AGPAT2 interaction with CDP-diacylglycerol synthases promotes the flux of fatty acids through the CDP-diacylglycerol pathway

Hoi Yin Mak, Qian Ouyang, Sergey Tumanov, Jiesi Xu, Ping Rong, Feitong Dong, Sin Man Lam, Xiaowei Wang, Ivan Lukmantara, Ximing Du, Mingming Gao, Andrew J. Brown, Xin Gong, Guanghou Shui, Roland Stocker, Xun Huang, Shuai Chen & Hongyuan Yang

AGPATs (1-acylglycerol-3-phosphate O-acyltransferases) catalyze the acylation of lysophosphatidic acid to form phosphatidic acid (PA), a key step in the glycerol-3-phosphate pathway for the synthesis of phospholipids and triacylglycerols. AGPAT2 is the only AGPAT isoform whose loss-of-function mutations cause a severe form of human congenital generalized lipodystrophy. Paradoxically, AGPAT2 deficiency is known to dramatically increase the level of its product, PA. Here, we find that AGPAT2 deficiency impairs the biogenesis and growth of lipid droplets. We show that AGPAT2 deficiency compromises the stability of CDP-diacylglycerol (DAG) synthases (CDSs) and decreases CDS activity in both cell lines and mouse liver. Moreover, AGPAT2 and CDS1/2 can directly interact and form functional complexes, which promote the metabolism of PA along the CDP-DAG pathway of phospholipid synthesis. Our results provide key insights into the regulation of metabolic flux during lipid synthesis and suggest substrate channelling at a major branch point of the glycerol-3-phosphate pathway.
living organisms need to store energy to survive in an ever-changing environment. For cells, energy is stored in the form of neutral lipids within lipid droplets (LDs), which are evolutionarily conserved organelles found in nearly all organisms. Mammals have developed adipocytes and adipose tissue, which specialize in energy storage. Adipocytes are derived from mesenchymal stem cells through a process called adipogenesis that is regulated by a transcriptional cascade. Understanding the fundamental mechanisms governing LD formation/growth (cellular lipid storage) and adipogenesis (systemic lipid storage) may provide better treatment strategies against obesity and its associated metabolic disorders.

The 1-acylglycerol-3-phosphate O-acyltransferases (AGPATs), also known as lysosphatidic acid acyltransferases (LPAATs) are intermediate enzymes in the biosynthesis of phospholipids and triacylglycerols (TAGs) through the glycerol-3-phosphate pathway. The first committed step is the acylation of glycerol-3-phosphate to form 1-acylglycerol-3-phosphate (also called lysosphatidic acid (LPA)). This reaction is catalyzed by glycero-3-phosphate O-acyltransferases (GPATs). AGPATs/LPAATs esterify the sn-2 position of LPA to make phosphatidic acid (PA), a critical intermediate that can be dephosphorylated into diacylglycerol (DAG) by PA phosphatases (PAPs, e.g., lipins), or converted into CDP-DAG by CDP-DAG synthases. Among the AGPAT isoforms, AGPAT2 stands out since null mutants of AGPAT2 are associated with Berardinelli-Seip congenital lipodystrophy type 1 (BSCL1/congenital generalized lipodystrophy, type 1 (CGL1), which is characterized by a near complete loss of adipose tissue, early onset of insulin resistance, diabetes, hypertriglyceridemia, and hepatic steatosis. Human AGPAT2 localizes to the endoplasmic reticulum (ER), and is expressed abundantly in adipose tissue, liver and pancreas. Importantly, AGPAT2 mice replicate most of the features of human BSCL1/CGL1, although the extent of fat loss and the degree of insulin resistance appear more severe in mice than in humans.

Exactly how AGPAT2 deficiency causes lipodystrophy, i.e., BSCL1, is unknown. While AGPAT2 catalyzes the acylation of LPA to PA, the level of PA was surprisingly and dramatically increased in AGPAT2-deficient cells and liver. In this connection, seipin, whose loss-of-function mutations cause BSCL1/congenital generalized lipodystrophy, is known to have acquired substantial neutral lipids. We also used seipin deficient cells as a control because seipin deficiency results in the formation of supersized LDs within 10 min of oleate treatment than wild type (WT) AGPAT2, but not a catalytic inactive mutant (H98A), rescued the LD phenotype when expressed in the knockdown cells (Fig. 1j). Moreover, the sizes of LDs were also increased when AGPAT2 was knocked down in 3T3 L1 adipocytes, a professional cell for fat storage (Supplementary Fig. 1f). Knocking down AGPAT2 did not seem to impact the viability of 3T3 L1 adipocytes (Supplementary Fig. 1g). To characterize the formation of supersized LDs in greater detail, we examined LD formation over 24 h of oleate treatment. Within 2 h of oleate addition, LDs in AGPAT2-depleted cells were larger than those in control cells, although almost all LDs were <2 µm in diameter (Supplementary Fig. 1h, i). Giant LDs first appeared in AGPAT2 knockdown cells ~8 h after adding oleate and became more prominent later. In contrast, giant LDs were extremely rare in control cells.

LDs are believed to originate from the ER via several steps. First, TAGs are synthesized within the two leaflets of the ER by diacylglycerol acyltransferases (DGATs). When newly synthesized TAG reaches a critical mass, it is thought to nucleate and bud from discrete sites of the ER as initial LDs. This nucleation and budding process may be controlled by both proteins such as seipin and/or ER membrane phospholipids and surface tension. Given that a negatively charged conical lipid, was dramatically increased in AGPAT2-deficient cells, we wondered if AGPAT2 deficiency may also impact the early steps of LD formation. To investigate this possibility, we tagged endogenous perilipin 3 (PLIN3) by genome engineering with mCherry as described. PLIN3 is an endogenous protein that indicates the earliest steps of LD formation whereas lipophilic dyes such as LipidTox/BODIPY only stain LDs that have acquired substantial neutral lipids. We also used seipin knockout cells as a control because seipin deficiency is known to impair early LD formation. In WT cells, when LD formation was induced by oleate addition, PLIN3 rapidly accumulated in small puncta that grew larger, and became BODIPY-positive within 20 min (Fig. 2a, b). In both seipin- and AGPAT2-deficient cells, there was a sharp increase in the number of PLIN3 puncta within 10 min of oleate treatment (Fig. 2a, b; Supplementary Fig. 2a). Moreover, there were much fewer BODIPY-positive LDs in AGPAT2-deficient cells within 10 min of oleate treatment than in WT cells (Fig. 2a, b; Supplementary Fig. 2b). In seipin-deficient cells, the delay in forming BODIPY-positive LDs is more...
pronounced as few PLIN3 puncta became BODIPY-positive after 18 min of oleate treatment (Fig. 2a, b; Supplementary Fig. 2b).

Reducing PA restores normal LD morphology in AGPAT2-deficient cells. We next aimed to determine the molecular basis for the aberrant LD biogenesis and formation under AGPAT2 deficiency. Knocking down DGAT1 or 2 with established siRNAs did not completely restore the normal size of LDs in AGPAT2-deficient cells (Fig. 3a, b)\(^3\). This suggests that mechanisms other than TAG synthesis may also be responsible for increased LD size. As mentioned above, an increase in whole cell PA in AGPAT2-deficient cells has been reported by different groups\(^1^1\)–\(^1^3\). This was further confirmed here by using a PA
Fig. 1 AGPAT2 depletion alters LD morphology. a The glycerol-3-phosphate (G-3-P) pathway for the synthesis of phospholipids and triacylglycerols. GPAT glyceraldehyde-3-phosphate dehydrogenase, AGPAT acylglycerol-3-phosphate acyltransferase, LPA lysophosphatidic acid, PA phosphatidic acid, DGDG diacylglycerol diacylglycerol, PS phosphatidylserine, PE phosphatidylethanolamine, PC phosphatidylcholine, TAG triacylglycerol, PG phosphatidylglycerol, PI phosphatidylinositol, CL cardiolipin. b Confocal imaging of LDs in control and HeLa, Huh7 and AML12 cells treated with AGPAT2 siRNA. The cells were treated with 20 nM siRNA for 24 h, followed by oleate treatment (400 μM) for 18 h. Blue represents DAPI staining, and green represents BODIPY staining. Bars: 5 μm. c, e, g Bar graphs show LD size distribution in HeLa, Huh7 and AML12 cells. Diameter of all detectable LDs in a cell was measured and represented by red (>3 μm), green (2–3 μm), white (1–2 μm) and black (0–1 μm) (n = 42–50 cells examined over 3 biologically independent experiments). d, f, h Quantification of LD diameters in HeLa, Huh7 and AML12 cells. Diameter of the largest LD in a cell was measured (mean ± SD; ***p < 0.0001, one-way ANOVA, n = 42–50 cells examined over three biologically independent experiments). i Human AGPAT2-mCherry or the catalytic dead mutant H98A was overexpressed in AGPAT2 siRNA. The cells were treated with 20 nM siRNA for 24 h, followed by oleate treatment (400 μM) for 18 h. Blue represents DAPI staining, and green represents BODIPY staining. Bars: 5 μm. e, g Bar graphs show LD size distribution in HeLa, Huh7 and AML12 cells. Diameter of all detectable LDs in a cell was measured (mean ± SD; ***p < 0.0001, one-way ANOVA, n = 42–50 cells examined over three biologically independent experiments). j Human AGPAT2-mCherry or the catalytic dead mutant H98A was overexpressed in AGPAT2 knocked-down HeLa cells. Cells were treated with 400 μM oleic acid for 18 h. Green represents BODIPY staining and red indicates mCherry expression. Bars: 5 μm. f Quantification of LD diameters after overexpressing vector, AGPAT2-mCherry, and AGPAT2-H98A-mCherry in AGPAT2 knockdown HeLa cells. Diameter of the largest LD in a cell was measured (mean ± SD; ***p < 0.0001, **p < 0.01, two-way ANOVA, n = 31–59 cells examined over three biologically independent experiments).

Reduced CDS protein expression and activity in AGPAT2-deficient cells. While examining the effect of CDS1/CDS2 expression on LD formation in AGPAT2-deficient cells, we noticed that the fluorescence intensity of CDS1/2, but not seipin, was much weaker in AGPAT2-deficient than WT HeLa cells (red fluorescence, Fig. 3c). We further verified this observation in Huh7 cells (Fig. 4a and Supplementary Fig. 4a). The loss of mCherry-CDS1/2 signal in AGPAT2-deficient cells suggests a possible reduction of CDS1/2 protein mass and activity. This could explain the increased PA in AGPAT2-deficient cells because CDS1/2-deficiency is known to result in the accumulation of PA and giant LDs.[15,30,32] We therefore examined the amount of CDS1 and CDS2 in WT and AGPAT2-deficient cells. Consistent with the imaging results, both HA-CDS1 and -CDS2 were reduced in AGPAT2-deficient cells (Fig. 4b–d). Moreover, the amount of endogenous CDS2 protein was significantly decreased in AGPAT2-deficient Huh7 cells (Fig. 4e). We were not able to quantify endogenous CDS1 due to the lack of a suitable antibody. The reduction in CDS1 and CDS2 was not due to transcriptional regulation, since CDS1/2 mRNA did not decrease in AGPAT2-deficient cells (Supplementary Fig. 4b). Similarly, AGPAT2 mRNA expression was not affected in CDS1/2 deficient cells despite a minor reduction in AGPAT2 protein (Fig. 4e and Supplementary Fig. 4b). The half-life of CDS2 is about 4–8 h in WT cells (Supplementary Fig. 4c–e). Under AGPAT2 deficiency, the steady state level of CDS1/2 was dramatically reduced and the effect of cycloheximide (CHX) became less clear (Supplementary Fig. 4c–e). Consistent with the reduction in protein expression, CDS activity was decreased by ~30% in AGPAT2-deficient cells relative to control cells (Fig. 4f). Moreover, overexpressing AGPAT2, CDS1 or CDS2 increased CDS activity (Fig. 4g and Supplementary Fig. 4f). Overexpressing AGPAT2 also caused a small but significant decrease of 14C oleate incorporation into TAG (Fig. 4h and Supplementary Fig. 4g). Finally, since there are five isoforms of AGPAT, we wonder if the loss of CDS1/2 was AGPAT2 specific. Knocking down AGPAT2, but none of the other AGPATs, consistently decreased CDS1/2 (Supplementary Fig. 4h–k). Together, these results support a specific functional relationship between AGPAT2 and CDS1/2.

AGPAT2 and CDS1/2 physically interact and form stable complexes. One possible explanation for the loss of CDS1/2 under AGPAT2 deficiency is that AGPAT2 may form a complex with CDS1/2 and promote their stability/activity. The interaction between AGPAT2 and CDS1/2 was therefore investigated. Human CDS1 and CDS2 both co-immunoprecipitated with human AGPAT2 with similar affinity (Fig. 5a, Supplementary Fig. 5a). As a control, seipin interacted with GPAT4, but not with AGPAT2 under the same conditions (Supplementary Fig. 5b). To further examine the AGPAT2-CDS interaction, we also expressed Strep-tagged AGPAT2 and Flag-tagged CDS1 or CDS2 in HEK293F cells, followed by a two-step affinity purification by anti-Flag and anti-Strep. A large amount of AGPAT2 and CDS1/2 co-purified as assessed by Coomassie blue, suggesting a direct and stable interaction (Fig. 5b and Supplementary Fig. 5c). To verify the interaction between AGPAT2 and CDSs, we tagged AGPAT2 with sfGFp and CDS2 with mScarlet at their respective genomic locus by CRISPR (Supplementary Fig. 5d). For unknown reasons, we were not able to tag CDS1 despite multiple attempts. Notably, knocking down AGPAT2 not only reduced the level of AGPAT2-sfGFp, but also that of CDS2-mScarlet, further demonstrating that AGPAT2 is required for the stability of CDS2 as shown in Fig. 4 (Supplementary Fig. 5d). Importantly, AGPAT2-sfGFp and CDS2-mScarlet coprecipitated (Fig. 5c). There are two isoforms of AGPAT2 and CDS2 appears to interact stronger with the longer isoform (Fig. 5c and Supplementary Fig. 5d). As a further proof of the dynamic interaction between AGPAT2 and CDS2, there appears to be enhanced colocalization between endogenous AGPAT2 and CDS2 when cells were cultured in low glucose media (Fig. 5d and Supplementary Fig. 5e). The enhanced colocalization was reversed upon adding back glucose. We further determined if the apparent interaction between AGPAT2 and CDS2 is unique since there are five AGPAT isoforms. Co-immunoprecipitation (co-IP) experiments using AGPAT1-5 and CDS1/2 identified AGPAT2 as the major AGPAT isoform to co-precipitate with CDS1/CDS2 (Fig. 5e–h; Supplementary Fig. 5f–i), consistent with its specific functional link with CDS1/2.

AGPAT2 promotes the flux of oleate through the CDP-DAG pathway. PA generated by AGPATs is a key branch point metabolite in the synthesis of phospholipids and TAGs: PA can be used directly by CDS1/CDS2 for the synthesis of CDP-DAG or by PAPs (e.g., lipins) for the synthesis of DAG. Substrate channeling often occurs at metabolic branch points[33,34]. AGPAT2

Sensor (GFP-PDE4A1) in AGPAT2 knockdown HeLa cells[31]: total fluorescence intensity of GFP-PDE4A1 in AGPAT2-deficient cells was higher than that in control cells, and GFP-PDE4A1 colocalized with calnexin, suggesting increased PA in the ER (Supplementary Fig. 3a, b). Among other possibilities, this increase in PA may underpin the formation of large LDs in AGPAT2-deficient cells. To examine this possibility, we overexpressed seipin, CDS1 and CDS2. Overexpressing any one of these three genes reduced cellular PA (Supplementary Fig. 3c, d) and almost completely abolished the formation of supersized LDs in AGPAT2-deficient cells (Fig. 3c–e). Together, these results suggest that increased PA is at least partially responsible for the abnormal LD formation in AGPAT2-deficient cells.
Fig. 2 AGPAT2 depletion impacted initial LD formation. a Control, seipin knockout (KO) and AGPAT2 knockdown HeLa cells deficient were starved in 1% LPDS for 16 h. Representative images show the localization pattern of endogenous PLIN3 (mCherry-tagged) and BODIPY puncta every two minutes after oleate addition (400 µM). Bars: 5 µm. b The number of PLIN3 and BODIPY puncta in control, seipin KO and AGPAT2 knockdown HeLa cells at indicated time points. Pair indicates colocalization of PLIN3 and BODIPY puncta. (mean ± SD; ****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05, two-way ANOVA, n = 15–20 cells examined over three biologically independent experiments).
Knocking down AGPAT2 reduced oleate incorporation into PI by 1.7-fold and to a lesser extent, into PG, while incorporation into TAG increased by ~40% (Fig. 6a). Conversely, overexpressing AGPAT2 increased oleate incorporation into PG (by ~100%) and PI (by ~30%) and reduced the flux to TAG by 3.3-fold (Fig. 6b).

As a control, overexpressing AGPAT1 had a modest effect on the rate of oleate incorporation into different lipids was shown in Supplementary Fig. 6c. The rate of 13C-oleate flux to PG and PI (Supplementary Fig. 6c). The rate of 13C-oleate incorporation into TAG by 3.3-fold (Fig. 6b).

AGPAT2-deficient liver has reduced CDS2 protein level and CDS activity in vivo. To confirm our findings on the specific functional connection between AGPAT2 and CDS1/2 in vivo, we generated a liver-specific AGPAT2 knockout mouse (A2LKO mice) by CRISPR/Cas9-mediated gene editing. The strategy used to disrupt Agpat2 in mice is shown in Supplementary Fig. 7a: two LoxP sites were introduced into the Agpat2 locus, sandwiching exons 2 and 4. Homozygous Agpat2fl/fl mice were then crossed with transgenic mice expressing Cre recombinase under the control of the albumin promoter. The resulting Agpat2fl/fl+Alb−creTg0 progeny was then crossed with Agpat2fl/fl mice to generate the A2LKO mice. Littermates lacking the Cre gene (Agpat2fl/fl) were used as controls and referred to as WT. The successful genomic disruption of Agpat2 was confirmed by the absence of the Agpat2 mRNA transcript and protein using western blotting and real-time quantitative PCR (Fig. 7a; Supplementary Fig. 7b). The level of PA in A2LKO liver increased almost three-fold (Fig. 7b), consistent with previous reports using other cell types/tissues.

Distinct from the global Agpat2-deficient (Agpat2−/−) mice, the A2LKO mice have normal brown and white adipose tissue weight (BAT and WAT) (Supplementary Fig. 7c, d). Food intake, body weight, liver and gastrointestinal weight of the A2LKO mice also appear normal (Supplementary Fig. 7e–h). Expression analyses showed little change in other AGPATs, CDS1/2 and most key metabolic genes in the liver of A2LKO mice except a mild (50%) increase in ACC2 (Supplementary Fig. 7i).

There was an increase of TAG but not total cholesterol (TC) in the liver of A2LKO mice fed either Chow or high fat diet (HFD) (Fig. 7c, d). There also appeared to be slightly more and larger LDs in A2LKO liver on Chow diet (Supplementary Fig. 7j, k). Notably, the liver of A2LKO mice on HFD appeared pale and enlarged, and the size of LDs increased dramatically, consistent with data from cell lines (Fig. 7e–h). Most importantly, the levels of hepatic CDS2 protein and hepatic CDS activity were significantly reduced in A2LKO mice (Fig. 7a and i). Moreover, oleate treatment of primary hepatocytes isolated from A2LKO mice resulted in enlarged LDs, which was reversed by...
overexpressing mCherry-CDS2 (cells with red signal) (Fig. 7j, k).

Overall, these results are consistent with data from cell lines and pull-down assays, and further support the functional relationship between AGPAT2 and CDS1/2.

Discussion

The mammalian genome encodes five putative AGPAT isoforms that catalyze a key step in the synthesis of phospholipids and TAGs: the acylation of LPA to PA. AGPAT2 is the only AGPAT isoform whose loss-of-function mutations are associated with BSCL1/CGL1. It has been puzzling that the direct product of AGPAT2, PA, is increased in AGPAT2-deficient cells. Our results from cellular studies, liver specific AGPAT2 knockout mice as well as biochemical analyses reveal that: 1. AGPAT2 regulates the biogenesis of cytoplasmic LDs; 2. The stability and activity of CDS enzymes are decreased in AGPAT2-deficient cells and mouse liver, contributing to the increased PA under AGPAT2 deficiency; 3. AGPAT2 and CDS1/2 form a stable functional complex possibly to channel PA for the synthesis of CDP-DAG and phospholipids of the CDP-DAG branch, e.g., PI and PG. Most importantly, our results imply substrate channeling at a major branch point of the glycerol-3-phosphate pathway.

The immediate product of AGPAT2, PA, sits at a metabolic branch point for the synthesis of all phospholipids and TAGs.
PA can be de-phosphorylated into DAG by PAPs (i.e. the lipins) for the synthesis of PC, PE, PS and TAG. PA can also be converted into CDP-DAG through the action of CDS1/2 for the synthesis of PI, and possibly PG and CL. Little is known about how the flux through this important metabolic branch point is regulated. Substrate channeling plays a key role in controlling flux at network branch points: the channeled metabolic intermediate is transferred directly from one enzyme to the next of the same biosynthetic pathway and is therefore prevented from being used by competing branch-point reactions. The channeling is often facilitated by the formation of specific enzyme assemblies/protein complexes. An AGPAT2–CDS1/2 complex would channel PA towards the synthesis of CDP-DAG, thereby decreasing its reaction with PAPs.
for DAG synthesis. Indeed, AGPAT2 is the only AGPAT isoform that specifically and directly interacts with CDS1 and CDS2. Strikingly, CDS proteins became unstable and CDS activity was significantly reduced under AGPAT2 deficiency, in both cell lines and mouse liver. Moreover, overexpressing AGPAT2 increased CDS activity, and reduced oleate incorporation into TAG. Consistently, metabolic flux analyses showed that the rate of oleate incorporation into PG/PI (the CDS branch) was increased upon overexpressing and decreased upon knocking down AGPAT2. It is also worth noting that the steady-state concentrations of PI and PG were significantly reduced in AGPAT2-deficient mouse embryonic fibroblasts, consistent with our present observations. Together, our data provide physical and functional evidence that AGPAT2 and CDS1/2 form complexes to promote the flux of PA into the CDP-DAG pathway. Without AGPAT2, CDS activity is reduced, causing accumulation of PA, aberrant LD formation and impaired adipogenesis. Although CDS1/2 and AGPAT2 can form rather stable complexes which can be co-purified in large quantity, we were unable to resolve the structures of the two complexes at this time despite multiple attempts. While CDS1 and CDS2 can both interact with AGPAT2, we were unable to detect a stable interaction between CDS1 and CDS2 (data not shown). Thus, AGPAT2 may associate with CDS1 or CDS2 separately, and the resulting distinct complexes may operate at different regions of the ER, and/or catalyze the synthesis of different CDP-DAG species, and/or function under different conditions. Future efforts are required to obtain insights into the structural details of the AGPAT2–CDS1/2 complexes and their specific functions.

The finding that AGPAT2′s product, PA, is increased in AGPAT2−/− cells and tissues has been confusing and paradoxical. We show here that the reduced CDS activity under AGPAT2 deficiency may be at least partially responsible for the increase in PA. Indeed, overexpressing CDS1/2 reduced the accumulation of PA and restore normal LD formation in AGPAT2−/− cells. Although CDS1/2 can clearly form functional complexes with AGPAT2, our results also suggest that CDS1/2 can use PA generated from other sources (e.g. other AGPATs, DAG kinases or phospholipase D) under AGPAT2 deficiency, CDS activity and the flux of oleate to PG and PI were significantly but only moderately reduced, suggesting that PA from other sources is available for use by the remaining CDS enzymes. There also appears to be increased flux of oleate to PA synthesis in AGPAT2−/− cells. Therefore, AGPAT2 deficiency may trigger a range of biochemical changes in addition to impaired CDS stability and activity, and the molecular basis for those changes requires further investigation.

Our results also reveal cell autonomous roles of AGPAT2 in LD formation. AGPAT2 deficiency delayed the maturation of initial LIDs and formed supersized LIDs after prolonged oleate treatment, phenotypes reminiscent of seipin deficiency. The delay in early LD lipidation in AGPAT2−/− cells is not as severe as that in seipin knockout cells. It should be noted that while seipin was knocked out, AGPAT2 was knocked down, because the AGPAT2 knock-out cancer cell lines were very sick in our hands and therefore not used. Nevertheless, these results further connect BSCL1 (AGPAT2) with BSCL2 (seipin) beyond adipogenesis. As PA has been implicated in seipin function, the increased PA in the ER may also underpin the effect of AGPAT2 deficiency on LD dynamics. Indeed, aberrant LD formation under AGPAT2 deficiency can be rescued by overexpressing CDS1/2 (consuming PA) or seipin (reducing PA production and increasing PA sequestration). Thus, our results highlight the role of non-bilayer lipids (e.g. PA) in LD biogenesis possibly by modulating the surface tension and curvature at sites of LD formation. To investigate the function of AGPAT2 in vivo, we generated the liver specific A2LKO. The LD phenotypes in AGPAT2−/− deficient liver and primary hepatocytes are consistent with those observed in cell lines. Importantly, CDS activity was significantly reduced in A2LKO liver, and the LD phenotype in AGPAT2−/− deficient primary hepatocytes was rescued by overexpressing CDS2. Overall, these results suggest that the reduced CDS activity in AGPAT2−/− deficient hepatocytes led to accumulation of PA in the ER, forming enlarged cytoplasmic LIDs. Although our data from biochemical assays, cell line and mouse studies strongly support a role for AGPAT2 to promote the synthesis of CDP-DAG branch of phospholipids, there are some limitations. We could not directly visualize the channeling of PA towards CDP-DAG synthesis in the AGPAT2−/− cells. This may be achievable after the structures of AGPAT2–CDS1/2 complexes are resolved. There is also some limitation in our flux studies because 13C-oleate can be incorporated into pre-existing phospholipids by the deacylation–recylation pathway. However, it should be noted that this pathway may be a minor contributor to 13C-oleate incorporation into phospholipids compared with the de novo synthesis pathway.

In summary, we provide strong evidence that AGPAT2 and CDS1/2 can form stable complexes which promote CDP-DAG synthesis. We also demonstrate a role for AGPAT2 in normal LD formation. Together, these results provide key insights into the regulation of the glycerol-3-phosphate pathway, unveil the molecular basis for the increase in PA under AGPAT2 deficiency and for the pathogenesis of BSCL1, and open future avenues of investigation on how the metabolism of PA is controlled at a major metabolic branch point.

Fig. 5 AGPAT2 and CDS1/2 physically interact. a Co-immunoprecipitation assay showing the interaction between AGPAT2-GFP and HA-CDS1/2 in HEK293E cells. n = 3 biologically independent experiments. b Streptagged AGPAT2 and Flag-tagged CDS1 or CDS2 were expressed in HEK293F cells alone or together as indicated, followed by either a one-step affinity purification by anti-Flag or anti-Strep or a two-step affinity purification by anti-Flag and then anti-Strep. The Coomassie blue-stained gel shows purification of CDS1, CDS2 and AGPAT2, and co-purification of CDS1/2 and AGPAT2. n = 3 biologically independent experiments. c Co-immunoprecipitation assay showing the interaction between endogenous AGPAT2-sfGFP and CDS2-mScarlet in HeLa cells. AGPAT2 or CDS2 was tagged at their genomic loci with sfGFP or mScarlet, respectively. Cells were treated with high glucose DMEM (basal) or low glucose DMEM for 48 h or re-incubation with high glucose DMEM (Refed) for another 24 h. Bars = 10 μm. n = 3 biologically independent experiments. d Confocal imaging of fixed HeLa cells showing AGPAT2 and CDS2 tagged at their genomic loci with sfGFP and mScarlet, respectively. e Co-immunoprecipitation of mCherry-tagged AGPAT1, 2, 3 and HA-tagged CDS1 from transfected HEK293E lysates. n = 3 biologically independent experiments. f Co-immunoprecipitation of mCherry-tagged AGPAT2, 4, 5 and HA-tagged CDS2 from transfected HEK293E lysates. n = 3 biologically independent experiments. g Co-immunoprecipitation of mCherry-tagged AGPAT2, 4, 5 and HA-tagged CDS2 from transfected HEK293E lysates. n = 3 biologically independent experiments. h Co-immunoprecipitation of mCherry-tagged AGPAT2, 4, 5 and HA-tagged CDS2 from transfected HEK293E lysates. n = 3 biologically independent experiments.
Incorporation were indicated as blue (increase) and red (decrease) arrows.

Methods

Antibodies, chemicals, plasmids, and primers. Antibodies, chemicals and plasmids with source and catalogue numbers are described in Supplementary Table 1. Primers used for cloning, for PCR and for knock-in (KI) are listed in Supplementary Table 2.

Cell culture, RNAi, and transfection. AML12, HeLa and Huh7 cells were grown in Dulbecco’s modified Eagle medium (DMEM) with 10% foetal bovine serum (FBS) and 1% penicillin–streptomycin–glutamine (PSG). Cells were maintained in 37 °C incubator with 5% CO₂. Medium was changed every 2 days. AML12 cells were grown in DMEM–nutrient mixture F-12 (DMEM/F12) with 10% FBS, 1% insulin–transferrin–selenium (ITS-G) and 40 ng/mL dexamethasone. 3T3-L1 cells were grown in DMEM with 10% Newborn calf serum (NCS) and 1% PSG.

Transient plasmid transfection was carried out by using Lipofectamine LTX. Plasmid DNA and Lipofectamine LTX were diluted in Opti-MEM™ I Reduced Serum Media separately, followed by 5 min incubation in room temperature. The mixture was incubated for another 10 min after mixed and added to the cells culture. Cells were harvested 48 h post transfection.

Transient small interfering RNA (siRNA) transfection was carried out by using Lipofectamine RNAiMAX. 20 nM siRNA and RNAiMAX (twice the volume of siRNA) were diluted in Opti-MEM™ I Reduced Serum Media separately, followed by 5 min incubation at room temperature. The mixture was incubated for another 20 min and added to cell culture. Cells were harvested 48 h post transfection.

Generation of KI cells with CRISPR/Cas12a-mediated genome editing. C-terminally mCherry-tagged PLIN3 was generated by CRISPR/Cas9 gene editing. HeLa cells were simultaneously transfected by Lipofectamine™ LTX Reagent with PLUS® Reagent (#15338100, Thermo Fisher Scientific) with MegaMer™ Single-Stranded DNA Fragments (Integrated DNA technologies) containing arms with 100-nucleotide-long homology upstream and downstream of the target site and gRNA targeting upstream of stop codon. The single-stranded DNA fragments and gRNA information are described in Supplementary Table 3. C-terminally superfolderGFP-tagged AGPAT2 and mScarlet-tagged CDS2 was generated by CRISPR/Cas12a gene editing method. A PCR cassette, a gene editing method. A PCR cassette, containing sgFPP/mScarlet, a Cas12a CRISPR RNA and ~100 bp homology arms, were amplified from pMax.Tag.P06 by Phusion™ high-fidelity DNA polymerase (#F-530L, Thermo Fisher Scientific). Primers were designed by http://www.pcreg- tagging.com/. HeLa cells were simultaneously transfected by Lipofectamine™ LTX Reagent with PLUS® Reagent (#15338100, Thermo Fisher Scientific) with equal amount of pcDNA3.1-hLScepT(TYCV) and PCR cassettes. For PLIN3-mCherry KI, CDS2-mScarlet KI and AGPAT2-sfGFP KI, cells were then selected with 2 µg/mL puromycin for 48 h and recovered for 1–2 weeks in the absence of puromycin. Single-cell FACs sorting was performed by BD FACSMelody™ Cell Sorter (BD Biosciences) at the flow cytometry (UNSW). 561 and 488 nm laser were used to sort mCherry and sfGFP-positive cells, respectively. To validate the insertion of tags, target regions were amplified by PCR and sequenced.

Viral stable transduction. Lentiv-293T® cells were used for lentiviral stable knockdown. Lentiv-293T® cells were plated at 2 × 10⁵ cells per 10 cm dish 24 h prior to transfection. Cells were transfected by using Lipofectamine LTX. For lentiviral production, 10 µg AGPAT2 shRNA, 2.970 µg pMD.G, 5.294 µg pMD.RRE and 1.848 µg pRSVre were transfected to Lentiv-293T® cells, followed by incubation in 37 °C with 5% CO₂ for 48 h. Media with virus were collected from LentiX-293T cells and filtered through a 0.45 µm filter, followed by the addition of 8 µg/mL polybrene. Lentivirus titre was tested by Lenti-X GoStix Plus. The filtered viral media was then added to the 3T3-L1 adipocytes and incubated for 24 h.

Adipocyte differentiation. To induce adipocyte differentiation, 3T3-L1 pre-adipocytes were grown in DMEM with 10% NCS and 1% PSG until 10% confluence. Two days post-confluence, differentiation was stimulated by using DMEM containing 10% FBS, 1% PSG and supplemented with insulin (10 µg/mL), dexamethasone (1 µM) and isobutylmethyxantine (IBMX) (0.5 mM). An additional 2 days later, cells were grown in DMEM/FBS/PSG with insulin (10 µg/mL) Media (DMEM/FBS/PSG) was refreshed every 2 days until the end of the differentiation.

Cell proliferation assay. 3T3-L1 cells were grown and differentiated in a 96-well plate. Cells were transduced with control and AGPAT2 shRNA lentivirus at day 6 of differentiation. Cell proliferation assay was performed at day 8 of differentiation by using CellTiter 96® AQueous One Solution (Promega) according to manufacturer’s protocol.

LDs studies. For fixed samples, cells were treated with 400 µM oleate-coupled BSA in DMEM/FBS/PSG for 18 h. Cells were rinsed twice with PBS and postfixed with 4% paraformaldehyde for 15 min at room temperature. Cells were rinsed three times with PBS before and after staining with freshly prepared 1 µg/mL BODIPY 493/503 (Thermo Fisher scientific) for 15 min. For immunofluorescence staining, cells were subsequently permeabilized with 0.2% TritonX-100 in PBS for 5 min at RT after fixation, and then blocked by incubation with 3% (w/v) BSA in PBS for 1 h at RT. Cells were than incubated with primary antibody and secondary antibody in 3% (w/v) BSA for 1 h at RT, with three 5 min PBS washes in between. Coverslips were then mounted onto slides by using ProLong® Gold Antifade Mountant with DAPI® (ThermoFisher Scientific). All images were obtained by ZEISS LSM 980 with Airyscan microscopy (Carl Zeiss, Jena, Germany) with ×63 Plan Apochromat (1.4 NA) oil objective. Acquired images were quantified by ImageJ software.
Fig. 7 Ablation of AGPAT2 from mouse liver reduces hepatic CDS activity. a Western blotting of MTP, CDS2 and AGPAT2 from primary hepatocytes isolated from WT (Agpat2<sup>f/f</sup>, n = 4) and liver-specific AGPAT2 knockout mice (A2LKO/Agpat2<sup>f/f</sup>, Alb-cre, n = 4). b Total PA levels of WT (n = 4) and A2LKO (n = 4) mice fed chow diet. c Liver TAG and d total cholesterol (TC) of WT (n = 6–9) and A2LKO (n = 5–7) mice fed chow or HFD. e Liver morphology and f the ratio of liver to body weight from WT and A2LKO mice fed HFD. Bars: 50 μm. g Distribution of LDs of different diameters as shown in (g). i CDS activity of WT (n = 4) and A2LKO liver (n = 4). j Primary hepatocytes from WT and A2LKO chow-fed mice were transfected with mCherry-CDS2 and stained with BODIPY. Dashed line indicates cells transfected with mCherry-CDS2. k Distribution of LDs according to diameters as shown in (j). b–d, f and f mean ± SD; two-tailed unpaired t test. *p < 0.05, **p < 0.01, ****p < 0.0001.
Live cell imaging. For live-cell imaging, HeLa cells were cultured in DMEM/10% FBS/PSG. Pre-LDs were removed by starving the cells with 1% LPDS in DMEM for 24 h. All live cell imaging experiments were performed at 37 °C, 5% CO2 in FluroBrite DMEM supplemented with 1% LPDS and Prolong Live Antifade (ThermoFisher Scientific). Equal volumes of media containing two times concentrated oleate–coupled BSA (100 µM) and BODIPY (1:5000) was added to the dishes immediately before image acquisition. HILO microscopy was performed using a Leica TCS SPE confocal microscope equipped with a high-sensitivity Andor Ikon EMCCD cameras fitted with a ×63, NA 1.4 Plan-Apochromat lens. Images were obtained every 1.26 s. Acquired live cell images were deconvoluted and quantified by custom MATLAB scripts.

SDS-PAGE and immuno-blotting. Cells were washed twice with ice-cold PBS and lysed by adding 0.1% SDS lysis buffer (1% Triton X-100, 0.1% SDS, 10 mM Tris pH 7.4, 150 mM NaCl, 0.1% NP-40). CDS1/2- and AGPAT2-Flag tagged proteins were eluted with a high-sensitivity Andor Ikon EMCCD cameras fitted with a ×63, NA 1.4 Plan-Apochromat lens. Images were obtained every 1.26 s. Acquired live cell images were deconvoluted and quantified by custom MATLAB scripts.

RNA extraction and quantitative real-time PCR. Total RNA was extracted using TRIzol™ reagent. Mammalian cells were grown in 6 cm Petri dishes. Cells were washed with ice-cold PBS once, followed by adding 1 mL TRIzol™ reagent. RNA extraction and quantitative real-time PCR analyses were performed using an Agilent 6560 ion mobility Q-TOF LC MS/MS analysis. For butanol extraction, cells were washed twice with PBS, 1% BSA, followed by incubation with primary antibody with appropriate dilution at 4 °C for 16 h. After primary antibody incubation, membranes were wash with TBST three times for 5 min each and then incubated with secondary antibody at 1:5000 dilution in TBST for 1 h at room temperature. Membranes were washed again with TBST three times for 5 min each and developed by using enhanced chemiluminescence and BioRad ChemiDoc XRS+ imaging system.

Immunoprecipitation. Protein lysates (1 mg) were mixed with 25 µL HA-tag agarose beads to a final volume of 500 µL per sample by using co-IP lysis buffer (25 mM HEPES pH 7.4, 150 mM NaCl, 0.1% NP-40) (3:2) for 30 min in a fume hood. After centrifuging at 100,000 × g for 10 min at 4 °C, the pellet was washed with ice-cold PBS once, followed by adding 1 mL TRIzol™ reagent and 30 µL 2× Protein loading buffer (with β-mercaptoethanol) for 5 min at room temperature. The mixture was then extracted by removing the supernatant and centrifuging at 100,000 × g for 1 h at 4 °C. 200 µM Egg PA was dried down by speedVac and resuspended in 125 mM Tris–HCl (pH 8.0), 250 mM KCl, 12.75 mM Tris–HCl, 100 mM NaCl, 0.2 mM ethylene glycol tetraacetic acid (EGTA), and 1/100 (v/v) protease inhibitor cocktail (EDTA-Free). Images were obtained every 1.26 s. Acquired live cell images were deconvoluted and quantified by custom MATLAB scripts.

CS1/2-AGPAT2 co-purification assay. The full-length CDNA of human CS1, CD2 and AGPAT2 were subcloned into the pCAG vector, tagged with an N-terminal Flag tag for CS1/2 and a C-terminal Strep tag for AGPAT2. The recombinant Flag-CD1, CD2 and AGPAT2-Strep were co-expressed in HEK293F cells. The cells were transiently transfected at a density of 2.0 × 106 cells per mL, using polyethyleneimine (PEI) (Polysciences). A 100-µL cell culture was transfected with 0.075 µg of CDS1 or CDS2 plasmid, and 0.075 µg of AGPAT2 plasmid. After 12 h, the cell culture was supplemented with 10 mM sodium butyrate, to boost protein expression. After another 48 h, the cells were collected by centrifugation and were resuspended in buffer containing 25 mM Tris pH 8.0, 150 mM NaCl, and protease inhibitor cocktails (Amresco). Membranes were solubilized at 4 °C for 2 h with 1% (v/v) GDN (Anatrace). After centrifugation at 20,000 × g for 45 min, the supernatant was added to anti-Flag G1 affinity resin (GenScript). The resin was rinsed with buffer W (25 mM Tris pH 8.0, 150 mM NaCl, 0.02% (v/v) GDN, 5 mM ATP, 5 mM MgCl2), and bound protein was eluted with buffer E1 (25 mM Tris pH 8.0, 150 mM NaCl, 0.02% (v/v) GDN, 200 µg/mL Flag peptide). The eluent was then applied to Strept Tactin resin (IBA Lifesciences). After rinsing with buffer W, bound protein was eluted with buffer E2 (25 mM Tris pH 8.0, 150 mM NaCl, 0.02% (v/v) GDN, 200 µg/mL Flag peptide). The eluant was analysed by SDS–PAGE and stained with Coomassie blue dyes.

CDS activity assay. CDS activity assay was performed as described32. Huh7 cells were resuspended in ice cold lysis buffer (50 mM Tris–HCl (pH 8.0), 50 mM KCl, 0.2 mM ethylene glycol tetraacetic acid (EGTA), and 1/100 (v/v) protease inhibitor cocktail) and lysed by 30 passes through homogenizer. Nuclear and unbroken cells were removed by centrifugation at 1000 x g for 10 min at 4 °C. Membrane fraction was then extracted by removing the supernatant and centrifuging at 100,000 × g for 1 h at 4 °C. 200 µM Egg PA was dried down by speedVac and resuspended in 125 mM Tris–HCl (pH 8.0), 250 mM KCl, 12.75 mM Tris–HCl, 100 mM NaCl, 0.2 mM ethylene glycol tetraacetic acid (EGTA), and 1/100 (v/v) protease inhibitor cocktail) and lysed by 30 passes through homogenizer. Nuclear and unbroken cells were removed by centrifugation at 1000 x g for 10 min at 4 °C. Membrane fraction was then extracted by removing the supernatant and centrifuging at 100,000 × g for 1 h at 4 °C. Live cell imaging, 12
Lipidomic data processing and analysis. Peak detection and lipid identification was performed using Agilent Lipid Annotator 1.0. The list of identified lipids was converted into .cvm file and used by MAVEN software as a library to analyse data acquired from stable isotope tracing experiment. Unlabelled and labelled intensities for each lipid were extracted using an unlabelled pool ($X_0$) of each lipid class was calculated by summing up the intensities of unlabelled ($M + 0$) lipids, a labelled pool ($X_1$) of each lipid class was calculated by summing up the intensities of $13$C-oleate-containing lipids ($M + 18$, $M + 36$ and $M + 54$). Fluxes through individual lipid classes were calculated using kinetic flux profiling approach, assuming cells were under metabolic steady state.

Analyses of liver PA. Lipids were extracted from mouse liver slices following a modified Bligh and Dyer’s protocol as previously described. The obtained lipid dome was quantified using a targeted lipidomic approach. Analyses of PA were conducted on a system comprising an Exion-UPLC coupled with a 6500 Plus QTRAP mass spectrometer (Sciex).

Neutral lipid extraction. Hela cells were grown in 10-cm dishes. 400 µM oleate was added to cells for 18 h when cells reached 80–90% confluence. A Triglyceride Assay Kit (Abcam) was used to determine the TAG levels according to the manufacturer’s protocol.

Generation of the A2LKO mice. The Ethics Committee at Model Animal Research Center of Nanjing University approved all animal procedures used in this study, which comply with all relevant ethical regulations. Mice were housed under a light/dark cycle of 12 h with free access to food and water unless otherwise stated. Liver specific Agpat2 knock out mice in C57Bl/6J background were generated by the transgenic facility at Nanjing University in collaboration with GenPharmatech Co. The CRISPR/Cas9-based strategy used to disrupt Agpat2 in mice is outlined in Fig. S7A: two Loxp sites were introduced into the Agpat2 locus, sandwiching exons 2 and 4. Briefly, sgRNA was transcribed in vitro and donor vector was constructed accordingly. Cas9, sgRNA and donor were microinjected into the fertilized eggs of C57BL/6J mice. Fertilized eggs were then transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6J mice. Homozygous Agpat2−/− mice were then crossed with transgenic mice expressing Cre recombinase under the control of the albumin promoter. The resulting Agpat2−/− Alb-creERT2 progeny was then crossed with Agpat2+/- mice to generate the A2LKO mice. Diet-induced obesity studies were carried out by feeding mice a HFD (60% calories from fat, Research Diets Di12492) for 15 weeks at the age of 8 weeks.

Triglycerol and cholesterol levels in liver. For Liver TAG levels, frozen liver chunks were sonicated in ethanolic KOH, and the extracts were subsequently neutralized. The resultant free glycerol was determined with the Free Glycerol Reagent (F6428, Sigma-Aldrich) using glycerol (Sigma-Aldrich) as standard for calculation. For liver TC levels, lipids were extracted with chloroform:isopropanol:NP-40 (7:1:0.1), and TC was measured after removal of organic solvent using LabAssay Cholesterol kit (Wako Chemicals, USA).

Primary mouse hepatocyte isolation and cell transfection. Primary mouse hepatocytes were isolated from 3-month-old mice using a collagenase-based method and cultured as previously described. Hepatocytes from preparations with a cell viability ≥90% were seeded in six-well plates at a density of 1 × 10⁶ per well in a plating medium (M199 with GlutaMAXTM supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.1% bovine serum albumin (BSA), 10% (v/v) foetal bovine serum, 10 mm N-isobutyl-N-(4-morpholin)pyridinium-1-iodide (BAPTA-AM; Thermo Fisher Scientific).

Immunofluorescence staining and imaging. Primary hepatocytes were fixed by 4% paraformaldehyde, followed by permeabilization with 0.1% Triton X-100 in PBS for 10 min. The cells and the frozen tissue sections were stained by DAPI and BODIPY for 30 min. Coverslips were then mounted onto slides by using 50% glycerol in PBS. All images were obtained by Olympus FV1000 laser scanning confocal microscope (Olympus, Tokyo, Japan) with x60 Plan Apochromat (1.4 NA) oil objective. Acquired images were quantified by ImageJ software.

Primer information for qPCR analysis of expression of target genes. All primers and protocols used for expression analyses of selected mouse liver genes were from a previous study, except CDS1 and CDS2 (Supplementary Table 3).

CDS activity assay using mouse liver. CDS activity assay were carried out as described above except 100 µg liver tissues were used and the reaction was incubated at 30°C for 30 min.

Statistics and reproducibility. Data were analysed via t-test for two groups, or via one-way or two-way ANOVA for multiple groups using the Prism software (GraphPad, San Diego, CA, USA). Differences were considered statistically significant at p < 0.05.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. A reporting summary for this article is available as Supplementary Information file. The main data supporting the findings of this study are available within the article and its Supplementary Figures. The exact P values for the data are also included within the Source Data file. Additional details on datasets and protocols that support the findings of this study will be made available by the corresponding author upon reasonable request. Source data are provided with this paper.

Received: 1 October 2021; Accepted: 10 November 2021; Published online: 25 November 2021

References
1. Blumsohn, N. J. & Cockcroft, S. CDP-Diaclylglycerol syntheses (CDP): gateway to phosphorylcholine and cardiopin synthesis. Front. Cell Dev. Biol. 8, 63 (2020).
2. Bradley, R. M. & Duncan, R. E. The lysophosphatidic acid acyltransferases (acylglycerophosphate acyltransferases) family: one reaction, many roles. Curr. Opin. Lipidol. 29, 101–115 (2018).
3. Bradley, R. M. & Duncan, R. E. The lysophosphatidic acid acyltransferases (acylglycerophosphate acyltransferases) family: one reaction, many enzymes, many roles. Curr. Opin. Lipidol. 29, 101–115 (2018).
4. CDS activity assay using mouse liver. CDS activity assay were carried out as described above except 100 µg liver tissues were used and the reaction was incubated at 30°C for 30 min.
5. Data availability. A reporting summary for this article is available as Supplementary Information file. The main data supporting the findings of this study are available within the article and its Supplementary Figures. The exact P values for the data are also included within the Source Data file. Additional details on datasets and protocols that support the findings of this study will be made available by the corresponding author upon reasonable request. Source data are provided with this paper.

Received: 1 October 2021; Accepted: 10 November 2021; Published online: 25 November 2021

References
1. Blumsohn, N. J. & Cockcroft, S. CDP-Diaclylglycerol syntheses (CDP): gateway to phosphorylcholine and cardiopin synthesis. Front. Cell Dev. Biol. 8, 63 (2020).
2. Bradley, R. M. & Duncan, R. E. The lysophosphatidic acid acyltransferases (acylglycerophosphate acyltransferases) family: one reaction, many enzymes, many roles. Curr. Opin. Lipidol. 29, 101–115 (2018).
3. Bradley, R. M. & Duncan, R. E. The lysophosphatidic acid acyltransferases (acylglycerophosphate acyltransferases) family: one reaction, many roles. Curr. Opin. Lipidol. 29, 101–115 (2018).
4. CDS activity assay using mouse liver. CDS activity assay were carried out as described above except 100 µg liver tissues were used and the reaction was incubated at 30°C for 30 min.
5. Data availability. A reporting summary for this article is available as Supplementary Information file. The main data supporting the findings of this study are available within the article and its Supplementary Figures. The exact P values for the data are also included within the Source Data file. Additional details on datasets and protocols that support the findings of this study will be made available by the corresponding author upon reasonable request. Source data are provided with this paper.
9. Agarwal, A. K. et al. AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. Nat. Genet. 31, 21–23 (2002).

10. Agarwal, A. K. et al. Human 1-acylglycerol-3-phosphate O-acyltransferase isoforms 1 and 2: biochemical characterization and inability to rescue hepatic steatosis in Agpat2−/− mice. J. Biol. Chem. 286, 37676–37691 (2011).

11. Cortes, V. A. et al. Molecular mechanisms of hepatic steatosis and insulin resistance in the AGPAT2-deficient mouse model of congenital generalized lipodystrophy. Cell Metab. 9, 165–176 (2009).

12. Cautivo, K. M. et al. AGPAT2 is essential for postnatal development and maintenance of white and brown adipose tissue. Mol. Metab. 5, 491–505 (2016).

13. Gale, S. E. et al. A regulatory role for 1-acylglycerol-3-phosphate-O-acyltransferase 2 in adipocyte differentiation. J. Biol. Chem. 281, 11082–11089 (2006).

14. Pagac, M. et al. SEIPIN regulates lipid droplet expansion and adipocyte development by modulating the activity of glycerol-3-phosphate acyltransferase. Cell Rep. 17, 1546–1559 (2016).

15. Shin, J. J. & Loewen, C. J. Putting the pH into phosphatidic acid signaling. Front. Cell. Dev. Biol. 7, 1546 (2019).

16. Pagac, M. et al. SEIPIN regulates lipid droplet expansion and adipocyte development by modulating the activity of glycerol-3-phosphate acyltransferase. Cell Rep. 17, 1546–1559 (2016).

17. Yan, R. et al. Human SEIPIN binds anionic phospholipids. Dev. Cell 47, 248–256 e244 (2018).

18. Sotlysák, K. et al. Nuclear lipid droplets form in the inner nuclear membrane in a seipin-independent manner. J. Cell Biol. 220, https://doi.org/10.1083/jcb.22005026 (2021).

19. Tsukahara, T. et al. Phospholipase D2-dependent inhibition of the nuclear hormone receptor PPARα by cyclic phosphatidic acid. Mol. Cell 39, 421–432 (2010).

20. Stapleton, C. M. et al. Lysocephosphatic acid activates peroxisome proliferator activated receptor-gamma in CHO cells that over-express glycerol-3-phosphate acyltransferase-1. PLoS ONE 6, e18093 (2011).

21. Shin, J. I. & Loewen, C. J. Putting the pH into phosphatidic acid signaling. BMC Biol. 9, 85 (2011).

22. Gao, M., Huang, X., Song, B. L. & Yang, H. The biogenesis of lipid droplets: lipids take center stage. Prog. Lipid Res. 75, 100989 (2019).

23. Chung, J. et al. LDAF1 and seipin form a lipid droplet assembly complex. Dev. Cell 51, 551–569 (2019).

24. Szymanski, K. M. et al. The lipophosphatidyl protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. Proc. Natl Acad. Sci. USA 104, 20890–20895 (2007).

25. Yamashita, A. et al. Glycerophosphate/acyglycerophosphate acyltransferases. Biology (Basel) 3, 801–830 (2014).

26. Walther, T. C., Chung, J. & Farese, R. V. Jr Lipid Droplet Biogenesis. Annu. Rev. Cell Dev. Biol. 33, 491–510 (2017).

27. Kassan, A. et al. Acyl-CoA synthetase 3 promotes lipid droplet biogenesis in ER microdomains. J. Cell Biol. 203, 985–1001 (2013).

28. Ben Mabrek, K. et al. ER membrane phospholipids and surface tension control cellular lipid droplet formation. Dev. Cell 41, 591–604 e597 (2017).

29. Wang, H. et al. Seipin is required for converting nascent to mature lipid droplets. eLife 5, https://doi.org/10.7554/eLife.16582 (2016).

30. Xu, Y. et al. CDP-DAG synthase 1 and 2 regulate lipid droplet growth through distinct mechanisms. J. Biol. Chem. 294, 16740–16755 (2019).

31. Kassan, N. et al. Comparative characterization of phosphatidic acid sensors and their localization during frustrated phagocytosis. J. Biol. Chem. 292, 4266–4279 (2017).

32. Qi, Y. et al. CDP-diacylglycerol synthases regulate the growth of lipid droplets and adipocyte development. J. Lipid Res. 57, 767–780 (2016).

33. Seree, P. A. Complexes of sequential metabolic enzymes. Annu. Rev. Biochem. 56, 89–124 (1987).

34. Lee, J. & Ridgway, N. D. Substrate channeling in the glycerol-3-phosphate pathway regulates the synthesis, storage and secretion of glycerolipids. Biochim. Biophys. Acta Mol. Cell. Biol. Lipids 1865, 158438 (2020).

35. Reue, K. & Dwyer, J. R. Lipin proteins and metabolic homeostasis. J. Lipid Res. 50(Suppl.), S109–S114 (2009).

36. Han, G. S., Wu, W. J. & Carman, G. M. The Saccharomyces cerevisiae Lipin homolog is a Mg2+–dependent phosphatidate phosphatase enzyme. J. Biol. Chem. 281, 9210–9218 (2006).

37. Sankella, S., Garg, A., Horton, J. D. & Agarwal, A. K. Hepatic gluconeogenesis is enhanced by phosphatidic acid which remains uninhibited by insulin in lipodystrophic Agpat2−/− mice. J. Biol. Chem. 289, 4762–4777 (2014).

38. Salo, V. T. et al. Seipin regulates ER-lipid droplet contacts and cargo delivery. EMBO J. 35, 2699–2716 (2016).