

A) rPsbO

MGLTFDEIQGLTYLQVKGSGIANTCPVLESGTTNLKELKAGSYKLENFCIEPTSFTVKEESQFKGGETEFVKT
LMTRLTYTLDDAMSGSFVKVSDGSAELKEDDGIDYAATTVQLPGGERVAFLFTIKQFDGKGTLDGIKGDFLVPSY
RGSSFLDPKGRGGSTGYDNAVALPARADAEELLKENVKITKALKGSAVFSVAKVDPVTGEIAGVFESI QPSD
TD LGAKPPKDIKVTGLWYAQLKLEHHHHHHH

B) rPsbQ

MGLTPVDLFDDRSVRDRGFDLIYEARDLDPQNVREGFTQARASLDETKKRVKESEARIDADLDVFIQKSYWTE
AREQLRRQVGTLRFDLNTLASTKEKEAKKAALGLRKEFIQAVEDLDFALREKDQASAACKLEITKAKLDSVLAA
VLEHHHHHHH

C) Δ 11-rPsbO

MGTYLQVKGSGIANTCPVLESGTTNLKELKAGSYKLENFCIEPTSFTVKEESQFKGGETEFVKTCLMTRLTYTL
DAMSGSFVKVSDGSAELKEDDGIDYAATTVQLPGGERVAFLFTIKQFDGKGTLDGIKGDFLVPSYRGSSFLDPK
GRGGSTGYDNAVALPARADAEELLKENVKITKALKGSAVFSVAKVDPVTGEIAGVFESI QPSD
TD LGAKPPKDI KVTGLWYAQLKLEHHHHHHH

D) Δ 18-rPsbO

MGSGIANTCPVLESGTTNLKELKAGSYKLENFCIEPTSFTVKEESQFKGGETEFVKTCLMTRLTYTLDDAMSGSF
KVGSDGSAELKEDDGIDYAATTVQLPGGERVAFLFTIKQFDGKGTLDGIKGDFLVPSYRGSSFLDPKGRGGSTG
YDNAVALPARADAEELLKENVKITKALKGSAVFSVAKVDPVTGEIAGVFESI QPSD
TD LGAKPPKDIKVTGLWYAQLKLEHHHHHHH

E) pro-CrCEP1

MGSSHHHHHHSSGLVPRGSHMASLQDRLLRAQHTQMLLEAQANPLGAFKEWAQTHSRSYVNDVAEFENRFKVL
ENLEYVLAYNARTTSHWLTLNHLADLSTPEYKSKLLGFDNQARVARNKLTGFRYEDVDAEALPPAIDWRKKNA
VAEVKNQGQCGSCWAFATTGSVEGINAIVTGSLSLSEQLVDCDTEQDKGCSGGLMDYAYAWIIKNKGINTEE
DYPYTAMDGQCDVAKMKRRVVTIDSIEDVPENDEVALKKAHAHQPVAVAI EADAKSFQLYGGGVYDDPTCGTSL
NHGVLVVGYGKDVTSYSGSNYIWKNSWGAEWGDAGYIRLKMGSTDAEGLCGIAMAPSYPVKTGPNPPTPGPTPG
PSPKPGPKPGPKPGPTPPGPVKCDDNECPNGSTCCCNEIFNMCQWGCCPMPKATCCDDHEHCCPADLPVCD
TDAGRCLPSAGVFLGSKPWAAKTPAVRRPRSTSLGGMAGRLAQKFMGGGRGFLRRGEPMN

F) pro-CrCEP2

MGSSHHHHHHSSGLVPRGSHMASLLSSADMLALAQVEPERAFLWATQHARTYSEGSPEYTRRLGVFADNVRAI
AEQNRNRTGITLALNEYADETWEEFAAKRLGLKISQEQLKAREARSSSSSSSSWRYAQVQTPAAVDWRKNAVT
QVKNQGQCGSCWAFSAVGSIEGANALATGQLVALSEQQLVDCDTASNMGCSGGLMDDAFKYVLDNGGIDTEEDY
SYWSGYGFGFWCNKRKQTDRPAVSIDGYEDVPTSEPALLKAVAGQPVAVAI CASANMQFYSSGVINSCEGLNH
GVLAVGYDTSKDAQPYWIVKNSWGSWGEQGYFRLKMGEQPKGLCGIASAASYAVKTSAVNKPVPMTCDMFGWT
ECGVGNTCSCSFSLFGWLCLWHDCCLADAVSCPD LKHCCPAGTTCNAAQGACIAADGASSTPWVDKTKAMVAN
TPAAHARQAEVEAAQARQEQQQQQRAAHALGEADMLLARGGGKGERKRIERIAPO

Figure S1: Amino acid sequences of the *E. coli*-produced recombinant proteins used in this study. A-D) Sequences of recombinant PsbO (rPsbO), recombinant PsbQ (rPsbQ) and the two truncated versions Δ 11-rPsbO and Δ 18-rPsbO as expressed from the pET28(b)+ vector. The sequences contain an additional Gly residue after the initial Met residue due to the NcoI restriction site and the C-terminal Leu-Glu dipeptide followed by a His₆-tag due to the 5' XhoI cleavage site. **E-F)** Sequences of recombinant proCrCEP1 and proCrCEP2 as expressed from the pET28/32 vector. The sequences contain additional N-terminal residues including a His₆-tag and a linker region due to cloning via the NheI restriction site. All additional sequences are coloured green.

Supplemental Table 1: Number of normalized total spectra identified for the top 10 proteins represented in the sample with the probe. Top three match the molecular weights of proteins observed in labelling experiments and are identified as proteins OEE3 (PsbQ), OEE2 (PsbP) and OEE1 (PsbO).

| <i>C. reinhardtii</i> _pulldown, Samples Report | | | | Quantitative Value: Normalized Total Spectra | |
|---|---|--------------------|--------|---|--------|
| # | Identified Proteins | Accession Number | MW | control | probe |
| 1 | oxygen evolving enhancer protein 3 [Chlamydomonas reinhardtii] | XP_001701331.1 | 22 kDa | 5.74 | 143.45 |
| 2 | Oxygen-evolving enhancer protein 2, chloroplastic; Short=OEE2; Flags: Precursor | P11471.1 (+1) | 26 kDa | 16.40 | 90.93 |
| 3 | oxygen-evolving enhancer protein 1 of photosystem II [Chlamydomonas reinhardtii] | XP_001694699.1 | 31 kDa | 8.20 | 56.35 |
| 4 | enolase [Chlamydomonas reinhardtii] | XP_001702971.1 | 52 kDa | 36.90 | 43.54 |
| 5 | plastocyanin, chloroplast precursor [Chlamydomonas reinhardtii] | XP_001702952.1 | 15 kDa | 27.06 | 35.86 |
| 6 | hypothetical protein CHLRE_01g042750v5 [Chlamydomonas reinhardtii] | PNW88748.1 (+1) | 89 kDa | 40.18 | 29.45 |
| 7 | Cluster of protein disulfide isomerase 1 [Chlamydomonas reinhardtii] (XP_001701755.1) | XP_001701755.1 [2] | 58 kDa | 34.44 | 29.45 |
| 8 | full-length thiazole biosynthetic enzyme [Chlamydomonas reinhardtii] | XP_001698672.1 | 37 kDa | 5.74 | 28.17 |
| 9 | Cluster of heat shock protein 70A [Chlamydomonas reinhardtii] (XP_001701326.1) | XP_001701326.1 [7] | 71 kDa | 29.52 | 26.89 |
| 10 | Cluster of elongation factor Tu (chloroplast) [Chlamydomonas reinhardtii] (NP_958362.1) | NP_958362.1 [6] | 46 kDa | 13.12 | 24.33 |

PsbO

XP_001694699.1 (100%), 30,580.6 Da

oxygen-evolving enhancer protein 1 of photosystem II [Chlamydomonas reinhardtii]

14 exclusive unique peptides, 14 exclusive unique spectra, 44 total spectra, 162/291 amino acids (56% coverage)

```

MALRAAQSAK  AGVRAARPNR  ATAVVCKAQK  VGQAAAAAAL  ATAMVAGSAN
ALTFDEIQGL  TYLQVKGSGI  ANTCPVLESQ  TTNLKELEKAG  SYKLENFCIE
PTSFVTVKES  QFKGGETEFLV  KTKLMTRLTY  TLDAMSGSFK  VGS DGS AELK
EDDGIDYAAAT  TVQLPGGERV  AFLFTIKQFD  GKGTLDNIG  DFLVPSYRGS
SFLDPKGRGG  STGYDNAVAL  PARADAEELL  KENVKIITKAL  KGS AVF S VAK
VDPVTGEIAG  VFESI QPSDT  DLGAKPKPKDI  KVTGLWYAQL  K

```

PsbP

P11471.1 (100%), 25,899.8 Da

RecName: Full=Oxygen-evolving enhancer protein 2, chloroplastic; Short=OEE2; Flags: Precursor

15 exclusive unique peptides, 18 exclusive unique spectra, 71 total spectra, 155/245 amino acids (63% coverage)

```

MATALCNKAF  AAPVVARPAS  RRSVVVRAS  GSDVSRRAAL  AGFAGAAALV
SSSPANAAAYG  DSANVFGKVT  NKSGFVPHYAG  DGFALLLPAL  WNPSKENDFP
GVILRYEDNF  DAVNNLVVIA  QDTDKKAIAD  FGSQDKFLES  VSYLLGKQAY
SGETQSEGGF  APNRVSAASL  LDVSTTTDKK  GKTYKYELL  VRSADGDEGG
RHQLIGATVG  SDNKL I I K I  QIGDKRWFKG  AKKEAMGAFD  SFTVV

```

PsbQ

XP_001701331.1 (100%), 21,824.7 Da

oxygen evolving enhancer protein 3 [Chlamydomonas reinhardtii]

16 exclusive unique peptides, 16 exclusive unique spectra, 112 total spectra, 122/199 amino acids (61% coverage)

```

MALASKVATR  PAVASRRGAV  VVRASGESRR  AVLGGLLASA  VAAVAPKAAL
ALTPVDLFDD  RSVDRDRGFDL  IYEARDLDLP  QNVREGFTQA  RASLDETKKR
VKESEARIDA  DLDVFIQKSY  WTEAREQLRR  QVGTLRFDLN  TLASTKEKEA
KKAALGLRKE  FIQAVEDLDF  ALREKDAQSA  AKKLEITKAK  LDSVLAAYL

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Figure S2: Amino acid sequences of proteins PsbO, PsbP and PsbQ. Identified peptides are denoted in bold font and coloured orange. Data was analyzed with the Scaffold Proteome Software.

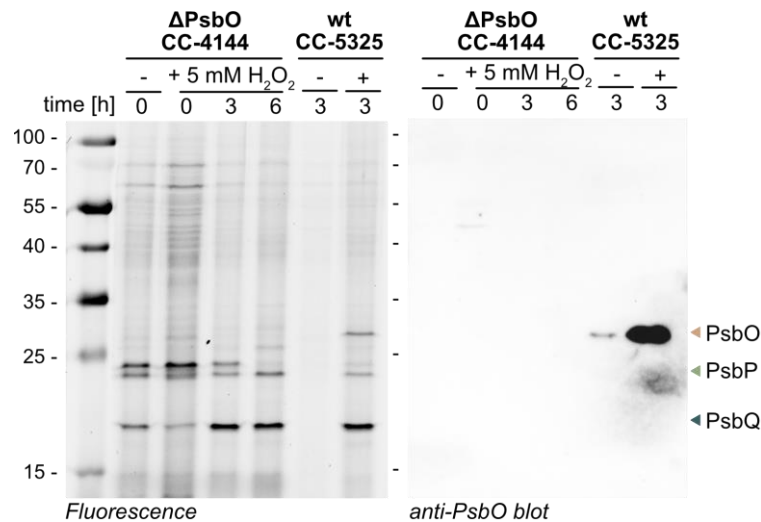


Figure S3: Labelling of soluble *Chlamydomonas* proteomes of the cell line lacking the protein PsbO (CC-4144 FUD44 mt+) with DK12. Soluble fractions of treated (+ 5 mM H₂O₂) and non-treated (-) wild-type (CC-5325) and ΔPsbO (CC-4144) *Chlamydomonas* cells, collected 0–6 h after administration of 5 mM H₂O₂ were labelled with 2 μM DK12 in 20 mM Hepes pH 8.0, 150 mM NaCl and 5 mM DTT. Following denaturation, alkyne-labelled proteins were coupled to picolyl azide via click chemistry. The samples were separated on SDS-PAGE under reducing conditions, the gel was scanned for Cy5 fluorescence (left) and analyzed by immunoblotting (right) using the anti-PsbO antibody. Labelling of the band corresponding to protein PsbO is absent in *Chlamydomonas* cell line samples lacking PsbO.

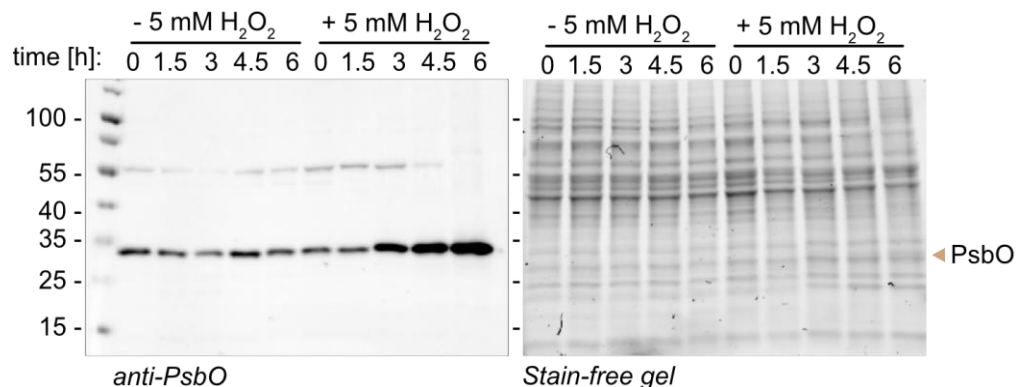


Figure S4: Presence of PsbO in treated and untreated soluble *Chlamydomonas* proteomes. Soluble proteomes of cells collected at different time points (0, 1.5, 3, 4.5, 6 h) after treatment with 5 mM H₂O₂ were separated on a stain-free SDS-PAGE, scanned to detect total proteins (right panel), and blotted to a PMSF membrane. PsbO was detected with primary Rabbit anti-PsbO antibodies, coupled to secondary anti-Rabbit-HRP antibodies (left panel). The position of PsbO is indicated with a sand-coloured arrow.

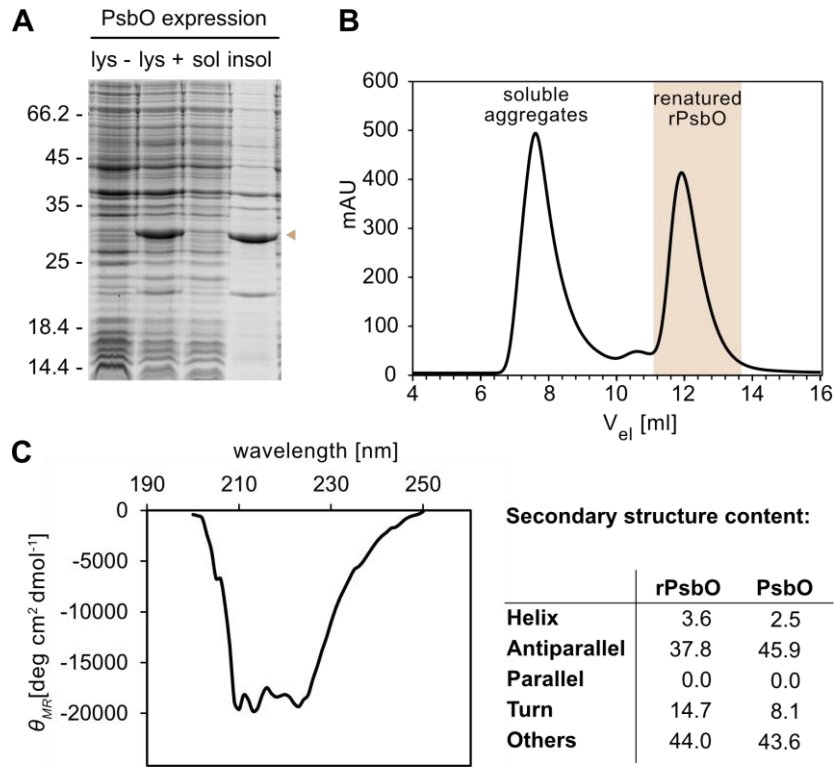


Figure S5: Purification of recombinant PsbO (rPsbO) from inclusion bodies. **A**) Lysate fractions before (lys-) or after (lys+) autoinduction and soluble (sol) or insoluble (insol) fractions of lysates after induction were separated on 12% SDS-PAGE gels. PsbO was present in the insoluble fraction (sand coloured arrow). **B**) Size exclusion chromatography of renatured rPsbO. Only proteins that eluted at an expected volume of a monomeric protein were collected ($V_{el} = 12$ ml). **C**) CD-spectroscopy of purified rPsbO. The circular dichroism spectrum of rPsbO at a concentration of 1 mg/mL in phosphate buffer was recorded at 25 °C using J-1500 Circular Dichroism Spectrometer. The data obtained were converted to mean residue ellipticity (θ_{MR}). The theoretical CD spectrum for *Chlamydomonas* PsbO was calculated using PDB2CD (Mavridis and Janes, 2017) based on the crystal structure of PsbO (PDB: 6KAC, entity 14), and the secondary structure contents for PsbO and rPsbO were estimated using BESTSEL (Micsonai et al., 2021). The secondary structure contents for rPsbO correspond to the ones calculated for PsbO, indicating that rPsbO is correctly folded. The minor deviations can be attributed to the His₆ tag at the C-terminus of rPsbO.

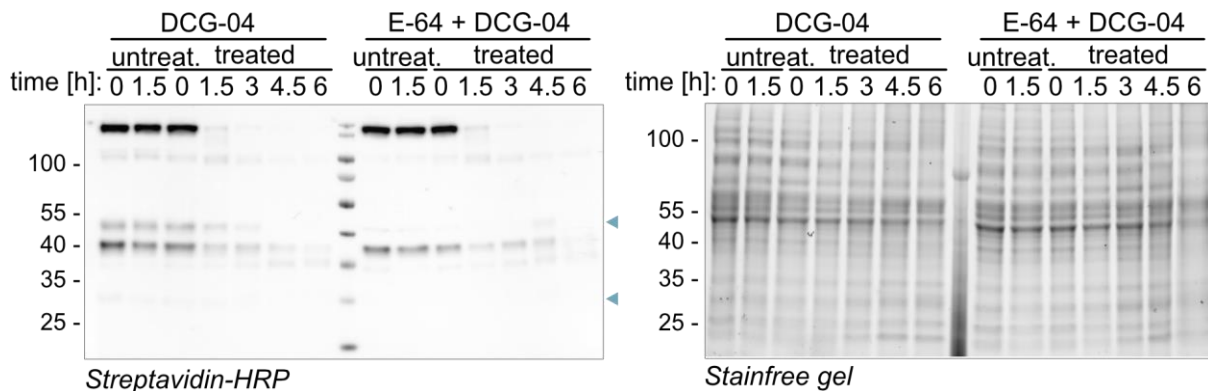


Figure S6: Activity of PLCPs in treated and untreated soluble *Chlamydomonas* proteomes. For all analysis, soluble proteomes of cells collected at different time points after treatment with 5 mM H₂O₂ (0, 1.5, 3, 4.5, 6 h) were used. **A**) DCG-04 labelling of *Chlamydomonas* extracts. Soluble proteomes were labelled with 2 μ M DCG-04 in 100 mM NaOAc pH 5.0, 150 mM NaCl, 5 mM DTT. Control samples were preincubated with 10 μ M E-64. The samples were separated on a stain-free SDS-PAGE, scanned (bottom panel) and blotted to a PMSF membrane and biotinylated proteins detected with streptavidin-HRP (upper panel). The positions of the two DCG-04 dependent bands are indicated with blue arrows.

| | | |
|----------------------|---|-----|
| C.reinhardtii_CrCEP2 | MQA---KFLALAL---AGLVGLS--CAHALSSADMLALAQVEPERAFGLWATQHART | 50 |
| C.reinhardtii_CrCEP1 | MALRLLGAAVVL-AAFASAGALQDRLLRAQH----TQMLEAQANPLGAFKEWAQTHSRS | 55 |
| A.thaliana_RD21A | MGFLKPTMAILFLAMVAVSSAVDMSIIISYDEKHGVSTTGGRSEAEVMSIYEAWLVKHGKA | 60 |
| A.thaliana_RD21B | MGFLKLSPMILLAMIGVSYAMDMSIIISYDENHHITTTSRSDSEVERIYEAWMVEHGKK | 60 |
| | * .: * : : . : : : * *: | |
| C.reinhardtii_CrCEP2 | YSE---GSPEYTRRLGVFADNVRAIAEQNRRTGITLALNEYADETWEEFAAKRLGLKIS | 107 |
| C.reinhardtii_CrCEP1 | YVN---DVAEFENRFKVVLENLEYVLAYNARTTSHWLTNLHLADLSTPEYKSKLLGFDNQ | 112 |
| A.thaliana_RD21A | QSQN--SLVEKDRRFEIFKDNLRFVDEHNEKNLSYRLGLTRFADLTNDEYRSKYLGAKE | 118 |
| A.thaliana_RD21B | KMNQNLGAEKDQRFEIFKDNLRFIDEHNTKNLSYKLGTRFADLTNEEYRSMYLGAKEPT | 120 |
| | : * .*: : :*: . * . . * * . ** : * : * * . | |
| C.reinhardtii_CrCEP2 | QEQLKAREARSSSSSSSSWRYAQ---VQTPAAVDWRKNAVTQVKNQGCGSCWAFSAVG | 164 |
| C.reinhardtii_CrCEP1 | ARVARN-----KLKTGFYEDVDAAELPPAIDWRKKNAVAEVKNQGCGSCWAFATTG | 165 |
| A.thaliana_RD21A | K---KG-----ERRTSLRYEARVGDELPSIDWRKKGAVAEVKDQGGCGSCWAFSTIG | 168 |
| A.thaliana_RD21B | K---R-----VLKTSDRYQARVGDA LPDSVDWRKEGAVADV KDGSCGSCWAFSTIG | 169 |
| | : : . ** * :*** :.***:***:*** *****: : * | |
| C.reinhardtii_CrCEP2 | SIEGANALATGQLVALSEQQLVDCDTASNMGCSGGLMDDAFKYVLDNGGIDTEEDYSYWS | 224 |
| C.reinhardtii_CrCEP1 | SVEGINAIVTGSLSVLSQELVDCDTEQDKGCSGGLMDYAYAWI IKKNGINTEEDYPYTA | 225 |
| A.thaliana_RD21A | AVEGINQIVTGDLITLSEQELVDCDTSYNEGCNGLMDYAFEFIIKNGGIDTDKDYPYKG | 228 |
| A.thaliana_RD21B | AVEGINKIVTGDLISLSEQELVDCDTSYNGCNGGLMDYAFEFIIKNGGIDTEADYPYKA | 229 |
| | ::** * :.***:***:***:*** : *.***** * : :.*** ** : * * . | |
| C.reinhardtii_CrCEP2 | GYGFGFWCNKRKQTRPAVSDIDGYEDVPTS-EPALLKAVAGQPVAVAIKASA-NMQFYSS | 282 |
| C.reinhardtii_CrCEP1 | MDGQ---CDV-AKMKRRVVTIDSIEDVPEDEVALKAAAHQPVAVAIADAKSFQLYGG | 281 |
| A.thaliana_RD21A | VDGT---CDQ-IRKNAKVVTIDSIEDVPTYSEESLKKAVAHQPI SIAIEAGGRAFLYDS | 284 |
| A.thaliana_RD21B | ADGR---CDQ-NRKNKVVTIDSIEDVPESEASLKKALAHQPI SVAIEAGGRAFLYSS | 285 |
| | * : * : . : .*:***:*** * : * * * * : :*** * . : : * : . | |
| C.reinhardtii_CrCEP2 | GVIN--SCEGLNHGVLAVGYDTS--DKAQPYWIVKNSWGGSWGEQGYFRLKMGEGPKG | 337 |
| C.reinhardtii_CrCEP1 | GVDPTCGTSLNHGVLVVGKDVTSYEDVPEDEVALKAAAHQPVAVAIADAKSFQLYGG | 341 |
| A.thaliana_RD21A | GIFDG-SCGTQLDHGVVAVGYGTE--NGKDYWIVRNSWGSWGESGYLRMARNIASSSG | 340 |
| A.thaliana_RD21B | GVFDG-LCGTELDHGVVAVGYGTE--NGKDYWIVRNSWGNRWGESGYIKMARNIEAPT | 341 |
| | * : : * * :***:***. . . ***** ** : * : : . * | |
| C.reinhardtii_CrCEP2 | LCGIASAASYAVKTSAVNK-----PVPTMCDMFGWTECGVG | 373 |
| C.reinhardtii_CrCEP1 | LCGIAMAPSYPVKTGPNPPTPGTPGPSKPGKPGKPGTPPGPVKCD--DNECPNG | 399 |
| A.thaliana_RD21A | KCGIAIEPSYPIKNGENPPNPGSPSPSP-----KPPTQCDS--YYTCPE | 384 |
| A.thaliana_RD21B | KCGIAMEASYPIKKGQNPNNPGSPSPSP-----KPPTTCDK--YFSCPES | 385 |
| | *** * * :.*** * . * * | |
| C.reinhardtii_CrCEP2 | NTCSCSFSLFGWLCLWHDCPLADAVSCPDCLKHCCPAGT-TCNAAQGACIAADGAS--ST | 430 |
| C.reinhardtii_CrCEP1 | STCCCVNEIFN-MCFQWGCCPMKATCCDDEHCCPADLPVCDTDAGRCLPSAGVFLGSK | 458 |
| A.thaliana_RD21A | NTCCCLFEYKG-YCFAWGCCPLEAATCCDDNYSCCPHEYPVCDLDQGTCLLSKNSPFSVK | 443 |
| A.thaliana_RD21B | NTCCCLYKYKG-YCFAWGCCPLEAATCCDDNYSCCPHEYPVCDVNRGTCILSKNSPFSVK | 444 |
| | .*** * . * :.***:*** * * * * .*: * * : . * | |
| C.reinhardtii_CrCEP2 | PWVDKTKAMVANTPAAHARQAEVEAAQARQEQQQQRAAHALGEADMLLARGGGKGERK | 490 |
| C.reinhardtii_CrCEP1 | PWAAKTPAVRRPRST-----SLGGMAGRLAQ-----KFMG-----GGRG | 492 |
| A.thaliana_RD21A | ALKRK-PA-----T-----PFW-----QGRK | 459 |
| A.thaliana_RD21B | ALKRT-PA-----I-----PFW-----KSRK | 460 |
| | . * . * | |
| C.reinhardtii_CrCEP2 | RIERIAQ*- | 498 |
| C.reinhardtii_CrCEP1 | FLRRGEPMN* | 501 |
| A.thaliana_RD21A | NIA*----- | 462 |
| A.thaliana_RD21B | HIA*----- | 463 |
| | : | |

Figure S7: Multiple sequence alignment showing similarity between proteases CrCEP1, CrCEP2 and granulin domain-containing proteases RD21A and RD21B from *A. thaliana*. The five structural elements are depicted as follows: the signal peptide in light gray, the autoinhibitory prodomain in red, the catalytic domain in blue, the proline-rich region in grey and the granulin domain in green. The remainder of the C-terminal sequence is depicted in light gray. The three catalytic aminoacid residues (Cys, His and Asn) are depicted in bold letters and denoted with black arrows. Sequence alignment was performed by Clustal Omega Multiple Sequence Alignment tool.

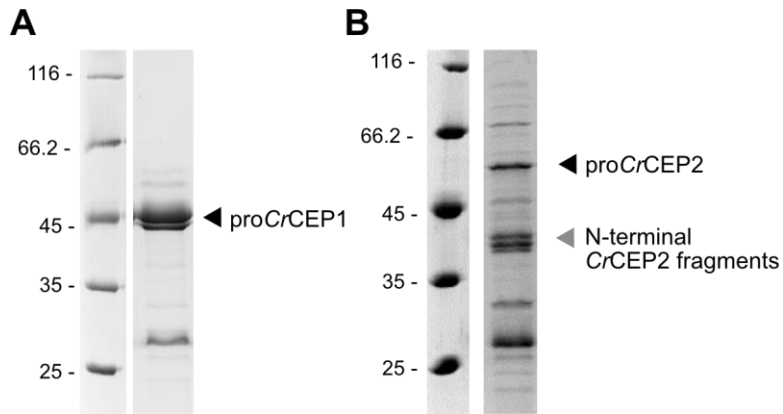


Figure S8: SDS-PAGE analysis of purified *CrCEP1* (A) and *CrCEP2* (B). The pro forms of both proteins are indicated by black arrows. The expected size of pro-*CrCEP1* is 52 kDa, but it migrated at a lower molecular weight of 45 kDa. The expected size of pro-*CrCEP2* was 51 kDa and it migrated at the expected molecular weight. Notably, *CrCEP2* displayed a lower degree of purity compared to *CrCEP1*.

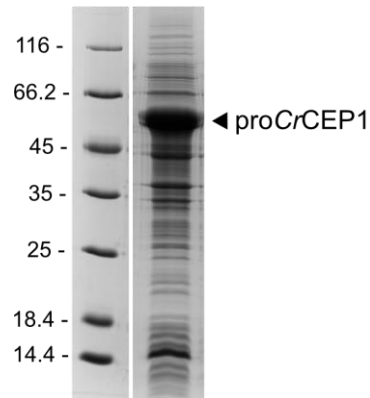


Figure S9: SDS-PAGE analysis of pro*CrCEP1* in the insoluble fraction. Black arrows indicate the pro form of *CrCEP1*. After expression, pro*CrCEP1* migrated at a considerably higher MW than after purification.

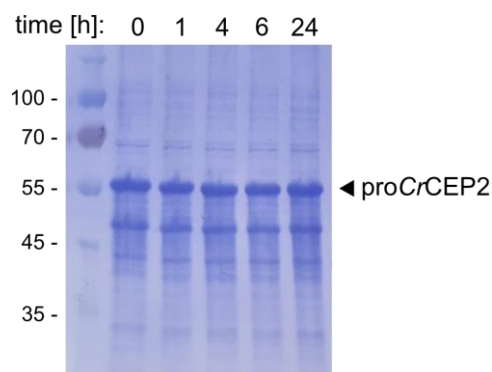


Figure S10: Analysis of the unsuccessful activation of *CrCEP2*. The proteins were separated on an SDS-PAGE gel, transferred to a PVDF membrane, and stained with Coomassie. Black arrow indicates the pro form of *CrCEP2*.

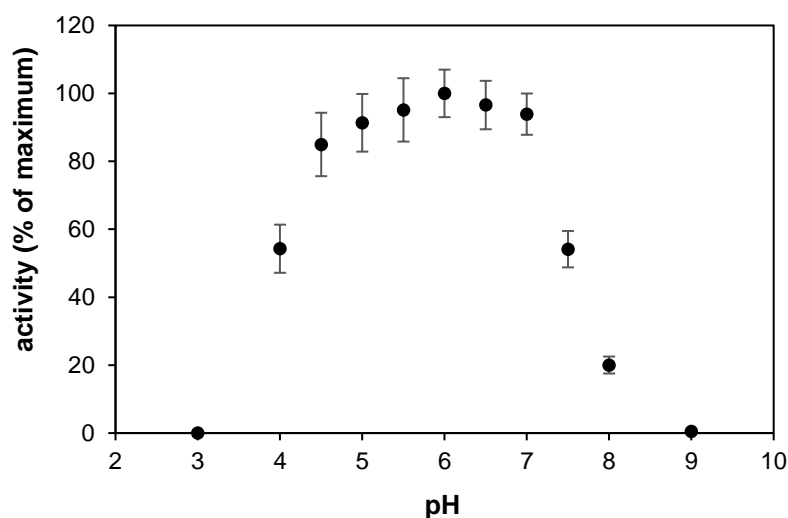


Figure S11: pH profile of recombinant *CrCEP1* (rCrCEP1). Assays were performed at 25 °C in 20 mM NaOAc, 20 mM MES, 20 mM Hepes (pH 3.0 – 9.0), 150 mM NaCl, 5 mM DTT, 10 μ M Z-Phe-Arg-AMC and 10 nM mature rCrCEP1.

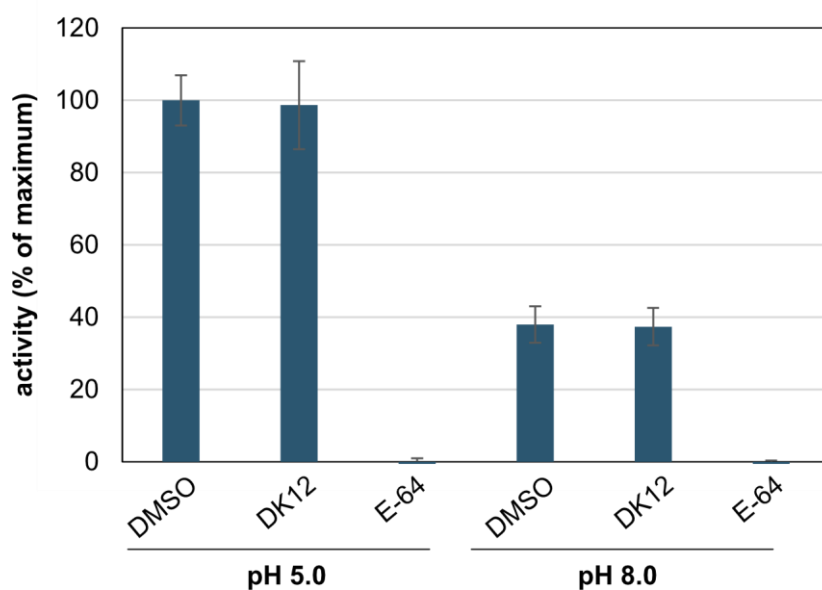


Figure S12: Kinetic measurements of rCrCEP1 in presence of the DK12 probe. Mature rCrCEP1 was preincubated for 20 min with either DMSO (positive control), 5 μ M DK12 or 5 μ M E-64 (negative control). Assays were performed at room temperature in 100 mM NaOAc pH 5.0 (left) or 20 mM Hepes, pH 8.0 (right), supplemented with 150 mM NaCl, 5 mM DTT, 5 μ M Z-Phe-Arg-MCA as substrate and 10 nM mature rCrCEP1.

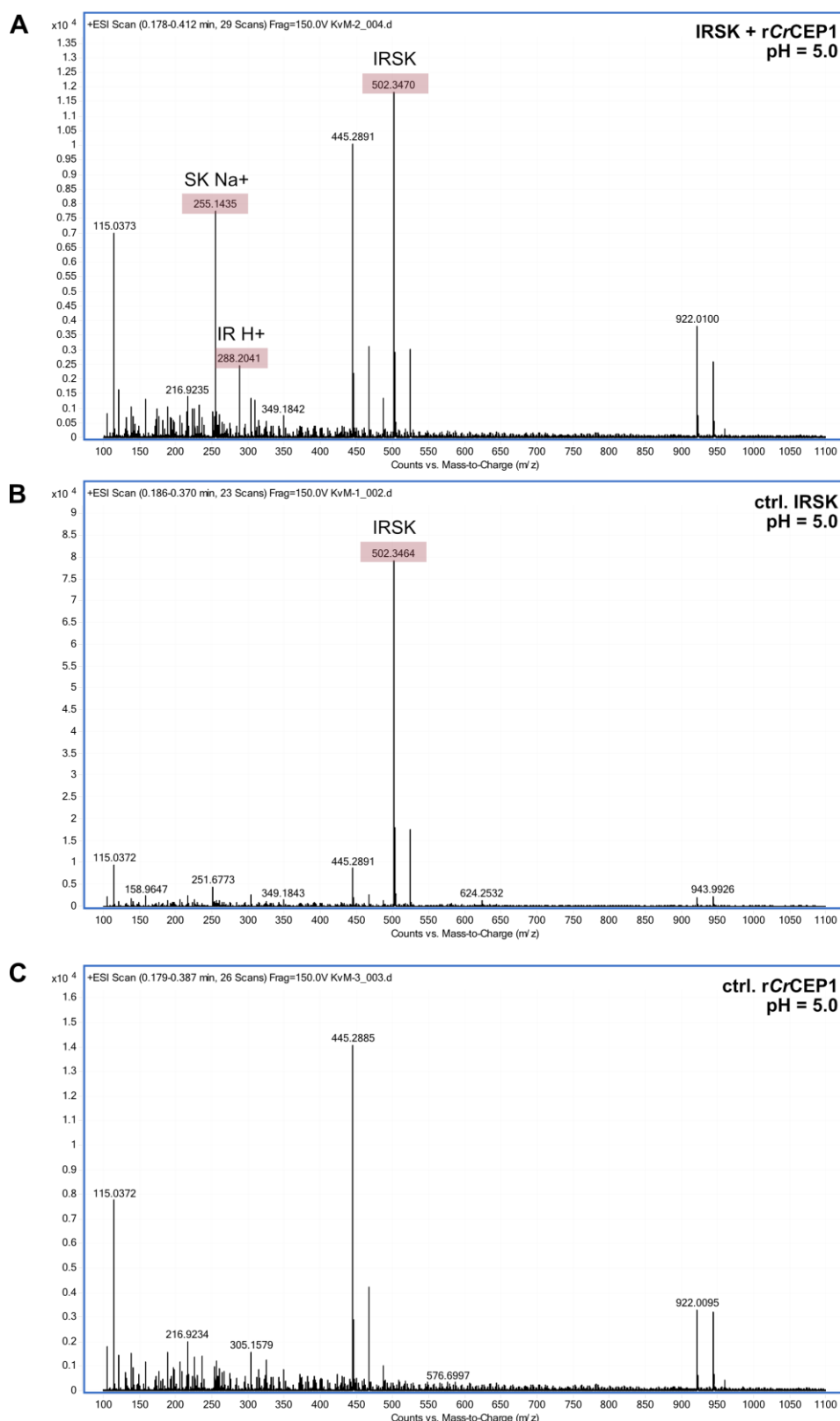
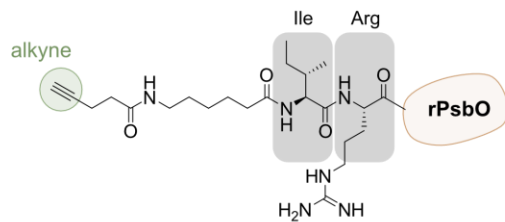
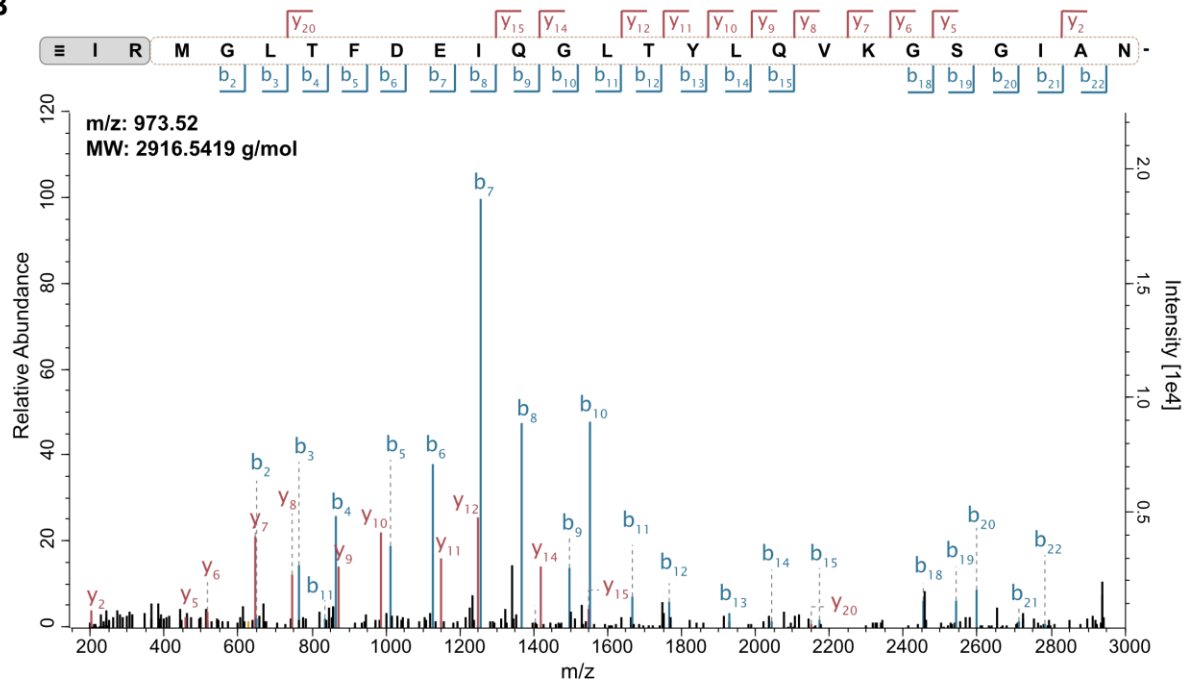


Figure S13: Mass spectra of the cleaved peptide Ile-Arg-Ser-Lys (IRSK). Mass spectra were recorded on an Agilent 6224 Accurate Mass TOF LC/MS chromatograph/spectrometer (Agilent Technologies, Santa Clara, CA, USA). **A)** The peptide IRSK was incubated with rCrCEP1 at pH 5.0 for 1 h at 25 °C, rCrCEP1 was removed with a 3 kDa cutoff spin column and the flow through analysed by MS. Red colour corresponds to spectra that match the MW of the IRSK peptide ($C_{21}H_{44}N_9O_5$, required (MH^+); $m/z = 502.34599$) or the two cleavage products IR ($C_{12}H_{26}N_5O_3$, required (MH^+); $m/z = 288.20302$) and SK ($C_9H_{20}N_4O_3$, required (MNa^+); $m/z = 255.14276$) **B-C)** The control samples containing only the IRSK peptide (**B**) or CrCEP1 (**C**) were incubated at pH 5.0 for 1 h at 25 °C, loaded onto 3 kDa cutoff spin columns and the flow through analysed by MS.

A \equiv IR modification:



B



C

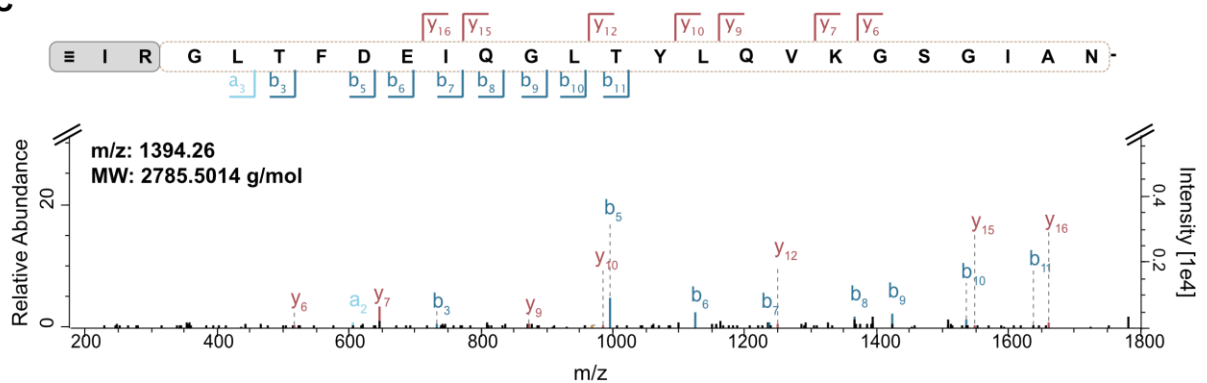


Figure S14: Identification of the \equiv IR modification on the N-terminus of rPsbO by tandem mass spectrometry. (A) Chemical formula of the \equiv IR modification as used in the MaxQuant search. (B) Peptide MS/MS fragmentation spectra of the \equiv IR labelled N-terminal peptide of rPsbO, including the initial methionine aminoacid residue. The sequence of the identified modified peptide is shown above the spectra. (C) Peptide MS/MS fragmentation spectra of the \equiv IR labelled N-terminal peptide of rPsbO, excluding the initial methionine aminoacid residue. The sequence of the identified modified peptide is shown above the spectra.

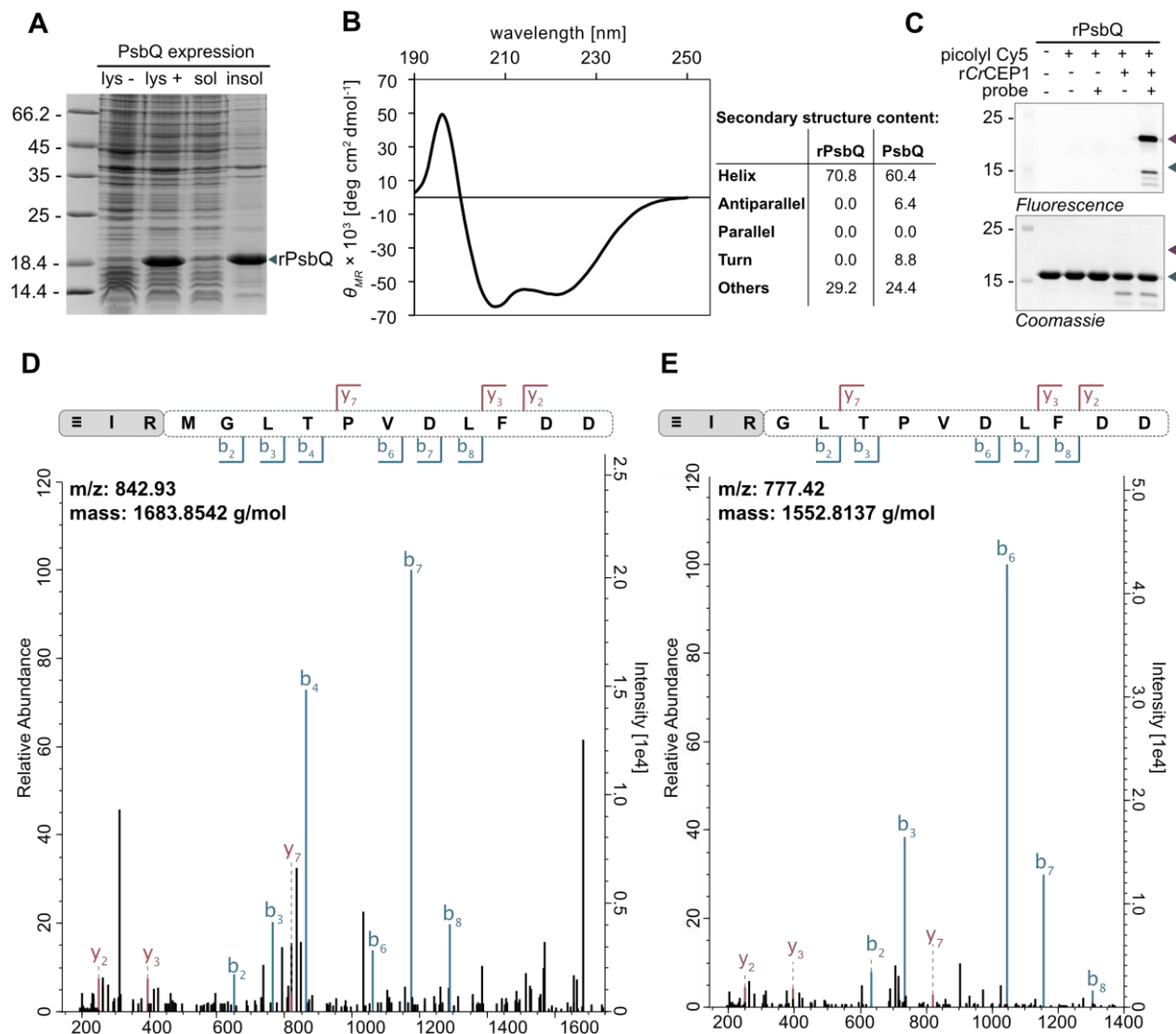


Figure S15: Recombinant PsbQ production, labelling and identification of the \equiv IR modification on the N-terminus of rPsbQ by tandem mass spectrometry. (A) Analysis of recombinant PsbQ expression in *E. coli*. Lysate fractions before (lys-) or after (lys+) autoinduction and soluble (sol) or insoluble (insol) fractions of lysates after induction were separated on 12 % SDS-PAGE gels. PsbQ was present in the insoluble fraction (blue coloured arrow). (B) CD-spectroscopy of purified rPsbQ. The circular dichroism spectrum of rPsbQ at a concentration of 1 mg/mL in phosphate buffer was recorded at 25 °C using J 1500 Circular Dichroism Spectrometer. The data obtained were converted to mean residue ellipticity (θ_{MR}). The theoretical CD spectrum for *Chlamydomonas* PsbQ was calculated using PDB2CD (Mavridis and Janes, 2017) based on the crystal structure of mature PsbQ (PDB: 6KAC, entity 16), and the secondary structure contents for PsbQ and rPsbQ were estimated using BESTSEL (Micsonai et al., 2021). The results indicate that rPsbQ is correctly folded. (C) *In vitro* labelling of rPsbQ with active rCrCEP1. Purified rPsbQ was labelled for one hour with 2 μ M probe DK12 in presence of active rCrCEP1 in 20 mM Hepes pH 8.0, 150 mM NaCl and 5 mM DTT. Alkyne-labelled proteins were coupled to picolyl Cy5, separated on an SDS-PAGE under reducing conditions, scanned for fluorescence (upper panel) and stained with Coomassie (bottom panel). rPsbQ is denoted with a blue-coloured arrow and \equiv IR modified rPsbQ with a violet arrow, respectively. The modified PsbQ migrates at a higher molecular weight. (D) Peptide MS/MS fragmentation spectra of the \equiv IR labelled N-terminal peptide of rPsbQ, including the initial methionine amino acid residue. The sequence of the identified modified peptide is shown above the spectra. (E) Peptide MS/MS fragmentation spectra of the \equiv IR labelled N-terminal peptide of rPsbQ, excluding the initial methionine amino acid residue. The sequence of the identified modified peptide is shown above the spectra.

References

- Mavridis, L., and Janes, R. W.** (2017). PDB2CD: a web-based application for the generation of circular dichroism spectra from protein atomic coordinates. *Bioinformatics* **33**:56–63.
- Micsonai, A., Bulyáki, É., and Kardos, J.** (2021). BeStSel: From Secondary Structure Analysis to Protein Fold Prediction by Circular Dichroism Spectroscopy. In *Structural Genomics: General Applications* (ed. Chen, Y. W.) and Yiu, C.-P. B.), pp. 175–189. New York, NY: Springer US.