# Single-Cell Gene Expression Profiling of the Tumor Immune Microenvironment (TIME) and Its Association with Immunotherapy Response in Syngeneic Mouse Models

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## Abstract

The TIME, comprised of the extracellular matrix and a milieu of both immune and non-immune cells, plays a critical role in tumor development, disease progression, and response and resistance to immunotherapy. Much of our understanding of the TIME's immunological features, cellular heterogeneity, and associations with immunotherapy response have come from the use of syngeneic mouse models representing various cancer cell types. To provide further characterization of the TIME in syngeneic mouse models commonly used for immunogenic drug discovery, CD45+ single-cell RNA sequencing was performed in 10 syngeneic mouse models representing 7 distinct cancer types (breast mammary carcinoma: 4T1, EMT6, MMTV-PyMT; colon carcinoma: CT26.WT, MC38; glioma: GL261; renal adenocarcinoma: Renca; lung carcinoma: LL2; melanoma: B16F10; and pancreatic adenocarcinoma: Pan02). Further, to unveil cellular subpopulations that correspond to immunotherapy response, anti-PD-(L)1 therapy efficacy studies were performed in the syngeneic models and correlations with the abundances of distinct immune cell populations were explored.

Across all syngeneic tumor models examined, seven major immune cell populations were identified and subpopulations within T cells, NK cells, innate lymphoid cells, and distinct myeloid cells were characterized. This included immune cells specific to particular models, such as an active NK subset in GL261 and an N2 tumor-associated neutrophil subset in LL2. The efficacy of anti-PD-(L)1 treatment was positively correlated with a unique population of interferon-primed tumor-associated macrophages. Additionally, a significant proportion of neutrophils was observed in our dataset. Neutrophil depletion experiments using Ly6G antibody treatment demonstrated a notable reduction in tumor burden within CT26WT models, but not in the EMT6 model.

These findings provide further characterization of these syngeneic lines to aid in rational model selection to facilitate translational relevance. This study also provides further insight into how immune cell composition and abundance within tumors may contribute to immune checkpoint blockade resistance.

## Overview of Immune Cell Populations Across Models

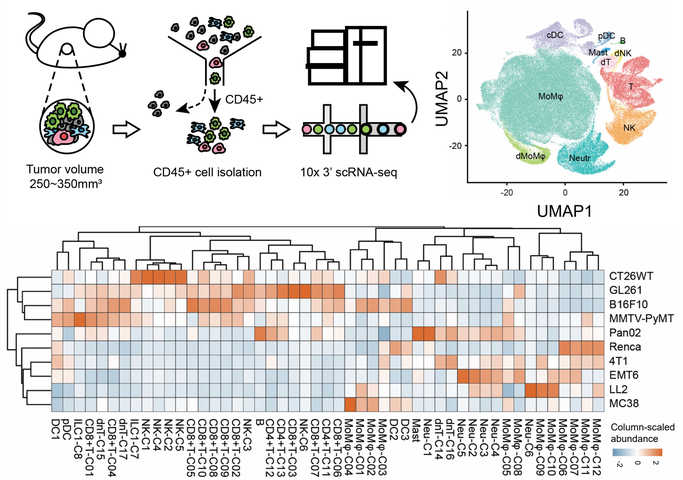


**Figure 1.** A total of 171,921 immune cells were analyzed after quality control, systematically categorizing various models into distinct TIME subtypes.

## Characterization of T-cell subtypes and states in syngeneic mouse tumors

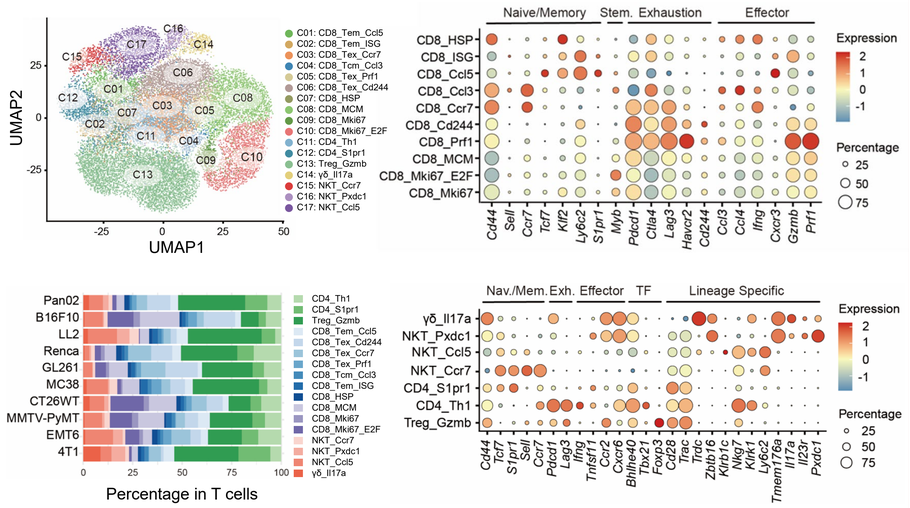


**Figure 2.** 17 distinct T cell subpopulations were identified, comprising 10 CD8+ subsets, 3 CD4+ subsets, and 4 CD8-CD4- subsets. The syngeneic model dataset exhibits a higher proportion of exhausted CD8+ T cells (40.84%), Treg cells (60.44%), proliferating T cells (20.61%), and NKT cells (11.09%) compared to human datasets.

## Neutrophil presence and function across different models

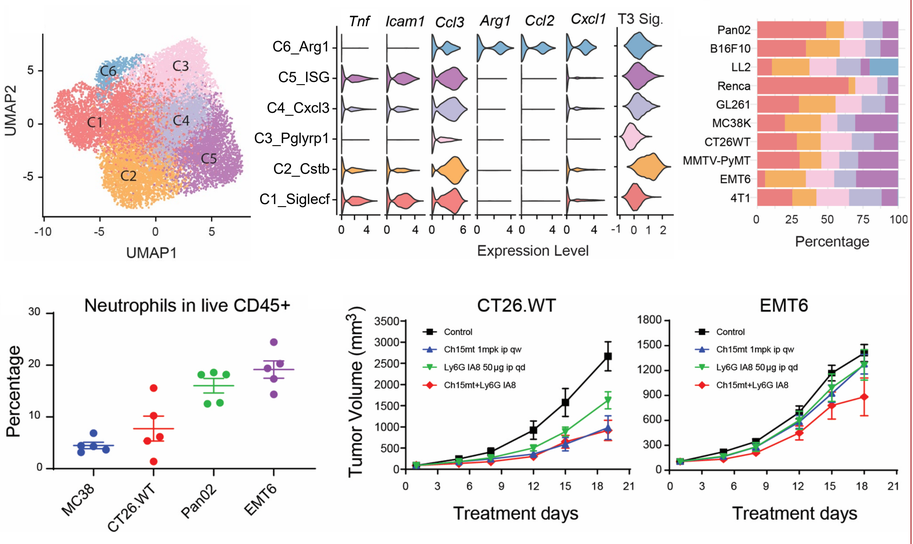


**Figure 4.** A significant population of neutrophils was observed across various models. The presence and function of these neutrophils was confirmed using flow cytometry (CD11b+CD115-Ly6G+) and by reducing their numbers with Ly6G antibody treatment.

## Functional states of intratumoral NK cells in syngeneic models

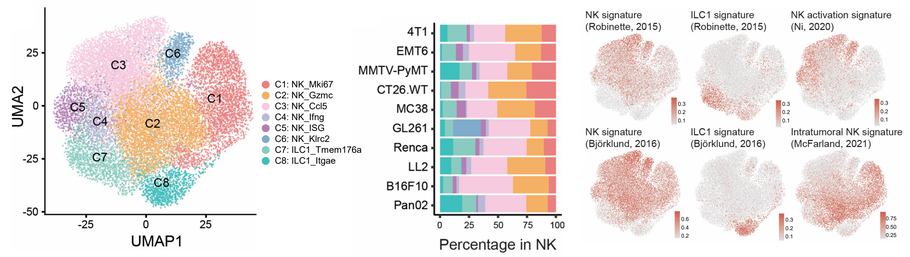


**Figure 3.** Intratumoral NK cells exhibited a diminished activation score, indicating a loss of their effector functions within the tumor microenvironment. Notably, a distinct cluster of adaptive NK-like cells was identified exclusively in the GL261 model.

## Molecular and Functional Diversity of Tumor-Infiltrating Macrophages

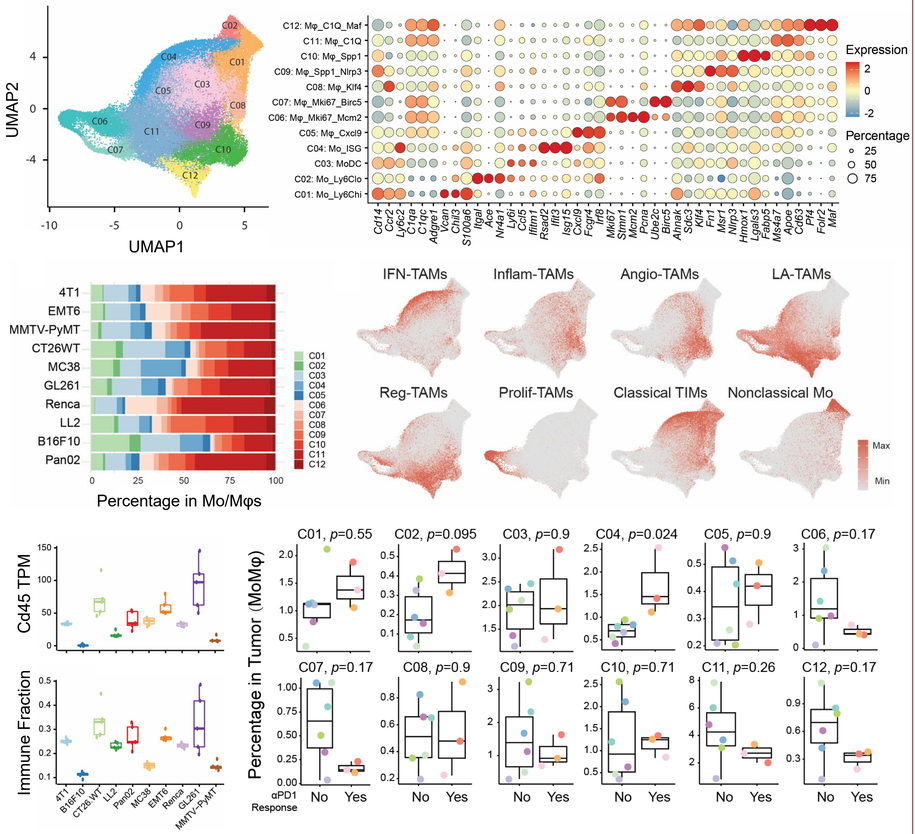


**Figure 5.** 12 transcriptional states of MoMφ population was identified in tumor microenvironment with distinct signatures. Higher interferon-primed TAMs (IFN-TAMs) in the syngenetic models linked to anti-PD-(L)1 treatment efficacy.