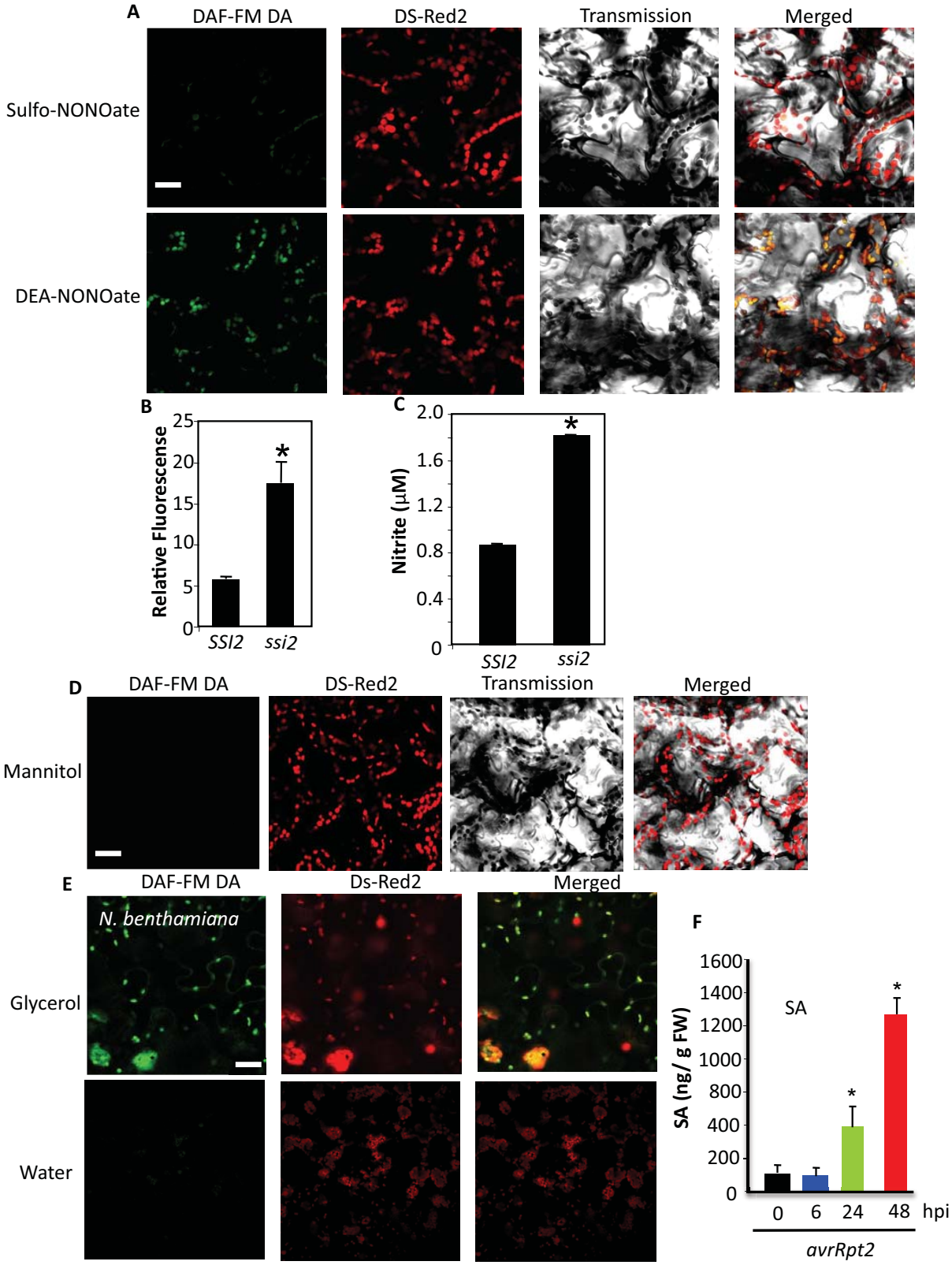
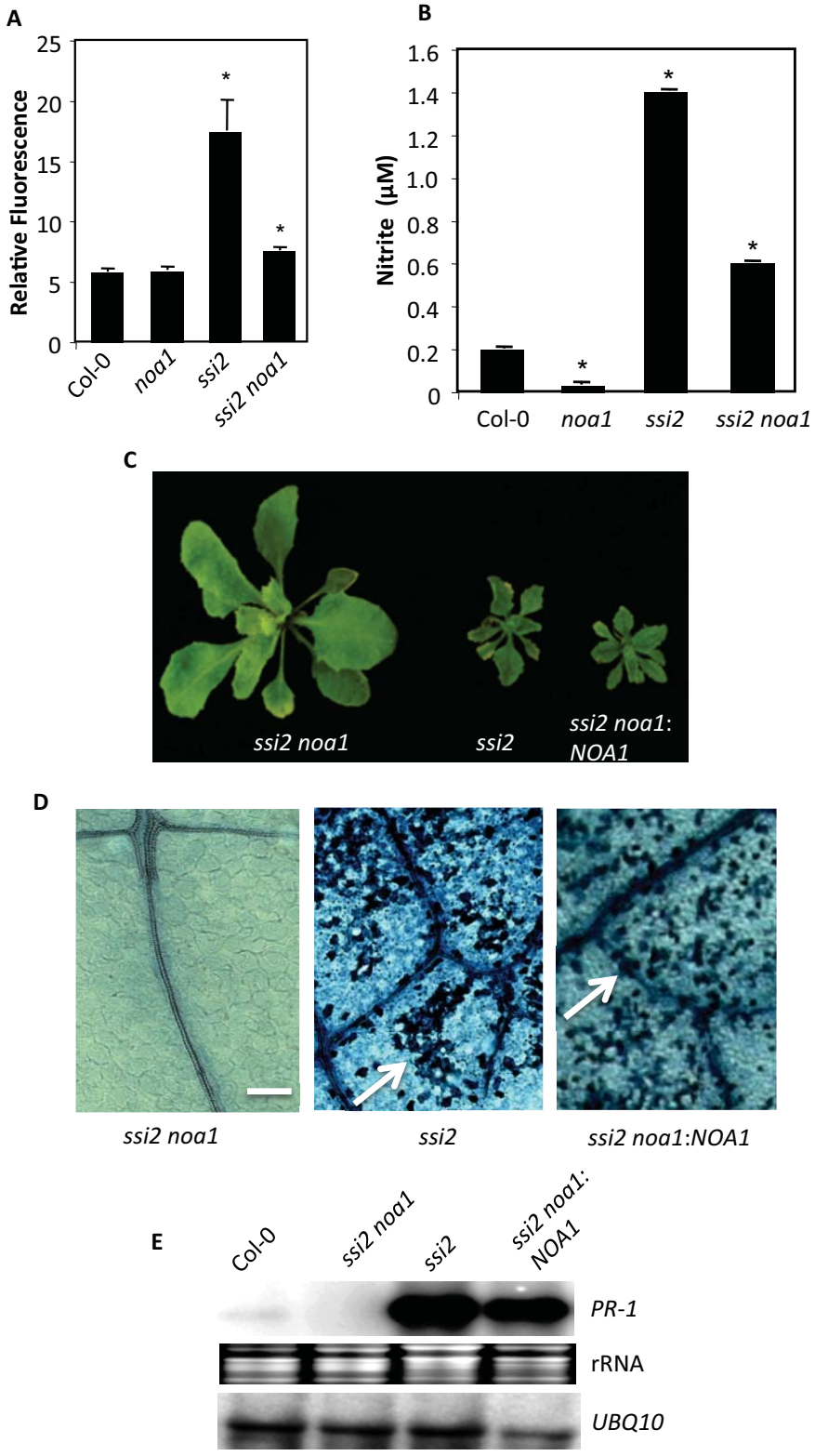
Supplemental Figure 1



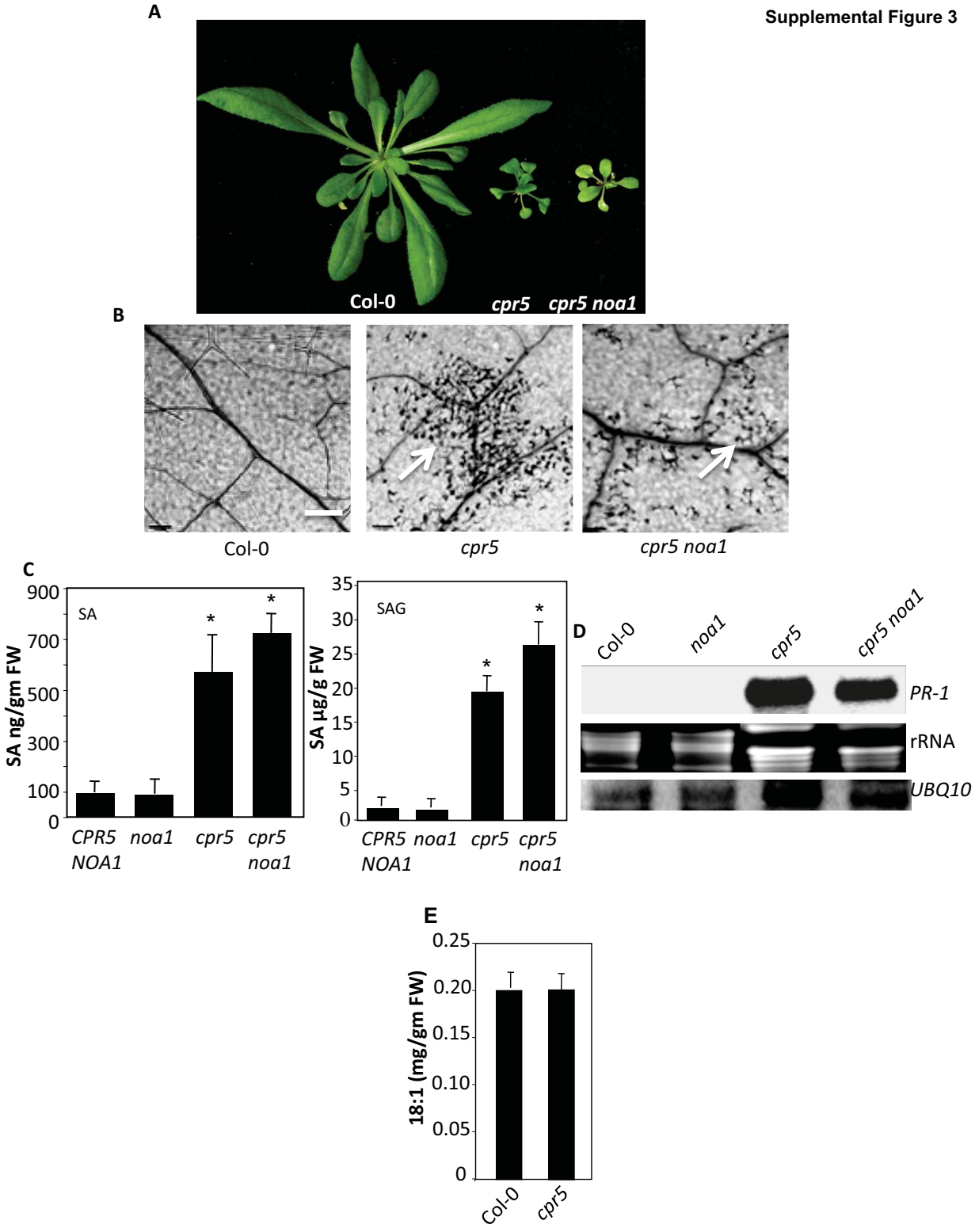
Supplemental Figure 1. The *ssi2* plants accumulate high levels of chloroplastic NO. (**A**) Confocal micrograph of DAF-FM DA stained leaves showing induction of NO in wild-type (Col-0) plants treated with 0.1 mM NO donor DEA-NONOate. Control plants were treated with 0.1 mM sulfo-NONOate and plants were analyzed 24 h post treatment. Scale bar, 20 μm. Chloroplast autofluorescence (red) was visualized using Ds-Red2 channel. At least four independent leaves were analyzed in two experiments with similar results. **(B**) Relative fluorescence in DAF-FM DA treated plants quantified using a fluorimeter. The error bars indicate SD (n=4). Asterisks denote a significant difference with wild-type Nössen (*SSI2*) (*t* test, P<0.05). DAF-FM fluorescence was quantified from tissue extracts prepared as described in the method section. (**C**) Levels of nitrite in the soil grown four-week-old plants. The nitrate levels were estimated using Griess assay. The error bars represent SD (n=4). Asterisks denote a significant difference with wild-type (*t* test, P<0.05). The experiment was carried out twice with similar results. (**D**) Confocal micrograph of DAF-FM DA stained Col-0 leaves 24 h post treatment with 50 mM mannitol. Scale bar, 20 μm. At least six independent leaves were analyzed in two experiments with similar results. Chloroplast autofluorescence (red) was visualized using Ds-Red2 channel. (**E**) Confocal micrograph of DAF-FM DA stained leaves showing subcellular location of NO in glycerol-treated *Nicotiana benthamiana* plants. Scale bar, 20 μm. Plants were treated with water or 50 mM glycerol and analyzed 24 h post treatments. At least four independent leaves were analyzed in two experiments with similar results. (**F**) A time course showing SA levels in pathogen inoculated plants wild-type Col-0 plants. Plants were inoculated with *avrRpt2 Pseudomonas syringae* and SA was measured from the inoculated leaves at indicated hours post inoculation (hpi). The error bars indicate SD (n=4). Asterisks denote a significant difference (*t* test, P<0.05).

Supplemental Figure 2



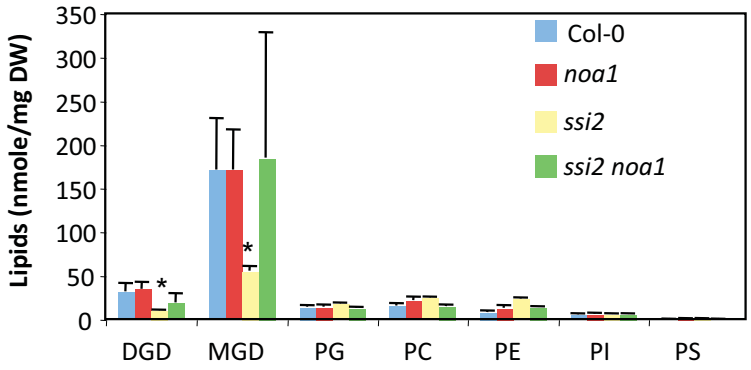
Supplemental Figure 2. Transgenic expression of *NOA1* restores *ssi2*-like phenotypes in *ssi2 noa1* plants. (**A**) Relative fluorescence in DAF-FM DA treated plants quantified using a fluorimeter. The error bars indicate SD (n=4). Asterisks denote a significant difference with wild-type Col-0 (*t* test, P<0.05). DAF-FM fluorescence was quantified from tissue extracts prepared as described in the method section. (**B**) Levels of nitrite in the soil grown four-week-old plants. The nitrate levels were estimated using Griess assay. The error bars represent SD (n=4). Asterisks denote a significant difference with wild-type (*t* test, P<0.05). (**C**) Morphological phenotype of four-week-old soil grown plants. (**D**) Microscopy of trypan blue stained leaves. Scale bars, 270 microns. Arrows indicate dead cells. At least six independent leaves were analyzed in two experiments with similar results. (**E**) RNA gel blot showing transript levels of *PR-1* gene. Ubiquitin mRNA (*UBQ10*) and ethidium bromide staining of rRNA were used as loading controls. Four independent *ssi2 noa1* T2 plants containing the *NOA1* transgene were analyzed and all showed *ssi2*-like levels of *PR-1* gene.

Supplemental Figure 3



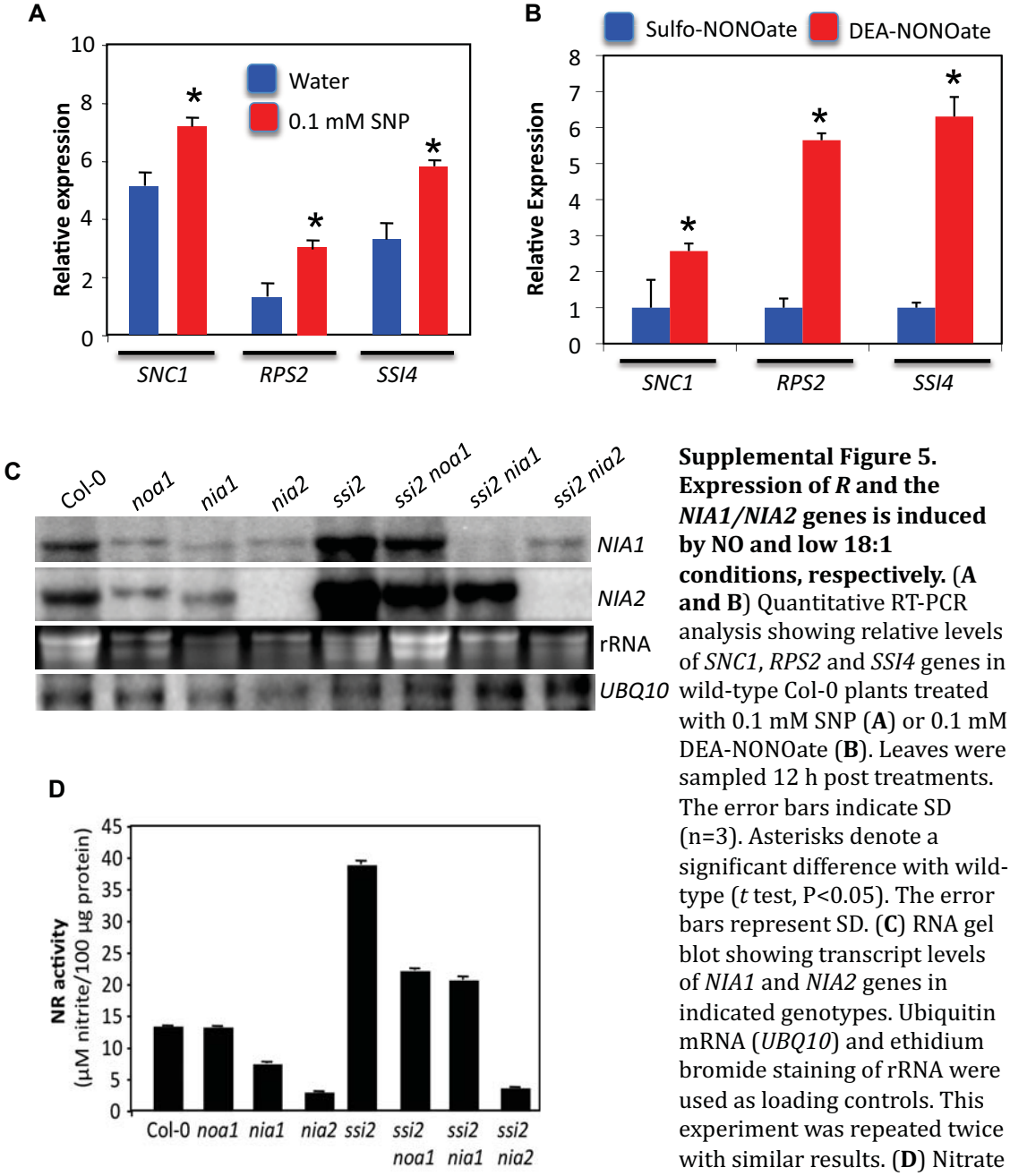
Supplemental Figure 3. A mutation in *NOA1* does not restore constitutive defense phenotypes in *cpr5* plants. (**A**) Morphological phenotype of four-week-old plants. (**B**) Microscopy of trypan blue stained leaves. Scale bars, 270 microns. Arrows indicate dead cells. At least four independent leaves of all genotypes were analyzed in two experiments with similar results. (**C**) SA and SAG levels in indicated genotypes. The error bars represent SD (n=3). Asterisks denote a significant difference with wild-type (*t* test, $P<0.05$). The experiment was repeated twice with similar results. (**D**) RNA gel blot showing transript levels of *PR-1* gene. Ubiquitin mRNA (*UBQ10*) and ethidium bromide staining of rRNA were used as loading controls. The experiment was repeated twice with similar results. (**E**) 18:1 levels in wild-type Col-0 and *cpr5* plants. The error bars represent SD (n=6).

Supplemental Figure 4



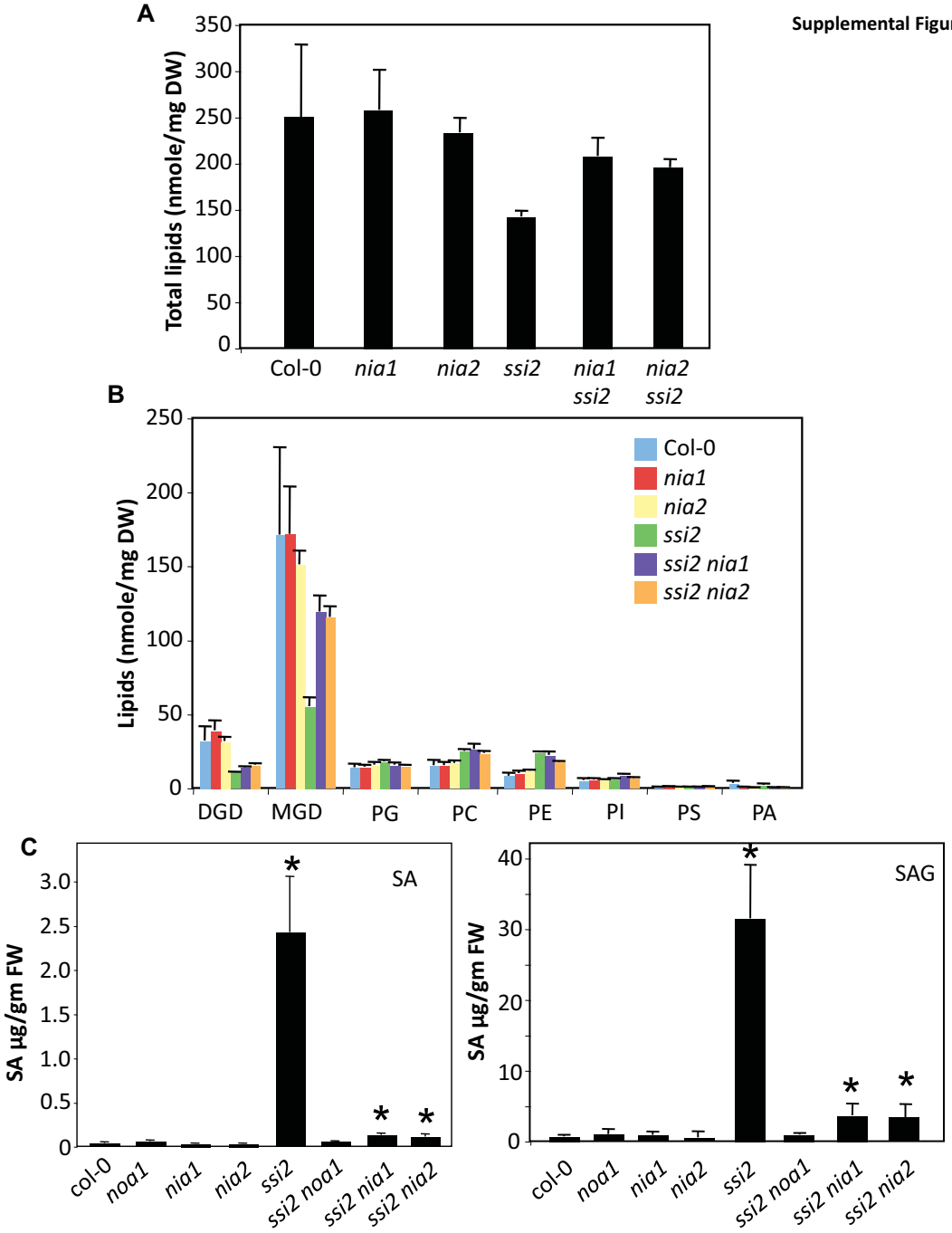
Supplemental Figure 4. Profile of total lipids extracted from wild-type (Col-0), *noa1*, *ssi2* and *ssi2 noa1* plants. The values are presented as an average of 5 replicates. The error bars represent SD (n=5). Asterisks denote a significant difference with wild-type (*t* test, P<0.05). Symbols for various components are: DGD, digalactosyldiacylglycerol; MGD, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethaloamine; PI, phosphatidylinositol; PS, phosphatidylserine.

Supplemental Figure 5



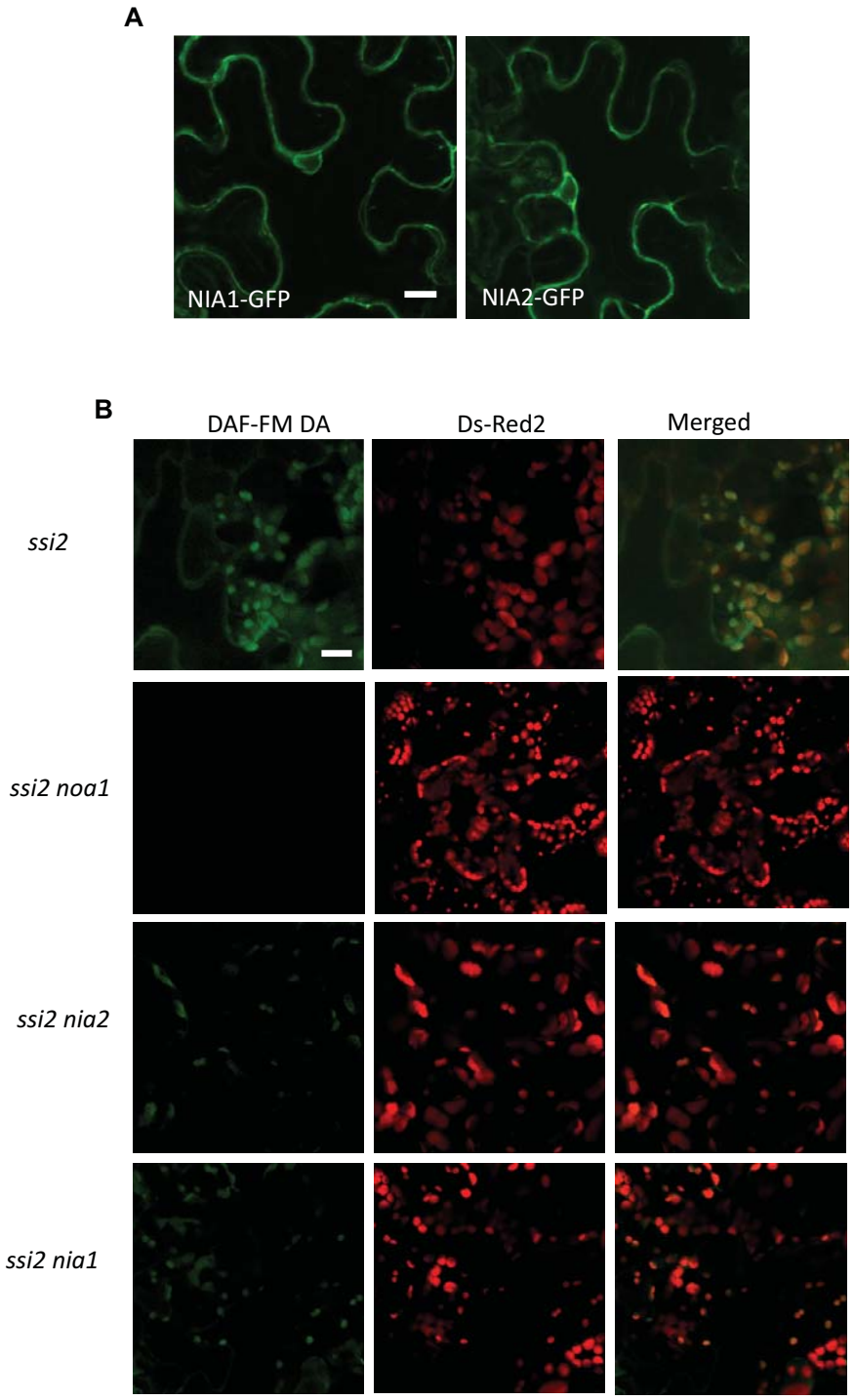
Supplemental Figure 5. Expression of *R* and the *NIA1/NIA2* genes is induced by NO and low 18:1 conditions, respectively. (**A and B**) Quantitative RT-PCR analysis showing relative levels of *SNC1*, *RPS2* and *SSI4* genes in wild-type Col-0 plants treated with 0.1 mM SNP (**A**) or 0.1 mM DEA-NONOate (**B**). Leaves were sampled 12 h post treatments. The error bars indicate SD (n=3). Asterisks denote a significant difference with wild-type (*t* test, P<0.05). The error bars represent SD. (**C**) RNA gel blot showing transcript levels of *NIA1* and *NIA2* genes in indicated genotypes. Ubiquitin mRNA (*UBQ10*) and ethidium bromide staining of rRNA were used as loading controls. This experiment was repeated twice with similar results. (**D**) Nitrate reductase activity in indicated genotypes. This experiment was repeated twice with similar results.

Supplemental Figure 6



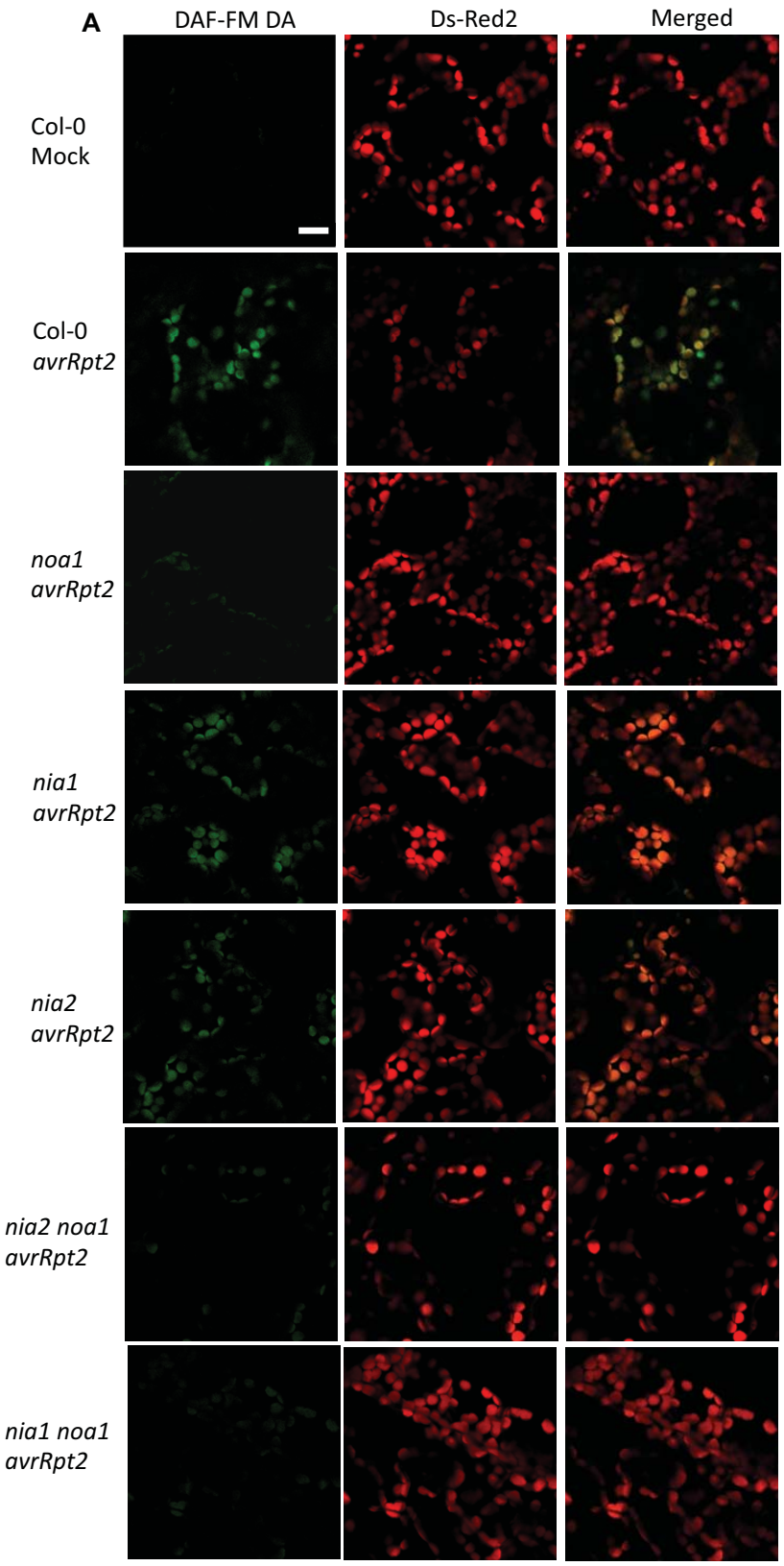
Supplemental Figure 6. Mutations in *NIA1* and *NIA2* partially restore *ssi2* phenotypes. (**A**) Total lipid levels in indicated genotypes. DW indicates dry weight. The error bars represent SD (n=5). (**B**) Profile of total lipids extracted from wild-type (Col-0), *nia1*, *nia2*, *ssi2*, *ssi2 nia1* and *ssi2 nia2* plants. The values are presented as an average of 5 replicates. The error bars represent SD (n=5). (**C**) SA and SAG levels in indicated genotypes. The error bars represent SD (n=3). Asterisks denote a significant difference with wild-type (*t* test, P<0.05).

Supplemental Figure 7

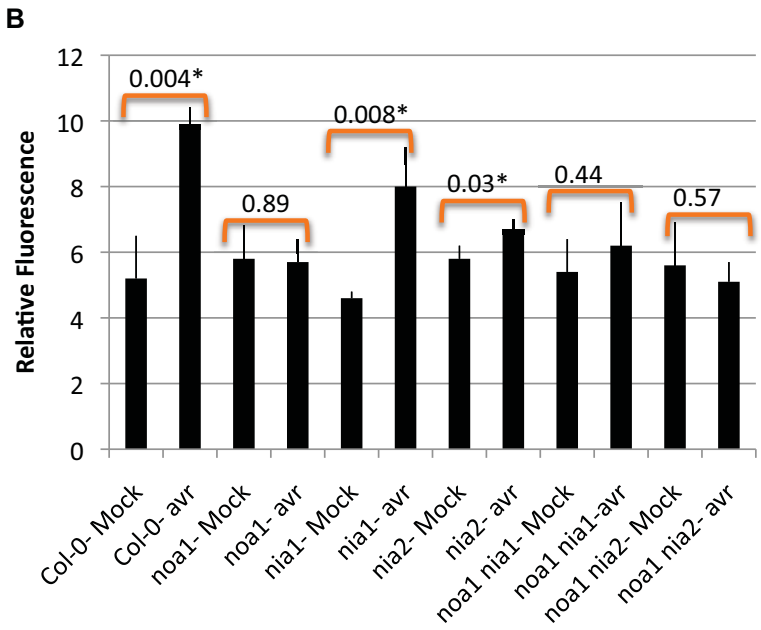


Supplemental Figure 7. NIA1 and NIA2 are extrachloroplastic proteins required for chloroplastic NO accumulation in *ssi2* plants. (**A**) Confocal micrograph showing localization of NIA1-GFP and NIA2-GFP protein in *N. benthamiana*. Scale bar, 5 µm. (**B**) Confocal micrograph of DAF-FM DA stained leaves showing subcellular location of NO in indicated genotypes. Chloroplast autofluorescence (red) was visualized using Ds-Red2 channel. Scale bar, 10 µm. At least six independent leaves were analyzed in two experiments with similar results.

Supplemental Figure 8

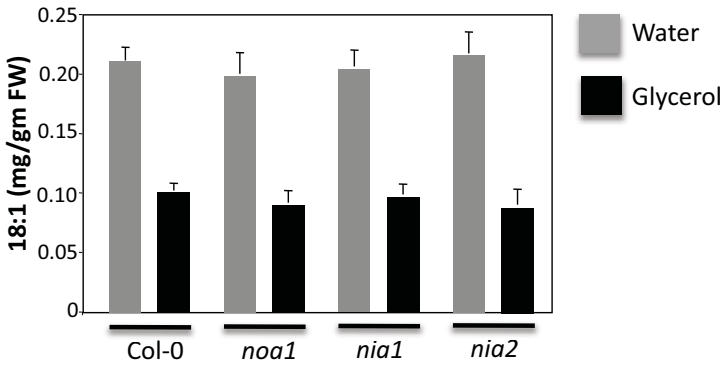


Supplemental Figure 8



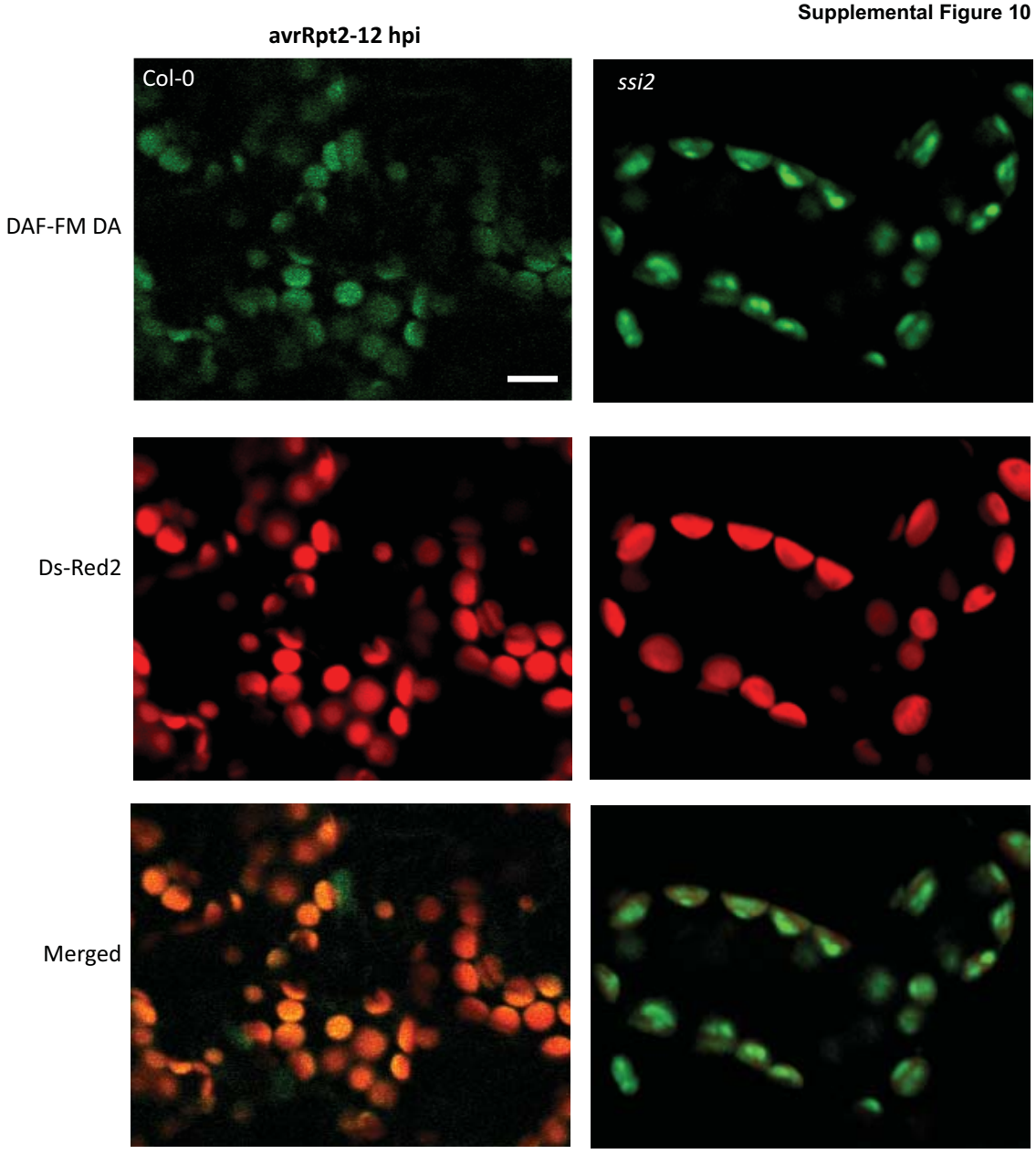
Supplemental Figure 8. The *noa1 nia* plants are compromised in pathogen induced NO accumulation. (**A**) Confocal micrograph showing pathogen-induced NO accumulation in indicated genotypes. Plants were inoculated with $MgCl_2$ (mock) or *avrRpt2* expressing *P. syringae*. Scale bar, 10 µm. At least four independent leaves were analyzed in two experiments with similar results. Chloroplast autofluorescence (red) was visualized using Ds-Red2 channel. (**B**) Relative fluorescence in $MgCl_2$ or pathogen inoculated leaves quantified using a fluorimeter. The error bars represent SD (n=3). Numbers above the bars indicate P value and asterisks denote a significant difference with mock-inoculated plants (*t* test).

Supplemental Figure 9



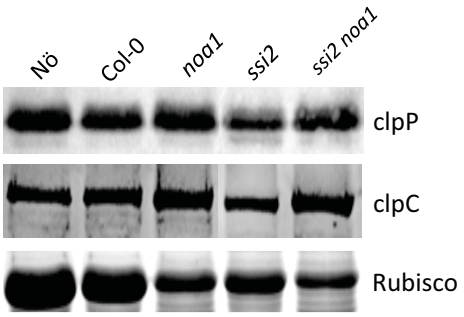
Supplemental Figure 9. The glycerol-treated Col-0, *noa1*, *nia1* and *nia2* plants show similar decrease in their 18:1 levels. 18:1 levels in water and glycerol treated plants. Error bars represent SD (n=6).

Supplemental Figure 10



Supplemental Figure 10. Subcellular localization of NO in *ssi2* and *avrRpt2* inoculated wild-type plants. Confocal micrograph of DAF-FM DA stained *ssi2* and *avrRpt2* inoculated wild-type (Col-0) plants. Scale bar, 5 µm. At least eight independent leaves were analyzed in three experiments with similar results. Chloroplast autofluorescence (red) was visualized using Ds-Red2 channel.

Supplemental Figure 11



Supplemental Figure 11. Levels of clpC and clpP in *ssi2* plants. Western blot showing levels of clpC, clpP levels in wild-type (*SSI2*), *noa1*, *ssi2* and *ssi2 noa1* plants. Ponceau-S staining of the Western blot was used as the loading control. The experiment was repeated twice with similar results.

Supplemental Figure 12

A

FABP1	NFEAFMKAIG-LPEFTVGEECE	IITNTMTLGDIVFKRISKR
FABP2	NYDKFMEKMG-VNVFELGVTFN	ELVQTYVYEGVEAKRIFKK
FABP3	NFDDYMKSLG-VGSFKLGVEFD	KLILTLTHGTAVCTRTYEK
FABP4	NFDDYMKEVG-VGSFILGQEFD	KLVVECVMKGVTSTRVYER
FABP5	GFDEYMKELG-VGSCTLGEKFE	KLVVECVMNNVTCTRIYEK
FABP6	NYDEFMKLLG-ISKFTVGKESN	KLVEVSTIGGVTYERVSKR
FABP7	NFDEYMKALG-VGSFQLGEEFD	KMVMTLTFGDVVAVRHYEK
FABP8	NFDDYMKALG-VGSFKLGQEFE	KMVAECKMKGVVCTRIYEK
FABP9	NFEDYMKELG-VNSFKLGEEFD	KMVVECKMNNIVSTRIYEK
NOA1	SHGHMITAVGGNGGYPGGKQFV	KLVDIVDFNGSFLARVRDL

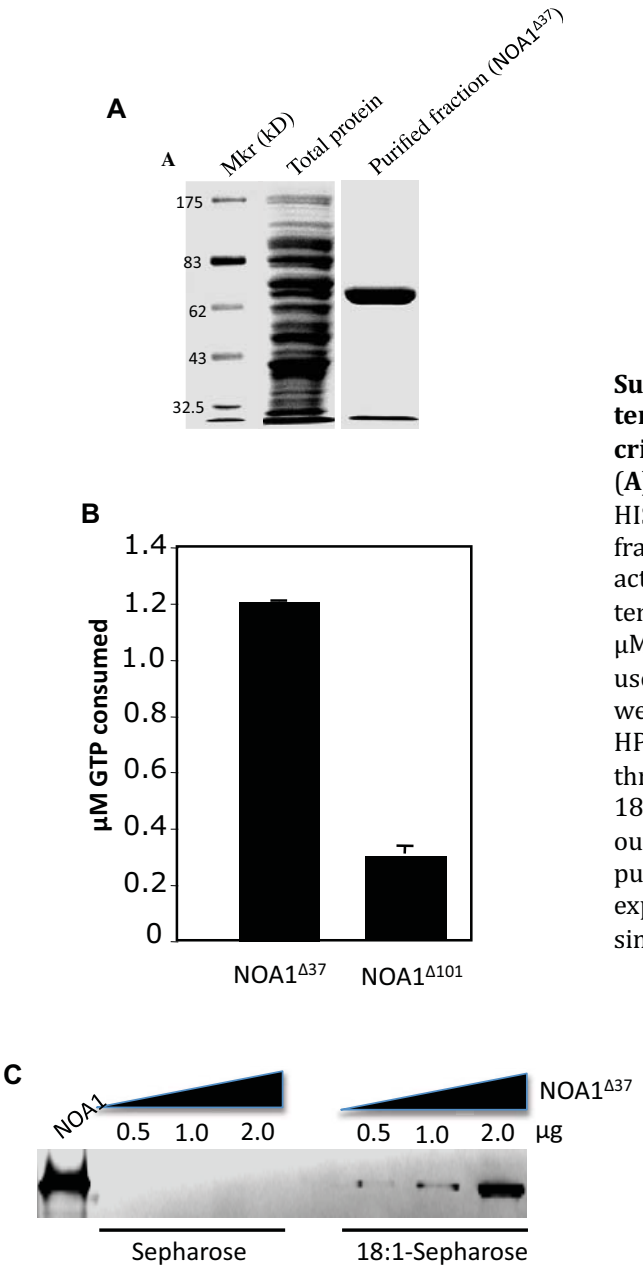
B

Arabidopsis, NOA1	LSHGHMITAVGGNGGYPGGKQ
A. lyrata	LSHGHMITAVGGNGGYSGGKQ
Nicotiana attenuata	LSHGHMITAVGGNGGYSGGKQ
N. benthamiana	LSHGHMITAVGGNGGYSGGKQ
Oryza sativa	LSHGHMITAVGGHGGYPGGKQ
Solanum tuberosum	LSHGHMITAVGGNGGYSGGKQ
Ricinis communis	LSHGHMITAVGGNGGYSGGKQ
Populus trichocarpa	LSHGHMITAVGGNGGYSGGKQ
Hordeum vulgare	LSHGHMVTAVGGHGGYPGGKQ
Vitis vinifera	LSHGQMITAVGGNGGYSGGKQ
Zea mays	LSHGHMVTAVGGHGGYPGGK

Arabidopsis, NOA1	KLVDIVDFNGSFLARVRDL
Nicotiana attenuata	KLVDIVDFNGSFLARVRDL
N. benthamiana	KLVDIVDFNGSFLARVRDL
Solanum tuberosum	KLVDIVDFNGSFLARVRDL
Brassica juncea	KLVDIVDFNGSFLARVRDL
Picea sitchensis	KLVDIVDFNGSFLARVRDL
Oryza sativa	KLVDIVDFNGSFLARVRD
Vitis vinifera	KLVDIVDFNGSFLAHVRDL
Hordeum vulgare	KLVDIVDFNGSFLARIRD
Medicago truncatula	KLVDVVDFNGSFLSRVRDL
Populus trichocarpa	KLVDVVDFNGSFLARLRDL

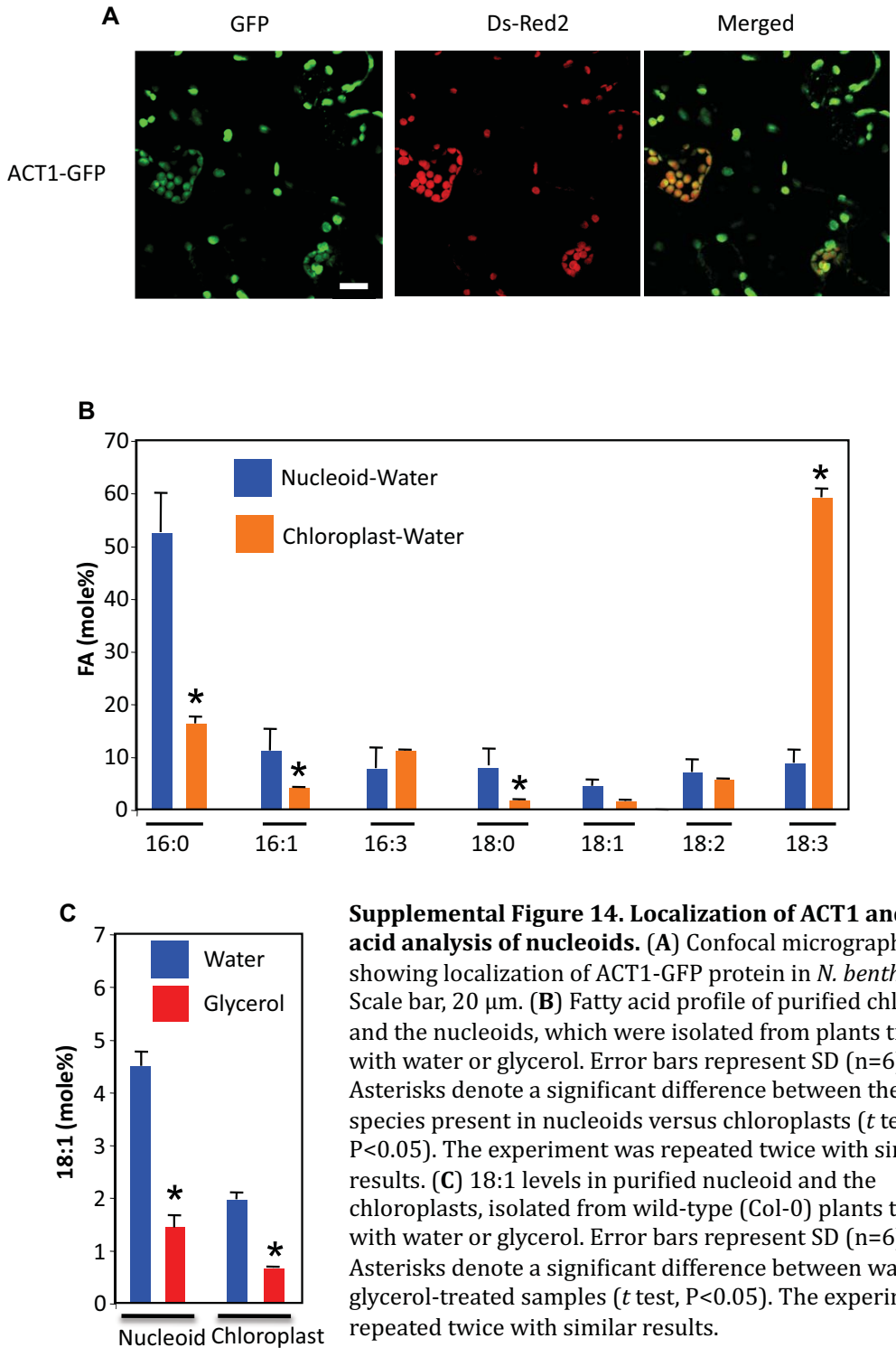
Supplemental Figure 12. Fatty acid binding properties of NOA1. (**A**) Amino acid alignment of conserved FA binding domains of mammalian FA binding proteins (FABP) and NOA1. The members of FABP family show 22-73% aa sequence similarity (Zimmerman and Veerkamp 2002). Identical residues are shaded in red. Residues common between NOA1 and most other FABPs are shaded in green. These domains in left and right panels represent aa 151-171 and 193-211 of NOA1 protein, respectively. Sequence alignment was carried out using ClustalW in the Megalign program of the DNASTAR package. (**B**) Amino acid alignment of putative FA binding domains of NOA1-like plant proteins.

Supplemental Figure 13



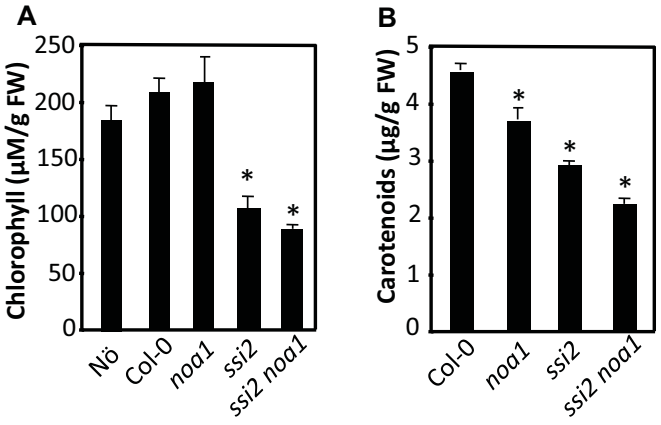
Supplemental Figure 13. N-terminal 37-101 amino acids are critical for NOA1 GTPase activity. (**A**) SDS-PAGE gel showing NOA1-HIS$^{\Delta 37}$ protein in total and purified fractions. (**B**) Comparison of GTPase activity of NOA1-HIS lacking N-terminal 37 or 101 amino acids. 100 µM GTP and 2 µM NOA1-HIS were used for the assay and levels of GDP were measured using reverse phase HPLC. The experiment was repeated three times with similar results. (**C**) 18:1 affinity chromatography carried out using 0.5, 1, or 2 µg of *E. coli* purified NOA1-HIS$^{\Delta 37}$ protein. The experiment was repeated twice with similar results.

Supplemental Figure 14



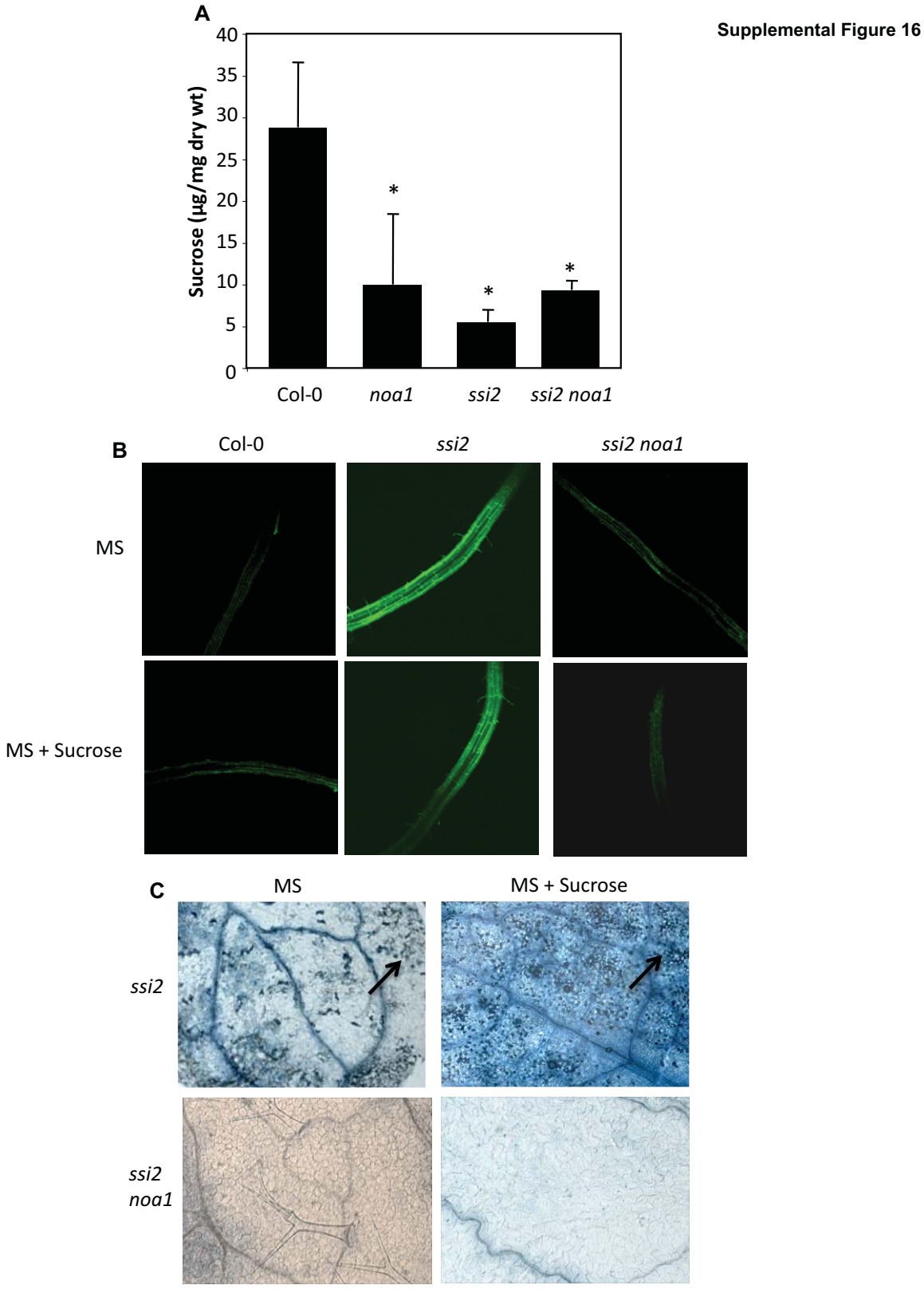
Supplemental Figure 14. Localization of ACT1 and fatty acid analysis of nucleoids. (**A**) Confocal micrograph showing localization of ACT1-GFP protein in *N. benthamiana*. Scale bar, 20 µm. (**B**) Fatty acid profile of purified chloroplasts and the nucleoids, which were isolated from plants treated with water or glycerol. Error bars represent SD (n=6). Asterisks denote a significant difference between the FA species present in nucleoids versus chloroplasts (*t* test, P<0.05). The experiment was repeated twice with similar results. (**C**) 18:1 levels in purified nucleoid and the chloroplasts, isolated from wild-type (Col-0) plants treated with water or glycerol. Error bars represent SD (n=6). Asterisks denote a significant difference between water- and glycerol-treated samples (*t* test, P<0.05). The experiment was repeated twice with similar results.

Supplemental Figure 15



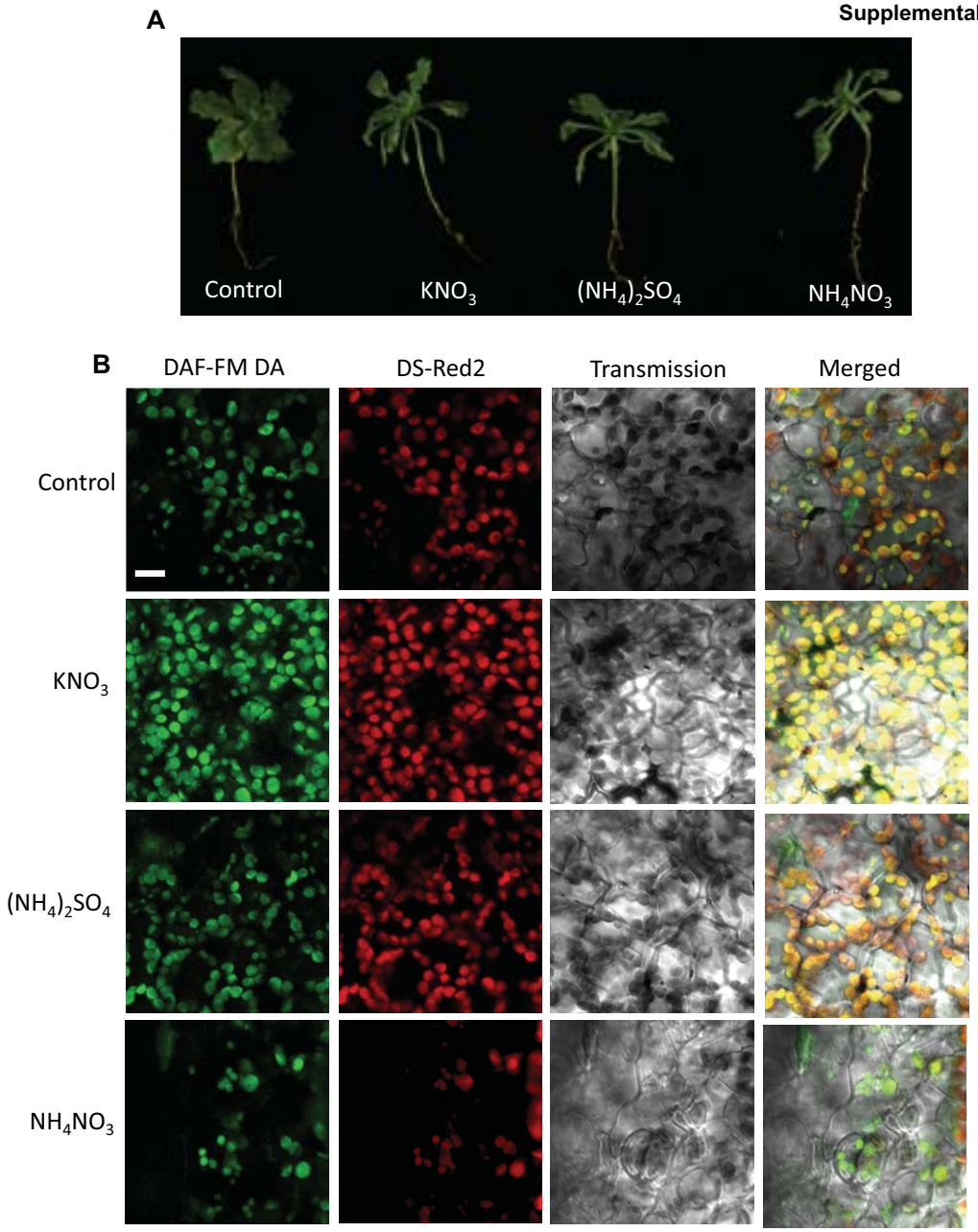
Supplemental Figure 15. Levels of chlorophyll and carotenoids in *ssi2* plants. (**A**) Levels of chlorophyll in four-week-old soil grown plants. Error bars indicate SD (n=4). Asterisks denote a significant difference with wild-type (*t* test, P<0.05). (**B**) Levels of carotenoids in four-week-old soil grown plants. Error bars indicate SD (n=4). Asterisks denote a significant difference with wild-type (*t* test, P<0.05).

Supplemental Figure 16



Supplemental Figure 16. Sucrose grown *ssi2 noa1* plants show wt-like phenotypes.
(**A**) Sucrose levels in wild-type (Col-0), *noa1*, *ssi2* and *ssi2 noa1* plants. Error bars represent SD (n=4). Asterisks denote a significant difference with wild-type (*t* test, P<0.05, n=3). (**B**) Confocal micrgraph showing NO-sensitive fluorescent staining of roots. Ten-day-old seedlings were grown with or without sucrose (~20 each) and stained with DAF-FM DA for 15 min prior to microscopy. (**C**) Microscopy of trypan blue stained leaves obtained from seedling grown on MS medium with or without sucrose. Scale bar, 270 microns. Arrow indicates dead cells.

Supplemental Figure 17



Supplemental Figure 17. *ssi2* plants grown without nitrogen accumulate elevated NO. (A) Typical morphological phenotypes of three-week-old *ssi2* plants grown on soil without external nitrogen source (control) or on soil containing 5 mM potassium nitrate, ammonium sulfate or ammonium nitrate. (B) Confocal micrograph of DAF-FM DA stained leaves showing relative NO levels in *ssi2* plants grown on soil without external nitrogen source (control) or containing potassium nitrate, ammonium sulfate or ammonium nitrate. Scale bar, 10 μm. At least four independent leaves were analyzed in two experiments with similar results. Chloroplast autofluorescence (red) was visualized using Ds-Red2 channel.

Supplemental Table 1. Transcrip levels of *NOA1*, *NIA1*, *NIA2* and *PR-1* in response to pathogen infections or exogenous application of SA. This data was obtained from the *Arabidopsis* gene expression browser (www.expressionbrowser.com; Zhang et al., 2010[a]). T and C indicate treatment and control, respectively.

Experiment: *Pseudomonas syringae* pv tomato avrRpm1 infiltration for 24 hr: infiltrated with $1x10^8$ cfu/ml *Pseudomonas syringae pv tomato avrRpm1*, 2 leaves per plant, 8 plants pooled, harvested after 24h

Name	T	C	Fold Change	p-value
AT2G14610 (PR-1)	6739	660	10.19	0.0036
AT1G77760 (NIA1)	471	408	1.15	0.273
AT1G37130 (NIA2)	8336	4734	1.76	0.0123
AT3G47450 (NOA1)	130	253	-1.94	0.0012

Experiment: SA treatment, SA vs Control

Name	T	C	Fold Change	p-value
AT2G14610 (PR-1)	3983	102	38.82	6.71E-5
AT1G77760 (NIA1)	8472	7695	1.1	0.4652
AT1G37130 (NIA2)	9797	9814	-1.0	0.9713
AT3G47450 (NOA1)	187	282	-1.5	0.0497

Experiment: Pst DC3000 infection 24 hr: Plants were inoculated by vacuum infiltration with *Pseudomonas syringae pv. tomato* strain DC3000 bacteria at a concentration of 10e6 cfu/ml. Inoculated leaf tissue from at least 15 plants was collected for RNA isolation.

Name	T	C	Fold Change	p-value
AT2G14610 (PR-1)	954	374	2.55	0.5541
AT1G77760 (NIA1)	1229	86	14.25	0.0837
AT1G37130 (NIA2)	3699	3149	1.17	0.4509
AT3G47450 (NOA1)	72	105	-1.44	0.2196

[a] Zhang M, Zhang Y, Liu L, Yu L, Tsang S, Tan J, Yao, W, Kang MS, An Y, Fan X. 2010. Gene Expression Browser: large-scale and cross-experiment microarray data integration, management, search & visualization. *BMC Bioinformatics* **11**: 433

Supplemental Table 2. Fold change in transcript levels of genes in *ssi2*, *ssi2 sid2, ssi2 act1* and *ssi2 eds1 sid2* plants compared to results from Col-0 (wild-type) plants. Genes showing 2-3, 3-4 and >4-fold activation are marked yellow, orange or red, respectively. Transcriptional profiling was performed using Affymetrix arrays.

	P Value	Gene ID	Gene Function	*ssi2* [a] / Col-0	*ssi2 sid2* [b] / Col-0	*ssi2 act1* [c] / Col-0	*ssi2 eds1 sid2* [d] / Col-0
1	0.028	AT5G60410	Small Ubiquitin like modifier (SUMO) E3 ligase (AtSIZ1)	2.49	1.52	1.52	0.61
2	0.00	AT5G04140	Ferredoxin-dependent glutamate synthase (Fd-GoGAT)	3.82	7.55	7.55	2.03
3	0.01	AT5G53460	NADH-dependent glutamate synthase	1.73	1.32	1.32	0.56
4	4.36E-05	AT5G56860	GATA family Zn finger transcription factor	-2.22	1.16	1.16	0.99
5	0.04	AT1G12110	NRT1.1 (Nitrate transporter)	-1.95	1.53	1.53	0.47
6	0.00	AT1G69850	NRT1.2 (Inducible component of low-affinity nitrate uptake)	3.36	1.96	1.96	0.75
7	0.00	AT1G32450	NRT1.5 (involved in xylem transport of nitrate)	2.34	2.11	2.11	0.5
8	4.95E-05	AT4G13510	Ammonium transport protein (AMT1)	3.73	1.94	1.94	0.89
9	0.00	AT1G64780	AMT1;2	14.91	76	1.55	3.03
10	0.00	AT1G37130	NIA2	9.65	8.27	1.2	10.04

[a] Low 18:1, constitutive defense, small morphology
[b] Low 18:1, constitutive defense, small morphology
[c] High 18:1, wild-type-like defense, wild-type-like morphology
[d] Low 18:1, wild-type-like defense, wild-type-like morphology

Supplemental Table 3. Primer sequences used for genotyping, real-time PCR, or generating clones for expression in *E. coli* and plants.

ORF/T-DNA	**Primers**
NOA1-overexpression Xho1-XbaI linkered	CAG CCT CGA GAT GGC GCT ACG AAC ACT CTC TGC ATC TAG ATC AAA AGT ACC ATT TGG GTC T
NOA1-genomic complementation-SalI-KpnI linkered	CAA GTC GAC CCC CAT AAA CCC TAG AAA TGG AAA CCC ACC GGT ACC CTG TTT CAT TTG TTG AAT TGT TGA TGT AG
NIA1-KO genotyping- LP and RP	TTC AAA CTC TTC GGT GCA ATC AGG AGA CAC GTG GAG TGT TTG
Lbb1	GCGTGGACCGCTTGCTGCAACT
NIA2-KO genotyping LP and RP	TGG CAT ATT CCT TCT TGA TGC AGT CAC AAA TGG TCC CAT ACG
ssi2-dCAPS-NsiI	TTG GTG GGG GAC ATG ATC ACA GAA GAT GCA AAG TAG GAC TAG CAC CTG TTT CAT CCC TAA
cpr5-dCAPS-BsmFI	GCG GTG TAT CGG GTA AAT TGT GTG TGC AAC GAA TTG CAA AAG GCA AAA CAC GTC
NOA1-localization attBI	AAAAAGCAGGCTTA ATGGCGCTACGAACACTCTCA AGAAAGCTGGGTA AAAGTACCATTTGGGTCTTAC
NOA1-HIS overexpression	AAAAAGCAGGCTTA ATGGCGCTACGAACACTCTCA AGAAAGCTGGGTATCAGTGGTGGTGGTGGTGGTGGTGGTG GTGGTGAAAGTACCATTTGGGTCTTAC
NOA1-*E. coli* expression-NheI-XhoI linkered (del	AGG GCT AGC ATG TGT AAA TCA ATA GCT AAT TCA GCG CTC GAG AAA GTA CCA TTT GGG TCT TAC

37)	
NOA1-*E. coli* expression-NheI-XhoI linkered (del 101)	AGG GCT AGC GAT ACC TCA GTC TCA TGT TGT
NIA1-localization-attBI	AAAAAGCAGGCTTA ATGGCGACCTCCGTCGATAAC AGAAAGCTGGGTA GAAGATTAAGAGATCCTCCTTCAC
NIA2-localization-attBI	AAAAAGCAGGCTTA ATGGCGGCCTCTGTAGATAAT AGAAAGCTGGGTA GAATATCAAGAAATCCTCCTTGAT
SSI2 localization-attBI	AAAAAGCAGGCTTA ATGGCTCTAAAGTTTAACCC AGAAAGCTGGGTA GAGCTGCACTTCTCTGT
β-tubulin	CGTGGATCACAGCAATACAGAGCC CCTCCTGCACTTCCACTTCGTCTT
RPS2	TCTTATCGTTGGCTGTGCTCAGGT ACGTATGGCCTTCAAGTCACCGAT
RPP5	AACAGACCGGCGAATTTGGAAAGG TCTGTGAGAGCTTGCACCCATCTT
SNC1	AACAGACCGGCGAATTTGGAAAGG GCAAGCTCTTCAATCATGGCTGCT
RPS4	GTTGGGATGCCCGGAATTGGTAAA ACTTGGACAACTCGCCTAAGAGCA
RPM1	TTGATGATGGCGATGCAAAGTGGG TTCCCTTGGGTGCATCAATCCCTA
RPP1	GCCGAGATCATGAAATGCAGGCAA GCGATTGTTGCCACATCCTCCAAA
SSI4	TCTTACGGGTGTTGCTGACCATGA TGTAGCCTTTCTCGTATTGCGCCT
Actin	ACACTGTGCCAATCTACGAGGGTT ACAATTTCCCGCTCTGCTGTTGTG