Suppementary Information

ONAC103 isoforms generated by alternative promoter coordinately regulate leaf senescence in rice

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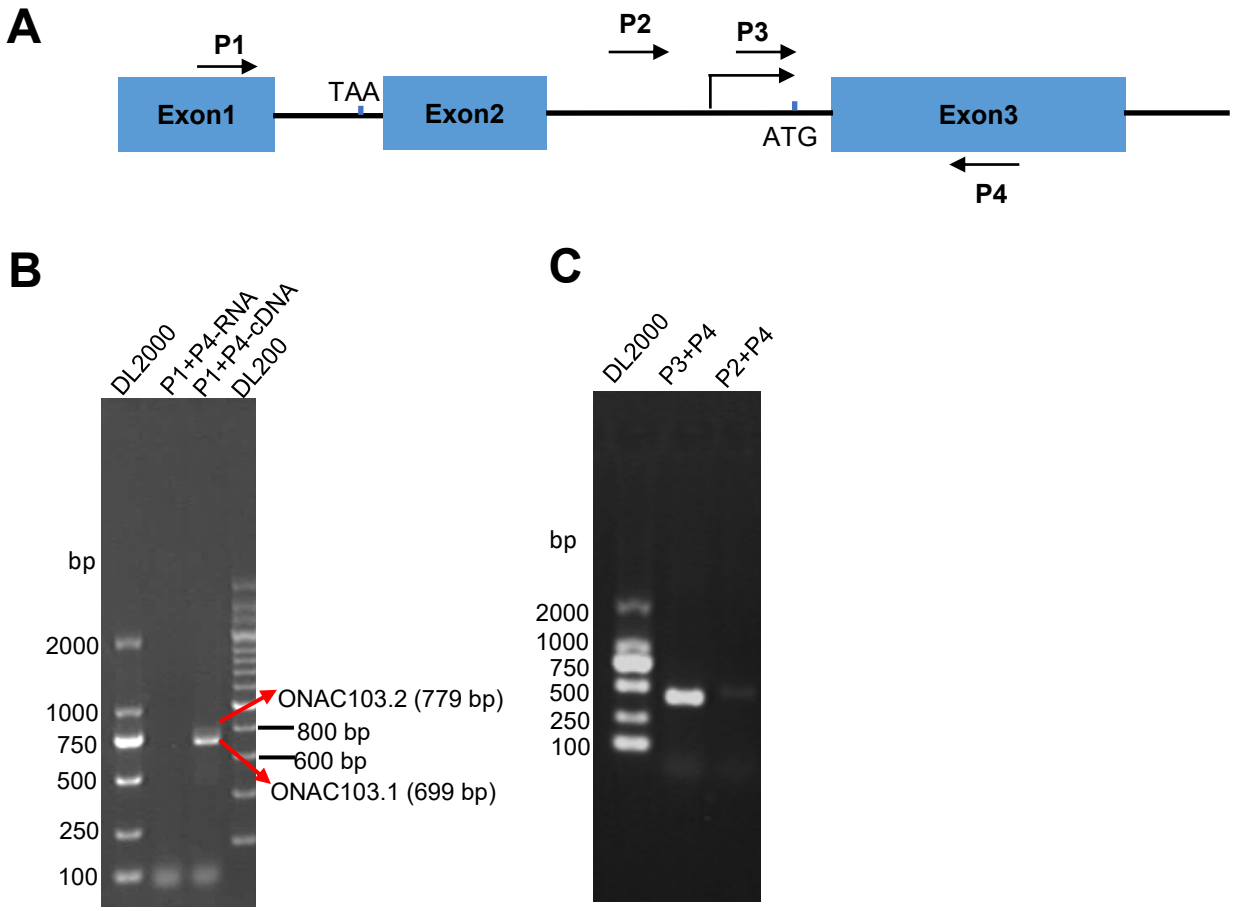

Figure S1. Identification of RNA isoforms generated by ONAC103. A. Primer sites for the semi-quantitative RT-qPCR; B. Two splice variants *ONAC103.1* and *ONAC103.2* were identified from the second leaf of 10-day old ZH11 seedlings. RNA was used as control to detect the possible DNA contamination; C. Identification of *ONAC103S* transcripts from the second leaf of 10-day old ZH11 seedlings.

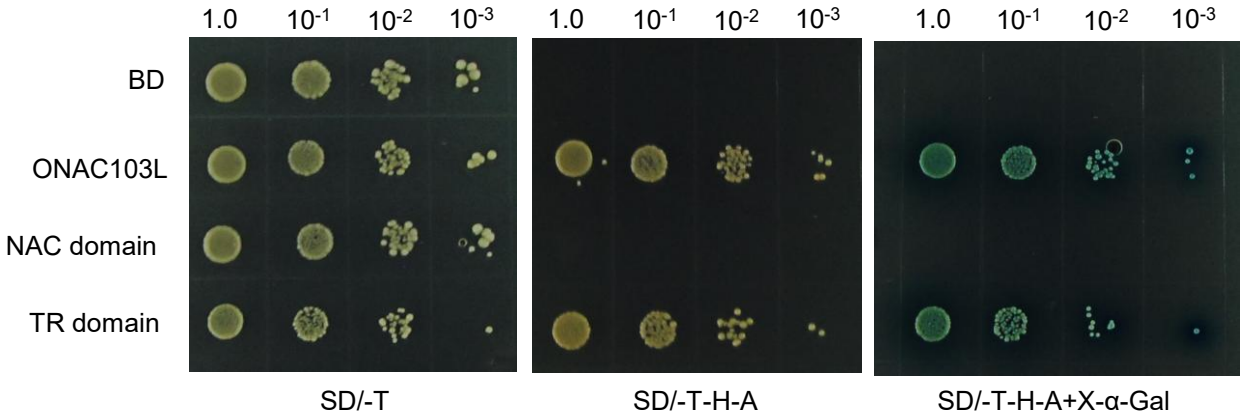

Figure S2 Transcriptional activation assay of ONAC103L in segments in yeast system. The pGBKT7 empty vector (BD) was used as negative control. ONAC103 full length, NAC domain and TR domain were cloned into pGBKT7 vector, respectively for self-activation assay. SD: basic medium, T: tryptophan, H: histidine, A: adenine.

A

ONAC103L：346 aa

MEMTMSSAAT SLPPGFRFHP TDEELILHYL RSRATAGQCP VPIIADVDIY KFDPWDLPSK
AVYGESEWYF FSPRDRKYPN GIRPNRAAGS GYWKATGTDK PIHDSATGES VGVKKALVFY
RGRPPKGTKT SWIMHEYRLA ADPLAAAANT YKPSSSSRFR NVSMRLDDWV LCRIYKKSGQ
ASPMMPPLAA DYDHDEPSGV LDDAYSFYAP PMISTTLIPK LPKIPSISEL FDEHALAQIF
DAAADPPADH HQHALAVHPS LNQLLGVGDN FLAECYPSTA STATVAGGKR KASPAGDYAG
GGHTPAKRLN GSCFDVAPQS VVGGLQATPS SVLAGLNHQM LPPQLF

ONAC103S: 199aa

MIGMTFLCSS IHLLNYVQLD DWVLCRIYKK SGQASPMMPP LAADYDHDEP SGVLDDAYSF
YAPPMISTTL IPKLPKIPSI SELFDEHALA QIFDAAADPP ADHHQHALAV HPSLNQLLGV
GDNFLAECYP STASTATVAG GKRKASPAGD YAGGGHTPAK RLNGSCFDVA PQSVVGGLQA
TPSSVLAGLN HQMLPPQLF

B

Predicted monopartite NLS by cNLS mapper

Protein	Position	Sequence	Score
ONAC103L	286	AGGKRKASP	2.0
	307	KRLNGSCF	3.0
ONAC103S	138	AGGKRKASP	2.0
	159	KRLNGSCF	3.0

Predicted bipartite NLS by cNLS mapper

Protein	Position	Sequence	Score
ONAC103L	288	GKRKASPAG DYAGGGHTP AKRLNGS	8.2
ONAC103S	141	GKRKASPAG DYAGGGHTP AKRLNGS	8.2

C

Predicted NES by LocNES

Protein	Position	Sequence	Score
ONAC103L	250-264	HHQHALAVHPSLNQL	0.186
	253-267	HALAVHPSLNQLLGV	0.146
ONAC103S	1-13	MIGMTFLCSSIHL	0.209
	103-117	HHQHALAVHPSLNQL	0.186
	106-120	HALAVHPSLNQLLGV	0.172

Figure S3 Sequence analysis of ONAC103L and ONAC103S.

A. The amino acid sequence of ONAC103L and ONAC103S. The underlined sequences indicate differences in ONAC103L and ONAC103S, and the predicted nuclear localization signal (NLS) and nuclear export signal (NES) sequences are highlighted by green and yellow colors, respectively.

B. Prediction of the NLSs in ONAC103L and ONAC103S. The NLS was predicted by cNLS mapper (https://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi), and the monopartite and bipartite NLSs with relatively high credibility were shown.

C. Prediction of the NESs in ONAC103L and ONAC103S. The NES was predicted by LocNES (http://prodata.swmed.edu/LocNES/LocNES.php). Only the NESs with top scores were shown.

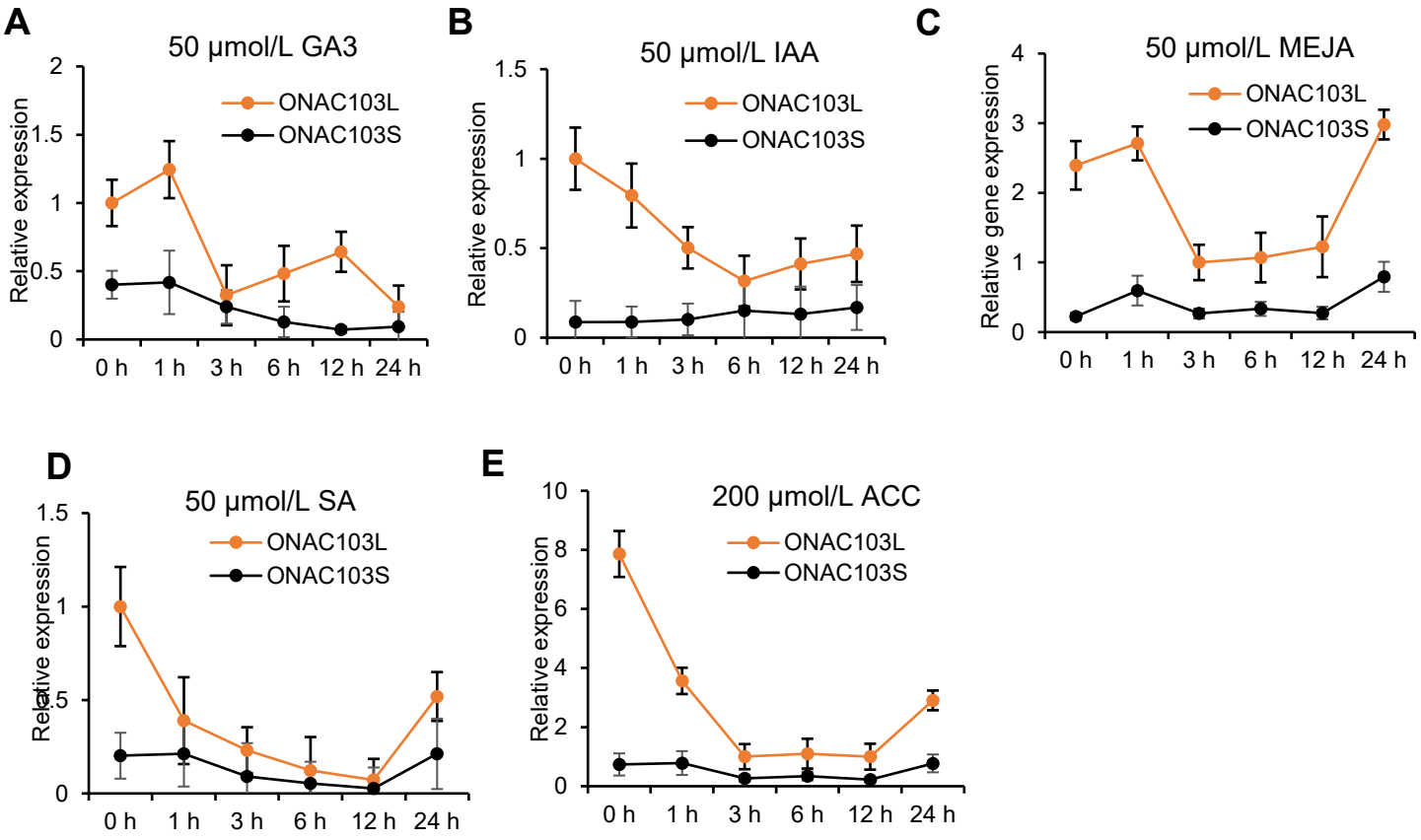

Figure S4 Time-course transcriptional changes of *ONAC103L* and *ONAC103S* in rice leaves under different phytohormone treatments. 11-day-old rice seedlings were treated with 50 μmol/L GA3 (A), 50 μmol/L IAA (B), 50 μmol/L MeJA (C), 50 μmol/L SA (D) and 50 μmol/L ACC (E), respectively. Leaves were sampled at each time point after treatment to examine the expression levels of *ONAC103L* and *ONAC103S* by RT-qPCR. Data are means ±SD (n = 3).

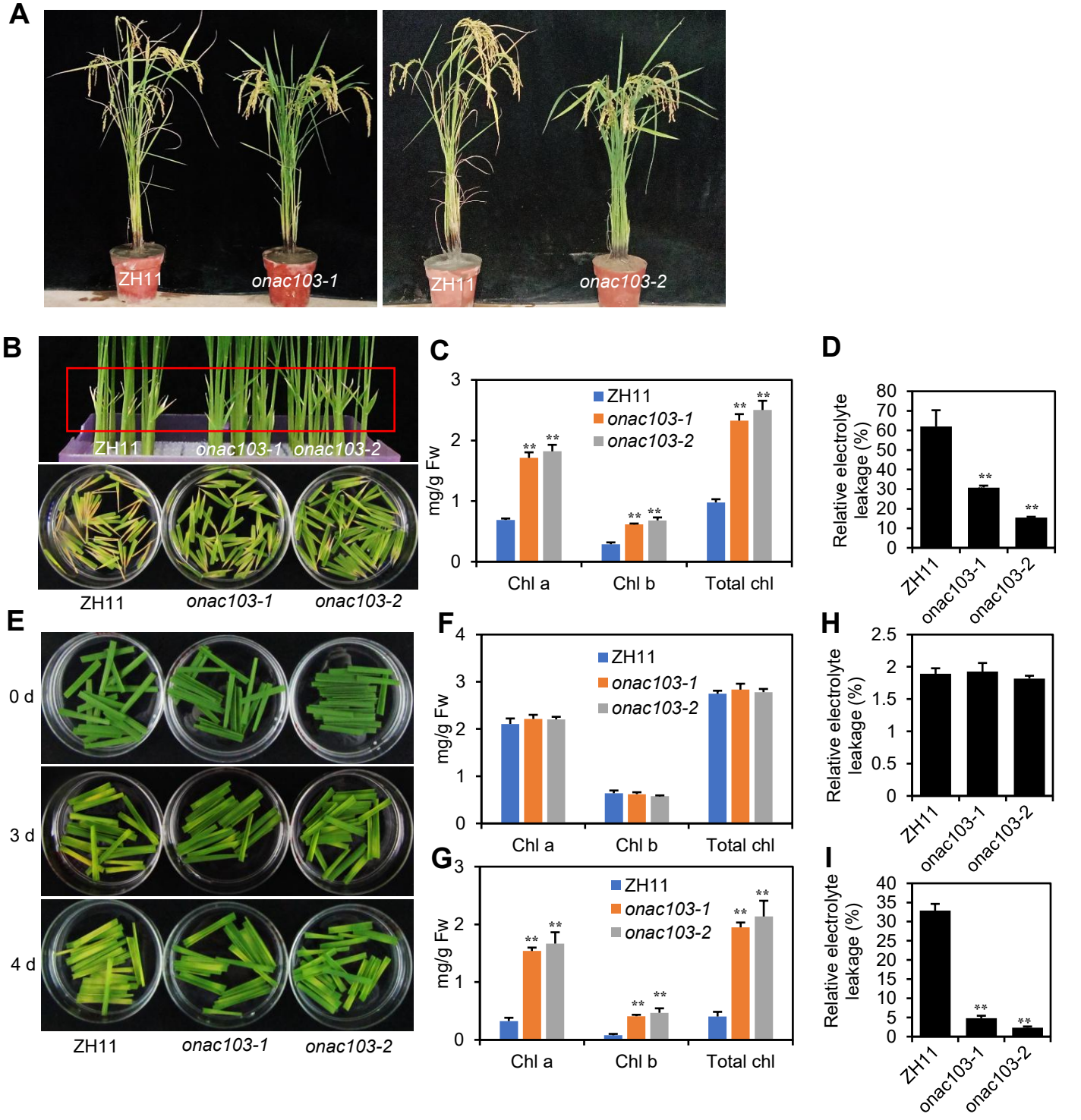

Figure S5 The *onac103* mutants show delayed leaf senescence phenotype. (A) Leaves of the *onac103* mutants showed delayed senescence compared to ZH11 at the ripening stage. (B) Comparison of the first leaf color of ZH11 and the *onac103* mutants grown for 24 days. The first leaves of each line were boxed (top), and they were detached for a group shot (below). (C) Determination of the chlorophyll content of the first leaves of each line grown for 24 days. (D) The relative electrolyte leakage rate of the first leaves of each line grown for 24 days. (E) Dark-induced senescence phenotype of the second leaves of each line. The second leaves of three-week-old seedlings of each line were induced by darkness and photographed on the 0th, 3rd and 4th day. (F, G) The chlorophyll content of the second leaves of each line before (F) and after dark induction (G). The second leaves of three-week-old seedlings of each line were induced by darkness, and the chlorophyll content was measured before dark induction (0 d) and on the 4th day after dark induction. (H, I) The relative electrolyte leakage rate of the second leaves of each line before (H) and after dark induction (I). The second leaves of three-week-old seedlings of each line were induced by darkness, and the relative electrolyte leakage rate was measured before dark induction and on the 4th day after dark induction. Data are means ± SD (n = 3). Statistical analysis was performed using Student's t-test. *, $P \leq 0.05$; **, $P \leq 0.01$.

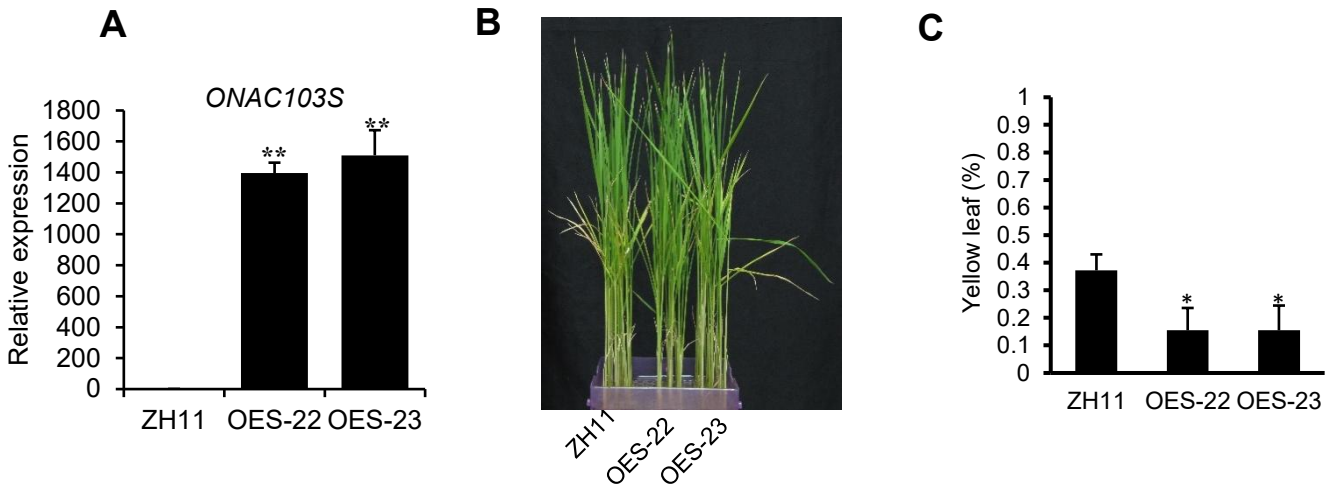

Figure S6 Phenotypes of *ONAC103S*-overexpression lines. A. Transcript level of *ONAC103S* in *ONAC103S*-overexpression lines by RT-qPCR. *OsUBQ5* was used as an internal control. B. Phenotype of *ONAC103S*-overexpression seedlings grown for 33 days in growth chamber. C. Chlorophyll contents of the second and third leaves harvested from ZH11 and *ONAC103S*-overexpression seedlings. Data are means ± SD (n = 3). Statistical analysis was performed using Student's t-test. * $P \leq 0.05$.

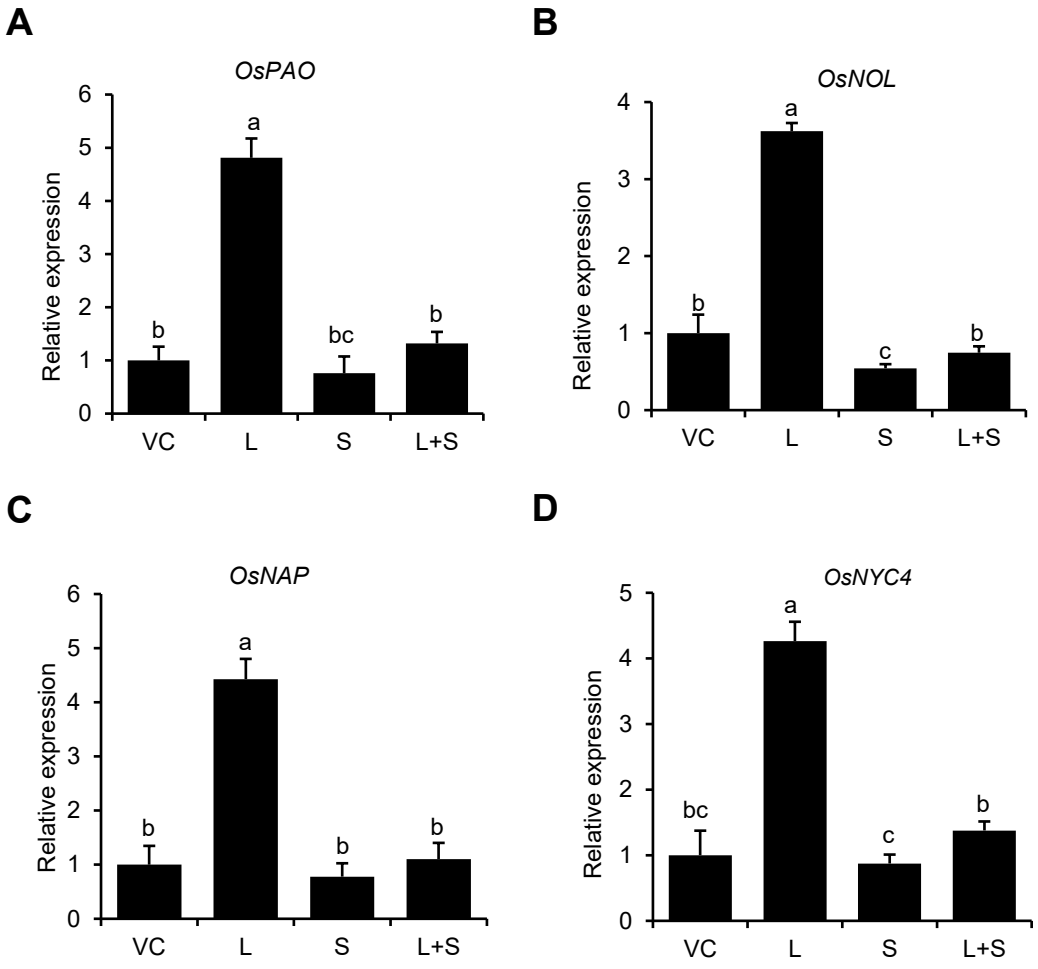

Figure S7 ONAC103S inhibited the transcriptional activation activity of ONAC103L towards its target genes. The *35S: ONAC103L* (L) and *35S: ONAC103S* (S) plasmids were both or individually transformed into rice protoplasts and incubated in darkness at 28 ℃ for 12-16 h. The protoplasts were collected and the expression levels of *OsPAO* (A), *OsNOL* (B), *OsNAP* (C) and *OsNYC4* (D) were examined by RT-qPCR. VC: Vector control. Data are means ±SD (n = 3). Two way ANOVA was performed to analyze the statistical significance between different samples.