Supplementary Information

A novel α-enolase-targeted drug delivery system for high-efficacy prostate cancer therapy

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1. Immunofluorescence analysis

The dissected tumours were first fixed within 4% paraformaldehyde over 48h, and then using 15% and 30% sucrose solution to dehydrate overnight, respectively. The frozen sections of tumours were cut into 8-μm-thick, adhered on coated slides, then fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.5% trixon-100 for 30 min at room temperature. After washing with PBS, the sections were immersed into 2% bovine serum albumin for 1 h to block nonspecific antibody binding. Next, stained with primary anti-α-enolase antibody (dilution ratio 1:50) at 4°C overnight. The sections were further stained with corresponding Alexa Fluor®647 conjugated secondary antibody (dilution ratio 1:200) at room temperature for 1 h. Finally, the slides were immersed in Hoechst 33258 for 10 min to stain the nuclei. The section images were taken using a confocal microscope (Nikon N-SIM, Japan).
Table S1. Median survival time of PC-3 bearing mice treated with saline and various doxorubicin formulations ($n=10$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Median (day)</th>
<th>Standard derivation</th>
<th>Increased survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>40.0</td>
<td>2.9</td>
<td>-</td>
</tr>
<tr>
<td>Dox</td>
<td>40.5</td>
<td>4.3</td>
<td>1.25%</td>
</tr>
<tr>
<td>PEG-lipo-Dox</td>
<td>46.0$^a$</td>
<td>7.5</td>
<td>15.0%</td>
</tr>
<tr>
<td>pHCT74-lipo-Dox</td>
<td>52.5$^{a,b,c}$</td>
<td>7.4</td>
<td>31.3%</td>
</tr>
</tbody>
</table>

$^a$ Compared to saline group, $p < 0.05$.

$^b$ Compared to Dox group, $p < 0.05$.

$^c$ Compared to PEG-lipo-Dox group, $p < 0.05$. 
Fig. S1 The MALDI-TOF mass spectrum (A) DSPE-PEG$_{2000}$-NHS. (B) DSPE-PEG$_{2000}$-pHCT74.
Fig. S2 Preparation and characterization of liposomes (A) Preparation schematic of pHCT74-Lipo-Dox. (B) Size and (C) Zeta potential distribution of PEG-lipo-Dox. (D) Size and (E) Zeta potential distribution of PEG-lipo-DiD. (F) Size and (G) Zeta potential distribution of pHCT74-lipo-DiD.
Fig. S3 Cellular uptake of pHCT74-Lipo-Dox (A) RWPE-1 cellular uptake of doxorubicin measured by flow cytometer (mean ± SD, n = 3). (B) Confocal images of RWPE-1 cellular uptake of liposomes. Bar indicates 30 µm.
**Fig. S4** The comparative uptake on RWPE-1 cells. The competitive inhibition of free peptide on doxorubicin uptake by preincubation with 1 mg/mL of free pHCT74 peptide for 1 h before RWPE-1 cells were exposed to the corresponding liposomes.