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SI Materials and Methods

Plant Materials and Growth Conditions. The prematurely senile 1 (ps1-D) mutant was identified by screening our transferred-DNA (T-DNA) population in the japonica Nipponbare background (1). For expression analysis, various organs were collected starting from the heading stage. To determine the fine expression pattern of Oryza sativa NAC (no apical meristem, Arabidopsis ATAF1/2, and cup-shaped cotyledon2)-like, activated by apetala3/pistillata (OsNAP), flag leaves were harvested every 7 d after leaf emergence, and seeds were collected every 2 d after flowering. For PS1 induction analysis, wild-type plants were hydroponically cultured with normal half-strength modified Hoagland medium after germination in the greenhouse for 10 d at 30 °C and with a 14-h light/24 °C, 10-h dark cycle. Whole 10-dold Nipponbare plants were immersed in nutrition solutions containing different kinds of phytohormones, including brassinosteroid (50 μm), gibberellin (50 μm), auxin (50 μm), 6-benzylaminopurine (50 μm), salicylic acid (100 μm), 1-aminocyclopropane-1-carboxylic acid (50 μ m), jasmonic acid (50 μ m), and abscisic acid (ABA) (50 μ m), and were maintained for 2 h in the dark under cold (4 °C) and alkaline ($pH = 11$) conditions with 200 mM mannitol and 200 mM NaCl. Twenty plants were collected for RNA isolation. All experiments were repeated three times independently.

Cloning PS1. Genetic linkage analysis was performed with the T-DNA insertion using the T_1 progenies of the *ps1-D* mutant. The flanking sequence of the T-DNA in ps1-D was isolated by SiteFinding PCR (2). (The primers used for SiteFinding PCR are listed in Table S5.)

Constructions for Rice Transformation. For the $OsNAP$ overexpression construct, a 1,179-bp section of full-length cDNA was amplified by PCR from the cDNA library of Nipponbare and was subcloned into the XmaI and XbaI sites of the binary vector pCAMBIA2300-Actin1 to generate the pAOS construct. To make the OsNAP RNAi construct, a 274-bp gene-specific fragment from bp 457–730 of the OsNAP ORF was amplified, and cloned into the SalI and PstI sites of the binary vector pCAMBIA2300-35S as described previously (5). For promoter analysis, the fragment ~1.9 kb upstream of the OsNAP 5' region was amplified and ligated into the pCAMBIA2391Z vector between SalI and EcoRI. The wild-type rice variety Nipponbare, was used as the recipient for all transformations by the Agrobacterium tumefaciens-mediated method (3). The primer sequences are listed in Table S6.

Constructions for Yeast One-Hybrid Assay. To generate activation domain OsNAP (AD-OsNAP), the full-length OsNAP was amplified using thermostable DNA polymerase from Thermococcus kodakaraensis (KOD) polymerase (Toyobo) and was subcloned into the EcoRI and XhoI sites of the binary vector pJG4-5 vector (Clontech). To generate SGRp::LacZ, NYC1p::LacZ, NYC3p:: LacZ, RCCR1p::LacZ, Osh36p::LacZ, Osh69p::LacZ, and OsI57p:: LacZ reporter constructs, the promoter fragments were amplified by PCR and then cloned into the pLacZi2μ vector. The primer sequences are listed in Table S6.

Expression Analysis. Total RNA was extracted using a TRIzol kit (Invitrogen) according to the manufacturer's instructions. RNA was reverse-transcribed using the ReverTra Ace quantitative PCR RT Master Mix Kit with gDNA remover (Toyobo). Quanti-

tative real-time PCR was performed with a Chromo4 real-time PCR detection system (Bio-Rad) according to the manufacturer's instructions. Rice *OsActin1* was used as an internal control. The primers used for quantitative RT-PCR (qRT-PCR) are listed in Table S6. Values are means \pm SD of three biological repeats. The Student t test was used for statistical analysis.

β-Glucuronidase Staining. β-Glucuronidase (GUS) staining was performed as described previously (4). Images were taken directly or with a stereomicroscope (SZX16; Olympus).

Chlorophyll Measurements. Chlorophyll was extracted from 50 mg of leaf tissue (fresh weight), and its content was determined by measuring the absorbance at 652 nm using a Tecan Infinite M200 multimode reader (Tecan Group Ltd) as described previously (5).

Transactivation Activity Assay in Yeast. The coding sequences of OsNAP and OsNAP fragments were obtained by PCR. Primers used are included in Table S6. The PCR products were digested by NdeI/EcoR, and then cloned into the vector pGBKT7. Three constructs with deletions in middle of the gene were made also, and the N-terminal parts (amino acids 1–64, 1–100, and 111– 142) of these constructs were fused to the C-terminal fragment (amino acids 181–392). The Matchmaker Gold Yeast Two-Hybrid system (Clontech) was used for the transactivation activity assay according to the manufacturer's protocol. Each yeast liquid culture was diluted serially to $OD_{600} = 0.6$, and 3 μ L of each dilution was inoculated onto tryptophan-, histidine-, and adenine-negative synthetic dropout medium.

Dark-Induced Leaf Senescence. Fully expanded flag leaves were excised carefully. Detached leaves were cut into ∼3-cm pieces and floated on 25 mL of water or 50 μM of ABA solution in Petri dishes with the adaxial side up. The samples were incubated at 28 °C in darkness for 5–10 d (5).

Yeast One-Hybrid Assay. Plasmids for the GAL DNA-binding domain fused with OsNAP were cotransformed with the LacZ reporter genes driven by SGR, NYC1, NYC3, RCCR1, Osh36, OsI57, and Osh69 promoter fragments (Clontech) into the yeast strain EGY48 using a standard transformation technique. Transformants were grown on proper drop-out plates containing X-Gal for blue color development (6). Representative data are shown from one of the three biological replicates, which yielded similar results.

ABA Analysis. Extraction and determination of ABA from agematched flag leaves at the heading stage was performed as described (7). Four independent biological repeats were performed.

Microarray Hybridization and Data Analysis. For the microarray experiment, RNAs from fully expanded flag leaves of wild-type and ps1-D plants were used for microarray hybridization (CapitalBio Corporation). Probe labeling and chip hybridization were carried out through the Affymetrix custom service following the standard protocol (www.affymetrix.com/). Normalization was performed according to the standard Affymetrix protocol to allow the comparison of the samples for each set of experiments. The differently expressed genes (DEGs) in wild-type and ps1-D plants were classified functionally using the biological process category of Rice Gene Ontology [\(www.geneontology.com\)](http://www.geneontology.com). The percentage shown in Table S1 refers to the ratio of genes relative

to the total up-regulated or down-regulated DEGs in each functional category. Three independent biological replicates were conducted for the wild-type and $ps1-D$ plants, and DEGs with log_2 ratios ≥ 1.00 or ≤ -1.00 (only GO Slim IDs with P values ≤ 0.5) were analyzed.

Element and Protein Measurements. Element and protein measurements were performed as described $(8, 9)$.

ChIP-PCR Assay.The wild-type rice (Nipponbare) and its transgenic calli harboring the *Pro35S:OsNAP-MYC* construct were used for ChIP assays according to the method described previously (10). Antibody to the MYC tag was used to immunoprecipitate OsNAP-MYC and associated DNA fragments. Immunoprecipitation was performed with anti-MYC antibody (A-MYC) or without antibody (No Ab). Fragments (2,000 bp) of sequences upstream of ATG were chosen as the promoter regions for designing primers. The promoter of *Ubiquitin* was used as a negative control. Three independent biological repeats were performed.

Phylogenetic Analysis. Protein sequences showing similarity to OsNAP were retrieved using the public BLAST on the National Center for Biotechnology Information Website [\(www.ncbi.nlm.](http://www.ncbi.nlm.nih.gov/) [nih.gov/](http://www.ncbi.nlm.nih.gov/)). Multiple alignments of the homologs were performed by Clustal X version 2.0 with the default parameters and manually adjusted (11).

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Field Cultivation of Rice. The rice plants were grown in a standard paddy field at the Experimental Station of the Institute of Genetics and Developmental Biology. The field planting followed a randomized complete-block design with three replicates; the plants were transplanted at a spacing of 20×20 cm. The area per plot was 4 m^2 . These plants then were grown under conventional cultivation conditions. Agronomic traits were measured using at least 10 plants randomly obtained from the center of the plot to avoid any irregularities present at the plot margins.

Accession Codes. Genes and their associated accession codes from GenBank/EMBL are as follows: Osh36 (AF251070), OsI57 (AF251076), Osh69 (AF251068), OsI85 (AF251075), OsNCED1 (AY838897), OsNCED2 (AY838898), OsNCED3 (AY838899), OsNCED4 (AY838900), and OsNCED5 (AY838901). Genes and their associated accession codes from Rice Annotation Project Database are as follows: OsNAP (LOC_Os03g21060), G1 (LOC_ Os03g21020), G2 (LOC_Os03g21030), G3 (LOC_Os03g21040), G4 (LOC_Os03g21050), G5 (LOC_Os03g21070), G6 (LOC_ Os03g21080), G7 (LOC_Os03g21090), G8 (LOC_Os03g21100), G10 (LOC_Os03g21110), SGR (LOC_Os09g36200), NYC1 (LOC_Os01g12710), NYC3 (LOC_Os06g24730), RCCR1(LOC_ Os10g25030), OsZEP (LOC_Os04g37619), OsABA8OX1 (LOC_ Os02g47470), OsABA8OX2 (LOC_Os08g36860), and OsABA8OX3 (LOC_Os09g28390).

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Fig. S1. Characterization of ps1-D mutants at the vegetative growth stage. (A) Phenotypes of the ps1-D mutant in the vegetative growth stage. (Scale bar: 20 cm.) (B) The upper five leaves from the main culm of ps1-D mutants and wild-type plants 60 d after transplanting. (C) Chlorophyll content of the leaves shown in B. Values are means \pm SD of 10 measurements. (D) Chlorophyll content of the leaves shown in Fig. 1D. Values are means \pm SD of 10 measurements. (E and F) Expression of the senescence markers Osh36 (E) and OsI57 (F) in the detached leaves of ps1-D mutants and wild-type plants incubated with water for 5 d in darkness. ** $P \le 0.01$; Student t test.

Fig. S2. Cloning of PS1. (A) Schematic representation of the T-DNA flanking region of the ps1-D mutant. LB and RB represent the left and right borders of the T-DNA, respectively. (B) Flanking sequence was confirmed by PCR using the primers indicated in A. Heter, heterozygous ps1-D mutant; Homo, homozygous ps1-D mutant. (C) The expression of PS1 was specifically activated in ps1-D mutants. G1–G4 are four genes located in the 50-kb region upstream of the T-DNA insertion site; G5-G10 are six genes located in the 50-kb region downstream of the PS1 gene. **P \leq 0.01; Student t test. (D) Overexpression of PS1 mimics the premature senescence phenotype of ps1-D mutants. (Scale bar: 20 cm.) (E) qRT-PCR analysis of PS1 transcript levels in wild-type plants and three lines overexpressing PS1 (OE1-OE3). (F) Protein sequence alignment of PS1 and homologs in Arabidopsis and wheat using Clustal W. Conserved amino acids are highlighted: White letters on a black background indicate amino acids that are conserved across all three samples (100% conservation); black letters on a gray background indicate amino acids that are conserved in two of our three samples (66% conservation).

Fig. S3. Transcriptional analysis of OsNAP. (A) Schematic structure of Va (1)/ NAP members in rice. Numbers indicate the position of amino acids. (B) (Left) Schematic structure of OsNAP protein. Numbers indicate the position of amino acids. (Right) Truncation analysis of transcriptional activation activity of OsNAP. Various truncated OsNAPs were constructed as bait vectors. BD, GAL4 DNA-binding domain. Empty control vector (BD) and GAL4 genes were used as negative and positive controls, respectively. All bait vectors were transformed into yeast strain Y2HGold (Invitrogen). Yeast was grown at 30 °C for 3 d on the synthetic dextrose agar plates lacking leucine and histidine and containing different concentrations of 3-amino-1,2,4-triazole, as indicated.

Fig. S4. Analysis of OsNAP expression. (A) Expression of OsNAP in various organs, including root, culm, leaf blade, leaf sheath, and panicle at the heading stage and endosperm 7 d after flowering. (B) Expression of OsNAP in leaves at different developmental stages. LS, leaf at the late stage of senescence, with >50% leaf area yellowing; NS, fully expanded, nonsenescent leaf; OS, leaf at the onset of senescence, with <5% leaf area yellowing; YL, young leaf approximately half the size of a fully expanded leaf. (C) Change over time in the OsNAP transcription levels of the seeds. Y1, young panicle (1–2 cm). (D) OsNAP expression in different leaves at the heading stage. L1–L5, leaf of plants numbered from top to bottom.

Fig. S5. Expression of ABA-related genes. **P \leq 0.01; Student t test. (A) Expression of ABA biosynthetic genes in wild-type and ps1-D mutant plants. (B) Expression of ABA metabolism genes in wild-type and ps1-D mutant plants.

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Fig. S6. Confirmation of OsNAP function by RNAi (Ri) transgenic lines. (A) Expression analysis of five orthologous members of OsNAP among wild-type, Ri1, and Ri2 transgenic lines. LOC_Os05g34310 was not detected in leaves. (B and C) Expression analysis of four chlorophyll degradation-related genes (CDGs) (SGR, NCY1, NCY3, and RCCR1) (B) and four other senescence-associated genes (SAGs) (Osh39, OsI57, Osh69, and OsI85) (C) in wild-type and OsNAP Ri transgenic lines. (D) Delay in the dark- and ABA-induced senescence phenotype of the OsNAP RNAi leaves. Detached flag leaves from the wild-type and OsNAP RNAi lines at the heading stage were incubated with continuous light (H₂O), darkness (H₂O), and ABA (continuous light). PT, Pretreatment. (Scale bar: 2 cm.) (E) Chlorophyll content of the detached leaves; labels are as in D. Values are means \pm SD of five measurements. (F-H) Expression of OsNAP (F), Osh36 (G), and OsI57 (H) in detached leaves; labels are as in D. *P \leq 0.05; **P \leq 0.01; Student t test.

Fig. S7. OsNAP binds directly to the promoter regions of nutrient transport-related genes. ChIP-PCR analysis of the promoter region of SAGs. The ChIP assays were performed using calli expressing the OsNAP-MYC fusion protein. Antibody to the MYC tag was used to immunoprecipitate OsNAP-MYC and associated DNA fragments. Immunoprecipitation was performed with or without (No Ab) anti-MYC antibody. Fragments (2,000 bp) of sequences upstream of ATG were chosen as the promoter regions for designing primers. The promoter of Ubiquitin was used as a negative control.

Table S1. Functional classification of DEGs in ps1-D

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DEGs, differentially expressed genes. Functional classification of the DEGs was performed using the biological process category of the Rice Gene Ontology [\(http://www.geneontology.com](http://www.geneontology.com)). Percentage refers to the ratio of genes in each functional category to the total up-regulated or down-regulated DEGs identified in the microarray experiment.

Locus	Annotation	Fold change
Up-regulated		Log2 ratio
LOC_Os07q33910	Transporter family protein	3.70
LOC_Os04q38680	Transmembrane amino acid transporter protein	2.52
LOC_Os12q02310	Lipid transport	1.98
LOC_Os06q05160	Sulfate transporter	1.77
LOC_Os01q65110	Proton-dependent oligopeptide transporter family protein	1.38
LOC_Os11g02389	Lipid transport	1.37
LOC_Os04q32920	Potassium transporter	1.21
LOC_Os02q57240	Probable voltage-gated potassium channel subunit beta	1.15
LOC_Os01q31940	Purine permease	1.14
LOC_Os03q40780	Transport protein-related	1.08
LOC_Os12q44100	Peptide transporter PTR2	1.06
LOC_Os12g02320	Transport	1.05
LOC Os07q15460	Metal transporter Nramp6	1.05
LOC_Os10g22039	Mitochondrial import inner membrane translocase subunit Tim17	1.03
Down-regulated		
LOC_Os06g22960	Aquaporin protein	-1.82
LOC_Os03q10640	Calcium-transporting ATPase, plasma membrane-type	-1.76
LOC Os02q07830	OsHKT6, high affinity potassium transporter	-1.63
LOC_Os06q38120	Low-affinity cation transporter	-1.54
LOC_Os07q26660	Aquaporin protein	-1.53
LOC_Os12g12934	Peptide transporter PTR3-A	-1.46
LOC Os02q21340	ABC-2 type transporter family protein	-1.41
LOC_Os02q27490	Bile acid sodium symporter family protein	-1.39
LOC_Os04q37990	Transporter family protein	-1.27
LOC_Os05q07870	Triose phosphate/phosphate translocator	-1.22
LOC_Os06q29790	Phosphate transporter 1	-1.20
LOC_Os10q28240	Calcium-transporting ATPase, plasma membrane-type	-1.18
LOC_Os12g08130	Amino acid transporter	-1.16
LOC_Os04g12499	Amino acid transporter protein	-1.08
LOC_Os04q55260	Thiamine-repressible mitochondrial transport protein THI74	-1.03
LOC_Os02q37160	Heavy metal transport/detoxification protein	-1.02

Table S3. Residual nitrogen, P, S, K, Mg, Fe, and Zn in flag leaves among wild type, ps1-D, OsNAP OE, and RNAi transgenic lines

Ri1 and Ri2 represent two independent OsNAP RNAi lines. Differences between the means of wild type and ps1-D, OE, or RNAi transgenic plants (Δ %) are reported in terms of change with respect to the wild type. Values are means \pm SD of ten biological replicates.

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Table S4. Agronomic traits of ps1-D and OsNAP RNAi transgenic lines

Ri1 and Ri2 represent two independent OsNAP RNAi lines. DWV, dry weight of vegetative organs. NPR stands for the rate of net photosynthesis (400 μmol•CO2•m^{−2}•s^{−1}), measured at the heading stage (0 DAF) and 15 d after flowering (15 DAF). Values are means \pm SD from at least 10 plants in Beijing. Asterisks indicate a statistically significant difference in comparison to the wild type (*P \leq 0.05).

SF SFP ₁	CACGACACGCTACTCAACACACCACCTCGCACAGCGTCCTCAAGCGGCCGCNNNNNNGCCT CACGACACGCTACTCAAC
SFP ₂	ACTCAACACACCACCTCGCACAGC
SP ₁	AGGCATCGATCGTGAAGTTTCTCATC
SP ₂ SP ₃	CATTTGGACGTGAATGTAGACACGTCG GCTTTCGCCTATAAATACGACGGATCG

Table S6. Primers used in this study for qRT-PCR, to generate DNA constructs, for ChIP-PCR, and for yeast one-hybrid assays

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Table S6. Cont.

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The underlined nucleotides indicate the restriction sites for cloning.