



Comprehensive immunophenotypic profiling sheds light on the dynamic interplay between immune cells in the 4T1 breast cancer model upon anti-PD1 and anti-CTLA4 immunotherapy

Christos Nikolaou, Krzysztof Brzezinka, Simon Heller, Wiebke Winkler, Stefan Kaulfuss, Carlo Stresemann, Alexandra Eichten, Oliver von Ahsen, Martin Lange

NUVISAN Innovation Campus Berlin GmbH | Muellerstr. 178 | 13353 Berlin | Germany

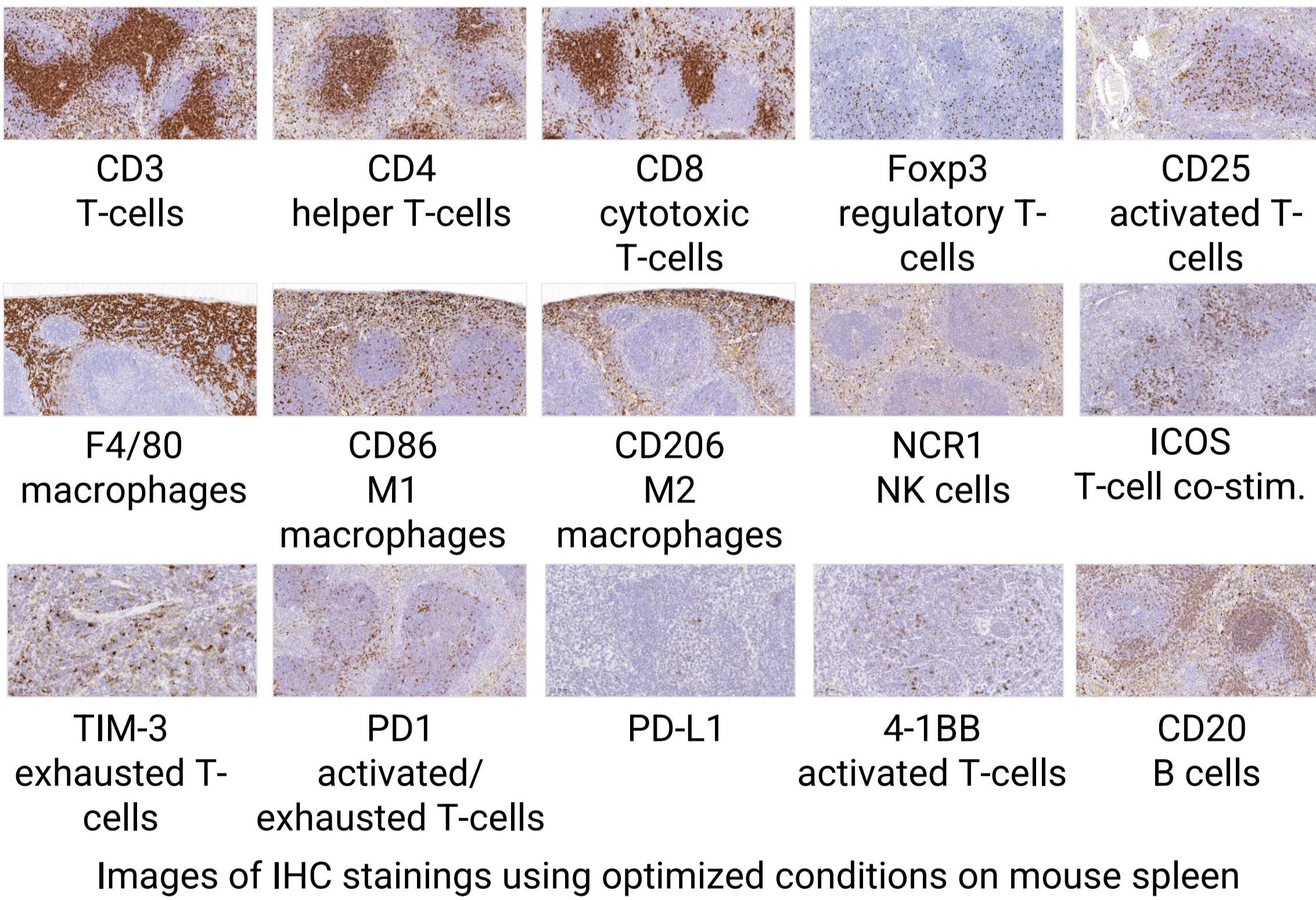
Introduction

Immuno-oncology has revolutionized cancer treatment by harnessing the immune system to target and eliminate tumor cells. The development of novel therapeutics requires robust preclinical models and immune cell assays.

In this study, we employed a combination of full spectrum flow cytometry (Cytek®) and spatial profiling by Immunohistochemistry (IHC) to investigate the impact of Immune checkpoint inhibitors (ICIs) anti-PD1 (programmed cell death protein 1) and anti-CTLA4 (cytotoxic T-lymphocyte-associated protein 4) treatments on the 4T1 breast cancer model. We comprehensively analyzed 25 surface markers on immune cells to gain insights into the dynamic changes induced by immunotherapy.

Methods

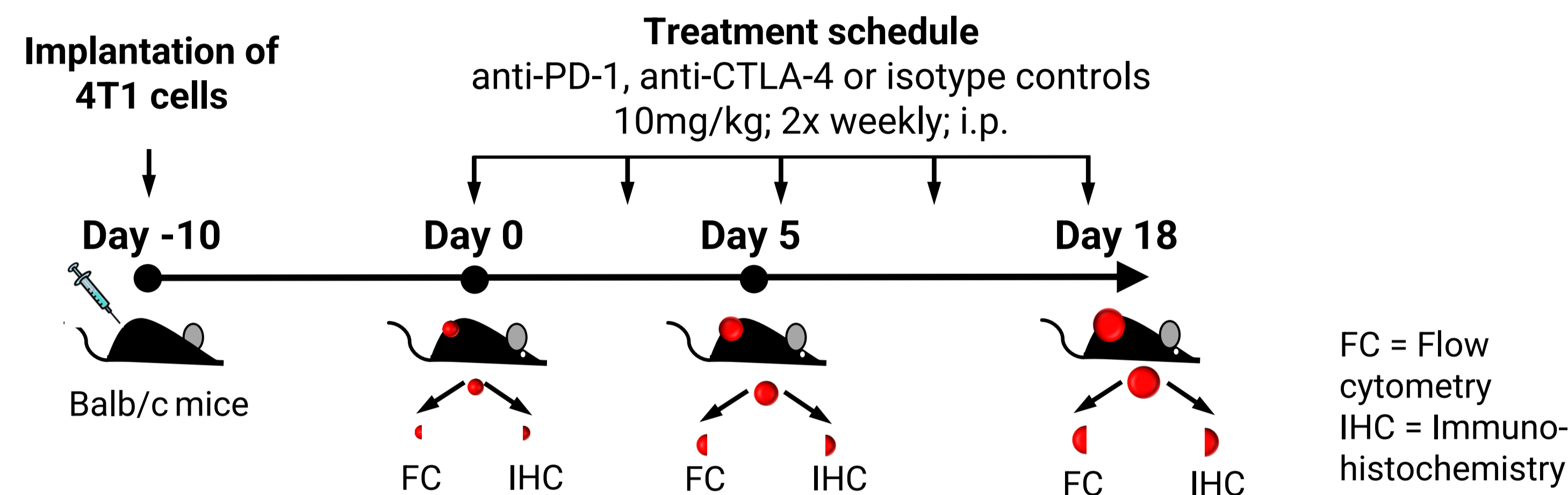
IHC assay development for immune cell markers was performed using commercially available antibodies and healthy mouse control and syngeneic mouse tumor tissues. Antigen retrieval and antibody dilutions were optimized for signal specificity.



Flow cytometry panel design: Epitope recovery was optimized by comparison of mechanical dissociation, enzymatic (collagenase 1, 4 or Dispase II) digest, fixation and freezing. Mouse immuno-panel consists of CD3, CD4, CD8, CD11b, CD19, CD25, CD38, CD44, CD45R, CD69, CD62L, CD137(4-1BB), CD206, TCRgd, SLAMF7, PD1, Ly6c, Ly6g, F4/80, CTLA4, NKp46, MHCII, CD45, Live/Dead and was measured on the Cytek® Aurora full spectrum cytometer.

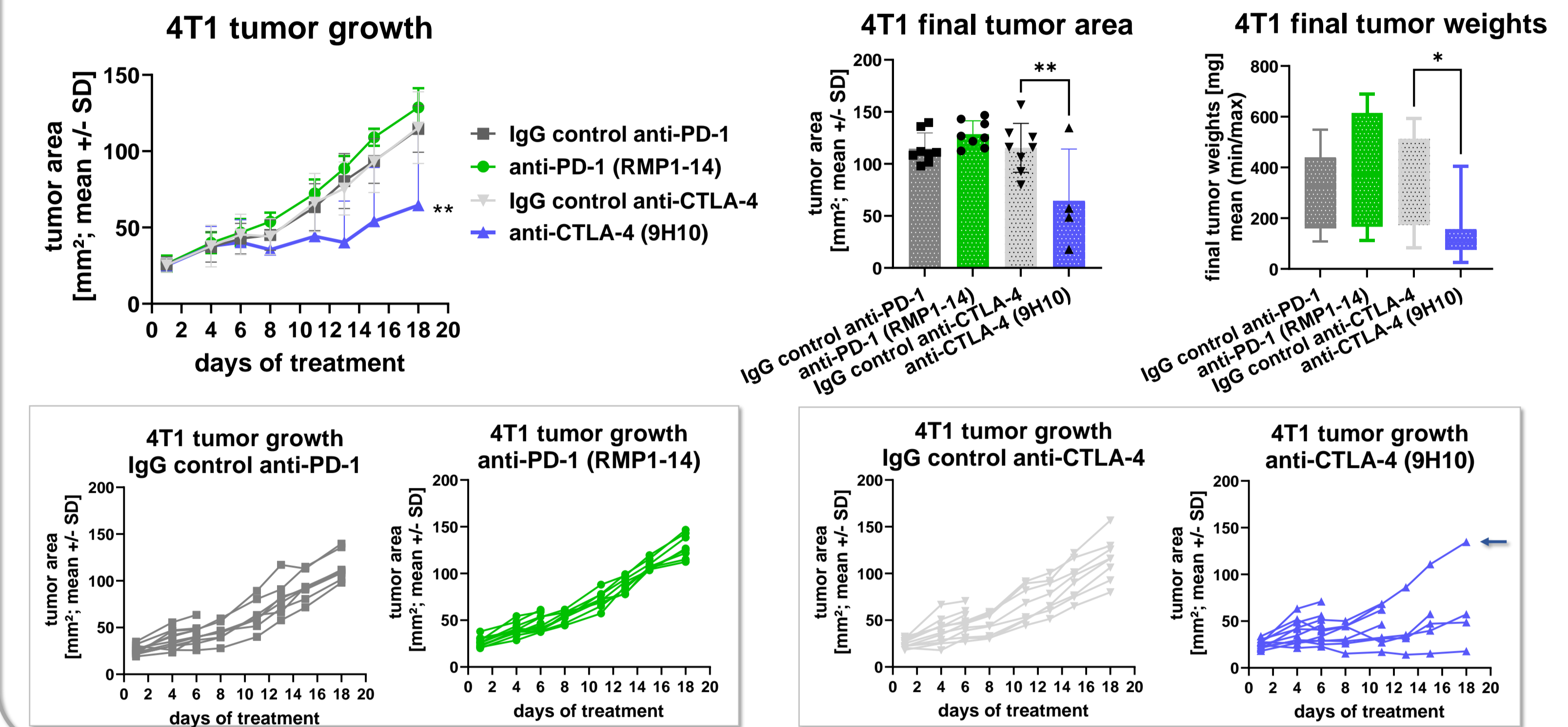
In vivo studies: 4T1 cells were implanted orthotopically into the mammary fat pad (fourth mamilla) of > 8-week-old female Balb/c mice. When tumor volume reached a predefined average size, animals were allocated to treatment groups by stratified randomization procedure and treatment started according to study plan with Immune checkpoint Inhibitors (a-PD-1 and a-CTLA-4, 10mg/kg vs. isotype controls, 2x weekly, i.p.). Animal experiments were conducted in accordance with animal welfare laws, approved by local authorities (State Office for Health and Social Affairs, Berlin, Germany).

In vivo study design of the murine 4T1 breast cancer model

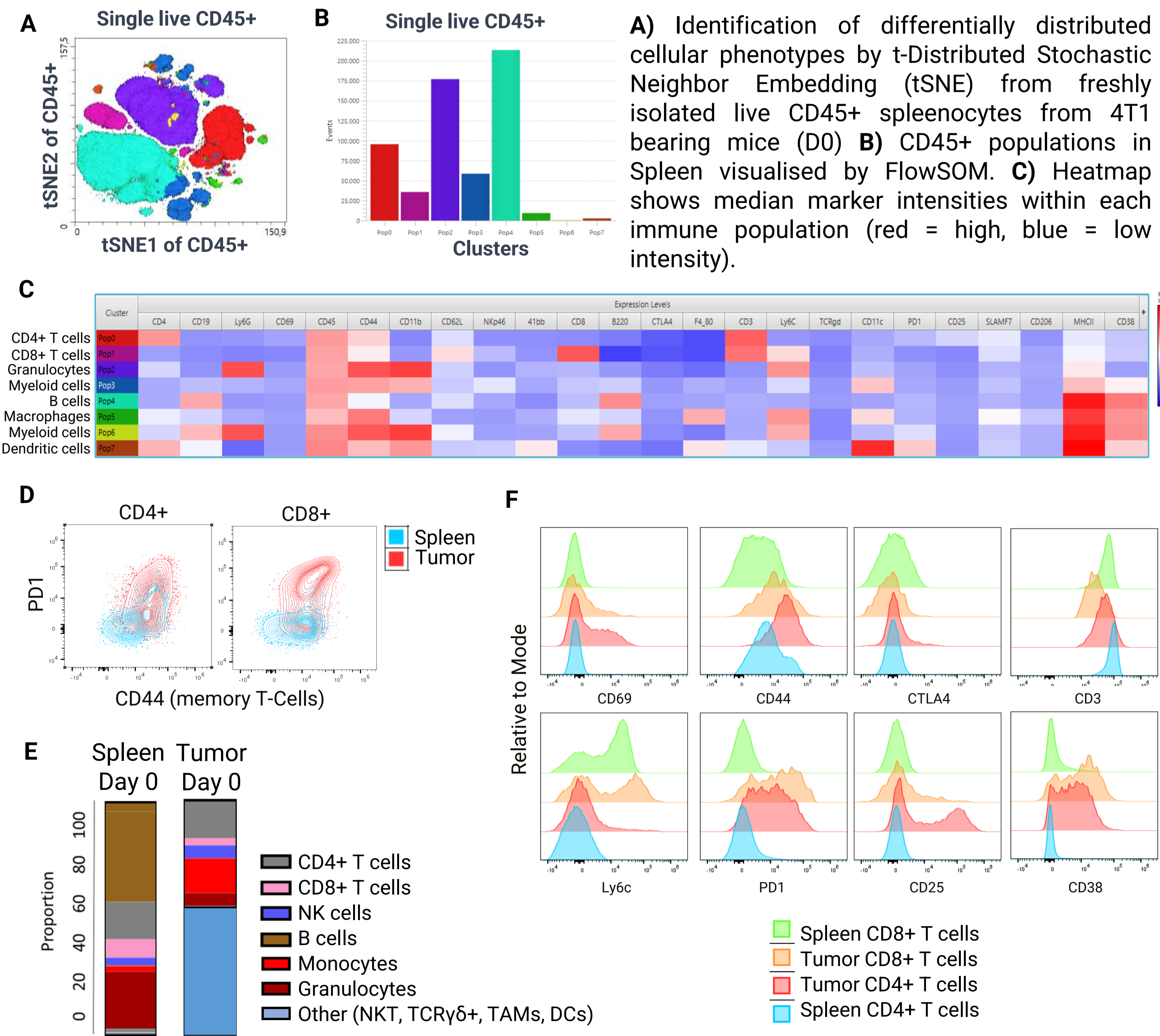


Results

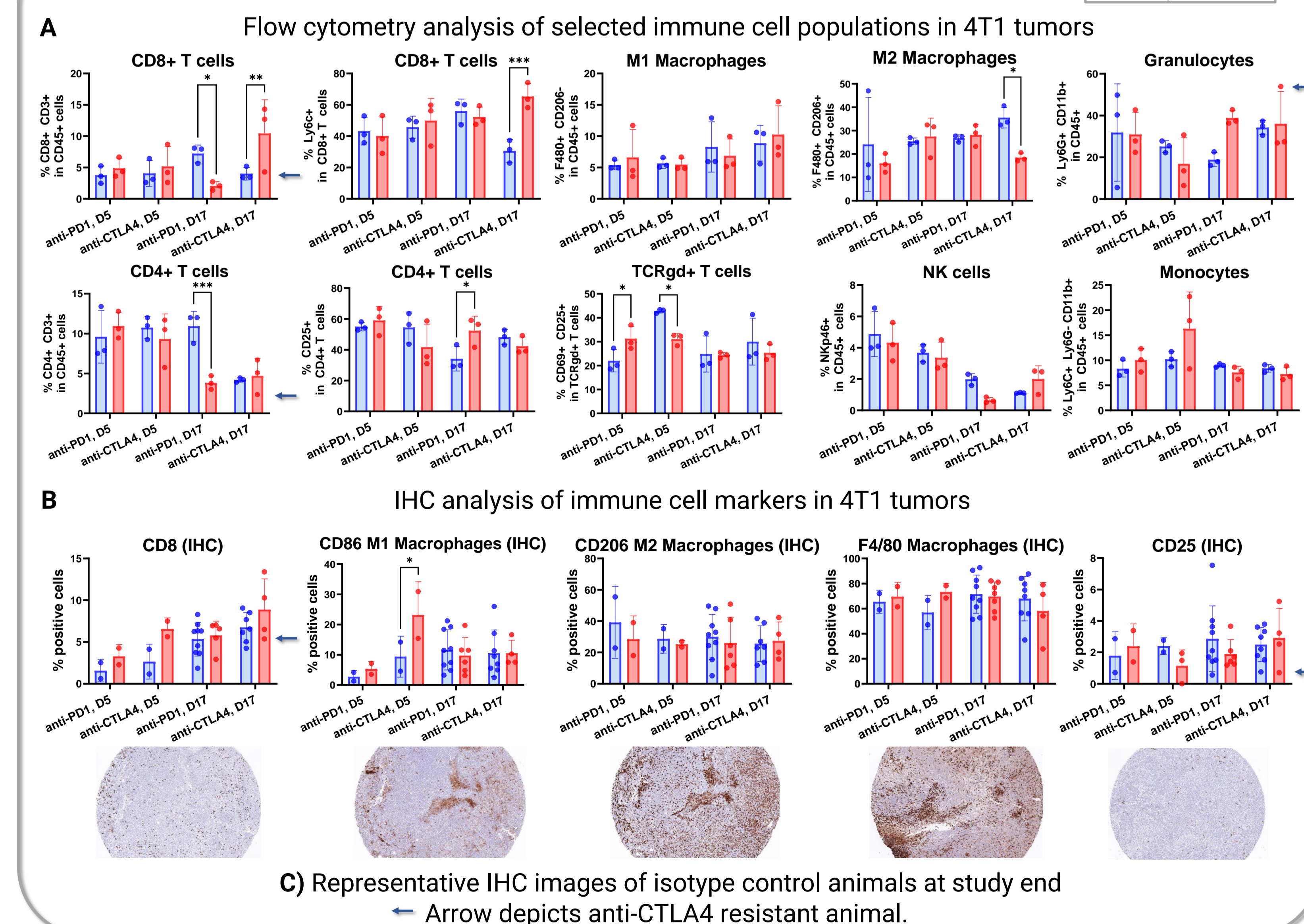
Anti-tumor efficacy of ICIs in the murine 4T1 breast cancer model



Immunophenotyping of spleen and 4T1 tumors



Immune cell profiling of 4T1 tumors treated with ICIs



Conclusion

Our comprehensive immunophenotypic analysis sheds light on the dynamic interplay between immune cells and the 4T1 breast cancer model during anti-PD1 and anti-CTLA4 immunotherapy. These findings provide valuable insights into the mechanisms underlying the therapeutic efficacy of immune checkpoint blockade in this aggressive cancer model.