

Supplementary materials

Downregulated TNF- α levels after cryo-thermal therapy drive Tregs fragility to promote long-term antitumor immunity

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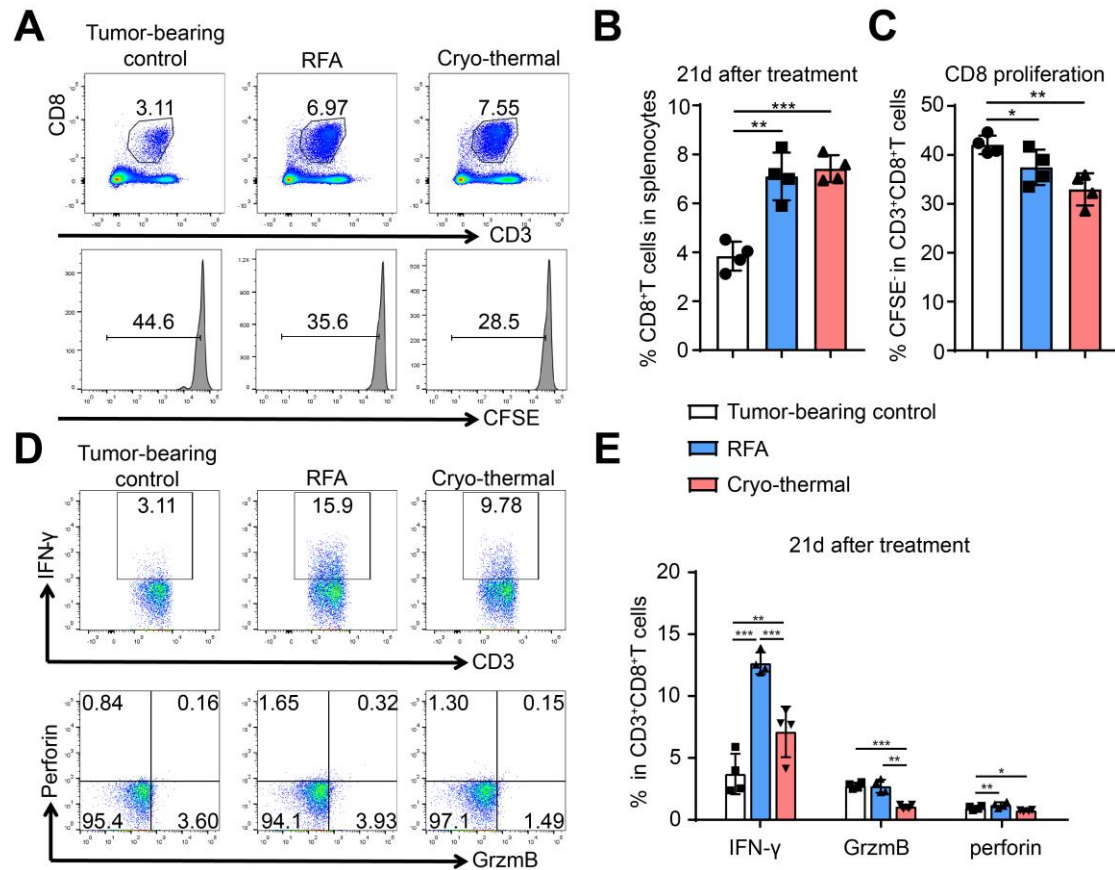


Figure S1. Cryo-thermal mediated antitumor immune response was not depended on CD8⁺ T cells. (A-C) The percentages of CD8⁺ T cells in splenic lymphocytes (B) and the proliferation (C) of CD8⁺ T cells were detected by flow cytometry on day 21 after cryo-thermal therapy or RFA treatment. For CD8⁺ T cells proliferation assay. Splenic CD8⁺ T cells were isolated from tumor-bearing control (39 days after inoculation), RFA or cryo-thermal (21 days after treatment) treated mice by using microbeads. The CD8⁺ T cells were labeled with CFSE and then cultured with α CD3 (1 ng/mL) for 24h. CD8⁺ T cell proliferation (CFSE-) were detected by flow cytometry. (D) The expression of IFN- γ , Granzyme B and perforin in splenic CD8⁺ T cells were detected by flow cytometry. *P < 0.05, **P < 0.01, ***P < 0.001. n = 4 for each group.

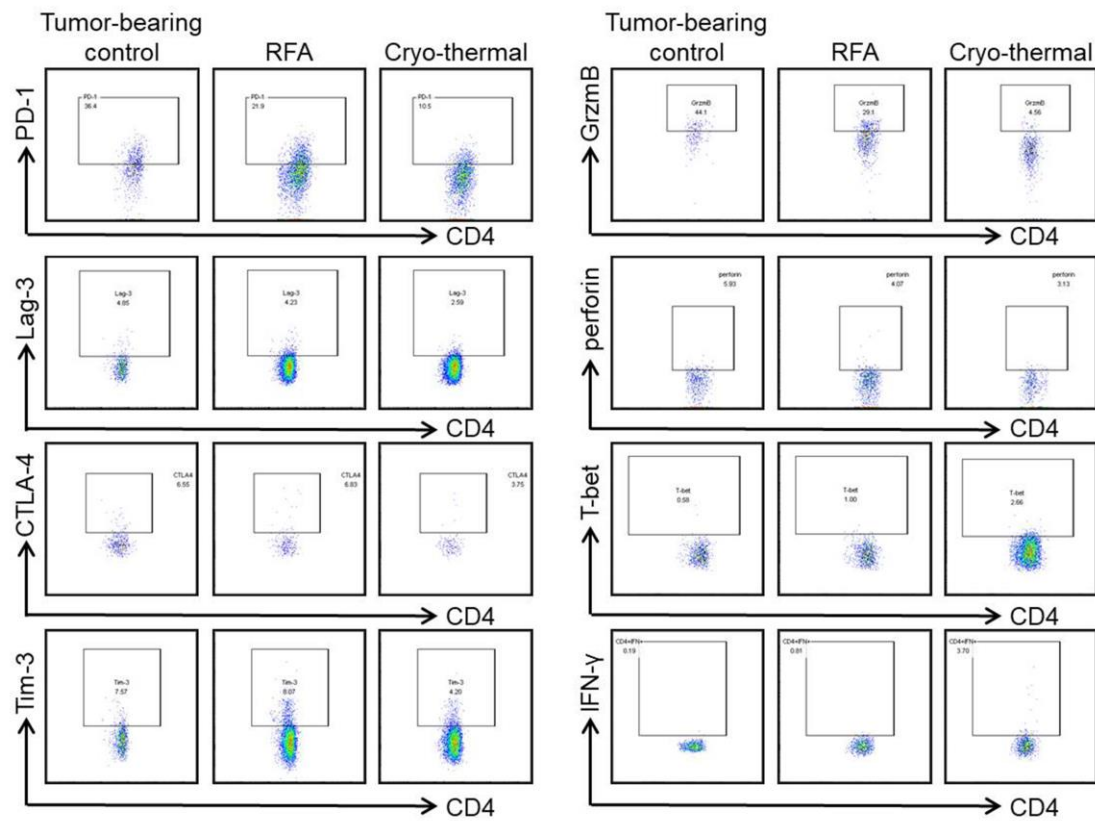


Figure S2. Cryo-thermal therapy drove the fragile phenotype of Tregs and down-regulated its suppressive function. Flow cytometry gating strategy for determination of PD-1, Lag-3, CTLA-4, Tim-3, Granzyme B, Perforin, T-bet and IFN-γ in Tregs. (Representative flow chart of Fig. 3).

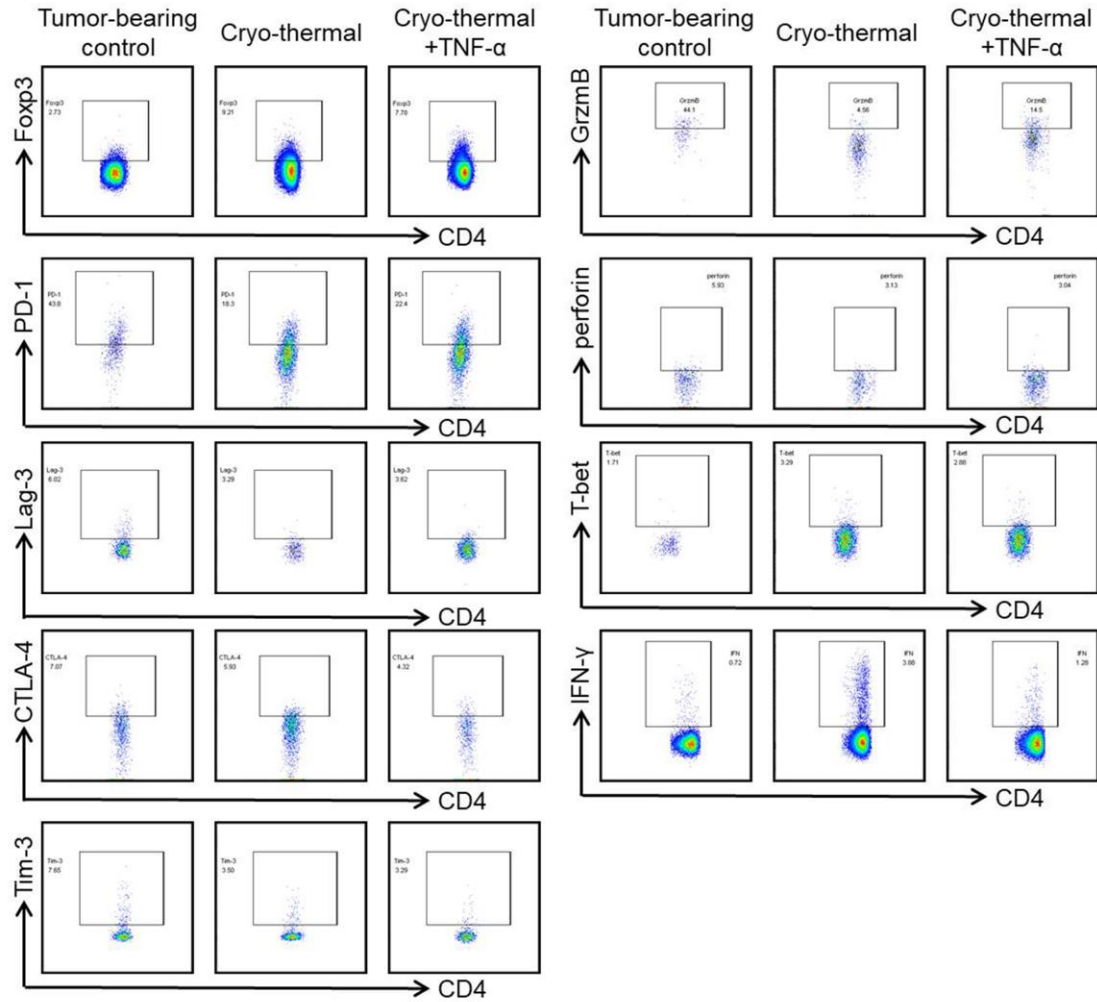


Figure S3. *In vivo* TNF- α supplement after cryo-thermal therapy damaged Tregs fragility and promoted tumor metastasis of mice. Flow cytometry gating strategy for determination of Tregs, and the expression of PD-1, Lag-3, CTLA-4, Tim-3, Granzyme B, Perforin, T-bet and IFN- γ in Tregs. (Representative flow chart of Fig. 5).

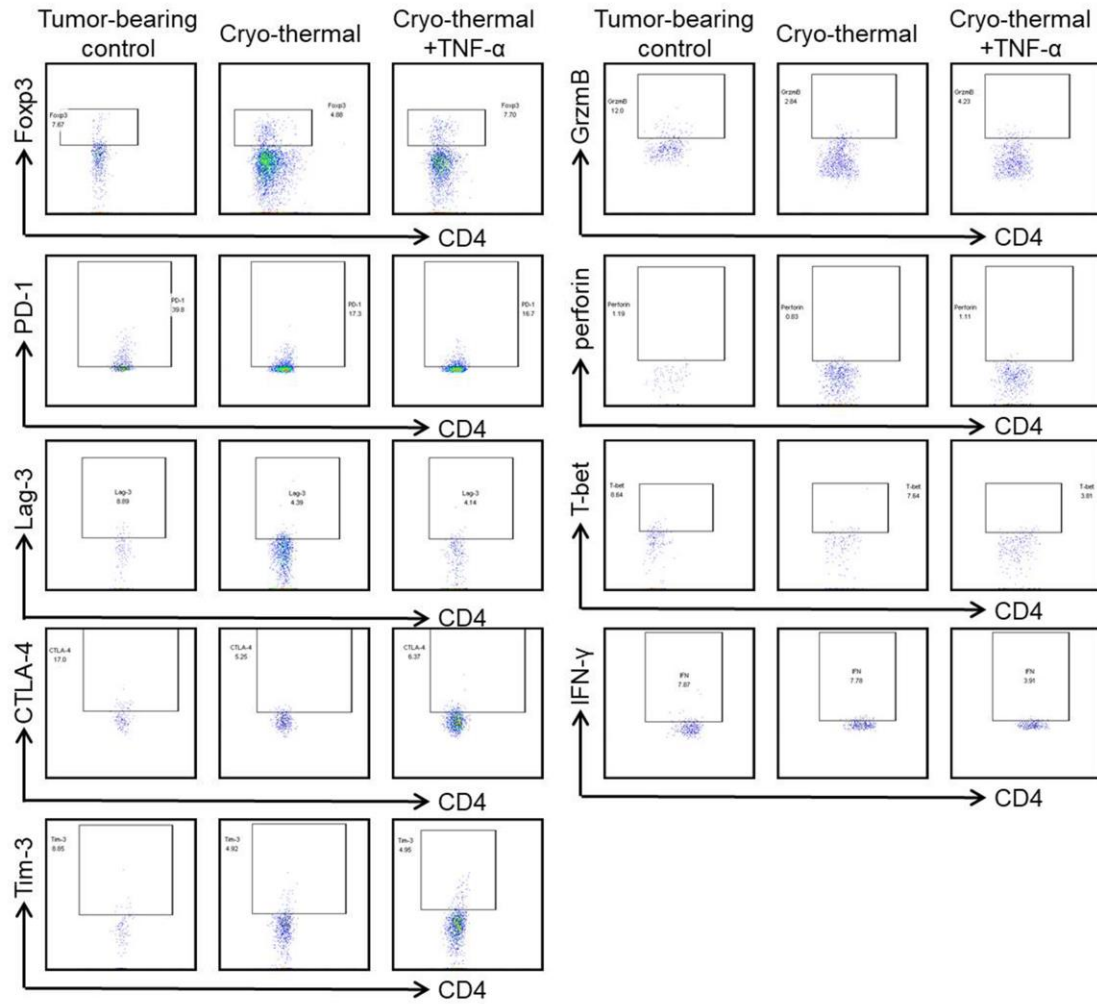


Figure S4. *In vivo* TNF- α supplement after cryo-thermal therapy damaged Tregs fragility and promoted tumor metastasis of mice. Flow cytometry gating strategy for determination of Tregs, and the expression of PD-1, Lag-3, CTLA-4, Tim-3, Granzyme B, Perforin, T-bet and IFN- γ in Tregs. (Representative flow chart of Fig. 6).

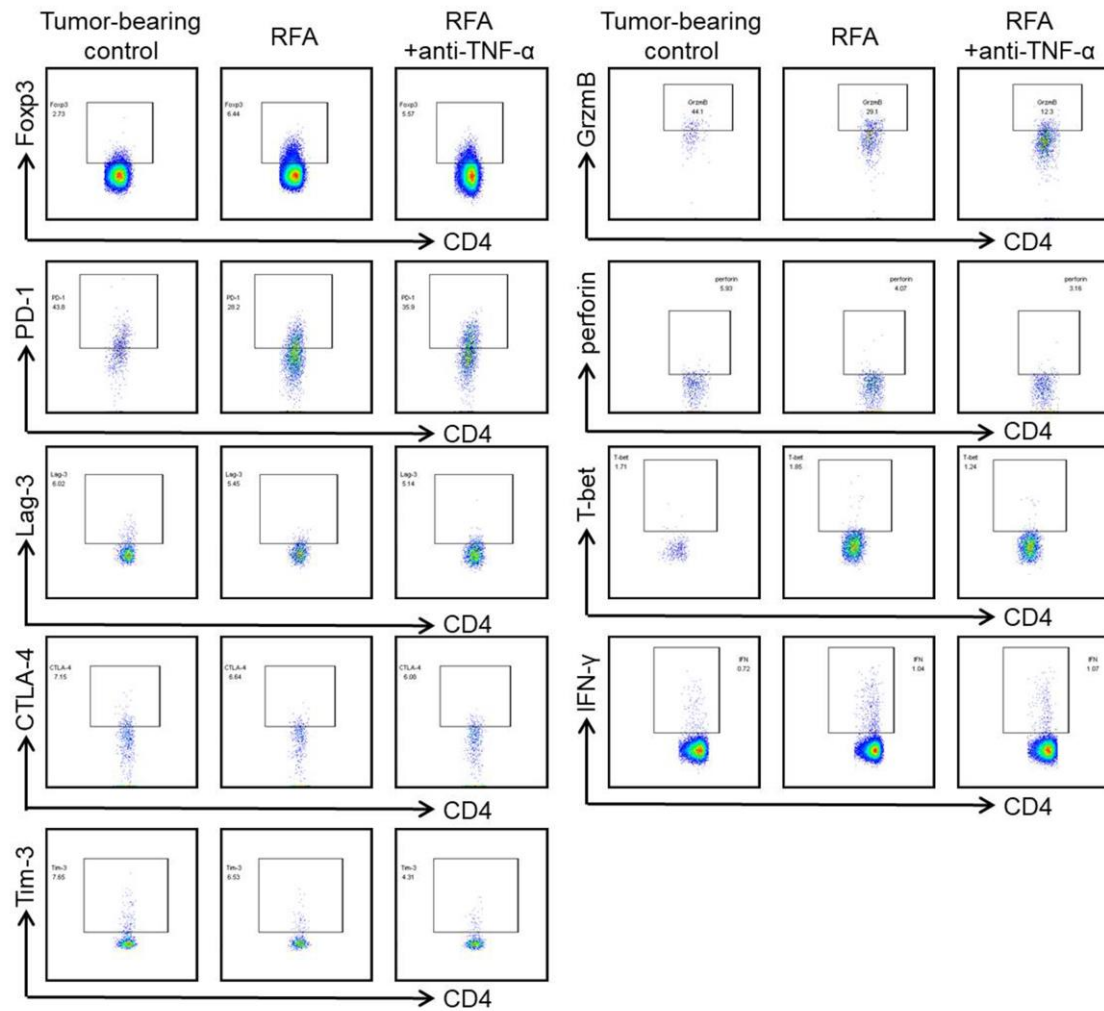


Figure S5. TNF- α neutralization after RFA treatment inhibited Treg function and suppressed tumor metastasis as well as promoted the long-term survival of mice. Flow cytometry gating strategy for determination of Tregs, and the expression of PD-1, Lag-3, CTLA-4, Tim-3, Granzyme B, Perforin, T-bet and IFN- γ in Tregs. (Representative flow chart of Fig. 7).

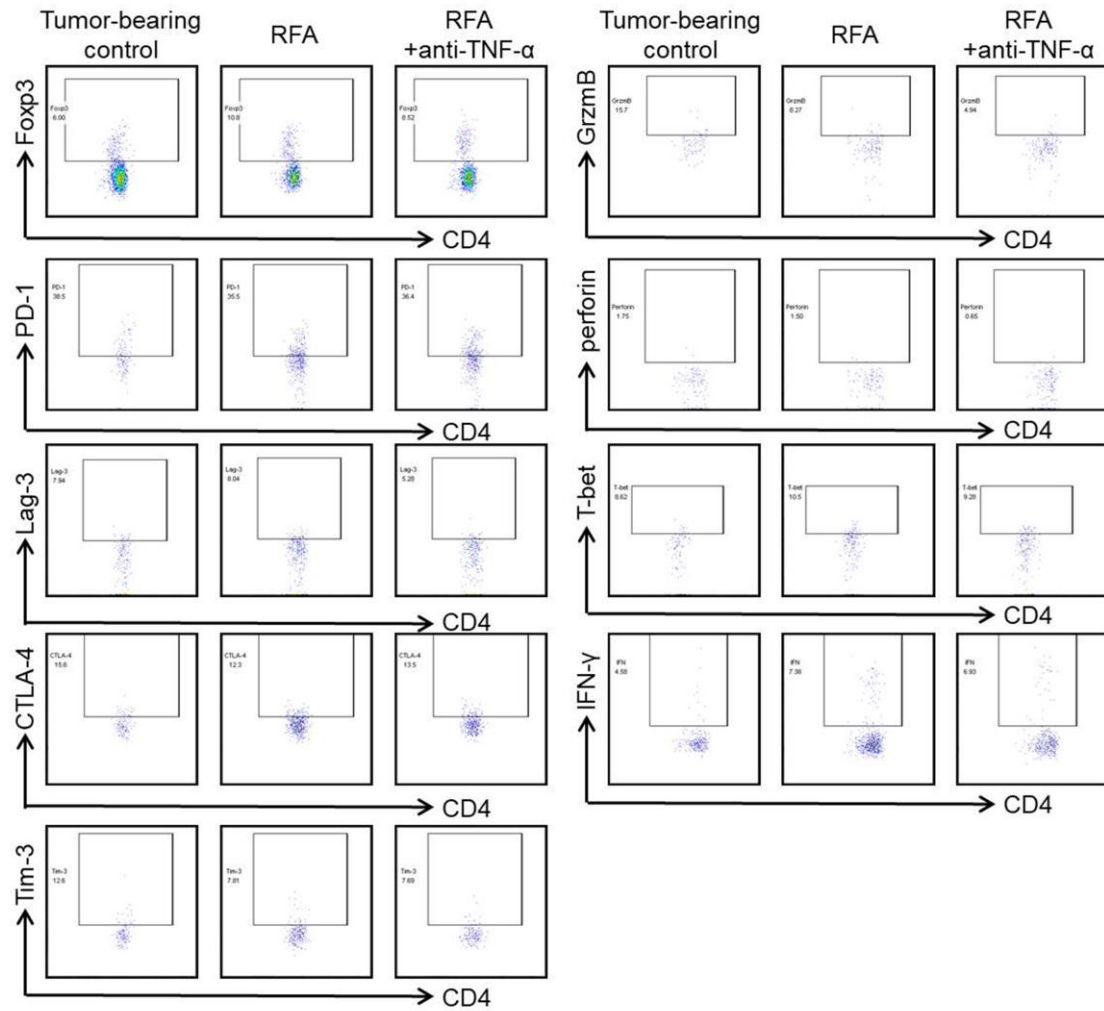


Figure S6. TNF- α neutralization after RFA treatment inhibited Treg function and suppressed tumor metastasis as well as promoted the long-term survival of mice. Flow cytometry gating strategy for determination of Tregs, and the expression of PD-1, Lag-3, CTLA-4, Tim-3, Granzyme B, Perforin, T-bet and IFN- γ in Tregs. (Representative flow chart of Fig. 8).