

Spatial and single-nucleus transcriptomics reveal the complexity of genomic imprinting in maize

Tao Li(李涛)¹, Yi Jiang(姜艺)¹, Mingyue Zhang(张明岳)², Kesen Zhu(朱克森)¹, Siqi Jiang(姜思奇)¹, Xuerong Yang(杨雪蓉)², Hongjun Liu(刘红军)^{2,3}, Jiechen Wang(王婕琛)⁴, Xiaomei Dong(董小妹)⁵ & Junpeng Shi(史俊鹏)^{1,#}

¹Guangdong Provincial Key Laboratory of Plant Stress Biology, School of Agriculture and Biotechnology, The Shenzhen Campus of Sun Yat-sen University, Sun Yat-sen University, Shenzhen, 518107, China

²State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an, Shandong, 271018, China

³Yazhouwan National Laboratory, Sanya, Hainan, 572024, China

⁴National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology & Ecology, Shanghai, 200032, China

⁵College of Bioscience and Biotechnology, Shenyang Agricultural University, Shenyang, Liaoning, 110866, China

[#]Correspondence: Junpeng Shi (shijp6@mail.sysu.edu.cn)

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Supplementary Figures

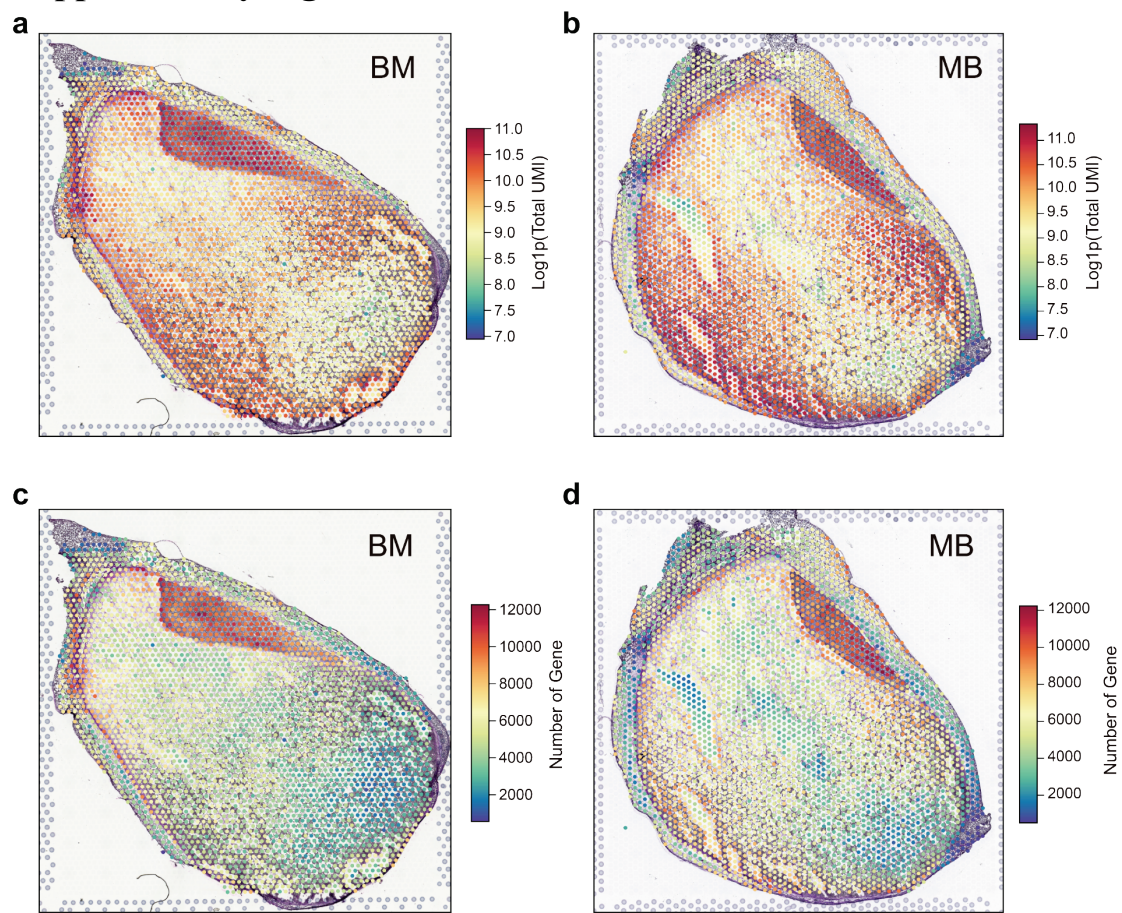


Fig. S1 Spatial overview of sequencing depth and gene detection.

a, b Spatial plot showing total UMI counts per spot in BM (a) and MB (b) samples. **c, d** Spatial plot showing the number of genes detected per spot in BM (c) and MB (d) samples.

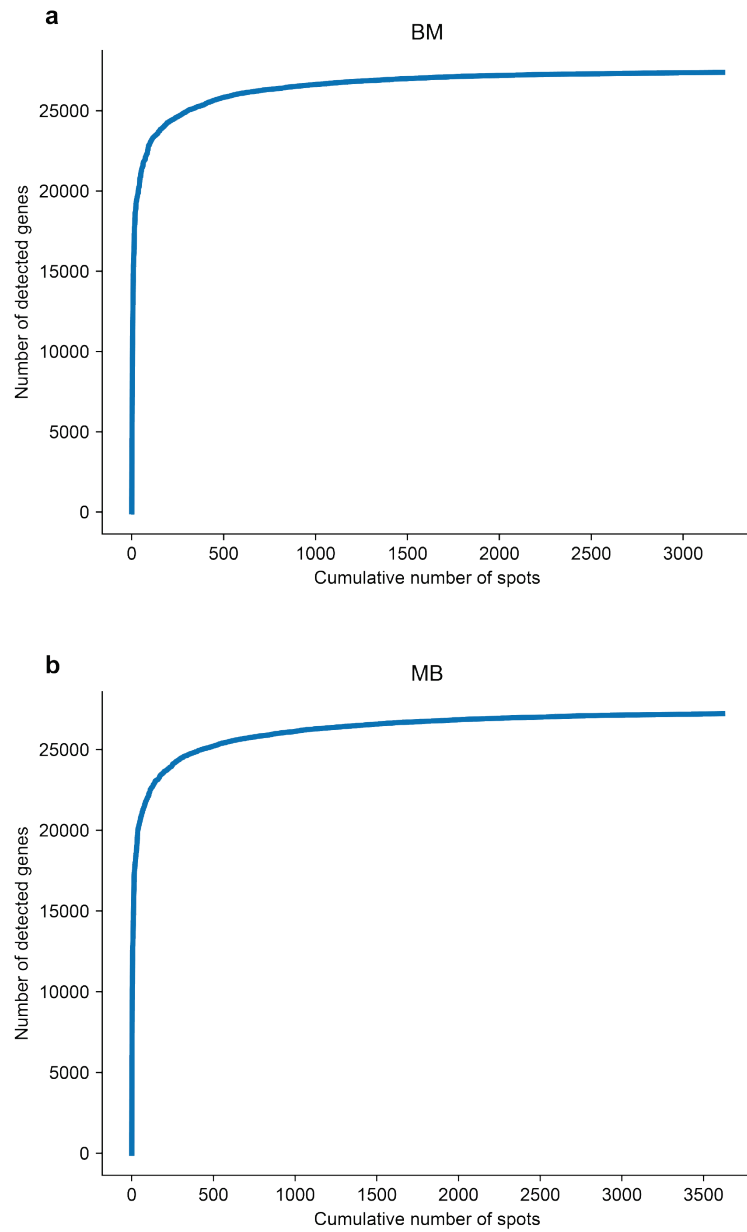


Fig. S2 Line plot showing the number of detected genes as the cumulative number of spots increases in BM (a) and MB (b).

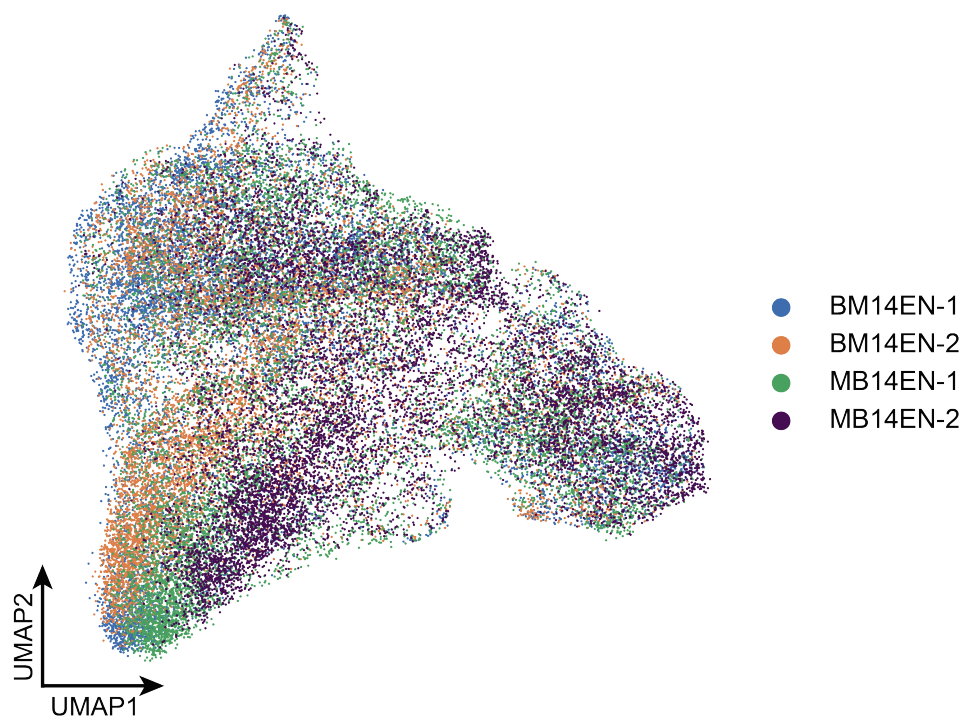


Fig. S3 UMAP of integrated snRNA-seq nuclei colored by 4 different samples (two replicates for both BM and MB).

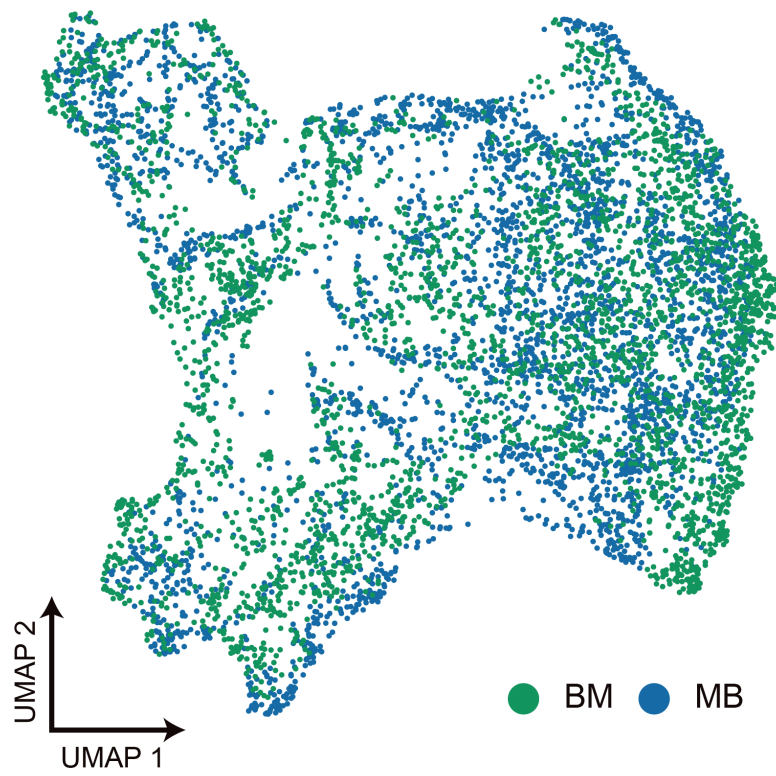


Fig. S4 UMAP of all spatial transcriptomic spots integrating BM and MB, with BM in green and MB in blue.

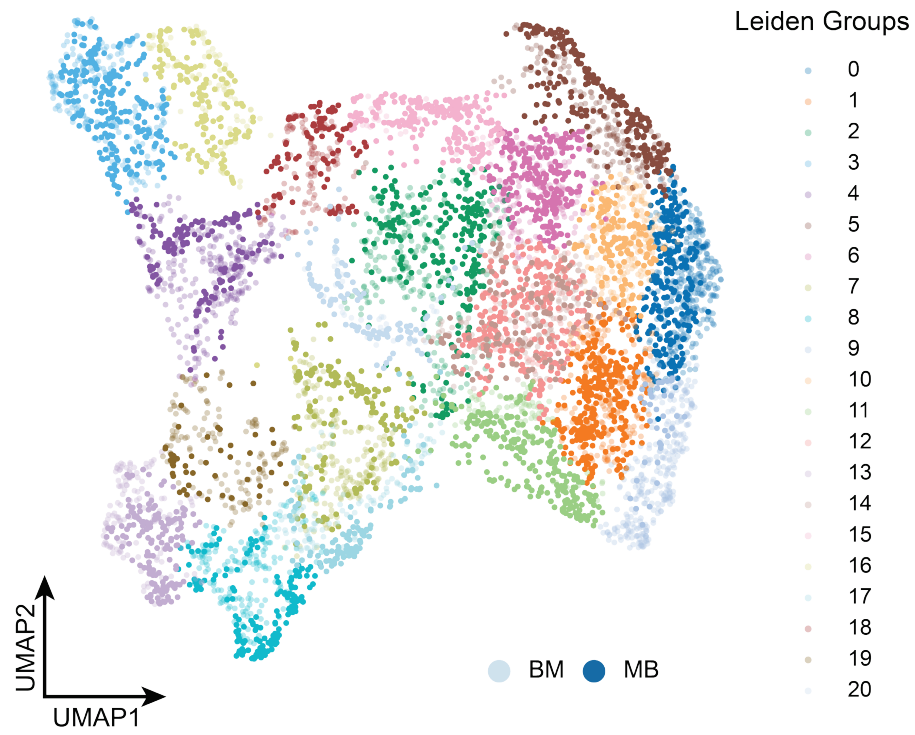


Fig. S5 UMAP of unsupervised clustering; BM shown in transparent, MB in solid color.

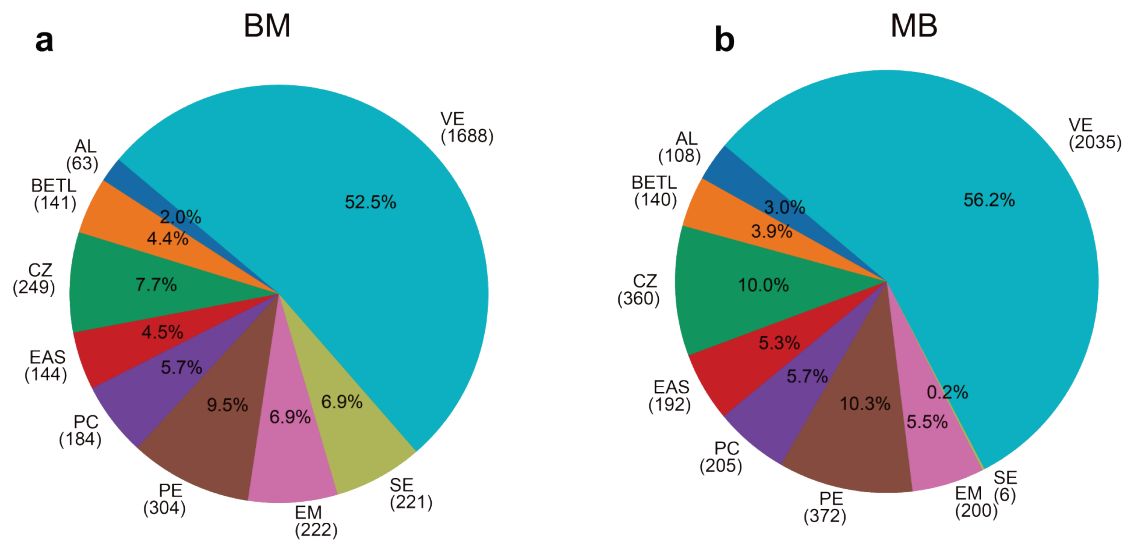


Fig. S6 Pie charts showing the proportion of spots for each cell type in BM (a) and MB (b) samples.

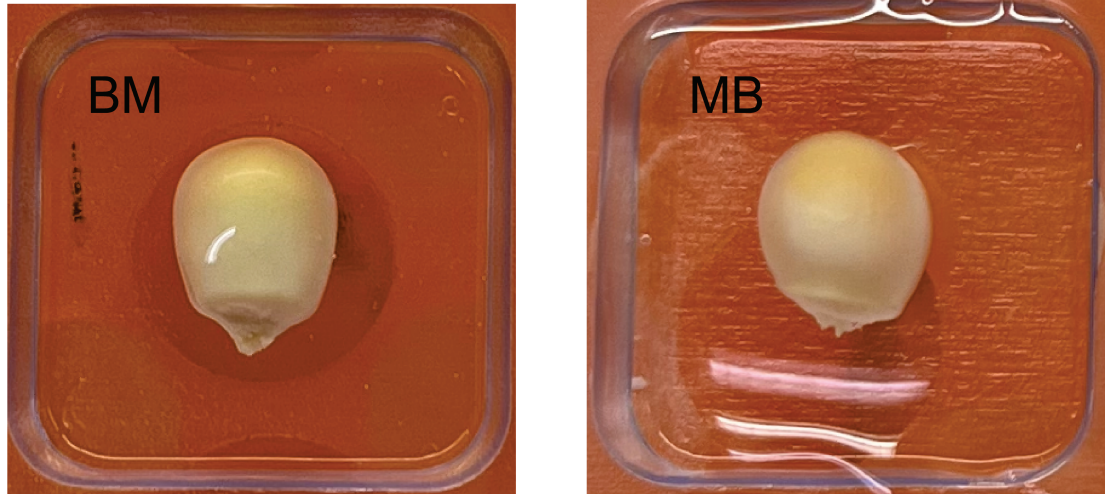


Fig. S7 The 14 DAP kernel phenotypes of BM and MB hybrids that were used for Visium spatial transcriptome sequencing.

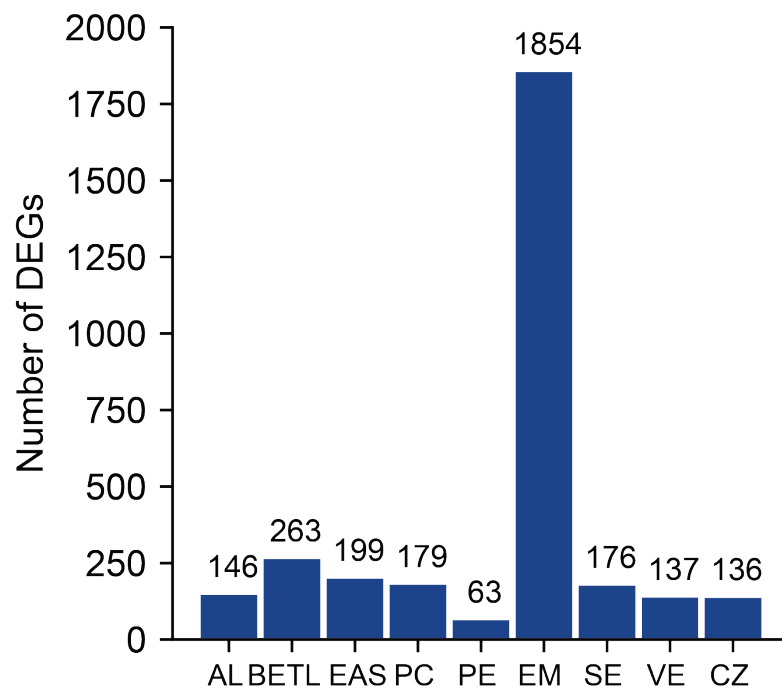


Fig. S8 Bar plot showing the number of DEGs for each cell type compared to other cell types.

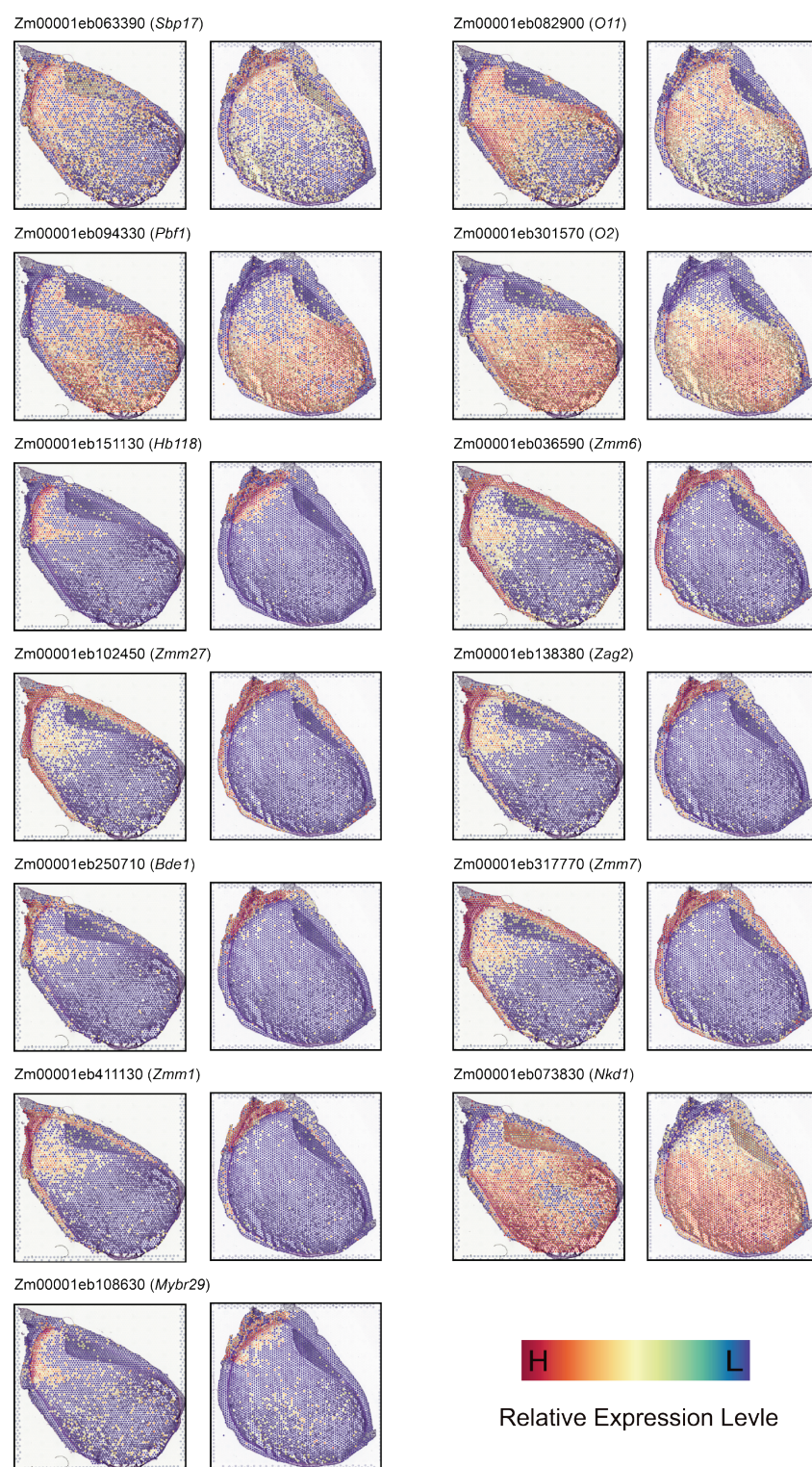
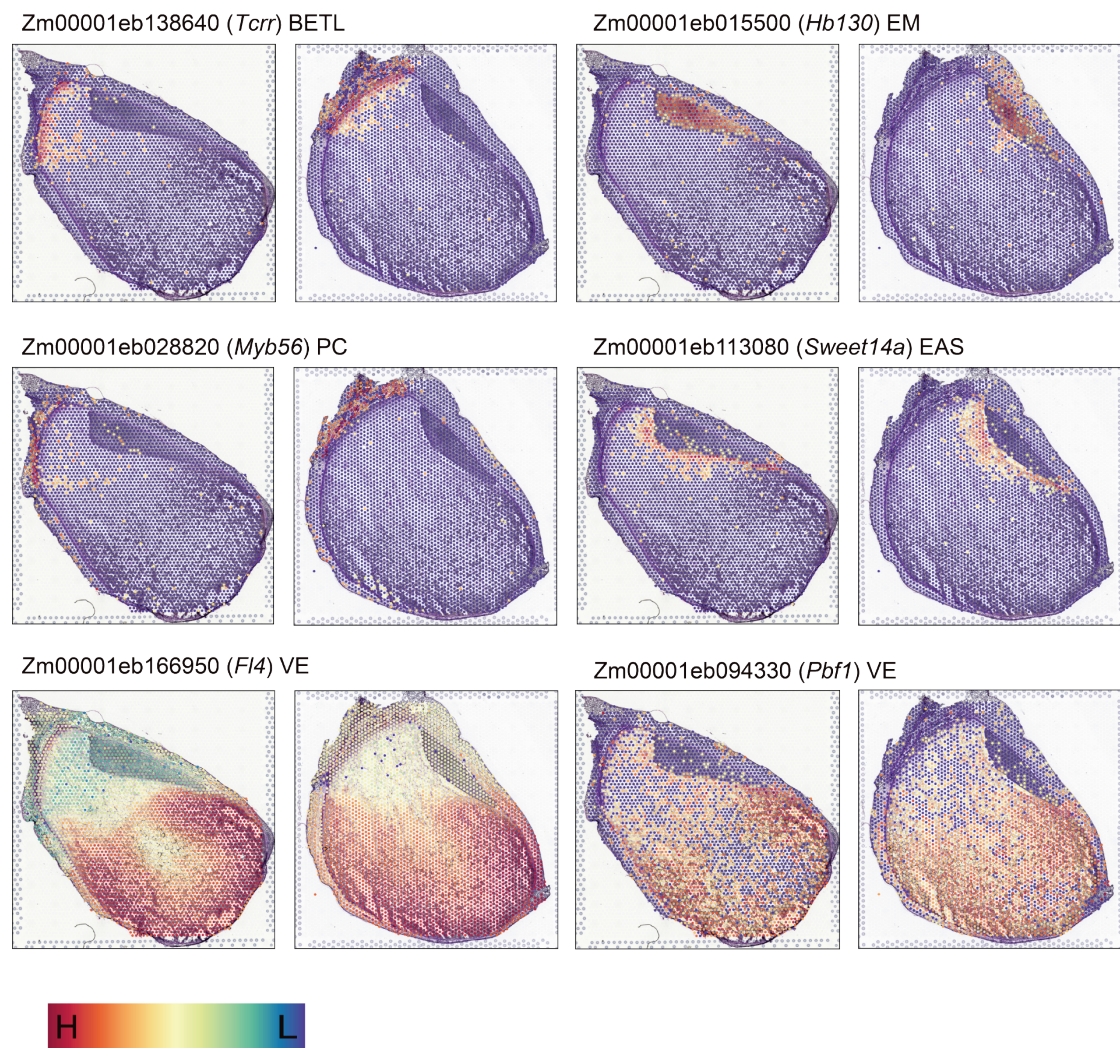


Fig. S9 Spatial expression patterns of transcription factor genes among DEGs.



Relative Expression Levle

Fig. S10 Spatial expression patterns of well-curated marker genes across different cell types.

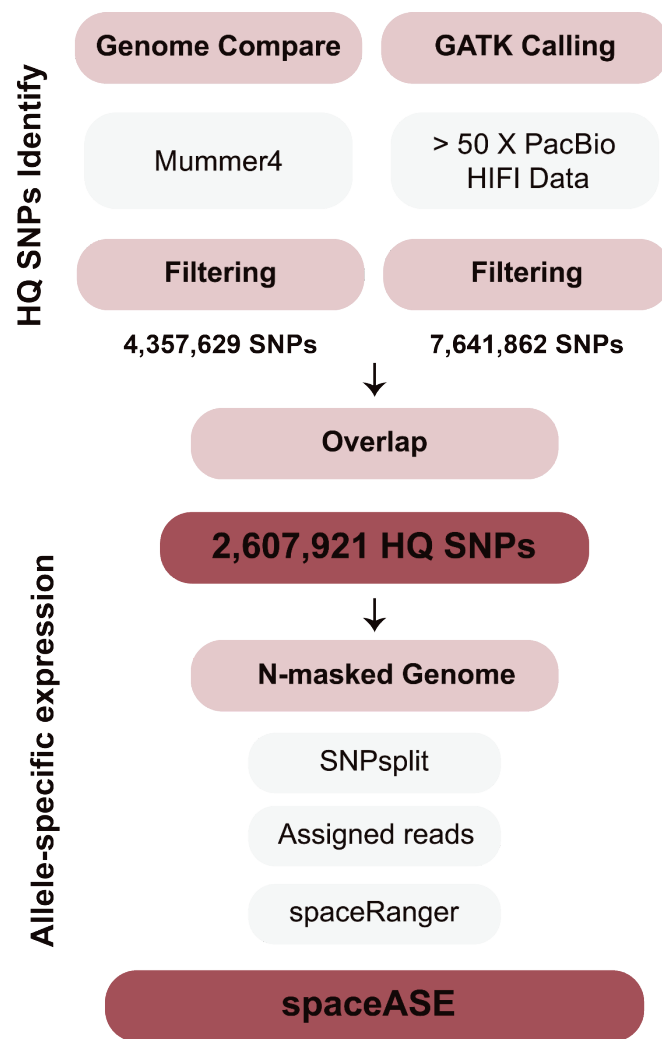


Fig. S11 The workflow of spaceASE to conduct allele-resolved spatial transcriptome analysis in B73 and Mo17 hybrids.

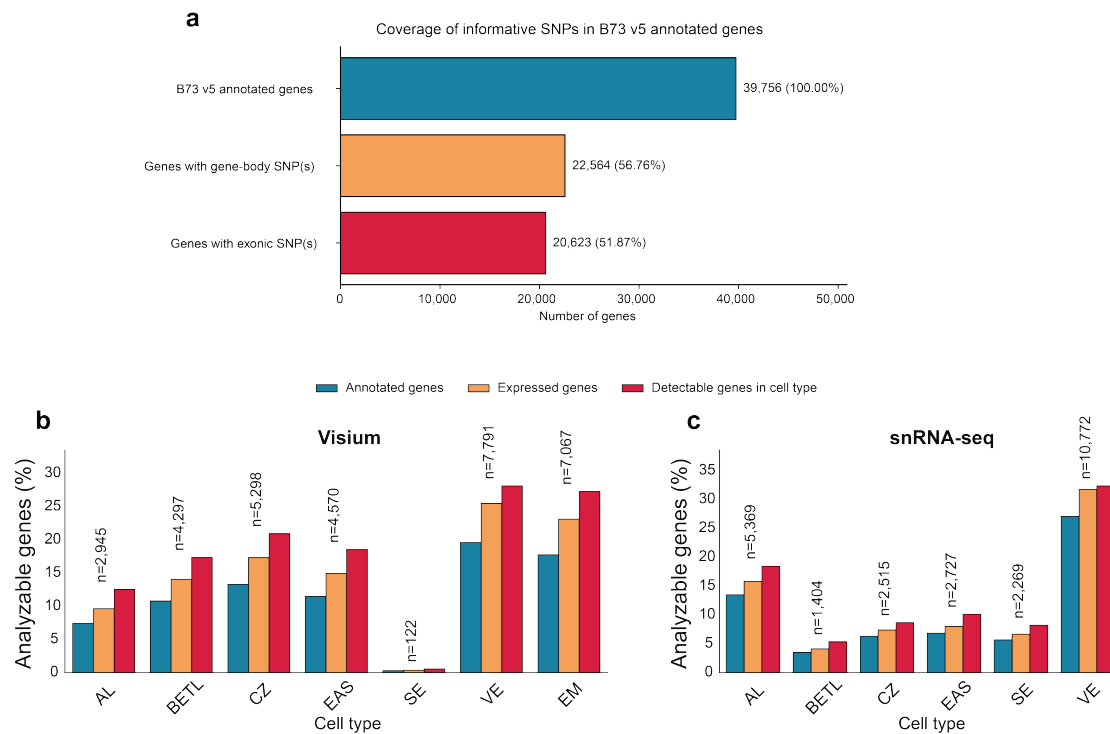


Fig. S12 Informative-SNP coverage and analyzable-gene proportions.

a Numbers and proportions of B73 v5 annotated genes containing informative SNPs in gene bodies or exons. **b** Visium analyzable genes by cell type, requiring ≥ 20 B73 + Mo17 reads in both reciprocal crosses. **c** snRNA-seq analyzable genes by cell type, requiring ≥ 20 B73 + Mo17 reads in all four libraries. Bars show proportions relative to annotated genes (annotated), genes detected across all Visium cell types (expressed), or genes detectable in the indicated cell type (cell-type-detectable genes); n indicates the number of analyzable genes.

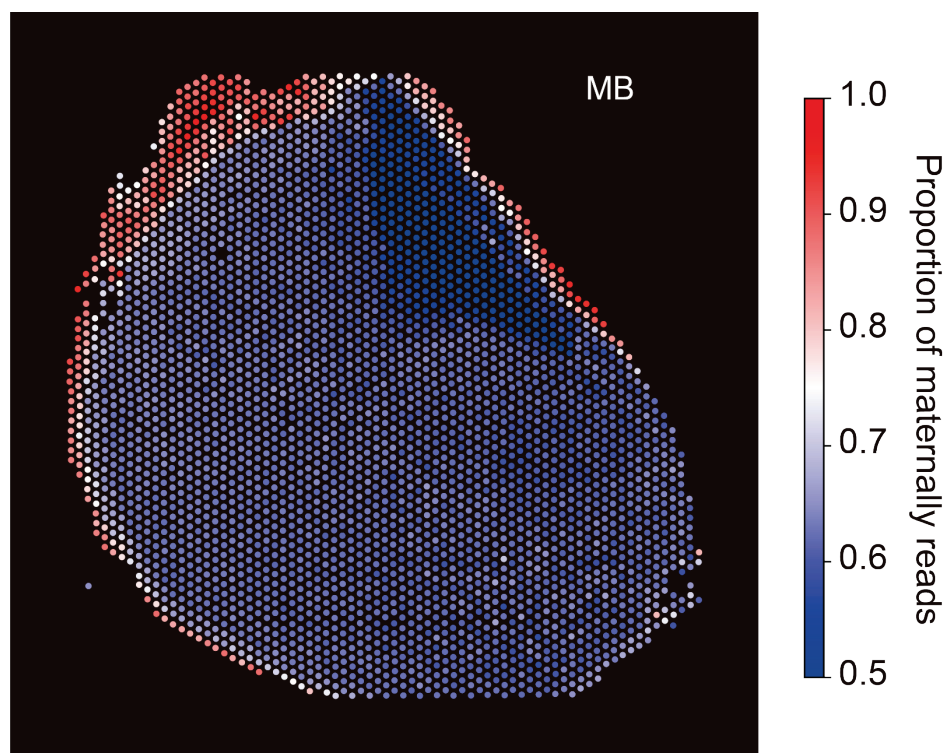


Fig. S13 Spatial plot showing the PMR value of each spot in the MB sample.

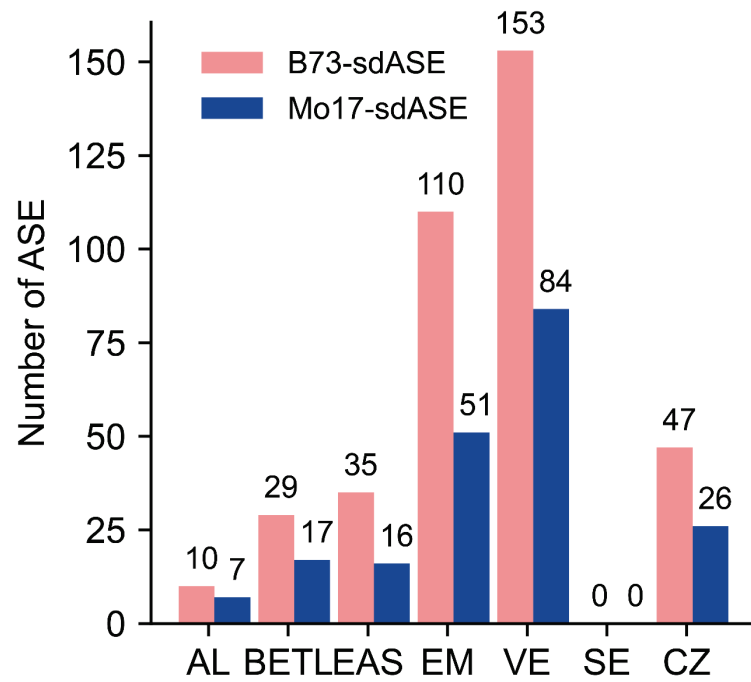


Fig. S14 Bar plot showing the number of B73-sdASE and Mo17-sdASE genes identified across different cell types.

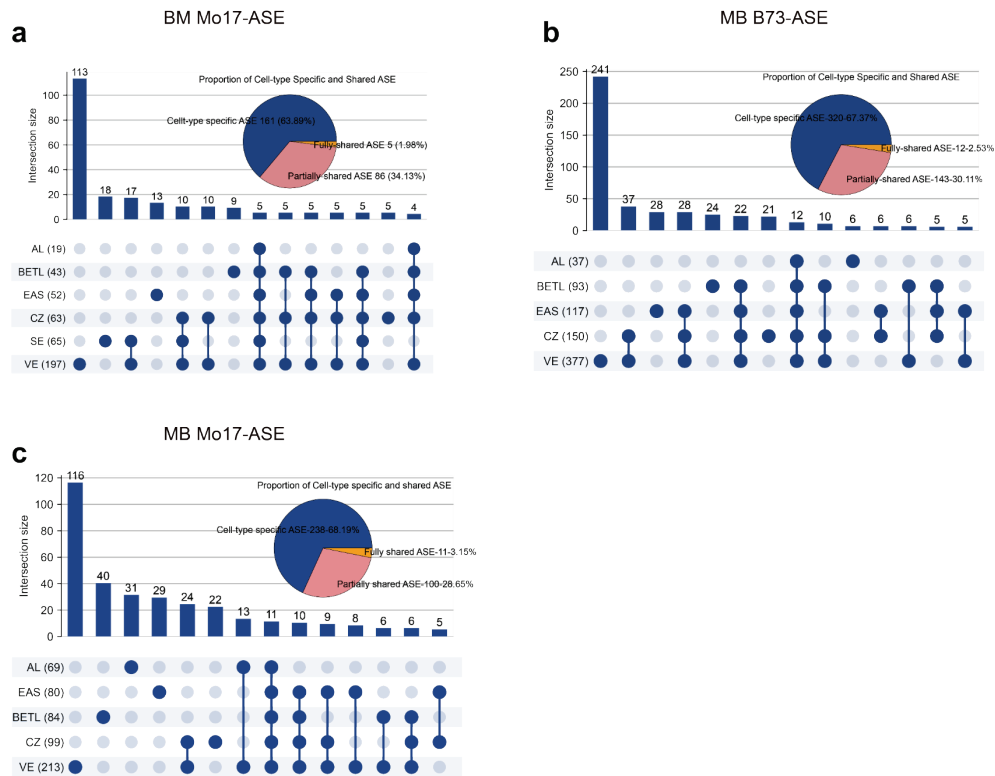


Fig. S15 UpSet plot and pie chart showing the overlap of ASE genes across different cell types.

a UpSet plot and pie chart showing the overlap of Mo17-ASE genes across different cell types in BM. **b** UpSet plot and pie chart showing the overlap of B73-ASE genes across different cell types in MB. **c** UpSet plot and pie chart showing the overlap of Mo17-ASE genes across different cell types in MB.

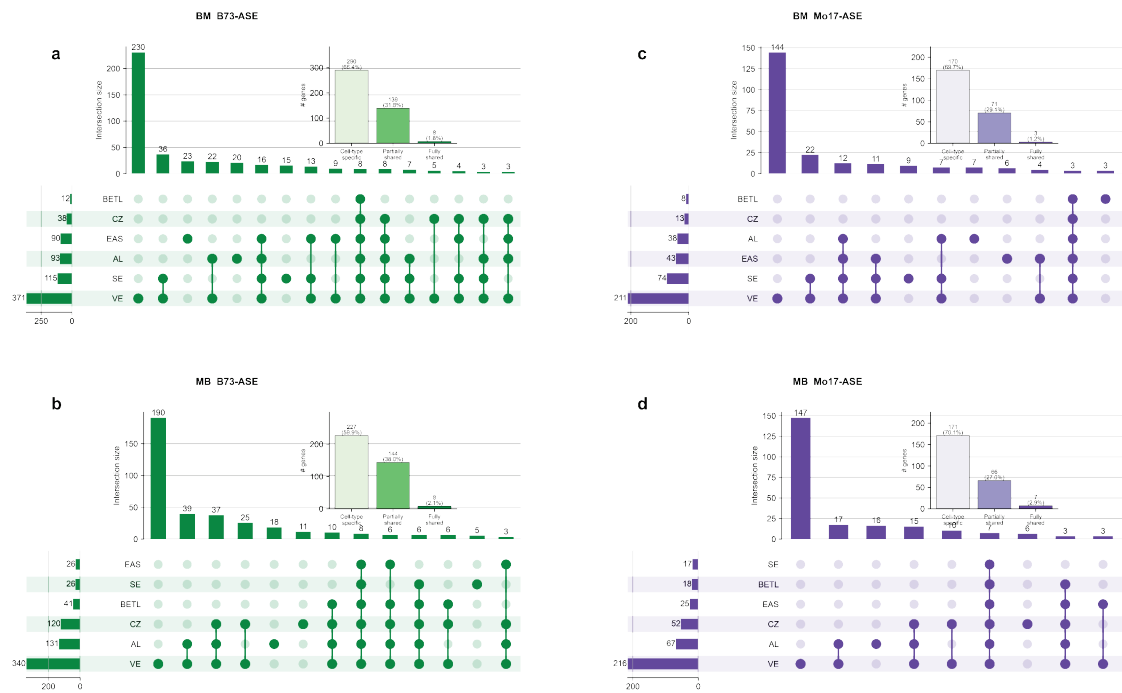


Fig. S16 UpSet plots and bar charts showing the overlap of ASE genes across different cell types in the snRNA-seq data. a UpSet plot and bar chart showing the overlap of B73-ASE genes across different cell types in BM. b UpSet plot and bar chart showing the overlap of B73-ASE genes across different cell types in MB. c UpSet plot and bar chart showing the overlap of Mo17-ASE genes across different cell types in BM. d UpSet plot and bar chart showing the overlap of Mo17-ASE genes across different cell types in MB. For each cross direction, ASE genes were called per cell type by intersecting the two replicates.

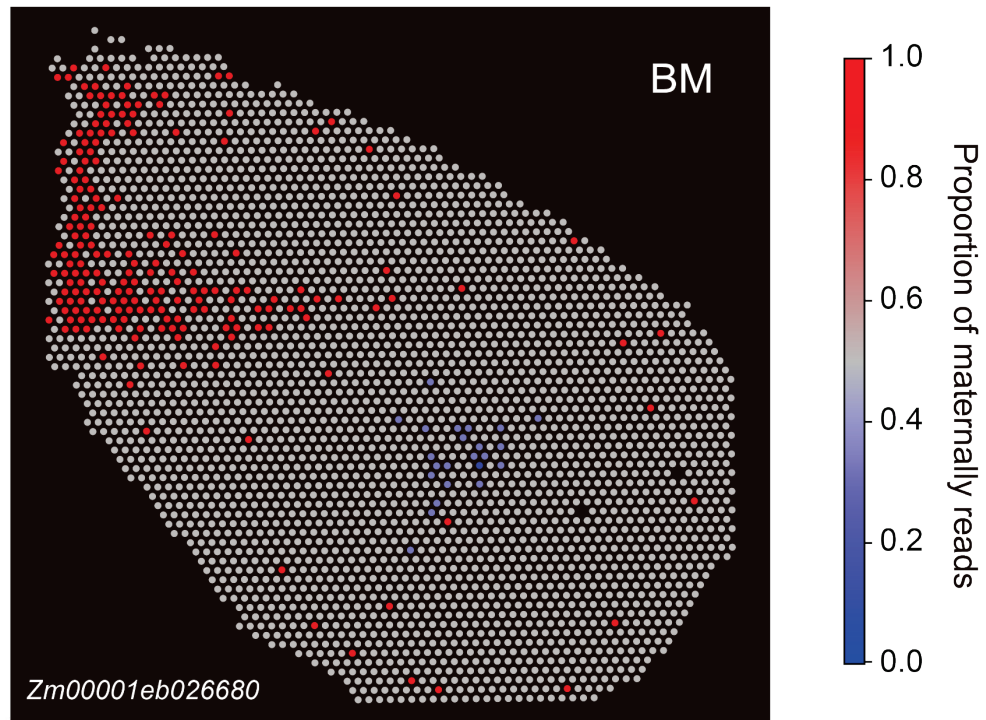


Fig. S17 Spatial PMR map shows antagonistic ASE directions of *Zm00001eb026680* in the BETL, CZ and SE of BM.

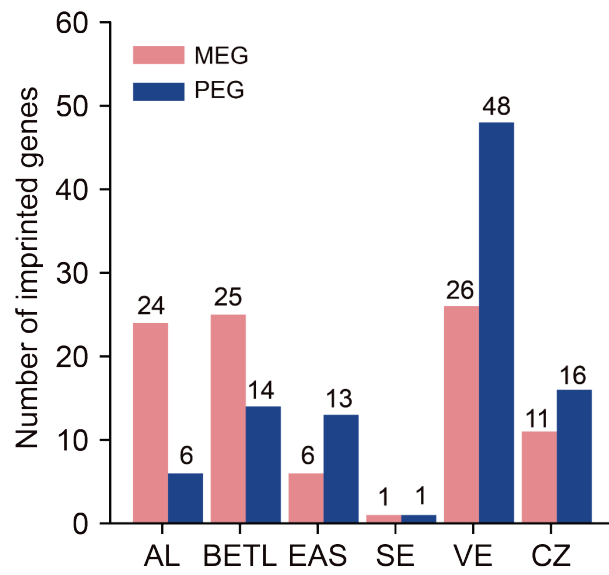
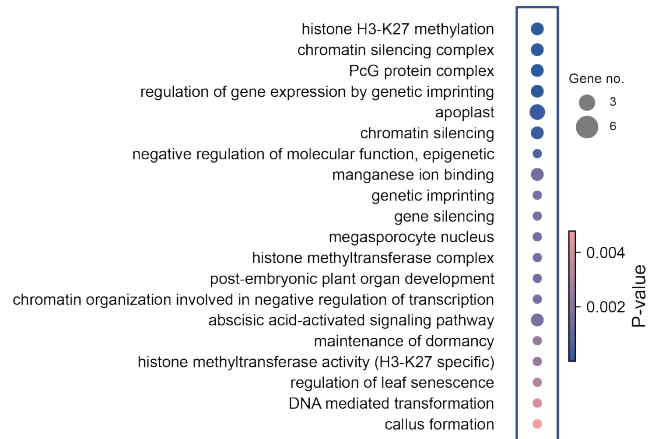


Fig. S18 Bar plot showing the number of unfiltered MEGs and PEGs identified in each cell type.

a

GO Maternally Expressed Genes

**b**

GO Paternally Expressed Genes

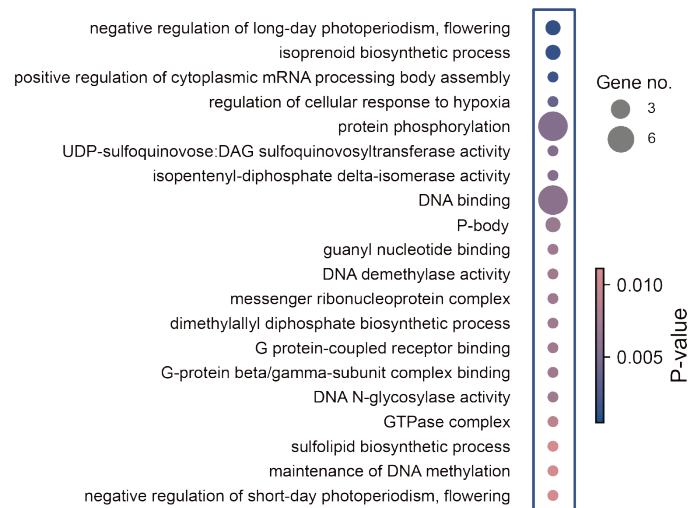


Fig. S19 Dot plot showing the terms of GO enrichment of MEGs (a) and PEGs (b).

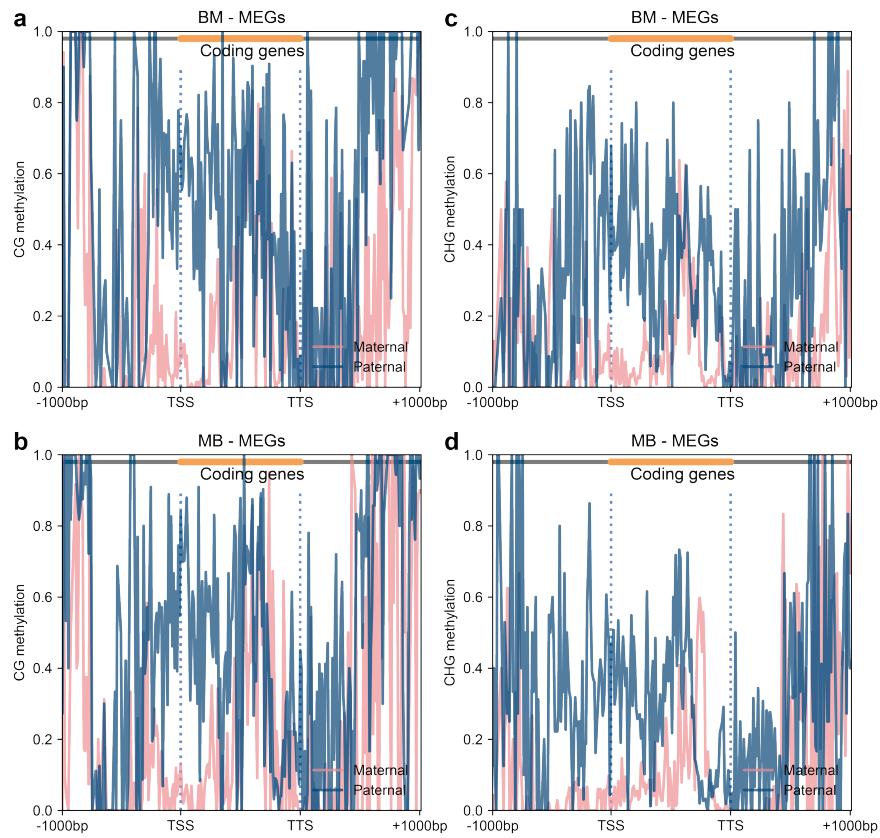


Fig. S20 a, b Metagene plot showing the CG methylation profiles for MEGs in BM and MB. c, d Metagene plot showing the CHG methylation profiles for MEGs in BM and MB.

Meg1

Score	Expect	Identities	Gaps	Strand
1227 bits(664)	0.0	664/664(100%)	0/664(0%)	Plus/Plus
Query 1		ATAGTATATATAAGTATACACTCTTCTAGGATCGGTCGTGAGGAGTTCCTTAACATTCT	60	
Sbjct 14183394		ATAGTATATATAAGTATACACTCTTCTAGGATCGGTCGTGAGGAGTTCCTTAACATTCT	14183453	
Query 61		TGCGACATGGAGTACAAAAAGAGGGTGGATGCGCTAGTGTTTTCTCTTTACTTCTCCTA	120	
Sbjct 14183454		TGCGACATGGAGTACAAAAAGAGGGTGGATGCGCTAGTGTTTTCTCTTTACTTCTCCTA	14183513	
Query 121		GGATACTTTGCTGCTCATGCGCATGGGAACGGTAAGTGAAACTATACAGACATGTGTGT	180	
Sbjct 14183514		GGATACTTTGCTGCTCATGCGCATGGGAACGGTAAGTGAAACTATACAGACATGTGTGT	14183573	
Query 181		GCATGCTTAGATAGTGTAGACAATTTAGAAGATGTTATTATATGATACCATGTGTATC	240	
Sbjct 14183574		GCATGCTTAGATAGTGTAGACAATTTAGAAGATGTTATTATATGATACCATGTGTATC	14183633	
Query 241		ATGGCGAATTGCTAATATATCAAAATCCCCTATGTTAAATTACTCAAATAATTGGACAT	300	
Sbjct 14183634		ATGGCGAATTGCTAATATATCAAAATCCCCTATGTTAAATTACTCAAATAATTGGACAT	14183693	
Query 301		ATCTACAAATGAGTAGATCTTTTGCTAAATAATTTGGGATGTAATTATTCTCGAGGCAT	360	
Sbjct 14183694		ATCTACAAATGAGTAGATCTTTTGCTAAATAATTTGGGATGTAATTATTCTCGAGGCAT	14183753	
Query 361		TTGTTGGTAACAGAACTCTTATCCTTTACCTTCTACTAGGTCATGTCACGGATGATGTCA	420	
Sbjct 14183754		TTGTTGGTAACAGAACTCTTATCCTTTACCTTCTACTAGGTCATGTCACGGATGATGTCA	14183813	
Query 421		ATGTTTCTGCTCCAGCTGAAGAAGGAATTTTGCAGAAAAAGAGCACAATGCGCTCAAG	480	
Sbjct 14183814		ATGTTTCTGCTCCAGCTGAAGAAGGAATTTTGCAGAAAAAGAGCACAATGCGCTCAAG	14183873	
Query 481		GGTTTCTCCATGCAAGATAACAAGTGTCTACTGTTGCATTGGGGCCGAACATCATGATT	540	
Sbjct 14183874		GGTTTCTCCATGCAAGATAACAAGTGTCTACTGTTGCATTGGGGCCGAACATCATGATT	14183933	
Query 541		GCTACTATACGATGGCTCAGTGTAGTCATGCATGCTTCTAATCAAAATTAAAGATCACTG	600	
Sbjct 14183934		GCTACTATACGATGGCTCAGTGTAGTCATGCATGCTTCTAATCAAAATTAAAGATCACTG	14183993	
Query 601		TTTTTATATACAATGTAATGGTAGGCAATGCTATTAATAATACATAAGGGAAATTTAGTT	660	
Sbjct 14183994		TTTTTATATACAATGTAATGGTAGGCAATGCTATTAATAATACATAAGGGAAATTTAGTT	14184053	
Query 661		TTGG 664		
Sbjct 14184054		TTGG 14184057		

Fig. S21 BLAST alignment of the *Meg1* gene to the Mo17 T2T genome sequence.

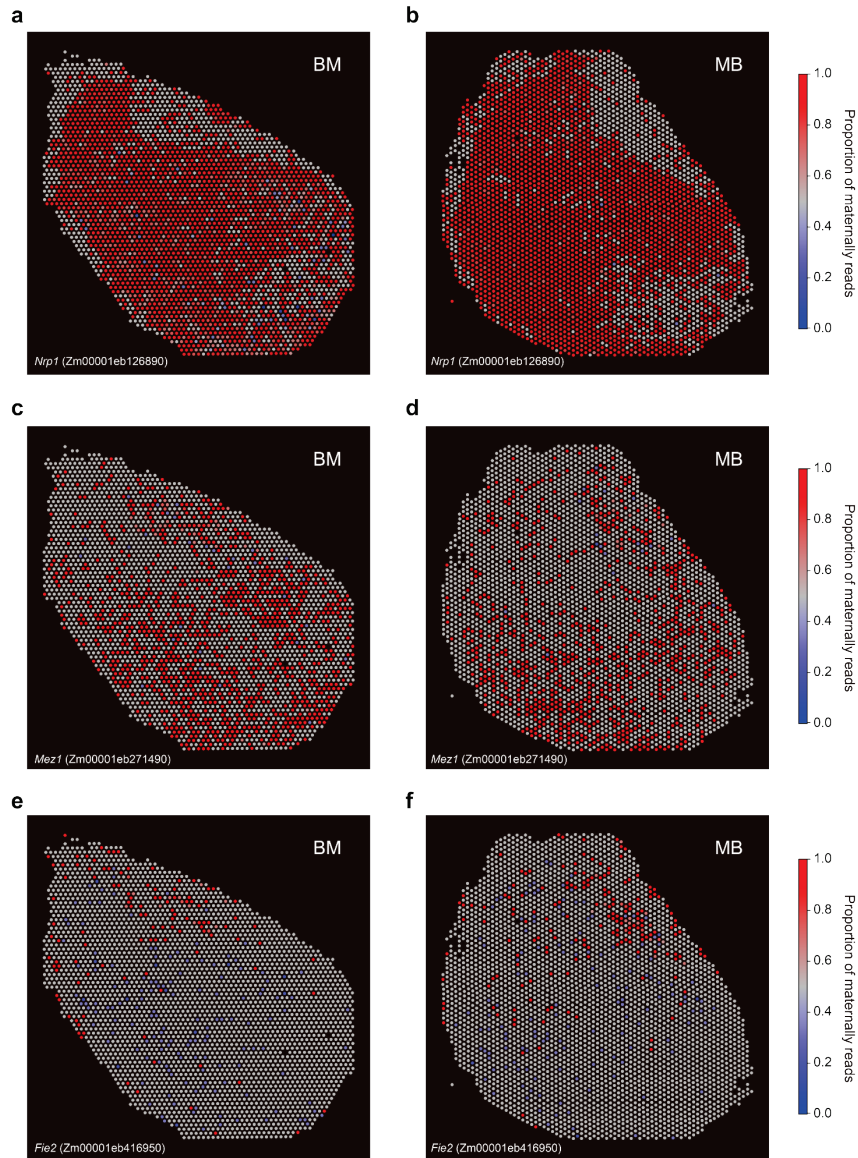


Fig. S22 Spatial PMR maps showing imprinting patterns.

a, b Spatial PMR maps showing imprinting patterns of the maternally expressed *Nrpl* (Zm00001eb126890) in BM (a) and MB (b). **c, d** Spatial PMR maps showing imprinting patterns of the maternally expressed *Mez1* (Zm00001eb271490) in BM (c) and MB (d). **e, f** Spatial PMR maps showing imprinting patterns of the *Fie2* (Zm00001eb416950) in BM (e) and MB (f).

Fie1 (Zm00001eb173090)

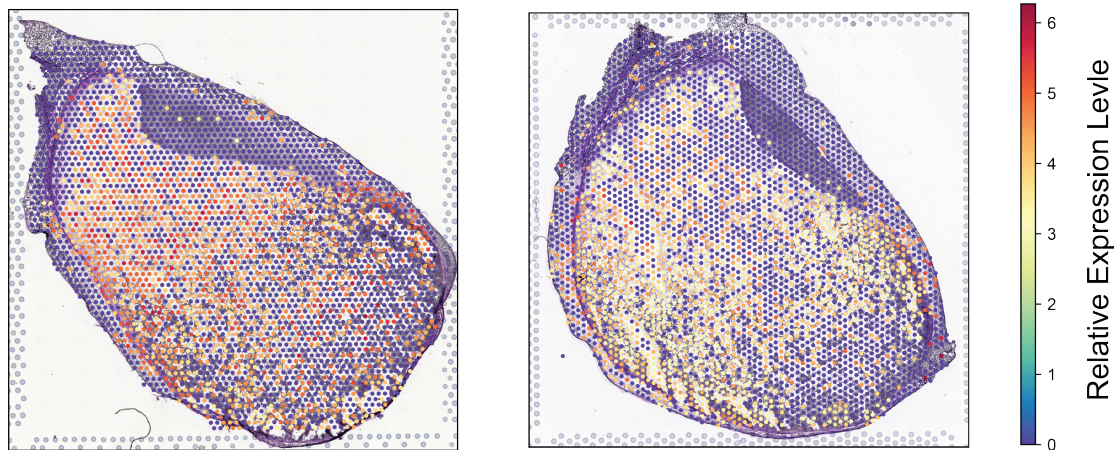


Fig. S23 Spatial expression patterns of the maternally expressed *Fie1* (Zm00001eb173090) in BM (left) and MB (right).

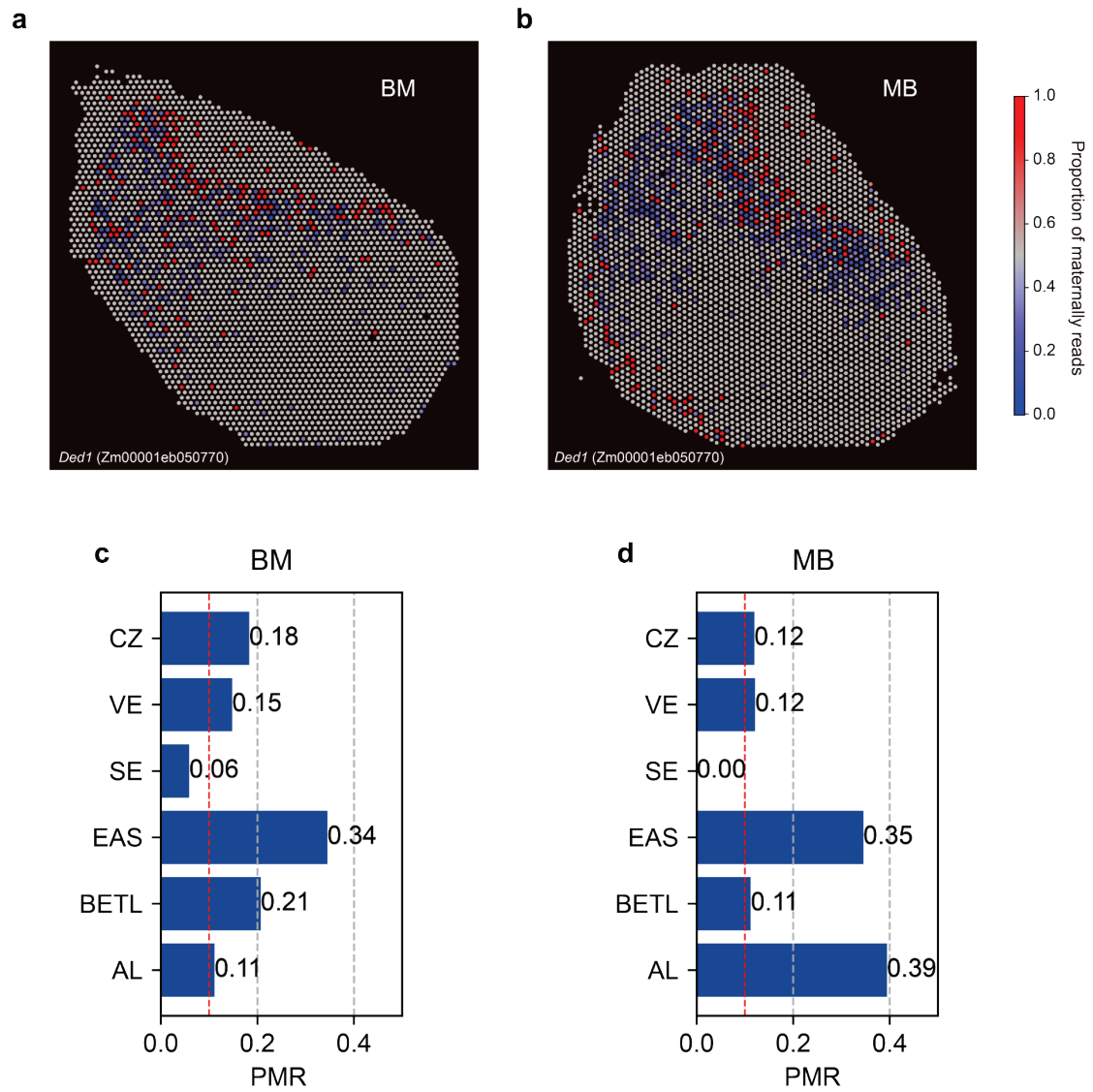


Fig. S24 PMR information of *Ded1*.

a, b Spatial PMR maps showing imprinting patterns of the *Ded1* (Zm00001eb050770) in BM (a) and MB (b). **c, d** PMR values of *Ded1* (Zm00001eb050770) across different cell types in BM (c) and MB (d).

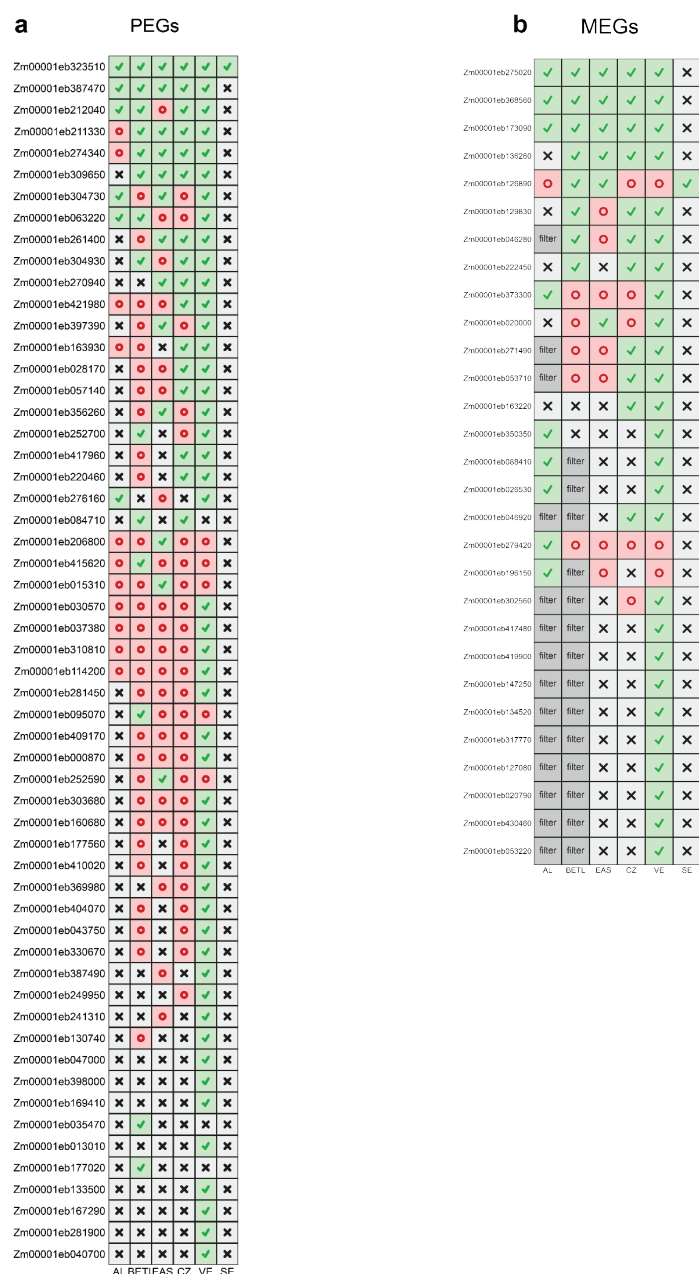


Fig. S25 Heatmap showing the imprinting status of PEGs (a) and MEGs (b) across six endosperm cell types. Green check marks indicate imprinted genes in each cell type, red circles denote non-imprinted genes with sufficient counts, black crosses represent genes with insufficient counts, and “filter” indicates genes excluded by filtering.

Ippi1 (Zm00001eb323510)

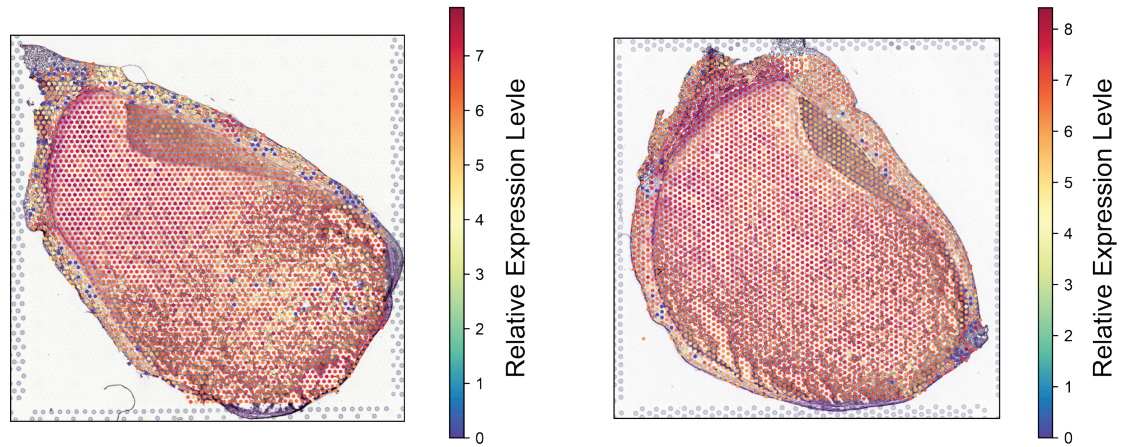


Fig. S26 Spatial expression patterns of the paternally expressed *Ippi1* (Zm00001eb323510) in BM (left) and MB (right).

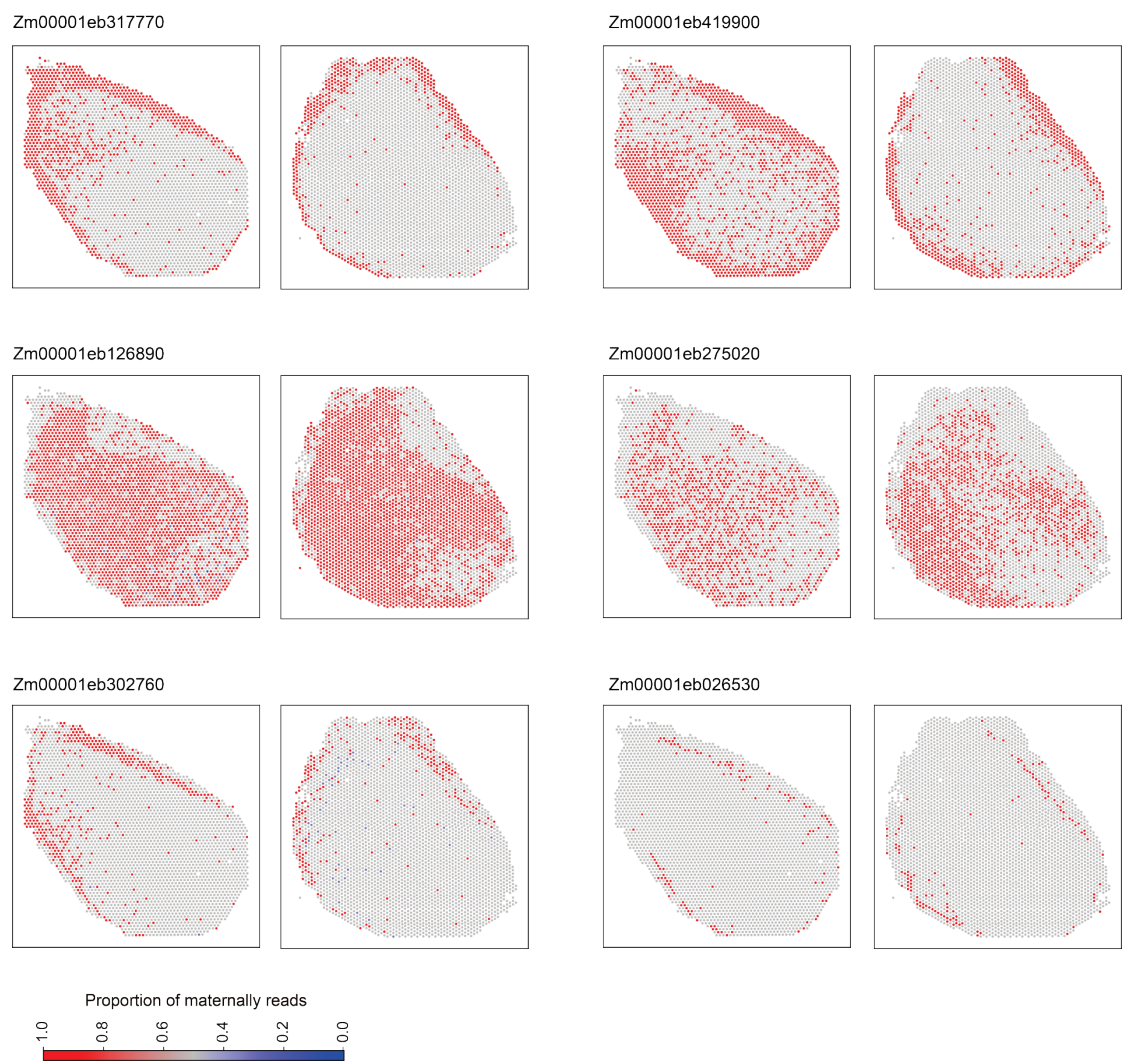


Fig. S27 Spatial PMR plots of six imprinted genes in the EM.

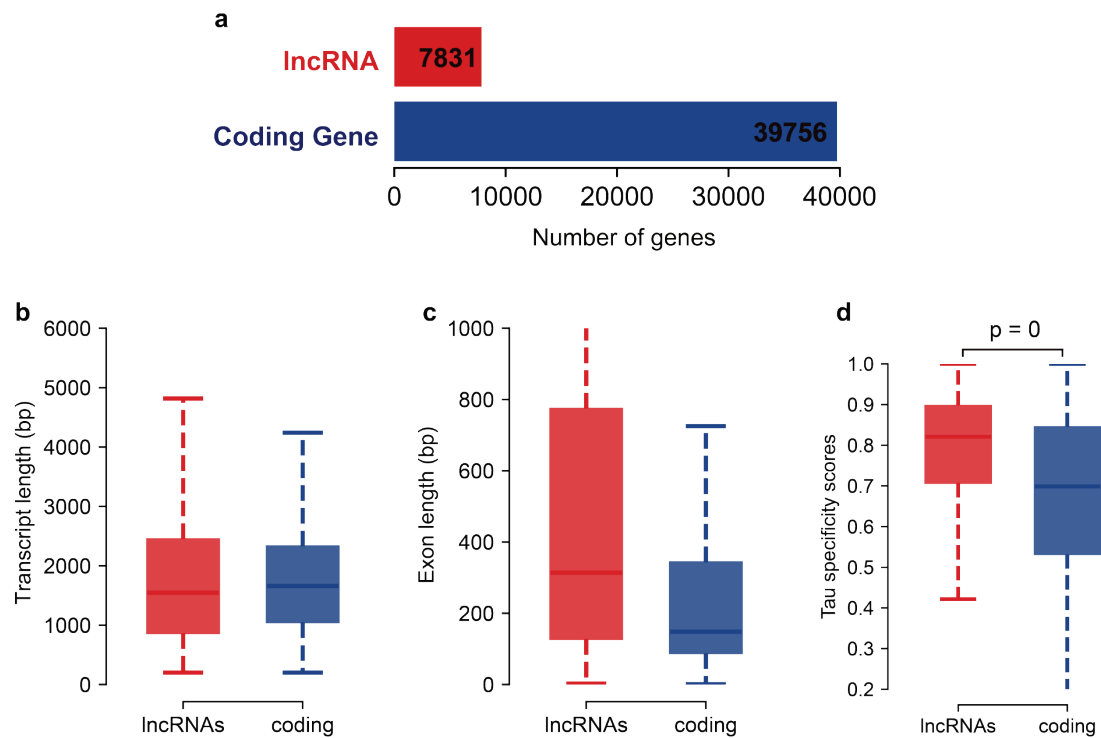


Fig. S28 Overview of coding genes and lncRNAs.

a Bar plot showing the numbers of identified lncRNAs and coding genes annotated in the reference genome. **b, c** Box plots showing transcript length (b) and exon length (c) of lncRNAs and protein-coding genes. **d** Box plot showing cell-type specificity of lncRNAs and coding genes.

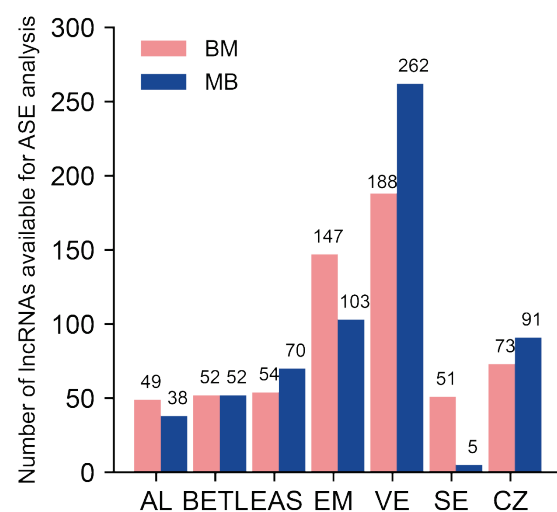


Fig. S29 Bar plot showing the number of lncRNAs available for ASE analysis in each cell type of BM and MB.

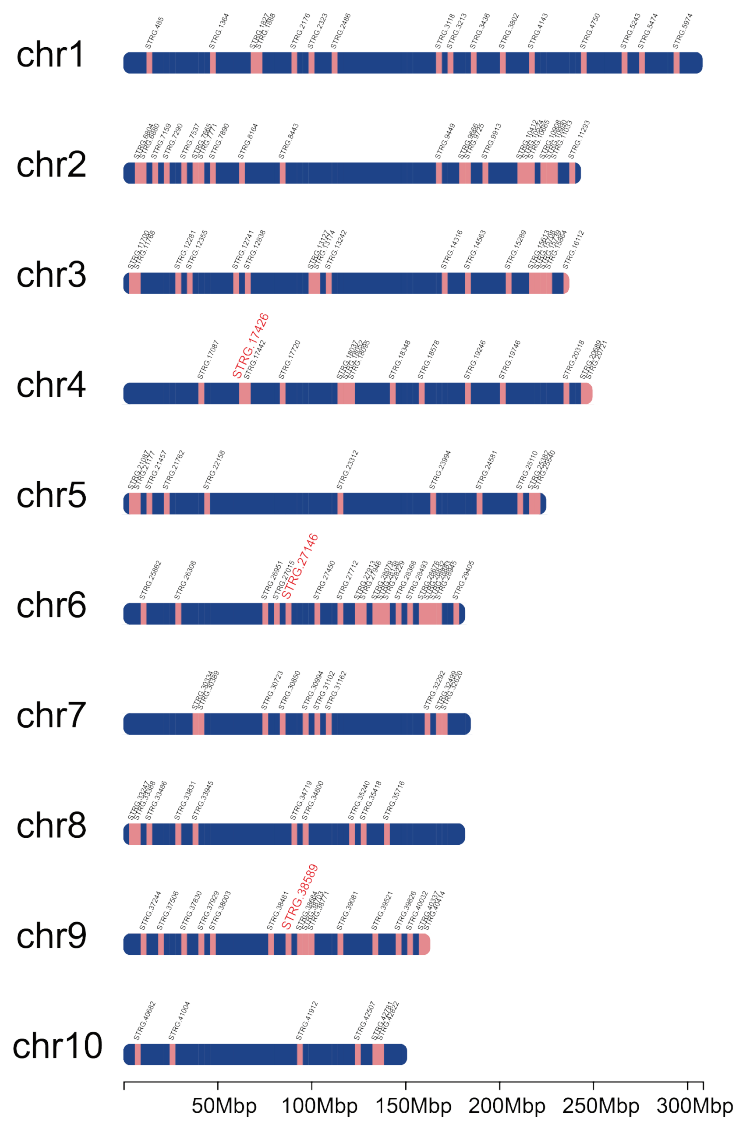


Fig. S30 Chromosomal distribution of ASE lncRNAs, with maternally expressed lncRNAs shown in red.

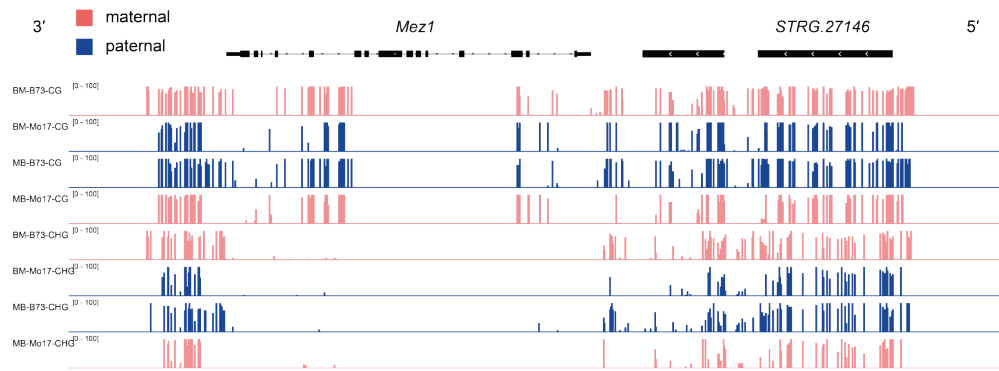


Fig. S31 IGV plot showing allele-resolved CG and CHG methylation levels of the *Mez1* and *STRG.27146* loci in reciprocal hybrid embryos. Red and blue tracks indicate maternal and paternal alleles, respectively.

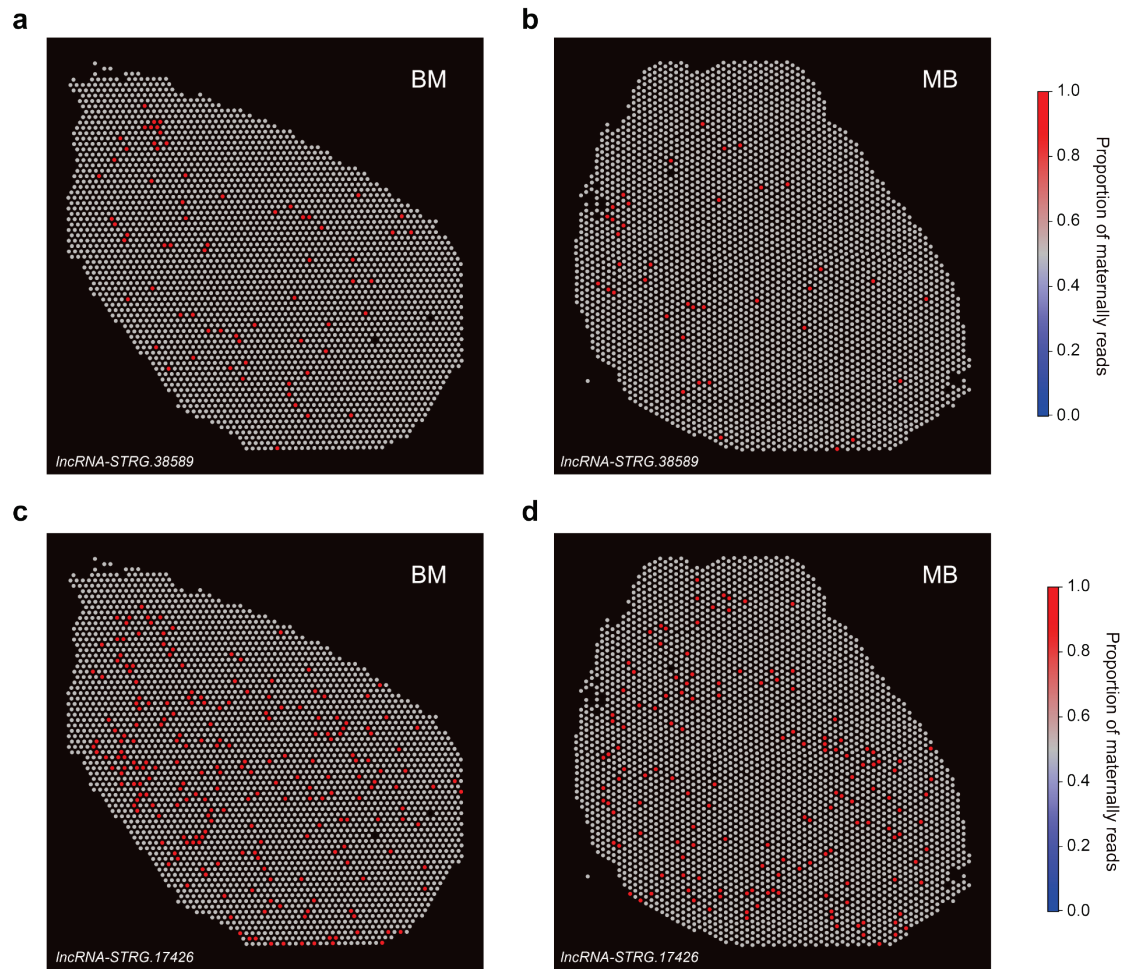


Fig. S32 Spatial imprinting status of lncRNAs.

a, b Spatial PMR maps showing imprinting patterns of the maternally expressed *lncRNA-STRG.38589* in BM (a) and MB (b). **c, d** Spatial PMR maps showing imprinting patterns of the maternally expressed *lncRNA-STRG.17426* in BM (c) and MB (d).

STRG.38589

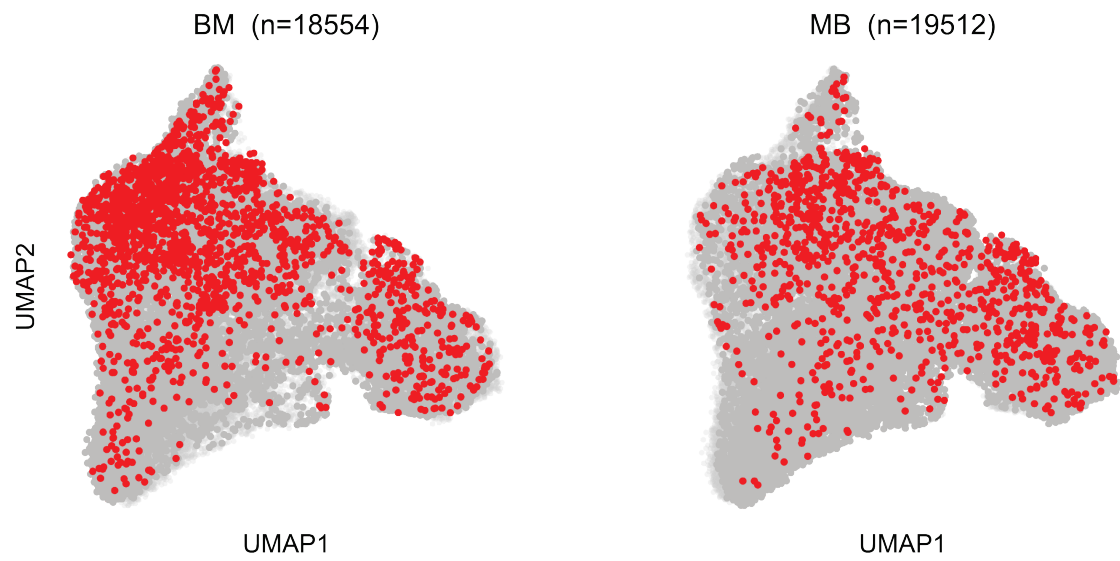


Fig. S33 snRNA-seq UMAP PMR plots showing imprinting status of the maternally expressed *STRG.38589* in BM and MB.

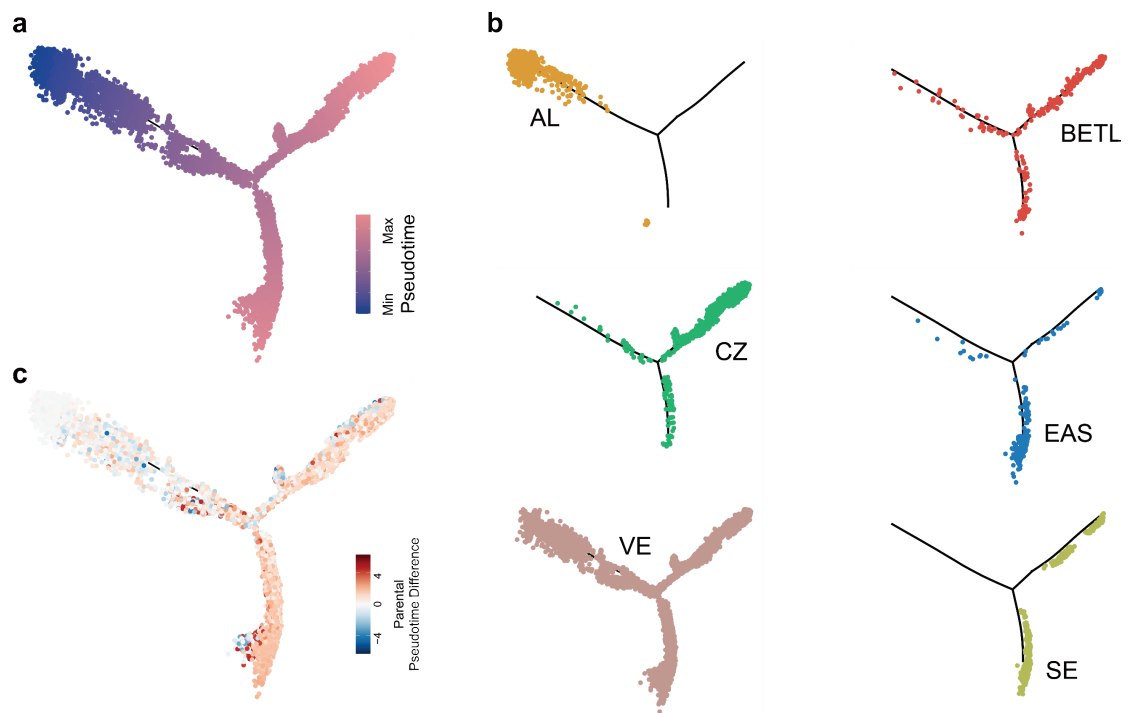


Fig. S34 Haplotype-resolved single-nucleus pseudotime analysis and developmental trajectory of maize endosperm in MB.

a Single-nucleus pseudotime analysis of MB endosperm nuclei using Monocle2. **b** Distribution of the six endosperm cell types (AL, BETL, CZ, EAS, VE and SE) along the snRNA-seq pseudotime trajectory in MB. **c** Parental pseudotime difference projected onto the MB snRNA-seq developmental trajectory. For each nucleus, the parental pseudotime difference was calculated as the maternal-allele-derived pseudotime minus the paternal-allele-derived pseudotime.