

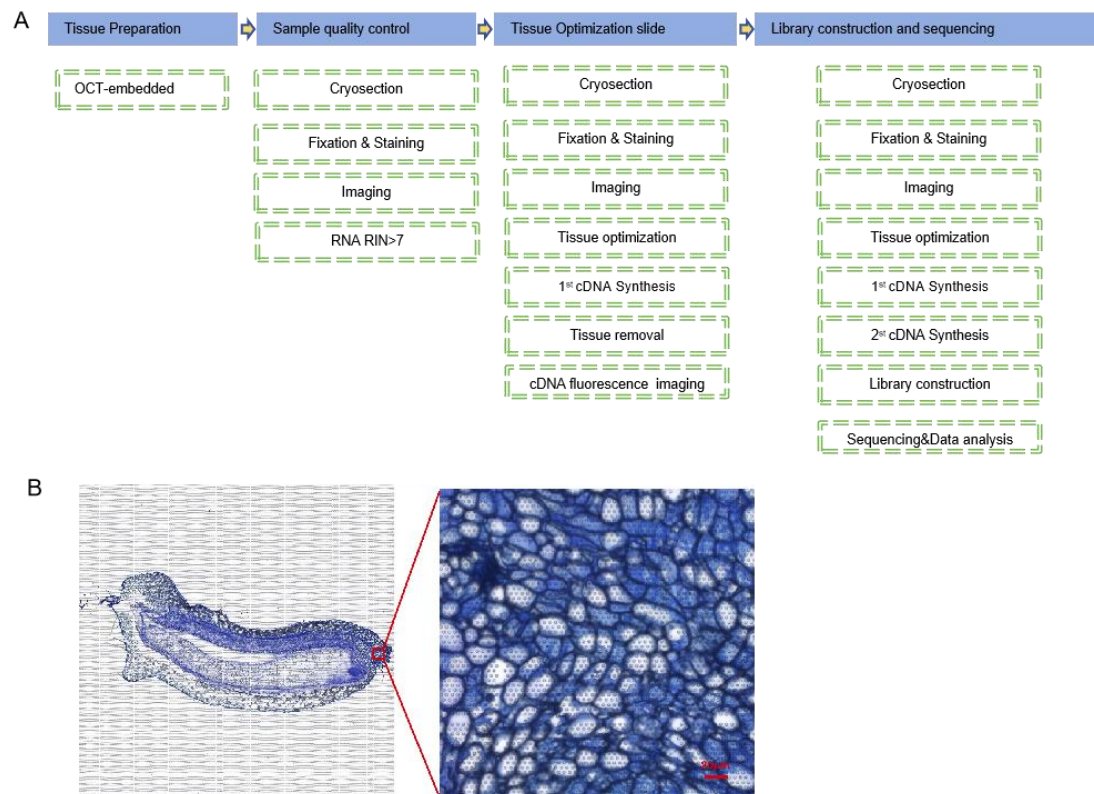


Fig. S1. Overview of spatial transcriptomics experiment on wheat development grains.

A. Spatial transcriptomics tissue protocol for wheat grains. B. The resolution of BMKMANU S1000, bar = 20 μm .

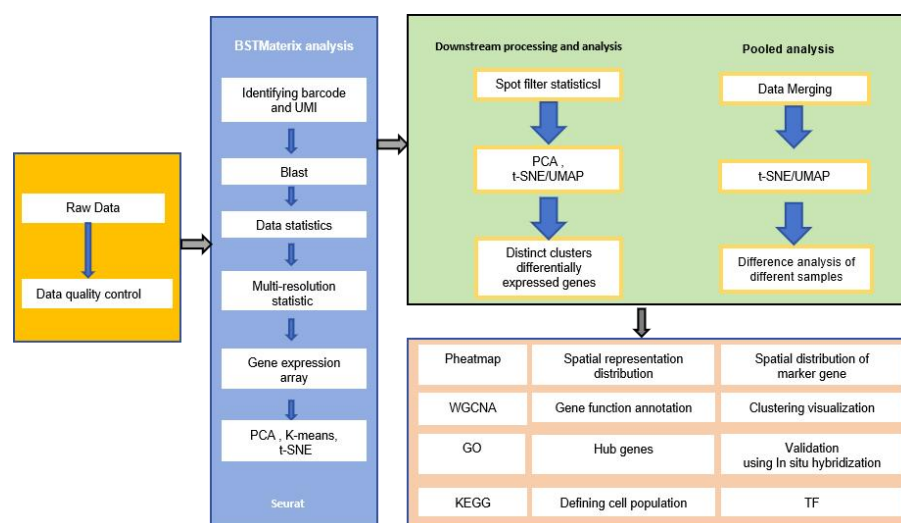


Fig. S2. The analysis pipeline for spatial transcriptomic study.

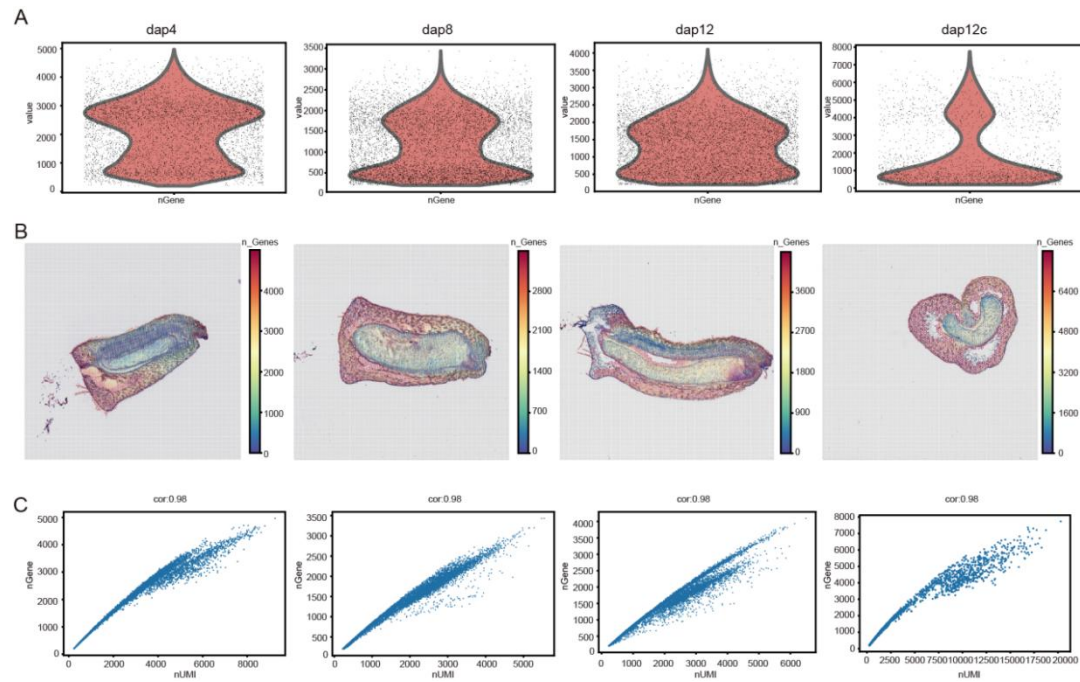


Fig. S3. The density of expressed genes and transcripts in a spot.

A. The number of expressed genes in a spot. The y-axis represents the number of genes expressed in a spot. B. The spatial distribution of expressed genes on the sample sections. The color changes gradually from green, yellow to red as the density gradually increases. C. Pearson correlation among UMI and genes.

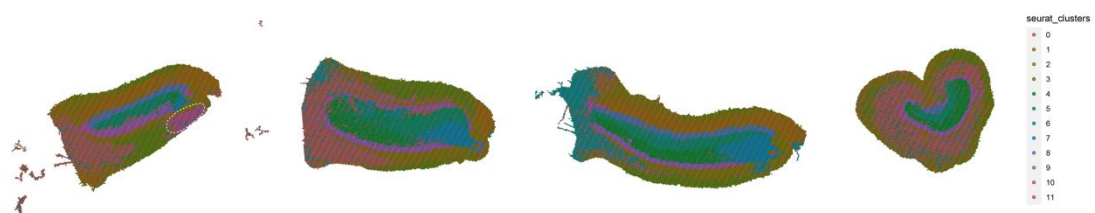


Fig. S4. Spatial visualization of the unbiased spot clustering for dap 4, 8 and 12 wheat sections. Merged bright field image and spatial clusters of the other three sections. White circle showed cluster 11.

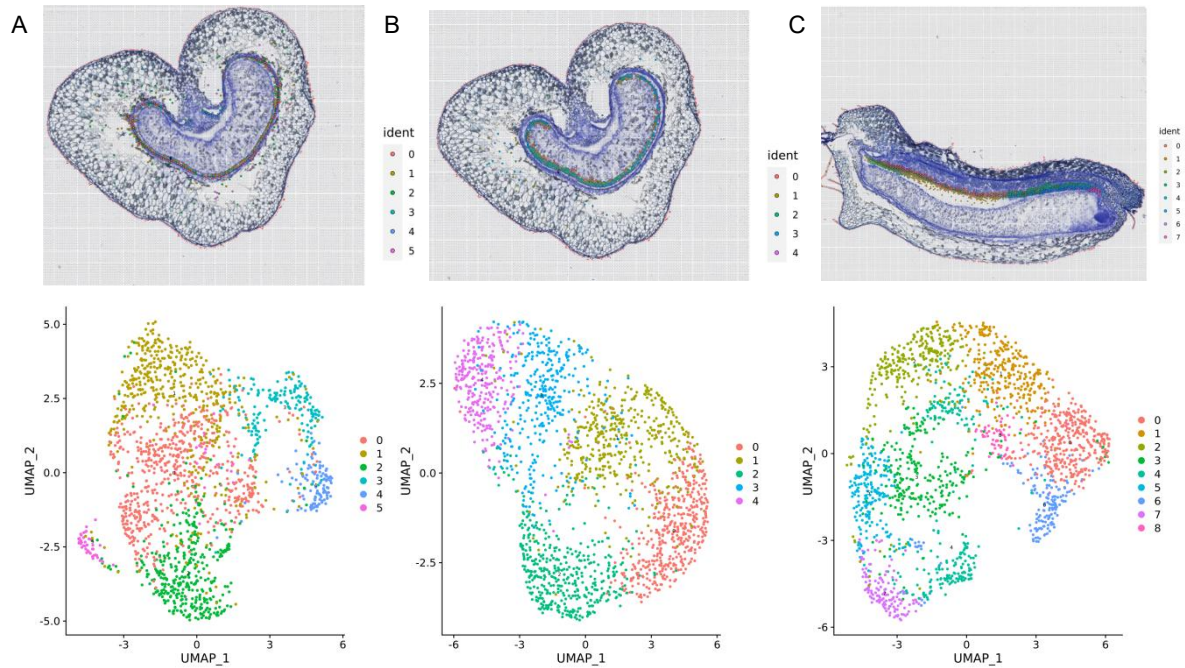


Fig. S5. The defined clusters after dimensional reduction.

A. The top row illustrates the six clusters identified through dimensional reduction, mapped onto tissue sections to reveal their specific localization within the aleurone layer, while the bottom row presents a UMAP visualization of the aleurone layer. B. The top row illustrates the five clusters identified through dimensional reduction, mapped onto tissue sections to reveal their specific localization within the sub-aleurone, while the bottom row presents a UMAP visualization of the sub-aleurone. C. The top row illustrates the nine clusters identified through dimensional reduction, mapped onto tissue sections to reveal their specific localization within the cavity fluid, while the bottom row presents a UMAP visualization of the cavity fluid.

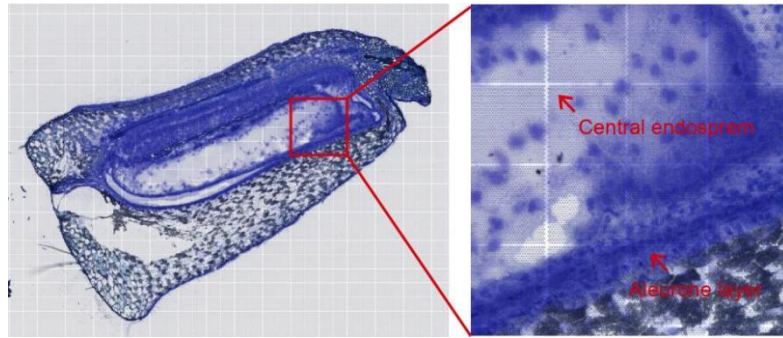


Fig. S6. Light microscopy of grain at dap 4.

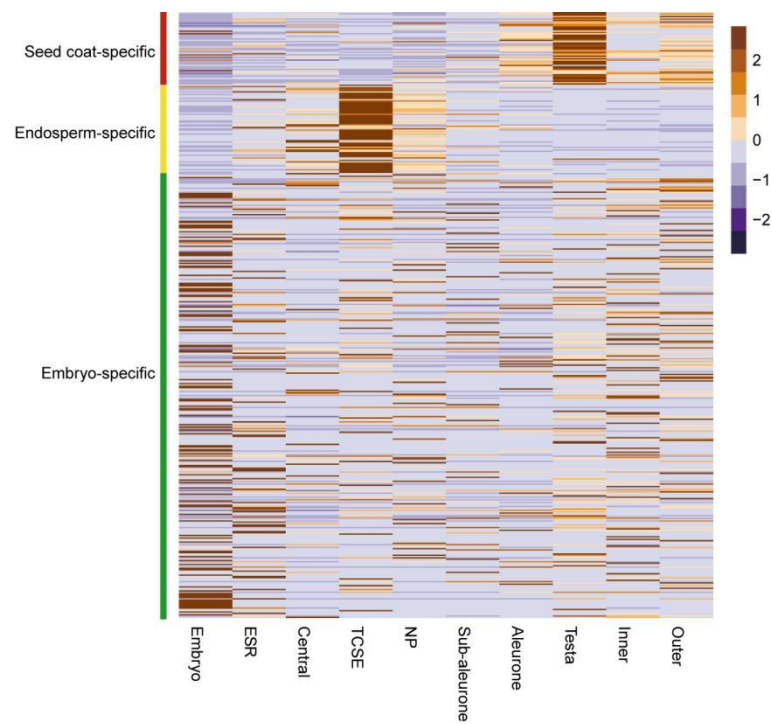


Fig. S7. Heat map shows the expression of known markers that are consistent with the previous studies. Y axis color showed tissues.

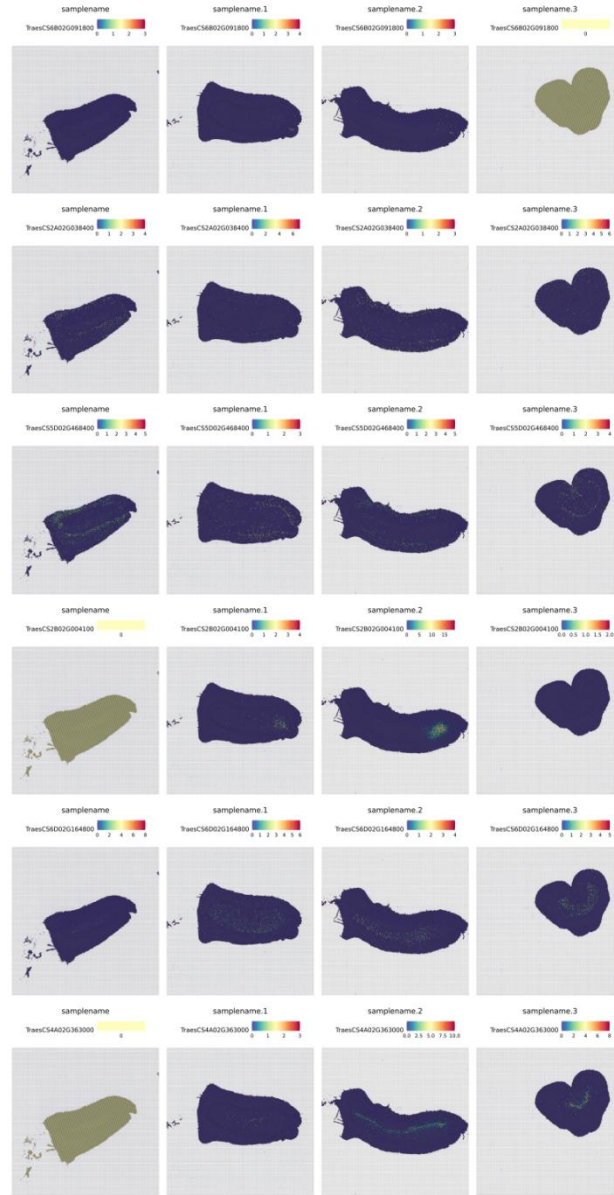


Fig. S8. Representatives of spatial visualization using known markers that are consistent with previous studies.

We selected two markers from three tissue due to unavailability from other research:

TaBBM-B1 (*TraesCS6B02G091800*) in embryo; *TaBGLU16-A1*

(*TraesCS2A02G038400*) and *TapsaD-D1* (*TraesCS5D02G468400*) in seed coat;

TaAMY2-B1 (*TraesCS2B02G004100*), *TaCYP71D7-A1* (*TraesCS4A02G363000*) and

TraesCS4A02G363000 in endosperm. Samplename is dap4, Samplename 1 is dap8,

Samplename 2 is dap12, Samplename 3 is dapc12.

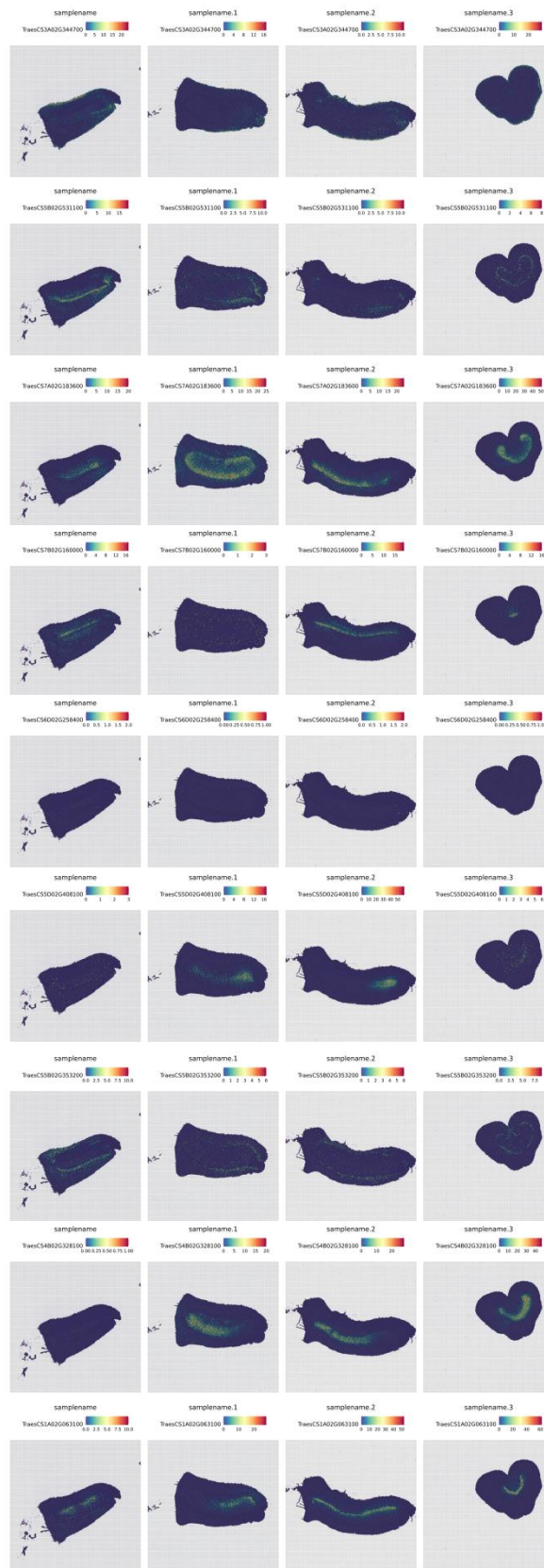


Fig. S9. Representatives of spatial visualization using newly defined markers.

We selected one marker from each compartment: *lipid transfer protein*

TraesCS3A02G344700 in pericarp, *non-specific lipid-transfer protein*

TraesCS5B02G531100 in aleurone layer, *protection and storage gene*

TraesCS7A02G183600 in sub-aleurone layer, *SWEET gene* *TraesCS7B02G160000* in

cavity fluid, *transcription factors* *TraesCS6D02G258400* in embryo,

gibberellin-regulated protein 1 *TraesCS5D02G408100* in ESR,

TraesCS5B02G353200 has been selected as the marker gene for this characteristic,

alpha-amylase *TraesCS4B02G328100* in central cells of starchy endosperm, *seed*

storage-related proteins *TraesCS1A02G063100* in TCSE. Samplename is dap4,

Samplename 1 is dap8, Samplename 2 is dap12, Samplename 3 is dapc12.

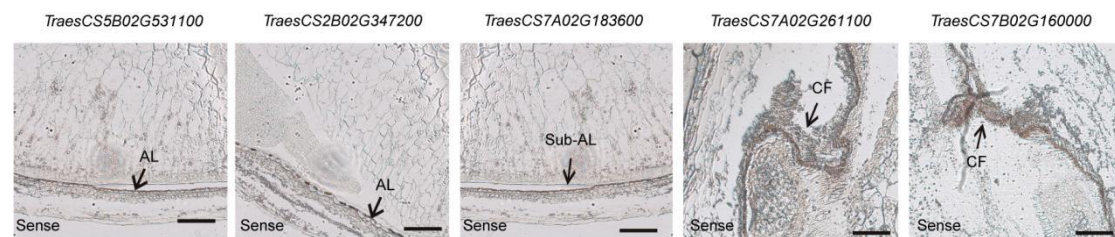


Fig. S10. The sense controls of the marker genes.

The sections were longitudinal. Scale bar = 100 μ m. Aleurone layer (AL),

sub-aleurone (Sub-AL), cavity fluid (CF).

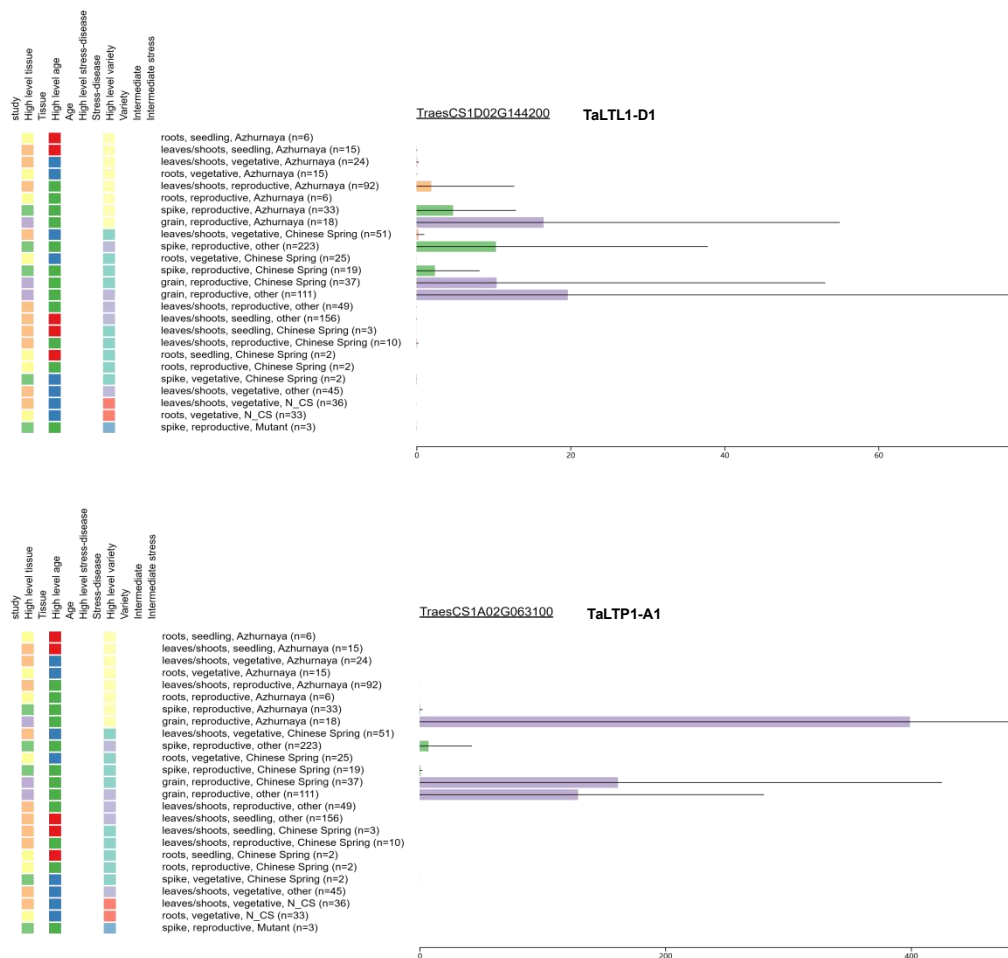


Fig. S11. RNA-seq analysis of the expression of *TaLTL1-D1* and *TaLTP1-A1*.

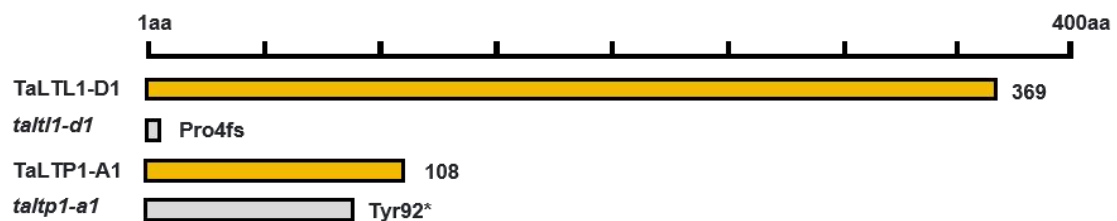


Fig. S12. Schematic diagram of mutants in KN9204 resulting in truncated proteins

(gray box). Scales indicate numbers of amino acid (aa) residues.

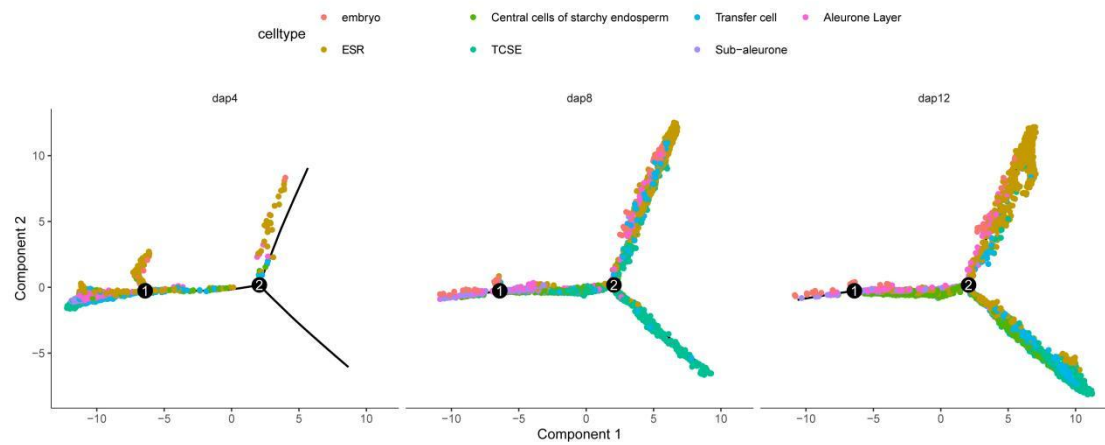


Fig. S13. Visualization of grain development along with pseudotime.

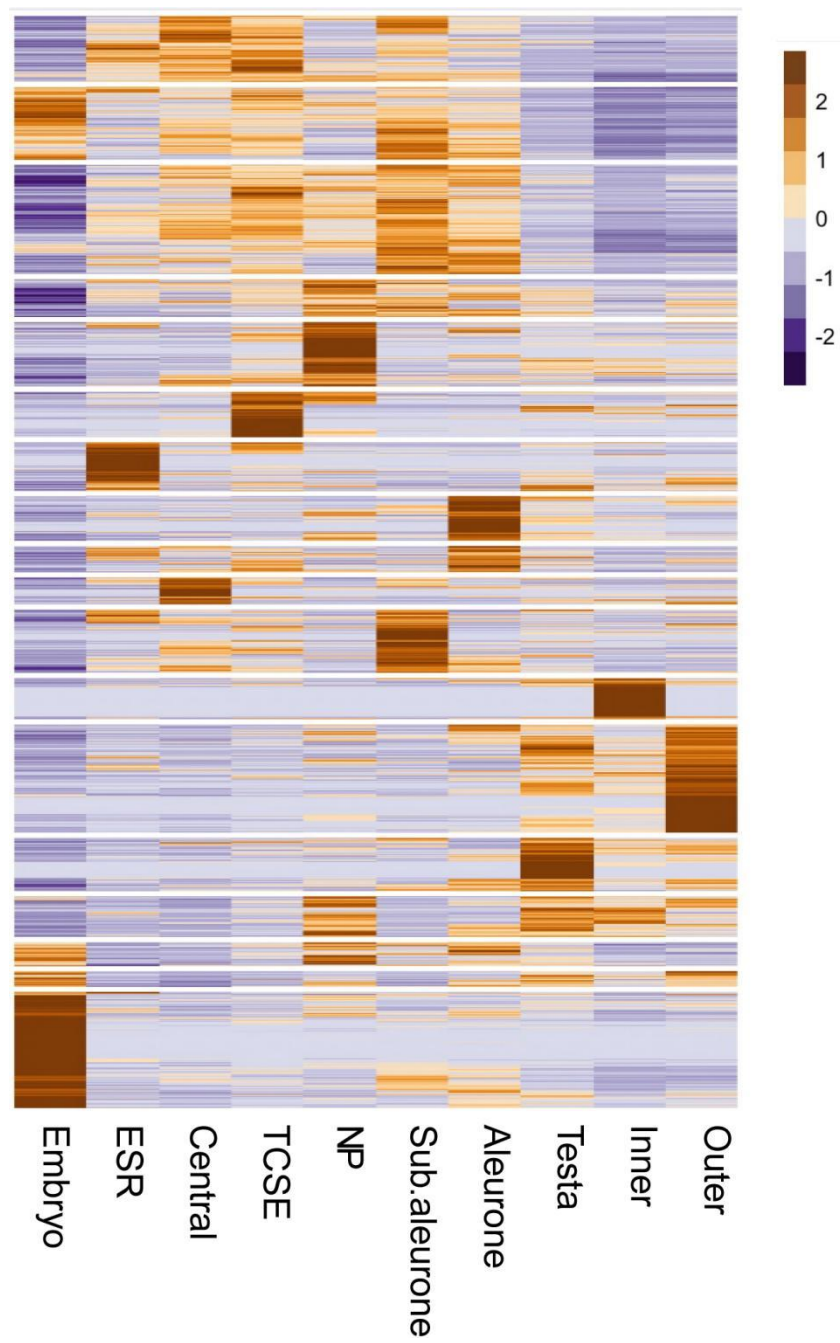


Fig. S14. Heat map showing the expression of TFs in ten grain cell types across three developmental stages.

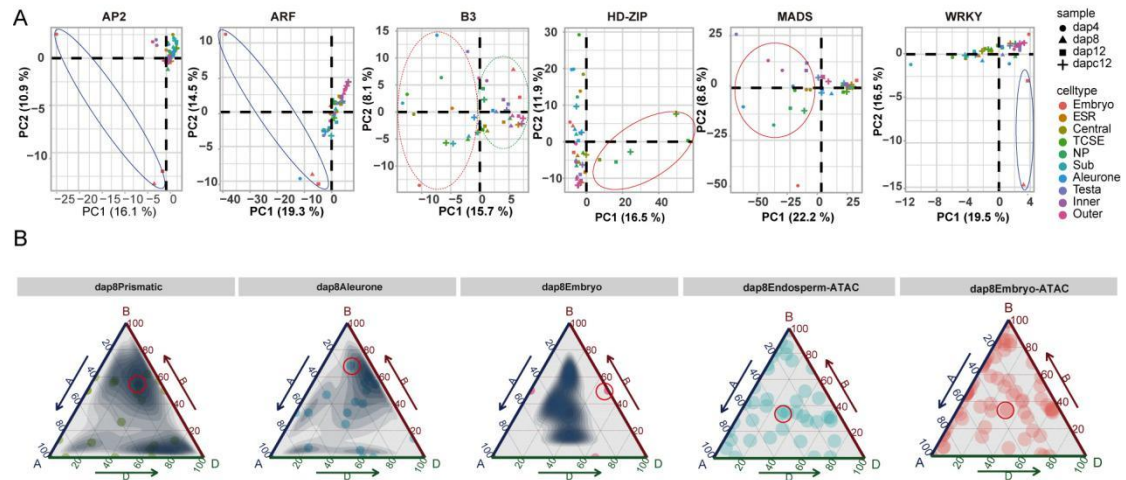


Fig. S15. Diverse expression patterns of TFs across cell types and stage.

A. TF family PCA in all stage of ten cell types. Blue solid circle showed embryo, red solid circle showed endosperm and seedcoat, red dashed circle showed dap 12, green dashed circle showed dap 4 and 8. B. Balanced homologs in B3 family show various unbalanced expression patterns in different cell clusters defined by stRNA-seq data. The ternary plot shows expression patterns of detected genes in stRNA-seq, red circle showed *TaABI3s*.

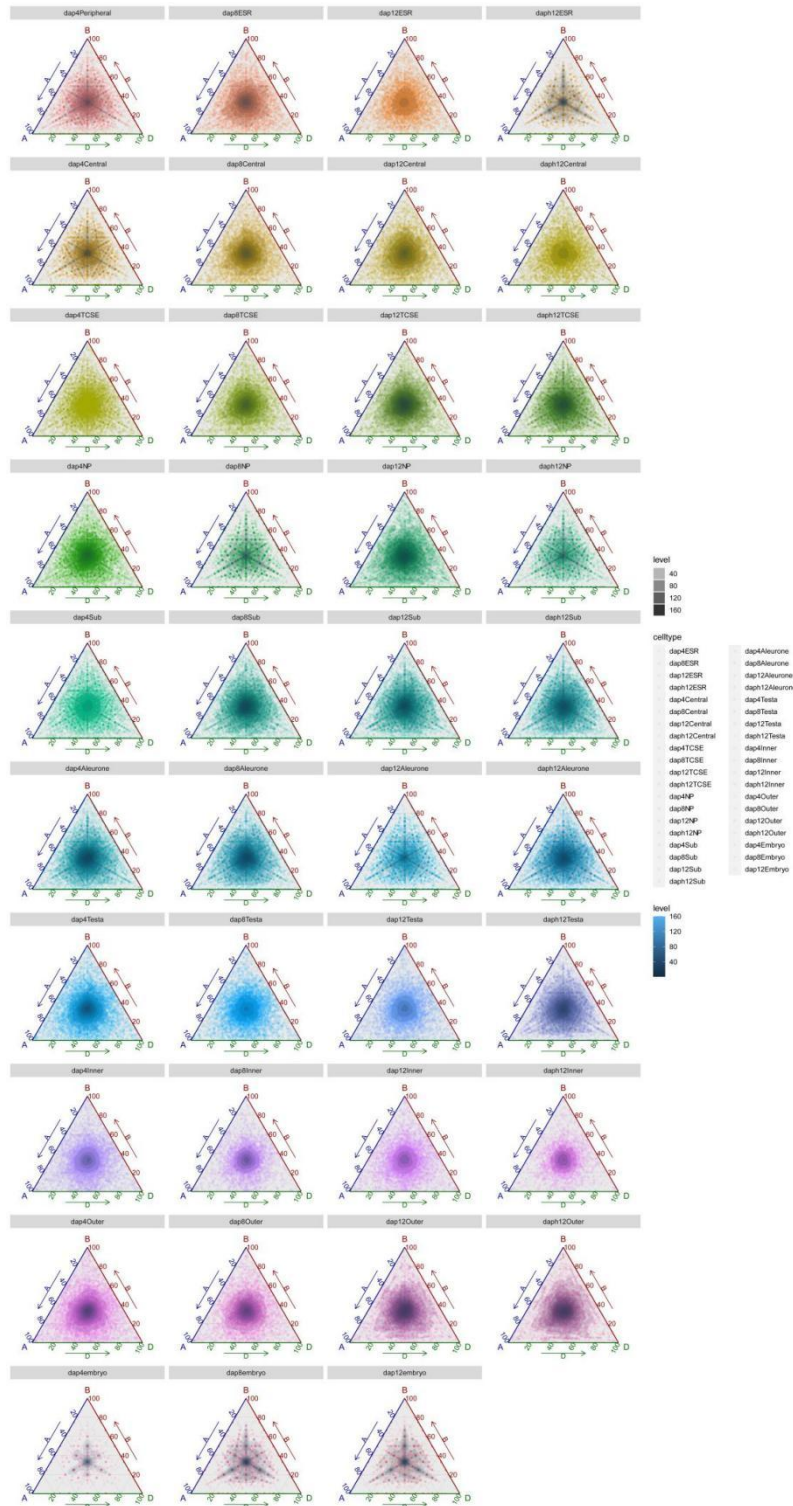


Fig. S16. Balanced homoeologs in genes expression show unbalanced expression patterns in ten grain cell types across three developmental stages defined by stRNA-seq data. The ternary plot shows expression pattern of detected genes in stRNA-seq.

