Supplemental information

Rice OsUBR7 modulates plant height by regulating histone H2B mono-ubiquitination and cell proliferation

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This file includes Supplemental Figures 1–17
Supplemental Figure 1. Genotyping the CRISPR/Cas9-edited T₀ rice plants and segregation of F₂ progeny from the cross between WT and osubr7 mutant.

(A) Genotypes and phenotypes of T₀ plants. The nucleotides highlighted in red show the targeted mutations. The underlined DNA sequences indicate protospacer adjacent motifs for single-guide RNAs.

(B) Segregation analysis of an F₂ family derived from crossing T₁-1 and a japonica line Nipponbare (NIP), showing that wild-type (≥ 75 cm) and osubr7 (≤ 67 cm) plant height phenotypes fit the 3:1 ratio based on a χ² test.

| T₀ plant | Targeted mutant alleles | Mutation type | Phenotype |
|----------|--------------------------|---------------|-----------|
| T1-1     | a1 GAGCCGACGTCACTCCGAGGAGTAC | homozygous    | semi-dwarf |
|          | a2 GAGCCGACGTCACTCCGAGGAGTAC |               |           |
|          | WTGAGCCGACGTCACTCCGAGGAGTAC |               |           |
| T1-2     | a1 GAGCCGACGTCACTCCGAGGAGTAC | homozygous    | semi-dwarf |
|          | a2 GAGCCGACGTCACTCCGAGGAGTAC |               |           |
|          | WTGAGCCGACGTCACTCCGAGGAGTAC |               |           |
| T1-3     | a1 GAGCCGACGTCACTCCGAGGAGTAC | heterozygous  | wild-type |
|          | a2 GAGCCGACGTCACTCCGAGGAGTAC |               |           |
|          | WTGAGCCGACGTCACTCCGAGGAGTAC |               |           |
| T1-4     | a1 GAGCCGACGTCACTCCGAGGAGTAC | biallelic     | semi-dwarf |
|          | a2 GAGCCGACGTCACTCCGAGGAGTAC |               |           |
|          | WTGAGCCGACGTCACTCCGAGGAGTAC |               |           |
| T1-5     | a1 GAGCCGACGTCACTCCGAGGAGTAC | heterozygous  | wild-type |
|          | a2 GAGCCGACGTCACTCCGAGGAGTAC |               |           |
|          | WTGAGCCGACGTCACTCCGAGGAGTAC |               |           |
| T1-6     | a1 GAGCCGACGTCACTCCGAGGAGTAC | biallelic     | semi-dwarf |
|          | a2 GAGCCGACGTCACTCCGAGGAGTAC |               |           |
|          | WTGAGCCGACGTCACTCCGAGGAGTAC |               |           |

| Parents | No. F₂ plant | Wild type | osubr7 type | χ² (3:1) |
|---------|--------------|-----------|-------------|---------|
| T1-1 ♂/NIP ♀ | 268 | 203 | 65 | 0.04 (P > 0.05) |
Supplemental Figure 2. Variations of agronomic traits between WT and osubi7 (T4) plants.

(A–D) The WT (Nipponbare) and osubi7 phenotypes of panicle (A), grain (B), flag leaf (C), and 28-day-old seedlings (D). Scale bars in (A), (C), and (D), 5 cm; Scale bar in (B), 1 cm.

(E–M) Comparison of panicle length (E), panicle (effective tiller) number (F), seed setting rate (G), grain number (H), grain length (I) and width (J), 1,000-grain weight (K), flag leaf length (L) and width (M), and seedling height (28-day-old, N) between WT and osubi7. Data are means ± SD (n = 30).

Asterisks represent significant difference determined by Student’s t-test at *P < 0.05, **P < 0.01, and ***P < 0.001. NS, not significant.
Supplemental Figure 3. Validation of OsUBR7 function in plant height regulation.

(A) Phenotype of two independent transgenic (T2) complementation lines (OsUBR7#5 and SD12#7) containing the OsUBR7 native genomic sequence. Scale bar, 20 cm.

(B and C) Plant height (B) and relative expression levels of the OsUBR7 transgene (C) in the transgenic complementation lines. Values are means ± SD (n = 30), and expression values are means ± SD (n = 3 biological repeats).

(D) The vector construct for OsUBR7-FLAG overexpression driven by the maize ubiquitin promoter (PUB) was transferred into the osubr7 background. T_NOS, the NOS terminator.

(E) Phenotypes of two independent transgenic (T2) OsUBR7-overexpression lines. Scale bar, 20 cm.

(F–H) Plant height (F), relative expression levels of the OsUBR7 transgene (G), and western blot detection of OsUBR7-FLAG protein (H) in the overexpression lines. UD, undetectable. The same blot was probed with anti-HSP82 antibody for a loading control (H). Plant heights are means ± SD (n = 30), and expression values are means ± SD (n = 3 biological repeats). Asterisks represent significant difference determined by Student’s t-test at ***P < 0.001.
Supplemental Figure 4. The UBR box protein family and phylogenetic analysis of UBR7 proteins.

(A) Diagrams of UBR box protein family members. UBR, UBR box; N, N domain; RING, RING finger; AI, autoinhibitory domain; CRD, cystein-rich domain; UBA, Ub association domain; PABC, poly(A)-binding protein C-terminal domain; HECT, homologous to the E6AP carboxyl terminus; PHD, plant homeodomain finger.

(B) Amino acid sequences of UBR7 proteins of the species were aligned using the Clustal W algorithm (MEGA X software). The tree was constructed by the neighbor-joining method after bootstrap analysis for 10,000 replicates. The conserved domains in UBR7 proteins were analyzed by NCBI CDD and visualized by TBtools software. The length of proteins can be inferred by the scale at the bottom.
Supplemental Figure 5. Alignment of the amino acid sequences of the UBR7 boxes in the proteins from twelve species.

Red line indicates the conserved UBR box and blue line indicates the conserved PHD. Asterisks represent conserved Cys and His residues in the UBR7 zinc finger.
Supplemental Figure 6. Comparison of the zinc finger structures between UBR7 and RING.

Cross brace topology depicting the organization in canonical RING zinc finger or UBR7 zinc finger.
Supplemental Figure 7. Phylogenetic analysis of rice E2 conjugases.

Amino acid sequences of 39 rice E2 conjugases and human UbcH5b (as a reference) were aligned using the Clustal W algorithm (MEGA X software). The tree was constructed by the neighbor-joining method after bootstrap analysis for 10,000 replicates. The result suggests that UBC18 is mostly similar to UbcH5b. Scale bar, the evolutionary distance.
Supplemental Figure 8. Amino acid sequence similarity between rice OsUBC18 and human UbcH5b and subcellular localization of OsUBC18.

(A) Amino acid sequence alignment of OsUBC18 and UbcH5b. The conserved cysteine, which is the active site for E2 enzyme activity, is highlighted in green. Asterisks represent the identical amino acids between OsUBC18 and UbcH5b.

(B) Co-localization of mCherry-OsUBC18 and GFP-OsUBR7 fusion proteins in rice sheath protoplasts. BF, bright field image. Scale bars, 10 μm.
### Supplemental Figure 9. Analysis of possible monoubiquitination sites by OsUBR7 in the rice H2B variants.

Comparison of the amino acid sequences of human H2B and rice H2B variants. The target lysine site K120 (K121 including the first Methionine in human H2B) for monoubiquitination in H2B by UBR7 in humans is highlighted in blue, and the candidate target lysines for monoubiquitination in the rice H2B variants by OsUBR7 are highlighted in green.

| H2B, 1 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 56 |
| H2B, 2 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 54 |
| H2B, 3 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |
| H2B, 4 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |
| H2B, 5 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |
| H2B, 6 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |
| H2B, 7 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |
| H2B, 8 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |
| H2B, 9 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |
| H2B, 1 Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |
| H2B, 1Os  | MAPKAECIKPAAEKPAACGHEE---KSAEKAPAKKKPAEKRLPAS-KASSKE--GGAGDKKGKRK       | 57 |

Comparison of the amino acid sequences of human H2B and rice H2B variants. The target lysine site K120 (K121 including the first Methionine in human H2B) for monoubiquitination in H2B by UBR7 in humans is highlighted in blue, and the candidate target lysines for monoubiquitination in the rice H2B variants by OsUBR7 are highlighted in green.
Supplemental Figure 10. Genome-wide distribution of H2Bub1 variation regions with up- and down-regulated peaks in osubr7 compared to WT (Nipponbare).

(A) For analysis of the ChIP-seq data (using an anti-H2Bub1 antibody, n = 2 biological repeats), according to the size of the chromosomes, windows of dynamic lengths were set, the number of differential H2Bub1 peaks in each window was counted, and the distribution of the peaks is plotted on the chromosomes.

(B) Venn diagrams of genes down-regulated in expression levels and genes down-regulated in H2Bub1 levels in osubr7 seedlings compared to WT.
Supplemental Figure 11. Diagrammatic presentation of ChIP-seq maps at some representative gene loci.

(A and B) The H2Bub1 levels at these loci in osubr7 and WT (Nipponbare) seedlings were detected by ChIP-seq using an anti-H2Bub1 antibody (n = 2 biological repeats). A subset of
cell cycle-related and pleiotropic genes with downregulated H2Bub1 levels (A) and the top ten genes with largely downregulated H2Bub1 levels (B) are shown.

Supplemental Figure 12. Gene ontology (GO) analysis of the H2Bub1-downregulated genes in osubr7 compared to WT (Nipponbare) seedlings.

All GO enrichment results were sorted according to p.adjust from smallest to largest, and the bubble charts were drawn with the top 20 GO enrichment functions.
Supplemental Figure 13. OsUBR7 affects the expression of numerous genes for cell cycle processes.

Relative expression of 19 cell cycle-related genes in WT (Nipponbare) and osubr7 seedlings (14-day-old). UFC1 was used as the control and the values of expression levels in WT were set to 1. Values are means ± SD (n = 3 biological repeats). Asterisks represent significant difference determined by Student’s t-test at *P < 0.05, **P < 0.01, and ***P < 0.001. NS, not significant.
Supplemental Figure 14. Haplotype variations of the OsUBR7 coding region in cultivated rice and several representative wild rice species.

The polymorphic sites causing amino acid alterations (AA alt.) are given.
| Haplotype | Polymorphic pattern of the promoter region at 45 sites | No. accession |
|-----------|-----------------------------------------------------|---------------|
|           | Site 1                                              | Site 20       | Site 45       |
| ph1       | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 48            | 1185          | 0             | 0             | 0             | 0             | 0             | 0             |
| ph2       | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 1029          | 17            | 0             | 0             | 0             | 0             | 0             | 0             |
| ph3       | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 1239          | 16            | 0             | 0             | 0             | 0             | 0             | 0             |
| ph4       | ACGGGGGCGGATAGGGGTAGGGGTAGGGGTAGGGGTAGGGGTAGGGGTAG | 292           | 16            | 0             | 0             | 0             | 0             | 0             | 0             |
| ph5       | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 19            | 220           | 0             | 0             | 0             | 0             | 0             | 0             |
| ph6       | ACGGGGGCGGATAGGGGTAGGGGTAGGGGTAGGGGTAGGGGTAGGGGTAG | 24            | 4             | 6             | 1             | 0             | 0             | 0             | 0             |
| ph7       | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 4             | 24            | 0             | 0             | 0             | 0             | 0             | 0             |
| ph8       | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 67            | 3             | 0             | 0             | 0             | 0             | 0             | 0             |
| ph9       | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 37            | 0             | 0             | 0             | 0             | 0             | 0             | 0             |
| ph10      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 90            | 0             | 0             | 0             | 0             | 0             | 0             | 0             |
| ph11      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 27            | 0             | 0             | 0             | 0             | 0             | 0             | 0             |
| ph12      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 12            | 1             | 0             | 0             | 0             | 0             | 0             | 0             |
| ph13      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 13            | 0             | 0             | 0             | 0             | 0             | 0             | 0             |
| ph14      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 2             | 0             | 0             | 0             | 0             | 0             | 0             |
| ph15      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph16      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 1             | 0             | 0             | 0             | 0             | 0             | 0             |
| ph17      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph18      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph19      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 5             | 0             | 0             | 0             | 0             | 0             |
| ph20      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 1             | 0             | 0             | 0             | 0             | 0             |
| ph21      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 1             | 0             | 0             | 0             | 0             | 0             |
| ph22      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 1             | 0             | 0             | 0             | 0             | 0             |
| ph23      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph24      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph25      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph26      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 3             | 0             | 0             | 0             | 0             | 0             |
| ph27      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 3             | 0             | 0             | 0             | 0             | 0             |
| ph28      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 3             | 0             | 0             | 0             | 0             | 0             |
| ph29      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 3             | 0             | 0             | 0             | 0             | 0             |
| ph30      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 1             | 0             | 0             | 0             | 0             | 0             |
| ph31      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 1             | 0             | 0             | 0             | 0             | 0             |
| ph32      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph33      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph34      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph35      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph36      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph37      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph38      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph39      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph40      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |
| ph41      | GGGCGGCTTGTAGGGACGGTTAGGGGTAGGGGTAGGGGTAGGGGTAG | 0             | 0             | 2             | 0             | 0             | 0             | 0             | 0             |

**Sum of accession No.**

2641 1485 33 21 19 11 15

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+ 1 ATG
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Supplemental Figure 15. Haplotype variations of the OsUBR7 promoter region in cultivated rice and several representative wild rice accessions.

(A) The haplotypes are based on nucleotide polymorphic patterns at the promoter sites (45 sites) as indicated in (B). "-" indicates a base deletion.

(B) The promoter/5'UTR region sequence of a japonica rice (Nipponbare). The polymorphic sites (1–45) among the cultivated and wild rice accessions are highlighted by red.
Supplemental Figure 16. Association analysis between *OsUBR7* haplotypes and plant height.

(A) The association between plant height and five dominant CDS haplotypes in *OsUBR7* of 503 rice accessions.

(B) The association between plant height and five dominant promoter haplotypes in *OsUBR7* of 445 rice accessions.

Different capital letters indicate significant difference (*P* < 0.01) with the others.
Supplemental Figure 17. Agronomic trait variations of the *osubr7* mutants in the T65 background.

(A) Phenotypes of the *osubr7*-T65 (T₃) lines and *japonica* WT line T65. Scale bar, 20 cm.

(B) Comparison of plant height between the *osubr7*-T65 lines and T65. Data are means ± SD (*n* = 30).

(C) Panicle phenotype of the *osubr7*-T65 lines and T65. Scale bar, 5 cm.

(D and E) Comparison of panicle (effective tiller) number per plant (D) and grain number per panicle (E) in main panicles between the *osubr7*-T65 lines and T65. Data are means ± SD (*n* = 30).

(F) Grain phenotypes of the *osubr7*-T65 lines and T65. Scale bar, 1 cm.

(G) Comparison of 1,000-grain weight between the *osubr7*-T65 lines and T65. Data are means ± SD (*n* = 3 biological repeats).

(H) Comparison of yield per plant between the *osubr7*-T65 lines and T65. Data are means ± SD.
\( n = 30 \).
Asterisks represent significant difference determined by Student’s \( t \)-test at \( **P < 0.01 \), and \( ***P < 0.001 \). NS, not significant.