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2
3 **Transcriptomics at maize embryo/endosperm interfaces identify a novel**
4 **transcriptionally distinct endosperm sub-domain adjacent to the embryo**
5 **scutellum (EAS).**

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19
20 **Running Title:** Maize embryo/endosperm interfaces transcriptomics

21
22 **One-sentence summary:** A previously undescribed population of endosperm cells adjacent to
23 the embryo scutellum shows transcriptomic enrichment in transport functions and is influenced by
24 embryo development.

25
26 The author responsible for distribution of materials integral to the findings presented in this
27 article in accordance with the policy described in the Instructions for Authors
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29 **Abstract**

30 Seeds are complex biological systems comprising three genetically distinct tissues nested
31 one inside another (embryo, endosperm and maternal tissues). However, the complexity of
32 the kernel makes it difficult to understand inter compartment interactions without access to
33 spatially accurate information. Here we took advantage of the large size of the maize kernel
34 to characterize genome-wide expression profiles of tissues at embryo/endosperm interfaces.
35 Our analysis identifies specific transcriptomic signatures in two interface tissues compared
36 to whole seed compartments: The scutellar aleurone layer (SAL), and the newly named
37 endosperm adjacent to scutellum (EAS). The EAS, which appears around 9 days after
38 pollination and persists for around 11 days, is confined to one to three endosperm cell layers
39 adjacent to the embryonic scutellum. Its transcriptome is enriched in genes encoding
40 transporters. The absence of the embryo in an *embryo specific (emb)* mutant can alter the
41 expression pattern of EAS marker genes. The detection of cell death in some EAS cells
42 together with an accumulation of crushed cell walls suggests that the EAS is a dynamic zone
43 from which cell layers in contact with the embryo are regularly eliminated, and to which
44 additional endosperm cells are recruited as the embryo grows.

45 **Introduction**

46 Cereal grains are not only essential for plant propagation, but are also high value
47 products which represent an important source of calories and proteins for human nutrition
48 and animal feed, as well as a coveted resource for bio-sourced industries. In maize, the
49 accumulation of oil in the embryo and of starch and protein in the endosperm requires the
50 development of adapted structures and a coordinated regulation and distribution of nutrient
51 flow from the mother plant. The development of the embryo, which will form the future
52 plant, and the endosperm, which will nourish the embryo during germination occurs in three
53 main phases (Berger, 1999; Dumas and Rogowsky, 2008; Lopes and Larkins, 1993). During
54 the first two weeks of early maize seed development, embryo and endosperm cells
55 differentiate into populations forming distinct tissues and organs (Leroux et al., 2014;
56 Randolph, 1936), including two storage organs, the scutellum of the embryo and the starchy
57 endosperm (*early development phase*). These two zygotic compartments then start to
58 accumulate large quantities of storage compounds during the following two to three weeks
59 (*filling phase*), while the surrounding maternal tissues provide or transport the necessary
60 nutrient supplies (Porter et al., 1987; Wu and Messing, 2014). During the final four weeks
61 (*maturation phase*), the kernel dehydrates and enters into quiescence prior to dispersal
62 (Sabelli and Larkins, 2009; Sreenivasulu and Wobus, 2013; Vernoud et al., 2005). These three
63 phases are determined by distinct genetic programs and characterized by distinct anatomical
64 and cytological features. Spatially, the maize kernel is organized like Russian dolls, the
65 embryo being enclosed within the endosperm, which is itself surrounded by the pericarp.

66 A closer look at the highly differentiated structure displayed by the maize embryo
67 shows that four days after pollination (DAP) two distinct parts can be distinguished: an apical
68 embryo proper and a basal suspensor that will degenerate at the end of early development
69 (Doll et al., 2017; Giuliani et al., 2002). At around 8 DAP the embryo proper generates, at the
70 abaxial side, a shield-shaped organ, the above-mentioned scutellum. The shoot apical
71 meristem develops on the adaxial side. Marking the apical pole of the future embryonic axis,
72 the shoot apical meristem will produce several embryonic leaves over time. The root apical
73 meristem differentiates within the embryo body defining the basal pole of the embryonic
74 axis. Shoot and root meristems will be surrounded by the protective coleoptile and
75 coleorhiza respectively (Bommert and Werr, 2001; Randolph, 1936; Vernoud et al., 2005).

76 The surrounding endosperm, which occupies 70% of the kernel volume at the end of the
77 early development (Leroux et al., 2014; Rousseau et al., 2015; Sabelli and Larkins, 2009; Zhan
78 et al., 2017), has been described as differentiating only four main cell types. The basal
79 endosperm transfer layer (BETL) and the aleurone layer (AL) are two peripheral cell types in
80 contact with maternal tissues. The embryo-surrounding region (ESR) is formed of small
81 densely cytoplasmic cells encircling the young embryo. Lastly, the starchy endosperm (SE)
82 corresponds to the central region of the endosperm, which subsequently accumulates huge
83 amounts of storage compounds before undergoing progressive programmed cell death. The
84 developing endosperm is surrounded by maternal tissues: the nutritive nucellus that
85 degenerates as the endosperm expands, and the protective pericarp, which comprises the
86 pedicel at the basal pole (Berger, 2003; Olsen, 2001; Sabelli and Larkins, 2009; Zhan et al.,
87 2017).

88 The parallel growth and profound developmental transformations of the three kernel
89 compartments highlight the need for constant coordination, which likely requires a complex
90 inter-compartmental dialog (Ingram and Gutierrez-Marcos, 2015; Nowack et al., 2010;
91 Widiez et al., 2017). Since maternal tissues, endosperm and embryo are symplastically
92 isolated, their apoplastic interfaces represent essential zones for this dialog (Diboll and
93 Larson, 1966; Van Lammeren, 1987). A good example to illustrate the importance and
94 specialisation of interfaces is carbon transport. Sugars must be transported from the
95 maternal tissues to the embryo for growth and fatty acid accumulation, passing through the
96 endosperm, which needs to retain part of the carbon for its own growth as well as the
97 biosynthesis of starch and storage proteins (Chourey and Hueros, 2017; Sabelli and Larkins,
98 2009). In maize, nutrients are unloaded from open ends of the phloem vessels into the
99 placento-chalazal zone of the maternal pedicel (Bezruczyk et al., 2018; Porter et al., 1987).
100 At the base of the endosperm, the BETL cells form dramatic cell wall ingrowths, thus
101 increasing the exchange surface (Davis et al., 1990; Kiesselbach and Walker, 1952). BETL cells
102 express a specific set of genes, including *MINIATURE1*, encoding a cell wall invertase, which
103 cleaves sucrose into hexoses. These are taken up by the sugar transporter SWEET4c, which
104 has been demonstrated to be the key transporter of sugar at the pedicel/endosperm
105 interface, since the defects in seed filling of the corresponding *sweet4c* mutant lead to a
106 miniature kernel phenotype (Cheng et al., 1996; Kang et al., 2009; Lowe and Nelson, 1946;
107 Sosso et al., 2015). The remaining endosperm interface with maternal tissues (initially the

108 nucellus and later on the pericarp) is the AL, which is not known to contribute to nutrient
109 exchange during seed development (Gontarek and Becraft, 2017).

110 The interface between the endosperm and the embryo is also developmentally
111 dynamic. At 3-6 DAP, the embryo is totally surrounded by ESR-type cells. As the embryo
112 expands, it emerges from the ESR, which consequently becomes restricted to the zone
113 surrounding the basal part (suspensor) of the embryo and ultimately disappears together
114 with the suspensor at the end of the early development phase (Giuliani et al., 2002;
115 OpsahlFerstad et al., 1997). From 8-9 DAP, the upper part (embryo proper) forms two new
116 interfaces: (1) At the adaxial side the embryo is enclosed by a single cell layer, which is called
117 the scutellar aleurone layer (SAL) in barley (Jestin et al., 2008). (2) At the abaxial side, the
118 embryo is brought into direct contact with central starchy endosperm cells (Van Lammeren,
119 1987). This interface is constantly moving due to the growth of the scutellum inside the
120 endosperm. On the embryo side of this interface, the epidermis of the scutellum has a
121 distinct morphology and gene expression pattern (Bommert and Werr, 2001; Ingram et al.,
122 2000). The dynamics of the endosperm/embryo interface, and the processes that occur
123 there, remain largely undescribed.

124 At many inter-compartmental interfaces, such as the BETL, the ESR or the AL, the
125 cells constitute readily identifiable tissues with distinctive and often striking cell
126 morphologies, and with defined organisations and established functions (except for the ESR)
127 (for review see Doll et al., 2017). In many cases specific sets of genes are expressed in these
128 tissues, as revealed by the identification and characterisation of marker genes, for example
129 of *MATERNALLY EXPRESSED GENE 1 (MEG1)*, *MYB-RELATED PROTEIN 1 (MRP1)* and *BETL1* to
130 4 (Cai et al., 2002; Gómez et al., 2002; Gutiérrez-Marcos et al., 2004; Hueros et al., 1999a,
131 1999b) in the BETL, *VIVIPARIOUS 1 (VP1)* in the AL (Suzuki et al., 2003), or *ESR1* to 3 in the
132 ESR (OpsahlFerstad et al., 1997).

133 Genome-wide gene expression studies at numerous developmental stages of whole
134 kernels and/or hand-dissected endosperm and embryo (Chen et al., 2014; Downs et al.,
135 2013; Li et al., 2014; Lu et al., 2013; Meng et al., 2018; Qu et al., 2016) have been
136 complemented by a recent transcriptomic analysis of laser-capture micro-dissected cell
137 types and sub-compartments of 8 DAP kernels (Zhan et al., 2015). However, even the latter
138 study did not address specifically the transcriptomic profiles of the embryo/endosperm

139 interfaces and did not answer the question of whether the endosperm at the
140 scutellum/endosperm interface is composed of cells with specific transcriptional identities.

141 In this study, we took advantage of the large size of the maize kernel to characterize
142 the genome-wide gene expression profile at embryo/endosperm interfaces at 13 DAP. RNA-
143 seq profiling revealed that endosperm cells in close contact with the embryo scutellum have
144 a distinct transcriptional signature allowing us to define a new endosperm zone named EAS
145 for Endosperm Adjacent to Scutellum, which is specialized in nutrient transport based on GO
146 (Gene Ontology) enrichment analysis. *In situ* hybridization shows that the EAS is confined to
147 one to three endosperm cell layers adjacent to the scutellum, whereas kinetic analyses show
148 that the EAS is present when the scutellum emerges at around 9 DAP and persists
149 throughout embryo growth, up to approximately 20 DAP. The detection of cell death in the
150 EAS together with impaired expression of EAS marker genes in an *embryo specific* mutant
151 suggests that the EAS is a developmentally dynamic interface influenced by the presence of
152 the neighbouring growing embryo.

153

154

155 Results

156 RNA-seq profiling of 13 DAP maize kernel compartments and 157 embryo/endosperm interfaces.

158 To obtain the gene expression patterns of embryo/endosperm interfaces in maize
159 kernels, six (sub)compartments were hand-dissected for transcriptomic analysis (Figure 1
160 and Supplemental Figure 1). The three whole compartments were: the maternal tissues
161 excluding the pedicel which were labelled pericarp (Per), the whole endosperm (End), and
162 the whole embryo (Emb) (Figure 1). The sub-compartments corresponding to three distinct
163 embryo/endosperm interfaces were the scutellar aleurone layer (SAL) (the single endosperm
164 cell layer at the adaxial side of the embryo), the apical scutellum (AS) (corresponding to the
165 embryo tip composed uniquely of scutellum tissues without the embryo axis), and a new
166 region that we named endosperm adjacent to scutellum (EAS) corresponding to several
167 layers of endosperm cells in close contact with the scutellum at the abaxial side of the
168 embryo (Figure 1 and Supplemental Figure 1). The tissues were collected from kernels of
169 inbred line B73 (used to establish the maize reference genome) at 13 DAP (embryo size of
170 about 2.5 mm), the earliest developmental stage at which hand dissection of these
171 embryo/endosperm interfaces was feasible, and also the transition from early development
172 to the filling phase. For each of the six samples, four biological replicates, each composed of
173 a pool of dissected tissues from two different plants, were produced (Supplemental Table 1).
174 A total of 24 RNA-seq libraries were constructed and sequenced in paired-end mode using
175 Illumina HiSeq2000 technology. The resulting reads (on average 62 million pairs per sample)
176 were checked for quality, cleaned and mapped to the current version of the B73 maize
177 reference genome (AGPv4). On average 95.8% ($\pm 0.4\%$) of the pairs were mapped, and on
178 average 78.3% ($\pm 5.3\%$) corresponded to annotated genes (Supplemental Figure 2A). Pairs
179 that mapped to multiple genes ($10.2\% \pm 5.3\%$) or to no gene ($5.2\% \pm 1.1\%$), as well as
180 ambiguous hits ($1.5\% \pm 0.6\%$) were filtered (Supplemental Figure 2A). A gene was considered
181 to be not expressed if it gives rise to less than 1 read per million. At least 25 000 genes were
182 found to be expressed per replicate, with the largest number found in the SAL (~30 000
183 genes expressed, Supplemental Figure 2A). The results generated for each replicate are
184 available in Supplemental Data Set 1. Venn diagrams were generated to visualize overlaps
185 between the sets of genes expressed in the three whole compartments (Per, Emb and End),

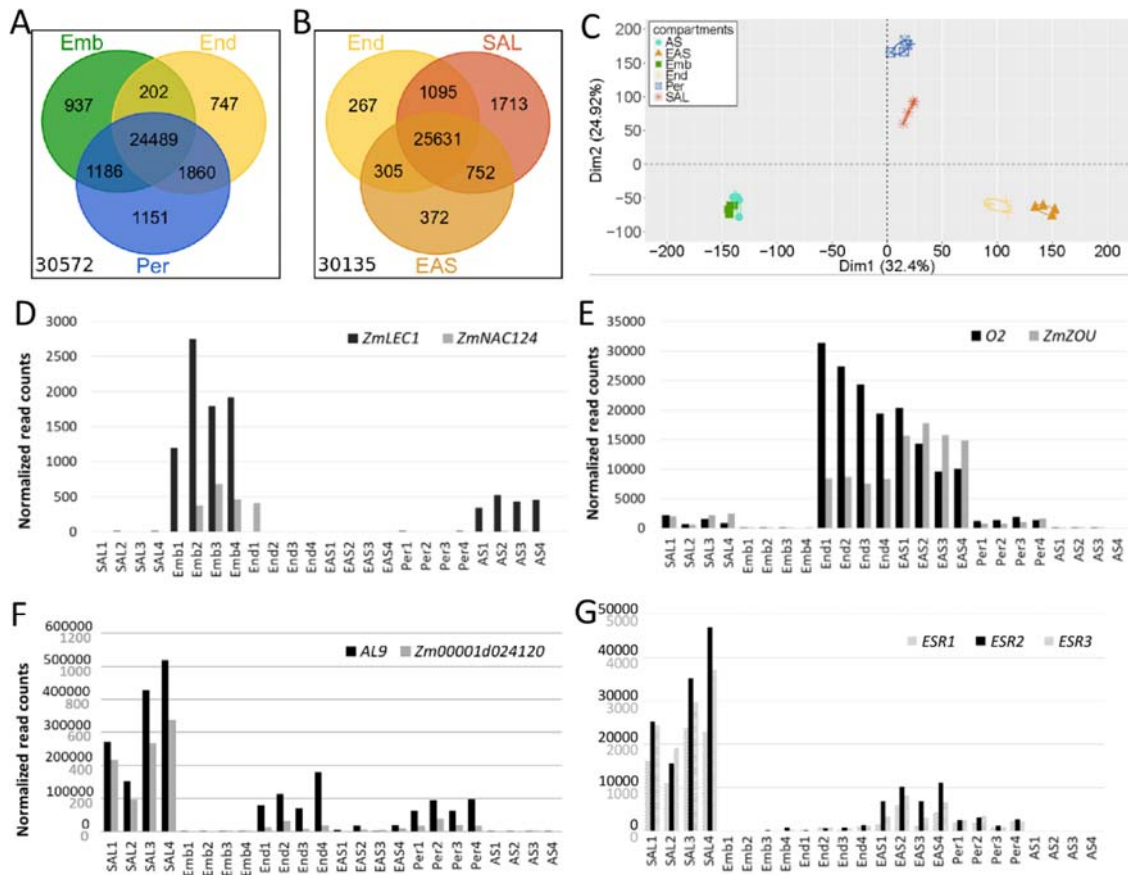


Figure 2. Validation of the RNA-seq approach.

(A) and (B) Venn diagrams. For each fraction, the number of genes expressed is indicated: (A) For End, Emb and Per; (B) For End, EAS and SAL. Total number of genes expressed for all three compartments analyzed is indicated below each Venn diagram. (C) Principal component analysis of the 24 RNA samples consisting of 4 biological replicates of Pericarp (Per), Apical Scutellum (AS), Embryo (Emb), Endosperm (End), Embryo Adjacent to Scutellum (EAS) and Scutellar Aleurone (SAL). (D) to (G) graphs represent the expression level (read counts were normalized using the trimmed mean of M-value (TMM) method) in the different samples of (D) the two embryo-specific genes *ZmLEC1* and *ZmNAC124*; (E) the two endosperm specific genes *O2* and *ZmZOU* (*O11*); (F) the two aleurone specific genes *AL9* and *Zm00001d024120* and the three *ESR* genes (*ESR1*, *ESR2* and *ESR3*). Grey and black Y-scales numbering in (F) are for *Zm00001d024120* and *AL9* expression level respectively, and in (G) for *ESR1* and *ESR3* (grey) and *ESR2* (black).

186 and between the sets of genes expressed in the End and the two endosperm sub-
 187 compartments (EAS and SA) (Figure 2A-B).

188 In order to assess the relationships between the different samples, a principal
 189 component analysis (PCA) was performed (Figure 2C). As expected, biological replicates
 190 grouped together, indicating experimental reproducibility. The PCA also revealed distinct
 191 sample populations corresponding to each (sub)compartment with the exception of the AS
 192 and Emb samples, which were partially superimposed (Figure 2C). Interestingly, the two
 193 endosperm interfaces SAL and EAS formed groups that were distinct both from each other,
 194 and from the whole endosperm samples. The EAS was more similar to the whole endosperm

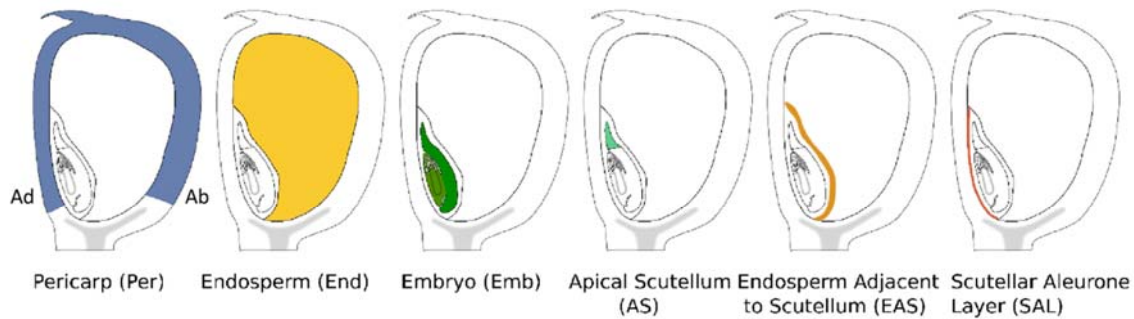


Figure 1. Scheme representing the six (sub)compartments hand-dissected for transcriptomics analysis at maize embryo/endosperm interfaces. Ad = adaxial, Ab = abaxial.

195 than the SAL, indicating a more similar transcriptomic landscape (Figure 2C). To explore
 196 potential contamination between tissues during the dissection process, the expression
 197 profiles of previously identified marker genes with tissue-specific expression patterns were
 198 investigated (Figure 2D-G). *ZmLEC1* (*Zm00001d017898*) and *ZmNAC124* (*Zm00001d046126*,
 199 named *ZmNAC6* by Zimmermann and Werr, 2005), two embryo-specific genes, were
 200 specifically expressed in the embryo samples in our dataset (Figure 2D). As expected,
 201 *ZmLEC1* was more strongly expressed in the Emb than in the AS sample (Zhang et al., 2002).
 202 Absence of *ZmNAC124* expression in the AS was consistent with the strong and specific *in*
 203 *situ* hybridisation signal for this gene in the basal part of the embryonic axis (Zimmermann
 204 and Werr, 2005). The two endosperm-specific genes *ZmZOU/O11* (*Zm00001d003677*) and
 205 *O2* (*Zm00001d018971*) were found to be strongly expressed in the End and EAS, and weakly
 206 in the SAL samples (Figure 2E) (Feng et al., 2018; Grimault et al., 2015; Schmidt et al., 1990).
 207 A weak expression in the Per sample was unexpected but consistent with other
 208 transcriptomics data (Sekhon et al., 2011), and could also reflect possible contamination of
 209 the Per samples with aleurone layer, since the aleurone layer has a tendency to stick to the
 210 pericarp (See discussion part). In addition, the preferential expression of *AL9* and
 211 *Zm00001d024120* genes in the aleurone was reflected by a stronger signal in SAL compared
 212 to End (Gomez et al., 2009; Li et al., 2014; Zhan et al., 2015). *AL9* (*Zm00001d012572*) and
 213 *Zm00001d024120* also showed a signal in the pericarp samples, again indicating a possible
 214 contamination of the Per samples by SAL (Figure 2F). The expression patterns of ESR marker
 215 genes (*ESR1*, *ESR2* and *ESR3*) were also evaluated in our samples. At 13 DAP, the ESR
 216 comprises a small endosperm region situated at the base of embryo, around the suspensor
 217 (Opsahl-Ferstad et al., 1997). We observed elevated expression of ESR markers in the SAL
 218 and to less extent in the EAS (Figure 2G). Previous *in situ* hybridizations of *ESR1* transcripts

219 showed that *ESR1* expression is restricted to the ESR and absent from the EAS and most if
220 not all of the SAL at both 12 and 14 DAP (Opsahl-Ferstad et al., 1997). However, the basal
221 part of the SAL is in direct contact with the ESR (Opsahl-Ferstad et al., 1997), and the
222 published data do not exclude the possibility that the *ESR1* signal might extend to the SAL in
223 this basal part. The apparent elevated expression of ESR marker genes in our SAL
224 transcriptomes may thus reveal contamination with adjacent ESR cells during dissection
225 and/or expression in the basal part of the SAL.

226 In order to compare our full transcriptomic data set with published RNA-seq data, we
227 used a unique, spatially resolved, maize kernel transcriptome (Zhan et al., 2015). Although
228 different (sub)compartments and developmental stages (8 DAP vs 13 DAP) were used, we re-
229 treated both RNA-seq raw data-set using the same bioinformatic pipeline and the same
230 genome version (see Material and Methods) in order to increase comparability. We then
231 performed a PCA on joint datasets. The first principal component (PC1) carries 43.7% of the
232 variance and clearly separates the two datasets (Supplemental Figure 3A). It may reflect a
233 “batch effect”, combination of biological effect of the age of sampling (8 DAP vs 13 DAP),
234 and technical differences between the two transcriptomes (growing environment, library
235 preparation, etc). The next components group together samples from the two datasets, and
236 still carries a relatively high fraction of the variance (26.9% and 9.7% for PC2 and PC3,
237 respectively). When PC2 was plotted against PC3, 13 DAP Emb is most similar to 8 DAP Emb
238 samples among the 8 DAP samples (Supplemental Figure 3B), indicating that although
239 important differences exist between these two datasets, these two embryo samples share
240 some similarities in their transcriptomic profiles. Likewise, the 13 DAP AS is most similar to
241 the 8 DAP Emb samples among 8 DAP samples (Supplemental Figure 3B). The 13 DAP SAL
242 groups most closely to the two 8 DAP samples BETL and ESR. Interestingly, the 13 DAP EAS
243 samples form an independent group that is closer to the two 8 DAP starchy endosperm
244 samples (which are the central starchy endosperm (CSE) and the conducting zone (CZ)),
245 among the 8 DAP samples (Supplemental Figure 3B).

246 In summary, we have generated RNA-seq profiles from 13 DAP maize kernel
247 compartments and embryo/endosperm interfaces. We have made this data available to the
248 community in a user-friendly format via the eFP Browser
249 (http://bar.utoronto.ca/efp_maize/cgi-bin/efpWeb.cgi?dataSource=Maize_Kernel) (Private

250 link for reviewers: [http://bar.utoronto.ca/~asher/efp_maize/cgi-](http://bar.utoronto.ca/~asher/efp_maize/cgi-bin/efpWeb.cgi?dataSource=Maize_Kernel)
251 [bin/efpWeb.cgi?dataSource=Maize_Kernel](http://bar.utoronto.ca/~asher/efp_maize/cgi-bin/efpWeb.cgi?dataSource=Maize_Kernel)) (See supplemental Figure 4 for examples).

252

253 **Preferentially expressed genes and biological processes associated with** 254 **specific maize kernel (sub)compartments.**

255 Differential expression analyses were performed between the 6 (sub)compartments
256 by comparing expression levels between pairs of tissues using a likelihood ratio test with p -
257 values adjusted by the Benjamini-Hochberg procedure to control false discovery rates (see
258 Material and Methods). Genes with both adjusted p -values lower than 0.05 and an
259 expression difference of 4-fold or greater ($\log_2(\text{Fold Change}) \geq 2$) were classed as
260 differentially expressed genes (DEGs) (Supplemental Table 2). The full lists of DEGs for the 15
261 inter-tissue comparisons performed are available in Supplemental Data Set 2.

262 To identify the biological processes associated with the DEGs, a gene ontology (GO)
263 analysis was performed. Due to the limited resources available, a new genome-wide
264 annotation of all predicted proteins was carried out and linked to GO terms (see Material
265 and Methods). In a first instance, GO terms enriched in the two zygotic compartments Emb
266 and End were identified by analysing DEGs upregulated in each compartment compared to
267 the two other main compartments (Table 1). The top ten GO terms enriched in the DEGs
268 upregulated in the embryo relative to endosperm and pericarp, showed a significant
269 enrichment in GO terms related to the cell cycle, DNA organization and cytoskeleton
270 organization, consistent with the extensive developmental and mitotic activity within the
271 embryo at this stage (Table 1). In contrast the GO terms enriched in the DEGs upregulated in
272 the endosperm relative to embryo and pericarp were linked to metabolic functions such as
273 nutrient reservoir activity and starch biosynthetic (Table 1). These enrichments were
274 consistent with the fact that the endosperm is a nutrient storage compartment where starch
275 and reserve proteins are synthesized (Nelson and Pan, 1995; Zheng and Wang, 2015).

276

277 **Enrichment for putative transporters at the endosperm/embryo interface**

278 Focusing in on the embryo/endosperm interfaces, DEGs between the three sub-
279 compartments (AS, SAL and EAS) and their whole compartments of origin were identified

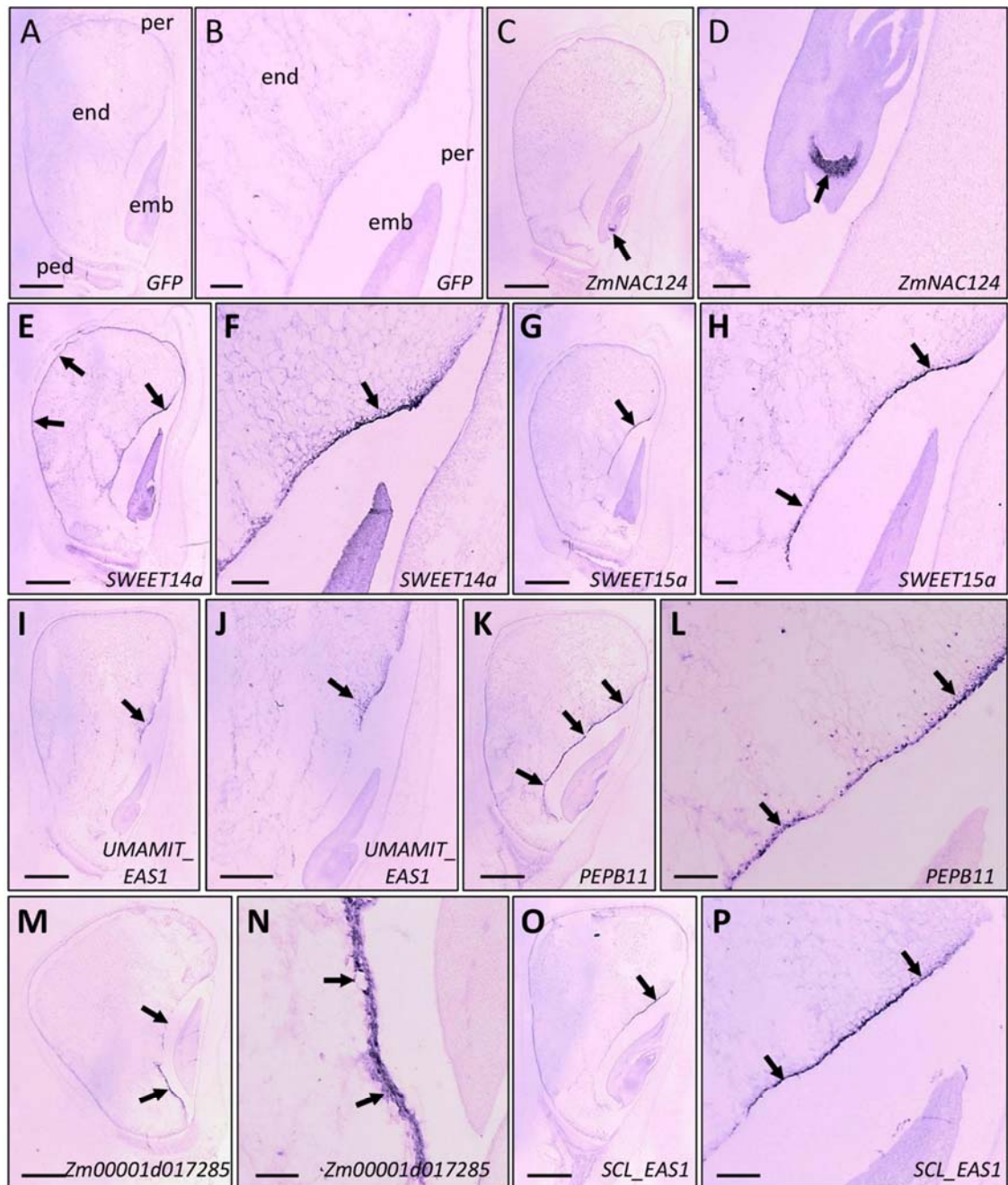


Figure 3. *In situ* hybridization on 13 DAP maize kernels probes detecting *GFP* (negative control) (A, B), *ZmNAC124* (positive control) (C, D), *SWEET14a* (E, F), *SWEET15a* (G, H), *UMAMIT_EAS1* (I, J), *PEPB11* (K, L), *Zm00001d017285* (M, N), *SCL_EAS1* (O, P). Scale bars correspond to 500 μ m in A, C, E, G, I, J, K, M, O and 1000 μ m in B, D, F, H, L, N, P. Arrows indicate main *in situ* hybridization signal. emb = embryo, end = endosperm, per = pericarp, ped = pedicel.

280 (Supplemental Table 2). 682 genes were found to be differentially expressed between AS
 281 and Emb according to the above criteria. Among them, 82 were more strongly and 600 more
 282 weakly expressed in AS compared to Emb samples (Supplemental Table 2). As expected,
 283 *ZmNAC124*, which is expressed in the coleorhiza (Figure 2D and Figure 3C, D) (Zimmermann

284 and Werr, 2005) was found among the genes showing reduced expression in the apical
285 scutellum. Only the GO term “DNA binding transcription factor activity” were found to be
286 significantly enriched in our analysis in the comparison of AS vs Emb (Table 2).

287 The comparison between the EAS and the End revealed 1 498 DEGs with 485 genes
288 showing stronger expression in the EAS than the End, and 1 013 genes with the inverse
289 profile (Supplemental Table 2). Among the genes more strongly expressed in the EAS, our
290 GO analysis revealed a significant enrichment in only one GO term (GO analysis on molecular
291 function terms at F3 level): “transmembrane transporter activity” (Table 2), which suggests a
292 stronger expression of transporter-encoding genes in the EAS compared to End.

293 Finally, 2 975 genes were found to be differentially expressed between SAL and End,
294 1 995 corresponding to genes more strongly expressed in the SAL, and 980 to genes with
295 lower expression levels in the SAL (Supplemental Table 2). Interestingly, in the first group our
296 GO analysis revealed an enrichment in two (out of 4) GO terms related to transport (Table
297 2).

298 A closer look at gene families encoding transporters amongst DEGs confirmed the
299 overrepresentation seen in the GO analysis and revealed differences between the SAL and
300 EAS. Among the genes that were at least 8 times more strongly expressed compared to End,
301 8.45 % (45/532) of the genes enriched in the SAL and 16.04 % (34/212) of the genes enriched
302 in the EAS have at least one orthologue in rice or in Arabidopsis that encodes a putative
303 transporter (Table 3). In the SAL, transcripts of genes encoding MATE (Multi-antimicrobial
304 extrusion protein), which have been implicated in a diverse array of functions (for review see
305 Upadhyay et al., 2019) and ABC (ATP-binding cassette) transporters were found to be the
306 most strongly enriched, whereas in the EAS, genes encoding transporters from the
307 MtN21/UMAMIT (Usually Multiple Acids Move In And Out Transporter), MtN3/SWEET
308 (Sugars Will Eventually be Exported Transporter), and ABC transporter-families were the
309 most represented. When looking at the putative molecules transported, a large number of
310 genes encoding putative amino acid transporters were found to show stronger expression in
311 the EAS than End samples, although genes encoding transporters for various other
312 molecules including sugars, heavy metals, phosphate, inorganic ions or nucleotides also
313 showed stronger expression (Table 3). Regarding the comparison of SAL vs End, transporters
314 mainly annotated as involved in amino acid and inorganic ions transport were identified
315 (Table 3). In summary, our work shows that both SAL and EAS cells strongly express putative

316 transporter-encoding genes, suggesting that these cells are characterised by an elevated
317 transmembrane transport of various molecules, and potentially mediate nutrient
318 repartitioning around the embryo. However, each tissue preferentially expresses different
319 classes of transporters, with MtN21/UMAMIT and MtN3/SWEET transporters involved in
320 amino acid and or sugar transport respectively, more likely to be enriched in the EAS.

321

322 **The EAS is restricted to one to three endosperm cell layers adjacent to the**
323 **scutellum.**

324 The SAL has both cellular and biochemical characteristics of the aleurone making it
325 inherently different from other endosperm tissues (Gontarek and Becraft, 2017; Zheng and
326 Wang, 2014). In contrast, EAS cells have not been reported to have distinct features that
327 allow them to be distinguished cytologically from SE cells, which compose the majority of
328 the volume of the endosperm (Van Lammeren, 1987). However, our transcriptomic analysis
329 suggests that these cells deploy a specific genetic program. In order to (1) confirm EAS
330 expression specificity and to (2) provide a more precise spatial resolution to define and
331 characterize this new region, *in situ* hybridizations were performed with a set of 6 genes
332 more than 10 fold enriched in the EAS transcriptome compared to the End transcriptome
333 (Supplemental Table 3, and Supplemental Figure 4 for two examples of eFP browser
334 pattern). Three of these genes encode putative transporters, namely *SWEET14a*
335 (*Zm00001d007365*) and *SWEET15a* (*Zm00001d050577*) encoding putative sugar transporters
336 of the SWEET family, and *Zm00001d009063*, called *UMAMIT_EAS1*, encoding a putative
337 amino acid transporter belonging to the *UMAMIT* family (Müller et al., 2015; Sosso et al.,
338 2015). The three remaining genes were *phosphatidylethanolamine-binding protein 11*
339 (*pebp11*, *Zm00001d037439*), a *serine carboxypeptidase-like* (*Zm00001d014983* or *SCL_EAS1*)
340 and *Zm00001d017285*, a gene with no name and unknown function (Supplemental Table 3).
341 The negative control chosen for *in situ* hybridizations, was an antisense probe generated
342 against a GFP-encoding ORF. The positive control was *ZmNAC124* (*Zm00001d046126*), which
343 is specifically expressed in the Emb compartment in our transcriptome (Figure 2D and
344 Supplemental Table 3) and which had previously been shown by *in situ* hybridization to be
345 expressed in specific embryonic tissues (Zimmermann and Werr, 2005). *In situ* hybridizations
346 were performed on 13 DAP kernels, the same stage as used for the transcriptome analysis.

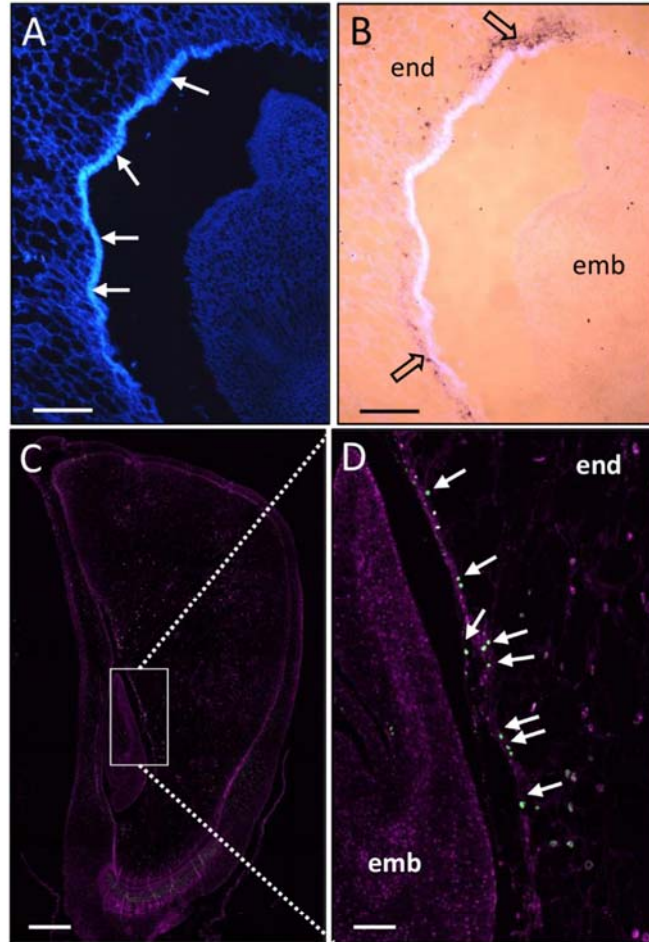


Figure 5. Crushed cell walls and cell death occurs in the EAS. **(A,B)** Calcofluor staining of cell walls of 13 DAP maize kernel sections **(A)** together with *in situ* hybridization with *SWEET15a* antisense probes **(B)** on sagittal section. Empty black arrow indicates *in situ* hybridization signal, while plain white arrows indicate the accumulation of crushed cell walls. **(C,D)** TUNEL labelling of 15 DAP kernels. Fluorescein labelling of the TUNEL positive nuclei is shown in green and propidium iodide counterstaining in purple. Arrows indicate the nucleus stained by TUNEL in the EAS. Scale bars correspond to 200 μm in **(A,B)** and 500 μm in **(C)** and 100 μm in **(D)**. emb = embryo, end = endosperm.

347 The 4 probes detecting *SWEET15a* (Figure 3G, H), *PEPB11* (Figure 3K, L), *Zm00001d017285*
 348 (Figure 3M, N) and *SCL_EAS1* (Figure 3O, P) gave a strong signal restricted to a few layers of
 349 endosperm cells immediately adjacent to the scutellum, with little or no expression detected
 350 elsewhere in the kernel. For the probe directed against *SWEET14a* the signal was strong in
 351 the EAS, but was also present, albeit more weakly, in other kernel tissues, especially in the
 352 embryo and aleurone (Figure 3E, F). The probe against *UMAMIT_EAS1* gave a weaker signal
 353 restricted to the apical part of the EAS region, consistent with the lower expression levels of
 354 this gene in our transcriptome data (Supplemental Table 3). However, the signal for
 355 *UMAMIT_EAS1* was specific to these EAS cells (Figure 3I, J). These results confirmed that EAS

356 cells have a specific transcriptional program and that this programme (and thus the EAS) is
357 restricted to 1 to 3 layers of endosperm cells adjacent to the scutellum as confirmed by
358 sagittal sections (Figure 5A, B).

359 **The EAS is a dynamic region reflecting the period of strong embryo growth.**

360 To evaluate the dynamics of gene expression in the EAS during kernel development,
361 *in situ* hybridizations were carried out on kernels at different developmental stages (9, 11,
362 14, 17 and 20 DAP) (Figure 4 and Supplemental Figure 5). The four probes giving a strong and
363 EAS-specific signal at 13 DAP (*SWEET15a*, *PEPB11*, *Zm00001d017285*, *SCL_EAS1*) were used
364 (Figure 4). In 9 DAP kernels, the probes for *PEPB11* and *SCL_EAS1* showed no signal, whereas
365 those for *SWEET15a* and *Zm00001d017285* gave a strong signal in the endosperm cells
366 adjacent to the apical part of the embryo (Figure 4 and Supplemental Figure 5). This signal
367 was restricted to a few layers of cells in the vicinity of the nascent scutellum. At this stage,
368 the basal part of the embryo was still surrounded by ESR cells and no signal was detected in
369 this region. At 11 DAP, all four probes tested gave a very strong signal in the layers of
370 endosperm cells adjacent to the scutellum. At 14 DAP and 17 DAP, the signal was still
371 detected and restricted to the cell layers in close contact with the embryo (Figure 4 and
372 Supplemental Figure 5). Finally, at 20 DAP the signal decreased for all four probes with a
373 total disappearance for *SWEET15a*. Together, these results revealed that the EAS
374 transcriptomic region was restricted to a defined time window. Its onset at 9 DAP was
375 concomitant with the formation of the scutellum, marking a switch in embryo/endosperm
376 interactions from an ESR/embryo to an EAS/scutellum interface. Its decline occurred around
377 20 DAP when rapid embryo growth comes to an end.

378 **EAS cells originate from the starchy endosperm (SE) and undergo cell death.**

379 Despite the preferential or specific expression of EAS marker genes, and consistent
380 with their SE-like morphology, EAS cells also showed some transcriptomic characteristics of
381 the SE such as a strong expression of genes encoding ZEIN storage proteins (Supplemental
382 Figure 6). The presence of *ZEIN* transcripts in the EAS region is supported by *in situ*
383 hybridization data (Woo et al., 2001). In order to perform a more global comparison, we
384 asked to which samples from the Zhan et al (2015) dataset (at 8 DAP) our EAS transcriptome
385 was most similar, using PCA (Supplemental Figure 3). Interestingly, on the third principal

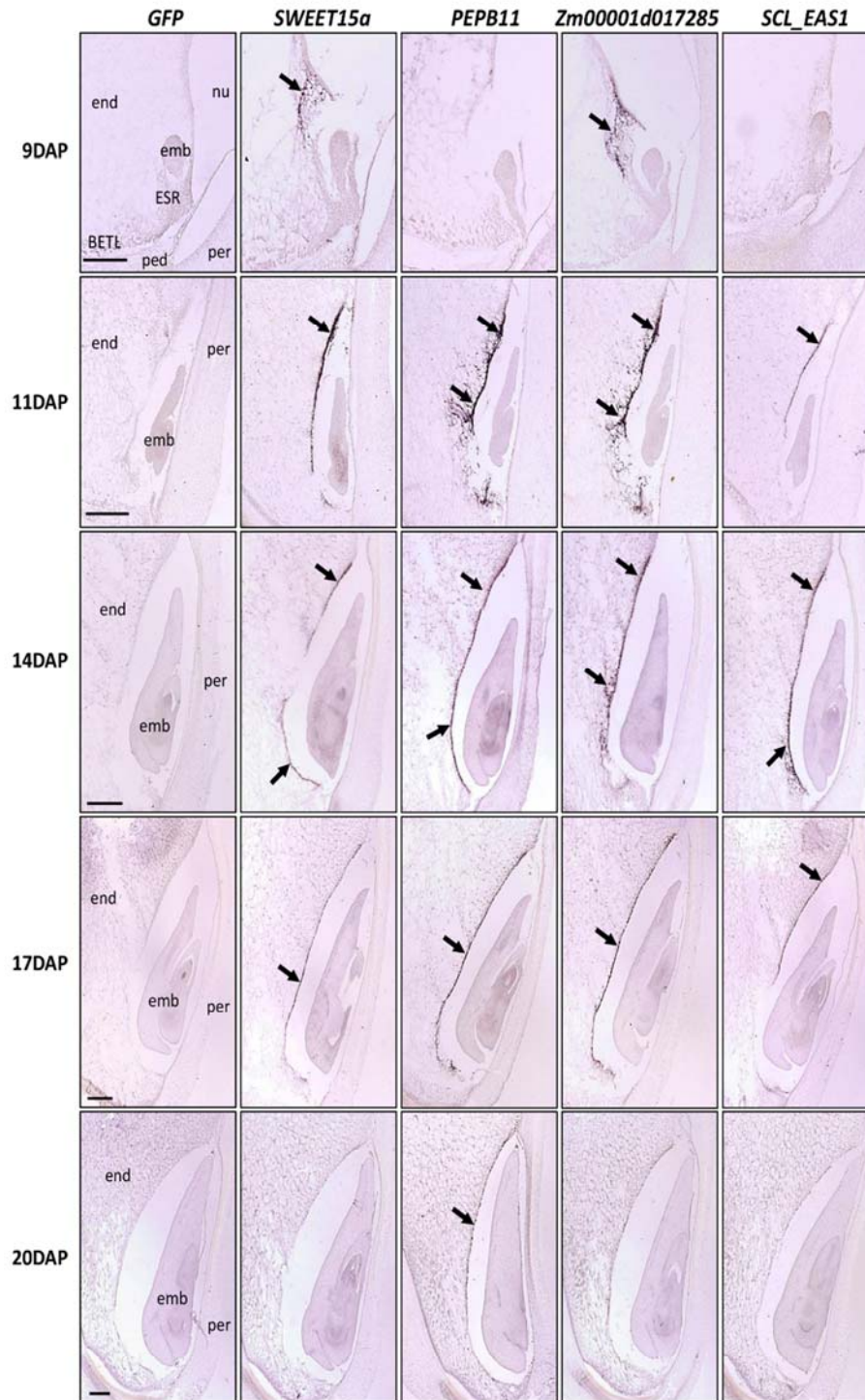


Figure 4. Legend is here after

Figure 4. *In situ* hybridization of 4 probes detecting EAS marker genes (*SWEET15a*, *PEPB11*, *Zm00001d017285*, *SCL_EAS1*) on kernel sections at different developmental stages. Probe detecting *GFP* was used as negative control. Pictures are zoom from Supplemental Figure 5, and scale bars correspond to 200 μ m for 9 DAP kernels and 500 μ m for the other stages. For each image the name of the probe is indicated at the top of the figure and the stage on the left. Arrows indicate main *in situ* hybridization signal. end = endosperm, emb = embryo, per = pericarp, nu = nucellus, ESR = embryo surrounding region, BETL = basal endosperm transfer layer, ped = pedicel.

387 regions at 8 DAP: the central starchy endosperm (CSE) and the conducting zone (CZ)
388 (Supplemental Figure 3B). As EAS cells, both CZ and CSE have no striking morphological
389 characteristics differentiating them from the starchy endosperm, strengthening the idea that
390 EAS originate from the starchy endosperm.

391 To address the question of EAS cell fate in proximity to the scutellum, sagittal
392 sections of the EAS/scutellum interface were both hybridized with an EAS-specific probe
393 (against *SWEET15a* transcripts) and stained with calcofluor to reveal cell walls (Figure 5A-B).
394 Accumulation of cell wall material occurred at the endosperm interface with the scutellum,
395 which may result from the compaction of crushed endosperm cells. Interestingly, *in situ*
396 hybridization signal for the EAS marker genes was found in the first uncrushed cell layer
397 (Figure 5B). An appealing model is that EAS cells are actually SE cells that are forced into
398 juxtaposition with the scutellum because of the invasive growth of the embryo into the SE
399 during kernel development (Figure 6), suggesting that the EAS program may not be fixed
400 within a static group of cells but instead be triggered as SE cells enter into contact with the
401 scutellum.

402 If this model is correct, EAS cells would be likely to be successively eliminated as they
403 come into contact with the embryo. Terminal deoxynucleotidyl transferase dUTP Nick End
404 Labelling (TUNEL) assays were performed on 15 DAP kernels to visualize DNA degradation as
405 a potential indicator of the presence of dying cells. However, it should be noted that TUNEL
406 signals are neither a fully reliable indicator of all forms of cell death, nor diagnostic of
407 specific cell death programmes (Charriaut-Marlangue and Ben-Ari, 1995; Labat-Moleur et al.,
408 1998). In addition to the PC and coleoptile regions, both of which had previously been shown
409 to give strong TUNEL signals (Giuliani et al., 2002; Kladnik et al., 2004), we also observed a
410 clear TUNEL positive signal in some EAS cells in close contact with the scutellum (Figure 5C,
411 D). This result is consistent with the possibility that a form of cell death occurs at this
412 interface. To clarify whether transcriptional activation of EAS specific genes is linked to the
413 initiation of known cell death programmes, the expression levels of genes associated with
414 programmed cell death in plants were analysed (Supplemental Figure 7) (Arora et al., 2017;
415 Fagundes et al., 2015). Surprisingly orthologues of none of the previously identified
416 programmed cell-death associated genes was found to be particularly up regulated in the
417 EAS compared to other samples. In addition, no enrichment of GO terms associated with

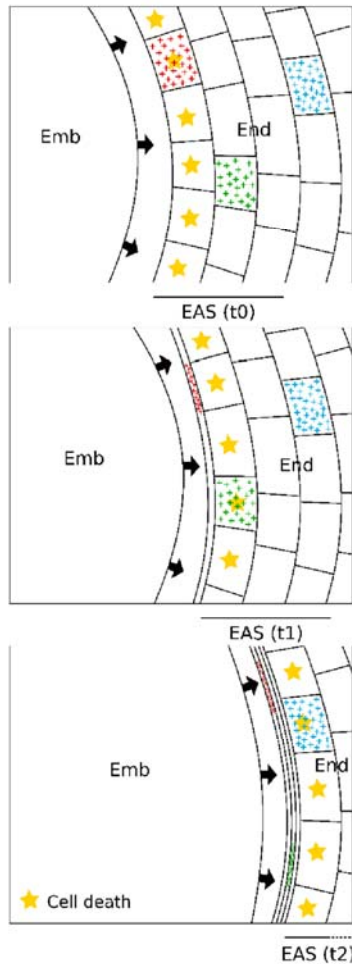


Figure 6. Scheme summarizing the EAS dynamic. Three different consecutive times points (t0, t1 and t2) are represented. Embryo scutellum invades (representing by arrows) the surrounding endosperm which enters in cell death (yellow stars). Three cells are labelled by a cross pattern to illustrates this dynamic. Emb = embryo scutellum, End = endosperm, EAS = endosperm adjacent to scutellum.

418 programmed cell death was found in the DEGs strongly expressed in the EAS relative to the
 419 End samples. Similar results were obtained when comparing genes strongly expressed in the
 420 EAS relative to the Emb, which remains alive (the GO term “programmed cell death”
 421 (GO:0012501) was slightly enriched (Ratio of 1.56) but in a not statistically significant
 422 manner (p-value=0.098)). These data suggested that either only a small proportion of EAS
 423 cells undergo cell death, or that crushing of EAS cells does not trigger a “classical”
 424 programmed cell death programme. A parallel could be drawn with accidental cell death
 425 (ACD) defined in animals, in which cells die as a result of their immediate structural
 426 breakdown due to physicochemical, physical or mechanical cues (Galluzzi et al., 2015).

427 **Impaired expression of some EAS marker genes in *emb* mutants.**

428 To test to what extent the proximity of the embryo/scutellum was required for EAS
 429 gene expression, the *embryo specific* mutation *emb8522* was used in an R-scm2 genetic
 430 background enhancing the early embryo deficient phenotype (Sosso et al., 2012). In this

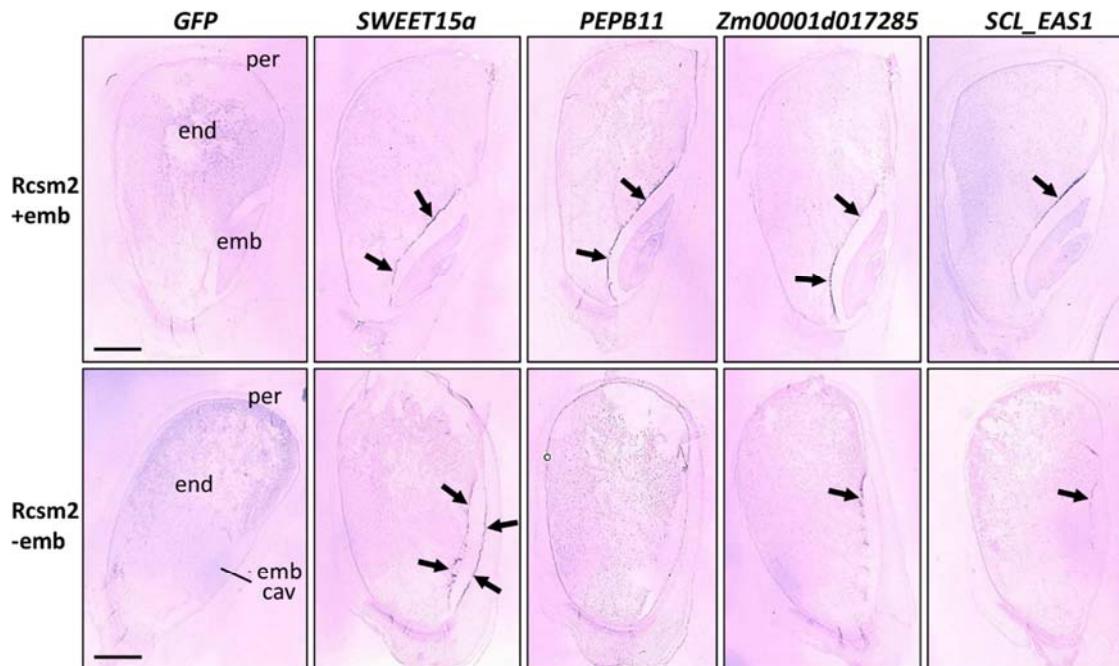


Figure 7. *In situ* hybridization with several probes marking the EAS on 13 DAP maize kernel sections of the R-scm2 genetic background. Probe detecting *GFP* was used as negative control. Kernels come from a self-pollination of a mother plant heterozygous for the *emb8522* mutation. Upper line corresponds to kernels with embryo (*emb8522* +/- or +/+), and the bottom line to kernels without embryo (*emb8522* -/-). Arrows indicate the main *in situ* hybridization signal. Scale bars correspond to 1000 μ m.

431 background the recessive *emb8522* mutation produced vestigial embryos composed of a
 432 small heap of cells. Nevertheless, a cavity corresponding to the size a normal embryo was
 433 generated that was only very partially occupied by the aborting embryo (Heckel et al., 1999;
 434 Sosso et al., 2012). Self-fertilization of heterozygous plants carrying the *emb8522* mutation
 435 was performed, and *in situ* hybridizations were carried out on 13 DAP sibling kernels with
 436 either phenotypically wild-type or mutant embryos, to visualize the transcripts of four EAS
 437 marker genes (Figure 7). Similar EAS specific expression patterns were observed in R-scm2
 438 kernels with embryos (Figure 7) to those observed in B73 kernels (Figure 3 and 4) for all
 439 genes tested, indicating a conservation of EAS cell identity in this genetic background. In *emb*
 440 kernels, the probes detecting *Zm00001d017285* and *SWEET15a* still showed a signal in the
 441 EAS region but with an altered distribution (Figure 7). In *emb* kernels, *Zm00001d017285*
 442 expression was found to be restricted to the apical part of the embryo cavity and *SWEET15a*
 443 expression expanded to the SAL, suggesting an inhibitory role of the normal embryo on
 444 *SWEET15a* expression in this tissue. Interestingly the two other EAS marker genes tested
 445 showed either only very weak expression (*SCL_EAS1*) or no expression (*PEPB11*) in *emb*

446 kernels, indicating a promoting effect of the normal embryo on the expression of these two
447 genes (Figure 7).

448

449

450 Discussion

451 Transcriptomes at embryo/endosperm interfaces

452 As in other flowering plants, seed development in maize is governed by specific
453 temporal and spatial genetic programs, distinguishing early development, filling and
454 maturation on one hand and embryo, endosperm and pericarp on the other (Chen et al.,
455 2014; Downs et al., 2013; Li et al., 2014; Lu et al., 2013; Meng et al., 2018; Qu et al., 2016).
456 Recently a transcriptome analysis on nucellus (including the fertilized embryo sac) increased
457 the temporal resolution and allowed unprecedented access to information regarding the
458 genetic control of early seed developmental (Yi et al., 2019). The most detailed spatial
459 analysis to date used Laser-Capture Microdissection (LCM) on 8 DAP kernels (Zhan et al.,
460 2015) to reveal the expression of specific populations of genes in the maternal tissues, the
461 embryo and the main endosperm cell types namely ESR, BETL, AL and SE (which was
462 subdivided in CSE and CZ). Although providing an extremely valuable resource, these studies
463 did not address the question of whether specific transcriptional domains exist at the
464 embryo-endosperm interface.

465 Both the endosperm and embryo are complex compartments with several
466 morphologically and functionally distinct domains (Olsen, 2004b; Sabelli and Larkins, 2009).
467 Because they undergo complex and coordinated developmental programmes, the interfaces
468 between the embryo and the endosperm represent important, and constantly changing
469 zones of exchange, both in term of nutrition and communication (Ingram and Gutierrez-
470 Marcos, 2015; Nowack et al., 2010; Widiez et al., 2017). In order to understand these
471 interactions two subdomains of the endosperm and one subdomain of the embryo were
472 hand-dissected: the scutellar aleurone layer (SAL) at the adaxial side of the embryo; the
473 starchy endosperm in close contact with the abaxial side of the embryo (EAS); and the
474 scutellum of the embryo (AS). 13 DAP kernels were chosen for our analysis because at this
475 stage the embryo has emerged from the ESR and is establishing new interactions with
476 endosperm. From a practical point of view, 13 DAP is also the earliest stage allowing reliable
477 hand dissection of the chosen interfaces.

478 Contamination with neighbouring tissues is an important issue in any dissection
479 experiment. For example, in Arabidopsis, an extremely valuable and globally very reliable
480 resource generated by LCM (Belmonte et al., 2013; Le et al., 2010) was recently shown to

481 contain some inter-compartment contamination, which caused problems for the
482 investigation of parental contributions to the transcriptomes of early embryos and
483 endosperms (Schon and Nodine, 2017). In our study, precautions were taken to limit inter-
484 tissue contamination by (i) washing each sample before RNA extraction (see material and
485 methods) and (ii) generating four biological replicates for each tissue. Marker gene analysis
486 confirmed the conformity of the samples with the exception of a potential minor
487 contamination of pericarp by the AL, suggested by the apparent expression of both the *AL9*
488 and *Zm00001d024120* aleurone marker genes and the endosperm marker genes
489 *ZmZOU/O11* and *O2* in the pericarp sample (Figure 2E, F). This could have been caused by
490 the tendency of the AL to stick either to the starchy endosperm or to the pericarp. In
491 addition, residual ESR tissues at 13 DAP might have contaminated both our SAL and EAS
492 samples (Figure 2G).

493

494 **The EAS, a novel endosperm subdomain likely involved in carbon and** 495 **nitrogen fluxes from the endosperm to the embryo**

496 Transcriptomic profiling of the two endosperm interfaces with the embryo (SAL and
497 EAS) revealed specific transcriptional signatures. While this could have been expected for
498 the cytologically distinct SAL, it was rather unexpected for the cell layers adjacent to the
499 abaxial side of the embryo, which do not present any obvious cytological differences to
500 other SE cells (Van Lammeren, 1987). Based on the observed enrichment of hundreds of
501 transcripts in these cell layers, they represent a novel subdomain of the endosperm which
502 we named “endosperm adjacent to scutellum” (EAS).

503 GO analysis revealed a significant enrichment in the GO category transmembrane
504 transporter activity for both the SAL and EAS, and additionally for lipid transporter activity
505 for SAL (Table 2). A closer look at DEGs for both EAS and SAL shows the presence of different
506 transporter gene families (Table 3). Interestingly, many *UMAMITs* and *SWEETs*, thought to
507 transport amino acids/auxin and sugars, respectively, were found enriched in the EAS.
508 *UMAMITs* and *SWEETs* are considered to be bi-directional transporters, although they tend
509 to act as exporters when located at the plasma membrane, exporting nutrients down
510 concentration gradients generated by sinks in adjacent tissues (Chen et al., 2012; Müller et
511 al., 2015). Two non-exclusive hypotheses could explain the elevated expression of

512 transporter-encoding genes in the EAS: either these cells actively take up nutrients that
513 arrive from the BETL via the SE and then export them into the apoplastic space surrounding
514 the growing embryo, or they are simply involved in recycling nutrients from dying
515 endosperm cells that are crushed by the growing embryo.

516 With regard to nutrient uptake on the embryo side, one might expect the expression
517 of genes encoding nutrient importers at the surface of the scutellum in order to take up
518 apoplastic metabolites. However, in our apical scutellum transcriptome (AS) we were not
519 able to detect differentially expressed importer-encoding genes with respect to the entire
520 embryo (Emb). While this could suggest that the regulation of importer activity does not
521 occur at the transcriptional level, it seems more likely that our transcriptomic comparison AS
522 vs Emb was not well designed for the identification of such genes, since the whole embryo is
523 mainly composed of scutellum tissues.

524 In the future a more detailed comparison of the gene expression profiles of the BETL
525 (import) and the EAS (export) regions could be informative. The BETL is an interface
526 specialized in nutrient transfer from maternal phloem terminals to the endosperm (Chourey
527 and Hueros, 2017). The hexose transporter *SWEET4c* is preferentially expressed in the BETL,
528 and loss of function of *sweet4c* results in the production of a shrivelled endosperm,
529 illustrating the critical importance of hexose transport in the BETL for normal endosperm
530 growth (Sosso et al., 2015). Interestingly, *sweet4c* is also found in the DEGs showing strong
531 expression in the EAS compared to the endosperm as a whole, possibly suggesting
532 commonalities between BETL and EAS function. EAS-specific knock-down of *SWEET4C* might
533 be one strategy to test this hypothesis and to address the question of possible redundancy
534 with *SWEET14a* and *SWEET15a*, also enriched in the EAS. Nonetheless, notable differences
535 exist between the EAS and the BETL. Firstly BETL cells have structural features including
536 dramatic cell wall ingrowths that make them unique in the endosperm (Chourey and Hueros,
537 2017; Leroux et al., 2014). In contrast EAS cells cannot be morphologically differentiated
538 from the SE (Van Lammeren, 1987). Secondly, the BETL represents a static interface,
539 contrary to the EAS which is displaced as the embryo scutellum expands (Figure 6) during the
540 most rapid growth-phase of the embryo (Chen et al., 2014).

541 **The EAS is a developmentally dynamic interface**

542 The detection of DNA fragmentation, a characteristic of cell death, in EAS cells (Figure
543 5C, D), together with an accumulation of cell wall material in this zone (Figure 5A, B)
544 suggested that endosperm cells are eliminated as the embryo grows. An important question
545 is whether this involves a genetically controlled cell-autonomous death or a more atypical
546 and passive cell death process caused by embryo growth. In the Arabidopsis seed, where
547 most of the endosperm degenerates during seed development, the expression of
548 developmental cell death marker genes such as *PLANT ASPARTIC PROTEASE A3 (AtPASPA3)*
549 or *BIFUNCTIONAL NUCLEASE 1 (AtBFN1)* has been detected at the embryo interface
550 (Fourquin et al., 2016; Olvera-Carrillo et al., 2015). In maize, less is known about molecular
551 actors involved in developmental cell death. To the best of our knowledge, cell death marker
552 genes have not been comprehensively identified in maize. Nevertheless a survey of putative
553 cell death marker genes derived from comparisons with other plant systems, showed their
554 expression in EAS cells, but without any significant enrichment compared to other
555 compartments (Supplemental Figure 7 and Supplemental Data Set 2). Although cell death in
556 the EAS could be triggered by the activation of unknown cell death-associated genes, a more
557 likely explanation for our observations could be a dilution of the transcriptional signal in the
558 EAS transcriptome, making it undetectable. This is supported by TUNEL staining, which
559 revealed a very localised signal limited to a few cells at the immediate interface with the
560 embryo (Figure 5C, D). In addition, previous cell death staining with Evans blue, did not
561 reveal any massive cell death in the EAS, further supporting the hypothesis of very localized
562 cell death events (Young and Gallie, 2000).

563 The precise spatial organisation of cell death and transporter expression remains
564 unclear but the expression of transporters might allow the recycling of nutrients from the
565 cells before they die. As these cells are SE in origin, they could already have initiated nutrient
566 storage at 13 DAP, as illustrated by substantial expression of *ZEIN* genes (Supplemental
567 Figure 6). Nutrient recycling could be an advantageous way for the plant to efficiently reuse
568 stored nutrients. Interestingly, in Arabidopsis the STP13 sugar transporter is upregulated in
569 several cell death contexts and the expression of many transporters increase during organ
570 senescence suggesting a function in nutrient recycling from dying cells (Graaff et al., 2006;
571 Norholm et al., 2006; Zhang et al., 2014). However, the precise role of transporters in
572 nutrient recycling remains poorly understood in plants.

573 **The importance of the embryo for the expression of EAS marker genes**

574 Since the EAS is a mobile interface, forming adjacent to the expanding scutellum, we
575 asked whether the presence/absence of the embryo influences the activation of EAS marker
576 genes (Figure 7). In *emb8522* mutant kernels which produce a seemingly empty, but
577 normally sized embryo cavity containing an aborted embryo (Heckel et al., 1999; Sosso et al.,
578 2012) the expression of different EAS marker genes was affected in different ways. The
579 *SWEET15A* gene was still expressed in EAS cells, but also became strongly expressed at the
580 opposite embryo/endosperm interface (SAL). Based on the precedent of the *SWEET4c*
581 transporter, which gene is induced by sugar (Sosso et al., 2015), it is possible that a similar
582 induction could occur in the case of *SWEET15a*. The absence of a normal embryo could lead
583 to a build-up of sucrose in the embryo cavity of *emb8522* mutants leading to such an
584 induction. In contrast, the expression domain of the *Zm00001d017285* marker gene is
585 reduced in *emb8522* mutants, with expression becoming restricted to the apical part of the
586 EAS. Finally, the expression of *PEPB11* and *SCL_EAS1* is dramatically reduced in *emb8522*
587 mutants compared to phenotypically wild-type kernels. Our results suggest that EAS-specific
588 gene expression could be a result of several independent factors, some of which could
589 originate from the endosperm, and others from the embryo. The mechanisms involved in
590 embryo cavity formation remain elusive, although a recent study showed that the SHOHA1
591 protein is required in the endosperm for the formation of the embryo cavity (Mimura et al.,
592 2018).

593 Interestingly, the expression of both *PEPB11* and *SCL_EAS1* initiates relatively late in
594 the EAS, whereas the expression of *SWEET15a* and *Zm00001d017285* initiates before 9 DAP.
595 This suggests the presence of at least two transcriptional programs in the EAS: one initiating
596 early and weakly influenced by the embryo and a second activated later, and more strongly
597 embryo-dependent. The generation of comparable transcriptomes at earlier developmental
598 stages could help us identify the key signals activating gene expression in the EAS, and
599 potentially pinpoint transcription factors regulating gene expression in this tissue. In parallel,
600 phenotypic analysis of loss-of-function mutants of genes enriched in the EAS is needed to
601 further elucidate the biological role of this novel endosperm subdomain.

602

603 **Material and Methods**

604 **Plant material, plant growth conditions**

605 A188 and B73 inbred lines were cultivated in the green house as described previously
606 (Rousseau et al., 2015). A188 inbred line depicted in supplemental figure 1 was cultivated in
607 a growth chamber as described previously (Doll et al., 2019). The *emb8522* mutant in the R-
608 scm-2 background (Sosso et al., 2012) and the B73 plants used for *in situ* hybridization were
609 grown in a field plot located at the ENS de Lyon, France.

610 **Isolation of maize kernel compartments**

611 Kernel (sub)compartments of the B73 inbred line were hand dissected and quickly
612 washed with HyClone Dulbecco's phosphate-buffered saline solution (ref. SH30378.02),
613 before freezing them in liquid nitrogen. For each (sub)compartment, four independent
614 biological replicates were produced (Supplemental Table 1). For each biological replicate, the
615 material comes from two independent, 13 day-old maize ears, i. e. a total of 8 different ears
616 was used for each (sub)compartment. Within each biological replicate, tissues from 4 to 84
617 kernels were pooled depending on the size of the considered (sub)compartment
618 (Supplemental Table 1).

619 **RNA extraction and RNA-seq**

620 Total RNAs were extracted with TRIzol reagent, treated with DNase using the Qiagen
621 “RNase-Free DNase Set”, and purified using Qiagen RNeasy columns according to the
622 supplier's instructions. RNA-seq libraries were constructed according to the
623 “TruSeq_RNA_SamplePrep_v2_Guide_15026495_C” protocol (Illumina®, California, USA).
624 Sequencing was carried out with an Illumina HiSeq2000 at the IG-CNS (Institut de
625 Génomique- Centre National de Séquençage). The RNA-seq samples were sequenced in
626 paired-end (PE) mode with a sizing of 260 bp and a read length of 2×100 bases. Six samples
627 were pooled on each lane of a HiSeq2000 (Illumina), tagged with individual bar-coded
628 adapters, giving approximately 62 millions of pairs per sample. All steps of the experiment,
629 from growth conditions to bioinformatics analyses, were managed in the CATdb database
630 (Gagnot et al., 2008, <http://tools.ips2.u-psud.fr/CATdb/>) with project ID
631 “NGS2014_21_SeedCom” according to the MINSEQE (minimum information about a high-
632 throughput sequencing experiment) standard (<http://fged.org/projects/minseqe/>).

633 **RNA-seq read processing and gene expression analysis**

634 RNA-seq reads from all samples were processed using the same pipeline from
635 trimming to counts of transcripts abundance as follows. Read quality control was performed
636 using the FastQC (S. Andrew, <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>).
637 The raw data (fastq files) were trimmed using FASTX Toolkit version 0.0.13
638 (http://hannonlab.cshl.edu/fastx_toolkit/) for Phred Quality Score > 20, read length > 30

639 bases, and ribosomal sequences were removed with the sortMeRNA tool (Kopylova et al.,
640 2012).

641 The genomic mapper TopHat2 (Langmead and Salzberg, 2012) was used to align read
642 pairs against the *Zea mays* B73 genome sequence (AGP v4, (Jiao et al., 2017)), using the gene
643 annotation version 4.32 provided as a GFF file (Wang et al., 2016). The abundance of each
644 isoform was calculated with the tool HTSeq-count (Anders et al., 2015) that counts only
645 paired-end reads for which paired-end reads map unambiguously one gene, thus removing
646 multi-hits (default option union). The genome sequence and annotation file used was
647 retrieved from the Gramene database (<http://www.gramene.org/> release 51, in September
648 2016 (Gupta et al., 2016)).

649 Choices for the differential analysis were made based on Rigai et al., 2018. To
650 increase the detection power by limiting the number of statistical tests (Bourgon et al.,
651 2010) we performed an independent filtering by discarding genes which did not have at least
652 1 read after a count per million analysis (CPM) in at least one half of the samples. Library size
653 was normalized using the method trimmed mean of M-values (TMM) and count distribution
654 was modelled with a negative binomial generalized linear. Dispersion was estimated by the
655 edgeR package (version 1.12.0, McCarthy et al., 2012) in the statistical software 'R' (version
656 2.15.0, R Development Core Team, 2005). Pairwise expression differences were performed
657 using likelihood ratio test and *p*-values were adjusted using the Benjamini-Hochberg
658 (Benjamini and Hochberg, 1995) procedure to control FDR. A gene was declared to have a
659 differential expression if its adjusted *p*-value was lower than 0.05. The FPKM value
660 (Fragments Per Kilobase of transcript per Million mapped reads) is used to estimate and
661 compare gene expressions in eFP Browser. This normalization is based on the number of
662 paired-end reads that mapped each gene taking into account the gene length and the library
663 size. This RNA-seq read processing method was used for all analyses presented in this
664 manuscript, except for the comparison of our RNA-seq with published RNA-seq, for which
665 the data were processed as described below.

666 **Comparison of our RNA-seq data-set with published RNA-seq data-set.**

667 For the comparison of our dataset with previously published RNA-seq data (Zhan et
668 al., 2015), the raw RNA-seq reads published by Zhan et al. (2015) were retrieved from NCBI
669 SRA (Leinonen et al., 2011) from Bioproject PRJNA265095 (runs SRR1633457 to
670 SRR1633478). That represents 53 millions of pairs of length 2×100 bases, for 22 samples. The
671 reads from the two datasets were processed using the same pipeline: quality control was
672 performed using FastQC version 0.11.7 (S. Andrew,
673 <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Sequencing adapters were
674 clipped using cutadapt v1.16 (Martin, 2011) sequencer artefacts were removed using FASTX
675 Toolkit version 0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/), and custom Perl scripts
676 were applied to trim regions of reads having an average Phred quality score (Ewing and
677 Green, 1998) lower than 28 bases over a sliding window of 4 bases. We noticed some
678 samples retrieved from SRA exhibited a high ribosomal RNA content. We built a maize rRNA

679 database by comparing sequences from Silva (Quast et al., 2013) and RFAM (Kalvari et al.,
680 2018) to the *Zea mays* B73 genome sequence ; we then used this custom database to filter
681 the RNA-seq reads with sortMeRNA version 2.1b (Kopylova et al., 2012). Reads shorter than
682 25 bases at the end of this processing, or with no mate, were discarded.

683 The genomic mapper HISAT2 v2.2.0 (Kim et al., 2015) was used to align read pairs
684 against the *Zea mays* B73 genome sequence (AGP v4; Jiao et al., 2017), using the gene
685 annotation version 4.40 provided as a GFF file (Wang et al., 2016); a first mapping pass was
686 performed with the complete set of read pairs to discover unannotated splicing sites, before
687 the per-sample mapping, with options “-k 10 --no-discordant --no-softclip” and allowing
688 introns of length 40 to 150,000 bp. Mapped reads were counted by gene (not distinguishing
689 isoforms) using FeatureCounts (Liao et al., 2014). The genome sequence and annotation file
690 used was retrieved from the Gramene database (<http://www.gramene.org/>, release 51, in
691 September 2016; Gupta et al., 2016).

692 Normalization, differential analysis and PCAs were performed with DESeq2 (Love et
693 al., 2014) under R version 3.6.2 (R Development Core Team, 2005). The PCAs were done
694 using the 1000 genes with the highest variance, after applying the variance stabilisation
695 transformation (VST) described by Anders and Huber, 2010 and implemented in DESeq2
696 v1.24.0. In parallel, FPKM values (Fragments Per Kilobase of transcript per Million mapped
697 reads) and confidence intervals were estimated using Cufflinks version 2.2.1 (Roberts et al.,
698 2011) with options “--frag-bias-correct --multi-read-correct --max-multiread-fraction 1”.

699

700 **Venn diagrams.**

701 For each compartment/sub-compartment, the mean of expression of the 4 samples
702 was calculated. If the value of the normalized read counts was equal or superior to 1, the
703 gene was considered as expressed. Venn diagrams were drawn using tools available at:
704 <http://bioinformatics.psb.ugent.be/webtools/Venn/>.

705

706 **Functional annotation of *Zea mays* transcriptome, GO term enrichment** 707 **analysis**

708 The *Zea mays* B73 genome sequence v4 (Jiao et al., 2017) and the gene annotation
709 v4.40 were used to predict transcript sequences using the gffread script from the Cufflinks
710 package v2.2.1 (Trapnell et al., 2013). In each isoform sequence, the putative ORFs were
711 identified using TransDecoder ((Haas et al., 2013)
712 <https://github.com/TransDecoder/TransDecoder/wiki>), and the amino acid sequence was
713 predicted. From 46 272 genes, 138 270 transcripts were predicted, leading to 149 699 amino
714 acid sequences.

715 The predicted protein sequences were annotated for functional domains with
716 InterProScan v5.27-66.0 (Jones et al., 2014) using databases Pfam v31.0 (Punta et al., 2012)
717 and Panther 12.0 (Mi et al., 2013). They were also compared to UniprotKB protein database
718 version 2017_12 (2019). The complete Swiss-Prot database of curated proteins was used,
719 (containing 41 689 plant sequences and 514 699 non-plant sequences) but only the plant
720 subset of the non-curated database TrEMBL, containing 5 979 810 sequences). The
721 comparison was carried out using WU-BlastP v2.0MP (Altschul et al., 1990) with parameters
722 “W=3 Q=7 R=2 matrix=BLOSUM80 B=200 V=200 E=1e-6 hitdist=60 hspsepqmax=30
723 hspsepsmax=30 sump postsw”. Blast output was filtered using custom Perl scripts to keep
724 only matches with \log_{10} (e-value) no lower than 75% of the best \log_{10} (e-value).
725 GeneOntology terms (Ashburner et al., 2000) associated to matched proteins were retrieved
726 from AmiGO (Carbon et al., 2009) with all their ancestors in the GO graph, using the SQL
727 interface. For each *Zea mays* protein, we kept the GO terms associated to all its matched
728 proteins or at least to 5 matched proteins. For each *Zea mays* gene, we merged the GO
729 terms of all its isoforms.

730 For subsets of genes selected based on their expression pattern, we used our GO
731 annotation to perform an enrichment analysis. The enrichment of a gene subset in a specific
732 GO term is defined as:

$$733 R = \frac{(\text{Genes annotated with the GO term in the subset})/(\text{Total genes in the subset})}{(\text{Genes annotated with the GO term among all expressed genes})/(\text{Total expressed genes})}$$

734 A hypergeometric test (R version 3.2.3; R Development Core Team, 2005) was applied to
735 assess the significance of enrichment/depletion of each subset (Falcon and Gentleman,
736 2007; Pavlidis et al., 2004). Custom Perl scripts using GraphViz (Ellson et al., 2001)
737 <https://graphviz.gitlab.io/>) were used to browse the GeneOntology graph and identify
738 enrichments or depletions that were both statistically significant and biologically relevant.
739 Only genes with at least one match on Uniprot and only GO terms with at least one gene in
740 the subset were considered for all those statistical tests.

741 Analysis of gene categories and orthology

742 Analysis of orthology to rice and Arabidopsis (Table 3) was based on Maize GDB
743 annotations (<https://www.maizegdb.org/>, Andorf et al., 2016). *ZEIN* genes were selected
744 based on a previous gene list (Chen et al., 2014, 2017) and on Gramene database
745 annotations (<http://www.gramene.org/>, Gupta et al., 2016). The list of cell death associated
746 genes was based on previously published lists (Arora et al., 2017; Fagundes et al., 2015).
747 Heat maps were drawn with the online Heatmapper tool (<http://www2.heatmapper.ca/>,
748 Babicki et al., 2016).

749 Kernel fixation and *in situ* hybridization

750 Kernels were fixed in 4% of paraformaldehyde (pH7 adjusted with H₂SO₄) for 2 h
751 under vacuum. For increased fixation efficiency, the two upper corners of the kernels were

752 cut and vacuum was broken every 15 min. Kernels were dehydrated and included with
753 Paraplast according to the protocol described by Jackson, 1991. Sections of 10-15 μm were
754 cut with a HM355S microtome and attached on Adhesion Slides Superfrost Ultra plus™
755 (ThermoFisher Scientific). RNA probes were amplified from genomic or cDNA (Supplemental
756 Table 4) and labelled by digoxigenin (DIG) using the T7 reverse transcriptase kit of Promega,
757 according to company instructions. RNA probes were then hydrolysed in carbonate buffer
758 (120 mM Na_2CO_3 , 80 mM NaHCO_3) at 60°C for various times depending on the probe length
759 (Supplemental Table 4) in order to obtain RNA fragments between 200 and 300 nucleotides
760 (Jackson, 1991).

761 For the pre-hybridization of the sections, the protocol described by Jackson in 1991
762 was followed with some slight changes: pronase was replaced by proteinase K (1 $\mu\text{g}\cdot\text{mL}^{-1}$,
763 ThermoFisher Scientific) in its buffer (100 mM Tris, 50 mM EDTA, pH8) and formaldehyde
764 was replaced by paraformaldehyde as described above. For each slide, 1 μL of RNA probe
765 was diluted in 74 μL of DIG easy Hyb buffer (Roche), denatured for 3 minutes at 80°C and
766 dropped on a section that was immediately covered by a coverslip. Hybridization was carried
767 out overnight at 50°C, in a hermetically closed box. Initial post hybridization treatments were
768 carried out using gentle shaking as follows: 0.1X SSC buffer (from stock solution 20X SSC (3M
769 NaCl, 300mM trisodium citrate, adjusted to pH7 with HCl)) and 0.5% SDS for 30 min at 50°C
770 to remove the coverslips. Two baths of 1 h 30 in 2X SSC buffer mixed with 50% of formamide
771 at 50°C and followed by 5 min in TBS buffer (400 mM NaCl, 0.1 mM Tris/HCl, pH7.5) at room
772 temperature. Slides were then incubated in 0.5% blocking reagent solution (Roche) for 1h,
773 followed by 30 min in TBS buffer with 1% BSA and 0.3% triton X100. Probes
774 immunodetection was carried out in a wet chamber with 500 μL per slide of 0.225 $\text{U}\cdot\text{mL}^{-1}$
775 anti-DIG antibodies (Anti-Digoxigenin-AP, Fab fragments, Sigma-Aldrich) diluted in TBS with
776 1% BSA and 0.3% triton X100. After 1 h 30 of incubation, slides were washed 3 times 20 min
777 in TBS buffer with 1% BSA, 0.3% triton and equilibrated in buffer 5 (100 mM Tris/HCl pH9.5,
778 100 mM NaCl, 50 mM MgCl_2). Revelation was performed overnight in darkness in a buffer
779 with 0.5 $\text{g}\cdot\text{L}^{-1}$ of nitroblue tetrazolium (NBT) and 0.2 $\text{g}\cdot\text{L}^{-1}$ of 5-Bromo-4-chloro-3-indolyl
780 phosphate (BCIP). Slides were finally washed 4 times in water to stop the reaction and were
781 optionally stained with calcofluor (fluorescent brightener 28, Sigma-Aldrich) and mounted in
782 entellan (VWR). Pictures were taken either with VHX900F digital microscope (Keyence) or for
783 magnification with Axio Imager 2 microscope (Zeiss).

784 **TUNEL staining**

785 Fifteen DAP kernels were fixed in PFA, included in Paraplast and sectioned as
786 described above. Paraplast was removed by successive baths in xylene (2x 5 min) and
787 samples were then rehydrated through the following ethanol series: ethanol 100% (5 min),
788 ethanol 95% (3 min), ethanol 70% (3 min), ethanol 50% (3 min), NaCl 0.85% in water (5 min)
789 and Dulbecco's Phosphate-Buffered Saline solution (PBS) (5 min). Sections were then
790 permeabilized using proteinase K (1 $\mu\text{g}/\text{mL}$, ThermoFisher Scientific) for 10 min at 37°C and
791 fixed again in PFA. Sections were washed in PBS and TUNEL staining was carried out with the

792 ApoAlert DNA Fragmentation Assay Kit (Takara) according to manufacturer's instructions.
793 Sections were then counter-stained with propidium iodide (1 $\mu\text{g}\cdot\text{ml}^{-1}$ in PBS) for 15 min in
794 darkness before being washed three times 5 min in water. Slides were mounted in Anti-fade
795 Vectashield (Vector Laboratories). The fluorescein-dUTP incorporated at the free 3'-hydroxyl
796 ends of fragmented DNA was excited at 520nm and propidium iodide at 620nm. Images
797 were taken on a spinning disk microscope, with a CSU22 confocal head (Yokogawa) and an
798 Ixon897 EMCCD camera (Andor) on a DMI4000 microscope (Leica).

799 **Data Deposition**

800 RNA-Seq raw data were deposited in the international repository GEO (Gene Expression
801 Omnibus, Edgar et al., 2002, <http://www.ncbi.nlm.nih.gov/geo>) under project ID GSE110060
802 (secure token for reviewers access: wxifegswbhabhcr). RNA-seq data as FPKM values is
803 available via the eFP Browser engine ([http://bar.utoronto.ca/efp_maize/cgi-](http://bar.utoronto.ca/efp_maize/cgi-bin/efpWeb.cgi?dataSource=Maize Kernel)
804 [bin/efpWeb.cgi?dataSource=Maize Kernel](http://bar.utoronto.ca/efp_maize/cgi-bin/efpWeb.cgi?dataSource=Maize Kernel)), which 'paints' the expression data onto images
805 representing the samples used to generate the RNA-seq data.

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817 been refused once for funding by the French granting agency ANR.

818 **Author contributions**

819 NMD and TW conceived and designed the experiments. TW performed samples dissections
820 (Supplemental Figure 1) and RNA extractions, JC performed RNA-seq library preparation and
821 sequencing, VB performed RNA-seq read processing and differential gene expression
822 analysis (Supplemental Dataset 1 and 2, Supplemental Figure 2, and Figure 1C), JJ performed
823 bioinformatics to create the GO database and provide scripts to analyses the GO, as well as
824 realized the comparison between published transcriptomes (Supplemental Figure 3), AG and
825 NDF performed TUNEL assay (Figure 5 C, D), NMD performed all other remaining
826 experiments. EE, AP and NJP contributed to the RNA-seq data accessibility via the eFP
827 Browser engine. NMD, PMR and TW analysed the data. NMD prepared tables and figures.

828 NMD, GI, PMR and TW wrote the manuscript. TW was involved in project management and
829 obtained funding.

830

831 **Declaration of Interests**

832 PMR is part of the GIS-BV (“Groupement d’Intérêt Scientifique Biotechnologies Vertes”).

833

834 **Tables**835 **Table 1**

GO term	Level (1)	DEGs/ total (2)	Enrichment (3)	p-value
DEGs Emb vs (End and Per): 1601 of 29845 genes				
GO:0010369 chromocenter	(C6)	8/13	11,47	2,11E-09
GO:0042555 MCM complex	(C3)	9/18	9,32	5,65E-08
GO:0003777 microtubule motor activity	(F9)	24/144	3,11	1,92E-07
GO:0007018 microtubule-based movement	(P4)	24/144	3,11	1,92E-07
GO:0006928 movement of cell or subcellular component	(P3)	24/145	3,09	2.20E-07
GO:0098687 chromosomal region	(C5)	13/50	4,85	2,34E-07
GO:0008092 cytoskeletal protein binding	(F4)	42/348	2,25	3,35E-07
GO:0003774 motor activity	(F8)	24/149	3,00	3,76E-07
GO:0031492 nucleosomal DNA binding	(F5)	7/16	8,15	5,89E-07
GO:0000786 nucleosome	(C4)	19/105	3,37	6,85E-07
DEGs End vs (Emb and Per): 818 of 29845 genes				
GO:0045735 nutrient reservoir activity	(F2)	11/47	8,54	3,59E-09
GO:0019252 starch biosynthetic process	(P8)	7/27	9,46	4,30E-07
GO:0019863 IgE binding	(F5)	3/4	27,36	5,60E-07
GO:0019865 immunoglobulin binding	(F4)	3/4	27,36	5,60E-07
GO:0004866 endopeptidase inhibitor activity	(F6)	9/55	5,97	2,17E-06
GO:0010466 negative regulation of peptidase activity	(P7)	9/55	5,97	2,17E-06
GO:0010951 negative regulation of endopeptidase activity	(P8)	9/55	5,97	2,17E-06
GO:0030414 peptidase inhibitor activity	(F5)	9/55	5,97	2,17E-06
GO:0052548 regulation of endopeptidase activity	(P7)	9/55	5,97	2,17E-06
GO:0061135 endopeptidase regulator activity	(F5)	9/55	5,97	2,17E-06

836

837 **Table 1:** Top ten GO terms (sorted by increasing on p-value) enriched in the differentially expressed
838 genes (DEGs) upregulated in one main compartment compared to the two others. Emb = embryo,
839 End =endosperm, Per = pericarp (1) Minimal depth of the GO term in the GO tree, 'P' = biological
840 process, 'F'=molecular function and 'C' = cellular component. (2) Number of genes associated with
841 the GO term in the DEGs list / Number of GO term annotated genes expressed in at least one sample.
842 (3) The enrichment is defined in the Material and Methods.

843

844 **Table 2**

GO term	Level (1)	DEGs/ total (2)	Enrichment (3)	p-value
DEGs AS vs Emb: 82 of 29845 genes				
GO:0003700 DNA binding transcription factor activity	(F3)	8/743	3,91	0,000202
DEGs EAS vs End: 485 of 29845 genes				
GO:0022857 transmembrane transporter activity	(F3)	26/1111	1,44	0,0256
DEGs SAL vs End: 1995 of 29845 genes				
GO:0008289 lipid binding	(F3)	24/183	1,96	0,000529
GO:0003700 DNA binding transcription factor activity	(F3)	70/743	1,41	0,00158
GO:0022857 transmembrane transporter activity	(F3)	97/1111	1,31	0,00305
GO:0005319 lipid transporter activity	(F3)	4/30	1,99	0,0468

845

846 **Table 2:** All GO terms from F3 (molecular function at level 3) significantly enriched in the
847 differentially expressed genes (DEGs) upregulated in a sub-compartment compared to its
848 compartment of origin. AS= Apical Scutellum, Emb = embryo, Embryo Adjacent to Scutellum (EAS),
849 End =endosperm, and SAL = Scutellar Aleurone. (1) Minimal Depth of the GO term in the GO tree, *F*
850 *stand for* "molecular function". (2) Number of genes associated with the GO term in the DEGs list /
851 Number of GO term annotated genes expressed in at least one samples. (3) The enrichment is
852 defined in the Material and Methods.

853

854 **Table 3**

Transporter family	Ratio SAL/End > 8	Ratio EAS/End > 8
MtN21/UMAMIT	1	5
MtN3/SWEET	0	3
AAP	1	2
MATE	7	1
ABC	3	4
GDU	1	2
VIT	0	2
Phosphate transporters	0	2
Other	32	13
Total number	45	34
% in the gene list	8.45%	16.04 %
Molecules putatively transported	Ratio SAL/End > 8	Ratio EAS/End > 8
Amino acids and/or auxin	7	12

Nucleotides	1	1
Heavy metal	3	3
Sugar	0	4
Phosphate	0	2
Other inorganic ions	5	2

855

856 **Table 3:** Number of genes encoding putative transporters in the DEGs upregulated in the SAL or in
857 the EAS compared to the End per family and per molecules putatively transported. Analysis was done
858 base on orthology to rice and Arabidopsis (see material and method section).

859

860 **Supplemental materials**

861 **Supplemental Table 1**

	Embryo (Emb)	Endosperm (End)	Pericarp (Per)	Scutellar Aleurone Layer (SAL)	Apical Scutellum (AS)	Endosperm Adjacent to Scutellum (EAS)
Rep-1	60	4	4	21	50	14
Rep-2	50	4	4	15	72	15
Rep-3	50	4	4	22	84	15
Rep-4	50	4	4	19	66	16

862

863

864 **Supplemental Table 1:** Number of kernels used for each of the four biological replicates. For each
865 replicate, the material comes from two independent 13 day old maize ears.

866

867 **Supplemental Table 2**

	AS vs Emb	EAS vs End	SAL vs End
Number of genes with a FDR \leq 0.05 in the comparison	11646	13161	16772
Number of DEGs	682	1498	2975
Number of DEGs upregulated in the sub-compartment	82	485	1995
Number of DEGs downregulated in the sub-compartment	600	1013	980

868

869 **Supplemental Table 2:** Number of genes differentially expressed between a sub compartment and its
870 compartment of origin. FDR = false discovery rate, DEGs = differentially expressed genes, AS = apical
871 scutellum, Emb = embryo, End = endosperm, SAL = scutellar aleurone Layer, EAS = endosperm
872 adjacent to scutellum.

873

874

875 **Supplemental Table 3**

Name	Gene id (AGPv4)	Mean SAL	Mean Emb	Mean End	Mean EAS	Mean Per	Mean AS
<i>SWEET14a</i>	Zm00001d007365	562	50	666	10876	22	36
<i>SWEET15a</i>	Zm00001d050577	3105	21	1935	31147	200	10
<i>UMAMIT_EAS1</i>	Zm00001d009063	3,25	0,5	45,5	467	3	0
<i>PEPB11</i>	Zm00001d037439	4586	24	3023	41507	265	13
<i>unknown</i>	Zm00001d017285	668	3	468	7271	85	2
<i>SCL_EAS1</i>	Zm00001d014983	692	449	2327	39382	4587	361
<i>ZmNAC124</i>	Zm00001d046126	0	482	0	0	0	0,5

876

877 **Supplemental Table 3:** Mean expression values (read counts were normalized using the trimmed
 878 mean of M-value (TMM) method) and gene IDs of genes selected for *in situ* hybridization.

879 **Supplemental Table 4**

Name	Probe size (bases)	Time of hydrolysis	Tem plate	Primer name	Primer sequence
<i>SWEET14a</i>	2157	47'45"	gDNA	<i>HIS_Sweet14a-F</i>	ATGGCTGGCCTGTCTCTACAG
				<i>HIS_Sweet14a-R3</i>	TGTAATACGACTCACTATAGGGCTCA TCCCTTTCGTATATAGCAG
<i>SWEET15a</i>	1343	45'15"	gDNA	<i>HIS_Sweet15-F</i>	ATGGCTTTCCTCAACATGGAG
				<i>HIS_Sweet15-R3</i>	TGTAATACGACTCACTATAGGGCCTA CGCTCCGCCCAATGAC
<i>UMAMIT_EAS1</i>	1241	45'40"	cDNA	<i>His_MtN21-1b_F4</i>	CAACGCTGAACCAGGTGCTGTTCTGT
				<i>His_MtN21-1b_R4</i>	TGTAATACGACTCACTATAGGGCGG AGGAACCAAATCAGATCATTCAAG
<i>PEPB11</i>	863	42'30"	cDNA	<i>His_ZCN11_F</i>	ATGCCTCTCATCGTCTTCCACTATC
				<i>His_ZCN11_R</i>	TGTAATACGACTCACTATAGGGCTGT AAGGCAAGTGACTTGATGACAC
<i>Zm00001d017285</i>	685	35'30"	cDNA	<i>His_SSE2_F</i>	AAAGCAACAATCCCTACATTCAAAC
				<i>His_SSE2_R</i>	TGTAATACGACTCACTATAGGGCAAC TCATTTTGAACCAAGCCATACAAC
<i>SCL_EAS1</i>	1024	41'50"	cDNA	<i>His_SCL_F</i>	ATGGCAAAAATGGCGATGGCGTCTCT
				<i>His_SCL_R</i>	TGTAATACGACTCACTATAGGGCTTC TTTGTCCCGACAAGCTTCATGATG
<i>ZmNAC124</i>	1488	45'50"	gDNA	<i>His_NAC_TF_Emb_F</i>	ATGCATCCAGGTGTTGGACCA
				<i>His_NAC_TF_Emb_R3</i>	TGTAATACGACTCACTATAGGGCTTA CTTGGGGAACCTGAGGAGA

880

881 **Supplemental Table 4:** Primers used in this study and conditions for RNA probes synthesis.

882

883 **Supplemental Data Set 1:** number of normalized read counts per gene annotated in the AGP
 884 v4 version of the B73 maize genome. Read counts were normalized using the trimmed mean
 885 of M-value (TMM) method (library size normalization).

886 **Supplemental Data Set 2:** Pairwise comparison of gene expression levels between the
 887 tissues. The 15 different comparisons are presented on different sheets. The genes with a
 888 statistical difference supported by a FDR < 0.05 are listed. Among them, the DEGs (*i.e.* genes
 889 with a $\log_2(\text{fold change}) \geq 2$) are highlighted in yellow.

890

891

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