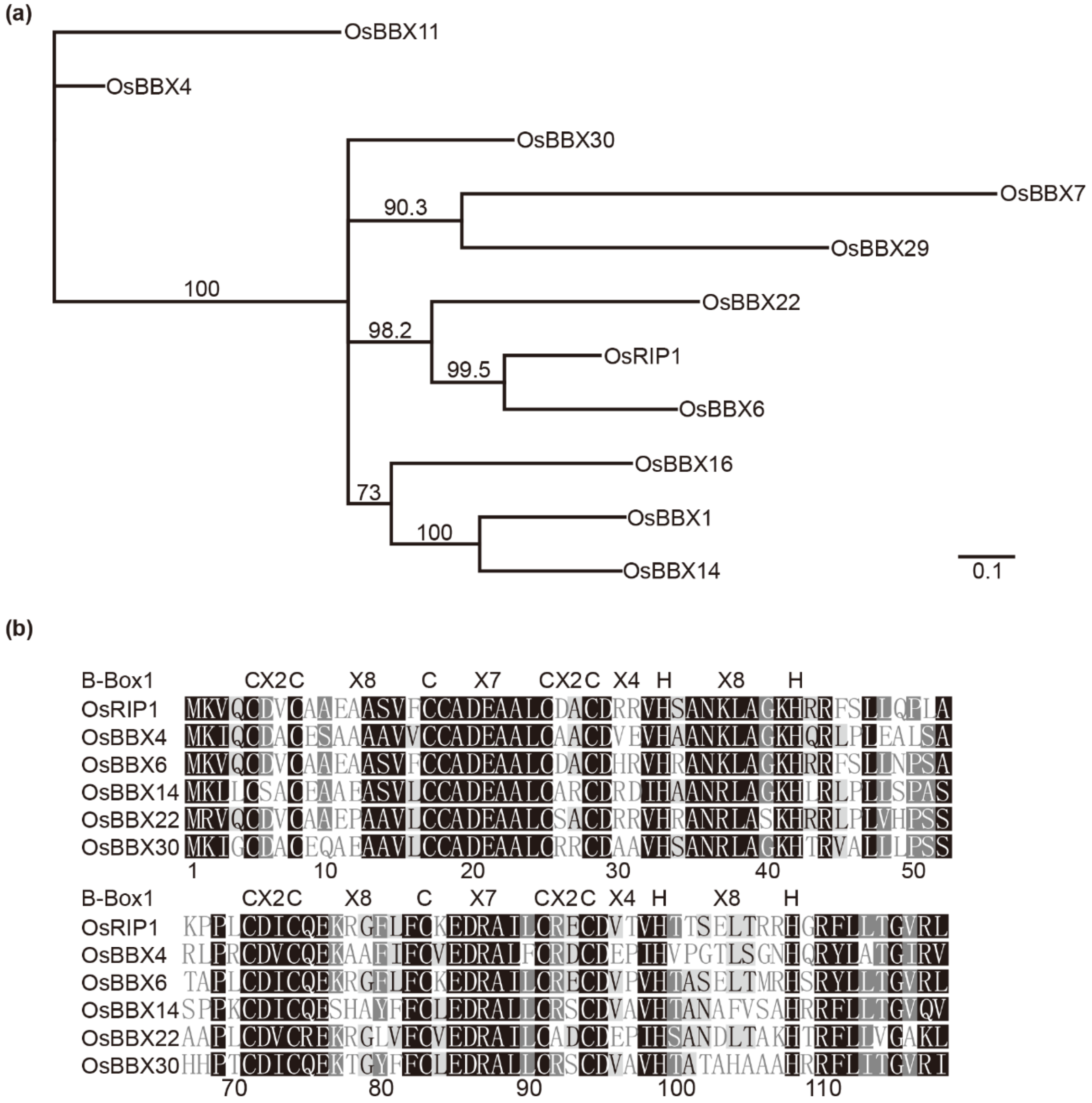
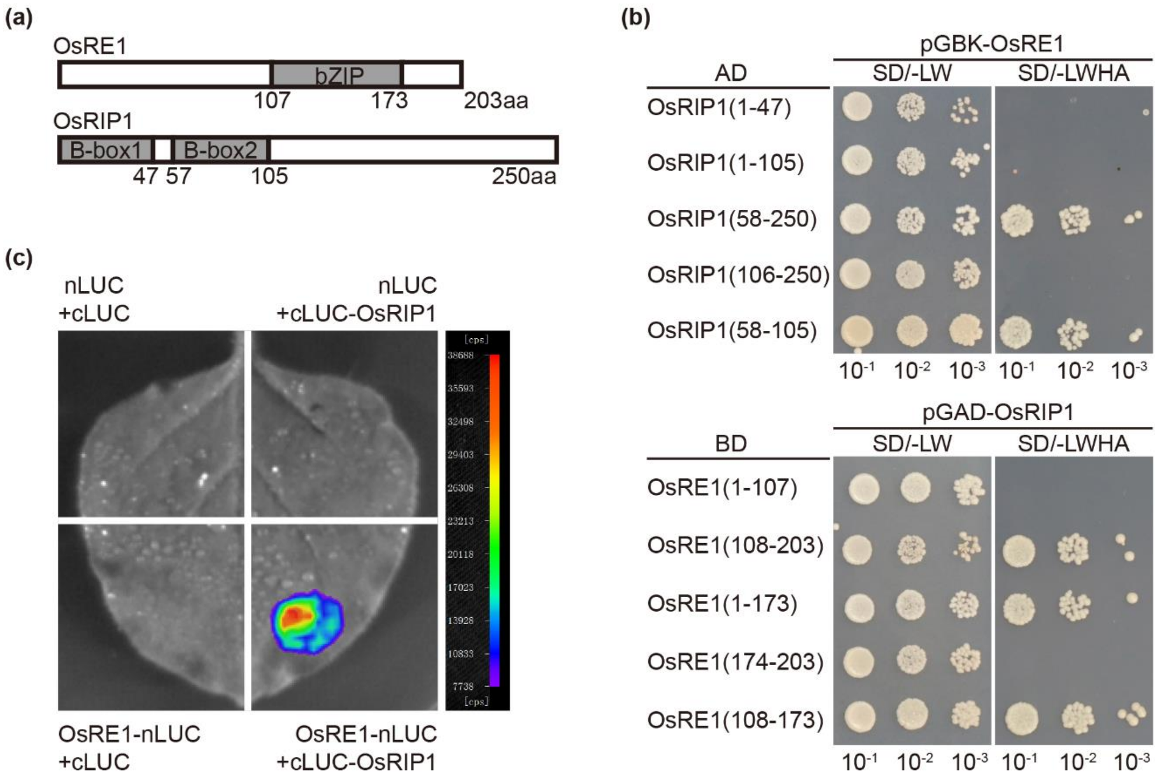


Supplemental Figure 1. Phylogenetic analysis of BBX family proteins in rice.

(a) Phylogenetic tree of OsRIP1 and its rice homologs. Tree was built using maximum likelihood analysis and a bootstrap of 1000 replications. Numbers on branches are consistency support rates for each clade. Scale bar indicates the average number of amino acid substitutions per site.

(b)Sequence alignments of the B-box 1 and B-box 2 domains. Identical, conserved and similar amino acid blocks are shaded in black, charcoal gray, and gray, respectively.

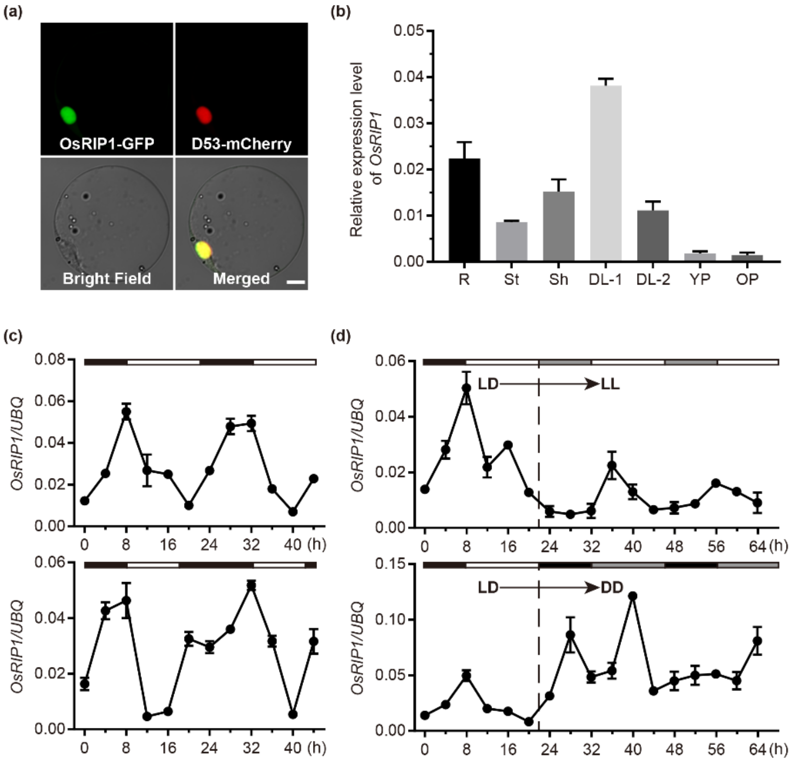


Supplemental Figure 2. OsRE1 physically interacts with OsRIP1.

(a) Schematic domain structures of OsRE1 and OsRIP1 proteins.

(b) Y2H assay showing the interaction analysis between series of truncated OsRE1 and OsRIP1 fragments. OsRE1 fragment (amino acids 108-173) and OsRIP1 fragment (amino acids 58-105) are necessary for the interaction between OsRE1 and OsRIP1. AD, activation domain. BD, binding domain.

(c) LCI assay showing the interaction of OsRE1 and OsRIP1 in leaves of *N. benthamiana*. The fluorescence signal intensity represents the level of interaction activity.



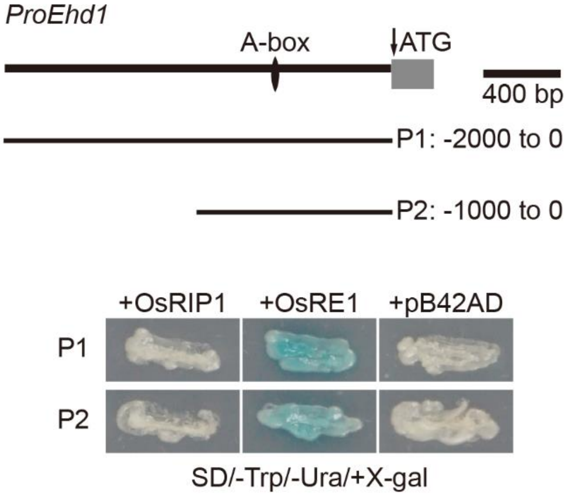
Supplemental Figure 3. Expression profiles and subcellular localization of OsRIP1**.**

(a) Subcellular localization of OsRIP1. D53-mCherry protein was used as a nuclear marker. Scale bar, 10 μm.

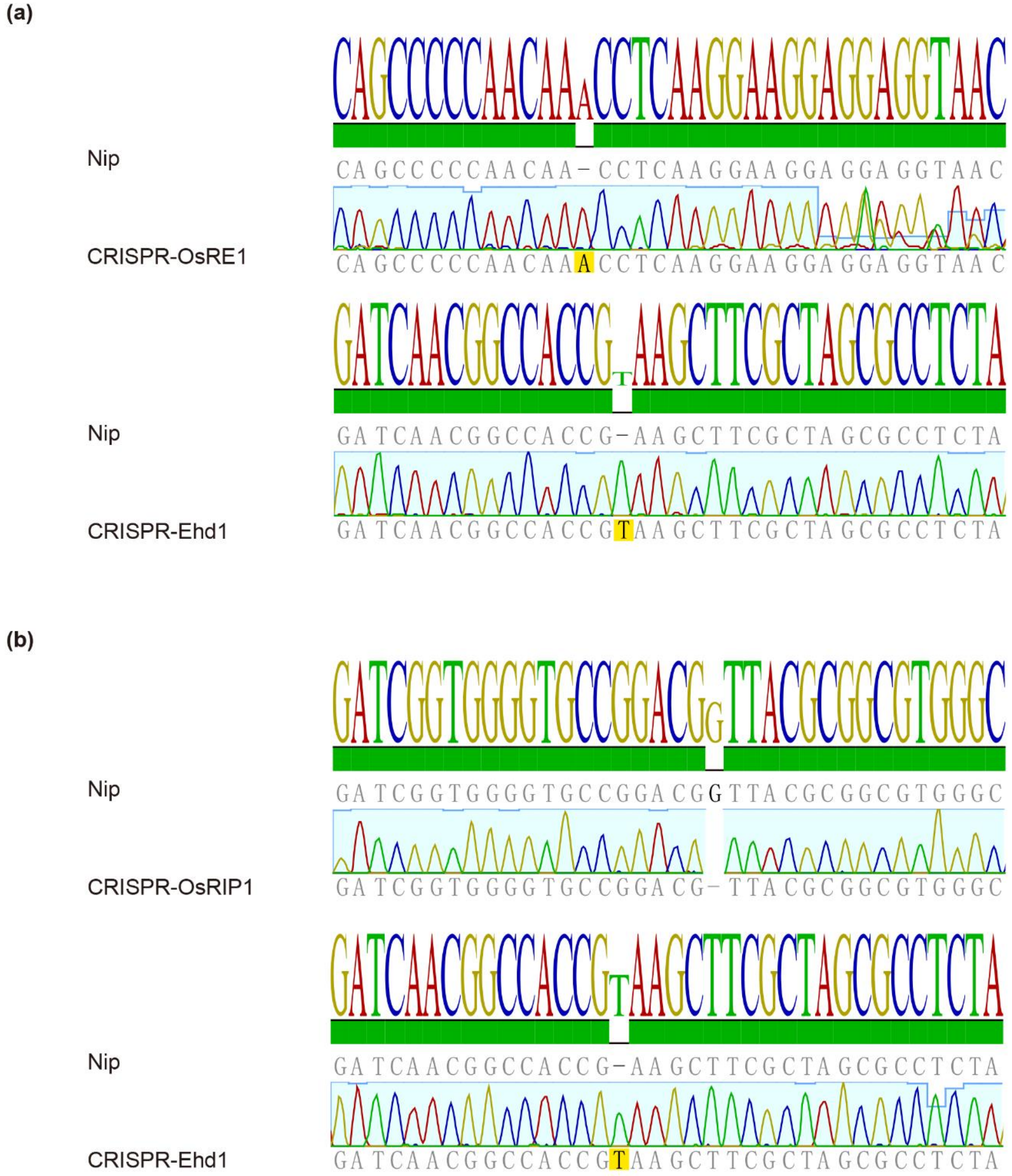
(b) qRT-PCR analysis of *OsRIP1* expression levels in different tissues. R, root; St, stem; Sh, leaf sheath; DL-1, uppermost leaves; DL-2, second uppermost leaves; YP, young panicles; OP, old panicles. Values are means ± SD; *n* = 3.

(c) Rhythmic expression of *OsRIP1* in second uppermost leaves under LD (14 h light/10 h darkness) and SD conditions (10 h light/14 h darkness). Rice *UBIQUITIN* gene was used as the internal control. Values are means ± SD; *n* = 3.

(d) Expression analysis of *OsRIP1* in second uppermost leaves under continuous light (LL) and continuous darkness (DD) conditions. The plants were grown in growth chambers under LD conditions for 30 days and then transferred to LL or DD conditions for 2 days. Black and white bars indicate darkness and light, respectively. gray bars represent the subjective darkness in DD and subjective light in LL. Rice *UBIQUITIN* gene was used as the internal control. Values are means ± SD; *n* = 3.



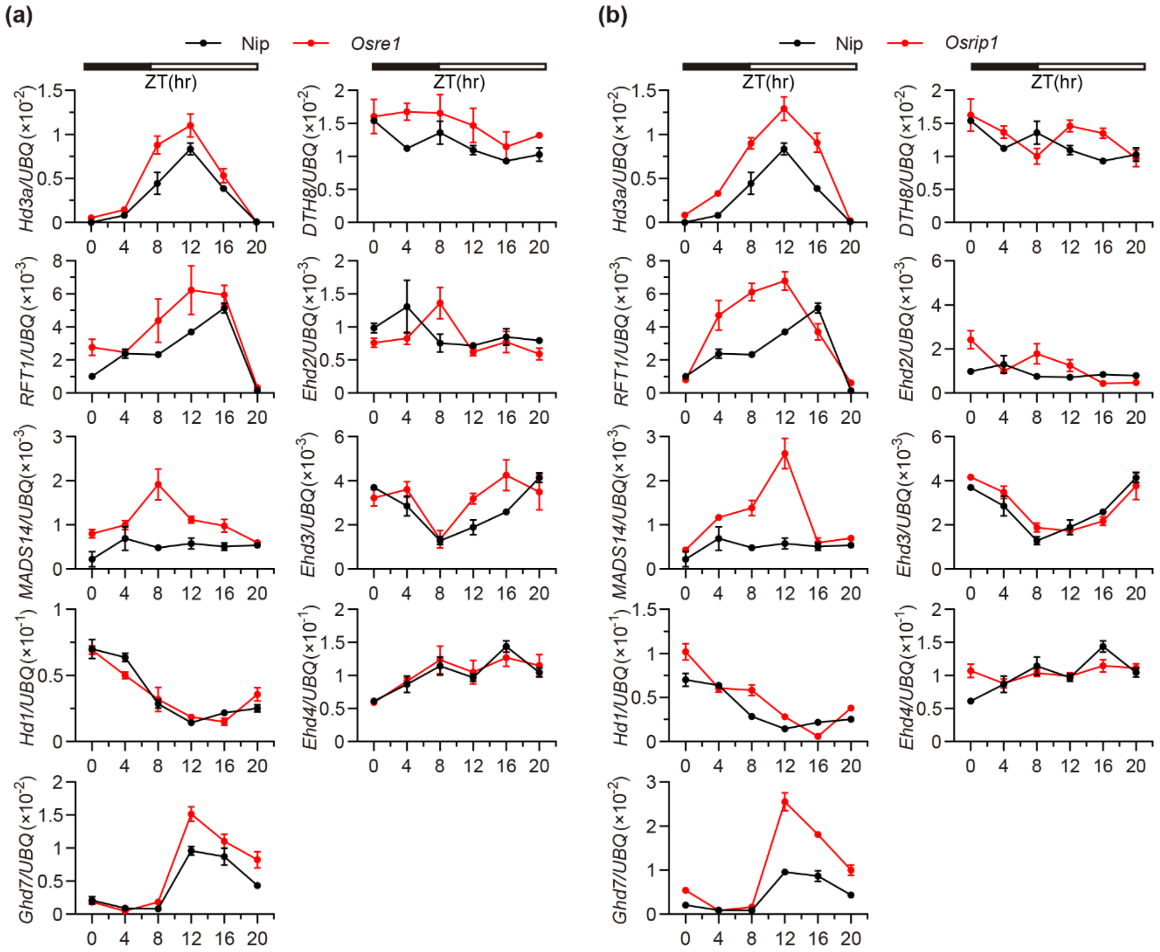
Supplemental Figure 4. OsRIP1 cannot bind to the promoter of *Ehd1*.in Y1H assay. The promoter of *Ehd1* was divided into two fragments (P1 to P2). An empty vector was used as the negative control. OsRE1 was used as the positive control. Yeast was grown on Synthetic Dropout (SD) medium containing X-gal and lacking Trp/Ura.



Supplemental Figure 5. Sequencing results of mutant sites in transgenic plants. Sequences were downloaded and aligned using Geneious (v4.8.5). Deleted nucleotides are depicted as dashes, inserted nucleotides are highlighted in yellow.

(a) Sequencing Results of Mutant Sites in *Osre1 ehd1* mutant.

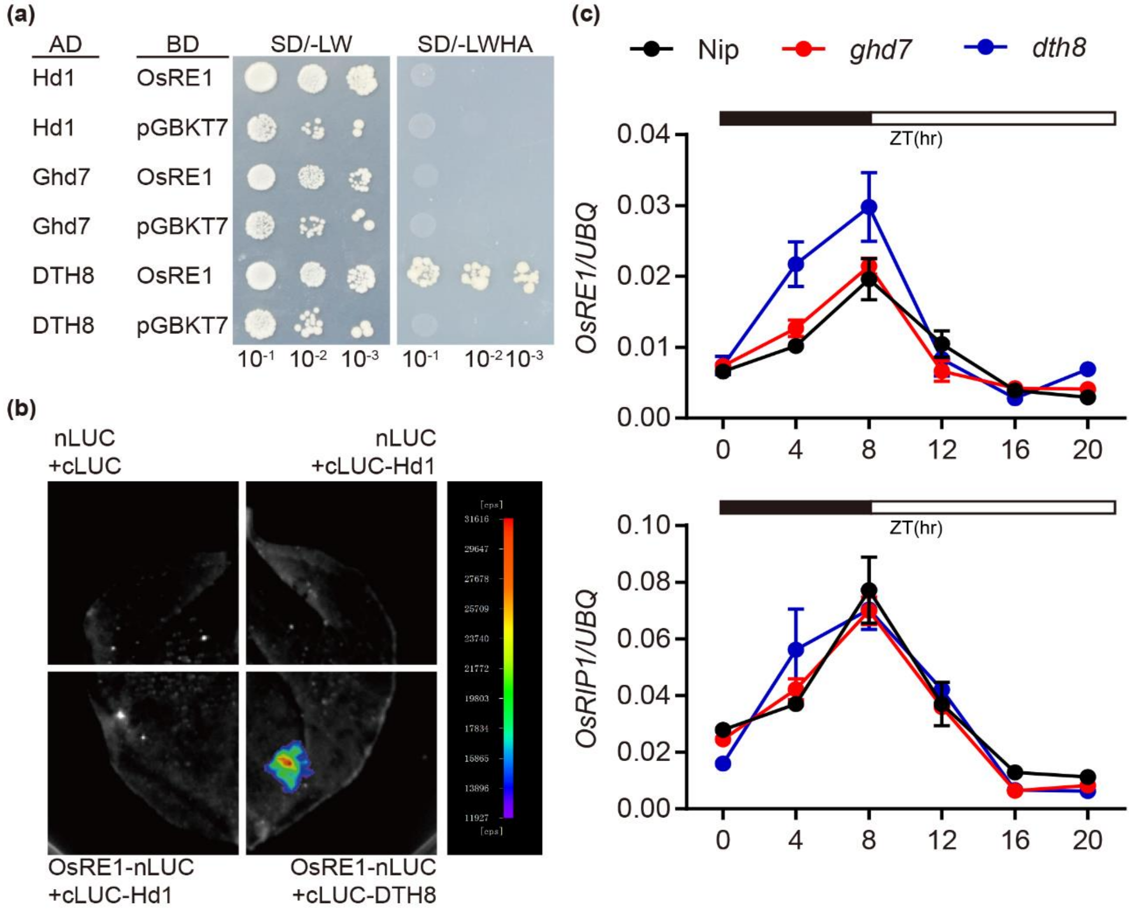
(b) Sequencing Results of Mutant Sites in *Osrip1 ehd1* mutant.



Supplemental Figure 6. Rhythmic expression patterns of genes related to heading date in Nipponbare, *Osre1* or *Osrip1* under LD conditions. Plants were grown in growth chambers under LD (14 h light /10 h darkness) conditions for 50 days. Black and white boxes denote dark and light periods, respectively. Rice *UBIQUITIN* gene was used as the internal control. Values are means ± SD; *n* = 3. ZT, Zeitgeber time.

(a) Expression patterns of genes related to heading date in Nip and *Osre1*.

(b) Expression patterns of genes related to heading date in Nip and *Osrip1*.



Supplemental Figure 7. Interaction relationships between OsRE1, OsRIP1 and several known *Ehd1* repressors.

(a) Y2H assay showed that OsRE1 interacts with DTH8, but not Hd1 or Ghd7. Yeast was grown at 30 ℃ for 3 days. The empty vector was used as a negative control.

(b) LCI assay showed that OsRE1 interacts with DTH8 in leaves of *N. benthamiana*. Hd1 was used as a negative control.

(c) Rhythmic expression patterns of *OsRE1* and *OsRIP1* in Nip, *ghd7* or *dth8* under LD conditions. Plants were grown in growth chambers under LD (14 h light /10 h darkness) conditions for 50 days. Black and white boxes denote dark and light periods, respectively. Rice *UBIQUITIN* gene was used as the internal control. Values are means ± SD; *n* = 3. ZT, Zeitgeber time.

Supplemental Table 1. Phenotypic data for Nipponbare, *Osre1* and *Osrip1* mutants.

Genotype	Condition	Days to flowering	Plant height, cm	Tiller No.	Panicle length, cm	Primary branches per panicle	Secondary branches per panicle	Grains per panicle	1000-grain weight, g
Nipponbare	NLD	122.8±0.7	107.21±0.64	17.45±2.42	24.16±0.26	12.43±0.24	29.29±0.44	163.2±3.95	26.68±0.65
Osre1	NLD	118.1±1.7**	106.89±0.55	16.12±2.48	23.41±0.38*	11.88±0.11*	26.47±0.62*	156.8±3.31	26.58±0.45
Osrip1	NLD	114.1±0.9**	106.42±0.88	16.19±2.82	23.95±0.45	12.36±0.18	28.23±0.73	160.2±5.35	26.37±0.48

Nipponbare, *Osre1* and *Osrip1* mutants were grown in the field under NLD conditions. Values are means ± SD; $n > 20$. *, $P < 0.05$, **, $P < 0.01$

Supplemental Table 2. Primers used in this study.

Primer (Forward)	Primer sequence
Construction	
PB42AD-OsRE1-F	TGCCTCTCCCGAATTCATGGCGGCGCAG
PB42AD-OsRE1-R	CGAGTCGGCCGAATTCGCTCTTGGTGAA
LacZ-P1-F	ATCTGTCGACCTCGAGTTAATTAAGATC
LacZ-P1-R	GAGCACATGCCTCGAGTATTATAACCTA
LacZ-P2-F	ATCTGTCGACCTCGAGTATTTATTTCCT
LacZ-P2-R	GAGCACATGCCTCGAGTAATGGTGGGTT
LacZ-P3-F	ATCTGTCGACCTCGAGAGTTTAAAACAC
LacZ-P3-R	GAGCACATGCCTCGAGTATTATAACCTA
LacZ-P4-F	ATCTGTCGACCTCGAGTATTTATTTCCT
LacZ-P4-R	GAGCACATGCCTCGAGTATTATAACCTA
OsRE1-Flag-F	ACGATGATAAGGGCGGTACCATGGCGGCGCAG
OsRE1-Flag-R	AGGCTACGTAGGATCCGCTCTTGGTGAA
OsRE1-CRISPR-F	AGATGATCCGTGGCACCTCCTTCCTTGAGGTTGTG TTTTAGAGCTATGC
OsRE1-CRISPR-R	GCATAGCTCTAAAACACAACCTCAAGGAAGGAGG TGCCACGGATCATCT
OsRIP1-Flag-F	ACGATGATAAGGGCGGTACCATGAAGGTGCAG
OsRIP1-Flag-R	AGGCTACGTAGGATCCTGCTTGTACTGG
OsRIP1-CRISPR-F	AGATGATCCGTGGCACCACGCCGCGTAACCGTC GTTTTAGAGCTATGC
OsRIP1-CRISPR-R	GCATAGCTCTAAAACGACGGTTACGCGGCGTGG TGCCACGGATCATCT
pAN580-OsRE1-F	CCCGGGGGATCCATGGCGGCGCAG
pAN580-OsRE1-R	CACCATGGATCCGCTCTTGGTGAA

Supplemental Table 2. Primers used in this study (Continued).

Primer (Forward)	Primer sequence
Construction	
pAN580-OsRIP1-F	CCCGGGGGATCCATGAAGGTGCAG
pAN580-OsRIP1-R	CACCATGGATCCTGCTTGTACTGG
pGAD-OsRE1-F	GAGGCCAGTGAATTCATGGCGGCGCAG
pGAD-OsRE1-R	ACCCGGGTGGAATTCGCTCTTGGTGAA
pGAD-OsRIP1-F	GAGGCCAGTGAATTCATGAAGGTGCAG
pGAD-OsRIP1-R	ACCCGGGTGGAATTCTGCTTGTACTGG
pGAD-OsRIP1-1-F	GAGGCCAGTGAATTCATGAAGGTGCAG
pGAD-OsRIP1-1-R	ACCCGGGTGGAATTCGAGGGAGAATCG
pGAD-OsRIP1-2-F	GAGGCCAGTGAATTCATGAAGGTGCAG
pGAD-OsRIP1-2-R	ACCCGGGTGGAATTCGAGGAGGAACCG
pGAD-OsRIP1-3-F	GAGGCCAGTGAATTCCAGAAGCCACCG
pGAD-OsRIP1-3-R	ACCCGGGTGGAATTCTGCTTGTACTGG
pGAD-OsRIP1-4-F	GAGGCCAGTGAATTCACCGGCGTGCGC
pGAD-OsRIP1-4-R	ACCCGGGTGGAATTCTGCTTGTACTGG
pGAD-OsRIP1-5-F	GAGGCCAGTGAATTCCAGAAGCCACCG
pGAD-OsRIP1-5-R	ACCCGGGTGGAATTCGAGGAGGAACCG
pGAD-Hd1-F	GAGGCCAGTGAATTCATGAATTATAAT
pGAD-Hd1-R	ACCCGGGTGGAATTCGAACCATGGAAC
pGAD-Ghd7-F	GAGGCCAGTGAATTCATGTCGATGGGA
pGAD-Ghd7-R	ACCCGGGTGGAATTCTCTGAACCATTG
pGAD-DTH8-F	GAGGCCAGTGAATTCATGAAGAGTAGG
pGAD-DTH8-R	ACCCGGGTGGAATTCATTAGTATTCCT
pGBK-OsRE1-F	ATGGAGGCCGAATTCATGGCGGCGCAG
pGBK-OsRE1-R	GATCCCCGGGAATTCGCTCTTGGTGAA

Supplemental Table 2. Primers used in this study (Continued).

Primer (Forward)	**Primer sequence**
Construction	
pGBK-OsRE1-1-F	ATGGAGGCCGAATTCATGGCGGCGCAG
pGBK-OsRE1-1-R	GATCCCCGGGAATTCGCCGGCGCTCCG
pGBK-OsRE1-2-F	ATGGAGGCCGAATTCGACAAGGAGCAG
pGBK-OsRE1-2-R	GATCCCCGGGAATTCGCTCTTGGTGAA
pGBK-OsRE1-3-F	ATGGAGGCCGAATTCATGGCGGCGCAG
pGBK-OsRE1-3-R	GATCCCCGGGAATTCCGTGTTCTTGAG
pGBK-OsRE1-4-F	ATGGAGGCCGAATTCACGGCGCACGCC
pGBK-OsRE1-4-R	GATCCCCGGGAATTCGCTCTTGGTGAA
pGBK-OsRE1-5-F	ATGGAGGCCGAATTCGACAAGGAGCAG
pGBK-OsRE1-5-R	GATCCCCGGGAATTCCGTGTTCTTGAG
YNE-OsRE1-F	AGTGGAGGATCCATGGCGGCGCAG
YNE-OsRE1-R	ACCTCCGGATCCGCTCTTGGTGAA
YCE-OsRIP1-F	CGTAGTCACGTGATGAAGGTGCAG
YCE-OsRIP1-R	GGACGTCACGTGTGCTTGTACTGG
His-OsRE1-F	GGCTGATATCGGATCCATGGCGGCGCAG
His-OsRE1-R	GACGGAGCTCGAATTCGCTCTTGGTGAA
MBP-OsRIP1-F	TTCAGAATTCGGATCCATGAAGGTGCAG
MBP-OsRIP1-R	CGACTCTAGAGGATCCTGCTTGTACTGG
OsRE1-GFP-F	CGAGCTGTACAGATCTATGGCGGCGCAG
OsRE1-GFP-R	GCCAAATGTTTGAACTGCAGGCTCTTGGTGAA
nLUC-OsRE1-F	GGTACCCGGGATCCATGGCGGCGCAG
nLUC-OsRE1-R	GAGATCTGGTCGACGCTCTTGGTGAA
cLUC-OsRIP1-F	CCCGGGGCGGTACCATGAAGGTGCAG
cLUC-OsRIP1-R	CTCTGCAGGTCGACTGCTTGTACTGG

Supplemental Table 2. Primers used in this study (Continued).

Primer (Forward)	Primer sequence
Construction	
pGreen-Ehd1-F	AGATCGAATTCCATGGTATTTATTTCCT
pGreen-Ehd1-R	TTGGCGTCTTCCATGGTATTATAACCTA
Transgenic plant DNA identification	
DT-OsRE1-Flag-F	CAGGAGAAGCAGCAGGTGAA
DT-OsRE1-Flag-R	TGAAATGGTGCTTCTTCCCG
DT-OsRIP1-Flag-F	GATTCTCCCTCCTCCAACCG
DT-OsRIP1-Flag-R	TCACCAGTACAAGCAGGGAG
DT-Osre1-F	CAGGAGAAGCAGCAGGTGAA
DT-Osre1-R	CCTGCCGCCACCGAATCG
DT-Osrip1-F	TCACGAGCAGATTGTTCCCT
DT-Osrip1-R	GATTCATCCAAGACACGCGT
ChIP-qPCR	
Ehd1-S1-F	AGTCAACATGCATGCCTTACTC
Ehd1-S1-R	TGTGTTAATTTGTAGAGGGACGT
Ehd1-S2-F	CCGTGTTTGATCCGATTCCA
Ehd1-S2-R	TGCGCGCTTTTGTAGTTTGA
Ehd1-S3-F	GTGAAATTGACCGGTGGCC
Ehd1-S3-R	TCTCATAGACGACACACCTCA
Ehd1-S4-F	GATCAACATTTCTCAGCAAAGGA
Ehd1-S4-R	CCAGGCAAAACAAACCAAACA
Ehd1-S5-F	TGCGGTTTCTAGGGTTAGCA
Ehd1-S5-R	CGAACACAACTTTTCCCGGT
RT-qPCR	
UBQ-F	GCTCCGTGGCGGTATCAT
UBQ-R	CGGCAGTTGACAGCCCTAG

Supplemental Table 2. Primers used in this study (Continued).

Primer (Forward)	Primer sequence
qEhd1-F	AACCCGGTCATCCTCCAT
qEhd1-R	TCATCTCTCACCTCATTTTCT
qOsRE1-F	AACAACACGCTCCGCCAGATAC
qOsRE1-R	TTGGTGAAATGGTGCTTCTTCCC
qOsRIP1-F	ATCTCCGAGTACCTCACCAAGAC
qOsRIP1-R	ACCCTGAAATAGCCCGTCTGAG
qHd1-F	CGTTTCGCCAAGAGATCAG
qHd1-R	AGATAGAGCTGCAGTGGAGAAC
qEhd2-F	CGACGACAATAGCTCGATCGC
qEhd2-R	GTGCATGGTCACGGAGCCTT
qEhd3-F	GACCACCTCGTCACCTACAAG
qEhd3-R	GAGTGTCCCTCCAGCTAATCC
qEhd4-F	CAGCCAGCGGAATCATCAC
qEhd4-R	CCAAATCCATCAGACCTACTCCT
qHd3a-F	GCTCACTATCATCATCCAGCATG
qHd3a-R	CCTTGCTCAGCTATTTAATTGCATAA
qMADS14-F	CAACCTCAAACAAGTTCCTC
qMADS14-R	TGCTGCTACATCCTCTATCC
qDTH8-F	CAGGAGTGCGTGTCGGAGTT
qDTH8-R	GGTCGTCGCCGTTGATGGT
qRFT1-F	TGACCTAGATTCAAAGTCTAATCCTT
qRFT1-R	TGCCGGCCATGTCAAATTAATAAC
qGhd7-F	GCTTGAACCCAAACACGG
qGhd7-R	CTCATCTCGGCATAGGCTT

Supplemental Table 3. Detailed information for rice BBX family members.

Gene name	Gene ID in phytozome	Gene name	Gene ID in phytozome
OsBBX1	*LOC_Os01g10580*	*OsBBX16*	*LOC_Os06g05890*
OsBBX4	*LOC_Os02g39360*	*OsBBX22*	*LOC_Os06g49880*
OsBBX6	*LOC_Os02g43170*	*OsBBX29*	*LOC_Os09g35880*
OsBBX7	*LOC_Os02g49230*	*OsBBX30*	*LOC_Os12g10660*
OsBBX11	*LOC_Os04g41560*	*OsRIP1*	*LOC_Os04g45690*
OsBBX14	*LOC_Os05g11510*		