Supplementary Methods

Constructs for CRISPR-Cas9 and transformation:

The vector *pPcas9-rU3-gRNA* from the ViewSolid Biotech was used, and two 20 bp fragments from the second exon of *NF-YB1* (sequence as showed in Figure 1C) was introduced into the vector, and then these plasmids were transformed into rice (Zhonghua 11) as described previously [1]. Principles for designing the target was described previously [2].

Constructs for RNAi and transformation:

RNA interference (RNAi) construct with a 350-bp fragment (nucleotides from 168 to 517 counted from ATG of coding sequence of *NF-YB1*) amplified from cDNA of caryopses of Zhonghua 11 with sequences carrying restriction enzyme sites *Kpn*I-*Spe*I (forward primer), *Bam*HI-*Sac*I (reverse primer) and cloned into the vector pEASY-Blunt Simple vector. The plasmid was then digested by *Kpn*I-*Bam*HI to obtain the sense fragment and by *Spe*I-*Sac*I to obtain the antisense fragment. These sense and antisense fragments were introduced into the target vector *pTCK303* together to generate the *pUbi::NF-YB1RNAi* construct. The protocols and principles for construct the RNAi vector as described previously [3].

Yeast one-hybrid experiments:

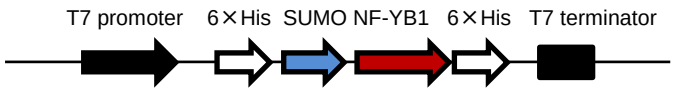
The cDNA sequence of the *NF-YB1* was amplified from cDNA isolated from caryopses of Zhonghua 11, and cloned into the vector *pB42AD* to generate *GAD-NF-YB1*. The 5' upstream regulatory sequences of *SUT1* (3 177 bp), *SUT3* (2 821 bp), *SUT4* (1 026 bp), *MST4* (3 074 bp) and *SUT2* (1 055 bp) were introduced into *pLacZ2u* to generate the LacZ reporter plasmids. These five constructs were transformed individually into yeast strain EGY48, together either with the *pB42AD* empty vector or *GAD-NF-YB1*. Yeast cells were grown on a synthetic dropout medium without tryptophan and uracil. 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside was used for color assay.

Luciferase (LUC) activity assay in rice protoplasts:

For the transient expression assay, the cDNA sequence of *NF-YB1* was amplified from cDNA of caryopses of Zhonghua 11, and cloned into the vector *pCambia1301-35S::tNOS* to get the effector construct; the 5' regulatory sequences of *SUT1*, *SUT3*, *SUT4*, *MST4* and *SUT2* (sequences are same as used in the yeast one-hybrid experiments described above) were introduced into the vector *pUC-35sLUC* to generate the reporter plasmids. *p35S::GUS* was used as an internal control. The effector (*p35S::NF-YB1::tNOS*) or the empty vector were co-transfected with one of the reporter constructs (*pSUT1::LUC*, *pSUT3::LUC*, *pSUT4::LUC*, *pMST4::LUC* or *pSUT2::LUC*) and *p35S::GUS*. Empty vector co-transfected with the reporter construct was normalized to 1, and luciferase activities in protoplasts co-transfected with the effector and these reporter constructs were relative to this value. The protoplasts were incubated in the dark for 12 hours before harvesting, and the LUC and GUS activities were determined with a luminometer / fluorometer (Promega). The protocols for isolating protoplasts and transfection were used as described previously [4].

Purification of His-NF-YB1 recombinant protein and EMSA:

The cDNA sequence of the *NF-YB1* was amplified from cDNA of Zhonghua 11, and cloned into the expression vector *pET28-SUMO-XF1024* to generate the *pHis-NF-YB1* expression vector, and the His-NF-YB1 protein was produced in the *Escherichia coli* BL21 (Transetta) strain and the recombinant protein were then purified by Ni-charged resin (Bio-Rad). The 59 bp fragments containing the CCAAT box (sequences showed below) were identified from the 1 kb 5' upstream region of *SUT1*, *SUT3* or *SUT4*, labeled with biotin. The unlabeled fragments were used as the competitive probes. *SUT4Δ* was made with a mutated CCAAT box (CCAAT changed to TGATA), which was used as a negative control. Then the recombinant protein with the labeled probes, or labeled probes with 200-folds, 500-folds unlabeled competitive probes were incubated on the ice for 20 minutes, the binding buffer used was Tris-HCl (pH 8.5). The electrophoresis was performed on 6% native polyacrylamide gel for 70 minutes, and then transferred to membrane, cross-linked, detected as described in the instruction book of the Light Shift Chemiluminescent EMSA Kit (Thermo Scientific). The rough map of the *pHis-NF-YB1* is illustrated below.



Supplementary information

Putative CCAAT elements identified in 5' upstream sequences of *SUT1*, *SUT3* and *SUT4*, and sequences used for EMSA: putative CCAAT elements are underlined, and sequences used for EMSA are colored in red.

The 5' upstream sequence of *SUT1*:

GGAGGAGTTAACAGCAAAGACAAGAAAGGATAATCGAGATCCTCTACAATACTATATGTGCAGTACTACTTACTGACATG
TGAATCAACTGTCAGTGAGTCGATATGTCGTGAACAGTACTGCACATGTATGGCACTACAAGAGTACTTGCATCGAAGGA
AGAAGCGTCAACCCCCCCCCCCCCCCCCGGGGCGCGGCGAGCACACGCGCTAGTCGCGGTAGCGCGCCCTGCGATTCTTC
CTTATCCATTCATGCATGCATGGCAGACCATTCCTCCATTCCATTTCGATCGATTTTAACTCCTCGGTATTGCAAACCCG
ATAGCCTCGGTATGCCAAATCTTGGTGTTACGGATGGATGCATGGATGATGTGGTTGACCAACCCGTCGTCAGTTGTTCA
TTGATCATACGCATCGGAGAAAGAAAGAGACTTTCCGTCTTTCTTTACCTTCCAATCCCCGTTGACTTGGATCATGTCAT
GCAGCTATCTGAGTGAATTGTTTAATTAAGCAAGAGACATTCCTCGCTTGCACTGCACCGGCCGGGCACAGAACAAATGT
CACTGTCGCGGTACTGAATTATTCAGACAATCTGGCTGCATCTCCTCATTTTACAGCATCAGTTAGACAAGCCACCTTTT
TTCCAGTAGTATGGGTTATGTGTAGGTTTGCTGCATACTCCTATATAGGAGTATCGCTTTAGGGAATCAATGTGCAGGCT
CCCATAGAGTTCCAGCTGAATGCTTCAGAAGGAATTGAATGTCCATACTGCAGATTTGTAGATGTAGATATCGAGAAGCA
TAGCCTTATATCTGCATGCATGGACTTGGATATTTCTCTCTTATCTGATAGCCCATGTATAGCTCTATATCTGCACAACA
CTTTACCATGCACATATATTTGCATCTAGCTACAACTCCGTCAGACATCTAGCTAGCTAGGAGGTGTGTGCAACAAGAAC
AATATCATATCTCACACCTTTTCCGAAAACTTCAATGTCAAAACCGCGGCATCATATCTCCATTGACGTGGTTTTCTCTT
TTTCCAGAGAAAAAAAAACATGCATGTTCAGTAGTACGAATTATAGACTCTACTGCAAATGCCAAGAAATATTAATTGGG
ACAAAAACGACGAAATGAACACTGTATTAACCCGATGCAAACTGTCTAAAAGTAGCGTTTAATTAAGCTGACCATTGCGA
CCGACCGACCGAATTGAATCGAGTCTCCAAAAGAAAAAAGAGAAAATGCCCTTCTCCTGCATGCATGCAGTCCTTGGTGC
AGCAGTGACCTCACTGAATTTTGTTTGTTCTTGGCTAGGGACTGCTTGGAATTGGATTTTAGTCTAGCAAAATTTGACTC
CAACATGGGAGCATGTGAGATATACATGTCATTGATCACATCACTATACTGCTGTGTATCTTTTCATACCAACTACCATA
ATATGGTAGCCCAAGTAGTGGAAATTAAAGAAAGCACAACTCCATATCTCGATCCATCTATCCCTTATCACTATATATAT
CTATATCATTATCCCGTAGCTATTTTACAATATCACAAATATATAAAAAGATTTAGTTTTTTTAAGGAGTGAATATGTTC
GTTTCAGTTTGACTATAATTTCCTGAACTTTTTTCAGTGACCGACCAGGCGACCACCCGTTACTAAGATCAGGGTAAAAT
GTGAGGCCGACATGTGTATTACTGGTCAATGGCAGAACTGAAATTCGAAAATGTATTGATGTATTATTCCTCTTTTTTTT
TTGTTCAAATTAAGATGACACGTAGGCTCCAGCAACCATCCACGTGGCGGCAGGTTGTGCACGTCACTCCATCTCCAACA
CATCAGCATTTTCAGCAAAGAAAGAAAAAACTGTAGTGGACTTTGCAGAAAACGCTGACGACGAGTACGTACGAATTAAT
GCCTTAATCATATGATATCTCCAAAGATTAATTAATAAGTGGCTTGCTTAATTTTGCTACTAATCGCTATCTCGACGTAG
CCAGAGAGCCATGCATGCATCACAGCGAGCCCACGGCCGCACGAGCACTCGCCAACGAATTCGCTAGGCGTACACCGTGA
ATGATTTGATGCGTTGATTACGGGTATTCATATTCCTTTATGAAAGGTTATTGTCAGACTTTTTTTATTCCACAAGATCG
ATCATACTACAAAGTTATTCTACAATAGTTTAGAACACTTATCCAGTTGTGTTAGAATATAATAATGATGGATGGATATG
TATGCCATATTAAACAATCTAAATTCCCCACAAAACATATAAAAGAACACTATAATAAACTATGGTTTATCCAACATGGA
CATATATTTAAATGAAGTGCGATCTCCGGTGCTCTTTACTGGTAGGATGAATGATGATAGAGATAAAAGCGTTTAACAAA
TATGGCCTCAAGCGAAATTCGTTATATTAATTAAATCAATGAAAACATTTACTGGATTAATAAAACTCCATGCTACTCCA
TTATAAATGAACGCACACCTATATATAGCAAAATTCCTATTTGCCAGTAGGTCCAATACTTCGGATCTGTTTTTTTTCT
TTTAAATATCCAAAATTGATTTTGGATAACTACTCGACAGTACAAACGAATTAAACCAGCTATTACAACGTCGAGTGGAT
TTAAAACACTCCTCTATTAAATTCACCTACAGAAAGTCGTTCCCGCTGAAATAATCGCACCGTCTAGAAGCTCGGCAAGC
GTGTCGCTAATCCGATACTAACTCCATTAATTCCATTTTCATTTCAATAATTGTTGAAGTTATTACTGCACTGGAAATAA
TAAAGGCAGGGGGGTGTAACTGGGTGTGTACAAAGTGTTGGTGAGCATAGCAGTTGGCAGGTGCACCACCCTTTATTATA

TTCCTCCTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCCCTCTTCCTCCCTTTAAATGCTTCGCC
TCTCTCGCTCGTCTCTCCAAACACAAACCCACCACCTCCTCCTCCTCCTCCCATCCAGCACGCGCCTCCTCTCTCGCGCG
GCTTTCCATTTCCATCTCCCCCTCCTCCTCCTACGTCTCCGCCGCTCCTCACTTCCTCCACTCGATTTCCTTTCTTGGCC
TCTCCTCCTCTGACACAGGGGTGTGCAGGTTTGTGTTTGTGCG

The 5' upstream sequence of *SUT3*:

GTTGTTAAATTTGTGGATGATTTTGTGAACGAGGATGATTTTATTGCTGAGTTCGGTTTGAAATCAATATTCAAACAGAA
AAAACAATGAAAAAGGAAAAAAAAAATACAGCCGGGCACGTGGCTTAGCCATCTTGTACCAACCGGGACTAAAGATCGAT
CTTTAGTCCGCGGTATTTAAACCAGAACTAAAGATAGAGATTTGTAGCCCCGGTTTACTACCCGGACTGAAGGAGGTTGC
AAACCGGGACTACAGAGGGTTTGTGCAGCAGTGGATATAACAAACATGCGTATGCTATGCAGATATGTAGCAACAAAGTT
CAGTTAATGGCTAGAAATCTATGGGCCTTCGCAATCTCCTAAAGTTTAAAATTTCACATTGATGTTACTGTAACTTTAGC
GAAAATTTAAAAAAGAATTAACACTGCTAGCCCTCGATCTCCAACATATAATTTTTAGTGTTTCCAACTCGATCAGCCAC
CGACCTGATCTAGTTATTTTCTGATAATTGAGCTACCACTGGTATATAAGCCATAGAAAAATAAATTAAGCAAAACCAAA
AATAACTCATAGGTAAAACTTTGGTGATGTCTATTTTTGACCTTTAAATTTAGTTTTTTTTTTGCCCTAAACTACAAACC
TGATTATTTTTTACCCTCGACCTTTTAAAACCGAAGAAATAACACTGAAACCATGTTTCACAGTGGGTTTTTGCTACATG
AACATCAATTAGGTGATGGTGATCTAATTACATTAGGAAAGTATGCATCAAAATGGTATAAGATATGAAAACCATCTTCA
TTTCAACAGAACCACTAAAAATATATTTATTTTTATCGAAGGTGTTATTTTTCTCATTTTGAAAGTTTGCTGAAAAATAG
AATCTGAGCAAAATTTGAGGTTAGCGGTTCAAATATTAAAGCTAGAAAATATACTATAATGAATTAACTCTAAAAATTAA
GTTTCAAAAATTATATTTTGGCTGCGCATAATAAGCCAATATACAGACGATGAAAGCCGTCGGTGGTTGCATATGCATAA
GCCTTTTTCATTGGAGATCCTCAGGTTGAAGATAACATTTTCAATTGGTTCTAGCTAGGATTTAAAAGAAGCTAGATGAA
GTGAAGTTCTACAACTTACAAGGTATCCCTGTCAAACAAATCTGGAGATTTTTGATTCAGAAAATATATAGTATTTTCGA
TCTCTCCTCCGACAGGGTCCCCCATTCAGCAATGAGCACTACAGAGTCAAATGAGCTCCTAGTGAATTTTTCTATAATCT
GCCGACCAGTTAGGTGCATGTAACCACAACTTGTGCTTTGCATTATTGTGTGAGCTCACAATAATTAATTTGACACAAGG
ATCGATTGACACTGATTTCACTAGTTAAAATTTTCTATTCTCAAATTTGATTGTAATATGTCAGTACCCGAACCTGCATG
TCACCTAGCTAGCATATATATTGTGCTTATTTATTTAATTTTGAAAAAACTAGGGAATAGTAGTGAAAAACCAGAGAAAC
AATGGATGGCGCTAGGAAAAACCGGTTTTTTAAGTAGTGAATAGTTCTGAAAATTAAGTTCCAATCGTTTTTTTTAAAAA
AGAAACAAAAGAACGTTTACAACCCCTGCACGAGTCACAAACTTAGGAGAGAAAGAAAGGACGAAGTTACGTTGAACGCA
AAGGTTTAAATTTAACTATATATCTAGGTATGTTATAAGGGTCTGGTAGGATTCGATTTTTATATATGTATTTTACATAT
TTAATCTATATTTTCAGTGTAAATAAATACTGCTTTGAGAAGCTAGCTGATTCAATCTCTCCTAGTTAACTAGTCGATCT
CGCACCAAAAAAAAATACAAAAGAAGAAATTAATTTCAATTTATGTGATATTAGTCCACAAGGATAGATTCAGCTATCAT
TTTCTTTGTTATTTCCTTCGTATGTAAGGGACACTTTAAAAATGCTTCGTAGGGGATACTTTTAGCTAGCTAGGTAGTTT
GGTGAGTTGGATAGTTACATATGAATTTTTTTTCTACCACATTCTGTCAGTGAGCTGGTTTACATTTCACCCTTGATTAT
GTATTTTTCTTCTGGTGAGTGGAAGGAATTGGATTTTTCAGCTTCACTCCCCATCAATTGTACACGATTATTTCTCAGTT
TTTAAGTTGGTGATCACATTGATGATTATTCCTTATAGATTACATCAAAACAGAACAGATCAAGGAAAGGCTTGGGCTCA
AATGGAAGCCCCATCCTAACTCTTTAGTTTACAGGCCAGCTGGCCCAGCTCAAGCCCACAATGAACTCTGCCTATTTTAG
TCCTTTATTATGAATGGATGTTCAATTGATTTTTGCAAAATGCAAATGTTTTCTCTATCATTCATTTTGTTCCAAAATTC
ATACCAGTAGAGCTACTCATATATGTAGGGAGGTGAATGGTTAATCAAATCCCAAAGCAATATATAGTAAGATAGATAAT
GTAAGACAAAACTGGAAAATTAAAGCCAATAATCTCAACTCTCAGTTAATTCATGTCCAAAAGTCCGAAAAACCCACAAA
CCAGAGCAGCACACAACATCAATAAATTCTTGAGGCAAAAGAAAAATGGCATTTAAGAAAATTAAAATATTGTGTCGATA
ATCATTTGGCTGGATAAACCCACTAAAAATAGGGATGCTCCAATGAGATCATGCGGCACAATGTCTCACCGCTCCCTCCT
CTCCTTGTTTCTCTCCATTCCATGCATCCACAAGTTCATCATATCCGTCGTCGTCTTCGTCTTCCTCAGTTTATCTCCAT
TTCTCCTCTTGCTTCAACTTC

The 5’ upstream sequence of *SUT4*:

TGGCAGACTACAGAGGAATGAATGGTCCATTGTTGGGGACATAGGGTGTGGCGTTTGATTTAGTTTGTTTTGTGGTGTAT
TGCTATCTGCTAATGTTAGTAGCTTAATTATGTACACTGTTGAGCTAAGTTGTCATTACTATAGAGCTCTCTCCTATACA
TGAGGGGAGGTGACATGATTTTGGTGTGCCTTTTTCTGTCGGTGGTGGTGCTGCTGGGTTGTGGTCATACAATCCAGGTG
CAGACATGATGTATTGTTTGCGAATTTATTATATTTTTTCAGTCTAACCCTGAGGAAAAGGAATCTTTGGTGGTGTGGAA
TAGGCAAAATACGTTGGGTGGCATAATGTTAGATGAAGTGATTGATACACAGGTGGTTGTAGAGGTAACGTTGAGTGATC
AAGTGCAGGCTTGCTGGAGCAAAAAGAAGATGATGCCTCACTCAGTCGTTGACGGCACAATCTAGCTAACCTGCTGCCAT
TGTGGTGATTCATGCCTCCTCTCCGAAGACTCTTTGACTTGTACTAATGGAGTGGACTGCTACCAAACACGCATGAATAG
ACGACCAGATCATCATACAATTCATCAAAATTAGCACTCCTAGTTTCAAACTACTTAACAAAGGAAATTCAACAAACTTC
ATTAAAATAGTGTAGTGTATTTTTTTCTTTAAAAAAAAAATTGCCAACGCCAATCGCGGAGTTTCACATGGAGGAGGGAAG
AATGGAATGAGCGCCAGGCGTCGCGTCGGCGCGCGCGTGAGTGCGTGATTTGTGTTGGTTGTCTTCCTCTTGGCTTCGCT
TCGCTGCTTTGCCGCGCCTTCGCCGAGGGAGGCCGACCAACGCATCAATCAAACACACAAGCACACCACGCGGACGCAGC
AGCAGGGGAGGAGACAATTTCCTATTCTTCCTCGCCCCGCGTCGCCTCGCCTGAGTCTGACTCTCCAAACGCCGACCAGT
GACGCCGCGAGCCTTGCCCCTTGCCCGCGCAGATCTCACCAAACCCTACCAGATCTGCGCCCCGCC

Supplementary information

References:

1. Hiei Y, Ohta S, Komari T, Kumashiro T. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J* 1994; **6**:271-282.
2. Shan QW, Wang YP, Li J, *et al*. Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat Genet* 2013; **31**:686-688.
3. Wang Z, Chen CB, Xu YY, *et al*. A practical vector for efficient knockdown of gene expression in rice (*Oryza sativa* L.). *Plant Mol Biol Rep* 2004; **22**: 409-417.
4. Yoo SD, Cho YH, Shen J. *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nat Protoc* 2007; **2**:1565-1572.