Supplementary Information for

Original article

Novel STING-targeted PET radiotracer for alert and therapeutic evaluation of acute lung injury

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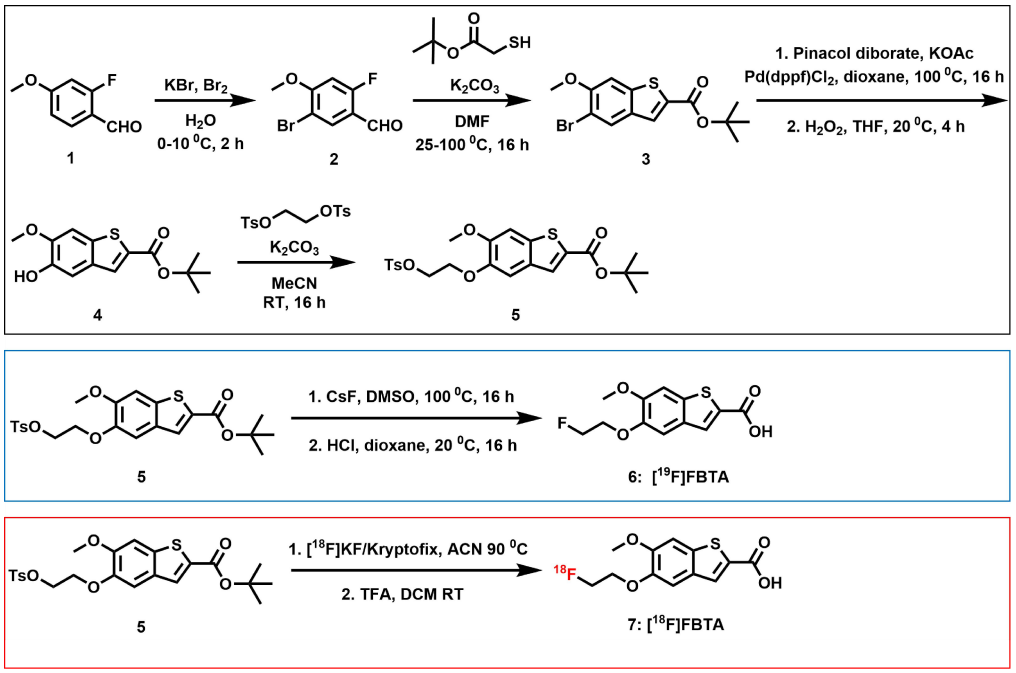


Figure S1 The chemical structures and synthetic routes of the labeling precursor (**5**), non-radioactive reference [^{19}F]FBTA (**6**) and ^{18}F-labeled compound [^{18}F]FBTA (**7**).

Synthesis and characterization

Synthesis of 2. At 0 °C, 2-fluoro-4-methoxybenzaldehyde (**1**, 9.2 g, 60 mmol) was added slowly to a solution of bromine (6.0 mL, 120 mmol) in methanol (40 mL), and the reaction mixture was stirred at 0-10 °C for 2 h. A solution of sodium bisulfite (24.3 g, 234 mmol) was slowly added to the reaction system at 0 °C, and the mixture was stirred at 0 °C for 30 min. The reaction mixture was filtered and washed with deionized water (3 × 25 mL), the organic phase was collected and the solvent was removed by vacuum to obtain 5-bromo-2-fluoro-4-methoxybenzaldehyde (**2**, 9.9 g, 42.5 mmol, 71%). The product can be used in subsequent step without purification. ^{1}H NMR (400MHz, DMSO-d_6): δ 10.03 (s, 1H), 8.00-7.95 (d, J = 7.5Hz, 1H), 7.30-7.25 (d, J = 13.0Hz, 1H), 3.99 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 185.96 (d, J = 4.4 Hz, 1C), 165.87, 163.31, 161.98 (d, J = 11.7 Hz, 1C), 133.06 (d, J = 3.7 Hz, 1C), 118.40 (d, J = 9.5 Hz, 1C), 107.07 (d, J = 2.9 Hz, 1C), 102.23 (d, J = 25.7 Hz, 1C), 58.10. MS (ESI) m/z: 233 $[M]^+$.

Synthesis of 3. At room temperature, potassium carbonate (17.1 g, 124 mmol) was added dropwise to **2** (9.6 g, 41.3 mmol) and tert-butyl 2-mercaptoacetate (6.5 mL, 41.3 mmol) in DMF (50 mL) under the protection of argon atmosphere, and then the temperature was raised to 100 °C. After stirring for 16 h, the mixture was cooled to room temperature and diluted with ether (500 mL) and washed with deionized water (3 × 200 mL), all the aqueous phase was collected and extracted with ether (3 × 200 mL). The organic phase was combined and washed with saturated

aqueous sodium chloride (50 mL). The organic phase was extracted, dried with magnesium sulfate, and the solvent was removed by vacuum to obtain tert-butyl 5-bromo-6-methoxybenzo[b]thiophene-2-carboxylate (**3**, 13.4 g, 39.0 mmol, 94 %). The product can be used in subsequent step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (s, 1H), 7.94 (s, 1H), 7.79 (s, 1H), 3.94 (s, 3H), 1.56 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 161.56, 155.05, 142.80, 133.88, 133.72, 129.79, 110.86, 105.73, 82.50, 57.12, 28.26. MS (ESI) m/z: 366 [M]+Na.

Synthesis of 4. Dimethylformamide (100 mL), **3** (13.0 g, 38 mmol), pinacol diborate (12.7 g, 50 mmol), potassium acetate (11.2 g, 114 mmol) and Pd(dppf)Cl_2 (1.4 g, 1.9 mmol) were added to the flask in sequence and reacted at 90 °C for 3 h under the protection of nitrogen. The mixture was cooled to room temperature and extracted with ethyl acetate (200 mL). The organic layer was washed with deionized water (3 × 200 mL) and concentrated. Then the concentrate was dissolved in tetrahydrofuran (50 mL), and then hydrogen peroxide (1.0 g, 30 mmol) was added, and the reaction was carried out at 20 °C for 4 h. The reaction was cooled to room temperature and extracted with ethyl acetate (200 mL). The organic layer was washed successively with water and saturated brine (3 × 200 mL), dried over anhydrous sodium sulfate, concentrated, and column chromatography (ethyl acetate: petroleum ether = 1:1) to obtain **4** (10.1 g, 36.0 mmol, 95 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.32 (s, 1H), 7.83 (s, 1H), 7.50 (s, 1H), 7.27 (s, 1H), 3.80 (s, 3H), 1.69 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 161.97, 150.39, 146.55, 134.27, 132.88, 132.42, 130.13, 109.96, 104.70, 81.90, 56.27, 28.32. MS (ESI) m/z: 280 $[M]^+$.

Synthesis of 5. To a solution of **4** (0.56 g, 2 mmol) and potassium carbonate (1.30 g, 4 mmol) in acetonitrile (20 mL) was added ethane-1,2-diylbis(4-methylbenzenesulfonate) (0.81 g, 2.2 mmol), reacted at room temperature for 16 h. Then the mixture was filtered, the solvent was removed under reduced pressure, and column chromatography (ethyl acetate: petroleum ether = 1:4) was performed to obtain **5** (0.8 g, 1.6 mmol, 80 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 2H), 7.70 (s, 1H), 7.28-7.09 (d, J = 13.0Hz, 4H), 4.36-4.34 (t, 2H), 4.21-4.18 (t, 2H), 3.86 (s, 3H), 2.39 (s, 3H), 1.53 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 162.03, 150.94, 147.00, 144.91, 137.09, 133.87, 132.93, 132.39, 132.26, 129.96, 129.82, 129.45, 129.30, 128.00, 109.38, 104.18, 103.97, 82.04, 68.05, 67.92, 67.17, 56.12, 28.26, 21.63. MS (ESI) m/z: 479 $[M]^+$.

Synthesis of [^{19}F]FBTA (6). **5** (0.24 g, 0.5 mmol) and cesium fluoride (0.38 g, 2.5 mmol) were added to dimethyl sulfoxide (10 mL), and reacted at 100 °C for 16 h. After the reaction liquid was cooled, it was extracted with water and ethyl acetate, and the organic phase was spin-dried. Dissolve it in dioxane (10 mL), add hydrochloric acid (1 mL) dropwise, and react at 20 °C for 16 h. The solvent was removed under reduced pressure and column

chromatography (ethyl acetate: petroleum ether = 5:1) to obtain [^{19}F]FBTA (0.1 g, 0.4 mmol, 95 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.19 (s, 1H), 7.91 (s, 1H), 7.60 (s, 1H), 7.50 (s, 1H), 4.84-4.70 (t, 2H), 4.29-4.28 (t, 2H), 4.22 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 164.03, 150.88, 147.65, 136.12, 132.59, 132.56, 130.47, 108.29, 104.99, 83.32, 81.67, 68.48, 68.29, 56.32. MS (ESI) m/z: 271 $[M+1]^+$.

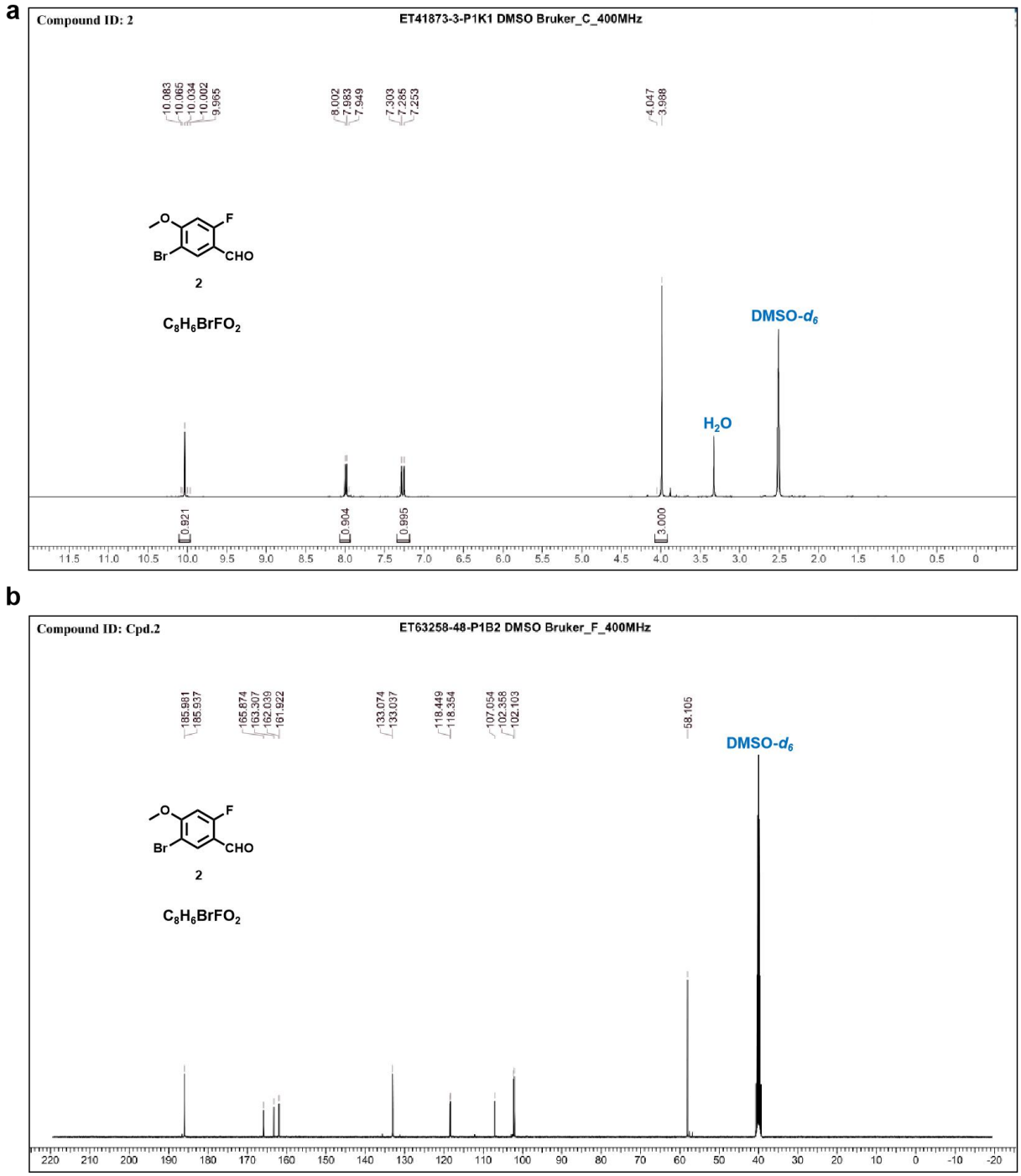


Figure S2 (**a**) ^{1}H-NMR and (**b**) ^{13}C-NMR spectrum of **2**.

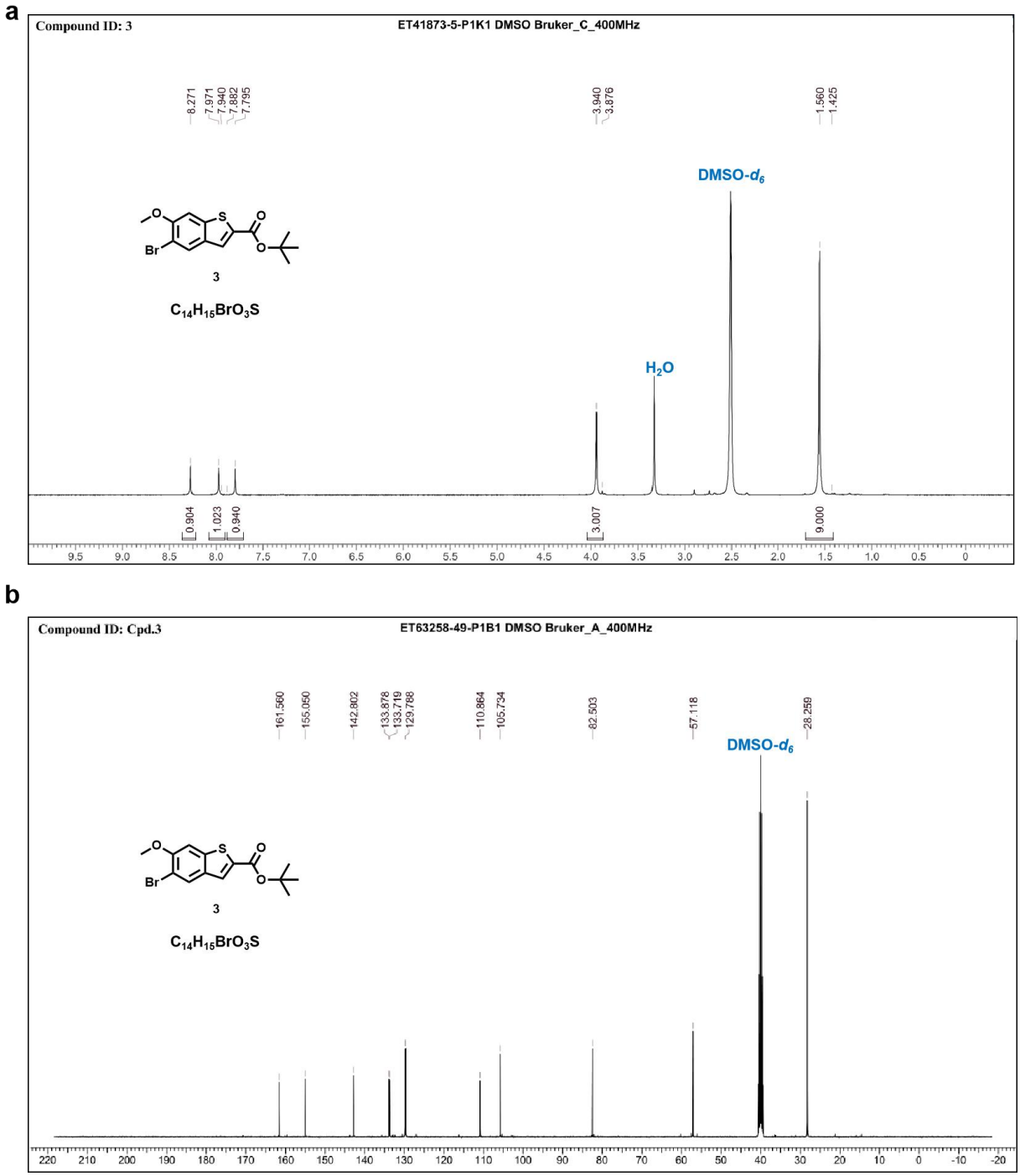
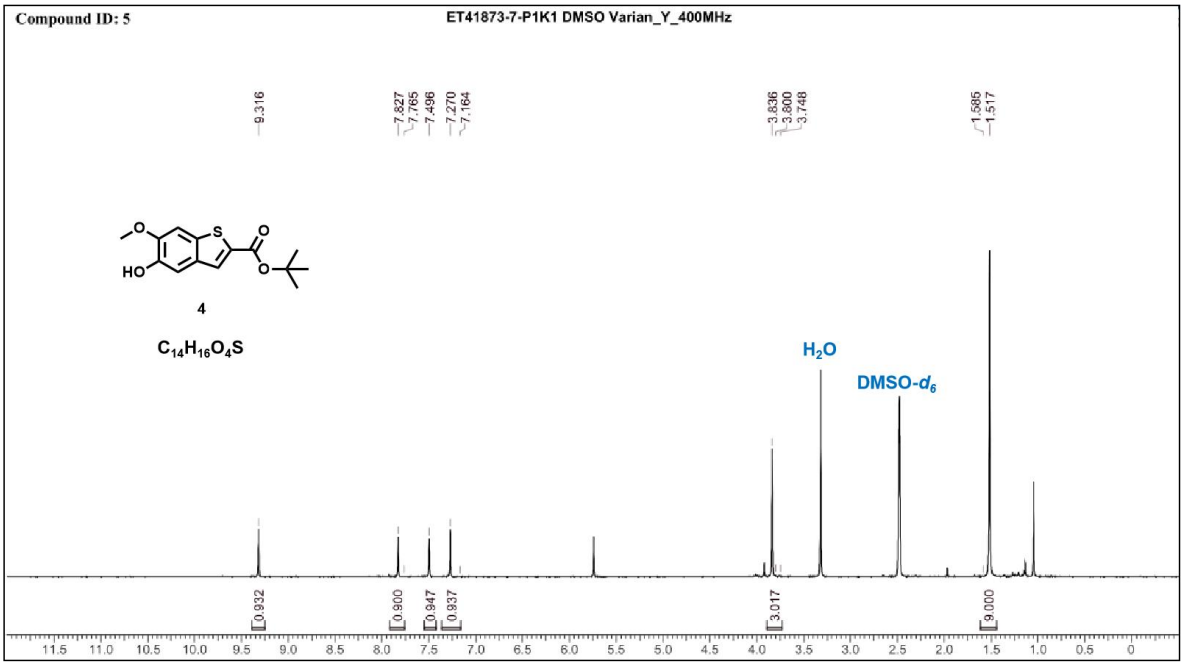


Figure S3 (**a**) ^{1}H-NMR and (**b**) ^{13}C-NMR spectrum of **3**.

a



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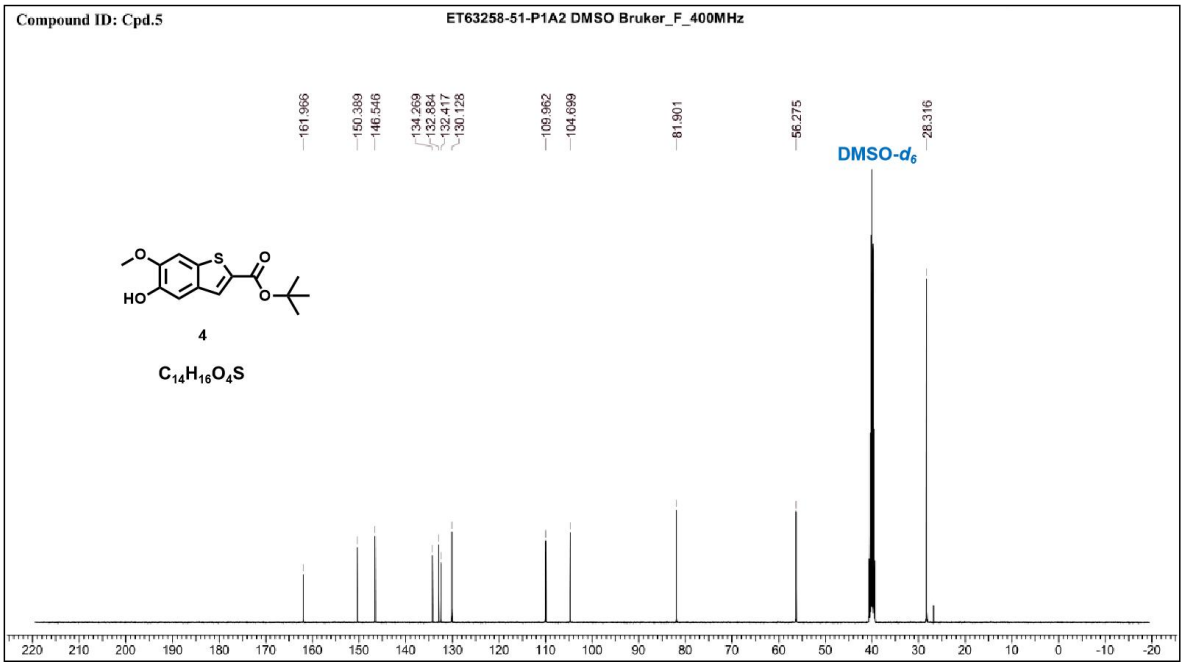


Figure S4 (**a**) ^{1}H-NMR and (**b**) ^{13}C-NMR spectrum of **4**.

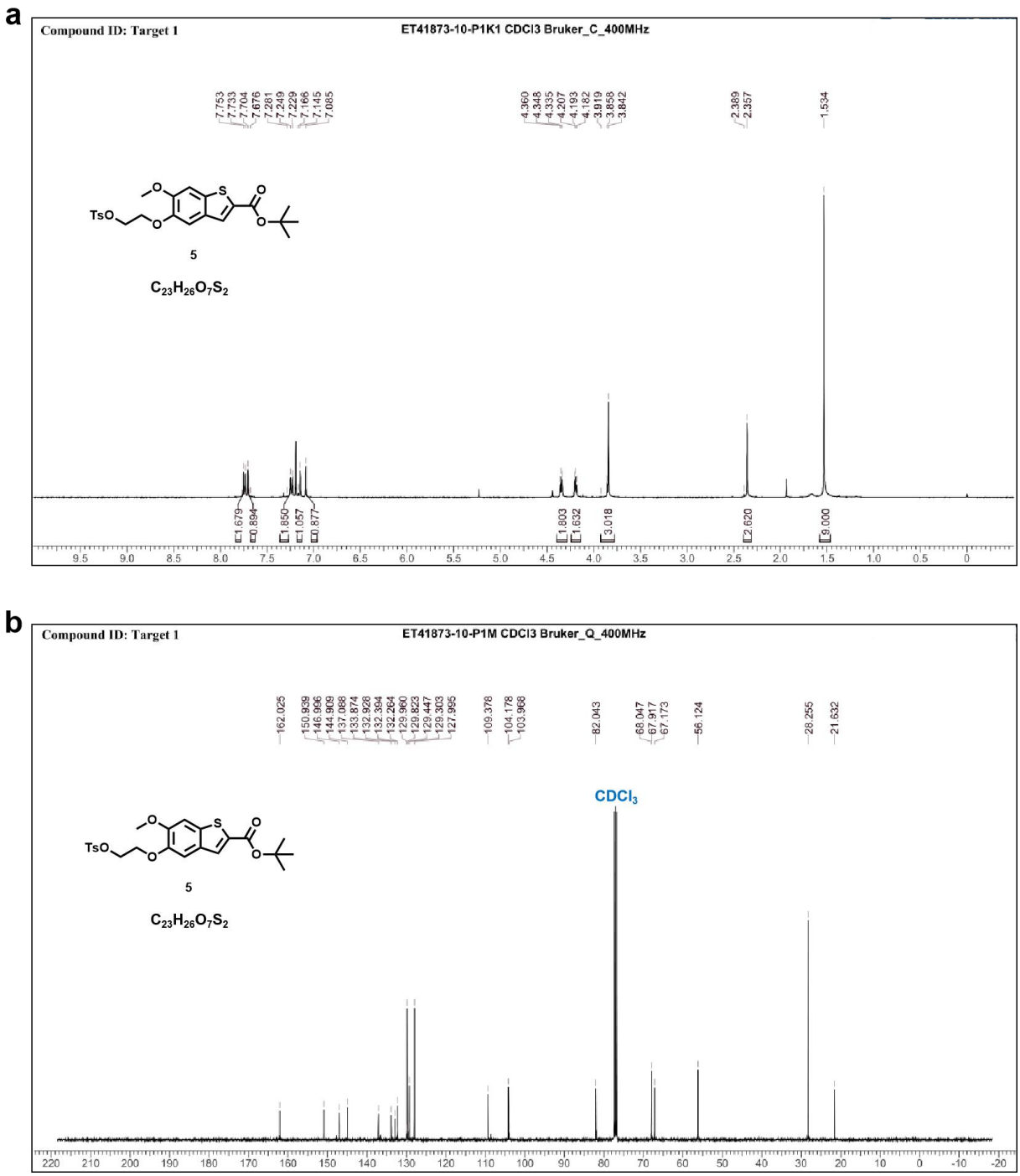


Figure S5 (**a**) ^{1}H-NMR and (**b**) ^{13}C-NMR spectrum of **5**.

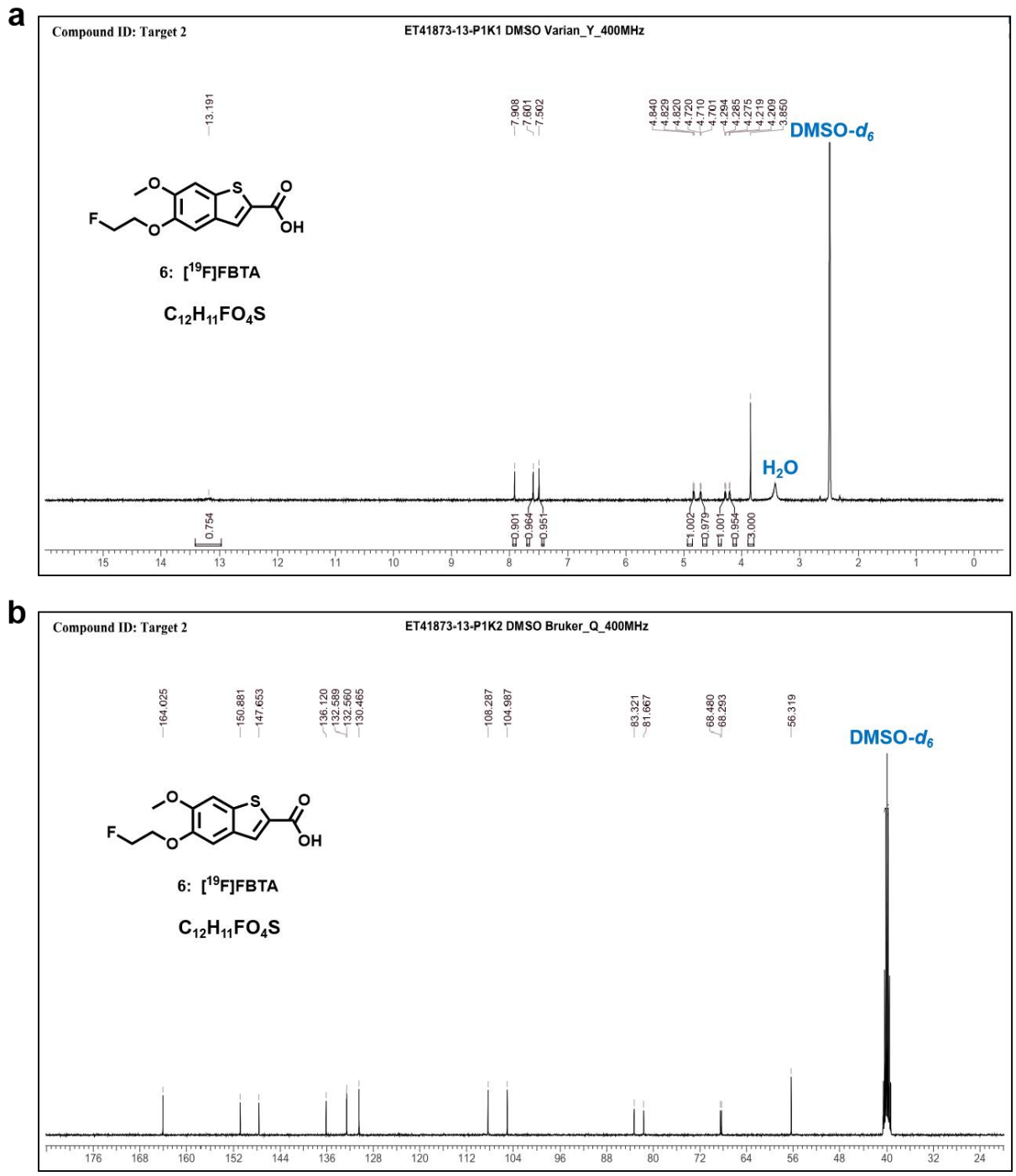


Figure S6 (**a**) ^{1}H-NMR and (**b**) ^{13}C-NMR spectrum of **6**.

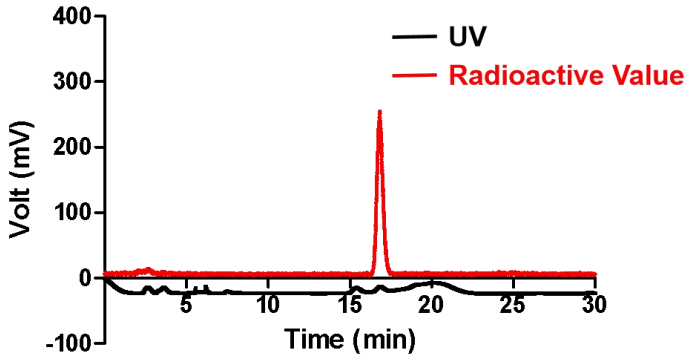
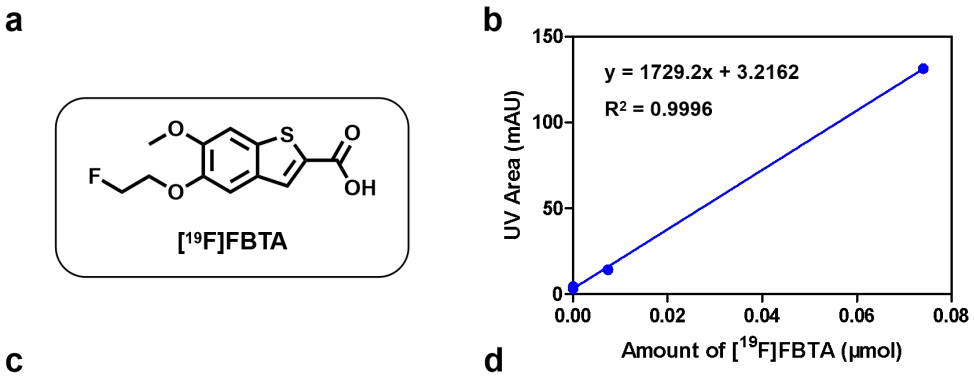


Figure S7 HPLC quality control (UV and radio traces) data of [^{18}F]FBTA.



Batch	Activity (MBq)	UV Area (mAU)	MA (GBq/μmol)
1	14.80	5.60	29.02
2	18.50	5.80	33.04
3	24.05	6.40	35.37
Mean	-	-	32.48
SD	-	-	2.87

Figure S8 (**a**) Chemical structure of non-radioactive compound [^{19}F]FBTA. (**b**) The standard curve of [^{19}F]FBTA. (**c**) HPLC analysis of [^{19}F]FBTA in different concentrations. (**d**) Calculation of the molar activity of [^{18}F]FBTA.

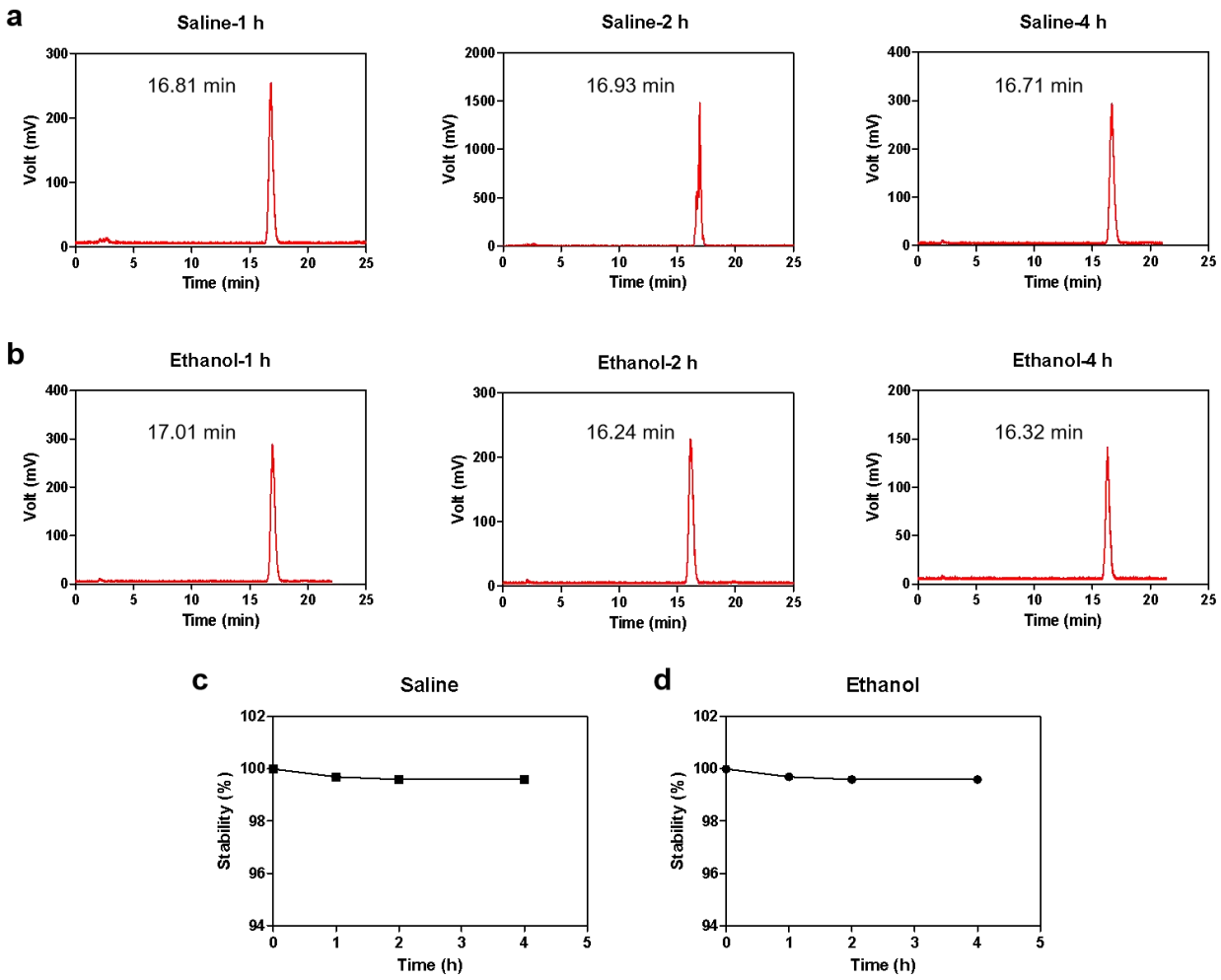


Figure S9 The radio-HPLC analysis of [^{18}F]FBTA incubated in (**a**) saline and (**b**) ethanol for 1 h, 2 h and 4 h. The radio-HPLC analysis of radiochemical purity of [^{18}F]FBTA in (**c**) saline and (**d**) ethanol.

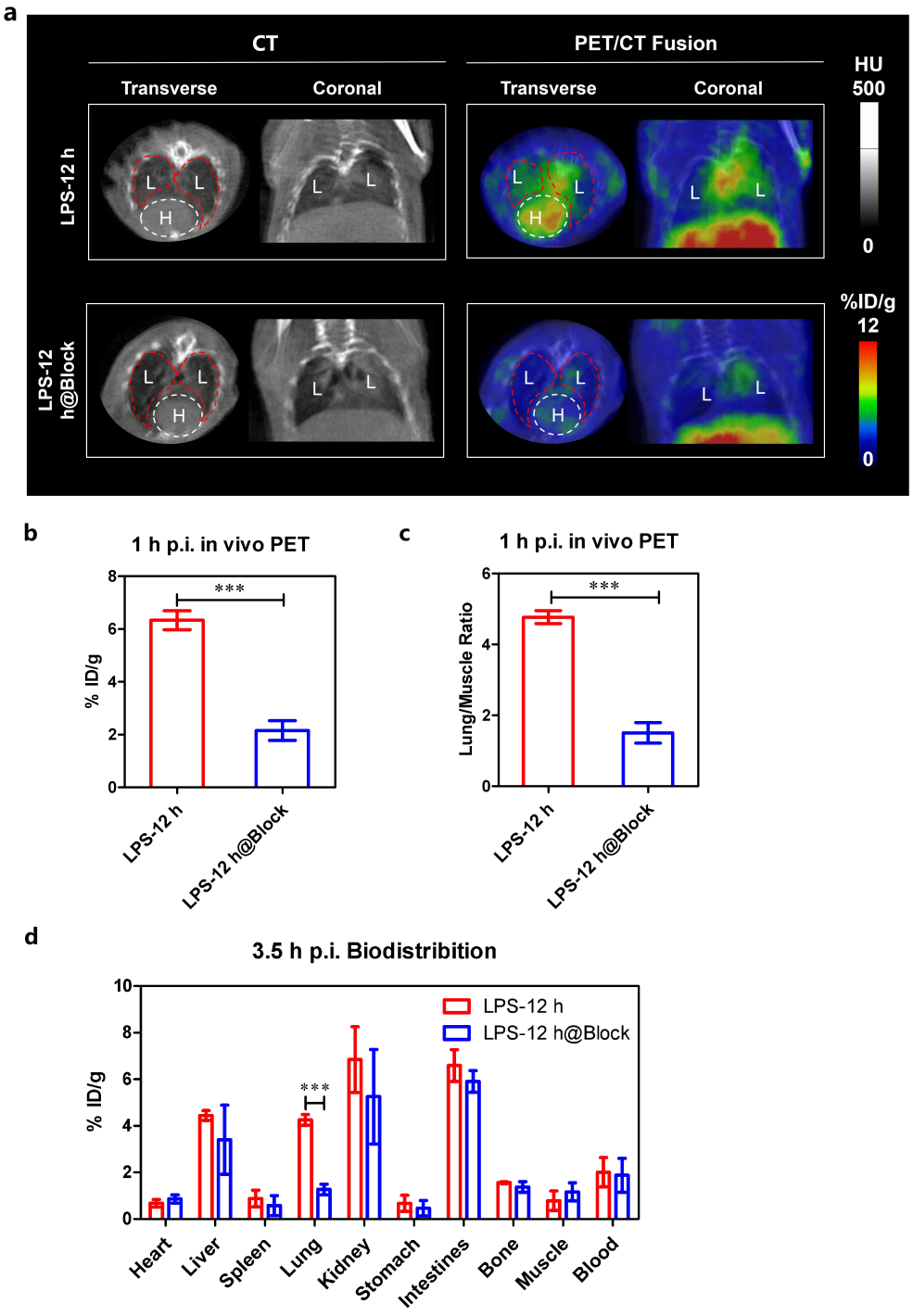


Figure S10 (**a**) Transverse and coronal PET/CT images of [^{18}F]FBTA (11.1~14.8 MBq) in ALI-12 h group with or without [^{19}F]FBTA co-injection. The lungs are indicated by red lines and hearts are indicated by white lines. L presents lung and H presents heart. (**b**) Quantification and comparison of [^{18}F]FBTA accumulation in the lungs with or without [^{19}F]FBTA co-injection at 1 h p.i.. PET data are shown as %ID/g ± SD (n = 3). (**c**) Lung to muscle ratios of the ALI-12 h group and the blocked group derived from PET quantification. (**d**) Biodistribution data of [^{18}F]FBTA in major organs of the two groups at 3.5 h p.i.. Data are shown as %ID/g ± SD (n = 3).

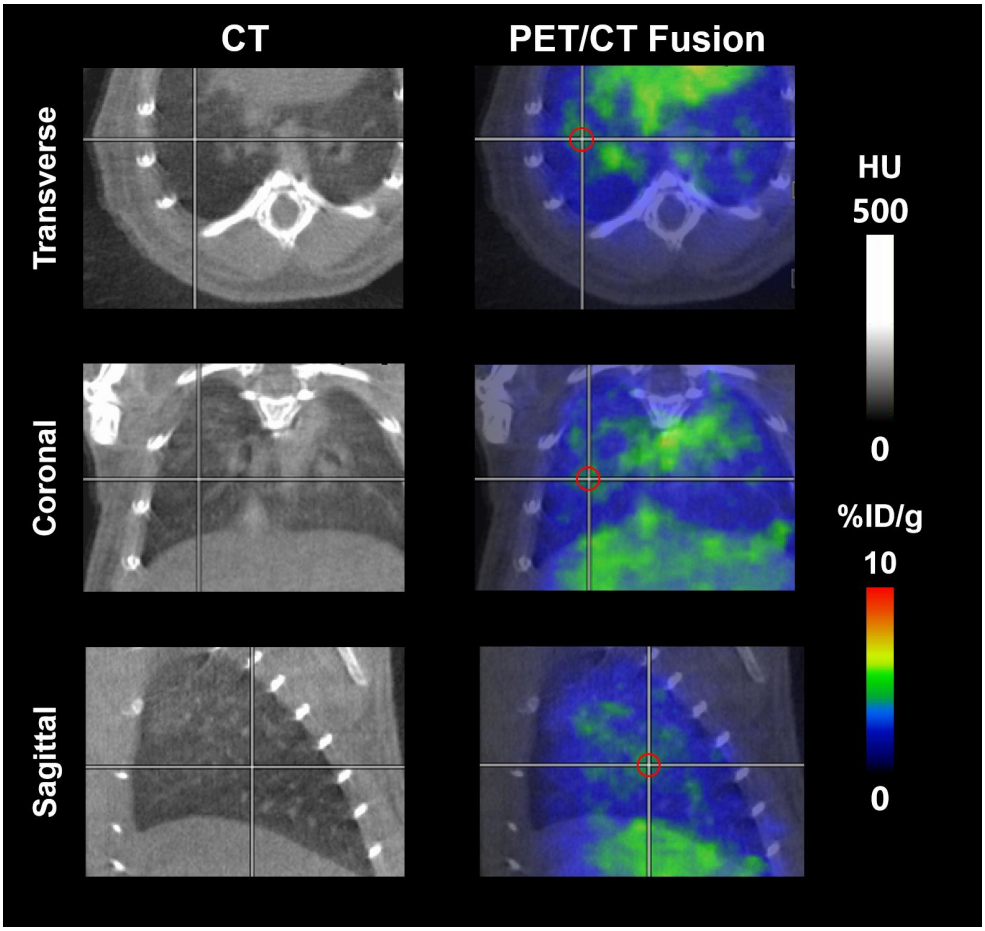


Figure S11 Transverse, coronal and sagittal PET/CT images of [^{18}F]FBTA (11.1~14.8 MBq) in LPS-induced mice for 2 h. The ALI lesions in lung are indicated by red circles.

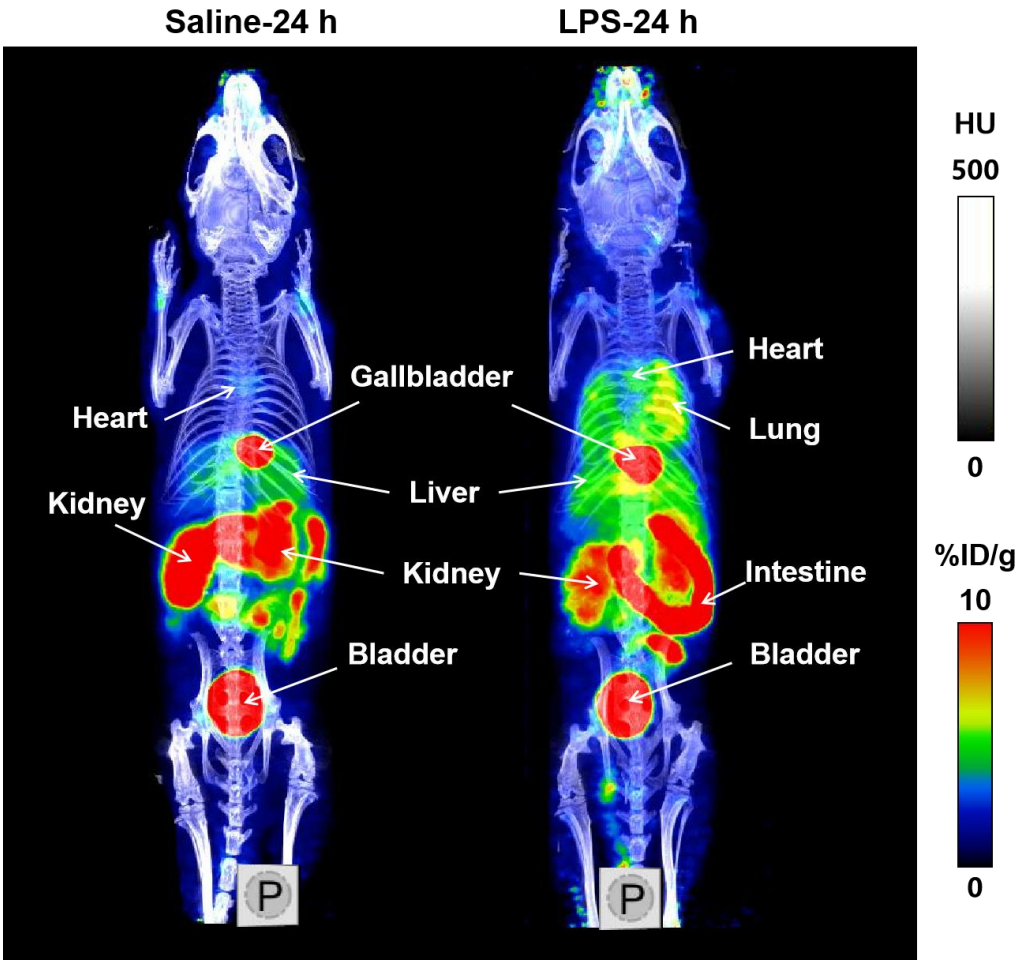


Figure S12 The whole body distribution maximum intensity projection (MIP) PET/CT images of [^{18}F]FBTA in saline group and ALI group at 1 h p.i.. [^{18}F]FBTA mainly accumulates in gallbladder, kidney, intestine and bladder.

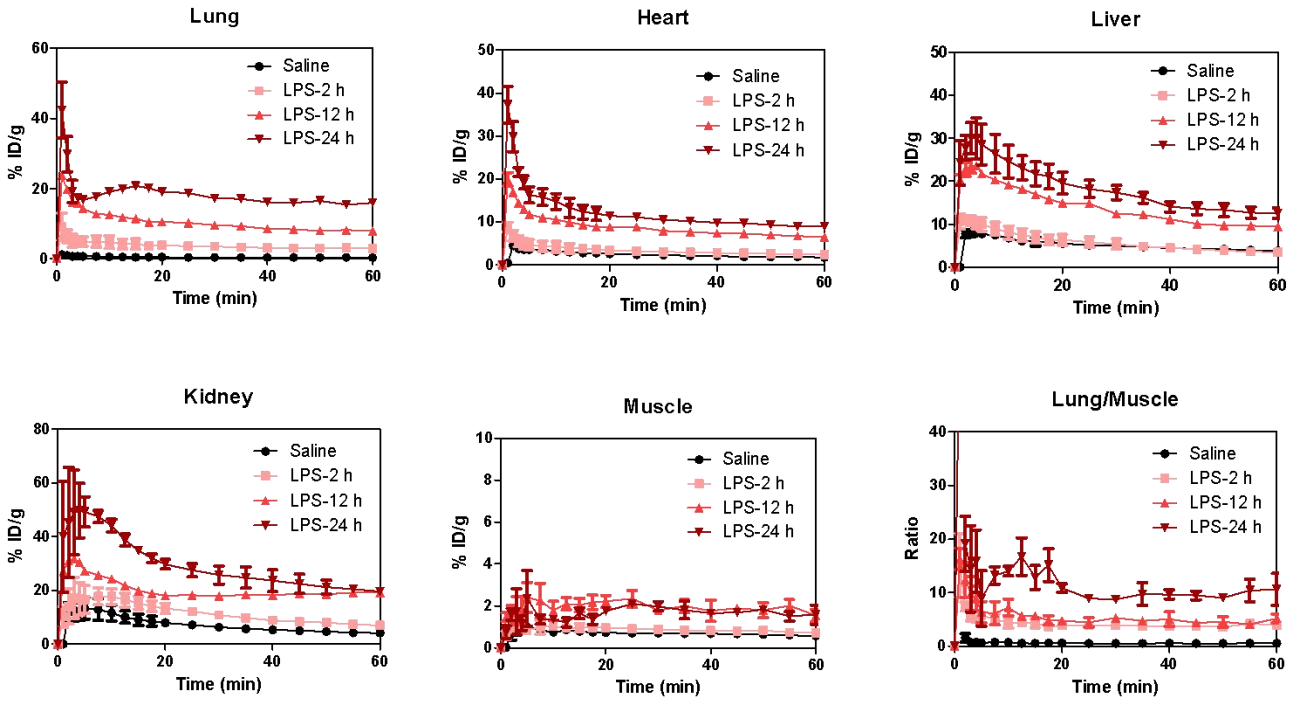


Figure S13 Time-activity curves of lung, heart, liver, kidney, muscle and lung to muscle ratio of control group and ALI groups injected with [^{18}F]FBTA. Data are shown as %ID/g ± SD (n = 3).

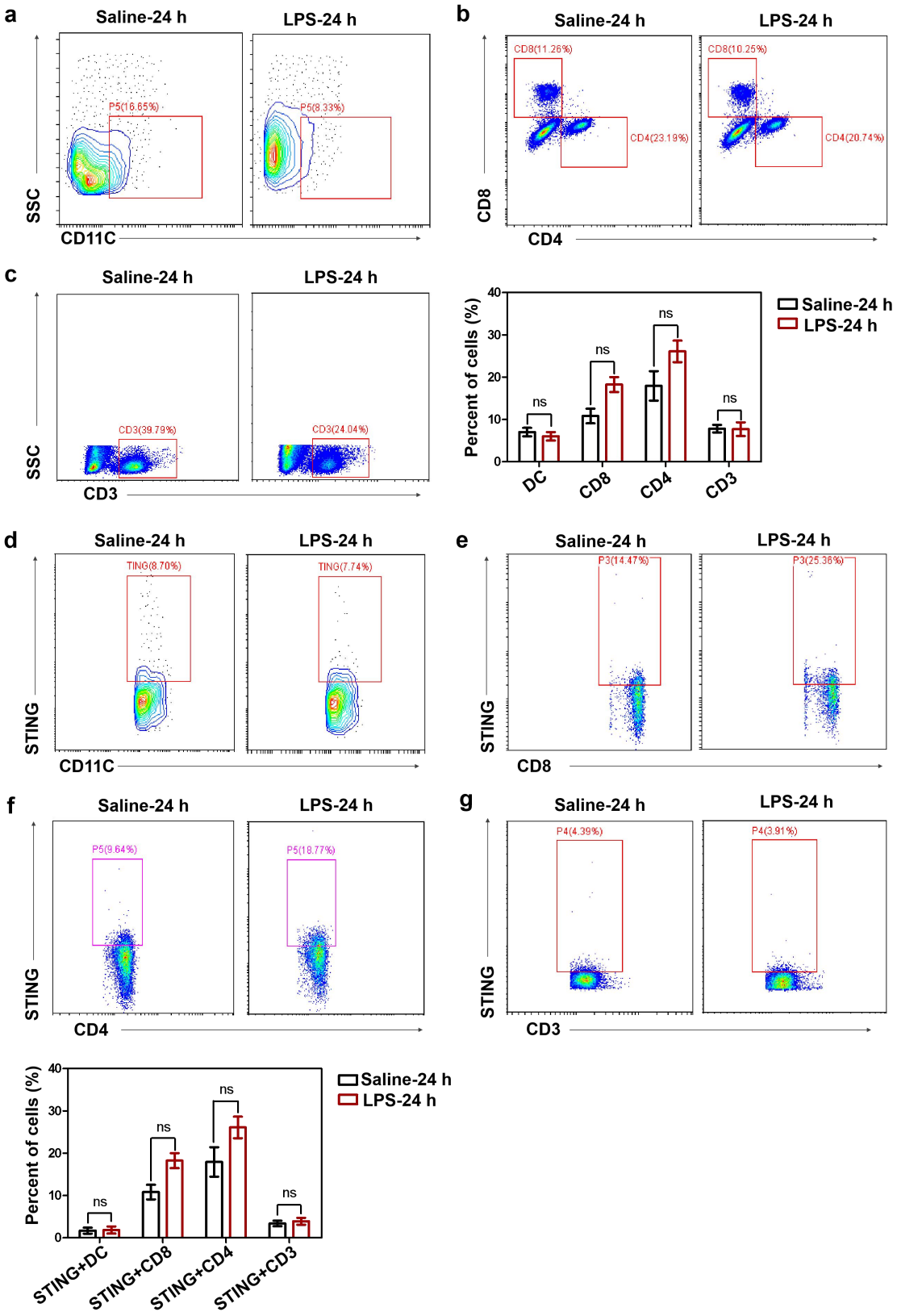


Figure S14 Flow cytometric plots and quantification of (**a**) DC, (**b**) $CD8^+$ T cells and $CD4^+$ T cells, (**c**) $CD3^+$ T cells in lungs and STING expression in (**d**) DC, (**e**) $CD8^+$ T cells, (**f**) $CD4^+$ T cells and (**g**) $CD3^+$ T cells of control group and ALI-24 h group.

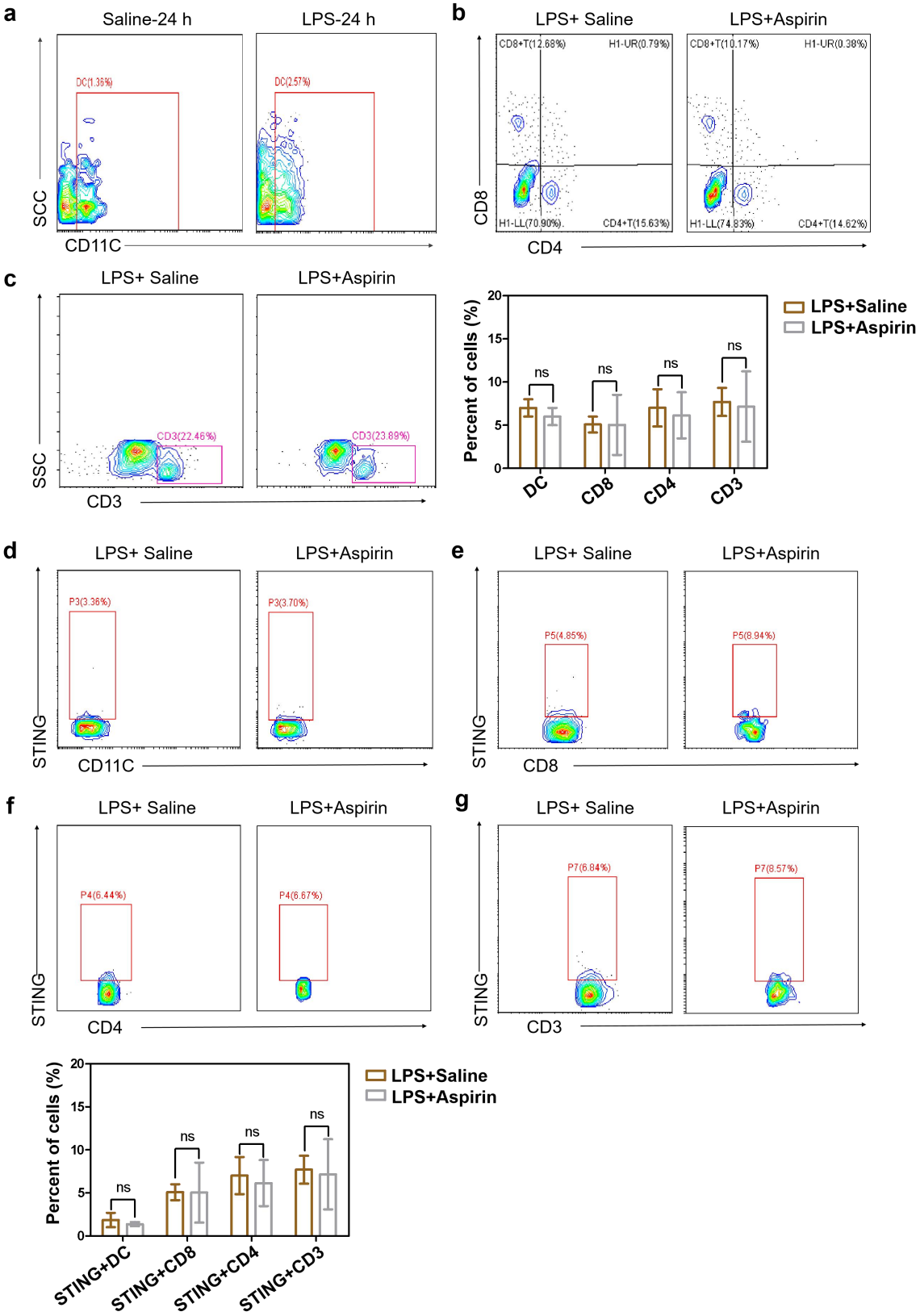


Figure S15 Flow cytometric plots and quantification of (**a**) DC, (**b**) $CD8^+$ T cells and $CD4^+$ T cells, (**c**) $CD3^+$ T cells in lungs and STING expression in (**d**) DC, (**e**) $CD8^+$ T cells, (**f**) $CD4^+$ T cells and (**g**) $CD3^+$ T cells of saline-treated and aspirin-treated ALI mice.

Table S1 Uptakes (%ID/g) of lung, heart, liver, kidney, muscle, lung to muscle ratio and lung to heart ratio derived from [^{18}F]FBTA-PET and [^{18}F]FDG-PET images at 1 h p.i..

	[^{18}F]FBTA				[^{18}F]FDG
Tissues	Saline	LPS-2 h	LPS-12 h	LPS-24 h	LPS-24 h
Lung	0.33±0.07	2.89±0.38	7.92±0.24	15.96±0.62	5.82±1.59
Heart	1.74±0.14	2.44±0.37	6.47±0.42	8.92±0.53	29.50±4.84
Liver	3.83±0.44	3.49±0.60	9.46±0.26	12.56±1.07	1.48±0.18
Kidney	4.10±0.19	6.95±0.17	19.14±0.85	19.55±0.75	9.02±0.85
Muscle	0.57±0.02	0.72±0.06	1.55±0.26	1.59±0.41	0.71±0.09
Lung/muscle	0.59±0.10	3.98±0.21	5.21±0.73	10.62±2.67	8.04±1.37
Lung/heart	0.19±0.04	1.21±0.30	1.23±0.04	1.79±0.05	0.20±0.04

Table S2 Biodistribution of [^{18}F]FBTA and [^{18}F]FDG in mice at 3.5 h p.i. (n = 3, %ID/g ± SD).

	[^{18}F]FBTA				[^{18}F]FDG
Tissues	Saline	LPS-2 h	LPS-12 h	LPS-24 h	LPS-24 h
Heart	0.29±0.19	0.36±0.10	0.34±0.02	1.41±0.26	29.13±5.12
Liver	2.40±0.37	2.92±0.17	4.63±0.37	8.26±0.29	0.66±0.16
Spleen	0.98±0.26	1.13±0.78	0.83±0.40	2.73±0.50	3.50±1.60
Lung	1.67±0.26	2.70±0.08	4.17±0.19	8.42±0.70	2.84±1.01
Kidney	4.37±1.20	6.99±2.56	11.14±1.12	13.27±2.04	3.07±0.94
Stomach	1.62±0.59	0.98±0.40	1.32±0.24	2.15±0.48	0.62±0.08
Intestines	6.97±0.42	6.61±0.51	6.53±0.48	6.93±0.53	1.10±0.21
Bone	2.34±0.23	1.46±0.46	2.05±0.81	2.59±0.29	0.91±0.03
Muscle	0.89±0.21	0.88±0.18	0.86±0.28	1.24±0.31	0.57±0.19
Blood	1.42±0.24	1.60±0.03	1.25±0.29	1.30±0.07	0.48±0.07
Lung/muscle	1.90±0.14	3.17±0.57	5.24±1.54	7.23±2.35	5.00±0.78
Lung/blood	1.18±0.02	1.69±0.08	3.49±0.76	6.23±0.19	6.10±2.45

Table S3 Biodistribution of [^{18}F]FBTA in ALI mice co-injected with or without [^{19}F]FBTA at 3.5 h p.i. (n = 3, %ID/g ± SD).

Tissues	LPS-12 h	LPS-12 h@[^{19}F]FBTA
Heart	0.68±0.13	0.87±0.16
Liver	4.44±0.17	2.23±0.53
Spleen	0.88±0.29	0.58±0.38
Lung	4.25±0.19	1.27±0.20
Kidney	6.84±1.15	5.25±1.81
Stomach	0.67±0.28	0.46±0.30
Intestines	6.59±0.56	5.90±0.42
Bone	1.56±0.03	1.37±0.20
Muscle	0.79±0.34	1.16±0.35
Blood	2.01±0.51	1.88±0.65
Lung/muscle	6.17±2.42	1.20±0.48
Lung/blood	2.25±0.67	0.74±0.27

Table S4 Biodistribution of [^{18}F]FBTA in ALI mice treated with saline or aspirin at 3.5 h p.i. (n = 3, %ID/g ± SD).

Tissues	LPS-saline	LPS-aspirin
Heart	2.49±0.52	2.03±1.39
Liver	7.90±2.14	8.83±3.72
Spleen	2.42±1.10	4.96±1.55
Lung	6.82±1.26	2.34±1.36
Kidney	11.46±3.72	12.51±4.19
Stomach	3.24±0.94	3.00±0.38
Intestines	5.44±1.04	8.35±2.19
Bone	2.61±0.29	2.48±0.18
Muscle	1.67±0.21	1.97±0.45
Blood	2.37±0.30	2.61±0.27
Lung/muscle	4.06±0.29	1.31±0.78
Lung/blood	2.95±0.78	0.89±0.52