

**Supplementary Figure S3** The multiple-sequence alignment of wild-type and mutation-type YTHDFB clade proteins used in this study. CRISPR-Cas9 system induced mutations lead to a serial of amino acid changes and early termination. Red box above the sequences shows the YTH domain.

[illegible]

**Ortho2FC**  
 oayrhu20-allele1  
 oayrhu20-allele2  
 VMDGKGGK MKASHAKH LNTSSKMS KKKKKKKK LAKNAAVA LGGKGVSS LKLT LGGG NGLSNNLP VYVCTPGYF SYVSPQVY MFGMVGVGG

**Ortho2FC**  
 oayrhu20-allele1  
 oayrhu20-allele2  
 SEVGSQYFA SPYQLGPSF GFFPMISPT TDFSSTVAE PPLLSTGTG SAVASRLNT SMKNYQMSG NTAPASQAP SGSPAVGRPQ QAYENESTNK PSNPDDAMS

**Ortho2FC**  
 oayrhu20-allele1  
 oayrhu20-allele2  
 RRDKSSLSL TVPVDASST KDGKSDGNG KEHVSQIQV TSGPMGSEG GQKATSNLT EKIMHPDQY NKVHPFVDHP DAKFFVIKSY SEDDVHK\$IK YNVWSSFTNG

**Ortho2FC**  
 oayrhu20-allele1  
 oayrhu20-allele2  
 NKRLDAAYS VOGRALGCP ILFFLSVNAS GQFCGVAEM GPVDFHKMD FWDQKQSGS FPKVHLWLD VNSTFRHIT LENNEKNPVY NSRDQELFP KSGTNMLKLF

**Ortho2FC**  
 oayrhu20-allele1  
 oayrhu20-allele2  
 KGDLPTSL DDFSFEYGR KAMLEEKRCR SGRNFVDVMY VPAFIAK\$V VAVGSEYEV KGQF\$SKDL SGDVEQNGA CEOPDKLQM KDILATEALK TGGGAFVGL

**Ortho2FC**  
 oayrhu20-allele1  
 oayrhu20-allele2  
 EHAKTNGSL DARVDHSEH CSCSNPFNG ERKPDLSLE VKLNGSORD SEADPGNLS EPNYSVKKG LPEEFCQNP SNFMKEGGAG TVEDRKSTKF VTKSGFPPSS

**Ortho2FC**  
 oayrhu20-allele1  
 oayrhu20-allele2  
 RVNKEAKNGN NEMARITTT VVKVGSVHK VNVVAGP\$SE IIGDENGLP

*OrthoFC*  
*oyrnf13o-alt1*  
*oyrnf13o-alt2*

M E M M M P E I Q V G N V V F A T A N P Y G V M T E V G S P T E W Y N Q S L G V D G D I Y F P G Q T E G T Q C M Y A A P D N G S G H P S Y S P Y P I N S F I V D G S Y L P L E Y V G D A A D D T Q C I V  
 V G N V V F A T A N P Y G V M T E V G S P T E W Y N Q S L G V D G D I Y F P G Q T E G T Q C M Y A A P D N G S G H P S Y S P Y P I N S F I V D G S Y L P L E Y V G D A A D D T Q C I V  
 M E M M M P E I Q V G N V V F A T A N P Y G V M T E V G S P T E W Y N Q S L G V D G D I Y F P G Q T E G T Q C M Y A A P D N G S G H P S Y S P Y P I N S F I V D G S Y L P L E Y V G D A A D D T Q C I V

*OrthoFC*  
*oyrnf13o-alt1*  
*oyrnf13o-alt2*

P S P Y V Y P T L P Y A H D N V L G N T T A P L H P P V Y V P L S P Y S T V S T N H A L P S V P V P A T K N D V I A N P I O S T I V T S K O F L D H A S D P K V O L R N P I L K K E L A D G S M P F V K P H T S Q

*OrthoFC*  
*oyrnf13o-alt1*  
*oyrnf13o-alt2*

A F S N F E R R S A A H S P Q E K F S V N N G S G F V G S V N V O R A A A I K F E P N L S G R I G S A P K M L S N V D G L G G A D K P C G Q S S A I L A K S Y T S R L S V G D P E G T I V I R C N Q Y S N D D

*OrthoFC*  
*oyrnf13o-alt1*  
*oyrnf13o-alt2*

L R V D Y P A K F F Y I K S I E D D V H K S K Y G V V S S S S G S K L D I A F K D A N R I A K R N S T K C P F L F F S V N G S G L F G C M A E M V G P D F H K D M D F W O D K W T G S F P Y R W I K V D V

*OrthoFC*  
*oyrnf13o-alt1*  
*oyrnf13o-alt2*

P N Y T L O H I L Q N N E N K P V T H S R D T Q E I P V Y P G I S M L K L A I K V K E C L D F M K Y E E D A R S K Y H F R R S L S H N A P D F V P V A O R R K O V D V R Q P K S G N V L I D R T P V I Q M N

*OrthoFC*  
*oyrnf13o-alt1*  
*oyrnf13o-alt2*

S V K P Q S N A I K P O D Q C L V V E K Q A S D G K E N R H O E N R N V R Q A N D K V V K T G T K O P Q A S T V K T S V D S K O Q Y W K K V E P G O N P N A V H G S S K A H E K H L N E S K A P E K H S N G A N C

*OrthoFC*  
*oyrnf13o-alt1*  
*oyrnf13o-alt2*

S S A T V S L K T A R E E T I V A R V S L A I S Y Q N S S V D V K I G S M P V L N K A N V

OrthoFSC  
orthofsc

WKEKSDPPAS AHSFLEDG F GERNNDNRN DAVGVDGDS AVGPCNSUKG GAGGCGMSIA NEDVNSLNRH GEFSSPSSNH AHSCHSDN DEUSGSGGV GURKSSSSSS

120 120 120 120 120 120 120 120 120 120

OrthoFSC  
orthofsc

PSFVSGVSGD EGVSGVSGD GEMVGVSGD GNVFVGVSGD FGVGVGVSGD FGVGVGVSGD GNVGVGVSGD ADFGVGVSGD IWSRGNNGS TAFTSSNGGS IEHHSIEDI PRANSMDET

120 120 120 120 120 120 120 120 120 120

OrthoFSC  
orthofsc

TSSSSWSDF NWGSLPRGM QYGDEIPSL SONYGARIPS LSRSGYDGE IPSLSRNCNY ASRSGYDGEI PSLSSRCHNS FSSRSGYDGE IPSLSRCHNA RSSRNYDGE

120 120 120 120 120 120 120 120 120 120

OrthoFSC  
orthofsc

IPSLNSDYQ CYQDIRPLHM RQWCHDAEAH PQLVNVRGAS HGNVRSDF VSSIATNRQF KMGTKHTVT RSDHHTIKN DVNCKHSDT LEQVRGPRM KLENATRSKI

120 120 120 120 120 120 120 120 120 120

OrthoFSC  
orthofsc

QEDIRSPLV RDOFNPDFI VEYEQAKFM IKSYSEDIH RGIKYNVRAS TPGHGNKLD AFEAQILK EGKKCKPVF FFSVNSSGQ VGLAEILGP DFKTKDMFK

120 120 120 120 120 120 120 120 120 120

OrthoFSC  
orthofsc

LDRNWFFPV THWIKDIPN RLFKHITLN NDNRIFTVSR DTQEIQLGLO LKMKIFKDY DOETSLDDF NFEYEKESAR CAKKGNAES THEARLFFG TGAHRSDDFK

120 120 120 120 120 120 120 120 120 120

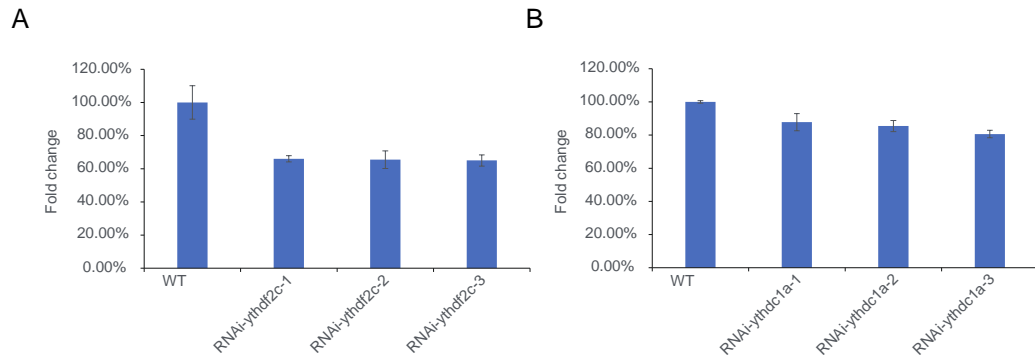
OrthoFSC  
orthofsc

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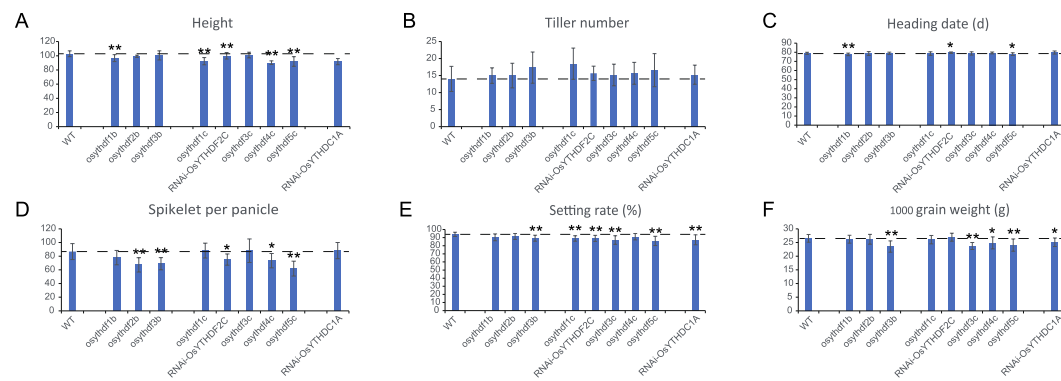
120 120

[illegible]

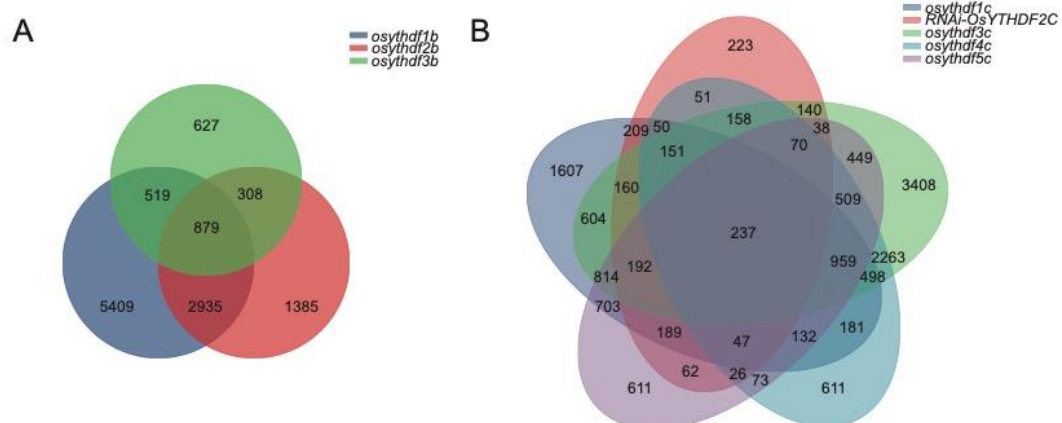
**Supplementary Figure S4** The multiple-sequence alignment of wild-type and mutation-type YTHDFC clade proteins (A-E) and OsYTHDC1A (F) used in this study. CRISPR-Cas9 system induced mutations lead to a serial of amino acid changes and early termination. Red box above the sequences shows the YTH domain.



**Supplementary Figure S5 (A-B)** Expression fold change of OsYTHDF2C (A) and OsYTHDC1A (B) mRNA in knockdown plants. The values obtained with wild type plants were referred to as 1 and shown as mean  $\pm$  SD.

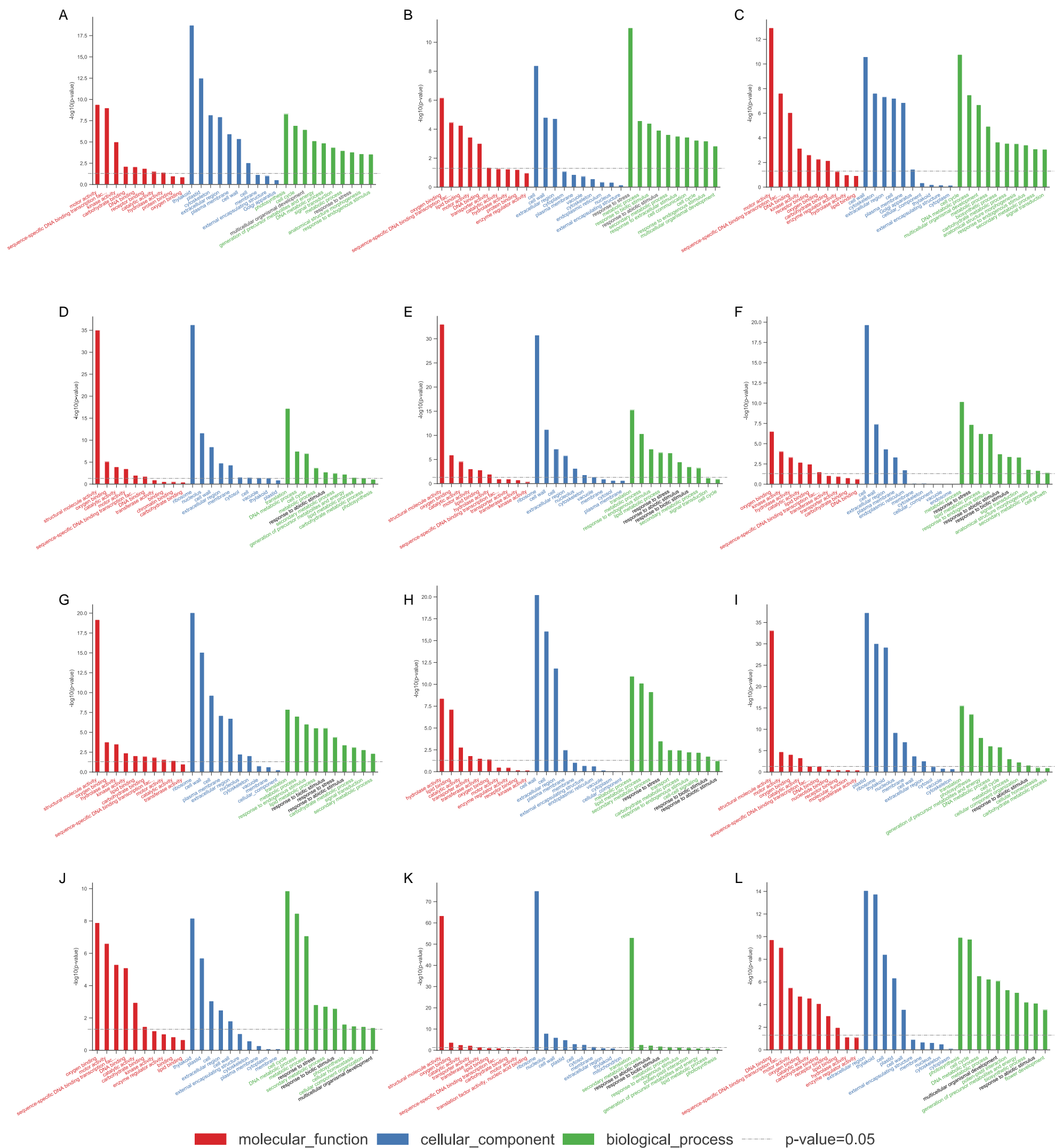


**Supplementary Figure S6** Quantification of several agricultural traits of mutants/RNAi plants except DFA clade mutants, including plant height (A), tiller number (B), heading date (C), spikelets number per panicle (D), setting rate (E), and 1000 grain weight (F). Values are shown as mean  $\pm$  SD. The significance of difference was determined by Student's-test (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ).

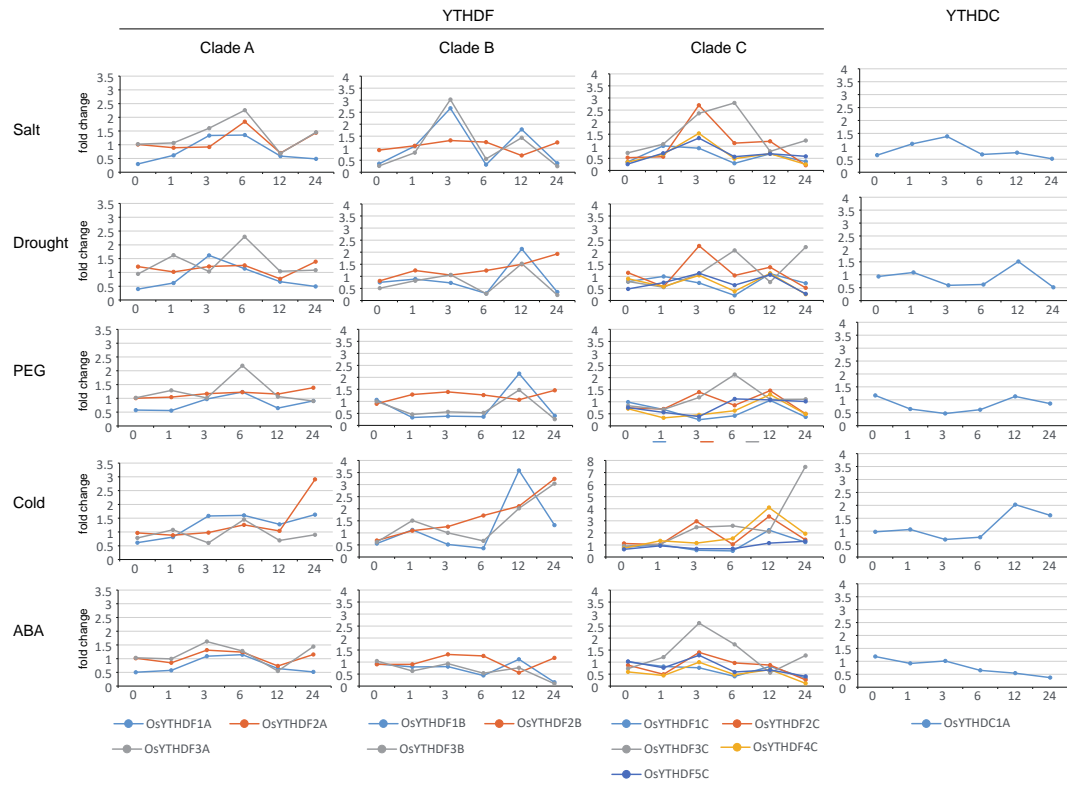


**Supplementary Figure S7** The Venn diagrams show unique and shared DEGs in YTHDFB (A) and YTHDFC (B) mutants.

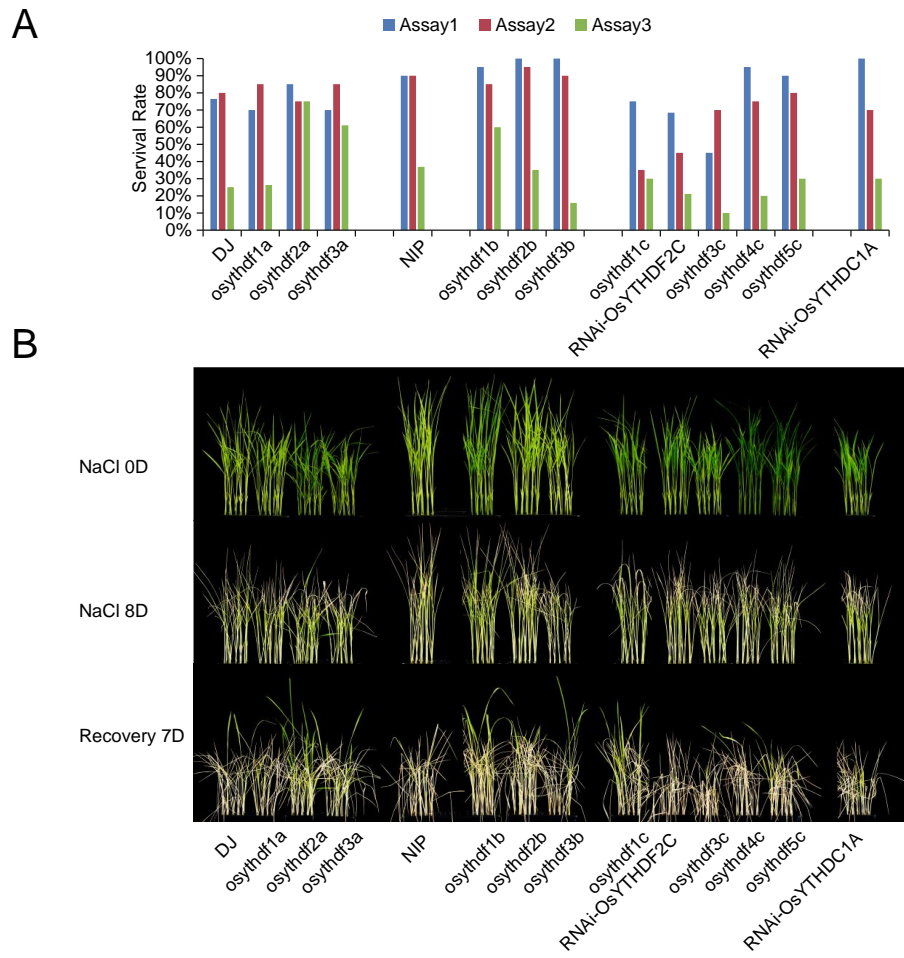




**Supplementary Figure S8** GO enrichment analysis of YTH mutants/knockdown plants. A-L represent for the GO enrichment results for genes differentially expressed in osythdf1a (A), osythdf2a (B), osythdf3a (C), osythdf1b (D), osythdf2b (E), osythdf3b (F), osythdf1c (G), RNAi-OsYTHDF2C (H), osythdf3c (I), osythdf4c (J), osythdf5c (K), and RNAi-OsYTHDC1A (L) compared with wild type. “Multicellular organismal development”, “Response to stress”, “Response to abiotic stimulus”, and “Response to biotic stimulus” were marked in black.



**Supplementary Figure S9** Expression patterns of OsYTH genes under different abiotic stresses (salt, drought, PEG, cold) and exogenous ABA application. Expression data are means of three independent biological replicate and normalized with the relative expression levels at the same treatment time under mock treatment.



**Supplementary Figure S10** OsYTH genes contribute to salt stress response progress. (A) percentage survival rates (Percentage is the ratio of number of plants with green new leaves to number of total plants) of WT/mutant seedlings after 150mM salt stress treatment in three assays. About 20 seedlings were used in each assay. (B) phenotypes of WT/mutant seedlings under salt stress for 8 days and recovery for 7 days (assay 3).