Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Data

In vivo senescence imaging nanoprobe targets the associated reactive oxygen species

Seung Koo Lee, Myung Shin Han, and Ching-Hsuan Tung**

Molecular Imaging Innovations Institute
Department of Radiology, Weill Cornell Medicine
413 East 69th Street, Box 290, New York, NY 10021, USA
E-mail: sel2013@med.cornell.edu; cht2018@med.cornell.edu

Figure S1. The size and zeta potential of D3. (a) The stepwise characterization of D3 and its precursor. The average size (gray bar) and zeta potential (black circle) during the D3 preparation. Each experiment was performed at least three times and the results were expressed as mean ± SD. (b) The TEM image of bare AuNPs and D3.

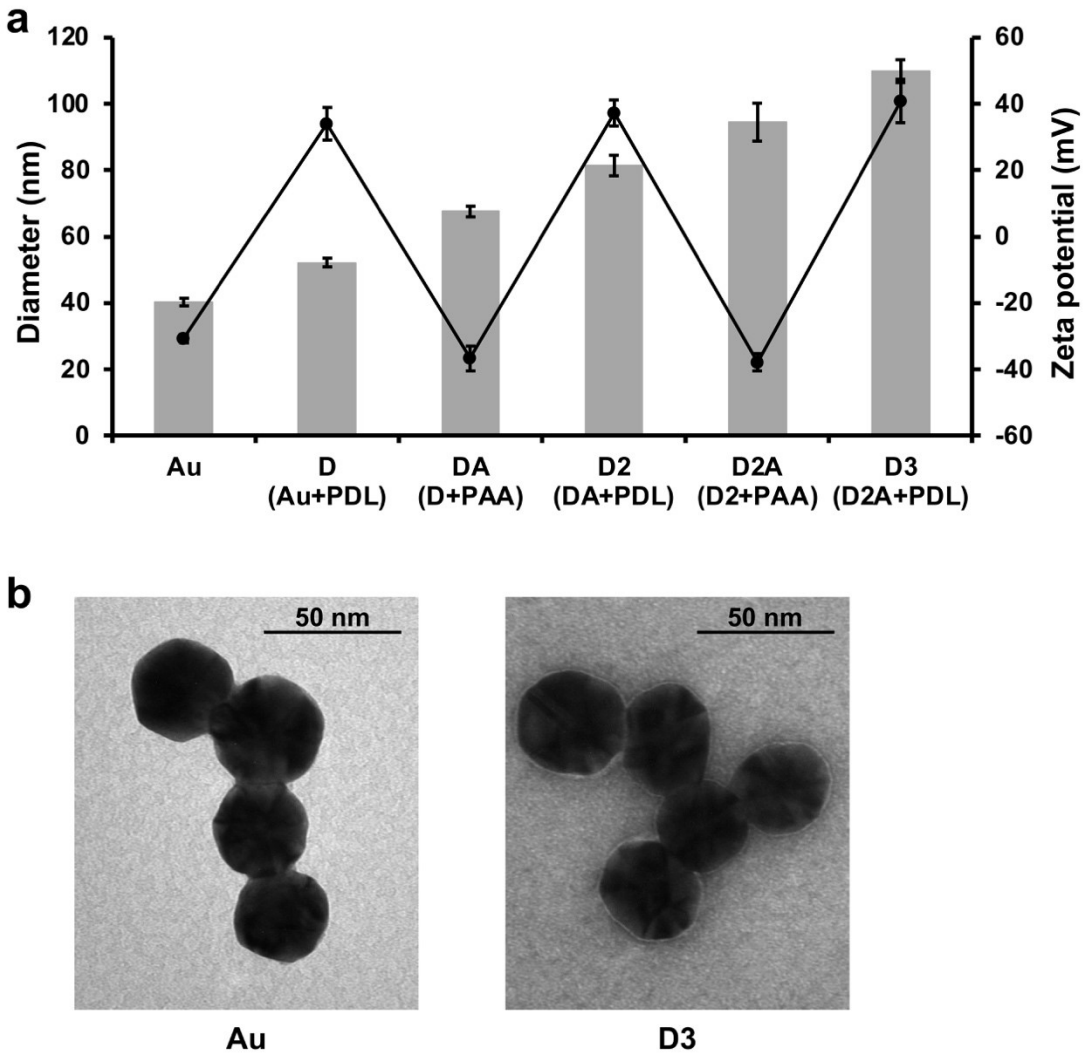

Figure S2. The cytotoxicity of D3. Cell viability was evaluated for seven days after D3 (0.12, 0.24, or 0.48 nmol) treatment in MDA-MB231 cells. The cell viability of the untreated MDA-MB231 cells was set as 100%. Each experiment was performed at least three times and the results were expressed as the median change in cell viability ± SD.

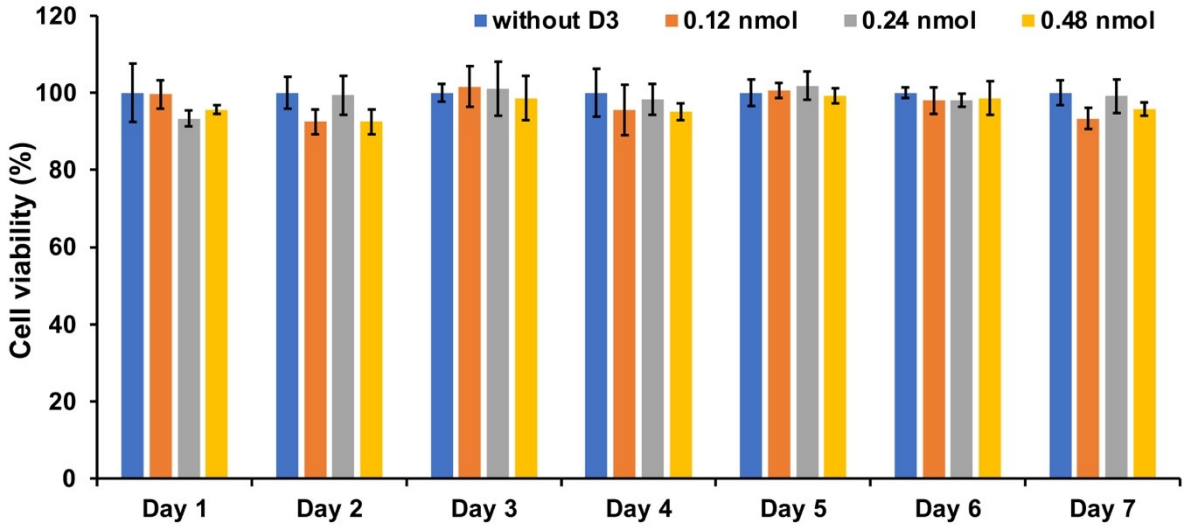

Figure S3. D3 activation by ROS in vitro. After incubation of MDA-MB231 with D3 (0.12 nmol), cells were cultured for 5 d. Then, cells were incubated with H_2O_2 (5 mM) and D3 signal was assessed via a fluorescence microscope (magnification: × 10). Scale bar, 400 μm. The fluorescence intensity of D3 was quantified using ImageJ and the fluorescence intensity of Day 1 was set as 1.

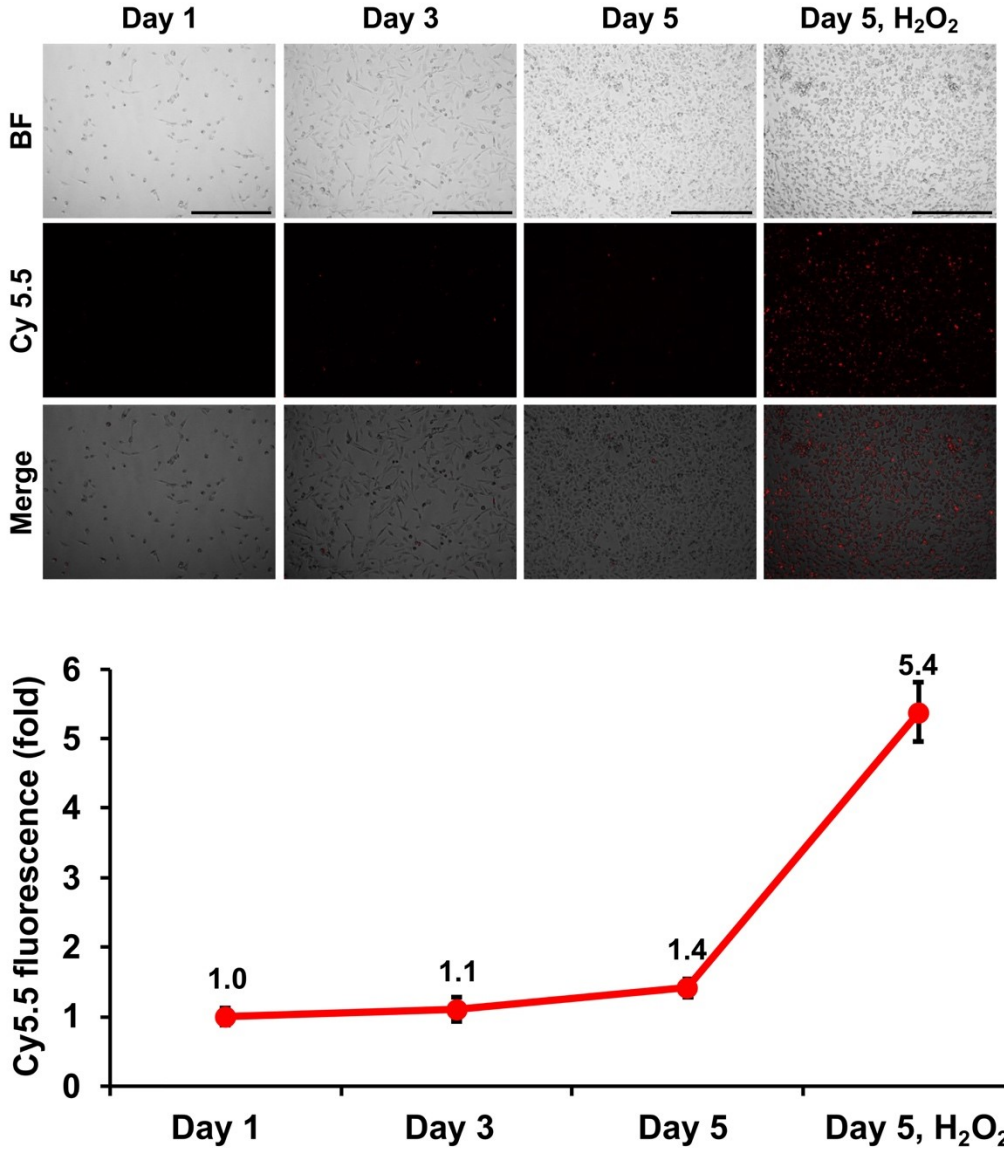

Figure S4. Characterization of pabociclib-induced cellular senescence. Various senescence detection assays, including X-gal staining, SPiDER-β-Gal staining, IF staining for p21 and γH2AX, mtSOX staining, and DCF-DA staining were performed in MDA-MB231 and SN_MB231 cells (magnification: × 20 or × 10). Scale bar, 200 μm. The percentage of X-gal–positive cells was determined as follows: number of X-gal–positive cells/number of attached cells × 100. The fluorescence intensity of β-Gal, p21, γH2AX, mtSOX and DCF-DA was quantified using ImageJ and the untreated MDA-MB231 cells' fluorescence intensity was set as 1.

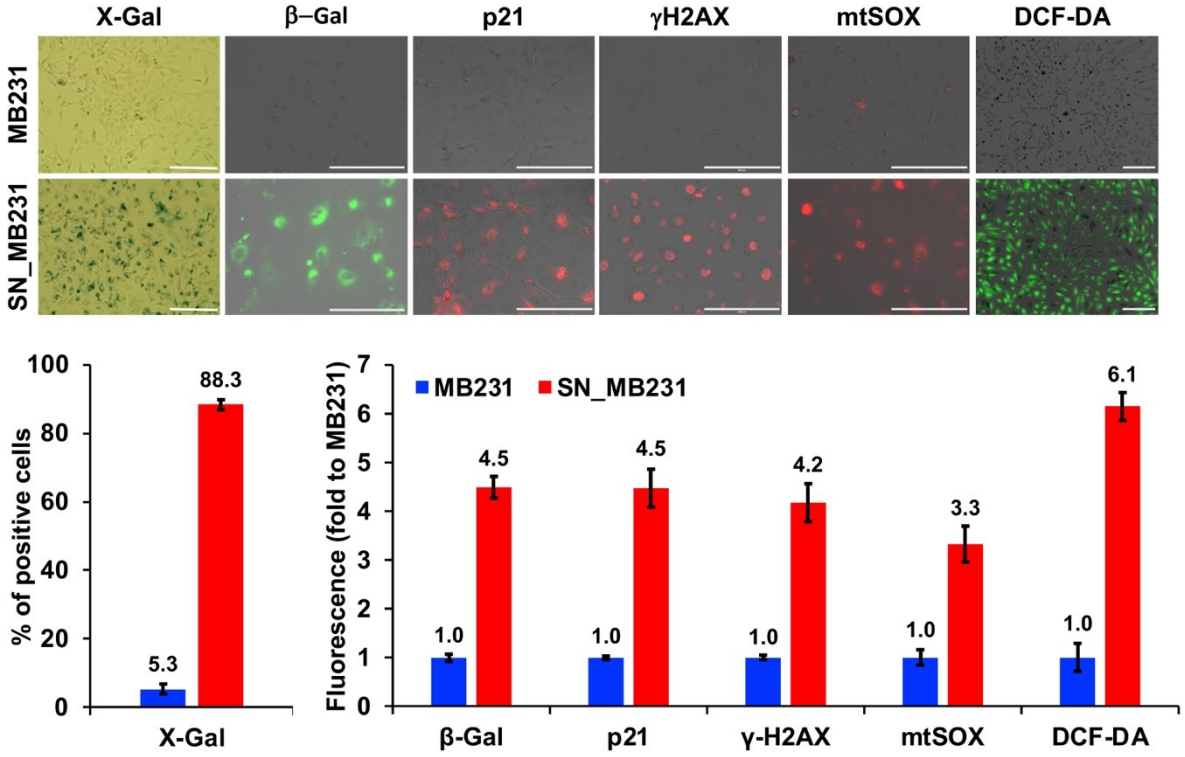

Figure S5. ROS production by palbociclib treatment. DCF-DA fluorescence intensity was measured using a plate reader (λ_{ex} = 485 nm, λ_{em} = 535 nm) during treatment with or without 5 μM of palbociclib in MDA-MB231 cells for 7 d. Each experiment was performed at least three times and the results were expressed as the median change in fluorescence ± SD.

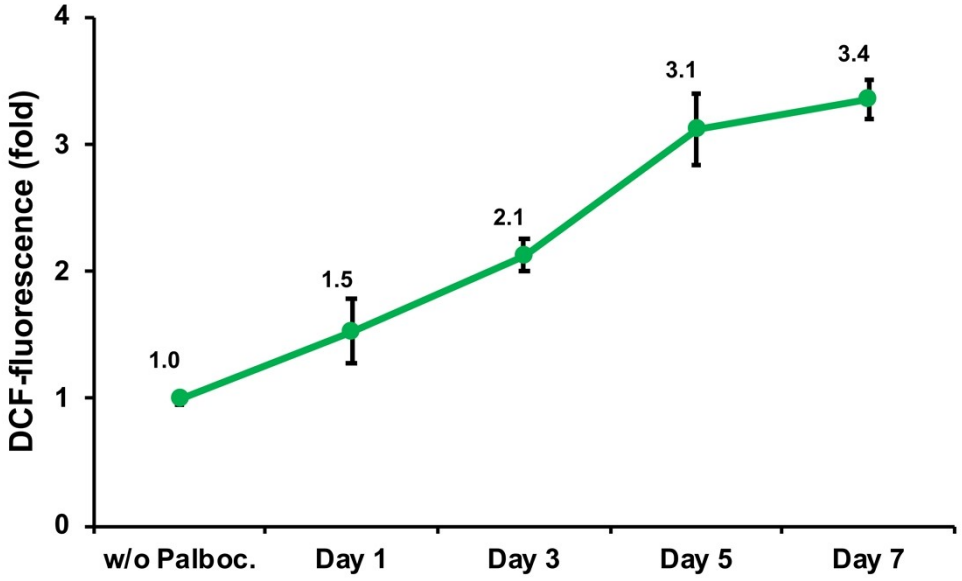

Figure S6. Senescent cell-specific detection of D3. Senescent MB231-GFP (SN_MB231-GFP) and MDA-MB231 (MB231) cells were cocultured at the ratios (SN_MB231-GFP:MB231) of 1:9, 2:8, 3:7, and treated with D3 (0.2 nmol). After 2 d, cells were imaged using a fluorescence microscope in the GFP and cy5.5 channels (magnification: × 10). Scale bar, 400 μm.

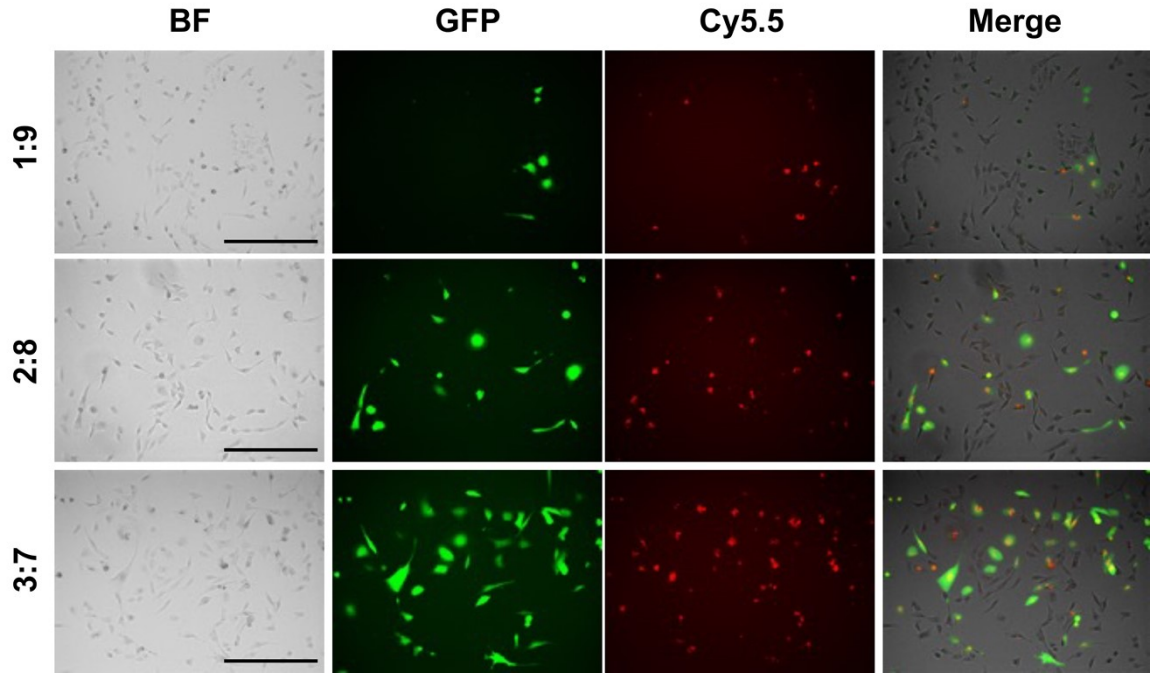

Figure S7. ROS production and D3 activation in young and old mouse fibroblast cells. (a) DCF-DA fluorescence intensities in different aged fibroblasts, from mouse embryo (NIH3T3), 4-month-old mouse (AG22816) and 12-month-old mouse (AG22858), were measured using a plate reader (λ_{ex} = 485 nm, λ_{em} = 535 nm) (b) After incubation with D3 (0.12 nmol) for 12 h, three kinds of cells were imaged using a fluorescence microscope (cy5.5 channels, magnification: × 10). Scale bar, 400 μm.