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RESEARCH

Sirtuin 3-mediated delactylation of malic enzyme 2 disrupts redox balance and inhibits colorectal cancer growth

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Abstract

Purpose Post-translational modifications, such as lactylation, are emerging as critical regulators of metabolic enzymes in cancer progression. Mitochondrial malic enzyme 2 (ME2), a key enzyme in the TCA cycle, plays a pivotal role in maintaining redox homeostasis and supporting tumor metabolism. However, the functional significance of ME2 lactylation and its regulatory mechanisms remain unclear. This study investigates the role of ME2 K352 lactylation in modulating enzymatic activity, redox balance, and tumor progression.

Methods Immunoprecipitation and western blotting were used to assess ME2 lactylation and its interaction with Sirtuin 3 (SIRT3). Mass spectrometry identified the lactylation site on ME2. Enzymatic activity was measured using NADH production assays. The functional effects of ME2 K352 lactylation were analyzed by measuring ROS levels, $NADP^+$/NADPH ratios, metabolic intermediates, and mitochondrial respiration parameters. Cell proliferation was evaluated via CCK-8 and colony formation assays. Xenograft tumor models and Ki-67 immunohistochemical staining were used to assess tumor growth and proliferation in vivo.

Results Mass spectrometry identified K352 as the primary lactylation site on ME2. Sodium lactate treatment enhanced ME2 lactylation and enzymatic activity, while SIRT3-mediated delactylation at K352 reduced ME2 activity, disrupting redox homeostasis. Cells expressing the K352R mutant exhibited elevated ROS levels, higher $NADP^+$/NADPH ratios, and altered levels of metabolic intermediates, including increased malate and lactate with reduced pyruvate. Additionally, re-expression of ME2 K352R in HCT116 cells significantly impaired proliferation and colony formation. In vivo, xenograft models demonstrated that ME2 K352R expression suppressed tumor growth, as evidenced by reduced tumor volume, weight, and Ki-67 staining.

Conclusions This study reveals that ME2 K352 lactylation is a critical regulatory mechanism that modulates enzymatic activity, mitochondrial function, and tumor progression. SIRT3-mediated delactylation of ME2 K352 disrupts redox homeostasis and inhibits tumor growth. These findings highlight the potential of targeting ME2 lactylation as a therapeutic strategy in cancer treatment.

Keywords Lactylation · Cancer metabolism · ME2 · SIRT3 · Mitochondrion · Posttranslational modification

1 Introduction

Cancer cells exhibit metabolic reprogramming, a cancer hallmark phenomenon known as the Warburg effect, characterized by a preference for aerobic glycolysis over oxidative phosphorylation even in the presence of oxygen [1]. This metabolic reprogramming supports rapid cell proliferation and survival in the tumor microenvironment [2, 3]. Understanding the molecular mechanisms driving tumor metabolism changes is essential for developing effective therapeutic strategies against cancer [4–6].

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Malic enzymes (MEs) catalyze the oxidative decarboxylation of malate to pyruvate, generating either NADPH or NADH in the process [7, 8]. In mammals, three isoforms of MEs have been identified: ME1, a cytosolic $NADP^+$-dependent enzyme; ME2, a mitochondrial isoform capable of utilizing both NAD^+ and $NADP^+$; and ME3, a mitochondrial $NADP^+$-specific enzyme. Among these, ME1 and ME2 are the predominant forms [9]. ME2 plays a central role in regulating TCA cycle flux, aligning metabolic activity with cellular demands for energy, biosynthetic precursors, and redox balance [8, 10]. It is particularly important in glutamine and lipid metabolism, which are critical for meeting the heightened metabolic needs of cancer cells [10]. Dysregulated ME2 expression has been implicated in various cancers, where it promotes tumor progression through multiple mechanisms. For example, 2-hydroxyglutarate (2-HG) produced by ME2 stabilizes mutant p53 and drives colorectal cancer (CRC) growth [11]. In addition, a recent study revealed that ME2 regulates $CD8^+$ T cell activation and antitumor immunity by modulating TCA cycle flux [12]. Despite its importance in cancer metabolism, the mechanisms controlling ME2 activity remain poorly undefined [13].

Post-translational modifications (PTMs) are critical regulators of protein function, influencing their activity, stability, and subcellular localization [14]. Recent studies have demonstrated that ME2 activity is intricately regulated by various PTMs. For example, we previously showed that succinylation can inhibit ME2 activity, subsequently suppressing mitochondrial respiration and cell proliferation in CRC [13]. In addition, PRMT1-mediated methylation at the R67 site activates ME2, enhancing redox homeostasis and driving the progression of hepatocellular carcinoma (HCC) [15]. However, the full spectrum of regulatory mechanisms controlling ME2 activity remains to be fully elucidated.

In this study, we uncover lactylation as a novel PTM of ME2, specifically at the K352 residue. This modification markedly enhances ME2 enzymatic activity, linking it to increased NADPH production and mitochondrial respiration. We also identify Sirtuin 3 (SIRT3), a mitochondrial deacetylase, as a delactylase for ME2. By removing lactylation, SIRT3 inhibits ME2 activity, disrupting redox balance and suppressing CRC cell growth. Our findings reveal a previously uncharacterized mechanism regulating ME2 activity, highlighting the dynamic interplay between lactylation and SIRT3-mediated delactylation. These insights not only enhance our understanding of tumor metabolism but also suggest that targeting ME2 lactylation could represent a promising therapeutic strategy for cancer.

2 Materials and methods

2.1 Cell lines and culture conditions

Human embryonic kidney 293T (HEK293T) and HCT116 cells were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle Medium (DMEM) (Eallbio), supplemented with 10% fetal bovine serum (VivaCell) and penicillin/streptomycin (Beyotime). Cells were cultured in a humidified incubator at 37 °C with 5% CO_2. Cell line authenticity was verified via short tandem repeat (STR) profiling, and all cultures were confirmed to be free of mycoplasma contamination.

2.2 Reagents and antibodies

Anti-Flag M2 magnetic beads (A2220, Sigma), nicotinamide (NAM, 72345, Sigma), and 3×Flag peptide (Beyotime). Antibodies used included β-actin (81115-1-RR, Proteintech), HA (51064-2-AP, Proteintech), ME2 (24944-1-AP, Proteintech), SIRT3 (15122-1-AP, Proteintech), Flag (M185-3, MBL), Pan-lactylation (PTM-1401, PTM), and Tom20 (sc-17764, Santa Cruz).

2.3 Plasmid construction

The cDNA encoding full-length human ME2 was cloned into Flag-tagged vectors (pRK7-Flag). Site-directed mutagenesis to generate point mutations in the ME2 constructs was performed using the Site-Directed Mutagenesis kit (C215-01, Vazyme).

2.4 Co-immunoprecipitation (Co-IP) and Western blotting

Cells were lysed in NP40 lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1-0.5% NP40, 1 mM PMSF, and protease inhibitors). For Co-IP, cell lysates were incubated with anti-Flag M2 magnetic beads at 4 °C for 4 h. The beads were washed three times with ice-cold NP40 buffer, and bound proteins were eluted by heating in 1× loading buffer at 100 °C for 10 min. The protein samples were resolved by SDS-PAGE, transferred to PVDF membranes, and blocked with 5% skim milk. Membranes were incubated with primary antibodies at 4 °C overnight. Protein bands were visualized using an ECL Kit (E412-02, Vazyme).

2.5 Immunofluorescence (IF) analysis

Cells were fixed, permeabilized, and incubated with primary antibodies (1:100 dilution) at 4 °C overnight, followed by fluorescence-conjugated secondary antibodies and

counterstained with DAPI to visualize nuclei. Images were captured using a confocal microscope.

2.6 Measurement of intracellular $NADP^+$/NADPH ratios

To evaluate intracellular $NADP^+$/NADPH ratios, cells were seeded into 6-well plates and incubated for 24 h prior to harvesting. The $NADP^+$/NADPH ratios were determined spectrophotometrically using the $NADP^+$/NADPH Assay Kit (S0179, Beyotime) following the protocols provided by the manufacturer.

2.7 Measurement of intracellular metabolite and ATP levels

Intracellular levels of pyruvate, malate, lactate and ATP were measured spectrophotometrically as we previously described [13]. Briefly, cells were plated in 96-well plates and incubated for 24 h before analysis. Specific assay kits were used for each metabolite: Pyruvate Content Assay Kit (BC2205, Solarbio), Malic Acid Content Assay Kit (BC5495, Solarbio), L-Lactic Acid Colorimetric Assay Kit (E-BC-K044-M, Eton), and ATP Content Assay Kit (BC0305, Solarbio). All measurements were conducted according to the manufacturer's instructions.

2.8 Cell proliferation

Cell proliferation assay was performed as we previously described [13].

2.9 Generation of ME2 reconstituted cell lines

To generate stable ME2 mutant cell lines, endogenous ME2 was depleted using a short hairpin RNA (shRNA) targeting sequence inserted into the lentiviral vector. For reconstitution, shRNA-resistant Flag-tagged wild-type (WT) ME2 and its K352R mutants were cloned into another lentiviral vector. Recombinant lentiviruses were packaged by Shanghai GenePharma Co., Ltd. (Shanghai, China) and used to infect HCT116 cells in the presence of 10 μg/mL polybrene to enhance viral transduction efficiency. Cells stably expressing shME2 were selected with 250 μg/mL hygromycin for 2 weeks. To establish reconstituted cell lines, shRNA-resistant Flag-tagged WT ME2 or K352R mutants were introduced into shME2-expressing cells, followed by 2 μg/mL puromycin selection for another 2 weeks. The efficiency of ME2 knockdown and re-expression was validated by western blot.

2.10 Tumor formation assays in nude mice

HCT116 cells stably expressing ME2 (WT and K352R) were subcutaneously injected into the same flank of athymic male BALB/c nude mice obtained from CavensBiogle (Changzhou, China) at 5 weeks of age ($n = 5$ for each group). The mice were sacrificed 3 weeks after injection, and tumor growth was measured.

2.11 Measurement of ME2 enzyme activity

Purified ME2 or mutant protein samples were added to reaction mixtures containing 50 mM MES (pH 6.5), 10 mM $MgCl_2$, 24 mM L-malate, and 1 mM NAD^+. ME2 activity was measured by monitoring the increase in absorbance at 340 nm, indicative of NADH production. Absorbance was measured using an EPOCH2 microplate reader (BioTek).

2.12 Statistical analyses

Data are presented as mean ± standard deviation (SD). Statistical comparisons were performed using a two-tailed Student's *t*-test or one-way analysis of variance (ANOVA) using GraphPad Prism 8.

3 Results

3.1 ME2 can be lactylated

Lactate is a critical substrate for lactylation and plays a pivotal role in promoting this PTM. To investigate whether ME2 can be lactylated, we first evaluated the effect of lactate on the ME2 lactylation. Using HEK293T cells expressing Flag-tagged ME2, we observed a significant increase in lactylation levels following treatment with sodium lactate, as confirmed by IP and immunoblotting assays (Fig. 1A). Also, endogenous IP results confirmed the presence of ME2 lactylation under physiological conditions (Fig. 1B). Furthermore, the lactylation of ME2 exhibited a dose-dependent response, with increasing sodium lactate concentrations correlating with higher lactylation levels (Fig. 1C). To investigate how lactate levels regulate ME2 lactylation, we employed pharmacological agents to modulate lactate production. Rotenone, an inhibitor of mitochondrial oxidative phosphorylation, was used to increase lactate production, while oxamate (OXA), an inhibitor of lactate dehydrogenase (LDH), was utilized to reduce lactate levels [16]. These agents were applied to enhance and suppress protein lactylation, respectively. Consistent with our hypothesis, treatment with oxamate significantly decreased ME2 lactylation, whereas rotenone enhanced lactylation levels of ME2

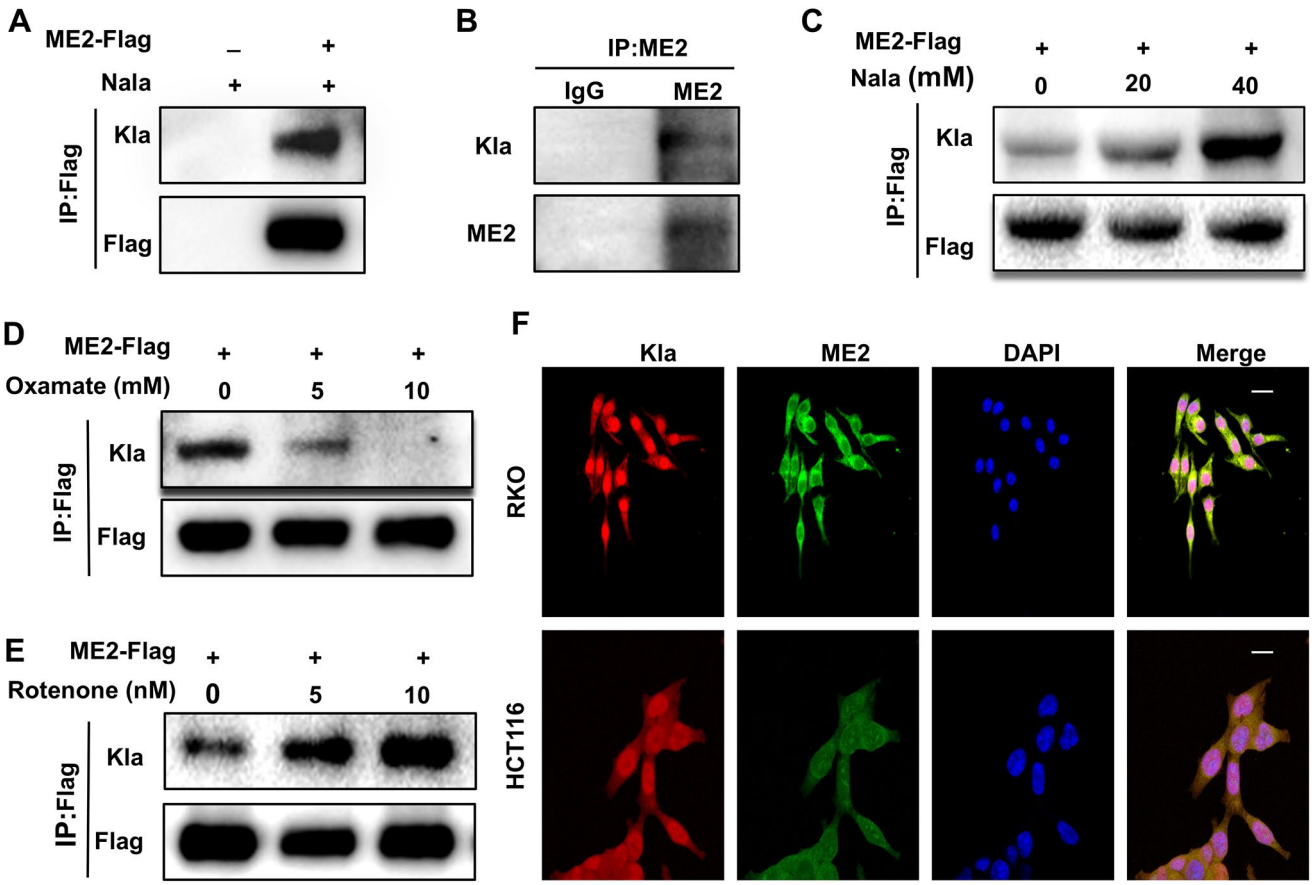


Fig. 1 ME2 can be lactylated. (**A**) IP and western blot analyses showing lactylation (Kla) of ME2 in HEK293T cells transfected with Flag-tagged ME2. Cells were treated with or without sodium lactate (Nala, 20 mM). (**B**) Endogenous lactylation of ME2 was analyzed via IP and western blot in HCT116 cells. (**C**) Dose-dependent increase in ME2 lactylation in HEK293T cells treated with escalating concentrations of sodium lactate. (**D**) Reduction of ME2 lactylation following treatment with oxamate. (**E**) Enhanced ME2 lactylation in HEK293T cells treated with increasing concentrations of Rotenone. (**F**) Immunofluorescence analyses of CRC cells(RKO and HCT116) showing colocalization of ME2 (green) and lactylation (red). Nuclear staining was performed with DAPI (blue), and merged images highlight their colocalization. (scale bar, 10 μm)

(Fig. 1D-E). In addition, IF analysis further confirmed the colocalization of lactylation and ME2 in CRC cells, further supporting the existence of lactylated ME2 in cancer cells (Fig. 1F). Collectively, these findings demonstrate that ME2 can be lactylated.

3.2 ME2 is lactylated at K352

To identify potential lactylation sites on ME2, we purified lactylated ME2 protein from HEK293T cells and performed mass spectrometry analyses. The results revealed a potential lactylation site at lysine 352 (K352) on ME2 (Fig. 2A). To further confirm the specificity of lactylation at K352, we generated site-directed mutants by substituting lysine with either arginine (K352R, mimicking the delactylated state) or glutamine (K352Q, mimicking negatively charged lactylated lysine). Flag-tagged wild-type (WT) ME2 and mutant constructs were expressed in HEK293T cells. The results showed that the lactylation levels of both K352R and K352Q mutants were significantly reduced compared to WT ME2, indicating that K352 is a critical lactylation site on ME2 (Fig. 2B). Furthermore, sequence alignment analyses revealed that K352 is a highly conserved residue across various species, underscoring its potential functional importance (Fig. 2C). To further confirm K352 as the main lactylated site of ME2, we transfected HEK293T cells with Flag-tagged WT ME2 or K352R mutant, followed by treatment with sodium lactate. The results demonstrated that sodium lactate treatment significantly enhanced the lactylation level of WT ME2, whereas no obvious changes were observed in the lactylation levels of K352R mutants (Fig. 2D). These findings confirm that K352 is the primary lactylation site on ME2.

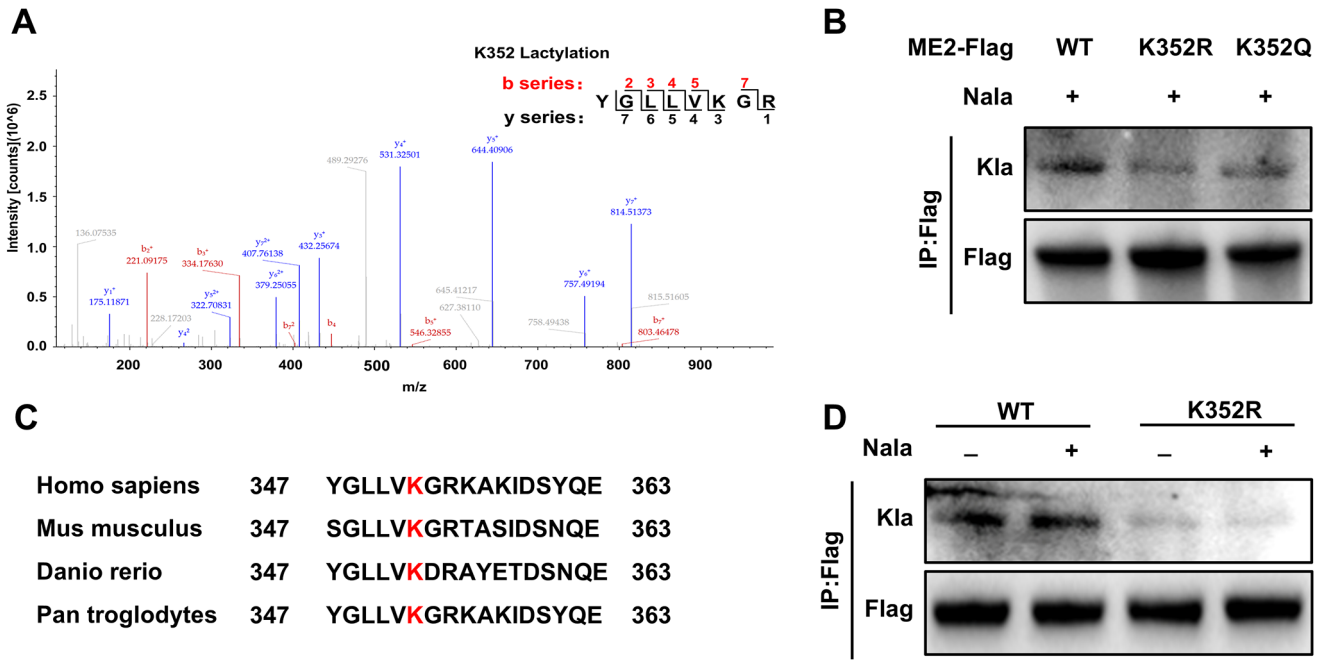


Fig. 2 ME2 is lactylated at K352. (**A**) Mass spectrometry analysis identifies K352 as a potential lactylation site on ME2. The peptide sequence containing the modified lysine is highlighted, with the lactylation modification indicated by a red asterisk. (**B**) Immunoprecipitation and western blot analyses of WT ME2 and mutants (K352R and K352Q) expressed in HEK293T cells, treated with Nala. Lactylation levels of the mutants are significantly reduced compared to WT ME2. (**C**) Sequence alignment of ME2 among different species, showing that K352 (highlighted in red) is a highly conserved residue. (**D**) Western blot shows that Nala enhances lactylation in WT ME2 but not in the K352R mutant

3.3 SIRT3 interacts with and delactylates ME2

The currently identified delactylation enzymes mainly include Class I histone deacetylases 1–3 (HDAC1–3) in the nucleus and Sirtuin 1–3 (SIRT1–3) in the cytoplasm [17]. Considering that ME2 is predominantly localized in the mitochondria, we first examined whether NAM (nicotinamide), a pan-inhibitor of Sirtuin family proteins, affects the lactylation of ME2. The results showed that NAM treatment significantly increased ME2 lactylation, suggesting that ME2 lactylation is regulated by members of the Sirtuin family (Fig. 3A). To identify the specific Sirtuin responsible for this regulation, we transfected HEK293T cells with expression plasmids of SIRT1–7 and investigated their effects on ME2 lactylation. Among them, only SIRT3 significantly reduced the lactylation level of ME2 (Fig. 3B). Using endogenous co-IP, we further confirmed a direct interaction between ME2 and SIRT3 in HCT116 cells (Fig. 3C). IF analysis also demonstrated clear colocalization of ME2 and SIRT3 in the mitochondria (Fig. 3D). In addition, SIRT3 overexpression significantly decreased ME2 lactylation, whereas its enzymatically inactive mutant (H248Y) had no effect (Fig. 3E). SIRT3-mediated delactylation was observed in WT ME2 but not in the K352R mutant, underscoring the importance of K352 in this regulation process (Fig. 3F–H). To further validate the critical role of the K352 site in SIRT3-mediated delactylation, we co-transfected HA-tagged SIRT3 with either WT ME2-Flag or its K352R mutant into HEK293T cells. The results showed that SIRT3 overexpression significantly reduced the lactylation level of WT ME2, but had no significant effect on the K352R mutant (Fig. 3F). Similarly, knockdown of SIRT3 using small interfering RNA (siRNA) increased the lactylation level of WT ME2, whereas no significant change was observed in the lactylation level of the K352R mutant, underscoring the importance of K352 in SIRT3-regulated delactylation of ME2 (Fig. 3G-H). These results demonstrate that SIRT3 interacts with ME2 and catalyzes its delactylation at K352.

3.4 Delactylation at K352 by SIRT3 inhibits ME2 activity

Given that ME2 is a metabolic enzyme with catalytic activity, we investigated whether ME2 lactylation regulates its enzymatic activity. Sodium lactate treatment significantly increased ME2 activity in a dose-dependent manner (Fig. 4A). However, the K352R mutant exhibited markedly reduced enzymatic activity compared to WT ME2, while the K352Q mutant showed no significant difference (Fig. 4B). SIRT3 knockdown enhanced the enzymatic activity of WT ME2 but had no effect on the K352R mutant (Fig. 4C-D). Conversely, overexpression of SIRT3 reduced ME2 activity,

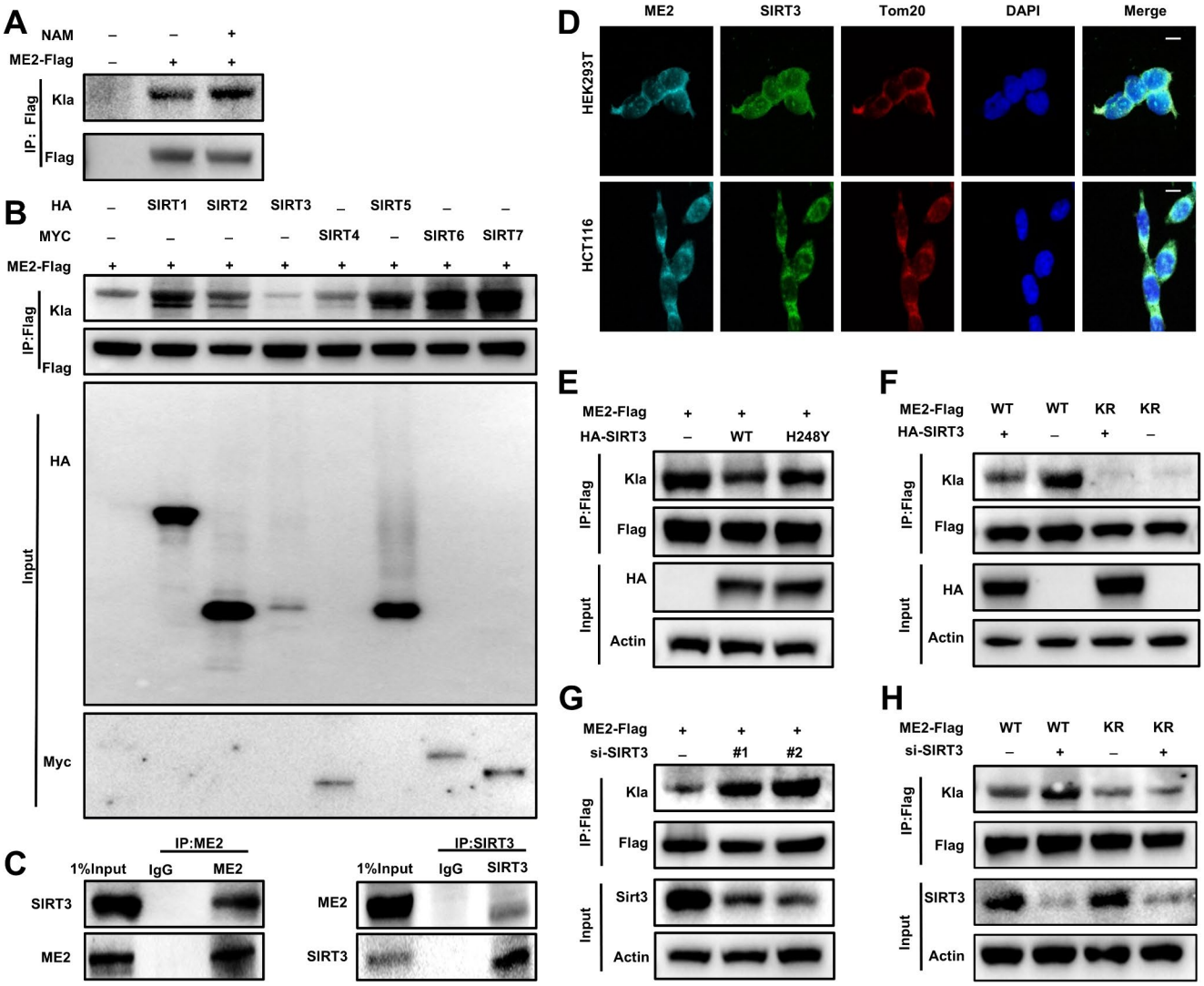


Fig. 3 SIRT3 interacts with and delactylates ME2. (**A**) ME2 lactylation levels significantly increase upon treatment with nicotinamide (NAM), a pan-inhibitor of the Sirtuins family. (**B**) Among Sirtuins family members (SIRT1–7), only SIRT3 significantly reduces ME2 lactylation levels in HEK293T cells co-transfected with ME2-Flag and SIRT1-7, as shown by immunoprecipitation and western blot analyses. (**C**) Co-IP confirms the endogenous interaction between ME2 and SIRT3 in HCT116 cells. (**D**) Immunofluorescence analyses shows colocalization of ME2 (cyan) and SIRT3 (red) in mitochondria (Tom20, red) in both HEK293T and HCT116 cells, with nuclei counterstained by DAPI (blue).(scale bar, 10 μm). (**E**) WT SIRT3, but not its enzymatically inactive mutant (H248Y), removes lactylation from ME2. (**F**) SIRT3 overexpression reduces lactylation in WT ME2 but has no effect on the K352R mutant. (**G, H**) SIRT3 knockdown by siRNAs enhances lactylation levels of WT ME2, while no significant change is observed in the K352R mutant

while SIRT3 H248Y mutant had no such effect (Fig. 4E). Importantly, SIRT3 did not alter the activity of the K352R mutant, further confirming the critical role of K352 in regulating ME2 activity through delactylation (Fig. 4F). These findings highlight that SIRT3-mediated delactylation at K352 plays a key role in modulating ME2 enzymatic activity.

3.5 ME2 K352 lactylation modulates mitochondrial respiration and redox homeostasis

To investigate the metabolic impact of ME2 K352 lactylation, we depleted endogenous ME2 in HCT116 cells and reconstituted them with shRNA-resistant Flag-tagged WT ME2 or ME2 K352R (Fig. 5A). Cells expressing the K352R mutant exhibited significantly elevated reactive oxygen species (ROS) levels compared to those expressing WT ME2 (Fig. 5B). The K352R mutant also showed an increased $NADP^+$/NADPH ratio, indicating disrupted redox balance (Fig. 5C). Furthermore, due to the reduced catalytic activity

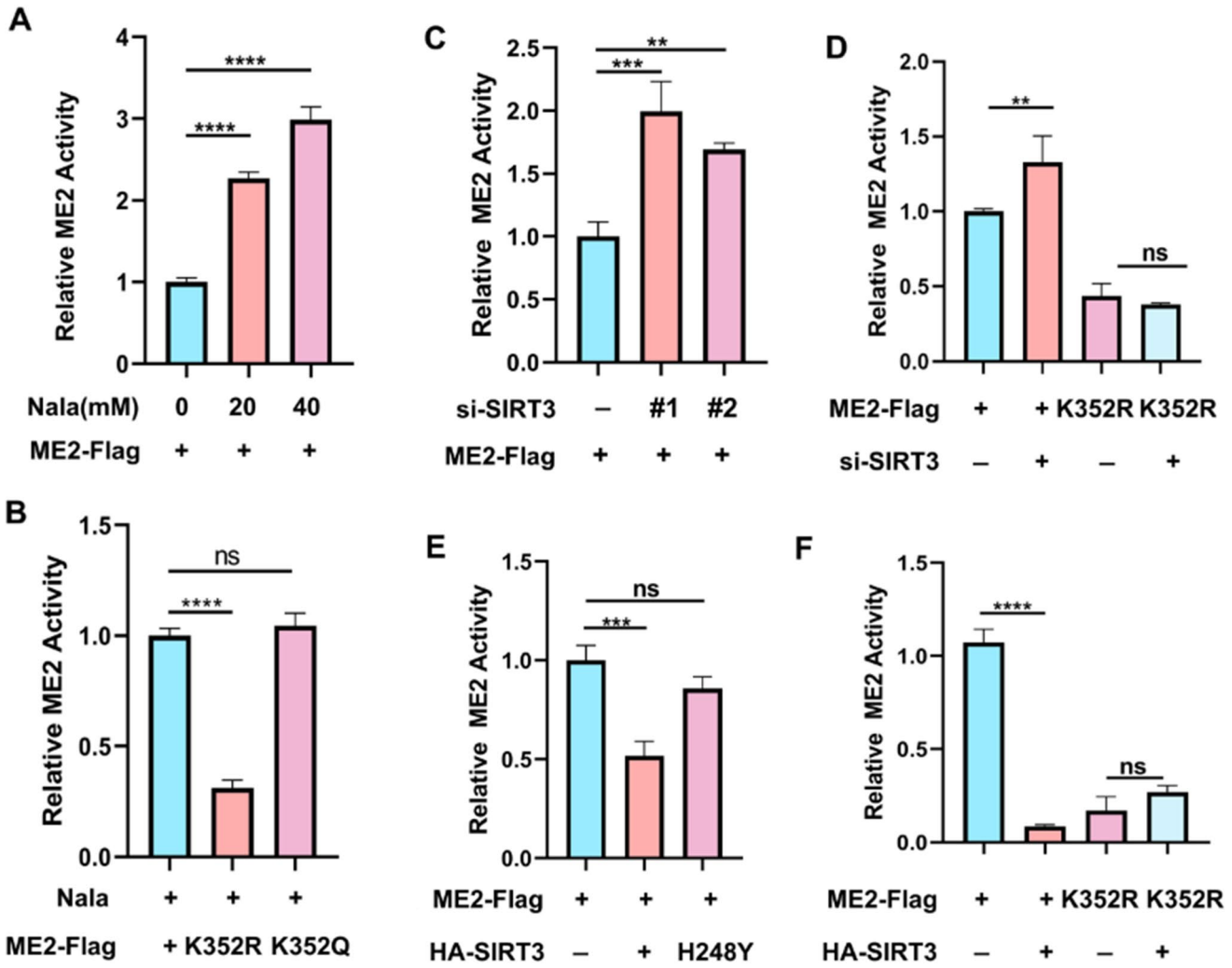
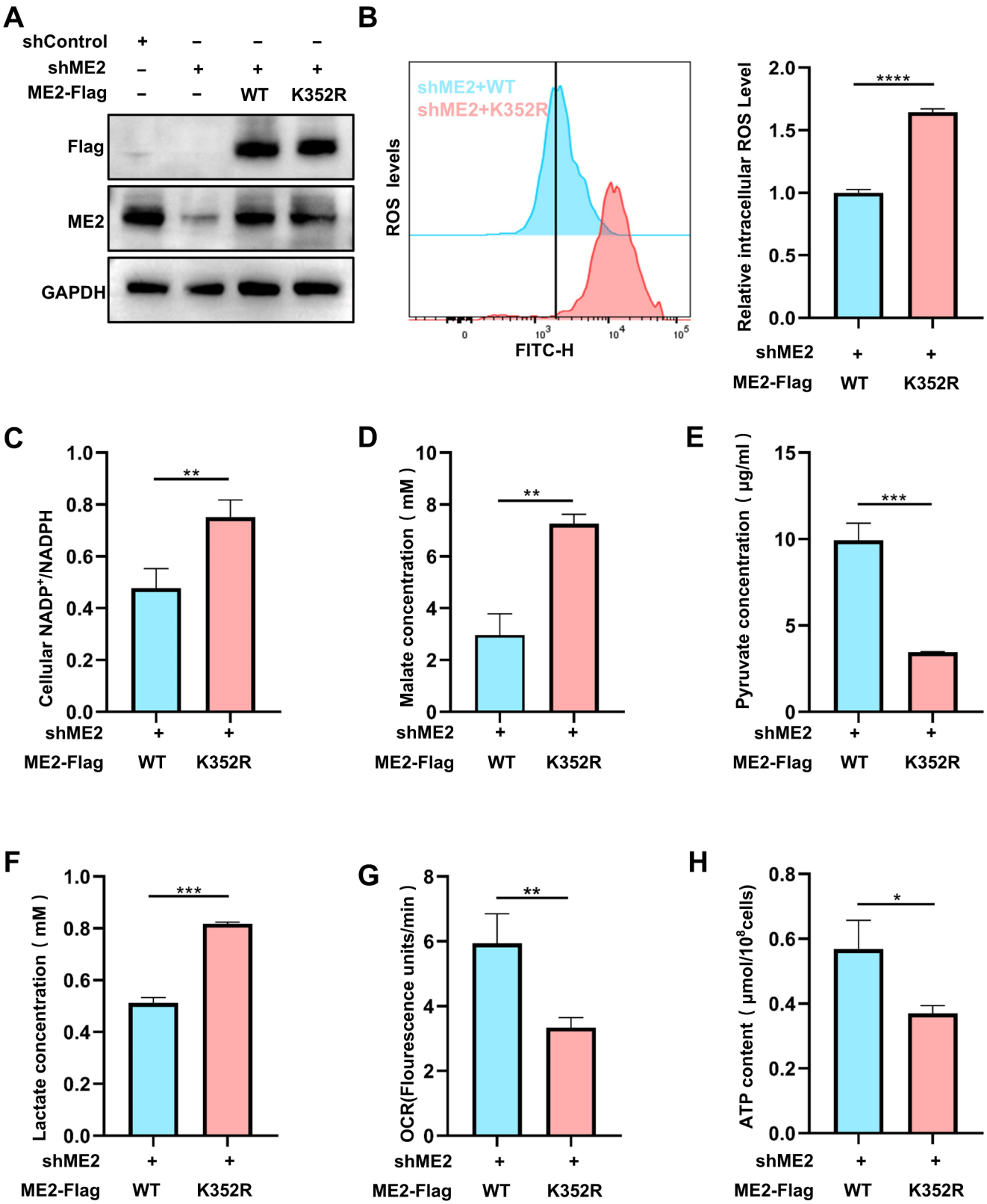


Fig. 4 Delactylation at K352 by SIRT3 inhibits ME2 activity. (**A**) ME2 enzymatic activity increases progressively with higher concentrations of sodium lactate in HEK293T cells transfected with ME2-Flag. (**B**) Enzymatic activity of ME2-Flag, K352R (delactylation mimic), and K352Q (lactylation mimic) mutants expressed in HEK293T cells. The K352R mutant shows significantly reduced activity compared to wild-type ME2-Flag, while the K352Q mutant exhibits no significant difference. (**C**) SIRT3 knockdown significantly enhances the enzymatic activity of wild-type ME2-Flag (**C**) but does not affect the activity of the K352R mutant (**D**). (**E**) SIRT3 overexpression reduces ME2 enzymatic activity, whereas its enzymatically inactive mutant (H248Y) has no significant effect in HEK293T cells. (**F**) Overexpression of SIRT3 reduces the enzymatic activity of wild-type ME2 but does not affect the activity of the K352R mutant in HEK293T cells. Data are mean ± s.d. of $n = 3$ independent replicates. P values were calculated using Dunnett's test after One-way ANOVA. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; n.s., not significant

of the K352R mutant, cells reconstituted with this mutant displayed higher malate levels and correspondingly lower pyruvate levels (Fig. 5D-E). Given the central role of ME2 in mitochondrial metabolism, we further investigated the impact of ME2 K352 delactylation on the balance between glycolysis and mitochondrial respiration by measuring lactate production, oxygen consumption rate (OCR), and ATP levels. Compared to WT ME2, re-expression of the K352R mutant resulted in increased lactate levels, alongside significantly reduced OCR and ATP production (Fig. 5F–H). Collectively, these findings highlight the critical role of ME2 K352 lactylation in maintaining mitochondrial respiration and cellular redox homeostasis.

3.6 ME2 K352 delactylation suppresses tomor growth

We next evaluated the role of ME2 K352 lactylation in tumor progression. In vitro, HCT116 cells expressing the K352R mutant exhibited significantly reduced proliferation and colony formation activities compared to WT ME2 (Fig. 5A-B). In vivo, xenograft tumor models showed that re-expression of ME2 K352R significantly suppressed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shME2 + +
ME2-Flag WT K352R
C
Cellular NADP+/NADPH
D
Malate concentration (mM)
E
Pyruvate concentration (μg/ml)
F
Lactate concentration (mM)
G
OCR(Flourescence units/min)
H
ATP content (μmol/10^8cells)

Fig. 5 ME2 K352 lactylation modulates mitochondrial respiration and redox homeostasis. (**A**) Western blot analyses of HCT116 cells depleted of endogenous ME2 (shME2) and reconstituted with shRNA-resistant Flag-tagged wild-type(WT) ME2 or the K352R mutant. (**B**) Measurement of ROS levels by flow cytometry in reconstituted HCT116 cells. (**C**) $NADP^+$/NADPH ratios in reconstituted HCT116 cells. The K352R mutant shows a higher $NADP^+$/NADPH ratio compared to WT ME2. (**D**) Malate levels are significantly increased in cells expressing the K352R mutant compared to WT ME2. (**E**, **F**) Compared to the WT ME2,the cells expressing K352R mutant exhibit reduced pyruvate levels but elevated lactate levels. (**G**) Oxygen consumption rate (OCR) is significantly lower in cells expressing ME2 K352R compared to WT ME2. (**H**) Cellular ATP levels are reduced in cells expressing the K352R mutant compared to WT ME2. Data are mean ± s.d. of n = 3 independent replicates. P values were calculated using Student'st-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$

tumor growth, as evidenced by reduced tumor volume and weight compared to WT ME2 (Fig. 5C–E). IHC staining for Ki-67, a marker of cell proliferation, further confirmed decreased proliferative activity in tumors expressing the K352R mutant compared to the WT ME2 group (Fig. 5F). Collectively, these findings underscore the tumor-suppressive role of ME2 K352 delactylation and highlight its critical function in regulating tumor metabolism.

4 Discussion

ME2 is a key regulator of cellular metabolism, catalyzing the conversion of malate to pyruvate while contributing to NADPH production and redox homeostasis. Its role in linking the tricarboxylic acid (TCA) cycle to metabolic processes such as glutamine metabolism and lipid synthesis underscores its importance in maintaining cellular metabolic flexibility, particularly under stress conditions [8, 10]. Beyond its functions in cancer cells, ME2 also plays a crucial role in immune regulation, particularly in $CD8^+$ T cell activation and anti-tumor immune defense. Loss of ME2 impairs $CD8^+$ T cell activation and weakens the anti-tumor immune response, highlighting its importance in immune metabolism [12]. We and others have highlighted the involvement of ME2 in tumorigenesis, where its dysregulation contributes to enhanced cancer cell survival and proliferation [11–13, 15, 18, 19]. However, the regulatory mechanisms governing ME2 activity remain incompletely understood.

The identification of lactylation as a PTM has expanded our understanding of protein regulation in various physiological and pathological contexts, including cancer metabolism [20]. Initially discovered using peptide immunoprecipitation combined with high-sensitivity HPLC-MS/MS, lactylation has been linked to epigenetic modulation and gene transcription regulation, particularly in cancer cells and macrophages [21, 22]. The Warburg effect, characterized by increased lactate production in cancer cells, provides the substrate for lactylation, establishing a direct link between metabolism and epigenetic regulation. In this study, we revealed for the first time ME2 is lactylated at K352, which enhances ME2 enzymatic activity, leading to increased NADPH production and mitochondrial respiration in CRC cells. Furthermore, we identified that SIRT3 interacts with and delactylates ME2 at K352, thereby inhibiting ME2 activity, disrupting redox balance and suppressing CRC growth.

Previous studies on lactylation have predominantly focused on histone lactylation and its impact on gene expression. For instance, Yu et al. uncovered a novel mechanism in ocular melanoma, demonstrating that histone lactylation upregulates YTHDF2 expression, which promotes m6A-mediated degradation of tumor suppressor genes PER1 and TP53, thereby driving tumor progression [23]. In addition, H3K18 lactylation (H3K18la) and H3K9 lactylation (H3K9la) have been shown to affect the effector functions of $CD8^+$ T cells through metabolic and epigenetic pathways [24]. In 2023, proteomic analyses of HCC revealed that lactylation also occurs extensively on non-histone proteins [25]. However, research investigating the lactylation of non-histone proteins, particularly metabolic enzymes, remains limited [26]. Our study builds upon this foundation by demonstrating that lactylation also regulates mitochondrial proteins, specifically ME2. The lactylation of ME2 at K352 enhances its enzymatic function, linking this PTM to increased NADPH production and redox balance—processes critical for sustaining cancer cell proliferation and survival. These findings provide a novel perspective on how lactylation influences cellular metabolism and tumor progression. In addition, we previously reported that ME2 activity is increased in CRC cells, and succinylation could regulate ME2 activity and inhibit its activity, thereby suppressing cancer cell survival and proliferation [13]. These data highlight the pivotal regulatory role of PTMs on the activity of metabolic enzymes such as ME2.

Studies on non-histone protein lactylation have demonstrated that this modification can either enhance or inhibit the functions of these proteins. For example, Ju et al. showed that AARS1 detects lactate and lactylates YAP, thereby maintaining its nuclear localization and promoting YAP activation [27]. In contrast, Yang et al. found that lactylation at K28 of adenylate kinase 2 suppresses its activity, thereby promoting the proliferation and metastasis of HCC cells [28]. These findings highlight the diverse roles of lactylation in regulating protein functions and underscore the complexity of its impact on cellular processes. In our study, the enhancement of ME2 activity by lactylation suggests that this modification plays a critical role in metabolic adaptation in tumor cells. Increased NADPH production

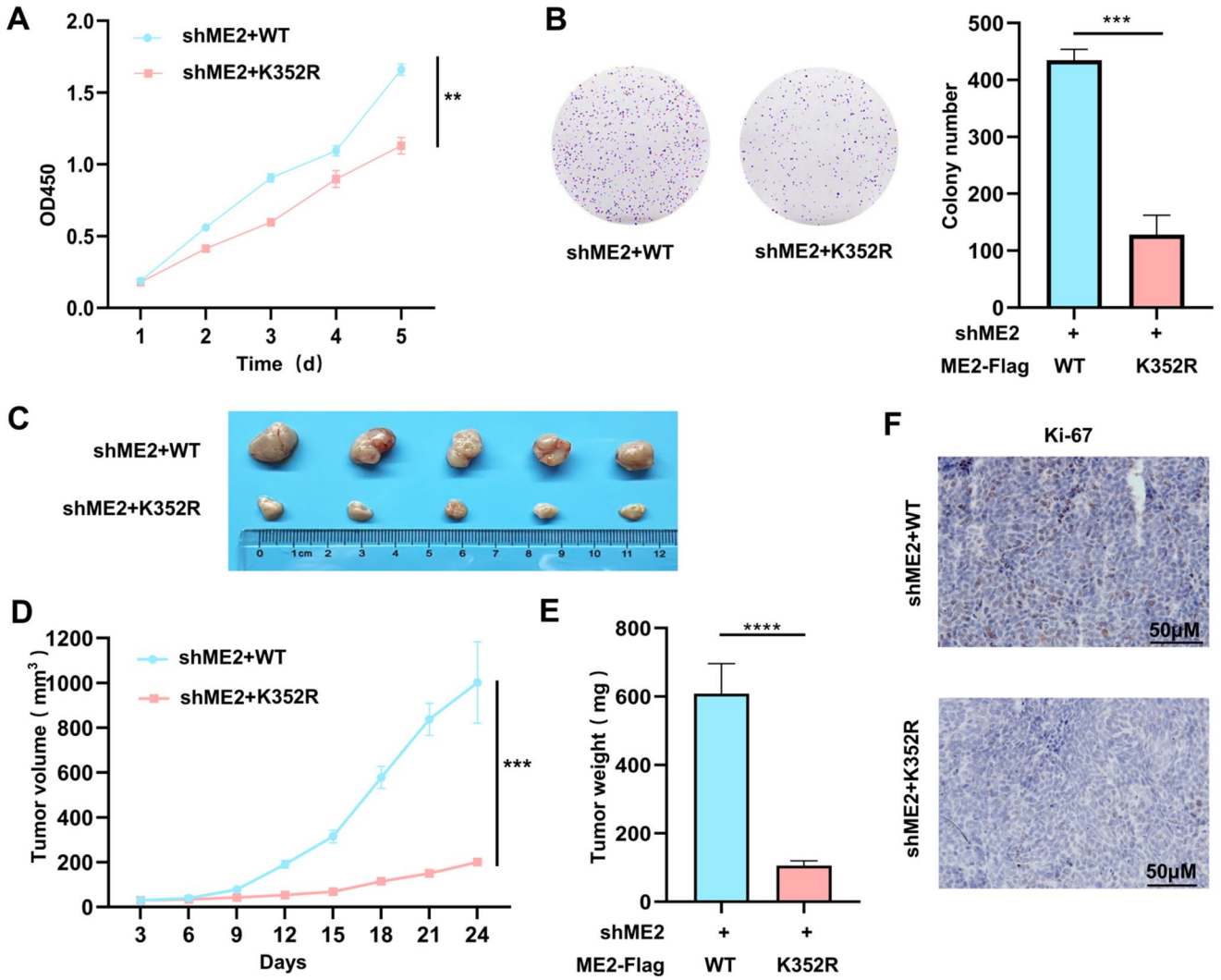

Fig. 6 ME2 K352 delactylation suppresses tumor growth. (**A**) CCK-8 assays showing reduced proliferation of HCT116 cells expressing the ME2 K352R mutant compared to wild-type(WT) ME2. (**B**) Colony formation assays demonstrating significantly fewer colonies in HCT116 cells reconstituted with ME2 K352R compared to WT ME2. Representative images (left) and quantified colony numbers (right) are shown. (**C**) Representative images of tumors from a mouse xenograft model. ME2-deficient HCT116 cells reconstituted with either WT ME2 or ME2 K352R were subcutaneously injected into mice. (**D**) Tumor growth curves showing significantly reduced tumor volume in the K352R group compared to the WT ME2 group. (**E**) Tumor weights from the K352R group are significantly lower than those in the WT ME2 group. (**F**) IHC staining for Ki-67, a proliferation marker, shows reduced expression in tumors expressing ME2 K352R compared to WT ME2. Data are mean ± s.d. of $n = 3$ independent replicates. P values were calculated using unpaired two-tailed t-tests. ***, $P < 0.001$; ****, $P < 0.0001$

and mitochondrial respiration, mediated by lactylated ME2, likely support cancer cell proliferation by maintaining redox balance and fueling biosynthetic pathways. This aligns with previous studies implicating ME2 in tumorigenesis, where its upregulation promotes metabolic flexibility and survival in the tumor microenvironment [11, 12, 18, 19, 29, 30]. However, the identification of lactylation as a regulatory mechanism expands the scope of ME2's functional modulation and offers novel insights into its role in cancer progression.

SIRT3's role as a delactylase of ME2 adds another dimension to its established function as a mitochondrial deacetylase and metabolic regulator. By removing lactylation from ME2, SIRT3 suppresses its enzymatic activity, disrupting NADPH production and redox homeostasis. This mechanism positions SIRT3 as a potential tumor suppressor in CRC. Previous studies have highlighted the tumor-suppressive functions of SIRT3 through its regulation of mitochondrial metabolism and oxidative stress [31–34]. Our findings further corroborate its critical role in counteracting oncogenic metabolic reprogramming. Interestingly, our previous study revealed that SIRT5 interacts with and desuccinylates ME2 at K346, increasing ME2 activity in CRC cells and promoting tumor growth [13]. These findings

demonstrated complex regulation between SIRTs and metabolic enzymes like ME2.

In conclusion, our study highlights lactylation as a critical PTM regulating ME2 activity and establishes its interplay with SIRT3-mediated delactylation as a key mechanism in CRC metabolism. These findings provide new insights into the regulatory networks of tumor metabolism and suggest potential therapeutic avenues targeting ME2 lactylation.

Author contributions C.Li, C.G, P.T and Z.H designed the research. C.L and C.G performed most of the experiments. Q.W and S.Y established the cell lines. C.G and H.J established mouse tumor model. Z.H and C.L supervised the project. C.L, C.G, and Z.H wrote the manuscript. All authors reviewed the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval All animal experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Clinical Research Ethics Committees of Jiangnan University (JN.No20240830b0201030[403]).

Competing interests The authors declare no competing interests.

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