Additional file 1

PD-L1-targeted co-delivery of two chemotherapeutics for synergistic suppression of skin cancer in vitro and in vivo

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Determination of the number of conjugates

Briefly, the total number of lipid molecules in the outer lipid layer of each LCP was estimated by Equation 1:

$$N_{lip}= \frac{4\pi(r+h)^2}{a} \qquad \text{(Equation 1)}$$

where r is the radius of CaP core, h is the thickness of lipid layer (taken as 5 nm) and *a* is the average area per lipid molecule (0.7 nm^2 for DOPC). Then, the number of LCP NPs per ml was calculated by Equation 2:

$$N_{\mathrm{LCP}}=\frac{\mathrm{Clip}\times \mathrm{NA}}{Nlip\times 1000} \qquad \text{(Equation 2)}$$

where C_{lip} is the molar concentration of DOPC, and NA is the Avogadro number. Finally, the number of PD-L1 antibody of folic acid ligands per LCP NP were determined by Equation 3:

$$N_{\mathrm{lig}}=\frac{\mathrm{Clig}\times \mathrm{Nlip}}{Clip} \qquad \text{(Equation 3)}$$

where C_{lig} is the molar concentration of the ligand.

Table S1. List of synthesised NPs

Nanoparticle code	Payload	Number of FA per NP	Number of PD-L1 per NP
OTS-ABZ-LCP	ABZ and OTS	0	0
Cy5-LCP	Cy5 dsDNA	0	0
Cy5-LCP-P40	Cy5 dsDNA	0	40
Cy5-LCP-P80	Cy5 dsDNA	0	80
Cy5-LCP-P160	Cy5 dsDNA	0	160
OTS-ABZ-LCP-P40	ABZ and OTS	0	40
OTS-ABZ-LCP-P80	ABZ and OTS	0	80
OTS-ABZ-LCP-P160	ABZ and OTS	0	160
OTS-ABZ-LCP-F50	ABZ and OTS	50	0
OTS-ABZ-LCP-F100	ABZ and OTS	100	0
OTS-ABZ-LCP-F200	ABZ and OTS	200	0
OTS-ABZ-LCP-F50P40	ABZ and OTS	50	40
OTS-ABZ-LCP-F50P80	ABZ and OTS	50	80
OTS-ABZ-LCP-F50P160	ABZ and OTS	50	160
OTS-ABZ-LCP-F100P40	ABZ and OTS	100	40
OTS-ABZ-LCP-F100P80	ABZ and OTS	100	80
OTS-ABZ-LCP-F100P160	ABZ and OTS	100	160

Table S2. The mean size of PD-L1/folic acid conjugated OTS-ABZ-LCPs

Sample name	Number mean size (nm)	Sample name	Number mean size (nm)
OTS-ABZ-LCP-F50	57.3	OTS-ABZ-LCP-F100P40	66.0
OTS-ABZ-LCP-F100	59.7	OTS-ABZ-LCP-F100P80	65.5
OTS-ABZ-LCP-F200	59.3	OTS-ABZ-LCP-F100P160	65.3
OTS-ABZ-LCP-P40	58.7	OTS-ABZ-LCP-F50P40	64.7
OTS-ABZ-LCP-P80	61.7	OTS-ABZ-LCP-F50P80	62.4
OTS-ABZ-LCP-P160	63.1	OTS-ABZ-LCP-F50P160	63.2
OTS-ABZ-LCP	58.0		

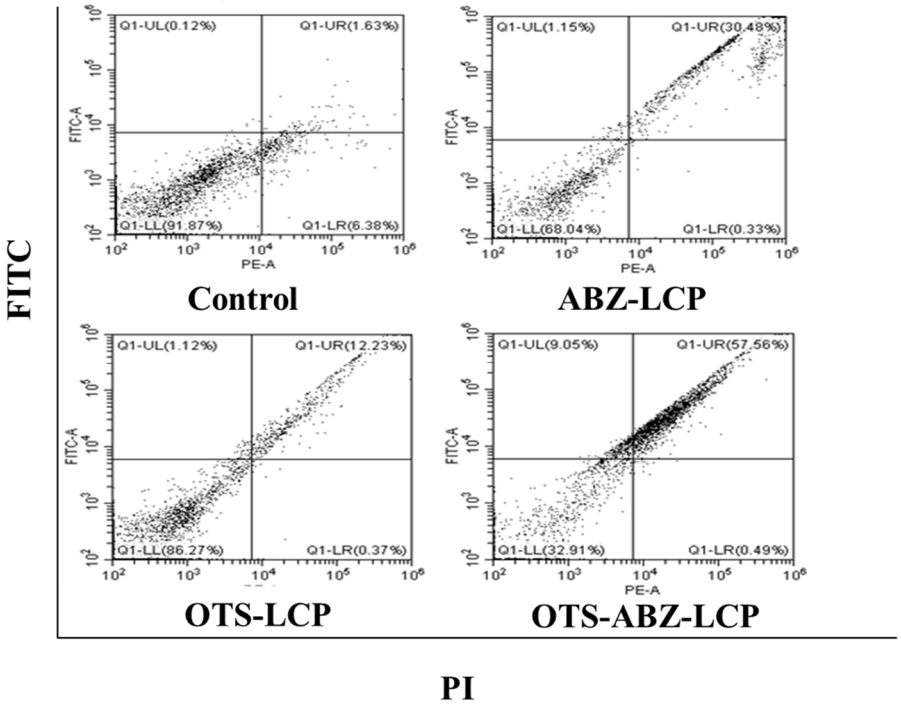

Figure S1. Annexin V-FITC/PI double staining analysis of apoptosis in B16F0 cells treated with ABZ-LCP, OTS-LCP and OTS-ABZ-LCP for 24 h (Annexin V and PI negative cells were considered as live, Annexin V positive and PI negative as early apoptotic, Annexin V and PI positive as late apoptotic and Annexin V negative and PI positive as necrotic cells).

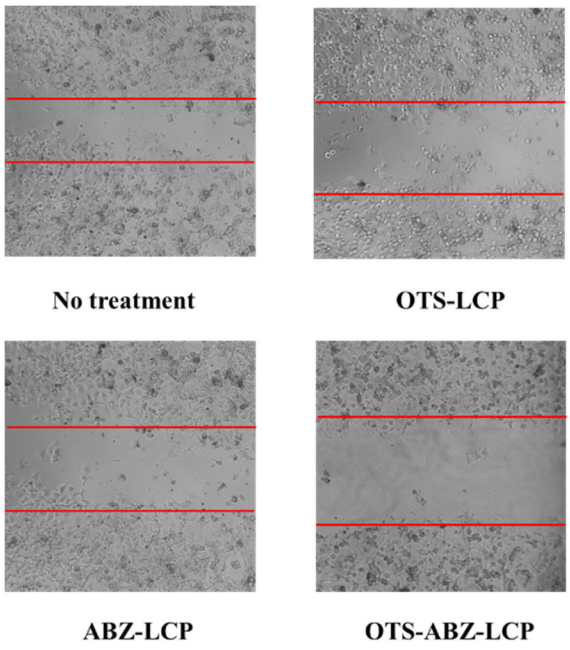

Figure S2. Relative migrated number of cells determined by wound healing assay for B16F0 cells treated with LCP formulations for 4 h. The equivalent concentration of ABZ and OTS was 2.5 μg/ml and 64 ng/ml, respectively.

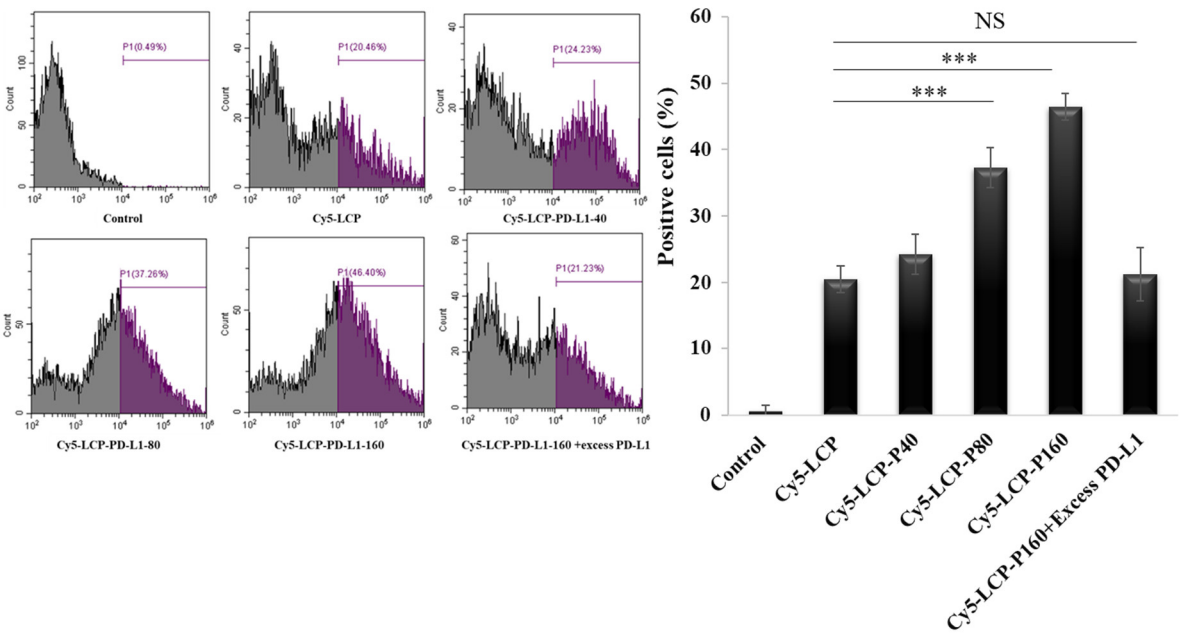

Figure S3. Cellular uptake of Cy5 dsDNA-loaded LCPs conjugated with 40, 80 or 160 PD-L1 antibodies per NP by B16F0 cells after 4 h.

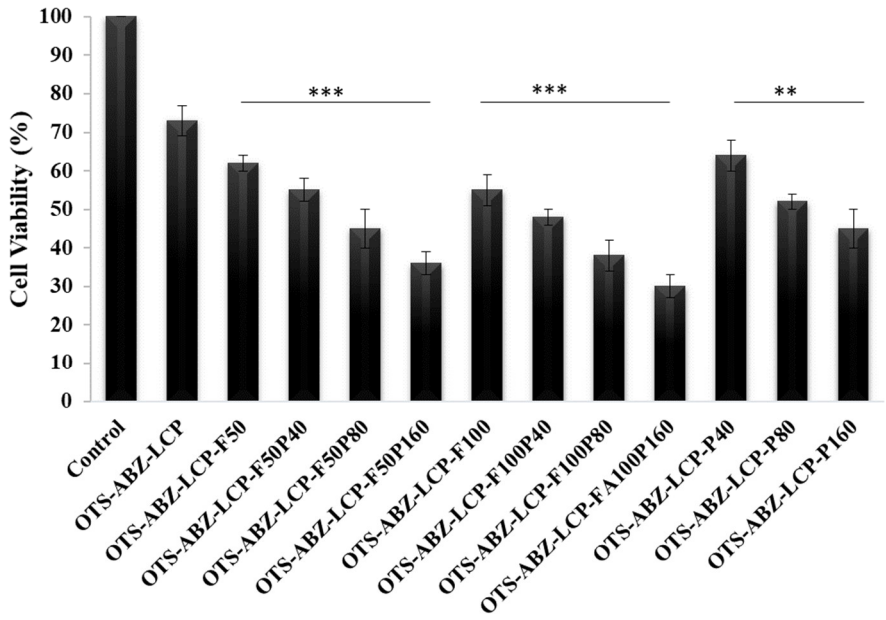

Figure S4. Cytotoxic effect of OTS-ABZ-LCP NPs dual conjugated with different numbers of folic acid and PD-L1 antibody (ABZ: 100 ng/ml; OTS964: 50 ng/ml).

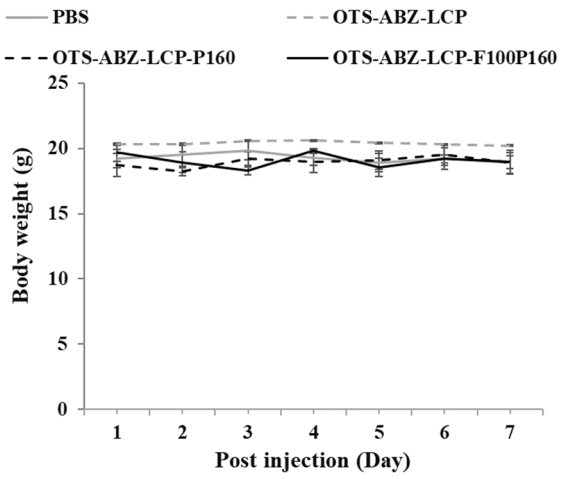

Figure S5. The average body weight of B16F0-bearing mouse treated with OTS-ABZ-LCP, OTS-ABZ-LCP-P160 and OTS-ABZ-LCP-F100P160 (equivalent amount of albendazole 2.5 mg/kg and 1 mg/kg OTS in all formulations) intraperitoneally injected 3 times every two days compared to that of PBS injection

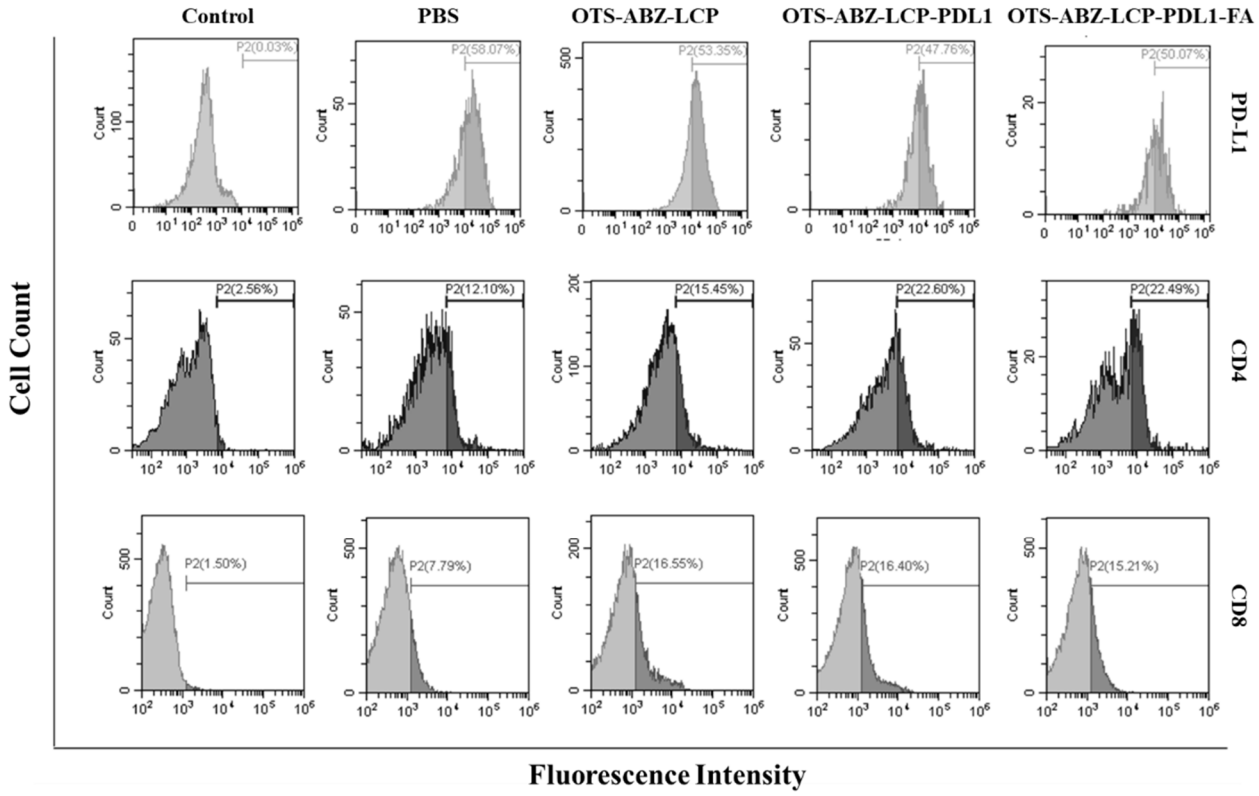

Figure S6. Flow cytometric analysis of PD-L1 expression, $CD4^+$ and $CD8^+$ in tumour population.

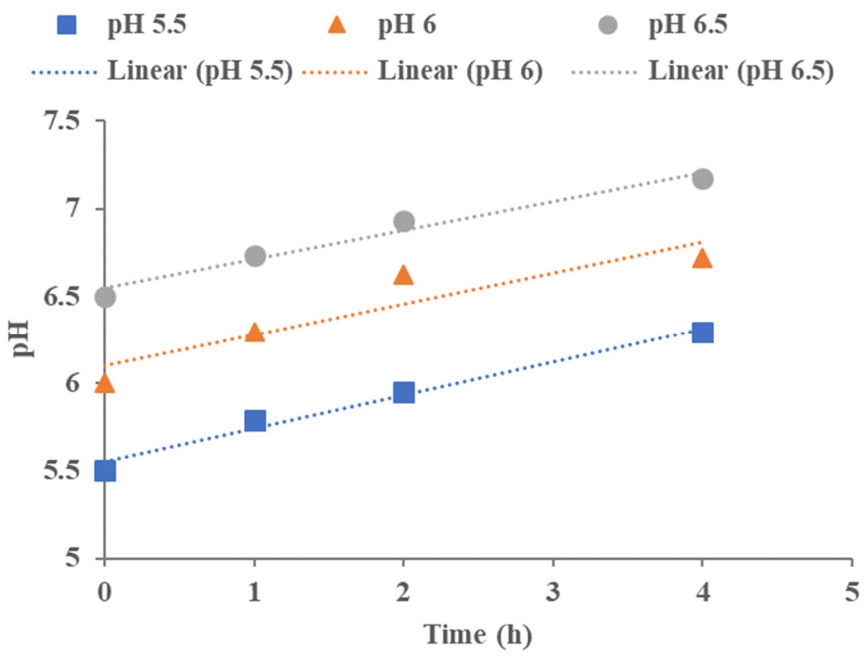

Figure S7. pH alteration by degradation of LCP NPs in DI water with set pH at 5.5, 6 and 6.5