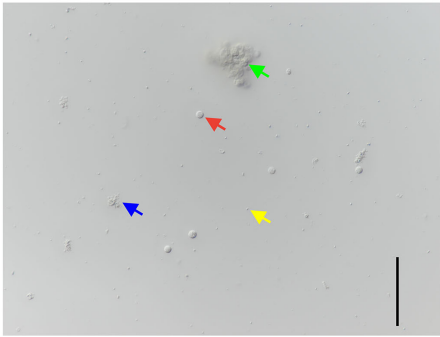


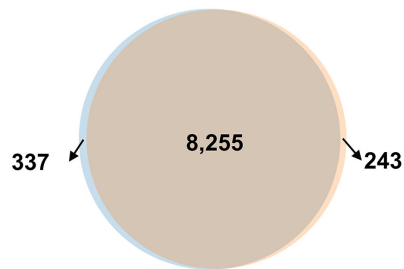
SUPPLEMENTARY FIGURES

A



B

HVGs_Without excluding
protoplasting-
responsive genes
(8,592)



HVGs_excluding
protoplasting-
responsive genes
(8,498)

C

| Meta-clusters without excluding protoplasting- responsive genes | Clusters excluding protoplasting- responsive genes |
|--|---|
| Meta-cluster 1 | No_Proto_cluster 1 |
| Meta-cluster 2 | No_Proto_cluster 2 |
| Meta-cluster 3 | No_Proto_cluster 3 |
| Meta-cluster 4 | No_Proto_cluster 4 |
| Meta-cluster 5 | No_Proto_cluster 5 |
| Meta-cluster 6 | No_Proto_cluster 6 |
| Meta-cluster 7 | No_Proto_cluster 7 |
| Meta-cluster 8 | No_Proto_cluster 8 |
| Meta-cluster 9 | No_Proto_cluster 9 |
| Meta-cluster 10 | No_Proto_cluster 10 |
| Meta-cluster 11 | No_Proto_cluster 11 |
| Meta-cluster 12 | No_Proto_cluster 12 |

Figure S1: Protoplasting-responsive genes do not affect clustering. Related to Figure 1.

(A) A representative picture after enzyme digestion shows cells (red arrow), debris (blue arrow), organelles released from broken cells (yellow arrow) and some occasional undigested tissue pieces (green arrow). Scale bar = 100 μ m. (B) Overlap of Highly Variable Genes (HVGs) identified with or without excluding protoplasting-responsive genes. (C) The river plot demonstrates that the meta-clusters are not changed after excluding protoplasting-responsive genes from the analysis. The left-sided grouping represents meta-clusters that are generated without excluding protoplasting-responsive genes (as in Figures 1B and 1C), and right-sided grouping represents meta-clusters generated by excluding protoplasting-responsive genes (No_Proto cluster 1 to No_Proto cluster12). Each cell between left-sided and right-sided groupings is connected with a grey line. The grey rectangles indicate complete match between groups of cells from each side. Protoplasting-responsive genes are identified by bulk RNA-seq (STAR methods) with three biological replicates.

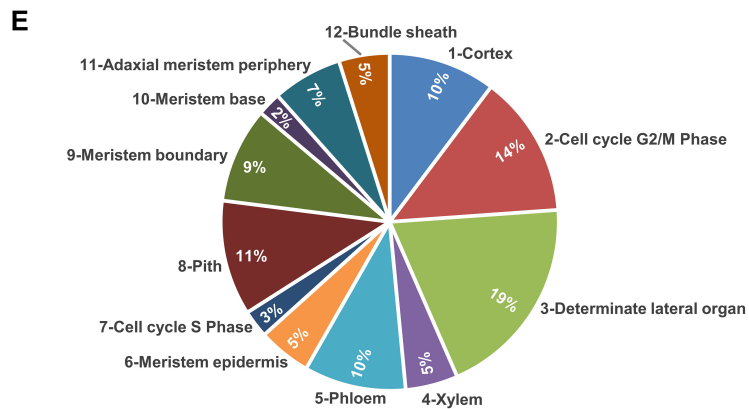
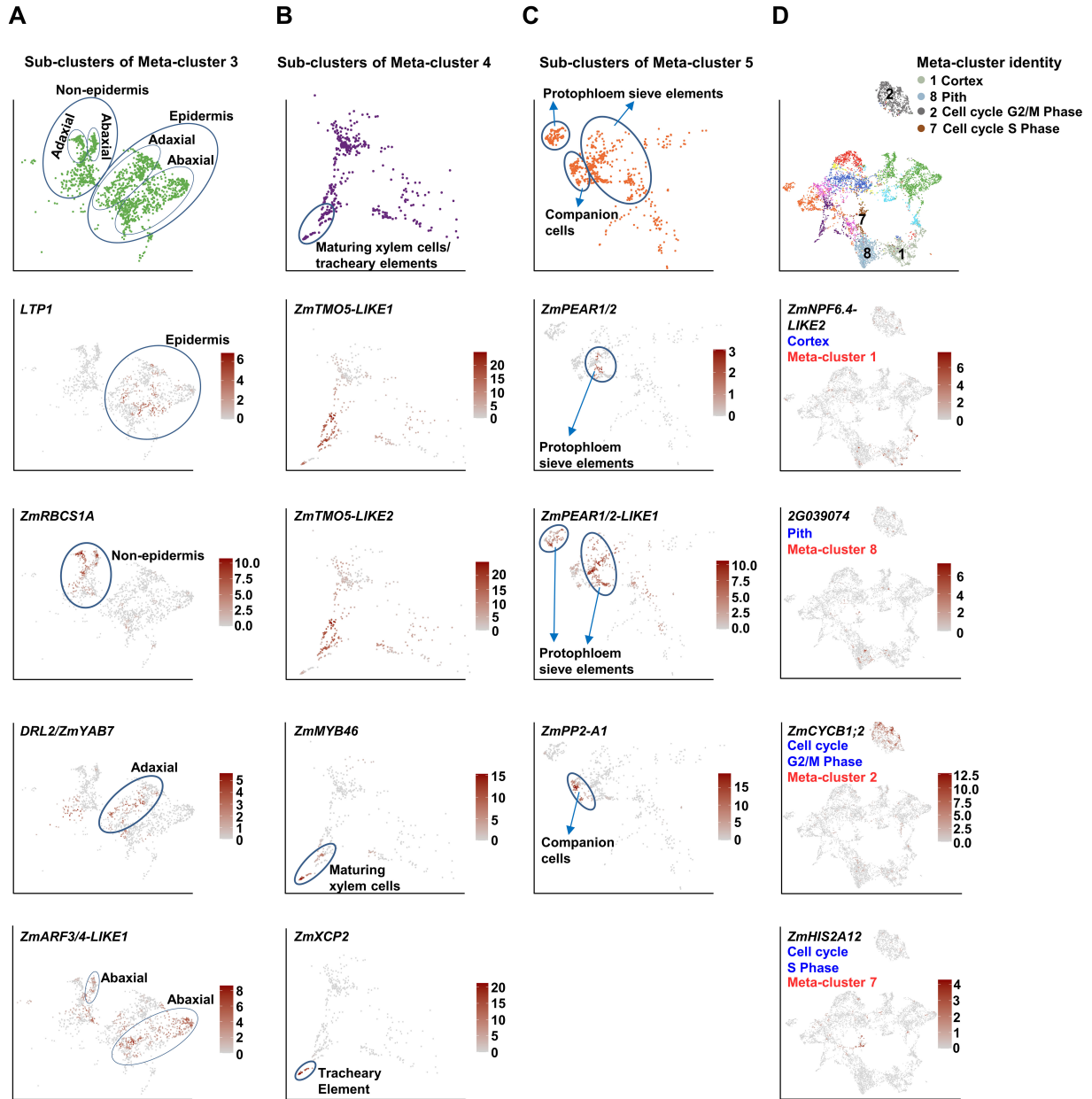


Figure S2: Sub-clusters of meta-clusters 3, 4, and 5 and markers for ground tissue and cell cycle meta-clusters. Related to Figure 1.

(A) Close-up view of meta-cluster 3 shows sub-clusters of epidermis and non-epidermis cells, and adaxial and abaxial cells, with markers shown in UMAPs: epidermis marker *LIPID TRANSFER PROTEIN1 (LTP1)*, *GRMZM2G126646*, non-epidermis marker *ZmRIBULOSE BIPHOSPHATE CARBOXYLASE SMALL SUBUNIT 1A (ZmRBCS1A)*, *GRMZM2G098520*, adaxial marker *DROOPING LEAF2/ZmYABBY7 (DRL2/ZmYAB7)*, *GRMZM2G102218*, and abaxial marker *ZmAUXIN RESPONSE FACTOR3/4-LIKE1 (ZmARF3/4-LIKE1)*, *GRMZM2G159399*. (B) Close-up view of meta-cluster 4 shows xylem markers and sub-cluster for maturing xylem cells/tracheary elements, with markers shown in UMAPs: xylem markers *ZmTARGET OF MONOPTEROS5-LIKE1 (ZmTMO5-LIKE1)*, *GRMZM2G072342* and *ZmTMO5-LIKE2*, *GRMZM2G526668*, maturing xylem marker *ZmMYB DOMAIN PROTEIN 46 (ZmMYB46)*, *GRMZM2G052606*, and tracheary element marker *ZmXYLEM CYSTEINE PEPTIDASE 2 (ZmXCP2)*, *GRMZM2G0367701*. (C) Close-up view of meta-cluster 5 demonstrates sub-clusters of protophloem sieve elements and companion cells, with markers shown in UMAPs: protophloem sieve element markers *ZmPHLOEM EARLY DOF 1/2 (ZmPEAR1/2)*, *GRMZM2G093725*, and *ZmPEAR1/2-LIKE1*, *GRMZM2G017470*, companion cell marker *ZmPHLOEM PROTEIN 2-A1 (ZmPP2-A1)*, *GRMZM2G073693*. (D) UMAP plots highlight two ground tissue meta-clusters, meta-cluster 1 (cortex) and meta-cluster 8 (pith), and two cell cycle-related meta-clusters, meta-cluster 2 (cell cycle G2/M Phase) and meta-cluster 7 (cell cycle S Phase), with markers listed below: cortex marker *ZmNITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER FAMILY 6.4-LIKE 2 (ZmNPF6.4-LIKE2)*, *GRMZM2G361652*, pith marker *GRMZM2G039074 (2G039074)*, cell cycle G2/M phase marker *ZmCYCLINB1;2 (ZmCYCB1;2)*, and cell cycle S phase marker *ZmHISTONE2A12 (ZmHIS2A12)*. Each dot represents a cell. (E) Pie chart shows the percentage of number of recovered cells across all replicates in each meta-cluster.

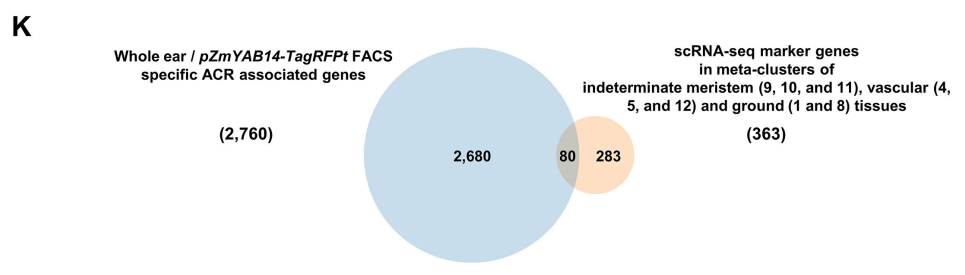
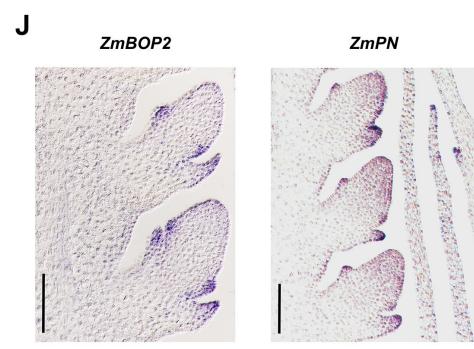
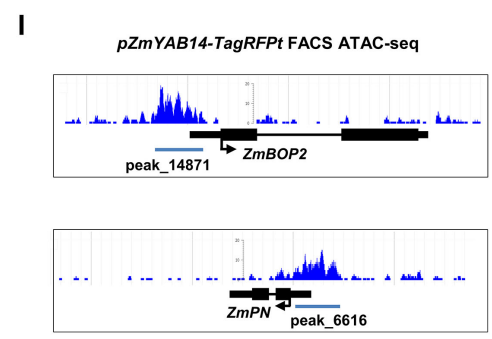
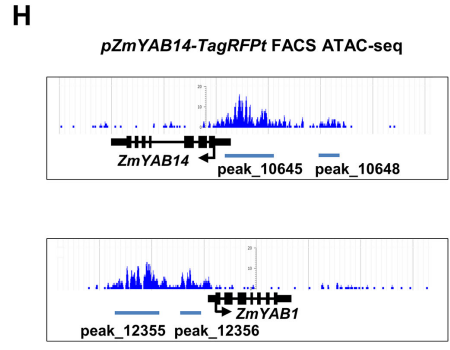
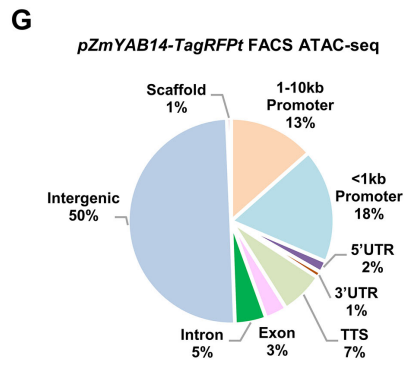
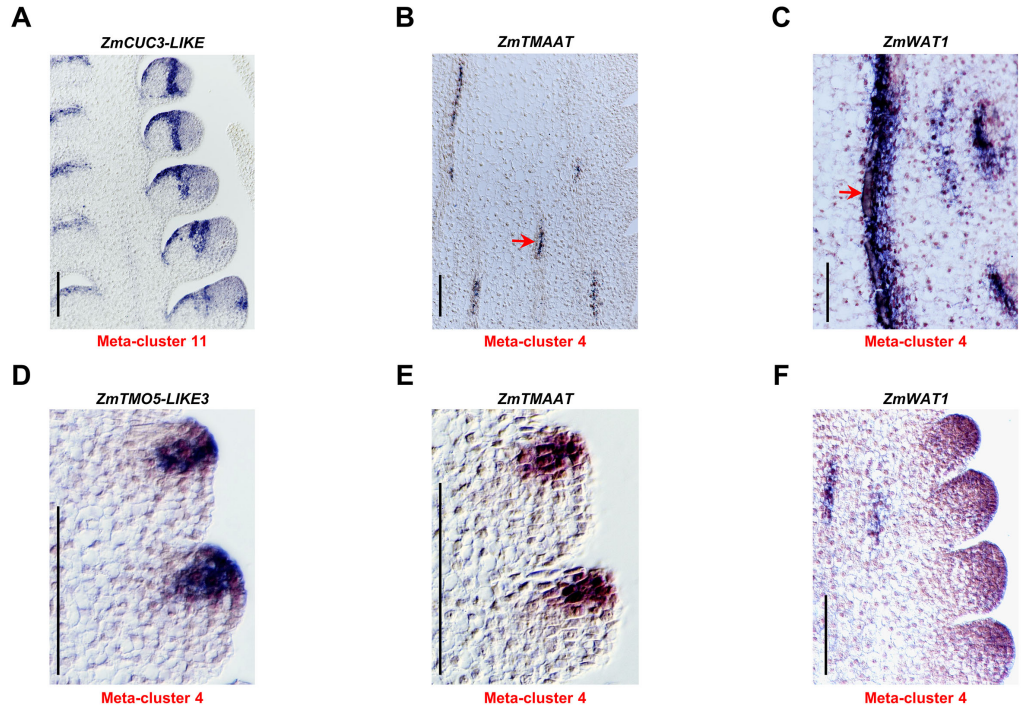
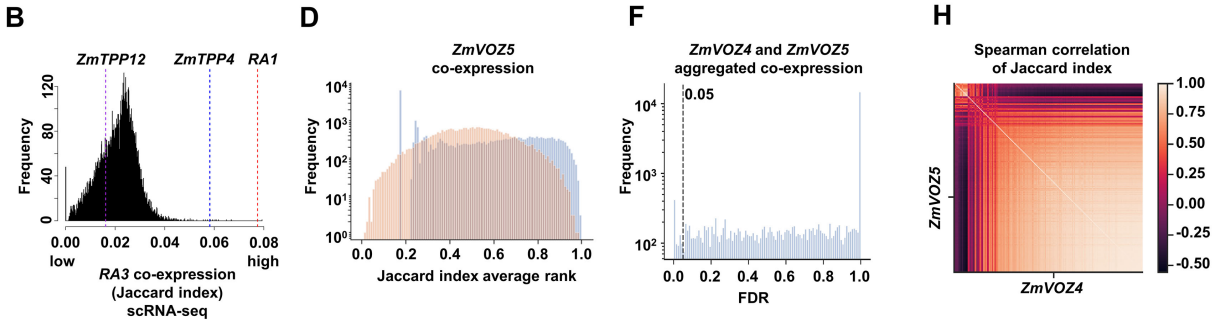
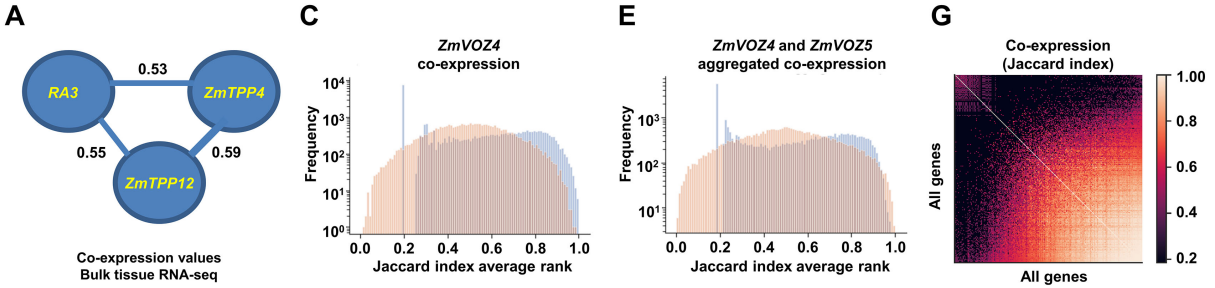
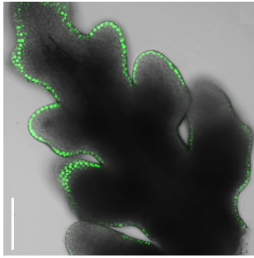


Figure S3: mRNA *in situ* for selected markers in meta-clusters 11 and 4 and integration of scRNA-seq and FACS ATAC-seq. Related to Figure 2.

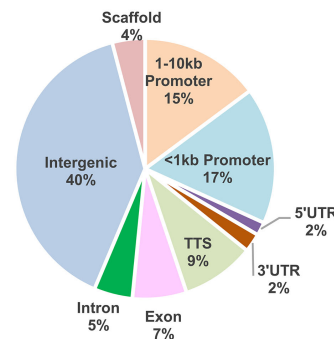
(A) mRNA *in situ* of meta-cluster 11 (adaxial meristem periphery) marker *ZmCUP-SHAPED COTYLEDON 3-LIKE* (*ZmCUC3-LIKE*), *GRMZM2G430522*; (B-C) mRNA *in situ* of meta-cluster 4 (xylem) markers, *ZmTRANSMEMBRANE AMINO ACID TRANSPORTER FAMILY PROTEIN* (*ZmTMAAT*), *GRMZM2G109865* (B) and *ZmWALLS ARE THIN 1* (*ZmWATI*), *GRMZM2G007953* (C), with red arrow indicating xylem vessels. (D-F) Examples of xylem markers identified from scRNA-seq meta-cluster 4 that also show meristem tip expression: *ZmTARGET OF MONOPTEROS5-LIKE3* (*ZmTMO5-LIKE3*), *GRMZM2G176141* (D); *ZmTMAAT* (E) and *ZmWATI* (F). Scale bar = 100 μ m. (G) Genome-wide distribution of *pZmYAB14-TagRFpT* FACS ATAC-seq accessible chromatin regions from one biological replicate. (H) Representative scRNA-seq meta-cluster 3 enriched genes from *ZmYAB* families have accessible chromatin in *pZmYAB14-TagRFpT* FACS sorted cells (Table S2), such as *ZmYAB14* (top panel) and *ZmYABI* (bottom panel). (I) scRNA-seq meta-cluster 3 enriched genes, *ZmBLADE ON PETIOLE2* (*ZmBOP2*), *GRMZM2G026556* (top panel), and *ZmPLANTACYANIN* (*ZmPN*), *GRMZM2G004012* (bottom panel) have accessible chromatin in their promoter-TSS regions in *pZmYAB14-TagRFpT* FACS sorted cells. (J) mRNA *in situ* shows *ZmBOP2* and *ZmPN* expression in determinate lateral organs. Scale bar = 100 μ m. (K) scRNA-seq marker genes in meristem (9, 10, and 11), vascular (4, 5, and 12) and ground tissue (1 and 8) meta-clusters are enriched for genes with open chromatin in whole ear but not in *pZmYAB14-TagRFpT* FACS sorted lateral organ cells, see text for details.



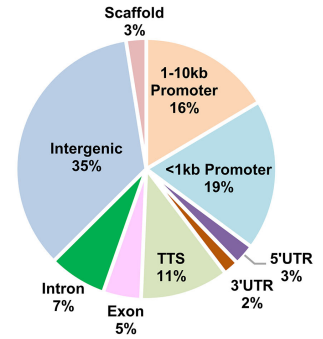
I *ZmHDZIV6*-YFP/*bd1;Tu*



K *ZmHDZIV6*-YFP ChIP-seq



L *ZmM16*-YFP ChIP-seq



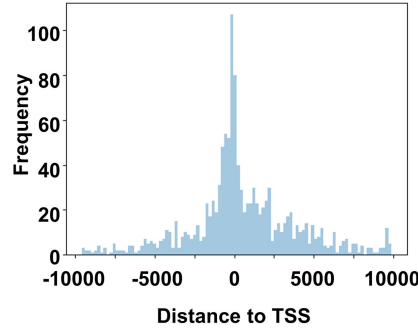
J *ZmHDZIV6*-YFP ChIP-seq



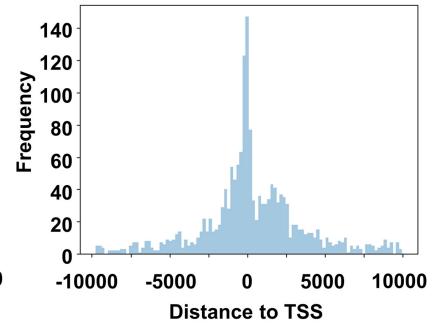
ZmM16-YFP ChIP-seq



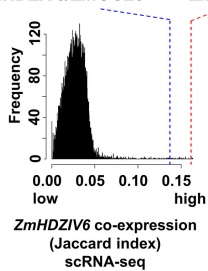
L *ZmHDZIV6*-YFP ChIP-seq



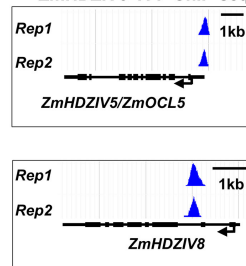
ZmM16-YFP ChIP-seq



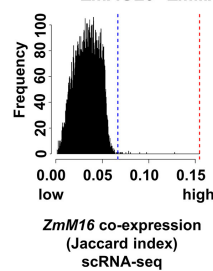
M *ZmHDZIV5*/*ZmOCL5* *ZmHDZIV8*



N *ZmHDZIV6*-YFP ChIP-seq



O *ZmAGL8* *ZmMADS7*



P *ZmM16*-YFP ChIP-seq

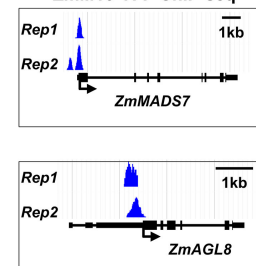


Figure S4: Application of scRNA-seq to predict genetic redundancy and build transcriptional regulatory networks. Related to Figure 3.

(A) Similar co-expression values of *RA3-ZmTPP4* (0.53), *RA3-ZmTPP12* (0.55) and *ZmTPP4-ZmTPP12* (0.59) from an aggregate network across 89 maize bulk tissue RNA-seq datasets. (B) Jaccard index co-expression values are higher between *RA3* and redundantly acting *ZmTPP4* than *ZmTPP12* in scRNA-seq datasets. X-axis represents co-expression values of *RA3* and all maize genes as reflected by the Jaccard index from low to high. Y-axis represents the frequency of each co-expression value. Colored dashed lines indicate corresponding gene co-expression values with *RA3*. (C-D) Empirical distribution (blue color) of *ZmVOZ4*, *GRMZM2G156016* (C) and *ZmVOZ5*, *GRMZM2G449165* (D) co-expression metrics. X-axis represents Jaccard index co-expression values. Y-axis represents log scaled frequency. The orange color indicates null distribution. (E-F) The overlap in co-expression distribution of *ZmVOZ4* and *ZmVOZ5* (E) identify 627 genes (Table S3) that are consistently co-expressed with both *ZmVOZ4* and *ZmVOZ5* (F) at cutoff at FDR < 0.05. (G) Pairwise similarity in co-expression partners using Jaccard index values ordered from lowest to the highest expression of all maize genes. (H) Spearman correlation of co-expression profiles between each pair of genes, indicating similarity in co-expression partners. *ZmVOZ4* and *ZmVOZ5* have Spearman correlation of 0.88 in their co-expression profiles. (I) Epidermis specific expression of *ZmHDZIV6*-YFP in the *bd1;Tu* line. Scale bar = 100 μ m. (J) Two biological replicates of *ZmHDZIV6*-YFP (top panel) and *ZmM16*-YFP (bottom panel) overlap significantly. (K) Genome-wide distribution of *ZmHDZIV6* (left panel) and *ZmM16* (right panel) bound peaks. (L) Bound peaks within ± 10 kb regions for both *ZmHDZIV6* (left panel) and *ZmM16* (right panel) are strongly enriched around transcription start sites (TSS). (M-N) *ZmHDZIV6* predicted direct targets, *ZmHDZIV8* and *ZmHDZIV5/OCL5*, identified by co-expression in scRNA-seq (M) and ChIP-seq (N). (O-P) Predicted *ZmM16* directly activated targets, *ZmMADS7* and *ZmAGAMOUS-LIKE 8* (*ZmAGL8*, *GRMZM2G079727*), identified by co-expression in scRNA-seq (O) and ChIP-seq (P). In the histogram of M and O, X-axis represents the co-expression values of *ZmHDZIV6* (M) or *ZmM16* (O) with all maize genes, reflected by the Jaccard index from low to high. Y-axis represents the frequency of each co-expression value. Dashed lines indicate co-expression values of predicted direct targets with *ZmHDZIV6* (M) or *ZmM16* (O).