Supplemental Materials

Table S1. Primers used in this study.

Primer pair	Sequences	Anneal. temp.	Amplicon
pC3tF pC3tR	GATATCGAATTCCTGCAGAATGGAGGCTAATGCGGGC TCCGCGGAAGGCCTCCTCACAAGCAGGTGAGGCC	68℃	CESA3 for ligation into pTFB1
pC3nF pC3nR	CCTCGGCCTCTCGAGATGGAGGCTAATGCGGGC AATTACATGACTCGAGGTTCACAAGCAGGTGAGGCC	68℃	CESA3 for ligation into pADSL-Nx
pC6tF pC6tR	GATATCGAATTCCTGCAGAATGGAGGCCAATGCGGGG TCCGCGGAAGGCCTCCTCAACAGTTTATCCCGCACTGC	68℃	CESA6 for ligation into pTFB1
pC6nF pC6nR	CCTCGGCCTCTCGAGATGGAGGCCAATGCGGGG AATTACATGACTCGAGGTCAACAGTTTATCCCGCACTGC	68℃	CESA6 for ligation into pADSL-Nx
pC7tF pC7tR	GATATCGAATTCCTGCAGAATGGAGGCGAATGCAGGG TCCGCGGAAGGCCTCCTCAACAGTTTATCCCGCACTG	68℃	CESA7 for ligation into pTFB1
pC7nF pC7tR	CCTCGGCCTCTCGAGATGGAGGCGAATGCAGGG AATTACATGACTCGAGGTTCAACAGTTTATCCCGCACTG	68℃	CESA7 for ligation into pADSL-Nx
pC8tF	GATATCGAATTCCTGCAGAATGGAGGCTAATGCGGGC TCCGCGGAAGGCCTCCTTACAAGCAGGTGAGGCC	68℃	CESA8 for ligation into pTFB1
pC8nF pC8nR	CCTCGGCCTCTCGAGATGGAGGCTAATGCGGGC AATTACATGACTCGAGGTTTACAAGCAGGTGAGGCC	68℃	CESA8 for ligation into pADSL-Nx
pC3gwF pC3gwR	CACCATGGAGGCTAATGCGGGCCTGGTTGCTG TCACAAGCAGGTGAGGCCGCACCGCACCAAG	68℃	CESA3 for ligation into pBIFc-2 and pBIFc-3
pC6gwF pC6gwR	CACCATGGAGGCCAATGCGGGGTTGGTGG TCAACAGTTTATCCCGCACTGCGACAGGTC	68℃	CESA6 for ligation into pBIFc-2 and pBIFc-3
pC7gwF pC7gwR	CACCATGGAGGCCAATGCGGGG TCAACAGTTTATCCCGCACTG	68℃	CESA7 for ligation into pBIFc-2 and pBIFc-3
pC8gwF pC8gwR	CACCATGGAGGCTAATGCGGGC TTACAAGCAGGTGAGGCC	68℃	CESA8 for ligation into pBIFc-2 and pBIFc-3

Table S2. Peptide antigens, designed to regions of each PpCESA, used to raise specific antibodies for each PpCESA isoform.

Peptide Antigen	Sequence
PpCESA3	CPDHDQEKSSSILSTKDIEKR
PpCESA8	CLDHDYEKSSPIMSTKDIEKR
PpCESA6/7	CVIRQESDGPRPLSN

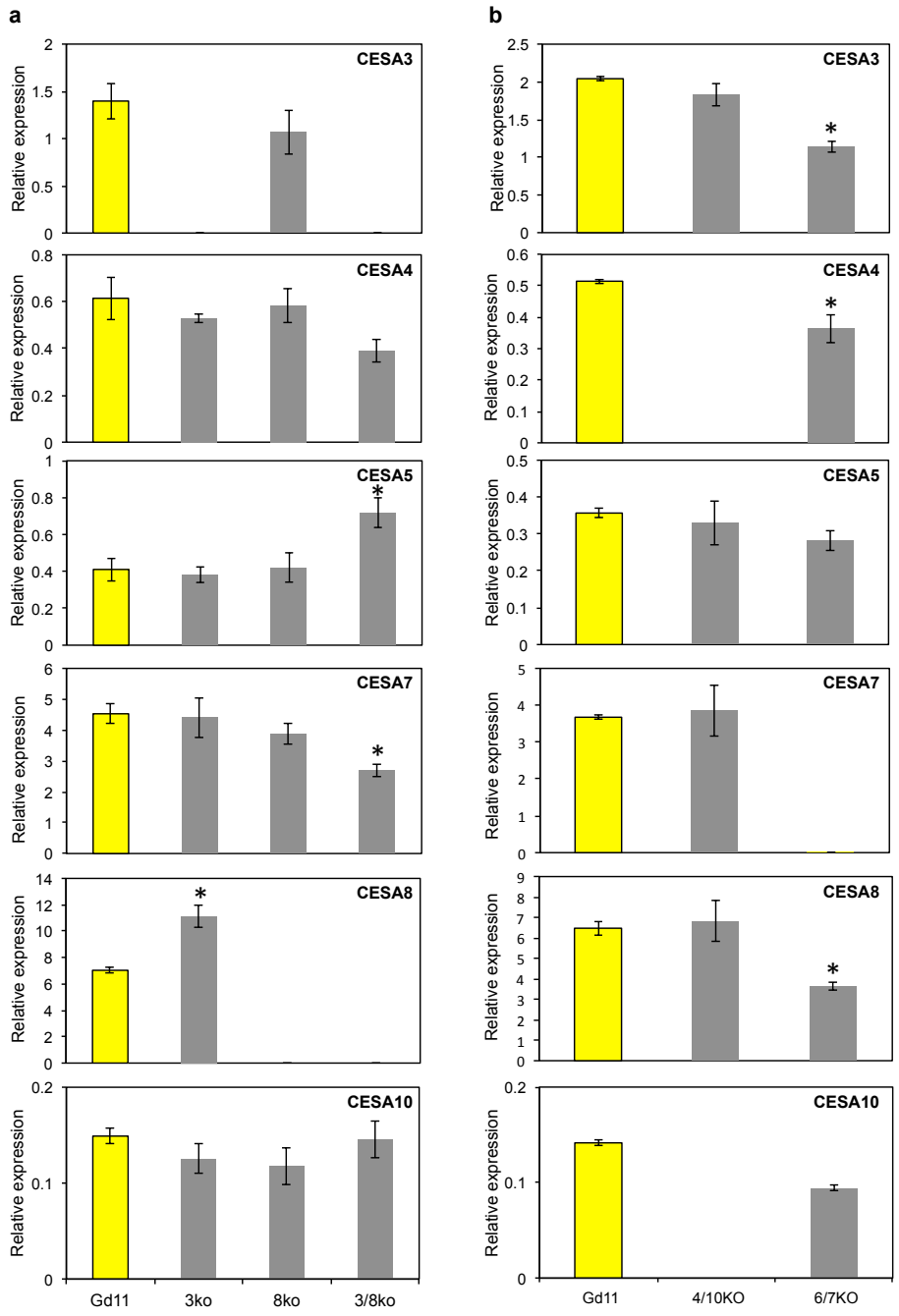

Figure S1: RT-qPCR analysis of *PpCESA* expression in the *ppcesa*KO mutants. (A) *PpCESA* expression levels relative to two reference genes (PpACT and PpVHP) in gametophores isolated from 21-day-old cultures of three clade A *ppcesa*KO mutants (*ppcesa3*KO*, ppcesa8*KO, and *ppcesa3/8*KO) and wild type GD11 (yellow bars). (B) Relative *PpCESAs* expression in gametophores isolated from 21-day-old cultures of two clade B *ppcesa*KO mutants (*ppcesa4/10*KO, and *ppcesa6/7*KO) and wild type GD11. Error bars indicate standard error of the mean (95% confidence interval) for 2^-ΔCt from 1 of 2 replicate experiments (n=3 independent genetic lines). Lines that differ from the wild type based on non-parametric statistical analysis (see Materials and Methods) are indicated by "*" ($p<0.05$). Missing bars indicate no measureable expression.

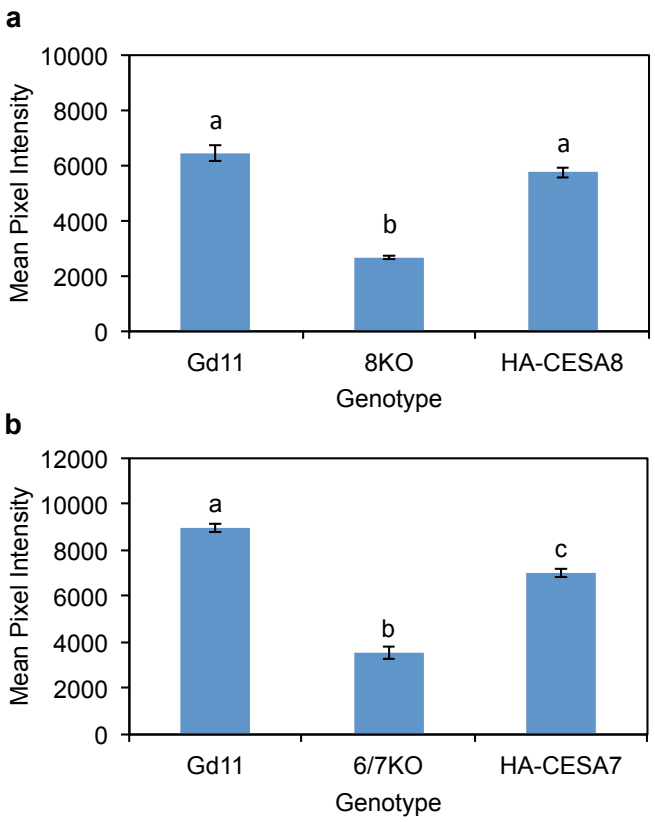

Figure S2: Quantitative analysis of S4B fluorescence intensity in leaf midribs of *P. patens* wild type (Gd11), *ppcesa* knockout lines, and *ppcesa* knockout lines expressing cognate *HA-PpCESA*s. (A) Fluorescence intensity is significantly reduced in *ppcesa8*KO-5B-lox (8KO) compared to GD11 wild type, and is fully restored in *ppcesa8*KO-5B-lox expressing *PpCESA8pro::HA-PpCESA8* (HA-CESA8). (B) Fluorescence intensity is also significantly reduced in *ppcesa6/7*KO-7A-lox (6/7KO) compared to wild type. Expression of *PpCESA7pro::HA-PpCESA7* restored *ppcesa6/7*KO-7A-lox (HA-CESA7) to 80% of wild-type level. Bars with different letters are significantly different as determined by ANOVA with Tukey multiple comparison of means ($p<0.05$).