Ammonia stimulates SCAP/Insig dissociation and SREBP-1 activation to promote lipogenesis and tumour growth

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Tumorigenesis is associated with elevated glucose and glutamine consumption, but how cancer cells can sense their levels to activate lipid synthesis is unknown. Here, we reveal that ammonia, released from glutamine, promotes lipogenesis via activation of sterol regulatory element-binding proteins (SREBPs), endoplasmic reticulum-bound transcription factors that play a central role in lipid metabolism. Ammonia activates the dissociation of glucose-regulated, N-glycosylated SREBP-cleavage-activating protein (SCAP) from insulin-inducible gene protein (Insig), an endoplasmic reticulum-retention protein, leading to SREBP translocation and lipogenic gene expression. Notably, 25-hydroxycholesterol blocks ammonia to access its binding site on SCAP. Mutating aspartate D428 to alanine prevents ammonia binding to SCAP, abolishes SREBP-1 activation and suppresses tumour growth. Our study characterizes the unknown role, opposite to sterols, of ammonia as a key activator that stimulates SCAP–Insig dissociation and SREBP-1 activation to promote tumour growth and demonstrates that SCAP is a critical sensor of glutamine, glucose and sterol levels to precisely control lipid synthesis.

Lipids form the basic structure of the plasma membrane and of all cellular organelle membranes, which makes gaining sufficient lipids a precondition for cell growth and proliferation1–4. Under physiological conditions, lipid levels are mainly regulated by SREBPs, a family of transcription factors that include three isoforms, SREBP-1a, SREBP-1c and SREBP-2 (refs. 5–7). SREBP-1c mainly regulates the expression of genes containing fatty acid synthesis, whereas SREBP-2 regulates cholesterol synthesis and uptake and SREBP-1a, which has the highest transcriptional activity and regulates all processes2,8–10. Recently, a series of studies from our group and others have demonstrated that SREBP-1 is highly activated in malignancies such as glioblastoma (GBM), liver, breast and colorectal cancers11–17. Nevertheless, the regulation mechanisms of SREBP-1 activation and lipid metabolism in cancer cells remain elusive.

SREBPs are synthesized as inactive precursors (~125kD) that are retained in the endoplasmic reticulum (ER) membrane and are activated through a tightly controlled ER–Golgi–nucleus translocation process9,10. SREBPs first bind to SCAP, which further binds to COPII-coated vesicles that transport the SCAP–SREBP complex from the ER to the Golgi18. In the Golgi, SREBPs are sequentially cleaved by site-1 and site-2 proteases, which release their N-terminal forms (~65kD) that then enter into the nucleus to activate lipogenic gene expression19–21. However, the trafficking of the SCAP–SREBP complex is suppressed by the ER-retention protein, insulin-inducible gene protein (Insig), which includes two isoforms, Insig-1 and Insig-2 (refs. 24,25). Insig binds to SCAP to retain the SCAP–SREBP complex in the ER19. Previous studies have revealed that cholesterol or 25-hydroxycholesterol (25-HC) can bind to SCAP or Insig to enhance their association, which mediates a negative feedback loop to modulate SREBP activation26–28; however, the key step activating the dissociation of SCAP from Insig for subsequent translocation remains unclear.

Our recent study demonstrated that glucose stimulates SREBP activation and lipogenesis by promoting SCAP N-glycosylation and stability26–31. In this study, we unexpectedly found that when glutamine is lacking, glucose alone is unable to activate SREBPs and lipogenesis despite low cholesterol levels and stable SCAP N-glycosylation. We unveiled that N-glycosylated SCAP requires the stimulation of ammonia released from glutamine to undergo sequential conformational changes to dissociate from Insig and promote SREBP translocation and lipogenesis. We identified the binding site of ammonia in the central location of SCAP transmembrane domain, including D428 and serine S326/S330 residues and demonstrated that the function of ammonia is prevented by 25-HC, which blocks access to its binding site on SCAP, thereby suppressing SCAP–Insig dissociation and SREBP activation. Our study further suggests that targeting the key molecular link between glutamine, glucose and lipid metabolism is a promising strategy for treating malignancies and metabolic syndromes.
Results
Glutamine is necessary for SREBP activation and lipogenesis.
In addition to glucose, cancer cells also consume large amounts of glutamine, the most abundant amino acid in human blood, and require dramatically elevated lipogenesis to promote tumour growth. Whether there is an intrinsic molecular connection between glutamine, glucose and lipid synthesis is unknown. To test this, we first conducted a transcriptome analysis using RNA sequencing in lung cancer H1299 cells to determine the response of lipogenic genes to absence versus presence of glutamine (Gln) or glucose (Gluc). Unexpectedly, neither glutamine nor glucose alone was able to activate the expression of genes regulating fatty acid and cholesterol synthesis and uptake, including SREBF1, SREBF2, ACAT1, ACACA, FASN, SCD1, HMGCR and LDLR, as compared to absence of both (Fig. 1a and Extended Data Fig. 1a). Notably, activation of lipogenic genes required the presence of both glutamine and glucose, but SCAP gene expression was not affected by either glutamine or glucose (Fig. 1a). These results were validated by real-time PCR (Fig. 1b).

We next examined whether glutamine is required for SREBP activation using multiple cancer cell lines. Western blot analysis showed that glucose alone failed to activate the cleavage of SREBP-1 and SREBP-2 (Fig. 1c). The expression of fatty acid synthase (FASN) and stearoyl-CoA desaturase-1 (SCD1), two key downstream targets of SREBP-1, was also not activated by glucose alone (Fig. 1c). Only the combination of glutamine and glucose was able to strongly induce cleavage of SREBP-1 and -2, as demonstrated by the dramatic increase of their N-terminal or C-terminal products and the upregulation of FASN and SCD1 expression (Fig. 1c). Furthermore, western blot analysis showed that glutamine in the presence of glucose activated SREBP-1 and SREBP-2 cleavage and FASN and SCD1 expression in a dose- and time-dependent manner (Extended Data Fig. 1b,c). Radioisotope assays showed that glutamine significantly increased lipid synthesis derived from glucose (Extended Data Fig. 1d). Moreover, SREBP activation by glutamine and glucose closely correlated with the high proliferation rate of all tested cancer cells, as compared to lack of cell proliferation under glucose or glutamine alone conditions (Extended Data Fig. 1e). Cell proliferation under these conditions was completely abolished by genetic inhibition of SREBP-1 via shRNA, whereas it was only slightly reduced by SREBP-2 knockdown (Extended Data Fig. 1f,g), demonstrating that SREBP-1 activation plays a major role in the cell proliferation.

As cholesterol has been demonstrated to be a critical negative regulator of SREBP activation, we examined whether cholesterol reduction is sufficient to activate SREBPs. Notably, severely reducing cholesterol levels by inhibiting its biosynthesis via atorvastatin in cholesterol-deficient medium failed to activate SREBP-1 and SREBP-2 cleavage in the absence of glutamine (Extended Data Fig. 1h). We previously demonstrated that EGFR–PI3K–Akt signaling promotes SREBP-1 activation by increasing glucose uptake and SCAP N-glycosylation. In the absence of glutamine, activating EGFR–PI3K–Akt signaling by EGF did not result in SREBP-1 activation, even when glucose was present (Extended Data Fig. 1i). We also examined whether other amino acids could play a critical role in SREBP-1 activation. The RPMI 1640 cell culture medium contains all 20 common amino acids. When removing only glutamine, with all other 19 amino acids and glucose remaining in the medium for lung cancer cell culture (H1299, H1975 and HCC4006), western blotting showed that SREBP cleavage and expression of FASN and SCD1 failed to be activated (Fig. 1c). We selected five amino acids, aspartate, asparagine, leucine, methionine and threonine, for confirmation. When lacking glutamine, none of these amino acids in combination with glucose was able to activate SREBP cleavage and FASN/SCD1 expression (Extended Data Fig. 1j). These data confirmed that glutamine is the key amino acid controlling lipogenesis activation.

We next examined whether the lack of glutamine would affect SCAP protein stability, thereby leading to the inactivation of SREBPs. Consistent with our previous study, western blot analysis showed that absence of glucose resulted in SCAP degradation and inactivation of SREBP-1 cleavage, as reflected by the lack of N-terminal SREBP-1 in the nuclear extracts (Fig. 1d; lane 1 and 3). In contrast, when glucose was present, removing glutamine had no influence on SCAP protein levels as compared to its levels when combining glutamine and glucose (Fig. 1d; lane 2 and 4); however, these data were puzzling as we could not detect any active N-terminal SREBP-1 in nuclear extracts in the absence of glutamine (Fig. 1d; lane 2 versus lane 4). Immunofluorescence (IF) staining confirmed that SREBP-1 was unable to move to the nucleus unless glutamine and glucose were both present (Fig. 1e).

We also examined SCAP N-glycosylation under the same culture conditions in HEK293T cells expressing green fluorescent protein (GFP)–SCAP, as we previously published. The data showed that in the presence of glucose, removing glutamine had no influence on SCAP N-glycosylation (Fig. 1f; lane 3/4 versus lane 7/8, top) and GFP–SCAP protein levels (Fig. 1f; lane 2 versus lane 4, bottom). Together, these data demonstrate that glucose-mediated SCAP N-glycosylation is necessary for SCAP stability but is not sufficient for activation of SREBPs and lipogenesis that require the presence of glutamine, suggesting the existence of an important intrinsic molecular link connecting glutamine and glucose to lipid metabolism.

Glutamine-released ammonia activates SREBPs and lipogenesis. Glutaminolysis is known to be highly activated in many cancers. In this process, glutamine is first deaminated by glutaminase (GLS) to release ammonia (NH₃) and produce glutamate. Glutamate is further converted to α-ketoglutarate (α-KG), which incorporates into the tricarboxylic acid cycle for energy production. By using the Bioprofile 100 Plus Analyzer, we detected NH₃, which is converted to NH₄⁺ in aqueous solution, and glutamate, in the medium from H1299 and U87 cells cultured in the presence of glutamine without glucose (12 h) (Fig. 2a; middle). NH₃ and NH₄⁺ are thereafter referred to as ammonia. In contrast, when cells were cultured in the presence of glucose but without glutamate, lactate and glutamate were detected (Fig. 2a; top). When combining glutamine and glucose, all three metabolites (NH₄⁺, glutamate and lactate) were detected (Fig. 2a; bottom).

We then examined which of the metabolites was involved in SREBP activation. Western blot analysis showed that neither glutamate, ammonia (derived from added NH₄Cl) or lactate alone, nor the combination of glucose with glutamate or lactate were able to activate SREBP-1 or SREBP-2 cleavage and promote FASN and SCD1 expression (Fig. 2b). In contrast, in the presence of glucose, ammonia-induced SREBP-1 and SREBP-2 cleavage, and FASN and SCD1 expression to a similar extent as the combination of glutamine and glucose did (Fig. 2b, lane 9 versus lane 6 and Extended Data Fig. 2a). The effects of ammonia were dose- and time-dependent (Fig. 2c,d). NaCl supplementation had no effect on SREBP cleavage and FASN/SCD1 expression (Extended Data Fig. 2a,b). IF imaging further showed that in the presence of glucose, ammonia markedly stimulated SREBP-1 translocation into the nucleus without the presence of glutamine (Fig. 2e), whereas glutamate, lactate or α-KG stimulation failed to do so (Extended Data Fig. 2c). We also confirmed that the glutamate and α-KG added to the medium were taken up by tumour cells, whereas their addition in combination with glucose had no effects on SREBP cleavage and downstream FASN/SCD1 expression (Extended Data Fig. 2d–f). Ammonia has previously been reported to upregulate autophagy. Western blot analysis showed that knockdown of ATG5 to block autophagy was unable to abolish glutamine- and ammonia-stimulated SREBP cleavage and FASN/SCD1 expression (Extended Data Fig. 2g), excluding the involvement of autophagy in ammonia-mediated SREBP activation.
Glutamine is necessary for SREBP activation and lipogenesis. a. Heat map of gene expression analyzed by RNA-seq in H1299 cells. H1299 cells were cultured in full RPMI 1640 medium supplemented with 5% FBS for 24 h. Cells were then washed with PBS once and placed in fresh serum-free medium with or without glutamine (4 mM) or glucose (5 mM) for 12 h before analysis. b. Real-time PCR analysis of gene expression in H1299 cells under the same culture condition shown above. The results are shown as mean ± s.e.m. (n = 3). SREBF1, the gene name of SREBP-1; ACLY, ATP citrate lyase; ACACA, acetyl-CoA carboxylase-α; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase-1; SREBF2, the gene name of SREBP-2; LDLR, low-density lipoprotein receptor; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase. c. Western blot analysis of whole lysates from different cancer cells under the same culture conditions as in a. U87, LN229, T98, M233, GBM30, HepG2 and MDA468 were cultured in DMEM and H1299, H1975 and HCC4006 in RPMI 1640 medium. P, precursor of SREBP; N, N-terminus of SREBP-1. C, C-terminus of SREBP-2. d. Western blot analysis of membrane (for SCAP) and nuclear extracts (for N-terminal SREBP-1) from different cancer cells under the same culture conditions as in a.c. Protein disulfide-isomerase (PDI), an ER-resident protein. e. Representative IF images of anti-SREBP-1 staining (red) in H1299 and U87 cells under the same culture conditions as in a.c. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) (blue). Scale bars, 10 μm. The nuclear intensity of SREBP-1 was quantified over 30 cells by ImageJ (mean ± s.e.m., n ≥ 30) (right). f. Western blot analysis of SCAP N-glycosylation (top) and total GFP-SCAP (bottom) in cell membrane fractions from HEK293T cells placed in serum-free DMEM medium with/without the presence of glutamine (4 mM) or glucose (5 mM) for 12 h. The numbers on the left side of the blot indicate the number of N-glycosylated residues on the SCAP protein (top). PNGase F, 1 Unit. Two-way (b) or one-way analysis of variance (ANOVA) (e) with Dunnett’s multiple comparisons were performed.
We next examined whether ammonia activated ERK or Akt/mTOR signaling. Western blot analysis showed that ammonia, from 0.1 mM to 10 mM, did not increase the levels of p-ERK, p-Akt and p-S6, an mTOR downstream target in the presence of glucose (Extended Data Fig. 2h), which excluded the involvement of ERK or Akt/mTOR in the ammonia-promoted SREBP activation.

RNA-sequencing analysis in H1299 cells confirmed that ammonia, similarly to glutamine, significantly activated the expression of genes controlling the fatty acid and cholesterol synthesis pathways as compared to glucose alone (Fig. 2f), which was further confirmed by real-time PCR (Fig. 2g).

We further examined whether ammonia affected glucose-regulated SCAP stability and N-glycosylation. Western blot analysis showed that in the presence of glucose, ammonia had no effect on both SCAP protein and N-glycosylation levels compared to glucose alone (Fig. 2h,i), whereas it dramatically activated SREBP-1 and SREBP-2 cleavage and increased FASN and SCD1 expression (Fig. 2h). We further examined whether SCAP N-glycosylation...
remained necessary for glutamine- and ammonia-induced activation of SREBPs. We used our previously established GFP-labeled SCAP mutant construct, where all three asparagine residues (N) 263, 590 and 641 (NNN), the sites for N-glycosylation, were replaced with glutamine (QQQ)28, which was co-transfected with full-length SREBP plasmids in HEK293T cells. Either glutamine or ammonia in the presence of glucose strongly activated the cleavage of SREBP-1a, SREBP-1c and SREBP-2 isoforms in wild-type (WT) SCAP (NNN) transfected cells (Extended Data Fig. 2i–k), whereas the activation was lost in cells transfected with the SCAP mutant (QQQ) (Extended Data Fig. 2i–k), demonstrating that SCAP N-glycosylation is required for glutamine or ammonia to activate SREBPs.

Together, these data demonstrate that ammonia, released by glutaminolysis, is a key activator of SREBP activation and lipogenesis, which requires the presence of glucose to maintain SCAP stability via its N-glycosylation.

Inhibiting glutaminolysis abolishes SREBP activation. We next validated the role of ammonia released from glutamine in the stimulation of SREBP activation and lipogenesis. We suppressed glutamine uptake with γ-glutamyl-p-nitroanilide (GPNA), an inhibitor of SLC1A5 (also named ASC2T) that is the major glutamine transporter29 and blocked glutaminolysis with CB-839, a GLS inhibitor. Metabolite analysis showed that both GPNA and CB-839 treatment dramatically reduced the levels of glutamate, ammonia and α-KG in cells and in cell culture medium (Fig. 3a and Extended Data Fig. 3a), which was associated with a significant reduction of glutamine consumption (Extended Data Fig. 3b). Both inhibitors dramatically suppressed glutamine-stimulated SREBP activation and FASN and SCD1 expression (Fig. 3b and Extended Data Fig. 3c,d). We then supplemented cells with ammonia (by adding NH4Cl), glutamate or α-KG to determine which metabolite was able to restore SREBP activation inhibited by CB-839. Western blot analysis showed that only ammonia strongly restored SREBP activation and FASN/SCD1 expression (Fig. 3c and Extended Data Fig. 3e). IF imaging showed that CB-839 blocked SREBP-1 nuclear translocation, which was successfully restored by adding ammonia, but not glutamate (Fig. 3d). We further examined the effects of inhibition of GLS by CB-839 on SREBP-1 activation in an H1299-derived xenograft mouse model. Consistent with the reduction of ammonia levels in tumour tissues (Extended Data Fig. 3f, left), immunohistochemistry (IHC) staining showed that SREBP-1 levels were significantly reduced in tumours from mice treated with CB-839 compared to the vehicle treatment group (Extended Data Fig. 3f, right).

We also genetically inhibited GLS using lentivirus-mediated shRNA to suppress the release of ammonia from glutamine. GLS knockdown significantly reduced glutamine consumption and inhibited glutamate, ammonia and α-KG production (Fig. 3e and Extended Data Fig. 3g,h), and strongly suppressed SREBP activation and FASN/SCD1 expression (Fig. 3f). Supplementation of ammonia, but not glutamate or α-KG, dramatically restored SREBP activation and FANS/SCD1 expression (Fig. 3g and Extended Data Fig. 3i,j), which was confirmed by IF imaging of SREBP-1 nucleus translocation (Fig. 3h). Pharmacological and genetic inhibition of GLS also dramatically reduced the appearance of the N-terminal form of SREBP-1 in nuclear extracts (Fig. 3i), whereas they did not alter SCAP protein and N-glycosylation levels (Fig. 3i–k). In contrast, genetically knocking down glutamate dehydrogenase (GDH), asparaginase (ASPG) or serine deaminase (SDS) had no effects on SREBP-1 cleavage and FASN/SCD1 expression (Extended Data Fig. 3k,l).

Together, these data confirm that ammonia is released from glutamine to activate SREBPs and lipogenesis, unveiling a glutamine–GLS–ammonia–SREBP activation axis that links glutaminolysis and lipogenesis.

GLS and SREBP-1 are highly correlated in human tumours. We next examined whether the connection between glutaminolysis and lipogenesis could be validated in human tissues. We first analyzed seven paired tumours versus adjacent normal human lung tissues, from individuals with adenocarcinoma (adeno), squamous and large-cell lung cancer by western blot. The data showed that all seven tumour tissues contained high levels of GLS and strong SREBP-1 expression and cleavage, together with dramatically increased FASN protein in comparison with adjacent normal lung tissues (Fig. 4a). We then examined multiple paraffin-embedded tumour versus adjacent normal lung tissues. IHC staining showed that GLS expression, and cytoplasmic and nuclear SREBP-1 staining were highly elevated in tumour tissues (T), whereas both were low in adjacent normal tissues (N) (Fig. 4b and Extended Data Fig. 4a). We then measured the ammonia levels in ten paired lung tissues using an ammonia assay kit. Consistent with the elevation of GLS expression and SREBP-1 activation (Fig. 4a, b and Extended Data Fig. 4a), the data showed that ammonia levels were significantly higher in tumours than in paired healthy tissues (Fig. 4c). We next examined a tissue microarray (TMA) containing 99 tumours and 50 matched adjacent healthy lung tissues from individuals with different types of lung cancer. IHC staining showed that over 90% of lung tumour tissues contained high level of GLS and strong SREBP-1 staining as compared to adjacent healthy lung tissues (Fig. 4d, e and Extended Data Fig. 4b–d). Pearson correlation analysis showed that GLS expression was strongly correlated with SREBP-1 staining (Fig. 4f). Genetic knockdown of GLS in a xenograft model gave the same result as SREBP-1 knockdown, dramatically suppressing tumour growth in a H1299 cell-derived xenograft mouse model (Extended Data Fig. 4e).

We also examined multiple GBM tissues and a TMA with 91 glioma samples. IHC staining showed that high GLS expression and strong SREBP-1 staining were associated in tumour tissues across low- to high-grade gliomas (Fig. 4g–j and Extended Data Fig. 4f–h). Kaplan–Meier plot analysis further showed that higher GLS expression and stronger SREBP-1 staining were associated with poorer survival in individuals with GBM (Fig. 4k). We also determined the presence of other amino acid deaminases, specifically ASPG and SDS by IHC. Staining showed that neither enzyme was detected in GBM tumour samples (Extended Data Fig. 4f), confirming the specific positive correlation between GLS and SREBP-1 in patient tissues.

Together, these large clinical sample analyses demonstrated that GLS expression is significantly correlated with SREBP-1 activation in human cancers, providing strong evidence in support of the molecular connection between glutaminolysis and lipogenesis under physiological conditions.

Ammonia binds SCAP to activate its dissociation from Insig. As neither glutamine nor ammonia affected SCAP stability and N-glycosylation (Figs. 1d, f and 2h, i), we wondered whether they activated SCAP–Inos dissociation to promote SREBP activation (Fig. 5a). We co-transfected GFP–SCAP and Myc tag-labeled Insig-1 (Myc–Insig-1) in HEK293T cells and then incubated these cells with glutamine or ammonia (NH4Cl) in the presence of glucose. IF imaging showed that Myc–Insig-1 co-precipitated with SCAP when cells were treated with glutamine or ammonia (Fig. 5a). Immunoblotting showed that SCAP and Insig-1 did not co-precipitate when cells were treated with glucose alone (Fig. 5b).

Confocal microscopy imaging showed that glutamine or ammonia stimulated GFP–SCAP trafficking to the Golgi, as demonstrated by the colocalization of GFP–SCAP with Giantin (red), a specific Golgi marker (see arrows in Fig. 5c and Extended Data Fig. 5a). In contrast, GLS inhibition with CB-839 completely restored the binding of SCAP to Insig-1, as shown by co-IP (Fig. 5d). Confocal microscopy imaging further showed that GLS inhibition abolished glutamine-promoted GFP–SCAP trafficking to the Golgi, whereas
the trafficking was fully restored by adding ammonia (Fig. 5e and Extended Data Fig. 5a), demonstrating that glutamine-released ammonia stimulates SCAP dissociation from Insig to trigger its trafficking.

To confirm the effects of ammonia on SCAP trafficking, we conducted an in vitro SCAP ER-budding assay48, where ammonia was added to cell culture or directly to purified ER membrane extracts. Western blot analysis of isolated membranes from H1299 cells and subsequent ER-budding vesicles showed that when glutamine or ammonia was absent, SCAP was unable to bud from the ER membrane into vesicles, while ammonia (NH$_4$Cl) or glutamate strongly stimulated SCAP budding from the ER membrane (Extended Data Fig. 5b). In contrast, the budding of ER–Golgi recycling protein ERGIC–53 from the ER membrane did not require ammonia or glutamine stimulation (Extended Data Fig. 5b). Western blot analysis further showed that ammonia or glutamine stimulation did not stimulate the budding of ER-resident proteins Grp94, ribophorin I and BIP (Extended Data Fig. 5b). Together, these data strongly demonstrate that ammonia specifically stimulates SCAP exit from the ER for subsequent translocation.

To elucidate how ammonia interacts with SCAP, we employed co-solvent molecular dynamics (MD) simulations40 to map out the potential ammonia binding sites in SCAP using the recently published cryo-EM structure of the SCAP–Insig transmembrane 580

![Image](https://example.com/image.png)
Domain\(^8\). Notably, when 25-HC was absent, NH\(_4\)\(^+\), not NH\(_3\), was found to occupy a large sphere in the transmembrane region next to the S6 helix of SCAP (Fig. 5f and Extended Data Fig. 5c). We closely inspected this site and found that it is formed by three key residues: aspartate D428 from the S6 helix, and serine S326 and S330 from the S3 helix (Fig. 5g, top). Of note, D428 and S326/S330 are evolutionarily highly conserved residues in SCAP (Extended Data Fig. 5d). NH\(_4\)\(^+\) formed stable hydrogen bonds with the side chains of D428, S326 and S330 throughout the simulations, with the following affinities, D428 > S330 > S326 (Fig. 5g, bottom and Extended Data Fig. 5e). Western blot analysis confirmed these binding predictions, showing that changing the negatively charged aspartate to neutral alanine (D428A) completely abolished NH\(_4\)\(^+\) or glutamine stimulation on SREBP-1 activation. The S330A mutant modestly reduced SREBP-1 activation, whereas the double S326A/S330A mutant strongly reduced it (Fig. 5h). In contrast, there was no effect on SREBP-1 activation when mutating valine 353 (from S4 helix) to glycine (V353G) (Fig. 5g,h and Extended Data

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**Fig. 4 | GLS and SREBP-1 are highly correlated in human tumours.** a, Western blot analysis of paired tumour (T) versus adjacent normal (N) lung tissues from individuals with adenocarcinoma (adeno), squamous cell carcinoma (squamous) and large-cell carcinoma (large) lung cancer. b, Representative IHC images of human tumour versus adjacent healthy lung tissues. Scale bars, 50 μm. c, Ammonia levels in paired human lung tumours versus adjacent healthy lung tissues. Significance was determined by unpaired and two-tailed Student’s t-test. d, Representative IHC images of anti-GLS and -SREBP-1 staining from a lung cancer TMA (n = 99) that contains 50 paired adjacent healthy lung tissues (d). Scale bars, 100 μm. The levels of GLS and SREBP-1 staining were quantified by ImageJ and shown by H score (e). Red lines in the graphs show mean ± s.e.m. Data were analyzed by using one-way ANOVA followed by comparisons with healthy control with Dunnett’s multiple comparisons adjustment. f, Correlation between GLS and SREBP-1 levels in tissues from lung cancer TMA shown in c. Correlation coefficient (R) and significance were determined by a two-sided Pearson correlation test. P < 0.0001. g–i, Representative IHC images of human GBM tissues (g) or different types of glioma in a glioma TMA (n = 91) (h). Scale bars, 100 μm. GLS expression and SREBP-1 staining in TMA were quantified by ImageJ and H score (i). Red lines in the graphs show mean ± s.e.m. (i). A2, astrocytoma grade II; AA, anaplastic astrocytoma, grade III. GBM, glioblastoma, grade IV; O2, oligodendroglioma, grade II; AO, anaplastic oligodendroglioma, grade III. j, Correlation between GLS and SREBP-1 staining in glioma TMA tissues shown in h,i. The correlation coefficient and significance were determined by a two-sided Pearson’s correlation test. P < 0.0001. k, Kaplan–Meier curves of the overall survival of individuals with GBM (n = 45) from the TMA (h), separated based on the quantification of GLS expression (mean = 199.78) or SREBP-1 levels (mean = 200.02) (i). Significance was determined by the log-rank test. P = 0.0042 for GLS and P = 0.0001 for SREBP-1 comparison.
Fig. 5 | Ammonia binds SCAP to activate its dissociation from Insig. a, Scheme of Insig-SCAP-SREBP complex in the ER. b, Co-IP and western blot (WB) analysis of cell lysates from HEK293T cells transfected with GFP-SCAP and Myc-Insig-1 after culturing in serum-free medium (5 mM glucose) with/without glutamine (4 mM) or NH₄Cl (4 mM) for 12 h. c, Representative confocal images of GFP-SCAP and the IF staining of Golgi marker Giantin in H1299 cells in response to glutamine SCAP (D428A) with Myc-Insig-1 as in i, Co-IP and WB analysis of the association of WT or mutant GFP-SCAP with Myc-Insig-1 (100 nM) for 12 h under serum-free medium containing 5 mM glucose. d, Representative confocal images of GFP-SCAP and Giantin in H1299 cells in response to glutamine (4 mM)/glucose (5 mM) or NH₄Cl (4 mM) stimulation and CB-839 (100 nM) for 12 h under serum-free medium. Scale bars, 10 μm. e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, x, y, z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, Ay, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC, CD, CE, CF, CG, CH, CI, CJ, CK, CL, CM, CN, CO, CP, CQ, CR, CS, CT, CU, CV, CW, CX, CY, CZ, DA, DB, DC, DD, DE, DF, DG, DH, DI, DJ, DK, DL, DM, DN, DO, DP, DQ, DR, DS, DT, DU, DV, DW, DX, DY, DZ, EA, EB, EC, ED, EE, EF, EG, EH, EI, EJ, EK, EL, EM, EN, EO, EP, EQ, ER, ES, ET, EU, EV, EW, EX, EY, EZ, FA, FB, FC, FD, FE, FF, FG, FH, FI, FJ, FK, FL, FM, FN, FO, FP, FQ, FR, FS, FT, FU, FW, FX, FY, FZ, GA, GB, GC, GD, GE, GF, GG, GH, GI, GJ, GK, GL, GM, GN, GO, GP, GQ, GR, GS, GT, GU, GV, GW, GX, GY, GZ, HA, HB, HC, HD, HE, HF, HG, HI, HJ, HK, HL, HM, HN, HO, HP, HQ, HR, HS, HT, HU, HV, HW,HX, HY, HZ, IA, IB, IC, ID, IE, IF, IG, IH, IJ, IK, IL, IM, IN, IO, IP, IQ, IR, IS, IT, IU, IV, IW, IZ, JA, JB, JC, JD, JE, JF, JG, JH, JJ, JK, JL, JM, JN, JO, JP, JQ, JR, JS, JT, JU, JV, JW, JZ, KA, KB, KC, KD, KE,KF, KG, KH, KI, KJ, KK, KL, KM, KN, KO, KP, KQ, KR, KS, KT, KU, KV, KW, KX, KY, KZ, LA, LB, LC, LD, LE, LF, LG, LH, LI, LJ, HK, HL, HM, HN, HO, HP, HQ, HR, HS, HT, HU, HV, HW, HX, HY, HZ, MA, MB, MC, MD, ME, MF, MG, MH, MI, MJ, MK, ML, MM, MN, MO, MP, MQ, MR, MS, MT, MU, MV, MW, MX, MY, MZ, NA, NB, NC, ND, NE, NF, NG, NH, NI, NJ, NK, NL, NM, NN, NO, NP, NQ, NR, NS, NT, NU, NV, NW, NX, NY, NZ, OA, OB, OC, OD, OE, OF, OG, OH, OI, OJ, OK, OL, OM, ON, OO, OP, OQ, OR, OS, OT, OU, OV, OW, OX, OY, OZ, PA, PB, PC, PD, PE, PF, PG, PH, PI, PJ, PK, PL, PM, PN, PO, PP, PQ, PR, PS, PT, PU, PV, PW, PX, PY, PZ, QA, QB, QC, QD, QE, QF, QG, QH, QI, QJ, QQ, QR, QS, QT, QU, QV, KW, QW, QX, QY, QZ, RA, RB, RC, RD, RE, RF, RG, RH, RI, RJ, RK, RL, RM, RN, RO, RP, RQ, RR, RS, RT, RU, RV, RW, RX, RY, RZ, SA, SB, SC, SD, SE, SF, SG, SH, SI, SJ, SK, SL, SM, SN, SO, SP, SQ, SR, SS, ST, SU, SV, SW, SX, SY, SZ, TA, TB, TC, TD, TE, TF, TG, TH, TI, TJ, TK, TL, TM, TN, TO, TP, TQ, TR, TS, TT, TU, TV, TW, TX, TY, TZ, UA, UB, UC, UD, UE, UF, UG, UH, UI, UJ, UK, UL, UM, UN, OU, UP, UQ, UR, US, UT, UU, UV, UW, UX, UY, UZ, VA, VB, VC, VD, VE, VF, VG, VH, VI, VJ, VK, VL, VM, VN, VO, VP, VQ, VR, VS, VT, VU, VW, VX, VY, VZ, WA, WB, WC, WD, WE, WF, WG, WH, WI, WJ, WK, WL, WM, WN, WO, WP, WQ, WR, WS, WT, WU, WV, WW, WX, WY, WZ, XA, XB, XC, XD, XE, XF, XG, XH, XI, XJ, XK, XL, XM, XN, XO, XP, XQ, XR, XS, XT, Xu, XV, XW, XY, XZ, YA, YB, YC, YD, YE, YF, YG, YH, YI, YJ, YK, YL, YM, YN, YO, YP, YQ, YR, YS, YT, YU, YV, YW, YX, YY, YZ, ZA, ZB, ZC, ZD, ZE, ZF, ZG, ZH, ZI, ZJ, ZK, ZL, ZM, ZN, ZO, ZP, ZQ, ZR, ZS, ZT, ZU, ZV, ZW, ZX, ZY, ZZ.
the membrane (Extended Data Fig. 6f), which might facilitate the binding of SCAP to COPII proteins and translocation of SCAP from the ER to the Golgi (detailed computational analyses provided in Supplementary Information).

To validate our computational analysis results, we compared ammonia binding to GFP–SCAP WT and to its D428A mutant after purifying these proteins from cell membranes (Fig. 5i,j). The results showed that the level of ammonia bound to GFP–SCAP WT was ~two-fold higher than that to control GFP protein, whereas the D428A mutation abolished the binding and returned the ammonia level to that seen with the control GFP (Fig. 5g). 25-HC addition significantly reduced the binding of ammonia to GFP–SCAP WT (Extended Data Fig. 5g, right), demonstrating that the presence of 25-HC blocks the binding of ammonia to SCAP (Extended Data Fig. 5g, left).

We next examined whether D428A mutation could abolish glutamine- and ammonia-triggered dissociation of SCAP from Insig-1 (Fig. 5k). Accordingly, GFP–SCAP trafficking to the Golgi stimulated by glutamine or ammonia was also abolished by the D428A mutation (Fig. S1 and Extended Data Fig. 7a). Moreover, the D428A mutation blocked glutamine- and ammonia-promoted SREBP-1a, SREBP-1c and SREBP-2 activation compared to WT SCAP transfection (Fig. 5m and Extended Data Fig. 7c). In addition, changing the D428 residue to glutamate (D428E), asparagine (D428N) or lysine (D428K) led to the same inhibitory effects (Extended Data Fig. 7b,d,e). In addition, changing the D428 residue to glutamate (D428E), asparagine (D428N) or lysine (D428K) led to the same inhibitory effects (Extended Data Fig. 7b,d,e).

Together, our experimental data plus the computational structural analyses reveal that ammonia stimulates SCAP dissociation...
from Insig by inducing conformational changes in the SCAP transmembrane domain via its interaction with the D428, S326 and S330 residues, eventually leading to SCAP/SREBP translocation and activation. D428A mutation abolishes ammonia binding to SCAP, thereby retaining association with Insig even under low sterol conditions. 25-HC blocks ammonia to reach its binding site, thereby suppressing SCAP–Insig dissociation and SREBP activation (Extended Data Fig. 6f).

**Disrupting ammonia–SCAP interaction suppresses tumour growth.** We next examined whether disrupting ammonia–SCAP interaction by changing D428 to alanine (D428A) in SCAP could affect tumour growth. GFP, GFP–SCAP WT or D428A mutant were transfected into H1299 lung cancer cells that stably express luciferase. Western blot analysis showed that WT SCAP dramatically increased SREBP-1 and SREBP-2 cleavage, which was abolished by the D428A mutation (Fig. 6a). These stably transfected cells were implanted into mice, and bioluminescence imaging showed at day 50 after implantation via tail vein injection that WT SCAP dramatically increased tumour growth in the lung area as compared to the control GFP group, whereas this increase was abolished by the D428A mutation (Fig. 6b,c). Gross lung images showed higher numbers of tumour lesions on the lung surfaces in the WT SCAP group than GFP and D428A mutant groups (Fig. 6d, left and Extended Data Fig. 8a). Hematoxylin and eosin (H&E) staining confirmed the dramatically increased number of tumour lesions in the lungs of WT SCAP group (Fig. 6d,e and Extended Data Fig. 8b). IHC staining showed that SREBP-1 was significantly elevated in lung tumour tissues in the WT SCAP group compared to the GFP group, whereas this increase was abolished by the D428A mutation (Fig. 6d,e).

We repeated those experiments with primary GBM30 cells (Fig. 6f). The stably transfected GBM cells were implanted into mice brains and tumour growth was examined by magnetic resonance imaging (MRI). The imaging showed that the tumour volume in the WT SCAP group was dramatically greater than in the control GFP and D428A mutation groups (Fig. 6g and Extended Data Fig. 8c). H&E staining confirmed that the tumour sizes in the different groups on day 17 were consistent with those detected by MRI (Fig. 6h, left and Extended Data Fig. 8d). IHC staining showed much stronger SREBP-1 staining in the WT SCAP group than in the other two groups (Fig. 6h,i). Moreover, the mice implanted with WT SCAP expressing cells had significantly shorter survival time than other two groups (Fig. 6j).

Together, these data demonstrate that disrupting the ammonia–SCAP interaction by mutating the D428 residue significantly suppresses SREBP-1 activation and tumour growth.

**Discussion**

SREBPs are spatially restricted to the ER membrane after synthesis6,8. The mechanisms triggering the exit of SREBPs from the ER have
so far remained unclear. In this study, we uncovered an unprecedented role of ammonia released from glutaminolysis, which acts as a key activator to trigger N-glycosylated SCAP dissociation from Insig by inducing dramatic conformational changes in the SCAP transmembrane domain through interaction with the D428, S326 and S330 residues, leading to SREBP activation and lipid synthesis (Fig. 5g and Extended Data Figs. 6f and 7). We also provided physiological evidence for the connection between glutaminolysis and lipogenesis by showing the molecular link between GLS expression and SREBP-1 activation in human lung cancer and glioma tissues. Moreover, our study demonstrated that the activation of SREBPs and lipogenesis by glutamine/ammonia also occurs in melanoma, liver and breast cancer cells in addition to lung cancer and GBM, suggesting that this is a common mechanism at play in a wide range of cancer types. We further unveiled the competitive role of 25-HC that blocks ammonia binding to SCAP, thereby retaining SCAP–Insig association and suppressing SREBP activation. Altogether, our study revealed an unanticipated role for ammonia in the regulation of SCAP–Insig dissociation, SREBP activation and lipid metabolism, and identified SCAP as the critical sensor connecting glutamine, glucose and lipid metabolism to promote tumour growth.

Ammonia released from amino acid deamination has long been considered as a toxic waste product\(^\text{15-19}\). A recent study showed that ammonia released from glutaminolysis can be utilized for pyrimidine synthesis in lung cancer cells\(^\text{20}\). Another recent study reported that ammonia could be recycled in breast cancer cells to synthesize different amino acids, for example, glutamate, aspartate and proline\(^\text{21}\). In contrast, our study uncovers that ammonia is a critical signaling molecule of lipid metabolism.

Our computational simulation analyses showed that NH\(_4\)\(^+\) enters the SCAP transmembrane domain to first bind to the D428 negatively charged side chain from the S6 helix and then engages with the core of the SCAP transmembrane domain and the NH\(_4\)\(^+\)-binding site comprising D428 and S326/S330 residues located next to the intersection between the two helices (Fig. 5f,g). Thus, NH\(_4\)\(^+\) binds in the center of the SCAP transmembrane domain, which enables the triggering of a propagating signal to induce large SCAP conformational changes, consequently the disruption of the interface between SCAP and Insig, finally leading to their dissociation (Fig. 7b and Extended Data Fig. 6f). Our data provide a molecular explanation for the effects of the D428A mutation on the stable association between SCAP and Insig under low sterol conditions\(^\text{22}\) as NH\(_4\)\(^+\) is unable to bind to SCAP to induce the necessary conformational changes for its dissociation from Insig (Extended Data Fig. 6f).

Our data also unveiled the competitive role of 25-HC, which serves as a roadblock to prevent the binding of NH\(_4\)\(^+\) to SCAP D428 and S326/S330 residues (Extended Data Fig. 6f). When the level of 25-HC decreases, the path to NH\(_4\)\(^+\) binding becomes open and NH\(_4\)\(^+\) can reach the cell site to exert its function (Fig. 5f,g and Extended Data Fig. 6f). Notably, SCAP was discovered 25 years ago, but the concept and understanding of its activation triggering dissociation from Insig have remained centered on the sterol-mediated negative feedback loop regulation model\(^\text{11}\). Our present study greatly extends beyond this model and shows that ammonia stimulation is indispensable for SCAP dissociation from Insig, suggesting a paradigm shift that significantly advances our understanding of the key regulation steps in SCAP/SREBP activation and lipid metabolism (Fig. 7 and Extended Data Fig. 6f; Supplementary Information).

**Limitation of the study.** Our present study offers multiple lines of strong computational and biochemical evidence for the binding of ammonia to SCAP to activate SREBPs, whereas we have not observed the direct biophysical binding between ammonia and SCAP D428 and S326/330 residues. To visualize the molecular details of ammonia–SCAP binding, resolving the cryo-EM structure of the Insig–SCAP–NH\(_4\)\(^+\) complex would be necessary, which is expected to provide the ultimate structural proof for the intriguing regulation of the Insig–SCAP complex by ammonia. In addition, our studies also raise the question of whether ammonia stays bound to SCAP upon dissociation from Insig and trafficking to the Golgi. These intriguing questions will require further intensive research.

**Methods**

The animal study was approved by The Institutional Animal Care and Use Committee at The Ohio State University (ref. 2011A00000864–R3–AM1). Gene expression analyses were approved by The Ohio State University Institutional Review Board (IRB) (ref. 2015C0067). There are additional methods included in Supplementary Information.

**Cell lines.** Individual lung tumour and adjacent healthy tissues, lung TMA containing 50 paired (tumours and matched adjacent healthy lung tissues) and 49 unpaired tumour tissues and individual GBM tumour tissues were from the Department of Pathology at The Ohio State University. All human tissues were collected from Ohio State University Hospitals under IRB and HIPPA-approved protocols and histologically confirmed. Glioma TMA with 91 tumours was from the University of Kentucky and IRB approval was obtained before study initiation. All samples were tested as negative for HIV and hepatitis B. TMA slides were stained using SREBP-1 (BD Pharmingen, 557036, 1:20 dilution) or GLS (Abcam, ab93434, 1:50 dilution) antibodies and then biotinylated horse anti-mouse IgG (Vector Labs, cat. no. BA-2000, 1:50 dilution) or biotinylated horse anti-rabbit IgG (Vector Labs, cat. no. BA-1100, 1:500 dilution) antibodies. The slides were scanned using ScanScope and analyzed using ImageScope v.11 software (Aperio Technologies). The staining intensity of tissues was graded as 0, 1+, 2+ or 3+. The H score was calculated using the following formula H score = (1 × (% cells with 1+) + 2 × (% cells with 2+)) × 3 × (% cells with 3+) × 100.

**Plasmids.** pCMV–Myc–Insig-1, pcDNA3.1-2 Flag–SREBP-1a (full length) and SREBP-1c (full length), pcDNA3.0–HA–SREBP-2 (full length) and pcDNA3.0–GFP–SCAP (QQQ) plasmids were obtained or cloned as previously described\(^\text{28}\). pcDNA3.0–GFP–SCAP WT plasmid was a gift from P. Mishchel (Stanford University) and human GBM primary cell, GBM30, was originally generated in I. Nakano’s laboratory at Ohio State University (OSU). The H1299-luc cell line was generated in our laboratory.

**Clinical samples.** Individual lung tumour and adjacent healthy tissues, lung TMA containing 50 paired (tumours and matched adjacent healthy lung tissues) and 49 unpaired tumour tissues and individual GBM tumour tissues were from the Department of Pathology at The Ohio State University. All human tissues were collected from Ohio State University Hospitals under IRB and HIPPA-approved protocols and histologically confirmed. Glioma TMA with 91 tumours was from the University of Kentucky and IRB approval was obtained before study initiation. All samples were tested as negative for HIV and hepatitis B. TMA slides were stained using SREBP-1 (BD Pharmingen, 557036, 1:20 dilution) or GLS (Abcam, ab93434, 1:50 dilution) antibodies and then biotinylated horse anti-mouse IgG (Vector Labs, cat. no. BA-2000, 1:50 dilution) or biotinylated horse anti-rabbit IgG (Vector Labs, cat. no. BA-1100, 1:500 dilution) antibodies. The slides were scanned using ScanScope and analyzed using ImageScope v.11 software (Aperio Technologies). The staining intensity of tissues was graded as 0, 1+, 2+ or 3+. The H score was calculated using the following formula H score = (1 × (% cells with 1+) + 2 × (% cells with 2+)) × 3 × (% cells with 3+) × 100.

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**Cell culture and transfection.** U87, U87EGFR, LN229, T98 (GBM cell line), M233 (melanoma), HeK293 (liver cancer), HEK293T (acs-4500), H1299 (CRL-5803), H1975 (CRL-5908), HCC4006 (CRL-2871) and MDA468 (HTB-132) cells were purchased from ATCC. M233 (CVCJ, D750) was purchased from RRID. U87/EGFR is a kind gift from P. Mishchel (Stanford University) and human GBM primary cell, GBM30, was originally generated in I. Nakano’s laboratory at Ohio State University (OSU). The H1299-luc cell line was generated in our laboratory.

**Western blot.** Cells were lysed with RIPA buffer containing a protease inhibitor cocktail and phosphatase inhibitors. The proteins were separated on 12% SDS–PAGE and transferred onto an ECL nitrocellulose membrane (1620112, Cellglo). Medium was supplemented with 5% HyClone FBS (SH30071.03, GE Healthcare) and 4 mM glutamine (25030-081, Life Technologies). GBM30, primary GBM patient-derived cells were maintained in DMEM/F12 (MT90090PB, Fisher) containing B-27 serum-free supplements (1x), heparin (2 mg ml\(^{-1}\)), epidermal growth factor (EGF) (50 ng ml\(^{-1}\)), collagen (2 mM) and fibroblast growth factor (50 ng ml\(^{-1}\)). All cell lines were cultured in a humidified atmosphere of 5% CO\(_2\) at 37°C. Transfection of plasmids was performed using X-tremeGENE HP DNA Transfection Reagent (06366263001, Roche) following the manufacturer’s instructions.

**Human tissue analyses.** Fold change analysis was performed on the publicly available data from the TCGA glioblastoma and melanoma datasets (available at cistrome.org).
expression), SREBP-2 (BD Pharmingen, 557036, 1:50 dilution) and SREBP-1 (IgG-2A4) (BD Pharmingen, 557036, 1:1,000 dilution). The tubes were washed 10 times with PBS, p-ERK (Cell Signaling, 9102S, 1:1,000 dilution), p-EGFR Y1086 (Invitrogen, 369700, 1:1,000 dilution), p-Akt Thr308 (Cell Signaling, 9275S, 1:1,000 dilution), p-EGFR Y1068 (Invitrogen, 369790, 1:1000 dilution), p90S6 (Cell Signaling, 2594S, 1:1,000 dilution) and p-ERK (Cell Signaling, 4370S, 1:1,000 dilution) were clarified by centrifugation at 16,000 × g for 15 min at 4 °C. The precipitated protein complex was washed with 1 ml buffer (25 mM Tris, pH 8.0, 150 mM NaCl and 1% (w/v) LMNG (DL14035, Biosynth Carbosynth)) containing a 2 μg/ml of anti-GFP (Roche, 11814460001, 1:1,000 dilution), anti-Flag-tag (Sigma, cat. no. 7074, 1:1,000–5,000 dilution) and anti-rabbit IgG (H+L) (Invitrogen, cat. no. 81-1620, 1:2,000 dilution) antibodies conjugated to horseradish peroxidase. Immunoreactivity was revealed using an ECL kit (RPN2106, GE Healthcare).

**Quantitative real-time PCR.** Total RNA was isolated with TRIzol according to the manufacturer’s protocol and complementary DNA was synthesized with the iScript cDNA Synthesis kit. Quantitative real-time PCR was performed with iQ SYBR Green Supermix using the Applied Biosystems (ABI) 7900HT Real-Time PCR System. Expression was normalized to the 36B4 housekeeping gene and calculated with the comparative method (2^-∆∆Ct).

**Isolation of microsomal membranes from H1299 cells for ER-budding assay.** H1299 cells were washed and scraped into 2 ml of ice-cold DPBS with protease inhibitors from duplicate 15-cm dishes. Cells were centrifuged at 1,800 × g for 5 min at 4 °C. The tubes were washed 20 times and centrifuged at 1,000 × g after aspiration of the supernatants. When needed, the tubes were thawed in a 37 °C water bath for 50 s and placed on ice. Each cell pellet was resuspended in 0.4 ml of Buffer B (10 mM HEPES-KOH (pH 7.2), 250 mM sorbitol, 10 mM KCl, 1.5 mM Mg(OAc), and protease inhibitors), passed through a 22-gauge needle 20–30 times and centrifuged for 5 min at 4°C. The supernatants were transferred to siliconized microcentrifuge tubes (121N66, Thermo Scientific) and centrifuged at 16,000 × g for 3 min at 4 °C. Subsequently, each pellet was resuspended in 0.5 ml of Buffer A and centrifuged again at 16,000 × g for 3 min at 4 °C. The microsomes for use in the in vitro vesicle-formation assay were obtained by dissolving the remaining pellet into 70–100 μl of Buffer A. The protein concentration was determined after a 5 μl of the microsomal suspension was added to 5 μl of a solution of 20% (w/v) of hexyl–β–d-glucopyranoside. Please also see previous study for the procedure.

**In vitro vesicle-formation assay.** Each reaction in a final volume of 80 μl contained 50 mM HEPES-KOH at pH 7.2, 250 mM sorbitol, 70 mM KOAc, 5 mM potassium EGTA, 2.5 mM Mg(OAc)2, 1.5 mM ATP, 0.5 mM GTP, 10 mM creatine phosphate, 4 U/ml creatine kinase, protease inhibitors, 37–80 μg protein of H1299 microsomes and 600 μg of rat liver cytosol. Reactions were carried out in siliconized 1.5 ml microcentrifuge tubes for 15 min at 37 °C, terminated by transfer of the tubes to ice and then followed by centrifugation at 16,000 × g for 3 min at 4 °C to obtain a medium-speed pellet (membrane fractions) and a medium-speed supernatant. The medium-speed supernatants were collected from each sample and centrifuged again at 132,527.16 × g for 40 min at 4 °C in a Beckman TL-A210.1 rotor to obtain a high-speed pellet (vesicle fractions). The vesicle and membrane fractions were each resuspended in 60 μl of the buffer (10 mM Tris-HCl at pH 7.6, 100 mM NaCl, 1% (w/v) SDS plus protease inhibitors, supplemented with 15 μl of the cocktail: 150 mM Tris-HCl at pH 6.6, 15% SDS, 2% (v/v) glycerol, 0.02% (w/v) bromophenol blue and 12.5% (v/v) 2-mercaptoethanol) and heated at 100 °C for 5 min. The vesicle and membrane fractions were subjected to 10% SDS–PAGE and analyzed by immunoblotting. The procedure is described previously.

**Mouse luminescence imaging.** Mice implanted with H1299 cells expressing luciferase were intraperitoneally injected with a luciferin (122796, PerkinElmer) solution (15 mg/ml in PBS, dose of 150 mg/kg). Bioluminescence images were acquired using an IVIS Lumina system and analyzed by Living Image software.

**Molecular dynamics simulations.** The cryo-EM structure of the Insg1/SCAP complex (Protein Data Bank ID, 6M49) was used as the initial structure for our simulations. The SCAP structure without 25-HC was prepared by replacing the partially unfolded 54 helix (residues 354–358) in the inactive conformation with a fully folded 54 helix, which was built with Modeller v.10.1 using PNC1 (PDB code: 5-val) and NPC2. The CHARMM-GUI membrane builder was used to build a membrane bilayer consisting of 366 hydrated palmitoyl–oleyl–phosphatidylcholine (POPC) molecules. Each system was solvated with approximately 34,000 TIP3P water molecules (a type of water used in simulations that represents three-site rigid water molecule with charges and Lennard-Jones parameters). Each system was then equilibrated with the CHARMM 36 force field used for the proteins, lipids and ions, while the ligand (25-HC) was parameterized using SwissParam. All simulations were performed at 310 K and the temperature was regulated with the v-rescale thermostat. The Parrinello–Rahman barostat was used to keep the pressure at 1 bar with a constant pressure of 0.2 ps. The isothermal compressibility was 4.5 x 10⁻¹⁰ bar⁻¹.

The pressure was coupled semi-isotopically, where the x and y directions were coupled together...
and the z direction was independently coupled. All bonds were constrained with the LINCS algorithm. The integration time step was 2 fs. The nonbonded long-range electrostatic interactions were calculated using the particle mesh Ewald method with a 14 Å cutoff. The van der Waals interaction also used a 14 Å cutoff. All simulations were carried out using Gromacs 2020 (ref. 19).

Each system was first energy minimized with the steepest-descent method with a maximum of 50,000 steps or the maximum force in the system reaching less than 100 kJ mol⁻¹ Å⁻¹. After energy minimization, a 500 ps equilibration simulation was performed with position restraints on the protein, lipids and ligands, which was followed by six 1 ns simulations with decreasing position restraints. Finally, one ~1-μs long production simulation without any restraints was run for each system, with trajectories saved every 100 ps (a total of ~10,000 frames for each simulation) for subsequent analysis.

Co-solvent mapping. With the Insg–SCAP complex embedded in a POPC bilayer described as above, we solvated each system with high concentrations of either NH₃ or NH₄⁺ to identify the potential binding sites for NH₃ or NH₄⁺ using MD simulations. A total of 1,000 NH₃ molecules were added to the system with a box size of 127×127×116 Å, yielding a final NH₃ concentration of 1 M. Independently, 600 NH₄⁺ molecules were added to the system, yielding a final NH₄⁺ concentration of 0.6 M. The force fields for NH₃ and NH₄⁺ were generated using CGenFF (98). All minimization and equilibration steps were the same as those in the standard MD simulations described above. Five sets of co-solvent mapping simulations were conducted; for each set, five independent replicas were performed, yielding a total of 25 simulations, each with a duration of 100 ns. A summary of all co-solvent mapping simulations performed is shown in Supplementary Table 2.

Simulation data analysis. The molecular visualization software VMD was used for visualization and structural parameter calculations (Supplementary Information).

Statistics and reproducibility. All figures, including western blots, metabolites analysis and mouse experiments, are representative of at least two biological replicates with similar results, unless stated otherwise. The quantification of tumour volume in mice brain measured by MRI was blinded. All other data collection and analysis were not performed blind to the conditions of the experiments. Data analysis was performed using GraphPad Prism v.8.0.2. Data distribution was assumed to be normal but this was not formally tested. No multiplicity was adjusted by the t test, unless specified. Data availability.

All data that support the findings of this study are available within the paper and its supplementary information files. RNA-seq data for Figs. 1a and 2f and Extended Data Fig. 1a are provided in Supplementary Data and the raw data are deposited in the Gene Expression Omnibus (accession no. GSE199089). Source data are provided with this paper.

Code availability

No custom codes were used during this study.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Glutamine activates SREBP-1 to promote cell proliferation. a, Heat map comparison of metabolic and overall pathways based on RNA-seq data from H1299 cells under glucose, glutamine or a combination of glucose and glutamine vs. both free conditions (12 h) using the bioinformatics Ingenuity Pathway Analysis (IPA). #NUM, no activity pattern available. b, c, Western blot analysis of cell lysates of cells stimulated with glutamine for 12 h (b) or with 4 mM glutamine at the indicated times (c) under serum-free conditions (glucose 5 mM). d, Lipids derived from 14C-labeled glucose (0.5 μCi, 2 h) in cells after culturing cells with/without glutamine (4 mM) for 12 h in serum-free medium containing 5 mM non-labeled glucose. The results are presented as mean ± SEM (n = 3). e, Proliferation of cancer cells cultured in medium supplemented with 1% dialyzed FBS with/without glutamine (4 mM) or glucose (5 mM) (mean ± SD, n = 3). f, g, Western blot analysis of cells after infection with shRNA-expressing lentivirus for 48 h and then placed in fresh medium (5 mM glucose) with/without glutamine (4 mM) for another 12 h (left panels). Cell proliferation was determined under 1% dialyzed FBS (right panels). The results are shown as mean ± SD (n = 3). h, Western blot analysis of cells after treatment with atorvastatin (5 μM) for 12 h in 5% lipoprotein-deficient serum (LPDS) containing 5 mM glucose with/without glutamine (4 mM). i, Western blot analysis of cells after stimulation with EGF (20 ng/ml) for 12 h in serum-free medium (5 mM glucose) with/without glutamine (4 mM). j, Western blot analysis of cells after incubation with/without aspartate (0.15 mM), asparagine (0.38 mM), leucine (0.38 mM), methionine (0.1 mM), threonine (0.17 mM) or glutamine (2 mM) for 12 h in HBSS buffer (containing 5.6 mM glucose) supplemented with essential amino acids. The dose selected for each amino acid is same as their concentration included in RPMI 1640 medium. Significance was determined by unpaired and two-tailed Student’s t-test (d) or two-way ANOVA with Dunnett’s (e) or Tukey’s (g) multiple comparisons adjustment.
Extended Data Fig. 2 | Ammonia activates SREBPs and lipogenesis. a, Western blot analysis of cells stimulated with glutamine, NH₄Cl, NH₄H₂O, NaCl, NaOH or NaNO₃ (all 4 mM) under serum-free medium (5 mM glucose) for 12 hr. b, Western blot analysis of cells stimulated with NaCl (12 hr) in the absence of glutamine under serum-free culture conditions containing 5 mM glucose. c, Representative IF images of cells after stimulation with glutamate (4 mM), α-KG (4 mM), lactate (10 mM) or glutamine (4 mM) for 12 hr under serum-free culture conditions (5 mM glucose). d, Western blot analysis of H1299 cells stimulated with glutamate (4 mM), glutamate (Glu, 4 mM), α-KG (4 mM), octyl-α-KG (OA-KG) (2 mM) or NH₄Cl (4 mM) for 12 hr under serum-free culture conditions (5 mM glucose). e-f, The levels of glutamate (e) and α-KG (f) in the cells were measured using the appropriate assay kits. The results (e and f) are presented as mean ± SEM (n = 3). g, Western blot analysis of H1299 cells stimulated with glutamine (4 mM) or NH₄Cl (4 mM) for 12 hr in the presence of glucose (5 mM) after ATG5 siRNA knockdown for 24 hr. h, Western blot analysis of cells stimulated with NH₄Cl at the indicated doses for 12 hr under serum-free culture conditions (5 mM glucose). i-k, Western blot analysis of membranes (for GFP-SCAP, PDI and SREBP precursors) and nuclear extracts (for N-terminal SREBPs and Lamin A) from HEK293T cells transfected with GFP (2 μg), GFP-SCAP wild-type (NNN) (2 μg) or its mutant QQQ (5 μg), obtained by replacing all three N-glycosylation residues asparagine (N) to glutamine (Q), together with full length Flag-SREBP-1a (i), -1c (j), or HA-SREBP-2 (k) for 24 hr and then stimulated with glutamine or NH₄Cl (all 4 mM) for another 12 hr under serum-free culture conditions (5 mM glucose). Significance was determined by unpaired and two-tailed Student t-test or one-way ANOVA with Dunnett’s multiple comparisons adjustment.
Extended Data Fig. 3 | Suppressing ammonia release from glutamine inhibits SREBPs. a, Relative metabolite levels in H1299 cells after treatment with GPNA (5 mM) or CB-839 (100 nM) for 12 hr under serum-free medium containing glutamine (4 mM) and glucose (5 mM) via using appropriate assay kits (mean ± SEM, n = 3). Cell culture conditions upon treatment are the same for the subsequent panels. b, Relative glutamine consumption of cells treating with GPNA (5 mM) or CB-839 (100 nM) for 12 hr (mean ± SEM, n = 3). c, d, Western blot analysis of cells treated with GPNA or CB-839 (48 hr). e, Western blot analysis of GBM30 cells treated with CB-839 (200 nM) for 12 hr with/without glutamine, glutamate or NH4Cl (all 4 mM). f, Ammonia measurement (left panel) in tumour tissues from H1299 cells (4 × 10⁶) derived xenograft model treated with CB-839 (30 mg/kg/mouse, i.p., twice per day for 3 days) when tumour size reached 200 mm³ (mean ± SEM, n = 6). Middle panel shows representative IHC images. Scale bars, 50 μm. The expression levels were quantified by using ImageJ to analyze 4 images per tumour (3 tumours/group) (mean ± SEM, n ≥ 2441 cells) (right panel). g, Relative glutamine consumption (12 hr) of cells in culture condition as (a) after infection with shRNA-expressing lentiviruses (48 hr) (mean ± SEM, n = 3). h, Relative metabolite levels (12 hr) in cells in culture condition as (a) measured by the appropriate assay kit after infection with shRNA-expressing lentiviruses (48 hr) (mean ± SD, n = 3). i, j, Western blot analysis of cells after infection with shRNA-expressing lentiviruses for 48 hr and then stimulated with 4 mM glutamate, α-KG or NH4Cl for 12 hr. k, l, Real-time qPCR (k) and Western blot (l) analysis of cells under serum-free medium containing 5 mM glucose and 4 mM glutamine for 12 hr after siRNA knockdown of glutamate dehydrogenase (GDH1/2), asparaginase (ASPG) or serine deaminase (SDS) (24 hr). The results (k,l) are presented as mean ± SEM (n = 3). Significance was determined by unpaired and two-tailed Student’s t-test or one-way ANOVA with Dunnett’s multiple comparisons adjustment.
Extended Data Fig. 4 | GLS is correlated with SREBP-1 in human tumour tissues. a, Representative IHC images of anti-GLS and -SREBP-1 staining in tumour vs. adjacent normal tissues from individuals with adenocarcinoma (Adeno) or squamous lung cancer. Scale bars, 50 μm. b, c, Representative IHC images of anti-GLS and anti-SREBP-1 staining from lung cancer TMA (b). Representative images of different levels of anti-GLS or anti-SREBP-1 staining and scoring are shown in (c). d, Comparison of GLS expression and SREBP-1 levels in 50 paired tumours vs. adjacent normal lung tissues from the lung cancer TMA based on H score. Significance was determined by an unpaired Student’s t-test. e, Genetic inhibition of GLS or SREBP-1 dramatically suppressed lung tumour growth in vivo. NSCLC H1299 cells were infected with shGLS- or shSREBP-1-expressing lentivirus for 48 hr and then were implanted (2 × 10^6 cells/mouse) into the flank of nude mice. The tumours were isolated from mice at 53 days post-implantation and were imaged (left panel) and weighed (right panel) for comparison. Data are shown as mean ± SEM (n = 6). Significance was determined by one-way ANOVA with Dunnett’s multiple comparisons adjustment. f, Representative IHC images of anti-GLS, anti-SREBP-1, anti-ASPG and anti-SDS staining in tumour tissues from patients with GBM. Scale bars, 50 μm. g, h, Representative images of anti-GLS and anti-SREBP-1 staining from glioma TMA (g). Representative images of different levels of anti-GLS or anti-SREBP-1 staining and scoring are shown in (h).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Ammonia binds to SCAP stimulating SCAP/SREBP activation. 
a, Representative confocal images of U87 cells in response to glutamine (4 mM), glucose (5 mM) or NH4Cl (4 mM) stimulation for 12 hr with/without CB-839 (100 nM) under serum-free culture conditions. Scale bars, 10 μm. 
b, In vitro SCAP ER-budding assay. H1299 cells were stimulated with/without glutamine (4 mM) or NH4Cl (4 mM) for 4 hr under serum-free medium (5 mM glucose). Microsomes were purified and incubated at 37 °C for 15 min or on ice (as time 0) with cytosol extracts from rat liver in the presence of ATP and GTP (left panel). Alternatively, microsomes purified from H1299 cells cultured with glucose (5 mM) alone (2 hr) were incubated with NH4Cl (1 mM) or NaCl (1 mM) at 37 °C or on ice together with liver extracts as above (right panel). The mixtures were centrifuged to separate budded vesicles from the ER membrane fractions, which were then analyzed by Western blot by using indicated antibodies. 
c, Co-solvent NH3 computational mapping of SCAP. 
d, Alignment of the SCAP protein fragment. 
e, A schematic model for the sequential binding of NH4+ to SCAP obtained from the co-solvent ammonia mapping and NH4+-bound SCAP simulations. 
f, Western blot analysis of HEK293T cells transfected with GFP, wild-type or different GFP-SCAP mutants together with full-length Flag-SREBP-1c for 24 hr and then stimulated with glutamine (4 mM) for 12 hr under serum-free conditions (5 mM glucose). 
g, Co-solvent ammonia mapping for SCAP bound with 25-HC. Right panel shows the biochemical analysis of GFP-SCAP-bound ammonia in HEK293T cells stimulated with NH4Cl (4 mM) for 2 hr with/without pretreatment with 25-HC (10 μg/ml, 1 hr) using an ammonia assay kit. Top panel shows by western blot that equal amounts of proteins were purified. The results are presented as mean ± SEM (n = 3). Significance was determined by unpaired and two-tailed Student’s t-test. 
h, i, Western blot analysis of H1299 cells cultured with NH4Cl (4 mM) (h) or glutamine (4 mM) (i) for 12 hr in serum-free medium (5 mM glucose) together with a cholesterol/25-hydroxycholesterol mixture (sterols).
Extended Data Fig. 6 | Ammonia binding induces SCAP dissociation from Insig. **a–d.** Comparison of the coupling, tilting and kink angles of S3, S5 and S6 helices during the 1 μs simulations of SCAP bound with NH₄⁺ vs. SCAP without bound NH₄⁺. In panel (a), S3 and S6 helices from the NH₄⁺ unbound simulation (in light gray) is aligned with the NH₄⁺ bound simulation (in dark gray). NH₄⁺, D428, S326 and S330 are shown in the stick representation. The coupling of the S3 and S6 helices was altered by the binding of NH₄⁺ (a). In the NH₄⁺ bound simulation, the S3 helix had a smaller tilting angle (b) and S5 and S6 helix had a larger tilting angle (c and d). Inset in panel (b) illustrates a helix tilting angle. Insets in panel (c) and (d) illustrate a helix kink conformation with the lower part of the helix aligned (white), and the top part of the helix showing a difference between NH₄⁺ bound and NH₄⁺ unbound SCAP. Only converged data from the last 500 ns of each simulation were used for the histogram analysis. **e.** Comparison of the interface contact area between SCAP and Insig during the simulations of the NH₄⁺ bound SCAP vs. the NH₄⁺ unbound SCAP. **f.** A schematic model for NH₄⁺ regulated SCAP activation. Left: Insig-SCAP binding in the absence of 25-HC and NH₄⁺. Top: Binding of 25-HC blocks NH₄⁺ binding to prevent SCAP activation (orange). Middle: Absence of 25-HC opens the channel, which permits the entry of NH₄⁺ to bind to D428 first, then to S326/S330 to form a stable binding site, leading to significant conformational changes of SCAP (red) and its dissociation from Insig for subsequent translocation and SREBP activation. Bottom: D428A mutant is unable to bind NH₄⁺, preventing NH₄⁺ from inducing conformational changes required for SCAP dissociation from Insig in the absence of 25-HC; thus, it cannot be activated by NH₄⁺.
Extended Data Fig. 7 | SCAP D428A mutation completely abolishes ammonia function. 

**a**, Representative confocal microscopy images of wild-type or mutant (D428A) GFP-SCAP in U87 cells compared to the Golgi marker Giantin (red) in response to glutamine or NH₄Cl stimulation in the presence of glucose. U87 cells were cultured on coverslips in DMEM medium supplemented with 5% FBS for 24 hr, followed by transfection with wild-type or mutant (D428A) GFP-SCAP plasmids for 24 hr. The transfected cells were washed with PBS once and incubated with glutamine (4 mM) or NH₄Cl (4 mM) for 12 hr in fresh serum-free DMEM medium with the presence of glucose (5 mM). Cell culture conditions prior to treatment are the same for subsequent panels. Scale bars, 10 μm.  

**b**, Western blot analysis of membrane and nuclear extracts from HEK293T cells transfected with GFP, GFP-SCAP wild-type or D428A mutant plasmids at the indicated doses together with full-length Flag-SREBP-1c for 24 hr and then placed in fresh serum-free DMEM medium containing glutamine (4 mM) and glucose (5 mM) for another 12 hr.  

**c**, Western blot analysis of membrane and nuclear extracts from HEK293T cells transfected with GFP, GFP-SCAP wild-type or mutant D428A, D428E (glutamate), D428N (asparagine), D428K (lysine) together with full-length Flag-SREBP-1c for 24 hr and then placed in fresh serum-free medium in the presence of glucose (5 mM) and glutamine (4 mM) for another 12 hr.  

**d, e**, Western blot analysis of membrane (for GFP-SCAP and SREBP precursors) and nuclear extracts (for N-terminal SREBPs) from HEK293T cells transfected with GFP, wild-type or mutant GFP-SCAP (D428A) together with full-length Flag-SREBP-1a (d) or HA-SREBP-2 (e) for 24 hr and then stimulated with glutamine (4 mM) or NH₄Cl (4 mM) in the presence of glucose (5 mM) under fresh serum-free medium.
Extended Data Fig. 8 | D428A mutation abolishes SCAP-promoted tumour growth. a, b, Gross and macroscopic images of mouse lungs (a) and H&E staining of lung sections (b) at day 50 after mouse implantation with H1299 cells expressing GFP, wild-type (WT) or mutant GFP-SCAP D428A. Framed images in red were presented in Fig. 6d as representatives. Scale bars, 2 mm. The number of nodules on mice lung sections was quantified by ImageJ (b, lower panel). Data are shown as mean ± SEM (n = 5). Significance was determined by one-way ANOVA with Dunnett’s multiple comparisons adjustment. c, MRI scans of mouse brain at day 12 after implantation of GBM30 cells stably transfected with GFP, wild-type or mutant (D428A) GFP-SCAP (3.5 × 10^3 cells/mouse). Yellow circles indicate tumour location. White arrows indicate injection site. Scatter plot shows tumour volume from MRI scans quantified from the outlined region-of-interest (ROIs) (right panel). The results are presented as mean ± SEM (n = 5). Significance was determined by unpaired and two-tailed Student’s t-test. d, H&E staining of mouse brain sections excised at day 17 after implantation of GBM30 cells as described in (c). Rectangle-framed images were used in Fig. 6h as representatives. Scale bars, 1 mm.
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Software and code

Policy information about availability of computer code

Data collection

The software used for data collection include:

- SM version 4.2, zeiss (https://www.zeiss.com/microscopy/us/downloads.html)
- Sequence Detection System v2.4, Applied Biosystems (https://www.thermofisher.com/order/catalog/product/4350490/4350490;)
- SPOT 5.2, SPOT IMAGING (https://www.spotimaging.com/software/)
- Modeller V10.1 (https://salilab.org/modeller/download_installation.html)
- CHARMM-GUI (https://charmm-gui.org/?doc= demo)
- Gromacs 2020 (https://manual.gromacs.org/documentation/2020/index.html)
- CgenFF (https://cgenff.umaryland.edu;)
- AMBER18 (https://ambermd.org/).

Data analysis

Data was analyzed using:

- GraphPad Prism 8.0.2, GraphPad (https://www.graphpad.com/scientific-software/prism/;), Fiji, imageJ (https://imagej.net/), and java 1.8.0_172 (64-bit) https://imagej.net/Downloads;)
- ImageScope v11.1.2.760, Aperio Technologies (https://www.leica Biosystems.com/digital-pathology/manage/apero-imagescope/;)
- MeV 9.0, http://mев.имр.рф/index.html; Ingenta Pathway Analysis (IPA), QIAGEN Bioinformatics (https://analysis.ingenuity.com/pa/installer/select;)
- Microsoft Office (Excel 2010, PowerPoint 2016, and word 2016;)
- VMD 1.9.3 (https://www.ks.uiuc.edu/Research/vmd/)
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All data that support the findings of this study are available within the paper and its supplementary information files. RNA data for Fig.1a, Fig.2f, and Extended Data Fig.1a are provided in supplementary data, and the raw data are deposited in the GEO repository (Accession: GSE199089; link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE199089) which is scheduled to be released on April 30, 2022.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
- No statistical method was used to determine sample sizes but sample size used was based on the results from our previous studies (Cheng et al., 2015a; Geng et al., 2016; Ru et al., 2016). All samples were included in the analysis.

Data exclusions
- No data were excluded from the analysis.

Replication
- All experiments were replicated at least twice independently. All attempts at replication were successful.

Randomization
- Among all experiments, samples/participants were randomly collected or allocated into groups. e.g., microscopic images were randomly collected, and mice were randomly grouped.

Blinding
- The quantification of tumor volume in mice brain measured by MRI imaging was blinded. Blinding is not applicable for other experiments, because the investigators who perform these experiments are same. However, we provided the proper control for each experiment.

Reporting for specific materials, systems and methods

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Materials & experimental systems

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology and archaeology |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |
| ☑   | Dual use research of concern |

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

Antibodies

Primary antibodies:

SCAP (9D5) (Santa Cruz: #sc-69836, 1:1000 for WB); PDI (H-17) (Santa Cruz: #sc-30932, 1:1000 for WB); Lamin A (H-102) (#sc-20680, 1:1000 for WB); SCAP antibody (Bethyl Laboratories: A303-554A, 1:1000 for WB); SREBP-2 (BD Pharmingen: #557037, 1:500 for WB); and SREBP-1 (IgG2A) (BD Pharmingen: 557036, 1:1000 for WB, 1:20 for IHC); SREBP-1 (Human Research Participants) (Abcam: #ab3259, 1:1000 for IF), GLS (Abcam: #ab93434, 1:1000 for WB and 1:50 for IHC); Giantin (Abcam: #ab24586, 1:1000 for IF); GFP (Roche: #11814460001, 1:1000 for WB), Flag-tag (Sigma: #F3165, 1:1000 for WB), p-EGFR Y1086 (Invitrogen: #369700, 1:5000 for WB); EGFR (Millipore: #05-1047, 1:1000 for WB); FASN (Cell Signaling: #3180S, 1:1000 for WB); SCD1 (M38) (Cell Signaling, #2438S, 1:500 for WB), HA-tag (C29F4) (Cell Signaling: #3724S, 1:1000 for WB); p-Akt Thr308 (Cell Signaling: #9275S, 1:1000 for WB); Ser473 (587F11) (Cell Signaling: #9801S, 1:1000 for WB); and HA-tag (M8) (Cell Signaling: #2178S, 1:1000 for WB).
Validation

All antibodies in this study were commercially purchased and validated. With multiple commercial options for each antibody, we selected those that had validating statements, multiple published references, clear instructions, and tested for its use for our intended purposes.

SCAP (9D5) (Santa Cruz: sc-69836), PDI (H-17) (Santa Cruz: sc-30932), Lamin A (H-102) (Santa Cruz: sc-20680), SCAP antibody (Bethyl Laboratories: #A303-554A), SREBP-2 (BD Pharmingen: #554737), and SREBP-1 (Cell Signaling: #2276) were validated in the previous studies (Cheng et al., 2015a; Gen et al., 2016; Ruet al., 2016; Guo et al., 2011).

GLS (Abcam: #ab93434) was validated in other previous studies (e.g., Hwang S, Yang S, Kim M, Hong Y, Kim B, Lee EK, Jeong SM. Mitochondrial glutamine metabolism regulates sensitivity of cancer cells after chemotherapy via amphiregulin. Cell Death Discov. 2021 Dec 20;7(1):395. doi: 10.1038/s41420-021-00792-7. PMID: 34924566; PMCID: PMC8685276; Paluschinski M, Jin C, Qvartskhava N, Görg B, Wammers M, Lang J, Lang K, Poschmann G, Stühler K, Häussinger D. Characterization of the scavenger cell proteome in mouse and rat liver. Biol Chem. 2021 Jul;402(9):1073-1085. doi: 10.1515/hsz-2021-0123. PMID: 34333885; Nguyen TL, Nokin MJ, Terés S, Tomé M, Bodineau C, Galmar O, Pasquet JM, Rousseau B, van Lempd S, Falcon-Perez JM, Richard E, Muzotte E, Rezvani HR, Priault M, Bouchecareilh M, Redonnet-Vernihet I, Calvo J, Uzan B, Piumo F, Fuentes P, Toribio ML, Khatib AM, Soubeiran P, Murdoch PDS, Durán RV. Downregulation of Glutamine Synthetase, not glutaminolysis, is responsible for glutamine addiction in Notch1-driven acute lymphoblastic leukemia. Mol Oncol. 2021 May;15(5):1412-1431. doi: 10.1002/1878-0261.12877. Epub 2021 Feb 13. PMID: 33314742; PMCID: PMC8096784). And we examined that the detected band migrated to the expected size, and loss of that specific band with gene-specific shRNA depletions, as well as its co-migration with product from its cDNA expressed from a plasmid. The antibody that were confirmed to specifically detect the protein of interest from a whole cell lysate were used for immunofluorescence (IF) and immunohistochemistry (IHC). Primary antibody specificity for IF was similarly determined by looking for loss or enhancement of signal with gene-specific shRNA or overexpression, respectively. Controls without any primary antibody was used to control for non-specific secondary antibody effects.

RIBOPHORIN I (Fisher: PIPAS25762) is validated for IHC, IF, WB, IHC-P. https://www.fishersci.com/shop/products/anti-ribophorin-i-polycyclonal-thermo-scientific-pierce-100ul-unlabeled/PIPAS25762

ERGIC-53/P58 (Sigma: E1031) is validated for IF, WB. https://www.sigmaaldrich.com/US/en/product/sigma/e1031

Grp94 (Cell Signaling: 20292S) is validated for IHC, IF, WB. https://www.cellsignal.com/products/primary-antibodies/grp94-d6x2q-xp-rabbit-mab/20292

ASPG (Sigma: #HPA069761) is validated for IHC, WB. https://www.sigmaaldrich.com/US/en/product/sigma/hpa069761

SDS (Lifespan Biosciences) is validated for IHC, IHC-P, IF, WB, Flo https://www.lsbio.com/antibodies/sds-antibody-clone-oti3d3-flow-if-immunofluorescence-ihc-wb-western-ls-c173534/180958
**Eukaryotic cell lines**

Policy information about cell lines

| Cell line(s) (See [Cell lines](#)) | U87 (HTB-14), T98 (CRL-1690), LN229 (CRL-2611), HepG2 (HB-8065), HEK293T (atcc-1500), H1299 (CRL-5803), H1975 (CRL-5908), HCC4006 (CRL-2871), and MDA468 (HTB-132) cells were purchased from ATCC, M233 (CCL-141) was purchased from RRD. U87/EGFR is a kind gift from Dr. Paul Vischel (Stanford) and human GBM primary cell, GBM30. Originally generated in Dr. Ichiro Nakano’s lab at OSU. GBM30-luc cells were generated in Dr. Balveen Kaur’s lab at OSU (currently working at UTHealth). H1299-luc cell line were generated in our lab. |

| Authentication | All cell lines used were authenticated by original sources and checked in lab for appropriate morphology. U87/EGFR was also authenticated by testing EGFR expression with western blot in our lab. H1299-luc and GBM30-luc were authenticated by testing luciferase expression in our lab. |

| Mycoplasma contamination | All cell lines were tested routinely for mycoplasma contamination either by PCR analysis or the mycoplasma detection kit. If mycoplasma was detected, the cell line was discarded and any results acquired from the contaminated cell lines were reconfirmed in mycoplasma negative cells. |

| Commonly misidentified lines (See [Cell lines](#)) | None were used. |

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**Animals and other organisms**

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Female Athymic nude (NCr-nu/nu) mice (6-8 weeks of age), and male Sprague-Dawley rats (350-400 g, 11-13 weeks of age) were used in the study. |

| Wild animals | NC wild animals were used in the study. |

| Field-collected samples | NC field-collected samples were used. |

| Ethics oversight | The research was approved by The Ohio State University Subcommittee on Research Animal Care, and all animal experiments were performed in accordance with guidance on the study protocol (2011A0000006-R3-AM1). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.