Development of a Gene Editing Platform For the Manufacture of CRISPR/Cas9 Engineered Tumor Infiltrating Lymphocytes (eTIL®)

KSQ THERAPEUTICS

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ABSTRACT

Background:
To improve the clinical activity of Tumor Infiltrating Lymphocyte (TIL) therapy, we developed engineered TIL (eTIL®) using CRISPR/Cas9 gene editing: KSQ-001EX is a single-edit eTIL product with SOCS1 inactivated; KSQ-004EX is a dual-edit eTIL product with inactivation of both SOCS1 and Regnase-1. Inactivation of SOCS1 and Regnase-1 is anticipated to enhance anti-tumor efficacy of TIL. These eTIL products will be evaluated in the clinic for the treatment of solid tumors.

To streamline and shorten the manufacture of eTIL, we established a next-generation ExPRESS manufacturing process. The ExPRESS process is initiated with either surgically resected tumors or core biopsies as starting material, with a pre-editing phase, followed by electroporation of CRISPR/Cas9 ribonucleoproteins for gene editing, and a post-editing expansion phase. This process eliminated the Rapid Expansion Phase (REP) as well as the requirement for feeder cells, resulting in a manufacturing process approximately 22 days in length. Due to the heterogeneous nature of tumor starting material, the number of TIL generated following the pre-editing expansion phase is variable, presenting a unique challenge to engineering of TIL with CRISPR/Cas9. Here, we describe the successful development of an eTIL editing platform.

Methods:
To account for inherent patient to patient variability several process development studies were performed to optimize the eTIL engineering step of the ExPRESS process. Viably-cryopreserved-tumor-fragments (VCTF) from different solid tumor indications were used as starting materials, and key parameters were assessed during the expansion and electroporation stages. Different means of improving sterility assurance and a wide range of editing parameters were experimentally tested. Following electroporation, engineered TIL were further expanded for an additional 10 days prior to cryopreservation. The growth profile and editing efficiency and functionality of KSQ-001EX and KSQ-004EX were characterized.

Results:
GMP-compatible gene editing process parameters were optimized and identified at full scale. Gene editing of eTIL by electroporation of Cas9/sgRNAs using a MaxCyte ExPERT GTx consistently achieved robust editing in both single and dual-edit settings within KSQ-001EX and KSQ-004EX eTIL, respectively. Robust eTIL editing efficiency was achieved while maintaining consistently high cell viability, in vitro functionality, and other process attributes.

Figure 1. ExPRESS: A Feeder Cell-Free Manufacturing Process for KSQ-001EX & KSQ-004EX

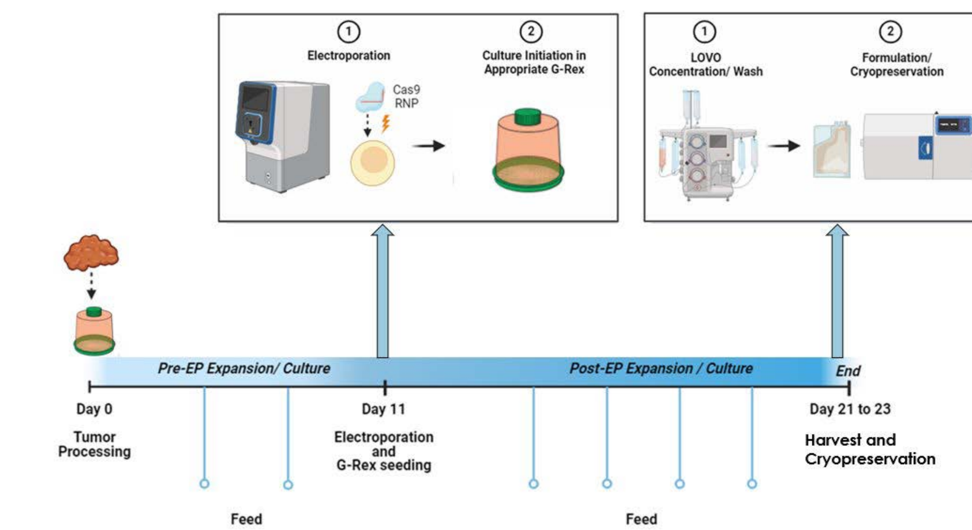

Figure 1: KSQ-001EX/KSQ-004EX manufacturing process consists of TIL activation and extravasation of the processed tumor for 11 days, followed by CRISPR engineering by electroporation (EP) and post-electroporation expansion of 10 to 12 days. TIL expansion through out the process is supported by IL-2.

Figure 2. Consistent Achievement of Minimum Pre-EP TIL Yield Across Donors and Tumor Types

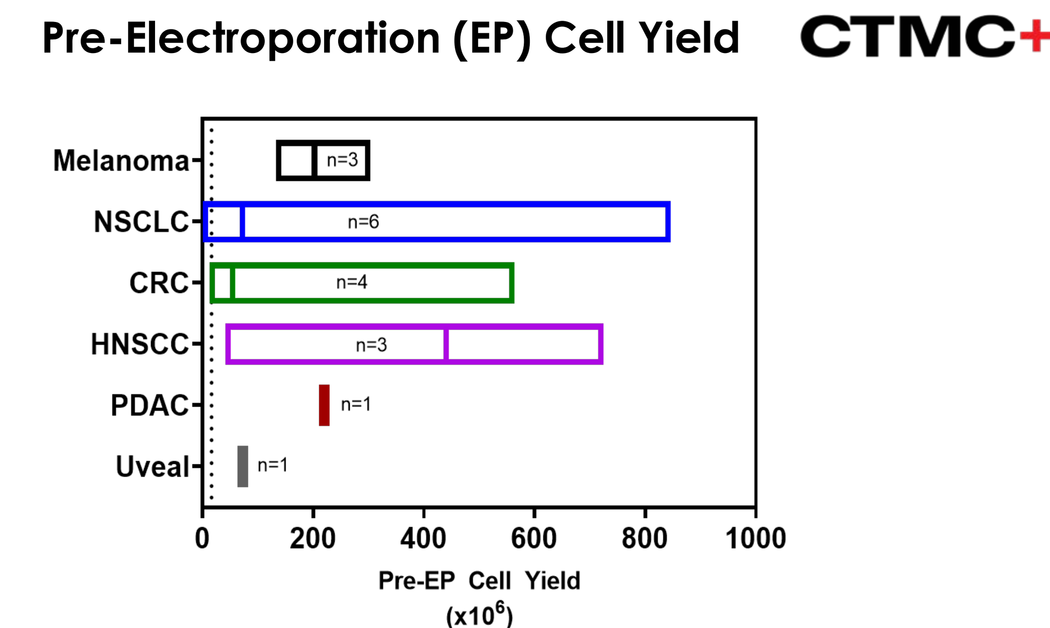

Figure 2: Full scale and extrapolated (scaled down) pre-EP yield. All melanoma and colorectal cancer patients were treated with systemic therapies prior to tumor harvest. The dotted line shows the minimum number of TIL (16×10^6) required to perform the electroporation (gene editing) step. The line in each bar shows the mean cell yield from the indication.

Figure 3. Engineering TIL in Culture Media Versus Buffer Improves Post-EP Expansion

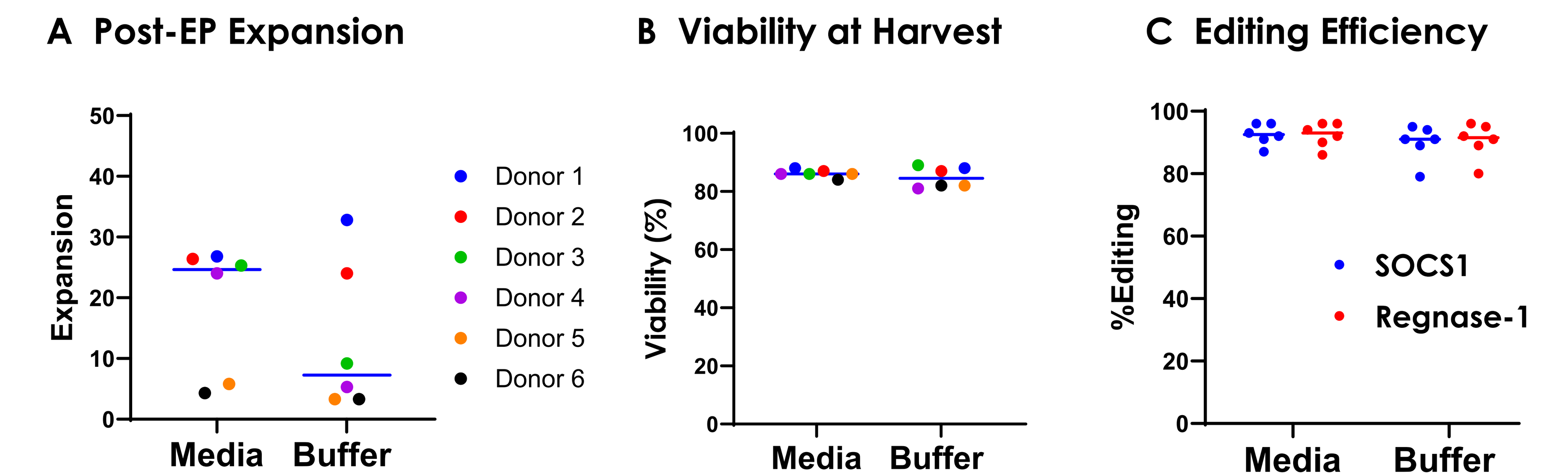

Figure 3: Pre-EP TIL were split in two, one half was suspended and edited in EP Buffer 1, the other half was suspended and edited in culture media. Post-EP TIL were cultured for 10 days and harvested. Cell counts were performed to calculate expansion and viability. DNA was extracted and editing efficiency was evaluated by ddPCR. **(A)** Five of six donors showed higher post-EP expansion when cells were suspended in culture media versus EP buffer during editing process. **(B)** Cell Viability at harvest was >80% in all samples. **(C)** Editing efficiency was >80% in all samples.

Figure 4. Cas9/sgRNA RNPs Are Stable and Drive High Editing Efficiency Six Hours After Complexation

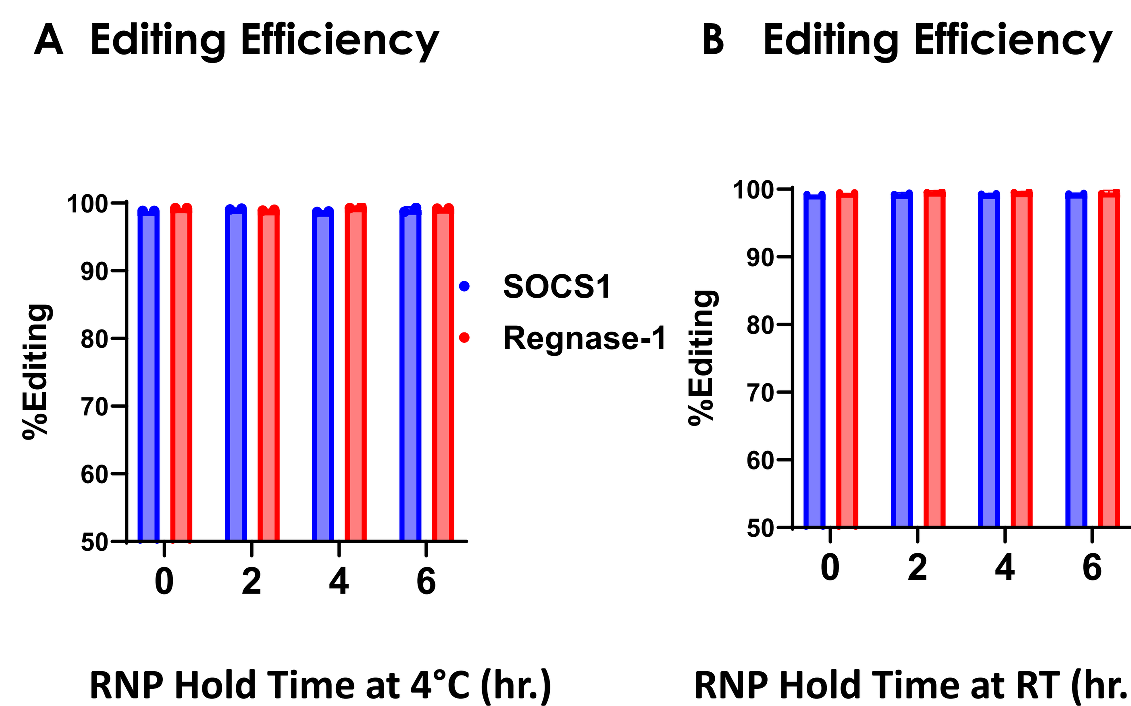

Figure 4: KSQ-004EX RNP were complexed in bulk and held at either 4°C or room temperature (RT). Editing was performed at T=0 hours, 2 hours, 4 hours, and 6 hours post RNP complexation. Post-EP TIL were cultured for 10 days. DNA was extracted from cell pellet from each condition. Editing efficiency was assessed by ddPCR. **(A)** High editing efficiency was achieved when RNP was held at 4°C and **(B)** room temperature for up to 6 hours.

Figure 5. Consistently High Editing Efficiency Achieved Across a Range of Cas9/sgRNA RNP

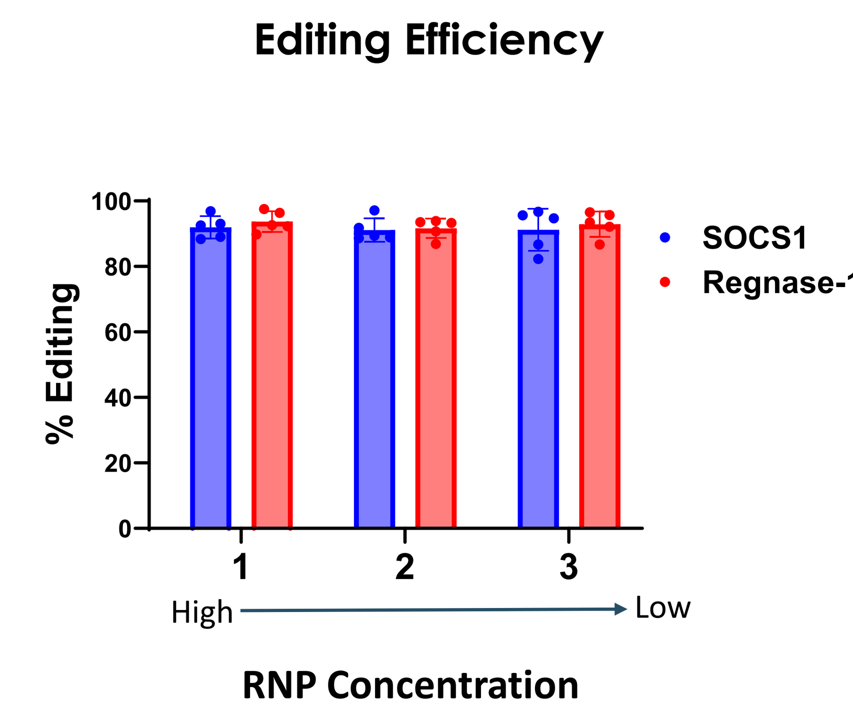

Figure 5: Three RNP concentrations were evaluated across five donors in KSQ-004EX. Both RNP 1 and RNP 2 achieved similarly high on-target editing efficiency whereas RNP 3 exhibited variability in some donors. RNP 2 dose was selected for clinical manufacture use.

Figure 6. Engineering TIL Across a Broad Range of Cell Concentrations Yields Consistent Expansion, Viability and Editing Efficiency

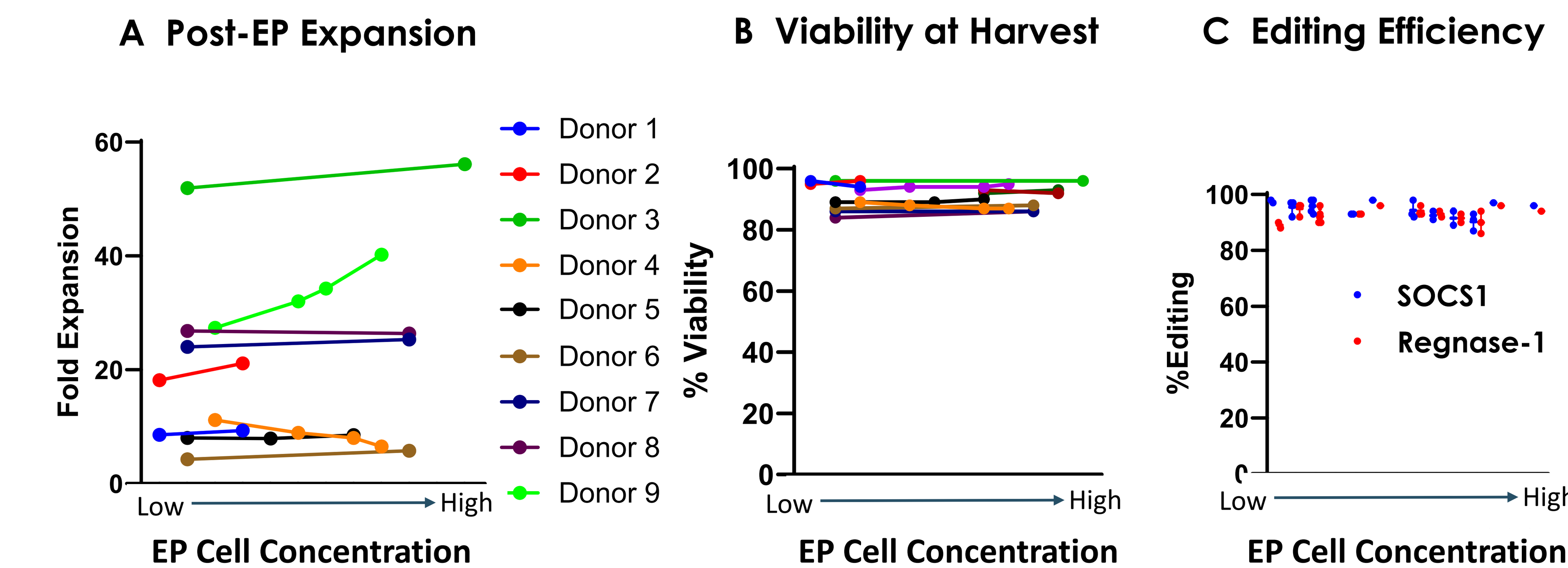

Figure 6: Multiple donors(n=9) were used for EP cell concentration titration. At least two cell concentrations were evaluated per donor based on the cell availability. Post-EP TIL were cultured for 10 days and harvested. Cell counts were performed to calculate expansion and viability. DNA was extracted and editing efficiency was evaluated by ddPCR. **(A)** Majority of donors showed slightly increased expansion at higher cell concentration. **(B)** Cell Viability at harvest was >80% in all samples. **(C)** Editing efficiency was >80% in all samples tested.

Figure 7. Robust Editing Efficiency, Viability and Functionality Achieved with High $CD3^+/CD8^+$ Cellularity in Scale Up Process Lock-Down Runs

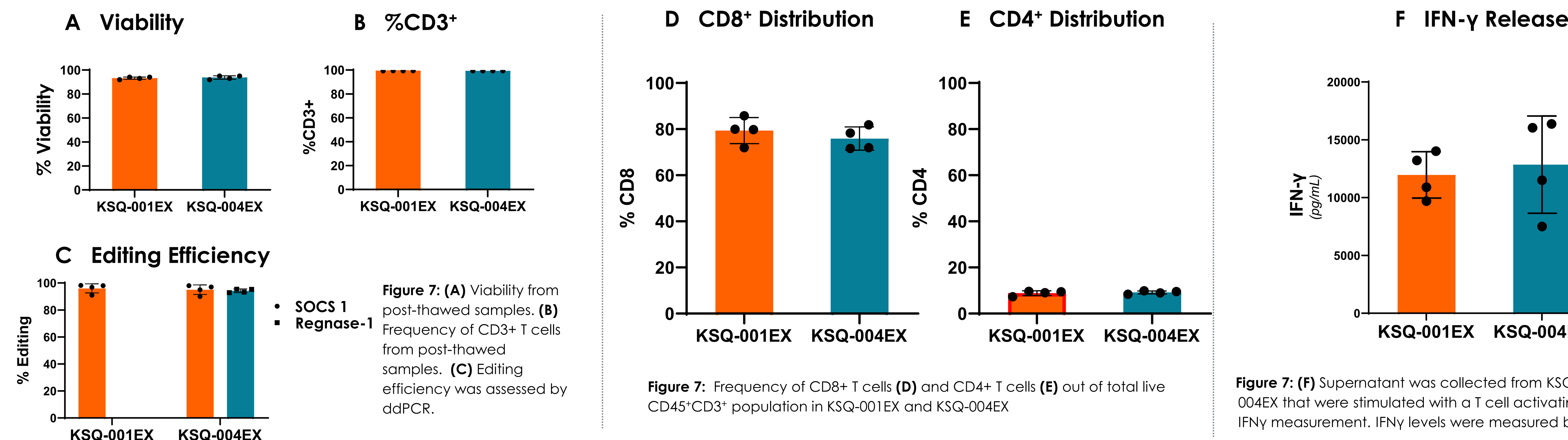

Figure 7: (A) Viability from post-thawed samples. **(B)** Frequency of CD3+ T cells from post-thawed samples. **(C)** Editing efficiency was assessed by ddPCR.

Figure 7: Frequency of CD8+ T cells **(D)** and CD4+ T cells **(E)** out of total live $CD45^+CD3^+$ population in KSQ-001EX and KSQ-004EX

Figure 7: (F) Supernatant was collected from KSQ-001EX and KSQ-004EX that were stimulated with a T cell activating reagent for 24hr for IFNγ measurement. IFNγ levels were measured by ELISA.

CONCLUSIONS

- With the goal of simplifying the manufacture of eTIL and achieving robust editing efficiency, we report the development of a robust, scalable gene editing platform that accounts for tumor variability in the manufacture of eTIL using a next generation ExPRESS manufacturing process that eliminates the need for feeder cells.
- Both KSQ-001EX and KSQ-004EX consist of high percentage of $CD8^+$ eTIL and release high levels of IFN-γ upon stimulation.