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Supplemental information

Diverse meibum lipids produced by Awat1 and Awat2 are important for stabilizing tear film and protecting the ocular surface

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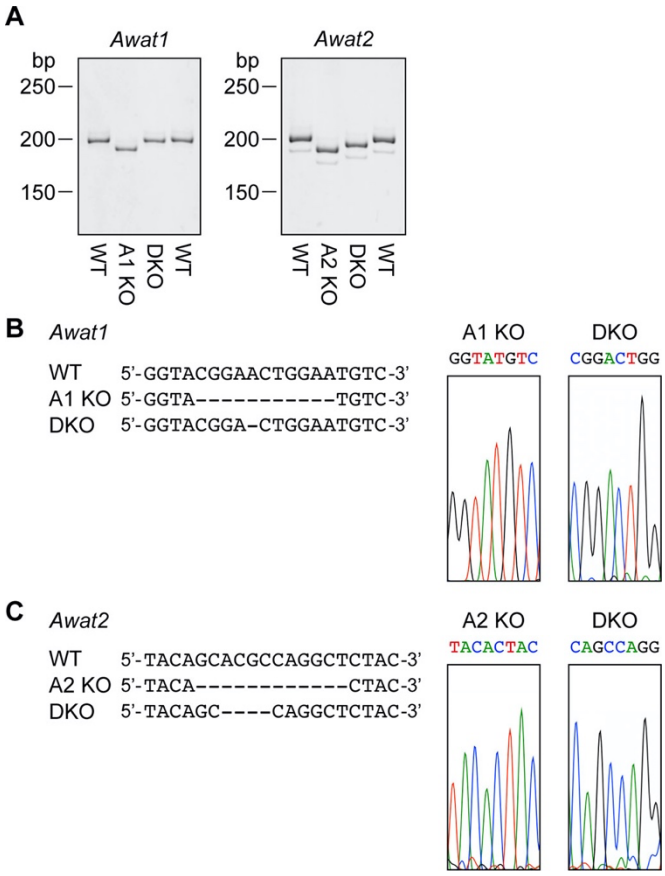

Figure S1. Gene Disruption of *Awat1* and/or *Awat2* in KO/DKO Mice. Related to Figures 1B and 1C.

(A) DNA fragments around the guide RNA target sequences in *Awat1* and *Awat2* were amplified by PCR using tail DNA from WT, *Awat1* KO, *Awat2* KO, and *Awat1 Awat2* DKO mice, separated by 16% polyacrylamide gel electrophoresis, and stained with ethidium bromide. bp, base pairs.

(B and C) Mutations in *Awat1* in *Awat1* KO and DKO mice (B) and *Awat2* in *Awat2* KO and DKO mice (C), determined by DNA sequencing. Sanger sequencing chromatograms of four bases each on the 5′ and 3′ ends of the deletion are shown.

A1 KO, *Awat1* KO; A2 KO, *Awat2* KO.

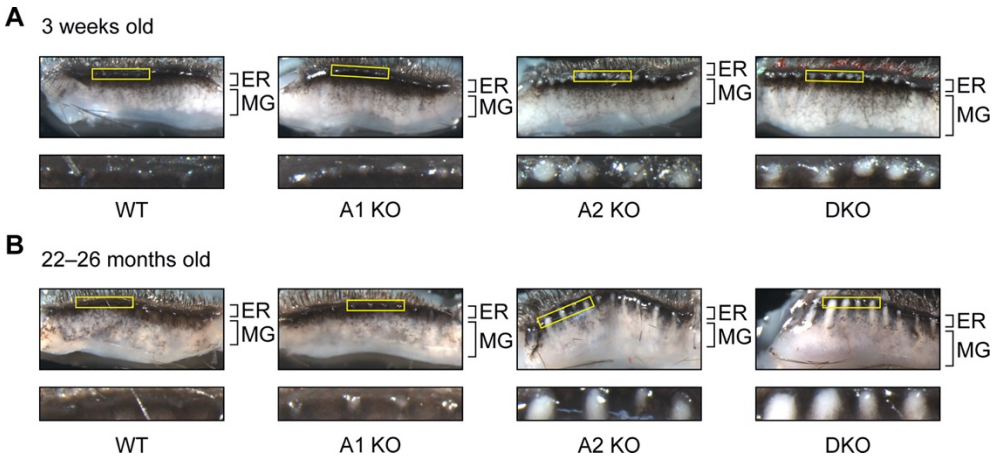

Figure S2. Meibomian Glands and Their Orifices in Young and Old Mice. Related to Figure 2B.

Upper eyelids from WT, *Awat1* KO, *Awat2* KO, and *Awat1 Awat2* DKO mice at 3 weeks old (A) and 22 or 26 months old (WT and *Awat1* KO mice, 22 months old; *Awat2* KO and DKO mice, 26 months old) (B), photographed under a light microscope. The lower images are magnifications of the yellow rectangles in the upper images and show the meibomian gland (MG) orifices.

A1 KO, *Awat1* KO; A2 KO, *Awat2* KO; ER, eyelid rim.

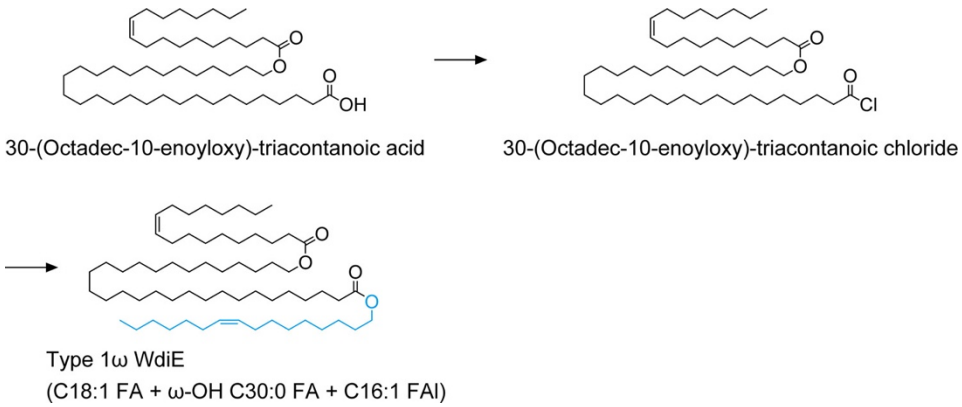

Figure S3. Synthesis scheme for type 1ω WdiE, (*O*-C18:1)-ω-OH C30:0–C16:1. Related to Figure 7.

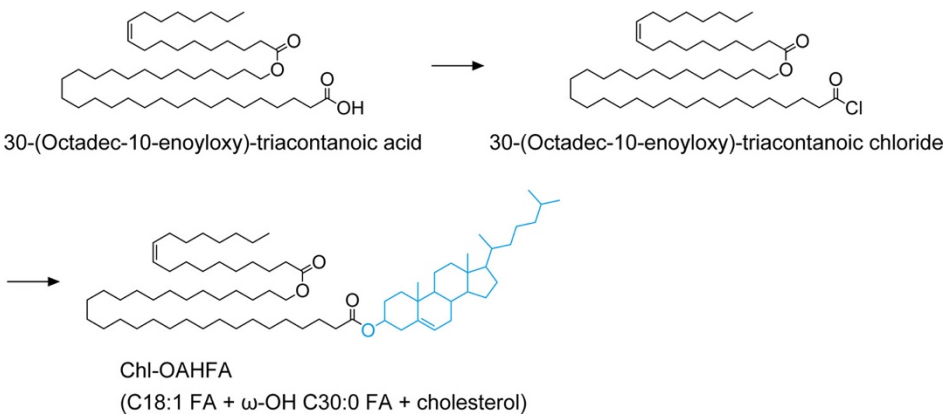

Figure S4. Synthesis scheme for Chl-OAHFA, (*O*-C18:1)–ω-OH C30:0–Chl. Related to Figure 8.

Table S1. Quantities of all WEs measured in this study. Related to Figures 4A and 4B.

FA	FAl	WT (pmol/mg)	A1 KO (pmol/mg)	A2 KO (pmol/mg)	DKO (pmol/mg)
C16:1	C16:1	ND	ND	ND	ND
C16:1	C17:1	ND	ND	ND	ND
C16:1	C18:1	ND	ND	ND	ND
C16:1	C19:1	ND	ND	ND	ND
C16:1	C20:1	0.09 ± 0.03	0.11 ± 0.06	0.13 ± 0.10	0.11 ± 0.11
C16:1	C21:1	ND	ND	ND	ND
C16:1	C22:1	ND	ND	ND	ND
C16:1	C23:1	ND	ND	ND	ND
C16:1	C24:1	0.42 ± 0.28	0.52 ± 0.45	0.13 ± 0.12	0.13 ± 0.10
C16:1	C25:1	ND	ND	ND	ND
C16:1	C26:1	6.67 ± 3.94	6.25 ± 6.36	0.10 ± 0.12	0.03 ± 0.02
C16:1	C27:1	0.42 ± 0.17	0.41 ± 0.31	0.00 ± 0.00	0.00 ± 0.00
C16:1	C28:1	8.37 ± 3.74	6.56 ± 6.08	0.04 ± 0.09	0.01 ± 0.02
C16:1	C29:1	0.63 ± 0.27	0.45 ± 0.46	0.00 ± 0.00	0.00 ± 0.00
C16:1	C30:1	26.71 ± 13.19	19.89 ± 18.23	0.04 ± 0.07	0.00 ± 0.01
C16:1	C31:1	1.82 ± 0.87	0.66 ± 0.51	0.00 ± 0.00	0.00 ± 0.00
C16:1	C32:1	54.18 ± 27.87	32.70 ± 33.74	0.20 ± 0.25	0.07 ± 0.08
C16:1	C33:1	2.70 ± 1.63	1.84 ± 2.14	0.00 ± 0.01	0.00 ± 0.00
C16:1	C34:1	7.63 ± 5.05	4.34 ± 5.58	0.02 ± 0.02	0.03 ± 0.03
C16:1	C35:1	0.28 ± 0.28	0.25 ± 0.29	0.03 ± 0.04	0.00 ± 0.00
C16:1	C36:1	0.08 ± 0.09	0.02 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
C16:1	C16:0	0.02 ± 0.03	0.03 ± 0.01	0.07 ± 0.12	0.03 ± 0.04
C16:1	C17:0	ND	ND	ND	ND
C16:1	C18:0	0.07 ± 0.07	0.12 ± 0.10	0.16 ± 0.10	0.14 ± 0.12
C16:1	C19:0	ND	ND	ND	ND
C16:1	C20:0	0.17 ± 0.16	0.17 ± 0.16	0.25 ± 0.14	0.16 ± 0.07
C16:1	C21:0	0.18 ± 0.23	0.18 ± 0.19	0.02 ± 0.03	0.02 ± 0.03
C16:1	C22:0	1.51 ± 2.74	0.36 ± 0.35	0.18 ± 0.26	0.06 ± 0.06
C16:1	C23:0	1.42 ± 2.07	0.60 ± 0.55	0.17 ± 0.25	0.06 ± 0.04
C16:1	C24:0	24.01 ± 12.26	21.29 ± 14.47	0.46 ± 0.56	0.30 ± 0.18
C16:1	C25:0	32.58 ± 14.77	34.34 ± 25.50	0.17 ± 0.21	0.07 ± 0.04

C16:1	C26:0	348.86 ± 144.37	261.96 ± 222.25	1.53 ± 1.79	0.31 ± 0.24
C16:1	C27:0	146.57 ± 47.58	100.79 ± 70.35	0.43 ± 0.68	0.14 ± 0.11
C16:1	C28:0	43.95 ± 17.31	26.26 ± 20.48	0.44 ± 0.44	0.18 ± 0.16
C16:1	C29:0	19.89 ± 7.01	11.42 ± 7.95	0.10 ± 0.09	0.02 ± 0.02
C16:1	C30:0	10.73 ± 5.19	6.50 ± 6.44	0.14 ± 0.11	0.06 ± 0.05
C16:1	C31:0	6.85 ± 3.82	3.69 ± 3.58	0.04 ± 0.04	0.04 ± 0.04
C16:1	C32:0	1.77 ± 1.43	1.12 ± 1.29	0.04 ± 0.05	0.04 ± 0.04
C16:1	C33:0	0.64 ± 0.39	0.47 ± 0.57	0.00 ± 0.01	0.01 ± 0.01
C16:1	C34:0	ND	ND	ND	ND
C16:1	C35:0	ND	ND	ND	ND
C16:1	C36:0	ND	ND	ND	ND
C16:2	C26:0	0.96 ± 0.49	0.88 ± 0.67	0.10 ± 0.02	0.07 ± 0.04
C18:2	C26:0	7.15 ± 2.75	4.32 ± 4.09	0.09 ± 0.06	0.07 ± 0.03
C16:1	C26:0	330.65 ± 121.26	234.02 ± 212.13	1.70 ± 1.58	0.73 ± 0.47
C17:1	C26:0	7.75 ± 3.29	4.89 ± 4.23	0.07 ± 0.08	0.02 ± 0.00
C18:1	C26:0	149.93 ± 52.51	80.66 ± 64.86	1.89 ± 1.47	1.18 ± 0.80
C19:1	C26:0	0.45 ± 0.17	0.21 ± 0.17	0.03 ± 0.01	0.02 ± 0.00
C20:1	C26:0	0.63 ± 0.44	0.31 ± 0.26	0.06 ± 0.02	0.09 ± 0.06
C21:1	C26:0	ND	ND	ND	ND
C22:1	C26:0	0.14 ± 0.10	0.07 ± 0.07	0.05 ± 0.03	0.11 ± 0.08
C23:1	C26:0	ND	ND	ND	ND
C24:1	C26:0	0.08 ± 0.04	0.07 ± 0.05	0.18 ± 0.10	0.36 ± 0.20
C25:1	C26:0	ND	ND	ND	ND
C26:1	C26:0	ND	ND	ND	ND
C16:0	C26:0	ND	ND	ND	ND
C17:0	C26:0	ND	ND	ND	ND
C18:0	C26:0	ND	ND	ND	ND
C19:0	C26:0	ND	ND	ND	ND
C20:0	C26:0	ND	ND	ND	ND
C21:0	C26:0	0.28 ± 0.03	0.16 ± 0.08	0.10 ± 0.03	0.07 ± 0.04
C22:0	C26:0	ND	ND	ND	ND
C23:0	C26:0	0.11 ± 0.02	0.07 ± 0.04	0.07 ± 0.02	0.06 ± 0.02
C24:0	C26:0	ND	ND	ND	ND
C25:0	C26:0	ND	ND	ND	ND

C26:0	C26:0	ND	ND	ND	ND

Values represent the means ± standard deviations (n = 5). ND, not detected.

Table S2. Quantities of all OAHFAs measured in this study. Related to Figures 5A and 5B.

FA	ω-OH FA	WT (×10^5 peak area/mg)	A1 KO (×10^5 peak area/mg)	A2 KO (×10^5 peak area/mg)	DKO (×10^5 peak area/mg)
C16:1	C16:1	ND	ND	ND	ND
C16:1	C17:1	ND	ND	ND	ND
C16:1	C18:1	ND	ND	ND	ND
C16:1	C19:1	ND	ND	ND	ND
C16:1	C20:1	ND	ND	ND	ND
C16:1	C21:1	ND	ND	ND	ND
C16:1	C22:1	ND	ND	ND	ND
C16:1	C23:1	ND	ND	ND	ND
C16:1	C24:1	0.14 ± 0.05	0.40 ± 0.12	0.01 ± 0.01	0.00 ± 0.00
C16:1	C25:1	ND	ND	ND	ND
C16:1	C26:1	0.12 ± 0.02	0.17 ± 0.12	0.05 ± 0.04	0.05 ± 0.05
C16:1	C27:1	ND	ND	ND	ND
C16:1	C28:1	ND	ND	ND	ND
C16:1	C29:1	ND	ND	ND	ND
C16:1	C30:1	0.67 ± 0.21	0.12 ± 0.10	0.44 ± 0.14	0.13 ± 0.11
C16:1	C31:1	0.39 ± 0.29	0.04 ± 0.04	0.08 ± 0.10	0.07 ± 0.02
C16:1	C32:1	13.62 ± 2.20	2.03 ± 0.39	9.96 ± 2.08	2.03 ± 0.57
C16:1	C33:1	7.70 ± 0.67	2.24 ± 0.62	3.32 ± 0.84	0.69 ± 0.18
C16:1	C34:1	24.24 ± 2.38	4.96 ± 1.23	19.23 ± 4.75	5.76 ± 2.27
C16:1	C35:1	14.66 ± 1.40	7.32 ± 1.90	4.09 ± 0.80	1.19 ± 0.26
C16:1	C36:1	4.04 ± 0.23	0.02 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
C16:1	C16:0	ND	ND	ND	ND
C16:1	C17:0	ND	ND	ND	ND
C16:1	C18:0	ND	ND	ND	ND
C16:1	C19:0	ND	ND	ND	ND
C16:1	C20:0	ND	ND	ND	ND
C16:1	C21:0	ND	ND	ND	ND
C16:1	C22:0	0.10 ± 0.04	0.21 ± 0.12	0.03 ± 0.02	0.01 ± 0.01
C16:1	C23:0	0.18 ± 0.06	0.25 ± 0.12	0.03 ± 0.06	0.01 ± 0.02
C16:1	C24:0	0.36 ± 0.13	0.55 ± 0.12	0.18 ± 0.05	0.21 ± 0.07
C16:1	C25:0	ND	ND	ND	ND

C16:1	C26:0	0.27 ± 0.14	0.25 ± 0.07	0.44 ± 0.11	0.78 ± 0.28
C16:1	C27:0	0.20 ± 0.04	0.07 ± 0.06	0.02 ± 0.01	0.02 ± 0.01
C16:1	C28:0	0.01 ± 0.01	0.01 ± 0.01	0.08 ± 0.10	0.20 ± 0.18
C16:1	C29:0	ND	ND	ND	ND
C16:1	C30:0	0.32 ± 0.05	0.16 ± 0.07	0.23 ± 0.09	0.34 ± 0.16
C16:1	C31:0	0.39 ± 0.07	0.02 ± 0.02	0.09 ± 0.07	0.09 ± 0.05
C16:1	C32:0	0.39 ± 0.12	0.14 ± 0.11	0.33 ± 0.15	0.34 ± 0.19
C16:1	C33:0	1.35 ± 0.26	0.34 ± 0.18	0.26 ± 0.05	0.17 ± 0.08
C16:1	C34:0	ND	ND	ND	ND
C16:1	C35:0	0.21 ± 0.07	0.09 ± 0.07	0.00 ± 0.00	0.02 ± 0.02
C16:1	C36:0	ND	ND	ND	ND
C16:2	C34:1	0.59 ± 0.36	0.03 ± 0.04	0.46 ± 0.32	0.03 ± 0.04
C18:2	C34:1	6.72 ± 3.60	3.93 ± 1.20	35.41 ± 16.75	35.63 ± 6.10
C16:1	C34:1	18.88 ± 5.78	2.95 ± 1.32	20.58 ± 9.28	7.02 ± 2.10
C17:1	C34:1	0.91 ± 0.79	0.10 ± 0.07	1.27 ± 0.88	0.37 ± 0.20
C18:1	C34:1	11.16 ± 2.87	2.67 ± 0.38	47.81 ± 20.55	26.12 ± 6.67
C19:1	C34:1	ND	ND	ND	ND
C20:1	C34:1	0.08 ± 0.05	0.03 ± 0.02	1.27 ± 0.91	0.91 ± 0.40
C21:1	C34:1	ND	ND	ND	ND
C22:1	C34:1	0.09 ± 0.03	0.01 ± 0.01	0.67 ± 0.32	0.33 ± 0.23
C23:1	C34:1	ND	ND	ND	ND
C24:1	C34:1	ND	ND	ND	ND
C25:1	C34:1	ND	ND	ND	ND
C26:1	C34:1	0.03 ± 0.02	0.01 ± 0.01	0.35 ± 0.35	0.08 ± 0.11
C27:1	C34:1	ND	ND	ND	ND
C28:1	C34:1	ND	ND	ND	ND
C29:1	C34:1	ND	ND	ND	ND
C30:1	C34:1	ND	ND	ND	ND
C31:1	C34:1	ND	ND	ND	ND
C32:1	C34:1	ND	ND	ND	ND
C33:1	C34:1	ND	ND	ND	ND
C34:1	C34:1	ND	ND	ND	ND
C35:1	C34:1	ND	ND	ND	ND
C36:1	C34:1	ND	ND	ND	ND
C16:0	C34:1	2.17 ± 0.96	0.81 ± 0.47	11.95 ± 5.07	5.14 ± 1.27

C17:0	C34:1	1.22 ± 0.36	0.08 ± 0.07	0.64 ± 0.14	0.36 ± 0.09
C18:0	C34:1	0.10 ± 0.11	0.15 ± 0.05	5.89 ± 3.04	2.15 ± 1.29
C19:0	C34:1	0.02 ± 0.01	ND	0.29 ± 0.27	0.08 ± 0.03
C20:0	C34:1	0.01 ± 0.01	0.01 ± 0.00	0.34 ± 0.21	0.08 ± 0.06
C21:0	C34:1	ND	ND	ND	ND
C22:0	C34:1	ND	ND	ND	ND
C23:0	C34:1	ND	ND	ND	ND
C24:0	C34:1	ND	ND	ND	ND
C25:0	C34:1	ND	ND	ND	ND
C26:0	C34:1	ND	ND	ND	ND
C27:0	C34:1	ND	ND	ND	ND
C28:0	C34:1	ND	ND	ND	ND
C29:0	C34:1	ND	ND	ND	ND
C30:0	C34:1	ND	ND	ND	ND
C31:0	C34:1	ND	ND	ND	ND
C32:0	C34:1	ND	ND	ND	ND
C33:0	C34:1	ND	ND	ND	ND
C34:0	C34:1	ND	ND	ND	ND
C35:0	C34:1	ND	ND	ND	ND
C36:0	C34:1	ND	ND	ND	ND

Values represent the means ± standard deviations (n = 4). ND, not detected.

Table S7. Quantities of all Chl-OAHFAs measured in this study. Related to Figure 8B.

OAHFA	WT (×10^5 peak area/mg)	A1 KO (×10^5 peak area/mg)	A2 KO (×10^5 peak area/mg)	DKO (×10^5 peak area/mg)
C32:3	ND	ND	ND	ND
C33:3	ND	ND	ND	ND
C34:3	ND	ND	ND	ND
C35:3	ND	ND	ND	ND
C36:3	ND	ND	ND	ND
C37:3	ND	ND	ND	ND
C38:3	ND	ND	ND	ND
C39:3	ND	ND	ND	ND
C40:3	ND	ND	ND	ND
C41:3	ND	ND	ND	ND
C42:3	ND	ND	ND	ND
C43:3	ND	ND	ND	ND
C44:3	ND	ND	ND	ND
C45:3	ND	ND	ND	ND
C46:3	ND	ND	ND	ND
C47:3	ND	ND	ND	ND
C48:3	0.92 ± 0.33	0.10 ± 0.12	0.04 ± 0.06	0.05 ± 0.07
C49:3	0.25 ± 0.07	0.07 ± 0.06	0.02 ± 0.01	0.02 ± 0.02
C50:3	2.10 ± 0.55	1.01 ± 0.11	0.65 ± 0.47	0.33 ± 0.38
C51:3	0.22 ± 0.08	0.12 ± 0.04	0.03 ± 0.03	0.04 ± 0.01
C52:3	1.43 ± 0.29	0.92 ± 0.13	0.75 ± 0.30	0.51 ± 0.13
C53:3	ND	ND	ND	ND
C54:3	0.54 ± 0.14	0.33 ± 0.22	0.48 ± 0.15	0.39 ± 0.06
C32:2	ND	ND	ND	ND
C33:2	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.05	0.26 ± 0.08
C34:2	ND	ND	ND	ND
C35:2	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.09	0.24 ± 0.16
C36:2	ND	ND	ND	ND
C37:2	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.07	0.17 ± 0.06
C38:2	ND	ND	ND	ND
C39:2	ND	ND	ND	ND

C40:2	ND	ND	ND	ND
C41:2	ND	ND	ND	ND
C42:2	ND	ND	ND	ND
C43:2	ND	ND	ND	ND
C44:2	ND	ND	ND	ND
C45:2	ND	ND	ND	ND
C46:2	0.54 ± 0.19	0.25 ± 0.09	0.14 ± 0.17	0.43 ± 0.19
C47:2	0.35 ± 0.19	0.04 ± 0.05	0.02 ± 0.01	0.04 ± 0.04
C48:2	5.54 ± 1.59	2.69 ± 0.26	1.85 ± 0.57	2.61 ± 0.22
C49:2	1.21 ± 0.40	0.59 ± 0.24	0.22 ± 0.04	0.60 ± 0.13
C50:2	10.53 ± 2.70	8.05 ± 0.41	5.45 ± 1.26	7.35 ± 1.21
C51:2	0.95 ± 0.33	0.84 ± 0.25	0.49 ± 0.16	0.83 ± 0.10
C52:2	6.13 ± 1.34	5.24 ± 1.53	3.54 ± 0.95	5.23 ± 0.83
C53:2	0.35 ± 0.13	0.29 ± 0.05	0.13 ± 0.04	0.29 ± 0.13
C54:2	3.24 ± 1.14	1.89 ± 0.88	1.98 ± 0.52	4.17 ± 0.44

Values represent the means ± standard deviations (n = 4). ND, not detected.

Table S8. Quantities of all CEs measured in this study. Related to Figure 8C.

FA	WT (pmol/mg)	A1 KO (pmol/mg)	A2 KO (pmol/mg)	DKO (pmol/mg)
C16:1	22.18 ± 32.88	22.27 ± 34.49	4.07 ± 5.69	1.14 ± 2.54
C17:1	0.85 ± 1.91	1.12 ± 2.50	0.89 ± 1.98	0.46 ± 1.02
C18:1	23.31± 17.68	16.51 ± 21.67	ND	2.17 ± 3.02
C19:1	ND	0.64 ± 1.43	ND	ND
C20:1	21.52± 15.05	8.07 ± 11.41	ND	1.74 ± 3.88
C21:1	3.71 ± 5.14	0.80 ± 1.79	0.64 ± 1.44	ND
C22:1	65.41± 22.55	55.12 ± 75.31	13.77 ± 13.99	6.63 ± 9.76
C23:1	ND	ND	ND	ND
C24:1	69.01± 24.86	67.33 ± 91.29	35.93 ± 21.57	40.41 ± 22.28
C25:1	ND	ND	1.65 ± 2.60	0.81 ± 1.81
C26:1	29.48± 20.23	25.45 ± 42.12	19.55 ± 17.15	47.85 ± 48.83
C27:1	ND	0.70 ± 1.58	0.91 ± 2.03	4.82 ± 8.58
C28:1	26.04 ± 27.98	27.26 ± 41.00	43.61 ± 22.48	49.36 ± 39.70
C29:1	1.21 ± 2.70	ND	0.82 ± 1.83	1.91 ± 2.75
C30:1	45.56 ± 40.67	24.33 ± 29.95	41.08 ± 16.82	87.86 ± 76.86
C31:1	ND	0.44 ± 0.99	1.90 ± 4.26	5.46 ± 10.35
C32:1	34.94 ± 50.99	19.74 ± 31.57	37.32 ± 28.30	78.49 ± 51.68
C33:1	6.24 ± 10.20	2.26 ± 3.37	3.01 ± 4.66	7.14 ± 7.11
C34:1	12.23 ± 15.49	0.82 ± 1.84	10.89 ± 7.55	15.45 ± 15.14
C35:1	0.69 ± 1.54	0.70 ± 1.57	0.89 ± 1.22	2.20 ± 2.26
C36:1	ND	0.92 ± 2.05	ND	ND
C16:0	127.67 ± 40.32	56.93 ± 72.17	75.48 ± 32.08	39.48 ± 32.54
C17:0	97.57 ± 23.59	48.53 ± 34.68	29.48 ± 27.62	21.80 ± 8.49
C18:0	60.36 ± 35.87	19.06 ± 38.81	12.69 ± 6.62	36.12 ± 24.53
C19:0	106.41 ± 20.52	54.38 ± 46.04	50.73 ± 22.57	36.13 ± 35.33
C20:0	456.30 ± 178.01	234.26 ± 152.02	296.93 ± 119.79	250.95 ± 89.87
C21:0	400.19 ± 234.69	213.39 ± 131.97	264.36 ±147.67	209.08 ± 72.41
C22:0	291.23 ± 258.19	141.82 ± 112.36	170.76 ± 112.92	114.98 ± 69.25
C23:0	208.05 ± 122.08	102.86 ± 109.70	140.15 ± 87.56	93.53 ± 27.23
C24:0	309.64 ± 176.15	166.68 ± 176.88	194.46 ± 62.38	171.31 ± 82.26
C25:0	465.79 ± 230.73	344.12 ± 375.26	274.93 ± 88.59	262.12 ± 117.22
C26:0	447.40 ± 290.43	262.46 ± 219.09	285.13 ± 102.06	294.55 ± 124.56

C27:0	252.27 ± 138.95	165.83 ± 153.67	163.98 ± 61.53	162.95 ± 86.84
C28:0	47.96 ± 52.54	30.56 ± 39.70	34.95 ± 41.68	39.81 ± 27.95
C29:0	42.21 ± 38.81	25.34 ± 26.43	20.34 ± 11.32	23.04 ± 27.12
C30:0	4.61 ± 6.56	4.58 ± 10.25	6.49 ± 8.24	1.82 ± 3.51
C31:0	5.36 ± 5.21	3.11 ± 5.49	3.74 ± 3.83	3.56 ± 3.47
C32:0	1.26 ± 2.09	ND	0.55 ± 1.22	ND
C33:0	ND	ND	0.42 ± 0.93	ND
C34:0	ND	ND	ND	ND
C35:0	ND	0.48 ± 1.08	1.68 ± 2.31	2.40 ± 5.36
C36:0	ND	ND	ND	0.27 ± 0.60

Values represent the means ± standard deviations (n = 5). ND, not detected.

Table S9. List of oligonucleotides used. Related to Figures 1B–E.

Oligonucleotide name	Sequence
Awat1-F1	5'-CACCGGTTCAGACTGGGTACGGAAC-3'
Awat1-R1	5'-AAACGTTCCGTACCCAGTCTGAACC-3'
Awat1-F2	5'-GGGTGAATAAGAGACCTGGGGTGG-3'
Awat1-R2	5'-TCCCTAGTCATTTGATATAATGTCTGC-3'
Awat1-F3	5'-GGGATCCAAACGCTAGACAGCTGAAACCATTC-3'
Awat1-R3	5'-CACATTGTAGCTACTTCTCTGAAGC-3'
Awat1-F4	5'-GGAGAAACAGAGGTATATGACCAGG-3'
Awat1-R4	5'-TCACAAGAATATCAGCTTCTGGGTGTTGG-3'
Awat2-F1	5'-CACCGGAGTGCAGATACAGCACGCC-3'
Awat2-R1	5'-AAACGGCGTGCTGTATCTGCACTCC-3'
Awat2-F2	5'-GGCTTTTTCTGCACACTAGGGTCGTG-3'
Awat2-R2	5'-CCTTACCCATGTTTAAGGGCTGTGC-3'
Awat2-F3	5'-GGGATCCCAAGATTGTCTCAGGTGCTAACCGC-3'
Awat2-R3	5'-AGTAAATACACACACACATGCATGC-3'
Awat2-F4	5'-GGAGAGACAGACCTCTATGACCAGC-3'
Awat2-R4	5'-TCAAACTATCACCAGCTCCTGGGTC-3'
Far1-F	5'-GATAATGTCAATATGTTAATGAACC-3'
Far1-R	5'-TCAGTATCTCATAGTGCTGGATGCTCG-3'
Far2-F	5'-TCCATGCTGGAGTATTTCATCAACC-3'
Far2-R	5'-TTGAACAAGGGACAAATGAAGAACC-3'
Soat1-F	5'-GCCGTCTTCGCCCTGTCGGCTGTGG-3'
Soat1-R	5'-CTAAAACACGTACCGACAAGTCCAGG -3'
Cyp4f39-F	5'-AGCATCTACGGGACCCACCACAACC-3'
Cyp4f39-R	5'-TGAGGGTAGAGGCTCTACATTGAGC-3'
Hprt-F	5'-GCTGACCTGCTGGATTACATTAAAG-3'
Hprt-R	5'-CTTAACCATTTTGGGGCTGTACTGC-3'

Table S10. Selected *m/z* values for WEs in MS/MS analysis. Related to Figures 4A and 4B.

FA	FAl	Precursor ion (Q1) $[M + H]^+$	Product ion (Q3) $[FA - OH]^+$
C16:1	C16:1	477.5	237.1
C16:1	C17:1	491.5	237.1
C16:1	C18:1	505.5	237.1
C16:1	C19:1	519.5	237.1
C16:1	C20:1	533.5	237.1
C16:1	C21:1	547.5	237.1
C16:1	C22:1	561.6	237.1
C16:1	C23:1	575.6	237.1
C16:1	C24:1	589.6	237.1
C16:1	C25:1	603.6	237.1
C16:1	C26:1	617.6	237.1
C16:1	C27:1	631.6	237.1
C16:1	C28:1	645.6	237.1
C16:1	C29:1	659.7	237.1
C16:1	C30:1	673.7	237.1
C16:1	C31:1	687.7	237.1
C16:1	C32:1	701.7	237.1
C16:1	C33:1	715.7	237.1
C16:1	C34:1	729.7	237.1
C16:1	C35:1	743.8	237.1
C16:1	C36:1	757.8	237.1
C16:1	C16:0	479.5	237.1
C16:1	C17:0	493.5	237.1
C16:1	C18:0	507.5	237.1
C16:1	C19:0	521.5	237.1
C16:1	C20:0	535.5	237.1
C16:1	C21:0	549.6	237.1
C16:1	C22:0	563.6	237.1
C16:1	C23:0	577.6	237.1
C16:1	C24:0	591.6	237.1
C16:1	C25:0	605.6	237.1

C16:1	C26:0	619.6	237.1
C16:1	C27:0	633.6	237.1
C16:1	C28:0	647.7	237.1
C16:1	C29:0	661.7	237.1
C16:1	C30:0	675.7	237.1
C16:1	C31:0	689.7	237.1
C16:1	C32:0	703.7	237.1
C16:1	C33:0	717.7	237.1
C16:1	C34:0	731.8	237.1
C16:1	C35:0	745.8	237.1
C16:1	C36:0	759.8	237.1
C16:2	C26:0	617.6	235.1
C18:2	C26:0	645.6	263.1
C17:1	C26:0	633.6	251.1
C18:1	C26:0	647.7	265.1
C19:1	C26:0	661.7	279.1
C20:1	C26:0	675.7	293.1
C21:1	C26:0	689.7	307.1
C22:1	C26:0	703.7	321.2
C23:1	C26:0	717.7	335.2
C24:1	C26:0	731.8	349.2
C25:1	C26:0	745.8	363.2
C26:1	C26:0	759.8	377.2
C16:0	C26:0	621.6	239.1
C17:0	C26:0	635.7	253.1
C18:0	C26:0	649.7	267.1
C19:0	C26:0	663.7	281.1
C20:0	C26:0	677.7	295.1
C21:0	C26:0	691.7	309.2
C22:0	C26:0	705.7	323.2
C23:0	C26:0	719.8	337.2
C24:0	C26:0	733.8	351.2
C25:0	C26:0	747.8	365.2
C26:0	C26:0	761.8	379.2

Table S11. Selected *m/z* values for OAHFAs in MS/MS analysis. Related to Figures 5A and 5B.

FA	ω-OH FA	Precursor ion (Q1) $[M + AMPP]^+$	Product ion (Q3) $[M + AMPP - (FA - OH)]^+$
C16:1	C16:1	673.5	419.0
C16:1	C17:1	687.5	433.0
C16:1	C18:1	701.5	447.0
C16:1	C19:1	715.5	461.0
C16:1	C20:1	729.5	475.1
C16:1	C21:1	743.5	489.1
C16:1	C22:1	757.6	503.1
C16:1	C23:1	771.6	517.1
C16:1	C24:1	785.6	531.1
C16:1	C25:1	799.6	545.1
C16:1	C26:1	813.6	559.1
C16:1	C27:1	827.6	573.2
C16:1	C28:1	841.6	587.2
C16:1	C29:1	855.7	601.2
C16:1	C30:1	869.7	615.2
C16:1	C31:1	883.7	629.2
C16:1	C32:1	897.7	643.2
C16:1	C33:1	911.7	657.3
C16:1	C34:1	925.7	671.3
C16:1	C35:1	939.8	685.3
C16:1	C36:1	953.8	699.3
C16:1	C16:0	675.5	421.0
C16:1	C17:0	689.5	435.0
C16:1	C18:0	703.5	449.0
C16:1	C19:0	717.5	463.1
C16:1	C20:0	731.5	477.1
C16:1	C21:0	745.6	491.1
C16:1	C22:0	759.6	505.1
C16:1	C23:0	773.6	519.1
C16:1	C24:0	787.6	533.1
C16:1	C25:0	801.6	547.1

C16:1	C26:0	815.6	561.2
C16:1	C27:0	829.6	575.2
C16:1	C28:0	843.7	589.2
C16:1	C29:0	857.7	603.2
C16:1	C30:0	871.7	617.2
C16:1	C31:0	885.7	631.2
C16:1	C32:0	899.7	645.3
C16:1	C33:0	913.7	659.3
C16:1	C34:0	927.8	673.3
C16:1	C35:0	941.8	687.3
C16:1	C36:0	955.8	701.3
C16:2	C34:1	923.7	671.3
C18:2	C34:1	951.7	671.3
C17:1	C34:1	939.7	671.3
C18:1	C34:1	953.7	671.3
C19:1	C34:1	967.7	671.3
C20:1	C34:1	981.8	671.3
C21:1	C34:1	995.8	671.3
C22:1	C34:1	1009.8	671.3
C23:1	C34:1	1023.8	671.3
C24:1	C34:1	1037.8	671.3
C25:1	C34:1	1051.8	671.3
C26:1	C34:1	1065.9	671.3
C27:1	C34:1	1079.9	671.3
C28:1	C34:1	1093.9	671.3
C29:1	C34:1	1107.9	671.3
C30:1	C34:1	1121.9	671.3
C31:1	C34:1	1135.9	671.3
C32:1	C34:1	1150.0	671.3
C33:1	C34:1	1164.0	671.3
C34:1	C34:1	1178.0	671.3
C35:1	C34:1	1192.0	671.3
C36:1	C34:1	1206.0	671.3
C16:0	C34:1	927.7	671.3
C17:0	C34:1	941.7	671.3

C18:0	C34:1	955.7	671.3
C19:0	C34:1	969.8	671.3
C20:0	C34:1	983.8	671.3
C21:0	C34:1	997.8	671.3
C22:0	C34:1	1011.8	671.3
C23:0	C34:1	1025.8	671.3
C24:0	C34:1	1039.8	671.3
C25:0	C34:1	1053.9	671.3
C26:0	C34:1	1067.9	671.3
C27:0	C34:1	1081.9	671.3
C28:0	C34:1	1095.9	671.3
C29:0	C34:1	1109.9	671.3
C30:0	C34:1	1123.9	671.3
C31:0	C34:1	1138.0	671.3
C32:0	C34:1	1152.0	671.3
C33:0	C34:1	1166.0	671.3
C34:0	C34:1	1180.0	671.3
C35:0	C34:1	1194.0	671.3
C36:0	C34:1	1208.0	671.3

Table S14. Selected *m/z* values for Chl-OAHFAs in MS/MS analysis. Related to Figure 8B.

OAHFA	Precursor ion (Q1) $[M + H]^+$	Product ion (Q3) $[Chl – H_2O]^+$
C32:3	873.8	369.4
C33:3	887.8	369.4
C34:3	901.9	369.4
C35:3	915.9	369.4
C36:3	929.9	369.4
C37:3	943.9	369.4
C38:3	957.9	369.4
C39:3	971.9	369.4
C40:3	986.0	369.4
C41:3	1000.0	369.4
C42:3	1014.0	369.4
C43:3	1028.0	369.4
C44:3	1042.0	369.4
C45:3	1056.0	369.4
C46:3	1070.1	369.4
C47:3	1084.1	369.4
C48:3	1098.1	369.4
C49:3	1112.1	369.4
C50:3	1126.1	369.4
C51:3	1140.1	369.4
C52:3	1154.1	369.4
C53:3	1168.2	369.4
C54:3	1182.2	369.4
C32:2	875.8	369.4
C33:2	889.9	369.4
C34:2	903.9	369.4
C35:2	917.9	369.4
C36:2	931.9	369.4
C37:2	945.9	369.4
C38:2	959.9	369.4
C39:2	974.0	369.4

C40:2	988.0	369.4
C41:2	1002.0	369.4
C42:2	1016.0	369.4
C43:2	1030.0	369.4
C44:2	1044.0	369.4
C45:2	1058.1	369.4
C46:2	1072.1	369.4
C47:2	1086.1	369.4
C48:2	1100.1	369.4
C49:2	1114.1	369.4
C50:2	1128.1	369.4
C51:2	1142.1	369.4
C52:2	1156.2	369.4
C53:2	1170.2	369.4
C54:2	1184.2	369.4

Table S15. Selected *m/z* values for CEs in MS/MS analysis. Related to Figure 8C.

FA	Precursor ion (Q1) $[M + H]^+$	Product ion (Q3) $[Chl – H_2O]^+$
C16:1	623.6	369.4
C17:1	637.6	369.4
C18:1	651.6	369.4
C19:1	665.6	369.4
C20:1	679.7	369.4
C21:1	693.7	369.4
C22:1	707.7	369.4
C23:1	721.7	369.4
C24:1	735.7	369.4
C25:1	749.7	369.4
C26:1	763.7	369.4
C27:1	777.8	369.4
C28:1	791.8	369.4
C29:1	805.8	369.4
C30:1	819.8	369.4
C31:1	833.8	369.4
C32:1	847.8	369.4
C33:1	861.9	369.4
C34:1	875.9	369.4
C35:1	889.9	369.4
C36:1	903.9	369.4
C16:0	625.6	369.4
C17:0	639.6	369.4
C18:0	653.6	369.4
C19:0	667.7	369.4
C20:0	681.7	369.4
C21:0	695.7	369.4
C22:0	709.7	369.4
C23:0	723.7	369.4
C24:0	737.7	369.4
C25:0	751.7	369.4

C26:0	765.8	369.4
C27:0	779.8	369.4
C28:0	793.8	369.4
C29:0	807.8	369.4
C30:0	821.8	369.4
C31:0	835.8	369.4
C32:0	849.9	369.4
C33:0	863.9	369.4
C34:0	877.9	369.4
C35:0	891.9	369.4
C36:0	905.9	369.4