Electronic Supplementary Information

Developing a far-red fluorogenic beta-galactosidase probe for senescent cell imaging and photoablation

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

Molecular Imaging Innovations Institute, Department of Radiology, Weill Cornell Medicine, New York, NY, USA.

# Equal contribution

* To whom correspondence should be addressed.

413 East 69th Street, Box 290, New York, NY 10021, USA. Phone: +1-646-962-2923; E-mail: cht2018@med.cornell.edu.

Figure S1. Synthetic scheme of Gal-MB.

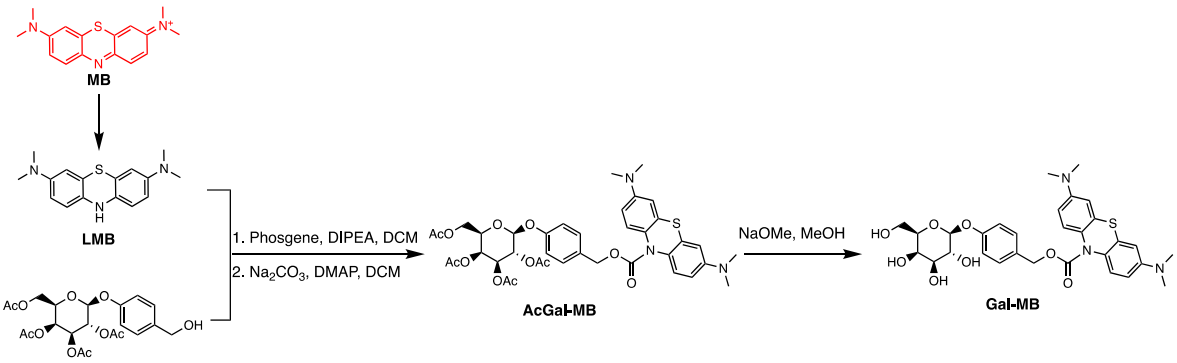
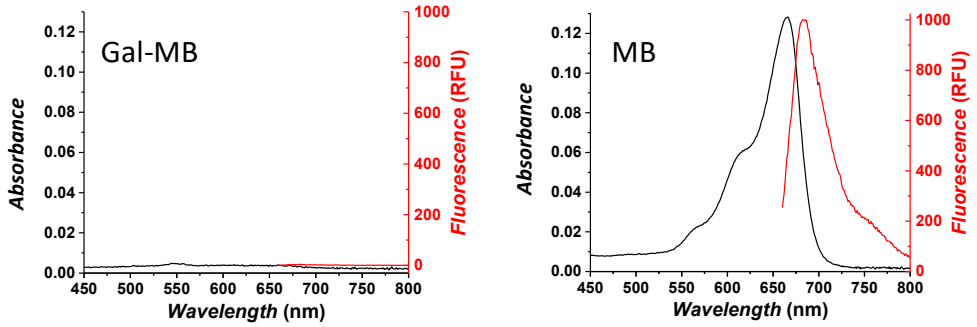


Fig. S2. Characterization of Gal-MB and MB. (A) Absorption and emission spectra. (B) LC-MS spectra of Gal-MB activation by β-gal. Gal-MB (300 μM, 1 mL) in PBS buffer (pH = 7.4) was treated with β-gal (10 U L^{-1}) at room temperature. The HPLC spectra (*left*) were recorded at different time points (0, 5, 15, and 60 min). MS spectra (*right*) confirmed that the peak at 5.90 min was Gal-MB (*m*/*z* = 598.5) and the peak at 5.77 min was MB (*m*/*z* = 284.2).

A.



B.

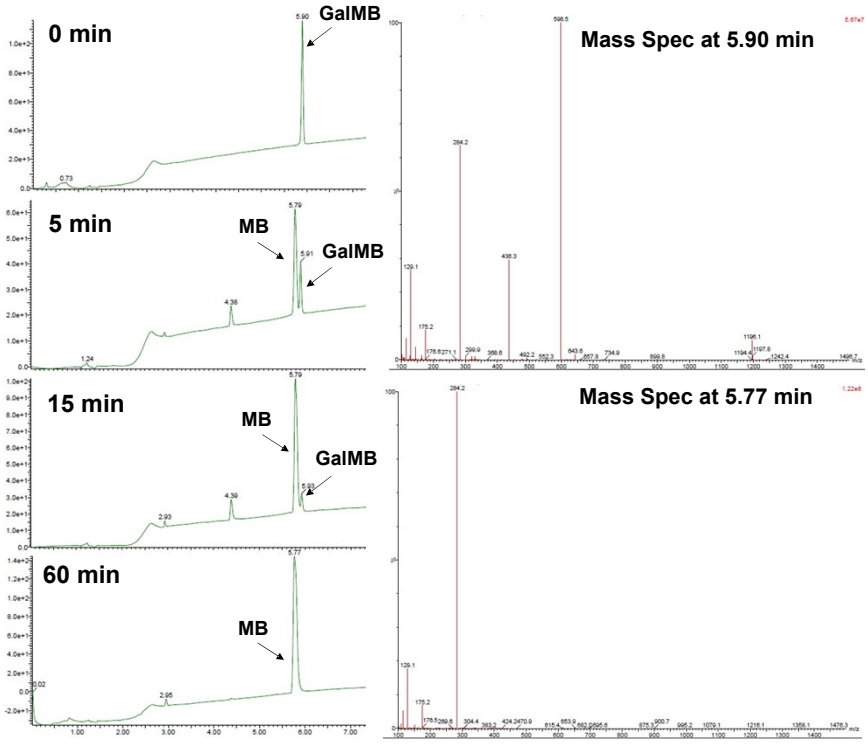


Figure S3. SA-β-gal dependent Gal-MB activation. A549 cells were irradiated with various dose of X-ray (0, 2, 5, or 10 Gy) and incubated in complete medium for 5 days. (A) X-ray-induced senescent cells were investigated by X-gal staining (magnification: × 20). (B) For SA-β-gal detection, the irradiated A549 cells (0, 2, 5, or 10 Gy) were incubated with Gal-MB (5 μM) for 1 h, washed and imaged using fluorescence microscope (magnification: × 40). Scale bar, 100 μm. (C) The Gal-MB fluorescence intensity was quantified using ImageJ.

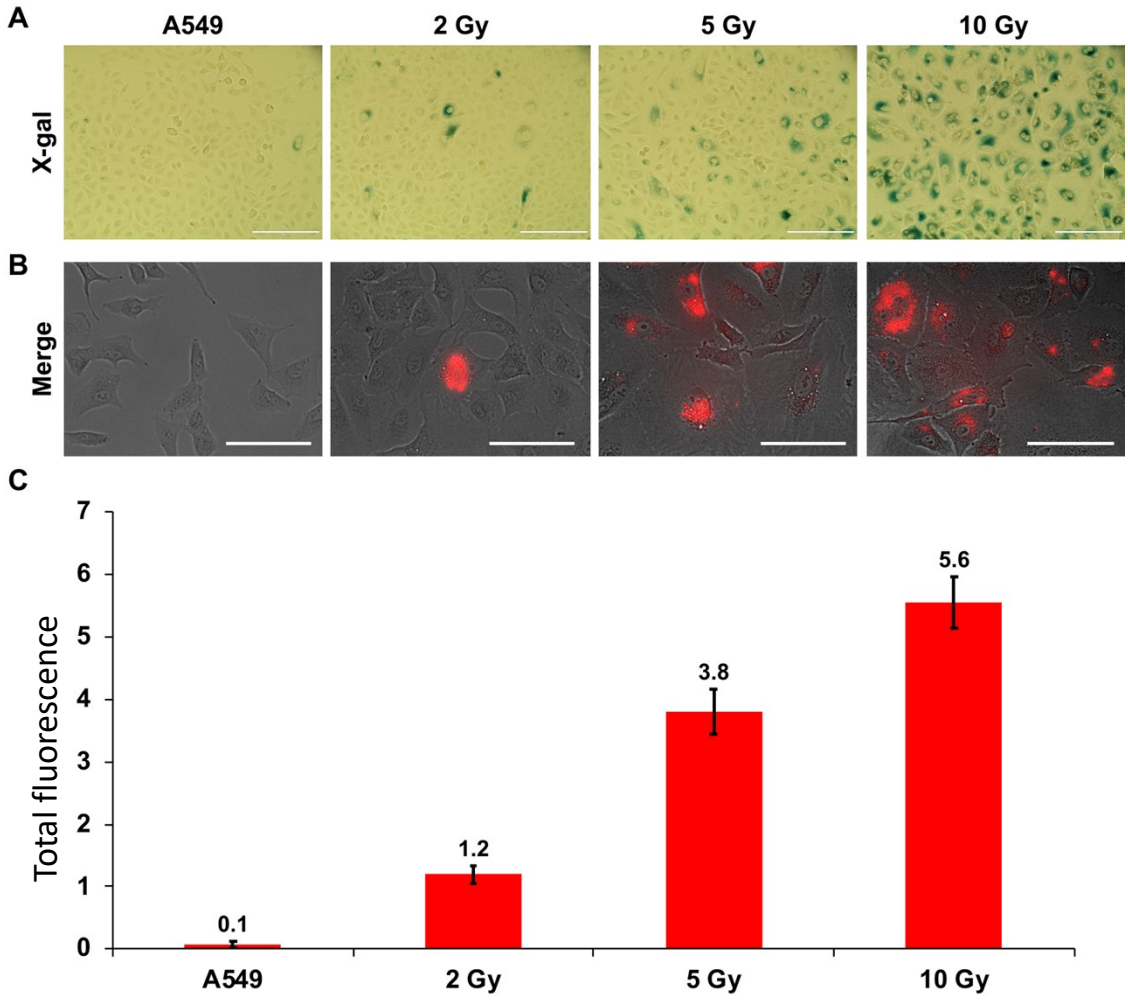


Figure S4. Gal-MB's photoablation effect in control MDA-MB231 cells. MDA-MB231 cells were incubated without or with Gal-MB (15 μM for 2 h), washed, and illuminated without or with 665 nm LED light (30 mW cm^{-2}) for 30 min. Cells were imaged with fluorescence microscope after 1 day (magnification: × 40). Scale bar, 100 μm.

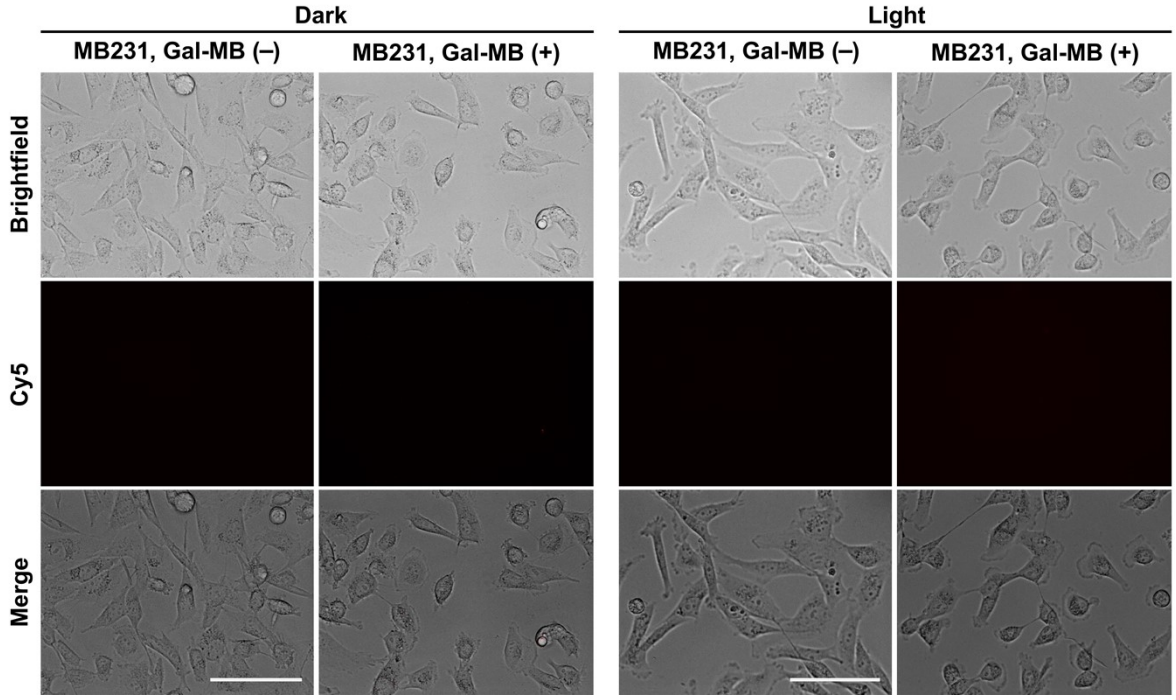
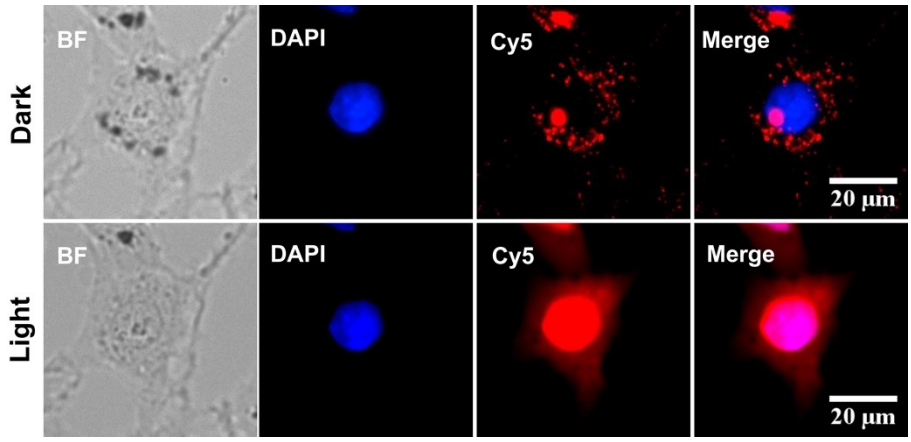
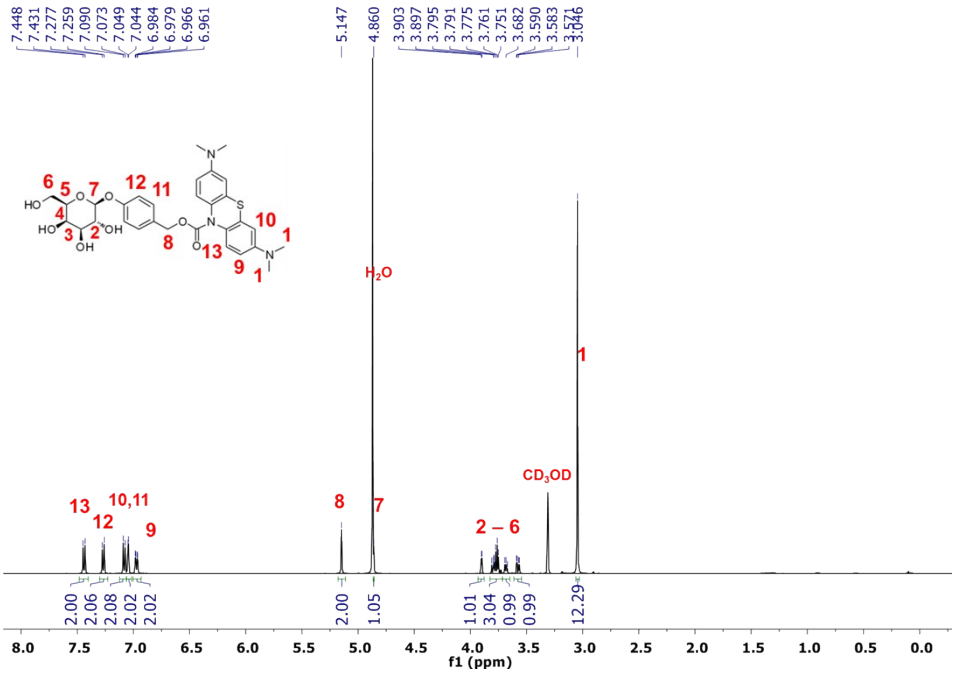


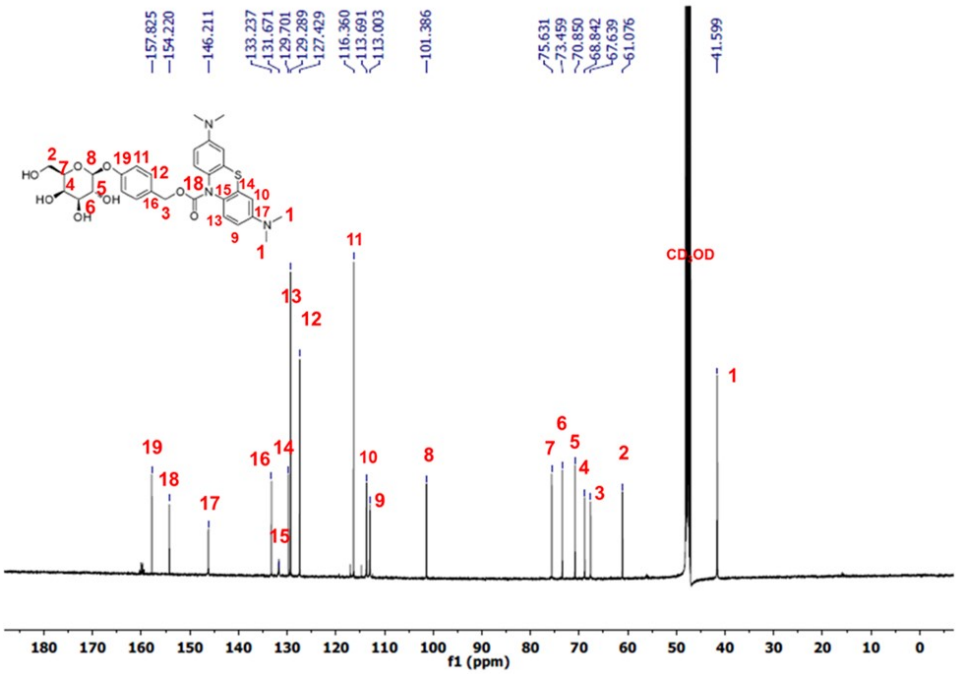
Figure S5. Light-induced redistribution of Gal-MB from lysosome to nucleus in C6/*LacZ* cells. Cells were treated with Gal-MB (20 μM) for 2 h, washed and captured before and after light illumination (magnification: × 40). Hoechst 33342 was added for nuclei staining.



^{1}H NMR of Gal-MB



^{13}C NMR of Gal-MB



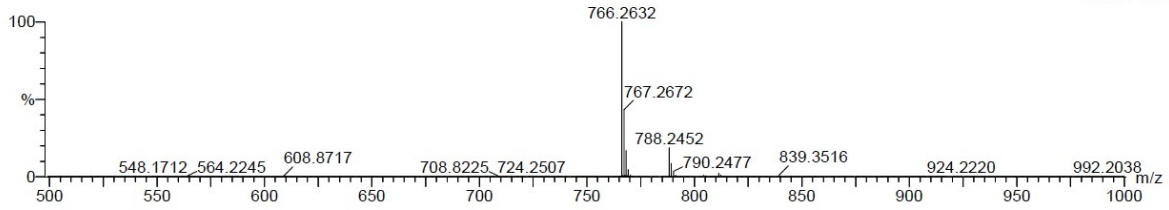
HRMS Spectrum of AcGal-MB

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
168 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-38 H: 0-44 N: 1-5 O: 1-12 S: 0-2

Ching Tung (Cornell) NMR Analytical Core Facility 11-Aug-2020
Zhenhua Chen LCT Premier XE 1::5::0
C38H43N3O12S
ZS_AcGalMB 32 (0.714) Cm (31:35) 1: TOF MS ES+
1.61e+005



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula

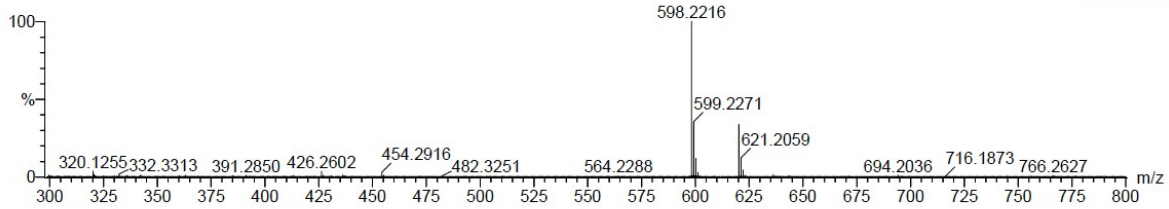
HRMS Spectrum of Gal-MB

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
64 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-30 H: 0-36 N: 1-3 O: 1-8 S: 0-2

Ching Tung (Cornell) NMR Analytical Core Facility 11-Aug-2020
Zhenhua Chen LCT Premier XE 1::0::7
C30H35N3O8S
ZS_GalMB 18 (0.407) Cm (17:22) 1: TOF MS ES+
7.63e+004



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula