Dual Targeting Nanoparticles for Epilepsy Therapy

Qinghong Hou [a, b, c‡], Lulu Wang [d‡], Feng Xiao [a], Le Wang [a], Xiaoyan Liu [a], Lina Zhu [c], Yi Lu *[d], Wenfu Zheng *[b], Xingyu Jiang *[a]

[a] Qinghong Hou, Feng Xiao, Le Wang, Xiaoyan Liu, Prof. Xingyu Jiang

Shenzhen Key Laboratory of Smart Healthcare Engineering, Guangdong Provincial Key Laboratory of Advanced Biomaterials, Department of Biomedical Engineering, Southern University of Science and Technology, Shenzhen 518055, P. R. China.

E-mail: jiang@sustech.edu.cn.

[b] Qinghong Hou, Prof. Wenfu Zheng

CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for NanoScience and Technology, Beijing 100190, P. R. China

E-mail: zhengwf@nanoctr.cn

[c] Qinghong Hou, Prof. Lina Zhu

Department of Chemistry, School of Science, Tianjin University, Tianjin 300350, P. R. China

[d] Lulu Wang, Prof. Yi Lu

CAS Key Laboratory of Brain Connectome and Manipulation, the Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences; Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Shenzhen 518055, P. R. China.

E-mail: luyi@siat.ac.cn

Experimental Methods

Materials

PLGA (lactide:glycolide = 75:25) was from Evonik Industries (Germany). 1,2-Dihexadecanoylrac-glycero-3-phosphocholine (DPPC), cholesterol, distearoyl phosphoethanolamine (DSPE)-polyethylene glycol (PEG) 2k, and DSPE-PEG2k-Mal

29 were from Avanti Polar lipids (USA). Dimethylformamide, trifluoroethanol,

30 lamotrigine, pilocarcinine hydrochloride, and urethane were from Aladdin.

31 Neurobasal medium, B27 supplement, penicillin/streptomycin (PS), F12/DMEM

32 medium, FBS and 1×PBS (and 10×PBS) were from Gibco. DiR was from Invitrogen.

33 DAPI and kainic acid (KA) monohydrate were from Sigma. Cell viability test kit and

34 DiI were from Shenzhen Kailian Biotechnology Co., Ltd. GFAP was from Abcam.

35 NeuN was from Millipore. Alexa Fluor 594 affinpure goat anti-rabbit IgG (HþL) and

36 Alexa Fluor 488 affinpure goat anti-rabbit IgG (HþL) were from Jackson

37 ImmunoResearch.

Synthesis of DSPE-PEG2k-D-T7 and DSPE-PEG2k-Tet1

39 DSPE-PEG2k-D-T7 was synthesized by conjugating DSPE-PEG2k-Mal to a cysteine

40 conjugated on D-T7 peptide (D-T7-Cys). Briefly, D-T7-Cys reacted with DSPE-

41 PEG2k-Mal (5:1 molar ratio) at RT under mild shaking for 24 h in PBS (Na_2HPO_4

42 and EDTA, pH = 7.2). The unreacted D-T7 peptides and the salts in the reaction

43 system were removed by an Amicon Ultra-4 ultrafilter (MWCO = 3KD, Millipore).

44 The DSPE-PEG2k-Tet1 was prepared in the same way. The final solution was freeze-

45 dried and stored at -20 ^oC until further use Liquid chromatography-quadrupole

46 orbitrap mass spectrometer (LC-MS, Q Exactive) of DSPE-PEG2k-Mal, D-T7-Cys,

47 Tet1-Cys and DSPE-PEG2k-D-T7, DSPE-PEG2k-Tet1 were performed to confirm

48 the successful synthesis.

Preparation and characterization of lipids@PL NPs with different surface coatings

51 The design and fabrication method of the microfluidic chip was the same as the

52 previous work.[1] In short, the microfluidic chip consisted of two stages, the first stage

53 consisted of three liquid inlets and a straight reaction channel. The second stage

54 consisted of a central liquid inlet and a spiral mixing channel. The three inlet channels

55 of the first stage were 100 μm wide and 60 μm high. The width and height of the

56 central liquid inlet channel and the spiral mixing channel of the second stage was 300

57 μm and 60 μm respectively [2]. We synthesized LTG-loaded PLGA NPs (lipids@PL)

58 with different surface coatings using a microfluidic chip. (Scheme 1). PLGA was

59 dissolved in DMF and TFE (v/v = 7:3) to form a 10 mg/mL PLGA solution. The

60 initial concentration of LTG in PLGA solution was 238 μg/mL. For lipids@PL, the

61 lipid solution was composed of DPPC, cholesterol, DSPE-PEG with a molar ratio of

62 80:16:11, For D-T7-lipids@PL, the lipid solution was composed of DPPC,

63 cholesterol, DSPE-PEG2k, DSPE-PEG2k-D-T7 with a molar ratio of 80:16:9:2. For

64 preparing Tet1-lipids@PL, the lipid solution was composed of DPPC, cholesterol,

65 DSPE-PEG2k, DSPE-PEG2k-Tet1 with a molar ratio of 80:16:9:2. For preparing D-

66 T7/Tet1-lipids@PL NPs with different D-T7/Tet1 ratios (2:1, 1:1, and 1:2), the lipid

67 solutions were composed of DPPC, cholesterol, DSPE-PEG2k, DSPE-PEG2k-D-T7

68 and DSPE-PEG2k-Tet1 with a molar ratio of 80:16:8:2:1, 80:16:8:1:1, and

69 80:16:8:1:2 respectively. The synthesis process of the nanoparticles on the

70 microfluidic channels is shown in Scheme 1. The flow rate of PLGA and lipids was 3

71 mL/h, and the flow rate of H_2O was 120 mL/h.

72   The microfluidic chip was composed of two parts. The first part of the chip was

73 composed of three inlets and a straight synthesis channel which was divided into two

74 curved channels to symmetrically connect the central inlet of the second part. The

75 mixed solution of PLGA and LTG flowed into the central inlet and was precipitated in

76 the water flowing from the flanking inlets to form a hydrophobic PLGA@LTG (PL)

77 core in the first part. In the second part, a central inlet connected to the two curved

78 channels from the first part and formed a single channel which extended and formed a

79 double spiral channel connecting to an outlet. In the second part, through the

80 hydrophobic attraction between the tail end of the lipids and the PLGA, the lipid

81 monolayer shell was assembled on the surface of the PL core to synthesize lipids@PL.

82 Targeting peptides conjugated to DSPE-PEG2k including D-T7-DSPE-PEG2k, and

83 Tet1-DSPE-PEG2k mixed with the lipids at certain ratios were introduced into the

84 central inlet of the second part to form lipids@PL nanoparticles with different

85 targeting molecules. In addition, we also synthesized D-T7/Tet1-lipids@PL NPs

86 using conventional methods.[3]

87   Dynamic light scattering (DLS, Zetasizer 3000HS) and transmission electron

88 microscopy (TEM, HT-7700) were used to characterize the morphology, size and size

89 distribution, and surface charge of the nanoparticles. Each sample was tested three

90 times separately. The samples for TEM were diluted and negatively stained with 2%

91 uranyl acetate.

92 Encapsulation efficiency of LTG by D-T7/Tet1-lipids@PL NPs

93 To determine the encapsulation efficiency of LTG by D-T7/Tet1-lipids@PL NPs, we

94 prepared different concentrations of LTG (0, 0.3125, 0.625, 1.25, 2.5, 5, 10, 20, 40,

95 80 μg/mL), and established the standard curve of LTG concentration by a UV-VIS

96 spectrophotometer (UV-2600 Shimadzu) with the excitation wavelength at 306 nm.

97 The D-T7/Tet1-lipids@PL NPs were filtered by a microsep (MWCO=10 KD,

98 Millipore) after synthesized by the microfluidics. The concentration of LTG in the

99 NPs was determined by the UV spectrophotometer.

100 LTG release from the D-T7/Tet1-lipids@PL NPs

101 To study the release of LTG in various conditions, the free LTG and D-T7/Tet1-

102 lipids@PL(2:1) NPs (238 μg/mL LTG) were added to dialysis bags (MW=3 KD,

103 Millipore). The bags were placed into PBS (pH=7.2, 3 mL), followed by an

104 incubation for 72 h on a shaking table at 37°C. We collected the dialysate at each

105 predetermined time point (0, 3, 6, 9, 24, 48, 72 h), and moved the dialysis bags into

106 fresh PBS (3 mL). The concentration of LTG was determined using a UV

107 spectrophotometer with the excitation wavelength at 306 nm. The cumulative release

108 rate at each time point was calculated according to the formula: release rate at time i

109 (%) = (sum of LTG concentration before i)/ (sum of LTG concentration at 72 h)

110 ×100%.

Quantification of encapsulation efficiency of DSPE-PEG-D-T7 and DSPE-PEG-Tet1 in D-T7/Tet1-lipids@PL NPs

To quantitively determine the encapsulation efficiency of DSPE-PEG-D-T7 and DSPE-PEG-Tet1 in the D-T7/Tet1-lipids@PL NPs, we labeled D-T7 peptide and Tet1 peptide with rhodamine B (RHB) and FITC respectively. We prepared different concentrations of LTG (0, 2.5, 5, 10, 50, 100, 200 μg/mL), and established the standard curve of D-T7-RHB and Tet1-FITC concentrations by a UV-vis spectrophotometer (UV-2600 Shimadzu) with the excitation wavelength at 554 nm and 488 nm, respectively. The D-T7/Tet1-lipids@PL NPs were filtered by a microsep (MWCO=10 KD, Millipore) after synthesized by the microfluidics. The concentration of D-T7 and Tet1 in the NPs was determined by the UV-vis spectrophotometer.

Cells and animals

Human neuroblastoma cells (SH-SY5Y) were from Kunming Cell Bank. Mouse cerebrovascular endothelioma cells (bEnd.3, KG593) were from Shenzhen Btek Technology Co., LTD. For cell culture, SH-SY5Y cells were cultured in RPMI 1640 medium (15-20 % FBS, 1% penicillin/streptomycin). bEnd.3 cells were cultured in DMEM (10 % FBS, 1% penicillin/streptomycin). For primary culture of hippocampal neurons, the neonatal rats of 0-3 days were decapitated.[4] The brain was taken out and placed in pre-cooled PBS. The hippocampus was extracted and digested by 0.25% trypsin in a 37 °C water bath for 15 min. The dissociated neurons were inoculated in confocal dishes or 12-well plate with DMEM/F12 medium (10 % FBS, 1 % penicillin/streptomycin). After the cells adhered to the wall, the F12 medium was replaced with the neurobasal medium (2 % B27, 1 % penicillin/streptomycin). The medium was changed every three days.

All protocols of animal experiments are approved by the Institutional Animal Care and Use Committee at the Southern University of Science and Technology (resolution number: SUSTC-JY2020124). All animals including male BALB/c nude mice and

138 male/female C57BL/6 mice (6-8 weeks) and neonatal rats were from Guangdong

139 Medical Laboratory Animal Center (Guangzhou, China).

Cellular internalization

141 For cellular uptake study, hippocampal neurons, bEnd.3 cells and SY5Y cells were

142 seeded into the confocal dishes with a density of 1×10^5 cells per dish, followed by the

143 addition of DiI-labelled nanoparticles. After co-incubation for 6 h at 37°C, the cells

144 were rinsed with pre-warmed PBS three times, and were fixed with 4% PFA for 15

145 min at 37°C. The cells were treated with PBS three times for 5 min each. The nuclei

146 were stained with DAPI for 10 min. The neurons were visualized by staining the

147 tubulin, whereas the SY5Y cells were visualized by staining the phalloidin. After

148 washing with PBS three times for 5 min each, we imaged the cells using a confocal

149 laser scanning microscopy (CLSM, A1, Nikon).

150 Uptake of DiI-labelled nanoparticles by hippocampal neurons, bEnd.3 cells and

151 SY5Y cells was quantified by a flow cytometry (FACScanto, BD Biosciences). The

152 hippocampal neurons, bEnd.3 cells and SY5Y cells were seeded in twelve-well plates

153 with a density of 1×10^5 cells per well. The cells were treated with DiI-labelled

154 nanoparticles for 6 h. The cells were washed with pre-warmed PBS three times, and

155 collected after trypsin treatment. Cells were quantitatively analyzed by the flow

156 cytometry.

Penetration experiment of D-T7/Tet1-lipids@PL NPs in *in vitro* BBB model

158 The hippocampal neurons were seeded into the lower chamber Transwell (Corning)

159 with a density of 5×10^4 cells per dish, and the bEnd.3 cells were seeded on 8.0 μm PC

160 membrane with a density of 1×10^5 cells. The penetration experiment of nanoparticles

161 started after the transendothelial electrical resistance (TEER) reached a stable state of

162 about 80 Ω. After co-cultivating various nanoparticles in the BBB model for 6 hours,

163 we rinsed the neurons three times with pre-warmed PBS, and collected the cells after

164 trypsin treatment. The cells were quantitatively analyzed by the flow cytometry.

165 ***In vivo* fluorescence imaging**

166 In order to explore the transcytosis ability and accumulation capacity of the BBB,

167 living fluorescence imaging technology (IVIS Spectrum, PerkinElmer) was used to

168 monitor the biodistribution of a near infrared (NIR) dye (DiR)-labelled nanoparticles

169 in nude mice. IVIS® Lumina Series III Imaging System can be used in two modes:

170 bioluminescence and fluorescence. We used the fluorescence mode in this study. The

171 focal length of the lens was 50 □m, and the light transmittance was 95%. The field of

172 view ranged from 5×5 cm to 12.5×12.5 cm. The lens size of the charge coupled

173 device (CCD) was 13.3×13.3 mm with a 1 million pixel. The imaging parameters we

174 set were: the field of view was 12.5×12.5 cm; the wavelength of excitation filter was

175 740 nm, the wavelength of emission filter was 790 nm; the binning factor was 4; the

176 luminescent exposure time was 0.5s; the subject height was 1.5cm; the f number was

177 2. The maximum excitation wavelength and emission wavelength of DiR was 748 nm

178 and 780 nm respectively. The nude mice were injected with DiR-labelled

179 nanoparticles (DiR=0.75 mg/mL) *via* tail vein, respectively. Fluorescence images

180 were obtained at 0.5, 2, 4, 6, 12, and 24 h. We selected the brain and the whole body

181 ROIs respectively, and measured the corresponding fluorescence intensity. The mice

182 were sacrificed 24 h after the injection, and their organs were collected and imaged by

183 using the *in vivo* imaging system.

184 **Immunohistochemistry**

185 PBS and various DiI-labelled D-T7/Tet1-lipids@ PL were injected to seven groups of

186 C57BL/6 mice (n = 3) *via* tail vein. The mice were sacrificed 6 h later. After

187 perfusion, the brain tissue was removed and immersed in paraformaldehyde (4% w/v).

188 A Leica CM1950 cryostat microtome was used to slice the brain into 35-μm thick

189 coronal slices. The slices were treated with PBS three times for 5 min each and

190 blocked in 10% normal goat serum for 1 h. The samples were then incubated with

191 primary antibodies (GFAP; NeuN) overnight at room temperature, washed with PBS

192 and incubated with secondary antibodies (Alexa Fluor 594-conjugated affinpure goat

anti-rabbit IgG (HþL); Alexa Fluor 488 affinpure goat anti-rabbit IgG (HþL) for 1 h. After washing with PBS three times for 5 min each, the sections were mounted onto gelatin-coated slides and cover slipped with a signal enhancer (ProLong Gold Antifade Reagent with DAPI). In addition, we performed immunofluorescence staining of blood vessels in the D-T7/Tet1-lipids@PL(2:1)-treated brains using CD31 antibody. The mounted slices were observed and imaged using an Olympus slide scanner (VS120).

Cytotoxicity assay

The cytotoxicity of various lipids@PL nanoparticles was assessed by CCK-8 assay. The hippocampal neurons and SY5Y cells were seeded into 96-well plates with a density of 5×10^4 cells per well in the neurobasal medium for 24 h prior to the experiment. The culture medium was substituted with 100 μL lipids@PL with different surface modification ligands with serial concentrations from 62.5 μg/mL to 1000 μg/mL for 24 h. Each concentration was replicated in 5 wells. the medium in each well was replaced with freshly medium with 1% CCK-8 for 1 h. the absorbance was measured by a microplate reader (Spark, Tecan) at 450 nm.

We used cell viability assay kit to characterize cytotoxicity. Neurons were seeded into confocal dishes with a density of 1×10^5 cells per dish, 100 μL lipids@PL nanoparticles with different surface modification ligands (1000 μg/mL) were added. After co-incubation for 24 h at 37 °C, the cells were washed with pre-warmed PBS two times, cell viability assay kit was used to characterize the toxicity of the nanoparticles to the neurons, and the cells were imaged using a confocal microscopy.

Hemolysis test

Mouse whole blood was collected in a heparin-treated centrifuge tube and diluted with PBS. The diluted blood was centrifuged at 2000 rpm for 5 min and the supernatant was removed. The red blood cells were suspended in PBS (pH 7.4) at a final concentration of 2 %. Subsequently, the cell suspension was incubated with

220 purified water (positive control), PBS (negative control) and nanoparticles of different

221 concentrations (62.5, 250, and 1000 μg/mL) at 37 °C for 4 h. The red blood cells were

222 centrifuged at 2000 rpm for 5 min, and 100 μL supernatant was transferred to a 96-

223 well plate. The sample was measured at 490 nm with a microplate reader (Spark,

224 Tecan).

In vivo electrophysiological recording for KA-induced acute epilepsy model

226 For the construction of KA-initiated epilepsy model, C57BL/6J mouse was

227 anesthetized with urethane (1.5 g/kg), and the head was fixed in a standard stereotaxic

228 frame (RWD). The cranium was exposed through a small midline scalp incision.

229 Holes were drilled through the skull, and a drug delivery electrode array was directed

230 towards the brain at the following stereotaxic coordinates: the electrode tip at

231 anteroposterior (AP) -2.06 mm, mediolateral (ML) -1.35 mm and dorsoventral (DV) -

232 2.10 mm for recording in the dorsal hippocampus (dHPC); The silica tubing at AP -

233 2.06 mm, ML -1.80 mm and DV -1.60mm for drug delivery in the dorsal

234 hippocampus. KA (0.3 mg/mL in PBS solution) was unilaterally injected into the

235 dorsal hippocampus via the drug delivery electrode array using a micropump.

236   Electrophysiological recordings were performed with a 64-channel neural

237 acquisition processor (Plexon). The local field potentials (LFPs) were sampled at 1

238 kHz, and bandpass was filtered at 1-500 Hz. Neural signal analyses were performed

239 using a Plexon NeuroExplorer software and a custom-designed Matlab (Mathworks)

240 code. Phosphate-buffered saline (PBS), LTG (2 mg/kg), lipids@PL (2 mg/kg), Tet1-

241 lipids@PL (2 mg/kg), D-T7-lipids@PL (2 mg/kg), D-T7/Tet1-lipids@PL (2:1) (2

242 mg/kg) and D-T7/Tet1-lipids@PL (2:1) (5 mg/kg) were intravenously injected 2 h

243 before the establishment of the KA model. LFPs were monitored and recorded in the

244 dorsal hippocampus of the mice after KA injection, and the signals were recorded for

245 2.5 h by the neural acquisition processor.

In vivo behavior recording for pilocarpine-induced acute epilepsy model

247 For the construction of pilocarpine-initiated epilepsy model, C57BL/6 mice were

248 divided into 4 groups, and injected intraperitoneally with methylscopolamine (1

249 mg/kg). 30 min later, pilocarpine (320 mg/kg) was intraperitoneally injected.

250 Approximately 10 min after the administration, persistent seizures were induced. We

251 injected PBS, lipids@PL (2 mg/kg), and D-T7/Tet1-lipids@PL(2:1) (2 mg/kg)

252 intravenously respectively 2 h before the construction of the model. We recorded the

253 real-time epileptic behavior for 40 min, 1.5 h, and 4 h by video.

254 **Treatment of D-T7/Tet1-lipids@PL NPs in a KA-induced chronic epilepsy model**

255 C57 BL/6J mice of 6-8 weeks were used for constructing chronic epilepsy models.

256 KA (0.3 mg/mL, 550 nL) was injected into the right dorsal hippocampus (AP -2.06

257 mm, ML -1.80 mm, DV -1.80 mm) *via* a 33-gauge metal needle connected with a

258 micro-syringe pump (UMP3/Micor4). Electrophysiological recordings and

259 immunohistochemistry were performed in epileptic mice treated with PBS, LTG, D-

260 T7/Tet1-lipids@PL(2:1) NPs for 6 weeks.

261 **Safety evaluation**

262 After the experiment, the mice were sacrificed and dissected, and the heart, liver,

263 spleen, lung, kidney, and brain were sliced for hematoxylin-eosin (H&E) staining. In

264 addition to H&E staining, blood biochemical indicators markers were evaluated. We

265 took 500 μL of blood from the eyeball, at 3500 rpm, 4°C, centrifuged for 5 min, took

266 out 100 μL of serum and used the automatic biochemical analyzer (MS-480) to test

267 the blood biochemical indicators. The parameters tested in this study included

268 glutamic-pyruvic transaminase (ALT), glutamic oxalacetic transaminase (AST),

269 (alkaline phosphatase) ALP, total protein (TP), serum albumin (ALB), carbamide

270 (urea). Similarly, take 100 μL of whole blood from the eyeball for routine blood

271 testing. The parameters tested in this study included white blood cells (WBC), red

272 blood cell (RBC), hemoglobin (HGB), mean RBC hemoglobin (MCH), red cell

273 distribution width (RDW), platelets (PLT), mean platelet volume (MPV), mean

274 corpusular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV).

275 **Statistics**

276 The data were expressed as mean ± SD. The statistical analysis of different

277 experimental groups was performed using Student's *t* test. $P<0.05$, $P<0.01$, $P<0.001$

278 were used as the standards of statistical significance.

279 **Results and Discussion**

D-T7 peptide
Sequence: D-HRPYIAHC

Tet1 peptide
Sequence: HLNILSTLWKYRC

DSPE-PEG2000-Mal

DSPE-PEG2000-D-T7

DSPE-PEG2000-Tet1
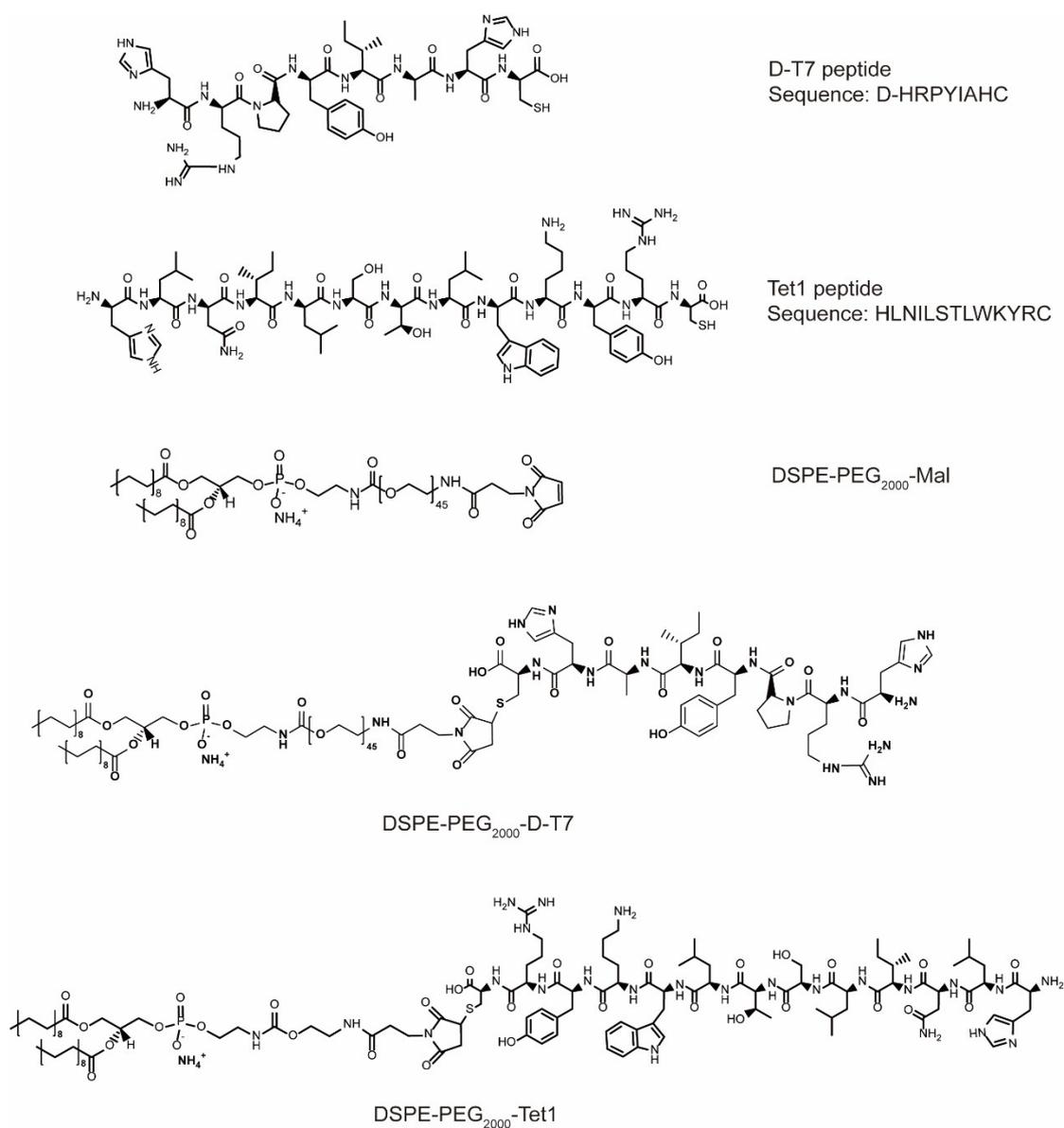
280

281 **Figure S1** Structural formula of the peptides and the DSPE-PEG-peptides.
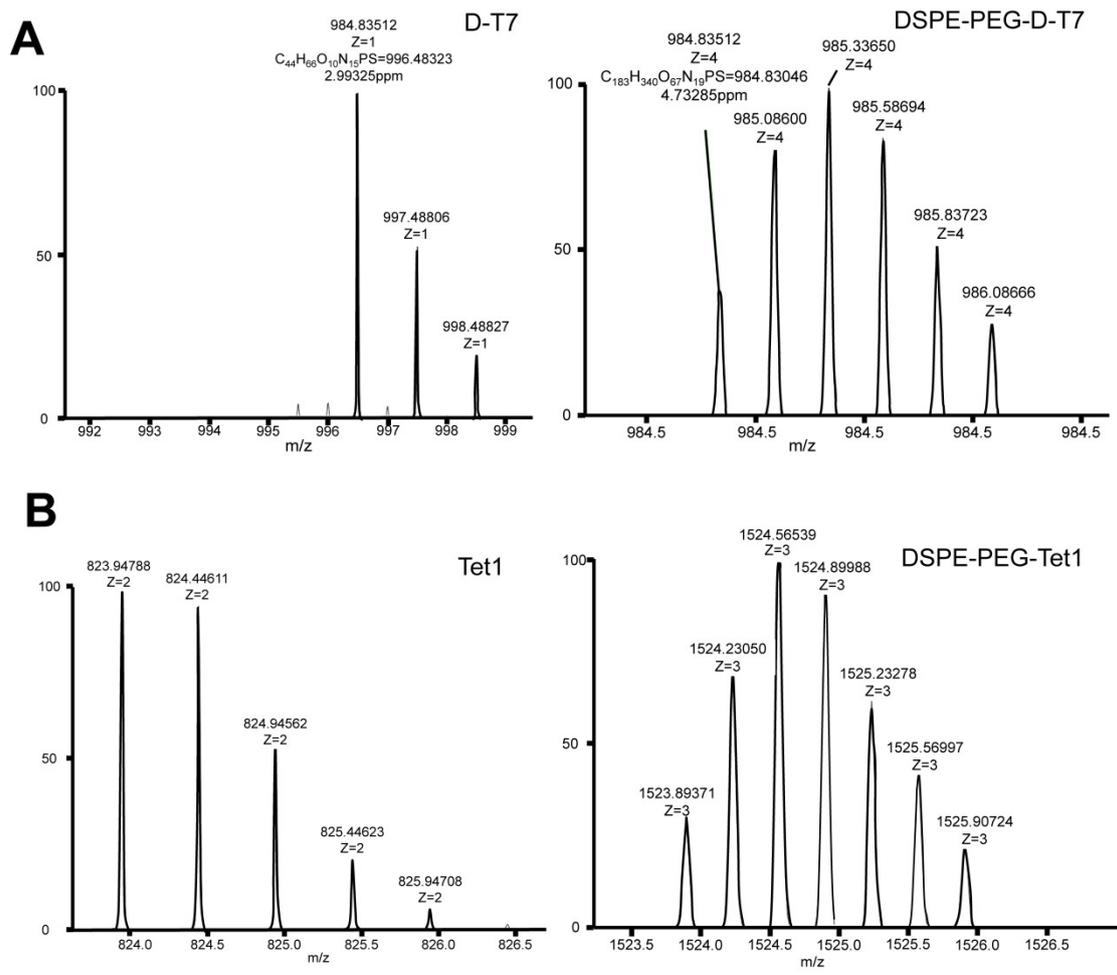
Figure S2 Mass spectra of DSPE-PEG-peptides. (A) Mass spectra of D-T7 and DSPE-PEG-D-T7. (B) Mass spectra of Tet1 and DSPE-PEG-Tet1.

A

	lipids@PL	D-T7-lipids@PL	Tet1-lipids@PL
Size (d.nm)	40.67±0.61	68.78±0.75	71.7±2.17
PDI	0.37±0.0068	0.24±0.006	0.37±0.015
Zeta	-28.70±1.91	-17.8±1.74	-16.3±0.9
	D-T7/Tet1-lipids@PL (1:1)	D-T7/Tet1-lipids@PL (1:2)	D-T7/Tet1-lipids@PL (2:1)
Size (d.nm)	58.89±0.88	71.86±0.31	68.93±0.59
PDI	0.22±0.0090	0.24±0.068	0.26±0.0053
Zeta	-10.48±2.08	-15.7±2.32	-15.17±0.63

D

	Size (d.nm)	PDI
Microfluidic technology	68.93 ±0.59	0.26 ±0.0053
Traditional method	124.30 ±1.18	0.12 ±0.014

285

286 **Figure S3** Characterization of various nanoparticles. (A-B) DLS characterization of
287 D-T7-Lipids@PL, Tet1-Lipids@PL, and D-T7/Tet1-Lipids@PL with different D-
288 T7/Tet1 ratios (1:2, 1:1, and 2:1). (C-D) DLS characterization of D-T7/Tet1-
289 lipids@PL(2:1) synthesized by microfluidic chip and traditional method.

290
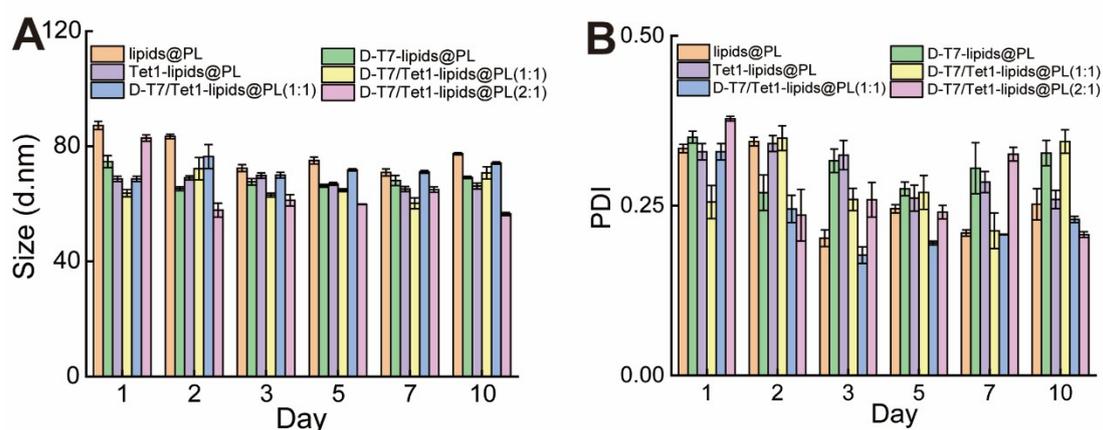
Figure S4 Stability test of various nanoparticles in 10 days characterized by DLS.

292
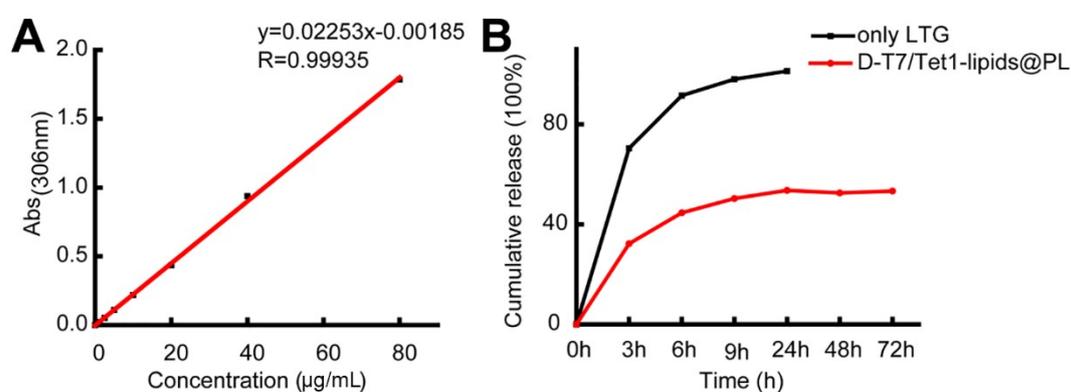
Figure S5 Drug release test of D-T7/Tet1-lipids@PL(2:1) NPs. (A) The standard curve of LTG absorbance at 360 nm. (B) Cumulative drug release of D-T7/Tet1-lipids@PL(2:1) NPs in PBS (pH 7.4).

	The content of DSPE-PEG-D-T7 in NPs solution (μg/mL)	The content of DSPE-PEG-D-Tet1 in NPs solution (μg/mL)	DSPE-PEG-D-T7 : DSPE-PEG-Tet1 (mol:mol)
D-T7-lipids@PL	104.46		
Tet1-lipids@PL		81.36	
D-T7/Tet1-lipids@PL(1:2)	22.84	65.82	1: 2.47
D-T7/Tet1-lipids@PL(1:1)	43.19	36.44	0.94: 1
D-T7/Tet1-lipids@PL(2:1)	57.33	43.05	1.55: 1

296
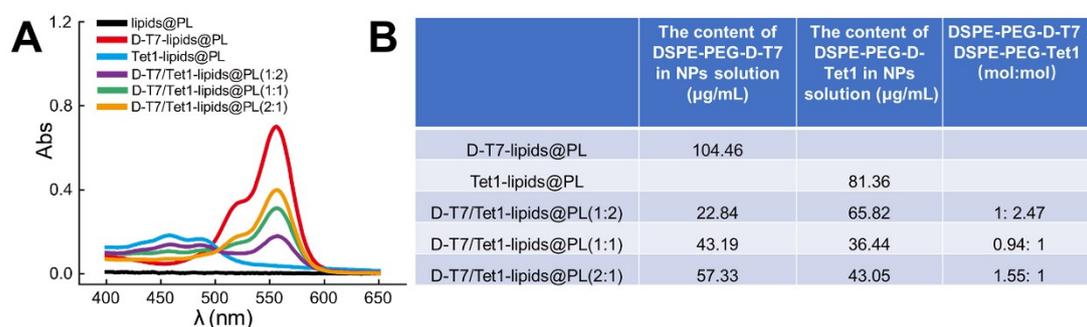
Figure S6 UV-vis spectra to characterize the DSPE-PEG-D-T7 and DSPE-PEG-Tet1 in various nanoparticles. A) UV-vis spectra of various NPs. B) The quantified data of each NP based on the UV-vis spectra.
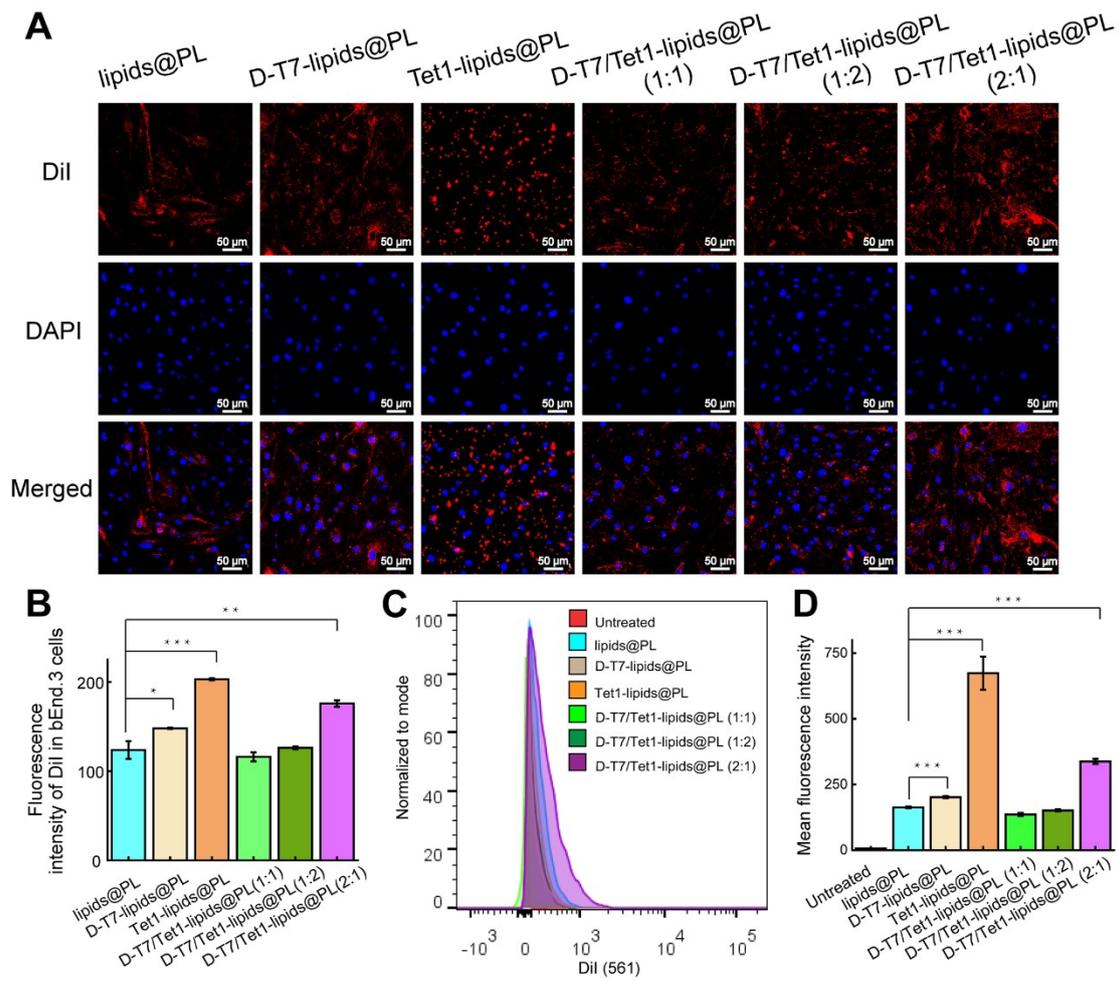
Figure S7 *In vitro* uptake of various NPs with different D-T7/Tet1 concentration titrations by bEnd.3 cells. (A) Confocal microscopy images of bEnd.3 cells incubated with various nanoparticles at 37 °C for 6 h. The nanoparticles are labeled by DiI. Nuclei are stained by DAPI (blue). Scale bars, 50 μm. (B) Quantitative analysis of the confocal fluorescence images of the nanoparticles internalized in the bEnd.3 cells using Image J. (C) Flow cytometry histogram of bEnd.3 cells incubated with various nanoparticles at 37 °C for 6 h. DiI is used to label the nanoparticles. (D) Data from the flow cytometry which shows the mean fluorescence intensity of the bEnd.3 cells incubated with various nanoparticles at 37 °C for 6 h. The nanoparticles are labelled by DiI. * $P<0.005$, *** $P<0.001$.
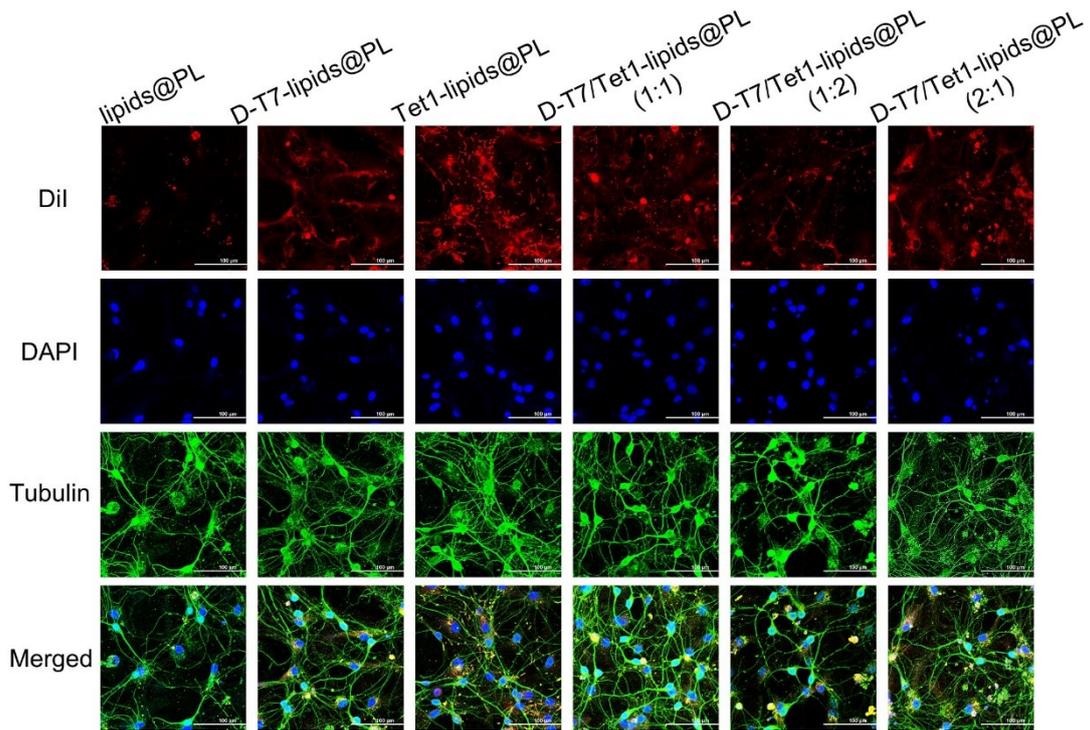
Figure S8 Confocal microscopy images of neurons incubated with various nanoparticles at 37 °C for 6 h. The nanoparticles were labeled by DiI. The nuclei were stained by DAPI (blue). The neurons were stained by tubulin (Green). Scale bars, 100 μm.

316
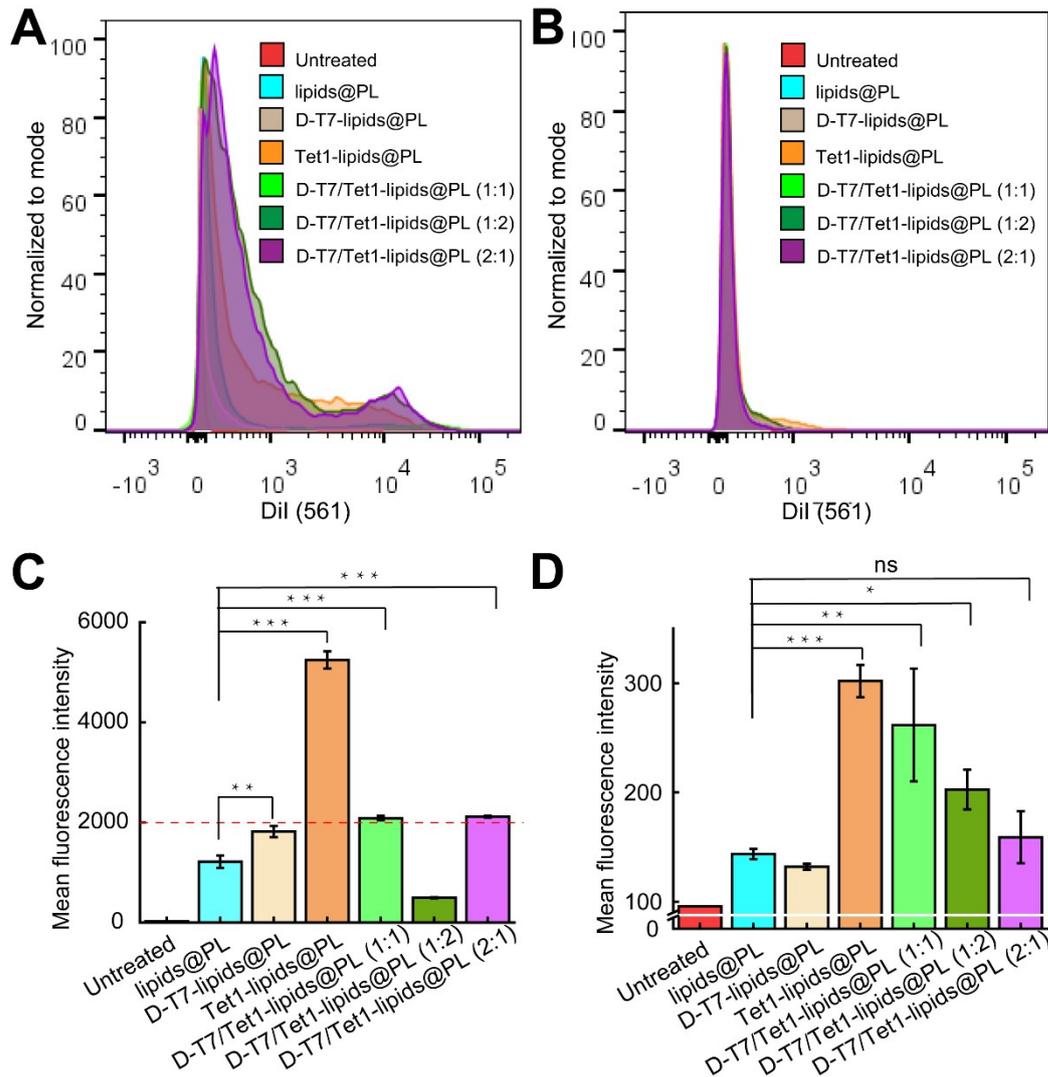
Figure S9 *In vitro* internalization of various NPs with different D-T7/Tet1

concentration titrations in hippocampal neurons and SY5Y cells. Flow cytometry

histogram of hippocampal neurons A) and SY5Y cells B) incubated with

nanoparticles at 37 °C for 6 h. DiI is used to label the nanoparticles. Data from flow

cytometry show the mean fluorescence intensity of the hippocampal neurons C) and

SY5Y cells D) incubated with nanoparticles at 37 °C for 6 h. * *P*<0.05, ** *P*<0.01,

*** *P*<0.001.

324
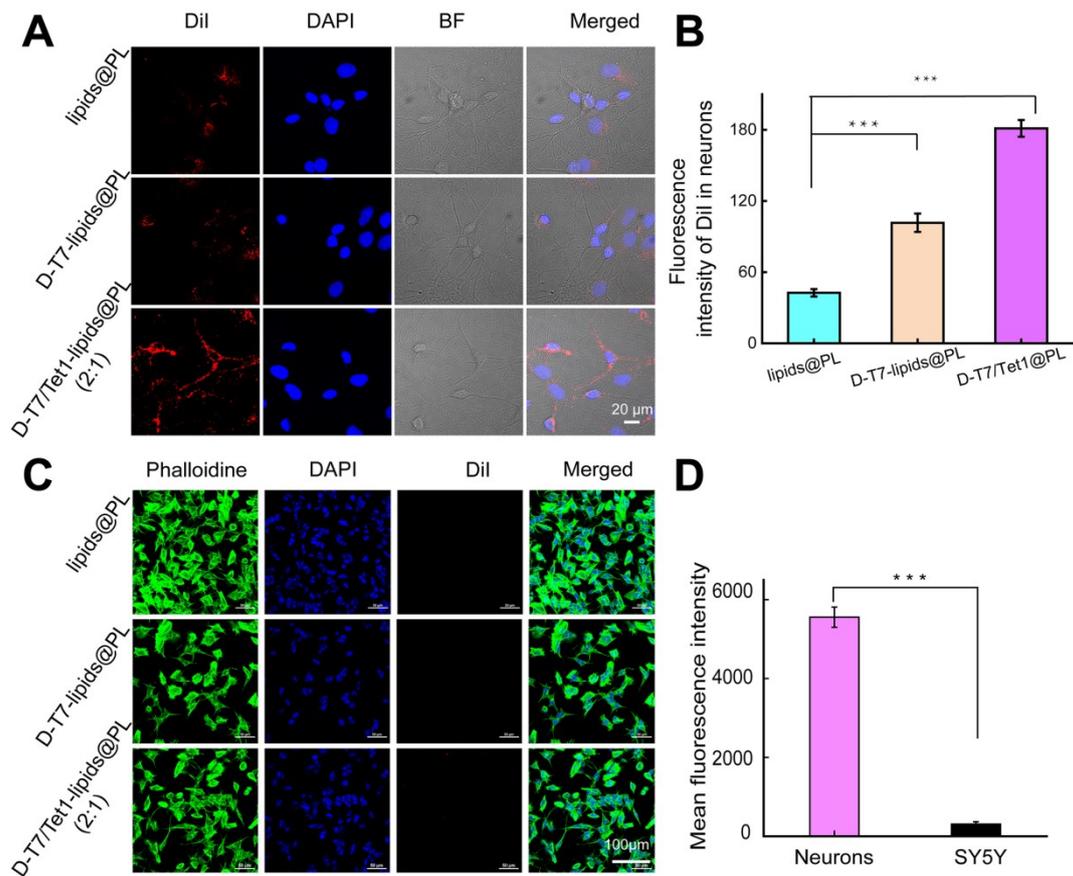
Figure S10 *In vitro* internalization of NPs by hippocampal neurons and SY5Y cells.
(A) Confocal microscopy images of hippocampal neurons incubated with lipids@PL,
D-T7-lipids@PL, and D-T7/Tet1-lipids@PL (2:1) at 37 °C for 6 h. The nanoparticles
are labeled by DiI. The cell nuclei are stained by DAPI (blue). Scale bars, 20 μm. (B)
Quantitative analysis of the fluorescence of the nanoparticles internalized in the
hippocampal neurons using Image J. (C) Confocal microscopy images of the SY5Y
cells incubated with various nanoparticles at 37 °C for 6 h. The nanoparticles are
labeled by DiI. The cell nuclei are stained by DAPI (blue). The SY5Y cells are
stained by phalloidin (green). Scale bars, 100 μm. (D) Data from flow cytometry
which shows the mean fluorescence intensity of the hippocampal neurons and SY5Y
cells incubated with the nanoparticles at 37 °C for 6 h. The nanoparticles are labelled
by DiI. *** *P*<0.001.

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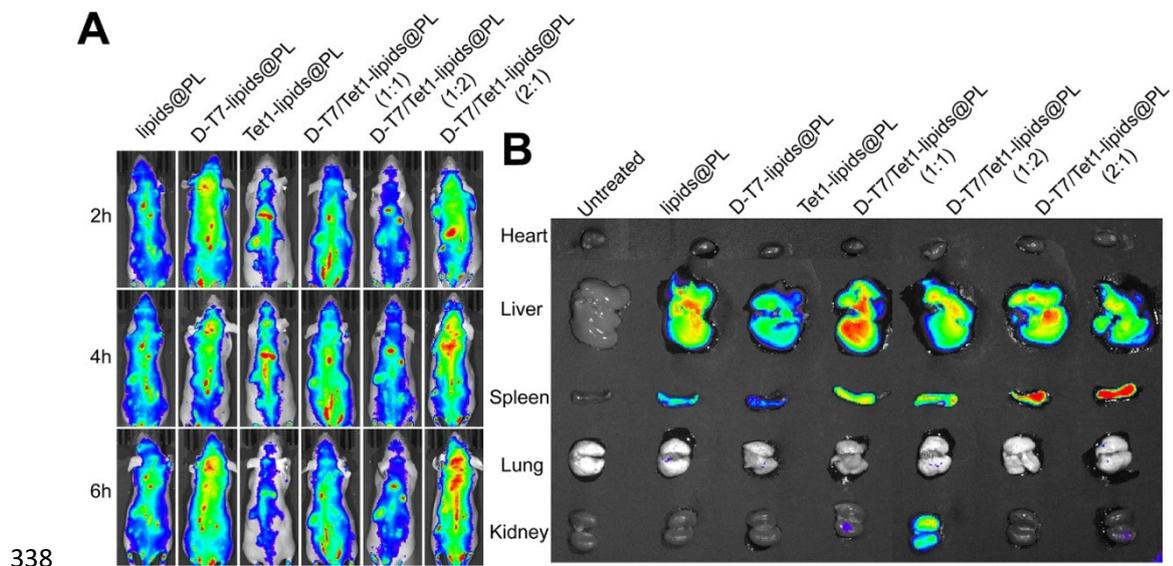
Figure S11 Fluorescence distribution of different NPs *in vivo*. (A) *In vivo* imaging shows the distribution of different NPs with different D-T7/Tet1 concentration titrations in mice at different time points. (B) Fluorescent imaging of the organs *ex vivo* 24 h after injection of DiR-labelled NPs.

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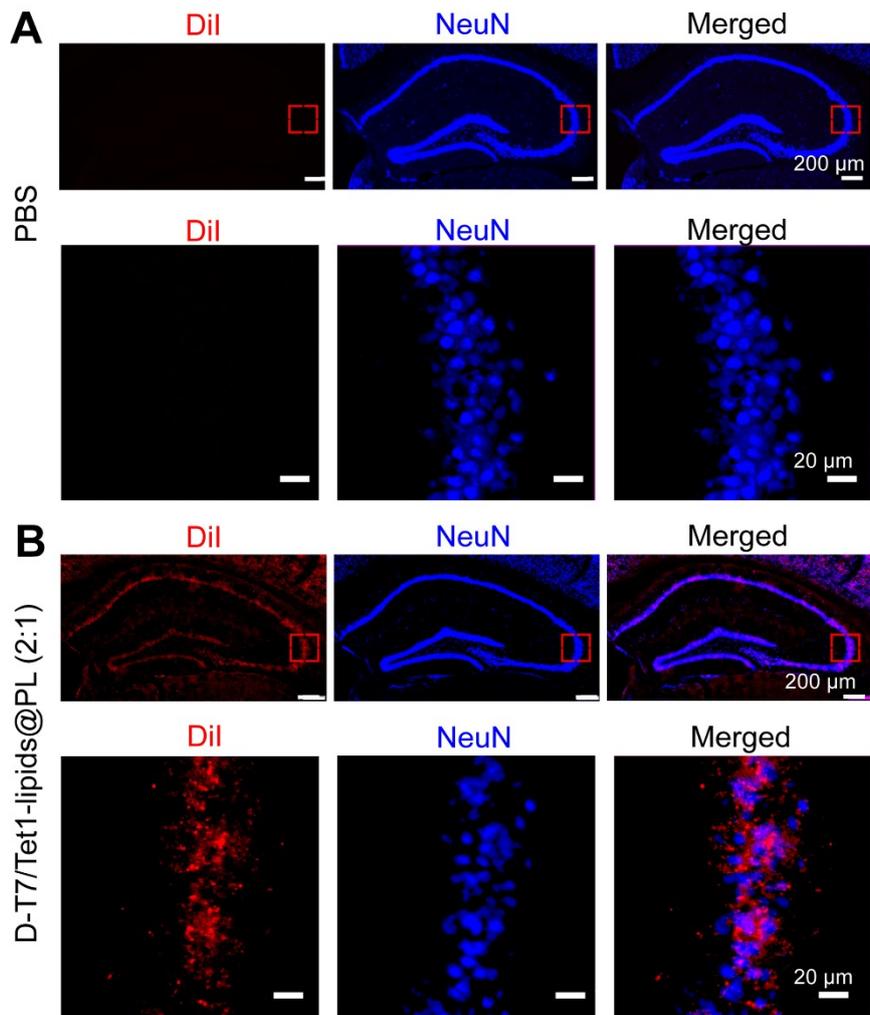
Figure S12 Immunofluorescence of mice brain dorsal hippocampus (dHPC) treated with D-T7/Tet1-lipids@PL(2:1) NPs labelled by DiI. The neuron nuclei are stained by NeuN (blue). Scale bar, 200 μm and 20 μm.
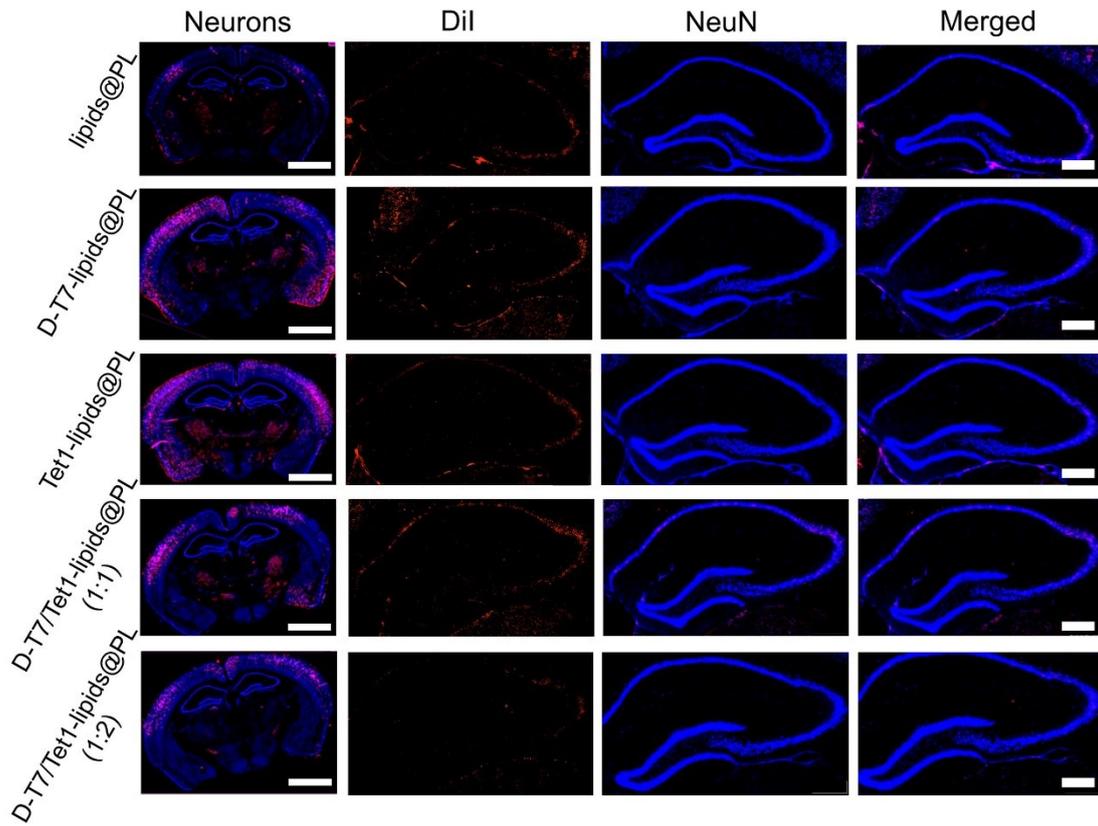
348
349 **Figure S13** Immunofluorescence of mice brain dorsal hippocampus (dHPC) treated

350 with various NPs labelled by DiI. The neuron nuclei were stained by NeuN (blue).

351 Scale bar, 2 mm and 200 μm.
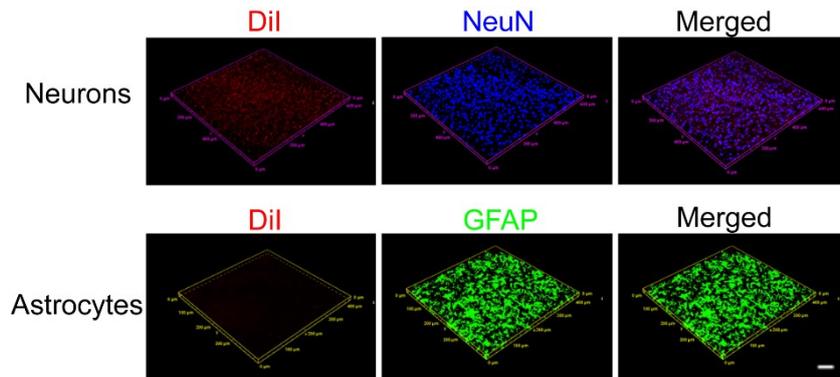
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353 **Figure S14** 3D images of immunofluorescence of mice brain treated with D-T7/Tet1-

354 lipids@PL(2:1) labelled by DiI. The neuron nuclei are stained by NeuN (blue), the

355 astrocytes are stained by GFAP (green). Scale bar, 100 μm.

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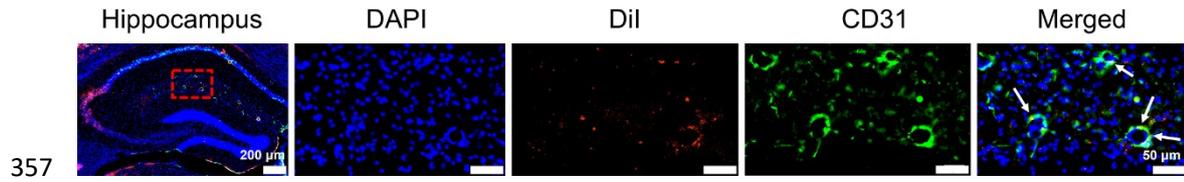
Figure S15 Immunofluorescence of mice brain treated with D-T7/Tet1-lipids@PL(2:1) NPs labelled by DiI. The cell nuclei were stained by DAPI (blue). The vascular endothelial cells were stained by CD31 antibody. Scale bar, 200 μm and 50 μm.
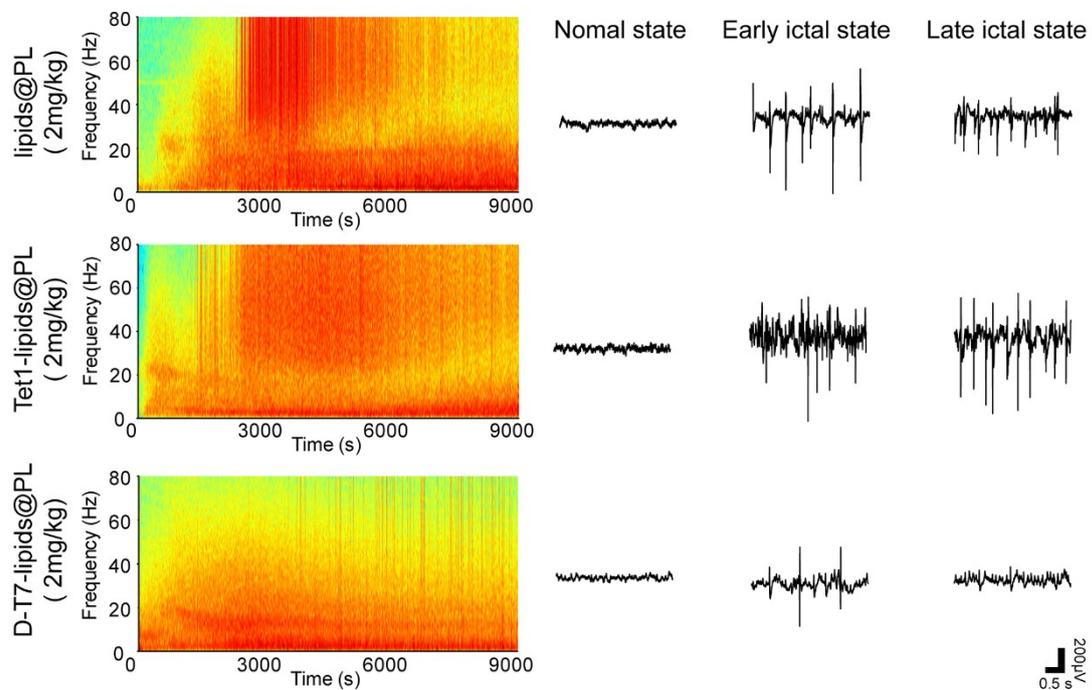
Figure S16 A 2.5-hour spectrogram of LFPs recorded in the dorsal hippocampus after modeling. Raw LFPs are recorded from the representative channels in the dorsal hippocampus. Representative raw LFPs of normal state, early ictal state and late ictal state are recorded in the dorsal hippocampus of mice after KA injection.
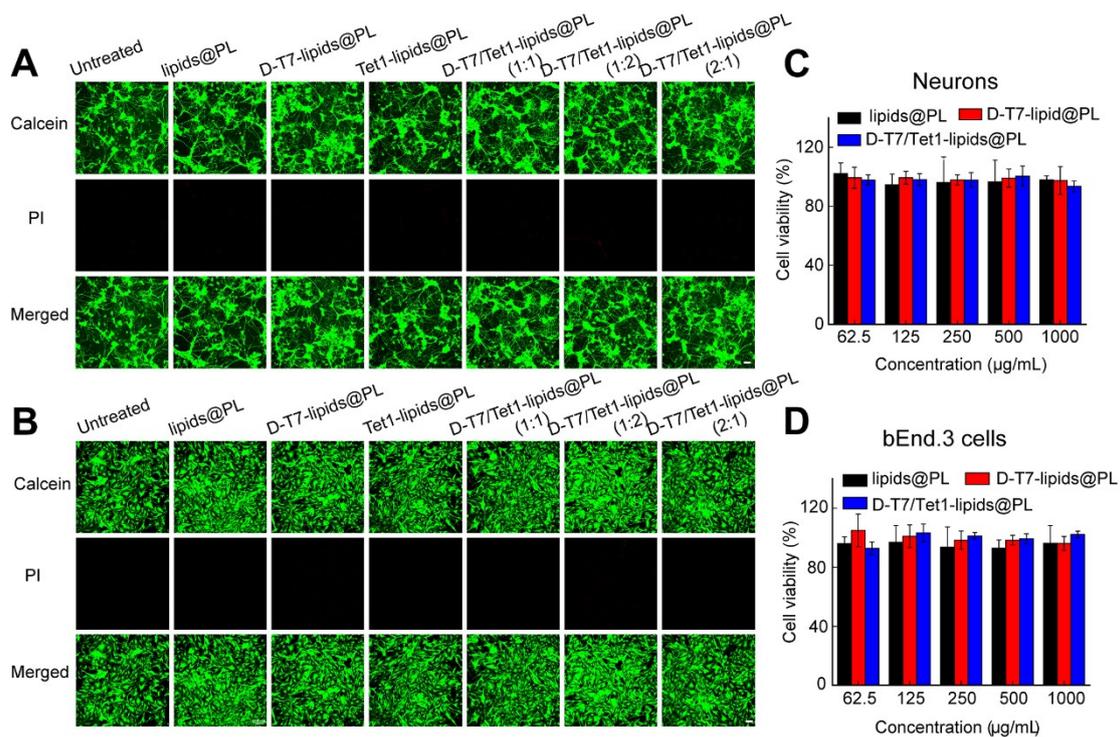
Figure S17 Cytotoxicity assay of the nanoparticles. Confocal microscopy image of hippocampal neurons A) and bEnd.3 cells B) co-cultured with various nanoparticles for 12 hours. The cells are stained by Calcein (green)/PI (red). Scale bar, 100 μm. The cell viability of hippocampal neurons C) and bEnd.3 cells D) after 24 h of incubation with the nanoparticles tested by CCK8 assay.
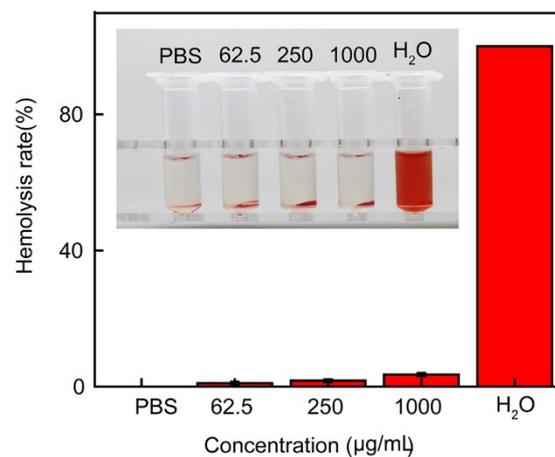
Figure S18 Hemolysis test of D-T7/Tet1-lipids@PL (2:1) nanoparticles at different concentrations. (n = 3).

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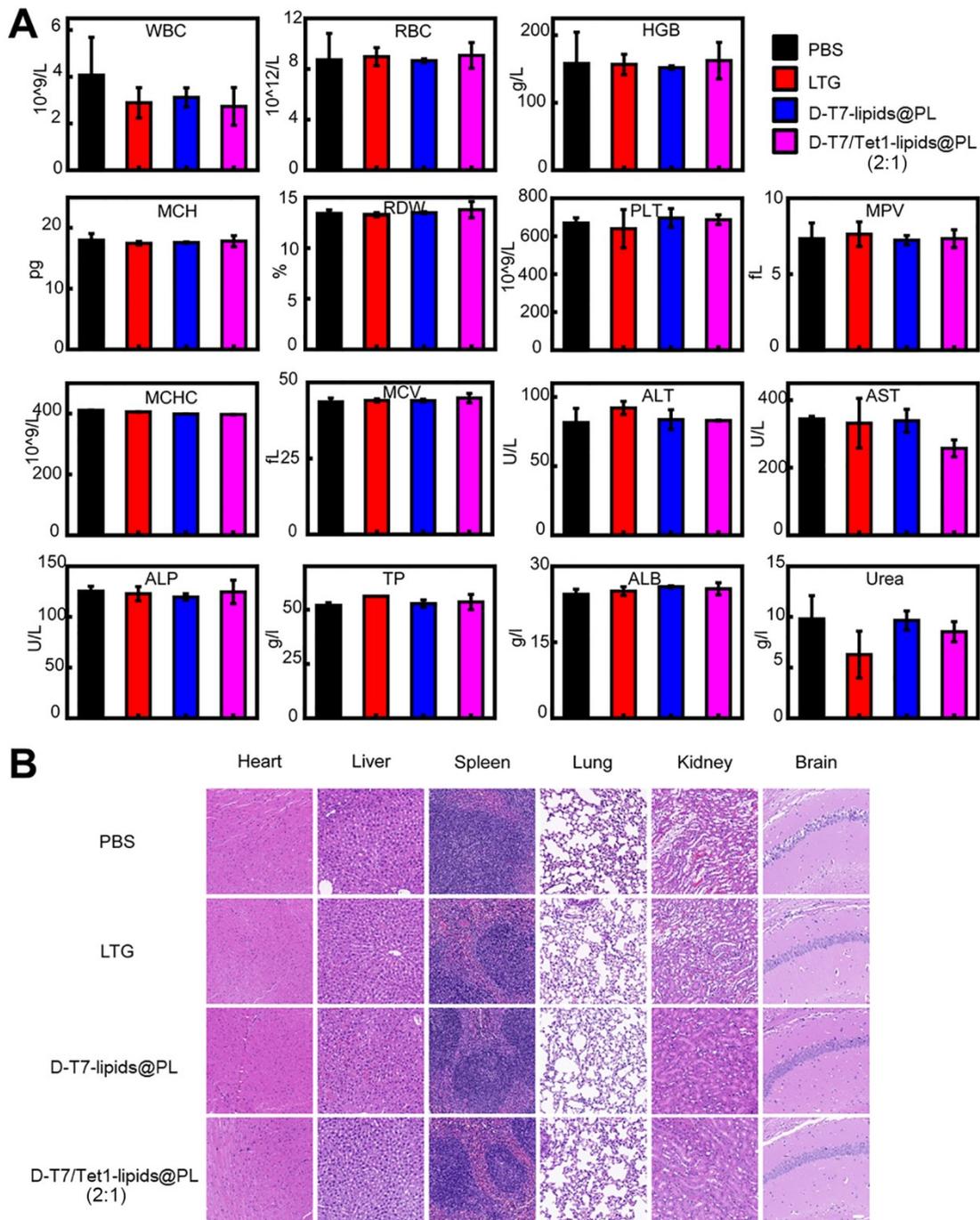
Figure S19 Biocompatibility test of various nanoparticles in an acute epilepsy mice model. (A) *In vivo* biosafety evaluation of various nanoparticles assessed by hematological markers and biochemical parameters in blood. (B) *In vivo* biosafety of various nanoparticles assessed by histopathological analysis. Scale bar, 50 μm. (n = 3).

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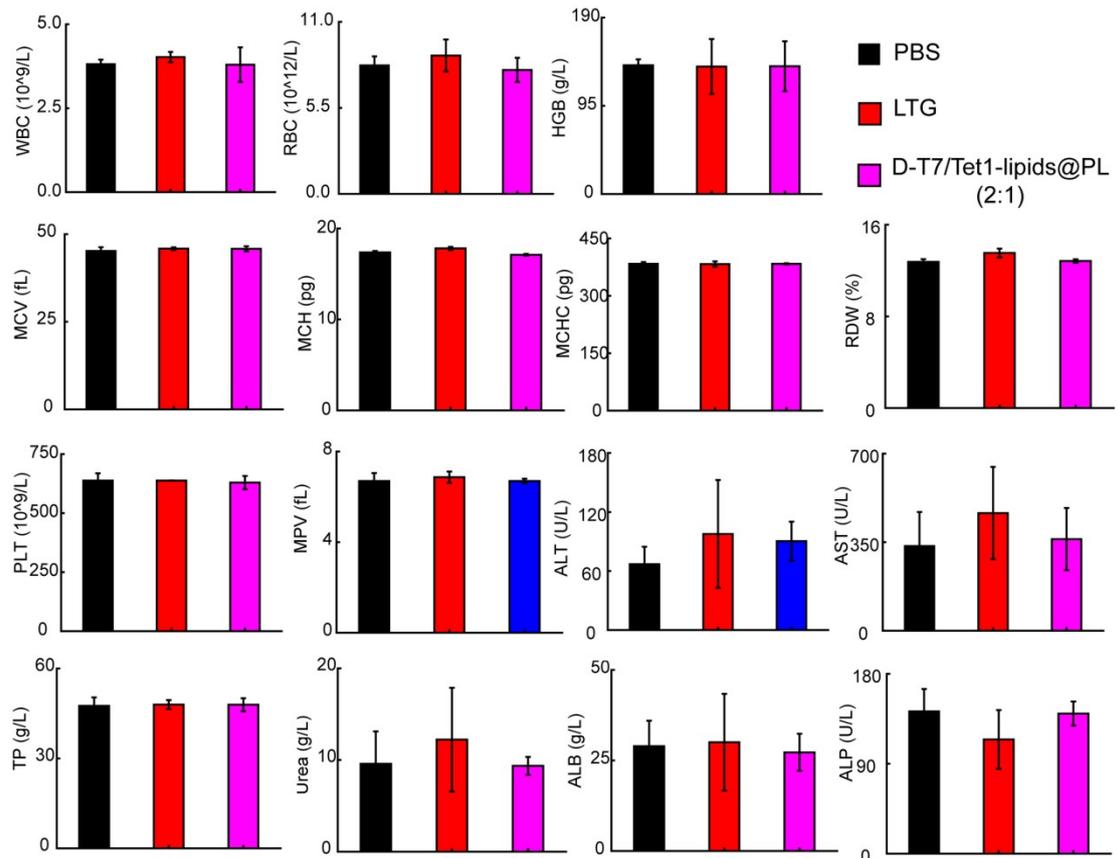
Figure S20 *In vivo* biosafety evaluation of various nanoparticles assessed by hematological markers and biochemical parameters in blood after 6 weeks of nanoparticles treatment in a chronic epilepsy model. (n = 3)
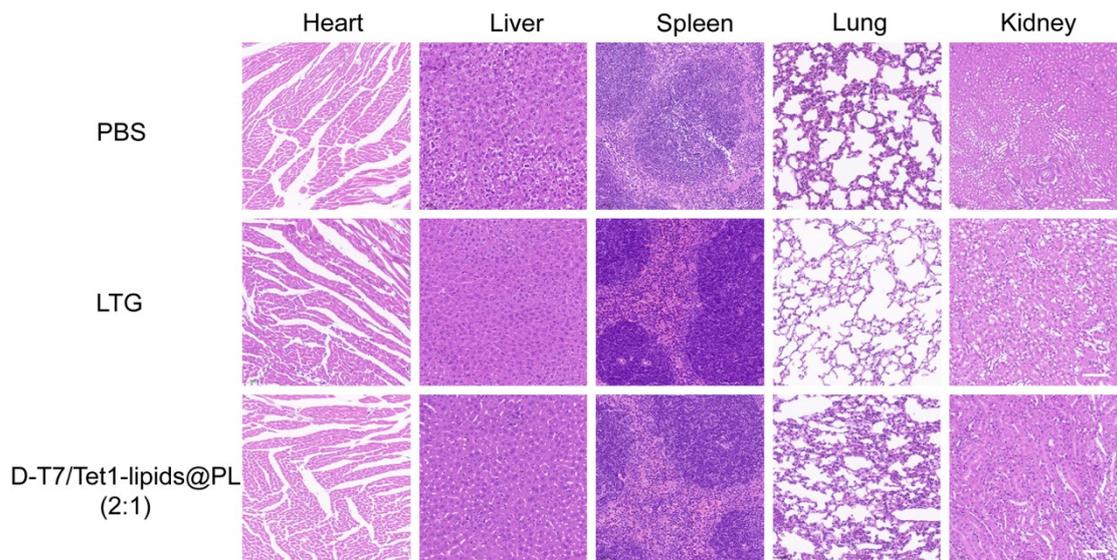
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387 **Figure S21** *In vivo* biosafety of various nanoparticles assessed by histopathological
388 analysis after 6 weeks of nanoparticles treatment in a chronic epilepsy model. Scale
389 bar, 50 μm.

390 **References**

391 1   L. Zhang, Q. Feng, J. L. Wang, S. Zhang, B. Q. Ding, Y. J. Wei, M. D. Dong, J.-
392 Y. Ryu, T.-Y. Yoon, X. H. Shi, J. S. Sun, X. Y. Jiang, *ACS Nano*, 2015, **9**, 9912-9921.
393 2   Q. Feng, L. Zhang, C. Liu, X. Y. Li, G. Q. Hu, J. S. Sun, X. Y. Jiang,
394 *Biomicrofluidics*, 2015, **9**, 052604.
395 3   L. Zhang, J. M. Chan, F. X. Gu, J. W. Rhee, A. Z. Wang, A. F. Radovic-Moreno,
396 F. Alexis, R. Langer, O. C. Farokhzad, *ACS Nano*, 2008, **2**, 1696-1702.
397 4   Y. Sun, Z. Huang, W. W. Liu, K. X. Yang, X. Y. Jiang, *Biointerphases*, 2012, **7**,
398 29.

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