Targeting tumors with IL-21 reshapes the tumor microenvironment by proliferating PD-1^{int}Tim-3^{-}CD8^{+} T cells

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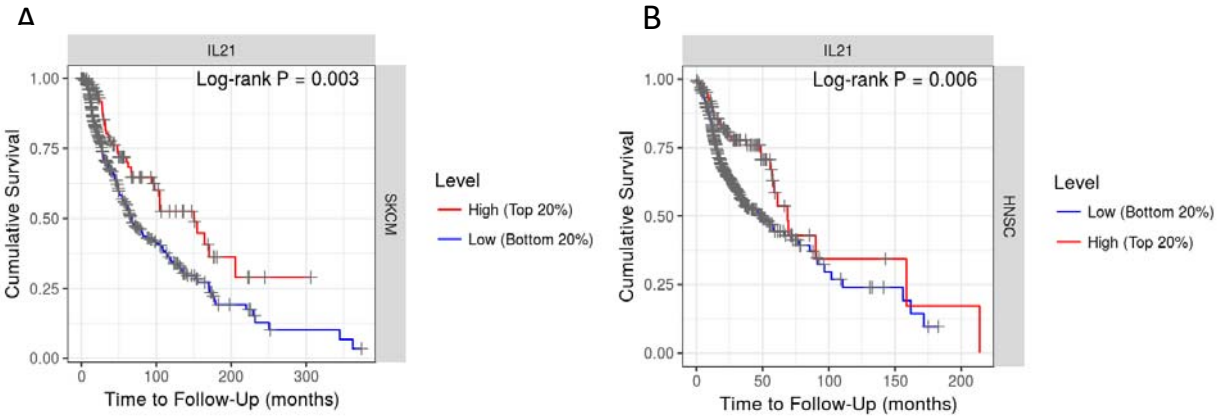
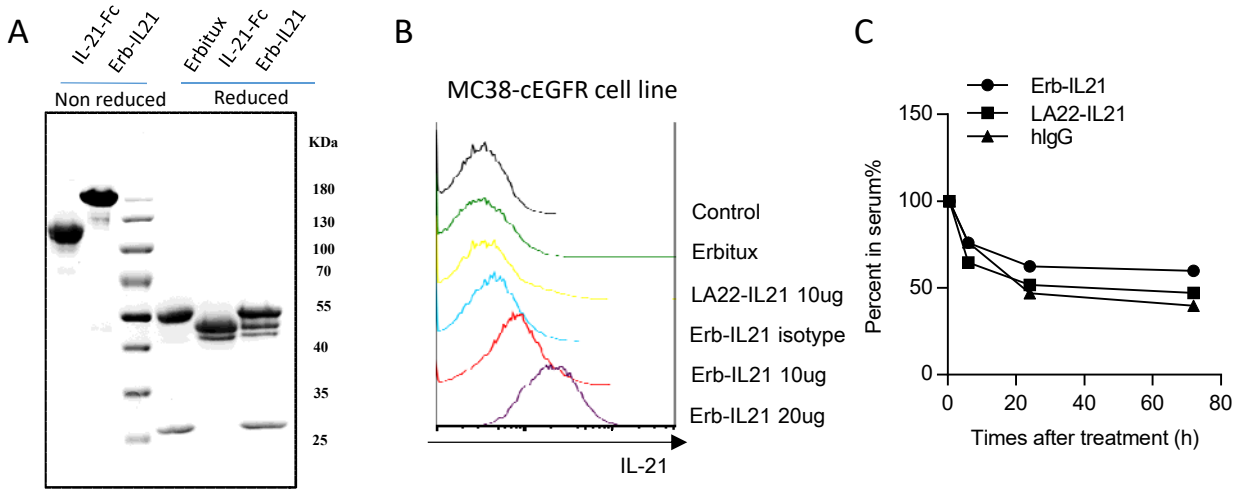


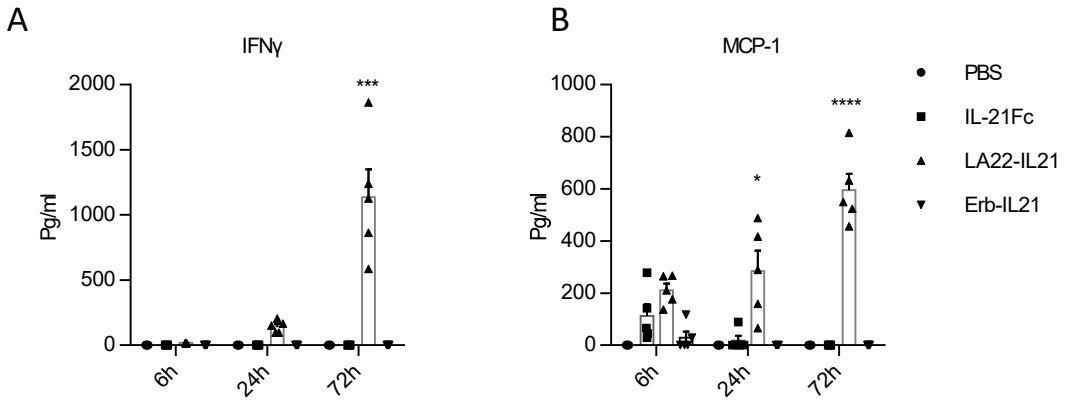
Supplementary Figure 1: Higher levels of IL-21 in tumors correlate with improved prognosis

(A) Cumulative survival curve of skin cutaneous melanoma (SKCM) from the TIMER data based on IL21 expression. (B) Cumulative survival curve of head-neck squamous cell carcinoma (HNSC) from the TIMER website based on IL-21 expression.



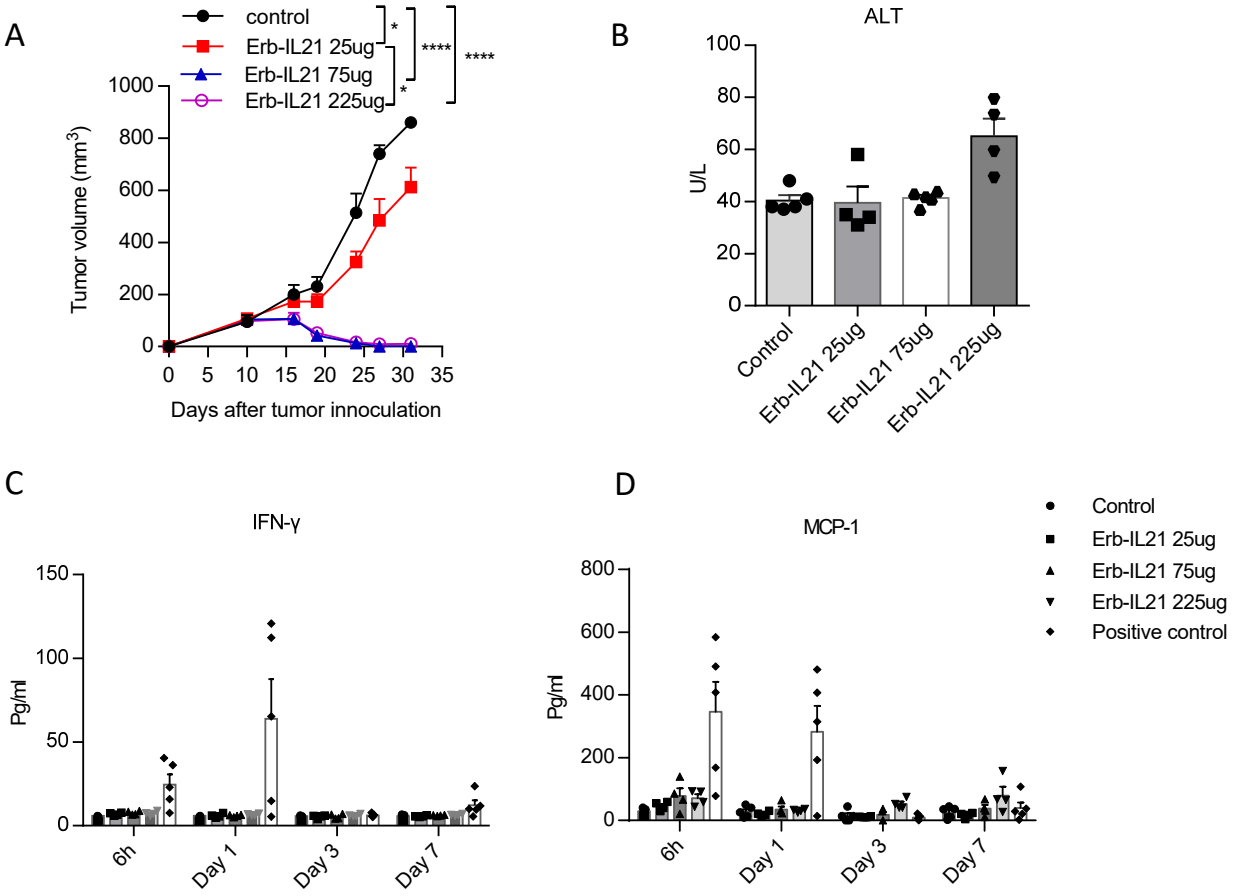
Supplementary Figure 2: The characterization of Ab-IL21 fusion proteins

(A) SDS PAGE of Erbitux, IL-21-Fc, and Erb-IL21. (B) MC38-cEGFR cells were incubated with Erbitux, LA22-IL21, or different concentrations of Erb-IL21, followed by anti-mouse IL21-APC staining. (C) Mice (n=3-4) were injected i.v with 10 µg of the indicated fusion proteins. Protein concentrations in serum at different time points were measured by ELISA. The mean SEM values are shown.



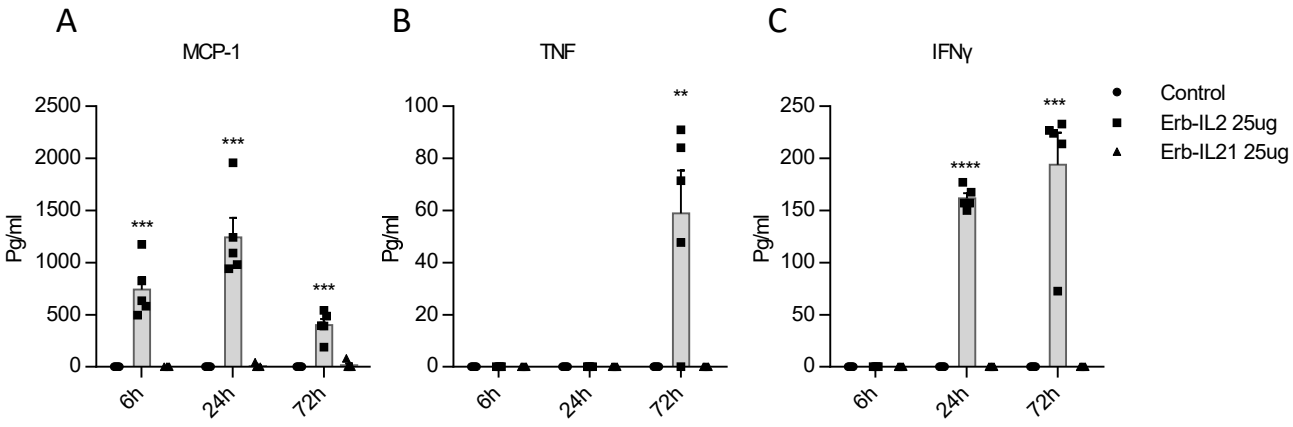
Supplementary Figure 3: ERB-IL21 has lower toxicity than LA22-IL21

(A) Tumor-bearing mice (n=5) were i.v injected with 30 µg IL-21-Fc, 75 µg LA22-IL21, or ERB-IL21 on day 10. At the indicated time points on the x-axis. The IFNγ concentration in serum was measured using a BD CBA kit. One of two representative experiments is shown. (B) Tumor-bearing mice (n=5) were i.v injected with 30 µg IL-21-Fc, 75 µg LA22-IL21, or ERB-IL21 on day 10. At the indicated time points, the MCP-1 concentration in serum was measured by a BD CBA kit. The mean SEM values are shown. Unpaired T-tests were used to analyze the other data, n.s (not significant). *P<0.05, **P<0.01, ***P<0.0001 and ****P<0.0001. One of two representative experiments is shown.



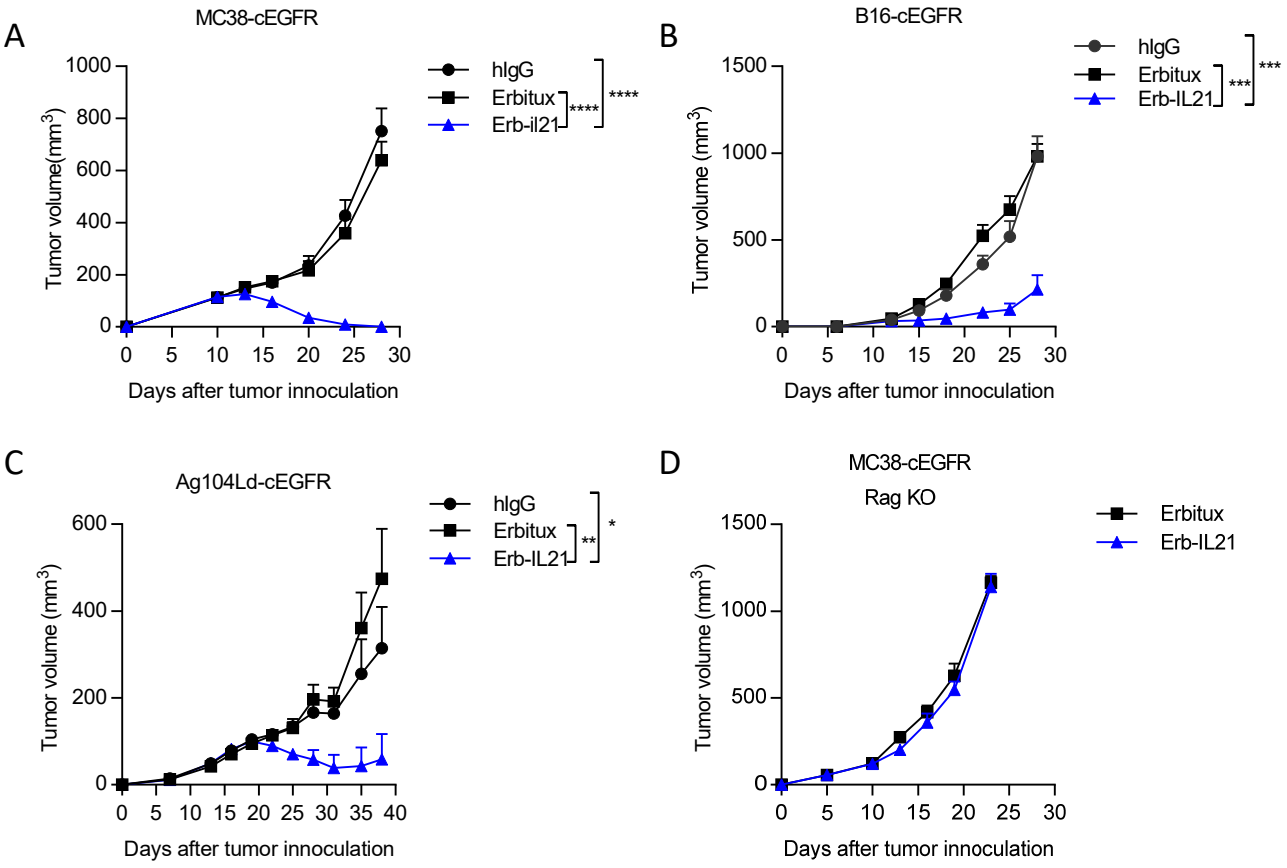
Supplementary Figure 4: Erb-IL21 treatment effect is dose-dependent.

C57BL/6 mice (n=4-5) were inoculated with 2.5x105 MC38-cEGFR cells, then i.p treated with the indicated ERB-IL21 on days 10, 13, and 16. Mice without treatment served as the control. (A) Tumor growth curve. (B) Serum ALT was measured on day 7 after the first treatment. (C, D) At the indicated time points, the concentration of the indicated cytokines in serum was measured by a BD CBA kit. The mean SEM values are shown. Two way ANOVA tests were used to analyze the tumor growth data and unpaired T-tests were used to analyze the other data. ns (not significant), *P<0.05, **P<0.01, ***P<0.0001 and ****P<0.0001. One of two representative experiments is shown.



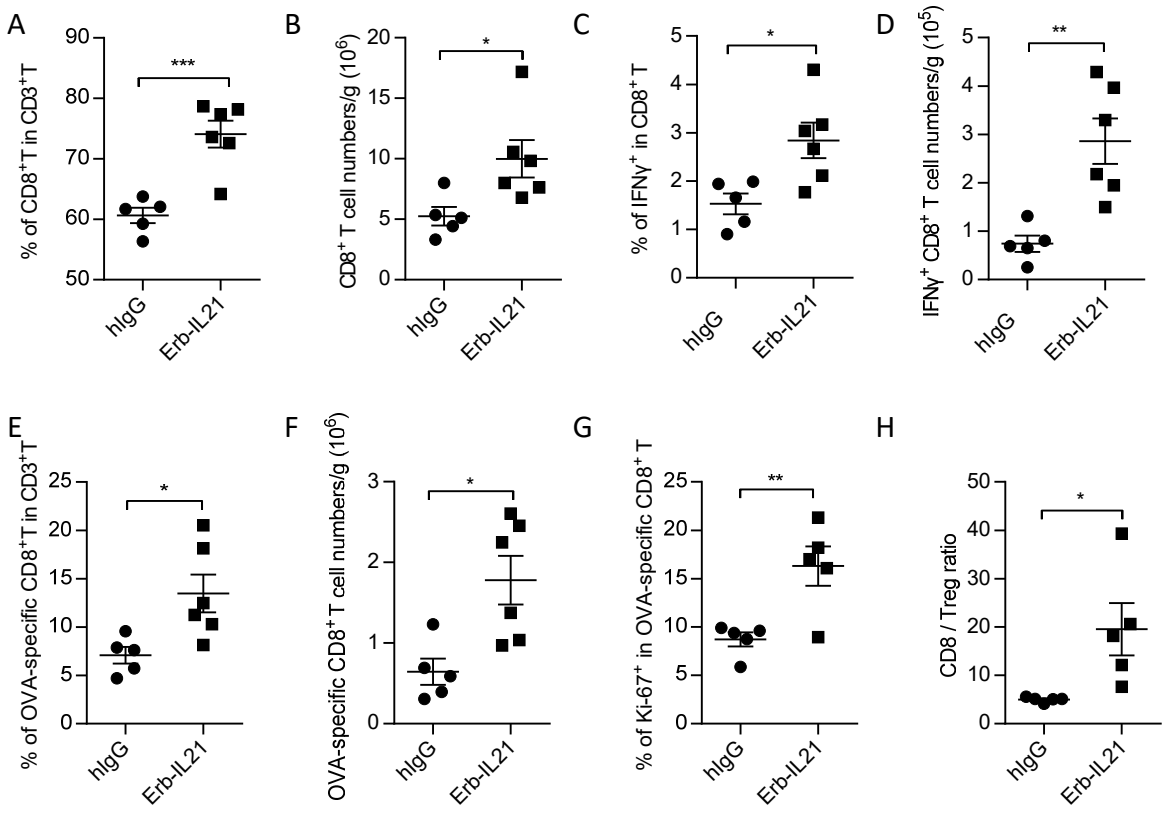
Supplementary Figure 5: Erb-IL21 is significantly less toxic than Erb-IL2

Tumor-bearing C57BL/6 mice (n=5) were inoculated with $3x10^5$ MC38-cEGFR cells on day 0 and were i.p treated with 25 µg Erb-IL21 or Erb-IL2 on days 11, 14, and 17. Mice without treatment are designated as the control. (A-C) At the indicated time points after the first treatment, the concentration of MCP-1, TNFα, and IFNγ in serum was measured by a BD CBA kit. The mean SEM values are shown. Unpaired T-tests were used to analyze the other data. ns (not significant), *P<0.05, **P<0.01, ***P<0.0001 and ****P<0.0001. One of two representative experiments is shown.



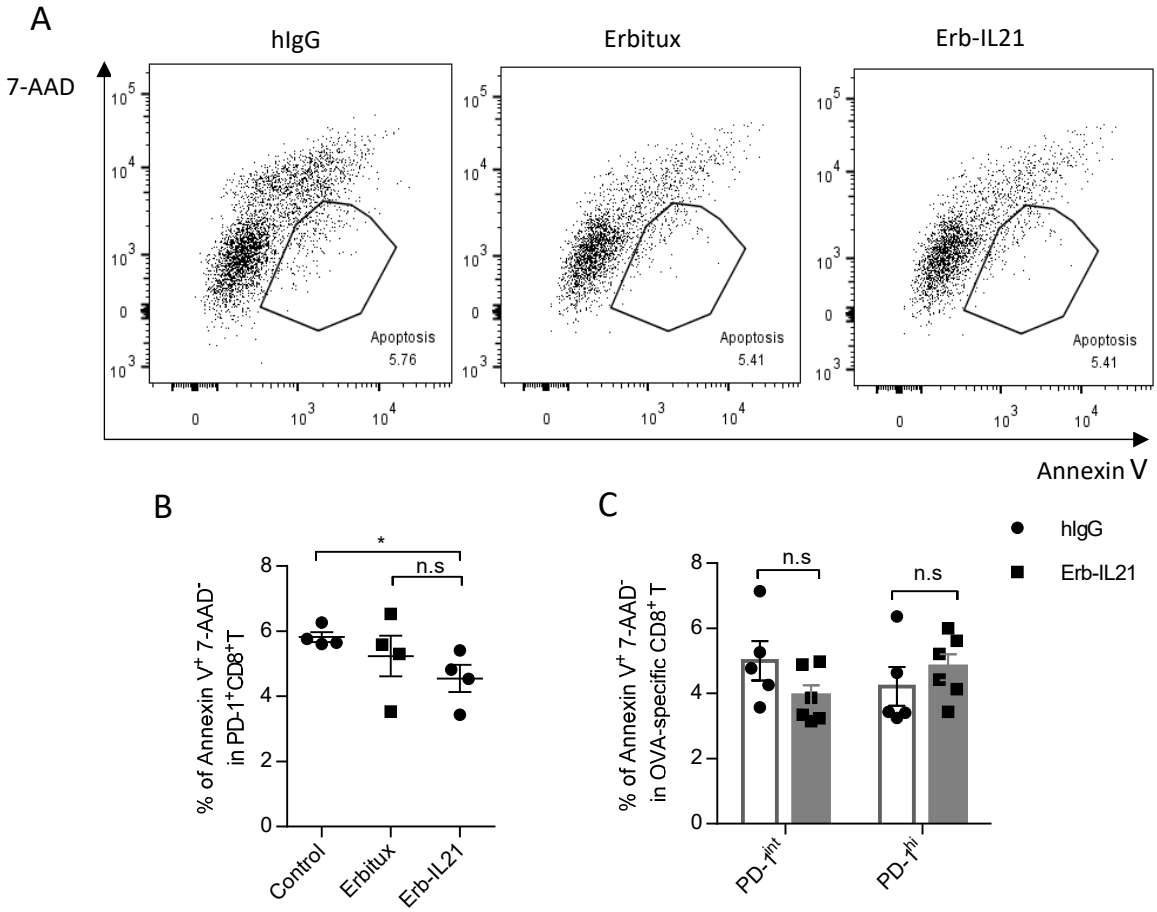
Supplementary Figure 6: Erb-IL21 enhances the antitumor effect in different tumor models and depends on the adaptive immune system

(A, B) C57BL/6 tumor-bearing mice (n=4-6) were i.p treated with 75 µg hIgG, Erbitux and Erb-IL21 on days 10, 13, and 16. (C) B6C3F1 mice (n=4-6) were I. P treated with 75 µg of hIgG, Erbitux and Erb-IL21 on days 13, 16, and 19. (D) Rag mice (n=4-5) were inoculated with $2.5x10^5$ MC38-cEGFR cells on day 0 and i.p treated with 75 µg hIgG, Erbitux or Erb-IL21 on days 10, 13, and 16. Tumor growth was measured and compared twice weekly. The mean SEM values are shown. Two-way ANOVA tests were used to analyze the tumor growth data, *P<0.05, **P<0.01, ***P<0.0001, and ****P<0.0001. One of two representative experiments is shown.



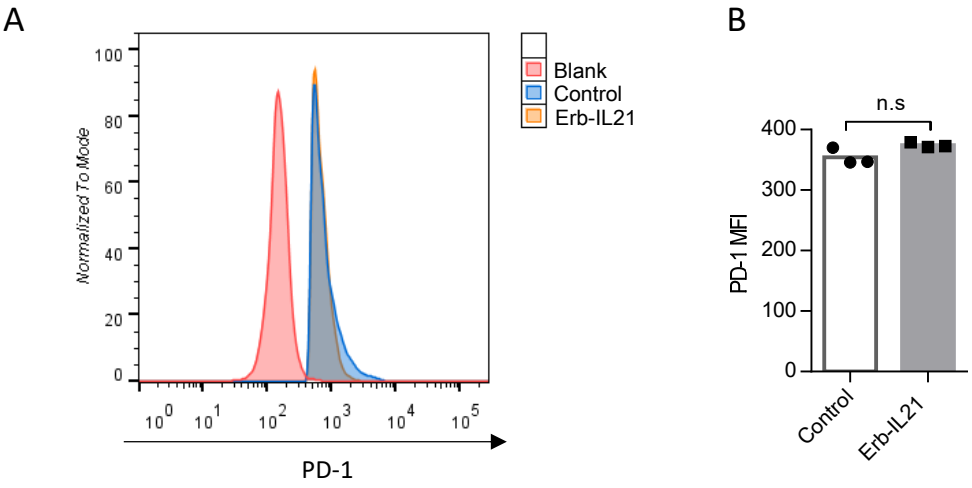
Supplementary Figure 7: IL-21 increases antigen-specific CD8$^+$ T cells in tumors.

C57BL/6 mice (n=5-6) were inoculated with 6-8x10^5 MC38-OVA cells and were i.t treated with 20 µg hIgG or Erb-IL21 on days 12 and 15. Five days after the first treatment, tumor tissues were analyzed by flow cytometry. (A) Percentage of CD8$^+$ T cells in CD3$^+$ T cells. (B) Total cell number of CD8$^+$ T cells per gram of tumor. (C) Percentage of IFNγ$^+$ in CD8$^+$ T cells. (D) Total cell number of IFNγ$^+$ CD8$^+$ T cells per gram of tumor. (E) Frequency of antigen-specific (OVA $^+$) in CD8 $^+$ T cells. (F) Total cell numbers of antigen-specific CD8$^+$ T cells per gram of tumor. (G) Percentage of Ki-67$^+$ in antigen-specific (OVA-specific) CD8$^+$ T cells. (H) The ratio of CD8$^+$ T cells to Treg cells in tumors. The mean SEM values are shown. Unpaired T-tests were used to analyze the other data, n.s. (not significant), *P<0.05, **P<0.01, ***P<0.0001 and ****P<0.0001. One of two representative experiments is shown.



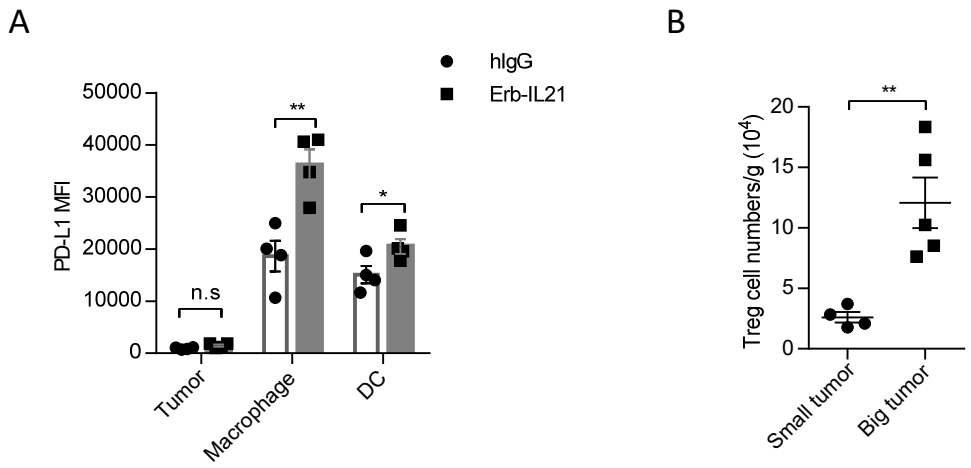
Supplementary Figure 8: IL-21 did not promote apoptosis of PD-1^{+}CD8^{+} T cells

(A, B) CD8^{+} T cells were sorted from mouse spleens and co-cultured with 0.2 µg anti-CD3 and 0.2 µg anti-CD28, with an additional 100 ng Erbitux or Erb-IL21 for 72 h, hIgG treatment as a control, n=3 independent wells. (C) C57BL/6 mice (n=5-6) were inoculated with 6-8x10^{5} MC38-OVA cells and were i.t treated with 20 µg hIgG or Erb-IL21 on days 12 and 15. Five days after the first treatment, T cells in the tumor tissues were analyzed by flow cytometry. Percentage of Annexin V^{+} 7-AAD^{-} of PD-1^{int} or PD-1^{high} antigen-specific CD8^{+} T cells was shown. The mean SEM values are shown. Unpaired T-tests were used to analyze the other data, n.s. (not significant), *P<0.05, **P<0.01, ***P<0.0001 and ****P<0.0001.



Supplementary Figure 9: PD-1 expression on PD-1$^+$CD8$^+$ T cells remains stable.

CD8$^+$ T cells were isolated from spleens of WT mouse by CD8 negative sorting kit, and cultured with anti-CD3 and anti-CD28 2ug/ml for 48h, followed by incubation with or without 100ng/ml Erb-IL21 for 48h, n=3 independent wells. (A) CD8$^+$ T cells were analyzed by flow cytometry. (B) MFI of PD-1 on CD8$^+$ T cells. MFI is defined as the geometric median fluorescence intensity. The mean SEM values are shown. Unpaired T-tests were used to analyze the other data, n.s. (not significant), *P<0.05, **P<0.01, ***P<0.0001 and ****P<0.0001.



Supplementary Figure 10: Advanced tumors express higher levels of immune inhibitory markers

(A) C57BL/6 mice (n=4-5) were inoculated with $2.5x10^5$ MC38-cEGFR cells and were i.p treated with 75 µg hIgG or Erb-IL21 on days 15, 18, 21. Seven days after the first treatment, PD-L1 on tumor cells, macrophages ($CD45^+CD11b^+F4/80^+Ly6C^-$), and DCs ($CD45^+CD11c^+F4/80^-Ly6C^-$) were analyzed by flow cytometry. (B) Treg cells in either small (<150mm3) or large (>250mm3) MC38-cEGFR tumors were analyzed by flow cytometry. The mean SEM values are shown. Unpaired T-tests were used to analyze the other data, n.s. (not significant), *P<0.05, **P<0.01, ***P<0.0001 and ****P<0.0001.