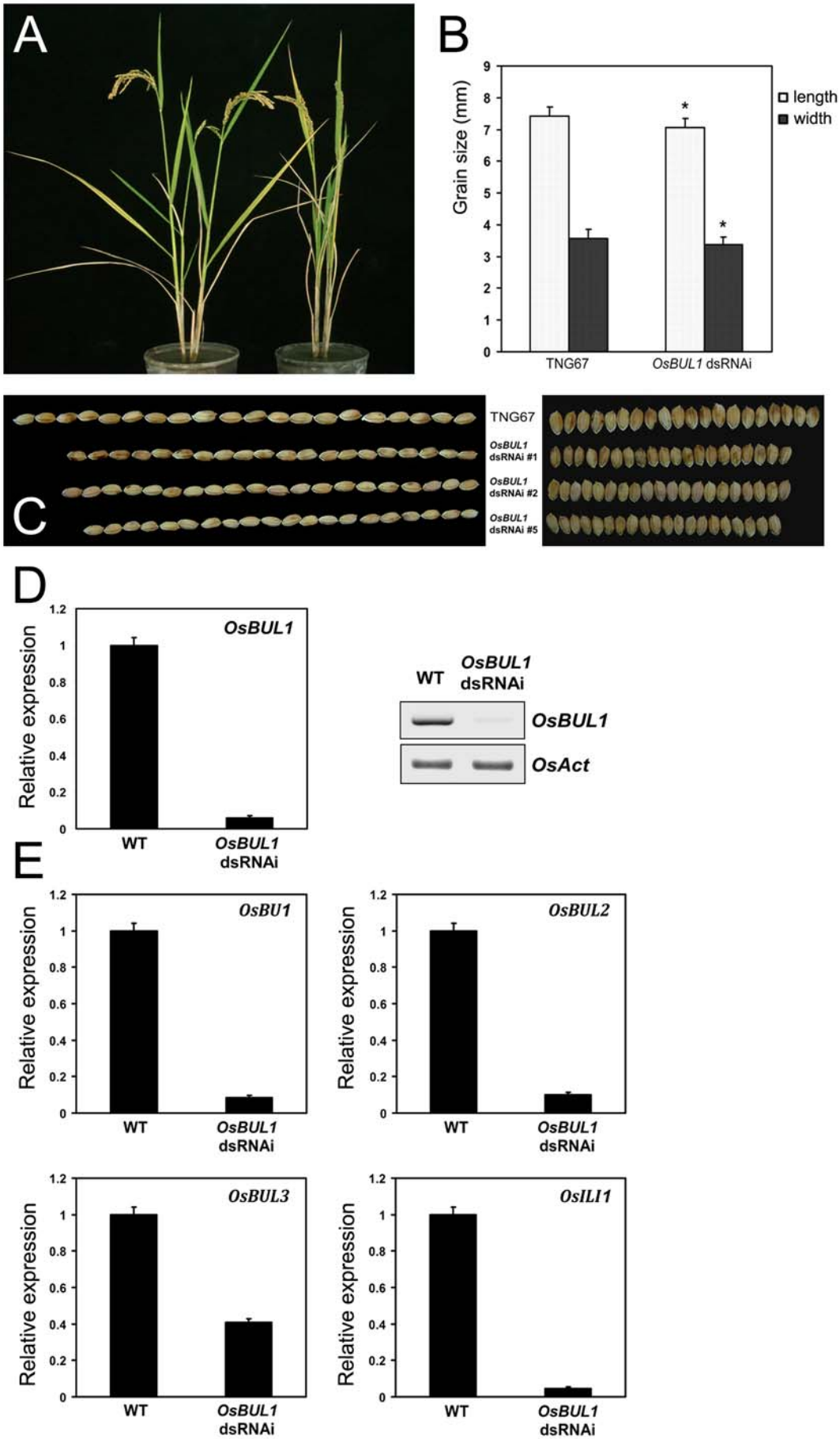
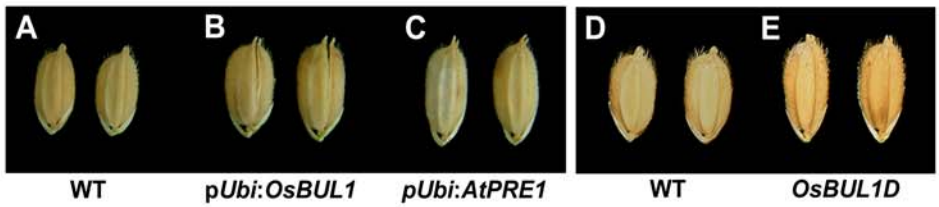
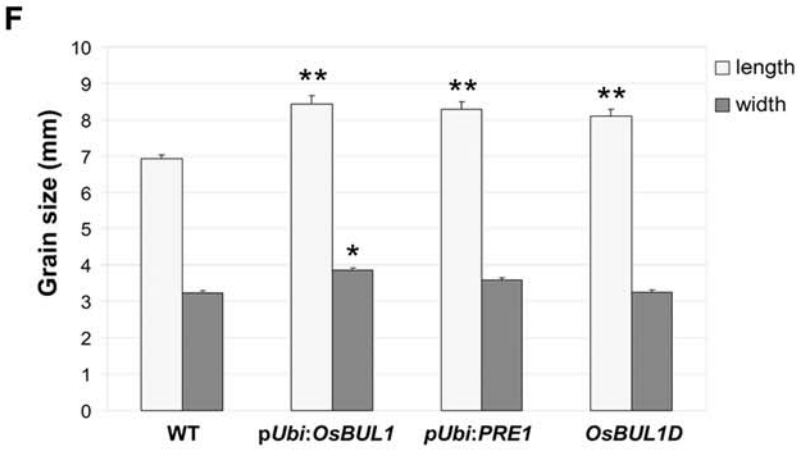
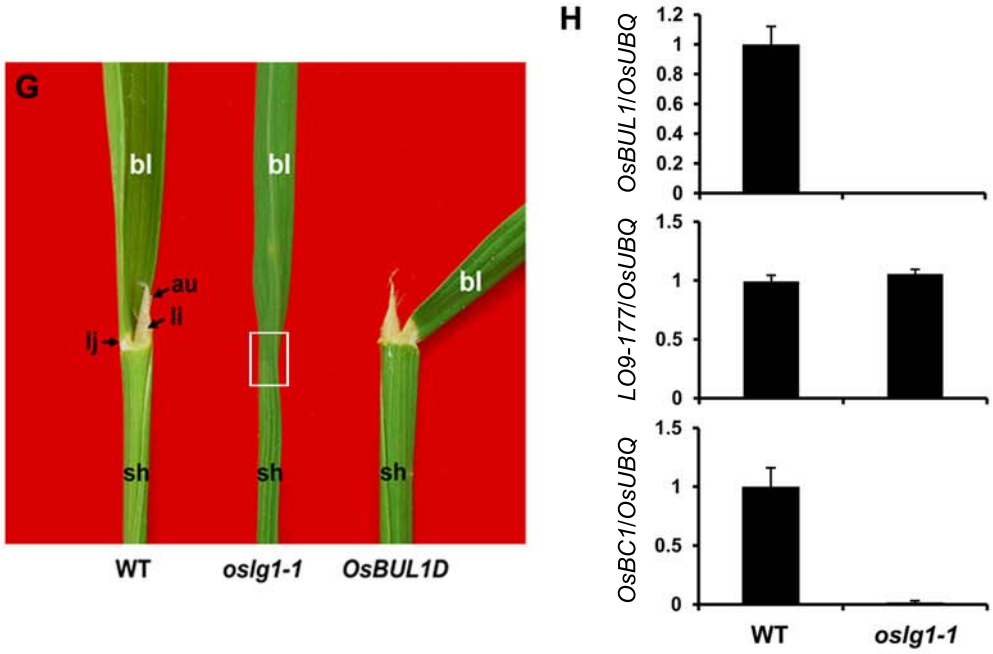


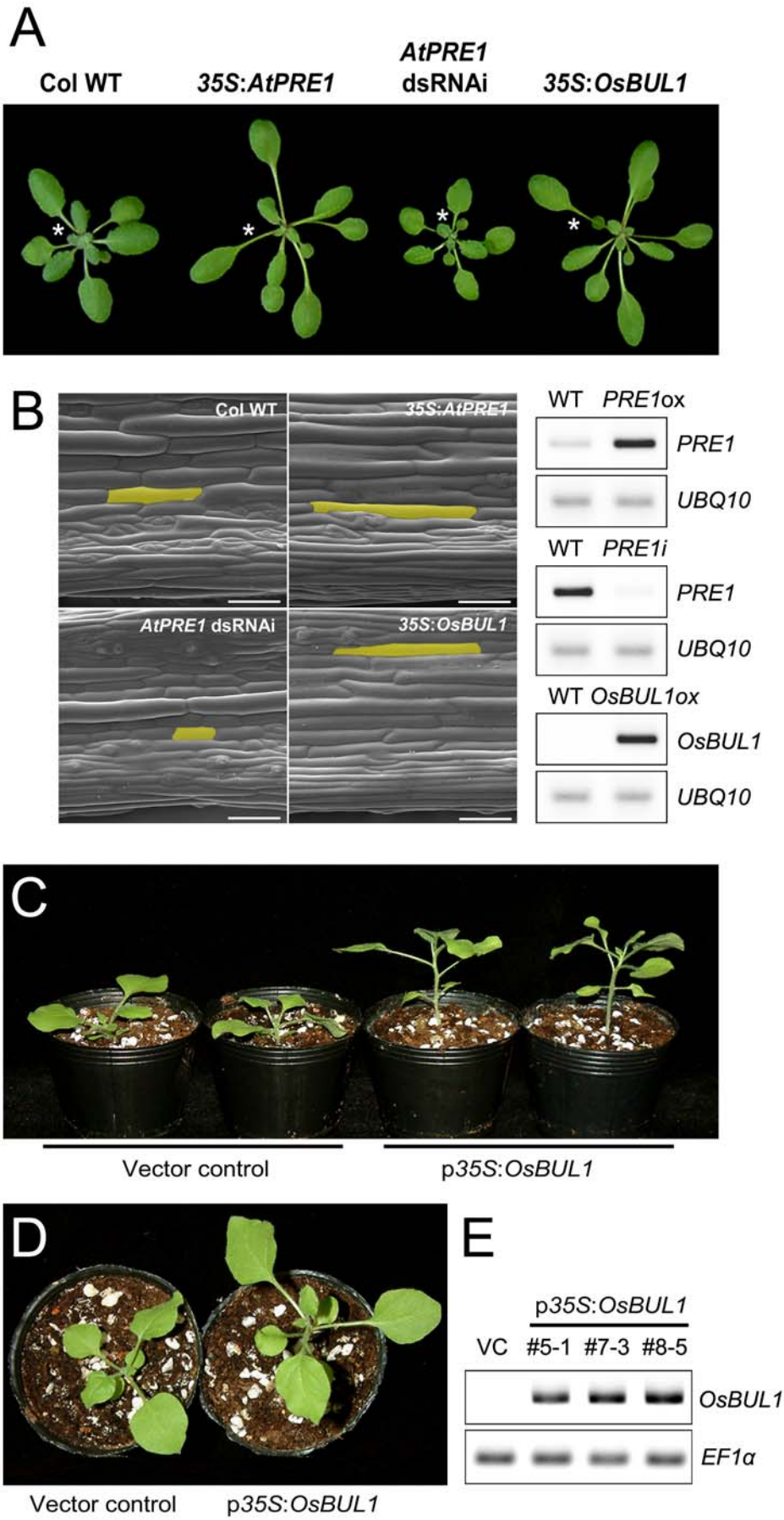
Supplemental Figure S1. *OsBUL1* dsRNAi lines phenocopy *OsBUL1* null mutation. (A to C) *OsBUL1* dsRNAi plants (right in A) have erect leaves and produced small grains (*, P < 0.001, Student's t test). (D, E) Endogenous *OsBUL1* transcripts were amplified with primers, 5'GTAGAAGAGGCTAGCTGTTGAC3' and 5'TCAGGAGCGGAGGATGCTG3'. (E) Since full-length ORF of *OsBUL1* was used for dsRNAi construction, expression level of homologous genes was also negatively affected. Reduced expression of *OsBUL1* homologous genes. Primers are as follows: (*OsBU1*) 5'CTCATCTCTTCTCATCTgTTCTTC3' and 5'ATCAGTAGTACACCGAGATGAGTA3', (*OsBUL2*) 5'TCGATCCTAGCTCTATCAGTAGCT3' and 5'AGTACATACCAAACAAGACAACCC3', (*OsBUL3*) 5'TCGGGGCGGCGAGCATCGGGCAGG3' and 5'GCTCCGACAGCCGCTCGCTCAGGT3', (*OsILI1*) 5'TCGAGCAGCCGGAGGTCGCGCTCA3' and 5'TGATGTAGCTGCACGTCTCCAGCA3'. Data are the average of three independent experiments and normalized by *OsAct*. Error bars indicate SD.





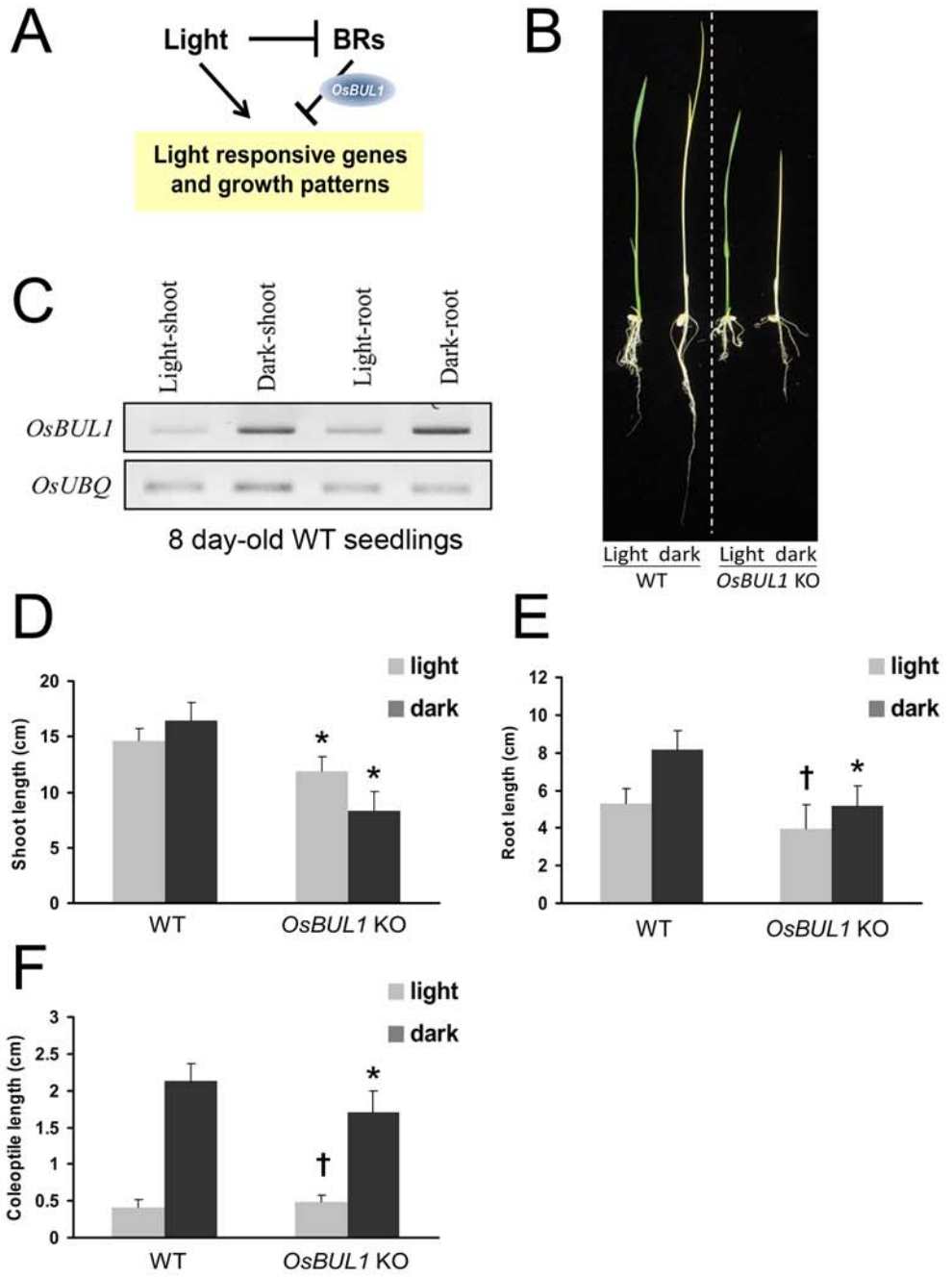


Supplemental Figure S2. Increased *OsBUL1* expression conferred larger grains and leaf angles in rice. (A to F) Larger grains by increased expression of *OsBUL1* or its *Arabidopsis* homologue, *PRE1*. All are japonica rice Dongjin cultivars (*, P < 0.001; **, P < 0.0001, Student's t test). (G, H) *oslg1-1* is a rice liguleless mutant that does not make a collar and expression of *OsBUL1* and *OsBC1* is hardly detected in the putative collar area of the mutant (marked by a white box). sh, leaf sheath; bl, leaf blade; lj, lamina joint; li, ligule; au, auricle.

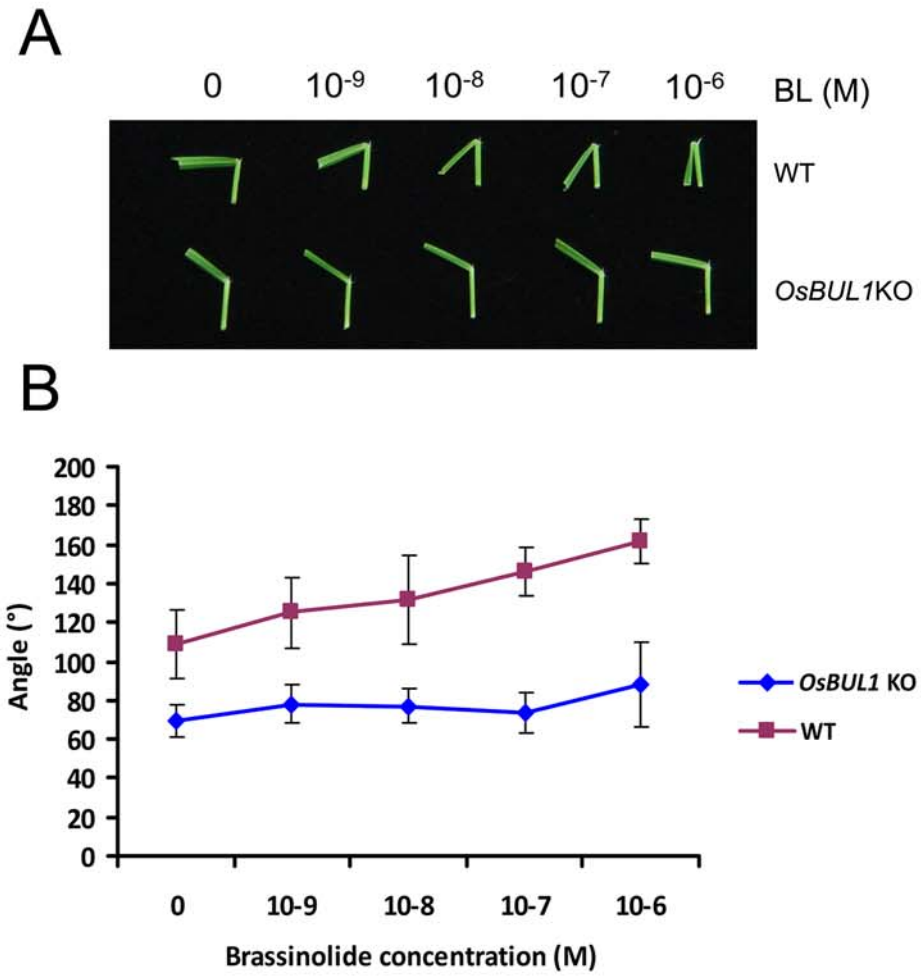


Supplemental Figure S3. Introduction of rice *OsBUL1* into dicot plants, *Arabidopsis* and tobacco. (A, B) Overexpression of *OsBUL1* or its *Arabidopsis* homologue, *PRE1* in *Arabidopsis* resulted in elongated petioles consisting of elongated cells while *PRE1*-dsRNAi *Arabidopsis* showed reduced petioles with small cells. Asterisks indicate petioles used for SEM in (B). Reduced expression of *AtPRE1* in the *AtPRE1*dsRNAi plants was confirmed by primers, 5' CTTGCCACATTGTTGAAC 3' and 5' GATTACATGGATAGGCTTGTCA 3'. (C, D, E) Transgenic tobacco containing p*35S*:*OsBUL1* also produced elongated internodes and petioles. *EF1α* was used as internal control for RT-PCR in tobacco with primers 5' AGGTCCAGTATGCCTGGGTGCTTGAC3' and 5' AGAATTCACAGGGACAGTTCCAATACCAC 3' (Segonzac et al., 2011).

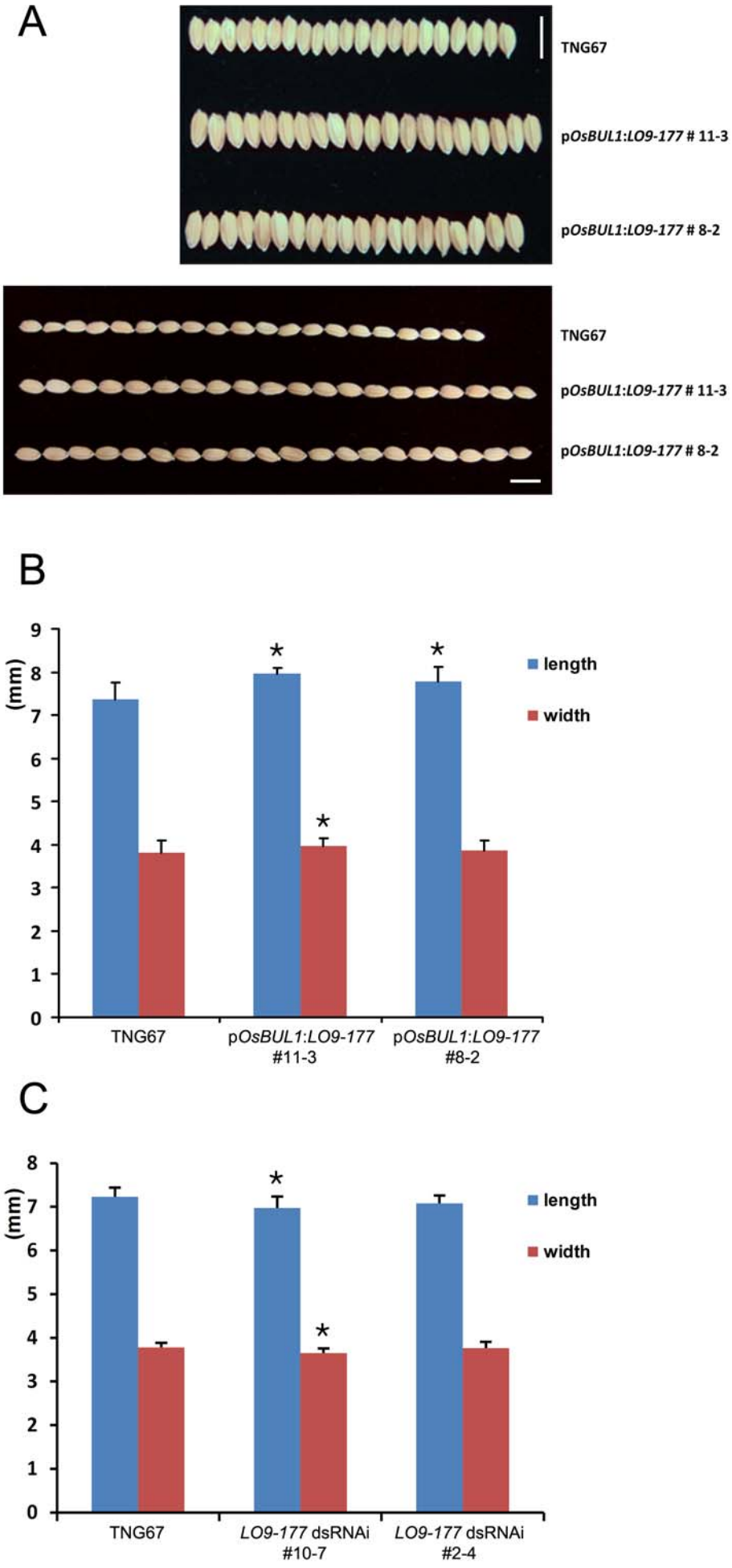
Segonzac C, Feike D, Gimenez-Ibanez S, Hann DR, Zipfel C, Rathjen JP (2011) Hierarchy and Roles of Pathogen-Associated Molecular Pattern-Induced Responses in Nicotiana benthamiana. Plant Physiology 156: 687-699



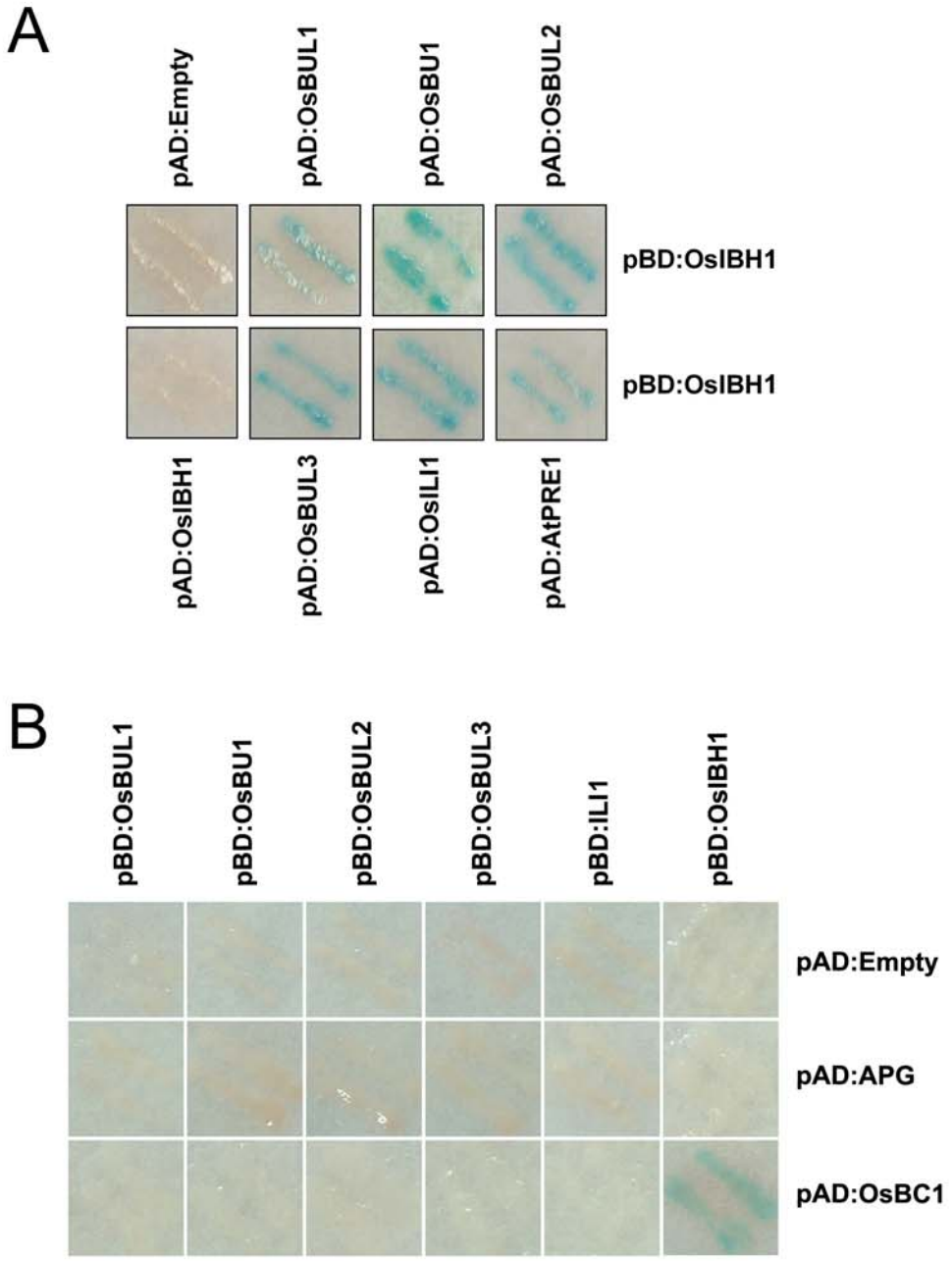
Supplemental Figure S4. Morphological changes of *OsBUL1*KO seedlings under light or dark conditions. (A) Simplified relationship among light, BR, *OsBUL1* and growth patterns. (B) Six-day old rice seedlings of WT (Hwayoung) and *OsBUL1*KO mutant under continuous light or dark conditions. (C) Expression of *OsBUL1* in shoots and roots under light and dark conditions. (D, E, F) Length of shoots (*, P < 0.0001, Student's t test), roots (*, P < 0.0005; †, P < 0.005, Student's t test) and coleoptiles (*, P < 0.005, †; P < 0.05, Student's t test) of WT and *OsBUL1*KO rice seedlings grown under light or dark conditions.



Supplemental Figure S5. Brassinolide (BL) response on WT and *OsBUL1*KO rice. (A, B) Lamina joint bending assays using different concentrations of brassinolide. Error bars indicate SD (n = 12).

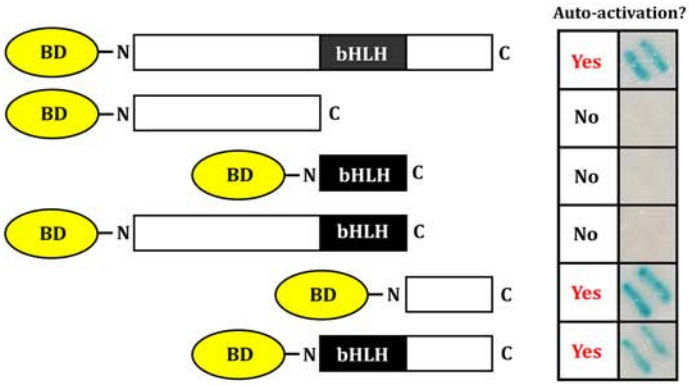


Supplemental Figure S6. Expression level of *LO9-177* is positively linked to grain size. (A, B) Length and width of grains gained from p*OsBUL1*:*LO9-177* transgenic plants are increased. Bars in (A) = 1 cm. (*, P < 0.001, Student's t test) (C) Conversely, reduced expression of *LO9-177* by dsRNAi approaches was likely to result in reduction of grain size (*, P < 0.001, Student's t test).

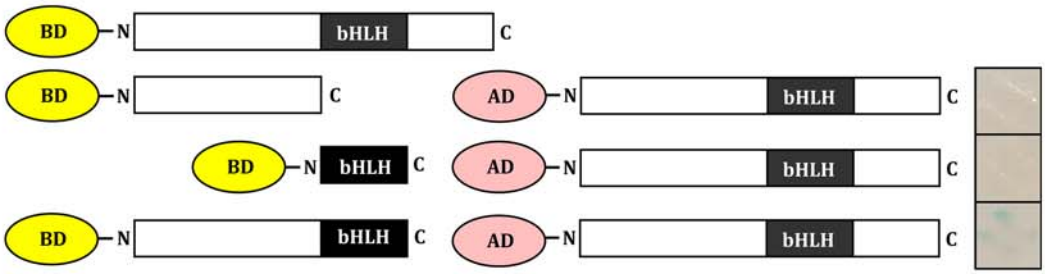


Supplemental Figure S7. Protein interactions using yeast two-hybrid systems. (A) OsIBH1, an atypical HLH protein interacts with another group of atypical HLH proteins including OsBUL1, OsBU1, OsBUL2, OsBUL3, OsILI1 and AtPRE1 but does not interact with itself to make homodimers. (B) Even though APG is a typical bHLH protein similar to OsBC1, only OsBC1 is able to interact with OsIBH1 showing molecular discrepancy between APG and OsBC1.

A



B



C

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MAGFSHLPQQ MEHGLITNNG FLFCHGSHGG AATTTAPAIP EDASMETSSV VLDTSPQDKK  60
RKPREEDTAS LNSAHSKEAK ENGRKRGGKK HSRDQMEEEA PQGFIHVRAR RGQATDSHSL  120
AERVRRERIS ERMRMLQALV PGCDKVTGKA LILDEIINYV QSLQNQVEFL SMRIASLSPV  180
LYGFGIDSDA FSDHSQKMEG MFHEAVAIPA SVLNRGSSPA QSHAIMDTSN TSPTPYTLQV  240
QGGSNNNSLS QDNGSYIMQT VGEPRQELFN QVVLNNYMCS FQ 282
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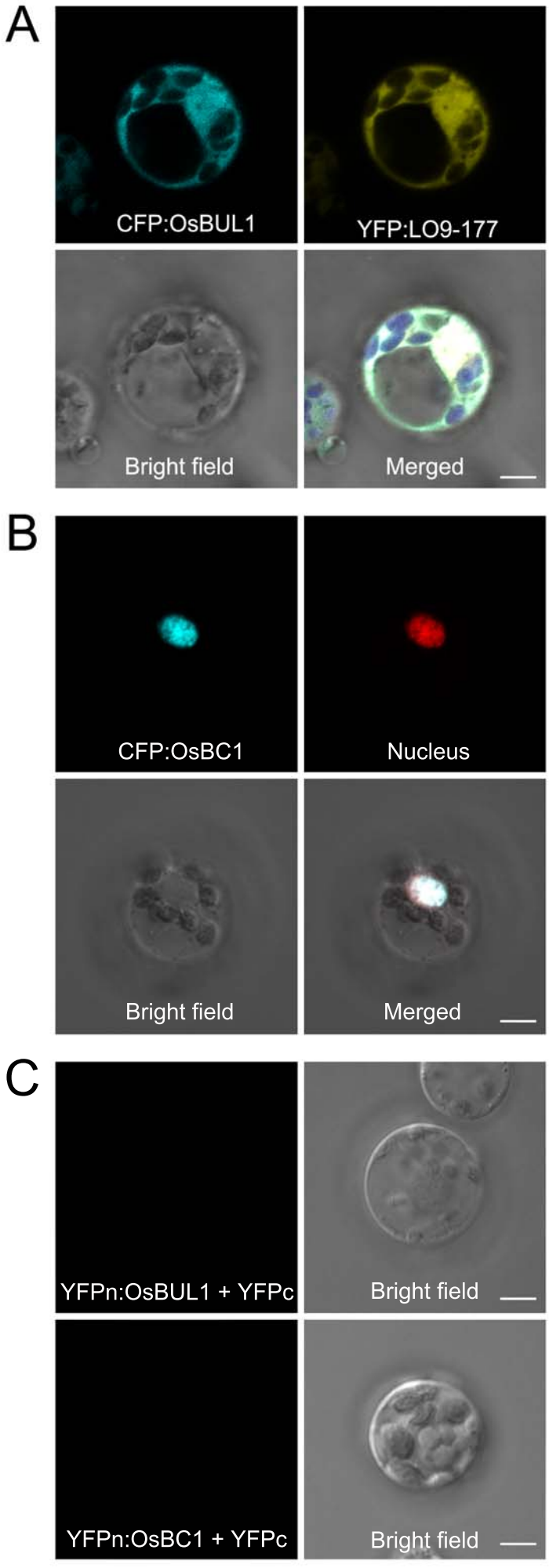
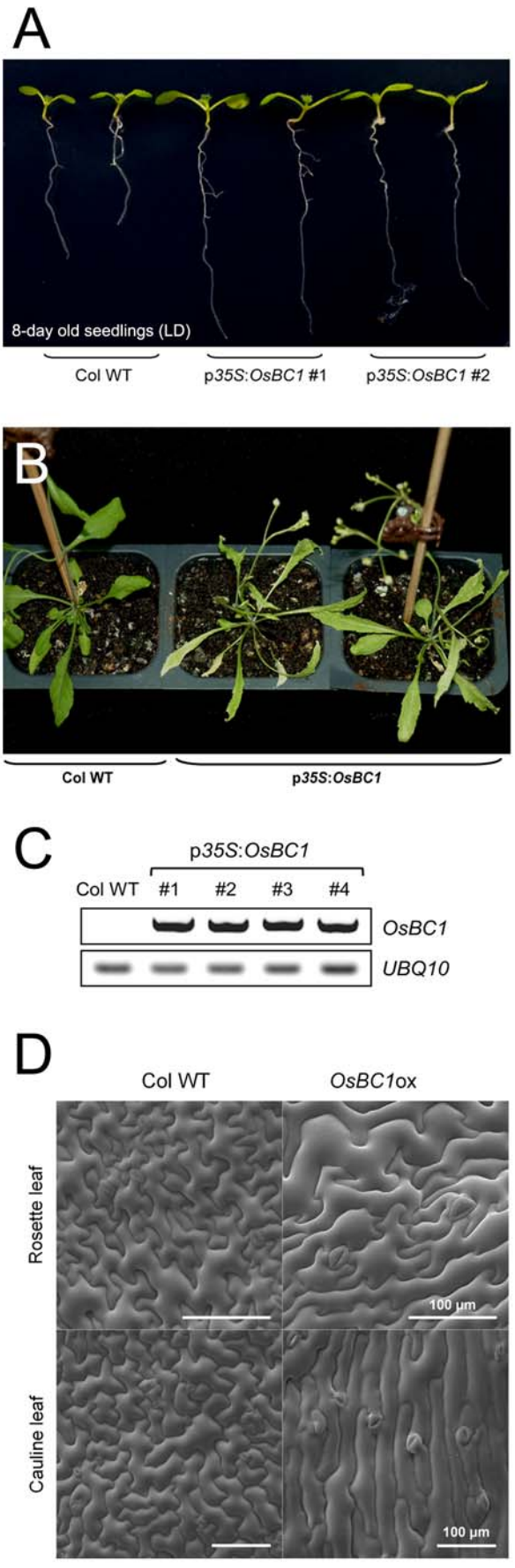
Supplemental Figure S8. OsBC1 has auto-transcriptional activation activity. (A) The carboxyl terminal of OsBC1 has transcriptional activation activity. BD presents GAL4 DNA binding domain and bHLH domain of OsBC1 is marked by a black box, respectively. Full-length and various truncated forms of OsBC1 were fused to BD domain in pBD vector and introduced into yeast cells and tested each of their transcriptional activities by x-gal filter assays. Carboxyl-terminal region of OsBC1 is necessary for transcriptional activity. (B) OsBC1 forms a homodimer through the amino region and the bHLH domain. Truncated forms of OsBC1 protein which do not show transcriptional activities were used as baits for testing homodimerization with full-length OsBC1. For OsBC1 homodimerization, both amino-terminal region and bHLH domain are required. (C) OsBC1 protein sequence. Amino acids corresponding to the bHLH domain are marked in bold. The red underlined region is the basic region and the red asterisks are conserved glutamic acid and arginine residues critical for binding to the E-box (CAGCTG) and G-box (CACGTG), which are the typical binding sequences of bHLH proteins (Toledo-Ortiz et al., 2003). Acidic amino acids with red color and glutamine residues with blue color are likely responsible for transcriptional activity of OsBC1 (Schwechheimer et al., 1998).

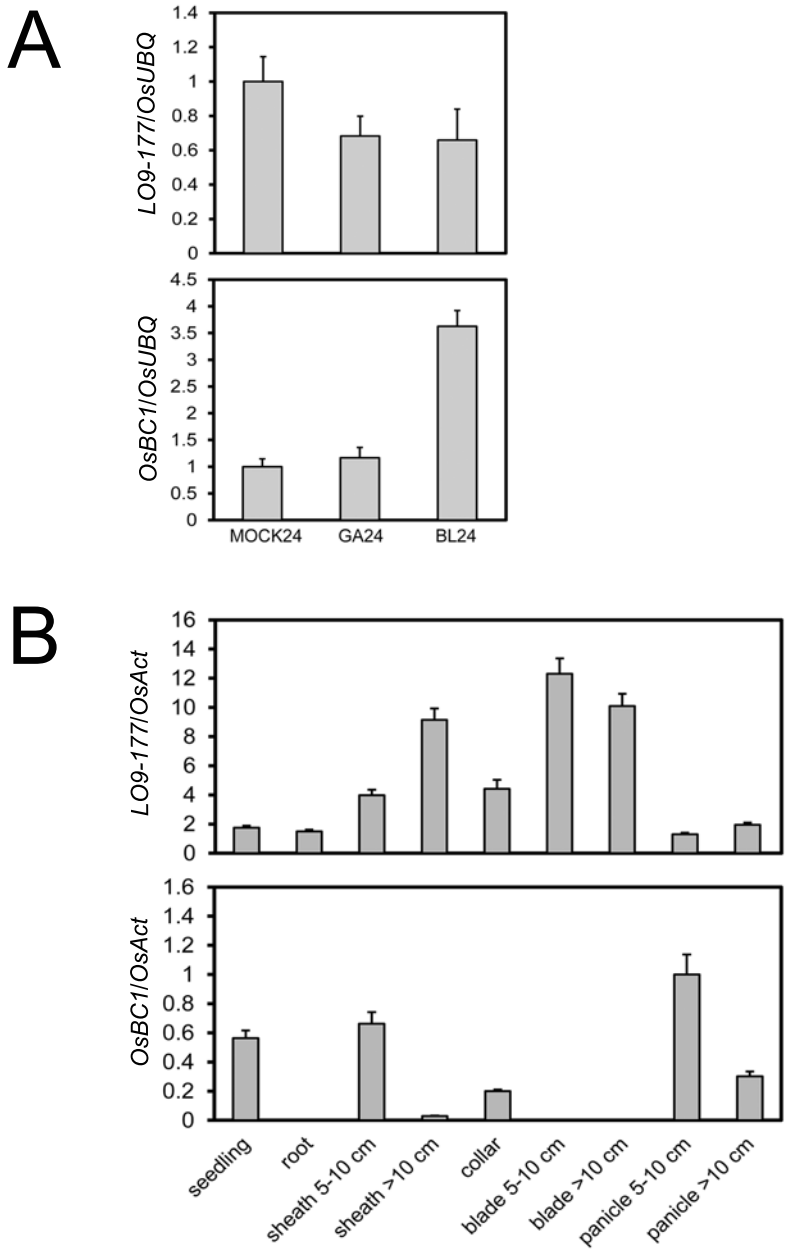
Schwechheimer C, Smith C, Bevan MW (1998) The activities of acidic and glutamine-rich transcriptional activation domains in plant cells: design of modular transcription factors for high-level expression. Plant Molecular Biology 36: 195-204.
Toledo-Ortiz G, Huq E, Quail PH (2003) The Arabidopsis Basic/Helix-Loop-Helix Transcription Factor Family. The Plant Cell 15: 1749-1770.



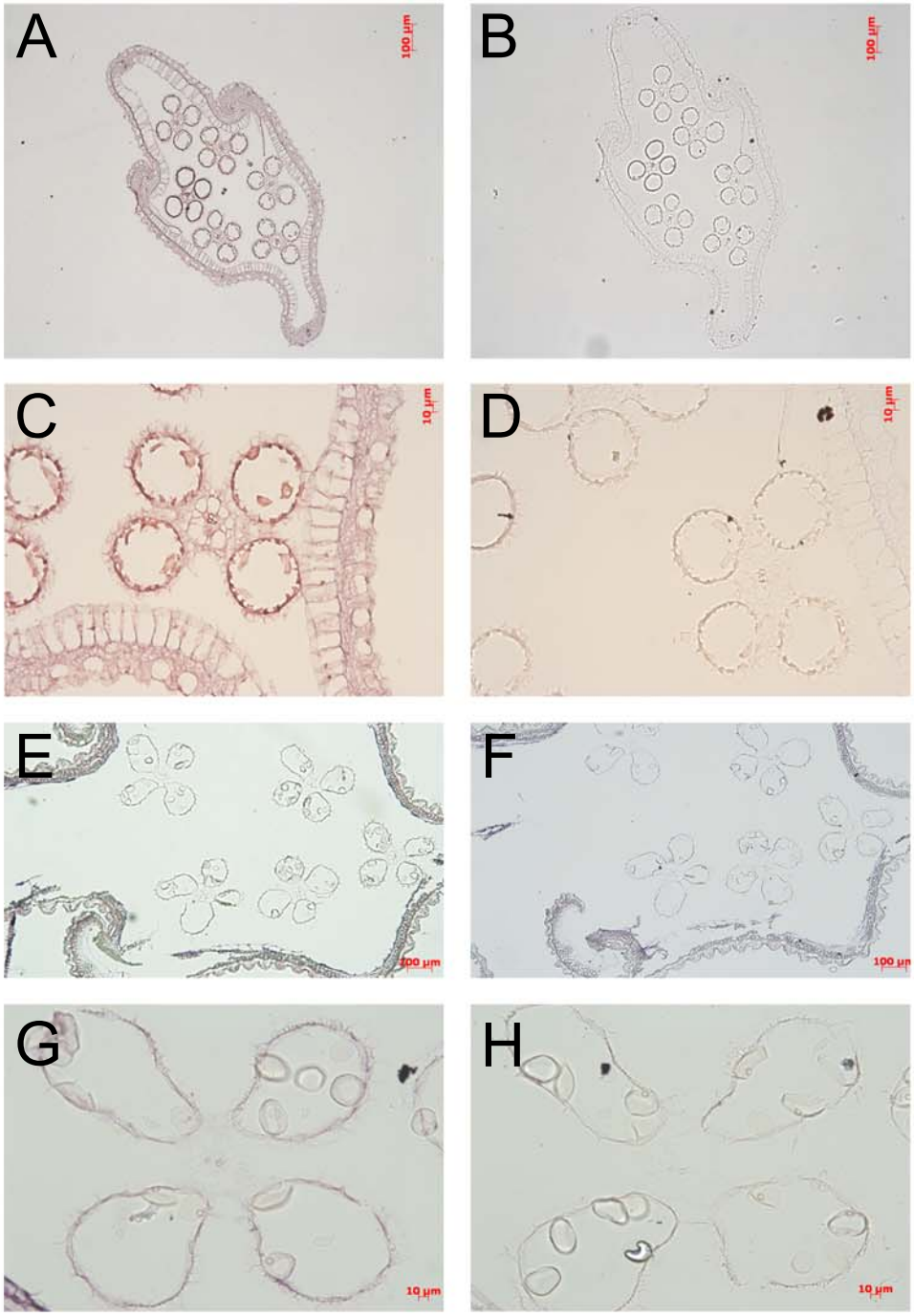
Supplemental Figure S9. Subcellular localization of proteins. (A) CFP:OsBUL1 and YFP:LO9-177 were co-transformed into rice protoplasts and they are co-localized in the cell. (B) CFP:OsBC1 and NLS:RFP marker were also co-transformed into rice protoplasts. OsBC1 is a nuclear protein. (C) Negative controls for BiFC assays shown in Fig. 4C and Fig. 6C. Bar = 5 μm.



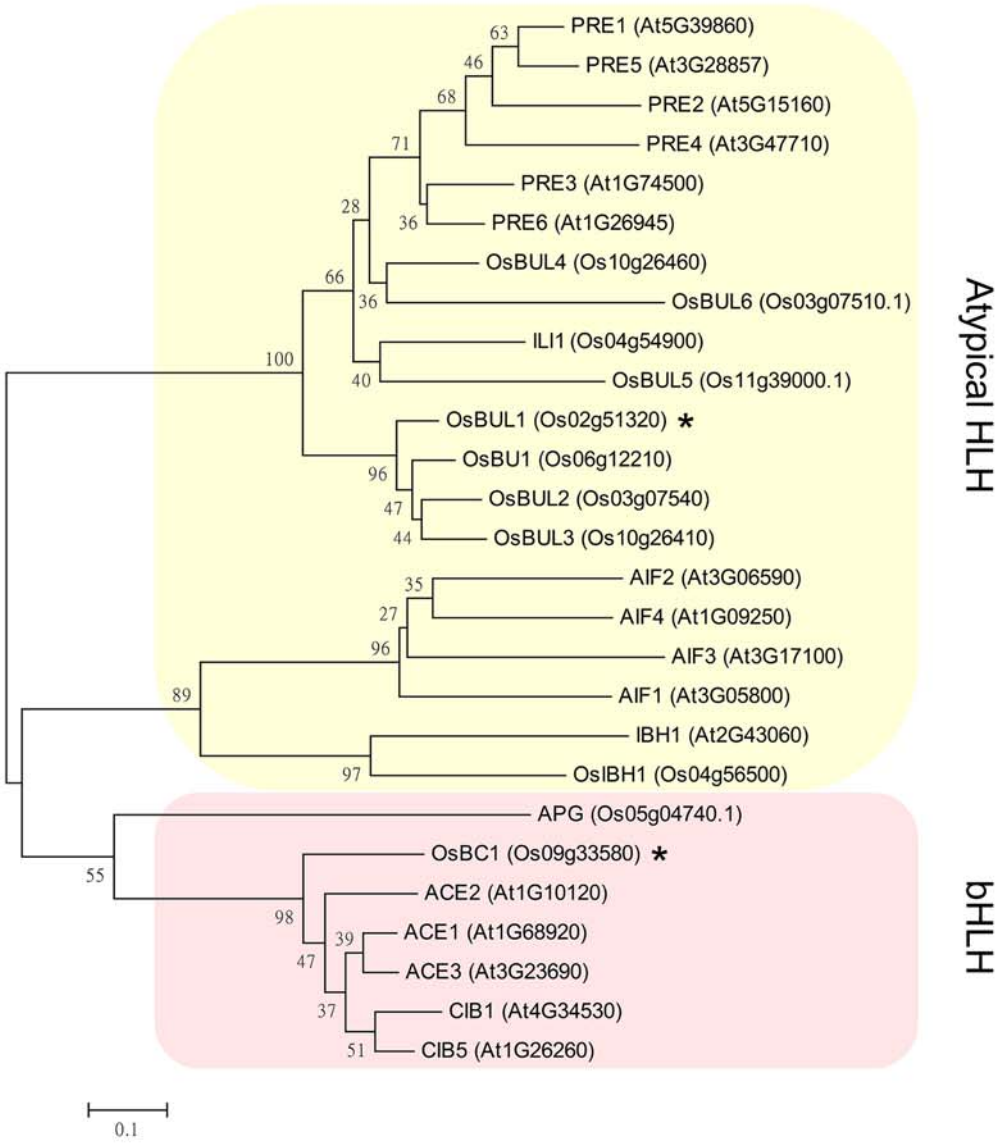
Supplemental Figure S10. Transgenic *Arabidopsis* overexpressing *OsBC1*. (A) Eight-day-old seedlings grown under long days. (B, C) *OsBC1*-overexpressing *Arabidopsis* showed narrower and longer leaves compared with WT. These phenotypes were observed both in rosette and cauline leaves. They were grown under long-day conditions (16h L). Expression level of *OsBC1* in transgenic and WT plants by RT-PCR. Total RNAs were extracted from cauline leaves and semi-quantitative RT-PCRs were carried out as in (C). *UBQ10* expression was shown as a control. (D) Epidermal cells of rosette and cauline leaves from *OsBC1*-overexpressing *Arabidopsis* together with Col WT.



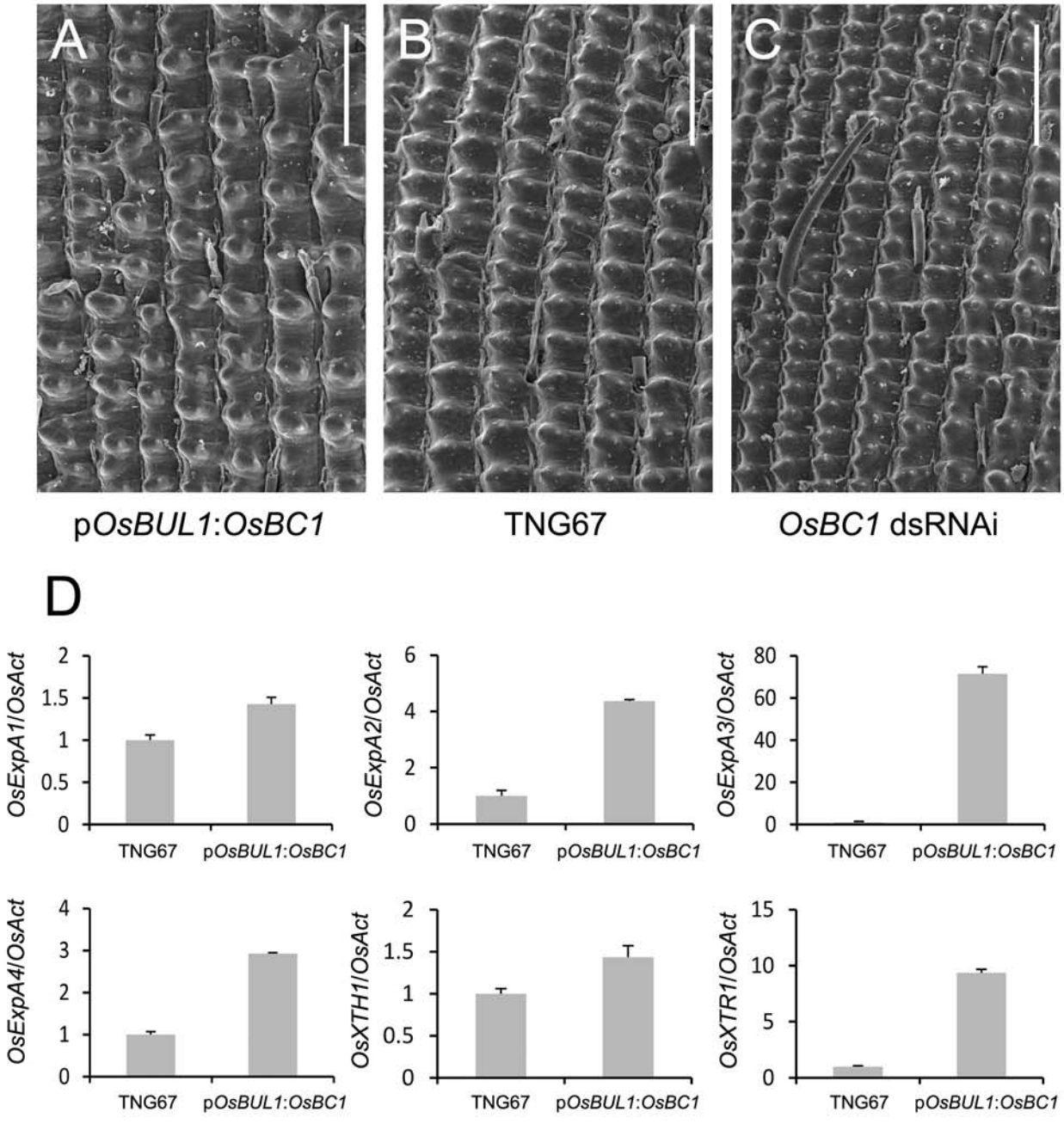
Supplemental Figure S11. Expression analyses of *LO9-177* and *OsBC1*. (A) Effect of phytohormones on the expression of *LO9-177* and *OsBC1*. Samples were harvested at the 24 h time point after treatment with gibberellin (GA3) and brassinolide (BL). Error bars indicate SD of three biological replicates. (B) Spatiotemporal expression patterns of *LO9-177* and *OsBC1*. Error bars indicate SD of three technical repeats.



Supplemental Figure S12. *In situ* hybridization of genes in spikelets. Expression of *LO9-177* (A, C) and *OsBC1* (E, G) in spikelets and anthers. Signals for *LO9-177* transcripts are observed in palea, lemma, filaments and anthers (A, C). Especially, strong expression signals are detected in the tapetum tissue (C). Weak signals for *OsBC1* transcripts are present in the endothecium and tapetum tissue of anthers (E, G). (B) and (D) are controls hybridized with *LO9-177* sense probes, and (F) and (H) are controls hybridized with *OsBC1* sense probes.



Supplemental Figure S13. A phylogenetic tree showing the relationships among atypical HLH and typical bHLH proteins. OsBUL1 and OsBC1 are marked with an asterisk. The tree was constructed using MEGA 5.1 software with the neighbor-joining method (1000-replicate bootstrapping). Bootstrap values are shown beside each node.



Supplemental Figure S14. *OsBC1* affects cell size in plants. Epidermal cells of grains produced from p*OsBUL1*:*OsBC1* (A), *OsBC1* dsRNAi (C) and WT (B) rice plants. Bar = 200 µm. (D) Genes involved in cell elongation/enlargement are increased in p*OsBUL1*:*OsBC1* plants. Data are the average of three independent experiments and normalized by *OsAct*. Error bars indicate SD.