A redox cycle with complex II promotes sulfide quinone oxidoreductase dependent H₂S oxidation

Roshan Kumar,¹ Aaron P. Landry,¹ Arkajit Guha,¹ Victor Vitvitsky,¹ Ho Joon Lee,² Keisuke Seike,³ Pavan Reddy,³ Costas A. Lyssiotis² and Ruma Banerjee*

Departments of ¹Biological Chemistry, ²Molecular and Integrative Physiology, and ³Internal Medicine, Michigan Medicine, University of Michigan, Ann Arbor, MI 48109

Short Title: H₂S induces complex II reversal

One Sentence Summary: Reversal of complex II sustains and prioritizes H₂S oxidation when respiration is poisoned.

*Corresponding author. Email: rbanerje@umich.edu
The dueling roles of H$_2$S as an endogenously synthesized respiratory substrate and as a toxin, raise questions as to how it is cleared when the electron transport chain is inhibited. Sulfide quinone oxidoreductase (SQOR) is a mitochondrial inner membrane flavoprotein that catalyzes the first step in the H$_2$S oxidation pathway and uses coenzyme Q (CoQ) as an electron acceptor. However, complex IV poisoning by H$_2$S inhibits complex III-dependent recycling of CoQH$_2$, which is needed to sustain H$_2$S oxidation. We have discovered that under these conditions, reversal of complex II activity using fumarate as an electron acceptor, establishes a new redox cycle with SQOR. The purine nucleotide cycle and the malate aspartate shuttle are sources of fumarate in H$_2$S treated cells, which accumulate succinate. Complex II knockdown decreases the efficiency of H$_2$S clearance and increases recovery time to the basal respiration rate in H$_2$S treated cells. In contrast, attenuation of complex I, which is a major competitor for the mitochondrial CoQ pool, has the opposite effects. Targeted knockout of complex II in murine intestinal epithelial cells that are routinely exposed to microbiota derived H$_2$S, decreases serum, urine, and fecal thiosulfate, a product of H$_2$S oxidation. Our study identifies a metabolic reprogramming response to H$_2$S that furnishes fumarate as an alternate electron acceptor and supports H$_2$S oxidation independent of complex IV activity. Complex II-linked redox cycling of SQOR has important implications for gut H$_2$S metabolism as colonocytes are routinely exposed to high concentrations of this gas derived from the microbiota.

**Keywords:** hydrogen sulfide, complex II, electron transport chain, coenzyme Q, fumarate, SDHA
The discovery of H$_2$S as an endogenously synthesized signaling molecule in mammals has fueled a growing literature on its physiological effects (1). Mechanistic insights into how H$_2$S modulates cellular responses are however, scarce (2, 3), and much attention has been focused on protein persulfidation, a reactive posttranslational modification of cysteine (4) that has been identified in hundreds of proteins (5, 6). On the other hand, the best characterized cellular effects of H$_2$S are its oxidation via a dedicated mitochondrial pathway (7), and its inhibition of complex IV (8) in the electron transport chain (ETC), leading to respiratory poisoning (Fig. 1a). The sulfide oxidation pathway begins with the conversion of H$_2$S to glutathione persulfide catalyzed by sulfide quinone oxidoreductase (SQOR), an inner mitochondrial membrane flavoprotein (9). Electrons released from H$_2$S oxidation are transferred to coenzyme Q (CoQ) and enter the ETC at the level of complex III, making H$_2$S an inorganic substrate for oxidative phosphorylation in mammals (10). The remainder of the pathway successively converts glutathione persulfide to thiosulfate and, in some cells, to sulfate (11). The role in signaling, if any, of the reactive sulfur species formed during H$_2$S oxidation remains to be fully elucidated (12). In this study, we report that a noncanonical redox circuit is established when complex IV is inhibited, via reversal of complex II activity, which allows continued oxidation of H$_2$S.

SQOR functions as a respiratory shield, sensitizing the ETC to H$_2$S poisoning when its activity is attenuated (13). At low H$_2$S concentrations however, SQOR activity increases respiration as measured by the oxygen consumption rate (OCR) (14). The dual potential to stimulate electron flux and inhibit the ETC, raises questions as to whether modulation of mitochondrial bioenergetics by H$_2$S is pertinent to its cellular signaling mechanism and fans out to other compartments via redox and metabolomic changes (15).

SQOR is one of several consumers of CoQ (Fig. 1a) and sulfide oxidation is impaired in CoQ deficiency (16). SQOR activity has the potential to cause a reductive shift in the CoQ pool, particularly at H$_2$S concentrations that partially or fully inhibit complex IV. H$_2$S also indirectly perturbs the NAD$^+$/NADH and FAD/FADH$_2$ couples that are connected to CoQ/CoQH$_2$ via the
ETC. We have previously demonstrated that H$_2$S induces a reductive shift in the NAD$^+$/NADH redox couple, creating an electron acceptor insufficiency that leads to uridine and aspartate deficiency and enhances reductive carboxylation (13). While uridine limitation results from the CoQ dependence of dihydroorotate dehydrogenase in the pyrimidine pathway (Fig. 1a), aspartate deficiency results in part from reduced flux through the TCA cycle and the NADH-linked malate-aspartate shuttle. Furthermore, H$_2$S stimulates the Warburg effect, enhancing glucose consumption and lactate production (17), and stimulates lipid biogenesis (18).

The effects of H$_2$S on the ETC itself has received scant attention (10, 17, 19). The observed increase in succinate and decrease in malate at H$_2$S concentrations that inhibit respiration was proposed to result from complex II reversal (10). While the same authors later proposed that H$_2$S induces reverse electron transfer through complex I (14), neither model was evaluated experimentally. A recent study on oligomycin-treated murine microglia reported increased OCR upon exposure to an H$_2$S donor and interpreted as evidence of reverse electron transfer through complex I (20). The known drivers of mitochondrial reverse electron transfer, which leads to reactive oxygen species (ROS) generation, are a high membrane potential and an over-reduced CoQ pool (21). Since respiratory poisons depolarize the membrane by limiting electron-coupled proton transfer (Fig. 1a), the premise for H$_2$S-induced reverse electron transfer is unclear. Furthermore, the study contradicted the reported lack of H$_2$S-induced ROS production (22).

Studies in our laboratory have focused primarily on colonic epithelial cells (13, 17, 18) that are routinely exposed to high concentrations of H$_2$S from gut microbiota, estimated to range from ~0.2 to 2.4 mM (23, 24). In this study, we report that rewiring within the ETC circuitry via complex II reversal, sustains H$_2$S oxidation under conditions of respiratory poisoning with fumarate serving as an electron acceptor. These results have important implications for understanding the mechanism by which intestinal epithelial cells respond to routine exposure to high H$_2$S levels derived from the microbiota and potentially, the role of H$_2$S in signaling a shift in energy metabolism.
Figure 1. The mitochondrial NADH pool influences the efficiency of H₂S oxidation in HT29 cells. (a) Scheme showing that multiple CoQ (Q) users compete with SQOR including complexes I and II, dihydroorotate dehydrogenase (DHOD), glycerol 3-phosphate dehydrogenase (G3PDH), proline dehydrogenase (PDH) and the electron transfer flavoprotein (ETF). (b) H₂S oxidation is enhanced in cells expressing mitochondrial but not cytoplasmic LbNOX versus the empty vector (EV) control. Rotenone (2 µM) enhanced H₂S clearance in control and cytoplasmic but not mitochondrial expressing LbNOX cells. (c) Disruption of complex I by NDUFS3 knockdown enhanced H₂S oxidation. (d) Mitochondrial expression of TPNOX accelerates H₂S oxidation, which is inhibited by NBD-Cl. The data are represent the SEM of 3 independent experiments (**p<0.001 and *p<0.05).

Results

SQOR catalyzes sulfide-dependent reduction of O₂

We examined whether O₂ can serve an alternate electron acceptor for SQOR since complex IV poisoning by H₂S should not restrict O₂ availability (Supplementary Fig. 1a). We found that in the presence of sulfide and sulfite but in the absence of CoQ, nanodisc-embedded SQOR (ndSQOR) (25) catalyzed O₂ consumption (Supplementary Fig. 1b). From the linear
dependence of OCR on O₂ concentration, a $k_{on}$ of $3370 \pm 290$ M$^{-1}$ s$^{-1}$ was estimated (Supplementary Fig. 1c). Oxygen ($k \sim 14$ min$^{-1}$ at 75 μM O₂) is however, a significantly less efficient electron acceptor than CoQ ($15 \times 10^3$ min$^{-1}$ at 75 μM CoQ) (26).

In the presence of a slight excess of sulfide (10 μM) and sulfite (15 μM), SQOR (7.5 μM) catalyzed the consumption of an equimolar concentration of O₂ (7.3 ± 0.6 μM). This reaction stoichiometry predicted that the products of O₂ reduction by SQOR could be either O₂$^-$ and FADH$^+$ or H₂O₂ and FAD. The equivalence between the concentration of H₂O₂ produced (7.6 ± 0.6 μM) and O₂ consumed is consistent with the two-electron reduction of O₂ by SQOR (Supplementary Fig. 1a). The concentration of H₂O₂ was significantly diminished (0.2 ± 0.1 μM) when catalase was added to the reaction mixture. The approximately 1:1:1 stoichiometry of sulfide added:O₂ consumed:H₂O₂ produced is consistent with electron transfer from FADH₂ to O₂ via a C4a-hydroperoxy FAD intermediate (Supplementary Fig. 1d), as proposed in other O₂-activating flavoenzymes (27).

**Complex I activity decreases the efficiency of H₂S oxidation**

Complex I-dependent oxidation of NADH with concomitant reduction of CoQ, is a major source of electron flux in the ETC and is expected to influence the efficiency of H₂S oxidation. We have previously reported that H₂S causes a reductive shift in the NAD$^+$/NADH ratio by inhibiting complex IV (13). H₂S oxidation was unaffected by the cytoplasmic, but significantly enhanced by the mitochondrial expression of the water forming NADH oxidase, LbNOX (28) (Fig. 1b).

Rotenone, a complex I inhibitor, increased H₂S oxidation in control and LbNOX but not mito-LbNOX cells (Fig. 1b). Knockdown of NDUFS3 (Supplementary Fig. 2), which is required for complex I assembly, increased H₂S oxidation (Fig. 1c). Collectively, these data demonstrate that the cellular H₂S oxidation capacity can be limited by the mitochondrial NADH pool.

The mitochondrial NADH and NADPH pools are interconnected via the activity of the electrogentic nicotinamide nucleotide transhydrogenase (NNT) located in the inner mitochondrial...
membrane. Cytoplasmic expression of TPNOX, a genetically encoded water forming NADPH oxidase (29), had no effect on H₂S oxidation, while mitochondrial expression enhanced clearance (Fig. 1d). The NNT inhibitor NBD-Cl (4-chloro-7-nitrobenzofurazan chloride) attenuated the mito-TPNOX effect, further demonstrating that the capacity for cellular H₂S oxidation is linked to the status of the mitochondrial NAD(P)H redox pool (Fig. 1d).

**Succinate accumulates in response to H₂S**

Metabolomics analysis after exposure to Na₂S (100 μM, 1 h) revealed a number of changes in glycolytic, TCA cycle (13) and purine metabolism intermediates in malignant HT29 cells (Fig. 1a,b). Interestingly, H₂S treatment led to ~5.5-fold higher levels of succinate. To test whether succinate accumulation resulted from reversal of complex II activity (Fig. 2c), we used dimethyl fumarate (DMF), a membrane permeable derivative of fumarate that increases intracellular fumarate concentration (30). DMF accelerated H₂S oxidation in 4 out of 5 colorectal carcinoma lines but not in RKO cells (Fig. 2d and Supplementary Fig. 3). Two other complex II inhibitors, dimethyl malonate and dimethyl itaconate, also inhibited H₂S clearance, while diethyl succinate did not (Supplementary Fig. 4). Knocking down SDHA (Supplementary Fig. 5), the complex II subunit that catalyzes the reversible oxidation of succinate to fumarate, reduced H₂S clearance (Fig. 2e). DMF shortened the recovery time for return to basal OCR following respiratory inhibition by H₂S in HT29 (Fig. 2f-h), HCT116, LoVo and DLD cells (Supplementary Fig. 6) but had no effect when SDHA was knocked down in HT29 cells (Supplementary Fig. 7). Together, these data are consistent with the model that H₂S oxidation is facilitated by reversal of complex II activity.

**The effect of complexes I and II on H₂S-dependent OCR**

To further test the influence of complexes I and II on the cellular response to H₂S, OCR was monitored in control versus NDUFS3 and SDHA knockdown cells. NDUFS3 knockdown
decreased basal OCR 2-fold (Fig. 3), consistent with complex I being a major entry point for electrons into the ETC. At a low concentration of H$_2$S (10 µM), OCR activation in NDUFS3 knockdown cells was robust, and the peak increase in OCR was higher than in control and

**Figure 2.** H$_2$S induces succinate accumulation through reversal of complex II activity. (a) Volcano plot showing metabolite changes in response to Na$_2$S (100 µM) treatment of HT29 cells for 1 h. (b) Heat map showing H$_2$S-induced changes in select metabolites in HT29 cells. (c) Scheme showing how complex II reversal can regenerate CoQ for H$_2$S oxidation. (d) DMF (100 µM) increases H$_2$S oxidation in EV HT29 cells. (e) SDHA knockdown in HT29 cells reduces H$_2$S oxidation. (f,g) The duration of respiratory inhibition in HT29 cells by H$_2$S is longer in the absence (f) versus presence (g) of DMF (200 µM). The red arrows indicate when H$_2$S (30 µM) was added. (h) Comparison of the time required by HT29 cells to return to the basal respiration rate ± DMF. The data in (d) and (e) represent the mean ± SEM of 3-4 independent experiments.
Figure 3. Complexes I and II influence H$_2$S-linked OCR. Comparison of OCR activation with H$_2$S (20 or 30 µM) in (a, d) scrambled, (b, e) SDHA knockdown, and (c, f) NDUFS3 knockdown HT29 cells. Red arrows indicate when H$_2$S was added. The traces are representative of 3-5 independent experiments.

SDHA knockdown cells (Supplementary Fig. 8). At a higher H$_2$S (20 µM) concentration, differences between the cell lines were clearly visible (Fig. 3a,b,c). While the NDUFS3 knockdown showed robust activation of OCR in response to H$_2$S, the control and SDHA knockdown cells showed signs of inhibition. The SDHA knockdown cells took a longer time to
recover basal OCR compared to controls. Following the first and second 20 μM H₂S injection, control and SDHA knockdown cells showed signs of partial and severe respiratory inhibition, respectively, in contrast to NDUFS3 knockdown cells. At a higher H₂S concentration (30 μM), control and SDHA knockdown cells responded with net inhibition of oxygen consumption in comparison to NDUFS3 knockdown cells, which exhibited a mixed response (Fig. 3d-f). These results indicate that the CoQ pool limits sulfide clearance and, in the absence of competition from complex I, cells clear sulfide more efficiently. The data also reveal that complex II has the opposite effect, i.e., it is advantageous for sulfide clearance, consistent with our model that complex II reversal supports H₂S oxidation by catalyzing CoQH₂ oxidation.

Malate-aspartate shuttle and PNC furnish fumarate in H₂S treated cells
Since the malate-aspartate shuttle and the purine nucleotide cycle (PNC) (Fig. 4a,b) are metabolic sources of fumarate in ischemic cells (21), we tested whether they also contribute to fumarate when the ETC is inhibited by H₂S. For this, GOT1 and GOT2 (glutamic-oxaloacetic aminotransferases 1 and 2) expressed in the cytoplasm and mitochondrion, respectively, were knocked down in HT29 cells (Supplementary Fig. 9). GOT1 but not GOT2 knockdown increased H₂S oxidation by ~38% compared to control cells (Fig. 4c). GOT1 knockdown also promoted H₂S clearance as reflected by the shorter recovery time to the basal respiration rate (Supplementary Fig. 10). Inhibition of adenylosuccinate lyase with AICAR (5-aminimidazole-4-carboxamide ribonucleotide) decreased H₂S clearance by ~50% (Fig. 4d), consistent with a role for the PNC in this process.

SDHA knockout in murine intestinal epithelial cells decreases H₂S oxidation
To assess the physiological relevance of our observation that H₂S clearance is supported by complex II working in reverse, we measured the impact of attenuating complex II on organismal H₂S metabolism. For this, mice harboring loxP-flanked Sdha were crossed to mice
Figure 4. The PNC and the malate-aspartate shuttle support fumarate driven H$_2$S oxidation. (a,b) Schemes showing that the malate-aspartate shuttle (a) and the PNC (b) are fumarate sources and that AICAR inhibits adenylosuccinate lyase (ASL). MDH1/2, FH, OAA and CII denote malate dehydrogenase 1/2, oxaloacetate, fumarate hydratase and complex II, respectively. (c) H$_2$S oxidation is stimulated in GOT1 knockdown but unaffected by GOT2 knockdown in HT29 cells. (d) AICAR (0.25 mM) inhibits H$_2$S clearance. The data in (c) and (d) represent the SEM of 3-4 independent experiments (*p<0.05).

expressing Cre recombinase under control of the villin promoter to specifically target intestinal epithelial cells, to generate Vil1-Cre Sdha$^{fl/fl}$ (Sdha$^{IEC}$) mice as described previously (31). The rationale for targeting intestinal epithelial cells is that they are routinely exposed to high concentrations of H$_2$S (23, 24) and actively oxidize sulfide (13). Thiosulfate, a stable product of H$_2$S oxidation (Fig. 5a), is a stable biomarker of H$_2$S metabolism. H$_2$S on the other hand, is difficult to measure due to its volatility and low steady-state concentrations in biological samples (32). Sdha$^{IEC}$ mice showed lower thiosulfate levels compared to control Sdha$^{fl/fl}$ (Fig. 5b-d)
revealing that the loss of complex II in intestinal cells caused local (feces) and systemic (serum and urine) perturbations in H$_2$S oxidation.

Figure 5. Villin$^{Cre}$SDHA$^{fl/fl}$ mice have reduced thiosulfate levels. (a) Scheme connecting H$_2$S oxidation to thiosulfate production. (b,c,d) Quantitation of thiosulfate levels in control and Villin$^{Cre}$Sdha knockout versus control (Sdha$^{fl/fl}$) mice in serum (b), urine (c) and feces (d). The data represent the SEM for samples collected from 4 mice in each group (*p<0.05).

Discussion

In this study, we have uncovered a new mechanism for clearing H$_2$S when its concentrations rise to levels that inhibit complex IV and preclude the use of O$_2$ as the terminal electron acceptor for SQOR-dependent H$_2$S oxidation. Such conditions might be relevant in the gut epithelium (where H$_2$S exposure is high) or in ischemia (where O$_2$ supply is cut off). Reversal of complex II activity under such conditions supports SQOR-dependent H$_2$S oxidation, using fumarate as an alternate electron acceptor.
Metabolomic changes in HT29 cells in response to H$_2$S provided clues to reprogramming driven changes that could potentially impact its clearance. Hypoxanthine and succinate, classic ischemic biomarkers (21, 33), also accumulate in response to H$_2$S (Fig. 2b). Ischemic succinate accumulation is derived from oxidative TCA cycle metabolism (34) as well as from complex II-catalyzed reduction of fumarate (21). Fumarate is derived via the malate-aspartate shuttle and the PNC (21). Since H$_2$S decreases the NAD$^+$/NADH ratio and stimulates reductive carboxylation of $\alpha$-ketoglutarate (13), the effect of the oxidative TCA cycle on H$_2$S clearance was not examined. The PNC and the malate aspartate shuttle both impacted H$_2$S clearance (Fig. 4c,d). The PNC is activated in response to a drop in the adenylate energy charge (35), and is consistent with lower ATP levels in H$_2$S-treated cells (17) as well as the observed increase in inosine, which is formed via deamination of adenosine.

Knockdown of GOT1, but not GOT2, increased the efficiency of H$_2$S clearance, suggesting that the cytoplasmic arm of the malate-aspartate shuttle is an important source of fumarate. H$_2$S leads to aspartate deficiency (13), potentially stimulating GOT1-catalyzed transamination of oxaloacetate to aspartate rather than the reverse, which is consistent with lower malate levels in H$_2$S-treated cells (Fig. 2b). In GOT1 knockdown cells, oxaloacetate should be more available for malate dehydrogenase catalyzed reduction to malate, which can be dehydrated to fumarate (Fig. 4a) by fumarate hydratase that is present in the cytoplasm and the mitochondrion (36). Cytosolic fumarate can potentially enter the mitochondrion via a dicarboxylate carrier (37).

Our studies support a model for efficient H$_2$S clearance by SQOR when the H$_2$S concentration is low with complexes I and II competing for the CoQ pool and complex III recycling CoQH$_2$ (Fig. 6a). However, when H$_2$S concentrations rise and inhibit complex IV, utilization of fumarate as an electron acceptor by complex II sustains recycling of CoQH$_2$ (Fig. 6b). Complex II catalyzes the reversible oxidation of succinate to fumarate (38) and exhibits similar $K_M$ values for both
Figure 6. Alternate redox cycles for disposing H$_2$S. (a,b) CoQH$_2$ formed during H$_2$S oxidation and by complexes I and II, enters the ETC at the level of complex III (a). When complex IV is inhibited by H$_2$S, blocking recycling of CoQH$_2$ by complex III, CoQH$_2$ can be oxidized by complex II, concomitant with fumarate reduction and succinate accumulation (b).

Substrates (39, 40). Under *in vitro* assay conditions, the ratio of succinate oxidation to fumarate reduction catalyzed by the succinate dehydrogenase component of complex II varies substantially with the electron acceptor, and ranges from ~0.1 to 50 for succinate:fumarate consumed (40). Under physiological conditions, flux through the forward versus reverse reaction is governed by the concentration of the respective substrates and by the potentials of the relevant redox couples. In the mitochondrial matrix (pH ~7.7), the standard redox potential for the fumarate/succinate couple (E’’ = +30 mV) is similar to that ubiquinone (+40-60 mV at pH 7.0, decreasing 60 mV per increase in pH unit (41)), but higher than of the FAD/FADH$_2$ couple (~79 mV (42, 43)). The reversibility of complex II in cells is supported by its ability to sustain proficient growth on fumarate as a terminal electron acceptor when expressed under anaerobic conditions in an *E. coli* strain lacking fumarate reductase (44). These data support the plausibility of
complex II reversal under conditions when the ETC is blocked, and the CoQ pool is over-
reduced.

Modulation of H$_2$S metabolism by complex I was demonstrated by its inhibition by rotenone
and by NDUFS3 knockdown; both enhanced H$_2$S clearance (Fig. 1b,c), as expected, and is
consistent with their increased sulfide-induced OCR compared to control cells (Fig. 3). On the
other hand, complex II inhibition (with DMM or DMI) or SDHA knockdown, decreased the
efficiency of H$_2$S clearance while DMF increased it (Fig. 2, Supplementary Figs. 3,4). Under
conditions of complete coupling, for every mole of sulfide oxidized by SQOR, ETHe1 and
complex IV are predicted to consume 1 and 0.5 mole of O$_2$, respectively. ETHe1 is a
mononuclear iron-dependent persulfide dioxygenase, which catalyzes the conversion of
glutathione persulfide to sulfite (45, 46). SDHA knockdown cells exhibited increased sensitivity
to H$_2$S-induced inhibition of OCR and took longer to recover, while DMF reduced the time to
recovery of the basal OCR (Figs. 2 and 3). Collectively, these results support our model of
complex II-dependent recycling of CoQH$_2$ (Fig. 6b). It is important to note however, that
interfering with complex II reduces but does not completely block H$_2$S consumption. Thus, other
mechanisms including SQOR-dependent reduction of O$_2$ (Supplementary Fig. 1) might
contribute to H$_2$S removal.

The significant decrease in thiosulfate upon silencing SDHA in murine intestinal epithelial
cells (Fig. 5) is notable for three reasons. It supports the physiological relevance of reverse
complex II activity for H$_2$S oxidation as loss of the canonical succinate oxidation activity would
be expected to stimulate SQOR-dependent H$_2$S oxidation by decreasing competition for the
CoQ pool. Second, the observed change in thiosulfate levels in Sdha$^{ΔIEC}$ mice reflect the impact
of complex II on endogenous sulfide metabolism. Third, changes in urine and serum thiosulfate
in Sdha$^{ΔIEC}$ mice reveal the systemic impact of altered H$_2$S metabolism at the host-microbe
interface, which warrants further study.
We speculate that H$_2$S-fueled succinate accumulation could have downstream metabolic effects. Succinate is a competitive inhibitor of $\alpha$-ketoglutarate-dependent dioxygenases and its accumulation could broadly impact histone and DNA methylations (47). Furthermore, succinylation, a posttranslational modification of proteins (48), could be enhanced by H$_2$S-driven succinate accumulation. Over >750 protein targets of succinylation have been identified, which are concentrated in mitochondria but also present in other compartments (49) and reversed by the NAD$^+$-dependent sirtuin, Sirt5 (50). Succinylation reportedly increases complex II activity (49). We speculate that succinylation could be enhanced by the opposing effects of H$_2$S on the succinate and NAD$^+$ pools, in an autocorrective loop for activating complex II and prioritizing its removal.

In summary, our study reveals that metabolic reprogramming leads to the establishment of a new redox cycle between SQOR and complex II, permitting sustained H$_2$S clearance. In addition to its relevance at the gut host-microbe interface, this circuitry could be important in the context of ischemia reperfusion injury. H$_2$S is cytoprotective when administered at the time of reperfusion, reducing infarct size, inhibiting myocardial inflammation and preserving mitochondrial integrity (51). The rapid reoxidation of succinate that accumulates in the ischemic phase, drives ROS production during reperfusion (21). We posit that the cytoprotective effects of H$_2$S could derive from its twin effects on complex IV inhibition and complex II reversal, thereby attenuating succinate-dependent ROS generation during reperfusion. Another cellular context in which H$_2$S mediated ETC rewiring might be relevant is during the transition from a quiescent to proliferative state. While quiescent cells primarily rely on the high energy yield of oxidative phosphorylation, proliferating cells increase aerobic glycolysis to meet their energy needs and redirect mitochondrial metabolism for macromolecular precursor synthesis (52). The potential for H$_2$S to function as an endogenous modulator of energy metabolism could be significant in this context and needs to be further understood.
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Author contributions-RK-kinetics of H$_2$S consumption, generation and analysis of TPNOX, NDUFS3 and SDHA knockdown cell lines thiosulfate in Sdha$^{-/}$IEC and control mice; APL-analysis of SQOR reactions; AG-OCR data generation and analysis; VV, HJL, CAL-metabolomics data generation and analysis; SK and PR-generation of Sdha$^{-/}$IEC mice; RK, APL and RB drafted the manuscript and all authors edited and approved the final version of the manuscript.

Supplementary Materials
Materials and Methods
Fig S1 – S10
Supplementary References

Data and materials availability-All data are available in the main text or the supplementary materials.
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