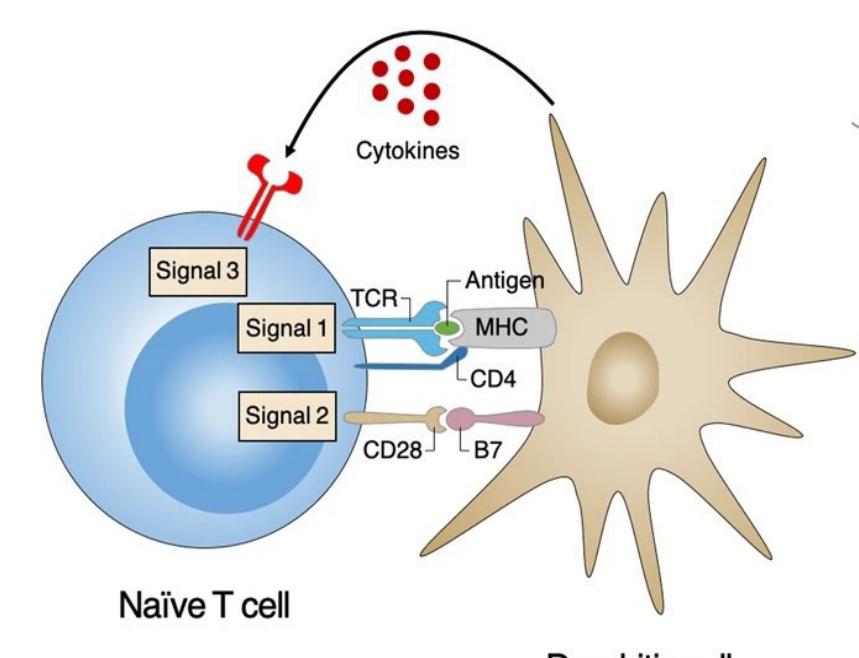
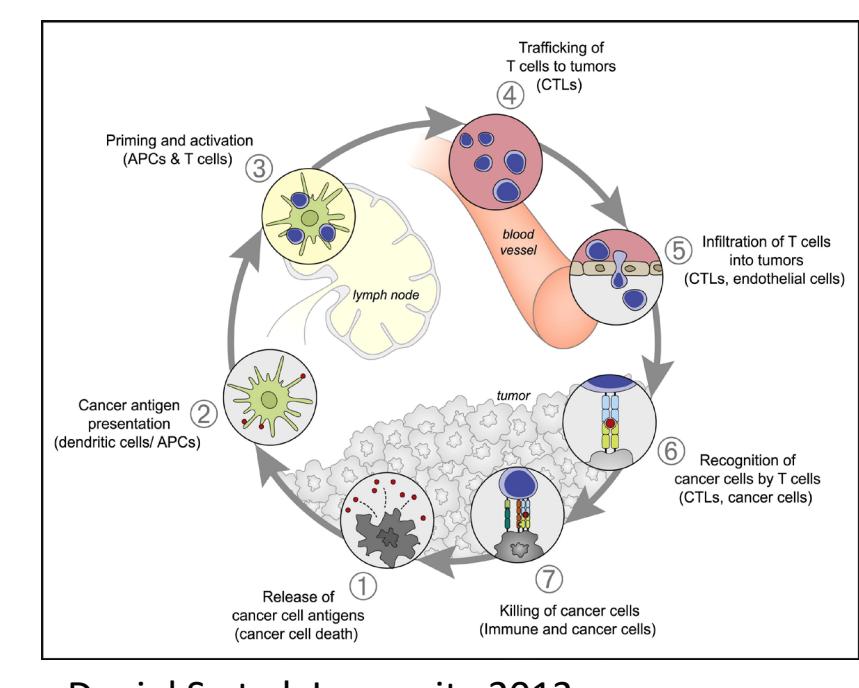


Abstract

The roles of Dendritic Cells (DCs) in the activation of different types of T cells have been extensively studied in various fields. It is well established that Conventional Dendritic Cells (cDCs) play a crucial role in the priming of T cells in the Draining Lymph Node (DLN). These T cells are then migrating to inflammatory tissues, where they are licensed by Monocyte-Derived Dendritic Cells (MoDCs). However, the exact process of T cell priming and licensing in the tumor setting is lacking. Our previous studies using a murine model have shown that MoDCs do not migrate to the DLN but instead remain at the tumor site, where they promote the cytotoxic activity of tumor-reactive CD8+ T cells. Furthermore, CD8+ T cells from the tumor exhibit higher cytotoxicity than those from the DLN. In this study, we provide new insights into the differential transcriptomic expression of CD8+ T cells and DCs in the DLN and the tumor microenvironment of mice bulk RNA-seq. We profiled the effect of priming CD8+ T cells with cDCs and licensing them with MoDCs. We also predicted the ligand-receptor interactions between MoDCs and licensed CD8+ T cells, identifying crucial interactions for the cytotoxic function of CD8+ T cells. Our study unveils the process through which CD8+ T cells become more cytotoxic and tumor-specific in a murine model, elucidating the importance of MoDCs in this process and revealing the exact interactions necessary for the full activation of CD8+ T cells.

Rationale

- DCs present cancer cell antigens to T cells in the lymph node.
- In previous studies, we found that MoDCs do not migrate to the DLN, but rather remain at the tumor site where they license the cytotoxic activity of tumor-reactive CD8+ T cells.
- The abundance of MoDCs is greater in the tumor compared to the DLN, where cDCs are abundant.
- T cells migrate to the tumor site where they are licensed and activated by MoDCs.
- Our in vivo experiments in mice show that CD8+ T cells from the tumor are more cytotoxic than CD8+ T cells from the DLN.
- We thus examined the ligand-receptor interactions between MoDCs and CD8+ T cells, showing enrichment of downstream analysis of different receptor genes.



The Data

In Vivo

In our in vivo analysis, we compared the transcriptional profiles of dendritic cells (DCs) and CD8+ T cells isolated from tumors and draining lymph nodes (DLN) via bulk RNA-seq. This allowed us to examine the differences in gene expression between these cell types in the tumor microenvironment and the DLN.

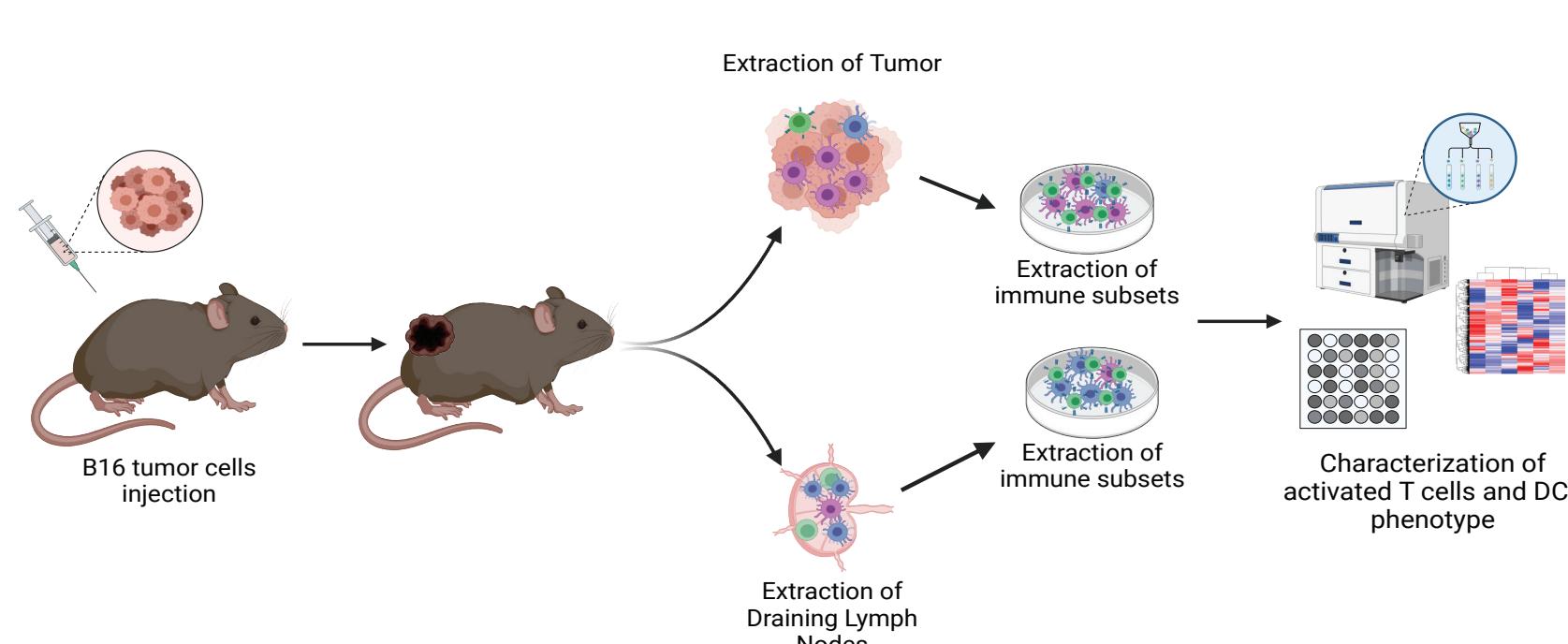


Figure 1 – CD8+ T cells from Tumor vs DLN in vivo

- A. CD8+ T cells from the tumor are more cytotoxic than T cells from the DLN.
B. The transcriptional profile of CD8+ T cells receptors isolated from the tumor or from DLN is different.

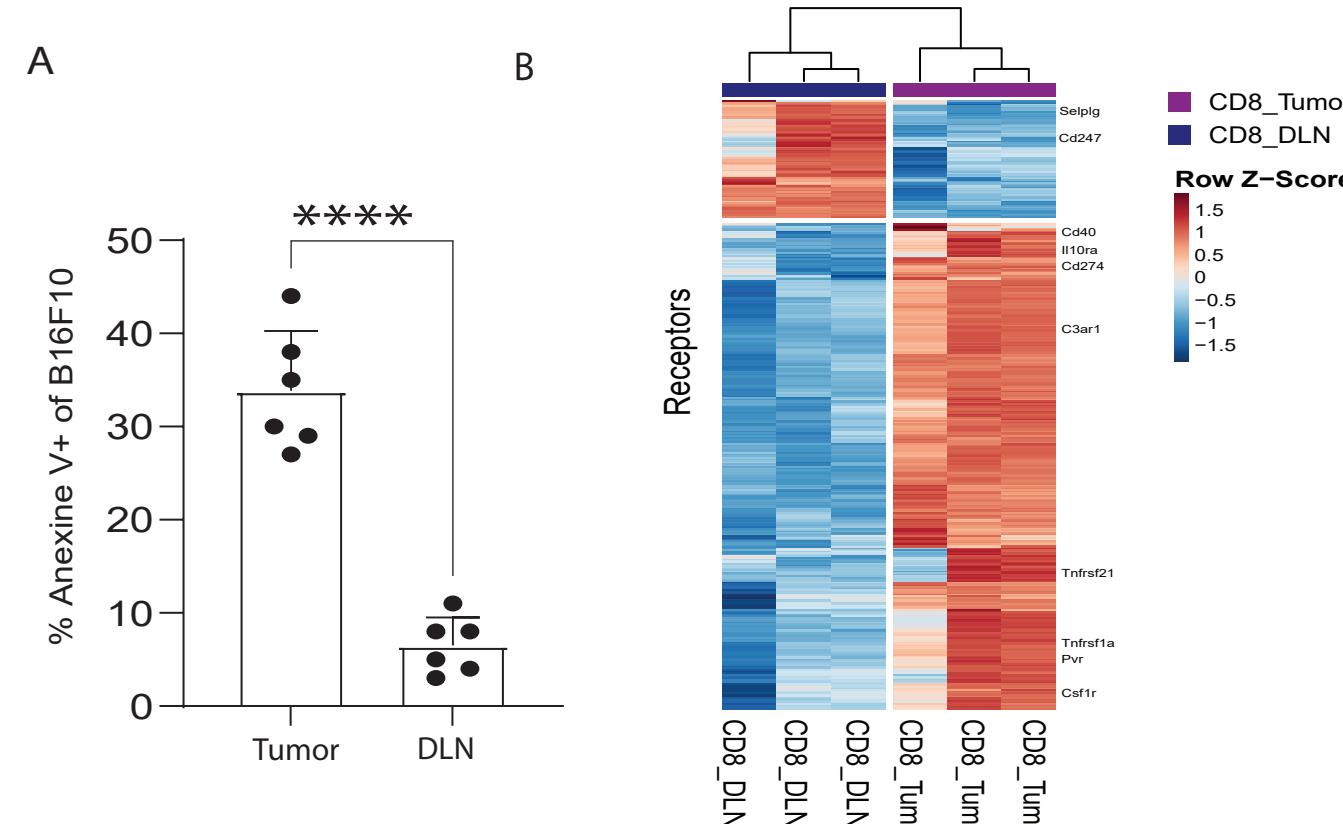


Figure 2 – DCs from Tumor vs DLN in vivo

- A. The abundance of MoDCs is greater in the tumor than in the DLN, where cDCs are more abundant.
B. The transcriptional profile of DCs in tumor differ from those in the DLN.

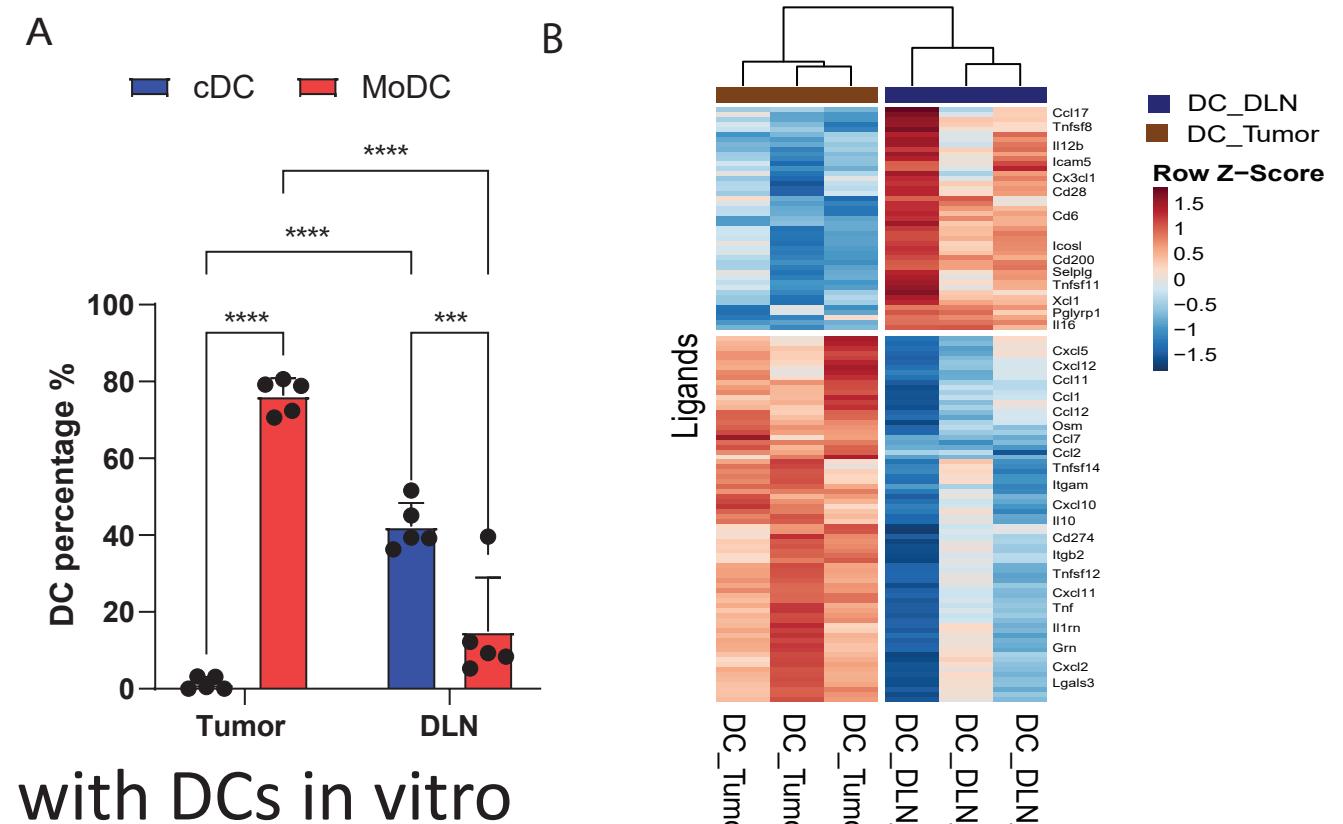
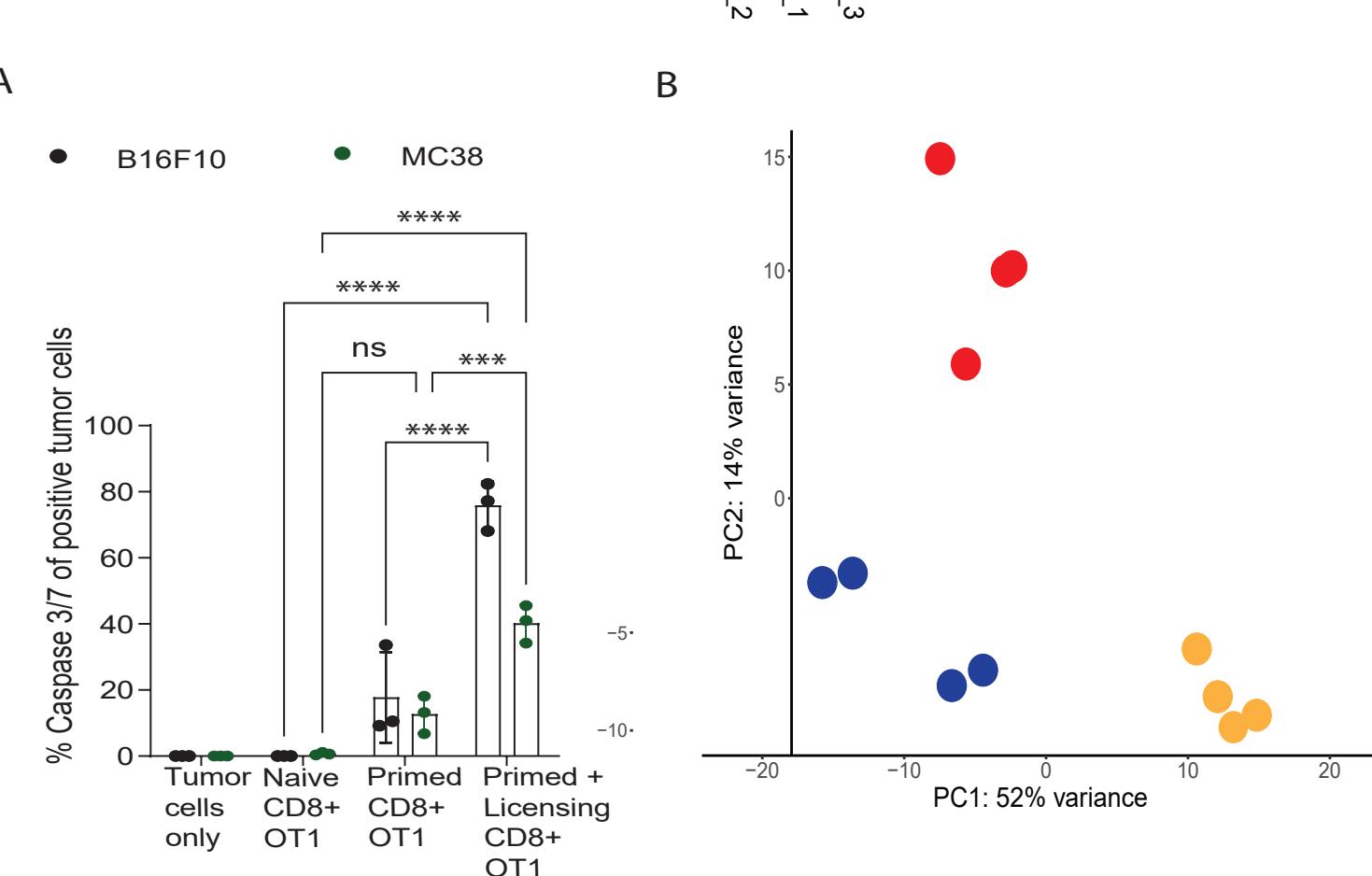


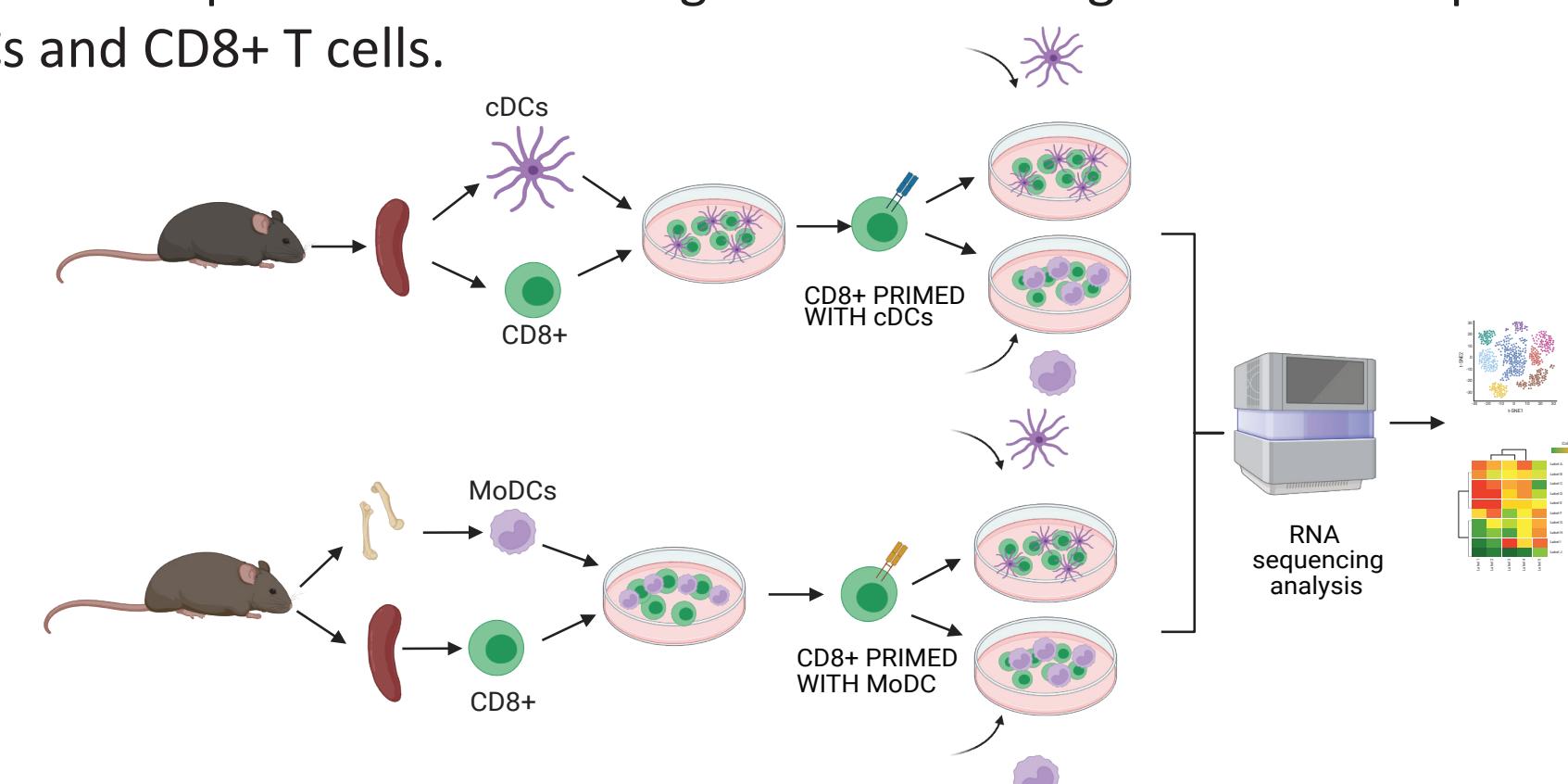
Figure 3 – CD8+ T cells priming and licensing with DCs in vitro

- A. In different cancer types (B16F10 and MC38) we see that for CD8+ T cells to become cytotoxic they need licensing with MoDCs in addition to priming with cDCs.
B. The transcriptional profile of CD8+ T cells after just priming differs from those after priming and licensing.



In Vitro

In our in vitro analysis, we examined the transcriptional profiles of various immune cell types, including MoDCs, cDCs, and CD8+ T cells, under different conditions via bulk-RNA seq. We compared activated and naïve states for both MoDCs and cDCs, and for CD8+ T cells, we looked at cells primed with different types of dendritic cells (cDCs and MoDCs) and examined the effects of licensing with either cDCs or MoDCs. This analysis allowed us to identify differential gene expression patterns and investigate the role of ligands and receptors in the interactions between MoDCs and CD8+ T cells.



Results

Figure 4 – MoDCs ligands and CD8+ T cells receptors interactions (Circos plot)

- A. Circos workflow illustration. Max flow algorithm was implemented on Protein-Protein Interactions (PPI) of bulk RNA-seq expression data.
B. The calculated DSA value represents how much a receptor impacts the cascading processes in the cell.
C. The uniquely up regulated ligand genes in activated MoDCs, taken for the circos plot in graph B.
D. Receptor genes Tnfrsf1b, Cxcr4 and Csf2rb are found to have high DSA values and play crucial role in activating CD8+ T cells at the tumor site.

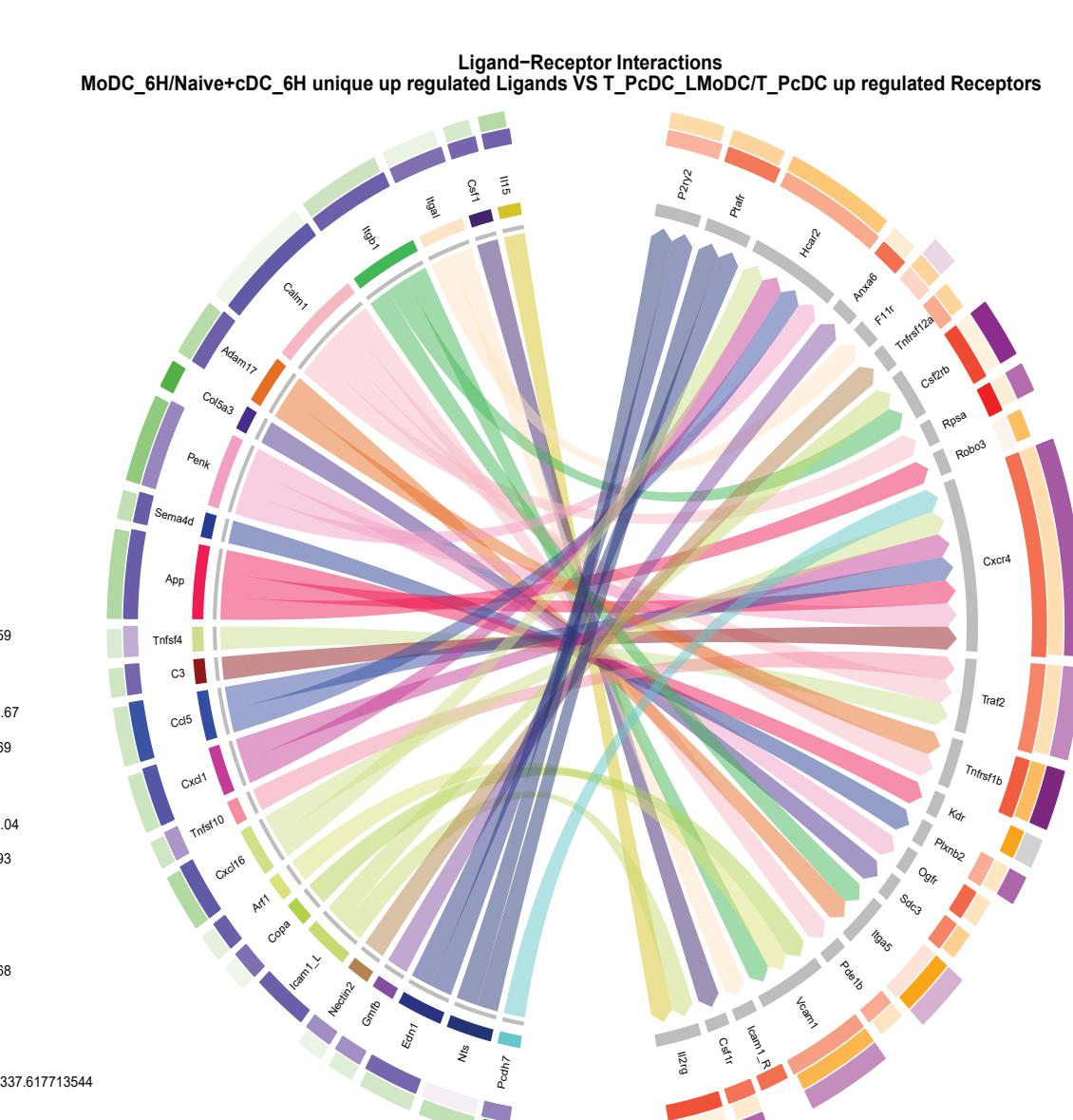


Figure 5 – Identification of enriched transcription factors in primed and licensed T cells

