Characterizing the impact of SOCS1 and Regnase-1 inactivation on tumor reactive T cells present in CRISPR/Cas9-engineered TIL (eTIL®) therapies derived from melanoma and NSCLC tumors through functional and single cell analyses

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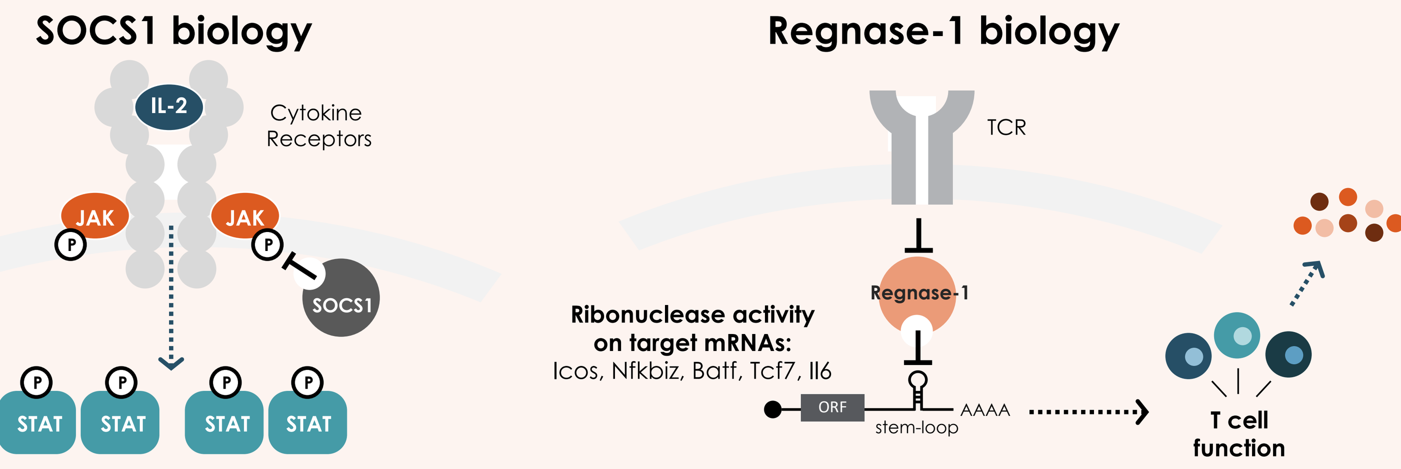
ABSTRACT 2196

Background



- Tumor Infiltrating Lymphocyte (TIL) therapy involves the isolation and ex vivo expansion of TIL for the treatment of solid tumors
- The T cell receptor (TCR) repertoires present within TIL drug products (DPs) are highly patient unique and include TCRs specific against tumor antigens at variable frequencies. The clinical activity of TIL is thought to be mediated in part by the anti-tumor functionality profile of TIL and the frequency of tumor reactive T cells (TRT) within DP
- Seeking to improve the clinical activity of TIL therapy, we developed KSQ-001EX and KSQ-004EX, two CRISPR/Cas9-engineered TIL (eTIL®) therapies wherein the genes encoding SOCS1 or SOCS1 in combination with Regnase-1 are inactivated, respectively. We have shown in preclinical models that inactivation of SOCS1 and SOCS1/Regnase-1 enhance the anti-tumor functionality of TIL, but the incidence of TRTs in eTIL DP and how inactivation of SOCS1 and Regnase-1 impact their functionality remains unclear
- In this study, we identified and characterized TRT in eTIL DP using two distinct but complementary methods

Methods

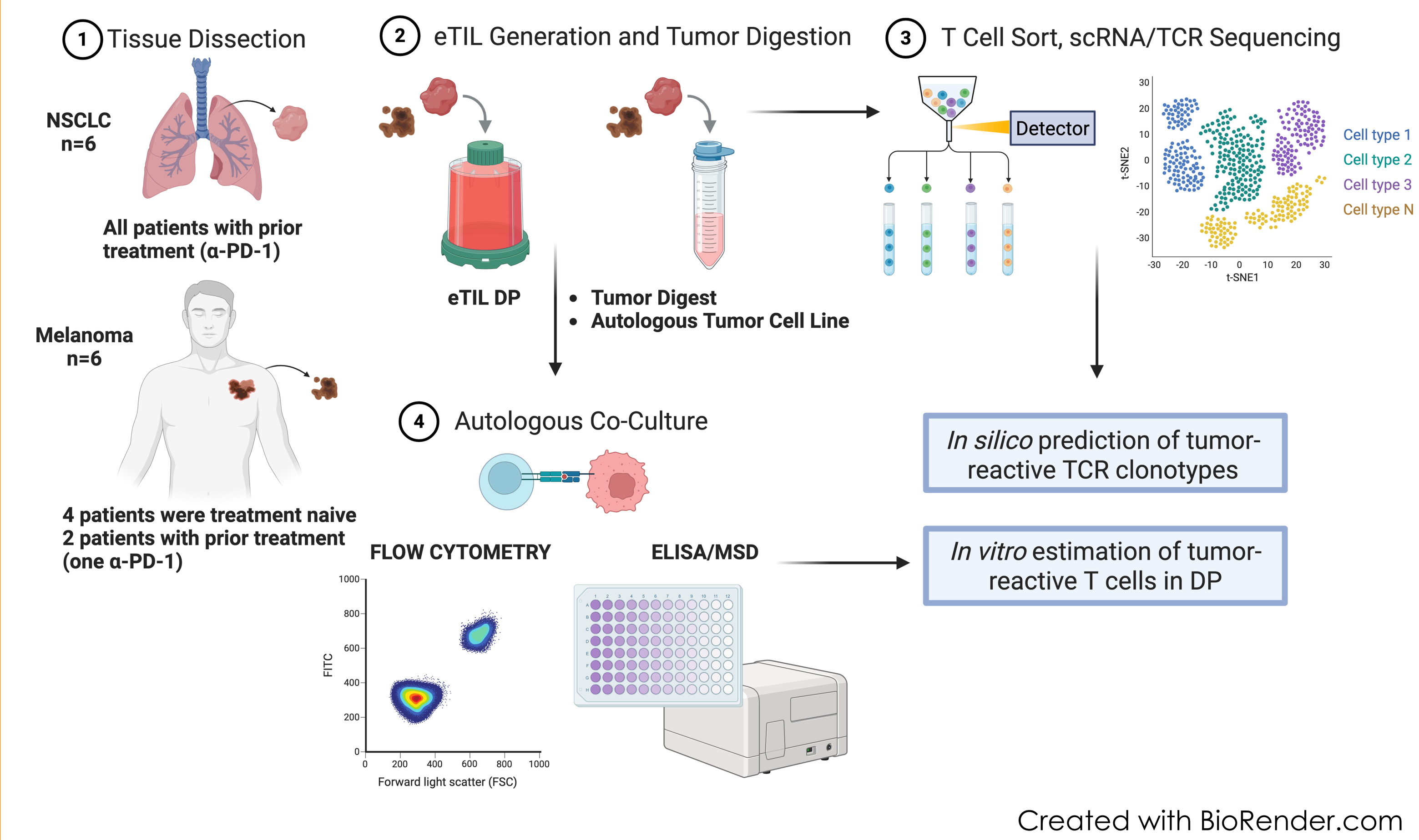


Figure 1: SOCS1 and SOCS1/Regnase-1 editing enhance TRT function but not frequency in melanoma-derived eTIL DPs

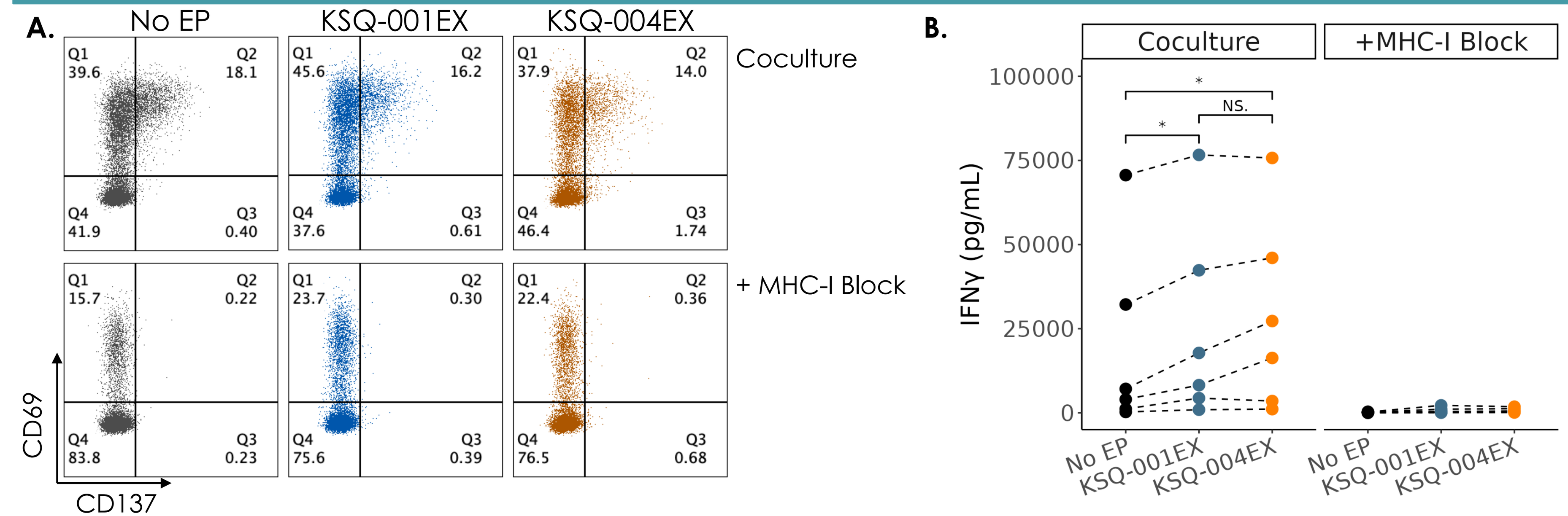


Figure 1. eTIL DPs were cocultured with autologous melanoma tumor cell lines with and without MHC-I block to assess TRT frequency and function. **(A)** SOCS1 and/or Regnase-1 inactivation did not significantly impact the frequency of CD69+CD137+ cells (representative data shown). **(B)** Consistent with the biology of SOCS1 editing, IFNγ production was enhanced after coculture in KSQ-001EX and KSQ-004EX compared to No EP controls in an MHC-dependent manner. *p < 0.05

Figure 2: scRNAseq reveals several distinct CD8 T cell subsets in NSCLC tumors

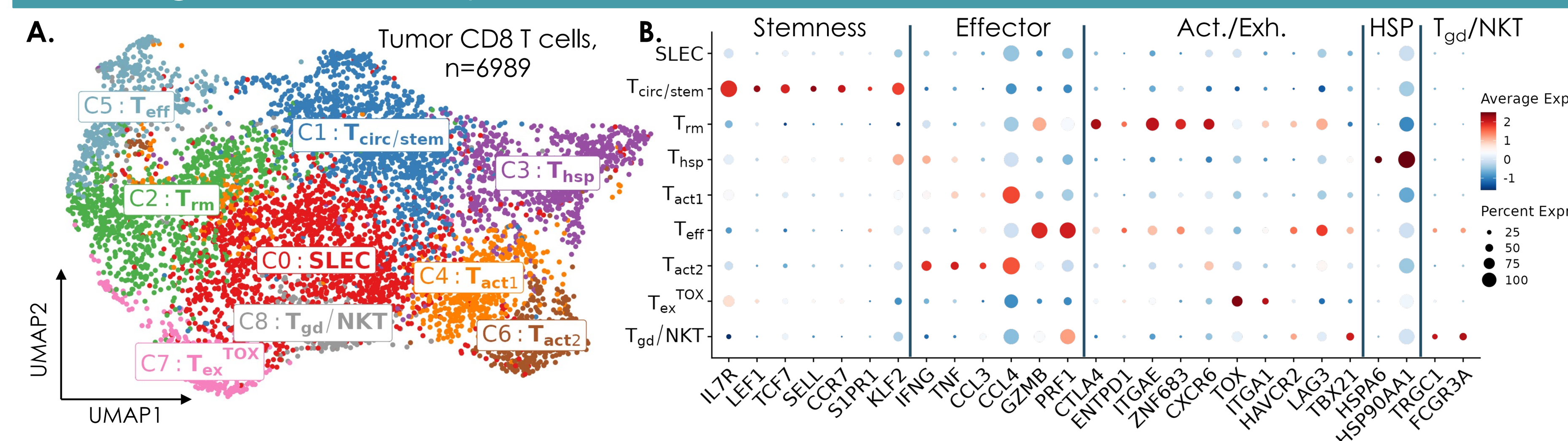


Figure 2. Single-cell RNA/TCR sequencing was performed on a 1:1 mixture of sorted CD4 and CD8 T cells isolated from surgically-resected NSCLC tumors. CD8 T cells were isolated using computational gating. **(A, B)** Location of CD8 T cell subsets on UMAP (A) annotated using expression of subset-defining transcripts (B). Clusters (C0 – C8) are numbered in order of decreasing size.

Figure 3: predTRT gene set scores identify TCR clones enriched in tumor relative to uninvolved lung and blood

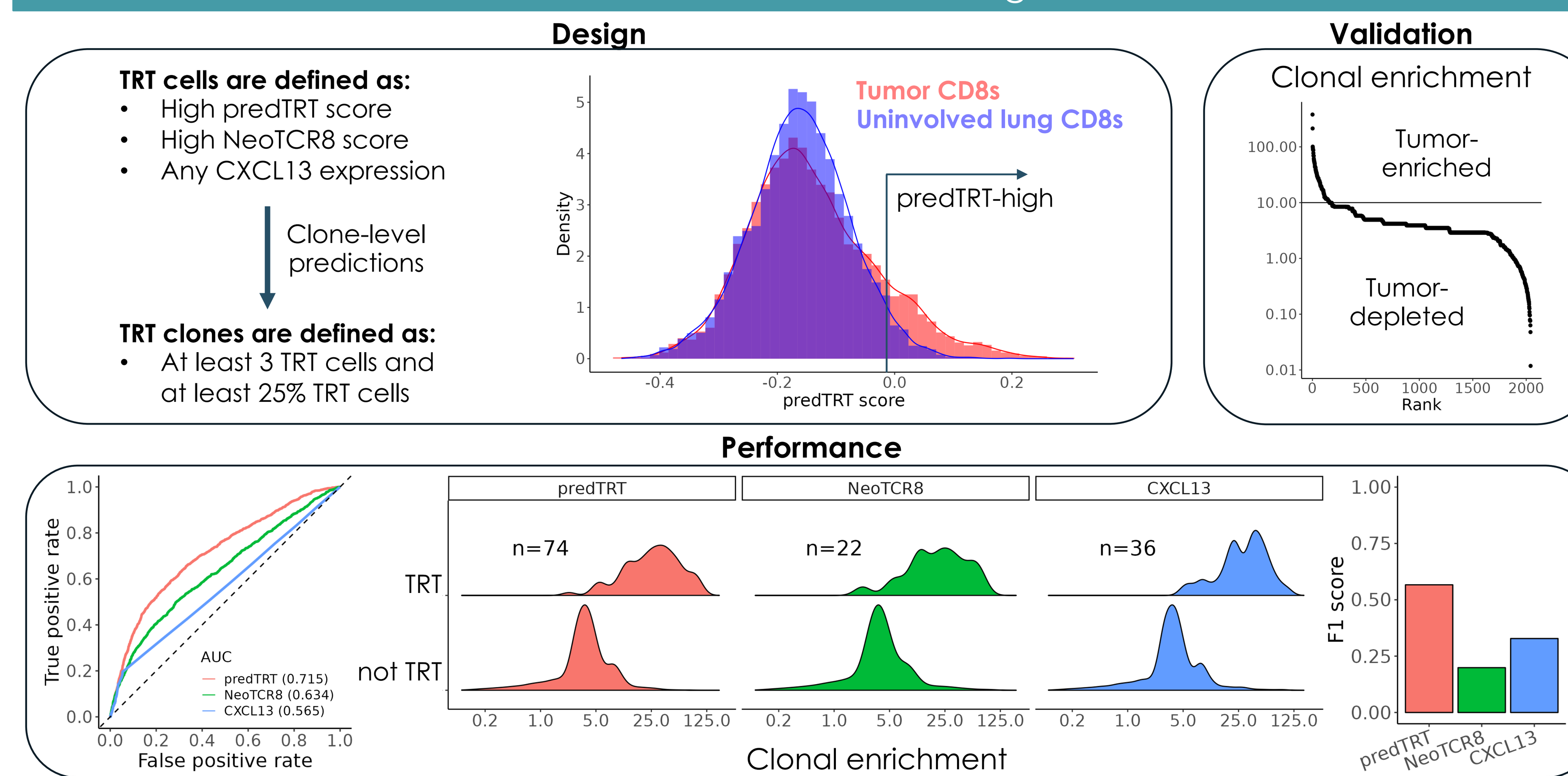


Figure 3. A custom TRT gene set (53 genes, predTRT) was constructed from genes common to 17 TRT-associated gene sets from 8 publications spanning several cancer types and evaluated against a single-gene model of CXCL13 expression[1] and the published gene set NeoTCR8[2]. Three attributes were independently used for TRT predictions; cell-wise gene set thresholds were set for each patient as the 95th percentile of scores in lung. Clones at least 10x enriched in tumor vs. lung or blood were used to validate the predicted TRT clones. AUC plot shows evaluation of cell-level scores as predictors of tumor-enriched clones. All prediction methods preferentially identified expanded clones in tumor; predTRT exhibited the greatest sensitivity and F1 score.

Figure 4: eTIL DP manufacturing preserves TRT clones; the effects of SOCS1 and Regnase-1 inactivation are seen broadly and within TRT clones

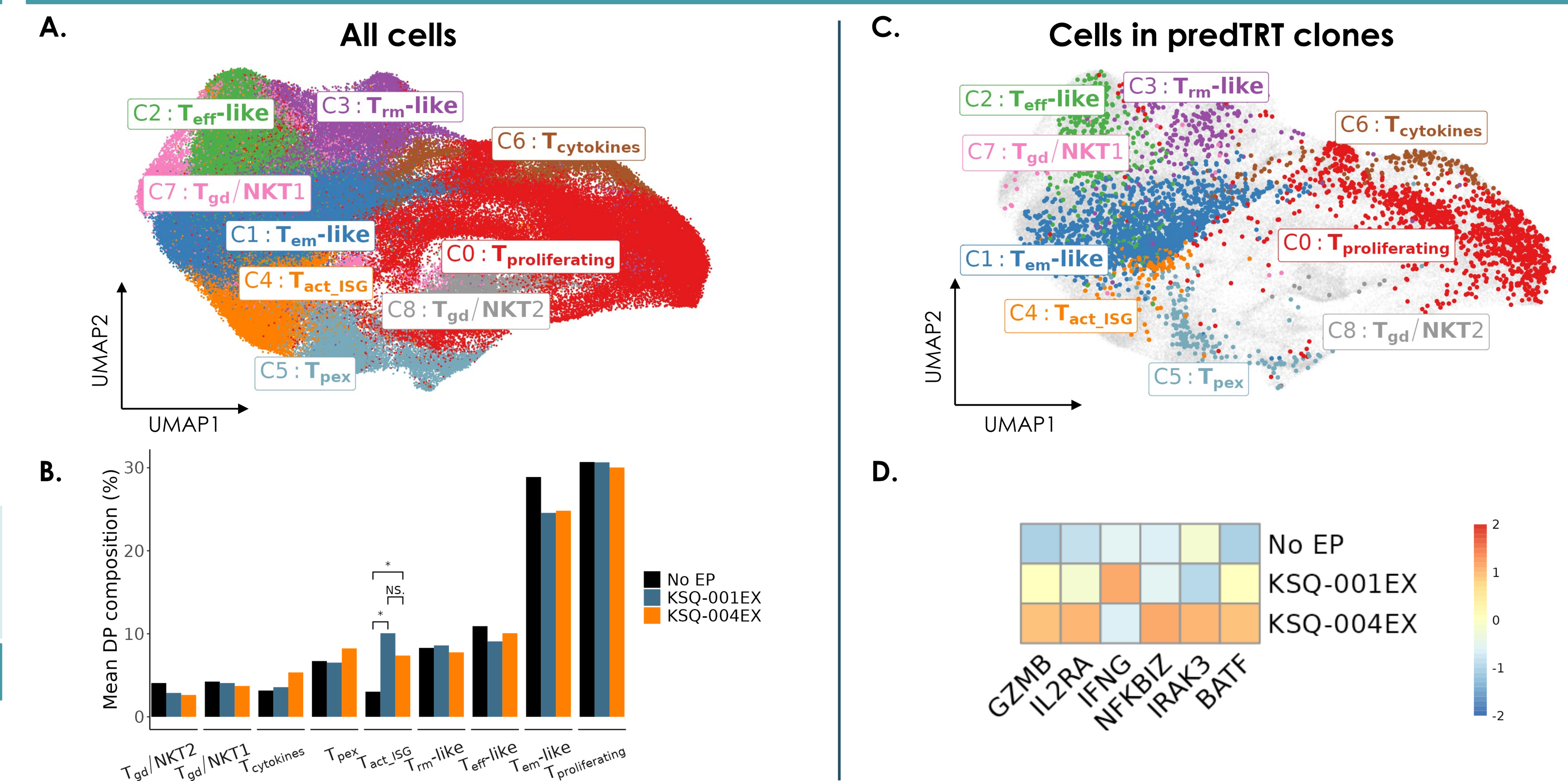


Figure 4. TRT clones identified in tumor starting material using the predTRT gene set were tracked to eTIL DPs. **(A)** Diversity of transcriptional states present in eTIL. **(B)** Consistent with the biology of SOCS1 and Regnase-1 inactivation, a greater fraction of cells in KSQ-001EX and KSQ-004EX were in an activated state with increased expression of interferon stimulated genes, and more cells from KSQ-004EX were found in a T cell state with elevated expression of cytokine genes. **(C)** eTIL manufacturing preserves TRT clones and they are overrepresented in T_{em}-like and proliferating clusters in eTIL DP. **(D)** Changes in the expression levels of genes known to be regulated by SOCS1 and/or Regnase-1 were observed within TRT clones across edits. *p < 0.05

Figure 5: Complementary methods report similar TRT frequencies in eTIL DPs

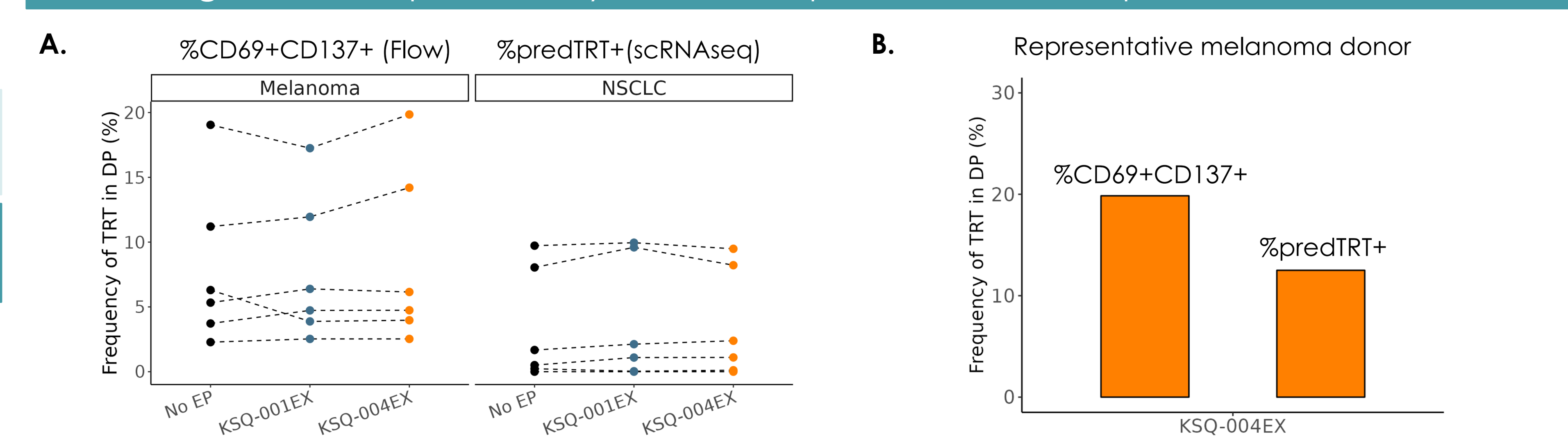


Figure 5. The fraction of TRT in eTIL DPs produced from melanoma and NSCLC tumor starting material was estimated using two methods: 1) %CD69+CD137+ by flow cytometry following coculture with autologous tumor cells and 2) *in silico* prediction of TRT clones in tumor starting material using single-cell RNA/TCR sequencing and tracking clones to eTIL DP. **(A)** The relative TRT frequencies predicted through these approaches are in-line with the expected frequencies of TRT in melanoma and NSCLC[3-5] and did not vary across edits. **(B)** For one melanoma sample, the %TRT in KSQ-004EX was estimated using both approaches.

Conclusions

- A custom TRT gene set (predTRT) was able to identify TCR clones that were enriched in tumor vs. matched uninvolved lung or blood
- Cells in predTRT clones were overrepresented in a T_{rm} transcriptional state in tumor starting material
- eTIL DP manufacturing preserves predTRT clones, where they preferentially occupy T_{em}-like and proliferating states
- Inactivation of SOCS1 and SOCS1 + Regnase-1 improves the antitumor functionality of cells in predTRT clones in eTIL
- The frequencies of TRT in eTIL DP were confirmed through complementary *in vitro* and *in silico* approaches

References

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Abbreviations

DP, drug product; IFNγ, interferon gamma; NKT, natural killer T cell; NSCLC, non-small cell lung cancer; SOCS1, suppressor of cytokine signaling 1; TCR, T cell receptor; T_{eff}, effector T cell; T_{em}, effector memory T cell; T_{gd}, gamma-delta T cell; TIL, tumor-infiltrating lymphocyte; T_{rm}, resident memory T cell; TRT, tumor-reactive T cell