Supplemental Data. Park et al., Integration of auxin and salt signals by a NAC transcription factor NTM2 during seed germination in Arabidopsis

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                               NAC
NTM1  MMKGLIGYRFSPTGEEVINHYLKNKLLGKYWLVDEAISEINILSHKPSKDLPKLARIQSE
NTM2  MVKDLVGYRFYPTGEELINHYLKNKILGKTWLVDEAISEINICSYDP-IYLPSLSKIKSD

NTM1  DLEWYFFSPIEYTNPNKMKMKRTTGSGFWKPTGVDREIRDKRGNGVVIGIKKTLVYHEGK
NTM2  DPVWYFFCPKEYTSAKKKVTKRTTSSGYWKATGVDRKIKDKRGNRGEIGIKKTLVYYEGR

NTM1  SPHGVRTPWVMHEYHITCLPHHKRKYVVCQVKYKGEAAEIS------YEPSPSLVSDSHT
NTM2  VPKGVWTPWVMHEYHITCLPQDQRNYVICQVMYKGEDGDVPSGGNNSSEPSQSLVSDSNT

NTM1  VIAITGEPEPELQVEQPGKENLLGMSVDDLIEPMNQQE----------------------
NTM2  VRATS---PTALEFEKPGQENFFGMSVDDLGTPKNEQEDFSLWDVLDPDMLFSDNNNPTV

NTM1  EPQGPHLAPNDDEFIRGLRHVDRGTVEYLFANEENMDGLSMNDLRIPMIVQQEDLSEWEG
NTM2  HPQAPHLTPNDDEFLGGLRHVNREQVEYLFANED--------------------------

NTM1  FNADTFFSDNNNNYNLNVHHQLTPYGDGYLNAFSGYNEGNPPDHELVMQENRNDHMPRKP
NTM2  -----FISR--------------------------------PTLSMTENRNDHRPKKA

NTM1  VTG-TIDYSSDSGSDAGSISTTSYQGTSSP-NISVGSSSRHLSSCSST---DSCKDLQTC
NTM2  LSGIIVDYSSDSNSDAESISATSYQGTSSPGDDSVGSSNRQFLQTGGDEILSSCNDLQTY

NTM1  TDPSIIS--------REIRELTQEVKQEIPRAVDAP--MNNESSLVKTEKKGLFIVEDAM
NTM2  GEPSISSSTRQSQLTRSIIRPKQEVKQDTSRAVDSDTSIDKESSMVKTEKKSWFITEEAM

NTM1  ERNRKKPRFIYLMKMIIG---------NIISVLLPVKRLIPVKKL----
NTM2  ERNRNNPRYIYLMRMIIGFILLLALISNIISVL---RNLNPAMKFDRER
                          TM
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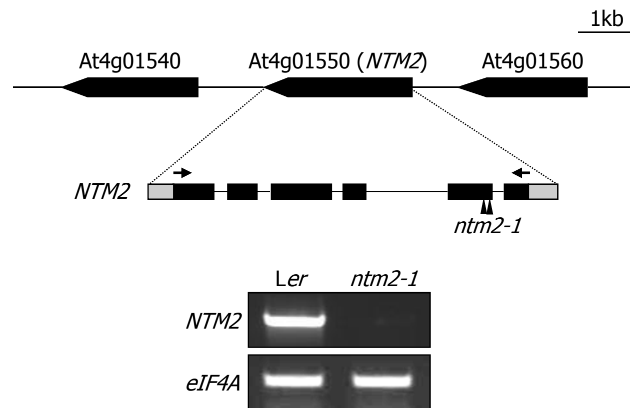
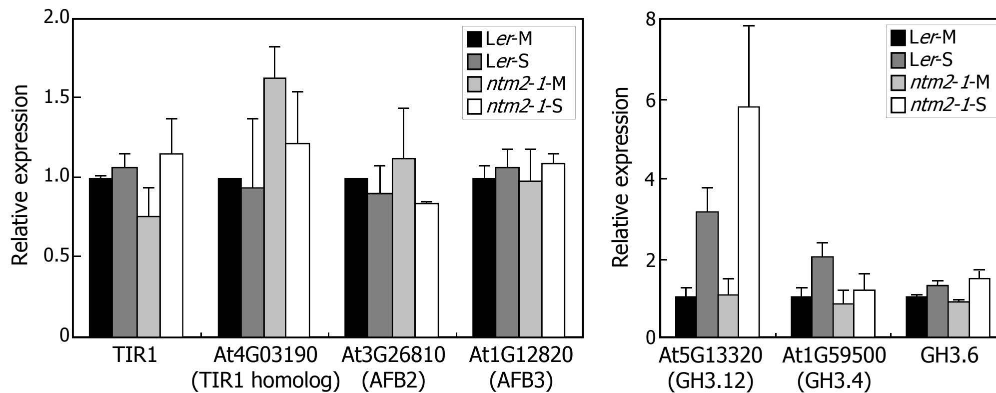
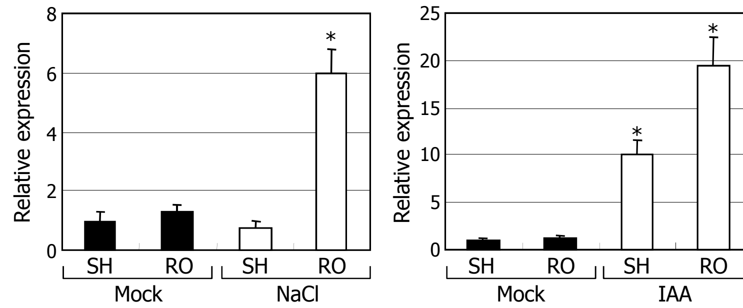
Supplemental Figure S1. Amino acid sequence alignment of NTM1 and NTM2 proteins. Amino acid sequences of NTM1 and NTM2 proteins were aligned using the ClustalW program (http://www.clustal.org/). Identical residues were boxed in black, and biochemically conserved residues were boxed in gray. The NAC domains were indicated. The putative transmembrane motifs (TMs) were underlined.



Supplemental Figure S2. Mapping of transposon insertion site and absence of *NTM2* gene expression in the *ntm2-1* mutant.
Arrowhead indicates the site of transposon insertion. Two copies of the transposon are inserted in the second exon of *NTM2* genome. In the *NTM2* gene structure, black boxes are exons, and gray boxes are untranslated regions. Transcript levels were examined by semi-quantitative RT-PCR using total RNA samples extracted from 2-week-old whole seedlings grown on 1/2 X Murashige and Skoog (MS)-agar plates (hereafter referred to as MS-agar plates). Arrows indicate the forward and reverse primers used for RT-PCR. A *eIF4A* gene was used as a control for RNA quality. kb, kilobase.

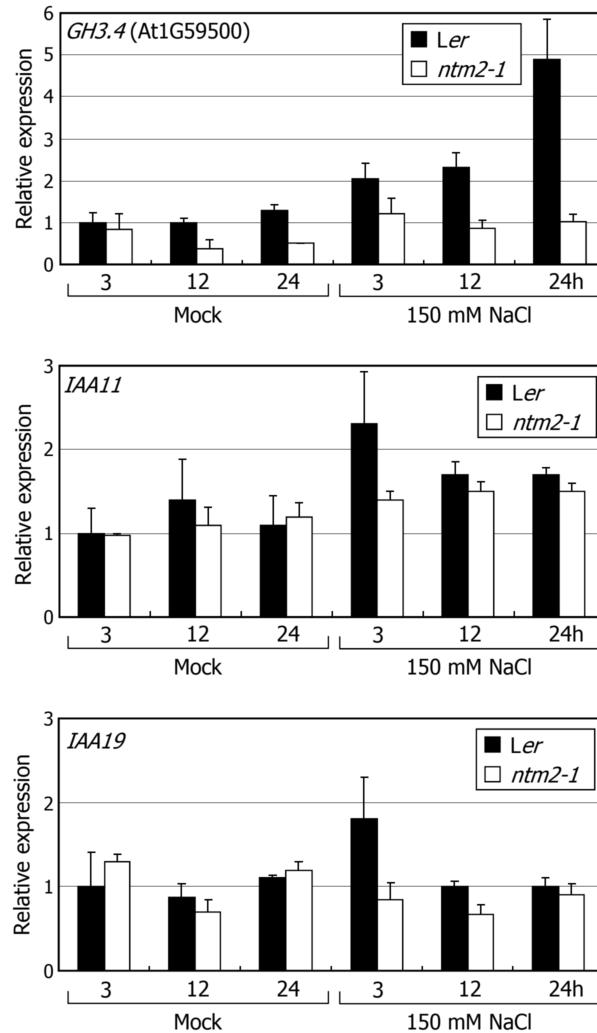


Supplemental Figure S3. Expression patterns of *TIR*-related and *GH3* genes under high salinity. Two-week-old plants grown on MS-agar plates were transferred to MS liquid cultures supplemented with 150 mM NaCl and soaked for 3h before harvesting plant materials. Whole plants were used for extraction of total RNA. Transcript levels were determined by quantitative real-time RT-PCR (qRT-PCR). Biological triplicates were averaged. Bars indicate standard error of the mean. M, mock; S, NaCl 150 mM.



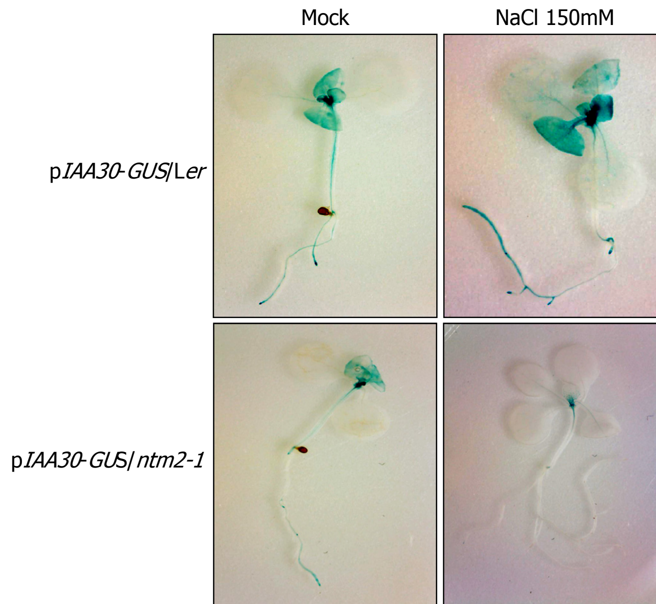
Supplemental Figure S4. Effects of high salinity on *IAA30* gene expression in the shoots (SH) and roots (RO).

Two-week-old plants grown on MS-agar plates were soaked for 6h in MS liquid cultures supplemented with 150 mM NaCl (A) or 20 μM IAA (B), and the shoot and root samples were harvested separately for extraction of total RNA. Transcript levels were determined by qRT-PCR. Biological triplicates were averaged. Bars indicate standard error of the mean. Statistical significance was determined by a student *t*-test (*P<0.01).

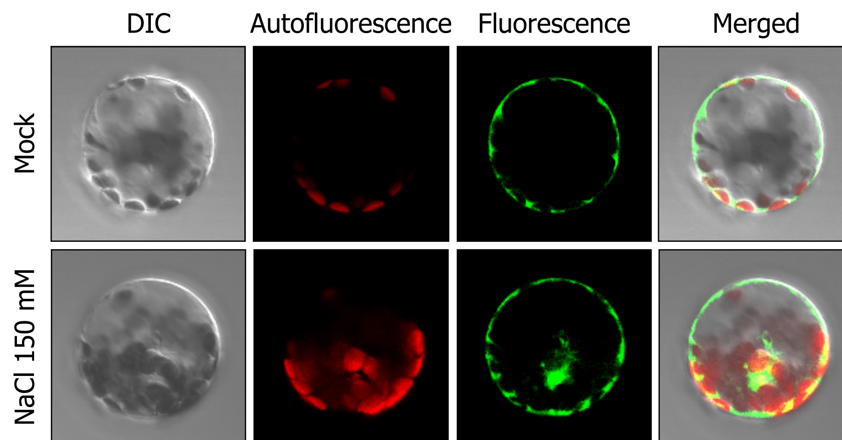


Supplemental Figure S5. Kinetic expression patterns of *IAA11*, *IAA19* and *GH3.4* genes under high salinity.

Two-week-old plants grown on MS-agar plates were soaked in MS liquid cultures containing 150 mM NaCl for the indicated time periods before harvesting plant materials. Whole plants were used for extraction of total RNA. Transcript levels were determined by qRT-PCR. Biological triplicates were averaged. Bars indicate standard error of the mean.



Supplemental Figure S6. Effects of high salinity on the promoter activities of *IAA30* gene.
A GUS-coding sequence was transcriptionally fused to the promoter sequence, consisting of approximately 1.9 kb upstream of the transcription start site of *IAA30* gene, and the p*IAA30-GUS* fusion construct was transformed into L*er* (p*IAA30-GUS*/L*er*) or *ntm2-1* mutant (p*IAA30-GUS*/*ntm2-1*). Ten-day-old transgenic plants expressing the p*IAA30-GUS* fusion were incubated for 6h in MS liquid cultures containing 150 mM NaCl and subject to GUS staining. In the p*IAA30-GUS*/L*er* transgenic plants, GUS activities were elevated in both the leaves and roots. In contrast, GUS activities were not elevated in the p*IAA30-GUS*/*ntm2-1* transgenic plants.



Supplemental Figure S7. High salinity-induced nuclear localization of NTM2.
The GFP-NTM2 construct was expressed transiently in *Arabidopsis* protoplasts for 12h, and the protoplasts were treated with 150mM NaCl for 2h and were visualized by differential interference contrast microscopy (DIC, upper left panel), autofluorescence (upper right panel), and fluorescence microscopy (lower left panel). The lower right panel is a merged image.

Supplemental Table S1. List of PCR primers used in this work.

Primer	Polarity	Usage	Sequence
eIF4a	F	qRT-PCR	5′-TGACCACACAGTCTCTGCAA
eIF4a	R	qRT-PCR	5′-ACCAGGGAGACTTGTTGGAC
TIR1	F	qRT-PCR	5′-CGCCTCTCTCTATCTGGCCT
TIR1	R	qRT-PCR	5′-GCATTTCCATCTTCTTGGCA
At4g03190	F	qRT-PCR	5′-ACTGATGGTATCGCTGCTATTG
At4g03190	R	qRT-PCR	5′-AGTTGAACTCTCTGGAAAATAGCTAAG
At3g26810	F	qRT-PCR	5′-CGTGCCTCGAAGGAGAAAC
At3g26810	R	qRT-PCR	5′-TTTGGAGACCTAGCAACAAGC
At1g12820	F	qRT-PCR	5′-TGATAAACTTTACCTCTACCGAACAG
At1g12820	R	qRT-PCR	5′-CCTAACATATGGTGGTGCATCTT
At5g13320	F	qRT-PCR	5′-GATGTTATATCGGCCCGAAC
At5g13320	R	qRT-PCR	5′-TTCCATGGCATCATCTTTTG
At1g59500	F	qRT-PCR	5′-AGGACGTCGGATTCAGACAGC
At1g59500	R	qRT-PCR	5′-TACTCCTCCATCTCCATCGTG
GH3.6	F	qRT-PCR	5′-AACAATGCGCCTCAGTTCAG
GH3.6	R	qRT-PCR	5′-ATAGTGGCCCGGGATAGATG
AUX/IAA2	F	qRT-PCR	5′-GAAGAATCTACACCTCCTACCAAAA
AUX/IAA2	R	qRT-PCR	5′-CACGTAGCTCACACTGTTGTTG
AUX/IAA3	F	qRT-PCR	5′-CAAAGATGGTGATTGGATGCT
AUX/IAA3	R	qRT-PCR	5′-TGATCCTTAGTCTCTTGCACGTA
AUX/IAA5	F	qRT-PCR	5′-TGAAGACAAAGATGGAGATTGG
AUX/IAA5	R	qRT-PCR	5′-GCACGATCCAAGGAACATTT
AUX/IAA11	F	qRT-PCR	5′-ATTGCTGGGATCAAGAGGAC
AUX/IAA11	R	qRT-PCR	5′-GTGGCCATCCCACAACTT
AUX/IAA13	F	qRT-PCR	5′-CATCTCCTCCTCGTTCAAGC
AUX/IAA13	R	qRT-PCR	5′-CTGTTCATCCTGTGTGACCCTA
AUX/IAA19	F	qRT-PCR	5′-TTGTATCAAATTGTGAGAGGAAAAA
AUX/IAA19	R	qRT-PCR	5′-CGTTATCTCAAGCCCGAGTC
AUX/IAA29	F	qRT-PCR	5′-TCCTCTGGAATCCGAGTCTTC
AUX/IAA29	R	qRT-PCR	5′-GGTGGCCATCCAACAACTT
AUX/IAA30	F	qRT-PCR	5′-TTCAATGCTTCAATCCTTTGG
AUX/IAA30	R	qRT-PCR	5′-AGCACGTGACTCTTCTCACTACA
NTM2	F	qRT-PCR	5′-CACATCACTTGTTTGCCTCAA
NTM2	R	qRT-PCR	5′-CCCTTATACATCACTTGGCAG
NTM2-B1	F	Subcloning	5′-AAAAAGCAGGCTGGATGGTGAAAGATCTGGTTGG
NTM2-B2	R	Subcloning	5′-AGAAAGCTGGGTGCTATCTCTCGCGATCAAACTT
GUS-NTM2-AscI	F	Subcloning	5′-TTGGCGCGCCGAAAGAAGACCCATAGACCGAAC
GUS-NTM2-SpeI	R	Subcloning	5′-GGACTAGTTCTCTCGCGATCAAACTTC
GUS-IAA30-BamHI	F	Subcloning	5′-CGCGGATCCTTGGTAATTATTATTATTAAAGAC
GUS-IAA30-Asc	R	Subcloning	5′-TTGGCGCGCCTGTAGTGATAAGCTCTTGAGATC

qRT-PCR primers were designed using the Primer Express Software installed into the Applied Biosystems 7500 Real-Time PCR System. The sizes of PCR products ranged from 80 to 150 nucleotides in length.