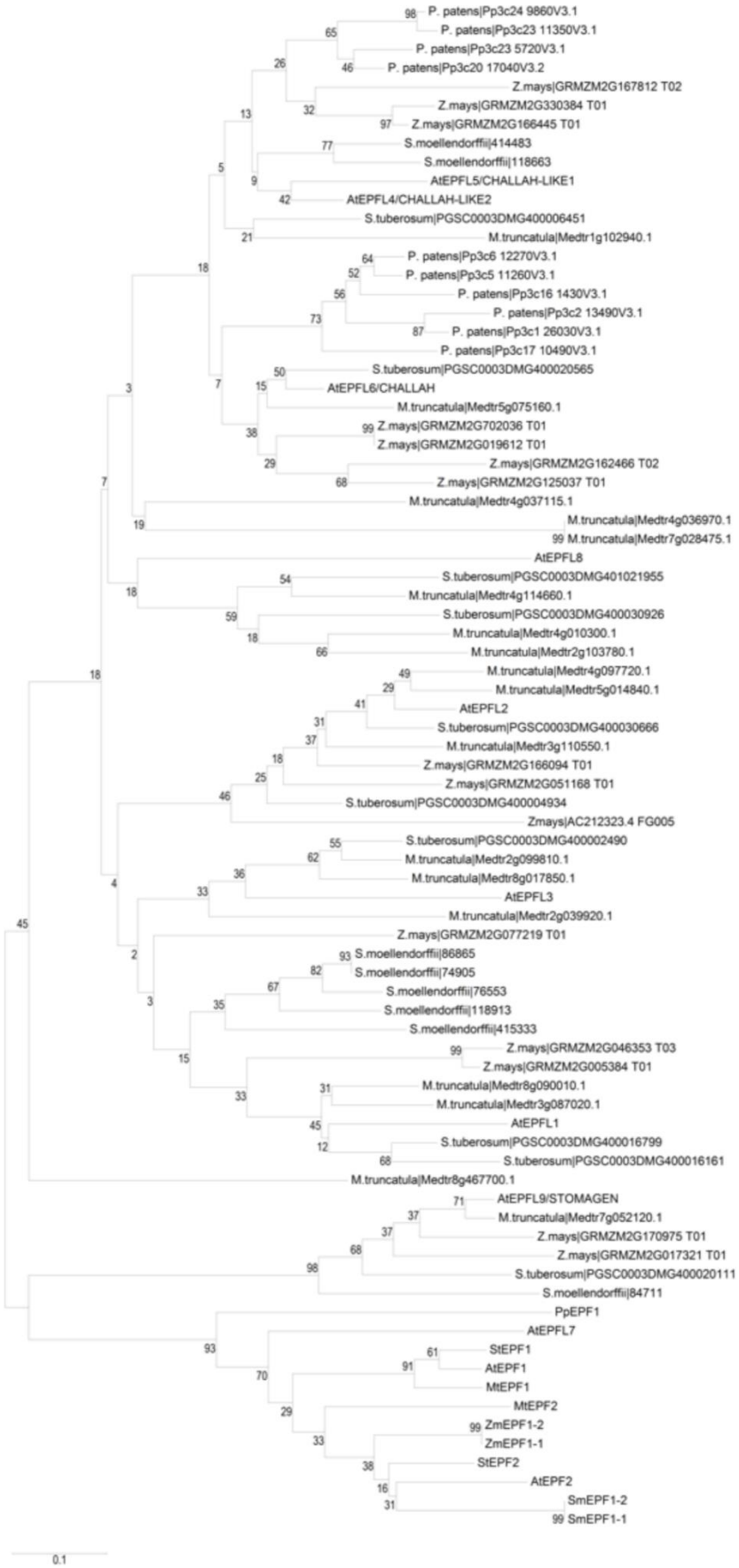
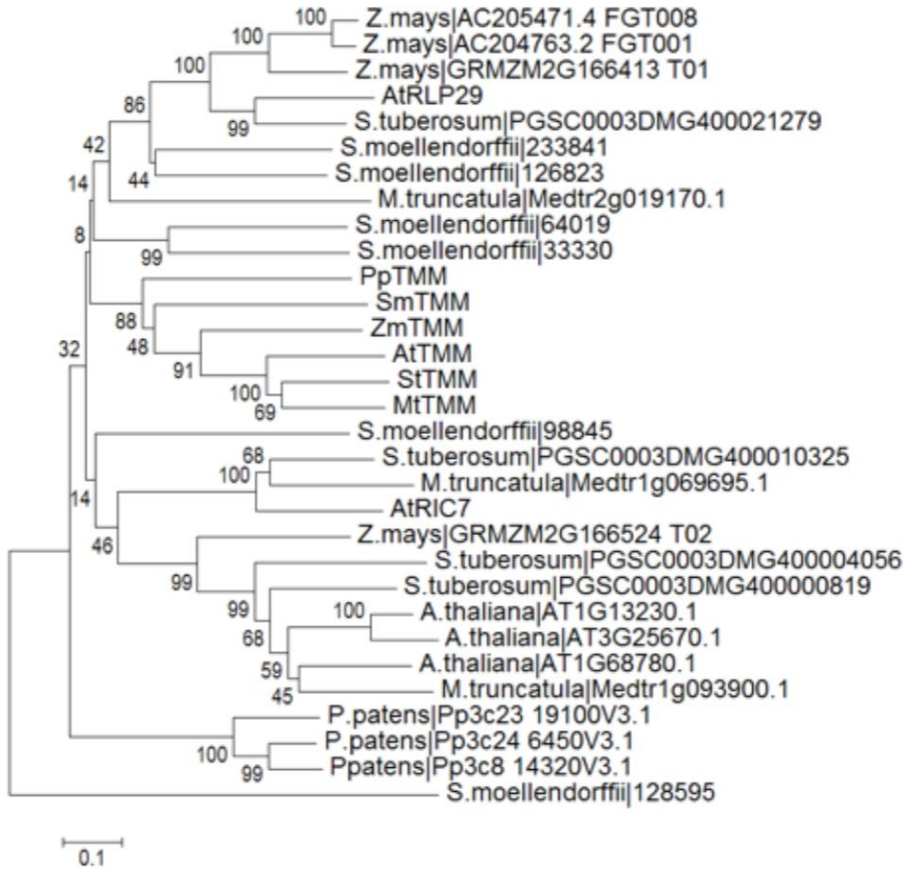
A



B



C

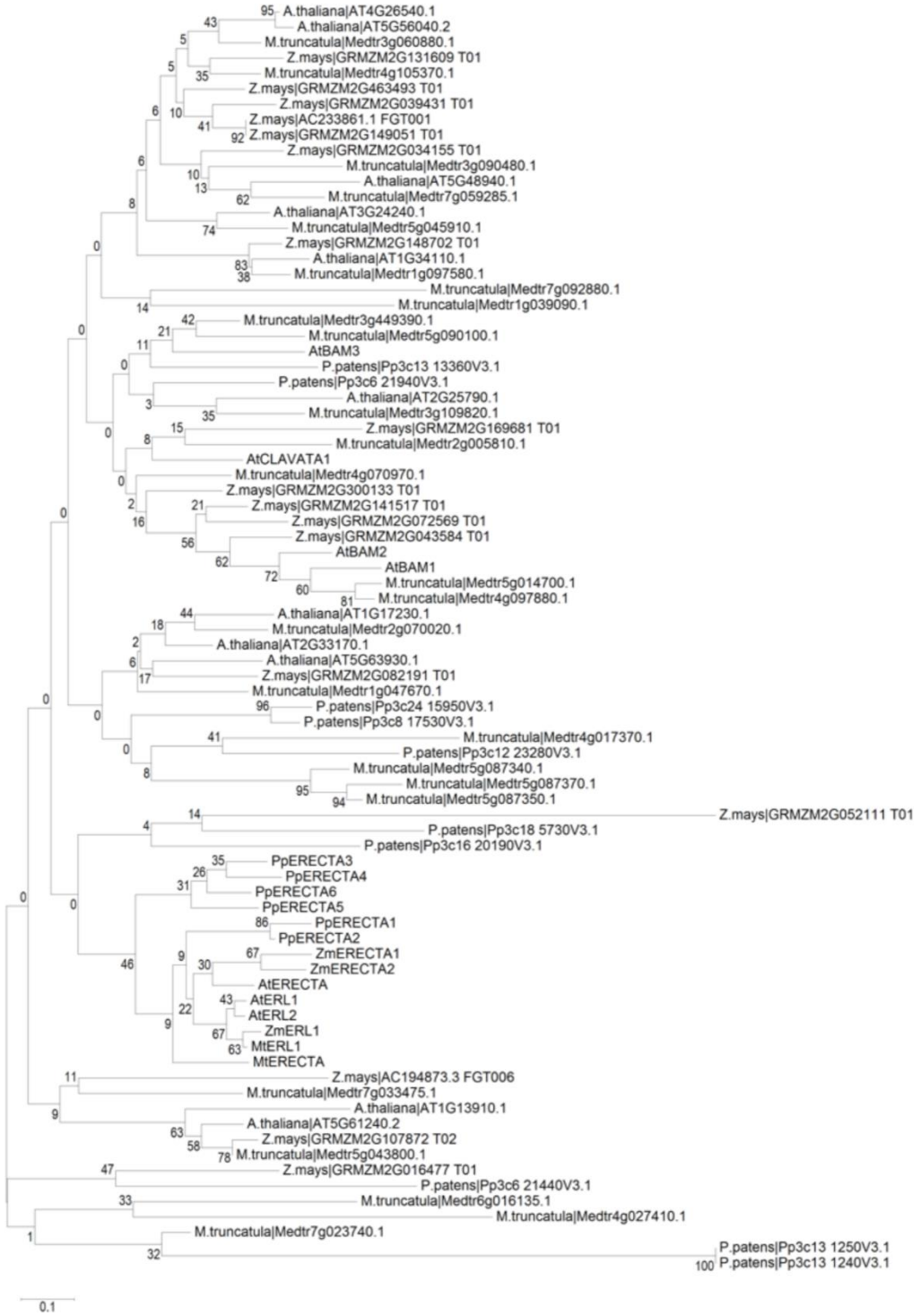


Fig. S1 Extended phylogenetic trees for EPF, TMM and ERECTA gene families
Evolutionary relationships of **(A)** Epidermal Patterning Factor (EPF) and Epidermal Patterning Factor-like (EPFL) genes; **(B)** TOO MANY MOUTHS (TMM) genes; and **(C)** ERECTA and ERECTA-like genes based on amino acid sequence alignments from selected land plant lineages. Gene family members related to A. thaliana EPF1, TMM and ERECTA from P. patens, S. moellendorffii, Z. mays, S. tuberosum, M. truncatula and A. thaliana (as indicated) were identified via phytozome gene family predictions and manual methods (Goodstein et al., 2012). For the ERECTA phylogeny S. moellendorffii and S. tuberosum have been emitted from the analysis due to the large number of gene representatives overall in this family. See Table S1 for accession numbers not indicated in the trees.

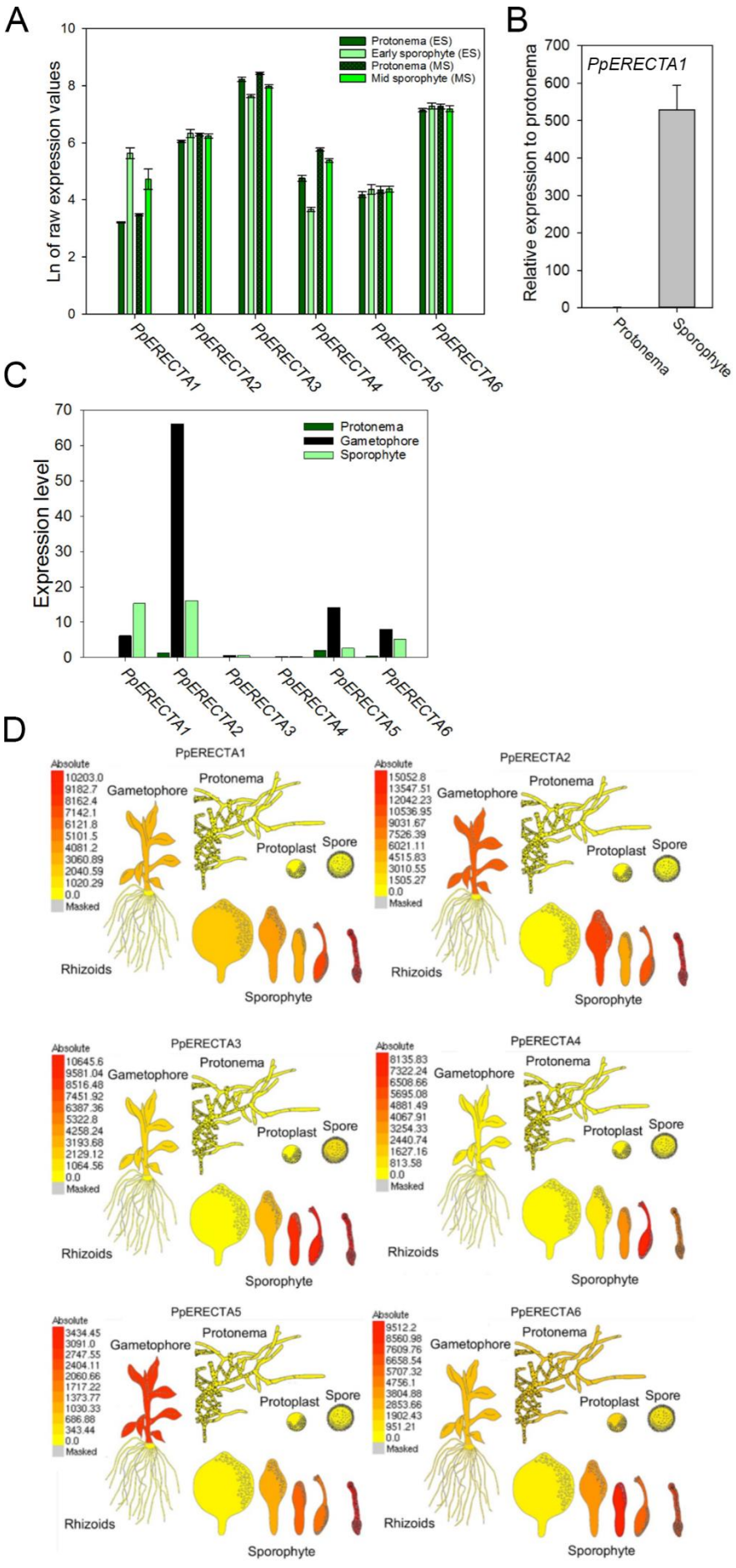


Fig. S2 Expression profiles of *PpERECTA* genes.

(A) Expression levels of 6 *PpERECTA* genes in early (ES) and mid-stage (MS) sporophytes derived from microarray data (O'Donaghue et al, 2013). The expression in each sporophyte stage can be compared with the transcript level in protonema taken from the equivalent colony stage.

(B) qPCR analysis of *PpERECTA1* expression in protonemal and sporophyte tissue.
Expression relative to an actin control gene has been set at 1 for the protonemal tissue, indicating a 500-fold relative increase in *PpERECTA1* expression in the sporophyte.

(C) Expression levels of 6 *PpERECTA* genes in protonema, gametophores and sporophytes. Data derived from the Phytozome gene atlas (vs 11) (Goodstein et al., 2012)

(D) Expression profiles of 6 *PpERECTA* genes derived from microarray data on the *P. patens eFP browser* (Ortiz-Ramirez et al., 2016) for spore, protoplast, protonemal, gametophyte and sporophyte tissue. Absolute expression values are provided to illustrate differential expression between related *PpERECTA* family members. See Table S1 for relevant accession numbers.

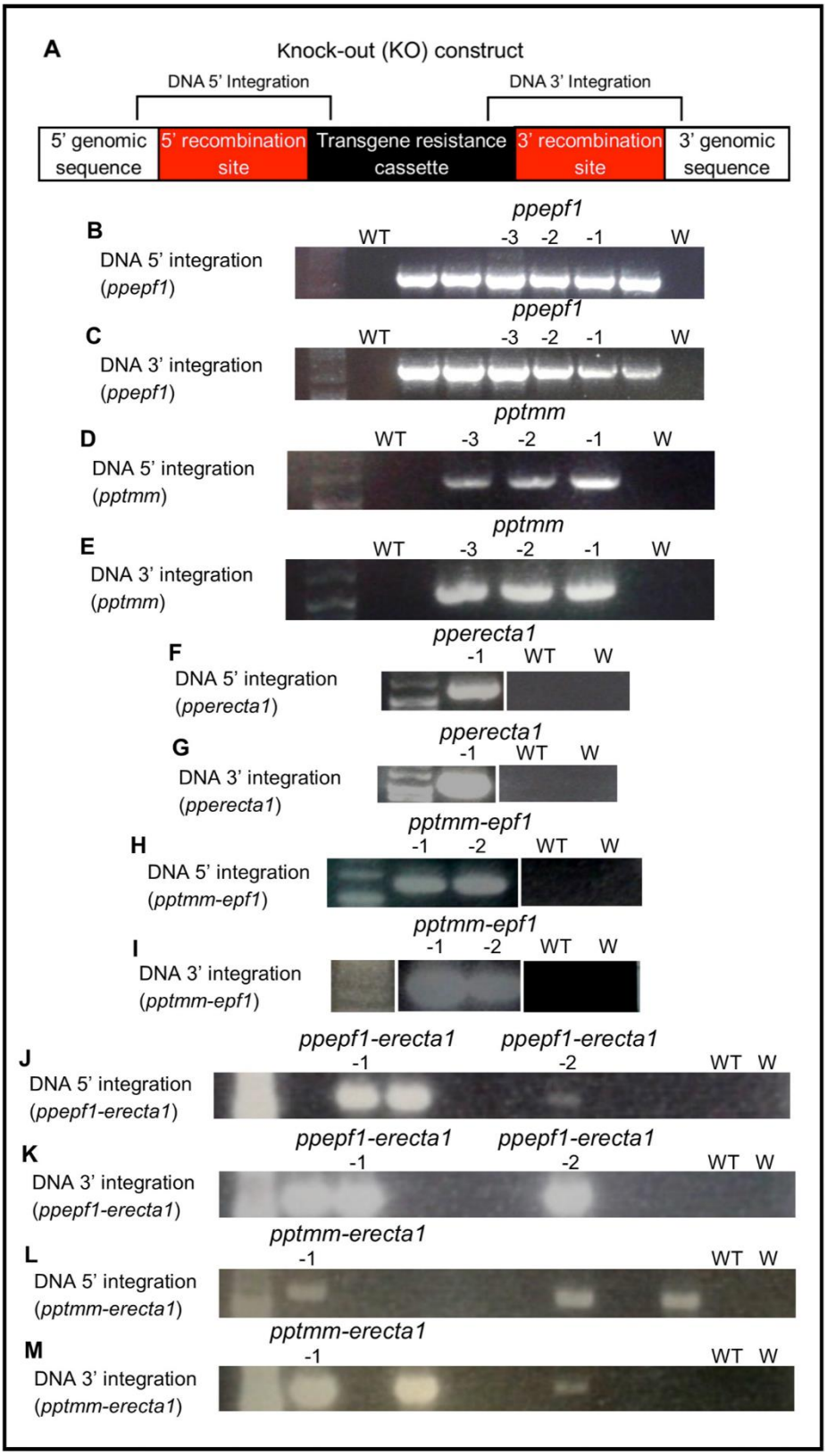


Fig. S3 Molecular analysis of gene knock-out lines

(A) Schematic of approach taken to confirm gene knock-outs. To verify 5' genomic integration, PCR was performed on mutant lines targeting a fragment spanning from the 5' genomic sequence to the transgene resistance cassette. To verify 3' genomic integration, PCR was performed to verify the presence of a fragment spanning from the transgene resistance cassette to the 3' genomic sequence. (B-M) Gel images of PCR products illustrating targeted integration of the KO construct at both the 5' and 3' regions of the genomic loci of mutants: *ppepf1* (B,C); *pptmm* (D,E); *pperecta1* (F,G); *pptmm-epf1* (H,I); *ppepf1-erecta1* (J,K); and *pptmm-erecta1* (L,M). Each lane with a number indicates an individual line taken forward and used for phenotypic analysis. Lanes showing a band but no number indicate potential KO lines obtained but not taken forward for phenotyping. WT refers to a Wild-type sample DNA and W refers to a water sample control. Ladders are included on the left of each gel shot but owing to variation in exposure are not always visible. Primers used are listed in Table S2.

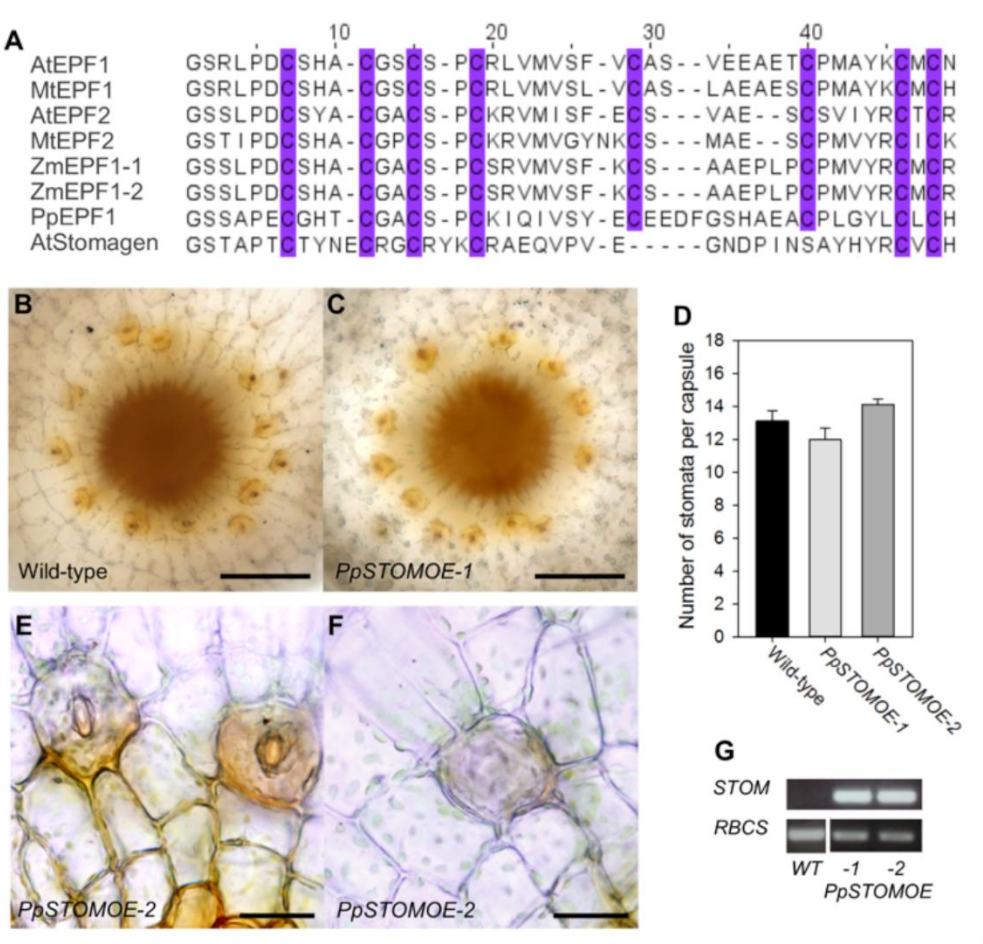


Fig. S4 Overexpression of *STOMAGEN* (*AtEPFL9*) does not disrupt stomatal patterning in *Physcomitrella patens*.

(A) Sequence alignment of EPF peptides. Conserved amino acids are highlighted in purple. (B,C) Bright field images of the base of the sporophyte from (B) WT and (C) PpSTOMOE-1 plants (D) Number of stomata per capsule in wild-type and two PpSTOMOE lines. No significant difference ($P< 0.05$) was found between the lines (oneway ANOVA with multiple comparisons corrected using a Dunnett's test, n=7). (E,F) Images showing abnormal cell division patterns at the base of sporophytes in PpSTOMOE lines. (G) RT-PCR analysis of *STOMAGEN* transcript accumulation (upper panel) in two PpSTOMOE lines and WT tissue, and transcript detection for a control RBCS gene (lower panel).

Scale bars: B,C= 100μm; E,F = 25μm; Error bars in D = s.e.m.

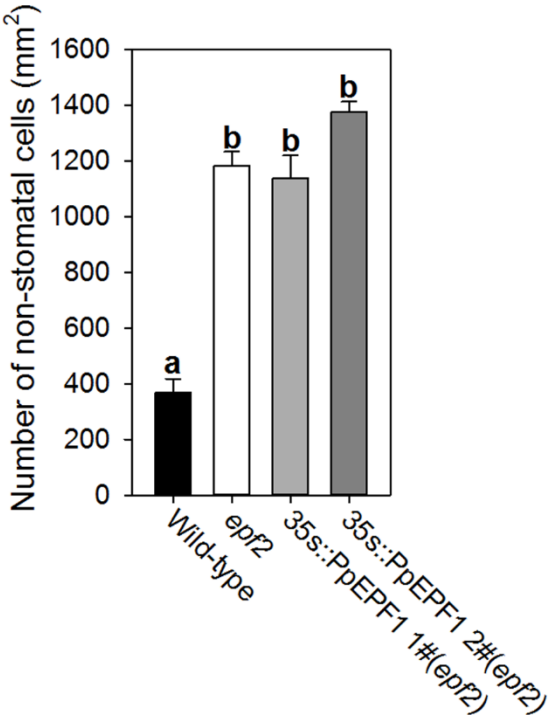


Fig. S5. Expression of *PpEPF1* in the Arabidopsis *epf2* background does not restore WT epidermal cell density. There is a significant increase in epidermal cell density relative to WT in both the *epf2* mutant and in two lines of the *epf2* mutant overexpressing *PpEPF1* (One-Way ANOVA with multiple comparisons corrected with a Dunnett test, n=6, $p < 0.001$ for columns indicated with different letters).

Table S1. Accession numbers relating to gene identifiers used in the phylogenetic analyses.
For *P. patens* both V3.3 and V1.6 identifiers are provided.

Gene identifier	Accession 1	Accession 2
PpEPF1	Pp3c6 27020V3.1	Pp1s279 24V6.1
PpCHALLAH-related1	Pp3c23_5720V3.1	Pp1s16_10V6.1
PpCHALLAH-related2	Pp3c24_9860V3.1	Pp1s196_93V6.1
PpCHALLAH-related3	Pp3c23_11350V3.1	Pp1s137_142V6.1
PpCHALLAH-related4	Pp3c17_10490V3.1	Pp1s105_161V6.1
PpCHALLAH-related5	Pp3c5_11260V3.1	Pp1s263_75V6.1
PpCHALLAH-related6	Pp3c6 12270V3.1	
PpCHALLAH-related7	Pp3c16_1430V3.1	Pp1s144_136V6.1
PpCHALLAH-related8	Pp3c2_13490V3.1	Pp1s30_64V6.1
PpCHALLAH-related9	Pp3c1 26030V3.1	Pp1s21_79V6.1
ZmEPF1-1	GRMZM2G177393	
ZmEPF1-2	GRMZM2G431783	
MtEPF1	Medtr2g090220.1	
MtEPF2	Medtr2g067510.1	
StEPF1	PGSC0003DMG400007864	
StEPF2	PGSC0003DMG400027541	
AtEPF1	AT2G20875.1	
AtEPF2	AT1G34245.1	
AtEPFL1	AT5G10310.1	
AtEPFL2	AT4G37810.1	
AtEPFL3	AT3G13898.1	
AtEPFL4/CLL2	AT4G14723.1	
AtEPFL5/CLL1	AT3G22820.1	
AtEPFL6/AtCHALLAH	AT2G30370.1	
AtEPFL7	AT1G71866.1	
AtEPFL8	At1g80133.1	
AtEPFL9/AtSTOMAGEN	AT4G12970.1	
AtCLAVATA3	AT2G27250.3	
PpTMM	Pp3c3_3780V3.1	Pp1s1_587V6
SmTMM	125817	
ZmTMM	GRMZM2G011401	
MtTMM	Medtr2g103940.1	
StTMM	PGSC0003DMG400028627	
AtTMM	AT1G80080.1	
AtRLP29	AT2G42800.1	
AtRIC7	AT4G28560.1	
PpERECTA1	Pp3c2_22410V3.1	Pp1s125_96V6.1
PpERECTA2	Pp3c1_17360V3.1	Pp1s63_16V6.1
PpERECTA3	Pp3c21_9500V3.1	Pp1s353_18V6
PpERECTA4	Pp3c18_10870V3.1	Pp1s19_291V6
PpERECTA5	Pp3c22_10630V3.1	Pp1s121_69V6
PpERECTA6	Pp3c19_15110V3.1	Pp1s20_166V6
ZmERECTA1	GRMZM2G463904	
ZmERECTA2	GRMZM5G809695	
ZmERL1	GRMZM2G082855	

MtERECTA	Medtr1g015530.1	
MtERL1	Medtr1g102500.1	
AtERECTA	AT2G26330.1	
AtERL1	AT5G62230.1	
AtERL2	AT5G07180.1	
AtBAM1	AT5G65700.1	
AtBAM2	AT3G49670.1	
AtBAM3	AT4G20270.1	
AtCLAVATA1	AT1G75820.1	

Table S2. Primers used in this study

Primer name	Sequence
5’ PpEPF1 3’ F	CTCTCACTCCTCAATACACGTG
5’ PpEPF1 3’ R	GCAACAAACGTCATTTCCAA
PpEPF1 KO CONSTRUCT F	AGCGCAATCCACATACGAAACT
PpEPF1 KO CONSTRUCT R	GGGTTGGGCGAAGGTTTTATATT
Flanking PpTMM 5’ F	GTGCATTAACGGTGCATTGAAA
Flanking PpTMM 5' R	GCATCTGACACGAAATGTCACAG
Flanking PpTMM 3’ F	TTCAACCTTCCCAATGCACCTAT
Flanking PpTMM 3’ R	CACTCATACTTTTGGACCGATGC
PpTMM KO CONSTRUCT F	GATGGAGGTGGTCCTACGAGAG
PpTMM KO CONSTRUCT R	GCGGATTGATAAATTGGCGTTA
Flanking PpERECTA1 5’ F	CTCGCTCTCTCTCTTCCTGG
Flanking PpERECTA1 5' R	ATCGCCATGACAGGGAGTAG
Flanking PpERECTA1 3’ F	TCCACTCCACTTCCCATTCT
Flanking PpERECTA1 3’ R	GGTGACTTCCTATCATGCGC
PpERECTA1 KO CONSTRUCT F	CTCGCTCTCTCTCTTCCTGG
PpERECTA1 KO CONSTRUCT R	GGTGACTTCCTATCATGCGC
PpEPF1 OE F	GCCTTATTGACATGGCTGCT
PpEPF1 OE R	TCAAGGGATGGGAAAGGATT
PpTMM OE F	ATTGTGGTAGTGTACGAGGTAGGC
PpTMM OE R	TTAGCACCTTGACATGATTACGA
AtSTOMAGEN OE F	AAGCATGAAATGATGAACATCAAG
AtSTOMAGEN OE R	TTATCTATGACAAACACATCTATAATGAT
M13 F	GTAAAACGACGGCCAGT
M13 R	CAGGAAACAGCTATGAC
PpEPF1 OE CONSTRUCT F	ACCATGAGCAACGAGCTGAA
PpEPF1 OE CONSTRUCT R	AACAGCACATAGGCCGACAA
PpTMM OE CONSTRUCT F	ACCATGAGCAACGAGCTGAA
PpTMM OE CONSTRUCT R	TGCCTCGGTAACATCTTCAGG
AtSTOMAGEN OE CONSTRUCT F	GGTCGATCTGGTTGTACTGAGG
AtSTOMAGEN OE CONSTRUCT R	AACAGCACATAGGCCGACAA
PpEPF1 RT-PCR F	CCGCGTCATACTTGGAACTG
PpEPF1 RT-PCR R	CAAGTAGCCCAACGGACAAG
PpEPF1 OE RT-PCR F	TCCAAGATAGAGACTGAGGGG
PpEPF1 OE RT-PCR R	TCCTCGCATTCATAGCTCACAA
PpTMM RT-PCR F	TGGCGCACAACAGATTCTCAGG
PpTMM RT-PCR R	AGCCTTCGTTGTTCTGCAGTCG
PpTMM OE RT-PCR F	CTCCAACAACCAAAGCGTCG
PpTMM OE RT-PCR R	AACGCTGGTTTTAAGCTGCC
PpERECTA1 RT-PCR F	TAAGCGAGAAGTACGTGGCA
PpERECTA1 RT-PCR R	GGATAACTGGGAGGTTTGCG
PpAtSTOMAGEN OE RT-PCR F	GTTCAAGCCTCAAGACCTCG
PpAtSTOMAGEN OE RT-PCR R	CCTTCGACTGGAACTTGCTC
PpRubisco RT-PCR F	TTGTGGCTCCTGTCTCTGTG
PpRubisco RT-PCR R	CGAGAAGGTCTCGAACTTGG
PpEPF1 comp AtEPF1 F	CACCATGGCCTTATTGACATGG
PpEPF1 comp AtEPF1 R	TCAAGGGATGGGAAAGGAT
pAtTMM F	CACCATACAATCCATGATGCTGCTT
pAtTMM R	CATTTCTTAGTTGTTGTTGTTGTGT
PpTMM comp AtTMM F	CACCATGATTGTGGTAGTGTACG
PpTMM comp AtTMM R	TTAGCACCTTGACATGATTACGAG

qPCR PpERECTA1 F	CTTCGGTATTGTGCTGCTGG
qPCR PpERECTA1 R	CTTCGCACACAACAACGCTA
qPCR Adenine phosphoribosyltransferase F	AGTATAGTCTAGAGTATGGTACCG
qPCR Adenine phosphoribosyltransferase R	TAGCAATTTGATGGCAGCTC
qPCR Small Ribosomal F	ACGGACATTGCATTTAAGACCT
qPCR Small Ribosomal R	GTCGATTACCTGTGGAGAAGAC
qPCR Large Ribosomal F	GACAGGCACAGGGTATTCCT
qPCR Large Ribosomal R	ATCTTCCGTCGTGTTGATCC