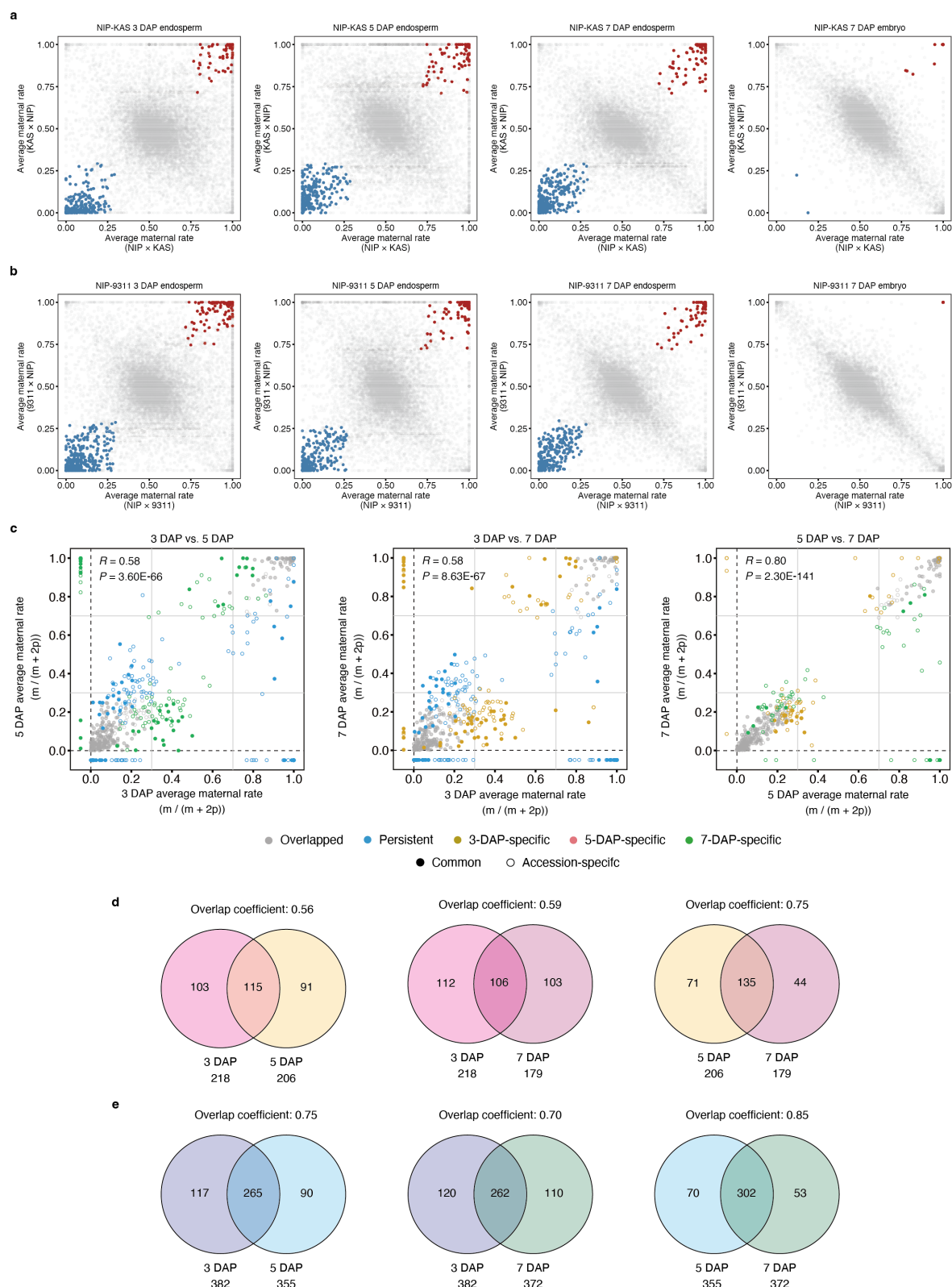


Multilayered epigenetic control of persistent and stage-specific imprinted genes in rice endosperm

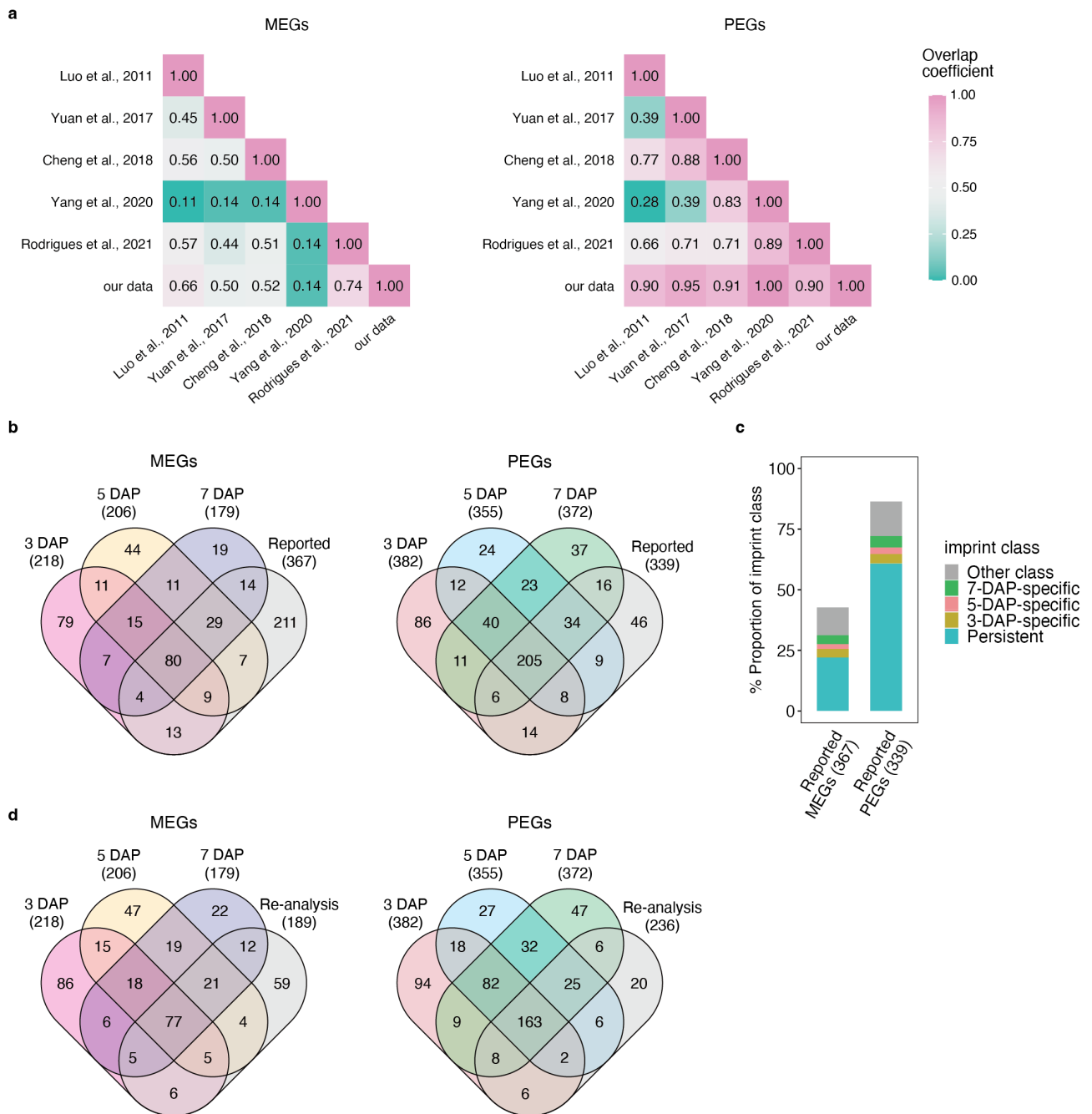
In the format provided by the
authors and unedited



Supplementary Fig. 1 (Supports Fig. 1). Identification of imprinted genes from the different stages of the F₁ endosperm and embryo.

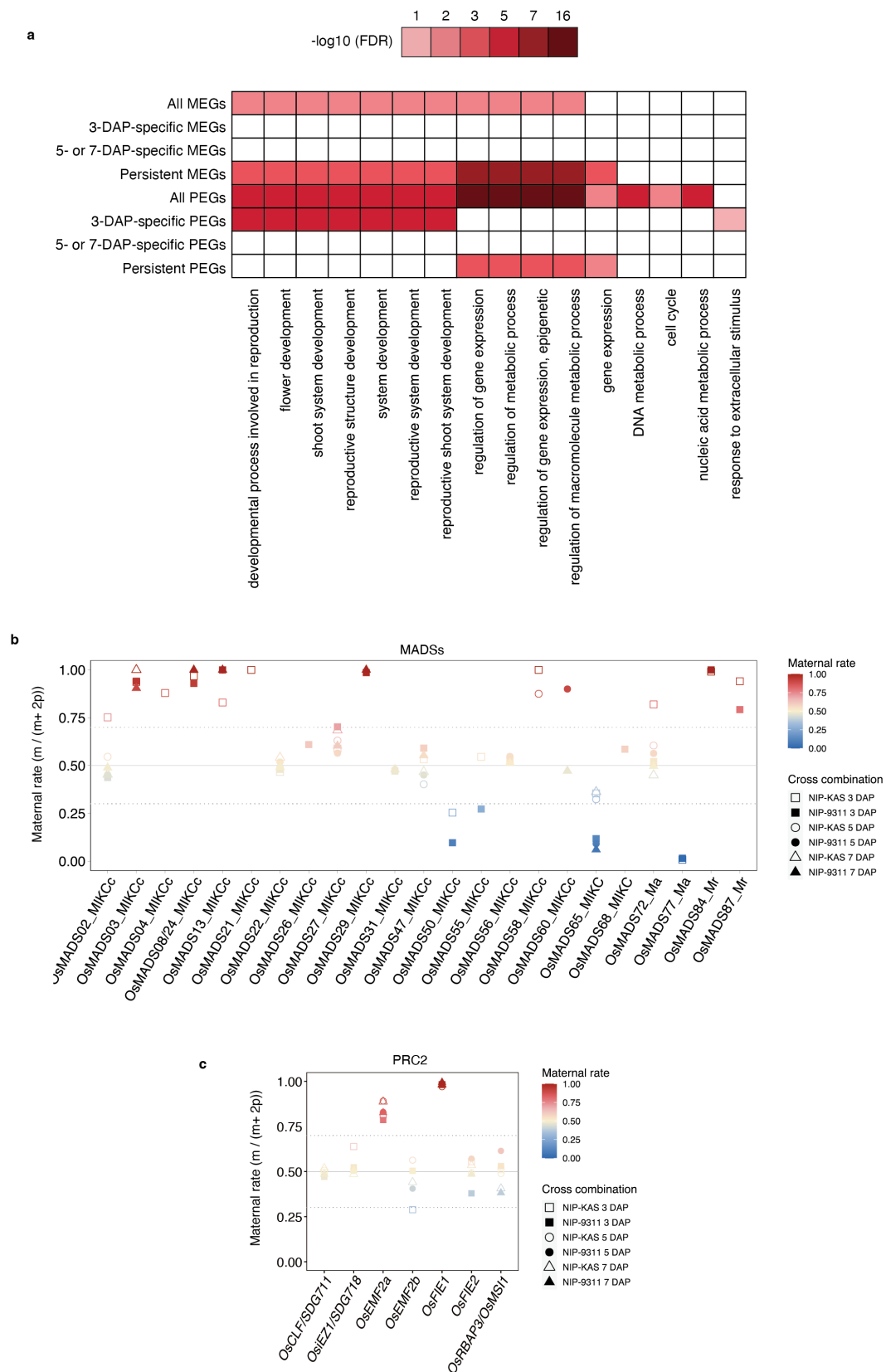
a,b, Scatterplots of maternal rates in the F₁ endosperm at 3, 5, and 7 DAP and in F₁ embryos at 7 DAP from two reciprocal crosses, NIP-KAS (**a**) and NIP-9311 (**b**). The red (MEGs) and blue (PEGs) points represent imprinted genes. Gray points are bi-allelically expressed genes. The two axes represent the average maternal rate of two replicates in each cross direction. **c**, Scatterplots of average maternal rates of the imprinted genes in the pairwise comparisons of 3 DAP vs. 5 DAP (left), 3 DAP vs. 7 DAP (middle), and 5 DAP vs. 7 DAP (right). Gray points represent imprinted genes identified at two comparative stages. Colored points represent stage-specific imprinted genes detected only in one of two stages: 3 (blue), 5 (green), or 7 (yellow)

DAP. Closed and open points show allele- and locus-specific imprinted genes, respectively. The genes plotted outside of the two stippled axes indicate expression in a single stage but no detectable expression in the other stage. The two axes represent average maternal rates ($\text{maternal}/[\text{maternal} + 2 \times \text{paternal}]$). Thresholds for maternal rates for MEGs (0.7) and PEGs (0.3) are indicated. R indicates Pearson's correlation coefficient between the two stages. **d,e**, Venn diagrams showing the number of overlapping imprinted genes, MEGs (**d**) and PEGs (**e**), common to two developmental stages: 3 DAP vs. 5 DAP (left), 3 DAP vs. 7 DAP (middle), and 5 DAP vs. 7 DAP (right). An overlap coefficient was used to quantify the overlap between the two gene sets.



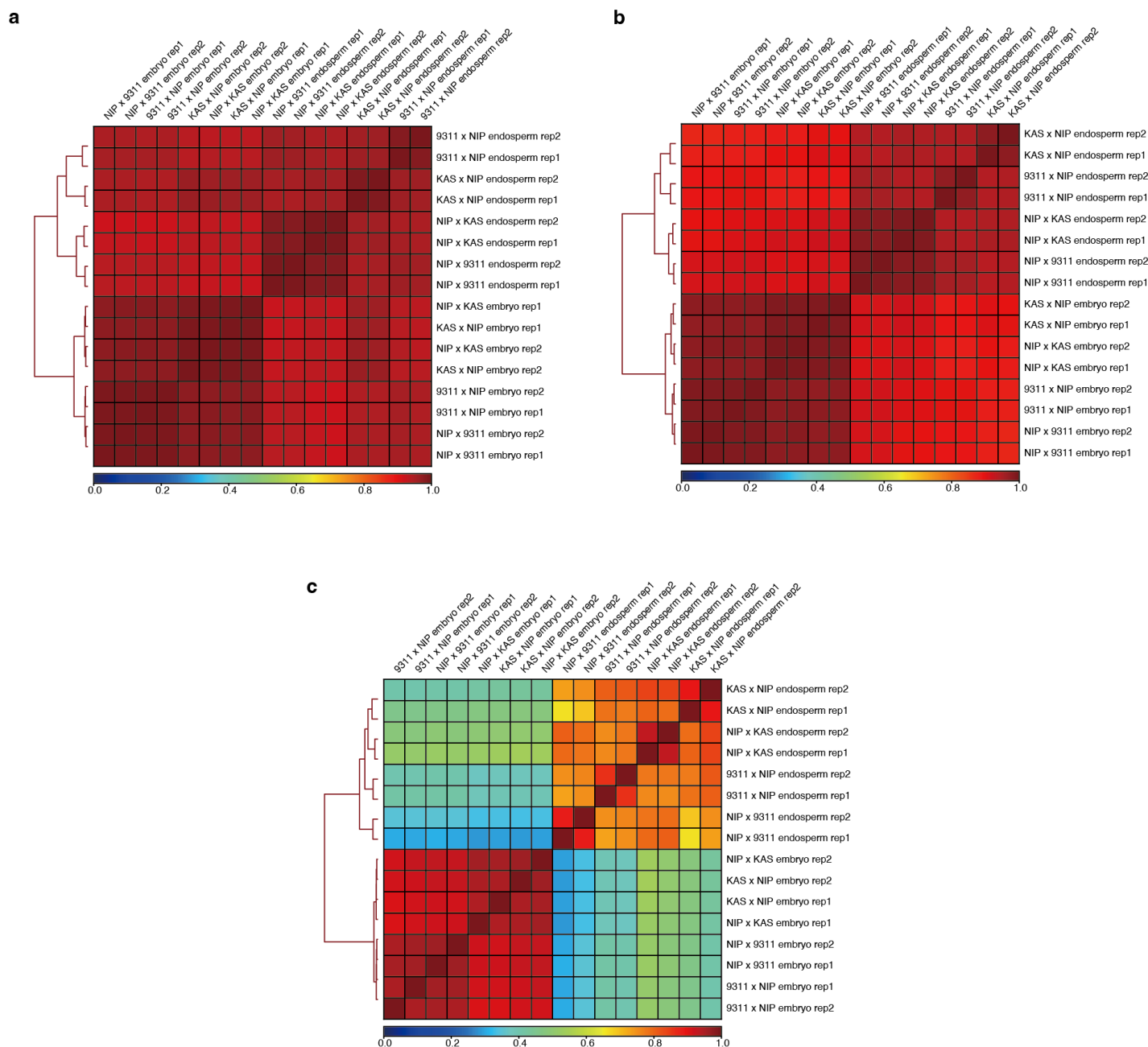
Supplementary Fig. 2 (Supports Fig. 1). Comparisons of imprinted genes among data sets in this study and the studies shown.

a, Heatmap representing the overlap coefficient of MEGs (left) and PEGs (right) between our data sets and five data sets reported in the literature. Cheng et al. (2018) used only imprinted genes with a proportion of maternal alleles >0.8 or <0.4 in a reciprocal cross. **b**, Venn diagrams showing the overlap of imprinted genes across our data sets and previously reported imprinted genes (number of genes). **c**, Proportions of different classes of imprinted genes among the imprinted genes overlapping between our data sets and previously reported imprinted genes. **d**, Venn diagrams showing the number of imprinted genes overlapping between our data sets and the imprinted genes of two available data sets from Yuan et al. (2017) and Rodrigues et al. (2021) re-analyzed using our criteria.



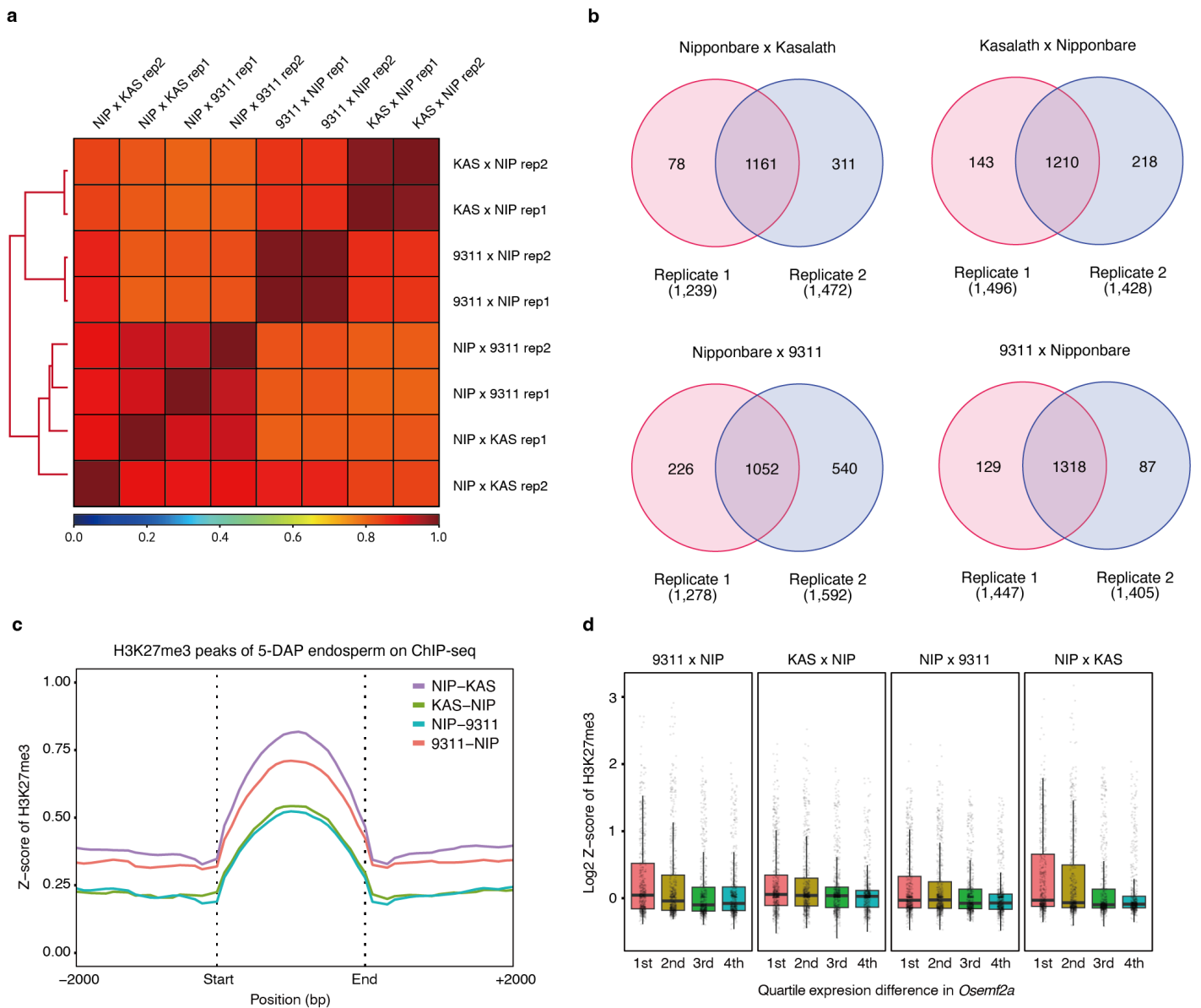
Supplementary Fig. 3 (Supports Fig. 1) Biological functions of imprinted genes.

a, Gene Ontology analysis of stage-specific and persistent imprinted genes. Heatmap of significantly enriched Gene Ontology terms for each class of imprinted genes. *P*-values were calculated using a hypergeometric test and adjusted by Bonferroni methods. **b,c**, Average maternal rates of genes encoding MADS-box transcription factors (**b**) and components of the PRC2 (**c**). Color gradient represents the maternal rate (maternal/[maternal + 2 × paternal]) of genes. Closed and open symbols represent developmental stages of endosperm in each cross combination.



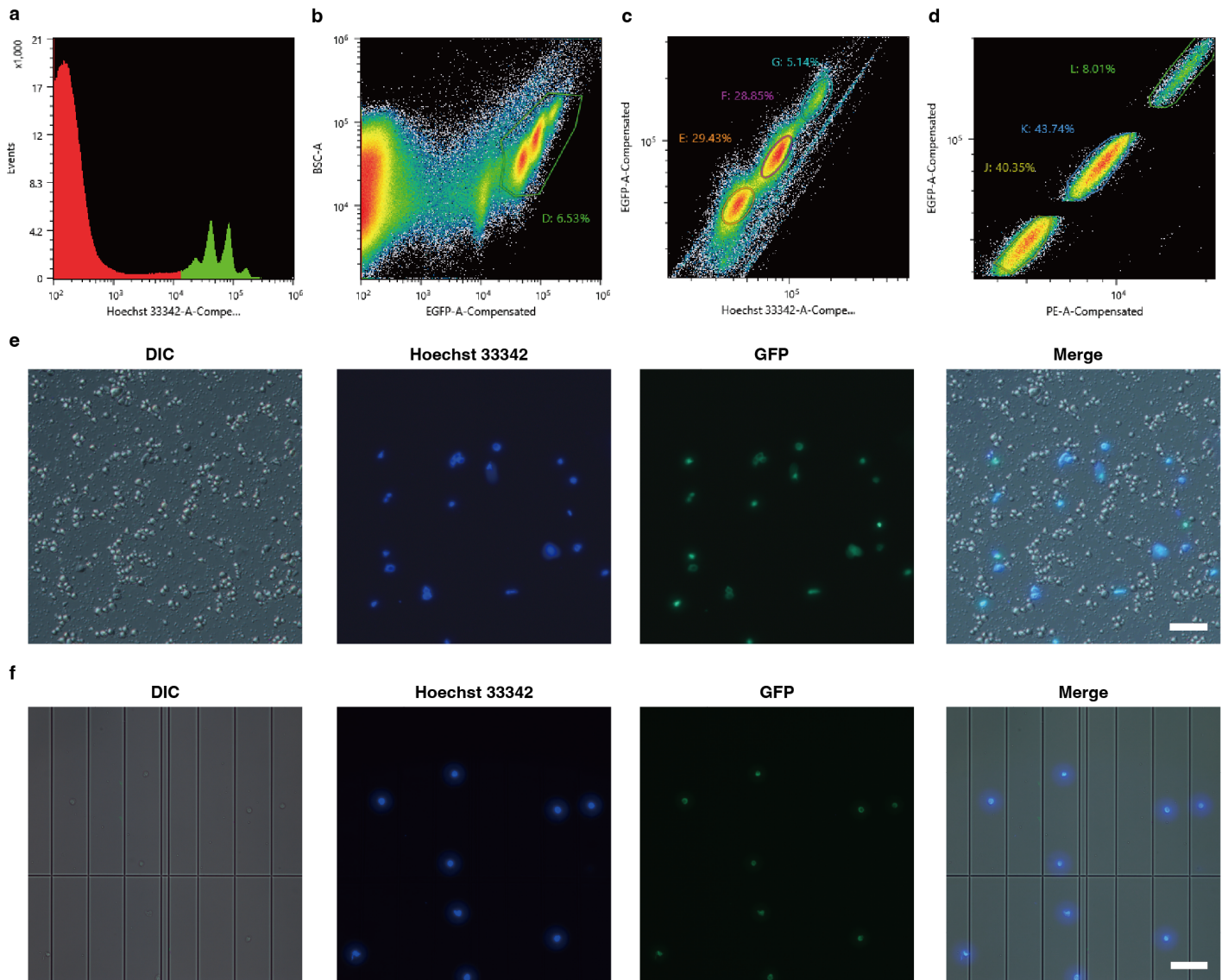
Supplementary Fig. 4 (Supports Fig. 2). Comparisons of whole-genome bisulfite sequencing data

a-c, Pearson correlation heatmap of whole-genome bisulfite sequencing data sets for CG (**a**), CHG (**b**), and CHH (**c**) contexts in the endosperm and embryo from two combinations of reciprocal crosses with two biological replicates.



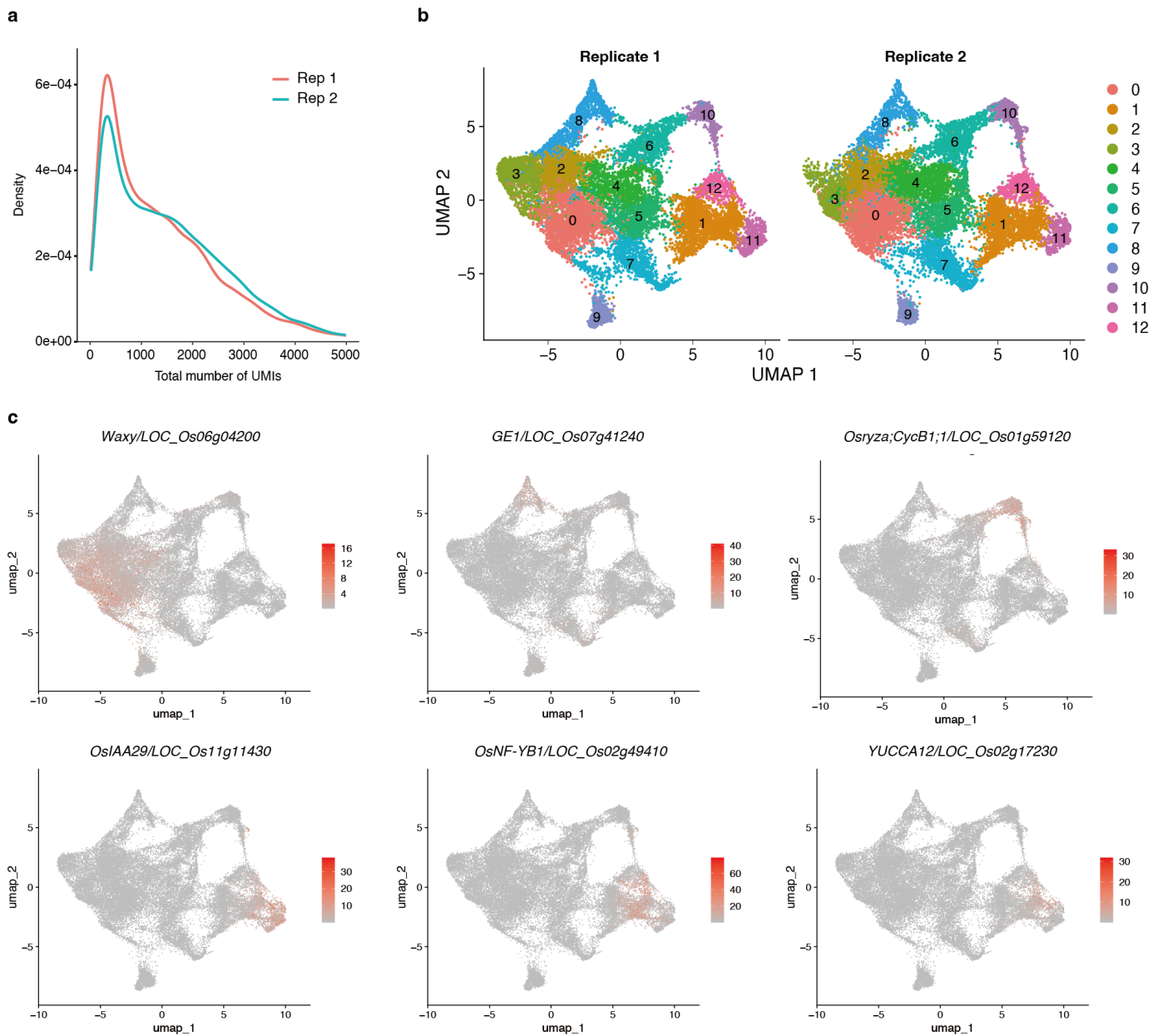
Supplementary Fig. 5 (Supports Fig. 4). Validation of CUT&RUN analysis for H3K27me3 marks in the endosperm at 5 DAP.

a, Pearson correlation heatmap of the CUT&RUN analysis for all data sets in endosperm at 5 DAP from two combinations of reciprocal crosses with two biological replicates. **b**, Venn diagrams showing H3K27me3-enriched genes detected in each replicate of two reciprocal crosses. Most of the H3K27me3-enriched genes overlapped between replicates. **c**, Meta plot showing average Z-scores of H3K27me3 levels located at peaks detected in previously reported ChIP-seq data of 5-DAP endosperm¹⁸. Colored lines represent different cross combinations. **d**, Average log₂ Z-scores of H3K27me3 levels assigned to quartile expression differences in up-regulated genes in *Osemf2a* endosperm compared with wild-type endosperm. The box plots show the median (line within the box), the lower and upper quartiles (box), margined by the largest and smallest data points that are still within the interval of 1.5 times the interquartile range from the box (whiskers); outliers are not shown. The expression levels of up-regulated genes in *Osemf2a* showed correlation with H3K27me3 levels of CUT&RUN analysis in this study.



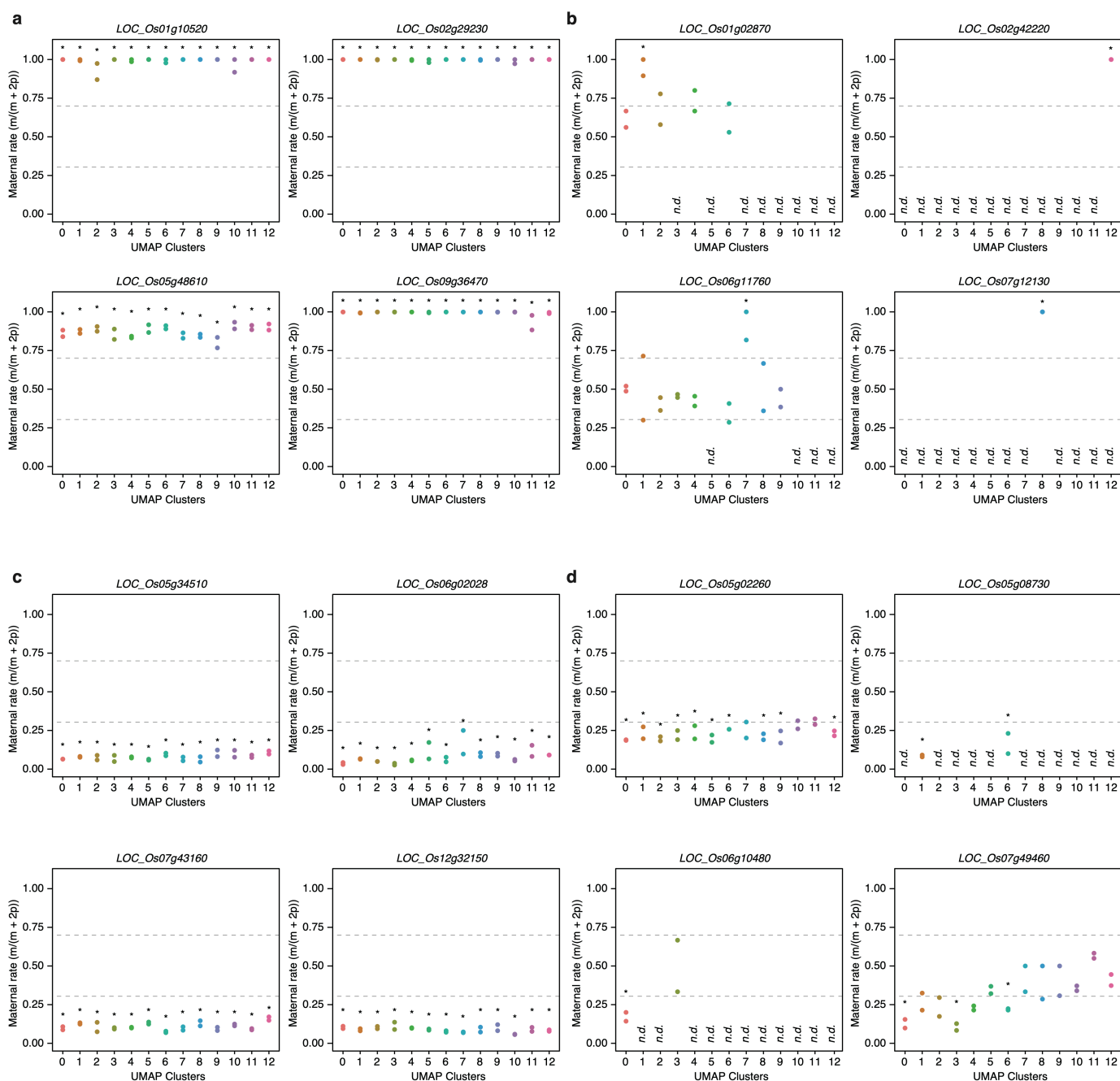
Supplementary Fig. 6 (Supports Fig. 4) Nuclei isolation from 5 DAP-endosperm using fluorescence-activated nuclei sorting (FANS).

a, Histogram showing all counted events based on the fluorescence of Hoechst 33342 staining. **b**, A population of endosperm nuclei (comprising $3n$, $6n$, and $12n$ nuclei) was gated by setting a parental gate (gate D) on the BSC (back scatter) vs. the fluorescence of GFP in the two-dimensional plane. **c,d**, Background noise was further removed according to the fluorescence of Hoechst 33342 vs. GFP (**c**, gates E, F, and G) and the fluorescence of GFP vs. PE (**d**, gates J, K, and L). The PE channel was used for the detection of contaminating debris. Purified endosperm nuclei were sorted from gates J, K, and L. **e,f**, Representative fluorescence images of endosperm nuclei before (**e**) and after FANS (**f**). Bars, 25 μm .



Supplementary Fig. 7 (Supports Fig. 4) Comparison of two biological replicates and expression profiles of representative endosperm domain-specific marker genes.

a, Density plot represents the distribution of Unique Molecular Identifiers (UMIs) per cell with two replicates. **b**, Comparisons of UMAP between two replicates. **c**, The expression of the endosperm domain-specific marker genes such as starchy endosperm; *Waxy/LOC_Os06g04200*⁷⁸, interfacing domain; *GE1/LOC_Os07g41240*⁷⁹, dividing cells; *Oryza;CycB1;1/LOC_Os01g59120*⁷⁷, dorsal aleurone facing vascular bundle; *OsIAA29/LOC_Os11g11430*⁷⁶, dorsal aleurone layer; *OsNF-YB1/LOC_Os02g49410*⁸⁰, dorsal and ventral aleurone; *OsYUCCA12/LOC_Os02g17230*⁷⁶. Plots shows individual cells and color represents the expression levels in each cell.



Supplementary Fig. 8 (Supports Fig. 4) Comparison of maternal rate for different class of imprinted genes in 13 UMAP clusters.

a-d, Maternal rate in pseudo-bulk data of UMAP clusters for persistent MEGs (**a**), 5-DAP-specific MEGs (**b**), Persistent PEGs (**c**) and 5-DAP-specific PEGs (**d**). In each class of imprinted genes, four genes showing significantly imprinted expression at least one UMAP cluster were selected. Different colors represent different UMAP clusters. Broken lines show threshold for maternal rate of imprinted genes. Asterisks indicate the significantly imprinted expression in each UMAP clusters.