

SUCLG2 contributes to platinum resistance in lung adenocarcinoma through enhancing succinylation of GAC and glutamine metabolism

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Abstract

Platinum-based drugs constitute first-line chemotherapy for lung adenocarcinoma (LUAD), yet resistance emerges in 50%–70% of patients, representing a major clinical challenge. Synthetase GDP-forming subunit β (SUCLG2), the beta subunit of succinyl-CoA synthetase, is a key enzyme in the tricarboxylic acid (TCA) cycle. Using untargeted proteomic profiling, we identified SUCLG2 as a potential contributor of platinum resistance in LUAD. Further knockout of SUCLG2 significantly enhanced cellular sensitivity to platinum-based drugs. Mechanistically, using targeted metabolomics, we found that SUCLG2 conferred platinum resistance by promoting glutamine metabolism and facilitating the succinylation of glutaminase (GAC). This post-translational modification elevated GAC activity, thereby inhibiting apoptosis in LUAD cells. SUCLG2 was found to be highly expressed in both LUAD tissues and platinum-resistant cell lines. Consistent with cellular findings, animal experiments confirmed that elevated SUCLG2 expression critically contributes to platinum resistance in vivo. Our findings provide new insights into the molecular mechanisms underlying platinum resistance in LUAD and suggest novel therapeutic strategies.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide, responsible for nearly one in five cancer fatalities [1, 2]. Among the various histological subtypes, lung adenocarcinoma (LUAD) constitutes the most prevalent form, representing approximately 55% of all lung cancer cases [3]. Although significant progress has been made with targeted therapies and immunotherapies, platinum-based chemotherapy remains the cornerstone of treatment for advanced LUAD [4, 5]. However, the therapeutic benefit of platinum-based drugs, including cisplatin and carboplatin, is often limited due to the emergence of drug resistance, which ultimately results in disease progression and poor prognosis [6, 7]. Therefore, elucidating the molecular mechanisms underlying platinum resistance in LUAD is critical for the development of more effective therapeutic strategies.

Among diverse metabolic pathways, glutamine metabolism plays a critical role in platinum resistance in cancer cells, as it fuels crucial survival mechanisms such as nucleotide synthesis, antioxidant defense, and energy production [8]. In ovarian cancer, platinum-resistant cells exhibit a significant dependency on glutamine metabolism, which is facilitated by the upregulation of glutaminase expression [9]. In addition, ACAT1 could mediate ME2 acetylation to induce platinum resistance in ovarian cancer by linking glutamine metabolism to lactate production. Glutaminase is a central enzyme in glutamine metabolism and catalyzes the initial step of its catabolism, the conversion of glutamine to glutamate. Its isoforms include renal-type glutaminase (GLS) and hepatic-type glutaminase 2 (GLS2)[10]. Of note, among the GLS isoforms, glutaminase C (GAC) has been identified as the predominant isoform in multiple cancers, including LUAD [11]. GAC markedly enhances glutamine catabolism, thereby promoting both tumor progression and resistance. Nevertheless, the precise mechanisms by which glutamine metabolism and GAC contributes to platinum resistance in cancer cells remains incompletely understood.

Succinyl-coenzyme A ligase (SUCL) is a key enzyme in the TCA cycle, catalyzing the conversion of succinyl-coenzyme A (CoA) to succinate. It consists of two subunits: an α subunit encoded by SUCLG1, and a β subunit encoded by either SUCLA2 (which produces ATP) or SUCLG2 (which produces GTP) [12]. Emerging evidence indicates that SUCLG2 may perform non-metabolic functions, such as participating in cell signaling and succinylation [13]. In lung cancer, SUCLG2 has been shown to regulate mitochondrial dysfunction and promote tumor development by modulating the succinylation of key proteins [12]. Additionally, SUCLG2 is mutated in 4.3% of patients with pheochromocytoma and paraganglioma, and its loss leads to aberrant respiration and an increased succinate-to-fumarate ratio [14]. However, the role and underlying mechanisms of SUCLG2 in platinum resistance of lung adenocarcinoma remain unexplored.

Succinylation, one of the most prevalent post-translational modifications (PTMs) in cancer cells [15]. It can alter the spatial structure and charged characteristics of substrate proteins, thereby regulating multiple cellular processes such as the TCA cycle, electron transport chain, glycolysis, fatty acid oxidation, and the urea cycle [16–19]. Accumulating evidence suggests that succinylation of metabolic enzymes not only modulates their catalytic activity but also contributes to tumor progression and therapy resistance [20, 21]. However, the specific role of succinylation in regulating GAC activity and its implications for chemotherapy resistance in LUAD remains poorly understood.

In this study, we identified elevated expression of SUCLG2 in both LUAD tissues and platinum-resistant cell lines. SUCLG2 knockdown (sh) markedly increased their sensitivity to platinum-based chemotherapy. Mechanistically, we demonstrated that SUCLG2 confers platinum resistance by augmenting glutamine metabolism and promoting the succinylation of GAC. This modification enhances GAC activity, which subsequently attenuates apoptosis in LUAD cells. Our findings uncover a novel mechanism through which SUCLG2-driven GAC succinylation and reprogrammed glutamine metabolism foster platinum resistance, highlighting the therapeutic potential of targeting the SUCLG2–GAC axis to counteract chemoresistance in LUAD.

2. Results

Elevated SUCLG2 expression in lung adenocarcinoma and its association with platinum resistance.

SUCLG2 plays a crucial role in tumor development. In order to investigate the foundational role in lung adenocarcinoma. We first examined its expression using TCGA data, which revealed higher SUCLG2 expression in LUAD compared to normal tissues (Figure. 1A). Subsequently, we analyzed SUCLG2 in 16 paired LUAD tumor and adjacent normal tissue. Western blot confirmed that SUCLG2 was significantly upregulated in tumor tissues (Figures. 1B and C). A similar increase was observed across multiple lung cancer cell lines relative to the normal bronchial epithelial cell line BEAS-2B (Figure. 1D and E). Patient survival was correlated with the SUCLG2 level. As shown in Fig. 1F, patients with low levels of SUCLG2 exhibited better survival than those with high levels of SUCLG2 ($P = 0.014$). These results indicated that

SUCLG2 is closely related to LUAD progression and patient survival. Having established the significant overexpression of SUCLG2 in LUAD.

Furthermore, we performed an untargeted proteomic analysis comparing A549-WT and SUCLG2-knockout (SUCLG2-KO) A549 cells, which revealed 370 upregulated and 359 downregulated proteins (Fig. 1G). KEGG and GO analyses indicated that SUCLG2 loss altered the expression of multiple proteins associated with drug resistance—specifically in pathways such as drug resistance: antineoplastic, cell growth and death, and positive regulation of the apoptotic process (Fig. 1H and I). Given the well-documented role of these pathways in conferring cisplatin resistance, we hypothesized that SUCLG2 may promote chemoresistance in LUAD by modulating these key cellular processes. To test this, we established a platinum-resistant A549 cell line (A549-R). CCK-8 assays showed that A549-R cells exhibited markedly increased resistance, with a higher IC₅₀ value for cisplatin (DDP) compared to wild-type (WT) A549 cells (Fig. 1J), confirming successful generation of the resistant line. Notably, SUCLG2 expression was significantly higher in A549-R cells than in WT A549 cells (Fig. 1K). These results suggest that SUCLG2 plays an important role in mediating platinum resistance in LUAD cells.

SUCLG2 promotes platinum resistance in LUAD cells by suppressing apoptosis

We investigated the functional impact of SUCLG2 on cisplatin sensitivity both in vitro and in vivo. First, SUCLG2 was overexpressed (OE) or knocked out (KO) in A549 and H1299 cells. Following DDP treatment, cell viability and death were assessed using CCK-8 and propidium iodide (PI) staining assays. SUCLG2 overexpression significantly increased resistance to DDP in both A549 and H1299 cells (Fig. 2A and B; Figure S1A), whereas SUCLG2 knockout enhanced their sensitivity to DDP (Figure. 2C and D; Figure S1B and C). Similarly, SUCLG2 OE conferred resistance to CBP (Figure. 2E and F; Figure S1D), and its KO increased sensitivity to CBP (Fig. 2G, H; Figure S1E, F). The role of SUCLG2 in platinum resistance was further validated in xenograft models. Tumors derived from sh-SUCLG2 A549 cells showed markedly reduced weight and volume after DDP or CBP treatment compared with those from control cells (Figure. 2I–N). Collectively, these in vitro and in vivo results demonstrate that SUCLG2 promotes platinum resistance in LUAD.

Given that evasion of apoptosis is a key mechanism of platinum resistance, and because our proteomic analysis implicated SUCLG2 in the regulation of cell death (Fig. 1I), we examined apoptosis levels in DDP-treated A549 and H1299 cells. SUCLG2 knockout increased the level of the apoptosis marker cleaved-caspase 3 (Figure. 2O and P). Consistently, flow cytometry analysis revealed that SUCLG2 knockout significantly elevated the apoptosis rate in both A549 and H1299 cells following treatment with DDP or CBP (Figure. 2Q–T). Taken together, these findings indicate that SUCLG2 promotes platinum resistance in LUAD cells by suppressing apoptosis.

SUCLG2 mediates platinum resistance by enhancing glutamine metabolism.

To explore the mechanism by which SUCLG2 promotes platinum resistance, we conducted targeted metabolomics analysis of mitochondrial metabolites in SUCLG2-sh and CTL-sh A549 cells. The results

revealed that SUCLG2-sh significantly increased glutamine levels, suggesting a role for SUCLG2 in regulating glutamine metabolism (Figure. 3A and B). Glutamine metabolism has been shown to play an important regulatory role in tumor resistance to platinum-based drugs. We further assessed the levels of glutamate and NH_4^+ , key downstream metabolites of glutamine metabolism, in SUCLG2-OE A549 and H1299 cells. Both metabolites were elevated in SUCLG2-OE cells compared to controls (Figure. 3C and D), indicating that SUCLG2 enhances glutamine metabolism flux. Given the established role of glutamine metabolism in platinum resistance, we examined the effect of glutamine deprivation on DDP and CBP sensitivity. Depriving A549 and H1299 cells of glutamine significantly increased their sensitivity to both DDP and CBP (Figure. 3E and F; Figure. S2A and B). Glutaminase C-IN-1 (C-968) and CB-839 are inhibitors of glutamine metabolism. We found that both inhibitors enhanced the sensitivity of LUAD cells to DDP and CBP (Figure. 3G-J). Importantly, SUCLG2-OE failed to confer resistance to DDP under glutamine-deficient conditions (Figure. 3K). Conversely, supplementing with glutamine restored platinum resistance in SUCLG2-KO A549 and H1299 cells (Figure. 3L-N). Apoptosis assays by flow cytometry (Figs. 3O-R and S2C-F) and Western blot (Figures. 3S and T) further confirmed that glutamine supplementation could rescue the platinum drug sensitivity of SUCLG2-KO A549 and H1299 cells. Collectively, these findings demonstrate that SUCLG2-driven platinum resistance is dependent on enhanced glutamine metabolism.

SUCLG2 promotes platinum resistance by enhancing GAC activity.

To elucidate how SUCLG2 enhances glutamine metabolism and platinum resistance, we assessed its impact on the expression and function of key glutamine metabolism enzymes. SUCLG2-OE did not alter the protein levels of GAC or GLUD1 in A549 or H1299 cells (Figure. 4A and B). However, SUCLG2 OE significantly increased GAC enzymatic activity in both A549 and H1299 cells, while SUCLG2 KO suppressed it (Figure. 4C and D). Consistent with a role in resistance, GAC activity was also elevated in A549-R cells compared to WT controls (Figure. 4E). These findings demonstrate that SUCLG2 increased the activity of GAC. GAC plays a critical role in platinum resistance. Given the critical function of GAC in platinum resistance, we overexpressed GAC in A549 and H1299 cells and observed a significant increase in resistance to DDP or CBP (Figure. 4F-I), whereas GAC KO attenuated resistance to DDP (Figure. 4J and K). Functional rescue experiments demonstrated that GAC OE partially reversed the enhanced platinum sensitivity induced by SUCLG2 KO in A549 cells (Figure. 4L and M). Taken together, these results indicate that SUCLG2 promotes platinum resistance primarily through the regulation of GAC activity.

SUCLG2 interacts with GAC and stabilizes it in its active homotetrameric form

To determine the mechanism through which SUCLG2 regulates GAC activity, we first investigated whether SUCLG2 binds to GAC. Co-immunoprecipitation (co-IP) assays confirmed a interaction between SUCLG2 and GAC in A549 cells (Figure. 5A and B). Furthermore, GST pull-down assays confirmed a direct physical interaction between SUCLG2 and GAC (Figure. 5C). Immunofluorescence staining further

demonstrate strong co-localization of SUCLG2 and GAC in both A549 and H1299 cells (Figure. 5D and E), supporting their functional association.

Next, we aimed to explore how SUCLG2 regulates GAC activity. Given that the homotetrameric form of GAC exhibits greater enzymatic activity than its monomeric form[22], We investigated whether SUCLG2 influences GAC homotetrameric. SUCLG2-OE increased the abundance of the GAC homotetrameric in both A549 and H1299 cells (Figure. 5F and G). Consistent with this, platinum-resistant A549-R cells showed elevated levels of homotetrameric GAC compared WT -A549 cells (Figure. 5H). Conversely, SUCLG2 KO reduced the formation of GAC homotetrameric in A549 and H1299 cells (Figure. 5I and J). Collectively, these results demonstrate that SUCLG2 enhances GAC activity by facilitating its assembly into the active homotetramer form.

SUCLG2 increases the succinylation level of GAC

Total succinylation of tumor cells has been reported to influence multiple oncogenic processes, including proliferation, glucose metabolism, redox homeostasis, tumor microenvironment remodeling and DNA damage response [10, 23–26]. Based on our previous finding that SUCLG2 modulates cellular succinylation [12], we hypothesized that SUCLG2 regulate GAC activity via succinylation. We first confirmed that GAC undergoes succinylation using co-IP (Figure. 6A). SUCLG2 OE significantly enhanced GAC succinylated in A549 cells compared to empty vector controls (Figure. 6B), whereas sh-SUCLG2 markedly reduced it (Figure. 6C). We further observed that treatment with DDP or CBP, which induces cell death, weakened the SUCLG2-GAC interaction and reduced GAC succinylation (Figure.6D and E). Conversely, enhanced SUCLG2-GAC interaction and increased GAC succinylation were observed in platinum-resistant A549-R cells compared to their wild-type counterparts (Fig. 6F and G). These results indicate that SUCLG2 promotes GAC succinylation, which may enhance GAC activity and contribute to platinum resistance.

Furthermore, we confirmed a physical interaction between SIRT5 and GAC (Figure. 6H and I). Additionally, we found that the desuccinylase SIRT5 reduces GAC succinylation, while SIRT5 KO enhances their modification (Figure. 6J and K), indicating that SIRT5 functions as a desuccinylase for GAC. Notably, SUCLG2 OE weakened the SIRT5-GAC interaction, whereas sh-SUCLG2 strengthened their binding (Figure. 6L and M). Furthermore, we found that SUCLG2 inhibited the binding of SIRT5 to GAC, as shown by His pull-down assay (Figure. 6N). To determine whether SUCLG2 affects GAC succinylation via its enzymatic activity, we constructed a SUCLG2 mutant with an inactive catalytic site (SUCLG2-S161A). However, the SUCLG2-S161A mutant failed to alter GAC succinylation levels or affect the interaction between GAC and SIRT5 (Figure. 6O and P). This indicated that SUCLG2 promotes the succinylation of GAC protein by competitively binding to SIRT5 through a non-enzymatic function. Furthermore, these results demonstrate that SUCLG2 enhances GAC succinylation and promotes platinum resistance by competitively inhibiting the binding of SIRT5 to GAC.

K311 is the primary succinylation site on GAC

To identify the major succinylation sites on GAC. We first used the bioinformatics platform PhosphoSitePlus (<https://www.phosphosite.org>) and predicted two potential succinylation sites: K164 and K311 (Figure. 7A). We then constructed corresponding mutants (GAC-K164R and GAC-K311R), and compared their succinylation levels to GAC-WT. The succinylation level of GAC-K311R was significantly reduced, while that of GAC-K164R remained unchanged (Figure. 7B; Figure. S3A). Moreover, Neither SUCLG2 OE nor SIRT5 sh affected succinylation of the GAC-K311R mutant (Figure. 7C; Figure. S3B). This finding suggests that SUCLG2 regulates the succinylation of GAC primarily at lysine 311.

Functional analyses revealed that, compared to GAC-WT, the GAC-K311R mutant exhibited impaired homotetramer formation and decreased enzymatic activity in both A549 and H1299 cells (Figure. 7D and E). In contrast, the GAC-K164R mutant did not affect GAC activity (Figure. S3C). Moreover, OE of GAC-K311R did not confer platinum resistance in A549 and H1299 cells treated with different concentrations of DDP or CBP (Figure. 7F and G; Figure. S3D and E). To further evaluate the therapeutic relevance of GAC succinylation, we generated A549 cells with endogenous GAC KO and reconstituted them with either GAC-WT or GAC-K311R. Flow cytometry analysis showed that GAC-K311R cells underwent significantly more apoptosis than GAC-WT cells after DDP or CBP treatment (Figure. 7H-K). In xenograft models, tumors derived from A549-GAC-K311R cells displayed reduced weight and volume following DDP or CBP administration compared to those from A549-GAC-WT cells (Figure. 7L-Q). Immunohistochemical staining of tumor tissues revealed lower expression of the proliferation markers Ki67 and PCNA in the GAC-K311R group (Figure. 7R), indicating enhanced platinum sensitivity. Collectively, these results demonstrate that succinylation at K311 is critical for GAC homotetramerization, enzymatic activity, and platinum resistance in LUAD.

In this study, we identified elevated expression of SUCLG2 in both LUAD tissues and platinum-resistant cell lines. sh-SUCLG2 significantly increased their sensitivity to platinum-based chemotherapy. Mechanistically, we demonstrated that SUCLG2 confers platinum resistance by augmenting glutamine metabolism and promoting succinylation of GAC. This post-translational modification enhances GAC enzymatic activity, which subsequently attenuates apoptosis in LUAD cells (Figure. 8).

3. Discussion

Platinum-based chemotherapy remains the backbone of systemic treatment for advanced LUAD, but inherent or acquired resistance is a major barrier to curative outcomes, with nearly 70% of patients experiencing disease progression within 12 months of treatment initiation [27]. This resistance not only limits therapeutic efficacy but also correlates with poor prognosis, making it a pressing unmet clinical need [28]. In this study, we identified SUCLG2 as a key driver of platinum resistance in LUAD. Its expression is markedly elevated in LUAD tissues and resistant cell lines. Importantly, our findings highlight SUCLG2 not only as a potential prognostic biomarker but also as a regulator of a novel metabolic axis—SUCLG2-mediated succinylation of GAC—that enables LUAD cells to evade platinum-induced apoptosis, offering new insights into metabolic mechanisms of chemoresistance.

SUCLG2 is traditionally recognized as a mitochondrial enzyme that catalyzes the conversion of succinyl-CoA to succinate in the TCA cycle, a role critical for maintaining energy production and metabolite homeostasis [29, 30]. Beyond this canonical function, emerging studies have begun to uncover its broader implications in cellular physiology and disease, particularly in cancer. For instance, SUCLG2 has been linked to mitochondrial dysfunction and metabolic reprogramming in lung adenocarcinoma [12]. In other cancer types, such as prostate cancer, it is upregulated by EGFR-LIFR signaling after androgen deprivation therapy, promoting neuroendocrine differentiation and tumor growth through regulating succinate metabolism and mitochondrial NDPK enzymatic activity [31]. These reports underscore SUCLG2 as more than a canonical metabolic enzyme, instead functioning as a multifaceted regulator of tumor biology. Our study extends these observations by demonstrating that SUCLG2 exerts noncanonical functions in chemotherapy resistance. While its metabolic role in the TCA cycle is indispensable for energy balance, we found that SUCLG2 confers platinum resistance through regulation of glutamine metabolism, independent of its role in ATP production. This is consistent with a growing body of evidence showing that metabolic enzymes often “moonlight” as modulators of signaling pathways and PTMs.

Metabolic reprogramming is a well-established hallmark of cancer and a critical contributor to drug resistance [32, 33]. Among the diverse metabolic adaptations, glutamine metabolism stands out as a key player in supporting tumor cell survival under therapeutic stress. Glutamine serves as a versatile substrate, fueling nucleotide synthesis, maintaining redox homeostasis via glutathione production, and replenishing TCA cycle intermediates to sustain energy production—all of which are critical for overcoming platinum-induced DNA damage and oxidative stress [10]. Our findings reveal that SUCLG2 acts as a regulator of glutamine metabolism in LUAD. Metabolomic profiling experiments demonstrated that SUCLG2 OE elevates intracellular glutamate levels, and promotes carbon flux from glutamine into the TCA cycle. Conversely, sh-SUCLG2 impairs glutamine catabolism. Functional assays further confirmed that the resistance phenotype conferred by SUCLG2 is dependent on active glutamine metabolism: glutamine deprivation sensitized SUCLG2-OE cells to platinum, while restoring glutamine supply rescued resistance in sh-SUCLG2 cells. These results align with prior studies highlighting glutamine metabolism play an important role in resistant tumors [34, 35]. For example, inhibition of GLS, the rate-limiting enzyme in glutaminolysis, has been shown to resensitize various cancers to chemotherapy [36]. Our work adds a new layer to this paradigm by identifying SUCLG2 as an upstream regulator that licenses glutamine metabolism to drive resistance, suggesting that targeting SUCLG2 could normalize this pathway and restore platinum sensitivity.

PTMs have emerged as critical regulators of metabolic enzyme function, allowing cells to rapidly adapt to environmental changes without altering gene expression [37, 38]. Succinylation, in particular, has gained attention for its ability to modulate metabolic pathways by altering protein structure, stability, and enzymatic activity [39, 40]. Our study identifies succinylation as a key mechanism through which SUCLG2 controls glutamine metabolism and resistance. Notably, we also identified a role for SIRT5, a mitochondrial desuccinylase, in this regulatory network. SUCLG2 and SIRT5 fine-tunes GAC

succinylation, suggesting a dynamic balance between succinylation (promoted by SUCLG2) and desuccinylation (mediated by SIRT5) that controls glutaminolysis.

We also demonstrated that SUCLG2 physically interacts with GAC, the predominant isoform of glutaminase in LUAD, and promotes its succinylation. This modification is functionally significant: GAC succinylation levels correlate with its enzymatic activity, and manipulation of SUCLG2 expression directly alters both succinylation and activity. Mechanistically, succinylation stabilizes GAC in its active oligomeric state—consistent with our observation that SUCLG2 enhances GAC oligomer formation, a structural prerequisite for catalytic function.

In summary, this study identifies SUCLG2 as a pivotal mediator of platinum resistance in LUAD through succinylation-dependent activation of GAC and subsequent enhancement of glutamine metabolism. Our findings provide mechanistic insight into adaptive tumor survival and suggest SUCLG2 as both a biomarker and therapeutic target.

Conclusions

In this study, we identified elevated expression of SUCLG2 in both LUAD tissues and platinum-resistant cell lines. sh-SUCLG2 significantly increased their sensitivity to platinum-based chemotherapy. Mechanistically, we demonstrated that SUCLG2 confers platinum resistance by augmenting glutamine metabolism and promoting succinylation of GAC. This post-translational modification enhances GAC enzymatic activity, which subsequently attenuates apoptosis in LUAD cells (Figure. 8).

4. Experimental Section

Antibodies and Reagents: Antibodies: The rabbit polyclonal antibodies against SUCLG2 (Proteintech, 14240-1-AP, 1:2000, RRID:AB_2197187), SIRT5 (Proteintech, 67257-1-Ig, 1:1000, RRID:AB_2882530), GAC (Proteintech, 12855-1-AP, 1:8000, RRID:AB_2110381), GLUD1 (Proteintech, 14299-1-AP, 1:20000, RRID:AB_2110515), β -Actin (Proteintech, 60008-1-Ig, 1:4000, RRID:AB_2289225) were ordered from Proteintech. The anti-succinyllysine rabbit polyclonal antibody (PTM-401, 1:500, RRID:AB_2687628) was ordered from PTM BIO.

Reagents

The SUCLG2 siRNA (SR305801) was ordered from Origene. Polyformaldehyde (P1110, 4%) was purchased from Solarbio.

Plasmids

PCR-amplified SUCLG2 was cloned into the pcDNA-His/v5.

Cell Culture and Transfection: Cell culture: The human lung epithelial cell line BEAS-2B (RRID:CVCL_0168) was purchased from the National Collection of Authenticated Cell Cultures and

cultured in RPMI 1640 (Gibco) supplemented with 10% FBS (SORFA), LUAD cell lines (A549 (RRID:CVCL_A549), H1299 (RRID:CVCL_XE62), H23 (RRID:CVCL_1547), HCC827 (RRID:CVCL_2063), H358 (RRID:CVCL_1559) and PC9 (RRID:CVCL_B7N9)) were purchased from the cell library of the Chinese Academy of Sciences were cultured in RPMI 1640 (Gibco) supplemented with 10% FBS (SORFA). HEK293T (RRID:CVCL_0063) cells were purchased from the National Collection of Authenticated Cell Cultures and cultured in DMEM (Dulbecco's Modified Eagle Medium, Gibco) supplemented with 10% FBS (SORFA). All cells were cultured under an atmosphere of 5% CO₂ at 37°C.

Transfection

Cells at 70–80% confluence were transfected with the plasmids using the SuperFectin DNA Transfection Reagent Kit (Pufei, 2103 – 100). For siRNA transfection, cells at 50% confluence were transfected using the siTran 2.0 siRNA Transfection Reagent (OriGene, TT320002), following the manufacturers' protocols.

Patient Samples: LUAD tissues and matched adjacent normal tissues were collected from the First Affiliated Hospital of Nanchang University. This study was approved by the ethics committee of the First Affiliated Hospital of Nanchang University with approval number [Approval No: (2024) CDYFYULK (10–020)]. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. All participants were informed about the purpose of the study, assured of confidentiality, and provided written consent prior to participation. Participation was voluntary, and respondents could withdraw at any time without consequence.

Cell Proliferation Assay: Cell growth assay: 3000–5000 cells/well were seeded in 24-well plates (three replicates/group). At specified time points, cells were fixed with 4% formaldehyde for 15 min, stained with 1% crystal violet, and solubilized in 10% acetic acid. Relative proliferation was quantified by measuring absorbance at 595 nm.

RT-PCR

Total RNA was extracted using the TRTGene reagent (Genestar P118-05), and complementary DNA (cDNA) was synthesized using the PrimeScript® RT Reagent Kit (DRR047A, Takara). Quantitative PCR (qPCR) was performed on an ABI StepOnePlus™ system using SYBR® Premix Ex Taq II (DRR820A, Takara). Gene expression was calculated via the $2^{-\Delta\Delta CT}$ method. Primer sequences are listed in Table S1.

Immunoprecipitation and Western Blot: Immunoprecipitation: Cells were washed with PBS and lysed in NP-40 buffer (150 mM NaCl, 20 mM β -glycerol phosphate, 1 mM Na orthovanadate, 20 mM NaF, 0.5% Nonidet P-40, 20mMHepes, pH 7.4) containing PMSF for 30min at 4°C. Lysates were incubated with Protein G-Agarose (Roche, 11 243 233 001) and primary antibodies overnight at 4°C. Beads were washed with PBS, resuspended in 2× loading buffer, and boiled for 10 min.

Western blot

Samples were separated by 10% SDS-PAGE, transferred to PVDF membranes (Millipore, IPVH00010), blocked with 5% skim milk (Solarbio D8340) for 1 h, and incubated with primary antibodies overnight at 4°C. Membranes were washed with TBS-T, incubated with the secondary antibody for 1 h, and visualized using ECL reagent (TIANGEN, PA112-01) with digital gel image analysis (TANON 5500) was used to visualize the proteins.

CRISPR-Cas9-Mediated Gene Disruption

SUCLG2 KO was performed using the lentiCRISPR-v2 system. sgRNAs targeting SUCLG2 were cloned into the lentiCRISPR-v2-Flag vector. Lentiviruses were produced in HEK-293T cells and used to infect A549 or H1299 cells. Infected cells were selected with 10 µg/mL puromycin, and single clones were expanded. KO efficiency was confirmed by western blot.

Immunofluorescence Assay

1×10^3 cells were seeded on cell slides (NEST, 801010) in 24-well plates and cultured for 24 h. Cells were fixed with 4% paraformaldehyde for 30 min, permeabilized/blocked with 3% BSA + 0.2% Triton X-100 in PBS for 1 h, and incubated with primary antibodies overnight. After washing, cells were incubated with fluorescent secondary antibodies for 1 h, counterstained with DAPI (SouthernBiotech, 0100 – 20), and imaged using a ZEISS laser confocal microscope.

In Vivo Xenograft Assay: All animal experiments were approved by the Institutional Animal Use and Care Committee of Nanchang University (Approval No: NCULAE-20240704001) and were conducted in accordance with the ARRIVE guidelines. Four-week-old male BALB/c-Nude mice were purchased from Gempharmatech Co., Ltd (Jiangsu, China). Mice were housed in a specific pathogen-free (SPF) facility at the Institute of Translational Medicine, Nanchang University, under controlled conditions (temperature: $22 \pm 2^\circ\text{C}$; humidity: $55 \pm 10\%$; 12-hour light/dark cycle) with ad libitum access to standard rodent diet and sterile water. To assess chemoresistance mechanisms, A549 cells (1×10^7) with various genetic manipulations—including control shRNA, sh-SUCLG2, GAC-WT, and GAC-K311R—were subcutaneously injected into mouse flanks. 7 days post-injection, mice received intratumoral injections of either DDP (0.1 mg/mL), CBP (0.2 mg/mL), or normal saline. For intratumoral injections and terminal procedures, mice were anesthetized using isoflurane (3% for induction, 1.5-2% for maintenance in oxygen). At the completion of the study (28 days post-injection), all mice were euthanized by carbon dioxide asphyxiation (gradual displacement rate of 30–70% of the chamber volume per minute). Tumors were dissected and photographed, with volume calculated as $(\pi/6 \times [\text{large diameter}] \times [\text{small diameter}]^2)$ and weight measured. Harvested tissues were processed for IHC analysis.

GLS1 activity was assessed using the GLS Activity Assay Kit (Solarbio, Beijing)

WT and SUCLG2-overexpressing A549 cells were lysed, and the supernatants were incubated with the assay reagents. After 1 h at 37°C, absorbance at 630 nm was measured.

Glutamic Acid(Glu) Content Assay(Solarbio, Beijing): Add 1 mL of Reagent 1 to 5 million cells, followed by ice-bath ultrasonic cell disruption (power: 200 W, sonication duration: 3 seconds, interval: 10 seconds, total time: 3 minutes). Then centrifuge at 10,000 rpm at 4°C for 10 minutes, collect the supernatant, and place it on ice for subsequent detection. Prepare the measurement system according to the kit instructions, mix thoroughly, incubate at 37°C for 30 minutes, and then measure the absorbance at 340 nm.

Blood Ammonia Content Assay(Solarbio, Beijing): Preheat the microplate reader at 37°C for 30 minutes in advance. Add 1 mL of extraction buffer to 5 million cells, followed by ice-bath ultrasonic cell disruption (power: 200 W, sonication time: 3 seconds, interval: 10 seconds, total duration: 3 minutes). Then centrifuge at 10,000 rpm at 4°C for 10 minutes, collect the supernatant, and place it on ice for subsequent detection. Prepare the measurement system according to the kit instructions, mix thoroughly, incubate in a 37°C water bath for 20 minutes, and then measure the absorbance at 630 nm.

Apoptosis and Cell Viability Assays

Apoptosis was evaluated by PI single staining. Control and SUCLG2-overexpressing or KO cells were treated with platinum drugs DDP (20 mM) or CBP (300 mM) for 48 hours. Cells were washed with PBS, incubated with PI/DAPI (1:1, 1:100 dilution) at 37°C for 30 min, and imaged under a fluorescence microscope.

Cell viability

Cell viability was measured using the CCK-8 assay. Briefly, 4000 cells per well were seeded in 96-well plates in RPMI-1640 medium containing 10% FBS. After 24 h, cells were treated with gradient concentrations of cisplatin or carboplatin for 24–48 h. CCK-8 reagent (1:20 dilution) was added for 2 h, and absorbance at 450 nm was measured.

Glutaraldehyde crosslink assay

WT and SUCLG2-modified A549 cells were lysed in NP-40 buffer, centrifuged, and supernatants were collected. Samples were incubated with 25% glutaraldehyde (1:500 dilution) at 4°C for 30 min, quenched with glycine (500 µM), and boiled with loading buffer for SDS-PAGE analysis.

Immunohistochemistry (IHC)

Paraffin-embedded sections (3 µm) were dewaxed, rehydrated, and treated with 3% hydrogen peroxide for 10 min. Antigen retrieval was performed in EDTA buffer (pH 9.0) for 24 min. Sections were blocked with 10% goat serum and incubated with the indicated antibodies.

Quantitative Proteomics analysis

The Quantitative Proteomics was performed and analyzed in PTM BIO (Table S2). Proteomic analysis was performed by extracting proteins from samples, followed by reduction with dithiothreitol, alkylation

with iodoacetamide, and tryptic digestion. The resulting peptides were, where applicable, labeled with TMT reagents, fractionated by high-pH reverse-phase HPLC, and subjected to affinity enrichment for post-translational modifications. Peptides were separated by nano-liquid chromatography and analyzed by LC-MS/MS using either a timsTOF Pro (4D-PASEF mode) or a Q Exactive HF-X mass spectrometer. The acquired MS/MS data were processed using the MaxQuant software suite against the appropriate species-specific database for protein identification and quantification. Bioinformatics analysis included Gene Ontology (GO) annotation, KEGG pathway enrichment, protein domain analysis, subcellular localization prediction, and protein-protein interaction network construction for differentially expressed proteins.

Flow Cytometry Sorting (FCS)

In the cell apoptosis assay, cells were treated with cisplatin or carboplatin. Following 48 hours of incubation, the cells were harvested, rinsed with PBS, and then resuspended in 500 μ L of 1 $\times$ binding buffer. The cell suspension was incubated with 5 μ L of annexin V/FITC and 5 μ L of propidium iodide (PI) at room temperature for 15 minutes. The apoptosis rate was determined using a CytoFLEX flow cytometer and analyzed with CytExpert 2.2 software. Both FITC+/PI⁻ and FITC+/PI⁺ cells were defined as apoptotic cells.

Targeted Mitochondrial Metabolomics

The Targeted Mitochondrial Metabolomics was performed and analyzed in Metware (Table S3). The sample extracts were analyzed using an LC-ESI-MS/MS system. Samples were prepared by resuspending the cell pellet, followed by extraction with precooled methanol, repeated freeze-thaw cycles, and centrifugation. The supernatant was processed through a Protein Precipitation Plate prior to analysis. Chromatographic separation was performed on an ACQUITY UPLC BEH Amide column using a gradient of water with 10 mM ammonium acetate/0.3% ammonium hydroxide and 90% acetonitrile/water. Mass spectrometric detection was conducted with an ESI source operating in both positive and negative ion modes, using multiple reaction monitoring (MRM).

Statistical Analysis: All data are presented as mean $\pm$ SD. Statistical analyses were performed using GraphPad Prism 8. Differences between groups were analyzed by Student's t-test. Statistical significance was defined as follows: ns: not significant ($p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of the First Affiliated Hospital of Nanchang University with approval number [Approval No: (2024) CDYFYYLK (10-020)]. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. All participants were informed about the purpose of the study, assured of confidentiality, and provided written consent prior to

participation. Participation was voluntary, and respondents could withdraw at any time without consequence.

All animal experiments were approved by the Institutional Animal Use and Care Committee of Nanchang University (Approval No: NCULAE-20240704001) and were performed in accordance with the ARRIVE guidelines.

Additional Sections:

ACAT1: Acetyl-CoA acetyltransferase 1	GLS: Glutaminase
ARRIVE: Animal Research: Reporting of In Vivo Experiments	GLS2: Glutaminase 2
BALB/c: Bagg Albino/c	GLUD1: Glutamate dehydrogenase 1
CBP: Carboplatin	GO: Gene Ontology
CCK-8: Cell Counting Kit-8	GTP: Guanosine triphosphate
cDNA: Complementary DNA	HCC: Hepatocellular carcinoma
Co-IP: Co-immunoprecipitation	HPLC: High-performance liquid chromatography
DAPI: 4',6-Diamidino-2-phenylindole	IHC: Immunohistochemistry
DDP: Cisplatin	IRB: Institutional Review Board
DMEM: Dulbecco's Modified Eagle Medium	KEGG: Kyoto Encyclopedia of Genes and Genomes
EDTA: Ethylenediaminetetraacetic acid	KO: Knockout
EGFR: Epithelial growth factor receptor	LC-MS/MS: Liquid chromatography-tandem mass spectrometry
FBS: Fetal bovine serum	LIFR: Leukemia inhibitory factor receptor
GAC: Glutaminase C	LUAD: Lung adenocarcinoma
ME2: Malic enzyme 2	OE: Overexpression
MRM: Multiple reaction monitoring	PBS: Phosphate-buffered saline
MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide	PCNA: Proliferating cell nuclear antigen
NSCLC: Non-small cell lung cancer	PCR: Polymerase chain reaction
PI: Propidium iodide	qPCR: Quantitative real-time polymerase chain reaction
PMSF: Phenylmethylsulfonyl fluoride	RPMI: Roswell Park Memorial Institute medium
PTM: Post-translational modification	SD: Standard deviation
sh: Short hairpin RNA	SIRT5: Sirtuin 5
SPF: Specific pathogen-free	SUCL: Succinyl-coenzyme A ligase
SUCLG2: Succinyl-CoA ligase GDP-forming subunit β	TCA: Tricarboxylic acid
TCGA: The Cancer Genome Atlas	TMT: Tandem mass tag
WB: Western blot	WT: Wild-type

Consent for publication

Not applicable.

Founding

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Author' contributions

QFH. and BTY. designed the study and H.W. implemented the design in practice. ZRW., HW., WXD., LW., JYC., JX., YGH., XPC.,YFS, YY., MLH., DFL.and LXB. designed and completed the experiments. LJ. and DL. collected clinical tissues. QFH. and HW. analyzed the data and wrote the manuscript. QFH. and BTY. revised the paper. This manuscript was approved by all authors.

Acknowledgements

Not applicable.

Conflict of Interest

The authors declare no conflicts of interest.

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Figures

Fig.1

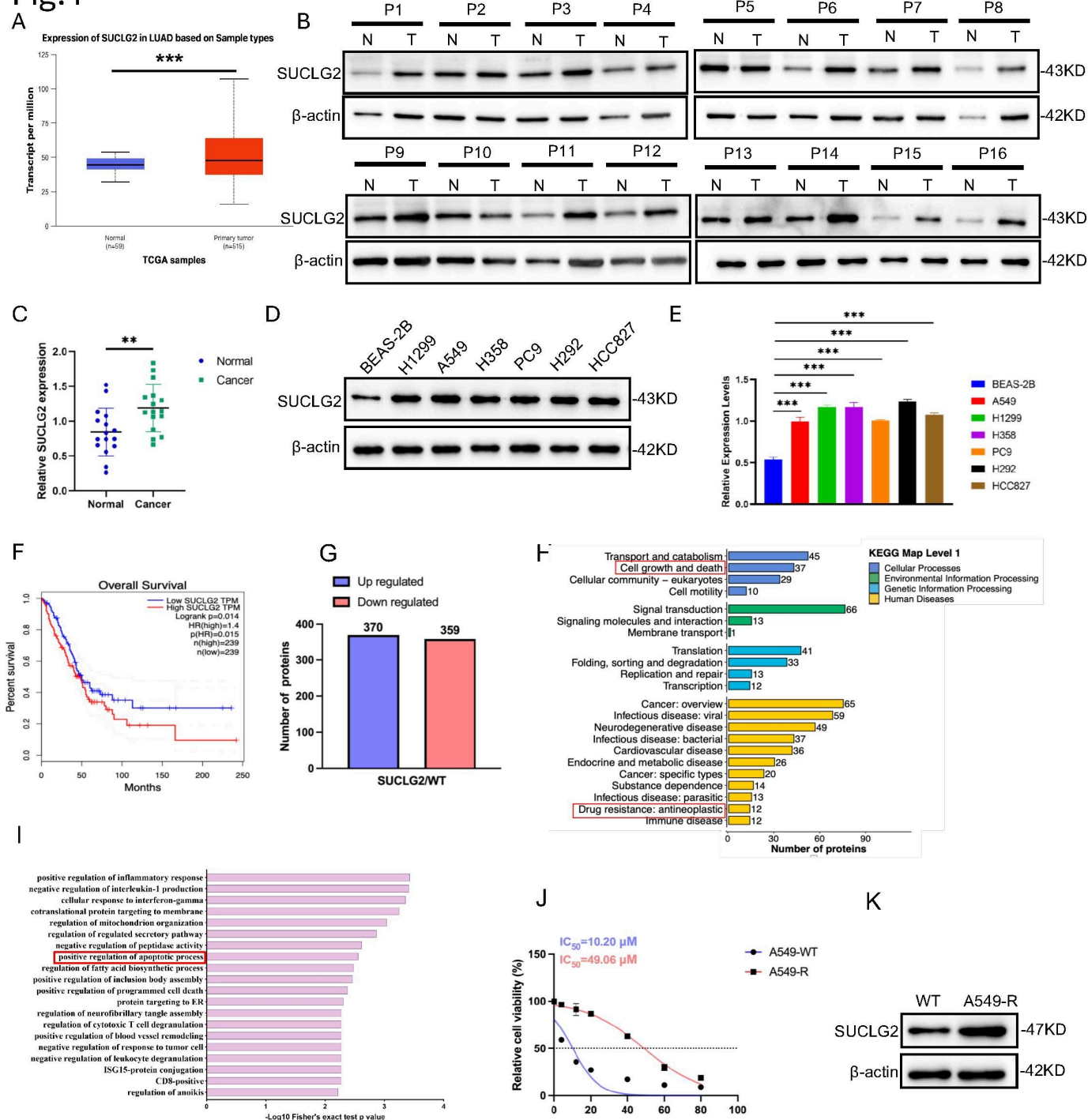


Figure 1

Elevated SUCLG2 expression in lung adenocarcinoma and its association with platinum resistance. (A) UALCAN (<https://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl>) analysis for SUCLG2 expression in LUAD tumor and normal tissues. (B and C) SUCLG2 protein levels were assessed by western blot in paired LUAD tissues (T) and adjacent normal tissues (N). Quantification was performed using ImageJ software (C), data represent mean $\pm$ SD from 16 independent experiments. ** $p < 0.01$. (D and E) SUCLG2

expression was analyzed in LUAD cell lines and normal bronchial epithelial cell line BEAS-2B via western bolt (D). Protein levels were quantified using ImageJ software (E), $***p < 0.001$. (F) GEPIA (<http://gepia.cancer-pku.cn>) were used to evaluate the impact of SUCLG2 on the survival of patients with LUAD. (G-I) SUCLG2-KO and WT A549 cells were sent to PTM Company for non-targeted proteomic analysis. Subsequently, KEGG and GO pathway enrichment analysis was performed on the proteomic profiles comparing the two cell lines. (J) A549-WT and A549-R cells were treated with varying concentrations of DDP for 24 h, and cell viability was measured by CCK-8 assay to determine the IC50 values. (K) The protein expression level of SUCLG2 in A549-WT and A549-R cells was assessed by Western blot analysis.

Fig.2

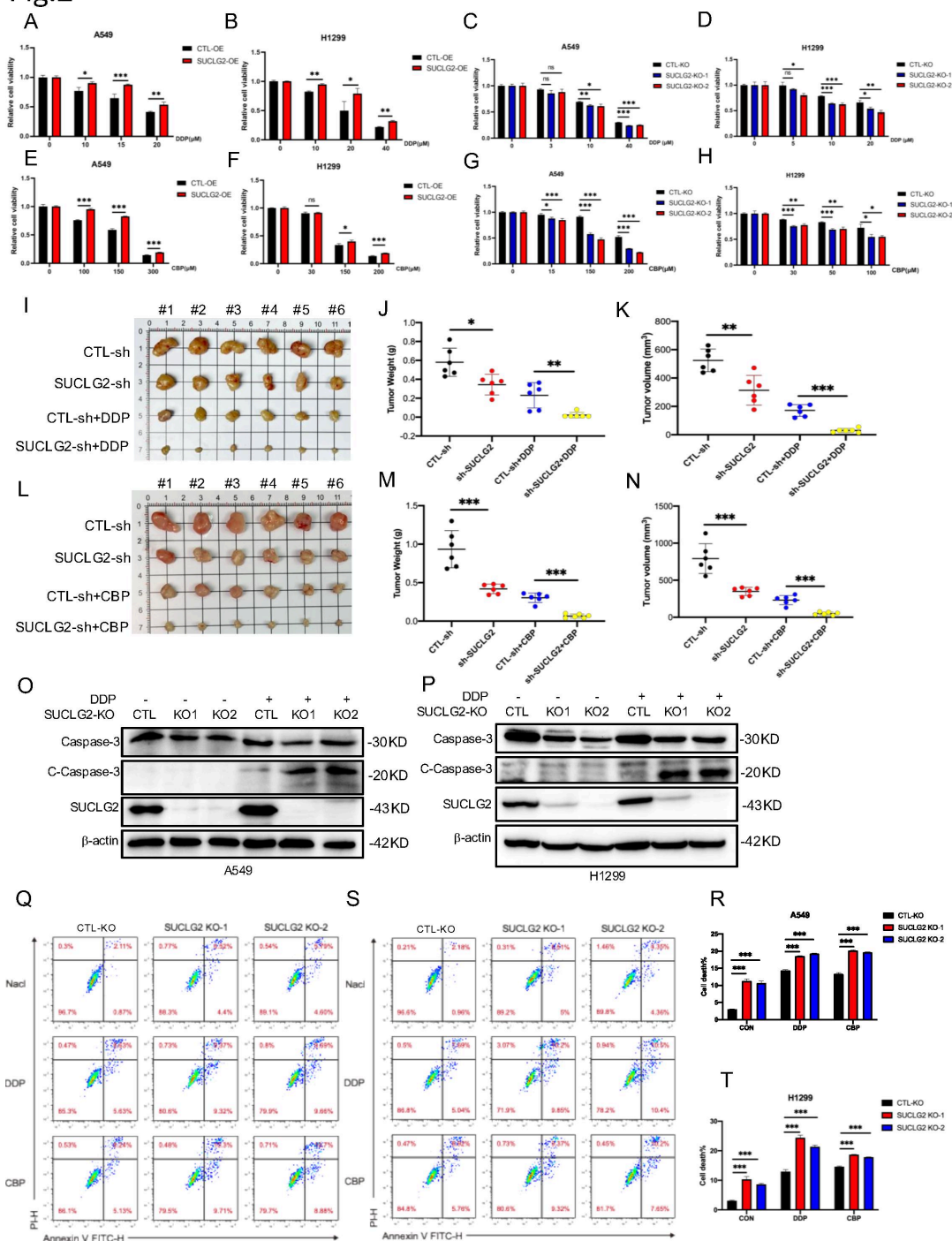


Figure 2

SUCLG2 promotes platinum resistance in LUAD cells by suppressing apoptosis. (A and B) SUCLG2-OE A549 (A) and H1299 (B) cells were treated with the indicated concentrations of DDP for 24 h, followed by cell viability analysis using CCK-8 assay. *p < 0.05; **p < 0.01; ***p < 0.001. (C and D) SUCLG2-KO A549 (C) and H1299 (D) cells were treated with the indicated concentrations of DDP for 24 h, followed by cell viability analysis using CCK-8 assay. Ns > 0.05, *p < 0.05; **p < 0.01; ***p < 0.001. (E and F) SUCLG2-OE

A549 (E) and H1299 (F) cells were treated with the indicated concentrations of CBP for 24 h, followed by cell viability analysis using CCK-8 assay. (G and H) SUCLG2-KO A549 (G) and H1299 (H) cells were treated with the indicated concentrations of CBP for 24 h, followed by cell viability analysis using CCK-8 assay. (I-K) SUCLG2-sh and CTL-sh A549 cells were subcutaneously injected into the nude mice (n = 6 per group). After 7 days, mice were treated with 0.1 mL DDP (0.1 mg/mL) or normal saline. Tumors were excised and photographed 28 days post-injection (I). Tumor weight (J) and volume (K) were measured. *p < 0.05; **p < 0.01; ***p < 0.001 (unpaired t-test). (L-N) SUCLG2-sh and CTL-sh A549 cells were subcutaneously injected into nude mice (n = 6 per group). After 7 days, mice were treated with 0.1 mL CBP (0.2 mg/mL) or normal saline. Tumors were excised and photographed 28 days post-injection (L). Tumor weight (M) and volume (N) were measured. ***p < 0.001 (unpaired t-test). (O and P) The expression levels of caspase-3 and cleaved-caspase-3 in SUCLG2-KO and control cells were assessed by Western blotting. (Q and R) Apoptosis levels in SUCLG2-KO and CTL-KO A549 cells treated with DDP or CBP were assessed by flow cytometry. Data represent mean $\pm$ SD of three independent experiments. ***p < 0.001. (S and T) Apoptosis levels in SUCLG2-KO and CTL-KO H1299 cells treated with DDP or CBP were assessed by flow cytometry. Data represent mean $\pm$ SD of three independent experiments. ***p < 0.001.

Fig.3

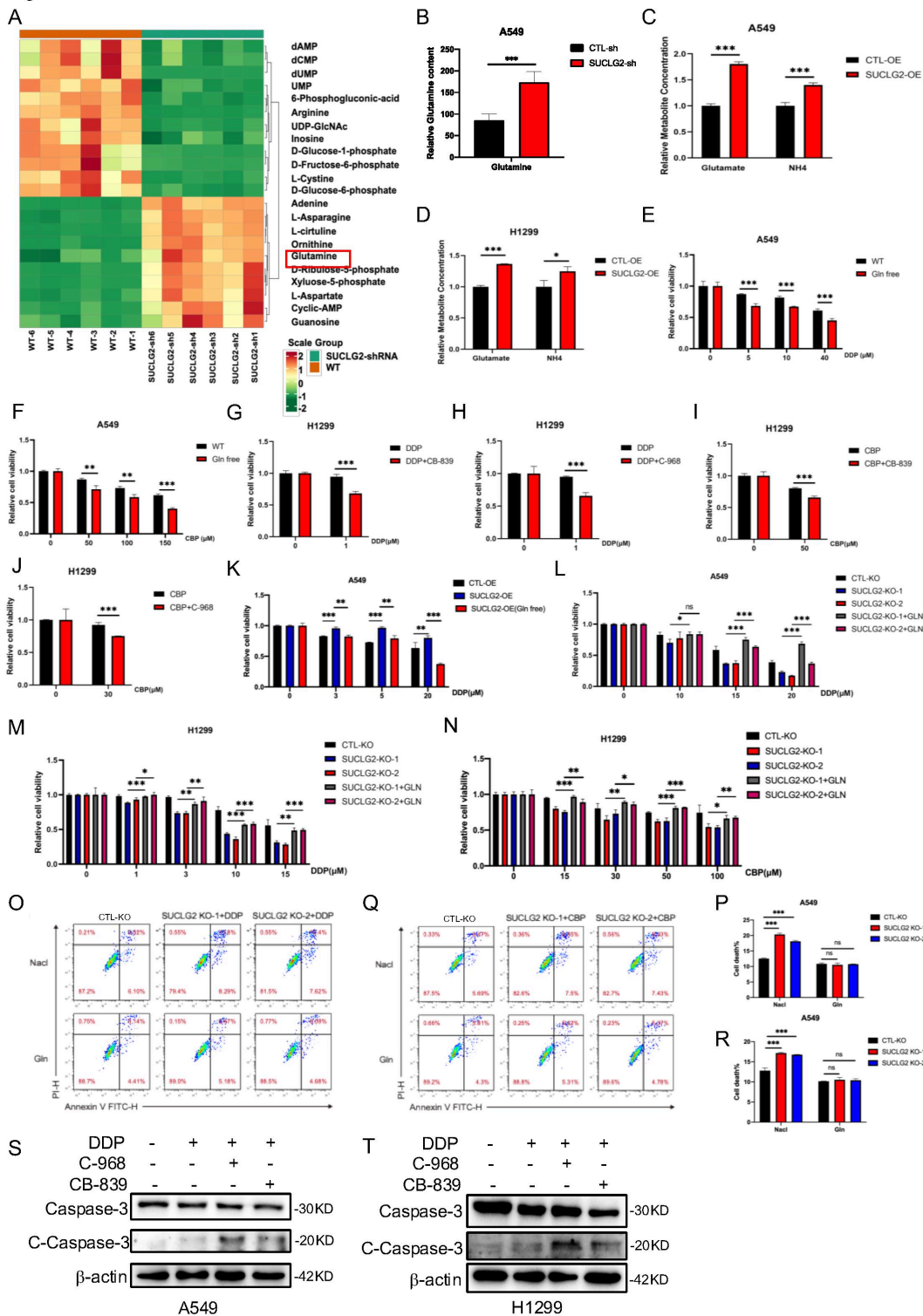


Figure 3

SUCLG2 mediates platinum resistance by enhancing glutamine metabolism. (A) Targeted mitochondrial metabolomics was performed in SUCLG2-sh and CTL-sh A549 cells (n = 6 per group). (B) Glutamine levels in SUCLG2-sh and CTL-sh A549 cells were quantified via targeted metabolomics. (C and D) Concentrations of glutamate and NH_4^+ in SUCLG2-OE A549 and H1299 cells were measured using commercial assay kits. (E and F) A549 cells cultured under glutamine-depleted or normal conditions

were treated with indicated doses of DDP (E) or CBP (F), followed by cell viability analysis using CCK-8 assay. (G and H) SUCLG2-KO H1299 cells were added with DDP and treated with CB-839 (G) or C-968 (H), followed by cell viability analysis using CCK-8 assay. (I and J) SUCLG2-KO H1299 cells were added with CBP and treated with CB-839 (I) or C-968 (J), followed by cell viability analysis using CCK-8 assay. (K) SUCLG2-OE A549 cells cultured under glutamine-deprived or normal conditions were treated with indicated doses of DDP, followed by cell viability analysis using CCK-8 assay. (L-N) SUCLG2-KO A549 and H1299 cells were supplemented with glutamine and treated with DDP or CBP, followed by cell viability analysis using CCK-8 assay. (O and P) Apoptosis in SUCLG2-KO and CTL-KO A549 cells treated with DDP and supplemented with glutamine was analyzed by flow cytometry. Data represent mean $\pm$ SD of three independent experiments. ns: not significant ($p > 0.05$); *** $p < 0.001$. (Q and R) Apoptosis in SUCLG2-KO and CTL-KO A549 cells treated with CBP and supplemented with glutamine was analyzed by flow cytometry. Data represent mean $\pm$ SD of three independent experiments. ns: not significant ($p > 0.05$); *** $p < 0.001$. (S and T) In A549 and H1299 cells treated with C-968 or CB-839, the expression levels of caspase-3 and cleaved-caspase-3 were assessed by Western blotting.

Fig.4

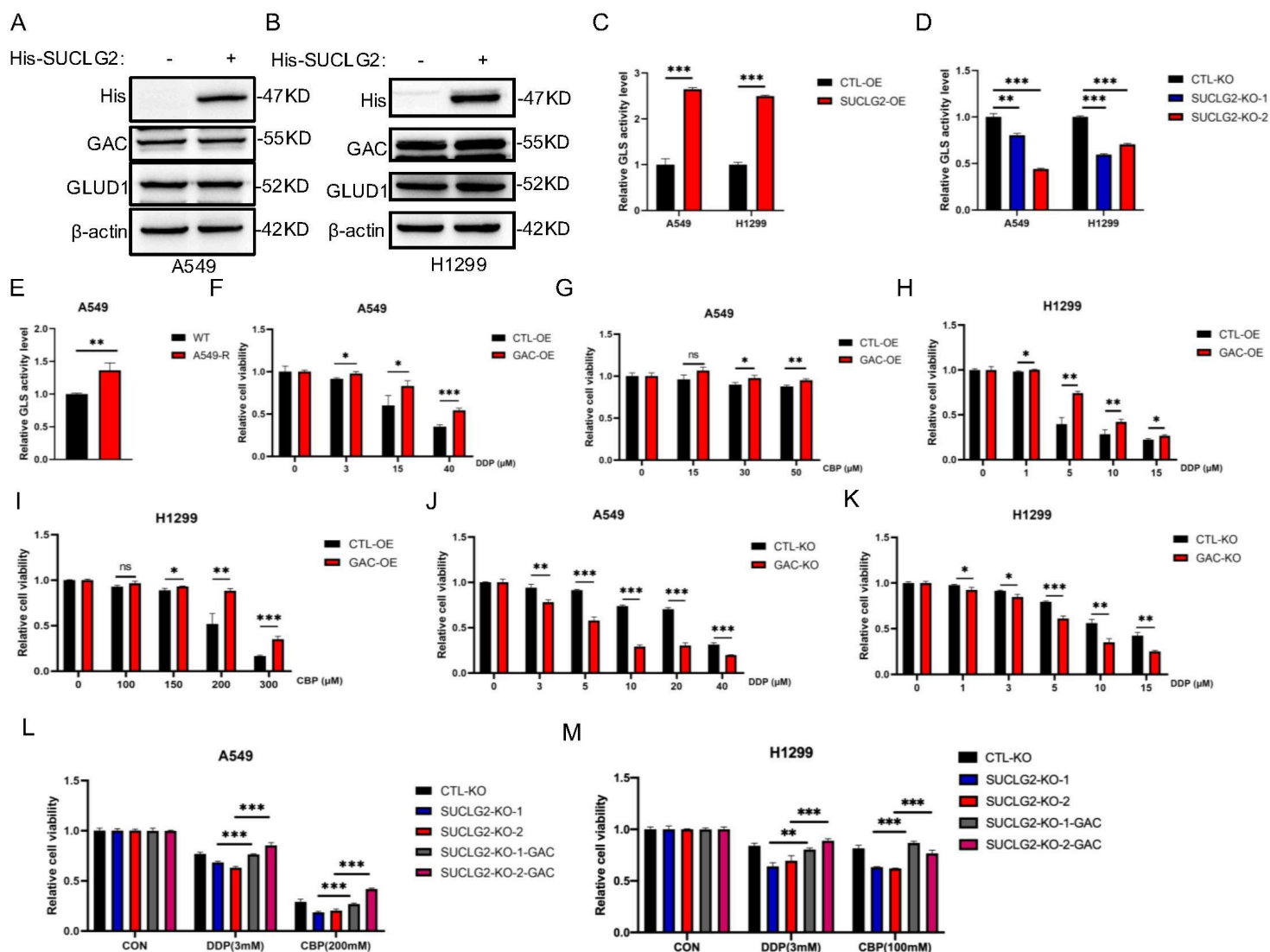


Figure 4

SUCLG2 promotes platinum resistance by enhancing GAC activity. (A and B) The expression of GLUD1 and GAC protein was analyzed by western blot in A549 and H1299 cells overexpressing SUCLG2. (C and D) GAC activity was measured using a GLS assay kit in A549 and H1299 cells following SUCLG2 OE (C) or KO (D), $***p < 0.001$. (E) GAC activity was compared between A549-WT and A549-R cells using a GLS activity assay kit. $**p < 0.01$. (F-I) GAC-overexpressing A549 and H1299 cells were treated with indicated concentrations of DDP or CBP, followed by cell viability analysis using CCK-8 assay. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. (J and K) GAC-KO A549 (J) and H1299 (K) cells were treated with indicated concentrations of DDP, followed by cell viability analysis using CCK-8 assay. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. (L and M) SUCLG2-KO and/or GAC-overexpressing A549 (L) and H1299 (M) cells were treated with DDP or CBP, followed by cell viability analysis using CCK-8 assay. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Fig.5

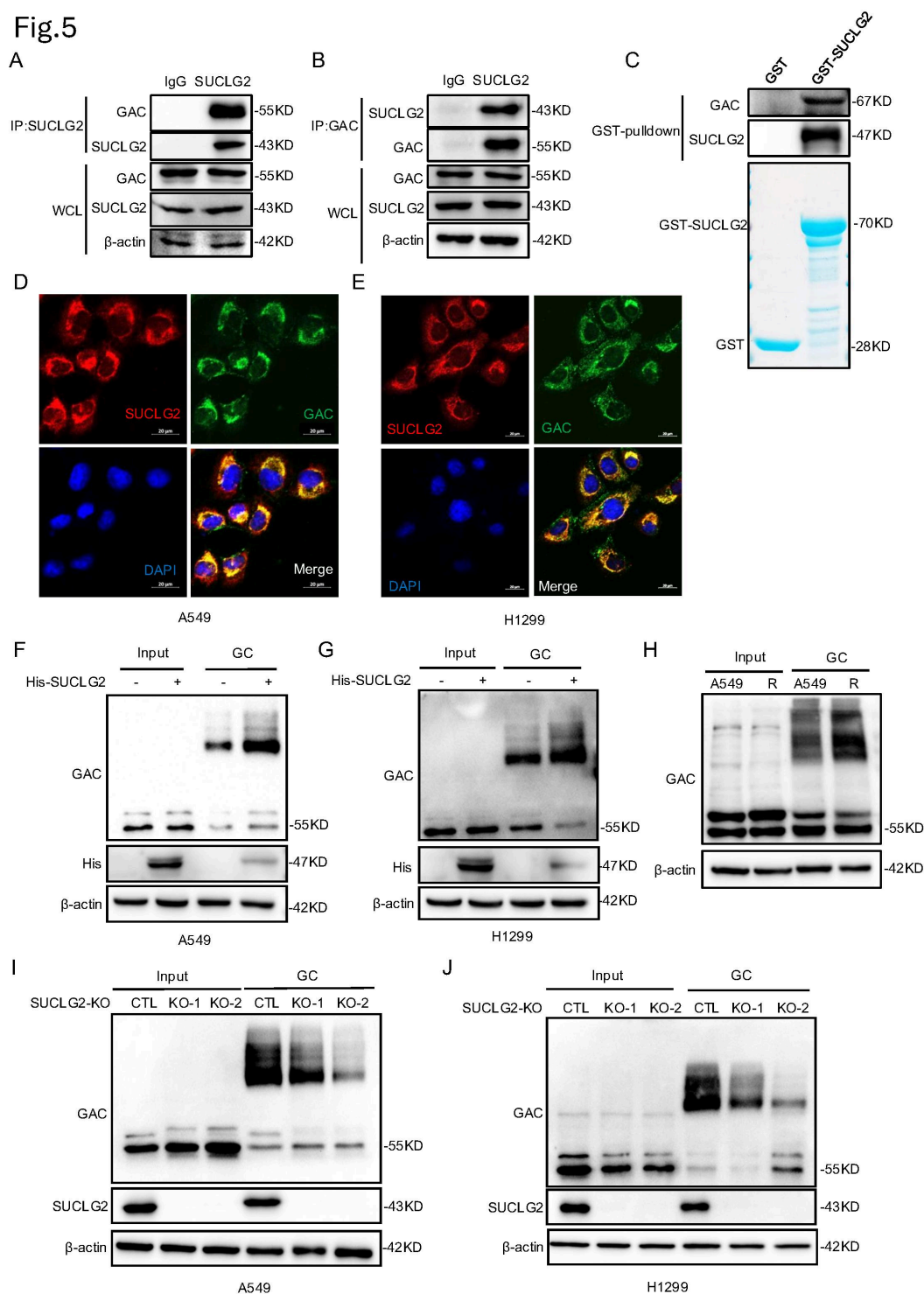


Figure 5

SUCLG2 interacts with GAC and stabilizes it in its active homotetrameric form. (A and B) Interaction between GAC and SUCLG2 in A549 cells was analyzed by co-IP and western blot. (C) The interaction between GST-SUCLG2 and GAC was examined in vitro using a GST pull-down assay. (D and E) Co-localization of SUCLG2 and GAC in A549 (D) and H1299 (E) cells was detected by immunofluorescence. (F and G) Cell lysates from His-SUCLG2-OE A549 (F) and H1299 (G) cells were crosslinked with or

without 0.025% glutaraldehyde and analyzed by western blot with anti-GAC antibody. (H) Cell lysates from WT-A549 and A549-R cells were crosslinked with or without 0.025% glutaraldehyde and analyzed by western blot with anti-GAC antibody. (I and J) Cell lysates from SUCLG2-KO A549 (I) and H1299 (J) cells were crosslinked with or without 0.025% glutaraldehyde and analyzed by western blot with anti-GAC antibody.

Fig.6

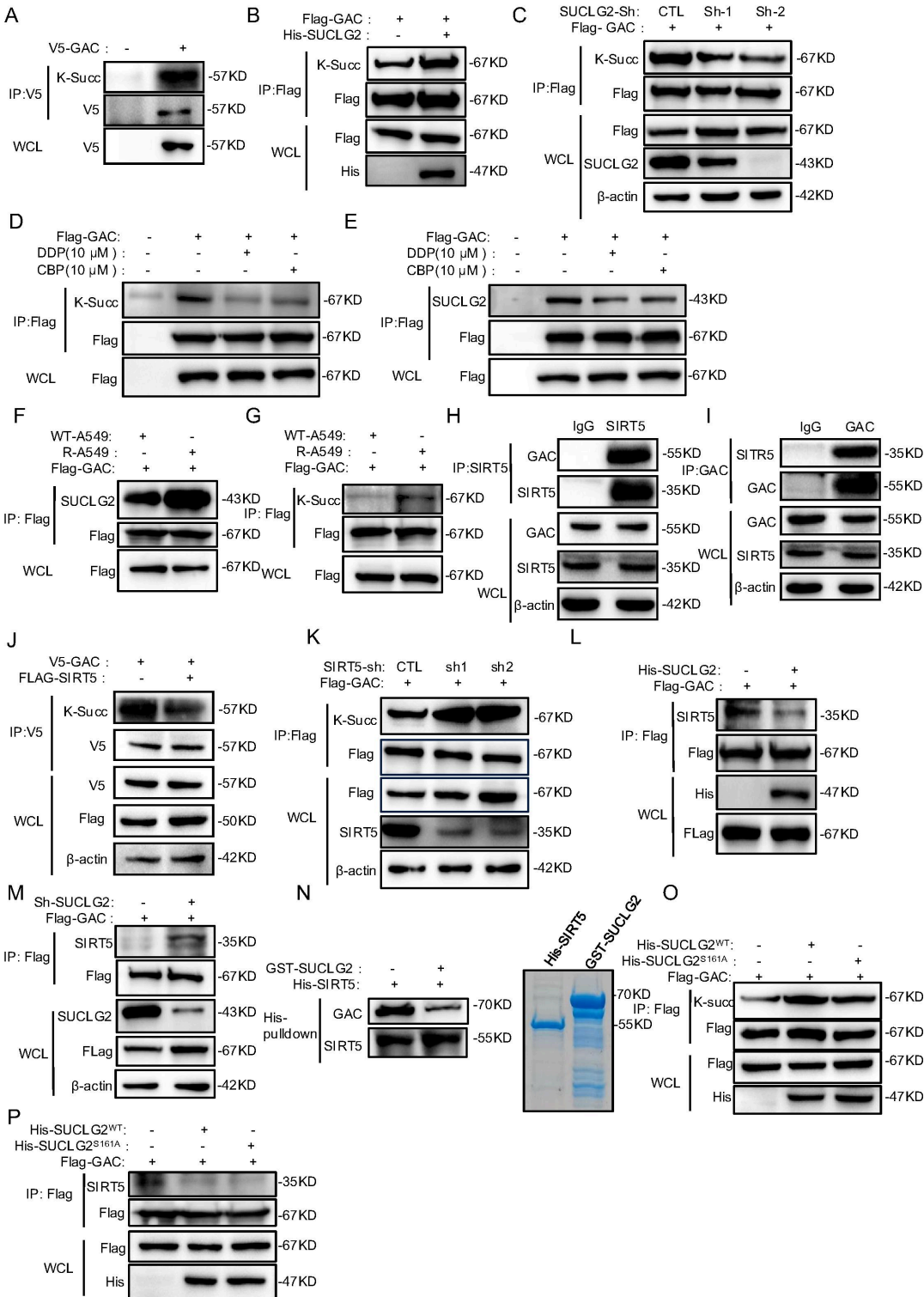


Figure 6

SUCLG2 increases the succinylation level of GAC. (A) Succinylation of GAC in A549 cells was assessed by co-IP and western blot. (B and C) GAC succinylation was detected in A549 cells following SUCLG2 OE (B) or sh (C) by co-IP and western blot. (D and E) After overexpressing GAC and treating A549 cells with indicated concentrations of DDP or CBP for 24 hours, GAC succinylation (D) and its binding to SUCLG2 (E) were analyzed by co-IP. (F) After overexpressing GAC in both A549 and A549-R cells, the interaction between SUCLG2 and GAC was assessed by co-IP followed by Western blot analysis. (G) GAC succinylation levels were compared between WT A549 and R-A549 cells. (H and I) Interaction between SIRT5 and SUCLG2 in A549 cells was analyzed by co-IP and western blot. (J and K) GAC succinylation was detected by co-IP and western blot in A549 cells following SIRT5 OE (J) or sh (K). (L) A549 cells were transfected with the indicated plasmids, and the binding between GAC and SIRT5 was detected by co-IP and western blot. (M) SUCLG2-sh A549 cells were transfected with flag-GAC plasmids, and the binding between GAC and SIRT5 was detected by co-IP and western blot. (N) SUCLG2 affect the interaction between His-SIRT5 and GAC was examined in vitro using a His pull-down assay. (O) GAC succinylation levels were compared between A549-SUCLG2-OE and A549-SUCLG2-S161A cells. (P) A549 cells were transfected with the indicated plasmids, and the binding between GAC and SIRT5 was detected by co-IP and western blot.

Fig.7

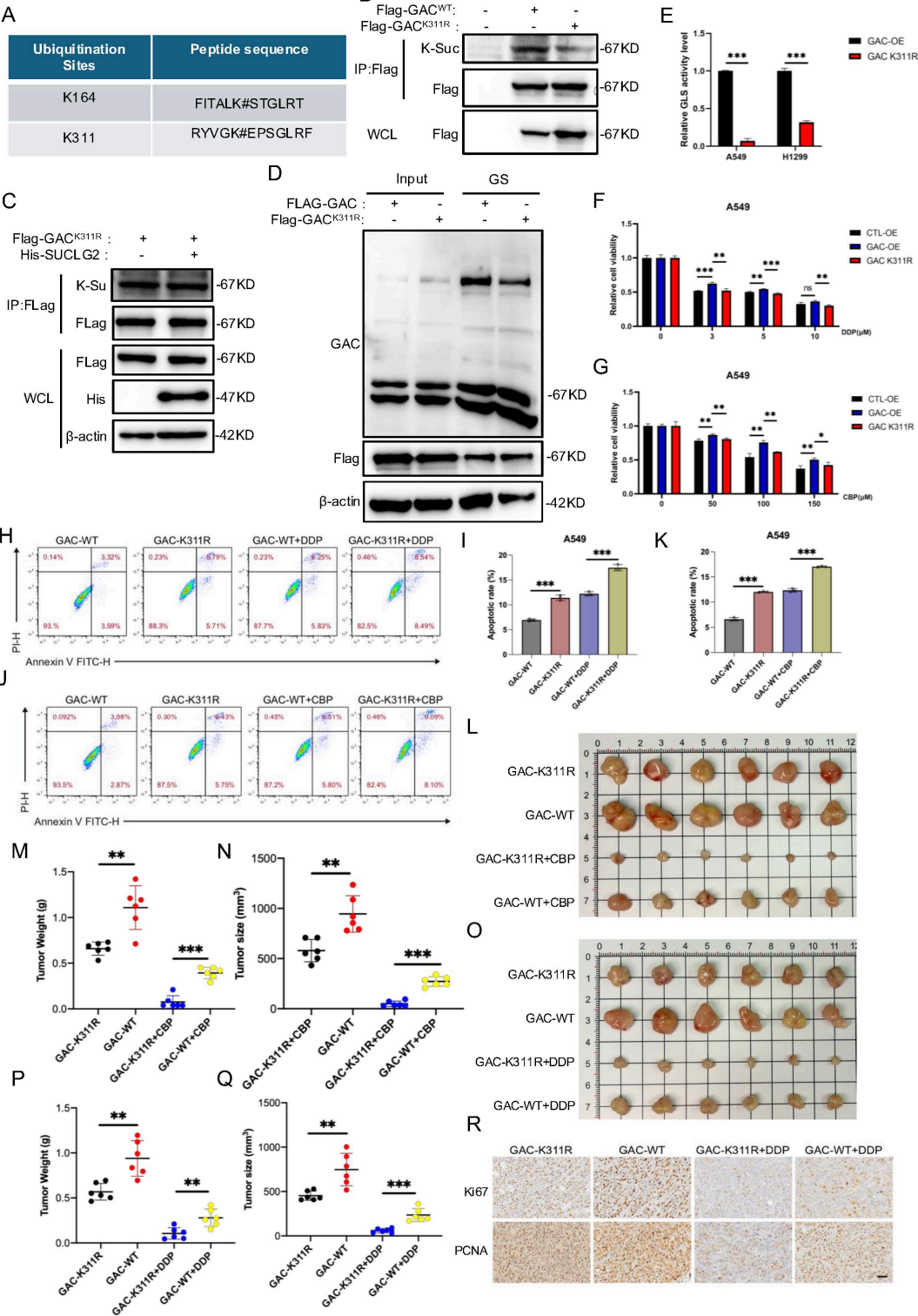


Figure 7

K311 is the primary succinylation site on GAC. (A) Predicted succinylation sites K164 and K311 on GAC. (B) Succinylation levels were detected by co-IP and western blot in A549 cells overexpressing GAC-WT or GAC-K311R. (C) Succinylation levels were analyzed by co-IP and western blot in A549 cells overexpressing both the SUCLG2 and GAC-K311R. (D) Cell lysates of A549 cells expressing GAC-WT or GAC-K311R were added with or without 0.025% glutaraldehyde and analyzed by western blot using an

Schematic model of SUCLG2 contributes to platinum resistance in lung adenocarcinoma through enhance succinylation of GAC and glutamine metabolism.

Supplementary Files

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- [TableS2SUCLG2targetedmetabolomicsdata.xlsx](#)
- [TableS3Primersequencesforconstructplasmids.docx](#)
- [TableS1SUCLG2proteomicsdata.xlsx](#)
- [SupplementalTextandFigures.doc](#)
- [FigureS2.pdf](#)
- [FigureS3.pdf](#)
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