

Using a tamoxifen inducible fate reporter to investigate the effect of CTLA-4 blockade on CD8⁺ T cell homeostasis

Yeung Man Tak¹, Cayman Williams², Benedict Seddon²

1. School of Biomedical Sciences, The University of Hong Kong 2. Institute of Immunity and Transplantation, University College London



Role of CTLA4 in controlling CD8⁺ T cells

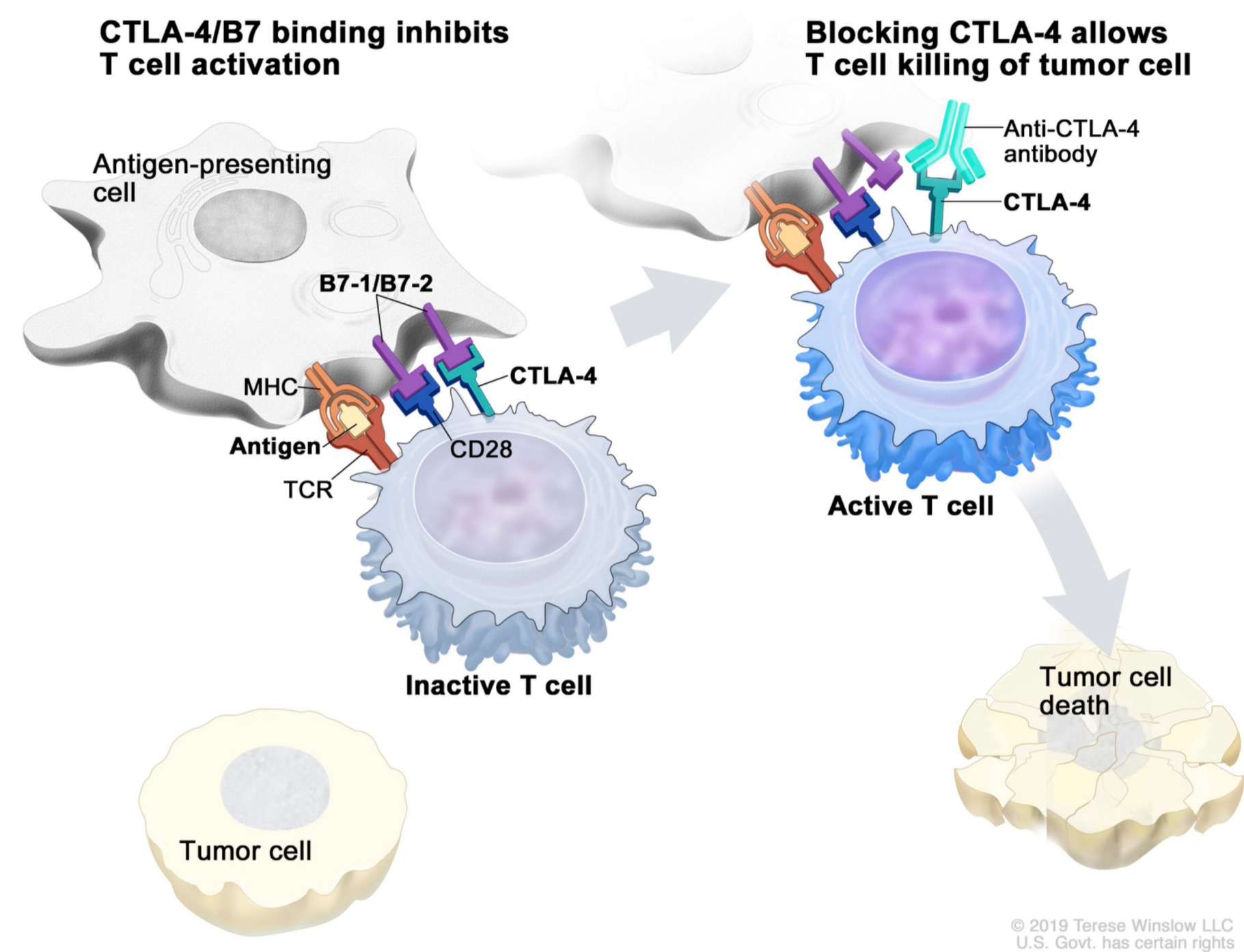


Figure 1. CTLA4 inhibits CD28 co-stimulation of T cells (National Cancer Institute, n.d.).

- CD28 co-stimulation is inhibited by CTLA4 function
- Anti-CTLA-4 blocking antibodies can be used to compromise CTLA4 function and enhance CD28 co-stimulation

Experimental Set-up

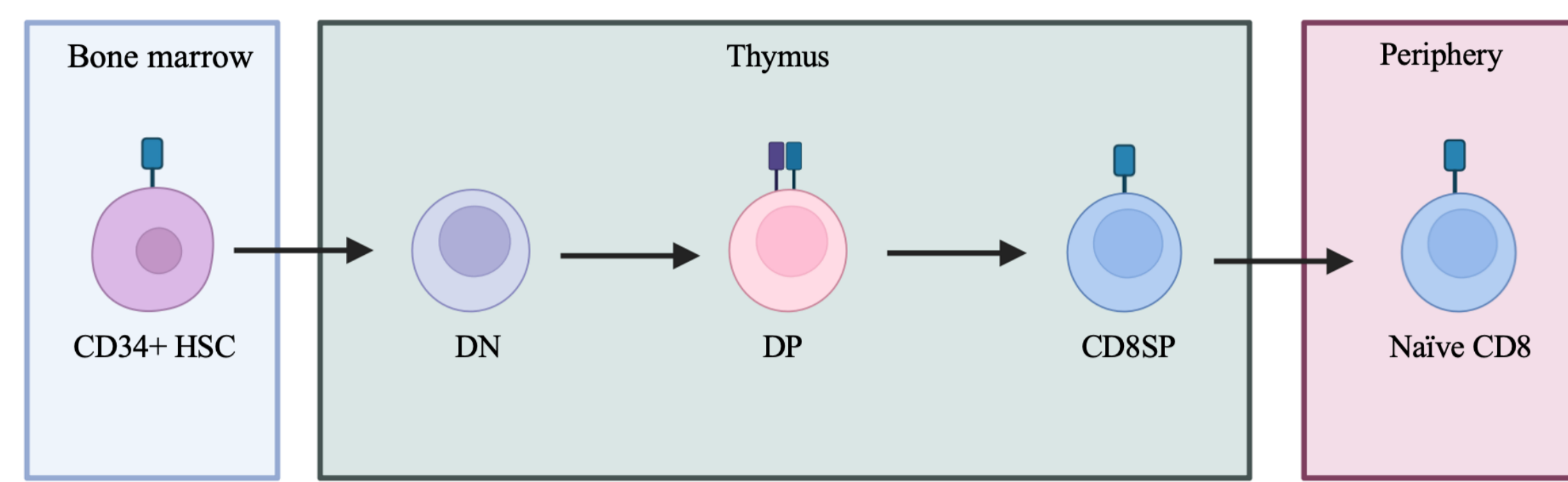


Figure 2. Developmental CD8 T cells in the thymus.

Bone marrow derived CD34⁺ hematopoietic stem cells migrate to the thymus and mature into double-negative (DN). These differentiate into double-positive (DP) T cells and undergo positive selection to become CD8 single-positive (CD8SP) cells. They are then released into the periphery as naive CD8⁺ T cells.

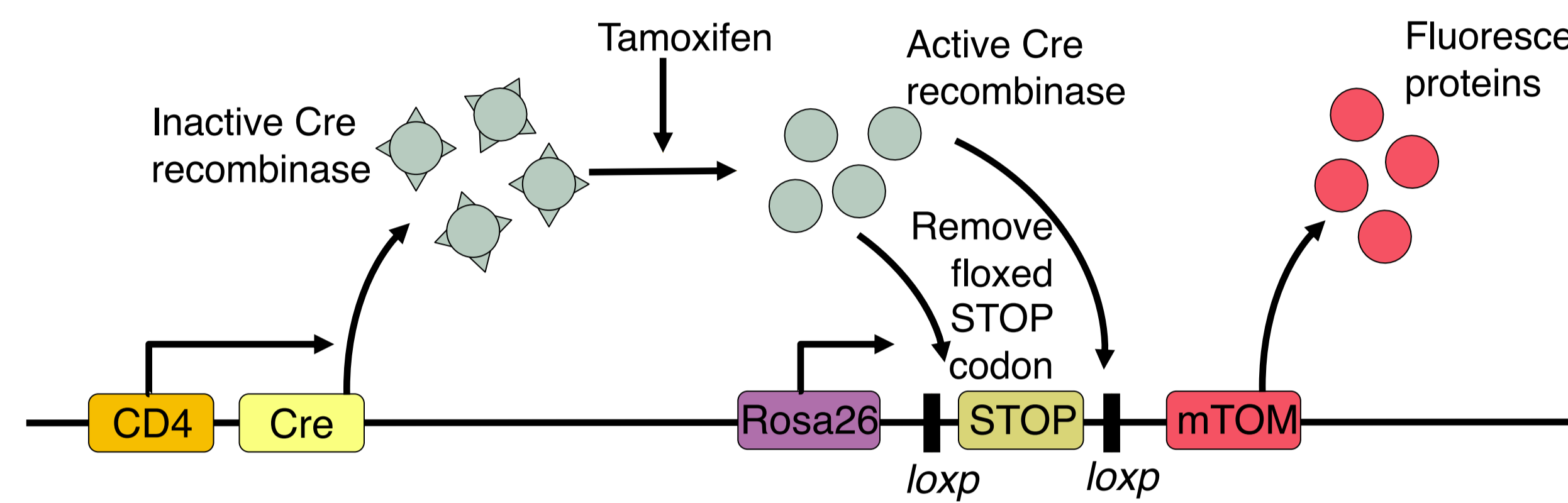


Figure 3. Graphical representation of CD4-CreERT2 Rosa26RmTOM mice.

CD4 express Cre producing Cre recombinase. Feeding of tamoxifen activated Cre recombinase which recognizes and excises the loxp-flanked STOP codon, enabling Rosa26 to express mTOM, producing fluorescent protein.

Tracking CD8⁺ T cell development in the thymus

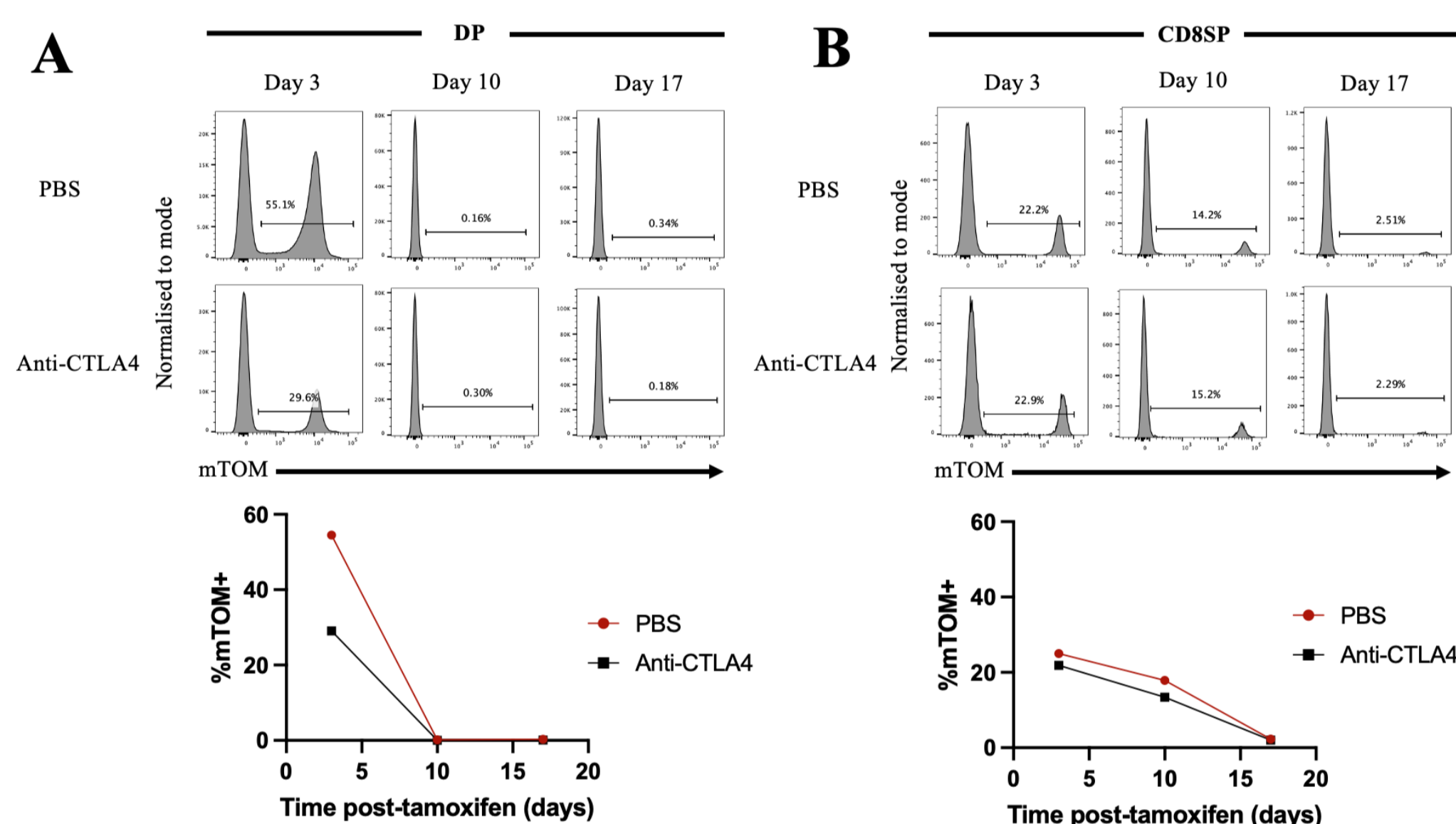


Figure 4. CTLA4 blockade does not impact developmental kinetics of CD8SP thymocytes. A) % DP thymocytes expressing mTOM over time treated mice. B) % CD8SP cells expressing mTOM over time in PBS and Anti-CTLA4

- Data suggest CTLA4 blockade does not impact CD8SP development
- Any consequences in the periphery are not due to upstream alterations in development

Developmental kinetics of CD8⁺ T cell population in lymph nodes

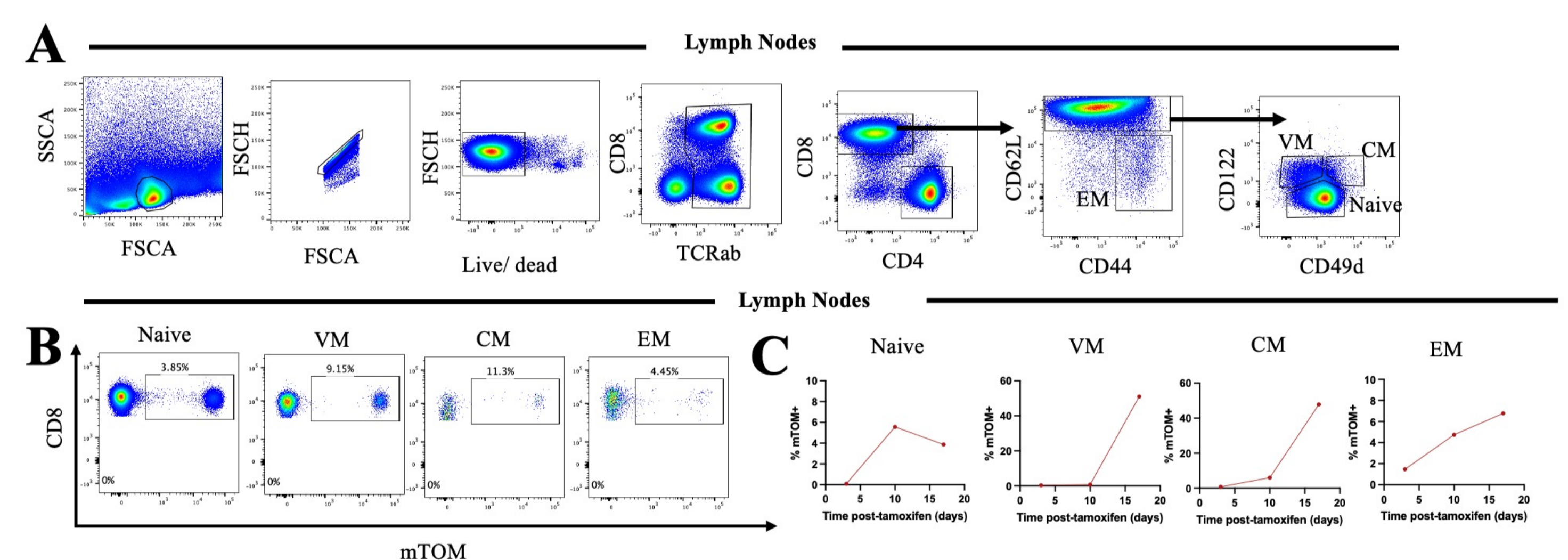


Figure 5. Tracking mTOM⁺ CD8⁺ T cell population in the periphery. A) FACS plots identifying mTOM⁺ CD8⁺ Naïve, CM, VM and EM cells in the lymph nodes. B) %mTOM⁺ CD8⁺ T cell subsets in lymph nodes and C) %mTOM⁺ CD8⁺ T cell subsets over time.

- Data suggest DP thymocytes mature into naïve T cells DP around day 10 while differentiate into further CD8 subsets at day 17
- Rapid increase in CM CD8 T cells likely due to rapid differentiation of Naïve precursor and a short half-life of the pre-existing cells (Measurement of further time points would be needed to confirm this)
- The modest increase in mTOM content over time suggest that the generation of EM is slower than that of CM, which is consistent with the literature

CTLA-4 Blockade impedes development of Memory CD8⁺ T cells in LN

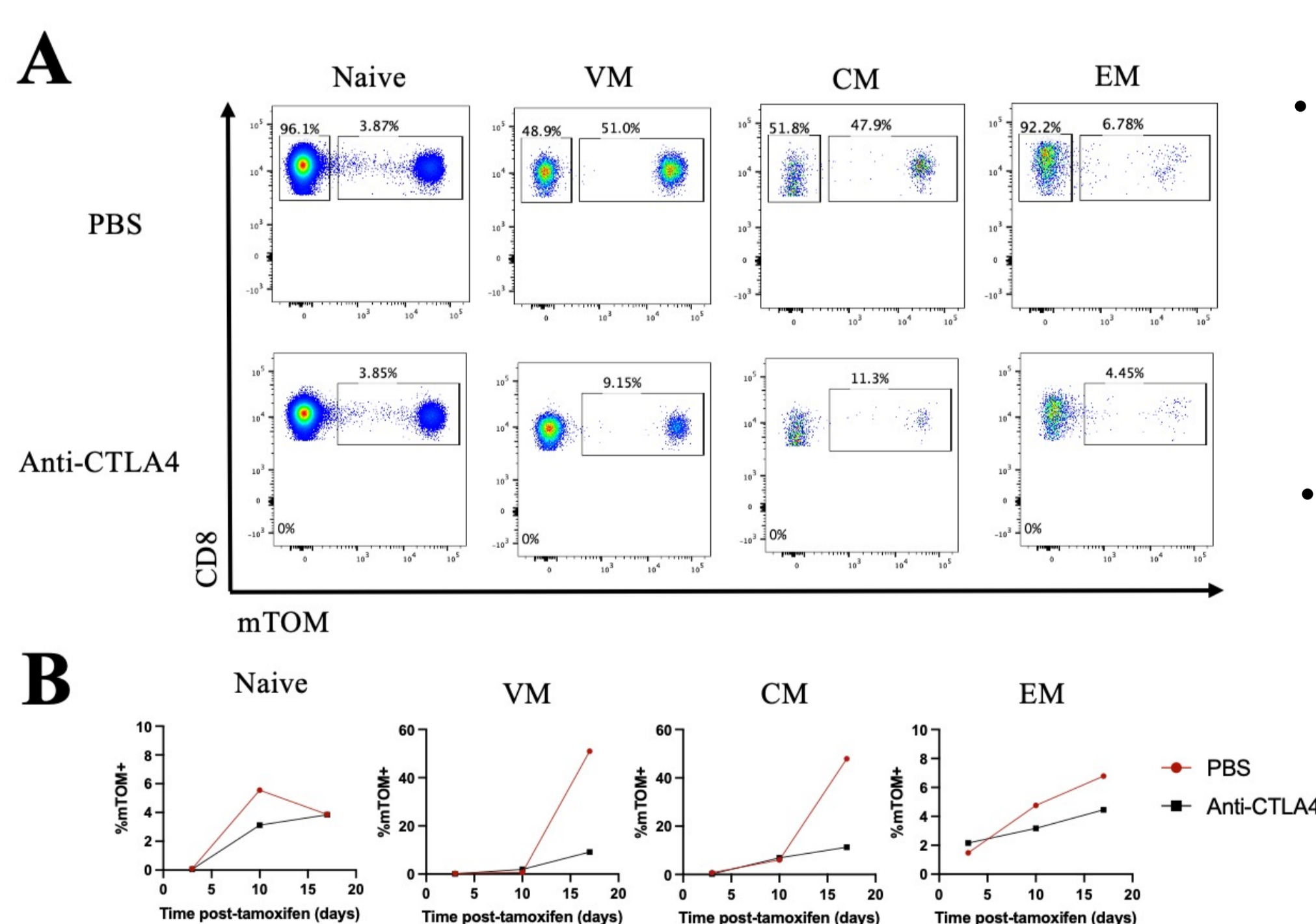


Figure 6. CTLA-4 selectively impacts development of CD8⁺ Memory T cell subsets. A) Plots showing %mTOM⁺ CD8⁺ T cell subsets in lymph nodes in PBS and anti-CTLA4 treated mice and B) shows the %mTOM⁺ CD8⁺ T cell subsets over time in response to PBS or anti-CTLA4.

- Data suggest that the generation of CD8⁺ T cell memory may be impaired in the presence of Anti-CTLA4 since % mTOM⁺ memory CD8⁺ T cell subsets by day 17 was lower in mice treated with anti-CTLA-4 compared to the PBS control
- Data also suggest that CTLA4 blockade does not influence the development of naïve CD8 T cells, as mTOM content in naïve CD8 T cells was similar at days 3 and 17, with 3% variation at day 10 likely due to experimental variability.

Discussion

- CD4-CreERT2 Rosa26RmTOM mice can be used to track CD8⁺ T cell in the thymus and periphery
- CD4-CreERT2 Rosa26RmTOM mice can be used to track the rate of generation of CD8⁺ T cell memory subsets in the periphery
- CTLA4 blockade does not appear to impact CD8SP development in the thymus
- CTLA4 blockade does not appear to impact the generation of Naïve CD8⁺ T cells in lymph nodes
- CTLA4 blockade impairs the generation of Memory CD8⁺ T cell subsets on lymph nodes

Conclusion

These data suggest that anti-CTLA-4 may impede the generation of memory CD8⁺ T cells.

Future work

- Repeating the experiment with larger sample size would offer more robust and reliable results to support the hypothesis, give more persuasive evidence with minimized variability and outlier data.
- Including additional time points would also aid future studies. Extending the duration of studies could reveal more long-term effects of Anti-CTLA4 on the development of CD8 T cells and therefore perhaps leading to more obvious phenotypes
- Exploring other models that inhibit CTLA-4 without using antibodies, like utilizing CTLA-4 knockout mice which would further strengthen the study by providing external validation using a completely different system to block CTLA-4.