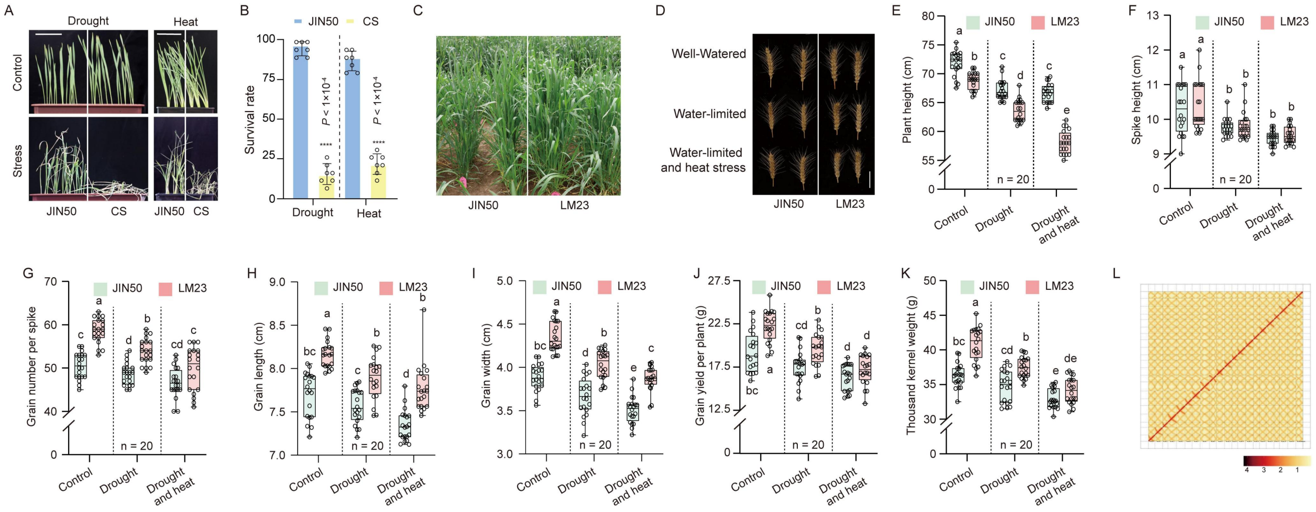
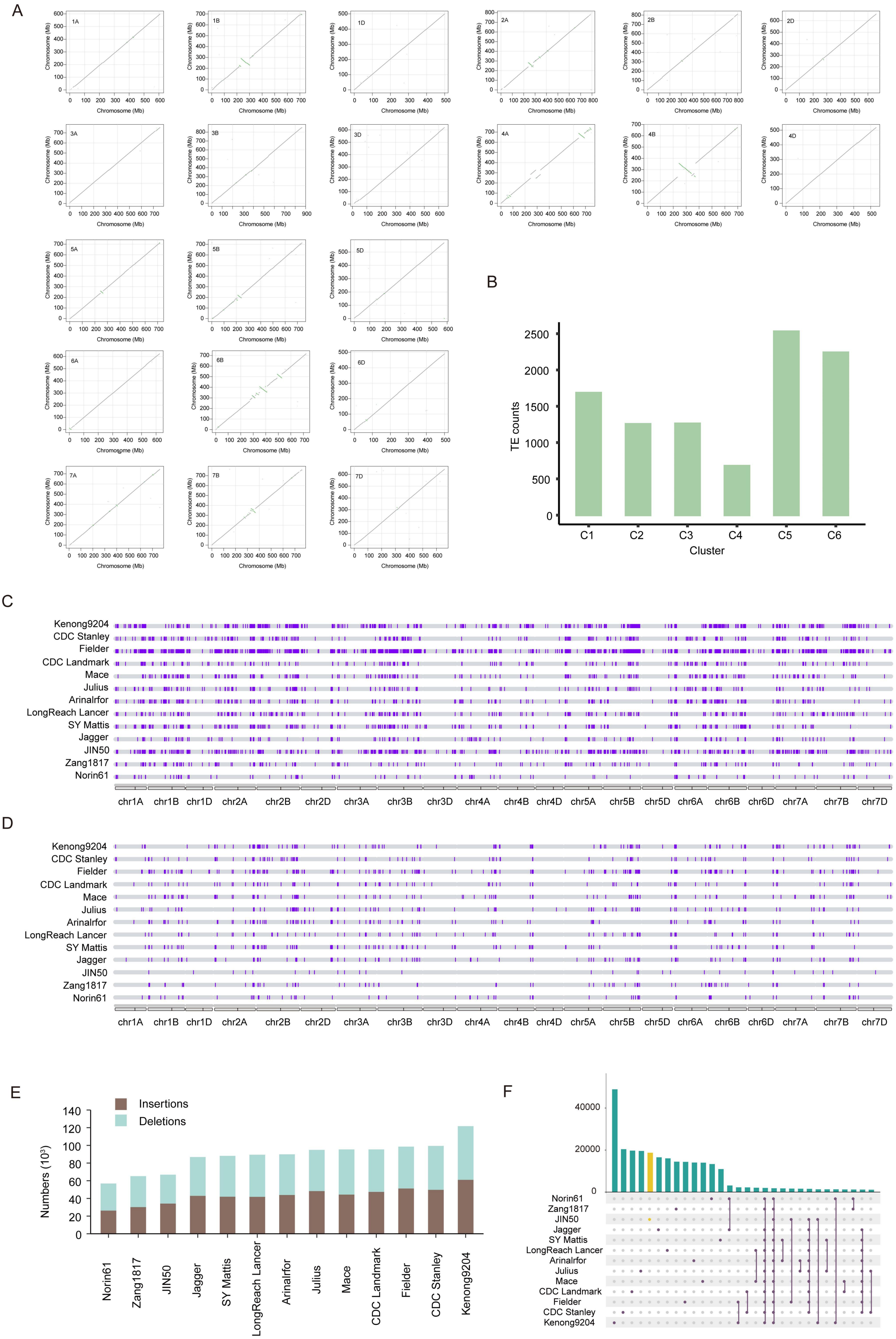
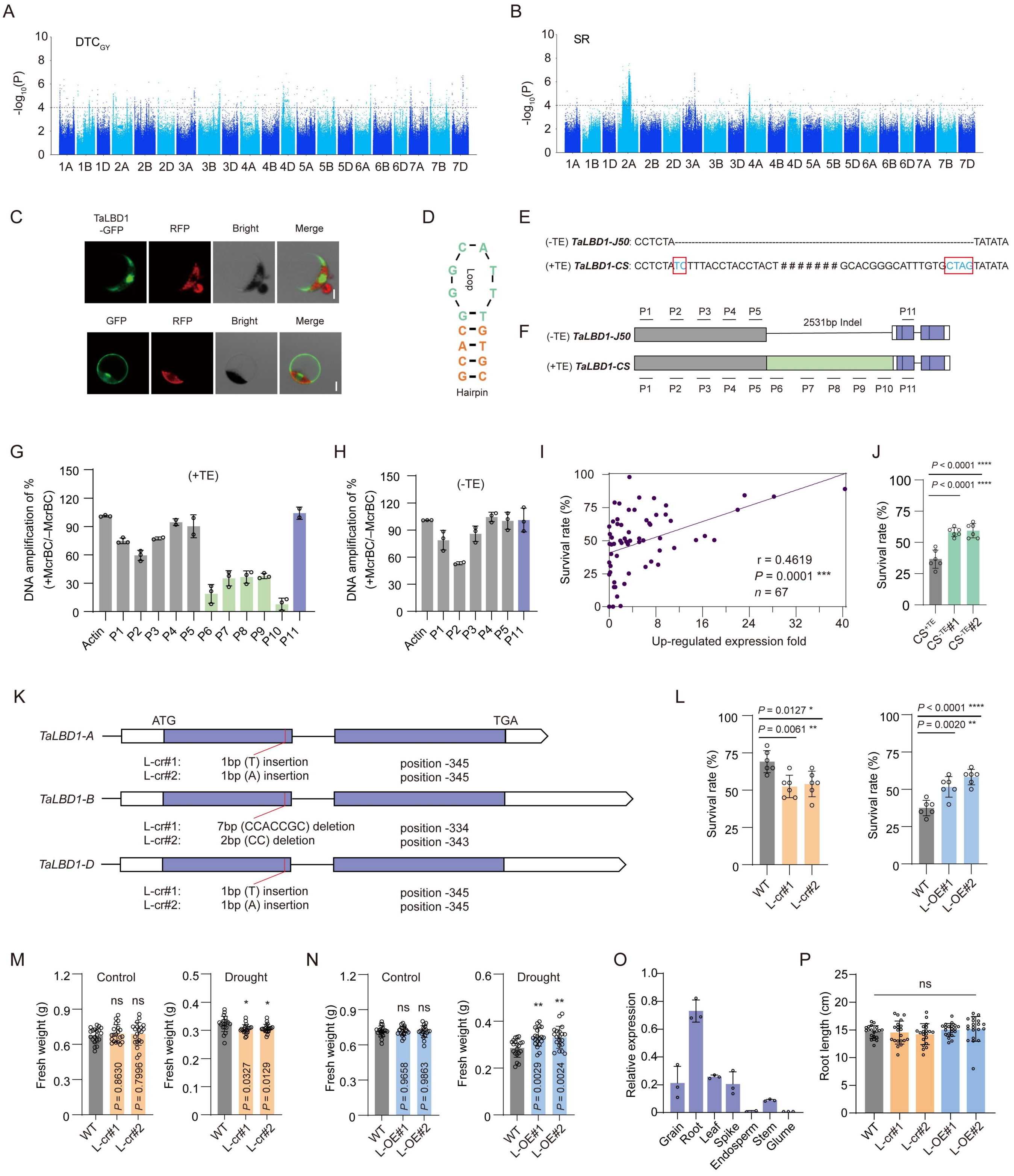


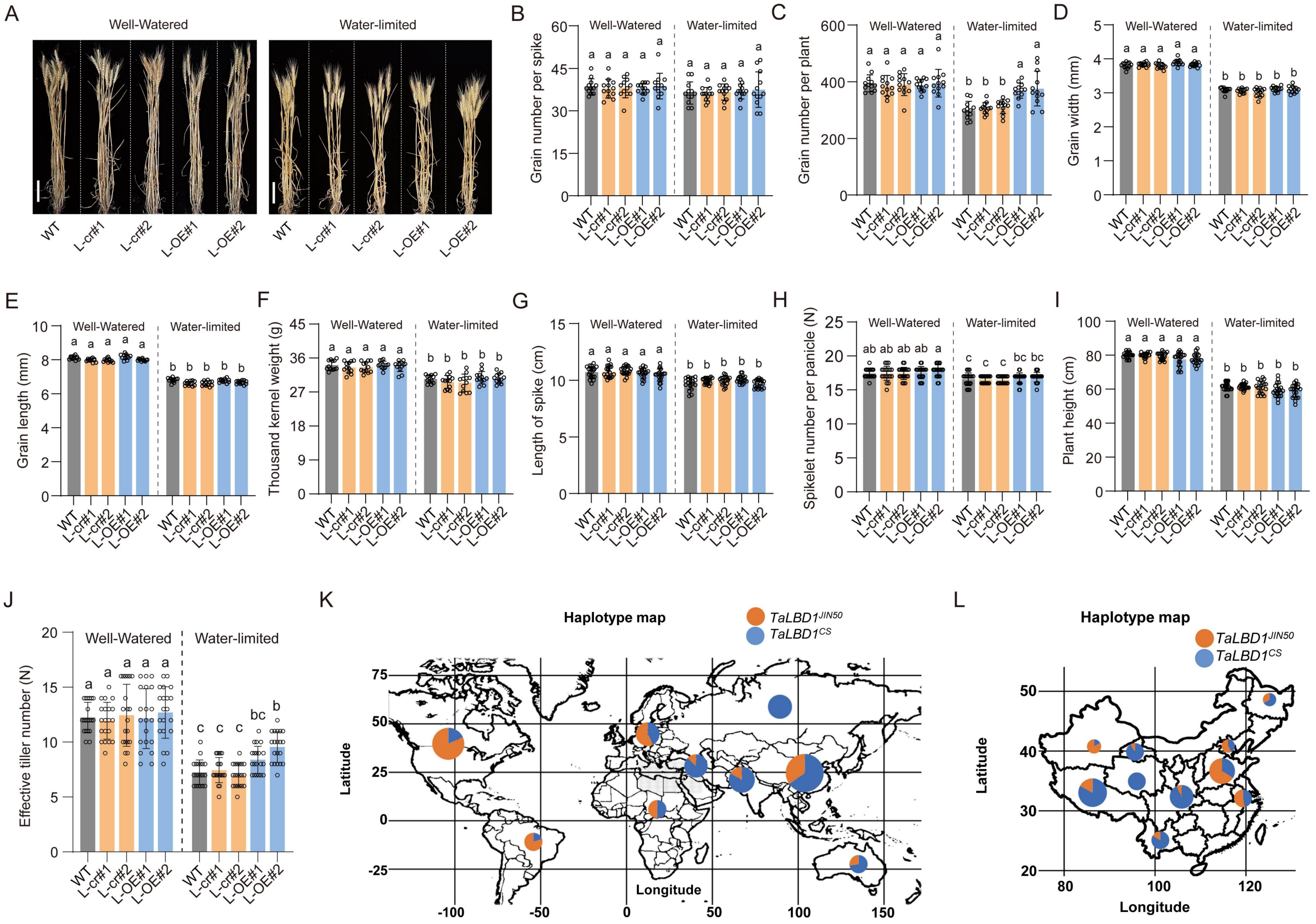
Extended Data Fig. 1 The phenotypes under stress and genome assembly of JIN50. A, Drought or heat stress tolerance assay in wheat seedlings of JIN50 and CS. Bar, 5 cm. **B**, Statistical analysis of the survival rate of JIN50 and CS under drought or heat conditions. **C**, Phenotypic analysis of JIN50 and LM23 in the field under normal conditions. **D**, Phenotypic analysis of JIN50 and LM23 in spikes under various conditions. Bar, 5 cm. **E-K**, Statistical analysis of plant height (E), spike height (F), grain number per spike (G), grain length (H), grain width (I), grain yield per plant (J), and Thousand kernel weight (K) for JIN50 and LM23. **L**, Chromosomal conformational capture (Hi-C) interaction matrix for the 21 chromosomes of JIN50. The data are means ± SEs. **** indicates significant differences at *P* < 0.0001 probability. Means with the same letter did not significantly differ at P < 0.05, according to Tukey's test.


A
1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
Chromosome (Mb)
Chromosome (Mb)
B
TE counts
2500
2000
1500
1000
500
0
C1
C2
C3
C4
C5
C6
Cluster
C
Kenong9204
CDC Stanley
Fielder
CDC Landmark
Mace
Julius
Arinalrfor
LongReach Lancer
SY Mattis
Jagger
JIN50
Zang1817
Norin61
chr1A
chr1B
chr1D
chr2A
chr2B
chr2D
chr3A
chr3B
chr3D
chr4A
chr4B
chr4D
chr5A
chr5B
chr5D
chr6A
chr6B
chr6D
chr7A
chr7B
chr7D
D
Kenong9204
CDC Stanley
Fielder
CDC Landmark
Mace
Julius
Arinalrfor
LongReach Lancer
SY Mattis
Jagger
JIN50
Zang1817
Norin61
chr1A
chr1B
chr1D
chr2A
chr2B
chr2D
chr3A
chr3B
chr3D
chr4A
chr4B
chr4D
chr5A
chr5B
chr5D
chr6A
chr6B
chr6D
chr7A
chr7B
chr7D
E
Insertions
Deletions
Numbers (10³)
140
120
100
80
60
40
20
0
Norin61
Zang1817
JIN50
Jagger
SY Mattis
LongReach Lancer
Arinalrfor
Julius
Mace
CDC Landmark
Fielder
CDC Stanley
Kenong9204
F
40000
20000
0
Norin61
Zang1817
JIN50
Jagger
SY Mattis
LongReach Lancer
Arinalrfor
Julius
Mace
CDC Landmark
Fielder
CDC Stanley
Kenong9204

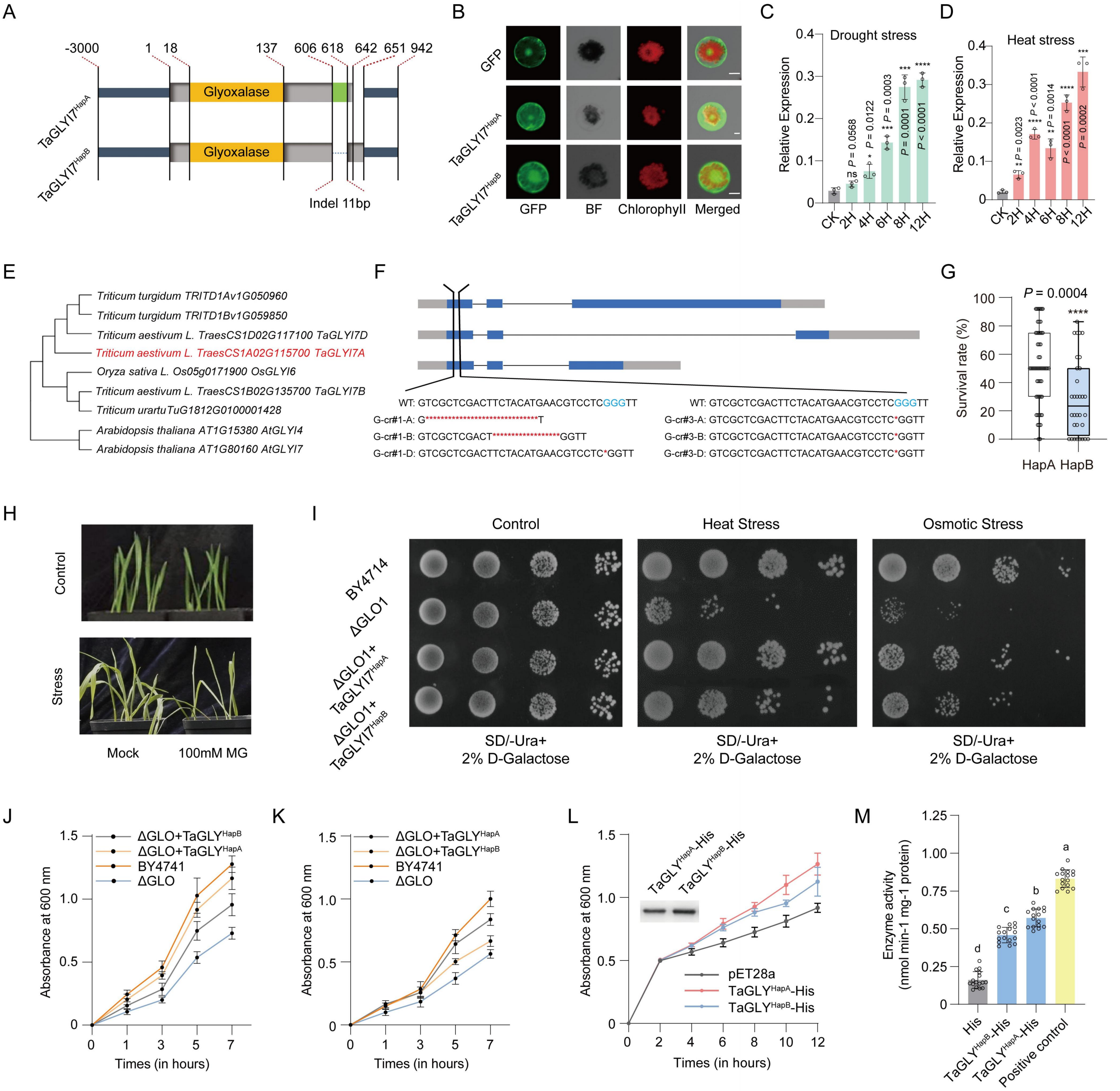
Extended Data Fig. 2 A, The SVs in JIN50, relative to CS genome. The x axis in each graph represents the JIN50 and the y axis in each graph represents the CS. **B**, The number of TEs in different cluster of genes associated with differential DNA methylation. **C**, The numbers of insertions and deletions in pan-genomes. **D**, The correlation analysis of all SVs between pan-genomes. **E**, The visualized analysis of SVs (>100kb) of insertions in pan-genomes. **F**, The visualized analysis of SVs (>100kb) of deletions in pan-genomes.


A
DTC$_{GY}$
-log$_{10}$(P)
1A 1B 1D 2A 2B 2D 3A 3B 3D 4A 4B 4D 5A 5B 5D 6A 6B 6D 7A 7B 7D
B
SR
C
TaLBD1-GFP
RFP
Bright
Merge
GFP
D
Loop
Hairpin
E
(-TE) TaLBD1-J50: CCTCTA------TATATA
(+TE) TaLBD1-CS: CCTCTATCTTTACCTACCTACT ####### GCACGGGCATTTGTGCTAGTATATA
F
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11
2531bp Indel
(-TE) TaLBD1-J50
(+TE) TaLBD1-CS
G
(+TE)
DNA amplification of % (+McrBC/−McrBC)
Actin
H
(-TE)
I
Survival rate (%)
Up-regulated expression fold
r = 0.4619
P = 0.0001 ***
n = 67
J
P < 0.0001 ****
CS^{+TE}
CS^{-TE}#1
CS^{-TE}#2
K
TaLBD1-A
ATG
TGA
L-cr#1: 1bp (T) insertion position -345
L-cr#2: 1bp (A) insertion position -345
TaLBD1-B
L-cr#1: 7bp (CCACCGC) deletion position -334
L-cr#2: 2bp (CC) deletion position -343
TaLBD1-D
L-cr#1: 1bp (T) insertion position -345
L-cr#2: 1bp (A) insertion position -345
L
P = 0.0127 *
P = 0.0061 **
P < 0.0001 ****
P = 0.0020 **
WT
L-cr#1
L-cr#2
L-OE#1
L-OE#2
M
Control
Drought
Fresh weight (g)
ns
P = 0.8630
P = 0.7996
P = 0.0327
P = 0.0129
N
P = 0.9658
P = 0.9863
P = 0.0029
P = 0.0024
O
Relative expression
Grain
Root
Leaf
Spike
Endosperm
Stem
Glume
P
Root length (cm)
ns

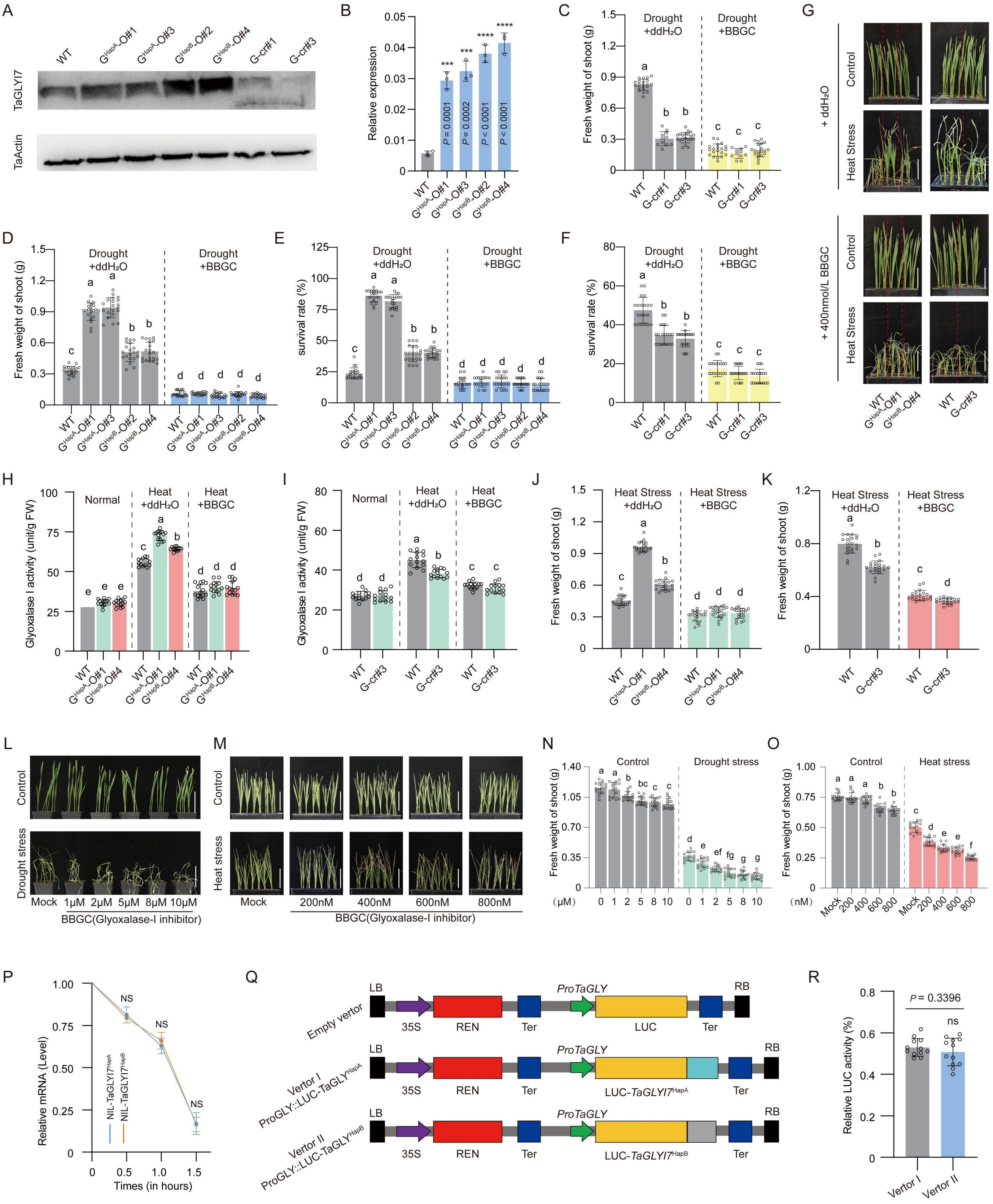
Extended Data Fig. 3 TaLBD1 regulates drought resistance of wheat. A, The GWAS analysis of Indels for drought resistance in wheat reproductive stage (DTC_{GY}). **B**, The GWAS analysis of InDels for drought resistance in wheat seedlings (SRs). **C**, Subcellular localization of TaLBD1. Wheat protoplasts and the leaves of *N. benthamiana* plants were transformed with either *35S:GFP* or *35S:TaLBD1-GFP* to observe a green fluorescent signal. Bars, 10 μm. **D and E**, The DNA sequence and structure (D) of the 2531-bp Helitron transposon inserted in the *TaLBD1*^{CS} promoter. The elements are characterized by 5′-TC and CTRR-3′ termini are indicated by the orange boxes and have a short palindromic motif located upstream of the 3′-terminus, with insertion occurring precisely between the 5′-A and T-3′ nucleotides at the host AT target sites (E). **F-H**, DNA methylation status was determined by treatment with McrBC, a methylation-sensitive restriction enzyme, followed by qPCR (McrBC-qPCR) analysis in the eleven regions of the genome sequence (P1-P11) of the *TaLBD1*. Genomic DNA was extracted from JIN50 and CS genotypes grown under well-watered conditions. **I**, Correlation analysis of the upregulated expression of *TaLBD1* and survival rates in different wheat varieties. **J**, Statistical analysis of the survival rates in NILs after drought treatment. **K**, CRISPR/Cas9-mediated mutations in there homoeologs of *TaLBD1*. The positions of target sites for CRISPR/Cas9 gene editing are indicated by red lines. **L**, Statistical analysis of the survival rates in KO, OE, and wild-type plants after drought treatment. **M** and **N**, Statistical analysis of the fresh weight in KO, OE and wild-type plants under control and drought treatment. **O**, The tissue expression pattern of *TaLBD1*. **P**, The root length between transgenic lines of *TaLBD1* and wild-type Fielder at seedling stage. Bar, 2 cm. The data are means ± SEs. *, **, ***, and **** indicate significant differences at $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$ probability.



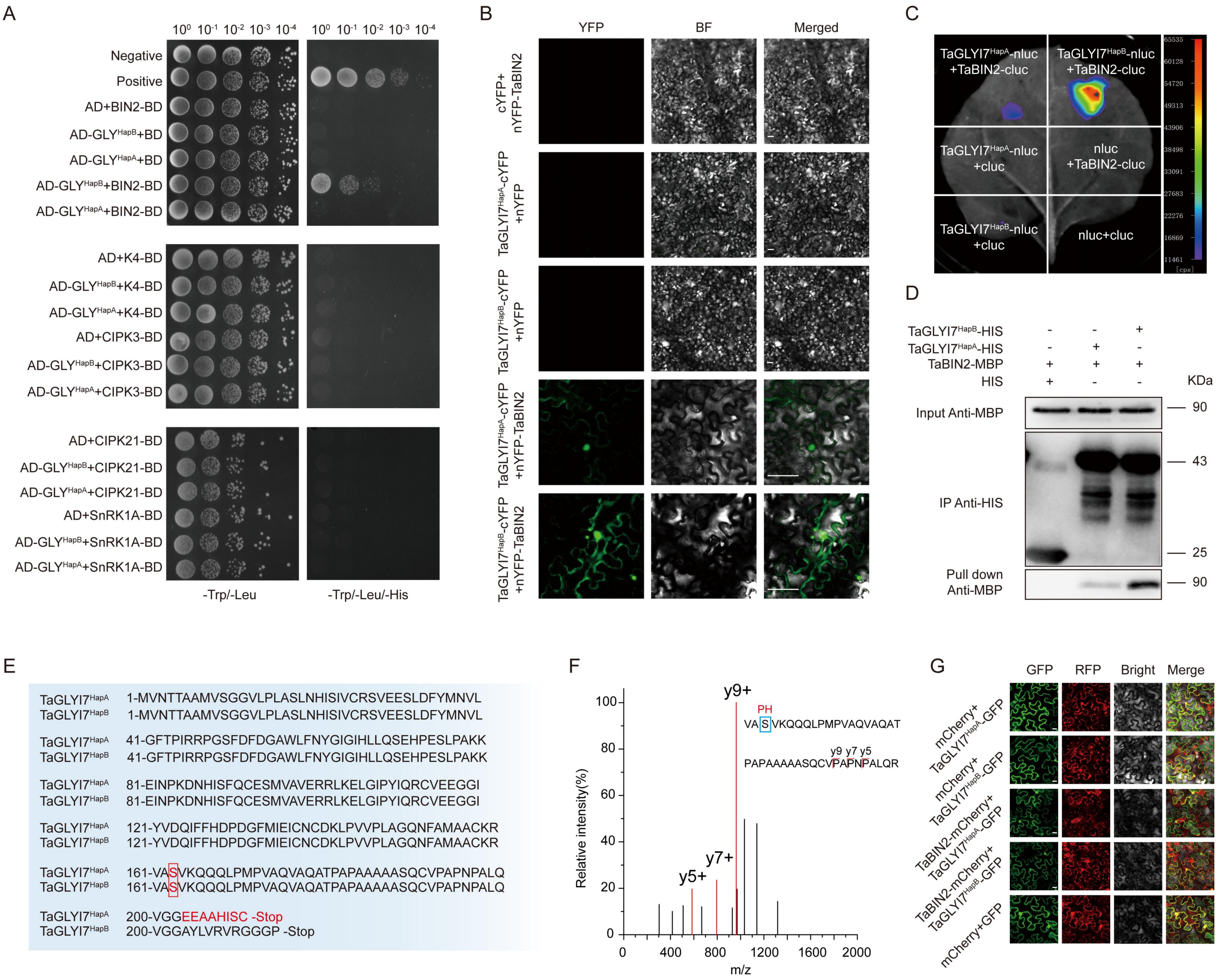
Extended Data Fig. 4 ***TaLBD1* enhances grain yields under the water-withheld condition. A**, Phenotypic analysis of *TaLBD1* OE, KO, and wild-type in plant height under the well-watered and water-limited conditions. Bar, 15 cm. **B-J**, Statistical analysis of the grain number per spike (B), grain number per plant (C), grain width (D), grain length (E), thousand kernel weight (F), length of spike (G), spikelet number per panicle (H), plant height (I), and effective tiller number (J) of different *TaLBD1* plants under diverse field conditions. **K** and **L**, Global (J) and China (K) distribution of two haplotypes of *TaLBD1*. Means with the same letter did not significantly differ at $P < 0.05$ according to Tukey's test.



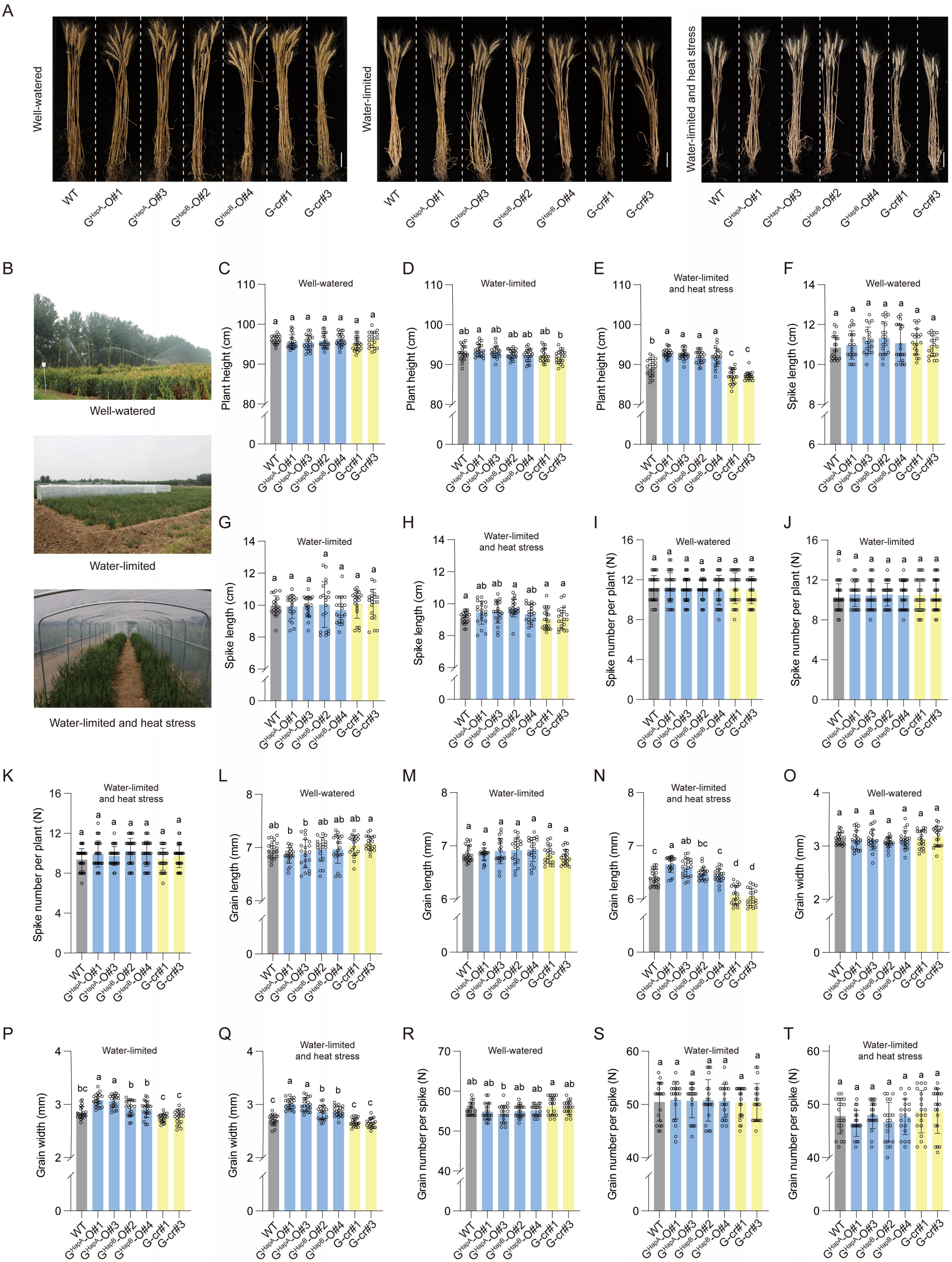
Extended Data Fig. 5 *TaGLYI7*, especially HapA, improves the drought and heat resistance of wheat by modulating the Glyoxalase activity. A, Schematic diagram of the *TaGLYI7*$^{\mathrm{HapA}}$ and *TaGLYI7*$^{\mathrm{HapB}}$, the region of green means the 11 bp indel. **B**, The subcellular localization assays of TaGLYI7$^{\mathrm{HapA}}$ and TaGLYI7$^{\mathrm{HapB}}$ in the wheat protoplast cells (Bar, 10 μm). BF, bright field. **C** and **D**, The expression pattern of *TaGLYI7* under drought stress (C) or heat stress conditions (D) in seedlings of wheat (n = 3). *TaACTIN* was used as the internal control. **E**, The phylogenetic tree of TaGLYI7. **F**, The gene structure and mutation sites of *TaGLYI7*. The symbol * indicates the nucleotide deletion. **G**, Heat tolerance distribution between *TaGLYI7*$^{\mathrm{HapA}}$ (n = 54 accessions) and *TaGLYI7*$^{\mathrm{HapB}}$ (n = 32 accessions). The bars within box plots represent the 25th percentiles, medians, and 75th percentiles. **H**, Phenotypic analysis of wild-type plants treated with 100 mM MG under normal conditions and stresses conditions. **I**, Complementation of a yeast *ΔGLO1* mutant with *TaGLYI7*. The yeast strain *ΔGLO1* was transformed with either the empty vector or with *TaGLYI7*$^{\mathrm{HapA}}$ and *TaGLYI7*$^{\mathrm{HapB}}$, respectively. Positive transformants and WT (BY4714) were grown on the SD/-Ura with 2% D-Galactose medium and treated with heat or osmotic stress. **J** and **K**, The growth rate of different yeast genotypes in heat conditions (J) and osmotic stress conditions (K) are indicated by the value of OD_{600} from (I). **L**, The growth rate of different *E.coli* types in heat conditions is indicated by the value of OD_{600}. **M**, The enzyme activity of different *E.coli* types in heat conditions. The data are means ± SEs. *, **, ***, and **** indicate significant differences at $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$ probability. Means with the same letter did not significantly differ at $P < 0.05$, according to Tukey's test.


A
WT
G^HapA-O#1
G^HapA-O#3
G^HapB-O#2
G^HapB-O#4
G-cr#1
G-cr#3
TaGLYI7
TaActin
B
Relative expression
0.05
0.04
0.03
0.02
0.01
0
***
***
****
****
P = 0.0001
P = 0.0002
P < 0.0001
P < 0.0001
C
Fresh weight of shoot (g)
Drought +ddH₂O
Drought +BBGC
G
+ ddH₂O
+ 400nmol/L BBGC
Control
Heat Stress
D
Fresh weight of shoot (g)
E
survival rate (%)
F
survival rate (%)
H
Glyoxalase I activity (unit/g FW)
Normal
Heat +ddH₂O
Heat +BBGC
I
Glyoxalase I activity (unit/g FW)
J
Fresh weight of shoot (g)
Heat Stress +ddH₂O
Heat Stress +BBGC
K
Fresh weight of shoot (g)
L
Control
Drought stress
Mock 1μM 2μM 5μM 8μM 10μM
BBGC(Glyoxalase-I inhibitor)
M
Control
Heat stress
Mock 200nM 400nM 600nM 800nM
BBGC(Glyoxalase-I inhibitor)
N
Fresh weight of shoot (g)
Control
Drought stress
(μM) 0 1 2 5 8 10 0 1 2 5 8 10
O
Fresh weight of shoot (g)
Control
Heat stress
(nM) Mock 200 400 600 800 Mock 200 400 600 800
P
Relative mRNA (Level)
Times (in hours)
NS
NIL-TaGLYI7^HapA
NIL-TaGLYI7^HapB
Q
Empty vertor
Vertor I ProGLY::LUC-TaGLYI^HapA
Vertor II ProGLY::LUC-TaGLYI^HapB
LB
RB
35S
REN
Ter
ProTaGLY
LUC
LUC-TaGLYI7^HapA
LUC-TaGLYI7^HapB
R
Relative LUC activity (%)
P = 0.3396
ns
Vertor I
Vertor II

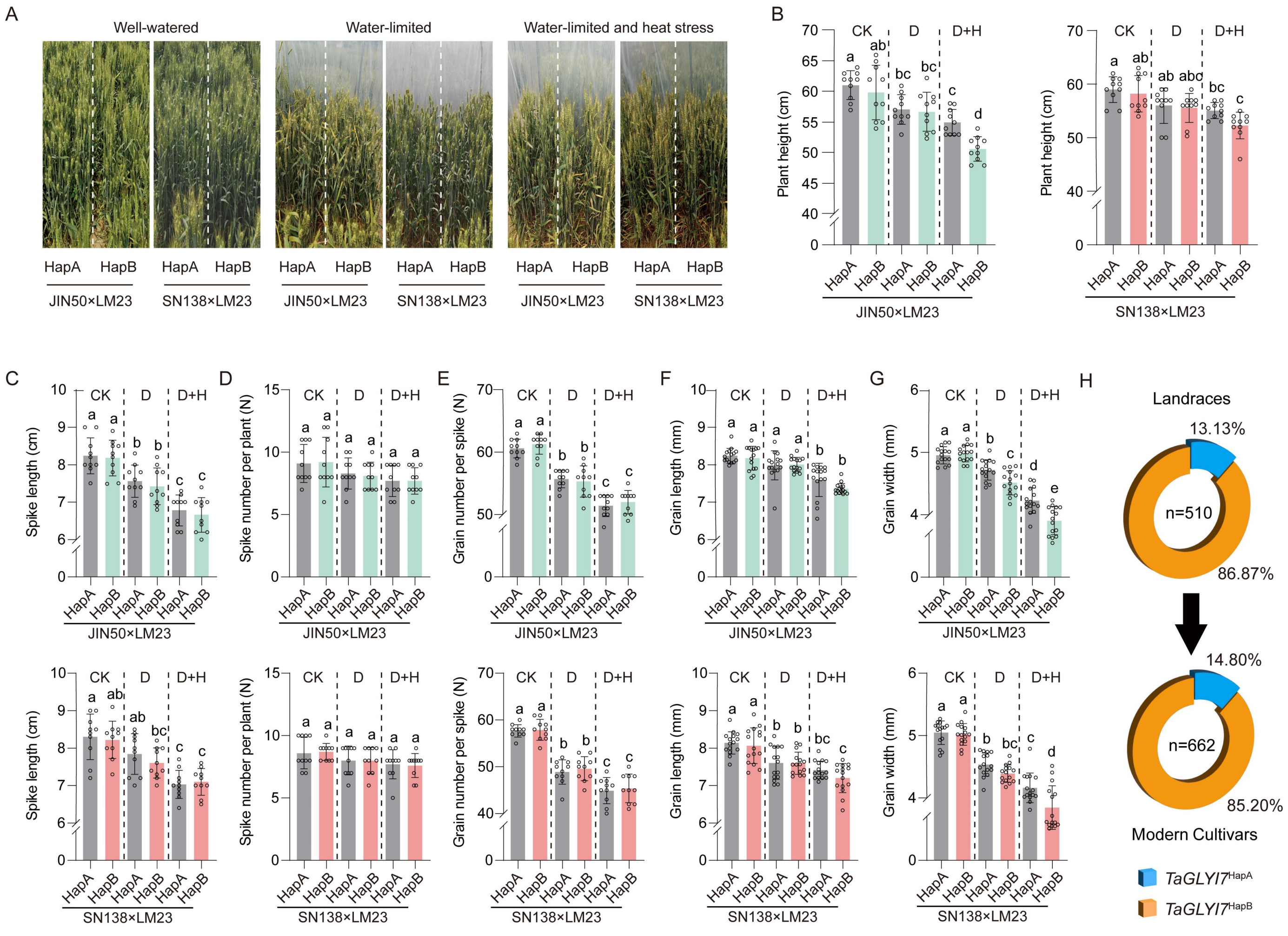
Extended Data Fig. 6 *TaGLYI7*$^{\text{HapA}}$ improves the drought and heat resistance of wheat by modulating the Glyoxalase activity. A, Relative protein levels of TaGLYI7 in transgenic over-expression and knock-out plants were shown. The protein levels of TaACTIN were used to normalize protein levels. **B**, Relative transcription levels of *TaGLYI7* in transgenic over-expression plants were shown. The expression of *TaACTIN* was used to normalize mRNA levels. **C** and **D**, Statistical analysis of the fresh weight of different *TaGLYI7* plants treated with BBGC under normal or drought conditions. **E** and **F**, Statistical analysis of the survival rate of different *TaGLYI7* plants treated with BBGC under normal or drought conditions. **G**, Phenotypic analysis of different *TaGLYI7* plants treated with BBGC under normal or heat stress conditions. Bar, 4 cm. **H** and **I**, Statistical analysis of the Glyoxalase I activity of different *TaGLYI7* plants treated with BBGC under normal or heat stress conditions. **J** and **K**, Statistical analysis of the fresh weight of different *TaGLYI7* plants treated with BBGC under normal or heat stress conditions. **L**, Phenotypic analysis of wild-type plants treated with different concentrations BBGC under normal and drought conditions. Bar, 5 cm. **M**, Phenotypic analysis of wild-type plants treated with different concentrations BBGC under normal and heat conditions. Bar, 5 cm. **N** and **O**, Statistical analysis of fresh weight of seedlings from (L) and (M), respectively. **P**, The mRNA degradation assay in NIL plants. **Q**, Schematic diagram of the transient expression vector with the promoter of *TaGLYI7* and the coding sequences of *TaGLYI7*$^{\text{HapA}}$ and *TaGLYI7*$^{\text{HapB}}$. **R**, Transient expression assays of different vectors from (Q). The data are means ± SEs. *** and **** indicate significant differences at $P < 0.001$ and $P < 0.0001$ probability. Means with the same letter did not significantly differ at $P < 0.05$, according to Tukey's test.



Extended Data Fig. 7 The stronger interaction between TaGLYI7^{HapB} and TaBIN2 leads to stronger phosphorylation and accelerated degradation of TaGLYI7^{HapB}. A-D, Y2H assays (A), split firefly luciferase complementation assays (B), bimolecular fluorescence complementation assays (C), and pull-down assays (D) show that *TaGLYI7*^{HapB} interacts stronger with TaBIN2 than *TaGLYI7*^{HapA}. **E**, The protein sequences of TaGLYI7^{HapA} and TaGLYI7^{HapB}. The red frame means the phosphorylation site. **F**, The predicted of the phosphorylation site in TaGLYI7. **G**, The co-localization of TaGLYI7^{HapA} and TaGLYI7^{HapB} with TaBIN2 in *N.benthamiana.*


A
Well-watered
Water-limited
Water-limited and heat stress
WT
GHapA_O#1
GHapA_O#3
GHapB_O#2
GHapB_O#4
G-cr#1
G-cr#3
B
Well-watered
Water-limited
Water-limited and heat stress
C
Well-watered
Plant height (cm)
a a a a a a
D
Water-limited
Plant height (cm)
ab a ab ab ab ab b
E
Water-limited and heat stress
Plant height (cm)
b a a a a c c
F
Well-watered
Spike length (cm)
G
Water-limited
Spike length (cm)
H
Water-limited and heat stress
Spike length (cm)
a ab ab a ab a a
I
Well-watered
Spike number per plant (N)
J
Water-limited
Spike number per plant (N)
K
Water-limited and heat stress
Spike number per plant (N)
L
Well-watered
Grain length (mm)
ab b b ab ab ab a
M
Water-limited
Grain length (mm)
N
Water-limited and heat stress
Grain length (mm)
c a ab bc c d d
O
Well-watered
Grain width (mm)
P
Water-limited
Grain width (mm)
bc a a b b c c
Q
Water-limited and heat stress
Grain width (mm)
c a a b b c c
R
Well-watered
Grain number per spike (N)
ab ab b ab ab a ab
S
Water-limited
Grain number per spike (N)
T
Water-limited and heat stress
Grain number per spike (N)

Extended Data Fig. 8 Phenotypic analysis and data statistics of *TaGLYI7* transgenic plants. A, Phenotypic analysis of *TaGLYI7* OE, KO, and wild-type in plant height. Bar, 10 cm. **B**, Images of different transgenic plants under well-watered, drought, and combined drought and heat conditions. **C**-**T**, Statistical analysis of the agronomic traits of different *TaGLYI7* plants under diverse field conditions. plant height (C-E), spike length (F-H), spike number per plant (I-K), grain length (L-N), grain width (O-Q), and grain number per spike (R-T). The data are means ± SEs. Means with the same letter did not significantly differ at $P < 0.05$, according to Tukey's test.



Extended Data Fig. 9 Phenotypic analysis and data statistics of *TaGLYI7* NIL plants. A, Phenotypic analysis of different NIL plants in the field under normal conditions, drought, and combined drought and heat stress conditions. **B-G**, Statistical analysis of the agronomic traits of different NILs under diverse field conditions. Plant height (B), spike length (C), spike number per plant (D), grain number per spike (E), grain length (F), and grain width (G). **H**, Distributions of the *TaGLYI7*$^{\text{hapA}}$ and *TaGLYI7*$^{\text{hapB}}$ alleles in landraces and cultivars. The data are means ± SEs. Means with the same letter did not significantly differ at $P < 0.05$, according to Tukey's test.