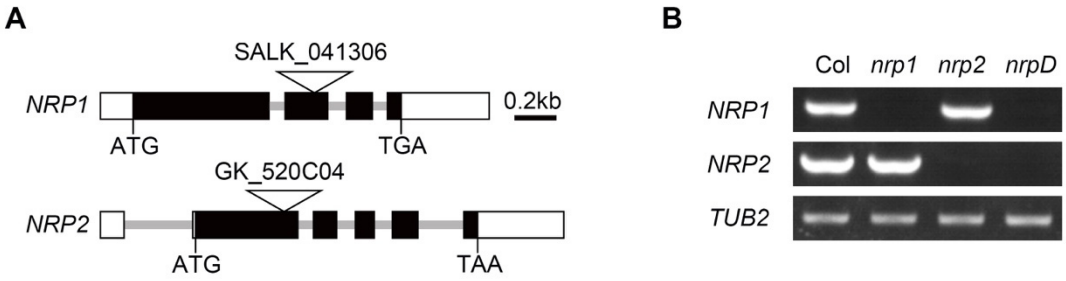
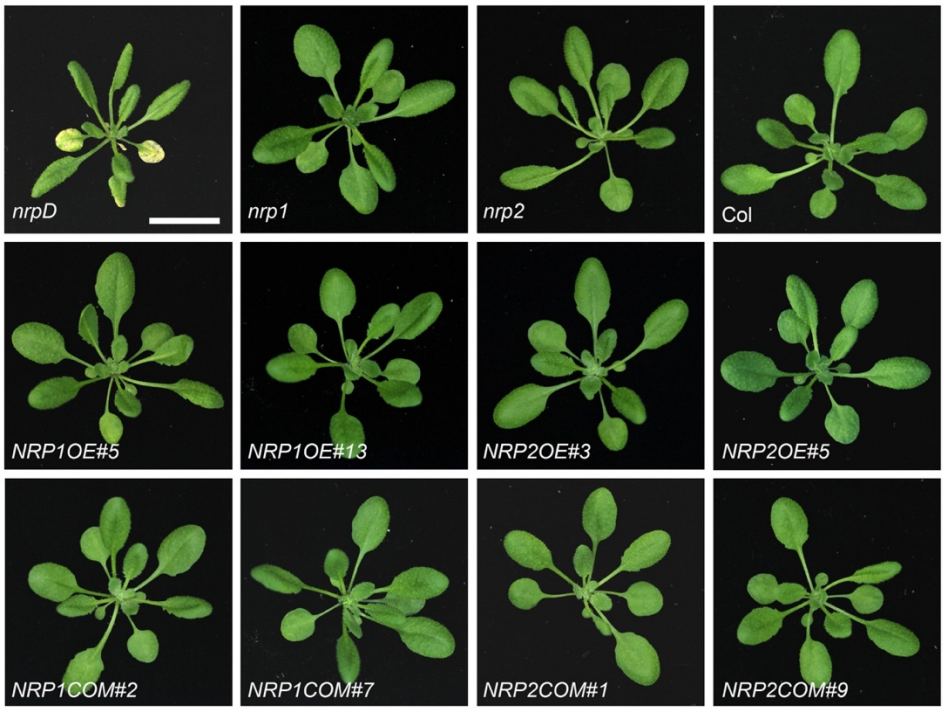
SUPPLEMENTAL INFORMATION

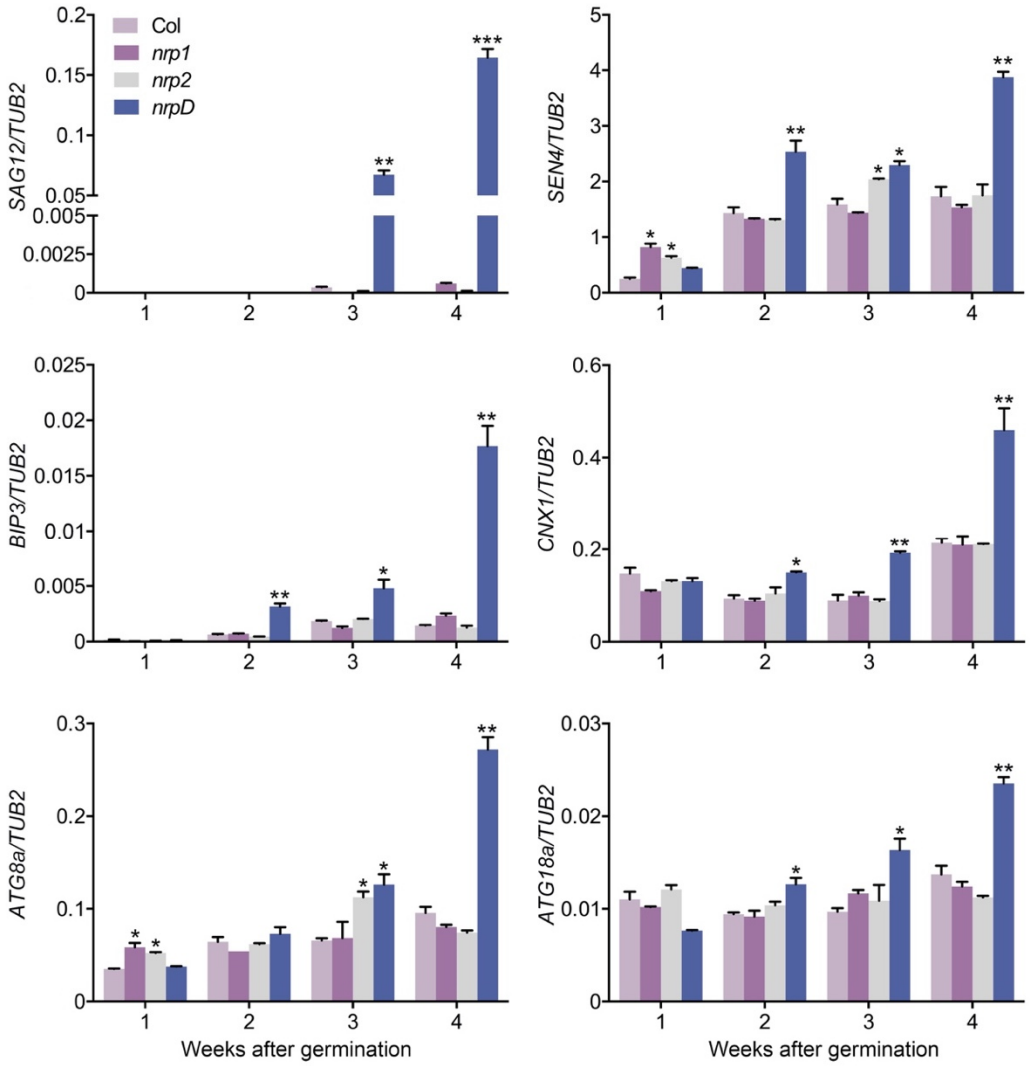
Supplemental Figures



Supplemental Figure S1. Schematic diagram and identification of T-DNA insertion mutants *nrp1* and *nrp2*. A, Gene structure of *NRP1* and *NRP2* with the location of T-DNA insertions. The position of the T-DNA inserts (*nrp1*, SALK_041306; *nrp2*, GK_520C04) is indicated by inverted triangles. Black boxes and grey lines denote exons and introns, respectively. White boxes indicate UTRs. B, RT-PCR was performed to detect the *NRP1* and *NRP2* expression in *Col, nrp1, nrp2, and nrpD.* *TUB2* was used as an internal control.

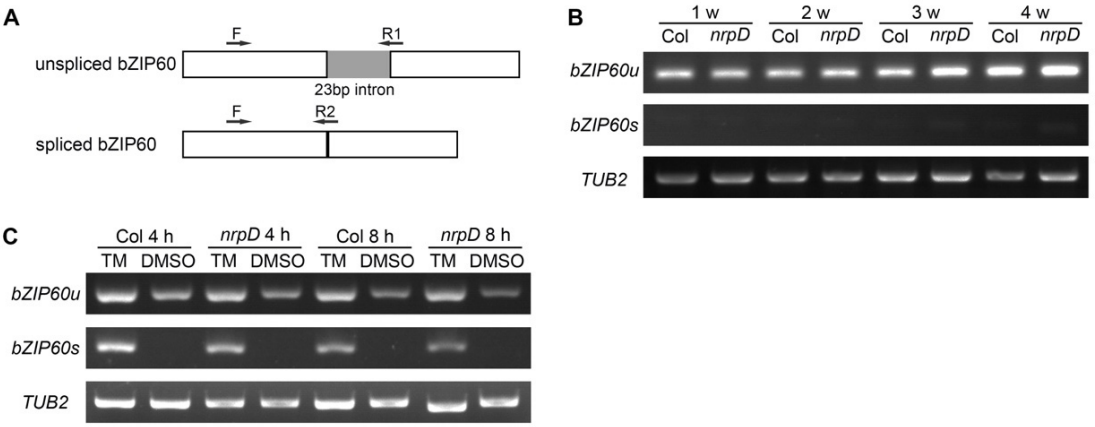


Supplemental Figure S2. Loss-of-function of *NRP1* and *NRP2* leads to precocious senescence. The seedlings were grown under LD conditions at 22°C for 21 days. Bar = 2 cm. OE, overexpression transgenic line; COM, complementation transgenic line.

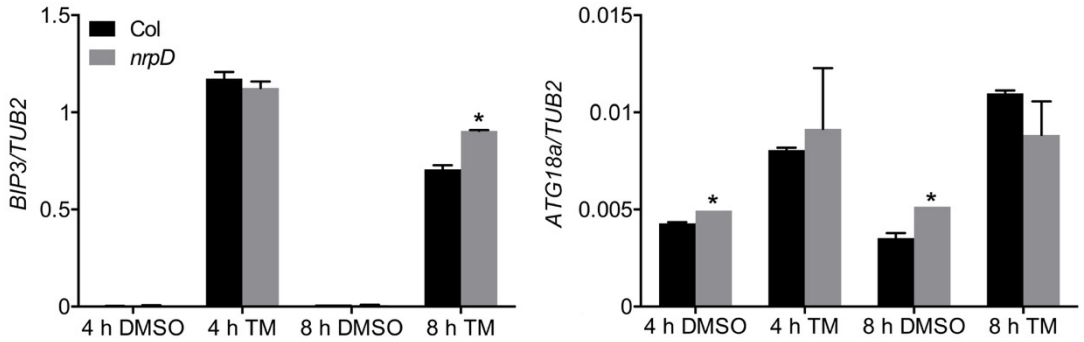


17

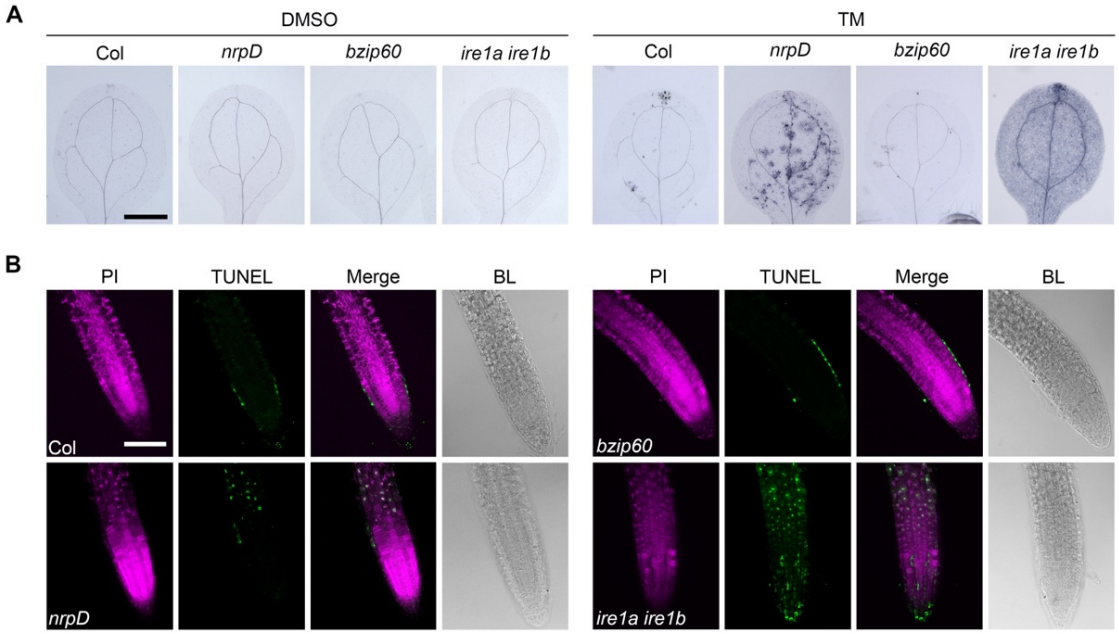
18 **Supplemental Figure S3.** The expression analysis of senescence, ER stress, and
19 autophagy-related marker genes in Col, *nrp1*, *nrp2*, and *nrpD* at different growth stages.
20 Seedlings were germinated on 1/2 MS medium and grown for 4 weeks. The whole
21 seedlings were collected for RNA extraction at indicated time. *TUB2* was used as an
22 internal control for calculation of relative gene expression level. Asterisks indicate
23 significant differences compared with Col (Student's *t*-test, **P* < 0.05; ***P* < 0.01;
24 ****P* < 0.001). Data represent means ± SD of biological triplicates.



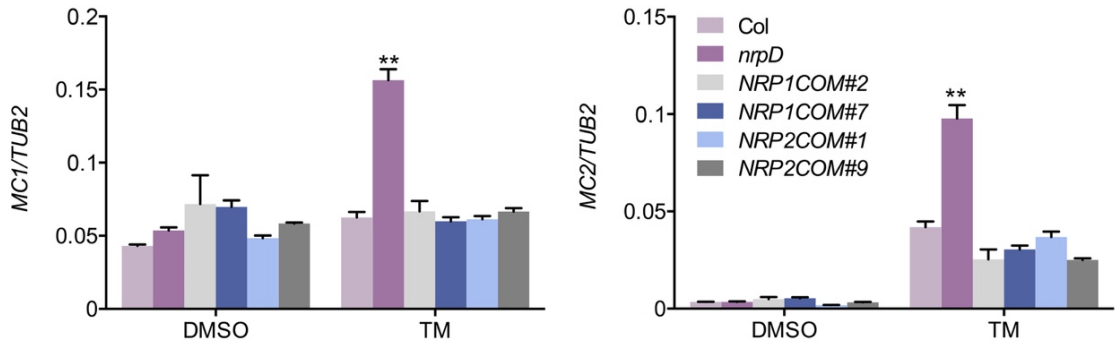
Supplemental Figure S4. *bZIP60* mRNA splicing analysis. A, Specific primers were used to specifically detect the unspliced/spliced *bZIP60* mRNA. F, forward primer for unspliced/spliced *bZIP60* RNA forms; R1, reverse primer 1 for unspliced *bZIP60* RNA forms; R2, reverse primer 2 for spliced *bZIP60* RNA forms. B, *bZIP60* mRNA splicing analysis under different growth stages. C, Tunicamycin (TM) induction of *bZIP60* mRNA splicing. *TUB2* was used as control. *bZIP60u*, unspliced *bZIP60*; *bZIP60s*, spliced *bZIP60*.



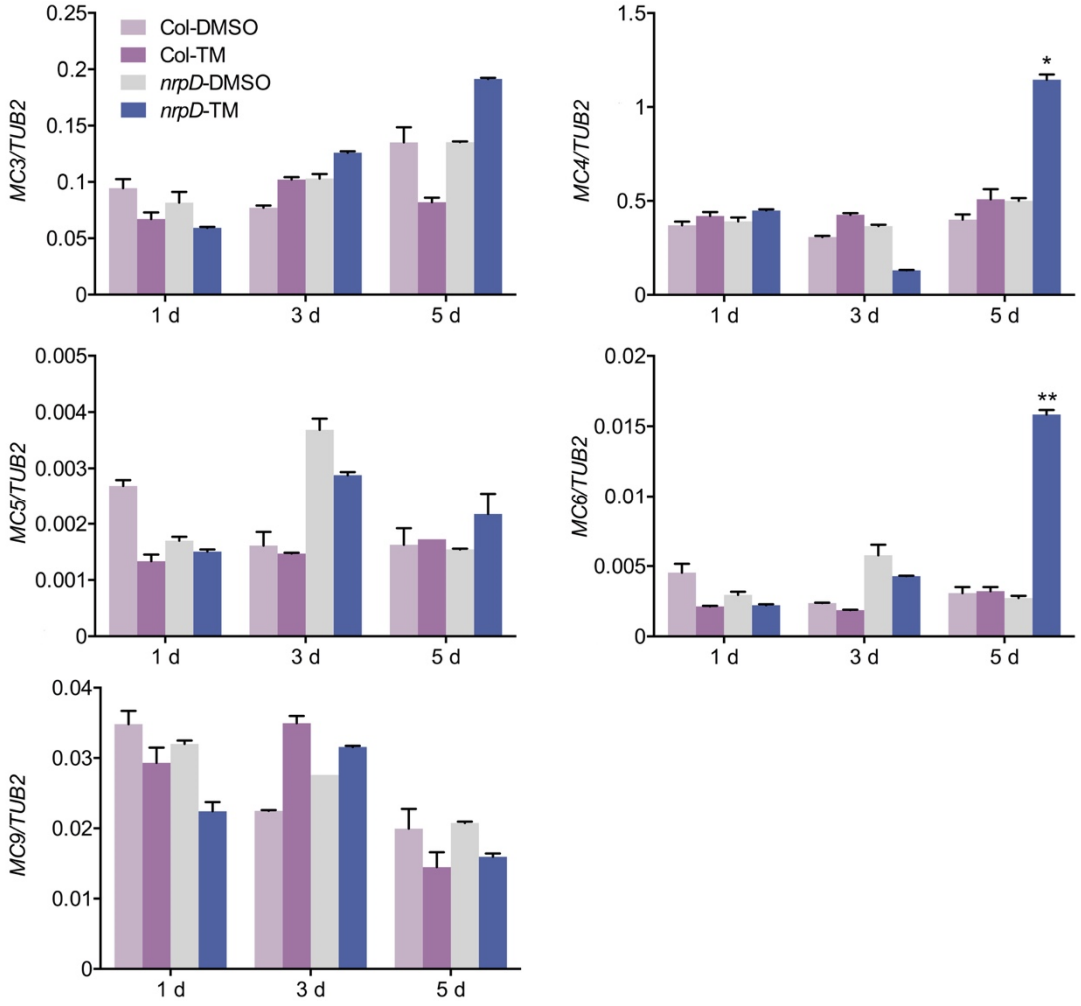
Supplemental Figure S5. The expression analysis of *BIP3* and *ATG18a* in Col and *nrpD*. Seven-day-old seedlings were treated with 1 μg/mL TM (DMSO as mock) for indicated time. *TUB2* was used as an internal control for calculation of relative gene expression level. Asterisks indicate significant differences compared with Col (Student's *t*-test, $*P < 0.05$). Data represent means ± SD of biological triplicates.



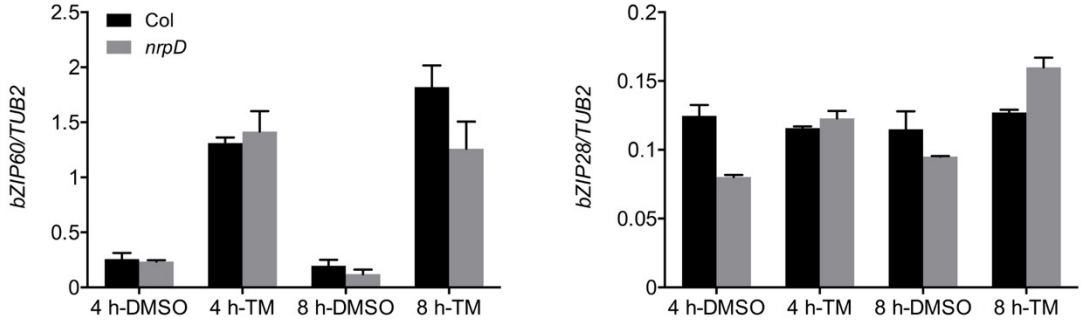
Supplemental Figure S6. The trypan blue staining and TUNEL assay of *bzip60* and *ire1a ire1b*. Seven-day-old seedlings were treated with TM (DMSO as mock) for 2 d and collected for trypan blue staining (A) or TUNEL labeling (B). Bar = 2 mm in (A), and 100 μm in (B). TM, tunicamycin; PI, propidium iodide; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick and labeling; BL, bright light.



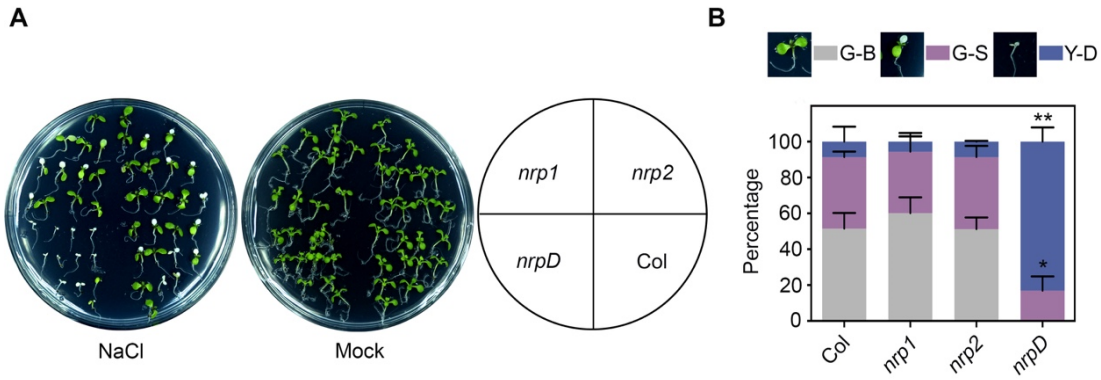
Supplemental Figure S7. The expression analysis of *MC1 and MC2* in Col and the complementation transgenic lines. Seven-day-old seedlings were treated with 1 μg/mL TM (DMSO as mock) for 5 d. *TUB2* was used as an internal control. Asterisks indicate significant differences compared with Col (Student's *t*-test, $^{**}P < 0.001$). Data represent means ± SD of biological triplicates.



Supplemental Figure S8. The expression analysis of MC genes in Col and *nrpD*. Seven-day-old seedlings were treated with 1 μg/mL TM (DMSO as mock) for indicated days. *TUB2* was used as an internal control for calculation of relative gene expression level. Asterisks indicate significant differences compared with Col (Student's *t*-test, **P* < 0.01; ***P* < 0.001). Data represent means ± SD of biological triplicates.



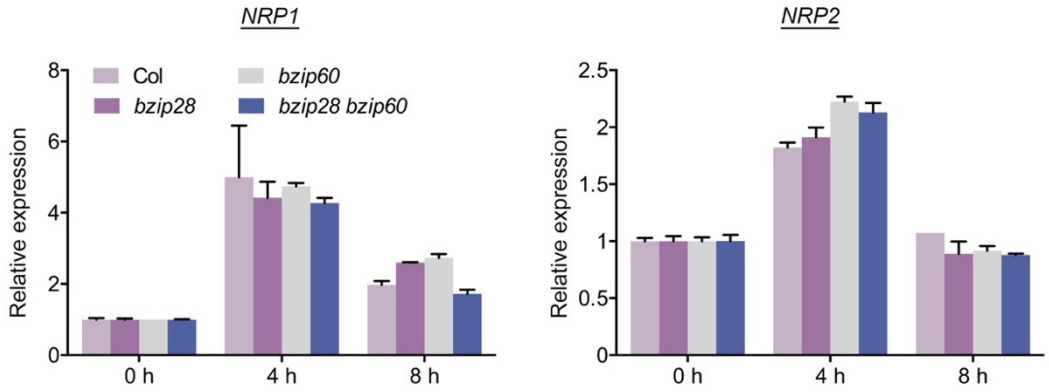
Supplemental Figure S9. The expression analysis of *bZIP60* and *bZIP28* in Col and *nrpD*. Seven-day-old seedlings were treated with 1 μg/mL TM (DMSO as mock) for indicated time. *TUB2* was used as an internal control for calculation of relative gene expression level. Data represent means ± SD of biological triplicates.



68

69 **Supplemental Figure S10.** The double mutant *nrpD* exhibits less tolerance to salt
70 stress. Seven-day-old seedlings were transferred to 1/2 MS medium with or without
71 150 mM NaCl for 7 d and the picture was taken (A). The percentages of green-big (G-
72 B), green-small (G-S), and yellow-dead (Y-D) seedlings were calculated (B). The
73 images next to the boxes display the phenotype of plants in the three groups. Asterisks
74 indicate significant differences compared with Col (Student's *t*-test, **P* < 0.01; ***P* <
75 0.001). Data represent means ± SD of biological triplicates.

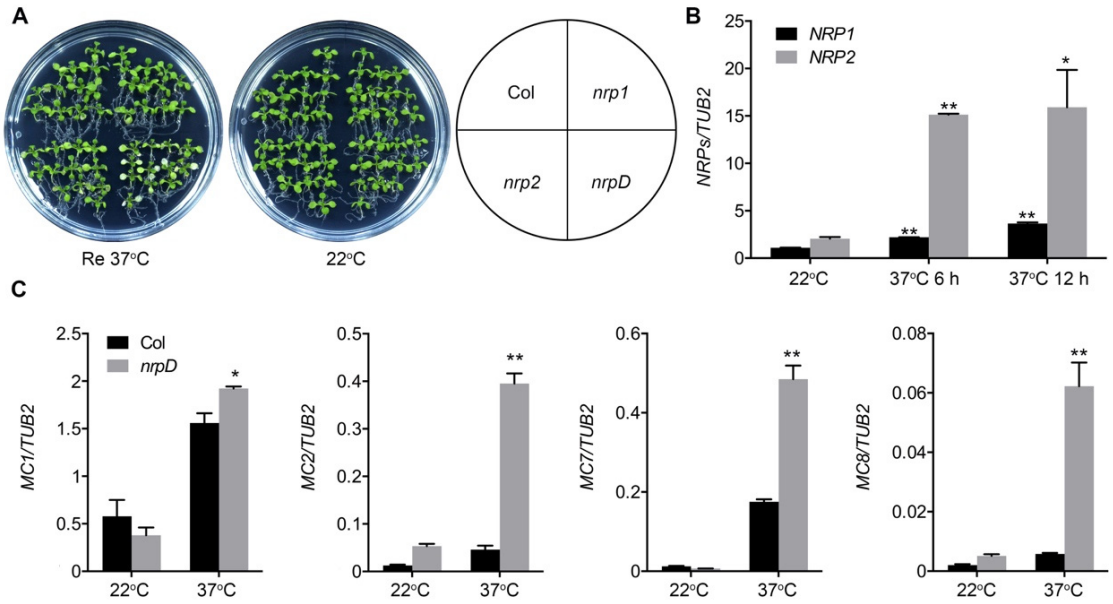
76



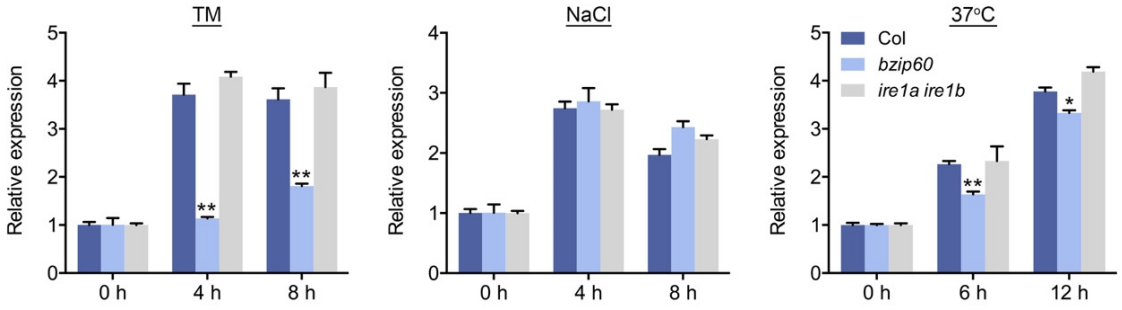
77

78 **Supplemental Figure S11.** The expression analysis of *NRP1*and *NRP2* in Col, *bzip28*,
79 *bzip60*, and *bzip28 bzip60* under salt stress. Seven-day-old seedlings were treated with
80 or without 150 mM NaCl. *TUB2* was used as an internal control. Data represent means
81 ± SD of biological triplicates.

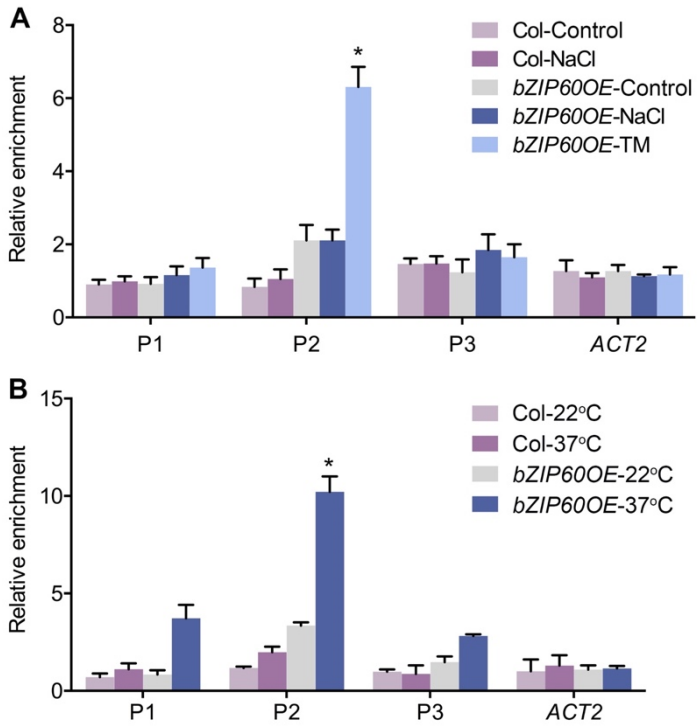
82



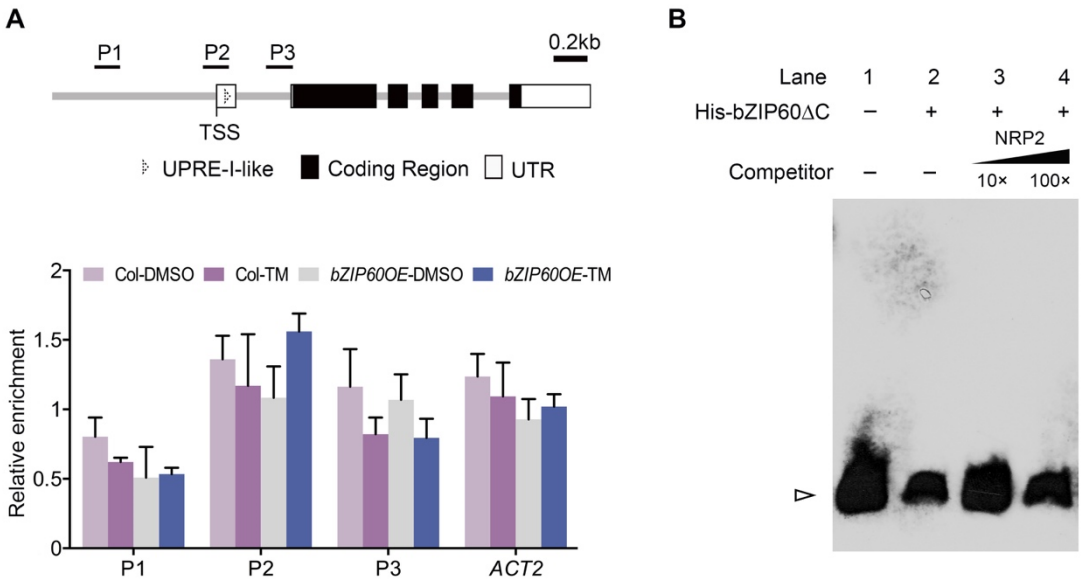
Supplemental Figure S12. *NRP1* and *NRP2* are involved in heat stress response. A, The double mutant *nrpD* exhibits less heat stress tolerance. Ten-day-old plants were incubated at 37°C for 1 d then grown at 22°C for 4 d. Re 37°C, recovered from 37°C. B, The expression analysis of *NRP1* and *NRP2* at 37ºC. C, The expression analysis of *MC* genes in Col and *nrpD* under heat stress. Ten-day-old plants were incubated at 37°C for 1 d. *TUB2* was used as an internal control for calculation of relative gene expression level. Asterisks indicate significant differences compared with 22ºC (B) or Col (C) (Student's *t*-test, **P* < 0.01; ***P* < 0.001). Data represent means ± SD of biological triplicates.



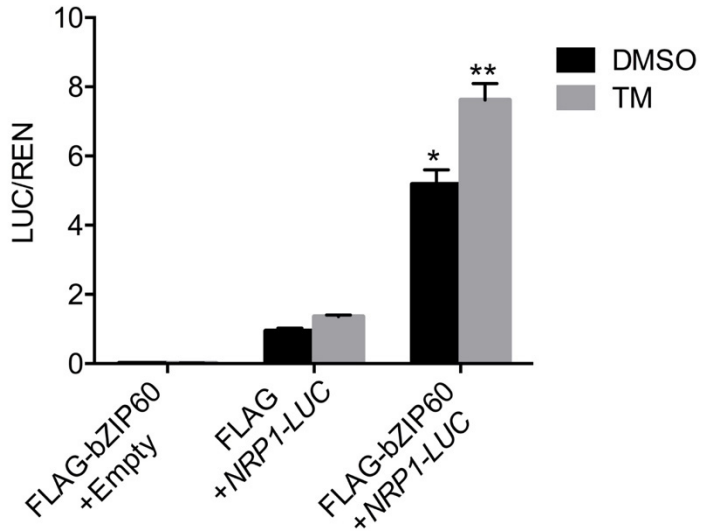
Supplemental Figure S13. The expression analysis of *NRP1* in *ire1a ire1b* mutant under ER stress, high salinity stress, and heat stress. Seven-day-old seedlings were treated with 1 μg/mL TM or 150 mM NaCl, and ten-day-old seedlings were incubated at 37°C. *TUB2* was used as an internal control. Asterisks indicate significant differences compared with Col (Student's *t*-test, $*P < 0.01$; $**P < 0.001$). Data represent means ± SD of biological triplicates.



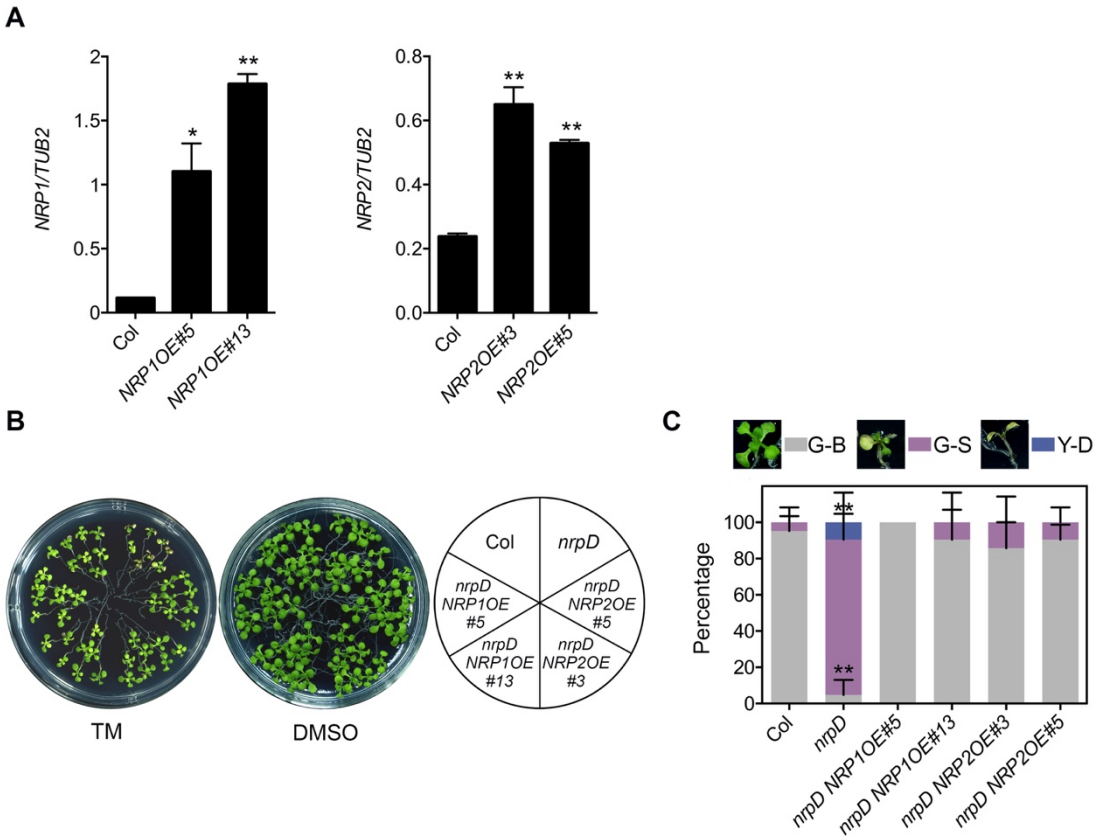
Supplemental Figure S14. ChIP analysis of bZIP60 binding to the *NRP1* promoter under salt and heat stress. Seven-day-old of *35S:FLAG-bZIP60* and Col seedlings were treated with 150 mM NaCl (A), and ten-day-old seedlings were incubated at 37ºC for 4 h (B), and harvested for ChIP assay. *35S:FLAG-bZIP60* seedlings treated with TM for 4 h were used as the positive control under salt stress (A). P1 to P3 represent the fragments for ChIP-qPCR amplification as described in Figure 4A. The enrichment of *ACT2* genomic fragment was used as the negative control. *PP2A* was used as an internal control. Data represent mean ± SD of triplicates. Asterisks indicate significant differences compared with the control (Student's *t*-test, $*P < 0.01$).



Supplemental Figure S15. ChIP and EMSA analysis of bZIP60 binding to the *NRP2* genomic regions. A, The upper panel shows schematic diagram of the *NRP2* genomic regions. P1 to P3 indicate fragments for ChIP-qPCR amplification. TSS, transcription start site. The lower panel shows ChIP analysis of bZIP60 binding to the *NRP2* genomic regions upon the precipitation with anti-FLAG antibody. Seven-day-old of *35S:FLAG-bZIP60* and Col seedlings were treated with 1 μg/mL TM (DMSO as mock) for 4 h and harvested for ChIP assay. The enrichment of *ACT2* genomic fragment was used as the negative control. *PP2A* was used as an internal control. Data represent mean ± SD of triplicates. B, EMSA experiment detecting the protein-DNA binding. The purified His-bZIP60ΔC was incubated with the biotin-labeled *NRP2* DNA fragments (40 bp) (Lane 1~4). The un-labeled *NRP2* DNA were use as cold competitors. Lane 3, 10× un-labeled *NRP2* (N2); lane 4, 100× un-labeled *NRP2*. White arrow head points to the free probes.

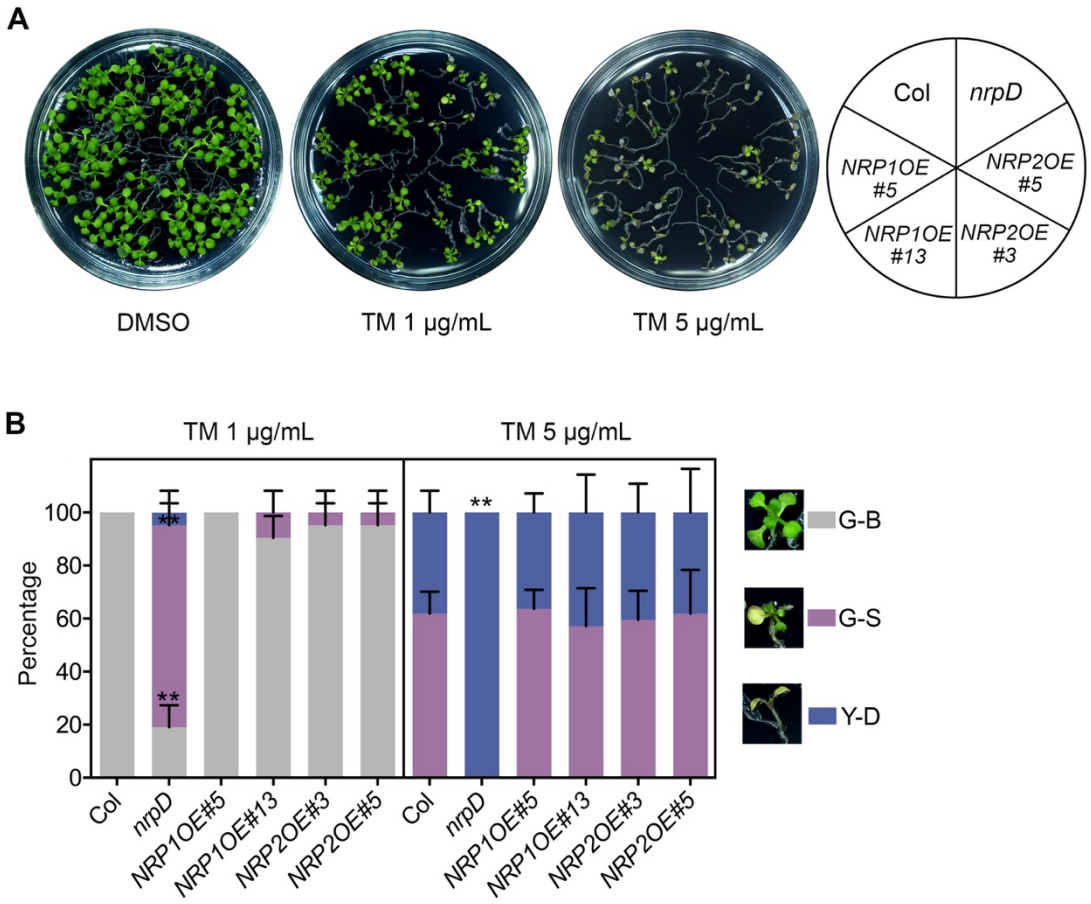


Supplemental Figure S16. Transient expression assay of the expression of *NRP1* regulated by bZIP60. The reporters *pNRP1:LUC* and empty *LUC* (without *NRP1* promoter, Empty) were co-transformed with *35S:FLAG-bZIP60* effectors (*35S:FLAG* (FLAG), as a control) into Arabidopsis *ire1a ire1b* mesophyll protoplasts, and then the protoplasts were treated with 1 μg/mL TM (DMSO as mock) for 1 h after cultured overnight. The LUC activity was calculated by relative LUC activity (LUC/REN). Asterisks indicate significant differences compared with FLAG+*NRP1-LUC* under DMSO or TM treatment, respectively (Student's *t*-test, *P < 0.01; **P < 0.001). The values are means ± SD of biological triplicates. REN, renilla luciferase.

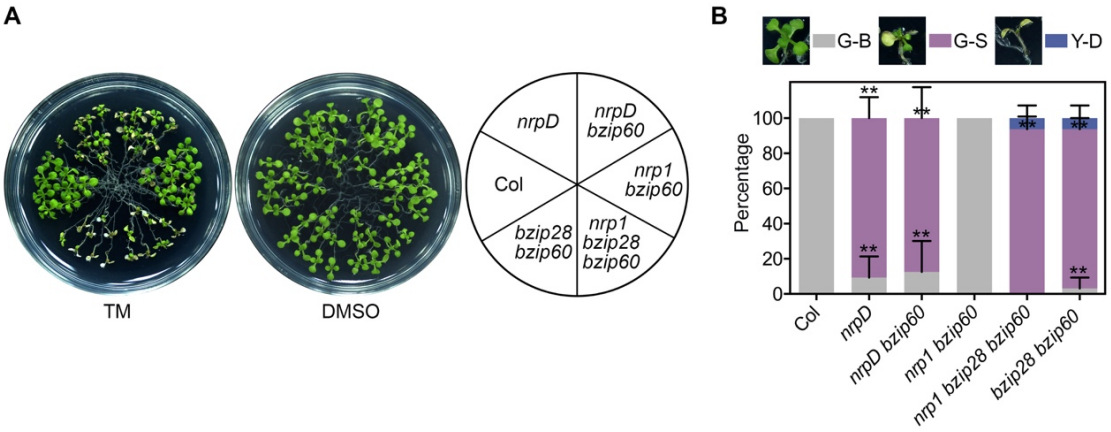


137

138 **Supplemental Figure S17.** The *NRP1/2* overexpression plants can rescue the *nrpD*
139 susceptibility to TM. A, The expression of *NRP1/2* in overexpression transgenic lines.
140 Seven-day-old seedlings were collected for RNA extraction. *TUB2* was used as an
141 internal control for calculation of relative gene expression level. B and C, TM
142 sensitivity of Col, *nrpD*, *nrpD NRP1OE#5*, *nrpD NRP1OE#13*, *nrpD NRP2OE#3*, and
143 *nrpD NRP2OE#5.* Seven-day-old seedlings were treated with 1 μg/mL TM (DMSO as
144 control) for 10 d and the picture was taken (B). The percentages of green-big (G-B),
145 green-small (G-S), and yellow-dead (Y-D) seedlings were calculated(C). The images
146 next to the boxes display the phenotype of plants in the three groups. A and C, Asterisks
147 indicate significant differences compared with Col (Student's *t*-test, $*P < 0.01$; $**P <$
148 0.001). Data represent means ± SD of biological triplicates.



Supplemental Figure S18. The TM sensitivity of *NRP1/2* overexpression plants is similar to that of Col wild type. Seven-day-old Col, *nrpD*, *35S:NRP1-6HA* (*NRP1OE#5* and *#13*), and *35S:NRP2-6HA* (*NRP2OE#3* and *#5*) seedlings were treated with 1 or 5 μg/mL TM (DMSO as mock) for 10 d and the picture was taken (A). The percentages of green-big (G-B), green-small (G-S), and yellow-dead (Y-D) seedlings were calculated (B). The images next to the boxes display the phenotype of plants in the three groups. Asterisks indicate significant differences compared with Col (Student's *t*-test, $^{**}P < 0.001$). Data represent means ± SD of biological triplicates.



159

160 **Supplemental Figure S19.** The TM sensitivity of *nrp1 bzip60*, *nrpD bzip60*, and *nrp1*
161 *bzip28 bzip60*. Seven-day-old seedlings were treated with 0.5 μg/mL TM (DMSO as
162 mock) for 10 d and the picture was taken (A). The percentages of green-big (G-B),
163 green-small (G-S), and yellow-dead (Y-D) seedlings were calculated (B). The images
164 next to the boxes display the phenotype of plants in the three groups. Asterisks indicate
165 significant differences compared with Col (Student's *t*-test, $^{**}P < 0.001$). Data
166 represent means ± SD of biological triplicates.

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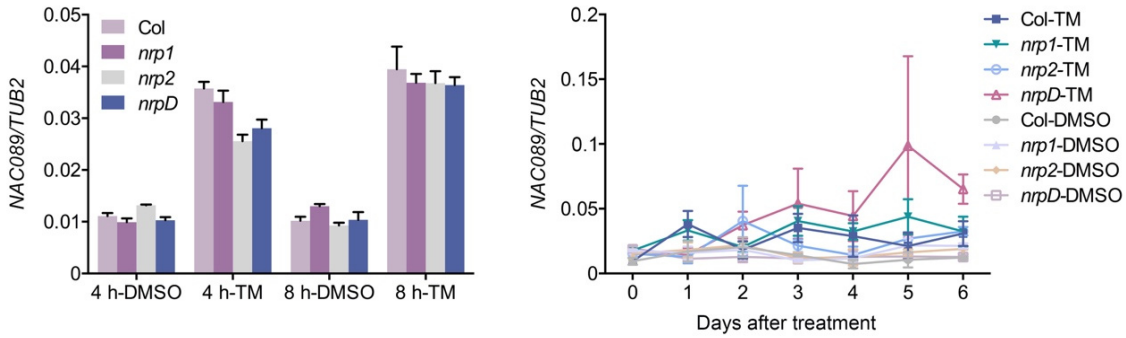
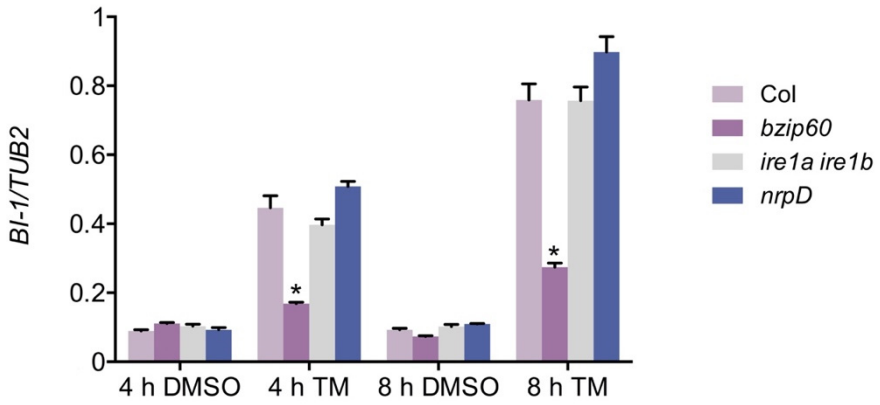


Supplemental Figure S20. The expression analysis of *NAC089* in Col, *nrp1*, *nrp2*, and *nrpD*. Seven-day-old seedlings were treated with 1 μg/mL TM (DMSO as mock) and collected for RNA extraction for indicated time. The *TUB2* was used as an internal control for calculation of relative gene expression level. Data represent means ± SD of biological triplicates.



Supplemental Figure S21. The expression analysis of *BI-1* in Col, *bzip60*, *ire1a ire1b*, and *nrpD*. Seven-day-old seedlings were treated with 1 μg/mL TM (DMSO as mock) and collected for RNA extraction for indicated time. The *TUB2* was used as an internal control for calculation of relative gene expression level. Data represent means ± SD of biological triplicates. Asterisks indicate significant differences compared with Col (Student's *t*-test, * $P < 0.001$).

Supplemental Tables

Supplemental Table S1. Primers used in this study.

The restriction sites used for gene cloning are marked in bold.

Primers for quantitative RT-PCR	
Gene name	**Primers sequence (5'-3')**
NRP1QPCR-F	CTTTTTGGCAGTTCAGCGAC
NRP1QPCR-R	TGGTGGCGGCTATGTCTAAG
NRP2QPCR-F	GACAGCTTCTGGCAATTAGG
NRP2QPCR-R	TCAGTGTAACCTTTGGAGAGAT
SEN4QPCR-F	CAATCCTCTGGAACCCTCAAAG
SEN4QPCR-R	GTGTACCAATTGGAGTTTGGTTTCG
SAG12QPCR-F	ACTGGTTTCAAAGGTGTCTCGGCAT
SAG12QPCR-R	ACGCCCAACAACATCCGCAGC
MC1QPCR-F	CAAGTGCATGCGTCACCTTC
MC1QPCR-R	TGGAAGACAAGTGAGTCGCC
MC2QPCR-F	TTTCACTTCTCCGGTCACGG
MC2QPCR-R	CACCTGAAGTCCTGTGGTCC
MC3QPCR-F	CTAGTGGACATAGCTCGCGG
MC3QPCR-R	CGCCGCATAAAACTGCTCTC
MC4QPCR-F	TGCCAAACCGATCAGACCTC
MC4QPCR-R	ACCTGGCTGCTGAGTAAACC
MC5QPCR-F	ATTACCCTGGAACCAAGGCG
MC5QPCR-R	TCACCCGGTTTAGCGGATTC
MC6QPCR-F	GATAAGCCTCGTCGAACGGT
MC6QPCR-R	TCACCCGATTTAGCCGGTTC
MC7QPCR-F	GAGAGTACCACGACGAAGCC
MC7QPCR-R	GATCTCGCGCGTCTCTACAT
MC8QPCR-F	TGGAGTTACGTGGCTGTGTC
MC8QPCR-R	AATCGCCAGATTGCCCTGAT
MC9QPCR-F	TGATGGCAAGGGATGTGCTT
MC9QPCR-R	GGTTGAGAAAGGAACGTCGC
NAC89QPCR-F	GAAGCGGAAGATGGATGGCT
NAC89QPCR-R	CACGCGCACAGAAGAAGAAC
TUB2QPCR-F	ATCCGTGAAGAGTACCCAGAT
TUB2QPCR-R	AAGAACCATGCACTCATCAGC
Primers for constructs	
Construct name	**Primers sequence (5'-3')**
35S:NRP1-6HA-F	GGC**GAATTC**ATGGAGTATAATAACAACAATCAGC

35S:NRP1-6HA-R	TAA**CCCGGG**AGGGTTTTGGTCAGCAAAAAT
35S:NRP2-6HA-F	TCA**AAGCTT**ATGGACAGCTTCTGGCAAT
35S:NRP2-6HA-R	TAT**CTGCAG**TGCAGAACCAGCTTGTTCG
pNRP1:NRP1-mVenus-F	GGC**GAATTC**ATGGAGTATAATAACAACAATCAGC
pNRP1:NRP1-mVenus-R	TAA**CCCGGG**AGGGTTTTGGTCAGCAAAAAT
pNRP2:NRP2-mVenus-F	TCA**AAGCTT**ATGGACAGCTTCTGGCAAT
pNRP2:NRP2-mVenus-R	TAT**CTGCAG**TGCAGAACCAGCTTGTTCG
pNRP1:LUC-F	GGC**GAATTC**AATGATCAAACAACTCT
pNRP1:LUC-R	CGC**ACTAGT**CTCTAACTCTCTGATTGATCT
pNRP2:LUC-F	CGG**CGAATT**CTACTTTTGTACTACGAAT
pNRP2:LUC-R	TTA**CCCGGG**ATCTCAAAAGGCTAG
35S:bZP60ΔC-FLAG-F	CCC**GGATCC**ATGGCGGAGGAATTTGGAAG
35S:bZP60ΔC-FLAG-R	ACGC**GTCGAC**AGACTCCTGCTTCGACATC
35S:FLAG-bZIP60-F	CCC**GGATCC**ATGGCGGAGGAATTTGGAAG
35S:FLAG-bZIP60-R	TAC**TCTAGA**CGCCGCAAGGGTTAAGATTTG
His-bZIP60-F	CCC**GGATCC**ATGGCGGAGGAATTTGGAAG
His-bZIP60-R	ACGC**GTCGAC**AGACTCCTGCTTCGACATC
Primers for ChIP assays	
Gene name	**Primers sequence (5'-3')**
ACT2-F	ACTCGTTTCGCTTTCCTTA
ACT2-R	CGGATCTAGAGACTCACCTTG
PP2A-F	TATCGGATGACGATTCTTCGTGCAG
PP2A-R	GCTTGGTCGACTATCGGAATGAGAG
NRP1P1-F	AACTGCTGTGAAACCTCCATTAG
NRP1P1-R	ACTTTAAGCCCATTCAATTACTGAT
NRP1P2-F	ACGCGTTTTTGGTGTTTGGT
NRP1P2-R	AGCATGTAGCAGCAGAGACG
NRP1P3-F	TGTGCTATTTTTGTGTGGGTGG
NRP1P3-R	GGGCAAGAAGTGAATAGGAGC
NRP2P1-F	TGAAGAATAAGTCAGCAATCGTAGA
NRP2P1-R	CTTTAAAAACAAAGTCTCTGCAGTC
NRP2P2-F	AAGATGAGGGGGTATTTGGGTAA
NRP2P2-R	GTTTCCTCGAAATCCGGCG
NRP2P3-F	TGTGTTCAGTTGTTGATTAGGGTTT
NRP2P3-R	ATCTCAAAAGGCTAGCATAAACAGA

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Supplemental Table S2. Probes used in this study.

The lower-case letters represent mutated bases; underlines represent the sequences of native/ mutated UPRE element.

Probes for EMSA	
Probe name	**sequence (5'-3')**
NRP1 probe	CGGAACAATTGCAATTATGACGTGGCAGACTTTTGAGAGG
NRP1-Mut1 probe	CGGAACAATTGCAATTAT**ctgca**GGCAGACTTTTGAGAGG
NRP1-Mut2 probe	CGGAACAATTGCAATTATGACGTG**t**CAGACTTTTGAGAGG
NRP2 probe	CTCTCATTCTCTCTTCTGACGTGTTTCCCTTCTTCGGTTT