Electronic Supplementary Information

An iridium(III) complex based luminogenic probe for high-throughput screening of hydrogen sulfide donors in living cells

Ke-Jia Wu[1], Wen Sun[2], Jian-Min Sun[1], Chang Lu[1], Ning Sun[1*], Chung-Hang Leung[3*], Yan Li[2*], Chun Wu[4*]

[1] Wuxi School of Medicine, Jiangnan University, Wuxi 214122, China

[2] Key Laboratory of Animal Biological Products & Genetic Engineering, Ministry of Agriculture and Rural, Sinopharm Animal Health Corporation Ltd., Wuhan 430023, China

[3] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China

[4] College of Science, Sichuan Agricultural University, Xin Kang Road, Yucheng District, Ya'an 625014, China

* Corresponding author:

ccwuchem@hotmail.com (Chun Wu); Email: keliyanust@163.com (Yan Li); sunning@jiangnan.edu.cn (Ning Sun); duncanleung@um.edu.mo (Chung-Hang Leung);

1. Chemicals and materials

Iridium chloride hydrate ($IrCl_3 \cdot xH_2O$) was purchased from Aladdin (China). Unless specified, all the reagents were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China) and used as received without further purification, and all aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm^{-1}) unless specified.

2. Supplementary Methods

2.1 Synthesis

Mass spectrometry was performed on Aglient 7250 GC/Q-TOF (USA). Deuterated solvents for NMR purposes were obtained from Armar and used as received. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (DMSO-d_6: ^{1}H, 2.50, ^{13}C, 39.52; acetone-d_6: ^{1}H, 2.05, ^{13}C, 29.8). Chemical shifts are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data were acquired and processed using standard Bruker software (Topspin).

2.2 Synthesis of complexes Ir-mito1

Ir-mito**1**: Complex Ir-mito**1** was synthesized according to reported literature with some modifications (*Nat Chem* 2019, *11*, 1041-1048; *Chem Sci* 2020, *11*, 11404-11412). Specifically, 2.1 equivalents of 4-chloro-2-phenylquinoline and 1 equivalent of $IrCl_3 \cdot xH_2O$ were mixed and further heated overnight at 120 °C in 2-methoxyethanol/H_2O (v/v, 3/1). Afterwards, the mixture was filtered and washed with excess deionized water and then diethyl ether for three times respectively to generate the dichloro-bridged dimer $[Ir(ppy)_2Cl]_2$. $[Ir(ppy)_2Cl]_2$ and 2.1 equivalents of 10-phenanthroline-5,6-dione were mixed together in DCM/MeOH (v/v, 1:1) for overnight reaction. After the completion of reaction, excess NH_4PF_6 was added to the reaction mixture for reaction of another 20 minutes. The reaction mixture was washed with

Milli-Q water and subsequently evaporated to obtain the crude products of Ir-mito**1**. The crude was purified using DCM/MeOH as eluent. Yield: 54%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.74 (s, 2H), 8.67 (d, *J* = 4.9 Hz, 2H), 8.63 (d, *J* = 7.8 Hz, 2H), 8.37 (d, *J* = 7.5 Hz, 2H), 8.16 (dd, *J* = 8.3, 1.0 Hz, 2H), 8.02 (dd, *J* = 7.9, 5.5 Hz, 2H), 7.56 (t, *J* = 7.3 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 2H), 7.29 – 7.17 (m, 4H), 6.94 – 6.86 (m, 2H), 6.69 (d, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 174.50, 170.18, 154.55, 152.25, 149.70, 147.99, 146.35, 145.21, 137.87, 134.77, 132.36, 131.21, 129.91, 128.05, 125.58, 125.44, 125.35, 123.53, 118.51. HRMS: Calcd. for $C_{42}H_{24}Cl_2IrN_4O_2$ $[M\text{-}PF6]^+$: 879.0906 Found: 879.0887.

2.3 Photophysical measurement

Emission spectra and lifetime measurements for complex Ir-mito**1** were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 335 nm) fitted with a 395 nm filter. Error limits were estimated: λ (±1 nm); τ (±10 %); φ (±10 %). UV/Vis absorption spectra were recorded on an Agilent Cary 8454 UV-Vis Spectrophotometer. The luminescence lifetime of the complex was measured by time-correlated single-photon counting (TCSPC), following excitation at 340 nm with a NanoLED light source. Luminescence quantum yields were determined using the method of Demas and Crosby with $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile (ACN) as a standard reference solution ($\Phi_r = 0.062$) and were calculated according to the following reported equation (1):

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r) \quad (1)$$

Where the subscripts s and r refer to the sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1–10^{-AL}$, where A is the absorbance at excitation wavelength and L is the optical path length.

2.4 LC-MS analysis

Analytical HPLC was performed on an Agilent 1260 series HPLC system (Agilent Technologies, Stockport, UK) equipped with a mass selective detector (MSD) and Agilent C18 column (Length 150 mm, Internal diameter 3.0 mm, particle size 2.7 ted by B = 1–10 softw, H_2O + 0.1 % formic acid; B: MeCN + 0.1 % formic acid. Flow rate:

0.5 mL/min. Gradient: 0.0 min, 30 % B; 10.0 min, 100 % B; 12.5 min, 100 % B.

2.5 Cell culture

Cells were cultivated in DMEM medium with 1% penicillin (100 units/mL)/streptomycin (100 μg/mL) and 10% fetal bovine serum (FBS). Cells were maintained at a density of 6×10^5 cell/mL in 5% CO_2 at 37 °C.

2.6 MTT assay

THLE-2 and HepG2 cells were seeded at 5000 cells per well in a 96-well plate and incubated overnight at 37 °C. The cells were treated with Ir-mito**1** at a final concentration as indicated for 12 h. Then 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was added to each well at a final concentration of 0.5 mg/mL for a further 4 h. After that, the medium was replaced with 100 μL DMSO. The viability of the cells was measured by recording the absorbance of each well at 490 nm after shaking the plate for 10 min at room temperature in the dark.

3. Supplementary Figure

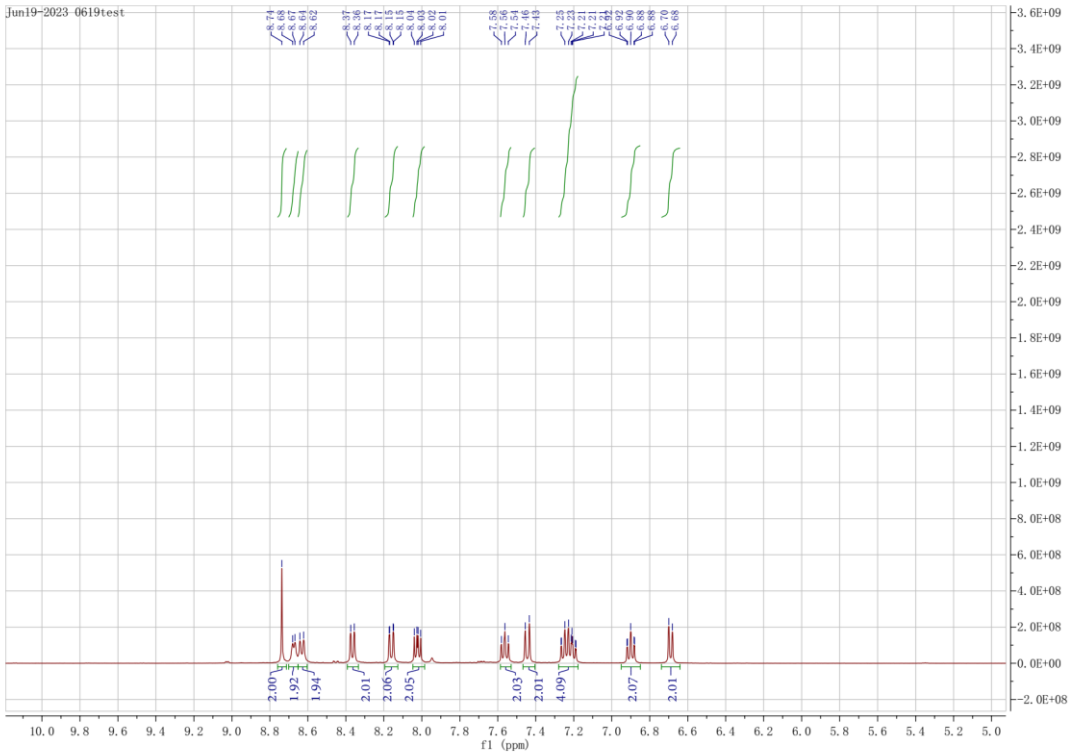

Figure S1 ^{1}H NMR spectra for Ir-mito**1.**

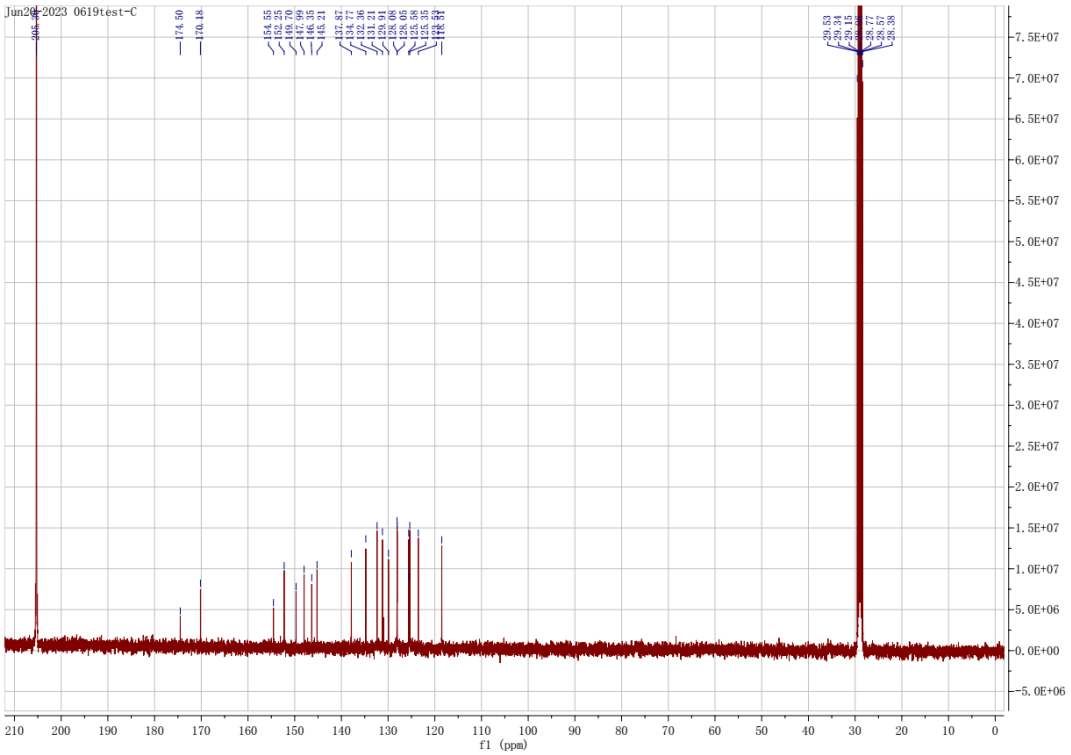

Figure S2 ^{13}C NMR spectra for Ir-mito**1.**

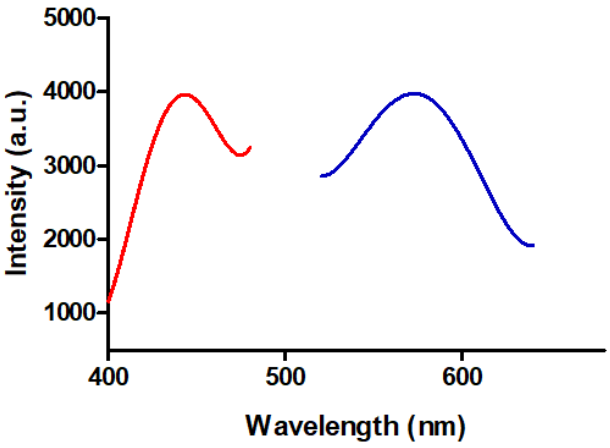

Figure S3 Excitation and emission spectra of Ir-mito**1** (10 μM) in PBS.

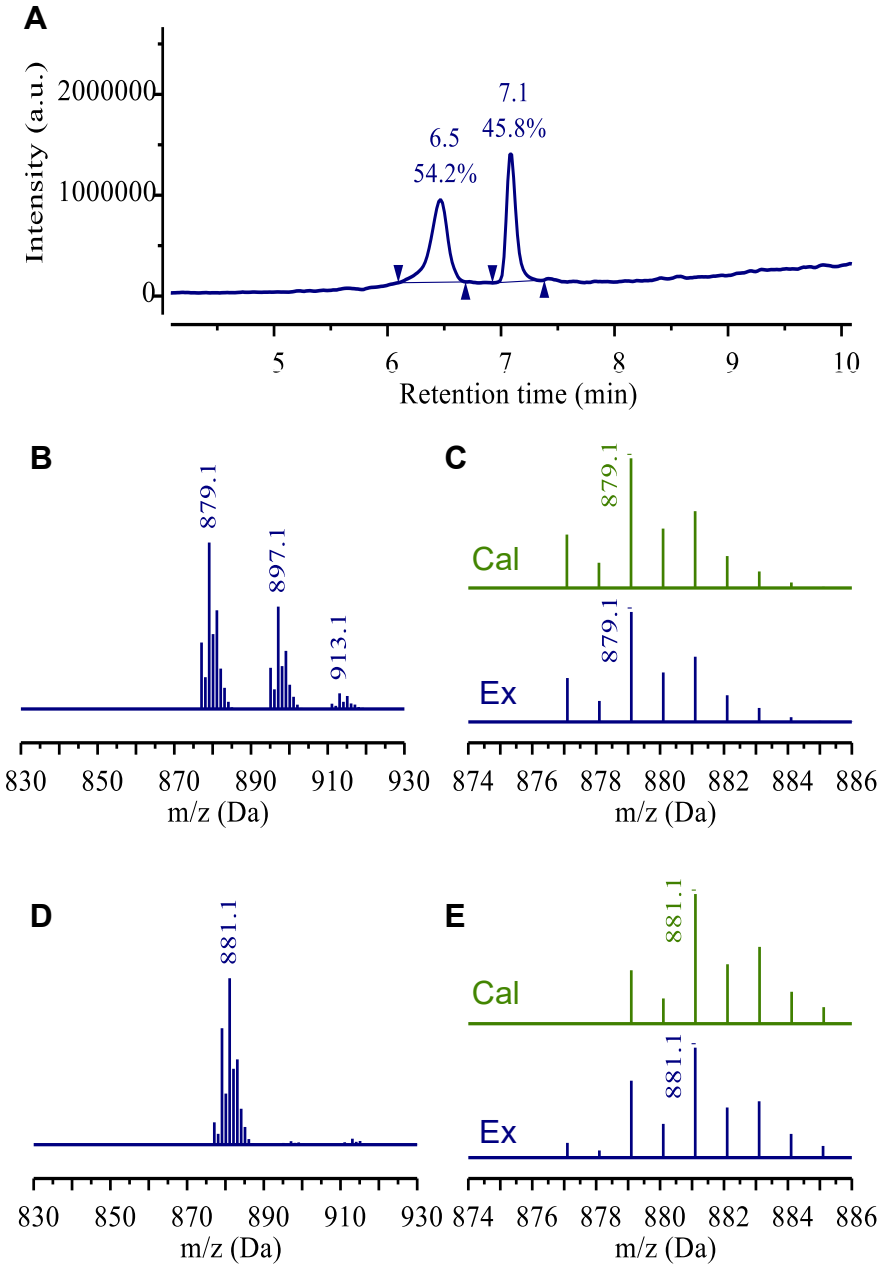

Figure S4 Mechanism of Ir-mito**1** response to H_2S. **(A)** LC-MS analysis of Ir-mito**1** in the presence of H_2S in PBS buffer. **(B)** Mass spectrum of the peak with retention time at 6.5 min. The peak at 879.1 refers to the signal of Ir-mito**1**. The peak at 897.1 and 913.1 refer to the hydroation forms of Ir-mito**1** with one and two H_2O, respectively. **(C)** Comprison of the calculated and experimental isotope distribution of Ir-mito**1**. **(D)** Mass spectrum of the peak with retention time at 7.1 min. The peak at 881.1 refers to the signal of Ir-mito**1** in the presence of H_2S. **(E)** Comprison of the calculated and experimental isotope distribution of Ir-mito**1** in the presence of H_2S.

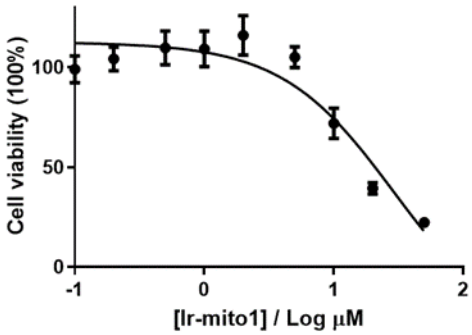

Figure S5 Cell viability of THLE-2 cells after treatment with Ir-mito**1** for 12 h, n=6.

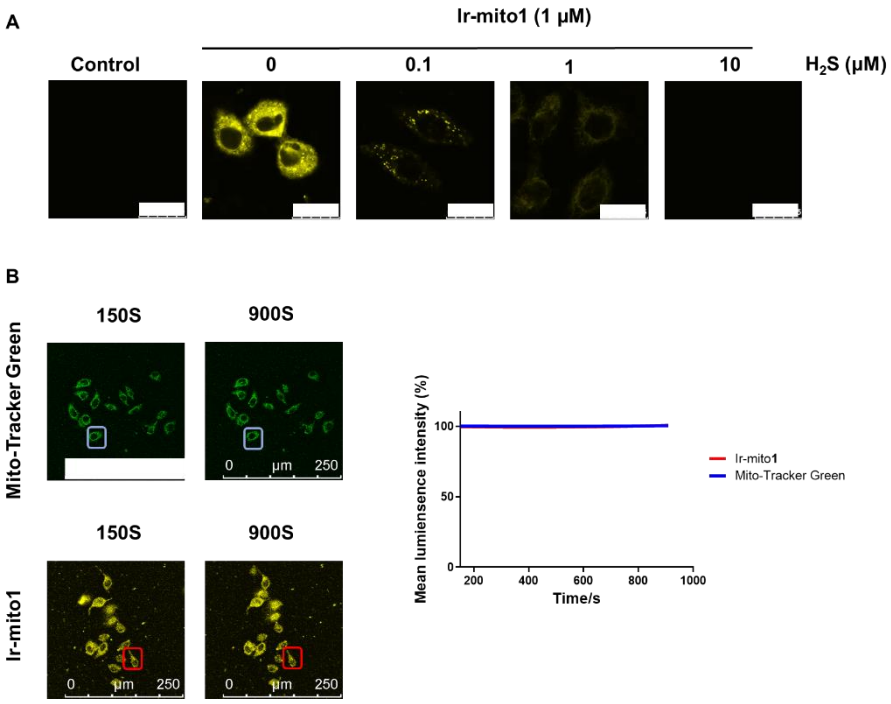

Figure S6 Ir-mito**1** is a promising H_2S probe with good biocompatibility. **(A)** Confocal imaging of cells incubated with Ir-mito**1** (0-10 μM, $\lambda_{ex}/\lambda_{emi}$ = 405/500−600 nm) for 1 h. Scale bar =25 μm. **(B)** Comparison of Mito-Tracker green and Ir-mito**1** for resistance to photobleaching. Confocal luminescence images of Ir-mito**1** ($\lambda_{ex}/\lambda_{emi}$ = 405/500−600 nm) or Mito-Tracker green ($\lambda_{ex}/\lambda_{emi}$ = 488/500−600 nm) in cells under continuous irradiation with different laser scan times (0−900 s). Scale bar = 250 μm. The relative mean luminescence intensity of Ir-mito**1** and Mito-Tracker green detected.

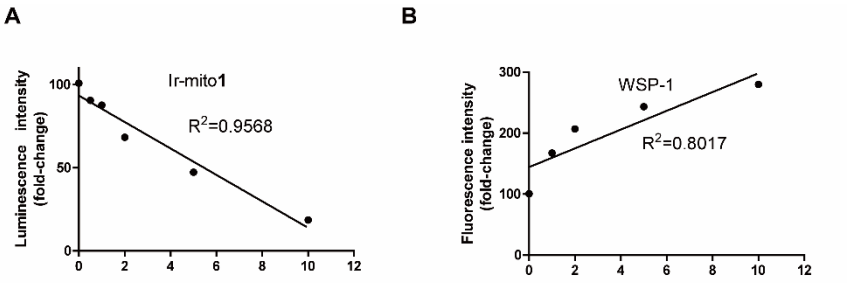

Figure S7 Detection ability of Ir-mito **1** compared to commercial H_2S probe**. (A)** The luminescence change of cells with Ir-mito**1** in TRES mode after treated with different concentration of H_2S for 1 h. **(B)** The fluorescence change of cells with WSP-1 in steady-state emission mode after treated with different concentration of H_2S for 1 h.

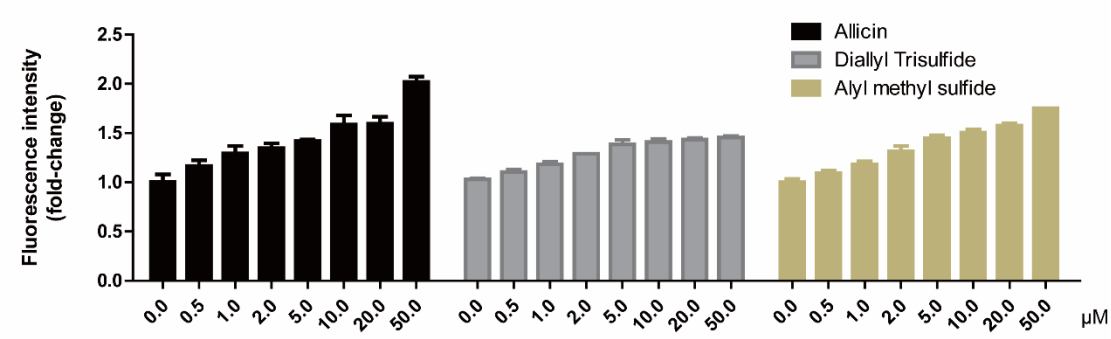

Figure S8 The fluorescence change of cells with WSP-1 after treated with different H_2S donors for 1 h, n=3.

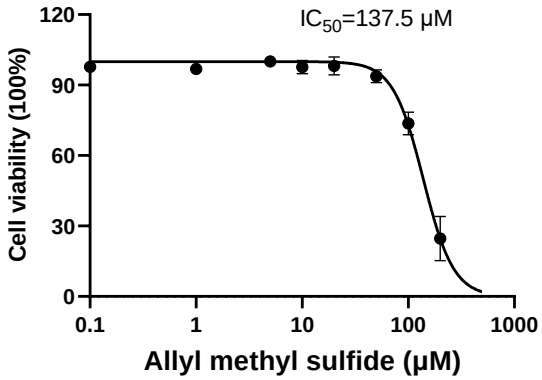

Figure S9 Cell viability of HepG2 cells after treatment with allyl methyl sulfide for 12 h, n=6.