## **Supporting Information**

Szabo et al. 10.1073/pnas.1306241110



**Fig. S1.** Effect of the CBS inhibitor AOAA (1 mM) and the CSE inhibitor PAG (3 mM) on H<sub>2</sub>S production in homogenates of a colorectal cancer and patientmatched normal colonic tissue. H<sub>2</sub>S production was measured in homogenates of a human colorectal cancer specimen by the methylene blue method. H<sub>2</sub>S production was stimulated in tissue or cell lysates by incubation at 37 °C (30 min) in presence of the L-cysteine (3 mM). H<sub>2</sub>S production was significantly higher in colon cancer tissues, compared with their corresponding controls. AOAA (1 mM) attenuated the H<sub>2</sub>S-producing activity of the tissue homogenates (<sup>#</sup>P < 0.05), whereas PAG (3 mM) had no significant effect. Data represent mean ± SEM of n = 3 determinations.

-<u>Δ</u> 0.3 μM NaHS 🖸 1 μM NaHS 🛛 💠 10 μM NaHS



Fig. S2. Biphasic effect of H<sub>2</sub>S on cellular bioenergetics in mitochondria isolated from HCT116 cells. Coupling experiments show that H<sub>2</sub>S at low concentration (0.3 μM) elevates oxygen consumption rate (OCR) in state 3 and state 3u respiration in mitochondria isolated from HCT116 cells (\*P < 0.05; \*\*P < 0.01), whereas at higher concentration (10 µM) it inhibits mitochondrial activity (##P < 0.01). (A) Individual extracellular flux analysis tracings. (B) Calculated ATP turnover data. (C) FCCP-stimulated maximal respiration values. Data represent mean  $\pm$  SEM of n = 3 determinations.

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**Fig. S3.** AOAA suppresses cellular bioenergetics in HCT116 cells. (*A*) Oxygen consumption rate (OCR) in HCT116 cells subjected to vehicle or AOAA (1 mM). AOAA significantly decreased basal OCR, calculated ATP production, maximal respiration, and spare respiratory capacity (\*P < 0.05 or \*\*P < 0.01). (*B*) AOAA significantly diminished the maximal glycolytic capacity and the glycolytic reserve capacity (\*P < 0.05 or \*\*P < 0.01). Data represent mean  $\pm$  SEM of n = 3 determinations.



**Fig. S4.** CBS silencing attenuates glycolysis in HCT116 cells. *Top* shows time-dependent Extracellular Flux Analysis values; *Bottom* shows the calculated bioenergetic parameters with statistical comparisons. The results show that shCBS significantly diminished the maximal glycolytic capacity and the glycolytic reserve capacity (\*P < 0.05 or \*\*P < 0.01 vs. shNT), whereas CSE silencing had no effect on the glycolytic parameters. Data represent mean  $\pm$  SEM of n = 4–5 determinations.



**Fig. S5.** Effect of AOAA on cellular bioenergetics in HCT116 cells transfected with nontargeting vector (shNT), with a CSE silencing vector (shCSE) or with a CBS silencing vector (shCBS). (*A*) Responses in nontargeting vector (shNT) treated cells; (*B*) responses in CSE silencing vector (shCSE) treated cells; (*C*) responses in CBS silencing vector (shCBS) treated cells; and (*D*) comparison of the various bioenergetic responses. Please note that AOAA causes a similar suppression of the bioenergetic responses in the shNT and the shCSE cells, whereas in the shCBS cells, where the bioenergetic response is already suppressed, the residual inhibitory effect of AOAA on cellular oxygen consumption is diminished. (\**P* < 0.05 shows significantly lower bioenergetic parameters in shCBS cells compared with shNT cells; "*P* < 0.05 shows significant effect of AOAA in either the shNT, shCBS, or shCSE cells, compared with the respective response in the absence of AOAA). Data represent mean ± SEM of *n* = 3 determinations.



**Fig. S6.** Effect of L-cysteine on cellular bioenergetics in NCM356 and HCT116 cells. Oxygen consumption rate (OCR) is shown in (A) NCM356 and (B) HCT116 cells in the absence or presence of L-cysteine (3  $\mu$ M). In NCM356 cells, L-cysteine did not affect basal OCR and slightly but significantly increased spare respiratory capacity. In HCT116 cells, L-cysteine increased both basal OCR, as well as spare respiratory capacity (\*P < 0.05; \*\*P < 0.01). Comparison of the relative enhancement of spare respiratory capacity in the two cell types is shown in (C): L-cysteine produces a larger relative stimulation of bioenergetic function in HCT116 cells than in NCM356 cells. Data represent mean  $\pm$  SEM of n = 3 determinations.

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**Fig. 57.** Pharmacological inhibition of CSE by PAG does not inhibit colon cancer growth in vivo. Effect of PAG treatment (50 mg/kg·d<sup>-1</sup>) on (*A*) tumor volume and (*B*) plasma H<sub>2</sub>S levels in Nu/nu Balb/C female mice (8–10 wk) injected s.c. in the right and left dorsum (10<sup>6</sup> cells per side with HCT116 cells). Consistent with the role of CSE in contributing to circulating H<sub>2</sub>S levels (1), PAG attenuated systemic H<sub>2</sub>S levels (*B*), but it failed to affect tumor growth (*A*). Data represent mean  $\pm$  SEM of n = 6 animals per group.

1. Yang G, et al. (2008) H<sub>2</sub>S as a physiologic vasorelaxant: Hypertension in mice with deletion of cystathionine gamma-lyase. Science 322(5901):587-590.

Patient sample	Age	Sex	Stage
CN052710	66	F	Normal margin of stage III
CN051310	60	М	Normal margin of stage III
CN041411	63	F	Normal margin of adenoma
CT121009	47	F	Chronic inflammation
CT121509	30	М	Stage II
CT041411	63	F	Adenoma
CN/CT060710	78	F	Stage II
CN/CT060310	88	F	Stage II
CN/CT071210	75	М	Stage II
CN/CT052912#24	28	М	Stage III

 Table S1. Clinicopathologic information of the human colon cancer samples used in the current study