SUPPLEMENTARY INFORMATION

The physical state of lipid substrates provides transacylation specificity for tafazzin

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Supplementary Results include 3 Tables and 7 Figures

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Supplementary Table 1. Effect of Triton X-100 on the transacylation reaction by purified tafazzin

Substrates	**PC:LPC**	**Transacylation product (nmol)**		
		Total Triton X-100 concentration (mM)		
		0.35	*0.7*	*3.5*
18:1-18:1-PC + 14:0-LPC	8:2	0.29±0.07	0.49±0.03	0.76±0.04
	2:8	2.76±0.20	3.95±0.41	0.74±0.05
18:2-18:2-PC + 14:0-LPC	8:2	0.44±0.02	0.80±0.04	7.41±0.91
	2:8	12.3±1.6	12.0±1.0	5.75±0.50

Purified tafazzin (40 μg) was added to the indicated lipid mixtures (total lipid: 100 nmol) and samples were incubated at 37 °C for 40 min. The amount of transacylation product was determined by mass spectrometry (duplicate measurements). Triton X-100 was present in the enzyme preparation at a concentration of 0.35 mM. When more Triton X-100 was added, the transacylation activity changed depending on the PC:LPC ratio. At a ratio of 8:2, where lipids formed liposomes, Triton increased transacylation activity, suggesting that tafazzin acts on micelles but not on bilayers. At a ratio of 2:8, where lipids themselves formed micelles, a low amount of Triton had no significant effect, indicating that Triton does not inhibit the enzyme. High concentration of Triton on the other hand, decreased the transacylation activity of 2:8 mixtures probably as a result of surface dilution.

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Supplementary Table 2. Transacylation reaction in liposomes prepared by extrusion

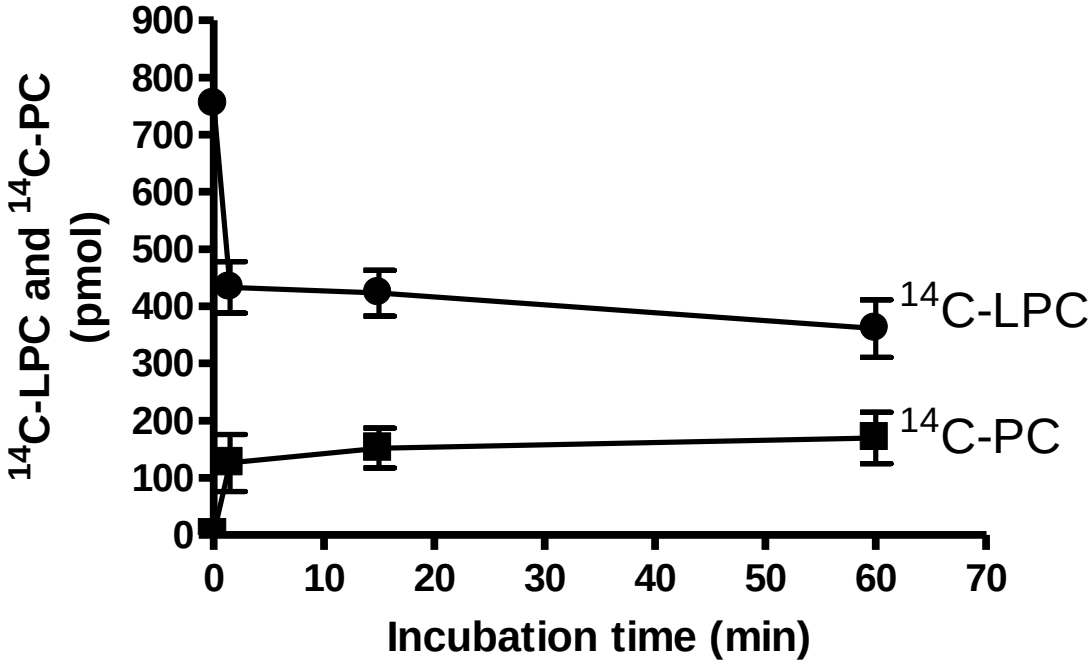
Lipids	Pore size (μm)	ULV (%)	Liposome diameter (nm)		Transacylation activity (nmol 14:0-18:2-PC)	
			Range	Mean ± s.d.	Ca^{2+} = 0	Ca^{2+} = 20 mM
PC/LPC	0.4	8	36-429	165±80	0.79±0.11	1.07±0.10
(8:2)	0.1	42	22-173	84±34	0.96±0.16	0.94±0.03
	0.03	91	16-83	41±14	1.18±0.12	1.03±0.08
CL/LPC	0.4	46	22-454	151±88	4.82±0.14	12.50±0.20
(8:2)	0.1	47	19-225	89±34	4.27±0.60	12.29±0.23
	0.03	52	24-123	67±19	4.96±0.03	11.66±0.99

Lipid mixtures (18:2-18:2-PC/14:0-LPC, 8:2 or bovine heart CL/14:0-LPC, 8:2) were hydrated in reaction buffer by repeated freezing and thawing and then passed through polycarbonate membranes with different pore sizes, using the Mini-Extruder provided by Avanti Polar Lipids (20 passes per sample). After extrusion, the size distribution of the liposomes was measured by cryo-electron microscopy. To this end, aliquots were plunge-frozen on holey carbon EM grids (Quantifoil R 2/2, supplied by SPI, West Chester, PA, USA) in liquid ethane/propane (37:63) cooled by liquid nitrogen. Samples were imaged in vitreous ice over holes by a 200-kV Philips CM 200 electron microscope equipped with an energy filter. Random images were collected and >100 vesicles were analyzed in each group. As expected, liposomes became smaller and more homogeneous when extruded through polycarbonate membranes with smaller pore sizes and, in the case of PC/LPC, the proportion of unilamellar vesicvles (ULV) increased. Transacylation activities were measured under standard conditions (100 nmol total lipid, 40 μg purified tafazzin, 37°C, 1 hour incubation, ± 20 mM $CaCl_2$) and expressed as mean values ± s.e.m. (N=4). The data demonstrate more transacylation in CL/LPC than in PC/LPC. Ca^{2+} increased transacylation in CL/LPC but not in PC/LPC. In PC/LPC, there was a small increase in transacylation when going from large multilamellar vesicles to small unilamellar vesicles ($P<0.05$).

Supplementary Table 3. CL-PC remodeling in mitochondrial model membranes

	CL : MLCL : LPC : PC 10 : 10 : 10 : 70			**CL : MLCL : LPC : PC 40 : 10 : 10 : 40**			**CL : MLCL : LPC : PC 70 : 10 : 10 : 10**		
dTAZ	-	+	+	-	+	+	-	+	+
$CaCl_2$	-	-	+	-	-	+	-	-	+
Molecular species (nmol)									
$(18:1)_4$-CL	12.9±1.1	12.6±1.0	11.6±0.6	42.3±1.9	42.5±0.3	42.0±1.3	71.0±2.3	71.4±3.6	71.5±2.7
18:1-$(18:2)_3$-CL	0±0	0±0	0.1±0	0±0	0±0	0.4±0	0±0	0±0	0.4±0.1
$(18:2)_4$-CL	0±0	0±0	1.0±0.1	0±0	0±0	3.4±0.1	0±0	0±0	1.5±0.2
$(18:1)_3$-MLCL	0.3±0.1	0.3±0.1	0.4±0.1	0.9±0.1	0.9±0.1	0.9±0	1.3±0.3	1.6±0.5	2.3±0.3
$(18:2)_3$-MLCL	6.8±0.5	7.0±0.8	6.9±0.4	6.9±1.9	6.6±1.6	3.3±1.3	7.7±0.4	7.0±0.8	4.3±0.3
18:1-LPC	21.3±2.0	16.4±1.8	13.3±1.5	14.9±3.0	12.2±3.5	3.7±1.9	12.1±0.2	10.7±1.0	6.1±0.7
18:2-LPC	2.4±0.2	7.2±0.5	11.0±0.9	1.8±0.1	3.9±0.2	15.1±1.3	0.3±0.1	0.9±0.2	6.2±0.5
$(18:1)_2$-PC	0.2±0	0.7±0.1	0.4±0.1	0.1±0	0.6±0.1	0.3±0.1	0±0	0.5±0.1	0.6±0.1
18:1-18:2-PC	4.1±0.6	5.7±1.0	7.2±1.2	2.6±0.4	3.0±0.3	6.7±0.5	0.2±0	0.4±0.1	3.4±0.4
$(18:2)_2$-PC	52.0±2.3	50.1±1.9	48.1±2.5	30.6±2.5	30.3±3.5	24.2±3.7	7.3±0.9	7.5±0.6	3.6±0.5

Membranes were produced in aqueous buffer by sonication of a mixture of $(18:1)_4$-CL, $(18:2)_3$-MLCL, 18:1-LPC, and $(18:2)_2$-PC. The molar ratio of the four lipids was varied as indicated. Each sample contained a total amount of 100 nmol lipids in 100 µl buffer. Incubations were performed in the presence (+) and absence (-) of 40 µg purified MBP-tagged Drosophila tafazzin (dTAZ) for 60 min at 37°C. $CaCl_2$ (20 mM) was added where indicated. Lipids were analyzed by mass spectrometry in positive and negative ion mode. Data are mean values with ranges of two experiments.



Supplementary Figure 1. Transacylation of ^{14}C-LPC to ^{14}C-PC by endogenous phospholipids in isolated mitochondria. Mitochondria were isolated from Sf9 cells expressing Drosophila tafazzin. An aliquot of mitochondria, corresponding to 50 μg protein, was incubated with 1 nmol of ^{14}C-LPC in a medium containing 50 mM Tris (pH 7.4), 0.5 mM EDTA, and 10 mM β-mercaptoethanol at 37°C. The amounts of ^{14}C-PC and ^{14}C-LPC were measured at different time points by liquid scintillation counting following separation by thin-layer chromatography. Data are means with standard error of mean of three experiments. They show that only a portion of ^{14}C-LPC was converted to ^{14}C-PC after the reaction reached its equilibrium.

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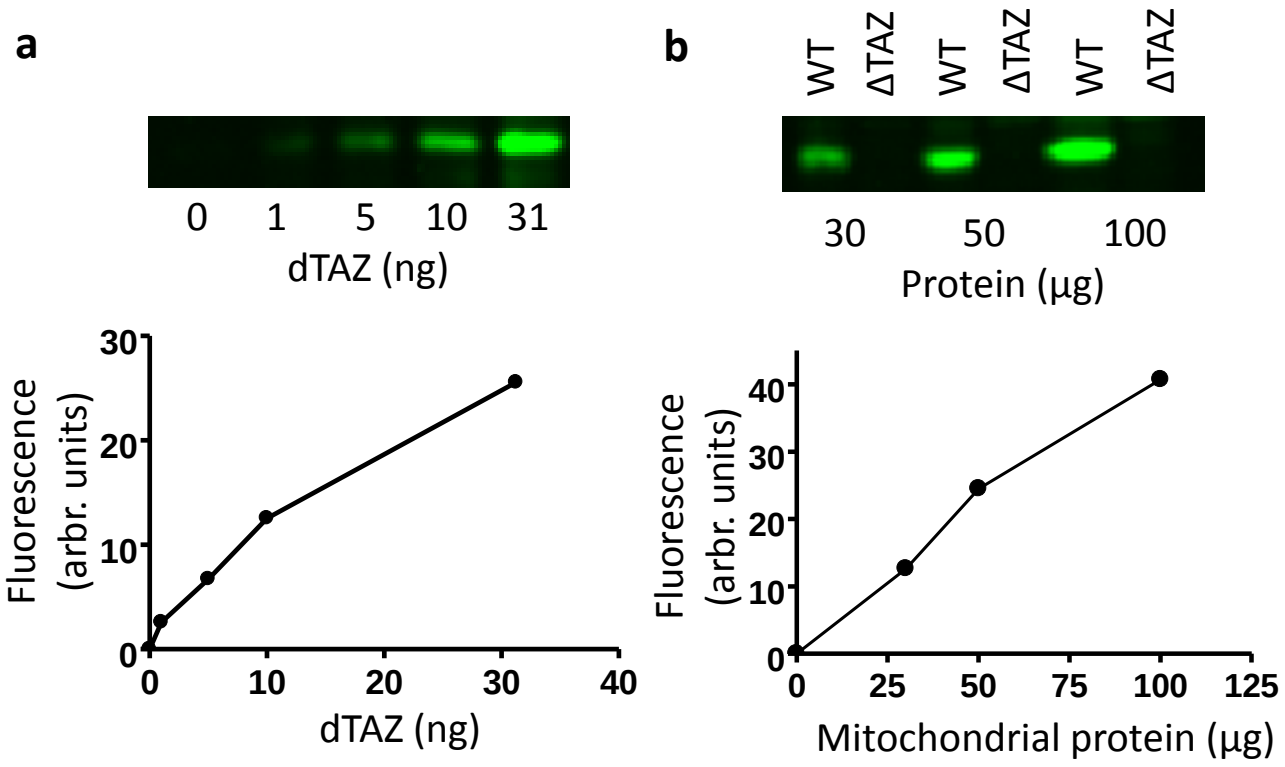

Supplementary Figure 2. Quantitative Western blot analysis of Drosophila tafazzin (dTAZ). Western blot analysis was performed with polyclonal anti-dTAZ from rabbit as primary antibody and LiCor GAR-IRDye800cw as secondary antibody. Fluorescence yield was determined with the LiCor 800 scanner. (**a**) The fluorescence signal was calibrated with purified MBP-tagged Drosophila tafazzin. (**b**) Different amounts of Drosophila mitochondria (wild-type, WT) were analyzed. The tafazzin deletion strain (ΔTAZ) is shown as negative control.

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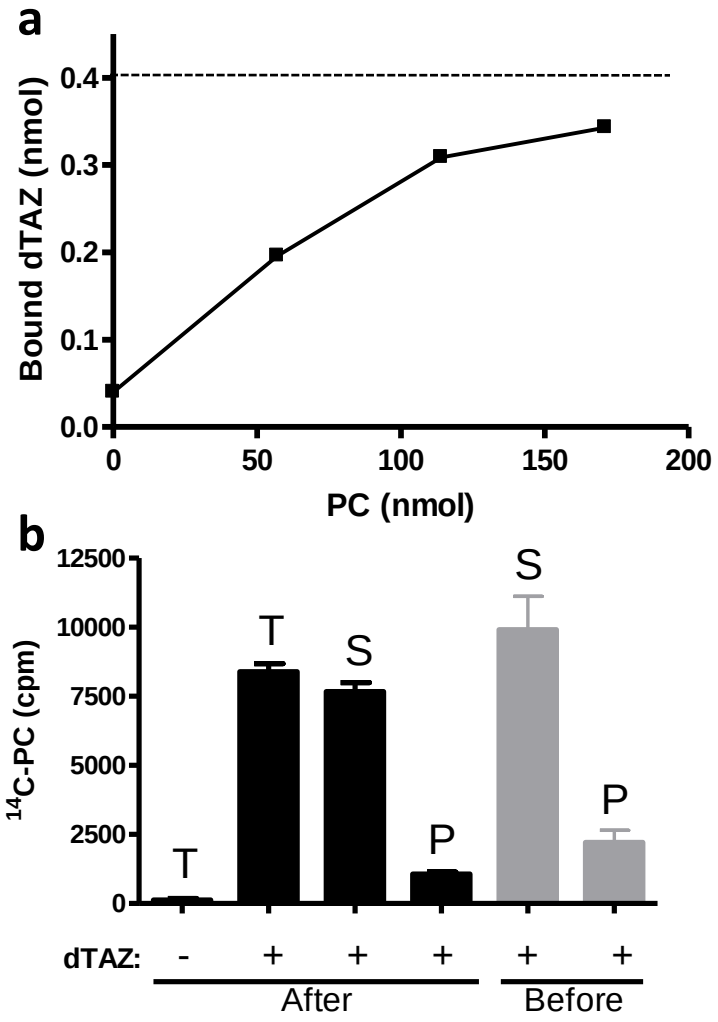

Supplementary Figure 3. Interaction of tafazzin with PC liposomes. (a) Tafazzin binds to liposomes. Liposomes were formed by sonication of dry 16:0-18:2-PC in aqueous buffer (0.05 M Tris, 0.5 mM EDTA, 10 mM β-mercaptoethanol, pH 7.4). Purified MBP-tagged Drosophila tafazzin (0.4 nmol) was incubated with different amounts of PC for 30 min, after which liposomes were collected by ultracentrifugation and tafazzin (dTAZ) was quantified in the pellet by Western blot analysis. The data show that tafazzin binds to liposomes almost quantitatively if liposomes are present in sufficient concentration (molecular ratio PC/dTAZ>300). (**b**) Tafazzin does not react with liposomes but with the 100,000 g supernatant. In each sample, 50 nmol PC and 1 nmol ^{14}C-LPC were dried, re-suspended in 200 µl aqueous buffer by sonication, and centrifuged at 100,000 g for 15 min. Both supernatant and pellet were collected and adjusted to the same volume. Purified MBP-tagged tafazzin (40 pmol, 3.3 µg) was added followed by incubation for 15 min at 37°C. ^{14}C-PC was measured in the total (T), the supernatant (S), and the pellet (P). Grey columns represent experiments, in which the enzyme was added before centrifugation. The data represent means with s.e.m. (N=3). They show that tafazzin reacts with substrates in the supernatant but not with substrates in the pellet.

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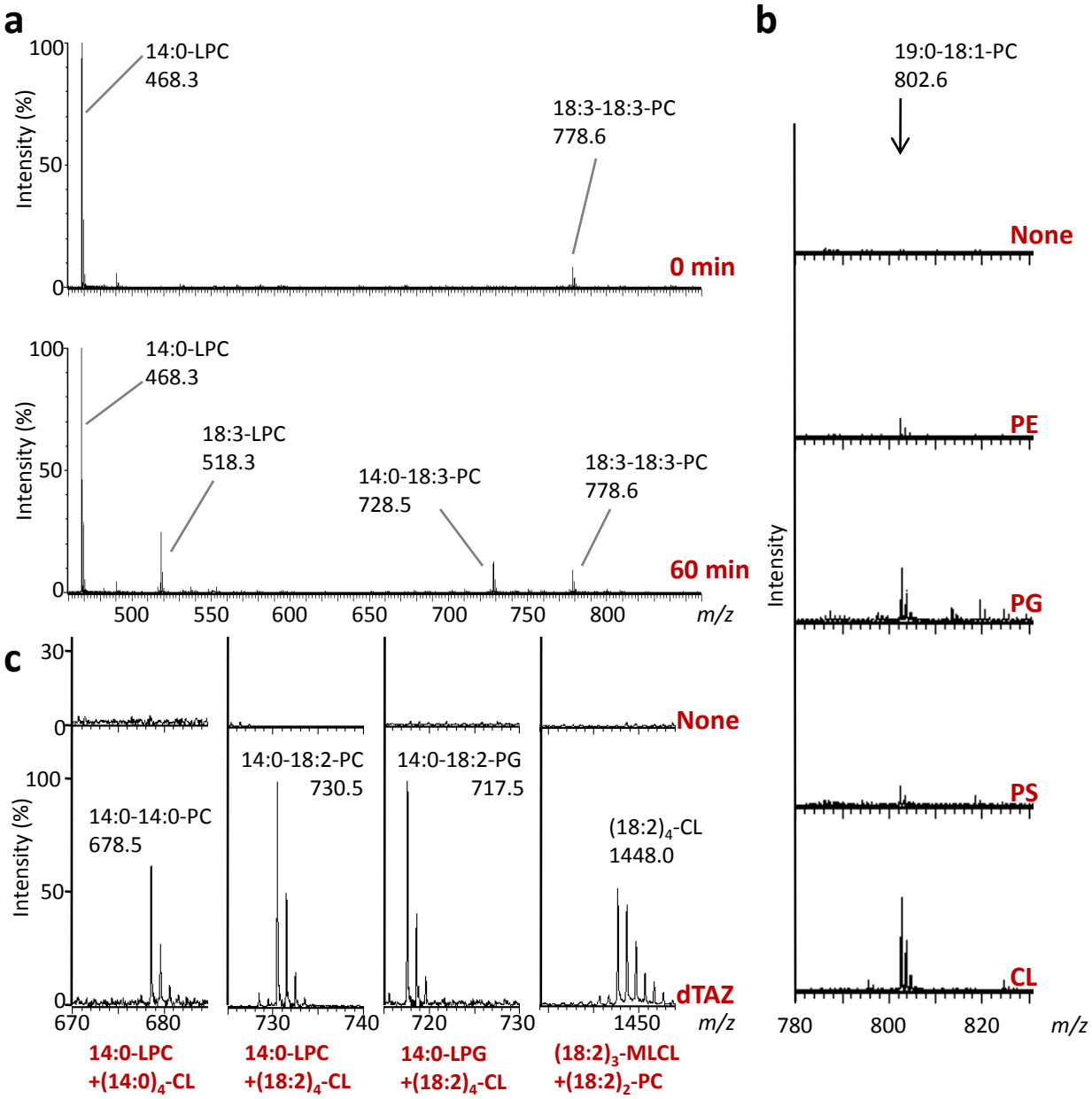

Supplementary Figure 4. Transacylations between various PLs and LPLs. Reactions were performed in 100 µl buffer containing 20 nmol PL, 80 nmol LPL, and 40 µg of purified tafazzin (dTAZ) at 37°C for 60 min. Samples were analyzed by MALDI-TOF MS in positive ion mode, except for the spectra showing PG and CL (panel C) that were analyzed in negative ion mode. Relevant sections of the mass spectra are shown. (**a**) 18:3-18:3-PC reacted with 14:0-LPC. Mass spectra were recorded before (0 min) and after (60 min) the reaction. (**b**) 19:0-LPC reacted with various PLs, including $(18:1)_2$-PE, $(18:1)_2$-PG, $(18:1)_2$-PS, and $(18:1)_4$-CL. The spectra, demonstrating the formation of 19:0-18:1-PC in the presence of each acyl donor, were normalized with respect to intensity. (**c**) Various PLs and LPLs were incubated in the presence or absence of dTAZ.

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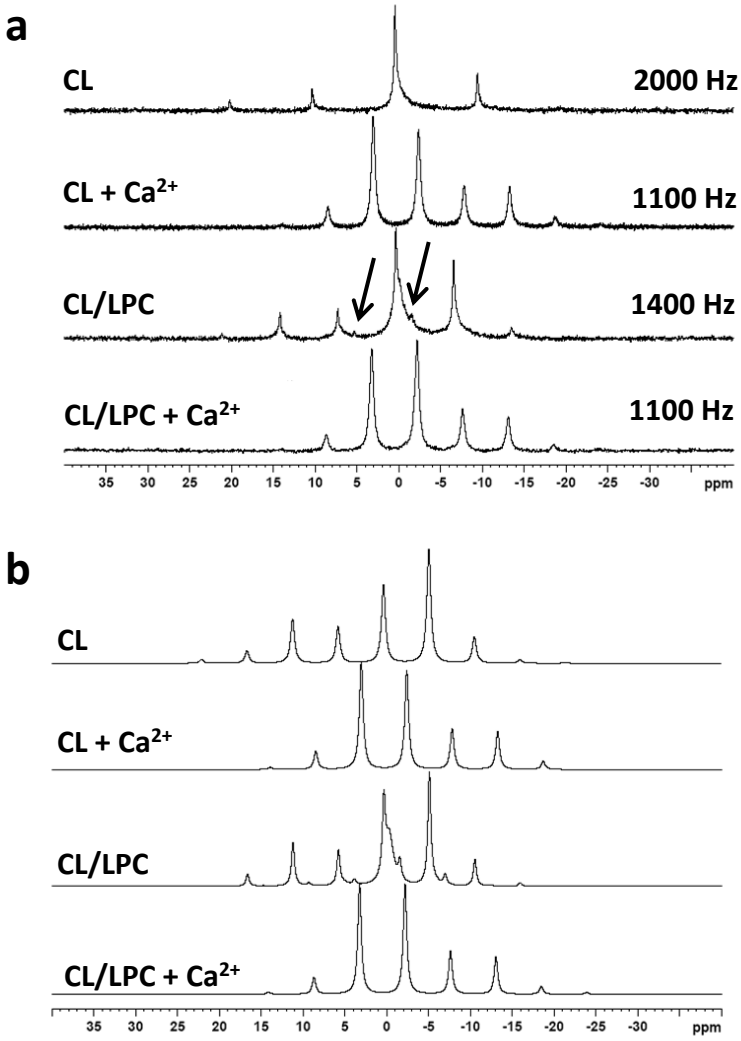
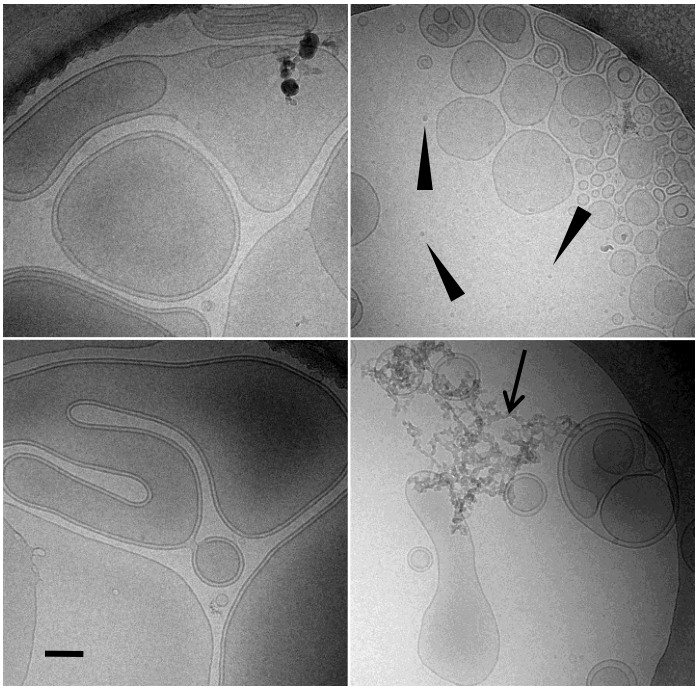


Supplementary Figure 5. MAS ^{31}P NMR spectra of CL/LPC mixtures. Spectra of bovine heart cardiolipin (CL) and of a mixture of CL with 14:0-LPC (9:1) were recorded in the absence and presence of 20 mM $CaCl_2$. (**a**) The panel shows stacked spectra with the spinning rate of each spectrum given at the right-hand side. Ca^{2+} induces a spinning side band pattern indicative of the presence of a hexagonal phase in both CL and CL/LPC. CL/LPC contains more than one spectral component (arrows) in the absence but not in the presence of Ca^{2+}. (**b**) The panel shows simulated spectra generated from the data of panel A. All simulations were performed for a uniform spinning rate (1100 Hz). Simulations clearly show the heterogeneous nature of the CL/LPC sample in the absence of Ca^{2+}.



Supplementary Figure 6. Cryo-electron microscopy of lipid preparations containing CL and CL/LPC. Bovine heart CL and bovine heart CL/14:0-LPC (8:2) mixtures were dried and sonicated in the same aqueous buffer used for enzyme assays. The total lipid concentration was 1 mM. Aliquots were withdrawn and plunge-frozen on holey carbon EM grids in liquid ethane cooled by liquid nitrogen. Samples were imaged in vitreous ice over holes in a 300-kV Joel electron microscope equipped with an energy filter. While CL preparations contained vesicles, CL/LPC (8:2) preparations contained vesicles as well as non-lamellar structures. The latter included isolated particles with a size of about 20 nm (arrow heads) and clusters of such particles (arrow). The vesicle diameter was generally smaller in CL/LPC than in CL. Scale bar: 100 nm.

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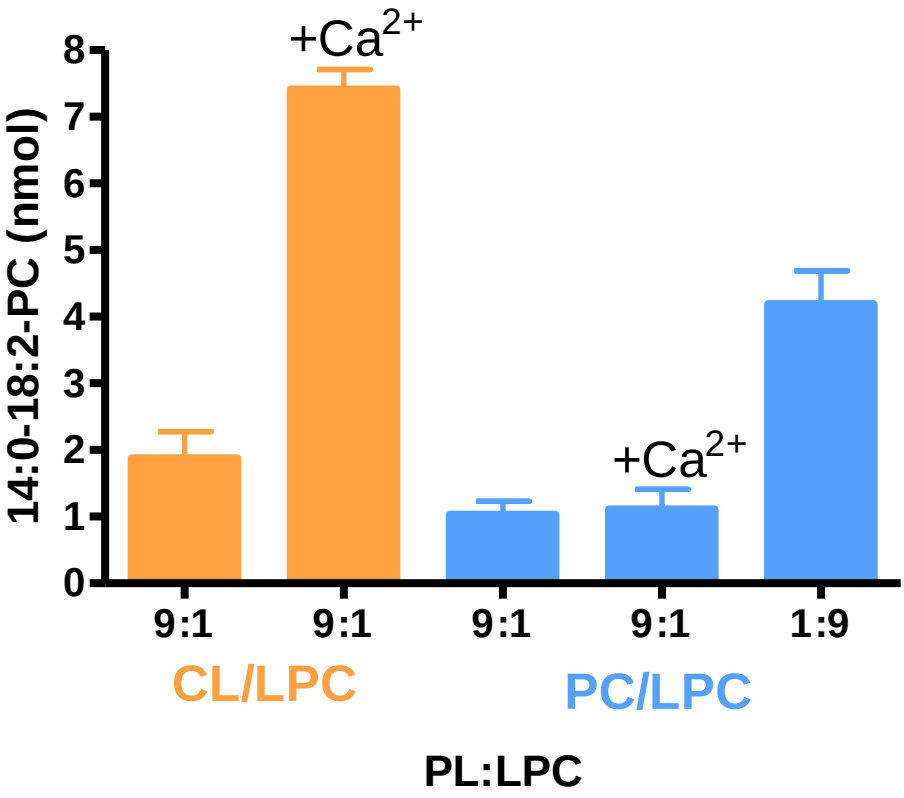

Supplementary Figure 7. Transacylations catalyzed by tafazzin expressed in Sf9 cells. The full-length isoform of Drosophila tafazzin was expressed in Sf9 insect cells and mitochondria were isolated. Aliquots of the mitochondrial preparation (50 µg protein) were incubated with 100 nmol substrates in 100 µl buffer at 37°C for 60 min in the absence or presence of 20 mM $CaCl_2$. Substrates included either bovine heart CL and 14:0-LPC or 18:2-18:2-PC and 14:0-LPC at the indicated PL/LPC ratios. Formation of 14:0-18:2-PC was measured by mass spectrometry. The data show that Sf9-derived mitochondrial tafazzin behaved similar to E. coli-derived purified tafazzin in the following ways: Ca^{2+} increased transacylation in CL/LPC but had no effect on PC/LPC; and transacylation was higher in PC/LPC if LPC was the dominant component.

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