

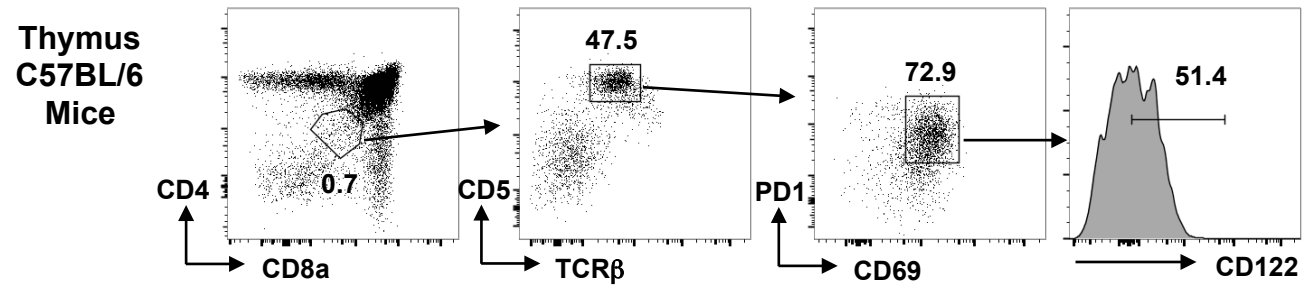
Increased TCR signal strength in DN thymocytes promotes development of gut $\text{TCR}\alpha\beta^{(+)}\text{CD8}\alpha\alpha^{(+)}$ intraepithelial lymphocytes

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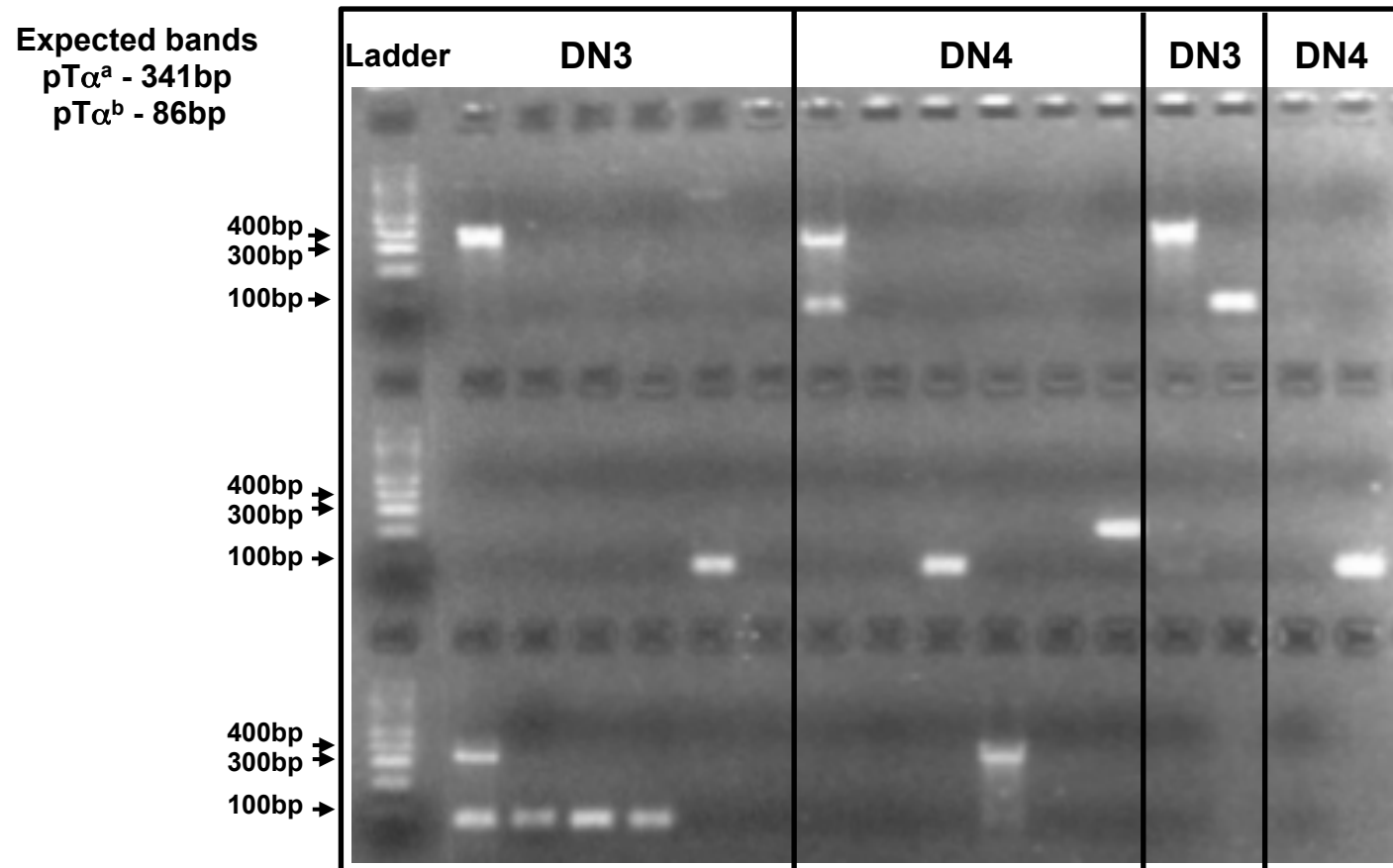
Supplementary Data S1



Supplementary Figure S1

Flow cytometry gating strategy used to identify thymic IEL progenitors (as described by *Mayans et al., Immunity 2014, 41 p207-218*)

Supplementary Data S2

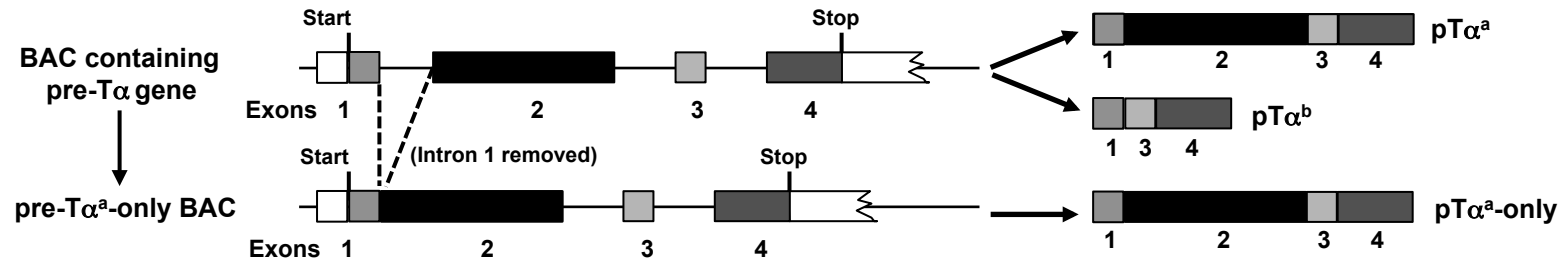


Supplementary Figure S2

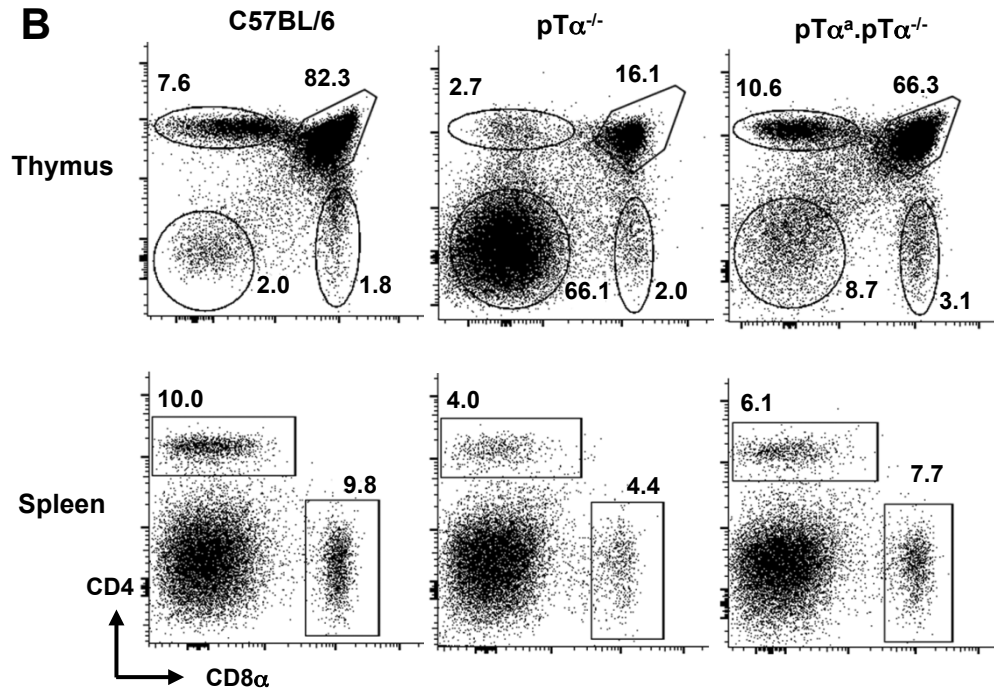
Agarose gel from single-cell PCR experiments demonstrating existence of cells expressing pT α^a only, pT α^b only, or both isoforms. Protocol and primers are described in methods. Expected bands are 341bp for pT α^a and 86bp for pT α^b . Bands were excised and sequenced to confirm identity.

Supplementary Data S3

A



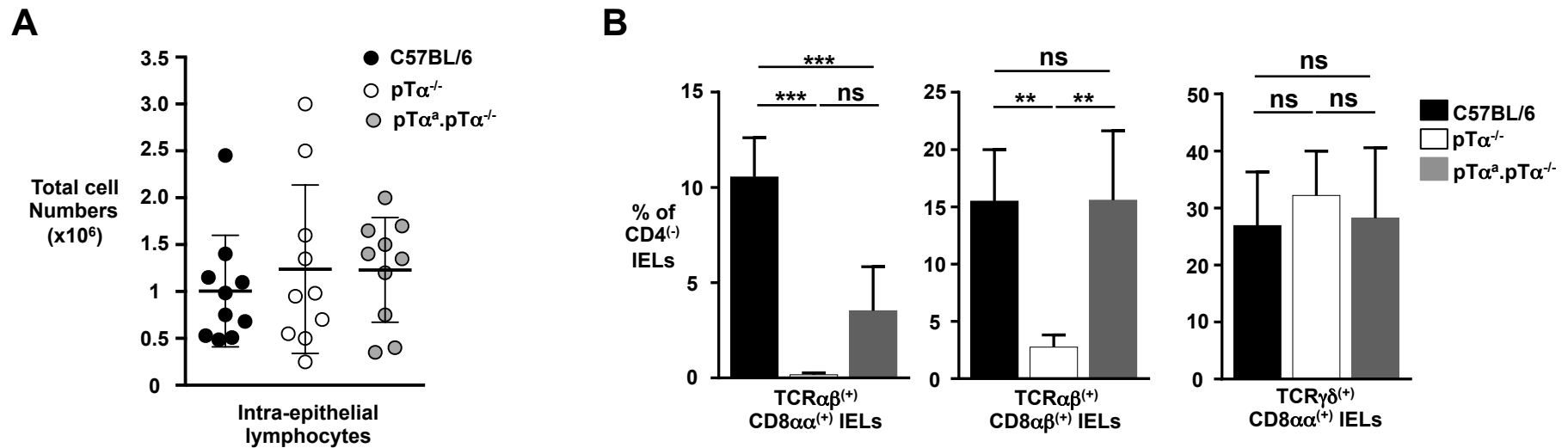
B



Supplementary Figure S3

(A) Schematic representation of the strategy used to generate pT α^a BAC-transgenic mice. (B) Representative CD4 versus CD8 flow cytometry plots from thymus and spleen of C57BL/6, pT α -deficient and pT α^a .pT $\alpha^{-/-}$ mice. Percentages of gated cells are indicated.

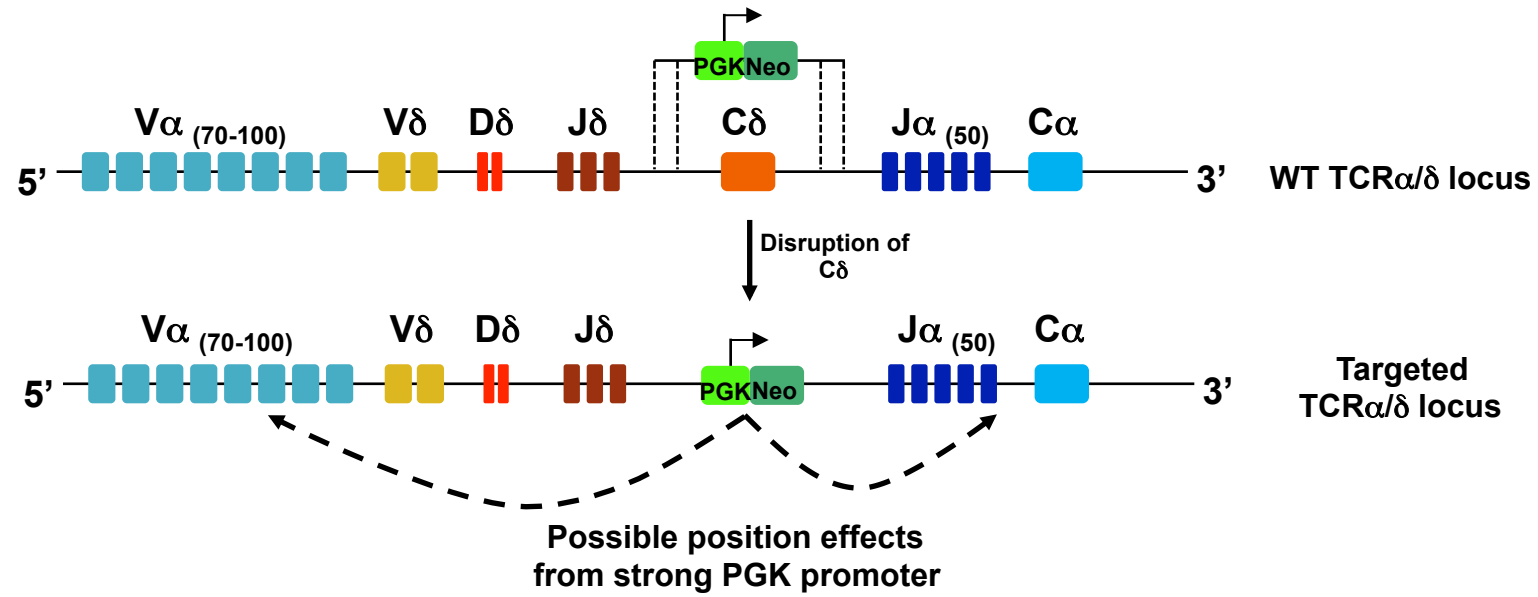
Supplementary Data S4



Supplementary Figure S4

(A) Total cell yield for IEL-preps from C57BL/6, $pT\alpha^{-/-}$ and $pT\alpha^a.pT\alpha^{-/-}$ mice. (B) Summary bar graphs ($n > 6$) of the percentages (of total $CD4^{(-)}$ IELs) of $TCR\alpha\beta^{(+)CD8\alpha\alpha^{(+)}$, $TCR\alpha\beta^{(+)CD8\alpha\beta^{(+)}$, and $TCR\gamma\delta^{(+)CD8\alpha\alpha^{(+)}$ IELs from C57BL/6, $pT\alpha^{-/-}$ and $pT\alpha^a.pT\alpha^{-/-}$ mice. *** $p < 0.001$, ** $p < 0.01$, ns is for not significant.

Supplementary Information S5



Supplementary Figure S5

Schematic overview of the targeted TCR δ locus in TCR $\delta^{-/-}$ mice.