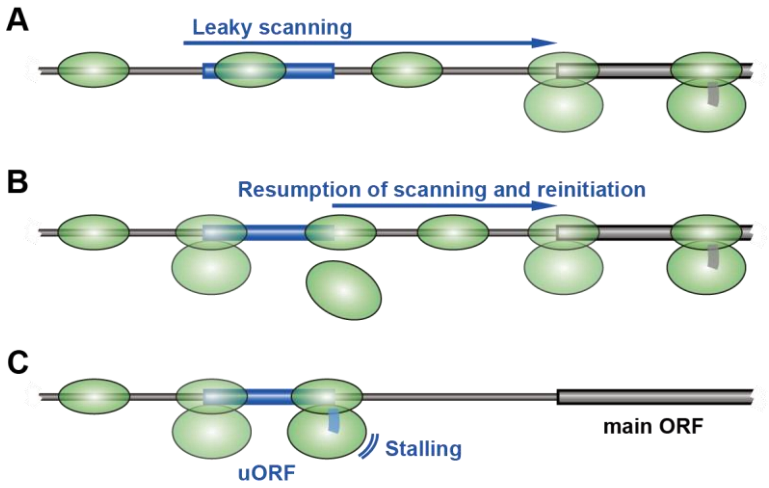
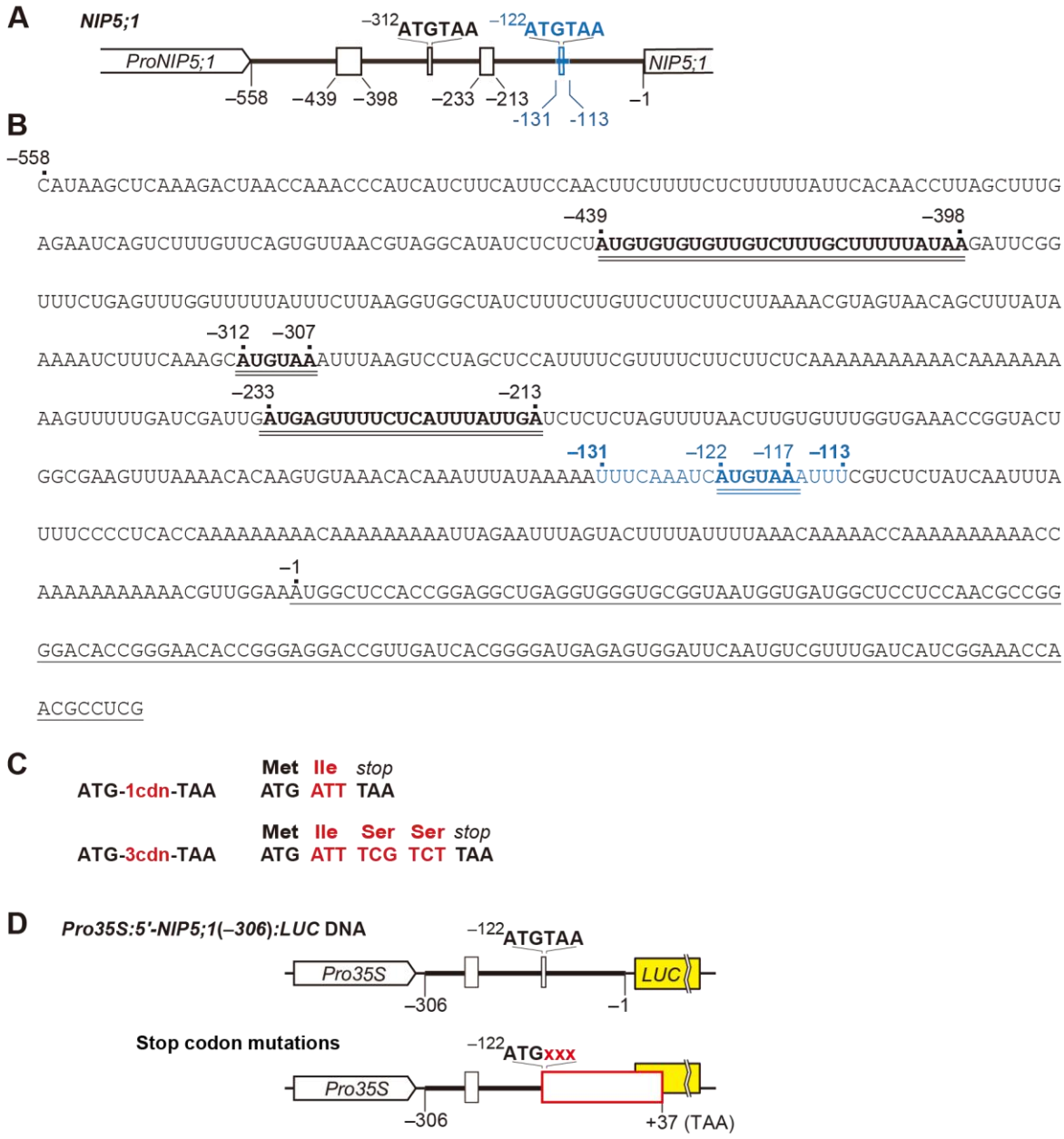


Supplemental Figure 1. General Effects of uORF on Translation of the Main ORF.

(A) Translation of the main ORF by leaky scanning at the uORF. AUG codon of the uORF is not recognized by the scanning 40S subunit (the 43S preinitiation complex), and the main ORF is translated.

(B) Translation of the main ORF by reinitiation of translation. After translation of uORF, 80S ribosome dissociates into 60S and 40S subunits, but the 40S subunit occasionally remains on the mRNA. In yeast, this process is reported to be more efficient in short uORFs than in longer ones (Rajkowitsch et al., 2004). The 40S subunit remaining on the mRNA resumes scanning for the downstream AUG codon, and reinitiate translation at the main ORF. The efficiency of reinitiation is higher if the spacer (the distance between the stop codon of the uORF and the AUG codon of the main ORF) is longer (Child et al., 1999; Zhou et al., 2010).

(C) If a ribosome stalls during translation of the uORF or at the stop codon of the uORF, translation of the main ORF is strongly inhibited.



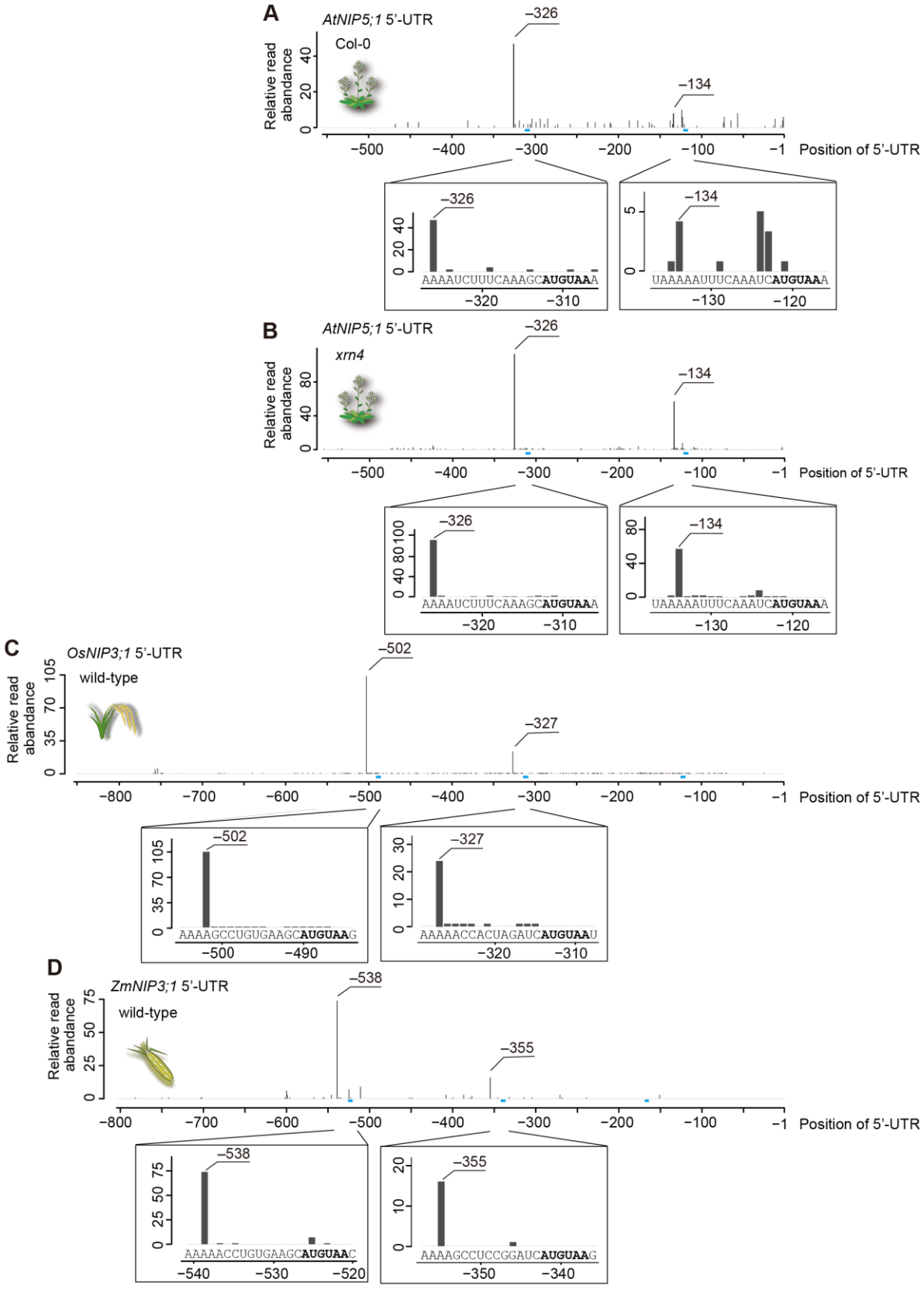
Supplemental Figure 2. Positions of uORFs, RNA Sequence of *NIP5;1* 5′-UTR and a Portion of *NIP5;1* Coding Region.

(A) Schematic representation of the *NIP5;1* 5′-UTR with positions of uORFs. Thick line represents 5′-UTR of *NIP5;1*. Open boxes represent uORFs. *NIP5;1* 5′-UTR contains four uORFs, including $^{-312}$AUG-stop and $^{-122}$AUG-stop. Nucleotide numbers are relative to the translation start site (+1).

(B) *NIP5;1* 5′-UTR sequence. uORFs are marked with double-underline and bold-face letters. The main ORF of *NIP5;1* is underlined. The blue bar/box in **(A)** and blue letters in **(B)** mark the region required for *NIP5;1* mRNA destabilization that we previously reported (Tanaka et al., 2011), which contains $^{-122}$AUG-stop.

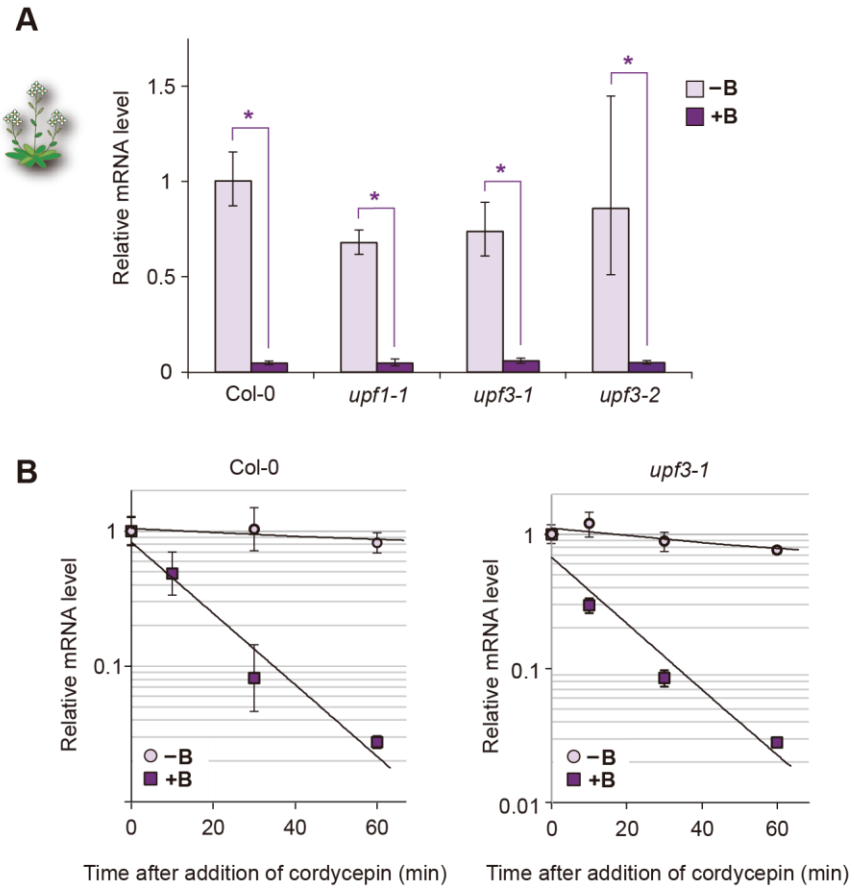
(C) Nucleotide and amino acid sequences of ATG-1cdn-TAA and ATG-3cdn-TAA constructs used in Figure 1B.

(D) Schematic representation of the stop codon mutations used in Figure 1B. The ORF started at $^{-122}$AUG is extended into the LUC reporter ORF in a different reading frame from the *LUC* ORF.


A
AtNIP5;1 5'-UTR
Col-0
Relative read abundance
−326
−134
Position of 5'-UTR
AAAAUCUUUCAAAGCAUGUAAA
UAAAAAUUUCAAAUCAUGUAAA
B
AtNIP5;1 5'-UTR
xrn4
C
OsNIP3;1 5'-UTR
wild-type
−502
−327
AAAAGCCUGUGAAGCAUGUAAG
AAAAACCACUAGAUCAUGUAAU
D
ZmNIP3;1 5'-UTR
wild-type
−538
−355
AAAAACCUGUGAAGCAUGUAAC
AAAAGCCUCCGGAUCAUGUAAG

Supplemental Figure 3. Position of 5′-Ends of mRNA Decay Intermediates in 5′-UTRs of *NIP5;1* and Its Rice and Maize Orthologs.

(A) to **(D)** Degradome datasets available at PARE database were analysed. The raw data were normalized to transcripts per 10 million and shown as relative read abundance. Blue bars represent the position of AUGUAA sequence. **(A)** and **(B)** Arabidopsis *NIP5;1* data for inflorescence tissues of wild-type (Col-0) **(A)** and *xrn4* mutant plants **(B)** grown on soil conditions (German et al., 2008). The plots show the distribution of mRNA decay intermediates in the 5′-UTR of *NIP5;1* (−1 to −558 nt). **(C)** and **(D)** Degradome datasets for *OsNIP3;1* in rice seedlings grown on hydroponic culture for 3 weeks (Li et al., 2010) **(C)** and for *ZmNIP3;1* in ears of maize grown in a controlled environment until ears are developed (Liu et al., 2014) **(D)**. The plots show the distribution of mRNA decay intermediates in the 5'-UTRs of *OsNIP3;1* (−1 to −859 nt) and *ZmNIP3;1* (−1 to −812 nt).



C

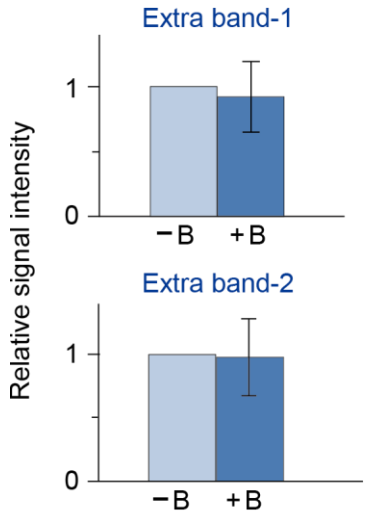
	NIP5;1 mRNA half-life ±SD (min)	
	−B	+B
Col-0	100 ± 33	12 ± 1
upf3-1	114 ± 41	12 ± 1

Supplemental Figure 4. B-Dependence of *NIP5;1* mRNA Accumulation and Half-Lives in NMD Mutants.

(A) mRNA accumulation of wild-type and NMD mutant, *upf1-1*, *upf3-1* and *upf3-3*, plants grown for 10 days under 100 µM B (+B) and 0.3 µM B (−B) conditions. Means ± SD of relative mRNA accumulation (n = 3) are shown. Asterisks indicate significant reduction under +B condition ($p < 0.05$).

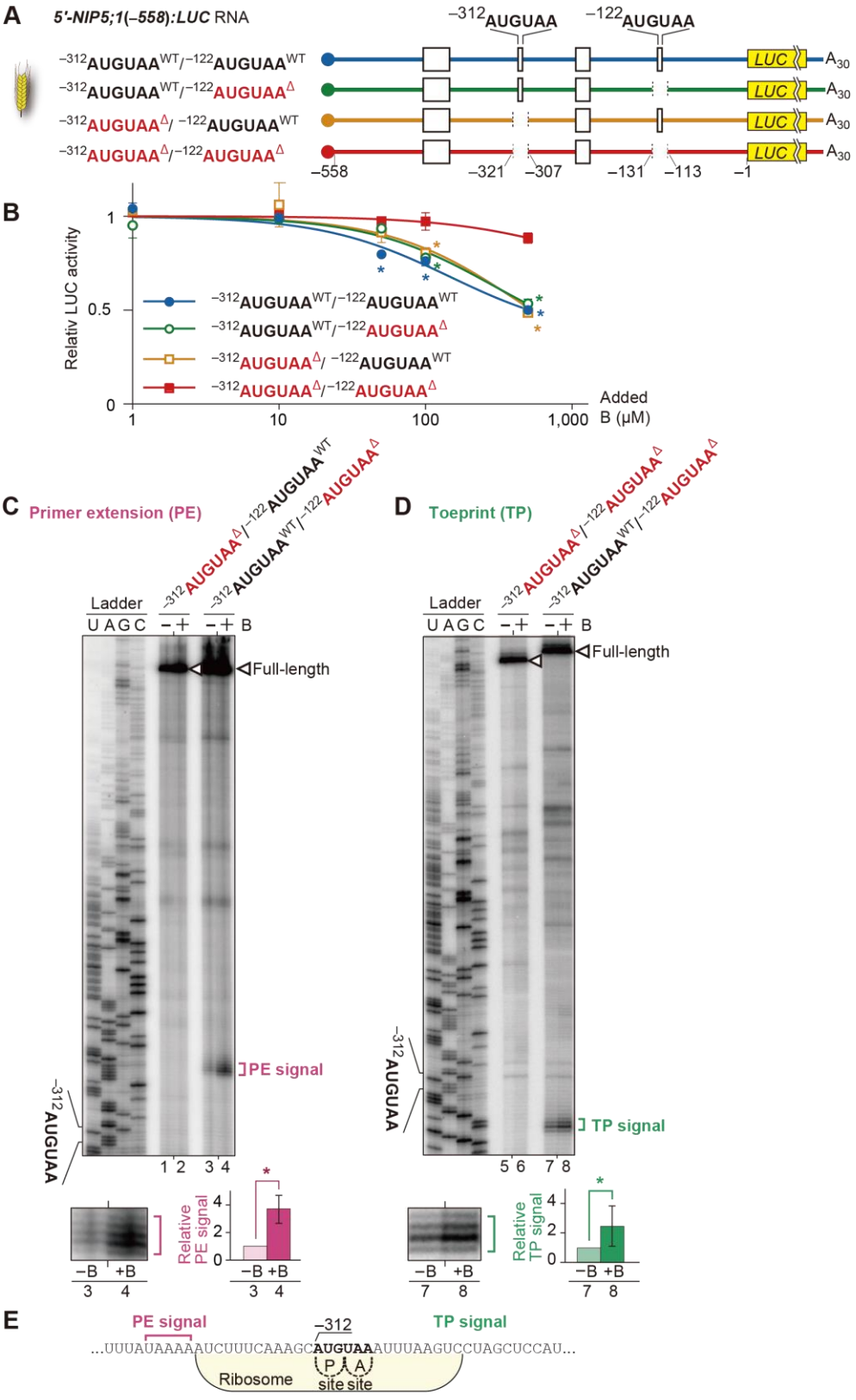
(B) mRNA degradation of wild-type and *upf3-1* mutant plants grown on solid media containing 0.3 µM B for 10 days. Plants were then transferred to hydroponic culture medium containing 100 µM B or 0.3 µM B conditions for 10 min, and then cordycepin, an inhibitor of transcription, was applied. Root samples were harvested 0, 10, 30, and 60 min after cordycepin application, and mRNA levels were measured by qRT-PCR. *NIP5;1* mRNA levels after cordycepin application were normalized to those at t=0. Means ± SD of relative mRNA accumulation (n = 3) are shown.

(C) *NIP5;1* mRNA half-lives were determined by linear regression of log-converted relative mRNA amounts.



Supplemental Figure 5. The Extra Bands in Primer Expression Assay Do Not Respond to B Conditions.

The intensities of the extra bands relative to the −B conditions are presented after normalizing the signal intensities with those of the full-length mRNA. Means ± SD are shown (n = 3).


A
5′-NIP5;1(−558):LUC RNA
−312AUGUAA
−122AUGUAA
−312AUGUAAWT/−122AUGUAAWT
−312AUGUAAWT/−122AUGUAAΔ
−312AUGUAAΔ/ −122AUGUAAWT
−312AUGUAAΔ/−122AUGUAAΔ
LUC
A30
−558
−321
−307
−131
−113
−1
B
Relativ LUC activity
1
0.5
0
1
10
100
1,000
Added B (μM)
−312AUGUAAWT/−122AUGUAAWT
−312AUGUAAWT/−122AUGUAAΔ
−312AUGUAAΔ/ −122AUGUAAWT
−312AUGUAAΔ/−122AUGUAAΔ
C
Primer extension (PE)
D
Toeprint (TP)
Ladder
U A G C
− + − + B
Full-length
PE signal
TP signal
−312AUGUAA
1 2 3 4
5 6 7 8
Relative PE signal
Relative TP signal
−B +B
3 4
7 8
E
PE signal
TP signal
−312
...UUUAUAAAAAUCUUUCAAAGCAUGUAAAUUUAAGUCCUAGCUCCAU...
Ribosome
P site
A site

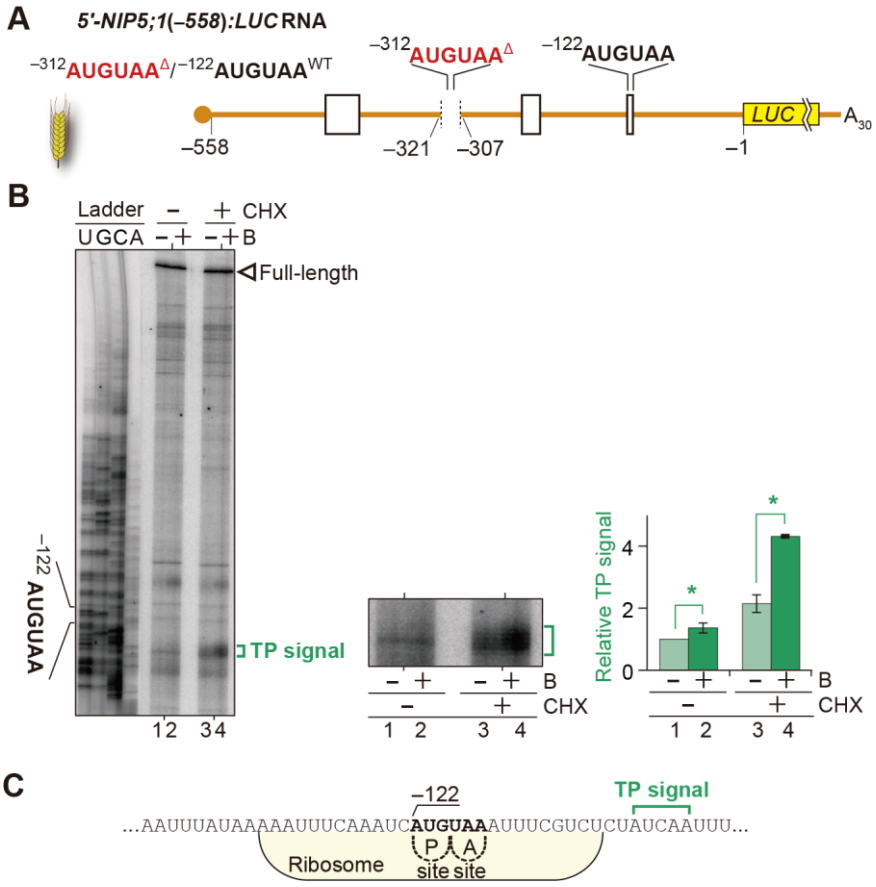
Supplemental Figure 6. Both $^{-312}$AUG-Stop and $^{-122}$AUG-Stop Are Responsive to B in WGE In Vitro Translation System.

(A) Schematic representations of 5′-*NIP5;1*(*−558*)*:LUC* RNA and its mutants in which $^{-312}$AUGUAA and/or $^{-122}$AUGUAA was deleted. Open boxes represent uORFs.

(B) RNAs were used for WGE in vitro translation assays at various B concentrations. Means ± SD of relative LUC activities (n = 3) are shown. Asterisks indicate significant reduction of relative reporter activities compared with $^{-312}$ATGTAA$^{\Delta}$/$^{-122}$ATGTAA$^{\Delta}$ ($p < 0.05$).

(C) and **(D)** Primer extension (PE) **(C)** and toeprint (TP) **(D)** analyses after in vitro translation in WGE with 300 µM B (+B) or without B supplementation (−B). Primer-2 (Supplemental Table 4) was used. Open arrowheads mark 5′-ends of the full-length RNA. Magenta and green brackets mark the PE and TP signals, respectively. The PE and TP signals are enlarged and their intensities relative to the −B conditions are presented after normalizing the signal intensities with those of the full-length mRNA. Means ± SD are shown (n = 3). Asterisks indicate significant difference ($p < 0.05$).

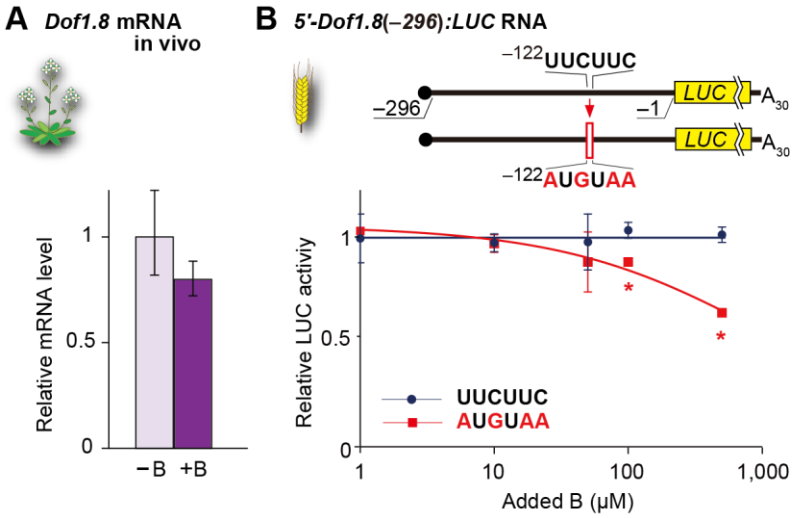
(E) Nucleotide sequence around $^{-312}$AUG-stop. Positions of PE (magenta) and TP signals (green) are marked. Ribosome occupation of RNA with AUG codons positioned at the P-site is shown.



Supplemental Figure 7. B-Dependent Toeprint Signals Are Strengthened by CHX Treatment.

(A) Schematic representations of *5′-NIP5;1*(−*558*)*:LUC* RNA carrying deletion of $^{-312}$AUG-stop ($^{-312}$ATGTAA$^{\Delta}$/$^{-122}$ATGTAA^{WT}). Open boxes represent uORFs.

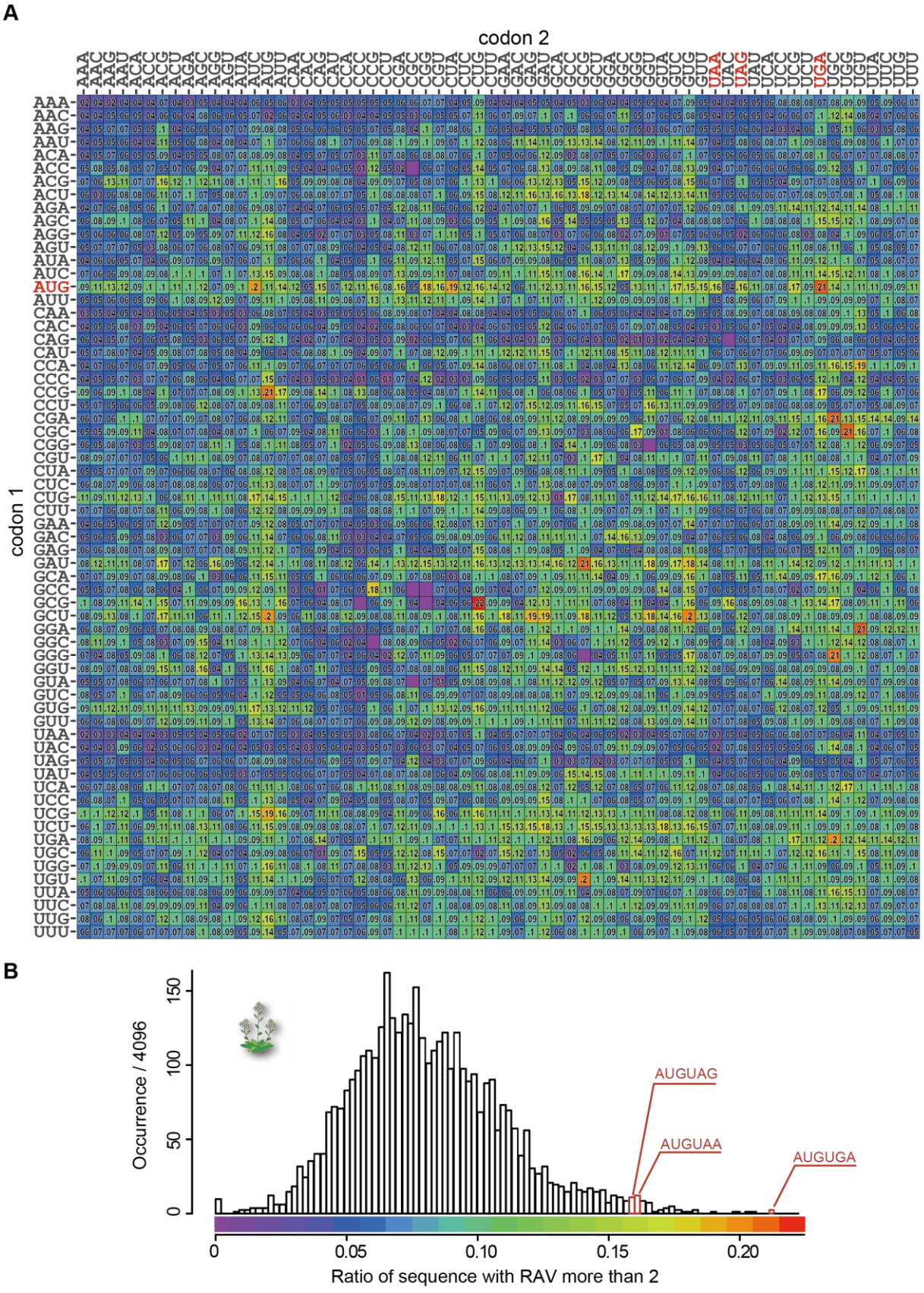
(B) Toeprint (TP) analyses after in vitro translation in WGE with 300 μM B (+B) or without B supplementation (−B) for 30 min. For the +CHX samples, CHX was added after the translation reaction. Open arrowheads mark 5′-ends of the full-length RNA. Green bracket marks the TP signals. The TP signals are enlarged and their relative signal intensities normalized with −B condition are shown. Means ± SD are shown (n = 3). Asterisks indicate significant difference ($p < 0.05$). Nucleotide sequence around $^{-122}$AUG-stop with positions of TP signals (green) are marked. Ribosome occupation of RNA with AUG codon positioned at the P-site is shown.



Supplemental Figure 8. B-Dependent Downregulation Conferred by Introduction of AUG-Stop into *Dof1.8* 5′-UTR.

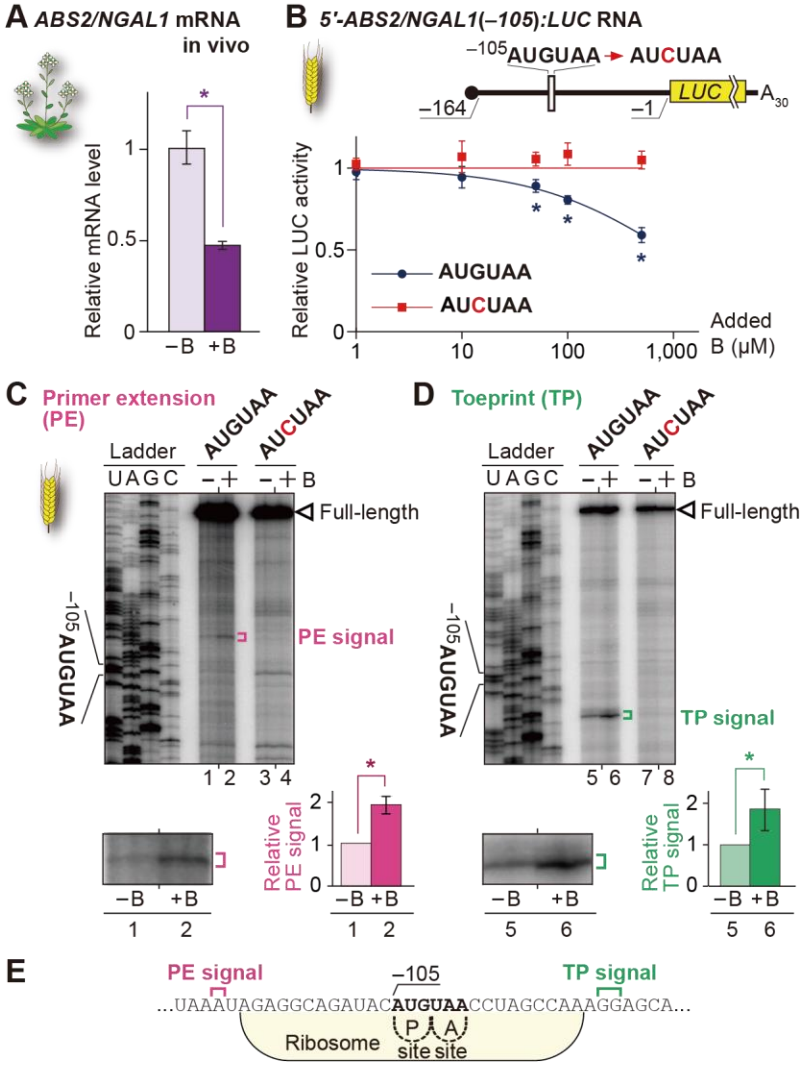
(A) mRNA accumulation in wild-type roots under 100 µM B (+B) and 0.3 µM B (−B) conditions. Means ± SD of relative mRNA accumulation (n = 3) are shown.

(B) Schematic representation of 5′-*Dof1.8*(*−296*)*:LUC* RNA and the effect of AUGUAA introduction on the B response. RNA carrying 5′-*Dof1.8*(*−296*)*:LUC* with or without AUGUAA was translated in WGE in the presence of various B concentrations. Means ± SD of relative LUC activities (n = 3) are shown. Asterisks indicate significant reduction of reporter activity in RNA having an AUGUAA than that in RNA having the original *Dof1.8* 5′-UTR sequence ($p < 0.05$).


A
codon 2
codon 1
B
Occurrence / 4096
0
50
100
150
AUGUAG
AUGUAA
AUGUGA
0
0.05
0.10
0.15
0.20
Ratio of sequence with RAV more than 2

Supplemental Figure 9 T**endency of Two Codon Combinations to Trigger Ribosome Arrest in 5′-UTRs.**

(A) and **(B)** Frequencies of ribosome arrest in each of the combination of two codons (with codon 1 in the P-site and codon 2 in the A-site) were evaluated by investigating ribosome footprint analysis datasets (SRR966474). For each instance of 2 codon combination in 5′-UTRs, the number of reads that start from 15 nt upstream of codon 1, which corresponds to the 5′-end of the ribosome stalled with the A-site on codon 1, was counted and normalized by average read count of the mRNA to cancel the read depth variation caused by different expression levels (designated as Ribosome Arrest Value, RAV). The numbers in **(A)** is the ratio of the instances with RAV greater than two to all instances of the two-codon combinations. The blank cells denote that no instance was found. The distribution of the ratios among all the two-codon combinations (64 x 64 = 4,096) is shown in **(B)**.



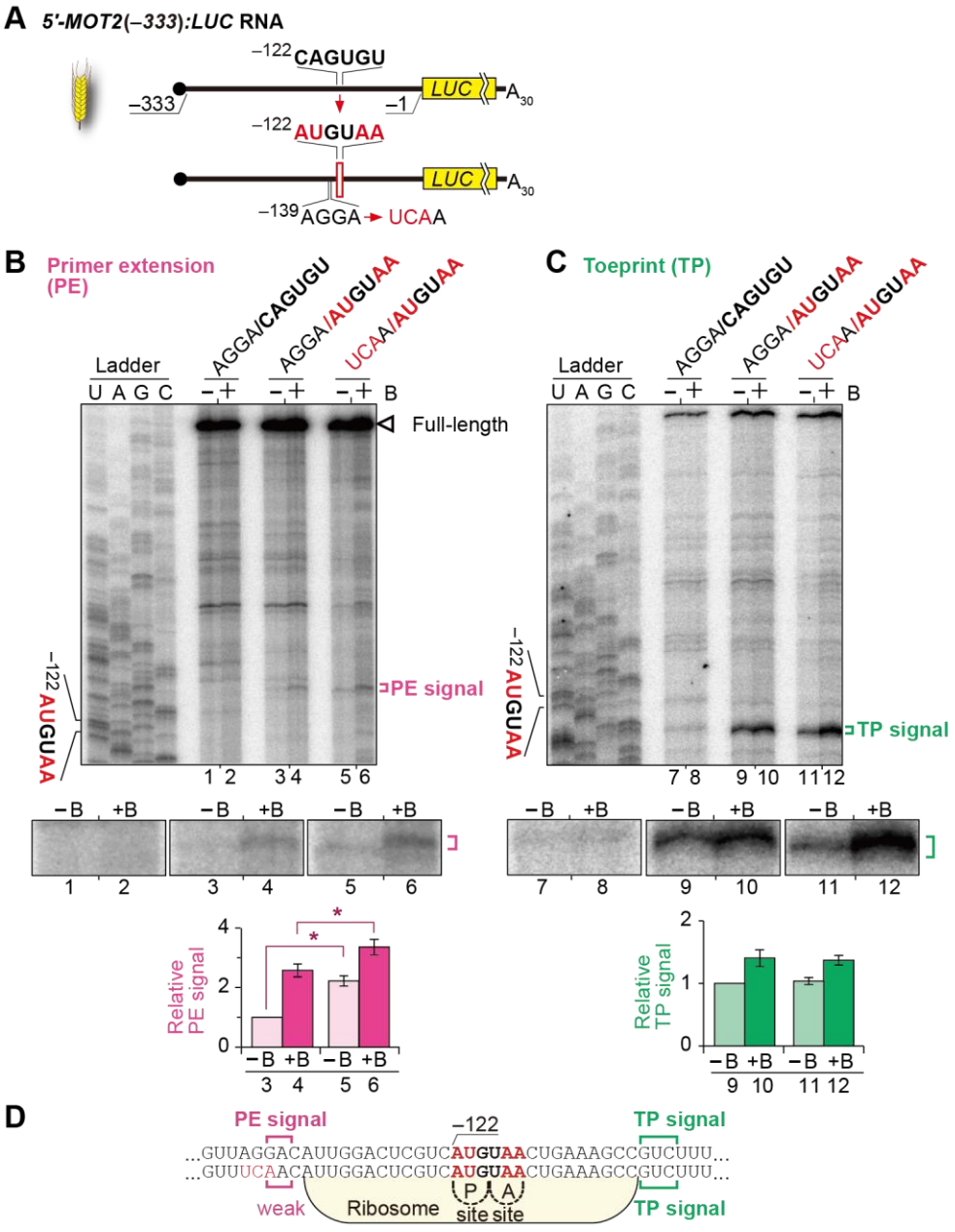
Supplemental Figure 10. Role of AUG-Stop in B-Dependent Downregulation of *ABS2/NGAL1*.

(A) mRNA accumulation in wild-type roots under 100 µM B (+B) and 0.3 µM B (−B) conditions. Means ± SD of relative mRNA accumulation (n = 3) are shown. An asterisk indicates significant reduction under +B condition ($p < 0.05$).

(B) Schematic representation of 5′-*ABS2/NGAL1*(−*105*):*LUC* RNA is shown. RNA carrying 5′-*ABS2/NGAL1:LUC* with or without mutation in AUGUAA was translated in WGE, and means ± SD of relative LUC activities (n = 3) are shown. Asterisks indicate significant reduction of reporter activity with RNA carrying AUGUAA ($p < 0.05$).

(C) and **(D)** Primer extension (PE) **(C)** and toeprint (TP) **(D)** analyses in WGE with 300 µM B (+B) or without B supplementation (−B). Signals are enlarged below the main image of AUGUAA lane, and means ± SD (n = 3) of relative intensities are shown. Asterisks indicate significant difference ($p < 0.05$).

(E) Nucleotide sequence around AUG-stop with positions of PE (magenta) and TP signals (green) are marked. Ribosome occupation of RNA with AUG codon positioned at the P-site is shown.

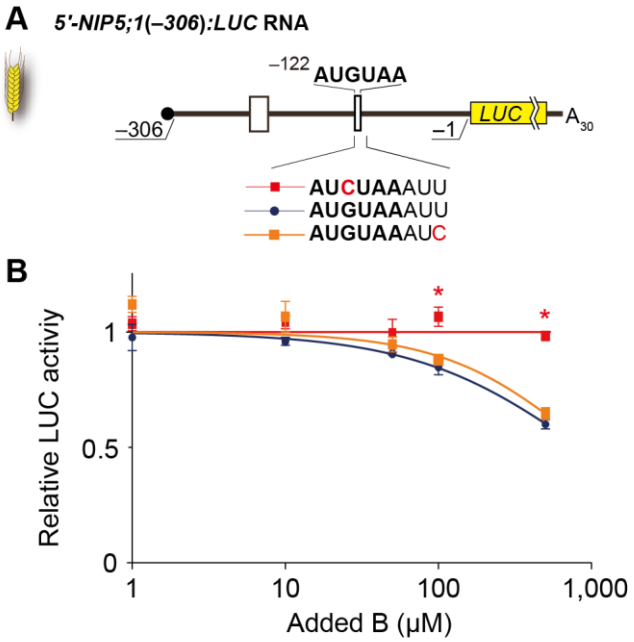

A
5′-MOT2(–333):LUC RNA
–122 CAGUGU
–333
–1
LUC
A30
–122 AUGUAA
LUC
A30
–139 AGGA → UCAA
B
Primer extension (PE)
AGGA/CAGUGU
AGGA/AUGUAA
UCAA/AUGUAA
Ladder
U A G C
– + B
Full-length
PE signal
–122 AUGUAA
1 2 3 4 5 6
–B +B
Relative PE signal
C
Toeprint (TP)
TP signal
7 8 9 10 11 12
Relative TP signal
D
PE signal
TP signal
–122
...GUUAGGACAUUGGACUCGUCAUGUAACUGAAAGCCGUCUUU...
...GUUUCAACAUUGGACUCGUCAUGUAACUGAAAGCCGUCUUU...
weak
Ribosome
P site
A site
TP signal

Supplemental Figure 11. B-Dependent mRNA Degradation Is Enhanced by Introduction of the Upstream Conserved Sequence into *MOT2* 5′-UTR.

(A) Schematic representation of 5′-*MOT2*(*−333*)*:LUC* RNA. Open boxes represent uORFs.

(B) and **(C)** RNA carrying 5′-*MOT2*(*−333*)*:LUC* with or without a mutation in AUGUAA or in the region 17–15 nt upstream of $^{-122}$AUG-stop was translated in WGE. Primer extension (PE) **(B)** and toeprint (TP) **(C)** analyses in WGE with 300 µM B (+B) or without B supplementation (−B). Signals are enlarged under the main image, and their relative intensities are shown. Means ± SD are shown (n = 3). Asterisks indicate significant differences ($p < 0.05$).

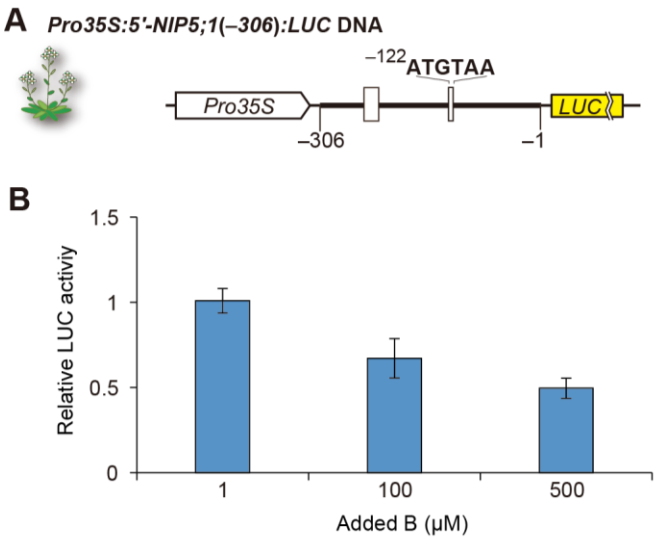
(D) Nucleotide sequence around $^{-122}$AUG-stop. Positions of PE (magenta) and TP signals (green) are marked. Red letter indicates the introduced mutations. Ribosome occupation of RNA having the AUG codon positioned at the P-site is shown.



Supplemental Figure 12. Nine nt Downstream of AUG-Stop Is not Involved in B-Dependent Downregulation in *NIP5;1* 5′-UTR in the In Vitro Translation Assay.

(A) Schematic representations of 5′-*NIP5;1*(−306)*:LUC* RNA and its mutants of $^{-312}$AUGUAA (AUCUAAAUU) or 9 nt downstream of $^{-312}$AUGUAA (AUGUAAAUC). Open boxes represent uORFs.

(B) RNAs were used for WGE in vitro translation assays at various B concentrations. Means ± SD of relative LUC activities (n = 3) are shown. Asterisks indicate significant difference of relative reporter activities compared with RNA carrying AUGUAAAUU ($p < 0.05$).



Supplemental Figure 13. B-Dependent Downregulation of *NIP5;1* under Different B Conditions.

(A) Schematic representation of *35S::5'-NIP5;1*(*–306*)*:LUC* DNA. Open boxes represent uORFs.

(B) Transfection experiments using Arabidopsis cultured cells. Transfected protoplasts were incubated under 500 μM B, 100 μM B or 1 μM B conditions. The LUC activity of each transfected cell extract was normalized with RLUC activity from the co-transfected internal control plasmid and shown as the relative LUC activity. Means ± SD of relative LUC activities are shown (n = 3).

Supplemental Table 1. List of B-Responsive Genes Carrying AUG-Stop in Their 5'-UTR.

AGI code	Gene name	Protein description	in vivo	in vitro translation[a]	
			qRT-PCR[b]	AUGUAA	AUCUAA
At4g10380	*NIP5;1*	NIP5;1 major intrinsic family protein	0.11 ± 0.12*	0.43 ± 0.01*	0.92 ± 0.04
At5g03190	*CPuORF47*	Conserved peptide upstream open reading frame 47	0.23 ± 0.03*	0.34 ± 0.03*	0.28 ± 0.06*
At2g36080	*ABS2/NGAL1*	AP2/B3-like transcriptional factor family protein	0.47 ± 0.02*	0.58 ± 0.01*	0.96 ± 0.02
At4g19370		Protein of unknown function	0.46 ± 0.11*	1.03 ± 0.04	n.t.[c]
At4g12420	*SKU5*	Cupredoxin superfamily protein	0.51 ± 0.01*	0.64 ± 0.02*	1.0 ± 0.02

[a] In vitro translation assay using RNAs carrying 5'-UTRs with AUG-stop (AUGUAA) sequence or with mutation in AUG-stop (AUCUAA) under 500 µM (+B) or 1 µM (–B) conditions. $FC_{(+B/-B)}$ was calculated. Asterisks indicate significant reduction under +B condition (n = 3).

[b] Total RNA was prepared from Col-0 roots that were grown under 100 µM (+B) or 0.3 µM (–B) B conditions for 10 days. qRT-PCR was performed and $FC_{(+B/-B)}$ was calculated. Asterisks indicate significant reduction under +B condition (n = 3).

[c] Not tested.

Supplemental Table 2. Sequence around the AUG-Stops of B-Responsive Genes.

Gene ID	Gene name	Sequence around AUG-stop[a]	AUG-stop[b]
At4g10380	*NIP5;1*	UUAUAAAAAUCUUUCAAAGC**AUGUAA**AUUUAAGUCCUAGCUCCAU	-312 **AUGUAA**
		AUUUAUAAAAAUUUCAAAUC**AUGUAA**AUUUCGUCUCUAUCAAUUU	-122 **AUGUAA**
At2g36080	*ABS2/NGAL1*	AUAUAAAUAGAGGCAGAUAC**AUGUAA**CCUAGCCAAAGGAGCAUUG	-105 **AUGUAA**
At4g12420	*SKU5*	CUUUUUCCCGAAUCUUGAUA**AUGUAA**AUUCACAACAAAUCUGUUU	-104 **AUGUAA**
Os10g36924 (*Oryza sativa*)	*NIP3:1*	UUGAGAAAAGCCUGUGAAGC**AUGUAA**GAACACACCAGUUUGUUCC	-490 **AUGUAA**
		UCUACAAAAACCACUAGAUC**AUGUAA**UUUUCGGCGAAAUCAUUCC	-314 **AUGUAA**
		UCUGCAAAAGCCAUCAAAUC**AUGUAA**UUAGUAGCUAAAAACCCAU	-125 **AUGUAA**
GRMZM2G176209 (*Zea mays*)	*NIP3:1*	UGGAGAAAAACCUGUGAAGC**AUGUAA**CUCCAGUCCCUGUCCAAAA	-526 **AUGUAA**
		UCAACAAAAGCCUCCGGAUC**AUGUAA**GUUUCGCCACCAUCAUCUU	-342 **AUGUAA**
		UCAGCAAAAACCAUCAAAUC**AUGUAA**UUAGUGUGCUCCCGCGUCC	-169 **AUGUAA**

[a] AUG-stops are marked with bold letters, the region 12–19 nt upstream of AUG-stop is underlined.

[b] Positions of AUG-stop from the main ORF are shown.

Supplemental Table 3. Plasmids Used in This Study and the Primers Used to Construct the Plasmids.

Plasmid	5'-UTR	Mutation	Reporter	Origin	Primers[a]	
					Forward	Reverse
Plasmids used for transient assay after transfection in Arabidopsis suspension cells						
pMT101	*NIP5;1* (−306)	$^{-122}$ATGTAA (WT)	LUC	this study	MT1f	MT12r
pMT104	*NIP5;1* (−306)	$^{-122}$TTGTAA	LUC	this study	MT4f	MT15r
pMT105	*NIP5;1* (−306)	$^{-122}$ATCTAA	LUC	this study	MT5f	MT16r
pMT107	*NIP5;1* (−306)	$^{-122}$ATGTAG	LUC	this study	MT7f	MT18r
pMT108	*NIP5;1* (−306)	$^{-122}$ATGTGA	LUC	this study	MT8f	MT19r
pMT109	*NIP5;1* (−306)	$^{-122}$ATG-1cdn-TAA[b]	LUC	this study	MT9f	MT20r
pMT110	*NIP5;1* (−306)	$^{-122}$ATG-3cdn-TAA[b]	LUC	this study	MT10f	MT21r
pMT141	*NIP5;1* (−306)	Deletion from −1 to −41	LUC	this study	MT1f	MT70r
pMT142	*NIP5;1* (−306)	Deletion from −1 to −65	LUC	this study	MT1f	MT71r
pMT143	*NIP5;1* (−306)	Deletion from −1 to −95	LUC	this study	MT1f	MT72r
pMT148	*NIP5;1* (−306)	TTC^{-122}ATGTAA	LUC	this study	MT80f	MT81r
pMT151	*NIP5;1* (−306)	$^{-122}$AAGTAA	LUC	this study	MT82f	MT90r
pMT152	*NIP5;1* (−306)	$^{-122}$ATGGGA	LUC	this study	MT83f	MT91r
pMT153	*NIP5;1* (−306)	$^{-122}$ATGCAA	LUC	this study	MT84f	MT92r
pMT154	*NIP5;1* (−306)	$^{-122}$ATGTAC	LUC	this study	MT85f	MT93r
pBI221-LUC+	vector sequence	'Vector 5'-UTR' (negative control)	LUC	Matsuo et al., 2001		
pKM75	vector sequence[c]	(internal control)	RLUC	this study	1832f	1801r
Plasmids used for construction of transgenic plants						
pMT100	*NIP5;1* (−306)	$^{-122}$ATGTAA (WT)	GUS	$P35S_{UTR+7}$-GUS in Tanaka et al., 2011		
pMT113	*NIP5;1* (−306)	$^{-122}$TTGTAA	GUS	this study	MT23f	MT24r
pMT145	*NIP5;1* (−558)	$^{-312}$ATGTAA$^{\Delta}$/$^{-122}$ATGTAA (WT)		this study	MT26f	MT35r
pMT146	*NIP5;1* (−558)	$^{-312}$ATGTAA$^{\Delta}$/$^{-122}$ATGTAA$^{\Delta}$		this study	MT27f	MT36r
pMT147	*NIP5;1* (−558)	$^{-312}$ATGTAA$^{\Delta}$/$^{-139}$CCCC/$^{-122}$ATGTAA		this study	MT73f	MT74r
pMT155	*NIP5;1* (−306)	$^{-122}$ATGGGA	GUS	this study	MT23f	MT24r
Plasmids used for transient assay after transfection in HeLa cells						
pMT139	*NIP5;1* (−231)	$^{-122}$ATGTAA (WT)	LUC	this study	MT68f	MT69r
pMT140	*NIP5;1* (−231)	$^{-122}$ATGTAA$^{\Delta}$	LUC	this study	MT68f	MT69r
pRL-SV40	vector sequence	(internal control)	RLUC	Promega		
Plasmids used for in vitro translation						
pMT114	*NIP5;1* (−558)	$^{-312}$ATGTAA^{WT} / $^{-122}$ATGTAA^{WT} (WT)	LUC	this study	MT25f	MT34r
pMT115	*NIP5;1* (−558)	$^{-312}$ATGTAA$^{\Delta}$ / $^{-122}$ATGTAA^{WT}	LUC	this study	MT26f	MT35r
pMT116	*NIP5;1* (−558)	$^{-312}$ATGTAA$^{\Delta}$ / $^{-122}$ATGTAA$^{\Delta}$	LUC	this study	MT27f	MT36r
pMT117	*NIP5;1* (−558)	$^{-312}$ATGTAA^{WT} / $^{-122}$ATGTAA$^{\Delta}$	LUC	this study	MT27f	MT36r
pMT125	*MOT2*	$^{-122}$CAGTGT (WT)	LUC	this study	MT32f	MT41r
pMT126	*MOT2*	$^{-122}$ATGTAA	LUC	this study	MT33f	MT42r
pMT127	*SKU5*	$^{-104}$ATGTAA (WT)	LUC	this study	MT43f	MT47r
pMT128	*SKU5*	$^{-104}$ATCTAA	LUC	this study	MT44f	MT48r
pMT129	*ABS2/NGAL1*	$^{-105}$ATGTAA (WT)	LUC	this study	MT45f	MT49r
pMT130	*ABS2/NGAL1*	$^{-105}$ATCTAA	LUC	this study	MT46f	MT50r
pMT131	*NIP5;1* (−306)	$^{-122}$ATGTAA (WT)	LUC	this study	MT51f	MT34r
pMT132	*NIP5;1* (−306)	$^{-122}$ATCTAA	LUC	this study	MT51f	MT34r
pMT144	*NIP5;1* (−306)	$^{-139}$CCCC	LUC	this study	MT73f	MT74r
pMT156	*NIP5;1* (−306)	$^{-122}$ATGTAAATC	LUC	this study	MT86f	MT94r
pMT157	*Dof1.8*	$^{-122}$TTCTTC (WT)	LUC	this study	MT87f	MT95r
pMT158	*Dof1.8*	$^{-122}$ATGTAA	LUC	this study	MT88f	MT96r
pMT161	*CPuORF47*	$^{-288}$ATGTAA (WT)	LUC	this study	MT52f	MT59r
pMT162	*CPuORF47*	$^{-288}$ATCTAA	LUC	this study	MT53f	MT60r
pMT163	*At4g19370*	$^{-38}$ATGTAA (WT)	LUC	this study	MT54f	MT61r
pMI27	vector sequence[d]	(internal control)	RLUC	Chiba et al., 2003		

[a] Primers used for construction. Sequence of the primers are found in Supplemental Table 4.

[b] Actual amino acid sequences are shown in Supplemental Figure 1C.

[c] Modified pBI221 sequence; 5'-ACACGGGGGACTCTAGACC-3'.

[d] pSP64 Poly(A) vector.

Supplemental Table 4. Primers Used in This Study.

Use	Name[a]	Sequence (5'-3')	Name[a]	Sequence (5'-3')	Remarks
Primer extension and toeprint analyses					
	Primer-1	TCGAGGCGTTGGTTTCCGATGATC			
	Primer-2	TCGCCAGTACCGGTTTCACCAAAC			
	ZW4	TCCAGGAACCAGGGCGTA			Wang and Sachs, 1997
qRT-PCR analysis					
	NIP5f	CACCGATTTTCCCTCTCCTGAT	NIP5r	GCATGCAGCGTTACCGATTA	*NIP5;1* mRNA
	At4g12420f	TCCTCTTGGTGTCCCTCAAC	At4g12420r	CAATGAAGAAGAAGTCCCTCGT	*SKU5* mRNA
	At2g36080f	CCGACTCTTATCGCCATGTT	At2g36080r	TCCATGTTCACTCCGAACAG	*ABS2/NGAL1* mRNA
	MOT2f	CGCCTTAGGATTTGGTTGTG	MOT2r	CTGCGACTCATCACTTGACC	*MOT2* mRNA
	At5g03190f	GGGTGGAAACCATACCTCCT	At5g03190r	TGCTCCATGATCACCAAAGA	*CPuORF47* mRNA
	At4g19370f	CGGTTCTATTGCTCCTGTCC	At4g19370r	TCATCAAGCCATCCTTCTCC	*At4g19370* mRNA
	eEF1αf	CCTTGGTGTCAAGCAGATGA	eEF1αr	TGAAGACACCTCCTTGATGATTT	*eEF1α* mRNA
	actin10f	GGTAACATTGTGCTCAGTGGTGG	actin10r	CTCGGCCTTGGAGATCCACATC	*Actin10* mRNA
	ubq10f	GGAGGTGGAGAGTTCTGACA	ubq10r	AGACCAAGTGAAGTGTGGAC	*Ubq10* mRNA
Plasmid construction[b]					
	1832f	GCTCTAGACCATGGTCATGACTTCGAAAGTTTA	1801r	GAATCAAGAACATTCATTTG	
	MT1f	GTTGGATCCATTTAAGTCCTAGCTC	MT12r	GAGCCTAGGTTCCAACGTTTTTTTTTTTG	
	MT4f	CAAATCTTGTAAATTTCGTCTCTATCAA	MT15r	GAGACGAAATTTACAAGATTTGAAATTTTTATAAATTTGTG	
	MT5f	CAAATCATCTAAATTTCGTCTCTATCAA	MT16r	GAGACGAAATTTAGATGATTTGAAATTTTTATAAATTTGTG	
	MT7f	CAAATCATGTAGATTTCGTCTCTATCAA	MT18r	GAGACGAAATCTACATGATTTGAAATTTTTATAAATTTGTG	
	MT8f	CAAATCATGTGAATTTCGTCTCTATCAA	MT19r	GAGACGAAATTCACATGATTTGAAATTTTTATAAATTTGTG	
	MT9f	GATTTAATCGTCTCTATCAATTTATTTC	MT20r	GACGATTAAATCATGATTTGAAATTTTTA	
	MT10f	CGTCTTAACTATCAATTTATTTCCCCTCAC	MT21r	GATAGTTAAGACGAAATCATGATTTGAAA	
	MT23f	CACCATTTAAGTCCTAGCTCCAT	MT24r	TTCCAACGTTTTTTTTTTTG	
	MT25f	GTGTCTAGACATAAGCTCAAAGACTAACCA	MT34r	GAGCCATGGCCAACGTTTTTTTTTTTGGT	
	MT26f	GCTTTATAAAAATCATTTAAGTCCTAGCTCCATTTTC	MT35r	CTAGGACTTAAATGATTTTTATAAAGCTGTTACTACG	
	MT27f	ACAAATTTATAAAAACGTCTCTATCAATT	MT36r	TAAATTGATAGAGACGTTTTTATAAATTT	
	MT32f	GTTTCTAGAGAGTTATAAACAATACAAACACTG	MT41r	GAGCCATGGGATTGGATCTAAAGTCAAAGC	
	MT33f	ACTCGTCATGTAACTGAAAAGCCGTCTTTTATCC	MT42r	CTTTTCAGTTACATGACGAGTCCAATGTCCTAAC	
	MT43f	TGCTCTAGATAGCCGTTCTCTTATGTCTATA	MT47r	CTGTCCATGGTTTCTTTTTTTTTCTCTCGCA	
	MT44f	GATAATCTAAATTCACAACAAATCT	MT48r	ATTTAGATTATCAAGATTCGGGAAAAAG	
	MT45f	TGCTCTAGAAGATAAATTTTCTCTTTCTT	MT49r	CTGTCCATGGTTGAAAGAGAGGGAGAGAGA	
	MT46f	ATACATCTAACCTAGCCAAAGGAGCAT	MT50r	AGGTTAGATGTATCTGCCTCTATTTAT	
	MT51f	GTGTCTAGAATTTAAGTCCTAGCTCCAT	MT59r	CTGTCCATGGTGATTTCTTCAAATACTTCA	
	MT52f	TGCTCTAGACTCTTCTTCCCCAAAAAAAA	MT60r	TCTTAGATGCTTGTAGAAATCTAAAAG	
	MT53f	GAGCATCTAAGACGAGATTTTGTTTC	MT61r	CTGTCCATGGAGTTTTTAGTGGACAAAATC	
	MT54f	TGCTCTAGAACCTCACATAGTCACATATC	MT69r	CCCAAGCTTGGGCCAACGTTTTTTTTTTTGGTTTTTTTTTTTG	
	MT68f	CCCAAGCTTAGTTTCTCATTTATTGATCT	MT70r	TATCCTAGGTAAAATAAAAGTACTAAATTCTAATTTTTTTTTTG	
	MT73f	ACACAAATTCCCCAAAATTTCAAATCATGTAAAT	MT71r	GCGCCTAGGTTTTTTTTTGTTTTTTTTTGGTGAGGGGA	
	MT78f	TAAAGTCGACAAAAATCAAGCCACTAACACG	MT72r	GAGCCTAGGAATAAATTGATAGAGACGAAATTTAC	
	MT80f	ATTTCAATTCATGTAAATTTCGTCTCTA	MT74r	TTGAAATTTTGGGGAATTTGTGTTTACACTTGTG	
	MT82f	CAAATCAAGTAAATTTCGTCTCTATCAA	MT79r	TAAAGTCGACACAACACATTACACATGCCATA	
	MT83f	CATGGGAATTTCGTCTCTATCAATTTAT	MT81r	CGAAATTTACATGAATTGAAATTTTTATAAATTTGTGTTTAC	
	MT84f	CAAATCATGCAAATTTCGTCTCTATCAA	MT90r	GAGACGAAATTTACTTGATTTGAAATTTTTATAAATTTGTG	
	MT85f	CATGTACATTTCGTCTCTATC	MT91r	GAGACGAAATTCCCATGATTTGAAATTTTTATAAATTTGTG	
	MT86f	TCATGTAAATCTCGTCTCTATC	MT92r	GAGACGAAATTTGCATGATTTGAAATTTTTATAAATTTGTG	
	MT87f	TGCTCTAGAAGTTCAGAGTGTGTCAGAGTCA	MT93r	GATAGAGACGAAATGTACATGA	
	MT88f	TTTTTTCGTCATGTAATTGCTTTTTCAAAAACCAG	MT94r	GATAGAGACGAGATTTACATGA	
			MT95r	CATGCCATGGGCTATGGAAATTTGCAG	
			MT96r	GAAAAAGCAATTACATGACGAAAAAAGAGAATTTAAACA	

[a] For qRT-PCR analysis and plasmid construction, forward and reverse primers are marked with a suffix "f" and "r", respectively.
[b] Restriction sites used for cloning the PCR-amplified fragments are underlined.

Supplemental Table 5. Relative *NIP5;1* mRNA Levels Obtained by Using Different Reference Genes.

Reference gene	Relative *NIP5;1* mRNA level[a] ± SD	
	+B	-B
eEF1α	1.0 ± 0.1	34.3 ± 6.9
Actin10	1.0 ± 0.1	33.1 ± 4.6
Ubq10	1.0 ± 0.1	36.4 ± 3.7

[a] qRT-PCR was performed with total RNA prepared from Col-0 roots that were grown under 100 μM (+B) or 0.3 μM (–B) conditions for 10 days (n = 3).