Support Information

Enhanced Anti-Tumor Activity of EGFP-EGF1-Conjugated Nanoparticles by a Multi-Targeting Strategy

Bo Zhang [a,1], Ting Jiang [a,1], Li Ling[b], Zhonglian Cao[c], Jingjing Zhao[b], Yanyan Tuo[b], Xiaojian She[b], Shun Shen[b], Xinguo Jiang[b], Yu Hu[a]*, Zhiqing Pang[b]*

[a]Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei, 430022, China;

[b]School of Pharmacy, Fudan University, Key Laboratory of Smart Drug Delivery, Ministry of Education, 826 Zhangheng Road, Shanghai, 201203, China;

[c]Instrumental Analysis Center of School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai, 201203, China

1: equal contribution to the work

* Corresponding author:

Yu Hu,

Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei, 430022, PR China.

Tel.: +86-27-85726335; fax: +86-27-85776343. E-mail address: dr_huyu@126.com (Y. Hu).

Zhiqing Pang,

School of Pharmacy, Fudan University, Key Laboratory of Smart Drug Delivery, Ministry of Education, 826 Zhangheng Road, Shanghai, 201203, China

Tel.: +86-21-51980069; fax: +86-21-51980069. E-mail address: zqpang@fudan.edu.cn

Table S 1 Analysis of A549 cells apoptosis induced by different PTX formulations (n=3)

Treatment	Annexin-FITC+ (%)	Annexin-FITC+ PI+ (%)	PI+ (%)	Total
Control	1.98±0.42	4.89±1.02	0.45±0.20	7.26±1.52
Taxol	6.71±0.70[a]	9.14±0.78[a]	1.62±0.10[a]	19.49±0.71[a]
NP-PTX	8.43±0.09[a]	10.84±1.42[a]	0.98±0.33	20.25±1.59[a]
ENP-PTX	10.13±1.34[ab]	12.47±0.39[ab]	1.41±0.29[a]	24.05±1.39[abc]

[a]$p < 0.05$, compared with Control.[b]$p < 0.05$, compared with Taxol.[c]$p < 0.05$, compared with NP-PTX.

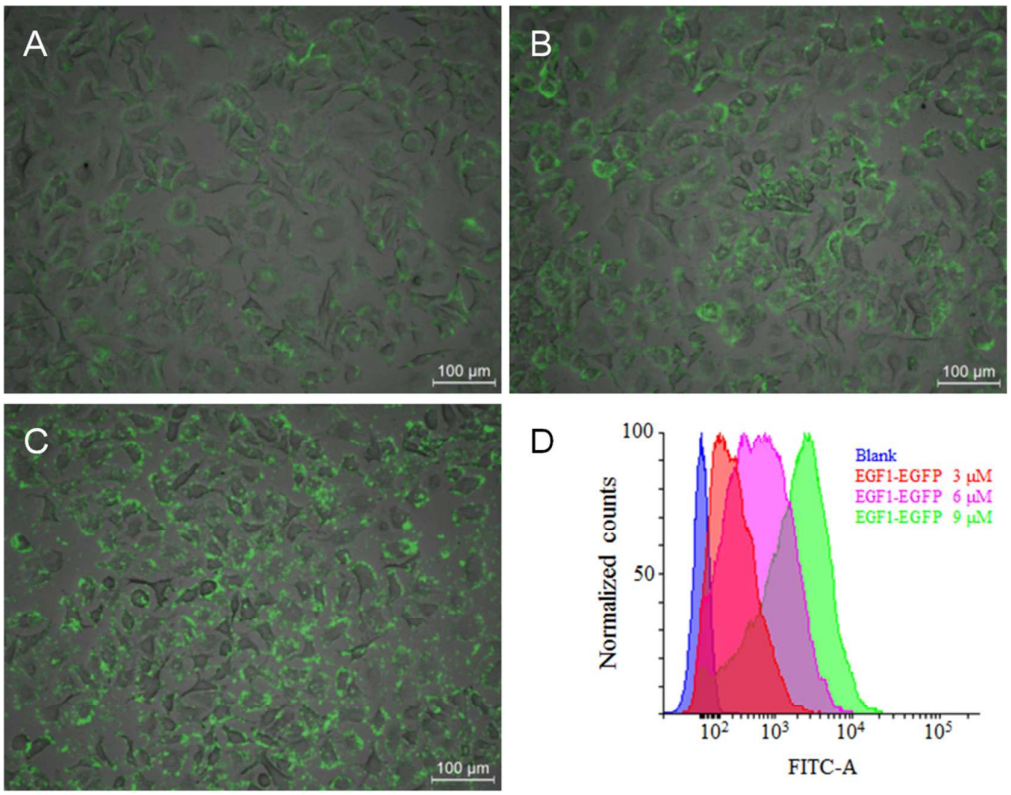

Figure S 1 EGFP-EGF1 protein binding to A549 cells evaluated by fluorescence microscopy qualitatively（A, B, C） and flow cytometry quantitatively（D) after incubation for 6 h (n=3). The protein concentration was 3 μM (A)，6 μM (B) and 9 μM (C)，respectively.

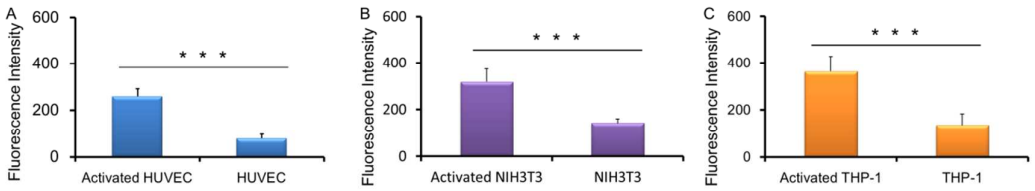

Figure S 2 EGFP-EGF1 protein binding to HUVEC (A), NIH3T3 (B) or THP-1 (C) activated with corresponding stimulating factor or not. The results were evaluated by flow cytometry quantitatively after incubation with 9 μM of EGFP-EGF1 protein for 6 h (n=3).

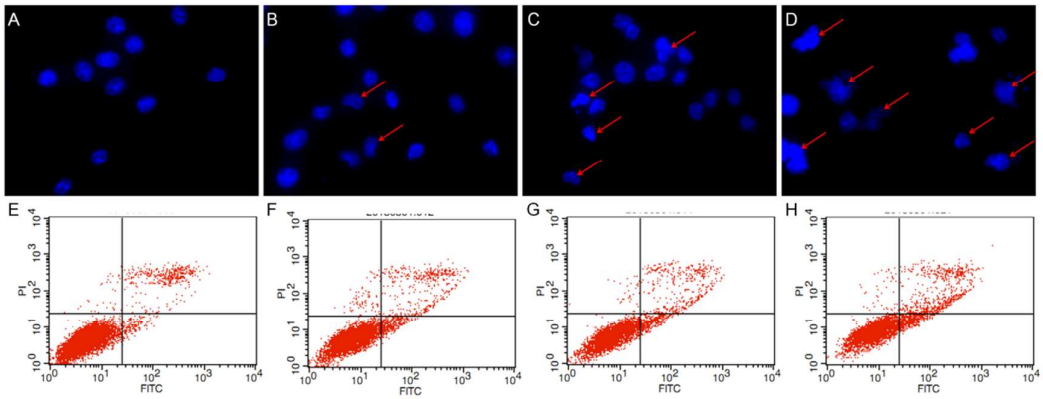

Figure S 3 Apoptosis of A549 cells induced by different PTX formulations including Taxol (B & F), NP-PTX (C & G) and ENP-PTX (D & H). A549 cells without drug treatment served as control (A & E). The results was analyzed qualitatively by fluorescence microscopy （A, B, C & D）and quantitatively by flow cytometry（E, F, G & H）. The cell nucleus was stained by Hoechst 33342. The red arrows indicated cell apoptosis. Original magnification: 200×.

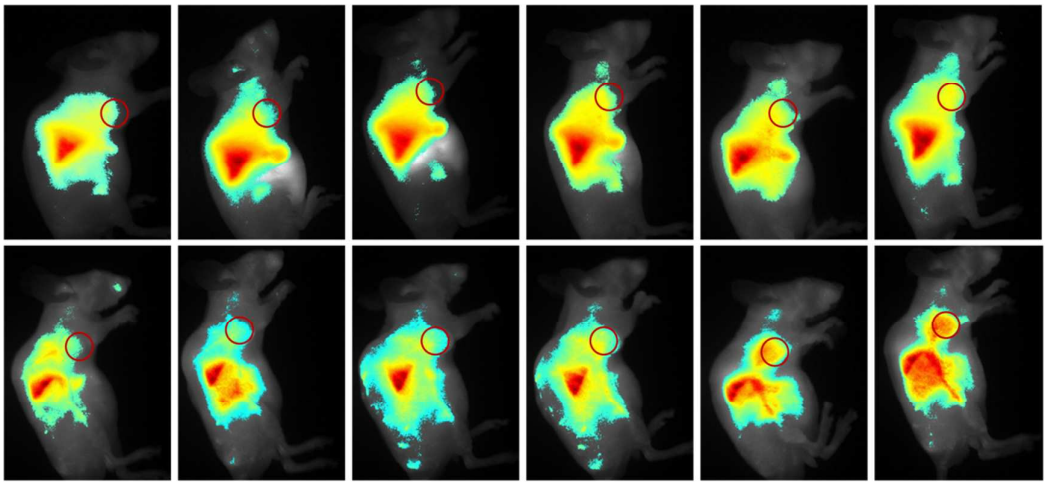

Figure S 4 *In vivo* imaging of A549 xenograft-bearing nude mice at different time points (1 h, 2 h, 4 h, 6 h, 8 h, 12 h) after administration with DiR-labeled NP (upper row) or ENP (lower row).

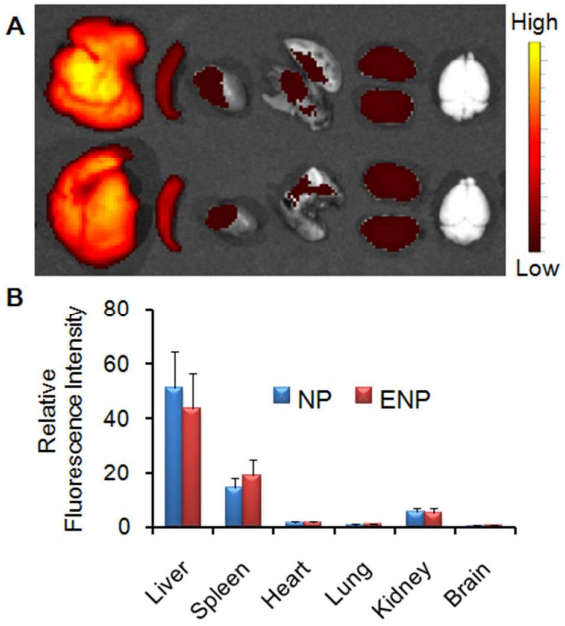

Figure S 5 Distributions of DiR-labeled NP (upper row) and ENP (lower row) in normal major organs (From left to right: liver, spleen, heart, lung, kidney, brain) analyzed by *in vivo* imaging system qualitatively (A) and semi-quantitatively (B).

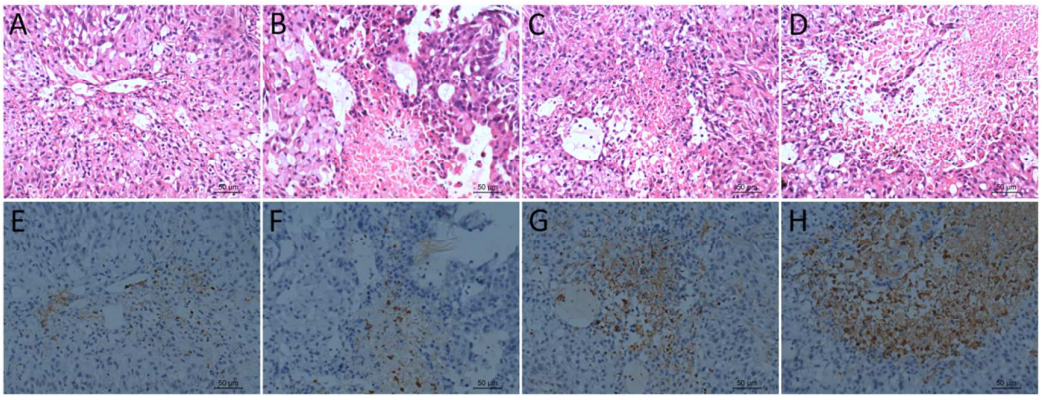

Figure S 6 H&E staining (A, B, C, D) and TUNEL staining (E, F, G, H) of tumor tissue sections obtained from animal models at day 18 after treatment for saline (A & E), Taxol (B & F), NP-PTX (C & G) and ENP-PTX (D & H)，respectively.