

**A) rPsbO**

MGLTFDEIQGLTYLQVKGSGIANTCPVLESGTTNLKELKAGSYKLENFCIEPTSFTVKEESQFKGGETEFVKTKLMTRLTYTL DAMSGSFKVGS DGS AELKEDDGI DYAATT VQLP GGERVAFLFTIKQFDGKGTLDGKGDFLVPSYRGSSFLDPKGRGGSTGYDNAVALPARADAEELLKENVKITKALKGSAVFSVAKVDPVTGEIAGVFESI QPSD TDLGAKPPKDIKVTGLWYAQLKLEHHHHHHH

**B) rPsbQ**

MGLTPVDLFDDRSVRDRGFDLIYEARDLDPQNVREGFTQARASLDETKKRVKESEARIDADLDVFIQKSYWTEAREQLRRQVGT LRFDLNLTASTKEKEAKKAALGLRKEFIQAVEDLDFALREKDQASAAKKLEITKAKLDSVLAAVLEHHHHHHH

**C) Δ11-rPsbO**

MGTYLQVKGSGIANTCPVLESGTTNLKELKAGSYKLENFCIEPTSFTVKEESQFKGGETEFVKTKLMTRLTYTL DAMSGSFKVGS DGS AELKEDDGI DYAATT VQLP GGERVAFLFTIKQFDGKGTLDGKGDFLVPSYRGSSFLDPKGRGGSTGYDNAVALPARADAEELLKENVKITKALKGSAVFSVAKVDPVTGEIAGVFESI QPSD TDLGAKPPKDIKVTGLWYAQLKLEHHHHHHH

**D) Δ18-rPsbO**

MGSGIANTCPVLESGTTNLKELKAGSYKLENFCIEPTSFTVKEESQFKGGETEFVKTKLMTRLTYTL DAMSGSFKVGS DGS AELKEDDGI DYAATT VQLP GGERVAFLFTIKQFDGKGTLDGKGDFLVPSYRGSSFLDPKGRGGSTGYDNAVALPARADAEELLKENVKITKALKGSAVFSVAKVDPVTGEIAGVFESI QPSD TDLGAKPPKDIKVTGLWYAQLKLEHHHHHHH

**E) pro-CrCEP1**

MGSSHHHHHHSSGLVPRGSHMASLQDRLLRAQHTQMLLEAQANPLGAFKEWAQTHSRSYVNDVAEFENRFKVVWLENLEYVLAYNARTTSHWLTLNHLADLSTPEYKSKLLGFDNQARVARNKLTGFRIYEDVDAEALPPAIDWRKKNAVAEVKNQGQCGSCWAFAT TGSVEGINAIVTGSLSLSEQELVDCDTEQDKGCSGGLMDYAYAWIIKKNKGINTEEDYPYTAMDGQC DVAKMKRRVVTIDSIEDVPENDEVALKKAHAHQPVAVAI EADAKSFQLYGGGVYDDPTCGTSLNHGVLVVG YGKDV TGS GS NYWIVKNSWGA EWGDAGYIRLKMGSTDAEGLCGIAMAPSYPVKTGPNPPTPGPTPGPSPKPGPKPGKPGPTPPGPVKCDDNECPNGSTCCC VNEIFNMCFQWGCCPMPKATCCDDHEHCCPADLPVCDTDAGRCLPSAGVFLGSKPWAAKTPAVRRPRSTSLGGMAGRLAQKFMGGGRGFLRRGEPMN

**F) pro-CrCEP2**

MGSSHHHHHHSSGLVPRGSHMASLLSSADMLALAQVEPERAFGLWATQHARTYSEGSPEYTRRLGVFADNVRAIAEQNRNTGITLALNEYADETWEEFAAKRLGLKISQEQLKAREARSSSSSSSSWRYAQVQTPAAVDWRKNAVTVQKNQGQCGSCWAFSAVGSIEGANALATGQLVALSEQQLVDCDTASNMGCSGGLMDDAFKYVLDNNGIDTEEDYSYWSGYGFGFWCNKRKQTDRPAVSDIGYEDVPTSEPAL KAVAGQPVAVAI CASANMQFYSSGVINS CCEGLNHGVLAVGYDTS DKAQPYWIVKNSWGGSWGEQGYFRLKMGE GPKGLCGIASAASYAVKTS AVNKPVPTMCDMFGWTECGVNTCSCSFSLFGWLCLWHDCCLADAVSCPDLKHCCPAGTTCNAAQGACIAADGASSTPWVDKTKAMVANTPAAHARQAEVEAAQARQEQQQQQRAAHALGEADMLLARGGGKGGGERKRIERIAPO

**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 Δ11-rPsbO and Δ18-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 His<sub>6</sub>-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 His<sub>6</sub>-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)

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M ALRAAQSAK AGVRAARPNR ATAVVCKAQQ VGQAAAAAAL ATAMVAGSAN
ALTFDEIQGL TYLQVKGSGI ANTCPVLESG TTNLKELKAG SYKLENFCIE
PTSFYVKEES QFKGGGETEFV KTKLMTRLTY TLDAMSGSFK VGS DGS AELK
EDDGDIDY AAT TVQLPGGERV AFLFTIKQFD GKGTLDN IKG DFLVPSYRGS
SFLDPKGRGG STGYDNAVAL PARADAEELL KENVKI TKAL KGS AVF SVAK
VDPVTGEIAG VFESI QPSDT DLGAKPKPKDI KVTGLWYAQL K

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#### 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)

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M A T A L C N K A F A A A P V A R P A S R R S A V V V R A S G S D V S R R A A L A G F A G A A A L V
S S S P A N A A Y G D S A N V F G K V T N K S G F V P Y A G D G F A L L L P A K W N P S K E N D F P
G V I L R Y E D N F D A V N N L V V I A Q D T D K K A I A D F G S Q D K F L E S V S Y L L G K Q A Y
S G E T Q S E G G F A P N R V S A A S L L D V S T T T D K K G K T Y Y K Y E L L V R S A D G D E G G
R H Q L I G A T V G S D N K L Y I I K I Q I G D K R W F K G A K K E A M G A F D S F T V V

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#### PsbQ

XP\_001701331.1 (100%), 21,824.7 Da

oxygen evolving enhancer protein 3 [Chlamydomonas reinhardtii]

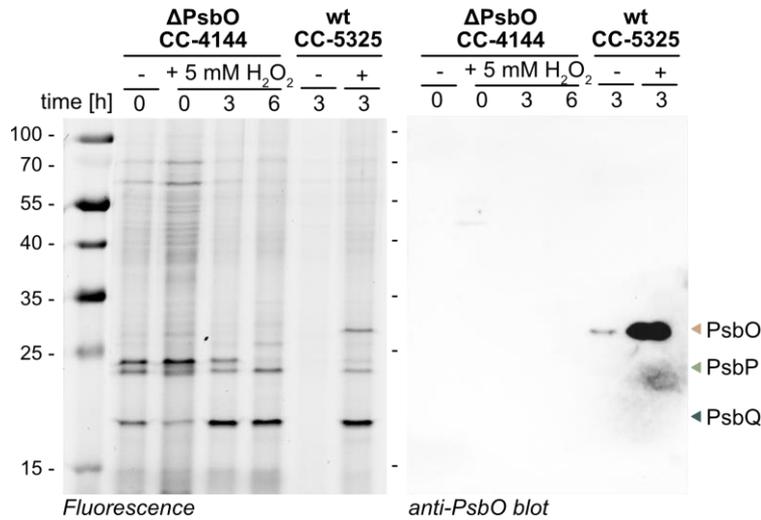
16 exclusive unique peptides, 16 exclusive unique spectra, 112 total spectra, 122/199 amino acids (61% coverage)

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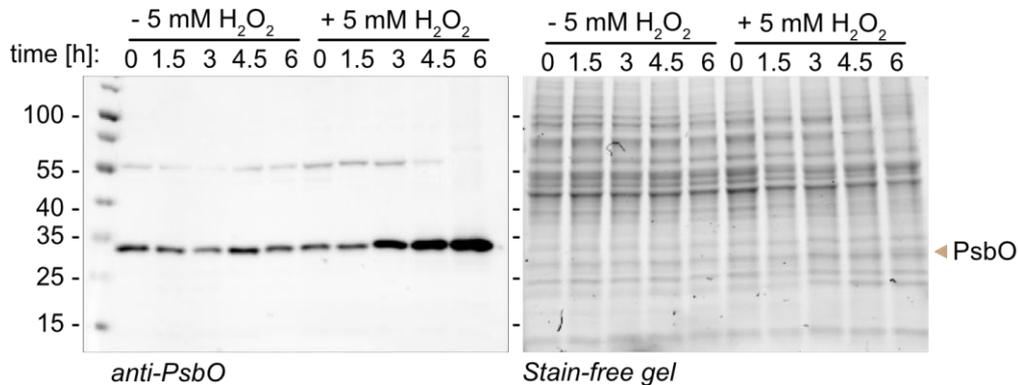
M A L A S K V A T R P A V A S R R G A V V V R A S G E S R R A V L G G L L A S A V A A V A P K A A L
A L T P V D L F D D R S V R D R G F D L I Y E A R D L D L P Q N V R E G F T Q A R A S L D E T K K R
V K E S E A R I D A D L D V F I Q K S Y W T E A R E Q L R R Q V G T L R F D L N T L A S T K E K E A
K K A A L G L R K E F I Q A V E D L D F A L R E K D Q A S A A K K L E I T K A K L D S V L A A V L

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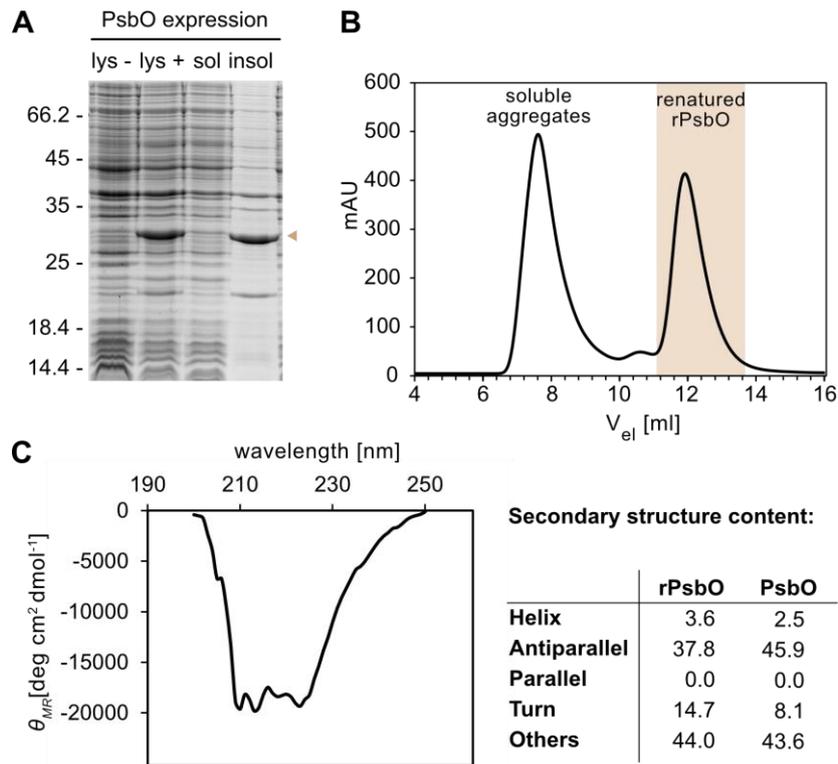
**Figure S2:** Amino acid sequences of proteins PsbO, PsbP and PsbQ. Identified peptides are denoted in bold font and coloured orange. Data was analyzed with the Scaffold Proteome Software.



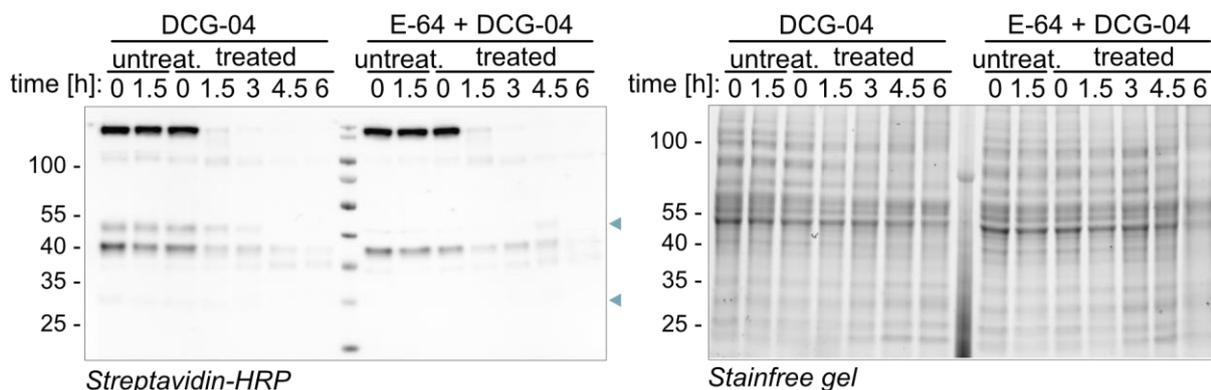
**Figure S3: Labelling of soluble *Chlamydomonas* proteomes of the cell line lacking the protein PsbO (CC-4144 FUD44 mt+) with DK12.** Soluble fractions of treated (+ 5 mM H<sub>2</sub>O<sub>2</sub>) and non-treated (-) wild-type (CC-5325) and ΔPsbO (CC-4144) *Chlamydomonas* cells, collected 0–6 h after administration of 5 mM H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> 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.



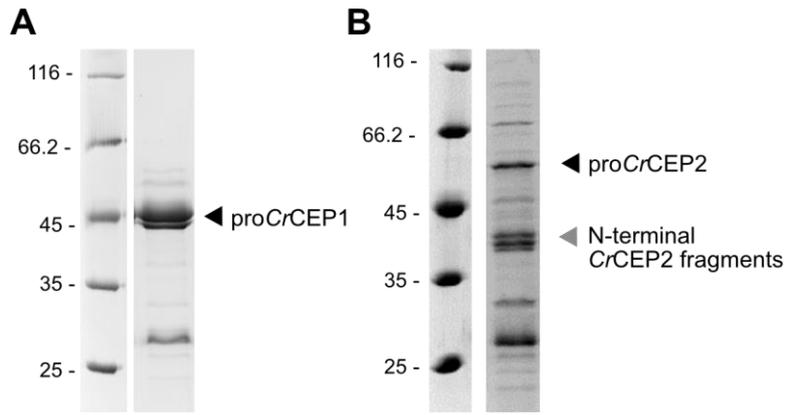
**Figure S5: Purification of recombinant PsbO (rPsbO) from inclusion bodies.** **A**) Lysate fractions before (lys-) or after (lys+) autoinduction and soluble (sol) or insoluble (insol) fractions of lysates after induction were separated on 12% SDS-PAGE gels. PsbO was present in the insoluble fraction (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 ( $\theta_{MR}$ ). 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 His<sub>6</sub> tag at the C-terminus of rPsbO.



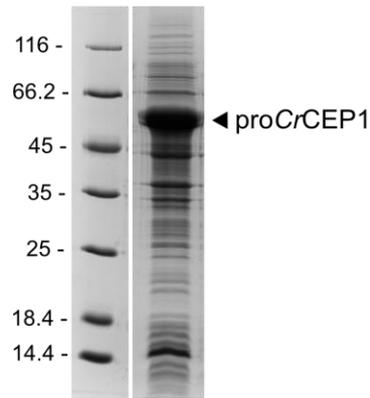
**Figure S6: Activity of PLCPs in treated and untreated soluble *Chlamydomonas* proteomes.** For all analysis, soluble proteomes of cells collected at different time points after treatment with 5 mM H<sub>2</sub>O<sub>2</sub> (0, 1.5, 3, 4.5, 6 h) were used. **(A)** DCG-04 labelling of *Chlamydomonas* extracts. Soluble proteomes were labelled with 2  $\mu$ M DCG-04 in 100 mM NaOAc pH 5.0, 150 mM NaCl, 5 mM DTT. Control samples were preincubated with 10  $\mu$ 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	MGFLKPTMAILFLAMVAVSSAVDMSIISYDEKHGVSTTGGRSEAEVMSIYEAWLVKHGKA	60
A.thaliana_RD21B	MGFLKLSPMILLLAMI GVSYAMDMSIISYDENHHITTESTRSDSEVERIYEAWMVHGGK	60
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C.reinhardtii_CrCEP2	YSE---GSPEYTRRLGVFADNVRAIAEQNRRNTGITLALNEYADETWEEFAAKRLGLKIS	107
C.reinhardtii_CrCEP1	YVN---DVAEFENRFKVVLENLEYVLAYNARTTSHWLTNLHLADLSTPEYKSKLLGFDNQ	112
A.thaliana_RD21A	QSQN--SLVEKDRRFEIFKDNLRFVDEHNEKNLSYRLGLTRFADLTNDEYRSKYLGAQME	118
A.thaliana_RD21B	KMNQNLGAEKDQRFEIFKDNLRFIDEHNTKNLSYKLGTRFADLTNEEYRSMYLGAKPT	120
	: * .*: : : : . * : . * * . * : * : * .	
C.reinhardtii_CrCEP2	QEQLKAREARSSSSSSSWRYAQ---VQTPAAVDWRKNAVTQVKNQCGSCWAFSAVG	164
C.reinhardtii_CrCEP1	ARVARN-----KLKTGFYEDVDAEALPPAIDWRKNAVAEVKNQCGSCWAFATG	165
A.thaliana_RD21A	K---KG-----ERRTSLRYEARVGDLEPESIDWRKKGAVAEVKDQGGCGSCWAFSTIG	168
A.thaliana_RD21B	K---R-----VLKTSDRYQARVGDALPDSVDWRKKGAVADVVDQGGCGSCWAFSTIG	169
	: : . ** * : : * : : * : : * : : * : : * : : *	
C.reinhardtii_CrCEP2	SIEGANALATGQLVALSEQQLVDCDTASNMGCSGGLMDDAFKYVLDNGGIDTEEDYSYWS	224
C.reinhardtii_CrCEP1	SVEGINAVTGSLSLSEQELVDCDTEQDKGCSGGLMDYAYAWI IKNKGINTEEDYPYTA	225
A.thaliana_RD21A	AVEGINQIVTGDILITLSEQELVDCDTSYNEGCNGLMDYAFEFIIKNGGIDTDKDYPYKG	228
A.thaliana_RD21B	AVEGINKIVTGDILISLSEQELVDCDTSYNGCNGGLMDYAFEFIIKNGGIDTEADYPYKA	229
	: : * * : . * : : * : : * : : * : : * : : * : : *	
C.reinhardtii_CrCEP2	GYGFGFWCNKRKQTRPAVSDIDGYEDVPTS-EPALLKAVAGQPVAVAIKASA-NMQFYSS	282
C.reinhardtii_CrCEP1	MDGQ---CDV-AKMKRRVVTIDSYEDVPEDEVALKAAAHQPVAVAIADAKSFQLYGG	281
A.thaliana_RD21A	VDGT---CDQ-IRKNAKVVTIDSYEDVPTYSEESLKKAVAHQFISIAIEAGGRAFLYDS	284
A.thaliana_RD21B	ADGR---CDQ-NRKNAKVVTIDSYEDVPESEASLKKALAHQFISVAIEAGGRAFLYSS	285
	* * : : . * : : * : : * : : * : : * : : * : : *	
C.reinhardtii_CrCEP2	GVIN--SCEGLNHGVLAVGYDTS--DKAQPYWIVKNSWGGSWGEGYFRLKMGEGPKG	337
C.reinhardtii_CrCEP1	GVDYDPTCGTSLNHGVLVVGKDVDTGSGSNYWIVKNSWGAEWGDAGYIRLKMGSTDAEG	341
A.thaliana_RD21A	GIFDG-SCGTQLDHGVVAVGYGTE--NGKDYWIVRNSWGWKSWGESGYLRMARNIASSSG	340
A.thaliana_RD21B	GVFDG-LCGTELDHGVVAVGYGTE--NGKDYWIVRNSWGNRWGESGYIKMARNIEAPTG	341
	* : : * * : : * : : * : : * : : * : : * : : * : : *	
C.reinhardtii_CrCEP2	LCGIASAASYAVKTSAVNK-----PVPTMCDMFGWTECGVG	373
C.reinhardtii_CrCEP1	LCGIAMAPSYPVKTGPNPPTPGTPGPSKPKGPKPGPTPPGPKVPCDD--DNECPNG	399
A.thaliana_RD21A	KCGIAIEPSYPIKNGENPNPNGPSPSPSI-----KPPTQCDS--YYTCPEP	384
A.thaliana_RD21B	KCGIAMEASYP IKKGQNPNGPSPSPSI-----KPPTTCDK--YFSCPEP	385
	*** * : : . * : : * : : * : : * : : * : : * : : *	
C.reinhardtii_CrCEP2	NTCCSFSFLFGWLCLWHDCCLADAVSCPDLKHCPCAGT--TCNAAQGACIAADGAS--ST	430
C.reinhardtii_CrCEP1	STCCCVNEIFN-MCFQWGCCPMPKATCCDDHEHCCPADLPVCDTDAGRCLPSAGVFLGSK	458
A.thaliana_RD21A	NTCCCLFEYK-YCFAWGCCPLEAATCCDDNYSCCPHEYPVCDLDQGTCLLSKNSPFSVK	443
A.thaliana_RD21B	NTCCCLYKYK-YCFCWGCCPLEAATCCDDNSCCPHEYPVCDVNRGTCMLSKNSPFSVK	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-----PFWA-----KSRK	460
	. * * : : * : : * : : * : : * : : * : : * : : *	
C.reinhardtii_CrCEP2	RIERIAQ*- 498	
C.reinhardtii_CrCEP1	FLRRGPMN* 501	
A.thaliana_RD21A	NIA*----- 462	
A.thaliana_RD21B	HIA*----- 463	
	: :	

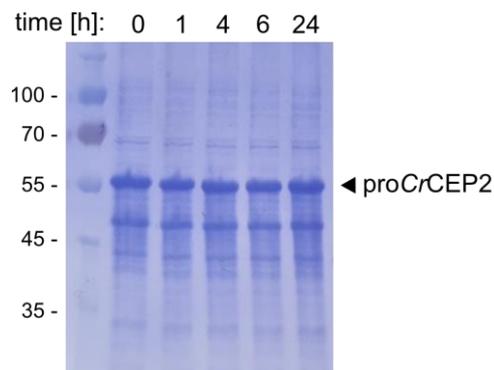
**Figure S7: Multiple sequence alignment showing similarity between proteases CrCEP1, CrCEP2 and granulin domain-containing proteases RD21A and RD21B from *A. thaliana*.** The five structural elements are depicted as follows: the signal peptide in light gray, the autoinhibitory prodomain in red, the 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.



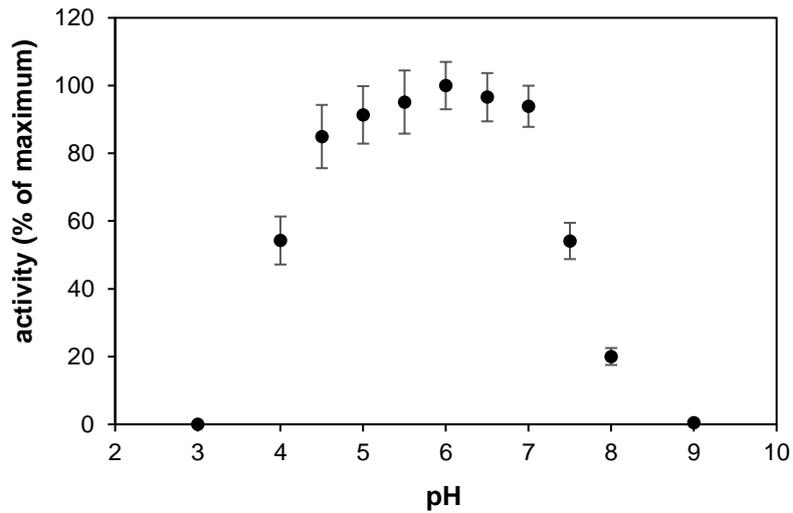
**Figure S8: SDS-PAGE analysis of purified *CrCEP1* (A) and *CrCEP2* (B).** The pro forms of both proteins are indicated by black arrows. The expected size of pro-*CrCEP1* is 52 kDa, but it migrated at a lower molecular weight of 45 kDa. The expected size of pro-*CrCEP2* was 51 kDa and it migrated at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



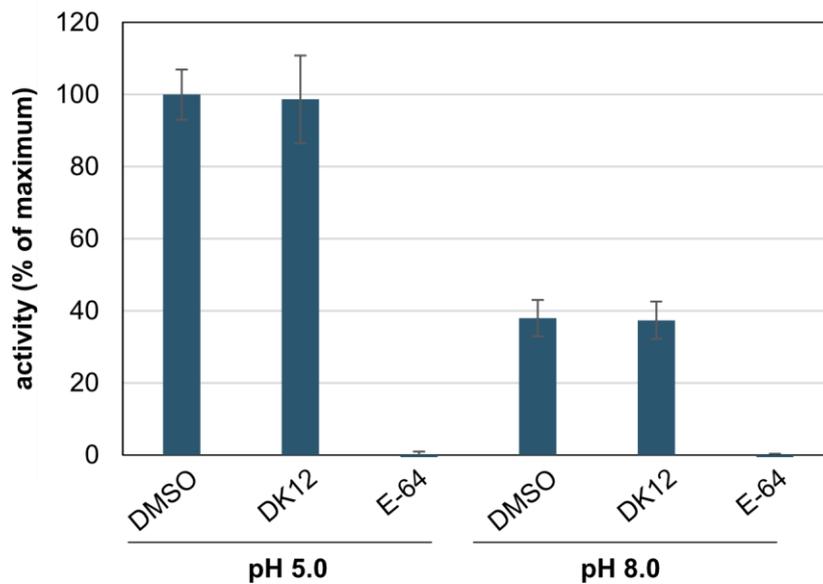
**Figure S9: SDS-PAGE analysis of pro*CrCEP1* in the insoluble fraction.** Black arrows indicate the pro form of *CrCEP1*. After expression, pro*CrCEP1* migrated at a considerably higher MW than after purification.



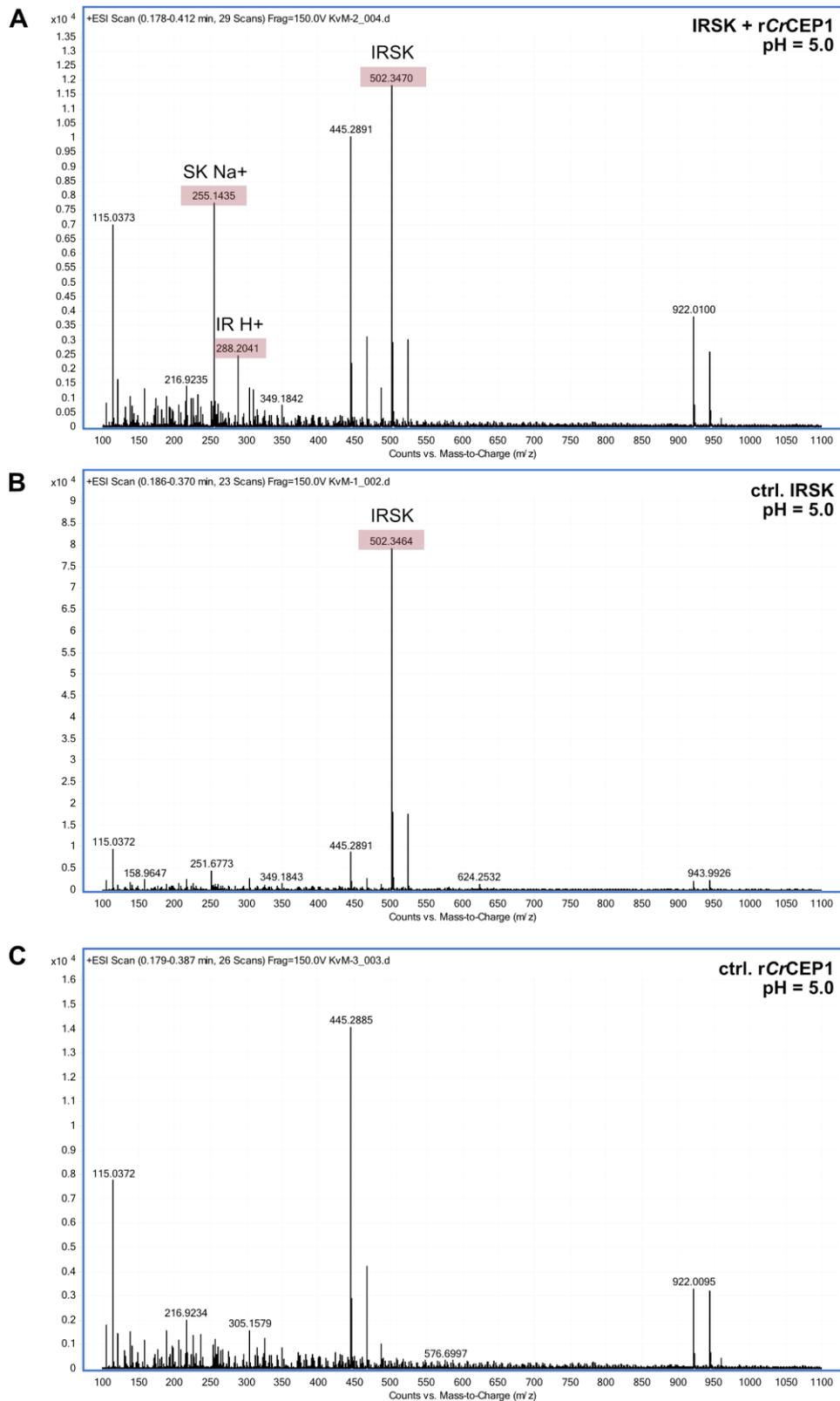
**Figure S10: Analysis of the unsuccessful activation of *CrCEP2*.** The proteins were separated on an SDS-PAGE gel, transferred to a PVDF membrane, and stained with Coomassie. Black arrow indicates the pro form of *CrCEP2*.



**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  $\mu$ M Z-Phe-Arg-AMC and 10 nM mature rCrCEP1.

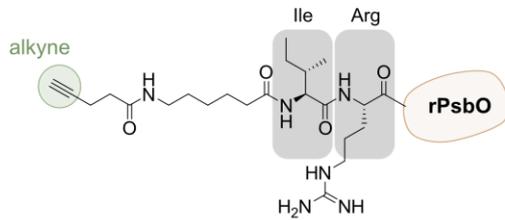


**Figure S12: Kinetic measurements of rCrCEP1 in presence of the DK12 probe.** Mature rCrCEP1 was preincubated for 20 min with either DMSO (positive control), 5  $\mu$ M DK12 or 5  $\mu$ M E-64 (negative control). Assays were performed at room temperature in 100 mM NaOAc pH 5.0 (left) or 20 mM Hepes,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

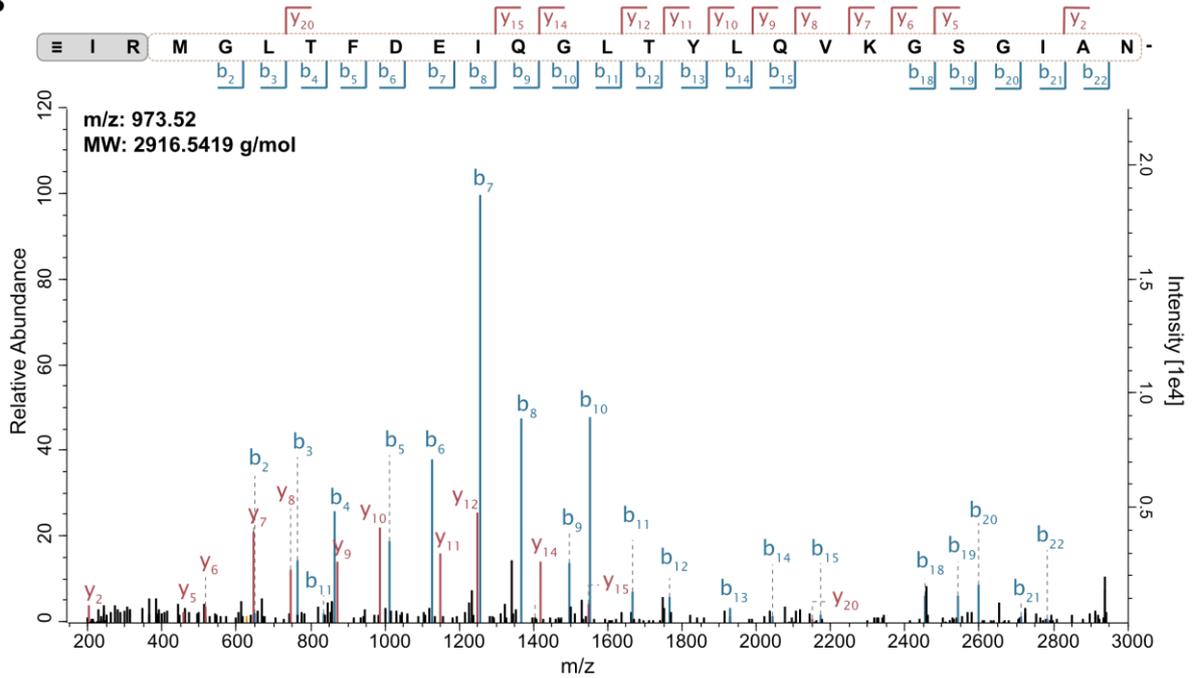


**Figure S13: Mass spectra of the cleaved peptide Ile-Arg-Ser-Lys (IRSK).** Mass spectra were recorded on an Agilent 6224 Accurate Mass TOF LC/MS chromatograph/spectrometer (Agilent Technologies, Santa Clara, CA, USA). **A)** The peptide IRSK was incubated with rCrCEP1 at pH 5.0 for 1 h at 25 °C, rCrCEP1 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 ( $MNa^+$ ): 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.

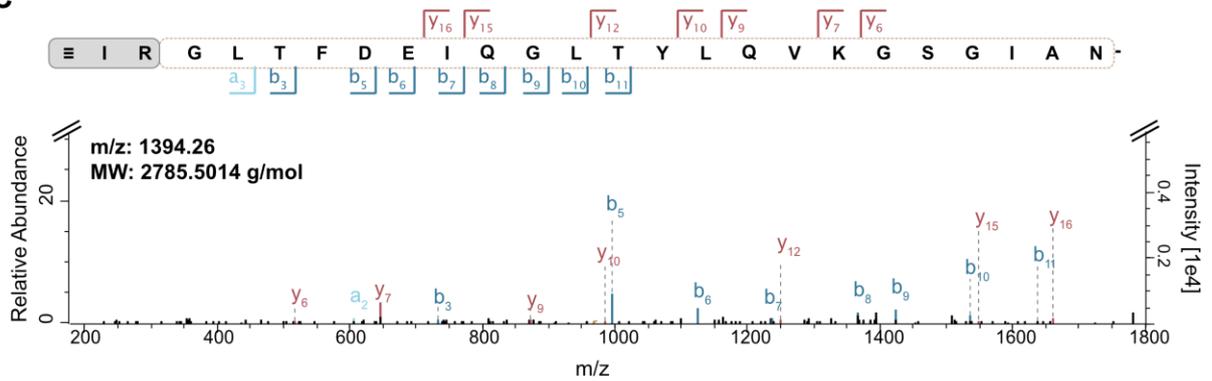
**A**  $\equiv$ IR modification:



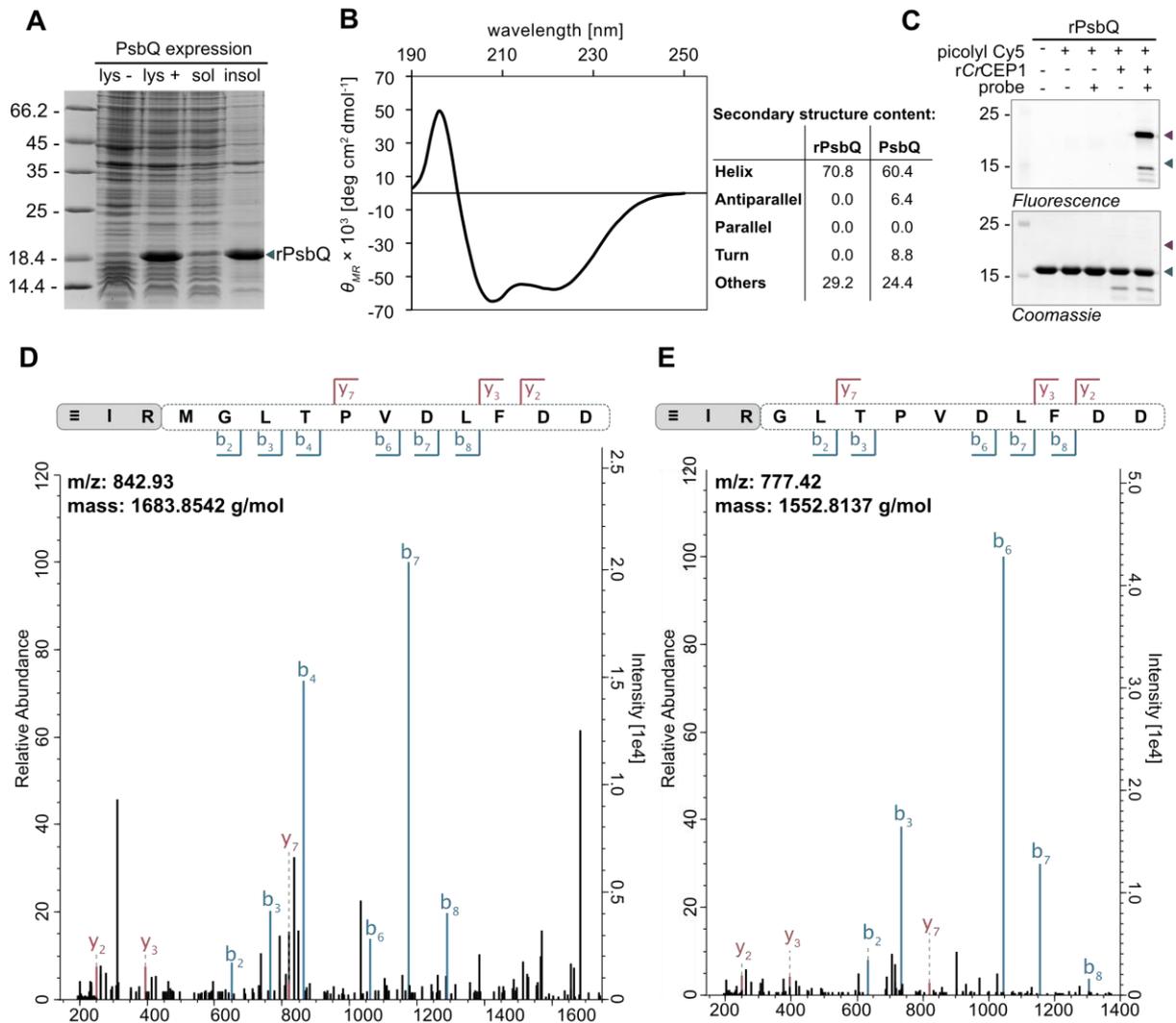
**B**



**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 ( $\theta_{MR}$ ). 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  $\mu$ 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

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