Supplementary Materials Figures for

H_2S Production by Reactive Oxygen Species in the Carotid Body Triggers Hypertension in a Rodent Model of Sleep Apnea

Guoxiang Yuan, Ying-Jie Peng, Shakil A. Khan, Jayasri Nanduri, Amritha Singh, Chirag Vasavda, Gregg L. Semenza, Ganesh K. Kumar, Solomon H. Snyder, Nanduri R. Prabhakar*

*Corresponding author. E-mail: nanduri@uchicago.edu

The PDF file includes:

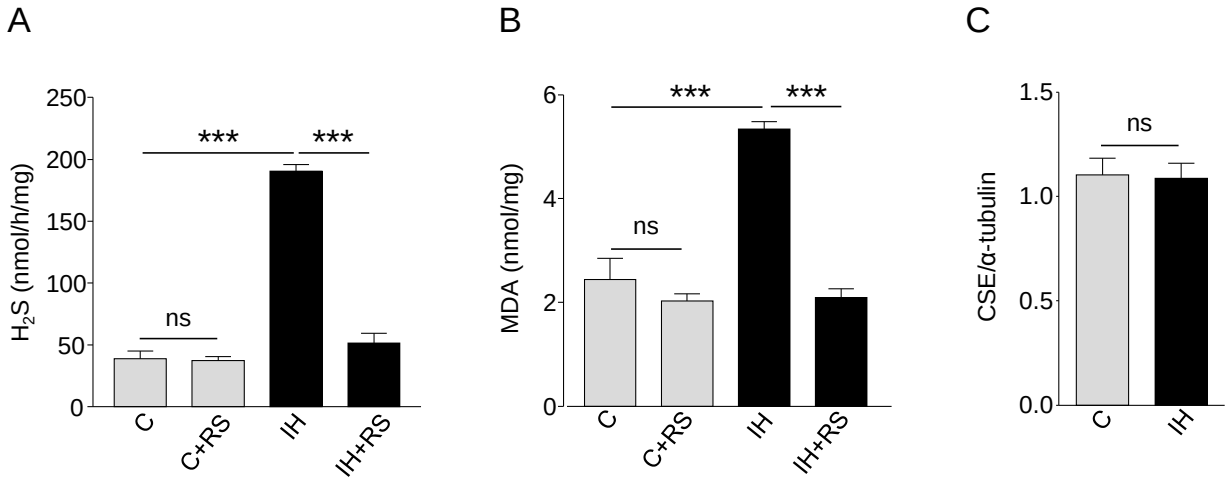

Fig. S1. Effect of IH on H_2S and MDA concentrations and CSE protein abundance in rat liver. (A to **B)** H_2S (**A**) and MDA (**B**) amounts in liver tissue of rats exposed to room air (C), room air with ROS scavenger (RS; C+RS), IH, or IH treatment with RS (IH+RS) (*n* = 3 rats for each treatment). (**C**) Densitometry analysis of CSE protein abundance in liver tissue of rats exposed to room air (C) or IH (*n* = 3 rats for each treatment) presented as ratio of CSE/α-tubulin (loading control). Data are presented as means ± SEM. ****p* < 0.001; ns, not significant.

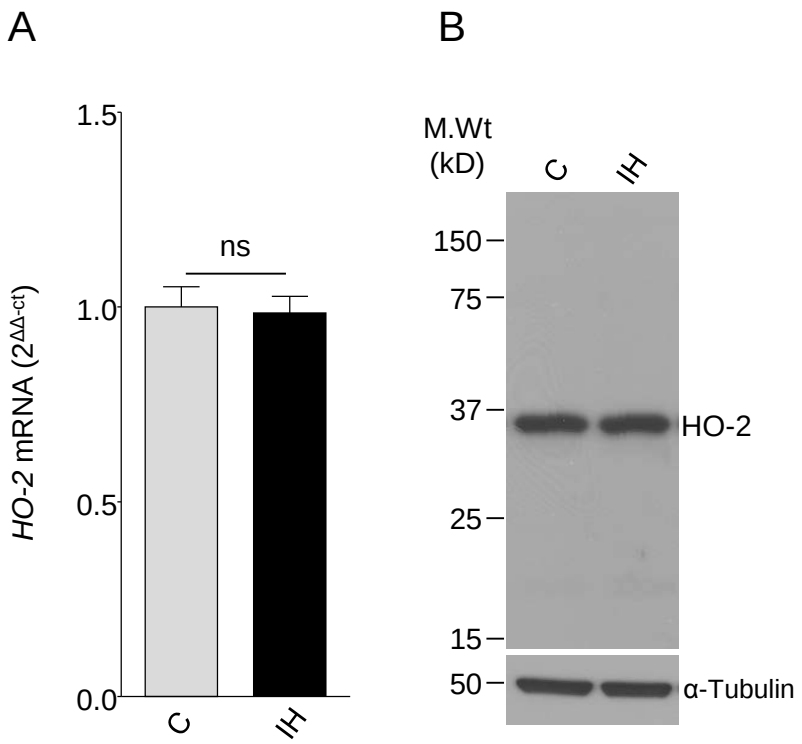

Fig. S2. Effect of IH on *HO-2* mRNA levels and protein abundance. (A) The ratio of *HO-2* mRNA to *18S* rRNA in carotid bodies of rats exposed to room air (C) or IH was determined by RT-qPCR and normalized to C (n = 5 for each treatment). Data are presented as means ±SEM. ns, not significant. (**B)** Representative immunoblot of HO-2 protein abundance in liver tissue from room air (C) and IH-exposed rats (n = 3 rats for each treatment). α-tubulin abundance was assayed as a loading control.

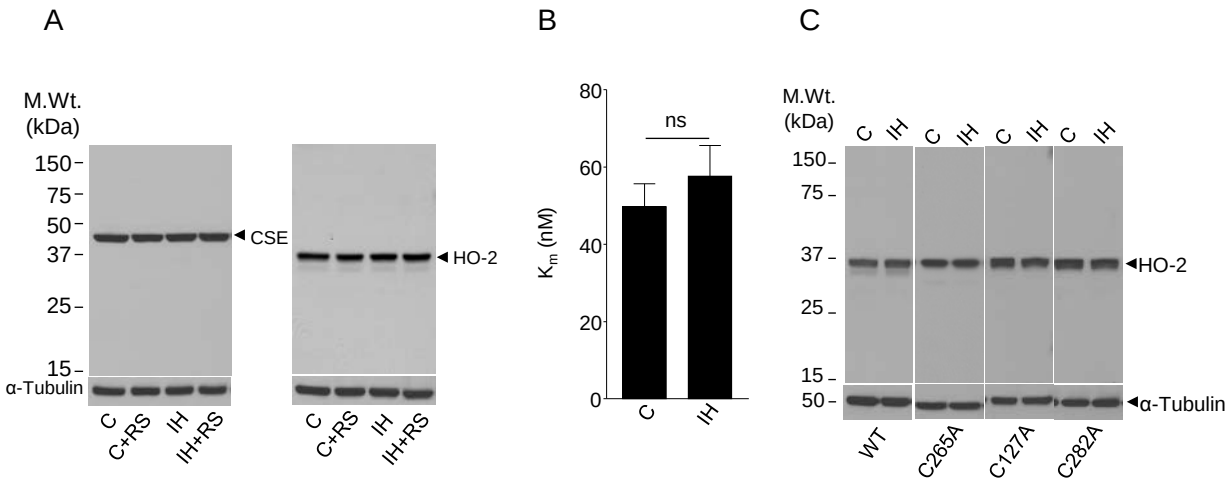

Fig. S3. Analysis of CSE and HO-2 protein abundance in HEK-293 cells. (A) Representative immunoblots of CSE and HO-2 protein abundance in CSE and HO-2-expressing HEK-293 cells exposed to room air (20% O_2), (C), room air with ROS scavenger (C+RS), IH, or IH with RS (IH+RS) (*n* = 5 independent experiments). **(B)** Hemin binding affinity (K_m) of HO-2 in HEK-293 cells expressing wild-type HO-2 treated with room air (20% O_2), (C) or IH (*n* = 4 experiments for each treatment). Data are presented as means ±SEM. ns, not significant. **(C)** HO-2 protein abundance in HEK-293 cells expressing either wild-type (WT) or mutant (C265A, C127A, or C282A) HO-2 exposed to room air (20% O_2), (**C**) or IH (*n* = 4 independent experiments for each treatment). α-tubulin abundance was assayed as a loading control.

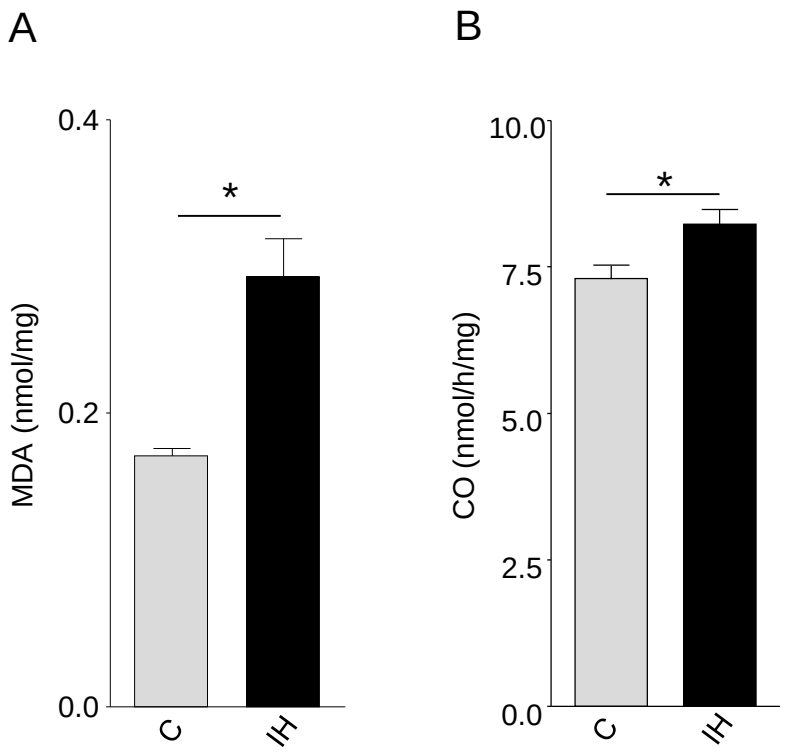

Fig. S4. Effect of IH on CO concentration in HO-1-expressing HEK-293 cells. (A) MDA and (**B**) CO concentrations in HO-1 expressing HEK-293 cells exposed to room air (20% O_2), (**C**) or IH (n = 4 independent experiments for each treatment. Data are presented as means ±SEM. *p < 0.05.

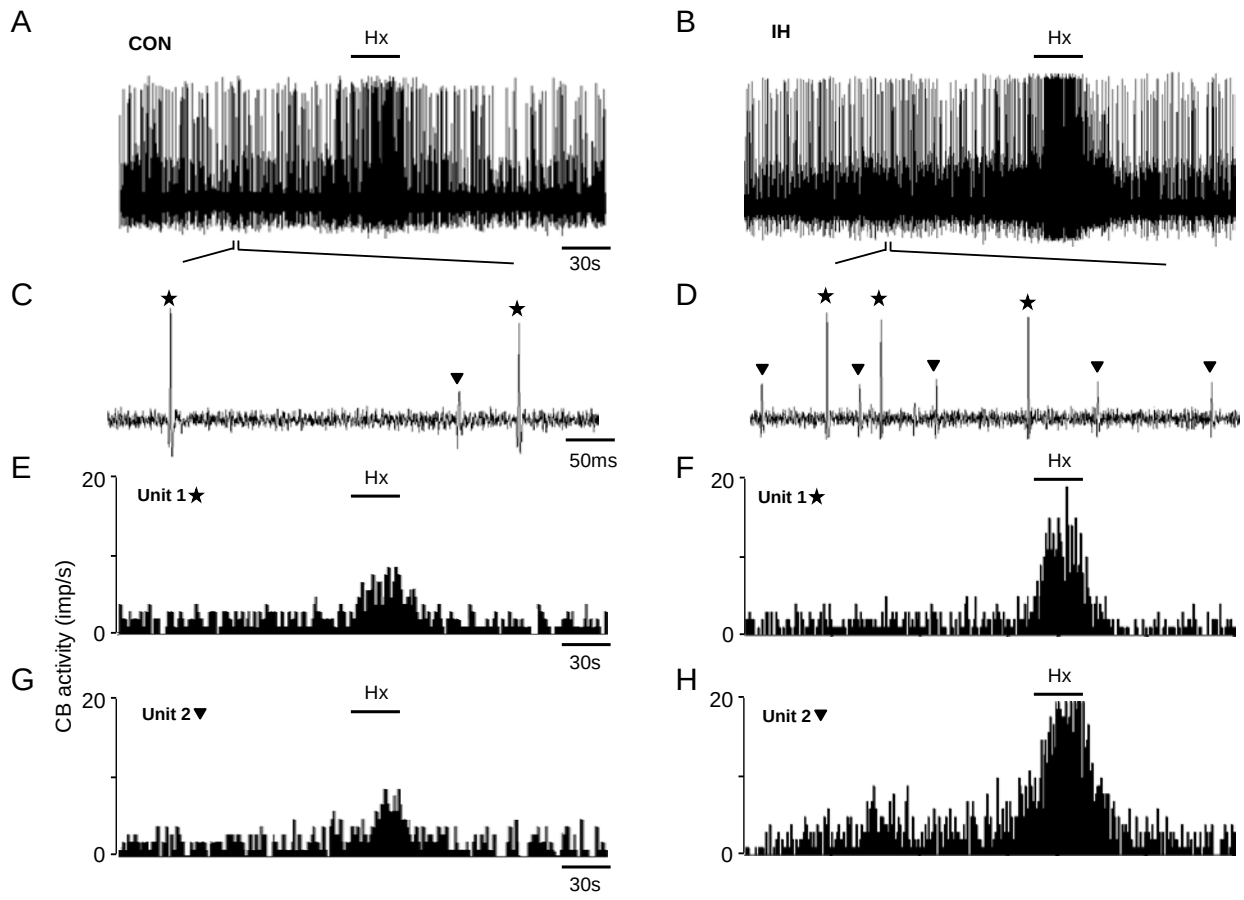

Fig. S5. Analysis of carotid body sensory nerve activity. (A to **B)** Upper panels show examples of raw action potentials recorded from the carotid sinus nerve of carotid bodies harvested from rats exposed to room air (CON, **A**) and intermittent hypoxia (IH, **B**) and their response to acute hypoxia (Hx). (**C** to **D**) Lower panels show identification of two "single units" with differing amplitudes in the control (**C**) and IH (**D**) treated carotid bodies. (**E** to **F**) Analysis of spike frequencies of Unit 1 (**E** and **F**) and Unit 2 (**G** and **H**).

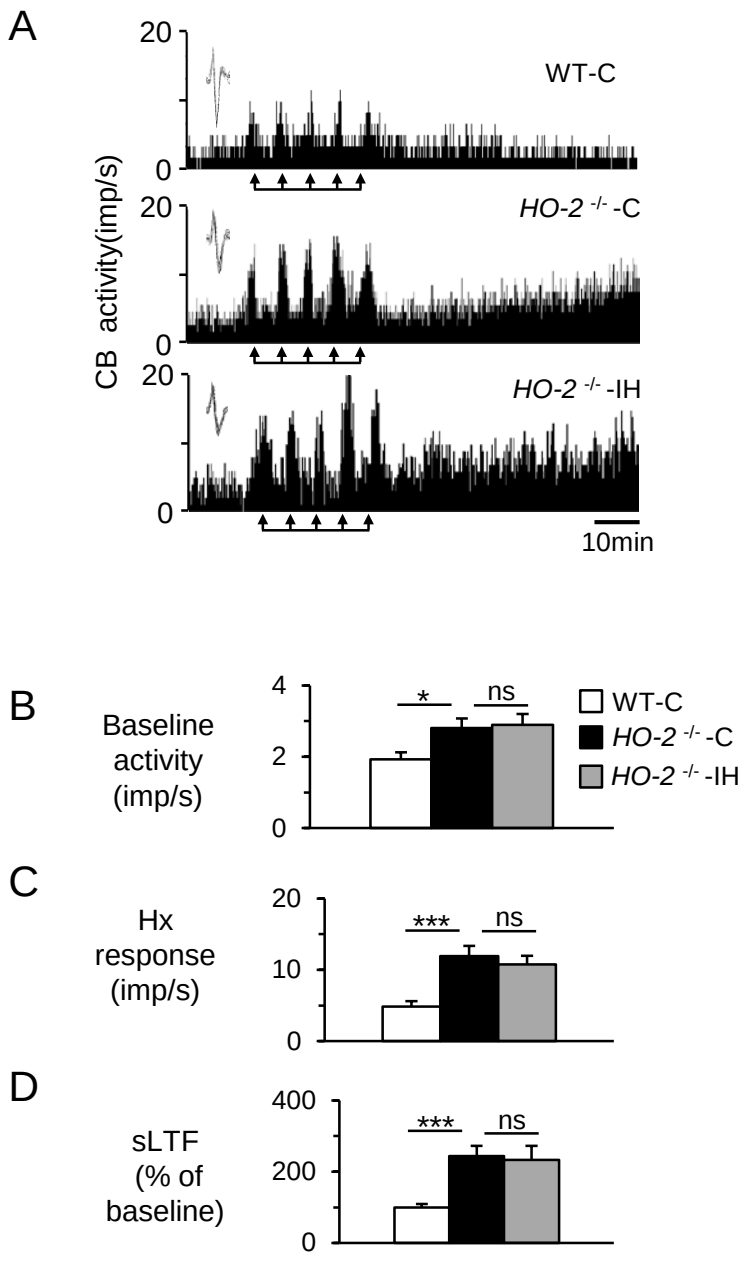

Fig. S6. Carotid body response to intermittent hypoxia in heme oxygenase-2 (HO-2) null mice (A) Examples of *ex vivo* carotid body sensory nerve responses during five 30-sec episodes of AIH (at arrows) and post-AIH for 60 min (sensory LTF; sLTF) from wild-type and HO-2 null mice exposed to room air (WT-C, *HO-2* $^{-/-}$ -C) or HO-2 null mice exposed to intermittent hypoxia (*HO-2* $^{-/-}$ -IH). (**B** to **D**) Average data of pre-AIH baseline activity (**B**), hypoxia (Hx) response (mean of 5 episodes of AIH; **C**), and sLTF (averaged over 60 min of post-AIH; **D**) (*n* =

6 experiments for each treatment per genotype; 12 single units from 6 carotid bodies for each treatment per genotype. Data are presented as means ± SEM. *$p < 0.05$; ***$p < 0.001$; ns, not significant.