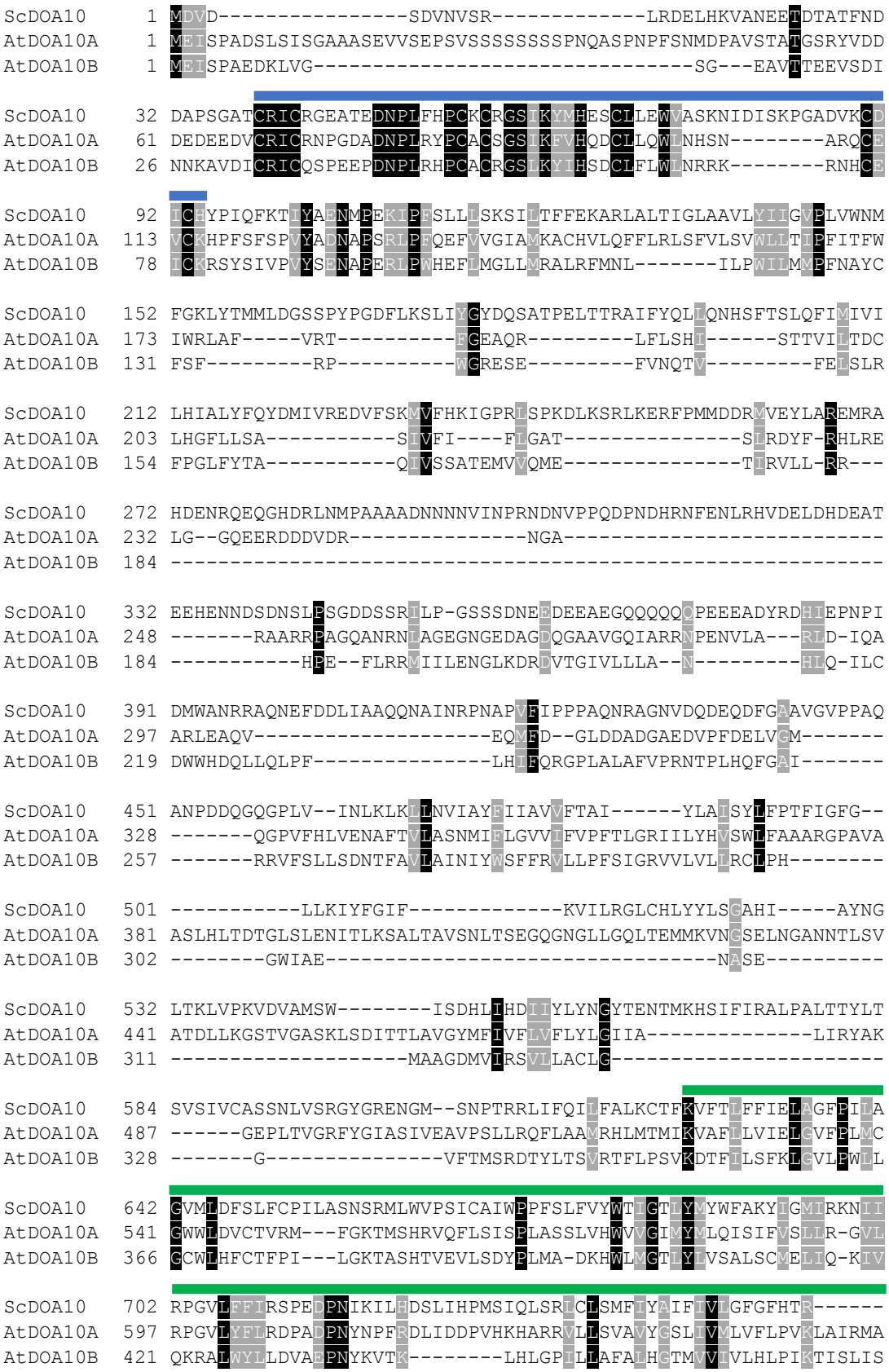
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AtDOA10A 1 MEISPADSLSISGAAASEVVSEPSVSSSSSSSSPNQASPNPFSNMDPAVSTATGSRYVDD
AtDOA10B 1 MEISPAEDKLVG----------SG---EAVTTEEVSDI
ScDOA10 32 DAPSGATCRICRGEATEDNPLFHPCKCRGSIKYMHESCLLEWVASKNIDISKPGADVKCD
AtDOA10A 61 DEDEEDVCRICRNPGDADNPLRYPCACSGSIKFVHQDCLLQWLNHSN--------ARQCE
AtDOA10B 26 NNKAVDICRICQSPEEPDNPLRHPCACRGSIKYIHSDCLFLWLNRRK--------RNHCE
ScDOA10 92 ICHYPIQFKTIYAENMPEKIPFSLLLSKSILTFFEKARLALTIGLAAVLYIIGVPLVWNM
AtDOA10A 113 VCKHPFSFSPVYADNAPSRLPFQEFVVGIAMKACHVLQFFLRLSFVLSVWLLTIPFITFW
AtDOA10B 78 ICKRSYSIVPVYSENAPERLPWHEFLMGLLMRALRFMNL-------ILPWILMMPFNAYC
ScDOA10 152 FGKLYTMMLDGSSPYPGDFLKSLIYGYDQSATPELTTRAIFYQLLQNHSFTSLQFIMIVI
AtDOA10A 173 IWRLAF-----VRT----------FGEAQR---------LFLSHI------STTVIITDC
AtDOA10B 131 FSF---------RP----------WGRESE---------FVNQTV---------FEISLR
ScDOA10 212 LHIALYFQYDMIVREDVFSKMVFHKIGPRLSPKDLKSRLKERFPMMDDRMVEYLAREMRA
AtDOA10A 203 LHGFLLSA-----------SIVFI----FLGAT---------------SLRDYF-RHLRE
AtDOA10B 154 FPGLFYTA-----------QIVSSATEMVVQME---------------TIRVLL-RR---
ScDOA10 272 HDENRQEQGHDRLNMPAAAADNNNNVINPRNDNVPPQDPNDHRNFENLRHVDELDHDEAT
AtDOA10A 232 LG--GQEERDDDVDR----------NGA----------
AtDOA10B 184 ----------
ScDOA10 332 EEHENNDSDNSLPSGDDSSRILP-GSSSDNEEDEEAEGQQQQQQPEEEADYRDHIEPNPI
AtDOA10A 248 -------RAARRPAGQANRNLAGEGNGEDAGDQGAAVGQIARRNPENVLA---RIDIQA
AtDOA10B 184 -----------HPE--FLRRMIILENGLKDRDVTGIVLLLA--N---------HIQILC
ScDOA10 391 DMWANRRAQNEFDDLIAAQQNAINRPNAPVFIPPPAQNRAGNVDQDEQDFGAAVGVPPAQ
AtDOA10A 297 ARLEAQV----------EQMFD--GLDDADGAEDVPFDELVGM-------
AtDOA10B 219 DWWHDQLLQLPF----------LHIFQRGPLALAFVPRNTPLHQFGAI-------
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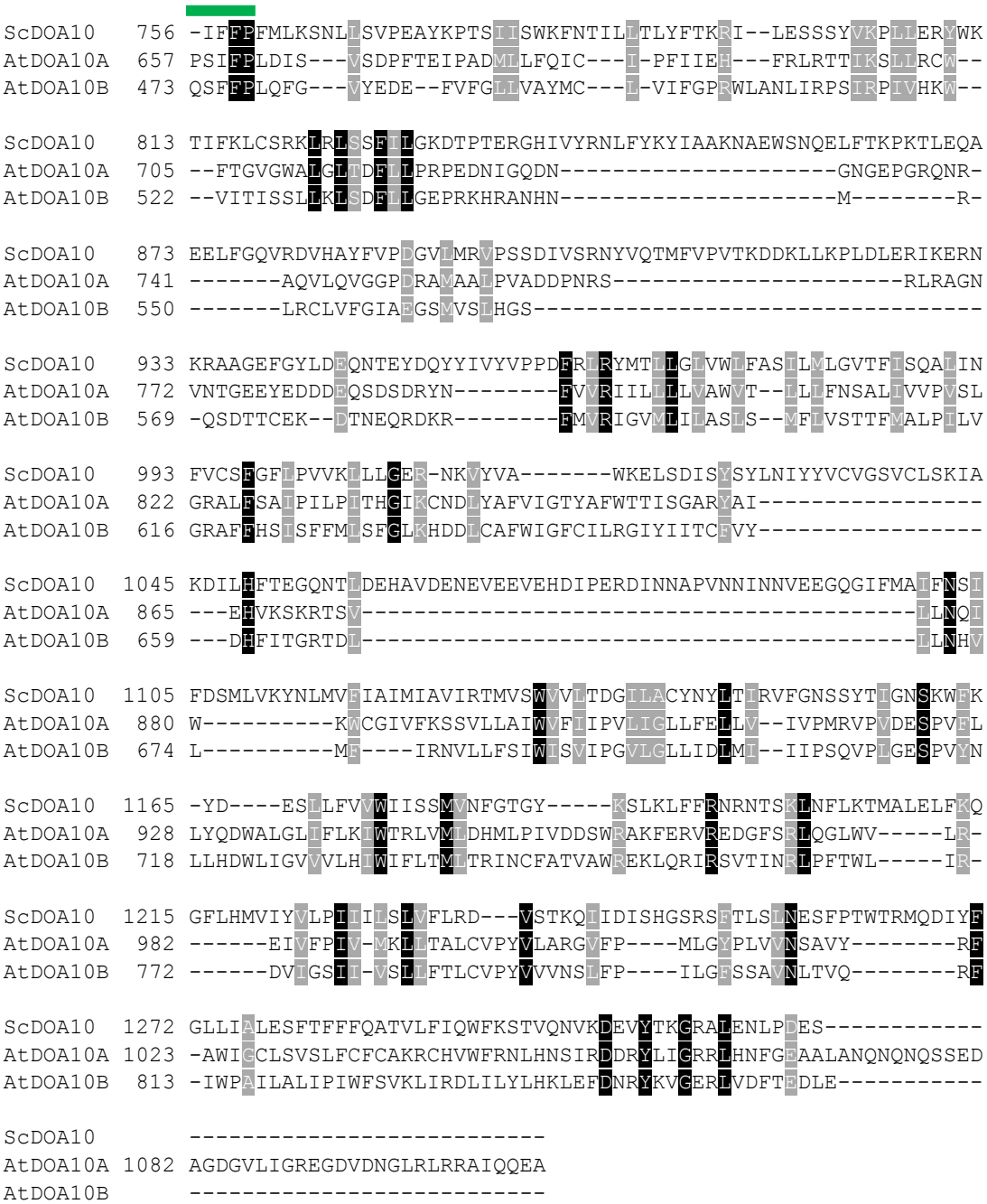
AtDOA10A 328 -------QGPVFHLVENAFTVLASNMIFLGVVIFVPFTLGRIILYHVSWLFAAARGPAVA
AtDOA10B 257 -------RRVFSLLSDNTFAVLAINIYWSFFRVLLPFSIGRVVLVLLRCLPH--------
ScDOA10 501 -----------LLKIYFGIF----------KVILRGLCHLYYLSGAHI-----AYNG
AtDOA10A 381 ASLHLTDTGLSLENITLKSALTAVSNLTSEGQGNGLLGQLTEMMKVNGSELNGANNTLSV
AtDOA10B 302 --------GWIAE----------NASE----------
ScDOA10 532 LTKLVPKVDVAMSW--------ISDHLIHDIIYLYNGYTENTMKHSIFIRALPALTTYLT
AtDOA10A 441 ATDLLKGSTVGASKLSDITTLAVGYMFIVFLVFLYLGIIA----------LIRYAK
AtDOA10B 311 ----------MAAGDMVIRSVLLACLG----------
ScDOA10 584 SVSIVCASSNLVSRGYGRENGM--SNPTRRLIFQILFALKCTFKVFTLFFIELAGFPILA
AtDOA10A 487 ------GEPLTVGRFYGIASIVEAVPSLLRQFLAAMRHLMTMIKVAFLLVIELGVFPLMC
AtDOA10B 328 -------G----------VFTMSRDTYLTSVRTFLPSVKDTFILSFKLGVLPWLL
ScDOA10 642 GVMLDFSLFCPILASNSRMLWVPSICAIWPPFSLFVYWTIGTLYMYWFAKYIGMIRKNII
AtDOA10A 541 GWWLDVCTVRM---FGKTMSHRVQFLSISPLASSLVHWVVGIMYMLQISIFVSLLR-GVL
AtDOA10B 366 GCWLHFCTFPI---LGKTASHTVEVLSDYPLMA-DKHWLMGTLYLVSALSCMELIQ-KIV
ScDOA10 702 RPGVLFFIRSPEDPNIKILHDSLIHPMSIQLSRICLSMFIYAIFIVLGFGFHTR------
AtDOA10A 597 RPGVLYFLRDPADPNYNPFRDLIDDPVHKHARRVLLSVAVYGSLIVMLVFLPVKLAIRMA
AtDOA10B 421 QKRALWYLLDVAEPNYKVTK--------LHLGPILLAFALHGTMVVIVLHLPIKTISLIS

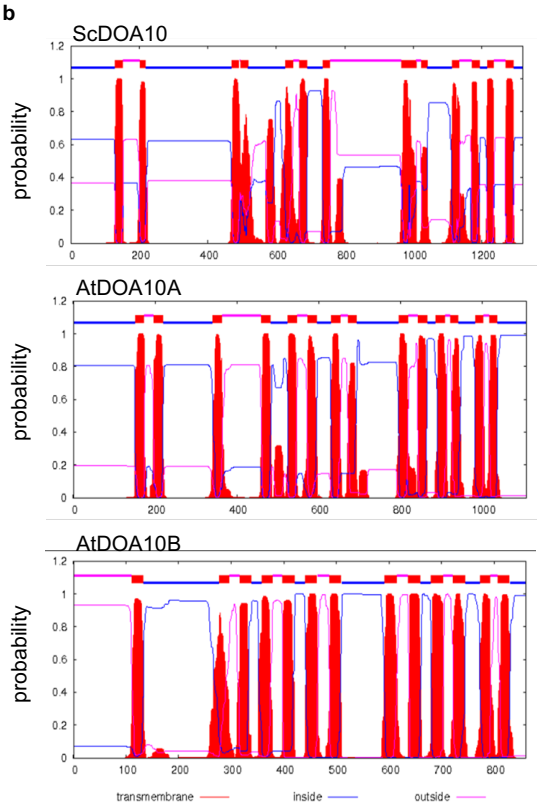


Supplemental Figure S1. Full length amino acid residue sequence alignment of ScDOA10, AtDOA10A and AtDOA10B. Blue bar denotes RING-CH domain, green bar conserved TD region. Sequences were obtained from NCBI and aligned using clustalW2 (now Clustal Omega: https://www.ebi.ac.uk/Tools/msa/clustalo/), then converted to the output shown using Boxshade 3.2 (https://embnet.vital-it.ch/software/ BOX_form.html), with the fraction of sequences that must agree for shading set to >0.5.

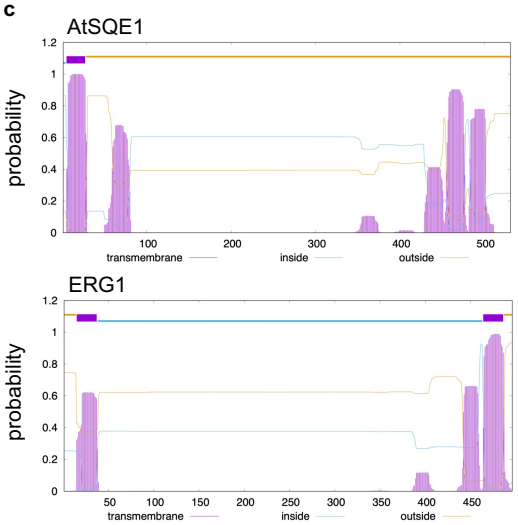
a

Prediction Model	Predicted number of Transmembrane domains		
	ScDoa10	AtDOA10A	AtDOA10B
TMHMM 2.0	14	14	13
TMpred	14	16	14
Phobius	16	16	14

b



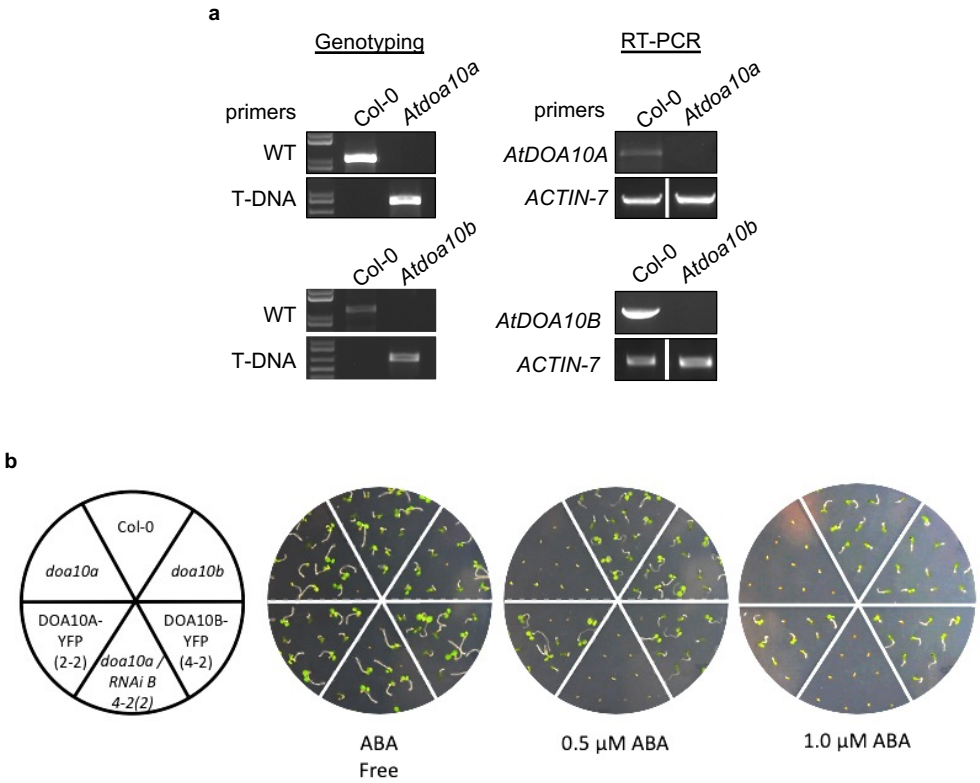
c



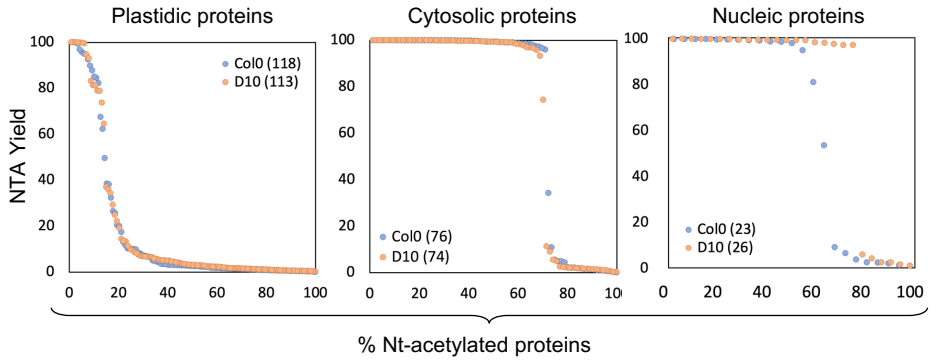
d

Protein	Prediction
*Sc*DOA10	ER
*At*DOA10A	ER
*At*DOA10B	ER
ERG1	ER
*At*SQE1	ER

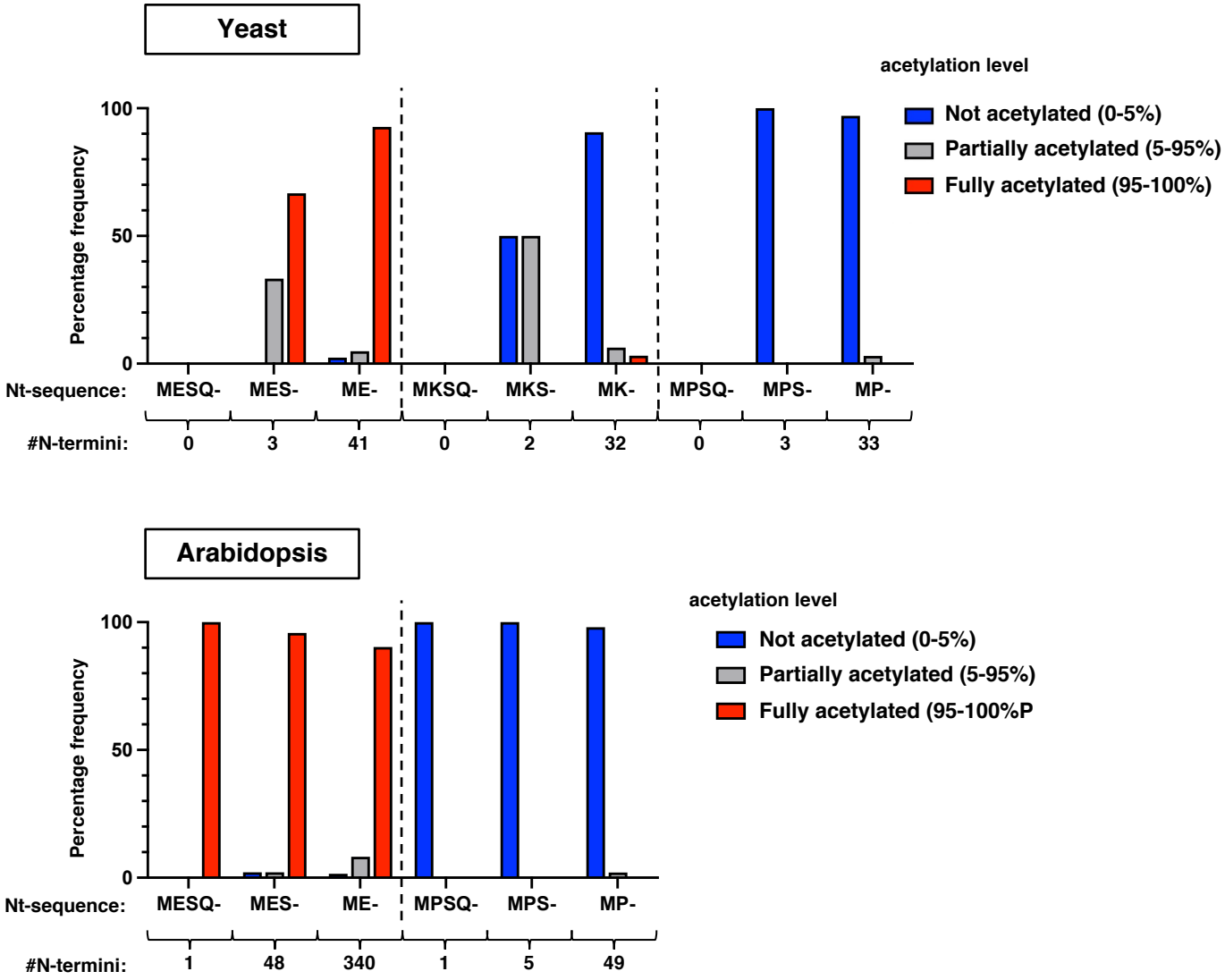
Supplemental Figure S2. Transmembrane and subcellular localization predictions for yeast and Arabidopsis DOA10 and SQE proteins. (**A**) Estimated number of TM domains in *At*DOA10A and B relative to *Sc*DOA10 using the three different prediction models shown. (**B**) TMHMM 2.0 predicted transmembrane topologies for *Sc* and *At*DOA10s. (**C**) TMHMM 2.0 predicted transmembrane topologies for AtSQE1 and ERG1. (**D**) Subcellular localization predictions for DOA10 and SQE proteins using Euk-mPLoC 2.0 (Chou and Shen 2010), which predicts eukaryotic protein localizations independent of the organism of origin.



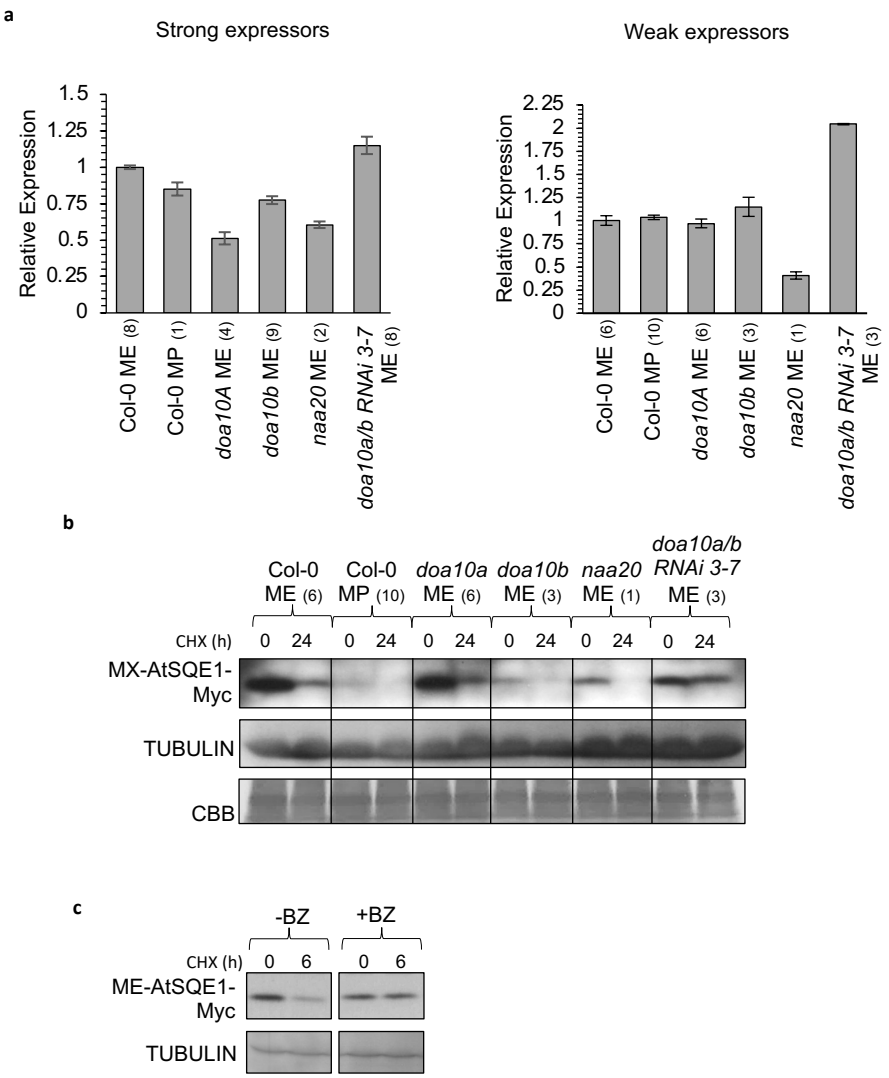
Supplemental Figure S3. *At*DOA10A and B mutant and complementation line characterization. (**A**) Genotyping PCR and RT-PCR confirming homozygous T-DNA insertion and absence of full-length mRNA expression in *Atdoa10a* and *Atdoa10b* T-DNA insertion lines. (**B**) seedling ABA-sensitivity assays showing ABA-hypersensitivity in *Atdoa10a* is reverted by complementation with *pDOA10A::AtDOA10A-YFP* (line 2-2).



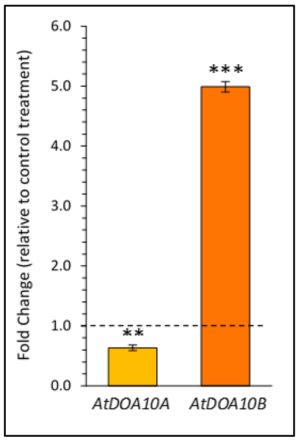
Supplemental Figure S4. Global NTA variation comparisons for plastidic, cytosolic and nuclear proteins in *Adoa10a/b RNAi 4-2* and Col-0. For each sample, the peptides were sorted in decreasing order of %NTA (quantitated only). Each N-ter entry was assigned a number corresponding to its relative position. These proteins numbers are plotted with matching %NTA for all acetylation positions. For nuclear N-termini the the low number of retrieved peptides introduces bias; looking at each individual N-terminus revealed no variation.



Supplemental Figure S5. Prevalence of Nt-acetylation in yeast and Arabidopsis Nt-peptides with the listed amino acid sequences**.** Data were compiled from the N-terminomics database NterDB (https://nterdb.i2bc.paris-saclay.fr/). The number of peptides for which Nt-acetylation has been quantified is given below the y-axis. Percentage frequency corresponds to the proportion of these peptides within each of the listed Nt-acetylation level categories: Not acetylated (0-5%), partially acetylated (5-95%) and fully acetylated (95-100%). For example, the single Arabidopsis MESQ Nt-peptide (matching ME-AtSQE1) for which data is available (AT5G38650) is 97.73% Nt-acetylated and therefore Arabidopsis MESQ peptides are shown as 100% fully acetylated. The single Arabidopsis MPSQ Nt-peptide (matching MP-AtSQE1) for which data is available (AT5G06830) is 0.69% Nt-acetylated and is therefore shown as 100% not acetylated. See also Supplemental Table 3.



Supplemental Figure S6. Expression and CHX-chase analysis of AtSQE1-Myc transgenic lines. (**A**) RT-qPCR of *35S::ME/MP-AtSQE-Myc* mRNA levels in the lines shown. Two independent lines for each transgene/genotype combination were analyzed and divided into "strong expressors" and "weak expressors". "Strong expressors" were analyzed further in Figure 7. Relative expression levels were calculated through normalisation to *AtACT7* and are the average of three biological repeats. (**B**) CHX chase of WT ME- and mutant MP-AtSQE1-Myc "weak expressors" in WT Col-0 and different mutant backgrounds. Tubulin and coomassie brilliant blue (CBB) loading controls are shown. (**C**) CHX chase of WT ME-AtSQE1-Myc in Col-0 +/- Bortezomib (BZ).



Supplemental Figure S7. Fold changes of *AtDOA10A* and *AtDOA10B* expression in 6-day old seedlings following 4-hour treatment with tunicamycin, as determined by qRT-PCR, relative to DMSO-treated seedlings. Fold changes were calculated according to the delta delta Ct method and data presented are the averages of 3 biological repeats (±SE). Statistical significance was determined using a student's t- test (** $p<0.01$; *** $p<0.001$).

Supplemental Table S1. List of primers used in this study.

Genotyping (Arabidopsis)		
doa10a_LP	AT4G34100	AATTTCTCCTCTGGCAAGCTC
doa10a_RP	AT4G34100	AAGAGTCACCCATGCAACAAG
doa10a_BP	AT4G34100	GGGCTACACTGAATTGGTAGCTC
doa10b_LP	AT4G32670	GGGGTGTCTCCTTAAAAGCAC
doa10b_RP	AT4G32670	TTTCCTTTTCTTTTGCGTGAG
doa10b_BP	AT4G32670	TGGTTCACGTAGTGGGCCATCG
DOA10_RT-PCR_Exon3_F	AT4G34100	CTCTGGCAAGCTCACTTGTTC
DOA10A_RT-PCR_Exon8_R	AT4G34100	GGCACGCATAAAGCCGTTAG
DOA10B_RT-PCR_Cloning_F	AT4G32670	CACCATGGAGATTTCTCCGGCG
DOA10B_RT-PCR_Cloning_RO	AT4G32670	CTCGAGATCTTCAGTGAAATCG
ACT7_F	AT5G09810	ATGGCCGATGGTGAGGATAT
ACT7_R	AT5G09810	GAGCACAATACCGGTTGTACG
DOA10A/B Cloning		
DOA10A_Cloning_F(pDNR)	AT4G34100	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCA TGGAGATTTCCCCGGCCGATTC
gDOA10A_Cloning_F(pDNR)	AT4G34100	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCG CATAATATACAAAAGGTCGTCC
DOA10A_Cloning_RO(pDNR)	AT4G34100	GGGGACCACTTTGTACAAGAAAGCTGGGTCAG CTTCTTGTTGGATTGCAC
DOA10A_Cloning_RC(pDNR)	AT4G34100	GGGGACCACTTTGTACAAGAAAGCTGGGTCCT AAGCTTCTTGTTGGATTGCAC
DOA10B_Cloning_F(TOPO)	AT4G32670	CACCATGGAGATTTCTCCGGCG
gDOA10B_Cloning_F(TOPO)	AT4G32670	CACCAAAGCTCAAGAAATCGGTACTC
DOA10B_Cloning_RO(TOPO)	AT4G32670	CTCGAGATCTTCAGTGAAATCG
SQE1_Cloning_F(TOPO)	AT1G58440	CACCATGGAGTCACAATTATGGAATTGG
SQE1_Cloning_RO(TOPO)	AT1G58440	TGAACATTTGGTTTCTCCAACTG
Confirmation of transgene expression (yeast)		
DOA10A_Cloning_F(TOPO)	AT4G34100	CACCATGGAGATTTCCCCGGC
DOA10A_Exon1_R	AT4G34100	GCACAAAACTCAACCGCAGA
DOA10B_Cloning_F(TOPO)	AT4G32670	CACCATGGAGATTTCTCCGGCG
DOA10B _Exon1_R	AT4G32670	TGCAAATCTCGCAATGGTTG
DOA10A_Exon5_F	AT4G34100	TCCGTGCTGGGAATGTCA
DOA10B_Exon6_F	AT4G32670	TATGTTTCTTGTTAGTACAACCTTCATG
eG/YFP_R		GCTGAACTTGTGGCCGTTTA
ScUBC6_F	YER100W	GATACTTGGAATCCTGGCTGGTCTGTCTC
ScUBC6_R	YER100W	AAAGGGTCTTCTGTTTCATCACCTGTATTTGC
Confirmation of transgene expression (Arabidopsis)		
SQE1_Cloning_F(TOPO)	AT1G58440	CACCATGGAGTCACAATTATGGAATTGG
HAtag_R	AT1G58440	ATAGGATCCTGCATAGTCCG
SQE1-Myc_qPCR_F	AT1G58440	GCGGAAGGAGTTAGGCAGAT
SQE1-Myc_qPCR_R	AT1G58440	ACCCGCTGTTATCAACCACTT
Tissue-specific / RNAi line expression analysis (qRT-PCR)		
DOA10A_qPCR_Exon8_F	AT4G34100	AATCTGGACTAGACTGGTAATGCT
DOA10A_qPCR_Exon8_R	AT4G34100	GGCACGCATAAAGCCGTTAG
DOA10B_qPCR_Exon1_F	AT4G32670	GGCGGAAGACAAACTCGTTG
DOA10B_qPCR_Exon1_R	AT4G32670	TGCAAATCTCGCAATGGTTG
DOA10B_qPCR_Exon7_F	AT4G32670	GGACTCAAACACGACGATCT
DOA10B_qPCR_Exon7_R	AT4G32670	AGCAAATCGGTTCTTCCCGTT
ACT7_qPCR_F	AT5G09810	CTGGAATGGTGAAGGCTGGT
ACT7_qPCR_R	AT5G09810	GTGCCTAGGACGACCAACAA

Generation of RNAi lines		
DOA10B_rnai3_cloF	AT4G32670	CACCGGAGACACCCCGAGTTCTTG
DOA10B_rnai3_cloR	AT4G32670	CAATCCAACCGTGTGGGAGA
DOA10B_rnai4_cloF	AT4G32670	CACCCCGGCGAAGCTGTTACTACA
DOA10B_rnai4_cloR	AT4G32670	TGGGAGACAGCGTAGGAGAA