Supporting Information

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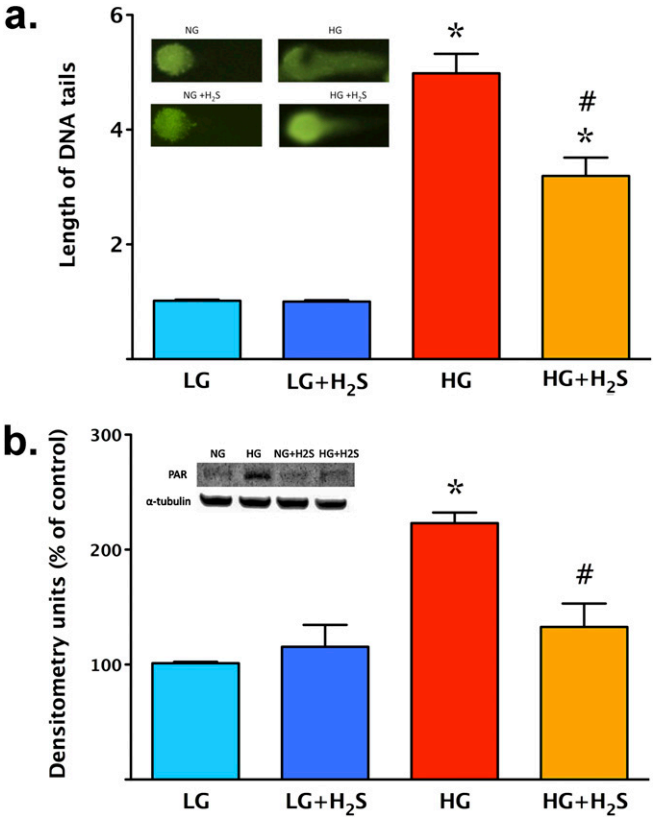

Fig. S1. Replacement of hydrogen sulfide (H_2S) attenuates cellular responses that lay downstream from hyperglycemic mitochondrial reactive oxygen species (ROS) production. (*A*) DNA strand breakage was measured in low (5.5 mM, LG) or high (40 mM, HG) glucose conditions at 7 d by using the Comet assay. High glucose induced an increase in DNA strand breakage compared with low glucose ($^*P < 0.05$), and H_2S (300 μM) afforded a significant suppression of this response ($^{\#}P < 0.05$). (*Inset*) Representative images are shown for the four respective groups (low/high glucose with and without 300 μM H_2S). (*B*) Activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) was measured by detection of the poly(ADP ribose) polymers by using Western blotting. High glucose induced an increase in PARP activation ($^*P < 0.05$), and H_2S (300 μM) afforded a suppression of this response ($^{\#}P < 0.05$). (*Inset*) Representative Western blot is shown for the four respective groups (low and high glucose with and without 300 μM H_2S) ($n = 5$).

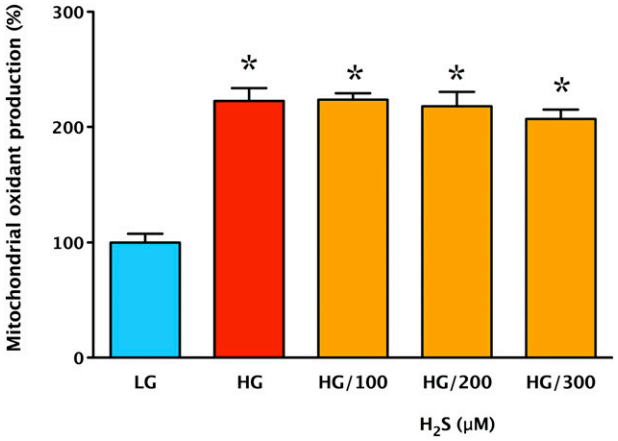

Fig. S2. Acute administration of H_2S at the end of the hyperglycemic period does not affect mitochondrial oxidant production in endothelial cells placed in high extracellular glucose. Mitochondrial ROS production was measured in low (5.5 mM, LG) or high (40 mM, HG) glucose conditions at 7 d by using the MitoSOX red method, and H_2S (100–300 μM) was administered for 1 h at the end of the experiment. High glucose increased MitoSOX red oxidation (*$P < 0.05$), but, when applied according to this protocol, H_2S failed to affect this response ($n = 4$).

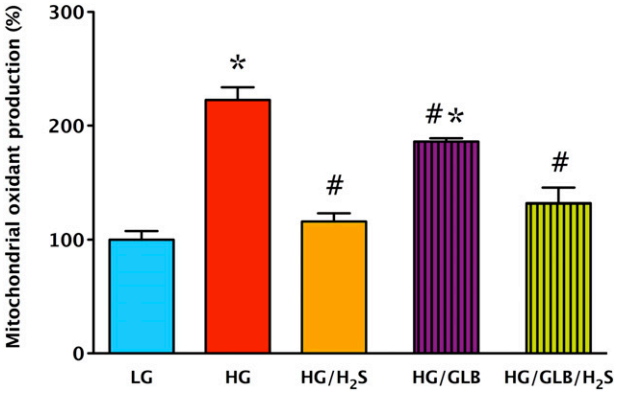

Fig. S3. The ATP-sensitive potassium (K_{ATP}) channel inhibitor glibenclamide does not prevent the protective effect of H_2S in endothelial cells placed in high extracellular glucose. Mitochondrial ROS production was measured in low (5.5 mM, LG) or high (40 mM, HG) glucose conditions at 7 d by using the MitoSOX red method in the presence or absence of H_2S (300 μM), with and without glibenclamide (10 μM, GLB) pretreatment. Glibenclamide slightly attenuated hyperglycemia-induced MitoSOX red oxidation but failed to influence the protective effect of H_2S on this response. *$P < 0.05$ indicates significant increases in MitoSOX red oxidation in high glucose compared with low glucose, and #$P < 0.05$ indicates significant suppression of this response by H_2S ($n = 4$).

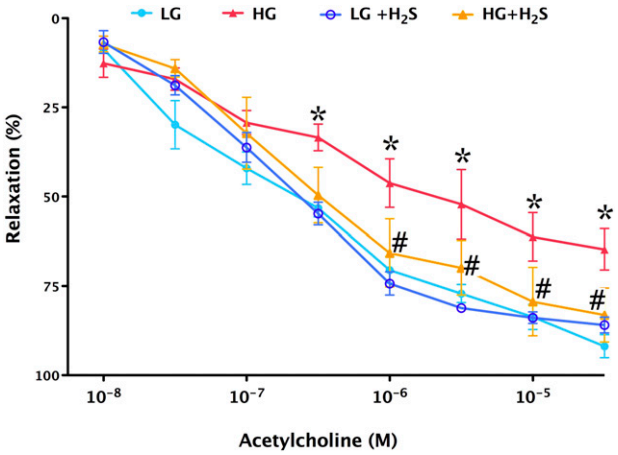

Fig. S4. H_2S protects against the development of diabetic endothelial dysfunction in rat aortic rings placed in elevated extracellular glucose. Rat aortic rings were incubated in low (5.5 mM, LG) or high (40 mM, HG) glucose for 72 h. H_2S (200 μM) was administered every 8 h. High glucose induced a suppression of endothelium-dependent relaxant responses (*$P < 0.05$), an effect that was prevented by H_2S (#$P < 0.05$; $n = 4$).

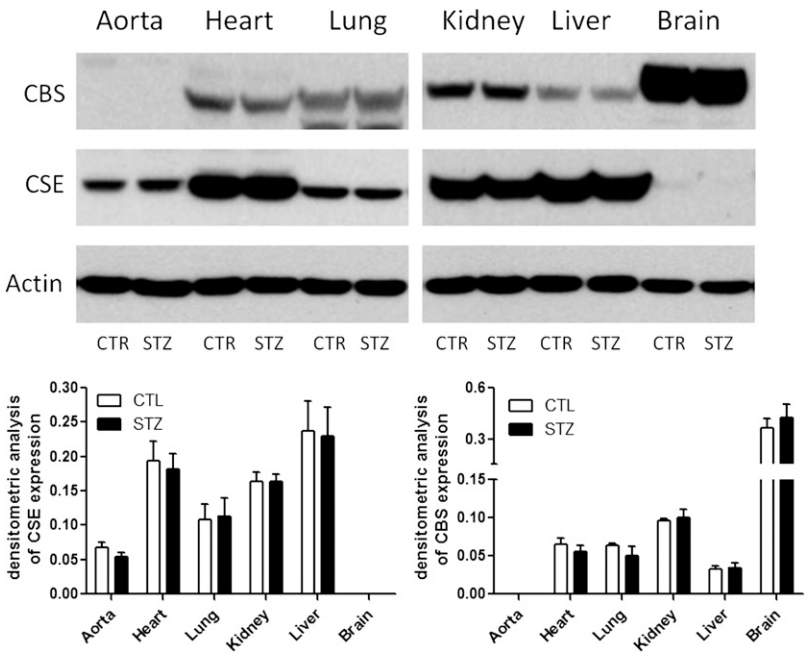

Fig. S5. Streptozotocin-induced diabetes for 6 wk does not affect the expression of cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS) in various tissues. Representative Western blots for CSE and CBS, loading control (actin), and densitometric analysis are shown in healthy control rats and in rats after 6 wk of streptozotocin-induced diabetes. Only CSE was detected in the thoracic aorta; CSE is more abundant than CBS in the heart, whereas only CBS was detected in the brain (n = 6).