Supporting Information

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SI Text

SI Materials and Methods. ***Synthetic materials and methods.*** Silica gel P60 (SiliCycle) was used for column chromatography. Analytical thin layer chromatography was performed using SiliCycle 60 F254 silica gel (precoated sheets, 0.25 mm thick). Fmoc-piperazine rhodol boronate was synthesized according to literature procedures (1). ^{1}H NMR spectra were collected in $CDCl_3$ or CD_3OD (Cambridge Isotope Laboratories) at 25 °C on a Bruker AV-300 spectrometer at the College of Chemistry NMR Facility at the University of California, Berkeley. Chemical shifts are reported in the standard δ notation of parts per million using the peak of residual proton signals of $CDCl_3$ or CD_3OD as an internal reference. Mass spectral analyses were carried out at the College of Chemistry Mass Spectrometry Facility at the University of California, Berkeley.

Peroxy Yellow-1 Methyl Ester, PY1-ME. Fmoc-piperazine rhodol boronate (15 mg, 0.02 mmol) was added to a vial and dissolved in 1.5 mL of acetonitrile. Two drops of piperidine were added to the solution and the mixture was stirred at room temperature for 30 min, after which the contents were evaporated under reduced pressure. Methyl N-succinimidyl adipate (Pierce, 10 mg, 0.04 mmol) and sodium bicarbonate (46 mg, 0.54 mmol) was added to the dry vial and the contents were dissolved in a mixture of 1.5 mL of acetonitrile and 2 mL of dichloromethane and stirred at room temperature for 6 h. The reaction mixture was then dried under reduced pressure. Purification by column chromatography (3:1 ethyl acetate/hexanes) furnished **PY1-ME** as an orange solid (5 mg, 35%). ^{1}H NMR ($CDCl_3$/5% CD_3OD, 300 MHz): δ 8.03 (1H, d, $J = 6.3$ Hz), 7.72 (1H, s), 7.64 (1H, t, $J = 6.6$ Hz), 7.60 (1H, t, $J = 6.6$ Hz), 7.41 (1H, d, $J = 7.8$ Hz), 7.11 (1H, d, $J = 6.3$ Hz), 6.79 (1H, d, $J = 7.8$ Hz), 6.70 (1H, s), 6.68 (1H, d, $J = 6.3$ Hz), 6.60 (1H, dd, $J = 2.1, 8.7$ Hz), 3.72–3.82 (2H, m), 3.67 (3H, s), 3.58–3.64 (2H, m), 3.19–3.29 (4H, m), 2.32–2.43 (4H, m), 1.66–1.75 (4H, m), 1.34 (12H, s). HR-FABMS: calculated for [M^+] 653.3034, found 653.3026.

Spectrophotometric Characterization of PY1-ME. PY1-ME features two major visible region absorptions ($\lambda_{abs} = 489$ nm, $\varepsilon = 18100\ M^{-1}\,cm^{-1}$; $\lambda_{abs} = 510$ nm, $\varepsilon = 18700\ M^{-1}\,cm^{-1}$) and a weak emission ($\lambda_{em} = 548$ nm, ($\Phi = 0.040$) in 20 mM Hepes, pH 7.0. The deprotected, phenol form of PYME has one major visible region absorption ($\lambda_{abs} = 515$ nm, $\varepsilon = 79900\ M^{-1}\,cm^{-1}$) and enhanced emission ($\lambda_{em} = 540$ nm, ($\Phi = 0.402$).

1. Dickinson BC, Chang CJ (2008) A targetable fluorescent probe for imaging hydrogen peroxide in the mitochondria of living cells. *J Am Chem Soc* 130:9638–9639.

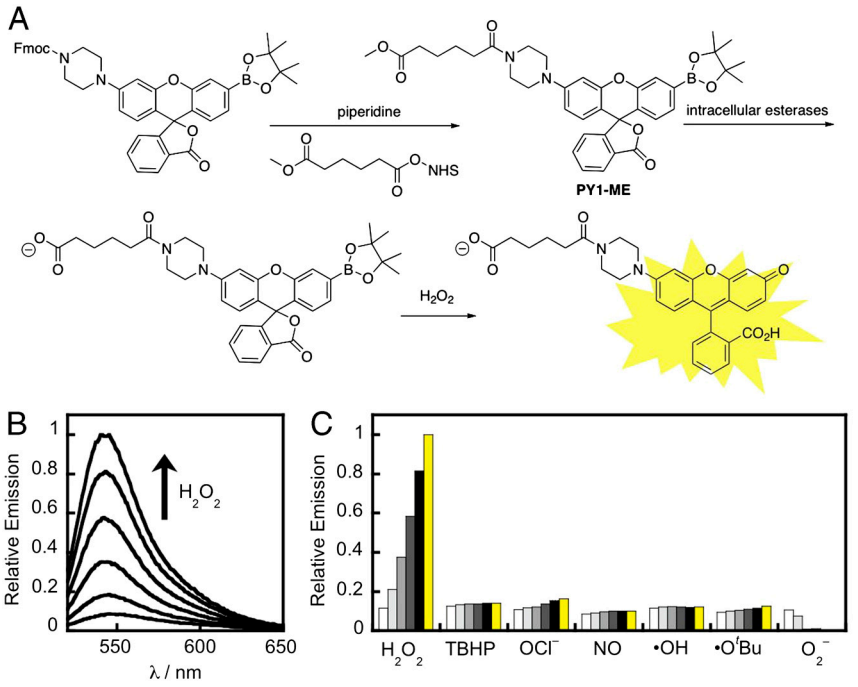

Fig. S1. Design and characterization of PY1-ME. (*A*) Design and synthesis of PY1-ME, a new fluorescent probe for the selective detection of H_2O_2. (*B*) Fluorescence turn-on response of 5 μM PY1-ME at 0, 5, 15, 30, 45, and 60 min after the addition of 100 μM H_2O_2. (*C*) Fluorescence responses of 5 μM PY1-ME to various reactive oxygen species (ROS). Bars represent relative responses at 0, 5, 15, 30, 45, and 60 min after addition of each ROS. Data shown are for 10 mM O_2^- (with 10 μM Catalase), 200 μM NO, and 100 μM for all other ROS.

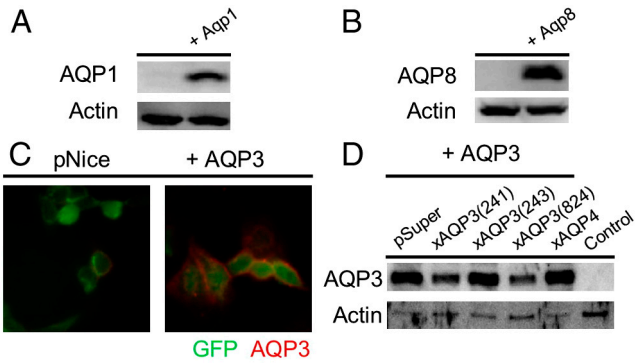

Fig. S2. (*A*) Western blot showing the overexpression of AQP1 in HEK 293 cells. HEK 293 cells were transfected with either control (pCMV6-XL4) or AQP1 expression vector ("+AQP1") and analyzed by Western blot. (*B*) Western blot showing the overexpression of AQP8 in HEK 293 cells. HEK 293 cells were transfected with either control (pCMV6-XL4) or AQP8 expression vector ("+AQP8") and analyzed by Western blot. (*C*) Immunocytochemistry of HEK 293 cells transfected with HyPer and AQP3 or a control vector. (*D*) Western blot showing overexpression and knockdown of AQP3 in HEK 293 cells. Lane 1: HEK 293 cells transfected with AQP3 and pSUPER. Lane 2: HEK 293 cells transfected with AQP3 and xAQP3 241 shRNA. Lane 3: HEK 293 cells transfected with AQP3 and xAQP3 243 shRNA. Lane 4: HEK 293 cells transfected with AQP3 and xAQP3 824 shRNA. Lane 5: HEK 293 cells transfected with AQP3 and xAQP4 801 shRNA. Lane 6: Untransfected control HEK 293 cells. Blots were stripped and reprobed for actin as a loading control.

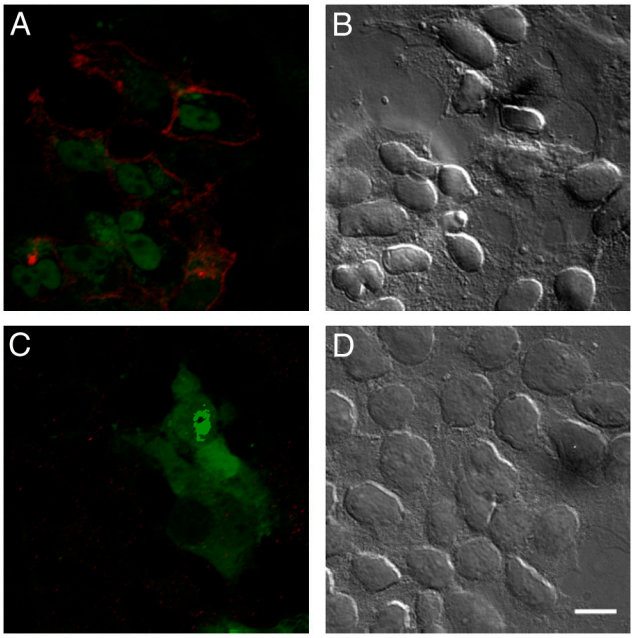

Fig. S3. Immunocytochemistry of HEK 293 cells transfected with HyPer and AQP3 or control vector. After transfection, cells were fixed, permeablized, and treated with anti-AQP3 antibody. AQP3 localization was visualized by addition of Cy-3 conjugated secondary antibody (red channel); YFP fluorescence from the HyPer chromophore, as a marker of transfection, is visualized in green. (*A*) HEK 293 cells expressing HyPer and AQP3. (*B*) DIC image of cells in A. (*C*) HEK 293 cells expressing HyPer and control vector. (*D*) DIC image of cells in D. Scale bar = 10 μm.

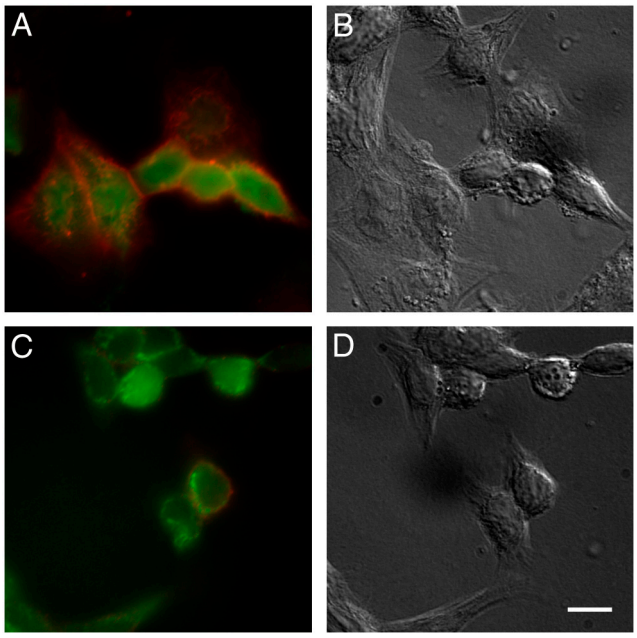

Fig. S4. Immunocytochemistry of HeLa cells transfected with HyPer and AQP3 or control vector. After transfection, cells were fixed, permeablized, and treated with anti-AQP3 antibody. AQP3 localization was visualized by addition of Cy-3 conjugated secondary antibody (red channel); YFP fluorescence from the HyPer chromophore, as a marker of transfection, is visualized in green. (*A*) HeLa cells expressing HyPer and AQP3. (*B*) DIC image of cells in A. (*C*) HeLa cells expressing HyPer and control vector. (*D*) DIC image of cells in D. Scale bar = 10 μm.

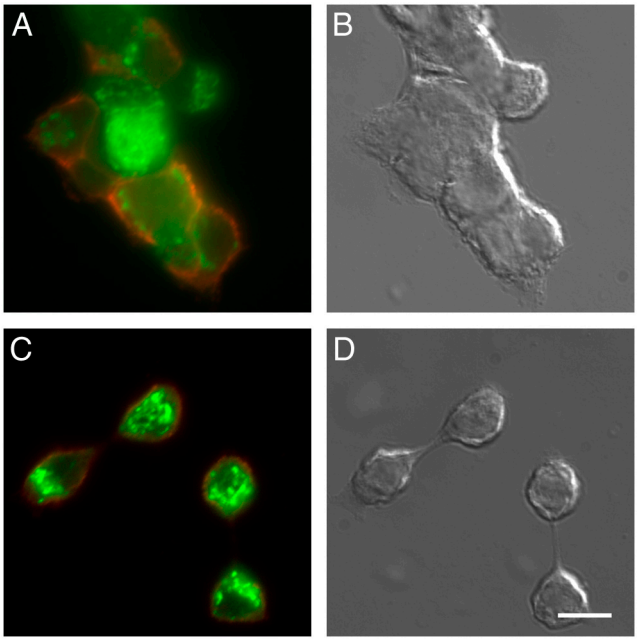

Fig. S5. Immunocytochemistry of HT-29 cells transfected with HyPer and AQP3 or control vector. After transfection, cells were fixed, permeablized, and treated with anti-AQP3 antibody. AQP3 localization was visualized by addition of Cy-3 conjugated secondary antibody (red channel); YFP fluorescence from the HyPer chromophore, as a marker of transfection, is visualized in green. (*A*) HT-29 cells expressing HyPer and AQP3. (*B*) DIC image of cells in A. (*C*) HT-29 cells expressing HyPer and control vector. (*D*) DIC image of cells in D. Scale bar = 10 μm.

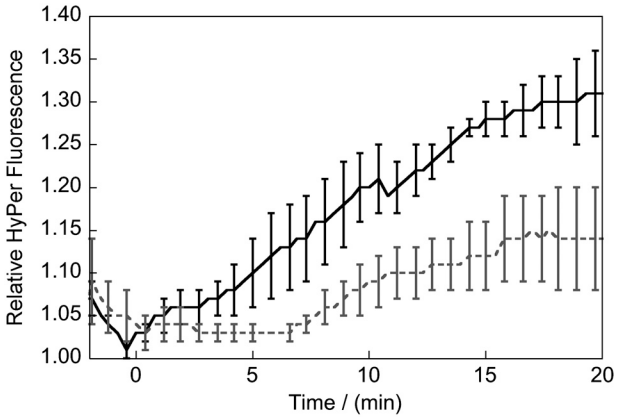

Fig. S6. Live-cell imaging of changes in HyPer fluorescence upon treatment of HEK 293 cells with 10 μM H_2O_2. HEK 293 cells transfected with HyPer and AQP3 (solid line) or control vector (dashed line) were treated with 10 μM H_2O_2 and the changes in HyPer fluorescence were monitored over time. Error bars are ±s.e.m. ($n = 3$).

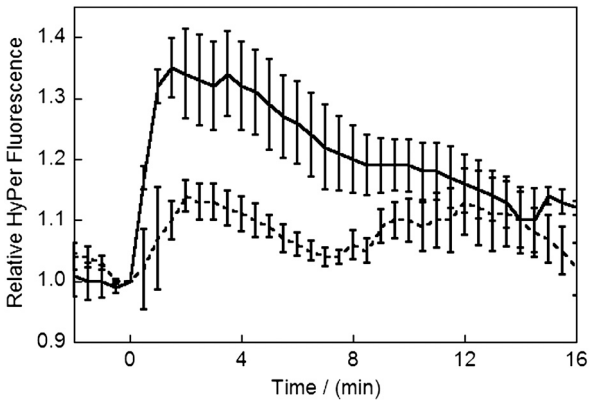

Fig. S7. Live-cell imaging of changes in HyPer fluorescence upon treatment of HT-29 cells with 100 μM H_2O_2. HT-29 cells transfected with HyPer and AQP3 (solid line) or pNice vector (dashed line) were treated with 100 μM H_2O_2 and the changes in HyPer fluorescence were monitored over time. Error bars are ±s.e.m. ($n = 3$).

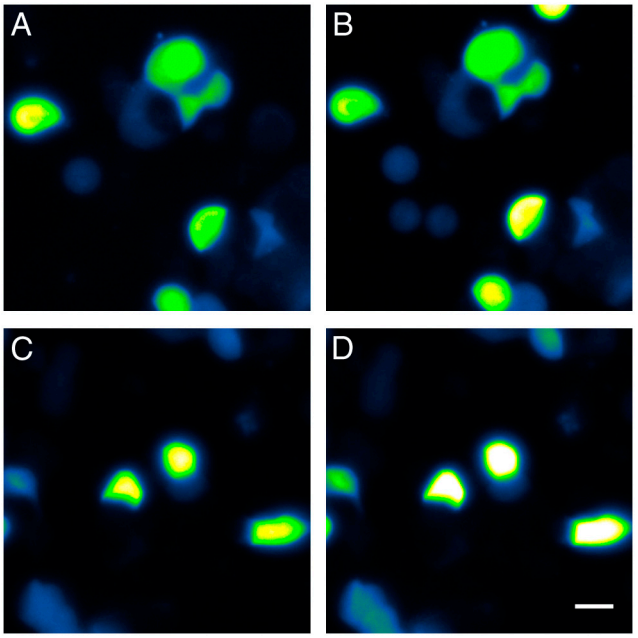

Fig. S8. Live-cell imaging of changes in HyPer fluorescence upon treatment of HT-29 cells with 100 μM H_2O_2. (*A*) HT-29 cells expressing HyPer and transfected with pNice as a control vector before addition of H_2O_2. (*B*) Cells from A, 4 min after addition of 100 μM H_2O_2. (*C*) HT-29 cells expressing HyPer and AQP3 before addition of H_2O_2. (*D*) Cells from C, 4 min after addition of 100 μM H_2O_2. Scale bar = 20 μm.