# SOCS1 and Regnase-1 Inactivation Enhances Functionality and Prevents Exhaustion of Tumor Reactive Clonotypes in KSQ-004EX eTIL

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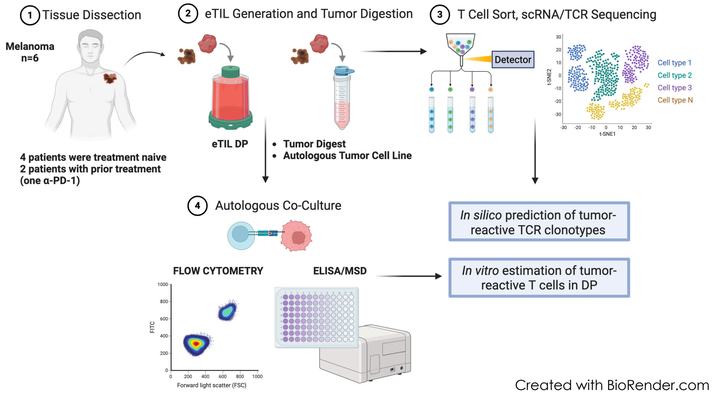
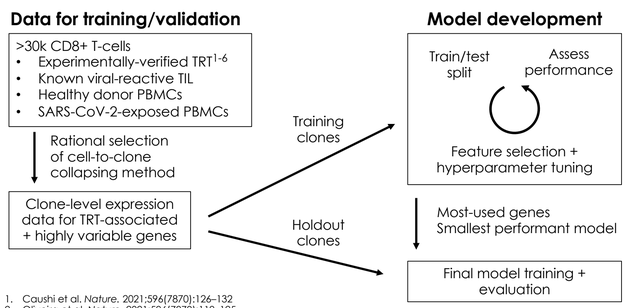
## Background

- Tumor Infiltrating Lymphocyte (TIL) therapy involves the isolation and *ex vivo* expansion of polyclonal TIL for the treatment of solid tumors
- The T-cell receptor (TCR) repertoires present within TIL drug products (DPs) are highly unique to each patient and include both tumor-reactive and bystander TCR clonotypes. The clinical activity of TIL is thought to be mediated both by the intrinsic functionality of T-cells 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
- In this study, we identified TRT in eTIL DP using two distinct but complementary methods and characterized the impact of dual SOCS1/Regnase-1 inactivation on their functionality and resistance to exhaustion in acute and chronic stimulation contexts

## Autologous Coculture




## TRACE: Clone-level TRT Classifier Built on scRNA/TCR-seq Datasets




1. Caushi et al. *Nature*. 2021;596(7870):126–132
2. Oliveira et al. *Nature*. 2021;596(7870):119–125
3. Hanada et al. *Cancer cell*. 2022;40(5):479-493
4. Lowery et al. *Science*. 2022;375(6583):877–884
5. Meng et al. *Science Transl Med*. 2023;15(722):eadh9562
6. Pétremand et al. *Nat Biotechnol*. 2025;43(1):134-142

## Figure 1: Clones exhibiting functional reactivity against autologous melanoma cell lines were also identified as TRT using our clone-level TRT classifier

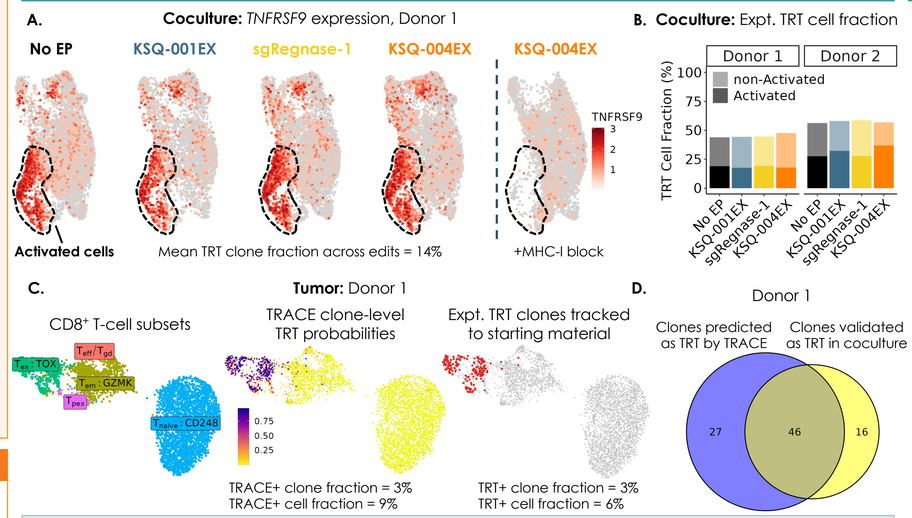



**Figure 1.** Predicted tumor-reactive (TRACE+) clones were confirmed by coculture of eTIL DP with autologous melanoma cell lines. **(A)** eTIL DPs were cocultured with an autologous tumor cell line with/without MHC-I blockade to confirm TCR-mediated reactivity to tumor. Experimentally-verified TRT clones were defined as those containing 1) ≥ 3 cells and 2) ≥ 10% of cells in a *TNFRSF9*-expressing activated state. **(B)** Bar plot showing the cumulative cell fraction of TRT clones and the subset of functionally activated cells in these clones across edits. **(C)** CD8⁺ T-cells in tumor starting material were identified using computational gating and used for TRACE predictions. Clone-level gene expression was used to predict clone-level reactivity. Experimentally-validated TRT clones were tracked back to starting material using alpha + beta TCR sequences. **(D)** Overlap between the set of experimentally validated TRT clones and those predicted as TRACE+, considering only clones detected in both samples.

## Figure 2: KSQ-001EX and KSQ-004EX exhibit increased functionality during autologous tumor coculture compared to unedited TIL

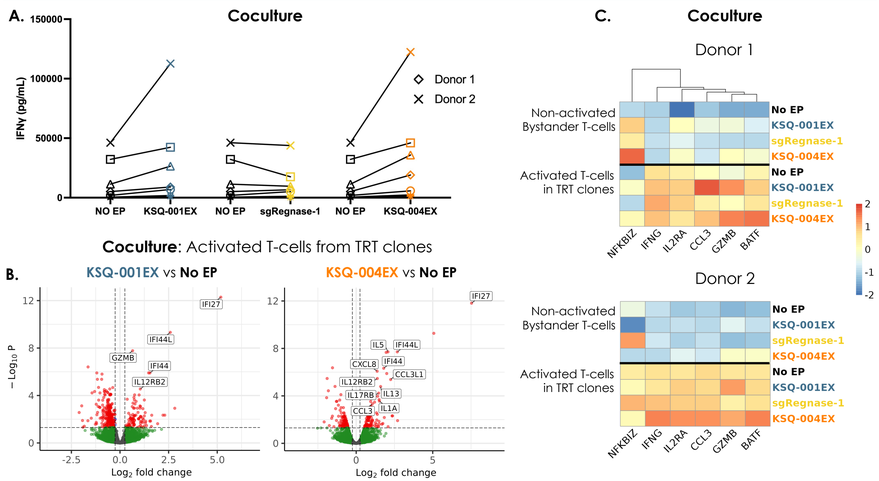



**Figure 2.** Inactivation of SOCS1 and/or Regnase-1 improves the anti-tumor function of TRT clones. **(A)** KSQ-001EX and KSQ-004EX exhibited increased IFN-γ levels in response to antigen stimulation, as measured by MSD of supernatant from overnight autologous co-cultures (n=7). **(B)** Volcano plot showing differential gene expression identified using edgeR on pseudobulked scRNA-seq data. In activated T-cells from TRT clones, interferon-stimulated genes are among some of the most upregulated in KSQ-001EX and KSQ-004EX compared to unedited controls. **(C)** Heatmap showing the impacts of SOCS1 and/or Regnase-1 inactivation on the expression levels of genes of interest in bystander T-cells and activated T-cells from TRT clones.

## Figure 3: KSQ-004EX exhibits increased resistance to exhaustion during serial restimulation with autologous tumor via increased cytotoxicity, proliferation, and cytokine production

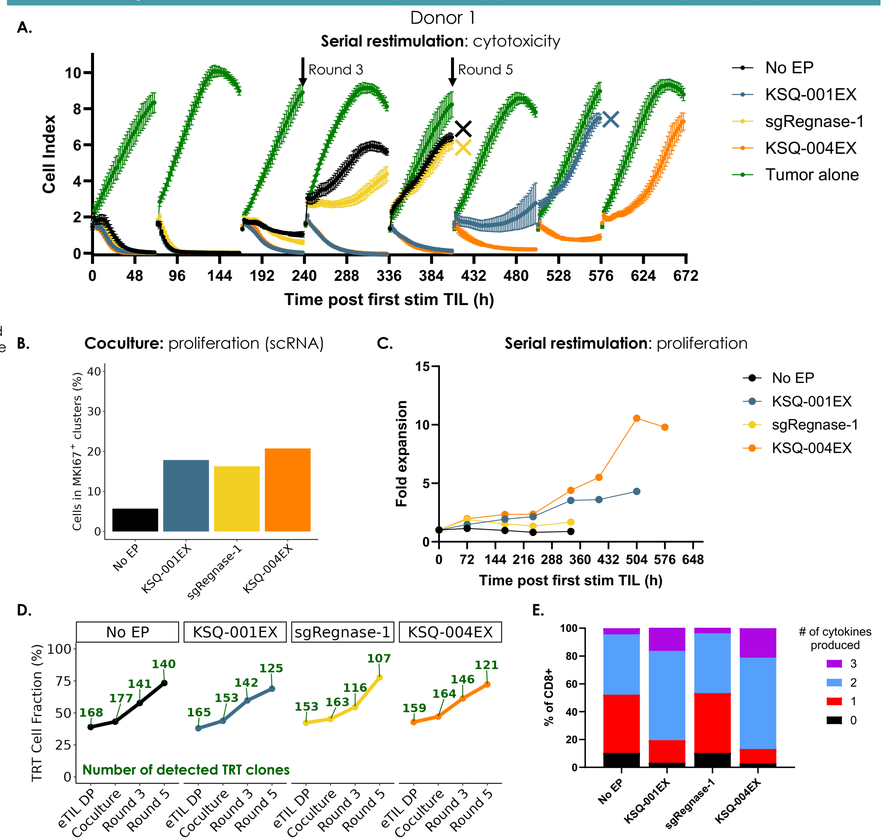



**Figure 3.** eTIL DPs were chronically restimulated with autologous tumor cells over 8 rounds. **(A)** Tumor cell growth was assessed by xCelligence to derive eTIL cytotoxicity. X's indicate where respective conditions were removed due to loss of tumor control. **(B)** Fraction of cells in *MKI67*+ clusters by scRNA-seq after 20h coculture. **(C)** Cumulative fold expansion of eTIL over the course of the serial restimulation assay. **(D)** The frequency of cells belonging to TRT clones during serial restimulation in all edit groups. **(E)** Intracellular cytokine staining was performed on serially restimulated TIL after Round 5 (~400h). Legend indicates how many of the three cytokines IFNγ, IL-2, and TNFα were produced in each cell.

## Conclusions

- We built TRACE, a clone-level ML classifier, to predict tumor-reactive T-cell clones in tumor starting material; a majority of predicted TRT clones were confirmed to be TRT by coculturing eTIL DP with an autologous melanoma cell line
- We observed the following impacts of SOCS1 and Regnase-1 inactivation in eTIL DP during autologous tumor coculture:
  - Upregulation of interferon-stimulated genes
  - Elevated expression of *GZMB*, *IFNG*, *CCL3*, and *BATF* in eTIL DP relative to unedited controls
- During chronic restimulation, the TRT cell fraction of eTIL DPs increased and KSQ-004EX demonstrated resistance to exhaustion relative to single-edited and unedited TIL via:
  - ✓ Sustained control of tumor growth
  - ✓ Enhanced proliferation
  - ✓ Retained polyfunctionality