The aged lymphoid tissue environment fails to support naïve T cell homeostasis

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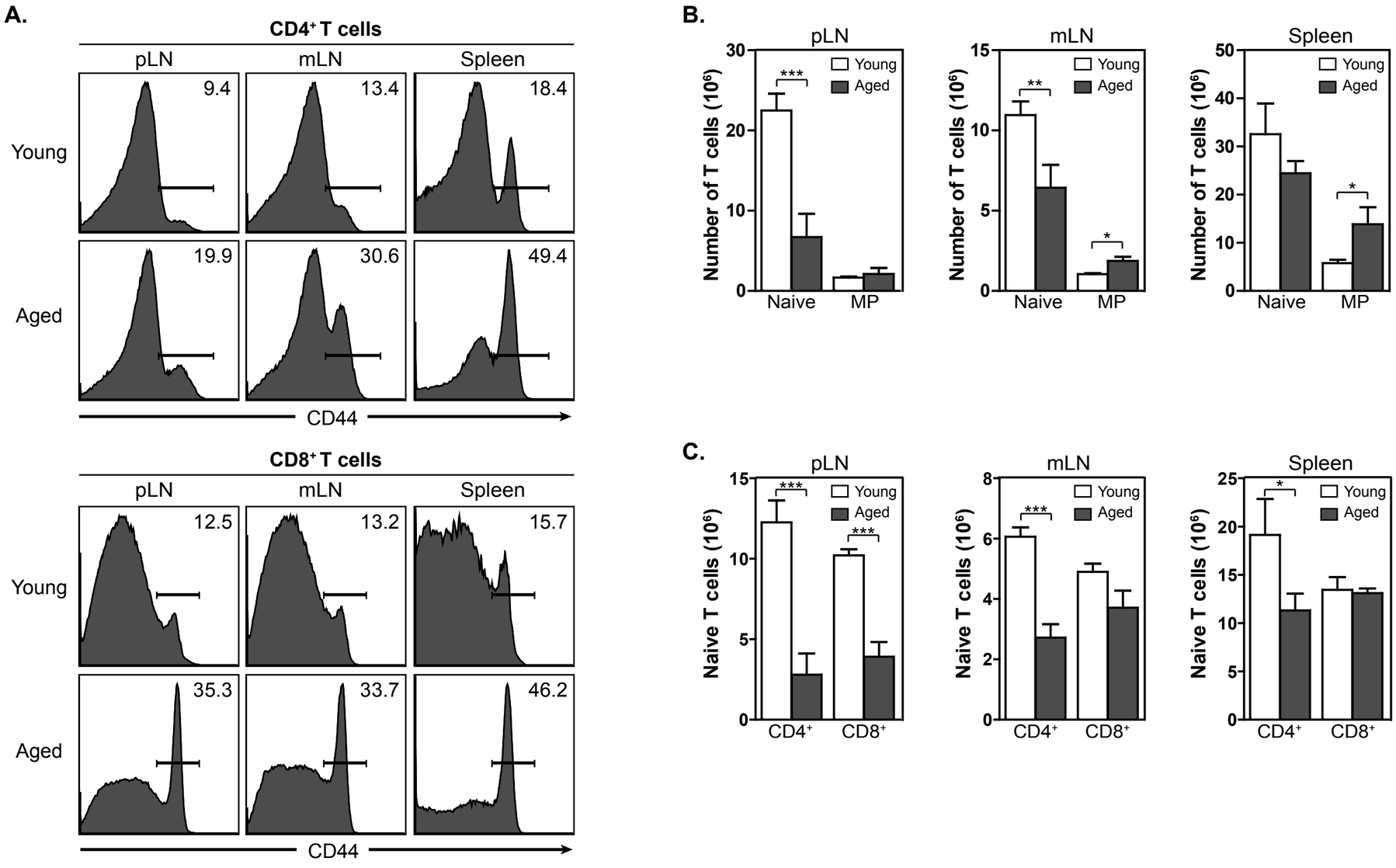
Supplementary Figure 1: The number of naïve T cells declines in aged mice. (**A-C**) Cells isolated from the pLN, mLN, and spleen of young and aged B6.Foxp3^{GFP+} mice were stained with anti-TCR-β, CD4, CD8, and CD44 antibodies and the number and phenotype of T cells was determined by FACS. **A**) Representative histograms show CD44 staining gated on TCR-β^{+} Foxp3^{GFP-} CD4^{+} or CD8^{+} T cells in young and aged mice. Numbers indicate the percentage of CD44^{hi} MP CD4^{+} or CD8^{+} T cells. **B**) Bars demonstrate the average number of naïve (CD44^{low}) or MP (CD44^{high}) T cells (±SD) recovered from young and aged mice (n=3 mice per group). **C**) Bars depict the recovery of naïve CD4^{+} or CD8^{+} T cells (±SD) from young and aged mice. *P<0.05, **P<0.01, and ***P<0.001 as determined by unpaired Student's T-test.

Supplementary Figure 2: Reduced T cell homeostatic proliferation in pLN, mLN, and spleen. (**A-B**) Naïve T cells from young B6.CD45.1^{+} mice were labeled with CTV and 1.5 X 10^{6} T cells were injected into irradiated young and aged B6.CD45.2^{+} hosts. Hosts were injected i.p. with 3 mg/kg FTY720 on 1 and 3 days after cell transfer. Proliferation of donor cells was analyzed 7 days after adoptive transfer. Histograms show the proliferation of CD4^{+} (**A**) or CD8^{+} (**B**) donor T cells in the indicated tissue of young and aged hosts. Histograms are representative of 2 independent experiments with 3-4 mice per group.

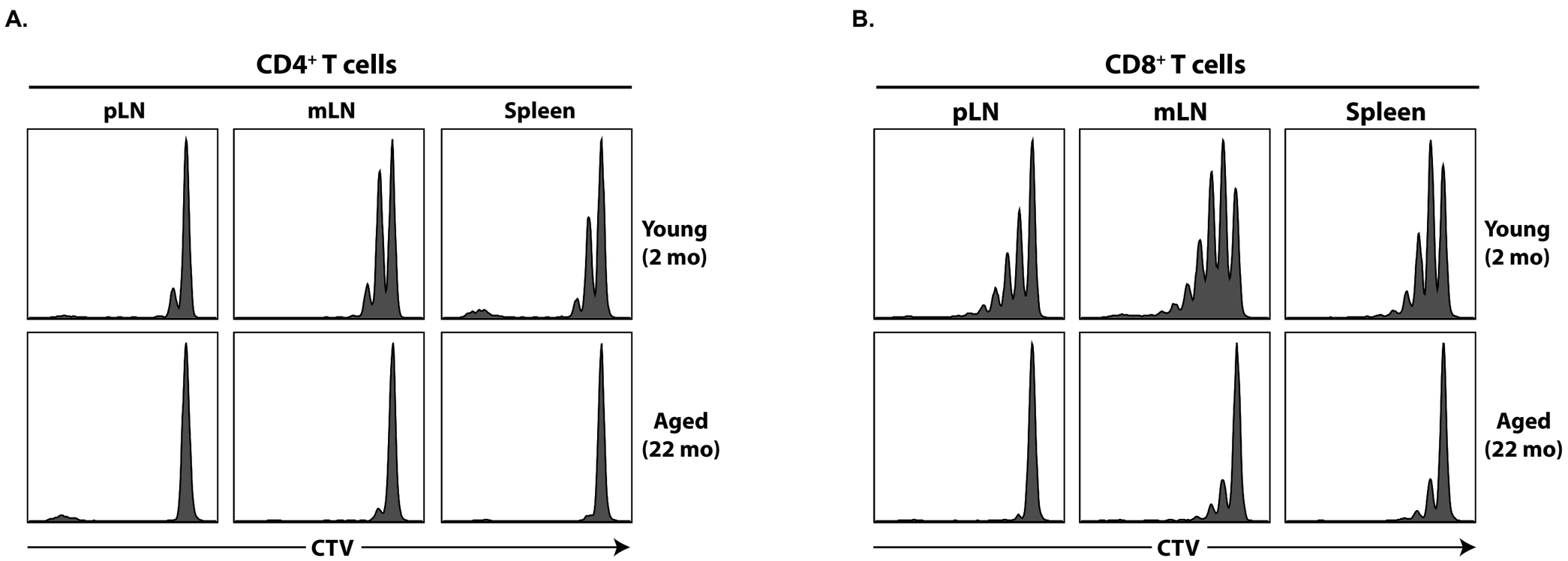
Supplementary Figure 3: Signaling defects in the mLN and spleen of aged mice. (**A-B**) Naïve T cells from young B6.CD45.1^{+} mice were injected into irradiated young (2 mo) and aged (22 mo) B6.CD45.2^{+} hosts. Phosphorylation of STAT5 (**A**) and CD127 expression (**B**) were analyzed in the

mLN and spleen one day later by flow cytometry. (**C**) Sorted MP $CD4^+$ and $CD8^+$ T cells were injected into irradiated young (2 mo) and aged (18 mo) hosts. One day later, donor cell pSTAT5 and CD127 levels were analyzed in the pLN by flow cytometry. Data is representative of 2 independent experiments with 2-5 mice per group. *P<0.05 and **P<0.01 as determined by unpaired Student's T-test.

Supplemental Figure 1:



Supplemental Figure 2:



Supplemental Figure 3: