr3-1	43.9±1.6	150 (15)	-0.68	0.50
	2014, experiment 1	WT	48.5±1.0	140 (15)		
		deg10-1	45.6±0.9	134 (15)	-2.00	0.06
	2014, experiment 2	WT	43.8±2.0	150 (15)		
		deg10-1	39.0±2.6	150 (15)	-1.52	0.14
		catr3-1	44.3±2.1	150 (15)	0.13	0.90

[a] Mean ± SE

[b] Indicates number (N) of counted siliques and plants. Whenever possible, 10 siliques per plant were analyzed.

[c] For comparison to WT reference (model intercept)