## **Supplemental Figures**



**Supplemental Figure S1: Background fluorescence check on FFPE maize shoot apex sections.** A-C) visualization of nuclei using DAPI-stain. A) Bright field image depicting maize shoot apex, B) DAPI signal highlighting nuclei and C) merged images. D-F) *DR5::RFP* signal, D) brightfield image of the maize SAM, E) *DR5::RFP* signal, F) merged images.



**Supplemental Figure S2: Quality control amplified spots in a longitudinal section of the maize SAM.** A) DAPI-stain depicting individual nuclei, B) Visualization of ISS spots using the anchor probe, C) merged image of DAPI and ISS spots and D) ISS spots of 90-genes are decoded and genes are assigned a specific color and symbol.



Supplemental Figure S3: Specific expression patterns visualized by ISS in a longitudinal section of the maize SAM. A) *KN1* expression is observed in the meristem and stem. B) *GA2OXIDASE1* (*GA2ox1*) is expressed at the base of developing leaves. C) *AGO18a* is expressed in the central zone of the meristem. D) *KNOX3* is specifically expressed at the boundary between stem and leaf. E) *BARELY ANY MERISTEM1d* (*BAM1d*) is expressed at the stem-leaf boundary and in young developing leaves. F) *JACALIN RELATED LECTIN* (*JRL*) is specifically expressed in mesophyll cells in the leaf and in the stem. G) *CUC2* is specifically expressed at the boundaries between stem and leaf. H) *TREHALOSE-6-PHOSPHATE PHOSPHATASE4* (*TTP4*) is specifically expressed at the base of the leaf.



**Supplemental Figure S4: Illustration of the SAM before and after segmentation.** A) Composite image of DAPI stain depicting nuclei and cell-wall stain depicting plant cell walls. B) Overlay obtained segmented cells on composite SAM image.



**Supplemental Figure S5: Evaluating the reproducibility of the ISS technology.** The count number for every gene was normalized relative to the total number of counts and the R<sup>2</sup> between the different sections was calculated.



**Supplemental Figure S6: Evaluating the expression pattern of PLA1 over different replicates.** A-B) PLA1 expression pattern in the first repeat (Pilot). C-D) PLA1 expression pattern in the first repeat (slide-A). E-F) PLA1 expression pattern in the first repeat (slide-B). Background signal are DAPI stained nuclei.



**Supplemental Figure S7: Validation of the ISS data with single gene mRNA** *in situ* hybridizations. (A-B) *GE1* is expressed in developing leaf primordia; C-D) *GIF2* is expressed in the vasculature; E-F) *GIF3* is expressed in young leaves and in vascular tissue; G-H) *GIF4* is expressed in the developing leaf primordia; I-J) *GRF10* is expressed in vascular tissue; K-L) *KLU-1* is expressed in developing vascular bundles; M-N) *KLU-2* is expressed is expressed in developing vascular bundles and the base of developing leaf primordia; O-P) *KN1* expression marks meristematic cells and Q-R) *VP8* is expressed in vascular tissue. Background signal in ISS pictures are DAPI stained nuclei.



Supplemental Figure S8: Histogram depicting the distribution of gene counts per cell over 12267 cells.



**Supplemental Figure S9: Expression pattern of** *HISTONE H4* **as obtained using ISS.** A) Expression in the shoot apex. B) Expression in a close-up of region marked by the white box in A. C) Expression in a close-up of region marked by the white box in A, with addition of a cell wall marker.

## Distribution of gene counts per cell



**Supplemental Figure S10: Comparison expression patterns of** *CUC2* **in the maize shoot apex**. A) Expression patterns of *CUC2* as obtained by amplification of probes targeting *CUC2*. B) Expression pattern of *CUC2* as observed by ISS.



**Supplemental Figure S11: Specific expression patterns obtained using ISS in the shoot apex.** Expression patterns of genes expressed in the boundary regions: *CUC2* (yellow) and *CUC3* (green), *D11-LIKE* (red), *BA1* (light blue), *BAF1* (purple), *TB1* (orange) and *GT1* (dark blue). B) Transverse section depicting expression of cell-specific marker genes: *OCL4* (yellow), *MWP4* (orange), *ARF3* (purple), *RLD1* (green) and *JRL* (blue). C-D) Zoomed regions of B. C) Zoom in of developing leaf primordia. D) Zoom in on the region of the maize SAM.



**Supplemental Figure S12: Phenotype of** *pla1* **mutant.** A) Image representing a WT and *pla1-2* mutant of the same age. B) The final leaf length of leaf 4 is decreased in *pla1-1* mutants. C) Quantitative measurement of number of leaves observed during a 11 day growth-period in *pla1-2* mutants. D) *pla1-2* mutants have a slightly reduced LER and a strong reduced LED. Black asterisk : significant difference P<0.05, Student's T-test. White asterisk: an additional leaf in the *pla1* mutant. N≥13. Error bars represent standard error.



**Supplemental Figure S13: Expression patterns of** *PLA1, KN1* and *YAB15* visualized through ISH. A) Expression pattern of *PLA1* is localized at the junction between the center of the ear and developing spikelets. B) *PLA1* accumulates at the boundary between differentiated lateral organs and meristematic cells in spikelets as depicted by *KN1* expression (C) and *YAB15* (D). GI: Glume; Pa: Palea; Ufm: Upper floral meristem; Lfm: Lower floral meristem; Le: Lemma.