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The *baseless* mutant links protein phosphatase 2A with basal cell identity in the brown alga *Ectocarpus*

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Summary

The first mitotic division of the initial cell is a key event in all multicellular organisms and is usually concomitant with the establishment of major developmental axes and cell fates. The brown alga *Ectocarpus* has a haploid-diploid life cycle that involves the development of two multicellular and independent generations, the sporophyte and the gametophyte. Each generation deploys a distinct developmental program autonomously from an initial cell, whose first cell division sets up the future body pattern. Here, we show that mutations in the *BASELESS* (*BAS*) gene result in multiple cellular defects during the first division of the initial cell and subsequently failure to produce basal structures (rhizoids and prostrate filaments) during both generations of the life cycle. Cloning-by-sequencing revealed that *BAS* encodes a type B" regulatory subunit of protein phosphatase 2A, and transcriptomic analysis of early developmental stages uncovered potential effector genes involved in setting up basal cell fate in this organism. The *bas* mutant phenotype is very similar to that observed in the *distag* (*dis*) mutants, which lack a functional TBCCd1 protein, at both the cellular and morphological levels. The high level of similarity of the *dis* and *bas* mutant phenotypes indicate that TBCCd1 and PP2A are two critical components of the cellular machinery that regulates the division of the initial cell and mediates the establishment of basal cell fate in the developing thallus.

35 Introduction

36 In most animals and plants key events during the first cell division lead to the establishment of
37 one or more major body axes, providing the foundation for the deployment of the subsequent
38 steps of multicellular developmental programs (reviewed in (Radoeva et al., 2019; Rose and
39 Gönczy, 2014). In animals, partitioning defective (PAR) proteins play a key role in establishing
40 the anterior/posterior body axis, whilst a number of pathways are involved in establishing the
41 dorsoventral axis (e.g. Mongera et al., 2019). In the land plant *Arabidopsis*, both the apical/basal
42 axis and apical/basal cell identities are established at the time of the first cell division, and
43 genetic analysis has identified two main pathways involved in this process (reviewed in Bayer et
44 al., 2017; Ueda and Berger, 2019). The first pathway involves SHORT SUSPENSOR (an interleukin-
45 1 receptor-associated kinase/Pelle-like kinase), YODA (a MAP kinase kinase), MPK3 and MPK6
46 (MAP kinases) and downstream transcription factors, which may include WRKY2 and
47 GROUNDED/RKD4. This pathway may also be influenced maternally through secreted peptide
48 factors such as EMBRYO SURROUNDING FACTOR1 and CLV3/ESR-RELATED8. The second
49 pathway involves auxin and consists of PIN-FORMED 7 (auxin efflux regulator),
50 MONOPTEROS/AUXIN RESPONSE FACTOR 5 (transcription factor) and BODENLOS/INDOLE-3-
51 ACETIC-ACID 12 (auxin response inhibitor). The establishment of zygote polarity is a pre-
52 requisite of the asymmetrical first cell division. This process involves movement of the nucleus
53 and other organelles, enlargement of the vacuole, and reorganization of microtubules (Kimata
54 et al., 2016; Ueda and Berger, 2019).

55 The mechanisms underlying the onset of early development from an initial cell in multicellular
56 plants and animals are relatively well understood, but research has lagged behind for the third
57 most complex group of multicellular eukaryotes, the brown algae. The brown algae offer an
58 interesting contrast to animals and plants because of their phylogenetic position and the fact
59 that they evolved complex multicellularity independently from those groups. Moreover, in
60 brown algae, the two generations are independent and often very distinct morphologically. The
61 same genome, therefore, regulates the set-up of two independent and distinct developmental
62 programs from two different types of initial cells, opening interesting questions about the
63 molecular control of alternation of generations (Arun et al., 2019; Cock et al., 2014; Coelho et
64 al., 2007; Coelho et al., 2011). Furthermore, gametophytes and sporophytes of brown algae
65 develop from single cells outside the parent organism, indicating that they likely establish
66 polarity in a cell-autonomous manner, without the involvement of factors delivered from the
67 parental tissues, simplifying the study of polarity, axis establishment and pattern formation.

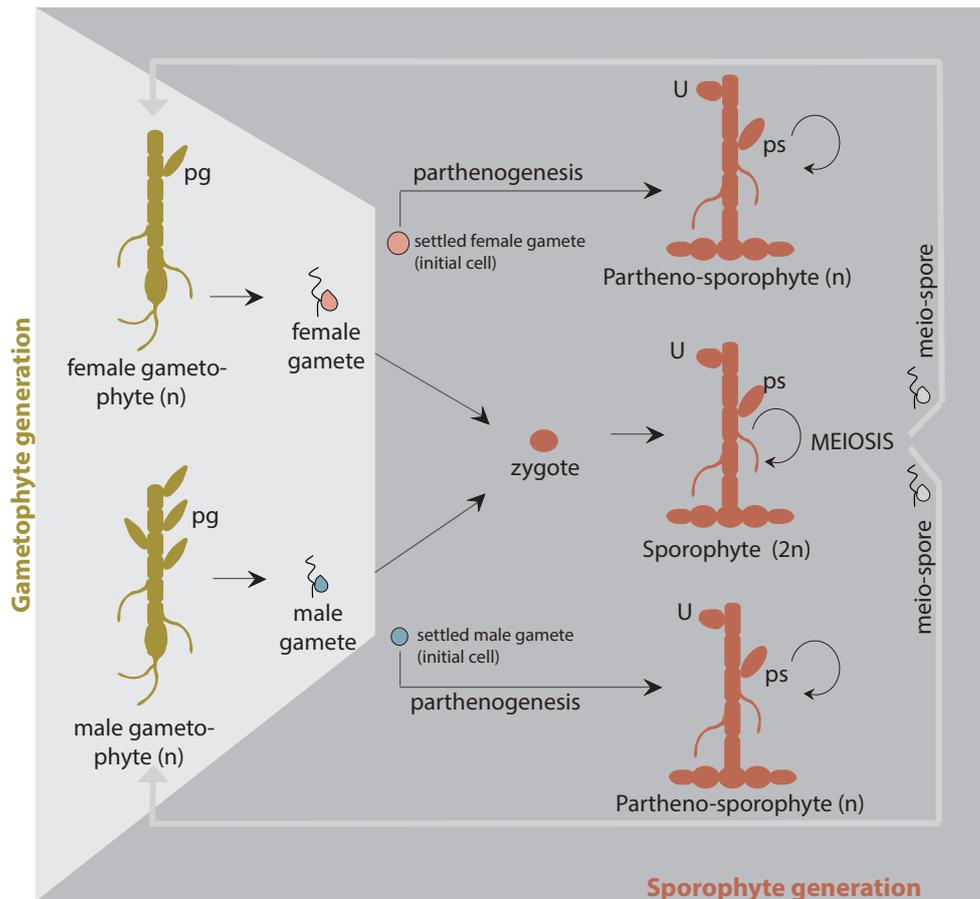
68 Investigations using the brown alga *Fucus* have shown that asymmetrical first cell division is
69 driven by apical-basal polarity established within the zygotic cell (Brownlee and Bouget, 1998;
70 Goodner and Quatrano, 1993); reviewed in Bogaert et al., 2022). The two daughter cells of this
71 first division divide to produce the apical and basal systems of the alga, the thallus and the
72 rhizoid, respectively (Brownlee and Bouget, 1998). Studies using *Fucus* zygotes have underlined
73 the role of Ca²⁺ asymmetries, mRNA distribution and position-dependent information from the
74 cell wall (involving an unknown diffusible apoplastic factor) in the determination of the fate of

75 the basal and apical systems (Berger et al., 1994; Bogaert et al., 2022; Bouget et al., 1996;
76 Brownlee and Bouget, 1998).

77 More recently, *Ectocarpus* has emerged as a suitable model to investigate the molecular
78 mechanisms underlying initial cell division and cell fate determination in the brown algae
79 (Bogaert et al., 2022). Advantages of this model include the availability of a range of genetic and
80 genomic tools (Avia et al., 2017; Badis et al., 2021; Bourdareau et al., 2021; Cock et al., 2017;
81 Coelho et al., 2020; Cormier et al., 2017; Umen and Coelho, 2019) but also, importantly, the
82 regularity of the first cell division that characterises the early stages of development of both the
83 gametophyte and sporophyte generations. In this organism, the gametophyte generation
84 exhibits an asymmetrical initial cell division that produces a basal rhizoid cell and an apical cell,
85 the latter further dividing to form the apical system of upright filaments. The upright filaments
86 bear the reproductive structures (plurilocular gametangia, which produce the gametes by
87 mitosis). In contrast, the initial cell of the sporophyte generation undergoes a symmetrical initial
88 cell division to produce two daughter cells with similar fates, both being components of the
89 basal system (Godfroy et al., 2017; Peters et al., 2008)(Figure 1). The apical system of the
90 sporophyte (upright filaments) are produced later, after an extensive system of basal filaments
91 has been established. Reproductive structures (unilocular and plurilocular sporangia) are
92 produced on the upright filaments.

93 Earlier work identified an *Ectocarpus* mutant, *distag* (*dis*), with abnormal cellular features during
94 the first cell division, and that was unable to develop basal systems (rhizoids in the
95 gametophyte, prostrate filaments in the sporophyte)(Godfroy et al., 2017). *dis* mutant alleles
96 exhibit a strong phenotype in the initial cell, with disordered microtubule networks, larger cell
97 size, altered Golgi structure and mispositioned nuclei and centrioles (Godfroy et al., 2017). The
98 cell division plane, however, is normal and the cellular defects are only observed during the
99 division of the initial cell, suggesting that *DIS* function may be specific to this cell. *DIS* encodes
100 a Tubulin Binding Cofactor C (TBCC) domain protein of the TBCCd1 class, with conserved roles
101 in animal and plants (André et al., 2013; Feldman and Marshall, 2009).

102 Here, we report the identification of the *BASELESS* (*BAS*) locus in *Ectocarpus*. *bas* mutants
103 exhibit phenotypes that closely resemble those of *dis* mutants, including an atypical initial cell
104 division that leads to failure to deploy a basal system in the adult organism, abnormal cellular
105 features such as disorganised microtubule cytoskeleton, loss of bipolar germination and more
106 extensive Golgi apparatus compared with wild-type cells. These phenotypic features are
107 underlie by important modifications in patterns of gene expression during very early stages of
108 development. *BAS* encodes a protein phosphatase 2A regulatory subunit type B" with EF-hand
109 domains. Together, our results are consistent with *BAS* being involved in a pathway that plays a
110 key role in initial cell division and basal cell fate determination during both the gametophyte
111 and sporophyte generations of the *Ectocarpus* life cycle.



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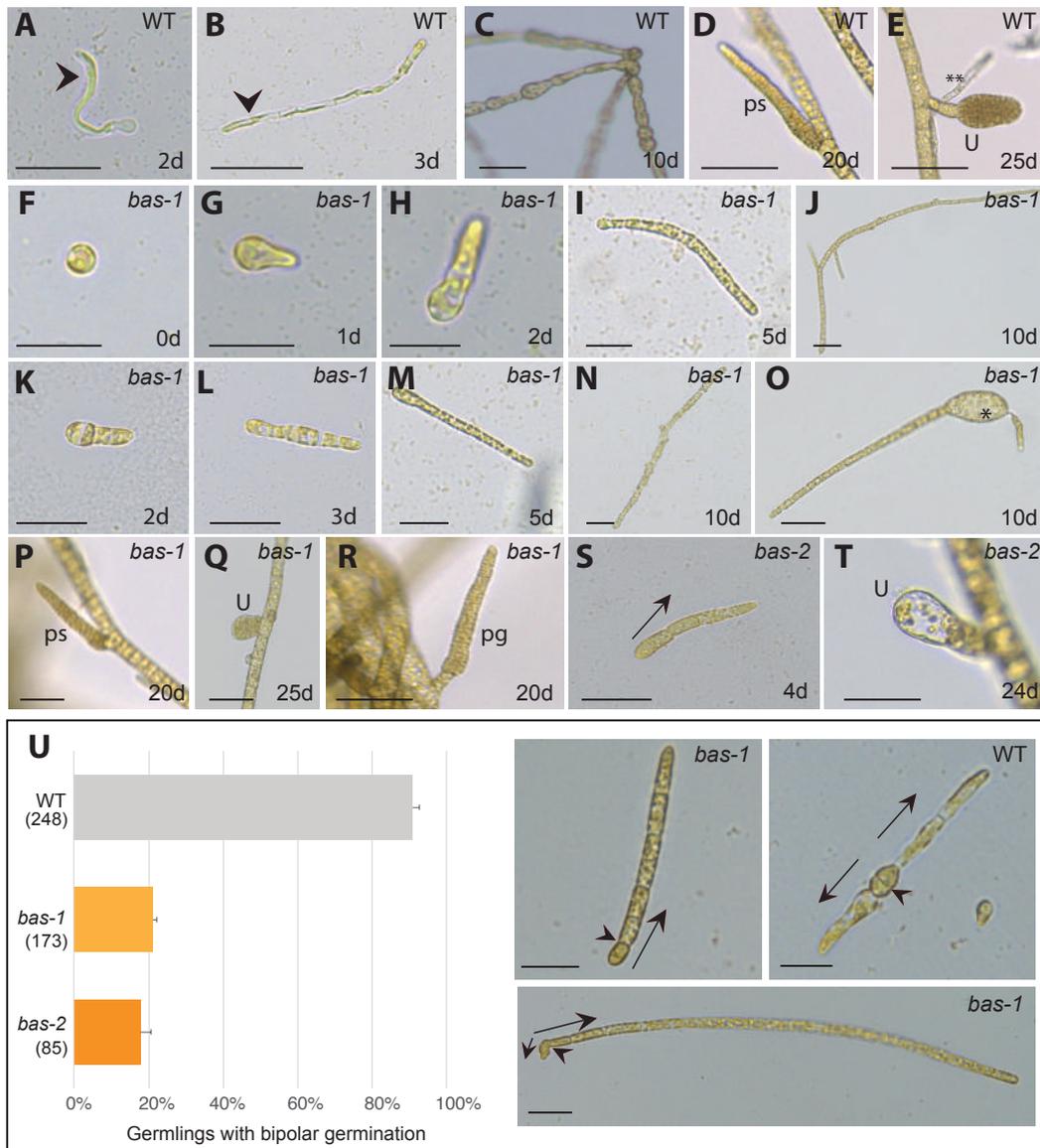
Figure 1. Schematic view of the life cycle of *Ectocarpus* sp.. Like most brown algae, *Ectocarpus* has a haploid-diploid life cycle with haploid, genetic sex determination (Coelho and Cock, 2020; Coelho et al., 2020). Male and female gametophytes produce male and female gametes, respectively, which are released into the seawater from plurilocular gametangia (pg). Gamete fusion produces a zygote which will initiate the (diploid) sporophyte generation. However, gametes that do not meet a gamete of the opposite sex settle and lose their flagella, and they may still function as initial cells of the sporophyte generation because they can develop parthenogenically into a (haploid) partheno-sporophyte. Note that there is no visible (morphological) difference between haploid and diploid sporophytes. Meiosis occurs in the sporophyte (in the unilocular sporangia, U), producing several hundred haploid meio-spores. These meio-spores are released into the seawater and develop into male or female gametophytes. Partheno-sporophytes and diploid sporophytes may also be maintained by production of mitospores in plurilocular sporangia (ps): released mitospores recycle the partheno-sporophyte generation (circular arrow in the scheme).

113 Results

114 **baseless** mutants lack a basal system during both the sporophyte and gametophyte generations

115 During the *Ectocarpus* gametophyte generation, the two cells derived from the division of the
 116 initial cell develop as two germ tubes, and establish a rhizoid (a basal, root-like organ) and an
 117 upright filamentous thallus (an apical, shoot-like organ) (Figure 2A, 2B). The first cell division of
 118 the sporophyte generation, in contrast, produces two daughter cells with similar morphology
 119 and equivalent cell fates (Figure 2C). These two cells then divide to produce a prostrate filament,
 120 which branches to establish the basal system and the apical system differentiates later in

121 development, growing up into the medium from the extensive, prostrate basal system.
 122 Reproductive structures are produced on the apical system (Figure 2D, 2E).



123

Figure 2. Phenotypes of *bas* mutants. A) Wild-type gametophyte germling, the arrowhead indicates the rhizoid cell (basal structure). B) Three-day old wild-type gametophyte, the arrowhead indicates the rhizoid. C) Wild-type sporophyte generation composed of round prostrate filaments firmly attached to the substrate. D) Wild-type plurilocular sporangium containing mitotic spores, produced after 20 days in culture. E) Wild-type unilocular sporangium (where meiosis takes place) produced after 25 days in culture. A secondary rhizoid is indicated by asterisks (**). (F-J) Development of the gametophyte generation of the *bas-1* mutant. (K-N) Development of the sporophyte generation of *bas-1* mutant. O) Occasionally, the mutant strains produced enlarged, abnormal cells (asterisk). P) Plurilocular sporangium on a *bas-1* mutant sporophyte. Q) Unilocular sporangium on a *bas-1* mutant sporophyte. R) Plurilocular gametangium on a fertile *bas-1* gametophyte. S) Initial cell division of a *bas-2* gametophyte. T) Aborted unilocular sporangium on a mature *bas-2* sporophyte (about 3 weeks after initial cell germination). U) Proportions of 10-day old *bas-1* and wild-type germlings that exhibited unipolar germination. Plots represent the mean and SE of five replicate cultures, the total number of germlings scored are indicated in brackets. The photographs are of representative *bas-1* and wild-type (WT) germlings, exhibiting uni- (one arrow) or bi-polar (two arrows) germination, respectively. Arrows indicate the direction of germination. Note that, in the *bas-1*

mutant, following the first cell division, one of the daughter cells continues to divide to produce an upright filament but division of the other daughter cell is arrested. Arrowheads indicate the division plane of the initial cell, which is perpendicular to the growth axis both in the *bas* mutants and in the wild type. *ps*, plurilocular sporangium; *pg*, plurilocular gametangium; *u*, unilocular sporangium. Scale bars=20 μ m.

124 A screen of a large population of individuals mutagenised by ultraviolet (UV) irradiation
125 identified two mutant strains (Ec800 and Ec801; Table S1) that failed to develop any of the basal
126 structures normally observed during either the gametophyte or the sporophyte generation of
127 wild-type strains (Figure 2). The screen used gametes, which in absence of fertilisation develop
128 into partheno-sporophytes, being thus initial cells of the sporophyte generation (Figure 1)
129 (Coelho et al., 2011; Godfroy et al., 2015; Godfroy et al., 2017; Peters et al., 2008). Initial cells
130 of Ec800 and Ec801 gametophytes immediately developed as apical upright filament cells and
131 no rhizoid cells were produced. Similarly, during the sporophyte generation, neither mutant
132 strain produced the network of prostrate basal filaments typical of the wild-type sporophyte
133 and, instead, the first divisions of the initial cell directly produced an upright filament (Figure
134 2).

135 In wild-type *Ectocarpus*, secondary rhizoids, which are analogous to the adventitious roots
136 produced from the stems of some land plants (Atkinson et al., 2014), are produced from apical
137 upright filament cells at a late stage of development (Figure 2E) (Peters et al., 2008). The Ec800
138 and Ec801 mutants did not produce secondary rhizoids (Figure 2, Figure S1). Hence, production
139 of all basal, attachment structures, both primary and secondary, was abolished in these
140 mutants. Taking into account these phenotypes, the Ec800 and Ec801 mutants were named
141 *baseless-1* (*bas-1*) and *baseless-2* (*bas-2*) respectively.

142 The establishment of reproductive structures on apical systems in both the gametophyte and
143 sporophyte generations was unaffected in the *bas-1* mutant, which was fully fertile after three
144 weeks in culture (Figure 2F-2S). In the *bas-2* mutant, the formation of the plurilocular sporangia
145 (which contain mito-spores) was unaffected whereas unilocular sporangia (where meiosis takes
146 place) aborted and no functional meio-spores were produced, preventing generation of
147 gametophytes (Figure 2T).

148 ***bas* mutants exhibit reduced bipolar germination compared with wild-type strains**

149 In wild-type *Ectocarpus*, the majority of the initial cells (91%) exhibited a bipolar pattern of
150 germination, with two germ tubes emerging from opposite poles of the initial cell (Figure 2U;
151 Peters et al., 2008). In contrast, only 21% of the initial cells of *bas-1* partheno-sporophytes
152 exhibited this bipolar pattern of germination, the remaining 79% undergoing unipolar
153 germination (Figure 2U). A proportion of the *bas-1* partheno-sporophytes that exhibited a
154 bipolar germination pattern produced one or more enlarged and abnormally shaped cells at the
155 extremity where the second germ tube would normally emerge, possibly corresponding to an
156 aborted germ tube (Figure 2O). Similar phenotypes were observed for *bas-2* partheno-
157 sporophytes (Table S2).

158 **Disorganisation of the microtubule cytoskeleton in *bas* mutant initial cells**

159 Mutations at the *DIS* locus strongly affect the organisation of the microtubule cytoskeleton
160 (Godfroy et al., 2017). Because of the similarity between the morphological phenotypes of *bas*
161 and *dis* mutants, we investigated the distribution of the microtubule network during early
162 development of *bas* mutants compared with wild-type germlings (Figure 3A-B). The
163 microtubule cytoskeleton was markedly disorganised in the *bas* mutants, with supernumerary
164 microtubule filaments and a disordered network (Figure 3C). This microtubule phenotype is
165 reminiscent of that of the *dis* mutant (Godfroy et al., 2017). Also, similarly to the *dis* mutant, we
166 did not detect any abnormalities in the positioning of the cell division plane during early
167 development; all *bas* initial cells produced a cell division plane perpendicular to the growing
168 axis (Figure 2U).

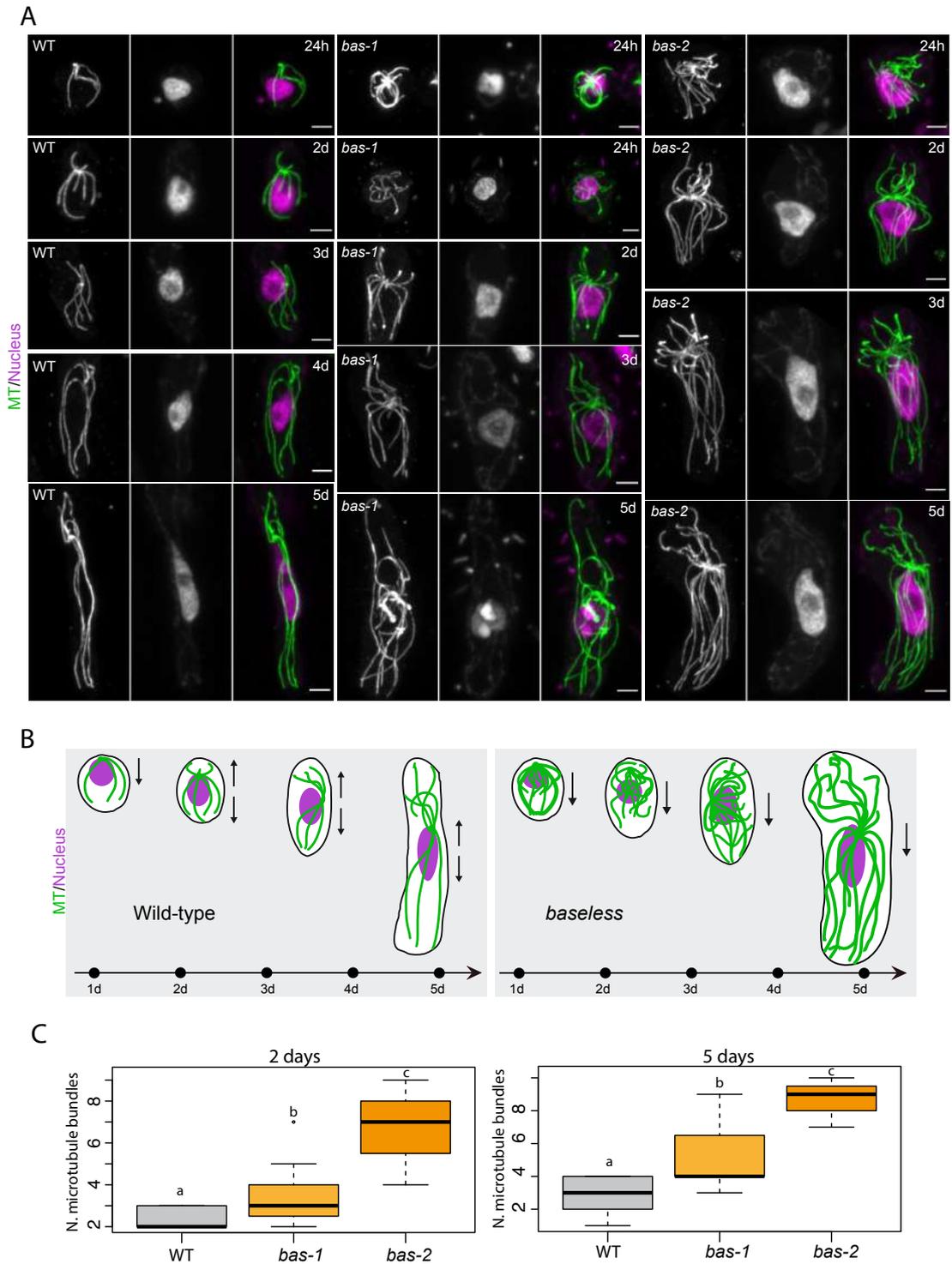


Figure 3. The organization of the microtubule cytoskeleton is affected in *bas* mutant germinating cells. A) Confocal maximum z-projections showing representative cells of wild type, *bas-1*, and *bas-2* partheno-sporophytes at several stages of early development (24h, 2-5 days). Microtubules (MT) were immune-stained with an anti-tubulin antibody (green). Nuclear DNA was counterstained with DAPI (mauve). Microtubule (MT) bundles were wavy and more abundant in both *bas-1* and *bas-2* mutant cells compared with the wild-type during the germination of the initial cell. B) Cartoons summarising the stages shown in A) in wild-type and *bas* mutants. C) Number of microtubule bundles during germination in wild-type (WT), *bas-1* and *bas-2* mutants at 2 days and 5 days after germination of the initial cell of the sporophyte generation.

170 **Ultrastructural analysis of *bas* initial cells**

171 Transmission electron microscopy (TEM) and Focused Ion Beam–Scanning Electron Microscopy
172 (FIB-SEM) were used to further characterise the cellular architecture of *bas* mutants. We
173 focused on the early development of the sporophyte generation, i.e., when unfused gametes
174 had started developing into partheno-sporophytes (2-5 cells), which is the stage when
175 conspicuous morphological differences were observed (Figure 2). This is also the stage when
176 *dis* mutants exhibit altered subcellular phenotypes, including significantly more abundant
177 cisternae and more fragmented Golgi compared with wild-type cells (Godfroy et al., 2017).

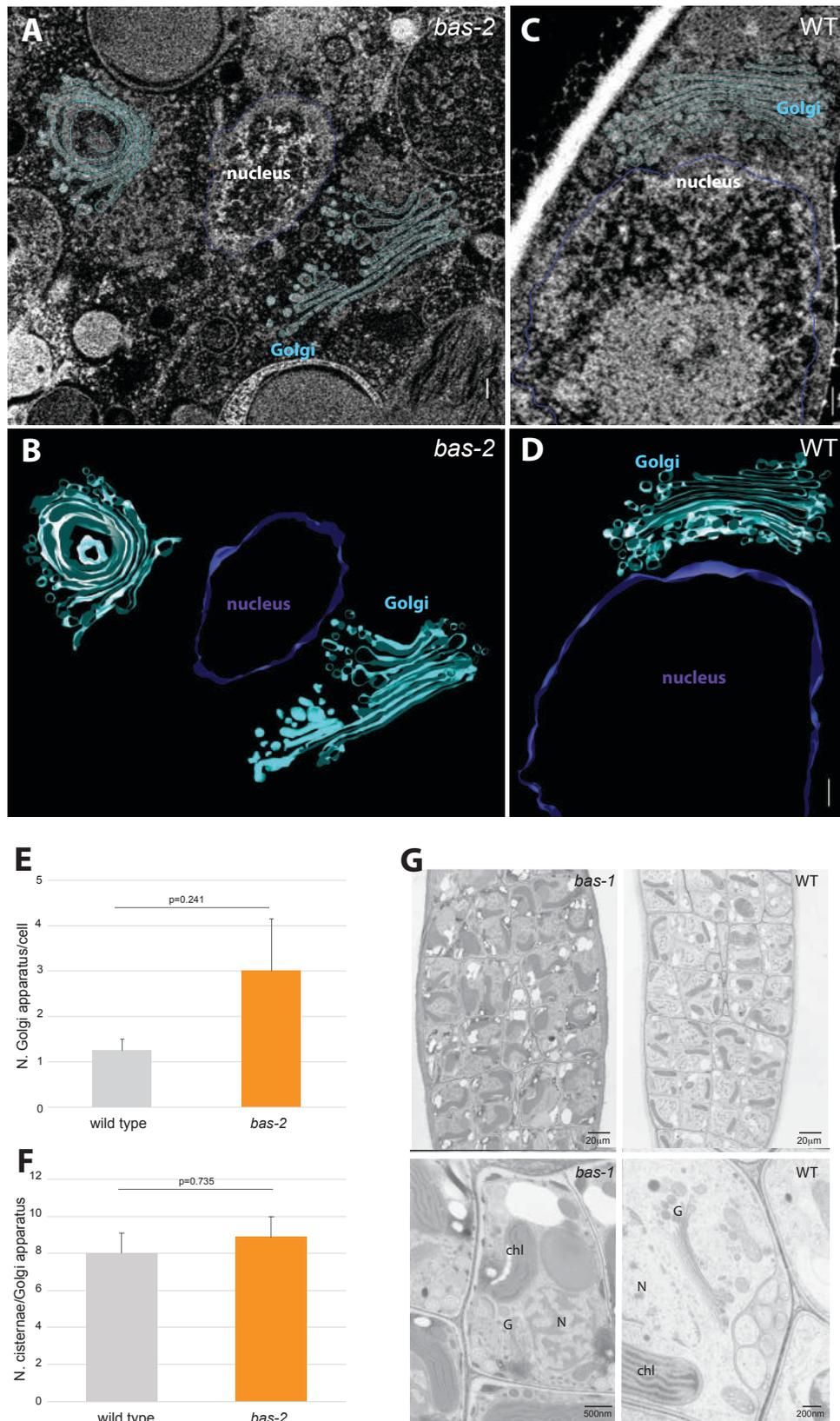
178 Morphometric analyses of the subcellular structures in *bas* mutant and wild-type indicated that
179 the Golgi apparatus were about twice as numerous in *bas* as in wild-type at the same
180 developmental stage, but this difference was not significant (Figure 4E; Table S3). We also
181 noticed that the number of cisternae per Golgi tended to be slightly higher in *bas* (Figure 4F,
182 Table S3). Therefore, *bas* exhibited a Golgi defect, but this defect appeared to be less
183 conspicuous than that of *dis* mutants. We did not observe any other organellar defects in *bas*
184 cells, such as abnormal structure or position of the nucleus, centrioles, mitochondria or
185 chloroplasts. Moreover, no visible defect, in particular at the Golgi, could be observed in the
186 gametes prior to their release from the plurilocular gametangia (Figure 4G). This indicates that
187 *bas* cellular defects are detectable only once the initial cell initiates germination.

188 **Genetic analysis of the BAS gene**

189 A male *bas-1* gametophyte (Ec800) was crossed with a wild-type female gametophyte (strain
190 Ec25; (Table S1 and Figure S2). The resulting sporophyte (Ec805) exhibited a wild-type pattern
191 of development, indicating that the *bas-1* mutation was recessive. A segregating family of 38
192 partheno-sporophyte individuals derived from this cross consisted of 16 and 22 phenotypically
193 wild-type and mutant individuals, respectively, consistent with a 1:1 segregation ratio and
194 Mendelian inheritance of a single-locus recessive mutation (Chi-square test with Yates
195 correction = 0.4767, p-value = 0.4899; Table S5). The *bas-1* mutation segregated with the
196 phenotype in the 38 individuals used for the phenotype segregation analysis (Table S4).

197 ***bas-1* and *bas-2* resemble *dis* mutants but are unaffected in the *DIS* gene**

198 The phenotypes of *bas-1* and *bas-2* strongly resembled that of the *dis* mutant (Godfroy et al.,
199 2017). The *dis* mutant also fails to produce any basal structures, during both the sporophyte
200 and gametophyte generations, and lacks secondary rhizoids, again during both generations.
201 Sporophytes resulting from crosses either between the *bas-1* strain Ec800 and strains carrying
202 either the *dis-1* or the *dis-2* allele had wild-type phenotypes (Figure S3 and Table S1), indicating
203 complementation, and therefore that the *DIS* gene was not mutated in the *bas-1* mutants.



204

Figure 4. Sub-cellular architecture of wild-type and *bas* (*bas-2*) germinating cells. 3D visualization with FIB-SEM (focused ion beam scanning electron microscopy) of representative wild-type (A-B) and *bas-2* mutant (C-D) developing sporophytes (2 cell stage), with Golgi (cyan) and nucleus (violet) highlighted. The scale bar represents 200 nm. E) Number of cisternae per Golgi apparatus in wild-type and *bas-2* developing sporophytes (2 cell stage). F) Number of Golgi apparatus per cell in wild-type and *bas-2* developing sporophytes (2 cell stage). G) Plurilocular

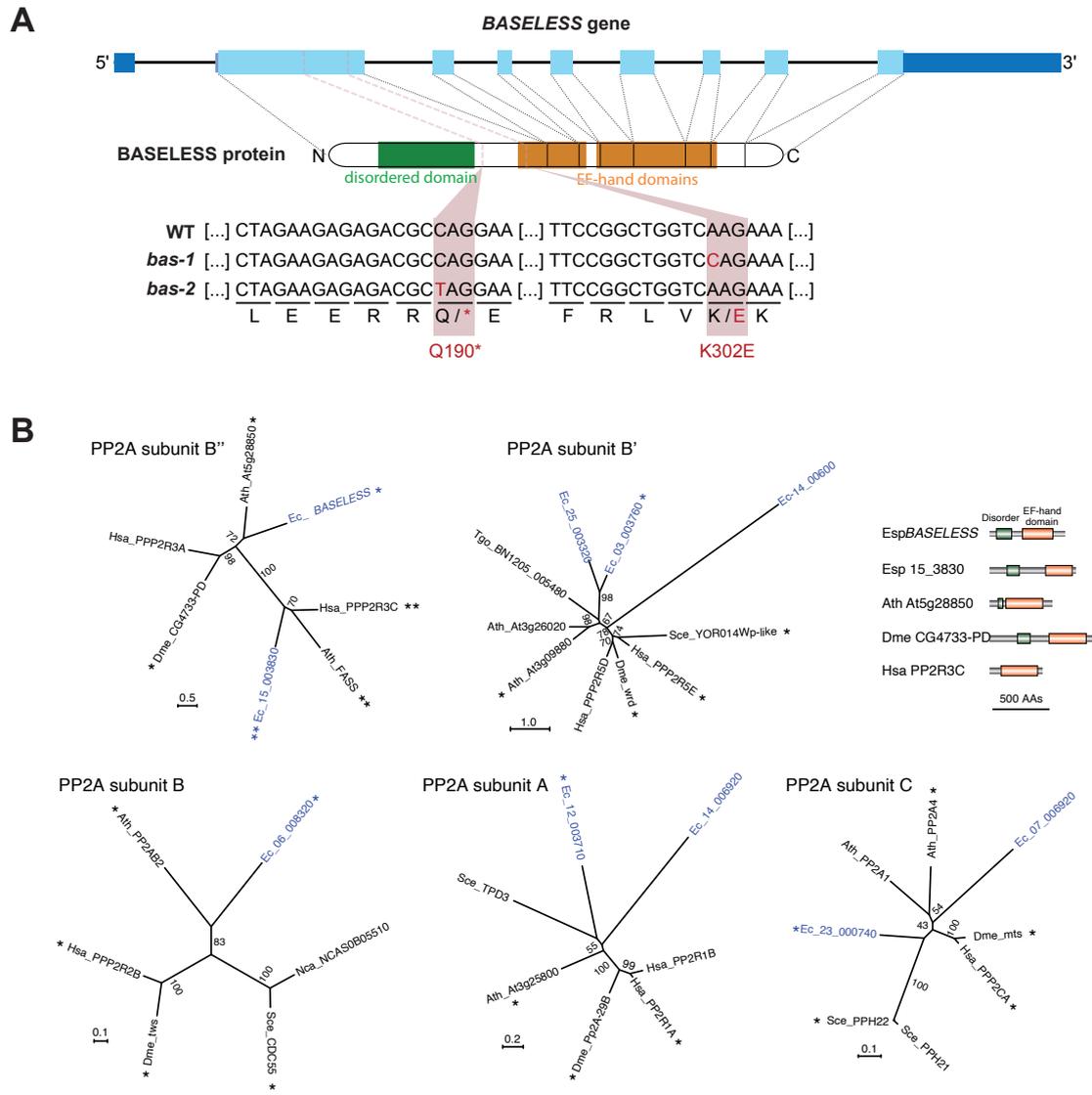
gametangia in bas-1 and WT, filled with gametes. Note that no difference in the ultrastructure could be observed in the mutant compared with the wild-type samples prior to the release of the gametes from the plurilocular gametangia. Chl: chloroplast, G: Golgi; N: nucleus.

205 **BAS encodes a protein phosphatase 2A type B'' regulatory subunit**

206 Genome resequencing identified a candidate locus on chromosome 21 for the location of the
207 *bas-1* and *bas-2* mutations (Figure 5). Whole genome resequencing (WGS) was carried out for
208 the Ec800 (*bas-1*) and Ec801 (*bas-2*) mutant strains and the data compared to the wild-type
209 *Ectocarpus* sp. strain Ec32 reference genome. More than 41,000 putative variants were
210 detected for each strain. Those variants were compared to a list of 567,532 variants called
211 during the analysis of 14 other mutant lines that showed a range of different phenotypes (Table
212 S5) and shared variants were eliminated. This approach allowed the identification of 827 and
213 769 variants that were unique to the Ec800 and Ec801 mutants, respectively. Quality filtering of
214 those variants (see methods for details) resulted in 118 and 67 putative mutations for the Ec800
215 and Ec801 strains, respectively, corresponding to one mutation every 1.7 to 3.0 Mb of genome.
216 Of these 185 putative mutations, 26 and 15 were in coding regions (CDS) (22%) in Ec800 and
217 Ec801, respectively. Only one gene (locusID: Ec-21_001770) contained a CDS mutation in both
218 strains. A single nucleotide transition from T to C, at position 2,806,985 was identified in the
219 *bas-1* mutant (strain Ec800) and a G to A transition at position 2,807,321 in the *bas-2* mutant
220 (strain Ec801) (Figure 5). The *bas-1* mutation segregated with the phenotype in the 38
221 individuals used for the phenotype segregation analysis (Table S6).

222 The Ec-21_001770 gene encodes a protein of 646 amino-acids similar to protein phosphatase
223 2A regulatory subunit type B'' proteins. This polypeptide contains three predicted functional
224 domains: a disordered region between positions 50 to 185 and two EF-hand domains at
225 positions 280 to 370 and 380 to 550. The *bas-1* mutation affects the first EF-hand, replacing a
226 positively charged lysine residue with a negatively charged glutamic acid (K302E). This
227 modification of electric charge may disrupt domain folding and/or function at least for the first
228 EF-hand. It is possible that the *bas-1* mutation leads to the production of a protein that is
229 partially active. The *bas-2* mutant carries a non-sense mutation that creates a premature stop
230 codon at position 190 of the protein. This mutation is predicted to result in the production of a
231 truncated protein that lacks both EF-hand domains (Figure 5A).

232 *BAS* is predicted to encode a protein phosphatase 2A regulatory B'' subunit. PP2A phosphatases
233 are protein complexes usually composed of three subunits, a catalytic C subunit, a scaffolding
234 A subunit and a regulatory B subunit (Wlodarchak and Xing, 2016). Most species have multiple
235 forms of each subunit and there are four distinct classes of the B subunit (B/B55/PR55,
236 B'/B56/PR61, B''/PR72/PR130 and B'''/Striatin), which are unrelated at the sequence level. An
237 analysis of the *Ectocarpus* sp. genome revealed that it encodes B, B' and B'' subunits, but not
238 B'''/Striatin (Figure 5B). The *BAS* protein is predicted to belong to the B'' class, homologous to
239 the PR130/PR72 human protein (Figure 5B).



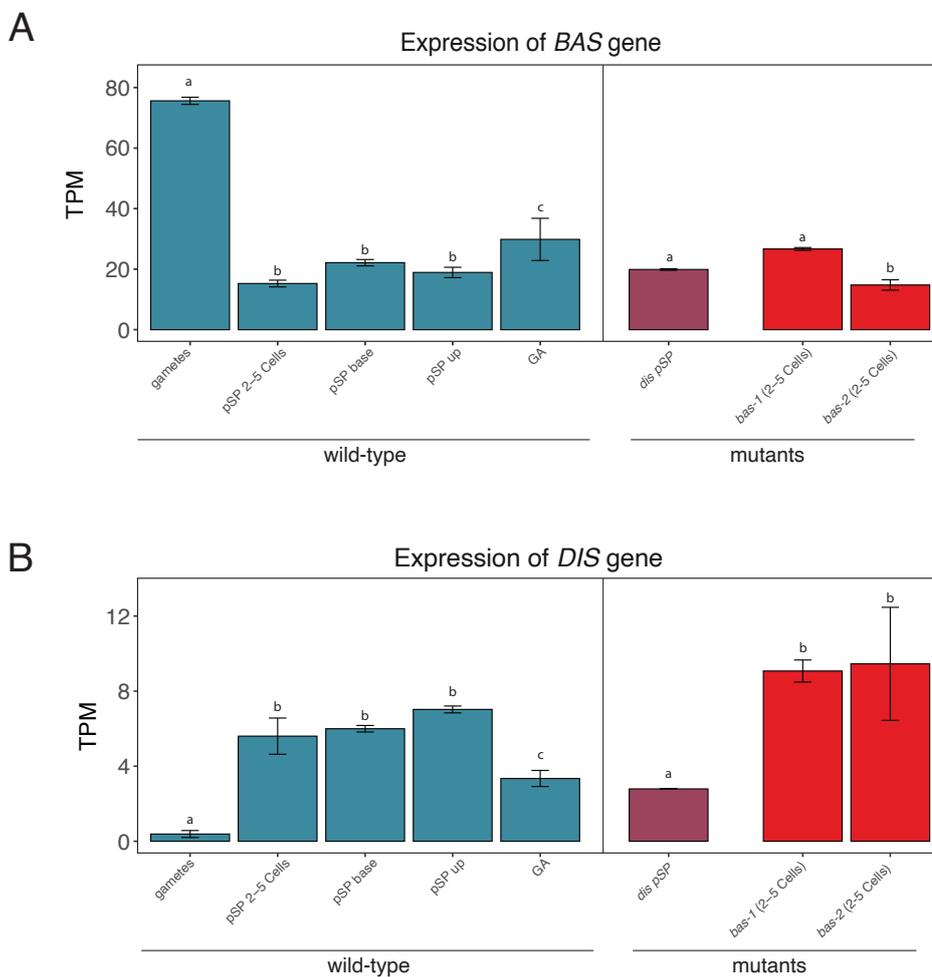
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Figure 5. Identification of mutations in the *BAS* gene and identification of protein phosphatase 2A subunits in *Ectocarpus*. A) Diagram showing the domain structure of the *BAS* gene, indicating the positions of the *bas-1* and *bas-2* mutations. The point mutation in exon 2 in *bas-1* results a lysine (K) being replaced with a glutamate (E) residue, whereas the point mutation in *bas-2* results in the introduction of a stop codon into the coding region of the gene (represented by an asterisk). Blue boxes indicate exons, with dark-blue representing untranslated regions and light-blue the coding region. B) *Ectocarpus* protein phosphatase 2A subunits. Unrooted maximum likelihood trees of PPA2A subunits (LG+G model). Only bootstrap (1000 repetitions) values of >50 are shown. *Ectocarpus* proteins are shown in blue. Asterisks and double asterisks indicate best species-to-species reciprocal Blastp matches with the corresponding *Ectocarpus* protein. The *Ectocarpus* genome does not encode an orthologue of PP2A subunit B''/striatin. The domain structures of five PP2A subunit B'' proteins are shown with the EF-hand domains in brown and disordered domains in green. AA, amino acid; Esp, *Ectocarpus* sp.; Tgo, *Toxoplasma gondii*; Ath, *Arabidopsis thaliana*; Hsa, *Homo sapiens*; Dme, *Drosophila melanogaster*; Sce, *Sccharomyces cerevisiae*; Nca, *Naumovozyma castelli*. *Ectocarpus* locusIDs are abbreviated as in the following example: Esp_14_3830, *Ectocarpus* sp. Ec-15_003830.

241

BAS expression during *Ectocarpus* life cycle and in other developmental mutantsz

242 RNA-seq data (Bourdareau, 2018; Coelho et al., 2011; Godfroy et al., 2017; Macaisne et al.,
 243 2017) were analysed to investigate *BAS* gene expression during the *Ectocarpus* life cycle. *BAS*
 244 transcripts were detected throughout development, during both the gametophyte and
 245 sporophyte generations (Figure 6A). The *BAS* transcript was most abundant at the gamete stage
 246 (after the release from plurilocular gametangia) and had decreased in abundance about one
 247 week after germination, at the 2-5 cell stage (Figure 6A; Tables S7). This pattern of expression
 248 is consistent with a role of *BAS* in the early divisions of the initial cells of the partheno-
 249 sporophyte generation provided that the transcript and/or protein persists in the initial cell
 250 during the first cell division. Interestingly, during the life cycle, the abundance of the *BAS*
 251 transcript was inversely related to the abundance of the *DIS* transcript, which was at a very low
 252 level in gametes but increased in abundance at later stages of development (Figure 6, Table S7,
 253 S8).



254

Figure 6. Abundance of the *BAS* and *DIS* transcripts (measured as transcripts per million, TPM) during the life cycle of *Ectocarpus* and in developmental mutants. A) *BAS* transcript abundance during several developmental stages of wild-type *Ectocarpus* and in *dis* and *bas* mutant strains. Note the high abundance of the *BAS* transcript in gametes. GA: gametophyte, pSP: partheno-sporophyte. Significant differences in expression (Tukey test) are indicated as different letters above the plots, and detailed statistics are presented in Table S14. B) Abundance of the *DIS* transcript in wild-type samples compared with developmental mutants. pSP: partheno-sporophyte; GA:

gametophyte; up, upright filaments of the pSP; base, basal system of the pSP; (2-5 Cells), early development (2-5 cell stage) of the partheno-sporophyte.

255 The similar phenotypes of *dis* and *bas* mutants suggest that the products of the two genes may
256 play roles in common cellular processes. We investigated the expression of *DIS* in a *bas*
257 background, and, conversely, the expression of *BAS* in a *dis* background. We noticed that in a
258 *bas* background, *DIS* expression in early stage (2-5 cell) was significantly higher (p-value=4.13E-
259 02 and p-value=7.66E-02 respectively for *bas-1* and *bas-2* comparison with wild-type at the 2-
260 5 cell stage, Figure 6B; Table S7), whereas no difference was observed in the levels of expression
261 of *BAS* in absence of *dis* gene product. Taken together, these analyses suggest that *BAS*
262 expression levels are not affected by *DIS*, but, conversely, *DIS* gene expression is disturbed by
263 mutations at the *BAS* locus. This observation, however, is unlikely to explain the phenotypic
264 similarity between *bas* and *dis* mutants.

265 **Analysis of the *bas* transcriptome**

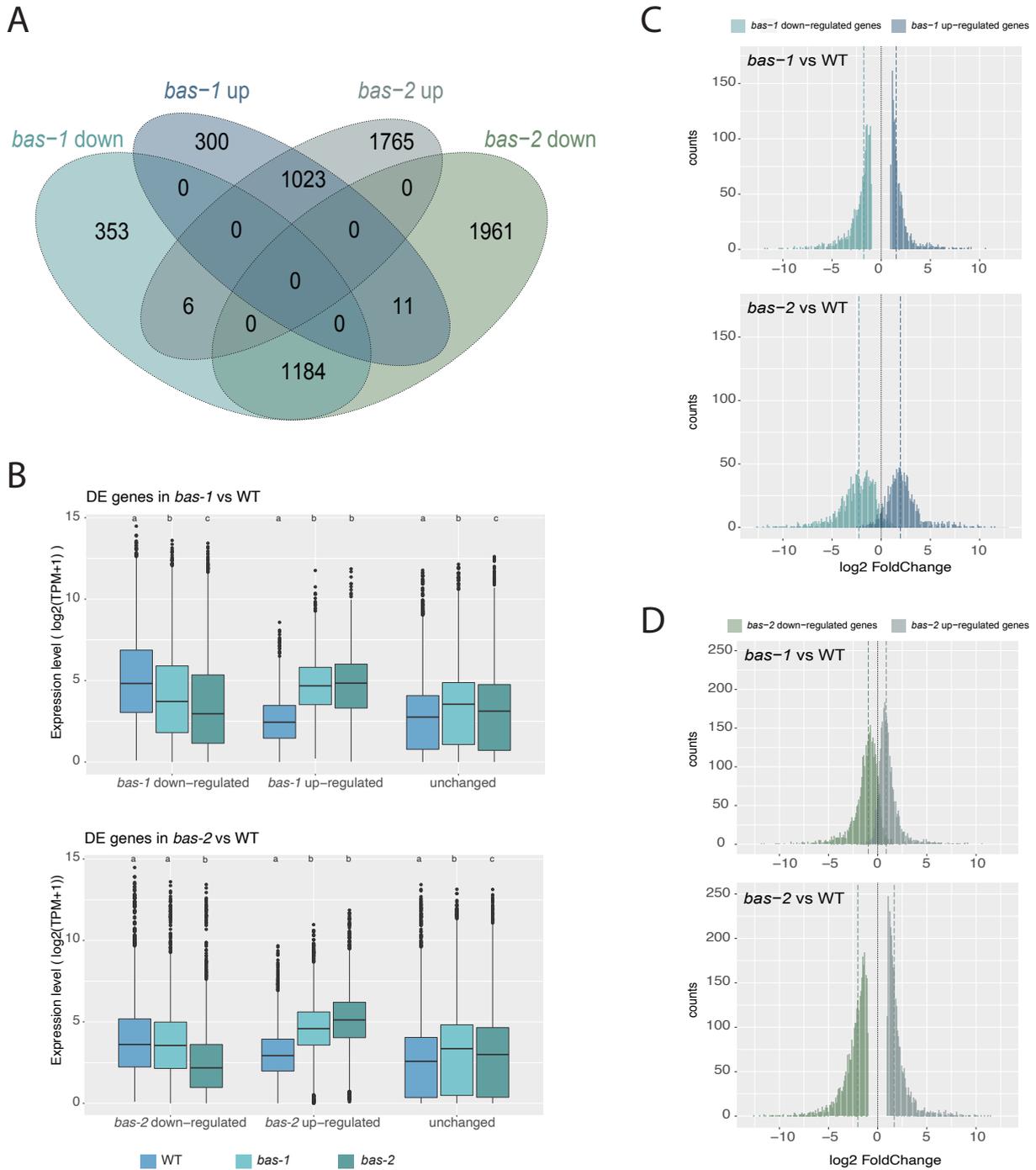
266 To further characterize the *bas* phenotype, an RNA-seq approach was employed to study gene
267 expression in the *bas-1* and *bas-2* mutants compared with wild-type. We focused on the 2-5
268 cell stage during gamete germination because the *bas* phenotype was prominent during the
269 early stages of development. Given the subtle differences in phenotype between *bas-1* and *bas-2*
270 (in particular the meiotic defect in *bas-2*) we also focused on comparing *bas-1* and *bas-2*
271 transcriptional patterns.

272 Overall, gene expression patterns in *bas-1* and *bas-2* were comparable, and both were different
273 from the wild-type samples (Figure S4-S5; Tables S7-S9). However, *bas-2* exhibited more
274 differences compared with wild-type samples (Figure S4-S5): 40% of the transcriptome was
275 differentially regulated in the comparison *bas-2* versus wild-type, whereas 20% of the genes
276 was differentially expressed (DE) between *bas-1* and wild-type (Figure S6, Table S8, see Material
277 and Methods for thresholds). Most genes were expressed under all conditions, only about 4%
278 of the genes were not expressed in any of the samples.

279 Differentially expressed (DE) genes exhibited very similar patterns of up and down regulation in
280 the two *bas* mutants compared to wild-type. Only 11 genes exhibited divergent expression
281 patterns, i.e., up-regulated in *bas-1* and down-regulated in *bas-2*, and six genes were up-
282 regulated in *bas-2* and down-regulated in *bas-1*. The vast majority of the DE genes in *bas-1* vs
283 WT were also differentially expressed in *bas-2* vs wild-type comparison (76.69% of the down-
284 regulated genes and 76.73% of the up-regulated genes) (Figure 7A, Table S9).

285 Down-regulated genes in *bas-1* compared to wild-type had, globally, lower expression levels in
286 *bas-2*; and up-regulated genes in *bas-1* were slightly more expressed in *bas-2* (Figure 7B).
287 Conversely, down-regulated genes in *bas-2* compared to WT had expression levels in *bas-1*
288 similar to wild-type samples; while up-regulated genes in *bas-2* had intermediate expression
289 level compare to wild-type and *bas-2* (Figure 7B, Table S9). Comparison of the fold-change
290 distribution of the DE genes from *bas-1* vs wild-type with those of the DE genes from *bas-2* vs

291 wild-type showed that DE genes exhibited greater fold changes in *bas-2* than in *bas-1* (Figure
 292 7C, D).



293

Figure 7. Differential gene expression analysis. A) Venn diagram of intersects of DE gene sets in *bas* mutants compare to wild-type. B) Boxplot representation of the distribution of gene expression levels (in \log_2 of TPM values +1) of the DE gene sets from comparisons of either *bas-1* or *bas-2* with wild-type. For each gene category, significant differences in expression level across strains, according to the Wilcoxon test, are indicated as different letters above the plots (details in Table S15). C-D) Histogram representation of the distribution of fold changes of the DE gene sets from comparisons of *bas-1* or *bas-2* with WT; vertical dashed bars indicate the medians of the distributions. DE genes in *bas-1* compared to wild-type (C); DE genes in *bas-2* compared to wild-type (D).

294 Overall, these observations indicate similar changes in transcriptome of both *bas* mutants, with
295 a stronger effect in *bas-2* than in *bas-1* compared to wild-type. Those results are coherent with
296 the predicted effects of the two mutations on the *BAS* protein. The *bas-1* mutation may result
297 in the production of a protein partially active whereas the *bas-2* mutation leads to a truncated
298 protein with probably no activity.

299 GO term enrichment analyses of DE genes revealed that functions related to photosynthesis
300 and metabolism were down regulated in both *bas* mutants whereas upregulated genes in
301 mutants were associated with intracellular protein transport transcription and protein synthesis
302 (Figure S7, Table S10). It is interesting to note that some of those functions can be linked with
303 the Golgi apparatus, which appears to be affected by the *bas* mutation (see above). Using the
304 HECTAR predictor (Gschloessl et al., 2008), we examined the enrichment of DE genes in
305 particular sub-cellular localizations. In coherence with GO term enrichment analyses, we
306 observed an enrichment in “chloroplast” localization of the down-regulated genes in both *bas*
307 mutants. We also observed a slight but strongly significant enrichment in proteins with a “signal
308 peptide” suggesting an impact of the *bas* mutation on the production of secreted proteins
309 (Table S11), which, again, may be consistent with defects observed in *bas* mutants at the level
310 of the Golgi. Finally, we noted that 13 out of the 26 predicted BASIC LEUCINE-ZIPPER (bZIP)
311 transcription factors in *Ectocarpus* are differentially expressed in *bas-2* mutant compared to
312 wild-type (Table S9).

313 We identified 26 and 41 genes that were exclusively expressed in the *bas-1* and *bas-2*
314 respectively compared with the wild-type (i.e., genes that had TMP=0 in wild-type samples).
315 Seventeen genes were silenced in the *bas-1* and 29 in *bas-2* compared with wild-type (Table
316 S12 and S13, see Material and Methods for thresholds). Most of those genes have unknown
317 functions and, due to the low numbers of genes, no significant functional enrichment could be
318 established. However, it is worth noting several transcription factors, which may be potential
319 effector genes: such as Ec-14_003940 and Ec-16_000350, which are silenced in *bas-1* and/or
320 *bas-2* and Ec-00_004890, which is activated in both mutants compared with the wild-type; and
321 numerous genes associated with “Cellular regulation and signalling” or “Membrane function
322 and transport”. Looking at the putative localisation of those proteins, we observed that about
323 30% of the genes specifically silenced in *bas-1* and/or *bas-2* have “signal peptide” or “signal
324 anchor” prediction, suggested a membrane or cell-wall localization, whereas only 16% of the
325 total predicted proteins in the genome of *Ectocarpus* present this signature (Table S12). This
326 two-fold enrichment is even higher than the “signal peptide” enrichment found in down-
327 regulated genes (Table S11).

328 Discussion

329 The *BAS* gene is involved in apical-basal axis formation in *Ectocarpus*

330 The two *Ectocarpus* mutant alleles identified in this study, *bas-1* and *bas-2*, lack basal structures
331 during both the gametophyte and the sporophyte generations of the life cycle. Analysis of the

332 initial cells of the *bas* mutant showed that the morphological phenotype was associated with
333 several cellular anomalies during germination and the first cell division, including
334 disorganisation of the microtubule network, an increase in the number of microtubule bundles
335 and Golgi apparatus and unipolar, rather than bi-polar, germination patterns. These
336 observations highlight a key role for the BAS protein during the development of *Ectocarpus*. In
337 particular, the BAS protein appears to operate during key cell divisions during development: the
338 (first) initial cell division and, at later stages of development, during meiosis. However, no
339 cellular defect was observed prior to initial cell divisions, suggesting that BAS operates only after
340 release of the initial cells from the reproductive structures. The meiotic defect observed in the
341 *bas-2* mutant suggests that the BAS protein may have also a role during meiotic cell division.
342 The absence of this meiotic defect in the *bas-1* may be explained by the production of a protein
343 with sufficient activity in *bas-1* to ensure its role during meiosis but not during the first cell
344 division. Higher penetrance of the *bas-2* mutation is also indicated by the greater proportion of
345 differentially expressed genes in *bas-2* compared to *bas-1*. Indeed, about 40% of the genome
346 was significantly mis-regulated in *bas-2* mutant during early stages of development,
347 demonstrating that mutations at the *BAS* locus can lead to broad, large-scale modifications to
348 the transcriptome. It is interesting to note that half of the bZIP transcription factors of
349 *Ectocarpus* are differentially expressed in *bas-2* compared to wild-type. This observation
350 suggests that BAS may part of a pathway involving additional regulatory proteins that drives the
351 establishment of apical- basal axis during the early development of *Ectocarpus*.

352 ***BAS* Encodes a PP2A protein with roles in cellular organization and development in animals and** 353 **plants**

354 *Ectocarpus* *BAS* is predicted to encode a PP2A phosphatase regulatory B'' subunit. In animals,
355 PP2A phosphatases are involved in diverse cellular processes and constitute a major component
356 of cellular serine/threonine phosphatase activity, dephosphorylating several hundred cellular
357 substrates (Reynhout and Janssens, 2019; Wlodarchak and Xing, 2016). PP2A has been
358 implicated in the reorganization of several cellular structures, playing key roles in nuclear
359 envelope breakdown during mitosis, and chromosome segregation via effects on assembly of
360 the mitotic spindle and attachment of cytoplasmic microtubules to kinetochores. It is also
361 involved in rearrangement of endoplasmic reticulum and the Golgi apparatus (reviewed in
362 (Wlodarchak and Xing, 2016; Wurzenberger and Gerlich, 2011). In particular, the PP2A B''
363 subunit PR130 associates with CG-NAP that localizes to centrosomes and the Golgi apparatus
364 (Takahashi et al., 1999), and restacking of newly formed Golgi cisternae requires
365 dephosphorylation of Golgi stacking proteins by PP2A (Tang et al., 2008). More broadly, the
366 animal PP2A B'' subunit is critical for cell-cell communication, cell adhesion, migration,
367 proliferation and differentiation during animal development (Creyghton et al., 2005;
368 Zwaenepoel et al., 2010). Altogether, these observations link animal PP2A to roles in cell
369 division, subcellular features and developmental pattern formation. In the multicellular brown
370 alga *Ectocarpus*, our results are consistent with a similar role for PP2A in subcellular
371 organisation, cell division and cell fate determination. Our results therefore provide an example

372 of protein functional conservation across eukaryotes that evolved multicellularity
373 independently, suggesting that the role of PP2A in multicellular development has likely been
374 preserved across very divergent lineages.

375 Consistent with a conserved role for PP2A in development across multicellular eukaryotes, the
376 *Arabidopsis* B''- δ/ϵ subunit of PP2A (At5g28900/At5g28850) interacts with BASIC LEUCINE-
377 ZIPPER (bZIP) transcription factors, and is implicated in leaf and root development as well as
378 mechanical stress response (Tsugama et al., 2019; Van Leene et al., 2016). The PP2A regulatory
379 B'' subunit FASS/TON2, is essential for the reorganisation of cortical microtubular arrays into a
380 dense preprophase band preceding cell division. FASS-containing PP2A complexes are targeted
381 to microtubules through an association with TONNEAU1 (TON1) and TON1-recruiting motif
382 protein (TRM) (Spinner et al., 2010). PP2A interacts with and dephosphorylates KATANIN, a
383 evolutionarily conserved microtubule-severing enzyme, to promote the formation of
384 circumferential cortical microtubule arrays in *Arabidopsis* (Ren et al., 2022). The *Ectocarpus* BAS
385 protein is related to the *Arabidopsis* FASS/TON2 protein but is orthologous to the AtB''- δ/ϵ
386 protein mentioned above (At5g28900/At5g28850). The microtubule phenotype we describe
387 here, where PP2A B'' disruption in brown algae is associated with microtubule disorganisation,
388 may further underline a conserved role for PP2A across plants and brown algae.

389 Golgi vesicle transport has been shown to play an important role in the establishment of the
390 *Fucus* zygote polar axis prior to the first cell division (reviewed in Bogaert et al., 2022; Shaw and
391 Quatrano, 1996)). It has been proposed that the selective targeting of Golgi vesicles to the
392 plasma membrane locally modifies the cell wall, participating in the establishment of the
393 asymmetry of the cell wall required for rhizoid differentiation (Bogaert et al., 2013; Bogaert et
394 al., 2022; Goodner and Quatrano, 1993). In this model, the cell wall plays a key role in the
395 establishment of the initial cell asymmetry, which is interesting with regard to the dramatic
396 changes in expression of numerous genes with putative "Membrane and transport" functions
397 in *bas* mutants.

398 In animals, the B'' class of PP2A B subunit is thought to be involved in Ca²⁺ signalling through
399 the presence of multiple EF-hand domains (Xu et al., 2010). Note that two EF-hand domains are
400 predicted in the BAS protein, and these EF-hands are absent or non-functional in *bas* mutants,
401 suggesting that Ca²⁺ binding may be disturbed. In the brown alga *Fucus*, Ca²⁺ gradients have
402 been shown to have a crucial function in zygote polarization and the establishment of the
403 apical/basal axis during first cell division (reviewed in (Bogaert et al., 2022; Brownlee and
404 Bouget, 1998). In particular, cytosolic free Ca²⁺ accumulates on the side of the growing (basal)
405 rhizoid, directly linking intracellular Ca²⁺ signaling and acquisition of basal cell identity. In this
406 context, we can speculate that the BAS protein may be a potential sensor of a pre-germination
407 Ca²⁺ signal.

408 **BAS and DIS may act in concert to mediate cell fate determination during the first cell division**

409 Similar morphological and cellular phenotypes were observed for *bas* and *dis* mutant strains
410 suggesting that *BAS* and *DIS* may be involved in similar cellular processes. However, some

411 differences between the mutants are also apparent, such as the less marked Golgi
412 fragmentation and no effect on nuclear positioning in early stages of development in *bas*, and
413 also a meiotic defect was only observed in *bas-2*.

414 *DIS* is predicted to encode a TBCCd1 protein. This protein shares similarity with TBCC, which is
415 a component of the complex (TBCE to TBCE) that mediates dimerization of α and β tubulin
416 subunits to form microtubules (Nithianantham et al., 2015; Tian et al., 1996). However, TBCCd1
417 lacks a conserved arginine residue that is essential for TBCC activity and is unable to
418 complement TBCC in yeast indicating that the two proteins may have different biochemical
419 functions (Goncalves et al., 2010). TBCCd1 has been localized to both the centrosome and the
420 Golgi in humans, *Chlamydomonas*, and trypanosomes and there is evidence that TBCCd1 plays
421 important roles in positioning organelles within the cells of these diverse organisms (André et
422 al., 2013; Feldman and Marshall, 2009; Goncalves et al., 2010). However, the molecular
423 mechanisms underlying these cellular phenotypes are unclear and they may not involve direct
424 effects on microtubule assembly (Goncalves et al., 2010).

425 Interestingly, analysis of human protein phosphatase interactions revealed that TBCCd1 is a
426 partner of PP2A regulatory subunit B'' (Huttlin et al., 2015; Huttlin et al., 2017; Yadav et al.,
427 2017). If this interaction also occurs in brown algae, it would provide a mechanism whereby DIS
428 and BAS could act within the same pathway involved in cell fate determination, with BAS
429 regulating the DIS protein. Further analysis of the biochemical functions of BAS and DIS will be
430 necessary to test this hypothesis.

431 To summarise, both TBCCd1 and PP2A have been linked to cytoskeleton and Golgi function and
432 both proteins have been shown to play important roles in the regulation of cellular architecture
433 in diverse eukaryotic systems. These observations are consistent with the pleiotropic cellular
434 phenotypes of both the *dis* and *bas* mutants. We suggest that the observed morphological and
435 cell fate (loss of basal cells) phenotypes of the *bas* and *dis* mutants are a consequence of cellular
436 defects during the first cell division, perhaps through disruption of the distribution of
437 hypothetical cell-fate-determining factors during this critical step of development (see model
438 proposed by (Godfroy et al., 2017). Combining information about the *Ectocarpus* DIS and BAS
439 proteins with observations of Ca^{2+} waves in the *Fucus* embryo, we can speculate that BAS may
440 be involved in sensing an intracellular Ca^{2+} signal which participates to the distribution of a cell-
441 fate determining factor through reorganization of cytoskeleton and Golgi function involving DIS.

442 **Methods**

443 **UV Mutagenesis and isolation of mutant strains**

444 Strain cultivation, genetic crosses, raising of sporophytes from zygotes, and isolation of meiotic
445 families were performed as described previously (Coelho et al., 2012a; Coelho et al., 2012b;
446 Coelho et al., 2020; Godfroy et al., 2017). *Ectocarpus* sp. (species 7, Montecinos et al., 2017)
447 gametes are able to develop parthenogenically to produce haploid partheno-sporophytes,
448 which are identical morphologically to the sporophytes that develop from diploid zygotes

449 (Bogaert et al., 2022; Bothwell et al., 2010; Peters et al., 2008). This phenomenon was exploited
450 to screen directly, in a haploid population, for mutants affected in early sporophyte
451 development. UV mutagenesis of gametes was performed as described previously (Coelho et
452 al., 2011; Godfroy et al., 2015; Godfroy et al., 2017) and mutant partheno-sporophytes lacking
453 basal structures were identified by visual screening under a light microscope. Table S1 describes
454 the strains used in this study.

455 **Genetic analysis of *bas* mutants**

456 Genetic crosses were performed as in (Coelho et al., 2012a). The *bas-1* mutant (Ec800) was
457 crossed with the outcrossing line Ec568 to generate a segregating population of 38 individuals.
458 Each of the 38 individuals was derived from a different unilocular sporangium (each unilocular
459 sporangium contains 50–100 meio-spores, derived from a single meiosis followed by at least
460 five mitotic divisions). The meio-spores germinated to produce gametophytes, which were
461 isolated and allowed to produce gametes which germinated parthenogenically. The resulting
462 partheno-sporophytes were then observed under a light microscope to determine whether
463 they exhibited the *bas* phenotype. The presence of the *bas-1* mutation was determined by
464 Sanger sequencing of PCR products (Forward: TGACGAATGATGCTAAACTGGA, Reverse:
465 GACAACGGAGCAGACGAAC) for each of the 38 individuals. The *bas-2* mutant (Ec801) was not
466 usable for crosses because it did not form functional unilocular sporangia, which are required
467 for gametophyte production.

468 **Identification of candidate mutations**

469 Genomic DNA from Ec800 and Ec801 strains was sequenced on an Illumina HiSeq4000 platform
470 (1/12th lane; 2x150nt paired-end; 8.50 and 7.95 Gbp of data, respectively; Fasteris, Switzerland).
471 After quality cleaning using Trimmomatic (Bolger et al., 2014), the reads were mapped onto the
472 *Ectocarpus* sp. reference genome (Cormier et al., 2017) using Bowtie2 (Langmead and Salzberg,
473 2012). Coverage depth and breadth were, respectively, 34x and 96.83% for Ec800 and 32x and
474 96.81% for Ec801. Variants were called and counted using bcftools mpileup
475 (<http://samtools.github.io/>). These variants were compared with a list of variants identified in
476 genome sequence data for 14 other *Ectocarpus* mutant lines in order to remove false positive
477 mutations due, for example, to errors in the reference genome sequence.

478 Variants unique to strains Ec800 and Ec801 were quality filtered based on coverage depth ($\pm 50\%$
479 of the genome mean), mapping quality (>20), variant quality (>50), variant frequency (>0.9) and
480 variant support in both sequencing directions. A custom python script allowed the identification
481 of variants in coding regions (CDS) and the effect of each CDS mutation on the predicted protein
482 was accessed manually (Table S5). A scheme of the approach is depicted in Figure S7.

483 **Immunostaining**

484 *Ectocarpus* samples were processed as described (Coelho et al., 2012c) using a protocol
485 adapted from (Bisgrove and Kropf, 1998). Briefly, *Ectocarpus* cells were settled on cover slips
486 and at appropriate times after settlement were rapidly frozen in liquid nitrogen and fixed in
487 2.5% glutaraldehyde and 3.2% paraformaldehyde for 1 h, then washed in PBS and treated with

488 5% triton overnight. Samples were then rinsed in PBS and 100 mM NaBH₄ was added for 4 h.
489 Cell walls were degraded with cellulase (1% w/v) and hemicellulase (4% w/v) for 1 h, and the
490 preparation was then rinsed with PBS and blocked overnight in 2.5% non-fat dry milk in PBS.
491 Samples were treated with an anti-tubulin antibody (1/200th, DM1A; Sigma-Aldrich) at 20°C
492 overnight and then treated with the secondary antibody (AlexaFluor 488-conjugated goat anti-
493 mouse IgG; Sigma-Aldrich; 1:1000 in PBS) at 20°C overnight. The preparation was rinsed with
494 PBS and blocked overnight in 2.5% non-fat dry milk in PBS and then treated with an anti-centrin
495 antibody (1/1000th anticentrin 1 ab11257; Abcam) at 20°C overnight, followed by the
496 secondary antibody (1/1000th AlexaFluor 555-conjugated goat anti-rabbit IgG; Sigma-Aldrich)
497 for 8 h. Samples were stained with 4',6-diamidino-2-phenylindole (DAPI; 0.5 µg/mL in PBS) for
498 10 min at room temperature and mounted in ProLong Gold (Invitrogen).

499 **Confocal Microscopy**

500 Confocal microscopy was conducted using an inverted SP8 laser scanning confocal microscope
501 (Leica Microsystems) equipped with a compact supply unit which integrates a LIAchroic scan
502 head, several laser lines (405 and 488 nm), and standard photomultiplier tube detectors. We
503 used the oil immersion lens HC PL APO 63×/1.40 OIL CS2. The scanning speed was set at 400 Hz
504 unidirectional. The pinhole was adjusted to one Airy unit for all channels. The spatial sampling
505 rate was optimized according to Niquist criteria, generating a 0.058 × 0.058 × 0.299-µm voxel
506 size (xyz). The Z-stack height fitted the specimen thickness. A two-step sequential acquisition
507 was designed to collect the signal from three or four channels. The first step recorded the anti-
508 tubulin fluorescence signal (excitation, 488 nm; emission, 530 nm) and the transmitted light.
509 The second step acquired the DAPI fluorescence signal (excitation, 405 nm; emission, 415–480
510 nm). Signal intensity was averaged three times. The Fiji software was used to optimize the raw
511 images, including maximum intensity projection and de-noising (3*3 median filter). For any
512 given data, both wild-type and mutant images were analysed simultaneously with similar
513 settings.

514 Tracking of microtubule bundles was performed on maximum intensity projections of z-planes
515 covering the whole thickness of the cells. We drew a line transversely, perpendicular to the
516 growth axis of the cell and crossing the nucleus. Peaks corresponding to the microtubule
517 bundles were then identified in plots of intensity profiles and counted, in order to estimate the
518 number of microtubule bundles in each cell. Note that in the *bas* mutants, due to the
519 disorganized nature of the microtubule network, average bundle numbers may be somewhat
520 underestimated. This is because this method is well adapted for tracking microtubule bundles
521 oriented with their long axis parallel to the image plane, but we may have missed bundles that
522 were perpendicular to the plane of the transection.

523 **TEM and FIB-SEM**

524 For transmission electron microscopy (TEM) and focused ion beam-scanning electron
525 microscopy (FIB-SEM), freshly released gametes were collected in cellulose capillaries and
526 cultivated in the capillaries in Provasoli-enriched seawater for 3 to 5 days at 14°C in the dark or

527 approximately 1 day at 14°C in 12 h light/12 h dark regime to produce two- to five-cell stage
528 partheno-sporophytes. Cells in capillaries were frozen at high-pressure (HPF Compact 03,
529 Engineering Office M. Wohlwend GmbH), freeze-substituted (AFS2, Leica Microsystems) with
530 0.2% OsO₄ and 0.1% uranyl acetate in acetone containing 5% H₂O as substitution medium
531 (Read et al., 2021) and embedded in Epon. Ultrathin sections were stained with uranyl acetate
532 and lead citrate and analysed with a Tecnai Spirit (Thermo Fisher Scientific) operated at 120 kV.

533 In order to identify a region of the sample containing algae at high density for FIB-SEM data
534 acquisition, a 3D X-ray tomogram of the resin block was acquired with a Bruker Skyscan 1272.
535 The region of interest was exposed using a 90° diamond trimming knife (Cryotrim 90, Diatome)
536 mounted on a Leica UC7 ultramicrotome. The sample was then attached on a stub using
537 conductive silver epoxy resin (Ted Pella) and gold sputter coated (Quorum Q150RS).

538 FIB-SEM imaging was performed with a Zeiss Crossbeam 540 or a Crossbeam 550, using Atlas
539 3D (FIBICS, Carl Zeiss Microscopy) for sample preparation and acquisition. After the deposition
540 of a protecting platinum coat on the surface above the region of interest, a 60 µm wide trench
541 was opened to identify and image several cells in parallel. During the stack acquisition, FIB
542 slicing was done with at 30 kV and 700 pA current. The datasets were acquired at 8 nm isotropic
543 voxel size with the SEM at 1.5 kV and 700 pA current, using a back-scattered electron detector
544 (ESB). After acquisition, the image stacks were acquired using the Fiji plugin “Linear stack
545 alignment with SIFT” (Lowe, 2004). We acquired images for a total of 5 cells for wild-type and 5
546 *bas-2* cells, and chose a representative image to present in Figure 4. Images containing Golgi
547 stacks were retrieved from aligned image stacks and cropped to reduce the image dimensions
548 for further segmentation with the IMOD software package (<https://bio3d.colorado.edu/imod/>).

549 **Phylogenetic trees**

550 Multiple alignments were generated with Muscle in MEGA7 (Kumar et al., 2016). Phylogenetic
551 trees were then generated with RAxML (Stamatakis, 2014) using 1000 bootstrap replicates and
552 the most appropriate model.

553 ***BAS* and *DIS* gene expression estimation during the *Ectocarpus* life cycle**

554 Expression levels of the *BAS* and *DIS* genes were investigated using TPM values obtained after
555 kallisto pseudo-mapping and calculation of the lengthScaledTPM using the tximport package in
556 R (see ‘Comparative Transcriptome Analyses’ section for details). Previously generated RNA-seq
557 data for wild type and *dis* samples (Table S7) was used for comparisons.

558 **Comparative transcriptome analyses**

559 RNA-seq analysis was performed to compare the abundances of gene transcripts in the mutants
560 *bas-1* and *bas-2* and wild-type sporophytes. RNA-seq datasets were generated from triplicate
561 samples of each genotype, and individuals were grown synchronously as described previously
562 in standard culture conditions (Coelho et al., 2012b; Cossard et al., 2022). Germlings were
563 filtered through a nylon mesh to recover only thalli at the 2-5 cell stage. Each replicate contained
564 between 10⁴ and 10⁶ individual germlings. Tissue samples were rapidly frozen in liquid nitrogen

565 and processed for RNA extraction. Total RNA was extracted from each sample using the Qiagen
566 Mini kit as previously described (Lipinska et al., 2015). For each replicate, cDNA was produced
567 by oligo(dT) priming, fragmented, and prepared for stranded 2× 150-bp paired-end sequencing
568 on an Illumina HiSeq 3000 platform.

569 Raw and cleaned read quality was evaluated using fastQC (v. 0.11.9) and mutliQC (v. 1.9). Raw
570 reads were trimmed and filtered based on quality score, and adapter sequences were removed
571 using Trimmomatic (v. 0.39). Transcript abundance was evaluate using kallisto (v. 0.46.2) via
572 pseudo mapping on CDS features. Then matrix of counts and TPMs for all samples, all replicates
573 were generated in R using tximport package.

574 A gene was considered to be expressed if the TPMmean was above the 5th percentile (as in
575 Cossard et al., 2022; Lipinska et al., 2019). About 4% of the genes in each sample had TPMmean
576 values under this threshold and were considered not to be expressed in our samples (Table S9).
577 Differential gene expression was analysed using DESeq2 (Love et al., 2014, 2). Genes were
578 considered to be differentially expressed when the log2 fold change was below or equal -1
579 (down-regulated, at least twice as weakly expressed) or above or equal to 1 (up-regulated, at
580 least twice as strongly expressed) and adjusted p-value below or equal to 0.01. Genes were
581 considered to be exclusively (uniquely) expressed in *bas* mutants when they were significantly
582 up-regulated compared to wild-type and the wild-type mean TPM was equal to 0. Conversely,
583 genes were considered to be specifically silenced in *bas* mutants when there were significantly
584 down-regulated compared to wild-type and their TPM means were equal to 0.

585 GO term and HECTAR localisation enrichment were carried out on R using the “clusterProfiler”
586 package (Yu et al., 2012).

587 Accession Numbers

588 Accession numbers are provided in Table S7.

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596 Author contribution

597 S.M.C, J.M.C and O.G conceived the project. O.G., M.Z. and S.M.C. performed the main
598 experimental work. O.G. performed bioinformatic analyses. J.M.C. carried out gene annotation
599 and phylogenetic analyses. Y.H., M.Z, A.H, A.F.P performed experiments. S.C. and D.S. generated
600 epifluorescence images. P.R. and K.H. generated and analysed Fib-Sem data. C.N and T.M

601 generated TEM images. O.G., J.M.C. and S.M.C. interpreted the data. S.M.C. wrote the
602 manuscript with input from all authors.

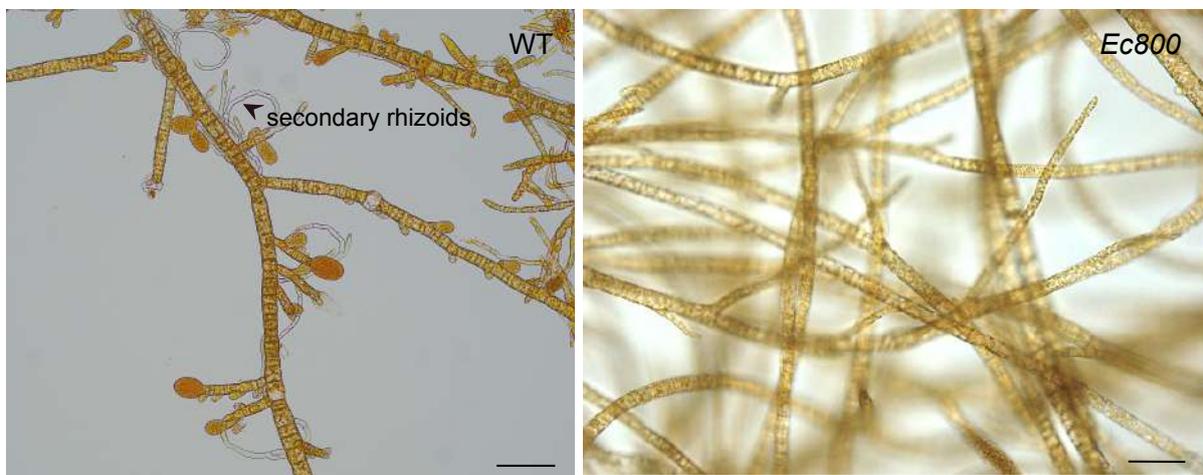
603

604 Supplemental Figures

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606

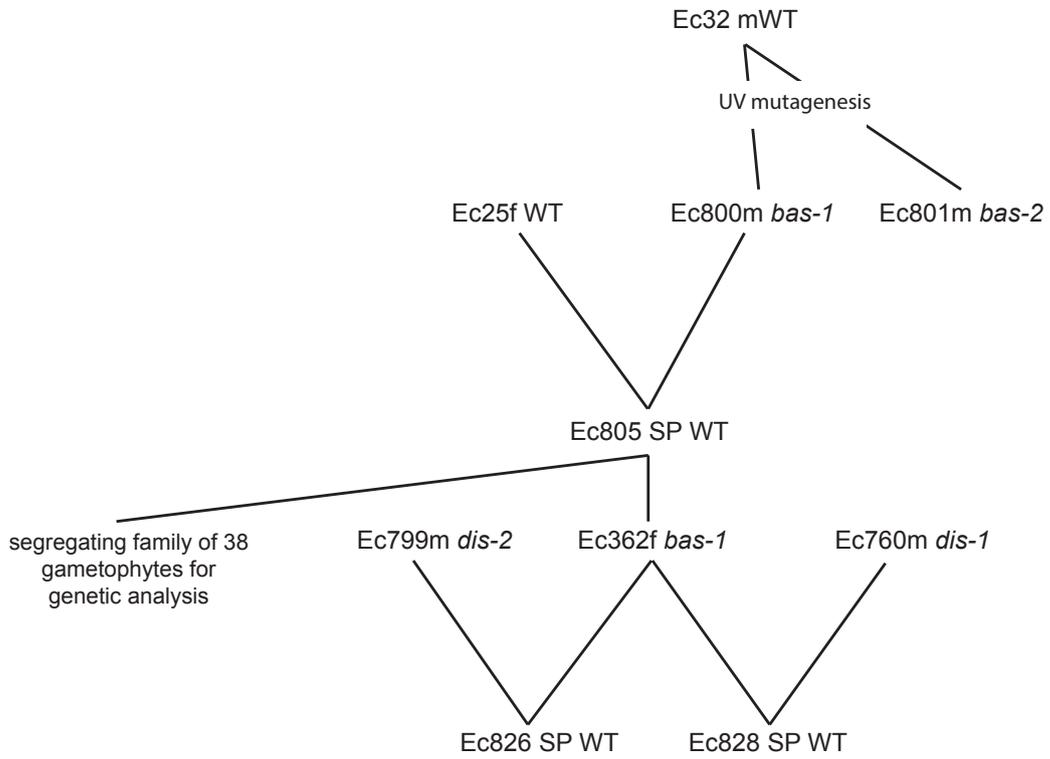
607 **Figure S1.** Absence of secondary rhizoids in apical filaments of mutant lines compared with wild-
608 type, after 3-weeks in culture. Scale=20 μm .



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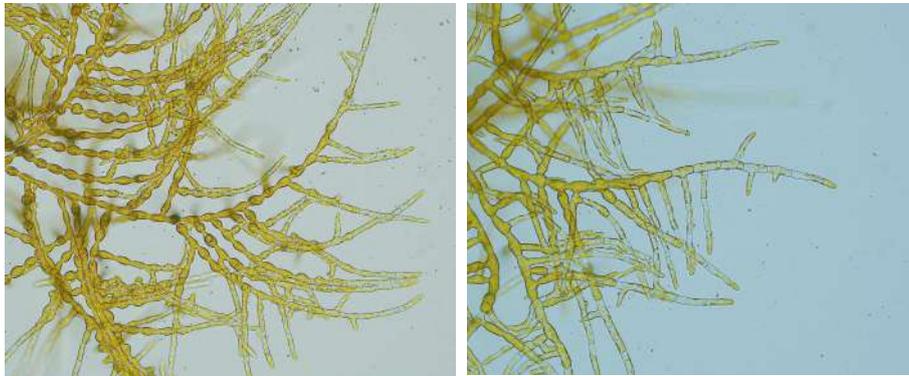
611 **Figure S2.** Pedigree of the *Ectocarpus* strains used in this study. SP, diploid, hybrid sporophyte;
612 WT, wild type; m, male; f, female.



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614

615 **Figure S3.** Morphological phenotypes of diploid sporophytes derived by crossing *dis-2* x *bas-1*
616 or *dis-1* x *bas-2*. Note that the diploid sporophyte derived from the cross has a wild-type
617 phenotype, indicating the two mutations complement each other. Scale=20 μm.



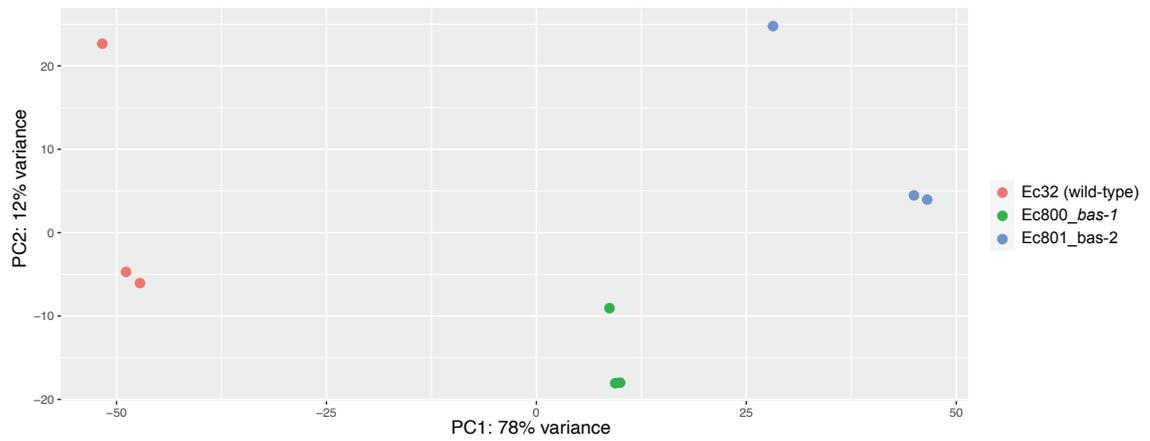
Ec760 (*dis-1*) x Ec800 (*bas-1*)

Ec799 (*dis-2*) x Ec800 (*bas-1*)

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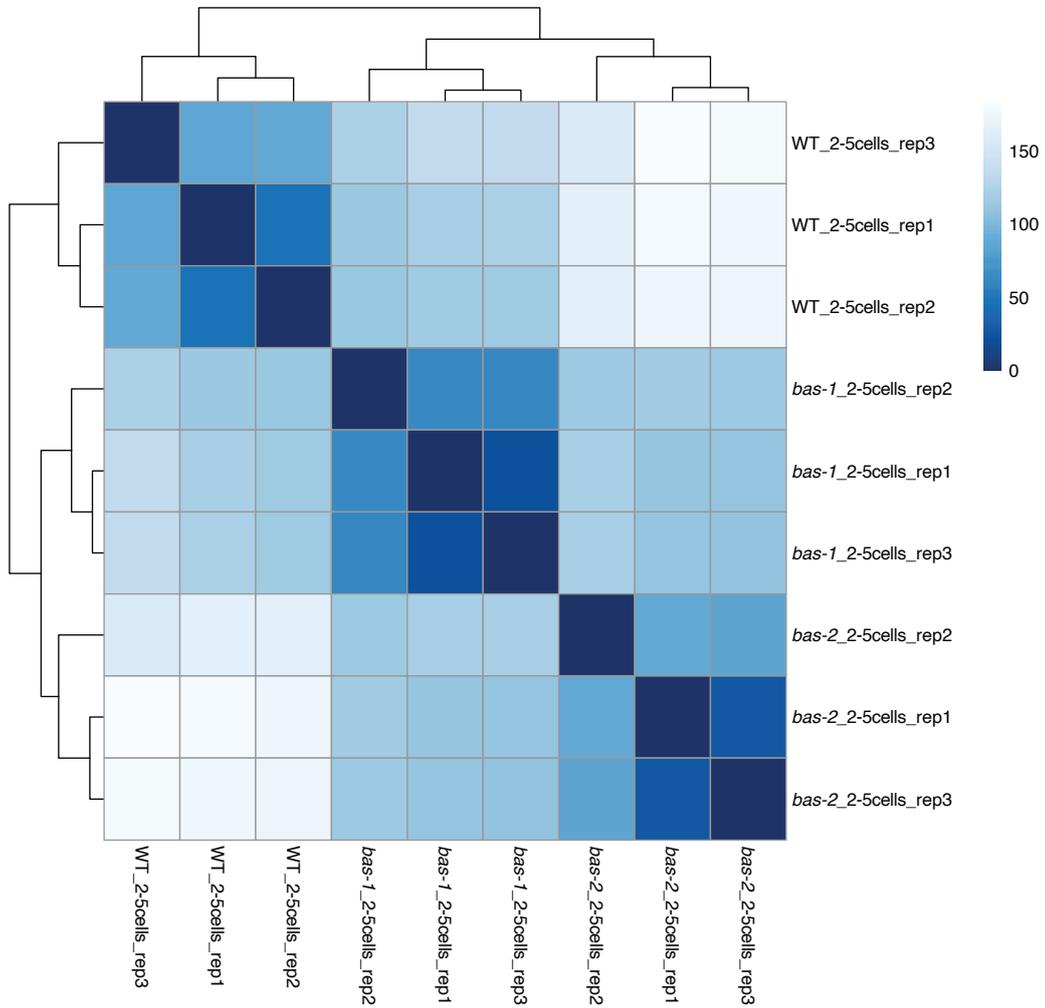
620 **Figure S4.** Principal component analysis (PCA) comparison of transcript abundance patterns for
621 all expressed genes across wild-type (WT), *bas-1* and *bas-2* replicate samples. The two
622 dimensions represent 78% and 12% of the variance. The analysis was carried out using
623 normalized counts generated by DESeq2 after Variance Stabilizing Transformation (VST).



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625

626 **Figure S5.** Between-sample correlation diagnostics of the RNA sequencing data. Heat map
627 representing the distance between replicates. Euclidian distances were calculating from
628 normalized counts generated by DESeq2 after Variance Stabilizing Transformation (VST). Details
629 of the samples are given in Table S7.

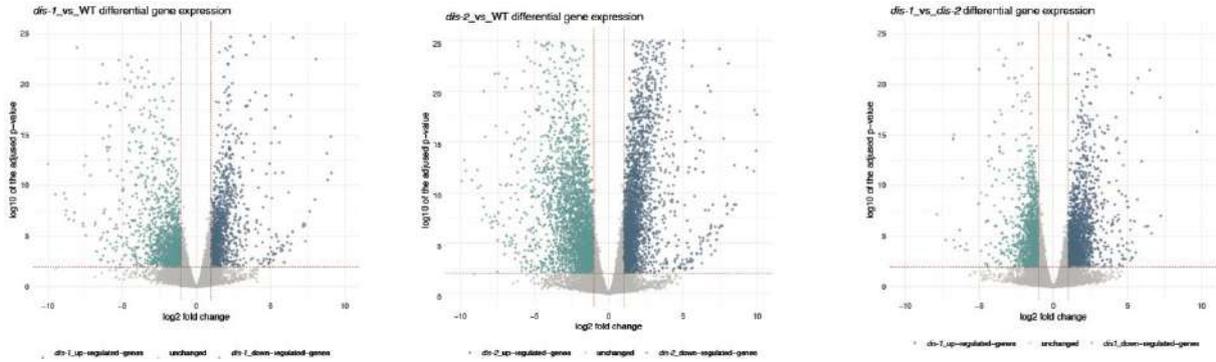


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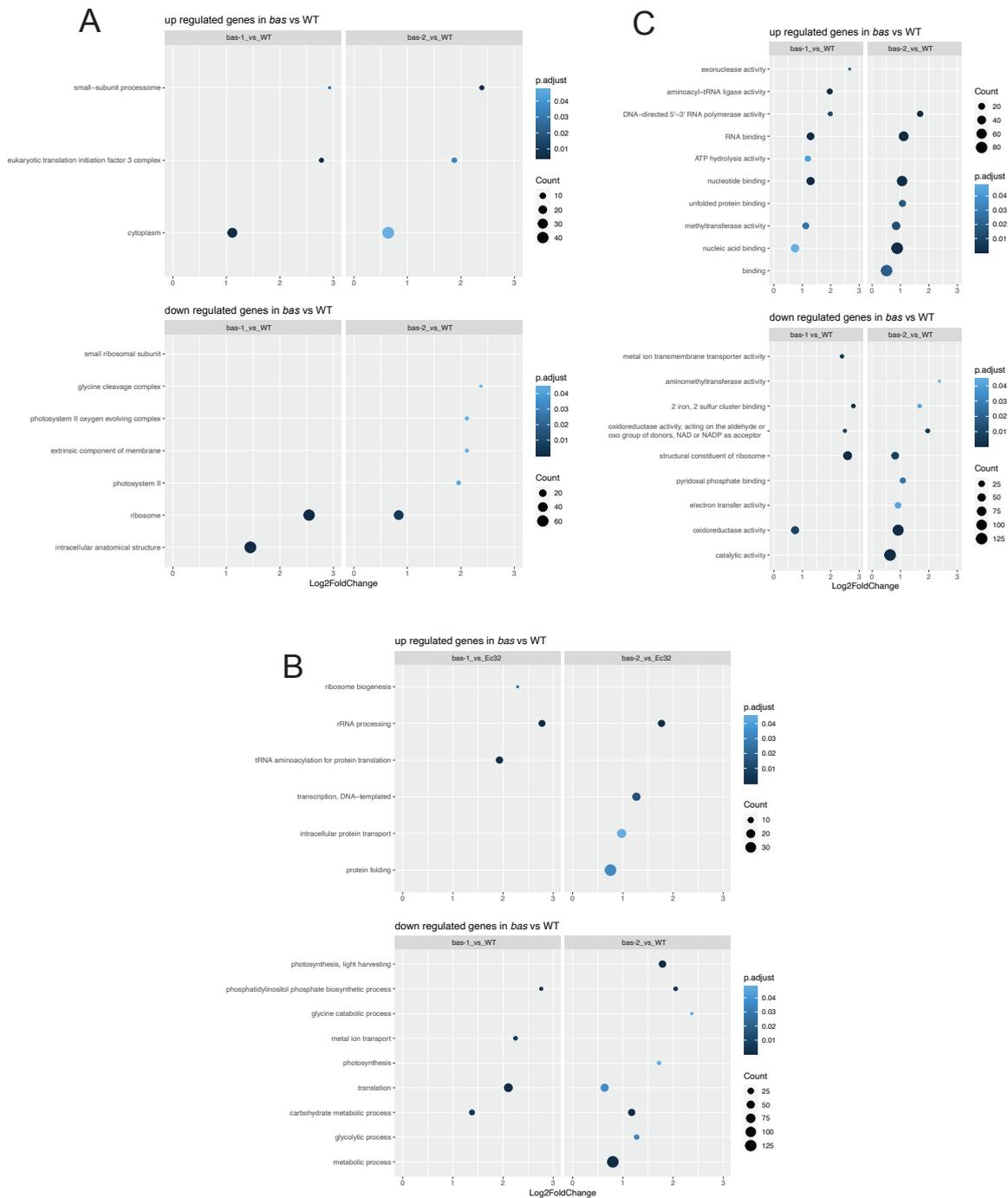
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633 **Figure S6.** Volcano plots of all genes in pairwise comparisons between wild-type (WT) and
634 mutants (*bas-1* and *bas-2*). The log₂ FC value was calculated based on the mean expression
635 level (TPM) for each gene. Each dot represents one gene. Blue represents upregulated genes
636 and green downregulated genes in each comparison.



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638

639 **Figure S7.** GO term enrichment observed in DE gene sets in *bas* mutants compared to WT. Dot
 640 plot representation is divided according to GO term ontology classes 'Cellular Component' (A),
 641 'Molecular Function' (B) and 'Biological Processes' (C).

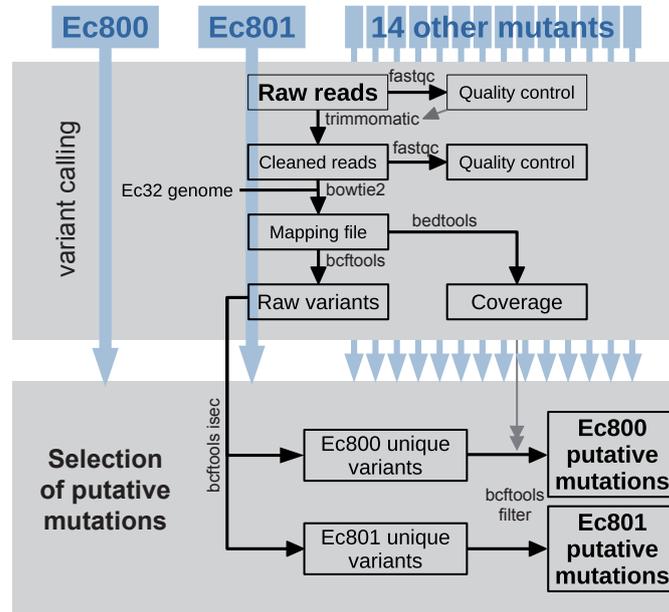


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644 **Figure S8.** Schematic diagram of the approach used to detect putative mutations in the
645 genomes of Ec800 and Ec801.

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