

SUPPLEMENTARY FILES

Table S1: Cellular parameters of *Tisochrysis lutea* (morphology (FSC and SSC), viability (FL1 - SYTOX), and chlorophyll content (FL3) using flow cytometry analysis (Mean \pm SD of the 3 balloons). Values for SYTOX are in %, values for FL3/SSC/FSC in arbitrary unit (a.u).

	FL1 – SYTOX ALIVE				FL3			SSC		FSC		
0	98	\pm	1	134	\pm	9	52	\pm	7	165	\pm	4
0.5	95	\pm	2	139	\pm	10	49	\pm	6	165	\pm	6
1	95	\pm	1	140	\pm	9	44	\pm	2	167	\pm	5
2	94	\pm	0	134	\pm	11	49	\pm	10	168	\pm	4
3	95	\pm	3	134	\pm	11	49	\pm	11	169	\pm	4
4	95	\pm	2	134	\pm	11	44	\pm	8	170	\pm	5
6	95	\pm	2	134	\pm	11	47	\pm	10	170	\pm	2
8	94	\pm	2	134	\pm	11	52	\pm	4	169	\pm	2
10	93	\pm	2	135	\pm	10	46	\pm	5	171	\pm	3
12	96	\pm	1	136	\pm	9	44	\pm	4	171	\pm	2
14	97	\pm	1	137	\pm	11	44	\pm	6	171	\pm	2
16	97	\pm	1	137	\pm	12	44	\pm	3	171	\pm	3
18	97	\pm	1	136	\pm	13	44	\pm	3	173	\pm	4
20	96	\pm	2	136	\pm	13	46	\pm	4	173	\pm	3
22	97	\pm	1	136	\pm	13	42	\pm	6	174	\pm	3
24	97	\pm	1	137	\pm	12	42	\pm	5	175	\pm	2

Table S5: Mean ratio of atomic enrichment (AE) for pairs of FA (FA_A vs FA_B) in the neutral lipids (NL) (mean \pm SD, n=9 sampling dates t₈ to t₂₄) for the two enriched balloons (T11, T12, T1 = *Tisochrysis lutea*). If the ratio is equal to or close to 1, A and B are assumed at equilibrium, and B is synthesized quickly from A; if the ratio is below 1, the transformation of B from A is possible but slow. Finally, if the ratio is above 1, A is not a main precursor of B, which has to be synthesized by a different pathway.

Fatty acid B/Fatty acid A	Neutral lipids			
	T11		T12	
	Mean	SD	Mean	SD
18:5n-3/18:4n-3	-	-	-	-
20:5n-3/18:5n-3	3.58	1.78	3.33	1.40
22:5n-3/20:5n-3	0.92	0.14	0.64	0.12
22:6n-3/22:5n-3	0.55	0.17	0.79	0.14
22:6n-3/20:5n-3	0.49	0.11	0.50	0.11
22:6n-3/22:5n-6	0.49	0.15	0.65	0.15

Table S6: List of the potential candidate protein sequences involved in *Tisochrysis lutea* PKS synthesis pathway. The suspected function of each protein has been assumed using the NCBI Conserved Domain Database (CDD) (Marchler-Bauer et al., 2017) by identifying the role of each domain recognized in the sequence. In columns KS/KR/DH/ER are written the number of domain corresponding to these functions in the studied sequences. **ACS:** Acetyl-CoA synthetase, **A_NRPS:** Adenylation domain of the non-ribosomal peptide synthetase (**NRPS**), **Croto:** crotonase/enoyl-CoA hydratase, **EntF:** Enterobactin non-ribosomal peptide synthetase or thioesterase domain of Type I PKS, **FAAL:** Fatty Acyl-AMP ligase, **GrsT:** alpha/beta hydrolase, **HM:** hydroxymethylglutaryl-CoA synthase, **MT:** methyltransferase, **PP:** PhosphoPantetheine-binding (= “swinging arm”), **Sulf:** sulfotransferase, **Thio:** thioesterase

NAME	4 DOMAINS	Cluster	KS	KR	DH	ER	OTHER DOMAINS	SUSPECTED FUNCTION
TISO_16054	No		0	0	1	0	No	3-hydroxyacyl-ACP dehydratase
TISO_09404	No		1	0	0	0	No	β -ketoacyl-ACP synthase II
TISO_19207	No		1	0	0	0	No	3-ketoacyl-CoA synthase
TISO_09037	No		0	1	0	0	No	β -ketoacyl-ACP reductase II
TISO_04539	Yes		7	1	1	1	PP+FAAL	Supposed involved in complex lipid synthesis
TISO_06404	Yes		4	3	2	1	PP+Thio	Thioesterase
TISO_06537	Yes		4	5	4	3	PP+Sulf+EntF	Thioesterase / Sulfotransferase
TISO_08010	No		1	0	0	0	No	β -ketoacyl-ACP synthase II
TISO_08047	Yes		17	18	16	14	PP+MT+Thio	Thioesterase / Methyltransferase
TISO_11097	Yes		6	5	4	4	PP+EntF	Peptide synthesis
TISO_14040	No		1	0	0	0	No	β -ketoacyl-ACP synthase II
TISO_14962	Yes	Cluster 1	7	8	5	4	PP+MT+Thio	Methyltransferase / Thioesterase
TISO_14968	Yes	Cluster 1	2	3	2	3	PP	FA synthesis
TISO_14973	No	Cluster 1	1	0	1	0	PP	Ketoacyl-synthase for n-3 PUFA
TISO_14975	Yes	Cluster 1	1	2	1	2	PP	FA synthesis
TISO_14977	Yes	Cluster 1	4	2	2	3	PP + ACS	Acetyl-CoA synthetase / FA synthesis
TISO_15188	No		1	1	0	0	PP+GrsT+A_NRPS	Peptide synthesis
TISO_16495	Yes		6	9	5	4	PP+FAAL+Sulf+EntF+Thio	Thioesterase / Sulfotransferase
TISO_27353	Yes	Cluster 2	2	1	1	1	PP+EntF	Thioesterase / Peptide synthesis
TISO_27354	No	Cluster 2	2	0	0	0	PP+FAAL	Supposed involved in complex lipid synthesis
TISO_30977	No		5	2	2	0	PP+MT+Croto+HM	Complex lipid synthesis
TISO_37254	No	Cluster 3	1	2	0	1	PP+Sulf+EntF	Thioesterase / Sulfotransferase
TISO_37256	No	Cluster 3	1	0	0	0	No	Claisen condensation
TISO_37258	No	Cluster 3	1	0	1	0	No	Claisen condensation / Dehydratase
TISO_37259	No	Cluster 3	0	0	0	0	PP	Phosphopantetheine binding
TISO_37260	Yes	Cluster 3	7	6	5	3	PP+FAAL	Supposed involved in complex lipid synthesis
TISO_37578	No	Cluster 4	1	0	0	0	No	Claisen condensation
TISO_37579	Yes	Cluster 4	3	2	2	1	PP	FA synthesis
TISO_37581	No	Cluster 4	1	0	0	0	PP	Claisen condensation / PP-binding
TISO_37631	Yes		4	3	4	3	PP	FA synthesis

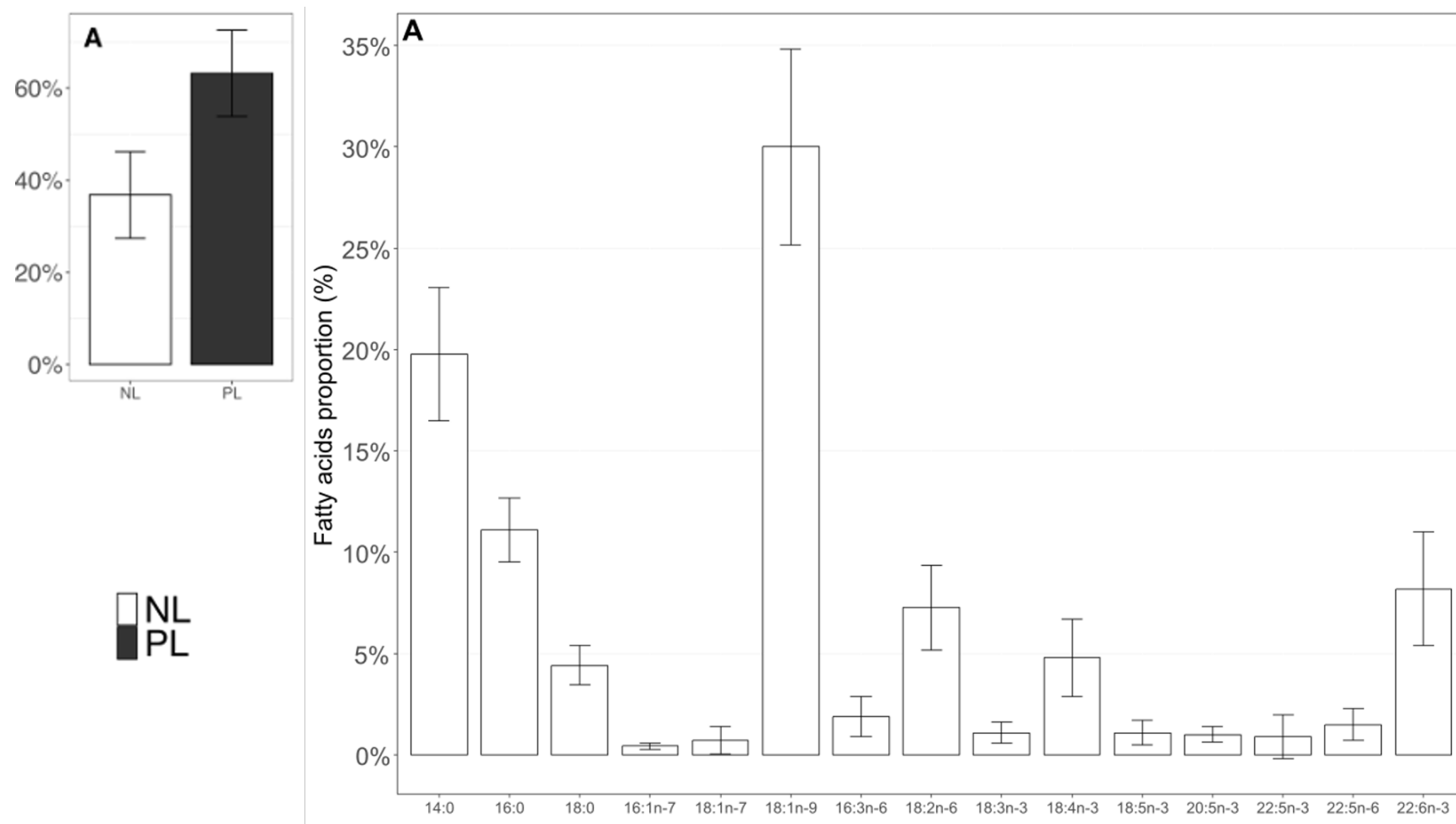


Figure S1: Proportions (%) of NL vs PL [A] and proportions (%) in average over the 24 hours of fifteen fatty acids in the NL fraction [B]

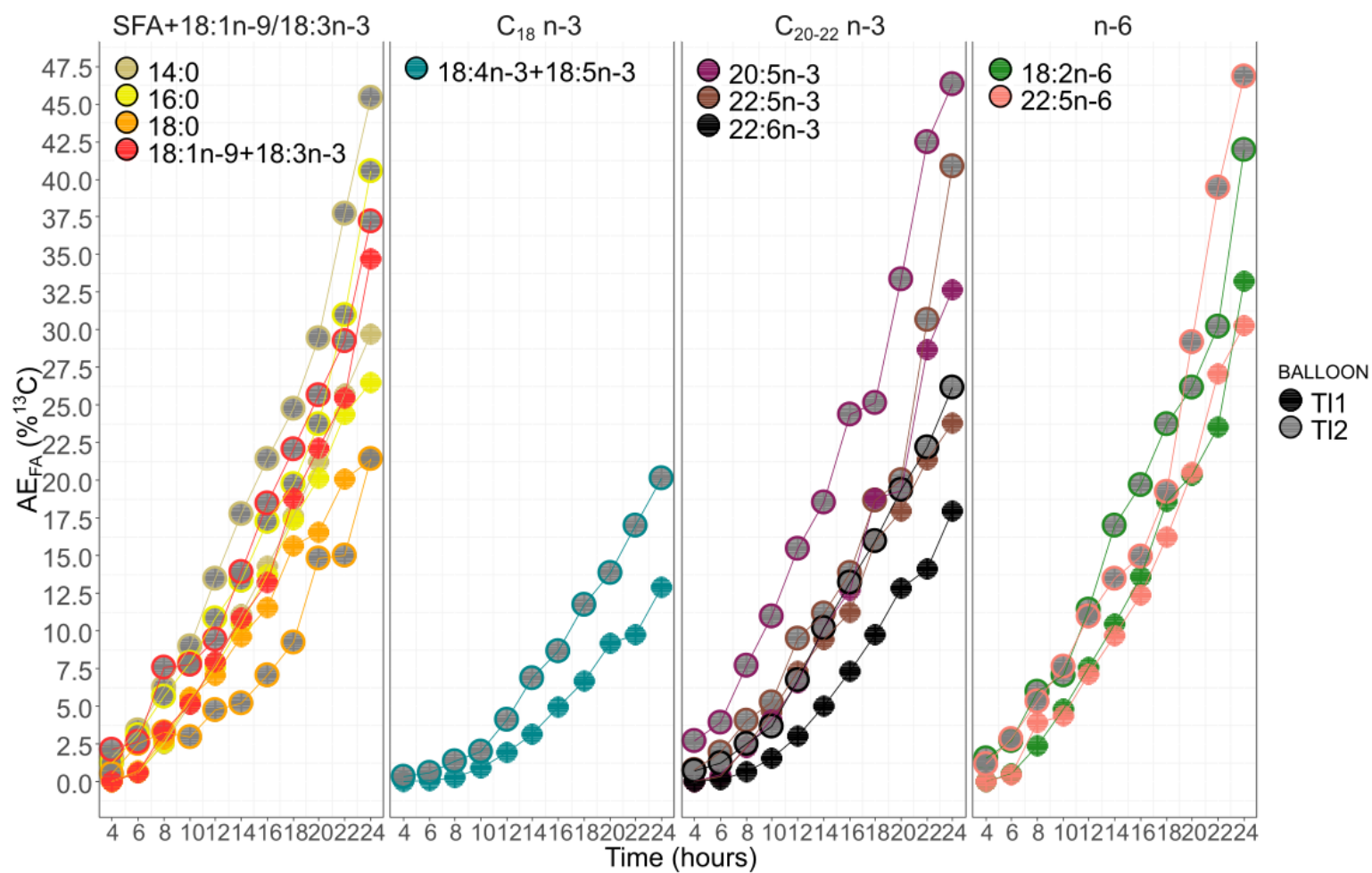


Figure S2: Atomic enrichment of 11 main fatty acids in the polar lipid (NL) fraction during a 24h ¹³C labelling experiment. T1: *Tisochrysis lutea*.