

Supplementary Material: Harnessing the IL-21-BATF Pathway in the CD8⁺ T Cell Anti-Tumor Response

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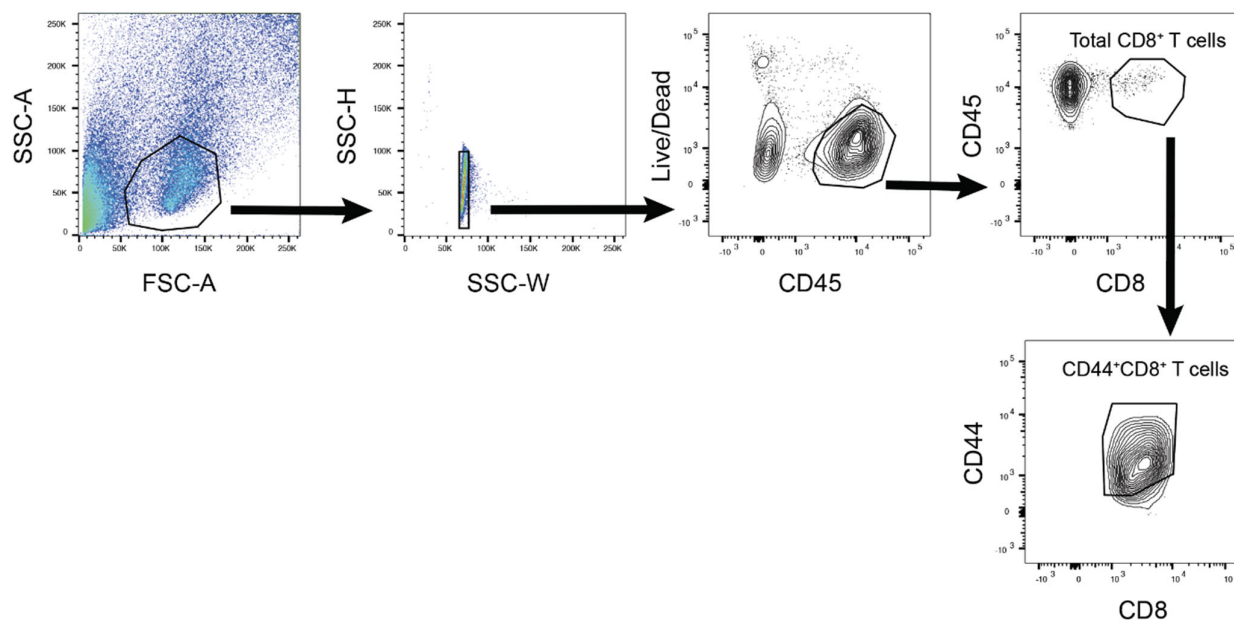


Figure S1. Gating strategy to assess tumor-infiltrating CD8⁺ T cells. Tumors of B16-GP33 tumor-bearing mice were harvested and immune cells were isolated. The flow cytometry panels above depict the gating strategy used to assess the CD8⁺ T cells in the tumor. First is the lymphocyte gate, followed by gating of single cells to exclude doublets, then gating on the live CD45⁺ leukocyte population. Then, the total CD8 T cell population is gated within the CD45 population, followed by looking at the activated CD44⁺ CD8⁺ T cell population.

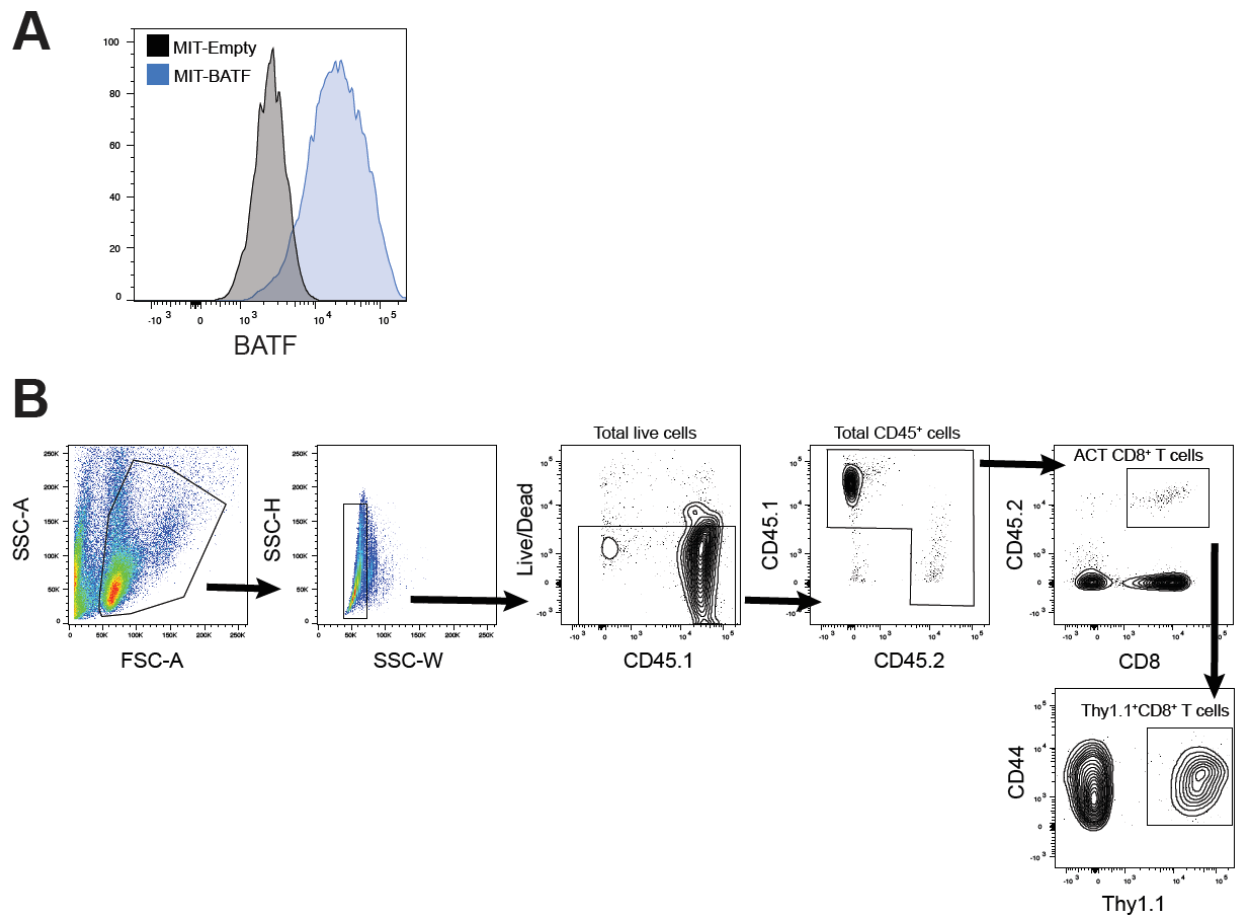


Figure S2. BATF retrovirally transduced CD8⁺ T cells. Pmel CD8⁺ T cells were activated and transduced with either MIT-Empty- or MIT-BATF-expressing retrovirus. **(A)** Forty-eight hours following transduction, flow cytometry was conducted to assess BATF expression levels in Thy1.1⁺ CD8 T cells. **(B)** Gating strategy to assess tumor-infiltrating CD8⁺ T cells in mice adoptively transferred with CD8 T cells retrovirally transduced with BATF. Tumors of B16-GP33 tumor-bearing mice (CD45.1 recipient mice) were harvested and immune cells were isolated. The flow cytometry panels above depict the gating strategy used to assess the CD8⁺ T cells in the tumor. First is the lymphocyte gate, followed by gating of single cells to exclude doublets, then gating on live cells. Total CD45⁺ cells were then selected and the adoptively transferred CD8 T cell population (CD45.2⁺) was selected. Thy1.1 expression of adoptively transferred cells was then assessed to identify retrovirally transduced CD8 T cells.

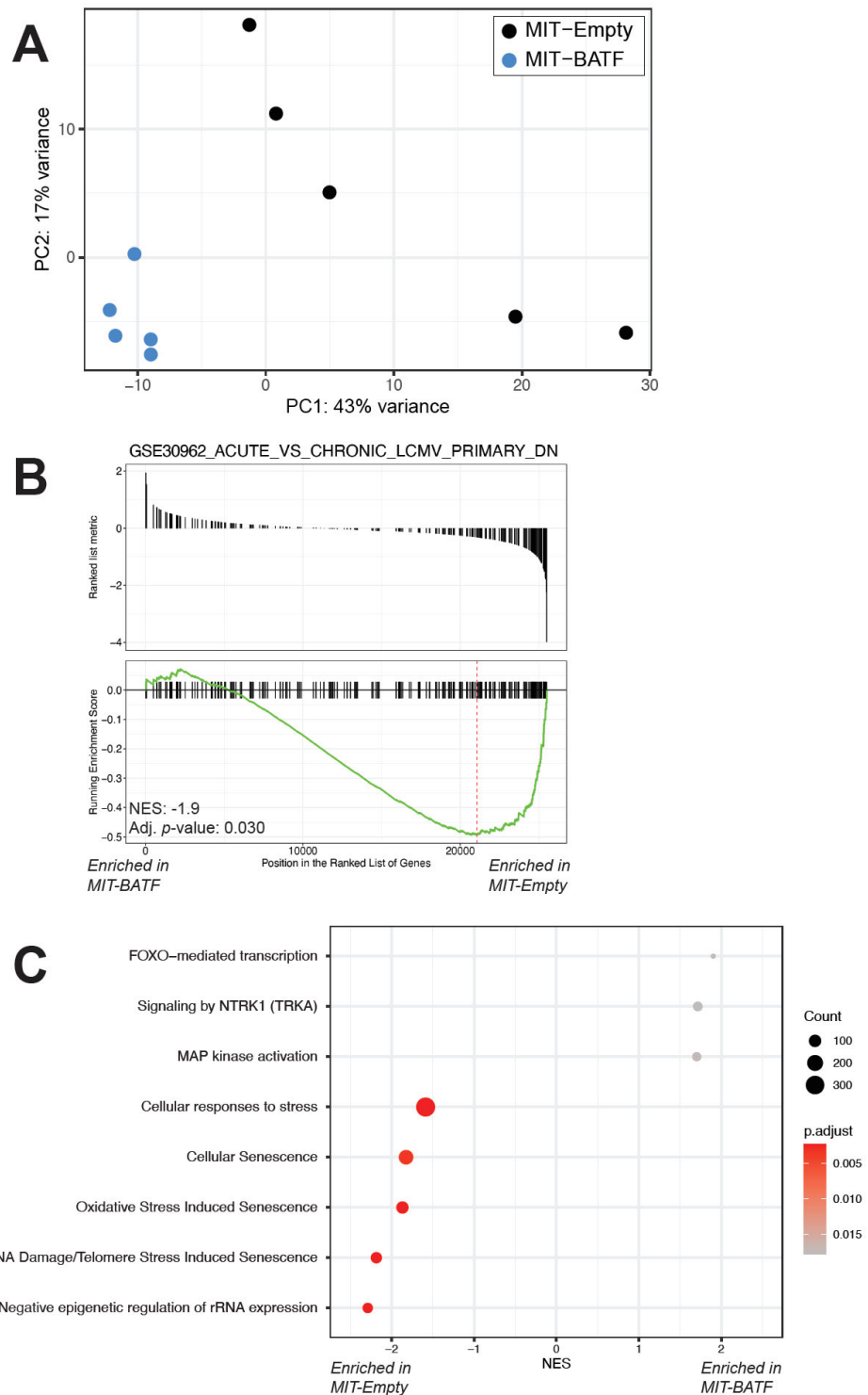


Figure S3. Tumor-infiltrating, adoptively transferred BATF-overexpressing CD8⁺ T cells are more resistant to cellular Scheme 16. F10 tumor-bearing mice were adoptively transferred with CD8⁺ T cells. One week following ACT, tumors were harvested and immune cells were isolated, stained, and FACS-sorted for Thy1.1⁺-expressing adoptively transferred CD8⁺ T cells. Bulk RNA sequencing was conducted on MIT-Empty and MIT-BATF cells. (A) Principle component analysis of samples. (B) Gene set enrichment analysis (GSEA) of genes downregulated in acute vs. chronic LCMV infection. (C) Reactome pathway analysis of relevant pathways. (*n* = 5 mice per group).