## Supplementary Information

## Sulfide catabolism ameliorates hypoxic brain injury

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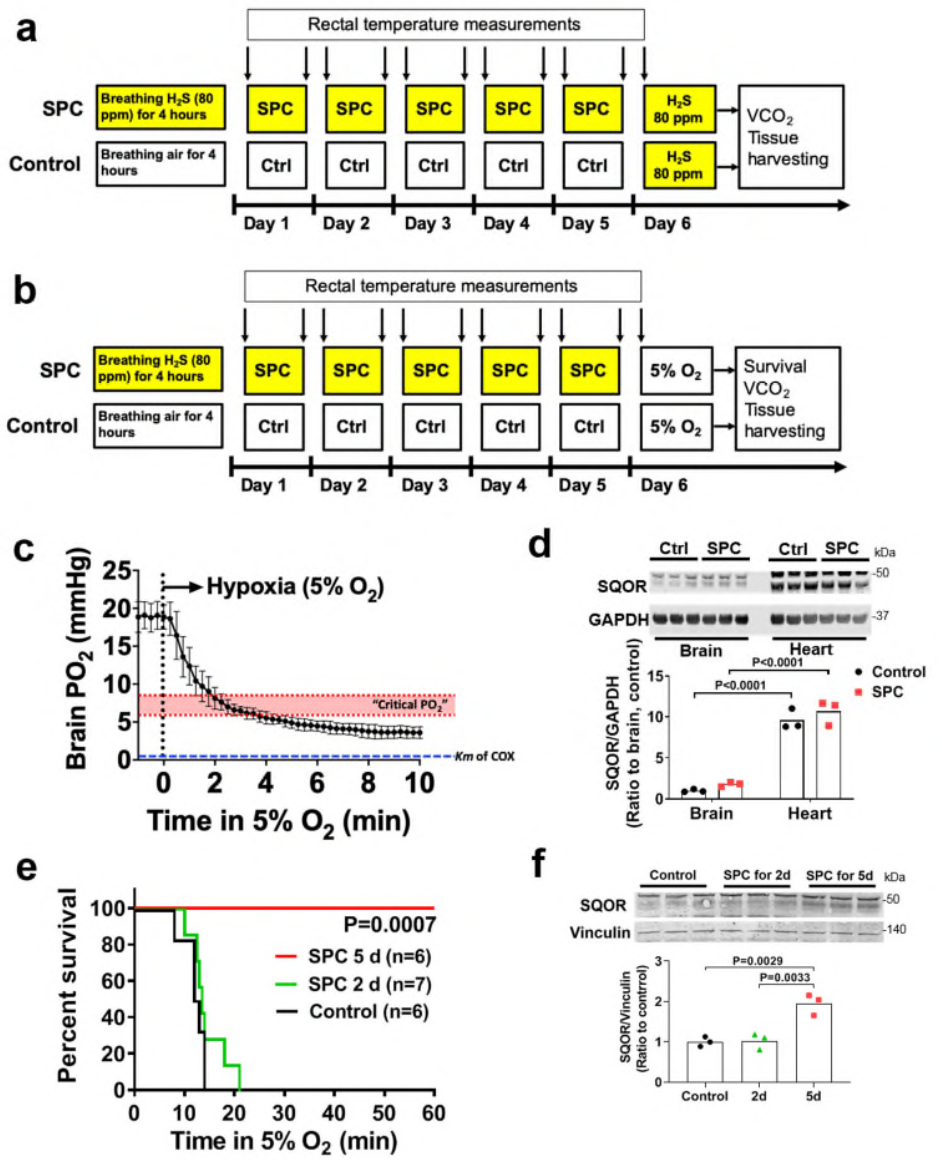
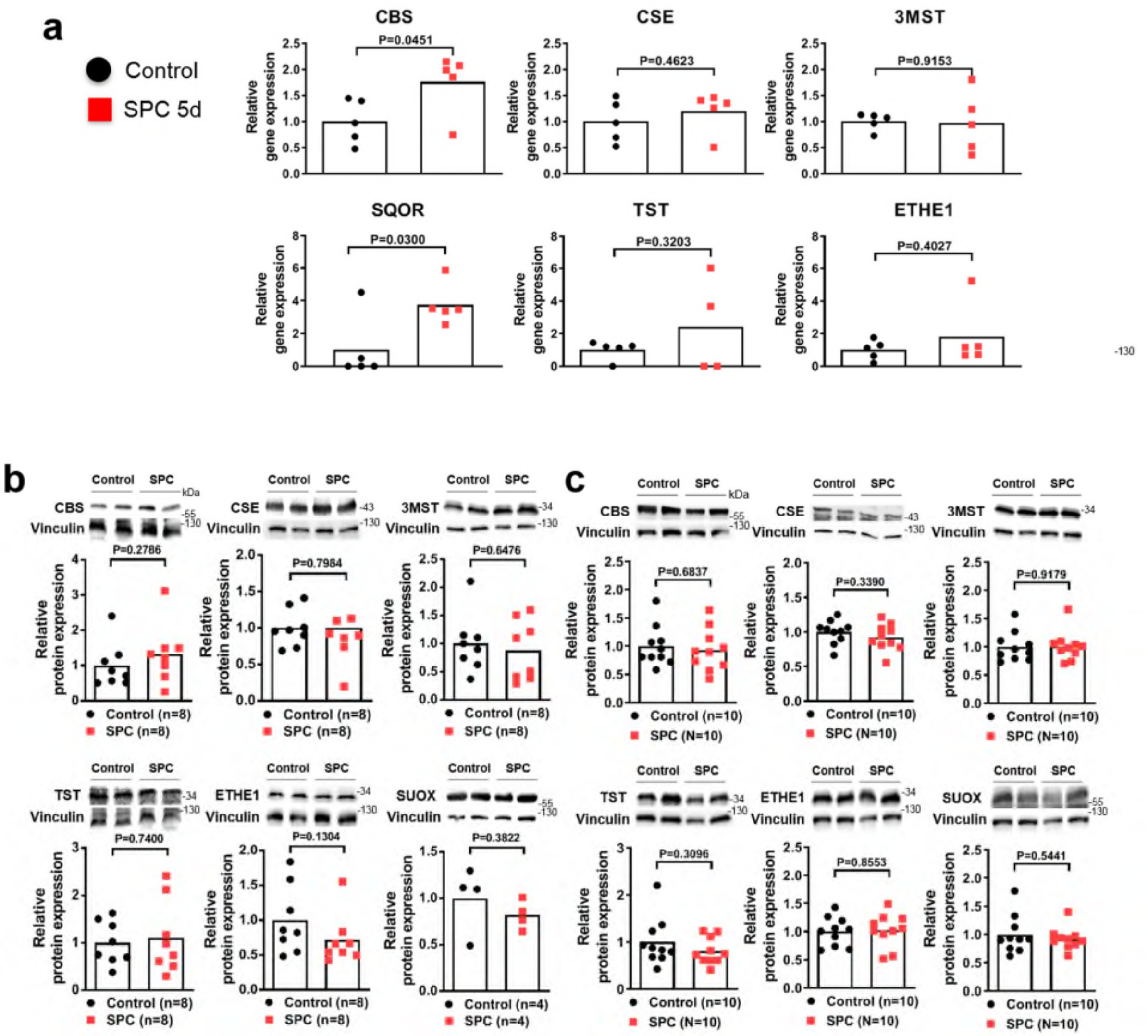
## Supplementary figures

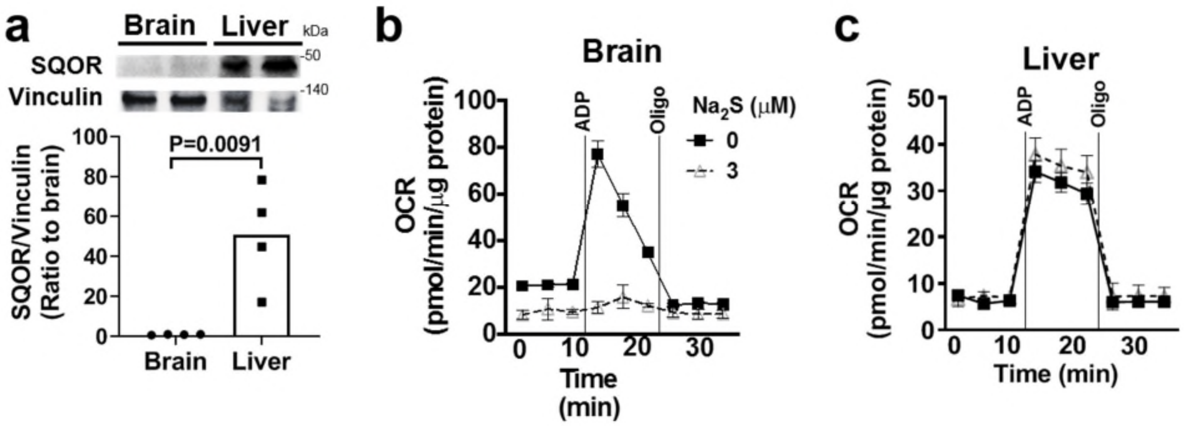



**Supplementary Fig. 1: Effects of sulfide pre-conditioning. a** Schematic representation of protocols of $H_2S$ inhalation after sulfide preconditioning (SPC) and **b** hypoxia breathing after SPC. **c** Change of brain tissue $PO_2$ in mice ventilated with 5% oxygen under isoflurane anesthesia (n = 3). Data are shown as mean ± SD. Critical $PO_2$ = 6-8 mmHg at which brain tissue ATP levels start decreasing rapidly. Km of COX; Km value of cytochrome c oxidase for oxygen. **d** Levels of SQOR in the brain and heart of control or SPC mice (n = 3 each). **e** Survival curve of control mice and mice treated with SPC 2 days or SPC 5 days in 5% oxygen. **f** Brain SQOR levels in control mice and mice treated with SPC 2 days (2d, n = 3) or SPC 5 days (5d, n = 3). Data are presented as mean± SEM or mean and individual values. Two-way ANOVA followed by Sidak's correction or One-way ANOVA followed by Tukey's correction was performed for **d** and **f**, respectively. Survival rates were estimated using the Kaplan-Meier method and log-rank test was used to compare the survival curves between groups for panel **e**.



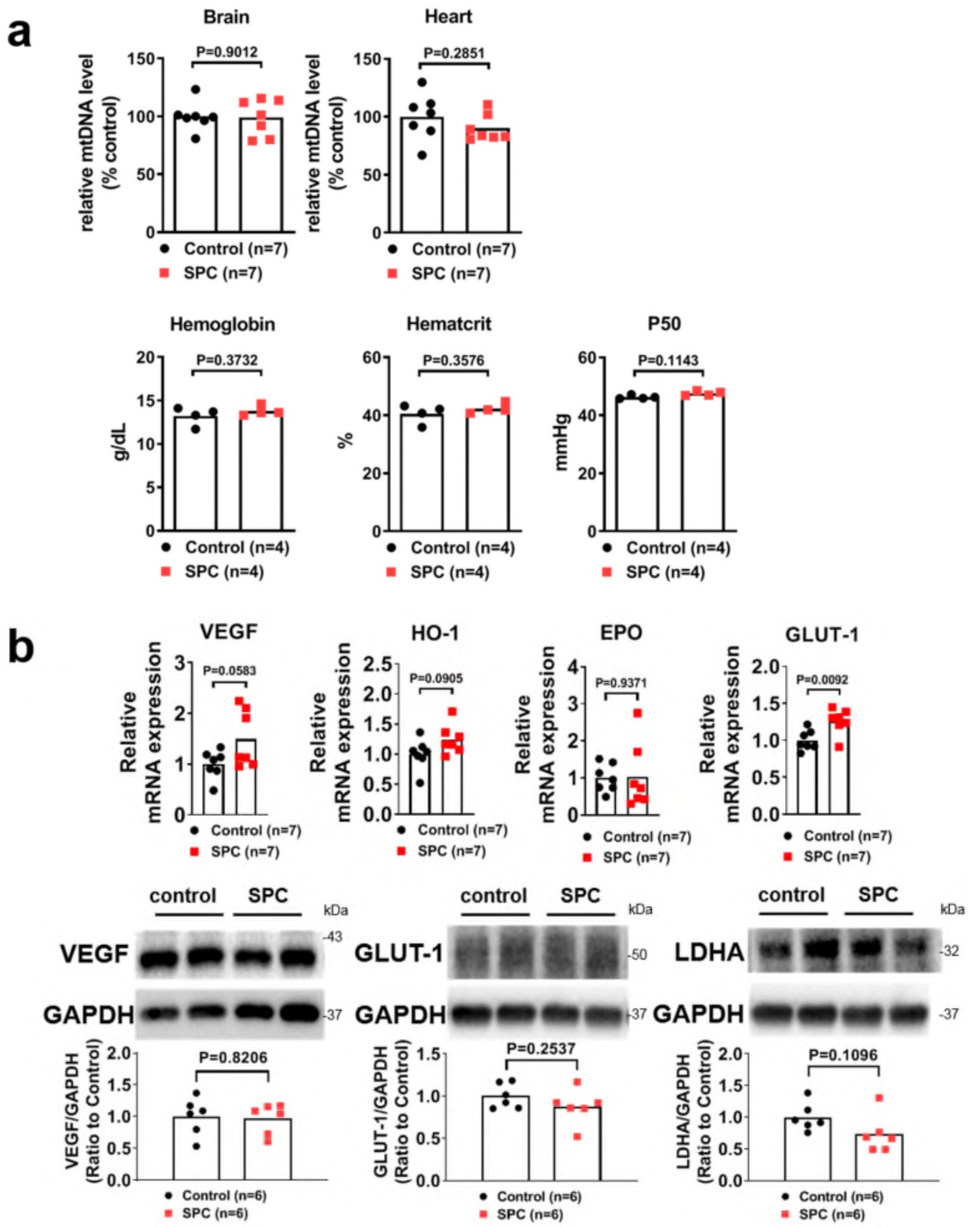

**Supplementary Fig. 2: Effects of sulfide pre-conditioning on levels of enzymes that metabolize sulfide**

**a** Relative mRNA levels of enzymes that synthesize or metabolize sulfide in brains in control mice and mice subjected to sulfide pre-conditioning (SPC). n = 5 each except TST. n = 5 and 4 for TST. CBS, cystathionine beta synthase. CSE, cystathionine gamma lyase. 3MST, 3-mercaptopyruvate sulfurtransferase TST, thiosulfate sulfurtransferase. ETHE1, ethylmaronic encephalopathy 1 (persulfide dioxygenase). **b** Representative immunoblot images and summary graphs of protein levels of enzymes in the brain that synthesize or metabolize sulfide in control mice and mice subjected to SPC. SUOX, sulfite oxidase. **c** Representative immunoblot images and summary graphs of protein levels of enzymes in the heart that synthesize or metabolize sulfide in control mice and mice subjected to sulfide SPC. Data are presented as mean and individual values. Two-tailed unpaired *t*-test was performed.



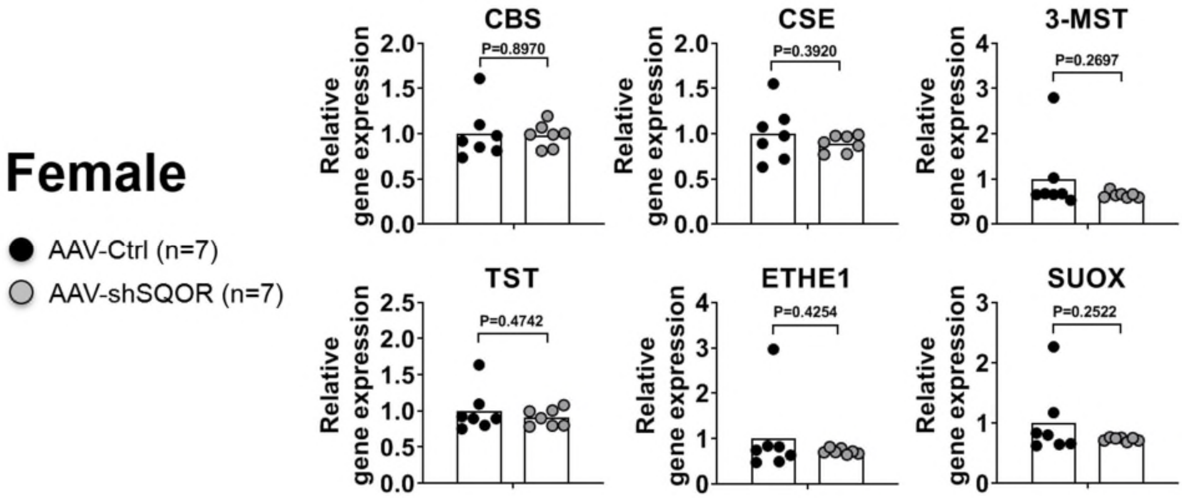

**Supplementary Fig. 3: Comparison of SQOR levels between brain and liver**

**a** Representative immunoblots and summary graph of the levels of SQOR in the brain and liver of naïve mice (n = 4 each). **b** Oxygen consumption rate (OCR) of isolated brain (n = 7 each) or **c** liver (n = 5 each) mitochondria from naïve mice with or without incubation with sulfide ($Na_2S$, 0, 3.0 µM). Data are presented as mean± SEM or mean and individual values. Two-tailed unpaired *t*-test was performed for **a**.

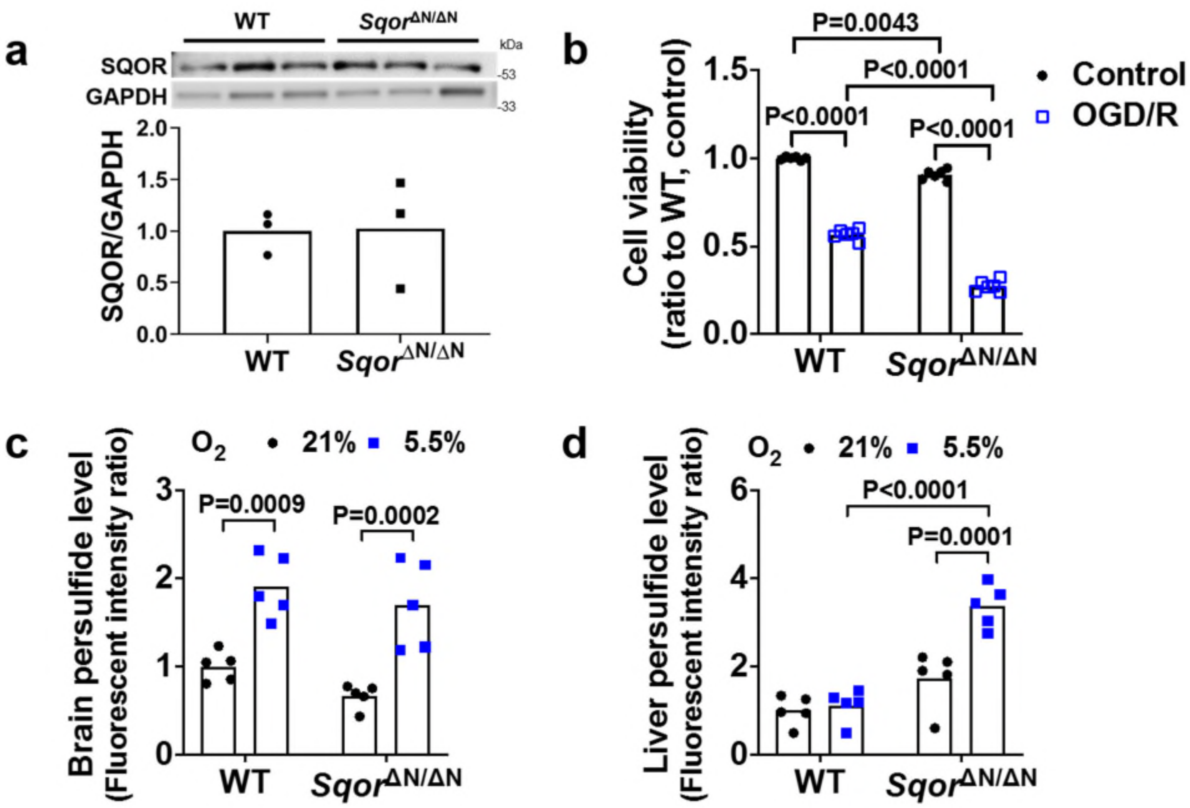



**Supplementary Fig. 4: Other potential effects of sulfide pre-conditioning**

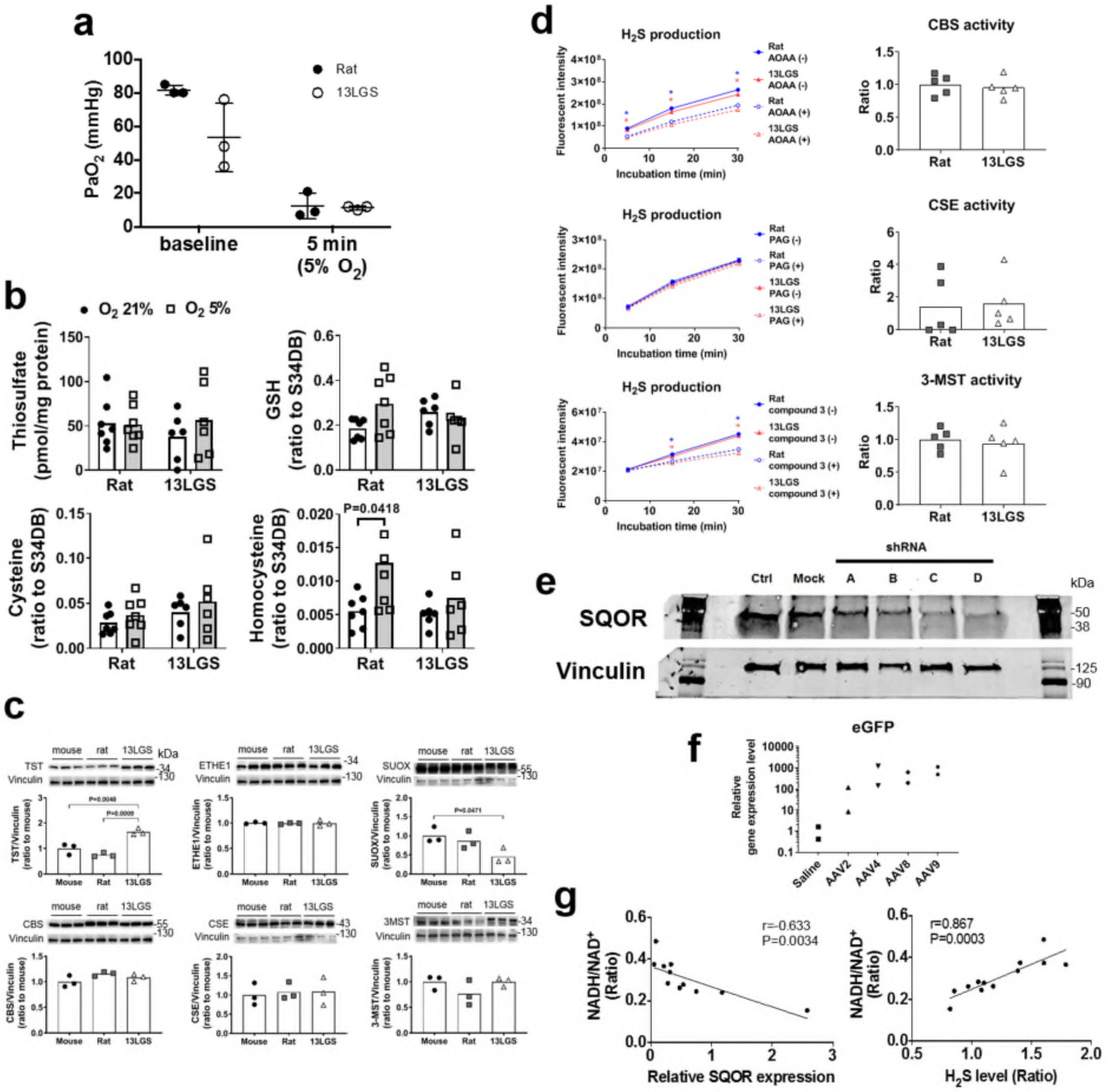
**a** Effects of sulfide pre-conditioning (SPC) on mitochondrial DNA levels and red blood cells. Relative mitochondrial DNA levels in the brain and heart in control mice and mice subjected to SPC. Hemoglobin levels, hematocrit, and P50 of oxygen dissociation curve in control mice and mice subjected to SPC. **b** Effects of SPC on hypoxia inducible factor 1-dependent signaling. Relative gene expression levels of vascular endothelial growth factor (VEGF), homoxygenase-1 (HO-1), erythropoietin (EPO), glucose transporter 1 (GLUT1) and lactate dehydrogenase A (LDHA) in the brain in control mice and mice subjected to SPC. Protein levels of VEGF and GLUT1 in the brain in control mice and mice subjected to SPC. Data are presented as mean and individual values. Two-tailed unpaired *t*-test was performed.



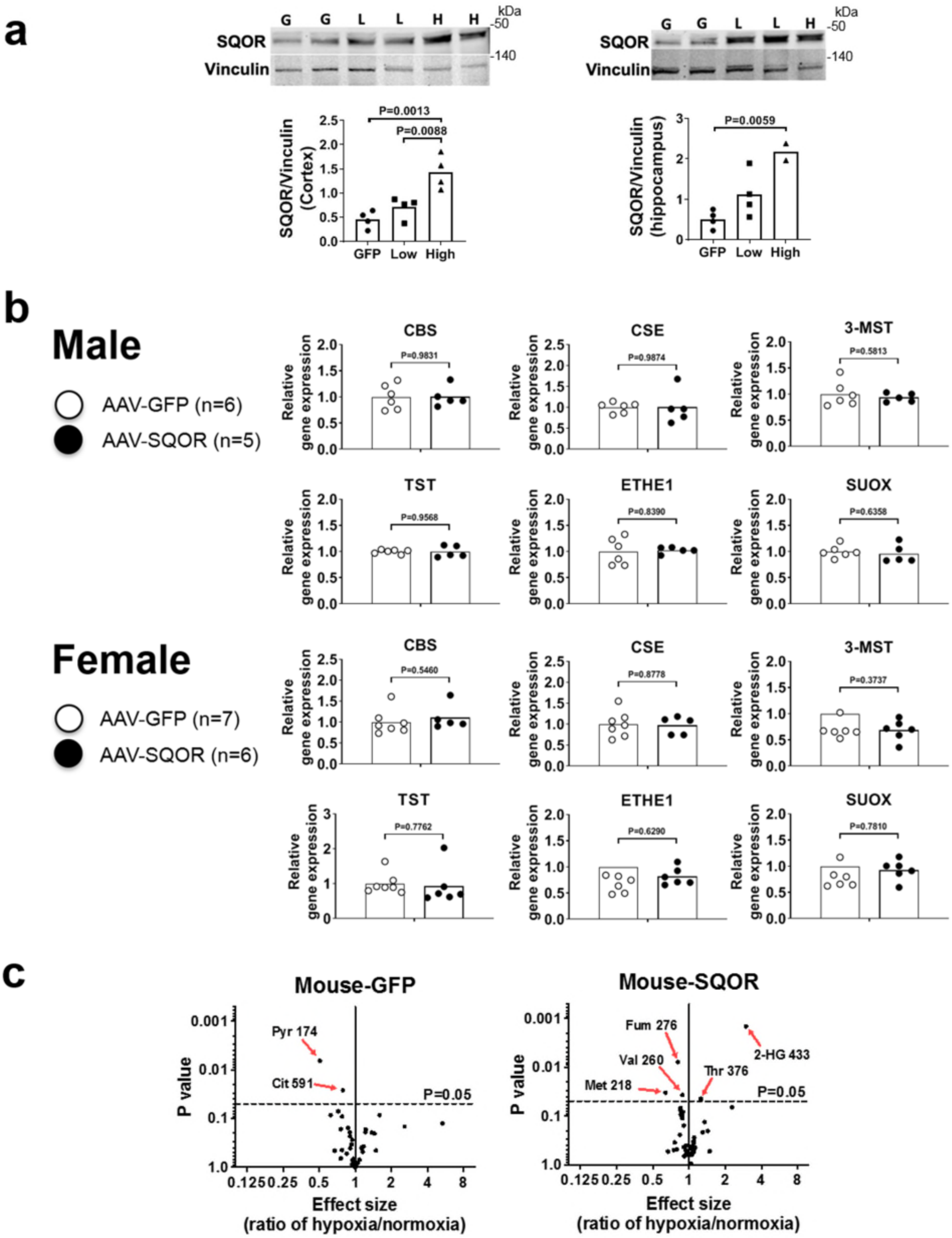

**Supplementary Fig. 5**: **Relative mRNA levels of enzymes that synthesize or metabolize sulfide in brains of adult female CD-1 mice infected with AAV-Ctrl or AAV-shSQOR.** CBS, cystathionine beta synthase. CSE, cystathionine gamma lyase. 3MST, 3-mercaptopyruvate sulfurtransferase. TST, thiosulfate sulfurtransferase. ETHE1, ethylmaronic encephalopathy 1 (persulfide dioxygenase). SUOX, sulfite oxidase. Data are presented as mean and individual values. Two-tailed unpaired *t*-test was performed.




**Supplementary Fig 6**: **Effects of exclusion of SQOR from mitochondria. a** Immunoblots and densitometric quantification of SQOR levels in whole cell lysates of WT or *Sqor*$^{\Delta N/\Delta N}$ MEF cells using GAPDH as a loading control (n = 3 each). **b** Cell viability of primary cortical neurons obtained from WT or *Sqor*$^{\Delta N/\Delta N}$ mice subjected to oxygen glucose deprivation and reoxygenation (OGD/R) (*n* = 6 each). **c** Persulfide levels in brain (n = 5 each) and **d** liver (n = 5 each) of *Sqor*$^{\Delta N/\Delta N}$ mice and their wild type littermates breathing 21% or 5% oxygen. Data are presented as mean and individual values. Two-tailed unpaired *t*-test was performed for **a**. Two-way ANOVA followed by Sidak's correction was performed for panel **b**-**d**.

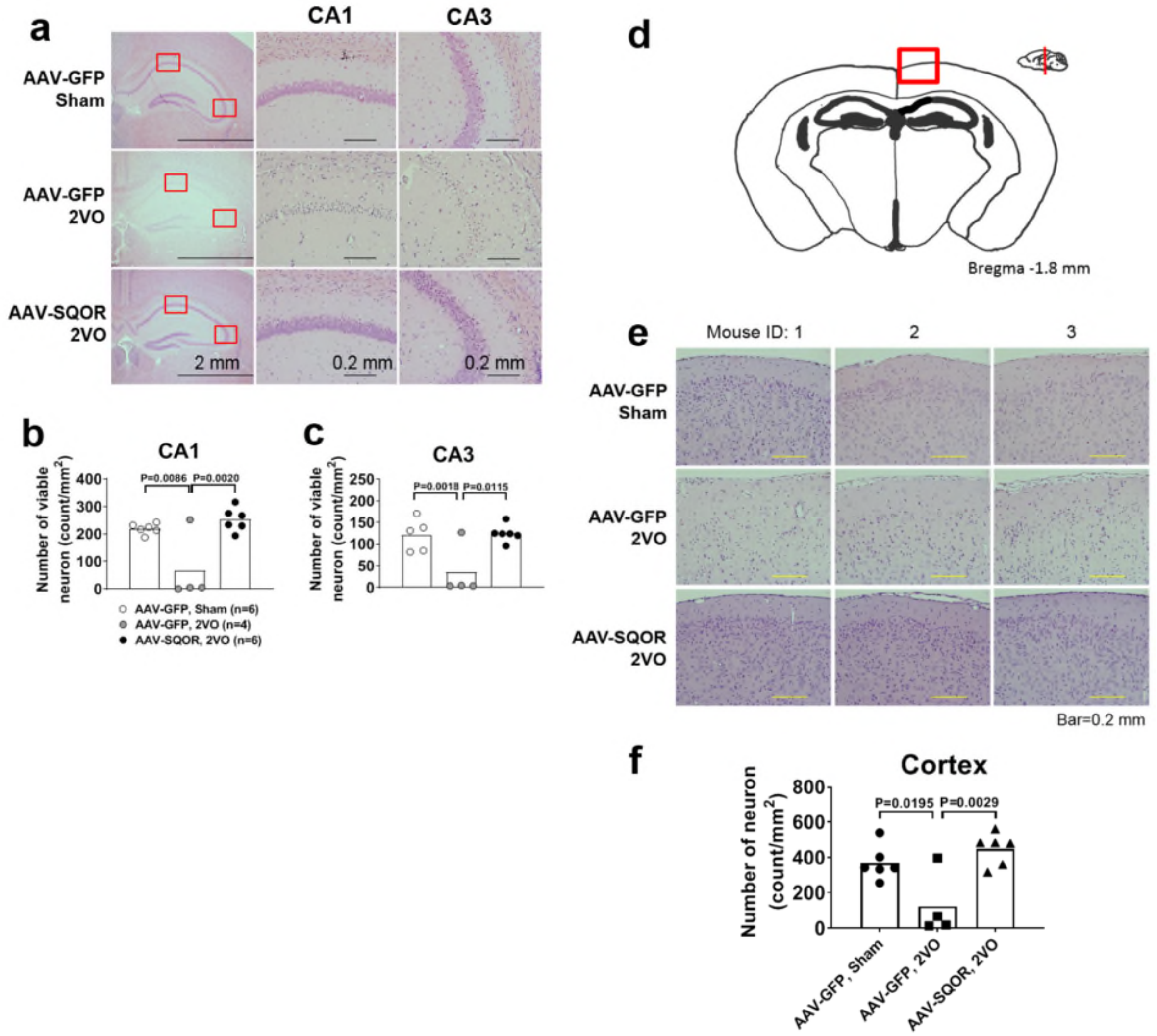



**Supplementary Fig. 7**: **13LG squirrel model**. **a** Changes in $PaO_2$ in rats and 13LG squirrels (13LGS) at baseline and after 5 min breathing 5% oxygen (n = 3 each). **b** Levels of thiosulfate, GSH, cysteine, and homocysteine in the brains of rats and 13LG squirrels after breathing 21% or 5% oxygen for 5 min (*n* = 7, 7, 6, 6). **c** Representative immunoblot images and summary graphs of protein expression levels of enzymes that synthesize or metabolize sulfide in brains of mouse, rat and 13LGS (n = 3 each). TST, thiosulfate sulfurtransferase. ETHE1, ethylmaronic encephalopathy 1 (persulfide dioxygenase). SUOX, sulfite oxidase. CBS, cystathionine beta synthase. CSE, cystathionine gamma lyase. 3MST, 3-mercaptopyruvate sulfurtransferase. **d** Enzyme activities of CBS, CSE, and 3MST in brains of rat and 13LGS (n = 5 each). AOAA, aminooxyacetic acid, CBS inhibitor; PAG, D,L-propargylglycine, CSE inhibitor; compound 3, 3MST inhibitor. **e** Knockdown effects of shRNA transfection on SQOR protein levels in primary myoblasts of 13LGS (representative image of 2 independent experiments). Protein levels of SQOR were measured at 48h after transfection by immunoblotting. **f** Brain GFP expression levels in 13LGS with ICV injection of AAV. To determine the effective AAV serotype to induce gene transfer in 13LGS, AAV serotype 2, 4, 8, or 9 was injected ICV and brain GFP expression levels were compared using qPCR after 1 week (n = 2 each). **g** Correlation between gene expression level of SQOR and NADH/$NAD^+$ ratio and between relative $H_2S$ level and NADH/$NAD^+$ ratio in the brain of 13LGS infected with AAV after breathing 5% oxygen for 5 min (n = 12). Data are presented as mean ± SEM or mean and individual values. Two-way ANOVA followed by Sidak's correction or one-way ANOVA followed by Tukey's correction was performed for **b** and **c**. Two-tailed unpaired *t*-test was performed for **d**. Two-tailed Pearson's coefficients were calculated for **g**.

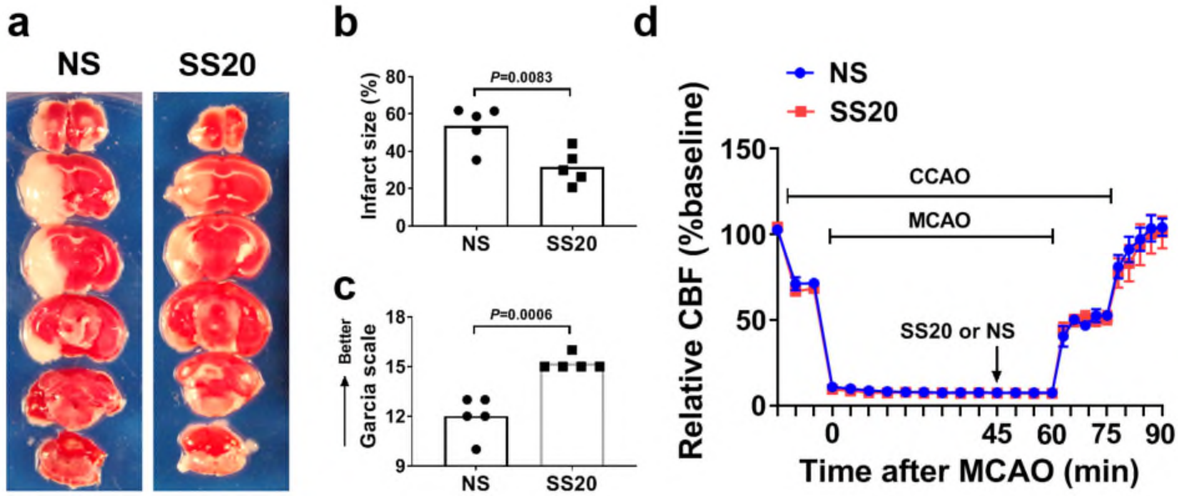



**Supplementary Fig. 8: Effects of SQOR expression in the brain of mice.** **a** SQOR protein levels in the cortex and hippocampus of the brains of mice that received AAV-GFP (G) or AAV-SQOR at

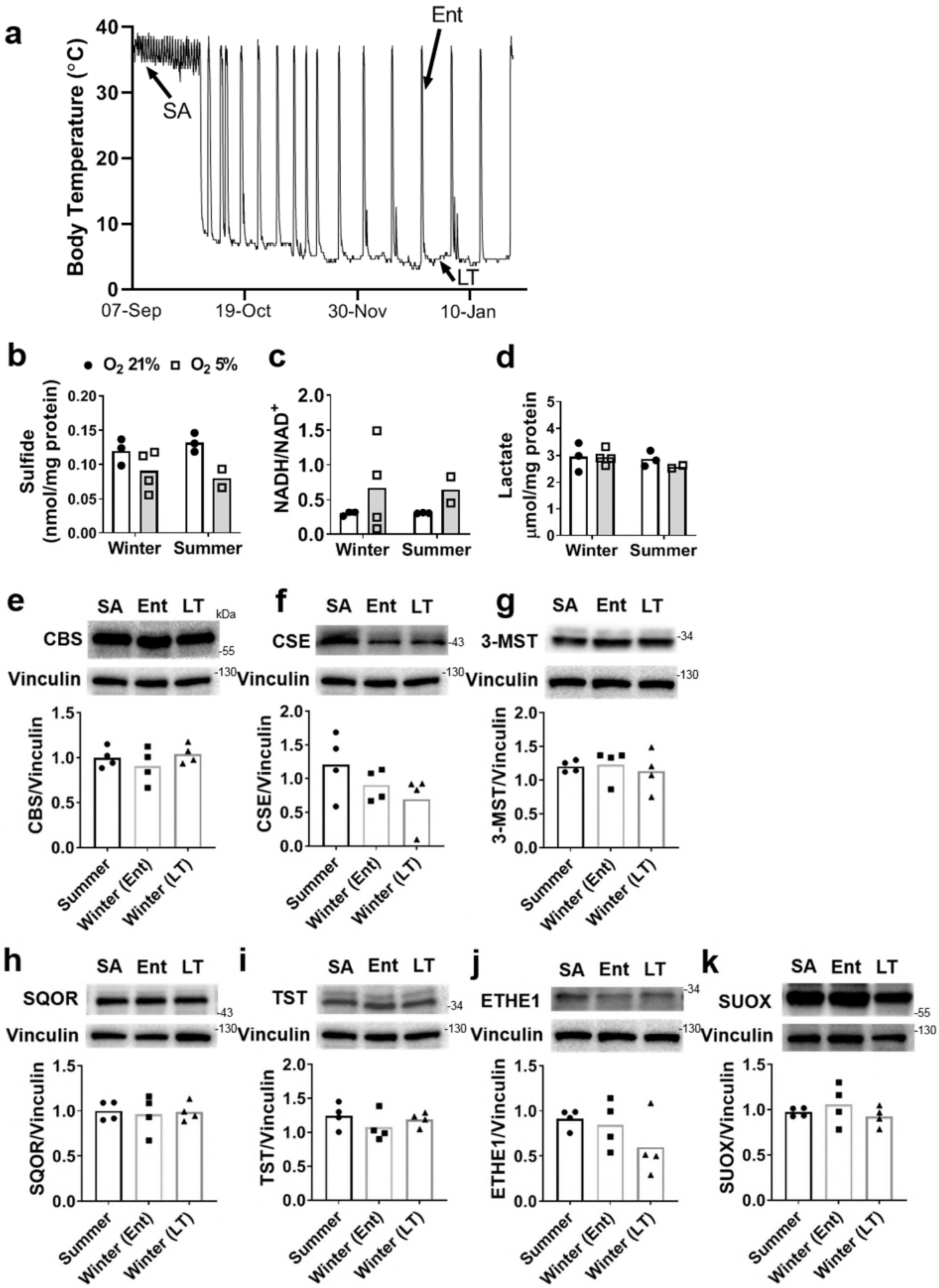
Low (L, $10^9$) or High (H, $10^{10}$) viral particles per hemisphere. **b** Relative gene expression levels of enzymes that synthesize or metabolize sulfide in brains of adult male and female CD-1 mice infected with AAV-GFP or AAV-SQOR. CBS, cystathionine beta synthase. CSE, cystathionine gamma lyase. 3MST, 3-mercaptopyruvate sulfurtransferase. TST, thiosulfate sulfurtransferase. ETHE1, ethylmaronic encephalopathy 1 (persulfide dioxygenase). SUOX, sulfite oxidase. **c** Volcano plots showing the changes in whole brain metabolite profiles in response to breathing 5.5 % oxygen in male CD-1 mice infected with AAV-GFP or AAV-SQOR. 2-HG, 2-hydroxyglutarate; Fum, fumarate; Lac, lactate; Met, methionine; Thr, threonine; Val, valine. Volcano plots were created using values in Table S4 and S5. One-way ANOVA followed by Tukey's correction was performed for **a**. Two-tailed unpaired *t*-test was performed for **b**.




**Supplementary Fig. 9: SQOR ameliorates ischemic brain injury in mice. A** Representative photomicrographs of H&E-stained brain sections focusing on hippocampal CA1 and CA3 regions of male mice transfected with AAV-GFP and subjected to sham surgery (n = 6) or 2VO and reperfusion (n = 4) or AAV-SQOR and subjected to 2VO and reperfusion (n = 6). Number of viable neurons in **b** CA1 (n = 6, 4, 6.) and **c** CA3 (n = 5, 4, 6.) regions of mice transfected with AAV-GFP or AAV-SQOR and subjected to sham surgery or 2VO. **d** Mouse brain atlas showing the part of the brain cortex examined. **E** Representative photomicrograph of H&E-stained brain sections focusing on brain cortex of male mice transfected with AAV-GFP and subjected to sham surgery (n = 6) or 2VO and reperfusion (n = 4) or AAV-SQOR and subjected to 2VO and reperfusion (n = 6). **f** Summary of the number of viable neurons in brain cortex in mice subjected to sham operation or 2VO with or without SQOR expression (n = 6, 4, 6). Data are presented as mean and individual values. One-way ANOVA followed by Tukey's correction was performed for panel **a**, **c**, and **f**.




**Supplementary Fig. 10**: **Sulfide scavenger ameliorates ischemic brain injury in mice. a** Representative images of brain sections stained with TTC, **b** summary of infarct volume, and **c** neurological functional score after 60 min of transient MCAO and 48h of reperfusion in mice treated with normal saline or specific sulfide scavenger SS-20. **d** Relative cerebral blood flow in mice treated with normal saline or SS-20 15 min after the onset of ischemia. n = 5 each. Data are presented as mean and individual values or mean ± SEM. Two-tailed unpaired *t*-test was performed for **b** and **c**.


a
Body Temperature (°C)
40
30
20
10
0
07-Sep
19-Oct
30-Nov
10-Jan
SA
Ent
LT
b
O2 21%
O2 5%
Sulfide (nmol/mg protein)
Winter
Summer
c
NADH/NAD+
Winter
Summer
d
Lactate µmol/mg protein
Winter
Summer
e
SA Ent LT
kDa
CBS
Vinculin
CBS/Vinculin
Summer
Winter (Ent)
Winter (LT)
f
SA Ent LT
CSE
Vinculin
CSE/Vinculin
g
SA Ent LT
3-MST
Vinculin
3-MST/Vinculin
h
SA Ent LT
SQOR
Vinculin
SQOR/Vinculin
i
SA Ent LT
TST
Vinculin
TST/Vinculin
j
SA Ent LT
ETHE1
Vinculin
ETHE1/Vinculin
k
SA Ent LT
SUOX
Vinculin
SUOX/Vinculin

**Supplementary Fig. 11: Sulfide metabolizing enzymes in summer and winter 13LG squirrels. a** Annual body temperature trace of a 13LG squirrels in the laboratory, housed in a 4°C hibernaculum during the winter season. SA, summer active; Ent, Entrance to torpor bout; LT, late torpor during deep torpor. Brain levels of **b** sulfide, **c** NADH/NAD+ ratio, and **d** lactate of 13LGS breathed air or 5%$O_2$ for 5 min in winter or summer. n = 3, 4, 3, 2. **e-k** Representative immunoblot images and summary graphs of protein expression for enzymes in the brain that synthesize or metabolize sulfide in 13LG squirrels at three timepoints across the hibernation cycle. n = 4 each. Sampling timepoints are defined by body temperature and time of year depicted in **a**; summer active (SA) animals were sacrificed in August during the summer homeothermic period, Entrance (Ent) animals were collected during entrance into a torpor bout when body temperature was 27-23°C, late torpor (LT) animals were collected during deep torpor with body temperature near 4°C, typically after 7-10 days when the animal's bout reached 80-95% of the duration of its previous torpor bout. CBS, cystathionine beta synthase. CSE, cystathionine gamma lyase. 3MST, 3-mercaptopyruvate sulfurtransferase. SQOR, sulfide:quinone oxidoreductase. TST, thiosulfate sulfurtransferase. ETHE1, ethylmaronic encephalopathy 1 (persulfide dioxygenase). SUOX, sulfite oxidase. Data are presented as mean and individual values. Two-way ANOVA followed by Sidak's correction or one-way ANOVA with Tukey's correction was performed for panel **b**-**d** and **e**-**k**, respectively.

**Supplementary tables**

**Supplementary Table 1.** Mean ion counts normalized with AUC of brain metabolites measured by GC-MS in rats. n = 7 in each $O_2$ concentration. P-values were obtained by multiple *t*-test.

| Ion species | 21% $O_2$ | 5% $O_2$ | p-value |
|---|---|---|---|
| Pyr_174 | 6.50849e-005 | 5.70086e-005 | 0.651026 |
| Lac_223 | 0.00207663 | 0.00326733 | 0.013447 |
| Lac_261 | 0.00243856 | 0.00505582 | 0.012296 |
| Ala 232 | 0.000296135 | 0.000397022 | 0.124424 |
| Ala 260 | 0.000253092 | 0.000332558 | 0.136735 |
| Gly 218 | 0.000385092 | 0.000387856 | 0.957862 |
| Gly 246 | 0.000325312 | 0.000323455 | 0.965953 |
| Fum 287 | 0.000226795 | 0.000354567 | 0.007616 |
| Ser 288 | 0.000570278 | 0.000590967 | 0.82665 |
| Ser 302 | 0.000314642 | 0.000327459 | 0.806602 |
| Ser 362 | 0.00051255 | 0.000538882 | 0.765693 |
| Akg 346 | 5.85783e-006 | 4.42474e-006 | 0.356106 |
| Mal 419 | 0.000273941 | 0.000322859 | 0.304859 |
| Asp 302 | 0.00154742 | 0.00146013 | 0.661361 |
| Asp 390 | 0.000617294 | 0.000589045 | 0.7384 |
| Asp 418 | 0.00133539 | 0.00125778 | 0.669871 |
| Glu 330 | 0.00413031 | 0.00330576 | 0.475835 |
| Glu 432 | 0.0072204 | 0.00665123 | 0.414885 |
| Gln 431 | 0.000738515 | 0.00119148 | 0.146789 |
| Cit 459 | 0.000174346 | 0.000137175 | 0.156331 |
| Cit 591 | 0.000116221 | 9.0486e-005 | 0.134994 |
| Pro 330 | 3.71348e-005 | 3.73476e-005 | 0.954313 |
| Pro 258 | 3.22401e-005 | 1.58194e-005 | 0.049187 |
| Val 260 | 3.44283e-005 | 4.1471e-005 | 0.285143 |
| Val 288 | 2.54186e-005 | 3.01869e-005 | 0.320469 |
| Leu 200 | 0.000102373 | 0.000134716 | 0.095964 |
| Leu 274 | 3.89868e-005 | 5.23065e-005 | 0.088079 |
| Leu 302 | 3.31687e-005 | 4.38997e-005 | 0.096732 |
| Leu 344 | 1.95873e-006 | 2.56482e-006 | 0.117771 |
| Lie 200 | 4.1567e-005 | 5.60327e-005 | 0.099981 |
| Lie 274 | 1.64376e-005 | 2.24511e-005 | 0.096407 |
| Lie 302 | 2.01567e-005 | 2.71345e-005 | 0.095806 |
| Lie 344 | 8.2475e-007 | 1.05877e-006 | 0.206482 |
| Thr 376 | 2.63361e-005 | 3.56543e-005 | 0.190794 |
| Thr 404 | 4.53716e-005 | 6.08905e-005 | 0.198932 |
| Met 218 | 2.3905e-005 | 2.86905e-005 | 0.35611 |

| | | | |
|---|---|---|---|
| Met 292 | 0.00210067 | 0.00133401 | 0.568965 |
| Met 320 | 1.736e-005 | 2.06092e-005 | 0.306421 |
| Phe 234 | 2.61922e-005 | 3.23615e-005 | 0.308761 |
| Phe 302 | 3.56892e-005 | 4.48983e-005 | 0.251851 |
| Phe 308 | 1.76954e-005 | 2.25202e-005 | 0.253396 |
| Phe 336 | 2.90238e-005 | 2.80851e-005 | 0.55359 |
| Cys 406 | 1.0156e-005 | 6.09951e-006 | 0.473979 |
| Cys 378 | 0.00014205 | 0.000559118 | 0.032777 |
| Tyr 302 | 0.000158229 | 0.0001915 | 0.422149 |
| Tyr 364 | 1.63481e-005 | 2.06229e-005 | 0.341308 |
| Tyr 466 | 2.28959e-005 | 2.8203e-005 | 0.379394 |
| KMV 258 | 3.10446e-005 | 2.57922e-005 | 0.051912 |
| KMV 216 | 8.5379e-007 | 7.39375e-007 | 0.471514 |
| KIC 258 | 3.4541e-005 | 3.05453e-005 | 0.176268 |
| KIC 216 | 1.113e-006 | 3.80472e-007 | 0.246484 |
| 2-HG 433 | 1.8076e-005 | 3.05519e-005 | 0.066508 |
| Suc 289 | 0.00018282 | 0.000270558 | 0.032552 |

**Supplementary Table 2.** Mean ion counts normalized with AUC of brain metabolites measured by GC-MS in 13LG squirrels. n = 4 and 6 in 21% and 5% $O_2$, respectively. P-values were obtained by multiple *t*-test.

| Ion species | 21% $O_2$ | 5% $O_2$ | p-value |
|---|---|---|---|
| Pyr_174 | 8.09277e-005 | 6.36684e-005 | 0.545939 |
| Lac_223 | 0.00197282 | 0.00285291 | 0.154553 |
| Lac_261 | 0.00309138 | 0.00444642 | 0.157632 |
| Ala 232 | 0.000296238 | 0.000378651 | 0.123836 |
| Ala 260 | 0.000252617 | 0.000317967 | 0.121951 |
| Gly 218 | 0.000284217 | 0.000326946 | 0.290805 |
| Gly 246 | 0.000239093 | 0.000272902 | 0.310442 |
| Fum 287 | 0.000331525 | 0.000369185 | 0.10473 |
| Ser 288 | 0.000499248 | 0.000467855 | 0.732694 |
| Ser 302 | 0.00027458 | 0.000257181 | 0.731903 |
| Ser 362 | 0.000450396 | 0.000423199 | 0.752393 |
| Akg 346 | 9.86086e-006 | 9.10528e-006 | 0.838412 |
| Mal 419 | 0.000352147 | 0.000413016 | 0.332212 |
| Asp 302 | 0.00105998 | 0.000783834 | 0.146989 |
| Asp 390 | 0.000425112 | 0.000312943 | 0.159099 |
| Asp 418 | 0.000907128 | 0.0006691 | 0.162115 |
| Glu 330 | 0.00424721 | 0.00427514 | 0.936842 |
| Glu 432 | 0.00671319 | 0.00676504 | 0.923456 |
| Gln 431 | 0.00114892 | 0.00115654 | 0.98526 |
| Cit 459 | 0.000276084 | 0.000226374 | 0.405498 |
| Cit 591 | 0.000183184 | 0.000150265 | 0.401444 |
| Pro 330 | 4.60997e-005 | 5.15606e-005 | 0.296345 |
| Pro 258 | 1.79219e-005 | 1.67094e-005 | 0.468645 |
| Val 260 | 3.60448e-005 | 4.34371e-005 | 0.239119 |
| Val 288 | 2.63329e-005 | 3.1435e-005 | 0.254951 |
| Leu 200 | 8.16983e-005 | 0.00010981 | 0.132769 |
| Leu 274 | 3.17085e-005 | 4.25038e-005 | 0.151474 |
| Leu 302 | 2.68247e-005 | 3.54497e-005 | 0.156856 |
| Leu 344 | 1.48254e-006 | 2.06859e-006 | 0.145074 |
| Lie 200 | 3.75479e-005 | 5.137e-005 | 0.091458 |
| Lie 274 | 1.51079e-005 | 2.06873e-005 | 0.09846 |
| Lie 302 | 1.83958e-005 | 2.50198e-005 | 0.088474 |
| Lie 344 | 6.94705e-007 | 1.0034e-006 | 0.10693 |
| Thr 376 | 2.4128e-005 | 2.82607e-005 | 0.551927 |
| Thr 404 | 4.10756e-005 | 4.78213e-005 | 0.566799 |
| Met 218 | 2.42847e-005 | 2.15023e-005 | 0.62984 |
| Met 292 | 0.00119967 | 0.00221315 | 0.625278 |
| Met 320 | 3.36892e-005 | 1.6461e-005 | 0.142129 |

| | | | |
|---|---|---|---|
| Phe 234 | 2.07365e-005 | 2.25621e-005 | 0.686096 |
| Phe 302 | 2.86321e-005 | 3.13527e-005 | 0.662265 |
| Phe 308 | 1.44024e-005 | 1.5513e-005 | 0.716458 |
| Phe 336 | 2.07673e-005 | 2.00113e-005 | 0.830452 |
| Cys 406 | 1.30451e-005 | 1.82628e-005 | 0.555365 |
| Cys 378 | 0.000802204 | 0.000968002 | 0.671621 |
| Tyr 302 | 0.000157799 | 0.000162267 | 0.906968 |
| Tyr 364 | 1.7974e-005 | 1.81237e-005 | 0.974549 |
| Tyr 466 | 2.33985e-005 | 2.8455e-005 | 0.361553 |
| KMV 258 | 2.53622e-005 | 2.42613e-005 | 0.730993 |
| KMV 216 | 4.9112e-007 | 6.33941e-007 | 0.350748 |
| KIC 258 | 1.5794e-005 | 2.51876e-005 | 0.34791 |
| KIC 216 | 1.2875e-006 | 3.79712e-007 | 0.088175 |
| 2-HG 433 | 6.77557e-005 | 8.63824e-005 | 0.356986 |
| Suc 289 | 0.000176539 | 0.000278869 | 0.013109 |

**Supplementary Table 3.** Target sequences of shRNA for SQOR knockdown in 13LGS.

| shRNA ID | Target sequence |
|---|---|
| **A** | 5'-ATCTTTACCTTCCCAAATACTCC-3' |
| **B** | 5'-AGCCTTTCTTCGGAAATTGTTTC-3' |
| **C** | 5'-GACTGGCTACAACCGTGTGAT-3' |
| **D** | 5'-GGCTACAACCGTGTGATTCTT-3' |

**Supplementary Table 4.** Mean ion counts normalized with AUC of brain metabolites measured by GC-MS in mice with AAV-GFP injection. n = 6 in each $O_2$ concentration. P-values were obtained by multiple *t*-test.

| Ion species | 21% $O_2$ | 5.5% $O_2$ | p-value |
|---|---|---|---|
| Pyr_174 | 0.000539 | 0.000272 | 0.006477 |
| Lac_223 | 0.003137 | 0.003823 | 0.167089 |
| Lac_261 | 0.004795 | 0.005805 | 0.188472 |
| Ala 232 | 0.000238 | 0.00027 | 0.412577 |
| Ala 260 | 0.000194 | 0.001031 | 0.125548 |
| Gly 218 | 0.00025 | 0.00025 | 0.988683 |
| Gly 246 | 0.000205 | 0.000158 | 0.441068 |
| Suc 289 | 0.000576 | 0.000675 | 0.374188 |
| Fum 287 | 0.000736 | 0.00057 | 0.090516 |
| Ser 288 | 0.000402 | 0.000406 | 0.932201 |
| Ser 302 | 0.000218 | 0.000217 | 0.991986 |
| Ser 362 | 0.000347 | 0.000351 | 0.933217 |
| Akg 346 | 5.39E-06 | 4.07E-06 | 0.656861 |
| Mal 419 | 0.000274 | 0.000266 | 0.793683 |
| Asp 302 | 0.00108 | 0.001118 | 0.839385 |
| Asp 390 | 0.00042 | 0.00044 | 0.79014 |
| Asp 418 | 0.000875 | 0.000912 | 0.811702 |
| Glu 330 | 0.004011 | 0.003874 | 0.667459 |
| Glu 432 | 0.006462 | 0.006238 | 0.666911 |
| Gln 431 | 0.001118 | 0.001096 | 0.901008 |
| Cit 459 | 0.000301 | 0.000216 | 0.070415 |
| Cit 591 | 0.000123 | 9.73E-05 | 0.026144 |
| Val 260 | 1.81E-05 | 2.68E-05 | 0.448056 |
| Val 288 | 1.49E-05 | 1.39E-05 | 0.338841 |
| Leu 200 | 0.000155 | 0.00014 | 0.192359 |
| Leu 274 | 3.82E-05 | 3.59E-05 | 0.86643 |
| Leu 302 | 4.69E-05 | 3.54E-05 | 0.164008 |
| Leu 344 | 2.71E-06 | 2.45E-06 | 0.227199 |
| ILe 200 | 0.000106 | 6.57E-05 | 0.085068 |
| ILe 274 | 6.19E-06 | 5.25E-06 | 0.518119 |
| ILe 302 | 3.08E-05 | 2.9E-05 | 0.272191 |
| ILe 344 | 1E-06 | 1.16E-06 | 0.480405 |
| Thr 376 | 1.36E-05 | 1.29E-05 | 0.687262 |
| Thr 404 | 2.32E-05 | 2.23E-05 | 0.745873 |
| Met 218 | 4.97E-05 | 6.79E-05 | 0.194199 |
| Met 292 | 0.003677 | 0.004161 | 0.392086 |
| Phe 234 | 6.56E-05 | 6.42E-05 | 0.929769 |
| Phe 302 | 6.76E-05 | 6.13E-05 | 0.406002 |
| Phe 308 | 0.000159 | 0.000138 | 0.154709 |
| Cys 406 | 3.62E-05 | 5.21E-05 | 0.20179 |

| | | | |
|---|---|---|---|
| Cys 378 | 0.000115 | 0.000297 | 0.14572 |
| Tyr 302 | 0.000248 | 0.000235 | 0.722719 |
| Tyr 364 | 4.19E-05 | 4.54E-05 | 0.700411 |
| Tyr 466 | 3.31E-05 | 5.27E-05 | 0.084794 |
| 2-HG 433 | 0.000121 | 8.08E-05 | 0.4538 |

**Supplementary Table 5.** Mean ion counts normalized with AUC of brain metabolites measured by GC-MS in mice with AAV-SQOR injection. n = 6 in each $O_2$ concentration. P-values were obtained by multiple *t*-test.

| Ion species | 21% $O_2$ | 5.5% $O_2$ | p-value |
|---|---|---|---|
| Pyr_174 | 0.000484 | 0.000437 | 0.435505 |
| Lac_223 | 0.002829 | 0.002998 | 0.549406 |
| Lac_261 | 0.004294 | 0.0045 | 0.631379 |
| Ala 232 | 0.000235 | 0.00025 | 0.588639 |
| Ala 260 | 0.000192 | 0.000203 | 0.616593 |
| Gly 218 | 0.000279 | 0.000234 | 0.242928 |
| Gly 246 | 0.000202 | 0.00016 | 0.481747 |
| Suc 289 | 0.000539 | 0.000507 | 0.623679 |
| Fum 287 | 0.000796 | 0.000642 | 0.007768 |
| Ser 288 | 0.000365 | 0.000376 | 0.602888 |
| Ser 302 | 0.000196 | 0.000205 | 0.462786 |
| Ser 362 | 0.000315 | 0.000332 | 0.416795 |
| Akg 346 | 7.77E-06 | 5.18E-06 | 0.533199 |
| Mal 419 | 0.000286 | 0.000277 | 0.626874 |
| Asp 302 | 0.001028 | 0.000871 | 0.067829 |
| Asp 390 | 0.000405 | 0.000345 | 0.092723 |
| Asp 418 | 0.000843 | 0.000712 | 0.074042 |
| Glu 330 | 0.003848 | 0.00337 | 0.095731 |
| Glu 432 | 0.006185 | 0.005424 | 0.110822 |
| Gln 431 | 0.001265 | 0.001192 | 0.503399 |
| Cit 459 | 0.000351 | 0.000319 | 0.669537 |
| Cit 591 | 0.000134 | 0.000127 | 0.401638 |
| Val 260 | 2.12E-05 | 1.87E-05 | 0.036663 |
| Val 288 | 1.5E-05 | 1.34E-05 | 0.082206 |
| Leu 200 | 0.000152 | 0.000164 | 0.409117 |
| Leu 274 | 4.51E-05 | 4.65E-05 | 0.928291 |
| Leu 302 | 4.65E-05 | 5.04E-05 | 0.365929 |
| Leu 344 | 2.65E-06 | 2.89E-06 | 0.329923 |
| ILe 200 | 6.81E-05 | 7.36E-05 | 0.334069 |
| ILe 274 | 6.44E-06 | 9.52E-06 | 0.52565 |
| ILe 302 | 2.99E-05 | 3.3E-05 | 0.278047 |
| ILe 344 | 1.22E-06 | 9.3E-07 | 0.348401 |
| Thr 376 | 1.43E-05 | 1.8E-05 | 0.044041 |
| Thr 404 | 2.49E-05 | 3.1E-05 | 0.045649 |

| | | | |
|---|---|---|---|
| Met 218 | 6.19E-05 | 3.94E-05 | 0.033052 |
| Met 292 | 0.002331 | 0.003339 | 0.201567 |
| Phe 234 | 6.11E-05 | 8.22E-05 | 0.133413 |
| Phe 302 | 6.81E-05 | 7.25E-05 | 0.500808 |
| Phe 308 | 0.000167 | 0.000151 | 0.155933 |
| Cys 406 | 2.02E-05 | 4.61E-05 | 0.065585 |
| Cys 378 | 0.000148 | 0.000111 | 0.496797 |
| Tyr 302 | 0.000258 | 0.000283 | 0.538845 |
| Tyr 364 | 3.72E-05 | 4.84E-05 | 0.21447 |
| Tyr 466 | 3.46E-05 | 3.88E-05 | 0.440309 |
| 2-HG 433 | 6.68E-05 | 0.000199 | 0.00145 |

**Supplementary Table 6. Breakdown of number of rats and 13LG squirrels used in each experiment.**

**Fig. 4b, sulfide level**

| *Normoxia* | | *Hypoxia* | | *Normoxia* | | | | *Hypoxia* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rat | | Rat | | 13LGS (winter) | | 13LGS (summer) | | 13LGS (winter) | | 13LGS (summer) | |
| Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 3 | 4 | 3 | 4 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 1 |

**Fig. 4c and d, lactate and NADH/NAD$^+$**

| *Normoxia* | | *Hypoxia* | | *Normoxia* | | | | *Hypoxia* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rat | | Rat | | 13LGS (winter) | | 13LGS (summer) | | 13LGS (winter) | | 13LGS (summer) | |
| Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 3 | 3 | 3 | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 1 |

**Fig. 4e and f, volcano plot**

| *Normoxia* | | *Hypoxia* | | *Normoxia* | | | | *Hypoxia* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rat | | Rat | | 13LGS (winter) | | 13LGS (summer) | | 13LGS (winter) | | 13LGS (summer) | |
| Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 3 | 4 | 3 | 4 | 1 | 2 | 1 | 0 | 2 | 2 | 1 | 1 |

**Fig. 4g and Supplementary Fig. 11, protein expression**

| Rat | | 13LGS (winter) | | 13LGS (summer) | |
|---|---|---|---|---|---|
| Male | Female | Male | Female | Male | Female |
| 1 | 2 | 1 | 2 | 0 | 0 |

**Fig. 4h and Supplementary Fig. 12, enzymatic activity**

| Enzyme | Rat | | 13LGS (winter) | | 13LGS (summer) | |
|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female |
| **SQOR** | 3 | 3 | 1 | 2 | 1 | 2 |
| **CBS** | 3 | 2 | 1 | 2 | 1 | 1 |
| **CSE** | 3 | 2 | 1 | 2 | 1 | 1 |
| **3-MST** | 3 | 2 | 1 | 2 | 1 | 1 |

**Fig. 4, i and j, mitochondrial OCR**

| Rat | | 13LGS (winter) | | 13LGS (summer) | |
|---|---|---|---|---|---|
| Male | Female | Male | Female | Male | Female |
| 1 | 5 | 0 | 0 | 2 | 4 |

**Fig. 4, l-o, SQOR expression, sulfide, persulfide and NADH/NAD$^+$**

| 13LGS (summer) | | | |
|---|---|---|---|
| Control | | SQOR KD | |
| Male | Female | Male | Female |
| 4 | 2 | 3 | 3 |

**Supplementary Table 7: List of primer sequences for real-time PCR in mice**

| | | |
|---|---|---|
| 18S | Forward | 5’-CGGCTACCACATCCAAGGAA-3’ |
| 18S | Reverse | 5’-GCTGGAATTACCGCGGCT-3’ |
| SQOR | Forward | 5’-TGGGGACCTTCAGGATCTAA-3’ |
| SQOR | Reverse | 5’-GGACTGGAGACAACAGTGACC-3’ |
| TST | Forward | 5’-CCAGCTGGTGGACTCTCG-3’ |
| TST | Reverse | 5’-GTGGCCCGAGTCTAGTCCT-3’ |
| ETHE1 | Forward | 5’-CTGTCATCTCCCGCCTCA-3’ |
| ETHE1 | Reverse | 5’-GCTCGAGTCTCCAAAGCAA-3’ |
| SUOX | Forward | 5’-TCTACCATGAGCATCGGTGT-3’ |
| SUOX | Reverse | 5’-CATCGAAGACCTCAGAGCCTA-3’ |
| CBS | Forward | 5'-CGGACTCCCCACATTATCAC-3‘ |
| CBS | Reverse | 5'-CACACTTGAGACCGGCATTC-3’ |
| CSE | Forward | 5'-CTTGCTGCCACCATTACGATT-3’ |
| CSE | Reverse | 5'-TCTTCAGTCCAAATTCAGATGCC-3’ |
| 3MST | Forward | 5'-TCACAGCCGCTGAAGTTACTG-3’ |
| 3MST | Reverse | 5'-CAGCATGTGGTCGTAGGGG-3' |
| VEGF | Forward | 5’-CCACGTCAGAGAGCAACATCA-3’ |
| VEGF | Reverse | 5’-TCATCTCTCCTATGTGCTGGCTTT-3’ |
| HO-1 | Forward | 5’-CTGACCCATGACACCAAGGAC-3’ |
| HO-1 | Reverse | 5’-AAAGCCCTACAGCAACTGTCG-3’ |
| EPO | Forward | 5’-CATCTGCGACAGTCGAGTTCTG-3’ |
| EPO | Reverse | 5’-CACAACCCATCGTGACATTTTC-3’ |
| GLUT-1 | Forward | 5’-AGCCCTGCTACAGTGTATCCT-3’ |
| GLUT-1 | Reverse | 5’-CCGACCCTCTTCTTTCATCT-3’ |
| COX1 | Forward | 5’-TGCTAGCCGCAGGCATTAC-3’ |
| COX1 | Reverse | 5’-GGGTGCCCAAAGAATCAGAAC-3’ |
| NDUFV1 | Forward | 5’-CTTCCCCACTGGCCTCAAG-3’ |
| NDUFV1 | Reverse | 5’-CCAAAACCCAGTGATCCAGC-3’ |

**Supplementary Table 8**

The optimum collision energy and precursor/production ions for thiol derivetives

| | Precursor Ion (m/z) | Production Ion (m/z) | Dwells (s) | Collision (v) |
|---|---|---|---|---|
| $S^{34}DB$ | 417.4 | 192.1 | 0.005 | 30 |
| Cysteine | 312.3 | 192.1 | 0.005 | 25 |
| HomoCys | 326.3 | 192.1 | 0.005 | 30 |
| Glutathione | 498.5 | 192.1 | 0.005 | 35 |