Supporting Information

LyP-1-Modified Multifunctional Dendrimers for Targeted Anti-Tumor and Anti-Metastasis Therapy

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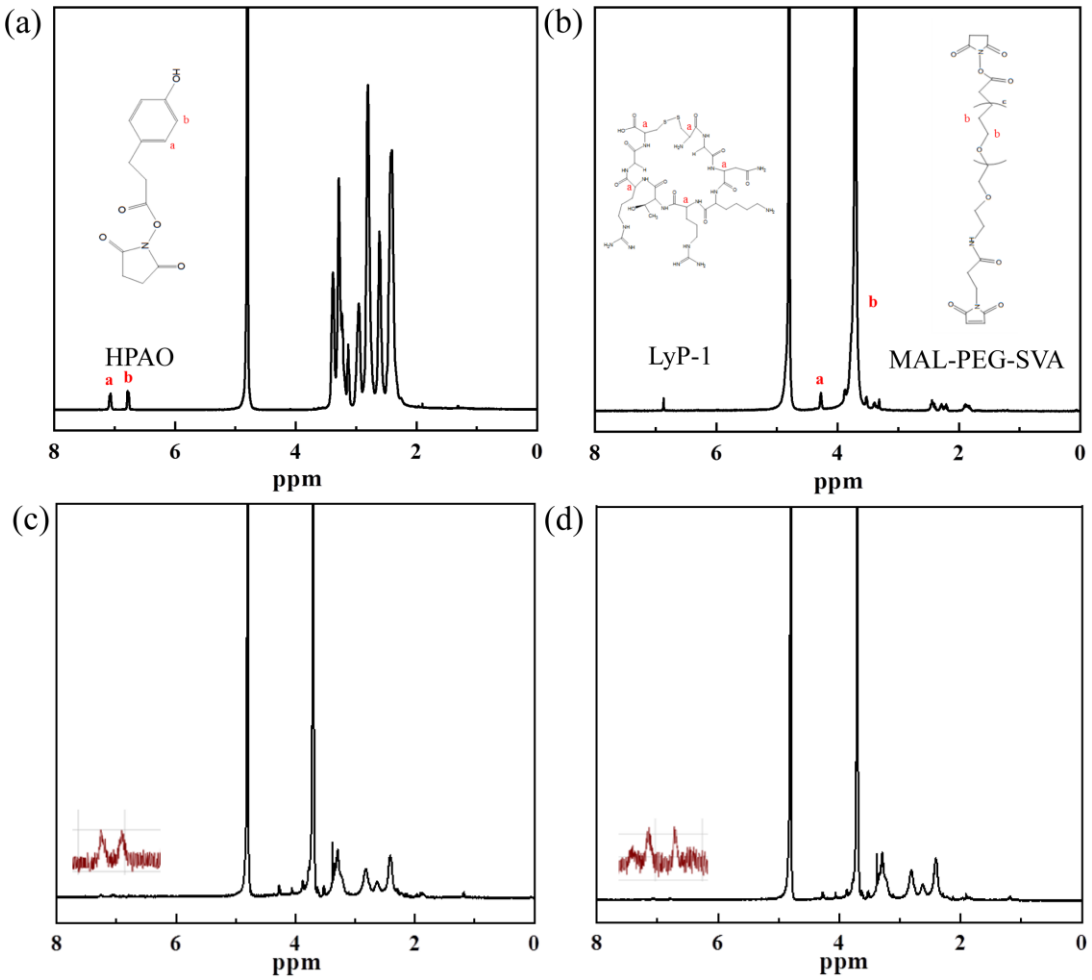

Figure S1. ^{1}H nuclear magnetic resonance spectra of G5.NH_2-HPAO (a), PEG-LyP-1-COOH (b), G5.NH_2-HPAO-(PEG-MAL) (c) and G5.NH_2-HPAO-(PEG-LyP-1) (d). As shown in the Figure S1a, the characteristic peaks at 2.3-3.4 ppm correspond to 1876 protons of -CH_2- groups on G5.NH_2, and the peaks at 6.8 and 7.1 ppm point to the 4 protons on the HPAO phenol. On the basis of these protons on G5.NH_2, the integral value of 4 protons on the HPAO can be calculated to be 36.89, and the number of the HPAO moieties modified onto each dendrimer is estimated to be 9.2. Similarly, the Figure S1b displays that the characteristic peaks of 5 protons on the LyP-1 peptide can be found at 4.3 ppm, and

the characteristic peaks at 3.6-3.8 ppm corresponds to 416 protons of PEG. Based on these protons on PEG, the integral value of 5 protons on the LyP-1 is 5.51, and the number of the LyP-1 modified onto each PEG is estimated to be 1.1. Finally, according to the NMR data of $G5.NH_2$-HPAO-(PEG-MAL) and $G5.NH_2$-HPAO-(PEG-LyP-1) in Figure S1c and S1d, there is some overlap in the characteristic peaks at 4.3 ppm between LyP-1 peptide and other structures of the NPs. Therefore, the number of the LyP-1 modified onto each dendrimer is calculated by $G5.NH_2$ and PEG. On the basis of 1876 protons on $G5.NH_2$, the integral value of 416 protons on the PEG is 13756.34, and the number of the LyP-1 modified onto each dendrimer is estimated to be 33.1.

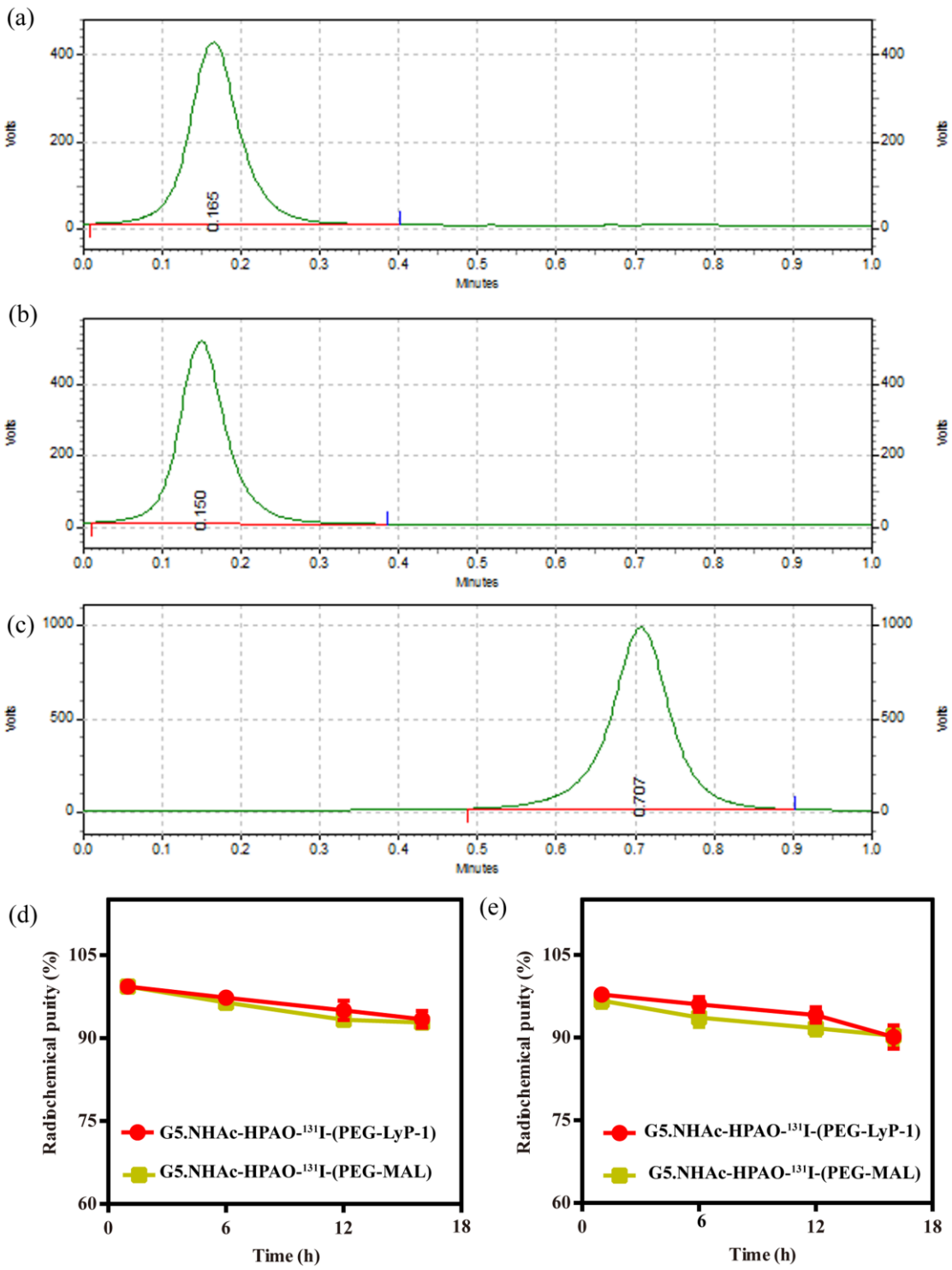

Figure S2. The ITLC results of G5.NHAc-HPAO-^{131}I-(PEG-LyP-1) (a) and G5.NHAc-HPAO-^{131}I-(PEG-MAL) (b) after PD-10 columns purification and unreacted ^{131}I ion (c); the radiochemical purities of

G5.NHAc-HPAO-^{131}I-(PEG-LyP-1) and G5.NHAc-HPAO-^{131}I-(PEG-MAL) were recorded in PBS (d) at room temperature and in FBS (e) at 37 °C for 1, 6, 12 and 16 h, respectively.

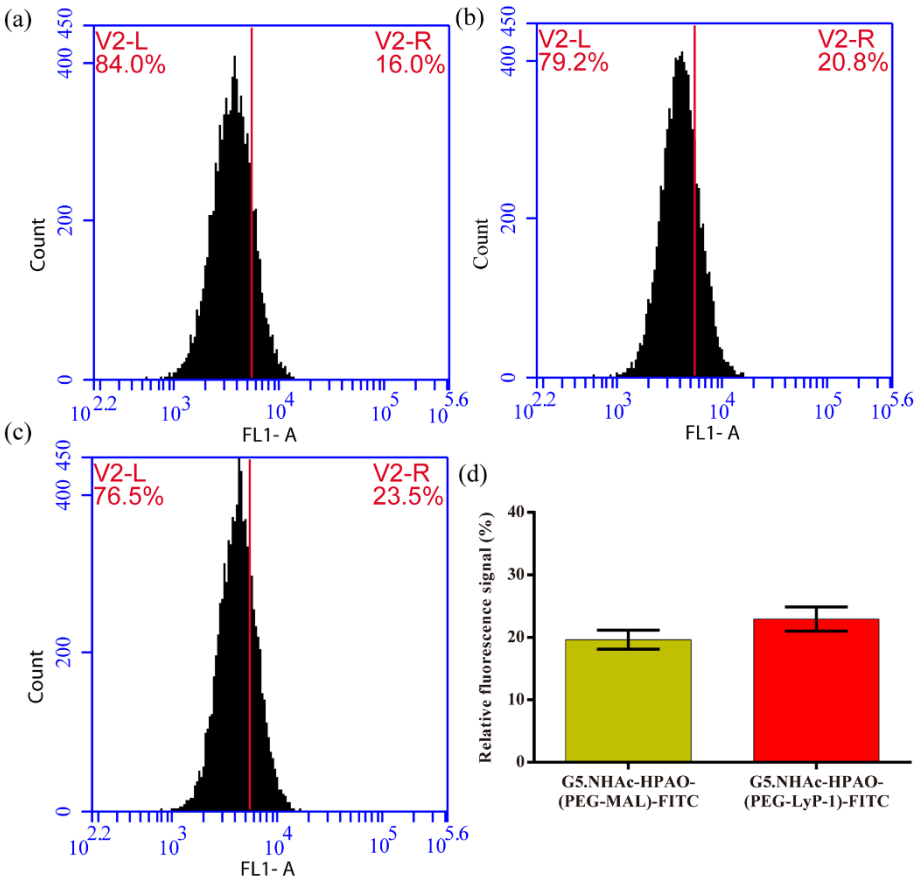

Figure S3. The targeting specificity study of G5.NHAc-HPAO-(PEG-LyP-1) to 4T1 cells with relatively low p32 expression by flow cytometry *in vitro*. 4T1 cells cultured with 10% FBS complete medium were incubated with PBS (a), G5.NHAc-HPAO-(PEG-MAL)-FITC (b), G5.NHAc-HPAO-(PEG-LyP-1)-FITC (c) at the concentration of 10 nM for 4 h. The relative FITC signal intensity was quantitative analyzed for comparison (d).

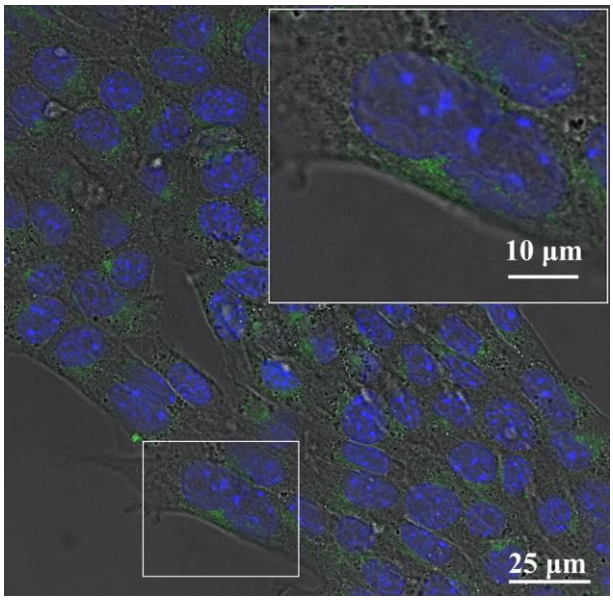

Figure S4. The combination image of bright filed and fluorescence microscopy of 4T1 cells treated with G5.NHAc-HPAO-(PEG-LyP-1)-FITC at the concentration of 10 nM for 4 h. The local magnification image was shown in white box.

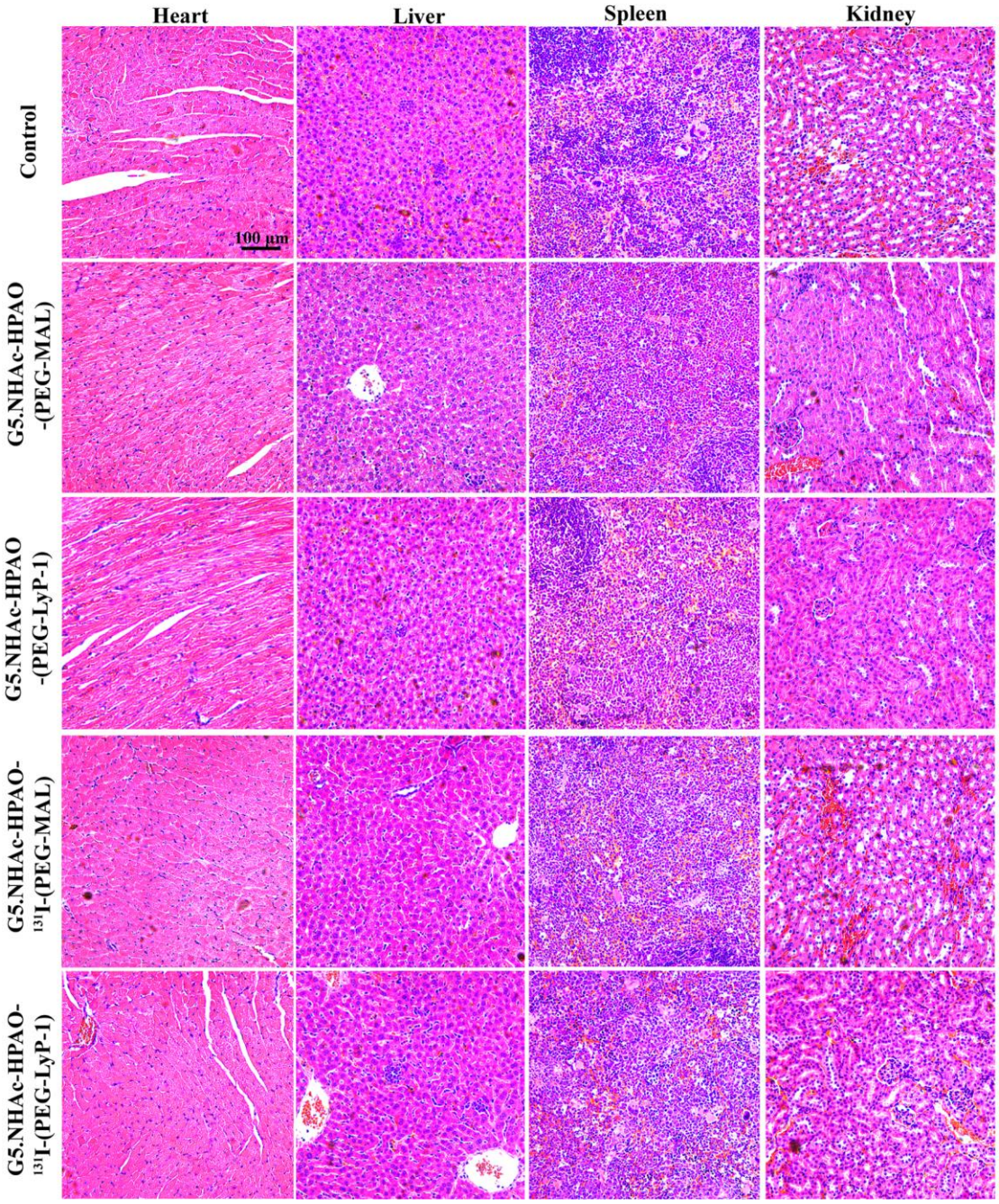

Figure S5. Toxicity of G5.NHAc-HPAO-^{131}I-(PEG-LyP-1) *in vivo*. Major organs including heart, liver, spleen and kidneys were harvest and performed histological study. Representative H&E staining of 4T1 tumor-bearing mouse after treatment with saline, G5.NHAc-HPAO-(PEG-MAL), G5.NHAc-HPAO-^{131}I-(PEG-LyP-1)

G5.NHAc-HPAO-^{131}I-(PEG-LyP-1) G5.NHAc-HPAO-^{131}I-(PEG-LyP-1). The scar bar = 100 μM.

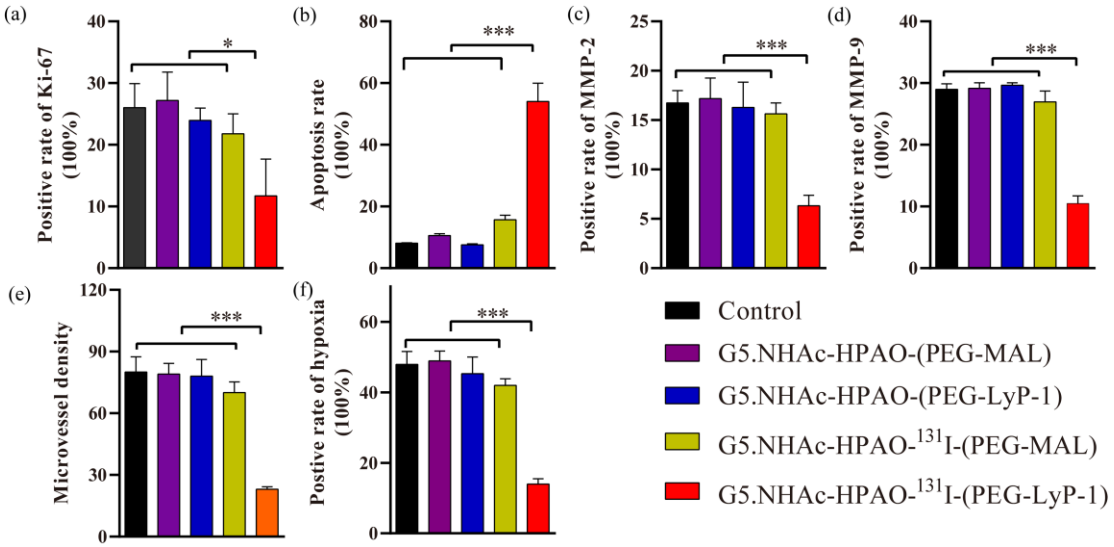

Figure S6. Quantitative analysis of molecular markers in tumor microenvironment of 4T1 tumor-bearing mice treated with saline, G5.NHAc-HPAO-(PEG-MAL), G5.NHAc-HPAO-(PEG-LyP-1), G5.NHAc-HPAO-^{131}I-(PEG-MAL), G5.NHAc-HPAO-^{131}I-(PEG-LyP-1), respectively. The quantitative positive rates (the percentage of positively stained cells/total cells) of Ki-67 and TUNEL were shown in histogram (a and b). The quantitative areal densities (the percentage of integrated optical density/total area) of MMP-2 and MMP-9 were shown in histogram (c and d). The quantitative microvessel density was assessed for comparison (e). The quantitative hypoxia was shown in histogram (f).* $P < 0.05$, *** $P < 0.001$.