A transposon insertion in the *OsUBC12* promoter enhances cold tolerance during germination in *japonica* rice (*Oryza sativa*)

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Fig. S1 Germination rates of 36 chromosome segment substitution lines (CSSLs) following 60 h at 15 °C (a) or 24 h at 30 °C (b). Values are mean ± standard error (SE) from three individual biological replicates (30 seeds per biological replicate).

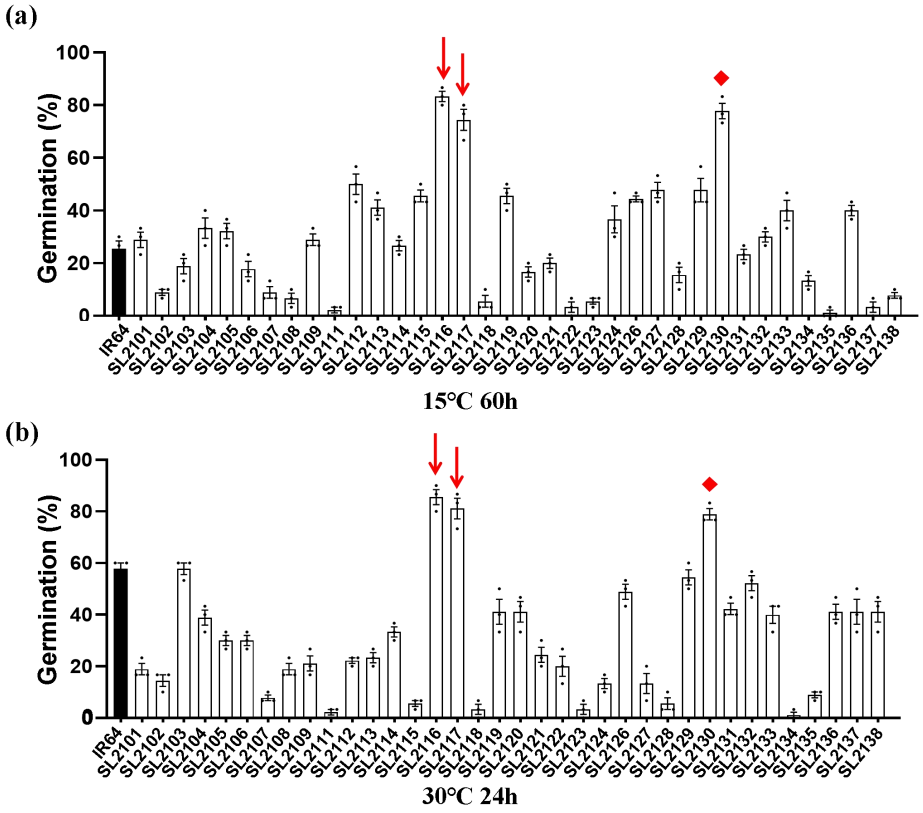
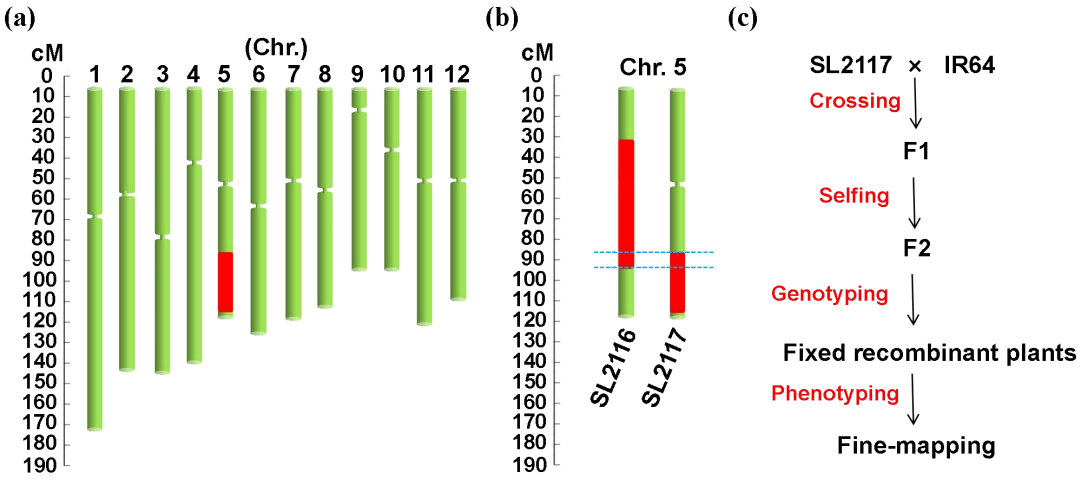


Fig. S2 Low-temperature germination phenotype of SL2117 segregated as a semi-dominant trait of higher resistance to low temperature during germination. **(a)** Graphical representation of the CSSL (SL2117) genotype. **(b)** Graphical genotypes of SL2116 and SL2117. Green region, genomic region from IR64; red region, genomic region from Koshihikari. **(c)** Schematic representation of the generation of an F_2 population from SL2117 × IR64. **(d)** Statistical analysis of the LTG phenotypes of F_2 plants using a χ^2 test.



(d)

Resistant	Semi-resistant	Sensitive	χ^2	P value
98	182	94	0.90	0.64

Fig. S3 Sequence comparison of the *OsUBC12* open reading frame between *japonica* Koshihikari and *indica* IR64. The red box indicates a nucleotide substitution.

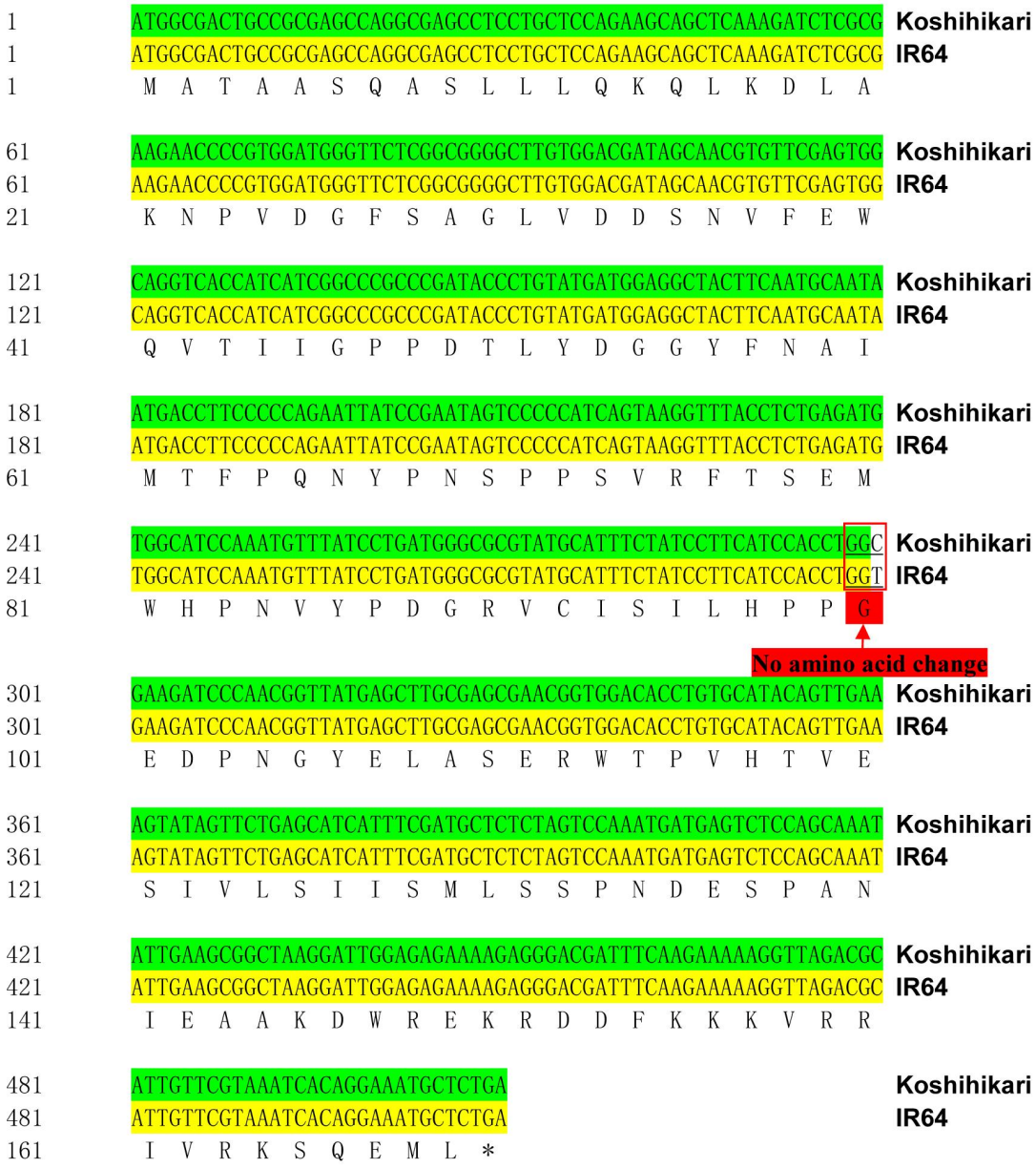


Fig. S4 Schematic representation of the recombinant vectors *p35S:REN–pOsUBC12^{IR64}:LUC* or *p35S:REN–pOsUBC12^{Koshihikari}:LUC* used in the dual-luciferase reporter assay.

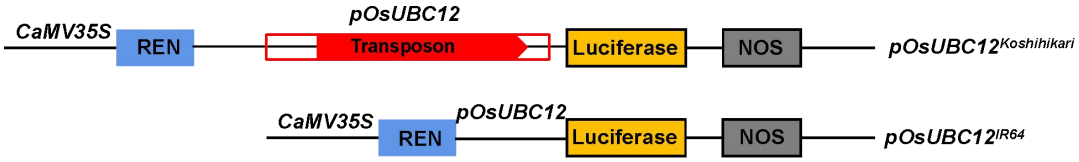


Fig. S5 Germination rates of SL2116, SL2117, *osubc12* mutants, *OsUBC12-OE*, *OsUBC12^{C92A}-OE*, and *OsSnRK1.1-OE* lines at 30 °C.

(a) Germination rates of SL2116 and SL2117 after 24 h at 30 °C. **(b)** Time-course germination of *osubc12* mutants at 30 °C. **(c)** Time-course germination of *OsUBC12-OE* and *OsUBC12^{C92A}-OE* lines at 30 °C. **(d)** Time-course germination of *OsSnRK1.1-OE* lines at 30 °C. Values are means ± SE from three individual biological replicates (30 seeds per biological replicate).

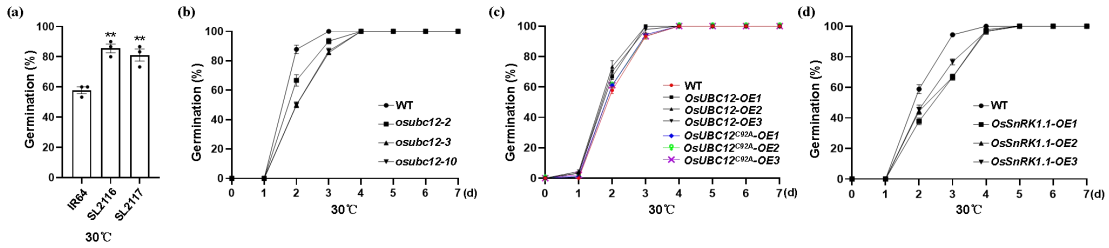


Fig. S6 Identification of *osubc12* mutants. The *OsUBC12* target sequences are indicated by red boxes.

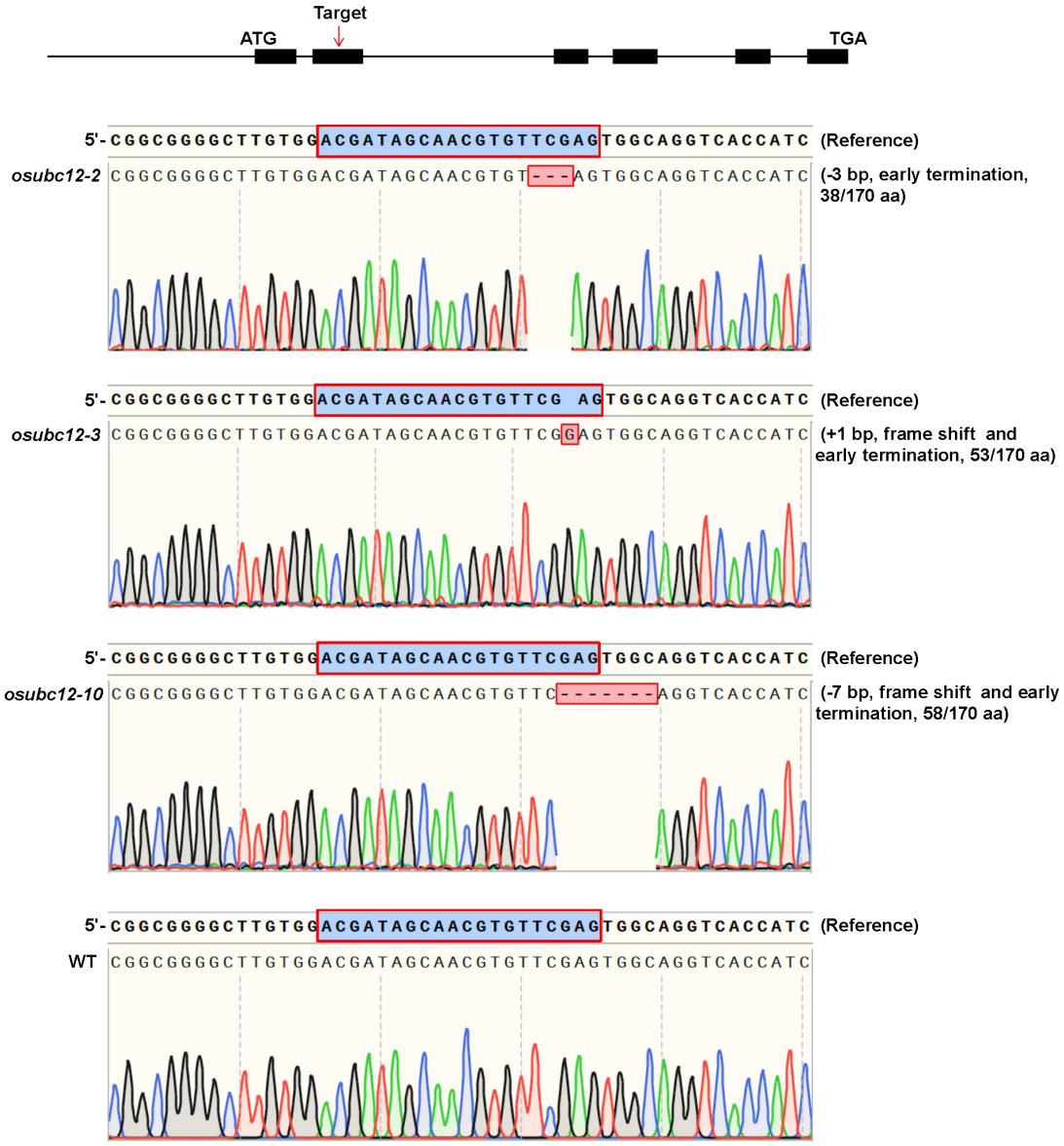


Fig. S7 Heat map showing the expression patterns of the genes differentially expressed between WT and the *osubc12* mutant as determined by RNA-seq analysis.

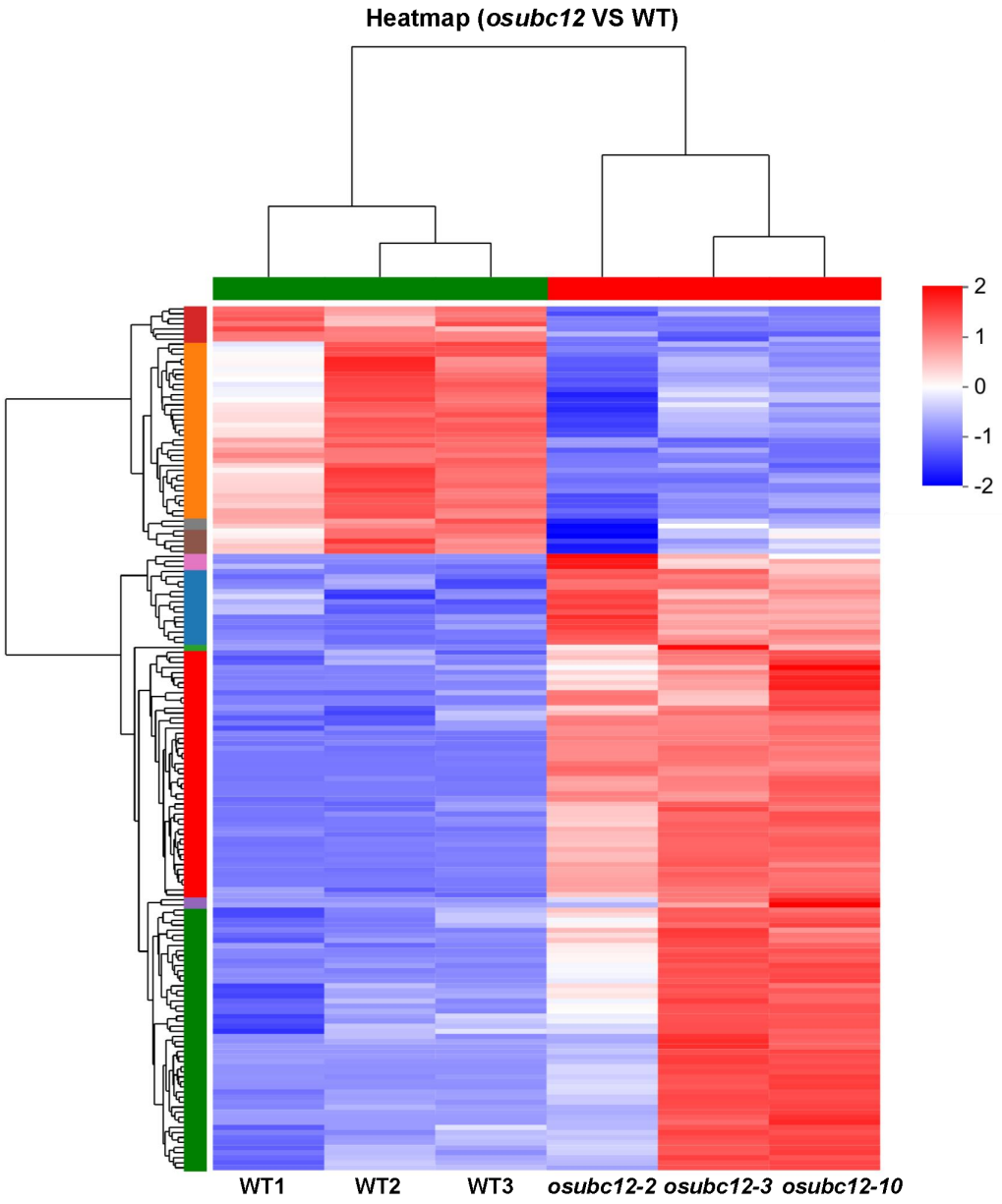


Fig. S8 Nucleotide and amino acid sequences of *OsUBC12*. The UBC domain is shaded in blue and the active-site cysteine residue in yellow.

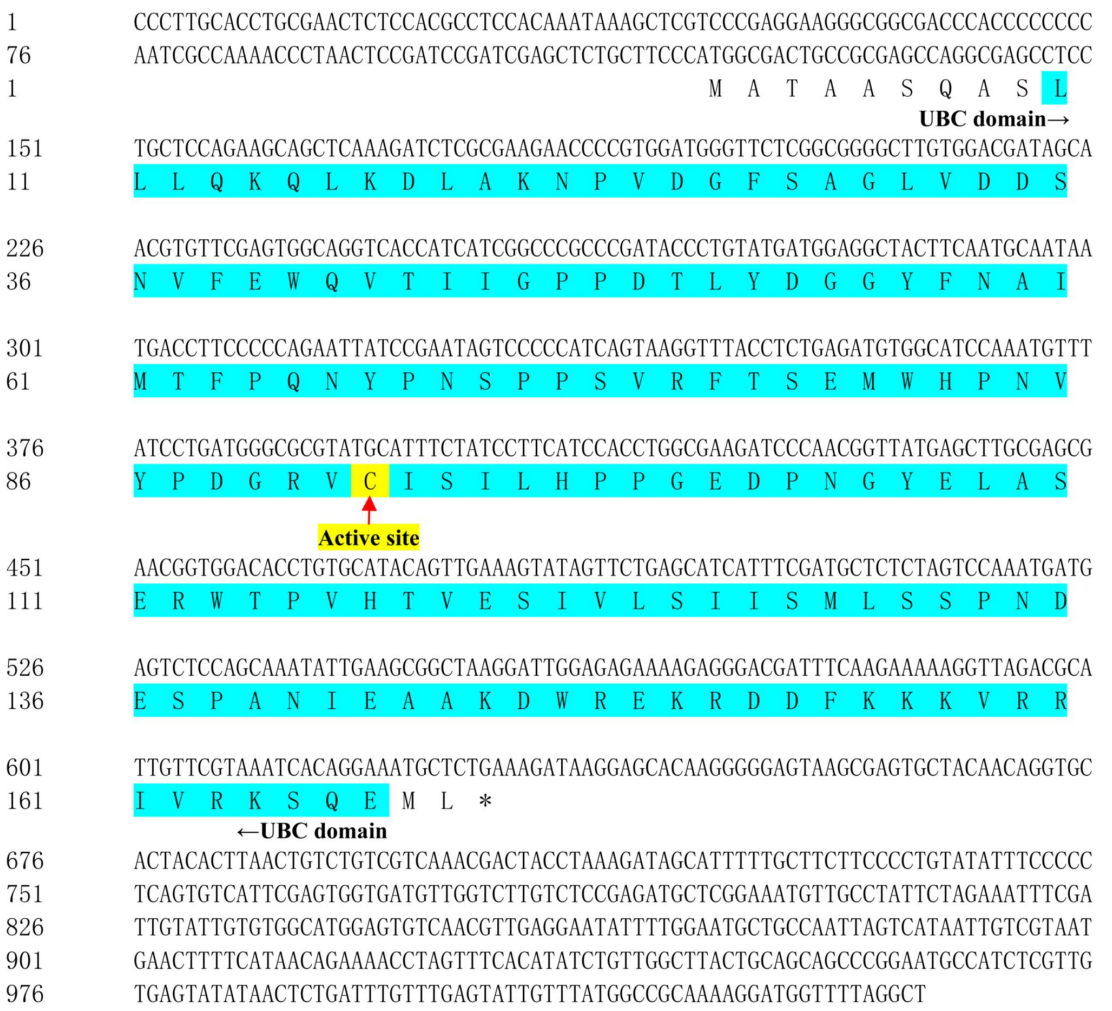


Fig. S9 Identification of *OsUBC12-OE* (a), *OsUBC12^{C92A}-OE* (b) and *OsSnRK1.1-OE* (c) lines by fluorescence screening. Scale bars, 0.5 cm.

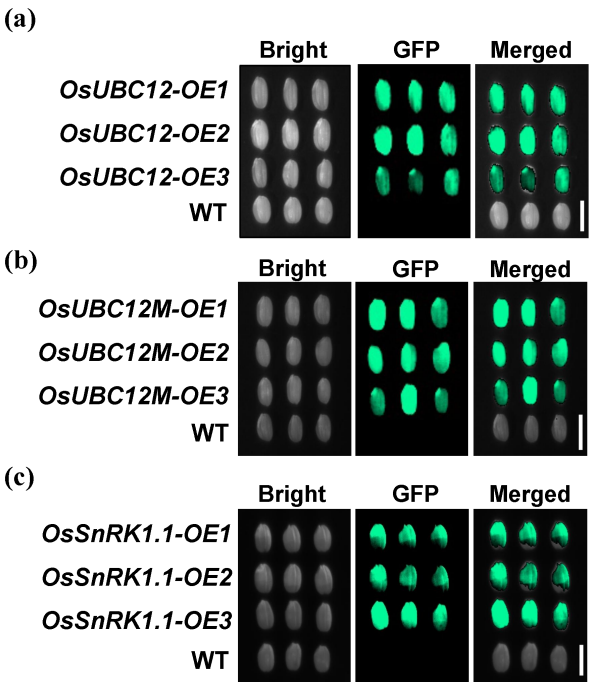


Fig. S10 Relative expression of *OsSnRK1.1* in seeds of *osubc12* mutants (a), *OsUBC12-OE*, *OsUBC12^{C92A}-OE* lines (b), or *OsSnRK1.1-OE* lines (c). The expression level of the control sample (WT) was set to 1. Values are means ± SE from three individual replicates (n = 3). The housekeeping gene *OsUBQ5* was used as an internal control to normalize the data.

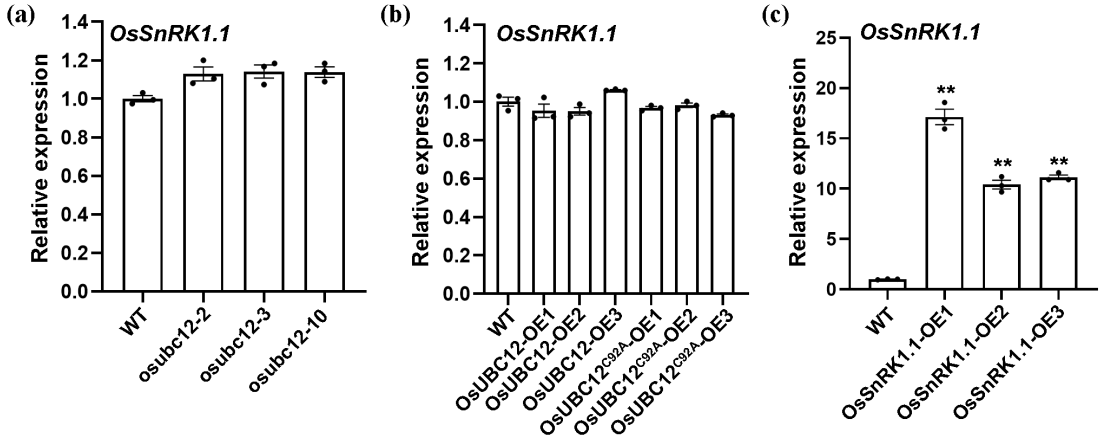
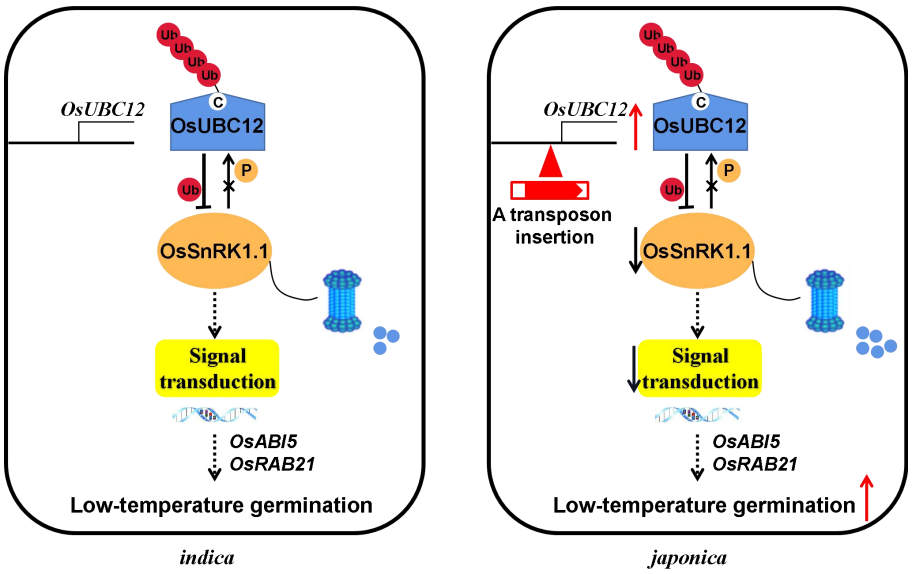


Fig. S11 Proposed working model of OsUBC12 in *indica* and *japonica* rice.

According to our model, OsSnRK1.1 functions as a downstream key regulator to enhance ABA signaling by upregulating the expression of ABA signaling-related genes such as *OsABI5* and *OsRAB21*, thus inhibiting LTG. OsUBC12, an E2 enzyme for Lys48-linked polyubiquitination, recruits and degrades OsSnRK1.1. Compared with *indica* rice, a transposon insertion in the *japonica OsUBC12* promoter activates the expression of *OsUBC12*. Increased OsUBC12 levels further promote the degradation of OsSnRK1.1, thereby weakening OsSnRK1.1-regulated ABA signaling and enhancing low-temperature germinability in *japonica* rice.



Supplemental Table S1 List of primers used in this study.

Specific primers for mapping

Primer name	Primers (5'-3')
5-20.34M	F: CGCGTGCTACCTTCCTAGTT
	R: TGCCATTAACCTAAGTTAGCATC
5-21.25M	F: GCTGACTTCACACTGCGATC
	R: GGCCACTTTCCAAACATCAG
5-21.65M	F: CATCTCGCCACCGTAGAAC
	R: CACAAGAGCACGGCTTGTTA
5-21.72M	F: CCTCTTCTCCAGGAGGGTACA
	R: GAACATTACATTCGCTCTCTCTCTC
5-21.95M	F: CAAACAGGCCCGCTCATT
	R: CACGTGCGACAACAACGTC
5-22.02M	F: TTATCATGTCATCATCAATGTGG
	R: TCATTCCGGTCCAAAGTCTC
5-22.21M	F: GCACCGAGAGAGACTTGGAC
	R: CGCTCTCCTCTCTCGTTTTC
5-22.59M	F: ATCCGGATTGGTCCAACAT
	R: GGATGACCCACATCATCATC
5-22.63MF	F: TCAGAGAGTAGCAGTGTAGC
	R: CTTTTTGTGTTTTCTTTCTAATAAA
5-23MF	F: TAGGCTTGCAGATTCCCTGT
	R: CCAATCTTGAAGAGGCTGCT
5-23.5M	F: TGAGATCGAAGGCGTAATCC
	R GCATCAGAGACCATGTGAGC
5-23.91M	F: GGATTTGTTCAAACTAACCAAAGAA
	R: GAAAATCCTACAAAACTCAGTTGTCA

Primers for quantitative real-time PCR

Primer name	Primers (5'-3')
OsUBC12-qPCR	F: CGGGGCTTGTGGACGATA
	R: CCAGGTGGATGAAGGATAGAAAT
OsABI5-qPCR	F: CATTTTCCTTGCCGCTAC
	R: AGTGTTACCTGTCGGTCGTTA
OsRAB21-qPCR	F: CTCGTCTGAGGATGATGGAATG
	R: CTGCTGCTCGCCCTTGTT
OsSnRK1.1-qPCR	F: AGATTGTTCATTCATCCCCATA
	R: CGTTACTCAAACCAAAGTCAGC
OsGAPDH -qPCR	F: CTTCATAGGAATGGAAGCTGCGGGTA
	R: CGACCACCTTGATCTTCATGCTGCTA
OsUBQ5 -qPCR	F: AGTGCGGCCTCACCTACGT
	R: CCGCCCCCAAAGAACAG

Primers for constructs in dual-luciferase reporter assay

Primer name	Primers (5'-3')
pOsUBC12^{IR64}-0800	F: GGGCCCCCCCTCGAGGTCGACTGAATGAAGGGGAAAAGTGTTCC R: CGCTCTAGAACTAGTGGATCCCCAGCTTTGATGAGTAATTTTGGG
pOsUBC12^{Koshihikari}-0800	F: GGGCCCCCCCTCGAGGTCGACTGAATGAAGGGGAAAAGTGTTCC R: CGCTCTAGAACTAGTGGATCCCCAGCTTTGATGAGTAATTTTGGG

Primers for constructs in rice genetic transformation

Primer name	Primers (5'-3')
The target sequence of *OsUBC12* in CRISPR/Cas9	ACGATAGCAACGTGTTCGAGR:
OsUBC12-U3 Cas9	F: ggcaCGATAGCAACGTGTTCGAG R: aaacCTCGAACACGTTGCTATCG
OsUBC12-sequence Cas9	F: TCGGTTCTTGATCTTGGCCC R: ACGGCAATATGGAATTCAGACA
OsUBC12-OE	F: cggggatcctctagagtcgacATGGATTACAAGGATGACGACGATAAGATGGCGACTGCCGCGAGC R: ctggcatgcctgcaggtcgacTCAGAGCATTTCCTGTGATTTACG
OsUBC12M-OE	F: cggggatcctctagagtcgacATGGATTACAAGGATGACGACGATAAGATGGCGACTGCCGCGAGC R: ctggcatgcctgcaggtcgacTCAGAGCATTTCCTGTGATTTACG
OsSnRK1.1-OE	F: cggggatcctctagagtcgac ATGGATGGAAATGCTAAAGGCG R: ctggcatgcctgcaggtcgacCTATAGAACCCTCAGCTTGGTAAGG

Primers for constructs in site-directed mutagenesis

Primer name	Primers (5'-3')
The target sequence of *OsUBC12* in site-directed mutagenesis	TTTATCCTGATGGGCGCGTATGCATTTCTATCCTTCATCCACC
OsUBC12M-mutagenesis	F: TTTATCCTGATGGGCGCGTAGCCATTTCTATCCTTCATCCACC R: GGTGGATGAAGGATAGAAATGGCTACGCGCCCATCAGGATAAA

Primers for constructs in ubiquitin-conjugating enzyme activity assays

Primer name	Primers (5'-3')
OsUBC12-His	F: ggcgatatcggatccgaattcTATGGCGACTGCCGCGAGC
	R: tgcggccgcaagcttgtcgacGAGCATTTCCTGTGATTTACGAAC
OsUBC12M-His	F: ggcgatatcggatccgaattcTATGGCGACTGCCGCGAGC
	R: tgcggccgcaagcttgtcgacGAGCATTTCCTGTGATTTACGAAC

Primers for constructs in yeast two-hybrid assays

Primer name	Primers (5'-3')
OsUBC12-BD	F: atggccatggaggccgaattcATGGCGACTGCCGCGAGC
	R: atgcggccgctgcaggtcgacTCAGAGCATTTCCTGTGATTTACG
OsRBX1-AD	F: gccatggaggccagtgaattc ATGGACAAGGGCGACGTCG
	R: cagctcgagctcgatggatcc CTAGTGACCATACTTCTGGAACTCCC
OsSnRK1.1-AD	F: gccatggaggccagtgaattcATGGATGGAAATGCTAAAGGCG
	R: cagctcgagctcgatggatccCTATAGAACCCTCAGCTTGGTAAGG
OsUBC12-AD	F: gccatggaggccagtgaattcATGGCGACTGCCGCGAGC
	R: cagctcgagctcgatggatccTCAGAGCATTTCCTGTGATTTACG
OsWRKY42 -AD	F: gccatggaggccagtgaattcATGTCGAGCAGAAGGTCGTCG
	R: cagctcgagctcgatggatccTCAGGAGCGGAGGATGCTG

Primers for constructs in pull-down assays and *in vitro* kinase assay

Primer name	Primers (5'-3')
OsSnRK1.1-His	F: ggcgatatcggatccgaattcT ATGGATGGAAATGCTAAAGGCG
	R: tgcggccgcaagcttgtcgacTAGAACCCTCAGCTTGGTAAGG
OsUBC12-GST	F: ccgcgtggatccccggaattcATGGCGACTGCCGCGAGC
	R: gtcacgatgcggccgctcgagTCAGAGCATTTCCTGTGATTTACG

Primers for constructs in LCI assays

Primer name	Primers (5'-3')
OsUBC12-nLUC	F: gagaacacgggggacgagctcATGGCGACTGCCGCGAGC
	R: cgcgtacgagatctggtcgacGAGCATTTCCTGTGATTTACGAAC
OsSnRK1.1-cLUC	F: ccggggcggtacccgggatccAATGGATGGAAATGCTAAAGGCG
	R: acgaaagctctgcaggtcgacCTATAGAACCCTCAGCTTGGTAAGG

Primers for transposon insertion detection

Primer name	Primers (5'-3')
Insertion detection	F: CGTCCAGTGCGTAAGATG
	R: CGAATAGGCGTAAACCAA

Supplemental Table S2 Expressed sequence tags (ESTs) from partial candidate genes encoding proteins isolated in the yeast two-hybrid (Y2H) screen of OsUBC12.

Accession number	Encoding protein	Clones
LOC_Os01g01700	Similar to RING-box protein 1A (regulator of cullins 1a) (OsRBX1)	1
LOC_Os03g17980	Serine/threonine protein kinase (OsSnRK1.1)	3
LOC_Os05g38550	Ubiquitin-conjugating enzyme 12 (OsUBC12)	1
LOC_Os02g26430	WRKY transcription factor 42 (OsWRKY42)	2
LOC_Os11g25800	Uncharacterized protein	1
LOC_Os02g48080	Extracellular kinase-1	1