**Abstract # 3843**

# Role of CD4/CD8 double positive T cells in modulating response to anti-PD-1 treatment in renal cell carcinoma

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## Background

Presence of CD4+CD8+ Double Positive T (DPT) cells in the tumor microenvironment has been correlated with good prognosis[1]. Renal cell carcinoma (RCC) is reported to have relatively much higher DPT cell sub-populations as compared to other cancer types[1,2]. DPT cells in RCC were reported to express high levels of programmed cell death protein-1 (PD-1) and therefore is considered a potential target for checkpoint based immunotherapy[2]. However, the clinical relevance of these DPT cells in determining response to anti-PD-1 has not yet been studied. Using the near native Farcast™ TruTumor RCC histoculture platform, we attempt to elucidate the role of DPT in anti-PD1 response modulation.

## Methods

**Patient tissue samples:** Fresh, surgically resected clear cell Renal Cell Carcinoma (ccRCC) tissue samples were collected from consented patients. A matched blood sample from the patient was also collected.

**Histo-Culture workflow:** The tumor sample was processed to generate thin explants, without enzymatic digestion, to retain the tumor microenvironment. Tumor explants were cultured with media and autologous plasma and cultured for 72 hrs. Explants were treated with anti-PD1 (Nivolumab:132 µg/ml) and media was replaced every 24 hours. Response was evaluated using H&E, IHC, cytokine release, flow cytometry and gene expression analysis using NanoString platform.

**Flow cytometry analysis:** The tumor explants were dissociated post culture with various treatments into single cells and stained with Live/Dead dye, and cocktail of immune cell lineage and activation marker antibodies. Data was acquired using BD LSR Fortessa Flow cytometer with appropriate compensation controls and analyzed using FlowJo software. DPT cells were gated and expressed as percentage of CD3.

**IHC:** Cleaved Caspase 3 IHC was performed with 4µm sections obtained from the FFPE block using Ventana IHC automated staining system. Scoring was performed by a certified pathologist.

**Multiplex Immunohistochemistry (mIHC)**: Using 4µm FFPE sections, mIHC (comprising anti-CD4, anti-CD8, anti-panCK and DAPI nuclear counterstain) was performed using Opal dyes (Akoya Biosciences) for detection. Data was analyzed using QuPath analysis software.

**NanoString Analysis:** The RNA extracted arm-wise from the explant TMA (Tissue Micro Array) FFPE block was quantified using Tape Station and 50ng of RNA based on DV200 concentration was used for running on the nCounter PanCancer IO 360 panel. Data was normalized and analyzed using the nSolver™ Data Analysis software for post treatment RNA samples.

**Cytokine Analysis:** The cultured supernatants at T0, T24, T48, T72 were tested for the presence of cytokines (IFN-g, Granzyme-B, Perforin) using Luminex MAGPIX instrument and data was analysed using MILLIPLEX™ Analyst software.

**Statistical analysis:** All data analysis and graphical representations were done using GraphPad Prism (Version 9). Mann-Whitney t-test was used to generate p-values. p-value significance is represented as *(p<0.05) **(p<0.01) ***(p<0.01). Heat maps were generated on GraphPad and Morpheus (https:/software.broadinstitute.org/morpheus).

## Farcast TruTumor Histoculture work-flow

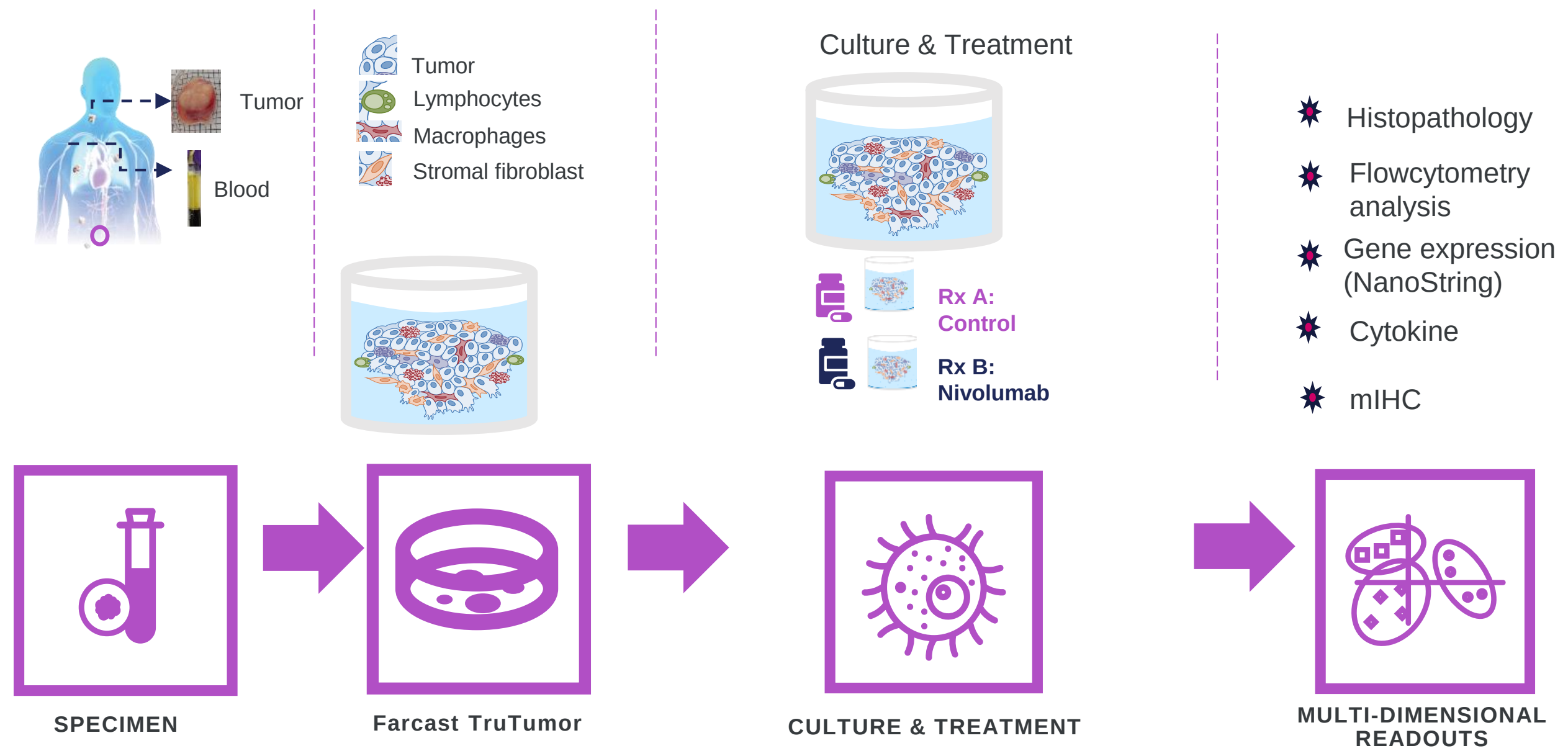

**Fig. 1:** Schematic representation of Farcast™ TruTumor Histoculture platform work-flow and downstream assays used for treatment response evaluation.

## Patient demography

| Parameters | Categories | Values (%) |
|---|---|---|
| Age | ≤ 60.5 | 9 (50%) |
| | ≥ 60.5 | 9 (50%) |
| Gender | Female | 2 (11.1%) |
| | Male | 16 (88.9%) |
| Grade | Grade 1 | 12 (66.7%) |
| | Grade 2 | 5 (27.7%) |
| | Grade 3 | 1 (5.6%) |
| Stage | I | 8 (44.5%) |
| | II | 4 (22.2%) |
| | III | 2 (11.1%) |
| | IV | 2 (11.1%) |
| | Unknown | 2 (11.1%) |
| Primary/Recurrent | Primary | 18 (100%) |
| Tumor Site | Left Renal mass | 9 (50%) |
| | Right Renal mass | 9 (50%) |

**Table 1:** Demography of patient (n=18) sample used for histoculture.

## RCC has relatively higher proportions of CD4+CD8+ DPT cells

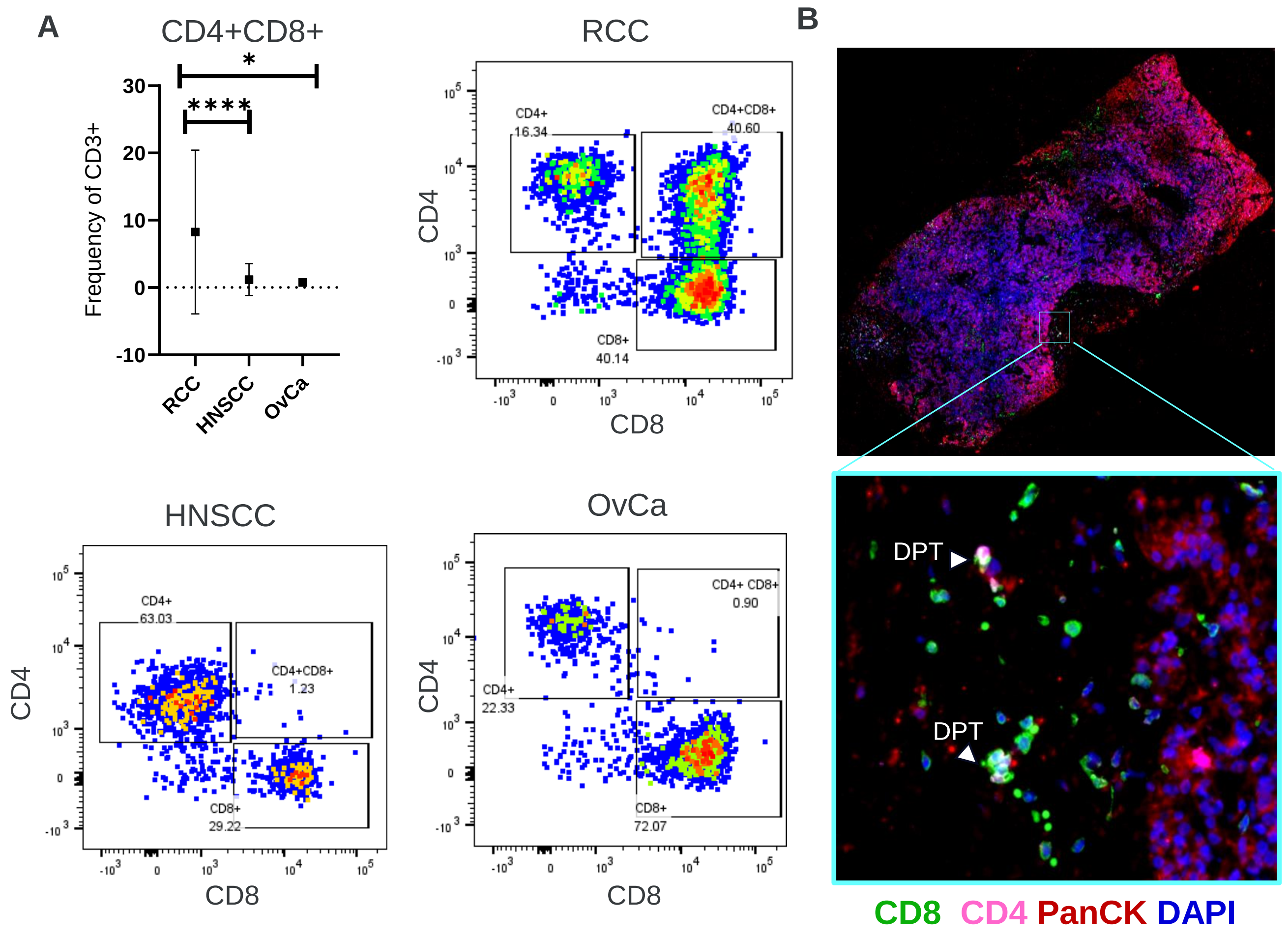

**Fig. 2:** **A.** Plot showing presence of DPT cell population in RCC, HNSCC and ovarian cancer (OvCa) samples (flowcytometry). **B.** Representative mIHC image showing presence of DPT cells in RCC microenvironment.

## DPT cells are preserved in culture

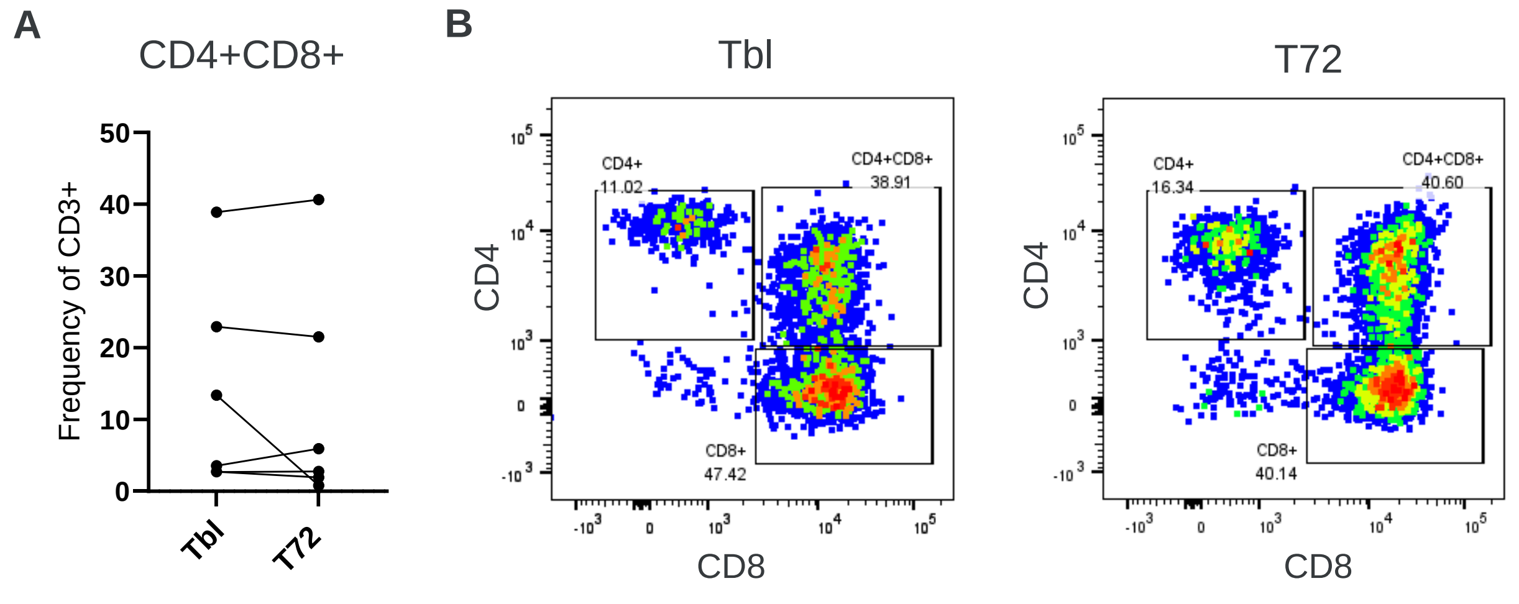

**Fig. 3:** **A.** Graph showing preservation of DPT cells in RCC. **B.** Representative image showing DPT cells at baseline (Tbl) and post-culture (T72).

## Segregation of RCC samples into low and high DPT sub-cohorts

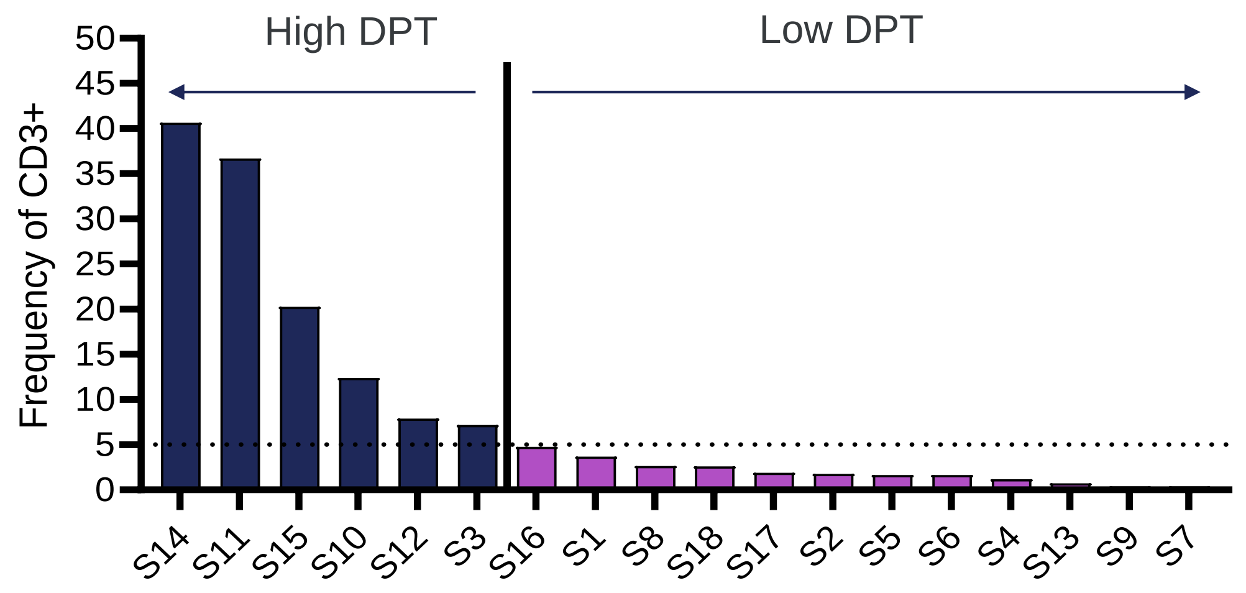

**Fig. 4:** Graph showing segregation of samples into high and low DPT sub-cohorts based on cut off of 5%.

## Both CD8+ CTLs and DPT exhibit higher exhaustion in high DPT sub-group

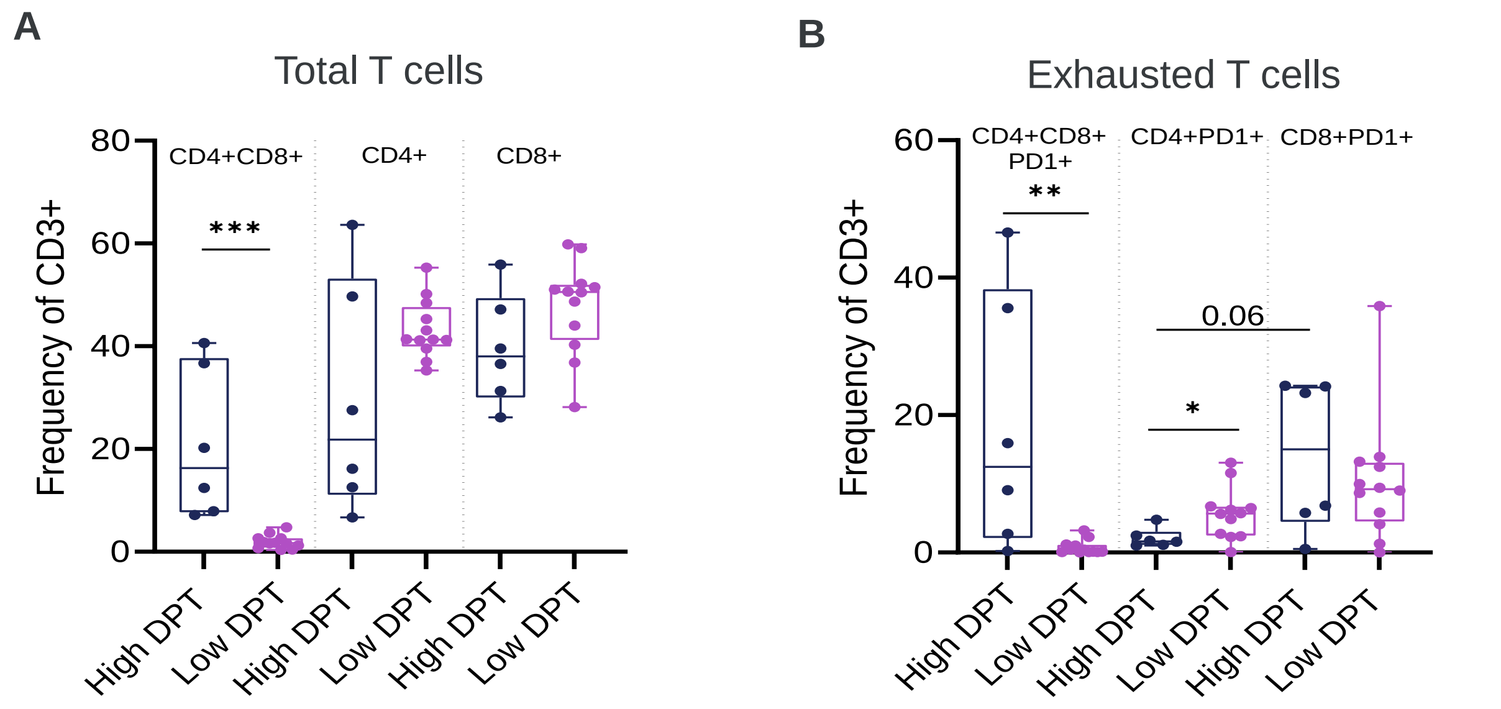

**Fig. 5:** **A.** Plot showing different T cell sub-populations in high and low DPT sub-cohorts. **B.** Exhaustion status of T cells in high and low DPT sub-cohorts.

## Higher response rate on anti-PD1 treatment in high DPT sub-group compared to low DPT sub-group

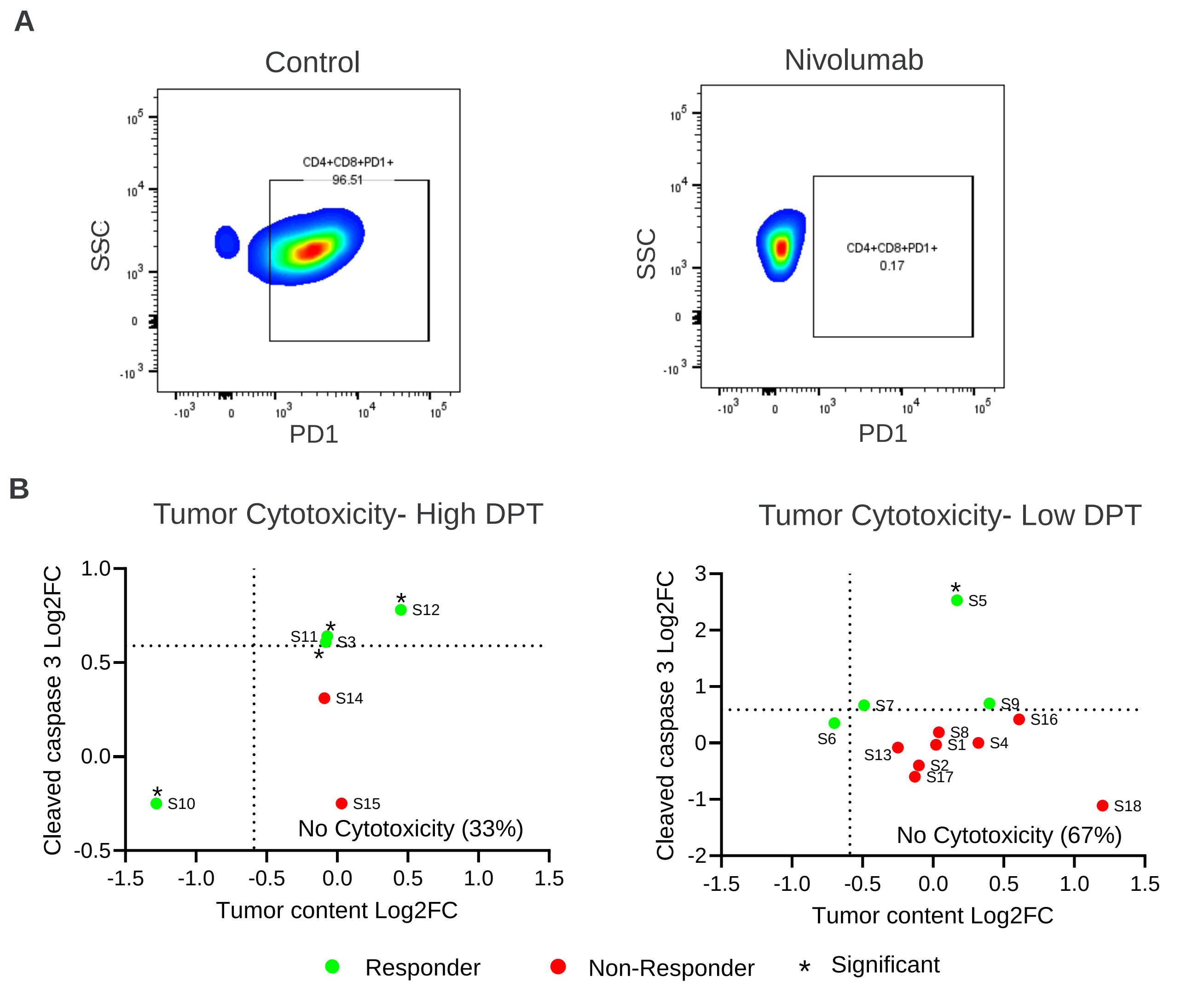

**Fig. 6:** **A.** Flowcytometry data showing masking of PD-1 receptor in nivolumab treated arm. **B.** Two-dimensional dot plot representation of fold change in tumor content and cleaved caspase 3 expression in tumor with respect to control.

## Improved cytotoxic response in high DPT sub-group is driven by activated single and double positive T cells

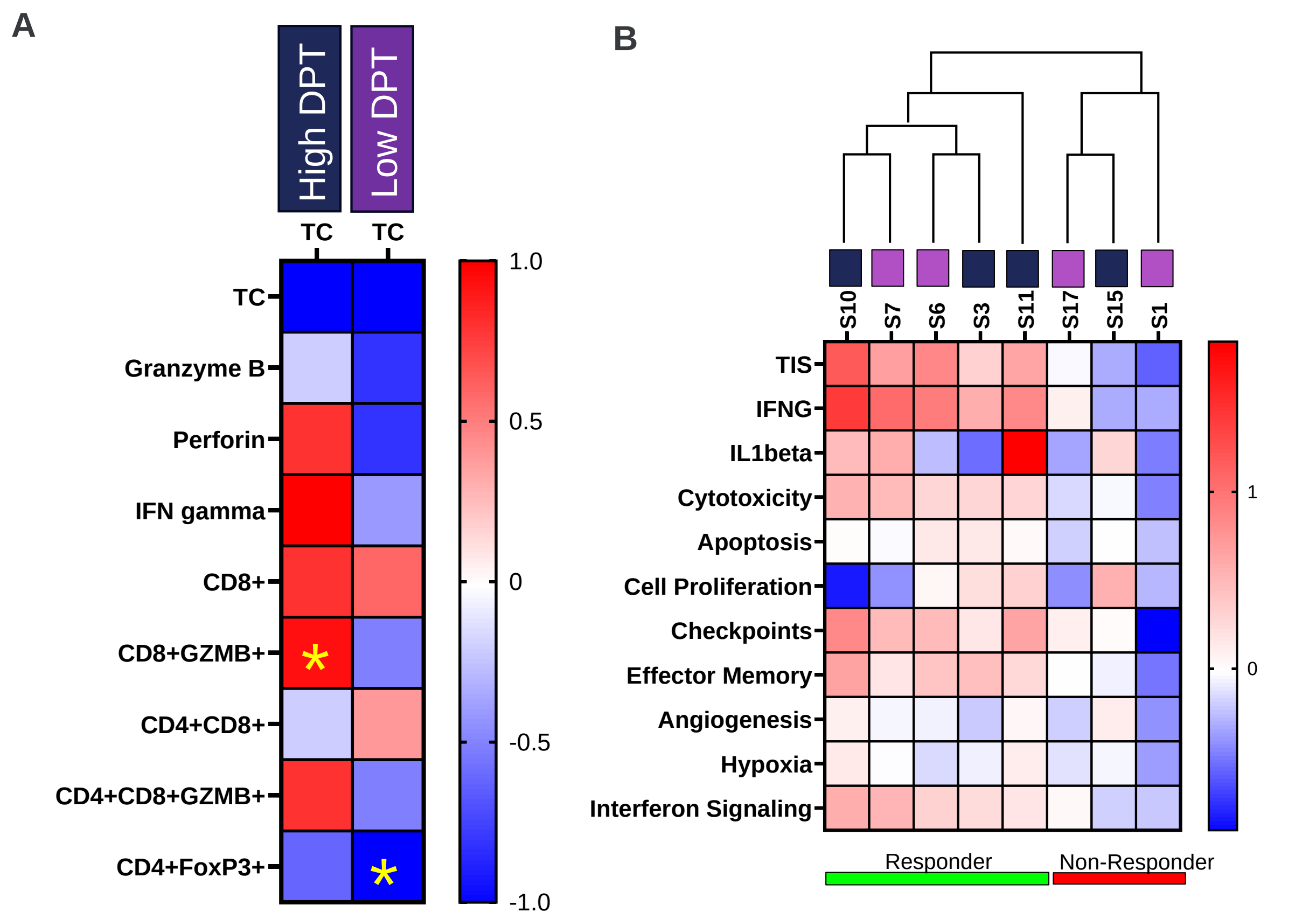

**Fig. 7:** **A.** Heat map showing correlation (Spearman's) across parameters in high and low DPT sub-cohorts. **B.** Heatmap showing gene expression signature on nivolumab treatment as Log2 fold change with respect to control.

## Non-response in high DPT sub-group is driven by TAMs

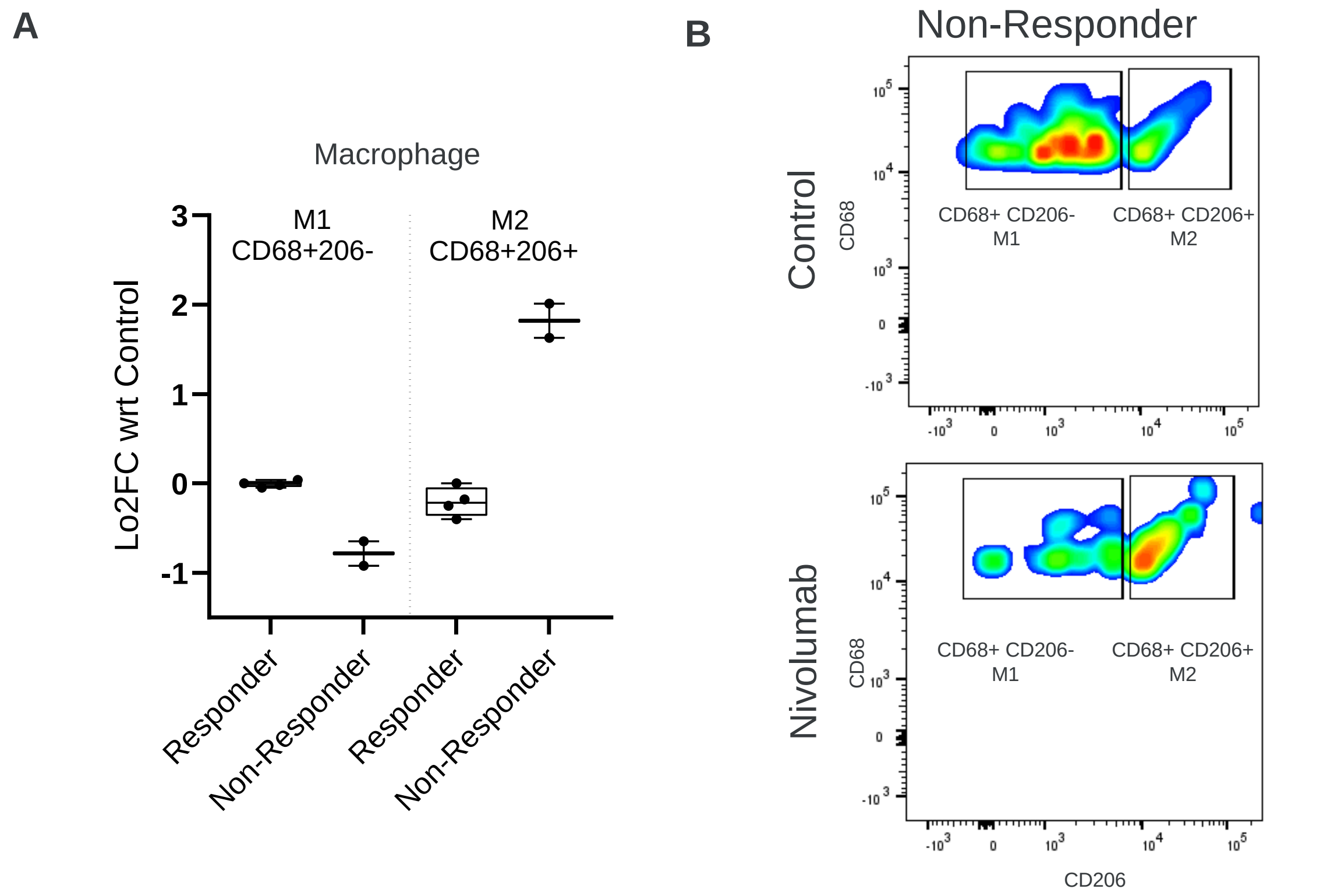

**Fig. 8:** **A.** Graph showing M1 and M2 macrophage subpopulation in high DPT responder and non-responder sub-groups. **B.** Representative image showing macrophage population in non-responder sample.

## Summary

- The Farcast™ TruTumor platform enables study of rare immune sub-populations within the tumor microenvironment and their modulation in response to drug treatment.
- Our data suggests that presence of high percentage of DPT cells favor better response to anti-PD1 therapy in RCC patients.

## References

1. Nishida, K., *et al.*, Int Immunol. 2020;32:347-357.
2. Menard, LC., *et al.*, Front Immunol. 2018;9:2728.

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