

LOC_0s01g15640.1 (NTL3) MESLRDMVLPPGFGFHPKDTELI SHYLKKKIHGQKIEYEI IPEVDIYKHEPWLPAKCDV
 LOC_0s01g15640.1 (*ntl3-1*) MESLRDMVLPPGFGFHPKDTELI SHYLKKKIHGQKIEYEI IPEVDIYKHE TMGFCKVRC
 LOC_0s01g15640.1 (*ntl3-2*) MESLRDMVLPPGFGFHPKDTELI SHYLKKKIHGQKIEYEI IPEVDIYKHEPWLPAKCDV

LOC_0s01g15640.1 (NTL3) PTQDNKWHFFAARDRKYPNGSRNRATVAGYWKSTGKDRAIKMGKQITGKTKTLVFHEGR
 LOC_0s01g15640.1 (*ntl3-1*) SNSG-----
 LOC_0s01g15640.1 (*ntl3-2*) PTQDNKWHFFAARDRKYPNGSRNRATVAGYWKSTGKDRAIKMGKQITGKTKTLVFHEGR

LOC_0s01g15640.1 (NTL3) PPTGRRTEWIMHEYYIDEREQACPMKDAYVLCRITKRNDWIPGNGNELDNSDPHPEPY
 LOC_0s01g15640.1 (*ntl3-1*) -----
 LOC_0s01g15640.1 (*ntl3-2*) PPTGRRTEWIMHEYYIDEREQACPMKDAYVLCRITKRNDWIPGNGNELDNSDPHPEPY

LOC_0s01g15640.1 (NTL3) DAPPSVISTEQLNPAAEVVGVEAAPVTVAEPDGVTTSAITANIPSPSDDINLDDWLNEL
 LOC_0s01g15640.1 (*ntl3-1*) -----
 LOC_0s01g15640.1 (*ntl3-2*) DAPPSVISTEQLNPAAEVVGVEAAPVTVAEPDGVTTSAITANIPSPSDDINLDDWLNEL

LOC_0s01g15640.1 (NTL3) FDPFFDPEQSLASADLSPDEQNVESNVGALAPKVEQDYSSPENENVDDTEYLLPEDVYN
 LOC_0s01g15640.1 (*ntl3-1*) -----
 LOC_0s01g15640.1 (*ntl3-2*) FDPFFDPEQSLASADLSPDEQNVESNVGALAPKVEQDYSSPENENVDDTEYLLPEDVYN

LOC_0s01g15640.1 (NTL3) ILHPGTDDFNMLQNPLDQYPIQYATDVWSGIQKEELWSPQANAEPQSNEAADNGIIRRY
 LOC_0s01g15640.1 (*ntl3-1*) -----
 LOC_0s01g15640.1 (*ntl3-2*) ILHPGTDDFNMLQNPLDQYPIQYATDVWSGIQKEELWSPQANAEPQSNEAADNGIIRRY

LOC_0s01g15640.1 (NTL3) RSMKTPETSVPQFKGKTQAKMRVGINKMATSSSESINQTIKFENSGRLVEHQKQAHDVA
 LOC_0s01g15640.1 (*ntl3-1*) -----
 LOC_0s01g15640.1 (*ntl3-2*) RSMKTPETSVPQFKGKTQAKMRVGINKMATSSSESINQTIKFENSGRLVEHQKQAHDVA

LOC_0s01g15640.1 (NTL3) STKRS DAGKPSTELSSNRGFLRGIRNAFAGCS DARWNMILVAGFAIGVAVVALHIGQRLG
 LOC_0s01g15640.1 (*ntl3-1*) -----
 LOC_0s01g15640.1 (*ntl3-2*) STKRS DAGKPSTELSSNRGFLRGIRNAFAGCSD SMEHDTCCGFRYWSRCGSASYRPTPW

LOC_0s01g15640.1 (NTL3) LSQRDQQT-----
 LOC_0s01g15640.1 (*ntl3-1*) -----
 LOC_0s01g15640.1 (*ntl3-2*) KPERSAAYLAFRFAGYWVSMFLAIWRNVNKLRLVGAEHLMYAPFLCWFCYTYLRFWS

LOC_0s01g15640.1 (NTL3) -----
 LOC_0s01g15640.1 (*ntl3-1*) -----
 LOC_0s01g15640.1 (*ntl3-2*) IFVLAS

Figure S1. Characterization of mutation in OsNTL3. Protein sequences of OsNTL3 in WT (NTL3) and mutants (*ntl3-1* and *ntl3-2*) derived from the CRISPR-CAS9 technology. The NAC DNA-binding domain is shown in red. The amino acids for a new C-terminus of NTL3 in *ntl3-2* mutant is boxed.

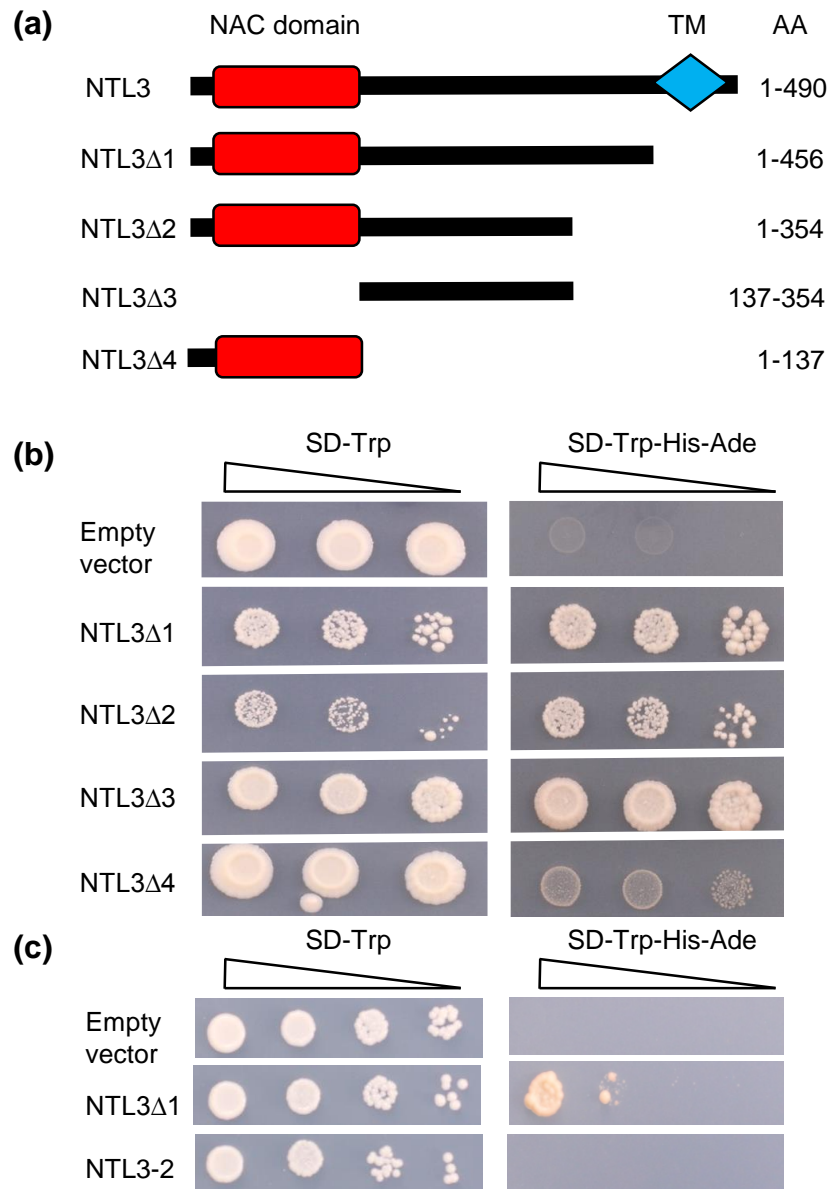


Figure S2. OsNTL3 has transcriptional activation activity. (a) Diagram showing various segments of OsNTL3 that were fused to the yeast GAL4 DNA-binding domain. The NAC domain and transmembrane domain (TM) were shown in rectangle and diamond, respectively. Amino acid (AA) positions were indicated for each segment. (b-c) Activation of the *His* and *Ade* reporter genes in yeast cells. Three different dilutions of yeast cells were spotted on nutritional selection medium. The mutated form of OsNTL3 (NTL3-2) was obtained from the *ntl3-2* rice mutant plants and used for comparison (c).

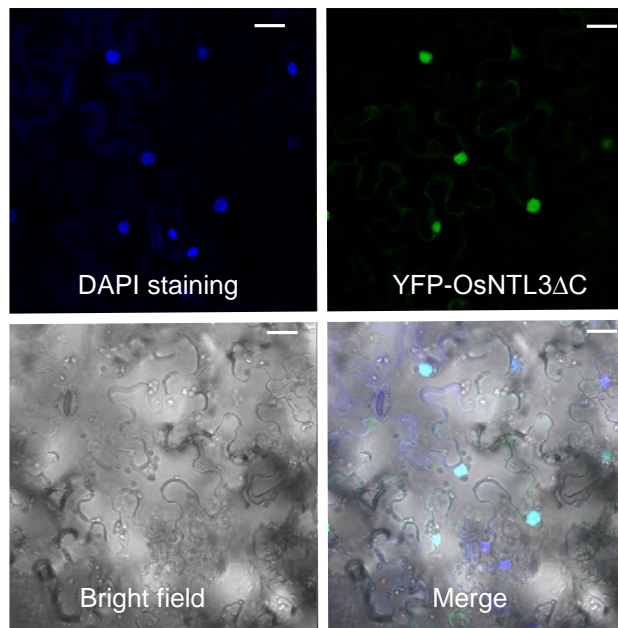


Figure S3. Subcellular localizations of YFP-OsNLT3 Δ C. The YFP-tagged truncated OsNLT3 devoid of the transmembrane domain were transiently expressed in tobacco leaves. DAPI staining was used to visualize the nuclei. Bar = 50 μ m.

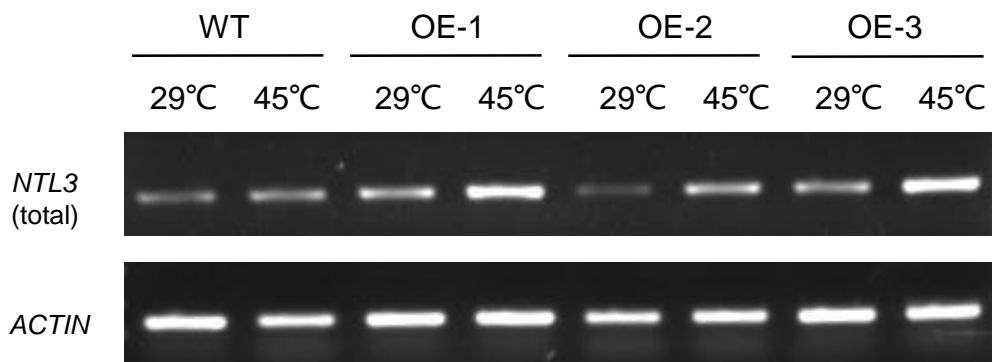


Figure S4. Validation of transgenic expression. Total RNA were extracted from the wild-type (WT) control plants and *NTL3 Δ C* overexpression (OE1-3) plants and the expression of total *OsNTL3* was detected by RT-PCR. *ACTIN* was used as an internal control.

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LOC_0s07g44950.1 (bZIP16) MAEPDLLAPFADLPFPPGDDFPDFPTLGDDAFALEDFDLDDLDFDFDVLFPDAPPVPT
LOC_0s07g44950.1 (bzip16) MAEPDLLAPFADLPFPPGDDFPDFPTGMTPSRWRISISTIWSTSMWISSRRMRRR---
*****
LOC_0s07g44950.1 (bZIP16) TSSSSAAGSPEAGTSSAGDGGSKNEESADSSSPSRSGSDGGGKDGKDDEAKRRARLVR
LOC_0s07g44950.1 (bzip16) -----

LOC_0s07g44950.1 (bZIP16) NRESAHQSRQRKKQYVEELEGKVKVMQATIADLTARISCVTAENAALKQQLGGAAGAGAA
LOC_0s07g44950.1 (bzip16) -----

LOC_0s07g44950.1 (bZIP16) APPPPMPYPVAVYPLPMPWIHPAYAMRGSQVPLVPIPRLKTQQPASTPEPPAKKARKTKK
LOC_0s07g44950.1 (bzip16) -----

LOC_0s07g44950.1 (bZIP16) VAGVSLGLLFLMMVCGCLVPAVNRMYGAAITGEGAAIVPSHHGRILAVEGPQNSVSNQV
LOC_0s07g44950.1 (bzip16) -----

LOC_0s07g44950.1 (bZIP16) DPKVPQNGSETLPALLYLPRNGKHVKINGNLVIKSI VASEKASSRLSNYGEKSGNQKKE
LOC_0s07g44950.1 (bzip16) -----

LOC_0s07g44950.1 (bZIP16) ETSLAIPGYVAPLEAGEVMDSAKGMNELMALAPGDGSIYREDDGMLPQWFSEAMSGPMLN
LOC_0s07g44950.1 (bzip16) -----

LOC_0s07g44950.1 (bZIP16) SGMCTEVFQFDLSPTTADANGIVPVYSGSVTNTSQNYTENLPSGPVQKVKNRRI SYSEAI
LOC_0s07g44950.1 (bzip16) -----

LOC_0s07g44950.1 (bZIP16) PLRGSTNDTDHFKAPPKNHSQSHAGRKPVSSVVSVLADPREASDRDGEGRISNSLSR
LOC_0s07g44950.1 (bzip16) -----

LOC_0s07g44950.1 (bZIP16) IFVVVLIDSVKYVTYSCVLPFKSHSPHL
LOC_0s07g44950.1 (bzip16) -----

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Figure S5. Characterization of mutation in *Os**b**ZIP16*. Protein sequences of *Os**b**ZIP16* in WT (*bZIP16*) and mutant (*bzip16*) derived from the CRISPR-CAS9 technology are aligned. The *bZIP* DNA-binding domain is shown in red.

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LOC_0s05g34050.1 (bZIP17) MAEPALLDPTAAFDLRLYPALHFDHELPLAGGGGGDDDDDLPLDGLFDFLPGDFSVEDFL
LOC_0s05g34050.1 (bzip17) MAEPALLDPTAAFDLRLYPALHFDHELPLAGGGGGDDDDDLPLTGSSSTCPAISPWRTSS
***** * *
LOC_0s05g34050.1 (bZIP17) LRSPERDDSGEGSAAGSGPTASPSSTTSASNSAVANGSGGEVKHEESDEGRSGGGDPK
LOC_0s05g34050.1 (bzip17) SGLRSGTTPARALLPDGPPPPRRRPPRRPTPPSPTAAAARSSTRSRMRGGAVGVTPS
** * * *
LOC_0s05g34050.1 (bZIP17) WSLKRKQASPGSSDAKCRRSRGDGDVSPSASASRTAVDSDEGGTVCEEEEDERRAARLM
LOC_0s05g34050.1 (bzip17) GA-----
-
LOC_0s05g34050.1 (bZIP17) RNRESAQLSRQKRKRYVEELEEKVKSMHSVINDLNSRISFVVAENATLRQQLSGGSVNCP
LOC_0s05g34050.1 (bzip17) -----
LOC_0s05g34050.1 (bZIP17) PPGVYPPAPIPGMHFPWMPGYAMRPPGSHVPLVPIRLKPPQVPSSKVVKKPESKKTVE
LOC_0s05g34050.1 (bzip17) -----
LOC_0s05g34050.1 (bZIP17) NKSkskTKTKKVASVLLGLLLIMLVFGAFIPGFNHNFGMCGQSDNAMFRNFGQSHARVL
LOC_0s05g34050.1 (bzip17) -----
LOC_0s05g34050.1 (bZIP17) SVSSQDKSSLNNSDMIGVDVGKMTGNTDGPgkKHQPAHNSSEILPALLYVPRNGKHVKIN
LOC_0s05g34050.1 (bzip17) -----
LOC_0s05g34050.1 (bZIP17) GNLIHsvLASEKAVAHKASKDDSDQSARDHKETSVAIARYLSLPGKDVNRQETSSADGP
LOC_0s05g34050.1 (bzip17) -----
LOC_0s05g34050.1 (bZIP17) LPQWFREGMEGPILNSGMCSEVFQFDISTASSNPGGIIPASPVVNSSSVNATEKIPAHA
LOC_0s05g34050.1 (bzip17) -----
LOC_0s05g34050.1 (bZIP17) AYHGKLNRRVMYNEAIPLTGKTANNTEPFNRTSESSSKLPDSKPASSVVVSVLADPREA
LOC_0s05g34050.1 (bzip17) -----
LOC_0s05g34050.1 (bZIP17) GNGDGDPRVSPKPLSKIFVVVLVDGVRVVTYSCTLPFKSSSPHLVN
LOC_0s05g34050.1 (bzip17) -----

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Figure S6. Characterization of mutation in *bZIP17*. Protein sequences of bZIP17 in WT (bZIP17) and mutant (*bzip17*) derived from the CRISPR-CAS9 technology are aligned. The bZIP DNA-binding domain is shown in red.

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LOC_0s06g41770.1 (bZIP74) MDVEFFADLDDLALLASFSSSAAAAGSGVSGLFAPSPPHDAEAGSPESVSSRRRPSPSREA
LOC_0s06g41770.1 (bzip74-1) MDVEFFADLDDLALLASFSSSAAAAGSGVSGLFAPSPPHDAEAGSPESVSSRRRPSPSREA
LOC_0s06g41770.1 (bzip74-2) MDVEFFADLDDLALLASFSSSAAAAGSGVSGLFAPSPPHDAEAGSPESVSSRRRPSPSREA
*****
LOC_0s06g41770.1 (bZIP74) ALSEIERFLMEEGPAAEEGVGAEDFFDALLVDGEEEEEEEEEGKSEAGGSTDGDGSGKEN
LOC_0s06g41770.1 (bzip74-1) ALSEIERFLIGGPRGGGGRRGGFLRRAARRRRGGGGRRGE-----
LOC_0s06g41770.1 (bzip74-2) ALSEIERAPRRRRGSARRISSTRCSSTAGRRRRKKRGRGVRRGEARMGIPGRRMRWLPR
***** *
LOC_0s06g41770.1 (bZIP74) EVATPDAEKEDVEAEVDGDDPMSKKKRRQMRNRDSAMKSREKMKMYVKDLETKSKYLEAE
LOC_0s06g41770.1 (bzip74-1) -----
LOC_0s06g41770.1 (bzip74-2) TRRRRMWRRRWMAMIP-----

LOC_0s06g41770.1 (bZIP74) CRRLSYALQCCAENMALRQSLKDRPVGAATAMQESAVLTETLPLVSLWLVSIVCLLP
LOC_0s06g41770.1 (bzip74-1) -----
LOC_0s06g41770.1 (bzip74-2) -----

LOC_0s06g41770.1 (bZIP74) VPGLPNRNPVARSSAGRDLATVTGKKTSEQQLEETLLHGRCKGSRARIKLDTGPFRL
LOC_0s06g41770.1 (bzip74-1) -----
LOC_0s06g41770.1 (bzip74-2) -----

LOC_0s06g41770.1 (bZIP74) AAAAC
LOC_0s06g41770.1 (bzip74-1) -----
LOC_0s06g41770.1 (bzip74-2) -----

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Figure S7. Characterization of mutation in *OsbZIP74*. Protein sequences of *OsbZIP74* in WT (bZIP74) and mutants (*bzip74-1* and *bzip74-2*) derived from the CRISPR-CAS9 technology are aligned. The bZIP DNA-binding domain is shown in red.

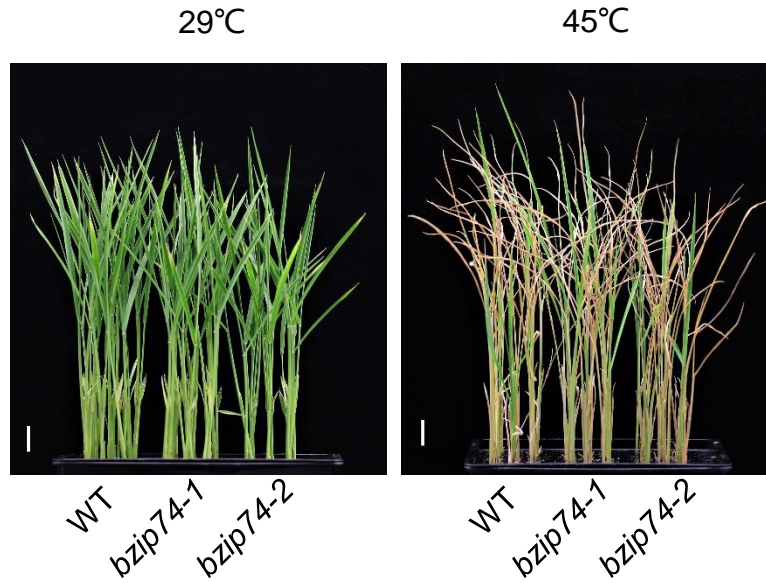


Figure S8. Loss-of-function of *OsbZIP74* does not affect heat stress sensitivity in rice. Eight-day old wild-type (WT) seedlings and two lines of targeted-gene-edited *OsbZIP74* (*bzip74-1* and *bzip74-2*) mutant seedlings grown at 29°C were transferred to 45°C for 4-5 days and then photographed after recovering at 29°C for 7 days. Bar = 1 cm.