S100A4 alters metabolism and promotes invasion of lung cancer cells by up-regulating mitochondrial complex I protein NDUF52

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It is generally accepted that alterations in metabolism are critical for the metastatic process; however, the mechanisms by which these metabolic changes are controlled by the major drivers of the metastatic process remain elusive. Here, we found that S100 calcium-binding protein A4 (S100A4), a major metastasis-promoting protein, confers metabolic plasticity to drive tumor invasion and metastasis of non-small cell lung cancer cells. Investigating how S100A4 regulates metabolism, we found that S100A4 depletion decreases oxygen consumption rates, mitochondrial activity, and ATP production and also shifts cell metabolism to higher glycolytic activity. We further identified that the 49-kDa mitochondrial complex I subunit NADH dehydrogenase (ubiquinone) Fe-S protein 2 (NDUF52) is regulated in an S100A4-dependent manner and that S100A4 and NDUF52 exhibit co-occurrence at significant levels in various cancer types as determined by database-driven analysis of genomes in clinical samples using cBioPortal for Cancer Genomics. Importantly, we noted that S100A4 or NDUF52 silencing inhibits mitochondrial complex I activity, reduces cellular ATP level, decreases invasive capacity in three-dimensional growth, and dramatically decreases metastasis rates as well as tumor growth in vivo. Finally, we provide evidence that cells depleted in S100A4 or NDUF52 shift their metabolism toward glycolysis by up-regulating hexokinase expression and that suppressing S100A4 signaling sensitizes lung cancer cells to glycolysis inhibition. Our findings uncover a novel S100A4 function and highlight its importance in controlling NDUF52 expression to regulate the plasticity of mitochondrial metabolism and thereby promote the invasive and metastatic capacity in lung cancer.

S100A4 (also known as metastasin-1, or fibroblast-specific protein-1 (FSP1)) is an established tumor metastasis-promoting protein and an epithelial-to-mesenchymal transition (EMT) marker. Accumulating evidence demonstrates that S100A4 is associated with the progression of a variety of cancers and is considered a valuable prognostic marker (1, 2) and therapeutic target (3). Despite the documented link between S100A4 and metastasis using experimental metastasis models and transgenic mouse models (1, 4–6), the underlying mechanisms of how it promotes metastasis remain to be fully elucidated.

Metabolic reprogramming is a hallmark of cancer (7). To successfully survive and colonize distant sites, the metastatic cancer cells undergo profound metabolic changes during the process of metastasis (8). Thus, metabolic reprogramming is considered a critical component of a metastatic phenotype (8). EMT is one of the mechanisms that confer the metastatic phenotype. Recent studies demonstrate that EMT induces the metabolic signature that associates with poor overall survival of patients with breast cancer (9). The metabolic switch to aerobic glycolysis, also known as the “Warburg effect,” is one of the most common metabolic reprogramming events occurring in cancer cells (8, 10). Impaired mitochondrial function has been found to contribute to tumorigenesis and is considered to be one of the major causes of the Warburg effect (11). Recent studies demonstrate that mitochondria in some tumors are intact and fully active and that mitochondrial oxidative phosphorylation is essential for tumor growth (12–15). In fact, a subset of tumor types is heavily

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The microarray data have been deposited to the GEO database and are available under accession ID GSE121628.

This article contains Table S1 and Figs. S1–S4.

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We observed that the medium from S100A4-depleted cells turned yellow faster compared with the control cells (Fig. 1C), suggesting that S100A4-depleted cells generate a more acidic environment. We measured medium pH from these cells and demonstrate that the medium pH from S100A4-depleted cells was substantially lower than the medium pH from shCont cells (Fig. 1D). This phenomenon is not due to an increase in cell number because knockdown of S100A4 decreases cell proliferation (23). To examine whether S100A4 depletion affects glycolysis, we measured the extracellular acidification rate (ECAR) in A549 cells. Fig. 1 (E and F) shows that S100A4 depletion increased glycolysis, suggesting that S100A4 depletion altered cancer cell metabolism in favor of glycolysis. In contrast, depletion of S100A4 decreased the glycolysis reserve capacity in response to 2-deoxy-D-glucose (2-DG), a glycolysis inhibitor. Similar results were obtained in H460 cells (Fig. S1F). To further validate this metabolic shift, we measured glucose consumption and lactate production in A549 and H460 (shCont versus shS100A4) using the commercially available kits. We found that both glucose consumption (Fig. 1G) and lactate production (Fig. 1H) were significantly increased in S100A4-depleted cells. We performed these assays on MDA-MB-231 cells with S100A4 knockdown and found that both glucose consumption and lactate production were similarly increased upon S100A4 knockdown (Fig. S1, A and C). In line with this observation, overexpression of S100A4 in H1299 cells decreased glucose consumption to a significant level, whereas lactate production was slightly decreased but did not reach statistical significance (Fig. S1, B and D). The suppression of glycolysis is further supported by the ECAR analysis (Fig. S1E). Collectively, these data demonstrate that depletion of S100A4 decreases the oxygen consumption rate and shifts cell metabolism to be more glycolytically active and that overexpression of S100A4 decreased glucose consumption and lactate production in cancer cells.

**NDUFS2 is regulated in an S100A4-dependent manner**

To identify the critical molecules downstream of S100A4 signaling that enhance lung cancer cells cellular respiration, we performed an Affymetrix GeneChip analysis on A549 cells with S100A4 modulation (data not shown; GEO database submission GSE121628). Interestingly, among genes that were altered by S100A4, we found that NDUFS2 was significantly downregulated upon S100A4 reduction. We further confirmed this regulation by quantitative real-time PCR (Q-PCR) (Fig. 2A) and immunoblot analysis (Fig. 2B) in A549, H460 (Fig. 2, A and B), and H358 cells (Fig. 2, C and D) with stable knockdown of S100A4. In contrast, H1299 cells with stable S100A4 overexpression increased NDUFS2 expression compared with GFP control cells (Fig. 2, E and F). We also assessed NDUFS2 expression in several types of cancer cell lines, including HeLa (cervical cancer), MDA-MB-231 (breast cancer), and Suit-2 (pancreatic cancer), with either S100A4 stable knockdown (HeLa and MDA-MB-231 cells) or S100A4 transient knockdown (Suit-2). We found that S100A4-dependent regulation of NDUFS2 was also present in these cell lines (Fig. S2). These data suggest that NDUFS2 regulation by S100A4 is a general phenomenon.

Nuclear DNA encodes seven core subunits, including NDUFS1, NDUFS2, NDUFS3, NDUFS7, NDUFS8, NDUFV1,
and NDUFV2. To test whether S100A4 modulation affects the expression levels of these subunits, we assessed the expression of these seven subunits in A549 and H1299 cells upon S100A4 modulation by Q-PCR. We found that NDUFS2 was the only nuclear DNA-encoded core subunit that was regulated in an S100A4-dependent manner (Fig. S3). We then further analyzed this regulation in lung cancer clinical samples by using database analysis (cBioPortal). Interestingly, significant co-occurrence between S100A4 and NDUFS2 amplification was observed in a variety of cancer types, including lung, breast, head and neck, and ovarian cancer (Table S1). Together, these results suggest that S100A4-dependent NDUFS2 regulation may play critical roles in S100A4-driven lung cancer invasive and metastatic capacity.

Knockdown of S100A4 decreases mitochondrial complex I activity and ATP production

Quantitative decrease or absence of specific subunits of the complex in various tissue samples correlates with complex I

Figure 1. Knockdown of S100A4 decreases cellular respiration and shifts cell metabolism toward glycolysis. A, cellular respiration in A549 cells with S100A4 knockdown (shS100A4) or control (shCont) was analyzed by a mitochondrial stress test using a Seahorse XF96 analyzer. OCR was measured prior to and after injection of 1 μM oligomycin, 0.6 μM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), and 1 μM antimycin A and rotenone combination (A&R). Representative data are shown. B, OCR for basal respiration, maximal respiration, ATP production, proton leak, and nonmitochondrial respiration from A was analyzed using Wave 2.1 and normalized to protein concentration. C and D, H460 cells were cultured in regular media for 5 days and assessed visually (C), or medium pH was measured using a pH meter (D). E and F, glycolysis in A549 shCont and shS100A4 cells was assessed by glycolysis stress test using a Seahorse XF96 Analyzer. ECAR was measured prior to and after injection of 10 mM glucose, 1 μM oligomycin, and 100 μM 2-DG. Representative data are shown in E. ECAR for glycolysis, glycolysis capacity, and glycolysis reserve capacity was analyzed using Wave 2.1 and normalized to protein concentration (F). G and H, cells (5 × 10^5) were seeded into a 6-well plate, and media were changed after 5 h and collected the next day for glucose and lactate assessment. Cells were counted, and the amount of glucose consumption (G) and lactate production (H) was normalized to cell number. *, p < 0.05. Error bars, S.E. (A, B, E, and F) or S.D. (D, G, and H).
deficiency (29). Select NDUFS2 mutations lead to mitochondrial complex I deficiency in neurodegenerative diseases (27). Therefore, we hypothesize that S100A4 affects mitochondrial complex I activity to promote the invasive capacity. To test this hypothesis, we first examined whether complex I activity is essential to the invasive capacity of lung cancer cells by treating A549 cells in 3D culture with 0.5 μM rotenone, a mitochondrial complex I inhibitor. We found that about 30% colonies in DMSO-treated control cells displayed an invasive growth pattern, whereas only 5% of colonies displayed invasive growth in rotenone-treated cells (Fig. 3, A and B). Likewise, colony size was significantly decreased after rotenone treatment (Fig. 3, A and C). We then used the commercially available kits to measure the activity of mitochondrial complex I and cellular ATP levels in cells with S100A4 modulation. We found that stable knockdown of S100A4 in A549 cells resulted in decreased mitochondrial complex I activity (Fig. 3D) and cellular ATP levels (Fig. 3E) with similar results in H460 (Fig. 3, F and G) and H358 cells (data not shown). In contrast, overexpressing S100A4 in H1299 cells led to enhanced complex I activity (Fig. 3H) and cellular ATP levels (Fig. 3I). Collectively, our results suggest that S100A4 promotes tumor-invasive growth through enhancing mitochondrial complex I activity.

**NDUFS2 mimics the effects of S100A4 on mitochondrial metabolism reprogramming and the invasive capacity**

Next, we addressed the molecular mechanisms underlying the shift from oxidative phosphorylation to glycolysis upon S100A4 depletion. Glucose supply and rate-controlling steps, such as glucose transporters and glycolytic enzymes, affect glucose flux. Accordingly, we first evaluated whether knockdown of S100A4 impacts glucose transporter levels, specifically the levels of Glut1 and Glut3 in several lung cancer cell lines. As shown in Fig. S4, we found that overexpressing S100A4 in H1299 cells did not significantly alter the expression levels of Glut1 and Glut3. Likewise, knockdown of S100A4 decreased Glut3 expression but did not alter Glut1 expression in A549 cells. In contrast, knockdown of S100A4 in H460 cells up-regulated Glut3 expression but down-regulated Glut1 expression.

We further examined whether levels of several rate-limiting enzymes in the glycolysis pathways are altered by using the Glycolysis Antibody Sampler kit, which includes hexokinases, phosphofructokinase, and pyruvate kinase. Among these major enzymes that kinetically control glycolysis, we found that H1299 cells overexpressing S100A4 had reduced hexokinase I and hexokinase II expression (Fig. 4A). In contrast, knockdown of S100A4 increased hexokinase expression in H460 and A549 cells at both protein (Fig. 4A) and mRNA levels (Fig. 4B). There were no significant and consistent changes in phosphofructokinase and pyruvate kinase expression upon S100A4 modulation. Because the association of hexokinase II with mitochondria is important to its function, we assessed the expression level of hexokinases in mitochondria. We isolated mitochondria from H460 shCont and shS100A4 cells, and we found that the expression levels of hexokinases in mitochondria were also increased upon S100A4 depletion (Fig. 4C). These data suggest that S100A4 depletion promoted glycolysis, likely through up-regulating expression of hexokinases.

To address the contribution of NDUFS2 to mitochondrial function and cell growth, we generated the stable knockdown of NDUFS2 in A549 cells (Fig. 5, A and B). To test how knockdown of NDUFS2 affects glycolysis, we performed a glycolysis stress test. As shown in Fig. 5C, knockdown of NDUFS2 increased glycolysis capacity and decreased glycolysis reserved capacity. Cells with NDUFS2 knockdown significantly increased glucose consumption (Fig. 5D) and lactate production (Fig. 5E). Using a mitochondrial stress test (Fig. 5F), we found that knockdown of NDUFS2 surprisingly increased the basal respiration but decreased maximal respiration and ATP-linked respiration. Likewise, knockdown of NDUFS2 decreased mitochondrial complex I activity (Fig. 5G) as well as the cellular ATP levels (Fig. 5H). We further monitored cell growth in two and three dimensions (Fig. 5, I and J). Similar to S100A4 knockdown, stable knockdown of NDUFS2 decreased cell proliferation in two dimensions (Fig. 5J) and decreased colony size in 3D growth (Fig. 5I). These
results demonstrate that reduction of NDUFS2 expression mimicked the effect of S100A4 depletion on proliferation, invasive growth, mitochondrial activity, and metabolic alteration. Thus, these observations demonstrate that S100A4-regulated mitochondrial activity and metabolic reprogramming, at least in part, is mediated through NDUFS2 up-regulation.

To further determine the functional contribution of NDUFS2 downstream of S100A4 to mitochondrial metabolism and the invasive capacity, we transfected a GFP-tagged NDUFS2 expression construct into H460 shS100A4 cells and sorted cells for GFP and then performed glucose consumption and 3D growth assays. As shown in Fig. 5K, re-expression of NDUFS2 in shS100A4 cells decreased glucose consumption, which was increased in response to S100A4 knockdown (Fig. 5L). Meanwhile, re-expression of NDUFS2 recovered 3D growth properties originally altered by S100A4 knockdown proportional to the degree of NDUFS2 re-expression (Fig. 5M). Collectively, the data suggest that NDUFS2 functions downstream of S100A4 to regulate mitochondrial metabolism to promote the invasive capacity driven by S100A4.

**NDUFS2 and S100A4 are critical for the effective establishment of lung metastases**

To test the impact of S100A4 and NDUFS2 on lung cancer cell metastatic capacity, we used a mouse xenograft model of...
spontaneous metastases. We implanted A549 cells with stable knockdown of S100A4 (shS100A4), NDUFS2 (shNDUFS2), or control cells (shCont) into the NSG mice subcutaneously. Tumor growth was monitored every 2 days until tumor burden on mice reached around 2000 mm³. To minimize the effect of tumor growth rate on the metastasis capacity, we set up an extra group of mice injected with shS100A4 cells (shS100A4 2nd) that was monitored for a longer time along with the shNDUFS2 group. As shown in Fig. 6A, tumor onset was delayed with knockdown of either S100A4 or NDUFS2 compared with the control, although tumor onset for S100A4 knockdown and NDUFS2 knockdown was similar. Tumor growth rate data showed that loss of S100A4 (shS100A4) or NDUFS2 (shNDUFS2) in cells resulted in reduced tumor growth rate compared with the control (shCont) cells. We further performed the mitochondrial complex I activity assay on the primary tumor tissue from the mice. The results showed that the mitochondrial complex I activity in tissues from the shS100A4- and shNDUFS2-injected group was dramatically decreased compared with the activity in the shCont cell–injected tumor tissues (Fig. 6B). IHC staining for S100A4 and Ki67 (Fig. 6C) further supported the effect of S100A4 and NDUFS2 on cell proliferation found in vitro. To assess the metastatic spread, we performed necropsy and examined organs such as lung, liver, and spleens for overt nodules. We did not observe changes in liver and spleens (data not shown). To examine the presence of Figure 5. NDUFS2 is critical to S100A4-driven biological effects. NDUFS2 was knocked down in A549 cells by three separate shRNAs, and the expression level of NDUFS2 was assessed by immunoblotting (A) or Q-PCR (B) and compared with control cells (Cont). ECAR (C), glucose consumption (D), lactate production (E), OCR (F), mitochondrial complex I activity (G), cellular ATP levels (H), cell proliferation (I), and 3D Matrigel culture colony size measurement (J) were assessed on cells as indicated. H460 shS100A4 cells transfected with the NDUFS2 construct were sorted by GFP and compared with H460 shCont and shS100A4 cells for glucose consumption (L) and 3D growth (M). The diameter of colonies (>100 colonies) from randomly chosen fields was measured, quantified for average individual colony volume. Data presented as mean ± S.E. (error bars) (C, F, J, and M) or S.D. (D, E, G, H, and L). *, p < 0.05; **, p < 0.001.
the microscopic metastases in lungs, we performed the hema-
toxylin and eosin (H&E), S100A4, and Ki67 staining in lung
tissues (Fig. 6D). We found that the shCont group had a higher
metastasis incidence rate than the shS100A4 or shNDUFS2
groups. Specifically, compared with shCont, the ratio of the
micrometastases incidence rate for shS100A4 (1st), shS100A4
(2nd), and shNDUFS2 groups were 5.95, 4.18, and 3.01, respec-
tively (p < 0.00001; Fig. 6E). We also measured the size of each
tumor focus in mice lungs for each group. We found that
knockdown of either S100A4 or NDUFS2 decreased the sizes of
the lung tumor foci about 10–15% compared with the control
group (Fig. 6F). Together, these in vivo data demonstrated that
NDUFS2 mimics the function of S100A4 for A549 cells to effec-
tively establish metastases in lung.

Cells with S100A4 suppression are sensitive to glycolysis
inhibition

Because knockdown of S100A4 shifts lung cancer cell metab-
olism toward glycolysis, we hypothesized that cells with
S100A4 knockdown depend on glycolysis; thus, modulation of
S100A4 should influence the vulnerability of lung cancer cells
to energy restriction with focus on glycolysis inhibition. To test
this hypothesis, we first addressed the impact of glycolysis inhi-
bition on lactate production in A549 cells. We treated A549
shCont and shS100A4 cells with 5 mM 2-DG. After 24 h, lactate
production was assessed as performed in Fig. 1. As shown in
Fig. 7A, 2-DG treatment decreased lactate production in shS100A4
cells compared with the shCont cells. Next, to examine the
impact of knockdown of S100A4 on the sensitivity of lung cells
to glycolysis inhibition, we treated A549 shCont and shS100A4
cells with 5 mM 2-DG for 3 days and then assessed cell viability
under glycolysis inhibition by either crystal violet staining or
direct cell count. We found upon 2-DG treatment that the per-
centage of cell viability was about 56% in shCont cells, whereas
in cells with S100A4 knockdown, levels dropped to 33% (Fig.
7B). Based on previous studies that niclosamide targets S100A4
signaling to inhibit proliferation and invasion of colon and lung
cancers (23, 24), we tested whether dual inhibition of
S100A4-mediated signaling by niclosamide and glycolysis by
2-DG would synergistically decrease cell survival. We treated
A549 cells with DMSO vehicle, 0.25 μM niclosamide, 5 mM
2-DG, or a combination of both inhibitors for 3 days and then
assessed cell survival by crystal violet staining. Similar to the
effect of glycolysis inhibition on S100A4 knockdown cells, we
found that cell viability was about 92% with niclosamide single
 treatment at 0.25 mM concentration and 56% with 5 mM 2-DG
treatment and that the combination treatment reduced the cell

Figure 6. Knockdown of S100A4 and NDUFS2 in A549 cells decreases lung metastases in vivo. A, cells (1 × 10^6), as indicated, in a 1:1 mixture of PBS and
growth factor–reduced Matrigel were implanted subcutaneously into NSG mice. Tumor growth was monitored, and volumes were calculated every 2 days.
Mice were sacrificed at week 12 (shCont and shS100A4 1st, blue arrows) and week 14 (shS100A4 2nd and shNDUFS2, black arrow). Three-color straight solid lines
are fitted tumor volume profiles, and the corresponding curved lines are observed mean tumor volume for each group. B, primary tumors from the indicated
cell line–injected groups were collected, snap-frozen, and homogenized. Tissue lysates (400 μg) were used for the mitochondrial complex I activity assay. C and
D, representative images from histologic assessments of primary tumor sections (C) or lung sections (D) with H&E staining or immunohistochemical staining
with antibodies against S100A4 and Ki67. Magnification is ×400 for H&E and S100A4 staining (scale bars, 100 μm) and ×200 for Ki67 staining (scale bars, 200 μm)
in C. Magnification is ×100 for images in D. Arrows in D indicate tumor foci in the lung. Scale bars, 400 μm in D. The total number of tumor foci was recorded
based on histology assessment. The incidence of tumor foci was calculated based on the observation time and presented in (E). The size of the tumor foci in the
indicated cell injection groups was measured by a digital micrometer. The average size of tumor foci in each group is presented in F. * , p < 0.05. Error bars, S.D.
viability to 24% (Fig. 7C). Altogether, these data demonstrate that cells with S100A4 knockdown are more susceptible to glycolysis inhibition.

**Discussion**

Reprogramming of energy metabolism is one of the hallmarks of cancer (7). Increasing evidence suggests that metastatic cancer cells undergo profound metabolic changes during the metastatic process (8, 30). It is recognized that these metabolic changes are due to the select pressures such as limited nutrients and the availabilities of oxygen (hypoxia and reoxygenation) that the metastatic cancer cells encounter due to the interaction of cancer cells with the tumor microenvironment as well as during the discrete steps of metastasis cascade (8, 31). This metabolic plasticity, which allows cancer cells to partially or completely reverse their metabolic switch phenotype (such as interchange between glycolysis and oxidative phosphorylation), is critical for metastatic cancer cells to survive during the metastasis process and eventually colonize in distant organs (8, 31). Our current data demonstrate that S100A4 is critical to the invasion and metastatic capacity in lung cancer cells, and modulation of S100A4 alters mitochondrial metabolism. We further observe that S100A4 promotes metastasis through the regulation of mitochondrial metabolism.

Mounting evidence supports the role of altered mitochondrial metabolism in metastatic cancer (8, 13, 30, 31). For example, invasive breast cancer cells display a shift toward OXPHOS metabolism via PGC-1α, a key regulator of mitochondrial biogenesis and metabolism, and circulation cancer cells have increased oxidative phosphorylation (13). Our studies on S100A4 in lung cancer demonstrate that S100A4 promotes lung cancer invasion and invasive growth (23), and S100A4 is required for the effective establishment of lung metastases in vivo (Fig. 6). Notably, mitochondrial complex I activity in primary tumor tissues from shCont cells was much higher compared with the tumor tissues from shS100A4 or shNDUFS2 cells (Fig. 6B). The effects of modulation of S100A4 on mitochondrial activity (Fig. 3) and metastatic capacity support the concept that cells with high S100A4 expression, such as A549, have the capacity to maintain mitochondrial activity and ATP production and promote the metastasis process. In line with these results, a known mitochondrial complex I inhibitor, rotenone, significantly decreased the invasive capacity of A549 cells (Fig. 3, A-C), confirming that mitochondrial activity is important to S100A4-driven invasion and metastasis.

Increased glucose metabolism is a common phenomenon due to metabolic reprogramming that cancer cells adapt to generate energy and metabolic intermediates to meet their high proliferation rate. As such, therapies targeting glycolysis have been tested clinically but were unsuccessful due to the systemic toxicity (32). We found that knockdown of S100A4 decreased cellular respiration but favored glycolysis (Fig. 1). Inhibition of oxidative phosphorylation led to elevated glycolytic metabolism (33). This event was not surprising because knockdown of S100A4 resulted in decreased mitochondrial complex I activity; thus, instead of entering into the mitochondrial TCA cycle, a large amount of pyruvate was converted to lactate, likely through lactate dehydrogenase (34). We further found that S100A4 depletion resulted in the up-regulation of hexokinase expression that contributed to the elevated glycolytic phenotype. Among the four isoforms of hexokinases, HK1 and HK2 were regulated in an S100A4-dependent manner, although the extent of the regulation of these two isoforms was cell type–specific. We found that in select cell lines, the glucose transporters were also regulated, although they were not universally regulated by S100A4 in different cell lines (Fig. S4), which would possibly contribute to the elevation of glycolysis in the specific cell lines. The functional consequences of this glycolysis reprogramming are intriguing because S100A4 knockdown dramatically decreased the incidence of lung metastases from our current in vivo experimental metastasis model (Fig. 6). In addition, we found that this glycolysis switch sensitized lung cancer cells to glycolysis inhibition. In support of our data, recent studies demonstrate that mitochondria-targeted drugs, such as Mito-CP, Mito-Q, and mitochondrial ETC blockers, can enhance the efficacy of the glycolysis inhibitor 2-DG in breast (35) and colon cancer (36). Similarly, combination treat-
S100A4 regulation of mitochondrial metabolism

ment of the mitochondrial complex I inhibitor metformin with 2-DG had a synergistic effect on NSCLC cells (37), thus supporting our findings that mitochondrial oxidative phosphorylation plays a critical role in S100A4-driven metastatic capability and that suppressing S100A4 decreases the metabolic plasticity.

In contrast to our work, a recent study using melanoma cells as the model reported that extracellular S100A4 stimulated cell migration and invasion, whereas it simultaneously activated glycolytic flux, suggesting that metabolic reprogramming from oxidative phosphorylation to glycolysis promotes the invasive phenotype (25). The difference in metabolic reprogramming seen in these two studies may be due to the cancer type–specific effects, which are a feature of cancer metabolism and should be considered when developing therapeutic targets (38, 39). Alternatively, these differences could originate from differences in the overall experimental objectives of these studies and the cellular localization of S100A4. Notably, S100A4 has extracellular, cytosolic, and nuclear functions. In the study by Bettum et al. (25), the authors sought to determine how S100A4 in the microenvironment impacted melanoma cells by treating cells with pure S100A4 protein in culture. They found that extracellular S100A4 administration altered differentiation gene expression profiles to promote glycolysis in the well-differentiated and poorly migratory cell line Melmet5, but not in the poorly differentiated Melmet1 cell line. In the poorly differentiated and highly invasive cell line, they found no impact of extracellular S100A4, which is a phenomenon we have seen in our cell lines (unpublished observation).² In our study, we sought to understand how intracellular expression of S100A4 in tumor cells impacts tumor biology and metabolism, based on our previous study (23). The effects of S100A4 on mitochondrial metabolism represent the action from both extracellular and intracellular S100A4, as we used genetic manipulations to modulate S100A4 expression. S100A4 is known to influence several key regulators in mitochondrial metabolism, such as p53 and NF-κB, to promote the invasive phenotypes (23, 40, 41). In several models of poorly differentiated NSCLC, we find that S100A4 up-regulates NDUSF2 at the mRNA and protein levels while suppressing the expression of hexokinases to mediate a metabolic switch. Extracellularly, S100A4 signals through RAGE, epidermal growth factor receptor, and Toll-like receptor-4 (TLR-4)–mediated pathways (23, 42). It is possible that, in poorly differentiated cells, these pathways are already activated by other ligands, thus nullifying the impact of extracellular S100A4. Considering these differences, it is reasonable that these two studies would find fundamentally different impacts of S100A4 signaling on metabolism, which suggests that S100A4 influences tumor metabolism differently depending on the state of differentiation and localization of its signaling. Further studies are needed to gain insight into the mechanisms governing these differences. Nevertheless, the results from the melanoma work provided additional evidence to support the role of S100A4 in promoting tumor dissemination through regulating the metabolic plasticity and add another layer of complexity of how S100A4 may contribute to the metabolic changes during tumor progression.

Our study identified the roles of S100A4 in promoting lung cancer invasion and metastasis and changing mitochondrial metabolism through NDUSF2 regulation. A recent study showed that high NDUSF2 expression is associated with worse overall survival in several types of cancer, including lung, breast, and ovarian cancer (28), suggesting the prognostic value of the mitochondrial complex I subunit in cancer progression. In line with this observation, we provided mechanistic links to further support the role of NDUSF2 in lung cancer progression. First, we showed that NDUSF2 is regulated in an S100A4-dependent manner in variety of cancer cell lines, including ovarian, pancreatic, breast, and lung cancer. Second, we found that knockdown of NDUSF2 mimics the biological effect in cells with S100A4 depletion, as demonstrated by decreased cell proliferation, invasive capacity, mitochondrial activity, and concomitantly up-regulated hexokinase expression, and decreases the incidence of tumor foci in lungs. Third, using cBioPortal Cancer Genomic database analysis (43, 44), we found that significant co-occurrence between S100A4 and NDUSF2 amplification was observed in a variety of cancer types, including lung, breast, head, and neck, and ovarian cancer (Table S1). It is not clear how the S100A4-mediated NDUSF2 expression affects the mitochondrial complex I activities to promote the metastasis process. Based on recent data showing that knockout of NDUSF2 in catecholaminergic cells impairs the ability of complex I to catalyze NADH oxidation, ubiquinone reduction, and proton transport (45), we speculate that metastatic cancer cells have better reoxygenation capacity with the existence of S100A4-mediated NDUSF2 regulation. It is possible that the S100A4 affects mitochondrial complex I assembly/stability, as discovered in neurodegenerative diseases with an NDUSF2 mutation. Finally, the effects of S100A4-mediated NDUSF2 regulation on mitochondrial structure, such as mitochondrial size and cristae organization, and mitochondrial dynamics, such as fusion and fission, are worthy of future investigation to advance our understanding of how S100A4 promotes cancer metastasis through regulation of mitochondrial energy metabolism.

In summary, we demonstrate a novel function of S100A4 on mitochondrial metabolism and the critical role of the S100A4-mediated NDUSF2 regulation in S100A4-driven lung cancer cell invasive and metastatic capacities. Through this work, we propose that the metabolic reprogramming upon targeting S100A4 offers a therapeutic window for enhancing the efficacy of glycolysis inhibition and provides the rationale for dual inhibition of S100A4 and glycolysis in advanced lung cancer cells that have high levels of S100A4.

Experimental procedures

Cell lines and reagents

Lung cancer cell lines were originally obtained from ATCC and were authenticated with short tandem repeat profile analysis by Genetica DNA Laboratories (Burlington, NC). Cell culture conditions were described previously (23) and detailed in the supporting information.

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² L. Liu, L. Qi, T. Knifley, D. W. Piecoro, P. Rychahou, J. Liu, M. I. Mitov, J. Martin, C. Wang, J. Wu, H. L. Weiss, D. A. Butterfield, B. M. Evers, K. L. O’Connor, and M. Chen, unpublished observation.
**Q-PCR, immunoblotting, and 3D culture**

Q-PCR, immunoblotting, and 3D culture were performed as described (23, 46) (see supporting information).

**Transient siRNA knockdown, stable lentiviral shRNA knockdown, cell line generation, and transfection**

For transient knockdown, siRNAs were electroporated into cells as described previously (47). In brief, cells (3 × 10⁶) were electroporated (400-V, 500-microfarad capacity) with either SMARTpool siRNA targeting S100A4 or nontargeting siRNA control (Dharmacon, Lafayette, CO) and used in experiments 72 h later. A549 and H460 cells with stable knockdown of S100A4 were generated as described previously using lentiviral shRNA constructs from Sigma (23). For stable reduction of S100A4 or NDUFS2, lentivirus construct pLKO.1-puro containing shRNA targeting human S100A4 (S100A4-#A6 or -#A8) or NDUFS2 (#1, #2, and #3) or containing nontargeting sequence was packaged into virus in 293T cells with Mission lentiviral packaging mix (Sigma-Aldrich) using polyethyleneimine based on a 1:3 ratio of DNA to polyethyleneimine. The viral supernatant was collected 48 h after transfection, as described previously (46). Then cells, as indicated, were infected with virus-containing medium, and stable transfectants were selected with puromycin (2 μg/ml).

The sequences for human S100A4 are as follows: 5′-CCGGCTCAACAATGCAGAATCTGATTTAGTTCTGATGTTGGACGTGTTTTTG-3′ (#A6) and 5′-CCGGCCATGATGTAACGAATTCTCGAGAATTCGTTATTGCTGACTCTTTTTG (#2), and CCGGCCCTCCGGGAGTCACTAATTCATCACTTTCTCGAGAAAGTTCTCGAGATCTTGTTTAGACACTGTGCGTTTTTG (#1), human NDUFS2 are CCGGCGCACAGTGTCTAAACAAGACACATCATGGCGTTTTTG-3′/H11032CCGCAATGATGTGTAACGAATTCTCGAGAATTCGTTATATGTTAGCTGTTTTTG-3′/H9262CGCCATGATGTGTAACGAATTCTCGAGAATTCGTTATTGCTGACTCTTTTTG (#A6) and 5′-CCGGCCATGATGTAACGAATTCTCGAGAATTCGTTATTGCTGACTCTTTTTG-3′/H11032CCGCAATGATGTGTAACGAATTCTCGAGAATTCGTTATTGCTGACTCTTTTTG (#2), and CCGGCCCTCCGGGAGTCACTAATTCATCACTTTCTCGAGAAAGTTCTCGAGATCTTGTTTAGACACTGTGCGTTTTTG (#A8). The sequences for human NDUFS2 are CCGGCCCTCCGGGAGTCACTAATTCATCACTTTCTCGAGAAAGTTCTCGAGATCTTGTTTAGACACTGTGCGTTTTTG (#A8). The sequences for human NDUFS2 are CCGGCCCTCCGGGAGTCACTAATTCATCACTTTCTCGAGAAAGTTCTCGAGATCTTGTTTAGACACTGTGCGTTTTTG (#A8).

Re-expression of NDUFS2 was achieved by transient transfection of the pCMV-NDUFS2-C-GFPspark (Sino Biological, Beijing, China) vector using Lipofectamin 2000 and sorted by GFP.

**Mitochondrial stress test and glycolysis stress test**

The Seahorse XF96 extracellular flux analyzer (Agilent) was used to perform the mitochondrial stress test and glycolysis stress test per the manufacturer’s protocol. Cells (2 × 10⁴ cells/well) were maintained for 24 h at 37 °C in a 5% CO₂ incubator. OCR was measured prior to and after injection of 1 μM oligomycin, 0.6 μM carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP), and a 1 μM antimycin A and rotenone combination. ECAR was measured prior to and after injection of 10 mM glucose, 1 mM oligomycin, and 100 mM 2-DG. Data were analyzed using Wave version 2.1 and normalized to protein concentration.

**Mitochondria isolation, glucose consumption and lactate production, mitochondrial complex I activity assay, and cellular ATP measurement**

Mitochondria fractionation was performed as described (48). Glucose consumption and lactate production, mitochondrial complex I activity, and cellular ATP levels were measured by using commercially available kits according to the manufacturers’ instructions, as detailed in the supporting information.

**S100A4 regulation of mitochondrial metabolism**

All animal studies were performed according to the procedure approved by the University of Kentucky Institutional Animal Care and Use Committee. A549 shCont, shS100A4, or shNDUFS2 cells (1 × 10⁶) were collected in 100 μl of PBS containing 50% growth factor reduced Matrigel (Corning) and implanted subcutaneously into NOD SCID γ mice (breeding pairs from Jackson Laboratory). Mouse weight and tumor growth were monitored every 2 days once the primary tumor was measurable. Tumor dimensions were measured using a caliper, and tumor volumes were calculated as V = (L × W × H)/2, where V = tumor volume, L = tumor length, W = tumor width, and H = tumor height. 8–10 weeks after implantation, mice were sacrificed. Tissues from the primary tumors and the other organs were collected, paraffin-embedded, and stained with either H&E or antibodies against S100A4 and Ki67 (Dako). Microscopic lung metastases were visualized by H&E staining and examined by a pathologist blinded to the experimental conditions. The number of total foci in each mouse was recorded, and the size of the measurable distinct tumor foci was measured by a digital micrometer. The number of lung tumor foci per mouse was divided by the observation time (days) to obtain the average incidence of lung tumor foci for each group.

**Statistical analysis**

For in vitro experimental metastasis study, a linear mixed effect model was fitted for the longitudinal tumor volume to study whether the mean tumor volume profiles are significantly changed between groups. The Welch two-sample t test was used to test the difference of the mean tumor foci size between groups. A Poisson model was used to model the tumor foci incidence (number) per day. Data from all other in vitro experiments were compared using a two-tailed unpaired Student’s t test. All experiments were performed at least three times, and representative data are shown and presented as mean ± S.D., unless stated otherwise. Difference between groups was considered significant at p < 0.05. TCGA copy number variation data of 13 cancer types were downloaded from cbioPortal (access date February 2017) (43, 44). A Fisher exact test was used to assess the co-occurrence between S100A4 and NDUFS2 amplification for each cancer type.

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S100A4 regulation of mitochondrial metabolism

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References

1. Bresnick, A. R., Weber, D. J., and Zimmer, D. B. (2015) S100 proteins in cancer. Nat. Rev. Cancer 15, 96–109 CrossRef Medline
2. HELFMAN, D. M., KIM, E. J., LUKANIDIN, E., and GRIGORIAN, M. (2005) The metabolism associated protein S100A4: role in tumour progression and metastasis. Br. J. Cancer 92, 1955–1958 CrossRef Medline
3. Mishra, S. K., Siddique, H. R., and Saleem, M. (2012) S100A4 calcium-binding protein is key player in tumor progression and metastasis: preclinical and clinical evidence. Cancer Metastasis Rev. 31, 163–172 CrossRef Medline
4. Grigorian, M., Ambarsumian, N., Lykkesfeldt, A. E., Bastholm, L., Elling, F., Georgiev, G., and Lukandin, E. (1996) Effect of mts1 (S100A4) expression on the progression of human breast cancer cells. Int. J. Cancer J. Int. Cancer 67, 831–841 CrossRef Medline
5. Maelandsmo, G. M., Hovig, E., Skrede, M., Engebretsen, O., Fløresen, V. A., Myklebost, O., Grigorian, M., Lukandin, E., Scott, K. J., and Fodstad, O. (1996) Reversal of the in vivo metastatic phenotype of human tumor cells by an anti-CAPL (mts1) ribozyme. Cancer Res. 56, 5490–5498 Medline
6. Takenaga, K., Nakamura, Y., and Sakiyama, S. (1997) Expression of antisense RNA to S100A4 gene encoding an S100-related calcium-binding protein suppresses metastatic potential of high-metastatic Lewis lung carcinoma cells. Oncogene 14, 331–337 CrossRef Medline
7. Hanahan, D., and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation. Cell 144, 646–674 CrossRef Medline
8. LEHUÉDÉ, C., DUPUY, F., RABINOVITCH, R., JONES, R. G., and SIEGEL, P. M. (2016) Metabolic plasticity as a determinant of tumor growth and metastasis. Cancer Res. 76, 5201–5208 CrossRef Medline
9. Bhownik, S. K., Ramírez-Peña, E., Arnold, J. M., Putluri, V., Sphyris, N., Michailidis, G., Putluri, N., AMBS, S., Sreekumar, A., and MANI, S. A. (2015) EMT-induced metabolite signature identifies poor clinical outcome. Oncotarget 6, 42651–42660 Medline
10. Lunt, S. Y., and VANDER HEIDEN, M. G. (2011) Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu. Rev. Cell Dev. Biol. 27, 441–464 CrossRef Medline
11. Koppenol, W. H., Bounds, P. L., and Dang, C. V. (2011) Otto Warburg’s contributions to current concepts of cancer metabolism. Nat. Rev. Cancer 11, 325–337 CrossRef Medline
12. Caro, P., Kishan, A. U., Norberg, E., Stanley, I. A., Chapuy, B., Facchini, S. B., Polak, K., Tondera, D., Gounarides, J., Yin, H., ZHOU, F., Green, M. R., and CHEN, I. (2015) Mitochondrial biogenesis and cancer: is glycolysis the main ATP supplier in all tumor cells? Mol. Cell. Biol. 30, 1303–1318 CrossRef Medline
13. Marin-Valencia, I., Yang, C., Mashimo, T., Cho, S., Baek, H., Yang, X. L., Rajagopalan, K. N., Maddie, M., Vemireddy, V., Zhao, Z., Cai, L., Good, L., Tu, B. P., Hanatanpa, K. J., Mickey, B. E., et al. (2012) Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain in vivo. Cell Metab. 15, 827–837 CrossRef Medline
14. Moreno-Sánchez, R., Rodríguez-Enríquez, S., Saavedra, E., Marín-Hernández, A., and Gallardo-Pérez, J. C. (2009) The bioenergetics of cancer: is glycolysis the main ATP supplier in all tumor cells? Biofactors 35, 209–225 CrossRef Medline
15. Alam, M. M., Lal, S., FitzGerald, K. E., and Zhang, L. (2016) A holistic view of tumor bioenergetics: mitochondrial function and respiration play fundamental roles in the development and progression of diverse tumors. Clin. Transl. Med. 5, 3 CrossRef Medline
16. Weinberg, S. E., and Chandel, N. S. (2015) Targeting mitochondria metabolism for cancer therapy. Nat. Chem. Biol. 11, 9–15 CrossRef Medline
17. WEN, S., ZHU, D., and HUANG, P. (2013) Targeting cancer cell mitochondria as a therapeutic approach. Future Med. Chem. 5, 53–67 CrossRef Medline
18. VAN, J. X., DING, K., and WANG, C. Y. (2012) Niclosamide, an old anthelminthic agent, demonstrates antitumor activity by blocking multiple signaling pathways of cancer stem cells. Chin. J. Cancer 31, 178–184 CrossRef Medline
19. FORETZ, M., GUIGAS, B., BERTRAND, L., POLLAK, M., and VIOLET, B. (2014) Metformin: from mechanisms of action to therapies. Cell Metab. 20, 953–966 CrossRef Medline
20. STEWART, R. L., CARPENTER, B. L., WEST, D. S., KNIFLEY, T., LIU, L., WANG, C., WEISS, H. L., GAL, T. S., DURBIN, E. B., ARNOLD, S. M., O’CONNER, K. L., and CHEN, M. (2016) S100A4 drives non-small cell lung cancer invasion, associates with poor prognosis, and is effectively targeted by the FDA-approved antihelminthic agent niclosamide. Oncotarget 7, 34630–34642 CrossRef Medline
21. SACK, U., WALThER, W., SCUDERIO, D., SELBY, M., KOBElt, D., LEMM, M., Fichtner, I., Schlag, P. M., SHOEMAKER, R. H., and STEIN, U. (2011) Novel effect of anthelmintic Niclosamide on S100A4-mediated metastatic progression in colon cancer. J. Natl. Cancer Inst. 103, 1018–1036 CrossRef Medline
22. BETTMUH, I. J., CORAD, S. S., BARKOVSKAYA, A., PETTERSEN, S. M., MOESTUE, S. A., VASILIAUSKAITE, K., TENSTAD, E., OTJORD, T., RISA, Ø., NYGaARD, V., MAELANDSMO, G. M., and PRASMICKAITE, L. (2015) Metabolic reprogramming supports the invasive phenotype in malignant melanoma. Cancer Lett. 366, 71–83 CrossRef Medline
23. ZHU, J., VINOTHKUMAR, K. R., and HIRST, J. (2016) Structure of mammalian respiratory complex I. Nature 536, 354–358 CrossRef Medline
24. NGU, L. H., NJITMANS, J., DISTELMAIER, F., VENSELAAR, H., VAN EMST-DRIE, S. E., VAN DEN BRAND, M. A., STOLtenborg, B. J., WIjNTJES, L. T., WILLEMs, P. H., VAN DEN HEUVel, L. P., SMEITINK, J. A., and RODENBURG, R. J. (2012) A catalytic defect in mitochondrial respiratory chain complex I due to a mutation in NDUFS2 in a patient with Leigh syndrome. Biochim. Biophys. Acta 1822, 168–175 CrossRef Medline
25. LI, L. D., SUN, H. F., BAI, Y., GOAO, S. P., JIANG, H. L., and JIN, W. (2016) Significant prognostic values of nuclear genes encoding mitochondrial complex I subunits in tumor patients. Neoplasma 63, 548–558 CrossRef Medline
26. MOREAITH, R. W., CLEETER, M. W., RAGAN, C. I., BATSWH, M. L., and LEHNGER, A. L. (1987) Congenital deficiency of two polypeptide subunits of the iron-protein fragment of mitochondrial complex I. J. Clin. Invest. 79, 463–467 CrossRef Medline
27. LeBleu, V. S., O’Connell, J. T., Gonzalez Herrera, K. N., Wikman, H., PANTel, K., HAigIS, M. C., de CARVALHO, F. M., DAMASCena, A., DomINGOS CHIEN, L. T., ROCa, R. M., ASARA, J. M., and KALLURI, R. (2014) PGC-1α mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. Nat. Cell Biol. 16, 992–1003, 1–15 CrossRef Medline
28. RAMANUJAN, V. K. (2015) Metabolic plasticity in cancer cells: reconnecting mitochondrial function to cancer control. J. Cell Sci. Ther. 6, 211 CrossRef Medline

7526 J. Biol. Chem. (2019) 294(18) 7516–7527
32. Pelicano, H., Martin, D. S., Xu, R. H., and Huang, P. (2006) Glycolysis inhibition for anticancer treatment. *Oncogene* **25**, 4633–4646 CrossRef Medline
33. Ward, P. S., and Thompson, C. B. (2012) Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. *Cancer Cell* **21**, 297–308 CrossRef Medline
34. Suhane, S., Kanzaki, H., Arumugaswami, V., Murali, R., and Ramanujan, V. K. (2013) Mitochondrial NDUFS3 regulates the ROS-mediated onset of metabolic switch in transformed cells. *Biol. Open* **2**, 295–305 CrossRef Medline
35. Cheng, G., Zielonka, J., Dranka, B. P., McAllister, D., Mackinnon, A. C., Jr., Joseph, J., and Kalyanaraman, B. (2012) Mitochondria-targeted drugs synergize with 2-deoxyglucose to trigger breast cancer cell death. *Cancer Res.* **72**, 2634–2644 CrossRef Medline
36. Fath, M. A., Diers, A. R., Aykin-Burns, N., Simons, A. L., Hua, L., and Spitz, D. R. (2009) Mitochondrial electron transport chain blockers enhance 2-deoxy-D-glucose induced oxidative stress and cell killing in human colon carcinoma cells. *Cancer Biol. Ther.* **8**, 1228–1236 CrossRef Medline
37. Hou, X. B., Li, T. H., Ren, Z. P., and Liu, Y. (2016) Combination of 2-deoxy-D-glucose and metformin for synergistic inhibition of non-small cell lung cancer: a reactive oxygen species and P-p38 mediated mechanism. *Biomed. Pharmacother.* **84**, 1575–1584 CrossRef Medline
38. Dang, C. V. (2012) Links between metabolism and cancer. *Genes Dev.* **26**, 877–890 CrossRef Medline
39. Elia, I., Schmieder, R., Christen, S., and Fendt, S. M. (2016) Organ-specific cancer metabolism and its potential for therapy. *Handb. Exp. Pharmacol.* **233**, 321–353 CrossRef Medline
40. Orre, L. M., Panizza, E., Kaminsky, V. O., Vernet, E., Gräsland, T., Zhivotovsky, B., and Lehtio, J. (2013) S100A4 interacts with p53 in the nucleus and promotes p53 degradation. *Oncogene* **32**, 5531–5540 CrossRef Medline
41. Grotterød, I., Maelandsmo, G. M., and Boye, K. (2010) Signal transduction mechanisms involved in S100A4-induced activation of the transcription factor NF-κB. *BMC Cancer* **10**, 241 CrossRef Medline
42. Fei, F., Qu, J., Zhang, M., Li, Y., and Zhang, S. (2017) S100A4 in cancer progression and metastasis: a systematic review. *Oncotarget* **8**, 73219–73239 CrossRef Medline
43. Gao, J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S. O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E., Cerami, E., Sander, C., and Schultz, N. (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **6**, pl1 CrossRef Medline
44. Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., Jacobsen, A., Byrne, C. J., Heuer, M. L., Larsson, E., Antipin, Y., Reva, B., Goldberg, A. P., Sander, C., and Schultz, N. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2**, 401–404 CrossRef Medline
45. Fernández-Agüera, M. C., Gao, I., González-Rodríguez, P., Pintado, C. O., Arias-Mayenco, I., García-Flores, P., García-Pergaño, A., Pascual, A., Ortega-Sáenz, P., and López-Barneo, J. (2015) Oxygen sensing by arterial chemoreceptors depends on mitochondrial complex I signaling. *Cell Metab.* **22**, 825–837 CrossRef Medline
46. Chen, M., Bresnick, A. R., and O’Connor, K. L. (2013) Coupling S100A4 to Rhokine alters Rho signaling output in breast cancer cells. *Oncogene* **32**, 3754–3764 CrossRef Medline
47. Chen, M., Sastry, S. K., and O’Connor, K. L. (2011) Src kinase pathway is involved in NFAT5-mediated S100A4 induction by hyposmotic stress in colon cancer cells. *Am. J. Physiol. Cell Physiol.* **300**, C1155–C1163 CrossRef Medline
48. Xiong, X., Wen, Y. A., Mitov, M. I., Oaks, M. C., Miyamoto, S., and Gao, T. (2017) PHLP2 regulates hexokinase 2-dependent glucose metabolism in colon cancer cells. *Cell Death Discov.* **3**, 16103 CrossRef Medline