A Palette of Fluorescent Probes with Varying Emission Colors for Imaging Hydrogen Peroxide Signaling in Living Cells

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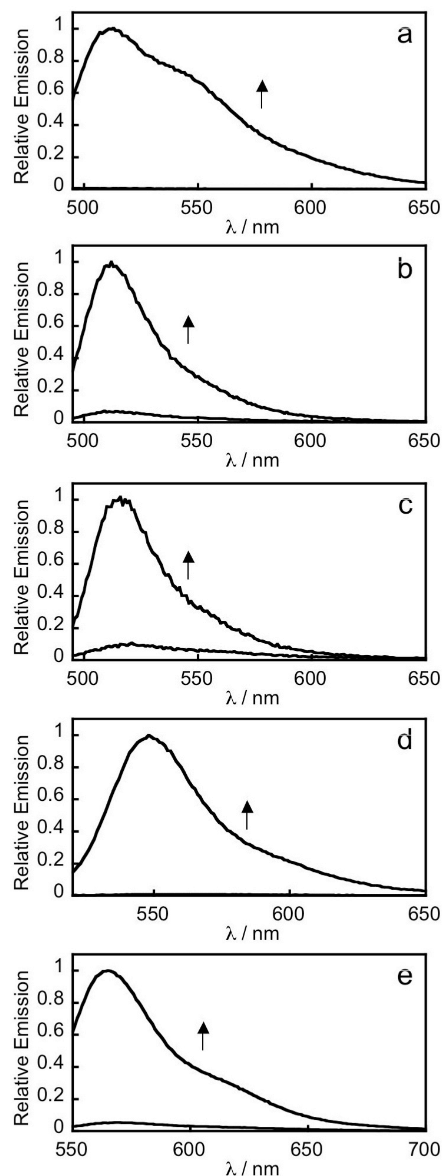

Figure S1. Complete fluorescence turn-on response of 5 μM PF2 (a), PF3 (b), PE1 (c), PY1 (d) or PO1 (e) to H_2O_2. Data were acquired at 25 ºC in 20 mM HEPES, pH 7, with excitation at λ = 488 nm for PF2 and PF3, λ = 490 nm for PE1, λ = 514 nm for PY1, and λ = 540 nm for PO1. Emission was collected between 493 and 750 nm for PF2 and PF3, 495 and 750 nm for PE1, 520 and 750 nm for PY1 and 545 and 750 nm for PO1. The emission spectra for the boronate-protected and deprotected forms of each dye are shown. In some cases, the boronate-protected form of the dye has an emission spectra that is not detectible in these plots.

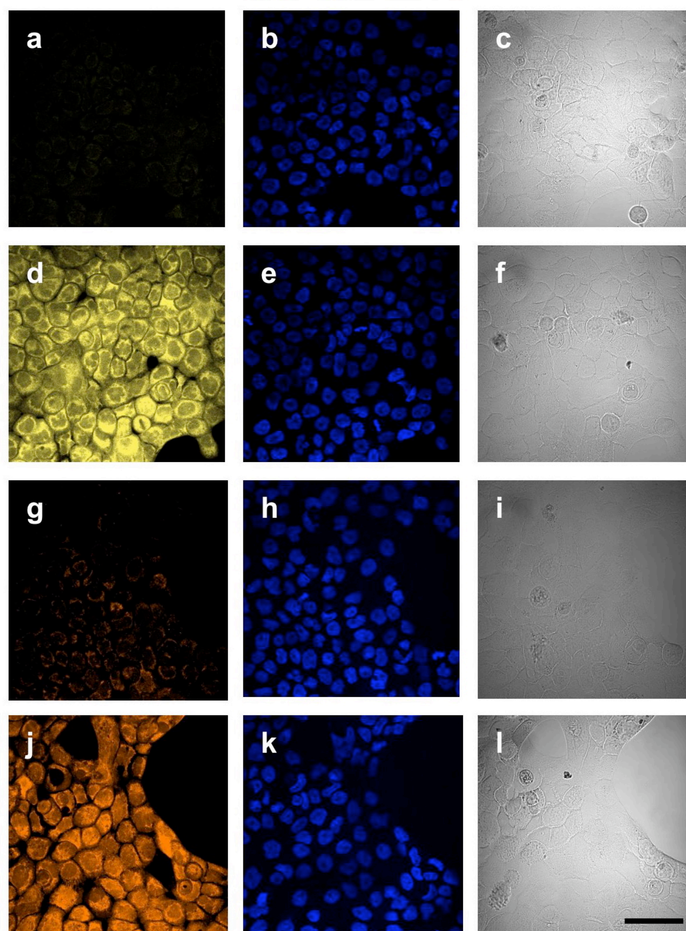

Figure S2. Confocal fluorescence images of H_2O_2 in live A431 cells under oxidative stress with PF2 and PF3-Ac with a nuclear stain and brightfields. A431 cells incubated with 10 μM PF2 for 40 min at 37 ºC and imaged for PF2 (a), Hoechst 33342 (b) and a brightfield (c). A431 cells incubated with 10 μM PF2 for 40 min at 37 ºC with 100 μM H_2O_2 added for the final 20 min and imaged for PF2 (d), Hoechst 33342 (e) and a brightfield (f). A431 cells incubated with 5 μM PF3-Ac for 40 min at 37 ºC and imaged for PF3-Ac (g), Hoechst 33342 (h) and a brightfield (i). A431 cells incubated with 5 μM PF3-Ac for 40 min at 37 ºC with 100 μM H_2O_2 added for the final 20 min and imaged for PF3-Ac (j), Hoechst 33342 (k) and a brightfield (l). 2 μM Hoechst 33342 added for the final 20 min of all experiments. 50 μm scale bare shown.

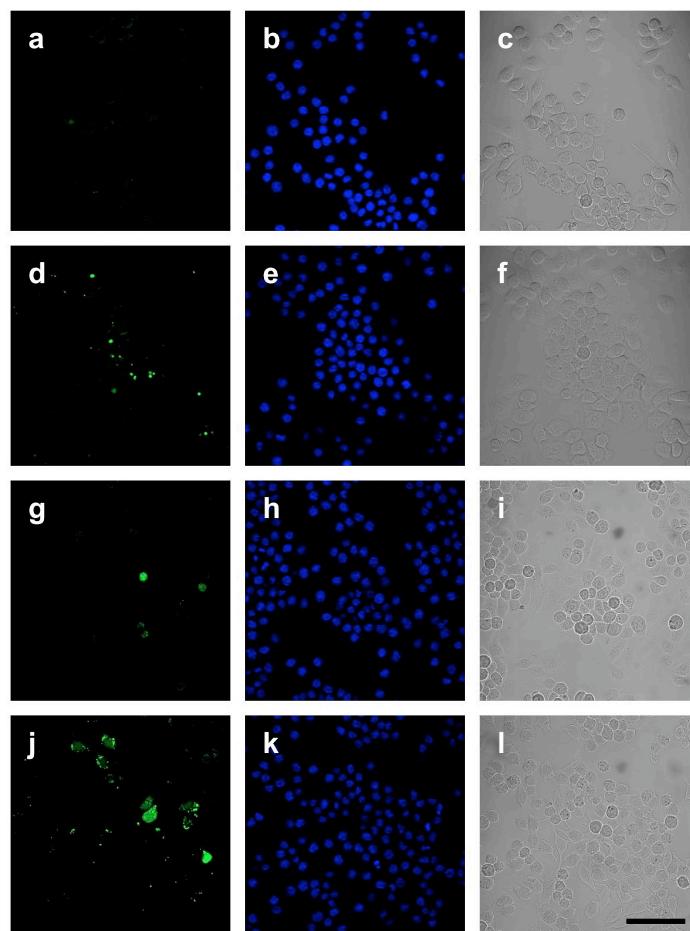

Figure S3. Confocal fluorescence images of H_2O_2 in live A431 cells under oxidative stress with PY1 and PO1 with a nuclear stain and brightfields. A431 cells incubated with 5 μM PY1 for 40 min at 37 ºC and imaged for PY1 (a), Hoechst 33342 (b) and a brightfield (c). A431 cells incubated with 5 μM PY1 for 40 min at 37 ºC with 100 μM H_2O_2 added for the final 20 min and imaged for PY1 (d), Hoechst 33342 (e) and a brightfield (f). A431 cells incubated with 5 μM PO1 for 40 min at 37 ºC and imaged for PO1 (g), Hoechst 33342 (h) and a brightfield (i). A431 cells incubated with 5 μM PO1 for 40 min at 37 ºC with 100 μM H_2O_2 added for the final 20 min and imaged for PO1 (j), Hoechst 33342 (k) and a brightfield (l). 2 μM Hoechst 33342 added for the final 20 min of all experiments. 50 μm scale bare shown.

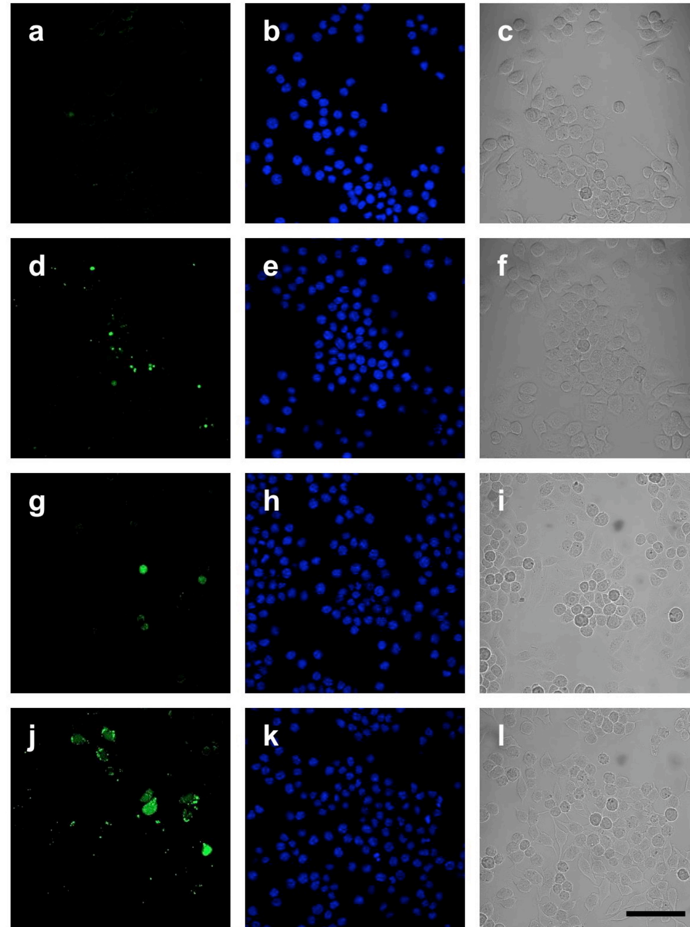

Figure S4. Confocal fluorescence images of PMA-induced H_2O_2 production in live RAW264.7 macrophages with PF2 and PF3-Ac with a nuclear stain and brightfield. Macrophages incubated with 10 μM PF2 for 60 min at 37 ºC and imaged for PF2 (a), Hoechst 33342 (b) and a brightfield (c). Macrophages incubated with 10 μM PF2 for 60 min at 37 ºC with 1 μg/mL PMA added for the final 40 min and imaged for PF2 (d), Hoechst 33342 (e) and a brightfield (f). Macrophages incubated with 5 μM PF3-Ac for 60 min at 37 ºC and imaged for PF3-Ac (g), Hoechst 33342 (h) and a brightfield (i). Macrophages incubated with 5 μM PF3-Ac for 60 min at 37 ºC with 1 μg/mL PMA added for the final 40 min and imaged for PF3-Ac (j), Hoechst 33342 (k) and a brightfield (l). 2 μM Hoechst 33342 added for the final 40 min of all experiments. 50 μm scale bare shown.

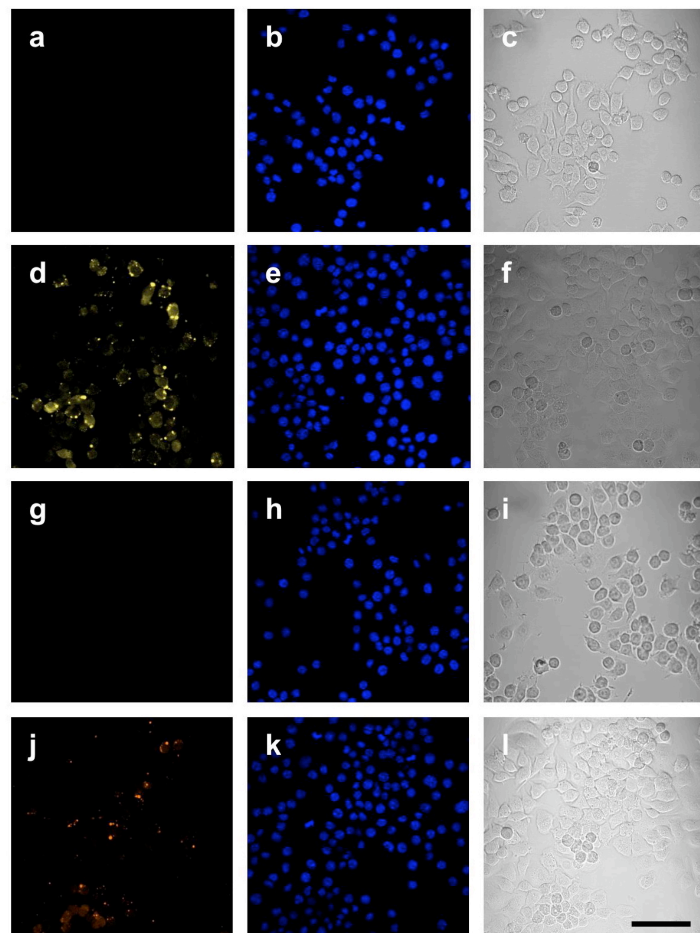

Figure S5. Confocal fluorescence images of PMA-induced H_2O_2 production in live RAW264.7 macrophages with PY1 and PO1 with a nuclear stain and brightfield. Macrophages incubated with 5 μM PY1 for 60 min at 37 ºC and imaged for PF2 (a), Hoechst 33342 (b) and a brightfield (c). Macrophages incubated with 10 μM PY1 for 60 min at 37 ºC with 1 μg/mL PMA added for the final 40 min and imaged for PY1 (d), Hoechst 33342 (e) and a brightfield (f). Macrophages incubated with 5 μM PO1 for 60 min at 37 ºC and imaged for PF3-Ac (g), Hoechst 33342 (h) and a brightfield (i). Macrophages incubated with 5 μM PO1 for 60 min at 37 ºC with 1 μg/mL PMA added for the final 40 min and imaged for PO1 (j), Hoechst 33342 (k) and a brightfield (l). 2 μM Hoechst 33342 added for the final 40 min of all experiments. 50 μm scale bare shown.

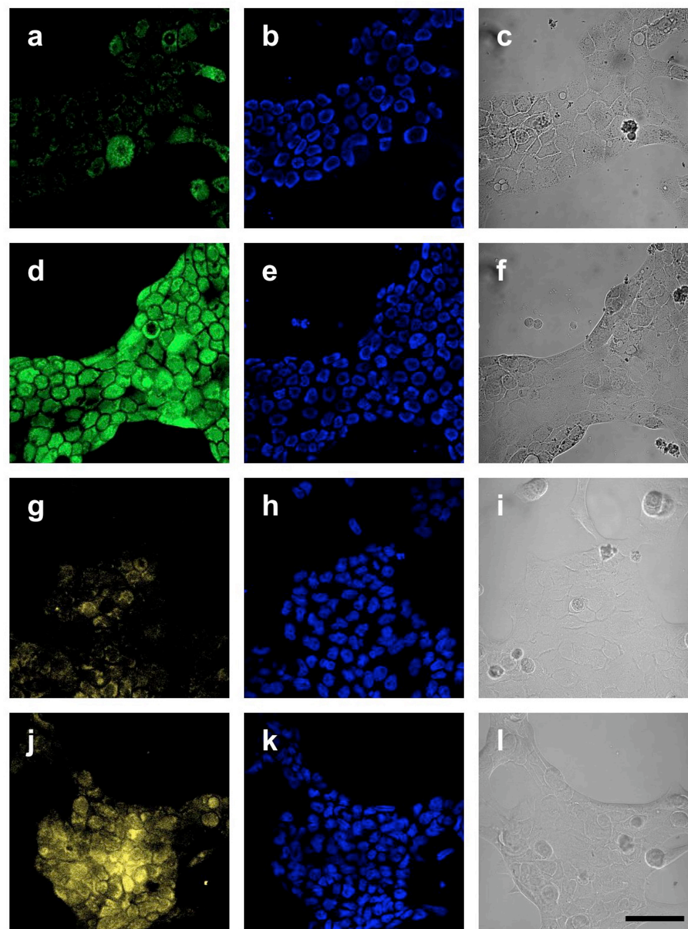

Figure S6. Confocal fluorescence images of EGF-induced H_2O_2 in live A431 cells with PF3-Ac and PY1 and a nuclear stain and brighfield. A431 cells incubated with 5 μM PF3-Ac for 60 min at 37 ºC and imaged for PF3-Ac (a), Hoechst 33342 (b) and a brightfield (c). A431 cells incubated with 5 μM PF3-Ac for 60 min at 37 ºC with 500 ng/mL EGF added for the final 40 min and imaged for PF3-Ac (d), Hoechst 33342 (e) and a brightfield (f). A431 cells incubated with 5 μM PY1 for 60 min at 37 ºC and imaged for PY1 (g), Hoechst 33342 (h) and a brightfield (i). A431 cells incubated with 5 μM PY1 for 60 min at 37 ºC with 500 ng/mL EGF added for the final 40 min and imaged for PY1 (j), Hoechst 33342 (k) and a brightfield (l). 2 μM Hoechst 33342 added for the final 40 min of all experiments. 50 μm scale bare shown.

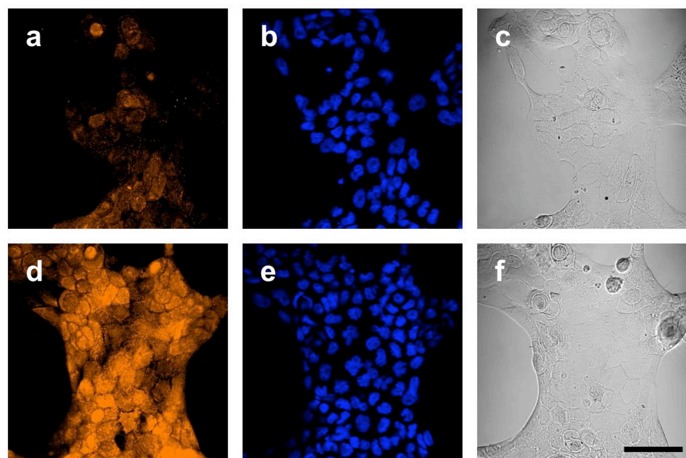

Figure S7. Confocal fluorescence images of EGF-induced H_2O_2 in live A431 cells with PO1 and a nuclear stain and brighfield. A431 cells incubated with 5 μM PO1 for 60 min at 37 ºC and imaged for PO1 (a), Hoechst 33342 (b) and a brightfield (c). A431 cells incubated with 5 μM PO1 for 60 min at 37 ºC with 500 ng/mL EGF added for the final 40 min and imaged for PO1 (d), Hoechst 33342 (e) and a brightfield (f).

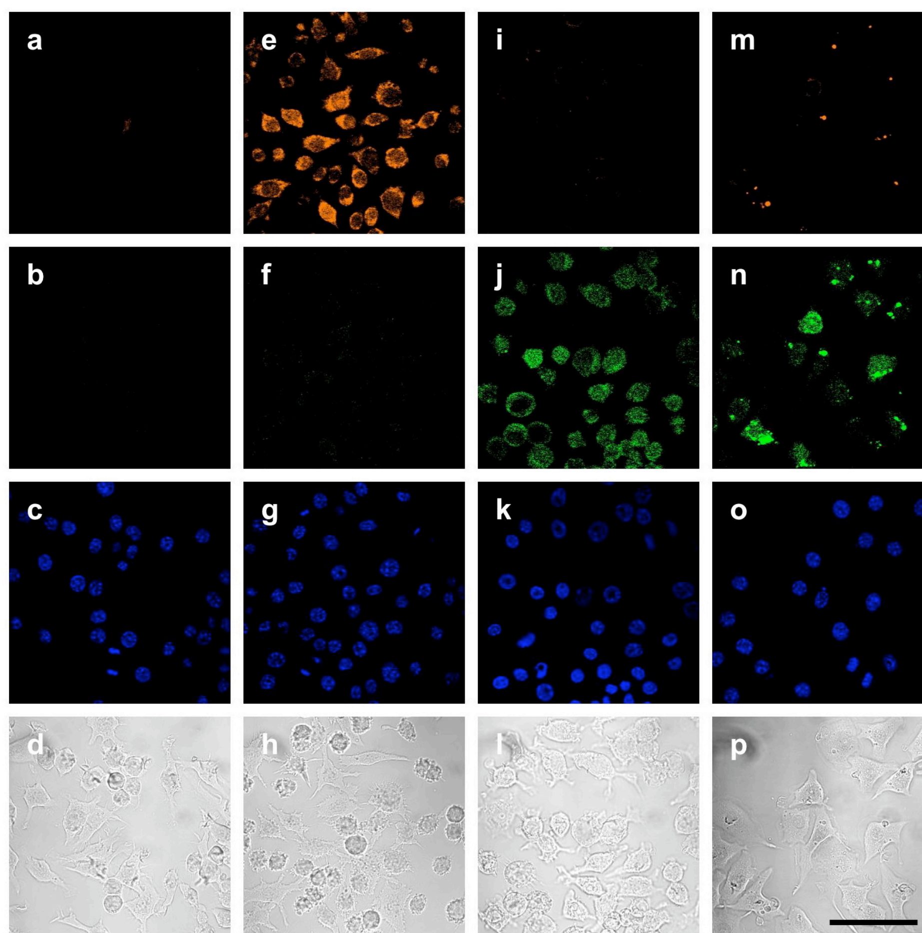

Figure S8. Confocal fluorescence images of PMA-induced ROS production in live RAW264.7 macrophages with PO1 and APF simultaneously with a nuclear stain and brightfield. Macrophages incubated with 5 μM PO1 and 5 μM APF for 40 min at 37 ºC and imaged for PO1 (a), APF (b), Hoechst 33342 (c), and a brightfield (d). Macrophages incubated with 5 μM PY1 and 5 μM APF for 40 min at 37 ºC with 50 μM H_2O_2 added for the final 20 min and imaged for PO1 (e), APF (f), Hoechst 33342 (g), and a brightfield (h). Macrophages incubated with 5 μM PO1 and 5 μM APF for 40 min at 37 ºC with 100 μM HOCl added for the final 20 min and imaged for PO1 (i), APF (j), Hoechst 33342 (k), and a brightfield (l). Macrophages incubated with 5 μM PO1 and 5 μM APF for 40 min at 37 ºC with 1 μg/mL PMA added for the final 20 min and imaged for PO1 (m), APF (n), Hoechst 33342 (o), and a brightfield (p). 2 μM Hoechst 33342 added for the final 20 min of all experiments. 50 μm scale bar shown.

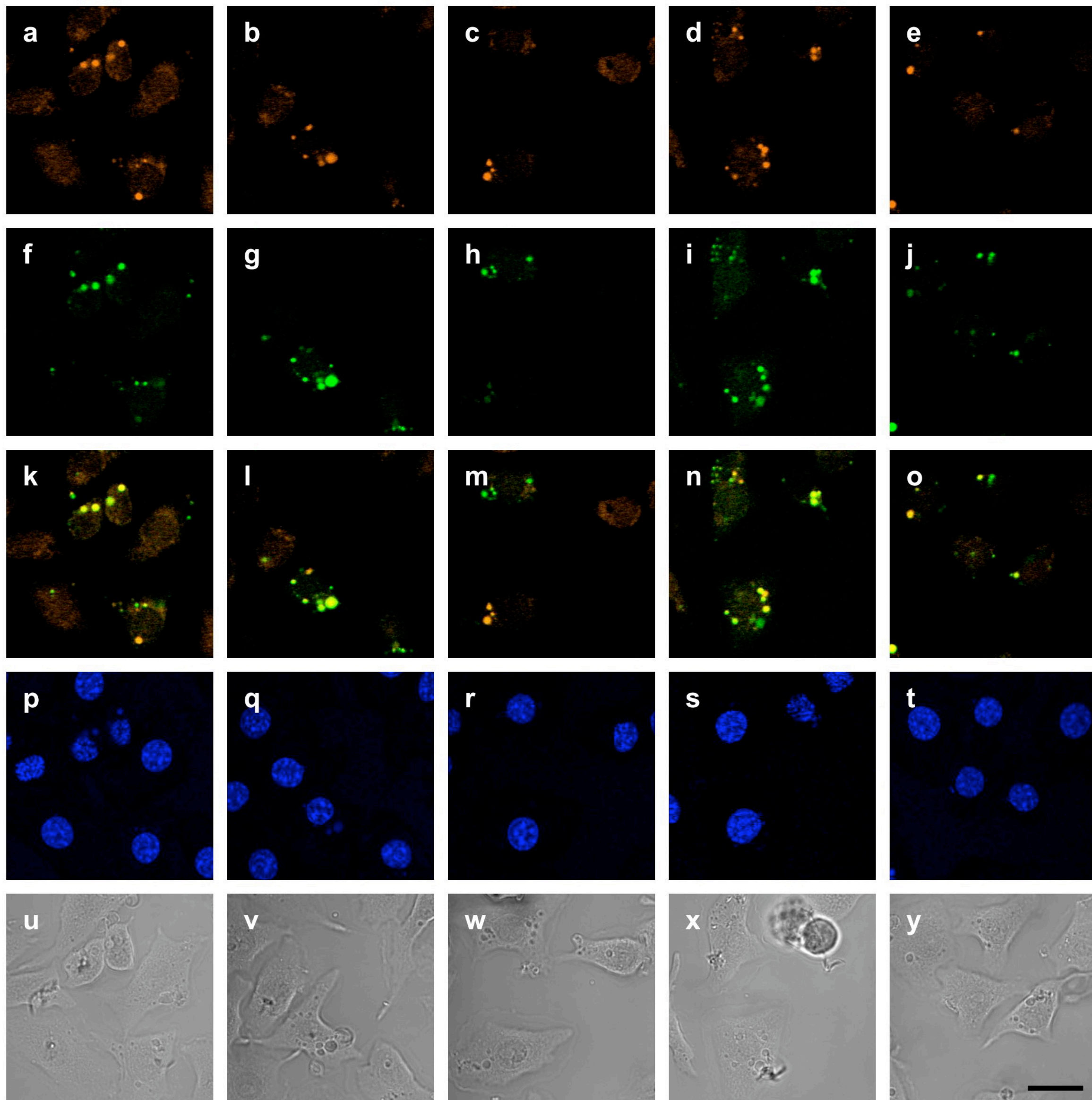

Figure S9. Confocal fluorescence images of PMA-induced ROS production in live RAW264.7 macrophages with PO1 and APF simultaneously with a nuclear stain and brightfield showing the various responses in ROS-production. Various regions of cells treated as in S6 showing signal from PO1 (a-e), APF (f-j), an overlay (k-o), Hoechst (p-t), and a brightfield (u-y). 20 μm scale bar shown.