

Figure S1. Enhancer-trap line N9322 and identification of genomic insertion site. (A) Suspensor and hypophysis expression at globular stage. (B) Insertion site of T-DNA determined by TAIL-PCR.

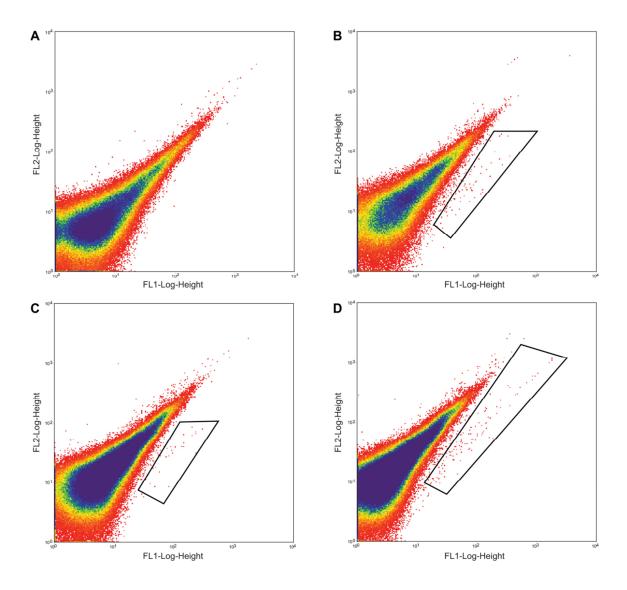


Figure S2. Scatter plots of FANS for GFP-tagged nuclear samples. (A) Mock sample. (B) Suspensor marker line pAT5G42200:n3xGFP. (C) Proembryo marker line pDRN:n2xGFP:DRN 3'UTR. (D) Whole embryo marker line pAT3G10010:nGFP. Fluorescent nuclei were detected by plotting the GFP channel (FL1, log, 513/17, x-axis) against auto-fluorescence (FL2, log, 575/25, y-axis) and drawing a gate around the GFP-positive events.

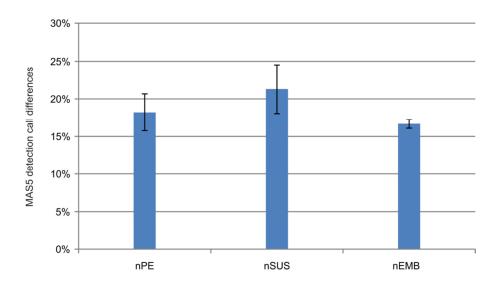


Figure S3. Percentage and standard deviation of MAS5 calls not correlating across three biological replicates. Replicates were compared to each other and the average percentage of calls not matching was calculated.

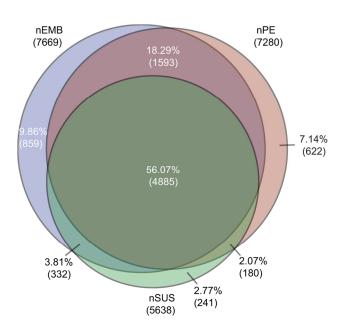


Figure S4. Venn diagram showing overlap of genes expressed in nuclei of the proembryo, suspensor, and whole embryo. For the analysis, only array elements with calls of 3x present (P) across all three biological replicates were used.

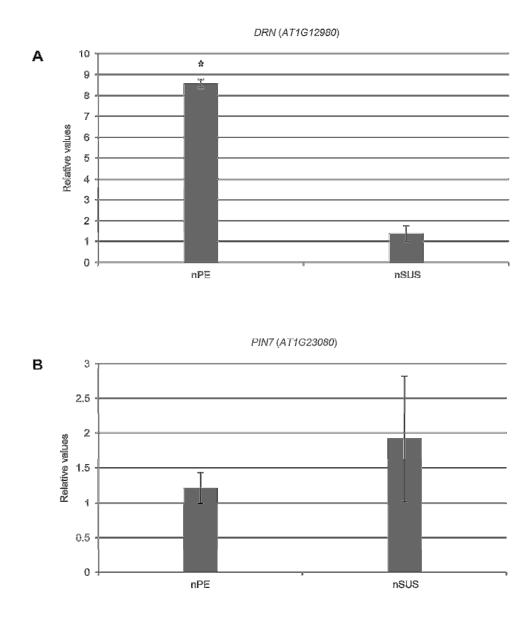


Figure S5. Relative mRNA levels detected by qRT-PCR analysis. (A) *PIN7* relative transcript levels are more abundant in nSUS compared to nPE. (B) *DRN* relative transcript levels are more abundant in nPE compared to nSUS. Average values and standard error are given for two biological replicates for nuclear RNA from both proembryo (nPE) and suspensor (nSUS). **P*<0.01 (Student's *t*-test).

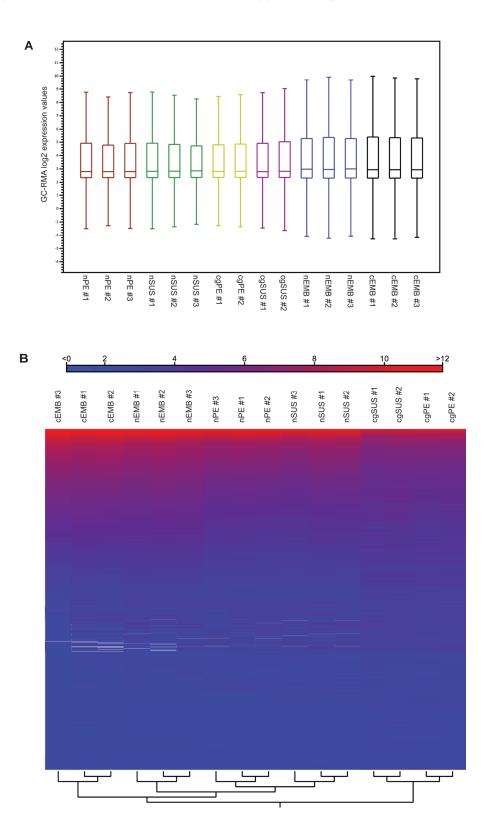


Figure S6. Quality analysis of biological replicates from different nuclear and cellular tissue types. (A) Box-plot analysis. (B) Hierarchical clustering analysis (Pearson correlation, complete linkage).

Table S1. gcRMA normalized, log2 transformed values for nuclear embryo, cellular embryo, and LCM cellular samples.

Table S2. Pearson correlation coefficient (PCC) analysis of nSUS, nPE, nEMB and cKAN1.

Table S3. List of nPE statistically enriched genes. FC, fold change. Pfp, percentage of false positive prediction.

Table S4. List of nSUS statistically enriched genes. FC, fold change. Pfp, percentage of false positive prediction.

Table S5. GO term enrichment analysis of nPE-enriched genes. Color code correspondingto color code in bar graph Figure 2A.

TableS6.GOtermenrichmentanalysisofnSUS-enrichedgenes.Colorcodecorresponding to color code in bar graph Figure 2B.

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Table S7. Identification of transcripts expressed in both nEMB and cEMB based on MAS5 3xP detection calls. Average MAS5 expression values of the three replicates are given (decreasing values from red to blue).

Table S8. Identification of transcripts only expressed in nEMB compared to cEMB based on MAS5 3xP detection calls. Average MAS5 expression values of the three replicates are given (decreasing values from red to blue).

Table S9. Identification of transcripts only expressed in cEMB compared to nEMB based on MAS5 3xP detection calls. Average MAS5 expression values of the three replicates are given (decreasing values from red to blue).

Table S10. Expression levels of reportedly endosperm-specific genes in nSUS and cgSUS. gcRMA normalized and log2 transformed values for all replicates are given (decreasing values from red to blue).

Table S11. Identification of proembryo-specific genes by comparing nPE and cgSEED.

Table S12. Identification of suspensor-specific genes by comparing nSUS and cgSEED.

Table S13. Oligonucleotides used in this study and fragment length for each promoter, *in situ* probe, and qRT-PCR product.

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