### Supplementary Table S1

**PCR primers used in this study**

| Primer name | Sequence          | 5'—GGATCCATGGTGGAAGAAAGCAAG-3' | 5'—GGATCCTGCAGCAAGATCCATG-3' | 5'—GGATCCTGCAGCAAGATCCATG-3' | 5'—GGATCCTGCAGCAAGATCCATG-3' |
|-------------|-------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| NbNSN1-F    |                   |                                 |                               |                               |                               |
| NbNSN1-R    |                   |                                 |                               |                               |                               |
| NbNSN1-N    |                   |                                 |                               |                               |                               |
| NbNSN1-N-R  |                   |                                 |                               |                               |                               |
| NbNSN1(C)   |                   |                                 |                               |                               |                               |
| NbNSN1(C)-R |                   |                                 |                               |                               |                               |
| EB2(F)      |                   |                                 |                               |                               |                               |
| EB2(R)      |                   |                                 |                               |                               |                               |
| EB2(N)      |                   |                                 |                               |                               |                               |
| EB2(N)-R    |                   |                                 |                               |                               |                               |
| EB2(C)      |                   |                                 |                               |                               |                               |
| EB2(C)-R    |                   |                                 |                               |                               |                               |
| NSN1(A)     |                   |                                 |                               |                               |                               |
| NSN1(A)-R   |                   |                                 |                               |                               |                               |
| NSN1-B      |                   |                                 |                               |                               |                               |
| NSN1-B-R    |                   |                                 |                               |                               |                               |
| NSN1-C      |                   |                                 |                               |                               |                               |
| NSN1-C-R    |                   |                                 |                               |                               |                               |
| NSN1-F      |                   |                                 |                               |                               |                               |
| NSN1-R      |                   |                                 |                               |                               |                               |
| UBC10-F     |                   |                                 |                               |                               |                               |
| UBC10-R     |                   |                                 |                               |                               |                               |
| α-tubulin-F |                   |                                 |                               |                               |                               |
| α-tubulin-R |                   |                                 |                               |                               |                               |
| NSN1(GFP)-F |                   |                                 |                               |                               |                               |
| NSN1(GFP)-R |                   |                                 |                               |                               |                               |
| NSN1-N(GFP)-F |              |                                 |                               |                               |                               |
| NSN1-N(GFP)-R |             |                                 |                               |                               |                               |
| ΔN(GFP)-F   |                   |                                 |                               |                               |                               |
| ΔN(GFP)-R   |                   |                                 |                               |                               |                               |
| ΔG(D)-F     |                   |                                 |                               |                               |                               |
| ΔG(D)-R     |                   |                                 |                               |                               |                               |
| NSN1(C(GFP)-F |                |                                 |                               |                               |                               |
| NSN1(C(GFP)-R |               |                                 |                               |                               |                               |
| ΔG1-3(F)-F  |                   |                                 |                               |                               |                               |
| ΔG1-3(F)-R  |                   |                                 |                               |                               |                               |
| NSN1(MBP)-F |                   |                                 |                               |                               |                               |
| NSN1(MBP)-R |                   |                                 |                               |                               |                               |
| NSN1(N(MBP)-F |              |                                 |                               |                               |                               |
| NSN1(N(MBP)-R |             |                                 |                               |                               |                               |
| RBD(MBP)-F  |                   |                                 |                               |                               |                               |
| RBD(MBP)-R  |                   |                                 |                               |                               |                               |

**VIGS constructs**

- Supplementary Table S1

- Realtime qRT-PCR for VIGS

- DEX-inducible RNAi constructs

- Realtime qRT-PCR for RNAi

- Controls for qRT-PCR

- GFP-tagging constructs

- MBP fusion constructs

### Supplementary Table S2

**Supplementary Table S2**

| Primer name | Sequence | 5'—GGATCCATGGTGGAAGAAAGCAAG-3' | 5'—GGATCCTGCAGCAAGATCCATG-3' |
|-------------|----------|---------------------------------|-------------------------------|
| NbNSN1(F)   |          |                                 |                               |
| NbNSN1(R)   |          |                                 |                               |
| NbNSN1(N)   |          |                                 |                               |
| NbNSN1(R)   |          |                                 |                               |
| NbNSN1(C)   |          |                                 |                               |
| NbNSN1(C)   |          |                                 |                               |
| EB2(F)      |          |                                 |                               |
| EB2(R)      |          |                                 |                               |
| EB2(N)      |          |                                 |                               |
| EB2(N)      |          |                                 |                               |
| EB2(C)      |          |                                 |                               |
| EB2(C)      |          |                                 |                               |
| NSN1(A)     |          |                                 |                               |
| NSN1(A)     |          |                                 |                               |
| NSN1-B      |          |                                 |                               |
| NSN1-B      |          |                                 |                               |
| NSN1-C      |          |                                 |                               |
| NSN1-C      |          |                                 |                               |
| NSN1-F      |          |                                 |                               |
| NSN1-R      |          |                                 |                               |
| UBC10-F     |          |                                 |                               |
| UBC10-R     |          |                                 |                               |
| α-tubulin-F |          |                                 |                               |
| α-tubulin-R |          |                                 |                               |
| NSN1(GFP)-F |          |                                 |                               |
| NSN1(GFP)-R |          |                                 |                               |
| NSN1-N(GFP)-F |        |                                 |                               |
| NSN1-N(GFP)-R |       |                                 |                               |
| ΔN(GFP)-F   |          |                                 |                               |
| ΔN(GFP)-R   |          |                                 |                               |
| ΔG(D)-F     |          |                                 |                               |
| ΔG(D)-R     |          |                                 |                               |
| NSN1(C(GFP)-F |        |                                 |                               |
| NSN1(C(GFP)-R |       |                                 |                               |
| ΔG1-3(F)-F  |          |                                 |                               |
| ΔG1-3(F)-R  |          |                                 |                               |
| NSN1(MBP)-F |          |                                 |                               |
| NSN1(MBP)-R |          |                                 |                               |
| NSN1(N(MBP)-F |       |                                 |                               |
| NSN1(N(MBP)-R |       |                                 |                               |
| RBD(MBP)-F  |          |                                 |                               |
| RBD(MBP)-R  |          |                                 |                               |
## Supplementary Table S1-continued

| Primer name | Sequence | BiFC constructs | Co-IP constructs | RT-PCR constructs |
|-------------|----------|----------------|-----------------|------------------|
| NSN1(YFP<sup>-</sup>)-F | 5'-GGATCCATGTTGAAACGGAGTAA-3' | | | |
| NSN1(YFP<sup>-</sup>)-R | 5'-GGATCCCTATTCTCTCAAGGCAA-3' | | | |
| PES(YFP<sup>-</sup>)-F | 5'-ACTAGTATGGCCAGACATTACA-3' | | | |
| PES(YFP<sup>-</sup>)-R | 5'-CCCGAGTGTAGTGTGTTAGTAC-3' | | | |
| PES(YFP<sup>-</sup>)-F | 5'-ACTAGTATGGCCAGACATTACA-3' | | | |
| PES(YFP<sup>-</sup>)-R | 5'-CCCGAGTGTAGTGTGTTAGTAC-3' | | | |
| EBP2(YFP<sup>-</sup>)-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| EBP2(YFP<sup>-</sup>)-R | 5'-CTCGAGCTCTTCTTGTGGCTT-3' | | | |
| RPL13(YFP<sup>-</sup>)-F | 5'-GGATCCATGTTGCTTCTAGTAC-3' | | | |
| RPL13(YFP<sup>-</sup>)-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| RPL14(YFP<sup>-</sup>)-F | 5'-GGATCCATGTTGCTTCTAGTAC-3' | | | |
| RPL14(YFP<sup>-</sup>)-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| RPS6(YFP<sup>-</sup>)-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| RPS6(YFP<sup>-</sup>)-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| RPL11(YFP<sup>-</sup>)-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| RPL11(YFP<sup>-</sup>)-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PES-Flag-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PES-Flag-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| NSN1-1A-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| NSN1-1A-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| EBP2-Myc-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| EBP2-Myc-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG12-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG12-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG13-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG13-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG13-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG13-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG121-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG121-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG27-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG27-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG27-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SAG27-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SIRK-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| SIRK-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| WRKY6-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| WRKY6-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PR1-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PR1-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| APG7-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| APG7-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| APG9-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| APG9-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| ATG18a-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| ATG18a-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PORA-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PORA-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PORB-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PORB-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PORC-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| PORC-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| Actin8-F | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
| Actin8-R | 5'-GGATCCATTCTTTGGAAAGAGA-3' | | | |
Supplementary Fig. S1. Protein sequence alignment of NSN1 with its homologs.

Amino acid sequences of *Arabidopsis* NSN1 (At3g07050) and its homologs from *Nicotiana benthamiana* (Nb NSN1: NbS00039776g0013), *Oryza sativa* (Os NSN1; Os01g0375000), *Homo sapiens* (NS; AAV74413), and *Saccharomyces cerevisiae* (Nug1; P40010) were aligned. The conserved GTPase motifs (G4, G1, and G3) and the “DAR” motif of unknown function are defined according to Matsuo *et al.* (2014) and marked with *overlines*. Motifs G2 and G5 are ill defined in this group of GTPases. The deletion point of NSN1ΔN is marked in red.
Supplementary Fig. S2. Expression profiles of *Arabidopsis NSN1* (At3g07050) based on the Genevestigator program (https://www.genevestigator.com/).

(A) *NSN1* transcript levels in various tissues relative to the transcript level in roots.

(B) Relative *NSN1* transcript levels in plants at various developmental stages.
Supplementary Fig. S3. Quantification of total chlorophyll, chlorophyll $a$, and chlorophyll $b$ contents in TRV and TRV:NbNSN1(F) leaves at 20 DAI.
**Supplementary Fig. S4.** Nucleolar localization of NSN1.

GFP-fused NSN1 was expressed in *N. benthamiana* leaves by agroinfiltration. GFP fluorescence in mesophyll protoplasts prepared from the infiltrated leaves was examined by confocal microscopy. Nuclei were visualized by DAPI staining. Chlorophyll autofluorescence is shown in red.
**Supplementary Fig. S5.** Subcellular localization of NSN1 mutants.

(A) Optical sectioning generated diverse fluorescent images of ΔN:GFP proteins in a single *N. benthamiana* leaf protoplast. Chlorophyll autofluorescence is shown in red.

(B) Expression of ΔN:GFP and NSN1-C:GFP proteins in the infiltrated leaves was determined by immunoblotting with anti-GFP antibody.
**Supplementary Fig. S6.** BiFC analyses.

BiFC was performed to visualize the interactions of NSN1 and its deletion mutants with PES (A) and EBP2 (B). YFP\(^N\) (Y\(^N\))- and YFP\(^C\) (Y\(^C\))-fusion proteins were expressed together in *N. benthamiana* leaves by agroinfiltration. YFP fluorescence in mesophyll protoplasts prepared from the infiltrated leaves was examined by confocal microscopy.
**Supplementary Fig. S7.** BiFC between NSN1 and PES mutants.

BiFC was performed with NSN1 and the N-terminal domain of PES (PES-N) or the PES mutant lacking the PES-N domain (ΔPES-N). YFP<sup>N</sup>- and YFP<sup>C</sup>-fusion proteins were expressed together in *N. benthamiana* leaves by agroinfiltration. YFP fluorescence in mesophyll protoplasts prepared from the infiltrated leaves was examined by confocal microscopy. *N*, nucleus; *nu*, nucleolus.
**Supplementary Fig. S8.** Immunoblotting with anti-RPL10a antibody to determine cellular levels of RPL10a proteins.

The 4th leaf above the infiltrated leaf of the VIGS plants at 14 DAI were used for the analysis. Coomassie blue-stained Rubisco large subunits (RbcL) were used as loading control.
Supplementary Methods S1

Virus-induced gene silencing (VIGS)

Functional genomics using Tobacco Rattle Virus (TRV)-based virus-induced gene silencing was carried out in *Nicotiana benthamiana* as described (Ahn et al., 2011; Cho et al. 2013). Various cDNA fragments of *N. benthamiana* NSN1 and EBP2 were polymerase chain reaction (PCR)-amplified and cloned into the pTV00 vector containing part of the TRV genome (Cho et al., 2013). The recombinant pTV00 plasmids and pBINTRA6 vector containing RNA1, which is required for virus replication, were separately transformed into *Agrobacterium tumefaciens* GV3101. The third leaf of *N. benthamiana* (3-week-old plants) was pressure-infiltrated with the *Agrobacterium* suspension. The fourth leaf above the infiltrated leaf was used for real-time quantitative reverse transcription (RT)-PCR to detect gene silencing.

Generation of dexamethasone (DEX)-inducible NSN1 RNAi lines in Arabidopsis

A 318-bp *NSN1* cDNA fragment was PCR amplified using NSN1-sense(F)/(R) primers containing XhoI and ClaI sites (5'-agacggttaacgaattcaacctg-3' and 5'-aactctccgctcatca gatctcc-3') for the sense construct and NSN1-antisense(F)/(R) primers containing SpeI and PstI sites (5'-agacggttaacgaattcaacctg-3' and 5'-aactctccgctcatca gatctcc-3') for the antisense construct. Using these constructs, DEX-inducible NSN1 RNAi transgenic *Arabidopsis* lines (ecotype Columbia-0) were generated as described (Ahn et al., 2011). Transgenic plants were selected on growth media containing hygromycin (30 mg/L). For induction of RNAi, dexamethasone (Sigma) was added to the medium to a final concentration of 10 or 20 μM in ethanol (0.033%) and tween 20 (0.01 % w/v) from 30 mM stock solution.

Agrobacterium-mediated transient expression

Agroinfiltration was carried out as described previously (Voinnet et al., 2003). Agrobacterial cultures (*Agrobacterium tumefaciens* GV3101 strain) containing various constructs fused to the CaMV35S promoter were adjusted to OD$_{600}$=0.6 in MES buffer (10 mM MES, pH 7.5, 10 mM MgSO$_4$). The suspension was incubated with acetosyringone for 2–3h at a final concentration of 150 μM, and infiltrated into leaves of wild-type *N. benthamiana* plants. In all experiments, *Agrobacterium* C58C1 carrying the 35S:p19 construct (Voinnet et al., 2003) was co-infiltrated to achieve maximum levels of protein expression. Expressed proteins
were analyzed at 48h post-infiltration.

Real-time quantitative RT-PCR
Real-time quantitative PCR was carried out using gene-specific primers as described previously (Cho et al., 2013).

Bimolecular fluorescence complementation (BiFC)
The cDNAs of EBP2, PES, and various ribosomal protein genes of Arabidopsis were PCR-amplified and cloned into the pSPYNE vector containing the N-terminal region of YFP (YFP\textsuperscript{N}; amino acid residues 1-155). Similarly, the coding region of NSNI and PES were cloned into pSPYCE vector containing the C-terminal region of YFP (YFP\textsuperscript{C}; residues 156–239). Various combinations of these pSPYNE and pSPYCE fusion constructs were agroinfiltrated together into the leaves of 3-week-old N.benthamiana plants as described (Cho et al., 2013). After 48 h, protoplasts were generated and YFP signal was detected using a confocal laser scanning microscope (Zeiss LSM510).

Measurement of in vivo \( \text{H}_2\text{O}_2 \) levels
NBT staining was carried out as described previously (Lee et al., 2013). \( \text{H}_2\text{DCFDA} \) staining was carried out as described previously (Ahn et al., 2011).

Immunoblotting
Protein extracts (30\( \mu \)g) were subjected to SDS-PAGE and immunoblotting as described (Ahn et al., 2011; Cho et al., 2013). Immunoblotting was performed using mouse monoclonal antibodies against the HA tag (Applied Biological Materials), Myc tag (ABM), Flag tag (Sigma), GFP (Clontech), and RPL10a (Santa Cruz Biotechnology). The membranes were then treated with horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (1:10,000; Invitrogen). Signals were detected on X-ray film (Kodak) using an ECL chemiluminescence kit (ELPIS-Biotech, Inc.).

Co-immunoprecipitation
HA-tagged NSN1 and Myc-tagged EBP2, or HA-tagged NSN1 and Flag-tagged AtPES were co-expressed in N. benthamiana leaves by agroinfiltration. After 48-h incubation, leaf extracts were prepared and co-immunoprecipitation was performed according to Ahn et al.
After SDS-PAGE, western blotting was carried out using the monoclonal anti-HA antibody (1:2500; ABM), anti-Flag antibody (1:10,000; Sigma), and anti-Myc antibody (1:10,000; ABM).

Purification of recombinant proteins
To purify recombinant proteins of NSN1 and its mutants for GTPase assays, the corresponding NSN1 cDNA fragments were PCR-amplified and cloned into the pMAL™c2 vector (New England Biolabs). The MBP fusion proteins were purified using amylose resin following the manufacturer's instructions (New England Biolabs). Purified NSN1 protein and its variants were concentrated using Amicon Ultra Centrifugal Filters (Millipore). To purify MBP:NSN1-N and MBP:RBD for RNA-binding assays, the NSN1 cDNA fragments corresponding to amino acid residues 1–174 and 374–400, respectively, were PCR-amplified and cloned into the pMAL™c2 vector, and the recombinant proteins were purified as described above.

GTPase assay
The turnover rate \( (k_{cat}) \) of recombinant proteins of NSN1 was measured as described previously (Im et al., 2011; Jeon et al., 2014). A reaction mixture containing 3 μM recombinant proteins and 1 mM GTP in GTPase assay buffer (20 mM Hepes, pH 8, 1 mM MgCl\(_2\), 0.5 mM DTT, and 1 mM NaN\(_3\)) was incubated at room temperature for 18 h. The released phosphate was quantified using the Biomol green reagent (Biomol Research Laboratories) according to the manufacturer’s protocol. The catalytic constant was derived from the equation \( k_{cat} = \frac{V_{max}}{C_{recombinant protein}} \).

Pulse-amplitude-modulation (PAM) fluorometry
Arabidopsis plants were grown for 2 weeks in MS medium containing either ethanol or 20 μM DEX, then transferred to soil, and sprayed with ethanol or 20 μM DEX for 3-4 days. The plants were incubated in the dark for 4 h, and then the fluorescence analysis was performed with the 5\(^{th} \) rosette leaf. The Fv/Fm ratio was determined as described (Kramer et al., 2004). Chlorophyll a fluorescence traces were measured using a FMS1 fluorometer (Hansatech instruments, Norfolk, England). \( F_m \) and \( F_0 \) are the maximum levels of fluorescence before and after the steady state during the actinic illumination, respectively. \( F_s \) is the steady-state fluorescence level. The intensities of saturating pulse and actinic lights were more than 5,000
µmol photons / m²·s and 600 µmol photons / m²·s, respectively. The quantum yield of photosystem II-mediated photosynthesis (ΦII) is defined as \( \Phi_{II} = \frac{F_s - F_m^\prime}{F_m^\prime} \).

**Statistical analyses**

Two-tailed Student’s \( t \)-tests were performed using the Minitab 16 program (Minitab Inc.; http://www.minitab.com/en-KR/default.aspx) to investigate the statistical differences between the responses of the samples. Significant differences between control and other samples were indicated by one \( (P \leq 0.05) \) or two \( (P \leq 0.01) \) asterisks.

**Supplementary References**

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