

Supplementary Materials

Recombinant His-tagged APX2

MGSSHHHHHSSGLVPRGSHMSPAVAAALDKALAKNIPKTKTAVALLAFHDAATFSAGAKDGGLNA
SIQYELDRPENFGLKRGWRRIEQVRADLKGTAAEGVVTADLVALAGAFVRLCGGPAIPLPIGRPVAAA
ARQDPPGRMPSENASAAELKANFAAKGLSVQEMVALSGAHTLGSKGFGDPVTFDNAYYVALLQKPWN
NTKDAMASMIPLSDHVLDDPDCLPVIQRYAADQDLFFRDFSAAAYIKMCGLGVAGWA

APX2 (Cre06.g285150)

MSTSVLSHRSLSNCTRHGNNRRACRVATHAKLRDLSQWRQEGSGTSEPAVAPELSRRVLLKLGAALPALA
AALAAATPPALLLAPLAAAEGPTLPSPAVAAALDKALAKNIPKTKTAVALLAFHDAATFSAGAKDGG
LNASIQYELDRPENFGLKRGWRRIEQVRADLKGTAAEGVVTADLVALAGAFVRLCGGPAIPLPIGRPVA
AAARQDPPGRMPSENASAAELKANFAAKGLSVQEMVALSGAHTLGSKGFGDPVTFDNAYYVALLQKP
WNNTKDAMASMIPLSDHVLDDPDCLPVIQRYAADQDLFFRDFSAAAYIKMCGLGVAGWA

(a)

10 20 30 40 50 60 70 80 90 100 110 120 130 140

R_Pp MALSCGSISSSSLCCLSPACT--PPASLACHFFPLRGVYLQLESRLHASSLGFNRNKL SANCYSGGIRASLDNSSPGDEKQVSRQALLAVLAFTI-----PGSQVFQQRFPQALAVEDEQM

R_Marp ---MAALGVR--QCASSAVFTQS--PMTSCIVPTVTLNCAQGRS--CCPSRVCAICGTDASENDTHAFKLQRRSLVVSILLP-----LLPAIINSDPGSTASAAEEAAV

R_Os ---MELTNVPHIA---ASSRFFSSCSRCTCRIRRSKGRTRIGASAGGRCS--SDSACSEPEEQQAD--FFGPISSLCYTRDFASVA-----LLPFLPHVHIASAAEPIDG

R_Zm ---MELNSIPYIASWSSSSSCFYYSRACRGTDRGKLVTKASAGGRKA--AD-SVCSQLSEQGTEDSISGPRSSWRIRRRDLASAI-----LLPFLPHVHISAAEYIDA

R_At ---MTTITASLVKTLFRCDSSFFSKFKCFESPAKTRLLSPATEKHVVR--SRAWIRCLSDDDPGSS--HVFVASRRKRVLLSTVQ-----LLSHMLPQN--GNAEITYP

R_Af ---METLFASMALPLPLFLIR--NCKPRKRTPLTLCFHRPIQO-----I--GKASVSS--PDTNFTRGILESLTYFT-----LLSLGEGISQ-KTAAETEQGLMGCEKKNMISDAF

R_Sc ---MAMSLSLPLQS--SCRKHTFSSVFCFRPRKFKQ-----P--GESQSF--SNMFSRDLLSLETSIM-----LLPLGQNIEN-KSAQAEQAG

R_Sm ---MAMLLAWTSSV--GALP-KIDGGRCA SCDCRD-----E--ARSP-----VVTFRFALLSSSSAA-----LLSS--KFALVSEDAK

R_Cr ---MSTSVLSHRSLSNCTRHGNFRRACR--VATHAKLRD-----LSQWRQEGSGTSEPAVAPELSRRVAVLKGAALP-ALAAALAAATFPALLLAPLAAAEQ-P

R_Vc ---MKIVHLI-CKIKCELLHAEV--VGVSLLCD-----GCHDRDQ--TKE--QPALSRRQVLGLSAPLGLGALIGVLPISQVLVATAPATAAISVP

R_Cs ---MQQAHRPAQ-CGAAFNPARRQQ--AAVHCSSA-----GAQPFSSQ--Q--VAAAFSRQLRLAAGLE-----LALLPALAAFLPAAARAFPG

R_Ca ---MASAASAGWLLVSSVSSWLSSEASFAERAGSLRRRLQRHGAVTAGAS--SQPKPKFPQFPQFPQAVVTRSLQRSSGLLLFMPAAAPVLTITVTRNGSNAARAVDAPS--FR

R_Micp ---MSSAALGALAPPRGAPVLGVARDRRGSRARAARSS--SDASSSSSS--STTRSSSPAPSGPTPRVATSRDVLAAALATL-----LASKTLVAPSSSTASVTASSA

R_Tp -----MNDK-----EREALQLAS

R_Pt -----FQLPNFQALDEVE-----S-----EKAQTING

Po_LAPX -----MGKSYPTVSPDYKKA-----

At_AFX1 -----MTKNYPTVSEDYKKA-----

Nt_sAFX -----AASDSAQ-----

Cr_AFX1 -----MQSARVSRRTARHTRP-----SCRPGRAVAVRSKVNVEQ-----

150 160 170 180 190 200 210 220 230 240 250 260 270 280

R_Pp -----KIELIQRELKVKLSKKG-----SAGVLRSLVPHDAGTFD--SSD-----NGGMNGSLLFELERPE--SAGLQRPV-VLQK-----AKKEIE--LAFPVSWADLIAVAGA

R_Marp -----RLEQIRAAVFKFTVKAK-----AAGVLRSLVPHDAGTFD--ATK-----QGMNGSIVLELERPE--NQGLNRSIKVLSK-----VKAELI--PDVEVSWADLIAVAGA

R_Os -----SIIQNGVRNLSVKV-----AAGMLRLVPHDAGTFD--AD-----KGMNGSIIYEDRPE--NTGLNRSIKVLSK-----AKEVID--LVQ-VSWADLIAVAGA

R_Zm -----SIIISGVRSVLTGKG-----AAGVLRSLVPHDAGTFE--GDG-----SGGMNGSIIYEDRPE--NIGLNRSIKILRK-----AKEGID--NVQKVSADLIAVAGA

R_At -----VMQNEIRKVVTKG-----AAGVLRSLVPHDAGTFE--DD-----HGGINGSIAYELERPE--NIGLNRSL-VLAK-----AKVKVD--EIQPVSWADMI SVAGS

R_Af LLYRNHLCHAITYSIQFLMFCMSNENYSENLKSIQTELAKTLKPR-----AAGMLRLVPHDAGTFD--KTN-----TGMNGSIIYELDRPE--NSGLDRSIKILAA-----VKDSIG--SSLSVSWADLIAVAGA

R_Sc ---NESCSGNALNTIQCELRRKVRKSK-----AAGMLRLVPHDAGTFD--EQ-----TGMNGSIIYELDRPE--NSGLDRSIKILAA-----HFQVVSADLIAVAGA

R_Sm ---DSRIREALRKVVSKQK-----APGLRLVPHDAGTFD--S-----GGGMNGSIIYELERPE--NAGLERSI VLNKA-----RGELE--GSLHVSADLIAVAGS

R_Cr ---TLPSFAVAALDKALAKNPKT-----TAVALLRLVPHDAGTFD--GAK-----DGLNASIQVELDRPE--NGLKRGWRIRIEQ-----VRADLKGTAEVGVTTADDLVALAGA

R_Vc ---VTPSEVKKAALAKALARAIPKAK-----TAVALLRLVPHDAGTFD--PPG-----NGLNASIQVELDRPE--NAGLKRGRIRIEQ-----VRVCMF--VCGVATDADDLVALAGA

R_Cs ---RYLTPEEQAAVDAFAATLPAK-----APVILRLVPHDAGTFD--AAG-----NGVNASIQVELDRPE--NGLKRGWRIRIEQ-----TAKKLGQTAELGSR-ADLIAVAGA

R_Ca ---SGVPKSEANATLYAALQSAIRETTSKGAPGVLRVPHDAGTFD--FAGAVL-----QGGPNGSIRFEDRPE--NEGLQRLPRVIAK-----TMASLKQLPNATVSADLIAVAGA

R_Micp ---FVDTPFIRAAALREALVANVVKTK-----APAVLRVPHDAGTFD--ASN-----DGMNGSVKYLEDRPE--SFLKRGGLGVLA-----TMDALRDT--PAAASLADVIALAGA

R_Tp -----CLVRKSTSDYLHDDP-----TLAGPFLRLVPHDAGTFD--SSSTFAFSGGPNGSIKYLEDRPE--NRGLSRPLKDIEQ-----IHEIV--RNINISLADVIALAGA

R_Pt ---KILRRERVQELARDT-----SLAGPFLRLVPHDAGTFD--SSNGFQ-----SGGPNGSIRFELERPE--NRGLSRPLHVEEA-----IHEGIN--KTYGISLADVIALAGA

Po_LAPX ---IEKAKRKLKRGFIAEK-----CAPLILRLVPHDAGTFD-----KTKTGGPFGTIKHQAEALHAGNNGDLIARLLEP-----IKEQFP-----IVSYADFYLAGV

At_AFX1 ---VEKCRRLKRLGIAEK-----CAPIMVRLVPHDAGTFD-----QSTTGGPFGTMRDFAEQAHGANGSIIHIALRLLEP-----IREQFP-----TISYADFYLAGV

Nt_sAFX ---LKSAREDIKELLKTKF-----CHPIMVRLVPHDAGTFD--YKNIIEE--WFGQGGANGSLFDFELHKGANAGLVNMLNLLK-----IKDKYS-----GVTYADFLQLASA

Cr_AFX1 ---LKALKAELIYINISRG-----CNPISVRLVPHDAGTFD--YKNIIEE--WFGQGGANGSLFDFELHKGANAGLVNMLNLLK-----IKDKYS-----GVTYADFLQLASA

290 300 310 320 330 340 350 360 370 380 390 400 410 420

R_Pp AAVLECDGPVPIVRLGRDLASGDPD-----GKMPEETLT-----ASELKRTFSQ-KGFSTQEMVALSAGHTIGNKG-----FGNP

R_Marp EAVSICGGPKIDVQLGRDLSSLPDPE-----GQMPEETLT-----GSQLEAFQA-KGFSTQELVALSAGHSVGGKG-----FGDP

R_Os ESVALCGGPEIPVRLGRDLSSADPA-----GKLPEETLD-----ATALKTLFSK-KGFSTQEMVLSAGHTIGGKG-----FGNP

R_Zm EAVALLCGGPEIPVRLGRDVSS-ADPS-----GKLPEETLD-----AASLKTFLSK-KGFSAQELVLSAGHTIGGKG-----FGSP

R_At EAVSICGGPTIPVRLGRDLASGDPD-----GKLPPETLS-----ASGLKECFK-KGFSTQELVALSAGHTIGSKG-----FGDP

R_Af EAVALLCGGPTIPVRLGRDLAVTPDPE-----GQLPEESLD-----ASNKKNFIA-KGFTTSELVALSAGHTIGSKG-----FGNP

R_Sc EIALCGGPTIPVRLGRDLAVTPDPH-----GELPESLD-----VLSKKNFIA-KGFTTSELVALSAGHTIGSKG-----FGNP

R_Sm EAIIVCGGPTIPVRLGRDLSSVADIQ-----GELPESLD-----AVALKIKFSQ-KGFSTQEMVALSAGHTIGSKG-----FGNP

R_Cr FAVRLCGGPAIPIPIGRVFAAAADQP-----PGRMFSENAS-----AEALKANFA-KGLSVQEMVALSAGHTIGSKG-----FGDP

R_Vc YAVRLCGGPDIALAGRPVFAAASDPD-----PDRMPGENFT-----AEQLKANFA-KGLSVQEMVALSAGHTIGSKG-----FGDP

R_Cs HAVRLTGGPQI-VAVGRQDAAADPD-----GRMPEDLSS-----AEQLLDFNA-KGLSARELIVLSGSH-LGSKG-----YGDP

R_Ca ESVALITGGPTFRVPIGRVDAQASAD-----GRMPGENWT-----AAQLKSHFA-KGFSTRELVALLAGHTIGFGK-----YGMP

R_Micp YAVELTGGPIVRLGRDVASADPE-----NRMADTLT-----GEGQRAHVR-AGFSTRELVALLAGHTIGGKG-----FGPE

R_Tp QAVQAARGPTISIRLGRDRAKADERNRNQLQSDTTRSLVDTLPSAGLD-----SEGRLRYFA-KGLSDKEFVALCGANDLGRHVITLLMPKACLKNTRECLNAPMLP-----FVAEEP

R_Pt VAVEQAAGPPTIPVRLGRDVASADPTQRTRETSVVAETMNPGLD-----ADGLRLYER-LGLSESEFVALSAGHSILGRHVITLLMPSCKLNTQCLKEAPTLLP-----FVSSSV

Po_LAPX VAVEITGGPEVFPFHGREDKPEP-----PPEGRLPDATKG-----SDHLRDVFKAMGLSDQDIALSGGHTIGAAHKEISGFEG-----FVTSNP

At_AFX1 VAVEITGGPDIPIPHGREDKPEP-----PPEGRLPDATKG-----CDHLRDVFAKMGSLDKDIALSAGHTIGRCHKDRSGFEG-----FVTSNP

Nt_sAFX TAIEEAGGPKIPMKYGRDVATEPEQC-----PPEGRLPDAGPP-----SPAQHLRDVFK-MGLNDKEIVALSAGHTIGRSPDRSGWKGPEKTYTKDGGP-----APG-----GGSTATGW

Cr_AFX1 TAIEASGGPKIPMYGRDKADGPEEC-----SPDGRPGAHPFADGSGSPAELHRLRVYR-MGLNDQDQIVLVSAGHTIGRARPDRSGFGAEKTYTKDVGPGTSTASPGATDRPVTPKPVQGGQTSWTNVN

430 440 450 460 470 480 490 500 510 520 530

R_Pp NLFDSNYFQILLQK--PWKIGPDD-----GNTSMIGLATRALADDEECLEWVRVYAADQGRFFTFDSAVYTKLVNT-GAR-WTPFQA-----

R_Marp YTFDNIYKILLQK--PWLTKENE-----TKMIGLSDRALPEDEECLEWVNAYAQDQLDFRDTFSAYMKVLDT-GVR-RL-----

R_Os NIFDINSYFKLLEK--PQFSS-----GMPAMVGLRDTWALTEDECLRWNIYLAQDQAKFFADFKDAIKLVNT-GAS-WRSA-----

R_Zm NIFDINSYFKLLEK--PQFSS-----GMAAMVGLRDTWALTEDECLRWIRIYAEQDQAKFFDFDFTYIKLVDS-GAS-WRTA-----

R_At VTFDNIYKILLK--PWTSS-----KMTSMVGLPSDHALVQDECLRWKRYAEDQDKFFEDFTNAYIKLVNS-GAK-WNML-----

R_Af LVFDINSYKILLK--PWTQI-DN-----EMSGMIGLPSDRLVDDDECFRLVTYAEDSKFFEDFSAAYIKLVNS-GAK-WRAGAT-----

R_Sc LVFDINSYKILLK--PWTQI-DN-----EISGMIGLPTDRALVDDDECLRWINCYANDESKFFEDFSAAYIKLVNS-GAI-WRIQPV-----

R_Sm LVFDINSYKILLK--PWSQD-DN-----KMSMIGLPSDRLVDSDEECLEWIKLYKRGDSKFIYADFTLAYTKTWACKGQS-YKTPDKFLDKA-----

R_Cr VTFDNIYVALLQK--PWNV-TDA-----MASMIGLPSDHLVDDPDCLEVIQRYAADQDLFFEDFSAAYIKMCGL-GVAGWA-----

R_Vc TRFDINSYKILLR--PWTNPDS-----MASMIGLPSDHLVDDPECLFYIERIYAEQDQADFFADFAAAYIKLTS-LGVPGWAV-----

R_Cs VTFDINTYKILLK--PWEVRSAE-----MAAHIGIPDHLVDNPTCRPFIEEYAADQQLFFERDFAAFAKMTL-GAR-WA-----

R_Ca DVFDINTYKILLK--PWNKSDA-----MAYMIGIPDHAVAEDTECLWQIYAADQQLFFEDFAAAYIKMTSA-GAV-WSV-----

R_Micp YVFDINSYKILLR--PWAATKSKKEELDASHIGLTSQKALATEPSLEYVRKVAEDQQLFFEDFAAAYIKLQTEQ-GAT-WR-----

R_Tp DTFDINSYKILLK--WNDR-----VALGEVAFIPDVLVDLVEGLRVRVYKHFADKAAFTSTFATAYIKLVEPGTTSVRY-----

R_Pt DRFDINSYFALMK--WNRS-----VFIGEVAFIPDVALVDKGLRVRVYKHFADQSLYRTFRAYIKLVENTATSVRY-----

Po_LAPX LIFDINSYFELLTG-----EKDGLLQSLKALITDSVFRPLVEKYAEDDEFFADYAEAHMLKSEL-G--FAEA-----

At_AFX1 LIFDINSYFELLTG-----EKDGLLQSLKALITDSVFRPLVEKYAEDDEFFADYAEAHMLKSEL-G--FADA-----

Nt_sAFX LKFDINSYFKDIKER-----RDEDLLVLPDAALEFDPFSFKYAEKYAADPEAFFKDYAEAHMLKSEL-GAK-FGPAEGFSLGSPAGAPEKFAAYKYSTGKD

Cr_AFX1 LEFDINSYFKDIKAK-----RDSLLVLPDADIFEDQFRPYAEKYAADQAFFADYCVSHQKSEL-GVE-WEGAPVTLD-----

10 20 30 40 50 60 70 80 90 100 110 120 130 140

R_Cr ---MSTSVLSHRSLSNCTRHGNFRRACR--VATHAKLR-----LSQWRQEGSGTSEPAVAPELSRRVAVLKGAALP-ALAA--ALAAATFPALL--L-----APLFAAAG

R_Vc ---MKIVHLI-CKIKCELLHAEV--VGVSLLCD-----GCHDRDQ--TKE--QPALSRRQVLGLSAPLGLGALIGVLPISQVLVATAPATAAISVP

R_Cs ---MQQAHRPAQ-CGAAFNPARRQQ--AAVHCSSA-----GAQPFSSQ--Q--VAAAFSRQLRLAAGLE-----LALLPALAAFLPAAARAFPG

R_Ca ---MASAASAGWLLVSSVSSWLSSEASFAERAGSLRRRLQRHGAVTAGAS--SQPKPKFPQFPQFPQAVVTRSLQRSSGLLLFMPAAAPVLTITVTRNGSNAARAVDAPS--FR

R_Micp ---MSSAALGALAPPRGAPVLGVARDRRGSRARAARSS--SDASSSSSS--STTRSSSPAPSGPTPRVATSRDVLAAALATL-----LASKTLVAPSSSTASVTASSA

R_Marp ---MAALGVR--QCASSAVFTQS--PMTSCIVPTVTLNCAQGRS--CCPSRVCAICGTDASENDTHAFKLQRRSLVVSILLP-----LLPAIINSDPGSTASAAEEAAV

R_Os ---MELTNVPHIA---ASSRFFSSCSRCTCRIRRSKGRTRIGASAGGRCS--SDSACSEPEEQQAD--FFGPISSLCYTRDFASVA-----LLPFLPHVHIASAAEPIDG

R_Zm ---MELNSIPYIASWSSSSSCFYYSRACRGTDRGKLVTKASAGGRKA--AD-SVCSQLSEQGTEDSISGPRSSWRIRRRDLASAI-----LLPFLPHVHISAAEYIDA

R_At ---MTTITASLVKTLFRCDSSFFSKFKCFESPAKTRLLSPATEKHVVR--SRAWIRCLSDDDPGSS--HVFVASRRKRVLLSTVQ-----LLSHMLPQN--GNAEITYP

R_Sm ---MAMLLAWTSSV--GALP-KIDGGRCA SCDCRD-----E--ARSP-----VVTFRFALLSSSSAA-----LLSS--KFALVSEDAK

R_Cr ---MSTSVLSHRSLSNCTRHGNFRRACR--VATHAKLR-----LSQWRQEGSGTSEPAVAPELSRRVAVLKGAALP-ALAA--ALAAATFPALL--L-----APLFAAAG

R_Vc ---MKIVHLI-CKIKCELLHAEV--VGVSLLCD-----GCHDRDQ--TKE--QPALSRRQVLGLSAPLGLGALIGVLPISQVLVATAPATAAISVP

R_Cs ---MQQAHRPAQ-CGAAFNPARRQQ--AAVHCSSA-----GAQPFSSQ--Q--VAAAFSRQLRLAAGLE-----LALLPALAAFLPAAARAFPG

R_Ca ---MASAASAGWLLVSSVSSWLSSEASFAERAGSLRRRLQRHGAVTAGAS--SQPKPKFPQFPQFPQAVVTRSLQRSSGLLLFMPAAAPVLTITVTRNGSNAARAVDAPS--FR

R_Micp ---MSSAALGALAPPRGAPVLGVARDRRGSRARAARSS--SDASSSSSS--STTRSSSPAPSGPTPRVATSRDVLAAALATL-----LASKTLVAPSSSTASVTASSA

R_Tp -----MNDK-----EREALQLAS

R_Pt -----FQLPNFQALDEVE-----S-----EKAQTING

Po_LAPX -----MGKSYPTVSPDYKKA-----

At_AFX1 -----MTKNYPTVSEDYKKA-----

Nt_sAFX -----AASDSAQ-----

Cr_AFX1 -----MQSARVSRRTARHTRP-----SCRPGRAVAVRSKVNVEQ-----

(b)

10 20 30 40 50 60 70 80 90 100 110 120 130 140

R_Cr ---MSTSVLSHRSLSNCTRHGNFRRACR--VATHAKLR-----LSQWRQEGSGTSEPAVAPELSRRVAVLKGAALP-ALAA--ALAAATFPALL--L-----APLFAAAG

R_Vc ---MKIVHLI-CKIKCELLHAEV--VGVSLLCD-----GCHDRDQ--TKE--QPALSRRQVLGLSAPLGLGALIGVLPISQVLVATAPATAAISVP

R_Cs ---MQQAHRPAQ-CGAAFNPARRQQ--AAVHCSSA-----GAQPFSSQ--Q--VAAAFSRQLRLAAGLE-----LALLPALAAFLPAAARAFPG

R_Ca ---MASAASAGWLLVSSVSSWLSSEASFAERAGSLRRRLQRHGAVTAGAS--SQPKPKFPQFPQFPQAVVTRSLQRSSGLLLFMPAAAPVLTITVTRNGSNAARAVDAPS--FR

R_Micp ---MSSAALGALAPPRGAPVLGVARDRRGSRARAARSS--SDASSSSSS--STTRSSSPAPSGPTPRVATSRDVLAAALATL-----LASKTLVAPSSSTASVTASSA

R_Marp ---MAALGVR--QCASSAVFTQS--PMTSCIVPTVTLNCAQGRS--CCPSRVCAICGTDASENDTHAFKLQRRSLVVSILLP-----LLPAIINSDPGSTASAAEEAAV

R_Os ---MELTNVPHIA---ASSRFFSSCSRCTCRIRRSKGRTRIGASAGGRCS--SDSACSEPEEQQAD--FFGPISSLCYTRDFASVA-----LLPFLPHVHIASAAEPIDG

R_Zm ---MELNSIPYIASWSSSSSCFYYSRACRGTDRGKLVTKASAGGRKA--AD-SVCSQLSEQGTEDSISGPRSSWRIRRRDLASAI-----LLPFLPHVHISAAEYIDA

R_At ---MTTITASLVKTLFRCDSSFFSKFKCFESPAKTRLLSPATEKHVVR--SRAWIRCLSDDDPGSS--HVFVASRRKRVLLSTVQ-----LLSHMLPQN--GNAEITYP

R_Sm ---MAMLLAWTSSV--GALP-KIDGGRCA SCDCRD-----E--ARSP-----VVTFRFALLSSSSAA-----LLSS--KFALVSEDAK

R_Cr ---MSTSVLSHRSLSNCTRHGNFRRACR--VATHAKLR-----LSQWRQEGSGTSEPAVAPELSRRVAVLKGAALP-ALAA--ALAAATFPALL--L-----APLFAAAG

R_Vc ---MKIVHLI-CKIKCELLHAEV--VGVSLLCD-----GCHDRDQ--TKE--QPALSRRQVLGLSAPLGLGALIGVLPISQVLVATAPATAAISVP

R_Cs ---MQQAHRPAQ-CGAAFNPARRQQ--AAVHCSSA-----GAQPFSSQ--Q--VAAAFSRQLRLAAGLE-----LALLPALAAFLPAAARAFPG

R_Ca ---MASAASAGWLLVSSVSSWLSSEASFAERAGSLRRRLQRHGAVTAGAS--SQPKPKFPQFPQFPQAVVTRSLQRSSGLLLFMPAAAPVLTITVTRNGSNAARAVDAPS--FR

R_Micp ---MSSAALGALAPPRGAPVLGVARDRRGSRARAARSS--SDASSSSSS--STTRSSSPAPSGPTPRVATSRDVLAAALATL-----LASKTLVAPSSSTASVTASSA

R_Tp -----MNDK-----EREALQLAS

R_Pt -----FQLPNFQALDEVE-----S-----EKAQTING

Po_LAPX -----MGKSYPTVSPDYKKA-----

At_AFX1 -----MTKNYPTVSEDYKKA-----

Nt_sAFX -----AASDSAQ-----

Cr_AFX1 -----MQSARVSRRTARHTRP-----SCRPGRAVAVRSKVNVEQ-----

(c)

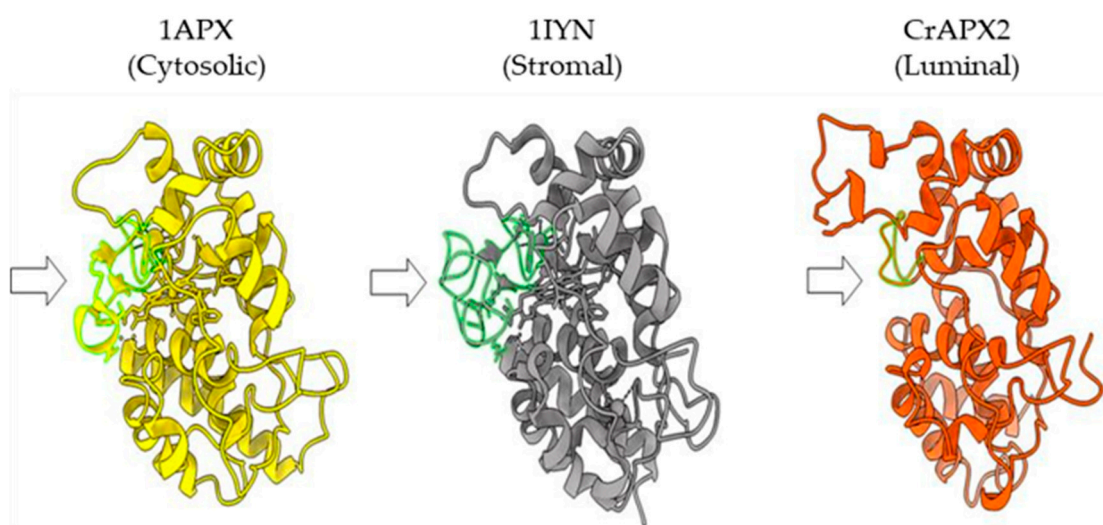
Figure S1. Amino acid sequence alignment of APX and APX-R showing conserved residues in representatives from algae, plants and diatoms.

(a) Sequence of recombinant His-tagged APX2 expressed in *E. coli*. The His-tag is underlined in blue and the sequence for cleavage by thrombin in violet. The sequence of APX2 (Cre06.g285150) from Phytozome is also shown and the targeting signal that has been removed in recombinant His-tagged APX2 is in gray.

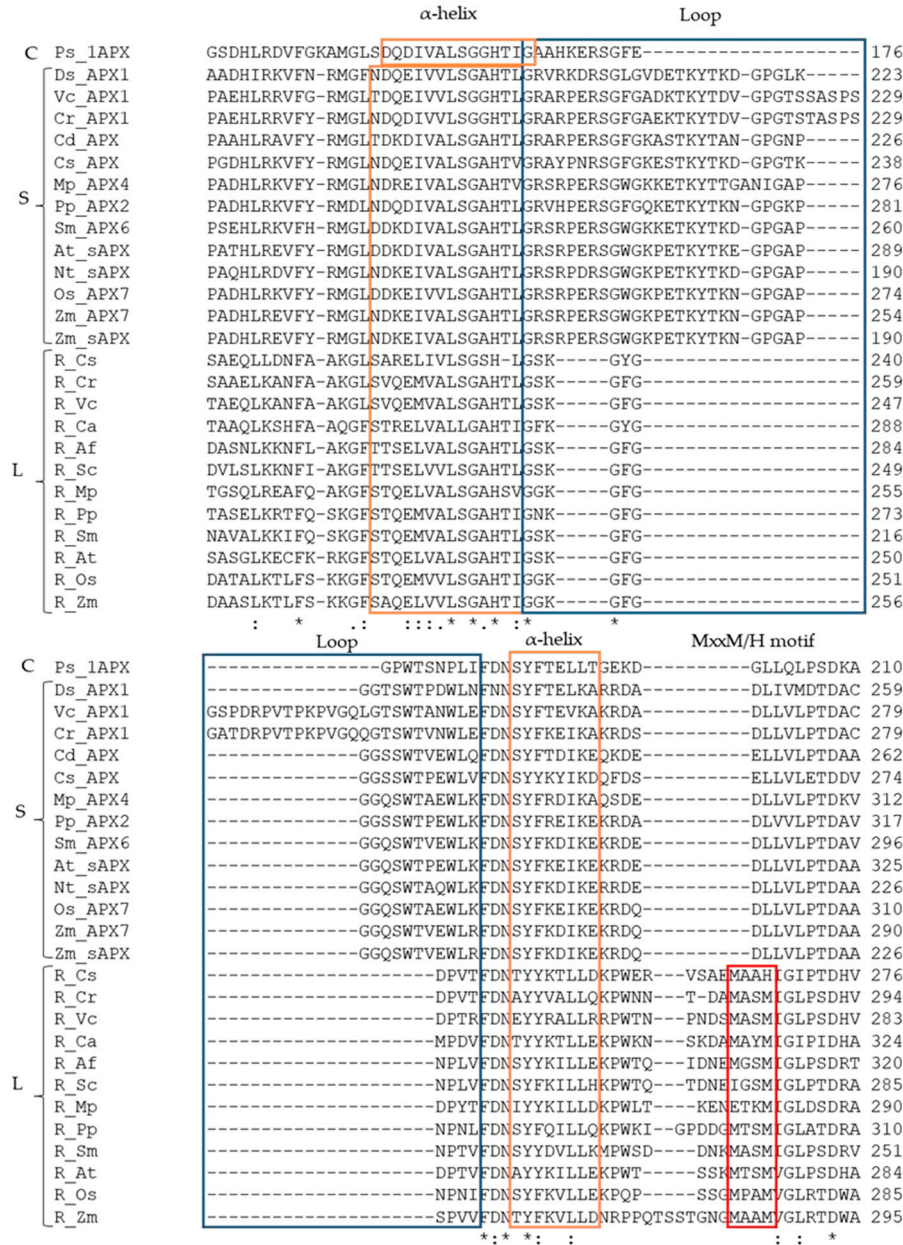
(b) The amino acid sequence alignment of APX-R from selected photosynthetic organisms (green algae, diatoms, plants) was performed using ClustalW (https://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html, accessed on 15 September 2023). The organisms were chosen based on [1] and the sequences retrieved from the RedOxiBase (<https://peroxibase.toulouse.inra.fr/>, accessed on 15 September 2023). The sequences for classical APXs were retrieved by blastp analysis using AtAPXs. Double arginine for TAT-motif is highlighted in bold (CrAPX2 as RedoxiBase reference: 56RR57, putative cleavage site is highlighted in yellow). Conserved amino-acid residues are highlighted in bold. The ones of the reference sequence from *Pisum sativum* Ps_1APX are in blue and bold, however, due to the alignment the exact position of these residues as described in [2] in the crystal structure has shifted (the essential active residues are **R38 -> R196**, **W41 -> W199**, **H42 -> H200**, **H163 -> H365**, **W179 -> W416**, **D208 -> D459**; the primary residue responsible for ascorbate binding is **R172 -> R374** together with the contribution of **C32 -> C190** and **K30 -> K185**; **H163 -> H365** and **H169 -> H371** are the heme-binding residues). In grey is highlighted the **MxxM or MxxH** motif typical of metal binding.

RedOxiBase Entry ID are the following: **R_Tp** *Thalassiosira pseudonana* APX-R 2559; **R_Pt** *Phaeodactylum tricornutum* APX-R 8208; **R_Micp** *Micromonas pusilla* APX-R 8359; **R_Cs** *Chlorella sorokiniana* APX-R 15183; **R_Cr** *Chlamydomonas reinhardtii* APX-R 7433; **R_Vc** *Volvox carteri* APX-R 8357; **R_Ca** *Chlorokybus atmophyticus* APX-R 7707; **R_Af** *Azolla filiculoides* APX-R 16514; **R_Sc** *Salvinia cucullata* APX-R 16550; **R_Marp** *Marchantia polymorpha* APX-R 5764; **R_Pp** *Physcomitrella patens* APX-R 5761; **R_Sm** *Selaginella moellendorffii* APX-R 7143; **R_At** *Arabidopsis thaliana* APX-R 3952; **R_Os** *Oryza sativa* APX-R 3961; **R_Zm** *Zea mays* APX-R 5183.

(c) ClustalW (https://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html, accessed on 15 September 2023) was used to perform the alignment of the APX-R from selected organisms (green algae, diatoms, plants). Sequences of APX-R were retrieved on RedOxiBase (<https://peroxibase.toulouse.inra.fr/>). Entry ID are the following: **R_Tp** *Thalassiosira pseudonana* APX-R 2559; **R_Pt** *Phaeodactylum tricornutum* APX-R 8208; **R_Micp** *Micromonas pusilla* APX-R 8359; **R_Cs** *Chlorella sorokiniana* APX-R 15183; **R_Cr** *Chlamydomonas reinhardtii* APX-R 7433; **R_Vc** *Volvox carteri* APX-R 8357; **R_Ca** *Chlorokybus atmophyticus* APX-R 7707; **R_Af** *Azolla filiculoides* APX-R 16514; **R_Sc** *Salvinia cucullata* APX-R 16550; **R_Marp** *Marchantia polymorpha* APX-R 5764; **R_Pp** *Physcomitrella patens* APX-R 5761; **R_Sm** *Selaginella moellendorffii* APX-R 7143; **R_At** *Arabidopsis thaliana* APX-R 3952; **R_Os** *Oryza sativa* APX-R 3961; **R_Zm** *Zea mays* APX-R 5183. Putative twin-arginine-translocation motif (TAT) is highlighted: double arginine (RR) for chloroplast translocation is highlighted in bold and the AxA cleavage site for luminal translocation is highlighted in yellow (as checked using TargetP-2.0, <https://services.healthtech.dtu.dk/services/TargetP-2.0/>, accessed on 30 October 2023). The AxA cleavage site is not clear for R_Micp, R_Zm, R_Af and R_Pp, and is lacking for R_At. Differences in the length of the hydrophobic region between the RR and the AxA cleavage site can be noticed between algal and land plant sequences. The members in the two diatoms **R_Tp** *Thalassiosira pseudonana* and **R_Pt** *Phaeodactylum tricornutum* do not present the TAT motif.



(a)



(b)

Figure S2. Chlamydomonas APX2 lacks the loop that faces the heme, and APX-R contains the MxxM/H sequence motif. (a) Structural comparison of classical cytosolic and chloroplastic APXs (respective PDB ID 1APX for *Pisum sativum* APX1; 1IYN for *Nicotiana tabacum* sAPX) with the predicted structure of Chlamydomonas APX2 [3]. The loop covering the heme propionyl tails present in the cytosolic and stromal APX enzymes is absent in CrAPX2, in which there is a solvent-exposed MxxM or MxxH motif. (b) Partial amino acid sequence alignment showing differences in the secondary structures (including the loop facing the heme or the MxxM or MxxH motif) between cytosolic (C), stromal (S) and luminal (L) isoforms (APX-R). Sequences of cytosolic and stromal isoforms were retrieved by a blastp search using stromal APX from *A. thaliana* (AtsAPX). The sequences of chloroplast APX isoforms used are the same as in Figure S1 and they are compared with the cytosolic isoform APX1 of *P. sativum*, from which the helices and loops are defined by crystal structure.

RedOxiBase Entry ID are the following: **R_Cs** *Chlorella sorokiniana* APX-R 15183; **R_Cr** *Chlamydomonas reinhardtii* APX-R 7433; **R_Vc** *Volvox carteri* APX-R 8357; **R_Ca** *Chlorokybus atmophyticus* APX-R7707; **R_Af** *Azolla filiculoides* APX-R 16514; **R_Sc** *Salvinia cucullata* APX-R 16550; **R_Mp** *Marchantia polymorpha* APX-R 5764; **R_Pp** *Physcomitrella patens* APX-R 5761; **R_Sm** *Selaginella moellendorffii* APX-R 7143; **R_At** *Arabidopsis thaliana* APX-R 3952; **R_Os** *Oryza sativa* APX-R 3961; **R_Zm** *Zea mays* APX-R 5183.

GenBank/NCBI accession numbers are as follows: **Ps_1APX** *Pisum sativum* 1APX XP_050898393.1; **Ds_APX1** *Dunaliella salina* APX1 QBY35580; **Vc_APX1** *Volvox carteri* APX1 XP_002953608.1; **Cr_APX1** *Chlamydomonas reinhardtii* APX1 XP_001701947.1; **Cd_APX** *Chlorella desiccata* APXhypot KAG7671850.1; **Cs_APX** *Coccomyxa subellipsoidea* APX XP_005643275.1; **Mp_APX4** *Marchantia polymorpha* APX4 PTQ42354.1; **Pp_APX2** *Physcomitrella patens* APX2 XP_024381690.1; **Sm_APX6** *Selaginella moellendorffii* APX6 XP_002986232.1; **At_sAPX** *Arabidopsis thaliana* sAPX NP_001319883.1; **Nt_sAPX** *Nicotiana tabacum* sAPX 1IYN_A; **Os_APX7** *Oryza sativa* APX7 NP_001389298.1; **Zm_APX7** *Zea mays* APX7 NP_001132683.1; **Zm_sAPX** *Zea mays* sAPX ONM17334.1.

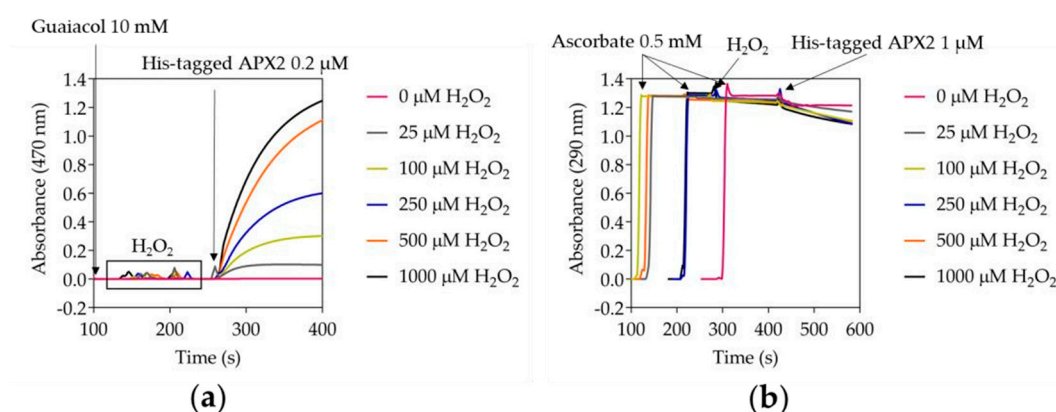


Figure S3. APX2 utilizes guaiacol, but not ascorbate, as electron donor. Peroxidase activity progress curves are shown. (a) Oxidation of guaiacol monitored at 470 nm as a function of time in the presence of recombinant APX2 and increasing H_2O_2 concentrations and (b) Oxidation of sodium ascorbate followed at 290 nm as a function of time in the presence of recombinant APX2 and increasing H_2O_2 concentrations. Arrows pointing at the position where guaiacol, ascorbate, H_2O_2 , and His-tagged APX2 were added.

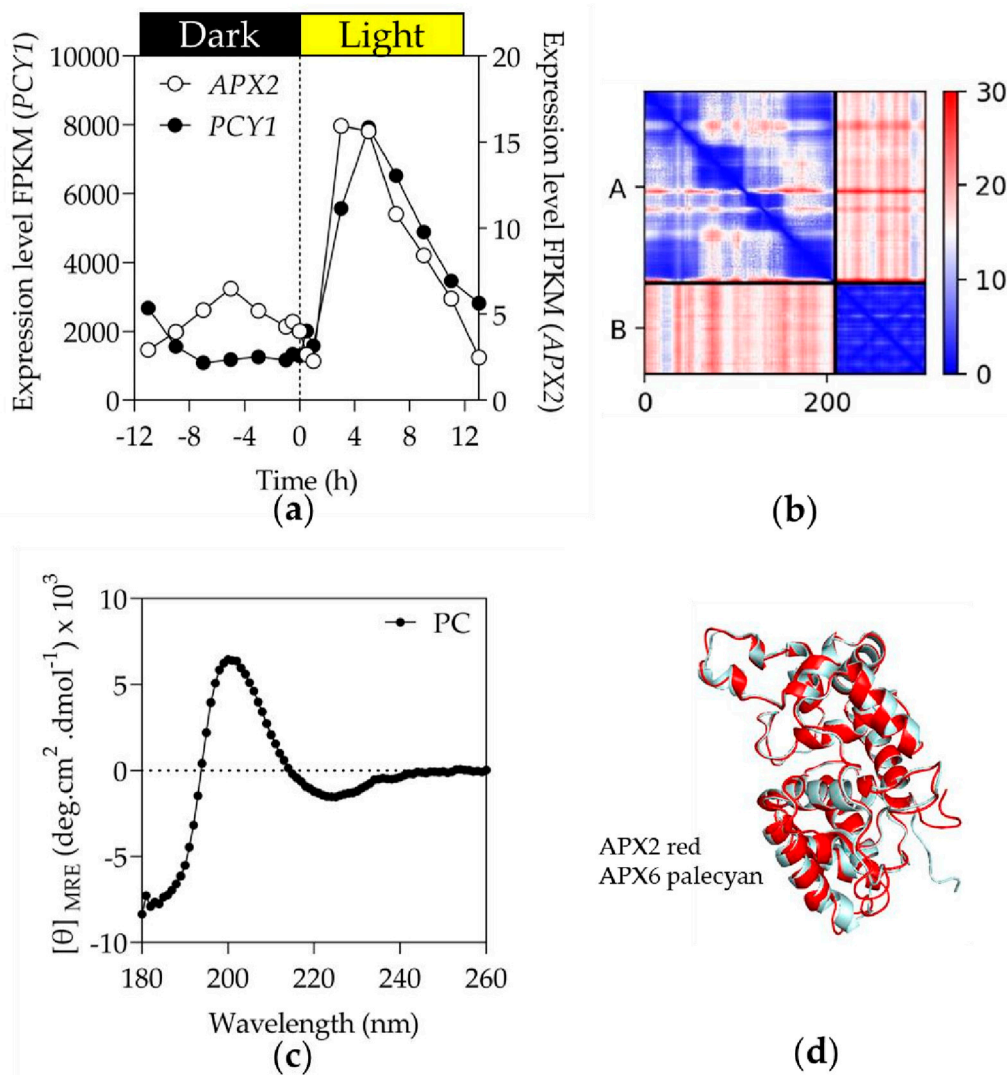


Figure S4. Further analyses of APX2 and plastocyanin. (a) Transcript levels of APX2 and plastocyanin (PCY1) in *Chlamydomonas* present similar dynamics changes in the light phase of asynchronized day-night culture. The time points -11 to 0 correspond to the dark phase, the time points 0 to 12 corresponds to the light phase, time points 12 to 13 correspond to the next dark phase. The data are retrieved from [4]. (b) AlphaFold2_multimer pairwise alignment diagram for the interaction of APX2 with apoplastocyanin. A represents all the residues of APX2 and B represents all the residues of apoplastocyanin. AF2 predicted five models and only the one ranked as number one is shown. The first quadrant shows the prediction error on the interactions within APX2 and the fourth quadrant the internal interaction error within apoplastocyanin. The blue color indicates high confidence, while the red color indicates low confidence (or high flexibility). The blue line marks in the second and third quadrant of the diagram show specific well predicted interactions between both proteins. (c) Circular dichroism spectrum of the recombinant plastocyanin. Circular dichroism between 180 and 260 nm of recombinant plastocyanin (PC, 50 μ M) in 20 mM phosphate buffer, pH 7.4 + 100 mM NaF shows the characteristic folded structure (recombinant plastocyanin was a kind gift from M. Hippler and Yuval Milrad (UMunster)). (d) Superimposition of predicted structure of APX2 of *Chlamydomonas* (red) and APX6 of *Arabidopsis* (palecyan) (predicted with AlphaFold2 powered by ColabFold) reveals that the two proteins are similar. The r.m.s.d. is 0.407 Å.

References

1. Dunand, C.; Mathé, C.; Lazzarotto, F.; Margis, R.; Margis-Pinheiro, M. Ascorbate peroxidase-related (APx-R) is not a duplicable gene. *Plant Signal. Behav.* **2011**, *6*, 1908–1913, <https://doi.org/10.4161/psb.6.12.18098>.
2. Raven, E.L. Understanding functional diversity and substrate specificity in haem peroxidases: what can we learn from ascorbate peroxidase? *Nat. Prod. Rep.* **2003**, *20*, 367–381, <https://doi.org/10.1039/b210426c>.
3. Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; et al. Highly accurate protein structure prediction with AlphaFold. *Nature* **2021**, *596*, 583–589, <https://doi.org/10.1038/s41586-021-03819-2>.
4. Strenkert, D.; Schmollinger, S.; Gallaher, S.D.; Salomé, P.A.; Purvine, S.O.; Nicora, C.D.; Mettler-Altmann, T.; Soubeyrand, E.; Weber, A.P.M.; Lipton, M.S.; et al. Multiomics resolution of molecular events during a day in the life of *Chlamydomonas*. *Proc. Natl. Acad. Sci.* **2019**, *116*, 2374–2383, <https://doi.org/10.1073/pnas.1815238116>.