1 **Title:** GPI-anchored SKU5/SKS are maternally required for integument

2 development in Arabidopsis

3 **Running title:** SKU5/SKS are required for integument development

4 **Authors:** Ke ZHOU[1,2] (zhouke0525@gmail.com)

5 **Affiliations**: 1. FAFU-UCR Joint Center for Horticultural Biology and Metabolomics, Haixia Institute of

6 Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

7 2. Institut des Sciences du Végétal, UPR2355, CNRS, Saclay Plant Sciences, 91198 Gif sur Yvette Cedex,

8 France.

9 **Corresponding author:** Ke ZHOU, (zhouke0525@gmail.com), FAFU-UCR Joint Center for Horticultural

10 Biology and Metabolomics, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry

11 University, Fuzhou, 350002, China

12 **CRCID:** https://orcid.org/0000-0002-5883-2550

13 **ABSTRACT**

14 Glycosylphosphatidylinositol-anchored proteins (GPI-APs) play crucial roles in various processes in

15 eukaryotes. In *Arabidopsis*, *SKS1*, *SKS2*, *SKS3* and *SKU5* from *SKU5/SKS* gene family could encode GPI-

16 anchored proteins, and they were recently reported to regulate cell polar expansion and cell wall synthesis

17 redundantly in roots. Here, we report that, they are also redundantly crucial for seed production and seed

18 morphogenesis in *Arabidopsis* through regulating maternal integument development. Their *loss-of-*

19 *functions* resulted in disrupted development of integuments that failed to protect embryo sacs from

20 exposure to external space due to physical restriction, and lead to female gametophytic abortion.

21 Interestingly, those less defective ovules could be fertilized and develop into seeds normally, however, their

22 seed morphogenesis was largely affected.

23 Our research made SKS1, SKS2, SKS3 and SKU5 be not only the first class of GPI-anchored proteins that

24 could regulate maternal integument development, but also the first class of proteins that could regulate cell

25 polar expansion in both root and integument cells besides several MAPK cascade components. Our study

26 also underlined the regulation by integument development in reproductive processes.

27 **KEY WORDS**

28 Glycosylphosphatidylinositol; GPI-anchored protein; GPI-AP; Integument Development; Seed

29   Morphogenesis; *Arabidopsis*

30   **INTRODUCTION**

31   In eukaryotes, various protein precursors could be modified during maturation in endoplasmic reticulum

32   and Golgi bodies, where their special hydrophobic carboxyl-terminal signal polypeptides could be cleaved

33   and catalyzed to be replaced by a common glycosylphosphatidylinositol (GPI) oligosaccharide structure. It

34   covalently links the lipid bilayer and the carboxyl-terminus of glycosylphosphatidylinositol-anchored

35   proteins (GPI-APs), which allows these proteins to be attached on outer surface of plasma membrane

36   (Kinoshita and Fujita, 2016; Oxley and Bacic, 1999; Stevens, 1995; Zhou, 2019b). Disruption of GPI

37   moieties biosynthesis resulted in broad and severe defections during various developmental processes

38   (Bundy et al., 2016; Gillmor et al., 2005; Kawagoe et al., 1996; Kinoshita, 2014).

39   In *Arabidopsis*, more than 300 GPI-APs from diverse families have been identified or predicted by

40   bioinformatics or proteomic tools (Borner et al., 2003; Takahashi et al., 2016), among which, only a small

41   proportion of them has been characterized of their involvement in cell wall components synthesis, catalytic

42   processes, signaling transduction, stress and pathogen response, and so on (Zhou, 2019b). But to date, no

43   particular GPI-AP has been reported to regulate integument development in plants.

44   *SKU5-Similar* (*SKU5/SKS*) gene family, named after *SKU5*, which has been reported to be involved in

45   twisting bias of root growth in *Arabidopsis*, contains 19 members (Rutherford and Masson, 1996; Sedbrook

46   et al., 2002). *SKS1*, *SKS2*, *SKS3* and *SKU5* (*GPI-SKU5/SKS*) within the same subgroup of this family could

47   encode GPI-anchored proteins, and they were reported by us to be redundantly essential for root

48   development through affecting anisotropic cell expansion in root and cell wall synthesis, and *SKS1* made

49   significant contribution during this process (Zhou, 2019a; Zhou, 2019c).

50   In this study, we further investigated the involvement of this subgroup of *GPI-SKU5/SKS* genes in

51   reproductive processes of *Arabidopsis*. *Loss-of-functions* redundantly and drastically reduced their seed

52   production, which was demonstrated to be due to maternal-dependent ovule abortions. In those aborted

53   ovules, the extension of their maternal integuments was disturbed and resulted in physical restriction to

54   female gametophytes, which lead to their exposure to out space and consequent female gametophytic

55   abortion. Although those less defective ovules could be fertilized and develop into seeds, their seed

56   morphogenesis was largely affected as the aberrant integument/seed coat development.

57   The interference on integument development seemed due to similar defective polar expansion of

58   integument cells as we described in their root cells previously, which is very surprising, as to our

59   knowledge, none of gene has been reported to interfere polar expansion on both root and integument cells

60   besides a series of MAPK components.

61   Our study firstly reported the crucial involvement of particular GPI-APs, GPI-SKU5/SKS proteins, in

62   reproductive processes through regulating maternal integument development, which increased functional

63   diversity of GPI-APs in *Arabidopsis*. Surprisingly, this group of GPI-APs could regulate cell polar

64   expansion in both root and integument the same as a series of MAPK components.

65   **MATERIALS AND METHODS**

66   **Pseudo-Schiff Propidium Iodide Staining**

67   Ovules and seeds were harvested and then fixed in greenfix solution (Diapath) for 1 to 3 h at room

68   temperature. Fixed samples were transferred into water and kept at 4°C for up to 7 d. The samples were

69   rinsed once more with water and incubated in a 1%SDSand 0.2 N NaOH solution at 37°C for 1 h. The

70   samples were rinsed in water and incubated in a a-amylase solution (0.4 mg/mL a-amylase, 20 mM NaPO4,

71   and 6.7mMNaCl, pH 6.9) at 37°C for 2 h. The samples were rinsed in water and incubated in a 12.5%

72   bleach solution (1.25% active Cl⁻) for 10 to 15 min. The samples were rinsed in water and then transferred

73   to a 1% periodic acid solution at room temperature for 20 min. The samples were rinsed in water and

74   incubated in a Schiff reagent and propidium iodide solution (100 mM sodium metabisulfite, 0.15 N HCl,

75   and 100 mg/mL propidium iodide) until plants were visibly stained. The samples were rinsed in water and

76   then incubated in achloral hydrate solution (4gchloral hydrate, 1mL glycerol, and 2 mL water) at room

77   temperature for 30min (Fiume et al., 2017; Xu et al., 2016). Finally, the samples were transferred onto

78   microscope slides for confocal laser scanning microscopy analysis (Leica SP5)

79   **Mature embryo sac observation**

80   The oldest closed flower buds of a given inflorescence was emasculated and harvested two days later. The

81   collected pistils were fixed in ethanol: acetic acid (9:1) solution overnight, and followed by two washes in

82   90% and 70% ethanol. Then they were cleared by a chloral hydrate:glycerol:water solution (8:1:2, w:v:v)

83   shortly before observed under DMI6000 microscope system with white light (Gross-Hardt et al., 2007;

84   Yadegari et al., 1994).

85   **Plant materials and growth conditions**

86   *Arabidopsis.Thaliana Wassilewskija* (*Ws*) and columbia accession was acquired as wild type. Mutants

87   acquired in the study were all in *Ws* background. *T-DNA* insertion lines, *sks1* (FLAG_521F09), *sks2*

88   (FLAG_415F09), *sku5* (FLAG_386B03) were obtained from Arabidopsis Thaliana Resource Center of

89   Institute Jean-Pierre Bourgin (IJPB) and *sks3* (SALK_067925). Seeds were surface sterilized and plated on

90   1/2MS medium containing 1% agar. After 48 hours vernalization at 4°C in dark, they were plated at 22°C

91   under continuous light. About 7 days growth, seedlings were transferred into soil in green house. Primers

92   for genotyping has been show in Table S1.

93 **RESULTS**

94 **Maternally affected seed production in *gpi-sku5/sks* mutants**

95 Characterization of various combinations of *null* mutations of members of the *GPI-SKU5/SKS* genes
96 revealed their severe defects in seed production, and this group of genes seemed play redundant roles in
97 this process (Fig.S1). Among *gpi-sku5/sks* triple and quadruple mutants, those with *SKS1* mutation
98 generated shorter mature siliques (Fig.1A) containing a large proportion of small, wrinkled and white
99 ovules that failed to develop into seeds (Fig.1B and 1C). Interestingly, together with quadruple mutant,
100 *sks1;sks2;sks3* triple mutant was nearly infertile due to those failures of ovule development, and bare seed
101 was produced.

102 To determine the origin of such a phenotype, we analyzed the segregation of the quadruple mutant allele in
103 the progeny of *sks1+/-;sks2;sks3;sku5* plants. A gametophytic- or zygotic-caused fertility due to *GPI-*
104 *SKU5/SKS* mutations would result in rare detection of quadruple mutant seeds. By contrast, 20.69% (n=116)
105 seedlings germinated from seeds generated by *sks1+/;sks2;sks3;sku5* plants were quadruple homozygous,
106 which suggested the major contribution made by maternal tissue in this process.

107 According to our previous quantitative PCR (Zhou, 2019c) and *in silico* data (Fig.S2 and S3), unlike the
108 specific expression of *SKS2* in reproductive organs, *SKS1*, *SKS3* and *SKU5* ubiquitously expressed at
109 different level among the whole developmental progresses. Surprisingly, in spite of the significant
110 contribution for reproduction, *SKS1* was expressed at relatively low level in reproductive organs.

111 Altogether, these data indicated that, *GPI-SKU5/SKS* genes played redundant and crucial roles in seed
112 production, potentially through regulating ovule or seed development, and *SKS1* made significant
113 contribution. By genetic analyses, defective maternal tissues were determined to be majorly responsible in
114 this process.

115

116

117

4

118
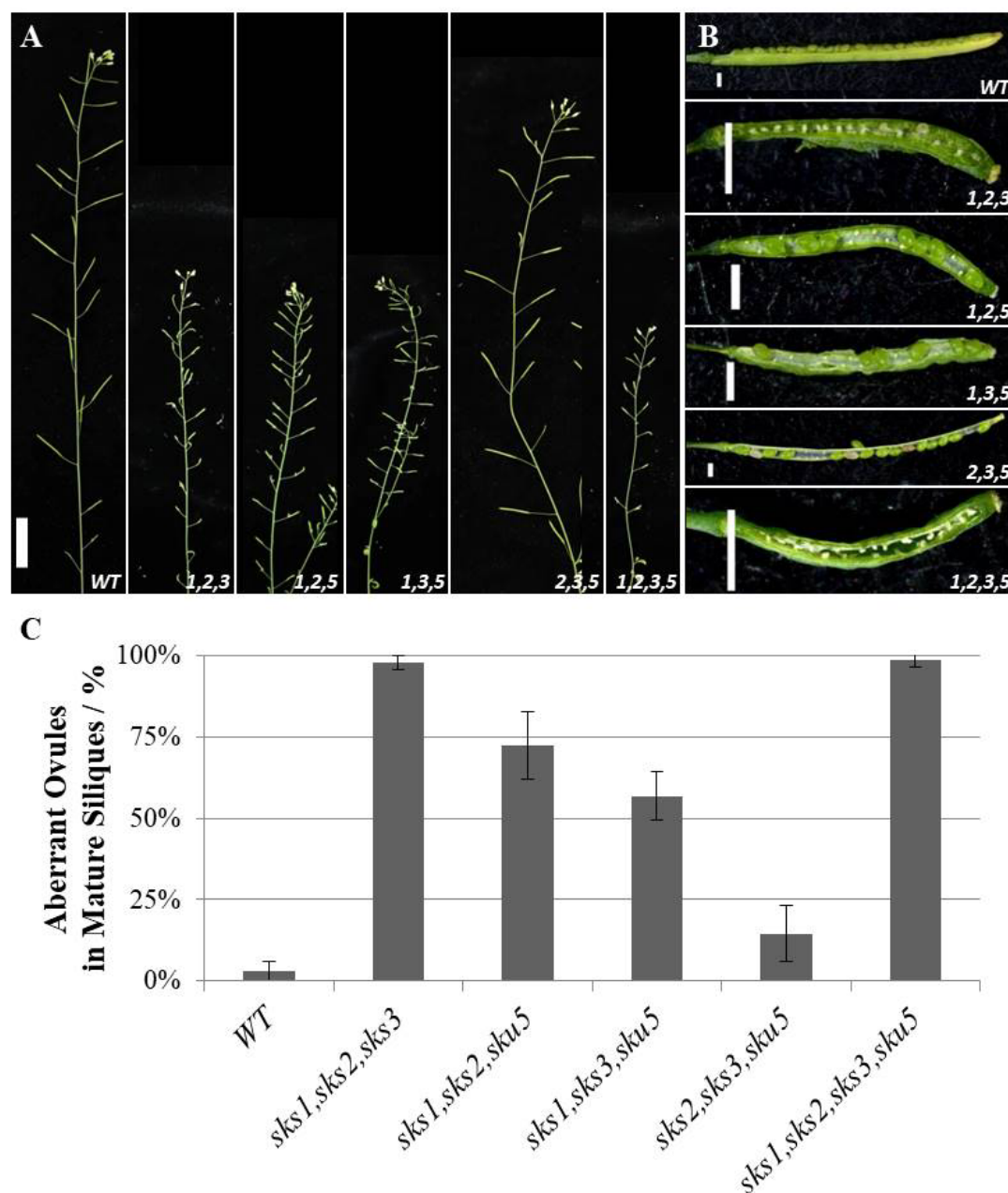
Fig1. Dramatic decreased seed production in *gpi-sku5/sks* mutant. (A), Mature siliques generated by wild-type and *gpi-sku5/sks* mutant lines. From Left to Right: Wild-type, *sks1,sks2,sks3*; *sks1,sks2,sku5*; *sks1,sks3,sku5*; *sks2,sks3,sku5* and *sks1,sks2,sks3,sku5*; bar, 2cm. **(B),** Dissected siliques from *wild-type* and *gpi-sku5/sks* mutants. From Top to Bottom: wild-type, *sks1,sks2, sks3*; *sks1,sks2,sku5*; *sks1,sks3,sku5*; *sks2,sks3,sku5* and *sks1,sks2,sks3,sku5*; bar, 1mm. **(C),** Proportion of white, small, and wrinkled undeveloped ovules among all ovules/seeds in mature green siliques from triple and quadruple *gpi-sku5/sks* mutants were shown. N=15 siliques, standard errors were indicated.

125

126

127   **Drastic decreased seed production was due to ovule abortion in *gpi-sku5/sks* mutants**

128   To further investigate the maternal origin of the *gpi-sku5/sks* mutant phenotype, the ovules from flowers

129   just blooming were collected and observed using the modified pseudo-Schiff propidium iodide (mPS-PI)

130   technique (Fiume et al., 2017; Xu et al., 2016). Surprisingly, more than 90% of the *sks1;sks2;sks3;sku5*

131   ovules failed to develop an embryo sac (Fig.2B, and 2I), and the rest showed an exposed embryo sac that

132   was not covered by the outer integument (Fig.2C and 2I), whereas both of them displayed irregular

133   maternal integuments. The same defective ovules were also observed, at lower frequency, in *gpi-sku5/sks*

134   triple mutant ovules (Fig.2D-2I) and the proportion of *sku5/sks* ovules that failed to form an embryo sac

135   matched that of aborted ovules in mature siliques in their respective mutant backgrounds (Fig.1C and Fig.

136   2I).

137   It demonstrated that, the drastic decreased seed production observed in *gpi-sku5/sks* mutants maternally

138   resulted from increased irregular ovules that failed to develop an embryo sac and the subsequent ovule

139   abortion, and *GPI-SKU5/SKS* genes were redundantly involved in these processes.

140

141
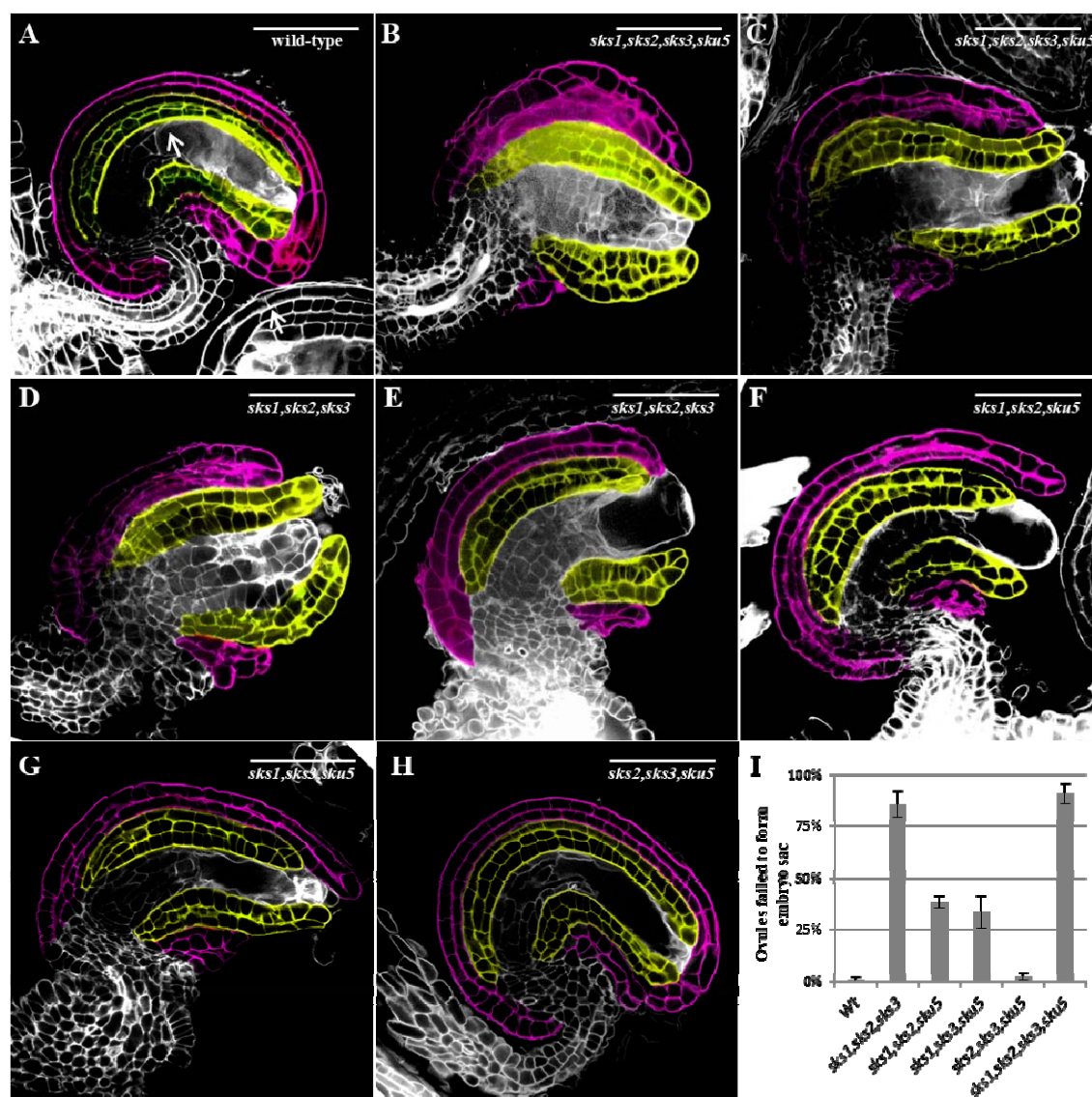
Fig2. Defective ovules generated by *gpi-sku5/sks* mutants with false colors. Ovules were collected from just flowering siliques from (**A**) wild-type; *(***B***) sks1,sks2,sks3,sku5* that failed to form an embryo sac; (**C**) *sks1,sks2,sks3,sku5* that generated an aberrant embryo sac; (**D**) *sks1,sks2,sks3* that failed to form an embryo sac; (**E**) *sks1,sks2,sks3* that generated an aberrant embryo sac; (**F**) *sks1,sks2,sks5*; (**G**) *sks1,sks3,sks5*; and (**H**) *sks2,sks3,sks5*. To better visualized, inner integuments are colored in yellow, and outer integuments are colored in purple; bar: 50μM. (**I**) Proportion of ovules with embryo sac formation failures generated by various combinations of *sku5/sks* mutants. N=3 siliques, and the standard error bars were indicated.

7

Female gametophytic abortion due to physical restriction resulted in ovule abortion in *gpi-sku5/sks* mutants

To follow the processes of their ovule abortion, the triple *null* mutants, *sks1;sks2;sku5*, which exhibited less defective ovules but be capable of generating reasonable seeds, were carefully studied. Our results showed a series of ovules and embryo sacs with various degrees of defections: slightly affected ovule of which integument could basically cover the embryo sac (Fig.3B); embryo sac were not well covered and partly exposed to external space (Fig.3C); embryo sac could barely covered by integument, but be exposed to external space as an outstretching bubble (Fig.3D); collapsing embryo sac at external space without protect by integument (Fig.3E); and ovule loss of embryo sac (Fig.3F).

It indicated that, the female gametophytic abortion, which resulted from physical restriction due to undeveloped maternal integument, should be the main reason of ovule abortion in *gpi-sku5/sks* mutants.
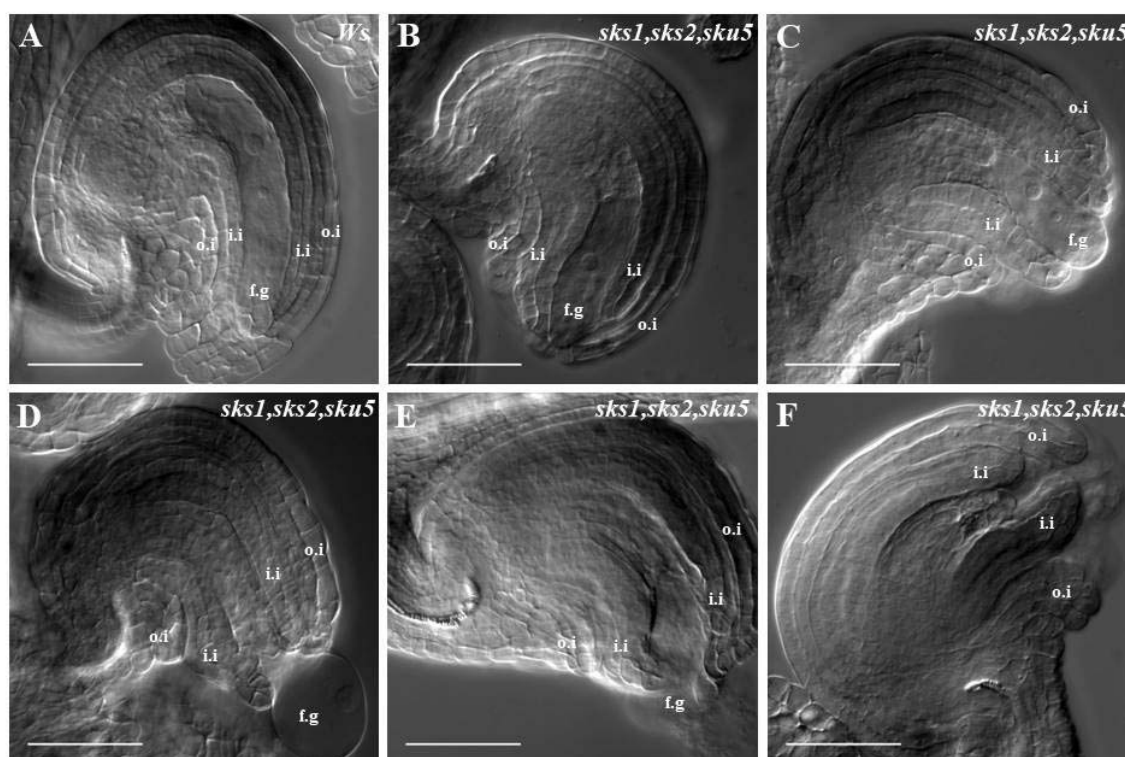

Fig3. Female gametophytic abortion in *gpi-sku5/sks* triple null mutant line. Ovules with mature embryo sacs were collected and observed as described in "materials and methods". **(A)**, wild-type; and a series of ovules and embryo sacs with various degrees of defections **(B-F)**; o.i: outer integuments, i.i: inner integuments, f.g: female gametophytes; bar, 50μm;

174 **Fertilization, embryonic development and seed formation in *gpi-sku5/sks* mutants**

175 Interestingly, those less affected ovules of *sks1;sks2;sku5* could be fertilized normally, however, due to

176 defective integument development, inner integument could not be fully covered and normally curved by

177 asymmetrically elongated outer integuments, which lead their embryos appeared mis-oriented (Fig.4A and

178 4B). Integuments develop into seed coats. Those mis-oriented embryos might develop at wrong position

179 and break through the limit of defective seed coat and grow partially at external space (Fig.4C-4E). These

180 defections certainly affected their seed morphogenesis largely, and exhibited irregularly shaped seed coat

181 epidermal cells (Fig.4C-4E).

182 It demonstrated that, the defections occurred on integuments of *gpi-sku5/sks* mutants also largely affect

183 their seed formation and seed morphogenesis.

184
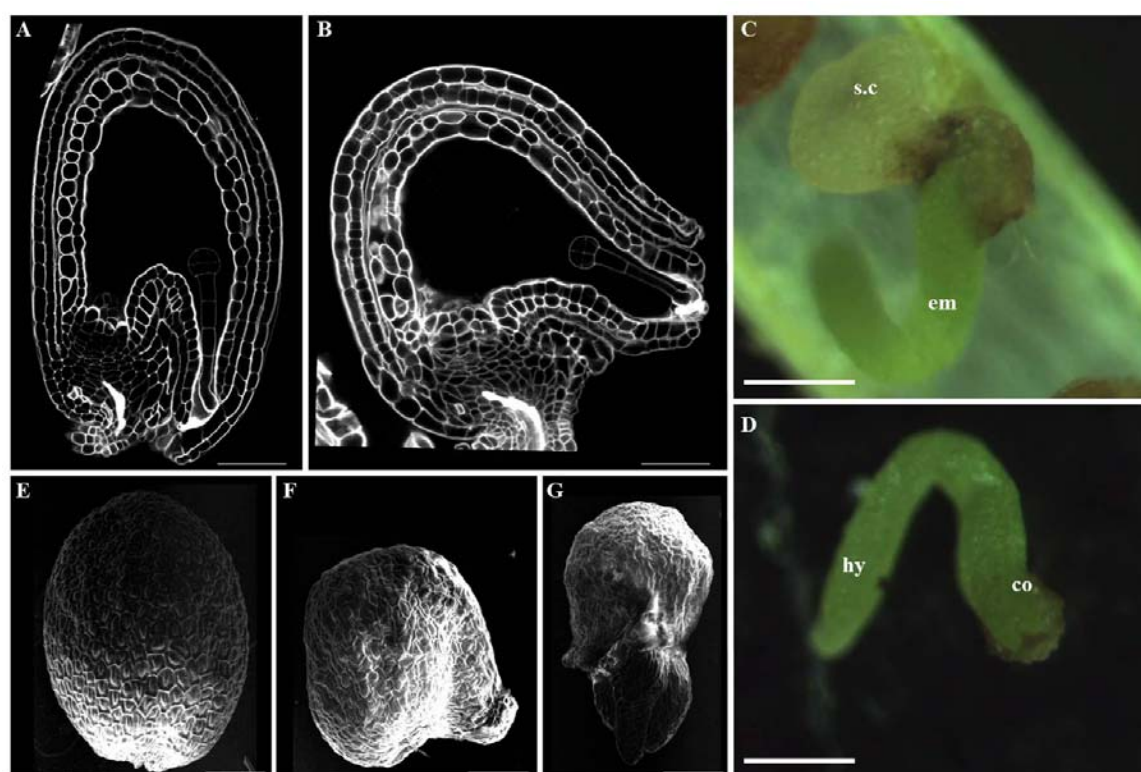
185 **Fig.4 Embryonic development and seed formation in *gpi-sku5/sks* triple null mutant line.** Embryos at pre-globular stage of (**A**)
186 Wild-type and (**B**) *sks1;sks2;sku5* stained by mPS-PI technique and observed under confocal SP8; bar:50μm; (**C**) aberrant seed and (**D**)
187 embryo separated from this seed generated by *sks1;sks2;sku5* mutant at mature embryo stage; s.c: seed coat; em: embryo; hy:
188 hypocotyl; co: cotyledon; bar: 100μm; dry seeds from wild-type (**E**) and *sks1;sks2;sku5* mutant (**F** and **G**) under Scanning Electronic
189 Microscopy; bar: 100μm.

190

191

192

193

9

194 **Maternal regulation of seed morphogenesis in *gpi-sku5/sks* mutant lines**

195 The same affected seed formation and morphogenesis were also found in other *gpi-sku5/sks* mutants that

196 generated defective integuments (Fig.5A-5F), which are irregularly shaped, and some time with tissue

197 protrusion (Fig.5H-5J). Interestingly, the affection on seed formation and morphogenesis was so strong that

198 all seeds generated by mutants with aberrantly developed integuments were irregularly shaped (Fig.5K).

199 To determine the contribution of maternal integument development regulated by *GPI-SKU5/SKS* genes in

200 this process, the segregation of *sks1* mutant allele in the progeny of *sks1+/-;sks2;sks3;sku5* plants was

201 characterized, which showed that, the aberrant seed formation and morphogenesis were only determined by

202 the affected integuments, but not embryonic reason (Fig.5L).

203 These data indicated that, integument development regulated by *GPI-SKU5/SKS* genes were crucial during

204 reproductive processes from gametophytic development to seed formation.

205

206

207

208

209

210

211

212
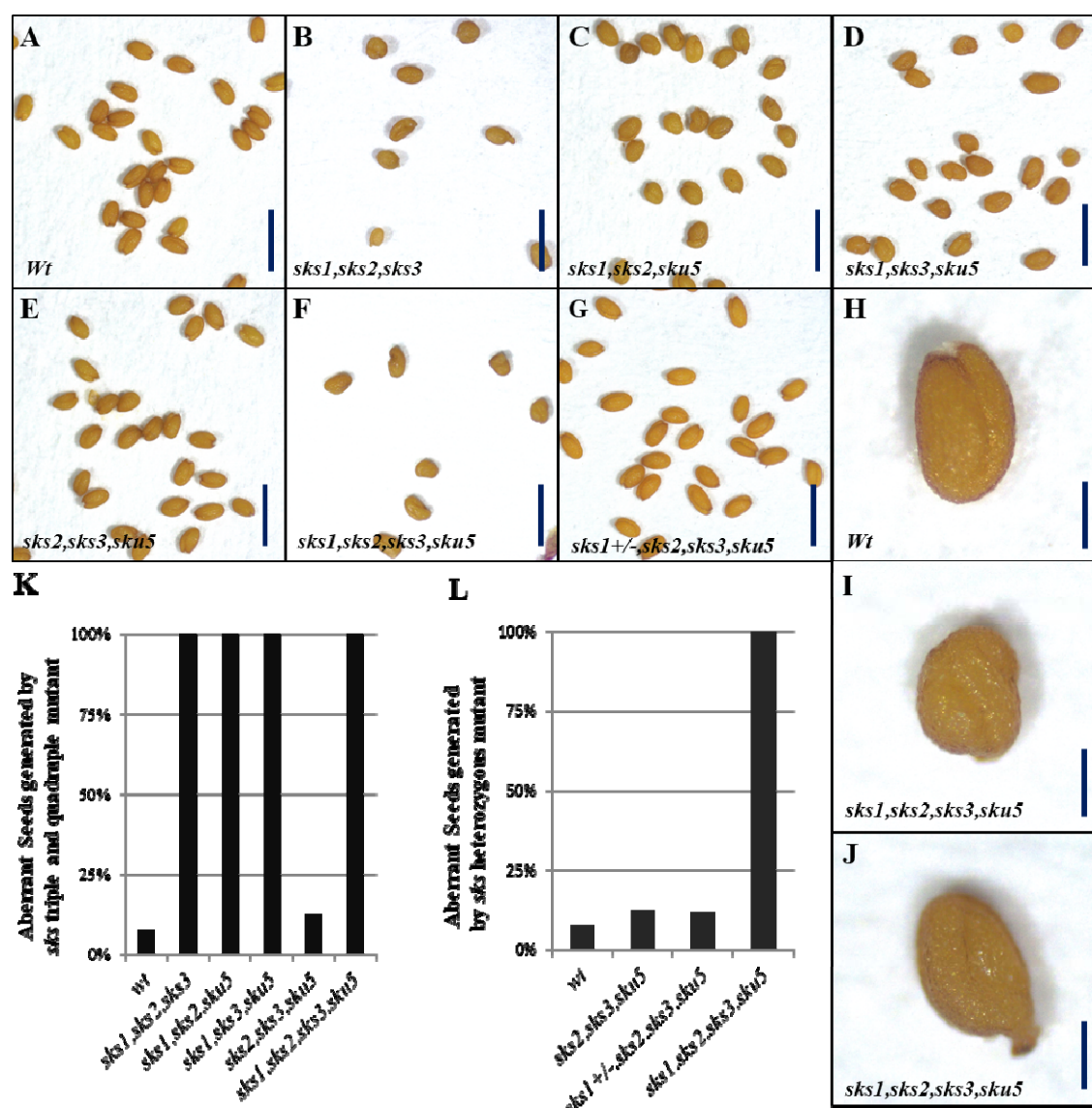
Fig5. Aberrant seed morphogenesis in *gpi-sku5/sks* mutant and seeds segregation generated by *sks1+/-,sks2,sks3,sku5* heterozygotes. Mature seeds generated by (**A**),wild-type (**B**), *sks1,sks2,sks3* (**C**), *sks1,sks2,sku5*; (**D**), *sks2,sks3,sku5* (**E**), *sks1,sks2,sks3,sku5* (**F**), *sks1+/-,sks2,sks3,sku5* (**G**), *sks1+/-,sks2,sks3,sku5*, bar: 1mm; typically seed morphogenesis of (**H**), wild-type and (**I-J**), *sku5/sks* quadruple mutant, bar,0.2mm; (**K**), proportion of aberrant seeds generated by all *sku5/sks* triple and quadruple mutants; (**L**), proportion of aberrant seeds generated by triple, heterozygous and quadruple *sku5/sks* mutants. wild-type (n=375); *sks1,sks2,sks3*(n=11); *sks1,sks2,sku5* (n=234)*; sks1,sks3,sku5* (n=322); *sks2,sks3,sku5* (n= 226), *sks1+/-,sks2,sks3,sku5* (n-347) and *sks1,sks2,sks3,sku5*(n=10).

220

221

222

223

224

11

225   **DISCUSSION**

226   *GPI-SKU5/SKS* **regulate maternal integument development in** *Arabidopsis*

227   Integuments were totally maternal tissues and composed by special double-layer cylinder structures, inner-

228   and outer- integument; after initiation on chalaza, the outer integument asymmetrically grew and eventually

229   covered the inner integument and embryo sac, which determined the curvature of inner integument and

230   shaped the ovule (Gallagher and Gasser, 2008; Gasser and Skinner, 2019; Kunieda et al., 2008; Villanueva

231   et al., 1999). Integument development was believed regulated by a series of signaling pathways, including

232   cell surface receptors (Jorda et al., 2016; Lee et al., 2015; Pillitteri et al., 2007; Terpstra et al., 2010), their

233   intracellular signaling MAP Kinases (Bush and Krysan, 2007; Lopez-Bucio et al., 2014; Meng et al., 2012;

234   Wang et al., 2008; Wang et al., 2007), and nucleus transcriptional factors (Elliott et al., 1996; Gaiser et al.,

235   1995; Gallagher and Gasser, 2008; Gasser and Skinner, 2019; Kunieda et al., 2008; Nole-Wilson and

236   Krizek, 2000; Villanueva et al., 1999). Although fertilization and seed production was largely affected

237   when GPI moieties biosynthesis was interrupted in *Arabidopsis* (Bundy et al., 2016; Gillmor et al., 2005),

238   the mechanism has not been revealed. Here, we firstly reported the involvement of particular GPI-anchored

239   proteins in seed production and seed morphogenesis through regulating integument development.

240   **Defective integument generated by** *gpi-sku5/sks* **mutants**

241   Through our genetic and microscopy analyses, both drastic decrease of seed production resulting from

242   ovule abortion and aberrant seed morphogenesis resulting from defective seed coat formation found in *gpi-*

243   *sku5/sks* mutants were determined to be due to irregularly developed integument. In those mutants, both

244   inner and outer integument were affected, however, defective outer integument, which was supposed to

245   cover the inner integuments, to enclose the embryo sac and to shape the ovules, contributed more in this

246   process. It made inner integument growing symmetrically, and embryo sac exposing to external space,

247   which resulted in loss of female gametophytic due to physical restriction and consequent ovule abortion.

248   Interestingly, those less affected ovules could be fertilized and develop into seeds, however, mis-

249   localization of embryos, or insufficient seed coat that both due to defective integuments would result in

250   post-embryonic abortion or aberrant seed morphogenesis. Here, our study clearly demonstrated the crucial

251   roles *GPI-SKU5/SKS* genes played during integument development, and how they affected seed production

252   and morphogenesis in *Arabidopsis*.

253

254

255

12

256 **Disturbed cell polar expansion in *gpi-sku5/sks* mutants**

257 In *gpi-sku5/sks* mutants, defective integuments seemed due to their disturbed cell polar expansion (Coen
258 and Magnani, 2018; Endress, 2011; Enugutti et al., 2012; Enugutti and Schneitz, 2013; Gaiser et al., 1995;
259 Scholz et al., 2019), where cells from both inner and outer integument seemed shorter, thicker, and
260 aberrantly shaped. It largely disturbed their elongation and extension, where *GPI-SKU5/SKS* played
261 redundant roles and *SKS1* made significant contribution. The same redundancy and significant contribution
262 of *SKS1* in root cell polar expansion were reported by us recently, which exhibited similar shorter, thicker
263 and aberrantly shaped cells (Zhou, 2019c).

264 But surprisingly, to our knowledge, none of gene has been reported to interfere polar expansion on both
265 root and integument cells besides a series of MAPK components, YODA(MAPKKK)-MEK4/5(MAPKK)-
266 MPK3/6(MAPK) cascade. And interruption of this cascade resulted in similar defective polar expansion in
267 both roots and integuments (Bush and Krysan, 2007; Lopez-Bucio et al., 2014; Smekalova et al., 2014;
268 Wang et al., 2008; Wang et al., 2007; Zhang et al., 2017).

269 **The potential involvement of GPI-SKU5/SKS proteins in signaling pathway**

270 As been reviewed recently, GPI-Anchored Proteins in *Arabidopsis* broadly participate in various biological
271 processes, including signaling transduction through associating with cell surface receptors and/or
272 extracellular ligands, to mediate developmental processes, stress and immune response, and so on (Zhou,
273 2019b). Interestingly, SKU5 was reported to directly interact with the synthesized functional Carboxyl-
274 terminal region of Auxin Binding Protein 1 (Shimomura, 2006), that was recently believed to be a potential
275 extracellular ligand that could bind Auxin and mediate extracellular auxin signaling through binding auxin
276 and being recognized by cell surface reporter TMK1, then activate quick auxin response (Cao et al., 2019;
277 Xu et al., 2014). Additionally, exogenous auxin could rapidly activate YODA(MAPKKK)-
278 MEK4/5(MAPKK)-MPK3/6(MAPK) cascade (Mockaitis and Howell, 2000; Pillitteri et al., 2007;
279 Smekalova et al., 2014), of which interruptions were reported to exhibit similar defective roots and
280 integuments as *gpi-sku5/sks* mutants.

281 Altogether, GPI-SKU5/SKS associate with Auxin-related extracellular polypeptide/ligand ABP1, which
282 could be recognized by cell surface receptor kinase TMK1 and activate intracellular signaling components;
283 and their *loss-of-functions* exhibit similar defections as interrupted intracellular MAPK cascade
284 components. It exhibits us a hypothesis that, GPI-SKU5/SKS may work as typical GPI-APs in signaling
285 transduction during regulation of cell polar expansion in both root and integument cells.

286

287

288 **Supplemental Materials**

289
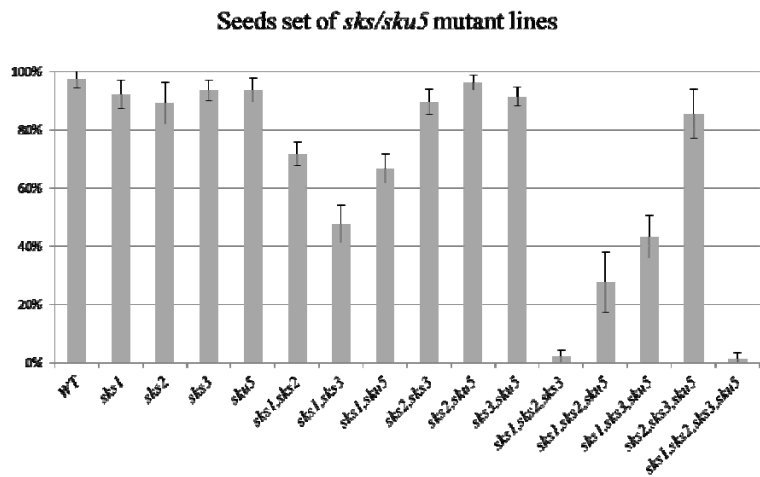
290   **Fig.S1. Seed sets of all *gpi-sku5/sks* mutants.** Proportion of mature seeds among all ovules/seeds in

291   mature green siliques from wild-type and *gpi-sku5/sks* mutants were shown. N=15 siliques, and standard

292   errors were indicated.

293
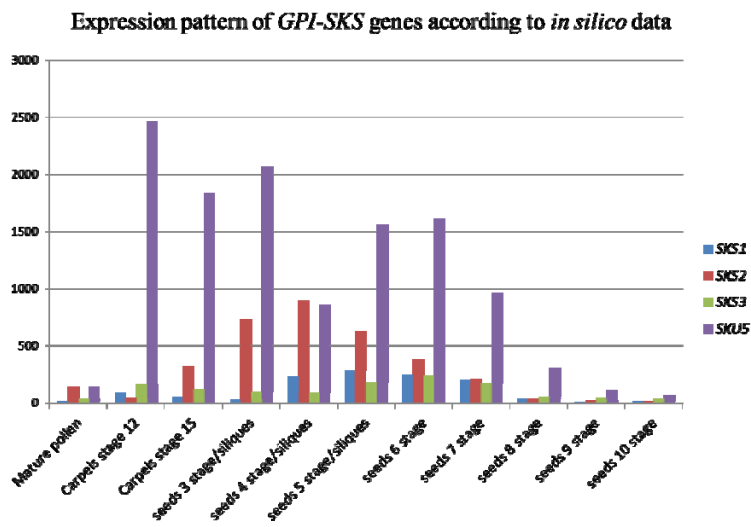
294   **Fig.S2 Expression level of *GPI-SKU5/SKS* genes in reproductive processes of *Arabidopsis*.** The expression patterns of *GPI-*

295   *SKU5/SKS* genes in reproductive organs based on microarray data from AtGenExpress Visualization Tool (AVT), were shown.

296   Different genes were show with different colors, different developmental stages were shown at X-axis and the relative expression

297   levels were shown at Y-axis.

298

299
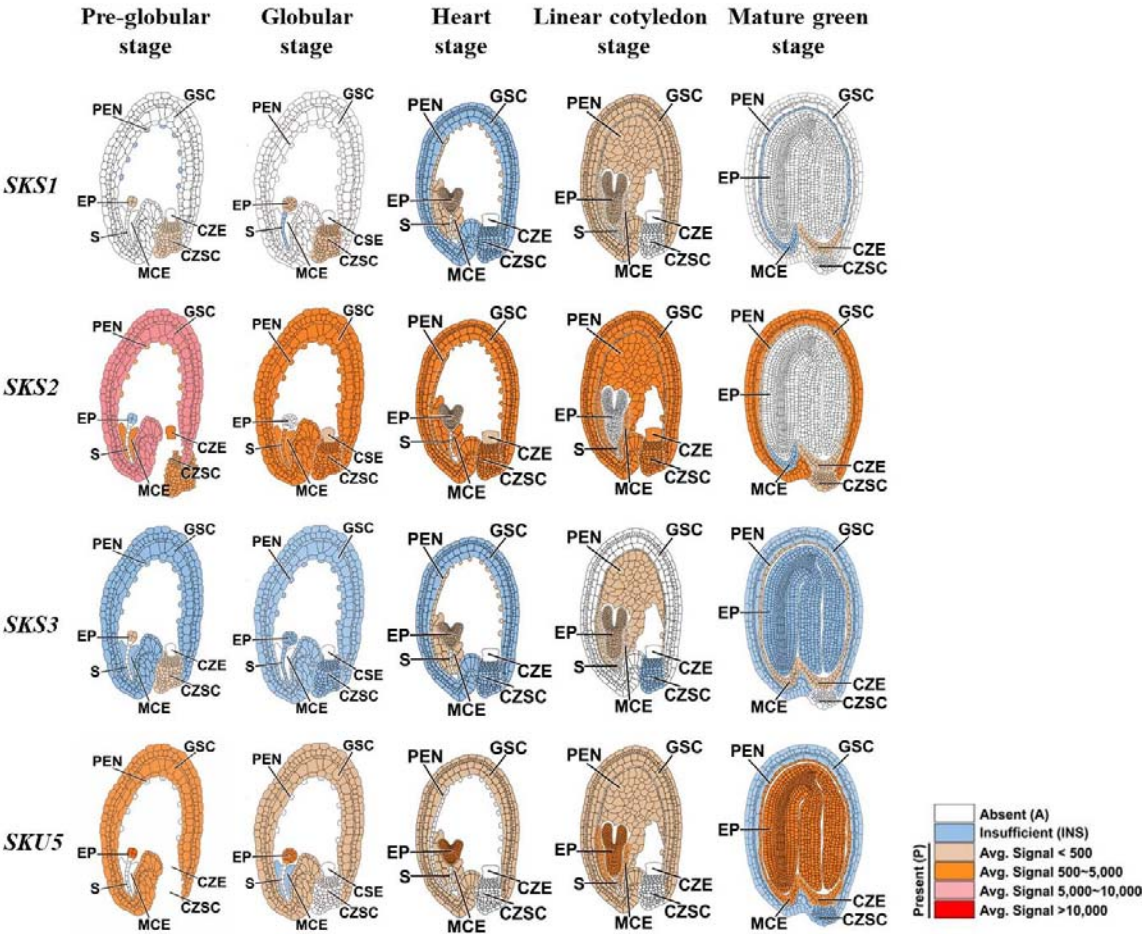
Fig.S3. Exprssion level of *GPI-SKU5/SKS* genes during embryo development of *Arabidopsis*.Expression pattern of *GPI-SKS* genes during different embryo developmental stages based on microarray data from database http://seedgenenetwork.net/ were shown in different colors, which represented their expression levels. PEN, Peripheral Endosperm; GSC, General Seed Coat; CZE, Chalazal Endosperm; CZEC, Chalazal Seed Coat; MCE, Micropylar Endosperm; S, Suspendsor; EP, Embryo Proper;

300
301
302
303

304

305

306

307

308

309

310

Primers for T-DNA insertion genotyping

SKS1-LP	GAATGGAGACTCAACACAGGC
SKS1-RP	TCGATTGAGAGATTCAATGGC
SKS2-LP	TTTGTTGCTGTCATTGCTTTG
SKS2-RP	TGAGAGTTTACCTCCACCGTG
SKS3-LP	GCAGTTTTGCTACAATCAGGC
SKS3-RP	TGGGAAAATGCTGTTTGGTAG
SKU5-LP	CTCGTGAATCCAATGCTCTTC
SKU5-RP	CAAAACGCGAGCTCTGATTAC
LB4	CGTGTGCCAGGTGCCCACGGAATAGT
RB4	TCACGGGTTGGGGTTTCTACAGGAC
LBa1	TGGTTCACGTAGTGGGCCATCG

311

312   **Table.S1 Primers being utilized for genotyping T-DNA insertions in *gpi-sku5/sks* mutants were shown**.

313

314

315

316

317

318

319

320   Borner, G.H., K.S. Lilley, T.J. Stevens, and P. Dupree. 2003. Identification of
321   glycosylphosphatidylinositol-anchored proteins in Arabidopsis. A proteomic and
322   genomic analysis. *Plant Physiol* 132:568-577.
323   Bundy, M.G., P.Z. Kosentka, A.H. Willet, L. Zhang, E. Miller, and E.D. Shpak. 2016. A
324   Mutation in the Catalytic Subunit of the Glycosylphosphatidylinositol
325   Transamidase Disrupts Growth, Fertility, and Stomata Formation. *Plant Physiol*
326   171:974-985.
327   Bush, S.M., and P.J. Krysan. 2007. Mutational evidence that the Arabidopsis MAP kinase
328   MPK6 is involved in anther, inflorescence, and embryo development. *J Exp Bot*
329   58:2181-2191.
330   Cao, M., R. Chen, P. Li, Y. Yu, R. Zheng, D. Ge, W. Zheng, X. Wang, Y. Gu, Z. Gelova, J. Friml,
331   H. Zhang, R. Liu, J. He, and T. Xu. 2019. TMK1-mediated auxin signalling regulates
332   differential growth of the apical hook. *Nature* 568:240-243.
333   Coen, O., and E. Magnani. 2018. Seed coat thickness in the evolution of angiosperms.
334   *Cell Mol Life Sci* 75:2509-2518.

335 Elliott, R.C., A.S. Betzner, E. Huttner, M.P. Oakes, W.Q. Tucker, D. Gerentes, P. Perez, and
336   D.R. Smyth. 1996. AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with
337   pleiotropic roles in ovule development and floral organ growth. *Plant Cell* 8:155-
338   168.
339 Endress, P.K. 2011. Angiosperm ovules: diversity, development, evolution. *Ann Bot*
340   107:1465-1489.
341 Enugutti, B., C. Kirchhelle, M. Oelschner, R.A. Torres Ruiz, I. Schliebner, D. Leister, and K.
342   Schneitz. 2012. Regulation of planar growth by the Arabidopsis AGC protein
343   kinase UNICORN. *Proc Natl Acad Sci U S A* 109:15060-15065.
344 Enugutti, B., and K. Schneitz. 2013. Genetic analysis of ectopic growth suppression
345   during planar growth of integuments mediated by the Arabidopsis AGC protein
346   kinase UNICORN. *BMC Plant Biol* 13:2.
347 Fiume, E., O. Coen, W. Xu, L. Lepiniec, and E. Magnani. 2017. Developmental patterning
348   of sub-epidermal cells in the outer integument of Arabidopsis seeds. *PLoS One*
349   12:e0188148.
350 Gaiser, J.C., K. Robinson-Beers, and C.S. Gasser. 1995. The Arabidopsis SUPERMAN Gene
351   Mediates Asymmetric Growth of the Outer Integument of Ovules. *Plant Cell*
352   7:333-345.
353 Gallagher, T.L., and C.S. Gasser. 2008. Independence and interaction of regions of the
354   INNER NO OUTER protein in growth control during ovule development. *Plant*
355   *Physiol* 147:306-315.
356 Gasser, C.S., and D.J. Skinner. 2019. Development and evolution of the unique ovules of
357   flowering plants. *Curr Top Dev Biol* 131:373-399.
358 Gillmor, C.S., W. Lukowitz, G. Brininstool, J.C. Sedbrook, T. Hamann, P. Poindexter, and C.
359   Somerville. 2005. Glycosylphosphatidylinositol-anchored proteins are required
360   for cell wall synthesis and morphogenesis in Arabidopsis. *Plant Cell* 17:1128-1140.
361 Gross-Hardt, R., C. Kagi, N. Baumann, J.M. Moore, R. Baskar, W.B. Gagliano, G. Jurgens,
362   and U. Grossniklaus. 2007. LACHESIS restricts gametic cell fate in the female
363   gametophyte of Arabidopsis. *PLoS Biol* 5:e47.
364 Jorda, L., S. Sopena-Torres, V. Escudero, B. Nunez-Corcuera, M. Delgado-Cerezo, K.U. Torii,
365   and A. Molina. 2016. ERECTA and BAK1 Receptor Like Kinases Interact to Regulate
366   Immune Responses in Arabidopsis. *Front Plant Sci* 7:897.
367 Kawagoe, K., D. Kitamura, M. Okabe, I. Taniuchi, M. Ikawa, T. Watanabe, T. Kinoshita, and
368   J. Takeda. 1996. Glycosylphosphatidylinositol-anchor-deficient mice: implications
369   for clonal dominance of mutant cells in paroxysmal nocturnal hemoglobinuria.
370   *Blood* 87:3600-3606.
371 Kinoshita, T. 2014. Biosynthesis and deficiencies of glycosylphosphatidylinositol. *Proc Jpn*
372   *Acad Ser B Phys Biol Sci* 90:130-143.
373 Kinoshita, T., and M. Fujita. 2016. Biosynthesis of GPI-anchored proteins: special
374   emphasis on GPI lipid remodeling. *J Lipid Res* 57:6-24.
375 Kunieda, T., N. Mitsuda, M. Ohme-Takagi, S. Takeda, M. Aida, M. Tasaka, M. Kondo, M.
376   Nishimura, and I. Hara-Nishimura. 2008. NAC family proteins NARS1/NAC2 and
377   NARS2/NAM in the outer integument regulate embryogenesis in Arabidopsis.
378   *Plant Cell* 20:2631-2642.

379   Lee, J.S., M. Hnilova, M. Maes, Y.C. Lin, A. Putarjunan, S.K. Han, J. Avila, and K.U. Torii.
380   2015. Competitive binding of antagonistic peptides fine-tunes stomatal
381   patterning. *Nature* 522:439-443.
382   Lopez-Bucio, J.S., J.G. Dubrovsky, J. Raya-Gonzalez, Y. Ugartechea-Chirino, J. Lopez-Bucio,
383   L.A. de Luna-Valdez, M. Ramos-Vega, P. Leon, and A.A. Guevara-Garcia. 2014.
384   Arabidopsis thaliana mitogen-activated protein kinase 6 is involved in seed
385   formation and modulation of primary and lateral root development. *J Exp Bot*
386   65:169-183.
387   Meng, X., H. Wang, Y. He, Y. Liu, J.C. Walker, K.U. Torii, and S. Zhang. 2012. A MAPK
388   cascade downstream of ERECTA receptor-like protein kinase regulates
389   Arabidopsis inflorescence architecture by promoting localized cell proliferation.
390   *Plant Cell* 24:4948-4960.
391   Mockaitis, K., and S.H. Howell. 2000. Auxin induces mitogenic activated protein kinase
392   (MAPK) activation in roots of Arabidopsis seedlings. *Plant J* 24:785-796.
393   Nole-Wilson, S., and B.A. Krizek. 2000. DNA binding properties of the Arabidopsis floral
394   development protein AINTEGUMENTA. *Nucleic Acids Res* 28:4076-4082.
395   Oxley, D., and A. Bacic. 1999. Structure of the glycosylphosphatidylinositol anchor of an
396   arabinogalactan protein from Pyrus communis suspension-cultured cells. *Proc*
397   *Natl Acad Sci U S A* 96:14246-14251.
398   Pillitteri, L.J., S.M. Bemis, E.D. Shpak, and K.U. Torii. 2007. Haploinsufficiency after
399   successive loss of signaling reveals a role for ERECTA-family genes in Arabidopsis
400   ovule development. *Development* 134:3099-3109.
401   Rutherford, R., and P.H. Masson. 1996. Arabidopsis thaliana sku mutant seedlings show
402   exaggerated surface-dependent alteration in root growth vector. *Plant Physiol*
403   111:987-998.
404   Scholz, S., J. Plessmann, B. Enugutti, R. Huttl, K. Wassmer, and K. Schneitz. 2019. The
405   AGC protein kinase UNICORN controls planar growth by attenuating PDK1 in
406   Arabidopsis thaliana. *PLoS Genet* 15:e1007927.
407   Sedbrook, J.C., K.L. Carroll, K.F. Hung, P.H. Masson, and C.R. Somerville. 2002. The
408   Arabidopsis SKU5 gene encodes an extracellular glycosyl phosphatidylinositol-
409   anchored glycoprotein involved in directional root growth. *Plant Cell* 14:1635-
410   1648.
411   Shimomura, S. 2006. Identification of a glycosylphosphatidylinositol-anchored plasma
412   membrane protein interacting with the C-terminus of auxin-binding protein 1: a
413   photoaffinity crosslinking study. *Plant Mol Biol* 60:663-677.
414   Smekalova, V., I. Luptovciak, G. Komis, O. Samajova, M. Ovecka, A. Doskocilova, T. Takac,
415   P. Vadovic, O. Novak, T. Pechan, A. Ziemann, P. Kosutova, and J. Samaj. 2014.
416   Involvement of YODA and mitogen activated protein kinase 6 in Arabidopsis post-
417   embryogenic root development through auxin up-regulation and cell division
418   plane orientation. *New Phytol* 203:1175-1193.
419   Stevens, V.L. 1995. Biosynthesis of glycosylphosphatidylinositol membrane anchors.
420   *Biochem J* 310 (Pt 2):361-370.
421   Takahashi, D., Y. Kawamura, and M. Uemura. 2016. Cold acclimation is accompanied by
422   complex responses of glycosylphosphatidylinositol (GPI)-anchored proteins in

423   Arabidopsis. *J Exp Bot* 67:5203-5215.

424 Terpstra, I.R., L.B. Snoek, J.J. Keurentjes, A.J. Peeters, and G. van den Ackerveken. 2010.
425   Regulatory network identification by genetical genomics: signaling downstream
426   of the Arabidopsis receptor-like kinase ERECTA. *Plant Physiol* 154:1067-1078.

427 Villanueva, J.M., J. Broadhvest, B.A. Hauser, R.J. Meister, K. Schneitz, and C.S. Gasser.
428   1999. INNER NO OUTER regulates abaxial- adaxial patterning in Arabidopsis
429   ovules. *Genes Dev* 13:3160-3169.

430 Wang, H., Y. Liu, K. Bruffett, J. Lee, G. Hause, J.C. Walker, and S. Zhang. 2008. Haplo-
431   insufficiency of MPK3 in MPK6 mutant background uncovers a novel function of
432   these two MAPKs in Arabidopsis ovule development. *Plant Cell* 20:602-613.

433 Wang, H., N. Ngwenyama, Y. Liu, J.C. Walker, and S. Zhang. 2007. Stomatal development
434   and patterning are regulated by environmentally responsive mitogen-activated
435   protein kinases in Arabidopsis. *Plant Cell* 19:63-73.

436 Xu, T., N. Dai, J. Chen, S. Nagawa, M. Cao, H. Li, Z. Zhou, X. Chen, R. De Rycke, H.
437   Rakusova, W. Wang, A.M. Jones, J. Friml, S.E. Patterson, A.B. Bleecker, and Z. Yang.
438   2014. Cell surface ABP1-TMK auxin-sensing complex activates ROP GTPase
439   signaling. *Science* 343:1025-1028.

440 Xu, W., E. Fiume, O. Coen, C. Pechoux, L. Lepiniec, and E. Magnani. 2016. Endosperm and
441   Nucellus Develop Antagonistically in Arabidopsis Seeds. *Plant Cell* 28:1343-1360.

442 Yadegari, R., G. Paiva, T. Laux, A.M. Koltunow, N. Apuya, J.L. Zimmerman, R.L. Fischer, J.J.
443   Harada, and R.B. Goldberg. 1994. Cell Differentiation and Morphogenesis Are
444   Uncoupled in Arabidopsis raspberry Embryos. *Plant Cell* 6:1713-1729.

445 Zhang, M., H. Wu, J. Su, H. Wang, Q. Zhu, Y. Liu, J. Xu, W. Lukowitz, and S. Zhang. 2017.
446   Maternal control of embryogenesis by MPK6 and its upstream MKK4/MKK5 in
447   Arabidopsis. *Plant J* 92:1005-1019.

448 Zhou, K. 2019a. The alternative splicing of SKU5-Similar3 in Arabidopsis. *Plant Signal*
449   *Behav* 1-4.

450 Zhou, K. 2019b. Glycosylphosphatidylinositol-Anchored Proteins in Arabidopsis and One
451   of Their Common Roles in Signaling Transduction. *Frontiers in Plant Science* 10:

452 Zhou, K. 2019c. GPI-anchored SKS proteins regulate root development through
453   controlling cell polar expansion and cell wall synthesis. *Biochem Biophys Res*
454   *Commun* 509:119-124.

455