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Supplemental information

MYC deficiency impairs the development of effector/memory T lymphocytes

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	Genes used to identify T cell populations
DN	Cd4- and CDd8-, Il2ra+, Ptcra+, Tcrb+, Tcf7+, Tcrg-C1+, Dntt+,Sell+
ISP	Cd4- and Cd8+, Ptcra+, Tcrb +, Tcf7+, Dntt+
DPblast (cycling)	Cd4+ and Cd8+, Cdk1+, Pcna+, Mki67+, Tcf7+, Ccnd3+, Dntt+, Trac+, Satb1+
DPsmall (exit of cell cycle)	Cd4+ and Cd8+,Ccnd3+, Cdk1-, Trac+, Tcf7+, Ccr9+, Dntt+, Rag1+, Satb1+
DP to SP	Cd69+, tox2+ , Tcf7+, Satb1+, Cd5+
Dying cells	Percentage of mitochrondrial mRNA
early SP	Cd4+ or Cd8+, Ccr7+, cd69+
CD4 naive	Cd4+, Ccr7+, Ccnd2+, Sell+
CD8 naive	Cd8+, Ccr7+, Ccnd2+, Sell+, Nkg7+
Treg	Foxp3+, Il2ra+, Ikzf2+
CD4 Eff/mem	Cd4+, Ccr7-, Ccnd2+
CD8 CTL	Cd8+, Gzma+, Ccl5+, Cxcr3+, Ccnd2+, Ly6c2+, Nkg7+, Ifng+
CD8 mem	Cd8+, Ccl5+, Sell+, Ccnd2+, Ly6c2+, Nkg7+
Tγδ	Tcrg-C1+, Tcrd+, Aes+, Anxa1+

Table S1. Gene markers used to annotate cell type, related to Figure 2.

Table of genes used to identify cell populations on the UMAP plot. Genes labelled as "+" are expressed in the population of interest while "-" labelled genes are not. The choice of these markers is partially based on markers previously described (Chopp et al., 2020; Mingueneau et al., 2013; Park et al., 2020) and on Immgen Database.

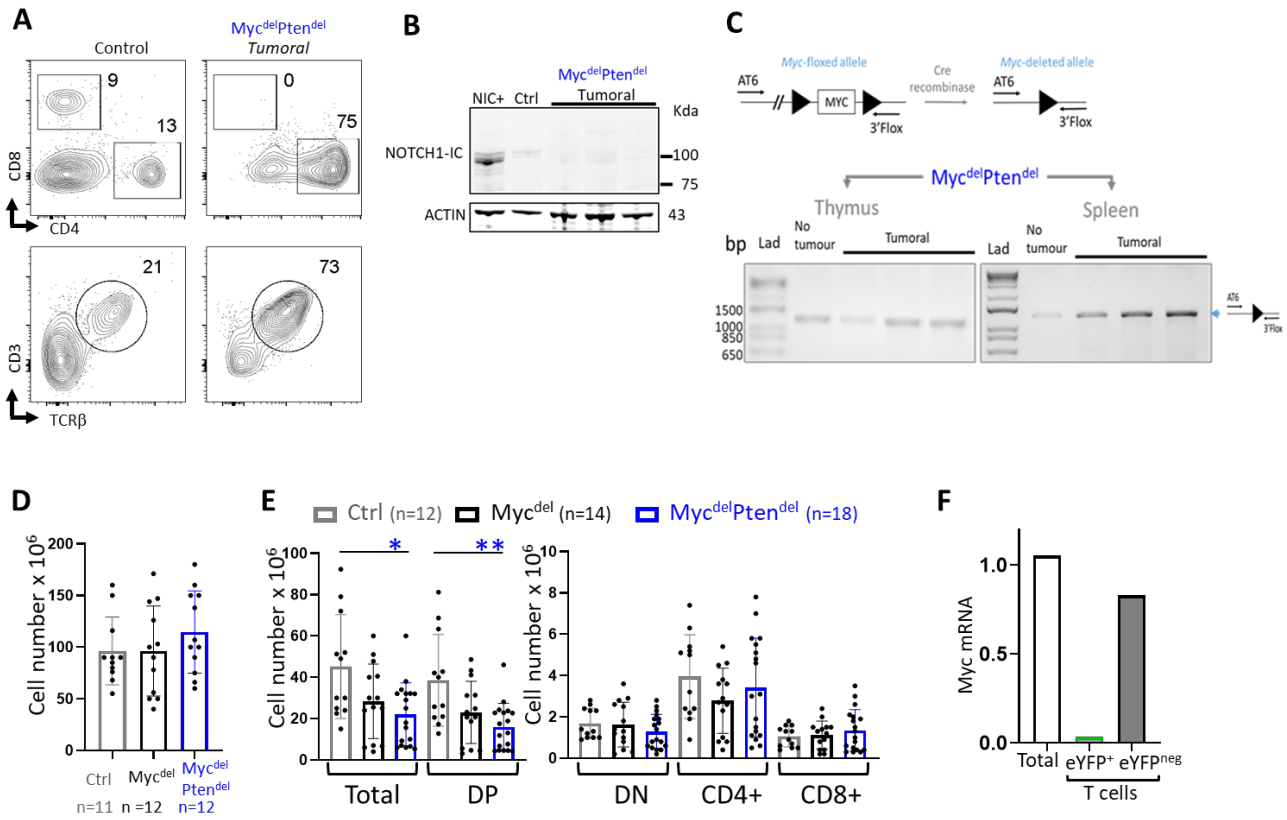

Figure S1. Impact of MYC deficiency on T cell development, related to Figure 1.

(A) Phenotype of tumors generated by *Myc*^{del}*Pten*^{del} mice in the spleen. CD4/CD8 (top plots) and CD3/TCRβ expression (bottom plots) were analysed by flow cytometry. Control spleen is shown (left plots). (**B**) Analysis of intracellular NOTCH1 (NOTCH1-IC) expression by immunoblot. Positive control for NOTCH1 hyperactivation (NIC+) corresponds to T-ALL cells from RAG1-deficient *Pten*^{del} mice (Gon et al., 2018); Ctrl corresponds to thymic cells from a Control mouse. T-ALL developed by the 3 *Myc*^{del}*Pten*^{del} mice were analyzed and none displays NOTCH1 activation. (**C**) Analysis of *Myc*-deletion. (Top) Schematic representation of Myc Floxed-allele before and after Cre recombination. Locations of the primers are indicated. (Bottom) Around 70 ng of thymic or splenic genomic DNA from each 3 tumoral *Myc*^{del}*Pten*^{del} mice and from one disease-free *Myc*^{del}*Pten*^{del} mice were amplified with AT6 and 3'Flox primers. Lane 'Lad' is the 1 kb DNA size marker. A PCR product is detected in tumoral samples suggesting that at least one *Myc*-allele has been deleted.(**D**) Absolute number of thymocyte in young (less than 10 weeks old) (**E**) and older mice (aged between 3 to 10 months) (**C**). In (**D**) total thymocyte number is shown. (**D & E**) Each dot represents a distinct mouse (number of mice analyzed is indicated). Error bars represent means with standard deviation (SD). Statistical significant differences were assessed using Mann-Whitney test: *P<0.05; ** P< 0.01). (**F**) Analysis of *Myc* mRNA expression by quantitative RT-PCR performed on cDNA obtained from total spleen and from sorted eYFP^{+} and eYFP^{neg} T cells. Transcripts levels were normalized to ABL. This quantification of *Myc* mRNA shows that *Myc* expression in total *Myc*^{del}*Pten*^{del} spleen is similar than eYFP negative sorted T cells, while *Myc* expression level is abrogated in eYFP^{pos} T cells. It confirms that in eYFP^{neg} cells, Cre is not expressed and thus *Myc* is not deleted. Splenic CD3+TCRβ+ T cells were sorted according to eYFP expression using a FACSAriaIII cytometer (BD).

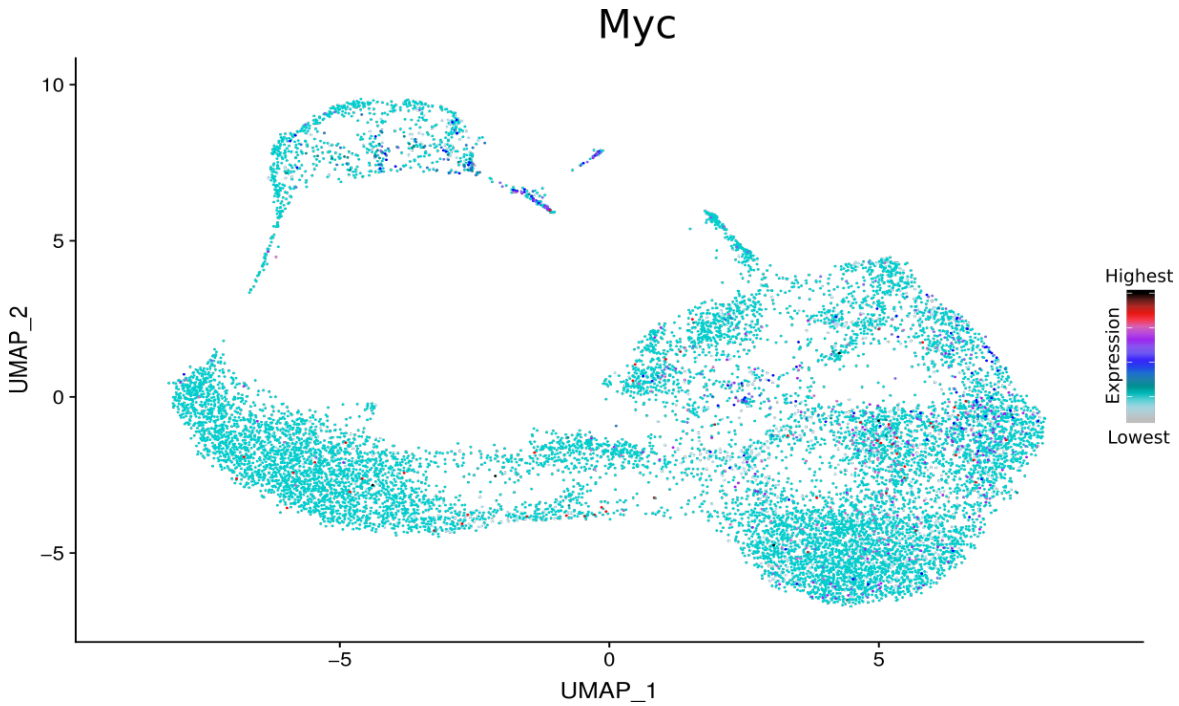

Figure S2. Analysis of *Myc* expression, related to Figure 2.

Myc mRNA expression is visualized on the UMAP plot which is described in Figure 2. Altough, *Myc* mRNA is not very well captured in our scRNAseq analysis, it is slightly detected in DN and ISP cells (clusters 22 and 21). This is consistent with previous bulk transcriptomic analysis which indicates that the highest *Myc* expression level is observed between DN3b and ISP stages (Mingueneau et al., 2013).

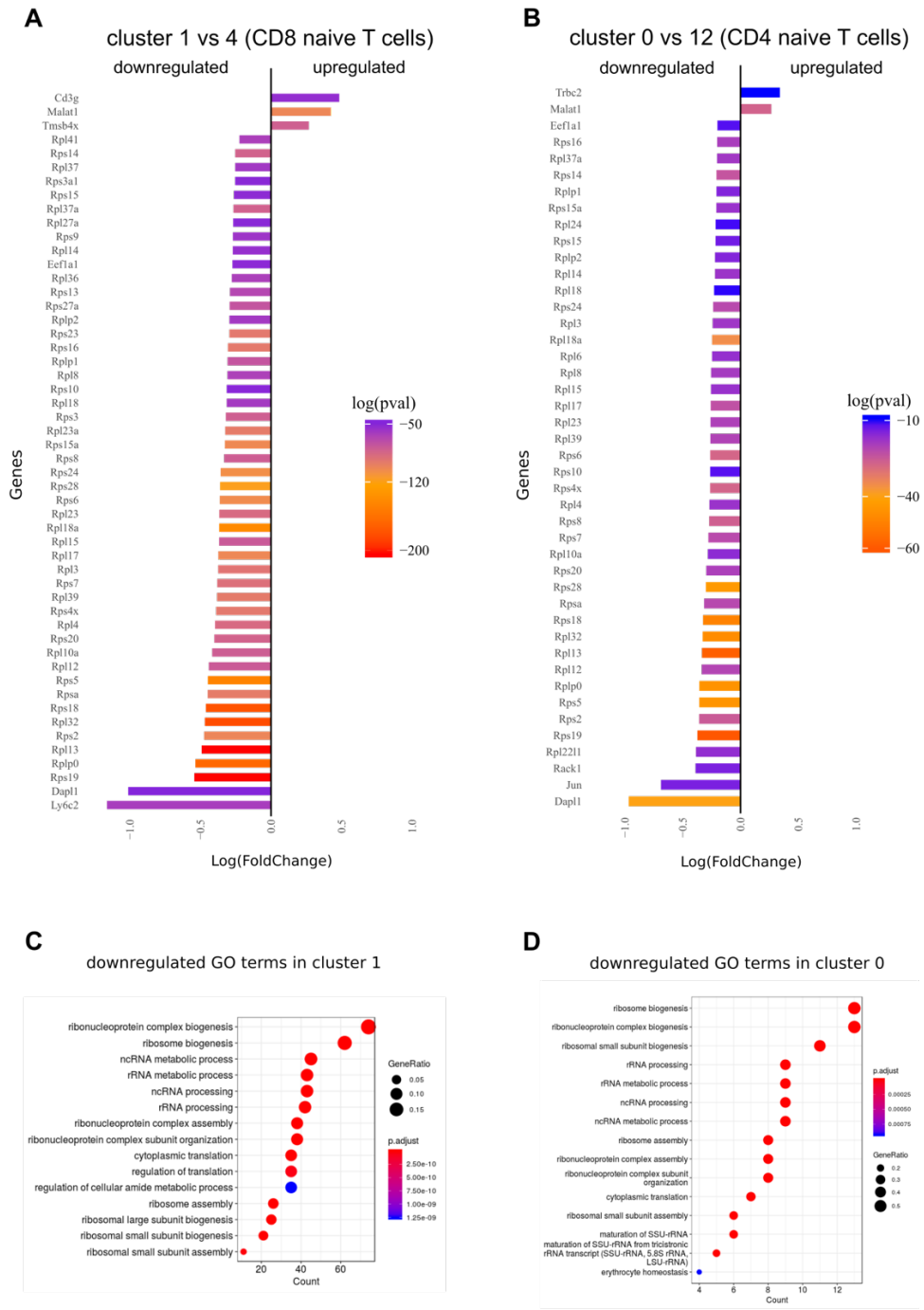

Figure S3. Differential gene expression analysis between MYC-deficient and MYC-proficient naïve T cell clusters, related to Figure 2.

Top differentially expressed genes in CD8 naïve T cells for cluster 1 (*Myc*^{del} / *Myc*^{del}*Pten*^{del}) versus cluster 4 (*Pten*^{del} / Control) (**A**), and in CD4 naïve T cells for clusters 0 (*Myc*^{del}/ *Myc*^{del}*Pten*^{del}) versus cluster 12 (*Pten*^{del} /Control) (**B**). Genes having a fold change > 0.2 and an adjusted p-value < 1e-50 (**A**) and < 1e-10 (**B**) are shown. The length of the bar represents the log(fold change) while the color represents the log(adjusted p-value). Top 15 enriched biological process GO terms for genes differentially expressed between cluster 1 versus cluster 4 (**C**), and cluster 0 versus cluster 12 (**D**). (**C & D**) Dot size represents gene ratio and dot color, adjusted p value.

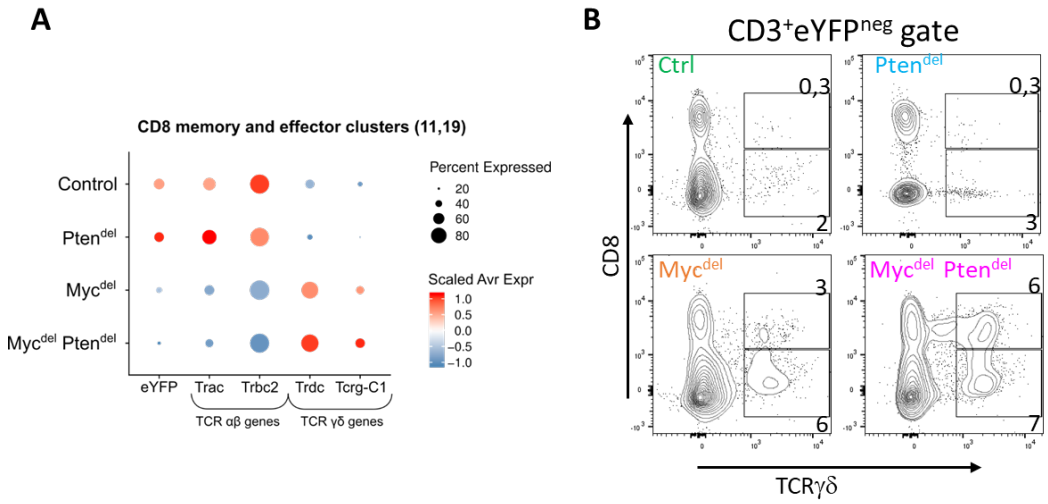

Figure S4. Increase of CD8$^+$TCR$\gamma\delta^+$ T cells in spleens of *Myc^{del}* and *Myc^{del}Pten^{del}* mice, related to Figure 4E.

(**A**) Dotplot showing the expression level of eYFP and TCR genes in memory (cluster 11) and effector (cluster 19) CD8 T cells. Color represents the scaled average expression of the gene of interest, while dot size indicates the proportion of cells expressing the gene of interest. (**B**) Typical FACS plots showing TCR$\gamma\delta$ and CD8 expression in CD3$^+$eYFP^{neg} gated cells. Genotype of the mice and percentages of cells in depicted gates are indicated.