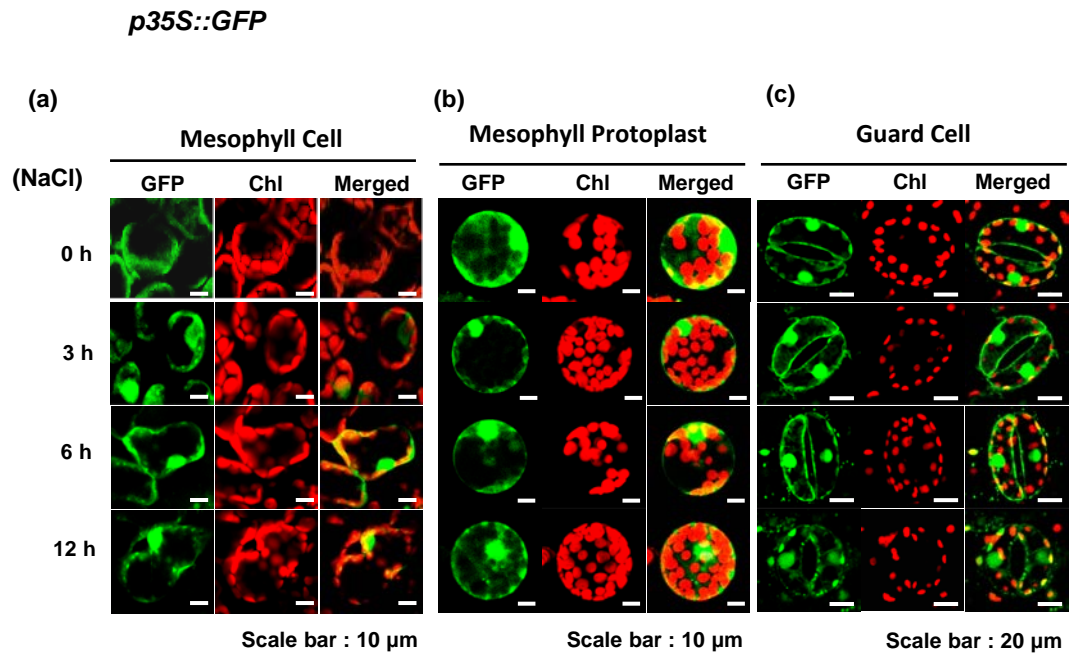**Figure S1**

p35S::GFP

Figure S1. Green fluorescent protein (GFP) fluorescence localization and immunoblot analysis in the cellular compartments of *p35S::GFP* transgenic plants under salt stress. (**a–c**) Confocal laser scanning microscopy (CLSM) images of (green) GFP fluorescence were observed in (a) mesophyll cells, (b) mesophyll protoplasts, and (c) guard cells of 6-week-old plants exposed to salt stress with 200 mM NaCl. CLSM images of (green) GFP fluorescence and (red) chlorophyll autofluorescence from chloroplasts are merged in the third column.
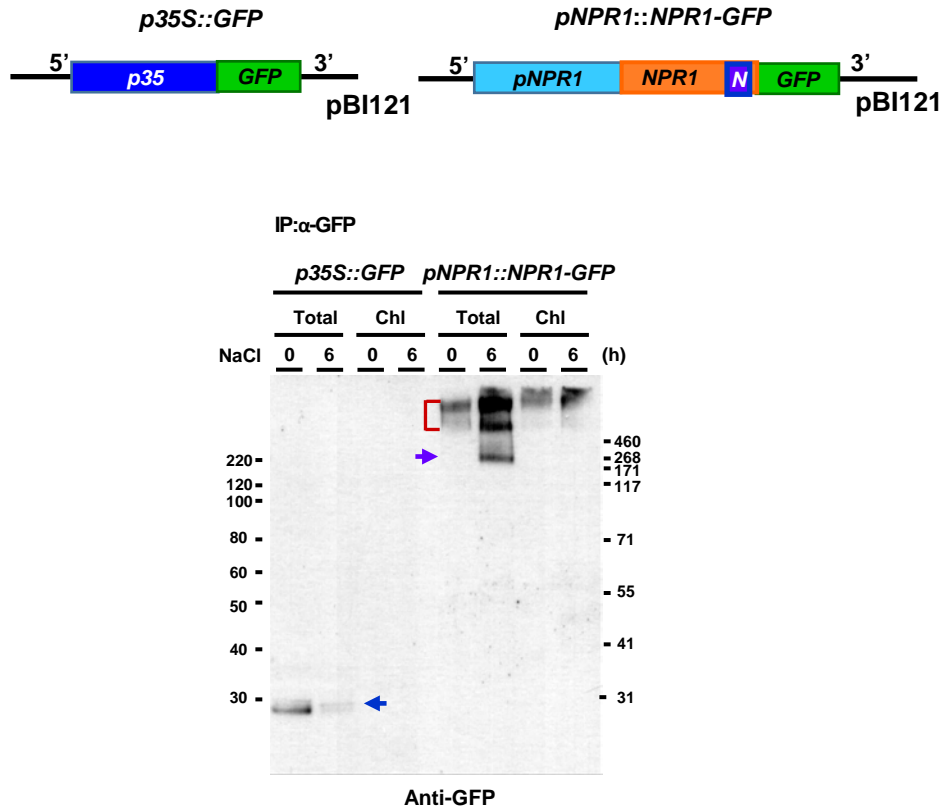
Figure S2

Figure S2. Immunoblots of the total and chloroplast protein fractions from *pNPR1::NPR1-GFP* under salt stress. After immunoprecipitating the protein fractions using GFP-Trap agarose, western blotting with anti-GFP antibody was performed. GFP-tagged nonexpressor of pathogenesis-related gene 1 (NPR1-GFP) oligomer (high molecular weight) and dimer (low molecular weight) are indicated with red square brackets. Purple arrow indicates GFP dimer. Blue arrow indicates GFP only in total protein fraction in *p35S::GFP* transformants.
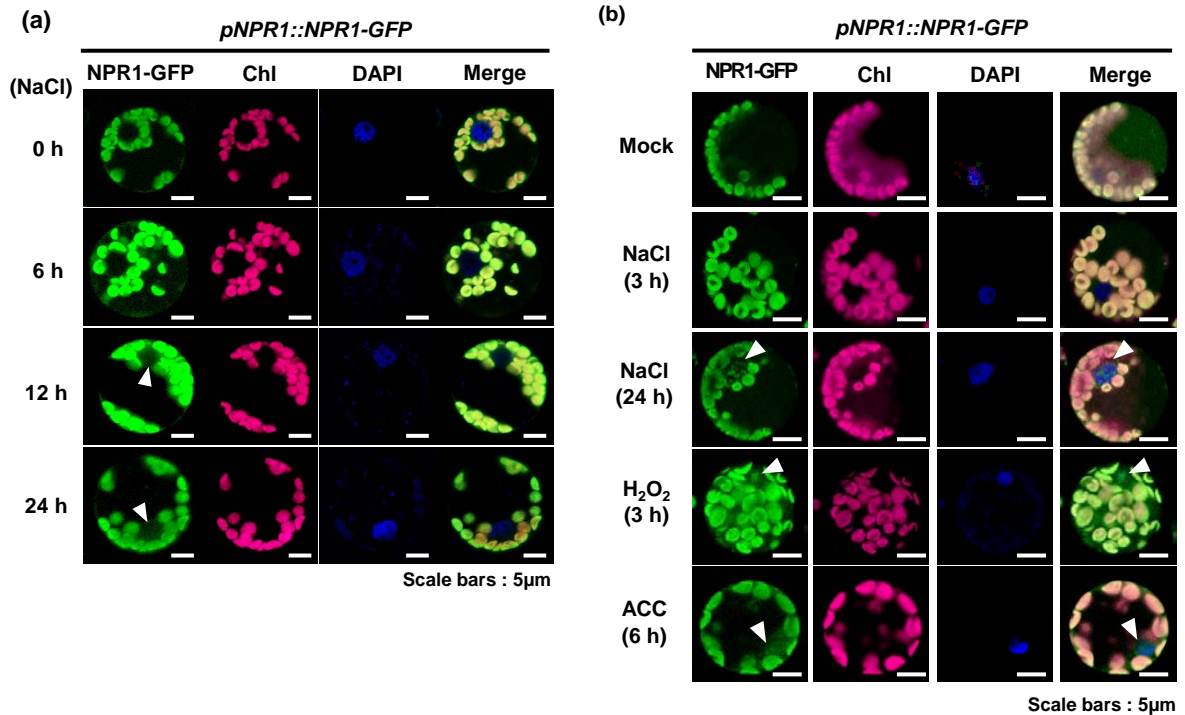
Figure S3

Figure S3. CLSM images of GFP fluorescence on mesophyll protoplasts after treatments with salt stress, H_2O_2 or ACC. **(a)** CLSM images of GFP fluorescence in mesophyll protoplasts from 6-week-old *pNPR1::NPR1-GFP* transgenic plants exposed to salt stress using 200 mM NaCl. (**b**) CLSM images of GFP fluorescence in mesophyll protoplasts from *pNPR1::NPR1-GFP* transgenic plants treated with 200 mM NaCl, 5 mM H_2O_2, or 1 mM 1-amino-1-cyclopropane carboxylic acid (ACC). Images of (green) GFP fluorescence and (magenta) chlorophyll autofluorescence from chloroplasts are merged in the fourth column. DAPI staining (blue) images are presented in the third column.
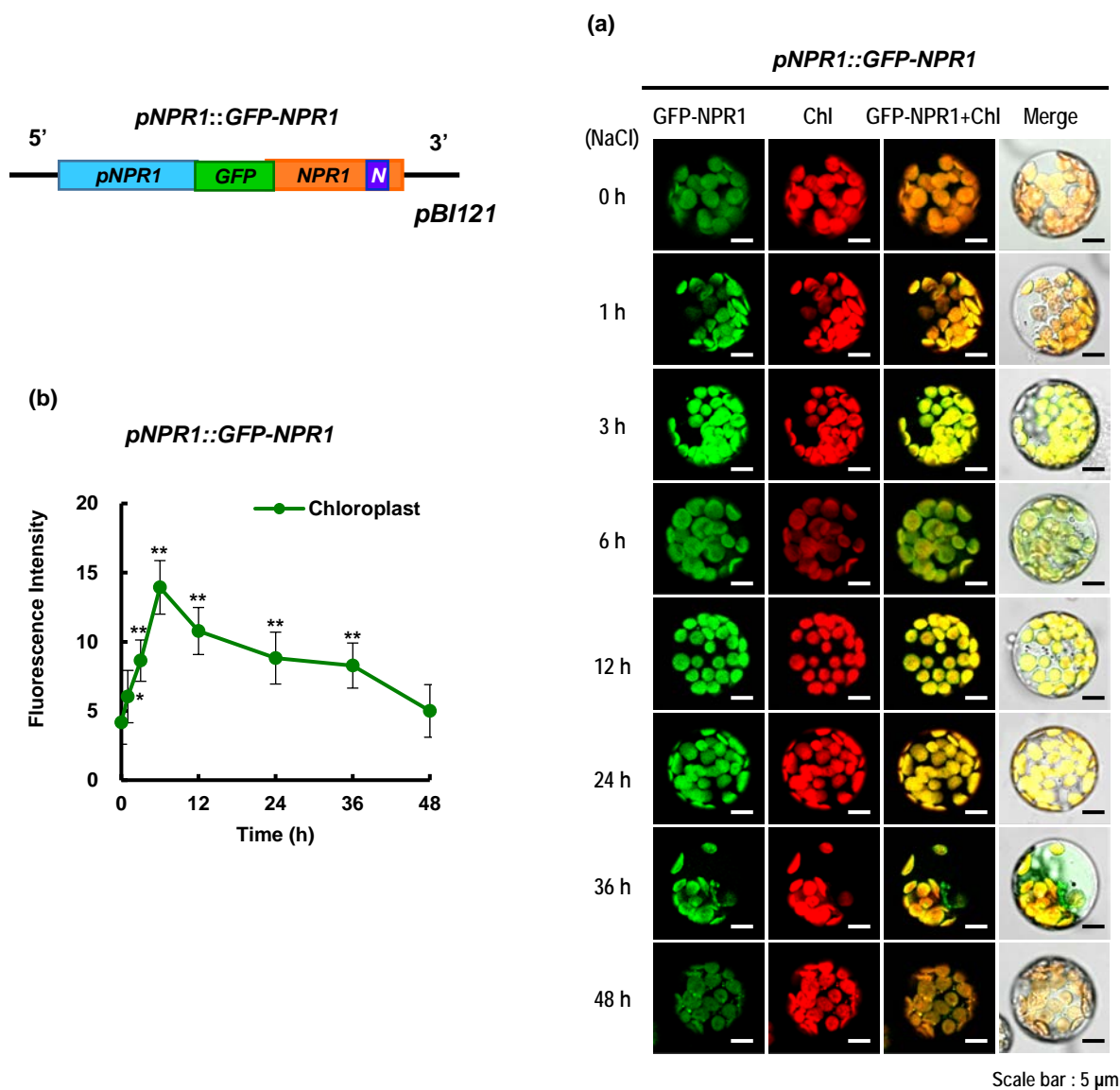
Figure S4

pNPR1::GFP-NPR1

(a)

pNPR1::GFP-NPR1

(NaCl)	GFP-NPR1	Chl	GFP-NPR1+Chl	Merge
0 h				
1 h				
3 h				
6 h				
12 h				
24 h				
36 h				
48 h				

Scale bar : 5 μm

(b)

pNPR1::GFP-NPR1

Figure S4. NPR1-GFP localization in salt stress. (**a**) GFP fluorescence localization in cellular compartments of mesophyll protoplasts after transient expression with *pNPR1::NPR1-GFP* construct. Confocal laser scanning microscopy (CLSM) images of (green) GFP fluorescence and (red) chlorophyll autofluorescence from chloroplasts are merged in the third column. The bright-field image is merged into the fourth column. (**b**) GFP intensity was quantified in the chloroplasts after transient *pNPR1::NPR1-GFP* (green line) expression under salt stress (n=30). Data are expressed as mean ± SD. An asterisk indicates a significant difference from 0 h (one asterisk (*P*<0.05) or two asterisks (P < 0.01)).

Figure S5

pNPR1::NPR1-GFP

pNPR1:: NPR1(△5'35aa)-GFP
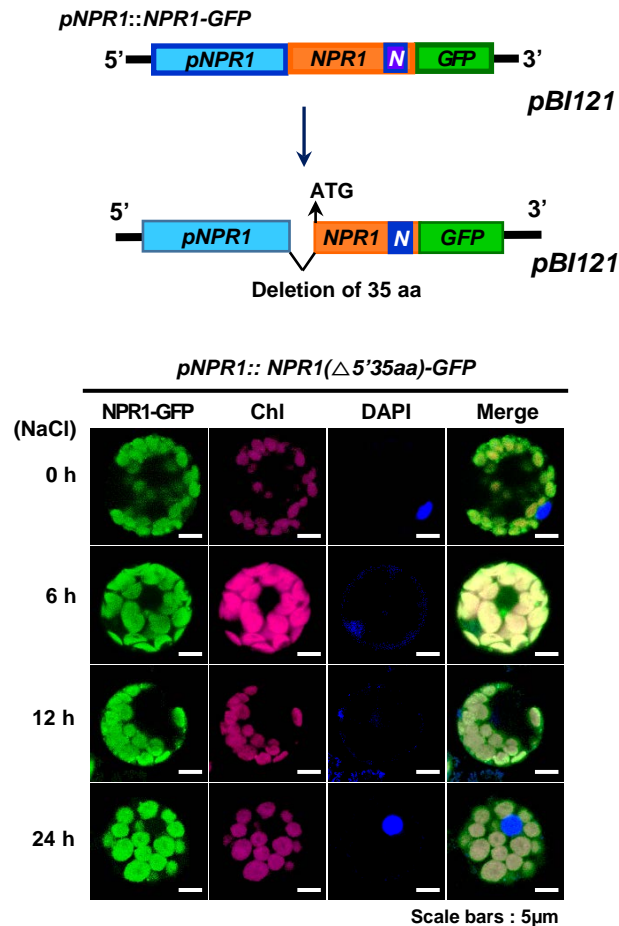
Scale bars : 5µm

Figure S5. Subcellular localization of *NPR1(△5'35aa)*-GFP. GFP-tagged nonexpressor of pathogenesis-related genes 1 (GFP–NPR1) localization in intracellular compartments of mesophyll protoplasts after transient *pNPR1::NPR1(△5'35aa)-GFP* expression. After deleting the N-terminal 35 amino acids from the NPR1 open reading frame, a deletion construct with the ATG start codon was constructed. GFP fluorescence localization in mesophyll protoplasts after transient *pNPR1::NPR1(△5'35aa)-GFP* construct expression. Confocal laser scanning microscopy (CLSM) images of (green) GFP fluorescence and (magenta) chlorophyll autofluorescence from chloroplasts are merged in the third column. The bright-field image is merged into the fourth column. Scale bars = 5 µm.
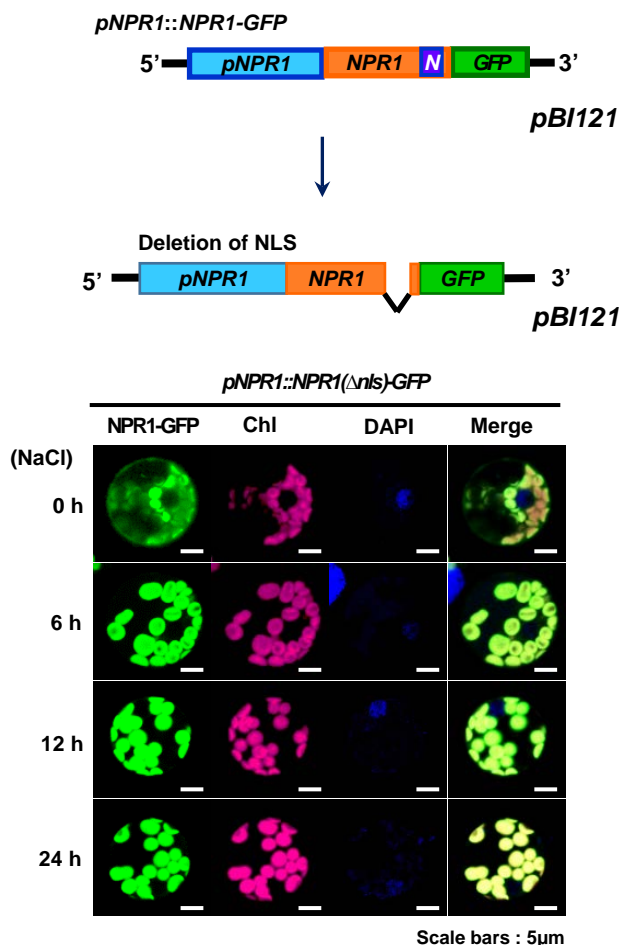
Figure S6

Figure S6. Subcellular localization of nuclear localization signal (NLS)-deleted NPR1-GFP mutant. (**a**) Deletion constructs of the NLS within the NPR1 open reading frame were subcloned in frame with a C-terminal GFP and under the control of native NPR1 promoter. NLS-deleted constructs were transiently expressed in mesophyll protoplast of wild-type (WT) tobacco plants. (**b**) CLSM images of GFP fluorescence in mesophyll protoplasts from plants transiently expressing *pNPR1::NPR1(ΔNLS)-GFP* under salt stress. Images of (green) GFP fluorescence and (magenta) chlorophyll autofluorescence are merged in the fourth column. DAPI staining (blue) images are presented in the third column. N indicates NLS.

Figure S7

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NtNPR1   ----MDNSRTAFSDSNDISGNSSICCIGGGMTEF---FSPETSPAEITSLKRLSETLESFDASLPEFD-YFADAKLVVPGA--DKEIPVHRCILSRSPFFKNLFCGK--------KE  101
AtNPR1   ---MDTTIDGPADSYETSSTSFVATDNTDSSIVYLAAEQVLTGPDVSALQLLSNSFESVFDS--P--DDFYSDAKLVLSD----GREVSFHRCVLSRSPFFKSALAAAKKEK--DSNN  106
CaNPR1   -----MDSRTAFSDSNDISGSSSICCIG-GMTES---FSPETSPAEITSLKRLSEILESFDSSPDFD-FFADAKLVVPI-----GKEIPVHRCIISRSPFFKNVFCGK--------ER   98
VvNPR1   ---MDYRAALSDSNDISGSSSICCIAATTESL-----SSEVSFPDISALRRLSENLESVPES--FEFD-FFTDARIVV-AG---GRFVPVHRCILARSVPFFAVLAGA--------RK   96
TcNPR1   -----MDNRNGFSDSNEISNNSSTCCIAAATNSETLASSEPLNTPDIAALQILSRNLESVPES--TDSFSLYSDAKIGL-SS---GREVPVHRCILSRSSVFKTVFSGL---------K  100
OsNPR1   MEPPTSHVTNAFSDSDSA---------------SVEEGGADADADVEALRRLSDNLAAAFRS--PEDFAFLADARIAVPGGGGGGDLLVHRCVLSRSPFLRGVFARRAAAAAGGGGE  102
AtNPR3   -----MATLTEPSSLSFTSS-HFSYGSIGSNH---FSSSSASNPEVVSLTKLSSNLEQLLSN--S--DCDYSDAEIIVD----GVPVGVHRCILARSKFFQDLFKKEKK----IS-K   97
AtNPR4   ----MAATAIEPSSISFTSS-HLSNPSPVVTT---YH-------SAANLEELSNLEQLLTN--F--DCDYTDAEIIIEEE---ANPVSVHRCVLAARSKFFLDLFKKDKD----S--S   92
GmNPR1   -----MAYSAEPSSSLSFTSSHLSNGSVSHNICPSYGSDPGPNLEAISLSKLSSNLEQLLIE--P--DCDYSDADLVVE-----GIPVSVHRCILASRSKFFHELFKREKG----SSEK  102
MhNPR1   -----MAHSAEPSSSLSFTSSPHLSNGSISHNLSC-SGSESVPSLEVISLSKLSSSLEQLLID--P--GCDYSDADIVVE-----GIPVGVHRCILASRSGFFRELFRREKG----SSGK  101
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NtNPR1   KNSSKVELKDV----MKEYEVSYDAVVSVLAYLYSGKVRPSPKDVCVDNECSHVACRPAVAFLVEVLYTSFTFQLSEAVDKFQRHLLDILDKAAADDVMMVLSVANICGKACDRLLSS  217
AtNPR1   TAAVKPEPKEI----ARDYEVGFDSVVTVLAYVYSSRVRPPPKGVSECADENCCFVACRPAVDFMLEVLYLAFIFKPELIITLYQRHLDLDVVDKVVIEDTLVIGKLANICGKACMKLLDR  222
CaNPR1   K--TKPELKEL----MKEYEASYDALVNVLAYLYSGKVRPSPKDVCVCDNECFHVACRPAVAFLVQVLYASFTFQLSEAVDKFQRHLGLDILNKAAADDVMMVLSVANICGKACERLLSS  212
VvNPR1   EKEAKPELKDL----AKPFDVGDSLVAVLGYLYSGRVGALPKSVCACVDDDCPHSACRPAVDEVMEVLYASFAFQLSEAVGLYQRRLMDILDKVASDDILVIGKVANLCGKACPRLLAR  212
TcNPR1   DRGAKPELKEL----ARDYEIGYDSLVAVLAYLYSGRVRSLPRGVCVCVDDDCFHLACRPAVDEVAEVLYAAFTFQVSEAISLYQRHLLDIKVEMDDILVLYVANMCGNTCERLLAK  216
OsNPR1   DGGERLELRELLGGGGEEVEVGYEALRLVLDYLYSGRVGDLPKAACLCVDEDCAEVGCHPAVAFMAQVLFAASTFQVAEITNLFQRRLLDVLDKVEVDNLLLILSVANLCNKSCMKLLER  222
AtNPR3   TEKPKYQLREMLPY----GAVAHEAFLYFLSYLYYTGRLKPFFLEVSTCVDPVCSHDCCRPAIDIVVQLMYASSVLQVPEVSSFQRRLCNFVEKTLVENVLPIIMVAFNCKL--TQLLDQ  211
AtNPR4   EKKPKYQMKDLLPY----GNVGREAFLHFLSYIYTGRLKPFFIEVSTCVDSVCAEDCCKPAIDFAVELMYASYVFQIPDLVSSFQRKLRNYVEKSLVENVLPILLVAFHCDL--TQLLDQ  206
GmNPR1   EGKLKYNMNDLLPY----GKVGYEAFLIFLGYVYTCKLKPSPMEVSTCLDNVCADACRPAINFAVRLMYASSIFQLPEAVSLFQRRLLNFIGKALVEDVIPILTVAFHCQS--NQLVNQ  216
MhNPR1   EDRPKYCMSDFLPY----GDVGYEAFLVFLSYVYTCKLKPSPVEVSTCVHNVCAEDACRPAINGVMBLMYAASIFQMPDLVSIFERRLLNFVGKALSDNVVPILLVAFHCQL--NQLIDQ  215
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NtNPR1   CIEIIVKSNVDIILDKALPHDIVKQITDSRAELGLQGPES----NGFPDKHVKRIHRALDSDDVELLQMLLREGHTTLDDAYALHYAVAYCDAKTTAELLDLALADVNHQNSRCYTVLH  333
AtNPR1   CKEIEIVKSNVDMVSLEESLPEELVLEIIDDRRKELGLEVPKV--------KKKVSNVHKAIDSDDHELVKLLLKEDHTNLDDACALHFAVAYCNVKLATDLIKLDLADVNHRNPRCYTVLH  334
CaNPR1   CIEIIVKSNVDIITLDKALPNDIVKQITDSRTELEIDLQGPVN----HGFPDKHVKRIHRALDSDDVELLRMLLKEGHTTLDDAYALHYAVAYCDAKITSELIDLALADVNHQNPRCYTVLH  328
VvNPR1   CIDIIIKSDVDLVALERALPQEMVKQITDSRLELGFEEPES----TNFPDKHVKRIHRALDSDDVELVRMLLKEGHINLDEASALHYAVAFGDAKITTELIDLGLADVNHKNHRCYTVLH  328
TcNPR1   CIETLVKSDVDIVTLDKALPYHTVKQIMDSRLELGIDKPEN----TGFPDKHVKRIHRALDSDDVELARMLLKEGHTNLDEASALHYAVAYCDAKITTELIDLGLADVNRRNSRCYTVLH  332
OsNPR1   CLDMVVRSNLDMIILEKSLPPDIVKQILDLARLSLGLISPEN---KCFPNKHVKRIHRALDSDDVELVRMLLTEGQLNLDDAFALHYAVNEHCDKTLTELLDLALADVNHRNPRCYTVLH  338
AtNPR3   CIERVARSDLYRFCIELEVPPEVAEKLIKQLRLISPQDEETSPKI-SEKLLERIGKILKALDSDDVELVKLLLTESDITLDQANGLHYSVVYSDPKVVAELIALDMGDVNYRNSRCYTVLH  330
AtNPR4   CIERVARSDLDRFCIEKELPLEVLEKIKQLRVKSV----NIPEV-EDKSIERTGKVLKAIDSDDVELVKLLLTESDITLDQANGIHYAVAYCDPKVVTQVFDLDMADVNFRNSRCYTVLH  321
GmNPR1   CIDRVARSDLQISIDQELPHELSQKVKLLRRKPQQDVENDASVVDALSLKRITRTHKAIDSDDVELVKLLLNESDITLDEANALHYAAYCDPKVVSEVLGLGLANVNLRNSRCYTVLH  336
MhNPR1   CVDRVARSDINDISLERGLPDEVVKTKILRRNYQQDSDPNLSPADPLHEKRIRRTHKAIDSDDVELVKPLLTESNITLDEANALHYAAYCDPKVVTEVIALGLADVNLRNSRCYTVLH  335
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NtNPR1   VAAMRKEPKTIVSLLTKGARPSDLTSDCRKAIQILKRLTRLVDFSKSPEEGKSASKDRLCTEILEQAERRDPLII-GEASVSLAMAGDDLRMKLLYLENRVGLAKLIFPMEAKVAMDIAQV  452
AtNPR1   VAAMRKEPOLILSLLEKGASASEALGLEGRTALMIAEQATMAVECNNIPEQCKHSLKGRLCVEILEQEDKREQIP-RDVPPSFAVKADELKMTLLDLENRVALAQRLLFPTEAQAAMEIAEM  453
CaNPR1   VAAMRKEPKIIVSLLTKGARPSDLTSDCRKALQILKRRALRLVDEIKSTEEGKSASKDRLCTEILEQAERRDPLII-GEASVSLAMAGDDLRKLLYLENRVGLAKLIFPNEAKVAMDIAQV  447
VvNPR1   IAAMRKEPKIIVSLLTKGARPIDFIPDDRNAIQTAKRLTRAVDYHKSTEEGKPSPKDLCVEVEQAERRDPLI-GEASSLAIAGDDLRMKLLYLENRVGLAKLLFPMEAKVAMDIAQV  447
TcNPR1   VAAMRKEPKIIVSLLTKGARPSDLTLDCRKAFQISKRLTRAADYYMSTEEGKASPKDRLCVETLEQAERRDPLII-GEASLSLAMAGDDLRKLLYLENRVGLAKLLFPMEAKVAMDIAKV  451
OsNPR1   IAARFREPKIIVSLLTKGARPADVVFDCRKAAEAAQEQMKQVDLPYGVTTQGGKRIPSPKDRLCTEILEQAERRDPDOL-GEASVSLAMAGESLRGRLLYLENRVALARIMPMEARVAMDIAQV  457
AtNPR3   FAAMKREPSIILSIIDKGANASEFTSDCRSAVNTLRRLTRNPKDYHTKTAKGRESSKARLCTDILEREIRKNPMVL-DTPMCSISMPEDLQMRLLYLEKRVGLAQLIFSPTEAKVAMDIGNV  449
AtNPR4   IAAMKREPTIIIPLDQKGANASEFEFDCRSAVNICRRLTRPKDYHTKTSR-KEPSIYKQLCIDILEREIRRNPLVSGDTPTCSHSMPEDLQMRLLYLEKRVGLAQLLFPAEAKVAMDVANV  440
GmNPR1   IAAMKREPSIIVSLLTKGACASDLTFDCRKSAVSICRRLTRPKDYHAKTEQGKETNKDRICIDVLEREMRRNPMA-GDACMSSHTMADDLHMKLLYLENRVAFARLFPSEAKLAMDIAHA  455
MhNPR1   IAVMRKEPSIIVLLLTKGARASELTSDCQSAVSICRRLTRPKDYHSKAEQOGQEANKDRICIDVLEREMRRNPMA-GDASISSQIMPDDLHMELLNLENRVALARLFPAEAKLAMVIAHA  454
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NtNPR1   DGTSEFPLASI-GK-KMANAQRTTVDLNEAPFKIKEEHLNRLRALSRTVELGKRFFPRCSEVLNKIMD---ADDLSEILYMGNDTAEERQLKKQRYMELQEIILSKAFTEDKEEFD-KTNH  566
AtNPR1   KGTCEPIVTSL-EPDRLTGTKRTSPGVKIAPFRILPEHQSRKALSKTVELGKRFFPRCSAVLLDQIMN---ADDLSEILMGNDTAEKRLQKKQRYMELQEILKKAFSEDNLPLG-NSSL  568
CaNPR1   DGTSEFPLASI-RK-KMADAQRTTVDLNEAPFKMKEEHLNRLMALSRIVELGKRFFPRCSEVLNKIMD---ADDLSEILYMGNDTFEERQLKKQRYMELQEIILKAFTEDKEEFA-KIN-  560
VvNPR1   DGTSEFTTAI-RPRNLADAQRTTVDLNEAPFRIKEEHLNRFRALSRTVDLGKRFFPRCSEVLNKIMD---ADDLSDLAYLGNGTEERLLKKRRYKELQDQLGKAFNEDKEEND-KSRI  562
TcNPR1   DGTSEFTLASI-NSNKLNDAQRTTVDLNEAPFRIQEEHLNRLRALSKTVELGKRFFPRCSEVLNKIMD---ADDLSQLACGNDTPEERLVKKQRMVELQODVLSKAFNEDKMEFD-RSTI  566
OsNPR1   DGLLEFNLGSG--ANPPPERQRTTVDLNESPFIMKEEHLARMTALSKTVELGKRFFPRCSNVLDKIMD---DET--DPVSLGRDTSA----EKRKRFHDEQDVLQKAFHEDKEEND-RSGL  566
AtNPR3   EGTSELTGLS---PPSSGLTGNLSQVDLNETPHMQTQRLLTRMVALMKTVETGRRFFPYGSEVLDKTVMAEYIDDILDDFHFEKGSTHERRLKRMRYRELKDDVQKAYSKDK--VARSCL  567
AtNPR4   EGTSECTGLLTPPPSNDTTENLGKVDLNETPYVQTKRMLTRMKALMKIVETGRRFFPSCYEVLDKTVMDQYMDEEIPDMSVPEKGKTVKERRQKRMRYNELKNDVKKAYSKDK--VARSCL  557
GmNPR1   ETTSEFAGLS-ASNSKGSNGNLREVDLNETPIVQNKRLLSRMEALTKTVEMGRRVFPHCSEVLDKF----MEDDIPDLFYLEKGQHEQRIKRTRFMELKDVHKAENKDKAEFS-RSGI  569
MhNPR1   ETSE-GA--A-PSSSKGSSGNLMEWDLNETPTVQSKRLHRLEALMKTVRLGRCYFPHCSEVLDKF----IDDDIPHLFYLEPGSSDEQKVKRRRFMELKEEVVQKAFGRDKABCN-LSGL  565
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NtNPR1   LSS-SCSSTSKGVDKENKLPFRK----  588
AtNPR1   TDSTSSTSKSTGGKRSNRKLSHRRR--  593
CaNPR1   VSS-SCSSTSKGVDKPNKLPFRK----  582
VvNPR1   -SS-SCSSTSLGFGRNNSRLSCKK---  584
TcNPR1   SS-SSSSKSIGVSRPNGKLTGSGRGG  591
OsNPR1   -SS-SCSSTSIGAIRPRR---------  582
AtNPR3   SASSSPSSSSIRDDLHNTT--------  586
AtNPR4   SSSSPA--SSLREALENPT--------  574
GmNPR1   SSSSS--SSSLRDSVVHYKARKV----  590
MhNPR1   PSSSS--TTSPKKIGANQKVREP----  586
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Figure S7. Alignment of nonexpressor of pathogenesis-related genes 1 ((NPR1) proteins from different plant species: *Nicotiana tabacum* (NtNPR1, KY402167), *Arabidopsis thaliana* (AtNPR1, ATU76707; AtNPR3, NM_123879.3: AtNPR4, AY785951), *Theobroma cacao* (TcNPR1, HM117159), *Oryza sativa* (Japonica cultivar-group) (OsNPR1, DQ450948), *Glycine max* (GmNPR1, NM001251729), *Capsicum annuum* (CaNPR1, DQ648785), *Vitis vinifera* (VvNPR1, XM002281439), and *Malus hupehensis* (MhNPR1, FJ598141). Two conserved protein–protein interaction motifs, a broad complex, tramtrack, bric a brac/poxvirus and zinc-finger (BTB/POZ)-like domain (pink bar), and an ankyrin-repeat domain (purple bar) are indicated. Nuclear localization signal is indicated with asterisks and a green open box. Two cysteines (Cys521 and Cys529) required for salicylic acid (SA)-dependent transcriptional activation in *Arabidopsis* are indicated in uppercase C in red. The putative NIMIN1 and NIMIN2 binding regions are indicated with asterisks and a black open box. The SA-binding region is indicated with asterisks and a red open box. Specific amino acids playing important roles in SA binding are indicated with red arrows. The conserved region (VDLNE) of ethylene-responsive element binding factor-associated amphipathic repression motif is indicated with a blue bracket. Red box (◼) in *Arabidopsis* NPR1 C156 indicates the redox sensor for oligomerization.

Figure S8
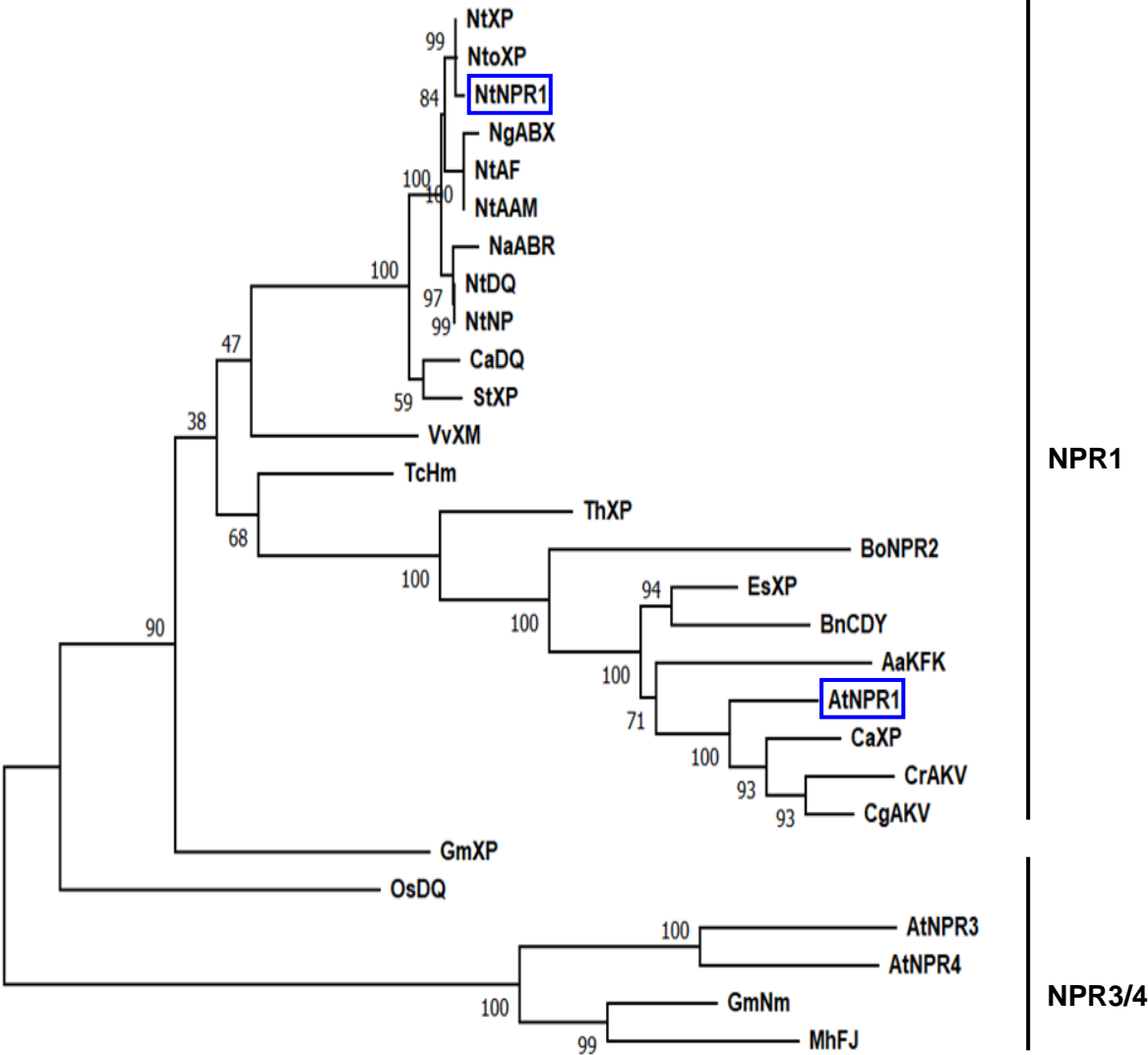
Figure S8. Phylogenetic tree constructed using the neighbor-joining method in MEGA version 11.0 based on the sequences of 28 NPR1 genes from different plants species. Amino acid sequence of each protein was downloaded from NCBI. Each number at nodes is the percentage of 1,000 bootstrap replicate support. Abbreviations and GenBank accession numbers are presented in parentheses after the species name: *Nicotiana tabacum* (NtNPR1, KY402167; NtAF, AF480488; NtAAM, AAM62410.1; NtXP, XP_016503804; NtNP, DQ837218.1 and NtDQ, DQ837218), *Nicotiana glutinosa* (NgABX, ABX71071.1), *Nicotiana tomentosiformis* (NtoXP, XP_009606361), *Nicotiana attenuate* (NaABR, ABR23001.1), *Solanum tuberosum* (StXP, XM_006357647), *Arabidopsis thaliana* (AtNPR1, ATU76707; AtNPR3, NM_123879.3: AtNPR4, AY785951), *Theobroma cacao* (TcHM, HM117159), *Oryza sativa* (japonica cultivar-group) (OsDQ, DQ450948), *Glycine max* (GmNM, NM001251729; GmXP, XP_003534926), *Capsicum annuum* (CaDQ, DQ648785), *Vitis vinifera* (VvXM, XM002281439), *Camelina sativa* (CaXP, XP_010430551), *Capsella rubella* (CrAKV, AKV91676.1), *Capsella grandiflora* (CgAKV, AKV91675.1), *Eutrema salsugineum* (EsXP, XP_006391648.1), *Brassica napus* (BnCDY, CDY34413.1), *Arabis alpine* (AaKFK, KFK36581.1), *Tarenaya hassleriana* (ThXP, XP_010545358.1), *Brassica oleracea* var. *oleracea* (BoNPR2, XM_013780436), and *Malus hupehensis* (MhFJ, FJ598141).

Figure S9

(a)

(b)

Ankyrin Repeats

Zn^{2+}

C

Salicylic-Acid-Binding Domain

BTB Domain

N

SWISS-MODEL
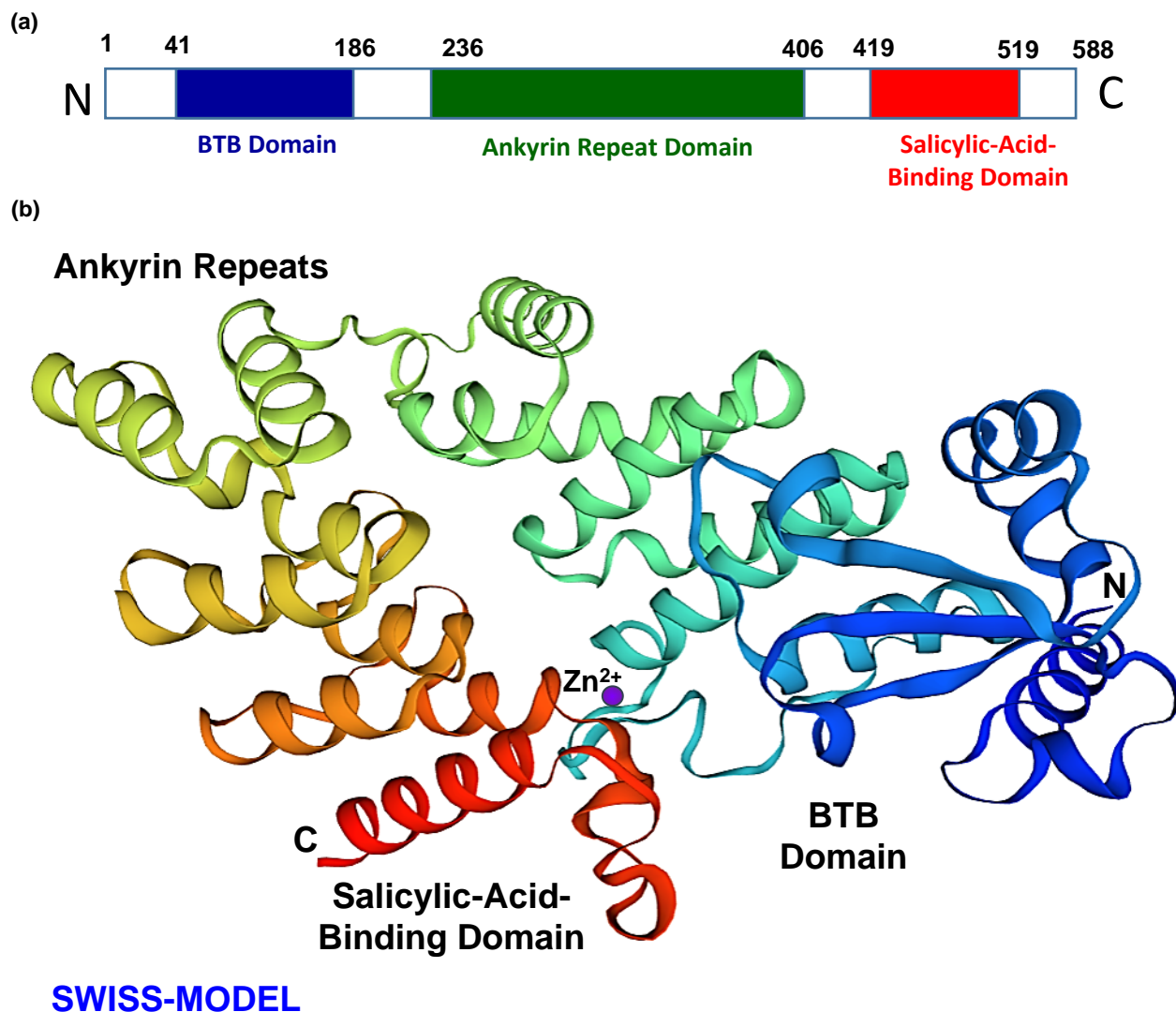
Figure S9. Information of functional domains and homology model NPR1 protein from *Nicotiana tabacum* (NtNPR1, KY402167) for functional analysis. (**a**) Domain map of the NtNPR1 protein to BTB domain, ankyrin repeats domain, and salicylic acid-binding domain using InterProscan for identifying the functional domains and protein families. (**b**) An NtNPR1 homology model (43–405 aa) was created with SWISS-MODEL at the highest score of global model quality estimate with the CryoEM structure of *Arabidopsis* NPR1.

Figure S10

Time

	0 h	6 h	24 h

pNPR1::NPR1-GFP

NaCl

SA

Scale bar : 20 μm
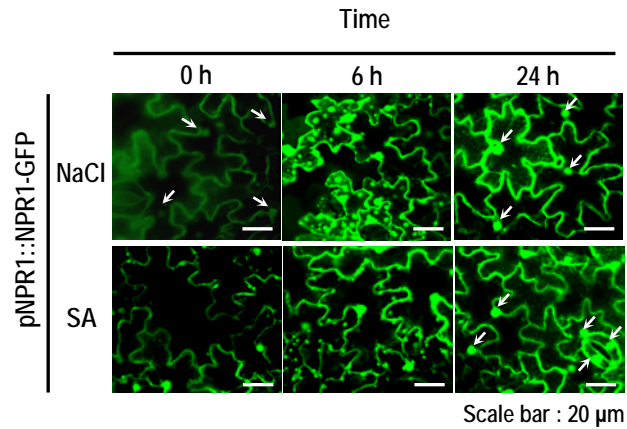
Figure S10. GFP fluorescence in the chloroplasts and nucleus of *p35S::cTP-NPR1-GFP* transgenic tobacco plants under salt stress. GFP-tagged nonexpressor of pathogenesis-related gene 1 (NPR1–GFP) localization in the pavement cells of abaxial leaf epidermis in *pNPR1::NPR1–GFP* transgenic plants under salt stress (upper) and salicylic acid (SA) treatment (middle) and *p35S::cTP-NPR1-GFP* transgenic plants under salt stress (lower). Arrows indicate NPR1 condensates.

Figure S11
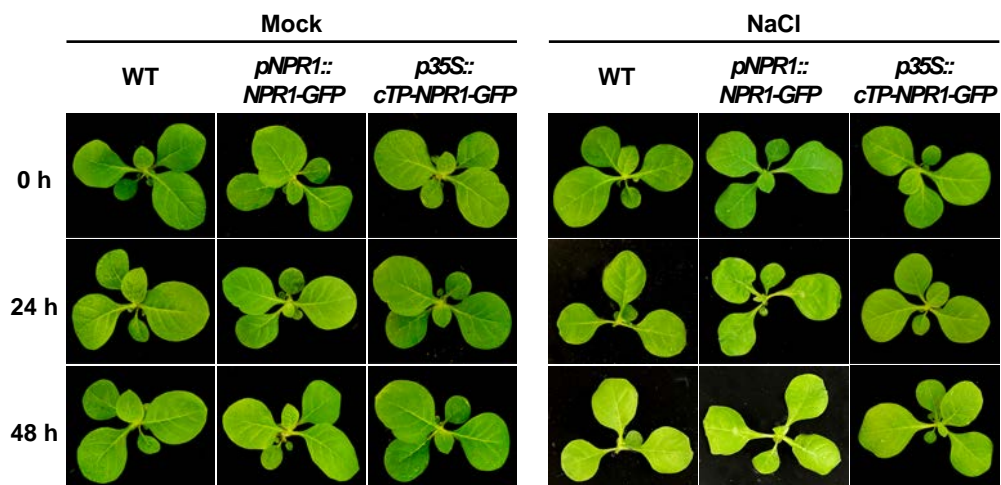
Figure S11. Phenotypes of WT and two transgenic tobacco lines under salt stress. Whole tobacco plants (WT, *pNPR1::NPR1-GFP*, and *p35S::cTP-NPR1-GFP*) were exposed to salt stress for 48 h.

Figure S12

(a)

(b)

NRIP1, WHY1, PRIN2, SAL1, ABI4, RRTF, ZAT10, FC2, EX1, EX2, GLK1, GLK2

Relative Expression Level
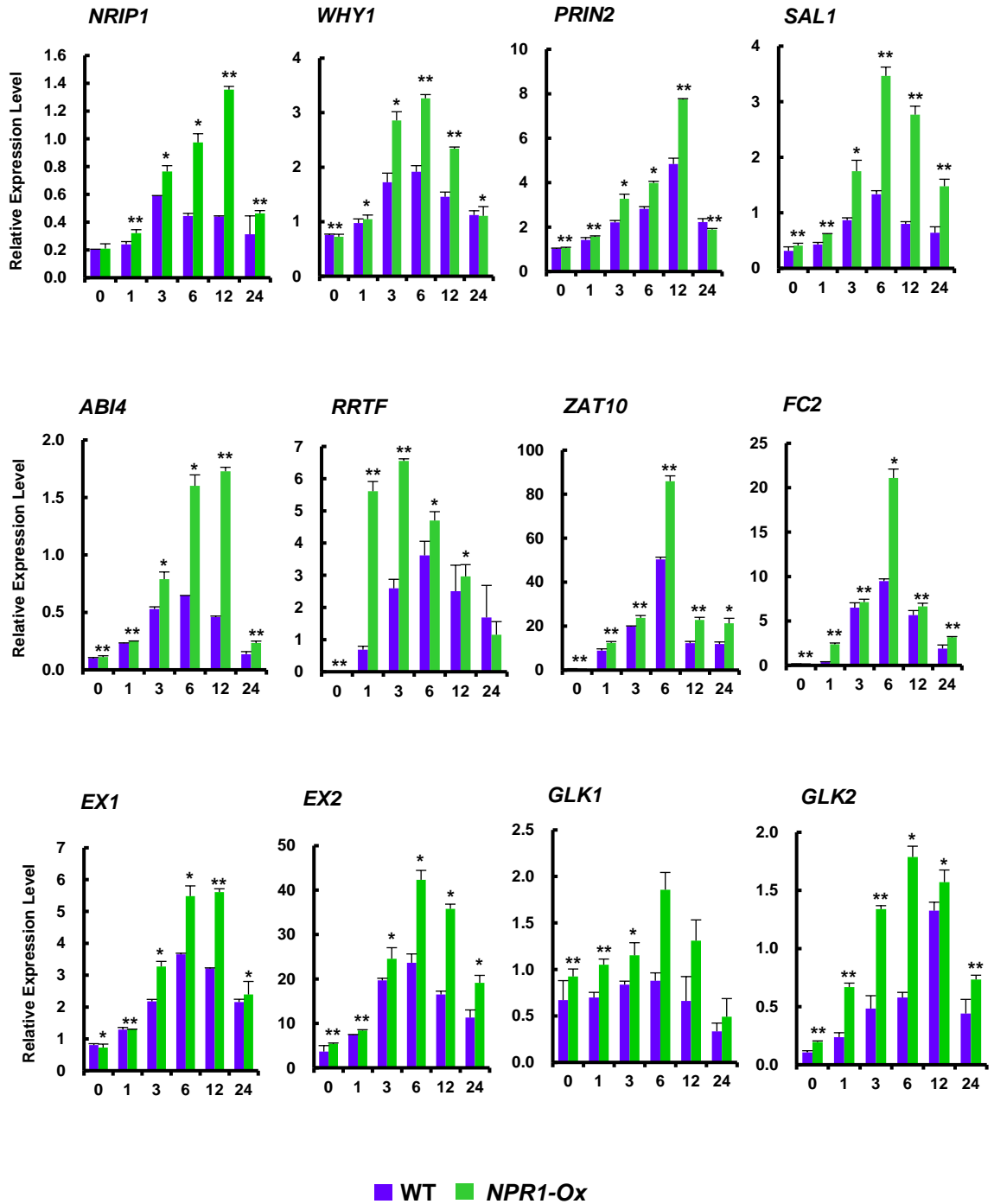
■ WT   ■ NPR1-Ox

Figure S12. Transcription of retrograde signaling-related genes involved in chloroplast development and abiotic/biotic stress response in WT and NPR1 overexpression (*NPR1-Ox*) transgenic tobacco plants under salt stress. Transcription profiles were determined using qRT-PCR in response to salt stress with 200 mM NaCl in WT and *NPR1-Ox* transgenic plant leaves and expressed relative to that of the reference gene β-actin. (**a**) Genes related to retrograde communication for chloroplast development: *GUN1*, genomes uncoupled1; *STN7*, serine/threonine-protein kinase7; *ChlI*, magnesium–protoporyphyrin chelatase subunit; *PC1*, plastoc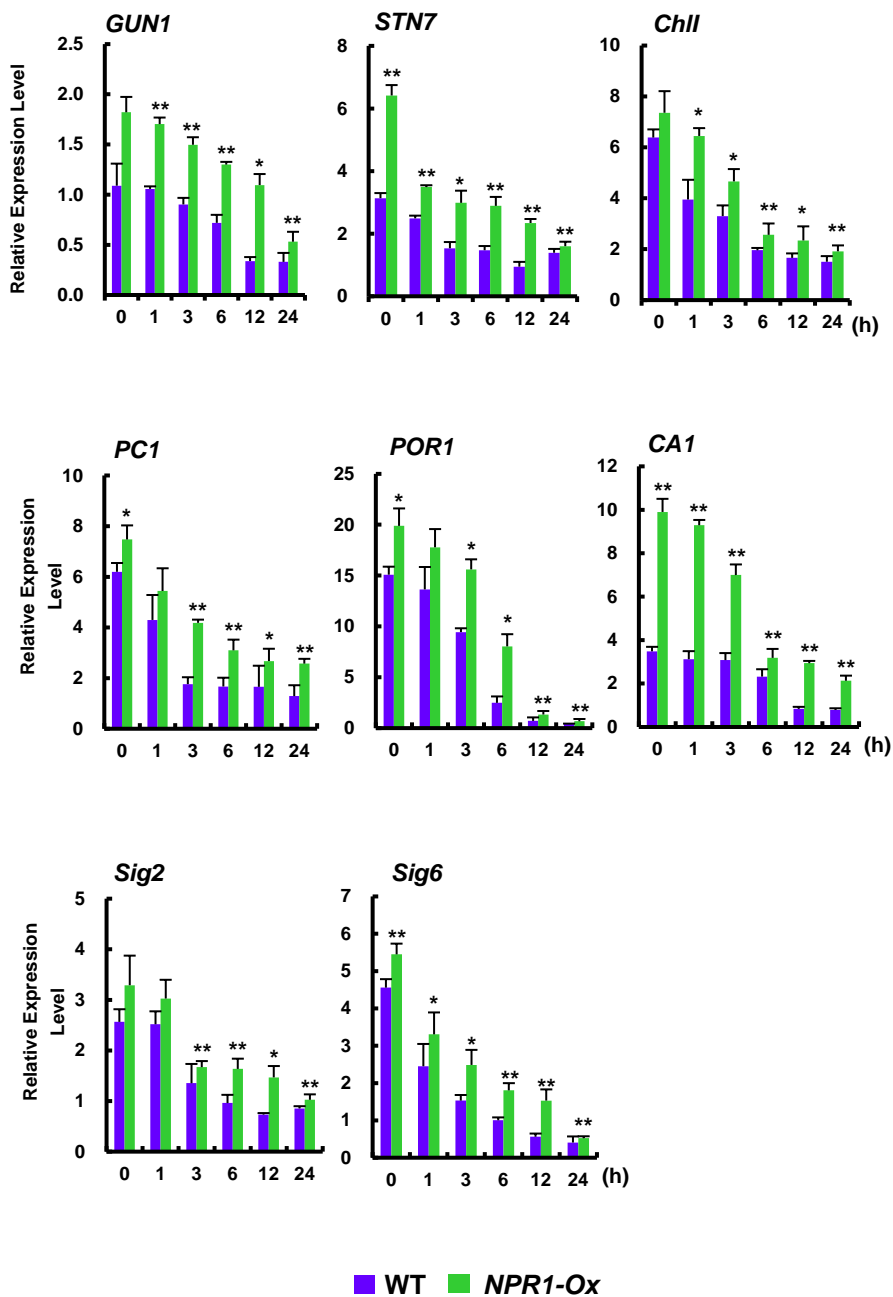yanin; NADPH:*POR1*, protochlorophyllide oxidoreductase; *CA1*, carbonic anhydrase 1; *Sig2*, chloroplast sigma factor 2; *Sig6*, chloroplast sigma factor 6. (**b**) Genes related to retrograde communication for operational signaling in response to biotic/abiotic stress: *NRIP1*, chloroplast NB-LRR receptor-interacting protein 1; *WHY1*, single-stranded DNA-binding protein WHIRLY 1; *PRIN2*, plastid redox insensitive 2; *SAL1*, 3′-phosphoadenosine 5′-phosphate (PAP) phosphatase; *ABI4*, abscisic acid-insensitive protein 4; *RRTF*, redox-responsive transcription factor; *ZAT10*, zinc-finger transcription factor 10; *FC2*, ferrochelatase; *EX1*, executer1; *EX2*, executer2; *GLK1*, golden 2-like1; *GLK2*, golden 2-like 2. Relative transcript expression levels are presented as mean ± SD. Asterisks indicate significant differences between the WT and transgenic plants in each case (*$P<0.05$ or **$P<0.01$).

Figure S13

(a)

(b)

Chloroplast-encoded genes
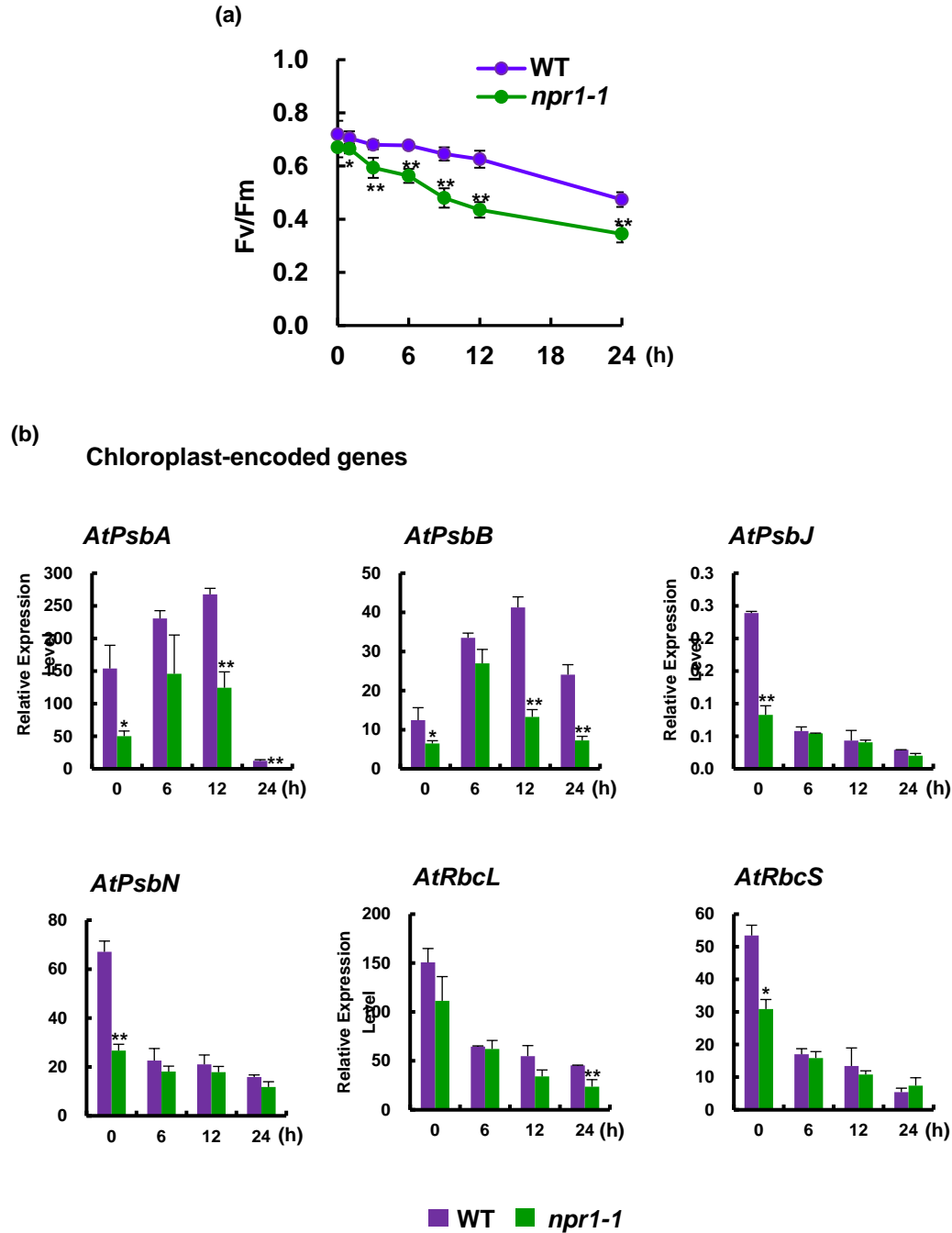
Figure S13. Kinetics of maximal photochemical efficiency and chloroplast-encoded gene transcription in WT and *npr1-1* mutant *Arabidopsis* plants under salt stress. (**a**) Maximal photochemical efficiency of photosystem II (PC II) (Fv/Fm) was measured in WT and *npr1-1* mutant *Arabidopsis* plants under salt stress. Fv/Fm values are expressed as mean ± SD. (**b**) Transcription levels in WT and *npr1-1* mutant *Arabidopsis* plants in response to 200 mM NaCl-induced salt stress were measured using qRT-PCR and expressed relative to that of the reference gene β-actin. Chloroplast-encoded genes: *AtPsbA*, photosystem II reaction center D1 protein; *AtPsaB*, *AtPsbJ*, PC II reaction center protein J; *AtPsbN*, PC II subunit; *AtRbc L*, RuBisCo large subunit; *AtRbc S*, RuBisCo small subunit. Relative transcript expression levels are presented as mean ± SD. Asterisks indicate significant differences between WT and *npr1-1* mutant *Arabidopsis* plants at the indicated time points (*$P<0.05$ or **$P<0.01$).

Figure S14
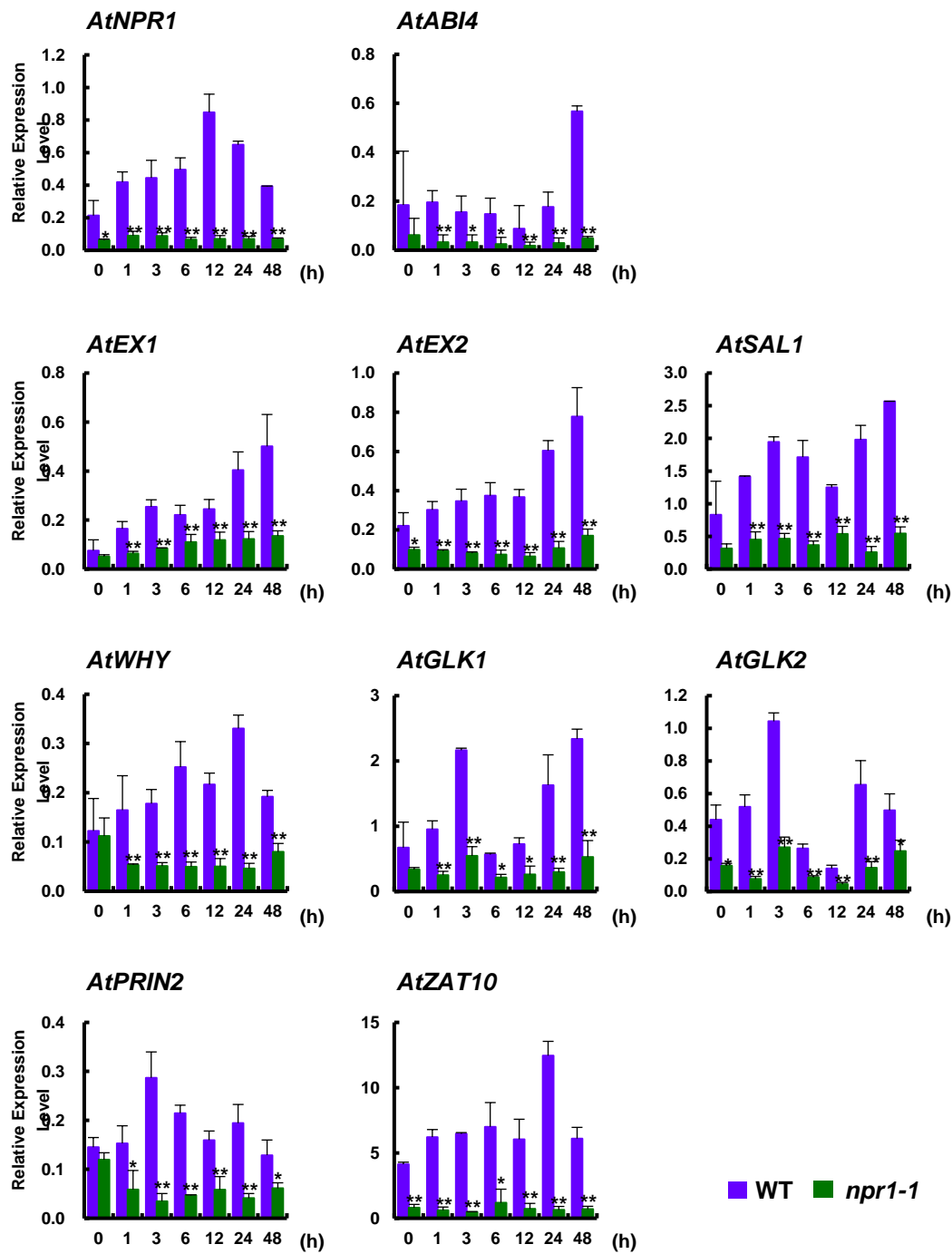
Figure S14. Transcription of retrograde signaling-related genes in response to abiotic/biotic stress in Col-0 WT and *npr1-1* mutant *Arabidopsis* plants under salt stress. Transcription profiles in the leaves of WT and *npr1-1* mutants in response to 200 mM NaCl-induced salt stress were determined using qRT-PCR and expressed relative to that of the reference gene β-actin. Genes related in retrograde communication for operational signaling in response to biotic/abiotic stress: *AtNPR1*, nonexpressor of pathogenesis-related genes 1; *AtABI4*, abscisic acid-insensitive protein 4; *AtEX1*, executer1; *AtEX2*, executer2; *AtSAL1*, 3′-phosphoadenosine 5′-phosphate (PAP) phosphatase; *AtWHY1*, single-stranded DNA-binding protein WHIRLY 1; *AtGLK1*, golden 2-like1; *AtGLK2*, golden 2-like 2; *AtPRIN2*, plastid redox insensitive 2; *AtZAT10*, zinc-finger transcription factor 10. Relative transcript expression levels are presented as mean ± SD. Asterisks indicate significant differences between WT and *npr1-1* mutant *Arabidopsis* plants at the indicated time points (*P<0.05 or **P<0.01).

Supplementary materials

The full scan of the entire original blots

The full scan of the entire original blots: Figure 1A
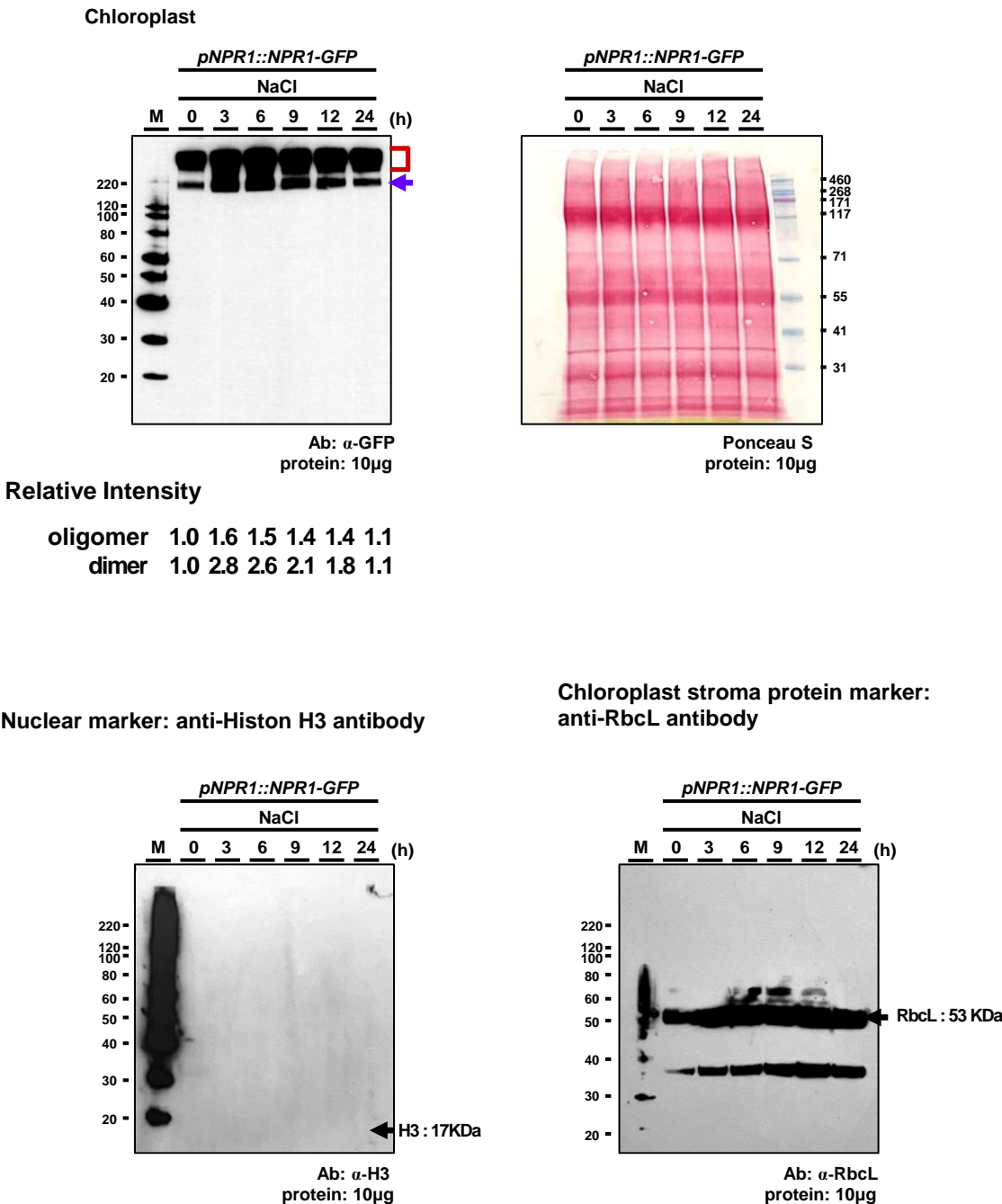
Chloroplast
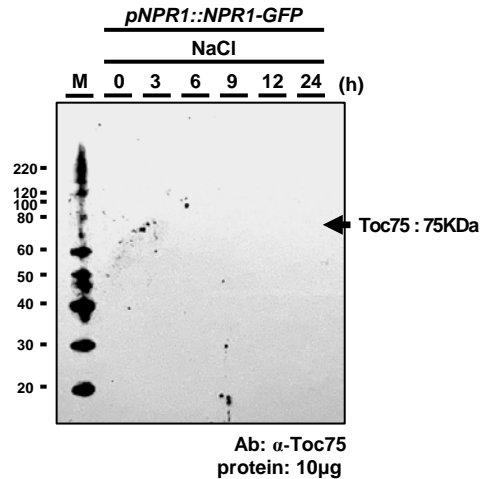
Ab: α-GFP
protein: 10μg

Ponceau S
protein: 10μg

Relative Intensity

oligomer	1.0	1.6	1.5	1.4	1.4	1.1
dimer	1.0	2.8	2.6	2.1	1.8	1.1

Nuclear marker: anti-Histon H3 antibody

Chloroplast stroma protein marker: anti-RbcL antibody

Ab: α-H3
protein: 10μg

Ab: α-RbcL
protein: 10μg

Chloroplast outer membrane marker: anti-Toc75 antibody

pNPR1::NPR1-GFP

NaCl

| M | 0 | 3 | 6 | 9 | 12 | 24 | (h) |

220 —
120 —
100 —
80 — ← **Toc75 : 75KDa**
60 —
50 —
40 —
30 —
20 —

Ab: α-Toc75
protein: 10μg

To ensure the reliability of our results, all experiments were repeated at least three times. Data from representative experiments were then reported after confirming consistency in the obtained results. To quantify band intensities, we utilized Image J software. Relative intensity was calculated by normalizing the band intensity values to the 0-hour treatment, which was assigned as 1.0.

The full scan of the entire original blots: Figure 1B

NPR1-GFP

Nucleus

pNPR1::NPR1-GFP

NaCl

| M | 0 | 3 | 6 | 9 | 12 | 24 | (h) |

220 —
120 =
100 =
80 —

← NPR1-GFP
: 92.3KDa

60 —
50 —

40 —

30 —

20 —

Ab: α-GFP
protein: 7μg

Relative Intensity

monomer   1.0 1.3 7.5 7.0 30.0 12.7

Marker of Nuclear proteins and equal loading: anti-Histon H3 antibody

pNPR1::NPR1-GFP

NaCl

| 0 | 3 | 6 | 9 | 12 | 24 | (h) |

220 —
120 =
100 =
80 —

60 —
50 —

40 —

30 —

20 —

← H3 : 17KDa

Ab: α-H3
protein: 7μg

Chloroplast stroma marker: anti-RbcL antibody

pNPR1::NPR1-GFP

NaCl

| M | 0 | 3 | 6 | 9 | 12 | 24 | (h) |

220 —
120 =
100 =
80 —

60 —
50 —

40 —

30 —

20 —

← RbcL : 53 KDa

Ab: α-RbcL
protein: 7μg

Chloroplast outer membrane marker: anti-Toc75 antibody

pNPR1::NPR1-GFP

NaCl

| M | 0 | 3 | 6 | 9 | 12 | 24 | (h) |

220 —
120 =
100 =
80 —

← Toc75 : 75KDa

60 —
50 —

40 —

30 —

20 —

Ab: α-Toc75
protein: 7μg

To ensure the reliability of our results, all experiments were repeated at least three times. Data from representative experiments were then reported after confirming consistency in the obtained results. To quantify band intensities, we utilized Image J software. Relative intensity was calculated by normalizing the band intensity values to the 0-hour treatment, which was assigned as 1.0.

The full scan of the entire original blots: Figure 1D

Chloroplast (6h)

Ab: α-GFP
protein: 20μg

Ponceau S
protein: 20μg

Relative Intensity

	Mock	NaCl	Mock	NaCl
oligomer	1.0	1.3	0.7	1.4
tetramer	1.0	2.2	0.9	3.9
dimer	1.0	144.6	1.0	216.2

Nucleus (12h)

NPR1-GFP
: 92.3KDa

Ab: α-GFP
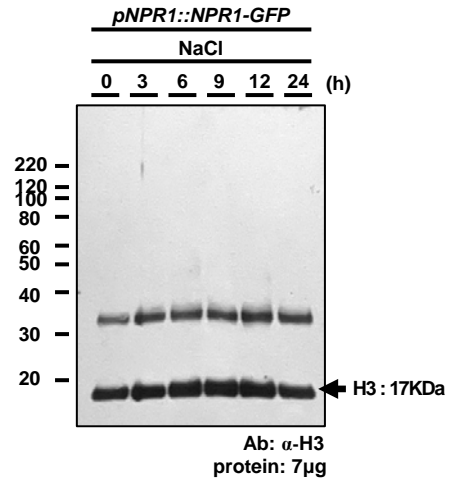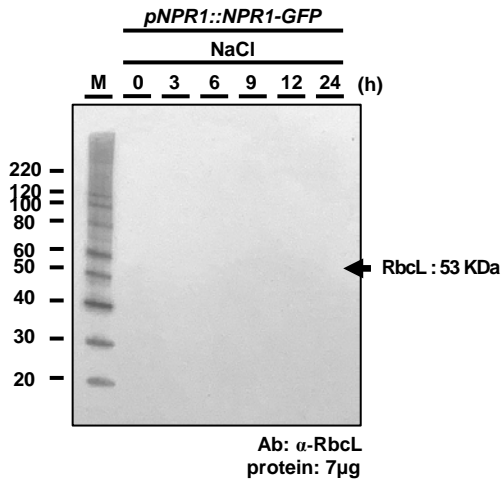protein: 7μg

Relative Intensity

monomer   1.0   0.8

To ensure the reliability of our results, all experiments were repeated at least three times. Data from representative experiments were then reported after confirming consistency in the obtained results. To quantify band intensities, we utilized Image J software. (left) Relative intensity was calculated by normalizing the band intensity values to the mock treatment of *pNPR1::NPR1-GFP*, which was assigned a value of 1.0. (right) Relative intensity of *p35S::cTP-NPR1-GFP* was calculated by normalizing the band intensity values to *pNPR1::NPR1-GFP*, which was assigned as 1.0.

The full scan of the entire original blots: Figure 1G and 1J

Chloroplast

	pNPR1::NPR1-GFP				p35S::cTP-NPR1-GFP			
	Mock	NaCl	ACC	H₂O₂	Mock	NaCl	ACC	H₂O₂

- 460
- 268
- 171
- 117
- 71
- 55
- 41

Ab: α-GFP
protein: 20μg

Relative Intensity

oligomer	1.0	1.4	2.0	2.1	2.0	2.5	2.0	2.2
tetramer	1.0	3.3	3.7	3.6	2.2	4.1	4.2	4.1
dimer	1.0	257	264.	209	1.1	538	291	120

Marker of chloroplast stroma proteins : anti-RbcL antibody

	pNPR1::NPR1-GFP				p35S::cTP-NPR1-GFP			
	Mock	NaCl	ACC	H₂O₂	Mock	NaCl	ACC	H₂O₂

- 460
- 268
- 171
- 117
- 71
- 55

RbcL : 53 KDa

Ab: α-RbcL
protein: 20μg

Nucleus

	pNPR1::NPR1-GFP				p35S::cTP-NPR1-GFP			
	Mock	NaCl	ACC	H₂O₂	Mock	NaCl	ACC	H₂O₂

220 —
120 =
100 =
80 —
60 —
50 —
40 —
30 —
20 —

NPR1-GFP : 92.3KDa

Ab: α-GFP
protein: 7μg
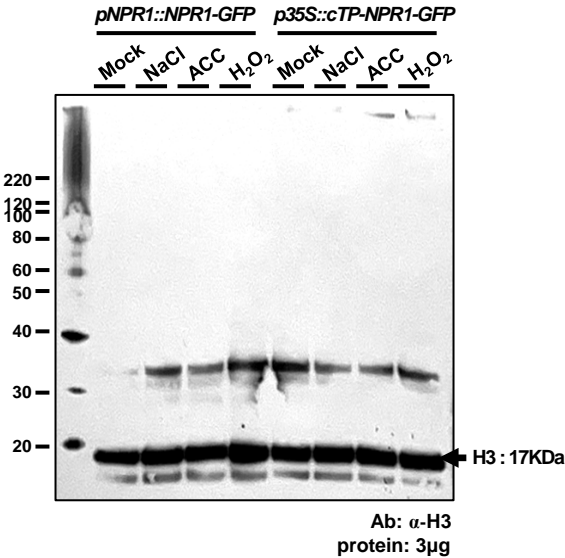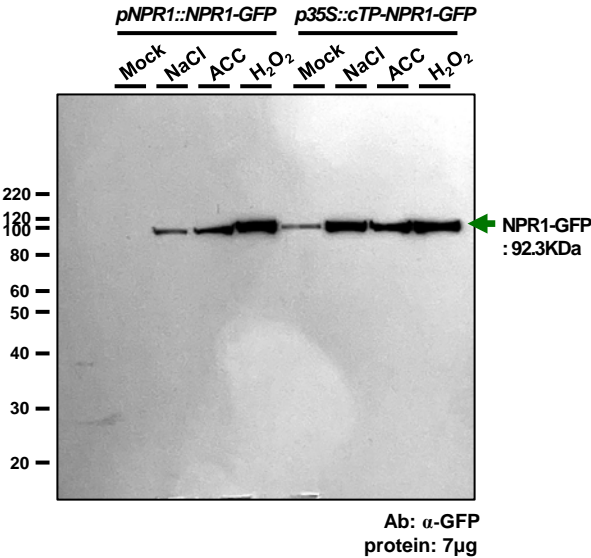
Relative Intensity

monomer	1.0	8.9	22.4	34.6.	1.5	26.6	28.0	40.2

Marker of nuclear proteins and Equal loading: anti-Histon H3 antibody

	pNPR1::NPR1-GFP				p35S::cTP-NPR1-GFP			
	Mock	NaCl	ACC	H₂O₂	Mock	NaCl	ACC	H₂O₂

220 —
120 =
100 =
80 —
60 —
50 —
40 —
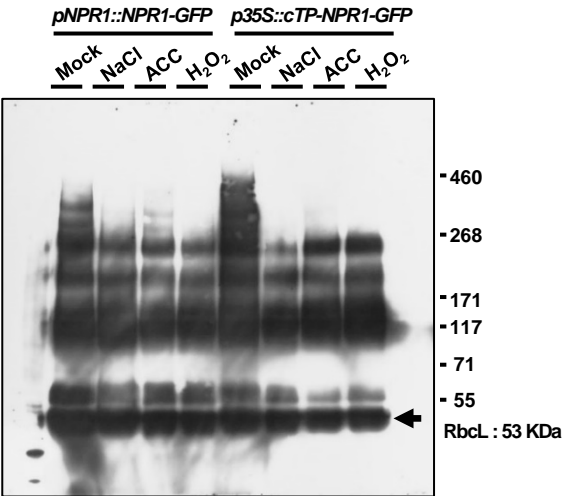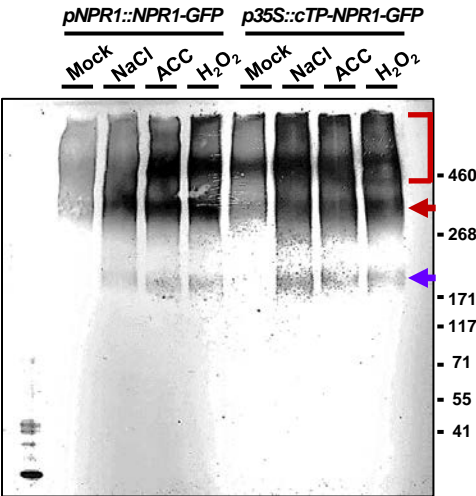30 —
20 —

H3 : 17KDa

Ab: α-H3
protein: 3μg

To ensure the reliability of our results, all experiments were repeated at least three times. Data from representative experiments were then reported after confirming consistency in the obtained results. To quantify band intensities, we utilized Image J software. Relative intensity was calculated by normalizing the band intensity values to the mock treatment of *pNPR1::NPR1-GFP*, which was assigned as 1.0.

The full scan of the entire original blots: Figure 3D

Cytoplasm(wt Chloroplast)
pNPR1::NPR1-GFP
NaCl

M 0 1 3 6 9 12 24 48 (h)

220 ·
120 · / 100 ·
80 · / 60 ·
50 ·
40 ·
30 ·
20 ·

460 / 268 / 171
117
71 / 55
41
31

Ab: α-GFP
protein: 10µg

Cytosol
pNPR1::NPR1-GFP
NaCl

M 0 1 3 6 9 12 24 48 (h)

220 ·
120 · / 100 ·
80 · / 60 ·
50 ·
40 ·
30 ·
20 ·

460 / 268 / 171
117
71 / 55
41
31

Ab: α-GFP
protein: 10µg

Relative Intensity

oligomer	1.0	1.4	1.6	1.7	1.2	1.1	0.9	0.9
dimer	1.0	21.5	227.9	236.7	33.2	9.6	5.2	0.0

Relative Intensity

oligomer	1.0	1.0	1.0	1.0	0.9	1.0	0.9	0.9
dimer	1.0	1.0	0.9	1.0	1.0	1.1	1.0	1.0

Cytoplasm(wt Chloroplast)
pNPR1::NPR1-GFP
NaCl

0 1 3 6 9 12 24 48 (h)

460 / 268 / 171 / 117
71
55
41
31

Ponceau S
protein: 10µg

Cytosol
pNPR1::NPR1-GFP
NaCl

0 1 3 6 9 12 24 48 (h)

460 / 268 / 171 / 117
71
55
41
31

Ponceau S
protein: 10µg

To ensure the reliability of our results, all experiments were repeated at least three times. Data from representative experiments were then reported after confirming consistency in the obtained results. To quantify band intensities, we utilized Image J software. Relative intensity was calculated by normalizing the band intensity values to the 0-hour treatment, which was assigned as 1.0.