Supporting Information

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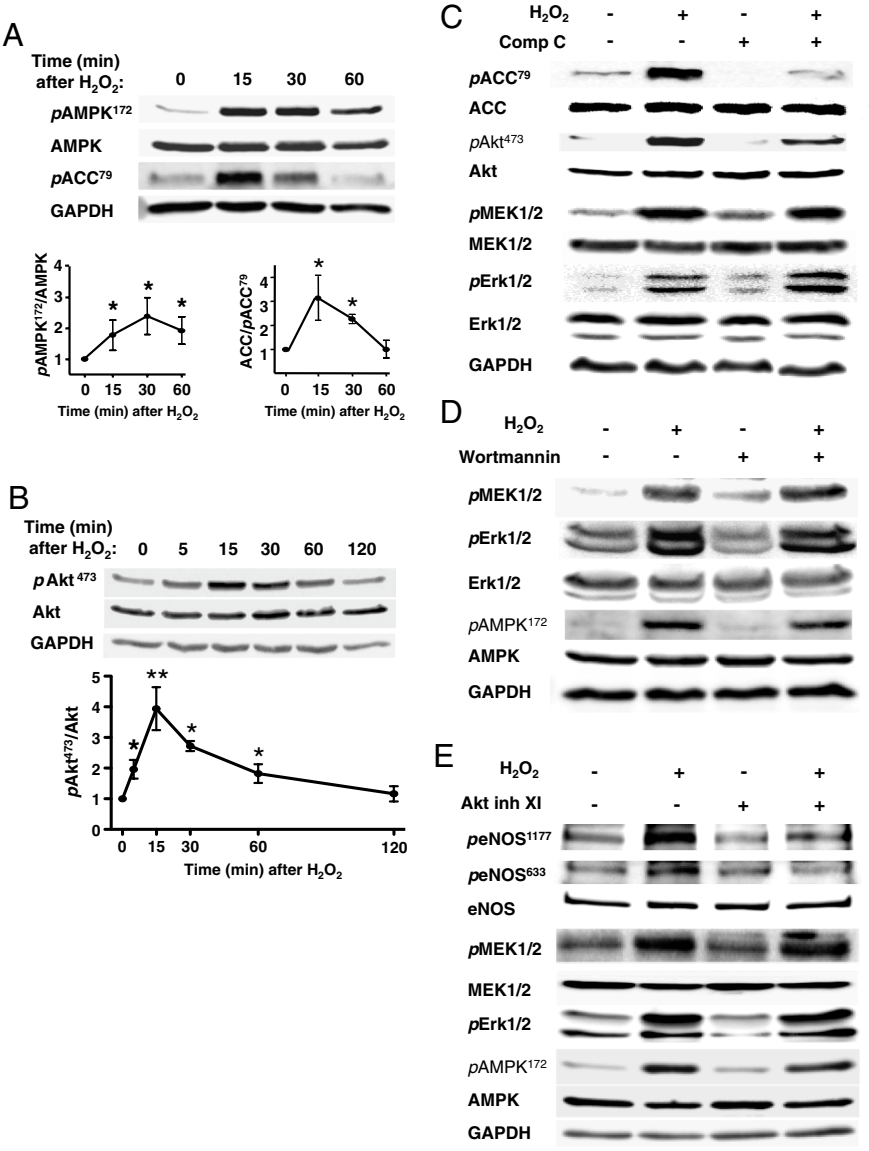

Fig. S1. H_2O_2-promoted phosphorylation of AMP-activated protein kinase (AMPK) and Akt in cardiac myocytes. (*A*) Time course for H_2O_2-stimulated AMPK phosphorylation at threonine 172 (pAMPK^{172}); a representative experiment is shown above and pooled data from three experiments are presented below; * indicates $p < 0.05$. (*B*) Time course for H_2O_2-induced Akt phosphorylation at serine 473 (pAkt^{473}); a representative experiment is shown above and pooled data from three experiments are presented below; * indicates $p < 0.05$ and ** indicates $p < 0.01$. (*C*) Results of immunoblot analyses performed in adult cardiac myocyte lysates prepared from cells incubated with the AMPK inhibitor compound C (Comp C, 20 μM, 30 min) before treatment with H_2O_2 (25 μM, 15 min). Immunoblots were probed with antibodies directed against phospho-acetyl-CoA carboxylase (ACC) Ser^{79}, phospho-Akt Ser^{473}, phospho-mitogen-activated protein kinase kinase–ERK1/2 (MEK1/2) Ser$^{217/221}$, phospho-Erk1/2 Thr^{202}/Tyr^{204}, ACC, Akt, MEK1/2, Erk1/2, or GAPDH, as indicated. The experiment shown is representative of three independent experiments that yielded similar results. (*D*) Results of immunoblot analyses performed in adult cardiac myocyte lysates prepared from cells incubated with the PI3K inhibitor wortmannin (1 μM, 30 min) before treatment with H_2O_2 (25 μM, 15 min). Immunoblots were probed with antibodies as indicated. The experiment shown is representative of three independent experiments that yielded similar results. (*E*) Results of immunoblot analyses performed in cell lysates prepared from cardiac myocyte that were incubated with the Akt inhibitor XI (Akt inh XI, 1 μM, 30 min) prior treatment with H_2O_2 (25 μM, 15 min); blots were probed with antibodies as shown. The experiment shown is representative of three independent experiments.

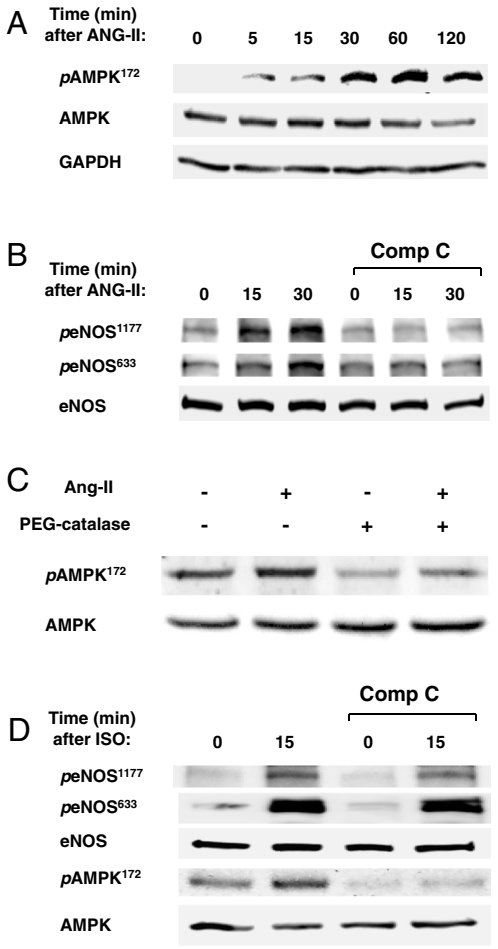

Fig. S2. (*A*) Angiotensin-II-promoted AMP-activated protein kinase (AMPK) phosphorylation. This figure shows the time course for angiotensin-II-mediated AMPK phosphorylation. Shown are the results of immunoblots analyzed in lysates prepared from adult cardiac myocytes treated with angiotensin II (ANG-II, 500 nM) for the indicated times. Cell lysates were analyzed in immunoblots probed using antibodies directed against phospho-AMPK Thr^{172}, AMPK, and GAPDH, as indicated. The experiment shown is representative of three independent experiments that yielded similar results. (*B*) Effects of the AMPK inhibitor compound C on angiotensin-II-promoted eNOS phosphorylation. This figure shows the results of immunoblot analyses performed in adult cardiac myocyte lysates prepared from cells incubated with the AMPK inhibitor compound C (Comp C, 20 μM, 30 min) before treatment with angiotensin II (ANG-II, 500 nM) for the indicated times. Immunoblots were probed with antibodies directed against phospho-endothelial isoform of nitric oxide synthase (eNOS) Ser^{1177}, phospho-eNOS Ser^{633}, or eNOS, as indicated. The experiment shown is representative of three independent experiments. (*C*) Effects of PEG-catalase on angiotensin-II-promoted AMPK phosphorylation. This figure shows the results of immunoblot analyses performed in adult cardiac myocyte lysates prepared from cells incubated with PEG-catalase before treatment with angiotensin II (ANG-II, 500 nM) for 15 min. Immunoblots were probed with antibodies directed against phospho-AMPK, as described in detail in *Materials and Methods*. (*D*) Effects of the AMPK inhibitor compound C on isoproterenol-promoted eNOS phosphorylation. *D* shows the results of immunoblot analyses performed in cell lysates prepared from cardiac myocyte that were incubated with the AMPK inhibitor compound C (Comp C, 20 μM, 30 min) before treatment with isoproterenol (ISO 100 nM, 15 min); blots were probed with antibodies as shown. The experiment shown is representative of three independent experiments.

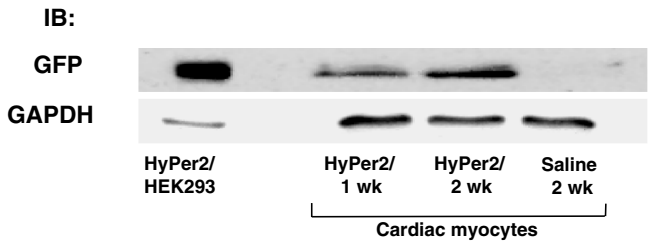

Fig. S3. Expression of the recently developed H_2O_2 biosensor HyPer2 in mouse cardiac myocytes. Shown is a representative immunoblot (IB) of cardiac myocytes isolated from mice 1 or 2 wk following tail vein injection of HyPer2 lentivirus; myocytes isolated from saline-injected mice serve as a negative control, and HEK293 cells infected with the HyPer2 lentivirus serve as a positive control. Immunoblots were probed with antibodies as indicated, directed against either GFP [Anti-Tag(CGY)FT antibody] to detect HyPer2, or with GAPDH antibodies as a loading control.

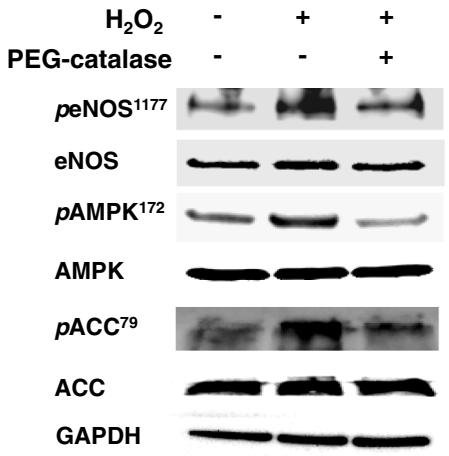

Fig. S4. Effects of PEG-catalase on H_2O_2-promoted increase in protein phosphorylation. This figure shows the results of immunoblot analyses performed in adult cardiac myocyte lysates prepared from cells either incubated or not with PEG-catalase (100 units/mL, 1 h) before treatment with H_2O_2 (25 μM, 15 min). Blots were probed with antibodies against phospho-endothelial isoform of nitric oxide synthase (eNOS) Ser^{1177}, phospho-AMP-activated protein kinase (AMPK) Thr^{172}, phospho-acetyl-CoA carboxylase (ACC) Ser^{79}, phospho-mitogen-activated protein kinase kinase–ERK1/2 (MEK1/2) Ser$^{217/221}$, phospho-Erk1/2 Thr^{202}/Tyr^{204}, ACC, MEK1/2, Erk1/2, or GAPDH as indicated. The experiment shown is representative of three independent experiments that yielded similar results.

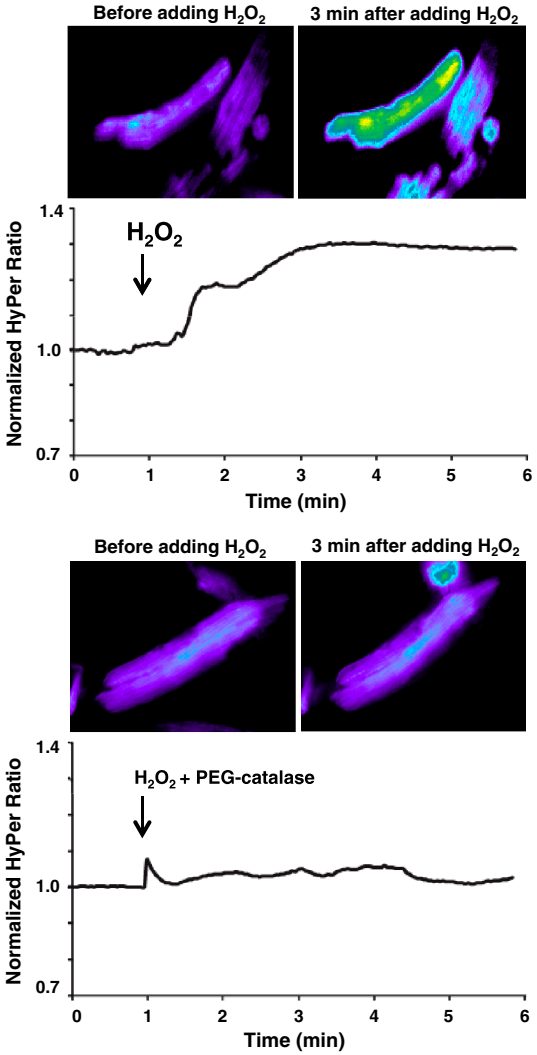

Fig. S5. Effects of PEG-catalase on H_2O_2-promoted increase in the recently developed H_2O_2 biosensor HyPer2 fluorescence. Adult mice were injected via the tail vein with lentivirus expressing the HyPer2 biosensor; 2-wk later the mice were euthanized following cardiac myocytes isolation. Cultured cells were treated with H_2O_2 (10 μM), in some studies myocytes were pretreated with PEG-catalase (100 units/mL) for at least 2 h before H_2O_2 treatment. (*Upper*) Representative HyPer2 images in isolated cardiac myocytes before adding H_2O_2 or 3 min after adding H_2O_2. (*Lower*) Representative fluorescence tracings analyzed following cell treatment with H_2O_2 (10 μM). The HyPer2 H_2O_2 image is determined as the YFP500/YFP420 excitation ratio; the grayscale is adjusted to improve contrast.

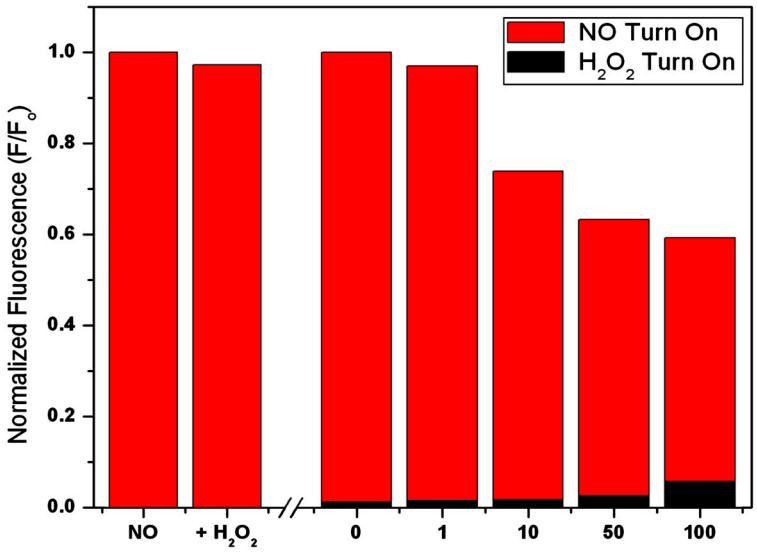

Fig. S6. Cu_2FL2A detects NO in the presence of H_2O_2. The two left bars show the integrated fluorescence of Cu_2FL2A after reaction with 1,300 equivalents of NO either after a 30 min incubation ("NO"), or followed by the addition of 100 equivalents of H_2O_2 and incubation for a further 30 min ("+H_2O_2"). The right bars shows the integrated fluorescence of Cu_2FL2A following the addition of the indicated amounts of H_2O_2 for 30 min (shown in black at each H_2O_2 concentration), followed by the addition of 1,300 equivalents of NO and incubation for a further 30 min (in red). The experimental conditions are described in detail *Materials and Methods*.