Natural variation in Tiller Number 1 affects its interaction with TIF1 to regulate tillering in rice

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Table S1 Nucleotide diversity and selection analysis of *TN1*

Supplemental Dataset 1 List of the 295 varieties used in this study.

Supplemental Dataset 2 The QTLs associated with tiller numbers.

Supplemental Dataset 3 The candidate gene in *qTN2*.

Supplemental Dataset 4 The differentially expressed genes between NIP and *tn1-1*.

Supplemental Dataset 5 The alternative 3’ splicing between NIP and *tn1-1* lines.

Supplemental Dataset 6 Primers used in the study.

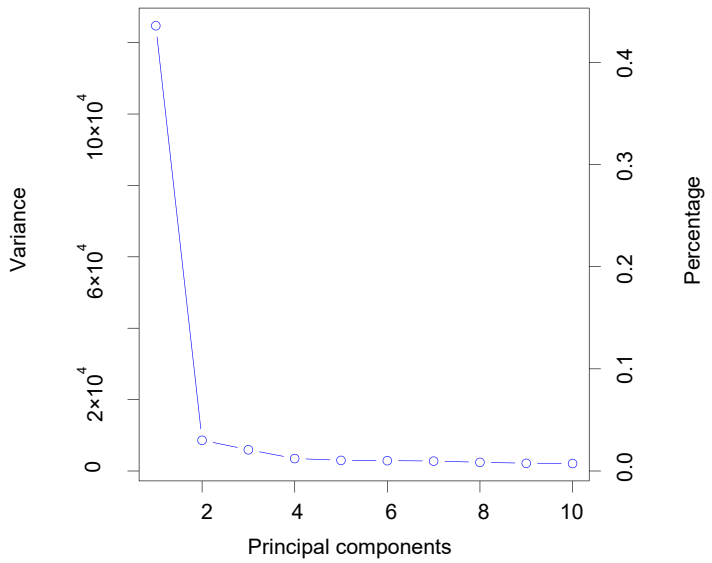

Figure S1 Population structure of 295 rice accessions

In total, 3,874,812 high-quality SNPs (MAF ≥ 5%, missing rate < 50%) were used to determine the population structure.

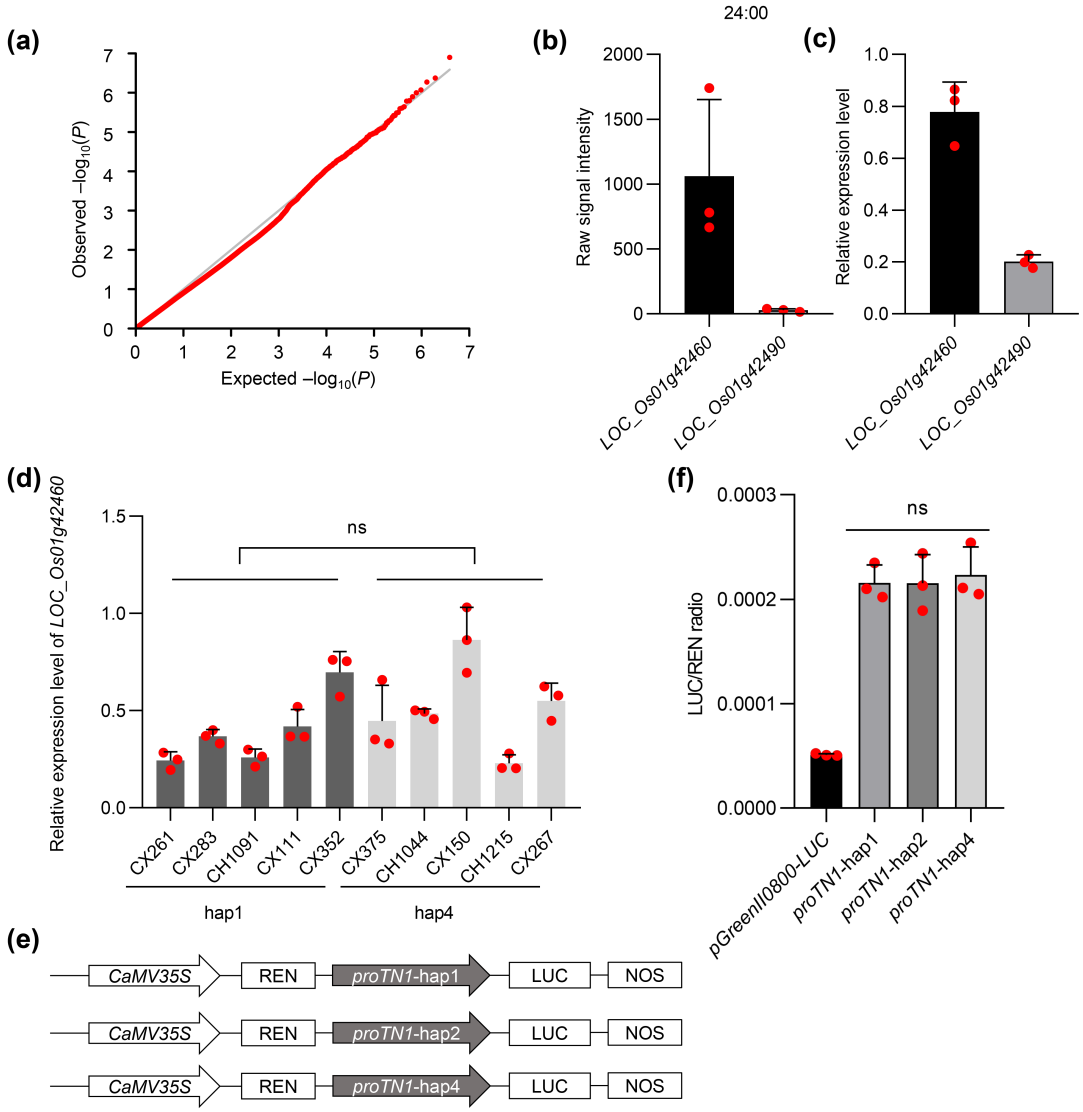

Figure S2 Functional variation analysis of *TN1*

(a) Quantile–quantile plots of the mixed linear model (MLM). (b) Expression levels of *LOC_Os01g42460* and *LOC_Os01g42490* in roots at 24:00 during the vegetative period; data were derived from RiceXpro (https://ricexpro.dna.affrc.go.jp/). (c) Expression levels of *LOC_Os01g42460* and *LOC_Os01g42490* at the base of tiller buds at 45 days after transplanting. (d) Expression levels of *LOC_Os01g42460* in germplasm materials; RNA was extracted from tiller buds at 30 days after transplanting. (e) Schematic diagram showing constructs used for the analysis of the 2.6 kb promoter. (f). Comparison of *proTN1*-hap1, *proTN1*-hap2, and *proTN1*-hap4. *P*-values in (c) and (e) were determined using two-tailed Student's *t*-test.

(a)

*LOC_Os01g42370*_hap

Sub	Hap	Exon			Promoter									No. of cvs.	No. of tiller
		5851	658	46	-408	-1025	-1031	-1067	-1068	-1167	-1440	-1478	-1721		
Ind	hap1	C	A	A	T	C	C	C	T	A	A	G	C	26	12.87±0.39^{a}
	hap2	C	G	C	C	T	A	T	C	A	A	A	T	4	11.58±0.34^{ab}
	hap3	C	G	C	C	T	A	T	C	G	A	A	T	12	10.92±0.54^{b}
	hap4	G	G	C	C	T	A	T	C	G	T	A	T	90	9.82±0.16^{c}
Jap	hap3	C	G	C	C	T	A	T	C	G	A	A	T	123	8.88±0.20
	hap4	G	G	C	C	T	A	T	C	G	T	A	T	3	7.13±0.89

*LOC_Os01g42380*_hap

Sub	Hap	Promoter			No. of cvs.	No. of tiller
		-1363	-1593	-1603		
Ind	hap1	A	C	A	116	10.06±0.15^{b}
	hap2	C	T	G	29	12.45±0.39^{a}
Jap	hap1	A	C	A	137	8.73±0.19

*LOC_Os01g42410*_hap

Sub	Hap	Promoter	No. of cvs.	No. of tiller
		-426		
Ind	hap1	A	30	12.6±0.37^{a}
	hap2	G	116	10.05±0.15^{b}
Jap	hap2	G	135	8.72±0.19

*LOC_Os01g42470*_hap

Sub	Hap	Promoter								No. of cvs.	No. of tiller
		-1271	-1154	-1038	-554	-470	-385	-381	-210		
Ind	hap1	G	A	A	C	G	A	A	C	97	9.81±0.16^{b}
	hap2	G	G	G	C	G	C	G	T	13	11.39±0.46^{a}
	hap3	T	G	G	T	A	C	G	T	25	12.63±0.40^{a}
Jap	hap1	G	A	A	C	G	A	A	C	2	7.55±1.24
	hap2	G	G	G	C	G	C	G	T	120	8.82±0.2

*LOC_Os01g42480*_hap

Sub	Hap	Promoter	No. of cvs.	No. of tiller
		-1394		
Ind	hap1	A	30	12.59±0.37^{a}
	hap2	T	118	10±0.15^{b}
Jap	hap2	T	136	8.71±0.19

*LOC_Os01g42490*_hap

Sub	Hap	Exon	Promoter		No. of cvs.	No. of tiller
		1700	-235	-250		
Ind	hap1	A	C	C	30	12.59±0.37^{a}
	hap2	G	A	A	81	9.85±0.17^{c}
	hap3	G	C	C	14	11.23±0.45^{b}
Jap	hap2	G	A	A	3	7±0.94
	hap3	G	C	C	131	8.8±0.20

(b)

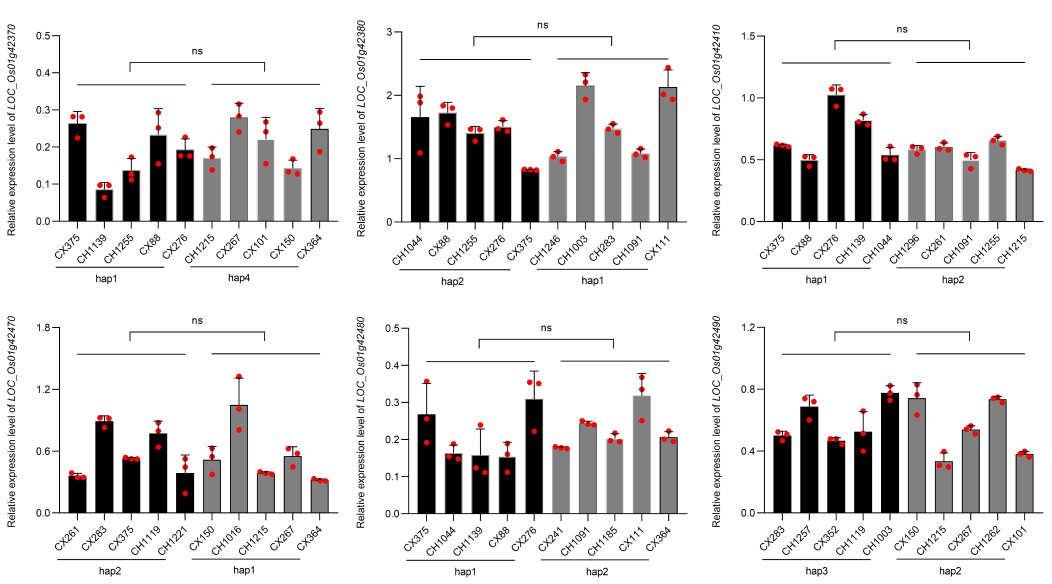

Figure S3 Haplotype and expression analysis of candidate gene in *qTN2*

(a) Haplotypes (hap) of *LOC_Os01g42370*, *LOC_Os01g42380*, *LOC_Os01g42410*, *LOC_Os01g42470*, *LOC_Os01g42480*, and *LOC_Os01g42490* in *qTN2* among germplasm materials; major and minor alleles are indicated in yellow and green, respectively. Data are presented as mean ± SE. Different letters indicate significant differences at $P < 0.05$

according to two-sided Student's *t*-test. (b) Expression levels of *LOC_Os01g42370*, *LOC_Os01g42380*, *LOC_Os01g42410*, *LOC_Os01g42470*, *LOC_Os01g42480*, and *LOC_Os01g42490* in *qTN2* among germplasm materials; RNA was extracted from tiller buds at 30 days after transplanting.

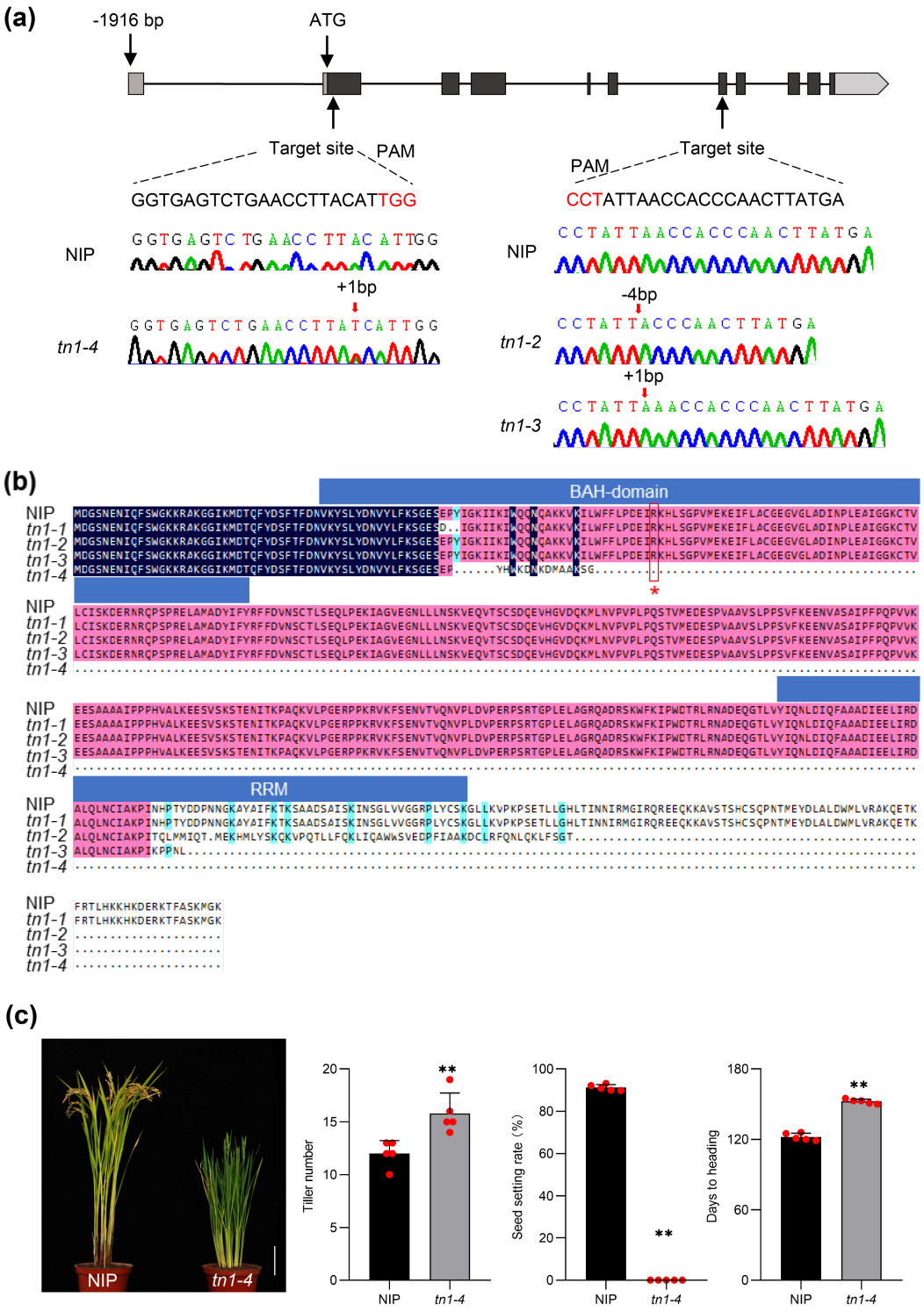

Figure S4 Target sites in *tn1-2*, *tn1-3*, and *tn1-4* lines and the phenotype of *tn1-4*

(a) A 4 bp fragment was deleted from line *tn1-2*, a 1 bp fragment was inserted into the target site of *TN1* in line *tn1-3* and *tn1-4*, respectively. (b) Amino acid sequence analysis of NIP, *tn1-1*, *tn1-2*, *tn1-3*, and *tn1-4* lines. The red frame

and asterisk represent the 83rd amino acid, which is a natural mutation, and the blue frame represents the BAH domain and RRM. (c) Phenotypes of NIP, *tn1-4* at the reproductive stage. *P*-values were determined using two-tailed Student's *t*-tests. $^{**}P < 0.01$. Data are presented as mean ± SD. ($n = 5$).

Figure S5 Comparison of tiller buds among the NIP, *tn1-1*, and *TN1*-OE lines

The samples were observed 3 weeks after sowing. Tiller buds of all lines grew normally. Tiller buds of NIP grew faster than those of *TN1*-OE but slower than those of *tn1-1*. Scale bar = 1 cm.

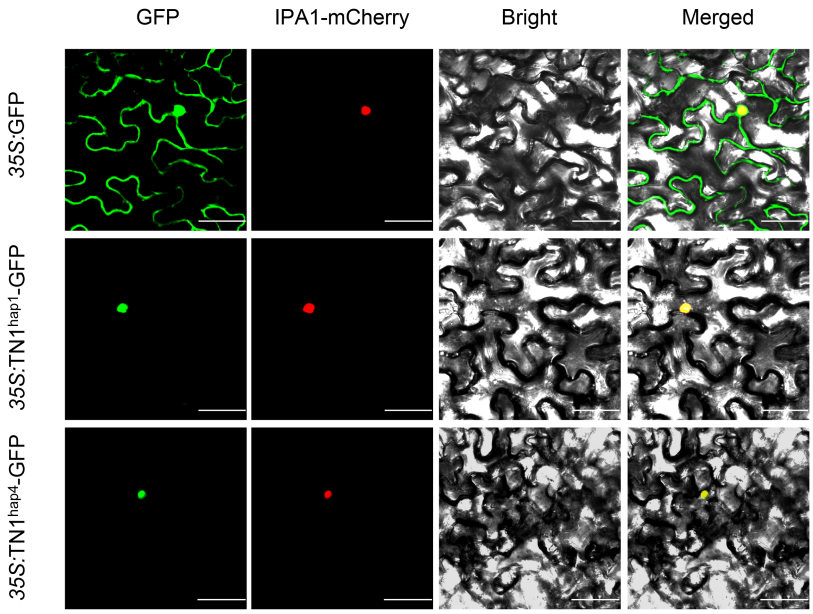

Figure S6 Subcellular localization of *TN1*-GFP fusion protein in tobacco leaves

Agrobacterium tumefaciens strain EHA105 was transfected with GFP plasmids containing different TN1 haplotypes, and the resultant mixed bacterial suspension was injected into tobacco leaves. After 48 hours, the GFP signal was captured by confocal microscopy (Zeiss LSM880). Scale bar = 50 μm.

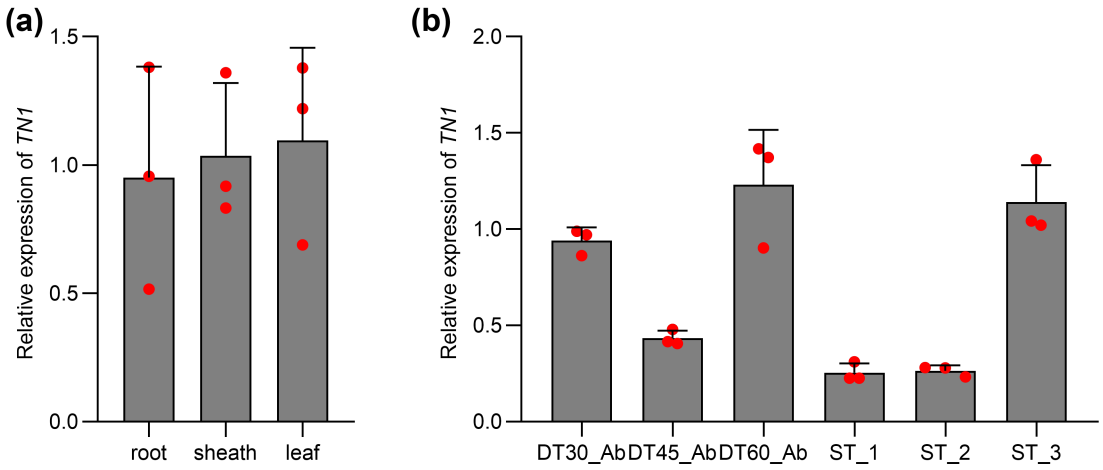

Figure S7 Expression pattern of *TN1* in different tissues of NIP, as determined by qRT-PCR

(a) Expression level of *TN1* in the root, leaf sheath, and leaf blade during vegetative growth. (b) Expression level of *TN1* in the base of tiller buds and stems. DT_30, DT_45, DT_60, ST_1, ST_2, and ST_3 represent developmental stages described in the Materials and Methods section. Data are presented as mean ± SD (*n* = 3 biologically independent samples).

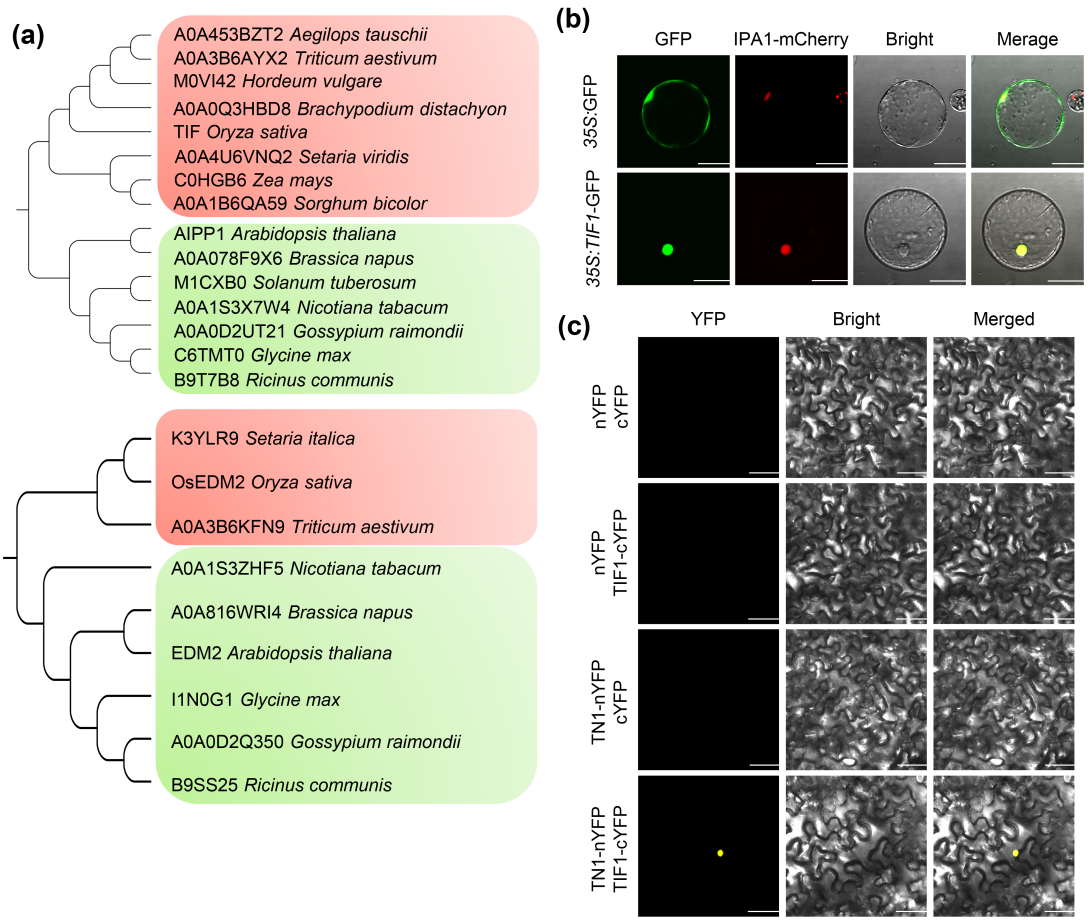

Figure S8 Phylogenetic analysis of TIF1 and OsEDM2 proteins and relationship of TN1 and TIF1 in tobacco leaves

(a) Phylogenetic analysis of TIF1 and OsEDM2 proteins. Monocots and dicots are indicated in orange and green, respectively. (b) Subcellular localization of the TIF1-GFP fusion protein in rice protoplasts (scale bar = 20 μm). (c) TN1 and TIF1 interacted with each other in tobacco leaves (scale bar = 50 μm).

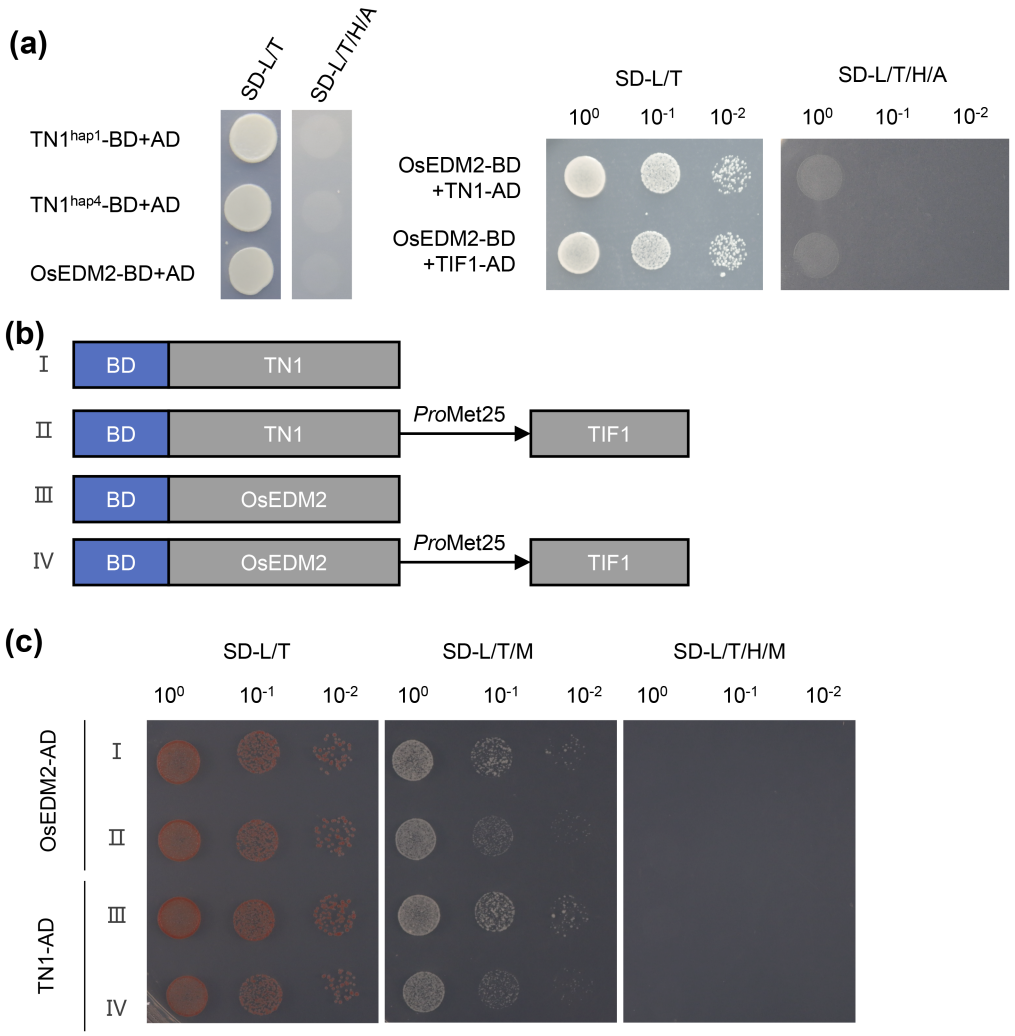

Figure S9 Interaction among TN1, TIF1, and OsEDM2

(a) Yeast two-hybrid (Y2H) assays showed that TIF1 cannot interact with TN1 and OsEDM2. (b) Schematics of vectors used in yeast three-hybrid assays. (c) Yeast three-hybrid (Y3H) assays showed that TN1, TIF1, and OsEDM2 could not form a complex.

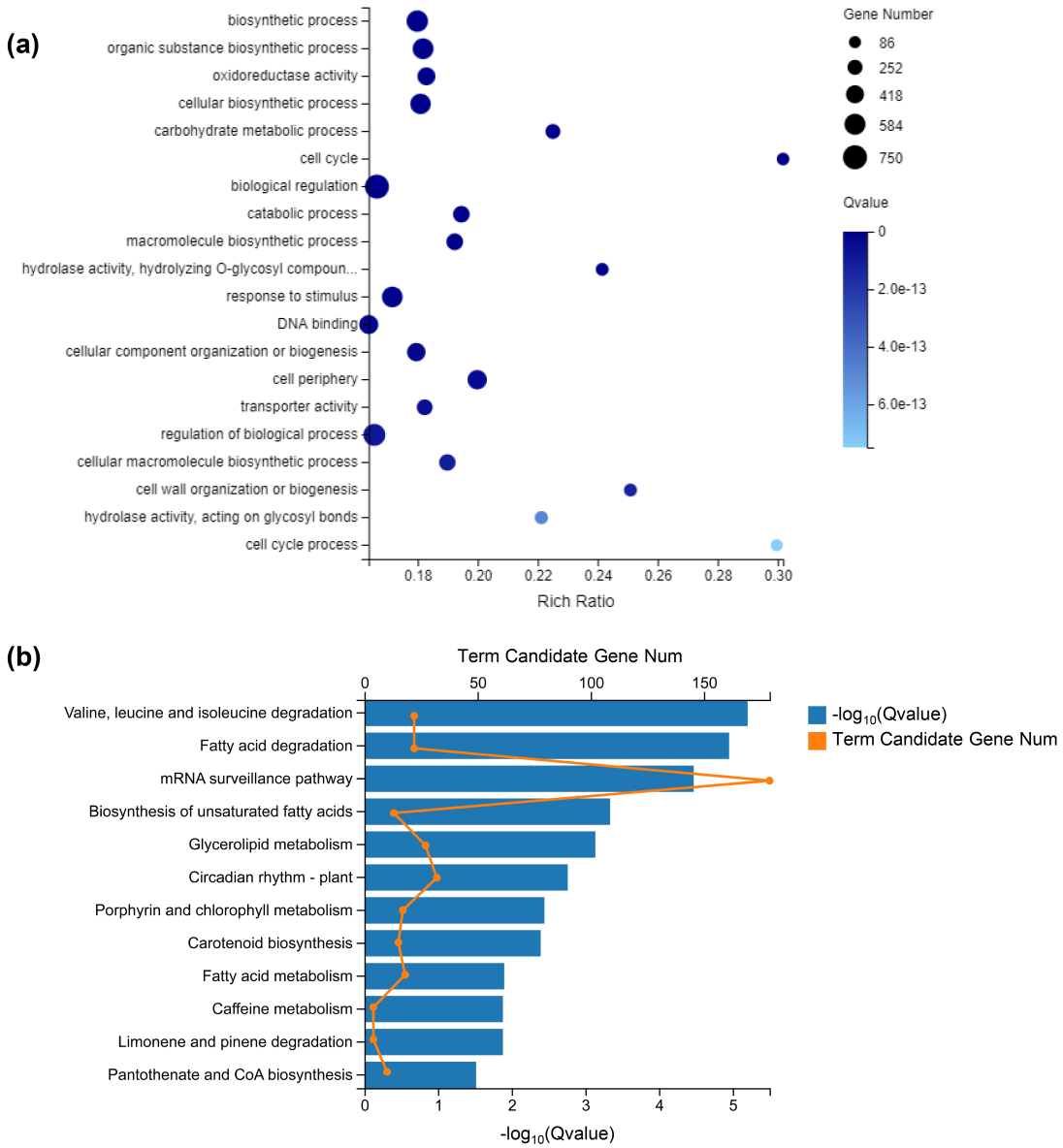

Figure S10 Enrichment analysis of 5,778 DEGs

(a) Statistics of gene ontology (GO) enrichment between NIP and *tn1-1* lines showing the top 20 GO terms according to the *Q*-value. (b) Statistics of the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment (*Q*-value < 0.05). [DEGs for the analysis were $|\log_2(tn1\text{-}1/\text{NIP})| \geq 1$, $P < 0.05$].

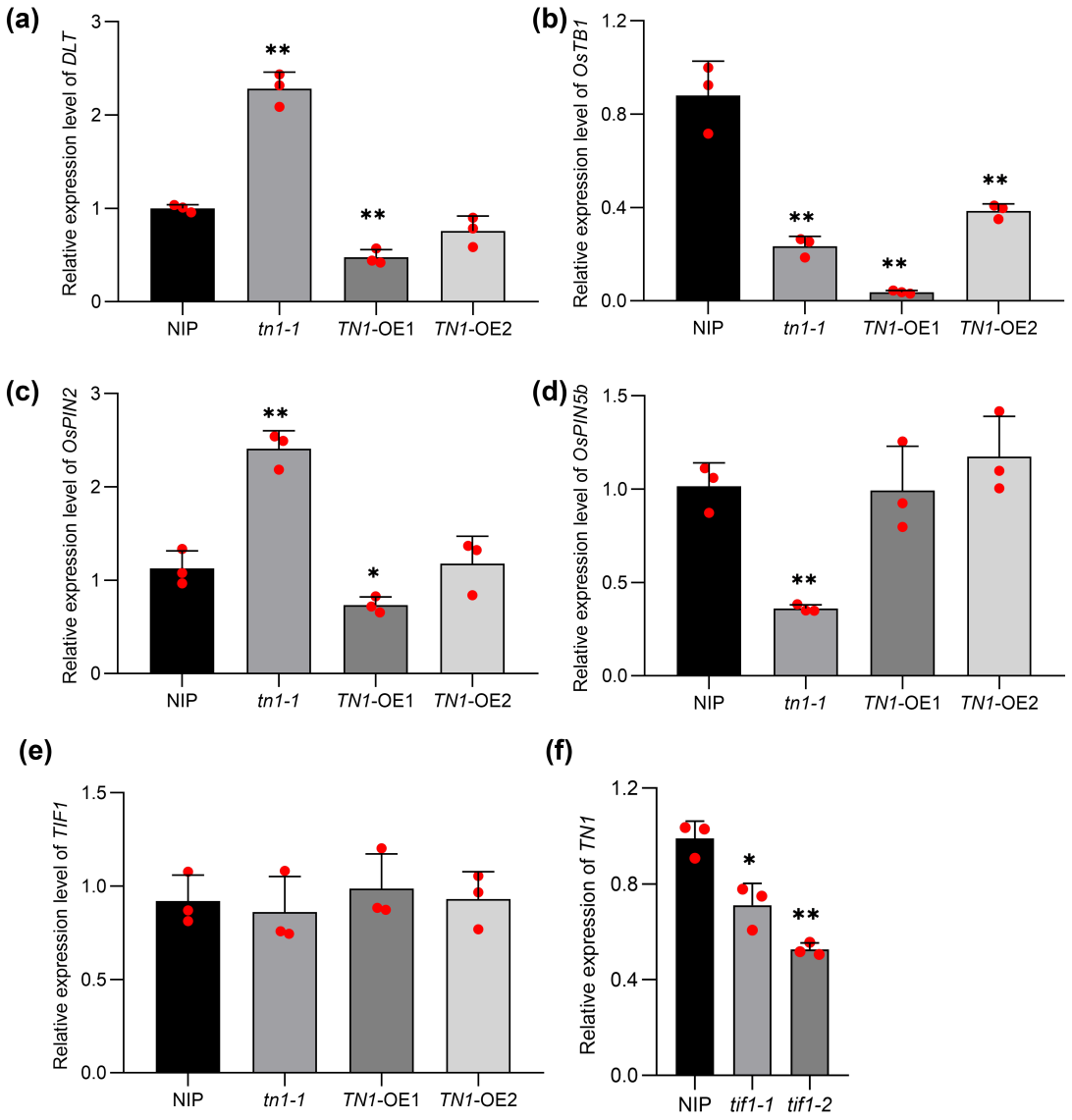

Figure S11 Expression levels of *DLT*, *OsTB1*, *OsPIN2*, *OsPIN5b*, and *TIF1* in NIP, *tn1-1*, and *TN1*-OE lines and expression level of *TN1* in *tif1* lines

(a-e) Expression levels of *DLT, OsTB1*, *OsPIN2*, *OsPIN5b*, and *TIF1* in the NIP, *tn1-1*, and *TN1*-OE lines. (f) TN1 expression in the *tif1* line. Data are presented as mean ± SD (*n* = 3 biologically independent samples).

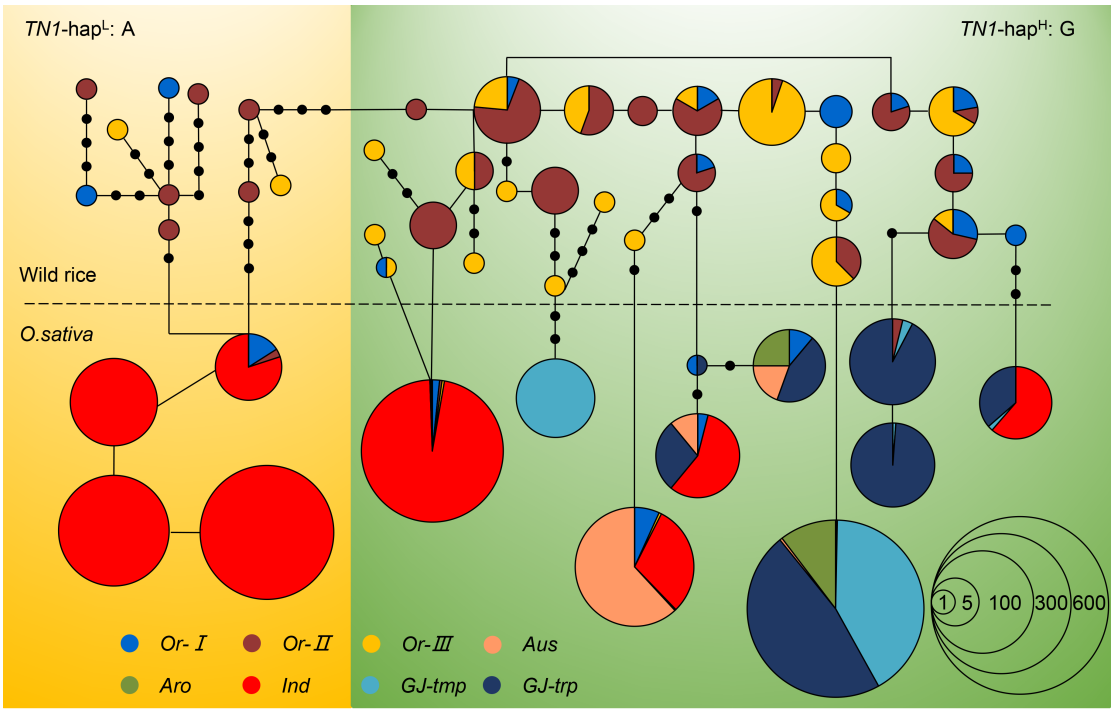

Figure S12 Evolutionary pattern of *TN1* based on minimum spanning

Minimum spanning tree of haplotypes, as created using methods described previously (Guo *et al.*, 2020).

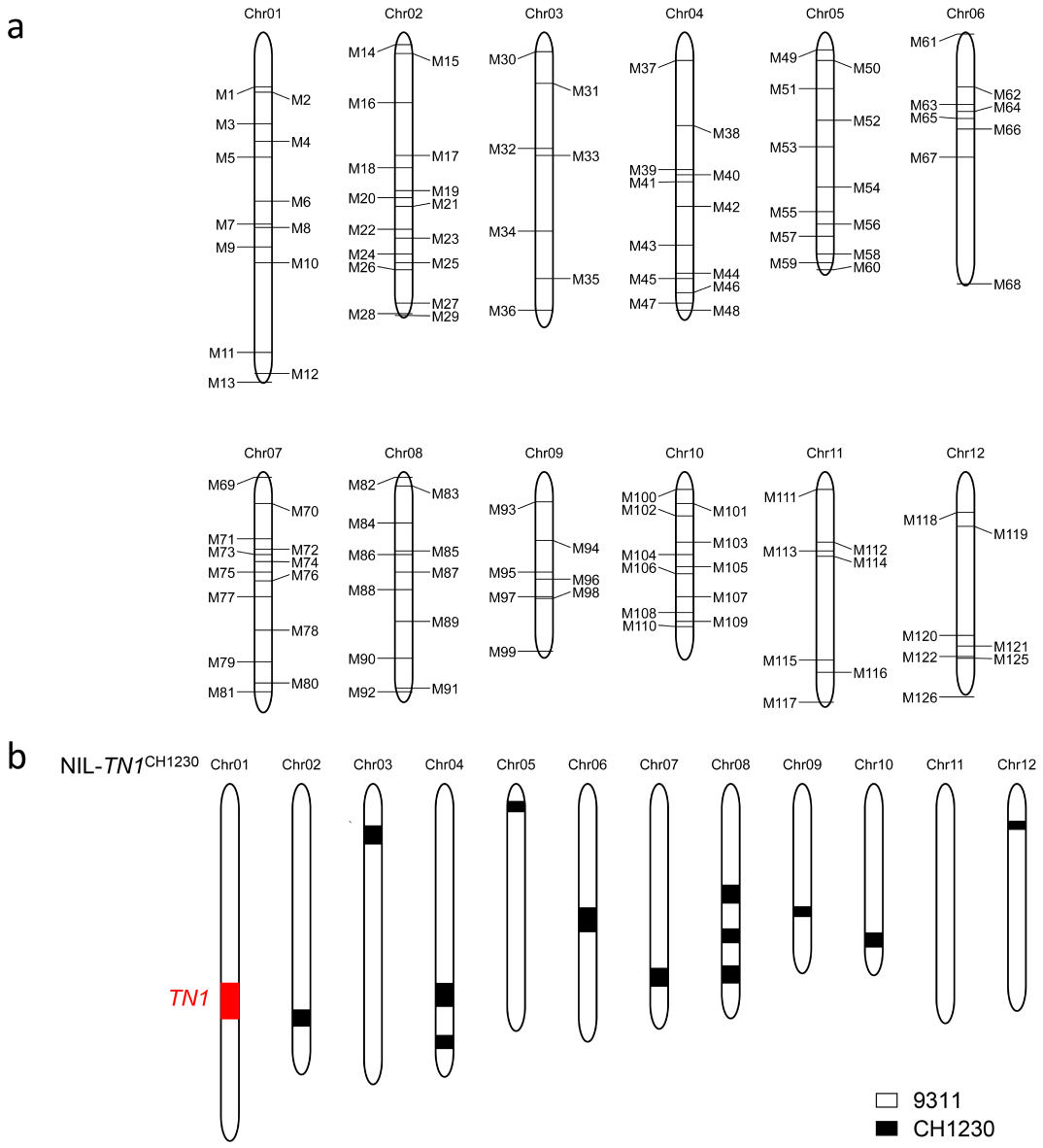

Figure S13 Genome constitution of NIL-*TN1*^{CH1230}

(a) A total of 126 polymorphism markers were used to genotype NIL-*TN1*^{CH1230}. (b) Graphical genotypes of NIL-*TN1*^{CH1230}. The red (*TN1*) and black regions indicate segments from CH1230, and the white regions indicate segments 93–11.

Sub	Hap	Promoter												Exon	No. of cvs.	No. of tiller
		-2000	-1958	-1776	-1627	-1597	-1487	-1106	-1020	-877	-817	-808	-277	+744		
Ind	*TIF1*-Hap1	A	G	T	G	T	G	C	C	G	T	G	G	G	60	11.21±0.29[a]
	TIF1-Hap2	A	G	T	G	T	A	C	C	G	T	G	G	A	7	10.76±0.79[a]
	TIF1-Hap3	G	A	A	A	C	A	A	G	A	G	A	A	A	66	10.01±0.25[b]
Jap	*TIF*-Hap1	A	G	T	G	T	G	C	C	G	T	G	G	G	8	7.86±0.80
	TIF-Hap2	A	G	T	G	T	A	C	C	G	T	G	G	A	123	8.73±0.20

Figure S14 Haplotype analysis of *TIF1*

Haplotype analysis of *TIF1* in a panel of 264 rice accessions. Data are presented as mean ± SE. Statistical significance was determined using two-sided Student's *t*-test. Different letters indicate significant differences in tiller number ($P < 0.05$).

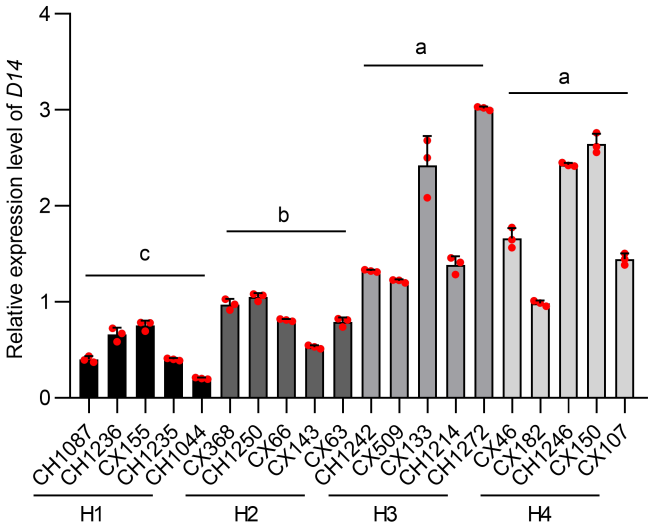

Figure S15 Expression levels of *D14* among different combination of *TN1* and *TIF1* haplotypes in natural germplasms. Data are presented as mean ± SD (*n* = 3).

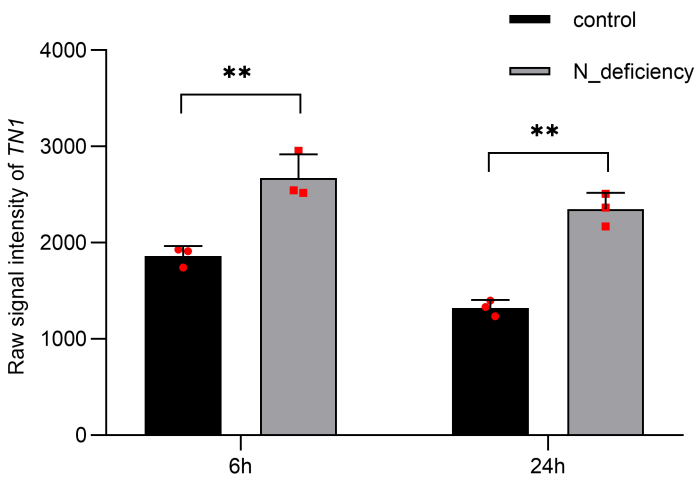

Figure S16 *TN1* is induced by nitrogen treatment

TN1 expression in roots with and without nitrogen treatment. Data were obtained from RiceXpro (https://ricexpro.dna.affrc.go.jp/).

Table S1 Nucleotide diversity and selection analysis of *TN1*

Taxon	n	L	S	H	π	Tajima'*D*
OrI	63	9258	27	22	0.13741	-0.66505
OrII	69	9258	14	13	0.0451	-1.79818
OrIII	89	9258	11	14	0.02823	-1.89521
Ind	1123	9258	24	18	0.05934	-0.41359
GJ-tmp	284	9258	34	7	0.04538	-1.18705
GJ-trp	472	9258	45	16	0.03673	-1.3057
Aus	196	9258	22	12	0.02694	-1.37571
Aro	75	9258	25	5	0.05701	-0.74775

Table S1 Nucleotide diversity and selection analysis of *TN1*

Nucleotide diversity and Tajima's *D* value in the *TN1* genome region and 2 kb promoter. n: total number of samples; L: average sequence length; S: number of segregating sites; H: number of haplotypes; π: average number of pairwise nucleotide differences per site based on the total number of polymorphic sites.