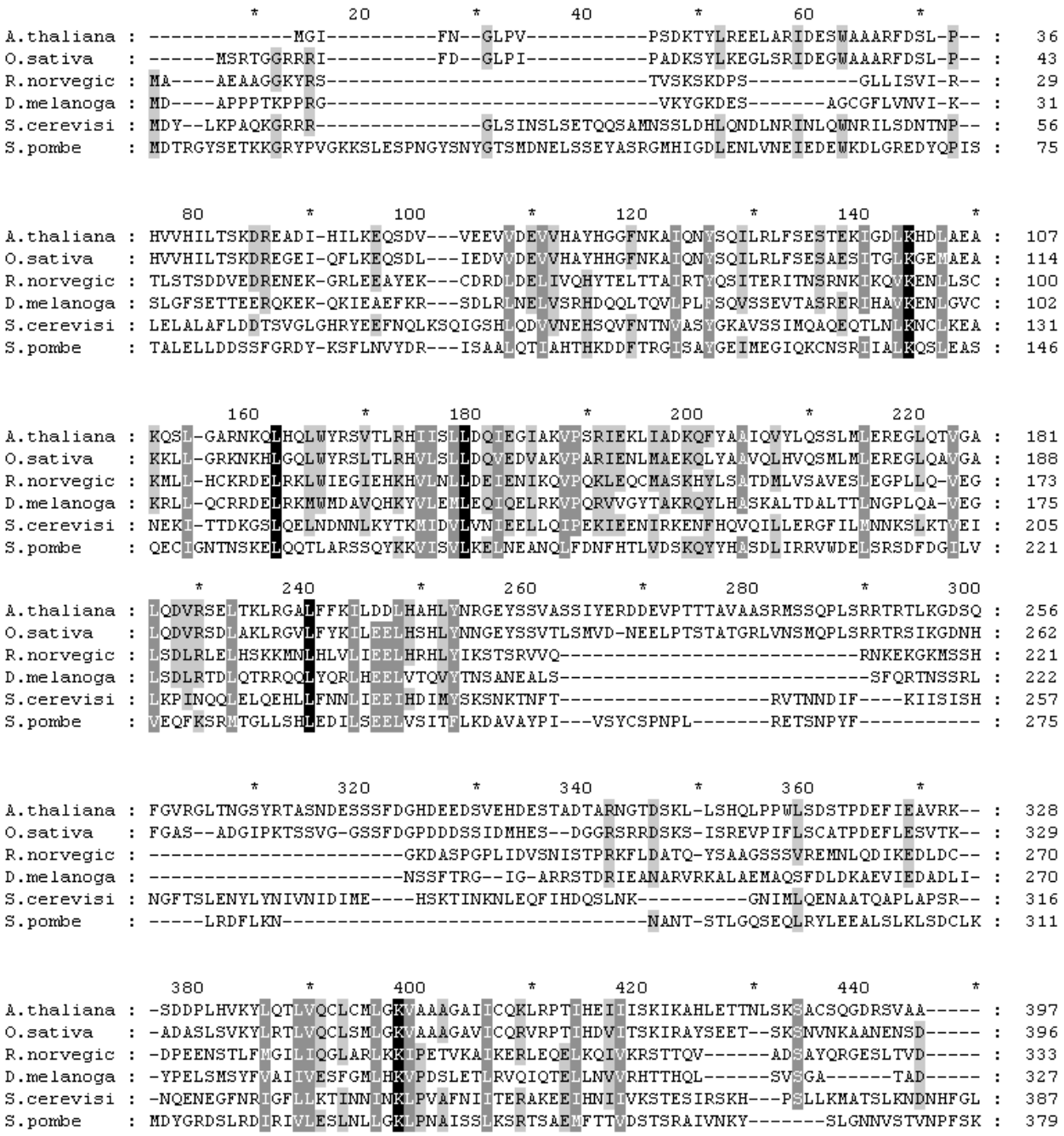
Supplemental Figure 1. Alignment of SEC8 amino acid sequences from a range of eukaryotic species, including plants, animals and fungi:
Arabidopsis thaliana (gi|24418673|sp|Q93YU5|SEC8_ARATH[24418673]), *Orysa sativa* (gi|50508194|dbj|BAD31511.1|[50508194] tr|Q69PQ1|Q69PQ1_ORYSA), *Ratus norvegicus* (gi|24418659|sp|Q62824|SEC8_RAT[24418659]), *Drosophila melanogaster* (gi|33860220|sp|Q9VNH6|SEC8_DROME[33860220]), *Saccharomyces cerevisiae* (gi|417762|sp|P32855|SEC8_YEAST[417762]), and *Schizosaccharomyces pombe* (gi|21542229|sp|O74562|SEC8_SCHPO[21542229]). White letters with black background = identity. White letters with dark grey back ground = 80% similarity. Black letters with light grey background = 60% similarity. Alignment by T-Coffee (Notredame, et al. 2000).



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                  460         *       480         *       500         *       520
A.thaliana : GLHLIKGQSEAYRLSKEKPQNGISNSGTHL-AVSPVSPLMAPGGKAQAAAKELLDSILDTIVKIFENHVVIGELL :  471
O.sativa   : VSHS-NGRAARYQLLKQKTKNGASLMASQL-VVSPISPAMAPTGDAQCAASQLLSAIFECLVDILENHITVGELL :  469
R.norvegic : -NQP-RLLLELLELLFDKFNAVASAHSIVL-GYLQDSVGTQPTQQEEIK-----------LYDMADVWVKIQDVL :  394
D.melanoga : -TNP---LLSLLEVIFKQFKAIAKTHSLLLKNYL--SVG-QKYSVVGPQ-----------PYDLTDFWAQAQSVL :  384
S.cerevisi : PVQD-ILSIILRECFWEIFLKLLYAIQCHR-AIFEMSNILQPTSSAKPA------------FKFNKIWGKLLDEI :  448
S.pombe    : SLYD-IG--------------------------------LHAETDREHTMISEFLTNLFTKLRCVLMHYRGISEFM :  422

                *       540         *       560         *       580         *       600
A.thaliana : EMKASQH--DINTPRSLPT-DVNWNTESEASQATGGYTISFPLTVL----QSECQQLICEILRATPEAA--SADA :  537
O.sativa   : EQKSSTEVDNANTPHMANG-DASWNPDSESSQATGGFTVAFSLSVV----QSECQQLLCEILRATPEAA--TADA :  537
R.norvegic : QMLLTEYLDM------KNT-RTASEPSAQLSYASTGREFA------------------------------------ :  427
D.melanoga : QLLLTDYLDI------QNA---AADESAQTGFSEPTSNIN------------------------------------ :  415
S.cerevisi : ELLLVRYIND------PEL---ISSNNGSIKPINGATNNA------------------------------------ :  479
S.pombe    : TKLETKTPKHASSSHKSSIMSVNSDPTSPKVSKFDTSDSTFPFDTLLQAFESEIRLMLKDYLISKEEYIENSGNF :  497

                      *       620         *       640         *       660         *
A.thaliana : AAQTAKLAKKAPKKDKRDAPEDGLTFTFRFTDAT--VSIS----NQGADLI-RQ------------GWGKRAPN- :  592
O.sativa   : AVQTARLANKDPVKEKRDGSE-GLSFAFRITDAA--ITAP----NEG------Q------------GW-RRNST- :  585
R.norvegic : ----AFFAKKKPQRPKNSL------FKFESSSHA--ISMSAYLREQRRELYSRS--------GELQGG-PDDNLI :  481
D.melanoga : ----SYFLRRKVPSTKRSM------FKFDKSSHVGTSNNSDAFKEHRRNASDASVDDNLAGQLGGSGK-GSTSGL :  479
S.cerevisi : ----PTLPKRKNPKIFSLE------YNIEDNSS--VKDQAFELKALLKDIFP--------GFSVSSNM-DLDSIY :  533
S.pombe    : VVGTEMSIYNLPGENEEDKLFD-VTNEIAVENK--SNAFYARINELVNEKAP----ELILNKSNA----SVSTIE :  561

                680         *       700         *       720         *       740         *
A.thaliana : ASLEGYGSAA------VLPEQGIYLAASIYRPVLQFTDKITSMLPKKHSQLVNDGLLTFTENFVKDHLLPTMFV- :  660
O.sativa   : VPQEGYGTAS------VIPDQGIFLAASVYRPVFEFMNKIGLMLPQKYWQLGNDGLLAFVNNFLKEHFLPAIFV- :  653
R.norvegic : -EGGGTK---------FVCKPGARNITVIFHPLLRFIQEIEHALGLGPAKQCL--LREFLTIYINKNIFLNQVLT- :  543
D.melanoga : FPHEKKQREK-----ILICTPDQSIITKVYLPLMGYIKEIENFMKCKPGQPCS--LHDFLDNYIKDTFLTKGHN- :  546
S.cerevisi : VKDESFEQDE------PLVPPSVFNMKVILDPFLLPTQSTSTIVPSVLTQNTIS-SLTFFDDYLNKSFLPKIQMT :  601
S.pombe    : LFSGSSKEIVRLAGHVVFVGPSVFHASSVLPQTVFPLEDSVSILKNPNIPPQ--FAVNFMKEFLRGSYIPQLYK- :  633

                      760         *       780         *       800         *       820
A.thaliana : -DYRKGVQQAISSAAAFRPRAHTTT-YTATVEKGRPILQGLLAIDLLAKEVLGWAQAMPKFATDLVKYVQTFLER :  733
O.sativa   : -DYRKCVQQAISSPAAFRPRVHATSVYSPLVENGRPVLQGLLAVDIIAKEVLGWVQLMPNYATELVEYVRTFLER :  727
R.norvegic : -EINKEIEGVTKTSDPLKILANADTMKV--LGVQRPLLQSTIIVEKTVQDLMNLMHDLSAYSDQFLNMVCVKLQE :  615
D.melanoga : RNLQLTIESLSKNQDAWRTIISPEEIKA--LNLSRPLLQSTVMVERRLMETKNLIQDLPCYSEDLLKMVCALLKA :  619
S.cerevisi : MDYLFTVEVESNNPYALEL-----------SDENHNIFKTALDFQRLFYNLLNVFNTANTFREKISYCILDLLNH :  665
S.pombe    : -FMSSHFDTIMKDVGAFQLHRDWKI-YSKI-----PIFKCHVAIVQYFHDLQDYLPIVALNLVEFYELLHTLLVR :  701

                  840         *       860         *       880         *       900
A.thaliana : TFERCRTSYME----AVL--EKLSYM---LIGR----HDIE-KLMRLDA--ASAC-----------------LPS :  775
O.sativa   : THERCRASYME----AVL--EKQSYI---LLSR----NDVE-SLMRLDP--ANLS-----------------LQN :  769
R.norvegic : YKDTCSTAYRG----IVQ--SEEKLV---ISASWAKDDDIS-RLLKSLPNWTNMAQ-PKQ------------LRP :  667
D.melanoga : YREICQAAYRG----IVQPDSEDKRI---YSVAWLKDEDIS-RFLKTLPNWTDLKT-YSQKSRHNRKLHRGSFEP :  685
S.cerevisi : FYNYYLGLFNS----LIG--TSDRHLTRKIITAWLQNGILM-DQEQKILNGDETLF-HEE------------SIE :  720
S.pombe    : FRNHCSDYLSDLCRTAVL--KEYKHV---NEDTEDVDDTVRVKLLHDDVTYPQFIKFLKQ------------KNP :  759

                      *       920         *       940         *       960         *
A.thaliana : TLG---HAVSHSEAVGTEVELSDLFLSLRPIKQDNLIRDDN-KLILLASLSDSLEYVADSIERL-GQAVPR---- :  841
O.sativa   : SFGQLDHSIPDAEAVEVEIELSDLLLDMCPIKQENLIHDDQ-KLILLASLSDSLEYLADSVERL-GESFIN---- :  838
R.norvegic : KREEEEDFIRA--AFGKESEVLIGNLGDKLIPPQDILRDVS-DLKALANLHESLEWLAGRTKSA-FSSLSA---- :  734
D.melanoga : SEEESPLQVQQ--RNIREAEMLTSNLGEGGITQQEILVEIS-VLKELAILQESMEWFSCRVSEF-ANDLRR---- :  752
S.cerevisi : LFKEIPHFYQAGK--GLSKSDLFNNLTLDTI------------LQFSASVLWILNWLPGLKKAINIDEVSQEPML :  781
S.pombe    : SLEGLNELCRM--ENKRLLQYEDRAITSEVKLPVSVLSKDSDLVNSVSYLHNSMEWFLQRCFSRFMNGSRRMNVL :  832

                  980         *      1000         *      1020         *      1040         *
A.thaliana : -------------VASQAEG--------NSRN------QAASPRNLASFADEYRKLATDCLKVLRVEMQLETVFH :  889
O.sativa   : -------------SSTMLENKNHIHQGRHTRS------TSAIPKSLASLANEYRRLAIDCVRVLRLEMQLETIYH :  894
R.norvegic : -------------S-QMLSPAQESHVNMDLPP-----VSEQIMQTLSELAKSFQDMADRCLLVLHLEVRVHCFHY :  790
D.melanoga : -------------PLVNGLNAVPAECGADIAV------KDGTIKVMTNLALEFDELANTCLLVLHLEVRVQCFHY :  808
S.cerevisi : DADRLRSSWTF--SESMDLNYSNPSSSPNSLGNLKILLDDKASKKFDETIDGFKTLKFKLITILRFNIRALCIYD :  854
S.pombe    : QQNQANFGGDFLPIDNLLGNNSDLMKGYVT--VCIELYTDFYKSAYKEVFDSLQRLQFDALLLIRMEVRLQYIHS :  905
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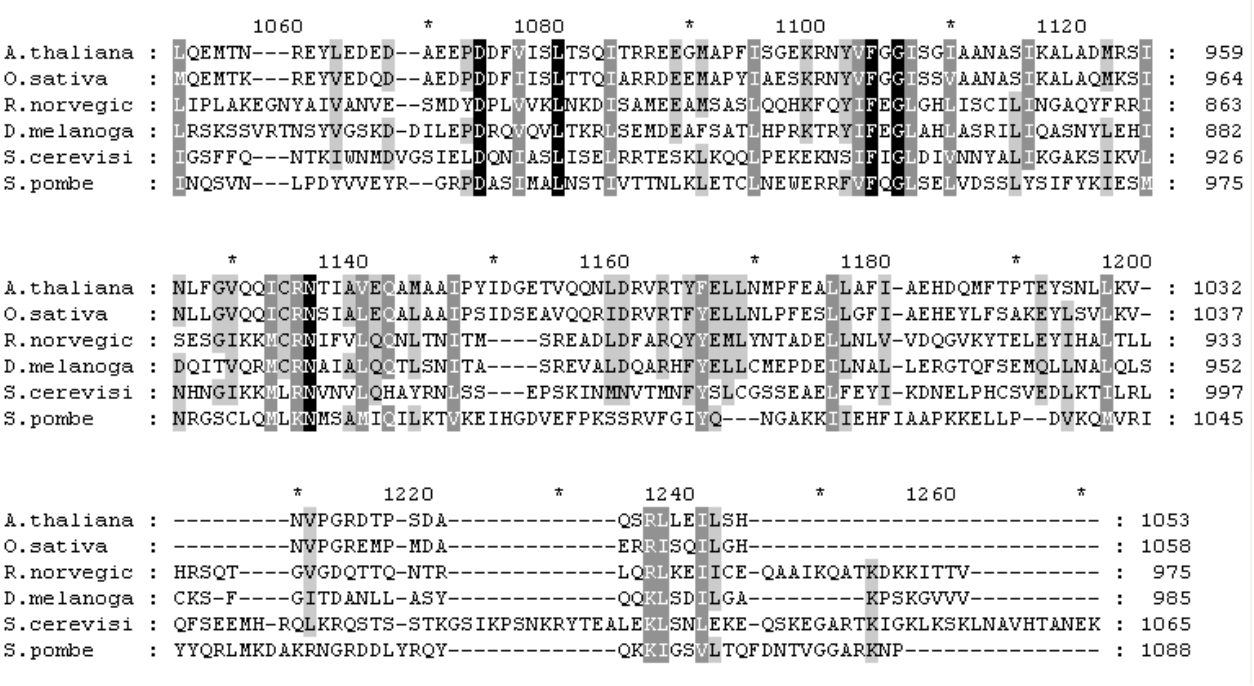
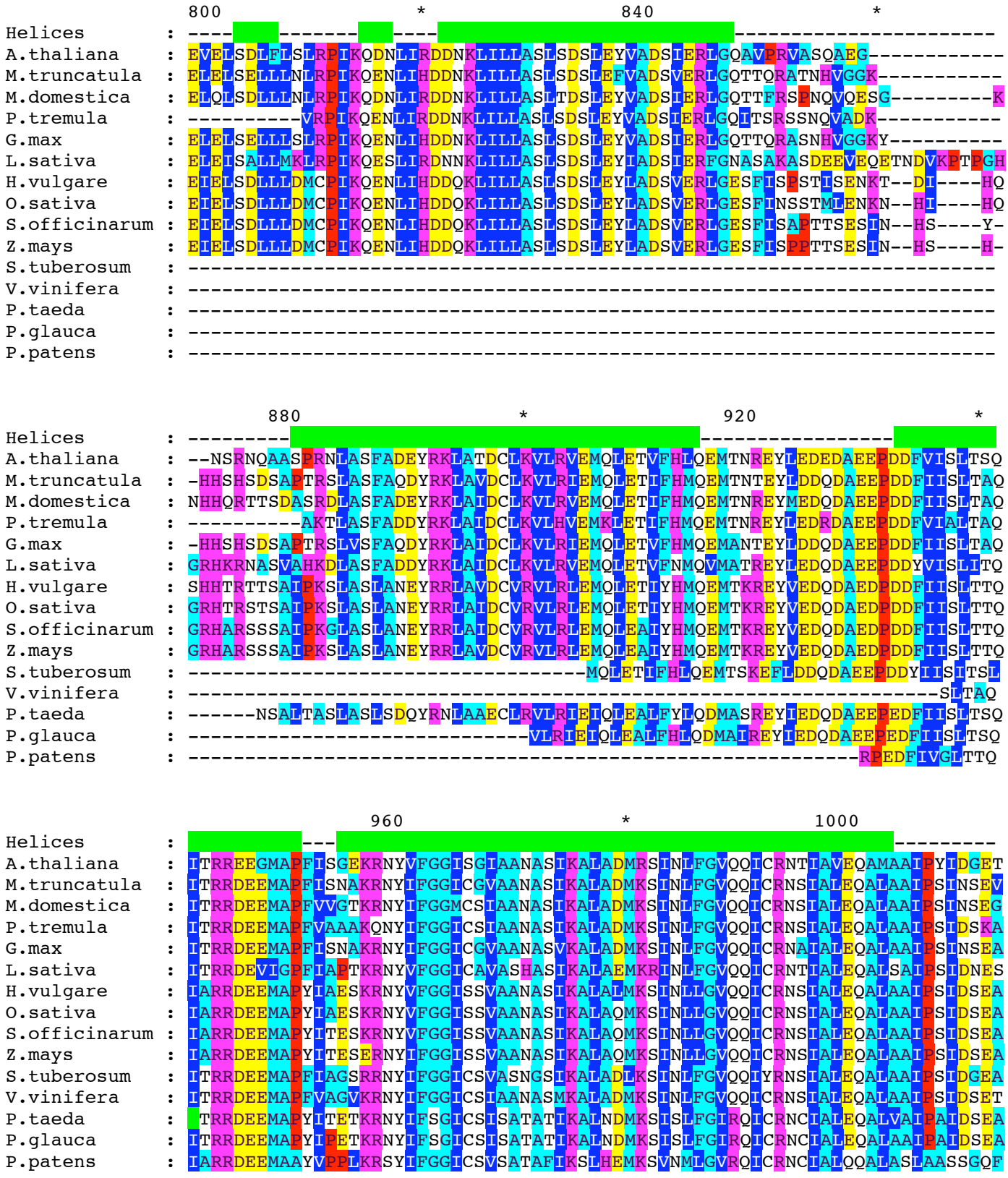


Supplemental Figure 2. (following page) The primary sequence of the C-terminal end of SEC8 orthologs is well conserved across a range of plant species including angiosperms (monocots and dicots), gymnosperms, and a moss. In the alignment, residues are color coded by property as indicated in the key to the right.
Green bars in upper row of alignment indicate sections of alpha helix structure predicted by PSIPRED analysis of *Arabidopsis thaliana* sequence (Jones, 1999; McGuffin et al., 2000). Some sections of the sequences are highly conserved with residues that suggest potentially important physical characteristics. For example, in the putative alpha helix at marker 840, every third or fourth residue is large and hydrophobic, while in between are negatively charged acidic residues. This suggests an alpha helix with one hydrophobic side and one negatively charged side that is highly conserved, but of unknown significance. The mutation of *sec8-m3* is an insertion in the coding sequence for this section of the protein.

Sequences were drawn primarily from contiguous sequences of ESTs found in the TIGR Eukaryotic Gene Orthologs (EGO**)** database for orthologous genes in eukaryotes. Plants with partial sequences for *SEC8* in the database form the tentative orthologous group 755210. These include *M. truncatula* (TC87864), *P. tremulosa* (TC9922), *G. max* (TC228872), *H. vulgare* (TC141913), *L. sativa* (TC14678), *S. officinarum* (TC59270), *Z. mays* (TC263314), *S. tuberosum* (TC105621), *V. vinifera* (TC43096), *S. tuberosum* (TC105621), and *P. glauca* (TC4881). Sequences derived by constructing contigs by hand include: *P. taeda* (contig of ESTs corresponding to GI #'s 805007, 11606574, 49447017), *P. patens* (EST corresponding to GI # 37842367), and *M. domestica* (contig of ESTs corresponding to GI #'s 48277362, 48385947, 48284063, 48488228, 50701353). Alignment by T-Coffee (Notredame, et al. 2000), coloring by the GeneDoc alignment editing and shading utility (http://www.psc.edu/biomed/genedoc/).


800
840
Helices
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M.truncatula
M.domestica
P.tremula
G.max
L.sativa
H.vulgare
O.sativa
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Z.mays
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960
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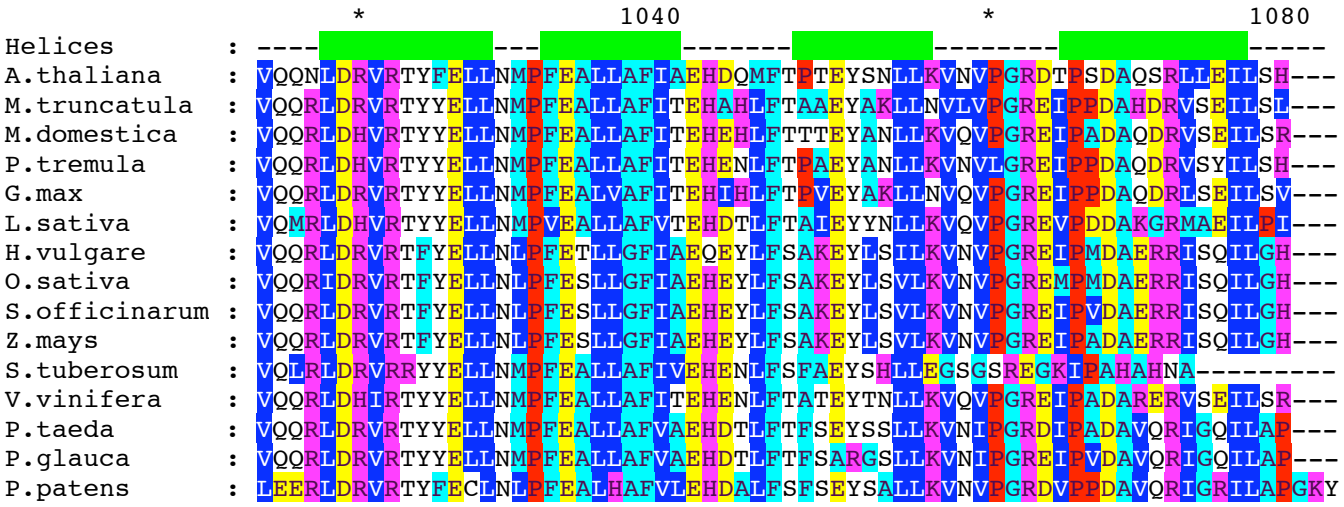

Supplemental Figure 3. Predicted C-terminal protein structure for mutant *SEC8* alleles, based upon sequencing of cDNA. Gray blocked residues represent non-wild-type sequence that has been added to otherwise truncated protein.

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SEC8    : MAAIPYIDGETVQQNLDRVRTYFELLNMPFEALLAFIAEHDQMFTPTEYSNLLKVNVPGRDTPSDAQSRLLEILSH
sec8-m4 : MAAIPYTQQNDAQCVTPGYIVV----------------------------------------------------
sec8-m5 : MAAIPYIDGETVQSESSETDNTLRTFLM-------------------------------------------------
sec8-m6 : MAAIPYIDGETVQQNLDRVRTYFELLNMPFEFKLKAGNDNLIMSGELRESRYDPRR--------------------
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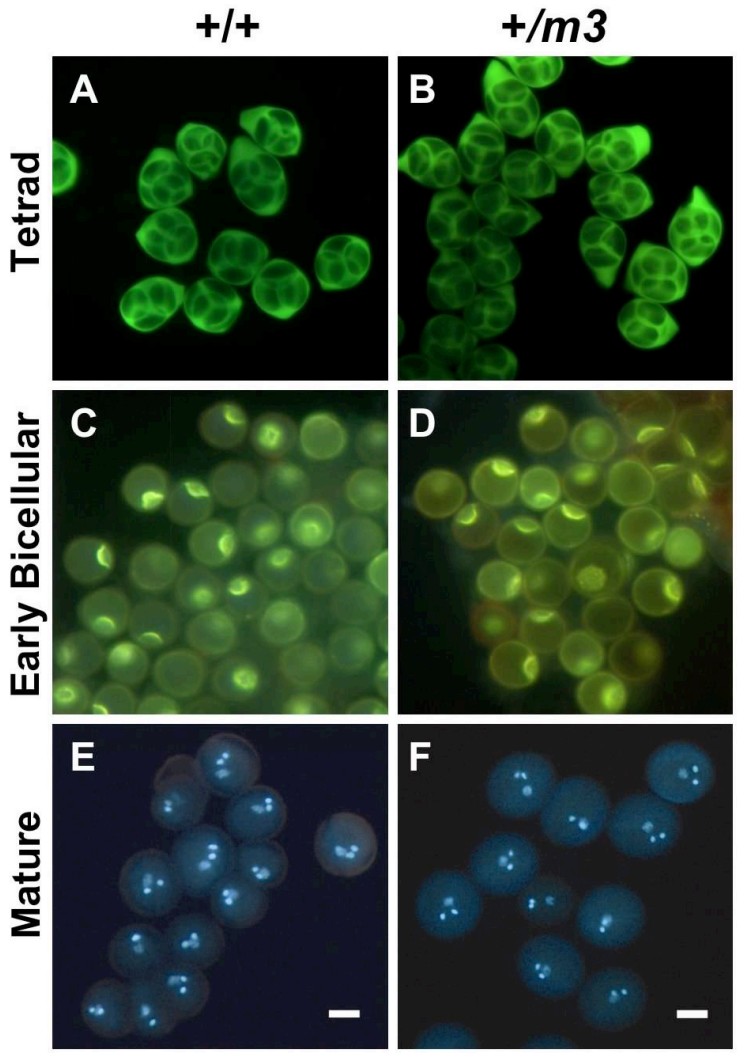
Supplemental Figure 4. Microscopic evaluation revealed no obvious *sec8-m3* mutant phenotype during microsporogenesis. Gametophytes from *sec8-m3* heterozygotes (right column) were compared to those from their homozygous wild-type siblings (left column). A, B, C, D, aniline blue staining to visualize developing cell walls at the tetrad and early bicellular stages; E, F DAPI staining to visualize the three nuclei in mature pollen grains. Bar = 10 μm.



Supplemental Table I.

Silique characteristics of *SEC8* mutant homozygotes and their wild-type siblings									
SEC8 genotype	N	Number of seeds per silique		Seed gaps per silique		Deformed seeds per silique		Silique Length (mm)	
		mean	std error	mean	std error	mean	std error	mean	std error
m4/m4	24	54.5	1.13	0.96	0.24	0.21	0.08	14.7	0.19
+/+	24	54.1	1.49	1.25	0.36	0.13	0.09	14.9	0.18
m5/m5	24	41.8	1.24	1.67	0.36	0.33	0.12	12.8	0.18
+/+	24	45.3	1.75	1.54	0.43	0.25	0.09	13.3	0.27
m6/m6	24	51.0	0.96	0.71	0.16	0.08	0.06	13.2	0.19
+/+	24	50.2	0.96	0.50	0.15	0.38	0.22	13.2	0.16

Supplemental Table II.
Primers used for PCR reactions in this study.

Primer	**Sequence (5' - 3' left to right)**	**Application**
At Sec8-F1	GGC TGT CAT CCT GGC AAA GC	Genotyping *sec8-m2*, RT-PCR WT expression
At Sec8-R1	CAT GCC TCC CAA TGA GCA TGT	Genotyping *sec8-m2*
At Sec8-F2	GGG GAG CCC TTT TCT TCA AA	Genotyping *sec8-m1*
At Sec8-R2	TGG CTA GAC AAA GCA ACT TCT GC	Genotyping *sec8-m1*
At Sec8-F3	CAC GTA GGG AGG AGG GAA TGG	Genotyping & Sequencing *sec8-m4*, *-m5*, *-m6*; RT-PCR Rxn B
At Sec8-R3	TGG CAA ACC AAA AGC CAA AAG	Genotyping *sec8-m5*, *-m6*
At Sec8-R5	CCT GCT TCT CCT TTA TGA TTT CAC C	Genotyping *sec8-m4*; RT-PCR Rxn B
At Sec8-F6	TGG AGC CAG TCT TAA ATG CAC C	Genotyping *sec8-m3*
At Sec8-R6	TGA CTC GCC ACA CGA GGA ACT	Genotyping *sec8-m3*; RT-PCR Rxn A
At Sec8-F11	AGG GAG CCG ATC TCA TTC GTC	RT-PCR Rxn A
At Act2-F1	TGG TGA TGA AGC ACA ATC CAA G	Control for Expression Analysis
At Act2-R1	TGG AAC AAG ACT TCT GGG CAT C	Control for Expression Analysis
At Act3-F1	TTC TCC TGC CGA GAG AAC GA	Control for Expression Analysis
At Act3-R1	ACG CTC AGC TGT TGT GGT GA	Control for Expression Analysis
LBb1	GTG GAC CGC TTG CTG CAA CT	Genotyping and Sequencing – T-DNA Left Border
OGUS 4	TCC AAA CGT AAA ACG GCT TGT CC	Sequencing – T-DNA Right Border

The PSIPRED server

- McGuffin LJ, Bryson K, Jones DT. (2000) The PSIPRED protein structure prediction server. Bioinformatics. 16: 404-405.

The PSIPRED secondary structure prediction method

- Jones DT. (1999) Protein secondary structure prediction based on position-specific scoring matrices. J. Mol. Biol. 292: 195-202.

The T-Coffee Alignment Tool

- Notredame C, Higgins D, Heringa J. (2000) T-Coffee: A novel method for multiple sequence alignments. J. Mol. Biol. 302, 205-217.