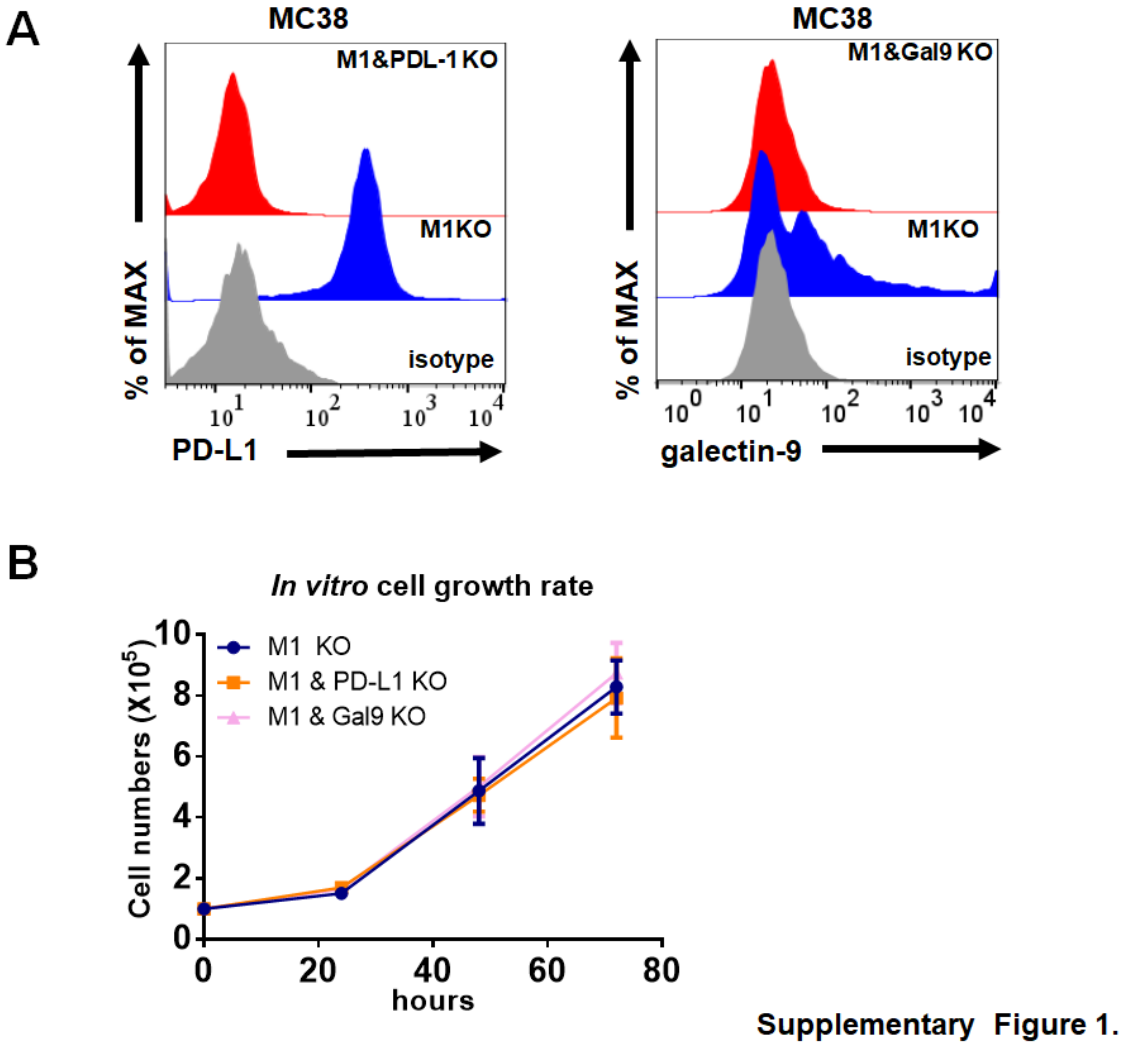
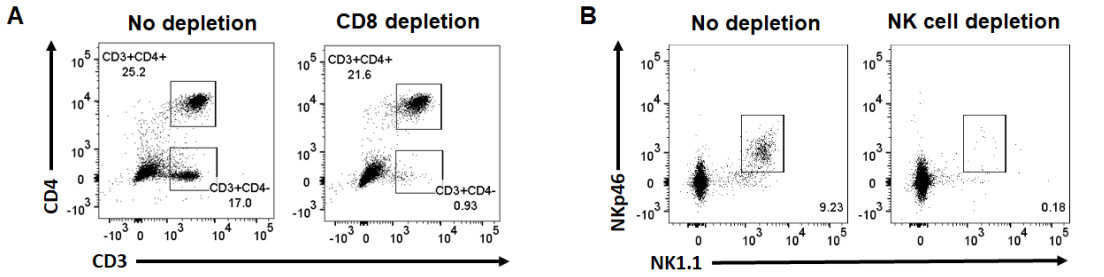
Supplemental Figures

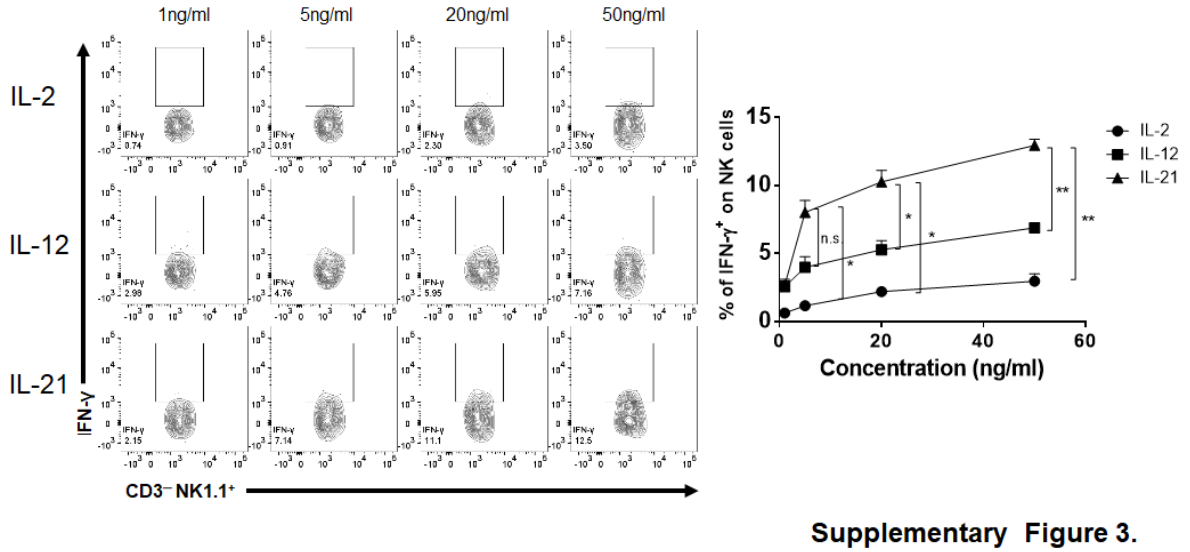


Supplementary Figure 1. PD-L1 or galectin-9 knock-out MC38 MHC class I-deficient tumor cells. (A) The expression levels of PD-L1 and galectin-9 on MC38 MHC class I-deficient tumor cells, MC38 MHC class I-deficient PD-L1 knock-out (M1 & PD-L1 KO) tumor cells or MC38 MHC class I-deficient galectin-9 knock-out (M1 & Gal9 KO) tumor cells were measured by flow cytometry. **(B)** *In vitro* tumor cell growth was measured after $1x10^6$ cells were cultured in a 100-pi cell culture dish. The data shown are from at least 2 individual experiments with similar results.



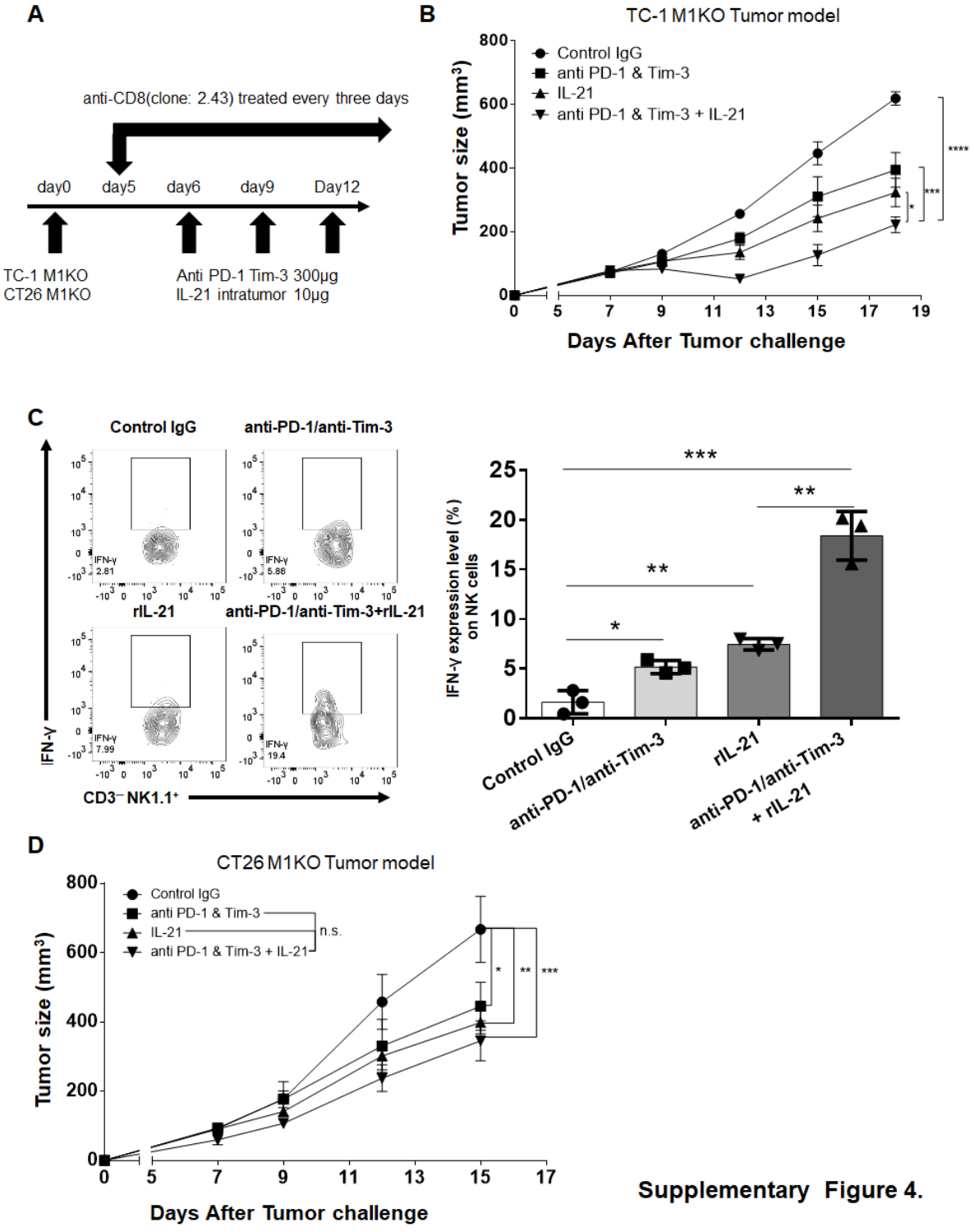
Supplementary Figure 2.

Supplementary Figure 2. Confirmation of CD8 T cell or NK cell depletion by depleting antibody treatment. Tumor-bearing mice were treated with anti-CD8 or anti-NK1.1 antibody every three days to deplete CD8 T cells or NK cells starting from day 5 after tumor injection. CD8 T cells ($CD3^+CD4^-$) **(A)** or NK cells ($NKp46^+NK1.1^+$) **(B)** in the TIL were analyzed by flow cytometry to confirm the depletion of each target cell population.



Supplementary Figure 3.

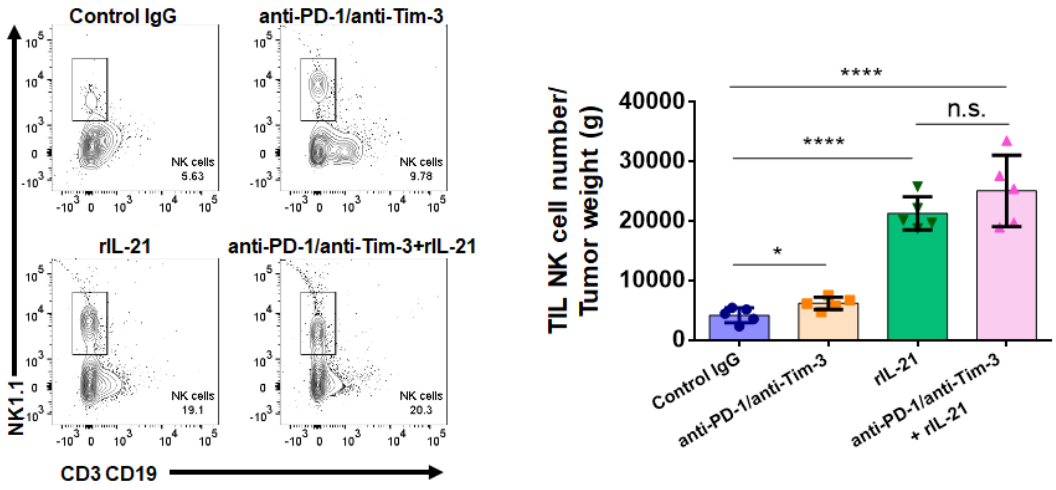
Supplementary Figure 3. Cytokine treatment increases IFN-γ secretion of NK cells in MHC class I-deficient tumors. rIL-2, rIL-12 or rIL-21 was added to the isolated intratumoral NK cells from the indicated mice bearing MHC class I-deficient tumors. IFN-γ was analyzed by flow cytometry. The data was analyzed by Student's t-test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. All values represent the mean ± s.e.m.



Supplementary Figure 4.

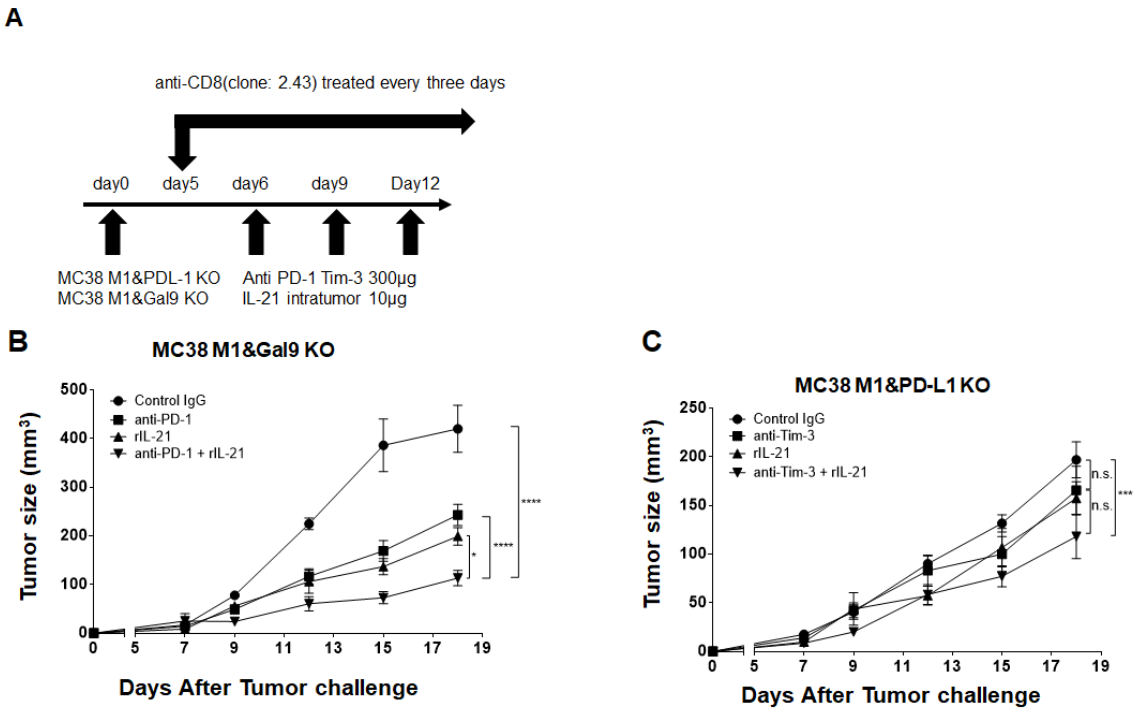
Supplementary Figure 4. Enhanced anti-tumor effects of the combination treatment of IL-21 and anti-PD-1/anti-Tim-3 therapy in TC-1 and CT26 tumor models. (A-D) TC-1 and CT26 MHC class I-deficient tumor-bearing mice (n=6) were treated with rIL-21 (10 μg/mouse) by intratumoral injection and/or anti-PD-1/anti-Tim-3 by i.p. injection. Each group

of mice was treated with anti-CD8 Ab to depletion of CD8 T cells every three days **(A-D). (C)** IFN-γ expression on NK cells was analyzed by flow cytometry. **(B and D)** Subcutaneous tumor growth was measured using a metric caliper 2-3 times per week **(D)**. The data in C was analyzed by Student's t-test. The data in B and D were analyzed by a two-way ANOVA with a Bonferroni multiple comparison test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. All values represent the mean ± s.e.m.



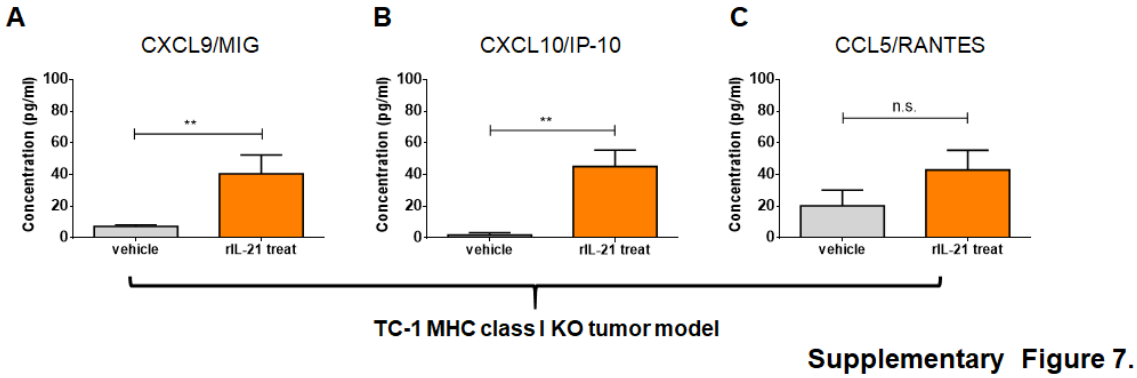
Supplementary Figure 5.

Supplementary Figure 5. The frequency and number of intratumoral NK cells in mice treated with combination or mono therapy. MC38 MHC class I-deficient tumor-bearing mice (n=6) were treated with rIL-21 (10 μg/mouse) by intratumoral injection and/or anti-PD-1/anti-Tim-3 antibodies by i.p. injection. Every group of mice was treated with anti-CD8 Ab to depletion of CD8 T cells. The percentage of intratumoral NK cells was analyzed by flow cytometry, and the number of intratumoral NK cells was calculated as ($= \frac{\%\ \text{of CD45.2+ NK1.1+ cells x No.of tumor infiltrating lymphocyte}}{Tumor\ \ weight\ \ (g)}$). The data was analyzed by Student's t-test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. All values represent the mean ± s.e.m.



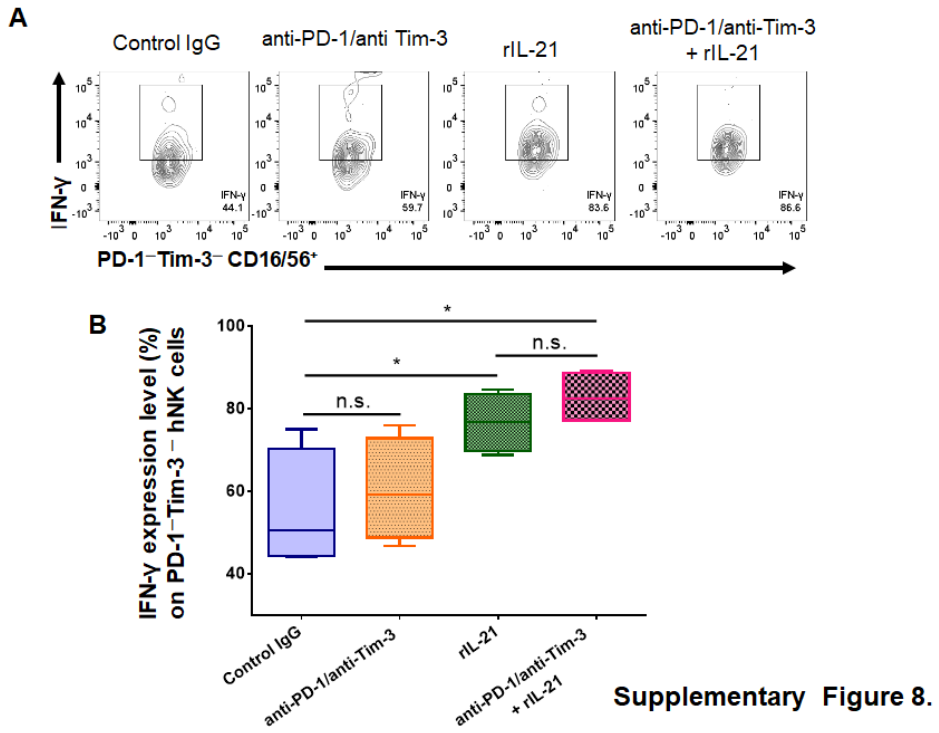
Supplementary Figure 6.

Supplementary Figure 6. IL-21 with checkpoint blockade additively foster anti-tumor effects in MC38 MHC class I & galectin-9 or PD-L1 knock out tumor models. (A-C) MC38 MHC class I and galectin- 9 or PD-L1 knock out tumor-bearing mice (n=6) were treated with rIL-21 (10 μg/mouse) by intratumoral injection and/or anti-PD-1 or anti-Tim-3 by i.p. injection. Every group of mice was treated with anti-CD8 Ab to depletion of CD8 T cells every three days **(A-C). (B and C)** The subcutaneous tumor growth was measured using a metric calliper 2-3 times per week. The data in B and C were analyzed by a two-way ANOVA with a Bonferroni multiple comparison test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. All values represent the mean ± s.e.m.



Supplementary Figure 7.

Supplementary Figure 7. IL-21 treatment induces chemokine production by TC-1 tumor cells. (A-C) TC-1 MHC class I-deficient tumor-bearing mice (n=6) were treated with rIL-21 (10 μg/mouse) by intratumoral injection. TC-1 MHC class I-deficient tumor cells were harvested from mice bearing the indicated tumors on Day 13. Tumor cells were cultured for two days prior to supernatant collection. The concentrations of the chemokines CXCL9 (A), CXCL10 **(B)** and CCL5 **(C)** in the culture supernatant of TC-1 MHC class I-deficient tumor cells were analyzed by ELISA. The data in A-C was analyzed by Student's t-test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. All values represent the mean ± s.e.m.



Supplementary Figure 8.

Supplementary Figure 8. Analysis of IFN-γ production in Tim-3⁻PD-1⁻ intratumoral NK cells from normal tissues of cancer patients. (A-B) Isolated immune cells from normal tissues of cancer patients incubated overnight in the presence or absence of rIL-21 (10 ng/ml) and/or anti-PD-1/anti-Tim-3 antibodies (1 µg/ml). IFN-γ expression of Tim-3⁻PD-1⁻ NK cells was analyzed by flow cytometry. The data were analyzed by Student's t-test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. The data are cumulative from five tumor tissues from colon (4 patients) cancer patients. All values represent the mean ± s.e.m