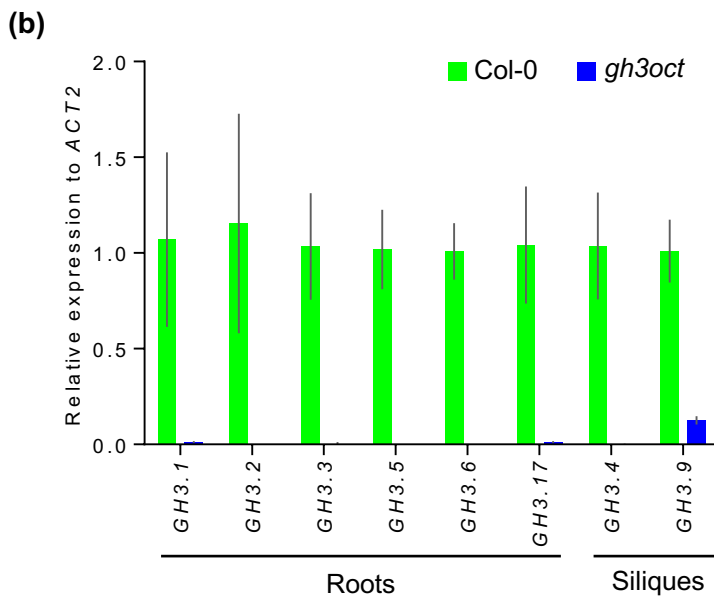
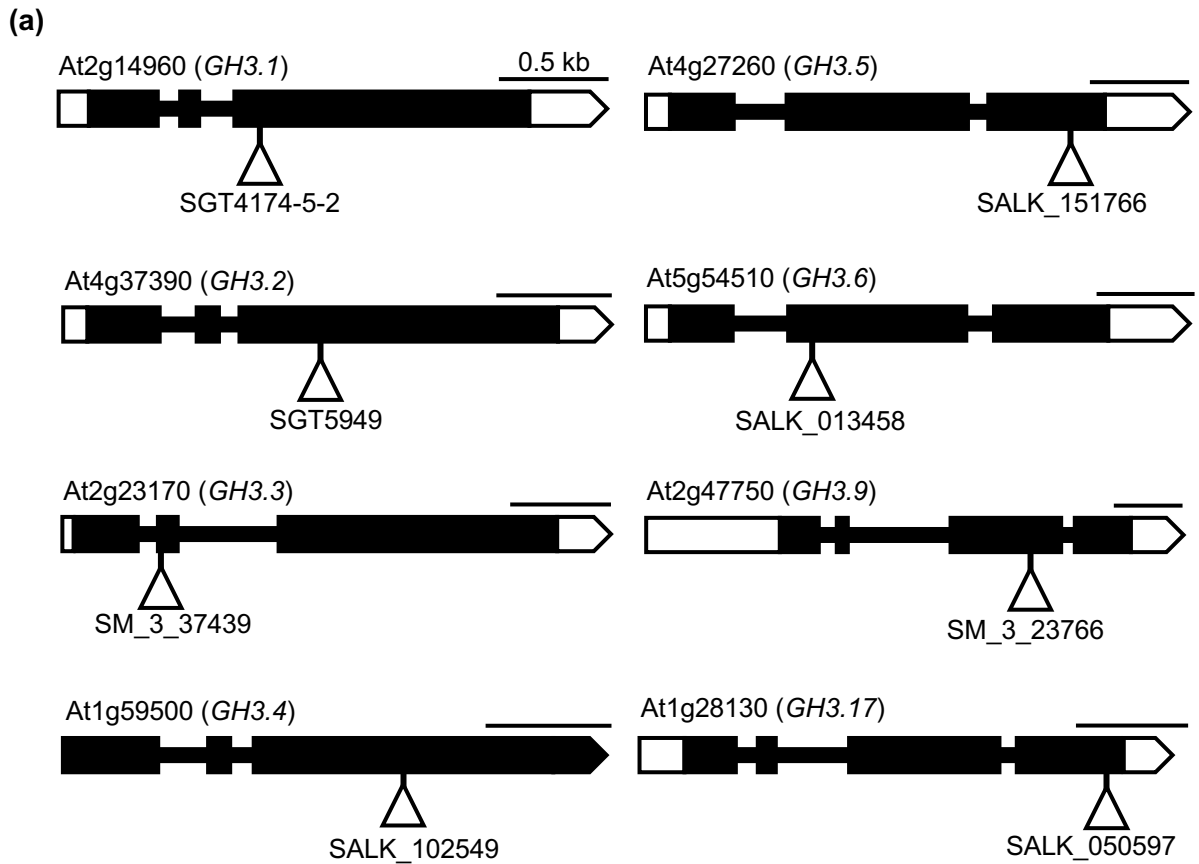


***New Phytologist* Supporting information**

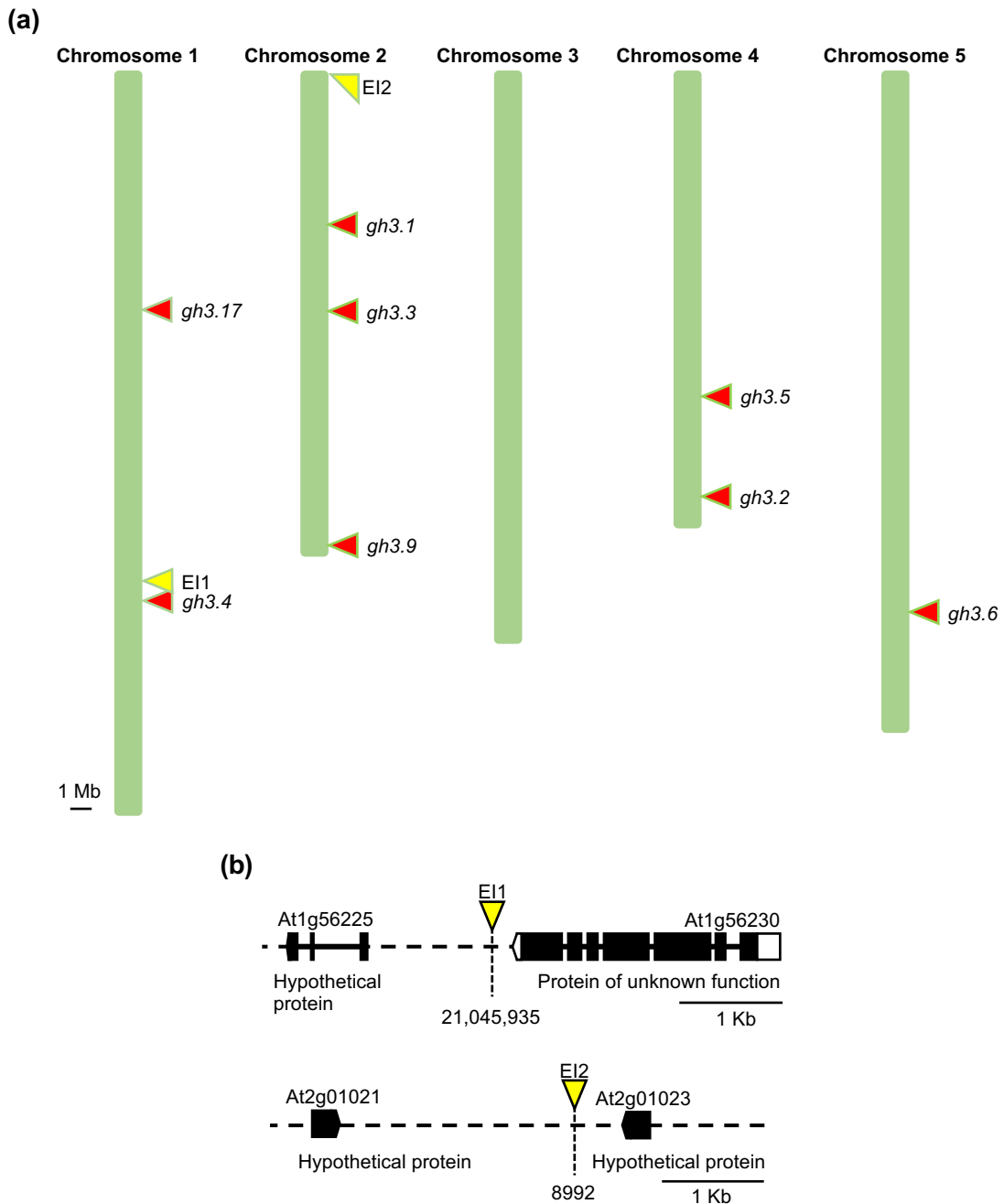
Inactivation of the entire Arabidopsis group II GH3s  
confers tolerance to salinity and water deficit

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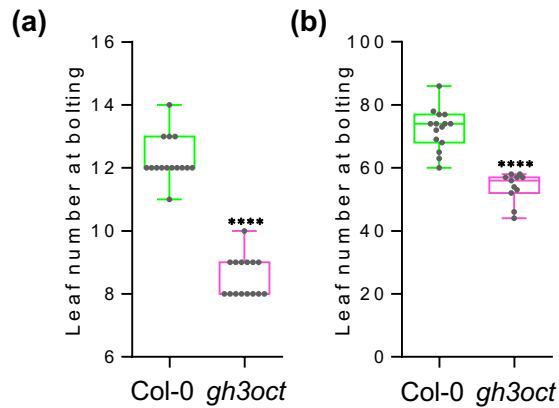
Article acceptance date: 5 March 2022



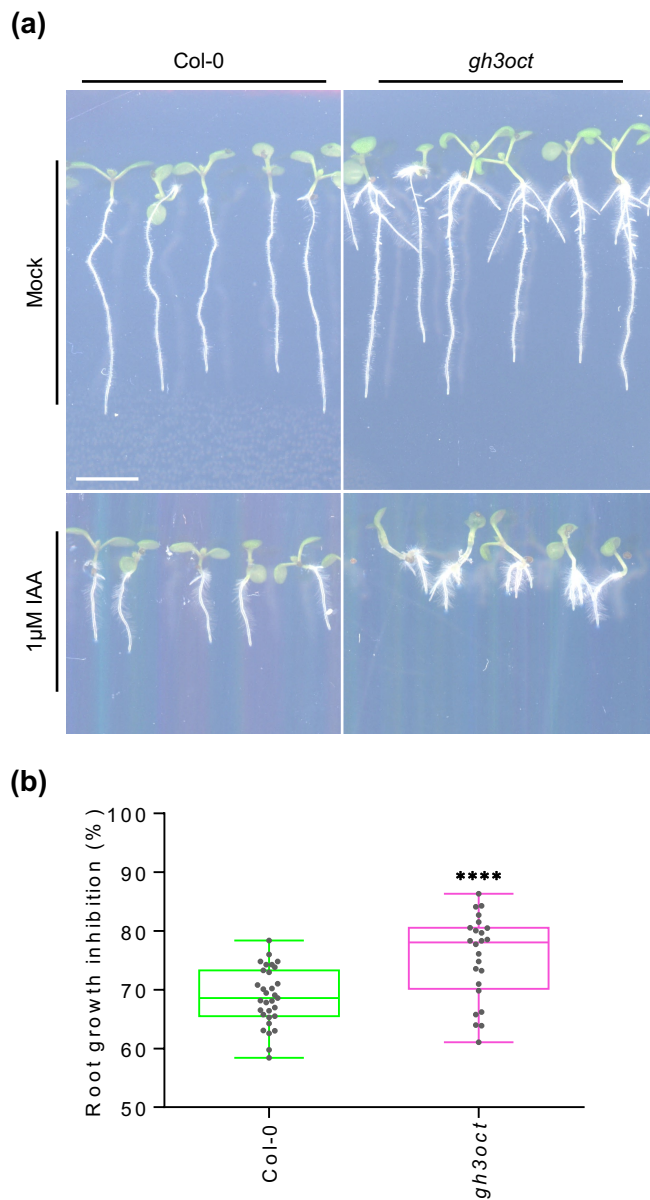
**Figure S1. The *gh3oct* mutant is an octuple knock-out.** (a) Structure of the *Arabidopsis thaliana* *GH3.1*, *GH3.2*, *GH3.3*, *GH3.4*, *GH3.5*, *GH3.6*, *GH3.9* and *GH3.17* genes with indication of the position of the insertions. Boxes and lines represent exons and introns, respectively. Open and black boxes represent untranslated and translated regions, respectively. Triangles indicate T-DNA/transposon insertions. Scale bars indicate 0.5 kb. (b) Relative expression of *GH3* genes in roots and siliques from 7- and 45-day-old *Arabidopsis thaliana* plants, respectively. No expression of *GH3.4* and *GH3.9* was detected in roots. Bars indicate relative expression in Col-0 and *gh3oct* plants calculated by using the  $2^{-\Delta\Delta CT}$  method. Error bars indicate the interval delimited by  $2^{-\Delta\Delta CT \pm SE}$ .



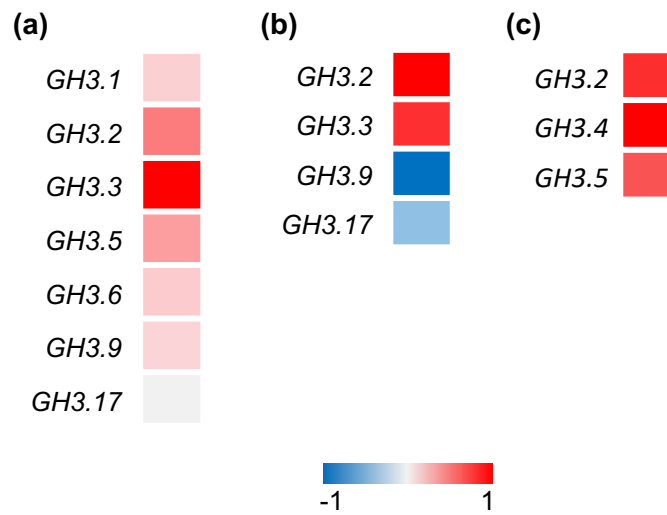
**Figure S2. The *gh3oct* mutant harbours two extra T-DNA insertions at intergenic regions.** (a) Map of the five *Arabidopsis thaliana* chromosomes with indication of the position of the ten insertions found using a tagged-sequence mapping strategy. Insertions disrupting *Arabidopsis thaliana* GH3s are shown in red. Yellow triangles represent the two extra insertions (EI) found. (b) Detailed locations of the two EI found. Open and black boxes represent untranslated and translated regions, respectively. Scale bars indicate 1 Mb (a) and 1 Kb (b).



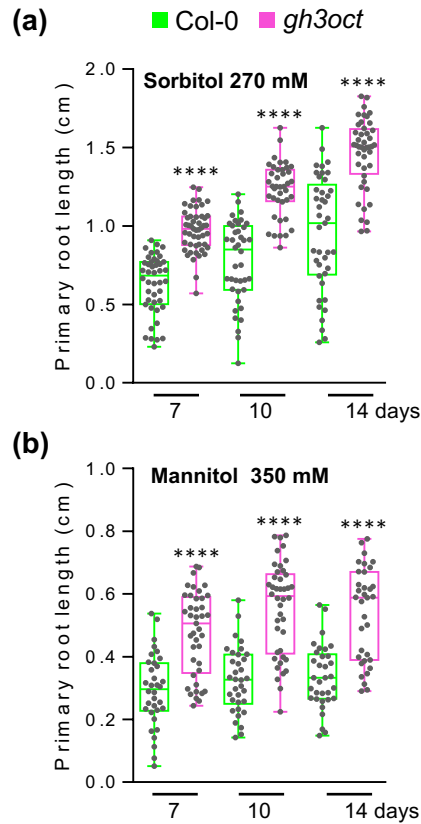
**Figure S3. The *gh3oct* is a photoperiod-independent early flowering mutant.** Flowering time determined as leaf number at bolting in *Arabidopsis thaliana* Col-0 and *gh3oct* plants grown under long- (a) and short-day (b) photoperiods. Box plots show the individual data points, the first and third quartile (bottom and top of the box), the median (line in the box), and the highest (top whisker) and lowest (bottom whisker) value. Asterisks indicate values significantly different from those of Col-0 in a two-tailed Student's *t*-test [\*\*\*\* $p < 0.0001$ ,  $n = 15$  (a), and  $n \geq 11$  (b)].



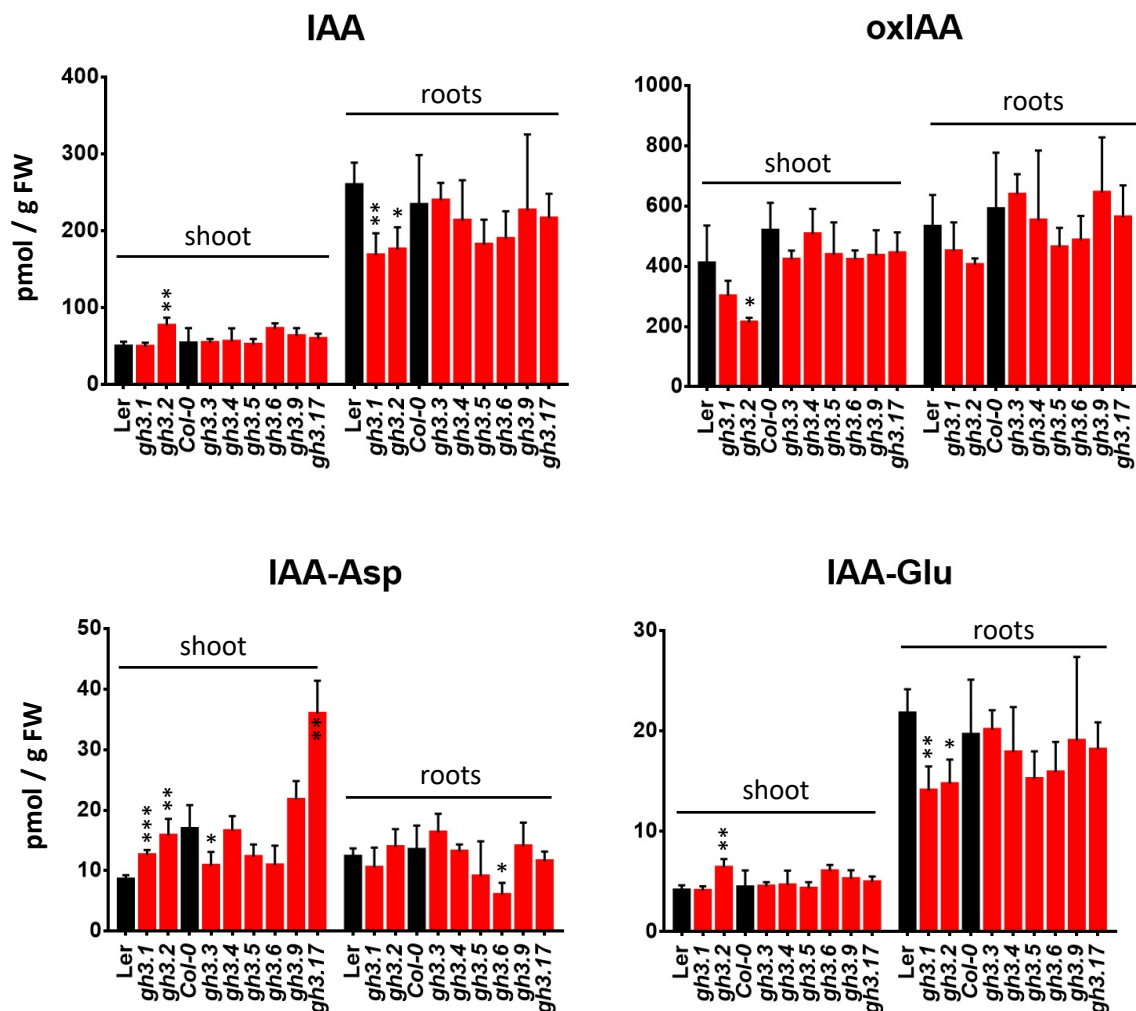
**Figure S4. Root growth in the *gh3oct* mutant is hypersensitive to IAA.** (a) *Arabidopsis thaliana* Col-0 and *gh3oct* seedlings on MS plates (top) and MS plates supplemented with 1  $\mu$ M IAA (bottom). Photographs were taken 7 days after stratification. Scale bar indicates 0.5 cm. (b) Percentage of root growth inhibition by 1  $\mu$ M IAA in 7-day-old seedlings. Box plots show the individual data points, the first and third quartile (bottom and top of the box), the median (line in the box), and the highest (top whisker) and lowest (bottom whisker) value. Asterisks indicate values significantly different from Col-0 in a two-tailed Student's *t*-test (\*\*\*\* $p$ <0.0001,  $n \geq 24$ ).



**Figure S5. Transcriptional response of *Arabidopsis thaliana* group-II GH3 genes to salinity according to published datasets.** Data were retrieved from RNA-seq datasets described in (a) Cai *et al.*, 2017 (b) Shen *et al.*, 2014 and (c) Jiang and Deyholos, 2006. Colour scale indicates normalized (a) RPKM and (b,c) log<sub>2</sub> fold change values.

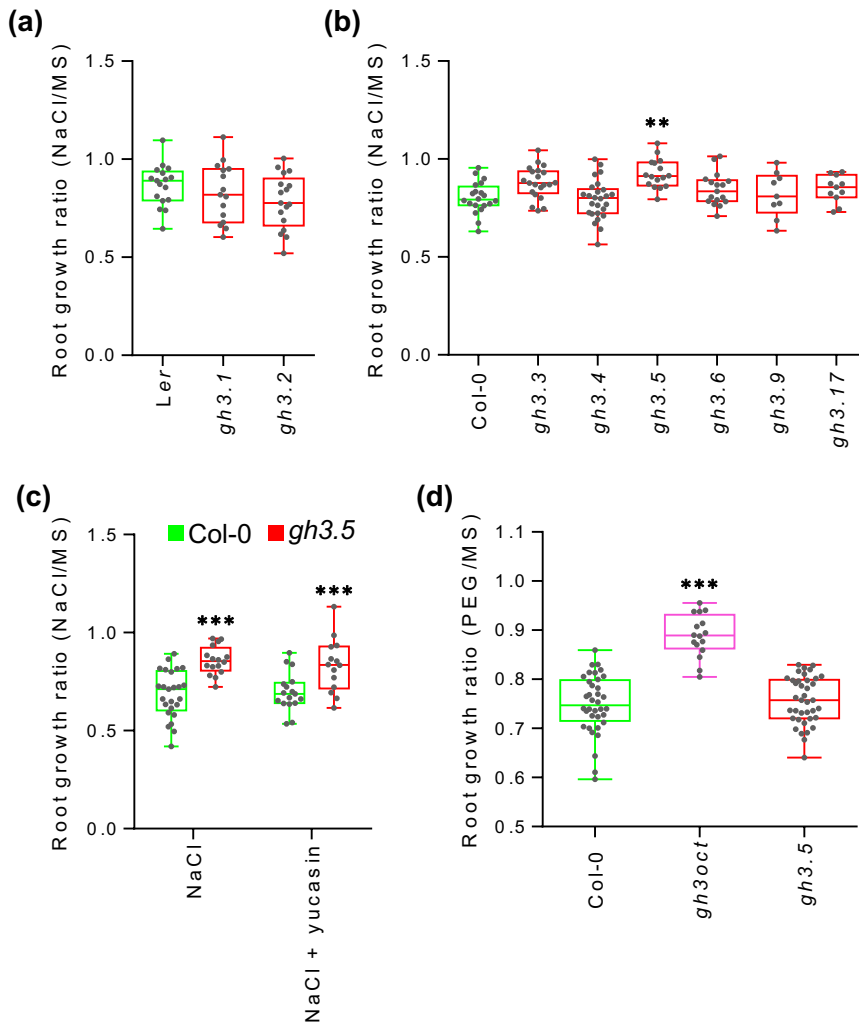


**Figure S6. Root growth in the *gh3oct* mutant is tolerant to different osmolytes.** Primary root length from *Arabidopsis thaliana* Col-0 and *gh3oct* seedlings grown on MS plates supplemented with (a) 270 mM sorbitol, and (b) 350 mM mannitol for 7, 10 and 14 days. Box plots show the individual data points, the first and third quartile (bottom and top of the box), the median (line in the box), and the highest (top whisker) and lowest (bottom whisker) value. Asterisks indicate values significantly different from those of Col-0 in a two-tailed Student's *t*-test (\*\*\*\* $p < 0.0001$ ,  $n \geq 33$ ).

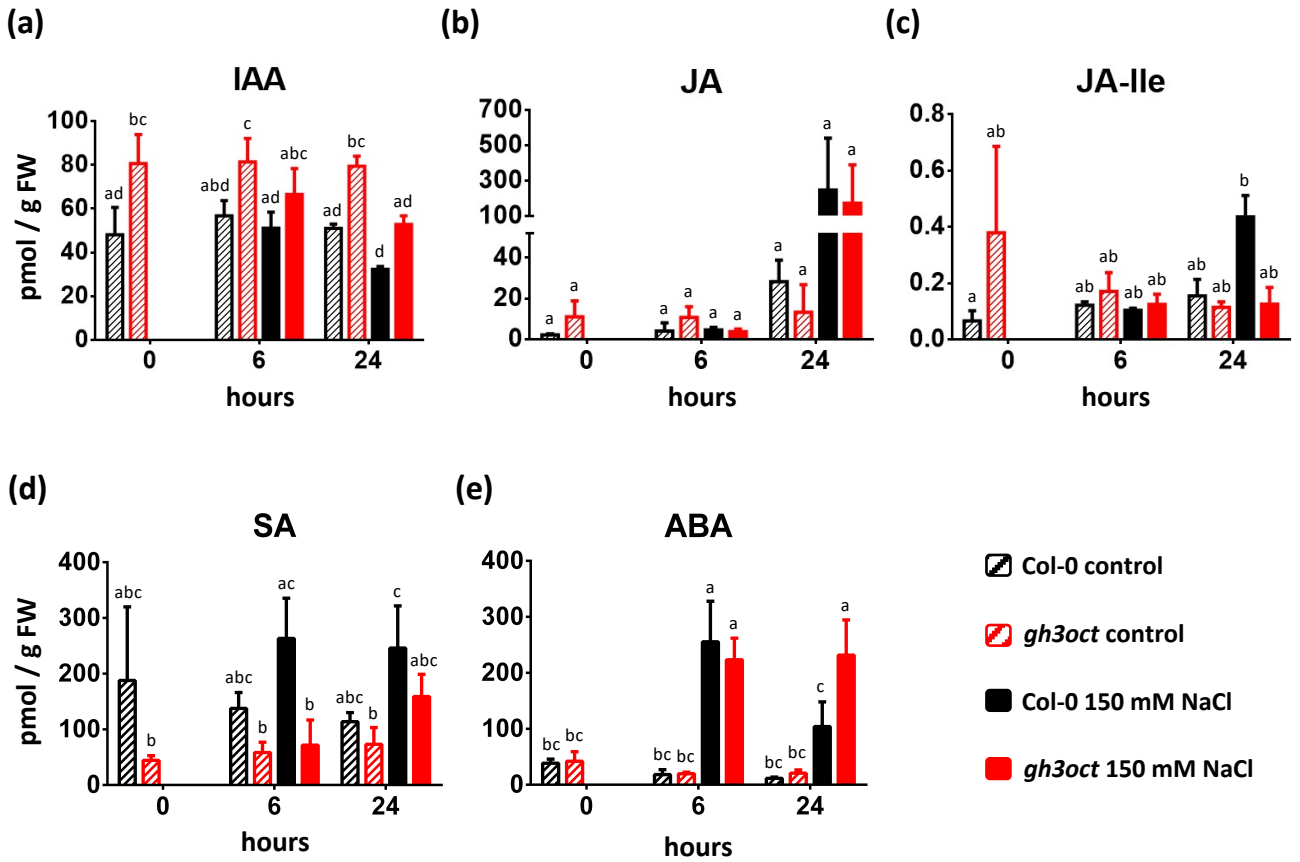


**Figure S7. Levels of IAA and IAA metabolites in shoots and roots from *Arabidopsis thaliana* group-II *gh3* single mutants.** Concentration of indole-3-acetic acid (IAA), 2-oxindole-3-acetic acid (oxIAA), indole-3-acetyl-aspartate (IAA-Asp) and indole-3-acetyl-glutamate (IAA-Glu) in rosettes and roots from 7-day-old *gh3* single mutants (red bars) and their correspondent wild-type background (black bars). Error bars indicate standard deviation. Asterisks indicate significantly different concentration values compared to the corresponding wild type in a two-tailed Student's *t*-test (\**p*<0.05; \*\**p*<0.01; \*\*\**p*<0.001; *n* = 4). FW: fresh weight.

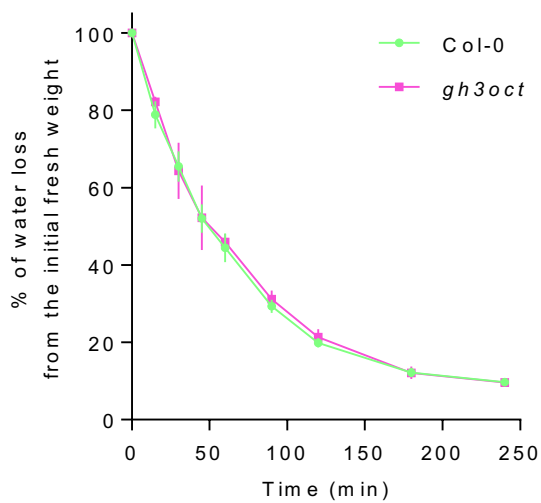




**Figure S8. Tolerance to salinity and water deficit of the *Arabidopsis thaliana* *gh3* single mutants.** (a,b) Root growth ratio of *gh3* single mutants in (a) *Ler* and (b) *Col-0* background grown in 50 mM NaCl. To calculate the root growth ratio, the root length from seedlings on NaCl plates was divided by the mean root length of seedlings on MS control plates. (c) Effects of the YUCCA inhibitor yucasin on the *gh3.5* salt-tolerant phenotype. Seedlings were normally grown for 4 days and then transferred for another 3 days to media plates containing 50 mM NaCl or 50 mM NaCl + 20  $\mu$ M of the YUCCA inhibitor yucasin. To calculate the ratio, the length of the root from seedlings on NaCl and NaCl + yucasin plates was divided by the mean root length of seedlings grown on control MS and MS + yucasin plates, respectively. (d) Root growth ratio of *Col-0*, *gh3oct* and *gh3.5* grown on media plates perfused with a 250 mg/L solution of PEG8000. 4-day-old seedlings were transferred to PEG-perfused or MS-perfused (control) plates and grown for another 2 days. To calculate the ratio, the root length from seedlings on PEG-containing plates was divided by the mean root length of seedlings on control plates. Box plots show the individual data points, the first and third quartile (bottom and top of the box), the median (line in the box), and the highest (top whisker) and lowest (bottom whisker) value. Asterisks indicate values significantly different from those of *Col-0* in a two-tailed Student's *t*-test (\*\* $p < 0.01$ , \*\*\* $p < 0.001$ ,  $n \geq 14$ ).



**Figure S9. Levels of IAA and stress-related phytohormones in response to salinity in the *gh3oct* mutant.** Concentration of (a) indole-3-acetic acid (IAA), (b) jasmonic acid (JA), (c) jasmonic acid-isoleucine (JA-Ile), (d) salicylic acid (SA) and (e) abscisic acid (ABA) in 9-day-old *Arabidopsis thaliana* Col-0 (black bars) and *gh3oct* (red bars) seedlings were determined 0, 6 and 24 hours after transferring to control MS plates (striped bars) or plates supplemented with 150 mM NaCl (solid bars). Error bars indicate the standard deviation. Different letters indicate significant differences ( $p < 0.05$ ) according to Tukey's post-hoc test. FW: fresh weight.



**Figure S10. Col-0 and *gh3oct* plants lose water at the same rate.** Aerial parts from 9-day-old *Arabidopsis thaliana* Col-0 and *gh3oct* seedlings were detached and immediately weighed ( $t = 0$  min). The weight of the samples was thereafter inspected at the indicated times. Data points represent the mean  $\pm$  standard deviation ( $n = 4$ ). No significant differences were found across the timepoints.

**Table S1.** Insertion lines combined to obtain the *gh3oct* mutant

Gene	AGI code	Insertion	Background
<i>GH3.1</i>	AT2G14960	SGT4174-5-2	<i>Ler</i>
<i>GH3.2</i>	AT4G37390	SGT5949-5-3	<i>Ler</i>
<i>GH3.3</i>	AT2G23170	SM.37350	Col-0
<i>GH3.4</i>	AT1G59500	SALK_102549	Col-0
<i>GH3.5</i>	AT4G27260	SALK_151766	Col-0
<i>GH3.6</i>	AT5G54510	SALK_013458	Col-0
<i>GH3.9</i>	AT2G47750	SM.23766	Col-0
<i>GH3.17</i>	AT1G28130	SALK_050597	Col-0

**Table S2.** Primer sets used in this work

Purpose	Primer name	Primer sequence (5' → 3')	
		Forward primer (F)	Reverse primer (R)
Genotyping	GH3.1	GGATCCATGGCGGTAGACTCCAAC	AGGACAGAAAGGCGGTCAAG
	GH3.2	GCTCCGACTCGTCCCAAAG	GTCGACCTAACGACGTCGTTCTGG
	Ds5'-1	ACGGTCGGGAAACTAGCTCTAC	
	GH3.3	CTATCGGTGACAGGCAGAGTC	AACCTCAGCATTTAGTCTTCACG
	GH3.9	ATGGGTTTCGCACTTAGGAAG	TCAACGACTTTATCACAGGGG
	3'-dSpm	TACGAATAAGAGCGTCCATTTTAGAGTGA	
	GH3.4	CAATGACGGGATTTTGATCAC	TGTGGAGCGGAATTATGAAAC
	GH3.5	AGGCCAGTGTTGTTGTCTTTG	TGGTCTTGAGCATAGATTCCG
	GH3.6	AAACCTAAACGATGCCTGAGG	CTCAGGCCAATGTTTCTCAAG
	GH3.17	GAAAAGTGAAAAGTGTGACGAG	CCTCTTGCTCACGGAGTACAC
	LBb1.3	ATTTTGCCGATTTTCGGAAC	
RT-qPCR	qGH3.1	GGACAACCTCGGTTGGACCAT	TGCACCTCTTGAGATTGCGT
	qGH3.2	TGCGTGAGCTTCACACCTAT	CTAAAACCGCACATCATCCG
	qGH3.3	CTCCGTGCCATTGGATTCCCT	ATCAGCCAGTTCTTGGTCCG
	qGH3.4	TGATCGGTGTGAGGCTTACG	TGGAGATACGTGTGGTGCAG
	qGH3.5	TGCTCCAATTATCGAGCTATTGA	TGTTTGTGACCAGGAACCCA
	qGH3.6	ACCTATGCTGGGCTTTACAGG	GCGGCATATGAAGCTGAACTG
	qGH3.9	CGACGATGAACAAGTCCCCT	GGTAACGGTACAATCCTGCGA
	qGH3.17	CATTTGTTAAGTTGCTAATTGGTGT	AGAGGACTTTGCTGAAAGTTTGT
	qACT2	CCGCTCTTTCTTTCCAAGC	CCGGTACCATTGTCACACAC

### Supplemental References

Cai R, Dai W, Zhang C, Wang Y, Wu M, Zhao Y, Ma Q, Xiang Y, Cheng B. 2017. The maize WRKY transcription factor ZmWRKY17 negatively regulates salt stress tolerance in transgenic *Arabidopsis* plants. *Planta* 246: 1215-1231.

Shen X, Wang Z, Song X, Xu J, Jiang C, Zhao Y, Ma C, Zhang H. 2014. Transcriptomic profiling revealed an important role of cell wall remodeling and ethylene signaling pathway during salt acclimation in *Arabidopsis*. *Plant Mol Biol* 86: 303-317.

Jiang Y, Deyholos MK. 2006. Comprehensive transcriptional profiling of NaCl-stressed *Arabidopsis* roots reveals novel classes of responsive genes. *BMC Plant Biol* 6: 25.