Supporting Information

for

Improving inhibitory effect of CXCR4 peptide antagonist in tumor metastasis by acetylated PAMAM dendrimer

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Note added after first publication: This Supplementary Information file replaces that originally published on 30th November 2018, in which the last two images in Fig. S2(c) were incorrect. This does not affect the conclusions of the paper.

1. The detection of CXCR4 expression level in cancer cells

The CXCR4 expression in MS-5, 4T1, MDA-MB-231 and MCF-7 was determined by flow cytometer. Briefly, 5×10^6 the cells were harvested and incubated with monoclonal anti-human FITC labeled CXCR4 antibody (BD, USA) or anti-mouse FITC labeled CXCR4 antibody (BD, USA) in the incubating buffer (PBS + 1% FBS). After incubating for 30 min at room temperature, the cells were washed three times and resuspended into 500 μL PBS. 1×10^4 cells were analyzed on the BD AccuriTM C6 system (BD, USA). FITC-labeled IgG was chosen as the isotype control.

Four cell lines including MS-5, MCF-7, MDA-MB-231 and 4T1 were chosen as cell models because of the different CXCR4 expression levels on the surface. The CXCR4 expression level for MCF-7, MDA-MB-231 and 4T1 was detected 32.0%, 51.9% and 52.6%, respectively, while MS-5 was the CXCR4-negative cell line. Results were shown in Fig. S1.

2. Stability assay of the P_{Ac80}-E5 complex

To detect the stability of the P_{AC80}-E5 complex, DLS assay was performed. The P_{AC80}-E5 (the molar ratio of P_{AC80}: E5 = 1: 3) was prepared with the E5 concentration of 1 μM in PBS buffer and deionized water. Then the size distribution of the complex was detected at 0 h, 4 h, 8 h and 24 h. The size distribution of free E5 peptide was detected with the same condition as control.

Meanwhile, the DLS assay was also used to evaluate the life time of the P_{AC80}-E5 complex *in vitro*. The P_{AC80}-E5 complex was dispersed in incubation medium (RIMP-1640 + 0.5% FBS) with the E5 concentration of 1 μM, Then the size distribution of the complex was detected at 0 h, 24 h and 48 h. In this experiment, the incubation medium was used to simulate the environment of the ascites. The life time of the P_{AC80}-E5 materials was determined through the size changes of the complex.

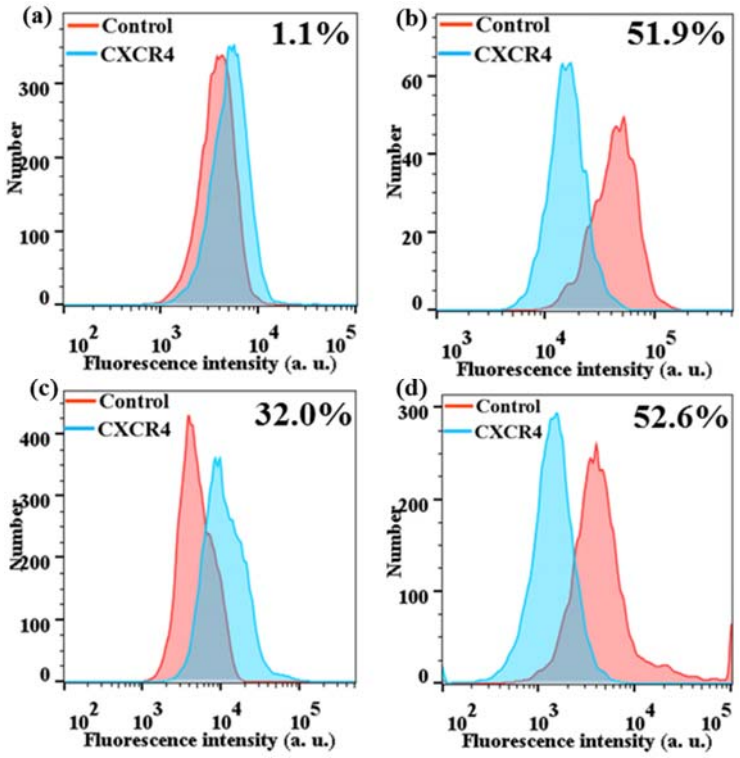

Fig. S1 The CXCR4 expression levels in (a) MS-5, (b) MDA-MB-231, (c) MCF-7 and (d) 4T1 cells. Results were measured by FCM analysis using FITC labeled anti-CXCR4 antibody.

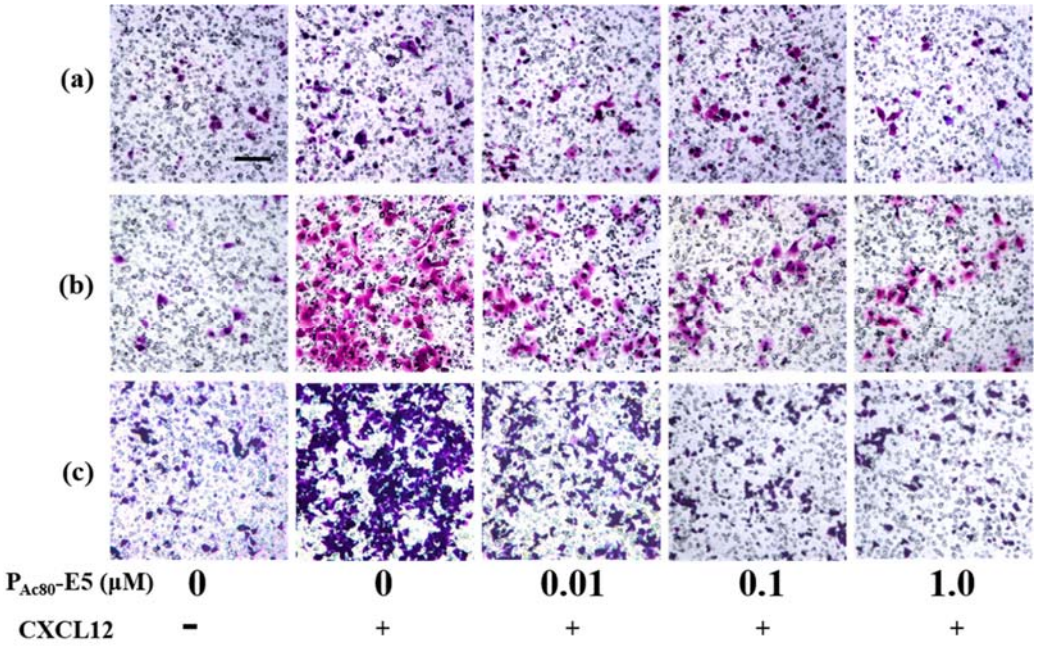

Fig. S2 Representative images of transwell assay showed the migration of (a) MCF-7, (b) MDA-MB-231 and (c) 4T1 cells after P_{AC80}-E5 treatment for 24 h in the presence or absence of CXCL12. Scale bar represents 100 μm.

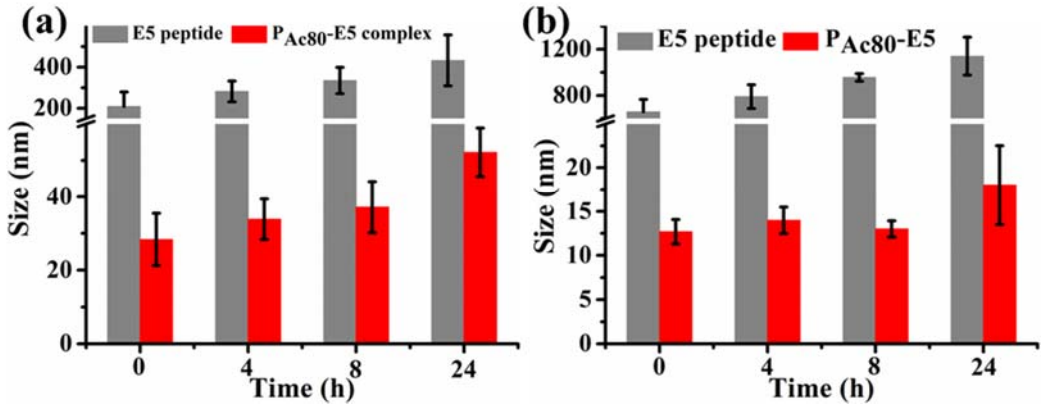

Fig. S3 Stability test of the P_{AC80}-E5 complex detected by DLS in (a) H_2O and (b) PBS buffer. The free E5 peptide was used as control.

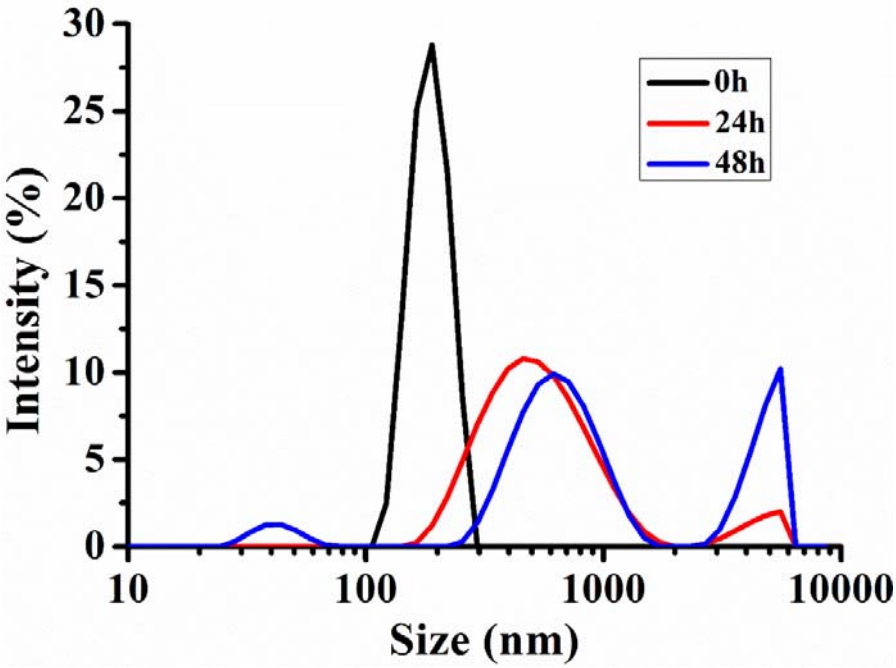

Fig. S4 The life time of P_{AC80}-E5 complex detected by DLS *in vitro* with the E5 concentration of 1 μM.