

A

NF-YB1 ATGGCAGGGAACAAAAGCGTGGTGGCAGGAACATGGATCAGGTCAA- GAAGGCGGCAGTGAGATCGGATGGGGT
nfyb1-1 ATGGCAGGGAACAAAAGCGTGGTGGCAGGAACATGGATCAGGTCAA- GAAGGCGGCAGTGAGATCGGATGGGGT
nfyb1-2 ATGGCAGGGAACAAAAGCGTGGTGGCAGGAACATGGATCAGGTCAA- GAAGGCGGCAGTGAGATCGGATGGGGT

NF-YB1 GGGAGGTAGTGCACCAACGCCGAGCTGC- CGATGGCCAACCTCGTACGCCTGATAAAGAAGGTGCTCCAGGGA
nfyb1-1 GGGAGGTAGTGCACCAACGCCGAGCTGC- CGATGGCCAACCTCGTACGCCTGATAAAGAAGGTGCTCCAGGGA
nfyb1-2 GGGAGGTAGTGCACCAACGCCGAGCTGC- CGATGGCCAACCTCGTACGCCTGATAAAGAAGGTGCTCCAGGGA

B

NF-YB1 MAGNKKRGRNMDQVKKA AVRSDGVGGSATNAELPMANL VRL IKKVLPGK AKIGGAAKGLTH
nfyb1-1 MAGNKKRGRNMDQVK EGGSEIGWGR*-----
nfyb1-2 MAGNKKRGRNMDQVKKA AVRSDGVGGSATNAEL LDGQPRTPDKEGAPRESEDRGSSQGSHP*

NF-YB1 DCAVEFVGFVGD EASEKAKAEHRRTVAPEDYLG SFGDL GFDRYVDPMDAY IHGYREFERAGG
nfyb1-1 -----
nfyb1-2 -----

NF-YB1 NRRVAPPPAAATPLTPGGPTFTDAELQFLRSVIPSRSDDEYSGSSPAIGGYGYGYGKNN*
nfyb1-1 -----
nfyb1-2 -----

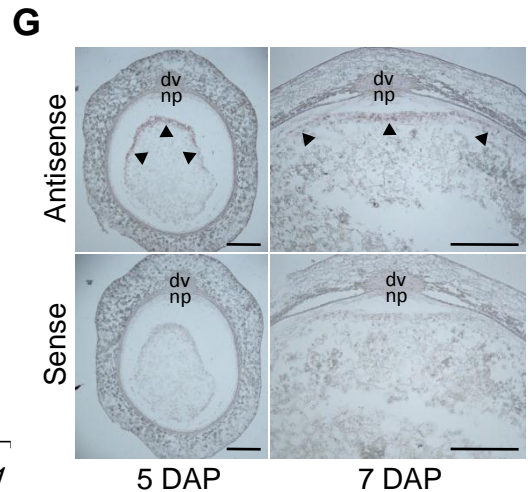
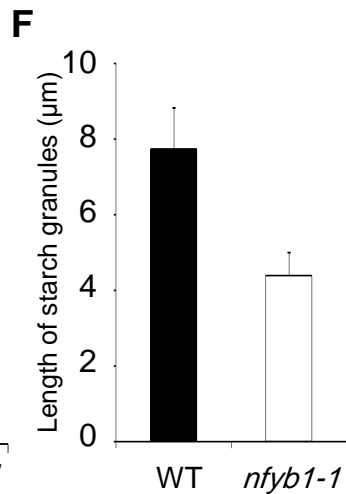
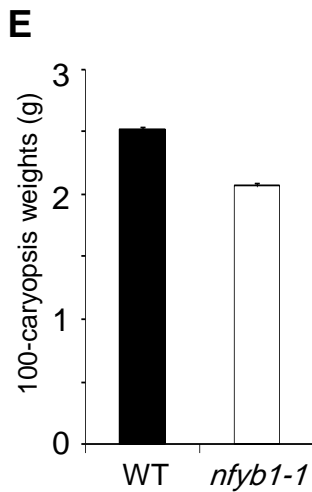
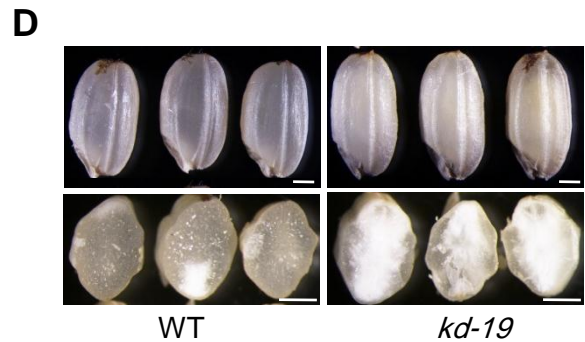
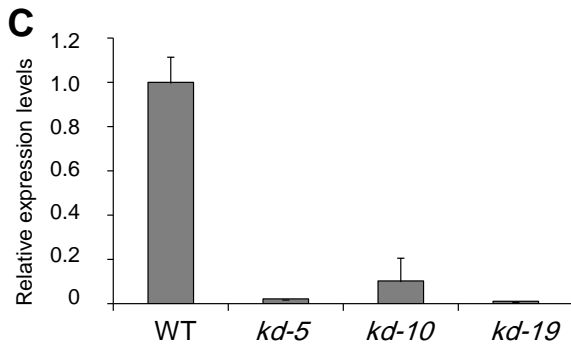


Figure S2 Genetic analyses of *NF-YB1* gene in rice grain filling.

(A and B) Genomic (A) and polypeptide sequences (B) of *NF-YB1* from the wildtype and *nfyb1-1* and *nfyb1-2* mutants generated using CRISPR-Cas9 technology, showing the presence of an A and a T insertions in *NF-YB1* from *nfyb1-1* and *nfyb1-2* mutants, respectively, leading to truncations of the NF-YB1 protein. *, stop codon.

(C) Real-time PCR to show that the expression of *NF-YB1* was down-regulated in caryopses collected at 11 DAP from 3 transgenic rice lines (*kd-5*, *kd-10* and *kd-19*) carrying a *pUbi::NF-YB1RNAi*, as compared to that in the wildtype. Data shown as means \pm SD ($n = 3$, $P < 0.01$).

(D) Down-regulation of *NF-YB1* led to production of rice grains with chalky endosperms. Morphologies of intact (upper panel) and cracked caryopses (lower panel) from the *kd-19* plants carrying *pUbi::NF-YB1RNAi* (right) and wildtype (left). Bars = 1 mm.

(E) Dry weights of 100 mature caryopses from the WT and *nfyb1-1*. Note that caryopses from *nfyb1-1* showed 18% reduction in dry weight. Data are shown as means \pm SD (100 seeds per sample, repeated 3 times; $P < 0.01$).

(F) The length of starch granules from the WT and *nfyb1-1* endosperms. The average length of WT and *nfyb1-1* starch granules along their long axes. Data are shown as means \pm SD ($n = 6$, $P < 0.01$).

(G) *In situ* hybridization in caryopses collected at 5 and 7 DAP, showing that *SUT3* is expressed specifically in dorsal aleurone (indicated by arrowhead). Note that no *SUT3* expression in maternal tissues, nor in starchy endosperm. dv, dorsal vascular bundle; np, nucellar projection. Bars = 200 μ m.