

1 **Spatiotemporal restriction of *FUSCA3* expression by class I BPC promotes ovule**  
2 **development and coordinates embryo and endosperm growth**

3  
4 Jian Wu <sup>1,4</sup>, Rosanna Petrella <sup>3</sup>, Sebastian Dowhanik <sup>1</sup>, Veronica Gregis <sup>3</sup> and Sonia  
5 Gazzarrini<sup>1,2,§</sup>

6  
7 <sup>1</sup> Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail,  
8 Toronto, ON Canada, M1C 1A4

9 <sup>2</sup> Department of Cell and Systems Biology, University of Toronto, 25 Willcocks Street, Toronto,  
10 ON Canada, M5S 3B2

11 <sup>3</sup> Università degli Studi di Milano, Dipartimento di Bioscienze, via Celoria 26 20133 Milano,  
12 Italia

13 <sup>4</sup> Beijing Key Laboratory of Development and Quality Control of Ornamental Crops,  
14 Department of Ornamental Horticulture and Landscape Architecture, China Agricultural  
15 University, Beijing, China

16 § Corresponding author: e-mail [gazzarrini@utsc.utoronto.ca](mailto:gazzarrini@utsc.utoronto.ca)

17  
18 **Short Title:** Class I BPCs repress *FUSCA3* during vegetative, reproductive and seed  
19 development

20

21

1

2 **ABSTRACT**

3

4 Spatiotemporal regulation of gene expression plays an important role in developmental timing  
5 in plants and animals. *FUSCA3* regulates the transition between different phases of  
6 development by acting as a link between different hormonal pathways in Arabidopsis.

7 However, the mechanisms governing its spatiotemporal expression patterns are poorly  
8 understood. Here, we show that *FUS3* is expressed in the chalaza and funiculus of the  
9 mature ovule and seed, but is repressed in the embryo sac, integuments and endosperm.

10 *FUS3* repression requires class I BASIC PENTACYSTEINE (BPC) proteins, which directly  
11 bind to the *FUS3* locus and restrict its expression pattern. During vegetative and reproductive  
12 development, derepression of *FUS3* in *bpc1/2* or *pML1:FUS3* misexpression lines results in  
13 dwarf plants carrying defective flowers and aborted ovules. Post-fertilization, ectopic *FUS3*  
14 expression in the endosperm increases endosperm nuclei proliferation and seed size and  
15 delays or arrests embryo development. These phenotypes are rescued in *bpc1/2 fus3-3*.

16 Lastly, class I BPCs interact with FIS-PRC2 (FERTILIZATION-INDEPENDENT SEED-  
17 Polycomb Repressive Complex 2), which represses *FUS3* in the endosperm. We propose  
18 that BPC1/2 promotes the transition from reproductive to seed development by repressing  
19 *FUS3* in ovule integuments. After fertilization, BPC1/2 and FIS-PRC2 repress *FUS3* in the  
20 endosperm to coordinate endosperm and embryo growth.

21

22

23

24



## 1 INTRODUCTION

2 Plants integrate endogenous and environmental signals to correctly time the expression of  
3 developmental genetic programs. During their life cycle, plants transition through three major  
4 phases of development: vegetative, reproductive and seed development. These phase  
5 transitions are characterized by large changes in gene expression, which depend on the  
6 action of conserved epigenetic machineries. Epigenetic changes are flexible and respond to  
7 developmental and environmental cues (Mozgova and Henning, 2015; Mozgova et al., 2015).

8 Reproductive development in seed plants starts with the production of female and male  
9 gametes and is followed by fertilization and seed development. During ovule development the  
10 maternal sporophytic integuments originate from the chalaza and enclose the female  
11 gametophyte (embryo sac), which contains two gametes: the haploid egg cell and the diploid  
12 central cell. The funiculus, connects the ovule to the placental region in the carpel (Drews and  
13 Koltunow, 2011; Gasser and Skinner, 2019). After fertilization of the central cell, the triploid  
14 endosperm nuclei undergo multiple rounds of division, which are followed by cellularization. In  
15 most Angiosperms the function of the endosperm is to nourish the developing embryo.  
16 Fertilization of the egg cell generates the diploid zygote, which divides asymmetrically  
17 producing two daughter cell lineages that form the apical embryo proper and the basal  
18 suspensor, respectively. The integuments will develop into the seed coat only after fertilization  
19 of the central cell (Lafon-Placette and Kohler, 2014; Dresselhaus et al., 2016; Gasser and  
20 Skinner, 2019). Auxin is a major player in establishing apical-basal polarity and patterning of  
21 the embryo, as well as regulating integuments and endosperm development (Figueiredo et  
22 al., 2015; Figueiredo et al., 2016; Robert et al., 2018; Lau et al., 2012; de Vries and Weijers,  
23 2017). In the absence of fertilization, seed development is repressed by the Polycomb-  
24 Repressive Complex2 (PRC2). In particular, the FIS-PRC2 complex represses autonomous  
25 endosperm development, while EMF-PRC2 and VRN-PRC2 prevent seed coat development  
26 prior to fertilization (Roszak and Kohler, 2011; Figueiredo and Kohler, 2018).

27 Seed maturation is characterized by cell expansion and very little cell division. During  
28 this stage of development the embryo accumulates storage compounds, acquires dormancy  
29 and establishes desiccation tolerance. These processes are largely controlled by the  
30 hormone abscisic acid (ABA), the B3 domain family of transcription factors, namely LEAFY  
31 COTYLEDON2 (LEC2), ABSCISIC ACID INSENSITIVE3 (ABI3) and FUSCA3 (FUS3), as well  
32 as the NF-YB subunits of the CCAAT-binding complex, LEC1 and LEC1-LIKE, which are  
33 collectively called LAFL (Sreenivasulu and Wobus, 2013). Genetic and spatiotemporal

1 expression analyses together with Chromatin immunoprecipitation (ChIP) and transcriptomic  
2 studies suggest that these genes play redundant as well as specific roles in promoting seed  
3 maturation, while repressing germination and vegetative development (Sreenivasulu and  
4 Wobus, 2013; Jia et al., 2014; Fatihi et al., 2016; Carbonero et al., 2017; Lepiniec et al.,  
5 2018). In particular, *FUS3* is a heterochronic gene, which was shown to promote seed  
6 maturation by increasing ABA levels while inhibiting vegetative growth and flowering by  
7 repressing gibberellins (GA) synthesis (Keith et al., 1994; Curaba et al., 2004; Gazzarrini et  
8 al., 2004). These hormones feed back by positively (ABA) and negatively (GA) regulating  
9 *FUS3* levels (Gazzarrini et al., 2004; Chiu et al., 2016). *FUS3* also inhibits vegetative phase  
10 change by repressing ethylene signaling (Lumba et al., 2012). Thus, *FUS3* regulates phase  
11 transitions by modulating hormones syntheses/signaling.

12 During germination the seed maturation program is repressed by epigenetic  
13 mechanisms, which leads to dormancy break and the transition to the next phase of  
14 development; these include: CHROMODOMAIN HELICASE DNA BINDING3 (CHD3)/PICKLE  
15 (PKL)-dependent chromatin remodeling; Polycomb Repressive Complex2 (PRC2)-mediated  
16 histone 3 lysine 27 trimethylation (H3K27me3); H2AK121ub monoubiquitination by the PRC1  
17 components RING-finger homologs AtBMI1A and AtBMI1B; and VIP1/ABI3/LEC (VAL)  
18 mediated recruitment of histone deacetylases (HDAC) and PRC complexes (Jia et al., 2014;  
19 Lepiniec et al., 2018). Mutations in these genes result in *LAFL* derepression, leading to  
20 expression of seed-specific traits and development of embryonic structures in severe  
21 mutants. Accordingly, ectopic expression of *LAFL* genes post-embryonically results in similar  
22 phenotypes (Lotan et al., 1998; Stone et al., 2001; Gazzarrini et al., 2004; Braybrook et al.,  
23 2006). Clearly, multiple pathways ensure a stable repression of the late embryogenesis  
24 program during vegetative growth.

25 Repression of *LAFL* genes has also been observed during early embryonic  
26 development. For example, *FUS3* is ectopically expressed in the endosperm of the PRC  
27 mutant *medea* (*mea*) (Makarevich et al., 2006), but the mechanism and function of *FUS3*  
28 repression in this tissue is unknown. *LAFL* expression is also regulated by post-transcriptional  
29 gene silencing; mutants that affect miRNA biogenesis show de-repression of *LAFL* genes in  
30 seedlings and early globular stage embryos (Vashisht and Nodine, 2014). This suggests that  
31 *LAFL* expression is tightly controlled and subjected to post-transcriptional and epigenetic  
32 regulation not only during vegetative growth, but also in specific seed tissues, although the  
33 regulation and role of *LAFL* expression during early embryogenesis is far from being fully

1 understood.

2 *FUS3* transcripts and protein are found as early as the globular stage embryo and  
3 become progressively restricted to the protoderm, root and cotyledon tips during mid-  
4 embryogenesis (Gazzarrini et al., 2004; Tsuchiya et al., 2004); however, its function during  
5 early embryogenesis is unknown. Recently, we have shown that *FUS3* plays a critical role  
6 also in reproductive development. The *fus3-3* loss-of-function mutant displays seed abortion,  
7 which is enhanced in plants grown at elevated temperature and dependent on *FUS3*  
8 phosphorylation (Chan et al., 2017; Tsai and Gazzarrini, 2012). Interestingly, *pML1:FUS3-*  
9 *GFP* plants that mis-express *FUS3* during reproductive development also show aborted  
10 siliques, suggesting that spatiotemporal expression of *FUS3* must be tightly regulated at this  
11 stage of development (Gazzarrini et al., 2004).

12 To further investigate the role of *FUS3* in reproductive development, we have  
13 characterized its localization pattern before and after fertilization. Prior to fertilization *FUS3* is  
14 transiently localized to the integuments and later confined to the chalaza and funiculus of  
15 mature ovules, while post-fertilization *FUS3* localizes to the seed coat, chalaza and funiculus,  
16 aside from the already known localization in the embryo. We show that class I BASIC  
17 PENTACYSTEINE (BPC) proteins interact the FIS PRC2 complex and bind to the *FUS3*  
18 chromatin. BPC1/2 repress *FUS3* in the stem, integuments of mature ovules, as well as in the  
19 endosperm of developing seeds. *FUS3* misexpression in the *bpc1-1* and *bpc1-1 bpc2*  
20 (*bpc1/2*) mutants reduces plant height, impairs the development of flowers, ovule and  
21 endosperm leading to seed abortion or arrested embryogenesis. Similar phenotypes are  
22 recapitulated in *pML1:FUS3-GFP* misexpression plants. Furthermore, the strong vegetative  
23 and reproductive phenotypes of *bpc1/2* double mutant can be partially rescued in the *fus3-3*  
24 background, strongly indicating that they are caused by ectopic *FUS3* expression. We  
25 propose that during reproductive development BPC1/2- and PRC2-mediated repression of  
26 *FUS3* is necessary for ovule development, while after fertilization *FUS3* repression in the  
27 endosperm by BPC1/2 and FIS-PRC2 coordinates endosperm and embryo growth. Hence,  
28 correct spatiotemporal expression of *FUS3* is required for the transition from plant  
29 reproduction to seed development and from pattern formation to seed maturation.

30

## 31 **RESULTS**

32 ***FUS3* localizes to reproductive organs before fertilization and is required for ovule**  
33 **development**

1 The *fus3-3* loss-of-function mutant displays seed abortion, which is enhanced at elevated  
2 temperature (Chan et al., 2017). To investigate the role of *FUS3* in reproductive development,  
3 we first determined *FUS3* localization pattern in flower buds using a *pFUS3:FUS3-GFP*  
4 translational reporter (Gazzarrini et al., 2004). However, no *FUS3*-GFP fluorescence was  
5 detected, likely due to the fast turnover rate of *FUS3* (Lu et al., 2010). We then used a  
6 *pFUS3:FUS3ΔC-GFP* reporter, which lacks the PEST instability motif of *FUS3* and allows  
7 detection of low *FUS3* protein levels (Lu et al., 2010). This reporter is non-functional (it  
8 doesn't rescue *fus3-3*), but recapitulates *FUS3* expression patterns determined by qRT-PCR,  
9 *pFUS3:GUS* and *pFUS3:GFP* reporters (Lu et al., 2010). Using the *pFUS3:FUS3ΔC-GFP*  
10 reporter, the *FUS3* protein was found to be localized to the pistil (septum, valves and  
11 funiculus) and ovules, in agreement with microarray data (Figure 1 A-F and Supplemental  
12 Figure 1A). In developing ovules *FUS3ΔC-GFP* was localized to the epidermis of the  
13 nucellus, the chalaza, and funiculus, while in mature ovules (FS12) it was localized to the  
14 chalaza and funiculus (Figure 1 C-F). After fertilization (6-48 hours after fertilization; HAF)  
15 *FUS3ΔC-GFP* was present in the funiculus, outer layer of the seed coat, chalaza and  
16 micropile; it was also localized to the embryo at early stages of embryogenesis (Figure 1G-L  
17 and Supplemental Figure 1B).

18 To further address the role of *FUS3* in reproduction, we monitored ovule development  
19 in *fus3-3* loss-of-function mutant and *pML1:FUS3-GFP* misexpression lines (Gazzarrini et al.,  
20 2004). *pML1:FUS3-GFP* was shown to rescue all *fus3-3* seed maturation defects, including  
21 desiccation intolerance, however misexpression during postembryonic development caused  
22 additional phenotypes (Gazzarrini et al., 2004). Strong *pML1:FUS3-GFP* lines show delayed  
23 vegetative growth and flowering, reduced plant height and aborted siliques, as previously  
24 described (Figure 2A; Gazzarrini et al., 2004; Lu et al., 2010). In addition, we found that in  
25 intermediate-to-strong *pML1:FUS3-GFP* lines *FUS3*-GFP was mislocalized to the  
26 endothelium, outer and inner integuments of developed ovules, while in aborted ovules *FUS3*-  
27 GFP surrounded the aborted embryo sac (Figure 2B). After fertilization, *pML1:FUS3-GFP*  
28 seeds showed *FUS3*-GFP mislocalization to the endosperm (Figure 2B). Moreover, by  
29 opening the developed siliques of intermediate-to-strong *pML1:FUS3-GFP* lines we also  
30 found that they contained aborted seeds or seeds with delayed development (Figure 2C, D).  
31 To determine if seed abortion in *fus3-3* and *pML1:FUS3-GFP* is the result of impaired ovule  
32 development, we analyzed ovules before fertilization and compared them with wild type  
33 (Figure 2E). The embryo sac of wild type ovules at FS12 stage contained the egg nucleus,

1 the secondary endosperm nucleus, the synergids, and was surrounded by inner and outer  
2 integuments. However, at FS12 stage the embryo sac of some *fus3-3* and *pML1:FUS3-GFP*  
3 lines was delayed at various stages, from FG1 to FG6, arrested or not fully wrapped by the  
4 integuments (Figure 2E). The arrest of female megagametogenesis resulted in seed abortion  
5 in *fus3-3* and more so in strong *pML1:FUS3-GFP* lines (Figure 2C, D).

6 Taken together, these results show that spatiotemporal localization of FUS3 is tightly  
7 regulated and that lack or misexpression of *FUS3* severely impairs embryo sac and  
8 integument development, indicating that spatiotemporal control of *FUS3* expression is  
9 required for proper ovule development.

10

### 11 **Class I BPC transcription factors bind to (GA/CT)<sub>n</sub> motifs in *FUS3*.**

12 To understand the mechanisms controlling the spatiotemporal patterns of *FUS3* expression,  
13 we identified upstream regulators of *FUS3* by yeast one-hybrid. To increase screening  
14 specificity, a short genomic region of 615bp upstream of the *FUS3* translation start (*pFUS3*)  
15 was screened against an Arabidopsis transcription factor library (Figure 3A; Mitsuda et al.,  
16 2010). About 200,000 yeast transformants were screened and 69 grew on selection plates.  
17 Sequencing of the cDNA inserts revealed that all colonies contained BPC3. BPCs are a small  
18 group of plant specific transcription factor with six genes and a pseudogene (BPC5) that are  
19 divided into 3 classes based on sequence similarity: class I (BPC1/2/3), class II (BPC4/5/6)  
20 and class III (BPC7) (Meister et al., 2004). We retested individually all class I BPCs (BPC1-3)  
21 and also included class II BPC4, which is not present in the cDNA library but it is highly  
22 expressed in embryos and flowers (Berger et al., 2011). The results show that all three class I  
23 BPCs bound to *pFUS3* by yeast one-hybrid, but not class II BPC4 (Figure 3A).

24 BPCs were shown to bind to (GA/CT)<sub>n</sub> cis elements in several plant species, with a  
25 preference for different numbers of repeats (Berger and Dubreucq, 2012; Simonini and Kater,  
26 2014). When all (GA/CT)<sub>n</sub> motifs of the *pFUS3* were mutated (*pFUS3<sup>MUT</sup>*), none of the class I  
27 BPCs interacted with the *FUS3* sequence, confirming binding specificity (Figure 3B;  
28 Supplemental Figure 2). To identify the binding location of BPCs on *pFUS3*, we generated  
29 truncations of approximately 200bp fragments (F1 to F3); the first exon/intron region  
30 containing 2 (GA/CT)<sub>n</sub> repeats (F4) was also tested (Figure 3C). In Y1H, BPC1 showed strong  
31 binding whereas BPC2/3 weak binding to the 5'UTR (F3) and first exon/intron regions (F4),  
32 where (GA/CT)<sub>n</sub> motifs are enriched (Figure 3D). BPC1/2/3/4 did not bind the promoter region  
33 further upstream, corresponding to the F1 or F2 truncations, where there is only one (GA)<sub>5</sub> or

1 no (GA/CT)<sub>n</sub> motif, respectively (Figure 3D). To determine if BPC1 also binds to the *FUS3*  
2 locus *in vivo* during reproductive development, we generated BPC1 overexpression lines and  
3 performed ChIP in inflorescences, which show that BPC1 binds to this region (Figure 3F).

4 Altogether, this indicates that class I BPCs bind to the 5'UTR and first intron/exon regions  
5 of *FUS3* in Y1H. Furthermore, BPC1 also binds to *FUS3 in vivo* during reproductive  
6 development.

7

### 8 **Class I BPCs repress *FUS3* during vegetative growth**

9 In a genome-wide study, BPC1 was found to interact with and recruit the conserved PRC2-  
10 complex subunit FIERY (FIE; Supplemental Figure 4) *in vivo* and trigger polycomb-mediated  
11 gene silencing in imbibed seeds (Xiao et al., 2017). We first analyzed ChIP-seq data from  
12 Xiao et al. (2017) and found that the first exon/intron and 5'UTR of *FUS3* was bound by  
13 BPC1, but not the ACTIN (*ACT2*) control, in seedlings (Figure 3E). Furthermore, this same  
14 region was bound by FIE and associated with H3K27me<sub>3</sub>, a repressive mark (Figure 3E).  
15 Lastly, BPC1/2 interact with EMBRYONIC FLOWER2 (*EMF2*), which belongs to the EMF-  
16 PRC2 complex involved in repressing the vegetative-to-reproductive and embryo-to-seedling  
17 phase transitions (Supplemental Figure 4; Xiao et al., 2017; Mozgova et al., 2015). This  
18 suggests that *FUS3* may be repressed in germinating seeds by BPC1 recruitment of EMF-  
19 PRC2. To confirm this, we mutated all BPC binding sites (GA/CT)<sub>n</sub> in the *FUS3* sequence  
20 (*pFUS3<sup>MUT</sup>*) and showed that *pFUS3<sup>MUT</sup>:GUS/GFP* is indeed derepressed post-embryonically  
21 in leaves and root tips (Figure 3G,H). Together with previous data showing that *FUS3* was  
22 strongly upregulated in *swinger curly leaf* (*swn clf*) (Makarevich et al., 2006), these results  
23 strongly suggest that BPC1 binds to and represses *FUS3* during vegetative development by  
24 recruiting the EMF-PRC2 complex.

25

### 26 **Class I BPCs form homo- or heterodimers and recruit FIS-PRC2**

27 Previous ChIP assays showed that in closed flowers the *FUS3* locus is also associated with  
28 the FIS-PRC2 complex component MEA and H3K27me<sub>3</sub> repressive marks, and that *FUS3* is  
29 upregulated in the endosperm of *mea/MEA* seeds at 3 days after flowering (DAF) (Makarevich  
30 et al., 2006). Given that BPC1 bind to the *FUS3* locus in closed flowers (Figure 3F), we  
31 hypothesized that *FUS3* may also be repressed during reproductive development by one or  
32 more class I BPCs through FIS-PRC2 recruitment. To test this hypothesis, we first determined  
33 if all class I BPCs interact *in planta* with the FIS-PRC2 complex, which acts during



1 gametophyte and endosperm development (Figure 4). All class I BPCs interacted with the  
2 unique components of this PRC2 complex, FIS2 and MEA, and also with the PRC2-shared  
3 component, MSI1, in BiFC assays; all but BPC3 also interacted with FIE (Figure 4). In  
4 agreement with previous Y2H results, class I BPCs also interacted with each other *in planta*,  
5 and BPC2 and 3 could also form homodimers (Figure S3; Simonini et al., 2012). No class I  
6 BPC member or FIS-PRC2 component interacted with FUS3, suggesting that these BiFC  
7 interactions are specific (Supplemental Figure 5). Lastly, given that BPC6 recruits PRC2 by  
8 interacting with LIKE HETEROCHROMATIN PROTEIN1 (LHP1; Hecker et al., 2015), we also  
9 tested the interaction between class I BPCs and LHP1 *in planta*. However, the results showed  
10 no interaction among them, suggesting class I and class II BPCs recruitment of the PRC2  
11 complex may differ (Supplemental Figure 6). We conclude that class I BPCs can form homo-  
12 and heterodimers and recruit the FIS-PRC2 complex *in planta*.

13 Class I BPCs were shown to be expressed in ovules (Monfared et al., 2011). To have a  
14 better understanding of the spatiotemporal expression pattern of class I BPCs during  
15 reproductive development and embryogenesis, we tracked their expression patterns before  
16 (FS4-12) and after (1-11DAF) fertilization using transcriptional or translational reporters. Class  
17 I BPCs had largely overlapping expression patterns before fertilization and they were all  
18 highly expressed in almost all tissues of developing ovules, while soon after fertilization BPCs  
19 were expressed in embryos from the globular to the cotyledon stage, as well as the  
20 endosperm and seed coat (Figure 5). BPC1 had a more restricted pattern before (chalaza  
21 and micropile) and after (chalaza, micropile, seed coat) fertilization. This suggests that class I  
22 BPCs act redundantly during ovule and embryo development. As previously shown, the FIS-  
23 PRC2 complex subunits FIS2 and MEA were only expressed in the central cell of developing  
24 ovules and in the endosperms at 2DAF (Supplemental Figure 7; Wang et al., 2006).

25 These data show that BPCs can interact with each other and with FIS-PRC2 to  
26 regulate gene expression. Given the specific localization of FIS and MEA to the central cell  
27 and endosperm, and *FUS3* derepression in the endosperm of *mea*/MEA, we conclude that  
28 aside from their role in silencing *FUS3* during vegetative growth through EMF-PRC2, class I  
29 BPCs repress *FUS3* during reproductive and seed development by recruiting FIS-PRC2 in the  
30 central cell and endosperm. Furthermore, BPCs may recruit sporophytic PRC2 (EMF/VRN  
31 PRC2) to repress *FUS3* in the integuments and seed coat.

32  
33 **Reproductive defects of *bpc1/2* are partially rescued by *fus3-3***

1 Previously, *bpc* mutants were shown to display pleiotropic phenotypes during vegetative and  
2 reproductive development (Monfared et al., 2011). Higher order *bpc1/2* and *bpc1/2/3* mutants  
3 are dwarf, have shorter or aborted siliques, display severe seed abortion and defects in  
4 embryo sac development, while most single *bpc* mutants resemble wild type, suggesting  
5 functional redundancy (Figure 6A-F; Supplemental Figure 8A-D; (Monfared et al., 2011).  
6 These phenotypes are remarkably similar to those shown by *pML1:FUS3* misexpression lines  
7 (Figure 2; (Gazzarrini et al., 2004). This suggests that *bpc1/2* phenotypes may be caused by  
8 ectopic expression of *FUS3*. To address the genetic relationship between class I BPCs and  
9 *FUS3*, we crossed *bpc1/2* with *fus3-3*. The *bpc1/2 fus3-3* triple mutant indeed showed partial  
10 rescue of these phenotypes, including plant height (Figure 6A,D), silique and seed abortion  
11 (Figure 6B,C,E,F), as well as embryo sac development (Figure 6H), supporting the hypothesis  
12 that *FUS3* is misexpressed in *bpc1/2*.

13 After fertilization, the endosperm of some *bpc1/2* mutants appeared very dense and  
14 some ovules were not fertilized (Figure 6H; Supplemental Figure 8E). In fertilized seeds, most  
15 *bpc1/2* also display delayed or arrested embryo development (Figure 6E,F,H; Supplemental  
16 Figure 8A,B,F,G). Overall, reproductive defects in higher order *bpc* mutants result in severe  
17 reduction of seed yield (Figure 6G). The *bpc1/2 fus3-3* triple mutant partially rescue  
18 endosperm and embryo development (Figure 6E,F,H). Thus, these data strongly suggest that  
19 BPCs repress *FUS3* during reproductive and seed development.

20

## 21 **BPC1/2 repress *FUS3* to promote inflorescence stem elongation, ovule and endosperm** 22 **development.**

23 To confirm a repressive role of BPCs on *FUS3* function, we analyzed *FUS3* expression level  
24 and patterns in *bpc1/2* mutants. We show that *FUS3* transcript level is indeed increased in  
25 *bpc1/2* inflorescence stem (Figure 7A). Consistent with the transcript analysis, *pFUS3:GUS*  
26 activity is also increased in *bpc1/2* inflorescence stem and flower buds (Figure 7B). In WT,  
27 low *FUS3* expression in the inflorescence stem is shown by transcriptomic data and detected  
28 with the *pFUS3:FUS3ΔC-GFP* sensitive reporter (Supplemental Figure 1). Together with  
29 previous findings showing that plant height is reduced in *pML1:FUS3-GFP* misexpression  
30 plants (Gazzarrini et al., 2004), while increased in the *fus3-3* mutant (Figure 6D), these results  
31 indicate that BPC1/2 downregulates *FUS3* in the stem to promote stem elongation.

32 During reproductive development *FUS3ΔC-GFP* is mislocalized to the integuments at  
33 the micropilar region of developing *bpc1-1* and *bpc1/2* ovules, while after fertilization ectopic



1 *pFUS3:GUS* activity and *FUS3ΔC-GFP* localization were detected in *bpc1* and *bpc1/2*  
2 endosperms (Figure 7B,C,D). Combined with the above functional analysis, these results  
3 show that before fertilization BPCs restrict *FUS3* expression to the funiculus and chalazal  
4 region of the ovule to promote ovule development, while after fertilization *FUS3* is repressed  
5 by BPCs in most of the endosperm to coordinate embryo and endosperm growth.

6 To analyze the repressive role of class I BPCs, we also crossed *pFUS3:FUS3:GFP*  
7 translational reporter with *bpc1/2* mutant. However, we were only able to isolate *bpc1-1*  
8 *pFUS3:FUS3:GFP* lines. As shown in Supplemental Figure 8 and previous research  
9 (Monfared et al., 2011) *bpc1-1* doesn't have any visible phenotype compared with wild type,  
10 nor does *pFUS3:FUS3:GFP*, which rescue the *fus3-3* mutant phenotypes (Gazzarrini et al.,  
11 2004; Chan et al., 2017). However, in *bpc1-1 pFUS3:FUS3:GFP* some flower buds were  
12 arrested and never opened, resembling *bpc1/2* mutant (Figure 8A). In those flower buds,  
13 petal and anther filament did not elongate and anthers were aborted, similar to *bpc1/2* double  
14 mutant (Figure 8A). Seed abortion was increased and delayed embryogenesis was evident in  
15 *bpc1-1 pFUS3:FUS3:GFP* plants (Figure 8B,C). The *bpc1-1 pFUS3:FUS3:GFP* plants were  
16 shorter compared with *bpc1-1*, *pFUS3:FUS3:GFP* or wild type and resembled the *bpc1/2*  
17 double mutant (Figure 8D and Figure 6A,D). Thus, our inability to isolate *bpc1/2*  
18 *pFUS3:FUS3:GFP* mutant may be due to the severe phenotype of such a mutant. The  
19 presence of the *pFUS3:FUS3:GFP* transgene enhanced the *bpc1-1* phenotype likely due to  
20 higher or ectopic *FUS3* expression. Accordingly, we could detect strong GFP fluorescence in  
21 the integuments, seed coat and funiculus of *bpc1-1 pFUS3:FUS3:GFP*, while  
22 *pFUS3:FUS3:GFP* showed no fluorescence in WT (in contrast to the stable *pFUS3:FUS3ΔC-*  
23 *GFP*). Furthermore, *FUS3:GFP* was mis-localized in *bpc1-1 pFUS3:FUS3:GFP* endosperm  
24 after fertilization (Figure 8E), in agreement with *pFUS3:FUS3ΔC-GFP* and *pFUS3:GUS*  
25 mislocalization in *bpc1-1* and *bpc1/2* endosperm (Figure 7). These results further support a  
26 repressive role of BPCs on *FUS3* expression in different tissues during reproductive and seed  
27 development.

28 Upon closer inspection of *bpc1/2*, *bpc1-1 pFUS3:FUS3:GFP* and *pML1:FUS3-GFP*  
29 ovules that were successfully fertilized we noticed that they had an increased number of  
30 endosperm nuclei, which correlated with an increase in seed size (Figure 9A,B,C,D;  
31 Supplemental Figure 9). In fertilized ovules, some embryos were delayed or arrested at  
32 various stages (globular to early torpedo) of development compared to wild type (Figure 9E;  
33 Supplemental Figure 9). Lastly, *bpc1/2* mutants also showed aberrant cell division patterns in

1 the embryo and suspensor, which resulted in defective embryos and were partially rescued by  
2 *fus3-3* (Figure 9E; Supplemental Figure 9). Collectively, these data show that repression of  
3 *FUS3* in the endosperm of developing seeds is required to coordinate endosperm and  
4 embryo growth.

5

## 6 **DISCUSSION**

7 PRC2 play important roles in balancing cell proliferation with differentiation and regulating  
8 developmental phase transitions in plants and animals. Recently, genome wide studies have  
9 shown that the plant-specific, class I BPC transcription factors bind Polycomb response  
10 elements (PREs), recruit EMF-PRC2 and trigger gene silencing during germination (Xiao et  
11 al., 2017). Similar to GAGA factors in *Drosophila melanogaster*, BPCs recognize (GA/CT)<sub>n</sub> cis  
12 elements, despite the lack of sequence similarity between these transcription factors,  
13 suggesting convergent evolution (Berger and Dubreucq, 2012). BPCs play essential roles  
14 during vegetative and reproductive development, as shown by the dwarf stature and severe  
15 seed abortion displayed by higher order *bpc* mutants, however the molecular mechanisms are  
16 largely unknown (Kooiker et al., 2005; Monfared et al., 2011; Simonini et al., 2012; Simonini  
17 and Kater, 2014;). Here we show that BPC1/2 interact with FIS-PRC2 and bind to the *FUS3*  
18 chromatin to restrict *FUS3* expression to specific tissues during reproductive and seed  
19 development. BPC-mediated spatiotemporal regulation of *FUS3* expression is required to i)  
20 suppress stem elongation during vegetative-to-reproductive phase change, ii) promote ovule  
21 development before fertilization and iii) coordinate embryo and endosperm development after  
22 fertilization (Figure 10). Several lines of evidence support these conclusions. First, Y1H show  
23 that class I BPCs bind to (GA/CT)<sub>n</sub> repeats around the *FUS3* transcription start, and ChIP  
24 assays in flower buds show that BPC1 binds *in vivo* to the *FUS3* chromatin. Mutations in  
25 these (GA/CT)<sub>n</sub> sites abolish BPCs binding and derepress *FUS3* during vegetative  
26 development. Furthermore, *FUS3* is upregulated in the inflorescence stem of *bpc1/2* dwarf  
27 plants, which is consistent with *fus3-3* tall plant and *ML1:FUS3-GFP* dwarf plant phenotypes,  
28 as well as *FUS3* role as repressor of vegetative-to-reproductive phase change (Gazzarrini et  
29 al., 2004; Lumba et al., 2012). Second, class I BPCs interact with FIS2-PRC2 complex *in*  
30 *planta*, and the *in vivo* BPC1-binding region on *FUS3* was shown to associate with MEA and  
31 H3K27me3 repressive marks (Makarevich et al., 2006), strongly suggesting BPC1 recruits  
32 FIS-PRC2 to repress *FUS3* during reproductive/seed development. Third, *FUS3* is transiently  
33 localized to the integuments during early ovule development and later restricted to the

1 funiculus and chalaza of mature wild type ovules. Ectopic and persistent expression of *FUS3*  
2 in the integuments of *bpc1/2* and *ML1:FUS3* mis-expression lines impairs integument and  
3 embryo sac development leading to seed abortion, which can be partially rescued in *fus3-3*  
4 *bpc1/2*. Last, after fertilization *FUS3* is localized to the funiculus, chalaza and outer  
5 integument, aside from its known localization to the embryo (Gazzarrini et al., 2004). Ectopic  
6 expression of *FUS3* in *bpc1/2* and *ML1:FUS3* endosperm leads to increased proliferation of  
7 the endosperm nuclei and delayed or arrested embryo development, which are rescued in  
8 *fus3-3 bpc1/2*. The latter phenotypes are also displayed by mutants in *FIS-PRC2* subunits  
9 (Kiyosue et al., 1999; Kohler and Grossniklaus, 2002). We conclude that BPCs recruit PRC2  
10 to restrict spatiotemporal *FUS3* expression during reproductive and seed development; this is  
11 required to regulate tissue development locally and modulate developmental phase  
12 transitions in Arabidopsis. The genomic sequences of *FUS3* orthologs in other species show  
13 conservation of (GA/CT)<sub>n</sub> repeats (Supplemental Figure 11), suggesting that similar  
14 mechanisms may regulate the expression of *FUS3*-like transcription factors in other species.

15

## 16 **Inflorescence stem elongation and flower development require repression of *FUS3* by** 17 **Class I BPCs**

18 During germination BPC1 directly binds to the genomic region of *FUS3* proximal to the  
19 transcription start, which is marked by H3K27me<sub>3</sub> repressive marks and associates with FIE  
20 (Figure 3; Xiao et al., 2017). Furthermore, *FUS3* is strongly expressed in *swn clf* seedlings  
21 (Makarevich et al., 2006), suggesting that during germination *FUS3* is repressed through  
22 BPC1-recruitment of EMF/VRN-PRC2. Here we show that mutations of all BPC binding sites  
23 on the *FUS3* promoter derepress *FUS3* in vegetative tissues, and that lack of BPCs results in  
24 ectopic *FUS3* expression in leaves, inflorescence stem and flower buds. Furthermore, ectopic  
25 *FUS3* in *bpc1/2*, *bpc1 pFUS3:FUS3-GFP* or *pML1:FUS3-GFP* leads to similar phenotypes,  
26 including reduced internode elongation and defective flowers (arrested flower bud  
27 development, flowers with a protruding carpel and shorter floral organs), suggesting *FUS3*  
28 inhibits the elongation of the stem and floral organs during flowering. Recently, deletion of a  
29 small region in the *FUS3* promoter near the BPC binding sites and corresponding to the  
30 PRC2 recruitment region, lead to ectopic *FUS3* expression in vegetative tissues (Roscoe et  
31 al., 2019). Thus, we propose that class I BPCs recruit VRN/EMF-PRC2 to repress *FUS3* post-  
32 embryonically, more specifically in germinating seeds, in vegetative and reproductive organs  
33 (Figure 10).

1           Although *bpc1/2* shows dramatic phenotypes during reproductive development,  
2 germination and early seedling development are not affected as it would be expected from  
3 derepression of embryonic genes. This may be due to functional redundancy within the BPCs  
4 family and the difficulty in isolating and characterizing higher order *bpc* mutant due to sterility  
5 (Monfared et al., 2011). However, the C1-2iD ZnF TF AZF1 associates with *LEC2*, *FUS3* and  
6 *ABI3* genomic regions that are also bound by BPC1 and that colocalize with FIE and PRC2  
7 H3K27me3 peaks, suggesting that PRC2-dependent *FUS3* and *LAF1* gene silencing during  
8 post-embryonic development requires BPC, ZnF and likely other factors (Xiao et al., 2017;  
9 Zhou et al., 2018). This is also consistent with the strong phenotype shown by telobox binding  
10 mutants (*trb1/2/3*), which is enhanced by mutations in PRC2 (Zhou et al., 2018). Given that  
11 42% of genome-wide FIE association regions were bound by BPC1 and AZF1, a  
12 combinatorial role for these transcription factors in recruiting PRC2 and triggering gene  
13 silencing has been proposed (Xiao et al., 2017; Zhou et al., 2018).

14

15           **BPC-mediated restriction of *FUS3* expression in developing ovules and seeds is**  
16           **required to promote ovule development and to coordinate endosperm and embryo**  
17           **growth**

18           During ovule development, the funiculus supplies nutrients and signaling molecules from the  
19 mother plant to the chalaza, initiates the integuments that grow around the nucellus and  
20 protect the developing female gametophyte (Schneitz et al., 1995). Our data show that during  
21 megagametogenesis *FUS3* is initially localized to the nucellus epidermis and tissues  
22 surrounding the nucellus, including the integuments and chalaza. However, BPC1/2 later  
23 repress *FUS3* in the integuments of mature ovules, and ectopic *FUS3* expression in *bpc1/2*  
24 inhibits integuments and embryo sac development, triggering ovule abortion. These  
25 phenotypes are recapitulated in *pML1:FUS3* misexpression lines, where the *pML1* promoter  
26 specifically drives expression of *FUS3* in the integuments and endothelium also in mature  
27 ovules, but rescued in *bpc1/2 fus3-3* mutant, strongly indicating that spatiotemporal restriction  
28 of *FUS3* localization is required for integuments, embryo sac and ovule development (Figure  
29 10). This is in agreement with previous finding showing that the integuments are required for  
30 female gametogenesis (Elliott et al., 1996; Klucher et al., 1996; Baker et al., 1997).

31           Following fertilization, the zygote together with the endosperm and the integuments  
32 develop in a coordinated manner to form the embryo and the seed coat of the mature seed.  
33 *FUS3* was previously shown to localize to developing embryos from globular to cotyledon

1 stages (Gazzarrini et al., 2004). Using the sensitive/stable FUS3dC-GFP reporter, we found  
2 that FUS3 localizes also to the funiculus, chalaza and outer seed coat of developing seeds,  
3 partially mirroring its expression pattern in ovules. In *bpc1/2* mutant or in *pML1:FUS3-GFP*  
4 misexpression lines, ectopic FUS3 localization to the endosperm increases cell proliferation  
5 resulting in enlarged endosperm and larger seeds at the expense of embryo development,  
6 which is typically delayed or arrested in *bpc1/2* and *pML1:FUS3-GFP* compared to WT. These  
7 phenotypes are reminiscent of some FIS-PRC2 mutant alleles of *mea* (Kiyosue et al., 1999).  
8 Given that *FUS3* is derepressed in *mea* endosperm and that MEA and H3K27me3 repressive  
9 marks associate in a repressive region of the *FUS3* locus where BPC1 also binds, we  
10 propose that BPC1/2 recruit FIS-PRC2 to repress *FUS3* in the endosperm (Makarevich et al.,  
11 2006); this is required to reduce the rate of endosperm nuclei proliferation, promoting  
12 endosperm differentiation and embryo growth (Figure 10).

13 In the absence of fertilization seed development is repressed by PRC2. FIS-PRC2  
14 represses autonomous central cell division in the ovule and regulate endosperm development  
15 after fertilization. The FIS-PRC2 specific subunits, MEA and FIS2, are targeted solely to the  
16 central cell in the ovule and endosperm in the seed, and thus are likely to participate in *FUS3*  
17 repression in these tissues (Luo et al., 2000; Wang et al., 2006). The MEA homolog SWN,  
18 which belongs to the VRN-PRC2 and FIS-PRC2 complexes, has a broader localization  
19 pattern, but plays a partially redundant function with MEA in repressing central  
20 cell/endosperm nuclei proliferation in the absence of fertilization (Wang et al., 2006). Thus,  
21 SWN may also be involved in repressing *FUS3* in the central cell/endosperm. In contrast,  
22 autonomous seed coat development in the ovule is repressed by the sporophytic complexes  
23 VRN-PRC2 and EMF-PRC2, which may be involved in repressing *FUS3* in the integuments  
24 (Kohler and Grossniklaus, 2002; Roszak and Kohler, 2011). In accordance, *FUS3* and other  
25 seed-specific genes were derepressed and showed reduced H3K27me3 repressive marks in  
26 siliques of a weak *curly leaf (clf)* allele, although the tissue specific expression was not  
27 investigated (Liu et al., 2016).

28 Although BPCs can recruit EMF- and FIS-PRC2 complexes for transcriptional  
29 silencing, BPCs were also shown to positively regulate a close *FUS3* family member, *LEC2*  
30 (Berger et al., 2011). This is in accordance with the role of GAGA binding proteins in animals,  
31 which have dual function of activators and repressors (Berger and Dubreucq, 2012).  
32 Interestingly, *FUS3* is expressed in the embryo and in specific sporophytic tissues of the ovule  
33 and seed (chalaza, funiculus, seed coat), where all class I BPCs are expressed. Thus, it will

1 be important to determine the mechanisms of BPCs activation and repression of *FUS3* and  
2 other *LAF1* genes during reproductive and seed development.

3 Collectively, these findings indicate that spatiotemporal restriction of *FUS3* expression  
4 is necessary for organ development and to allow the transition between various phases of  
5 development. An important question is how does *FUS3* regulate tissue development and  
6 phase transitions. *FUS3* was shown to be a nexus in hormone synthesis; by controlling the  
7 ABA/GA ratio, *FUS3* promotes seed maturation while inhibiting germination and flowering,  
8 with ABA and GA acting as positive and negative regulators of *FUS3* protein levels,  
9 respectively (Gazzarrini et al., 2004; Lu et al., 2010; Chiu et al., 2012). A positive feedback  
10 regulatory loop has been established also between auxin and *FUS3* in the embryo, whereby  
11 *FUS3* promotes auxin synthesis and auxin induces *FUS3* (Gazzarrini et al., 2004). Several  
12 studies have shown that *LAF1* genes are involved in regulating auxin biosynthesis, which also  
13 ties to their role in somatic embryogenesis (Lepiniec et al., 2018). Given that auxin is required  
14 for the synchronized growth of the fruit, the different tissues within the seed (integuments,  
15 endosperm and embryo) and that *FUS3* localization patterns in ovules and seeds largely  
16 mirror those of auxin, we propose that *FUS3* may regulate auxin level/localization and that  
17 auxin may in turn regulate *FUS3* expression/activity (Gazzarrini et al., 2004; Figueiredo et al.,  
18 2015; Figueiredo et al., 2016; Larsson et al., 2017; Robert et al., 2018). Reduced auxin  
19 accumulation in the chalaza and funiculus of *fus3-3* or increased auxin levels in the  
20 integuments and endosperm of *pML1:FUS3* or *bpc1/2* would impair ovule and seed  
21 development resulting in seed abortion and delayed embryo development, respectively, as  
22 shown by delayed endosperm cellularization and embryo growth arrest triggered by auxin  
23 overproduction in the endosperm (Figueiredo and Kohler, 2018; Batista et al., 2019; Robert,  
24 2019).

25 In conclusion, mutations affecting FIS-PRC2 or PRE binding TF BPCs cause severe  
26 seed abortion, however the molecular mechanisms are still poorly understood (Monfared et  
27 al., 2011; Wang and Kohler, 2017; Figueiredo and Kohler, 2018). Here we show that BPC1/2-  
28 mediated spatiotemporal restriction of *FUS3*, a target of the PRC2 complex, is required for the  
29 development of ovule and seed tissues and to regulate developmental phase transitions.

30

## 31 **MATERIAL AND METHODS**

### 32 **Plant material**



1 T-DNA insertion lines *bpc1-1* (SALK\_072966C), *bpc2* (SALK\_090810), *bpc1-1/bpc2* (*bpc1/2*;  
2 CS68700), and *bpc1-1/bpc2/bpc3-1* (CS68699), and an EMS mutant *bpc3-1* (CS68805) were  
3 previously described (Monfared et al., 2011). T-DNA insertion lines *bpc1\_salk*  
4 (SALK\_101466C), *bpc2\_salk* (SALK\_110830C), *bpc3\_sail* (SAILseq\_553\_B09.0) were  
5 obtained from ABRC. All primers used for genotyping are listed in the Supplemental Table 1.  
6 The *pFIE:FIE:GFP*, *pMSI1:MSI1:GFP*, *pMEA:MEA:YFP* and *pFIS2:GUS* reporter lines were  
7 previously described (de Lucas et al., 2016). The *pML1:FUS3-GFP* construct previously  
8 described (Gazzarrini et al., 2004) was transform into *fus3-3* loss-of-function mutant (Keith et  
9 al., 1994). *pFUS3:FUS3ΔC-GFP* construct previously described was transformed into *Col-0*  
10 (Lu et al., 2010). *pFUS3:FUS3-GFP* construct was previously described (Gazzarrini et al.,  
11 2004). For transgenic plants carrying the (GA/CT)<sub>n</sub> mutant promoter reporter [*pFUS3(1.5kb)*,  
12 1.5kb upstream of *FUS3* coding sequence with or without mutated (GA/CT)<sub>n</sub> motifs (shown in  
13 Supplemental Figure 2)] was PCR amplified (primers listed in Supplemental Table 1) and  
14 cloned into pCAMBIA1391-GUS and pCAMBIA1391-GFP vectors by restriction enzyme  
15 digestion (*Hind* III and *Bam*H I). Eight to ten transgenic lines per constructs were selected on  
16 MS containing 30mg/L hygromycin plates and analyzed for GUS staining or GFP  
17 fluorescence. Sterilized Arabidopsis seeds were germinated on half-strength Murashige and  
18 Skoog (MS) medium, transferred to soil and grown under 16/8h light/darkness 22°C/18°C.  
19 Frequencies of seed phenotypes displayed by various genotypes were calculated with half  
20 dissected siliques (n=10); experiments were repeated three times with similar results and one  
21 is shown. Total seed yield per plant was calculated with 5 plants per pot, experiments were  
22 repeated three times.

23

## 24 **Yeast one-hybrid screening**

25 Yeast one-hybrid library screening and one-on-one retests were performed as described by  
26 Deplancke et al. (2006), with some modifications. To construct the baits, 615bp of the *FUS3*  
27 genomic sequence upstream of the translation start [*pFUS3(0.6kb)*; base pairs -615 to +1], or  
28 the *pFUS3* with the mutated (GA/CT)<sub>n</sub> motif [*pFUS3<sup>MUT</sup>(0.6kb)*] or the truncated *pFUS3* (F1 to  
29 F4) were PCR-amplified and recombined into the pDEST-HISi-2 vector by Gateway cloning.  
30 Mutagenesis of (GA/CT)<sub>n</sub> motifs on *pFUS3<sup>MUT</sup>(0.6kb)* was generated by PCR-driven overlap  
31 extension (Heckman and Pease, 2007) with primers listed in Supplemental Table 1. The  
32 linearized vectors (digested by *Xho*I) were then transformed into the yeast strain YM4271(a)  
33 using the LiAc/SS carrier DNA/PEG method (Gietz and Schiestl, 2007). Transformed yeast

1 colonies were tested for background expression of the *HIS3* reporter and the appropriate 3-  
2 aminotriazole (3-AT) concentration was selected. An *Arabidopsis thaliana* transcription factor  
3 library (Mitsuda et al., 2010) was transformed into the yeast strain EGY48( $\alpha$ ) by  
4 electroporation. The initial screening was performed by mating YM4271(a) containing the bait  
5 *pFUS3* (0.6kb) with EGY48( $\alpha$ ) containing the library on YPD plates overnight. Colonies were  
6 selected on medium without Ura, His and Leu, supplemented with 20mM 3-AT (SDA-Ura-His-  
7 Leu + 20mM 3-AT). Plasmids isolated from 69 out of 200,000 CFU harbored *BPC3*. To test the  
8 binding preference of BPC1-3 on the *FUS3* promoter, BPCs were PCR-amplified and  
9 recombined into pDEST-GAD424 by Gateway. The recombined vectors were then  
10 transformed into yeast strain EGY48( $\alpha$ ). A single transformed YM4271(a) colony containing  
11 different truncated or mutated promoters [F1 to F4 and *pFUS3<sup>MUT</sup>* (0.6kb), described above]  
12 was used for mating with EGY48( $\alpha$ ) containing BPCs. Mating and selection procedures were  
13 described in Wu et al. (2018). The interaction was judged by the growth of yeast on selection  
14 media on the third day.

15

### 16 **Bimolecular fluorescence complementation (BiFC) assay**

17 The CDS of *BPCs*, *FIE*, *MSI1*, *MEA* and *FIS2* were cloned into BiFC vectors pB7WGYN2  
18 (YNE) or pB7WGYC2 (YCE) (Tsuda et al., 2017) by Gateway. These recombined vectors  
19 were transformed into *Agrobacterium tumefaciens* strain GV2260 and infiltrated into *Nicotiana*  
20 *benthamiana* leaves as described previously (Duong et al., 2017). At least three biological  
21 replicates were performed.

22

### 23 **Differential interference contrast (DIC) microscopy**

24 Pistils at FS12 or siliques were dissected and immersed in fixing solution (9:1, ethanol:acetic  
25 acid, v/v) for 2h before washing them twice with 90% ethanol. The siliques were then cleared  
26 with clearing solution (2.5g/ml chloral hydrate and 30% glycerol) overnight. Images were  
27 taken by a Zeiss Axioplan 2 microscope equipped with DIC optics. The quantification of seed  
28 size and endosperm nuclei are performed by Image J software.

29

### 30 **Confocal microscopy**

31 To observe the expression of GFP signal in transgenic *Arabidopsis*, fresh tissues was  
32 dissected and mounted on the slides with 10% glycerol. Visualization was done with a Zeiss  
33 LSM510 confocal microscope (488 nm excitation and a 515-535 nm band pass filter).



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33

## **GUS staining**

The *pBPC3:GUS* line was previously described (Monfared et al., 2011). The promoter regions of *BPC1/2* described in Monfared et al. (2011) were PCR-amplified and transformed into the pGWB3 vector to generate *pBPC1:GUS* and *pBPC2:GUS*. Several transformed homozygous lines were selected on kanamycin and hygromycin plates and analyzed and two lines were selected for further analysis. The GUS staining assays were performed as previously described (Wu et al., 2019) with some modifications. The concentration of ferri/ferrocyanide used for *pBPC3:GUS* was 2mM, while 5mM was used for *pBPC1:GUS* and *pBPC2:GUS*. To detect low expression of *FUS3* in inflorescences, leaves or flowers of *pFUS3(1.5 kb):GUS* and *pFUS3<sup>MUT</sup> (1.5kb):GUS* lines, ferri/ferrocyanide was not included in the buffer. Cleared tissues were imaged by DIC microscopy using Zeiss Axioplan 2.

## **Glutaraldehyde staining**

To visualize ovule/seed structures, whole pistils/siliques at FS12 or 1-2DAF were fixed in 3% paraformaldehyde in PBS for 15min at room temperature and rinsed twice with PBS. The treated tissues were stained in 5% glutaraldehyde in PBS at 4°C overnight in the dark. Tissues were washed 3 times with PBS and cleared for about 1 to 2 weeks with ClearSee buffer (Kurihara et al., 2015). The images were photographed with a Zeiss LSM510 confocal microscope (530nm excitation and a 560nm long pass filter).

## **Gene expression assay**

RNA was extracted using the RNeasy Plant Mini Kit (Qiagen). About 1µg of RNA was used for reverse transcription. Quantitative real-time PCR was performed using Step One Plus real-time PCR system (Applied Biosystems) with SYBR premix. *PP2AA3* was chosen as the internal reference gene. Primers used are listed in Supplemental Table 1. Three biological replicates were performed.

## **ChIP assay**

To generate *35S:BPC1-RFP*, the *BPC1* coding sequence was first cloned into pDONR221 (Life Technologies) and subsequently transferred to pB7RWG2 (Flanders Interuniversity Institute for Biotechnology, Gent, Belgium). Arabidopsis plants were transformed with the *35S:BPC1-RFP* using the *Agrobacterium tumefaciens*-mediated floral dip method (Clough

1 and Bent, 1998). Transformant plants were sown on soil and selected by BASTA; the  
2 presence of the construct was assessed by genotyping and analysis of RFP expression.  
3 Arabidopsis plants were directly sown on soil and kept under short-day conditions for 2 weeks  
4 (22°C, 8h light and 16h dark) and then moved to long-day conditions (22°C, 16h light and 8h  
5 dark). ChIP assays were performed as described by Gregis et al. (2009) using for BPC1-RFP  
6 an anti-RFP V<sub>H</sub>H coupled to magnetic agarose beads RFP-trap\_MA® (Chromotek). Real-time  
7 PCR assays were performed to determine the enrichment of the fragments. The detection  
8 was performed in triplicate using the iQ SYBR Green Supermix (Bio-Rad) and the Bio-Rad  
9 iCycler iQ Optical System (software version 3.0a), with the primers listed in Supplemental  
10 Table 1. ChIP-qPCR experiments and relative enrichments were calculated as reported by  
11 Gregis et al. (2009).

12

### 13 **Accession Numbers**

14 Sequence data from this article can be found in the Arabidopsis Genome Initiative or  
15 GenBank/EMBL databases under the following accession numbers: *FUSCA3* (At3g26790),  
16 *BPC1* (AT2G01930), *BPC2* (AT1G14685), *BPC3* (AT1G68120), *BPC4* (AT2G21240), *FIE*  
17 (AT3G20740), *MSI1* (AT5G58230), *MEA* (AT1G02580), *FIS2* (AT2G35670) and *LHP1*  
18 (AT5G17690).

19

### 20 **SUPPLEMENTAL DATA**

21

22 **Supplemental Figure 1 *FUS3* expression profile in reproductive tissues and stem**  
23 **epidermis.** The images were generated by the eFP browser ([www.bar.utoronto.ca](http://www.bar.utoronto.ca)) using  
24 microarray data (Schmid et al., 2005; Suh et al., 2005; Swanson et al., 2005; Dean et al.,  
25 2011). **A**, *FUS3* expression profile in the pistil and different stages of flowers. **B**, The  
26 expression profile of *FUS3* in the seed coat at the heart, bending and walking embryo stage.  
27 **C**, The expression pattern of *FUS3* in the stem epidermis. **D**, *FUS3* transcripts are higher at  
28 the top of the inflorescence stem compared to bottom (\*: p<0.05; student t-test). **E**,  
29 *pFUS3:FUS3ΔC-GFP* fluorescence appears stronger in the epidermis of internodes closer to  
30 the flower buds and weaker at the bottom of the stem. Identical confocal settings were used.

31

32 **Supplemental Figure 2 Location of (GA/CT)<sub>n</sub> mutated in the *pFUS3* genomic region**  
33 **used in Y1H.** The (GA/CT)<sub>n</sub> motifs that were mutated in the *FUS3* sequence upstream of the

1 start codon (base pairs -1 to -615) are highlighted in yellow. Mutated G/C residues are  
2 marked in red. Telo boxes binding sites of AZF1 (Xiao et al., 2017) are marked in blue. The  
3 repressive region (126bp) that associated with MEA and H3K27me3 repressive marks  
4 (Makarevich et al., 2006), and was deleted in promoter studies Roscoe et al. (2019) is  
5 underlined.

6

7 **Supplemental Figure 3 Class I BPC family members form homo- or hetero- dimers.**

8 BPC1, BPC2 and BPC3 form heterodimers with each other. Only BPC2 and BPC3 form  
9 homodimers. Lack of interaction between FUS3 and BPCs or FIS-PRC2 in BiFC assays is  
10 shown as the negative control in Supplemental Figure 5.

11

12 **Supplemental Figure 4 PRC2 complexes in Arabidopsis.** There are three different types of

13 PRC2 in Arabidopsis depending on subunit composition - VRN-PRC2, EMF-PRC2 and FIS-  
14 PRC2-, which regulate vernalization, vegetative development and female gametophyte/seed  
15 development, respectively (Mozgova et al., 2015).

16

17 **Supplemental Figure 5 FUS3 protein does not interact with FIS-PRC2 complex and**

18 **Class I BPC family members using BiFC.** Negative control showing that FUS3 does not

19 interact with FIE, MSI1, MEA, FIS2, BPC1, BPC2 or BPC3 in *N. benthamiana* by BiFC.

20

21 **Supplemental Figure 6 Class I BPC proteins do not interact with LHP1 using BiFC.**

22 Class I BPC proteins do not interact with LHP1 in *N. benthamiana* by BiFC.

23

24 **Supplemental Figure 7 Expression patterns of FIS-PRC2 in ovules and embryos.**

25 FIS2 complex (FIE, MSI1, MEA and FIS2) expression/localization patterns in ovules before  
26 fertilization (FG4 and FG7), 1DAP seeds and embryos at globular, heart, torpedo and  
27 cotyledon stages. Ant: antipodals; cc: central cell; chl: chalaza; cze: chalazal endosperm; ec:  
28 egg cell; fu: funiculus; ii: inner integuments; mce: micropilar endosperm; oi: outer integuments;  
29 pen: peripheral endosperm; pge: pre-globular embryo; sdc: seed coat; sus: suspensor; syn:  
30 synergids cell. Pink dashed lines represent the outline of the embryo sac. The  
31 ferro/ferricyanide used in GUS staining buffer was 2mM for *pFIS2:GUS*.

32

1 **Supplemental Figure 8 Class I *bpc* mutants show delayed megagametogenesis, seed**  
2 **abortion and delayed embryogenesis. A**, Class I BPC mutants show seed abortion and  
3 delayed embryogenesis phenotypes. The white asterisks indicate aborted seeds; the yellow  
4 asterisks represent delayed embryogenesis seeds. **B**, The frequencies of seed phenotypes in  
5 10 peeled half-side of *bpc* mutants siliques. Three biological repeats were performed and one  
6 representative result is shown. **C**, Seed yield of WT and *bpc* mutants. The error bars  
7 represent the SD of three biological replicates (\*:  $p < 0.05$ ; student t-test was used) **D**, FS12  
8 ovules of Class I BPC mutants showing aborted embryo sac and delayed  
9 megagametogenesis. **E**, Class I BPC mutants show condensed endosperm and unfertilized  
10 egg cell with the degenerated synergid cell at 2DAF. **F**, Arrested embryos in *bpc1/2* with an  
11 enlarged seed at 3DAP. **G**, At mature stage (11DAP), some *bpc1/2* embryos were arrested at  
12 torpedo stage. The pink arrow points to the aborted embryo sac. Ant: antipodals; cc: central  
13 cell; cze: chalazal endosperm; ec: egg cell; es: embryo sac; mce: micropilar endosperm; nu:  
14 nuclei; pen: peripheral endosperm; dsyn: degenerated synergid cell; z: zygote. Pink dashed  
15 lines outline the embryo sac at FS12; the yellow dash lines outline the embryo sac at 2DAF.

16

17 **Supplemental Figure 9 Overexpression of *FUS3* results in embryo defect and over-**  
18 **proliferation of the endosperm nuclei.** Seeds of (A) Wildtype, (B) *bpc1-1*, (C, D) *MFG*, (E,  
19 F) *bpc1-1 FFG* and (G-L) *bpc1/2* at 3 DAP. Pink dashed lines represent the outline of the  
20 embryo. The yellow dashed line represents the embryo sac. White arrows indicate the  
21 abnormal suspensors. White, yellow or red asterisk indicates the aborted seed, arrested  
22 embryo or defective embryo, respectively.

23

24 **Supplemental Figure 10 Frequencies of seed phenotypes. A, B** The total number of  
25 seeds displaying various phenotypes was calculated in 10 peeled siliques (half side) of **A**, WT,  
26 *bpc1/2* and *bpc1/2 fus3* mutants, and **B**, WT, *bpc1-1* and *bpc1-1 FFG* mutants. Three  
27 biological repeats were performed, and two are shown here. See also Figures 6 and 8.

28

29 **Supplementary Figure 11 Conserved (GA/CT)<sub>n</sub> motifs in orthologous *FUS3* genes.**

30 Accession number of *FUS3* orthologous genes: *OsLFL1* (LEC2 and FUSCA3-like protein 1;  
31 GenBank: EF521182.1) from *Oryza sativa*, *BnFUS3* (NCBI: XM\_013792060) from *Brassica*  
32 *napus*, *GmFUS3* (Gene ID: LOC100813055) from *Glycine max*.

33

1 **Supplemental Table 1 Primers used in this study.**

2

3 **ACKNOWLEDGMENT**

4 We thank C.S. Gasser (UC Davis) for *pBPC3:GUS* reporter; F. Parcy for *pFUS3:GUS*; SM  
5 Brady (UC Davis) and M. De Lucas (Durham University) for *pFIE:FIE:GFP*, *pMSI1:MSI1:GFP*,  
6 *pMEA:MEA:YFP* and *pFIS2:GUS* reporter lines as well as *FIE* and *MSI1* vectors; C. Koehler  
7 (The Swedish University of Agricultural Sciences) and R. Yadegari (University of Arizona) for  
8 *MEA/pBluescript II KS* and *FIS2/pGBKT7* vectors. JW was supported by the National Natural  
9 Science Foundation projects (grants 31701952) and China Postdoctoral Council scholarships.  
10 V.G. was supported by the Ministero dell'Istruzione, dell'Università e della Ricerca MIUR,  
11 SIR2014 MADSMEC, Proposal number RBSI14BTZR. R.P. was supported by the Doctorate  
12 School in Molecular and Cellular Biology, Università degli Studi di Milano Fellowship. This  
13 work was funded by a Natural Sciences and Engineering Research Council of Canada  
14 Discovery Grant to SG.

15

16 **AUTHOR CONTRIBUTIONS**

17 JW and SG conceived the study and wrote the paper. JW conducted most of the experiments.  
18 SD helped with the identification of higher order mutants. RP and VG conducted ChIP assays.  
19 All read and approved the manuscript.

20

21 **FIGURES**

22

23 **Figure 1. FUS3 localization in developing ovules and during early stages of seed**  
24 **development.**

25 Confocal images showing pFUS3:FUS3 $\Delta$ C-GFP localization in Arabidopsis. **(A)** Valve and **(B)**  
26 septum of the pistil. **(C-F)** Developing ovules during female megasporogenesis **(C)** and  
27 megagametogenesis at stages FG1-FG7 **(D-F)**. FUS3 $\Delta$ C-GFP fluorescence was localized to  
28 the nucellar epidermis **(C)**, inner and outer integuments **(C,D)**, funiculus, chalazal **(C,F)**. **(G-J)**  
29 seeds at 6 hours to 2 days (6HAP to 2DAP) after pollination. FUS3 $\Delta$ C-GFP fluorescence was  
30 localized to the seed coat, chalaza and funiculus **(G-J)**. **(K)** Suspensor and 16-cell stage  
31 embryo proper. **(L)** 32-cell stage embryo proper. chl: chalaza; es, embryo sac; fun, funiculus;  
32 ii: inner integument; megaspore mother cell; ne, nucellar epidermis; nu: nucellus; oi: outer

1 integument; sept, septum. Red, autofluorescence from chlorophyll. Purple dashed lines  
2 represent the outline of embryo sac. Scale bars, 10 $\mu$ m.

3

#### 4 **Figure 2. *FUS3* is required for ovule development.**

5 **A**, Aborted silique (asterisks) in *fus3-3 pML1:FUS3-GFP* (*MFG*) overexpression lines. **B**,  
6 pML1:FUS3-GFP localization to the integuments and endothelium of ovules at flower stage  
7 12 (FS12), and outer layer of the seed coat and endosperm (inset) of 2DAP seeds. (i)  
8 developed ovule; (ii) aborted embryo sac; (iii, iv). outer layer of the seed coat and the  
9 endosperm (inset) in 2DAP seeds Bar, 10 $\mu$ M. **C**, Aborted seeds (white asterisk) and delayed  
10 embryogenesis (yellow asterisk) in *MFG* and *fus3-3* siliques. **D**, The distribution of seeds in  
11 peeled, half sides siliques of WT, *MFG* and *fus3-3* (n= ten siliques/genotype). **E**, DIC images  
12 of WT, *MFG* and *fus3-3* FS12 ovules. Pink dashed lines outline the embryo sac. Ant: anti-  
13 antipodals; ec: egg cell; es: embryo sac; et: endothelium; fm: functional megaspore; ii, inner  
14 integument; nu: nuclei; oi, outer integument; syn: synergid cell nuclei. Bars represent 10 $\mu$ m

15

#### 16 **Figure 3. Class I BPCs bind to the *FUS3* genomic region proximal to the transcription** 17 **start site.**

18 **A**, BPC1/2/3 bind to a *FUS3* genomic region of 615bp upstream of the translation start  
19 [*pFUS3*(0.6 kb); -615 to +1 base pairs]. **B**, BPC1/2/3 do not bind the *FUS3* genomic  
20 sequence carrying mutations in (GA/CT)<sub>n</sub> motifs [*pFUS3*<sup>MUT</sup>(0.6 kb)]. Colonies in **A** and **B**  
21 were selected on -ura-his-leu medium (-UHL) with or without 5 or 20mM 3-AT. **C**, Distribution  
22 of (GA/CT)<sub>n</sub> motifs in *FUS3* genomic sequence (-615 to +434). **D**. Binding specificity of  
23 BPC1/2/3 to truncated *FUS3* genomic sequences shown in C (F1 to F4). **E**, Bowser view of  
24 chromatin occupancy of FIE, BPC1, AZF1 and H3K27me3 at *FUS3* and *ACT2* (negative  
25 control) in 30-h-old seedlings using ChIP-seq data from Xiao et al. (2017). Numbers indicate  
26 peak Significant peaks (Q < 10<sup>-10</sup>) according to MACS2 are marked by horizontal bars. **F**.  
27 Real-time PCR analysis of ChIP assay using chromatin extracted from 35S:*BPC1-RFP* and  
28 *Col-0* (negative control) inflorescences and primers for the F3 region of *pFUS3*. Antibodies  
29 against the RFP tag were used in the IP. Error bars represent the propagated error value  
30 using three biological replicates (\*: p<0.05; student t-test). **G**, *pFUS3*(1.5kb):*GUS* and  
31 *pFUS3*<sup>MUT</sup>(1.5kb):*GUS* stain in 10-days-old seedlings; numbers refer to the number of  
32 transgenic lines displaying the same GUS stain pattern as shown in **G**. **H**, *pFUS3*(1.5kb):  
33 *GFP* and *pFUS3*<sup>MUT</sup>(1.5kb):*GFP* fluorescence in the leaf tip of 15-days-old seedlings.



1

2 **Figure 4. Class I BPC family members intact with FIS-PRC2 complex.**

3 The interaction between Class I BPC family members and FIS complex in *N. benthamiana* by  
4 Bimolecular Fluorescence Complementation (BiFC). Lack of interaction between FUS3 and  
5 BPCs or FIS-PRC2 in BiFC assays is shown as the negative control (Supplemental Figure 5).

6

7 **Figure 5. Expression patterns of Class I BPCs in ovules and embryos.**

8 Class I BPC1s expression patterns in ovules before pollination at flower stages FG4 and  
9 FS12; in seeds at 1 day after pollination (DAP); and in embryos at globular, heart, torpedo  
10 and cotyledon stages. Ant: antipodals; cc: central cell; chl: chalaza; cze: chalazal endosperm;  
11 ec: egg cell; fu: funiculus; ii: inner integuments; mce: micropilar endosperm; oi: outer  
12 integuments; pen: peripheral endosperm; pge: pre-globular embryo; sdc: seed coat; sus:  
13 suspensor; syn: synergids cell.

14

15 **Figure 6. Partial rescue of *bpc1/2* stunted growth, aborted ovules and seeds in *fus3-3*  
16 *bpc1/2*.**

17 **A**, The stunted growth of *bpc1/2* was partially rescued in *bpc1/2 fus3-3*. **B, C**, *bpc1/2 fus3-3*  
18 partially rescues *bpc1/2* reduced silique elongation. Scale bar, 1cm. **D**, Quantification of the  
19 plant height. Five biological replicates were performed. Each replicate consisted of five plants  
20 per genotype. **E, F**, *fus3-3* partially rescues *bpc1/2* severe seed abortion. The white asterisk  
21 in **E** represents aborted seed, while the yellow asterisk represents the delayed  
22 embryogenesis seeds. **F**, Frequencies of seed phenotypes in *bpc1/2 fus3-3* mutants. The  
23 total number of sees was calculated in 10 peeled siliques (half side). Three biological repeats  
24 were performed with similar results and one is shown (see also Supplemental Figure 10A). **G**,  
25 The seed yield of *bpc* mutants. Error bars represent the SD of three biological replicates (n=5).  
26 n.s.: no significant difference. (\* p<0.05; \*\* p<0.01; \*\*\*\* p<0.0001); student t-test was used. **H**,  
27 *fus3-3* partially rescues the embryo sac defects of *bpc1/2*. The image was taken at 1DAP.  
28 Scale bar, 20µm. Numbers refer to the number of embryos displaying the phenotype shown.

29

30 **Figure 7. BPC1/2 negatively regulate FUS3 expression in reproductive organs and  
31 seeds.**

32 **A**, qRT-PCR showing increased *FUS3* transcript level in *bpc1/2* inflorescence stem. Error  
33 bars represent the SD of three biological replicates (\* p<0.05; student t-test). **B**, GUS staining

1 in the inflorescence stem, flower buds, septum and seed (2DAF) of *pFUS3:GUS* and *bpc1/2*  
2 *pFUS3:GUS* lines. The GUS staining was enhanced in the inflorescence stem and septum,  
3 while ectopically expressed in the endosperm of *bpc1/2*. **C, D** *pFUS3:FUS3ΔC-GFP* and *bpc1*  
4 *pFUS3:FUS3ΔC-GFP* ovules were images before (C) and two days after (D) fertilization by  
5 confocal microscopy. FUS3ΔC-GFP was localized to the chalaza region of developing WT  
6 ovules before fertilization, while ectopically localized to the integuments at the micropilar  
7 region of *bpc1-1* and of *bpc1/2* ovules (FS12) and to the endosperm of 2DAF *bpc1-1* seeds.

8

9 **Figure 8. Ectopic FUS3 expression negatively impacts reproductive organ development.**

10 **A**, Introduction of a *pFUS3:FUS3-GFP* (*FFG*) transgene in *bpc1-1* mutant results in arrested  
11 flower buds that never open (white asterisk), similar to *bpc1/2* double mutant. The arrested  
12 flower buds in *bpc1-1 FFG* have underdeveloped petals, non-elongated filaments and aborted  
13 anthers, similar to *bpc1/2*. *pML1:FUS3-GFP* (*MFE*) also show shorter filaments and  
14 underdeveloped anthers, but flower buds open prematurely. **B**, *bpc1-1 FFG* mutant caused  
15 aborted seeds and delayed embryogenesis. Aborted seeds (white asterisk) and delayed  
16 embryogenesis (yellow asterisk) are shown. **C**, Frequencies of seed phenotypes. The total  
17 number of seeds was calculated in ten siliques (half side). Three biological repeats were  
18 performed, and one representative is shown (see also Supplemental Figure 10B). **D**, *bpc1-1*  
19 *FFG* plants display stunted growth. The error bar represents SD of three biological replicates  
20 (n=5). (\*\*: p<0.01; student t-test was used). **E**, *FUS3* is mis-expressed in the integument (ii)  
21 and increased in the funiculus (iv) of *bpc1-1* at FS12. Two days after fertilization (2DAF),  
22 *FUS3-GFP* is increased in the seed coat (vi) and mis-expressed in the endosperm (viii) at 2  
23 DAF.

24

25 **Figure 9. BPC1/2 negatively regulate endosperm nuclei proliferation and seed size by**  
26 **repressing FUS3.**

27 **A**, Whole-mount clearing, **B**, seed size, **C**, quantification of endosperm nuclei, **D** seed size  
28 versus number of endosperm nuclei and **E**, frequencies of embryo phenotypes of wild-type  
29 (*Col-0*), *pML1:FUS3-GFP* (*MFG*), *bpc1-1*, *bpc1-1FFG*, *bpc1/2*, *fus3-3* and *bpc1/2 fus3-3*  
30 seeds at 6DAP. Over-proliferation of endosperm nuclei and larger seed size in the *bpc1/2*,  
31 *bpc1-1FFG* and *MFG* lines, and partial rescue in *bpc1/2 fus3-3*. Images were taken 6DAP.  
32 Scale bar, 100μm. **B, C** Ectopic expression of *FUS3* in *MFG*, *bpc1-1 FFG*, and *bpc1/2* leads  
33 to enlarged seed size **B**), increased endosperm nuclei proliferation **C**) and density **D**), which



1 is partially rescued in *bpc1/2 fus3-3*. **E**, Ectopic expression of *FUS3* in *bpc1/2*, *bpc1-1FFG*  
2 and *MFG* results in delayed embryogenesis; *bpc1/2* defective embryos are partially rescued  
3 by *fus3-3*. (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*\*  $p < 0.0001$ , student t-test was used).

4

5 **Figure 10. Spatiotemporal restriction of *FUS3* expression by *BPC1/2* during**  
6 **reproductive and seed development.**

7 Model depicting spatiotemporal expression of *FUS3* and its role in the regulation of  
8 vegetative-to-reproductive and gametophytic-to-sporophytic phase transitions. During the  
9 vegetative-to-reproductive phase change, *FUS3* is repressed by *BPC1/2* in the inflorescence  
10 stem to allow stem elongation. During ovule development, *FUS3* becomes restricted to the  
11 funiculus and chalaza through *BPC1/2*-mediated repression in the integuments; this is  
12 required to promote integument and embryo sac development. After fertilization, *FUS3* is  
13 localized to the embryo, seed coat, chalaza and funiculus, but is repressed in the endosperm  
14 by *BPC1/2* to decrease endosperm nuclei division and promote embryo development. In the  
15 stem, *BPC1/2*-mediated *FUS3* repression may be orchestrated by *EMF-PRC2*, which  
16 interacts with *BPC1/2* and represses *FUS3* postembryonically (Liu et al., 2016; Xiao et al.,  
17 2017). *FUS3* repression in the integuments may require sporophytic *VRN/EMF PRC2*. After  
18 fertilization, *FIS-PRC2* represses *FUS3* in the endosperm (Makarevich et al., 2006).

19

20 **REFERENCES**

- 21 Baker SC, Robinson-Beers K, Villanueva JM, Gaiser JC, and Gasser CS. 1997. Interactions  
22 among genes regulating ovule development in *Arabidopsis thaliana*. *Genetics* **145**:  
23 1109-1124
- 24 Batista RA, Figueiredo DD, Santos-Gonzalez J, and Kohler C. 2019. Auxin regulates  
25 endosperm cellularization in *Arabidopsis*. *Genes Dev* **33**: 466-476
- 26 Berger N, and Dubreucq B. 2012. Evolution goes GAGA: GAGA binding proteins across  
27 kingdoms. *Biochim Biophys Acta* **1819**: 863-868
- 28 Berger N, Dubreucq B, Roudier F, Dubos C, and Lepiniec L. 2011. Transcriptional regulation  
29 of *Arabidopsis* *LEAFY COTYLEDON2* involves *RLE*, a cis-element that regulates  
30 trimethylation of histone H3 at lysine-27. *Plant Cell* **23**: 4065-4078
- 31 Braybrook SA, Stone SL, Park S, Bui AQ, Le BH, Fischer RL, Goldberg RB, and Harada JJ.  
32 2006. Genes directly regulated by *LEAFY COTYLEDON2* provide insight into the  
33 control of embryo maturation and somatic embryogenesis. *Proc Natl Acad Sci USA*  
34 **103**: 3468-3473
- 35 Carbonero P, Iglesias-Fernandez R, and Vicente-Carbajosa J. 2017. The AFL subfamily of B3  
36 transcription factors: evolution and function in angiosperm seeds. *J Exp Bot* **68**: 871-  
37 880
- 38 Chan A, Carianopol C, Tsai AYL, Varatharajah K, Chiu RS, and Gazzarrini S. 2017. SnRK1  
39 phosphorylation of *FUSCA3* positively regulates embryogenesis, seed yield, and plant

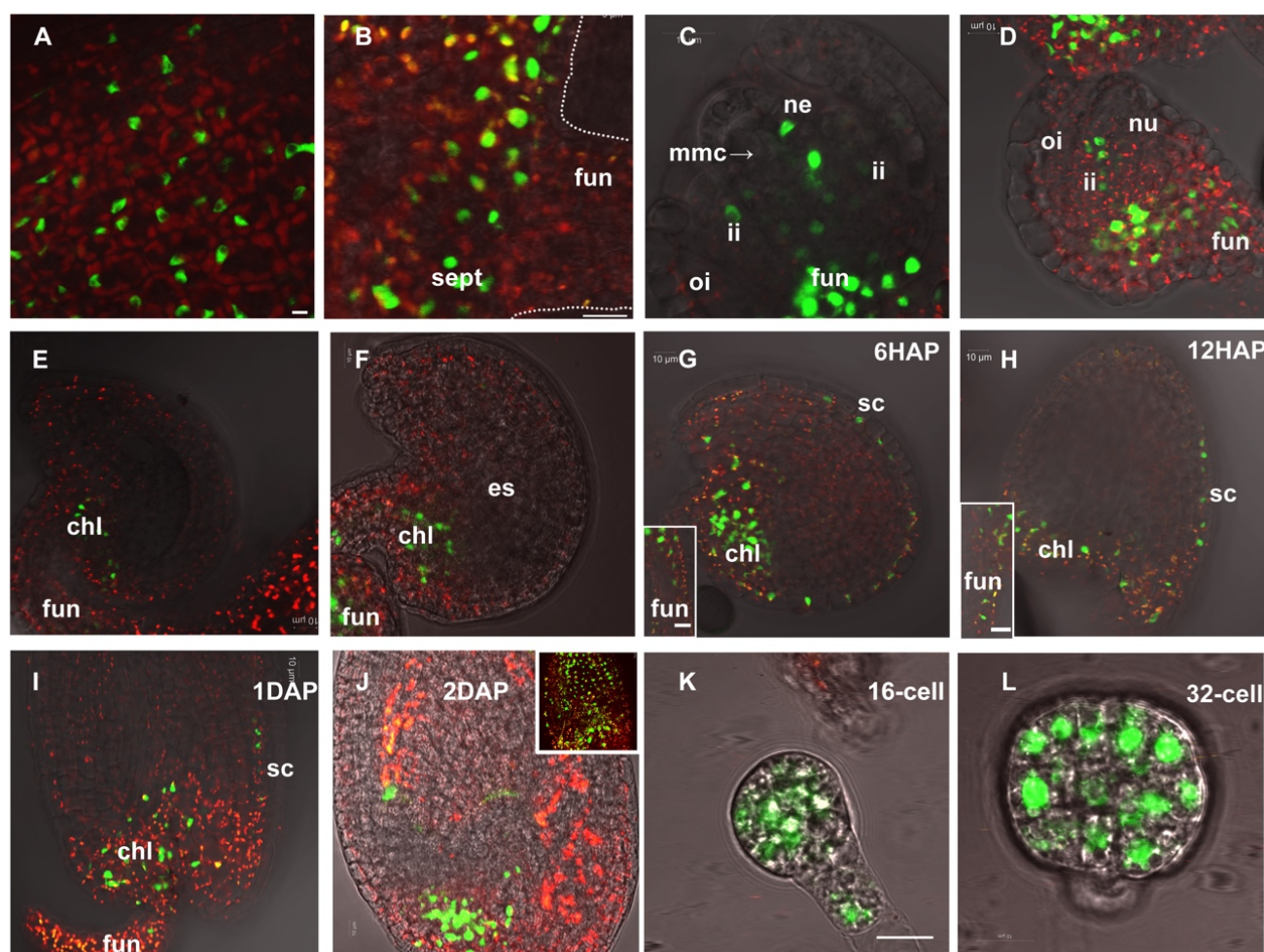
- 1 growth at high temperature in Arabidopsis *J Exp Bot* **68**: 5981-5981
- 2 Chiu RS, Pan S, Zhao R, Gazzarrini S. 2016. ABA-dependent inhibition of the ubiquitin  
3 proteasome system during germination at high temperature in Arabidopsis. *The Plant*  
4 *Journal* **88**: 749–761
- 5 Curaba J, Moritz T, Blervaque R, Parcy F, Raz V, Herzog M, Vachon G. 2004. AtGA3ox2, a  
6 key gene responsible for bioactive gibberellin biosynthesis, is regulated during  
7 embryogenesis by LEAFY COTYLEDON2 and FUSCA3 in Arabidopsis. *Plant Physiol.*  
8 **136**:3660-9.
- 9 Clough SJ, and Bent AF. 1998. Floral dip: a simplified method for Agrobacterium-mediated  
10 transformation of Arabidopsis thaliana. *Plant J* **16**: 735-743
- 11 de Lucas M, Pu L, Turco G, Gaudinier A, Morao AK, Harashima H, Kim D, Ron M, Sugimoto  
12 K, Roudier F, et al. 2016. Transcriptional Regulation of Arabidopsis Polycomb  
13 Repressive Complex 2 Coordinates Cell-Type Proliferation and Differentiation. *Plant*  
14 *Cell* **28**: 2616-2631
- 15 de Vries SC, and Weijers D. 2017. Plant embryogenesis. *Curr Biol* **27**: R870-R873
- 16 Dean G, Cao Y, Xiang D, Provart NJ, Ramsay L, Ahad A, White R, Selvaraj G, Datla R, and  
17 Haughn G. 2011. Analysis of gene expression patterns during seed coat development  
18 in Arabidopsis. *Mol Plant* **4**: 1074-1091
- 19 Deplancke B, Vermeirssen V, Arda HE, Martinez NJ, and Walhout AJ. 2006. Gateway-  
20 compatible yeast one-hybrid screens. *CSH Protoc* **2006**: doi: 10.1101/pdb.prot4590
- 21 Dresselhaus T, Sprunck S, and Wessel GM. 2016. Fertilization Mechanisms in Flowering  
22 Plants. *Curr Biol* **26**: R125-139
- 23 Drews GN, and Koltunow AM. 2011. The female gametophyte. *Arabidopsis Book* **9**: e0155
- 24 Duong S, Vonapartis E, Li CY, Patel S, and Gazzarrini S. 2017. The E3 ligase ABI3-  
25 INTERACTING PROTEIN2 negatively regulates FUSCA3 and plays a role in cotyledon  
26 development in Arabidopsis thaliana. *J Exp Bot* **68**: 1555-1567
- 27 Elliott RC, Betzner AS, Huttner E, Oakes MP, Tucker WQ, Gerentes D, Perez P, and Smyth  
28 DR. 1996. AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic  
29 roles in ovule development and floral organ growth. *Plant Cell* **8**: 155-168
- 30 Fatihi A, Boulard C, Bouyer D, Baud S, Dubreucq B, and Lepiniec L. 2016. Deciphering and  
31 modifying LAFL transcriptional regulatory network in seed for improving yield and  
32 quality of storage compounds. *Plant Sci* **250**: 198-204
- 33 Figueiredo DD, and Kohler C. 2018. Auxin: a molecular trigger of seed development. *Genes*  
34 *Dev* **32**: 479-490
- 35 Figueiredo DD, Batista RA, Roszak PJ, and Kohler C. 2015. Auxin production couples  
36 endosperm development to fertilization. *Nat Plants* **1**: 15184
- 37 Figueiredo DD, Batista RA, Roszak PJ, Hennig L, and Kohler C. 2016. Auxin production in the  
38 endosperm drives seed coat development in Arabidopsis. *eLife* **5**
- 39 Gasser CS, and Skinner DJ. 2019. Development and evolution of the unique ovules of  
40 flowering plants. *Curr Top Dev Biol* **131**: 373-399
- 41 Gazzarrini S, Tsuchiya Y, Lumba S, Okamoto M, and McCourt P. 2004. The transcription  
42 factor FUSCA3 controls developmental timing in Arabidopsis through the hormones  
43 gibberellin and abscisic acid. *Devel Cell* **7**: 373-385
- 44 Gietz RD, and Schiestl RH. 2007. Large-scale high-efficiency yeast transformation using the  
45 LiAc/SS carrier DNA/PEG method. *Nat Protoc* **2**: 38-41
- 46 Gregis V, Sessa A, Dorca-Fornell C, and Kater MM. 2009. The Arabidopsis floral meristem  
47 identity genes AP1, AGL24 and SVP directly repress class B and C floral homeotic  
48 genes. *Plant J* **60**: 626-637
- 49 Grossniklaus U, Vielle-Calzada JP, Hoepfner MA, and Gagliano WB. 1998. Maternal control

- 1 of embryogenesis by MEDEA, a polycomb group gene in Arabidopsis. *Science* **280**:  
2 446-450
- 3 Hecker A, Brand LH, Peter S, Simoncello N, Kilian J, Harter K, Gaudin V, and Wanke D. 2015.  
4 The Arabidopsis GAGA-Binding Factor BASIC PENTACYSTEINE6 Recruits the  
5 POLYCOMB-REPRESSIVE COMPLEX1 Component LIKE HETEROCHROMATIN  
6 PROTEIN1 to GAGA DNA Motifs. *Plant Physiol* **168**: 130-141
- 7 Heckman KL, and Pease LR. 2007. Gene splicing and mutagenesis by PCR-driven overlap  
8 extension. *Nat Protoc* **2**: 924-932
- 9 Jia H, Suzuki M, and McCarty DR. 2014. Regulation of the seed to seedling developmental  
10 phase transition by the LAFL and VAL transcription factor networks. *Wiley Interdiscip*  
11 *Rev Dev Biol* **3**: 135-145
- 12 Keith K, Kraml M, Dengler NG, and McCourt P. 1994. fusca3: A Heterochronic Mutation  
13 Affecting Late Embryo Development in Arabidopsis. *Plant Cell* **6**: 589-600
- 14 Kiyosue T, Ohad N, Yadegari R, Hannon M, Dinneny J, Wells D, Katz A, Margossian L,  
15 Harada JJ, Goldberg RB, et al. 1999. Control of fertilization-independent endosperm  
16 development by the MEDEA polycomb gene in Arabidopsis. *Proc Natl Acad Sci USA*  
17 **96**: 4186-4191
- 18 Klucher KM, Chow H, Reiser L, and Fischer RL. 1996. The AINTEGUMENTA gene of  
19 Arabidopsis required for ovule and female gametophyte development is related to the  
20 floral homeotic gene APETALA2. *Plant Cell* **8**: 137-153
- 21 Kohler C, and Grossniklaus U. 2002. Epigenetic inheritance of expression states in plant  
22 development: the role of Polycomb group proteins. *Curr Opin Cell Biol* **14**: 773-779
- 23 Kooiker M, Airoidi CA, Losa A, Manzotti PS, Finzi L, Kater MM, and Colombo L. 2005. BASIC  
24 PENTACYSTEINE1, a GA binding protein that induces conformational changes in the  
25 regulatory region of the homeotic arabidopsis gene SEEDSTICK. *Plant Cell* **17**: 722-  
26 729
- 27 Kurihara D, Mizuta Y, Sato Y, and Higashiyama T. 2015. ClearSee: a rapid optical clearing  
28 reagent for whole-plant fluorescence imaging. *Devel* **142**: 4168-4179
- 29 Lafon-Placette C, and Kohler C. 2014. Embryo and endosperm, partners in seed  
30 development. *Curr Opin Plant Biol* **17**: 64-69
- 31 Larsson E, Vivian-Smith A, Offringa R, and Sundberg E. 2017. Auxin Homeostasis in  
32 Arabidopsis Ovules Is Anther-Dependent at Maturation and Changes Dynamically  
33 upon Fertilization. *Front Plant Sci* doi: 10.3389/fpls.2017.01735
- 34 Lau S, Slane D, Herud O, Kong J, and Jurgens G. 2012. Early embryogenesis in flowering  
35 plants: setting up the basic body pattern. *Annu Rev Plant Biol* **63**: 483-506
- 36 Lepiniec L, Devic M, Roscoe TJ, Bouyer D, Zhou DX, Boulard C, Baud S, and Dubreucq B.  
37 2018. Molecular and epigenetic regulations and functions of the LAFL transcriptional  
38 regulators that control seed development. *Plant Reprod* **31**: 291-307
- 39 Liu J, Deng S, Wang H, Ye J, Wu HW, Sun HX, and Chua NH. 2016. CURLY LEAF Regulates  
40 Gene Sets Coordinating Seed Size and Lipid Biosynthesis. *Plant Physiol* **171**: 424-436
- 41 Lotan T, Ohto M, Yee KM, West MAL, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg  
42 RB, and Harada JJ. 1998. Arabidopsis LEAFY COTYLEDON1 is sufficient to induce  
43 embryo development in vegetative cells. *Cell* **93**: 1195-1205
- 44 Lu QS, dela Paz J, Pathmanathan A, Chiu RS, Tsai AYL, and Gazzarrini S. 2010. The C-  
45 terminal domain of FUSCA3 negatively regulates mRNA and protein levels, and  
46 mediates sensitivity to the hormones abscisic acid and gibberellic acid in Arabidopsis.  
47 *Plant J* **64**: 100-113
- 48 Lumba S, Tsuchiya Y, Delmas F, Hezky J, Provart NJ, Shi Lu Q, McCourt P, and Gazzarrini S.  
49 2012. The embryonic leaf identity gene FUSCA3 regulates vegetative phase transitions

- 1 by negatively modulating ethylene-regulated gene expression in Arabidopsis. *BMC Biol*  
2 doi: 10.1186/1741-7007-10-8
- 3 Luo M, Bilodeau P, Dennis ES, Peacock WJ, and Chaudhury A. 2000. Expression and parent-  
4 of-origin effects for FIS2, MEA, and FIE in the endosperm and embryo of developing  
5 Arabidopsis seeds. *Proc Natl Acad Sci USA* **97**: 10637-10642
- 6 Makarevich G, Leroy O, Akinci U, Schubert D, Clarenz O, Goodrich J, Grossniklaus U, and  
7 Kohler C. 2006. Different Polycomb group complexes regulate common target genes in  
8 Arabidopsis. *EMBO Rep* **7**: 947-952
- 9 Meister RJ, Williams LA, Monfared MM, Gallagher TL, Kraft EA, Nelson CG, and Gasser CS.  
10 2004. Definition and interactions of a positive regulatory element of the Arabidopsis  
11 INNER NO OUTER promoter. *Plant J* **37**: 426-438
- 12 Mitsuda N, Ikeda M, Takada S, Takiguchi Y, Kondou Y, Yoshizumi T, Fujita M, Shinozaki K,  
13 Matsui M, and Ohme-Takagi M. 2010. Efficient Yeast One-/Two-Hybrid Screening  
14 Using a Library Composed Only of Transcription Factors in Arabidopsis thaliana. *Plant*  
15 *Cell Physiol* **51**: 2145-2151
- 16 Monfared MM, Simon MK, Meister RJ, Roig-Villanova I, Kooiker M, Colombo L, Fletcher JC,  
17 and Gasser CS. 2011. Overlapping and antagonistic activities of BASIC  
18 PENTACYSTEINE genes affect a range of developmental processes in Arabidopsis.  
19 *Plant J* **66**: 1020-1031
- 20 Mozgova I, Köhler C, Hennig L. 2015. Keeping the gate closed: functions of the polycomb  
21 repressive complex PRC2 in development. *Plant J.* **83**: 121-32.
- 22 Mozgova I, Hennig L. 2015. The polycomb group protein regulatory network. *Annu Rev Plant*  
23 *Biol.* **66**: 269-96.
- 24 Robert HS. 2019. Molecular Communication for Coordinated Seed and Fruit Development:  
25 What Can We Learn from Auxin and Sugars? *Int J Mol Sci* doi: 10.3390/ijms20040936
- 26 Robert HS, Park C, Gutierrez CL, Wojcikowska B, Pencik A, Novak O, Chen JY, Grunewald  
27 W, Dresselhaus T, Friml J, et al. 2018. Maternal auxin supply contributes to early  
28 embryo patterning in Arabidopsis. *Nat Plants* **4**: 548-553
- 29 Roscoe TJ, Vaissayre V, Paszkiewicz G, Clavijo F, Kelemen Z, Michaud C, Lepiniec L,  
30 Dubreucq B, Zhou DX, and Devic M. 2019. Regulation of FUSCA3 expression during  
31 seed development in Arabidopsis. *Plant Cell Physiol* **60**: 476-487
- 32 Roszak P, and Kohler C. 2011. Polycomb group proteins are required to couple seed coat  
33 initiation to fertilization. *Proc Natl Acad Sci USA* **108**: 20826-20831
- 34 Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D,  
35 and Lohmann JU. 2005. A gene expression map of Arabidopsis thaliana development.  
36 *Nat Genet* **37**: 501-506
- 37 Schneitz K, Hülskamp M, and Pruitt RE. 1995. Wild-type ovule development in Arabidopsis  
38 thaliana: a light microscope study of cleared whole-mount tissue. *Plant J* **7**: 731-749
- 39 Simonini S, and Kater MM. 2014. Class I BASIC PENTACYSTEINE factors regulate  
40 HOMEBOX genes involved in meristem size maintenance. *J Exp Bot* **65**: 1455-1465
- 41 Simonini S, Roig-Villanova I, Gregis V, Colombo B, Colombo L, and Kater MM. 2012. Basic  
42 pentacysteine proteins mediate MADS domain complex binding to the DNA for tissue-  
43 specific expression of target genes in Arabidopsis. *Plant Cell* **24**: 4163-4172
- 44 Sreenivasulu N, and Wobus U. 2013. Seed-Development Programs: A Systems Biology-  
45 Based Comparison Between Dicots and Monocots. *Annu Rev Plant Biol* **64**: 189-217
- 46 Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB, and Harada  
47 JJ. 2001. LEAFY COTYLEDON2 encodes a B3 domain transcription factor that  
48 induces embryo development. *Proc Natl Acad Sci USA* **98**: 11806-11811
- 49 Suh MC, Samuels AL, Jetter R, Kunst L, Pollard M, Ohlrogge J, and Beisson F. 2005.



- 1 Cuticular lipid composition, surface structure, and gene expression in Arabidopsis stem  
2 epidermis. *Plant Physiol* **139**: 1649-1665
- 3 Swanson R, Clark T, and Preuss DJSPR. 2005. Expression profiling of Arabidopsis stigma  
4 tissue identifies stigma-specific genes. *Sex Plant Reprod* **18**: 163-171
- 5 Tsai AY, Gazzarrini S. 2012. AKIN10 and FUSCA3 interact to control lateral organ  
6 development and phase transitions in Arabidopsis. *The Plant Journal* **69**: 809–21.
- 7 Tsuchiya Y, Nambara E, Naito S, and McCourt P. 2004. The FUS3 transcription factor  
8 functions through the epidermal regulator TTG1 during embryogenesis in Arabidopsis.  
9 *Plant J* **37**: 73-81
- 10 Tsuda K, Abraham-Juarez MJ, Maeno A, Dong Z, Aromdee D, Meeley R, Shiroishi T,  
11 Nonomura KI, and Hake S. 2017. KNOTTED1 Cofactors, BLH12 and BLH14, Regulate  
12 Internode Patterning and Vein Anastomosis in Maize. *Plant Cell* **29**: 1105-1118
- 13 Vashisht D, and Nodine MD. 2014. MicroRNA functions in plant embryos. *Biochem Soc Trans*  
14 **42**: 352-357
- 15 Zhou Y, Wang Y, Krause K, Yang T, Dongus JA, Zhang Y, Turck F. 2018. Telobox motifs  
16 recruit CLF/SWN-PRC2 for H3K27me3 deposition via TRB factors in Arabidopsis. *Nat*  
17 *Genet.* 50:638-644
- 18 Wang D, Tyson MD, Jackson SS, and Yadegari R. 2006. Partially redundant functions of two  
19 SET-domain polycomb-group proteins in controlling initiation of seed development in  
20 Arabidopsis. *Proc Natl Acad Sci USA* **103**: 13244-13249
- 21 Wang G, and Kohler C. 2017. Epigenetic processes in flowering plant reproduction. *J Exp Bot*  
22 **68**: 797-807
- 23 Wu J, Wu W, Liang J, Jin Y, Gazzarrini S, He J, and Yi M. 2019. GhTCP19 Transcription  
24 Factor Regulates Corm Dormancy Release by Repressing GhNCED Expression in  
25 *Gladiolus*. *Plant Cell Physiol* **60**: 52-62
- 26 Wu J, Jin Y, Liu C, Vonapartis E, Liang J, Wu W, Gazzarrini S, He J, and Yi M. 2018.  
27 GhNAC83 inhibits corm dormancy release by regulating ABA signaling and cytokinin  
28 biosynthesis in *Gladiolus hybridus*. *J Exp Bot* **70**: 1221-1237
- 29 Xiao J, Jin R, Yu X, Shen M, Wagner JD, Pai A, Song C, Zhuang M, Klasfeld S, He C, et al.  
30 2017. Cis and trans determinants of epigenetic silencing by Polycomb repressive  
31 complex 2 in Arabidopsis. *Nat Genet* **49**: 1546
- 32

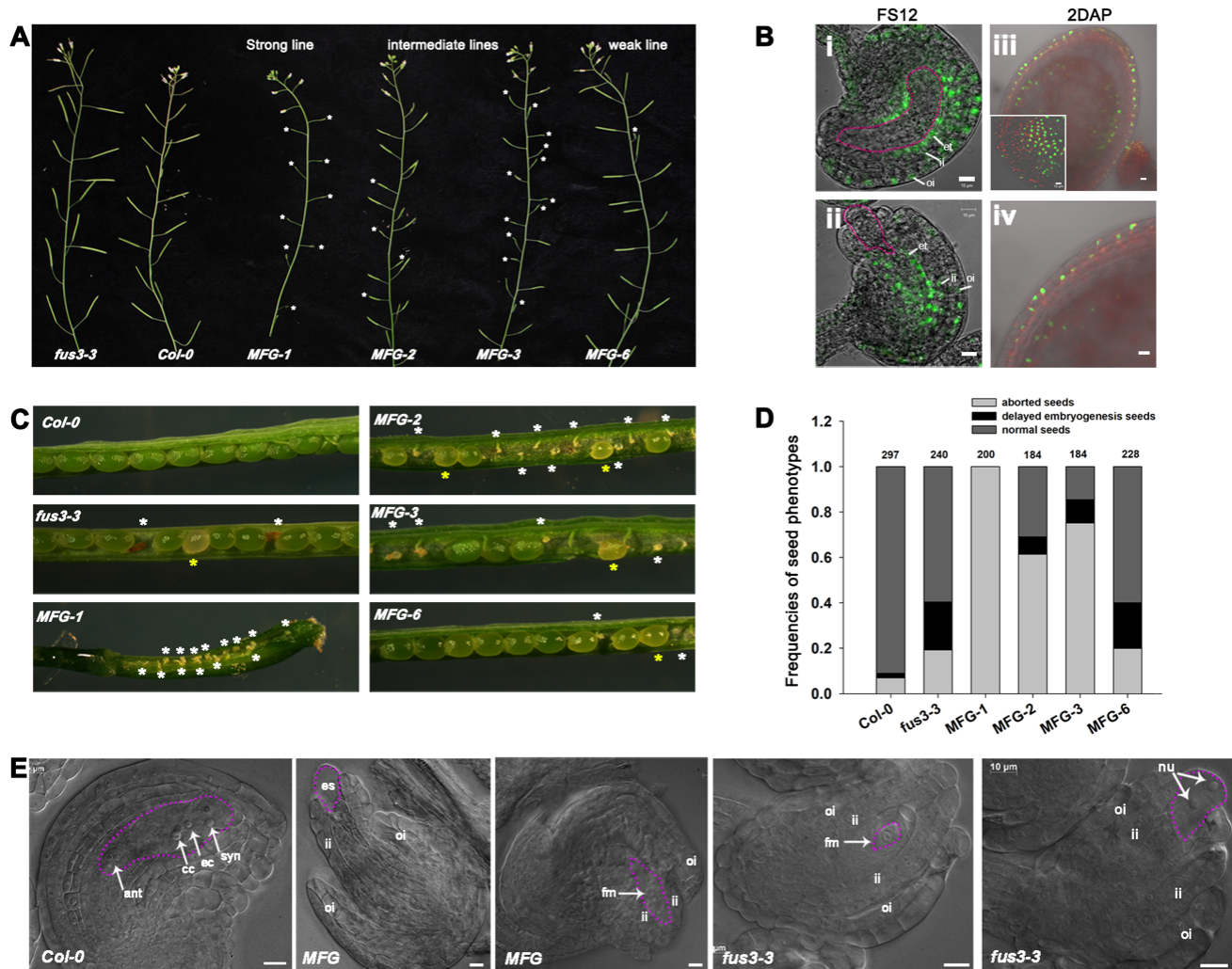


1  
2

3 **Figure 1. FUS3 localization in developing ovules and during early stages of seed**  
4 **development.**

5 Confocal images showing pFUS3:FUS3ΔC-GFP localization in Arabidopsis. (A) Valve and (B)  
6 septum of the pistil. (C-F) Developing ovules during female megalporogenesis (C) and  
7 megagametogenesis at stages FG1-FG7 (D-F). FUS3ΔC-GFP fluorescence was localized to  
8 the nucellar epidermis (C), inner and outer integuments (C,D), funiculus, chalazal (C,F). (G-J)  
9 seeds at 6 hours to 2 days (6HAP to 2DAP) after pollination. FUS3ΔC-GFP fluorescence was  
10 localized to the seed coat, chalaza and funiculus (G-J). (K) Suspensor and 16-cell stage  
11 embryo proper. (L) 32-cell stage embryo proper. chl: chalaza; es, embryo sac; fun, funiculus;  
12 ii: inner integument; megaspore mother cell; ne, nucellar epidermis; nu: nucellus; oi: outer  
13 integument; sept, septum. Red, autofluorescence from chlorophyll. Purple dashed lines  
14 represent the outline of embryo sac. Scale bars, 10μm.

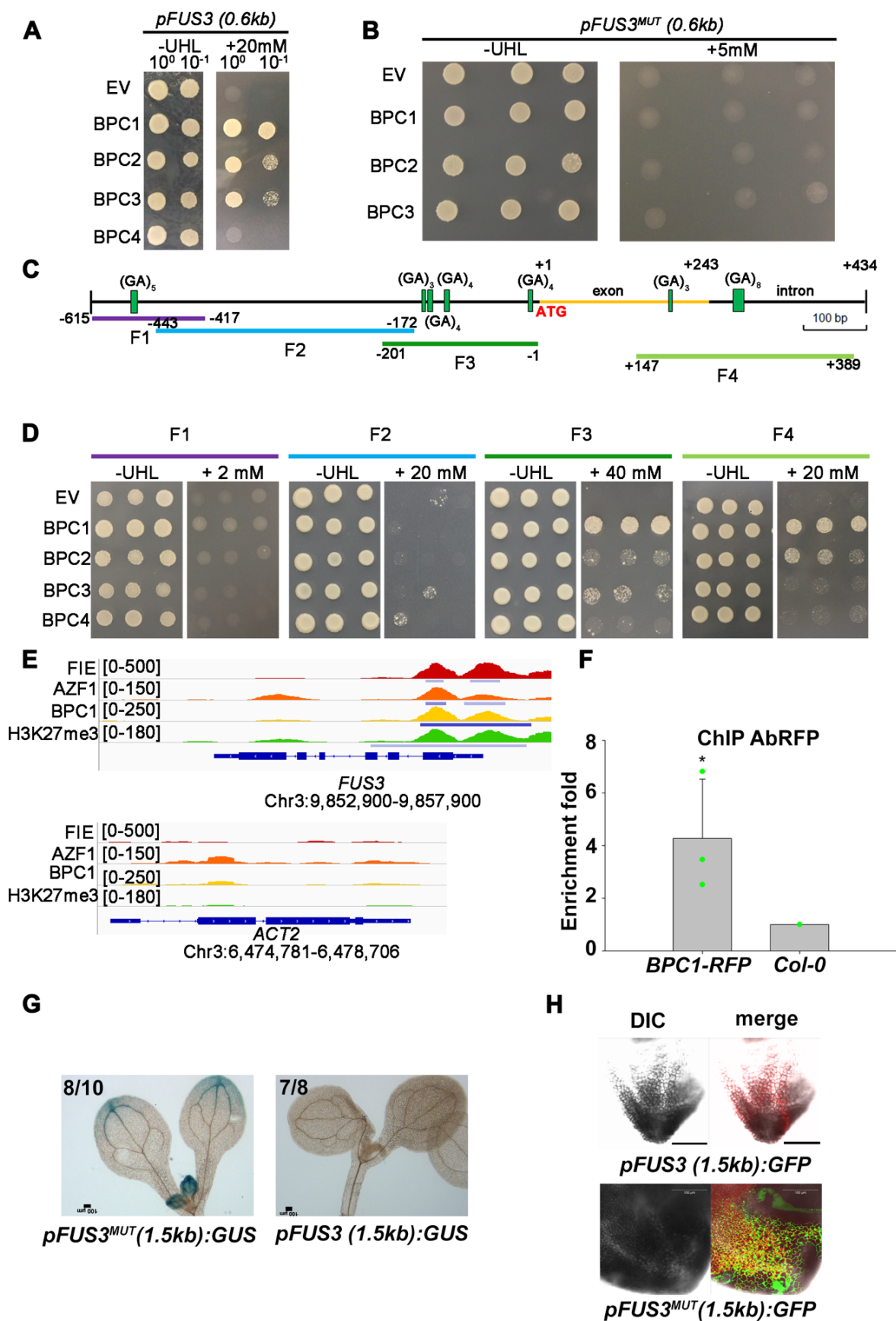
15



1  
2 **Figure 2. *FUS3* is required for ovule development.**

3 **A**, Aborted silique (asterisks) in *fus3-3 pML1:FUS3-GFP* (*MFG*) overexpression lines. **B**,  
4 *pML1:FUS3-GFP* localization to to the integuments and endothelium of ovules at flower stage  
5 12 (FS12), and outer layer of the seed coat and endosperm (inset) of 2DAP seeds. (i)  
6 developed ovule; (ii) aborted embryo sac; (iii, iv). outer layer of the seed coat and the  
7 endosperm (inset) in 2DAP seeds Bar, 10 $\mu$ M. **C**, Aborted seeds (white asterisk) and delayed  
8 embryogenesis (yellow asterisk) in *MFG* and *fus3-3* siliques. **D**, The distribution of seeds in  
9 peeled, half sides siliques of WT, *MFG* and *fus3-3* (n= ten siliques/genotype). **E**, DIC images  
10 of WT, *MFG* and *fus3-3* FS12 ovules. Pink dashed lines outline the embryo sac. Ant: anti-  
11 antipodals; ec: egg cell; es: embryo sac; et: endothelium; fm: functional megaspore; ii, inner  
12 integument; nu: nuclei; oi, outer integument; syn: synergid cell nuclei. Bars represent 10 $\mu$ m.



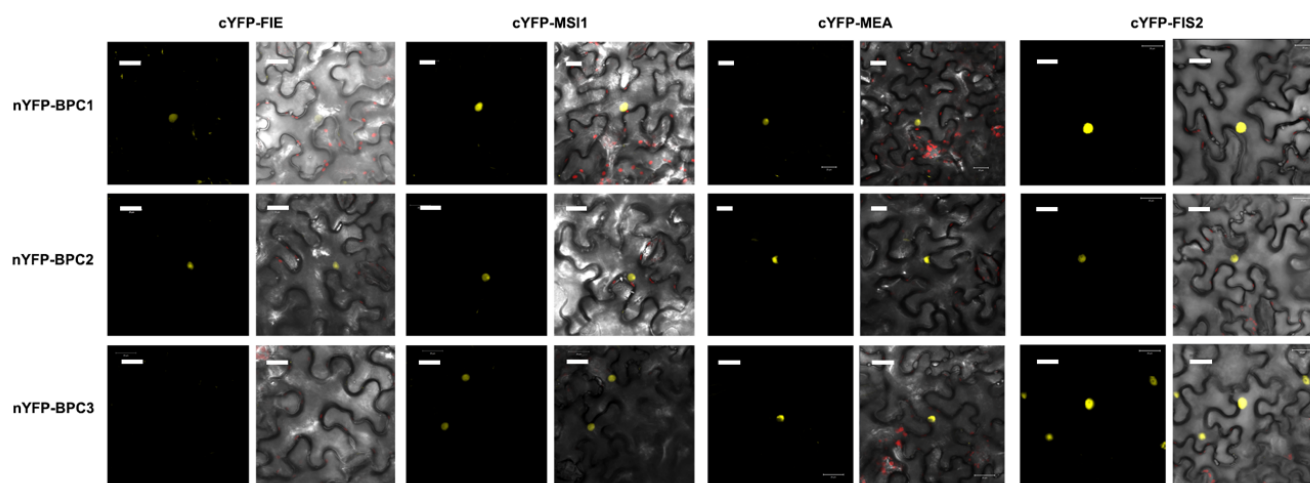




1 **Figure 3. Class I BPCs bind to the *FUS3* genomic region proximal to the transcription**  
2 **start site.**

3 **A**, BPC1/2/3 bind to a *FUS3* genomic region of 615bp upstream of the translation start  
4 [*pFUS3*(0.6 kb); -615 to +1 base pairs]. **B**, BPC1/2/3 do not bind the *FUS3* genomic  
5 sequence carrying mutations in (GA/CT)<sub>n</sub> motifs [*pFUS3<sup>MUT</sup>*(0.6 kb)]. Colonies in **A** and **B**  
6 were selected on -ura-his-leu medium (-UHL) with or without 5 or 20mM 3-AT. **C**, Distribution  
7 of (GA/CT)<sub>n</sub> motifs in *FUS3* genomic sequence (-615 to +434). **D**. Binding specificity of  
8 BPC1/2/3 to truncated *FUS3* genomic sequences shown in C (F1 to F4). **E**, Bowser view of  
9 chromatin occupancy of FIE, BPC1, AZF1 and H3K27me3 at *FUS3* and *ACT2* (negative  
10 control) in 30-h-old seedlings using ChIP-seq data from Xiao et al. (2017). Numbers indicate  
11 peak Significant peaks ( $Q < 10^{-10}$ ) according to MACS2 are marked by horizontal bars. **F**.  
12 Real-time PCR analysis of ChIP assay using chromatin extracted from *35S:BPC1-RFP* and  
13 *Col-0* (negative control) inflorescences and primers for the F3 region of *pFUS3*. Antibodies  
14 against the RFP tag were used in the IP. Error bars represent the propagated error value  
15 using three biological replicates (\*:  $p < 0.05$ ; student t-test). **G**, *pFUS3*(1.5kb):*GUS* and  
16 *pFUS3<sup>MUT</sup>*(1.5kb):*GUS* stain in 10-days-old seedlings; numbers refer to the number of  
17 transgenic lines displaying the same GUS stain pattern as shown in **G**. **H**, *pFUS3*(1.5kb):  
18 *GFP* and *pFUS3<sup>MUT</sup>*(1.5kb):*GFP* fluorescence in the leaf tip of 15-days-old seedlings.

19  
20  
21

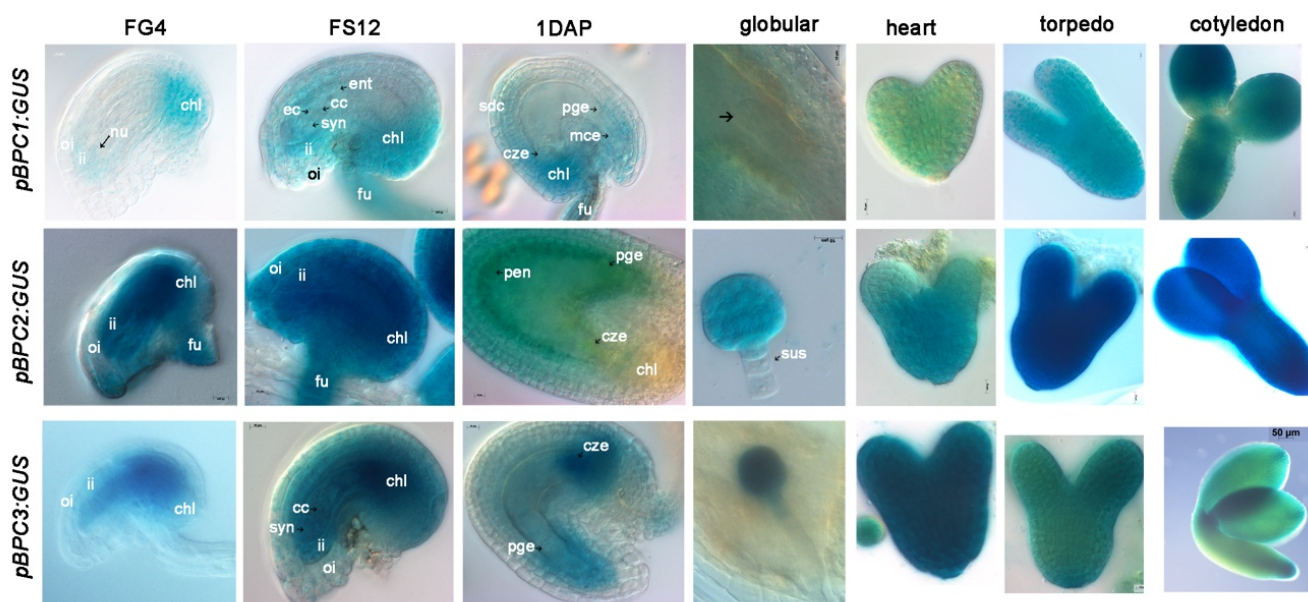


1  
2

3 **Figure 4. Class I BPC family members intact with FIS-PRC2 complex.**

4 The interaction between Class I BPC family members and FIS complex in *N. benthamiana* by  
5 Bimolecular Fluorescence Complementation (BiFC). Lack of interaction between FUS3 and  
6 BPCs or FIS-PRC2 in BiFC assays is shown as the negative control (Supplemental Figure 5).

7  
8

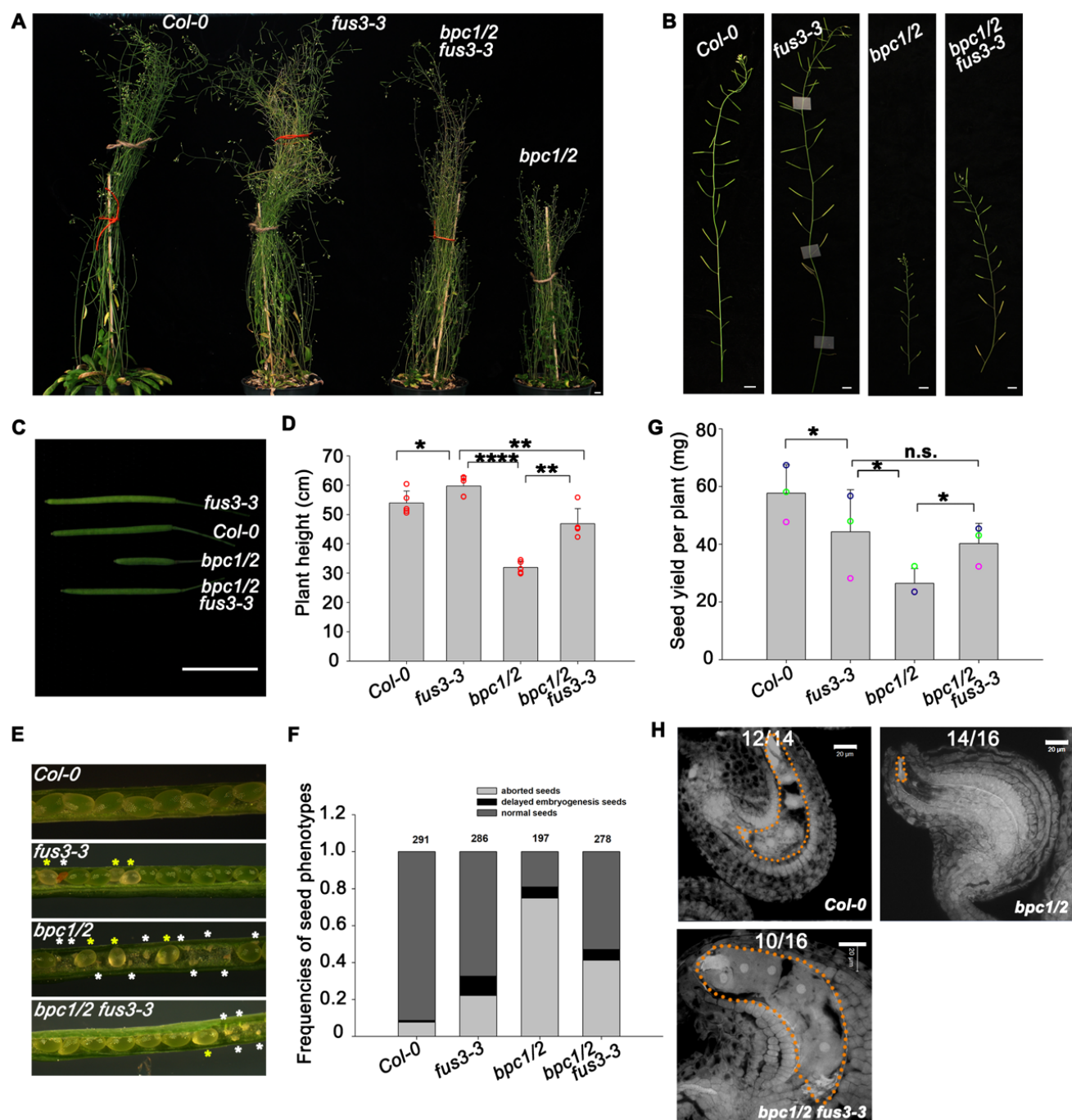


1  
2

3 **Figure 5. Expression patterns of Class I BPCs in ovules and embryos.**

4 Class I BPC1s expression patterns in ovules before pollination at flower stages FG4 and  
5 FS12; in seeds at 1 day after pollination (DAP); and in embryos at globular, heart, torpedo  
6 and cotyledon stages. Ant: antipodals; cc: central cell; chl: chalaza; cze: chalazal endosperm;  
7 ec: egg cell; fu: funiculus; ii: inner integuments; mce: micropilar endosperm; oi: outer  
8 integuments; pen: peripheral endosperm; pge: pre-globular embryo; sdc: seed coat; sus:  
9 suspensor; syn: synergids cell.

10  
11  
12



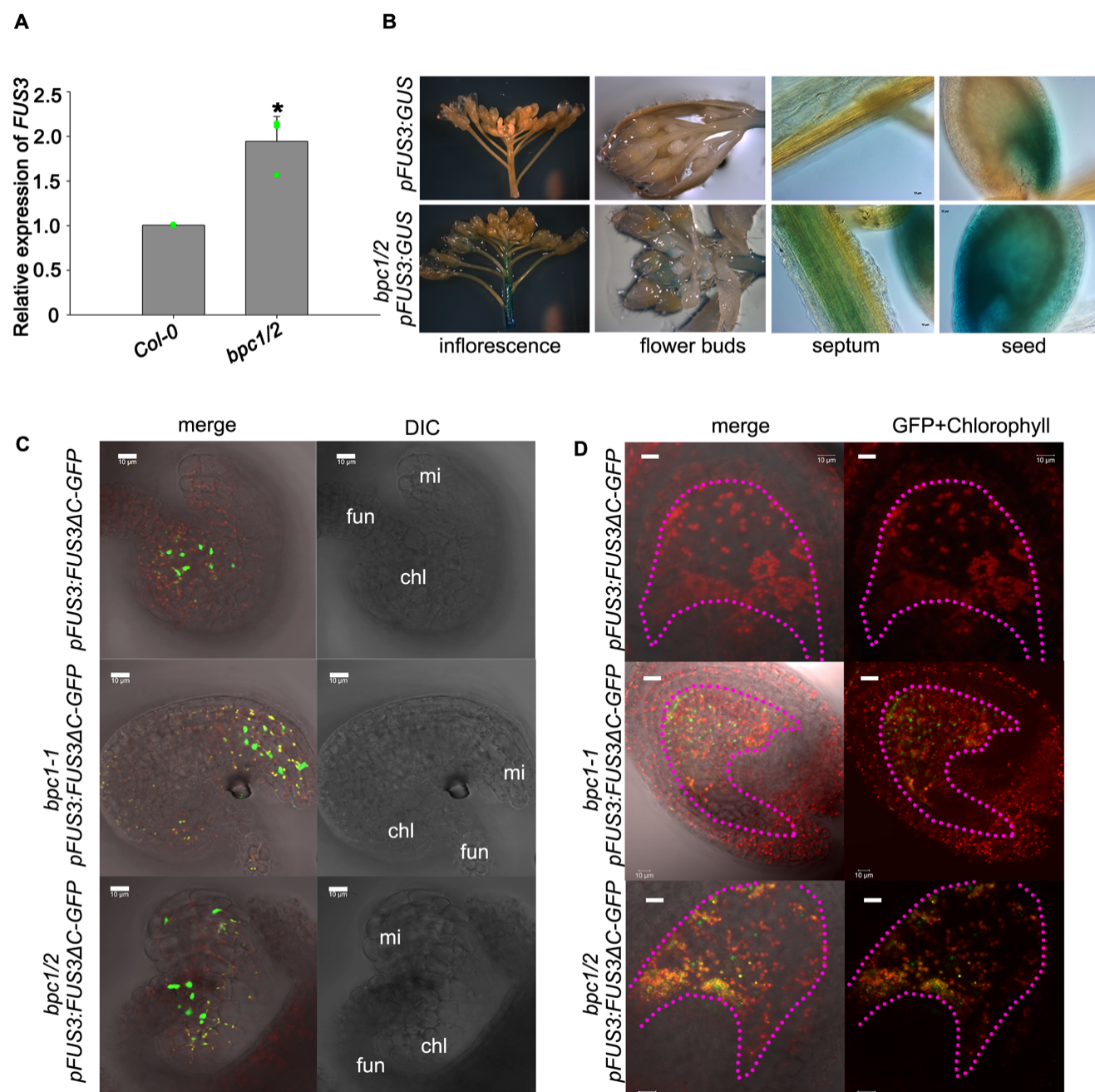
1  
2  
3  
4  
5  
6  
7  
8  
9

**Figure 6. Partial rescue of *bpc1/2* stunted growth, aborted embryos and arrested seeds in *fus3-3 bpc1/2*.**

**A**, The stunted growth of *bpc1/2* was partially rescued in *bpc1/2 fus3-3*. **B**, **C**, *bpc1/2 fus3-3* partially rescues *bpc1/2* reduced silique elongation. Scale bar, 1cm. **D**, Quantification of the plant height. Five biological replicates were performed. Each replicate consisted of five plants per genotype. **E**, **F**, *fus3-3* partially rescues *bpc1/2* severe seed abortion. The white asterisk in **E** represents aborted seed, while the yellow asterisk represents the delayed embryogenesis

1 seeds. **F**, Frequencies of seed phenotypes in *bpc1/2 fus3-3* mutants. The total number of  
2 sees was calculated in 10 peeled siliques (half side). Three biological repeats were performed  
3 with similar results and one is shown (see also Supplemental Figure 10A). **G**, The seed yield  
4 of *bpc* mutants. Error bars represent the SD of three biological replicates (n=5). n.s.: no  
5 significant difference. (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*\*  $p < 0.0001$ ); student t-test was used. **H**, *fus3-3*  
6 partially rescues the embryo sac defects of *bpc1/2*. The image was taken at 1DAP. Scale bar,  
7 20 $\mu$ m. Numbers refer to the number of embryos displaying the phenotype shown.  
8



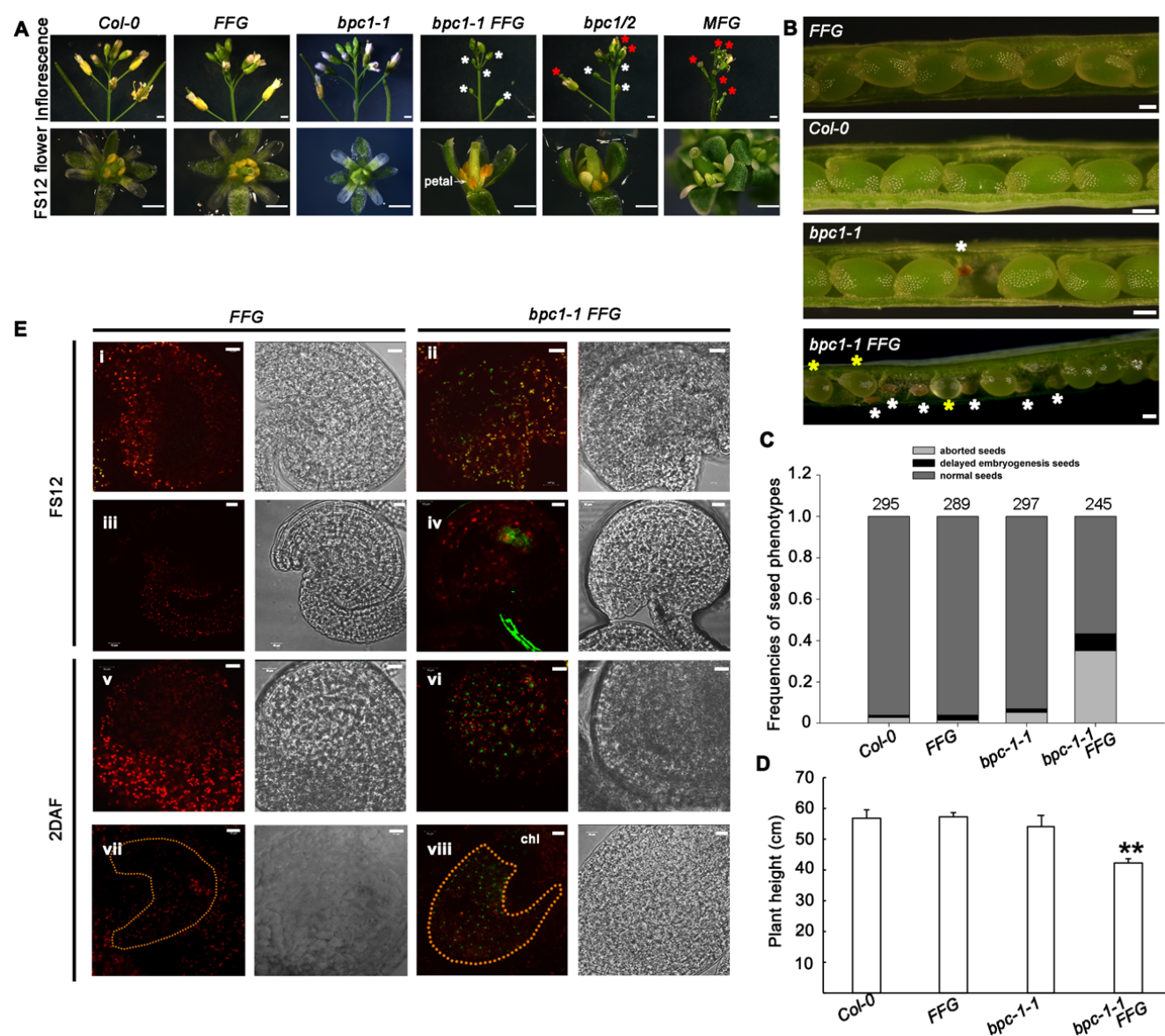


1  
2

3 **Figure 7. BPC1/2 negatively regulate *FUS3* expression in reproductive organs and**  
4 **seeds.**

5 **A**, qRT-PCR showing increased *FUS3* transcript level in *bpc1/2* inflorescence stem. Error  
6 bars represent the SD of three biological replicates (\*  $p < 0.05$ ; student t-test). **B**, GUS staining  
7 in the inflorescence stem, flower buds, septum and seed (2DAF) of *pFUS3:GUS* and *bpc1/2*  
8 *pFUS3:GUS* lines. The GUS staining was enhanced in the inflorescence stem and septum,  
9 while ectopically expressed in the endosperm of *bpc1/2*. **C**, **D** *pFUS3:FUS3ΔC-GFP* and *bpc1*  
10 *pFUS3:FUS3ΔC-GFP* ovules were images before (C) and two days after (D) fertilization by

1 confocal microscopy. FUS3 $\Delta$ C-GFP was localized to the chalaza region of developing WT  
2 ovules before fertilization, while ectopically localized to the integuments at the micropilar  
3 region of *bpc1-1* and of *bpc1/2* ovules (FS12) and to the endosperm of 2DAF *bpc1-1* seeds.  
4



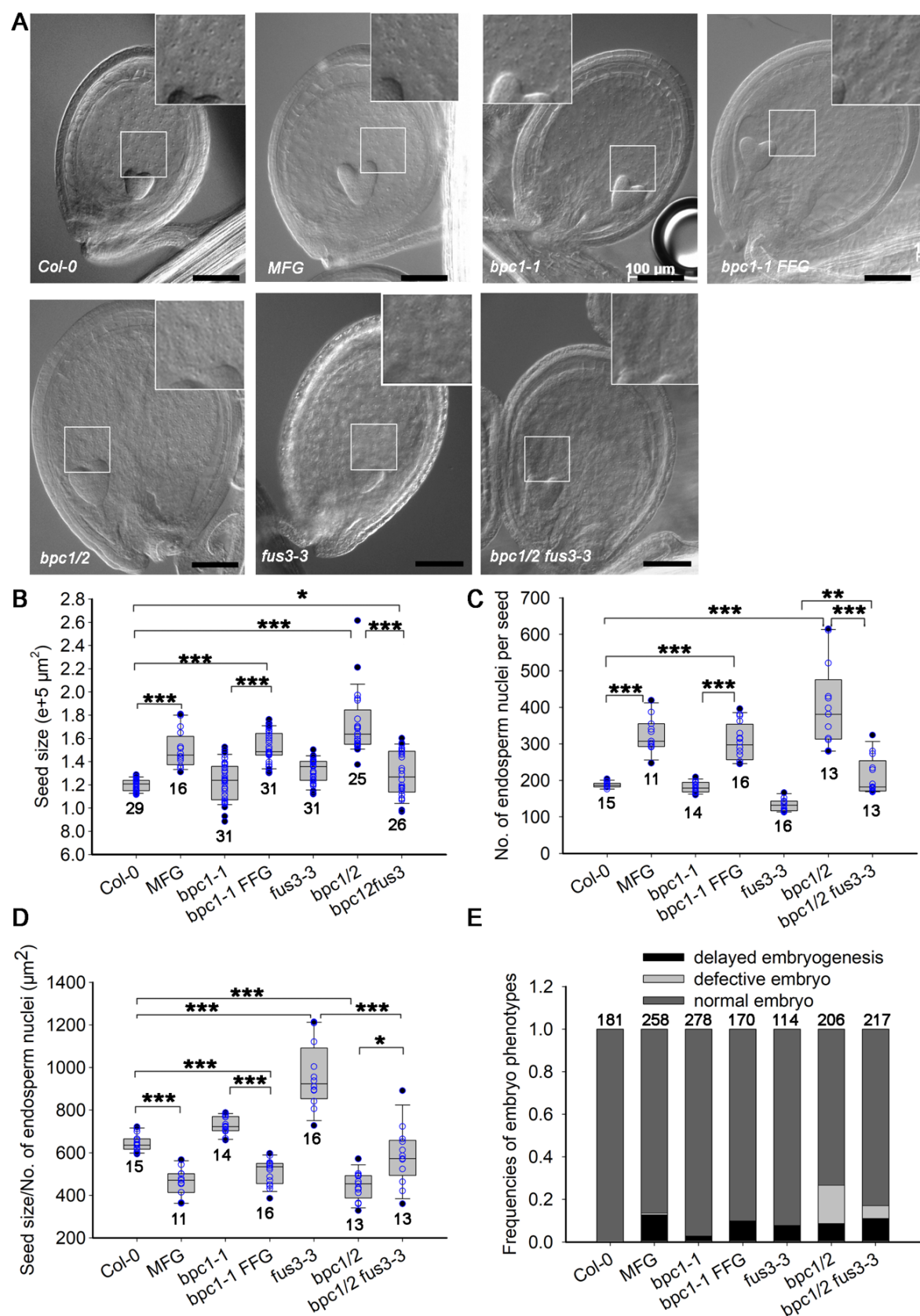
1  
2

3 **Figure 8. Ectopic *FUS3* expression negatively impacts reproductive organ development.**

4 **A**, Introduction of a *pFUS3:FUS3-GFP* (*FFG*) transgene in *bpc1-1* mutant results in arrested  
5 flower buds that never open (white asterisk), similar to *bpc1/2* double mutant. The arrested  
6 flower buds in *bpc1-1 FFG* have underdeveloped petals, non-elongated filaments and aborted  
7 anthers, similar to *bpc1/2*. *pM1:FUS3-GFP* (*MFE*) also show shorter filaments and  
8 underdeveloped anthers, but flower buds open prematurely. **B**, *bpc1-1 FFG* mutant caused  
9 aborted seeds and delayed embryogenesis. Aborted seeds (white asterisk) and delayed  
10 embryogenesis (yellow asterisk) are shown. **C**, Frequencies of seed phenotypes. The total  
11 number of seeds was calculated in ten siliques (half side). Three biological repeats were  
12 performed, and one representative is shown (see also Supplemental Figure 10B). **D**, *bpc1-1*

1 *FFG* plants display stunted growth. The error bar represents SD of three biological replicates  
2 (n=5). (\*\*: p<0.01; student t-test was used). **E**, *FUS3* is mis-expressed in the integument (**ii**)  
3 and increased in the funiculus (**iv**) of *bpc1-1* at FS12. Two days after fertilization (2DAF),  
4 *FUS3*-GFP is increased in the seed coat (**vi**) and mis-expressed in the endosperm (**viii**) at 2  
5 DAF.  
6



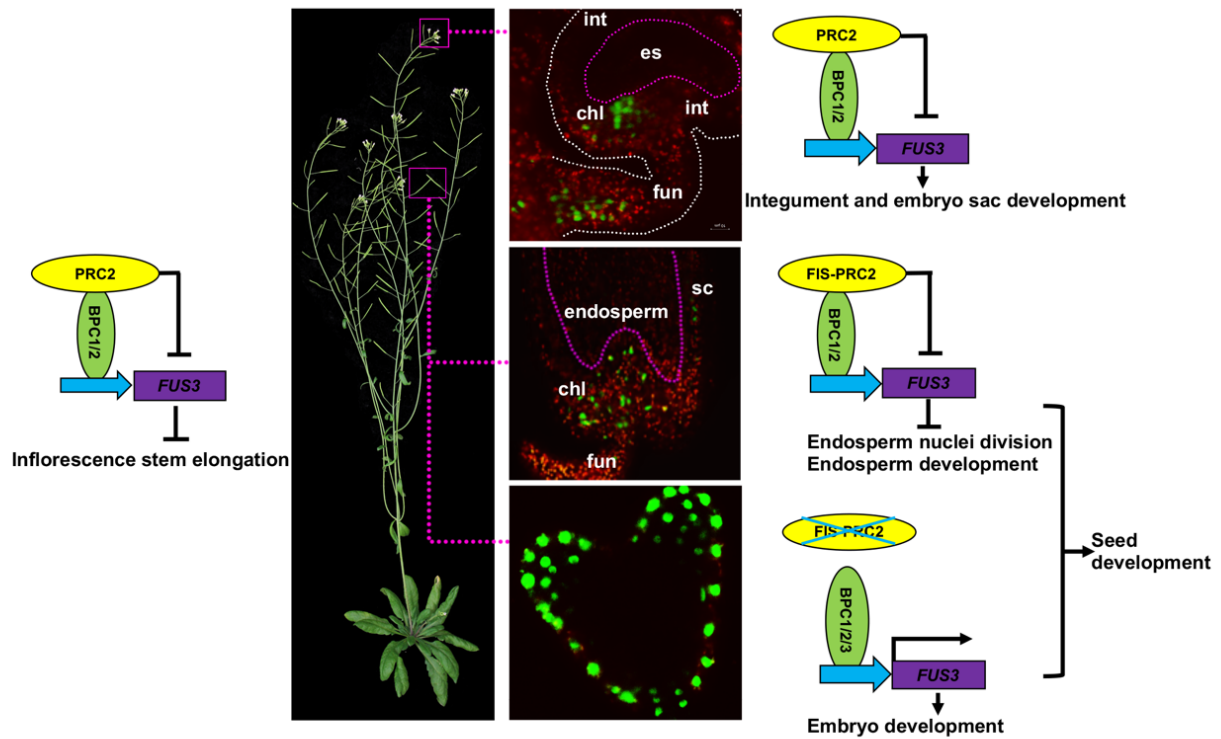


1  
2 **Figure 9. BPC1/2 negatively regulate endosperm nuclei proliferation and seed size by**  
3 **repressing *FUS3*.**



1 **A**, Whole-mount clearing, **B**, seed size, **C**, quantification of endosperm nuclei, **D** seed size  
2 versus number of endosperm nuclei and **E**, frequencies of embryo phenotypes of wild-type  
3 (*Col-0*), *pML1:FUS3-GFP (MFG)*, *bpc1-1*, *bpc1-1FFG*, *bpc1/2*, *fus3-3* and *bpc1/2 fus3-3*  
4 seeds at 6DAP. Over-proliferation of endosperm nuclei and larger seed size in the *bpc1/2*,  
5 *bpc1-1FFG* and *MFG* lines, and partial rescue in *bpc1/2 fus3-3*. Images were taken 6DAP.  
6 Scale bar, 100µm. **B**, **C** Ectopic expression of *FUS3* in *MFG*, *bpc1-1 FFG*, and *bpc1/2* leads  
7 to enlarged seed size **B**), increased endosperm nuclei proliferation **C**) and density **D**), which  
8 is partially rescued in *bpc1/2 fus3-3*. **E**, Ectopic expression of *FUS3* in *bpc1/2*, *bpc1-1FFG*  
9 and *MFG* results in delayed embryogenesis; *bpc1/2* defective embryos are partially rescued  
10 by *fus3-3*. (\* p<0.05; \*\* p<0.01; \*\*\*\* p<0.0001, student t-test was used).

11  
12



1  
2

3 **Figure 10. Spatiotemporal restriction of *FUS3* expression by *BPC1/2* during**  
 4 **reproductive and seed development.** Model depicting spatiotemporal expression of *FUS3*  
 5 and its role in the regulation of vegetative-to-reproductive and gametophytic-to-sporophytic  
 6 phase transitions. During the vegetative-to-reproductive phase change, *FUS3* is repressed by  
 7 *BPC1/2* in the inflorescence stem to allow stem elongation. During ovule development, *FUS3*  
 8 becomes restricted to the funiculus and chalaza through *BPC1/2*-mediated repression in the  
 9 integuments; this is required to promote integument and embryo sac development. After  
 10 fertilization, *FUS3* is localized to the embryo, seed coat, chalaza and funiculus, but is  
 11 repressed in the endosperm by *BPC1/2* to decrease endosperm nuclei division and promote  
 12 embryo development. In the stem, *BPC1/2*-mediated *FUS3* repression may be orchestrated  
 13 by EMF-PRC2, which interacts with *BPC1/2* and represses *FUS3* postembryonically (Liu et al.,  
 14 2016; Xiao et al., 2017). *FUS3* repression in the integuments may require sporophytic  
 15 VRN/EMF PRC2. After fertilization, FIS-PRC2 represses *FUS3* in the endosperm (Makarevich  
 16 et al., 2006).

17