The TaBBM-TaWRKY41-TacAGPS1a module affects starch synthesis and grain quality by regulating embryo and endosperm development in wheat

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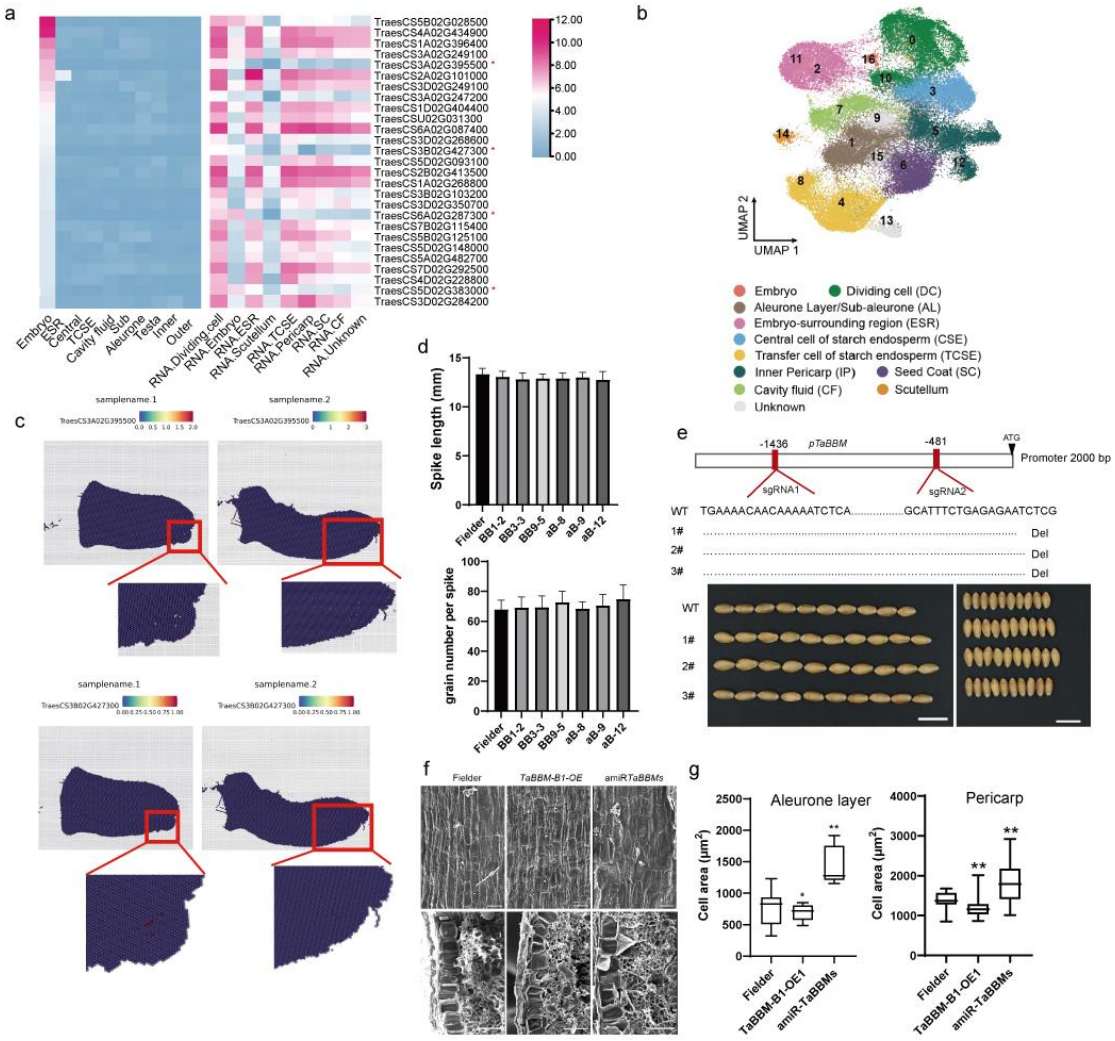


Supplementary Fig. 1 Identification of embryo and ESR expressed TF in wheat.

(a) The expression heatmap of ST and SN transcriptomics genes during grain development in wheat. The color of heatmap represents the normalized TPM value of genes expression. *Possible candidate genes.

(b) Visualization of grain cell types (states) via UMAP. The dots indicate individual cells, while the colours represent the respective cell types.

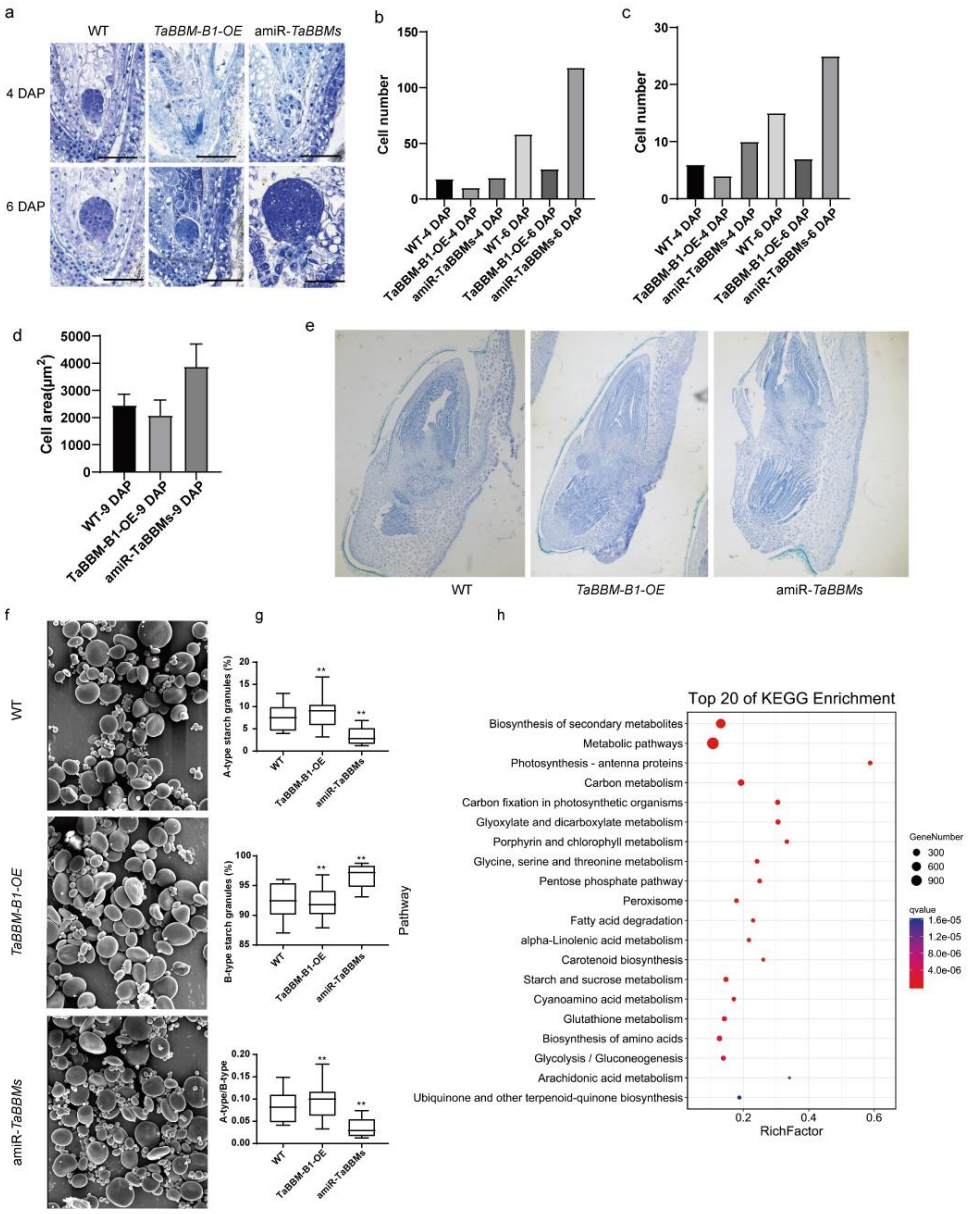
(c) Representatives of spatial visualization using *TaBBM-A1* and *TaBBM-B1*.

(d) Spile length and grain nμmber per spike between Fielder, *TaBBM-B1*-OE and amiR-TaBBMs plants. Values are means ± SD from six independent biological replicates, and *P*-values were determined using two-tailed Student's *t*-test. **P < 0.01.

(e) CRISPR/Cas9 target sites in TaBBM-B1 and sequencing confirmation of mutant. The symbols "-" indicate the nucleotide deletion, base nμmbers of deletion are shown behind. Representative seed morphology images of *tabbm*-KO and WT(Fielder). Bars = 1cm.

(f) The histological section of aleurone layer and pericarp. Scale bar = 50 μm.

(g) Statistical results of aleurone layer and pericarp cells area. For each sample, at least 15 cells were analyzed. *: *P*<0.05, **: *P*<0.01.



Supplementary Fig. 2 Phenotypic analysis of Fielder, *TaBBM-B1*-OE and amiR-*TaBBMs* embryo.

(a) Light microscopy observations of Fielder, TaBBM-B1-OE and amiR-*TaBBMs* embryo during grain development. Scale bar = 100 μm.

(b) Cell nμmber of Fielder, *TaBBM-B1*-OE and amiR-*TaBBMs* early embryo.

(c) Dividing cell nμmber of Fielder, *TaBBM-B1*-OE and amiR-TaBBMs early embryo.

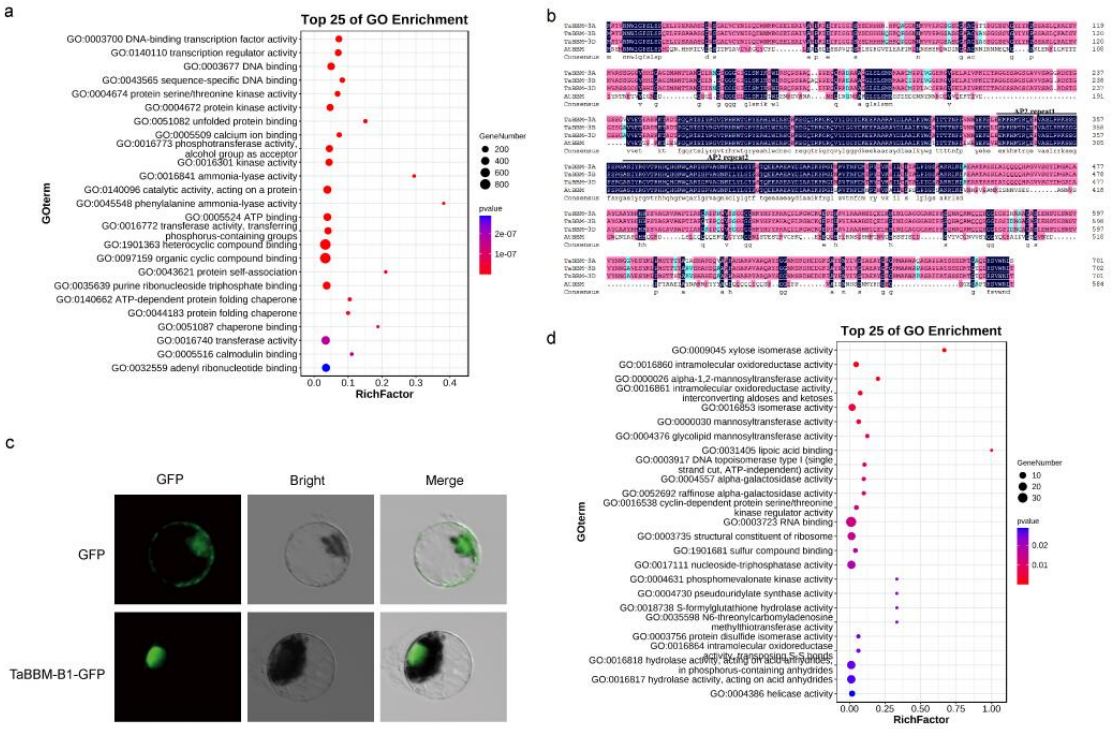
(d) Cell area of Fielder, *TaBBM-B1*-OE and amiR-*TaBBMs* 9 DAP embryo.

(e) Light microscopy observations of Fielder, *TaBBM-B1*-OE and amiR-*TaBBMs* mature embryo. Scale bar = 100 μm.

(f) Scanning electron micrographs of purified endosperm starch. Bar = 10 μm.

(g) A-type and B-type granule volμme (% of total starch), plots show the mean from the analysis of n = 5 to 3 replicate starch extractions, each from grains from a separate plant.

(h) KEGG enrichment analysis of common DEGs in amiR-*TaBBMs*. FDR, false discovery rate; DEGs, differential expressed genes.



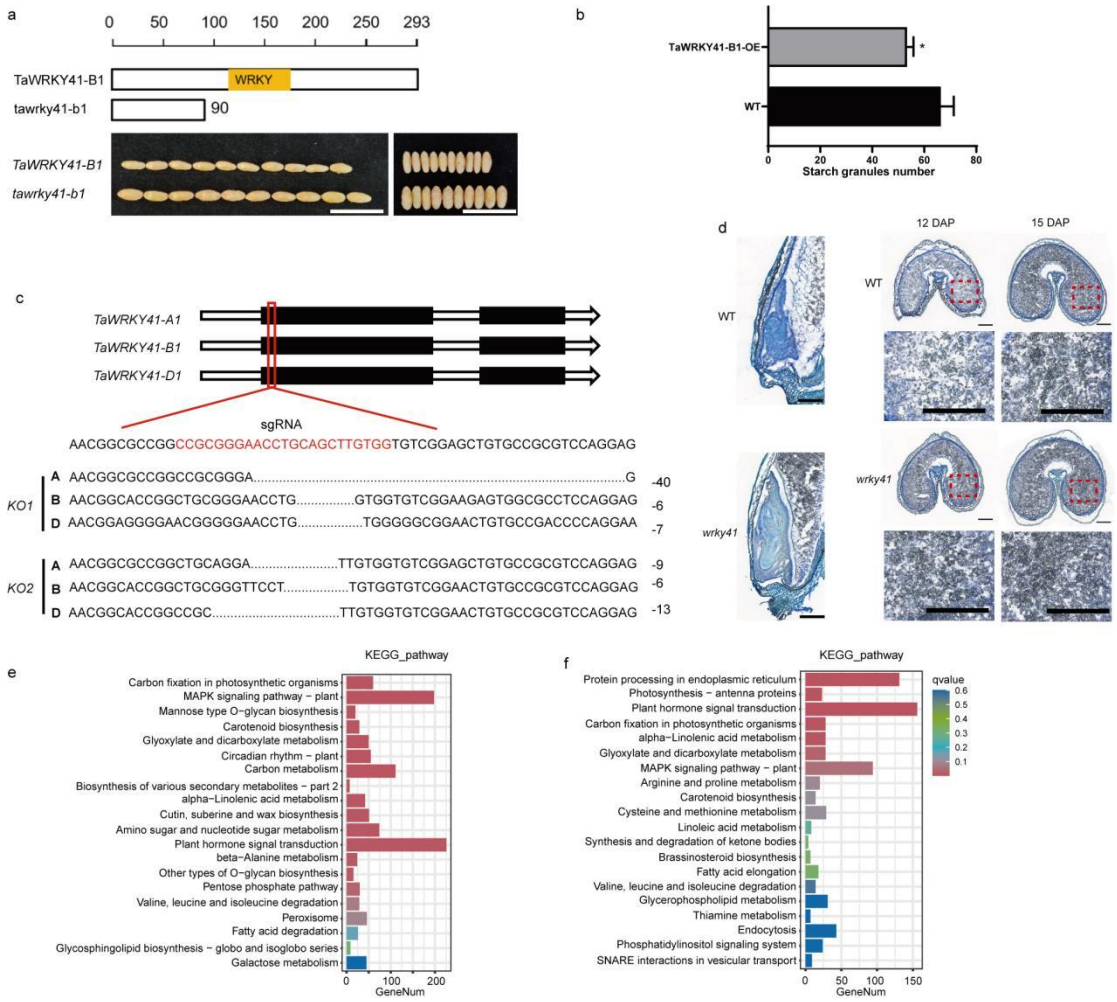
Supplementary Fig. 3 Overview of RNA-seq data for Fielder, *TaBBM-B1*-OE and amiR-*TaBBMs* embryo.

(a) GO enrichment analysis of common DEGs in *TaBBM-B1*-OE and amiR-TaBBMs. FDR, false discovery rate; DEGs, differential expressed genes. Venn diagram showing overlapping DEGs between *TaBBM-B1*-OE and amiR-*TaBBMs* genotypes.

(b) Alignment of TaBBMs and AtBBM amino acid sequences..

(c) Subcellular localization of TaBBM-B1. GFP and TaBBM-B1-GFP fusions under the control of the Cauliflower mosaic virus *35S* promoter were transiently expressed in wheat proplasts. Eighteen hours after transformation, the fluorescence signal of GFP was observed under a confocal laser scanning microscope. GFP (green), bright-field images, and an overlay of the merged fluorescence are shown in each panel. Scale bar=10 μm.

(d) GO enrichment analysis of common DEGs in TaBBM-B1 DAP-seq. FDR, false discovery rate; DEGs, differential expressed genes.



Supplementary Fig. 4 TaWRKY41-B1 negatively regulates wheat grain size.

(a) (d)Mature seeds of wild type (WT) and tabbm double mutant plants. Scale bar = 1 cm.

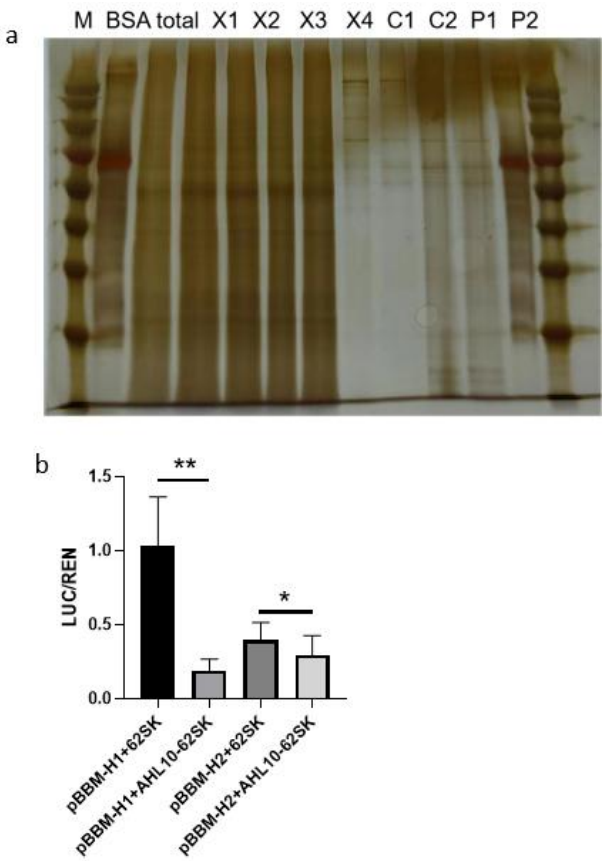
(b) The triangles represent starch granules, the nμmber was manually calculated per 75 μm^2. Scale bar, 100 μm. The data are shown as mean ± SD (n = 10). Statistical significance was determined by Student's *t* test. *, $P < 0.05$; **, $P < 0.01$.

(c) Schematic of *TaWRKY41* homeolog genomic structures showing the target sites and PAMs for single guide RNAs used for mutagenesis by CRISPR-Cas9 in the Fielder background. Sequences of Fielder and two recovered mutant alleles (designated KO1 and KO2) are shown (bottom).

(d) Light microscopy observations of Fielder and *TaWRKY41*-KO grain during grain development. Scale bar = 200 μm.

(e) KEGG enrichment analysis of common DEGs in *TaWRKY41*-B1-OE 8 DAP grain. FDR, false discovery rate; DEGs, differential expressed genes.

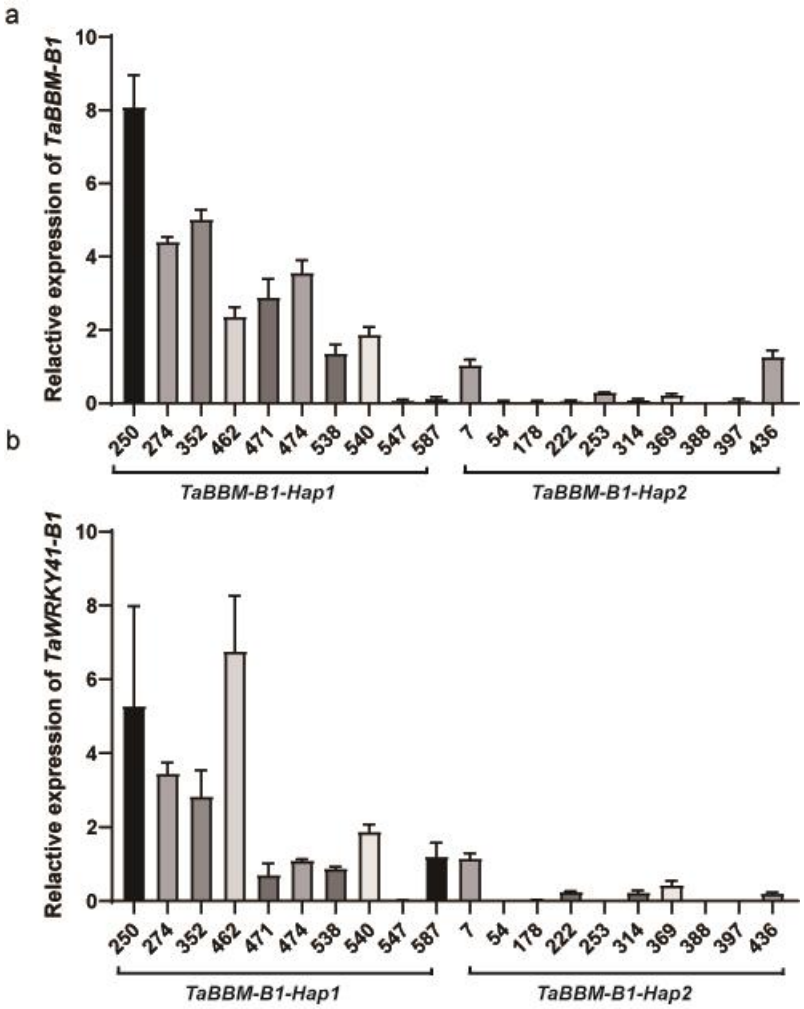
(f) KEGG enrichment analysis of common DEGs in *TaWRKY41*-B1-OE 12 DAP grain. FDR, false discovery rate; DEGs, differential expressed genes.



Supplementary Fig. 5 Screening of upstream regulatory transcription factors of *TaBBM-B1*.

(a) Promoter Pull-Down Assay. Scale bar = 1 cm. M: maker, Total: Total protern, X1-X4: wash, C1-C2: empty bease, P1-P2: *TaBBM-B1* promoter.

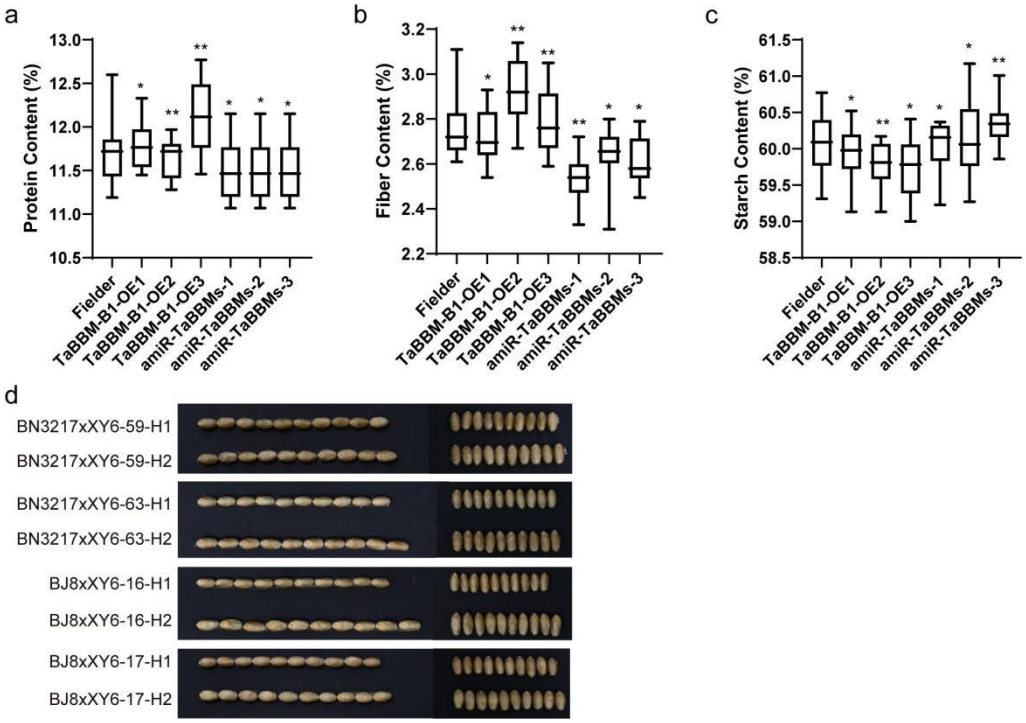
(b) Transient expression assays in N. benthamiana leaves show that TaAHL10-B1 suppresses *TaBBM-B1*. Relative luciferase activity is expressed as the LUC/REN ratio.



Supplementary Fig. 6 Expression in *TaBBM-B1-Hap1* and *TaBBM-B1-Hap2* alleles.

(a) The *TaBBM-B1* relative expression of *TaBBM-B1*-Hap1 and *TaBBM-B1-Hap2* alleles were analyzed in natural germplasm at the 4 DAP grain. Values are presented as means ± SDs (n=3).

(b) The *TaWRKY41-B1* relative expression of *TaBBM-B1-Hap1* and *TaBBM-B1-Hap2* alleles were analyzed in natural germplasm at the 4 DAP grain. Values are presented as means ± SDs (n=3).



Supplementary Fig. 7 TaBBM-B1 regulates wheat grain quality.

(a-c) Quantification of grain agronomic traits related traits between the WT plants, *TaBBM-B1*-OE and amiRTaBBMs lines. Student's t-test was used to determine the difference significance between WT, *TaBBM-B1*-OE and amiR-*TaBBMs*. *, $P \leq 0.05$, **, $P \leq 0.01$, Data represent mean±SD (n = 15 biological replicates).

(d) Recombinant inbred line grain size. Scale bar = 1 cm.