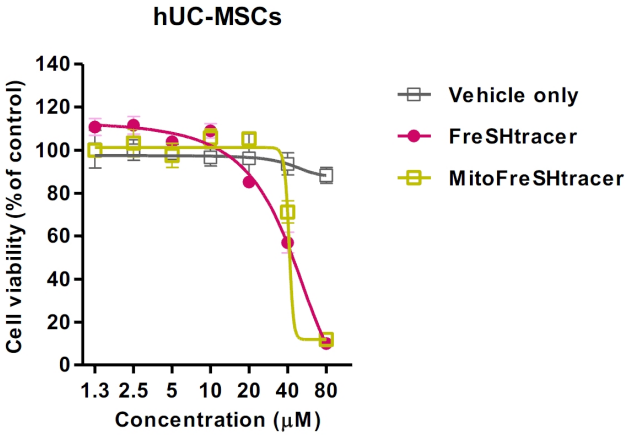

Fig. S1. Effects of FreSHtracer and MitoFreSHtracer on hUC-MSC viability. hUC-MSCs (5×10^3 cells/well; $n=3$ independent biological replicates) were cultured for 18 h in 96-well plates, and then treated for 24 h with either vehicle (dimethyl sulfoxide [DMSO]) or the indicated concentrations of FreSHtracer or MitoFreSHtracer. Cell viability was analyzed using a WST-1 assay. The LD50 values for FreSHtracer and MitoFreSHtracer were calculated as 34.2 ± 4.77 μM and >41.1 μM, respectively, using GraphPad Prism 5. All error bars represent the mean$\pm$SEM.

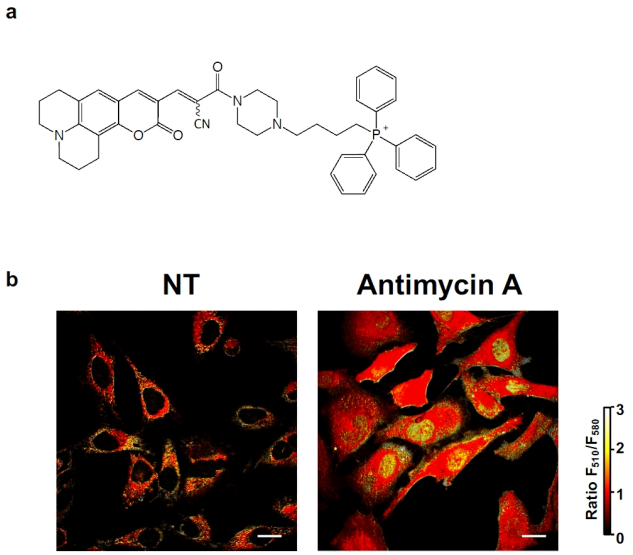

Fig. S2. Loss of mitochondrial staining pattern of triphenylphosphonium-conjugated FreSHtracer. (FreSHtracer-PPh_3) in antimycinA-treated HeLa cells. (a) Structure of FreSHtracer-PPh_3. (b) Pseudocolor images of the FR following treatment with antimycin A in FreSHtracer-PPh_3-loaded HeLa cells. HeLa cells were loaded with 10 μM FreSHtracer-PPh_3 for 1.5 h, washed with PBS, and then treated with 100 μM for 75 min, following confocal microscopic determination of the FR. Scale bars, 20 μm.

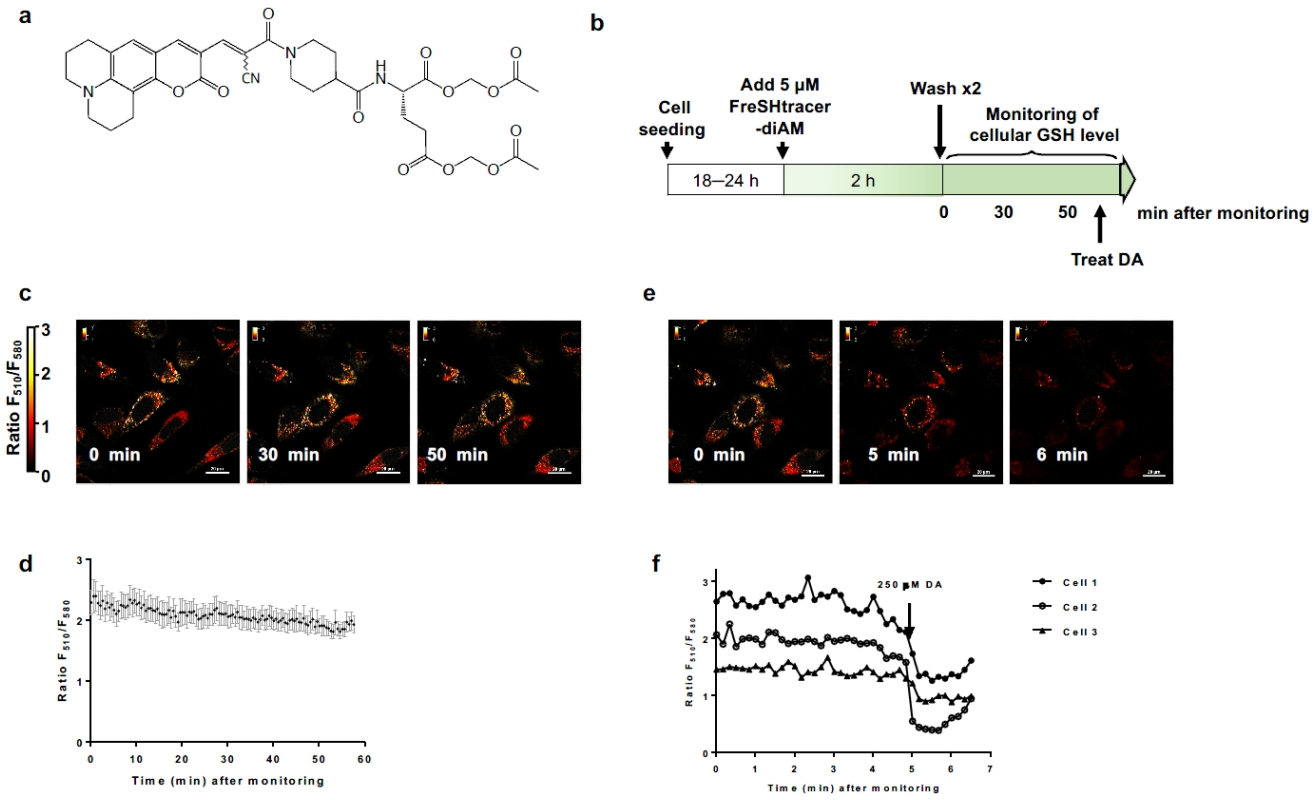

Fig. S3. GSH monitoring using di-acetoxymethyl ester-conjugated FreSHtracer (FreSHtracer-diAM) in living HeLa cells. (a) Structure of FreSHtracer-diAM. (b) Scheme of the experimental procedure for the monitoring of GSH in HeLa cells using FreSHtracer-diAM. (c, e) Pseudo-color images of the FR in FreSHtracer-diAM-loaded HeLa cells. (d, f) Time course of FR changes within HeLa cells (d; $n=6$). DA, diamide.

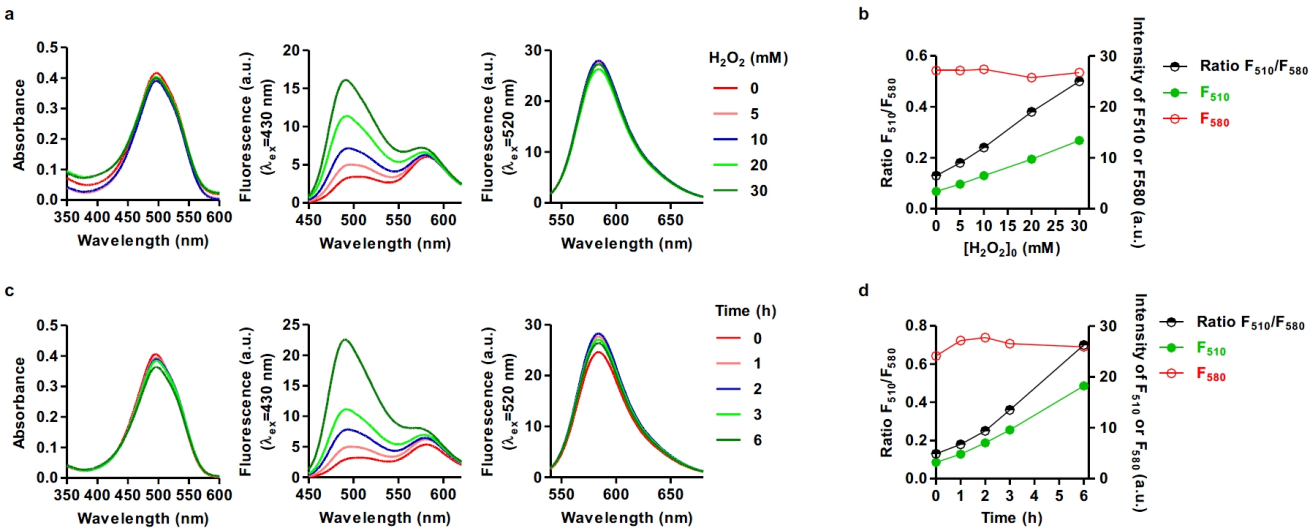

Fig. S4. Direct effects of an extremely high concentration of H_2O_2 on FreSHtracer fluorescence response. (a, b) FreSHtracer was reacted with the indicated concentrations of H_2O_2 for 1 h and analyzed using a UV-visible spectrometer and fluorometer. FreSHtracer emission spectra were obtained by excitation at 430 and 520 nm (a). The sensor emission ratio was calculated from the intensities at F_{510} and F_{580} (b). (c, d) FreSHtracer was reacted with 5 mM H_2O_2 for the indicated times and analyzed using the methods in a and b. All reactions were carried out using 10 μM [FreSH-tracer]$_0$ in 10 mM phosphate buffer containing 150 mM NaCl and 2% DMSO, pH 7.4, at room temperature. F_{510}, Ex430-Em$_{510}$; F_{580}, Ex520-Em580.