

Figure S1. Gating strategy of flow cytometry for intracellular staining. Percentages of IFN- γ ⁺ among CD4⁺ T cells and IFN- γ ⁺ among CD8⁺ T cells analysed in this article.

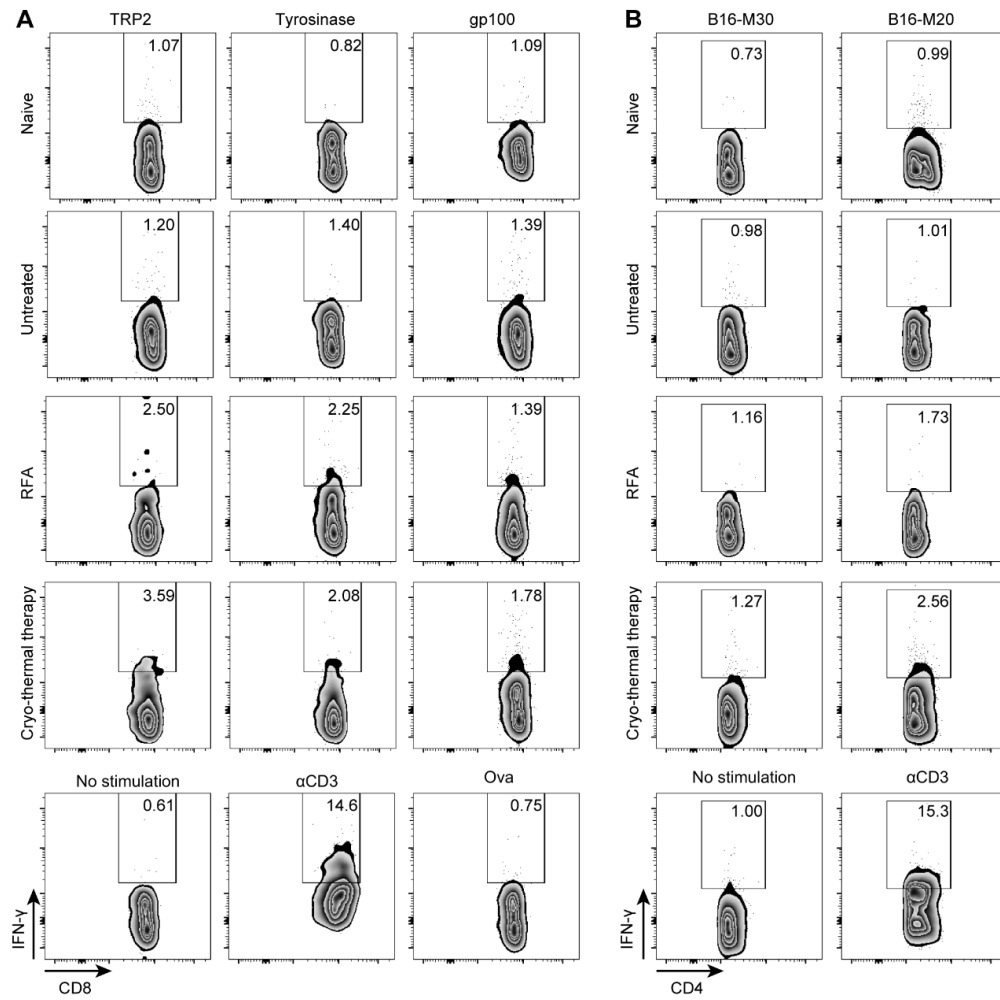


Figure S2. Represented flow cytometry graph of IFN- γ^+ cells in CD8 $^+$ T cells and CD4 $^+$ T cells in Figure 2. Cells were gated on CD8 $^+$ T cells (A) or CD4 $^+$ T cells (B).

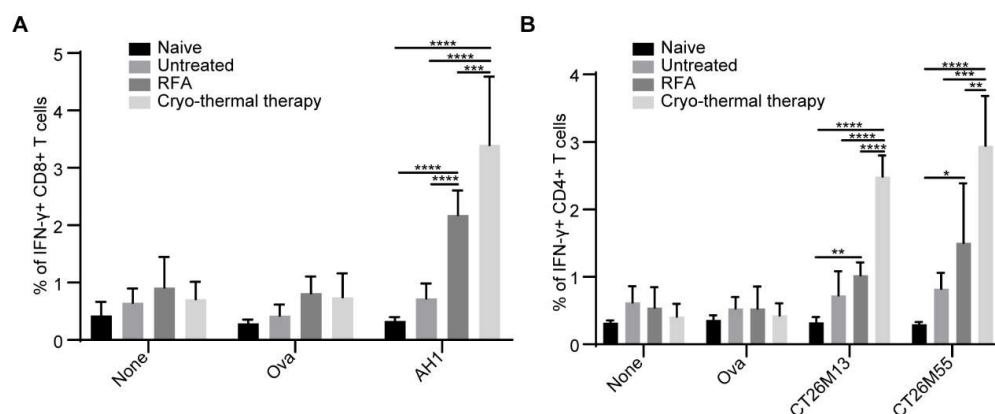


Figure S3. Frequency of IFN- γ ⁺ cells in CD8⁺ and CD4⁺ T cells. On day 14 after RFA or cryo-thermal therapy, splenocytes were obtained from naïve mice, untreated mice and treated mice in CT26 model. (A) Each 1×10^5 splenocytes were co-incubated with 10 μ g/ml AH1, Ova (negative control), and medium (blank control) respectively to measure IFN- γ ⁺ CD8⁺ T cells. AH1₆₋₁₄ is the H2-Ld-restricted epitope which is expressed in CT26. (B) Each 1×10^5 splenocytes were co-incubated with 10 μ g/ml CT26M13, CT26M55, and medium (blank control) respectively to measure IFN- γ ⁺ CD4⁺ T cells. CT26M13 and CT26M55 are two neoepitopes found in CT26. Mean percentages of IFN- γ ⁺ CD8⁺ T cells (A) and IFN- γ ⁺ CD4⁺ T cells (B) in each group were shown (data for bar graphs were calculated using two-way ANOVA, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$), $n = 4$ for each group.

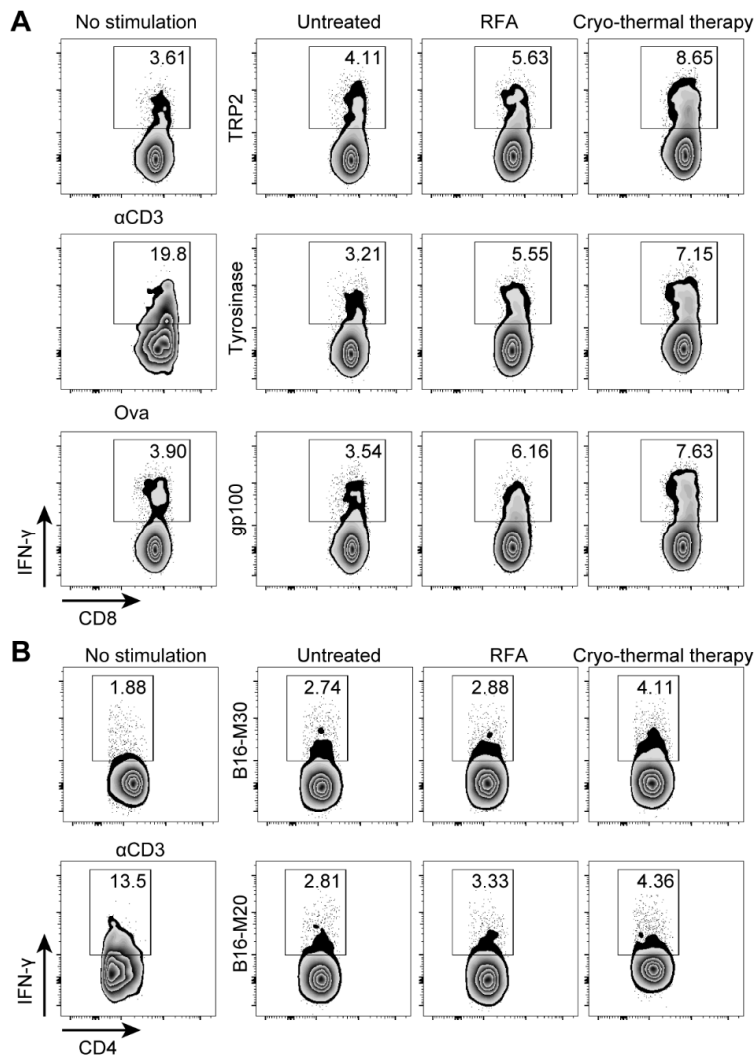


Figure S4. Represented flow cytometry graph of IFN- γ ⁺ cells in CD8⁺ T cells and CD4⁺ T cells after expansion in Figure 3. Cells were gated on CD8⁺ T cells (A) or CD4⁺ T cells (B).

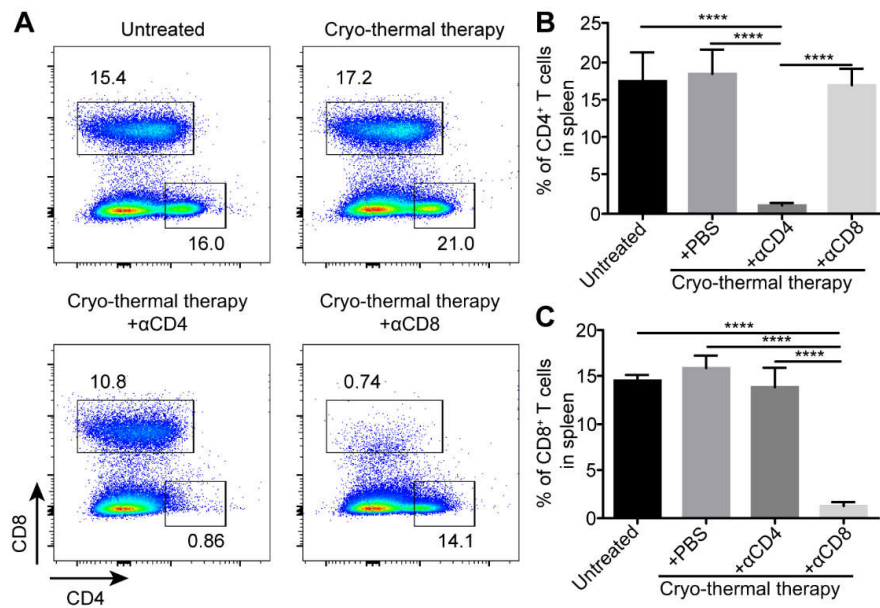


Figure S5. Consequent of depletion of CD8⁺ T cells or CD4⁺ T cells. (A) Represented flow cytometry graph of the frequency of CD8⁺ T cells and CD4⁺ T cells in each group. (B) Quantitative statistics of frequency CD4⁺ T cells. (C) Quantitative statistics of frequency of CD8⁺ T cells. All data were shown as mean ± SD. n = 4 per group. **P < 0.01, ****P < 0.0001. Data for graphs were calculated using one-way ANOVA.

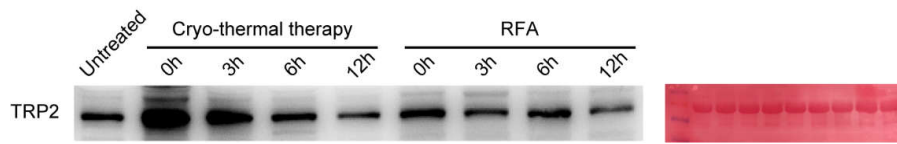


Figure S6. Western blot of TRP2 in tumour interstitial fluid. Tumours were harvested at 0, 3, 6, 12, and 24 hour after treatments. Left: Western blot analysis of released TRP2 in tumour interstitial fluid from different treatments (tumour-bearing untreated group; cryo-thermal group; RFA group). Right: Ponceaux staining of quantifying the same amount of proteins of 50 μ g in all the samples.

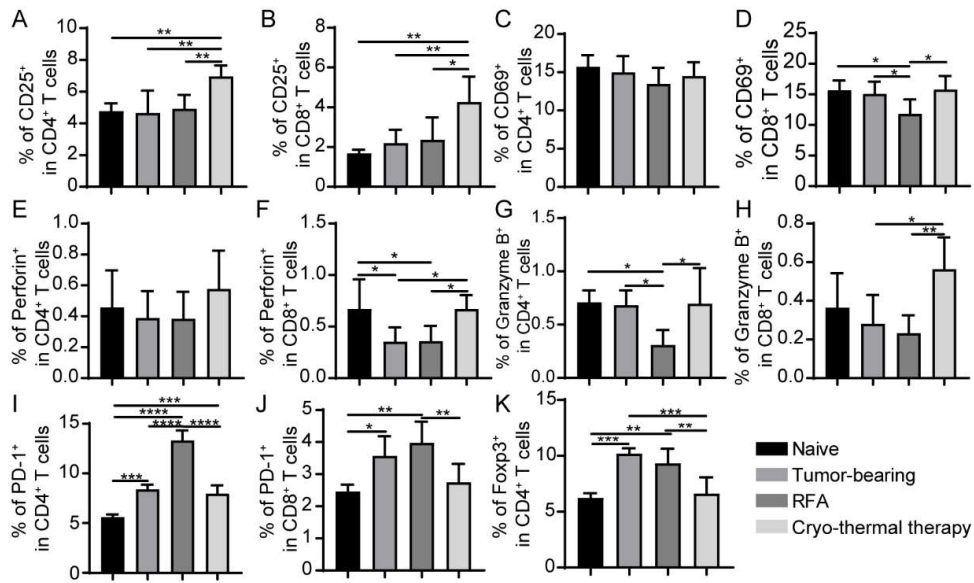


Figure S7. Frequency of CD25⁺, CD69⁺, perforin⁺, granzyme B⁺, PD-1⁺ cells in CD8⁺ and CD4⁺ T cells (A-J) and foxp3⁺ in CD4⁺ T cells (K). On day 14 after RFA or cryo-thermal therapy, splenocytes were obtained from naïve mice, untreated mice and treated mice to analyse by flow cytometry.