Supporting Information

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SI Materials and Methods

Reverse Transcription PCR (RT-PCR) Analysis. Total RNA fractions were prepared from seedlings, siliques or seed tissues (whole seeds, embryos or endosperms) using the Wizard SV total RNA Isolation kit (Promega) or the RNeasy Micro Kit (QIAGEN). The dissection of embryos (*em*) and endosperms (*es*) from seeds at 7 DAP was performed as described by Kinoshita et al. (1). First strand cDNA was synthesized using the SuperScriptII First-Strand Synthesis system for RT-PCR (Invitrogen Japan). PCR was performed using the primer sets shown in Table S4.

Complementation Analysis. The plasmid pDM121 was constructed by introducing the reading frame cassette A from the Gateway Vector Conversion System (Invitrogen Japan), the *BiP1* cDNA (produced by RT-PCR), and the *NOS* terminator sequence into pCambia1300 (CAMBIA). The promoter regions of *BiP1*, *DD29*, *DD31*, *DD45*, *DD65*, and *FIE* were amplified from *Arabidopsis* genomic DNA by PCR using the primers shown in Table S4. The PCR products were cloned into the pENTR/D-TOPO vector (Invitrogen) and then inserted upstream of the *BiP1* cDNA in pDM121 using LR clonase II (Invitrogen). The resulting plasmids were introduced into the *b1*/+ *b2*/*b2* line using the floral dip method (2) and *Agrobacterium tumefaciens* strain GV3101. Transformants were selected on Murashige-Skoog plates containing 50 μg/mL hygromycin. Pistils of each transgenic line were pollinated with wild-type pollen, and ratios of viable and aborted seeds were scored.

***GUS* Expression Analysis.** The cloning of the promoter region of *BiP1* is described above. The promoter regions of the *BiP2* and *BiP3* genes were amplified by PCR from *Arabidopsis* genomic DNA using primers shown in Table S4. The PCR products were cloned into the pENTR/D-TOPO vector (Invitrogen Japan). Each promoter was inserted upstream of the *GUS* reporter gene in pGWB233 (3) (a gift from Tsuyoshi Nakagawa, Shimane University, Matsue, Japan) using LR clonase II (Invitrogen Japan). The resulting plasmids were introduced into the *A. tumefaciens* strain GV3101 by electroporation. *Arabidopsis* was transformed as described above. GUS activity was analyzed as follows. Pollinated pistils or siliques were fixed with ice-cold 90% (vol/vol) acetone for 20 min, washed once with staining buffer (25 mM sodium phosphate, pH 7.2, 20 mM $KFe(CN)_2$, 20 mM $KFe(CN)_3$ and 2% (vol/vol) Triton X-100) and incubated overnight in staining buffer containing 2 mM 5-bromo-4-chloro-3-indolyl-D-glucuronide at 37 °C in the dark. The GUS-stained materials were dehydrated with an ethanol series, postfixed in 50% (vol/vol) ethanol, 10% (vol/vol) acetic acid, and 5% (vol/vol) formaldehyde, and then cleared with 70% ethanol. The samples were rehydrated and immersed in 70% (wt/wt) chloral hydrate, 8.8% (wt/wt) glycerol. Images of the pistils were captured using an SZX7 stereo microscope (Olympus) equipped with a Pro600ES CCD camera (Pixera), and the seeds were photographed using a BX51 microscope (Olympus) equipped with a DP70 cooled CCD camera (Olympus).

Pollen Analysis. The Alexander staining of pollen grains and pollen germination assays were performed as described previously (4, 5). For the staining of nuclei in pollen grains, mature pollen grains were stained with 1 μg/mL 4′,6-diamidino-2-phenyl indole (DAPI) solution for 1 min and then washed in sterilized water. Images of 0.5 μm sections were captured using a CSU10 confocal laser scanning system (Yokogawa Electric) mounted on a BX60 microscope (Olympus) combined with 405 nm laser irradiation.

1. Kinoshita T, et al. (2004) One-way control of *FWA* imprinting in *Arabidopsis* endosperm by DNA methylation. *Science* 303:521–523.
2. Clough SJ, Bent AF (1998) Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16:735–743.
3. Nakagawa T, et al. (2007) Improved Gateway binary vectors: High-performance vectors for creation of fusion constructs in transgenic analysis of plants. *Biosci Biotechnol Biochem* 71:2095–2100.
4. Alexander M (1969) Differential staining of aborted and nonaborted pollen. *Stain Technol* 44:117–122.
5. Bovid LC, McCormick S (2007) Temperature as a determinant factor for increased and reproducible in vitro pollen germination in *Arabidopsis thaliana*. *Plant J* 52: 570–582.

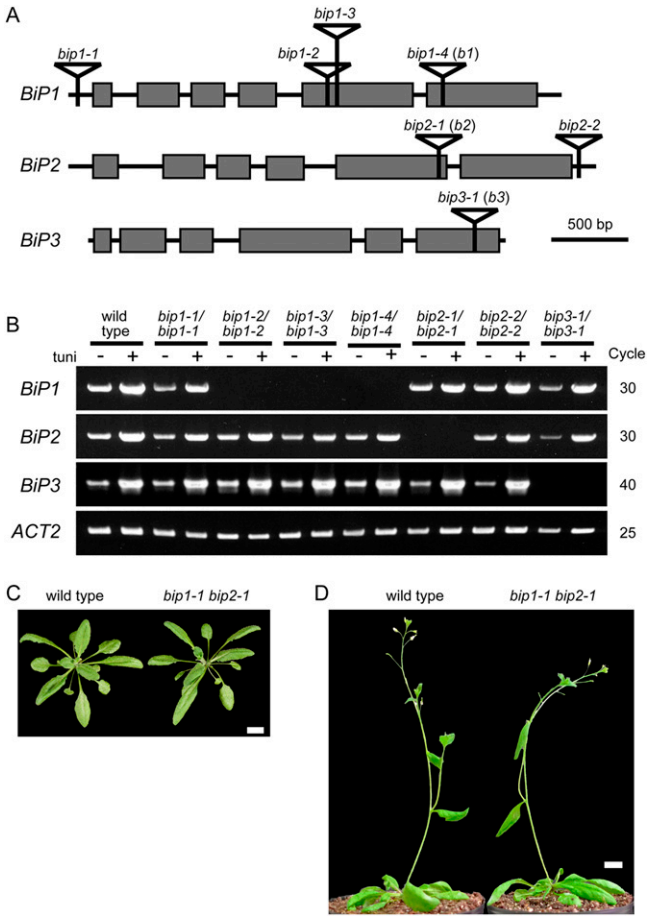

Fig. S1. T-DNA insertion mutants of *Arabidopsis BiP* genes. (*A*) Gene structures of *BiP1*, *BiP2*, and *BiP3*. Gray boxes and lines represent exons and introns, respectively. The T-DNA insertion sites are shown by triangles. (*B*) RT-PCR analysis of *BiP* gene expression in wild-type and *bip* mutants. Total RNA was prepared from 10-day-old seedlings of wild-type and homozygous *BiP* mutants treated with (+) or without (–) 5 μg/mL tunicamycin for 5 h. RT-PCR reactions were performed, with the numbers of cycles shown on the right. (*C*) Five-week-old soil-grown wild-type and *bip1-1 bip2-1* double homozygous plants. (*D*) Six-week-old soil-grown wild-type and *bip1-1 bip2-1* double homozygous plants. (Scale bars, 1 cm.)

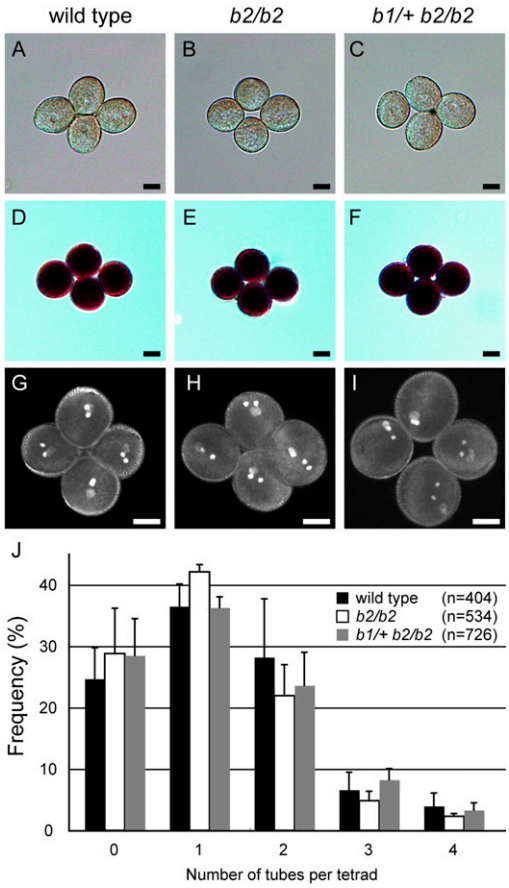

Fig. S2. The *bip1 bip2* double mutations do not affect the development or germination of pollen. (*A*–*C*) Light microscopy was used to examine the morphology of tetrads from *qrt1*/*qrt1* (*A*, wild-type), *b2*/*b2 qrt1*/*qrt1* (*B*, *b2*/*b2*), and *b1*/+ *b2*/*b2 qrt1*/*qrt1* (*C*, *b1*/+ *b2*/*b2*) plants. (*D*–*F*) Alexander staining of tetrads from wild-type (*D*), *b2*/*b2* (*E*), and *b1*/+ *b2*/*b2* (*F*) plants. (*G*–*I*) DAPI staining of tetrads from wild-type (*G*), *b2*/*b2* (*H*), and *b1*/+ *b2*/*b2* (*I*) plants. (Scale bars, 10 μm.) (*J*) Frequencies (%) of wild-type (black bars, *n* = 404), *b2*/*b2* (white bars, *n* = 534), and *b1*/+ *b2*/*b2* (gray bars, *n* = 726) tetrads with zero to four pollen tubes after in vitro pollen germination. The values represent the means of three different experiments with standard deviations. (Scale bars, 10 μm.)

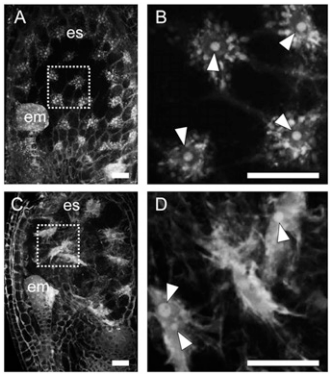

Fig. S3. Abnormal nuclear proliferation observed in the *b1 b2* seeds. The *b2* (*A* and *B*) and *b1 b2* (*C* and *D*) seeds were analyzed by CLSM at 3 DAP. (*B* and *D*) show magnifications of the regions indicated with boxes in (*A* and *C*). Arrowheads indicate endosperm nuclei. em, embryo; es, endosperm. (Scale bars, 20 μm.)

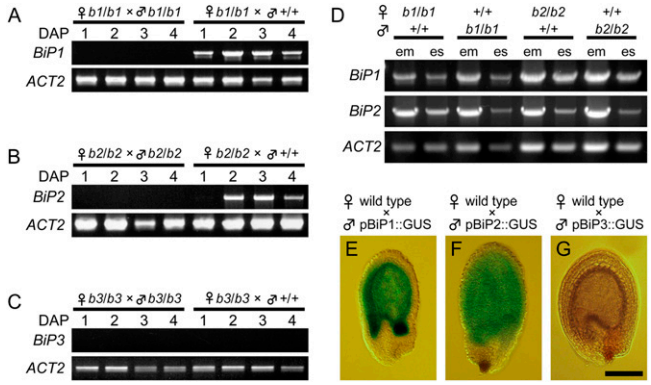

Fig. S4. Expression of paternally derived *BiP* genes during early seed development. (*A*) Pistils of *b1*/*b1* plants were pollinated with pollen from *b1*/*b1* or wild-type (+/+) plants. Total RNA was prepared from seeds at 1–4 DAP, and expression of the *BiP1* gene was analyzed by RT-PCR. (*B*) Expression of the *BiP2* gene was analyzed as in (*A*) except that *b2*/*b2* plants were used instead of *b1*/*b1* plants. (*C*) Expression of the *BiP3* gene was analyzed as in (A) except that *b3*/*b3* plants were used instead of *b1*/*b1* plants. (*D*) Paternally derived *BiP1* and *BiP2* are expressed both in embryo and the endosperm. Cross-pollination was performed between wild-type (+/+) plants and *b1*/*b1* or *b2*/*b2* plants. Total RNA was isolated from dissected embryo (*em*) and endosperm (*es*) fractions 7 DAP and was subjected to RT-PCR analysis. (*E*–*G*) Wild-type pistils were pollinated with pollen expressing GUS from the *BiP1* promoter (*pBiP1::GUS*, *E*), the *BiP2* promoter (*pBiP2::GUS*, *F*), or the *BiP3* promoter (*pBiP3::GUS*, *G*). Seeds were subjected to GUS staining at 3 DAP. (Scale bar, 50 μm.)

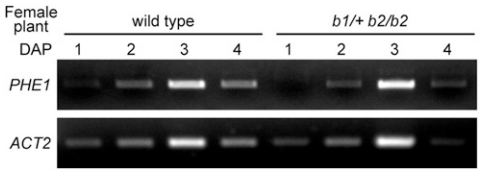

Fig. S5. *PHERES1* is not up-regulated in *bip1 bip2* mutant seeds after pollination with wild-type pollen. Wild-type (+/+ +/+) or *b1*/+ *b2*/*b2* pistils were pollinated with wild-type pollen. Total RNA was prepared from fertilized siliques at 1–4 DAP and expression of the *PHERES1* (*PHE1*) gene was analyzed by RT-PCR.

Table S1. Percentages of aborted seeds in *bip1 bip2* double mutants

Female	Male	Aborted seeds	Total seeds	F1 seed abortion (%)
+/+ +/+	+/+ +/+	12	2306	0.5
b1/b1 +/+	*b1/b1* +/+	8	904	0.9
+/+ *b2/b2*	+/+ *b2/b2*	8	954	0.8
b1/+ *b2/b2*	*b1*/+ *b2/b2*	303	692	43.8
b1/b1 b2/+	*b1/b1 b2*/+	374	823	45.4
b1/+ +/+	+/+ +/+	6	311	1.9
+/+ +/+	*b1*/+ +/+	1	301	0.3
+/+ *b2*/+	+/+ +/+	4	146	2.7
+/+ +/+	+/+ *b2*/+	3	226	1.3
b1/+ *b2/b2*	+/+ +/+	83	186	44.6
+/+ +/+	*b1*/+ *b2/b2*	1	240	0.4
b1/b1 b2/+	+/+ +/+	61	127	48.0
+/+ +/+	*b1/b1 b2*/+	5	595	0.8
bip1-1/bip1-1 b2/b2	*bip1-1/bip1-1 b2/b2*	5	690	0.7
bip1-1/+ *b2/b2*	*bip1-1*/+ *b2/b2*	2	549	0.4
bip1-2/+ *b2/b2*	*bip1-2*/+ *b2/b2*	335	707	47.4
bip1-3/+ *b2/b2*	*bip1-3*/+ *b2/b2*	331	712	46.5
bip1-1/bip1-1 b2/+	*bip1-1/bip1-1 b2*/+	5	532	0.9
bip1-2/bip1-2 b2/+	*bip1-2/bip1-2 b2*/+	349	774	45.1
bip1-3/bip1-3 b2/+	*bip1-3/bip1-3 b2*/+	385	838	45.9
bip1-1/+ +/+	+/+ +/+	0	316	0
+/+ +/+	*bip1-1*/+ +/+	3	355	0.8
bip1-2/+ +/+	+/+ +/+	0	82	0
+/+ +/+	*bip1-2* +/+	4	123	3.3
bip1-3/+ +/+	+/+ +/+	1	104	1.0
+/+ +/+	*bip1-3*/+ +/+	3	90	3.3
bip1-1/+ *b2/b2*	+/+ +/+	1	82	1.2
+/+ +/+	*bip1-1*/+ *b2/b2*	1	490	0.2
bip1-2/+ *b2/b2*	+/+ +/+	248	524	47.3
+/+ +/+	*bip1-2*/+ *b2/b2*	2	148	1.4
bip1-3/+ *b2/b2*	+/+ +/+	174	370	47.0
+/+ +/+	*bip1-3*/+ *b2/b2*	2	314	0.6
bip1-1/bip1-1 b2/+	+/+ +/+	0	261	0
+/+ +/+	*bip1-1/ bip1-1 b2*/+	2	622	0.3
bip1-2/bip1-2 b2/+	+/+ +/+	75	169	44.4
+/+ +/+	*bip1-2/bip1-2 b2*/+	3	389	0.8
bip1-3/bip1-3 b2/+	+/+ +/+	172	386	44.6
+/+ +/+	*bip1-3/bip1-3 b2*/+	1	143	0.7

Table S2. Transmission of *BiP* alleles to F1 progenies after reciprocal crosses between mutant and wild-type plants

Genetic cross		F1					
Female	Male	Genotype	%	Genotype	%	*P* value for 1:1 segregation*	*n*
b1/+ +/+	+/+ +/+	*b1*/+ +/+	53.0	+/+ +/+	47.0	0.41	181
+/+ +/+	*b1*/+ +/+	*b1*/+ +/+	47.1	+/+ +/+	52.9	0.49	140
+/+ *b2*/+	+/+ +/+	+/+ *b2*/+	55.7	+/+ +/+	44.3	0.24	106
+/+ +/+	+/+ *b2*/+	+/+ *b2*/+	50.8	+/+ +/+	49.2	0.86	132
b1/+ *b2*/*b2*	+/+ +/+	*b1*/+ *b2*/+	2.5	+/+ *b2*/+	97.5	1.31×10^{-31}*	157
+/+ +/+	*b1*/+ *b2*/*b2*	*b1*/+ *b2*/+	15.0	+/+ *b2*/+	85.0	8.41×10^{-19}*	160
b1/*b1* *b2*/+	+/+ +/+	*b1*/+ *b2*/+	0	*b1*/+ +/+	100	1.80×10^{-20}*	86
+/+ +/+	*b1*/*b1* *b2*/+	*b1*/+ *b2*/+	27.8	*b1*/+ +/+	72.2	1.48×10^{-12}*	255

*Validation by χ^2 test. * Significant difference with the probability value $P < 0.01$.

Table S3. Complementation of the seed abortion phenotype in *bip1 bip2* mutant female gametophytes

Transgene (target cell)	Line no.	Aborted seeds (%)	*P* value for aborted:viable = 1:1*	*P* value for aborted:viable= 1:3*	*n*
pBiP1::BiP1	1	22.0	1.16×10^{-7}*	0.73	218
pDD29::BiP1	1	49.5	0.91	1.63×10^{-8}*	275
(antipodal cells)	2	45.6	0.38	1.99×10^{-6}*	136
	3	32.3	4.02×10^{-4}*	0.09	130
	4	40.6	0.06	3.27×10^{-4}*	286
pDD31::BiP1	1	47.7	0.64	1.67×10^{-7}*	128
(synergid cells)	2	48.1	0.71	9.00×10^{-8}*	216
	3	43.5	0.19	1.98×10^{-5}*	368
	4	54.8	0.34	6.07×10^{-12}*	230
pDD45::BiP1	1	48.4	0.75	6.21×10^{-8}*	64
(egg cell)	2	40.7	0.06	2.84×10^{-4}*	140
	3	45.5	0.37	2.10×10^{-6}*	426
	4	42.7	0.15	4.15×10^{-5}*	524
pDD65::BiP1	1	29.5	4.09×10^{-5}*	0.30	78
(central cell)	2	25.0	5.74×10^{-7}*	1.00	152
	3	26.4	2.36×10^{-6}*	0.75	125
	4	24.7	4.35×10^{-7}*	0.95	283
pFIE::BiP1	1	22.7	4.78×10^{-8}*	0.60	185
(central cell)	2	24.0	1.93×10^{-7}*	0.81	146

Each *bip1-4*/+ *bip2-1*/*bip2-1* line that was also hemizygous for the transgene shown in the table was pollinated with wild-type pollen, and the percentage of aborted seeds was analyzed.
*Validation by χ^2 test. *Significant difference with the probability value $P < 0.01$.

Table S4. Primers used in this study

	Gene	Direction	Sequence (5′->3′)
RT-PCR primers	*BiP1*	Forward	TCGATCTATAGAGACGAAACAAAAA
		Reverse	CTTTATCGATGTAATTTGATGC
	BiP2	Forward	GACCTATTTAAGCATCCTAACTT
		Reverse	CTAGAGCTCATCGTGAGACTC
	BiP3	Forward	ATGATTTTTATCAAGGAAAACAC
		Reverse	TTATACGATTCTATAACTCATCG
	ACT2	Forward	TGGTGATGAAGCACAATCCAAG
		Reverse	TGGAACAAGACTTCTGGGCATC
	PHE1	Forward	TTTTACGTCAAAGGATCGCC
		Reverse	GGTGACGGTAGCGAGACAAT
Primers used in cloning of promoter regions	*DD29*	Forward	CACCAGATCTGTCTTCGATTTCG
		Reverse	CGTATAGAATTGATTTTTTATAATTCACATCTAA
	DD31	Forward	CACCTTCTAGATATATTATTGTAATTTCTACGAG
		Reverse	TTTTTTTATGGATGTAAGAATACTTTTAGTATTG
	DD45	Forward	CACCTCTTTGTCACCGTCACTC
		Reverse	TATTCTTTCTTTTTGGGGTTTTTGTTTTG
	DD65	Forward	CACCATCTATATATAATGATAATCTGACATATTC
		Reverse	ATCCTTTTCTACTTTGTTTTTGTTTTTG
	FIE	Forward	CACCACTATAGAAAAGTTCAAGAAAATCAA
		Reverse	TCGATATTCGAAATCTCTCTCTCTG
	BiP1	Forward	CACCAGGAGGTTGAGAGAGAAGATAGAC
		Reverse	ATCGGAAACTTTTGCGTACGATCTCTC
	BiP2	Forward	CACCTGTATTGTAAAAGCCCTTAGCGTTAC
		Reverse	ATCGGAAACTTTTGCGTACGATCTCTCTTT
	BiP3	Forward	CACCCAAACATAGCACCGAACGACTTACT
		Reverse	TTTTCGTTGTTGAGAACTCTTCTTCGAT