PDL1-targeted synthetic receptor engineering for T cell immunotherapy in solid tumor

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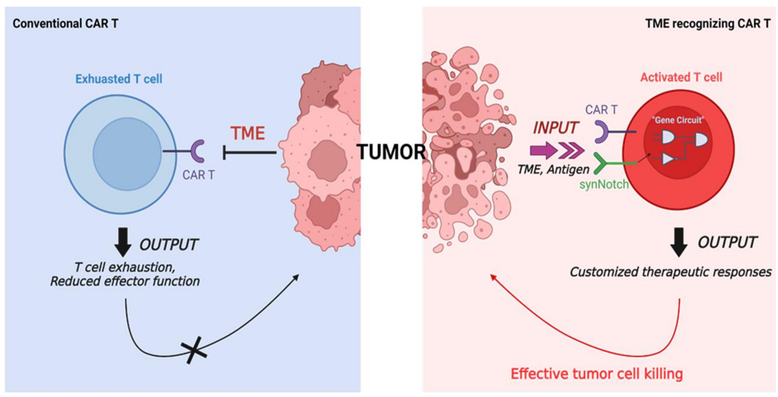
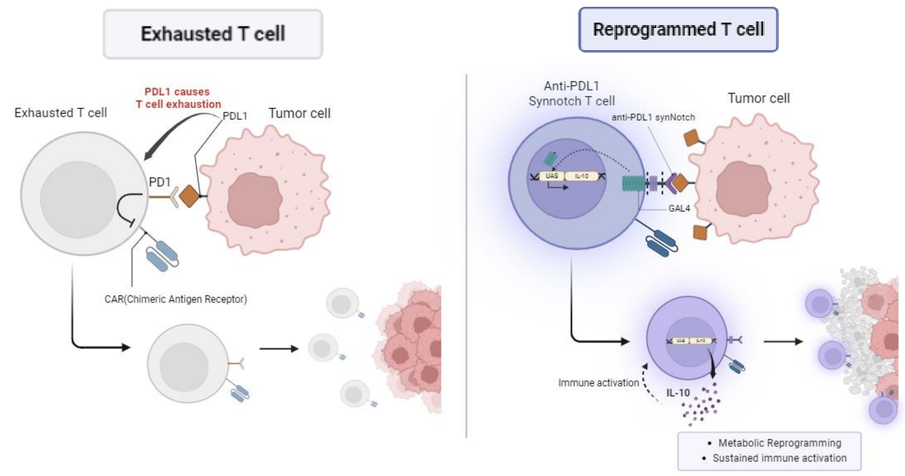
Introduction

Figure 1 l Schematic of overcoming solid TME by Synnotch signaling.



CAR T cell therapy has demonstrated significant potential, particularly in hematological malignancies, but its efficacy in solid tumors is hindered by the **immunosuppressive tumor microenvironment (TME).** Within the TME, immune checkpoints such as PD-1 induce T cell exhaustion, limiting their ability to sustain an anti-tumor response. This study introduces a synNotch-based CAR T platform designed to **overcome these challenges by recognizing TME-specific antigens** and incorporating a genetic circuit for IL-10 cytokine secretion. This system **enhances immune activation**, supports effector molecule production, and **inhibits PD-1 signaling**, thereby preventing T cell exhaustion and enabling a more durable anti-tumor response, even in the presence of immunosuppressive signals.

Mechanism



The proposed mechanisms for engineering SynNotch CAR T cells that resist exhaustion and maintain effector functions in **immunosuppressive tumor microenvironments (TME).**

When SynNotch binds its target antigen (PD-L1), it undergoes cleavage, releasing an intracellular transcription factor that translocates to the nucleus. There, it binds to the Upstream Activation Sequence (UAS) promoter region, triggering IL-10 gene expression. As a result, engineered T cells produce and secrete IL-10**. IL-10 expression activates the functions** of metabolic reprogramming and **sustained immune activation.**

Experimental Methods

Figure 2 l Confirmation of anti-PD-L1 SynNotch and tBFP reporter gene expression

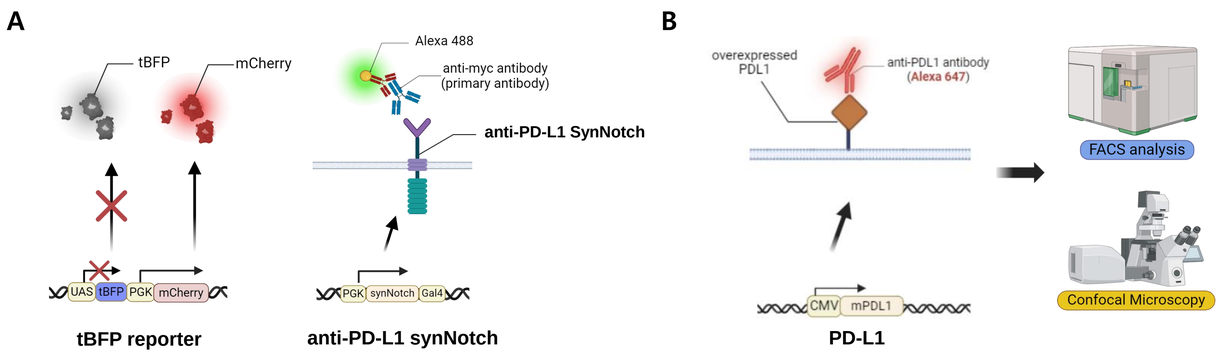
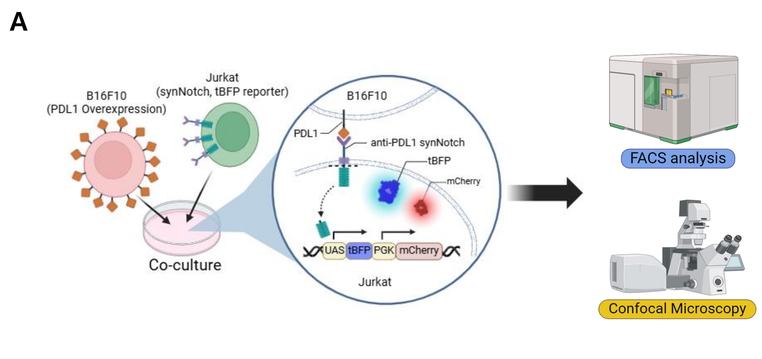


Figure 2 l

A) Confirmation of tBFP reporter and synNotch transduction via antibody staining. Two plasmids were synthesized using a lentivector plasmid, one tBFP reporter (UAS-tBFP-PGK-mCherry) and synNotch (PGK-anti-PDL1-synNotch-Gal4) plasmid. Lenti-X HEK293T was used as host. Lentivirus was harvested and transduced to Jurkat T cell line. ICC was conducted with anti-myc (primary antibody) and anti-mouse-488 (secondary antibody). Fluorescence data was obtained through Confocal Microscopy and FACS analysis.

B) Confirmation of PD-L1 transduction via antibody staining. A plasmid for PDL1(pHR-CMV-mPDL1) was synthesized using Lenti-X HEK293T as a host. After harvest, the lentivirus was transduced to the B16F10 cell line. ICC was conducted with anti-PDL1 antibody expressing Alexa 647 signal. Fluorescence data were obtained through Confocal Microscopy and FACS analysis.

Figure 3 l Co-culture for confirming selective binding of anti-PDL1 synNotch T cells to PDL1 overexpressing tumor cells.



Cell line	PDL1 O.E.	Synnotch
B16F10 + Jurkat	X	X
B16F10 PD-L1 O.E. + Jurkat	O	X
B16F10 + Synnotch Jurkat	X	O
B16F10 PD-L1 O.E. + Synnotch Jurkat	O	O

Figure 3 l

A) Co-culture Schematic. Jurkat cell (synNotch+, tBFP reporter+) and B16F10 (PD-L1 overexpression condition)
B) Co-culture condition table. (E:T ratio = 1:5)

Figure 4 l Mouse primary CD8+ T cell isolation and Repetitive stimulation assay

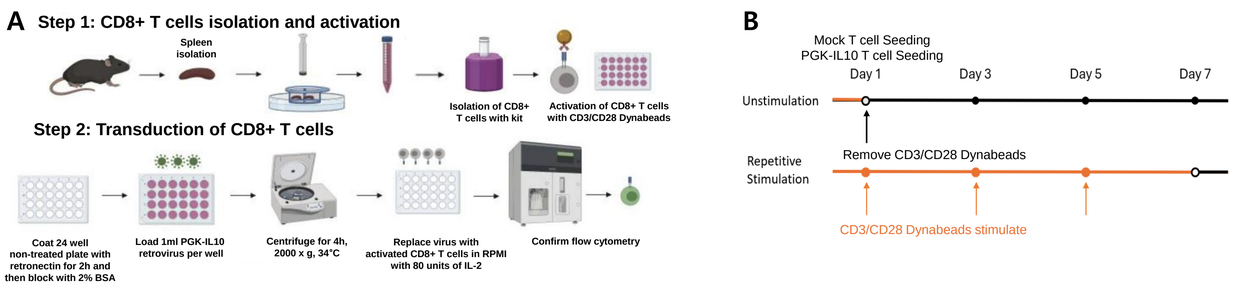


Figure 4 l

A) Mouse primary CD8+ T cells isolation, activation and PGK-IL10 retroviral transduction. T cells with PGK-IL10 integration were sorted and used for the experiment.

B) Repetitive stimulation timeline. On Day 1, CD3/CD28 Dynabeads were removed, and for the repetitive stimulation group, Dynabeads were added every two days. Exhaustion marker analysis was performed on Day 7, followed by cytokine measurement two days later.

Results

Figure 5 l Effector cells and Target cells express each component after lentiviral transduction

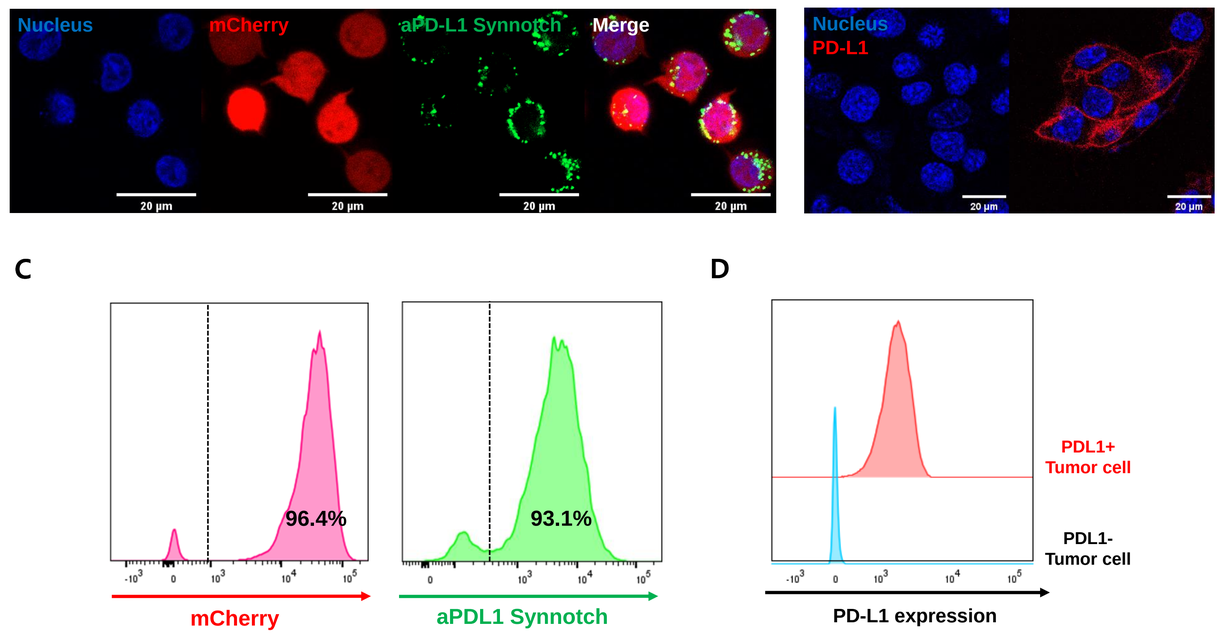


Figure 5 l

A) Confirmation of anti-PD-L1 synNotch expression on the membrane of Jurkat T cells by confocal microscopy. Cells were stained with DAPI (Nucleus) and anti-myc (primary antibody) and anti-mouse Alexa fluor 488 (secondary antibody).

B) Confirmation of PD-L1 expression on the membrane of B16F10 tumor cells by confocal microscopy. Cells were stained with DAPI (Nucleus) and anti-PD-L1 APC conjugated antibody (primary antibody).

C) Confirmation of anti-PD-L1 synNotch expression on the membrane of Jurkat T cell by Flow cytometry.

D) Confirmation of PD-L1 expression on B16F10 Tumor cells by Flow cytometry. FACS result shows that expresses each fluorescence component accordingly. (PD-L1- and PD-L1+ tumor cells).

Figure 6 l Jurkat T-cell expresses tBFP selectively when co-cultured with PDL1 overexpressing B16F10 tumor cells.

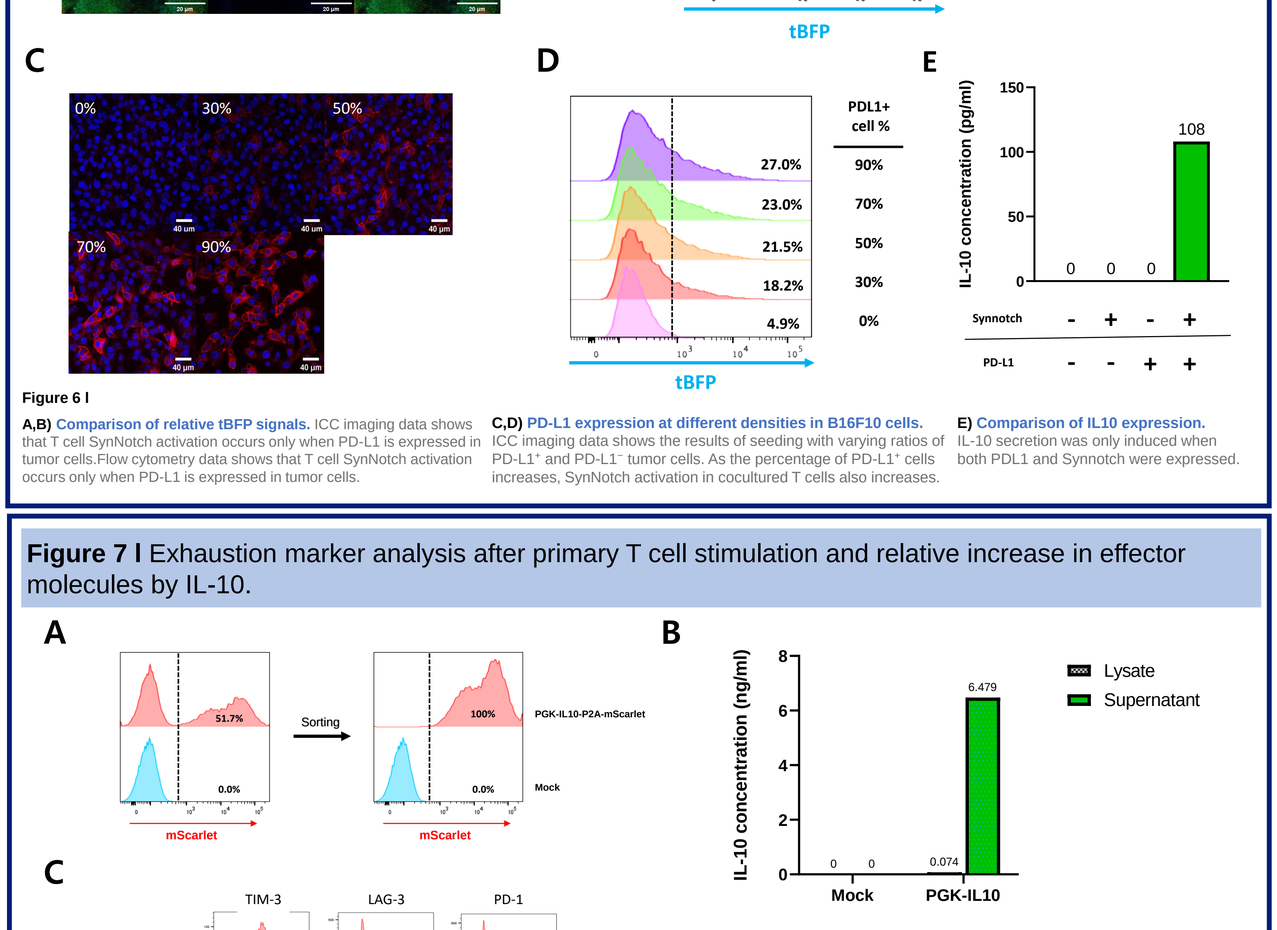


Figure 6 l

A,B) Comparison of relative tBFP signals. ICC imaging data shows that T cell SynNotch activation occurs only when PD-L1 is expressed in tumor cells.Flow cytometry data shows that T cell SynNotch activation occurs only when PD-L1 is expressed in tumor cells.

C,D) PD-L1 expression at different densities in B16F10 cells. ICC imaging data shows the results of seeding with varying ratios of PD-L1$^+$ and PD-L1$^-$ tumor cells. As the percentage of PD-L1$^+$ cells increases, SynNotch activation in cocultured T cells also increases.

E) Comparison of IL10 expression. IL-10 secretion was only induced when both PDL1 and Synnotch were expressed.

Figure 7 l Exhaustion marker analysis after primary T cell stimulation and relative increase in effector molecules by IL-10.

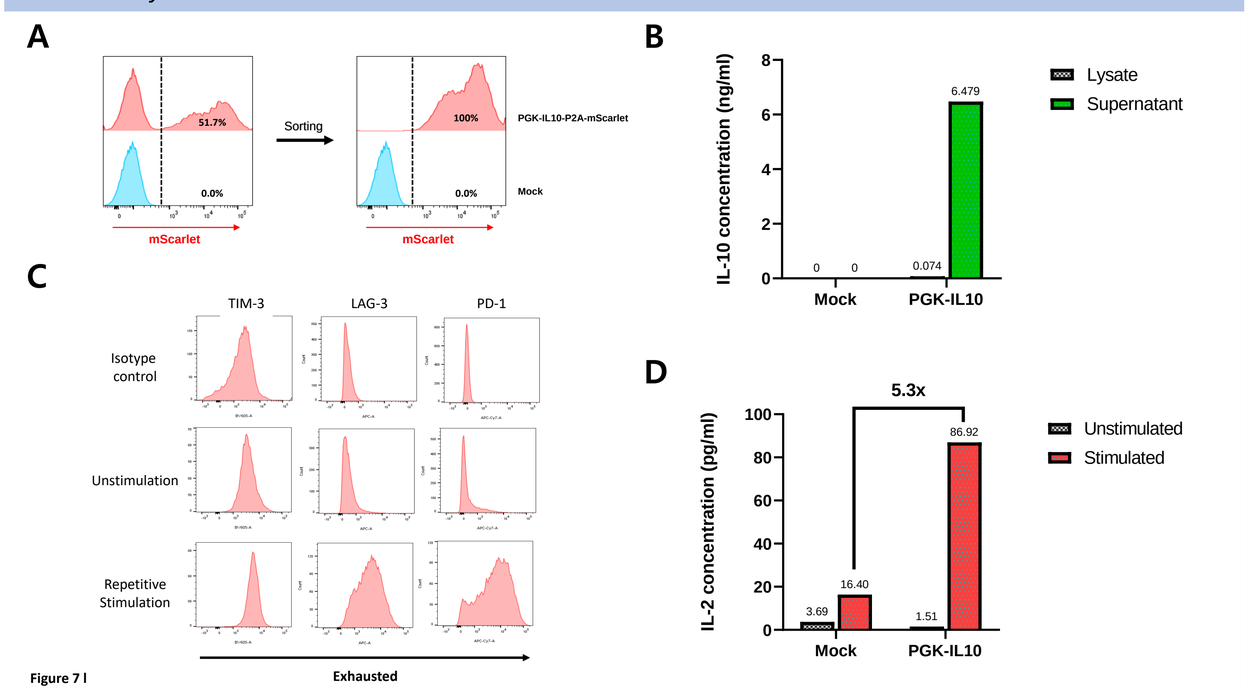


Figure 7 l

A) Validation of PGK-IL10 expression in primary mouse T cells. Following isolation, transduction resulted in 51.7% expression of PGK-IL10. Subsequently, sorting was performed using the reporter fluorescent protein mScarlet, and only PGK-IL10-expressing cells were used for the experiment.

B) IL-10 ELISA Results. IL-10 was detected only in the supernatant of primary T cells expressing IL-10 under the PGK promoter. In contrast, IL-10 in the cytosol was not detected, as confirmed by lysate measurements.

C) Exhaustion marker analysis after primary T cell stimulation. Repetitive stimulation group shows increased expression of TIM-3, LAG-3, and PD-1 compared to the unstimulated group.

D) IL-2 ELISA Results. Cytokine secretion was measured after sorting PD-1$^+$ LAG-3$^+$ cells from the repetitive stimulation group. IL-2 expression was higher in the stimulation group compared to the unstimulated group, and the PGK-IL10-expressing group showed a 5.3-fold increase in IL-2 levels.

Conclusion & Further Study

Our study demonstrates that SynNotch-based engineered **T cells can selectively recognize PD-L1-expressing tumor cells and sustain immune activation** through **IL-10 secretion.** This system effectively enhances effector molecule production and reduces exhaustion marker expression, thereby overcoming immunosuppressive signals in the tumor microenvironment. However, some limitations remain, including slight **leakage from the UAS promoter** and the potential for further **optimization of activation efficiency**. Addressing these issues will improve the precision and efficacy of the system. For future studies, we aim to evaluate this approach in **in vivo tumor models**. Specifically, we will assess whether **SynNotch-driven IL-10 secretion significantly enhances anti-tumor cytotoxicity** while maintaining T cell functionality within the immunosuppressive tumor microenvironment. These findings will provide valuable insights for the clinical translation of SynNotch-based T cell therapies.