

Suppl Figure 1: Tumor progression and health condition of mice treated with PEDD nelitolimod and CPIs

A. Eight- to twelve-week-old C57/BL6 mice were inoculated with 10^6 MC38-Luc cells/mouse and after 7 days mice were treated Sys α -PD-1. PBS served as Veh control and was administered via PV. Fold change of the tumor burden was calculated based on D0 baseline bioluminescence. The differences between these two groups were statistically different. B. Weight of individual mice was monitored from the day of tumor inoculation (D-7) to the day of sacrifice (D10). The percentage weight change of mice was plotted here. C. Sequential IVIS images of each mouse from D0 to D10 were assembled. D. Representative Trichrome Mason- stained image of LM tissue of Sys group to demonstrate small nodules of fibrotic tumors.

Suppl Figure 2: Gating strategy for flow cytometry analysis

A. Gating strategy to evaluate MDSC, M/G MDSC, Dendritic Cells, B, T cells and M1-like & M2-like macrophages from CD45⁺, isolated from LM. B. Ratio of M1 and M2-like macrophages. n denotes the number of mice per group. Data presented as mean \pm SEM and n is mentioned in the individual graph.

Suppl Figure 3: Liver function test

Circulatory level of ALP (A) and ALT (B), n denotes number of mice per group. Normal ranges for C57/BL6 ALP: 16-200 U/L, ALT: 22-133 U/L.

Suppl Figure 4: Targeted transcriptomic analysis (Volcano plot and cell score)

Total RNA was isolated from the CD45⁺ cells of LM, and NanoString analysis was performed using murine myeloid and innate panel. A. The volcano plot illustrated the log₂ fold changes of gene expression in α-PD-1 Ctrl vs. Veh (top-left); nelitolimod vs. Veh (top-right); SQ vs. Veh (bottom-left); Sys vs. Veh (bottom-right). B. The graph shows nelitolimod mono or combination therapy enhanced cytotoxic T cells in the tumor. Each group contains at least three samples to perform NanoString analysis.

Suppl Figure 5: Tumor progression of treated mice until day 35

The tumor progression of the mice that were undergoing survival study was monitored and reported as a fold change in the tumor burden as compared to D0. Each dot of the corresponding color represents the tumor progression of each mouse of the respective groups.