Seed coat-derived brassinosteroids non-cell autonomously regulate endosperm development

Extended materials

Figure S1

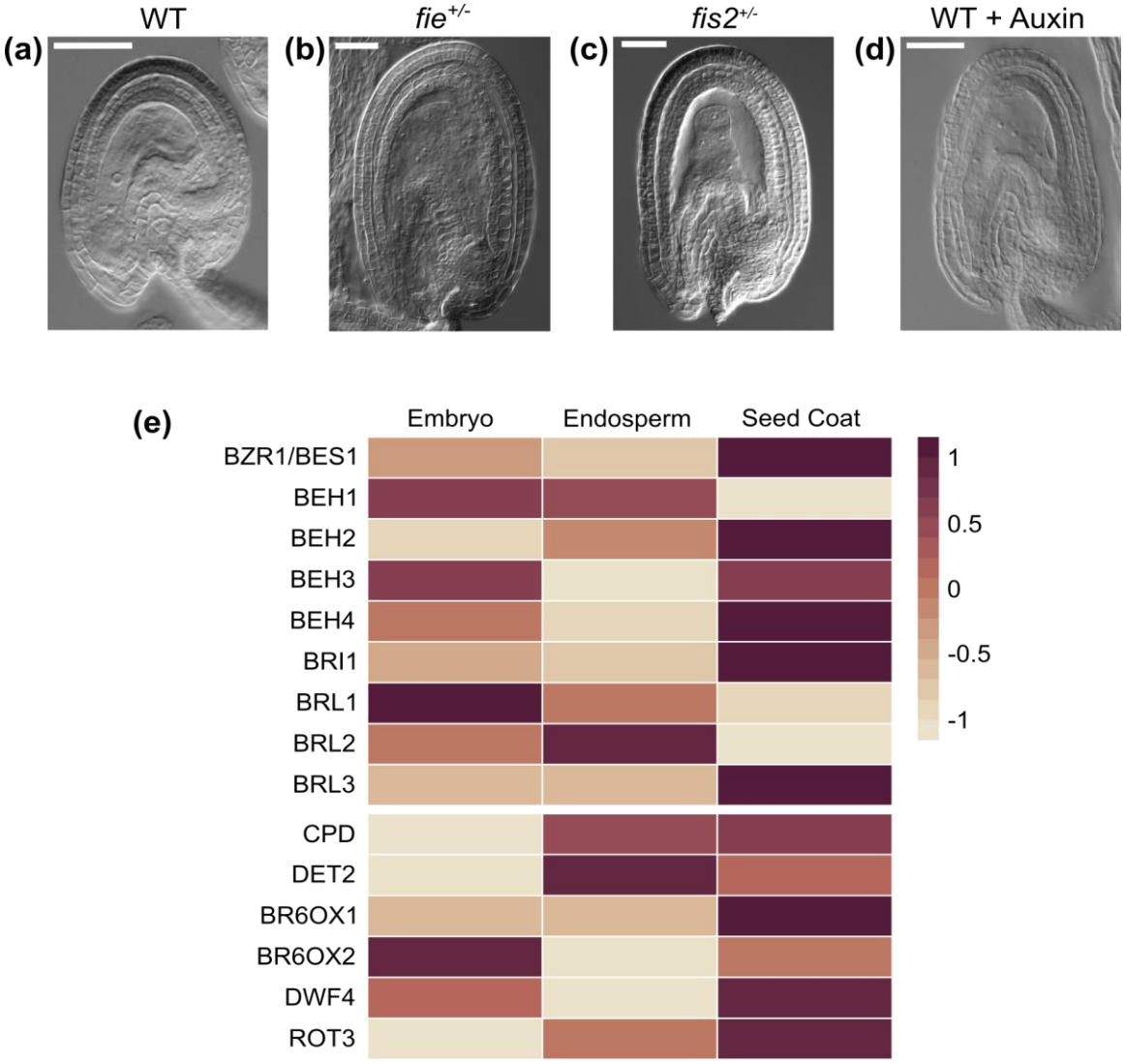

Figure S1. **BR are putative regulators of seed development**. Unfertilized WT ovule (a) and autonomous seeds of *fie*$^{+/-}$ (b) and *fis2*$^{+/-}$ (c) at 5 DAE, and WT autonomous seed (d) at 3 DAT after treatment with 100 µM of exogenous auxin. Scale bars, 50 µm. (e) Relative expression of BR signalling and biosynthetic genes in the embryo, endosperm and seed coat at 3 DAP. Z-score normalization was performed to center the mean and set the distribution of values to a SD of 1.

Figure S2

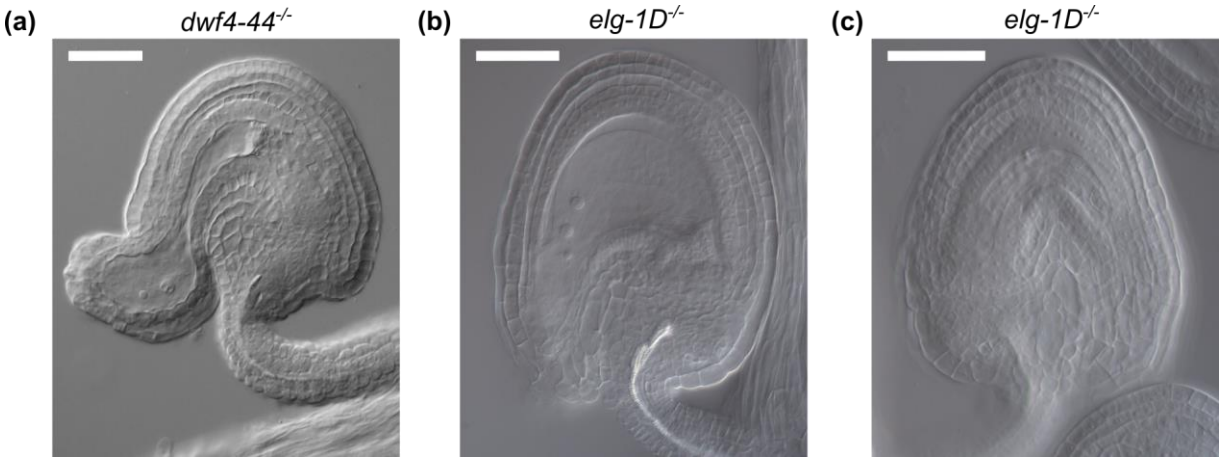

Figure S2. **Examples of malformed ovules of mutants impaired in BR function**. *dwf4-44*$^{-/-}$ (a) ovule with underdeveloped outer integuments and *elg-1D*$^{-/-}$ ovules with unfused polar nuclei (b) and persistent nucellus (c) at 5 days after emasculation (DAE). Scale bars, 50 µm.

Figure S3

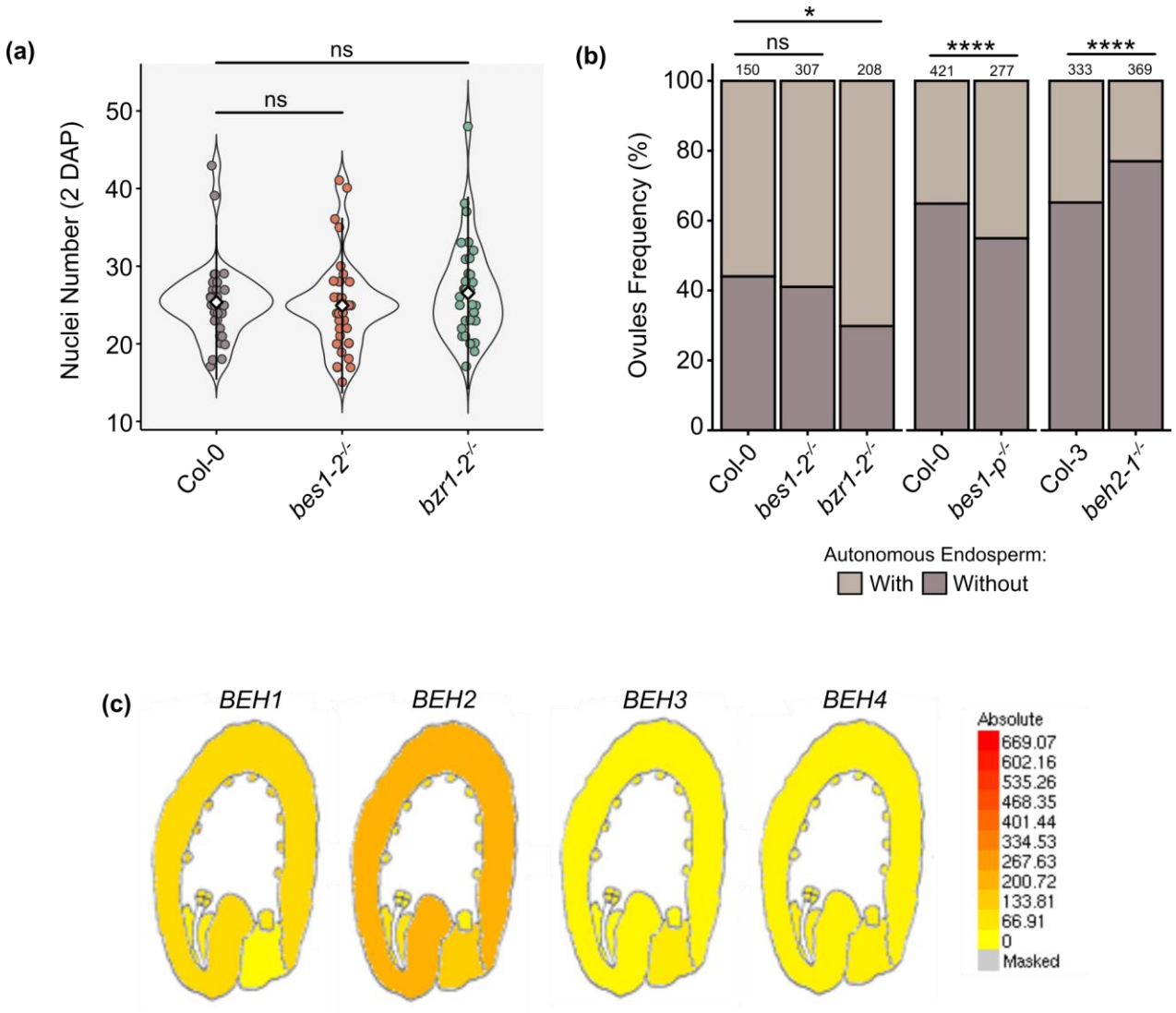

Figure S3. **BZR1/BES1 family of TFs and endosperm development**. (a) Endosperm nuclei number at 2 days after pollination (DAP) for BR mutants and WT. At least 35 seeds were assayed per sample. Error bars represent standard deviation. Significance of differences were determined by one-way analysis of variance (ANOVA). (b) Percentage of BR mutant and WT ovules with (neutral) and without (purple) autonomous endosperm development 3 days after exogenous auxin application. The values on top of each bar indicate the number of ovules analysed. Significance of differences were determined by chi squared test. (c) expression map of *BES1/BZR1* homologs *BEH1* , *BEH2* , *BEH3* and *BEH4* (taken from eFP Browser). **** p<0.0001, *** p<0.001, ** p<0.01 and * p <0.05.

Figure S4

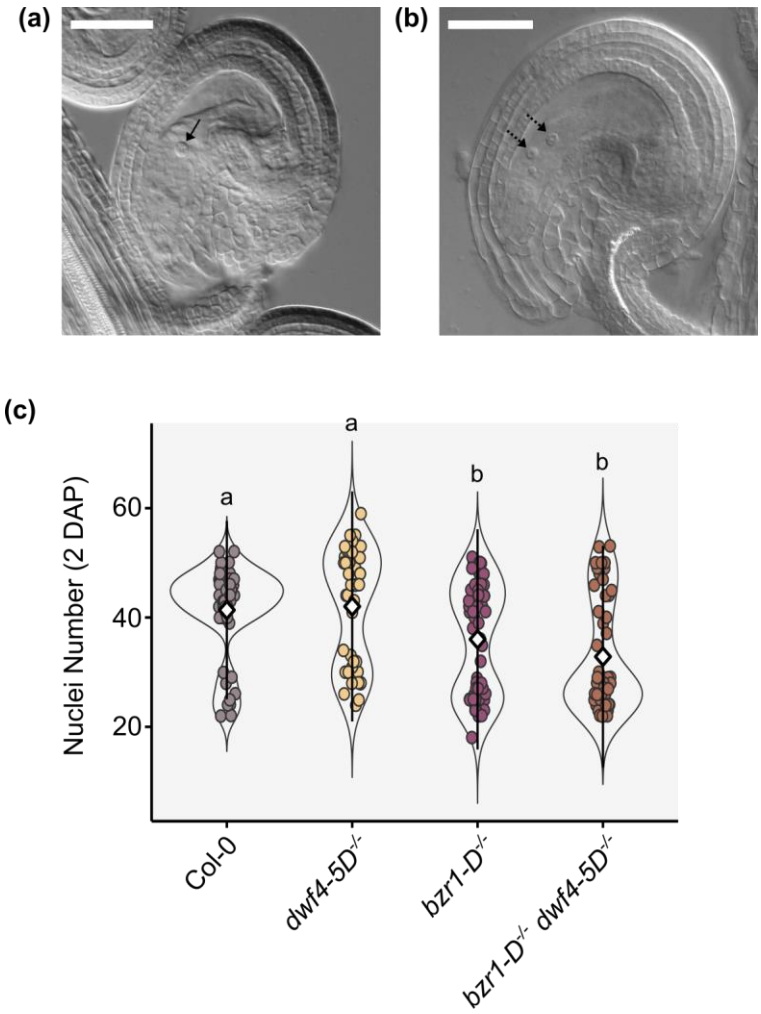

Figure S4. **Constitutive BR signalling affects ovule and seed development**. WT (a) and *bzr1-D*$^{-/-}$ (b) ovule showing unfused polar nuclei at 5 DAE. Solid arrows indicate central cell nucleus and dotted arrows indicate polar nuclei. Scale bars, 50 µm. (c) Endosperm nuclei number for WT, *dwf4-5D*$^{-/-}$, *bzr1-D*$^{-/-}$ and the double mutant *bzr1-D*$^{-/-}$ dwf4-5D$^{-/-}$ at 2 DAP. At least 46 seeds were assayed per sample. Error bars represent standard deviation. Letters indicate significance of differences determined by Tukey's HSD test after one-way ANOVA test.

Figure S5

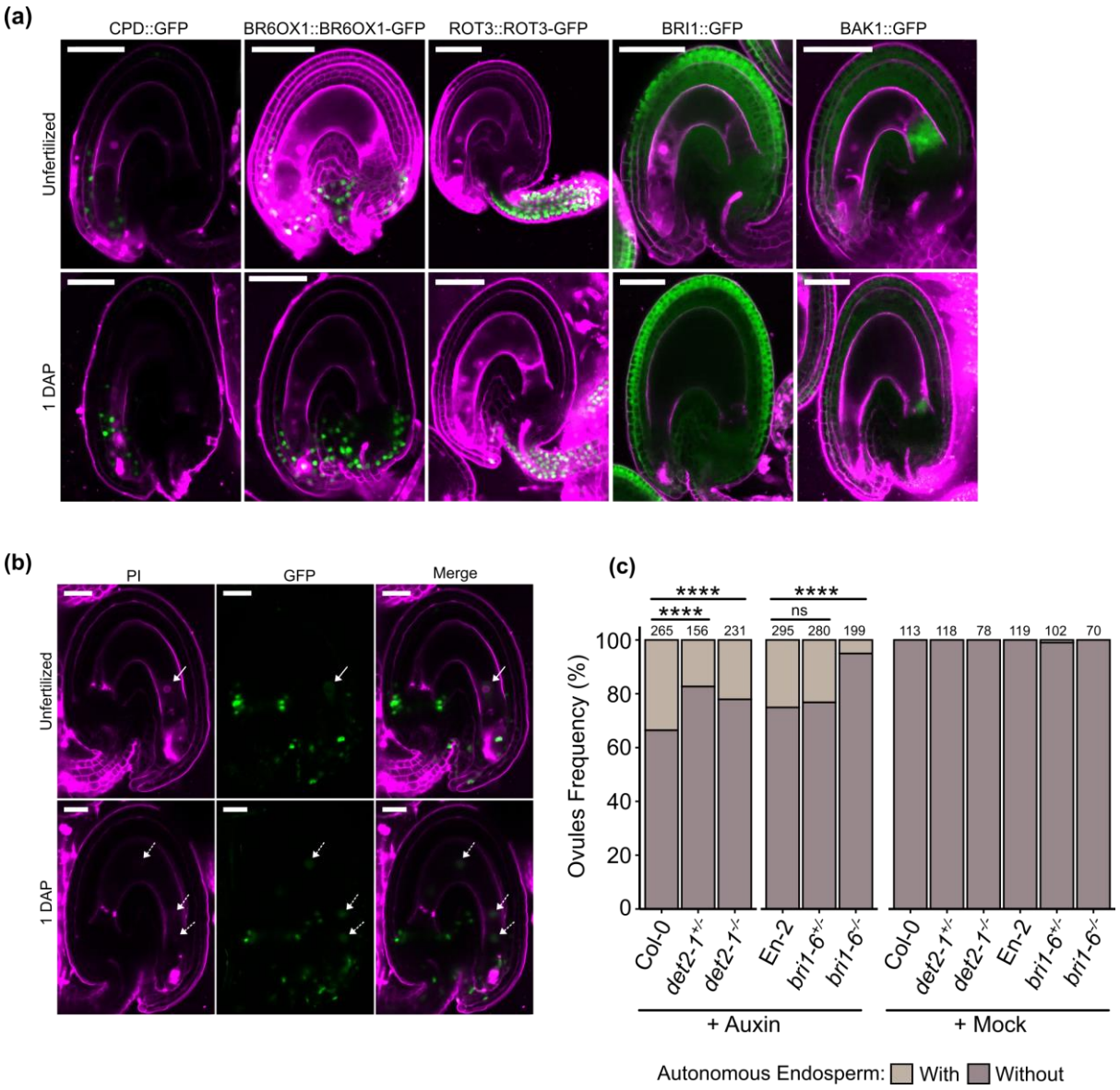

Figure S5. **BR effect on endosperm development is sporophytic**. (a) Expression pattern of *CPD::GFP, BR6OX1::BR6OX1-GFP, ROT3::ROT3-GFP, BRI1::GFP* and *BAK1::GFP* in unfertilized ovules (upper row) and seeds at 1 DAP (bottom row). Ovules and seeds were stained with propidium iodide (in magenta). Scale bars, 50 µm. (b) Representative image of an ovule (upper panels) and seed at 1 DAP (lower panels) showing *BR6OX2::BR6OX2-GFP* expression in the central cell and endosperm, respectively. Solid arrows indicate central cell nucleus and dotted arrows point to endosperm nuclei. Ovules and seeds were stained with propidium iodide (in magenta). Scale bars, 25 µm. (c) Percentage of *det2-1*$^{+/-}$, *bri1-6*$^{+/-}$, and respective WTs, ovules producing autonomous endosperm development at 3 days after exogenous auxin application. The values on top of each bar indicate the number of ovules analysed. Significance of differences were determined by chi squared test. **** p<0.0001, *** p<0.001, ** p<0.01 and * p <0.05.

Figure S6

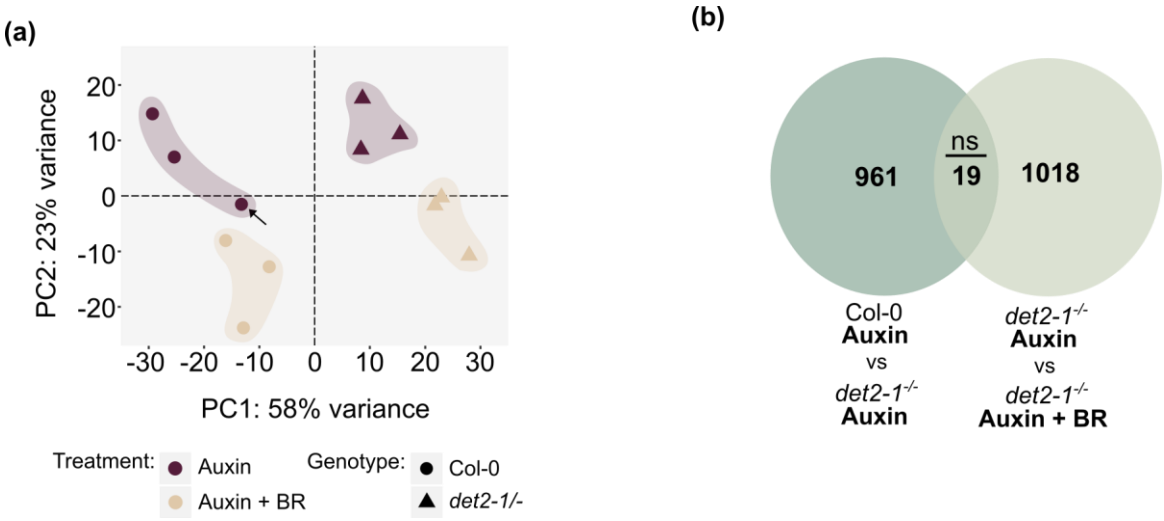

Figure S6. **RNAseq analysis of BR treatments**. (b) Principal component analysis of the samples used for the RNAseq experiment, showing PC1 and PC2. The sample indicated with an arrow was removed from the final analysis, since it did not cluster with the other two replicates. (d) Venn diagram showing overlapping genes that are downregulated in WT versus *det2-1*$^{-/-}$ ovules treated with auxin and upregulated in *det2-1*$^{-/-}$ ovules treated with auxin and BR versus auxin. The overlap between datasets was not statistically significant as determined by a hypergeometric test. **** p<0.0001, *** p<0.001, ** p<0.01 and * p<0.05.

Figure S7

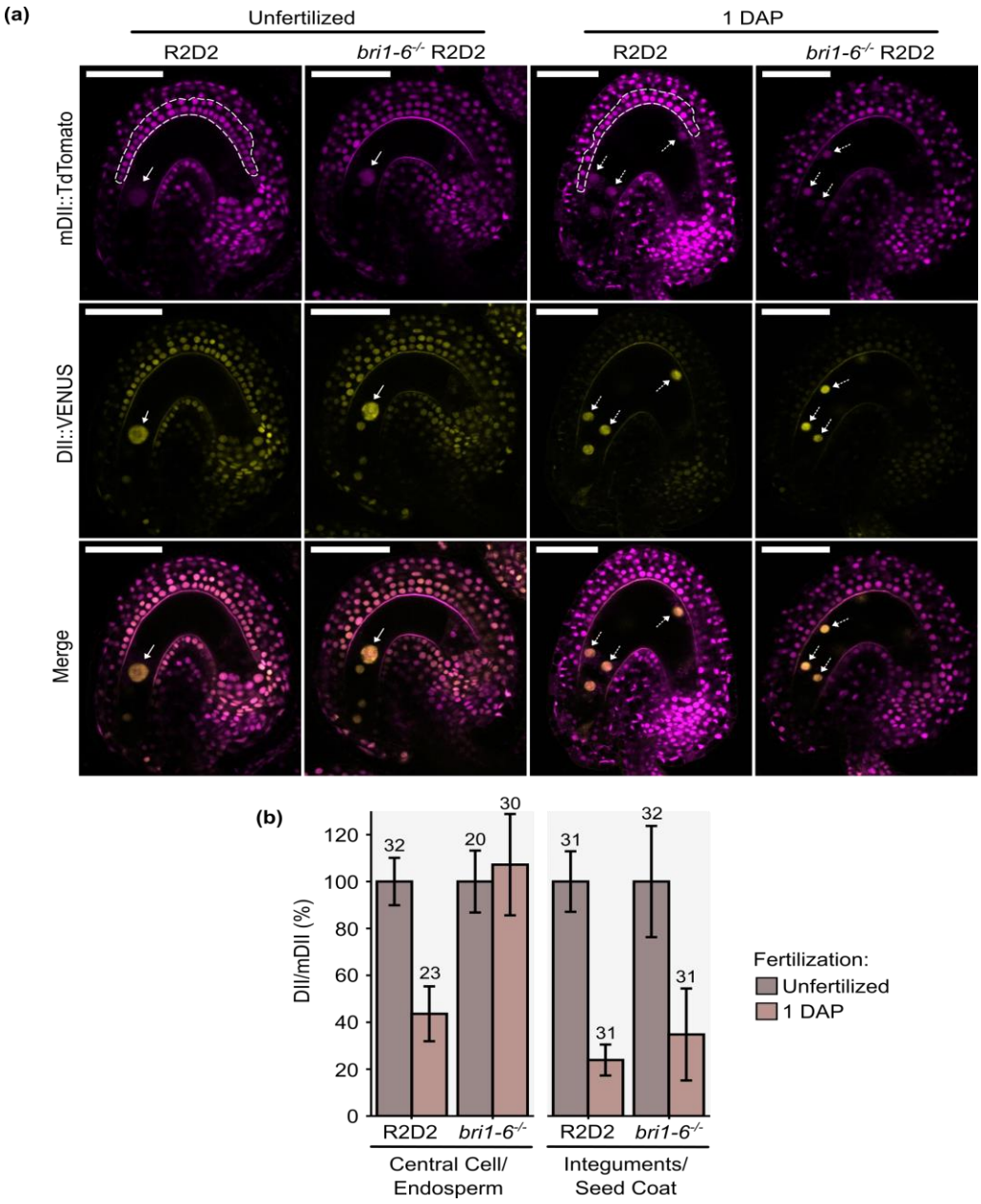

Figure S7. **Auxin activity is reduced in *bri1-6* endosperm**. (a) Representative microscopy images of WT and *bri1-6*$^{-/-}$ unfertilized ovules and seeds at 1 DAP expressing the R2D2 auxin sensor. Solid arrows indicate the central cell nucleus and dotted arrows point to endosperm nuclei. Dashed lines surround the nuclei of the integuments and seed coat layers used for quantification. Scale bars, 50 µm. (b) Quantification of DII-Venus/mDII-ntdTomato signal in R2D2 and *bri1-6*$^{-/-}$ seeds before and after fertilization. The values on top of each bar indicate the number of ovules/seeds analysed. Error bars represent standard deviation.

Figure S8

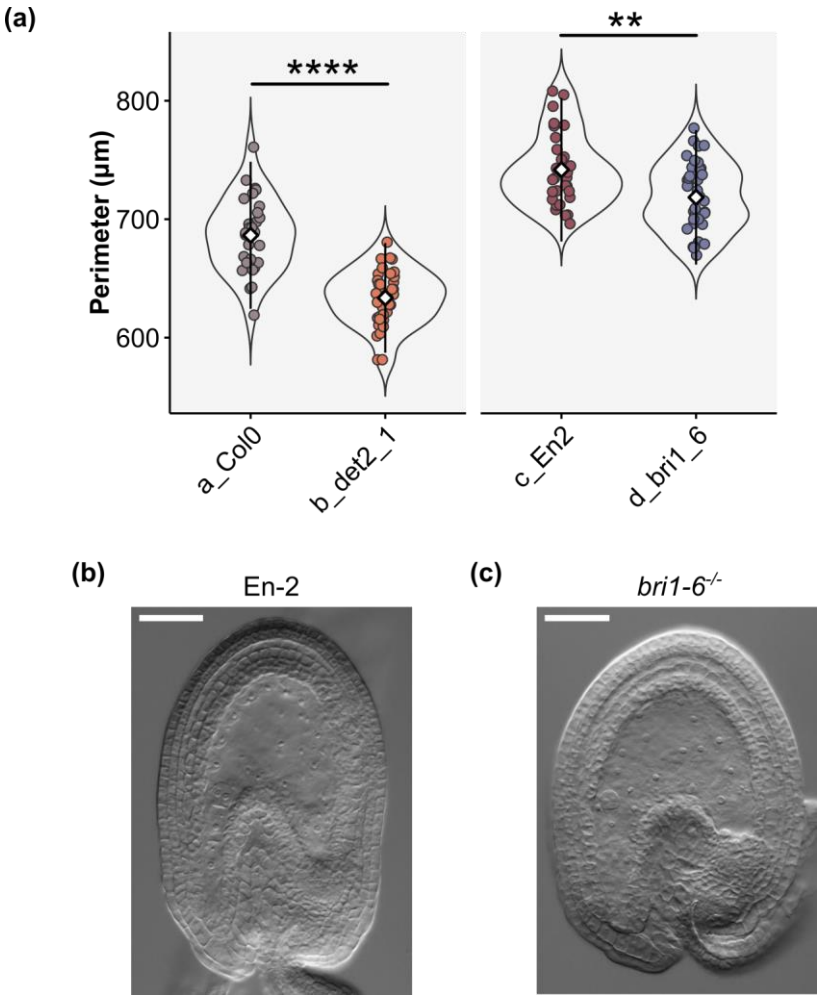

Figure S8. ***det2-1* and *bri1-6* seeds are smaller than the WT**. (a) Perimeter of *det2-1*$^{-/-}$ and *bri1-6*$^{-/-}$ seeds comparatively to the respective WT at 2 DAP. At least 30 seeds were analysed per sample. Significance of differences were determined by one-way ANOVA. Representative seed of En-2 (b) and *bri1-6*$^{-/-}$ (c) at 2 DAP. Scale bars, 50 µm. **** p<0.0001, *** p<0.001, ** p<0.01 and * p<0.05.

Figure S9

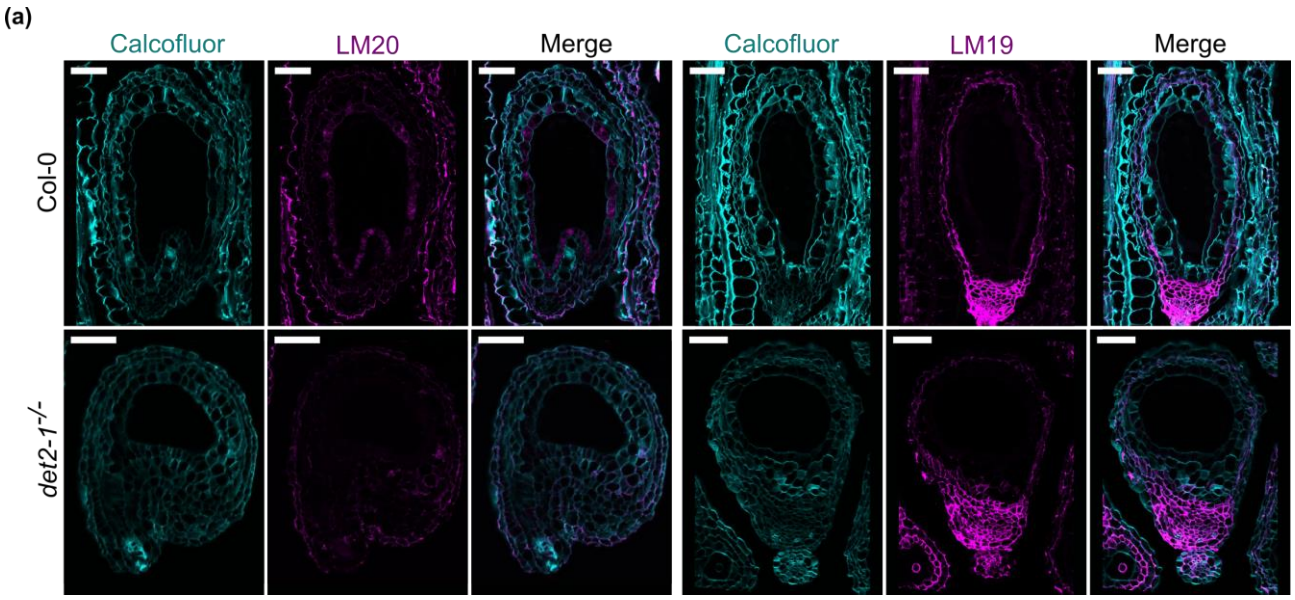

Figure S9. ***det2-1* seed coats have altered cell wall compositions**. (a) Labelling of cell wall components in WT (upper row) and *det2-1*$^{-/-}$ (lower row) at 4 DAP. Cyan is calcofluor white staining and purple is immunolabelling with either LM19 or LM20 antibodies. Scale bars, 50 µm.

Figure S10

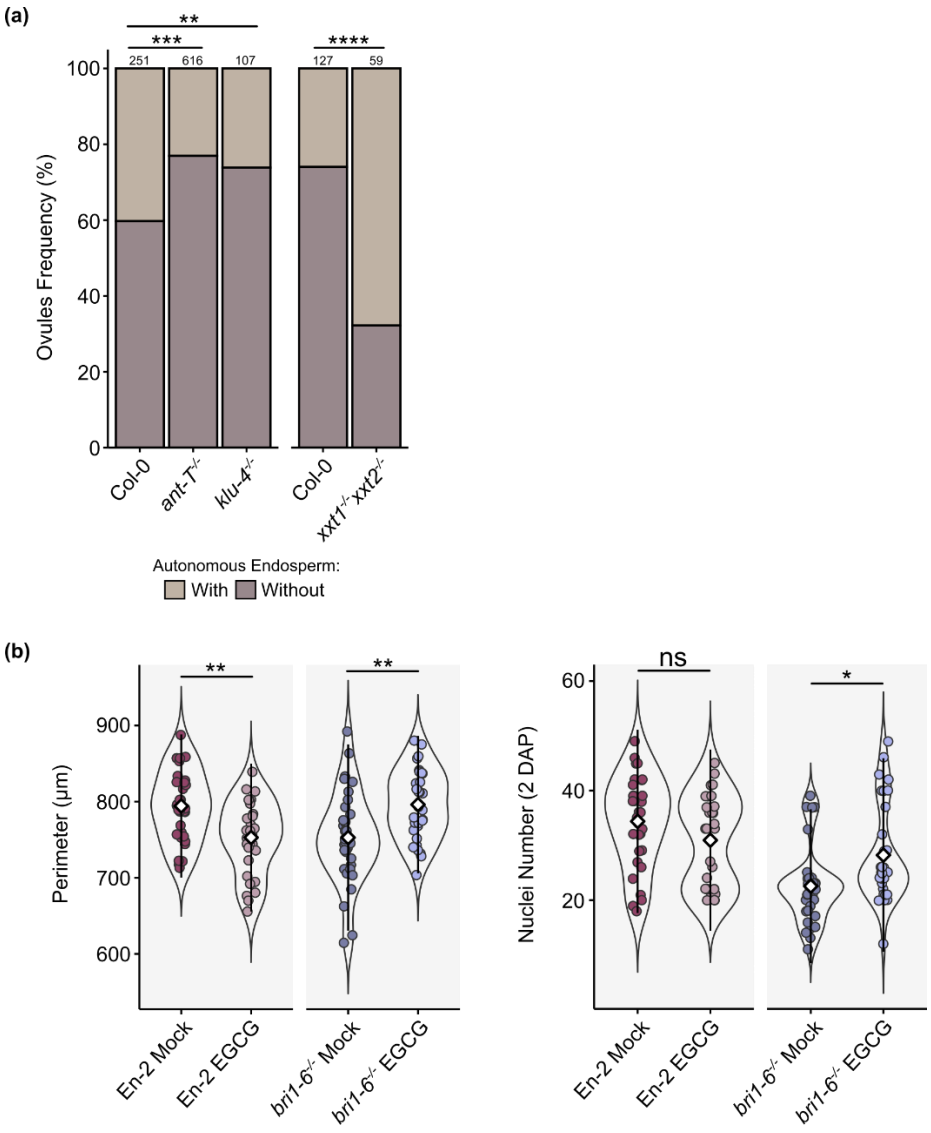

Figure S10. **Smaller seed sizes correlate with less proliferative endosperms**. (a) Percentage of ovules starting autonomous endosperm development in *ant-T*$^{-/-}$, *klu-4*$^{-/-}$ and *xxt1*$^{-/-}$ *xxt2*$^{-/-}$ and respective WT at 3 DAT. The values on top of each bar indicate the number of ovules analysed. Error bars represent standard deviation. Significance of difference was determined by chi-squared test. (b) Seed perimeter (left) and endosperm nuclei number (right) of mock and EGCG-treated En-2 and *bri1-6*$^{-/-}$. Significance of differences was determined by ANOVA. *** p<0.0001, *** p<0.001, ** p<0.01 and * p<0.05.

Table S2. Primer sequences used for cloning.

Gene	Region	Sequence (5’ → 3’) *	
BRI1 (AT4G39400)	Promoter	fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGAACGTCTGTGTTAGTC
		rv	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCTCAAGAGTTTGTGAGAGAG
BAK1 (AT4G33430)	CDS	fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCCACTCAAAAATAACAG
		rv	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTATCCTCAAGAGATTAAAAAC
DET2 (AT2G38050)	Promoter	fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTGACCACCCTACTCTCTCGGT
		rv	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTTCGGGTTATGGAATTGGGG

* Primer adapters are underlined.