

Supplementary Note 1 Egg cell size in *ΔBELL1*

The diameter of egg cells of WT and the deletion lines *ΔBELL1-2*, *ΔBELL1-4*, and *ΔBELL1-9* was measured from micrographs (**Fig. 1k, Supplementary Table 1**). The developmental status of the respective archegonia was recorded as well: closed archegonia with the apical cell still intact are not fertilized as sperms are mechanically prevented from entry. Open archegonia with a degraded apical cell are potentially fertilized; however the likelihood was decreased by not adding water to the plants. Moss sperms require free water in order to be able to swim to the archegonium. No signs of zygote development, such as a wavy surface, elongation⁴³ or cell division occurred. From this we conclude that egg cells either were not fertilized or if fertilized, were arrested in development. Both the size and the variance of *ΔBELL1* egg cells were increased. The mean WT egg cell (n = 9 egg cells) size was 16.61 μm with a standard deviation of 1.14. The mean *ΔBELL1* (n = 27) egg cell size was 19.32 μm with a standard variation of 3.02, with a maximum diameter of 26.39 μm (*ΔBELL1-4*). A Welch t-test yielded a P value of 0.0004 which indicates significant (P < 0.001) differences. The volume of a sphere depends on the diameter raised to the third power. Therefore, the biggest *ΔBELL1* egg cell has four times ($26.39^3 / 16.61^3$) the volume of the mean WT egg cell volume.

Supplementary Note 2 Germination of spores from apogamous sporophytes

To assess the germination rate of spores from apogamous sporophytes the content of individual spore capsules was placed on Knop agar plates and cultivated under standard conditions for nine days. It was not possible to distribute the spores of the *BELL1oe* apogamous sporophytes evenly with forceps or in an aliquot of water as this is usually done. Not only did the tetrads of spores not separate (**Fig. 21** insert), but also the entire content of the spore sac stuck together. After nine days the majority of spores of all ten analysed WT

capsules germinated. The content of five of the six (line 7), two of the ten capsules (line 27), and four of the six capsules (line 31) analysed did not germinate at all. The number of spores which germinated was low in all *BELL1oe* lines and could not be further quantified as the spores stuck together.

Supplementary Note 3 Ploidy of apogamous sporophytes

As the individual sporophytes did not yield enough material for nuclei measurement via flow cytometry, the relative amounts of nuclear DNA were estimated from micrographs of DAPI-stained nuclei according to⁴⁴. This allows the determination of relative DNA amounts of individual cells. As non-dividing cells have a fixed amount of DNA, one expects the individual data points to fall into categories with relative values differing by factor 2. As cells are either at G1 or G2 phase of the cell cycle, these categories are 1C, 2C and 4C. Gametophytic cells (e.g. leaves) are haploid (1n) and either 1C when in G1 or 2C when in G2. Correspondingly, sporophytic cells are diploid (2n) and therefore are 2C when in G1 and 4C in G2. In addition, a proportion of plant cells undergo endoreduplication^{27,28}. Depending on the method used, the individual value obtained from each nucleus may be imprecise. Therefore, we analysed many nuclei (49-73 nuclei per line). Relative DNA amounts of nuclei were measured from WT and apogamous sporophytes and from WT leaves as a control for haploid (1n) gametophytic cells.

Size distributions of DNA amounts were similar between WT and apogamous sporophytes and twice the amount of the controls (haploid WT leaf cells) (**Supplementary Fig. 13**). The relative values of the sporophyte nuclei ranged from 511 to 10730. The bulk of the nuclei split into a group from about 750 to 1250 and a second group from about 1500 to 2400. We reason that the individual data points fluctuate around the “true” values resulting in the range of values observed by us.

To find the relative (“true”) values in an unbiased way, hierarchical clustering was performed. Analysis of all data revealed three distinct clusters of sporophytic cells corresponding to 2C (2n, G1, 750 to 1250), 4C (2n, G2, 1500 to 2400), and a cluster of endoreduplicated cells (>2500). Correspondingly, the gametophytic cells were in two clusters, 1C (1n, G1) and 2C (1n, G2) (**Supplementary Fig. 14**). Median values of these clusters were similar in the sporophytes and twice the amount of the gametophytic cells (**Supplementary Table 3**), revealing that the fully functional apogamous sporophytes are diploid.

Supplementary methods

Plant material and culture conditions

Chloronemal tissue was cultivated in liquid cultures which were disrupted weekly. Density was adjusted to 200 mg/L dry weight. 7d after the last disruption the material was harvested for RNA isolation. For isolation of RNA from caulonema-enriched tissue, the plants were grown for 21d under standard conditions and then placed for 14d in the dark in a vertical position.

The formation of aposporous protonema was induced by injuring sporophytes with forceps. The injured sporophytes were then cultivated on solid medium and protonemata spontaneously developed.

WT calyptrae were removed with forceps once they detached from the leafy gametophytes carrying the developing sporophytes. On each colony about equal numbers of calyptrae were removed from developing sporophytes or left undisturbed, respectively. After two weeks, once the spore capsules were fully expanded, they were analysed under a dissecting microscope. For analysis, the calyptrae of the undisturbed sporophytes were

removed to allow maximum comparability of the samples. From the acquired images the sporophyte tips were outlined using a cursor.

Transcriptional analysis

For RT-PCR total RNA was extracted using the TRIZOL® Reagent (Life Technologies, Carlsbad, CA, USA) and reversely-transcribed using oligo d(T)16 primers with Superscript III reverse transcriptase (Life Technologies). Expression analysis via RT-PCR of *P. patens* *BELL1* was performed with gene-specific primers BELL1_3_fw 5'-GGCGATACCGATTTTGGTGC-3' and BELL1_3_rev 5'-GCCCATTGCTCATAGTTGCG-3'. The expression level of the constitutively expressed gene coding for the 60S ribosomal protein L21 (primers C45_fw 5'-GGTTGGTCATGGGTTGCG-3' and C45_rev 5'-GAGGTCAACTGTCTCGCC-3') was used to monitor amounts of input RNA.

For quantitative Real-Time PCR of the *PpBELL* genes total RNA was extracted using the SV Total RNA Isolation System (Promega, USA) from 6 days old protonemata. The cDNA was synthesized from 2 µg of total RNA with the SuperScript® III First-Strand Synthesis System (Life Technologies) using oligo d(T)20 primers. The quantitative Real-Time PCR was performed in a StepOne Thermal Cycler (Applied Biosystems, Foster City, CA, USA) using SYBR Green to monitor dsDNA synthesis. The amount of cDNA for each gene was quantified with a log-linear regression curve of the threshold cycle and the amount of standard template prepared from a cDNA clone. *PpBELL1* was amplified with the primers BELL1_f 5' TGGACCATTTTGAAGGAAGG 3' and BELL1_r 5' GCTCATAGTTGCGGAGGATT 3', *PpBELL2* with BELL2_f 5' GCCAATTGTTTGTTCAGTAGGTT 3' and BELL2_r 5' AGATGGCACAACATGACCAC 3', *PpBELL3* with BELL3_f 5' CTGCCTCAACACTGAACAGAA 3' and BELL3_r 5'

AGATCTCTGGAACCGTCTGGC 3', and *PpBELL4* with BELL4_f 5' CACAACGTTGAGCATGATTTC 3' and BELL4_r 5' CAGGATGTAAAGCACTGGACCT 3'. The transcripts coding for Histone H3 (Pp1s1963_1V6.1 and Pp1s3_594V6.1) served as control and were amplified with PpHistone3_100_f 5' GGAGGAGTGAAGAAGCCACA 3' and PpHistone3_143_r f 5' TGCGAATCTCCCGAAGAG 3'. The transcript coding for the TATA-binding protein was amplified with the primers TATA_qf 5' GATCTAGCTATAAGCCTGATCTACCG 3' and TATA_qr 5' CAGGAGCAGGGAGAGATTTG 3'. The bar chart depicts means of three technical replicates with standard deviations as error bars.

For quantitative Real-Time PCR of the *PpKNOX* (*MKN*) genes, total RNA was extracted with cetyltrimethylammonium bromide (CTAB) buffer containing 2% 2-mercaptoethanol⁴⁵. Contaminants were removed by two extractions with chloroform: isoamyl alcohol (24:1). The nucleic acids were precipitated with lithium chloride at a final concentration of 2.5 M overnight at 4 °C. After two washing steps the RNA was resuspended in water and DNaseI (RNase-Free DNase Set, Qiagen, Hilden, Germany) treatment was performed according to the manufacturer's instructions. Next, the RNA was purified using the RNeasy Plant Mini Kit (Qiagen). After column purification, a DNaseI treatment (TURBO™ DNase, Life Technologies) was performed and a sample of the RNA was run on an agarose gel to confirm integrity. The RNA was reversely-transcribed with the TaqMan® Reverse Transcription Reagents (Life Technologies). In order to monitor DNA contamination of the samples, 200 ng of each DNaseI-treated RNA sample were used as a no reverse transcriptase control by omitting the reverse transcriptase. The quantitative Real-Time PCR was performed using the SensiMix™ SYBR No-ROX Kit (Bioline, Luckenwalde, Germany) in a LightCycler 480II (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). *PpMKN2* was amplified with the primers MKN2_qf 5' CACTGGAGCTGCGAAGGTAA 3' and MKN2_qr 5' CCGTTGGAGGTGCCATAGAA 3', *PpMKN1* with MKN1_qf2 5'

GCGGCTGATTCAGGAGACA 3' and PpMKN1_qr2 5' TCCGCTTGCGTTGGTTTATG 3', and *PpMKN6* with MKN6_qf2 5' GGGGCTATGGTCCTCTTGTG 3' and MKN6_qr2 5' CCCGCTCCATTAAACTACGC 3'. The transcripts coding for TATA-binding protein (primers TATA_qf and TATA_qr), and 60S ribosomal Protein L21 (primers C45_qf 5' ACGCACCGGCATCG 3' and C45_qr 5' TGCTTGTTTCATCACGACACCAA 3') were used as reference for relative quantification.

For all quantitative Real-Time PCR analyses efficiency between 1.90 and 2.09 of each primer set was determined with a logarithmic dilution of a DNA mix. For all primer sets the generation of specific PCR products was confirmed by melting curve analyses. Quantification of the target relative to the abundance of the reference genes was calculated according to⁴⁶.

Determination of transgene copy numbers via quantitative Real-Time PCR

Transgene copy numbers in each tested line were estimated via quantitative Real-Time PCR according to⁴⁷. Genomic DNA was isolated from chloronema cultures using the innuPREP Plant DNA Kit (Analytic Jena AG, Jena, Germany) according to the manufacturer's instructions. Integrity of DNA was verified on an agarose gel. *PpBELL1* was amplified using the gene-specific primers BELL1_f and BELL1_r. The single copy gene *CLF* was used as a reference for normalization with two primer sets (pPCLF_5915_qf 5'-AGCAATGTCCGTGCCTACTT-3', pPCLF_5981_qr 5'-TTGTAAGAATCACTCACCCACAG-3' and pPCLF_7739_qf 5'-GTATTGGCGATCCCACTCTT-3', pPCLF_7804_qr 5'-GCATAAAATAGGTCACAGATTGAGG-3'). The number of transgene copies was determined by comparing relative values of the tested transgene with the relative values of the single copy *PpBELL1* gene in WT.

Flow cytometry

Flow cytometric analysis was performed according to⁴⁸.

Determination of relative nuclear DNA content

The relative amount of nuclear DNA of individual nuclei was calculated from microscopic images of 4',6-diamidino-2-phenylindole (DAPI)-stained tissue⁴⁴. Gametophores and sporophytes were fixed in 50 % v/v ethanol, 3.7 % v/v formaldehyde and 5 % v/v acetic acid. After replacement of the fixative with water, whole mount samples of leaves, embryos, and spore sacks, respectively, were desiccated on polylysine-coated slides. The samples were rehydrated in phosphate buffered saline containing 1 mg/L DAPI (Merck KGaA, Darmstadt, Germany). Using a Nikon Eclipse 80i fluorescent microscope (Tokyo, Japan) with a 60x air objective equipped with a Nikon DS-Vi1 CCD camera grayscale images of nuclei were captured. All images were acquired with an exposure time of 33 ms and with the same settings to obtain a constant background.

The nucleus was segmented using a cursor and its area and the grayscale values of the fluorescent pixels were determined from the microscopic images using ImageJ (<http://rsbweb.nih.gov/ij/>). The Integrated Optical Density (i. e. the summed up grayscale values of each nucleus) was calculated to obtain the relative amount of DNA. Hierarchical Clustering of the relative values was performed with the Free Statistics Software v1.1.23-r7 (http://www.wessa.net/rwasp_agglomerativehierarchicalclustering.wasp/)⁴⁹.

Microscopic analysis

For the assessment of sperm viability (**Supplementary Fig. 7**), antheridia were placed in 100 μ l tap water containing 10 μ g/ml propidium iodide. Phase contrast and epifluorescent images of the same view were captured and merged with GIMP 2.8 (<http://www.gimp.org/>).

Movies of WT and $\Delta PpBELL1$ were composed from time series of 50 micrographs obtained at 100 ms intervals. Movies were generated utilizing ImageJ and run at 0.5x speed.

Bimolecular fluorescence complementation analysis

Protein interaction of BELL and KNOX proteins was examined by bimolecular fluorescence complementation (BiFC) as previously described¹³. The coding sequences of *PpMKN2*, *PpMKN4*, *PpMKN5*, *PpMKN1*, and *PpMKN6* were amplified with the primer pairs MKN2-SpeI-f 5'-ACTAGTATGGAGCAGCAAACCCCGTC-3', MKN2-SpeI_r 5'-ACTAGTCTACTCCAGATGACCTCTGCATT-3', and MKN4_SpeI_f 5'-ACTAGTATGGGAGGGAAGGAGGAAG-3', MKN4-SpeI_r 5'-ACTAGTTTACTGCTCCAGATAACCCTCA-3', and MKN5_SpeI_f 5'-ACTAGTATGGCCAGGTCATCGGATA-3', MKN5-SpeI_r 5'-ACTAGTCTACTCCAGATGACCATTGC-3', and MKN1_SpeI_f 5'-ACTAGTATGATGGCTTTCAACAATTTATCACATG-3', MKN1_SpeI_r 5'-ACTAGTTCACCTTTTCCGTTTGCTCTTCAGC-3', and MKN6_SpeI_f 5'-ACTAGTATGACGTTTCGACAATGACTT-3', and MKN6_SpeI_r 5'-ACTAGTTTACCTCTTTCGTACTTTATTCTTCA-3'. Coding sequences of the MKNs were cloned C-terminally of the sequence coding for the N-terminal fragment of YFP (YN) into the *SpeI* site of the plasmid pSY 736⁵⁰. *PpBELL1* was amplified with primers containing overlapping regions to the plasmid pSY 735⁵⁰: Bell1_gib_YC_f 5'-GTTCTGACTATGCGTCGACATATGAGCTCACTAGTATGGATTCTTCAGAGCCT

TTGC-3' and Bell1_gib_r

5'-GATTTTTCGGACTCTAGAGGATCCAGATCTGACTAGTTCAACTGGTAACAAAC

TCATGATGA-3' for the cloning of the sequences C-terminally of the sequence coding for the C-terminal fragment of YFP (YC). The amplified fragments and the plasmids, linearized with *SpeI*, were combined in a 4:1 molecular ratio for Gibson⁵¹ assembly. 5 µl of DNA was added to 15 µl 1.33x assembly master mixture prepared according to⁵¹, incubated at 50 °C for 60 minutes and directly used for transformation of chemically competent cells.

Agrobacterium tumefaciens strain GV3101/pMp90 containing plasmids of interest were transiently co-expressed in *N. benthamiana* leaves via the leaf injection procedure⁵⁰. Image annotation was performed with a LSM 780 NLO confocal microscope (Zeiss) and Adobe Photoshop 7.0 (Mountain View, CA). Negative controls with vectors bearing only YN or YC alone were carried out in every experiment to verify the specificity of the interactions.

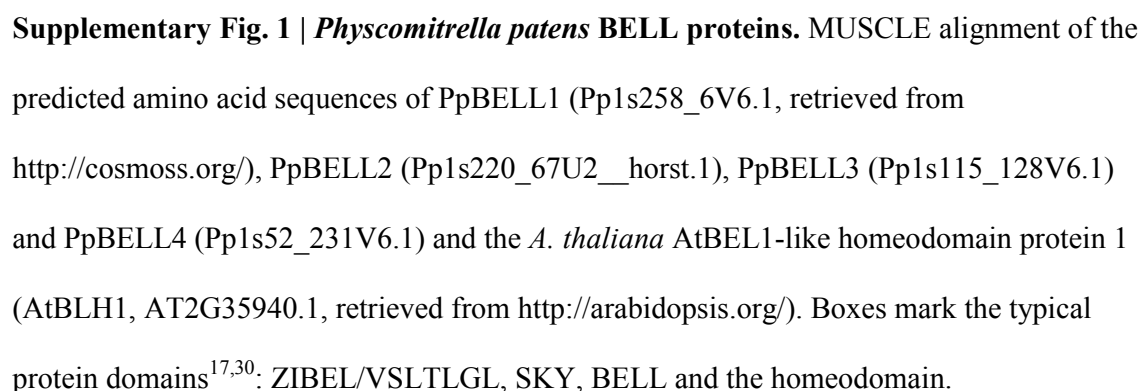
Bioinformatics methods

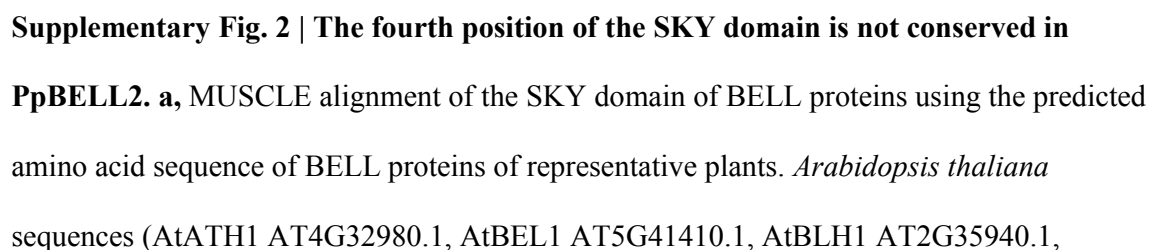
MUSCLE alignment⁵² of amino acid sequences was performed using

<http://www.ebi.ac.uk/Tools/msa/muscle/>.

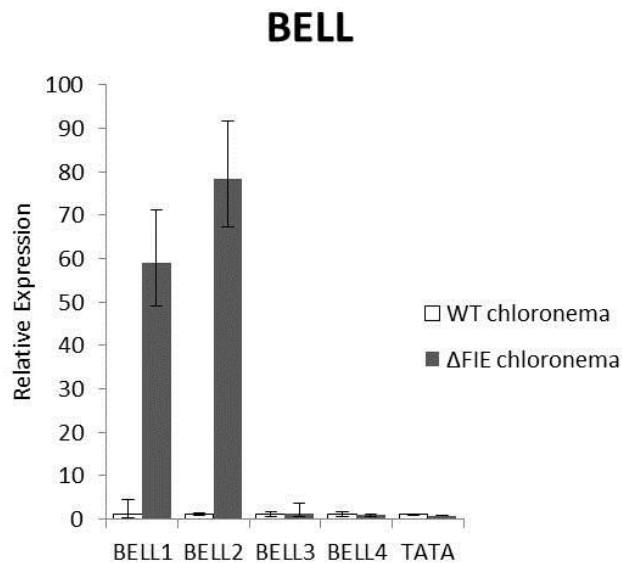
Helical wheel projections of the SKY domains were created with

<http://emboss.bioinformatics.nl/cgi-bin/emboss/pepwheel>.

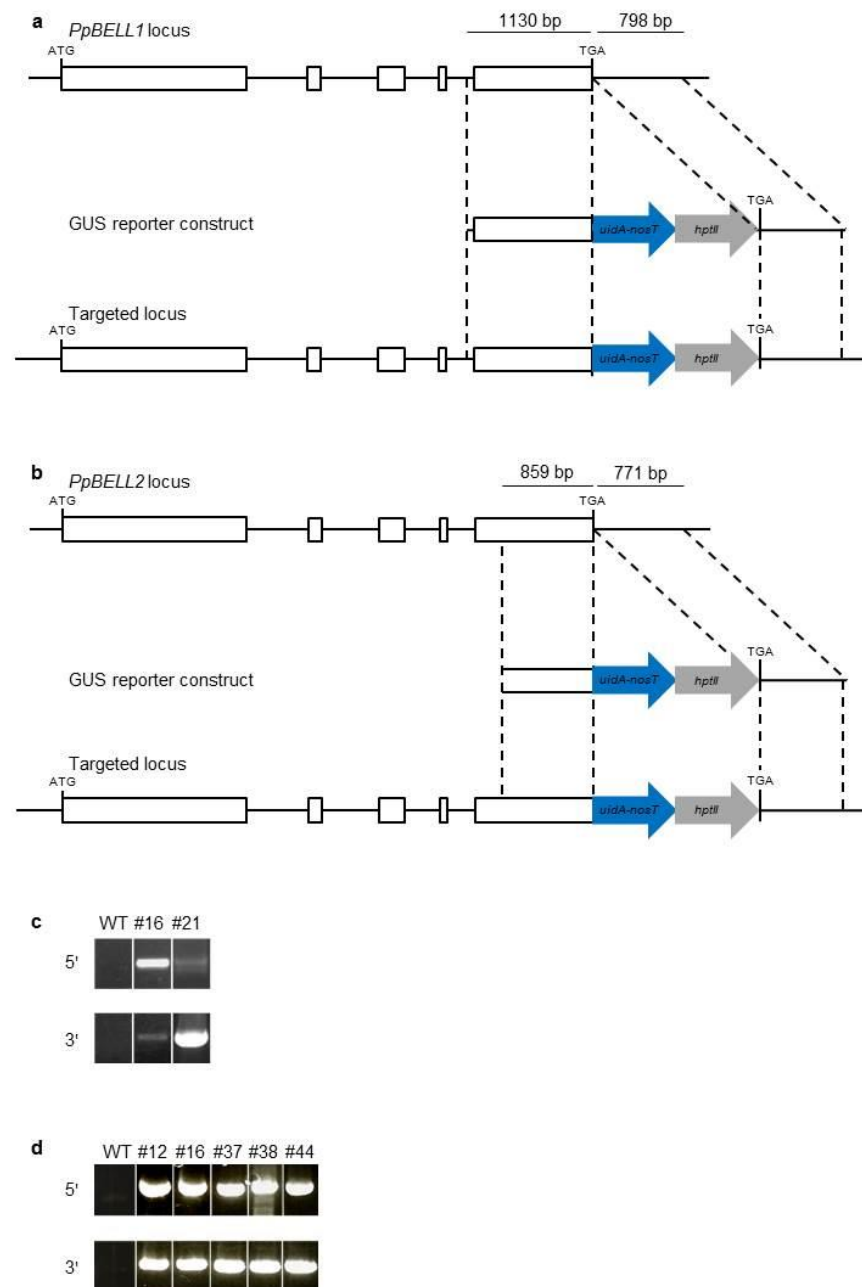




AtBLH3 AT1G75410.1, AtBLH5 AT2G27220.1, AtBLH6 AT4G34610.1, AtBLH7 AT2G16400.1, AtBLH10 AT1G19700.1, AtBLH11 AT1G75430.1, AtPNF AT2G27990.1, AtPNY (AtBLR) AT5G02030.1, AtSAW1 AT4G36870.1, AtSAW2 AT2G23760.1) were obtained from <http://www.arabidopsis.org/>. Rice (Oz), poplar (Potri), and *Selaginella moellendorffii* (Sm) sequences were retrieved from <http://www.phytozome.net/> and are displayed with the IDs used there. Refer to **Supplementary Fig. 1** for the accessions of *Physcomitrella patens* (Pp) BELL sequences. A partial sequence of a *Gnetum gnemon* (Gg) BELL was obtained from <https://www.ncbi.nlm.nih.gov/genbank/> (GgMELBEL1 AJ318871.1). **b** and **c**, Helical wheel projections of the SKY domains of PpBELL1 (**b**) and PpBELL2 (**c**). The hydrophobic face is indicated as a blue arc, the hydrophilic face as a red arc. The arrow in **c** points to the glutamic acid (E) residue at the fourth position of the SKY domain of PpBELL2.



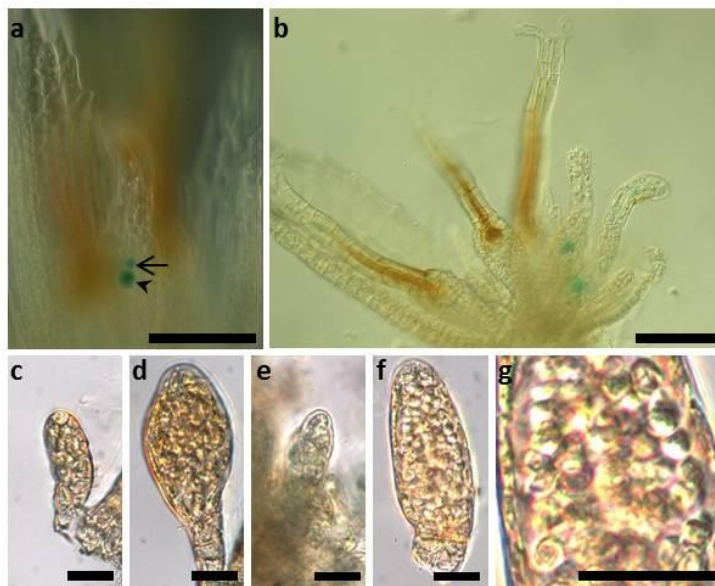
Supplementary Fig. 3 | Expression analysis via quantitative Real-Time PCR of *PpBELL* genes in WT and a FIE null mutant (ΔFIE). Transcripts of *PpBELL* genes *BELL1*, *BELL2*, *BELL3* and *BELL4* were analysed in WT and ΔFIE chloronemata samples. Bars depict means of three technical replicates with standard errors. Signals for *BELL3* and *BELL4* were below the background in all samples. The transcripts coding for Histone H3 (Pp1s1963_1V6.1 and Pp1s3_594V6.1) were used as reference for relative quantification. The transcript coding for the TATA-binding protein (Pp1s246_34V6.1) served to validate the accuracy of the data analysis.



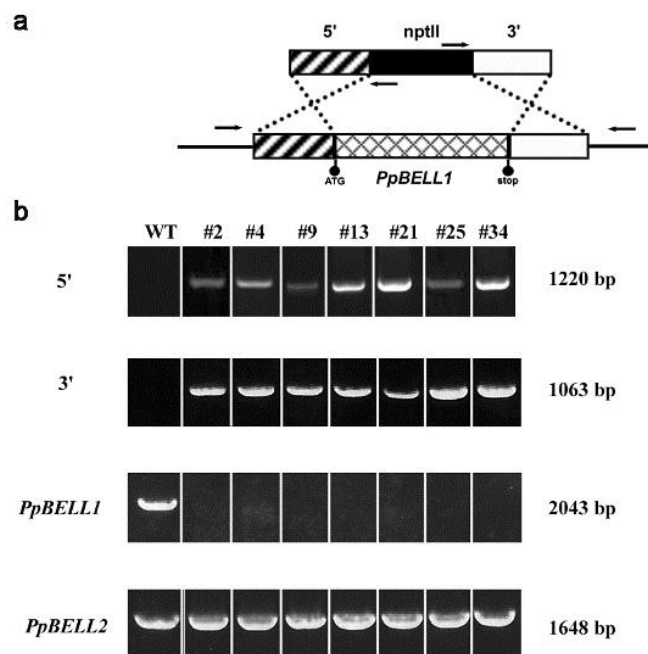
Supplementary Fig. 4 | Construction of *PpBELL1*:GUS and *PpBELL2*:GUS reporter

lines. Schematic depiction of *PpBELL1* (a) and *PpBELL2* (b) genomic loci on top, in the middle the construct used for in-frame insertion of the *uidA* (GUS) reporter gene, and at the bottom the modified genomic loci after integration of the construct. GUS reporter constructs containing the GUS CDS followed by the nos terminator (*uidA-nosT*; blue arrow) and an *hptII* selection cassette (grey arrow) are flanked by the 5' genomic region and the 3' un-translated

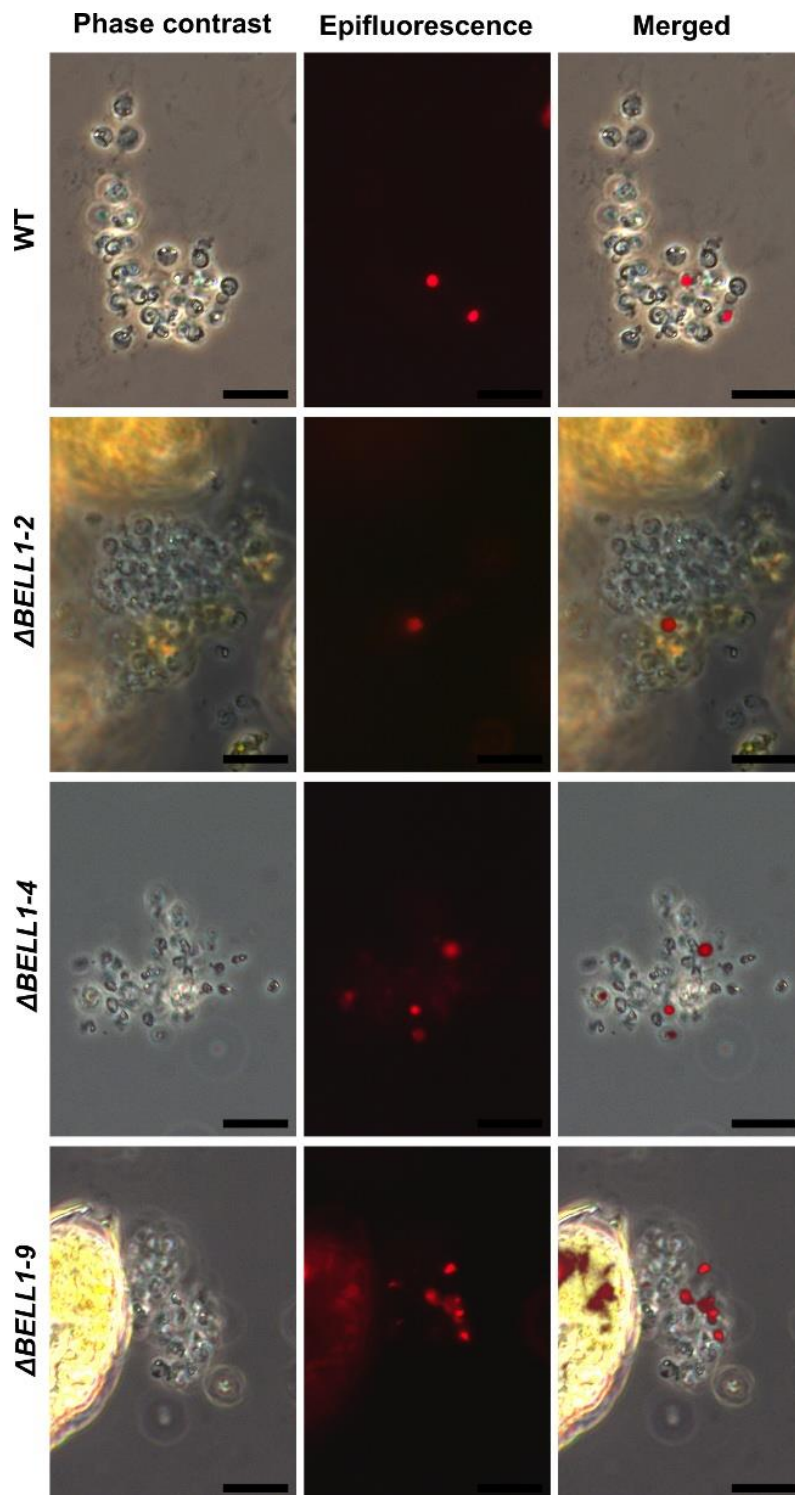
region of the respective genes. The *PpBELL* start and stop codons are indicated. Boxes and lines represent exons and introns, respectively. **(c, d)** Correct integration at the genomic loci of *PpBELL1:GUS* and *PpBELL2:GUS* reporter constructs were verified via PCR. Correct 5' and 3' integration of the GUS CDS at the *PpBELL1* genomic locus in line 16 and 21 (c). Correct 5' and 3' integration of the GUS CDS at the *PpBELL2* genomic locus in lines 12, 16, 37, 38 and 44 (d).



Supplementary Fig. 5 | GUS staining indicative for protein accumulation of *PpBELL1:GUS* in line BELL1:GUS-21. Absence of GUS staining in *PpBELL1:GUS* antheridia. **a and **b**, Archegonia of line BELL1:GUS-21. Arrowhead indicates egg cell, arrow indicates ventral canal cell. **c**, Early and **d**, late stage antheridia of line BELL1:GUS-16. **e**, Early and **f**, late stage antheridia of line BELL1:GUS-21. **g**, Magnification of **f**, with spermatozooids visible. Scale bars: 100 μ m in **a**, **b**; and 20 μ m in **c**-**g**.**

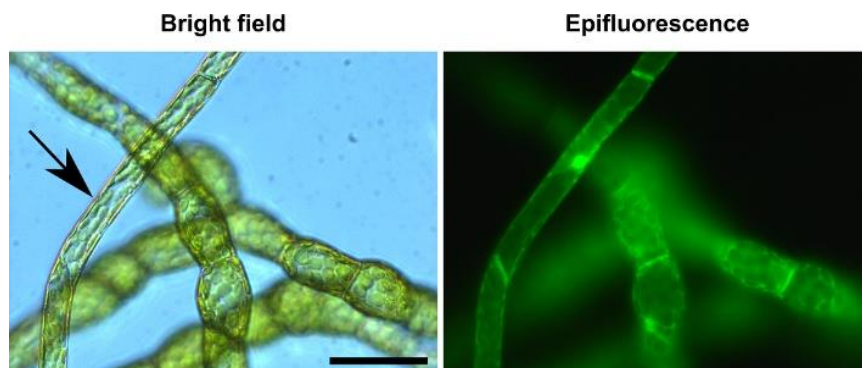


Supplementary Fig. 6 | Construction of *PpBELL1* null mutants. (a) Schematic representation of *PpBELL1*-KO construct and its predicted integration to the genome. The bottom line represents the endogenous genomic locus, the upper line represents the plasmid construct, and the dotted lines point to the site at which the cassette is directed to integrate into the endogenous locus following homologous recombination. The arrows indicate the position of primers used to detect the 5' and 3' integration of the recombinant DNA, respectively. (b) PCR verification of cassette integration at 5' and 3' homologous regions, *PpBELL1* absence and *PpBELL2* presence. (*nptII*, *nptII* selection cassette).

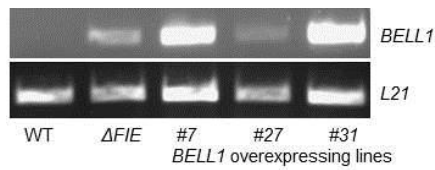


Supplementary Fig. 7 | Sperms of $\Delta BELL1$. First column: Phase contrast micrographs of sperm masses of WT $\Delta BELL1-2$, -4 , and -9 . Middle column: Epifluorescent micrographs of propidium iodide-stained dead cells, showing that the majority WT and $\Delta BELL1$ sperms are viable. Last column: Merged image of the phase contrast and epifluorescent images. Scale

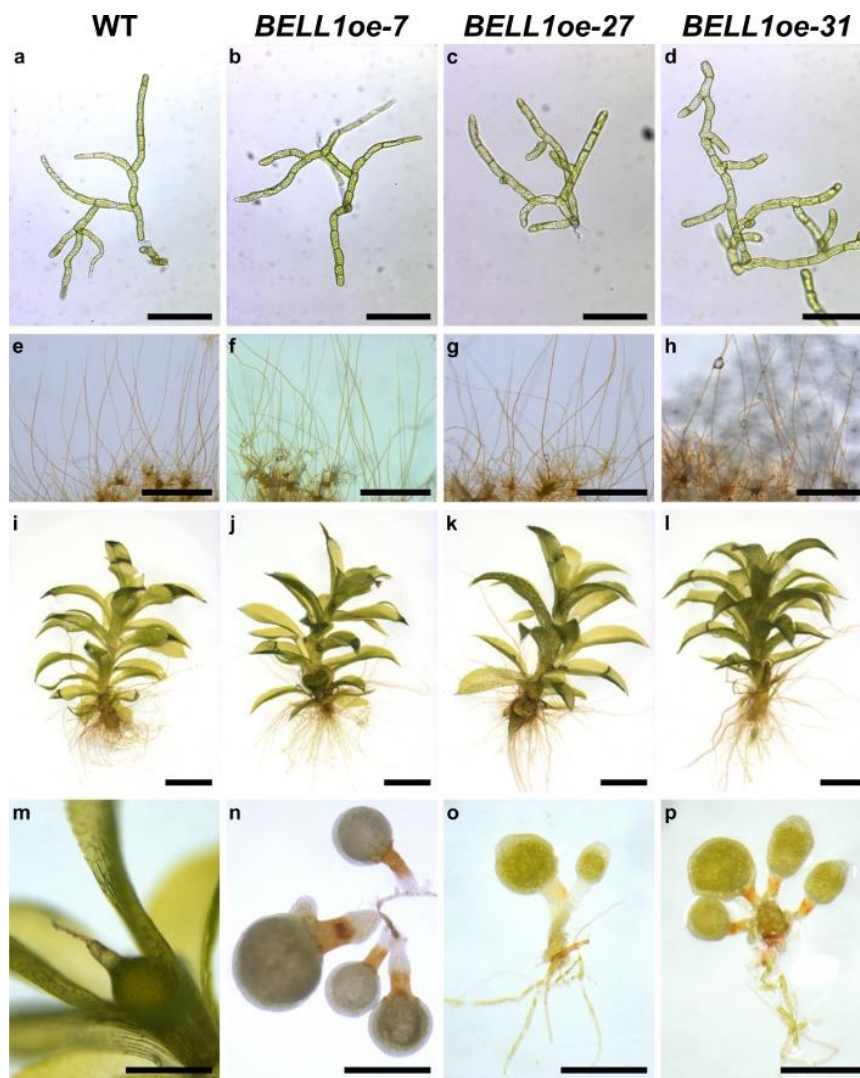
bars: 20 μm . The number of analysed antheridia was 9 (WT), 13 (*ΔBELL1* line 2), 3 (*ΔBELL1* line 4), and 11 (*ΔBELL1* line 9), respectively.



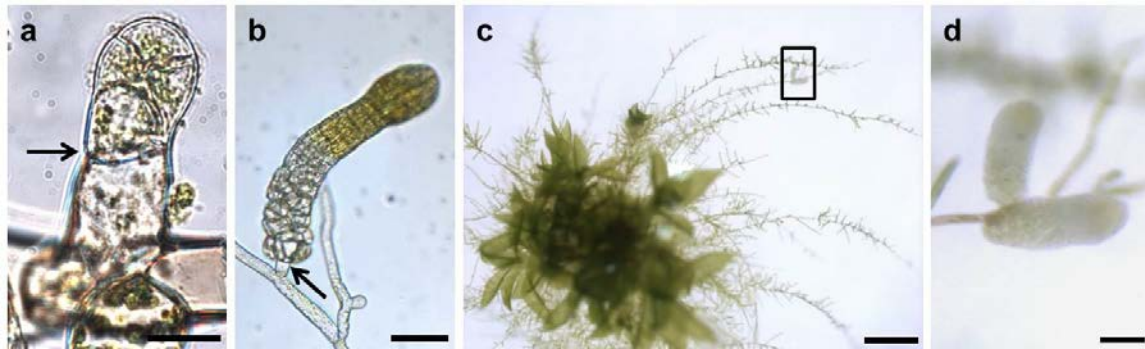
Supplementary Fig. 8 | Constitutive expression of GFP driven by the Actin5 promoter *Ppact5*. Bright field image of chloronemata and caulonemata (arrow) of a transgenic moss line expressing GFP under the control of the Actin5 promoter. Epifluorescent image of the identical sample showing fluorescence of the Green Fluorescent Protein GFP in all cells, chloronemata as well as caulonemata. Scale bar: 50 μm .



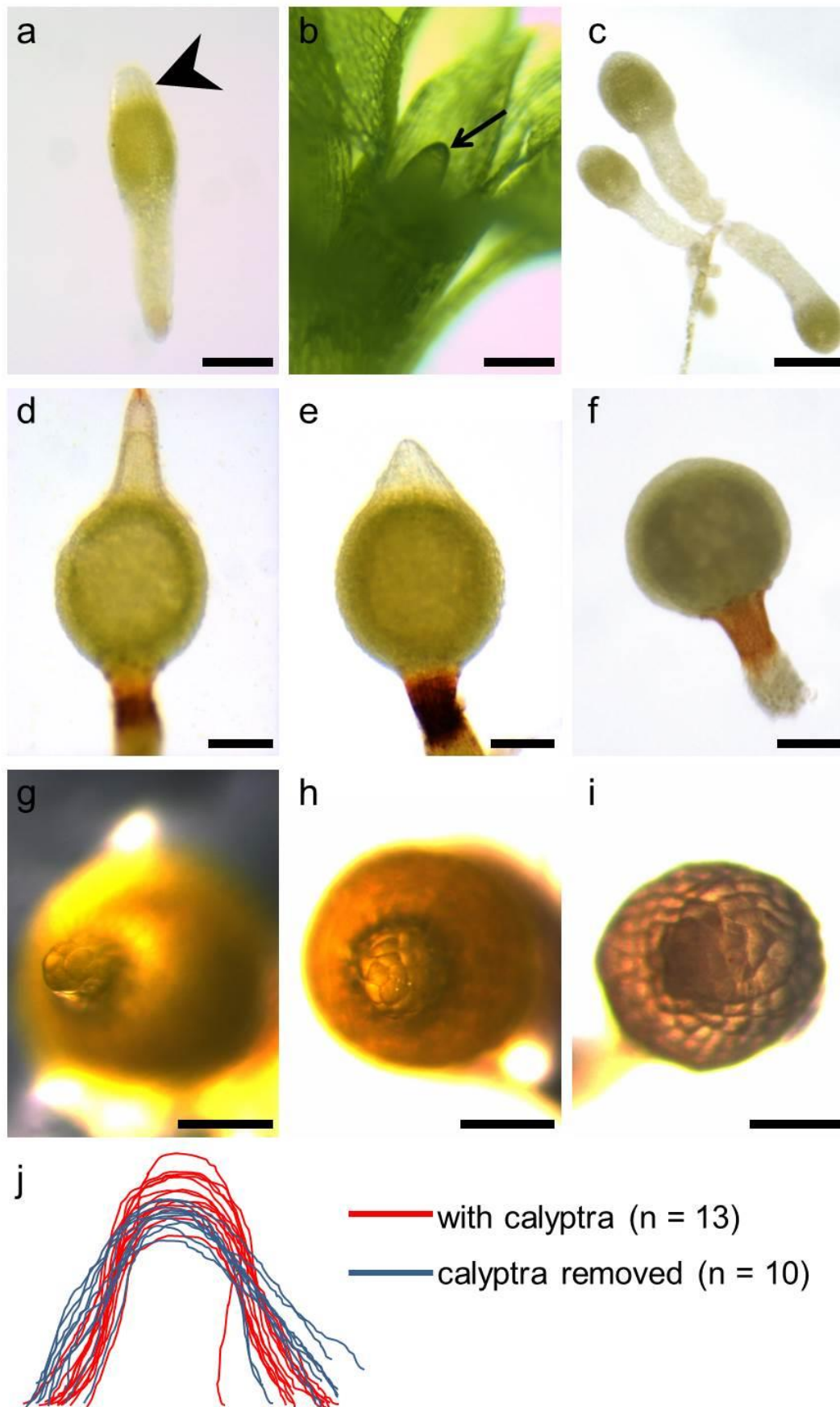
Supplementary Fig. 9 | Analysis of *PpBELL1oe* lines. Expression analysis via RT-PCR of *PpBELL1* in WT, ΔFIE and overexpressor lines *BELL1oe-7*, -27 and -31. *L21* (Pp1s107_181V6.1) coding for the 60S ribosomal protein was used to monitor amounts of input RNA.



Supplementary Fig. 10 | Protonema, leafy shoots, and apogamous sporophytes of *PpBELL1oe* lines. **a-d**, Chloronemata from liquid cultures 7 days after the last disruption. **e-h**, Negatively gravitropic grown caulonemata after 14 days incubation in the dark. **a-h**, Representative images of the samples used for quantitative Real-Time PCR of KNOX genes (Supplementary Fig. 12). **i-l**, Individual leafy shoots of four-week-old plants. **m**, WT spore capsule on a leafy shoot. **n-p**, *PpBELL1oe* apogamous sporophytes on caulonema filaments. Scale bars: 200 μ m in a-d; 2 mm in e-l; 500 μ m in m-p.

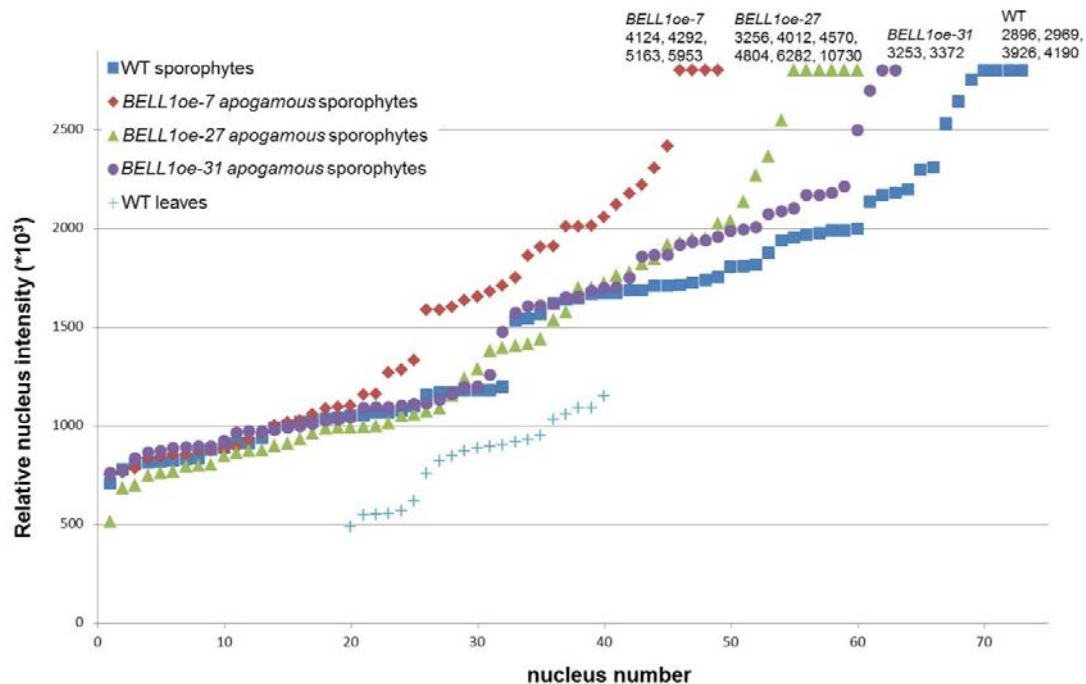


Supplementary Fig. 11 | *BELL1oe* apogamous sporophytes develop instead of side branches. **a**, Early *BELL1oe* apogamous sporophyte, the first division plane (arrow) is perpendicular to the longitudinal axis, indicative of side branch formation²⁶. **b**, *BELL1oe* apogamous sporophyte, the first cell (arrow) branching of the caulonema cell is filamentous. **c**, *BELL1oe* apogamous sporophytes (box) on caulonemata at the edge of a gametophyte colony, apart from the sporophytes only filamentous side branches are present on the protruding filaments. No leafy gametophytes have yet developed on the protruding filaments. **d**, Magnification of box in c. Scale bars: 20 μm in a; 100 μm in b, d; 1 mm in c.

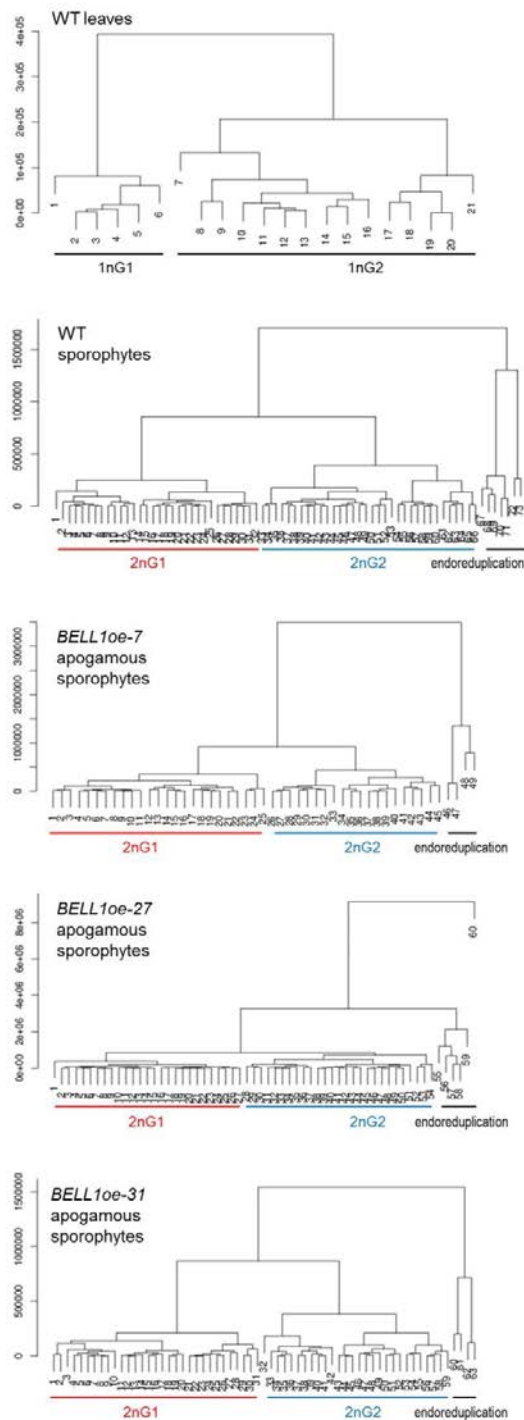


Supplementary Fig. 12 | Influence of the calyptra on the WT sporophyte shape.

Calyptrae were removed from developing sporophytes as soon as they detached from the leafy gametophyte. **a**, Developmental stage of WT embryos at calyptras removal. Note the presence of significant amount of apical tissue (arrowhead). **b**, WT embryo (arrow) with its calyptra removed on the intact plant. On intact plants it is impossible to judge the amount of apical tissue already present. **c**, *BELL1oe* apogamous embryos without apical tissue. **d**, WT sporophyte with calyptra. **e**, WT sporophyte with the calyptra removed. **f**, *BELL1oe* apogamous sporophyte that had developed freely in the medium. **g-i**, Apical views of sporophytes, developed with calyptra (g), calyptra removed (h), and *BELL1oe* apogamous sporophyte (i). **j**, Outlines of apical tips of WT sporophytes developed with (n = 13) and without (n = 10) calyptra. Scale bars: 200 μ m.

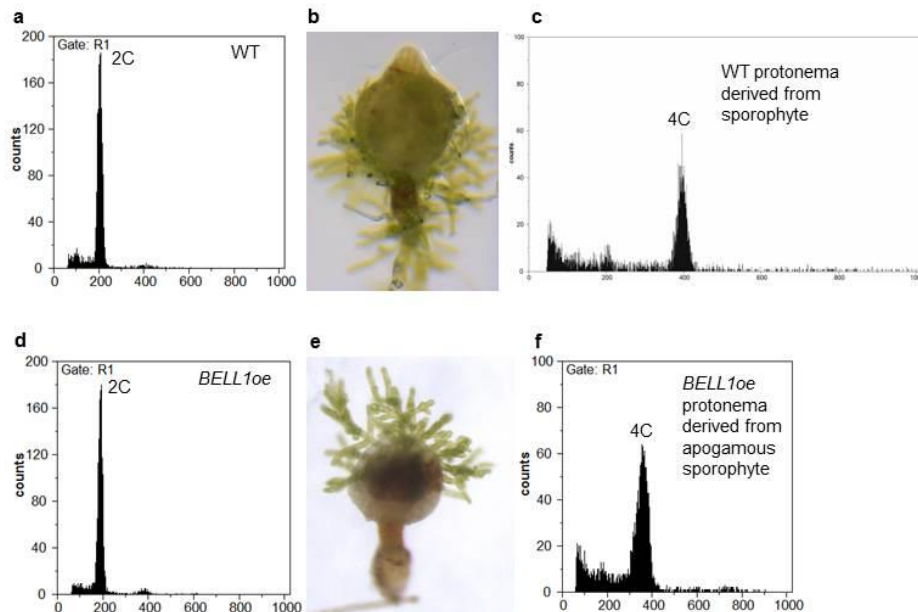


Supplementary Fig. 13 | Relative DNA content of WT sporophyte (n = 73) cells, WT leaf (n = 21) cells and *PpBELL1oe* apogamous sporophyte cells from lines 7 (n = 49), 27 (n = 60) and 31 (n = 63). Relative values were obtained by quantification of fluorescent pixels of DAPI-stained nuclei of whole-mount tissues and were plotted in ascending order. Relative DNA contents of WT haploid leaf cells were used as control for ploidy level. Data points from WT haploid leaf cells were moved to the right along the x-axis for better visibility. The analysis is described in **Supplementary Note 2** (n= sample size).

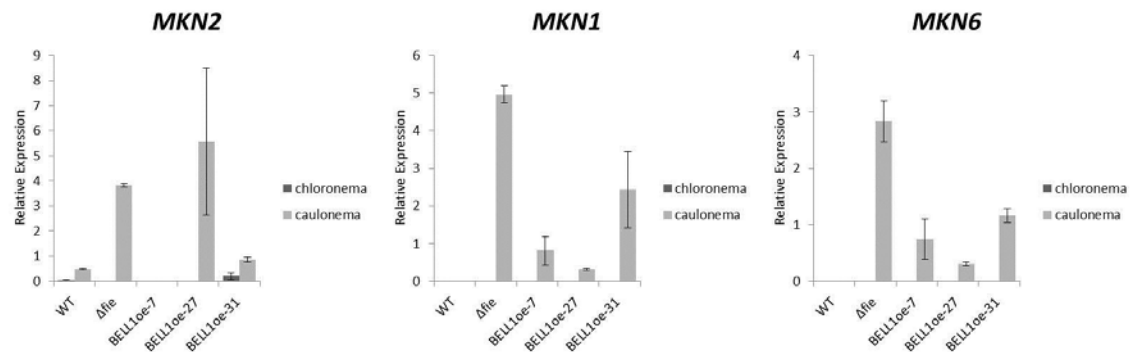


Supplementary Fig. 14 | Hierarchical clustering of relative nuclear DNA amounts of WT leaf cells, WT sporophyte cells and apogamous sporophyte cells from lines *PpBELL1oe-7*, *-27* and *-31*. Depiction of hierarchical clustering. According to their values, the clusters are named 1nG1 (leaf cells, haploid, G1-phase), 1nG2 (leaf cells, haploid, G2-phase), 2nG1 (sporophyte cells, diploid, G1-phase, red labelling), 2nG2 (sporophyte cells, diploid, G2-

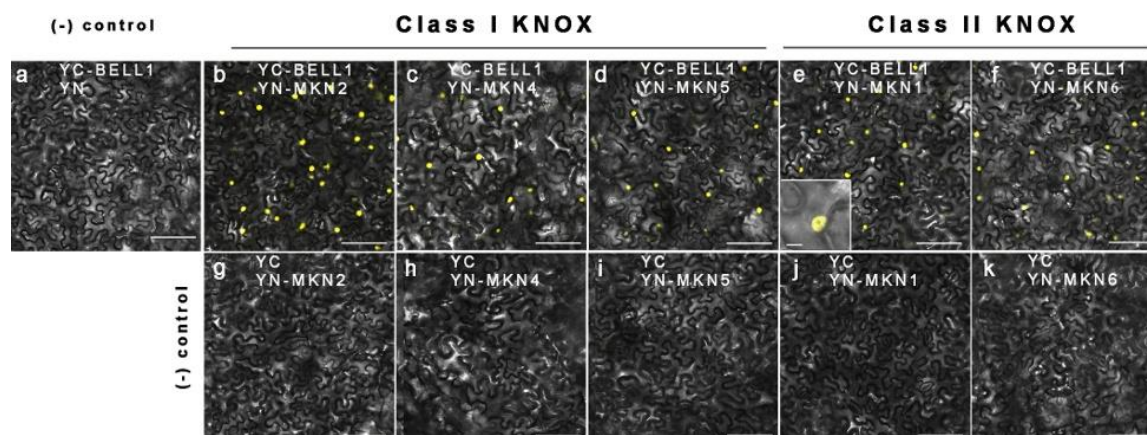
phase, blue labelling) and endoreduplication (sporophyte cells, black labelling). The analysis is described in **Supplementary Note 2**.



Supplementary Fig. 15 | Ploidy measurement of protonema cells. The DNA content of nuclei in WT protonemata (a), *BELL1oe* protonemata (d), and protonemata regenerated from a regular WT sporophyte (b, c) and an apogamous *BELL1oe* sporophyte (e, f) was determined by flow cytometry. The dominant peak at 200 fluorescent units represents haploid cells in the G2 phase of the cell cycle (2C) in the original haploid protonemata (a, d). The protonemata regenerated from sporophytes (c, f) are diploid and in G2 (4C).

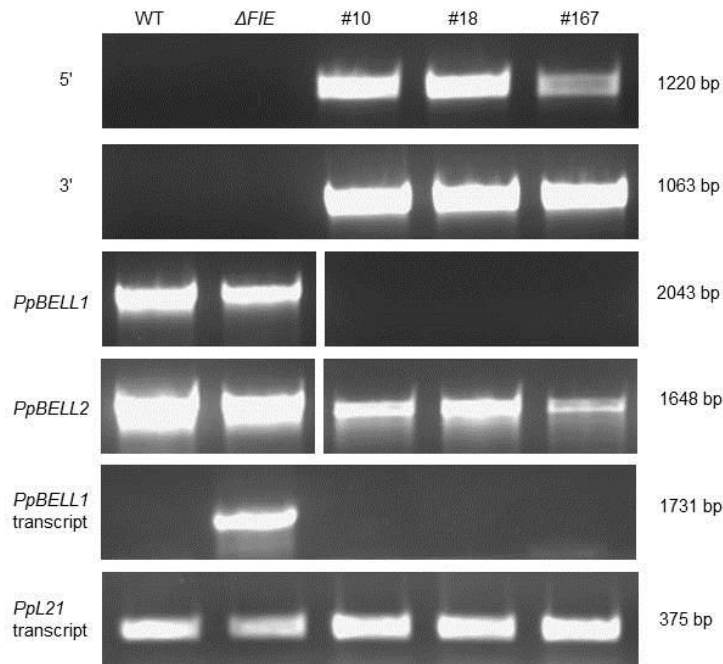


Supplementary Fig. 16 | Expression analysis via quantitative Real-Time PCR of *MKN* (*PpKNOX*) genes. Transcripts of the *PpKNOX1* genes *MKN2* and the *PpKNOX2* genes *MKN1* and *MKN6* were amplified from WT, Δ *FIE* and *BELL1oe-7*, -27, -31 plants enriched in chloronemata or caulonemata, respectively. Bars depict averages of two biological replicates with standard errors. The transcripts coding for TATA-binding protein (Pp1s246_34V6.1) and 60S ribosomal Protein L21 (Pp1s107_181V6.1) were used as reference for relative quantification.

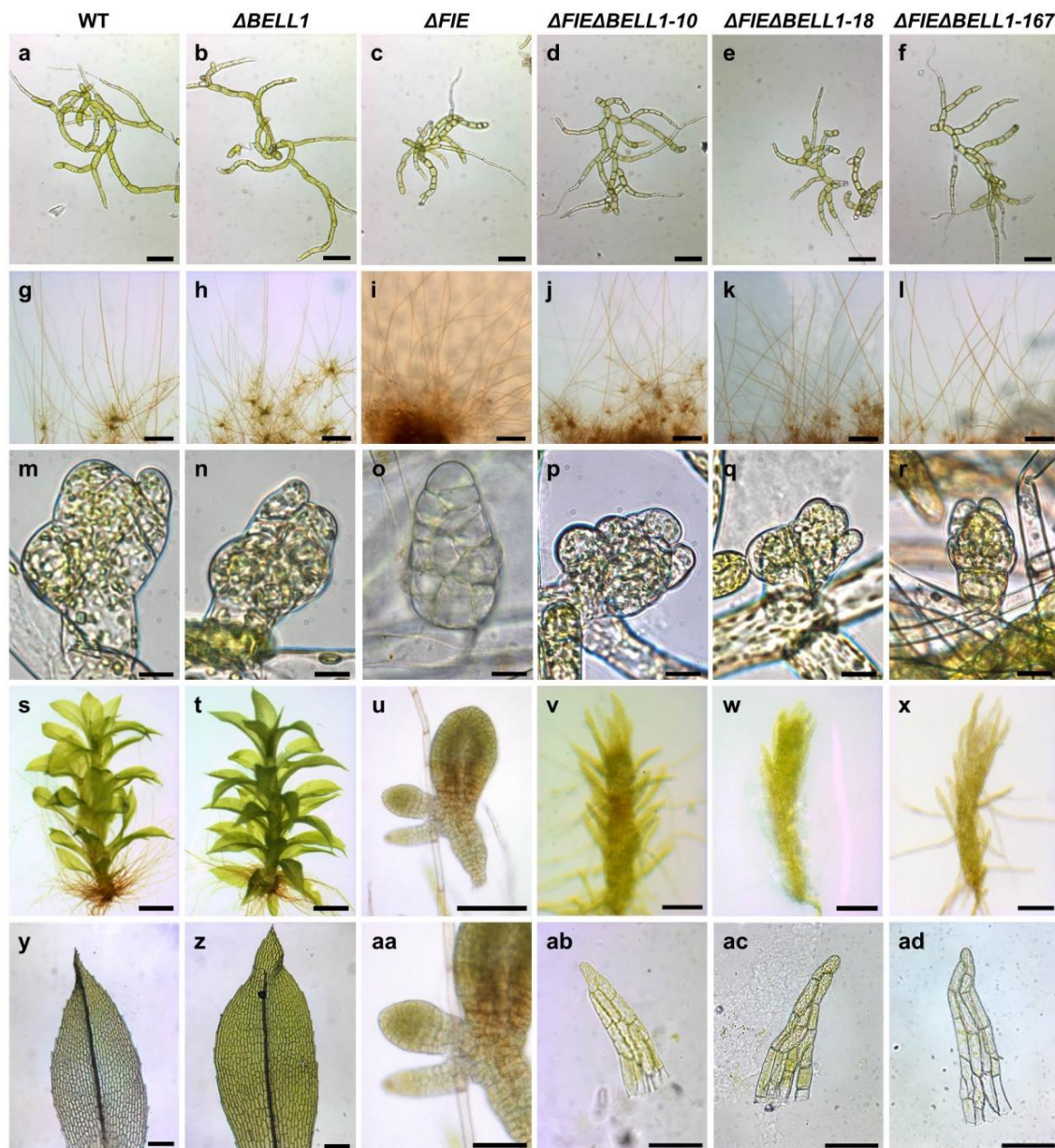


Supplementary Fig. 17 | PpBELL1 and MKN (PpKNOX) proteins interact *in planta*.

BiFC analysis of *in planta* interaction between YC-PpBELL1 and the following: **a**, YN-empty serving as negative control. **b-f**, YN-MKN2, 4, and 5 members of the class I KNOX family, MKN1 and 6 members of the class II KNOX family, respectively. Insert in panel e presents a typical fluorescent image from the nucleus which is excluded from the nucleolus. **g-k**, No signal is observed between YN-MKN2, 4, 5, 1, or 6 with YC serving as negative control. Localization was determined in leaf epidermis of *Nicotiana benthamiana*. YFP fluorescence from single confocal sections is overlaid with Nomarski Differential Interference Contrast (DIC) images. Scale bars: 100 μm; 20 μm in insert in e.



Supplementary Fig. 18 | Molecular analysis of $\Delta FIE\Delta BELL1$ mutant lines #10, #18 and #167. Rows 1 and 2: PCR verification of 5' and 3' integration of the gene disruption cassette in the $\Delta FIE\Delta BELL1$ mutant lines. Row 3: Absence of the *PpBELL1* gene in the double mutants. Row 4: Presence of the *PpBELL2* gene in the double mutants. Row 5: RT-PCR confirming the absence of the *PpBELL1* transcript in the mutant lines. Row 6: RT-PCR confirming presence of *PpL21* transcripts coding for the 60S ribosomal protein as positive control to monitor amounts of input RNA.



Supplementary Fig. 19 | Morphology of $\Delta FIE\Delta BELL1$ mutants lines -10, -18, and -167 in comparison to WT, $\Delta BELL1$, and ΔFIE . **a-f**, Chloronemata from liquid cultures 7 days after the last disruption. **g-l**, Caulonemata after 14 days incubation in the dark. **m, n, p-r**, Gametophyte buds in WT, $\Delta BELL1$, and $\Delta FIE\Delta BELL1$ mutants. **o**, Early ΔFIE sporophyte-like structure. **s, t, v-x**, Individual leafy shoots of four-week-old plants. **u**, ΔFIE branched sporophyte-like structure. **y, z, ab-ad**, Individual leaves. **aa**, Side branch of a ΔFIE sporophyte-like structure (magnification of **u**). Scale bars: 100 μm in **a-f**; 1 mm in **g-l, s, t**; 20 μm in **m-r**; 200 μm in **u-z**; 100 μm in **aa-ad**.

Supplementary Table 1 | Egg cell sizes in WT and three different *ABELL1* lines. The egg cell diameters were measured from micrographs of WT (n = 9 egg cells), *ABELL1-2* (n = 13), *ABELL1-4* (n = 3), and *ABELL1-9* (n = 11).

Egg cell diameters in μm				
WT	state of archegonium	<i>ABELL1</i>	state of archegonium	<i>ABELL1</i> line
14.43	closed	15.47	open	<i>ABELL1-2</i>
15.67	open	15.63	open	<i>ABELL1-2</i>
15.74	closed	15.82	open	<i>ABELL1-2</i>
16.76	closed	15.9	open	<i>ABELL1-9</i>
16.93	open	16.22	closed	<i>ABELL1-9</i>
17.02	open	16.32	open	<i>ABELL1-4</i>
17.3	open	16.77	open	<i>ABELL1-2</i>
17.34	closed	16.79	open	<i>ABELL1-2</i>
18.27	open	17.03	closed	<i>ABELL1-9</i>
		17.18	closed	<i>ABELL1-9</i>
		17.23	open	<i>ABELL1-9</i>
		18.04	open	<i>ABELL1-2</i>
		18.79	open	<i>ABELL1-2</i>
		19.78	closed	<i>ABELL1-9</i>
		19.84	open	<i>ABELL1-2</i>
		20.03	closed	<i>ABELL1-9</i>
		20.12	open	<i>ABELL1-2</i>
		20.26	open	<i>ABELL1-2</i>
		20.54	open	<i>ABELL1-2</i>
		20.55	open	<i>ABELL1-9</i>
		21.04	open	<i>ABELL1-2</i>
		21.85	open	<i>ABELL1-9</i>
		23.28	open	<i>ABELL1-2</i>
		23.47	closed	<i>ABELL1-9</i>
		23.7	closed	<i>ABELL1-4</i>
		23.73	open	<i>ABELL1-9</i>
		26.39	closed	<i>ABELL1-4</i>

Supplementary Table 2 | Number of *BELL1* transgene copies in the *BELL1oe* lines. The number of transgene copies was measured using quantitative Real-Time PCR. The number of *BELL1* copies (one endogenous copy and transgene copies) was determined in *BELL1oe* lines compared to the single copy in WT. Table displays the means of three technical replicates and the standard deviation.

	# copies	standard deviation	transgene copies
WT	1.1	0.04	0
<i>BELL1oe-7</i>	3.1	0.4	2-3
<i>BELL1oe-27</i>	1.6	0.09	1
<i>BELL1oe-31</i>	1.6	0.06	1

Supplementary Table 3 | Median values of clusters shown in Supplementary Fig. 14. The median values for clusters 1nG1 and 1nG2 were determined for WT and for clusters 2nG1 and 2nG2 were determined for WT and the lines *BELL1oe-7*, -27 and -31.

cluster	median values and standard deviations							
	WT		<i>BELL1oe-7</i>		<i>BELL1oe-27</i>		<i>BELL1oe-31</i>	
1nG1	549	38						
1nG2	912	109						
2nG1	1019	139	960	163	895	137	994	124
2nG2	1778	217	1904	249	1758	347	1889	211

Supplementary Movie 1 | Sperm masses from WT. Boxes mark motile sperms in focus.

Video runs at 0.5x speed. Scale bar: 20 μm .

Supplementary Movie 2 | Sperm masses from *ABELLI-2*. Boxes mark motile sperms in focus. Video runs at 0.5x speed. Scale bar: 20 μm .**Supplementary references**

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