

Using a Tamoxifen-Inducible fate reporting to investigate the effect of CD4 T Cell age on Responses to Influenza

Namir Khalil,¹ Cayman Williams,² Benedict Seddon²

1. UCL Medical School, University College London 2. The Institute of Immunity and Transplantation, University College London



The Role of CD4+ T cells in the Immune Response

Experimental Set-Up

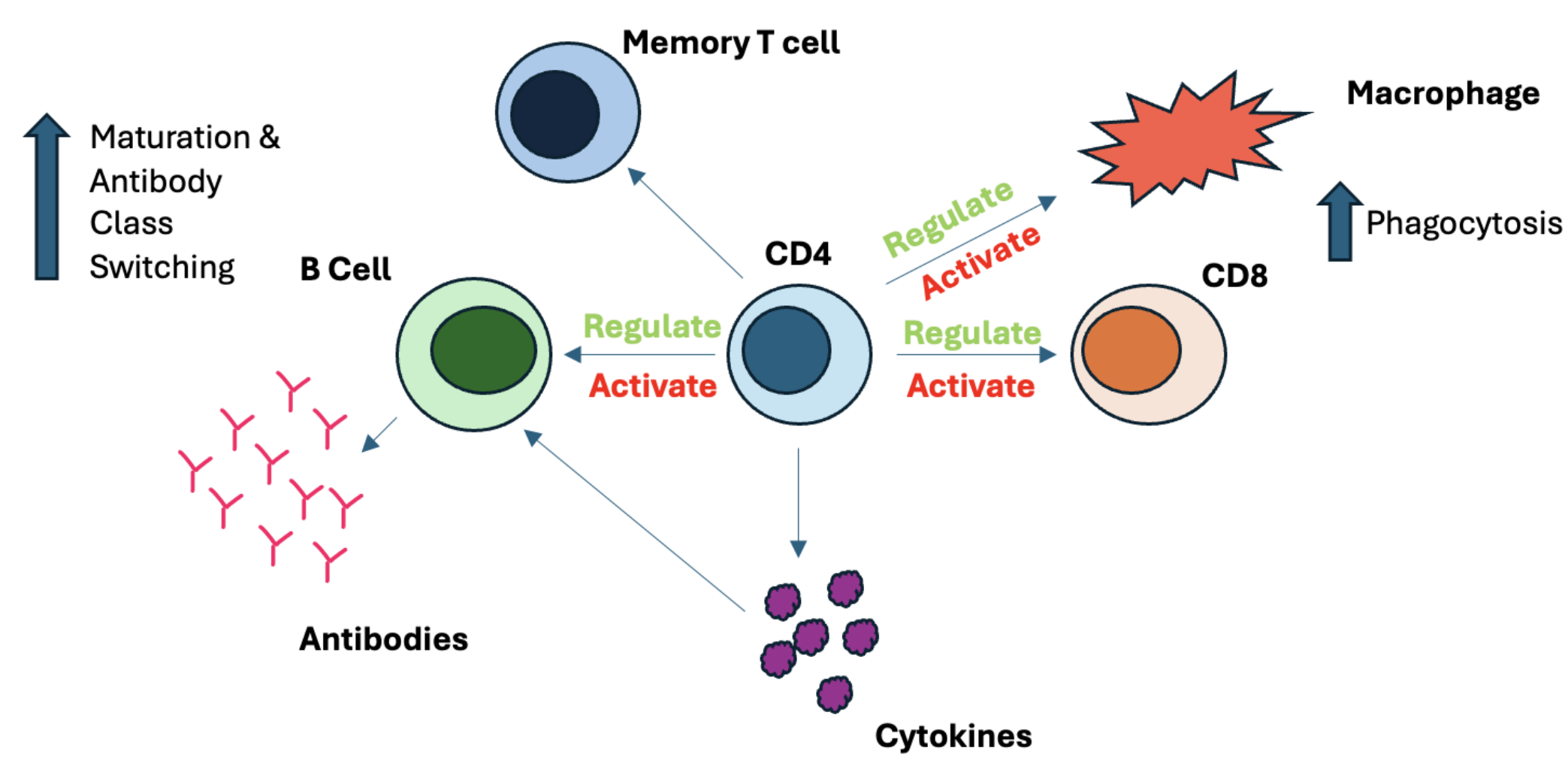


Figure 1: CD4+ T cells activate and regulate many different immune cells as part of the response to Influenza

- CD4+ T cells activate CD8+ T cells, B cells and Macrophages
- CD4+ T cells release various cytokines that influence the activity of other immune cells
- After an infection, CD4+ T cells differentiate into memory T cells to provide long lasting immunity
- CD4+ Regulatory T cells maintain immune tolerance and prevent autoimmune disease by suppressing excessive immune responses

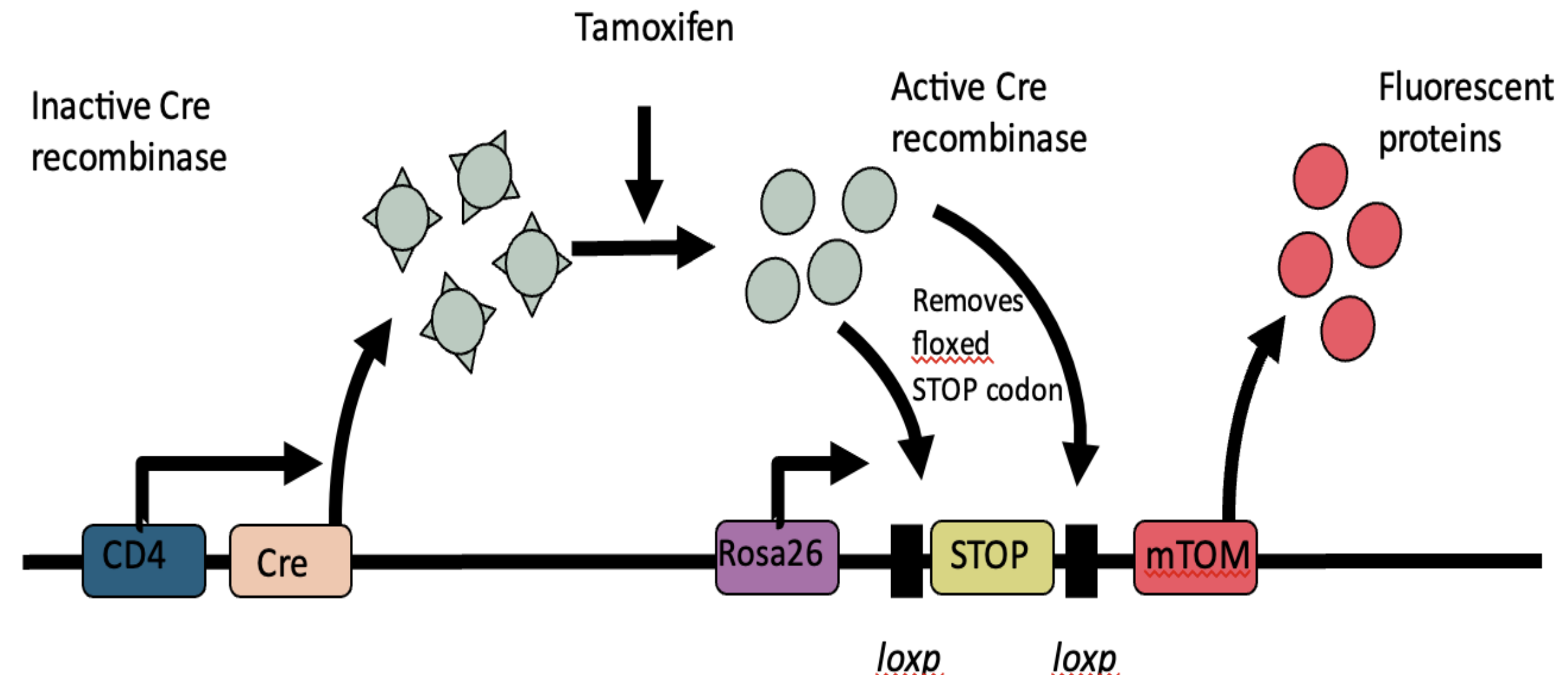


Figure 2: A diagram showing the translation sequence of CD4+ T cells in mice, tamoxifen activating cre recombinase and mtom being translated

- Mice were given the tamoxifen feed on day 0
- Tamoxifen activates Cre recombinase which removes the STOP codon at the loxp sites causing expression of mtom
- Mtom expression allows fluorescent proteins to be synthesised, labelling CD4+ T cells which will be our older population
- Mice were given either a PBS or intranasal flu dose on day 56 and were culled 7, 14, 21 and 28 days after (Exp 1, 2, 3, 4).
- The Lungs, Mediastinal Lymph Nodes, Spleen and Peripheral Lymph Nodes were harvested

A Flu Response was demonstrated in the Lungs and MLN

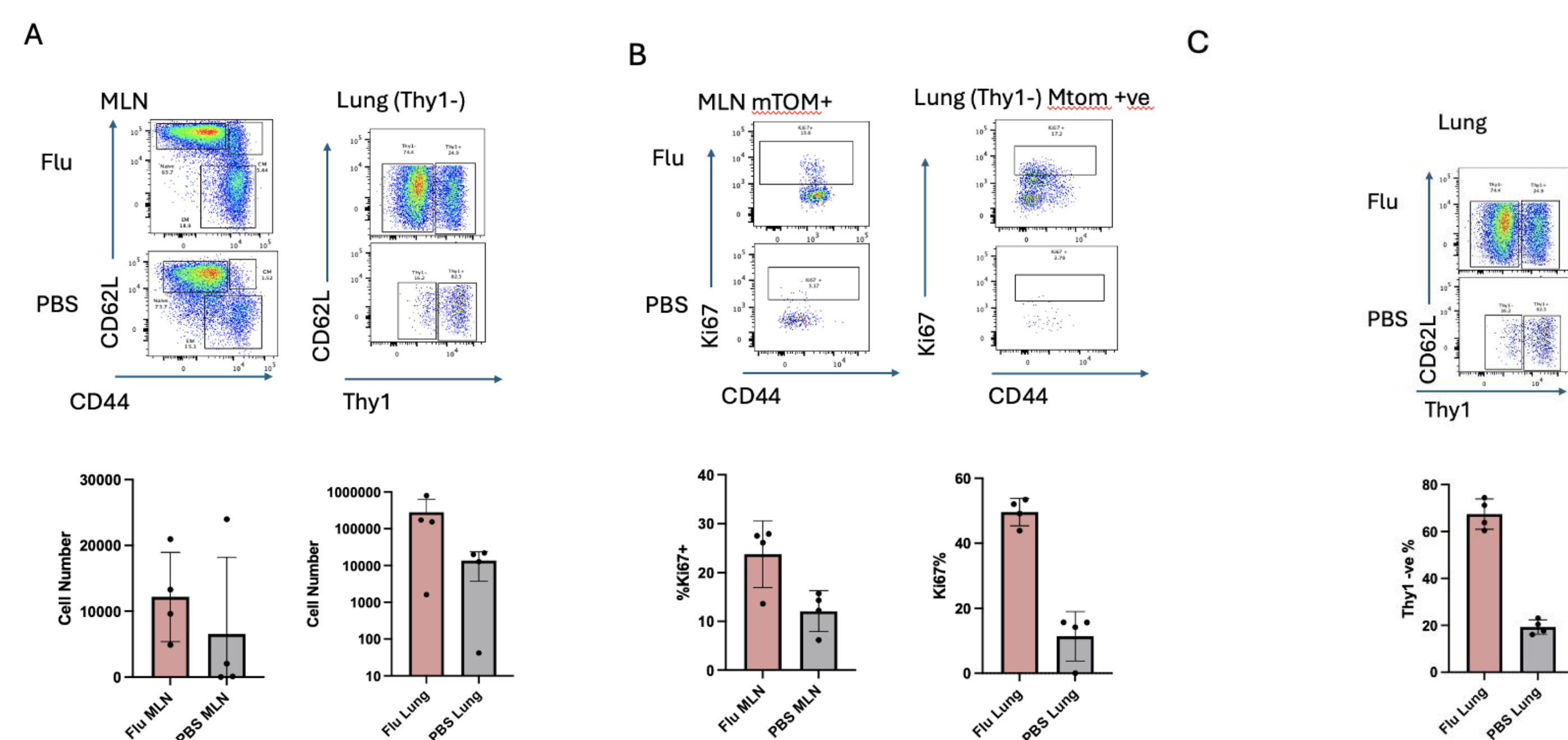


Figure 3: CD4+ EM cell counts, Ki67 expression and protection from Thy1 staining identify T cells that are responding to flu infection. A) FACS plots and graphs showing number of CD4+ EM T cells in the lungs and MLN B) FACS plots and graphs showing the frequency of CD4+ EM expressing Ki67 in the lungs and MLN C) FACS plot and graph showing the frequency of CD4+ EM protected from Thy1 staining in the MLN and lungs of PBS treated and flu infected mice n=4.

- These graphs and FACS plots show higher CD4+ EM cell counts in the Flu lung and Flu MLN samples, suggesting there has been a response
- Higher Ki67+ expression among Flu lung and Flu MLN CD4+ EM cells suggesting increased proliferation in the infected mice indicating a response
- Higher number of Thy1- CD4+ EM cells in the flu lung. These are the EMs in the lung tissues clarifying a response has occurred in the lungs themselves

The Spleen and LN served as controls within the infected Mice

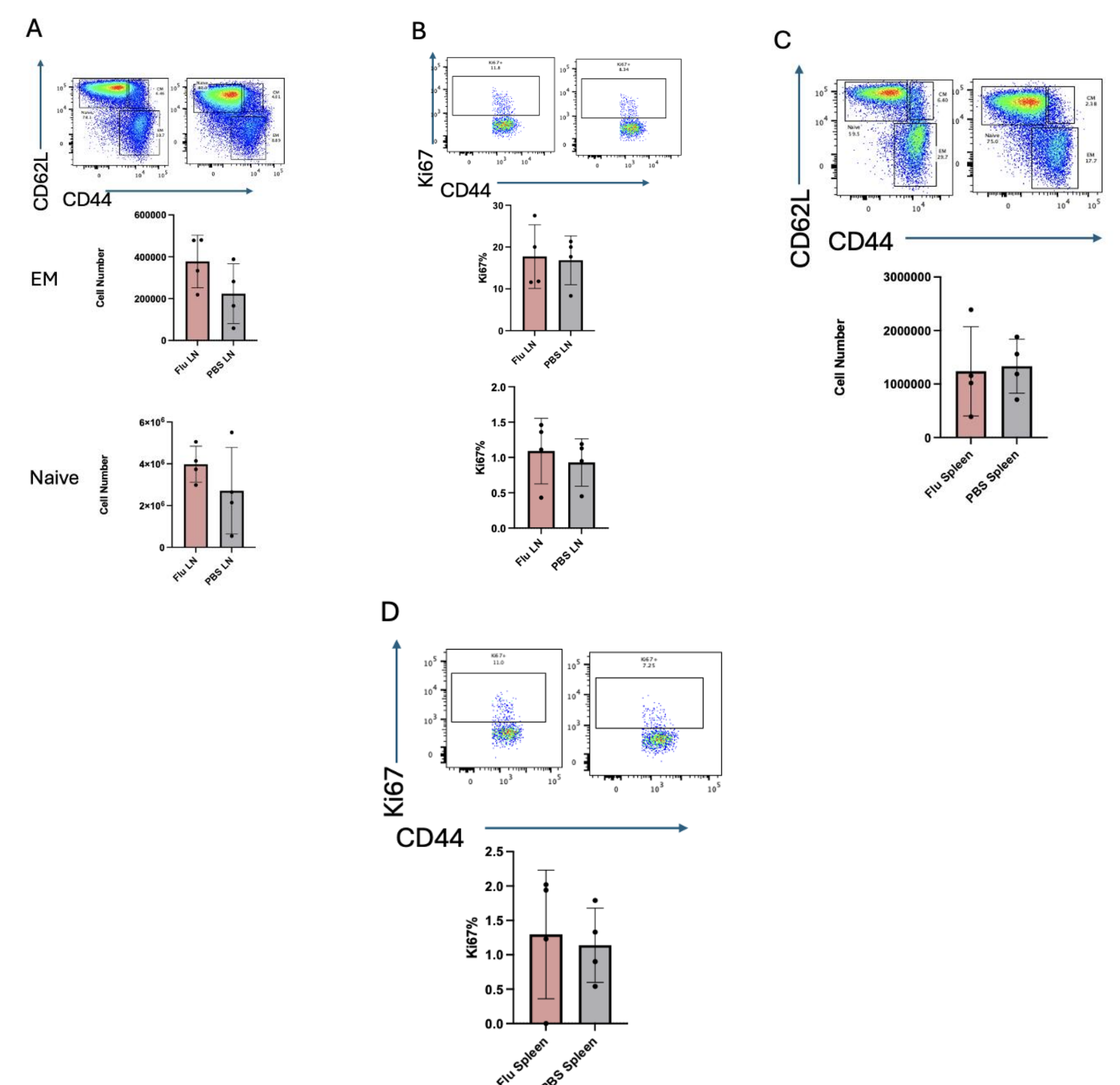


Figure 4: CD4+ EM and CD4+ naive cell counts and Ki67 expression identifying T cells that are not responding to flu. A) FACS plots and Graphs showing number of CD4+ EM and naive T cells in the LN and Spleens. B) FACS plots and graphs showing Ki67+ EM and Ki67+ Naive cell frequency in the LN. C) FACS plots and graph showing the number of CD4+ EM in the spleen. D) FACS plots and graphs showing CD4+ Naive cell Ki67 expression in the spleen n=4.

- Minimal to no difference between PBS and Flu spleen and LN EM and naive cell counts, suggesting a lack of response in these organs
- Minimal to no difference between PBS and Flu spleen and LN EM and naive cell Ki67+ expression suggesting a lack of increased proliferation and therefore a lack of response

Are Flu Specific CD4+ EMs derived from younger or older cells

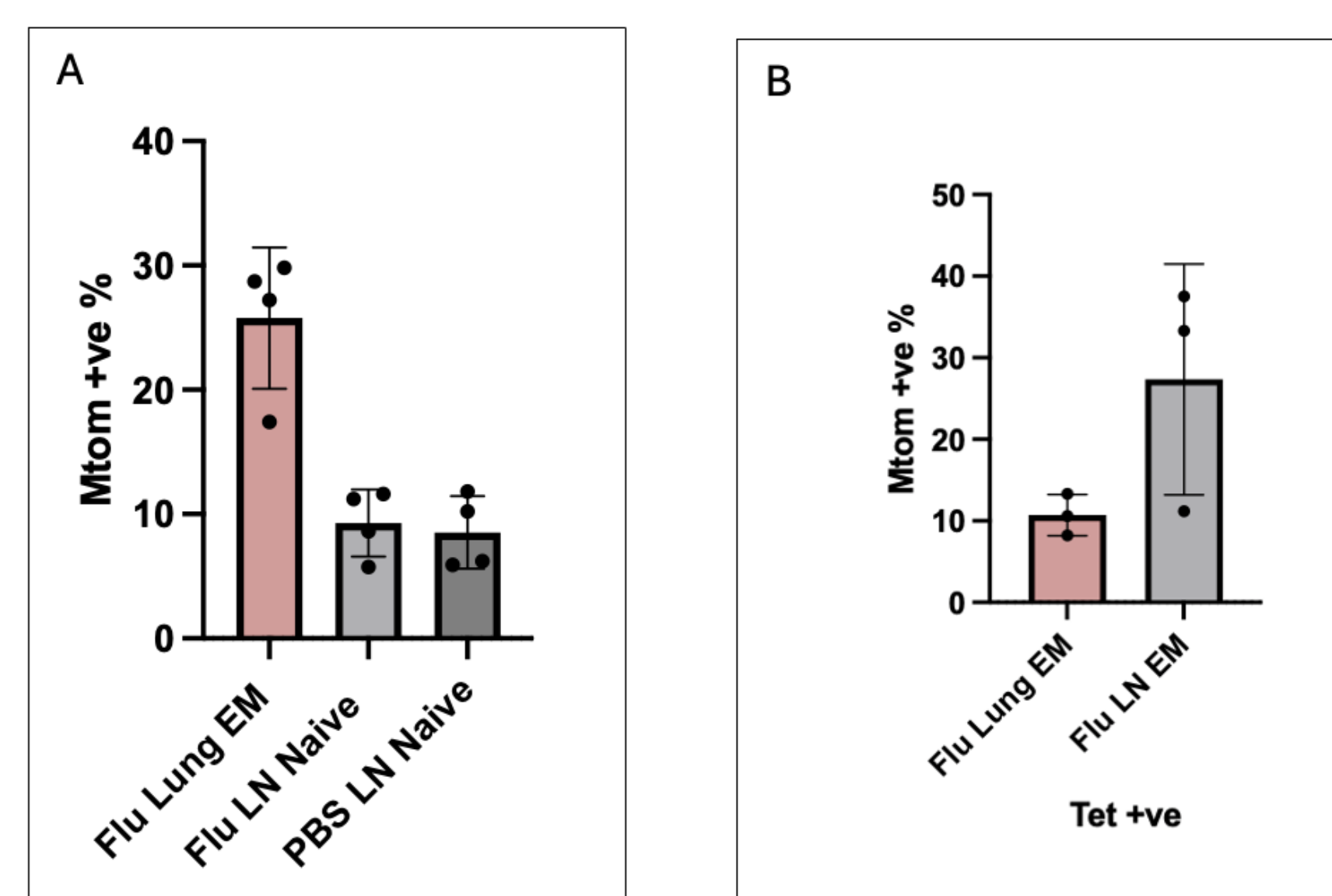


Figure 5: Mtom+ frequencies among CD4+ EM T cells in the lungs and Naive CD4+ T cells in the LN as well as among Tetramer positive lung and LN EMs. A) Graph showing Mtom+ frequency among Flu lung CD4+ EM and LN CD4+ naive. B) Graph showing Mtom+ frequency of tetramer positive CD4+ EMs in the flu lung and LN

- Initial observations showed a higher proportion of mtom+ EMs in the lung than mtom+ naive cells in the LN hinting at improved response by older CD4+ cells
- However, equal proportion of mtom+ Tetramer+ (flu-specific) EMs in the lung as in the LN naive cells therefore indicating that the older cells did not have a varied response to flu

Discussion

- Older (mtom+) CD4+ naive cells gave rise to a higher number of CD4+ EM cells relative to the younger (mtom-) CD4+ naive cells
- However, the older CD4+ naive cells gave rise to the same proportion of flu specific EMs as the younger naive CD4+ cells. This tells us that in fact the older cells are not necessarily responding better and so age doesn't then have an effect on the response.
- The next question to ask is how much are the extra mtom+ non flu-specific EMs contributing to the response

Conclusion

The data collected is overall suggesting that the age of CD4+ T cells does not have an effect on the response to influenza