

Supplementary information

Aggregation-Induced Emission Luminogens for Image-Guided Surgery in Non-Human Primates

Danni Zhong^{1,2‡}, Weiyu Chen^{3,8‡}, Zhiming Xia^{2, 4,‡}, Rong Hu^{5,‡}, Yuchen Qi^{2,‡}, Bo Zhou²,
Wanlin Li², Jian He², Zhiming Wang⁵, Zujin Zhao⁵, Dan Ding⁶, Mei Tian¹¹, Ben Zhong
Tang^{5,7*}, and Min Zhou^{1,2,9,10*},

¹Eye Center, the Second Affiliated Hospital, Zhejiang University School of Medicine,
Hangzhou 310009, China.

²Institute of Translational Medicine, Zhejiang University, Hangzhou, 310009, China.

³The Fourth Affiliated Hospital, Zhejiang University School of Medicine, Yiwu, 320000,
China.

⁴Department of Nuclear Medicine, the Second Affiliated Hospital, Zhejiang University
School of Medicine, Hangzhou 310009, China.

⁵NSFC Center for Luminescence from Molecular Aggregates, SCUT-HKUST Joint
Research Institute, State Key Laboratory of Luminescent Materials and Devices,
South China University of Technology, Guangzhou, 510640 China

⁶Key Laboratory of Bioactive Materials, Ministry of Education, and College of Life
Sciences, Nankai University, Tianjin 300071, China

⁷Department of Chemistry, Hong Kong Branch of Chinese National Engineering
Research Center for Tissue Restoration and Reconstruction, State Key Laboratory of
Neuroscience and Division of Biomedical Engineering, The Hong Kong University of
Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong, China

⁸Molecular Imaging Program at Stanford, Department of Radiology, Stanford
University, Stanford, CA 94305-5427, USA

⁹Cancer Center, Zhejiang University, Hangzhou 310009, China.

¹⁰State Key Laboratory of Modern Optical Instrumentations, Zhejiang University,
Hangzhou, 310058, China

¹¹Department of Nuclear Medicine, Shandong Provincial Hospital Affiliated to
Shandong First Medical University, Jinan, 250021, China.

‡These authors contributed equally to this work.

*Corresponding authors. Email: zhoum@zju.edu.cn; tangbenz@ust.hk

Content of Experiments, Tables and Supplementary Figures

33

34

35 Table. 1. Post-inspection of the rhesus macaque.

36 Table. 2. Changes in the bodyweight of rhesus macaque 1-day before and 180-day
37 after operations.

38 Table. 3. Systematical Comparison of ICG dye and AIEgens.

39 Table. 4. Advanced performances and limitations of AIEgens and typical contrast
40 agents.

41

42 Fig. S1. Characterizations of folic-AIEgen.

43 Fig. S2. Cell viabilities of different cells (HEK293, HL7702, 4T1, and fibroblast cells)
44 after incubation with series concentrations of folic-AIEgen for 24 h,
45 respectively.

46 Fig. S3. Folic-AIEgen for targeting cell imaging.

47 Fig. S4. Histological images of the major organs from the mice at 30 days after
48 surgical operation.

49 Fig. S5. Histological images of the major organs from the rabbits at 30 days after
50 surgical operation.

51 Fig. S6. Histological images of the major organs from the mice at 30 days after
52 different treatments.

53 Fig. S7. Blood test results (a-l) for the rhesus macaques before and after the surgical
54 operation.

55 Fig. S8. Specific tumor targeting ability of folic-AIEgen in the intraperitoneal SKOV3
56 xenograft mouse model.

57 Fig. S9. Histological images of the major organs from the tumor-bearing mice at 30
58 days after surgical operation.

59 Fig. S10. Blood test results for the SKOV3 tumor-bearing mice after the intravenous
60 injection.

61 Fig. S11. Targeted imaging of folic-AIEgen in metastatic liver tumor.

62 Fig. S12. White light and bioluminescence images of intraperitoneal SKOV3 and Hela
63 tumors bearing mice after surgery.

64 Fig. S13. Survival rate of intraperitoneal SKOV3 and Hela tumors bearing mice with or
65 without surgery.

66 Fig. S14. Comparison of fluorescence characteristics and stability between ICG and
67 folic-AIEgen.

68 Fig. S15. Schematic illustration of ICG and folic-AIEgen guided SLN dissection in
69 nude mice.

70 Fig. S16. Biodistribution of ICG and folic-AIEgen in nude mice after intravenous
71 administration.

72

73

74

Behaviors check list	Results
Eating	Normal
Drinking	Normal
Urination	Normal
Defecation	Normal
Sleeping	Normal
Activity	Normal
Grooming	Normal
Neurological	Normal

75

76

77 **Supplementary Table 1. Post-inspection of the rhesus macaque.** No
78 abnormalities were observed in behaviors of rhesus macaque after operations,
79 including eating, drinking, urination, defecation, sleeping, activity, grooming, and
80 neurological status.

81

82

83

84

85

Date	Body weight (Kg)
Before Surgery	5.80
Post-Surgery: Day 1	5.88
Post-Surgery: Day 7	5.98
Post-Surgery: Day 30	6.22
Post-Surgery: Day 90	6.38
Post-Surgery: Day 180	6.55

86

87

88 **Supplementary Table 2. Changes in the bodyweight of rhesus macaque 1-day**
89 **before and 180-day after operations.** Bodyweight of the monkey was monitored

90 1-day before and 180-day after the surgical operation. Over the whole period, no
 91 greater fluctuations were observed after 180-day treatment (6.55 kg) than before
 92 treatment (5.80 kg).

93
 94
 95

Properties	ICG, NIR fluorescence ~850 nm	AI Egens, visible fluorescence ~540 nm
Component	Small organic dyes (<1 KD)	Fluorescent nanoparticles (Diameter: ca 20 nm)
Dispersibility	Dispersed in sterile water but easily aggregated in physiological saline	Well dispersed in both sterile water and physiological saline
Fluorescent stability	<ol style="list-style-type: none"> 1. Decrease to <40% of initial fluorescence intensity within 24 h at 37 °C; 2. Rapid photobleaching to ~52% in 105 min under continuous laser irradiation 	<ol style="list-style-type: none"> 1. Remain stable during 3-day storage; 2. Possess superior photostability, preserving ~80% during 105 min continuous laser irradiation
Real-time surgical operation	<ol style="list-style-type: none"> 1. NIR fluorescence imaging system, including NIR excitation light, collection optics, filtration, NIR camera, color camera; 2. Assisted by surgeon's guidance due to undetectable NIR fluorescence seen by naked eyes 	Portable UV lamp
Medical equipment cost	High cost (~100,000 US dollars)	Low cost (100-500 US dollars)
Medical training	Long-term training time	No additional training Simple operation procedure

96
 97
 98
 99
 100
 101
 102
 103
 104
 105
 106
 107
 108
 109

Supplementary Table 3. Systematical Comparison of ICG dye and AI Egens.

110

Typical example	Method	Diameter	Advanced performances	Limitations
^{99m} Techneium nanocolloids	Nuclear imaging	50~3000 nm	Detect deep-seated targets (~ 25 mm)	<ol style="list-style-type: none"> 1. Large sizes; 2. Slow clearance from injection site; 3. Slow physiologic transport within lymphatics
ICG	MR fluorescence imaging	<1 KD	Detect superficially located targets (~5 mm)	Extravasation and nonspecific tissue scattering
Evans blue	Visible blue color	<1 KD	Aid visual identification under white-light illuminations	<ol style="list-style-type: none"> 1. Limited tissue penetration; 2. Lose visibility when intraoperative bleeding; 3. Extravasation and nonspecific tissue scattering
AIEngens	Naked eye visible fluorescence	~20 nm	<ol style="list-style-type: none"> 1. Aid visual identification under UV-light illuminations; 2. Visualize the draining lymphatics in real time 	Limited tissue penetration

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130

131

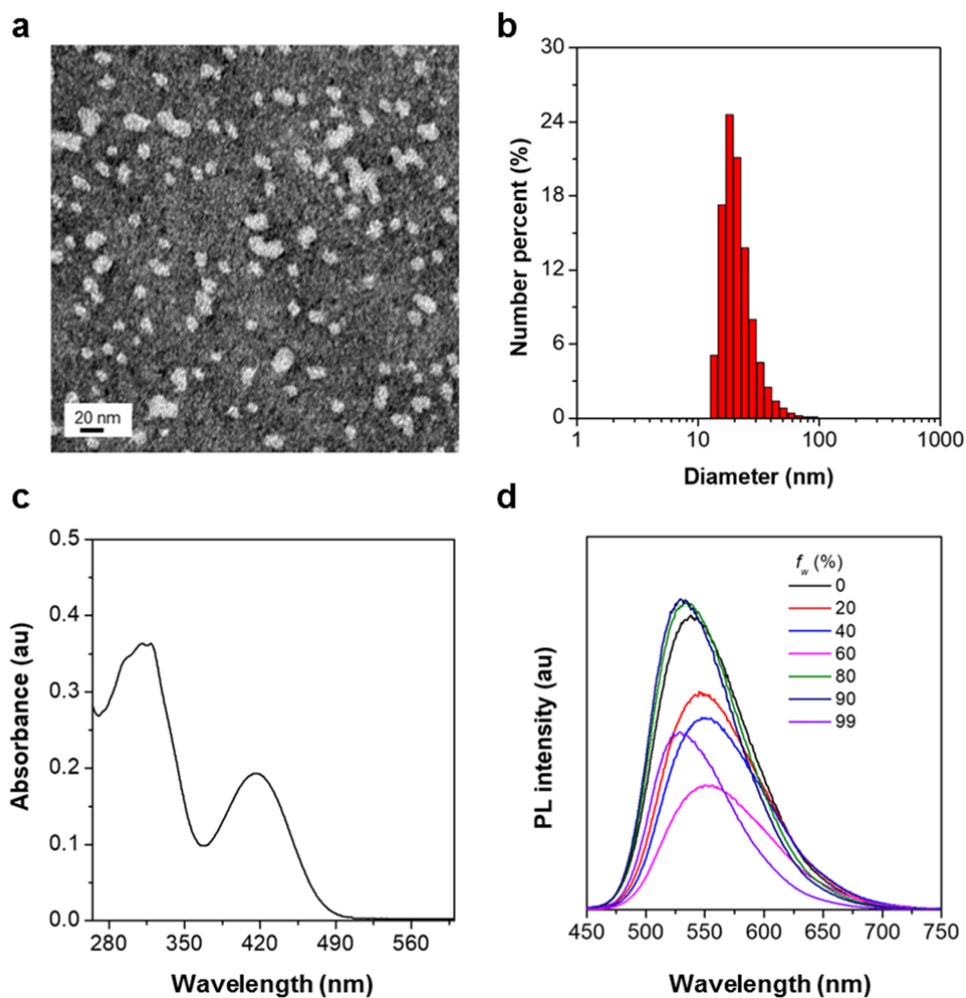
132

133

134

135

Supplementary Table 4. Advanced performances and limitations of AIEngens and typical contrast agents.



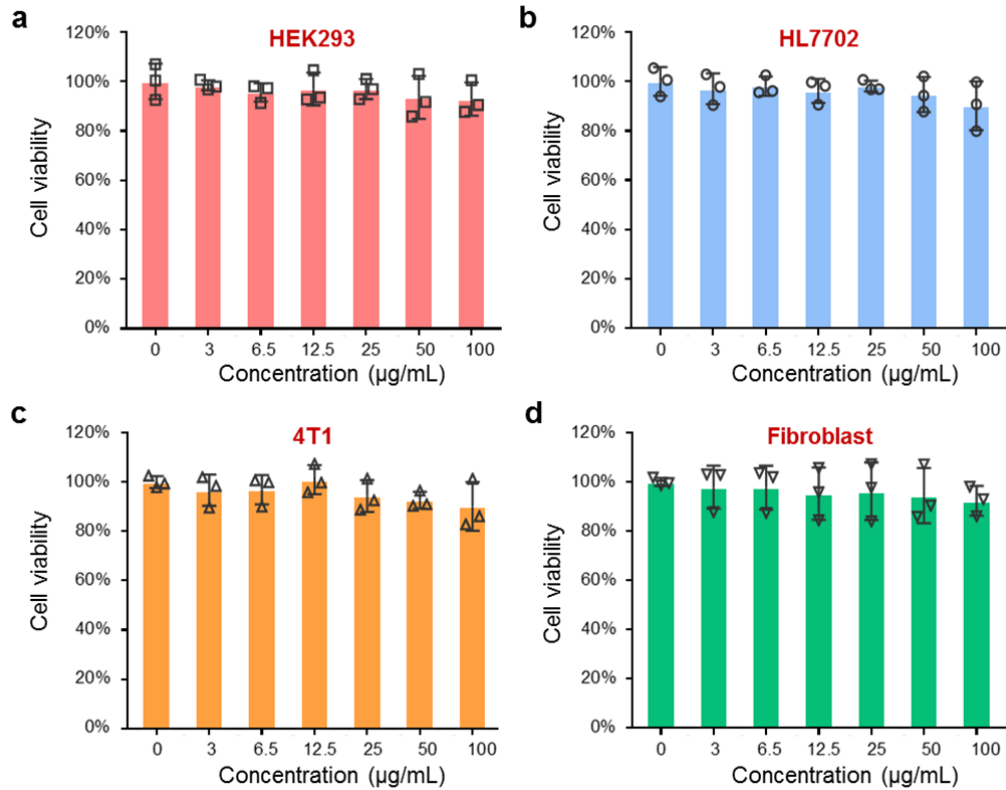
136

137

138 **Supplementary Figure 1. Characterizations of folic-AIEgen.** (a) Transmission
 139 electron microscopy (TEM) image. **The experiment was repeated at least three times.**
 140 (b) DLS, and (c) UV-vis spectra of the as-prepared folic-AIEgen. (d)
 141 Photoluminescence of AIEgen in tetrahydrofuran with different fraction of water.

142

143



144

145

146 **Supplementary Figure 2. Cell viabilities of different cells (HEK293, HL7702, 4T1,**
 147 **and fibroblast cells) after incubation with series concentrations of folic-AIEgen**
 148 **for 24 hours, respectively. No significance was observed in terms of cell viability**
 149 **among four types of cells** incubated with different concentrations of folic-AIEgen. **Data**
 150 **are means ± SD, n = 3.**

151

152

153

154

155

156

157

158

159

160

161

162

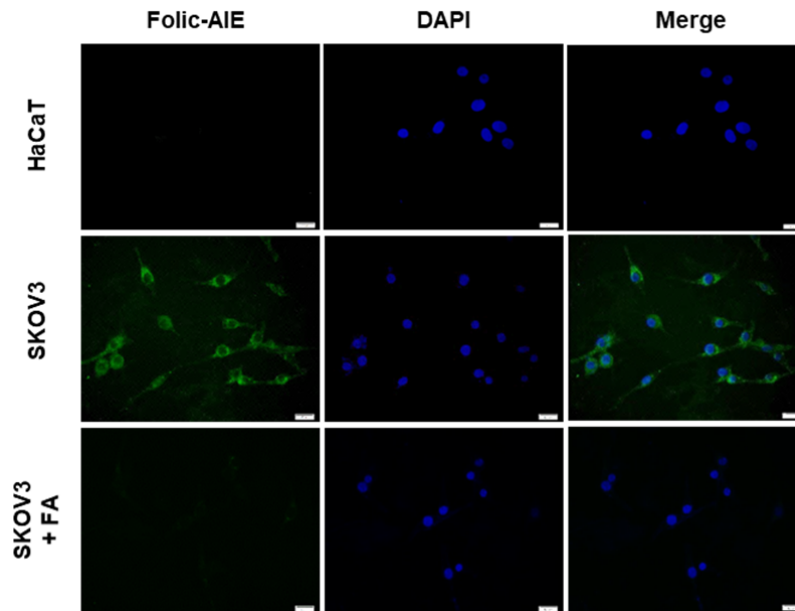
163

164

165

166

167



169

170

171 **Supplementary Figure 3. Folic-AIEgen for targeting cell imaging.** SKOV3 cells
 172 with over-expressing FR displayed a significant enrichment of folic-AIEgen after
 173 incubation (middle) and free folic acid competitively inhibited the uptake of
 174 folic-AIEgen (bottom). **HacaT** cells (with a middle expression of FR) did not tend to
 175 internalize folic-AIEgen nanoparticles after 24-hour incubation (top). Scale bars = 50
 176 μm . **The experiment was repeated at least three times.**

177

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

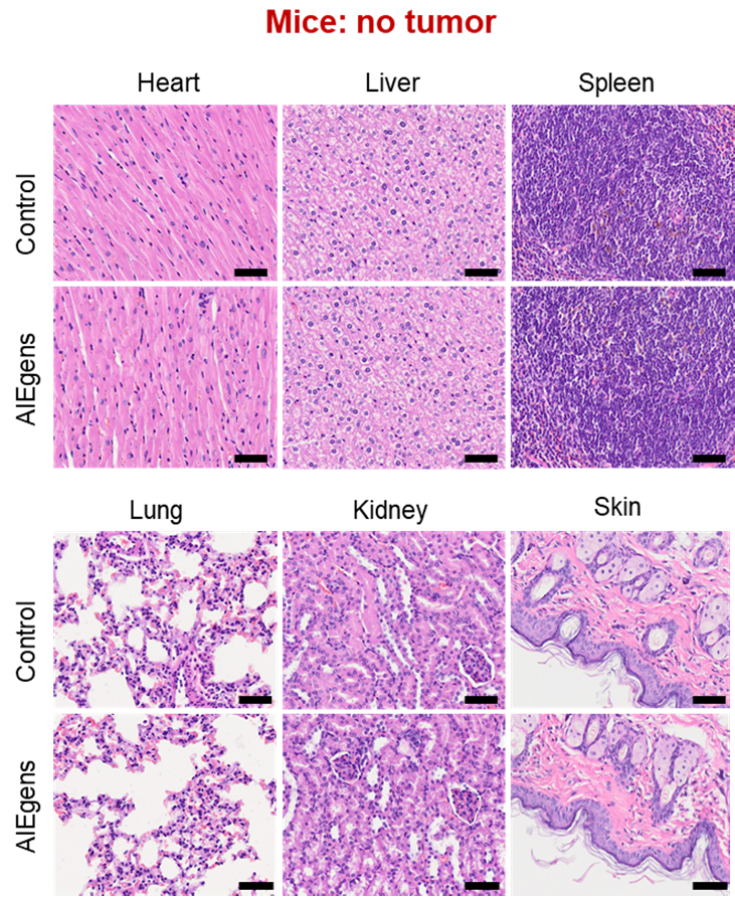
193

194

195

196

197
198

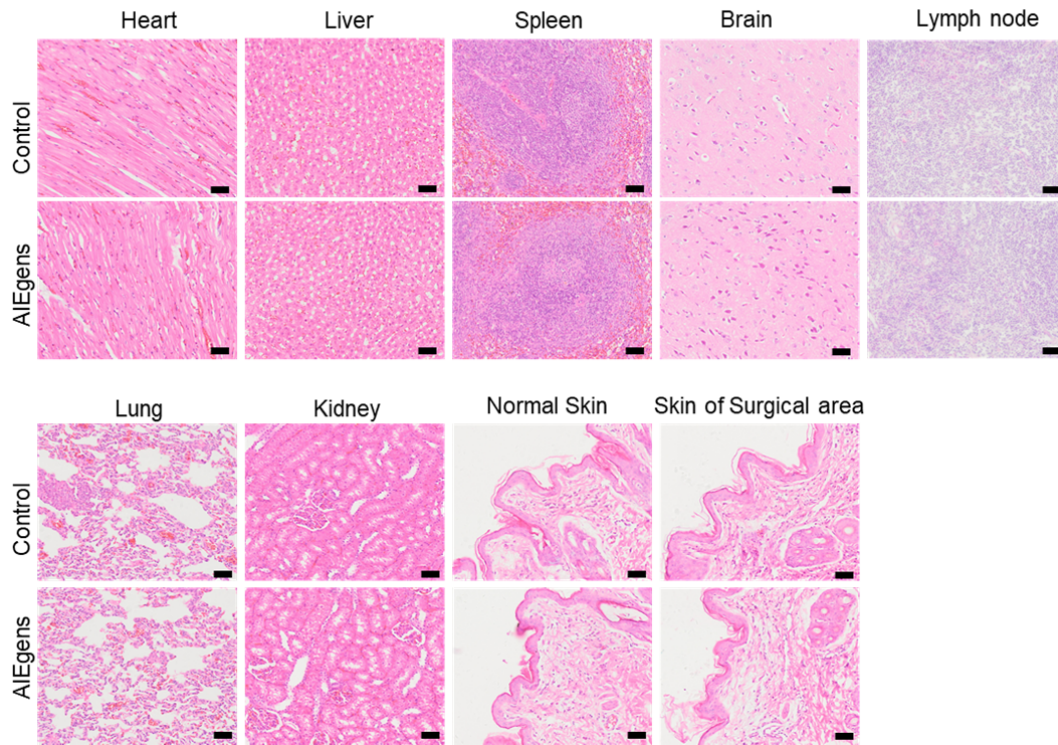


199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216

Supplementary Figure 4. Histological images of the major organs from the mice at 30 days after surgical operation. Representative H&E staining images of heart, liver, spleen, lung, kidney, and skin. Scale bar = 50 μ m. The experiment was repeated at least three times.

217

Rabbit: no tumor



218

219

220 **Supplementary Figure 5. Histological images of the major organs from the**
221 **rabbits at 30 days after surgical operation.** Representative **H&E staining** images of
222 heart, liver, spleen, lung, kidney, normal skin, and skin of surgical area. Scale bar = 50
223 μm . **The experiment was repeated at least three times.**

224

225

226

227

228

229

230

231

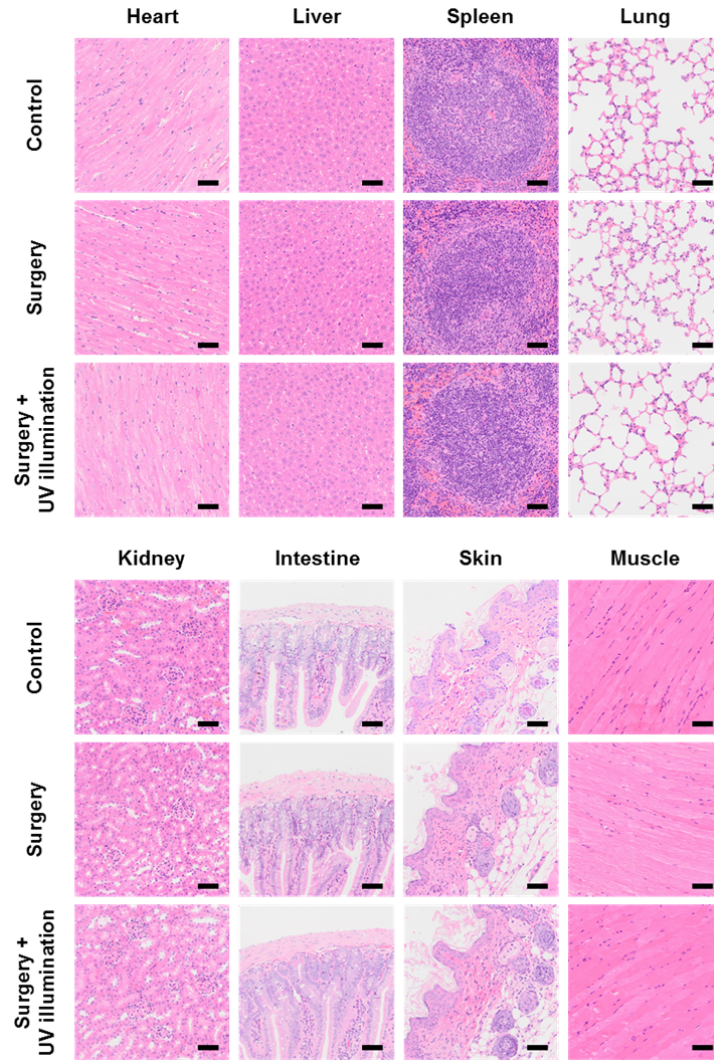
232

233

234

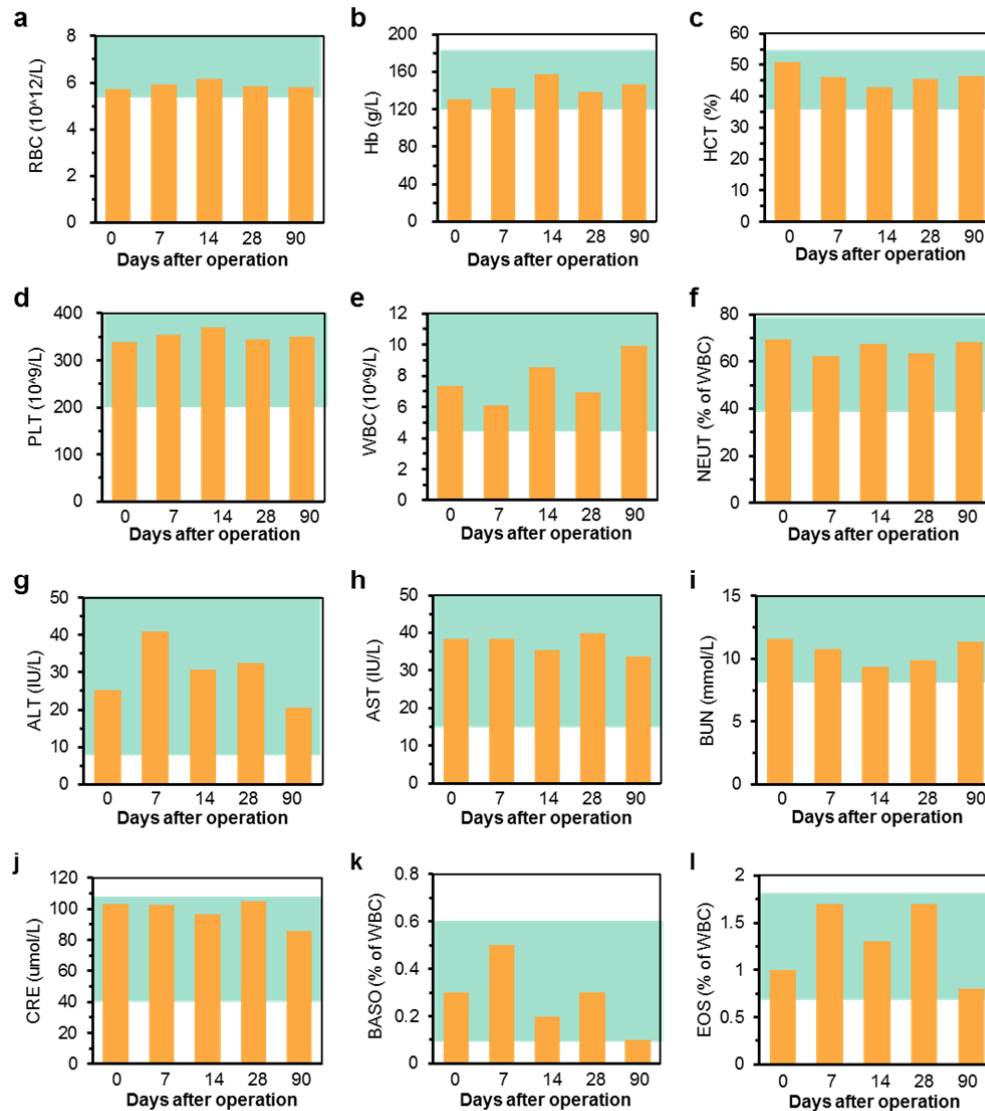
235

236



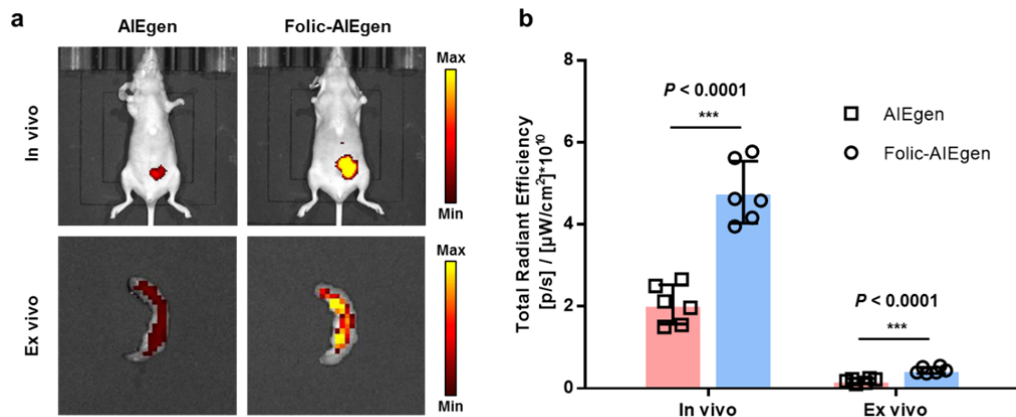
237
 238
 239
 240
 241
 242
 243
 244
 245
 246
 247
 248
 249
 250
 251
 252
 253

Supplementary Figure 6. Histological images of the major organs from the mice at 30 days after different treatments. Representative H&E staining images of heart, liver, spleen, lung, kidney, intestine, skin and muscle (n = 6). Scale bar = 50 μm. The experiment was repeated at least three times.



254
 255 **Supplementary Figure 7. Blood test results (a-l) for the rhesus macaques before**
 256 **and after the surgical operation.** There were no abnormalities in liver and kidney
 257 function, complete blood count and immune system over the 90-day post-treatment.
 258 Abbreviations: haemoglobin, Hb; red blood cell count, RBC; haematocrit, Hct; platelet
 259 count, PLT; white blood cell count, WBC; neutrophil granulocyte, NEUT; lymphocyte,
 260 LY; monocyte, MONO; eosinophil granulocyte, EOS; basophil granulocyte, BASO;
 261 alanine transaminase, ALT; aspartate transaminase, AST; blood urea nitrogen, BUN;
 262 creatinine, CRE. The regions rendered in GREEN represent the normal range. Before
 263 the operation, the animal is subject to blood testing as the normal control.

264
 265
 266
 267
 268



269

270

271

272

273

274

275

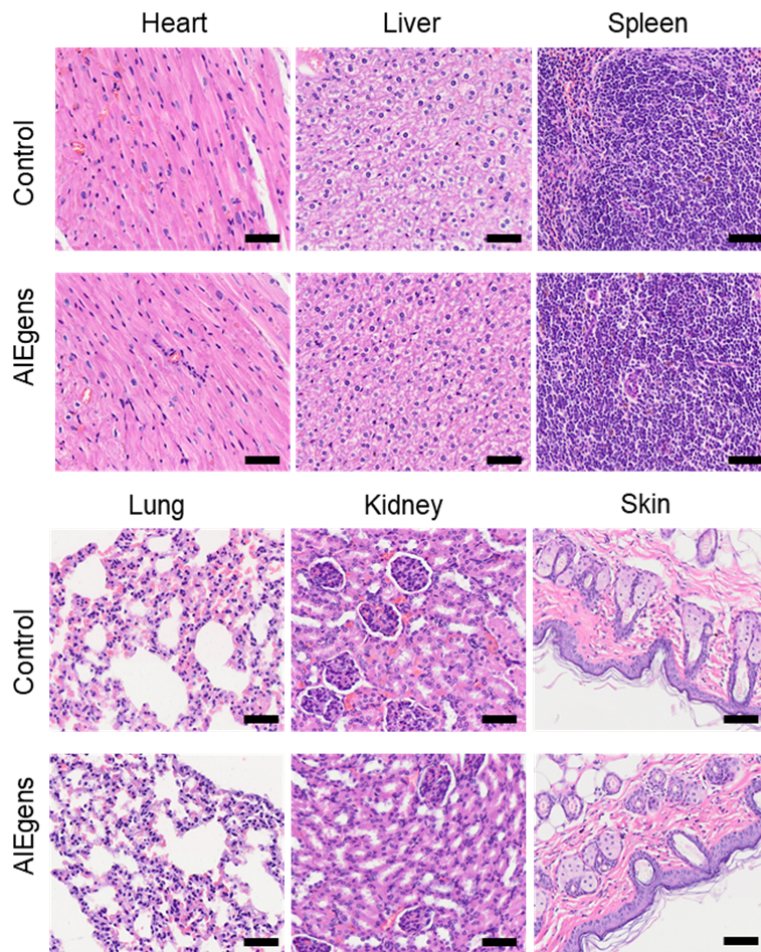
276

277

278

Supplementary Figure 8. Specific tumor targeting ability of folic-AIEgen in the intraperitoneal SKOV3 xenograft mouse model. (a) In vivo fluorescence imaging of mice (top) 24 hours after intraperitoneal injection of AIEgen or folic-AIEgen (100 μL, 40 μg/mL) and Ex vivo fluorescence imaging of the intestine tissues with tumors collected from mice (bottom) (n = 6). (b) Quantitative analysis of the fluorescence signals of the in vivo and ex vivo images. Data are means ± SD, n = 6, Student's two-tailed t test, *** $P < 0.001$.

Mice: SKOV3 tumor-bearing



280

281

282 **Supplementary Figure 9. Histological images of the major organs from the**283 **tumor-bearing mice at 30 days after surgical operation. Representative H&E**284 **staining images of heart, liver, spleen, lung, kidney, and skin. Scale bar = 50 μm. The**285 **experiment was repeated at least three times.**

286

287

288

289

290

291

292

293

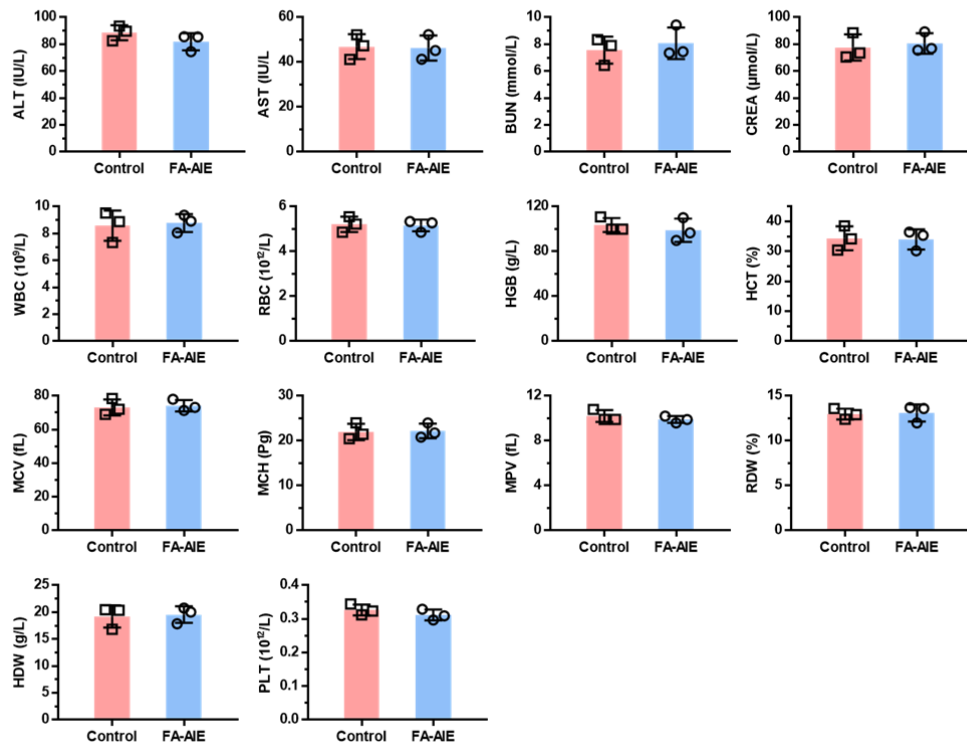
294

295

296

297

Mice: SKOV3 tumor-bearing



298

299

300

301

302

303

304

305

306

307

308

309

310

311

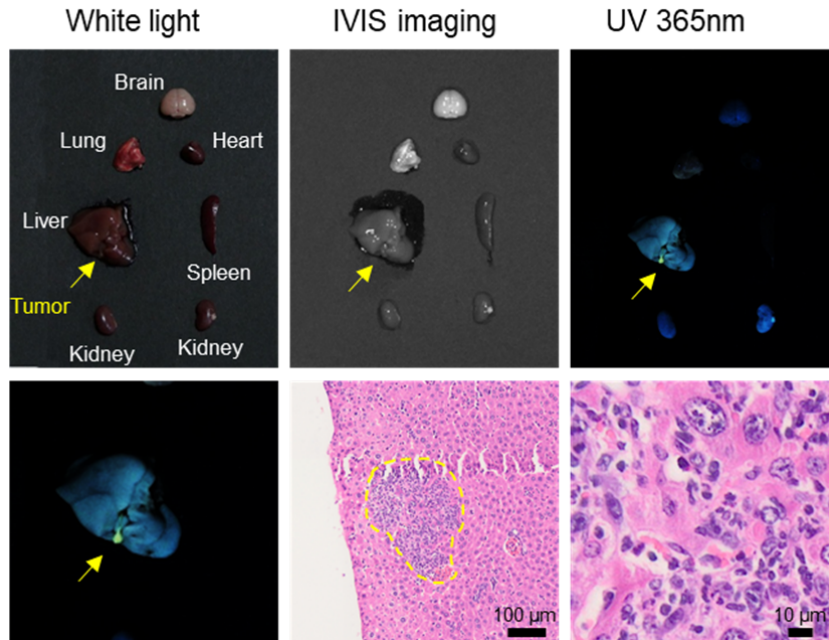
312

313

314

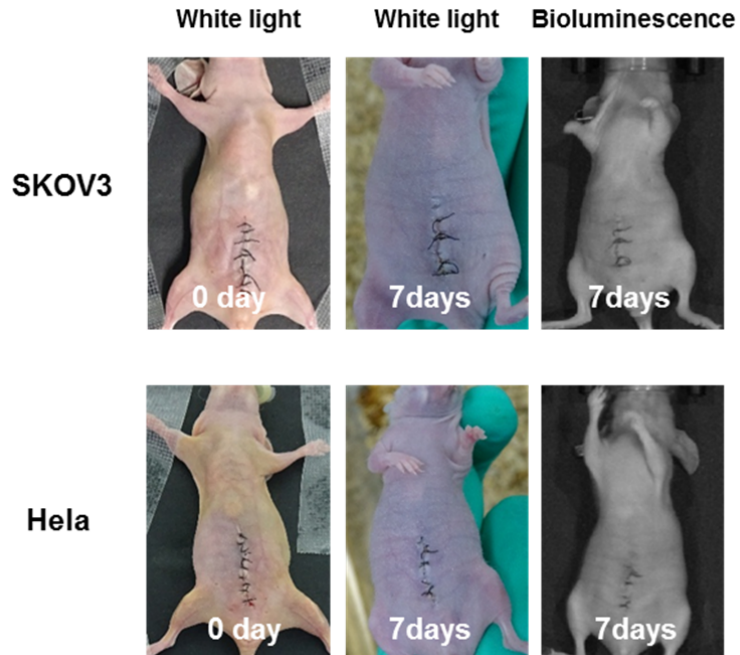
315

Supplementary Figure 10. Blood test results for the SKOV3 tumor-bearing mice after intraperitoneal injection of folic-AIEgen. There were no abnormalities in liver and kidney function, complete blood count and immune system over the 90-day post-treatment. Abbreviations: folic-AIEgen, FA-AIE, haemoglobin, Hb; red blood cell count, RBC; haematocrit, Hct; platelet count, PLT; white blood cell count, WBC; neutrophil granulocyte, NEUT; lymphocyte, LY; monocyte, MONO; eosinophil granulocyte, EOS; basophil granulocyte, BASO; alanine transaminase, ALT; aspartate transaminase, AST; blood urea nitrogen, BUN; creatinine, CRE. The regions rendered in GREEN represent the normal range. Before the operation, the animal is subject to blood testing as the normal control. **Data are means ± SD, n = 3.**



316
 317
 318
 319
 320
 321
 322
 323
 324
 325
 326
 327
 328
 329
 330

Supplementary Figure 11. Targeted imaging of AIEgens in metastatic liver tumor. Ex vivo images of major organs in Hela metastatic tumor-bearing mice under white light, IVIS imaging system, and UV light (top). Ex vivo image of the liver with tumor under UV light and representative H&E staining images (bottom). Yellow arrow and yellow circle: metastatic tumor. The experiment was repeated at least three times.



331

332

333 **Supplementary Figure 12. White light and bioluminescence images of**
 334 **intraperitoneal SKOV3 and Hela tumors bearing mice after surgery. 7 days after**
 335 **surgery, there were no signs of recurrence observed via bioluminescence** imaging.

336

337

338

339

340

341

342

343

344

345

346

347

348

349

350

351

352

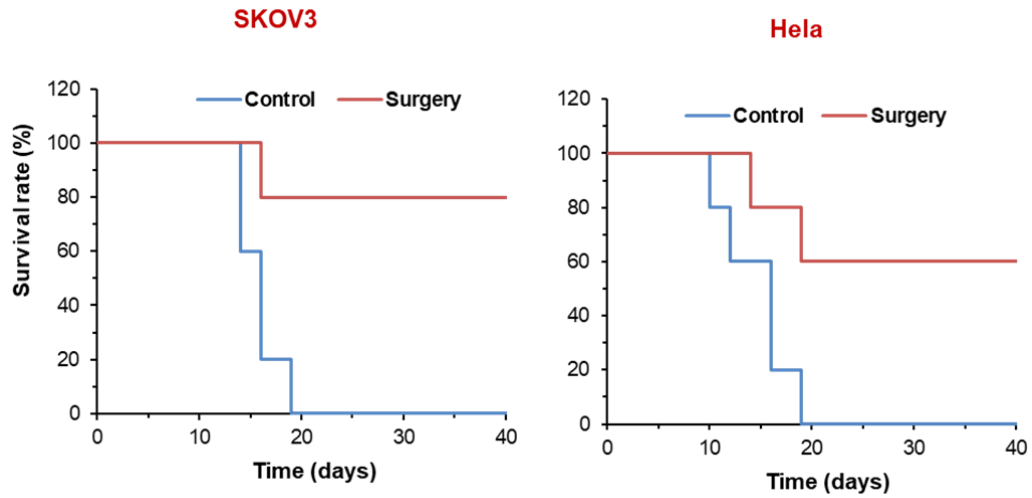
353

354

355

356

357



358

359

360 **Supplementary Figure 13. Survival rate of intraperitoneal SKOV3 and Hela**
 361 **tumors bearing mice with or without surgery.**

362

363

364

365

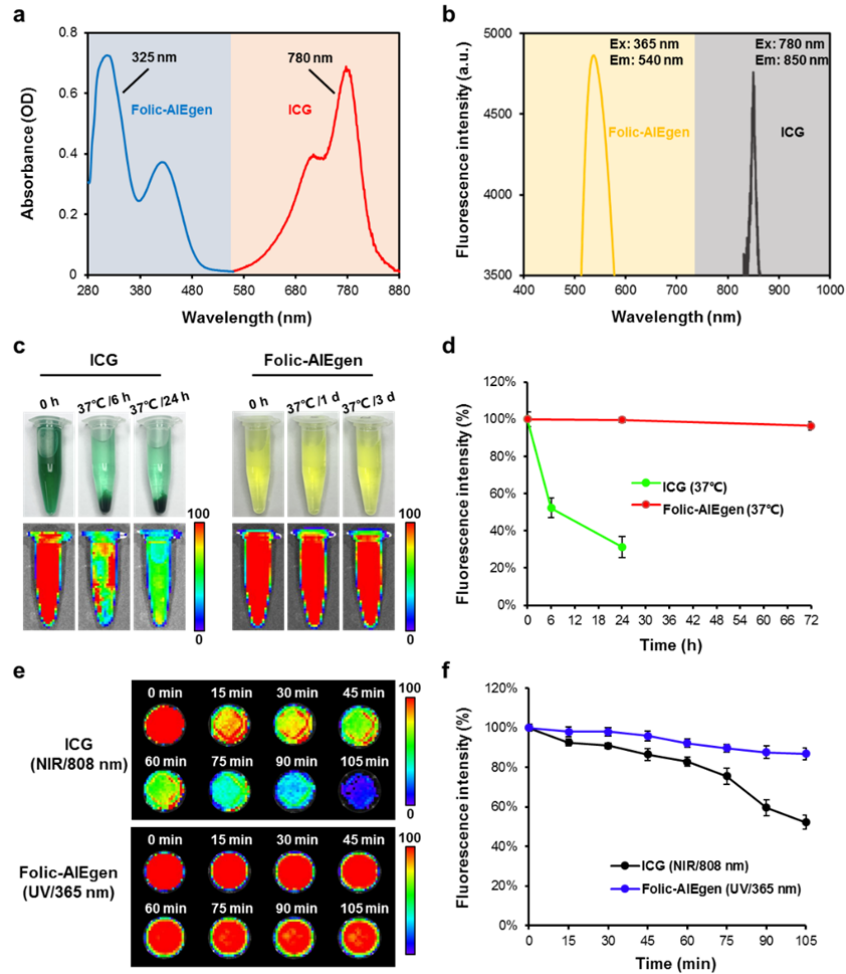
366

367

368

369

370



371

372

373

374

375

376

377

378

379

380

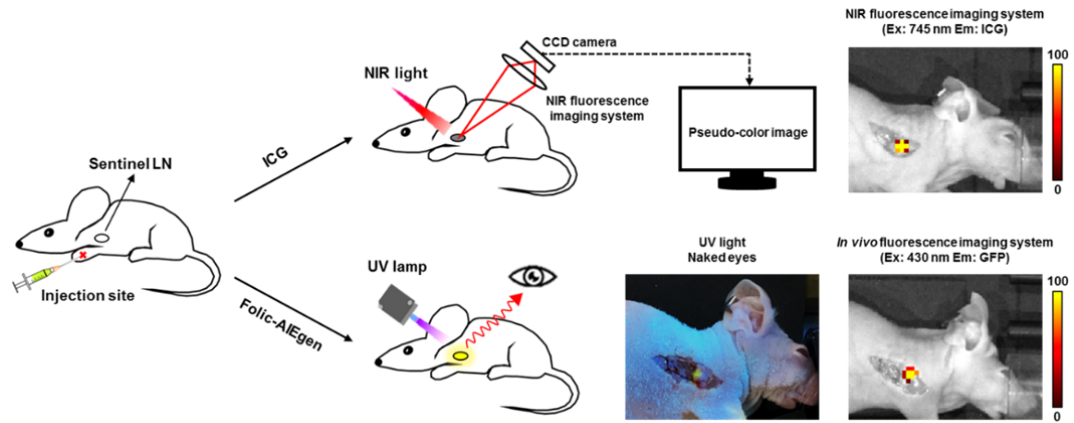
381

382

383

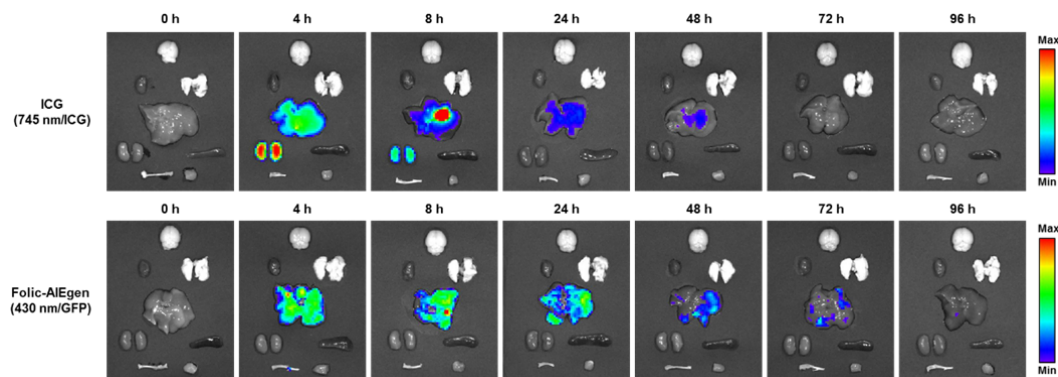
384

Supplementary Figure S14. Comparison of fluorescence characteristics and stability between ICG and folic-AIEgen. (a) UV-vis spectra and (b) fluorescence spectra of ICG and folic-AIEgen. (c) Time-dependent photographs (top) and fluorescence imaging (bottom) of ICG (250 $\mu\text{g}/\text{mL}$) and folic-AIEgen (40 $\mu\text{g}/\text{mL}$) in PBS at 37 $^{\circ}\text{C}$. (d) Quantitative analysis of the fluorescence signals of ICG and folic-AIEgen in fluorescence imaging. Data are means \pm SD, $n = 3$. (e) Time-dependent fluorescence imaging of ICG (NIR, 808 nm, 250 $\mu\text{g}/\text{mL}$) and folic-AIEgen (365 nm, 40 $\mu\text{g}/\text{mL}$) subjected to continuous laser irradiation. (f) Quantitative analysis of the fluorescence signals of ICG and folic-AIEgen in fluorescence imaging. Data are means \pm SD, $n = 3$.



385
 386
 387
 388
 389
 390
 391
 392
 393
 394

Supplementary Figure S15. Schematic illustration of ICG and folic-AIEgen guided SLN dissection in nude mice. NIR fluorescence imaging of ICG (250 µg/mL, 25 µL) administered mice with skin removal was imaged by NIR fluorescence imaging system. Photographs and fluorescence imaging of folic-AIEgen (40 µg/mL, 25 µL) administered mice with skin removal were recorded under UV light and IVIS imaging system, respectively.



395

396

397

398

399

400

401

402

403

404

Supplementary Figure 16. Biodistribution of ICG and folic-AIEgen in nude mice after intravenous administration. Ex vivo fluorescence imaging of major tissues (brain, heart, lung, liver, kidney, spleen, bone and muscle) of mice (n = 6) collected at different time points after intravenous injection with ICG (250 µg/ml, 100 µL) or folic-AIEgen (40 µg/ml, 100 µL). Images in different groups were required under the corresponding instrumental conditions (Ex: 745 nm/Em: ICG for ICG group, Ex: 430 nm/Em: GFP for folic-AIEgen group).