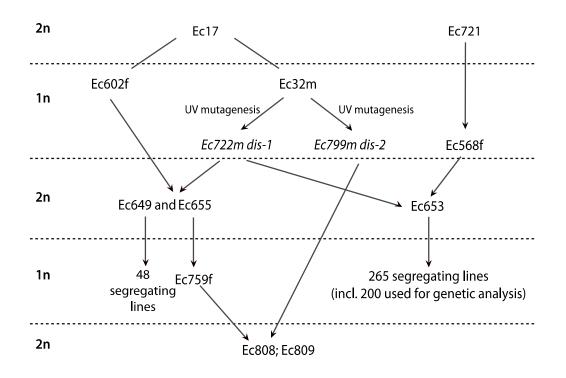
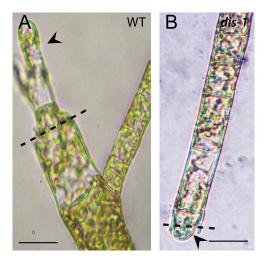


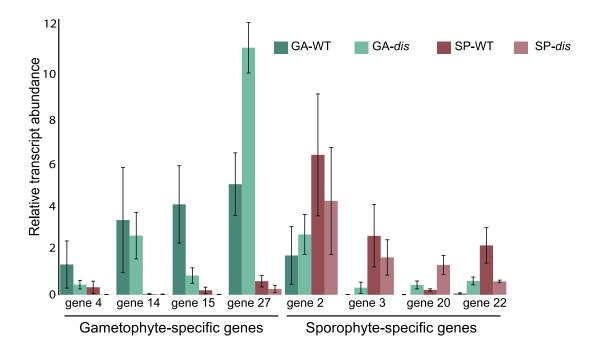
Supplemental Figure 1. Basal and apical systems in brown algae and land plants and the life cycle of *Ectocarpus* sp. (A) Basal (red), secondary basal (orange) and apical (olive green) structures in the brown algae *Ectocarpus* sp. and *Fucus* sp., compared with analogous structures in angiosperms (Arabidopsis). Note that the cartoons are not drawn to scale. (B) The haploid-diploid life cycle of *Ectocarpus* sp. GA: gametophyte; pSP: partheno-sporophyte; SP: diploid (zygote-derived) sporophyte. Olive green, apical systems of the sporophyte and gametophyte generations; red and orange, basal systems of the sporophyte and gametophyte generations; purple, plurilocular gametangia (gamete production); light green, plurilocular sporangia (mito-spore production); dark green, unilocular sporangia (meio-spore production); grey, gametes and spores. Note that the developmental patterning of the partheno-sporophyte is indistinguishable from that of the diploid sporophyte. Asterisks indicate stages of the life cycles used in the RNA-seq analysis. Supports Figure 1.



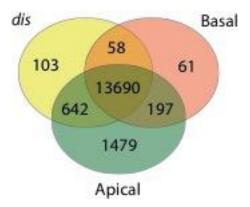
Supplemental Figure 2. Pedigree of the strains used in this study. Supports Figure 1.



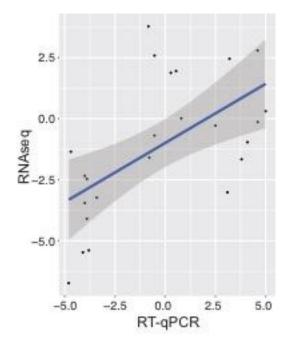
Supplemental Figure 3. Cell regeneration in dis and wild type filaments. (A) Regeneration of rhizoids from wild type sporophyte apical cells 48h after cutting the filament by microdissection. The dashed line indicates the position of the cut, the arrow heads the regenerating cell. (B) *dis-1* individuals do not regenerate rhizoids in response to wounding: *dis-1* filament 24h after microdissection; Size bar=10  $\mu$ m. The same results were obtained for *dis-1* and for *dis-2*: 100% of the micro-dissected cells regenerated as apical cells, in contrast to the wild type where 100% of the cells regenerated as rhizoids. The images shown are representative of the 82 individuals that were analysed in three independent experiments. See also Figure 2.



Supplemental Figure 4. Expression patterns, measured using RT-qPCR, of generation-specific marker genes (Peters et al., 2008) during the gametophyte and sporophyte generation of *dis-1* individuals compared with the wild type. The graph shows means of triplicate samples and the error bars indicate standard deviations. There was no significant difference between the levels of expression of generation marker genes in *dis-1* and wild type individuals (one tail t-test for samples of unequal variance). Supports Figure 2.

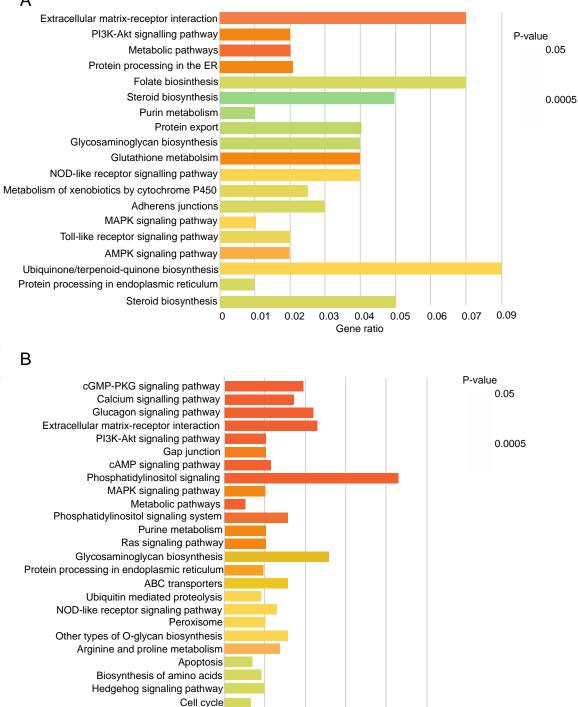


Supplemental Figure 5. Venn diagram comparison of the set of genes expressed in *dis-1* with those expressed in wild type basal (Basal) and apical (Apical) filaments (TPM>1). See also Figure 3.



Supplemental Figure 6. Correlation between RNA-seq and RT-qPCR results for a set of 12 differentially expressed genes selected based on their differential expression in at least one of the pairwise comparisons (*dis* vs apical; *dis* vs basal; apical vs base) shown in Figure 3A. Transcript abundance was measured in wild type basal and apical filaments and in whole thalli of the *dis-1* mutant using the two methods. For the RNA-seq data (duplicate samples), log2 fold change in transcript abundance was calculated in DESeq2 for the following tissue comparisons: apical/basal, *dis-1*/basal. For the RT-qPCR data, Ct values were normalized in relation to a control gene that is stably expressed throughout all the samples (Ec-09\_003710, an actin-related gene) and mean transcript abundances for triplicates were then used to calculate log2 fold change for the same two tissue comparisons. The plot shows the correlation between the calculated ratios for the RNA-seq and RT-qPCR datasets. Spearman's correlation: R2 = 0.619, p-value = 0.0012. See also Figure 3.

## A



Supplementary Figure 7. Kegg pathway enrichment analysis for the set of genes differentially expressed in *dis-1* versus basal system (A) and *dis-1* versus apical system (B). See also Supplemental Table 11 and Figure 3.

0.2

0.3

Gene ratio

0.4

0.5

0.1

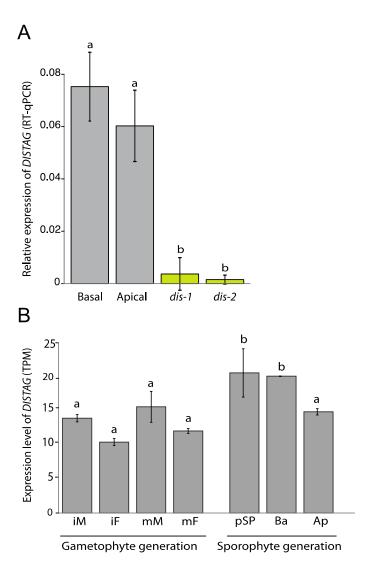
Pentose and glucuronate interconversions

Valine, leucine and isoleucine degradation

Carbon metabolism Nucleotide excision repair

0

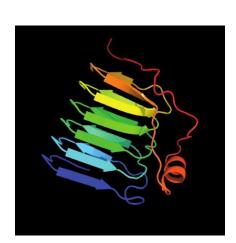
mTOR signalling pathway Pyramidine metabolism



Supplemental Figure 8. Patterns of expression of DIS. (A) Expression of DIS in wild type parthenosporophyte apical and basal systems compared with whole *dis-1* and *dis-2* mutant parthenosporophytes. The y axis represents the Ct values that were normalized to the control gene (2^(deltaCt)). (B) Expression of the DIS gene during the gametophyte (GA) and sporophyte (SP) generations. iM: immature male GA, iF: immature female GA, mM: mature male GA, mF: mature female GA, pSP: whole partheno-sporophyte, Ap: partheno-sporophyte upright filaments, Ba: partheno-sporophyte basal filaments. The graph shows means and the error bars indicate standard deviations. Different letters above the bars indicates significant differences (t-test, padjvalue<0.005). See also Figure 3.

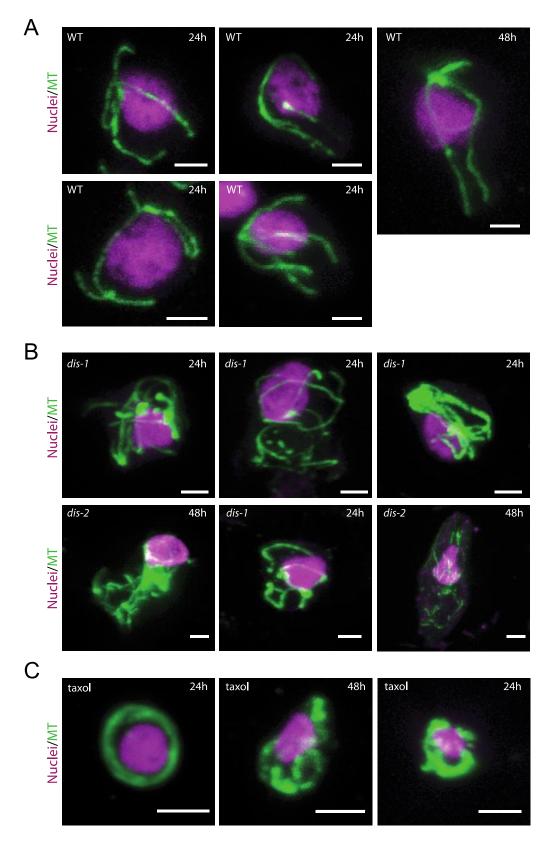
Ec-05_001860 dis-2 SyTECcd1 CrTBCcd1 ArTBCcd1a ArTBCcd1a HsTECcd1 HsTECcd1 HsTECcd1 HsTECcd1 HsTECcd1 HsTECc ArTBCC CrTBCC CrTBCC CrTBCC CrTBCC CrEC VCRP2 VCRP2 VCRP2 HsTP25 HsTP25	LRDLQVNCCQESYVYVLSAVRFATVMGCKDCTVVIGAAAGMVRVEECERMQ LRDLQVNCCQESYVYVLSAVRFATVMGCKDCTVVIGAAAGMVRVEECERMQ LRDLQVNYCQESYVYVLSAVRFATVMGCKDCTVVIGAAAGMVRVEECERMQ HGDVRYTDCHEAVIYLLAPLQAAFISACSDCTIVVGAVGRMLRVERCDKVT LGDVRITDCHEAVIYLLAPLQAAFISACSDCTIVVGAVGRMLRVERCDKVT SGLSKVANCHDSVIYLLAPLRYATVHGCSDTTIVLGAVGKAIKVEHCERVH GRSVKVANCHDSVIYLLAPLRYATVHGCSDTIVLGAVGKAIKVEHCERVH GRSVKVANCHDSVIYLLAPLRYATVYGCSDSTIVLGAVGKAIKVEHCERVH GRSVKVANCHDSVIYLLAPLRYATVYGCSDSTIVLGAVGKAIKVEHCERVH GRSVKVANCHDSVIYLLAPLRYATVYGCSDSTIVLGAVGKAIKVEHCERVH GRSVKVANCHDSVIYLGPLKYVSYFGCSDSIVLGAVGKAVRVELCERVQ GAHKIHRCNESFIYLLSPLRSVTIEKCRNSIFVLGPVGTIHHISCDNVK SSSYTVEKCSQTSFVTSDLPHTRITQLVNCTVALGPVGGVLYIDRCENTD DKDFNVADLDDCKVTIHHVLGALRLRRLTSCRVVCGPVRGPIYVEGCNCV GRSVLIGLTRCTVVLGRNTLFIKTAHSCKLCGPVSTSVFIEDCSDCV IGEFTLSDLDSCQVKLTGTVNAFFLHRLKKCSVTGFVIGSILIDDVEDCV GGDFVLLGLTRCTVVLLGRLRALRAGLRGCTVVAGPVTGACFLDDVRDCT GGDFVLLGLTRCTVVLLGRLRALRAGLRGCTVVAGSVTGDVDFDKST GGDFVLLGLTRCTVVLLGRLRALRAGLRGCTVVAGSVTGSVFIENCTGCV GQPFDLTSLNRCEVALLDRSEAVQVDELDCCRVFIAACVDSVFVRNCTGCV GQCVLEYLTNCTVYLDLDSITVDDCEGGELIIAACGSVFFRNCNMT RNGFKLDTLHDCEVYLLDHTSQVQDDCVNCKIFIGPIDGSVFFRNCRCMT GQGFFULTSLNRCEVALLDHSEAVQVDELDCCRVFIAACVDSVFVRNCTGCV GQCFIVECSECEVYLLDHTSQVQDDCVNCKIFIGPIDGSVFFRNCRMT GGGVFLDTLHDCEVYLLDHTSQVQDDCVNCKIFIGPIDGSVFFRNCRMT GQCFIVECSECEVYLLDHTSQVQDDCVNCKIFIGPIDGSVFFRNCRMT GQCFIVECSECEVYLLDHTSQVQDDCVNCKIFIGPIDGSVFFRNCRMT GQCFILDTLHDCEVYLLDHTSQVQDDCVNCKIFIGPIDGSVFFRNCRMT GQCFILQCENCNIYIFDHSATTIDDCTNCIIFLGPVKGSVFFRNCRCK
T = 05 001060	LVTCCRRLTVTNSLECIFPVFVATPPVLCGDNRACQFAPYNS
dis-2	LVTCCRRLTVTNSLECIFPVFVATPPVLCGDNRAC*
SjTBCCd1	LVTCCRRLTVTNSLECIFPVFVATPPVLSGDNRACQFAPYNS
CrTBCCd1	VIAATSRLLVSSCHECVFNVGTPRPPCLIGDNRFLKLGPYNT
VcTBCCd1 AtTBCCd1a	VIAATNRLVASCHECCFNVGTPRGPVLGDNHKLQVAPYNT
AtTBCCd1b	VIVATKRICIANCRECVFFLGVNQRPLIVGDNHKLQVAPYNT
MpTBCCd1	LIVASARCIANCRECLFNLGVNQRPLVIGDNHNLQVAPYNT VIAVCHRLSISSTTGCIFHVLTPTRPLILSGNQTVTFAPFHT
HsTBCCd1	
TbTBCCd1	ISALCTAVVVNRCRNVNIFICTNSPPVLCQLEGSENSENVRFAPYNS
Ec-08_006490 SjTBCC	IVTAGRQL <b>R</b> IHESRHVDFYVLVASGPIIEDCSGLRFAPAGL IVAAGRQL <b>R</b> IHDSQGVDFYVAVSSGPIIEDCSALRFAPSGL
HSTBCC	LAVACQQLRIHSTKDTRIFLQVTSRAIVEDCSGIQFAPYTW
AtTBCC	LVLASHQI <b>R</b> IHCARKSDFYLRVRSRPIIEDSNGVRFAPYCL
MpTBCC	LVLASHQIRIHSTKSTDFYVRVRSRPIVEYTTKNRFAPYAF
CrTBCC VcTBCC	LALATYQV <b>R</b> IHRAHATDLFLRVRSKPIIEHSAGIRVAPWPA LSLATYQV <b>R</b> VHRTHATDLFLRARSKPIIEHSTGIRVAPWVA
TbTBCC	VYVACHQLRLKNCTGSDMYVSCASTPIIECCTGMRFGPYGCWTGI
Ec-19_004040	FTVACKQLRTRDCEDCEFRLYCKTEPIIETSHGMTFAPFNG
SjRP2a	FTVACKQLRTRDCTDCEFRLYCKTEPIIETSTRLSFGQFNG
TbRP2 CrRP2	VHVACKQL <b>R</b> TRDCEYITLHIFATTDPVVESSHHINFKPFYI LCVAARQL <b>R</b> TRDCTRLDIGACTP
VcRP2	LCVAARQL <b>R</b> FRDCTRLDIGACTPETSTGIVFSCWRG
Ec-21 002520	VVVACQQFRARDCTDCTFFLFSATQPVIESSVGLRFGCYTL
SjRP2b	
HsRP2	CTLACQQF <b>R</b> /RDCRKLEVFLCCATQPIIESSSNIKFGCFQW
Ec-05 001860	CYPRHKAHLQRAGLLGEEEDEVPANLWGNPVEASMM
SjTBCCd1	CYPRHKAHLMRAGLLGDGED-VPANLWGSPVEASMM
CrTBCCd1	RYEKQAAHAREVGLRCDLPNRFDSPVVLLG
VcTBCCd1	HHHRDARDLHLHLHTPRTGKDRKSLAG
AtTBCCd1a AtTBCCd1b	FYSHLEEHMREVGLEPTINKWDKPLALGAV FYSHLEEHITEVGIQPTINKWNESLALGAI
MpTBCCd1	FYPKLEADLAQVGVDATVNSWDRLLTLGLI
HsTBCCd1	HYPMLEDHMARTGLATVPNYWDNPMVVCRE
TbTBCCd1	HYSTLEEHLANSGVNPMMNLWNVGIPSPHC
Ec-08_006490 SjTBCC	RYPEYQHHLQAAGLEEDAVTNAWADVKDFKWH EYPQHRAHLQEAGLADVTNAWEDVKDFKWH
HSTBCC	SYPEIDKDFESSGLDRSKNNWNDVDDFNWL
AtTBCC	DYKGIDEDLKTAGLEEETNNWANVDDFRWL
MpTBCC TbTBCC	SYLGIEEDLRAANLAEETGLWEKVDDFRWL
CrTBCC	LHSSIGEHYNSHEEWLKCLGEIEDLQRAGEMYKTVDDFQWL LLPGPEAQPRLAELLGRHMLGEETGCWQQVEDFGWI
VcTBCC	AVAPEPRLERLLQANMLGEETGCWQQVDDFGWI
Ec-19_004040	AYVGHAEHLREAGLMTPNLWFGVYDFNDE
SjRP2a TbRP2	TTPNLWFGVYDFNDE
TBRP2 CrRP2	RLPGLQASFKSARLDPKTNRFVHVYDFTED GWAEGVHHFSDAKLDPTKNTWMQVYDFNSK
VcRP2	DPAKNTWRQVYDFNRT
Ec-21_002520	DYFSLAGQFAAAKLSVWNNRWSQIHNFTGG
SjRP2b	
HsRP2	YYPELAFQFKDAGLSIFNNTWSNIHDFTPV

А

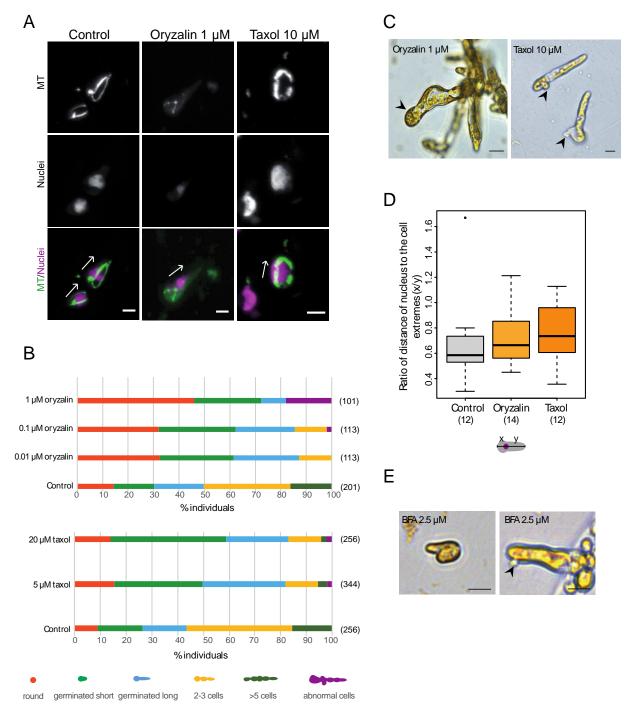


В

Supplemental Figure 9. (A) Alignment of the TBCC domains of diverse TBCCd1 (green), TBCC (orange) and RP2 (blue) proteins. The conserved arginine residue in TBCC and RP2 proteins is indicated in red. The arginine residue that has been proposed to carry out the same function in TBCCd1 is indicated in purple. Ectocarpus sp. proteins are labelled with their LocusIDs. The TBCC domain of the predicted *Ectocarpus* sp. *dis-2* mutant protein is also shown with the stop codon introduced by the mutation indicated with an asterisk. At, Arabidopsis thaliana; Cr, Chlamydomonas reinhardtii; Hs, Homo sapiens; Mp, Marchantia polymorpha; Tb, Trypanosoma brucei; Vc, Volvox carteri. (B) Predicted secondary structure representation of the overall fold of the TBCC domain of the Ectocarpus sp. DIS/TBCCd1 protein, as predicted by Phyre2 (Kelley et al., 2015). The model was based on the PDB 2BX6 (Kuhne et al. 2006), and the alignment covers residues 458-604 of the DIS/TBCCd1 protein with a sequence identity of 19% and 100% confidence. Supports Figure 4.



Supplemental Figure 10. Additional representative confocal maximum z-projections of untreated wild type cells (A), of untreated *dis-1* and *dis-2* mutant cells (B) and of wild type cells treated for 4h with 10  $\mu$ M taxol (paclitaxel) (C) at several stages of early development of the initial cell (24h and 48h after release). Cells were labelled with anti-tubulin antibody (green) and stained with DAPI (purple). Note that the microtubule bundles of *dis* mutant cells are wavy and more numerous compared with the wild type initial cells. MT, microtubules. Size bars = 2  $\mu$ m. See also Figure 5 and Figure 6.



Supplemental Figure 11. Effect of oryzalin and taxol (paclitaxel) treatment on the microtubule cytoskeleton and early development. (A) Anti-tubulin antibody staining of wild type parthenosporophyte initial cells treated for 24 hours with the microtubule polymerisation inhibitor oryzalin (1 uM), taxol (10 uM) or with 0.002% DMSO as a control (Control). Images are representative of 67 cells for oryzalin and 97 cells for taxol treatment. The arrow in the lower panel shows the direction of the germination. Size bar=5 µm. (B) Oryzalin treatment (0.01-1 µM) for 24h and taxol treatment for 4h significantly affected cell elongation and cell division. Number of cells counted is indicated in brackets. (C) Ectocarpus sp. filament showing abnormally shaped cells (arrowhead) seven days after recovery from a 24h-treatment with 1 µM oryzalin and 4h-treatment with taxol 10 µM. Size bar=20 µm. Arrows indicate the direction of the germination. (D) 24h-treatment of wild type Ec32 germinating cells with oryzalin (1 µM) or 4h-treatment with taxol (10 µM) did not affect nuclei positioning. Number of individuals used are indicated in brackets. (E) Abnormal cells after four days after recovery from a 24h-treatment with 2.5 µM Brefeldin A (BFA). Representative germinating initial cell showing an abnormal morphology (left panel). Right panel shows a representative germinating initial cell with cytoplasmic leakage (arrow head). Size bar=5 µm. See also Figure 6 and Supplemental Table 16.