Phosphorylated sphingolipids ceramide-1-phosphate (C1P) and sphingosine-1-phosphate (S1P) have emerged as key regulators of cell growth, survival, migration, and inflammation. C1P produced by ceramide kinase is an activator of group IVA cytosolic phospholipase A2 (cPLA2α), the rate-limiting releaser of arachidonic acid used for pro-inflammatory eicosanoid production, which contributes to disease pathogenesis in asthma or airway hyper-reactivity, cancer, atherosclerosis and thrombosis. To modulate eicosanoid action and avoid the damaging effects of chronic inflammation, cells require efficient targeting, trafficking and presentation of C1P to specific cellular sites. Vesicular trafficking is likely but non-vesicular mechanisms for C1P sensing, transfer and presentation remain unexplored. Moreover, the molecular basis for selective recognition and binding among signalling lipids with phosphate headgroups, namely C1P, phosphatidic acid or their lyso-derivatives, remains unclear. Here, a ubiquitously expressed lipid transfer protein, human GLTPD1, named here CPTP, is shown to specifically transfer C1P between membranes. Crystal structures establish C1P binding through a novel surface-localized, phosphate headgroup recognition centre connected to an interior hydrophobic pocket that adaptively expands to ensheathe differing-length lipid chains using a cleft-like gating mechanism. The two-layer, α-helically-dominated 'sandwich' topology identifies CPTP as the prototype for a new glycolipid transfer protein fold subfamily. CPTP resides in the cell cytosol but associates with the trans-Golgi network, nucleus and plasma membrane. RNA interference-induced CPTP depletion elevates C1P steady-state levels and alters Golgi cisternae stack morphology. The resulting C1P decrease in plasma membranes and increase in the Golgi complex stimulates cPLA2α release of arachidonic acid, triggering pro-inflammatory eicosanoid generation.

During screening of the NCBI human genome database, we noted an in silico predicted transcript (GenBank NP_001025056.1; glycolipid transfer protein domain-containing protein 1; GLTPD1) encoding a protein sharing sequence identity (17%) with glycolipid transfer protein (GLTP). Although annotation indicated glycolipid binding and transport activity, Lys and Arg substitutions occurred at key positions (N52, N1–1–α8) are numbered from amino (N) to carboxy (C) termini. We validated GLTPD1 mRNA transcript expression in human tissues, finding widespread occurrence and relatively elevated transcript levels in placenta, kidney, pancreas and testis (Fig. 1a). Cloning and heterologous expression revealed that purified GLTPD1 (GenBank JN542538) transfers anthrylvinyl-C1P (AV-C1P) between 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer vesicles in a protein concentration-dependent fashion (Fig. 1b, c and Supplementary Fig. 1b, d) requiring acceptor membranes (Supplementary Fig. 1c). Testing of lipid specificity revealed no transfer of galactosylceramide (GalCer), lactosylceramide (LacCer), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidic acid (PA) or ceramide (Cer) by GLTPD1 (Fig. 1b, c). Slow-down of AV-C1P transfer by potential lipid ligands (nonfluorescent) showed no competition effect by S1P (Fig. 1d and Supplementary Fig. 1e, f) and a transfer rate of ~4 C1P molecules per min per protein molecule at 37 °C. We have designated GLTPD1 as ceramide-1-phosphate transfer protein (CPTP).

The crystal structure of human CPTP and 160-C1P complex (1.9 Å, Supplementary Table 1) revealed a two-layered, all α-helical topology (Fig. 1e–g) homologous with the GLTP fold. αN, α1 and α2 form one layer, α4, α5 and α8 form another layer, and α3, α6 and α7 localize along the periphery of the two-layer core. A positively-charged surface cavity for anchoring the lipid headgroup (Fig. 1h) extends through a

**Figure 1 | CPTP lipid transfer activity and architecture.** a, CPTP mRNA transcript levels in various human tissues. bp, base pairs. b, Lipid transfer in vitro by Förster resonance energy transfer. c, Initial lipid transfer rates for panel d, Competition against CPTP-mediated AV-C1P transfer by nonfluorescent lipids. Kinetic traces are shown in Supplementary Fig. 1e–g. Data in c and d represent the mean ± s.d. of three independent experiments. e, 160-C1P chemical formula. f, g, Two views of CPTP structure (ribbon) with bound 160-C1P (space-filling). α-helices (cyan), 3 helices (light blue), loops (orange) and bound 160-C1P (yellow, red, blue for carbon, oxygen, nitrogen, respectively). α-helices (αN and α1–α8) are numbered from amino (N) to carboxy (C) termini. h, Surface electrostatics of CPTP with bound 160-C1P showing positive (blue) and negative (red) charged residues.
The hydrophobic pocket is lined by ~25 nonpolar residues, mainly Phe, Leu, Val and Ile (Fig. 2c and Supplementary Fig. 2b) that prevent water entry while ensheathing the ceramide aliphatic chains. Mutation of L43, L118 or L146 to positively-charged Arg, or V57 or V158 to high polarity Asn compromises hydrophobic pocket functionality and strongly diminishes C1P transfer (Fig. 2d). More conservative mutation (for example, W117A) only moderately reduces C1P transfer, whereas F42A near the pocket bottom stimulates C1P transfer. Mutation near the entry portal (I53N) or in the flexible α1-2 loop (F50R) is well tolerated (75–80% active) (Fig. 2d). Ceramide entry is oriented by hydrogen bonding of the lipid amide oxygen and nitrogen with H150 and D56, respectively. Hydrogen bond disruption between lipid amide nitrogen and D56 (D56V) moderately slows C1P transfer, but H150 mutation (H150L) abolishes activity. Superpositioning of apo- and 16:0-C1P–CPTP structures (root mean squared deviation (r.m.s.d.) 1.4 Å) shows K60, R106 and R110 nearly identically positioned in the positively-charged surface cavities. Yet, large conformational differences exist for I53, W36, W119 and F52 (Fig. 2e) due to closer packing of certain α-helices in apo-CPTP (Supplementary Fig. 2d, e). Many Leu and Phe are repositioned, reducing the solvent accessible volume (40 Å³) (Supplementary Table 4) and effectively collapsing the hydrophobic pocket (Fig. 2f, g) compared to the 16:0-C1P–CPTP complex (364 Å³).

CPTP adaptability for different C1P species is reflected in structures of CPTP complexed with C1P containing differing-length acyl chains (Figs 2h–k, 3a–f and Supplementary Fig. 4c–f). Two lipid-binding conformational modes are apparent. In ‘sphingosine-in’ mode, both ceramide chains occupy the hydrophobic pocket, whereas only the acyl chain occupies the pocket in the ‘sphingosine-out’ mode. For the 12:0-C1P–CPTP complex (Fig. 2h–j), both binding modes occurred in the same asymmetric unit (Fig. 2i, j and Supplementary Figs 3c, d and 4a, b) enabling comparison (Fig. 2k) under the closest possible conditions. The lipid phosphate headgroups and amide groups bind exactly as in the 16:0-C1P–CPTP complex (Supplementary Figs 2b and 4a, b).

In sphingosine-out mode (Fig. 2j and Supplementary Fig. 3d), a bend in sphingosine at C6 is stabilized by hydrophobic interactions with V153, V154 and the D56 CB atom, enabling outward projection. Sphingosine cross-bridging interactions with F50, I149, A157 and V153 of neighbouring, symmetry-related CPTP stabilize further (Supplementary Fig. 4b). Solvent accessible pocket volumes reflect the altered sphingosine location, that is, 261 Å³ for sphingosine-out versus 329 Å³ for sphingosine-in (Supplementary Table 4). In 18:1-C1P–CPTP complex (Fig. 3a, b), the cis-double bond kink in the acyl chain increases separation from the sphingosine chain, maximally expanding the pocket (stereo view; Supplementary Fig. 4c, d) although leaving the overall chain length in the pocket similar to 16:0-C1P. Accordingly, the solvent accessible volume of the hydrophobic pocket of 18:1-C1P–CPTP is larger (387 Å³) (Supplementary Table 4) than in 16:0-C1P–CPTP where slightly closer packing by the saturated acyl chain decreases the solvent accessible volume (364 Å³). Shortening the acyl chain length reduces solvent accessible pocket volumes to 104 Å³ for 8:0 and 263 Å³ for 2:0 (Supplementary Table 4). Structures for 2:0-C1P–CPTP (sphingosine-in) and 8:0-C1P–CPTP (sphingosine-out) are detailed in Supplementary Figs 3a, b and 4e, f and Supplementary Discussion.

The functional consequences of CPTP hydrophobic pocket structural adaptability become clear upon transfer analyses. Pocket expansion accommodates ceramide aliphatic chains in ‘molecular ruler’-like fashion with CPTP adaptability limits optimized for 16:0- or 18:1-C1P species which are particularly effective competitors at slowing the AV-C1P transfer rate (Fig. 3g), consistent with maximal pocket expansion and optimal fit (Supplementary Table 4). It is noteworthy that C1P containing long lignoceryl (24:0) acyl chains are not very effective competitors, suggesting poor accommodation in the hydrophobic pocket.
because of adaptation limitations. Also, 16:0-C1P with dihydrosphingosine base competes less effectively than 16:0-C1P with naturally prevalent sphingosine base.

Structure determination of the di12:0-PA–CPTP complex elucidated the molecular basis of phosphatic acid non-transfer (Supplementary Fig. 5a–h and Supplementary Table 1). Phosphatic acid occupies the same binding site and its phosphate group interacts with the same binding site and its phosphate group interacts with the same sphingosine base.

CPTP architecture not only represents a new motif for specific binding of phosphosphingolipids, but is previously unknown for any lipid phosphate recognition site. Figure 5a–h shows the regions between \( \alpha \)–1 and \( \omega \)–2, with the nonpolar acyl chain always inside of the pocket, with the nonpolar acyl chain always inside regardless of sphingosine being in or out, supports a concerted mechanism of action in which the acyl chain enters first and leaves last during membrane interaction (Supplementary Fig. 6a–h and Supplementary Discussion). The conformational adaptability of the inherently flexible, single-cavity, hydrophobic pocket of CPTP contrasts with lipid cavities in fatty acid binding proteins which use \( \beta \)-barrels/\( \alpha \)-cups to generate a large, solvent-filled binding site that remains conformationally fixed whether or not occupied by fatty acid. A single, fixed, lipid binding cavity also is characteristic of START lipid binding domains in PC transfer protein19 and CERT (ref. 20), which uses an \( \alpha \)-/\( \beta \)-fold built around an incomplete U-shaped \( \beta \)-barrel to bind ceramide20 (Supplementary Fig. 7 and Supplementary Discussion).

In the human genome, the differing origins of CPTP and GLTP are clear. CPTP (214 amino acids) is encoded by a three-exon transcript originating from GLTPD1 on chromosome 1 ( locus 1p36.33). GLTP (209 amino acids) is encoded by a five-exon transcript originating from GLTPD1 on chromosome 1 ( locus 1q24.11)21. The shared folding topology encoded by GLTPD1 and GLTP, despite only limited sequence homology (Supplementary Fig. 8a–e) and different lipid specificity, provides a striking example of evolutionary convergence and emphasizes the structural premium placed by eukaryotes on conservation of this fold22–24. The related architectures of CPTP and GLTP, but with naturally evolved and remarkably different lipid headgroup specificity (Supplementary Discussion), suggest that the term ‘sphingolipid transfer protein (SLTP) superfamily’ might better reflect the existence of the two major subfamilies: CPTP, with selectivity for ceramide-linked phosphates; and GLTP, with selectivity for ceramide-linked sugars.

In cells, CPTP tracked by monospecific antibody or fluorescent epitope tag enhanced green fluorescent protein (EGFP) is localized in the cytosol but also associates with perinuclear membranes (for example, Golgi/trans-Golgi network (TGN)/endosomes), nuclei and plasma membranes (Fig. 3i–n and Supplementary Fig. 9). No localization to mitochondria, lysosomes or the endoplasmic reticulum is detected. CPTP co-localization with TGN-46 verified interaction with the TGN, a site where ceramide kinase (CERK) generates C1P (refs 3, 26–28) and led us to propose a C1P regulatory/sensing role for CPTP during CERK-mediated metabolic/signalling events. Short interfering RNA approaches have validated the role of C1P in CPTP localization and metabolism signaling.
RNA (siRNA)-induced CPTP downregulation (~90%; Supplementary Fig. 10a) elevated both 16:0-C1P and 24:1-C1P (~4-fold) (Fig. 4a) and fragmented the Golgi cisternal stacks (Supplementary Fig. 11). RNAi-induced C1P changes were partially rescued with moderately-active R110L and K60N, but not with inactive K60A and R106L mutants (Fig. 4b). CPTP overexpression in the absence of RNAi decreased 16:0-C1P and 24:1-C1P. K60A or R106L overexpression had the opposite effect (Supplementary Fig. 12) and fragmented the Golgi cisternal stacks (not shown) consistent with a dominant-negative effect. CPTP depletion measurably decreased sphingosine and S1P (Supplementary Fig. 10b, c), 14:0-, 22:0-, 24:0- and 24:0-sphingomyelin, 16:0-monohexosylceramide, and 24:0-Cer, but modestly increased (Supplementary Fig. 10b, c), 14:0-, 22:0-, 24:1- and 24:0-sphingomyelin, decreased 16:0-C1P and 24:1-C1P. K60A or R106L overexpression mutants (Supplementary Fig. 14). Parallel siRNA-induced downregulation of CERK (Supplementary Fig. 10), the only established producer of C1P in mammals, decreased 16:0-C1P (Fig. 4a), arachidonic acid (Fig. 4c), and eicosanoids (Fig. 4d–f) elevated by CPTP depletion.

Figure 4h depicts a model showing how CPTP could regulate pro-inflammatory eicosanoid generation. In mammals, the only established pathway for C1P production is through phosphorylation of ceramide by CERK at the cytoplasmic surface of the TGN.28 CERK contains nuclear localization/export signals and traffics to the plasma membrane by microtubule-driven vesicles in response to hyperosmotic shock.3 To produce C1P, CERK uses ceramide delivered from its endoplasmic reticulum synthetic site to the Golgi by either CERT (ref. 27) or possibly by vesicular trafficking. C1P elevation by CERK is known to activate soluble cPLA2α by enhancing translocation to the TGN,26,27 where cPLA2α action releases arachidonic acid needed by eicosanoid producers such as COX-1 or COX-2. siRNA-induced CPTP depletion elevates C1P in the Golgi complex and nucleus, but lowers C1P plasma membrane levels. We propose that CPTP prevents excess C1P accumulation after production by CERK, thereby regulating cPLA2α action.

Because CERK-generated C1P induces Group IVA cPLA2α activity which releases arachidonic acid to produce pro-inflammatory eicosanoids,26,27, CPTP involvement was assessed. In siRNA-induced CPTP-depleted cells, arachidonic acid increased (Fig. 4c) consistent with C1P accumulation at the Golgi/TGN that activates cPLA2α (ref. 26). Also elevated were major arachidonic acid metabolites generated by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) pathways, that is, PGE2, PGF2α, 6-keto-PGF1α (COX, Fig. 4d); 5HETE, 8HETE, 12HETE (LOX, Fig. 4e); 11,12 EET (CYP, Fig. 4f). By contrast, arachidonic acid and eicosanoid levels decreased upon overexpression of wild-type CPTP but not the K60A or R106L mutant (Supplementary Fig. 14). Parallel siRNA-induced downregulation of CERK (Supplementary Fig. 10), the only established producer of C1P in mammals, decreased 16:0-C1P (Fig. 4a), arachidonic acid (Fig. 4c), and eicosanoids (Fig. 4d–f) elevated by CPTP depletion.
diminishing arachidonic acid release and downstream generation of pro-inflammatory eicosanoids. One destination for CPTP cargo is the plasma membrane. Models involving CPTP in catabolic C1P generation by sphingomyelinase D are less plausible because mammalian cells lack this enzyme6 (Supplementary Discussion).

Previously, the only identified mechanism for regulating CERK-mediated production of C1P was by control of ceramide availability through ceramide transfer protein22. It is noteworthy that siRNA-induced CPTP depletion yields the highest increase in endogenous C1P reported to date, mostly as 16:0-C1P, and dramatically alters Golgi cisternal stack morphology indicating CPTP-mediated transport is essential for maintaining proper Golgi organization by safeguarding localized C1P levels. The ensuing stimulation in eicosanoid production triggered by elevated C1P in the Golgi complex potentially implicates CPTP in, as of yet unidentified, disease states associated with inflammation.

METHODS SUMMARY

CPTP was cloned and expressed in BL21 (DE3) Star cells using a PET-SUMO vector. Lipid intervesicular transfer assays were performed by Förster resonance energy transfer. X-ray diffraction data were collected on crystals of mouse apo-CPTP and of human CPTP with bound C1P containing acyl chains of differing length, for example, 2:0, 8:0, 12:0, 16:0 and 18:1, as well as di-12:0 PA. For phasing, single-wavelength anomalous dispersion data were collected for Se-Met-labelled, 8:0-C1P–CPTP crystal complex at Se peak wavelength (Supplementary Table 2) enabling other structures to be solved by molecular replacement. Data collection, processing, structure solution and refinement are described in the Methods. Protein docking with membranes was performed using the Orientation of Proteins with Membranes modelling. Epifluorescence microscopy images of fixed BSC-1 cells were captured by labelling with anti-CPTP antibody and anti-TGN-46, anti-GM130, anti-p230, anti-Rab5 or anti-Rab9 followed by secondary antibodies coupled to Alexa Fluor 488, Alexa Fluor 594, or Alexa Fluor 660 and counterstaining with DAPI (4′,6-diamidino-2-phenylindole). Reverse transcription PCR and quantitative PCR were performed using the逆转录酶QuantitativePCR, PCR and sequencing reactions using the appropriate primers. Analysis of cytosolic C1P and its role in eicosanoid synthesis was performed using the PORC3 (Supplementary Discussion).

Full Methods and any associated references are available in the online version of the paper.

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Author Contributions D.S.W. carried out all structural analyses and provided evidence for C1P binding by CPTP, generated all CPTP point mutants and wrote text. R.K.D. conducted sRNA CPTP knockdown, rescue, and all lipid analyses and wrote text. X.2.o cloned wild-type CPTP and did PCR analyses of CPTP transcript distribution in human tissues. X.2.o. did CPTP transfer rate analyses. S.K.M. prepared CPTP RNAi constructs for microscopy and CPTP overexpression constructs for lipidomics analyses. J.G.M. synthesized all fluorescent lipids. L.M. contributed to structural data interpretation. E.H.H. did fluorescence microscopy of CPTP localization in fixed and living cells and contributed the write-up. C.E.C. directed sRNA knockdown, rescue and related lipidomics analyses and finalized the write-up. R.E.B. directed CPTP structural analyses and finalized the write-up. R.E.B. directed functional analyses after the initial CPTP discovery in his laboratory, finalized the write-up and coordinated and integrated all section write-ups.

Author Information Atomic coordinates and structure factors for human CPTP crystal complexes with various lipids and mouse apo-CPTP have been deposited in the Protein Data Bank. Accession codes are: 2:0-C1P–CPTP (4KBS), 8:0-C1P–CPTP (4KF6), 12:0-C1P–CPTP (4KB5), 16:0-C1P–CPTP (4KB4), 18:1-C1P–CPTP (4KBN), di12:0-PA–CPTP (4KBS) and mouse apo-CPTP (4KBR). Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to R.E.B. (reb@umn.edu), E.H.H. (ehinhclife@umn.edu), C.E.C. (cechafant@uc.edu) or D.J.P. (pateid@mskcc.org).
METHODS

Protein expression and purification. GLTPD1 ORFs encoding human and mouse (GenBank JN542538 & NP_077792.2) CPTP were cloned in PET-SUMO vector (Invitrogen) and expressed in BL21 (DE3) Star cells (Invitrogen). Soluble CPTP tagged N-terminally with His6-SUMO was affinity-purified by Ni-NTA chromatography followed by ubiquitin-like protein 1 (Ulp1) SUMO protease digestion overnight at 4°C to release the His6-SUMO tag. Affinity repurification by Ni-NTA chromatography was followed by FPLC gel filtration chromatography. t-selenomethionine (Se-Met)-labelled protein for ab initio phasing was produced by feedback inhibition of the methionine synthesis pathway. Mutants were constructed by QuikChange Site-Directed Mutagenesis (Stratagene) and verified by sequencing.

CPTP lipid transfer activity involving membrane vesicles. Intermembrane lipid transfer by CPTP was measured in real time for FRET between donor POPC vesicles, containing 1 mole % AV-lipid (acyl chain omega-labelled with anthrylvinyl fluorophore, that is, (11E)-12-(9-arylmethylenyl)-11-dodecenoyl, and 1.5 mole % 1-acyl-2[9-(3-perylnoyl)-nonanoyl]-3-sn-glycero-3-phosphocholine (Per-PC) and POPC acceptor vesicles at 10-fold excess. In competition assays, donor vesicles also contained competitor lipids at 0.5, 1.0 and 2.0 mole % (ref. 30). Briefly, CPTP addition produces an exponential increase in AV-emission intensity (425 nm) as the protein transports AV-C1P away from the donor vesicles (creating separation from the ‘nontransferable’ Per-PC) and delivers to the POPC acceptor vesicles present in tenfold excess. The time-dependent increase in 425 nm emission relative to signal in the absence of CPTP reflects lipid transfer kinetics. In the absence of acceptor vesicles, no transfer is observed. The initial lipid transfer rate, vs, is obtained by nonlinear regression analyses (see Supplementary Methods).

Crystalization and structure determination. Crystalization hits from initial screens were optimized by the hanging drop vapour diffusion method and systematically varying pH and individual component concentrations (Supplementary Table 3). For data collection, crystals were flash frozen (100K) in reservoir solutions containing 20% (v/v) ethylene glycol. Diffraction data sets were collected on 24-ID-C and 24-ID-E beamlines at the Advanced Photon Source (APS) and X29 beamline at Brookhaven National Laboratory. All crystals belonged to different crystal forms. For phasing, single-wavelength anomalous dispersion (SAD) data were collected for Se-Met-labelled, 8:0-C1P–CPTP crystal complex at Se peak wavelength (Supplementary Table 2; see Supplementary Methods). Use of SeMet for data collection, refinement and SAD phasing are provided in Supplementary Tables 1 and 2.

Epithelial microsopy analyses. BSC-1 cells on coverslips were fixed in −20°C methanol and labelled with anti-CPTP (Santa Cruz Biotechnology, sc247014), and Golgi markers anti-TGN46, anti-GM 130 and anti-p230, or endosome markers anti-Rab5 and anti-Rab9 (Cell Signaling) followed by secondary antibodies coupled to Alexa Fluor 488, Alexa Fluor 594 or Alexa Fluor 660. Cells were counter-stained with DAPI, mounted in 10% PBS, 90% glycerol, imaged using a Leica DM RXA2 microscope with a ×63 1.4 NA APO C objective, a Leica DMRX2, and Leica DMRX2 digital camera, and Image software and analysed as intensity scattergrams with measured correlation coefficients. Time-lapse images of living cells expressing EGFP–CPTP were captured with a Leica DM RXA2 microscope stand equipped with a Yokogawa CSU-10 spinning disk confocal head of living cells expressing EGFP–CPTP were captured with a Leica DM RXA2 fluorescence (Image Quant system, GE Healthcare).

Intracellular sphingolipid analyses. Cell lipids were harvested using an improved Bligh-Dyer protocol (Supplementary Methods). Sphingolipids were separated by HPLC (Prominence HPLC system, Shimadzu) using a Kinetich-C18 column (20 mm × 2.6 mm; Phenomenex) and eluted using a linear gradient (solvent A, methanol:formic acid (99:1) in 5 mM ammonium formate; solvent B, methanol:formic acid (99:1) in 5 mM ammonium formate, 20–100% B (3.5 min) and at 100% B (4.5 min); flow rate of 0.4 ml min−1, 60°C). ESI-MS/MS (API 4000 QTRAP instrument; Applied Biosystems, MDS Sciex) was used to detect C1P (ref. 29), ceramide, sphingosine, S1P, sphingomyelin, and monohexosyl ceramide under positive ionization (see Supplementary Methods).

Eicosanoid analysis. Eicosanoids were analysed as detailed by the Lipid Maps Consortium. Culture media (4 ml) from siRNA was combined with 10% methanol:water:formic acid (99:4:1) in 5 mM ammonium formate, spiked with C1P (ref. 29), methylated eicosanoids at 100 pg ml−1. 10 ng total: m/z 604.5 (d6)6 ketopGPE2, (d6)5 PGD2, (d6)4 PGF2, (d6)3 PGD2, (d6)3 5-hydroxyeicosatetraenoic acid (5HETE), (d6)3 15-hydroxyeicosatetraenoic acid (15HETE), (d6)14,15 epoxyeicosatrienoic acid and (d6)12 arachidonic acid. Samples and vial rinses (5% MeOH; 2 ml) were applied to Strata-X SPE columns (Phenomenex), previously washed with methanol (2 ml) and then H2O (2 ml). Eicosanoids eluted with isopropanol (2 ml), were dried in vacuo and reconstituted in EtOH:H2O (50:50; 100 ml) before HPLC ESI-MS/MS analysis (see Supplementary Methods).

Subcellular fractionation. Subcellular fractionation was performed by multi-step centrifugation as detailed and characterized previously with minor modifications (see Supplementary Methods). Fraction enrichment was validated by SDS–PAGE/western blotting (Supplementary Fig. 13b) using organellar markers for: nuclei (anti-lamin AC), trans-Golgi (anti-TGN46), ER (anti-protein disulphide isomerase (PDI)) and plasma membrane (anti-caveolin-1).

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