

## Electronic Supplementary Information

### Medical Fluorophore 1 (MF1), a benzoquinolizinium-based fluorescent dye, as an inflammation imaging agent

Sang Bong Lee<sup>†,a</sup>, Ye Ri Han<sup>†, a</sup>, Hui-Jeon Jeon<sup>†, a</sup>, Chul-Ho Jun<sup>c, d, e</sup>, Sang-Kyoon Kim<sup>b</sup>, Jungwook Chin<sup>a</sup>, Su-Jeong Lee<sup>a</sup>, Minseon Jeong<sup>a</sup>, Jae-Eon Lee<sup>b, f</sup>, Chang-Hee Lee<sup>c, d, e</sup>, Sung Jin Cho<sup>\*, a</sup>, Dong-Su Kim<sup>\*, a</sup>, and Yong Hyun Jeon<sup>\*, b</sup>

<sup>a</sup>New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation, 80 Chembok-ro Dong-gu Daegu, Republic of Korea. E-mail : dongsukim1127@dgmif.re.kr., sjcho@dgmif.re.kr.

<sup>b</sup>Laboratory Animal Center, Daegu-Gyeongbuk Medical Innovation Foundation, 80 Chembok-ro Dong-gu Daegu, Republic of Korea. E-mail : jeon9014@gmail.com.

<sup>c</sup>Department of Chemistry, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea.

<sup>d</sup>Center for NanoMedicine, Institute for Basic Science (IBS), Seoul, Republic of Korea.

<sup>e</sup>Yonsei-IBS Institute, Yonsei University, Seoul 03722, Republic of Korea.

<sup>f</sup>Department of Biomaterials Science, College of Natural Resources and Life Science/Life and Industry Convergence Research Institute, Pusan National University, Pusan, Republic of Korea.

†These authors contributed equally.

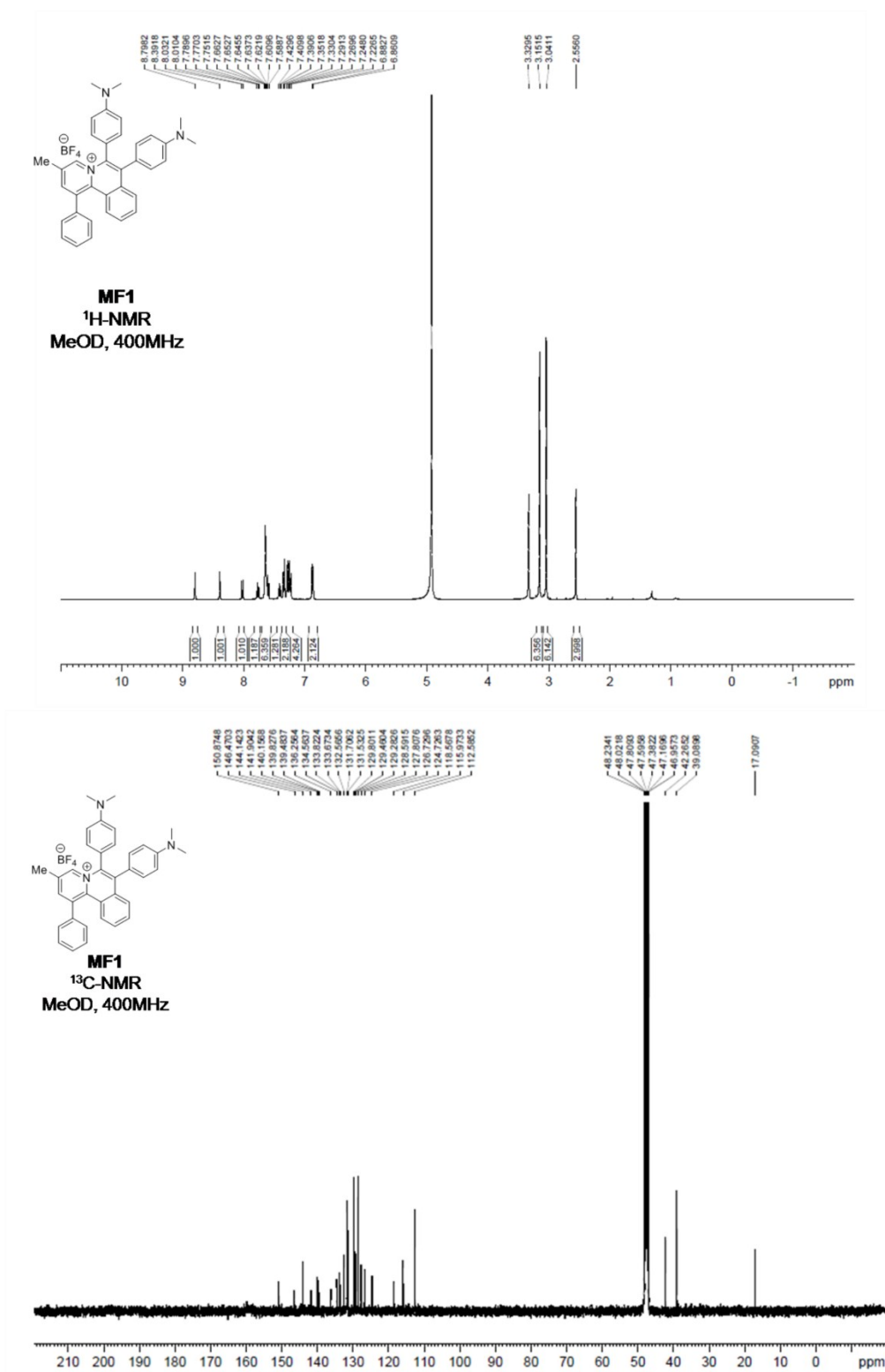
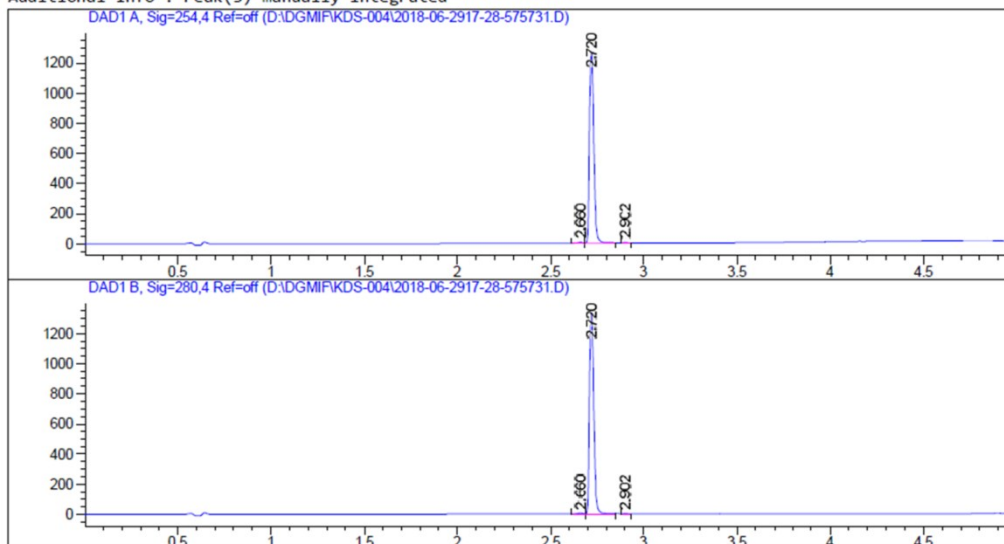


Fig. S1. <sup>1</sup>H and <sup>13</sup>C NMR spectra of MF1.

=====  
Acq. Operator : SYSTEM  
Sample Operator : SYSTEM  
Acq. Instrument : 1290HPLC                      Location : P1-F-01  
Injection Date : 6/29/2018 5:29:36 PM                      Inj Volume : 2.000 µl  
  
Acq. Method : C:\CHEM32\1\METHODS\DGMIF\JINA\20180312.M  
Last changed : 5/30/2018 9:45:38 AM by SYSTEM  
Analysis Method : C:\CHEM32\1\METHODS\DGMIF\JINA\20180312.M  
Last changed : 7/13/2018 2:40:08 PM by SYSTEM  
Additional Info : Peak(s) manually integrated



=====  
Area Percent Report  
=====

Sorted By : Signal  
Multiplier : 1.0000  
Dilution : 1.0000  
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.660	BV	0.0283	12.76570	6.67008	0.6310
2	2.720	VV	0.0242	2003.41260	1283.48877	99.0232
3	2.902	VV	0.0279	6.99704	3.72083	0.3458

Totals :                                      2023.17534 1293.87967

Fig. S2. HPLC spectrum of MF1.

# ==== Shimadzu LabSolutions Browser Report ====

Data File Name: **MF1**  
Method File Name: std 30% (100-800)\_4.5 min.lcm  
Acquired by: System Administrator  
Date Acquired: 2018-06-29 □ □ 5:27:18  
Sample Name:  
Sample ID:  
Sample Type: Unknown  
Level#: 0  
Detector: PDA, MS  
Comment:

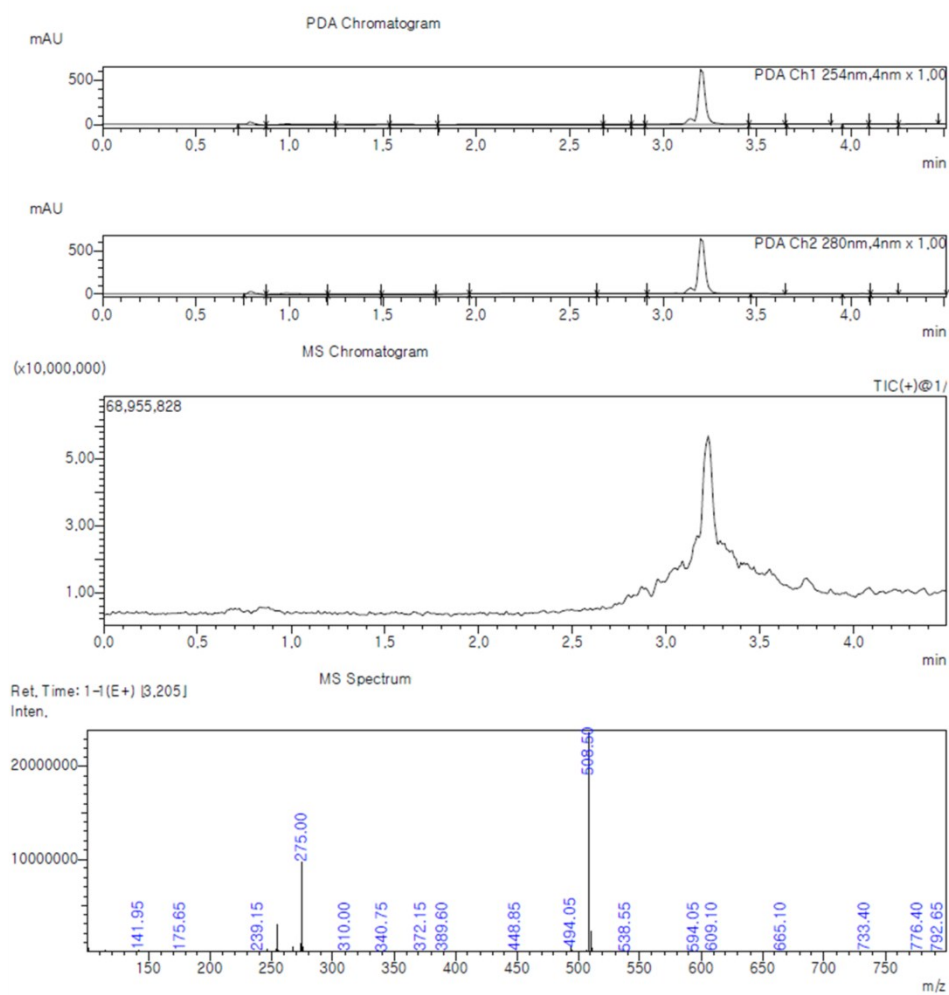
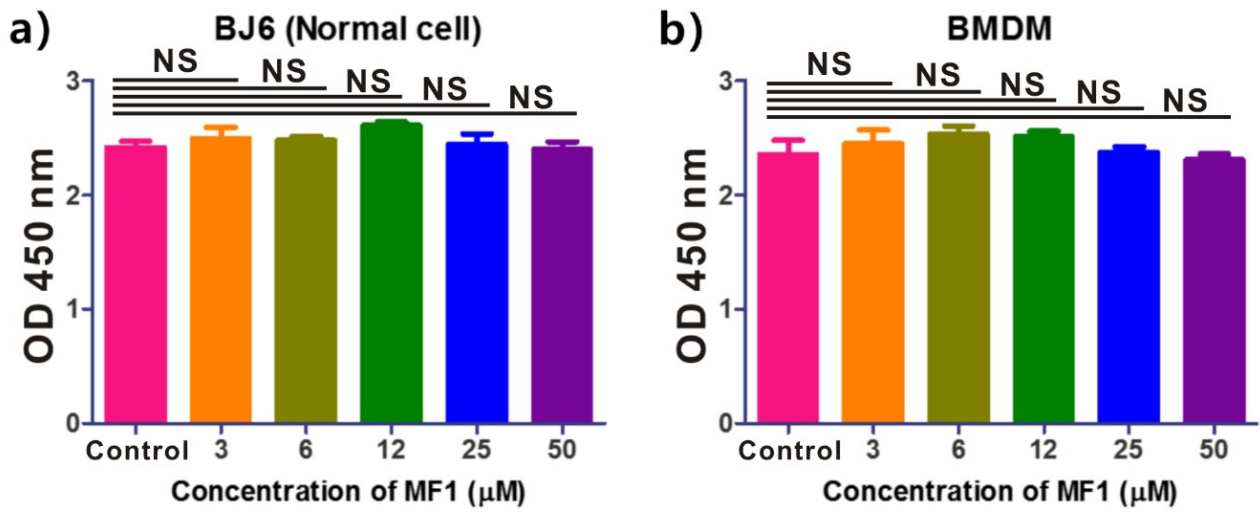
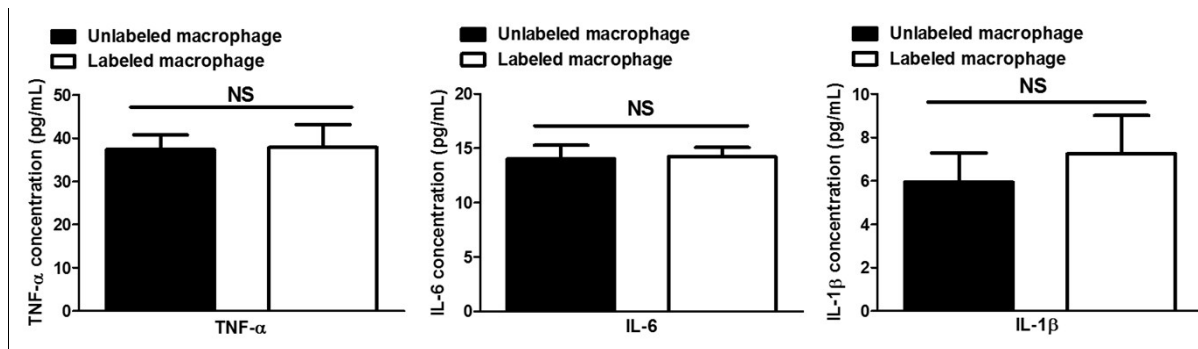


Fig. S3. LC mass spectrum of MF1.

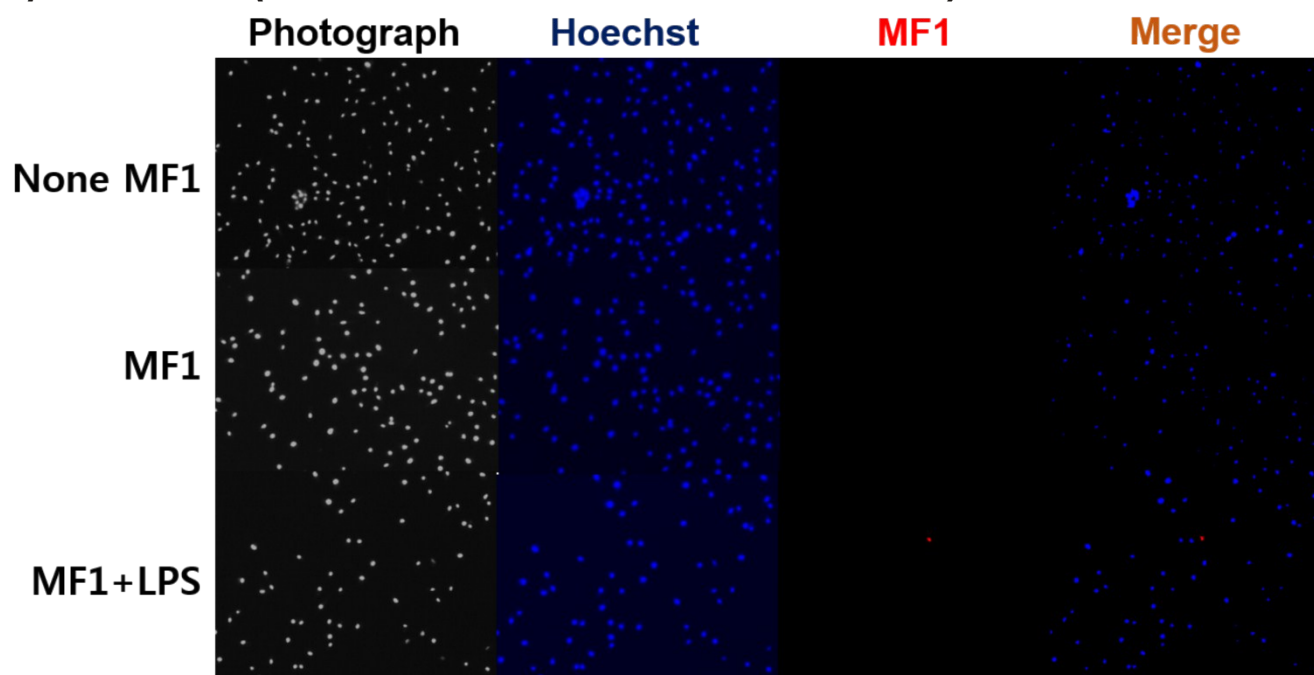


**Fig. S4.** Effect of MF1 on cell viability. (a, b) Cell proliferation in BJ6 cells (a) and BMDMs (b) with or without MF1 addition.

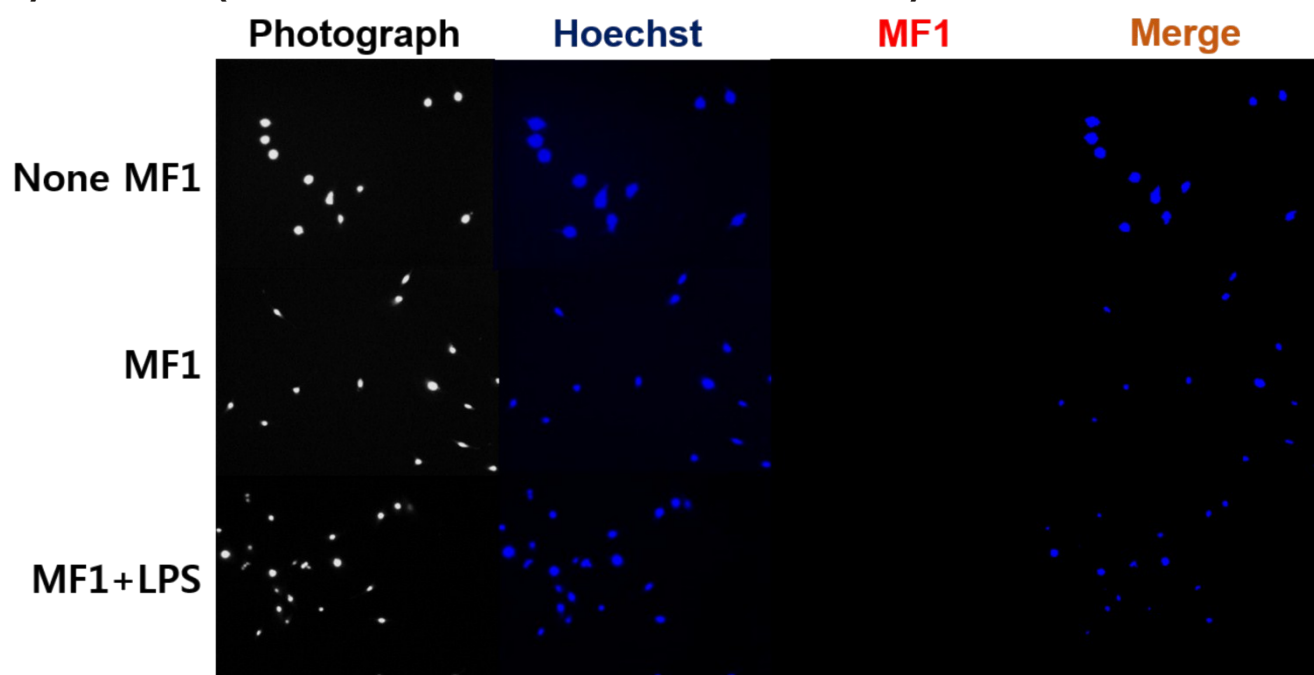


**Fig. S5.** TNF $\alpha$ , IL-6, and IL-1 $\beta$  levels in unlabeled and labeled BMDMs.

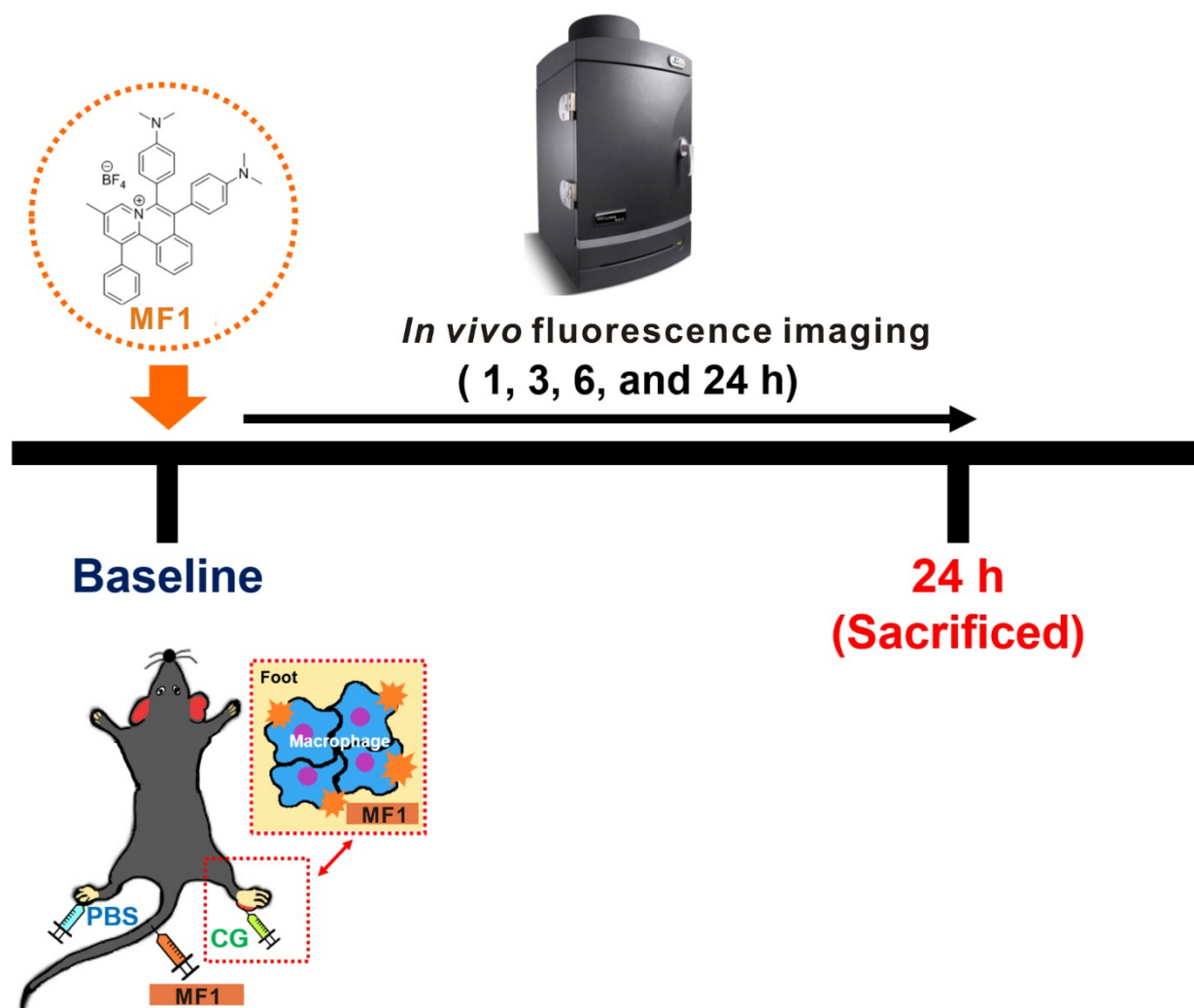
### a) 3T3-L1 (Normal fibroblast cell line)



### b) L929 (Normal fibroblast cell line)

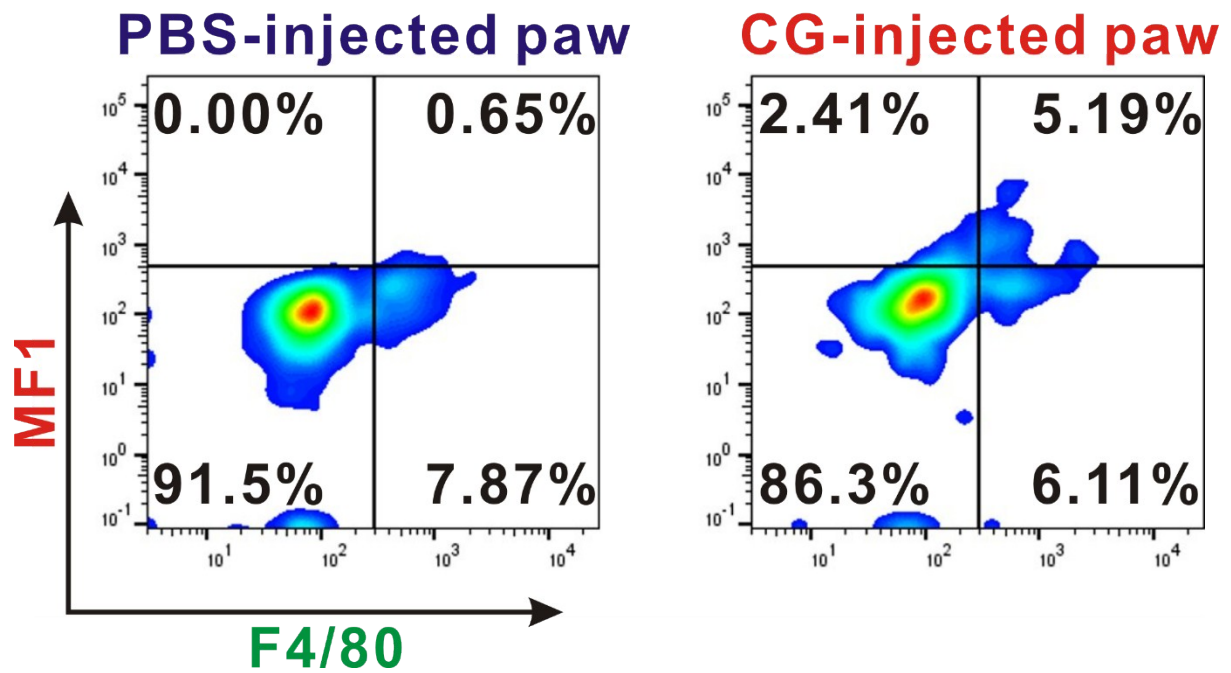


**Fig. S6.** Uptake of MF1 by a) 3T3-L1 and b) L929 cells. Intact and LPS-induced cells were incubated with MF1 at 37 °C for 1 h. Fluorescent microscopy was performed to determine cellular uptake level of MF1.



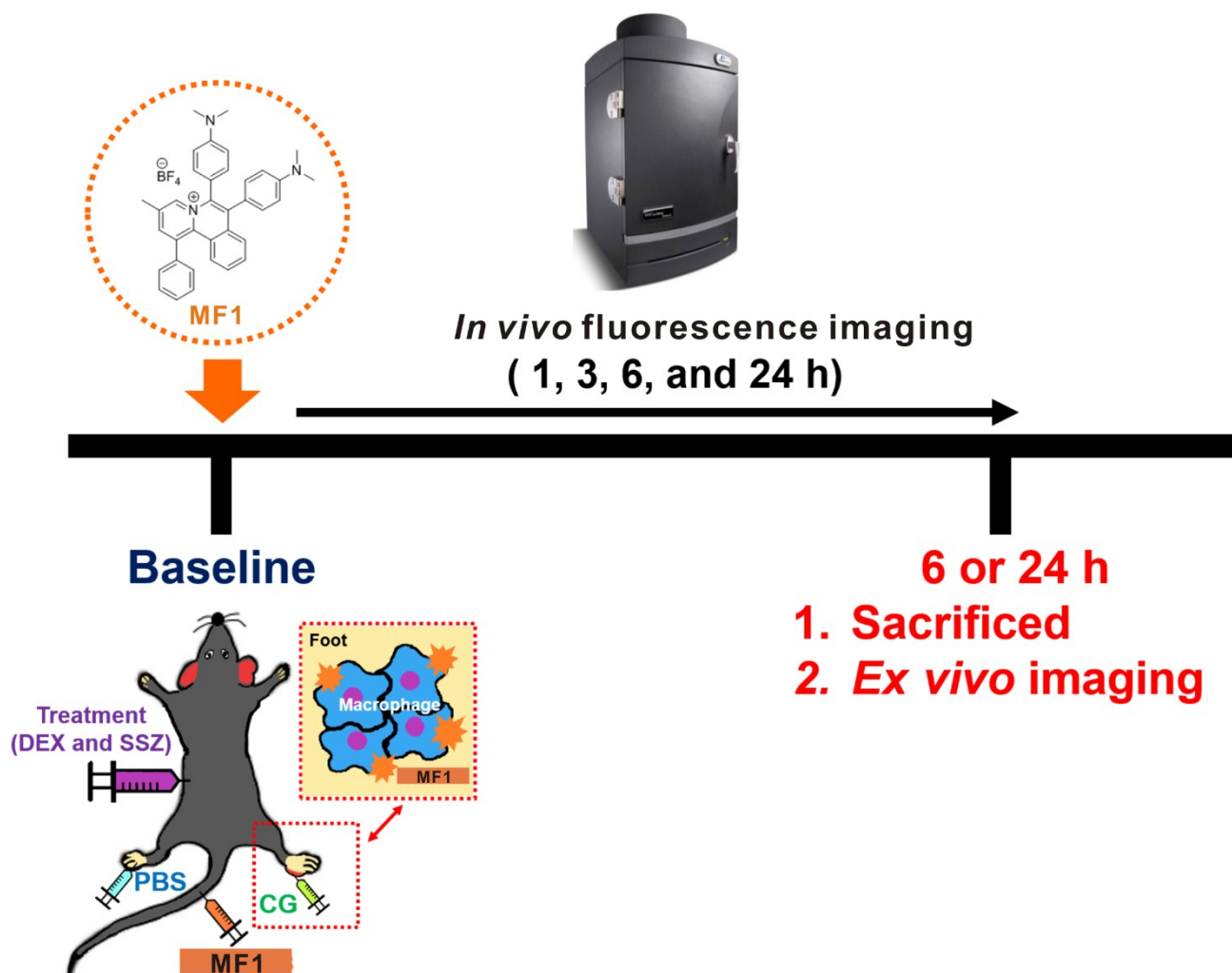
**Fig. S7.** Scheme for *in vivo* imaging of acute inflammation using MF1. PBS or CG solution were injected into the left and right foot pads, respectively, of immunocompetent mice. The mice were then intravenously injected with MF1, and dye uptake in inflammatory lesions was visualized by *in vivo* FLI at indicated times.

## Inflammation lesion (tissue)

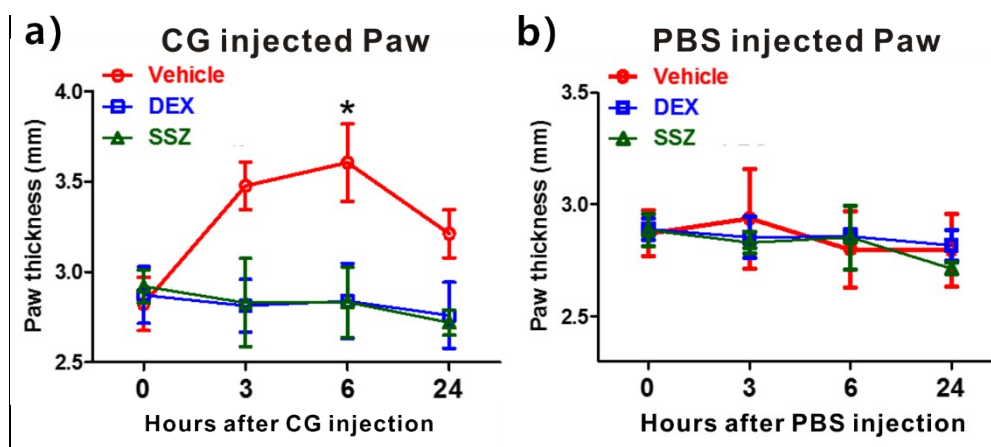


**Fig.S8.** FACS analysis revealing levels of F4/80+ and MF1+ cells in PBS-injected paw and CG-injected-paw at 6h post-injection MF1-injection.





**Fig. S9.** Scheme for evaluating physiological response to anti-inflammatory drugs using MF1. Mice were divided into three vehicle, DEX, and SSZ groups (n = 6 mice each). Acute inflammation was induced with 1% CG; immediately afterward, the mice were injected with a single dose of 10 mg/kg DEX, SSZ, or vehicle. *In vivo* FLI was performed at indicated times to visualize MF1 uptake. Mice were then sacrificed and organs were removed for *ex vivo* imaging.



**Fig. S10.** Measurement of paw thickness after PBS or CG injection with or without DEX or SSZ treatment. Changes in hind paw thickness were measured.

**Table. S1.** Full optical characterization data of MF1

Molecular weight	595.4926 g/mol
Extinction coefficient	18,977 M <sup>-1</sup> Cm <sup>-1</sup>
Absorbance maximum	470 nm
Emission maximum	688 nm
Stokes shift	218 nm
Quantum yield <sup>a</sup>	0.1%

<sup>a</sup>Relative quantum yield.