Supplementary Fig.1

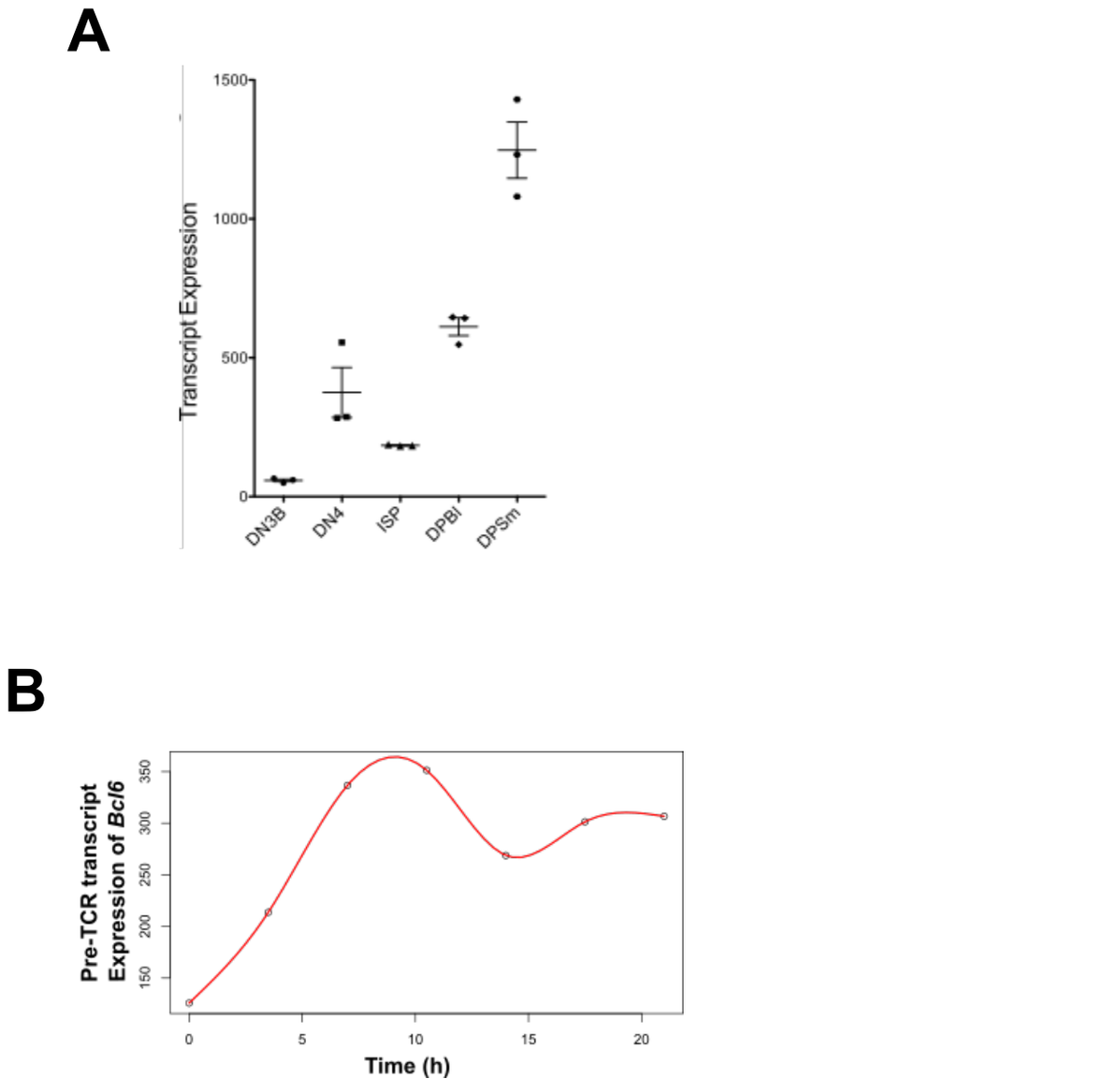

Figure S1 (Figure S1): *Bcl6* is upregulated following pre-TCR signal transduction and regulates foetal thymocyte development on E15.5

(A) *Bcl6* transcript expression in sorted thymocyte populations from the Immgen database (GSE15907): DN3B, DN4, ISP DPblast (DPBl) and DPsmall (DPSm).

(B) Transcript expression of *Bcl6* in anti-CD3 treated Rag1-/- thymocytes plotted against time (hours), where t=0 is when the stimulus was added in FTOC, determined by microarray (E-MTAB-3088).

Supplementary Fig.2

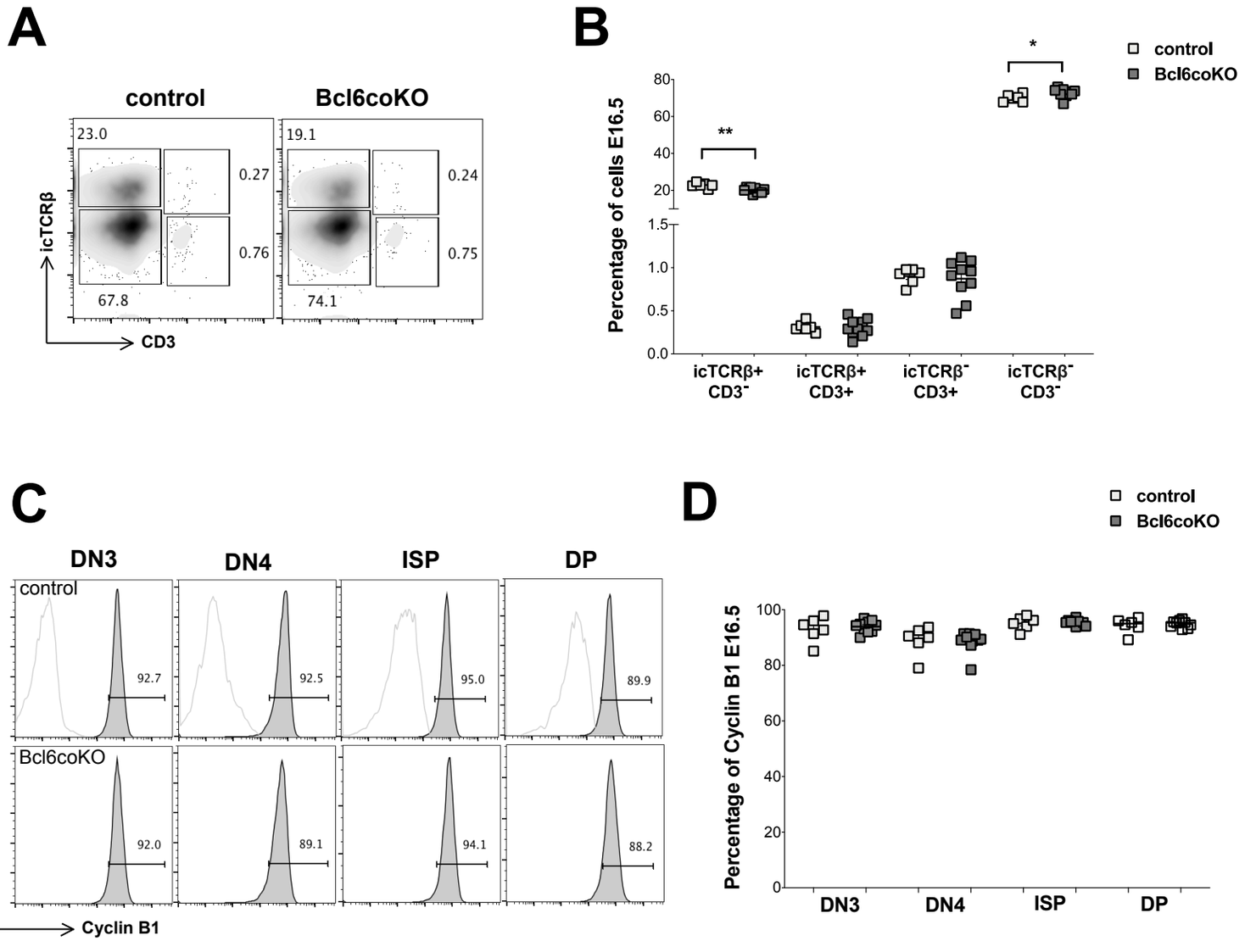

Figure S2 (Figure S2): CD3+TCRβ- cells (γδ T-cells) and intracellular Cyclin B1 expression in E16.5 Bcl6coKO and control thymus

(A-B) Flow cytometry analysis of icTCRβ and cell surface CD3 expression gated on DN cells (CD4-CD8-). (A) Density plots show representative staining of E16.5 foetal thymus from control and Bcl6coKO, giving percentage of cells in the regions shown. (B) Scatter plot shows percentages of cells from control (n=6, light squares) and Bcl6coKO (n=10, dark squares) thymus giving significance by student's t-test for icTCRβ+CD3- ($p<0.05$) and icTCRβ-CD3- ($p=0.05$), where each point represents an individual embryo. There was no significant difference in the proportion of icTCRβ-CD3+ cells which represent the γδ T-cell population between control and Bcl6coKO.

(C-D) Flow cytometry analysis of intracellular Cyclin B1 expression in E16.5 thymocyte populations. (C) Histograms shows representative intracellular anti-Cyclin B1 staining gated on DN3 (CD3-CD44-CD25+), DN4 (CD3-CD44-CD25-) and ISP (CD3-CD4-CD8+) and DP (CD4+CD8+) populations in E16.5 foetal thymus from control and Bcl6coKO, giving the percentage of cells in the marker shown, and the negative control (isotype-matched) staining as a faint overlay. (D) Scatter plot shows percentages of cells in the different thymocyte subsets from control (n=6, light squares) and Bcl6coKO (n=10, dark squares) thymus, where each point represents an individual embryo.

Supplementary Fig.3

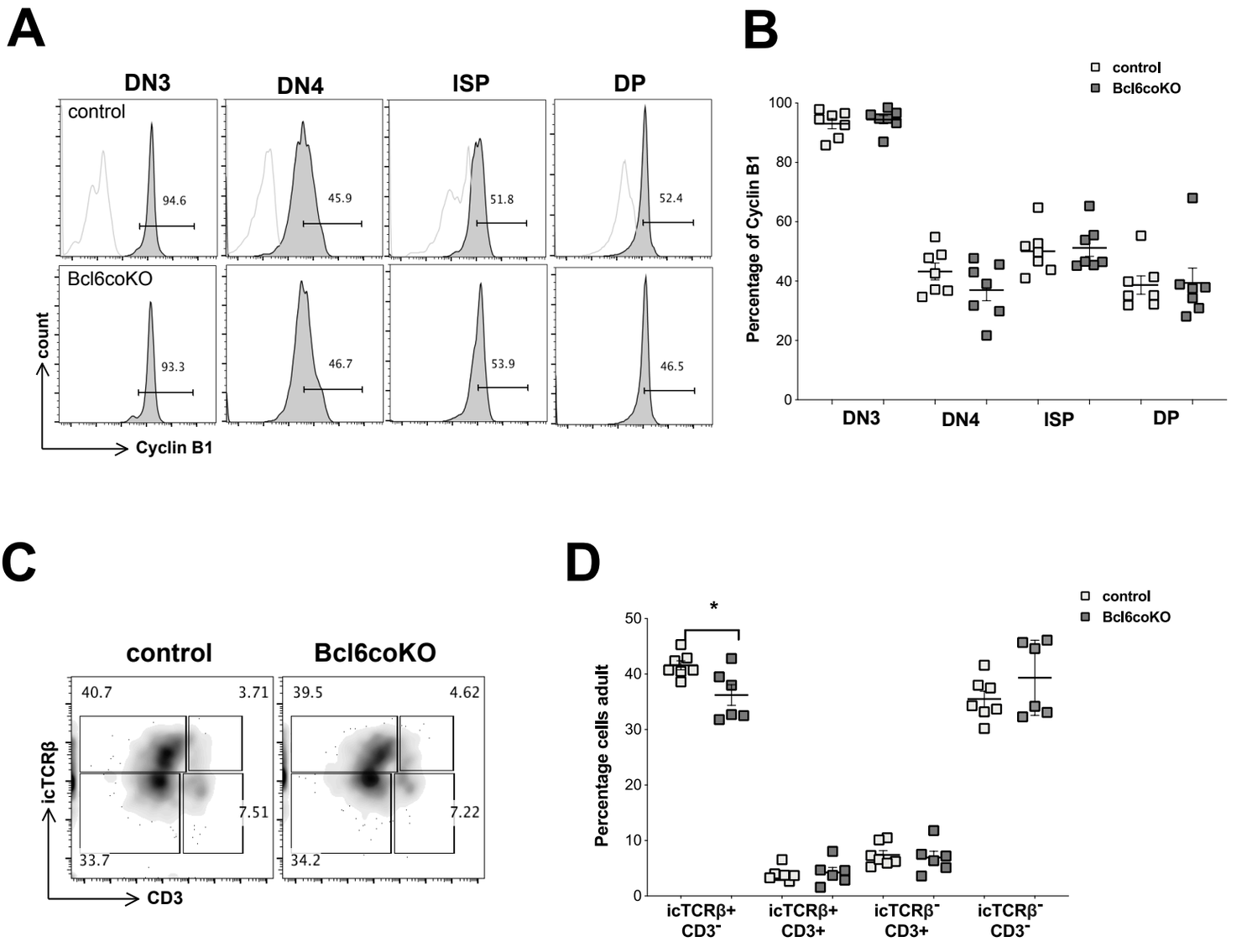

Figure S3 (Figure S3): Intracellular Cyclin B1 expression and CD3+TCRβ- cells (γδ T-cells) and in adult Bcl6coKO and control thymus

(A-B) Flow cytometry analysis of intracellular Cyclin B1 expression in adult thymocyte populations. (A) Histograms shows representative intracellular anti-Cyclin B1 staining gated on DN3 (CD3-CD44-CD25+CD4-CD8-), DN4 (CD3-CD44-CD25-CD4-CD8-) and ISP

(CD3-CD4-CD8+) and DP (CD4+CD8+) populations in adult thymus from control and Bcl6coKO, giving the percentage of cells in the marker shown, and the negative control (isotype-matched) staining as a faint overlay. (B) Scatter plot shows percentages of cells in the different thymocyte subsets from control (n=7, light squares) and Bcl6coKO (n=7, dark squares) thymus, where each point represents an individual mouse.

(C-D) Flow cytometry analysis of icTCRβ and cell surface CD3 expression gated on DN cells (CD4-CD8-). (C) Density plots show representative staining, giving the percentage of cells in the regions shown. (D) Scatter plot shows percentages of cells from control (n=6, light squares) and Bcl6coKO (n=10, dark squares) thymus giving significance by student's t-test for icTCRβ+CD3- ($p<0.05$), where each point represents an individual embryo. There was no significant difference in the proportion of icTCRβ-CD3+ cells which represent the γδ T-cell population between control and Bcl6coKO.

Table S1: Antibodies used for flow cytometry

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