

Supplemental Material Online

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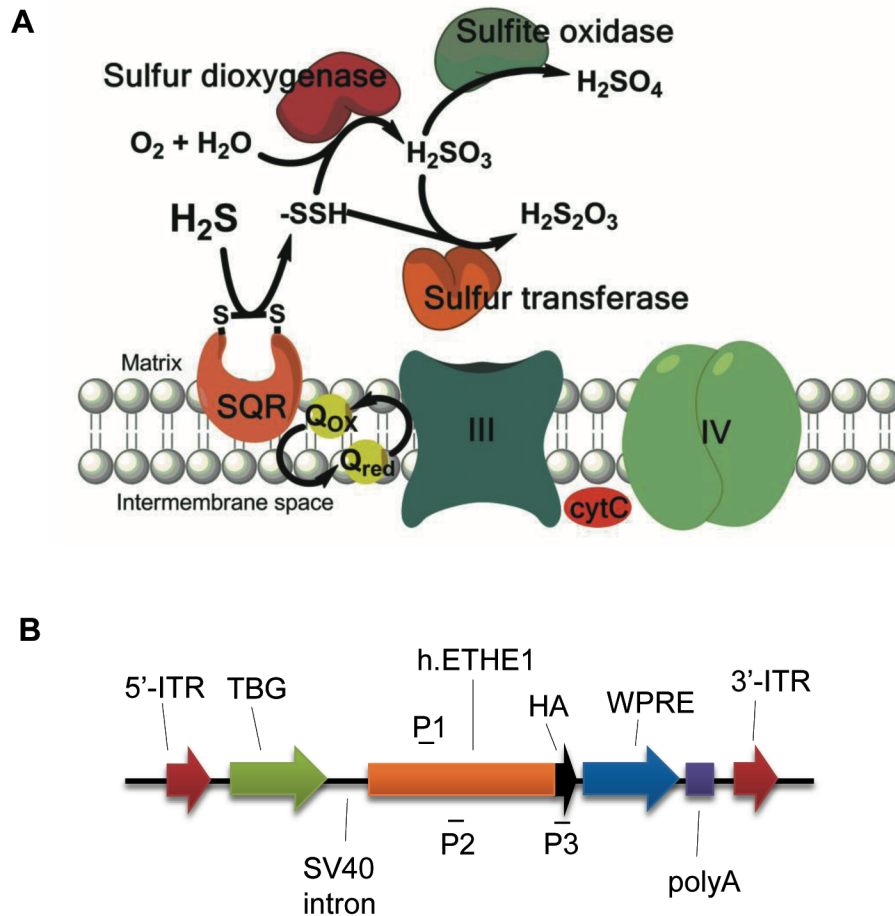
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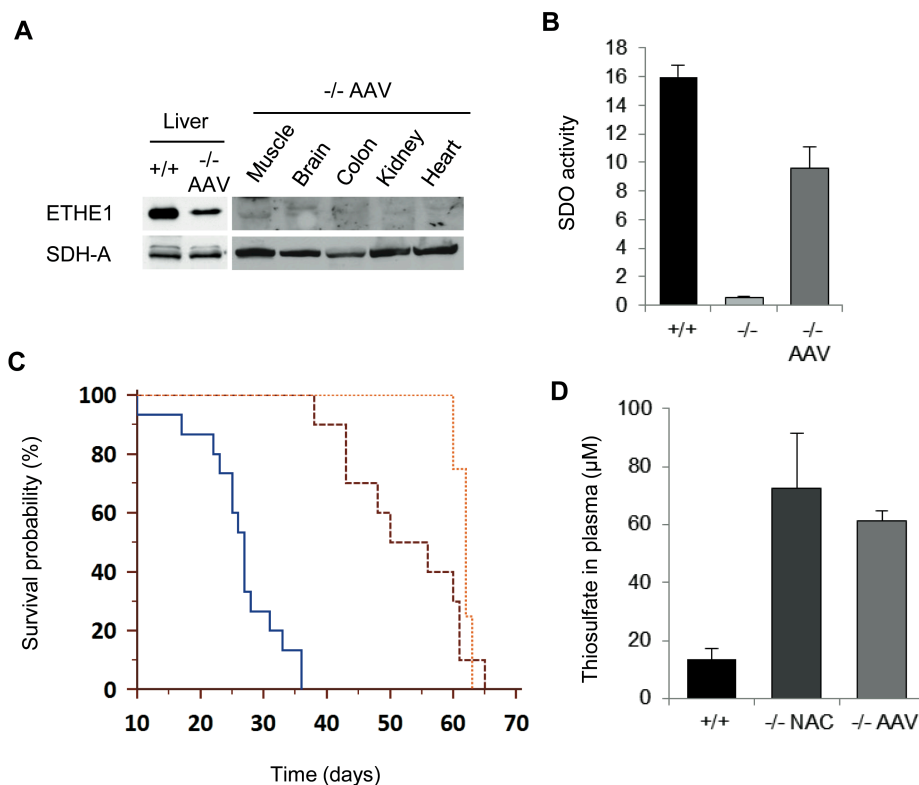
Supplemental Table I: COX/CS activities in skeletal muscle and brain.

Supplemental Figure 1



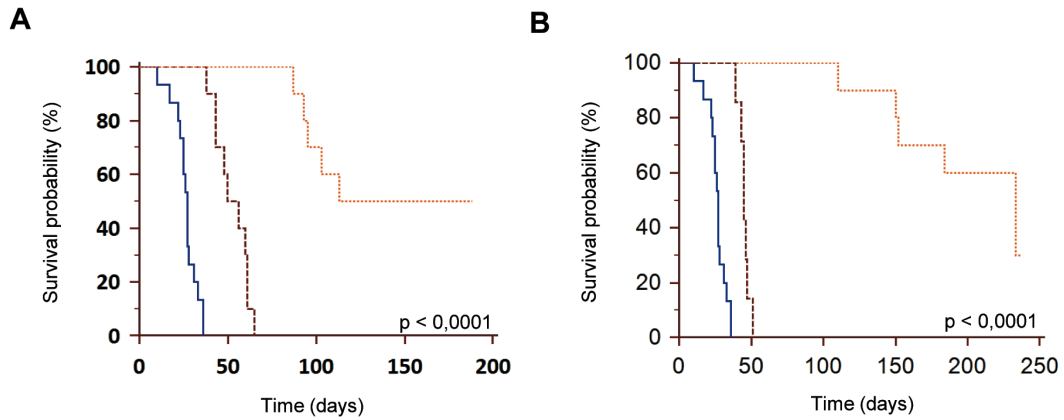
Supplemental Figure 1. Explanatory schemes. (A) Proposed mitochondrial pathway of H₂S disposal according to Hildebrandt and Grieshaber, 2008. SQR: sulfide:quinone oxidoreductase; Qox: oxidized coenzyme Q; Qred: reduced coenzyme Q; III: complex III; IV: complex IV; cytC: cytochrome C; the Sulfur dioxygenase is encoded by *Ethel*. (B) AAV2/8-TBG-*h.Ethel*^{HA} construct. ITR: inverted terminal repeat; TBG: thyroxine-binding globulin promoter; WPRE: Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element; P1-P3: primers used for PCR amplification.

Supplemental Figure 2



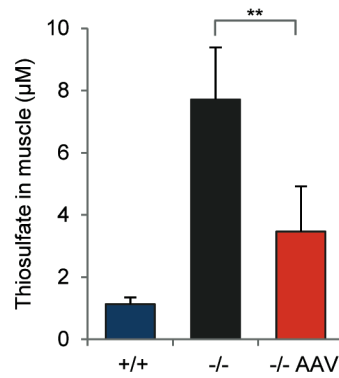
Supplemental Figure 2. Characterization of *Ethel1*^{-/-} mice infected with 4x10¹² vg/Kg AAV2/8-TBG-*h.Ethel1*^{HA}. **(A)** Liver-specific expression of the *h.Ethel1*^{HA} gene product using an anti-Ethel1 antibody; SDH-A is used as a protein load standard. **(B)** SDO activity, expressed as nmol O₂/min/mg) in *Ethel1*^{+/+} (+/+), *Ethel1*^{-/-} (-/-), and AAV-treated *Ethel1*^{-/-} (-/- AAV) liver homogenates. **(C)** Kaplan-Meier survival probability curve. Significance was assessed by the log-rank test. Blue line: *Ethel1*^{-/-} mice; Red line: NAC-treated *Ethel1*^{-/-} mice; Yellow line: AAV+NAC-treated *Ethel1*^{-/-} mice; the curves of NAC-treated and AAV+NAC-treated *Ethel1*^{-/-} mice do not differ significantly; the difference with the untreated group is p<0.0001. **(D)** Plasma thiosulfate: the levels in AAV+NAC-treated *Ethel1*^{-/-} (-/- AAV) mice do not significantly differ from those in NAC-only treated (-/- NAC) animals. The difference with the untreated group is p<0.0001 (unpaired, two-tail Student's *t* test). See main text for details.

Supplemental Figure 3



Supplemental Figure 3. Kaplan-Meier survival probability curves. **(A)** Blue line: untreated *Ethel*^{-/-} mice (n=15); Red line: NAC-treated *Ethel*^{-/-} mice (n=10); Yellow line: AAV+NAC-treated *Ethel*^{-/-} mice (n=10). NAC was administered throughout the experiment. **(B)** Blue line: untreated *Ethel*^{-/-} mice; Red line: NAC-treated *Ethel*^{-/-} mice (n=7); Yellow line: AAV+NAC-treated *Ethel*^{-/-} mice (n=10). NAC was suspended at P28. The two curves of NAC-treated *Ethel*^{-/-} mice are not significantly different.

Supplemental Figure 4



Supplemental Figure 4. Thiosulfate in skeletal muscle. Blue bar: *Ethe*^{+/+} control mice (n=3); Black bar: untreated *Ethe1*^{-/-} mice (n=3); Red bar: AAV-treated *Ethe1*^{-/-} mice (n=5). ** Unpaired, two-tail Student's *t* test p= 0.0006.

Supplemental Table I

Tissue	Genotype	COX/CS*
Muscle	+/+	99.7 ± 5.0
	-/-	22.7 ± 2.1
	-/- AAV	46.4 ± 6.0
Brain	+/+	60.7 ± 5.5
	-/-	16.7 ± 2.5
	-/- AAV	32.8 ± 4.7

Values are expressed as means ± SD. +/+ : *Ethe*^{+/+} control mice (n=3); -/- : untreated *Ethe1*^{-/-} mice (n=3); -/- AAV: AAV-treated *Ethe1*^{-/-} mice (n=5)

*Student's *t* test between each group in each tissue p<0.001