

Supplementary Information

Bioinformatic analysis

alr2269N	TEPRVLVSEVLVRFQSQQLTPELETQVYNVIRTPGRTTTRSQLQEDINAIFGTGFFSNV	60
2QDZ	-----	0
ptToc75	-----	0
alr2269N	QASPEDTPLGVRVSVFIVQPNPVLSKVEIQANPGTNVPSVLP---QATADEIFRAQYGKIL	117
2QDZ	-----	0
ptToc75	-----NMPIQRVVVPNST--VLPN---EVI-QLAANRAG-VVGHPL	34
alr2269N	NLRDLQEGIKELTKRYQDQGYVLANVVGAPQVSENGVVTLQVAEGVVENISVRFRNKEGQ	177
2QDZ	-----	0
ptToc75	QSTSIQSFAKSLKEWYIRKGYILHSVTGATLQSETGTAITIVQEPAVSKDPVGITFCKEM	94
alr2269N	DVNEQQQPIRGRTQDYIITREVELKPGQVFNNT-V-QKDLQRVFGTGLFE-D-V-NVSL	232
2QDZ	-----	0
ptToc75	VVDTFVPTAGHTRPNRIASALGLSPGNPFQ---W-DGIRWQQIASSGIFAQ-V-LRATP	148
alr2269N	DPGTDPTKVVVVNVVERS-----	251
2QDZ	-----QLLPGARDLNRIDDRQRKEQLQAAAAAAAAATSGHTVTVHAVD	41
ptToc75	QP-MQDGTVQLHILATEA-----	165
alr2269N	-----	251
2QDZ	LDFGVEGRFLFDPAPLVQDYLNRPDNEQLFLLVKALSAAALYDRGYATSIVTFVPPGVVDG	101
ptToc75	-----	165
alr2269N	-----	251
2QDZ	VLKLVVEWGRIKGLWIDGKPLEGTRDRMMVFSAMPGWQDKVLNVFDIDQAIYNINNGGKT	161
ptToc75	-----	165
alr2269N	-----	251
2QDZ	GNITIVPADEYGYSLDLQLQRRALPRVSLGMDNSGPGTPENG---RYKYNASVTANDL	217
ptToc75	-----PTRHLEYGLGKS-----LYTGSWEGELDFEHINL	194
alr2269N	-----	251
2QDZ	LGLNDTLGLYIGNRY--RDAGHD-AERNYDLMYSVPL--GR--TRLDLQTGYSTYRNL	270
ptToc75	LGGGESLGLSVRRG--TK-----DAEPSIRLSFTDSKFG--LGGGYDLEVFSDFIGDQP	244
alr2269N	-----	251
2QDZ	KTRYGQ-----YQSAGNSRSFGLKATRLLYRDRSQFSVYGGKLRQNKNYLAGTRLD	323
ptToc75	EEMQEGELPPDYDHSILDRKGATVRLRNPICSRIVRNSVASGSLERTATKTGLH---ET	301
alr2269N	-----	251
2QDZ	VSSKHYSDVTVGMQYSTQRGANAYFGDLSFTRGVGV-----GNVSRFN	366
ptToc75	IGSTTLAVGPFVKRLPYDARS-SIDAKCSLGRFLFAASSTTEEANALLPPLKLPYTSAT	360
alr2269N	-----	251
2QDZ	GSLAWTRYMALA--GQPIQWASQLGFQYSRQQ-----LLNSYQITAGAAAAAGAAAA	418
ptToc75	ATTRQIFPLLASFGPGGRPLILALKHSI---TTATPNLPRHEAKAQGIAHNIRGSSING	416
alr2269N	-----	251
2QDZ	AASGDSGVYLSNLTVPVQFSLGKQASVAPFVGADV GALKSNHPDARTIRMAGLAAGVR	478
ptToc75	RVA--SALTGTTELRVPIDIPVVQIRQDTNVVFFGDWLF AAKDS-QSSFRRKSCVGVGVR	473
alr2269N	-----	251
2QDZ	FDLPYARMSFTYSK-----PVGAQPGGAPRAPVWLYINAGLSF	516
ptToc75	KSVQGIPLKYDLCYSSDDGK-----FQSAFGLGADDFD	506

Supplemental Figure 1. Structural analysis of ptOmp85

Multiple alignment of the N-terminal part of alr2269, the crystal structure of FhaC (PDB:2QDZ) and Toc75 from *Phaeodactylum tricorutum* constructed with MAFFT v6.624b (38), manually adjusted and fed to Modeller v8.2 for constructing the homology model.

		1220	1230	1240	1250	1260	1270	1280	1290	1300	1310														
Nmen/1-797	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Ecol/1-810	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Clar/1-738	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Ddes/1-893	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Rrub/1-778	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Cpha/1-821	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Tthe/1-822	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Fnuc/1-678	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Ana1/1-833	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
AthaV/1-732	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
AthaIII/1-818	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
AthaM/1-524	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Xlae/1-468	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Ptri/1-690	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Cmer/1-840	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Cmer_Sam50/1-541	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Tpse/1-804	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Ehux/1-636	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----														
Tgon/1-1425	592	VSGVSA	LPATPP	POTPARLLV	PAGHFRV	GFAAS	GERRL	GAGTM	GEATI	ERRK	GLYVNL	GLAGSASR	GSDSSRL	PSSLA	EASDT	SHV	SEKRN	EDAP	PHRK	692					
		1320	1330	1340	1350	1360	1370	1380	1390	1400	1410														
Nmen/1-797	464	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	484				
Ecol/1-810	464	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	484				
Clar/1-738	457	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	477				
Ddes/1-893	604	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	624				
Rrub/1-778	480	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	500				
Cpha/1-821	513	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	534				
Tthe/1-822	493	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	517				
Fnuc/1-678	378	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	399				
Ana1/1-833	509	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	529				
AthaV/1-732	445	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	465				
AthaIII/1-818	502	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	523				
AthaM/1-524	197	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	218				
Xlae/1-468	166	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	186				
Ptri/1-690	393	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	410				
Cmer/1-840	433	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	455				
Cmer_Sam50/1-541	171	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	200				
Tpse/1-804	451	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	469				
Ehux/1-636	349	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	412				
Tgon/1-1425	693	ESQSW	GDKVRNAL	GMLOPP	RFA	PTTTT	TSVAMV	SADDVA	LL	PSRER	GSVL	TDP	SAHDVAKVA	GGVRFQF	SESSRQF	GFLP	PTRLA	PTW	GFEASS	FA	GV	791			
		1420	1430	1440	1450	1460	1470	1480	1490	1500	1510														
Nmen/1-797	485	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	509				
Ecol/1-810	485	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	507				
Clar/1-738	478	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	495				
Ddes/1-893	625	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	642				
Rrub/1-778	501	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	521				
Cpha/1-821	535	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	559				
Tthe/1-822	518	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	553				
Fnuc/1-678	400	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	413				
Ana1/1-833	530	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	563				
AthaV/1-732	466	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	483				
AthaIII/1-818	524	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	549				
AthaM/1-524	219	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	239				
Xlae/1-468	187	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	206				
Ptri/1-690	411	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	439				
Cmer/1-840	456	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	468				
Cmer_Sam50/1-541	201	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	241				
Tpse/1-804	470	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	502				
Ehux/1-636	413	LLSAVA	PRAAG	LLVGG	QLS	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	488				
Tgon/1-1425	792	YTPYA	AADAAA	AFA	PLTSS	GSASTR	PGSRP	SRVSRDR	PLPL	PGLAG	FSPR	PHV	TARSL	SLS	SLV	SRVSAFA	AKLV	PRPL	LRDES	SRE	SQR	CREV	KETV	S	892
		1520	1530	1540	1550	1560	1570	1580	1590	1600	1610														
Nmen/1-797	510	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	528				
Ecol/1-810	508	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	526				
Clar/1-738	496	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	510				
Ddes/1-893	643	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	657				
Rrub/1-778	522	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	536				
Cpha/1-821	560	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	577				
Tthe/1-822	554	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	568				
Fnuc/1-678	414	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	441				
Ana1/1-833	564	TNGQDR	PRVT	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	588				
AthaV/1-732	484	GNQPD	NSSLTI	GRV	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	507				
AthaIII/1-818	550	PGVEEV	PP	IWDRA	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	580				
AthaM/1-524	240	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	250				
Xlae/1-468	207	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	220				
Ptri/1-690	440	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	461				
Cmer/1-840	469	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	481				
Cmer_Sam50/1-541	242	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	255				
Tpse/1-804	503	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	511				
Ehux/1-636	489	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	496				
Tgon/1-1425	893	AALASVS																							

Supplemental Figure 2. Alignment of Omp85 sequences for the calculation of the phylogenetic tree presented in Figure 1B

Representation of the alignment of all Omp85 sequences used in the phylogenetic analysis (Fig. 1B) created with JalView (Waterhouse, A.M., Procter, J.B., Martin, D.M.A, Clamp, M. and Barton, G. J. (2009) *Bioinformatics* 25, 1189-1191) and colored with the "Clustal-X" color scheme.

A

>gi|30687572|ref|NP_189102.2| PL-SP1 [Arabidopsis thaliana]
MMVMISLHFSTPPLAFLKSDSNRFLKPNPNFNIQFTPKSQLLFPQRLNFNTGTNLNRRRLSCYGIKDSSETTKS
APSLDSGDGGGGDGGDDDKGEVEEKNRFLPEWLDFTSDDAQTVFVAIAVSLAFRYFIAEPRYIPSLSMYPTFDVG
DRLVAEKVSYYFRKPCANDIVIFKSPVVLQEVGYTDADVFIKRIVAKEGDLVEVHNGKLMVNGVARNEKFILEPP
GYEMTPIRVPENSVFVMGDNRNNSYDSHVWGPLPLKNIIGRSVFRYWPPNRVSGTVLEGGCAVDKQ

>estExt_gwp_gw1.C_chr_10974| Phatr2:17972 [Phaeodactylum tricornutum]
MPTISDHTHACRAANLASSPQRRMVLALLALCLSLVAPSVTAERPSTPPAVRSSVFVSSRRTKISWADASSRRKQI
LGMSDNKNDSPVDRKPASEPGLKASLRNKWEDETPASEPDAGFLEGIKRWFNSDEGREVDKTYFISLFLALLLR
TIIIEPRFIPSLSMYPTFEVGDQLAVEKVTKRIKPFYRTEVVVFQPPQAFRDIVENQYGDKSKGKEALIKRIVAVE
GDKVEIKNGKLLINDIEQEEAYTAEDAQYAFGPVRVPPENVLVLGDNRNHSLDGHIWGFLEPTKNVIGRAVAVYVW
PWRVGNNGMF

B

```

      *           20           *           40           *
A.thaliana : MMVMISLHFE STPPLAFLKSDSN-----SRFLKPNPNFNIQFTP----- : 38
P.tricornu : -MPTISDHTHACRAANLASSPQRRMVLALLALCLSLVAPSVTAERPSTPPA : 49

      60           *           80           *           100
A.thaliana : -KSQLLFPQRLNFNTGTNLNRRRLSCYGIKDSSE--TTKSAPSLDSGDGG : 85
P.tricornu : VRSSVFVSSRRTKISWADASSRRKQILGMSDNKNDSPVDRKPASEEGLKA : 99

      *           120           *           140           *
A.thaliana : GGDGGDDDKGEVEEKNRFLPEWLD--FTSD----DAQTVFVAIAVSLAFR : 129
P.tricornu : SLRNKWEDETPASEPDAGFLEGIKRWFNSDEGREVDKTYFISLFLALLLR : 149

      160           *           180           *           200
A.thaliana : YETIAEPRYIPSLSMYPTFDVGDRLVAEKVSYYFRKPCANDIVIFKSPVVL : 179
P.tricornu : ETIIIEPRFIPSLSMYPTFEVGDQLAVEKVTKRIKPFYRTEVVVFQPPQAF : 199

      *           220           *           240           *
A.thaliana : QEVGY-----TDADVFIKRIVAKEGDLVEVHNGKLMVNGVARNEKFIL : 222
P.tricornu : RDIVENQYGDKSKGKEALIKRIVAVEGDKVEIKNGKLLINDIEQEEAYTA : 249

      260           *           280           *           300
A.thaliana : EPPGYEMTPIRVPENSVFVMGDNRNNSYDSHVWGPLPLKNIIGRSVFRYW : 272
P.tricornu : EDAQYAFGPVRVPPENVLVLGDNRNHSLDGHIWGFLEPTKNVIGRAVAVYVW : 299

      *
A.thaliana : PPNRVSGTVLEGGCAVDKQ : 291
P.tricornu : PEWRVG----NGGMF---- : 310
```

Supplemental Figure 3. Identification of the putative PL-SP1 homologue in *P. tricornutum*

A. The two sequences which were compared are shown. The predicted signal peptide is shown in blue and the predicted transit peptide in red. *B.* The alignment of *A. thaliana* plastidic type I signal peptidase 1 and the respective homologue from *P. tricornutum* is shown. Note that the conserved functional domain starts at ~aa120.