

# Photo-induced crosslinked and anti-PD-L1 peptide incorporated liposomes to promote PD-L1 multivalent binding for effective immune checkpoint blockade therapy

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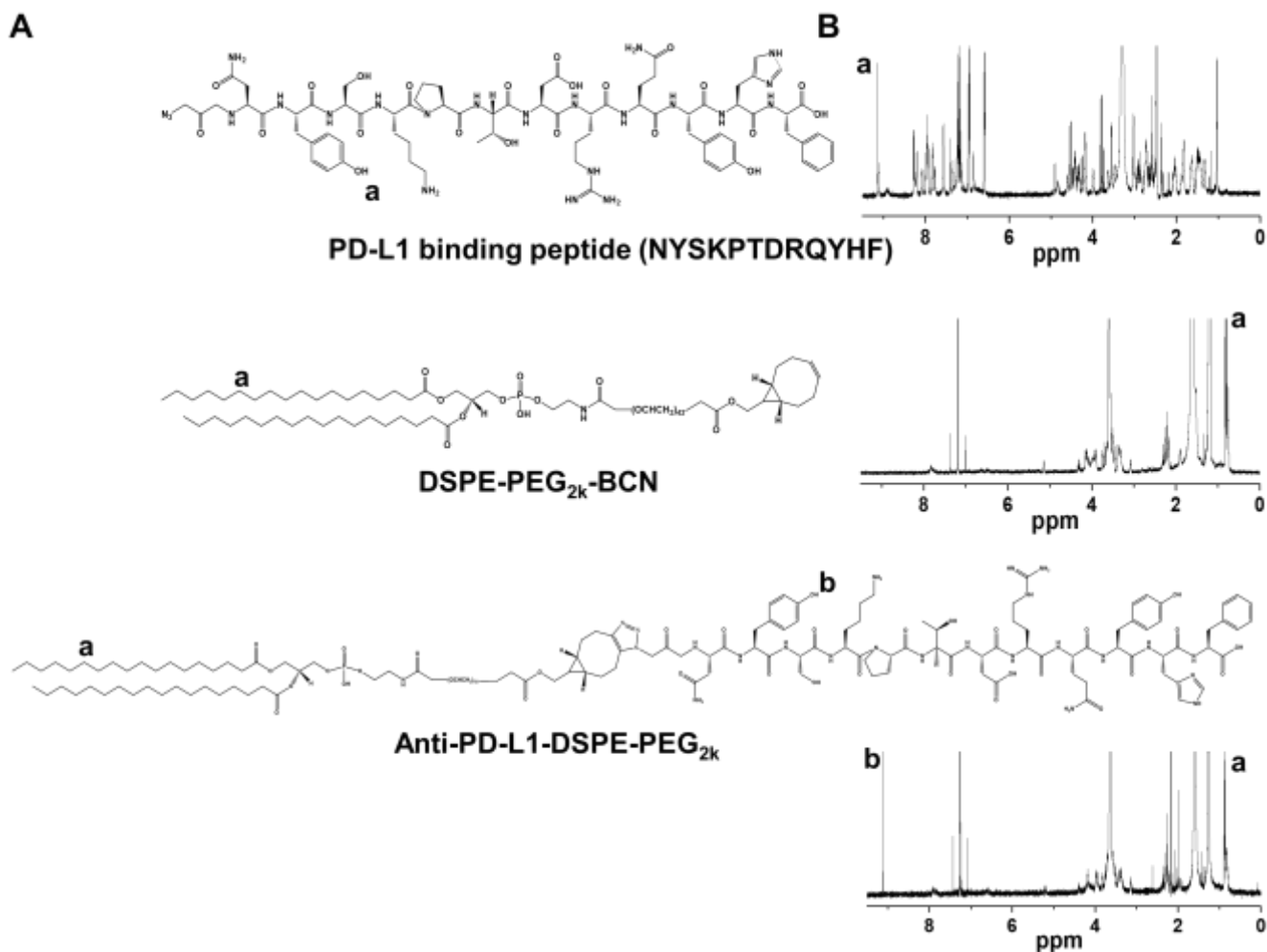
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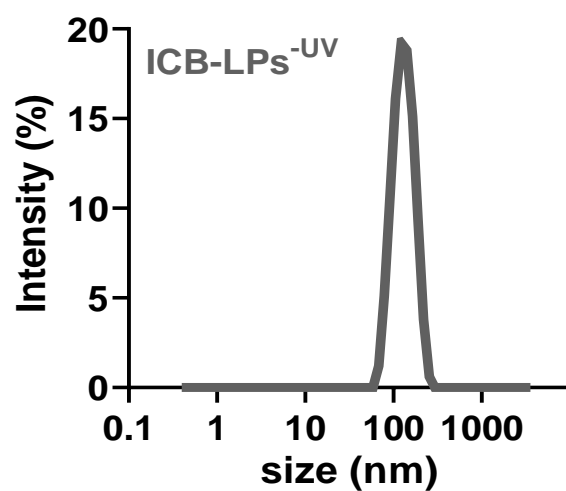
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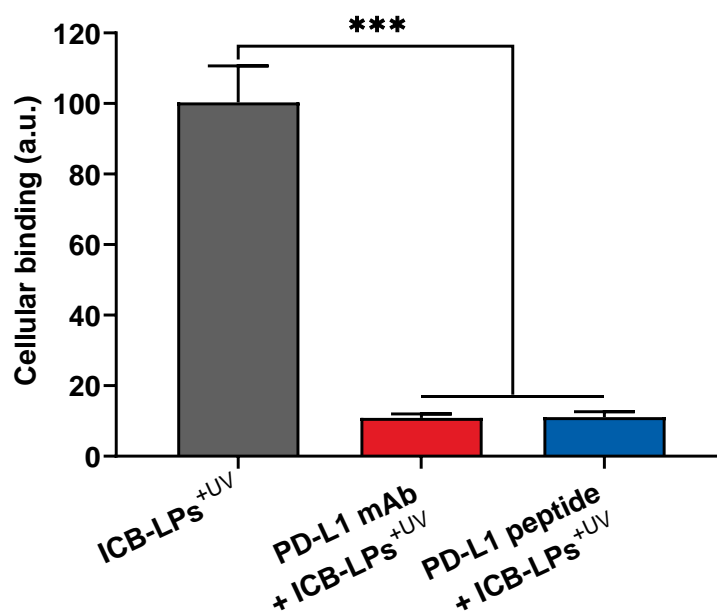
**Figure S1** (A) Synthetic scheme to prepare anti-PD-L1-DSPE-PEG<sub>2k</sub>. (B) <sup>1</sup>H NMR spectrum of PD-L1 binding peptide, DSPE-PEG<sub>2k</sub>-BCN and anti-PD-L1-DSPE-PEG<sub>2k</sub>.



**Figure S2** Size distribution of ICB-LPs<sup>-UV</sup> in saline (1 mg/mL).

**Table S1** Diameter, PD-L1 binding peptide contents, formulation efficiency and zeta potential of PEG-LPs, ICB-LPs<sup>-UV</sup> and ICB-LPs<sup>+UV</sup>

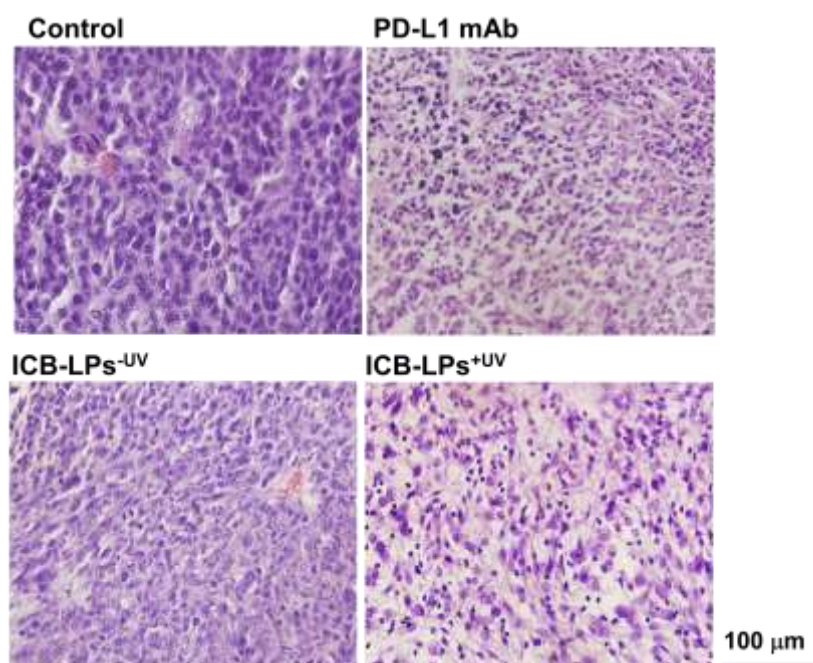
|                              | Diameter (nm) | PD-L1 binding peptide contents (mol%) | Formulation efficiency (%) | Zeta potential (mV) |
|------------------------------|---------------|---------------------------------------|----------------------------|---------------------|
| <b>PEG-LPs</b>               | 105.8 ± 1.23  | -                                     | -                          | -42.2 ± 0.289       |
| <b>ICB-LPs<sup>-UV</sup></b> | 134.3 ± 4.82  | 9.76 ± 0.147                          | 97.6 ± 0.15                | -2.97 ± 0.175       |
| <b>ICB-LPs<sup>+UV</sup></b> | 157.7 ± 1.04  |                                       |                            | -3.31 ± 0.219       |



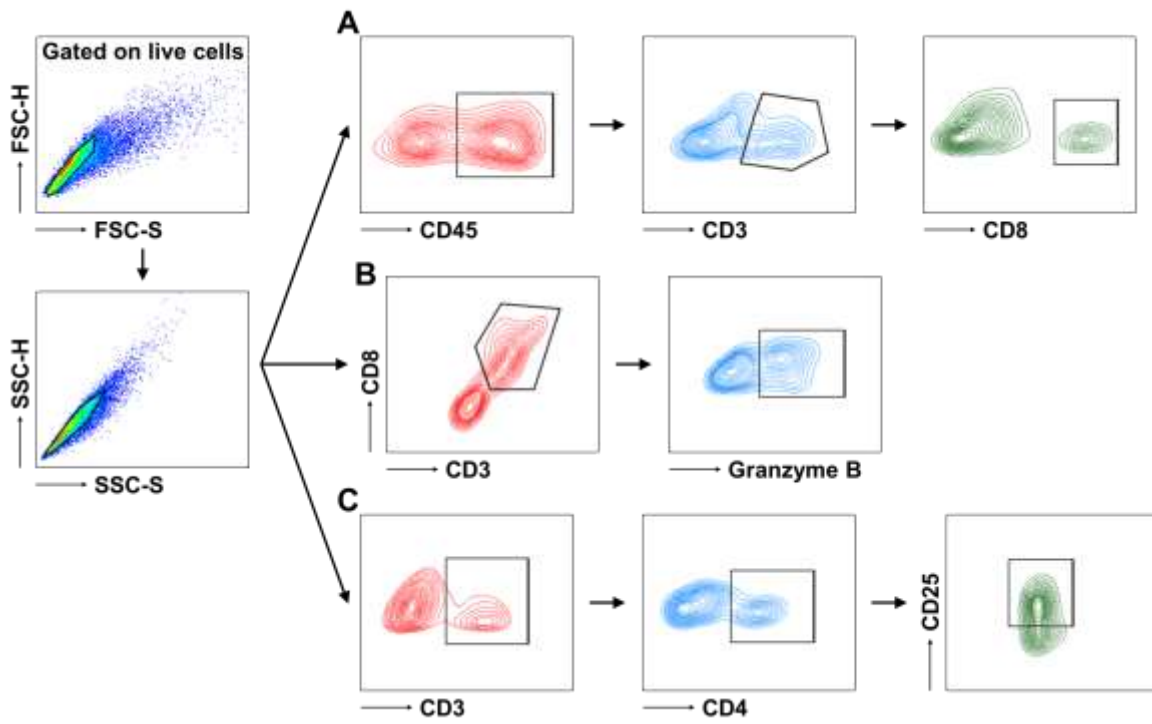
**Figure S3** Cellular binding of ICB-LPs<sup>+UV</sup> in the naive, or PD-L1 mAb- or PD-L1 peptide-treated CT26 cancer cells. Data are presented as mean ± SD (*n* =5); \*\*\**P*<0.001 vs. Control or indicated.

**Table S2** Pharmacokinetics (PK) parameters of area under the curves (AUC) and half-life ( $t_{1/2}$ ) of PEG-LPs, ICB-LPs<sup>-UV</sup> and ICB-LPs<sup>+UV</sup> in BALB/c mice. DiD-labeled PEG-LPs, ICB-LPs<sup>-UV</sup> and ICB-LPs<sup>+UV</sup> (1 mg/kg) were intravenously injected into BALB/c mice, and blood samples were collected from mice *via* cardiac puncture after deep anesthesia at pre-determined times.

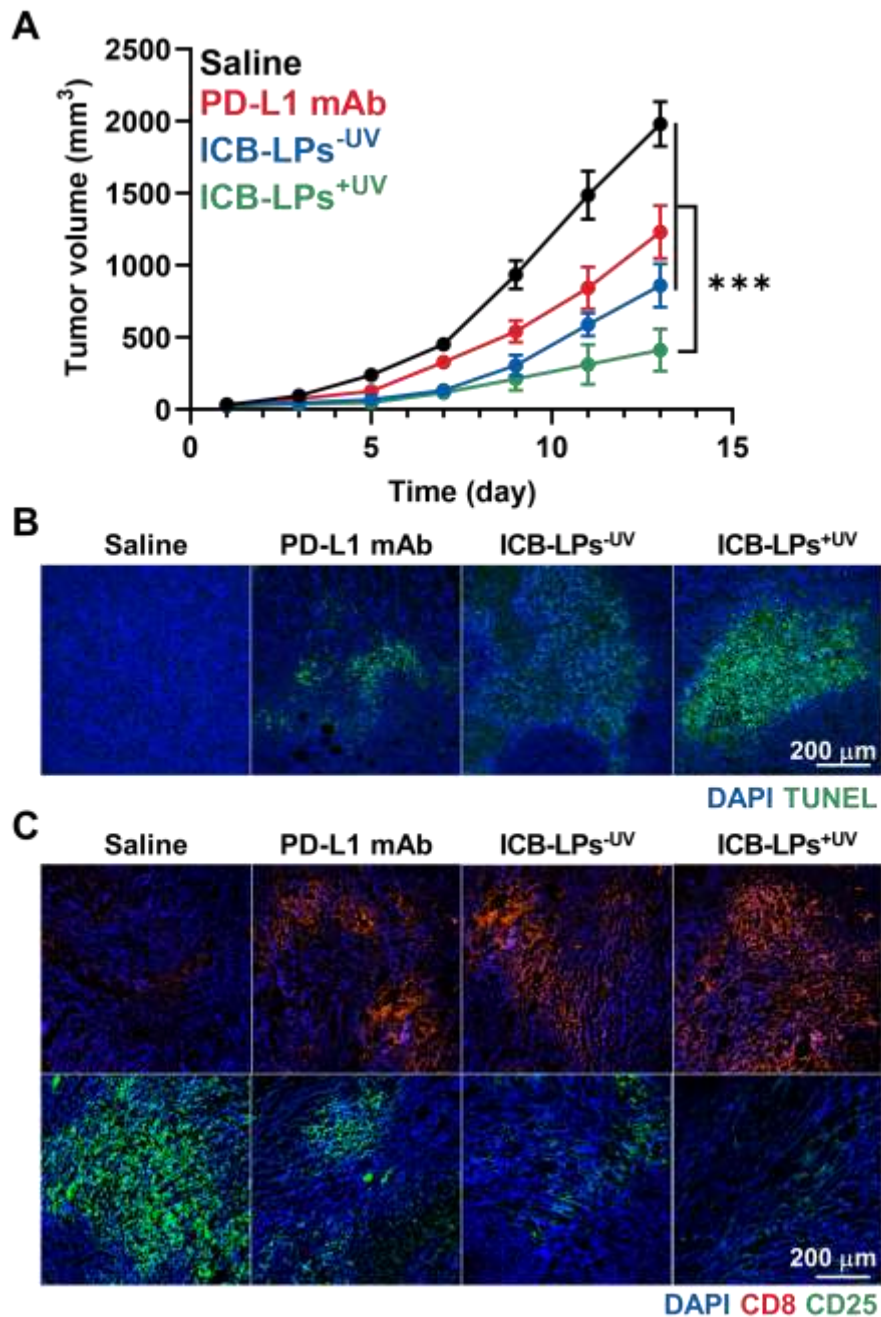
|                        | Area under the curves (AUC) | Half-life ( $t_{1/2}$ ) |
|------------------------|-----------------------------|-------------------------|
| PEG-LPs                | 84.18 ± 12.62               | 4.12 ± 0.42             |
| ICB-LPs <sup>-UV</sup> | 129.1 ± 17.03               | 6.55 ± 1.1              |
| ICB-LPs <sup>+UV</sup> | 199.6 ± 17.84               | 18.3 ± 2.1              |



**Figure S4** Tumor tissues stained with H&E on 18 days after treatment with saline, PD-L1 mAbs, ICB-LPs<sup>-UV</sup> or ICB-LPs<sup>+UV</sup>.



**Figure S5** The flow cytometric gating strategy to analyze the (A) cytotoxic T lymphocytes (CTLs; CD45<sup>+</sup>CD3<sup>+</sup>CD8<sup>+</sup>), (B) granzyme B (GrzB)-secreting CD8<sup>+</sup> T cells and (C) regulatory T cells (Treg; CD3<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup>) within the tumor tissues. FSC x SSC gating was used to obtain live cells and singlets based on granularity and size.



**Figure S6** (A) Tumor growth of 4T1 breast tumor-bearing mice during treatment ( $n=5$ ). Saline, PD-L1 mAbs (10 mg/kg), or equivalent 1 mg/kg dose of ICB-LPs<sup>-UV</sup> or ICB-LPs<sup>+UV</sup> were intravenously injected into the mice once every 3 days. (B, C) Tumor tissues stained with (B) TUNEL, or (C) CD8 or CD25 on 18 days after treatment with saline, PD-L1 mAbs, ICB-LPs<sup>-UV</sup> or ICB-LPs<sup>+UV</sup>. Data are presented as mean  $\pm$  SD ( $n = 5$ ); \*\*\* $P < 0.001$  vs. Control or indicated.