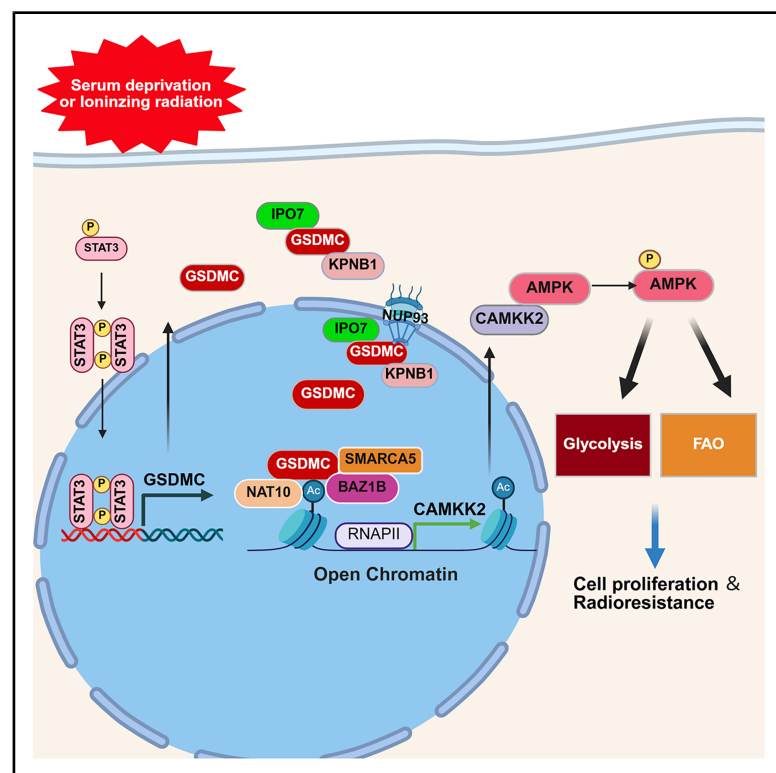


Gasdermin C reprograms metabolism through the CAMKK2-AMPK axis to promote lung adenocarcinoma progression and radioresistance

Graphical abstract



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In brief

Ren et al. uncover that GSDMC is upregulated in patient with LUAD and correlates with poor prognosis. Under nutrient stress or ionizing radiation, phosphorylated STAT3 at Ser727 transcriptionally induces GSDMC. Nuclear GSDMC activates CAMKK2-AMPK to reprogram metabolism and promote radioresistance, serving as a promising therapeutic target.

Highlights

- GSDMC upregulation in LUAD links to poor prognosis via p-STAT3 Ser727 under stress
- Nuclear GSDMC forms a complex with NAT10/BAZ1B/SMARCA5 to regulate gene expression in LUAD
- GSDMC activates CAMKK2-AMPK to enhance glycolysis and fatty acid oxidation for LUAD growth



Article

Gasdermin C reprograms metabolism through the CAMKK2-AMPK axis to promote lung adenocarcinoma progression and radioresistance

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SUMMARY

Metabolic reprogramming is a hallmark of lung adenocarcinoma (LUAD) progression and therapeutic resistance. Gasdermin C (GSDMC) is frequently upregulated in patients with LUAD and correlates with poor prognosis. Under nutrient stress or ionizing radiation, phosphorylated STAT3 at Ser727 transcriptionally induces GSDMC, which translocates into the nucleus via the IPO7-KPNB1-NUP93 complex. In the nucleus, GSDMC functions as a scaffold molecule, recruiting NAT10 to mediate histone H3 acetylation and recruiting BAZ1B/SMARCA5 to modulate chromatin remodeling and chromatin accessibility. These changes facilitate CAMKK2-AMPK pathway activation. The GSDMC-CAMKK2-AMPK axis promotes metabolic reprogramming toward glycolysis and fatty acid oxidation, supporting LUAD cell proliferation and survival. Importantly, GSDMC contributes to LUAD radioresistance through the STAT3-GSDMC-CAMKK2-AMPK axis, and targeting any component of this pathway enhances radiotherapy sensitivity in preclinical models. Our findings identify a regulatory role for GSDMC in LUAD progression via metabolic reprogramming and support its potential as a preclinical candidate target to improve radiotherapy response.

INTRODUCTION

Metabolic reprogramming is a hallmark of cancer, with tumor cells exhibiting distinct metabolic adaptations to support their growth and survival. While normal cells primarily rely on oxidative phosphorylation for energy production, cancer cells preferentially utilize aerobic glycolysis even under oxygen-rich conditions.^{1,2} Tumor cells dynamically reprogram their energy metabolism to adapt to nutrient availability, optimizing ATP production from glucose and lipids. In eukaryotes, ATP serves as the primary energy currency, and its hydrolysis to ADP/AMP triggers metabolic stress responses. Cells monitor energy levels through AMP-activated protein kinase (AMPK), which is activated by AMP/ADP binding.³ Once activated, AMPK promotes catabolic pathways (e.g., glycolysis, lipid oxidation) while inhibiting anabolic processes, thereby restoring energy balance and maintaining cellular homeostasis.^{4–6} This shift of metabolism, along with reprogrammed lipid metabolism, provides tumor cells with the necessary energy and biosynthetic precursors while reducing oxidative stress.^{7–9} Fatty acid metabolites further serve as clinical biomarkers and correlate with poor prognosis.¹⁰ Given

the pivotal role of metabolic rewiring in tumor progression, identifying key regulatory genes remains crucial for developing targeted therapies.

The gasdermin (GSDM) family comprises six human genes (GSDMA-E and PJVK), initially identified for their role in pyroptosis.^{11–15} Among these, GSDMD is the most extensively studied, undergoing caspase-mediated cleavage to release its N-terminal fragment (GSDMD-N), which permeabilizes the plasma membrane, triggering pyroptosis and inflammatory cytokine release (e.g., IL-1 β , IL-18).^{15,16} GSDMC, in contrast, is cleaved by caspase-6/8 but exhibits distinct functions.^{17–19} It is linked to metastatic melanoma and is highly expressed in multiple cancers (breast, lung, renal cancers, etc.), promoting tumor progression and immune evasion.^{20–26} However, the precise mechanisms by which GSDMC promotes tumor progression remain unclear.

In this study, we elucidate how GSDMC contributes to tumor progression in lung adenocarcinoma (LUAD). We demonstrate that GSDMC, which is frequently upregulated in LUAD tissues and cell lines and linked to poor prognosis, is transcriptionally activated by STAT3 (via Ser727 phosphorylation) under



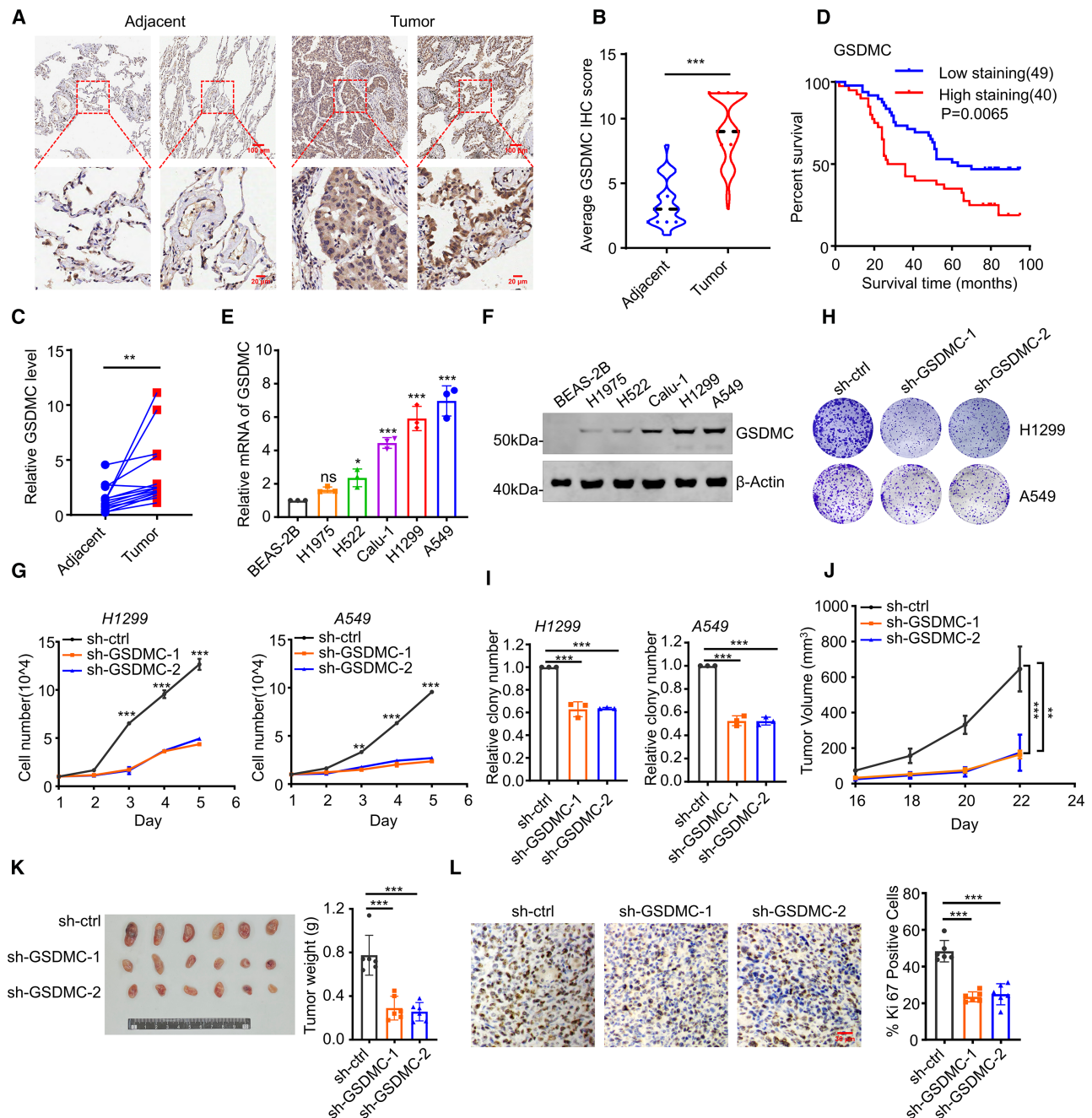


Figure 1. GSDMC facilitates cell growth and tumor progression in LUAD

(A and B) Representative IHC images show GSDMC exhibiting relatively higher expression in LUAD compared to adjacent normal tissue ($n = 89$). (Top: low magnification, scale bars: 100 μ m; bottom: high magnification, scale bars: 20 μ m).

(C) qPCR assays measuring GSDMC expression in LUAD tumor and adjacent normal tissues ($n = 15$).

(D) Association between GSDMC expression level and survival probability of patients with LUAD from (A and B).

(E and F) qPCR (E) and WB (F) assays measuring GSDMC expression in LUAD and normal cell lines.

(G) Growth curves of cells from H1299 and A549 cells transduced with sh-ctrl, sh-GSDMC-1 or sh-GSDMC-2.

(H and I) Colony formation (H) and quantification (I) of cells from (G).

(J and K) Comparison of the growth of A549 xenografts expressing sh-ctrl v.s. sh-GSDMC-1 or sh-GSDMC-2 (J). Comparison of final tumor weights after dissection (K). Data represent a single experiment; $n = 6$ mice per group.

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metabolic (serum deprivation [SD]) or genotoxic (ionizing radiation [IR]) stress. Full-length GSDMC translocates to the nucleus via the IPO7-KPNB1-NUP93 complex in a K41 nuclear localization signal (NLS)-dependent manner. In the nucleus, GSDMC acts as a scaffold protein associated with epigenetic regulation. For the purposes of this study, we define epigenetic regulation as heritable changes in gene expression independent of DNA sequence alterations, specifically focusing on histone acetylation and chromatin remodeling that modulate chromatin accessibility and transcriptional activity. GSDMC recruits NAT10 to mediate multi-site acetylation of histone H3 and recruits BAZ1B and SMARCA5 to modulate chromatin remodeling and chromatin accessibility. These epigenetic changes support CAMKK2 upregulation and subsequent AMPK activation, driving glycolytic and fatty acid oxidation (FAO) metabolic rewiring to fuel tumor growth. Notably, GSDMC contributes to radioresistance via the STAT3-GSDMC-CAMKK2-AMPK axis, and pathway targeting enhances radiotherapy sensitivity. Our findings expand the current understanding of GSDMC by revealing its non-canonical nuclear function that couples stress adaptation to malignant progression and therapeutic response in LUAD.

RESULTS

GSDMC facilitates cell growth and tumor progression in LUAD

To probe GSDMC expression landscapes in LUAD, we performed immunohistochemistry (IHC) staining on tissues from 89 patients with LUAD, including both tumor tissues and adjacent non-tumor tissues. The IHC results revealed that intense GSDMC immunoreactivity, including nuclear staining, was observed in 89 LUAD specimens, contrasting with only focal weak positivity in matched para-cancerous non-neoplastic tissues (Figures 1A and 1B). To validate high GSDMC expression in LUAD, we analyzed 15 paired tumor and adjacent normal tissues, finding significantly higher GSDMC mRNA levels in LUAD tissues (Figure 1C). In relation, patients with LUAD with higher GSDMC expression had poorer prognosis (Figure 1D). To investigate the expression of GSDMC in cells, we compared its expression in a panel of LUAD-derived cell lines with that in normal bronchial epithelial cells, also revealing significantly higher GSDMC expression in LUAD cells (Figures 1E and 1F). Briefly, these data indicate that GSDMC is commonly upregulated in patients with LUAD, and this observation suggests a potential association with poor prognosis in the clinic.

To investigate the role of GSDMC in tumorigenesis, two LUAD cell lines (H1299 and A549) were used for silencing GSDMC (Figures S1A and S1B). As expected, silencing GSDMC inhibited the proliferation (Figure 1G) and cloning efficiency (Figures 1H and 1I) of LUAD cells. To further verify these effects, we ectopically expressed GSDMC and observed a remarkable increase in cell proliferation and cloning efficiency (Figures S1C–S1F). Following these analyses, we expanded our experiments to an

in vivo setting, nu/nu mice xenografts. As expected, knockdown of GSDMC resulted in inhibition of tumor growth (Figures 1J and 1K) while its enforced expression produced the opposite effect (Figures S1G and S1H). Likewise, we observed a consistent correlation in the quantitative analysis of the cell proliferation marker Ki67 staining in xenograft tumor tissues (Figures 1L and S1I). Consequently, these findings indicate that GSDMC enhances LUAD growth both *in vitro* and *in vivo*.

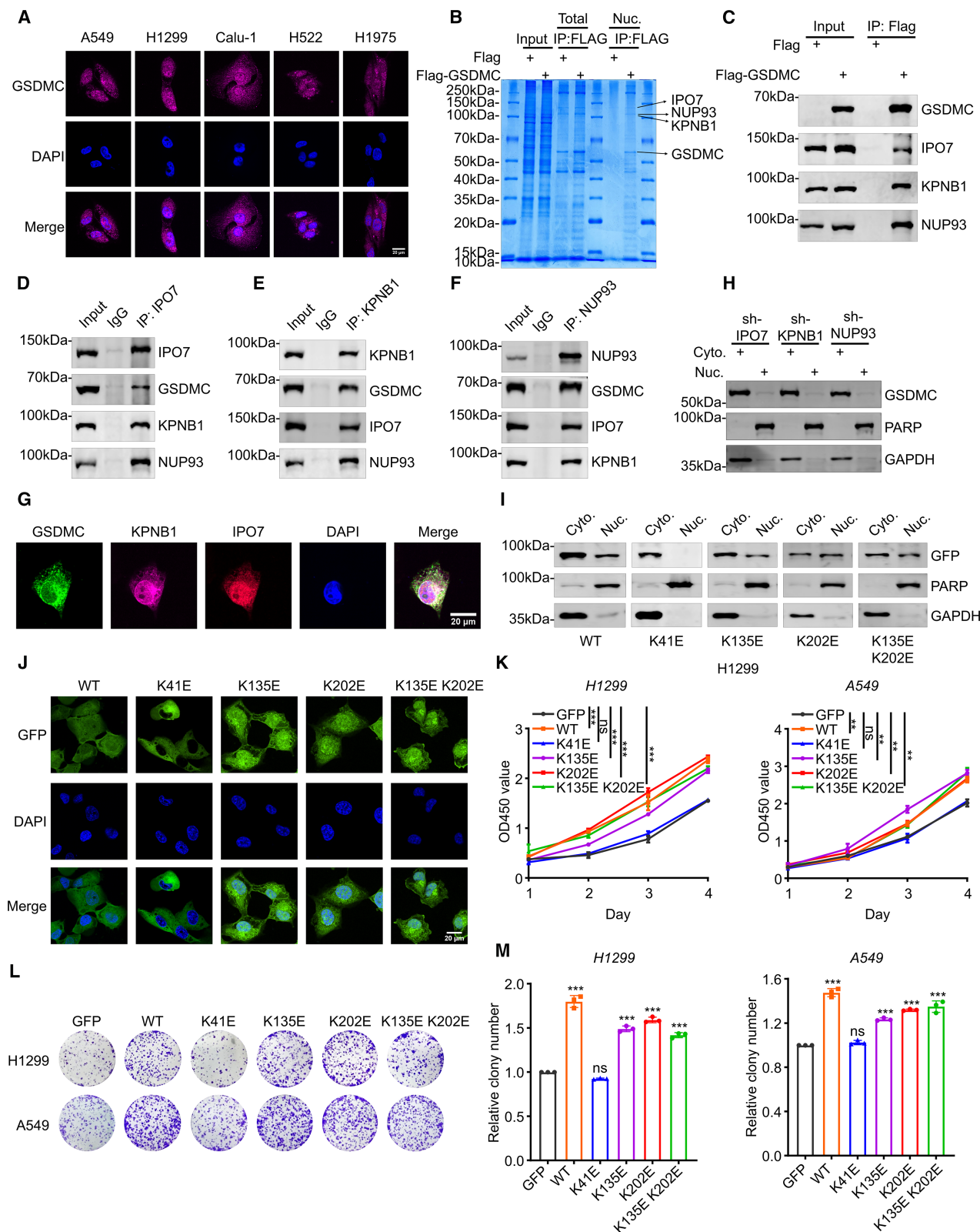
Nuclear translocation of GSDMC is mediated by Importin family proteins

We sought to clarify the subcellular localization of GSDMC, immunofluorescence (IF) analysis of LUAD cell lines confirmed GSDMC nuclear localization (Figure 2A). Notably, cytosolic/nuclear fractionation assays revealed that both the full-length GSDMC and its 35-kDa cleaved fragment localized to the nucleus (Figure S2A). Although prior studies have documented this phenomenon for the full-length protein, its nuclear function remains uncharacterized.²⁵ To investigate GSDMC nuclear translocation and function, we performed co-immunoprecipitation (coIP) coupled with MS in GSDMC-overexpressing cells, identifying 634 potential interacting proteins (Figure 2B; Table S1). Notably, we identified nuclear transport factors importin-7 (IPO7), importin β -1 (KPNB1), and nucleoporin 93 (NUP93) (Figure S2B).^{27–29} Subcellular fractionation showed nuclear and cytoplasmic localization of NUP93, IPO7, and KPNB1 (Figure S2C). CoIP assays demonstrated direct binding between GSDMC and each of IPO7, KPNB1, and NUP93 (Figures 2C–2F). IF staining further supported their co-localization (Figures 2G and S2D). To assess interactions, we performed pull-down assays, which confirmed direct binding of GSDMC to IPO7, KPNB1, and NUP93 (Figures S2E–S2G). Knockdown of IPO7, KPNB1, or NUP93 (Figure S2H) blocked GSDMC nuclear translocation (Figure 2H), indicating that all three are required for nuclear import. Together, these findings reveal a mechanism by which GSDMC undergoes nuclear translocation in an IPO7/KPNB1/NUP93-dependent manner.

To test whether GSDMC nuclear import depends on its predicted NLS, we generated cell lines expressing GSDMC with mutated NLS sequences. Among three predicted NLS motifs (Figure S2I), only mutation of the K41 site (K41E-GSDMC) abolished nuclear localization, as shown by immunoblotting and IF (Figures 2I, 2J, S2J, and S2K). Notably, GSDMC retained nuclear localization even when both K135 and K202 sites were mutated (Figures 2I, 2J, S2J, and S2K), demonstrating that the K41 NLS is essential for nuclear entry. Subsequently, we sought to investigate how nuclear translocation of GSDMC influences the tumorigenesis of LUAD cells. The data revealed that when GSDMC failed to enter the nucleus, LUAD cells exhibited reduced proliferation and cloning efficiency (Figures 2K–2M). Collectively, the nuclear import of GSDMC required the cooperative action of IPO7, KPNB1, and NUP93 in a manner depending on its canonical NLS.

(L) Ki67 staining from (K) and quantification. Scale bars: 30 μ m.

Data information: (B and C) are mean $\pm$ SD, two-tailed paired Student's *t* test; (D) log rank test; (E, I, K, and L) one-way ANOVA with Tukey's multiple comparison post-test; (G and J) two-way ANOVA with Bonferroni's multiple comparison post-test; (E, G, and I) *n* = 3 biological replicates. ns, not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001.



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The epigenetic regulatory function of GSDMC

To elucidate the nuclear role of GSDMC, we performed MS-based profiling to identify its interacting partners. MS analysis of GSDMC-interacting proteins identified histones, with its specific binding to histone H3 confirmed by immunoblotting (Figures 3A and S3A). GSDMC overexpression increased acetylation at multiple histone H3 sites (H3K9, H3K14, H3K18, and H3K23) (Figures 3B and S3B). Notably, N-acetyltransferase 10 (NAT10), which has been linked to histone acetylation,³⁰ was detected in the MS data (Figure S3A). CoIP verified the GSDMC-NAT10 interaction and showed enhanced NAT10 binding to histone H3 in GSDMC-overexpressing cells (Figures 3A, 3C, and 3D). IF confirmed their nuclear co-localization (Figure 3E). To further evaluate the role of GSDMC in histone H3 acetylation, we performed a rescue experiment in which GSDMC was overexpressed after NAT10 knockdown. The results showed that ectopic GSDMC overexpression did not restore histone H3 acetylation in NAT10-depleted cells (Figure S3C), suggesting that the ability of GSDMC to promote histone H3 acetylation relies on NAT10. Collectively, these results indicate that GSDMC acts as a scaffold protein that associates with epigenetic regulatory components by recruiting NAT10, thereby supporting multi-site acetylation of histone H3.

To explore how histone H3 acetylation contributes to transcription regulation, we performed MS analysis and identified bromodomain adjacent to zinc finger domain 1B (BAZ1B) and SNF2-related chromatin remodeling ATPase 5 (SMARCA5) as candidate regulators. BAZ1B, an acetyl-lysine reader protein, binds to acetylated H3, whereas SMARCA5, an ATPase subunit of chromatin remodeling complexes, modulates nucleosome structure to support transcriptional activation.^{31–33} CoIP and IF assays confirmed interactions among GSDMC, BAZ1B, and SMARCA5 (Figures 3F–3H and S3D). Importantly, coIP and IF analysis revealed co-localization of GSDMC, BAZ1B, and acetylated histone H3 on chromatin (Figures 3I, 3J, and S3E), indicating that BAZ1B recognizes acetylated histone H3 marks and may help recruit the remodeling complex.

To further explore the potential mechanism by which GSDMC regulates downstream gene expression, we performed ATAC-seq to assess chromatin accessibility in control and GSDMC-overexpressing cells. Analysis of ATAC-seq libraries showed typical read enrichment at peak centers in both groups, consistent with the quality of the chromatin accessibility profiling

(Figure 3K). Using a threshold of $|\log_2FC| > 1$ and $-\log_{10}p > 10$, differential peak analysis identified 3,234 increased and 3,284 decreased peaks, indicating widespread changes in chromatin accessibility upon GSDMC overexpression (Figure S3F). Genomic annotation showed that these differential peaks were mainly distributed in promoters, intergenic regions, and introns, supporting the possibility that GSDMC may influence both proximal and distal gene regulation (Figure 3L). GO enrichment analysis showed that these peaks were associated with pathways including histone H3-K36 methylation, lipid metabolism, MAPK signaling, and calcium homeostasis (Figure 3M). Together, these ATAC-seq findings provide supportive evidence that GSDMC may modulate chromatin accessibility, consistent with its downstream regulatory effects.

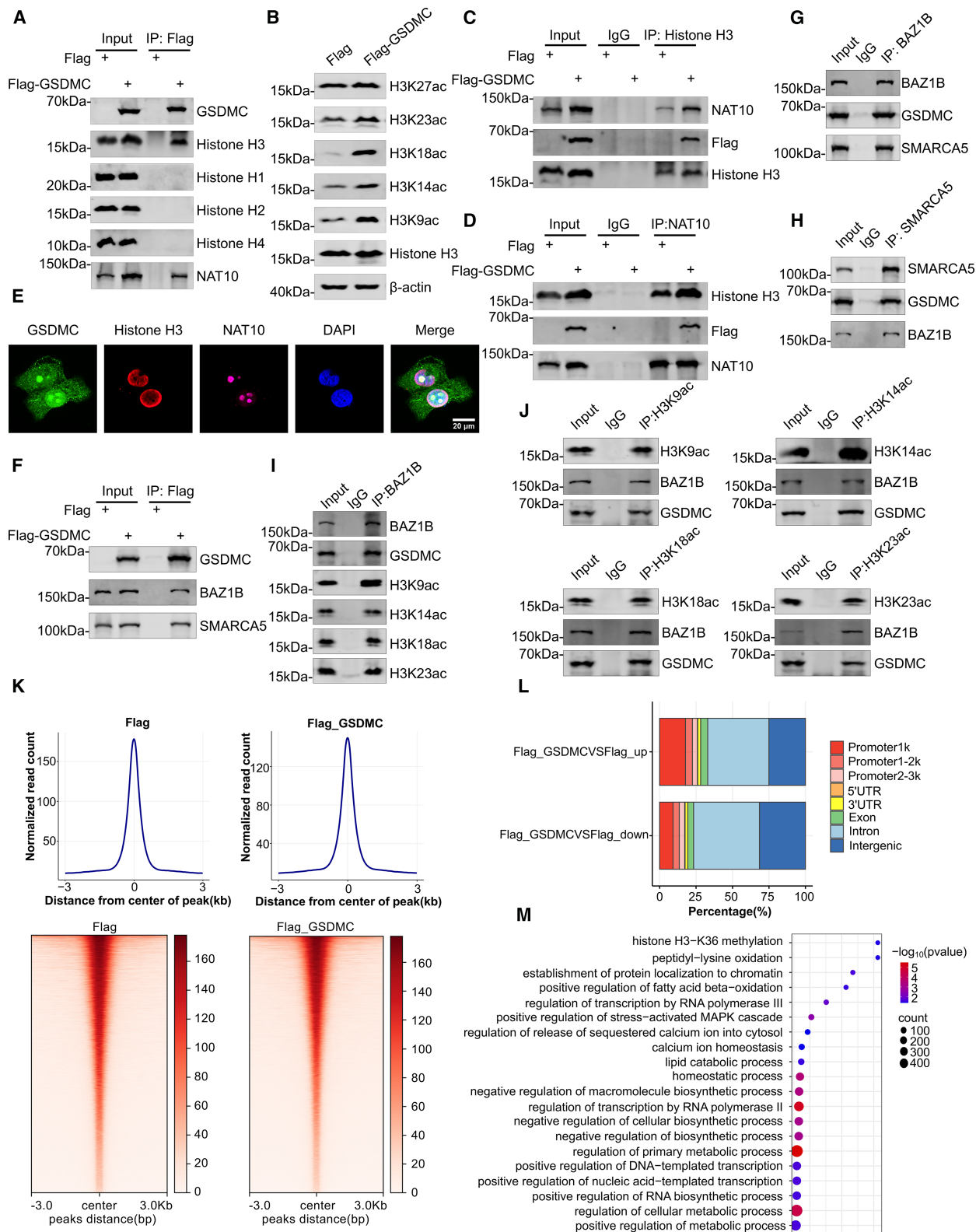
GSDMC contributes to cellular metabolic reprogramming

For investigating the regulatory role of GSDMC in cell progression, we performed RNA-seq in GSDMC-silenced cells (Figures S1A and S1B). Scatterplot analysis revealed significant downregulation of multiple genes upon GSDMC knockdown (Figure S4A). GO enrichment analysis indicated that silencing GSDMC suppressed metabolic pathways, including catabolism, glucose metabolism, fatty acid metabolism, as well as cell proliferation and adhesion (Figure 4A). The latter two are consistent with previous findings.^{25,26} Consistently, the GSEA further confirmed downregulation of macromolecule catabolic processes (Figure 4B), while fatty acid synthesis-related pathways were notably enriched in GSDMC-depleted cells (Figure S4B). Together, these results suggest that GSDMC silencing disrupts macromolecule catabolism and promotes fatty acid synthesis.

To assess the potential metabolic role of GSDMC, we measured glycolysis and mitochondrial respiration in H1299 cells. GSDMC silencing decreased ECAR but increased oxygen consumption rate (OCR), while its overexpression had the opposite effect (Figures 4C, 4D, S4C, and S4D), suggesting GSDMC promoted glycolysis but its impact on mitochondrial respiration required further investigation. Non-targeted metabolomics analysis of GSDMC-depleted cells revealed significant changes in 110 metabolites (56 upregulated vs. 54 downregulated, Figure 4E). Enriched pathways included fatty acid metabolism, with key intermediates such as glycerol 3-phosphate, arachidonic acid, N-acetyl-d-glucosamine, and niacinamide markedly elevated (Figures 4F and S4E). Lipids and lipid-like molecules

Figure 2. Nuclear translocation of GSDMC is mediated by Importin family proteins

(A) IF of GSDMC, and nucleus staining (DAPI) in A549, H1299, Calu-1, H522, and H1975 cells. Scale bars: 20 μ m.
(B) IP of FLAG-tagged recombinant GSDMC from H1299 cells was performed to profile candidate proteins interacting with GSDMC. Coomassie blue-staining compares FLAG-GSDMC v.s. control FLAG, with the highlighted bands indicating the recovered GSDMC, IPO7, NUP93, and KPNB1 using MS.
(C) IP assays using FLAG antibody analysis of IPO7, GSDMC, KPNB1, and NUP93 in H1299 cells transduced with FLAG or FLAG-GSDMC.
(D–F) IP assays using IPO7 (D), KPNB1 (E), or NUP93 (F) antibody, analysis of IPO7, GSDMC, KPNB1, and NUP93 in H1299 cells transduced with FLAG-GSDMC.
(G) IF of FLAG-GSDMC, IPO7, and KPNB1 and nucleus staining (DAPI) in H1299 cells. Scale bars: 20 μ m.
(H) WB analysis of nuclear and cytoplasmic extracts of H1299 cells transduced with sh-IPO7, sh-KPNB1, and sh-NUP93.
(I) WB analysis of nuclear and cytoplasmic fractions from H1299 cells expressing GFP-GSDMC or its NLS mutants (K41E, K135E, K202E, or K135E/K202E).
(J) GFP fluorescence labeling of H1299 cells from (I). Scale bars: 20 μ m.
(K) Growth curves of H1299 and A549 cells expressing GFP-GSDMC or its NLS mutants (K41E, K135E, K202E, or K135E/K202E).
(L and M) Colony formation (L) and quantification (M) of cells from (K).
Data information: (K) two-way ANOVA with Bonferroni's multiple comparison post-test; (M) one-way ANOVA with Tukey's multiple comparison post-test; (K and M) $n = 3$ biological replicates. ns, not significant, ** $p < 0.01$, *** $p < 0.001$.



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emerged as the most altered categories (Figure S4F), collectively indicating that GSDMC depletion enhances lipogenesis.

To further confirm this phenomenon, we performed Nile red staining and observed increased lipid droplet accumulation in GSDMC-depleted cells, while GSDMC overexpression reduced lipid droplets (Figures 4G and S4G). Non-targeted lipidomics revealed that 32 of 44 detected lipids were significantly upregulated upon GSDMC knockdown (Figure 4H). Consistent with this, triglyceride (TG) quantification showed elevated intracellular TG levels after GSDMC depletion, with opposite effects observed upon GSDMC overexpression (Figures 4I and S4H). To delineate the regulatory role of GSDMC in FAO, cells were treated with exogenous palmitate, a free fatty acid. Silencing of GSDMC significantly impaired FAO, whereas its overexpression enhanced FAO activity (Figures 4J and S4I). Together, these findings indicate that GSDMC promoted both glycolysis and FAO in LUAD cells while silencing GSDMC enhanced fatty acid synthesis.

GSDMC drives metabolic reprogramming through CAMKK2-AMPK axis activation

GSDMC orchestrates metabolic reprogramming by bidirectionally regulating glycolysis and fatty acid metabolism. Depletion of GSDMC suppresses glycolysis but enhances fatty acid synthesis, while its overexpression promotes both glycolysis and FAO. Meanwhile, GSDMC overexpression increases AMPK α -Thr172 phosphorylation (Figure 5A), indicating AMPK activation. This leads to downstream phosphorylation of ACC1-Ser79 (inhibiting fatty acid synthesis)^{34,35} and PFKFB3-Ser461 (promoting glycolysis)³⁶ (Figure 5A), whereas GSDMC knockdown reduces these phosphorylation events (Figure S5A). These findings suggested that GSDMC drove metabolic reprogramming through AMPK-dependent signaling. To investigate how GSDMC activates AMPK, we first confirmed that neither overexpression nor knockdown of GSDMC-altered AMPK subunit protein or mRNA levels (Figures 5A, S5A, and S5B). Since prior studies indicated that AMPK activation typically requires direct binding to regulatory subunits.^{37–40} Thus, we performed coIP assays but found no interaction between GSDMC and AMPK subunits (Figure 5B). This suggests that GSDMC enhances AMPK phosphorylation indirectly, potentially through an upstream regulatory pathway.

We next investigated whether GSDMC activates AMPK through known upstream kinases. Although LKB1 is a well-es-

tablished AMPK activator that phosphorylates Thr172,³⁷ GSDMC overexpression still enhanced AMPK phosphorylation in LKB1-deficient cells (HeLa and H460,⁴¹ Figures S5C and S5D). We then tested two alternative kinases, CAMKK2 and TAK1, which also directly phosphorylate AMPK.^{38,39} Knockdown experiments in H1299 cells revealed that only CAMKK2 depletion abolished GSDMC-induced AMPK activation (Figure 5C). This was confirmed in LKB1-knockdown H1299 cells (Figure 5C) and replicated in A549 cells (Figure S5E), where only silencing CAMKK2 impaired AMPK phosphorylation. Together, these results demonstrate that GSDMC promotes AMPK α -Thr172 phosphorylation specifically through CAMKK2 but not LKB1 or TAK1.

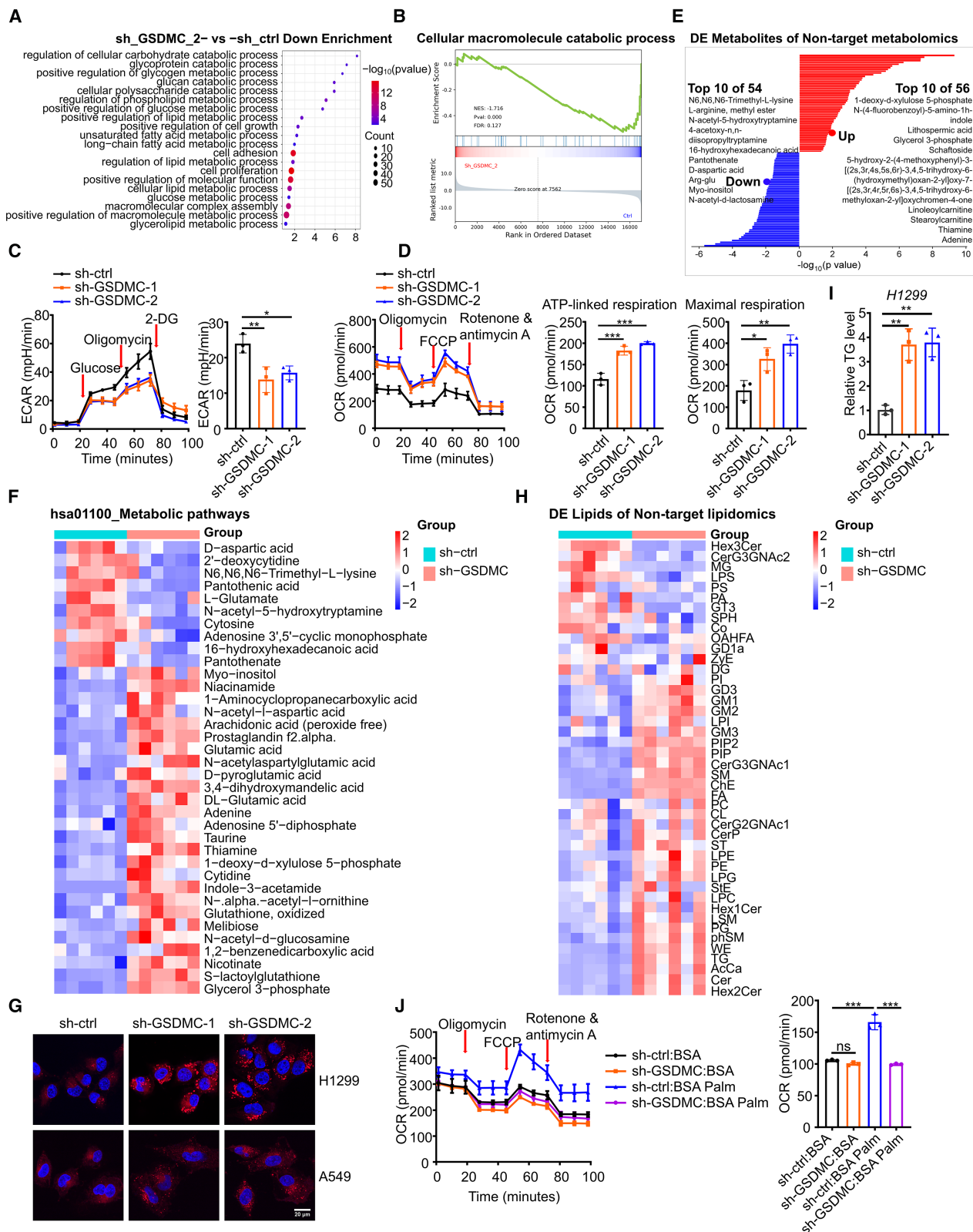
We next explored how GSDMC regulates CAMKK2. GSDMC overexpression increased both CAMKK2 protein and mRNA levels (Figures 5D and 5E), while GSDMC knockdown reduced them (Figures S5F and S5G), suggesting transcriptional regulation. ATAC-seq revealed enhanced chromatin accessibility at the CAMKK2 promoter in GSDMC-overexpressing cells (Figure 5F). Further luciferase reporter assays using three CAMKK2 promoter fragments confirmed increased transcriptional activity upon GSDMC overexpression (Figures 5G and 5H). To further confirm transcriptional regulation of CAMKK2 by GSDMC, we found that nuclear localization-deficient GSDMC failed to alter CAMKK2 mRNA levels (Figures 5I and S5H). Consistently, protein analyses showed no changes in CAMKK2 expression or AMPK signaling when GSDMC nuclear translocation was blocked (Figures 5J and S5I). Taken together, our findings support a model in which GSDMC enhances CAMKK2 transcription via chromatin-associated mechanisms, thereby contributing to AMPK phosphorylation and subsequent metabolic reprogramming in LUAD cells.

STAT3 positively regulates GSDMC expression responding to SD

Our previous findings demonstrated the role of GSDMC in activating AMPK, a key sensor in energy metabolism. To investigate how metabolic stress affects GSDMC expression, we tested various nutrient-deficient conditions. Only SD significantly upregulated GSDMC expression, and this effect was reversed by serum restoration (Figures 6A, 6B, S6A, and S6B). Consistently, SD induced GSDMC upregulation at the protein level without triggering pyroptotic cleavage, and this treatment further enhanced GSDMC expression in the nucleus (Figures 6C and

Figure 3. The epigenetic regulatory function of GSDMC

- (A) IP assays using FLAG antibody analysis of GSDMC, Histone H1, Histone H2, Histone H3, Histone H4 and NAT10 in H1299 cells transduced with FLAG or FLAG-GSDMC.
(B) WB analysis of indicated proteins in H1299 cells transduced with FLAG or FLAG-GSDMC.
(C and D) CoIP of Histone H3(C) or NAT10 (D) in H1299 cells transduced with FLAG or FLAG-GSDMC.
(E) IF of FLAG-GSDMC, Histone H3 and NAT10 and nucleus staining (DAPI) in H1299 cells. Scale bars: 20 μ m.
(F) IP assays with FLAG antibody analysis of GSDMC, BAZ1B, and SMARCA5 in H1299 cells transduced with FLAG or FLAG-GSDMC.
(G and H) IP assays with BAZ1B (G) or SMARCA5 (H) antibody, analysis of GSDMC, BAZ1B, and SMARCA5 in H1299 cells transduced with FLAG-GSDMC.
(I) IP assays with BAZ1B antibody analysis of GSDMC, BAZ1B, and H3K9/14/18/23ac in H1299 cells transduced with FLAG-GSDMC.
(J) IP assays with H3K9/14/18/23ac antibodies, respectively, to analyze the binding of BAZ1B to H3K9/14/18/23ac in H1299 cells transduced with FLAG-GSDMC.
(K) Heatmap and pileup of ATAC-seq signals. The heatmap and pileups are centered on the ATAC-seq peak (upstream 3 kb and downstream 3 kb of the peaks).
(L) Peak distribution across genomic features for the FLAG-GSDMC vs. FLAG groups.
(M) GO enrichment analysis from ATAC-seq.



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S6C). To identify transcriptional regulators of GSDMC under SD, we analyzed University of California, Santa Cruz (UCSC) Genome Browser and ARCHS4 (All RNA-seq and ChIP-seq Sample and Signature Search 4) databases, revealing SOX2, ELF3, STAT3, TAL1, JUND, and JUN as candidates (Figure S6D). Knockdown experiments in H1299 cells showed that only STAT3 and TAL1 silencing reduced GSDMC expression (Figures 6D and 6E). Further validation confirmed STAT3, but not TAL1, as the key transcription factor under SD (Figure 6F). Similar results were observed in A549 cells (Figures S6E–S6G). Collectively, these findings indicate that STAT3 serves as the primary transcriptional regulator of GSDMC during SD.

As a response to stimuli, STAT3 is activated by phosphorylation at tyrosine (Y705) or serine (S727) residues, which is triggered by upstream signals like cytokines, JAKs, and kinases (e.g., SRC, ABL).⁴² Under SD, both STAT3 and AMPK signaling were activated (Figure S6H). Knockdown of STAT3 in SD-treated cells phenocopied GSDMC depletion, reducing AMPK activity, while GSDMC overexpression partially rescued this effect (Figure 6H). Since STAT3-Y705 regulates GSDMC under hypoxia,¹⁷ we analyzed the GSDMC promoter and identified two putative STAT3 binding sites (BD1 and BD2) (Figure 6I). Reporter assays revealed that only BD1, not BD2 (the hypoxia-responsive site), mediated GSDMC upregulation under SD (Figure 6J). Following these, we speculated that STAT3-Y705 did not regulate GSDMC transcription under the SD condition. To confirm our speculation, we generated plasmids encoding STAT3 mutants (STAT3-Y705F and STAT3-S727F) and observed that specifically the STAT3-S727F mutation abrogated GSDMC induction under SD conditions (Figures 6K and 6L). Collectively, these results suggest that under SD conditions, STAT3-S727 contributes to the transcriptional expression of GSDMC by binding to the BD1 site, thereby modulating cellular metabolism and promoting cell survival. In contrast, under hypoxia, STAT3-Y705 regulates GSDMC transcription by binding to the BD2 site, activating the pyroptosis program and inducing cell death (Figure 6M). Although both stimuli induce GSDMC expression, they mediate distinct cell outcomes.

Silencing GSDMC improves the radiosensitivity of LUAD

Our IHC analysis revealed significantly higher GSDMC expression in tumor tissues compared to adjacent non-tumor tissues from 89 patients with LUAD (Figures 1A and 1B). Notably, high GSDMC expression correlated with poorer patient survival (Figure 1D). We then investigated whether standard LUAD treat-

ments affect GSDMC expression.⁴³ Both radiotherapy (X-ray) and chemotherapy (5-Fu and cisplatin) upregulated GSDMC without inducing its cleavage (Figure S7A). Furthermore, our analysis of GSEA enrichment from RNA-seq of GSDMC knockdown cells revealed that the cellular response to irradiation was reduced following GSDMC knockdown (Figure 7A). Functional studies showed that GSDMC knockdown enhanced radiosensitivity in A549 cells (Figure 7B) but did not affect chemosensitivity to 5-Fu or cisplatin (Figure S7B). The differential treatment responses suggested that additional unknown mechanisms might be involved in chemotherapy resistance. To validate these findings, we extended our study to an *in vivo* setting using nu/nu mice xenografts. As expected, knockdown of GSDMC enhanced the radiosensitivity of the tumors in these xenografts (Figures 7C and 7D).

Subsequently, we aimed to investigate the mechanism underlying the upregulation of GSDMC expression following irradiation. qPCR analysis showed that GSDMC transcription was upregulated after irradiation, and we found that silencing STAT3 reduced irradiation-induced GSDMC upregulation (Figure 7E). To further test this, we irradiated A549 cells and found that S727-phosphorylated STAT3 was significantly upregulated (Figure 7F). To confirm whether irradiation activates AMPK signaling via GSDMC, we irradiated GSDMC-silenced cells and then detected AMPK signaling. Consistently, GSDMC silencing blocked the radiation-induced phosphorylation of AMPK α (Thr172), ACC1 (Ser79), and PFKFB3 (Ser461) (Figure 7F). To sum up, these results suggest that radiation may promote transcriptional expression of GSDMC via STAT3 phosphorylation.

Thereafter, we aimed to reconfirm the importance of GSDMC in radiosensitivity. The results showed that silencing STAT3 also enhanced radiosensitivity, whereas restoring GSDMC expression counteracted this effect (Figure 7G). Moreover, to explore whether GSDMC regulates radiosensitivity via the CAMKK2-AMPK signaling pathway, we tested the radiosensitivity of CAMKK2 or AMPK α knockdown cell lines, respectively, and found that silencing CAMKK2 or AMPK α could increase radiosensitivity (Figures 7H and S7C). To further ascertain the roles of STAT3, CAMKK2, and AMPK in radiosensitivity, we also performed radiosensitivity tests using their respective inhibitors, which demonstrated that all these inhibitors enhanced radiosensitivity (Figure 7I). To sum up, these findings suggest that activation of the STAT3-GSDMC-CAMKK2-AMPK pathway after irradiation may contribute to the radioresistance of LUAD.

Figure 4. GSDMC contributes to cellular metabolic reprogramming

(A and B) RNA-seq data from H1299 cells transduced with sh-ctrl or sh-GSDMC-2 were analyzed for functional annotation using GO (A) and GSEA (B) enrichment analysis.

(C) Representative ECAR traces and quantification during glycolysis stress tests in H1299 cells transduced with sh-ctrl, sh-GSDMC-1, or sh-GSDMC-2.

(D) Representative OCAR traces and quantification during cell mitochondrion stress tests in H1299 transduced with sh-ctrl, sh-GSDMC-1, or sh-GSDMC-2.

(E) Different expression metabolites of non-targeted metabolomics data ($n = 6$) in H1299 cells transduced with sh-ctrl or sh-GSDMC-2.

(F) Metabolic pathways enrichment of non-target metabolomics in H1299 cells from (E).

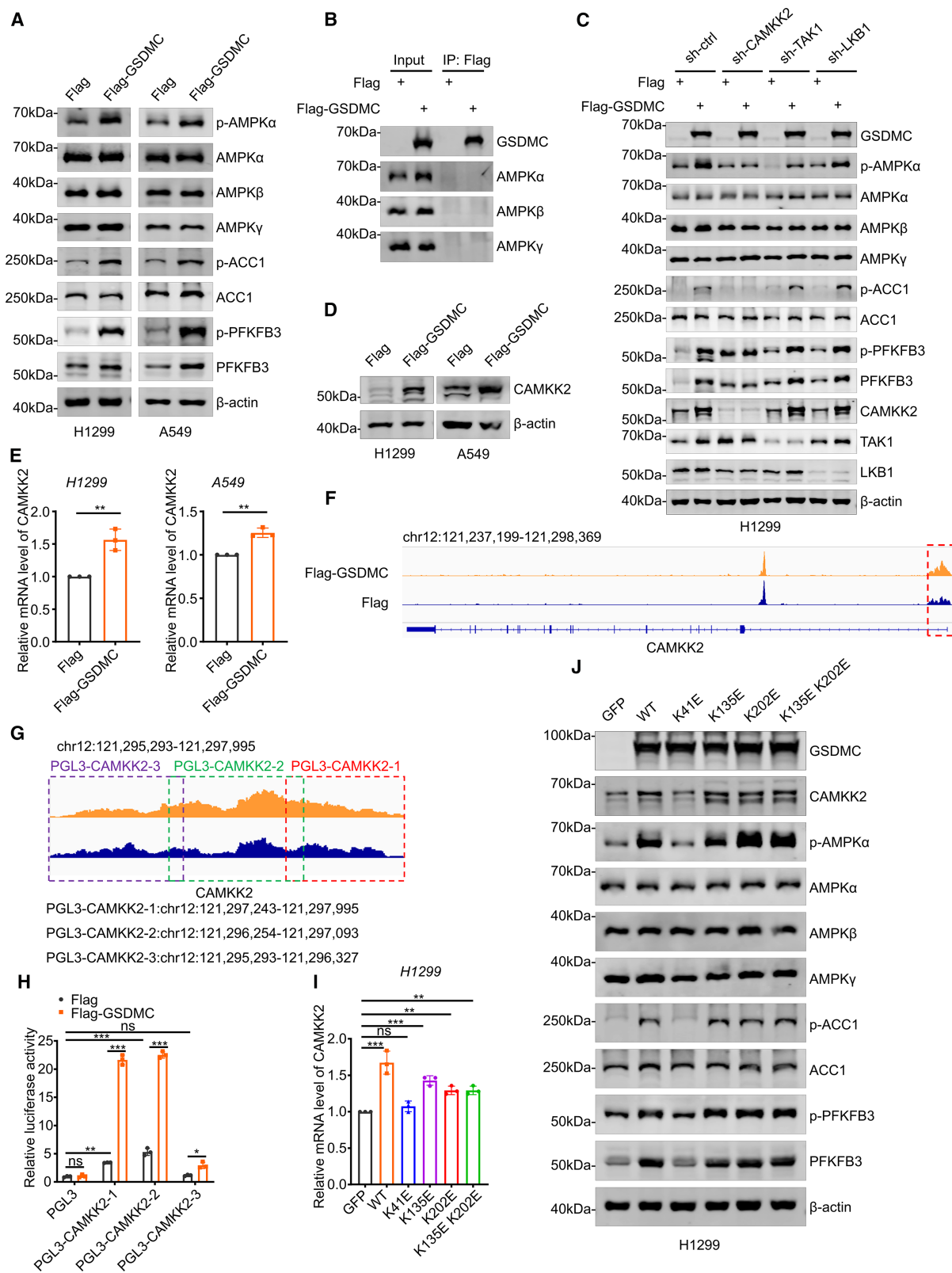
(G) Nile red (red) and DAPI (blue) staining of cells transduced with sh-ctrl, sh-GSDMC-1, or sh-GSDMC-2. Scale bars: 20 μ m.

(H) The difference of 44 lipids in non-targeted lipidomics data ($n = 6$) in H1299 cells transduced with sh-ctrl or sh-GSDMC-2.

(I) Total TG levels measured in H1299 cells transduced with sh-ctrl, sh-GSDMC-1, or sh-GSDMC-2.

(J) Seahorse FAO assays in H1299 cells transduced with sh-ctrl or sh-GSDMC-2 with or without the addition of exogenous fatty acids.

Data information: (C, D, I, and J) one-way ANOVA with Tukey's multiple comparison post-test; $n = 3$ biological replicates. ns, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



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Finally, to investigate the relationship among STAT3, GSDMC, and CAMKK2 in patients with LUAD, we analyzed their expression in tumor tissues from 30 LUAD cases. Most samples showed elevated STAT3, GSDMC, and CAMKK2 levels compared to normal tissues (Figure S7D). Correlation analysis further revealed positive associations between each pair of these molecules (Figure S7E). These findings suggest that GSDMC contributes to LUAD progression, potentially promoting tumor cell proliferation in coordination with STAT3 and CAMKK2.

DISCUSSION

Tumor cells exhibit metabolic reprogramming to support their rapid proliferation and survival.¹ A key feature is the shift to aerobic glycolysis, which provides ATP and biosynthetic intermediates through the pentose phosphate and serine metabolism pathways.² The resulting lactate accumulation creates an acidic microenvironment that further promotes tumor progression.⁴⁴ Additionally, tumor cells alter lipid metabolism to enhance uptake, synthesis, storage, and oxidation of lipids, ensuring a supply of essential biomolecules under nutrient stress. This metabolic flexibility allows cancer cells to adapt dynamically to environmental challenges, optimizing their survival and growth.⁷ Given the complexity and variability of tumor metabolic pathways, understanding the molecular regulators that orchestrate metabolic balance is critical for unraveling tumor progression mechanisms and identifying potential therapeutic targets. In this study, we explored the functions of GSDMC in metabolism, stress adaptation, and radioresistance in LUAD. Our findings provide additional insights into the epigenetic and signaling mechanisms of GSDMC, which may contribute to a better understanding of LUAD pathogenesis.

Our findings suggest a complex role of GSDMC in regulating cellular metabolic homeostasis, as GSDMC silencing and overexpression led to distinct metabolic profiles. Both GSDMC silencing and overexpression increased maximal mitochondrial respiration and ATP production, suggesting a non-linear regulatory role of GSDMC in mitochondrial function. GSDMC overexpression enhanced catabolic processes, including glycolysis and FAO, while potentially increasing respiratory chain activity or uncoupling, leading to elevated OCR. Conversely, GSDMC knockdown shifted metabolism toward anabolism, reducing glycolysis and FAO. The persistent rise in OCR under both conditions suggests compensatory mitochondrial biogenesis or alternative respiratory pathway activation to meet elevated energy demands. Additional unidentified factors may further

contribute to the observed OCR increase. These results highlight the complex regulatory role of GSDMC in cellular metabolic adaptation.

We identified SD as an inducer of GSDMC expression, distinct from other metabolic stresses such as glutamine or glucose deprivation, indicating stress-specific regulatory mechanisms for GSDMC. This SD-induced GSDMC upregulation is mediated by STAT3 activation as part of the JNK-dependent stress response, further characterized by a stimulus-specific regulatory mode: SD or irradiation drives GSDMC expression through STAT3-S727 phosphorylation (rather than the hypoxia-associated Y705 phosphorylation), which binds to a STAT3-binding site (BD1) on the GSDMC promoter, distinct from the hypoxia-responsive BD2 site. Our findings provide further insights into GSDMC-mediated stress adaptation in cancer cells by identifying a distinct, stress-specific regulatory mechanism of GSDMC expression that is dependent on STAT3. Since SD recapitulates the metabolic challenges of tumor growth (e.g., reduced ATP production and elevated oxidative stress^{45,46}), GSDMC upregulation likely facilitates cellular stress adaptation and survival, a conclusion supported by our *in vivo* data confirming that GSDMC promotes tumor growth in mice.

A major finding of this study is the characterization of full-length GSDMC as a nuclear scaffold protein with associated epigenetic activities, expanding the conventional view of GSDMC as a predominantly cytoplasmic protein. GSDMC translocates to the nucleus via an IPO7/KPNB1/NUP93-dependent mechanism, with the K41 NLS being essential for this process. As a non-enzymatic scaffold molecule, GSDMC interacts with NAT10, histone H3, and BAZ1B/SMARCA5 to support multi-site H3 acetylation and chromatin remodeling. Rescue experiments confirmed that this H3 acetylation is largely dependent on NAT10, supporting the notion that GSDMC acts as a specific scaffold component rather than a non-specific adapter. ATAC-seq analysis further demonstrated that GSDMC broadly modulates chromatin accessibility across LUAD cells, with GO enrichment analysis linking these changes to tumor-relevant pathways, including lipid metabolism and MAPK signaling. These observations are consistent with a model in which GSDMC modulates chromatin accessibility to influence downstream gene expression programs.

We further show that GSDMC-associated AMPK activation promotes LUAD growth *in vitro* and *in vivo*, complementing previous studies on AMPK-mediated metabolic reprogramming in lung cancer by revealing a distinct epigenetic mechanism contributing to AMPK activation that differs from classical energy

Figure 5. GSDMC drives metabolic reprogramming through CAMKK2-AMPK axis activation

- (A) WB analysis of indicated proteins in H1299 and A549 cells expressing FLAG or FLAG-GSDMC.
(B) IP analysis using anti-FLAG antibodies in H1299 cells transfected with FLAG or FLAG-GSDMC.
(C) WB of indicated proteins in H1299 cells expressing sh-ctrl, sh-CAMKK2, sh-TAK1, or sh-LKB1 following transfection with FLAG or FLAG-GSDMC.
(D and E) WB (D) and qPCR (E) analysis of CAMKK2 and β -actin (loading control) in H1299 cells transduced with FLAG or FLAG-GSDMC.
(F) Genome browser tracks of CAMKK2 ATAC-seq profiles across chromosome 12 (chr12:121,237,199-121,298,369).
(G) Genome browser tracks of CAMKK2 ATAC-seq profiles amplify across chromosome 12 (chr12:121,295,293-121,297,995).
(H) Luciferase reporter assays in H1299 cells transduced with FLAG or FLAG-GSDMC after co-transfection by the indicated plasmids for 48 h.
(I) qPCR analysis of CAMKK2 and β -actin (loading control) in H1299 cells expressing GFP-GSDMC or its NLS mutants (K41E, K135E, K202E, or K135E/K202E).
(J) WB analysis of indicated proteins in H1299 cells from (I).

Data information: (E) are mean $\pm$ SD, two-tailed paired Student's *t* test; (H) two-way ANOVA with Tukey's multiple comparison post-test; (I) one-way ANOVA with Tukey's multiple comparison post-test; (E, H and I) *n* = 3 biological replicates. ns, not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

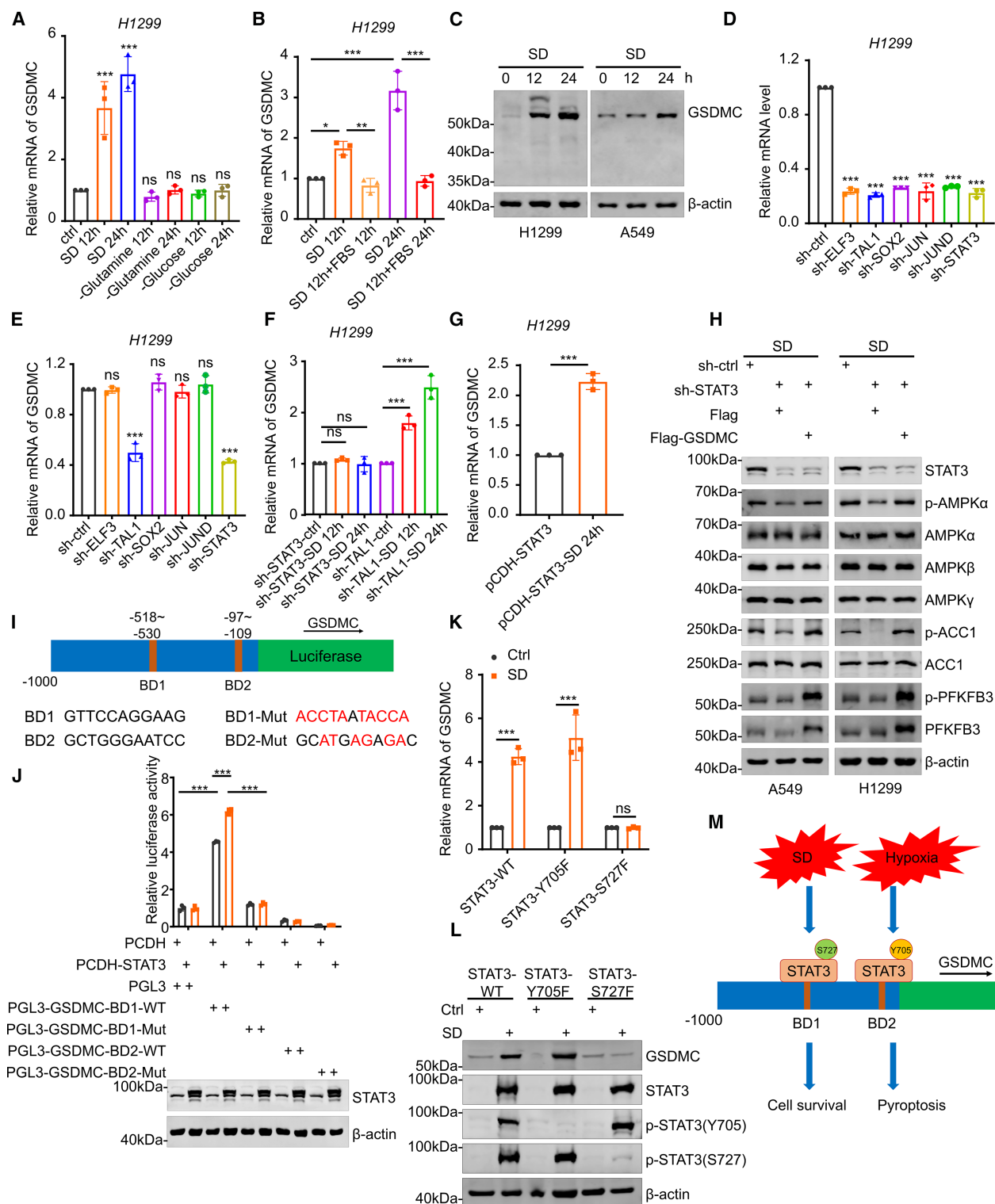


Figure 6. STAT3 positively regulates GSDMC expression responding to SD

(A) GSDMC abundance was determined by qPCR in H1299 cells under the indicated stress conditions including SD, glutamine deprivation, or glucose deprivation.

(B) qPCR analysis of GSDMC conducted in H1299 cells as per (A) under the indicated culture conditions.

(C) WB analysis of GSDMC and β-actin (loading control) in H1299 and A549 cells after SD.

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stress-dependent induction.^{47–49} Mechanistically, GSDMC nuclear activities follow a sequential regulatory cascade where GSDMC facilitates H3 acetylation and chromatin remodeling, which in turn enhances accessibility at the CAMKK2 promoter and supports its transcriptional activation, leading to subsequent AMPK α -Thr172 phosphorylation. These findings uncover a previously unrecognized epigenetic mechanism that acts upstream of the CAMKK2-AMPK pathway and reveal a non-canonical mode of activation for this key metabolic regulator. Our data suggest that GSDMC-mediated CAMKK2-AMPK signaling may promote a bidirectional metabolic reprogramming program in LUAD by concurrently enhancing both glycolysis and FAO. This process supports metabolic flexibility in the nutrient-poor tumor microenvironment and identifies a STAT3-GSDMC-CAMKK2-AMPK signaling cascade that contributes to cellular stress adaptation (Figure 7J).

Notably, this STAT3-GSDMC-CAMKK2-AMPK axis is activated by irradiation and represents a mechanism contributing to LUAD radioresistance. Irradiation induces STAT3-S727 phosphorylation, which upregulates GSDMC and thereby supports the CAMKK2-AMPK axis to cf. radioresistance. Functional validation showed that targeting components of this axis via gene silencing or clinically available inhibitors significantly enhances LUAD radiosensitivity. This axis differs from classic STAT3/AMPK resistance mechanisms in three key aspects: its distinct activation mode, which is epigenetically associated with GSDMC; its functional specificity, which confers both proliferation and radioresistance; and its potential therapeutic advantage, as GSDMC-associated precise AMPK blockade may help reduce systemic off-target effects.

Clinically, GSDMC is significantly upregulated in tumor tissues from patients with LUAD compared with adjacent normal tissues, correlates with increased proliferation and poor patient prognosis, and is weakly expressed in normal bronchial epithelial cells. These observations suggest that GSDMC may serve as a tumor-associated prognostic indicator and a candidate therapeutic target with favorable potential for limited off-target effects. Furthermore, GSDMC expression is associated with unfavorable prognosis in LUAD, and co-expression of STAT3, GSDMC, and CAMKK2 is associated with poorer clinical outcomes. These findings raise the possibility that GSDMC expression could be explored as a candidate indicator of radiosensitivity in future clinical investigations: tumors with high GSDMC expression may exhibit greater radioresistance and might

benefit from radiotherapy combined with pathway inhibitors, whereas those with low GSDMC expression may be more responsive to conventional radiotherapy.

In summary, our study enriches the current understanding of GSDMC and LUAD pathogenesis by characterizing GSDMC as a nuclear scaffold protein with epigenetic regulatory roles, revealing a previously unrecognized chromatin-associated mechanism upstream of the CAMKK2-AMPK pathway, and uncovering the STAT3-GSDMC-CAMKK2-AMPK cascade as a critical regulator of LUAD radioresistance. These findings clarify the stress-specific regulatory mechanism of GSDMC and highlight a tumor-specific metabolic-epigenetic axis that may have potential translational relevance for LUAD therapy. Importantly, our results suggest that combining radiotherapy with inhibitors targeting this axis could represent a promising approach to mitigate radioresistance and improve clinical outcomes for patients with LUAD, an avenue that merits further preclinical and clinical exploration.

Limitations of the study

While we have demonstrated that GSDMC-mediated histone H3 lysine acetylation in the nucleus, its other potential roles in chromatin remodeling warrant further investigation. Furthermore, the observed GSDMC-histone H3-NAT10 ternary complex orchestrates the histone H3 hyperacetylation at multiple lysine residues; however, resolving its architectural determinants necessitates high-resolution crystallographic characterization. Finally, the precise serum constituents governing GSDMC transcriptional regulation remain to be elucidated, despite established mechanistic evidence of STAT3-mediated GSDMC biogenesis.

RESOURCE AVAILABILITY

Lead contact

Further information and request for reagents and resources should be directed to and will be fulfilled by the lead contact, Wei Han (hanw@hfcas.ac.cn).

Materials availability

All unique/stable reagents generated in this study are available from the [lead contact](#) with a completed materials transfer agreement as applicable.

Data and code availability

- Raw data for RNA sequencing, ATAC sequencing, untargeted metabolomics, and untargeted lipidomics are available in the GSA DataSets repository under accession numbers HRA012923, HRA018202, OMIX010825, and OMIX010826, respectively.

(D) qPCR analysis of SOX2, ELF3, STAT3, TAL1, JUND, and JUN mRNA levels in H1299 cells with knockdown of respective genes.

(E) qPCR analysis of GSDMC and β -actin (loading control) in H1299 cells from (D).

(F) qPCR analysis of GSDMC and β -actin (loading control) in H1299 cells transduced with sh-STAT3 or sh-TAL1 after SD.

(G) qPCR analysis of GSDMC and β -actin (loading control) in H1299 cells transduced with pCDH-STAT3 after SD.

(H) WB analysis of indicated proteins in A549 and H1299 cells with STAT3 knockdown (sh-STAT3) or FLAG-GSDMC overexpression under SD conditions.

(I) Schematic of the putative STAT3 binding domains in the GSDMC promoter and the strategy for dual-luciferase reporter assays. The consensus and mutant sequences for STAT3 binding are shown.

(J) Dual-luciferase reporter assays in A549 cells transfected with indicated plasmids and SD for 24 h.

(K) qPCR analysis of GSDMC and β -actin (loading control) in A549 cells expressing STAT3 mutants after SD.

(L) WB analysis of GSDMC, STAT3, phosphorylated STAT3 (Y705/S727), and β -actin (loading control) in A549 cells (from K).

(M) Schematic diagram of STAT3-mediated transcriptional activation of GSDMC under different stimulatory conditions.

Data information: (A, B, D, and E) one-way ANOVA with Tukey's multiple comparison post-test; (F, J, and K) two-way ANOVA with Tukey's multiple comparison post-test; (G) are mean $\pm$ SD, two-tailed paired Student's *t* test; (A, B, D, E, F, G, J, and K) *n* = 3 biological replicates. ns, not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

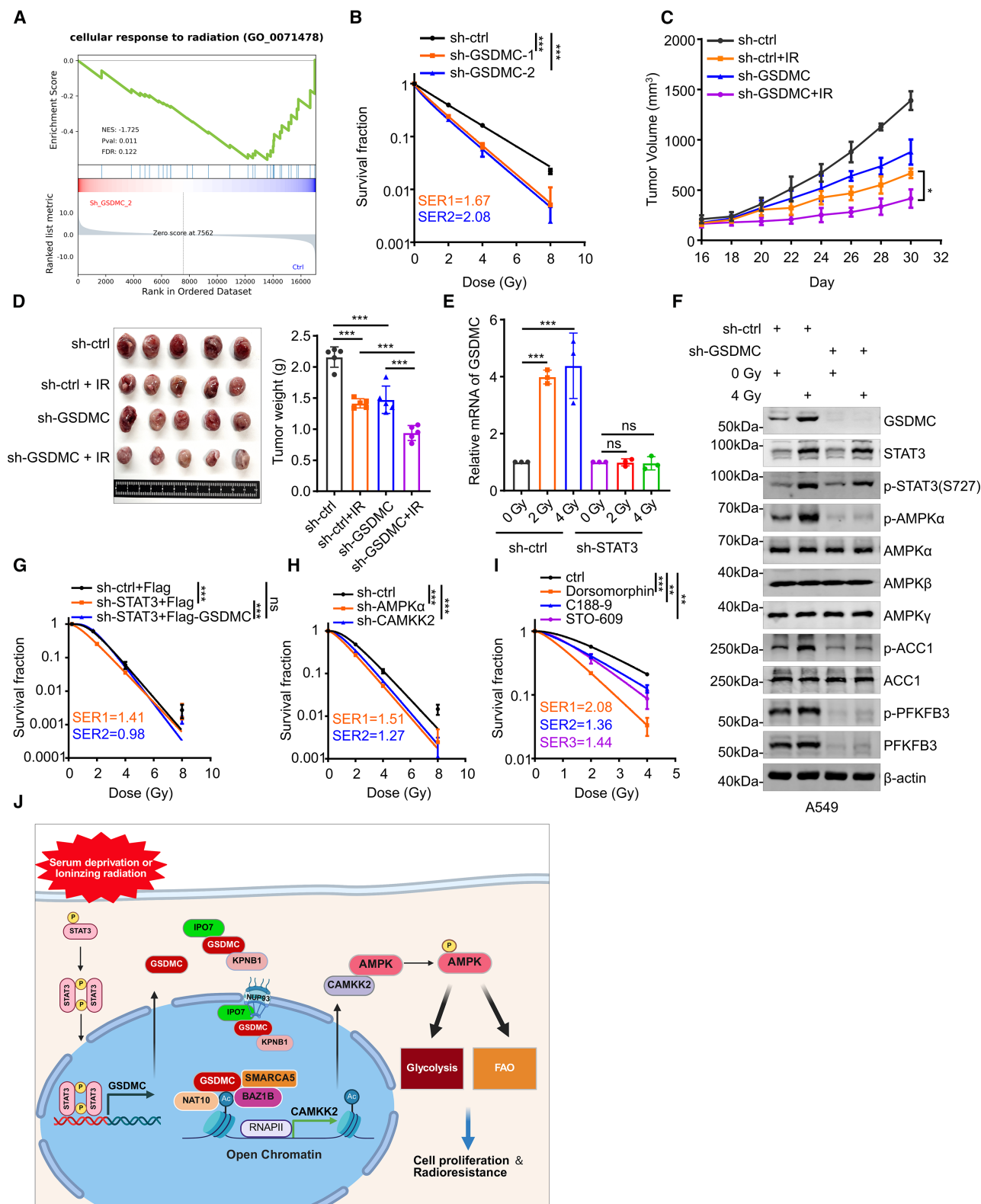


Figure 7. Silencing GSDMC improves the radiosensitivity of LUAD

(A) GSEA enrichment analysis of cellular response to radiation in RNA-seq from H1299 cells transduced with sh-ctrl or sh-GSDMC-2.

(B) Survival fraction of A549 cells transduced with sh-ctrl, sh-GSDMC-1, or sh-GSDMC-2 after X-ray irradiation.

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- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

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AUTHOR CONTRIBUTIONS

D.R., Y.L., M.L., and W.H. conceived and designed the study. D.R., Y.L., and M.L. performed all parts of the experiment and data analysis. D.R., Y.L., and M.L. contributed equally to the study. L.W. and K.N.Y. provided the necessary suggestions and technical supports. W.J. and H.X. participated in the construction of plasmids. D.R., Y.L., M.L., and W.H. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS](#)
 - Cell lines and clinical tissues
 - Xenograft model
- [METHOD DETAILS](#)
 - RNA interference and gene overexpression
 - Nile red staining
 - Cytosolic/nuclear fractionation
 - Dual-luciferase reporter assays
 - ECAR, OCAR, and FAO rate Measurements
 - RNA-seq
 - RNA extraction and qPCR assays
 - Untargeted metabolomics methods
 - Untargeted lipidomics
 - ATAC-seq
 - Irradiation and sensitization enhancement ratio (SER)
 - Recombinant protein purification and pull-down assays
 - Immunohistochemistry (IHC)
 - Western blot and Co-IP

- IP-mass spectrometry (MS)
- IF staining
- [QUANTIFICATION AND STATISTICAL ANALYSIS](#)
 - Statistical analysis and reproducibility

SUPPLEMENTAL INFORMATION

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(C and D) Comparison of radiosensitivity between A549 xenograft tumors expressing sh-ctrl or sh-GSDMC-2 (C). Final tumor weights after dissection are shown (D). Data represent a single experiment; $n = 5$ mice per group.

(E) qPCR analysis of GSDMC and β -actin (loading control) in A549 cells transduced with sh-ctrl or sh-STAT3 cells at 72 h after X-ray irradiation.

(F) WB analysis of A549 cells transduced with sh-ctrl or sh-GSDMC at 72 h after X-ray irradiation.

(G) Survival fraction of A549 cells bearing sh-ctrl or sh-STAT3 transfected with FLAG or FLAG-GSDMC plasmids after X-ray irradiation.

(H) Survival fraction of A549 cells transduced with sh-ctrl, sh-CAMKK2, or sh-AMPK α after X-ray irradiation.

(I) Survival fraction of A549 cells treated with various inhibitors including STAT3 inhibitor (C188-9), CAMKK2 inhibitor (STO-609), or AMPK inhibitor (Dorsomorphin) after X-ray irradiation.

(J) Schematic of GSDMC regulating metabolism reprogramming to promote LUAD progression. Created with BioRender (<https://biorender.com/>).

Data information: (B, G, H, and I) two-way ANOVA test was applied. Data are presented as mean $\pm$ SD; (C) two-way ANOVA with Bonferroni's multiple comparison post-test; (D) one-way ANOVA with Tukey's multiple comparison post-test; (E) two-way ANOVA with Tukey's multiple comparison post-test; (B, E, G, H, and I) $n = 3$ biological replicates. ns, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
GSDMC (WB 1:1000, IHC 1:1000)	Proteintech	Cat#30469-1-AP; RRID: AB_3086329
GSDMC (IF 1:1000)	Thermo Fisher Scientific	Cat#PA5-116594; RRID: AB_2901225
p-AMPK (WB 1:1000)	Cell Signaling Technology	Cat#2535; RRID: AB_331250
AMPK α (WB 1:1000)	Cell Signaling Technology	Cat#5831; RRID: AB_10622186
AMPK β (WB 1:1000)	Proteintech	Cat#10308-1-AP; RRID: AB_513239
AMPK γ (WB 1:1000)	Proteintech	Cat#10290-1-AP; RRID: AB_2268781
p-S727-STAT3 (WB 1:1000)	Cell Signaling Technology	Cat#9134; RRID: AB_331589
p-Y705-STAT3 (WB 1:1000)	Cell Signaling Technology	Cat#9145; RRID: AB_2491009
STAT3 (WB 1:1000, IHC 1:100)	Proteintech	Cat#10253-2-AP; RRID: AB_2302876
p-ACC1 (WB 1:1000)	Proteintech	Cat#29119-1-AP; RRID: AB_2918238
ACC1 (WB 1:1000)	Proteintech	Cat#21923-1-AP; RRID: AB_11042445
p-PFKFB3 (WB 1:500)	Abcam	Cat#ab202291; RRID: AB_3698749
PFKFB3 (WB 1:1000)	Proteintech	Cat#13763-1-AP; RRID: AB_2162854
CAMKK2 (WB 1:1000, IHC 1:300)	Proteintech	Cat#11549-1-AP; RRID: AB_2259441
TAK1 (WB 1:1000)	Proteintech	Cat#12330-2-AP; RRID: AB_2140101
LKB1 (WB 1:1000)	Proteintech	Cat#10746-1-AP; RRID: AB_2271311
PARP (WB 1:1000)	Proteintech	Cat#13371-1-AP; RRID: AB_2160459
GAPDH (WB 1:1000)	Proteintech	Cat#60004-1-Ig; RRID: AB_2107436
NUP93 (WB 1:1000, IP 1:500)	Proteintech	Cat#13077-1-AP; RRID: AB_2251835
NUP93 (IF 1:50)	Santa Cruz Biotechnology	Cat#sc-374399; RRID: AB_10990113
KPNB1 (WB 1:1000, IP 1:500, IF 1:100)	Proteintech	Cat#10077-1-AP; RRID: AB_2133977
IPO7 (WB 1:1000, IP 1:500)	Proteintech	Cat#28289-1-AP; RRID: AB_2881106
IPO7 (IF 1:100)	Santa Cruz Biotechnology	Cat#sc-365231; RRID: N/A
β -actin (WB 1:20000)	Proteintech	Cat#66009-1-Ig; RRID: AB_2782959
GFP (WB 1:1000)	Proteintech	Cat#66002-1-Ig; RRID: AB_11182611
DYKDDDDK tag (IP 1:500, WB 1:2000, IF 1:1000)	Proteintech	Cat#Biotin-66008-4; RRID: AB_3086560
DYKDDDDK tag (IF 1:100)	Cell Signaling Technology	Cat# MA1-142; RRID: AB_2536846
Histone H1.2 (WB 1:1000)	Proteintech	Cat#19649-1-AP; RRID: AB_10694432
Histone H3 (WB 1:1000)	Proteintech	Cat#17168-1-AP; RRID: AB_2716755
Histone H3 (IF 1:50)	Elabscience	Cat#E-AB-22003; RRID: N/A
Histone H4 (WB 1:1000)	Proteintech	Cat#16047-1-AP; RRID: AB_2118625
Histone H2A.X (WB 1:1000)	Zenbio	Cat#R26852; RRID: N/A
Acetyl-Histone H3 (Lys14) (IP 1:500)	Abclonal	Cat#A27265; RRID: N/A
Acetyl-Histone H3 (Lys14) (WB 1:1000, IF 1:50)	Zenbio	Cat#R27466; RRID: N/A
Acetyl-Histone H3 (Lys9) (WB 1:1000, IF 1:50)	Zenbio	Cat#340016; RRID: N/A
Acetyl-Histone H3 (Lys9) (IP 1:500)	Abclonal	Cat#A21107; RRID: AB_3714809
Histone H3K23ac (WB 1:1000, IF 1:50)	Proteintech	Cat#39132; RRID: N/A
Histone H3K23ac (IP 1:500)	Abclonal	Cat#A18154; RRID: AB_2861944
Histone H3K18ac (WB 1:1000, IF 1:50)	Proteintech	Cat#39756; RRID: N/A
Histone H3K18ac (IP 1:500)	Abclonal	Cat#A20735; RRID: N/A
Acetyl-Histone H3 (Lys27) (WB 1:1000)	Cell Signaling Technology	Cat#8173; RRID: AB_10949503
NAT10 (WB 1:1000, IF 1:100)	Proteintech	Cat#13365-1-AP; RRID: AB_2148944

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
BAZ1B (WB 1:1000, IF 1:100)	Proteintech	Cat#68361-1-Ig; RRID: N/A
SMARCA5 (WB 1:1000, IF 1:100)	Proteintech	Cat#13066-1-AP; RRID: N/A
GST-Tag (WB 1:1000)	Cell Signaling Technology	Cat#2625; RRID: AB_490796
Ki67 (IHC 1:4000)	Proteintech	Cat#27309-1-AP; RRID: AB_2756525
DAPI	Beyotime	Cat#P0131; RRID: N/A
IRDye® 800CW Goat anti-Rabbit IgG (H + L) (WB 1:10000)	Li-COR	Cat#926-32211; RRID: N/A
IRDye® 680CW Goat anti-Mouse IgG (H + L) (WB 1:10000)	Li-COR	Cat#926-68070; RRID: N/A
Goat anti-Rabbit IgG (H + L) Cross-Adsorbed Secondary Antibody (Alexa Fluor® 488) (IF 1:1000)	Thermo Fisher Scientific	Cat#A-11008; RRID: N/A
Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody (Alexa Fluor® 594) (IF 1:1000)	Thermo Fisher Scientific	Cat#A21203; RRID: N/A
Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (IF 1:1000)	Thermo Fisher Scientific	Cat#A-31573; RRID: N/A
Donkey anti-Rat IgG H&L (Alexa Fluor® 488) (IF 1:1000)	Abcam	Cat#ab150153; RRID: N/A

Chemicals, peptides, and recombinant proteins

Dorsomorphin	MedChemExpress	HY-13418A; CAS: 866405-64-3
C188-9	MedChemExpress	HY-112288; CAS: 432001-19-9
STO-609	MedChemExpress	HY-19805; CAS: 52029-86-4
5-Fluorouracil	MedChemExpress	HY-90006; CAS: 51-21-8
Cisplatin	MedChemExpress	HY-17394; CAS: 15663-27-1
L-Carnitine	MedChemExpress	HY-B0399; CAS: 541-15-1
Puromycin	Beyotime	ST551-10mg; CAS: 58-58-2

Critical commercial assays

DMEM	Gibco	12800082
RPIM-1640	Gibco	61870044
TRIzol	Invitrogen	AM9738
Nile red	Sigma-Aldrich	72485
RIPA buffer	Beyotime	P0013B
Cytosolic/nuclear fractionation kit	Beyotime	P0028
Triglyceride (TG) Content Assay kit	Boxbio	AKFA003M
Dual-luciferase reporter assays kit	Vazyme	DL101-01
Glycolysis Stress Test Kit	Agilent	103020-100
Cell Mito Stress Test Kit	Agilent	103015-100
Seahorse XF Palmitate Oxidation Stress Test Kit	Agilent	103693-100
Hifair® III 1st Strand cDNA Synthesis SuperMix for qPCR	YEASEN	11141ES60
Hieff® SYBR Green Master Mix	YEASEN	11202ES03
Mag-MK GST purification	Sangon Biotech	C650031-0025
LipoMax DNA Transfection Reagent	Sudgen	32012
ClonExpress II One Step Cloning Kit	Vazyme	C112-01

Deposited data

RNA-seq raw data	This paper	GSA database: HRA012923
ATAC-seq raw data	This paper	GSA database: HRA018202

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Untargeted metabolomics raw data	This paper	GSA database: OMIX010825
Untargeted lipidomics raw data	This paper	GSA database: OMIX010826
Experimental models: Cell lines		
H1299	ATCC	CRL-5803
A549	ATCC	CRM-CCL-185
Calu-1	ATCC	HTB-54
H522	ATCC	CRL-5810
H1975	ATCC	CRL-5908
HeLa	ATCC	CRM-CCL-2
HEK-293T	ATCC	CRL-11268
BEAS-2B	Shanghai Cell Bank (CAS, Shanghai, China)	GNHu27
Oligonucleotides		
shRNA sequence, see Table S2	This paper	N/A
qRT-PCR primer, see Table S3	This paper	N/A
Recombinant DNA		
pSin-3xFLAG-empty	This paper	N/A
pSin-3xFLAG-GSDMC	This paper	N/A
pCDH-empty	This paper	N/A
pCDH-STAT3	This paper	N/A
pLVX-EGFP-C1-empty	This paper	N/A
pLVX-EGFP-GSDMC-WT	This paper	N/A
pLVX-EGFP-GSDMC-K41E	This paper	N/A
pLVX-EGFP-GSDMC-K135E	This paper	N/A
pLVX-EGFP-GSDMC-K202E	This paper	N/A
pLVX-EGFP-GSDMC-K135E K202E	This paper	N/A
pGEX-5X-3-empty	This paper	N/A
pGEX-5X-3-GSDMC	This paper	N/A
pGL3-Basic	This paper	N/A
pGL3-BD1-WT	This paper	N/A
pGL3-BD1-Mut	This paper	N/A
pGL3-BD2-WT	This paper	N/A
pGL3-BD2-Mut	This paper	N/A
pLKO.1-puro	Sigma	SHC002
Software and algorithms		
GraphPad Prism 8	GraphPad Software	https://www.graphpad.com/
ImageJ	NIH, USA	https://imagej.nih.gov/ij/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Cell lines and clinical tissues

H1299, H1975, BEAS-2B and Calu-1 cells were cultured in RPMI-1640 medium, while H522, A549, H460, HeLa and HEK293T cells were cultured in DMEM. All cell lines were maintained in medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, at 37°C in a humidified incubator with 5% CO₂. All cell lines used in this study were authenticated by short tandem repeat (STR) profiling to confirm their identity, and were routinely screened for mycoplasma contamination using a PCR-based detection method; all cell lines tested negative for mycoplasma throughout the experiments.

Human LUAD samples, paired adjacent non-tumor tissues and complementary DNA (cDNA) were obtained from Outdo Biotech (#HLugA180Su11, #HLugA060PG03, #cDNA-HLugA030PG01). All samples were allocated to experimental groups based on their pathological diagnosis, with paired tumor and non-tumor tissues analyzed in parallel. The study was approved by the Human Research Ethics Committee of Hefei Cancer Hospital, Chinese Academy of Sciences (CAS), with written informed consent obtained from all participants, in accordance with the Declaration of Helsinki guidelines. Due to limited demographic data provided by the

commercial biobank, we were unable to assess the influence of sex or gender on the study outcomes, which represents a limitation of this work.

Xenograft model

Four-week-old male Balb/c nude mice (Nanjing Meiris Biotechnology) were raised and housed in a specific pathogen-free (SPF) laboratory environment with a standard 12 h light/12 h dark cycle, constant temperature and humidity, and free access to sterile food and water. All *in vivo* experimental procedures were reviewed and approved by the Hefei Institutes of Physical Science Ethics Committee and performed in strict accordance with institutional animal care guidelines. For tumor formation assays, A549 cells (1×10^7) with GSDMC knockdown/overexpression or corresponding control cells were injected subcutaneously into the right flank of nude mice. Tumor tissues were isolated and weighed at 20–22 days after cell inoculation. For radiosensitivity experiments, mice with A549 xenograft tumors reaching 100–200 mm³ were subjected to localized 12 Gy X-ray irradiation, with non-tumor tissues of the whole body tightly shielded by lead plates. Tumor volume was measured every two days using a vernier caliper. Humane endpoints were strictly implemented: mice were euthanized once the tumor volume exceeded 1,500 mm³, or when obvious weight loss, cachexia and mental depression occurred. All tumor tissues were harvested and measured 14 days after radiation treatment.

METHOD DETAILS

RNA interference and gene overexpression

Lentiviruses were generated in HEK293T cells using either the pLKO.1/psPAX2/pMD2.G system (for knockdown) or the pSin-3XFlag/pCDH/pLVX-EGFP/psPAX2/pMD2.G system (for overexpression; at a 4:3:1 ratio). After filtration through a 0.22 μ m membrane, transduced cells were selected with 5 μ g/mL puromycin (Beyotime, #ST551-10mg). Targeting short hairpin RNA (shRNA) sequences are provided in [Table S2](#).

Nile red staining

Cells were washed with PBS, fixed in 4% formaldehyde, and stained with Nile red (0.05 mg/mL) for 10 min. Nuclei were counterstained with DAPI (#P0131, Beyotime) and imaged with a confocal microscope (Olympus SpinSR10).

Cytosolic/nuclear fractionation

Nuclear and cytoplasmic proteins were extracted with a commercial kit (#P0028, Beyotime). Cells were lysed in cytoplasmic buffer A, vortexed, and incubated on ice (15 min). Cytoplasmic buffer B was added, followed by brief vortexing and centrifugation (12,000 $\times$ g, 5 min) to collect the cytosolic fraction. Pellets were washed with PBS, resuspended in nuclear buffer, incubated on ice (30 min), and centrifuged (12,000 $\times$ g, 10 min) to isolate nuclear proteins. PARP and GAPDH served as controls.

Dual-luciferase reporter assays

Dual-luciferase reporter assays (Vazyme #DL101-01) were performed per the manufacturer's protocol. Cells were co-transfected with promoter plasmids and a renilla luciferase control. After 48 h, firefly luciferase activity was measured and normalized to renilla activity.

ECAR, OCAR, and FAO rate Measurements

Metabolic analysis was performed on a Seahorse XFe24 analyzer (Agilent). Glycolysis (ECAR) was measured after sequential glucose (10 mM), oligomycin (2 μ M) and 2-DG (100 mM) treatment (#103020-100). Mitochondrial respiration (OCR) was assessed after oligomycin (2 μ M), FCCP (2 μ M) and rotenone/antimycin A (1 μ M) (#103260-100). Data were analyzed with Seahorse Glycolysis and Mito Stress Test packages.

FAO rates were measured with the Seahorse XF Mito Stress Test. After cell adherence, the medium was replaced with glucose-, pyruvate-, glutamine- and GlutaMAX-free medium supplemented with 0.5 mM glucose, 0.5 mM carnitine, 1 mM GlutaMAX, and 1% FBS, and the cells were cultured for a further 24 h. Before the assay, cells were washed and incubated for 1 h in FAO assay medium (111 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl₂, 2 mM MgSO₄, 1.2 mM NaH₂PO₄, 2.5 mM glucose, 0.5 mM carnitine, 5 mM HEPES, pH 7.4) in a non-CO₂ incubator. BSA-conjugated palmitate (50 μ M final) was added, and data were analyzed with the Seahorse Mito Stress Test software.

RNA-seq

RNA-seq was performed in H1299 cells with GSDMC knockdown versus control cells. Total RNA was extracted by using Trizol, and sequencing was conducted on an Illumina NovaSeq 6000 (SEQHEALTH, China). Differentially expressed genes (FDR < 0.05, $|\log_2 FC| \geq 1$) were identified with DESeq2 and analyzed for KEGG pathways, GO functions, and GSEA.

RNA extraction and qPCR assays

Total RNA was extracted with TRIzol (Invitrogen), reverse-transcribed with Hifair® cDNA Synthesis SuperMix (YEASEN, #11120ES60), and analyzed by qPCR (Hieff® SYBR Green Master Mix, YEASEN, #11202ES03). Primer sequences are listed in [Table S3](#).

Untargeted metabolomics methods

H1299 sh-GSDMC and sh-ctrl cells were analyzed by UPLC-Q-TOF/MS (Aptbiotech) using an Agilent 1290 UHPLC-AB Sciex TripleTOF 6600 system. QC samples ensured analytical stability. Metabolites with VIP > 1 and $p < 0.05$ (Student's t-test) were considered significant.

Untargeted lipidomics

H1299 sh-GSDMC and sh-ctrl cells were subjected to lipid extraction (MTBE method) and analyzed by UPLC-Q-TOF/MS (Aptbiotech). Samples were redissolved in 90% isopropanol/acetonitrile and analyzed with a UHPLC Nexera LC-30A coupled to a Q-Exactive Plus mass spectrometer (Thermo Scientific) in positive/negative modes. Lipids were identified and quantified with LipidSearch 4.1, and statistical significance was determined by Student's t-test ($p < 0.05$).

ATAC-seq

Flag-GSDMC or Flag-transduced H1299 cells (5×10^5) were lysed, and nuclei were processed with transposase (37°C, 30 min). Libraries were purified (MinElute PCR Purification Kit), sequenced on Illumina NovaSeq 6000 (PE150) by Rainbow Genome, and analyzed using IGV (Broad Institute) for visualization.

Irradiation and sensitization enhancement ratio (SER)

The cells were irradiated with an X-ray irradiator (XHA600D, SHINVA, Zibo, China) at a dose rate of 1.88 Gy/min. The SER was evaluated at a 37% survival fraction (SF). Two-way ANOVA was applied for statistical significance analysis. Each experiment was carried out in triplicate or more independent replicates.

Recombinant protein purification and pull-down assays

GST-GSDMC was purified from *E. coli* by using Mag-MK GST purification (Sangon Biotech, #C650031-0025). After overexpression in HEK293T cells, the proteins were incubated with GST-GSDMC (4°C, 4 h), washed three times, and heated for analysis.

Immunohistochemistry (IHC)

Serial FFPE tissue sections (5 mm) were immunohistochemically stained with the indicated primary antibodies. The intensity of staining was measured with the IHC profiler plugin in ImageJ software. Antibodies and dilutions used are shown in the [key resources table](#).

Western blot and Co-IP

Cells were lysed in RIPA buffer with protease inhibitors, and after centrifuged, the supernatants were separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 5% milk, membranes were incubated with indicated primary antibodies overnight, then incubated with Li-COR fluorescent secondary antibodies for 1 h at room temperature. Signals were detected with an Odyssey CLx Imaging System.

For co-IP, cells were lysed in IP buffer (0.5% Triton-X100, 0.5% NP-40, 1.5 mM MgCl₂, 150 mM NaCl, 20 mM Tris-HCl and 10% glycerol). Lysates were incubated overnight at 4°C with primary antibody, followed by protein A/G beads (2 h, 4°C), washed three times with IP buffer, and heated at 97°C for 10 min. Antibody details are shown in [key resources table](#).

IP-mass spectrometry (MS)

Flag-GSDMC was expressed in H1299 cells and immunoprecipitated with anti-FLAG antibody and protein A/G beads. After washing, samples were heated, separated by 10% Tris-Glycine SDS-PAGE and Coomassie-stained. Target bands were trypsin-digested for MS analysis (Aptbiotech, China).

IF staining

Cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.5% PBST, and blocked with 5% BSA (1 h). After incubation with primary and fluorescent secondary antibodies, nuclei were stained with DAPI (Beyotime, #P0131) and imaged with an Olympus SpinSR10 confocal microscope. Antibody details are shown in [key resources table](#).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis and reproducibility

All experiments were repeated independently at least three times. The data are presented as means $\pm$ SD. Comparisons between two groups were performed by using a two-tailed Student's *t*-test, while multiple-group comparisons were assessed by one-way ANOVA (Tukey's post hoc test) or two-way ANOVA (Tukey's or Bonferroni's post hoc test for bivariate analyses). A *p*-value <0.05 was considered statistically significant (**p* <0.05 , ***p* <0.01 , ****p* <0.001 ; ns, not significant). Statistical analysis and graphing were performed by using Microsoft Excel and GraphPad Prism 8.0.

Cell Reports, Volume 45

Supplemental information

**Gasdermin C reprograms metabolism
through the CAMKK2-AMPK axis to promote lung
adenocarcinoma progression and radioresistance**

Delong Ren, Yunyun Li, Mingquan Lu, Wuqiao Jiang, Hao Xu, Lijun Wu, K.N. Yu, and Wei Han

Figure S1. Related to Figure 1

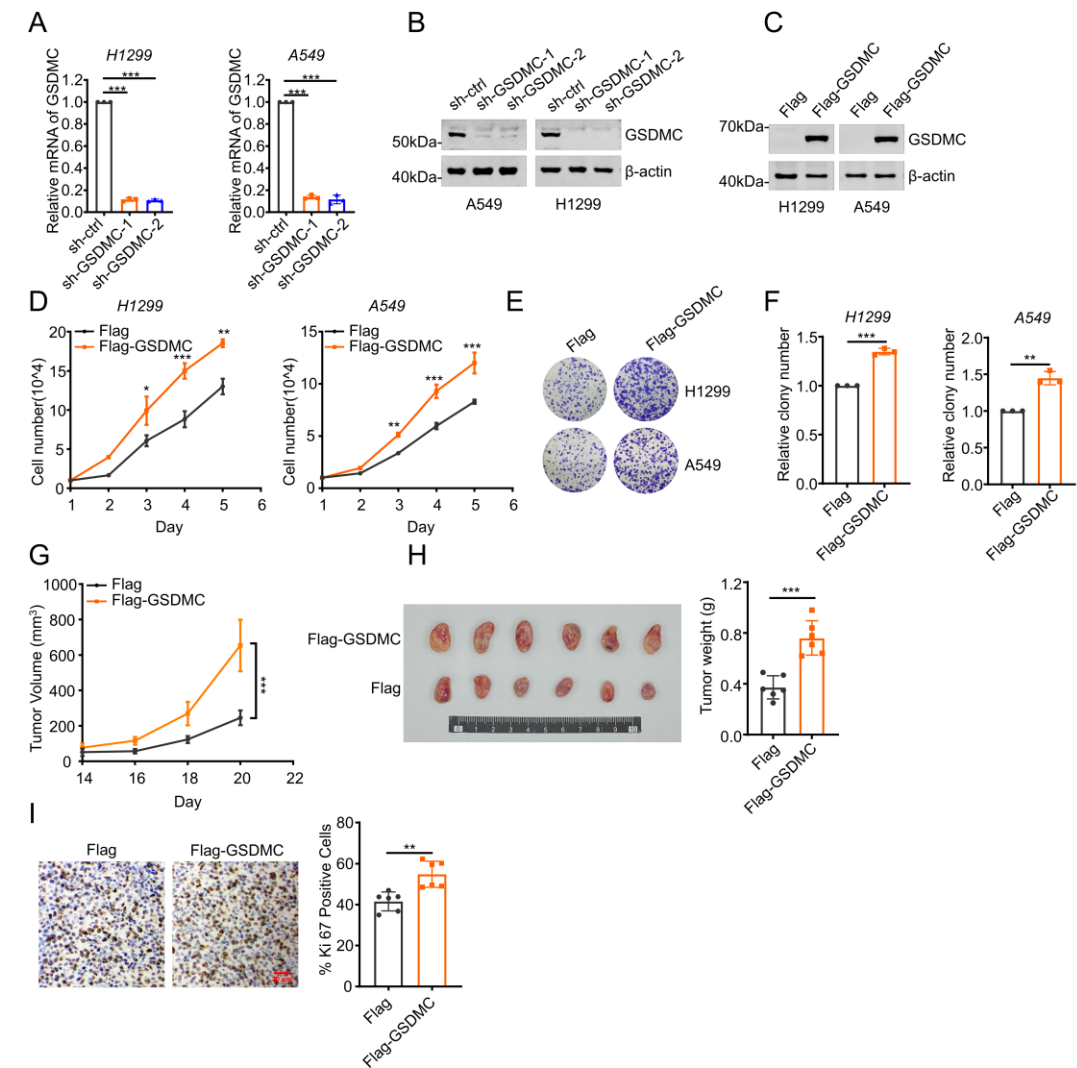


Figure S1. Related to Figure 1

(A and B) qPCR (A) and WB (B) analysis of GSDMC and β -actin (loading control) in H1299 and A549 cells transduced with sh-ctrl or two independent shRNAs targeting GSDMC (sh-GSDMC-1 and sh-GSDMC-2).

(C) WB analysis of GSDMC and β -actin (loading control) in H1299 and A549 cells transduced with Flag or Flag-GSDMC.

(D) Growth curves of cells from (C).

(E and F) Colony formation (E) and quantification (F) of cells from (C).

(G and H) Comparison of the growth of A549 xenografts expressing Flag or Flag-GSDMC (G). Comparison of final tumor weights after dissection (H). Data represent a single experiment; n = 6 mice per group.

(I) IHC of Ki 67 from (H) and quantification. Scale bar: 30 μ m.

Data information: (A) one-way ANOVA with Tukey's multiple comparison post-test; (D and G) two-way ANOVA with Bonferroni's multiple comparison post-test; (F, H and I) are mean $\pm$ SD, two-tailed paired Student's t test; (A, D and F) n = 3 biological replicates. *p < 0.05, **p < 0.01, ***p < 0.001.

Figure S2. Related to Figure 2

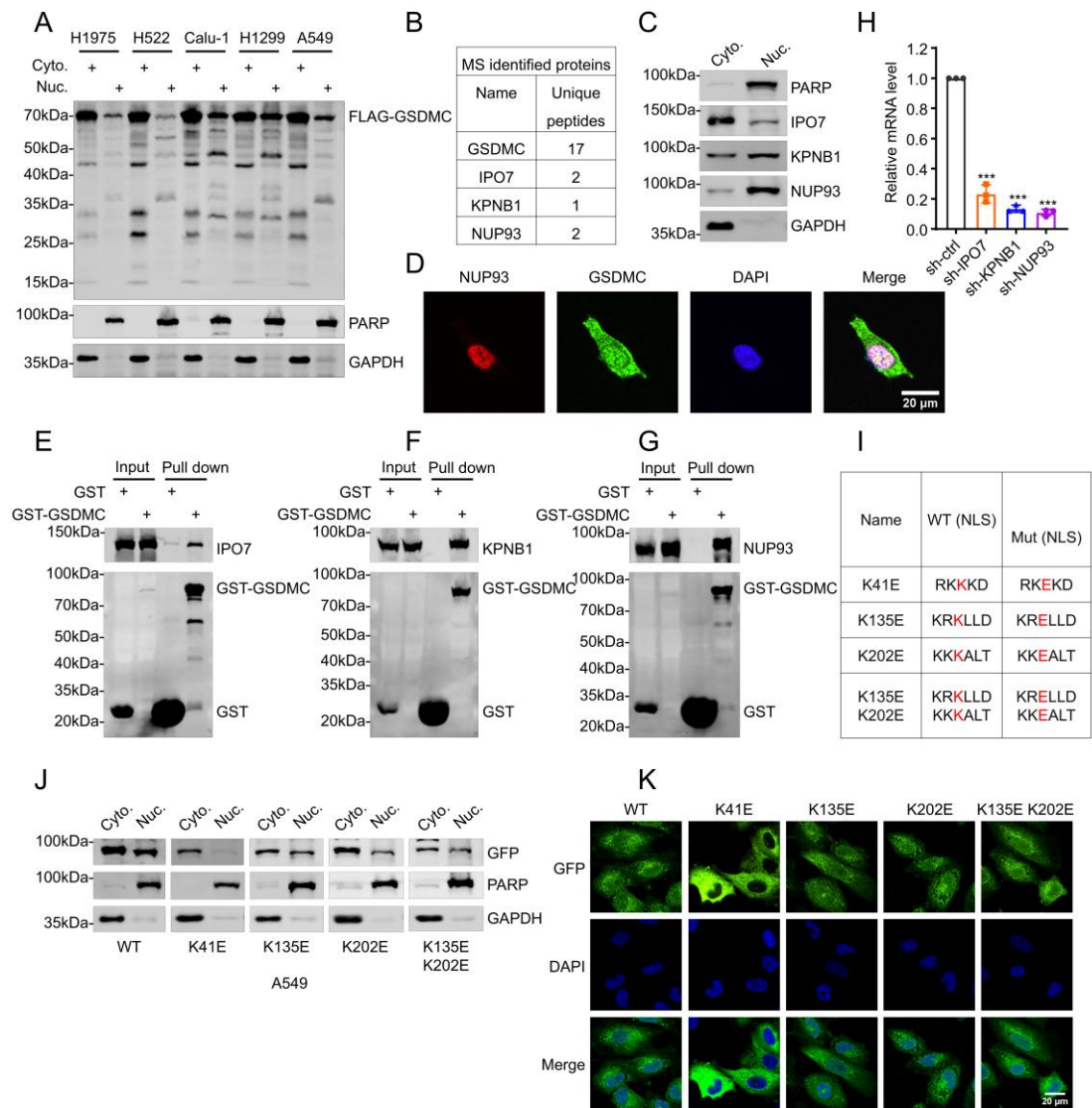


Figure S2. Related to Figure 2

(A) WB analysis of extracted nuclear and cytoplasmic fractions after enforced expression of Flag-GSDMC in A549, H1299, Calu-1, H522 and H1975 cells.

(B) MS identified proteins GSDMC, IPO7, KPNB1 and NUP93.

(C) WB analysis of nuclear and cytoplasmic extracts of H1299 cells.

(D) IF of FLAG-GSDMC, NUP93 and nucleus staining (DAPI) in H1299 cells. Scale bar: 20 μ m.

(E-G) Pull-down assays were conducted with IPO7 (E), KPNB1 (F) or NUP93 (G) overexpression in HEK293T cells against the indicated GST-tagged-GSDMC proteins purified from *Escherichia coli*.

(H) qPCR analysis of IPO7, KPNB1, NUP93 and β -actin (loading control) in H1299 cells transduced with sh-ctrl, sh-IPO7, sh-KPNB1 or sh-NUP93.

(I) Mutations in NLS prediction of GSDMC.

(J) WB analysis of nuclear and cytoplasmic fractions from A549 cells expressing GFP-GSDMC or its NLS mutants (K41E, K135E, K202E or K135E/K202E).

(K) GFP fluorescence labeling of A549 cells from (J). Scale bar: 20 μ m.

Data information: (H) one-way ANOVA with Tukey's multiple comparison post-test; n = 3 biological replicates. ***p < 0.001.

Figure S3. Related to Figure 3

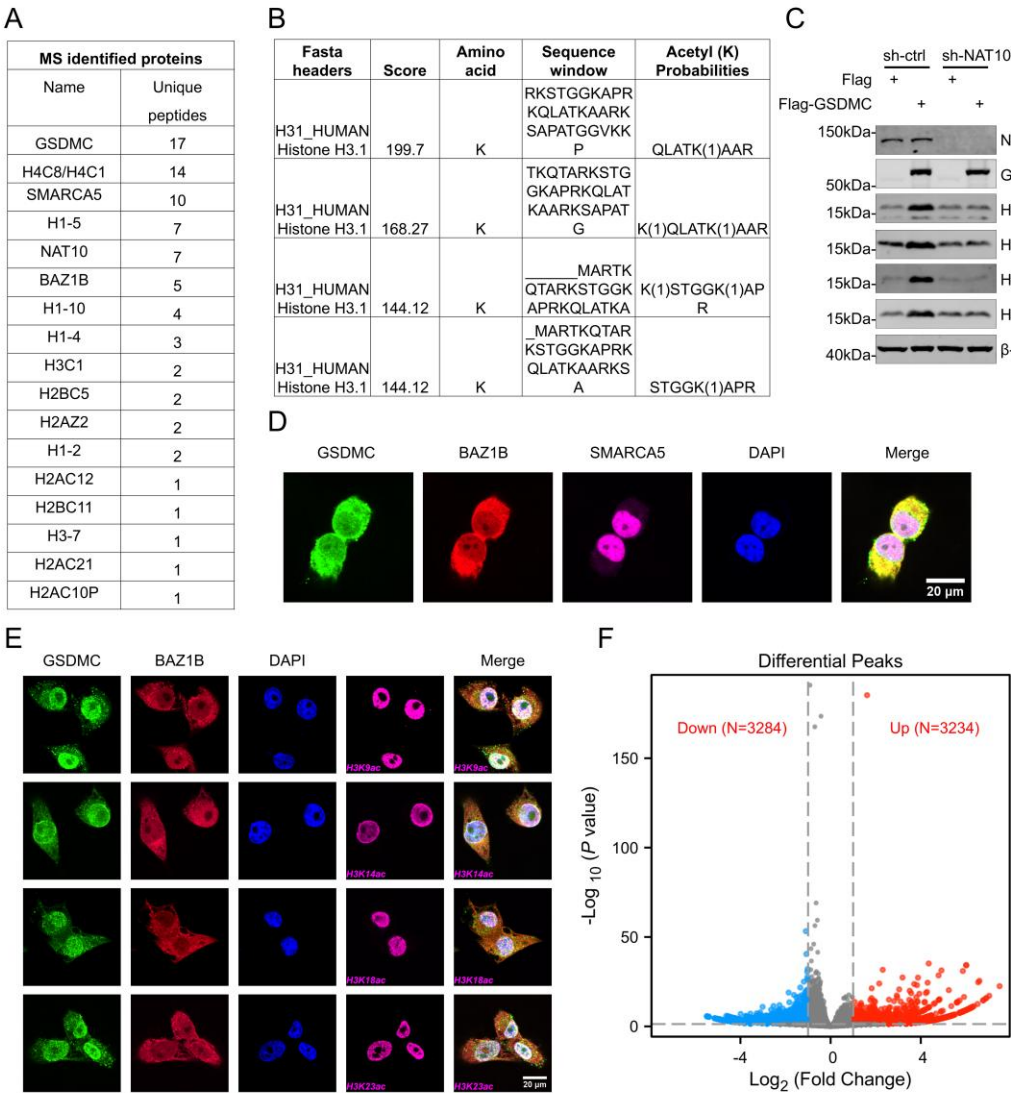


Figure S3. Related to Figure 3

(A) MS identified proteins.

(B) MS identified Histone H3 acetylation modification sites.

(C) WB of indicated proteins in H1299 cells expressing sh-ctrl or sh-NAT10 following transfection with Flag or Flag-GSDMC.

(D) IF of FLAG-GSDMC, BAZ1B and SMARCA5 and nucleus staining (DAPI) in H1299 cells. Scale bar: 20 μ m.

(E) IF of FLAG-GSDMC, BAZ1B and H3K9/14/18/23ac in H1299 cells (DAPI: nuclei). Scale bar: 20 μ m.

(F) Volcano plot visualization of differentially accessible chromatin regions (peaks) identified by ATAC-seq.

Figure S4. Related to Figure 4

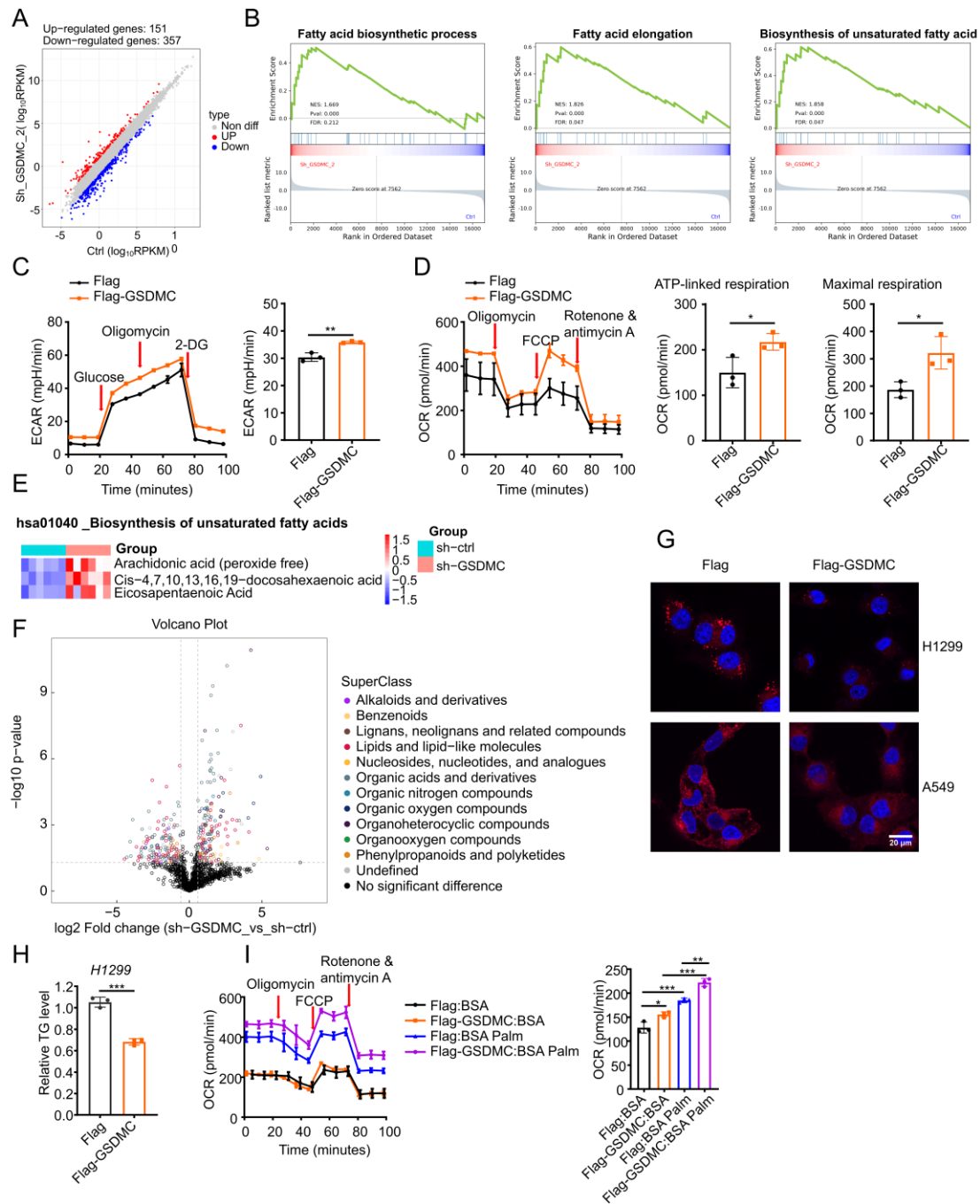


Figure S4. Related to Figure 4

- (A) Scatter plot of RNA-seq in H1299 cells transduced with sh-ctrl or sh-GSDMC-2.
- (B) GSEA enrichment analysis of fatty acid biosynthetic process, fatty acid elongation and biosynthesis of unsaturated fatty acids in RNA-seq from (A).
- (C) Representative ECAR traces during glycolysis stress tests in H1299 cells transduced with Flag or Flag-GSDMC.
- (D) Representative OCAR traces during cell mitochondrion stress tests in H1299 transduced with Flag or Flag-GSDMC.
- (E) Biosynthesis of unsaturated fatty acids enrichment of non-target metabolomics in H1299 cells.
- (F) Volcano plot of non-target metabolomics (n = 6) in H1299 cells transduced with sh-ctrl or sh-GSDMC-2.
- (G) Nile red (red) and DAPI (blue) staining of H1299 and A549 cells transduced with Flag or Flag-GSDMC. Scale bar: 20 μ m.
- (H) Total TG levels measured in H1299 cells transduced with Flag or Flag-GSDMC.
- (I) Seahorse FAO assay in H1299 cells transduced with Flag or Flag-GSDMC with or without addition of exogenous fatty acids, and the maximum respiratory capacity of cells induced by FCCP was quantitatively analyzed.

Data information: (C, D and H) are mean $\pm$ SD, two-tailed paired Student's t test; (I) one-way ANOVA with Tukey's multiple comparison post-test; (C, D, H and I) n = 3 biological replicates.

*p < 0.05, **p < 0.01, ***p < 0.001.

Figure S5. Related to Figure 5

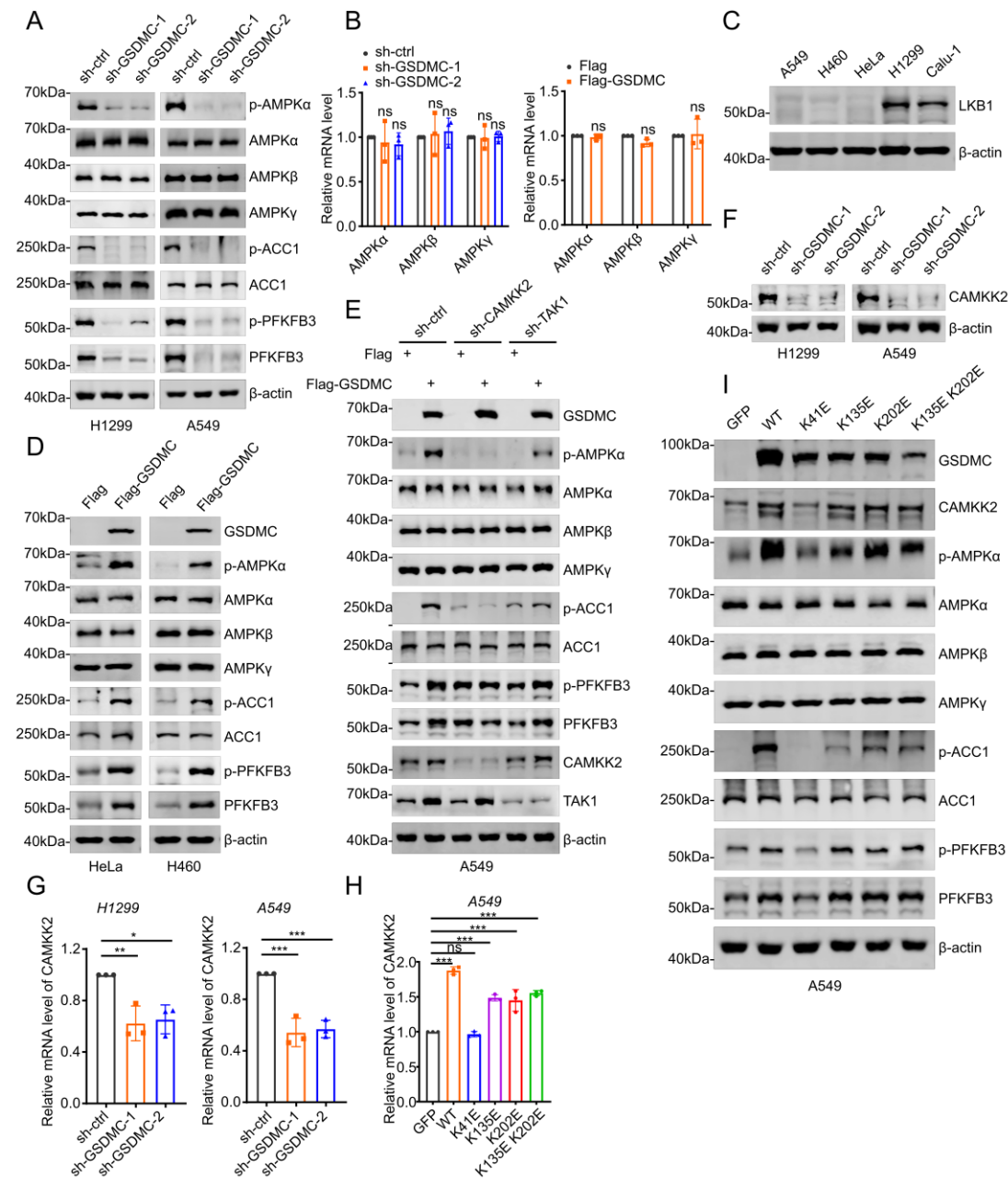


Figure S5. Related to Figure 5

(A) WB analysis of protein expression levels in H1299 and A549 cells transduced with sh-ctrl, sh-GSDMC-1 or sh-GSDMC-2.

(B) qPCR analysis of AMPK subunits (α , β , γ) and β -actin (loading control) in H1299 cells with GSDMC knockdown (sh-GSDMC-1/2) or overexpression (Flag-GSDMC).

(C) WB analysis of LKB1 and β -actin (loading control) in HeLa, H460, A549, H1299 and Calu-1 cells.

(D) WB of indicated proteins in HeLa and H460 cells expressing Flag or Flag-GSDMC.

(E) WB analysis of indicated proteins in A549 cells expressing sh-ctrl, sh-CAMKK2 or sh-TAK1 following transfection with Flag or Flag-GSDMC.

(F and G) WB (F) and qPCR (G) analysis of CAMKK2 and β -actin (loading control) in H1299 and A549 cells transduced with sh-ctrl, sh-GSDMC-1 or sh-GSDMC-2.

(H) qPCR analysis of CAMKK2 and β -actin (loading control) in A549 cells expressing GFP-GSDMC or its NLS mutants (K41E, K135E, K202E or K135E/K202E).

(I) WB analysis of indicated proteins in A549 cells from (H).

Data information: (B, G and H) one-way ANOVA with Tukey's multiple comparison post-test; (B, G and H) n = 3 biological replicates. ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

Figure S6. Related to Figure 6

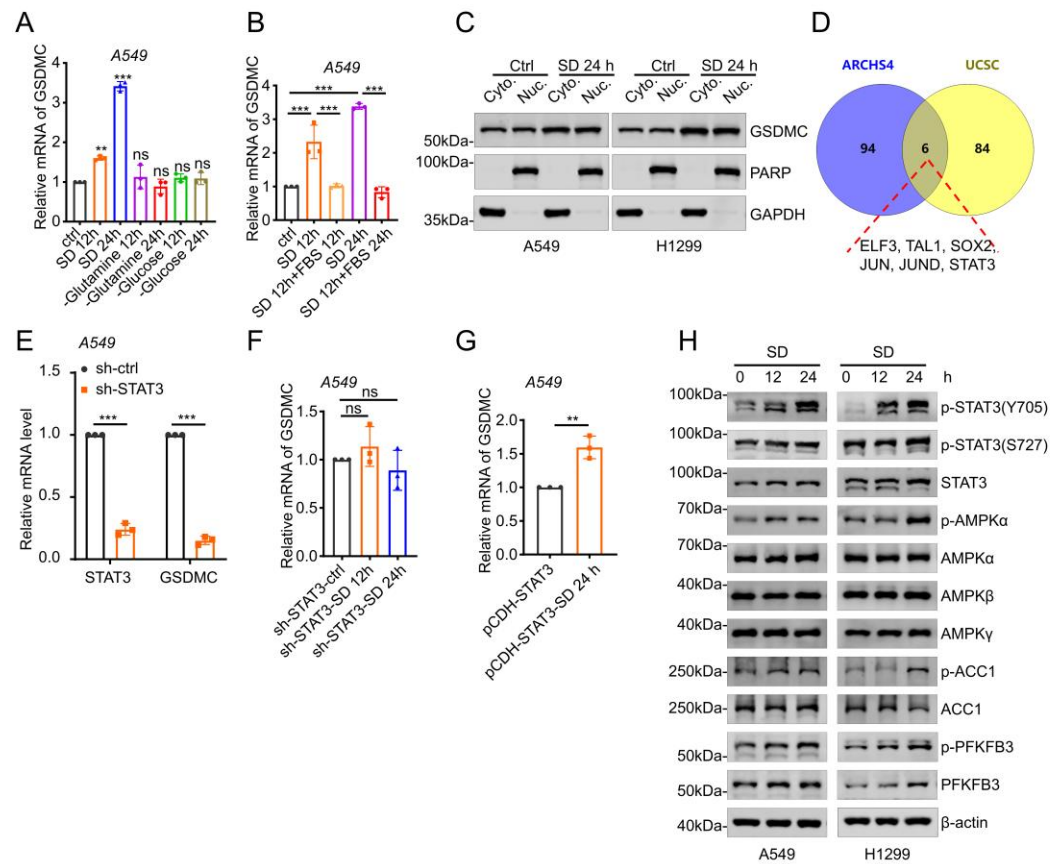


Figure S6. Related to Figure 6

(A) GSDMC abundance was determined by qPCR in A549 cells under the indicated stress conditions including SD, no glutamine or no glucose.

(B) qPCR analysis of GSDMC conducted in A549 cells as per (A) under the indicated culture conditions.

(C) WB analysis of nuclear and cytoplasmic extracts from A549 and H1299 cells after SD treatment.

(D) Venn analysis of upstream transcription factors of GSDMC on ARCH4 and UCSC websites.

(E) qPCR analysis of STAT3, GSDMC and β -actin (loading control) in A549 cells transduced with sh-ctrl or sh-STAT3.

(F) qPCR analysis of GSDMC and β -actin (loading control) in A549 cells transduced with sh-STAT3 after SD.

(G) qPCR analysis of GSDMC and β -actin (loading control) in A549 cells transduced with pCDH-STAT3 after SD.

(H) WB analysis of indicated proteins in A549 and H1299 cells cultured under serum-free conditions for 0, 12 or 24 h.

Data information: (A, B and F) one-way ANOVA with Tukey's multiple comparison post-test; (E) Two-way ANOVA with Tukey's multiple comparison post-test; (G) are mean $\pm$ SD, two-tailed paired Student's t test; (A, B, E, F and G) n = 3 biological replicates. ns, not significant, **p < 0.01, ***p < 0.001.

Figure S7. Related to Figure 7

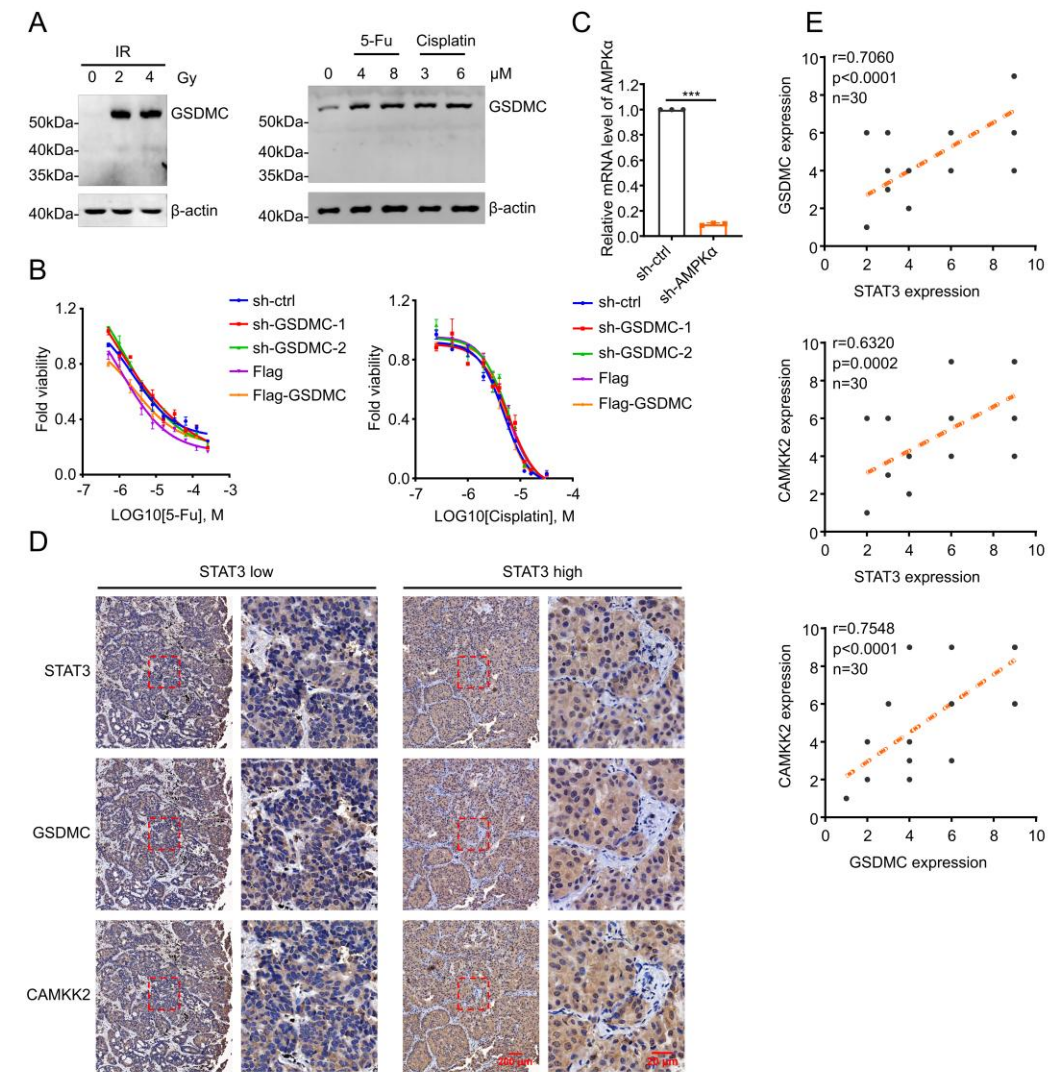


Figure S7. Related to Figure 7

(A) WB analysis of GSDMC and β -actin (loading control) in A549 cells at 72 h post X-ray irradiation or chemotherapy (5-FU/cisplatin) treatment.

(B) Sensitivity to 5-FU and cisplatin assayed in A549 cells.

(C) qPCR analysis of AMPK α and β -actin (loading control) in A549 cells transduced with sh-ctrl or sh-AMPK α .

(D) Representative IHC images showing STAT3, GSDMC and CAMKK2 expression in LUAD tumor tissues (n = 30). (Left panels: low magnification, scale bars: 200 μ m; right panels: high magnification, scale bars: 20 μ m.)

(E) Correlation analyses between GSDMC and the expression of the indicated proteins from (D).

Data information: (C) are mean $\pm$ SD, two-tailed paired Student's t test; (E) R, Pearson correlation coefficients (r) and p values. (C) n = 3 biological replicates. ***p < 0.001.