## Supplemental figures and legends

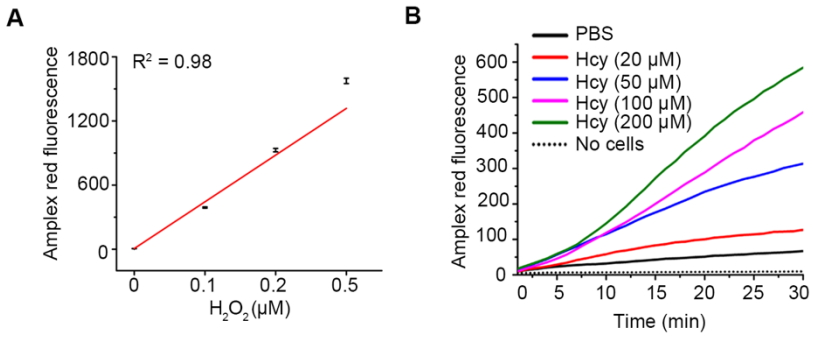


**Figure S1. Detection of $H_2O_2$ using the Amplex red hydrogen peroxide/peroxidase assay**

(A) Amplex red fluorescence intensity was linearly dependent on the amount of $H_2O_2$ at very low levels.

(B) Measurement of $H_2O_2$ released from HUVECs pre-treated with different concentrations of Hcy. The Amplex red fluorescence changes were traced for 30 min.

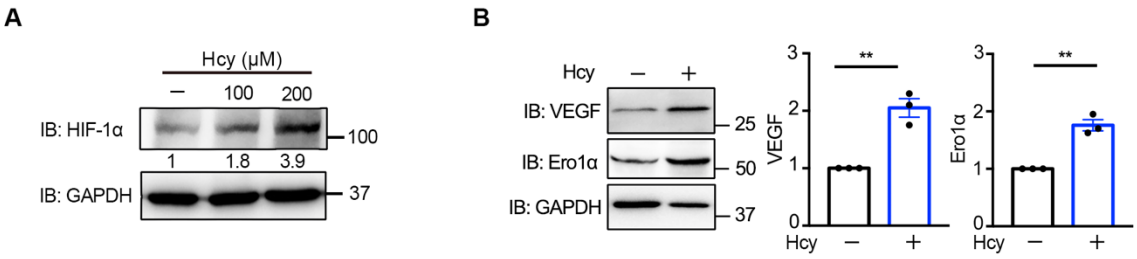


**Figure S2. Hcy upregulates the expression of HIF-1α**

(A) HUVECs were treated with different concentrations of Hcy for 12 h. Cell lysates were subjected to immunoblotting for HIF-1α expression. The relative density was quantified as indicated below, normalized to GAPDH.

(B) HUVECs were treated without or with Hcy (200 μM) for 4 h, and the expression of VEGF and Ero1α was detected by immunoblotting. Representative blots were shown. Data were represented as mean ± SEM from three independent experiments, **p < 0.01 via two-tailed Student's *t*-test.

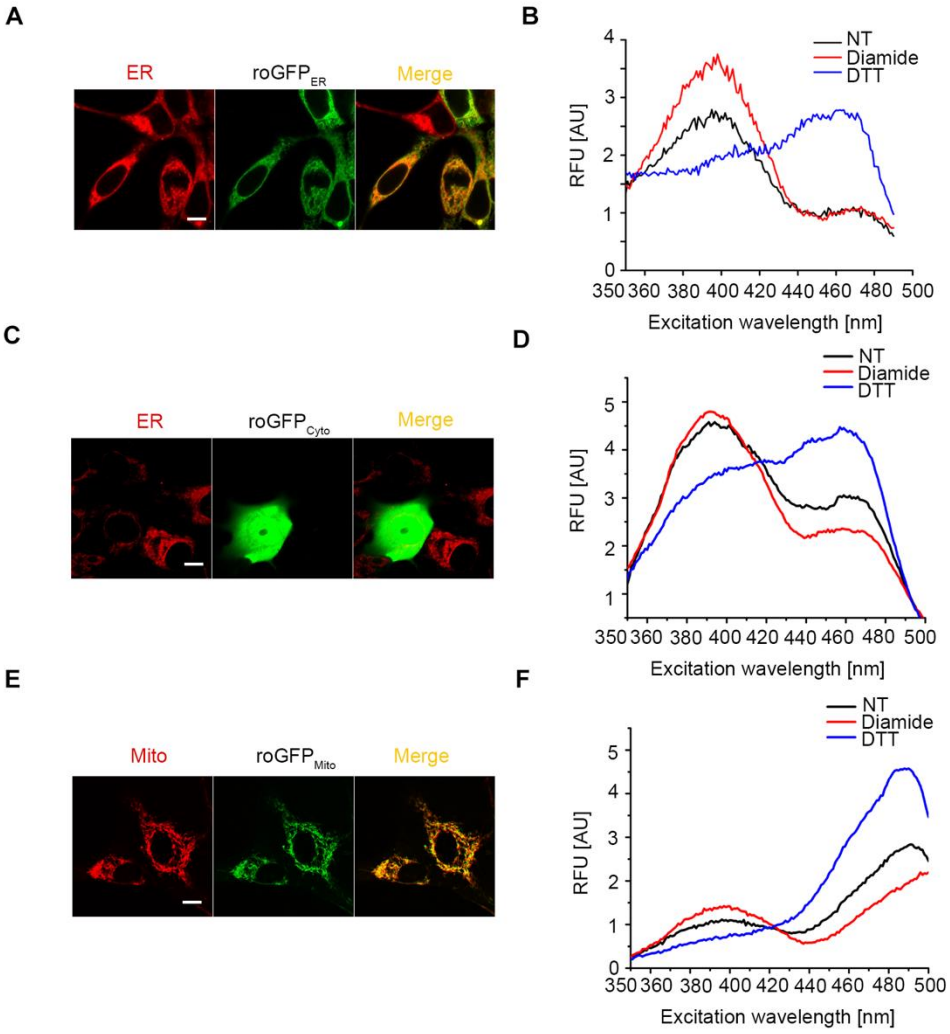


**Figure S3. Detection of redox states in different cellular compartments in HUVECs**

(A) 239T cells were transduced with roGFP$_{ER}$ for 24 h. Immunofluorescence analysis of ER tracker (red, Ex = 568 nm) and roGFP$_{ER}$ (green, Ex = 488 nm). Scale bars, 5 μm.

(B) The excitation fluorescence spectra of HUVECs expressing roGFP$_{ER}$ treated without (NT) or with 0.5 mM diamide or 10 mM DTT were recorded at an emission wavelength of 515 nm.

(C) HUVECs were transduced with roGFP$_{Cyto}$ for 24 h. Immunofluorescence analysis of ER tracker (red, Ex = 568 nm) and roGFP$_{Cyto}$ (green, Ex = 488 nm). Scale bars, 10 μm.

(D) The excitation fluorescence spectra of HUVECs expressing roGFP$_{Cyto}$ treated without (NT) or with 0.5 mM diamide or 5 mM DTT were recorded at an emission wavelength of 525 nm.

(E) HUVECs were transduced with roGFP$_{Mito}$ for 24 h. Immunofluorescence analysis of Mito tracker (red, Ex = 568 nm) and roGFP$_{Mito}$ (green, Ex = 488 nm). Scale bars, 10 μm.

(F) The excitation fluorescence spectra of HUVECs expressing roGFP$_{Mito}$ treated without (NT) or with 0.5 mM diamide or 5 mM DTT were recorded at an emission wavelength of 525 nm.

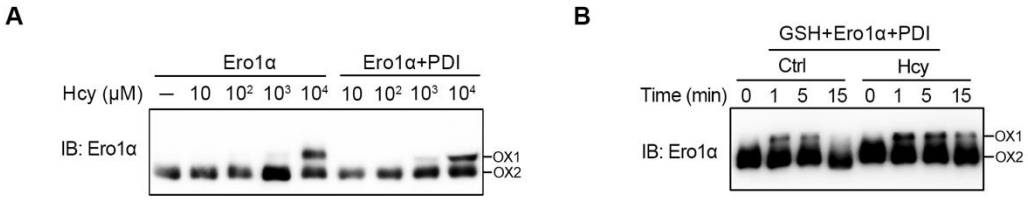


**Figure S4. Hcy activates Ero1α *in vitro***

(A) Ero1α (1 μM) with or without PDI (10 μM) was incubated with different concentrations of Hcy at 25 ℃ for 10 min and then analyzed by nonreducing SDS-PAGE and immunoblotting.

(B) Ero1α (1 μM) with 10 μM PDI and 1 mM GSH was incubated with or without 200 μM Hcy at 25 ℃ for different times as indicated, and then analyzed by nonreducing SDS-PAGE and immunoblotting.

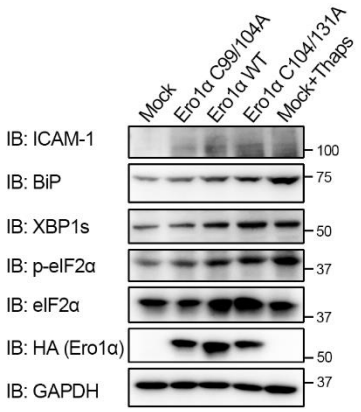


**Figure S5. Hyperactive Ero1α triggers UPR signaling**

HUVECs were transduced with Ero1α WT, Ero1α C99/104A (inactive) or Ero1α C104/131A (hyperactive) for 48 h. Mock-transduced cells were treated with 5 μM Thaps for 12 h as a positive control. The expression levels of BiP, XBP1s, p-eIF2α, eIF2α and Ero1α (HA-tag) were determined by immunoblotting.

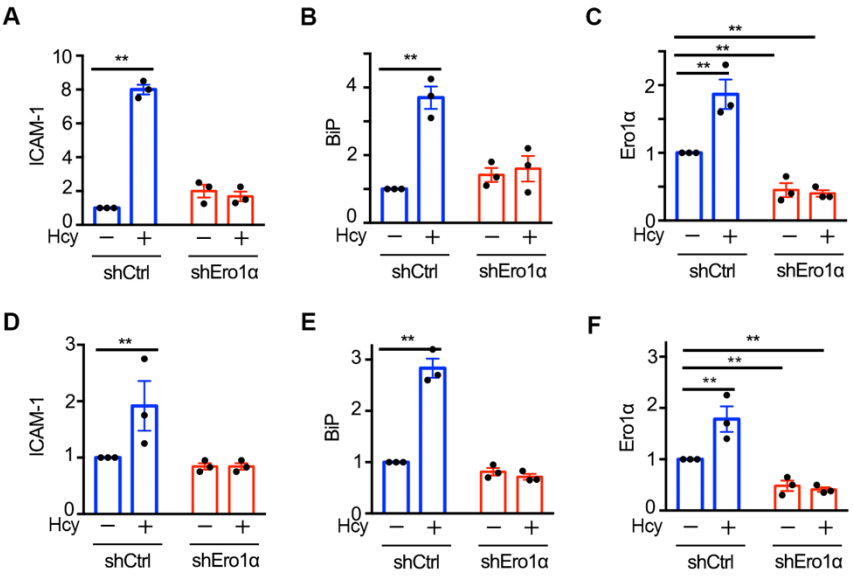


**Figure S6. Statistical analysis of *ex vivo* aortic ring assays and *in vivo* external carotid artery injection assays with Ero1α knockdown**

(A-C) Statistical analysis of ICAM-1 (A), BiP (B) and Ero1α (C) in Figure 5D.

(D-F) Statistical analysis of ICAM-1 (D), BiP (E) and Ero1α (F) in Figure 5E.

Data were represented as mean ± SEM from three biological replicates, **p < 0.01 via two-way ANOVA, Tukey's multiple comparisons test.

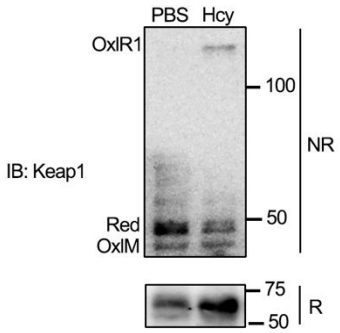


**Figure S7. Hcy increases the oxidation of Keap1 in HUVECs**

HUVECs were treated without or with 200 μM Hcy for 4 h, quenched with 5 mM NEM for 10 min, and then the lysates were analyzed by immunoblotting under nonreducing (NR) or reducing (R) conditions. The oxidized slow (OxlR1), fast (OxlM) and reduced (Red) Keap1 species were indicated according to [1].

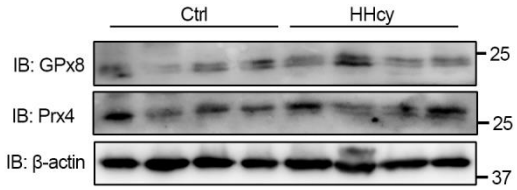


**Figure S8. The protein levels of GPx8 and Prx4 in the thoracic aortas of mice**

Lysates prepared from the thoracic aortas of normal (Ctrl) or HHcy mice were subjected to immunoblotting to detect the expression of GPx8 and Prx4.

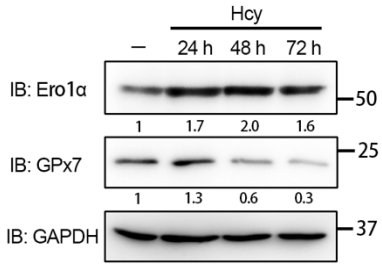


**Figure S9. Chronic Hcy treatment downregulates the expression of GPx7**

HUVECs were treated with 200 μM Hcy for the indicated times. The cell lysates were subjected to immunoblotting analysis for Ero1α and GPx7.

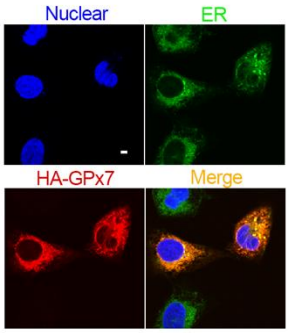


**Figure S10. The ER localization of HA-GPx7**

293T cells were transduced with lentiviral HA-GPx7 and subjected to immunofluorescence analysis (red, Ex = 568 nm). Calreticulin (green, Ex = 488 nm) and Hoechst (blue, Ex = 405 nm) were used to mark the ER and nuclei, respectively. Scale bars, 5 μm.

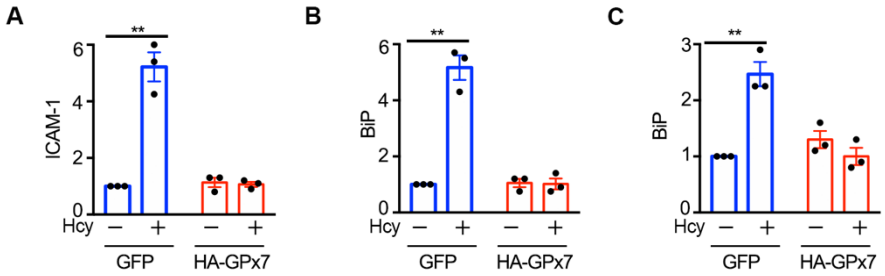


**Figure S11. Statistical analysis of *ex vivo* aortic ring assays and *in vivo* external carotid artery injection assays with GPx7 overexpression**

(A, B) Statistical analysis of ICAM-1 (A) and BiP (B) in Figure 7F.

(C) Statistical analysis of BiP in Figure 7G.

Data were represented as mean ± SEM from three biological replicates, **p < 0.01 via two-way ANOVA, Tukey's multiple comparisons test.

**Supplemental tables**

**Table S1. Primers for qPCR**

| Gene | Forward Primer (5'–3') | Reverse Primer (5'–3') |
|---|---|---|
| *GPX7* | GACCAGCACTACCGAGCCCT | CCCCTACCACCTTTCCATCT |
| *PDI* | GATGACCAAGTACAAGCCCGAATC | TGTCGGCACTGGCAGGAAAG |
| *ERO1A* | GCCAGGTTAGTGGTTACTTGG | GGCCTCTTCAGGTTTACCTTGT |
| *GPX8* | CCGCCCAAGCAAGGAAGTAG | TCTAACCAGAGCTGCTATGTCAG |
| *PRX4* | CTGTTGATTCACAGTTTACCCATTT | ATTATTGTTTCACTACCAGGTTTCC |
| *ERO1B* | TTCTGGATGATTGCTTGTGTGAT | GGTCGCTTCAGATTAACCTTGT |
| *NRF2* | TCAGCGACGGAAAGAGTATGA | CCACTGGTTTCTGACTGGATGT |
| *β-ACTIN* | CCTGTACGCCAACACAGTGC | ATACTCCTGCTTGCTGATCC |

**Table S2. Primers for ChIP-PCR**

| Name | Forward Primer (5'–3') | Reverse Primer (5'–3') |
|---|---|---|
| HRE1 | TCACGCGGCGGGAAGGAACCGGT | AGCTTGTCGCCCCACGCTT |
| HRE2 | GATCTCGGTTCACTGTAACCTCTGTCTTCCAGG | TGATGAATGATAACAGGTGCTGGAAAGGATG |

**Reference**

[1] S. Fourquet, R. Guerois, D. Biard, M.B. Toledano, Activation of NRF2 by nitrosative agents and H2O2 involves KEAP1 disulfide formation, J. Biol. Chem. 285 (2010) 8463-71.