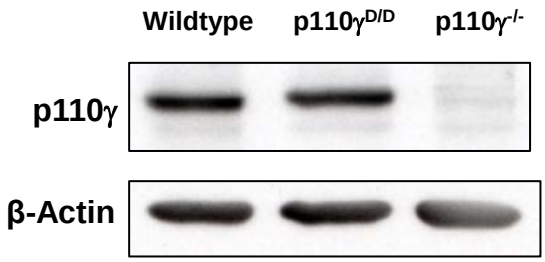
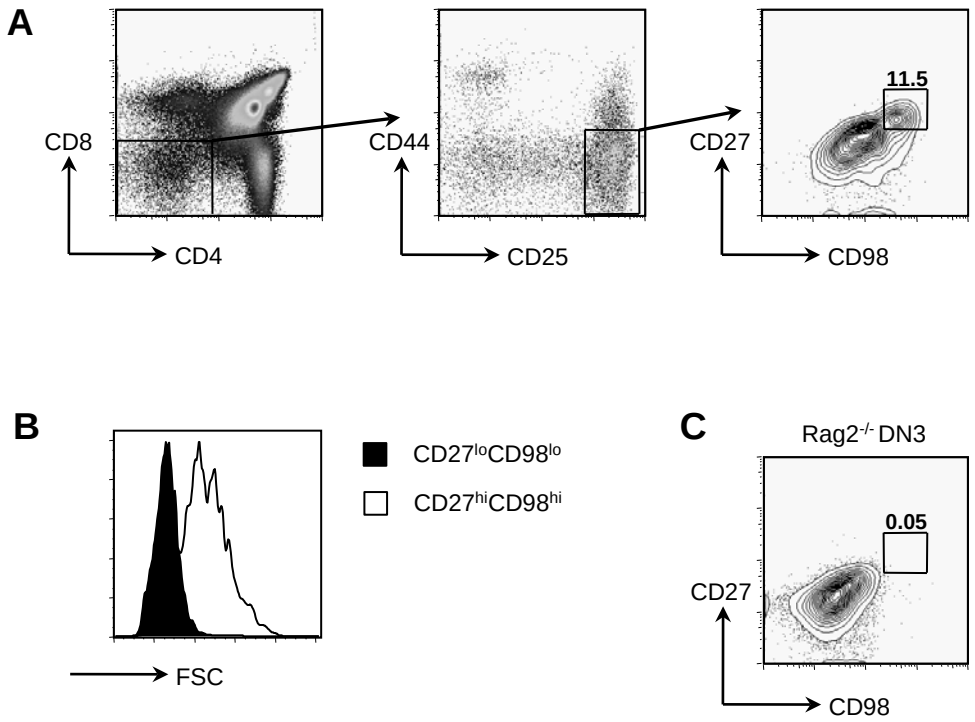


Supp. Figure 1 Janas et.al



Supp. Figure 2

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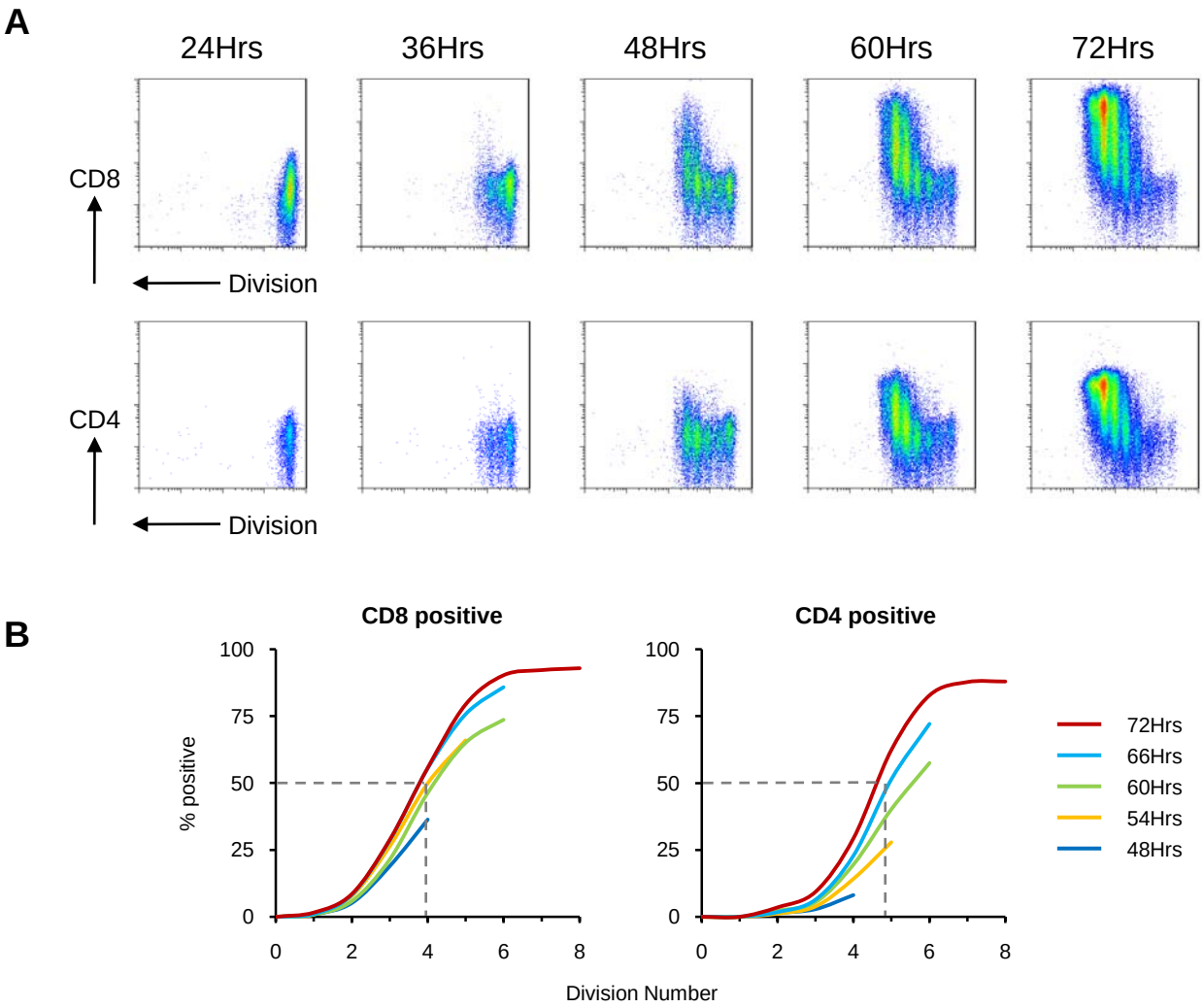

Supp. Figure 3

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Supplementary Figure Legends

Supplementary Figure 1. p110γ expression in thymocytes. Western blot analysis of p110γ expression in thymic lysates from WT, $p110^{D/D}$ or $p110\gamma^{-/-}$ mice.

Supplementary Figure 2. Expression of CD27 and CD98 on DN3 thymocytes. (**A**) FACS profiles from a wildtype mouse showing coincidence of CD98 and CD27 expression in DN3 thymocytes. The $CD98^{hi}CD27^{hi}$ cells represent a minority of the DN3 population and are termed DN3b. (**B**) Forward Scatter FACS profiles show that $CD98^{hi}CD27^{hi}$ DN3b cells are larger than the $CD98^{lo}CD27^{lo}$ DN3a population, reflecting their growth and proliferation. (**C**) TCRβ rearrangement is required for the generation of DN3b cells as this population is absent in $Rag2^{-/-}$ mice.

Supplementary Figure 3. In vitro analysis of DN3 differentiation to DP T cells. (**A**) FACS analysis showing division versus CD8 or CD4 expression on WT DN3a ($CD25^{hi}CD98^{lo}$) cells cultured on OP9-DL1 stroma for various time periods. (**B**) Graphic representation of CD8 and CD4 expression versus division number demonstrating division-dependent and time-independent differentiation of DN3 cells to DP. The graphs show the onset of CD8 expression occurring approximately one division prior to the expression of CD4. Graphs represent the mean from three independent experiments.