

Supplementary data

Dually Regulating the Proliferation and the Immune Microenvironment of Melanoma *via* Nanoparticle-Delivered siRNA Targeting Onco-Immunologic CD155

Yan Wang, Ying-Li Luo, Yi-Fang Chen, Zi-Dong Lu, Yue Wang, Anna Czarna, Song Shen, Cong-Fei Xu,* Jun Wang**

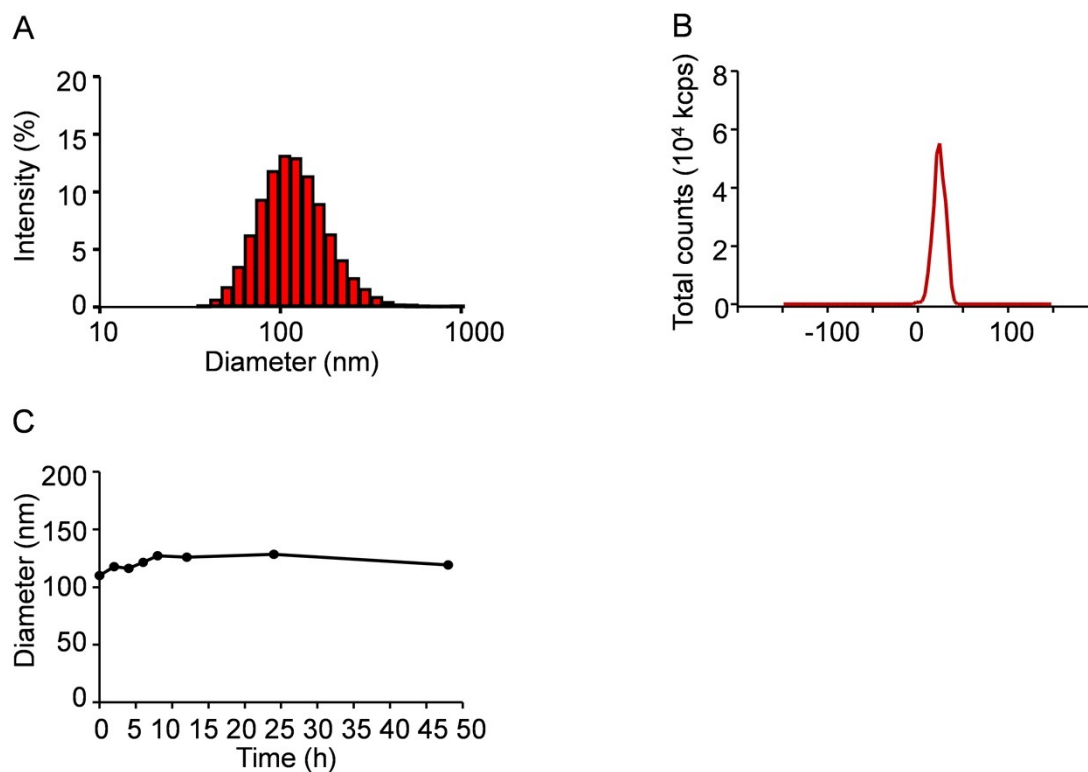


Fig S1. The characterizations of CLAN_{Cy5-siRNA}. (A-C) Size distribution (A), zeta potentials (B), and serum stability (C) of CLAN_{Cy5-siRNA}.

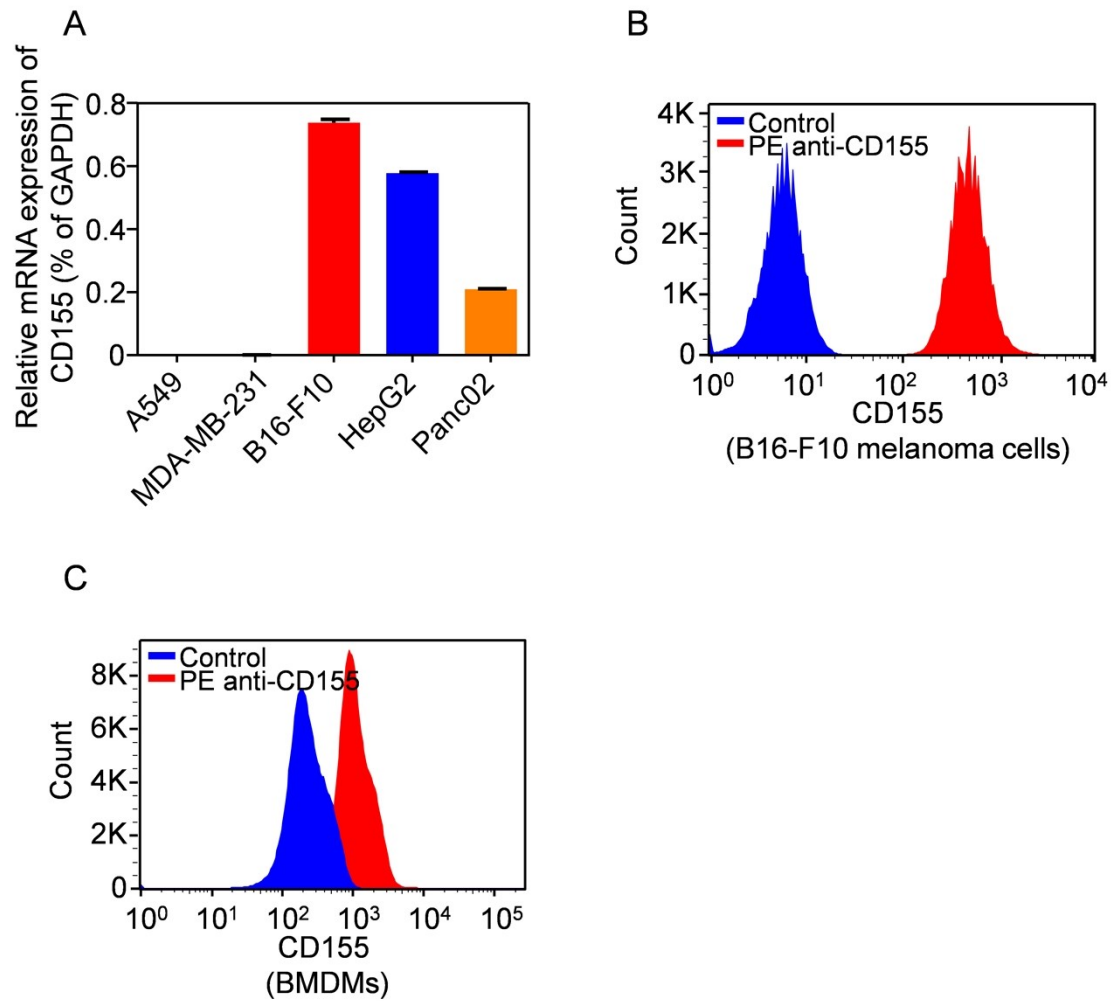


Fig S2. (A) The relative mRNA expression of CD155 in different tumor cell lines. (B and C) Flow cytometry analysis of CD155 expression in B16-F10 melanoma cells (B) and BMDMs (C). CD155 was labelled with PE anti-mouse CD155 antibody.

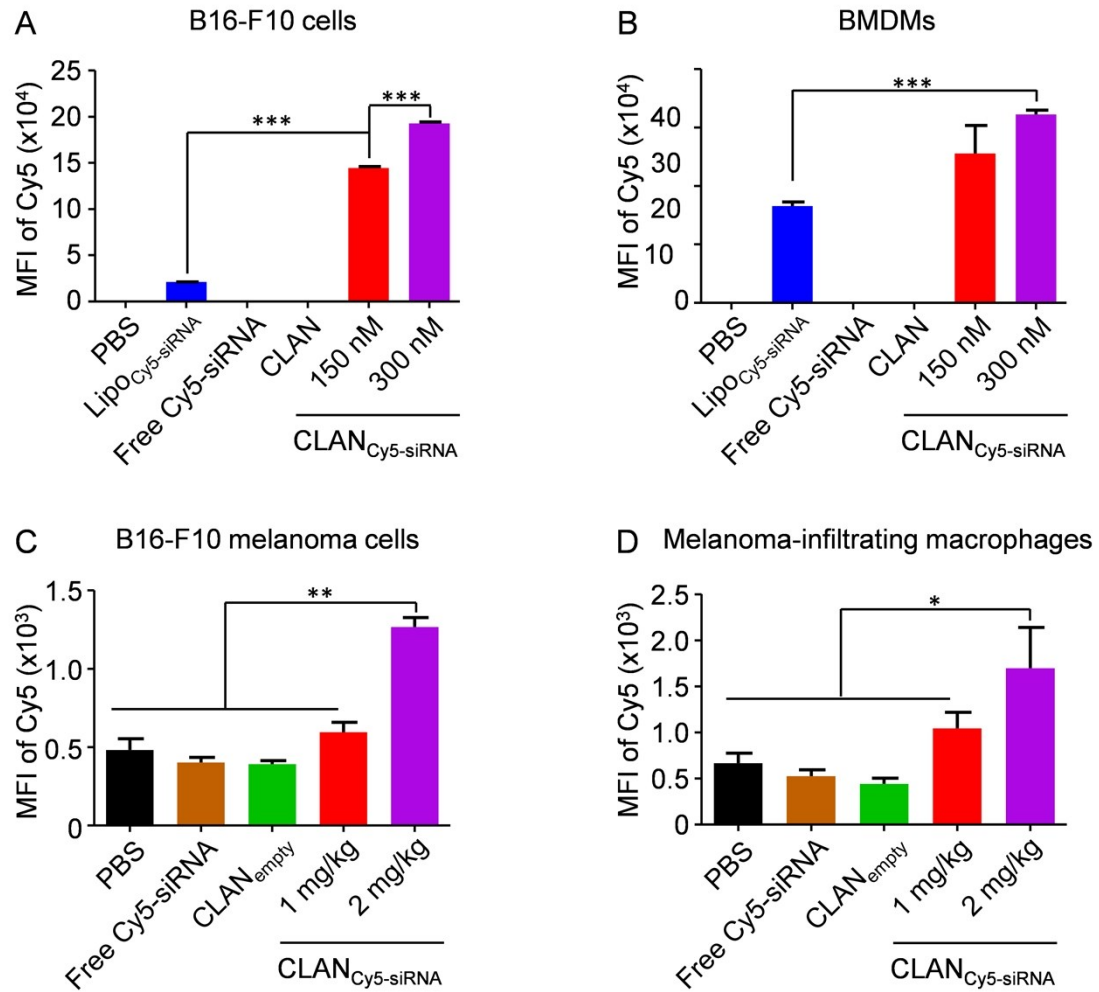


Fig S3. Cellular uptake of CLAN_{Cy5-siRNA} by melanoma cells and macrophages *in vitro* and *in vivo*. (A, B) MFI of Cy5 fluorescence in B16-F10 cells (A) and BMDMs (B) after transfection of CLAN_{Cy5-siRNA} or other controls *in vitro*. (C, D) MFI of Cy5 fluorescence in B16-F10 melanoma cells and melanoma-infiltrating macrophages after intravenous injection of CLAN_{Cy5-siRNA} or other controls. Data are shown as the means \pm SD (n = 3), one-way ANOVA with post-hoc analysis, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

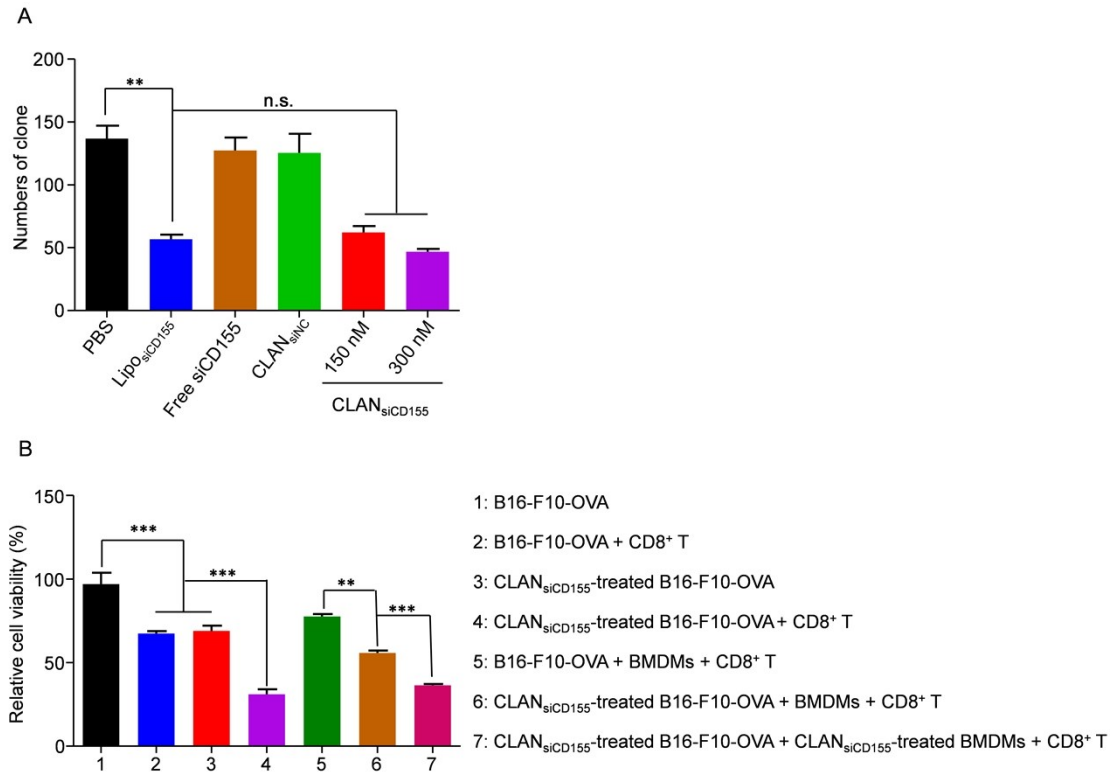


Fig S4. Cell clone formation assay of B16-F10 cells and the cytotoxicity of antigen-specific CD8⁺ T cells to B16-F10-OVA cells. (A) Statistical analysis of the numbers of B16-F10 cell clone in Fig. 3F. (B) The relative cell viability of B16-F10-OVA cells after co-culture with CD8⁺ T cells. B16-F10-OVA cells or BMDMs were treated or not with CLAN_{siCD155} before co-culture. Data are shown as the means \pm SD (n = 3), one-way ANOVA with post-hoc analysis, ** $P < 0.01$, *** $P < 0.001$ and n.s. $P > 0.05$.

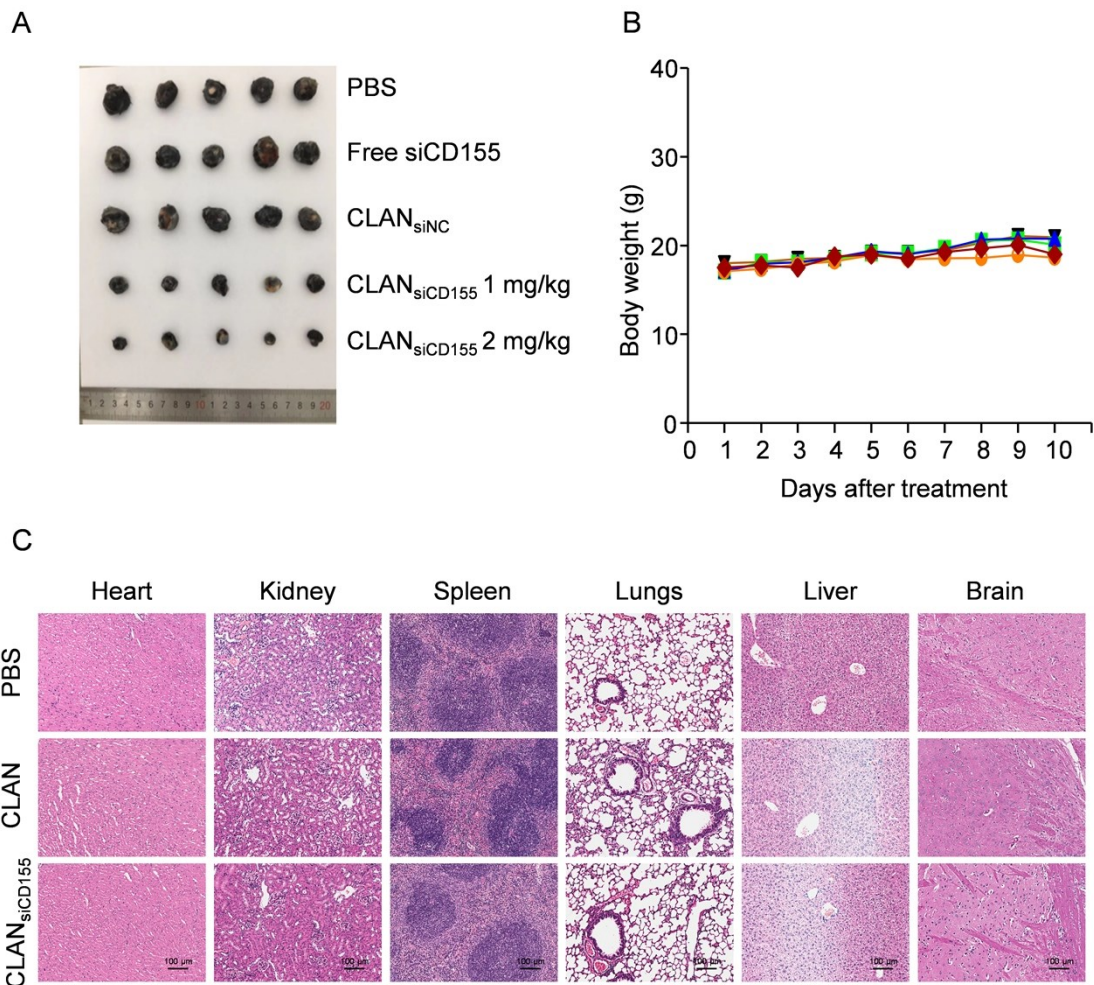


Fig S5. (A) Tumor images of melanoma isolated from B16-F10-bearing mice at the end of the treatments. (B) Body weight change of B16-F10-bearing mice during the treatments. Data are shown as the means \pm SD ($n = 5$). (C) H&E analysis of heart, kidney, spleen, lungs, liver and brain tissue after the treatment of CLAN or CLAN_{siCD155}.

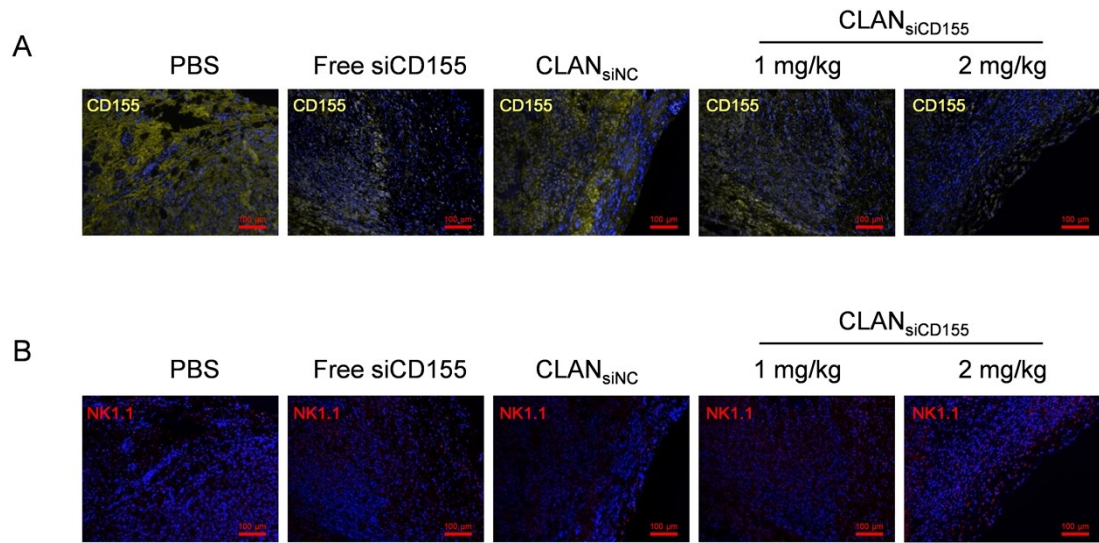


Fig S6. Original staining images of CD155 (A) and NK1.1 (B) from multispectral immunofluorescence assay.