Supporting information for

Versatile Histochemical Approach to Detection of Hydrogen Peroxide in Cells and Tissues Based on Puromycin Staining

Clive Yik-Sham Chung,[†,‡,§] Greg A. Timblin,[‡] Kaoru Saijo[‡] and Christopher J. Chang[†,‡,§,]*

[†]Department of Chemistry, [‡]Department of Molecular and Cell Biology, and [§]Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, California 94720, United States

*To whom correspondence should be addressed. Email: chrischang@berkeley.edu

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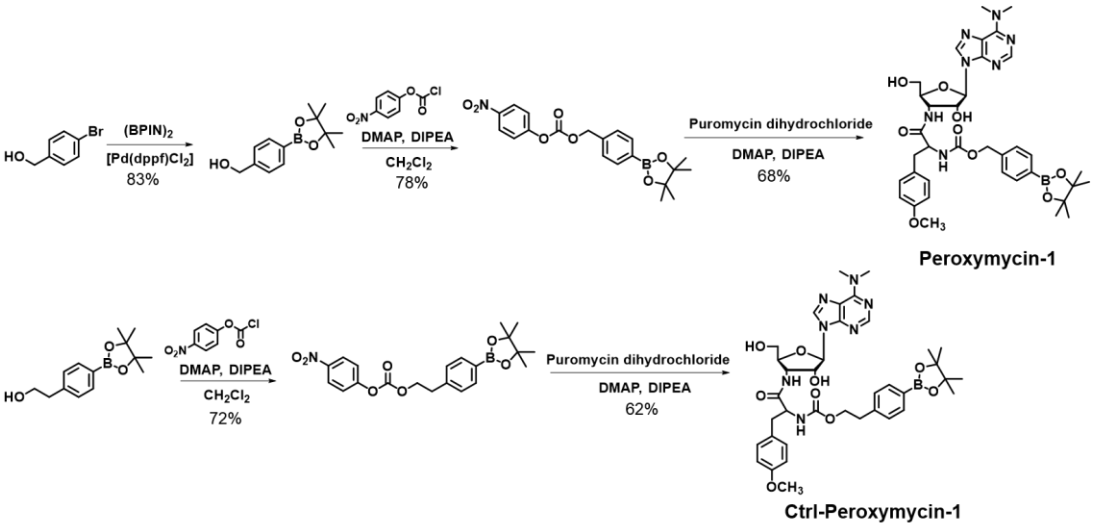

Scheme S1 Synthetic scheme of Peroxymycin-1 and Ctrl-Peroxymycin-1.

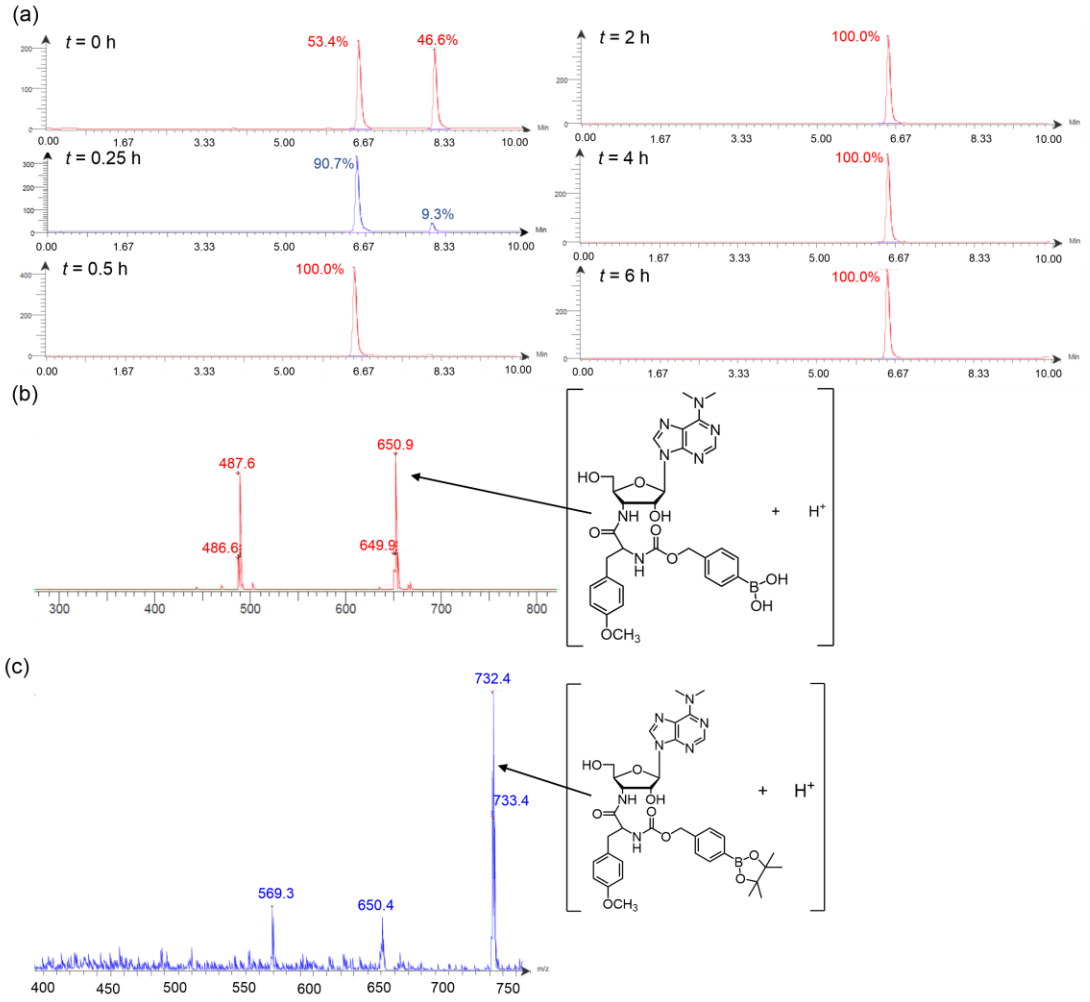

Figure S1 Stability of Peroxymycin-1 in the phosphate buffer (20 mM, pH 7.4)-methanol solution mixture (2:1, v/v) with 3 vol% DMSO. (a) LC chromatograms of Peroxymycin-1 (0.3 mM) in the solution mixture at different time intervals. MS of the peak with retention time of (b) 6.46 min and (c) 8.06 min respectively.

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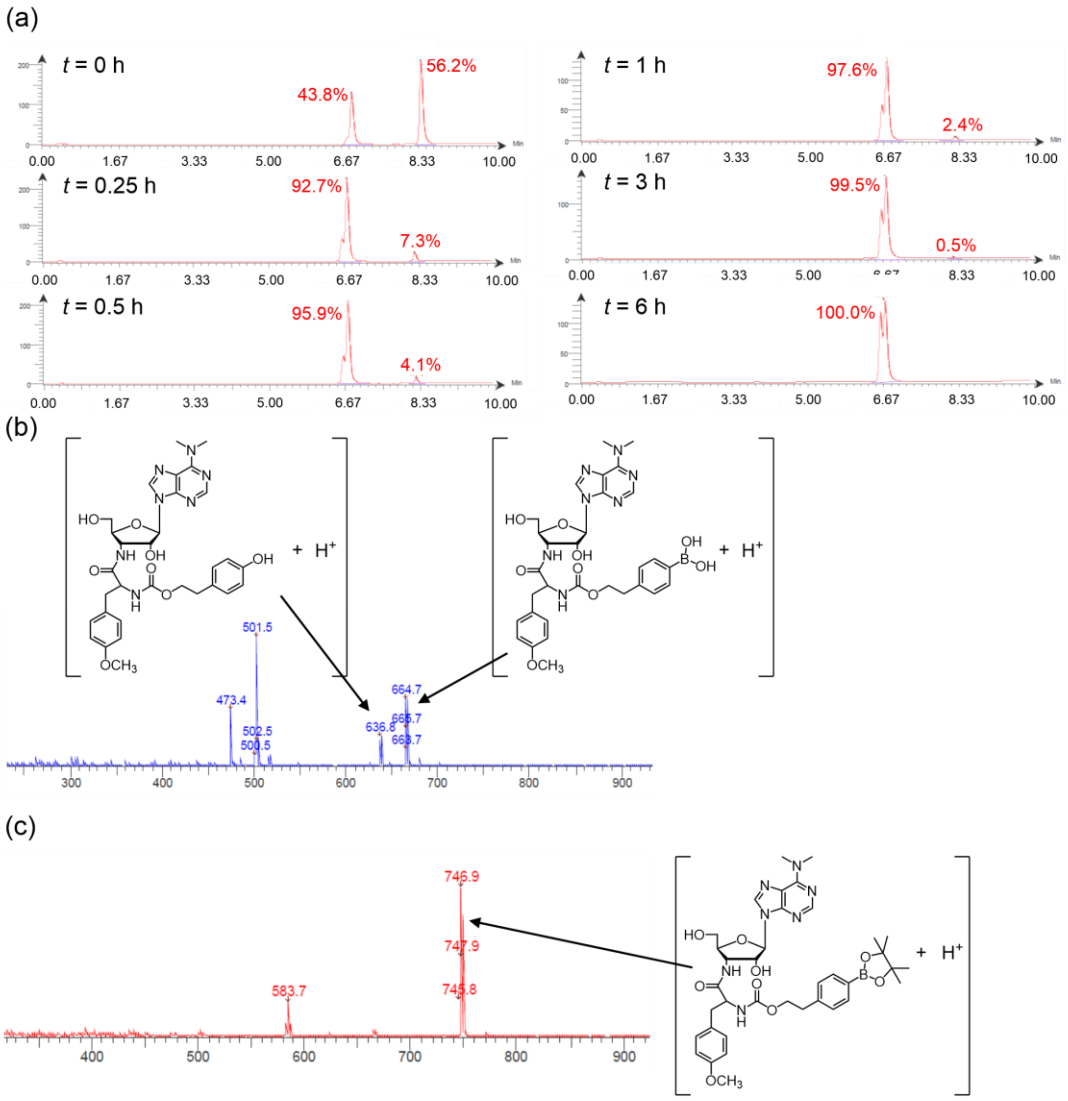

Figure S2 (a) LC chromatograms of the reaction mixture of Ctrl-Peroxymycin-1 (0.3 mM) and H_2O_2 (0.1 mM) in phosphate buffer (20 mM, pH 7.4)-methanol solution mixture (2:1, v/v) with 3 vol% DMSO at different time intervals. (b) MS of the two peaks with retention time ranging from 6.50 to 6.80 min. (c) MS of the peak with retention time of 8.22 min.

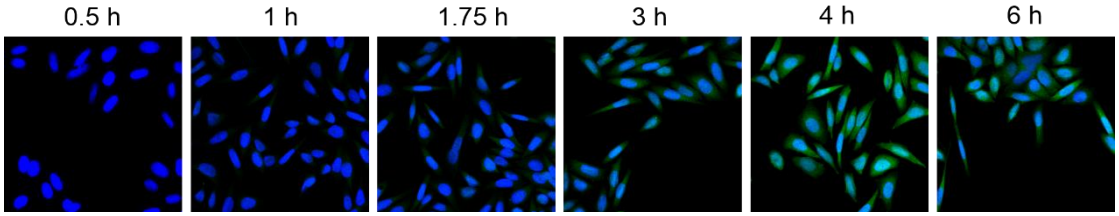

Figure S3 Overlaid images of Hoechst (blue) and Alexa488 (green) channels of HeLa cells treated with Peroxymycin-1 (1 μM) for indicated time intervals. The cells were then washed, fixed, stained and imaged.

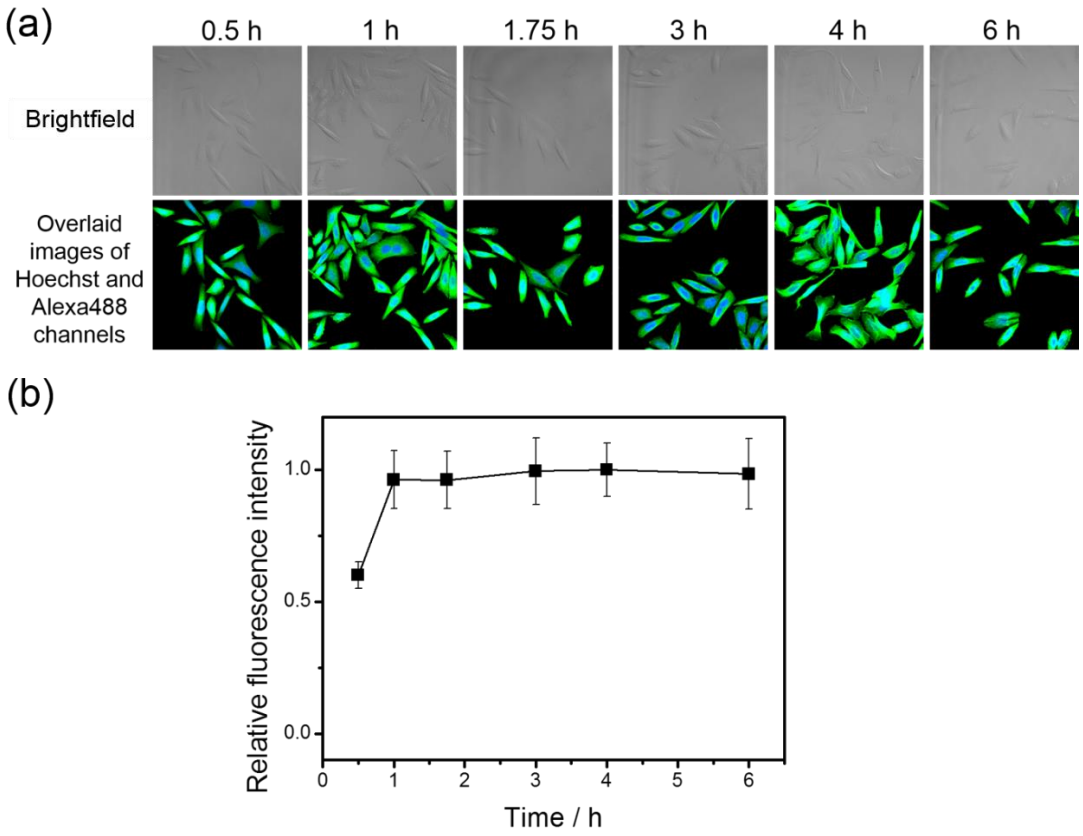

Figure S4 (a) Confocal fluorescence microscopy images of HeLa cells treated with puromycin (1 μM) for indicated time intervals. The cells were then washed, fixed, stained and imaged. (b) Normalized cellular fluorescence intensities of the HeLa cells as determined by ImageJ. Error bars denote SD (n = 5).

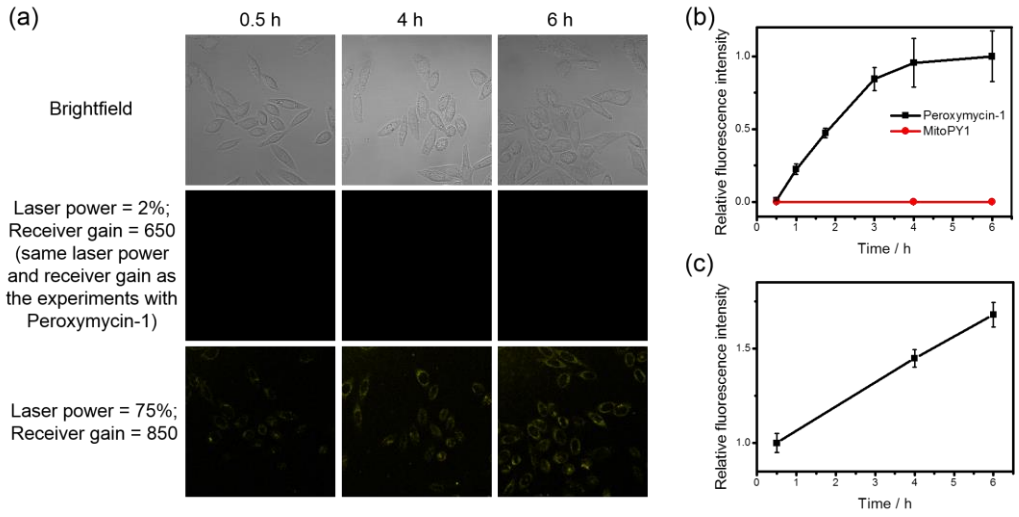

Figure S5 (a) Confocal fluorescence microscopy images of HeLa cells stained with MitoPY1 (5 μM) in full medium for indicated time intervals. The medium was then replaced by PBS solution and imaged using same laser power and receiver gain as the experiments of Peroxymycin-1, or with higher laser power and receiver gain. (b) Relative cellular fluorescence intensities of the HeLa cells stained by Peroxymycin-1 and MitoPY1 using same laser power and receiver gain, as determined by ImageJ. (c) Significant increase in fluorescence intensity from MitoPY1-stained HeLa cells over time at higher laser power and receiver gain, indicating that MitoPY1 is capable of detecting basal H_2O_2 level in HeLa cells under this experimental setting. Error bars denote SD (n = 5).

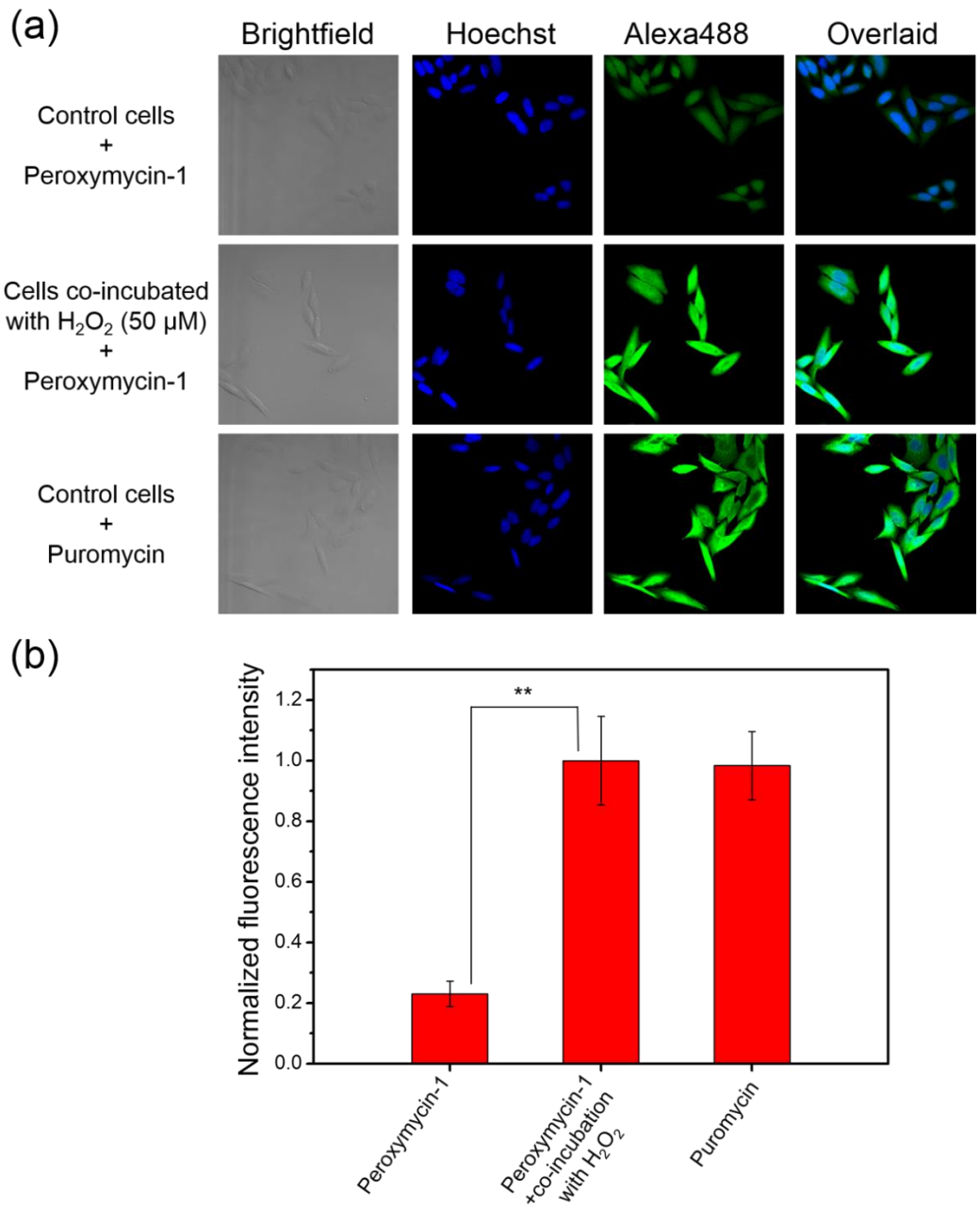

Figure S6 (a) Confocal fluorescence microscopy images of HeLa cells treated with Peroxymycin-1 and puromycin (1 μM), respectively, for 4 h, with or without co-incubation with H_2O_2 (50 μM) for the last 2 h. The cells were then washed, fixed, stained and imaged. All the images were recorded using the same imaging parameters with the Alexa488 channel. (b) Cellular fluorescence intensities of the HeLa cells as determined by ImageJ. Error bars denote SD (n = 5). ** $p < 0.01$.

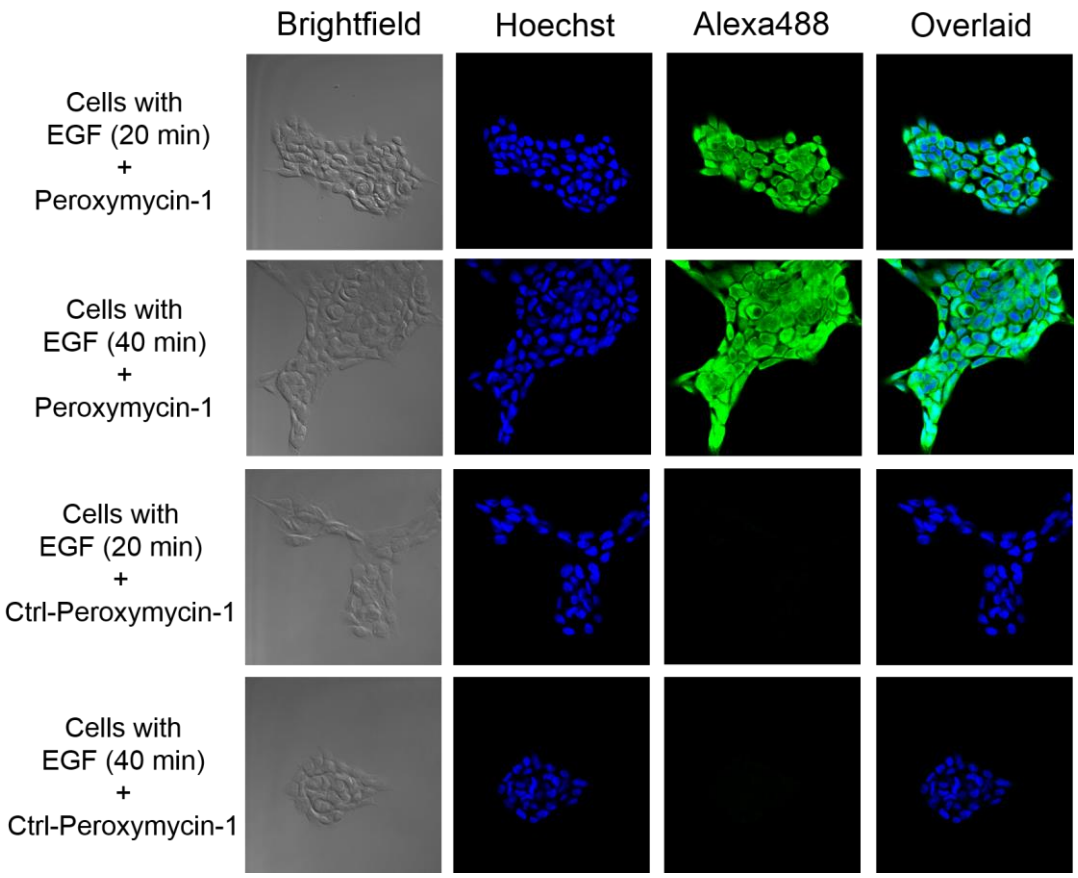

Figure S7 Confocal fluorescence microscopy images of A431 cells stained with Peroxymycin-1 or Ctrl-Peroxymycin-1 (1 μM), respectively, for 4 h, with or without pretreatment with EGF (100 ng/mL) for indicated time intervals. The cells were subsequently washed, fixed, stained and imaged. All the images were recorded using the same imaging parameters with the Alexa488 channel.

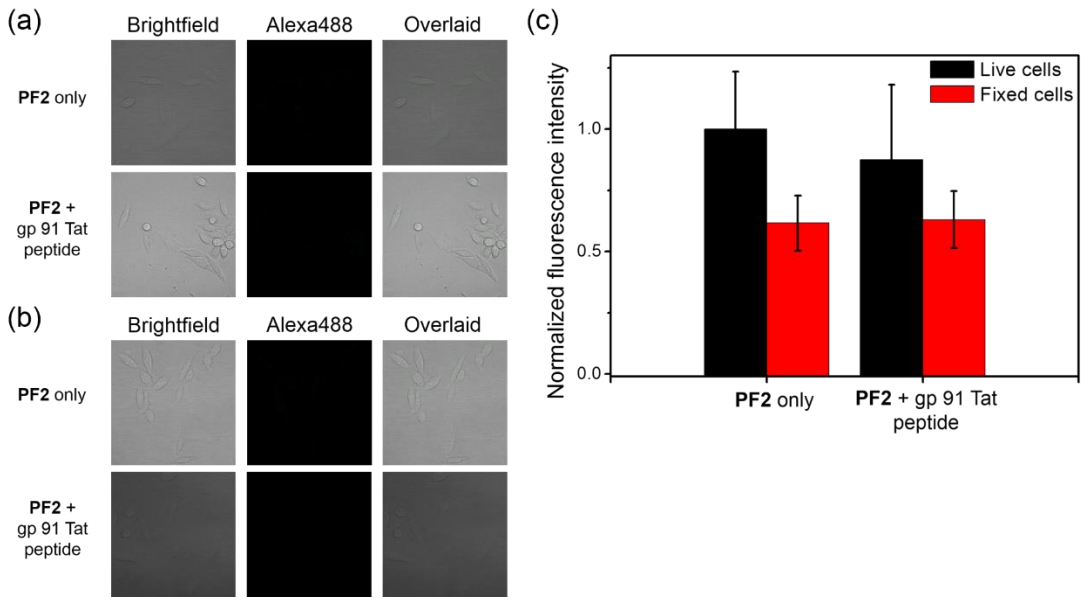

Figure S8 Confocal fluorescence microscopy images of HeLa cells, without pre-treatment or with pre-treatment with gp91 Tat peptide (100 μM) for 30 min and subsequent washing by PBS, stained with PF2 (10 μM) for 4 h. The cells were then (a) imaged in PBS solution, or (b) fixed by 4% paraformaldehyde solution and imaged in PBS solution. (c) Cellular fluorescence intensities of the HeLa cells as determined by ImageJ. Error bars denote SD (n = 5).

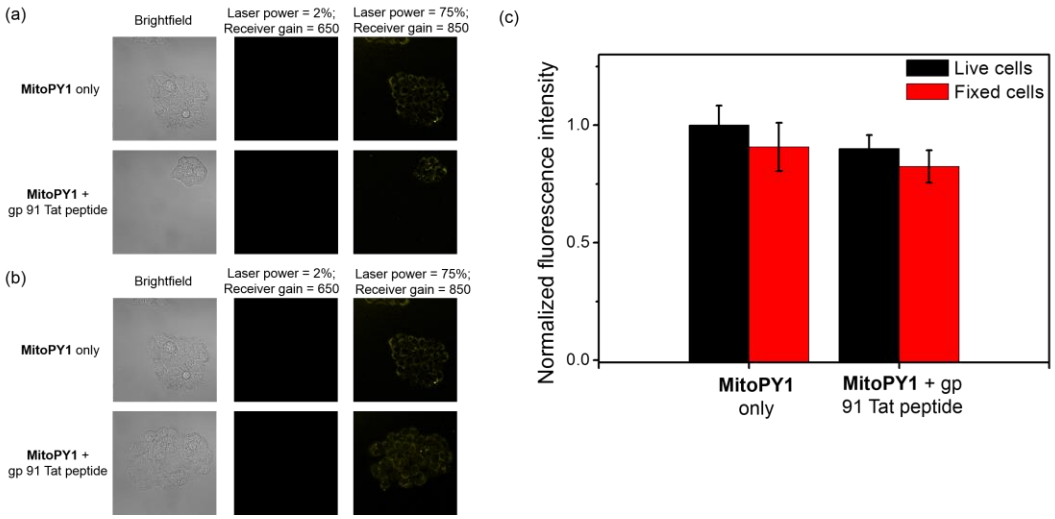

Figure S9 Confocal fluorescence microscopy images of A431 cells, without pre-treatment or with pre-treatment with gp91 Tat peptide (100 μM) for 30 min, treated with MitoPY1 (5 μM) and gp91 Tat peptide (100 μM) for 4 h. The cells were then (a) imaged in PBS solution, or (b) fixed by 4% paraformaldehyde solution and imaged in PBS solution using same laser power and receiver gain as the experiments of Peroxymycin-1, or with higher laser power and receiver gain. (c) Cellular fluorescence intensities of the HeLa cells imaged at higher laser power and receiver gain, as determined by ImageJ. Error bars denote SD (n = 5).

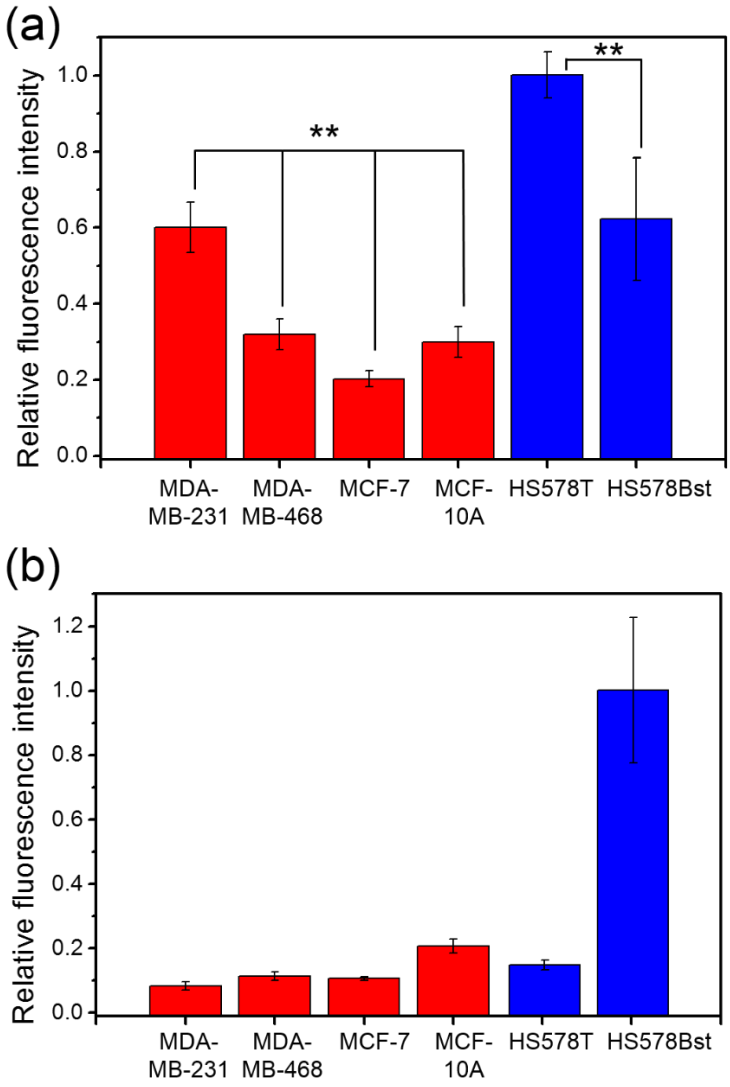

Figure S10 Fluorescence intensity from cells treated by (a) Peroxymycin-1 and (b) puromycin (1 μM) respectively, for 4 h. Error bars denote SD (n = 5). ** $p < 0.01$.

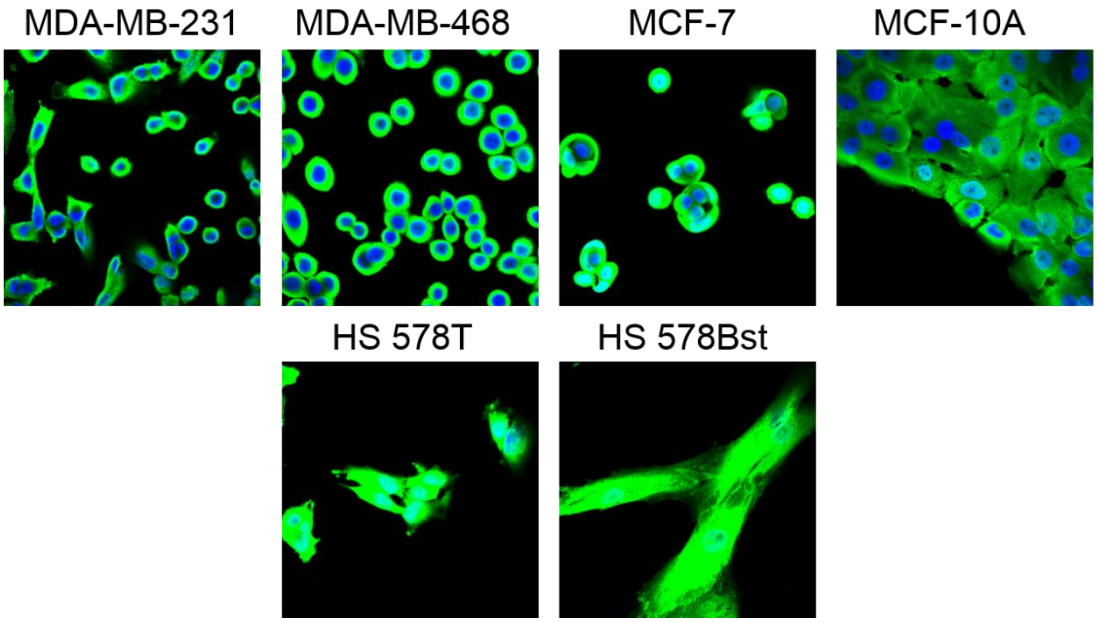

Figure S11 Confocal fluorescence microscopy images of breast normal cells (HS 578Bst), non-tumorigenic breast epithelial cells (MCF-10A), breast cancer cells (MDA-MB-468 and MCF-7), invasive breast cancer cells (HS 578T) and highly metastatic breast cancer cells (MDA-MB-231) treated with puromycin (1 μM) for 4 h. The cells were then washed, fixed, stained and imaged. All the images were recorded using the same imaging parameters.

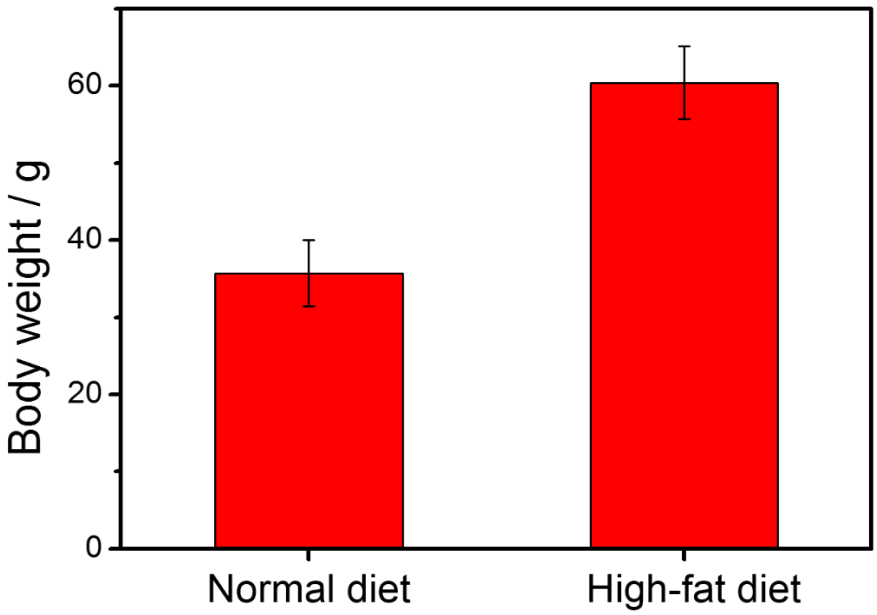

Figure S12 Body weight of mice fed with normal chow (NC) or high-fat diet (HFD) monitored at the end of feeding (20 wk).

Figure S13 Photograph of representative liver tissues harvested from mice fed with normal chow (NC; left) and high-fat diet (HFD; right) respectively, showing ectopic lipid deposition in livers of HFD mice.