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Supplementary Materials for

Depletion of H_2S during obesity enhances store-operated Ca^{2+} entry in adipose tissue macrophages to increase cytokine production

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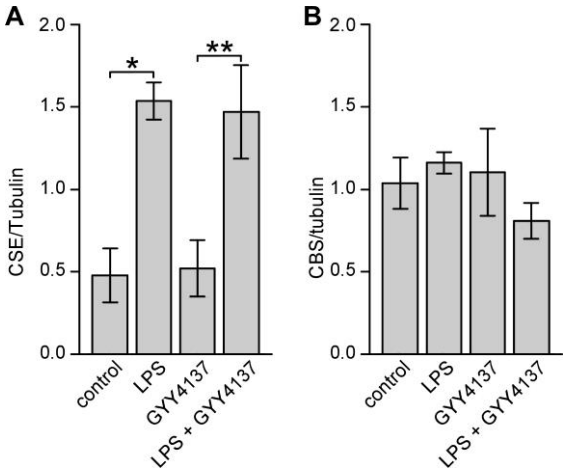

Fig. S1. LPS increases the abundance of CSE but not CBS. (**A** and **B**) The relative abundances of CSE (A) and CBS (B) proteins, normalized to the abundance of tubulin, were determined by densitometric analysis of Western blots of RAW264.7 cells treated with LPS in the presence and absence of GYY4137, as shown in Fig. 2C. Data are means ± SEM of three independent experiments. **P* = 0.005 and ***P* = 0.008 by one-way ANOVA.

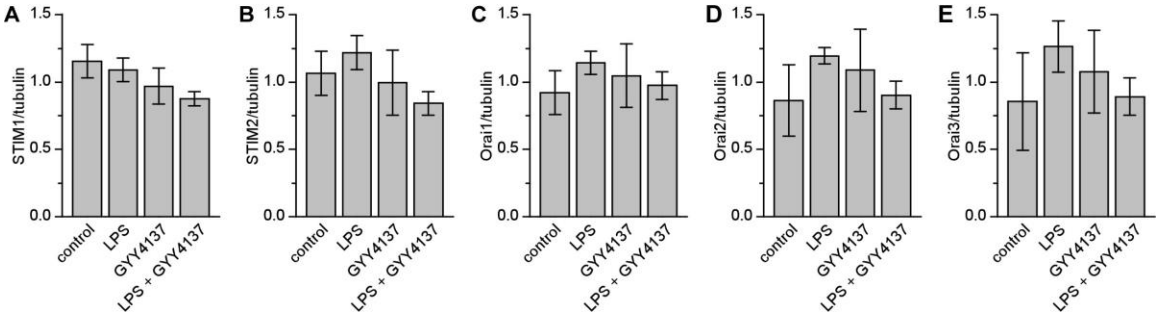

Fig. S2. LPS has no effect on the abundances of STIM and Orai isoforms. (**A** to **E**) RAW264.7 cells that were left unstimulated or were stimulated with LPS in the presence and absence of GYY4137 were analyzed by Western blotting as depicted in Fig. 3G. The abundances of STIM1 (A), STIM2 (B), Orai1 (C), Orai2 (D), and Orai3 (E), relative to that of tubulin, were determined by densitometric analysis. Data are means ± SEM of three independent experiments. $P > 0.05$ by one-way ANOVA.

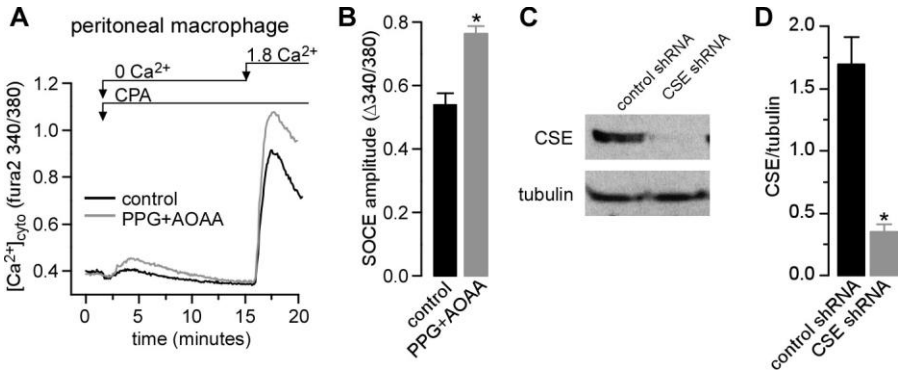

Fig. S3. Inhibition of endogenous H_2S production increases SOCE. (**A**) Typical traces recorded in peritoneal macrophages and depicting SOCE in the presence or absence of 0.4 mM PPG and 0.4 mM AOAA. (**B**) SOCE amplitudes in control and PPG/AOAA-treated peritoneal macrophages. Data are means ± SEM of five to seven independent samples from four mice. **P* = 0.014 by unpaired *t* test. (**C**) Lysates of RAW264.7 cells stably expressing either control shRNA or CSE-specific shRNA were analyzed by Western blotting with antibodies against the indicated proteins. Western blots are representative of four experiments. (**D**) Quantification of CSE abundance relative to that of tubulin as assessed by densitometric analysis of Western blots represented by the experiment shown in (C). Data are means ± SEM of four independent samples. **P* < 0.001 by unpaired *t* test.

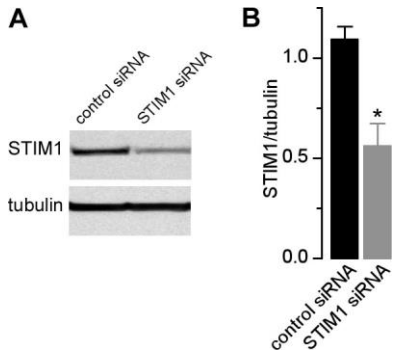

Fig. S4. Analysis of the knockdown of STIM1 in RAW264.7 cells. (**A**) Lysates of RAW264.7 cells transiently transfected with control siRNA or STIM1-specific siRNA were analyzed by Western blotting with antibodies against the indicated proteins. Western blots are representative of three experiments. (**B**) Quantification of the abundance of STIM1 relative to that of tubulin as assessed by densitometric analysis of Western blots represented by the experiment shown in (A). Data are means ± SEM of three independent samples. **P* = 0.02 by unpaired *t* test.

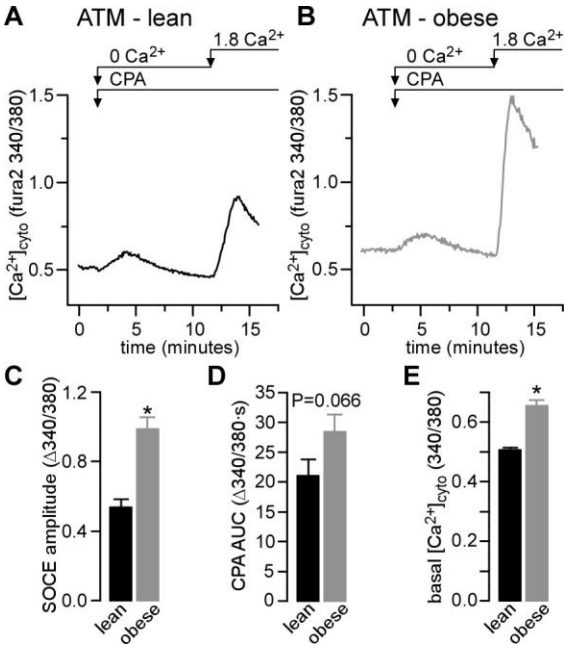

Fig. S5. SOCE is enhanced in ATMs from obese mice. (**A** and **B**) Representative SOCE traces made in ATMs isolated from lean (A) and obese (B) mice during the SOCE measurement protocol. (**C** to **E**) Summary of basal $[Ca^{2+}]_{cyto}$, SOCE amplitude, and ER Ca^{2+} content [estimated as the area under the curve (AUC)] during the transient increase in $[Ca^{2+}]_{cyto}$ in response to CPA. Data are means ± SEM of three mice of each group. **P* < 0.001 by unpaired *t* test.

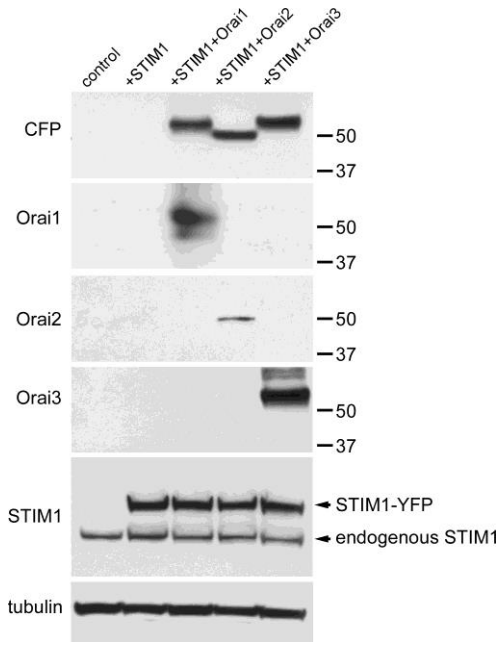

Fig. S6. Western blotting analysis of STIM1-YFP and Orai-CFP proteins in transfected HEK 293 cells. Lysates from untransfected HEK 293 cells (control), HEK 293 stably expressing STIM1-YFP alone (+STIM1), or HEK 293 cells stably expressing STIM1-YFP and transiently transfected with plasmids encoding CFP-tagged Orai1, Orai2, or Orai3, as indicated, were subjected to Western blotting analysis with antibodies specific for CFP, Orai1, Orai2, Orai3, STIM1, and tubulin. Western blots are representative of three independent experiments.

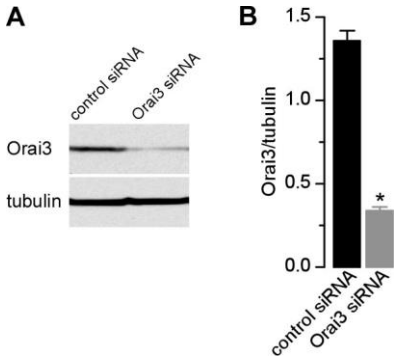

Fig. S7. Analysis of the knockdown of Orai3 in RAW264.7 cells. (**A**) Lysates of RAW264.7 cells transiently transfected with control siRNA or Orai3-specific siRNA were subjected to Western blotting analysis with antibodies against the indicated proteins. Western blots are representative of three experiments. (**B**) Quantification of the abundance of Orai3 relative to that of tubulin as assessed by densitometric analysis of Western blots represented by the experiment shown in (A). Data are means ± SEM of three independent samples. **P* < 0.001 by unpaired *t* test.