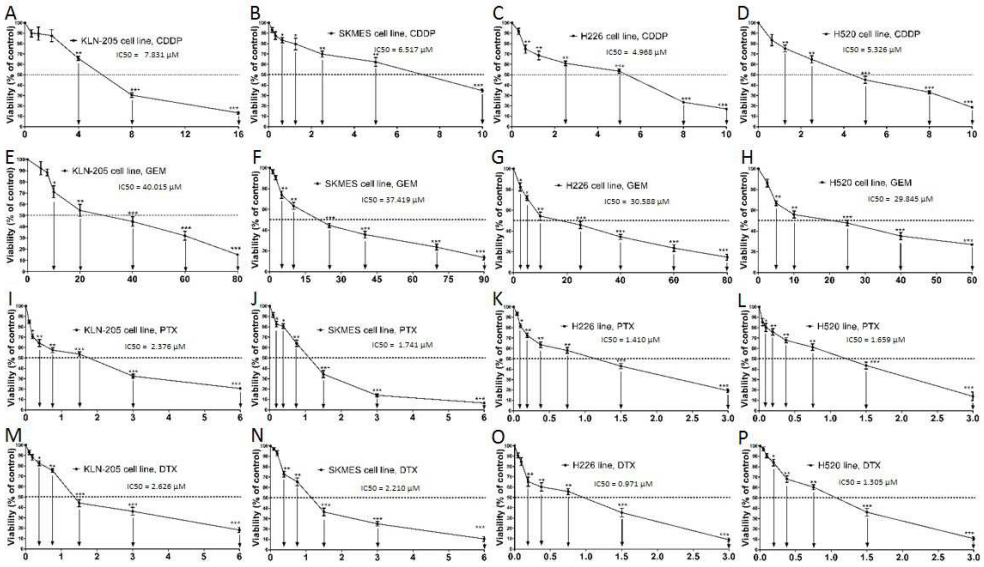
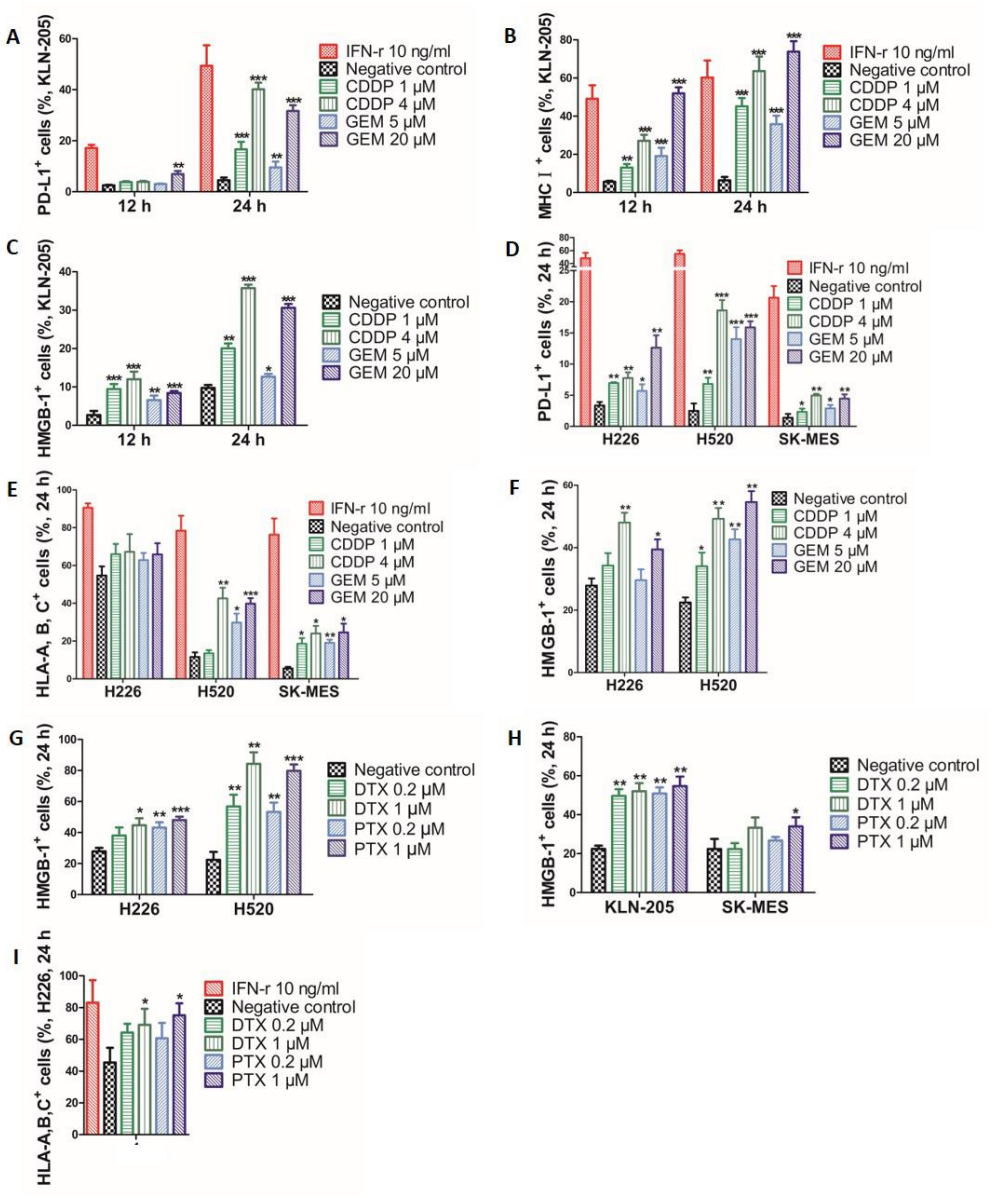
Supplementary Figures and Table

Contents:

Supplementary Figure S1-S6

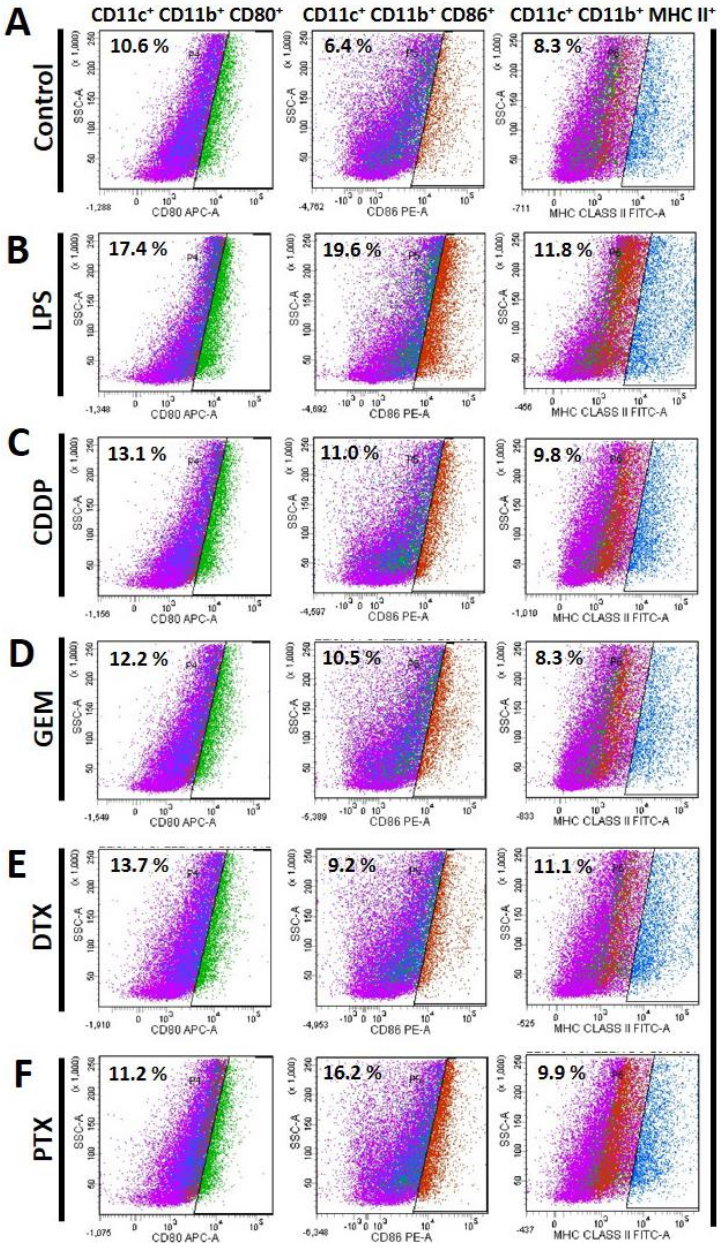


Supplementary Figure S1: Viability curves of SQCLC cell lines after treatment with different chemotherapeutic drugs, representing their comparative cytotoxicity. CDDP (cisplatin), GEM (gemcitabine), PTX (paclitaxel), DTX (docetaxel). **P* < 0.05; ***P* < 0.01; ****P* < 0.001.



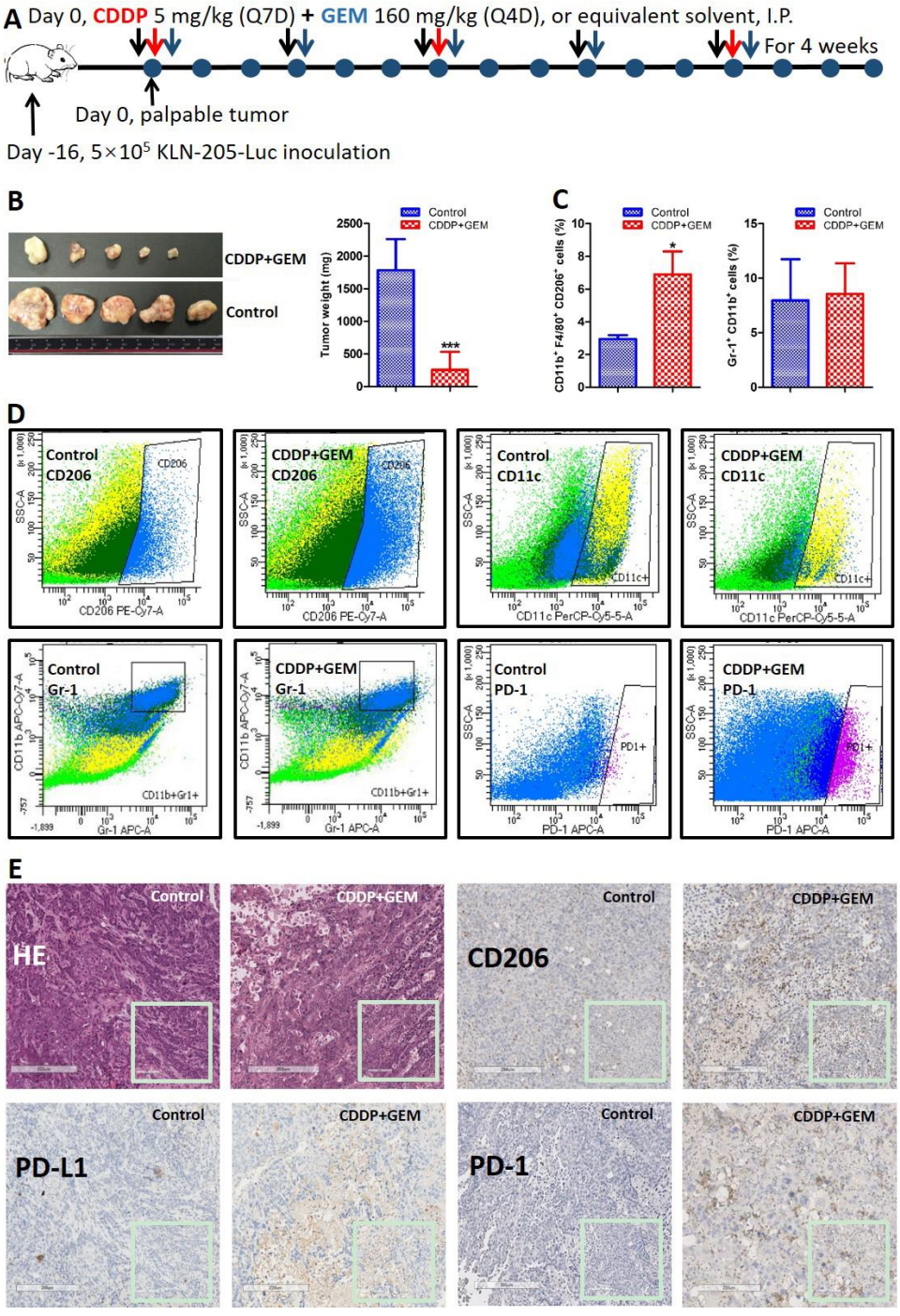
Supplementary Figure S2: Low-dose chemotherapy can induce immunogenic cell death (ICD) *in vitro*. The changes of several ICD markers after low-dose chemotherapeutics drugs were assessed. As the positive control we used 10 ng/ml interferon gamma (IFN-γ), which upregulates the expressions of PD-L1, MHC-Class I, and HLA-A,-B,-C. As the negative control, we utilized an equivalent volume of the solvent which was used to dissolve chemotherapy agents. A–C Percentage of murine SQCLC KLN-205 cells expressing (A) cell surface PD-L1, (B) cell surface

MHC-Class I, and (C) cytoplasmic HMGB-1, by flow cytometric analysis, after treatment with CDDP, GEM, or controls for 12 or 24 h. (D–F) Percentage of human SQCLC cell lines (H226, H520, SK-MES) expressing (D) cell surface PD-L1, (E) cell surface HLA-A,-B,-C, and (F) cytoplasmic HMGB-1 by flow cytometric analysis, after incubating with indicated CDDP, GEM, or controls for 24 h. (G-I) Percentage of SQCLC cells expressing cytoplasmic HMGB-1 (G-H) and HLA-A, -B, -C (I) detected by flow cytometric analysis, after incubating 24 h with indicated DTX, PTX, or controls. Data are presented as mean ± SD; **P* < 0.05, ***P* <0.01, ****P* < 0.001 for all other treatments vs. negative control.



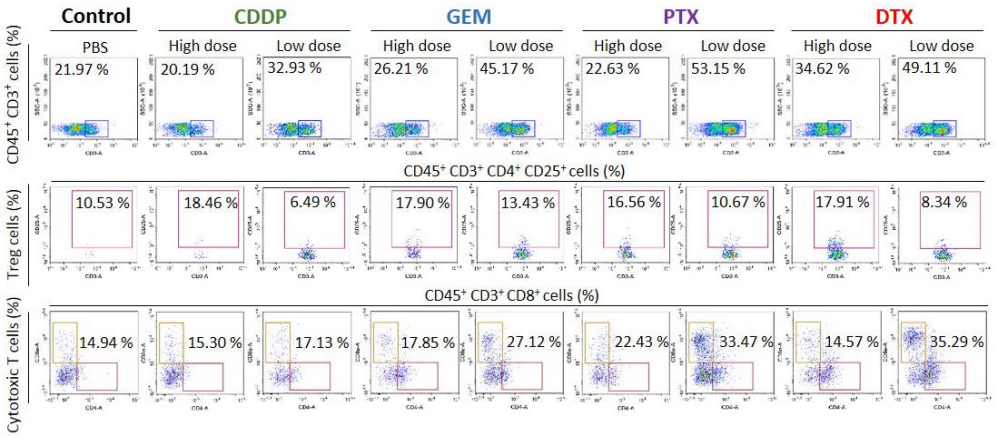
Supplementary Figure S3: KLN-205-Luc cells were treated with low-dose chemotherapeutic agents for 30 h, after which the conditioned culture media were dialyzed to remove any residual chemotherapeutics. Immature BMDCs were then incubated with the dialyzed media for a further 24 h, and the expression of the mature DC surface markers $CD11c^+$ $CD11b^+$ $CD80^+$, $CD11c^+$ $CD11b^+$ $CD86^+$, $CD11c^+$ $CD11b^+$ MHC class II^+ was assessed by flow cytometric analysis

(representative images).

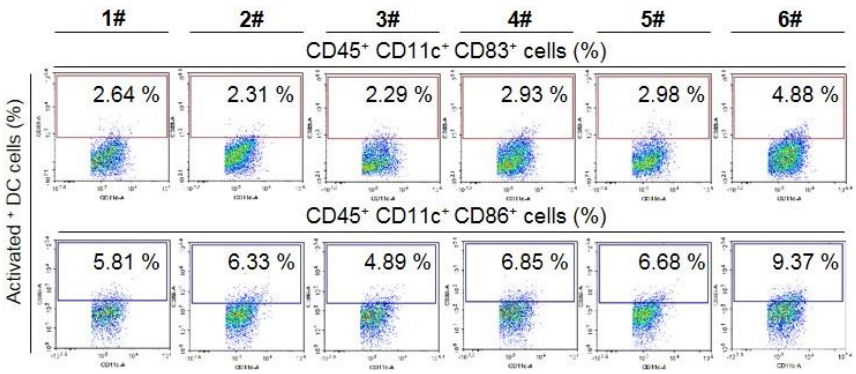


Supplementary Figure S4: High-dose chemotherapy inhibits tumor growth, but also results in significant immunosuppression *in vivo*. (A) Chemotherapy treatment scheme. (B) High-dose

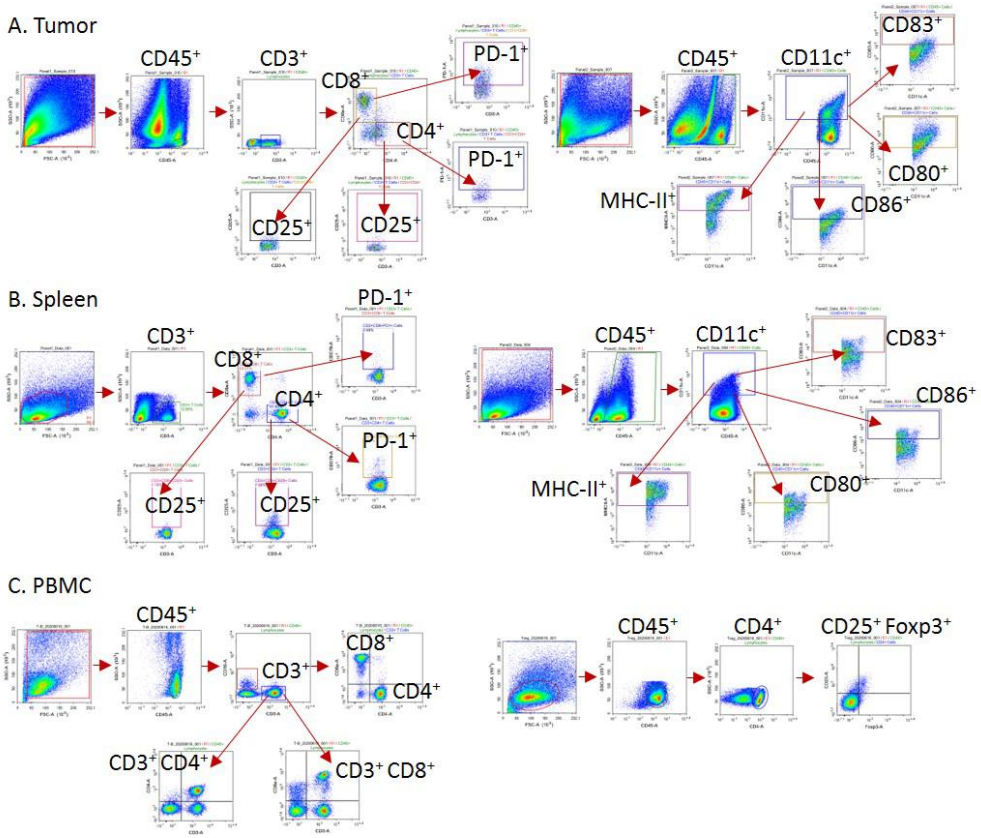
CDDP (5 mg/kg, Q7D) plus GEM (160 mg/kg, Q4D) significantly inhibited tumor growth (P < 0.001) compared with the negative control. (C-E) High-dose CDDP plus GEM increased the percentage of $CD11b^+$ $F4/80^+$ $CD206^+$ cells (immunosuppressive type II TAMs) and PD-1/$PD\text{-}L1^+$ cells. Data are presented as mean ± SD. N.S. no significance, *P < 0.05; **P < 0.01; ***P < 0.001.



Supplementary Figure S5: High-dose SQCLC mono-chemotherapy agents cause greater immunosuppression compared to low-dose regimens *in vivo*. Mice received an intraperitoneal injection of low-dose CDDP 2.8 mg/kg, high-dose CDDP 8.4 mg/kg, low-dose GEM 60 mg/kg, high-dose GEM 240 mg/kg, low-dose PTX 11 mg/kg, high-dose PTX 33 mg/kg, low-dose DTX 11 mg/kg, high-dose DTX 33 mg/kg, or vehicle, respectively. After 30 h, tumors were harvested for flow cytometric analysis (representative images).



Supplementary Figure S6: Combined with anti-PD-1 mAb, sequential low-dose CDDP exerts more obvious synergistic antitumor effects than MTD CDDP. Single-cell spleen suspensions were used for flow cytometric analysis. The representative images of $CD11c^+$ $CD83^+$ cells and $CD11c^+$ $CD86^+$ cells after treatment. 1#: Negative control; 2#: Anti-PD-1 mAb alone; 3#: MTD CDDP (8.4 mg/kg, Q14D) alone; 4#: MTD CDDP (8.4 mg/kg, Q14D) combined with anti-PD-1 mAb alone; 5#: Low-dose CDDP (2.8 mg/kg, Q7D) alone; 6#: Low-dose CDDP (2.8 mg/kg, Q7D) alone combined with anti-PD-1 mAb alone.



Supplementary Figure S7: Gating strategy for flow cytometric analysis of tumor and spleen-infiltrating lymphocytes, as well as PBMCs.